UNIVERSITY OF CALIFORNIA, SAN DIEGO

Marine-Derived Heterocycles: Structural, Synthetic and Biological Investigations

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Chemistry

Colin K. Skepper

Committee in charge:

Professor Tadeusz F. Molinski, Chair
Professor Michael D. Burkart
Professor William Fenical
Professor Judy E. Kim
Professor Jerry Yang

2009

The Dissertation of Colin K. Skepper is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

Chair

University of California, San Diego

2009

DEDICATION

For my wife Jamie.

For patiently supporting me as I pursued my goals, keeping me sane and reminding me what is most important in life. I couldn't have done it without you. I love you!

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ac	acetyl
Aq	aqueous
Bn	benzyl
Boc	*t*-butoxycarbonyl
Bu	butyl
CAN	ceric ammonium nitrate
CSA	camphor sulfonic acid
DCC	*N*,*N*,-dicyclohexylcarbodiimide
DCM	dichloromethane
DIBAL	diisobutylaluminum hydride
DMAP	*N*,*N*-dimethylaminopyridine
DME	1,2-dimethoxyethane
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin Periodinane
DMSO	Dimethylsulfoxide
Et	Ethyl
FT-IR	fourier transform infrared
HMPA	hexamethylphosphoramide
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HWE	Horner-Wadworth-Emmons reaction
IR	infrared

LC	liquid chromatography
LAH	lithium aluminium hydride
MIC	minimum inhibitory concentration
Me	methyl
MHz	megahertz
MOM	methoxymethyl
MS	mass spectrometry
NaHMDS	sodium hexamethyldisilazide
NBS	*N*-bromosuccinimide
NMM	*N*-methylmorpholine
NMO	4-methylmorpholine-*N*-oxide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
*i*Pr	isopropyl
PG	protecting group
Piv	pivalate
Ph	phenyl
PMB	*p*-methoxyphenyl
PPTS	pyridinium *p*-toluene sulfonate
Pyr	pyridine
TBAF	tetrabutylammoniumfluoride
TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsilyl

Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	*p*-toluenesulfonyl
UV	ultraviolet

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

ACKNOWLEDGEMENTS

First and foremost I want to thank my advisor, Prof. Ted Molinski, for his support, guidance and advice over the years. My experience in this lab has been nothing short of transformative, inspired by Dr. Molinski's passion not only for natural products but for the pursuit of broad-minded, rigorous science. I'm extremely grateful to have had the unique opportunity to work on all facets of marine natural products, from collecting sponges and tunicates in the Bahamas to isolation and structure elucidation to total synthesis. Not many graduate students receive training as thorough and diverse as this!

This dissertation could not have been written without my personal support network. Thank you to my wife Jamie for being so patient and supportive throughout. I could write a second thesis thanking you properly, but words alone would not do it justice. I am eternally grateful to my parents and my brother Nick who have always supported me and continued to do so when I decided to move to the other side of the world. Thanks for visiting so frequently! Thank you also to my mother in law Sherry for taking such good care of Jamie and I.

I am extremely grateful to Dr. Michael Burkart, Dr. William Fenical, Dr. Judy Kim and Dr. Jerry Yang for serving on my doctoral committee following my transfer from UC Davis, for the advice and guidance along the way and for taking the time to read this dissertation.

I wish to thank all the members of the Molinski Lab who have made this such a great place to work. Dr. Mako Masuno and Dr. Evan Rogers in particular spent a great deal of time discussing my projects with me and offered no end of

valuable advice. I am especially grateful to Mako for his guidance on the phorbaside configuration project (Chapter 2 of this dissertation) and to Evan who was our lab's resident technical and instrumental expert for many years and a remarkably patient teacher. I want to thank Dr. Doralyn Dalisay who obtained all of the antifungal data presented in Chapter 3 of this dissertation and whose bright and cheerful personality we all miss. Thanks to Dr. Tim Quach who worked with me on the total synthesis of enigmazole A (Chapter 4 of this dissertation) and made it possible to finish the project in 2 years. Tim's assistance in turning back the tide of country music in the lab is also gratefully acknowledged. Thanks to Brandon Morinaka for all the helpful discussions on NMR, HPLC, LCMS and CD, for keeping the lab running and having a sense of humor. I want to extend my gratitude to Dr. John MacMillan and Dr. Sarah Lievens who helped me settle into the lab in Davis and generously gave their time and advice. Thanks to Jonel Saludes, Jill Basinger, Lu Yang, Julie Pigza and Bea Flores who have all in their own unique way helped to make my grad school experience so enjoyable. I've also had the privilege of working with two very talented undergraduate researchers, David Sze and Kristy Elbel, who both demonstrated remarkable aptitude and enthusiasm for organic chemistry and were a genuine pleasure to work with.

Thanks to Dr. Anthony Mrse for assistance with NMR, Dr. Yongxuan Su for MS analyses and assistance with GC, Dr. Jeffery DeRopp (UC Davis) for assistance with NMR, Dr. James C. Fettinger (UC Davis) for the X-Ray crystal structure of compound **2.24a** and Dr. John berg for assistance with chiral GC.

Thanks to Dr. Kirk Gustafson for providing the NMR spectra and a natural sample of enigmazole A and for helpful discussions. Some of the MS data presented in this dissertation was obtained by the UC Riverside Mass Spectrometry Facility. MS analysis of advanced intermediates in the total synthesis of enigmazole A (Chapter 4) was performed by the Scripps Research Institute Mass Spectrometry Facility.

Chapter 2 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; MacMillan, J. B.; Zhou, G. –X.; Masuno, M. N.; Molinski, T. F. "Chlorocyclopropane Macrolides from the Marine Sponge *Phorbas* sp. Assignment of the Configurations of Phorbasides A and B by Quantitative CD" *J. Am. Chem. Soc.* **2007**, *129*, 4150-4151. The dissertation author was the primary researcher/author on this paper.

Chapter 3 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592-2597. The dissertation author was the primary researcher/author on this paper.

Chapter 3 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; Dalisay, D. S.; Molinski, T. F. "Synthesis and Antifungal Activity of (–)-*Z*-Dysidazirine", *Org. Lett.* **2008**, *10*, 5269-5271. The dissertation author was the primary researcher/author on this paper.

Chapter 3 is, in part, currently being prepared for submission for publication of the material. Skepper, C. K.; Dalisay, D. S.; Molinski, T. F. "Antifungal Structure-Activity-Relationships of Long Chain 2*H*-Azirine

Carboxylates". The dissertation author was the primary researcher/author of this material.

Chapter 4 is, in part, currently being prepared for submission for publication of the material. Quach, T.; Skepper, C. K.; Molinski, T. F. "Synthesis of C1-C16 of Enigmazole A: A Hetero-Diels-Alder Approach" and Skepper, C. K.; Molinski, T. F. "Total Synthesis of Enigmazole A Using an Oxazole Grafting Approach". The dissertation author was the primary researcher and anuthor of this material.

VITA

2003 Bachelor of Biotechnology (1st Class Hons.) University of Queensland

2004-2005 Teaching Assistant, University of California, Davis

2005-2009 Research Assistant, University of California, San Diego

2009 Doctor of Philosophy, University of California, San Diego

PUBLICATIONS

1. Skepper, C. K.; Quach, T.; Molinski, T. F. "Total Synthesis of Enigmazole A Using an Oxazole Grafting Approach." *J. Am. Chem. Soc.* **2009** manuscript in preparation.

2. Skepper, C. K.; Quach, T.; Molinski, T. F. "Synthesis of C1-C16 of Enigmazole A: a Hetero-Diels-Alder Approach." *Org. Lett.* **2009** mansuscript in preparation.

3. Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. "A Tetrachloro Polyketide Hexahydro-1*H*-isoindolone, Muironolide A, from the Marine Sponge *Phorbas sp.* Natural Products at the Nanomole Scale." *J. Am. Chem. Soc.* **2009**, *131*, 7552-7553.

4. Skepper, C. K.; Dalisay, D. S.; Molinski, T. F. "Synthesis and Antifungal Activity of (−)-(*Z*)-Dysidazirine." *Org. Lett.* **2008**, *10*, 5269-5271.

5. MacMillan, J. B.; Zhou, G. -X.; Skepper, C. K.; Molinski, T. F. "Phorbasides A-E, Cytotoxic Macrolide Glycosides from the Marine Sponge *Phorbas* sp. CD Determination of *C*-Methyl Sugar Configurations." *J. Org. Chem.* **2008**, *73*, 3699-3706.

6. Skepper, C. K.; Molinski, T. F. "Long Chain 2*H*-Azirines with Heterogeneous Terminal Halogenation from the Marine Sponge *Dysidea fragilis*." *J. Org. Chem.* **2008**, *73*, 2592-2597.

7. Makarieva, T. N.; Dmitrenok, P. S.; Zakharenko, A. M.; Denisenko, V. A.; Guzii, A. G.; Li, R.; Skepper, C. K.; Molinski, T. F.; Stonik, V. A. "Rhizochalins C and D from the Sponge *Rhizochalina incrustata*. A Rare *threo*-Sphingolipid and a Facile Method for Determination of the Carbonyl Position in α,ω-Bifunctionalized Ketosphingolipids." *J. Nat. Prod.* **2007**, *70*, 1991-1998.

8. Skepper, C. K.; MacMillan, J. B.; Zhou, G. -X.; Masuno, M. N.; Molinski, T. F. “Chlorocyclopropane Macrolides from the Marine Sponge *Phorbas* sp. Assignment of the Configurations of Phorbasides A and B by Quantitative CD.” *J. Am. Chem. Soc.* **2007**, *129*, 4150-4151.

9. Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. “Ene-yne Tetrahydrofurans from the Sponge *Xestospongia muta*. Exploiting a Weak CD Effect for Assignment of Configuration.” *Org. Lett.* **2007**, *9(10)*, 1975-1978.

10. Masuno, M. N.; Young, D. M.; Hoepker, A. C.; Skepper, C. K.; Molinski, T. F. “Addition of Cl_2C: to (−)-Menthyl Acrylate Under Sonication-Phase-Transfer Catalysis. Efficient Synthesis of (+)- and (−)-(2-chlorocyclopropane)methanol.” *J. Org. Chem.* **2005**, *70*, 4162-4165.

FIELDS OF STUDY

Major Field: Chemistry

Studies in Marine Natural Products Synthesis, Structure, Isolation
Professor Tadeusz F. Molinski

ABSTRACT OF THE DISSERTATION

Marine-Derived Heterocycles: Structural, Synthetic and Biological Investigations

by

Colin K. Skepper

Doctor of Philosophy

University of California, San Diego, 2009

Professor Tadeusz F. Molinski, Chair

This dissertation describes synthetic, structural and biological studies on three different groups of heterocyclic marine natural products. Chapter 2 describes the assignment of the absolute configuration of phorbasides A and B, cytotoxic macrolide glycosides from the marine sponge *Phorbas* sp. The synthesis of a series of ene-yne chlorocyclopropane model compounds is described; the circular dichroism spectrum for each model is presented and

compared to the spectra obtained for the natural products. The relationship between configuration and structure of the extended ene-yne chromophore and the observed Cotton effect is discussed.

Chapter 3 describes i) The characterization of three new antazirine analogues from the marine sponge *Dysidea fragilis*; ii) The total synthesis of (*Z*)-dysidazirine, a related 2*H*-azirine natural product isolated in 1988 from *Dysidea fragilis*, and iii) The synthesis of a series dysidazirine analogues. The antifungal activity of all compounds against a panel of clinically-relevant pathogenic fungi is reported. The data is used to develop a basic Structure-Activity-Relationship for antifungal activity of long-chain 2*H*-azirine carboxylates. Implications for the mechanism of action of (*Z*)-dysidazirine are discussed.

Chapter 4 details the first total synthesis of the cytotoxic polyketide macrololide enigmazole A, isolated from the marine sponge *Cynachyrella enigmatica*. Enigmazole A was synthesized in 22 steps (longest linear sequence) and 0.41% overall yield from known compounds. The development of a method for preparation of functionalized oxazol-2-yl zinc reagents by direct zinc insertion is described. Oxazol-2-yl zincates were found to undergo palladium catalyzed Negishi coupling and copper catalyzed acylation reactions. The application of this methodology to the preparation of the oxazole-containing side chain of enigmazole A is presented.

CHAPTER 1

STRUCTURE, SYNTHESIS AND BIOLOGY OF HETEROCYCLIC MARINE NATURAL PRODUCTS WITH ANTIFUNGAL AND ANTI-CANCER ACTIVITY

1.1 Marine Natural Products as a Source of Drug Leads

Natural products have a long history as a source of pharmaceutical agents, particularly in the areas of oncology and infectious disease.[1] In the early to mid 20th century the natural world was the preeminent source of drugs, producing some of the original blockbusters – penicillin, aspirin, quinine, morphine, tetracycline antibiotics to name just a few. These drugs and others contributed to an increase in mean life expectancy for US residents from 47 years (1900) to 77 years (2000).[2] Recent statistics attest to the fact that natural products continue to be a major source of new pharmaceutical agents.[3] Between 1981-2002 there were 877 small molecule new chemical entities (NCEs) registered, and of these 33% were either natural products or natural product-derived. Of all anticancer drugs launched between 1940-2002, 40% were natural products or natural product-derived.[4]

The propensity of natural products to modulate biological function with high potency and by exquisite mechanisms of action is likely no accident.[5] It has been argued that natural products are the result of millions of years of molecular evolution, structures that have been gradually adapted, *via* natural selection, to bind to specific proteins, enzymes or receptors.[6] Nature is, it would seem, the original combinatorial chemist.[7]

Despite this, major pharmaceutical companies have for the most part abandoned or curtailed natural products research over the past 20 years.[8] Advances in cell biology (in particular the sequencing of the human genome) have helped identify numerous new targets (ie. enzymes and receptors) for drug development. In response, resources have been redirected to the production of libraries of purely synthetic organic small molecules that can be screened easily in high-throughput, target-based assays. Such libraries are intended to sample large portions of chemical space and are usually designed to incorporate compounds with 'drug-like properties'. Rather than the expected explosion of new drug leads, the result has been very much the opposite. The number of NCEs registered in 2001 was 37 – a 20 year low. That same year saw 16 New Drug Applications (NDAs), down from 24 the previous year.[4,9]

The pharmaceutical industry is struggling to meet the demand for new drugs in a wide variety of indications, however this review will deal with only two: cancer and fungal infection (mycoses). There is little need to stress the impact of cancer on the population of the Western world. According to the American Cancer Society In the US alone 1 479 350 new cases of invasive cancer are expected to be diagnosed in 2009. Around 562 340 people are expected to die of cancer this year – more than 1500 people per day. This makes cancer the second leading cause of death in America, behind heart disease.[10] Given the widespread occurrence of cancer there is an increasing need for targeted chemotherapeutics that circumvent the serious side effects

associated with current drugs that, while effective, are frequently highly toxic. Taxol® (paclitaxel, **1.1**), for example, is undeniably one of the major success stories of natural products in cancer chemotherapy.[11] The anticancer effects of **1.1** are due to stabilization of microtubules and promotion of tubulin polymerization, interrupting cell division and resulting in cell death. These effects are most significant in rapidly dividing cells, however there is no inherent selectivity for cancerous *vs* healthy cells. Pharmaceutical research is now focused on finding compounds targeting mutated proteins and abberant cellular processes unique to cancer cells.

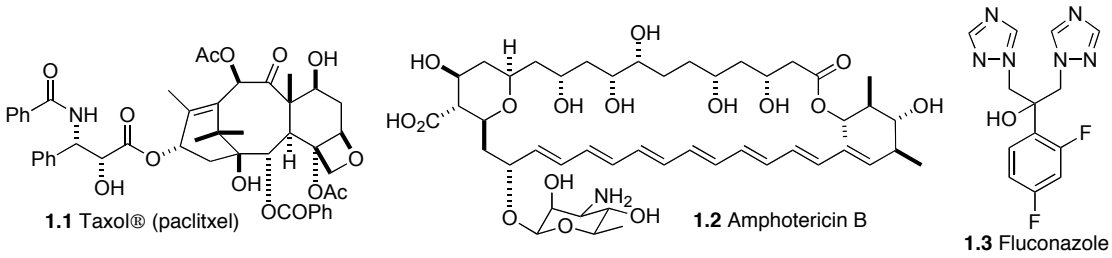


Figure 1.1 Structures of the anticancer drug taxol (paclitaxel) and antifungal drugs amphotericin B and fluconazole

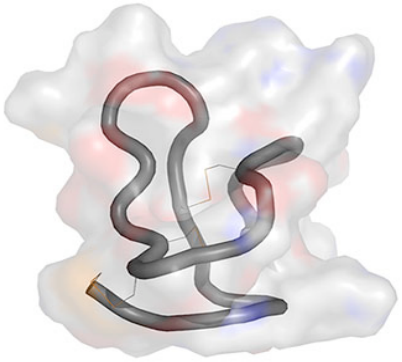
The effect of fungal infection on human health is perhaps less widely understood. Fungal infections have become a serious and increasing threat to the health of immunocompromised patients, particularly those undergoing organ or bone marrow transplant operations, cancer chemotherapy or suffering from AIDS. In fact, 90% of AIDS patients in 1997 had suffered from oropharyngeal or oesophageal candidiasis at some point since acquiring the disease.[12] The widespread emergence of aggressive strains of *Candida* (*C.*

albicans, *C. krusei*, *C. glabrata*, *Aspergillus*) has been battled quite successfully with traditional drugs such as amphotericin B and newer azole drugs such as fluconazole. Fluconazole has become one of the most successful and widely prescribed antifungal drugs. Alarming resistance to azole drugs has been increasingly observed in part *because* of their success.[13] By one estimate up to a third of AIDS patients harbor an azole-resistant *Candida* strain.[14] Furthermore, up to 80% of AIDS patients experience recurrence of fungal infection within 3 months of treatment with azole drugs.[12]

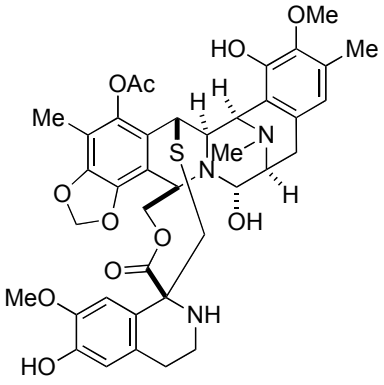
1.2 Recent Successes and Barriers in Marine Natural Products

These dilemmas argue that a return to the natural world as a source of lead compounds is called for. Indeed, natural products seem to be enjoying something of a renaissance.[15] Marine natural products, in particular, represent an under-exploited source of lead compounds with novel structures and modes of action. Although the study of marine natural products has been around for 50 years, the first true marine drug, ziconotide (Prialt® **1.4**) was approved recently as an intrathecal analgesic for the treatment of chronic pain. Ziconotide is synthetic ω-conotoxin MVIIA, a naturally-ocurring peptide originally isolated from the venom of the cone snail *Conus magnus*.[16] The anti-cancer drug ET-743 (Yondelis® **1.5**), isolated from the sea squirt *Ecteinascidia turbinata*,[17] was approved in Europe in 2007 for the treatment of

soft tissue sarcoma becoming the second marine natural product approved for clinical use.



1.4 Ziconotide



1.5 ET-743

Figure 1.2 Structures of ziconotide (taken from 3Dchem.com) and ET-743

Since the 1960s the study of marine natural products has benefited enormously from advances in chromatography, analytical technology, spectroscopy and biological assays. In the academic setting it is now possible to identify, characterize and assign a structure to a novel compound available in *nano-mole* quantities in a matter of weeks.[18] From a pharmaceutical viewpoint, however, marine natural products are cumbersome. Structure determination of an unknown compound is a time-intensive exercise that is not economically competitive in the fast-paced drug discovery research environment. Crude natural product extracts are complex mixtures that are not always amenable to target based high-throughput screens. The source organisms live in environments that require scuba equipment, at the very

least, for collection. The compounds are frequently found in minute amounts, often precluding extensive *in vitro* testing and *in vivo* evaluation. Marine invertebrates and their associated flora are generally unculturable, meaning that access to hit compounds largely depends on re-collection of the source organism. Natural production, however is not necessarily consistent over time or by location making large scale procurement undependable.

1.3 The Role of Organic Synthesis

The study of marine natural products is, by necessity, an interdisciplinary and collaborative effort between chemists, marine biologists and molecular biologists. The supply issue has made marine natural products a particularly vigorous area of activity for synthetic organic chemists. In some cases, total synthesis is the only viable means by which to obtain more of the compound in question. Synthetic chemists also have the ability to design and produce non-natural analogues that help define the minimum pharmacophore for activity. The synthesis of simpler analogues that retain the activity of the parent molecule is crucial when the natural product is too complex to synthesize in quantities sufficient for clinical studies. Furthermore, the complex architecture of many marine natural products has fueled the discovery and development of new synthetic methods, providing impetus for innovation in organic synthesis as a field in its own right. An excellent example is the antitumor natural compound halichondrin B (**1.6**), a highly complex polyether toxin originally isolated from the sponge *Halichondria okadai* by Uemura and

coworkers.[19] Halichondin B was shown to inhibit cell growth at nanomolar concentrations (IC_{50} ~ 1×10^{-9} M) by binding to tubulin.[20] Despite a monumental total synthesis of **1.6** by Kishi *et al*.[21] clinical development had to be supported by massive collections of the sponge *Lissodendoryx* n. sp. 1 off the coast of New Zealand.[22] A total of 1 tonne of the sponge yielded 310 mg of **1.6**. This situation was relieved by the discovery of simplified halichondrin analogues, such as eribulin mesylate (**1.7**) that displayed comparable activity to **1.6**. A scalable synthesis of the new analogue **1.7** allowed it to progress into Phase I clinical trials in 2002. The results from Phase I and II trials in patients with breast and non-small cell lung cancer were recently reported and appear promising.[23]

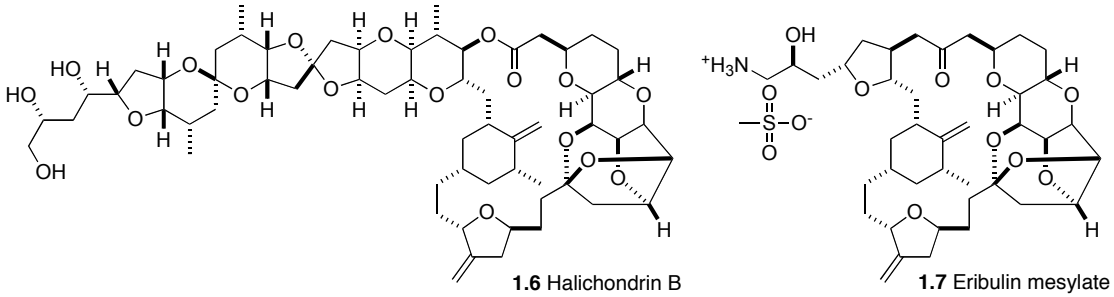


Figure 1.3 Structures of halichondrin B (**1.6**) and analogue eribulin mesylate (**1.7**)

1.4 Heterocyclic Marine Natural Products

Heterocycles of all kinds are widespread in marine natural products, from the small (e.g. azirines, aziridines, oxiranes) to the very large (e.g. macrolides). 2,4-Disubstituted oxazole rings in particular are frequently found in marine natural products that display anti-cancer and antifungal activity.

Oxazoles are five membered heterocycles containing a nitrogen and oxygen atom. The ring is numbered 1-5 starting at oxygen and the term “1,3-oxazole” is sometimes used to explicitly denote the positions of the heteroatoms. For unsubstituted oxazole the relative acidity of the 3 protons decreases in the order C2>C5>C4. The pKa of the C2 proton is ~20, but can vary depending on substitution. Oxazoles are weakly basic (comparable to pyridine), with pKa of the conjugate acid ~2 compared with ~7 for imidazole. Oxazoles are also weakly aromatic, although less so than thiazole.

The following review examines a series of well-known oxazole-containing marine natural products. Elements of structure and biological activity are discussed, as well as the contributions of organic synthesis in each case. Particular attention will be paid to the methods used for preparation of 2,4-disubstituted oxazoles. Many excellent methods are available for the synthesis of oxazoles, however this review is not intended to provide an exhaustive coverage.[24]

1.5 Calyculin A

Calyculin A (**1.8**) is a highly unusual polyketide isolated from the marine sponge *Discodermia calyx* by Fusetani and coworkers in 1986.[25] The planar structure and relative configuration was assigned by X-ray crystallography, however the absolute configuration was not assigned until 1991. Fusetani *et al*. obtained fragment **1.9** from acid hydrolysis of **1.8** and determined the

configuration based on interpretation of the CD spectrum.[26] Shiori and coworkers synthesized *ent*-**1.9** soon afterwards and showed it to be the antipode of naturally-derived **1.9**, confirming the original assignment.[27] Calyculin A exhibited potent cytotoxicity (IC_{50} 1.75 ng/mL, L1220 cells), was shown to selectively inhibit protein phosphatase 1 and 2A (IC_{50} 0.5-1 nm and 2 nm respectively) and induced tumor growth on mouse skin. Interestingly, Calyculin A was found to display similar phosphatase inhibition and tumor promotion to okadaic acid.[28] Both calyculin A and okadaic acid have become valuable tools for the study of protein-serine/threonine phosphatases.

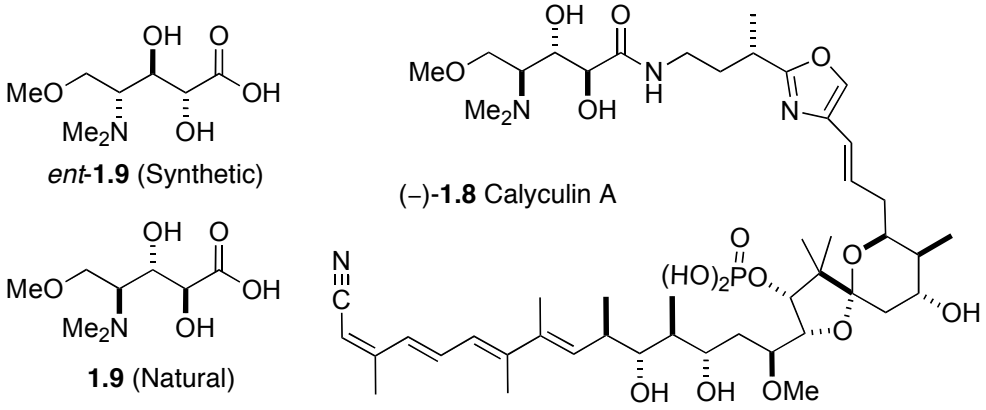
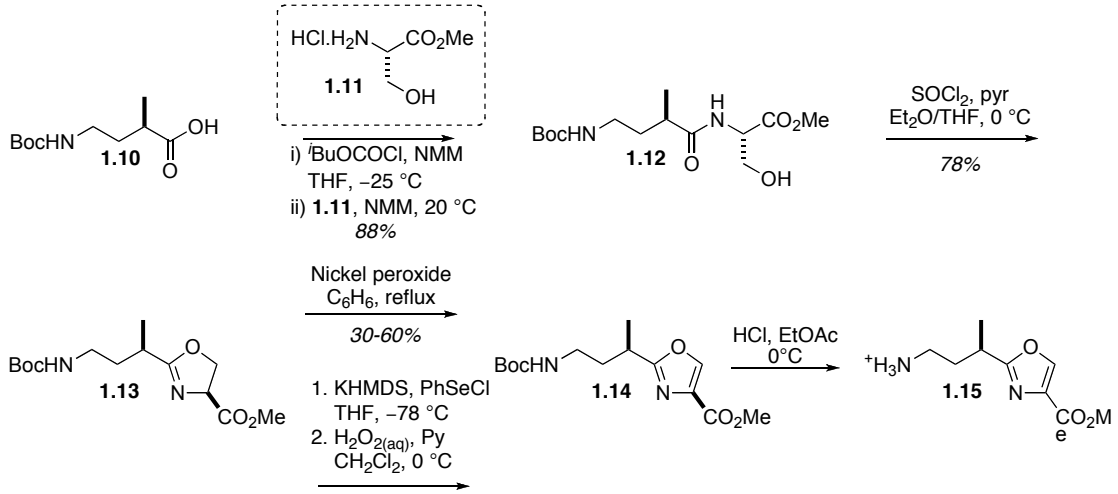


Figure 1.4 Structure of Calyculin A (**1.8**) and degradation product **1.9**

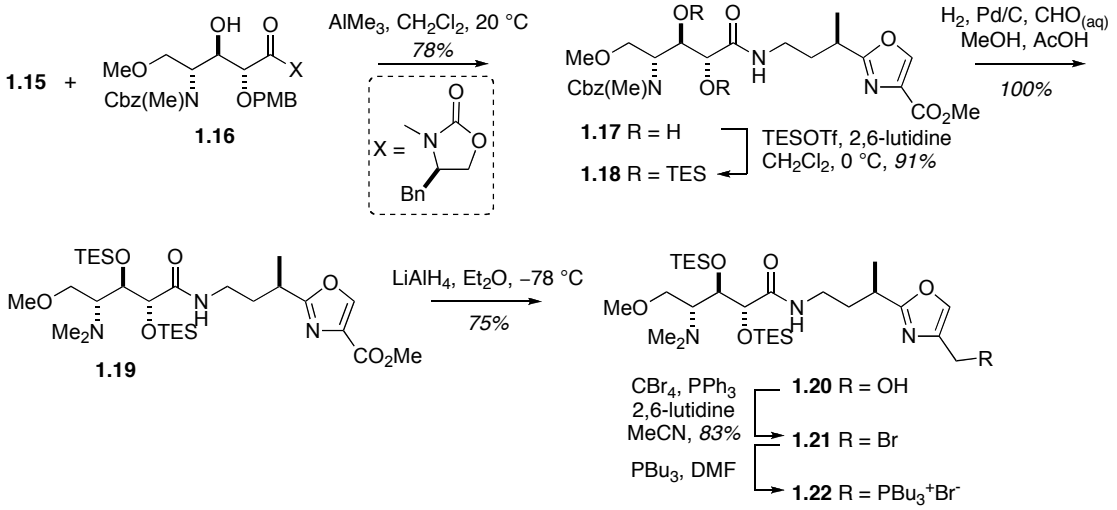
Calyculin A has densely functionalized structure containing spiroacetal, oxazole, nitrile and amino acid moieties. The unusual structure and bioactivity of **1.8** has made it an attractive target for synthesis. Evans *et al*. completed the first total synthesis of (+)-**1.8** in 1992.[29]



Scheme 1.1 Synthesis of oxazole **1.15**

Preparation of the oxazole-containing portion began with formation of dipeptide **1.12** by coupling L-serine methyl ester (**1.11**) with the mixed anhydride derived from acid **1.10** (Scheme 1.1). Cyclodehydration of **1.12** ($SOCl_2$, pyridine) produced oxazoline **1.13** in good yield. Initially **1.13** was oxidized to oxazole **1.14** with nickel peroxide, a reagent developed by Meyers for dehydration of partially reduced nitrogenous heterocycles in 1979 and widely used since for preparation of oxazoles from oxazolines.[30] In this case, however, nickel peroxide lead to highly variable yields of **1.14**. It was found that enolization of **1.13** (KHMDS), quenching with PhSeCl and oxidation/elimination of the resulting selenide gave **1.14** in a reproducible 57% yield. Deprotection of the primary amine then gave **1.15** which was in turn coupled with imide **1.16** in the presence of 3.75 equivalents of $AlMe_3$, resulting in concomitant loss of the PMB group (Scheme 1.2). Diol **1.17** was subsequently protected as its bis-TES ether **1.18**. Catalytic hydrogenation in

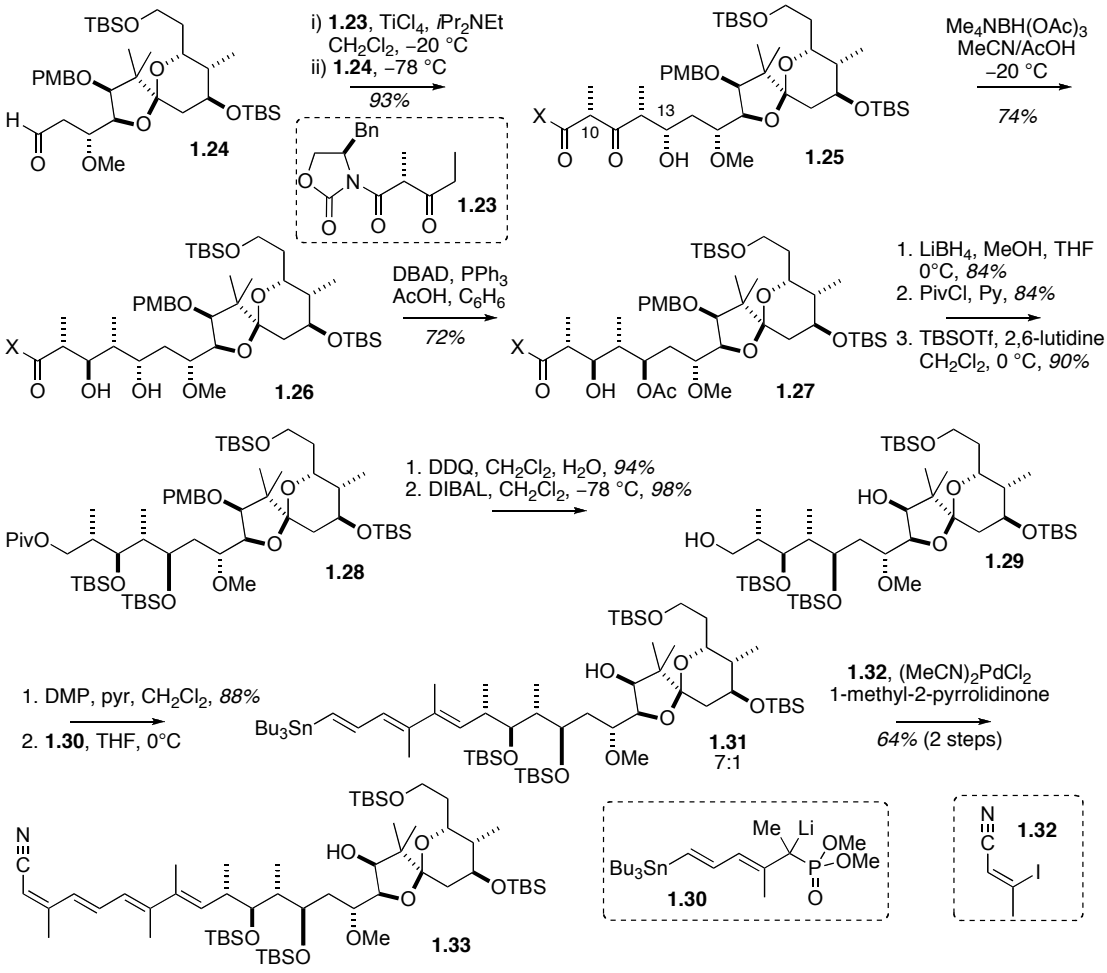
the presence of formaldehyde gave dimethylamine **1.19** which was converted in three steps to the phosphonium bromide **1.22**.



Scheme 1.2 Synthesis of phosphonium bromide **1.22**

The southern half of **1.8** was elaborated from advanced spiroketal fragment **1.24** (Scheme 1.3). Addition of the titanium enolate of **1.23** to aldehyde **1.24** produced **1.25** as a single diastereomer in excellent yield. Directed reduction of the ketone ($Me_4NBH(OAc)_3$) then gave the *anti*-1,3-diol **1.26**. The C13 (calcyulin numbering) stereocenter was selectively inverted under Mitsonobu conditions to give the correct relative configuration of the C10-C13 stereotetrad. Reduction ($LiBH_4$) of **1.27** removed both the oxazolidinone auxiliary and the acetate group from the C13-OH. A series of protecting group interchanges lead to diol **1.29**; the primary alcohol was subsequently oxidized selectively (Dess-Martin Periodinane). Addition of lithio-phosphonate **1.30** gave triene **1.31** as a 7:1 mixture of double bond isomers

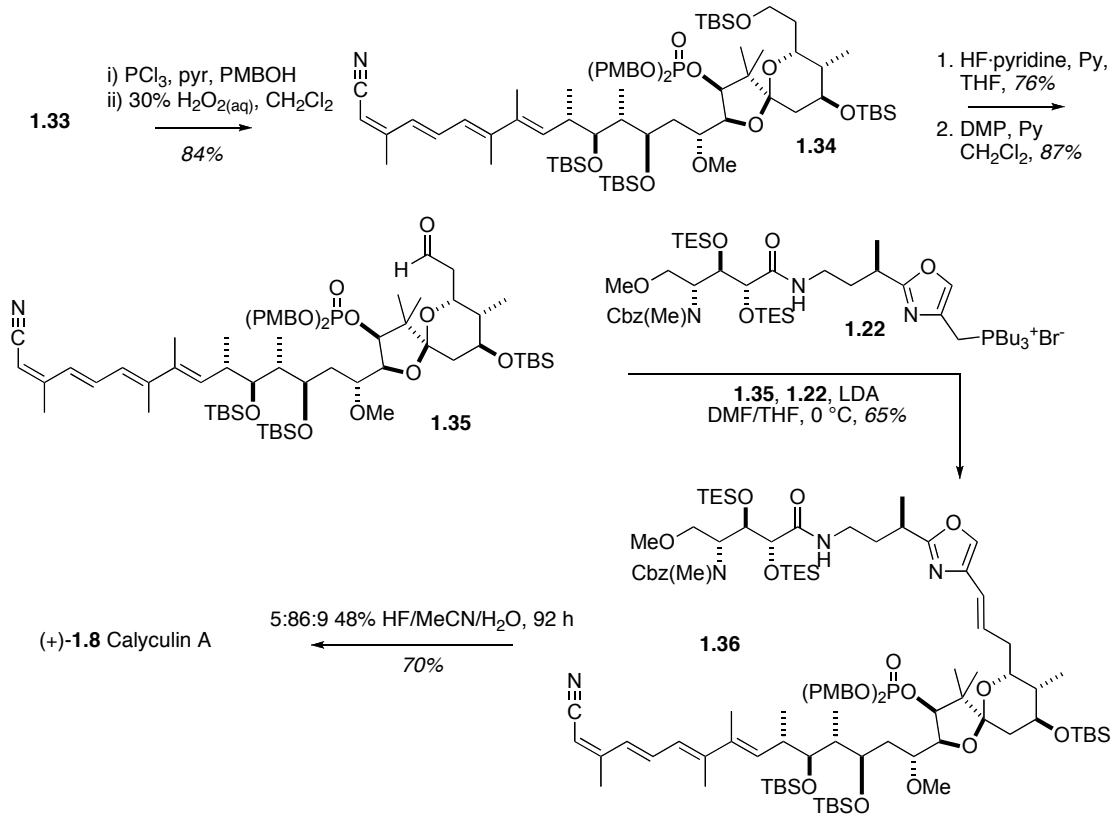
that was carried directly into the Stille coupling with vinyl iodide **1.32** yielding tetraene **1.33** after chromatographic separation of the minor olefin isomer.



Scheme 1.3 Synthesis of the southern hemisphere of calyculin A

The synthesis of **1.8** was completed as follows (Scheme 1.4). The protected phosphate ester was introduced by treating **1.33** with PCl_3 in the presence of *p*-methoxybenzyl alcohol and pyridine to give **1.34** (Scheme 1.4). Removal of the primary TBS group was effected with HF·pyridine followed by oxidation with Dess-Martin periodinane to furnish key aldehyde **1.35**. Wittig

reaction with the ylide derived from **1.22** then gave fully protect calyculin A. The Wittig reaction was remarkably selective for the *E* product; none of the isomeric *Z*-olefin was observed by NMR. Finally, **1.36** was treated with aqueous HF to afford (+)-**1.8** in 70% yield.

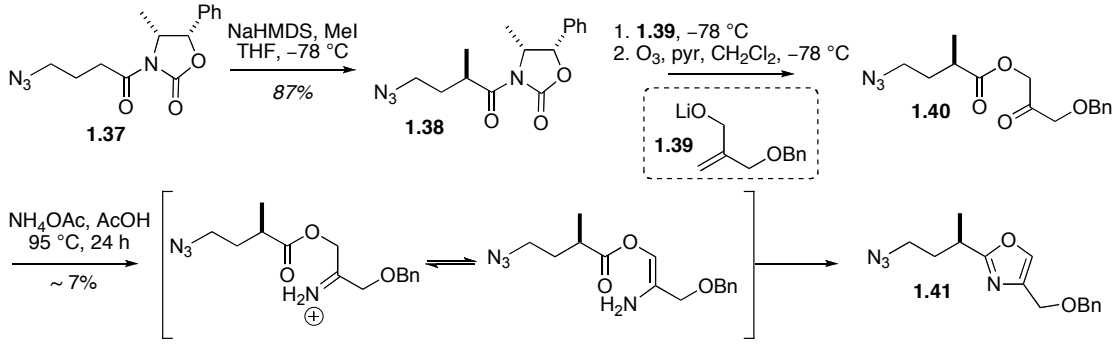


Scheme 1.4 Completion of Evans' synthesis of (+)-calyculin A

The final deprotection required prolonged exposure to HF due to remarkable resistance of the C11 silyl group to removal. All other protecting groups were removed within 24 hours, producing a spot corresponding to mono-TBS calyculin A which surprisingly co-eluted with natural **1.8** by TLC. The relatively non-polar nature of **1.8** has been ascribed to a tight H-bond

network involving the phosphate group that forces **1.8** to adopt a highly folded conformation. It was postulated that the folded nature of **1.8** renders the C11 TBS group relatively inaccessible to external reagents, explaining its resistance to deprotection.

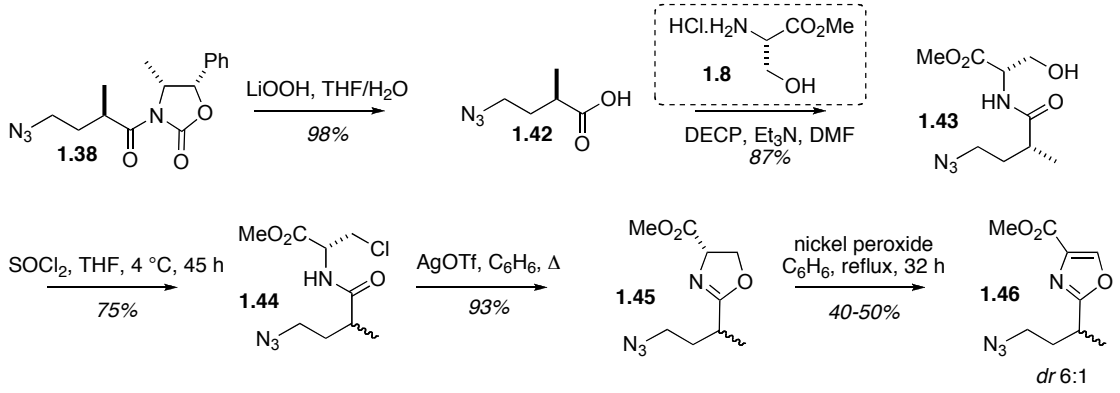
Smith *et al*. completed a total synthesis of (+)-**1.8** in 1999 using a conceptually similar approach involving Wittig reaction of a phosphonium salt analogous to **1.22** with an advanced spiroketal aldehyde fragment.[31] Preparation of the requisite oxazole fragment, however, proved troublesome and highlights some of the complications involved in the cyclodehydration/oxidation approach.



Scheme 1.5 Smith's initial approach to the oxazole ring of (+)-**1.8**

The initial approach to the oxazole-containing fragment of **1.8** began with a diastereoselective alkylation of imide **1.37** to give **1.38** (Scheme 1.5). The oxazolidinone auxiliary was displaced with alkoxide **1.39** to give **1.40**, however all attempts at Davidson cyclization (NH_4OAc, AcOH) were unsuccessful.[32] Smith postulated that the poor outcome could be explained a

lack of regioselectivity in formation of the desired enamine necessary for cyclization.

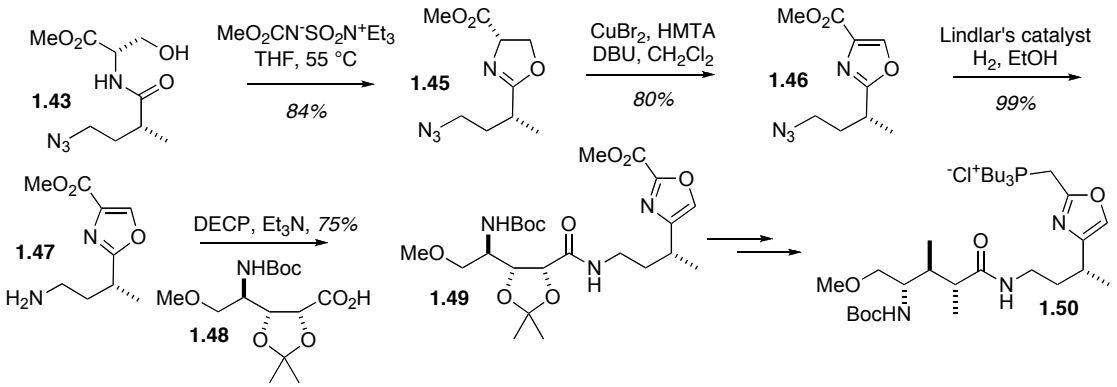


Scheme 1.6 Smith's 1st generation approach to oxazole **1.46**

As an alternative, imide **1.38** was hydrolyzed to give acid **1.42** which was in turn coupled with L-serine methyl ester hydrochloride to furnish **1.43** (Scheme 1.6). Treatment of **1.43** with $SOCl_2$ gave primary chloride **1.44** which was subjected to cyclodehydration (AgOTf) affording oxazoline **1.45** in excellent yield. Oxidation with nickel peroxide then provided oxazole **1.46**. Subsequent ^{13}C NMR analysis indicated that **1.46** was a mixture of diastereomers (*dr* 6:1). HPLC analysis of **1.43** and **1.44** indicated that epimerization was occurring during the chlorination step, however further epimerization during the oxidation step could not be ruled out.

In the end, oxazole **1.46** was obtained successfully using several modifications of the above route. Cyclodehydration of **1.43** with Burgess reagent[33] (as described by Wipf *et al.*[34]) led to the desired oxazoline **1.45** which was oxidized using Barrish-Singh conditions[35] to afford **1.46** with little or

no epimerization (Scheme 1.7). Reduction of the primary azide led to amine **1.47** which was coupled to acid **1.48**. The resulting amide **1.49** was elaborated in several steps to provide the key phosphonium salt **1.50**.



Scheme 1.7 Smith's 2nd generation approach to oxazole **1.46** and elaboration to key phosphonium salt **1.50**

1.6 Bengazole A

Bengazoles A and B (**1.51** and **1.52**) are unique bisoxazoles first isolated by Crews *et al.* in 1988 from the marine sponge *Jaspis* sp. collected in Fiji.[36] Bengazole A was originally identified as having anthelminthic activity against the rodent parasitic worm *Nippostrongylus brasiliensis*. Molinski and coworkers later re-isolated **1.51** and **1.52** (along with homologues bengazoles C-G) from a Great Barrier Reef collection of *Jaspis* sp. The absolute configuration of bengazole A was subsequently solved by a combination of Mosher's ester analysis and circular dichroism. Furthermore, broth dilution assay showed that the bengazoles are potent antifungal agents with activity against *C. albicans* comparable to amphotericin B (MIC ~ 1 μg/mL).[37] Interestingly, the *in vitro* antifungal activity of **1.51** and **1.52** is attenuated in the

presence of ergosterol, a feature shared by amphotericin B.[38] Amphotericin B exerts its activity by binding non-covalently to ergosterol, the yeast homologue of cholesterol and a major component of fungal cell membranes. The resulting complexes form pores in the fungal cell wall, leading to leakage of ions, membrane depolarization and cell lysis. Thus, it seems that bengazoles exert their activity with a mechanism of action similar to amphotericin B despite the fact that their structures are entirely different. It should be noted that at this time the cellular target of bengazoles is unknown.

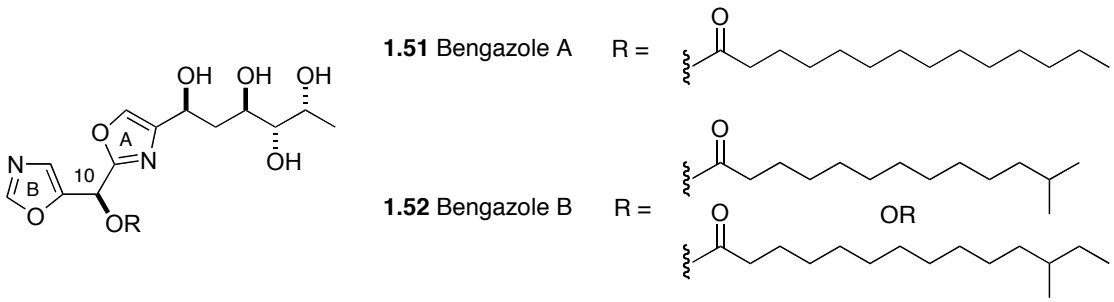
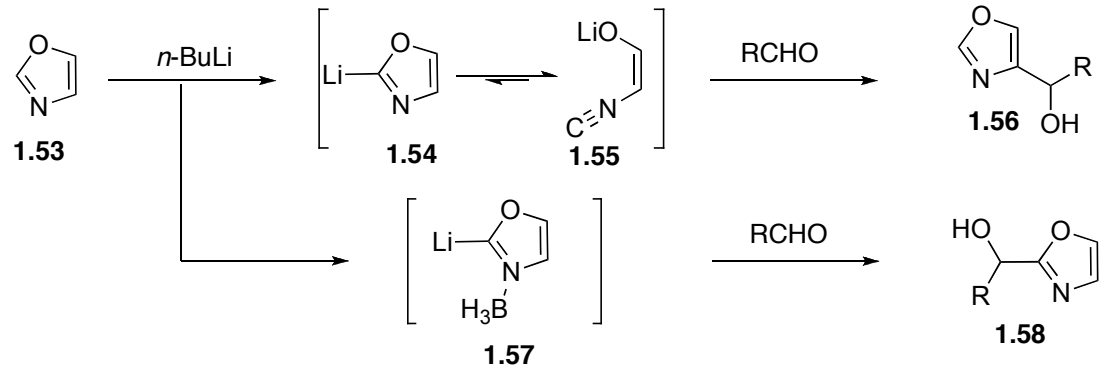


Figure 1.5 Structures of bengazoles A and B

The bengazoles are structurally quite unique, consisting of two oxazole rings attached to an isolated stereocenter (C10, bengazole numbering). Oxazole ring A is 2,4-disubstitued with a carbohydrate-like segment (C1-C6) appended at C4 (oxazole numbering) while ring B is substituted only at C5. Bengazole A has been the subject of two total syntheses and several synthetic studies. The first total synthesis of **1.51**, accomplished by Molinski *et al*.,[39] made use of consecutive metallations of the parent oxazole ring, a conceptual

departure from the standard cyclodehydration/oxidation of *N*-acylserine precursors.

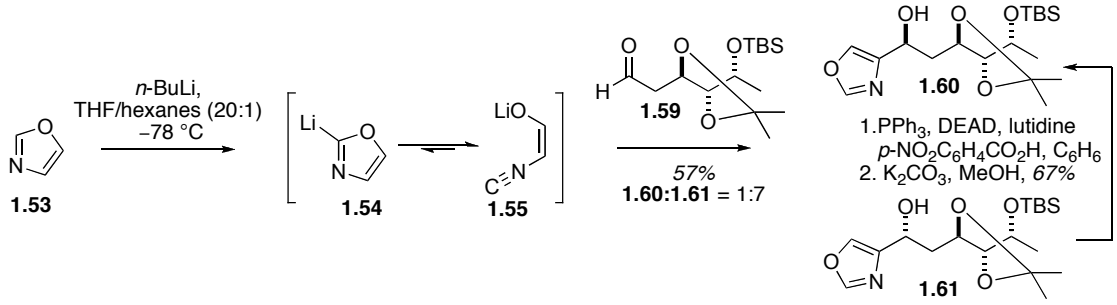
Oxazole **1.53** is deprotonated readily at C2 with 1 equivalent of *n*-BuLi (Scheme 1.8). It has been observed that 2-lithiooxazoles exist in equilibrium with the ring-open isonitrile form.[40] The products obtained from addition of 2-lithiooxazole to electrophiles is dependant upon the substituents on the oxazole ring and the nature of the electrophile. Hodges *et al*.[40c] observed that 2-lithiooxazole **1.54** reacts with aldehydes *via* isonitrile **1.55**, to give almost exclusive net addition at C4 (ie. **1.56**). Vedejs later found that the addition of borane suppresses the ring opening of 2-lithiooxazole, presumably through coordination with nitrogen, directing addition to C2.[41]



Scheme 1.8 The ambident nucleophilicity of 2-lithiooxazole

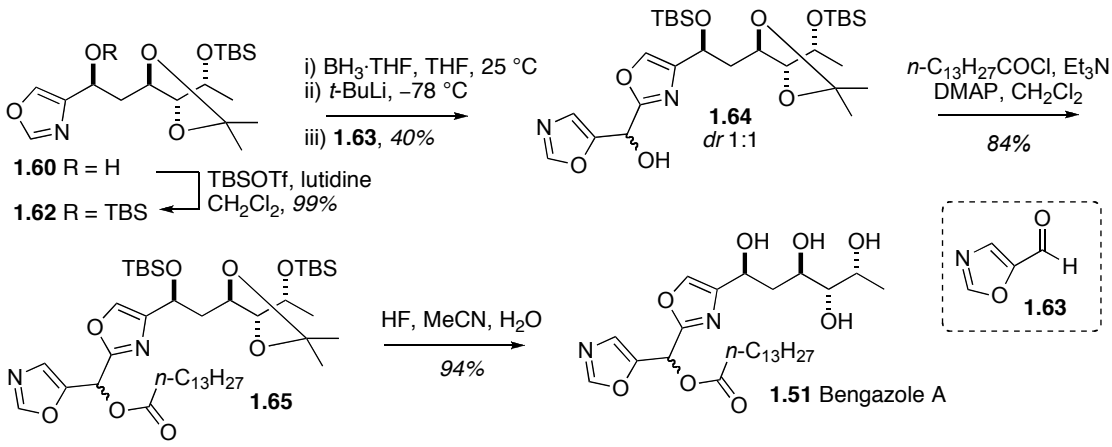
Consistent with these findings Molinski and coworkers found that deprotonation of oxazole **1.53** with *n*-BuLi followed by addition of aldehyde **1.59** led to exclusive C4 addition, providing **1.60** and **1.61** as a 1:7 mixture favoring the unwanted diastereomer **1.61** (Scheme 1.9). The solvent

composition proved critical to the outcome of this reaction. Increasing the percentage of hexane (1:2.4 → 1:4.3 hexane/THF) resulted in diminished yield and selectivity. This outcome can be rationalized on the basis of chelation-controlled addition of 2-lithiooxazole *via* coordination to the β-alkoxy substituent of **1.59**. The unwanted diastereomer **1.61** could be converted to **1.60** using a 2-step procedure of Mitsonobu inversion followed by methanolysis.



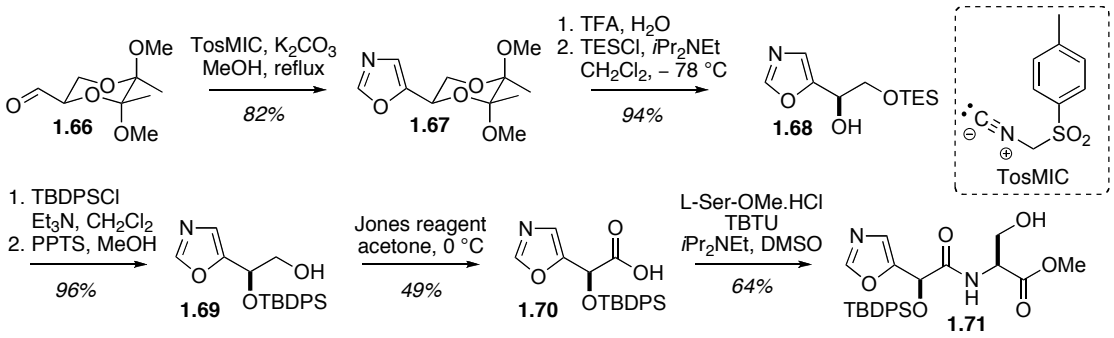
Scheme 1.9 Molinski's synthesis of bengazole A: regioselective addition of 2-lithiooxazole to aldehyde **1.59**.

The newly formed alcohol of **1.60** was protected as its TBS ether (Scheme 1.10). Deprotonation of oxazole **1.62**, this time in the presence of BH_3•THF, followed by addition of oxazole-5-carboxaldehyde (**1.63)** led to formation of **1.64** with complete regioselectivity for addition at the C2 position of the oxazole ring of **1.62**. The resulting mixture of diastereomers (*dr* 1:1) could not be separated by HPLC. Acylation of the new alcohol with myristoyl chloride followed by global deprotection (HF) gave bengazole A **1.51** in good yield as an inseparable mixture of epimers at C10.



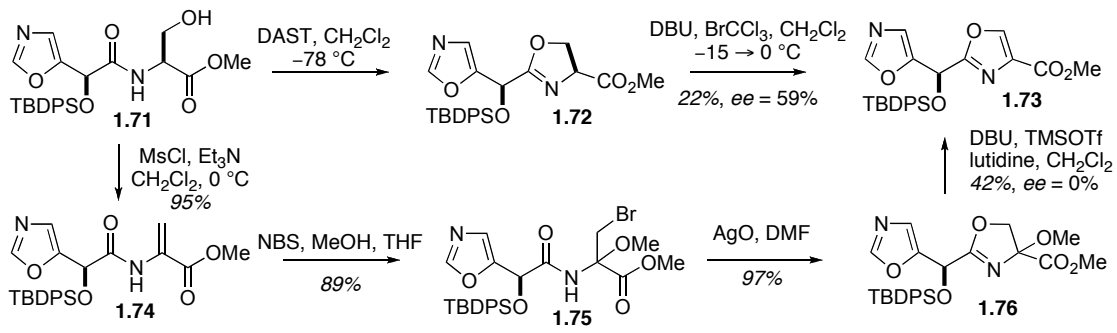
Scheme 1.10 Completion of Molinski's synthesis of bengazole A

Ley *et al.* recently completed the second total synthesis of bengazole A.[42] Their approach involved setting the configuration of the C10 stereocenter early in the synthesis, a risky strategy that demanded careful optimization of the oxazole-forming steps to avoid epimerization. Thus, a Schöllkopf-type oxazole preparation[43] was employed using a butane-2,3-diacetal protected glyceraldehyde equivalent **1.66** (Scheme 1.11). Treatment of **1.66** with *p*-toluenesulfonylmethyl isocyanide (TosMIC) in the presence of base gave oxazole **1.67** as a single diastereomer. Acid cleavage of the acetal and protection of the primary alcohol gave **1.68** in excellent yield. Protecting group interchange then furnished the free primary alcohol **1.69** which was oxidized carefully to unstable carboxylic acid **1.70**. Amide coupling with L-serine methyl ester gave **1.71** in 64% yield.



Scheme 1.11 Ley's synthesis of bengazole A: preparation of the first oxazole

Several methods were screened for formation of the second oxazole ring without epimerization of the sensitive C10 stereocenter (Scheme 1.12). Cyclodehydration of **1.71** using conditions developed by Wipf (DAST, −78 °C) gave oxazoline **1.72**.[44] Subsequent oxidation using Williams' conditions ($BrCCl_3$, DBU) produced the desired bisoxazole **1.73** (22% yield over 2 steps), but with significant epimerization (*ee* = 59%).[45] In an alternate route, treatment of alcohol **1.71** with mesyl chloride and triethylamine smoothly produced alkene **1.74** which was brominated efficiently in the presence of NBS in MeOH. Non-basic cyclization with silver oxide produced oxazoline **1.75** in excellent yield, however elimination of MeOH in the presence of DBU and TMSOTf resulted in completely racemic **1.73**.

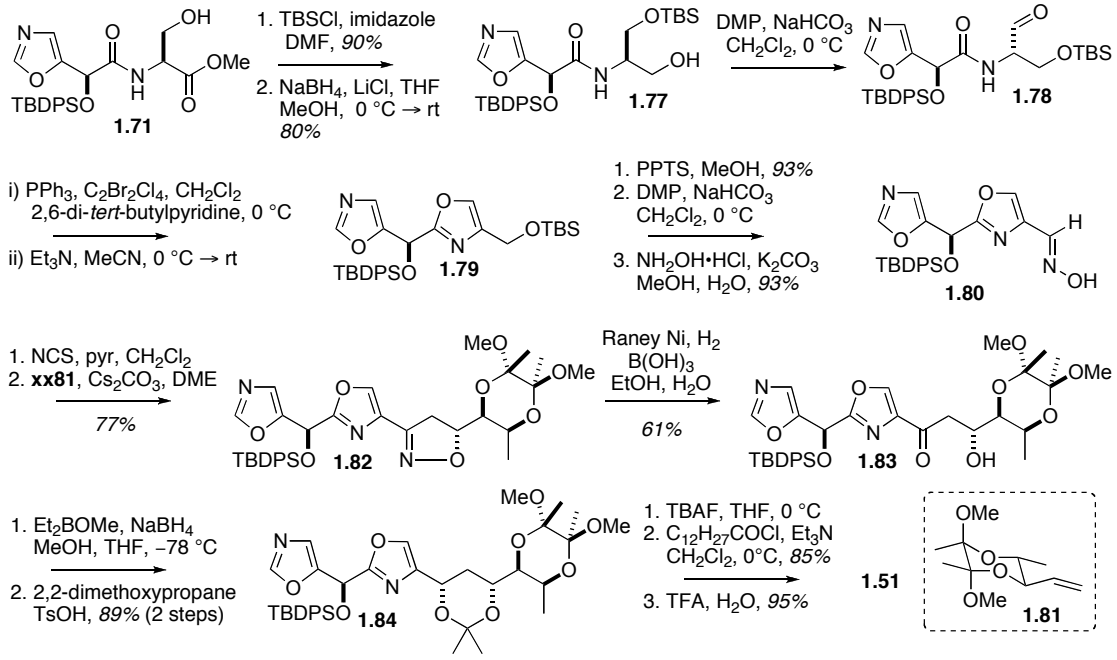


Scheme 1.12 Ley's synthesis of bengazole A: attempted formation of the second oxazole ring

In the end, the necessary bisoxazole fragment was synthesized by protection of the hydroxyl function of **1.71** and reduction of the methyl ester to give **1.77** (Scheme 1.13). Oxidation (Dess-Martin periodinane) led to aldehyde **1.78** which underwent a Robinson-Gabriel oxazole forming reaction using conditions developed by Panek and Wipf.[46,47] The use of triethylamine in the elimination step was crucial (use of DBU caused significant epimerization), furnishing bisoxazole **1.79** in good yield and optical purity >98%.

Removal of the primary TBS group (PPTS, MeOH) followed by oxidation (Dess-Martin periodinane) and condensation with hydroxylamine hydrochloride gave oxime **1.80**. Chlorination of the oxime (NCS) followed by treatment with base (Cs_2CO_3) produced the corresponding nitrile oxide which underwent 1,3-dipolar cycloaddition with alkene **1.81** to give 1.**82** embodying the full carbon skeleton of bengazole A. Reductive cleavage of isoxazoline **1.82** gave ketone **1.83** (61%) which was reduced stereoselectively to give a 1,3-diol which was in turn protected as the acetonide (89% over 2 steps, *dr* =

14:1). Finally, removal of the TBDPS protecting group (TBAF) and acylation with myristoyl chloride produced fully protected bengazole A (**1.84**), which after global deprotection (TFA, H_2O) gave the natural product as a single diastereomer.



Scheme 1.13 Ley's synthesis of bengazole A: successful formation of the second oxazole ring and completion of the synthesis.

1.7 Mycalolide A

The trisoxazole family of marine macrolides is an intriguing class of compounds that has captured the imagination of both biologists and chemists. The family includes (but is not limited to) mycalolide A (**1.85**),[48] ulapualide A (**1.86**),[49] halichondramide (**1.87**)[50] and kabiramide C (**1.88**)[51] (Figure 1.6). While **1.85** and **1.87** were isolated from sponges (*Halichondria* sp. and *Mycale* sp. respectively) **1.86** and **1.88** were obtained from the egg masses of

nudibranchs. Scheuer's original isolation of ulapualide A was spurred by the observation that the ostensibly defenseless egg masses of *Hexabranchus sanguineus* were avoided by predators. Faulkner and co-workers later established that *H. sanguineus* obtains trisoxazole macrolides from its diet of the sponge *Halichondria* sp., concentrating them in the dorsal mantle and egg masses. Some of the compounds are chemically modified, producing a diverse suite of trisoxazoles that confers protection from predation.[52]

Compounds **1.85-88** are characterized by a macrolide ring containing three contiguous 2,4-disubstiuted oxazoles, a relatively non-polar side chain and potent *in vitro* anticancer and antifungal activity. Fusetani and coworkers found that the mycalolide B (not shown) causes rapid depolymerization of actin filaments, crucial components of the mammalian cell cytoskeleton required for maintenance of cell shape, cell motility and cytokinesis.[53] In a recent study, Shaw and Pattenden showed that ulapualide displays similar actin depolymerizing activity.[54] In a seminal study, Rayment *et al.* obtained the crystal structure of kabiramide C in complex with G-actin (the monomeric protein component of actin filaments). Crystal structure data revealed that kabiramide C likely severs actin filaments by first binding weakly to an external surface on F-actin (polymeric form of G-actin), then inserting its aliphatic side chain between two monomers and thereby destabilizing the monomer interaction. Remarkably, **1.88** binds to actin at exactly the same site as gelsolin, a native protein that serves to modulate actin polymerization *in vivo*.

The surprisingly long-lived kabiramide C-G-actin complex was also shown to cap growing actin filaments. Marriot *et al.* dubbed Kabiramide C and related compounds "biomimetics of Gelsolin".[55] Thus, it appears that trisoxazole macrolides exert their cytotoxicity *via* a common mechanism of action, although certain members of the family bind more specifically to actin than others.[56]

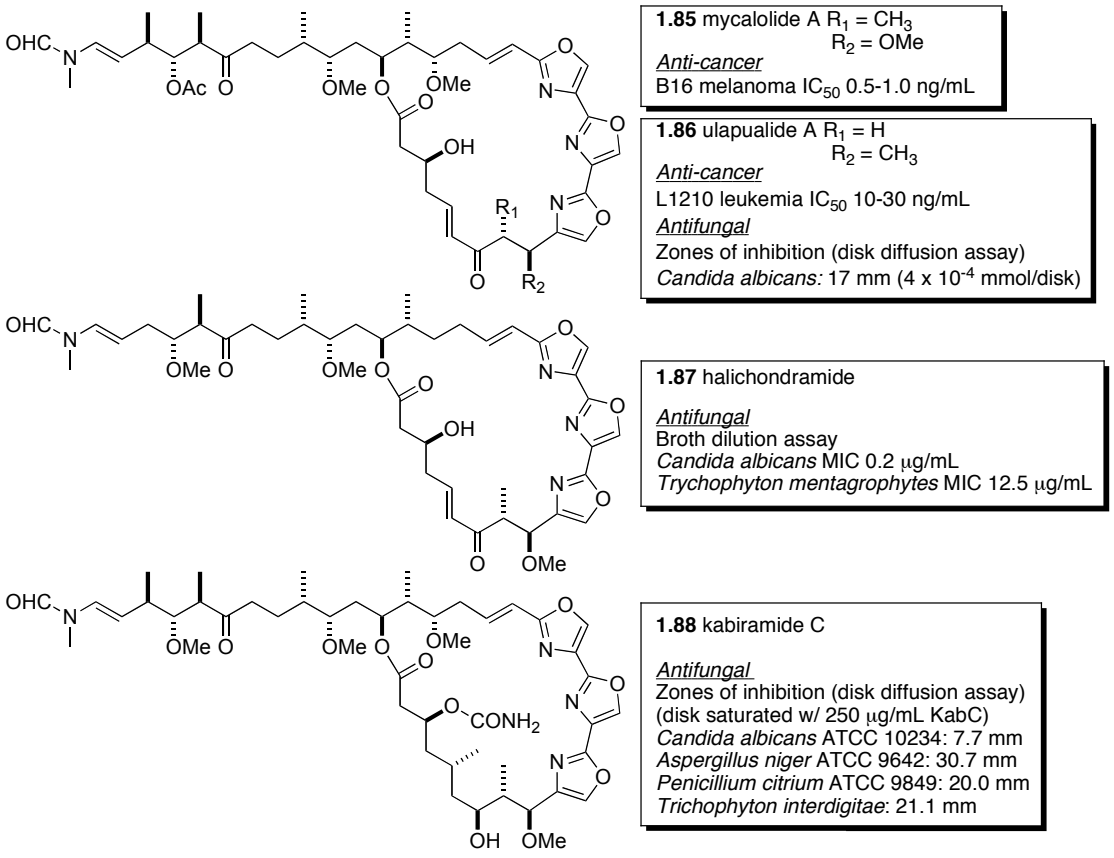
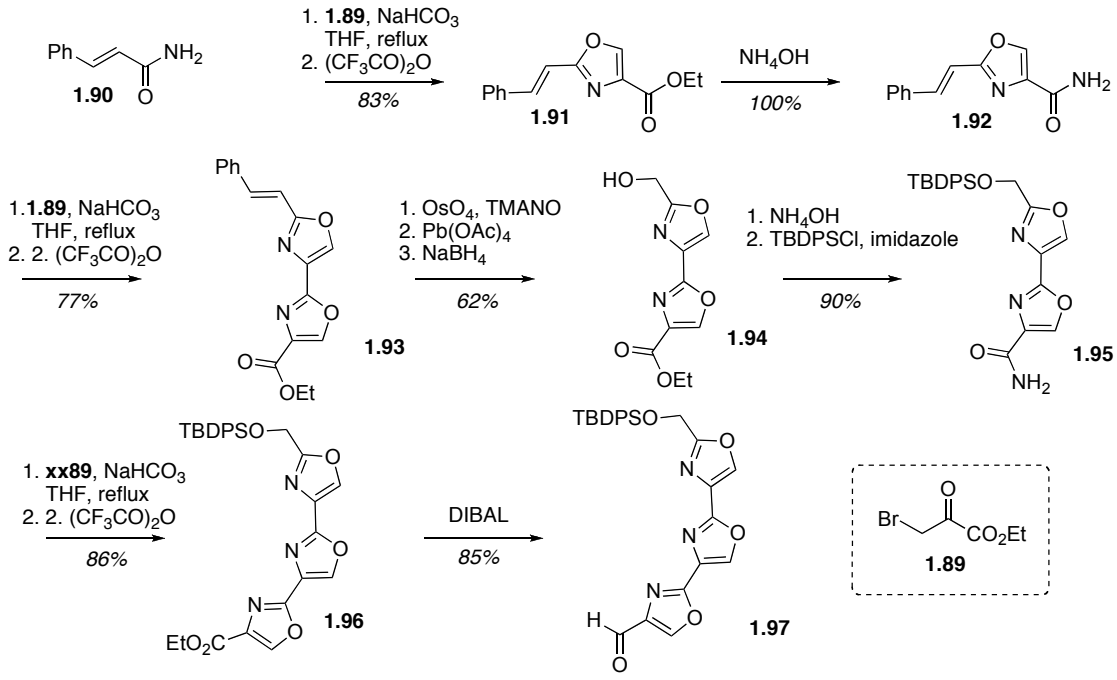


Figure 1.6 Structures and biological activity summary of trisoxazole macrolides mycalolide A (**1.85**), ulapualide A (**1.86**), halichondramide (**1.87**) and kabiramide C (**1.88**).

Given their intricate structures and potent bioactivity the trisoxazole macrolides have attracted a great deal of attention from synthetic chemists. It

is somewhat remarkable that only two total syntheses of trisoxazole natural products have been reported – mycalolide by Panek *et al.* [57] in 2000 and ulapualide by Pattenden *et al.* in 2007.[58]

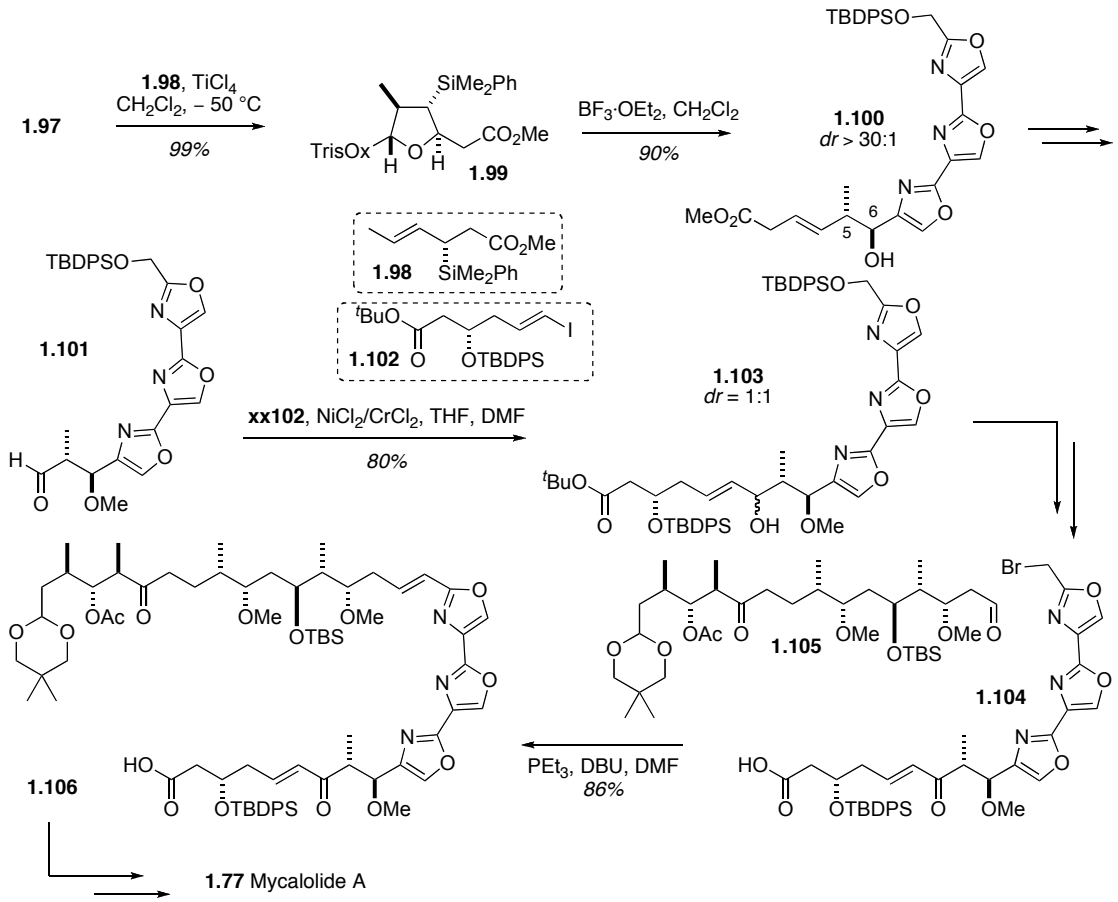
Panek's approach to the trisoxazole fragment of mycalolide A utilized sequential Hantzch-type condensations between ethyl bromopyruvate (**1.89**) and an amide.[59] Beginning with cinnamamide **1.90**, condensation with **1.89** in the presence of base produced an intermediate hydroxyl-substituted oxazoline that underwent dehydration in the presence of trifluoroacetic anhydride to give oxazole **1.91** (Scheme 1.14). Conversion of the ethyl ester to the amide (NH_4OH) set the stage for a second condensation with **1.89** to give bisoxazole **1.93** in good yield. At this stage the styrene olefin was oxidatively cleaved to give the aldehyde which was reduced to the primary alcohol **1.94**. The ester was once more converted to the corresponding amide, and the primary alcohol protected as its TBDPS ether (**1.95**). A third Hantzch-type condensation with **1.89** gave the trisoxazole **1.96** in excellent yield. Reduction with DIBAL then furnished the requisite aldehyde **1.97**.



Scheme 1.14 Panek's synthesis of the trisoxoazole fragment of mycalolide A

Diastereoselective crotylation of aldehyde **1.97** with crotyl silane **1.98** furnished tetrahydrofuran intermediate **1.99**, which was ring-opened in the presence of BF_3•OEt_2 to give homoallylic alcohol **1.100** (Scheme 1.15). The configuration of the newly formed methyl-branch stereocenter is set by the configuration of crotyl silane **1.98**, while use of a chelating Lewis acid ($TiCl_4$) results in the 5,6-*anti* relative configuration. Alcohol **1.100** was converted in several steps to the aldehyde **1.101** which underwent Kishi-Nozaki vinyl iodide addition with **1.102**, providing **1.103** as an inconsequential mixture of diastereomers (*dr* = 1:1).[60] Several more steps led to primary bromide **1.104** in preparation for the crucial Wittig coupling with aldehyde **1.105**. Use of triethylphosphine with DBU as base proved to be crucial, as other attempts

with tributylphosphine and stronger bases such as LDA and KHMDS were unsuccessful. In the event, *E* olefin **1.106** was obtained in excellent yield and was converted in short order to mycalolide A following macrolactonization and deprotection.

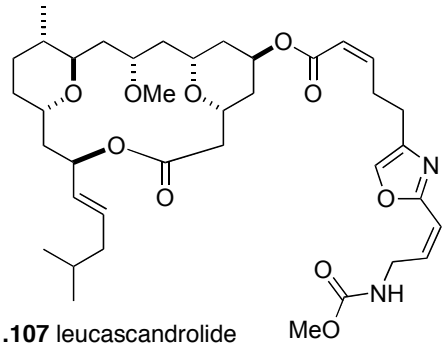


Scheme 1.15 Completion of Panek's synthesis of mycalolide A

1.8 Leucascandrolide A

Leucascandrolide A (**1.107**) is a polyketide macrolide from the calcerous sponge *Leucascandra caveolata* isolated by Pietra *et al*. in 1996.[61] Leucascandrolide exhibited potent cytotoxicity and antifungal activity (see

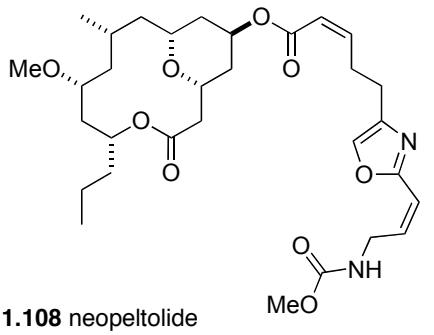
Figure 1.7). Recently, a closely related macrolide, neopeltolide (**1.108**), was isolated from a deep-water specimen of the sponge *Deadalopelta* sp.[62] Neopeltolide exhibited cytotoxicity and antifungal activity comparable to that of leucascandrolide (Scheme 1.7).



1.107 leucascandrolide

Anticancer
KB oral carcinoma IC_{50} 0.05 μg/mL
P388 murine leukemia IC_{50} 0.25 μg/mL

Antifungal
Zones of inhibtion (disk diffusion assay):
20 mm (10 μg/disk)(*C.albicans*)



1.108 neopeltolide

Anticancer
A549 lung adenocarcionoma IC_{50} 1.2 nM
NCI/ADR-RES ovarian carcinoma IC_{50} 5.1 nM
P388 murine leukemia IC_{50} 0.56 nM

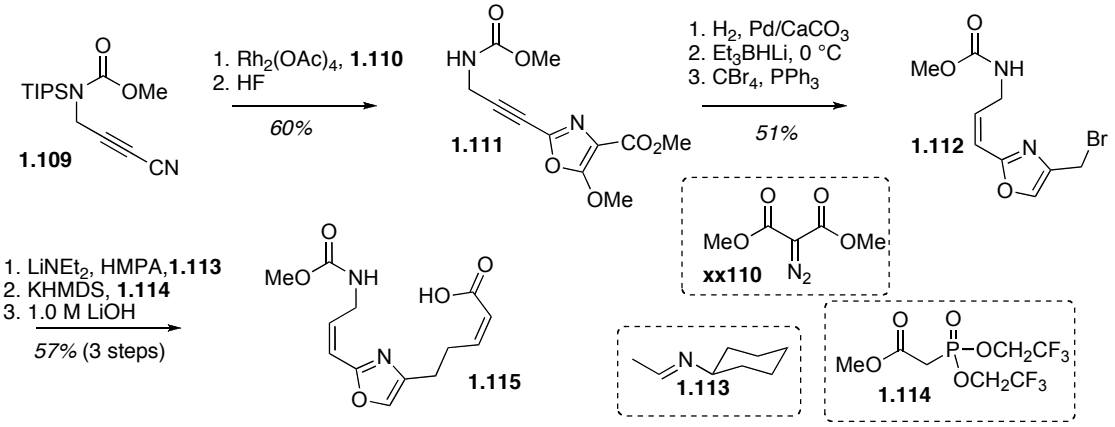
Antifungal
Broth dilution assay
MIC_{50} 0.625 μg/mL (*C.albicans*)

Figure 1.7 Structure of the related cytotoxic macrolide leucascandrolide and neopeltolide

Both leucascandrolide A and neopeltolide have generated enormous interest amongst synthetic organic chemists. Attempts to re-isolate **1.107** from later collections of *L. caveolata* have proved fruitless, suggesting that leucascandrolide may be produced by microbes living in association with the sponge, rather than the sponge itself.[63] Pietra *et al*. described the presence of

extensive dead tissue in the original sample of *L. caveolata*, perhaps indicative of the presence of an opportunistic (as opposed to symbiotic) microbial colony.

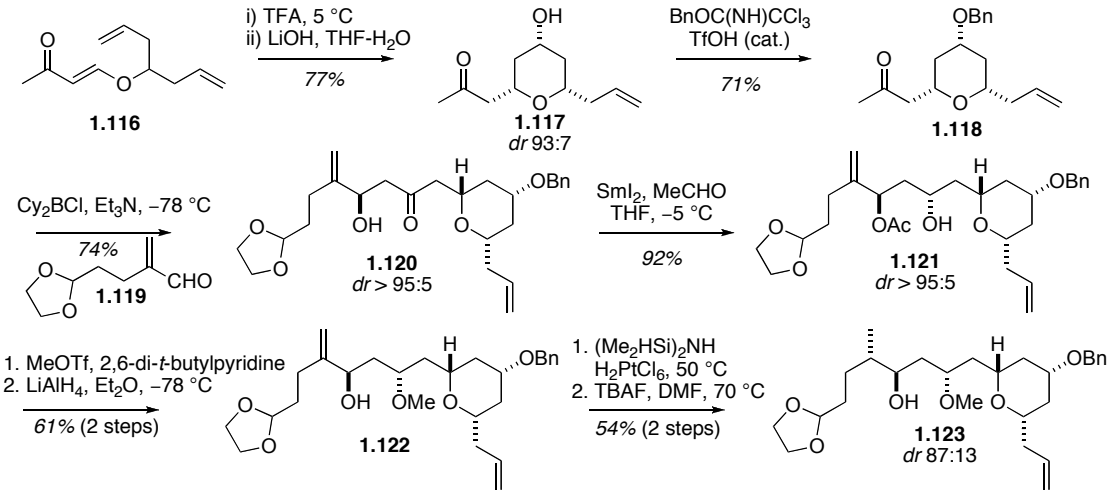
To date, **1.107** has been the subject of total syntheses from the groups of Leighton, Kozmin, Carreira, Paterson, Panek and Rychnovsky.[64] In particular, the efforts of the Kozmin group demonstrate the power of organic synthesis in solving issues of supply in marine natural products and thereby enabling key biological studies.



Scheme 1.16 Kozmin's approach to the oxazole side chain of leucascandrolide A

Kozmin's first synthesis of leucascandrolide A utilized Helquist's acyl carbene approach[65] for preparation of the unusual oxazole side chain (Scheme 1.16).[66] Reaction of alkynyl nitrile **1.109** with diazomalonate **1.110** in the presence of the rhodium catayst $Rh_2(OAc)_4$ gave the fully substituted oxazole **1.111** in good yield following removal of the TIPS group (HF). Hydrogenation of the alkyne with Lindlar's catlyst gave the desired *Z* olefin, followed by super hydride reduction of the methyl ester with concomitant

removal of the oxazole C5 OMe group. Finally, conversion of the primary alcohol to the bromide (PPh_3, CBr_4) gave **1.112**. The terminal bromide was displaced with the lithium enolate derived from imine **1.113** to give, after workup, the 2-carbon-extended aldehyde. Still-Gennari olefination of this aldehyde followed by saponifcation (LiOH) gave carboxylic acid **1.115**.

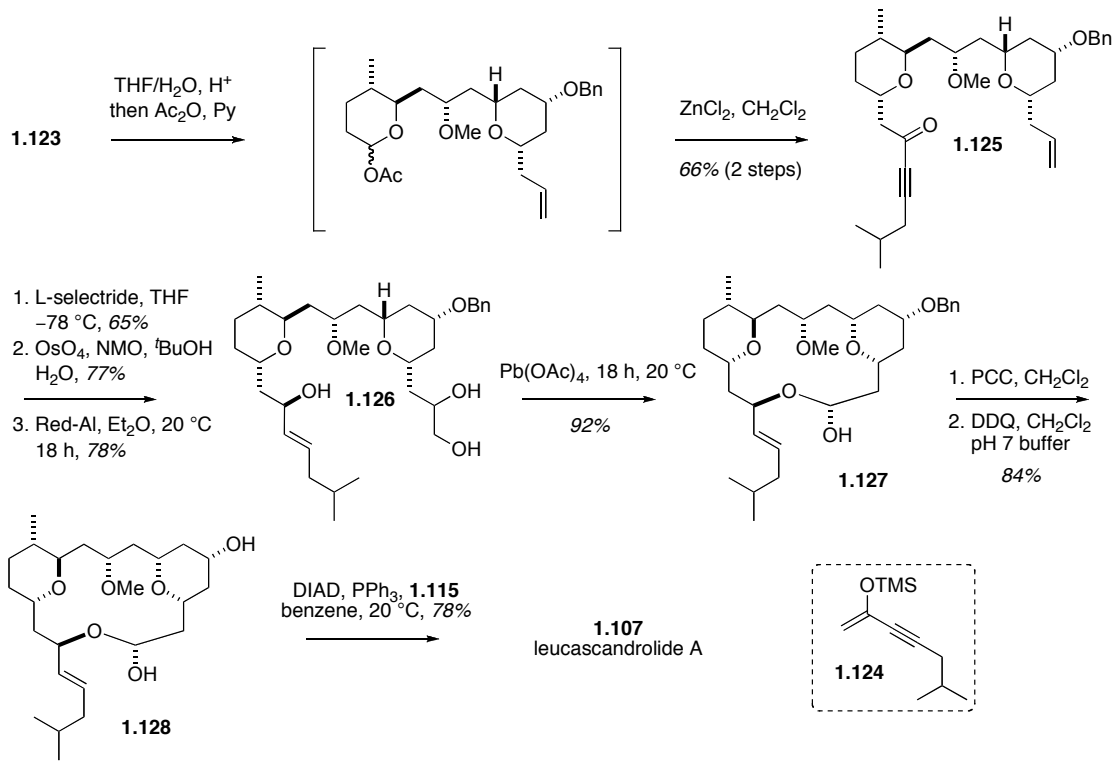


Scheme 1.17 Kozmin's synthesis of the macrolide portion of leucascandrolide

Synthesis of the macrolide portion began with a highly diastereoselective Prins cyclization of **1.116** to give the all-equatorial pyran **1.117** (Scheme 1.17). Protection of the free alcohol as its benzyl ether gave **1.118**. Paterson aldol reaction[67] of the boron enolate of **1.118** with aldehyde **1.119** gave **1.120** with excellent 1,5-*anti* stereoinduction (*dr*>95:5). Evans-Tischenko reduction[68] of the resulting ketone gave **1.121**; methylation of the newly formed alcohol and reductive removal of the acetate group to then gave

1.122. Hydrosilylation of the *exo* methylene gave an intermediate silacycle that was treated with TBAF to give advanced intermediate **1.123**.

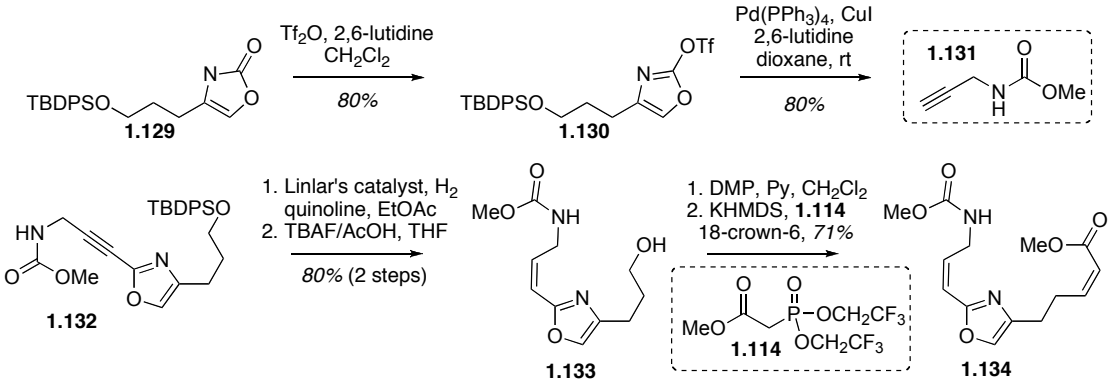
Removal of the acetonide group was effected with acid in aqueous THF to give the corresponding aldehyde (Scheme 1.18). Cyclization and acetylation provided the intermediate lactol acetate. Alkylation with **1.124** in the presence of $ZnCl_2$ resulted in formation of the desired pyran **1.125** as a single diastereomer. Reduction of the ketone with L-selectride proceeded with moderate selectivity (*dr* 2.5:1) giving the desired diastereomer in 65% isolated yield. Dihydroxylation of the terminal alkene (OsO_4, NMO) and reduction of the alkyne (Red-Al) yielded triol **1.126**. Remarkably, oxidative cleavage of the 1,2-diol ($Pb(OAc)_4$) resulted in macrocyclization to give a stable hemiacetal (**1.127**) which was oxidized with PCC to give the macrolide **1.128** following oxidative benzyl deprotection (DDQ). Finally, introduction of the oxazole side chain was effected *via* a Mitsonobu inversion of the hydroxyl group in **1.128** with acid **1.115** to yield (±)-leucascandrolide A in 78% yield.



Scheme 1.18 Completion of Kozmin's synthesis of leucascandrolide A

Panek and coworkers developed a unique approach to the oxazole side chain of leucascandrolide A utilizing Sonogashira coupling of 2-trifloyloxazoles (Scheme 1.19).[69] Treatment of oxazolidinone **1.129** with triflic anhydride gave 2-trifloyloxazole **1.130**. Sonogashira coupling with alkyne **1.131** gave the desired product **1.132** in excellent yield. Choice of solvent proved critical, as use of DMF (instead of dioxane) led to significantly reduced yields (55%). Interestingly, use of triethylamine in DMF led only to decomposition of the unstable trifloyloxazole. Panek *et al*. have extended this methodology to include 4-trifloyloxazoles, 4-trifloylthiazoles and 2-trifloylisoxazoles and have also shown that 4-trifloyloxazoles also undergo efficient Stille cross

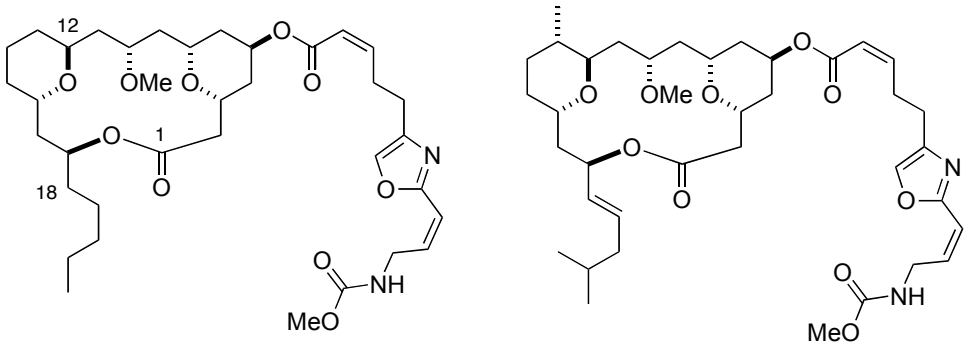
couplings.[70] This latter methodology was applied to a synthesis of the C26-C31 fragment of phorboxazole A.[71] Reduction of alkyne **1.132** with Lindlar's catalyst and removal of the primary TBDPS group then gave gave alcohol **x133**. Oxidation (Dess-Martin periodinane) and Still-Genari olefination gave **1.134** in good yield, completing an efficient and novel preparation of the leucascandrolide side chain.



Scheme 1.19 Panek's synthesis of the leucascandrolide side chain.

Kozmin *et al*. recently completed the synthesis of a simplified analogue of leucascandrolide A (**1.135**).[72] Strategic modifications were made such that **1.135** would adopt a similar conformation to the parent natural product (ie. removal of the C12 and C21 methyl groups and removal of the C18-C19 side olefin). The oxazole-containing side chain was retained, as it appears to be necessary for activity. Interestingly, the two enantiomers of leucascandrolide (obtained by separation of the originally-synthesized racemic material) show quite similar bioactivity profiles, so **1.135** was prepared in racemic form using

a very similar approach to that described previously for **1.107**. The structural simplifications meant that **1.135** could be synthesized in only 15 steps (longest linear sequence) and 24 total steps compared with 18 linear and 34 total steps for **1. 107**.



1.135 simplified leucascandrolide analogue **1.107** leucascandrolide

Figure 1.8 Structure of the rationally-designed leucascandrolide analogue

Analogue **1.135** exhibited almost identical cytotoxicity (A549 lung tumor, PC3 prostate tumor and HCT-116 colon tumor cells) and antifungal activity (*Saccharomyces cerevisiae*) to the natural product. Analogue **1.135** was screened against a library of 4900 haploid yeast strains featuring single deletions of non-essential genes. Analysis of growth rates revealed several strains with supersensitivity to **1.135** when grown on 2% galactose. One of these strains contained a deletion of SNF4, a gene that encodes the regulatory unit of the yeast homologue of AMPK. In mammals, AMPK is activated in cases of low ATP concentration due to consumption or inhibited production. In yeast, SNF4 is required for growth in the absence of glucose.

This suggested that **1.135** might inihibit 'non-glucose' ATP production – in other words, oxidative phosphorylation. Extensive studies eventually revealed that cytochrome bc_1 complex is the target of **1.135** and by extension, leucascandrolide. Both analogue **1.135** and neopeltolide were shown to reduce the activity of purified bovine heart mitochondrial cytochrome bc_1 complex by 50% at a concentration of 6 nm. Cytochrome bc_1 complex is also referred to as Complex III of the mitochondrial electron transport chain. It is a transmembrane protein responsible for reduction of cytochrome C ($Fe^{3+} \rightarrow Fe^{2+}$), a proton-coupled reaction that contributes to the proton gradient across the mitochondrial membrane. This gradient is crucial for ATP production.

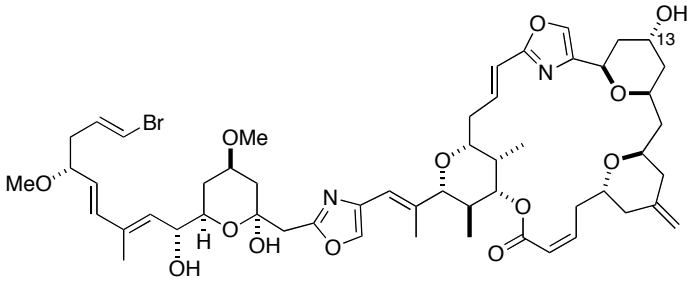
Leucascandrolide A represents a rare story in marine natural products spanning isolation, initial biological investigation, total synthesis, medicinal chemistry and finally identification of an enzyme target. Inhibition of cytochrome bc_1 complex seems to account for the potent antifungal activity of leucascandrolide (and neopeltolide), however the contribution of cytochrome bc_1 inhibition to cancer cell toxicity is not clear and will no doubt be the subject of future research.

1.9 Phorboxazoles A and B

Phorboxazoles A and B (**1.136** and **1.137**) are unprecedented macrocyclic natural products from the marine sponge *Phorbas* sp. collected off the coast of Western Australia.[73] A single collection of *Phorbas* sp. has yielded

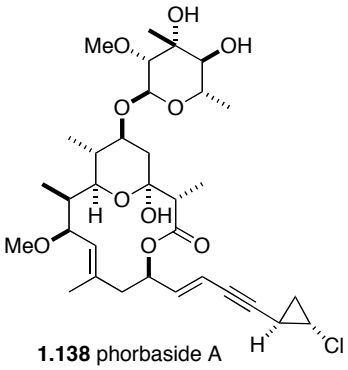
a striking array of polyketide secondary metabolites, including phorbaside A (**1.138**) and muironolide (**1.139**).[74,75] Phorboxazoles A and B displayed comparable potent activity against *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* (see Figure 1.9), however it was the cytotoxicity that proved most striking. Both **1.136** and **1.137** were evaluated against the National Cancer Institute 60 Tumor Cell panel and demonstrated a mean GI_{50} value of $< 1.58 \times 10^{-10}$ M with particularly strong activity against solid tumors (see Figure 1.9). These values place phorboxazoles amongst the most cytotoxic compounds yet discovered.

The phorboxazoles feature a striking array of 5 pyran and 2 oxazole rings; 3 of these rings (2 pyrans and 1 oxazole) are embedded in a 25-membered macrolide ring and the other two in the C24-C46 side chain that terminates with an *E*-vinyl bromide. The structural complexity and phenomenal activity of **1.136** and **1.137** have stimulated more activity amongst synthetic chemists than any of the natural products discussed thus far, resulting in six total syntheses of phorboxazole A[76] and four of phorboxazole B.[77] These syntheses have inspired the development of several original methodologies for the preparation of 2,4-disubstituted oxazoles. Presentation of all of the details of these impressive syntheses is not possible, so discussion will be limited to preparation of oxazole-containing fragments only.

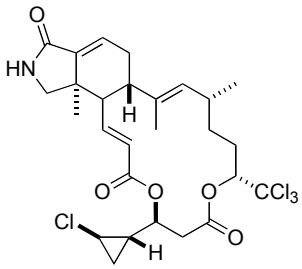


1.136 phorboxazole A
1.137 phorboxazole B (*epi*-C13)

Antifungal
Zones of inhibtion (disk diffusion assay):
1.136: 12 mm (1 μg/disk), 9 mm (0.1 μg/disk) (*S. cerevisiae*)
20 mm (1 μg/disk), 13 mm (0.1 μg/disk) (*S. carlsbergensis*)
1.137: 11 mm (1 μg/disk), 8 mm (0.1 μg/disk) (*S. cerevisiae*)
16 mm (1 μg/disk), 10 mm (0.1 μg/disk) (*S. carlsbergensis*)
Anti-cancer
HCT116 colon tumor GI_{50} 4.36×10^{-10} M
HT29 colon tumor GI_{50} 3.31×10^{-10} M
MCF7 breast tumor GI_{50} 5.62×10^{-10} M



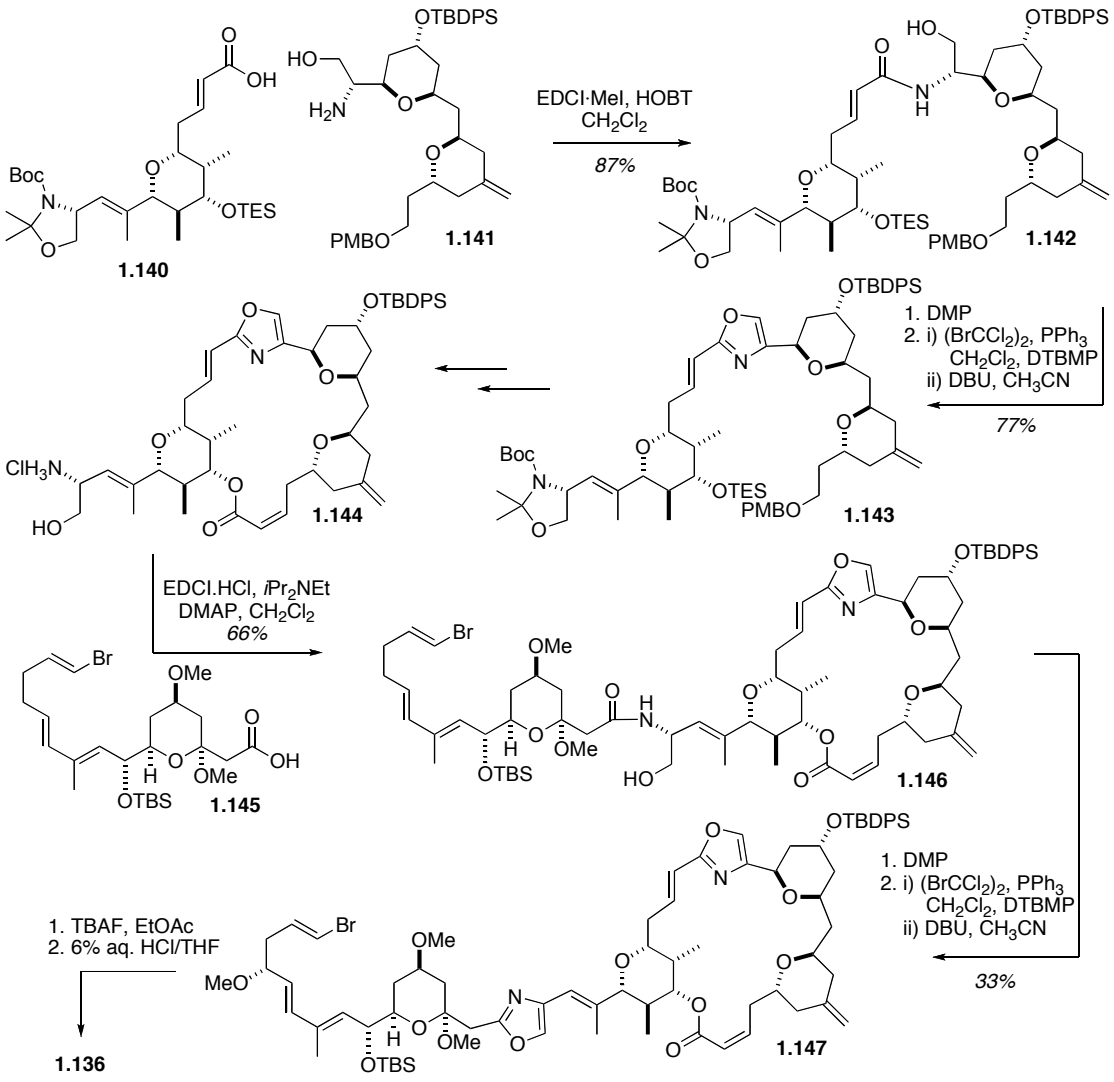
1.138 phorbaside A



1.139 muironolide A

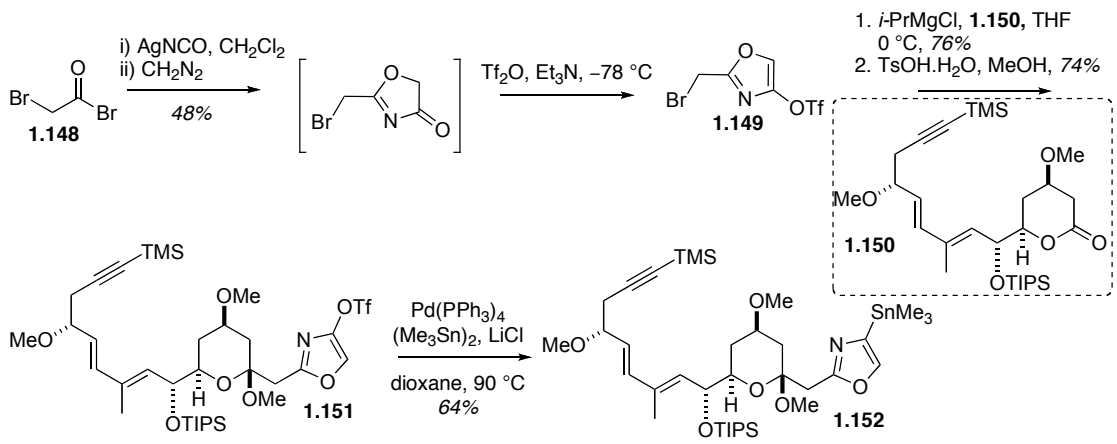
Figure 1.9 Structure and bioactivity of phorboxazoles A and B (**1.136** and **1.137**) and structures of phorbaside A (**1.138**) and muironolide A (**1.139**).

Forsyth *et al.* completed the first total synthesis of phoboxazole A[76a] utilizing a strategy that involved installation of the oxazole rings at a late stage *via* the Robinson-Gabriel-type cyclodehydration/oxidation method developed by Wipf (Scheme 1.20).[47] Thus, amide coupling between amino-alcohol **1.141** and acid **1.140** proceeded smoothly to give **1.142** which was oxidized to give the corresponding α-amido aldehyde. Cyclodehydration ($(BrCCl_2)_2$/PPh_3/2,6-di-*t*-butyl-4-methylpyridine) gave the intermediate bromooxazoline which eliminated HBr upon treatment with DBU to furnish the oxazole **1.143** in good yield.



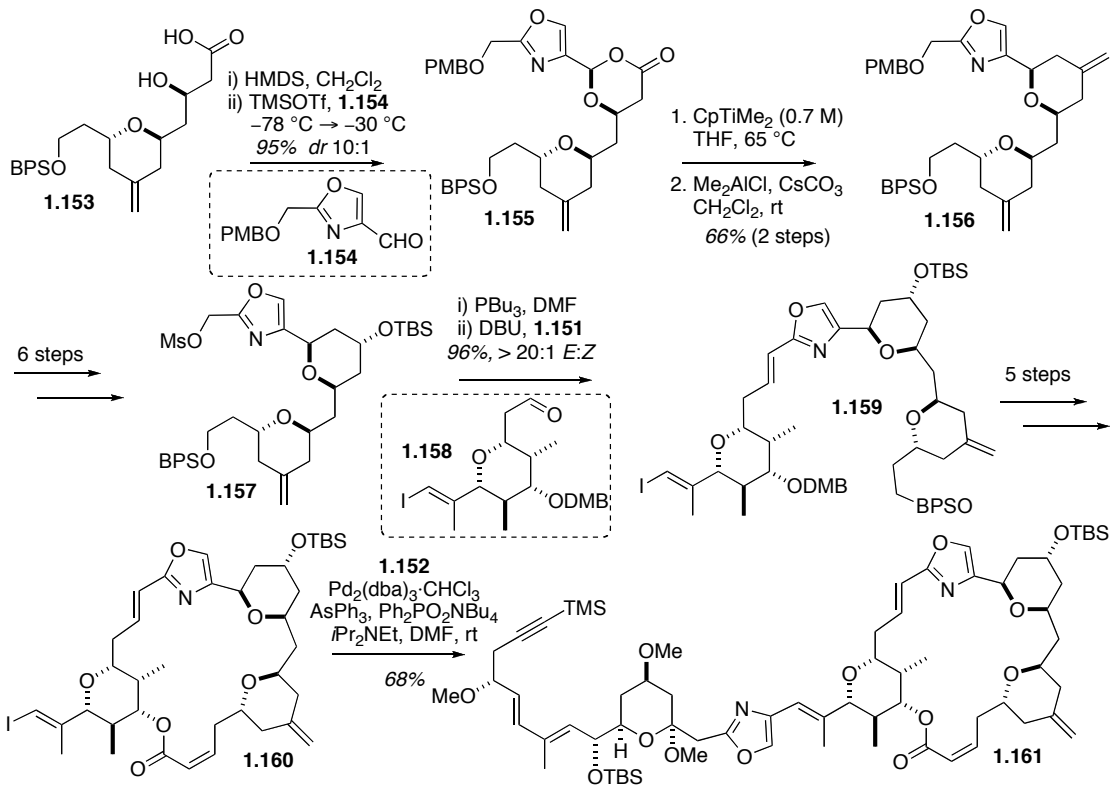
Scheme 1.20 Forsyth's synthesis of phorboxazole A.

A further 7 steps led to **1.144**, embodying the macrocyclic framework of phorboxazole A. Amide coupling with acid **1.145** provided serine-amide **1.146** which was subjected to the same cyclodehydration procedure as previously described to form the second oxazole ring in moderate yield (33%). A two-step deprotection protocol then furnished the natural product in a total of 34 steps (longest linear sequnce).



Scheme 1.21 Smith's approach to the C24-C46 side chain of phorboxazole A

The Smith group has developed a synthesis of phorboxazole A and a series of analogues that has been refined over 10 years and several generations.[76b,c,g,j,k] In the most recent generation, the oxazole side chain was prepared using a bifunctional oxazole linchpin (**1.149**) prepared using a variation of a reaction developed by Sheehan *et al*.[78] (Scheme 1.21). Specifically, reaction of bromo acetylbromide **1.148** with silver isocyanate formed, *in situ*, the corresponding acyl isocyanate which, upon treatment with diazomethane, furnished an intermediate oxazolone. Enolization and trapping with trifluoromethanesulfonic anhydride gave trifloyloxazole **1.149**.[79] Halogen-magnesium exchange (*i*PrMgCl) proceeded rapidly to provide the corresponding Grignard reagent that underwent nucleophilic addition to lactone **1.150** to give, after formation of the mixed methyl acetal, **1.151**. Finally, palladium cross coupling with $(Me_3Sn)_2$ provided the desired stannane **1.152**.



Scheme 1.22 Smith's second generation endgame toward phorboxazole A

Preparation of the macrocyclic portion of phorboxazole A was centered on a key Petassis-Ferrier rearrangement (Scheme 1.22). Union of oxazole aldehyde **1.154** with β-hydroxy acid **1.153** gave dioxanone **1.155** in good yield (95%) and diastereoselectivity (*dr* 10:1). Olefination of the carbonyl in **1.155** with the Petassis-Tebbe reagent (Cp_2TiMe_2) generated the corresponding enol-acetate in good yield provided that the reaction was run in the presence of ethyl pivalate to act as a scavenger for excess Cp_2TiMe_2. The key Petassis-Ferrier rearrangement proceeded in good yield (~80%) in the presence of Cs_2CO_3, an additive necessary to prevent loss of the PMB group. Six further steps provided intermediate **1.157**.

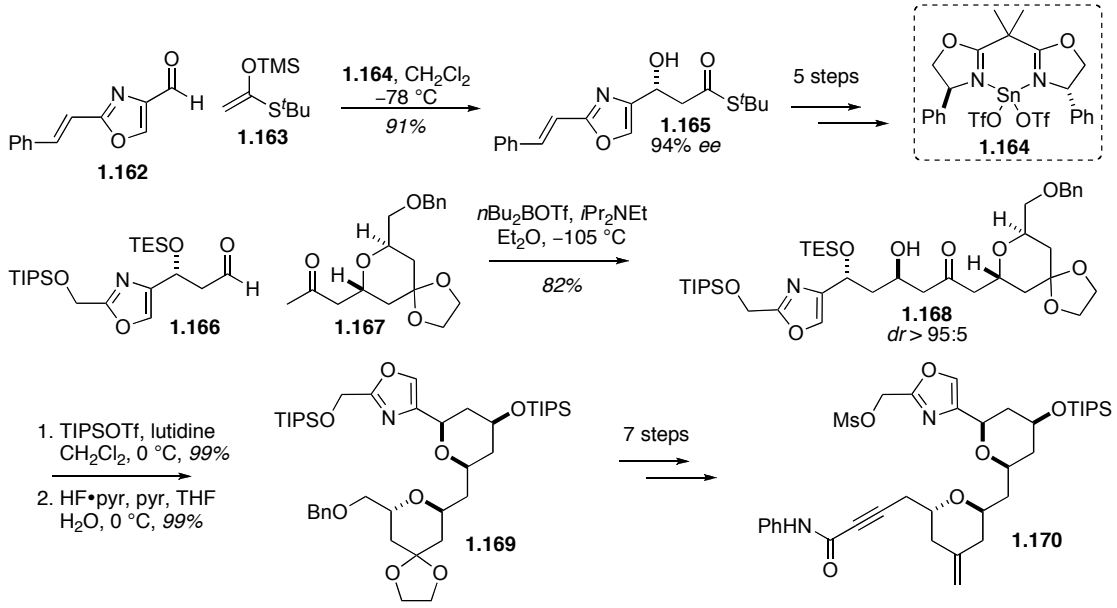
The subsequent Wittig reaction with **1.158** was found to proceed poorly when the ylide was prepared by treating the phosphonium salt derived from **1.157** with strong bases such as LiHMDS. Alternatively, formation of the phosphonium salt *in situ* (PBu_3) and reaction with aldehyde **1.158** in the presence of DBU furnished olefin **1.159** in excellent yield (96%) and selectivity (>20:1 *E*/*Z*). This is the same strategy developed by Evans for the synthesis of calyculin A.[29] Five further steps provide macrocycle **1.160**. The final key carbon-carbon bond-forming step involved Stille coupling of oxazole stannane **1.157** with the advanced vinyl iodide **1.160**. In the event the coupling proceeded in good yield to give **1.161** representing the entire carbon skeleton of phorboxazole A.

Smith's refined synthetic route to phorboxazole A has enabled the preparation of a series of structural analogues.[76k] Biological evaluation of these analogues has revealed several interesting features:

1. The C13 hydroxyl group is not required for activity (although analogues without this hydroxyl group were not as active as **1.136**
2. The C2-C3 *Z*-olefin is required for activity. Analogues with the corresponding *E*-olefin displayed significantly reduced activity.
3. Replacement of the terminal bromide with chloride gives an analogue "chlorophorboxazole A" somewhat more active than **1.136**, displaying sub-nanomolar activity against a range of tumor cells.

In 2000, Evans *et al.* reported the first total synthesis of phorboxazole B.[77a-c] Their synthesis focused on the application of asymmetric aldol reactions to set the configuration of the majority of the chiral centers in **1.137**. In particular, they pioneered several asymmetric aldol reactions involving oxazole aldehydes, a strategy that had not been widely employed previously. For example, addition of silylketene acetal **1.163** to oxazole aldehyde **1.162** was carried out in the presence of the bisoxazoline ("box") catalyst **1.164** to give **1.165** in excellent yield and high *ee* (91% yield, 94% *ee*) (Scheme 1.23). This aldol addition is significant because it the first example of the use of the "box" catalysts with an oxazole aldehyde. Such catalysts display selectivity only with aldehydes that have ability to chelate to a Lewis acid (tin in this case). Evans *et al.* rationalized that **1.162**, with a nitrogen atom at the α position, might behave like a chelating aldehyde and indeed the out come of the reaction seems to support this hyporthesis. Several standard functional group manipulations led to aldehyde **1.166** which underwent a highly diastereoselective aldol addition with the boron enolate derived from methyl ketone **1.167**. Evans *et al.* had previously shown that that methyl ketones with a β-alkoxyl group (such as **1.167**) undergo aldol additions to chiral aldehydes with high levels of 1,5-*anti* asymmetric induction.[80] The outcome of the aldol addition is controlled entirely by the configuration of the β-alkoxyl stereocenter of the ketone, regardless of the configuration of the aldehyde. Silyl protection of the newly formed alcohol (TIPSCl, imidazole) followed by selective removal

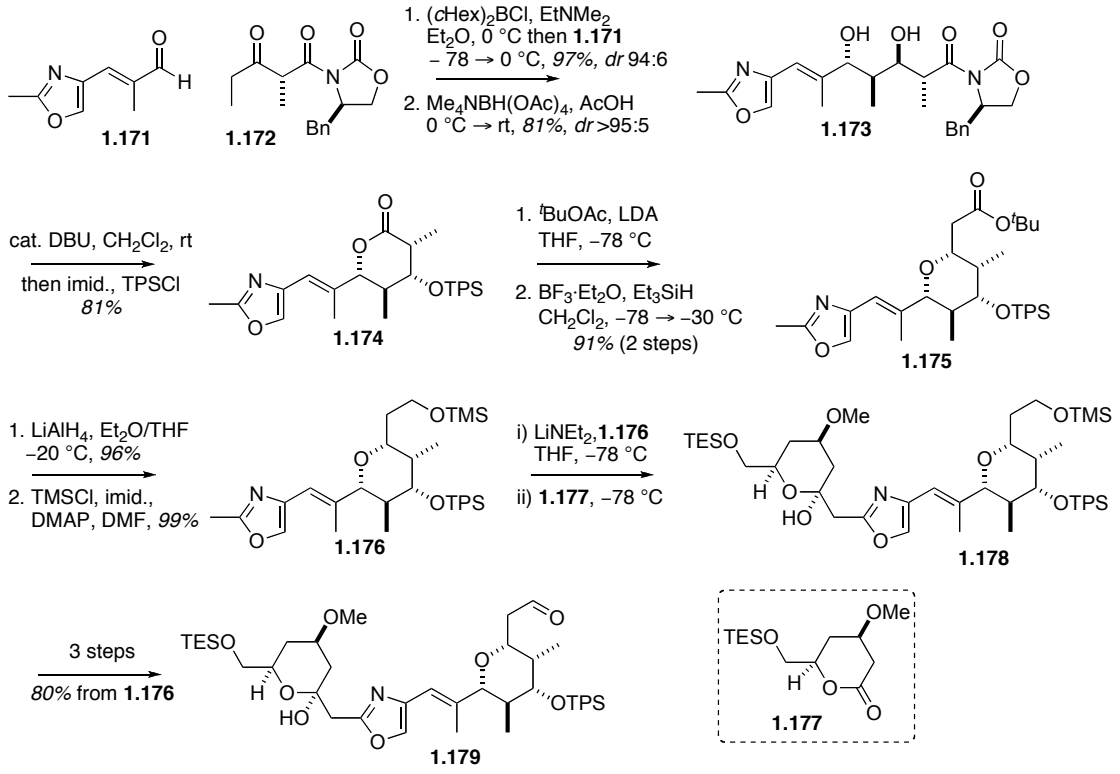
of the TES group (HF·pyridine) furnished bis-pyran **1.169**. A further 7 steps provided key intermediate **1.170**.



Scheme 1.23 Evan's synthesis of phorboxazole B

The synthesis continued with a diastereoselective aldol addition of the boron enolate derived from **1.172** to oxazole aldehyde **1.171** to give the desired *anti* product **1.173** in 97% yield. Reduction of the ketone ($Me_4NBH(OAc)_3$) was directed by the free hydroxyl group to provide the 1,3-*anti* diol **1.173**.[81] In the presence of a catalytic amount of DBU **1.173** cyclized to form the lactone **1.174**. Addition of the enolate derived from *tert*-butylacetate and reduction of the resulting hemiketal ($BF_3{\cdot}Et_2O$, Et_3SiH) provided the 2,6-*syn* pyran **1.175**. Reduction of the ester and protection of the primary alcohol then yielded **1.176**. The next step required selective metalation of the oxazole methyl substituent, however attempts to do so with

LDA were hindered by the comparably kinetic acidity of the oxazole C5 proton. Remarkably, deprotonation with $LiNEt_2$ afforded, after low temperature equilibration the desired lithiated species that was alkylated with lactone **1.177**.[82] The product **1.178** was isolated as a single diastereomer that was converted in three steps to key intermediate **1.179**.



Scheme 1.24 Evan's synthesis of phorboxazole B

While numerous syntheses of of phorboxazole A and B have been reported, the mechanism of action of the natural products remains elusive. Several anticancer natural products (eg. Taxol® and halichondrin B) are known to interfere with microtubule formation which usually leads to cell cycle

arrest in the M phase when cellular division takes place. Phoboxazoles, on the other hand, do not appear to affect microtubules and furthermore have been shown to induce S phase arrest in Burkitt lymphoma CA46 cells.[73c] DNA replication takes place during S phase, suggesting that phorboxazoles may have a very unique mechanism of action. Forsyth and La Clair recently published findings from experiments in which HeLa cells were treated with fluorescently labeled phorboxazole A.[83] Analysis of cell lysates by affinity chromatography and gel electrophoresis indicated that labeled phorboxazoles associated with cytokeratins KRT1, KRT9 and KRT10. Repetition of the analysis using resins coated with an anti-KRT10 antibody indicated that phorboxazoles induce association of KRT10 and cyclin dependent kinase 4 (cdk4) a protein crucial for G1-S phase cell cycle progession.

1.10 Conclusions

At the beginning of the 21st century the battle against infectious diseases and cancer remain far from won. The current demand for new lead compounds against such diseases indicates that natural products should be re-prioritized in the context of the pharmaceutical industry. In the academic setting, the study of marine natural products as a source of anti-cancer and antifungal drug leads remains vibrant, engaging isolation chemists and spectroscopists, synthetic chemists and biologists. New frontiers in marine natural product discovery are opening up through advances in instrumentation

that allow isolation and structure elucidation on the nanomole scale and investigation of previously unstudied source organisms, such as obligate marine actinomycetes.[84]

Natural product synthesis has traditionally served to help elucidate or confirm structures, alleviate supply problems and stimulate advances in fundamental organic chemistry. Since the days of R. B. Woodward synthesis has developed into a 'mature science'; the question is no longer 'if' a particular structure can be synthesized, but 'when'. The role of synthesis in the study of natural products is therefore evolving. In the current age of target-based drug discovery it is increasingly important to define the mechanism of action of bioactive natural products. Synthetic chemistry is positioned to make valuable contributions in this context. The work of Kozmin with leucascandrolide is a case in point: synthesis provided a means of obtaining the natural product (which is no longer available from the sponge source) and also allowed access to a rationally designed, simplified analogue which in turn facilitated elegant mechanism of action studies. Synthesis can also provide access to 'tagged' analogues of natural products. The work of Forsyth and La Clair in preparing a fluorescent analogue of phorboxazole A (**1.136**) exemplifies this approach.[83] The tagged analogue was visualized in HeLa cells using fluorescence microscopy, revealing cell uptake kinetics, subcellular localization and the effect on cell cycle progession of **1.136**. Tagged natural products can also be used to identify specific protein or enzyme targets *via* affinity pull-down

(immunoprecipitation) experiments, as recently demonstrated by Fenical and La Clair with ammosamides A and B.[85] Engaging in such multidisciplinary endeavors enables organic synthesis to contribute to advances in fundamental fundamental disease biology.

The following chapters present research on several bioactive, heterocyclic natural products from marine sponges including phorbasides A and B, 2*R*-*Z*-dysidazirine and enigmazole A. While organic synthesis plays a role in each chapter, the goals are different. In the case of Phorbasides A and B organic synthesis and circular dichroism were used to assign the absolute configuration of the ene-yne chlorocylopropane side chain.

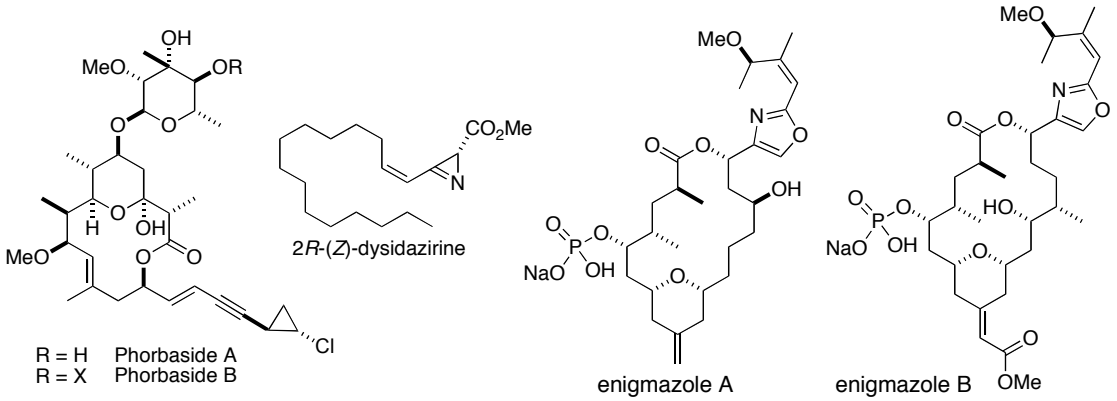


Figure 1.10 Structures of the heterocyclic marine natural products that constitute the subject matter of the following chapters: phorbasides A and B, 2*R*-(*Z*)-dysidazirine and enigmazoles A and B.

In the case of 2*R*-(*Z*)-dysidazirine total synthesis of the natural product and preparation of a series of analogues facilitated the development of a Structure-Activity-Relationship for the antifungal activity of long-chain 2*H*-azirine carboxylates. Finally, in the case of enigmazoles a total synthesis of

enigmazole A was designed and implemented as part of a larger goal of defining the biological mechanism of action and minimum pharmacophore of enigmazole B. These studies also resulted in the development of a procedure for direct formation of functionalized oxazol-2-yl zinc reagents by zinc insertion.

References

(1) a) *Natural Products: Drug Discovery and Therapeutic Medicine* Zhang, L.; Demain, A. L. Eds. Humana Press: New Jersey, 2005. b) *Anticancer Agents from Natural Products* Cragg, G. M.; Kingston, D. G. I.; Newman, D. J. Eds. CRC Press: Boca Raton, 2005.

(2) Lederberg, J. *Science* **2000**, *288*, 287-293.

(3) a) Butler, M. S. *Nat. Prod. Rep.* **2005**, *22*, 162-195. b) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Disovery* **2009**, *8*, 69-85.

(4) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022-1037.

(5) Clardy, J.; Walsh, C. *Nature* **2004**, *432*, 829-837.

(6) Williams, D. H.; Stone, M. J.; Hauck, P. R.; Rahman, S. K. *J. Nat. Prod.* **1989**, *52*, 1189-2108.

(7) Verdine, G. L. *Nature* **1996**, *384* (Supp.), 11-13.

(8) Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discovery* **2005**, *4*, 206-220.

(9) Class, S. *Chem. Eng. News* **2002**, *80*, 39-49.

(10) American Cancer Society: *Cancer Facts and Figures 2009*. Atlanta: American Cancer Society; 2009.

(11) Mekhail, T. M.; Markman, M. *Exp. Opin. Pharmacother.* **2002**, *3*, 755-766.

(12) Alexander, B. P.; Perfect, J. R. *Drugs* **1997**, *54*, 657-678.

(13) a) White, T. C.; Holleman, S.; Dy, F.; Mirels, L. F.; Stevens, D. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1704-1713. b) Lyons, C. L.; White, T. C. *Antimicrob. Agents Chemother.* **2000**, *44*, 2296-2303. c) Georgopapadakou, N. H. *Curr. Opin. Microbiol.* **1998**, *1*, 547-557. d) White, T. C.; Marr, K. A.; Bowden, R. A. *Clin. Microbiol. Rev.* **1998**, *11*, 382-402.

(14) Law, D.; Moore, C. B.; Wardle, H. M.; Ganguli, L. A.; Keaney, M. G.; Denning, D. W. *J. Antimicrob. Chemother.* **1994**, *34*, 659-686.

(15) Paterson, I.; Anderson, E. A. *Science* **2005**, *310*, 451-453.

(16) Olivera, B. M.; Gray, W. R.; Zeikus, R.; McIntosh, J. M.; Varga, J.; Rivier, J.; de Santos, V.; Cruz, L. J. *Science* **1985**, *230*, 1338-1343.

(17) a) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Stroh, J. G.; Keifer, P. A.; Sun, F.; Li, L. H.; Martin, D. G. *J. Org. Chem.* **1990**, *55*, 4512-4515. b) Wright, A. E.; Forleo, D. A.; Gunawardana, G. P.; Gunasekera, S. P.; Koehn, F. E.; McConnell, O. J. *J. Org. Chem.* **1990**, *55*, 4508-4512.

(18) Molinski, T. F. *Curr. Opin. Drug Discov. Dev.* **2009**, *12*, 197-206.

(19) a) Uemura, D.; Takahashi, K.; Yamamoto, T. *J. Am. Chem. Soc.* **1985**, *107*, 4796-4798. b) Hirata, Y.; Uemura, D. *Pure App. Chem.* **1986**, *58*, 701-710.

(20) a) Bai, R. L.; Paull, K. D.; Herald, C. L.; Malspeis, L.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1991**, *266*, 15882-15889. b) Dabydeen, D. A.; Burnett, J. C.; Bai, R.; Verdier-Pinard, P.; Hickford, S. J. H.; Pettit, G. R.; Blunt, J. W.; Munro, M. H.; Gussio, R.; Hamel, E. *Mol. Pharmacol.* **2006**, *70*, 1866-1875.

(21) Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162-3164.

(22) Munro, M. H. G.; Blunt, J. W.; Dumdei, E. J.; Hickford, S. J. H.; Lill, R. E.; Li, S.; Battershill, C. N.; Duckworth, A. R. *J. Biotechnol.* **1999**, *70*, 15-25.

(23) a) Vahdat, L. T.; Pruitt, B.; Fabian, C. J.; Rivera, R. R.; Smith, D. A.; Tan-Chiu, E.; Wright, J.; Tan, A. R.; DaCosta, N. A.; Chuang, E.; Smith, J.; O'Shaughnessy, J.; Shuster, D. E.; Meneses, N. L.; Chandrawansa, K.; Fang, F.; Cole, P. E.; Ashworth, S.; Blum, J. L. *J. Clin. Oncol.* **2009**, *27*, 2954-2961. b) Goel, S.; Mita, A. C.; Mita, M.; Rowinsky, E. K.; Chu, Q. S.; Wong, N.; Desjardins, C.; Fang, F.; Jansen, M.; Shuster, D. E.; Mani, S.; takimoto, C. H. *Clin. Cancer Res.* **2009**, *15*, 4207-4212. c) Tan, A. R.; Rubin, E. H.; Walton, D. C.; Shuster, D. E.; Wong, Y. N.; Fang, F.; Ashworth, S.; Rosen, L. S. *Clin. Cancer Res.* **2009**, *15*, 4213-4219. d) Blum, J.; Forero, L.; Heiskala, M. K.; Meneses, N.; Chandrawansa, K.; Fang, F.; Shapiro, G.; Fields, S. Z.; Silberman, S.; Vahdat, L. *J. Clin. Oncol.* **2006**, *24* (Suppl. 18), 653. e) Blum, J. L.; Pruitt, B.; Fabian, C. J.; Rivera, R. R.; Shuster, D. E.; Meneses, N. L.; Chandrawansa, K.; Fang, F.; Fields, S. Z.; Vahdat, L. **2007**, *25* (Suppl. 18), 1034. f) Spira, A. I.; Ianotti, N. O.; Savin, M. A.; Neubauer, M.; Gabrail, N. Y.; Yanagihara, R.; Datta, K. K.; Zang, E. A.; Fields, S. Z.; Das, A. *J. Clin. Oncol.* **2007**, *25* (Suppl. 18), 7546.

(24) *Oxazoles: Synthesis, Reactions and Spectroscopy* Palmer, D. C. Ed. Wiley: New Jersey, 2003

(25) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 2780-2781.

(26) Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1991**, *32*, 5605-5606.

(27) Hamada, Y.; Tanada, Y.; Yokokawa, F.; Shioiri, T. *Tetrahedron Lett.* **1991**, *32*, 5983-5986.

(28) a) Ishihara, H.; Martin, B. L.; Brautigan, D. L.; Karaki, H.; Ozaki, H.; Kato, Y.; Fusetani, N.; Watabe, S.; Hashimoto, K.; Uemura, D.; Hartshorne, D. J. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 871-877. b) Suganuma, M.; Fujiki, H.; Furuya-Suguri, H.; Yoshizawa, S.; Yasumoto, S.; Kato, Y.; Fusetani, N.; Sugimura *Cancer Res.* **1990**, *50*, 3521-3525.

(29) Evans, D. A.; Gage, J. R.; Leighton, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 9434-9453.

(30) Evans, D. L.; Minster, D. K.; Jordis, U.; Hecht, S. M.; Mazzu, Jr. A. L.; Meyers, A. I. *J. Org. Chem.* **1979**, *44*, 497-501.

(31) Smith, A. B.; Friestad, G. K.; Barbosa, J.; Bertounesque, E.; Hull, K. G.; Iwashima, M.; Qiu, Y.; Salvatore, B. A.; Spoors, P. G.; Duan, J. J. –W. *J. Am. Chem. Soc.* **1999**, *121*, 10468-10477; e) Smith, A. B.; Friestad, G. K.; Barbosa, J.; Bertounesque, E.; Duan, J. J. –W.; Hull, K. G.; Iwashima, M.; Qiu, Y.; Spoors, P. G.; Salvatore, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 10478-10486.

(32) Davidson, d. Weiss, M.; Jelling, M. *J. Org. Chem.* **1937**, *2*, 328-334.

(33) Burgess, E. M.; Penton, H. R.; Taylor, E. A.; Williams, W. M. *Org. Synth.* **1977**, *56*, 40.

(34) Wipf, P.; Miller, C. P. *Tetrahedron Lett.* **1992**, *33*, 907-910.

(35) Barrish, J. C.; Singh, J.; Spergel, S. H.; Han, W. –C.; Kissick, T. P.; Kronenthal, D. R.; Mueller, R. H. *J. Org. Chem.* **1993**, *53*, 4494-4496.

(36) Adamczeski, M.; Quiñoà, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110*, 1598-1602.

(37) Searle, P. A.; Richter, R. K.; Molinski, T. F. *J. Org. Chem.* **1996**, *61*, 4073-4079.

(38) Molinski, T. F. *J. Nat. Prod.* **1993**, *56*, 1-8.

(39) Mulder, R. J.; Shafer, C. M.; Molinski, T. F. *J. Org. Chem.* **1999**, *64*, 4995-4998.

(40) a) Schröder, R.; Schöllkopf, U.; Blume, E.; Hoppe, I. *Justus Liebigs Ann. Chem.* **1975**, 533-546. b) Jacobi, P. A.; Ueng, S.; Carr, D. *J. Org. Chem.* **1979**, *44*, 2042-2044. c) Hodges, J. C.; Patt, W. C.; Connolly, C. J. *J. Org. Chem.* **1991**, *56*, 449-452.

(41) Vedejs, E. Monahan, S. D. *J. Org. Chem.* **1996**, *61*, 5192-5193.

(42) a) Bull, J. A.; Balskus, E. P.; Horan, R. A. J.; Langner, M.; Ley, S. V. *Angew. Chem. Int. Ed.* **2006**, *45*, 6714-6718. b) Bull, J. A.; Balskus, E. P.; Horan, R. A. J.; Langner, M.; Ley, S. V. *Chem. Eur. J.* **2007**, *13*, 5515-5538.

(43) a) Schöllkopf, U.; Schröder, R. *Angew. Chem Int. Ed.* **1971**, *10*, 333. b) van Leusen, A. M.; Hoogenboom, B. E.; Siderius, H. *Tetrahedron Lett.* **1972**, 2369.

(44) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165-1168.

(45) Williams, D. R.; Lowder, P. D.; Gu, Y. –G.; Brooks, D. A. *Tetrahedron Lett.* **1997**, *38*, 331.

(46) Panek, J. S.; Beresis, R. T. *J. Org. Chem.* **1996**, *61*, 6496-6496.

(47) a) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604-3606. b) Wipf, P.; Sungtaek, L. *J. Am. Chem. Soc.* **1995**, *117*, 558-559.

(48) Fusetani, N.; Yasumuro, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1989**, *30*, 2809-2812.

(49) Roesener, J. A.; Scheuer, P. J. *J. Am. Chem. Soc.* **1986**, *108*, 846-857.

(50) a) Kernan, M. R.; Faulkner, D. J. *Tetrahedron Lett.* **1987**, *28*, 2809-2812. b) Kernan, M. R.; Molinski, T. F.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 5014-5020.

(51) Matsunaga, S.; Fusetani, N.; Hashimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 847-849.

(52) Pawlik, J. R.; Kernan, M. R.; Molinski, T. F.; Harper, M. K.; Faulkner, D. J. *J. Exp. Mar. Biol. Ecol.* **1988**, *119*, 99-109.

(53) a) Hori, M.; Saito, S.; Shin, Y. Z.; Ozaki, H.; Fusetani, N.; Karaki, H. *FEBS Lett.* **1993**, *322*, 151-154. b) Saito, S.; Watabe, S.; Ozaki, H.; Fusetani, N.; Karaki, H. *J. Biol. Chem.* **1994**, *269*, 29170-29714.

(54) Vincent, E.; Saxton, J.; Baker-Glenn, C.; Moal, I.; Hirst, J. D.; Pattenden, G.; Shaw, P. E. *Cell. Mol. Life Sci.* **2007**, *64*, 487-497.

(55) Tanaka, J.; Yan, Y.; Choi, J.; Bai, J.; Klenchin, V. A.; Rayment, I.; Marriot, G. *Proc. Natl. Acad. Sci.* **2003**, *100*, 13851-13856.

(56) Wada, S.; Matsunaga, S.; Saito, S.; Fusetani, N.; Watabe, S. *J. Biochem.* **1998**, *123*, 946-952.

(57) Liu, P.; Panek, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 11090-11097.

(58) Pattenden, G.; Ashweek, N. J.; Baker-Glenn, C. A. G.; Walker, G. M.; Yee, J. G. K. *Angew. Chem. Int. Ed.* **2007**, *46*, 4359-4363.

(59) Liu, P.; Celatka, C. A.; Panek, J. S. *Tetrahedron Lett.* **1997**, *38*, 5445-5448.

(60) a) Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644-5646. b) Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048-6050.

(61) D'Ambrosio, M.; Guerriero, A; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1996**, *79*, 51-60.

(62) Wright, A. E.; Botelho, J. C.; Guzmán, E.; Harmody, D.; Linley, P.; McCarthy, P. J.; Pitts, T. P.; Pomponi, S. A.; Reed, J. K. *J. Nat. Prod.* **2007**, *70*, 412-416.

(63) D'Ambrosio, M.; Tatò, M.; Pocsfalvi, G.; Debitus, C.; Pietra, F. *Helv. Chim. Acta* **1999**, *82*, 347-353.

(64) a) Hornberger, K. R.; Hamblett, C. L.; Leighton, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 12894-12895. b) Wang, Y.; Janjic, J.; Kozmin, S. A. *J. Am. Chem. Soc.* **2002**, *124*, 13670-13671. c) Fettes, A.; Carreira, E. M. *J. Org. Chem.* **2003**, *68*, 9274-9283. d) Paterson, I.; Tudge, M. *Tetrahedron* **2003**, *59*, 6833-6849. d) Su, Q.; Panek, J. S. *Angew Chem. Int. Ed.* **2005**, *44*, 1223-1225. e) Van Orden, L. J.; Patterson, B. D.; Rychnovsky, S. D. *J. Org. Chem.* **2007**, *72*, 5784-5793.

(65) Connell, R.; Scavo, F.; Helquist, P. *Tetrahedron Lett.* **1986**, *27*, 5559-5562.

(66) Kozmin, S. A. *Org. Lett.* **2001**, *3*, 755-758.

(67) Paterson, I.; Gibson, K. R.; Oballa, R. M. *Tetrahedron Lett.* **1996**, *37*, 8585-8588.

(68) Evans, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1990**, *112*, 6447-6449.

(69) Dakin, L. A.; Langille, N. F.; Panek, J. S. *J. Org. Chem.* **2002**, *67*, 6812-6815.

(70) Langille, N. F.; Dakin, L. A.; Panek, J. S. *Org. Lett.* **2002**, *4*, 2485-2488.

(71) Schaus, J. V.; Panek, J. S. *Org. Lett.* **2000**, *2*, 469-471.

(72) Ulanovskaya, O. A.; Janjic, J.; Suzuki, M.; Sabharwal, S. S.; Schumacker, P. T.; Kron, S. J.; Kozmin, S. A. *Nat. Chem. Biol.* **2008**, *4*, 418-424.

(73) a) Searle, P. A.; Molinski, T. F. *J. Am. Soc.* **1995**, *117*, 8126-8131. b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422-9423. c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879-7880.

(74) a) MacMillan, J. B.; Xiong-Zhou, G.; Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 3699-3706. b) Skepper, C. K.; MacMillan, J. B.; Zhou, G. –X.; Masuno, M. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129*, 4150-4151.

(75) Dalisay, D. S.; Morinaka, B. I.; Skepper, C. K.; Molinski, T. F. *J. Am. Chem. Soc.* **2009**, *131*, 7552-7553.

(76) a) Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 5597-5598. b) Smith, A. B. III; Verhoest, P. R.; Minbiole, K. P.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 4834. c) Smith, A. B. III; Minbiole, K. P.; Verhoest, P. R.; Schelhaas, M. *J. Am. Chem. Soc.* **2001**, *123*, 10942-10953. d) González, M. A.; Pattenden, G. *Angew. Chem. Int. Ed.* **2003**, *42*, 1255-1258. e) Pattenden, G.; González, M. A.; Little, P. B.; Millan, D. S.; Plowright, A. T.; Tornos, J. A.; Ye, T. *Org. Biomol. Chem.* **2003**, *1*, 4173-4108. f) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Angew. Chem. Int. Ed.* **2003**, *42*, 1258-1262. g) Smith, A. B. III; Razler, T. M; Ciavarri, J. P.; Hirose, T.; Ishikawa, T. *Org. Lett.* **2005**, *7*, 4399-4402. h) White, J. D.; Lee, T. H.; Kunttiyong, P. *Org. Lett.* **2006**, *8*, 6039-6042. i) White, J. D.; Lee, T. H.; Kunttiyong, P. *Org. Lett.* **2006**, *8*, 6043-6046. j) Smith, A. B. III; Razler, T. M.; Ciavarri, J. P.; Hirose, T.; Ishikawa, T.; Meis, R. M. *J. Org. Chem.* **2008**, *73*, 1192-1200. k) Smith, A. B. III; Razler, T. M.; Meis, R. M.; Pettit, G. R. *J. Org. Chem.* **2008**, *73*, 1201-1208.

(77) a) Evans, D. A.; Cee, V. J.; Smith, T. E.; Fitch, D. M.; Cho, P. S. *Angew. Chem. Int. Ed.* **2000**, *39*, 2533-2536. b) Evans, D. A.; Fitch, D. M. *Angew. Chem. Int. Ed.* **2000**, *39*, 2536-2540. c) Evans, D. A.; Fitch, D. M.; Smith, T. E.; Cee, V. J. *J. Am. Chem. Soc.* **2000**, *122*, 10033-10046. d) Li, D. –R.; Zhang, D. –H.; Sun, C. –Y.; Zhang, J. –W.; Yang,

L.; Chen, J.; Liu, B.; Su, C.; Zhou, W. –S.; Lin, G. –Q. *Chem. Eur. J.* **2006**, *12*, 1185. e) Lucas, B. S.; Gopalsamuthiram, V.; Burke, S. D. *Angew. Chem. Int. Ed.* **2007**, *46*, 769-772.

(78) Sheehan, J. C.; Izzo, P. T. *J. Am. Chem. Soc.* **1949**, *71*, 4059.

(79) Smith, A. B. III; Minbiole, K. P.; Freeze, S. *Synlett* **2001**, *11*, 1739-1742.

(80) Evans, D. A.; Coleman, P. J.; Côté, B. *J. Org. Chem.* **1997**, *62*, 788-789.

(81) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560-3578.

(82) Evans, D. A.; Cee, V. J.; Smith, T. E.; Santiago, K. J. *Org. Lett.* **1999**, *1*, 87-90.

(83) Forsyth, C. J.; Ying, L.; Chen, J.; La Clair, J. J. *J. Am. Chem. Soc.* **2006**, *128*, 3858-3869.

(84) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666-673.

(85) Hughes, C. C.; MacMillan, J. B.; Gaudêncio, S. P.; Fenical, W.; La Clair, J. J. *Angew. Chem. Int. Ed.* **2009**, *48*, 728-732.

CHAPTER 2

SYNTHETIC AND CHIROPTICAL STUDIES ON THE ENE-YNE CHLOROCYCLOPROPANE SIDE CHAIN OF PHORBASIDES A AND B

2.1 Ene-yne Chlorocyclopropane Marine Natural Products

In 1996 and 1997 Minale and co-workers reported the isolation of a unique family of cytotoxic macrolides callipeltosides A,[1] B[2] and C[2] (**2.1-2.3**) from the marine sponge *Callipelta* sp. collected near New Caledonia. Callipeltoside A displayed moderate cytotoxicity toward NSCLC-N6 and P388 cells (IC_{50} = 11.26 and 15.26 μg/mL respectively) and induced blockade of NSCLC-N6 cells in the G1 phase.

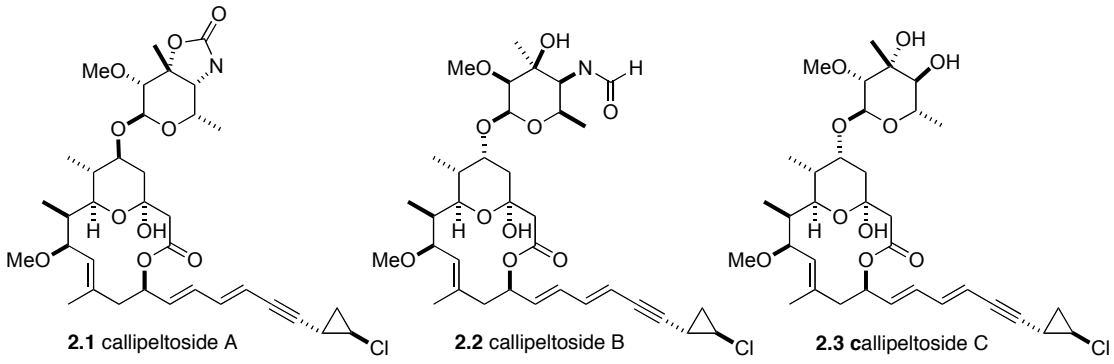
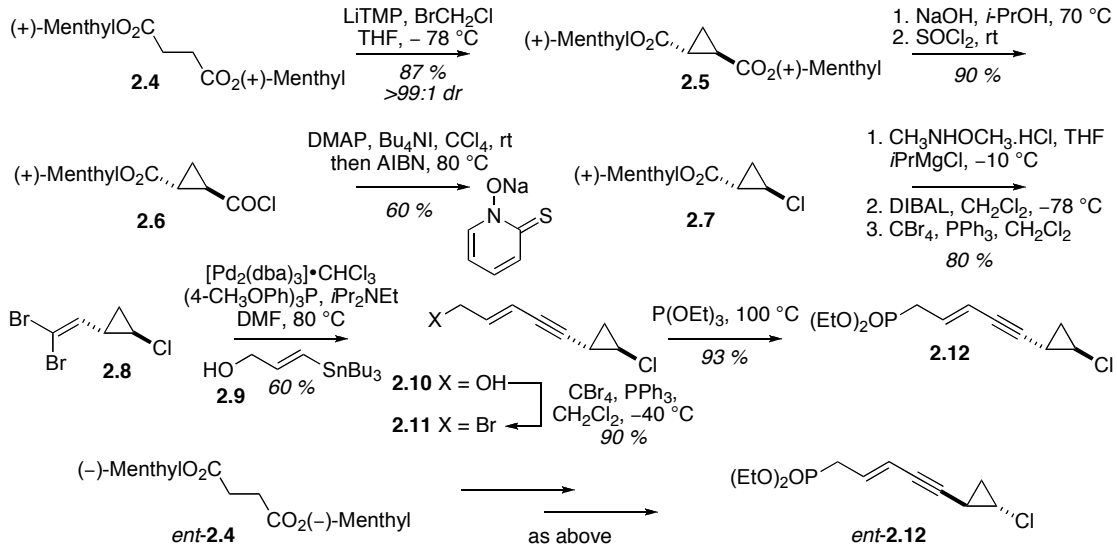


Figure 2.1 Structures of callipeltosides A-C

Callipeltoside A (**2.1**) possessed several unique structural elements, including a previously undescribed sugar moiety (callipeltose) attached by an α-*O*-glycoside linkage at C5 and a diene-yne chlorocyclopropane side chain appended at C13.

The diene-yne chlorocyclopropane side chain presented a problem for stereochemical assignment. Compound **2.1** consists of three isolated spin systems: the sugar portion, the macrolide portion and the chlorocyclopropane ring. The relative configuration of the macrolide and sugar portions were assigned by interpretation of ROESY and NOE difference NMR data. Several key NOE correlations allowed the relative configuration of the sugar to be relayed to the macrolide ring. The relative configuration of the *trans*-chlorocylcopropane ring ($J_{H20\text{-}21}$ = 3.1 Hz), however, could not be related to the remainder of the molecule due to the intervening diene-yne chromophore. Consequently there were four possible configurations for callipeltoside A.

Callipeltosides quickly garnered significant interest from the organic synthesis community due to their unique structures and the need for a complete configurational assignment. This attention has resulted in total syntheses first by the group of Trost[3] and followed by Evans,[4] Paterson,[5] Panek[6] and MacMillan.[7] The Trost group prepared two possible diastereomers of **2.1** differing only in configuration of the cyclopropane ring and showed them to be indistinguishable by ^{1}H and ^{13}C NMR. Optical rotation, however, proved different in both sign and magnitude thereby allowing an unambiguous assignment of the correct relative and absolute configuration of **2.1**.

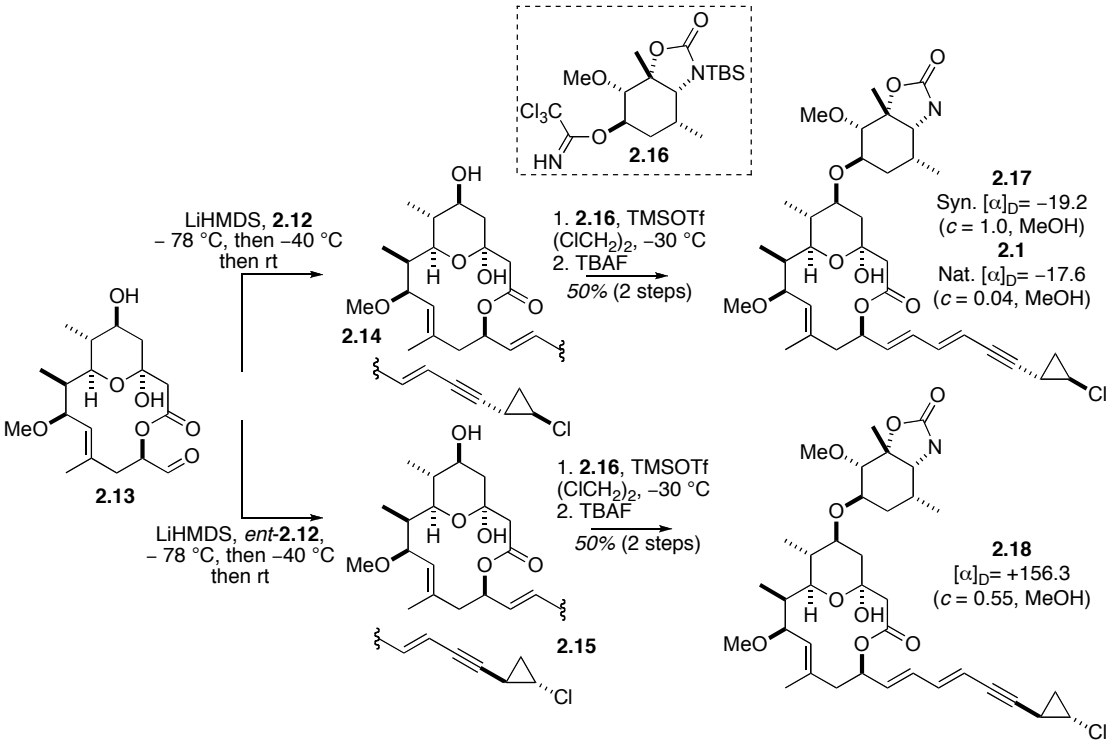


Scheme 2.1 Trost's preparation of the ene-yne chlorocyclopropane side chain of callipeltoside A

The Trost group's approach to the diene-yne side chain of **2.1** is depicted in Scheme 2.1. Cyclopropanation of dimenthyl succinate **2.4** by deprotonation and sequential double alkylation gave **2.5** with excellent diastereoselectivity. Saponification of one ester group followed by treatment of the liberated carboxylic acid with thionyl chloride yielded acyl chloride **2.6**. Barton-Crich-Motherwell decarboxylation in CCl_4 then provided the desired chlorocyclopropane **2.7** in reasonable yield. The remaining ester substituent was converted to the corresponding aldehyde by reduction of the corresponding Weinreb amide. Treatment of the aldehyde under Corey-Fuchs homologation conditions then gave vinyl dibromide **2.8** which was subjected to Stille coupling with vinyl stannane **2.9** to yield **2.10**. A two-step sequence

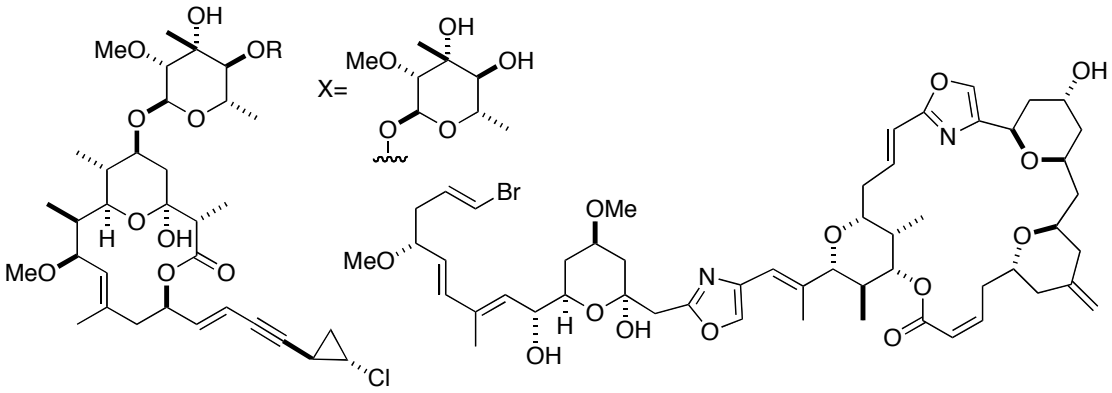
furnished phosphonate **2.12** in high yield. Repetition of this sequence starting from *ent*-**2.4** provided the antipodal phosphonate *ent*-**2.12**.

Macrolide fragment **2.13** was subsequently coupled to both **2.12** and *ent*-**2.12** to give **2.14** and **2.15** respectively. Glycosidation and final deprotection provided two diastereomers **2.17** and **2.18** which were *indistinguishable* by NMR. Fortunately the two compounds exhibited very different values for $[\alpha]_D$ (−19.2 for **2.17** and +156.3 for **2.18**) providing a clear match between **2.17** and with the natural product ($[\alpha]_D$ −17.6)[1] and defining the absolute configuration as shown.



Scheme 2.2 Trost's synthesis of callipeltoside A (**2.17**) and unnatural diastereomer **2.18**. The optical rotation of **2.17** matched that of **2.1** confirming the configuration as shown.

In 2004 our group isolated a family of cytotoxic macrolide glycosides, represented by phorbasides A (**2.19**) and B (**2.20**)[8] from the marine sponge *Phorbas* sp., the same sponge that yielded phorboxazoles A (**2.21**) and B (**2.22**)[9]. Phorbasides are closely related to callipeltoside A, and to date are the only other natural products known that possess the ene-yne chlorocyclopropane motif. Phorbaside A displays moderate cytotoxicity toward HCT-116 cells (IC_{50} = 30 μM).



2.19 R = H Phorbaside A
2.20 R = X Phorbaside B

2.21
2.22 *epi*-C13

Figure 2.2 Structures of phorbasides A and B and phorboxazoles A and B

Assignment of configuration for **2.19** and **2.20** is complicated by the same problem encountered for callipeltoside A – insulation of the *trans*-chlorocyclopropane ring from the remainder of the molecule. Although the configuration of callipeltoside A was known, the structures of phorbasides A and B were sufficiently different to render comparison by $[\alpha]_D$ equivocal. We therefore sought an alternative method of assignment that would not require a synthesis of the entire natural product.

The ene-yne side chain represents the only UV-chromophore in **2.19** and **2.20.** Since the σ-bonds of cycloproprane rings exhibit increased π-character we expected significant hyperconjugation between the ene-yne π system and the chlorocyclopropane. Therefore, we anticipated that this inherently asymmetric chromophore could be selectively interrogated by circular dichroism (CD). Our strategy involved CD comparison of a series of stereo-defined synthetic model compounds with the natural products, an approach that led to the unambiguous assignment of the absolute configuration of phorbasides A and B.

2.2 Introduction to Circular Dichroism

As Nakanishi pointed out, chirality is a widespread property of organic natural products from macromolecules such as proteins, polysaccharides and DNA to small molecule from marine, plant and microbial sources.[10] The study of natural products has helped fuel the development of increasingly sophisticated techniques for organic structure elucidation. With modern NMR techniques it is now possible to assign planar structures and (in some cases) relative configuration to new compounds available in only *nano-mole* quantities.[11] The assignment of absolute configuration, however, remains challenging at any scale. Methods based upon NMR (e.g. Mosher's ester analysis) demand relatively large quantities of compound due to the inherent insensitivity of the technique and the need for prior derivatization. X-Ray crystallography is only applicable to the few natural products that form X-Ray

quality crystals. Optical rotation, the oldest form of chiroptical analysis, is highly insensitive and gives limited information on molecular structure. In contrast, circular dichroism (CD) often exhibits excellent sensitivity (comparable to UV absorption spectroscopy) and gives rise to information-rich spectra for compounds with a suitable chromophore.[12]

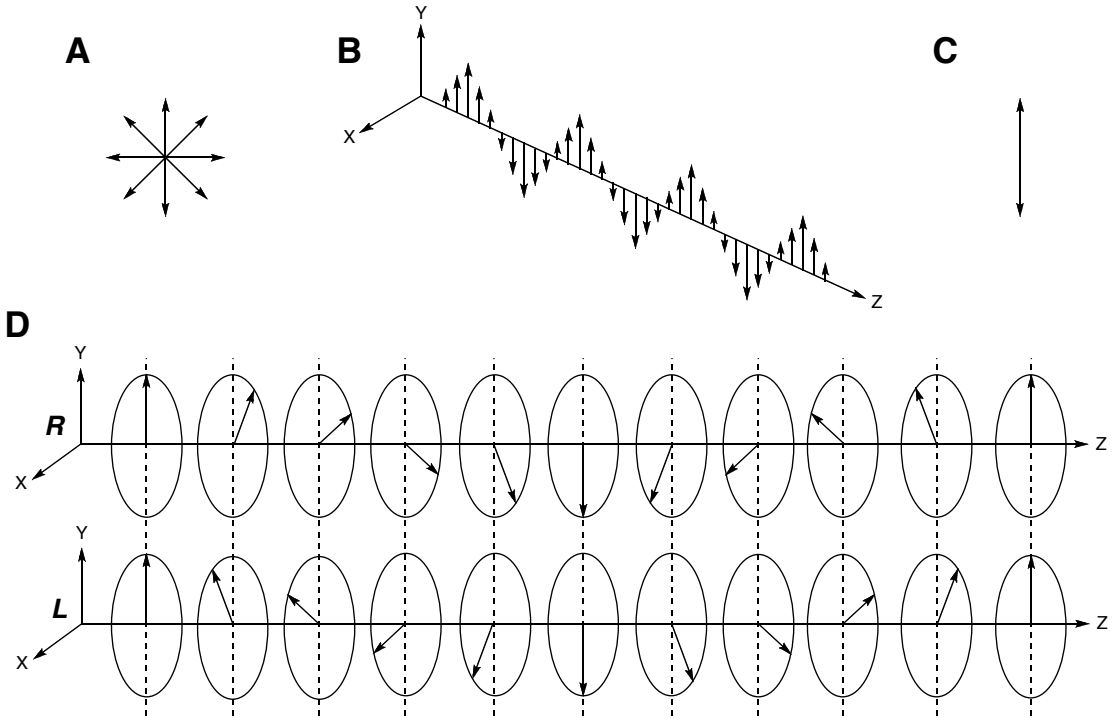


Figure 2.3 A) Random orientation of the electric field vector of natural light. B) Oscillation of the electric field vector of plane polarized light. C) Plane of oscillation of the electric field depicted in (B) as observed when viewed along the direction of propagation (Z axis). D) Rotating electric field vector of Left and Right circularly polarized light.

Chiroptical spectroscopy (optical rotation, optical rotatory dispersion [ORD] and CD) relies on the interaction of chiral molecules with polarized light, usually in the UV-visible spectrum. Light, or electromagnetic radiation, is composed of two oscillating electric and magnetic fields that propagate

perpendicular to one another. The plane of electric field oscillation is randomly oriented (Figure 2.3A), however if natural light is passed through a polarizer it emerges as “plane-polarized light” (Figure 2.3B and 2.3C).

Plane-polarized light can be resolved into two counter-rotating electric field vectors, **L** and **R**, which are equal in magnitude (Figure 2.3D). The sum of the vectors at any point gives a resultant vector that lies in the YZ plane of propagation. Plane polarized light that passes through a photoelastic modulator crystal (modulated at a given frequency, ~20 kHz) emerges alternating between net **L** and **R** electric field vectors.

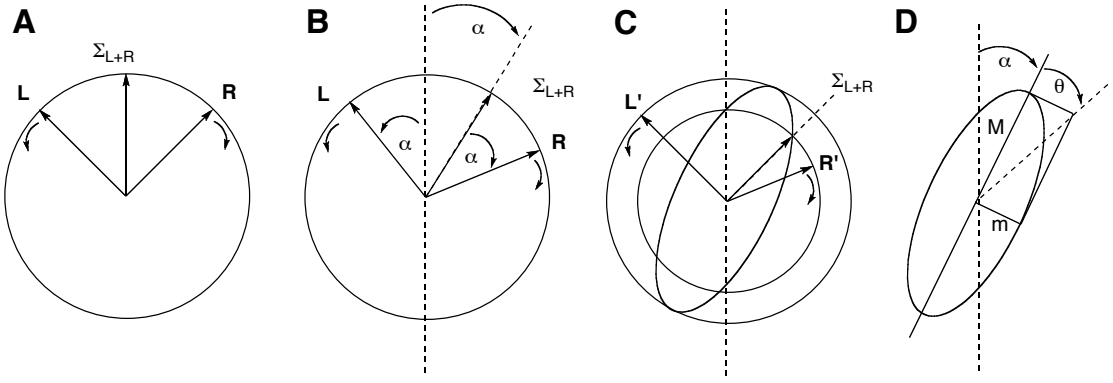


Figure 2.4 A) Counter-rotating electric field vectors **L** and **R** give a resultant vector Σ_{L+R} that oscillates in the vertical direction. B) Left and Right circularly polarized light pass through a chiral medium with different velocities, emerging out of phase and producing a rotation of the plane of oscillation of the resultant vector Σ_{L+R}. C) Left and Right circularly polarized light are absorbed to a different extent by asymmetric or asymmetrically perturbed chromophores; the resultant vector Σ_{L+R} now traces out an ellipse with minor and major axes m and M respectively. D) The ellipticity angle θ can be calculated from the geometry of the ellipse.

Left and right circularly polarized light exhibit different refractive indices when passing through a chiral medium due to different retardation of **L** and **R**.

The net vector lies in a plane rotated by α, the "optical rotation" (Figure 2.4B). If the chiral medium contains a chromophore capable of absorbing UV light then **L** and **R** are absorbed to a different extent (ie. $\varepsilon_L \neq \varepsilon_R$). The two vectors of the transmitted light, **L'** and **R'** are no longer equal in magnitude and the resultant vector traces out an ellipse instead of oscillating in a plane (Figure 2.4C). From this ellipse one can derive the ellipticity angle θ from simple trigonometry (θ = arctan m/M, Figure 2.4D). The value of θ is typically only a few millidegrees for most compounds in dilute solution.

A CD spectrum plots the difference in absorption between left and right circularly polarized light ($\Delta\varepsilon$) as a function of wavelength. The value of $\Delta\varepsilon$ is related to molar ellipticity [θ] according to equations **1** and **2** (where *M* = molecular weight).

$$[\theta] = \theta M/100 \qquad \text{Equation 1}$$

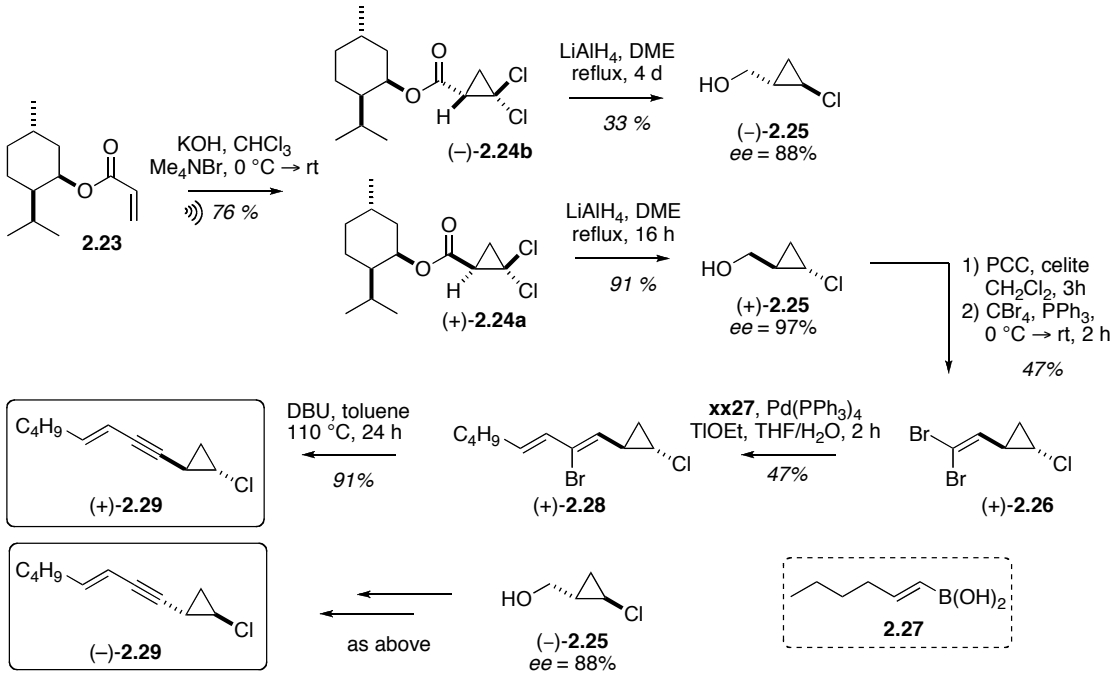
$$\Delta\varepsilon \approx [\theta]/3300 \qquad \text{Equation 2}$$

When $\Delta\varepsilon \neq 0$ (usually at λ_{max}) the resulting peak or trough in the spectrum is referred to as a "Cotton effect" after the French physicist Aime Cotton who first discovered the phenomenom.

2.3 Synthesis of Model Compounds

Our approach to the synthesis of the ene-yne chlorocylopropane side chain of phorbasides A and B relied upon the phase-transfer-catalyzed dichlorocyclopropanation of menthyl acrylate **2.23** under sonication conditions,

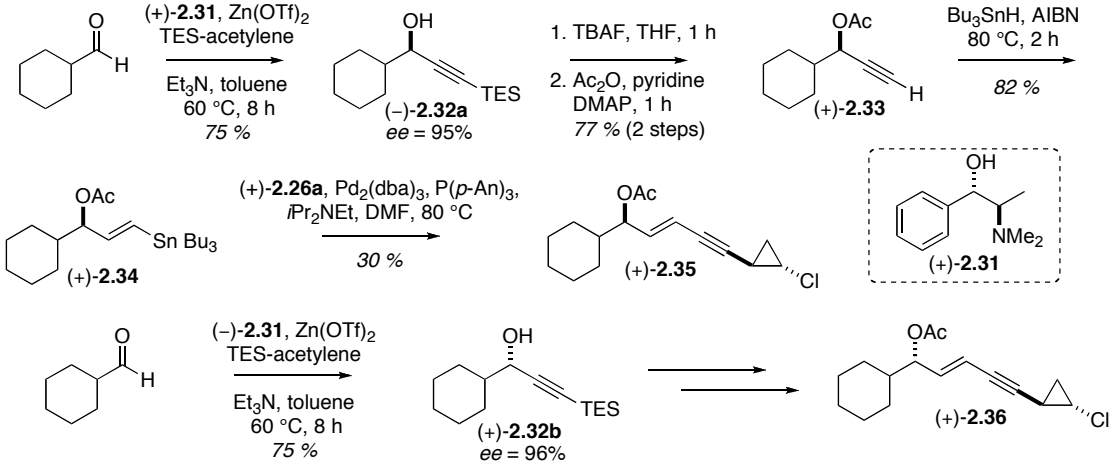
a methodology developed earlier in our laboratories.[13] Crystalline diastereomer (+)-**2.24a** was separated from (−)-**2.24b** by fractional crystallization (pentane, −80 ˚C). Pure diastereomers were reduced ($LiAlH_4$, refluxing DME) to provide the known alcohols (+)- and (−)-**2.25**.[14]



Scheme 2.3 Synthesis of ene-yne chlorocyclopropane model compounds (+)- and (−)-**2.29**

Oxidation of alcohol (+)-**2.25** (PCC, celite) followed by Corey-Fuchs homologation[15] of the resulting aldehyde (CBr_4, PPh_3) gave vinyl dibromide (+)-**2.26**.[3,4,14] Suzuki coupling of (+)-**2.26** with boronic acid **2.27** provided the all-*trans* diene (+)-**2.28** stereospecifically. Elimination of HBr (DBU, toluene, 110 ˚C) furnished the desired ene-yne chlorocylopropane model (+)-**2.29** (*ee* = 97%). Repetition of the same sequence starting with antipodal alcohol (−)-**2.25** led to (−)-**2.29** (*ee* = 88%).

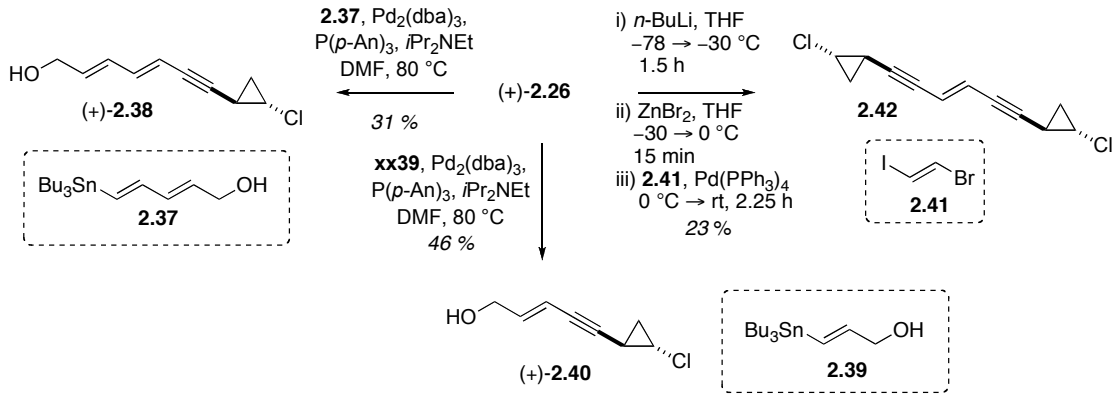
We next targeted compounds **2.35** and **2.36** featuring an allylic acetate group that we anticipated would mimic the influence of the C13 stereocenter upon the CD spectrum of the natural products (Scheme 2.4). *N*-methylephedrine-mediated nucleophilic addition of TES-acetylene to cyclohexane carboxaldehyde in the presence of $Zn(OTf)_2$ provided propargyl alcohol (−)-**2.32a** in good yield (75%) and with high enantioselectivity (determined by Mosher's ester analysis).[16,17] Removal of the silyl group (TBAF, THF) and acetylation of the free alcohol gave (+)-**2.33**. The alkyne was converted to (*E*)-vinyl stannane (+)-**2.34** under radical conditions (Bu_3SnH, AIBN) which was used in a Stille coupling with vinyl dibromide (+)-**2.26**. This provided the desired model (+)-**2.35**. Repetition of this sequence starting from (+)-**2.32** yielded (+)-**2.36**.



Scheme 2.4 Synthesis of ene-yne chlorocyclopropane model compounds (+)-**2.35** and (+)-**2.36**

Finally, in order to examine the effect of polar substituents compounds (+)-**2.38** and (+)-**2.40**[3,4] were prepared from (+)-**2.26** by Stille coupling with the

appropriate vinyl stannane (Scheme 2.5). Diene-yne (+)-**2.38** was of particular interest for its relevance to assignment of configuration of the callipeltoside family. An additional dimeric model compounds (+)-**2.42** was prepared by coupling of the alkynyl zincate derived from (+)-**2.26** with the bifunctional linchpin **2.41**.[18]



Scheme 2.5 Synthesis of model compounds (+)-**2.38**, (+)-**2.40** and (+)-**2.42**

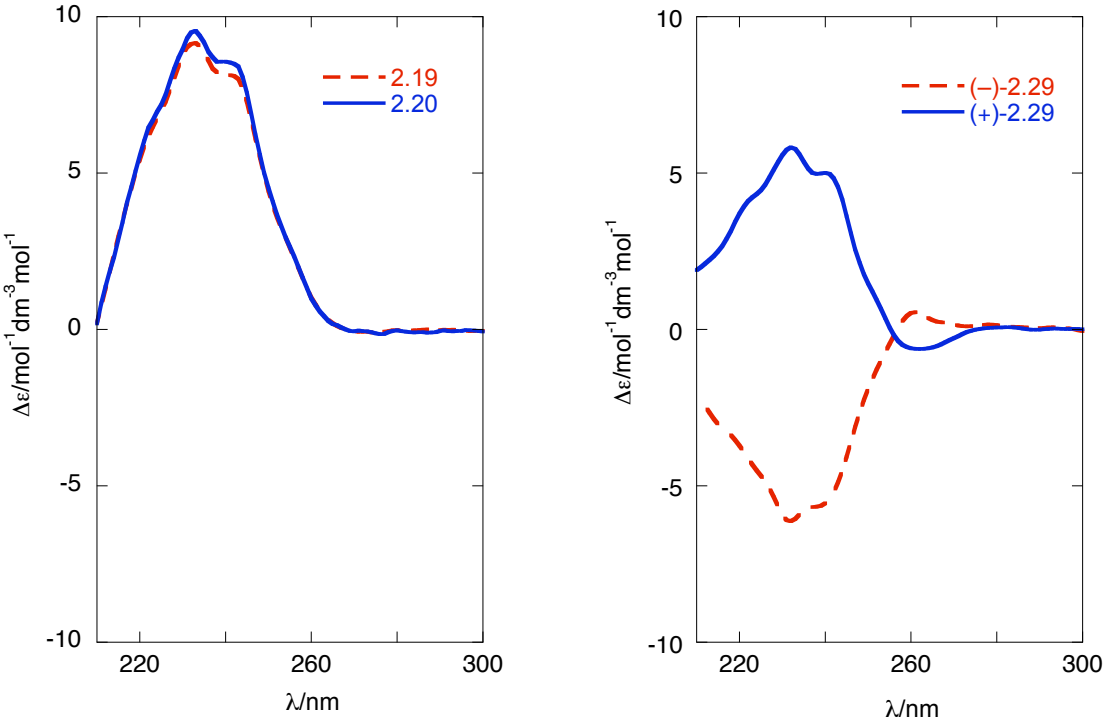


Figure 2.5 CD spectra (MeOH, 25 ˚C) of phorbasides A and B (**2.19** and **2.20**) and model compounds (+)- and (−)-**2.29** ((−)-**2.29** corrected for %*ee*).

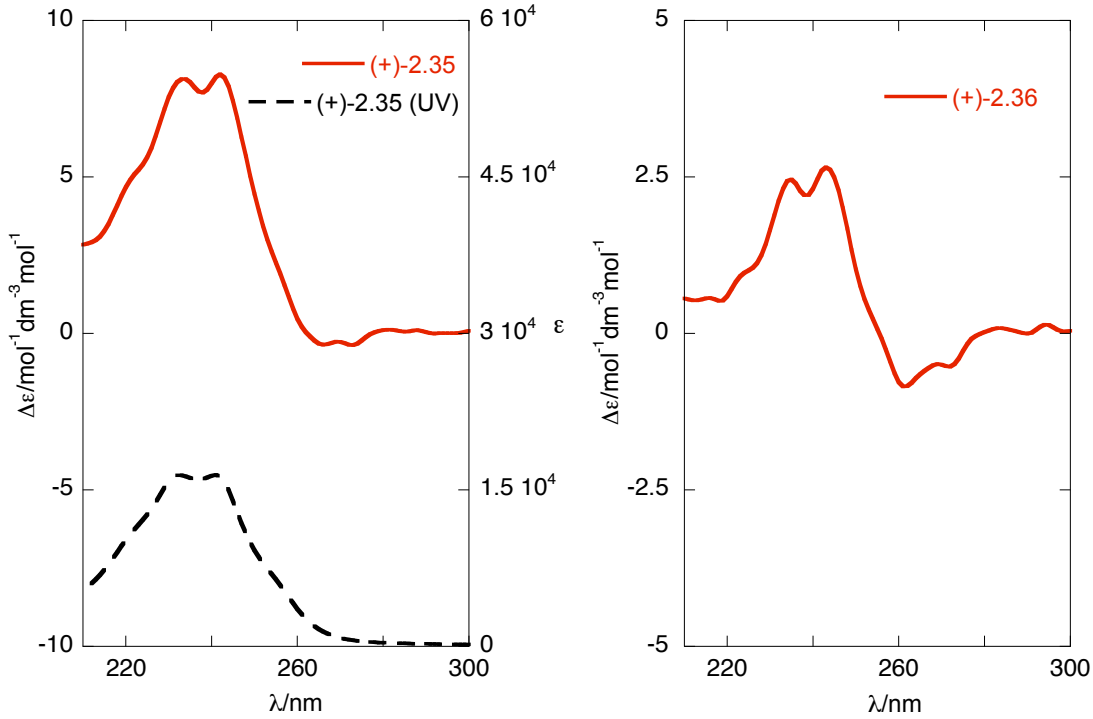


Figure 2.6 CD spectra (MeOH, 25 ˚C) of (+)-**2.35** (shown with UV spectrum) and (+)-**2.36**.

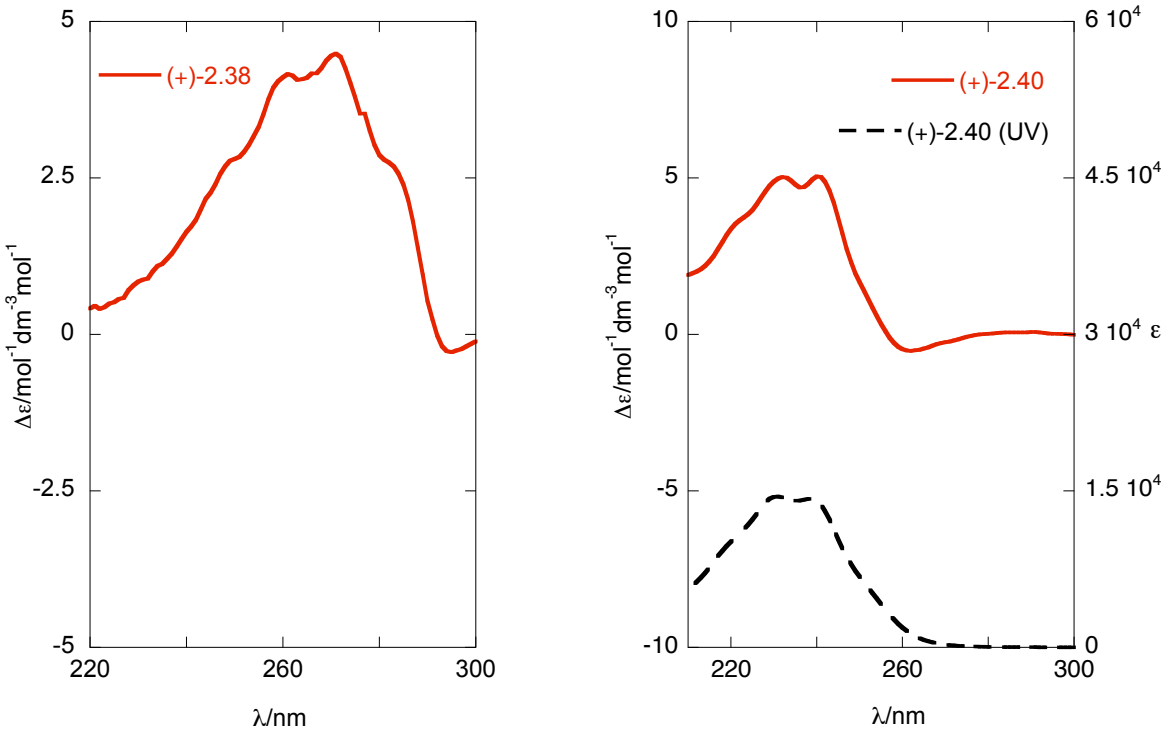


Figure 2.7 CD spectra (MeOH, 25 ˚C) of (+)-**2.38** and (+)-**2.40** (shown with UV spectrum of (+)-**2.40**).

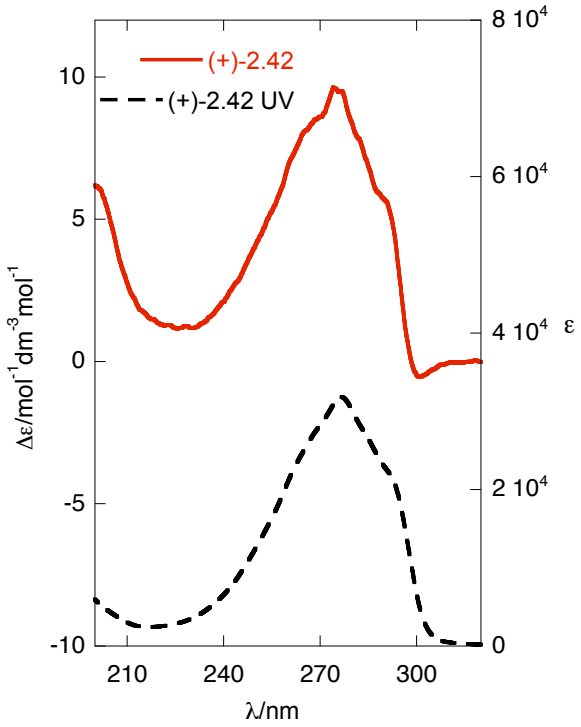


Figure 2.8 CD and UV spectrum (MeOH, 25 ˚C) of (+)-**2.42**.

CD spectra of all six model compounds were compared to those of phorbasides A and B (**2.19** and **2.20**, Figure 2.5). The simplest models, (+)- and (−)-**2.29** Cotton effects of equal magnitude and opposite sign, as expected. The shape and sign of the CE observed for (+)-**2.29** clearly matches that observed for **2.19** and **2.20**, indicating that the configuration of the chlorocyclopropane ring in the natural products can be assigned as 18*R*, 19*S*. Interestingly, the magnitude of the signal observed for (+)-**2.29** was substantially smaller than that for **2.19** and **2.20**.

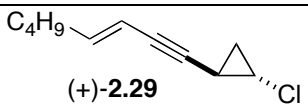
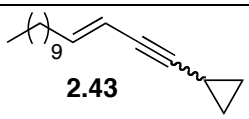
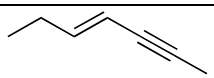
Models (+)-**2.35** and (+)-**2.36** (with an allylic acetoxyl at the opposing terminus of the chromophore) possess the natural configuration at the cyclopropane ring but differ in configuration at the allylic stereocenter (C13 phorbaside numbering). Model (+)-**2.35** displayed a CE of the same sign as (+)-**2.29** but almost double in magnitude, matching the natural spectra very closely (Figure 2.6). Diastereomeric (+)-**2.36**, with mismatched configuration at C13, produced a CE of the same sign but approximately half the magnitude of (+)-**2.29**. Thus, (+)-**2.35** possesses the same configuration as phorbasides A and B at C13, C18 and C19. *This data effectively relays configurational information from the isolated chlorocyclopropane ring across the ene-yne chromophore to the remainder of the macrolide in phorbasides A and B.* The complete configuration of **2.19** and **2.20** can be stated as (2*S*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*,13*R*,18*R*,19*S*).

Examination of the CD spectra of (+)-**2.38** and (+)-**2.40** indicates that a polar substituent at the ene-yne terminus has almost no effect on the observed CE (Figure 2.7). Diene-yne (+)-**2.39** showed a red-shifted CE (λ_{max} = 272 nm) of similar shape and sign to (+)-**2.29**. Model (+)-**2.40**, on the other hand, produced a CE almost indistinguishable from (+)-**2.29**. The dimeric model **2.42** also showed a red-shifted CE (λ_{max} = 272 nm) with magnitude approximately double that of (+)-**2.29**, (+)-**2.38** and (+)-**2.40** (Figure 2.8). Therefore, the two chlorocylcopropane rings of **2.42** appear to contribute to the CD spectrum in an additive fashion.

The extent of hyperconjugation from the ene-yne to the cyclopropane ring deserves some comment. Literature UV data for (*E*)-hept-4-en-2-yne indicates an absorption maximum of λ_{max} = 223 nm (ether)[19] compared to λ_{max} = 232 nm for (+)- and (−)-**2.29**. Since these chromophores have no lone pairs of electrons (rendering solvent effects negligible) a red shift in λ_{max} of ~9 nm for the ene-yne chromophore is strong evidence for hyperconjugation. The UV spectrum of synthetic **2.43** (λ_{max} = 232 nm, hexanes or MeOH) was identical to (+)- or (−)-**2.29**, indicating that the chlorine atom does not participate in hyperconjugation (Table 2.1). Based upon a red-shift of $\Delta\lambda_{max}$ = 9 nm, we estimate that the E-gap between the HOMO and the LUMO for 1-cyclopropyl-4-ene-2-ynes to be reduced by ~4.9 kCal/mol compared with 1-methyl-4-ene-2-ynes. Further evidence for hyperconjugation can bee seen in the DFT-calculated frontier molecular orbitals of the ene-yne chlorocyclopropane

chromophore. The calculated HOMO and LUMO for model **2.44** clearly extend to the cyclopropane ring (see Figure 2.9).

Table 2.1 UV absorption comparison for (+)-**2.29**, **2.41** and (*E*)-hept-4-en-2-yne

 (+)-**2.29**	 **2.43**	 (*E*)-hept-4-en-2-yne
λ_{max} (nm)	**λ_{max} (nm)**	**λ_{max} (nm)**
234 (*i*-octane) 232 (MeOH)	232 (hexanes) 232 (MeOH)	223 nm (Et_2O)

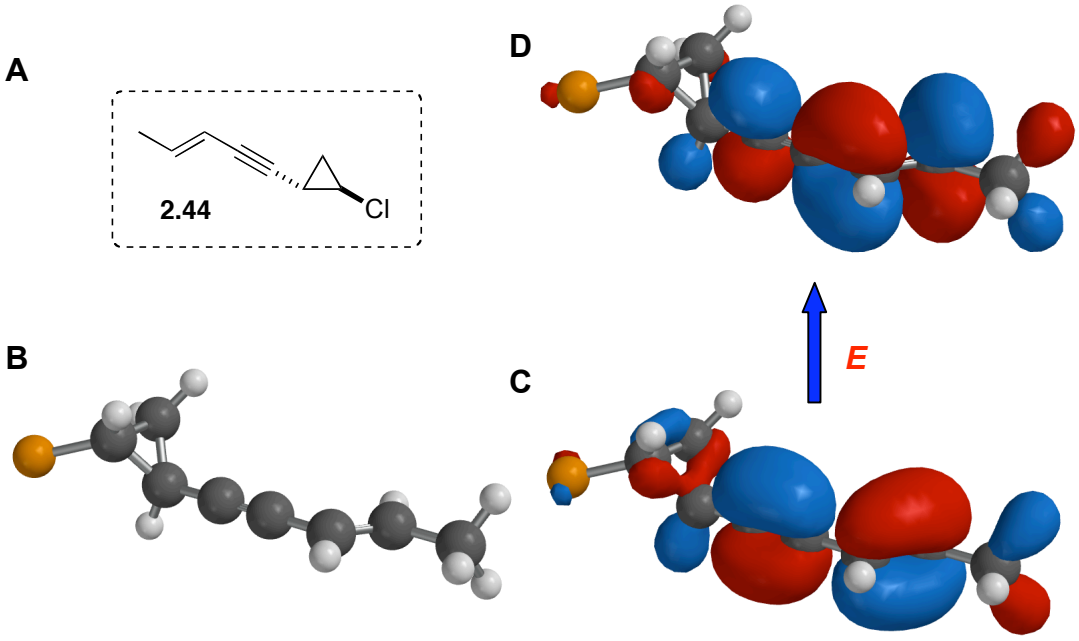


Figure 2.9 a) (1*R*)-1-chloro-2-((*E*)-pent-3-en-1-yn-1-yl)cyclopropane (**2.44**). b) a ball and stick representation of **2.44**. c) Highest Occupied Molecular Orbital (HOMO) of **2.44** and c) Lowest Unoccupied Molecular Orbital of **2.44**. Calculated performed by Tadeusz F. Molinski using Spartan '08, density functional theory, B3LYP 6-31G(D).

2.4 Conclusions

The absolute configuration of phorbasides A and B was assigned unambiguously by comparison of the natural products to model compounds of defined configuration by CD. The CD spectra of **2.19** and **2.20** is dominated by a positive CE that can be assigned to the asymmetrically perturbed ene-yne chromophore. The sign and magnitude of the CE is influenced by the configuration at C18 and C19 but also C13. Thus CD allows relay of configurational information from the cyclopropane ring to the distal terminus of the ene-yne chromophore, or C13 of the natural products. The configuration of the ene-yne chlorocyclorpane side chain of **2.19** and **2.20** can be stated as 13*R*,18*R*,19*S* and the total configuration as (2*S*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*,13*R*,18*R*,19*S*). These studies will be useful for assignment of configuration of new members of the callipeltoside/phorbaside family.

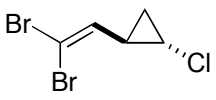
Chapter 2 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; MacMillan, J. B.; Zhou, G. –X.; Masuno, M. N.; Molinski, T. F. “Chlorocyclopropane Macrolides from the Marine Sponge *Phorbas* sp. Assignment of the Configurations of Phorbasides A and B by Quantitative CD” *J. Am. Chem. Soc.* **2007**, *129*, 4150-4151. The dissertation author was the primary researcher/author on this paper.

2.5 Experimental Section

Enantiomeric Purity of (+)- and (−)-2.25

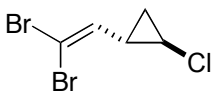
Samples of alcohols (+)-**2.25** and (−)-**2.25** (*ca*. 10 mg) were converted to their corresponding 2-naphthoate esters (2-naphthoyl chloride, Et_3N, DMAP, CH_2Cl_2, rt). Analysis of the esters by chiral HPLC (Chiralpak AD, 0.25:99.75 *i*-PrOH/hexanes, 1.5 mL/min, UV 254 nm) gave separations for (+)-**2.25** (t_R 20.6 min) and (−)-**2.25** (t_R 24.9 min). %ee's were determined from peak integrations. %ee = 97 % for (+)-**2.25**; %ee = 88 % for (−)-**2.25**.

(1*S*,2*R*)-1-chloro-2-(2,2-dibromovinyl)cyclopropane, (+)-2.26



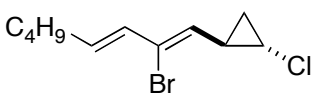
$[\alpha]_D^{28}$ +67.9 (*c* 2.25, CH_2Cl_2) [lit.[7a] $[\alpha]_D$ +80.1 (*c* 1.40, CH_2Cl_2)]; IR, ^{1}H NMR and ^{13}C NMR ($CDCl_3$) were in agreement with literature values.[3,4,14]

(1*R*,2*S*)-1-chloro-2-(2,2-dibromovinyl)cyclopropane, (−)-2.26



$[\alpha]_D$ –55.5 (*c* 0.71, CH_2Cl_2), uncorrected for optical purity; [lit.[7c] –80.5 (*c* 0.71, CH_2Cl_2); lit.[7e] –70.9 (*c* 0.65, $CHCl_3$), lit.[7a] –80.3 (*c* 0.77, CH_2Cl_2)]; IR, ^{1}H NMR and ^{13}C NMR ($CDCl_3$) were in agreement with literature values.[3,4,14]

(1*R*,2*S*)-1-((1*Z*,3*E*)-2-bromoocta-1,3-dienyl)-2-chlorocyclopropane, (+)-2.28

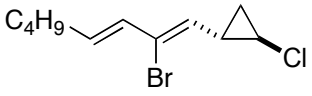


A mixture of (+)-**2.26** (50 mg, 0.26 mmol) and (*E*)-hex-1-enylboronic acid (168 mg, 1.31 mmol) in THF (4.9 mL) and water (1.6 mL) was subjected to three freeze-pump-thaw cycles. Tetrakis(triphenylphosphine)palladium (0) (60 mg, 0.052 μmol) was added as a suspension in degassed THF (0.35 mL), and the mixture stirred at 25 ˚C for 10 minutes. Thallium ethoxide (33 μL, 0.47 mmol) was added which produced a yellow precipitate. The resulting suspension was stirred at 25 ˚C for 1 hour, then diluted with pentane (10 mL) and aqueous NH_4Cl (1 M, 4 mL). The entire mixture was then filtered through Celite (3×1 mL pentane wash), and a further 8 mL of 1M NH_4Cl was added. The layers were separated, and the aqueous layer extracted with pentane (5 mL). The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure prior to purification by silica gel chromatography (pentane) to afford (+)-**2.28**, (23.6 mg, 47%) which was used immediately in the next step.

$[\alpha]_D^{28}$ +80.7 (*c* 0.16, CH_2Cl_2); IR (neat) ν 2957, 2926, 2871, 2857, 1465, 1457, 1433, 1377, 1365, 1311, 1285, 1262, 1246, 1180, 1124, 1086, 1067, 1038, 950, 935, 875, 833, 805, 685 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.04 (dt, 1H, *J* = 15.0, 7.0 Hz), 5.93 (dd, 1H, *J* = 15.0, 0.8 Hz), 5.22 (d, 1H, *J* = 9.0 Hz), 3.01 (ddd, 1H, *J* = 7.4, 4.3, 3.2 Hz), 2.28 (dddd, 1H, *J* = 9.6, 9.1, 6.3, 3.0

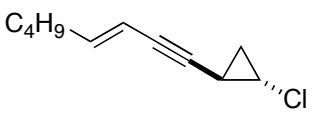
Hz), 2.13 (q, 2H, *J* = 6.8 Hz), 1.33 (m, 5H), 1.07 (dt, 1H, *J* = 7.6, 6.4 Hz), 0.88 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ135.9 (CH), 130.3 (CH), 128.4 (CH), 126.0 (C), 34.1 (CH), 31.8 (CH_2), 31.3 (CH_2), 25.0 (CH), 22.2 (CH_2), 18.2 (CH_2), 13.9 (CH_3).

(1*S*,2*R*)-1-((1*Z*,3*E*)-2-bromoocta-1,3-dienyl)-2-chlorocyclopropane, (−)-2.28



$[\alpha]_D^{28}$ –77.0 (*c* 0.22, CH_2Cl_2); IR, ^{1}H NMR and ^{13}C NMR as above.

(1*S*,2*R*)-1-chloro-2-((*E*)-oct-3-en-1-ynyl)cyclopropane, (+)-2.29

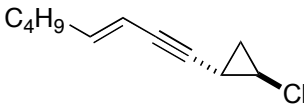


(+)-**2.28** (19.4 mg, 0.074 mmol) was dissolved in anhydrous toluene (690 µL) in a screw cap vial fitted with a silicon/Teflon seal. DBU (20 µL,1.37 mmol) was added and the vial evacuated and purged with N_2 three times. The degassed solution was then heated with stirring for 24 hours at 110 ˚C. The reaction mixture was diluted in pentane (3 mL), washed with water (2×3 mL) and saturated $CuSO_{4(aq)}$ (1 mL). The organic phase was then dried (Na_2SO_4) and concentrated under reduced pressure. Purification by silica gel chromatography yielded (+)-**2.29** (12.2 mg. *volatile!* 91% determined from NMR integrations), which was further purified by Si HPLC (pentane, 10×250 mm, 2 mL/min, 230 nm) prior to CD analysis.

$[\alpha]_D^{30}$ +222.8 (*c* 0.18, $CHCl_3$); IR (neat) ν 3020, 2956, 2927, 2871, 2859, 2221 (weak), 1724 (weak), 1466, 1433, 1377, 1256, 1097, 1043, 1000,

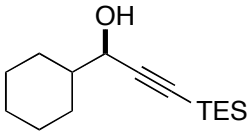
955, 930, 886, 856, 803, 688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.06 (dt, 1H, *J* = 15.9, 7.1 Hz), 5.38 (dq, 1H, *J* = 15.9, 1.6 Hz), 3.14 (ddd, 1H, *J* = 6.6, 5.0, 3.3 Hz), 2.06 (dq, 2H, *J* = 7.2, 1.3 Hz), 1.74 (m, 1H), 1.4-1.2 (m, 6H), 0.86 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9 (CH), 109.0 (CH), 86.9 (C), 77.2 (C), 34.2 (CH), 32.7 (CH_2), 30.8 (CH_2), 22.1 (CH_2), 19.1 (CH_2), 13.8 (CH), 11.9 (CH_3); UV (MeOH) λ_{max}= 232 nm (ε 18177); CD (MeOH) λ_{max}= 232 nm ($\Delta\varepsilon$ +5.82); HREIMS *m/z* 182.0861 (calcd. for $C_{11}H_{15}N_1Cl_1$ 182.0862).

(1*R*,2*S*)-1-chloro-2-((*E*)-oct-3-en-1-ynyl)cyclopropane, (−)-2.29



$[\alpha]_D^{30}$ –230.5 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} 232 nm (ε 18177); CD (MeOH) λ_{max} = 232 nm ($\Delta\varepsilon$ −6.13); HREIMS *m/z* 182.0861 (calcd. for $C_{11}H_{15}N_1Cl_1$ 182.0862).

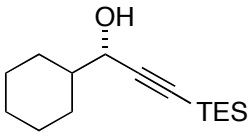
(*R*)-1-Cyclohexyl-3-triethylsilyl-2-propyn-1-ol, (−)-2.32



$Zn(OTf)_2$ mediated addition of TES-acetylene to cyclohexane carboxaldehyde was carried out according to the procedure of Carreira.[16]

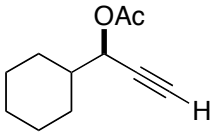
$[\alpha]_D^{28}$ −3.8 (*c* 1.97, $CHCl_3$); LRESIMS *m/z* 275.19 $[M+Na]^+$; IR, ^1H and ^{13}C NMR ($CDCl_3$) were in agreement with literature values.[16] ee = 95% as determined by Mosher ester analysis.

(*S*)-1-Cyclohexyl-3-triethylsilyl-2-propyn-1-ol, (+)-2.32



$[\alpha]_D^{27}$ +5.0 (*c* 1.84, $CHCl_3$); LRESIMS 275.17 $[M+Na]^+$; IR, ^1H and ^{13}C NMR ($CDCl_3$) were in agreement with literature values.[16] ee = 96% as determined by Mosher ester analysis.

(*R*)-1-cyclohexylprop-2-ynyl ethanoate, (+)-2.33

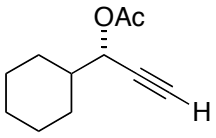


Tetrabutylammonium fluoride trihydrate (3.54 g, 11.2 mmol) was dissolved in THF (32 mL). A solution of (−)-**2.32** (1.89 g, 7.49 mmol) in THF (5 mL) was added dropwise to the mixture, and after 1 hour the dark yellow solution was poured into saturated $NH_4Cl_{(aq)}$ (50 mL). Diethyl ether (50 mL) was added, and the layers were separated. The organic layer was washed with saturated $NH_4Cl_{(aq)}$ (50 mL) followed by water (50 mL), and combined aqueous layers were extracted twice with diethyl ether (50 mL). Combined organic layers were dried (Na_2SO_4) and solvent evaporated under reduced pressure. Crude product was purified by flash chromatography (SiO_2, 1:4 Et_2O/hexane) to yield (*R*)-1-cyclohexyl-2-acetoxypropyn-2-ol (1.12 g) as a white solid. ^1H and ^{13}C NMR analysis showed the product to be contaminated with triethylsilyl fluoride, which could not be removed chromatographically, so the mixture was carried directly into the next step.

(*R*)-1-Cyclohexyl-2-propyn-1-ol was combined with acetic anhydride (4.14 g, 41 mmol), pyridine (3.21 g, 41 mmol) and a single crystal of DMAP and stirred for one hour, at which time excess acetic anhydride and pyridine were removed by bulb-to-bulb distillation. The crude product was passed through a plug of silica gel (1:9 Et_2O/hexane) to yield (+)-**2.33** (1.04 g, 77% over 2 steps) as a clear colorless oil.

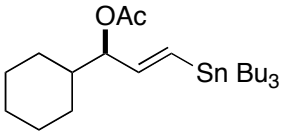
$[\alpha]_D^{27}$ +64.4 (*c* 1.80, $CHCl_3$); IR (neat) ν 2929, 2856, 1743, 1452, 1371, 1230, 1020, 979 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.17 (dd, 1H, *J* = 6.0, 2.1 Hz), 2.41 (d, 1H, *J* = 2.1 Hz), 2.06 (s, 3H), 1.88-1.57 (m, 6H), 1.31-1.00 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0 (C), 80.2 (CH), 74.0 (C), 67.9 (CH), 41.5 (CH), 28.4 (CH_2), 27.9 (CH_2), 26.1 (CH_2), 25.7 (CH_2), 25.6 (CH_2), 20.9 (CH_3); LRESIMS *m/z* 203.07 $[M+Na]^+$; HRCIMS (NH_3) *m/z* 198.1500 $[M + NH_4^+]^+$ (calcd. for $C_{11}H_{20}NO_2$ 198.1494).

(*S*)-1-cyclohexylprop-2-ynyl ethanoate, (−)-2.33



$[\alpha]_D^{27}$ −65.5 (*c* 1.50, $CHCl_3$); HRCIMS (NH_3) *m/z* 198.1487 $[M + NH_4^+]^+$ (calcd. for $C_{11}H_{20}NO_2$ 198.1494). IR, ^1H NMR and ^{13}C NMR as above.

(*R*)-1-Cyclohexyl-1-acetoxy-3-tributylstannyl-prop-2-ene, (+)-2.34

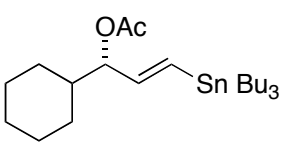


(+)-**2.33** (0.466 g, 2.59 mmol) was combined with tri-*n*-butyltin hydride (0.98 g, 3.36 mmol) in a dry 20 mL

scintillation vial. AIBN (0.043 g, 0.26 mmol) was added, and the vial was sealed and heated to 80 ˚C with stirring for 2 hours. The mixture was allowed to cool, then diluted in hexane (1 mL) and passed through a silica gel column (1:39 Et_2O/hexane) to give semi-pure product (1.10 g), which was further purified by Si HPLC (5:95 Et_2O/hexane, 9 mL/min, RI detection) to give (+)-**2.34** (1.01 g, 82%) along with the internal stannane (~13%), which proved to be inseparable.

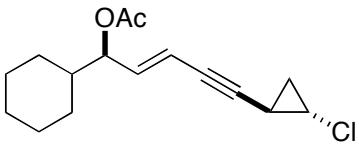
$[\alpha]_D^{29}$ +35.4 (*c* 2.22, $CHCl_3$); IR (neat) ν 2954, 2925, 2871, 2853, 1745, 1603 (weak), 1464, 1451, 1367, 1235, 1017, 988 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.09 (dd, 1H, *J* = 19.1, 0.9 Hz), 5.82 (dd, 1H, *J* = 19.1, 6.3 Hz), 4.99 (m, 1H), 2.05 (s, 3H), 1.80-1.58 (m, 6H), 1.58-1.40 (m, 6H), 1.40-1.22 (m, 6H), 1.20-0.75 (m, 11H), 0.86 (t, 9H, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (C), 144.5 (CH), 131.4 (CH), 81.2 (CH), 41.5 (CH), 29.1 (CH_2), 28.6 (CH_2), 28.5 (CH_2), 27.2 (CH_2), 26.4 (CH_2), 25.9 (CH_2), 21.3 (CH_3), 13.7 (CH_3), 9.50 (CH_2); HRDCIMS (NH_3) *m/z* 473.2433 $[M+H]^+$ (calcd. for $C_{23}H_{45}O_2Sn$ 473.2441).

(*S*)-1-Cyclohexyl-1-acetoxy-3-tributylstannyl-prop-2-ene, (−)-2.34



$[\alpha]_D^{29}$ −36.1 (*c* 1.91, $CHCl_3$); HRCIMS (NH_3) *m/z* 473.2455 $[M + H]^+$ (calcd. for $C_{23}H_{45}O_2Sn$ 473.2441). IR, ^1H NMR and ^{13}C NMR as above.

(1*R*, 2*S*)-1-chloro-2-((*E*)-(5*R*)-5-Cyclohexyl-5-acetoxy-pent-3-en-1-ynyl)cyclopropane, (+)-2.35



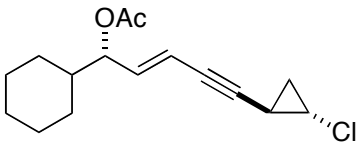
To a dry 3.8 mL vial was added (+)-**2.26a** (0.05 g, 0.19 mmol), (+)-**2.34** (0.099 g, 0.21 mmol), degassed DMF (0.50 mL, subjected to 3 freeze-pump-thaw cycles) and freshly distilled $(i\text{-Pr})_2$NEt (0.037 g, 0.29 mmol). This mixture was then subjected to 3 freeze-pump-thaw cycles.

A suspension of tris-(4-methoxyphenyl)phosphine (0.041 g, 0.12 mmol) in degassed DMF (0.25 mL) was added, followed by tris-(dibenzylideneacetone)dipalladium(0) (0.018 g, 0.019 mmol). The mixture was heated to 80 ˚C for 8 hours then diluted in ethyl acetate (5 mL) and passed through a short plug of Celite followed by two washes of ethyl acetate (5 mL). The combined organic fractions were washed with water (3×20 mL), and combined aqueous layers re-extracted with ethyl acetate (3×20 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (5:95 Et_2O/hexane) followed by Si HPLC (5:95 Et_2O/hexane, 2 mL/min, 254 nm) gave (+)-**2.35** (16.1 mg, 30%).

$[\alpha]_D^{24}$ +199.9 (*c* 0.65, CH_2Cl_2); UV (MeOH) λ_{max} 242nm (ε 16 544); IR (neat) ν 2929, 2854, 2222, 1736, 1451, 1370, 1234, 1044, 1017, 975, 953, 924, 688 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (dd, 1H, *J* = 15.9, 7.3 Hz), 5.57 (dt, 1H, *J* = 15.9, 1.4 Hz), 5.00 (t, 1H, *J* = 7.1 Hz), 3.14 (ddd, 1H, *J* = 6.2,

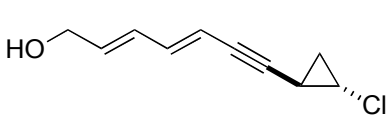
5.5, 3.2 Hz), 2.02 (s, 3H), 1.78-1.58 (m, 6H), 1.57-1.44 (m, 1H), 1.28-1.06 (m, 5H), 1.04-0.86 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2 (C), 139.6 (CH), 112.5 (CH), 89.9 (C), 77.8 (CH), 76.2 (C), 41.5 (CH), 34.1 (CH), 28.48 (CH_2), 28.42 (CH_2), 26.2 (CH_2), 25.8 (CH_2), 21.1 (CH_3), 19.1 (CH_2), 11.8 (CH); CD (MeOH) λ_{max} = 242 nm (Δε +8.28), 234 nm (Δε +8.13); LRCIMS (NH_3) *m/z* 238 $[M-OAc+NH_3]^+$, 221 $[M-AcO+H]^+$, 185 $[M-AcO-Cl]^+$.

(1*R*, 2*S*)-1-chloro-2-((*E*)-(5*S*)-5-Cyclohexyl-5-acetoxy-pent-3-en-1-ynyl)cyclopropane (+)-2.36



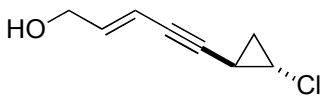
$[\alpha]_D^{26}$ +89.8 (*c* 0.18, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (dd, 1H, *J* = 16.0, 7.2 Hz), 5.57 (dt, 1H, *J* = 16.0, 1.0 Hz), 5.00 (t, 1H, *J* = 7.2 Hz), 3.14 (ddd, 1H, *J* = 6.2, 5.6, 3.2 Hz), 2.02 (s, 3H), 1.78-1.60 (m, 6H), 1.56-1.46 (m, 1H), 1.28-1.06 (m, 5H), 1.0-0.88 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C), 139.6 (CH), 112.5 (CH), 89.9 (C), 77.8 (CH), 76.2 (C), 41.6 (CH), 34.1 (CH), 28.49 (CH_2), 28.43 (CH_2), 26.2 (CH_2), 25.8 (CH_2), 21.1 (CH_3), 19.1 (CH_2), 11.8 (CH); UV (MeOH) λ_{max} 242 nm (ε 15,594); CD (MeOH) λ_{max} = 243 nm (Δε +2.65), 235 nm (Δε +2.46); LRCIMS (NH_3) *m/z* 238 $[M-OAc+NH_3]^+$, 221 $[M-AcO+H]^+$, 185 $[M-AcO-Cl]^+$.

(2*E*,4*E*)-7-((1*R*,2*S*)-2-chlorocyclopropyl)hepta-2,4-dien-6-yn-1-ol (+)-2.38



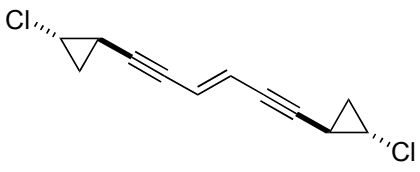
$[\alpha]_D^{22}$ +247.3 (*c* 0.09, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (dt, 1H, *J* = 15.7, 11.0 Hz), 6.26 (dd, 1H, *J* = 14.9, 10.6 Hz), 5.88 (dt, 1H, *J* = 14.9, 5.8 Hz), 5.53 (d, 1H, *J* = 15.7 Hz), 4.20 (t, 2H, *J* = 5.6 Hz), 3.16 (ddd, 1H, *J* = 6.3, 5.5, 3.3 Hz), 1.78 (m, 1H), 1.33 (t, 1H, *J* = 6.0 Hz), 1.29-1.23 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5 (CH), 134.2 (CH), 129.9 (CH), 111.2 (CH), 91.6 (C), 77.6 (C), 63.1 (CH_2), 34.4 (CH), 19.4 (CH), 12.2 (CH_2); UV (MeOH) λ_{max} = 272 nm (ε 26 141), CD (MeOH) λ_{max} = 271 nm (Δε +4.49), 261 nm (Δε +4.16)

(*E*)-5-((1*R*,2*S*)-2-chlorocyclopropyl)pent-2-en-4-yn-1-ol, (+)-2.40[7a-c,14]



$[\alpha]_D^{26}$ +238.2 (*c* 0.31, CH_2Cl_2), (lit.[14] $[\alpha]_D^{26}$ +226 (*c* 0.7, CH_2Cl_2)); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 (dt, 1H, *J* = 16.1, 5.1 Hz), 5.66 (dq, 1H, *J* = 16.1, 1.8 Hz), 4.17 (dd, 2H, *J* = 5.1, 1.8 Hz), 3.15 (ddd, 1H, *J* = 6.3, 5.7, 3.2 Hz), 1.80-1.72 (m, 1H), 1.43 (br s, 1H), 1.28-1.22 (m, 2H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.5 (CH), 110.2 (CH), 89.5 (C), 76.4 (C), 62.9 (CH_2), 34.1 (CH), 19.1 (CH), 11.8 (CH_2); UV (MeOH) λ_{max} = 232 nm (ε 14 510); CD (MeOH) λ_{max} = 240 nm (Δε +5.05), 232 nm (Δε +5.03).

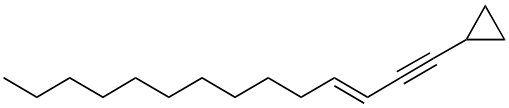
(*E*)-1,6-bis((1*R*,2*S*)-2-chlorocyclopropyl)hexa-3-en-1,5-diyne



n-BuLi (0.153 mmol, 61 μL of a 2.5 M solution in hexanes) was added to a mixture of (+)-**2.26a** (20 mg, 0.077 mmol) in degassed THF (250 μL) at −78 ˚C. The mixture was stirred at −78 ˚C for 1 hour then warmed to −30 ˚C over 0.5 hours. $ZnBr_2$ (10 mg, 0.046 mmol) was added as a solution in THF (100 μL). The resulting mixture was stirred at −30 ˚C for 15 minutes, then warmed to 0 ˚C at which time $Pd(PPh_3)_4$ (8.8 mg, 7.7 μmol) and **2.41** (8.9 mg, 0.038 mmol) were added as a mixture in THF (175 μL followed by 100 μL rinse). After stirring 2 hours at 0 ˚C the mixture was warmed to room temperature for 15 minutes, then diluted with saturated $NH_4Cl_{(aq)}$ and extracted with pentane (4 × 1 mL). Combined pentane extracts were washed with saturated $NaHCO_{3(aq)}$ and brine, then dried (Na_2SO_4) and concentrated carefully under reduced pressure. Silica gel chromatography (pentane) followed by SiO_2 HPLC (pentane, 3 mL/min) gave **2.42** (2 mg, 23%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (t, 2H, *J* = 0.8 Hz), 3.16 (ddd, 2H, *J* = 6.4, 5.6, 3.2 Hz), 1.78 (m, 2H), 1.30-1.26 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 120.5 (CH), 93.9 (C), 34.2 (CH), 19.3 (CH_2), 12.0 (CH); UV (MeOH) λ_{max} = 277 nm (ε 31 826); CD (MeOH) λ_{max} = 274 nm (Δε +9.64).

(*E*)-tetradec-3-en-1-ynylcyclopropane (2.43)



Conditions adapted from those developed by Johnson *et al.*[20] To a 20 mL scintillation vial under N_2 was added anhydrous, degassed THF (3.4 mL), (*E*)-1-iodododec-1-ene (100 mg, 0.34 mmol, 4:1 *E*/*Z*), ethynylcyclopropane (34 mg, 0.51 mmol), $Pd(PPh_3)_2Cl_2$ (24 mg, 0.034 mmol) and CuI (13 mg, 0.068 mmol). The vial was flushed with N_2, and the mixture was cooled to 0 ºC in an ice/water bath. $(i\text{-Pr})_2NH$ (0.14 mL, 1.02 mmol, freshly distilled from KOH) was added and the resulting yellow-brown mixture was stirred for 2 hours. At this time the mixture was poured in aqueous HCl (0.05 M, 50 mL) and extracted with hexanes (3 × 50 mL). The combined hexane layers were dried (Na_2SO_4) and evaporated under reduced pressure. Silica gel chromatography (hexanes) gave (*E*)-tetradec-3-en-1-ynylcyclopropane as a clear oil (52 mg, 66% overall, 82% based upon quantity of (*E*)-1-iodododec-1-ene in starting material).

UV (MeOH) λ_{max} 232 nm (ε 13 615); IR (neat) ν 2959, 2925, 2854, 2212 (weak), 1461, 1359, 1055, 1032, 953, 893, 810 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.01 (dt, 1H, *J* = 15.6, 7.0 Hz), 5.38 (dq, 1H, *J* = 15.6, 1.5 Hz), 2.03 (qd, 2H, *J* = 7.4, 1.5 Hz), 1.36-1.26 (m, 2H), 1.23 (s, 14H), 0.85 (t, 3H, *J* = 6.8 Hz), 0.78-0.71 (m, 2H), 0.70-0.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.7 (CH), 109.6 (CH), 91.6 (C), 74.6 (C), 32.9 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 29.57

(CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 22.7 (CH_2), 14.1 (CH_3), 8.37 (CH_2), 0.055 (CH); HREIMS m/*z* 232.2186 (calc. for $C_{17}H_{28}$ 232.2186).

References

(1) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085-11088.

(2) Zampella, A.; D'Auria, M. V.; Minale, L. *Tetrahedron* **1997**, *53*, 3243-3248.

(3) a) Trost, B. M.; Dirat, O.; Gunzner, J. L. *Angew. Chem. Int. Ed. Eng.* **2002**, *41*, 841-843; b) Trost, B. M. Gunzner, J. L.; Dirat, O.; Rhee, Y. H. *J. Am. Chem. Soc.* **2002**, *124*, 10396-10415.

(4) Evans, D. A.; Hu, E.; Burch, J. D.; Jaeschke, G. *J. Am. Chem. Soc.* **2002**, *124*, 5654-5655.

(5) Paterson, I.; Davies, R. D. M.; Heimann, A. C.; Marquez, R.; Meyer, A. *Org. Lett.* **2003**, *5*, 4477-4480.

(6) Huang, H.; Panek, J. S. *Org. Lett.* **2004**, *6*, 4383-4385.

(7) Carpenter, J.; Northrup, A. B.; Chung, D.; Wiener, J. J. M. Kim, S. –G.; MacMillan, D. W. C. *Angew. Chem. Int. Ed. Eng.* **2008**, *47*, 3568-3572.

(8) Skepper, C. K.; MacMillan, J. B.; Zhou, G. -X.; Masuno, M. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129*, 4150-4151.

(9) (a) Searle, P.A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126. (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422. (c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879.

(10) *Circular Dichroism: Principles and Applications*, Nakanishi, K.; Berova, N.; Woody, R. W. Eds., VCH Publishers, New York, NY, USA 1994.

(11) a) Molinski, T. F. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 197-206. b) Molinski, T. F. *Nat. Prod. Rep.* **2009**, submitted.

(12) *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Lightner, D. A., Gurst, J. E., Wiley-VCH, USA, 2000.

(13) Masuno, M. N.; Young, D. M.; Hoepker, A. C.; Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2005**, *70*, 4162.

(14) Olivo, H. F.; Velaquez, F.; Trevisan, H. C. *Org. Lett.* **2000**, *2*, 4055-4058.

(15) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *36*, 3769-3772.

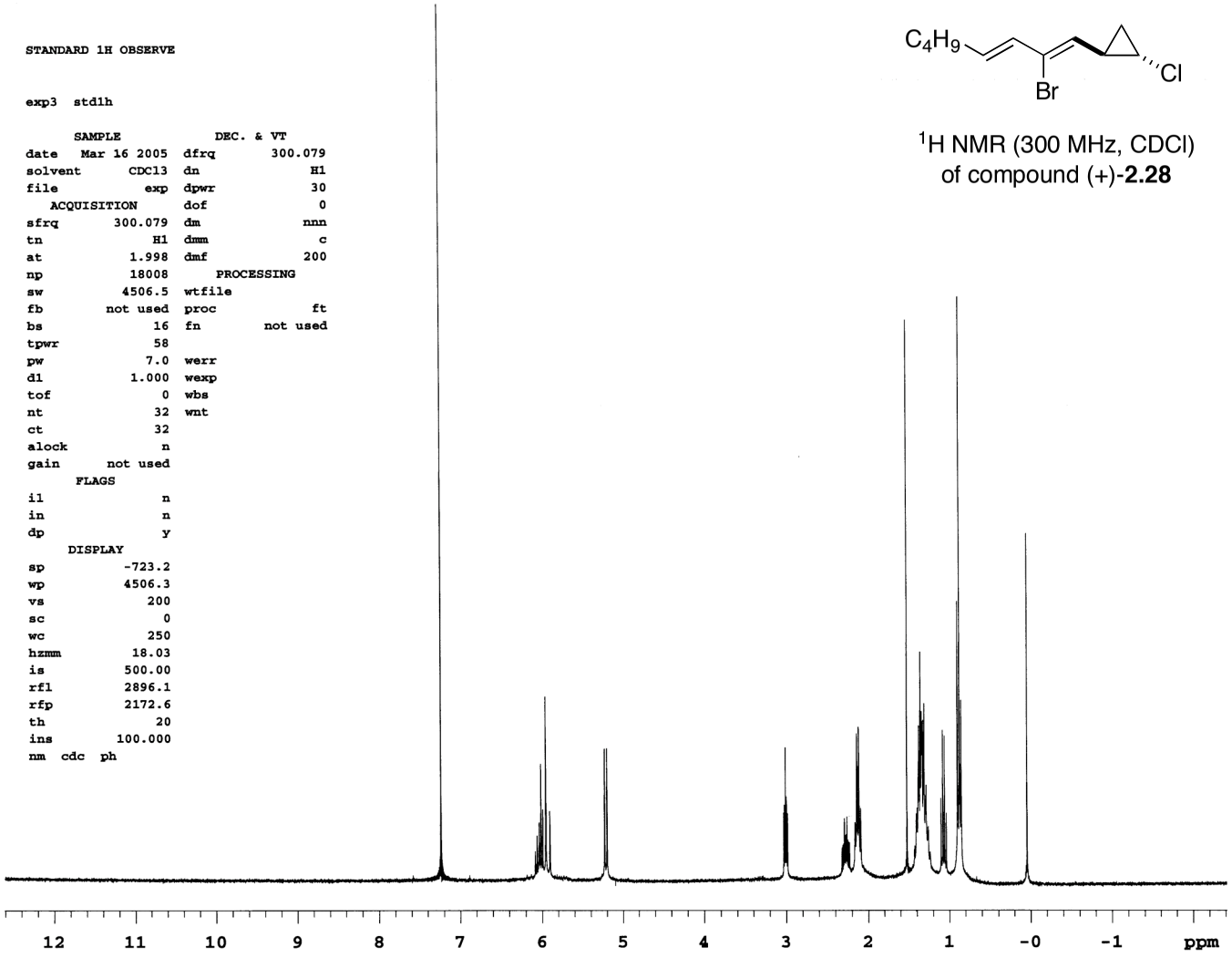
(16) Anand, N. K.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 9687-9688.

(17) Ohtani, I.; Kusuki, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**,*113*, 4092-4096.

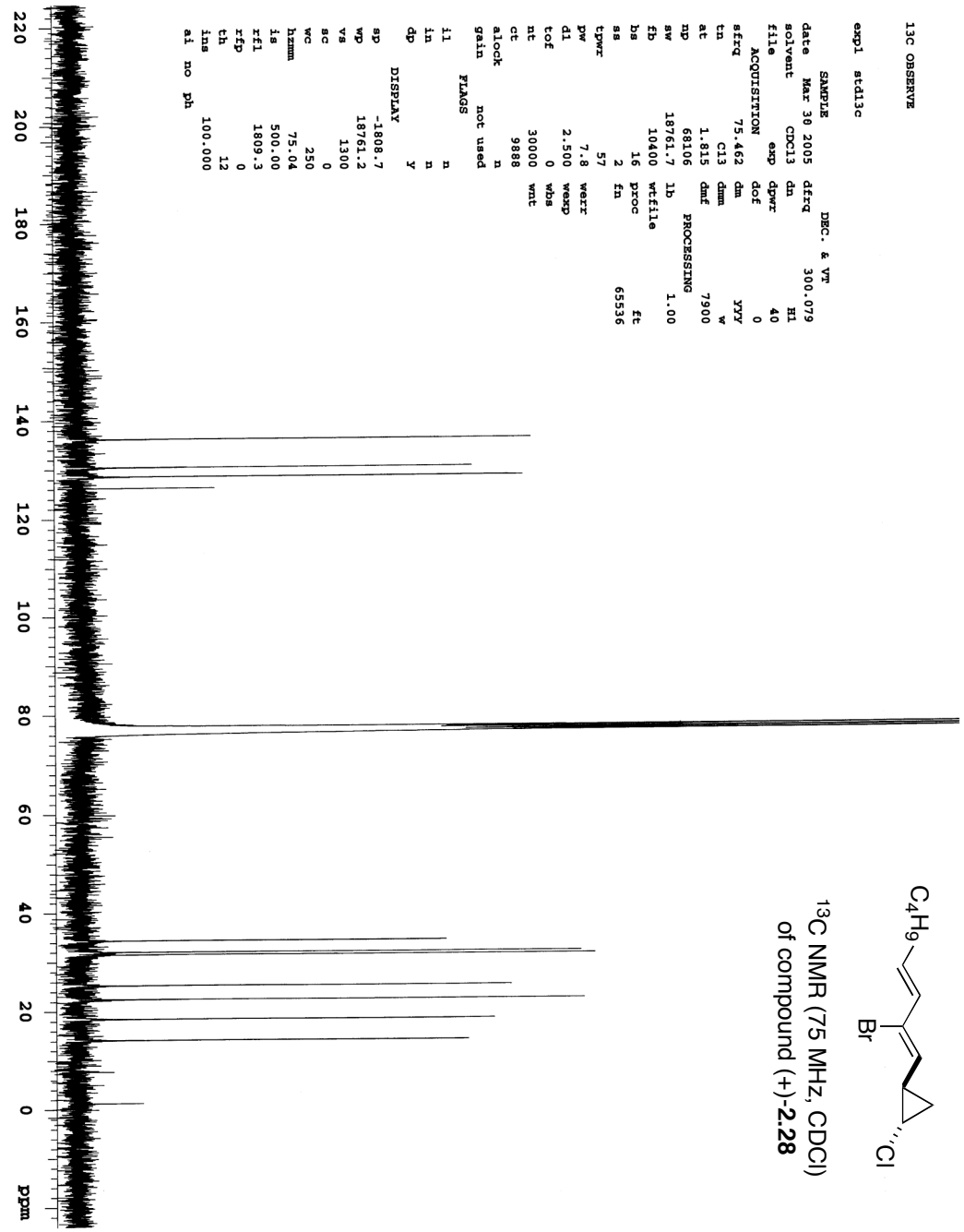
(18) a) Negishi, E.; Alimardanov, A.; Xu, C. *Org. Lett.* **2000**, *2* 65-67. b) Negishis, E.; Hata, M.; Xu, C. *Org. Lett.* **2000**, *2*, 3687-3689. c) Métay, E..; Hu, Q.; Negishi, E. *Org. Lett.* **2006**, *8*, 5773-5776.

(19) *UV Atlas of Organic Compounds Vol. 1*, Plenum, N.Y. 1966.

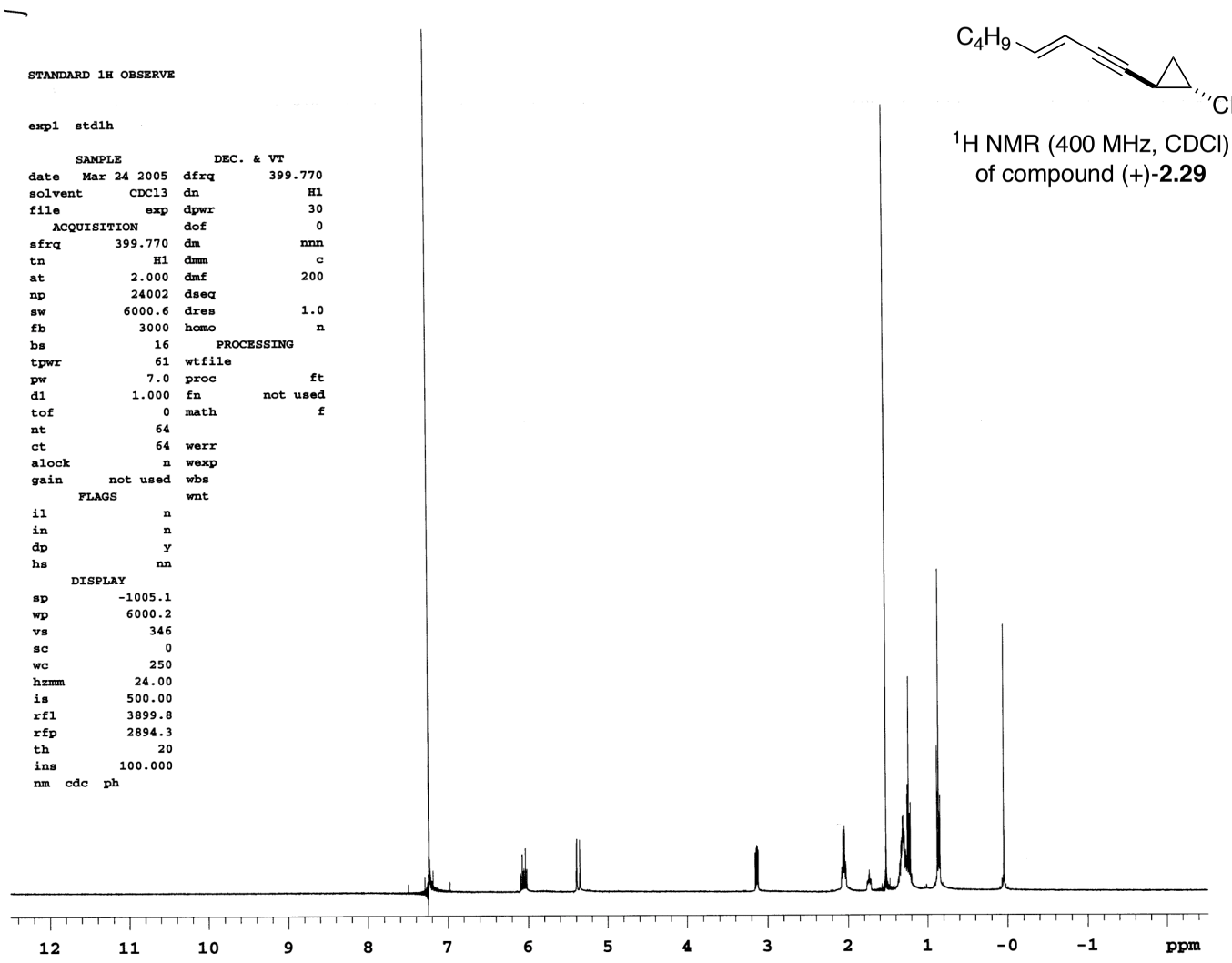
(20) Miller, M. W.; Johnson, C. R. *J. Org. Chem.* **1997**, *62*, 1582-1583.



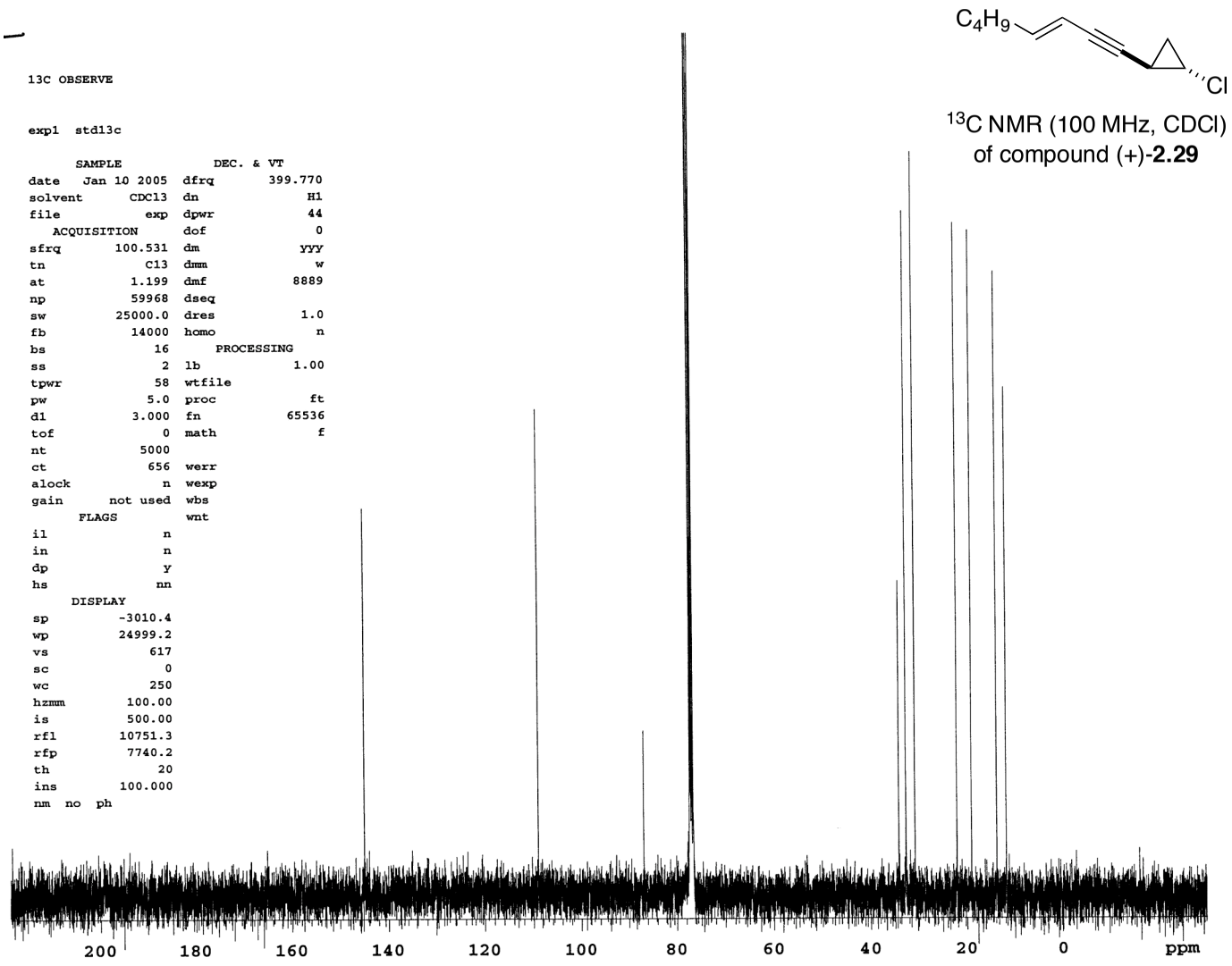
^{1}H NMR (300 MHz, CDCl) of compound (+)-**2.28**



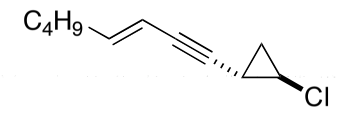
^{13}C NMR (75 MHz, CDCl) of compound (+)-**2.28**



^{1}H NMR (400 MHz, CDCl) of compound (+)-**2.29**

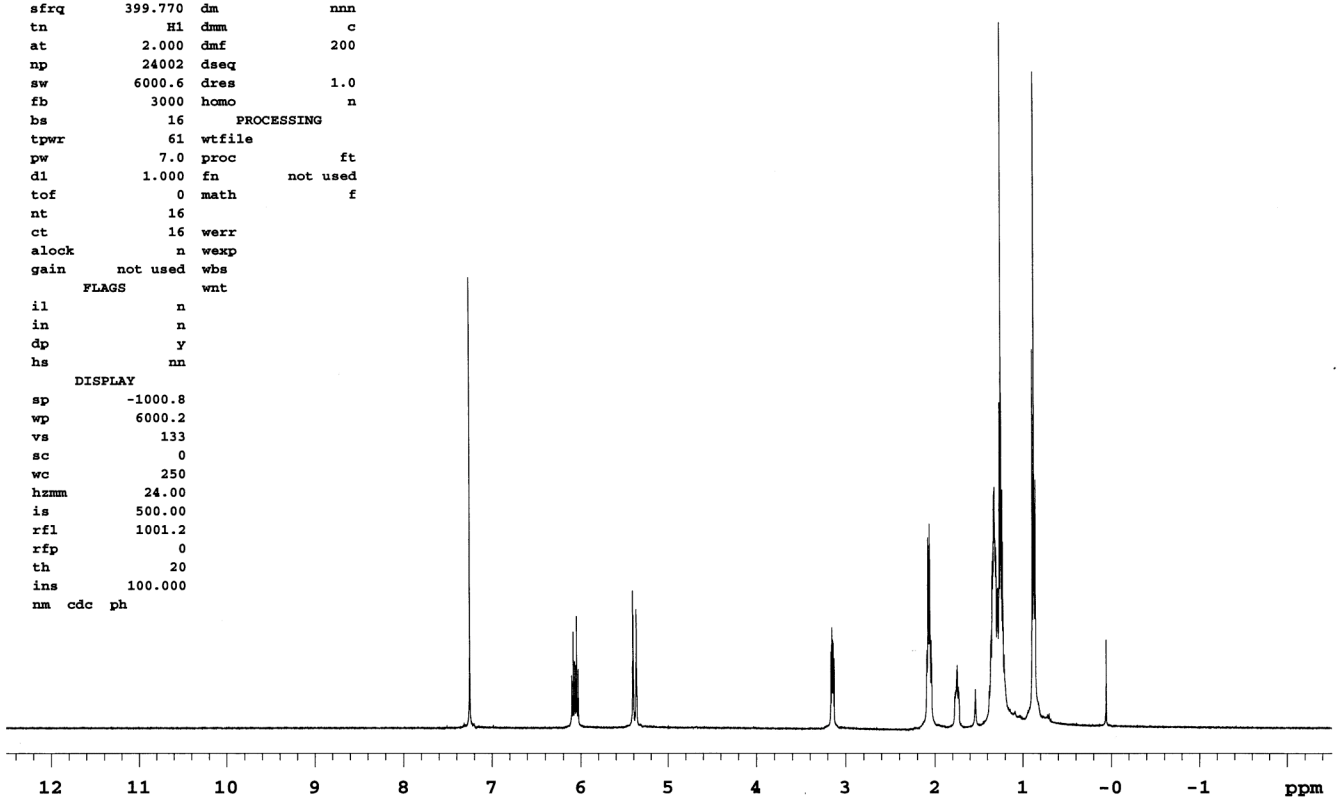


^{13}C NMR (100 MHz, CDCl)
of compound (+)-**2.29**



^{1}H NMR (400 MHz, CDCl) of compound (–)-**2.29**

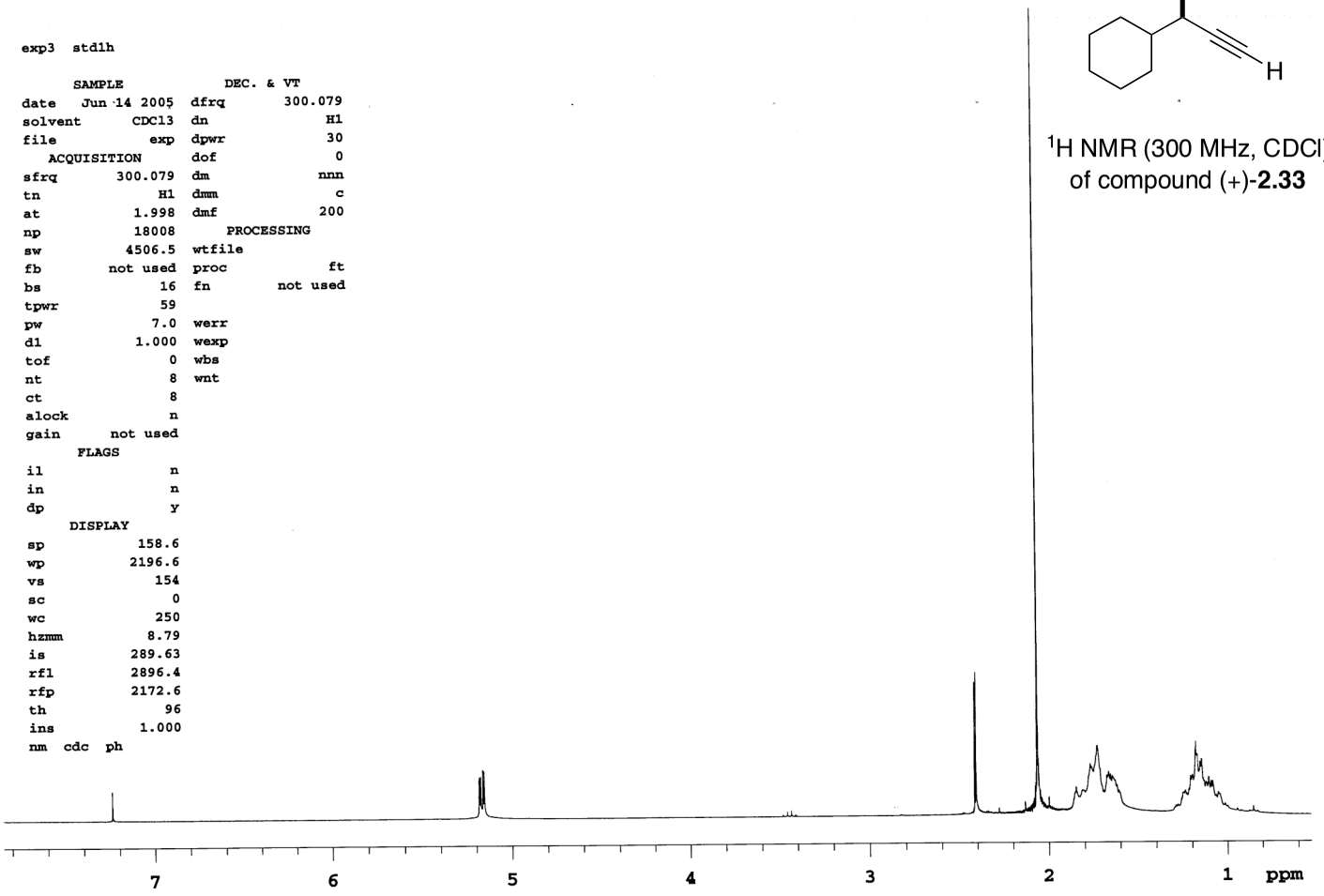
```
STANDARD 1H OBSERVE


exp1  std1h

      SAMPLE            DEC. & VT
date    Jun  5 2005  dfrq        399.770
solvent       CDCl3  dn             H1
file            exp  dpwr           30
   ACQUISITION       dof             0
sfrq        399.770  dm            nnn
tn               H1  dmm             c
at            2.000  dmf           200
np            24002  dseq
sw           6000.6  dres          1.0
fb             3000  homo            n
bs               16       PROCESSING
tpwr             61  wtfile
pw              7.0  proc            ft
d1            1.000  fn         not used
tof              0  math            f
nt              16
ct              16  werr
alock            n  wexp
gain      not used  wbs
      FLAGS         wnt
il               n
in               n
dp               y
hs              nn
     DISPLAY
sp          -1000.8
wp           6000.2
vs              133
sc                0
wc              250
hzmm          24.00
is            500.00
rfl          1001.2
rfp               0
th               20
ins         100.000
nm  cdc  ph
```



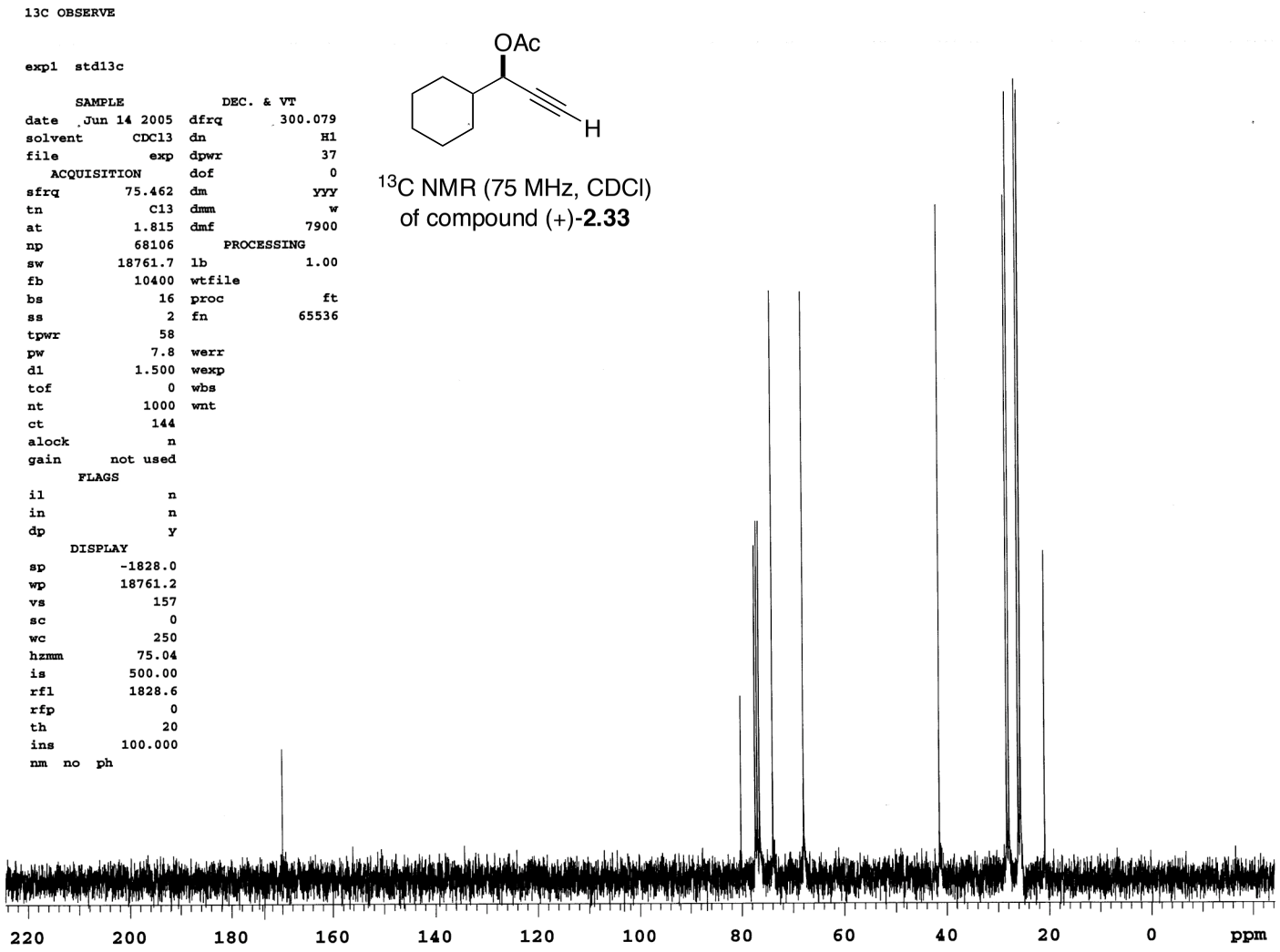
STANDARD 1H OBSERVE

exp3 std1h

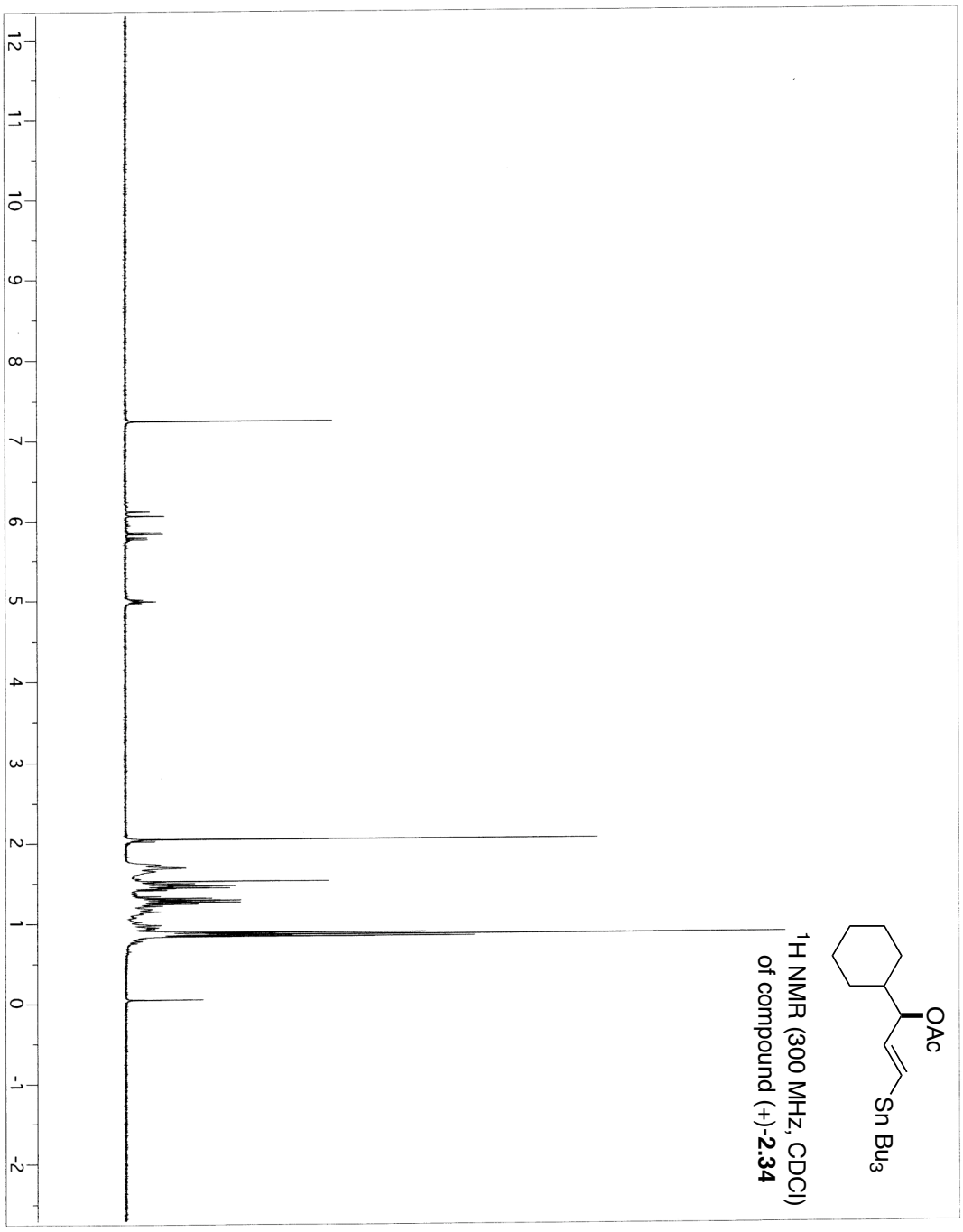
SAMPLE		DEC. & VT	
date	Jun 14 2005	dfrq	300.079
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.079	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	18008	PROCESSING	
sw	4506.5	wtfile	
fb	not used	proc	ft
bs	16	fn	not used
tpwr	59		
pw	7.0	werr	
d1	1.000	wexp	
tof	0	wbs	
nt	8	wnt	
ct	8		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	158.6		
wp	2196.6		
vs	154		
sc	0		
wc	250		
hzmm	8.79		
is	289.63		
rfl	2896.4		
rfp	2172.6		
th	96		
ins	1.000		
nm cdc ph			



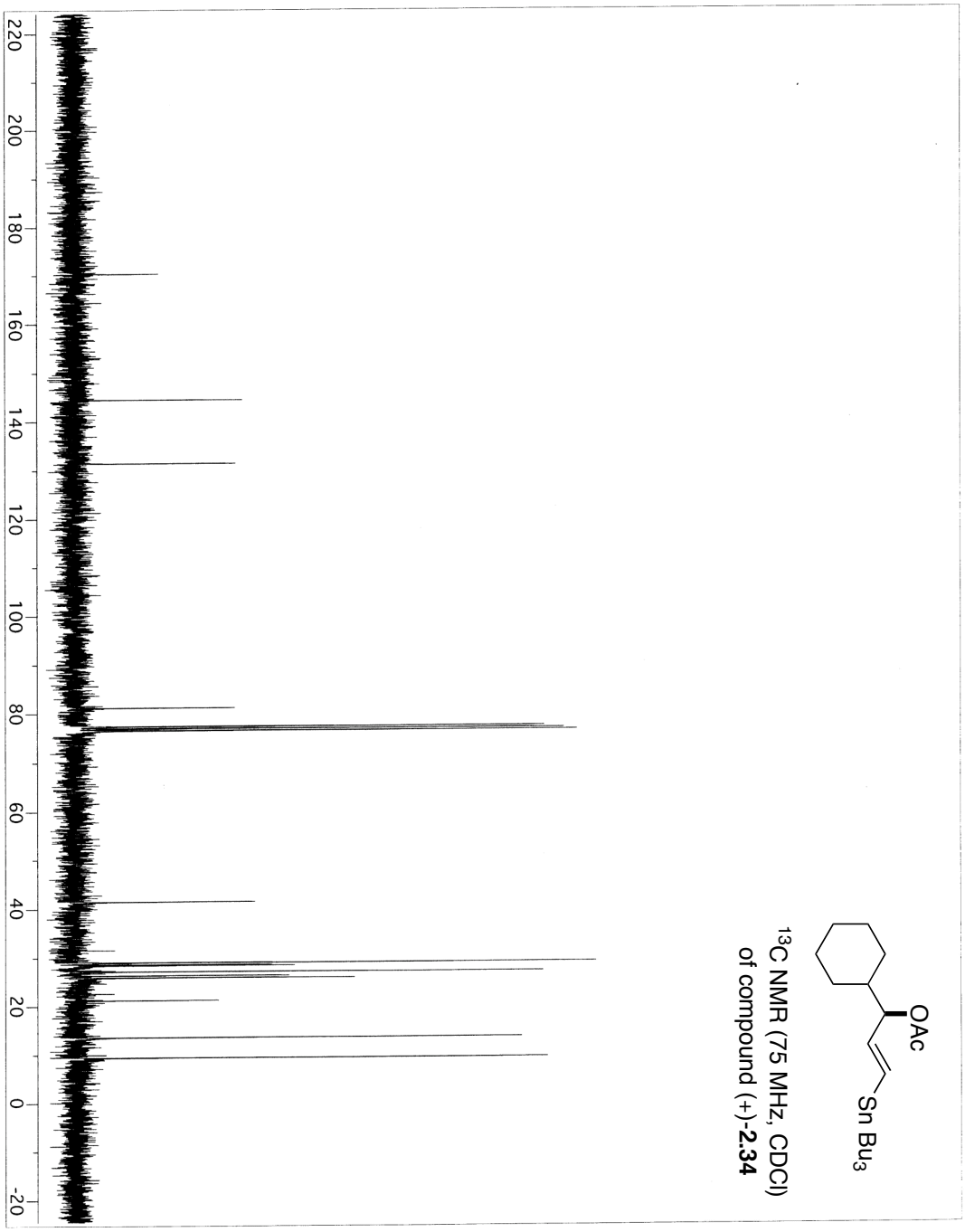
^{1}H NMR (300 MHz, CDCl) of compound (+)-**2.33**



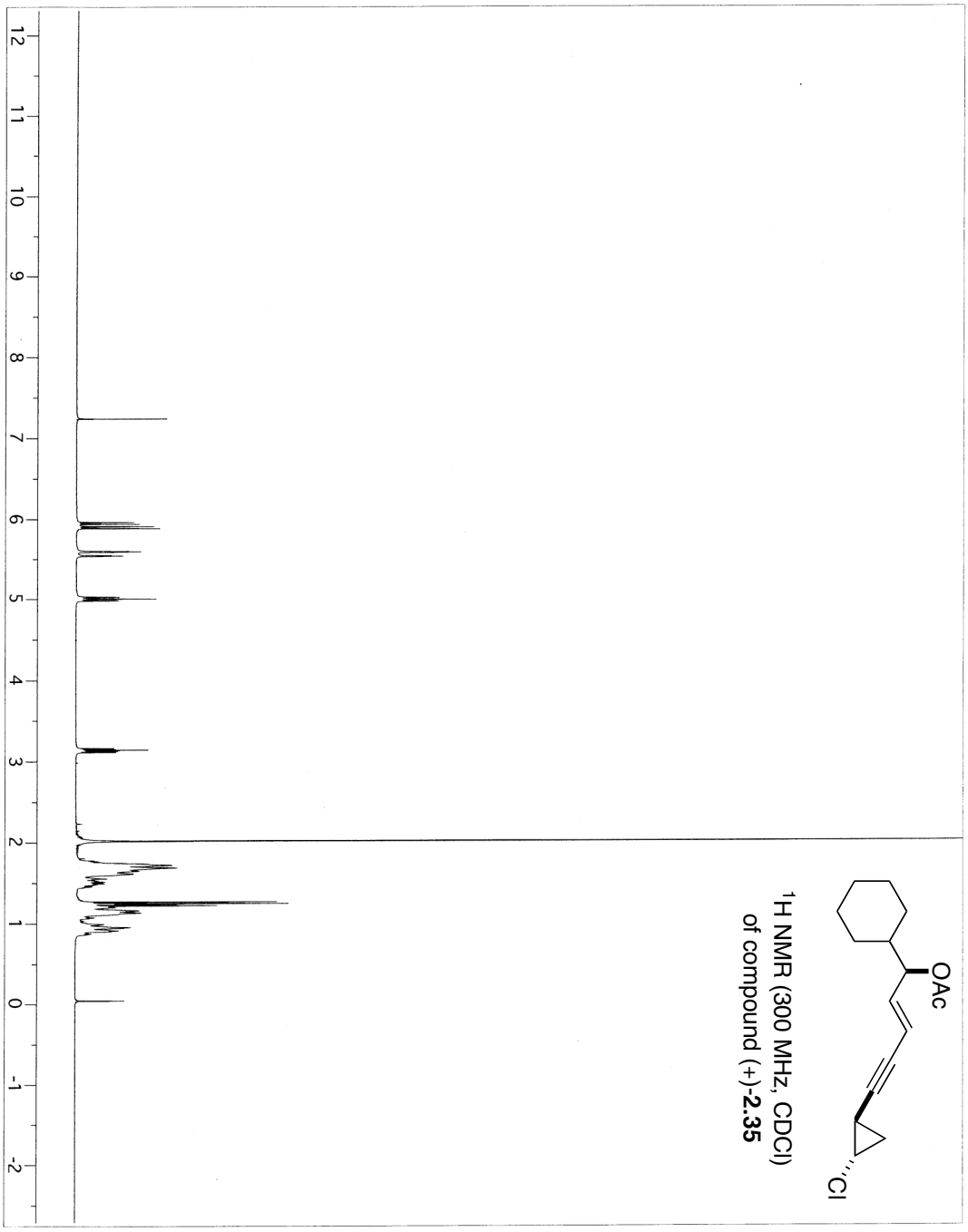
^{13}C NMR (75 MHz, CDCl) of compound (+)-**2.33**



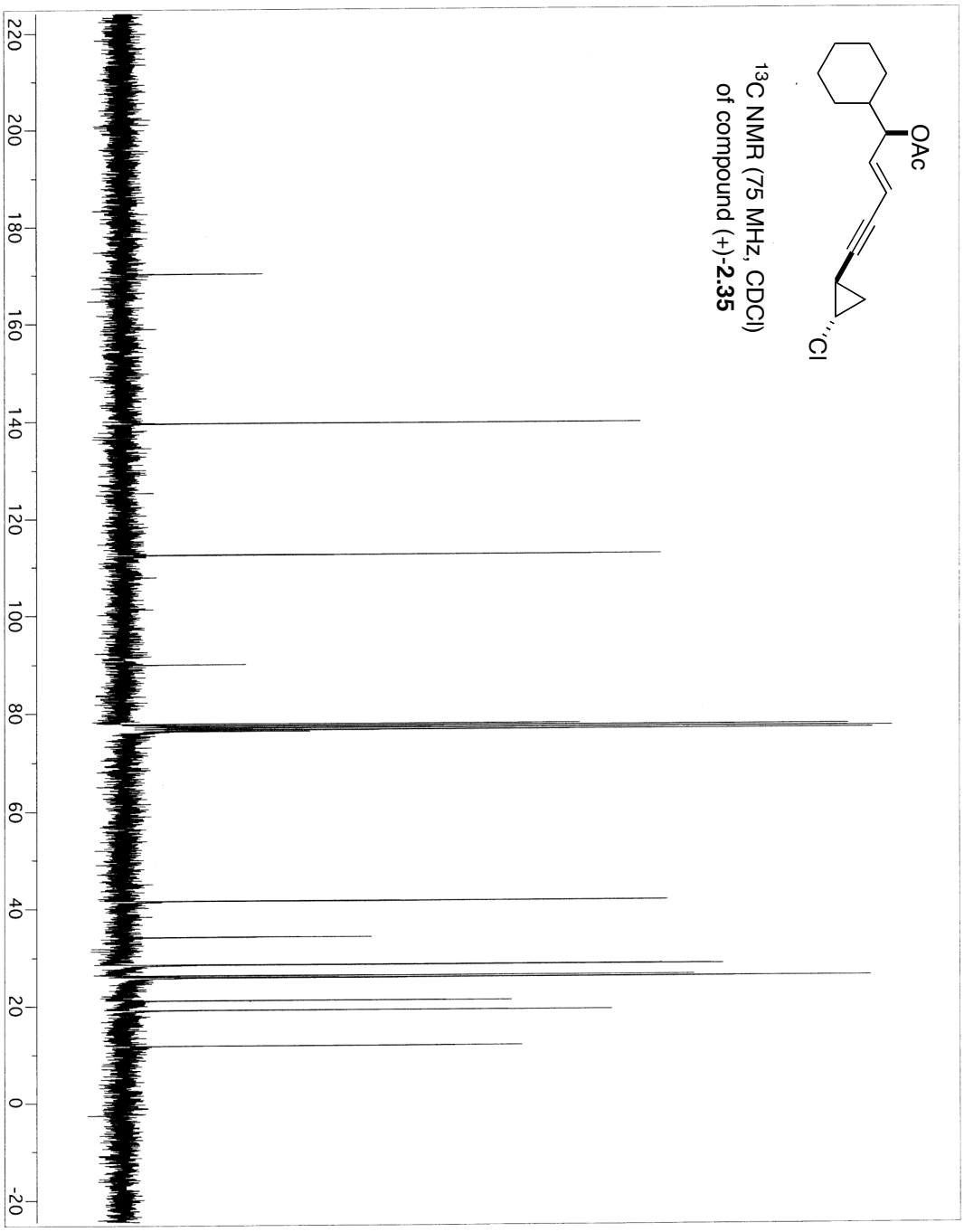
^{1}H NMR (300 MHz, CDCl) of compound (+)-**2.34**

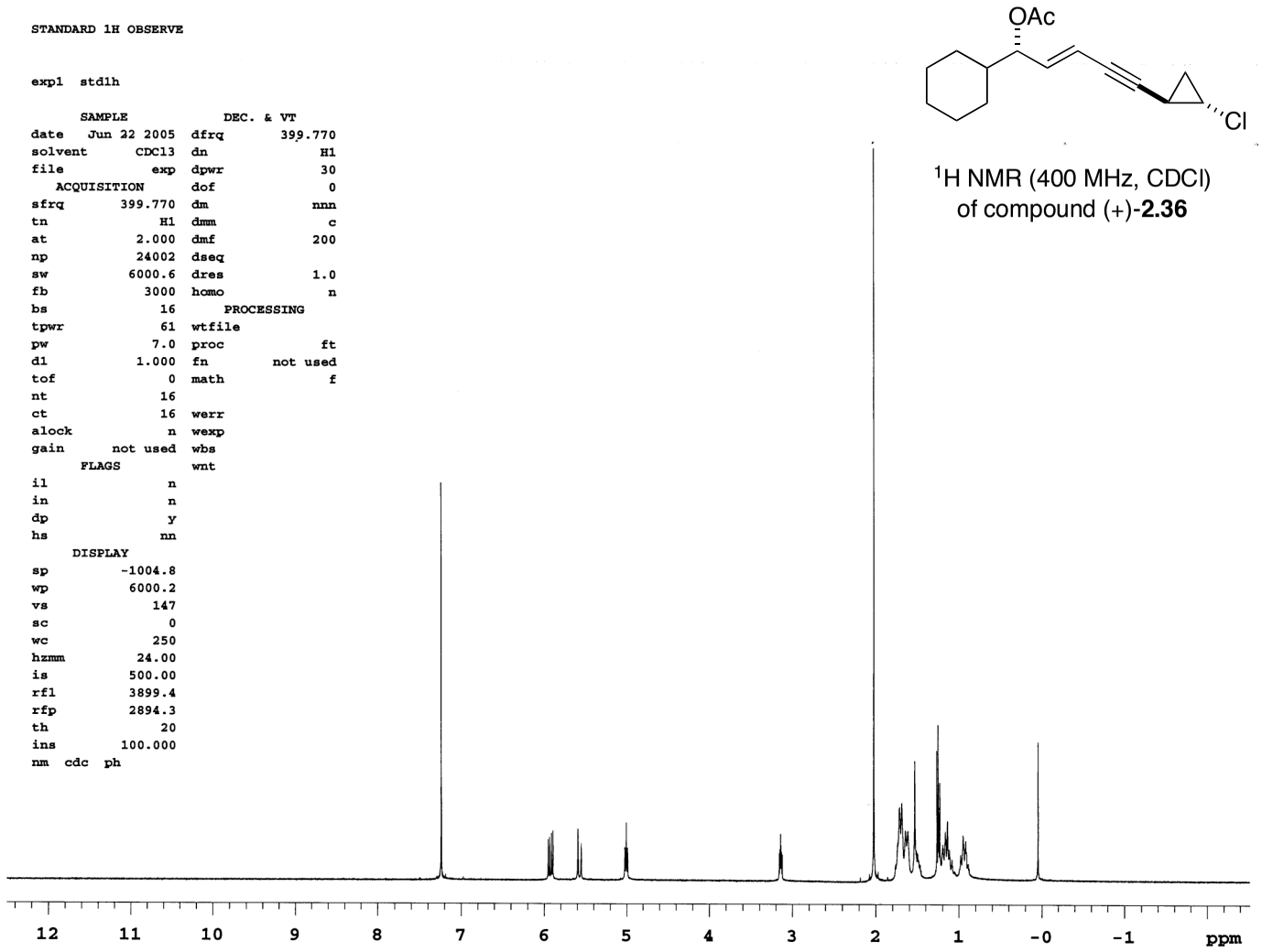


^{13}C NMR (75 MHz, CDCl) of compound (+)-**2.34**

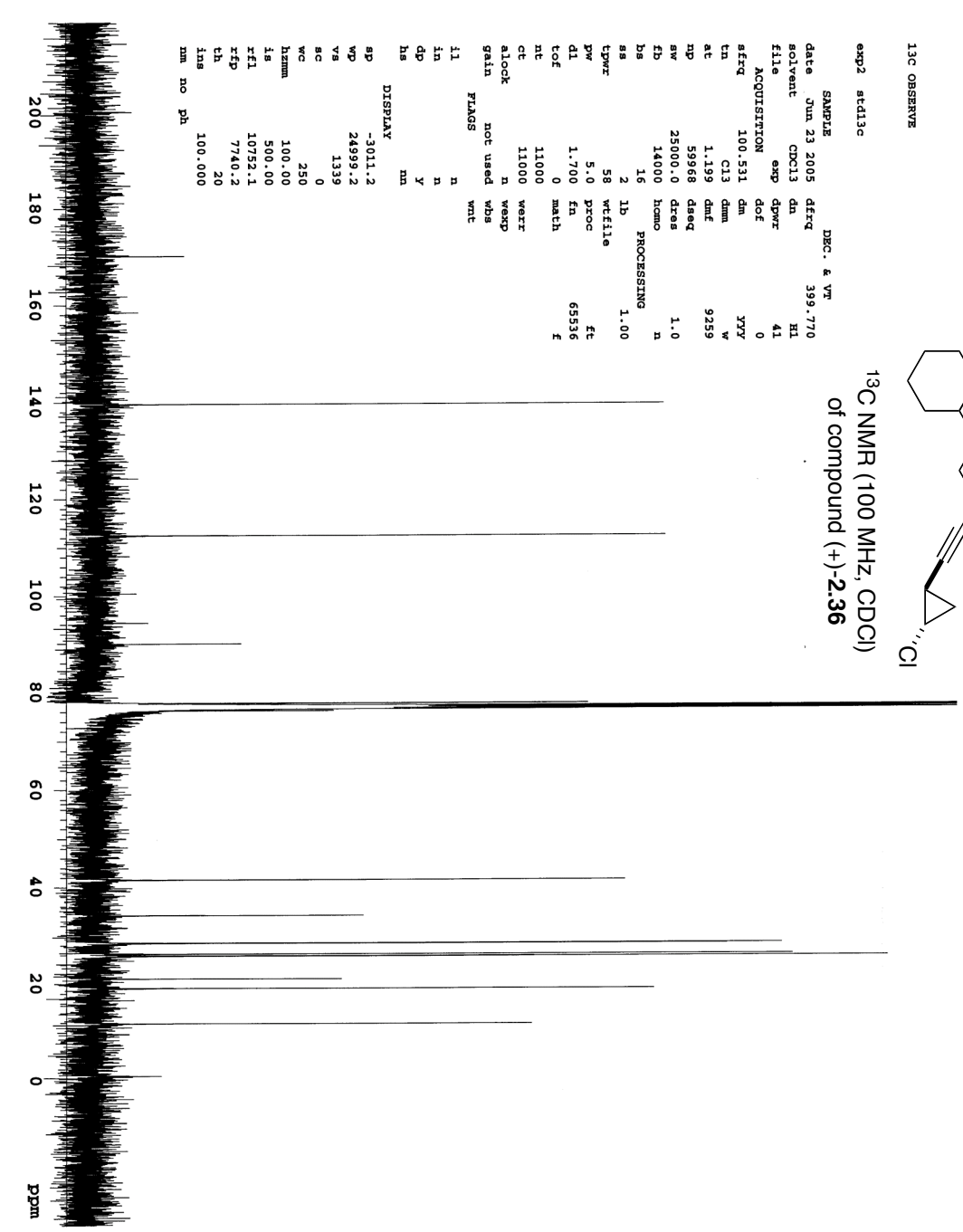


^{1}H NMR (300 MHz, CDCl)
of compound (+)-**2.35**

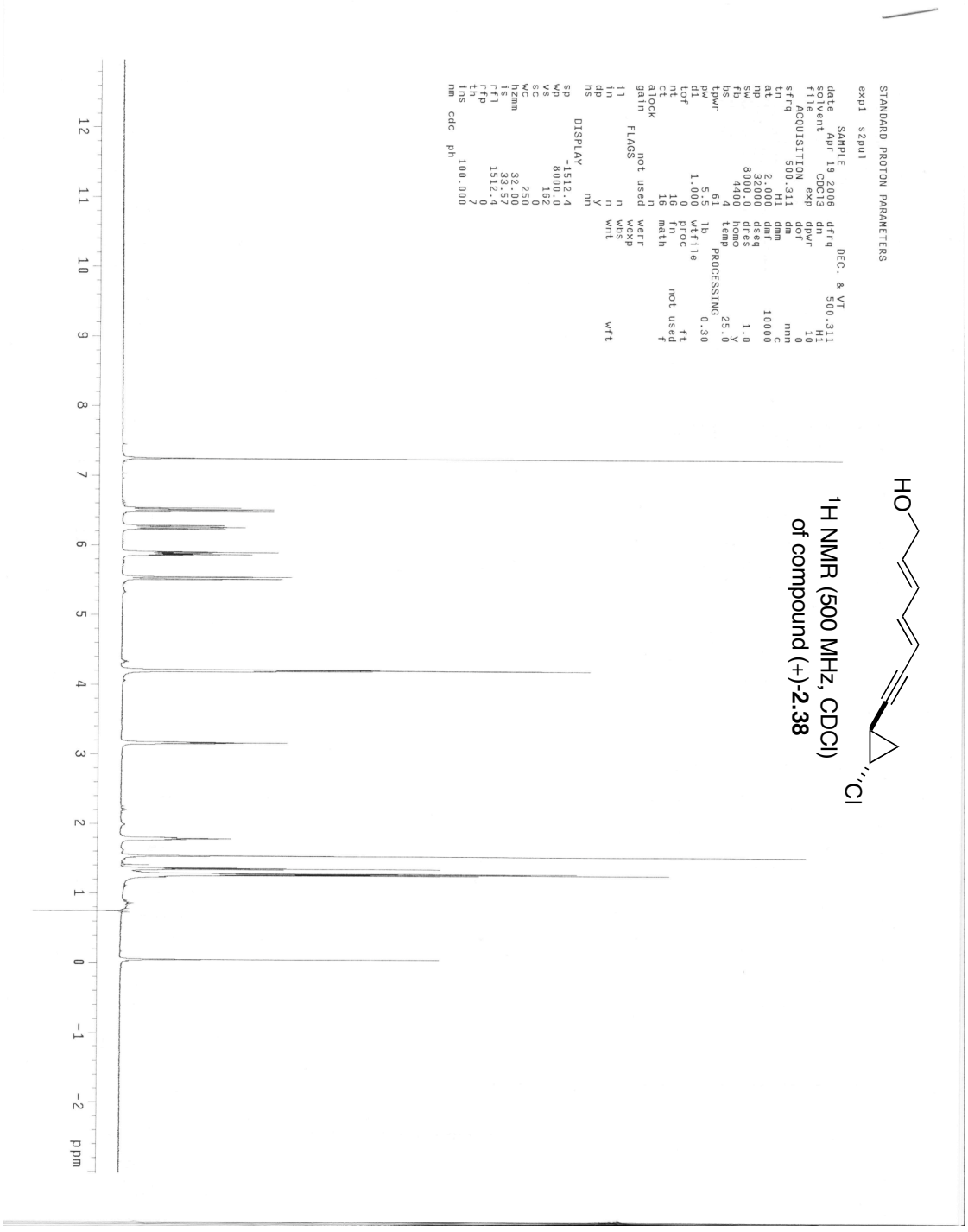

OAc
Cl
^{13}C NMR (75 MHz, CDCl)
of compound (+)-**2.35**
220
200
180
160
140
120
100
80
60
40
20
0
-20

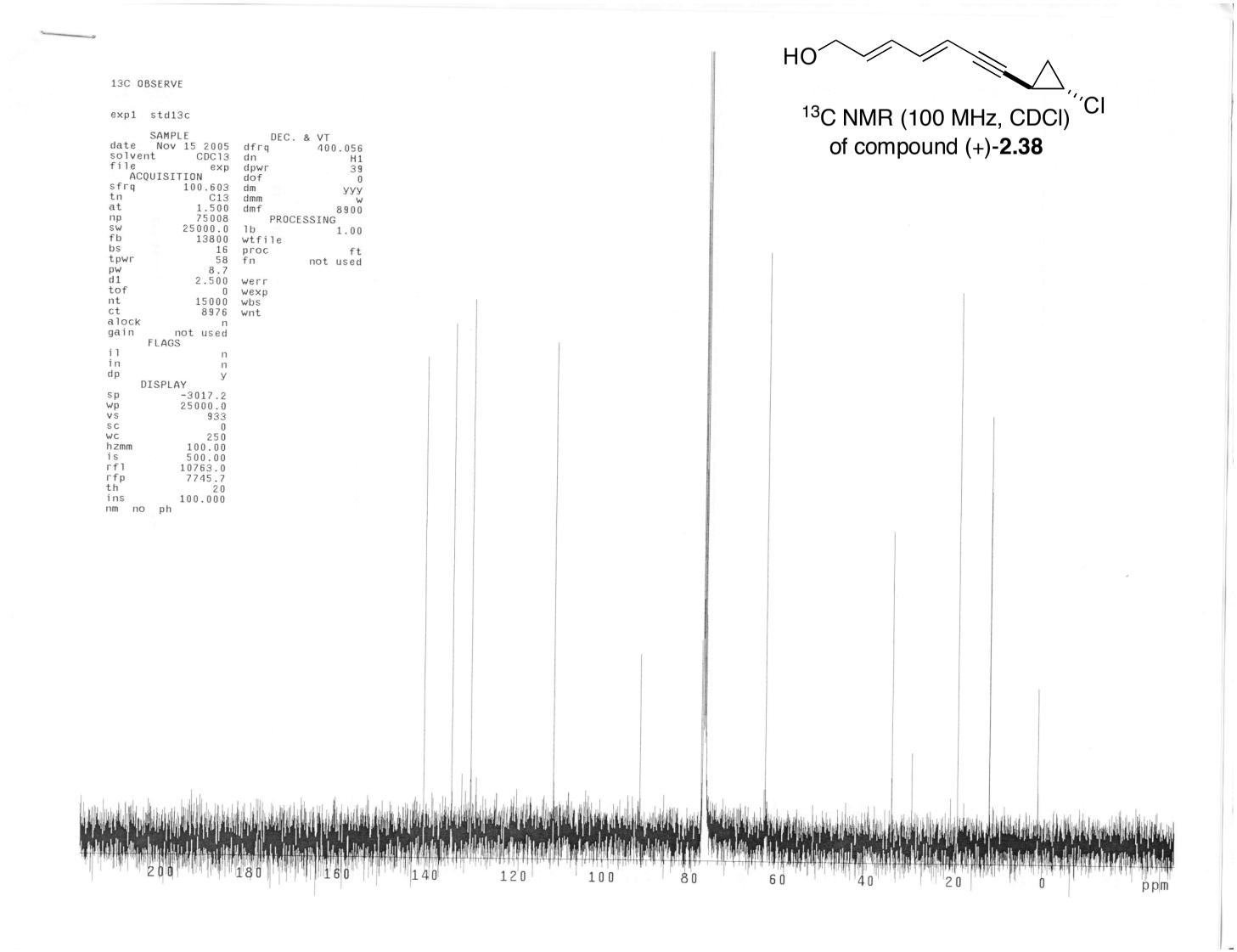


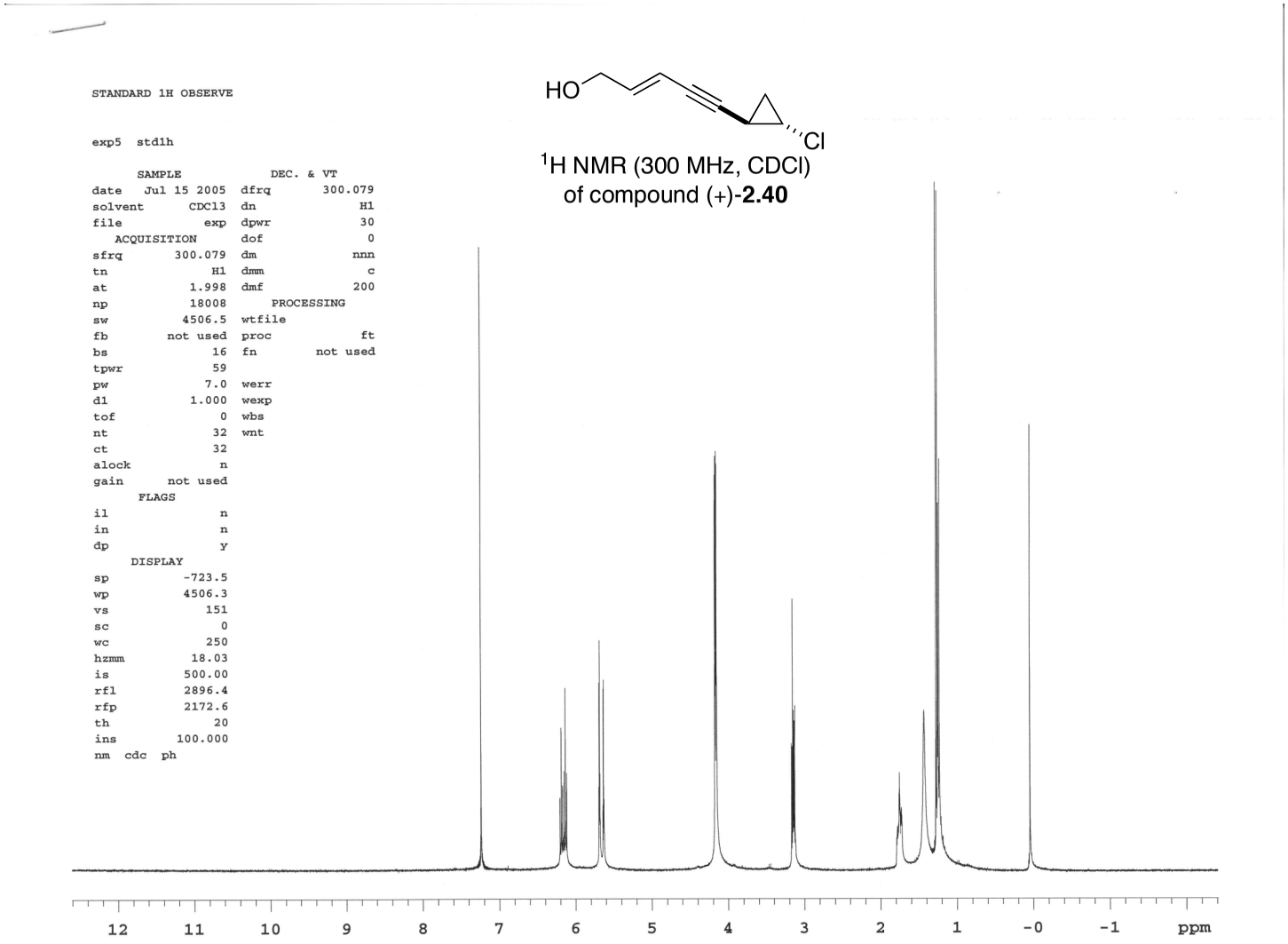
^{1}H NMR (400 MHz, CDCl) of compound (+)-**2.36**



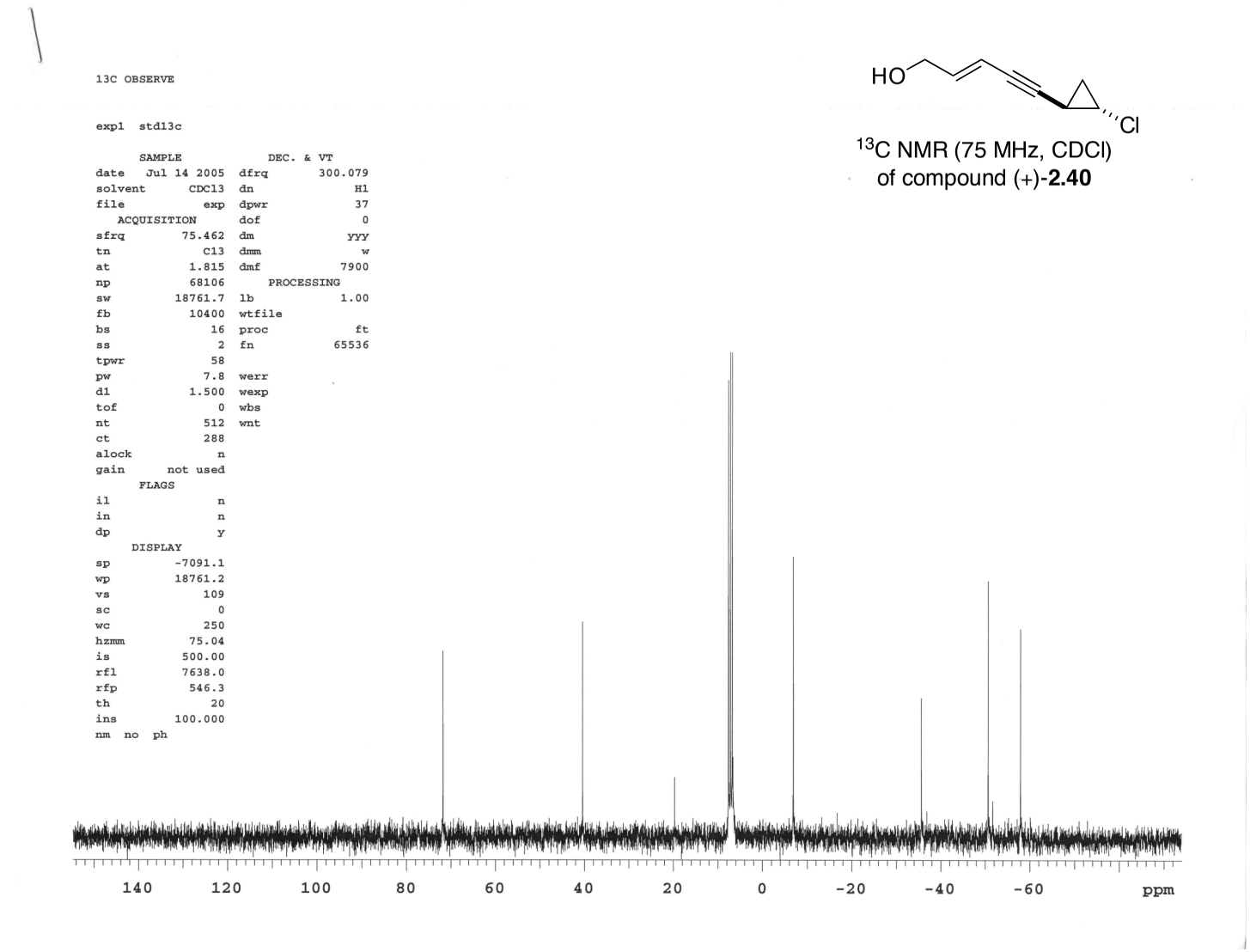
^{13}C NMR (100 MHz, CDCl) of compound (+)-**2.36**


HO
Cl
^{1}H NMR (500 MHz, CDCl)
of compound (+)-**2.38**
STANDARD PROTON PARAMETERS
exp1 s2pul
SAMPLE DEC. & VT
date Apr 19 2006 dfrq 500.311
solvent CDCl3 dn H1
file exp dpwr 10
ACQUISITION dof 0
sfrq 500.311 dm nnn
tn H1 dmm c
at 2.000 dmf 10000
np 32000 dseq
sw 8000.0 dres 1.0
fb 4400 homo y
bs 4 temp 25.0
tpwr 61 PROCESSING
pw 5.5 lb 0.30
d1 1.000 wtfile
tof 0 proc ft
nt 16 fn not used
ct 16 math
alock n
gain not used werr
FLAGS wexp
il n wbs
in y wnt wft
dp y
hs nn
DISPLAY
sp -1512.4
wp 8000.0
vs 162
sc 250
wc 32.00
hzmm 33.57
is 1512.4
rfl 0
rfp 7
th 100.000
ins
nm cdc ph
12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm

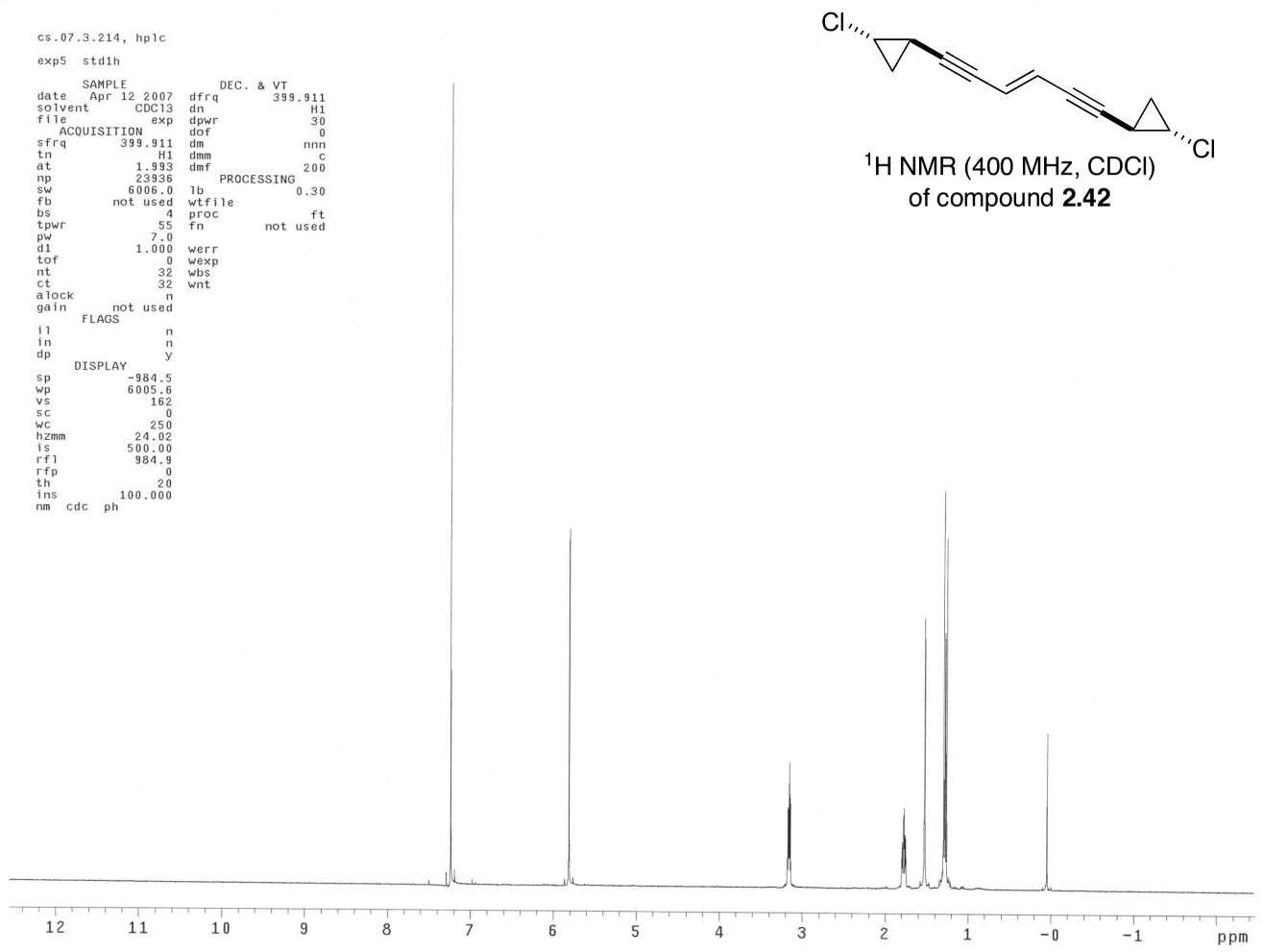

13C OBSERVE
exp1 std13c
SAMPLE
date Nov 15 2005
solvent CDCl3
file exp
ACQUISITION
sfrq 100.603
tn C13
at 1.500
np 75008
sw 25000.0
fb 13800
bs 16
tpwr 58
pw 8.7
d1 2.500
tof 0
nt 15000
ct 8976
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -3017.2
wp 25000.0
vs 933
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10763.0
rfp 7745.7
th 20
ins 100.000
nm no ph
DEC. & VT
dfrq 400.056
dn H1
dpwr 39
dof 0
dm yyy
dmm w
dmf 8900
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
HO
Cl
13C NMR (100 MHz, CDCl)
of compound (+)-2.38
200
180
160
140
120
100
80
60
40
20
0
ppm



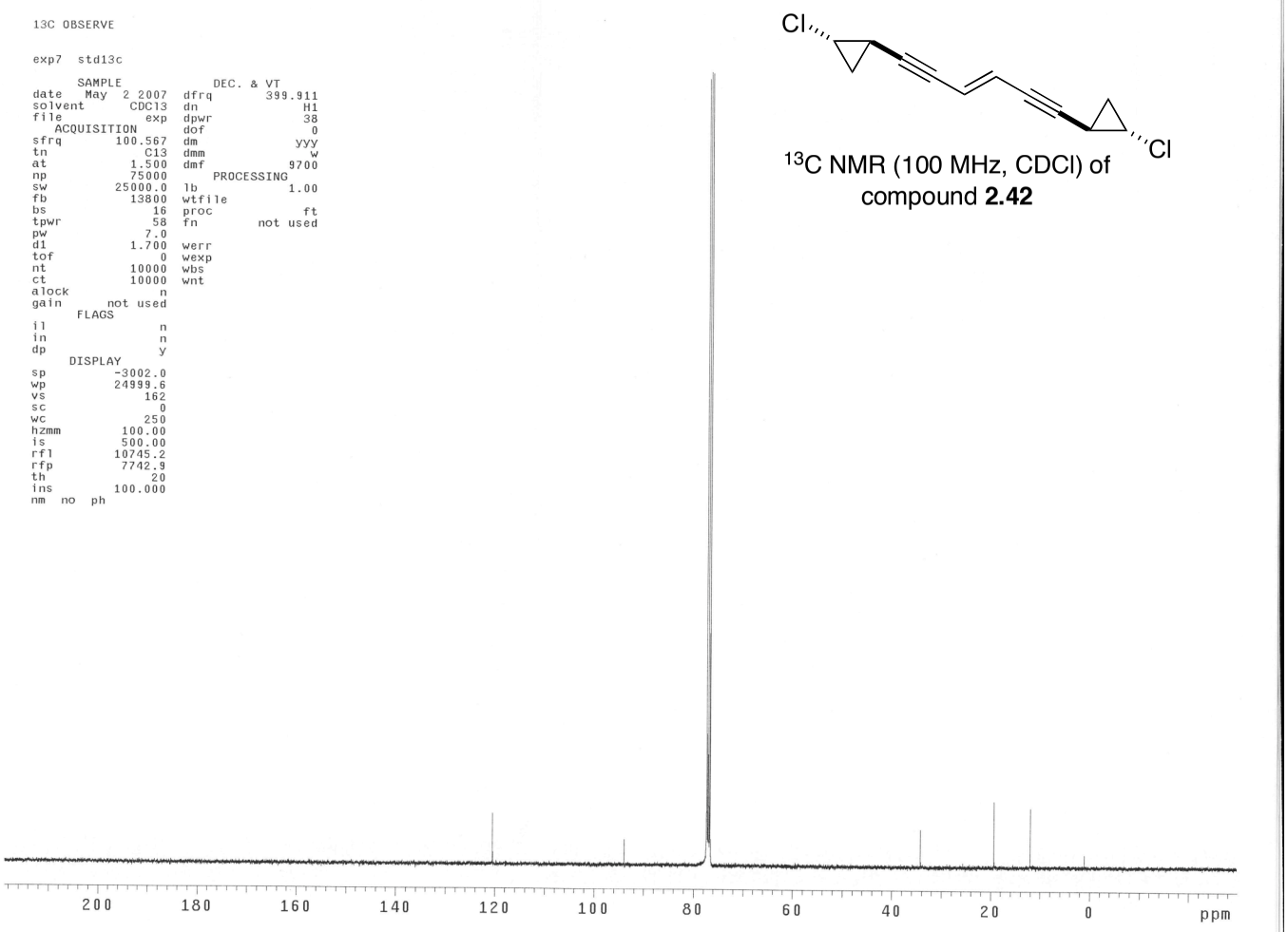
^{1}H NMR (300 MHz, CDCl) of compound (+)-**2.40**



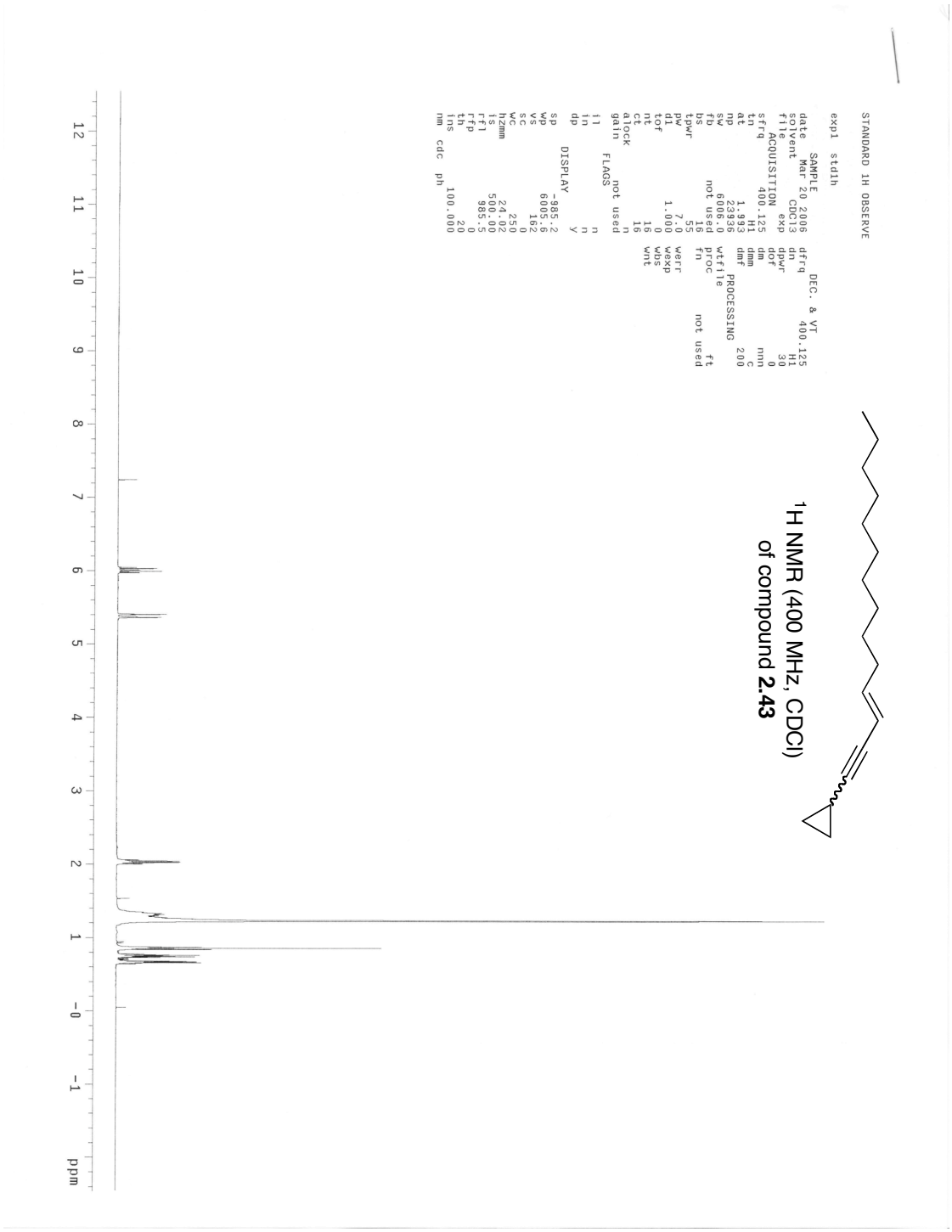
^{13}C NMR (75 MHz, CDCl)
of compound (+)-**2.40**



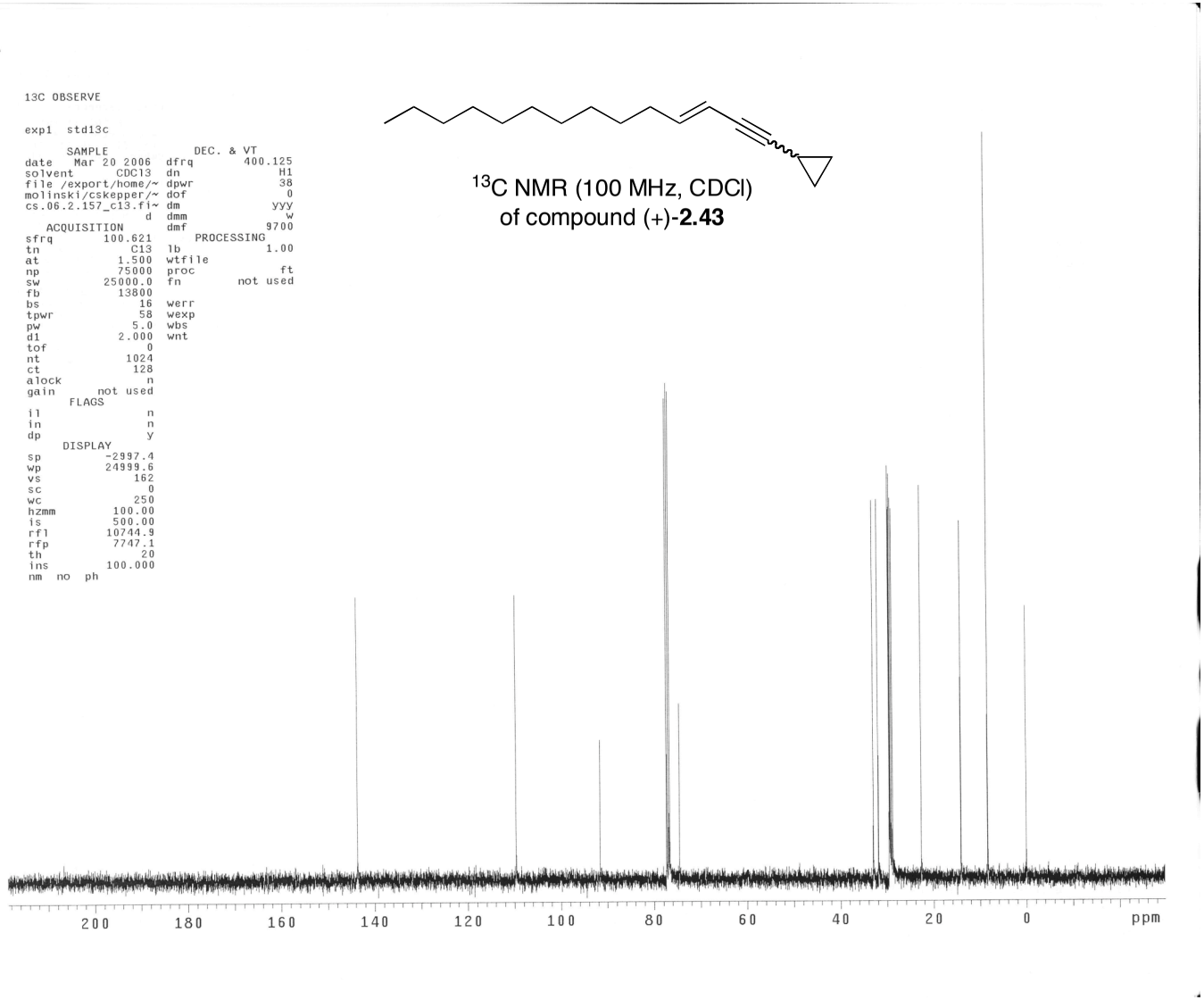
^{1}H NMR (400 MHz, CDCl) of compound **2.42**



^{13}C NMR (100 MHz, CDCl) of compound **2.42**



^{1}H NMR (400 MHz, CDCl)
of compound **2.43**


13C OBSERVE
exp1 std13c
SAMPLE
date Mar 20 2006
solvent CDCl3
file /export/home/~
molinski/cskepper/~
cs.06.2.157_c13.fi~
d
ACQUISITION
sfrq 100.621
tn C13
at 1.500
np 75000
sw 25000.0
fb 13800
bs 16
tpwr 58
pw 5.0
d1 2.000
tof 0
nt 1024
ct 128
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -2997.4
wp 24999.6
vs 162
sc 0
wc 250
hzmm 100.00
is 500.00
rfl 10744.9
rfp 7747.1
th 20
ins 100.000
nm no ph
DEC. & VT
dfrq 400.125
dn H1
dpwr 38
dof 0
dm yyy
dmm w
dmf 9700
PROCESSING
lb 1.00
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
13C NMR (100 MHz, CDCl)
of compound (+)-2.43
200
180
160
140
120
100
80
60
40
20
0
ppm

CHAPTER 3

EVALUATION OF THE ANTIFUNGAL ACTIVITY OF SYNTHETIC AND NATURAL LONG-CHAIN 2*H*-AZIRINE CARBOXYLATES

3.1 A History of 2*H*-Azirine Marine Natural Products

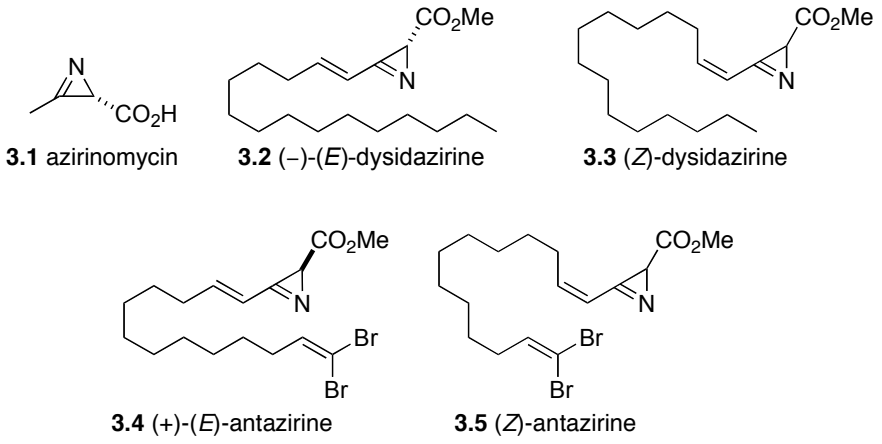
Azacyclopropene rings are highly strained three-membered heterocycles that have historically been confined to the realm of physical-organic chemistry. The heat of formation (ΔH_f) for a 2*H*-azirine ring is more than twice that of the saturated analogue (aziridine) and surpassed only by the anti-aromatic 1*H*-azirine. It is remarkable not that the list of 2*H*-azirine natural products is so short, but that Nature produces such compounds at all!

1*H*-azirine ΔH_f^o = 98.4 kcal/mol | 2*H*-azirine ΔH_f^o = 65.7 kcal/mol | aziridine ΔH_f^o = 30.2 kcal/mol | oxirane ΔH_f^o = -12.6 kcal/mol

Figure 3.1 Heats of formation of common three-membered heterocycles.[1]

The first 2*H*-azirine natural product isolated, azirinomycin (**3.1**) was in fact not of marine origin but came from *Streptomyces aureus*.[2] Like all azacyclopropene natural products isolated since, **3.1** is substituted at C2 with a carboxylate group. Azirinomycin displayed potent, broad-spectrum antibiotic activity. It was not until 1988 that the first marine-derived 2*H*-azirine was discovered, (2*R*)-(*E*)-dysidazirine ((−)-**3.2**) from the sponge *Dysidea fragilis* (Montagu, 1818) collected in Fiji.[3] Compound (−)-**3.2** also showed antibiotic activity (*P. aeruginosa*) as well as cytotoxicity (IC_{50} 0.27 μg/mL, L1220 cells)

and anti-fungal activity (MIC 4 μg/disk, *Candida albicans*, *Saccharamyces cerevisiae*). The 2*H*-azirine ring of (−)-**3.2** was conjugated to an *E*-alkene and featured a carboxylate subsituent at the azirine C2 position. The configuration at C2 was defined unambiguously as *R* by chemical degradation, derivatization and circular dichroism. Molinski and Ireland noted that (*E*)-dysidazirine possessed a C18 carbon backbone - the same carbon chain number as sphingosine, a common long-chain base found in eukaryotic cell membranes, suggesting a possible biogenetic relationship.



3.1 azirinomycin **3.2** (−)-(*E*)-dysidazirine **3.3** (*Z*)-dysidazirine

3.4 (+)-(*E*)-antazirine **3.5** (*Z*)-antazirine

Figure 3.2 Structures of all known 2*H*-azirine natural products prior to 2006. The configuration of **3.3** and **3.5** was unassigned in Faulkner's original report.

In 1995 Faulkner *et al.* reported the isolation of four long-chaing 2*H*-azirines from the same sponge, *D. fragilis*, this time collected in Micronesia.[4] Both geometrical isomers of dysidazirine, (+)-**3.2** and **3.3**, were characterized along with brominated analogues (+)-(*E*)- and (*Z*)-antazirine, (+)-**3.4** and **3.5**. Interestingly, the sample of (*E*)-dysidazirine in this case exhibited an $[\alpha]_D$ of

+47.2 compared with −165 reported by Molinski and Ireland. It appeared that this new sample of (+)-**3.2** was in fact enriched in the 2*S* enantiomer which was confirmed by comparison to the original sample by circular dichroism. The brominated analogue (+)-**3.4** also appeared to be enriched in the 2*S* enantiomer based on optical rotation ($[\alpha]_D$ = +10.3). Chiroptical data was not reported for **3.3** or **3.5**. The antazirines were reported to be inactive against a panel of microorganisms.

3.2 Isolation of Three New Antazirine Derivatives from *D. fragilis.*

In 2008 we reported the isolation of three new antazirine derivatives (+)-**3.6**, (+)-**3.7** and (−)-**3.8** along with (+)-**3.4** and (+)-**3.5** from another sample of *D. fragilis* collected in Pohnpei, Micronesia.[5] In contrast to the findings of Faulkner *et al*. (*Z*)-antazirine (+)-**3.5** was the most abundant of the five 2*H*-azirines isolated. No trace of (*E*)- or (*Z*)-dysidazirine was observed.

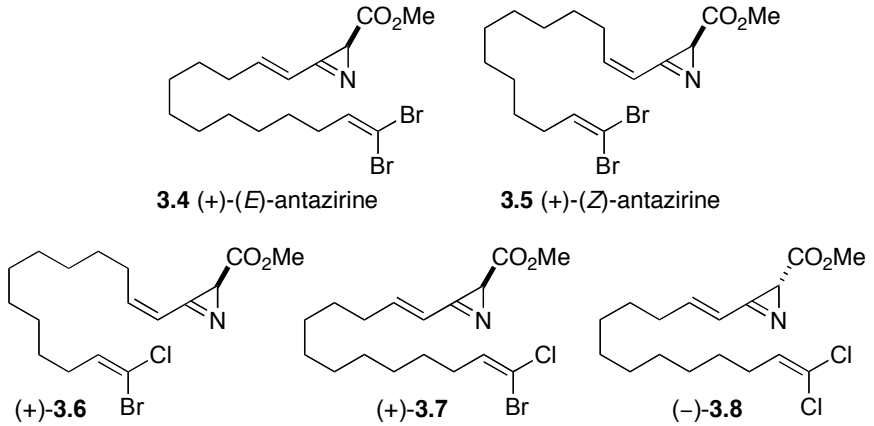


Figure 3.3 Structures of antazirines (+)-**3.4** and (+)-**3.5** and new analogues (+)-**3.6**, (+)-**3.7** and (−)-**3.8** isolated from *D. fragilis* collected in Pohnpei, Micronesia.

The new compounds **3.6**-**3.8** differed from the known antazirines only in the halogenation pattern at the chain terminus featuring a 1-bromo-1-chlorovinyl or 1,1-dichlorovinyl group. Literature searches indicate that (+)-**3.6** and (+)-**3.7** are the first natural products from a marine invertebrate to contain the 1-bromo-1-chlorovinyl group.[6]

The absolute configuration of (+)-**3.4**-**3.7** and (−)-**3.8** was assigned by comparison of $[\alpha]_D$ values with (−)-**3.2**. Surprisingly, each compound exhibited a completely different value for optical rotation ranging from −4.1 for (−)-**3.8** to +98.9 for (+)-**3.5** (Table 3.1). Chiral HPLC subsequently confirmed that each compound was a mixture of enantiomers, with enantiomeric excess proportional to the magnitude of specific rotation (Figure 3.4).

Table 3.1 Enantiomeric excess, optical rotation and configuration of **3.4**-**3.8**

Cmpd	Abundance[a]	% *ee*[b]	$[\alpha]_D$[c]	Configuration
3.4	0.016	30	+16.7[d]	2*S*,4*E*-
3.5	0.100	78	+98.9	2*S*,4*Z*-
3.6	0.037	72	+96.9	2*S*,4*Z*,15*Z*-
3.7	0.018	4	+8.9	2*S*,4*E*,15*Z*-
3.8	0.020	9	−4.1	2*R*,4*E*

[a] % dry weight of sponge. [b] Determined by chiral HPLC (Chiralpak AD), see Figure 3.4. [c] Recorded in *n*-hexane at 24 °C. [d] lit. +10.2.[4] [e] in MeOH, lit. –165.[3]

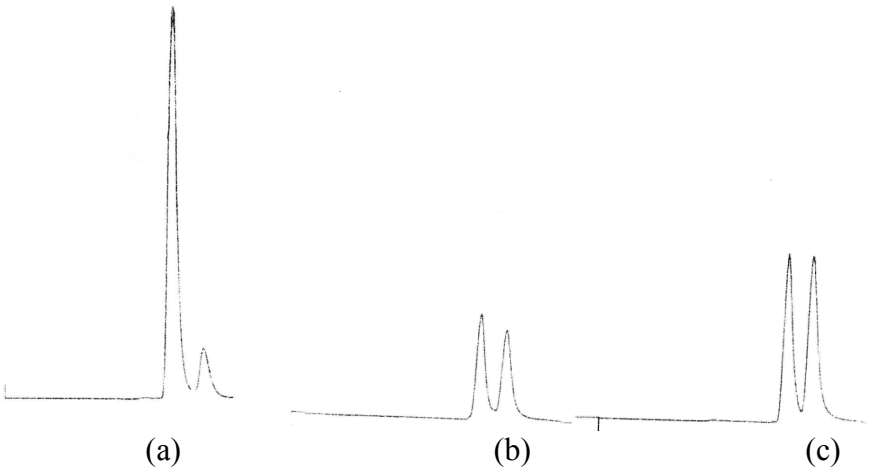
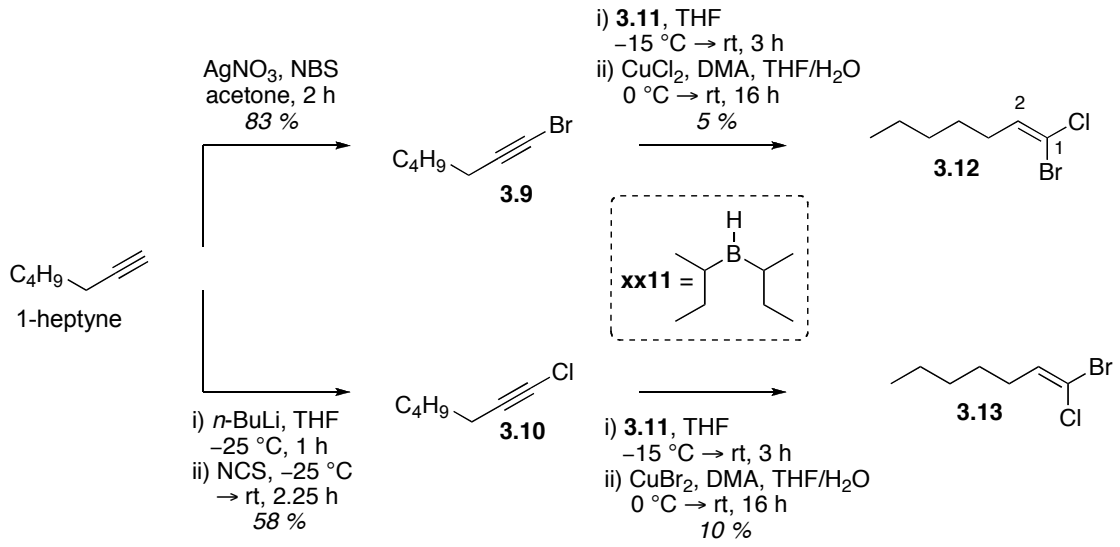


Figure 3.4 Chiral HPLC (Chiralpak AD, 85:15 hexanes/*i*-PrOH or 1:1 hexanes*/i*-PrOH, 0.5 mL/min) of (+)-**3.6** (a), (+)-**3.7** (b) and (−)-**3.8** (c).

Faulkner *et al*. had previously postulated that antazirines might occur naturally as non-racemic mixtures of enantiomers, however the mechanism by which enantiopurity is eroded was unclear. Irradiation of (+)-**3.5** with UV light (275W Sunlamp, Rose Bengal, *i*-octane, 0 ˚C) failed to induce epimerization over the course of 3 hours. Insatead, olefin isomerization was observed resulting in formation of small amounts (~10-20%) of the 4*E* isomer. Re-examination of a 19 year old original sample of natural (*E*)-dysidazirine[7] by chiral HPLC, however, indicated *ee*=22%, significantly lower than the original estimate of 89% and suggested spontaneous epimerization during storage at −20 ˚C (see Experimental Section for Chiral HPLC traces).



Scheme 3.1 Synthesis of 1-bromo-1-chloroalkene model compounds **3.12** and **3.12.**

Assignment of the geometry of the terminal olefin in (+)-**3.6** and (+)-**3.7** was deceptively non-trivial. Although stereodefined synthetic 1-bromo-1-chloro alkenes are known insufficient data was reported to allow an unambiguous comparison with the natural products. Using methodology developed by Masuda *et al*. two stereodefined model compounds **3.12** and **3.13** were prepared. Bromination or chlorination[8] of 1-heptyne led to **3.9** and **3.10** respectively. Hydroboration of **3.9** and **3.10** followed by displacement with chloride (for **3.9**) or bromide (for **3.10**) gave models **3.12** and **3.13.** The overall conversion, although low, provided sufficient quantity of compound for comparison to the natural products (Scheme 3.1).[9]

Remarkably, **3.12** and **3.13** are indistinguishable by ^{1}H NMR. Comparison by ^{13}C NMR, however, showed significant differences at C1 and

C2 with the largest difference observed for C1. The ^{13}C NMR data of (+)-**3.6** and (+)-**3.7** clearly matched model **3.12**, defining the terminal olefin geometry as 15*Z* (Figure 3.5).

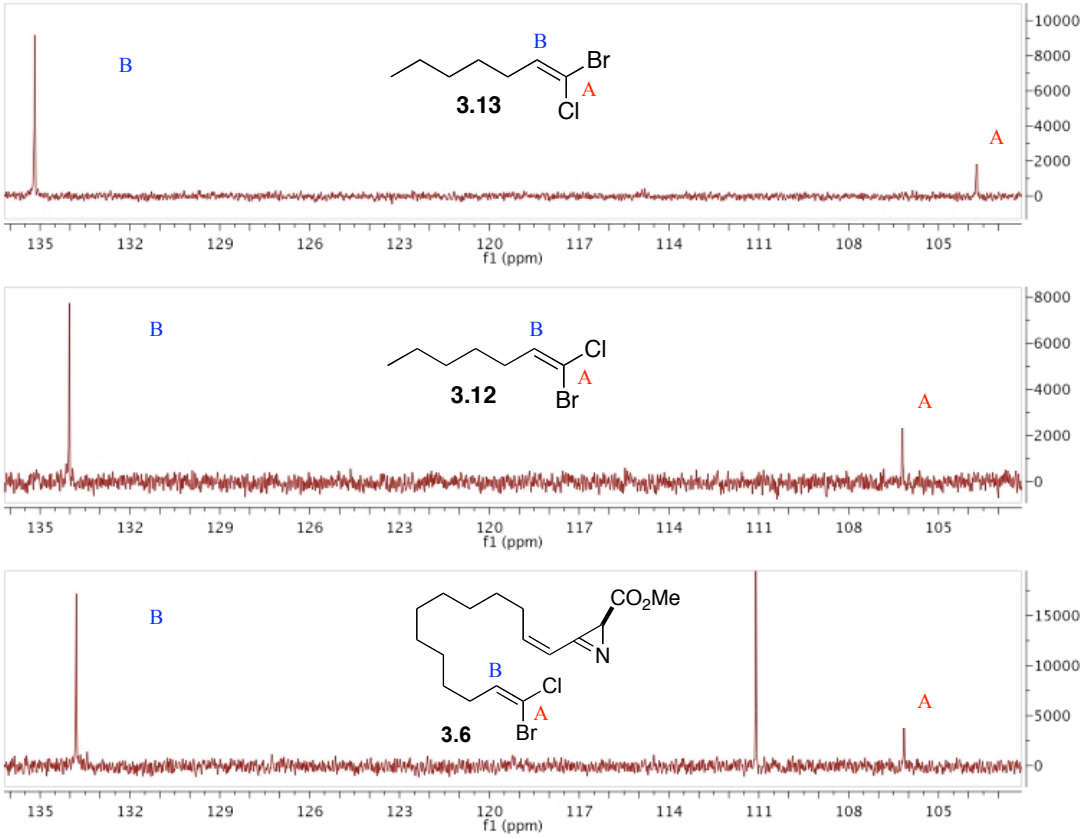


Figure 3.5 ^{13}C NMR comparison of model compounds **3.12** and **3.13** against the natural product **3.6** (100 MHz, $CDCl_3$).

All five antazirines (+)-**3.4-3.7** and (−)-**3.8** showed moderate cytotoxicity against HCT-116 cells (Table 3.2) but were *completely inactive* against a panel of yeast cells. This result is in stark contrast with (*E*)-dysidazirine which was reported to show quite potent antifungal activity (MIC 4 μg/disk, disk diffusion assay).[3]

Table 3.2. *In vitro* cytotoxicity data of (+)-**3.4**-**3.7** and (−)-**3.8** against HCT-116 cells.[a]

Compound	IC_{50} (μg/mL)	IC_{50} (μM)
(+)-**3.4**	8.5	19.6
(+)-**3.5**	7.9	18.2
(+)-**3.6**	5.3	12.6
(+)-**3.7**	5.9	15.2
(−)-**3.8**	8.6	24.8

a, Cells were grown for 20 h (37 ˚C, 5% CO_2), treated with drug then grown for 2.5 days. Cell viability was measured by the MTS endpoint (soluble formazan dye): MTS= (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt.

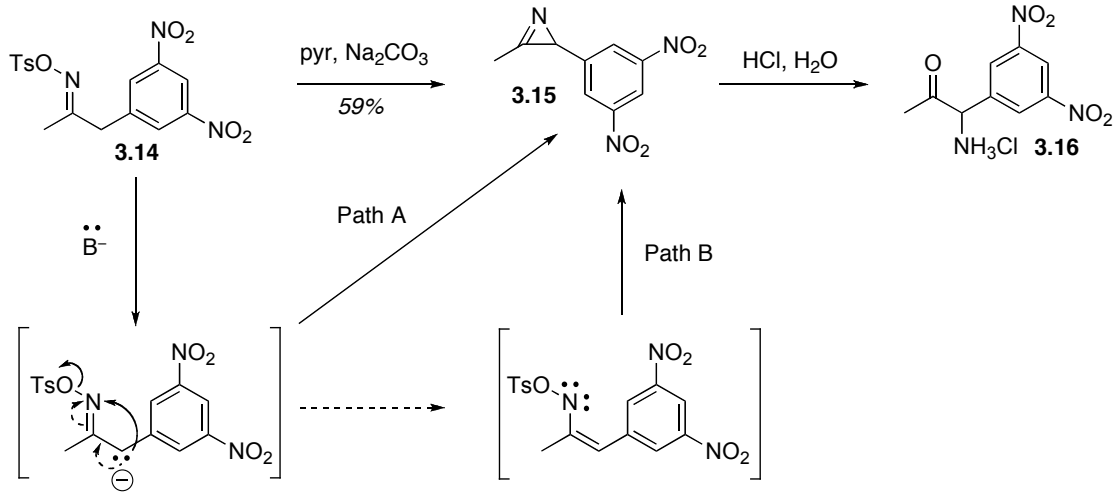
Structurally, (−)-**3.2** and **3.4-3.8** differ only in olefin configuration, chain length and terminal substitution. In order to evaluate the effect of these structural elements on the antifungal activity of long-chain 2*H*-azirines a synthetic project was initiated targeting the synthesis of (*Z*)- and (*E*)-dysidazirine along with a range of analogues.

3.3 Asymmetric Synthesis of 2*H*-Azirines

The preparation of 2*H*-azirines has been extensively studied since the mid-1960s due to interest in the ring strain inherent to these three-membered heterocycles and the wide variety of reactions they undergo. 2*H*-Azirines are precursors to larger heterocycles and open chain amino compounds and also exhibit extensive photochemistry.[10]

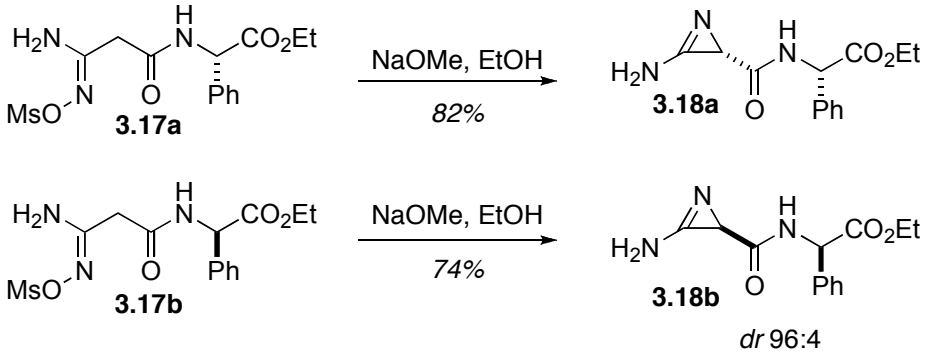
Strategies for the synthesis of 2*H*-azirines fall into three general categories: 1) Intramolecular cyclization (e.g. the classic Neber reaction). 2) Intermolecular reaction between a carbene and nitrile or nitrene and alkyne. 3) Formation from a pre-exisisting heterocycle (e.g. oxidation of aziridines or ring contraction of isoxazoles).[11] Asymmetric syntheses of 2*H*-azirines usually rely on variations of the Neber reaction or on the oxidation/elimination reaction of stereodefined azridines.

Neber *et al.* described the first synthesis of an azirine in 1932 *en route* to α-aminoketones.[12] Treatment of oxime tosylate **3.14** with base resulted in formation of 2*H*-azirine **3.15** which upon treatment with HCl provided the α-aminoketone **3.16**. Further characterization of the intermediate 2*H*-azirine was later carried out by Cram who provided support for the structure of **3.15**.[13] The mechanism of the Neber reaction was proposed to involve either a concerted nucleophilic displacement of the leaving group on nitrogen (Path A, Scheme **3.2**) or by electrocyclization of a vinylnitrene (Path B, Scheme **3.2**).[14]



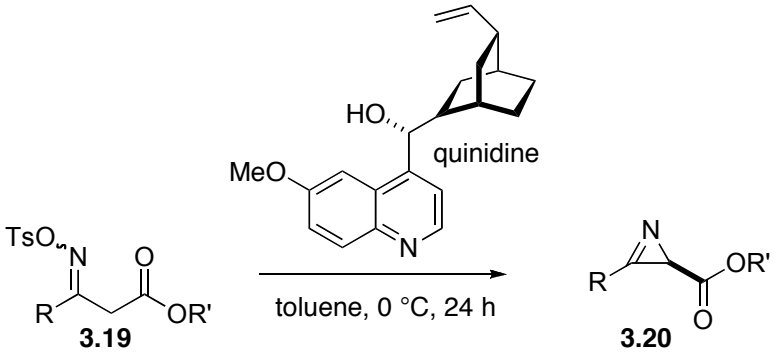
Scheme 3.2 Neber's synthesis of the first azirine in 1932

Variations on the Neber reaction have been used extensively since then for the preparation of 2*H*-azirines. Probably the first asymmetric Neber reaction was attempted by Cram *et al*. who treated **3.14** with brucine in 1953 with the aim of producing **3.15** in an optically active form.[13] While good yields of **3.15** were obtained the product was racemic. It was not until 1993 that Piskunova *et al*. prepared the first synthetic optically active 2*H*-azirine by treating *N*-acyl phenylglycine derivatives **3.17a,b** with base producing the desired azirines **3.18a,b** with high *dr* (96:4).[15]



Scheme 3.3 First synthesis of an optically active azirine by Neber reaction.

Table 3.3 Preparation of optically active 2*H*-azirines by Neber reaction catalyzed by quinidine



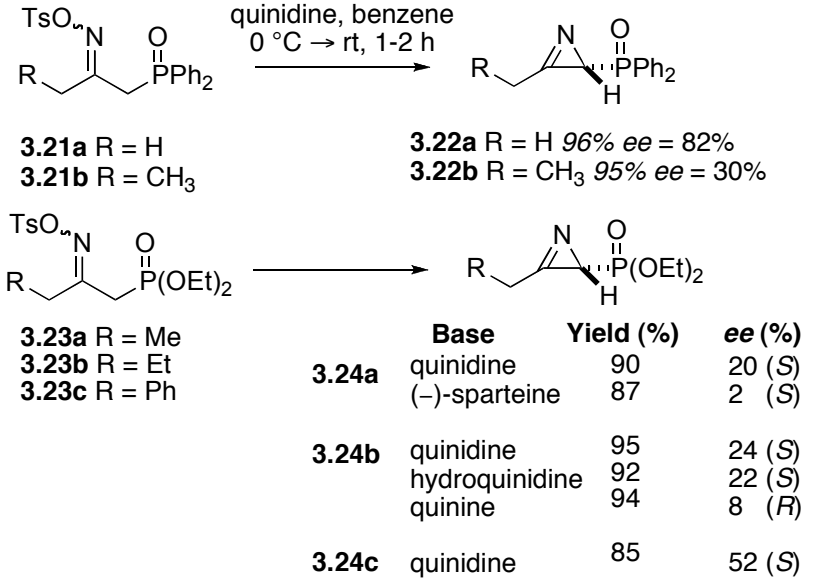
3.19 **3.20**

R	R'	Yield (%)	*ee* (%)
Me	Me	40	81
Me	Et	43	82
Me	^{t}Bu	29	44
n-Pr	Et	72	80
Bz	Et	85	80

Zwanenburg *et al*. showed that the Neber reaction of achiral oxime tosylates (**3.19)** is catalyzed by chiral amine bases to give enantio-enriched azirines (**3.20)**.[16] The best results were obtained with cinchona alkaloids such as quinidine which produced 2*H*-azirines optically enriched in the 2*R* enantiomer (*ee* = 44-82%). It is interesting to note that the pseudoenantiomer quinine produces 2*H*-azirines with the opposite configuration at C2, albeit with somewhat lower *ee* (55-57%). It was proposed that the observed enantioselectivity is dependent upon a hydrogen bond between the OH of the amine base and the tosylate moiety of the substrate. When the reactions were carried out in hydroxylic solvents or with amine bases that lack an OH (e.g.

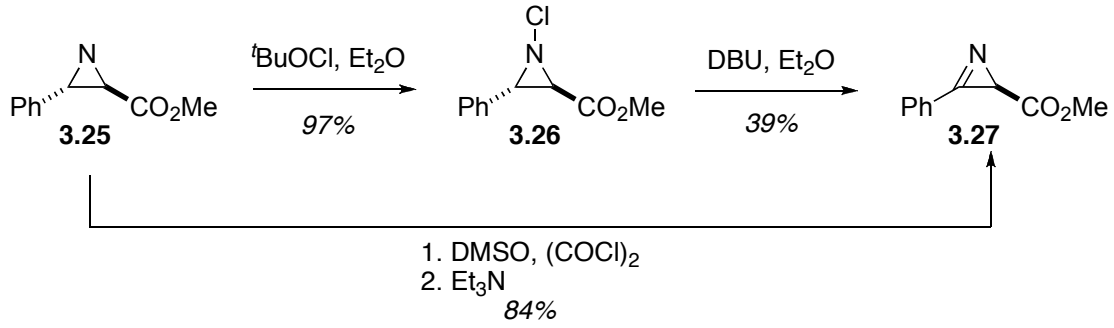
(−)-sparteine, brucine, strychnine) 2*H*-azirines of markedly lower %*ee* were obtained.

Palacios *et al.* extended this methodology to the formation of azirines of low %*ee* substituted at C2 with either phophonates or phosphine oxides (Scheme 3.4).[17]



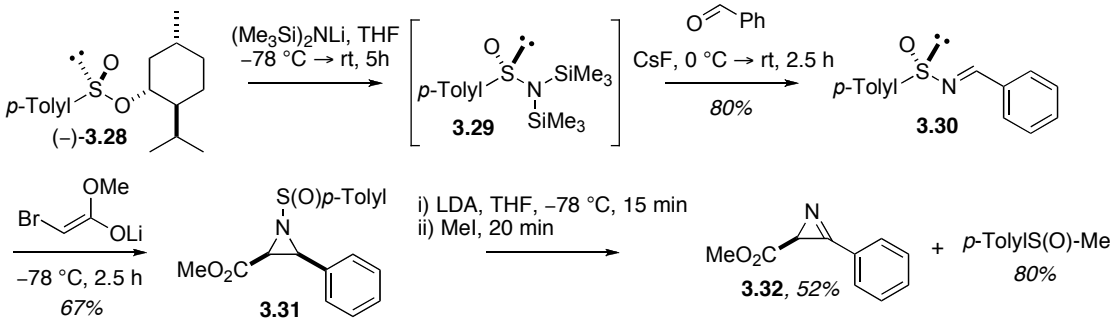
Scheme 3.4 Preparation of optically-enriched 2*H*-azirine-2-phosphonates and 2*H*-azirine-2-phosphine oxides by asymmetric Neber reaction.

Optically active 2*H*-azirines have also been prepared from optically pure aziridines. Zwanenburg *et al*. successfully converted optically enriched aziridine carboxylic ester **3.25** into the *N*-chloro derivative **3.26** in high yield (**Scheme 3.5**).[18] Subsequent dehydrohalogenation produced the desired 2*H*-azirine-2-carboxylic ester **3.27** in modest yield (39%). Remarkably, however, a modified Swern oxidation protocol converts aziridine **3.25** into azirine **3.27** directly in high yield (84%).



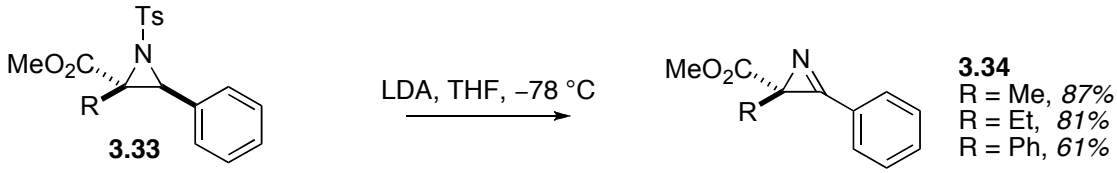
Scheme 3.5 Preparation of enantio-enriched 2*H*-azirine-2-carboxylate esters by Swern oxidation of aziridines or dehydrohalogentation of *N*-chloroaziridines.

The Davis group developed a route to highly enantiopure 2*H*-azirines that involves elimination of sulfenic acid from chiral, enantiopure *N*-sulfinylaziridine-2-carboxylate esters.[19] Treatment of Andersen's reagent (−)-**3.28** ((1*R*,2*S*,5*R*)-(−)-menthyl (*S*)-*p*-toluenesulfinate) with LiHMDS followed by benzaldehyde gave *N*-*p*-toluenesulfinimine **3.30** (Scheme 3.6). Reaction of **3.30** with the (*E*)-enolate of methyl bromoacetate gave *N*-*p*-toluenesulfinylaziridine **3.31**. Elimination of sulfinic acid with LDA then gave the desired 2*H*-azirine-2-carboxylate ester **3.32** in moderate yield (42%, *ee* ≥ 95%). The addition of MeI in the elimination step improved the yield of **3.32** slightly and allowed the isolation of a high yield of *p*-TolylS(O)Me, presumably formed by the trapping of eliminated sulfinic acid by MeI. No trace of the isomeric 2*H*-azirine-3-carboxylate ester was observed.



Scheme 3.6 Davis' synthesis of optically pure 2*H*-azirine-2-carboxylate esters by elimination of sulfinic acid from *N*-sulfinylaziridine-2-carboxylate esters.

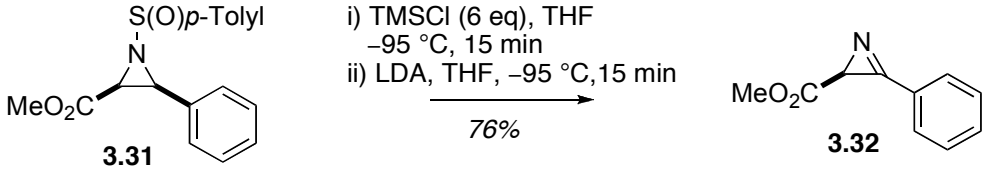
The apparent selectivity for deprotonation of the less acidic C3 proton of aziridine **3.31** was initially puzzling. The high isolated yield of *p*-TolylS(O)Me, however, suggested that sulfinic acid was being eliminated by deprotonation at both C2 and C3. The authors speculated that deprotonation at C2 of **3.31** led to an unstable intermediate that decomposed to form polar, oligomeric material leaving **3.32** as the only observed 2*H*-azirine product. This hypothesis was supported by the observation that treatment of *N*-*p*-toluenesulfinylaziridines of type **3.33**, in which there is no C2 proton, led to formation of 2*H*-azirines **3.34** in good yield (Scheme 3.7).



Scheme 3.7 Formation of 2*H*-azirine-2-carboxylate esters by elimination of sulfinic acid from *N*-sulfinylaziridine-2-carboxylate esters that lack a C2 proton.

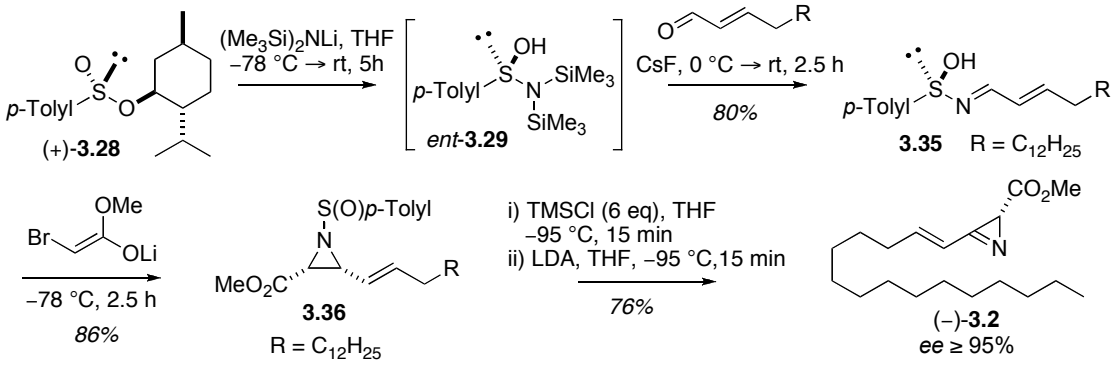
The Davis group eventually found that addition of TMSCl to *N*-*p*-toluenesulfinylaziridine **3.31** prior to addition of LDA dramatically improved the

yield of 2*H*-azirine formation, but only at −95 ˚C (Scheme 3.8). No difference in yield was observed with or without TMSCl at −78 ˚C.



Scheme 3.8 Improved procedure for formation of 2*H*-azirine-2-carboxylate esters by elimination of sulfinic acid from *N*-sulfinylaziridine-2-carboxylate esters.

This improved procedure was applied to the first total synthesis of 2*R*-(*E*)-dysidazirine (−)-**3.2**. Starting with (+)-**3.28** *N*-*p*-toluenesulfinimine **3.35** was obtained in good yield (Scheme 3.9). Aziridine formation proceeded smoothly to give **3.36** which was treated sequentially with TMSCl and LDA to furnish synthetic (−)-**3.2**. NMR studies with the chiral shift reagent Eu(hfc)$_3$ showed that (−)-**3.2** was formed with *ee* ≥ 95%.

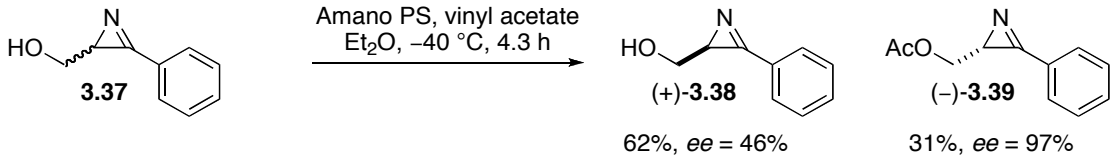


Scheme 3.9 Davis' total synthesis of (−)-(*E*)-dysidazirine.

Interestingly, the specific rotation for synthetic (−)-**3.2** (−186.4) was significantly higher than that observed for the natural product (−165),

suggesting that natural (−)-**3.2** was originally isolated with an optical purity of ~89%.

Finally, enantioenriched 2*H*-azirines have been prepared by enzymatic kinetic resolution. Sakai *et al*. demonstrated that (±)-phenyl-2*H*-azirine-2-methanol can be successfully resolved using the commercially available lipase Amano PS at low temperatures in ether (Scheme 3.10).[20] At the time this was the first report of a low temperature (<0 ˚C) enzymatic resolution.



Scheme 3.10 Enzymatic resolution of (±)-phenyl-2*H*-azirine-2-methanol.

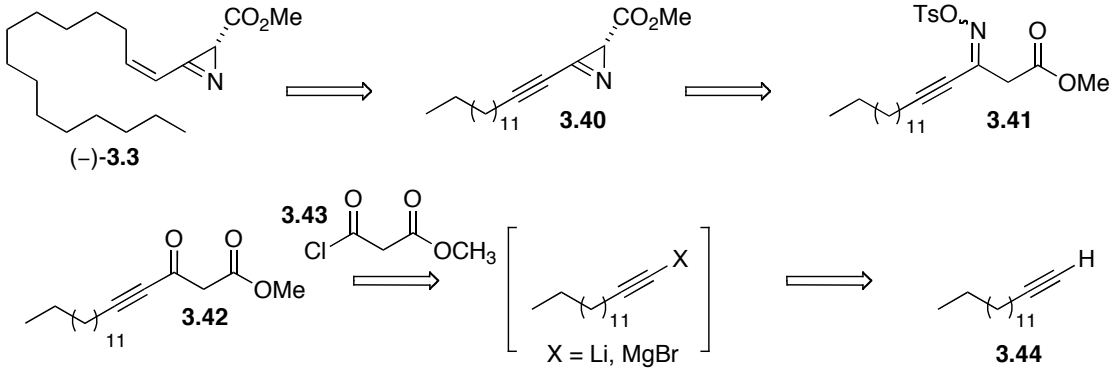
3.4 Total Synthesis of (−)-(*Z*)-dysidazirine

At the time of isolation (2006) of there was no clear explanation for the lack of antifungal activity exhibited by (+)-**3.6**, (+)-**3.7** and (−)-**3.8** compared with (*E*)-dysidazirine. Faulkner's report that (*Z*)-dysidazirine displayed no antimicrobial activity was also puzzling.[4] We thus set out to define the important structural features for dysidazirine antifungal activity. In the first step toward this goal, a flexible synthetic route toward (−)-**3.3** was designed for which no previous synthesis had been reported.

In designing a synthesis of (−)-**3.3** the critical factor was choice of an appropriate asymmetric azirine-forming reaction. Davis' approach toward (−)-**3.2** was clearly successful,[19] however the difficulty of adapting this method to

the required (*Z*) geometry of the C4-C5 olefin dictated choice of an alternative method. In the end Zwanenburg's quinidine-mediated cyclization was selected for it's mild conditions and ease of implementation and likelihood of approximating the natural optical enrichment.[16]

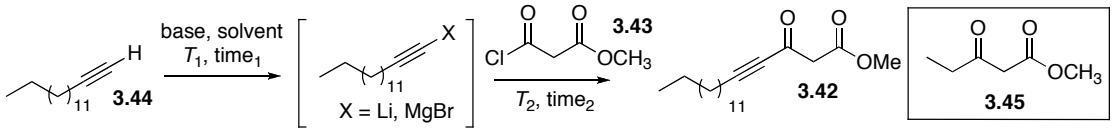
It was envisaged that the (*Z*)-olefin could be introduced at the final step by selective reduction of the corresponding alkyne **3.40**. Disconnection of the 2*H*-azirine ring leads to oxime tosylate **3.41** which in turn could be obtained from keto-ester **3.42**. Finally, addition of acetylene **3.44** to methyl malonyl chloride would provide **3.42**.



Scheme 3.11 Restrosynthetic analysis of (−)-(*Z*)-dysidazirine

The addition of metallated pentadecyne **3.44** to methyl malonyl chloride **3.43** was accomplished most effectively when the deprotonation step was carried out using EtMgBr (Table 3.4) providing **3.42** in good yield (70%). When the deprotonation step was carried out with *n*-BuLi low and variable yields of **3.42** were obtained. This is presumably due to the lowered basicity of the Grignard reagent or aggregation of the corresponding lithium acetylide.

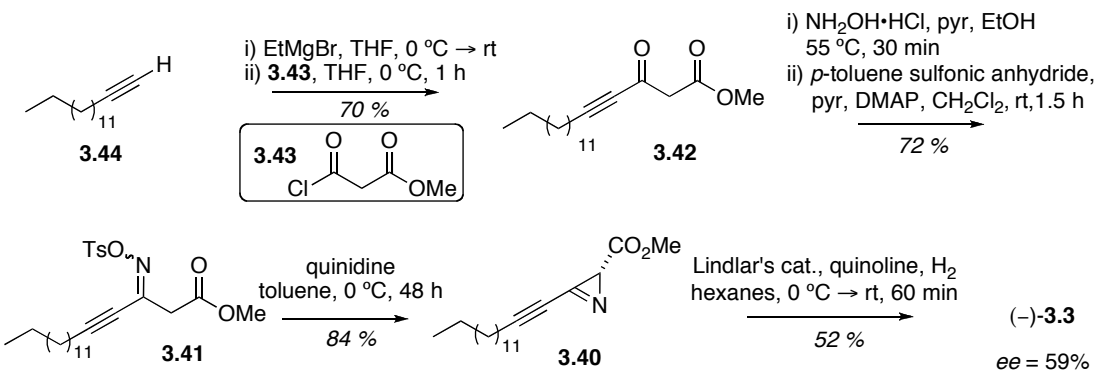
Table 3.4 Optimization of addition of pentadecyne to methyl malonyl chloride.



Entry	Base	Solvent	T_1 (˚C)	time$_1$ (min)	eq. 3.43	T_2 (˚C)	time$_2$ (min)	Yield (%)
1	*n*-BuLi	THF	0	90	0.5	−78 → 0	150	0[a]
2	*n*-BuLi	Et_2O	−20	60	0.5	−60 → 0	120	14[a]
3	EtMgBr	Et_2O	0 → r.t.	80	0.5	0	40	0[b]
4	EtMgBr	THF	0 → r.t.	120	0.8	0	50	19
5	EtMgBr	THF	0 → r.t.	150	0.5	0	60	70

[a] Estimated from crude NMR. [b] Compound **3.45** was isolated in 35% yield.

Conversion of keto-ester **3.42** to the corresponding oxime tosylate was accomplished in two steps without purification of the intermediate oxime to provide **3.41** as a mixture of isomers (Scheme 3.12). The key cyclization proceeded smoothly in the presence of a stoichiometric quantity of quinidine to give the desired 2*H*-azirine **3.40** in 84% yield. The final step required careful monitoring to avoid over-reduction of the azirine ring but provided (*Z*)-dysidazirine in reasonable yield. Analysis of (−)-**3.3** by chiral HPLC (Chiralpak AD, 90:10 hexanes/*i*-PrOH) revealed *ee*=59%, comparable to that observed for natural long-chain 2*H*-azirines.



Scheme 3.12 Total synthesis of 2*R*-(*E*)-dysidazirine

3.5 Synthesis of Dysidazirine Analogues

To examine the relative importance of terminal substitution and conjugate unsaturation on antifungal activity a series of analogues were prepared (Figure 3.6), including (−)-**3.2** (to examine the effect of olefin geometry), (−)-**3.40** (olefin→alkyne), (−)-**3.46** (terminal substitution), (−)-**3.47** (removal of olefin) and (−)-**3.48** and (−)-**3.49 (**chain length).

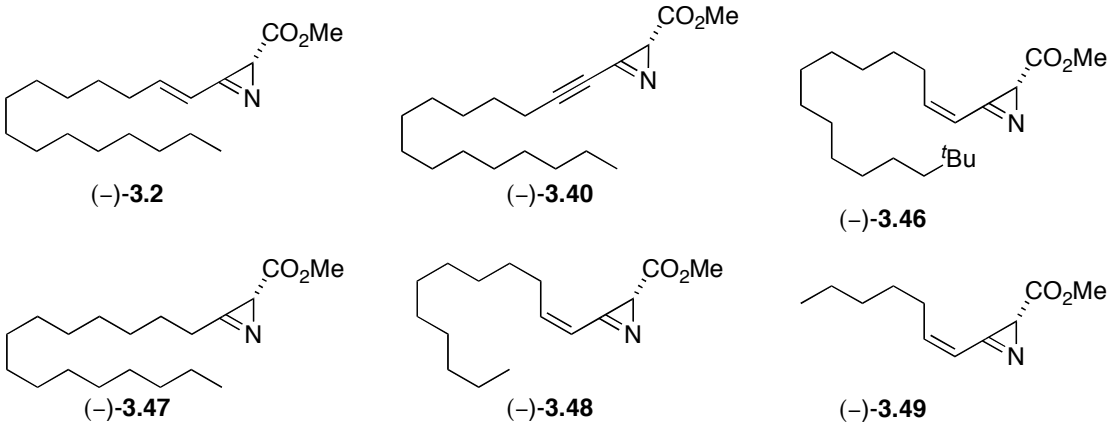
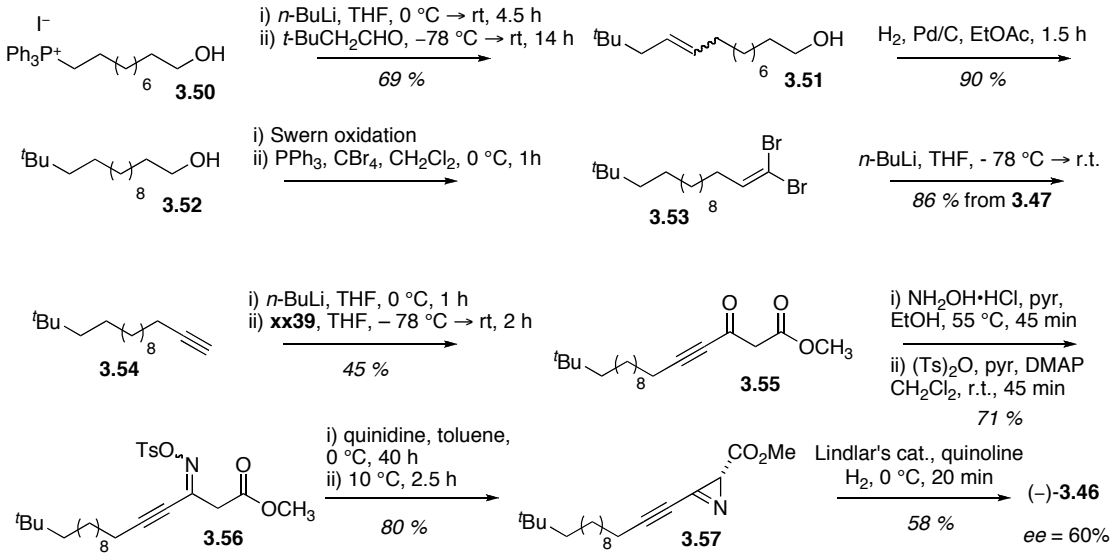


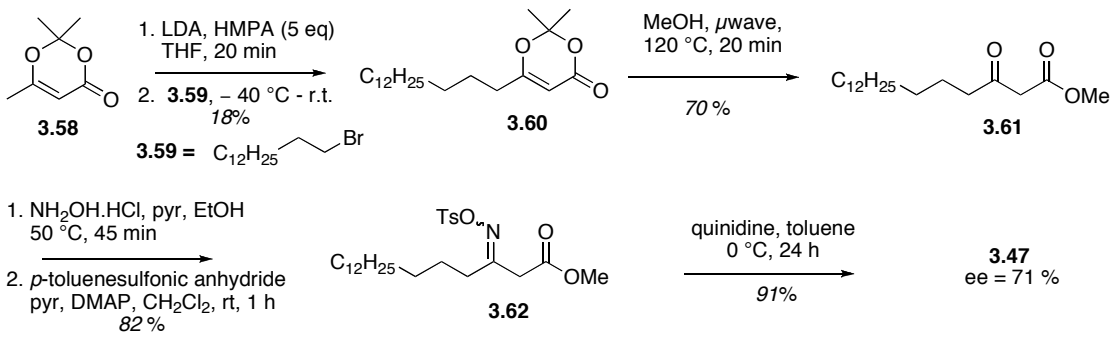
Figure 3.6 Targeted Long-Chain 2*H*-azirine Analogues

Preparation of (−)-**3.46**, with a terminal *tert*-butyl group, began with Wittig reaction between phosphonium iodide **3.50** and 3,3-dimethylbutanal to give alkenol **3.51** as a mixture of double bond isomers. Hydrogenation gave saturated alcohol **3.52** which was oxidized to the corresponding aldehyde under Swern conditions and submitted directly to Corey-Fuchs homologation[21] providing vinyl dibromide **3.52.** Treating **3.53** with 2 equivalents of *n*-BuLi furnished the desired alkyne **3.54**. In this case, addition of the lithio-alkyne derived from **3.54** to methyl malonyl chloride provided keto-ester **3.55** in reasonable yield (45%). Conversion of the ketone to the oxime tosylate once again proceeded in good yield (71%) over 2 steps to give **3.56**. Quinidine-mediated cyclization furnished the 2*H*-azirine **3.57** (80%) which was reduced carefully with Lindlar's catalyst yielding analogue (−)-**3.46** (58%, *ee* = 60%).



Scheme 3.13 Synthesis of analogue (−)-**3.46**

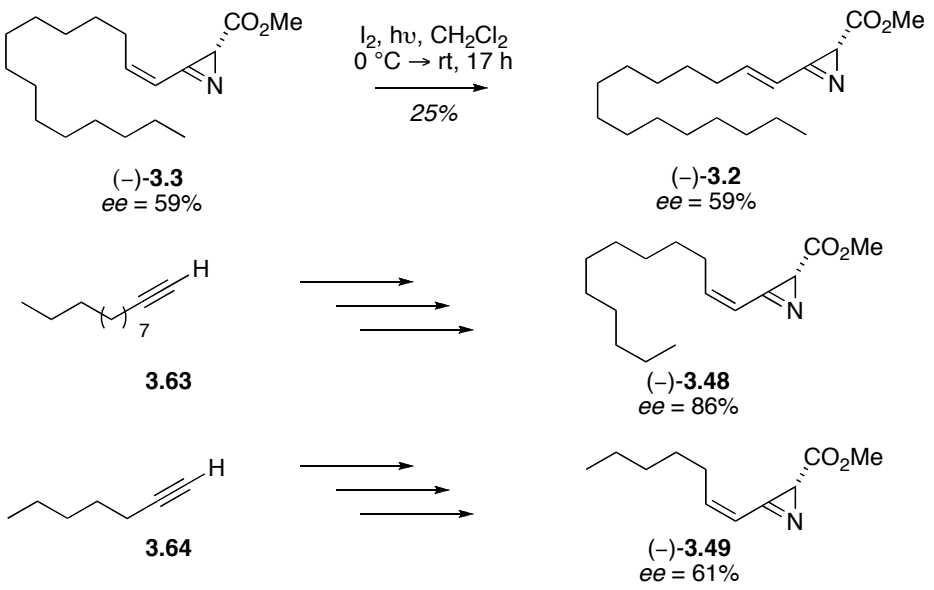
Model **3.47**, which lacks the C4-C5 olefin of dysidazirine, was synthesized starting from dioxinone **3.58**. Deprotonation of **3.58** with LDA to give the extended enolate followed by alkylation with 1-bromotetradecane in the presence of HMPA yielded the desired product **3.60** (18%) accompanied by an approximately equal amount of the product derived from alkylation at C2 of **3.58**. Thermolysis of **3.60** gave keto-ester **3.61** which was converted to oxime-tosylate **3.62** and then 2*H*-azirine (−)-**3.47** as described previously.



Scheme 3.14 Synthesis of long-chain 2*H*-azirine analogue (−)-**3.47**

(−)-(*E*)-dysidazirine was prepared by isomerization of 2*R*-(*Z*)-dysidazirine under photochemical conditions (Scheme 3.15). Using I_2 as a photosensitizer (−)-**3.3** was irradiated with a 275W Sunlamp for a total of 17 hours providing a 25% isolated yield of (−)-**3.2**. The disappointing yield is most likely ascribed to decomposition of dysidazirine under these conditions, suggested by the presence of a significant amount of unidentified polar by-products in the crude reaction mixture. Notably, the enantiomeric excess of synthetic (−)-**3.2** was found to be 59%, identical to the starting material, (−)-**3.2.** This underscores the fact that conjugated 2*H*-azirines do not epimerize photochemically to any measurable extent.

The final two analogues, (−)-**3.48** and (−)-**3.49** were synthesized from the appropriate alkynes (**3.63** and **3.64** respectively) using the same sequence as described for the preparation of (−)-**3.2** (see Experimental Section for details)**.**



Scheme 3.15 Synthesis of (−)-**3.2** and analogues (−)-**3.48** and (−)-**3.49**

3.6 Antifungal Activity of (−)-(*Z*)-dysidazirine and Analogues

(−)-**3.3**, (±)-**3.3**, (−)-**3.2,** (−)-**3.40** and (−)-**3.46-49** were tested against a panel of clinically-relevant human fungal pathogens including *Candida albicans* (ATCC, UCD-FR1 and 96-489), *Candida glabrata*, *Candida krusei*, *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *gattii* (Figure 3.7). Of these species, *C. glabrata*, *C. krusei* and *C. albicans* UCD-FR1 and 96-489 are resistant to fluconazole, one of the most widely prescribed antifungal drugs. The results are displayed using MIC_{50}, the concentration (in μg/mL) required to inhibit the growth of 50% of the cells tested in a broth micro-dilution assay (see Figures 3.7, 3.8 and 3.9).[22]

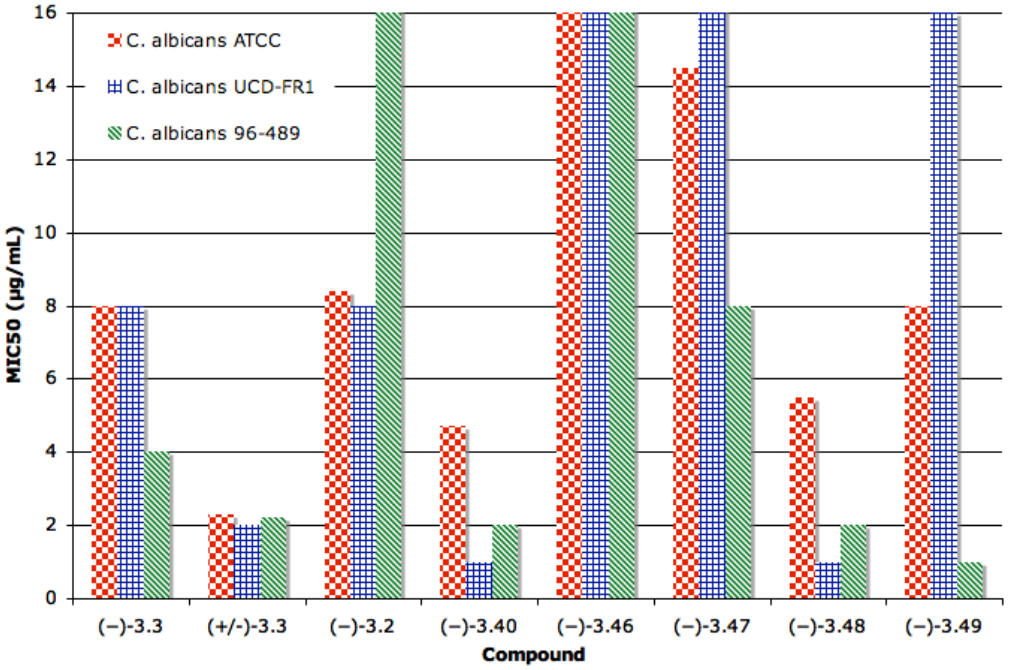


Figure 3.7 Antifungal data for synthetic long-chain 2*H*-azirine carboxylate esters against *C. albicans* ATCC, *C. albicans* UCD-FR1 and *C. albicans* 96-489.

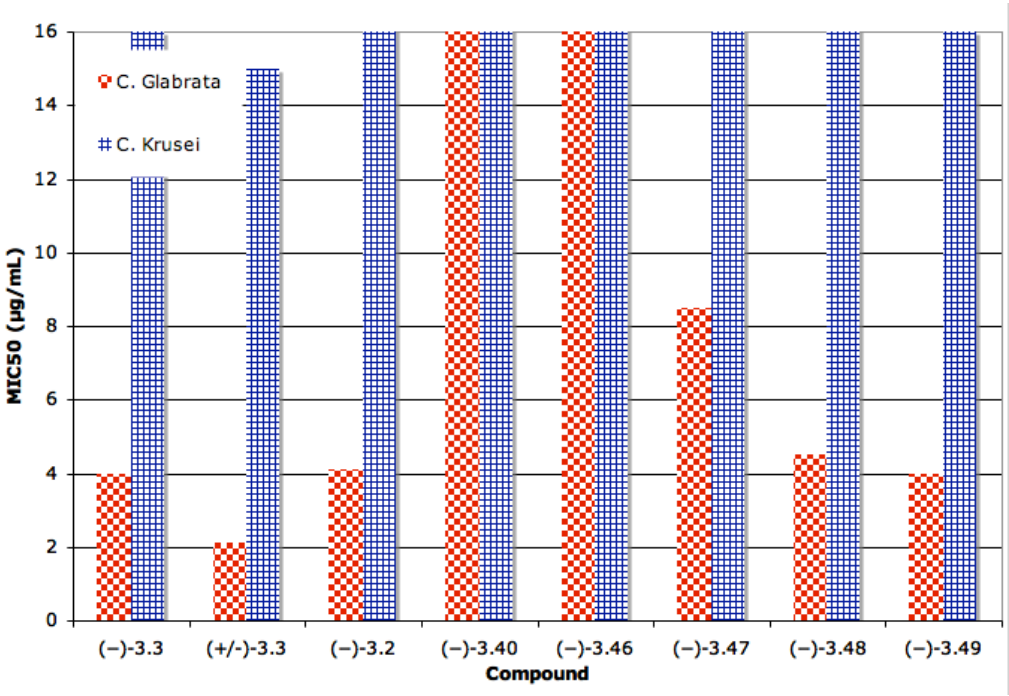


Figure 3.8 Antifungal data for synthetic long-chain 2*H*-azirine carboxylate esters against *C. glabrata* and *C. krusei*.

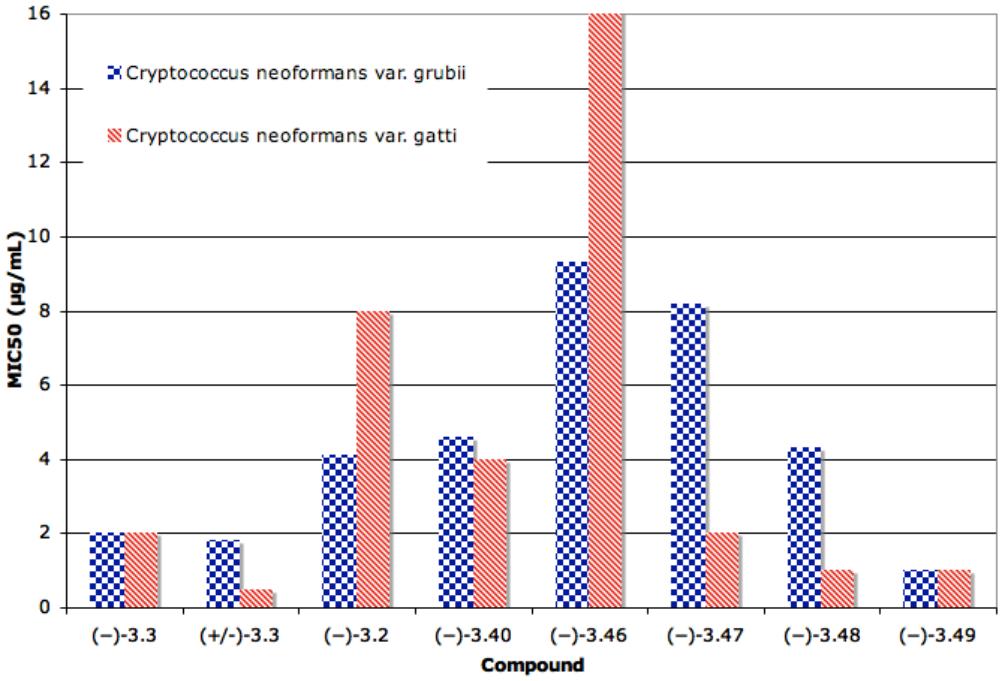


Figure 3.9 Antifungal data for synthetic long-chain 2*H*-azirine carboxylate esters against *Cryptococcus neoformans* var. grubii and gatti.

For comparison purposes the widely-used antifungal drugs amphotericin B and fluconazole typically display MIC values of ~0.1-0.3 μg/mL against susceptible *Candida* strains. By comparison, the MICs for 'resistant' strains such as *C. glabrata* and *C. krusei* are much higher (MIC 16-32 μg/mL).

The above data confirms that the dysidazirines have potent fungistatic activity as originally described by Molinski and Ireland. Interestingly, *C. krusei* is the only strain that shows broad resistance to dysidazirine and its analogues (Figure 3.8) while the other fluconazole resistant strains (*C. glabrata*, *C. albicans* UCSD-FR1 and 96-489) are susceptible. The two strains of

Cryptococcus neoformans (gatti and grubii) are overall the most susceptible to these compounds.

Compounds (−)-**3.3** and (−)-**3.2** were generally the most active compounds against each cell type. While (−)-**3.3** and (−)-**3.2** displayed comparable activity against *C. albicans* ATCC and UCD-FR1, *C. glabrata* and *C. krusei*, (−)-**3.3** was more active against *C. albicans* 96-489 and *Cryptococcus neoformans* var. gattii and grubii. Thus, a *Z*-olefin confers a small increase in anti-fungal activity over the corresponding *E*-olefin.

It has previously been suggested that configuration at C2 is crucial for antifungal activity of long-chain 2*H*-azirine-2-carboxylate esters given Faulkner's report that natural (+)-**3.2** was inactive against a panel of microorganisms. In these studies, however, (±)-**3.3** showed comparable activity to its optically enriched counterpart (−)-**3.3.** Against all three strains of *C. albicans* (±)-**3.3** is slightly *more* active. Thus it seems that C2 configuration is not in fact crucial, although without a genuine sample it is not possible to comment on the specific activity of (+)-**3.2**.

Remarkably (−)-**3.46**, with the *tert*-butyl chain terminus, is almost completely inactive. Terminal substitution, even with simple methyl groups, severely abrogates antifungal activity of long-chain 2*H*-azirine-2-carboxylate esters. Activity is maintained for the unbranched alkyne analogue (−)-**3.40** but decreases markedly for the saturated compound (−)-**3.47**, which lacks the C4-C5 olefin. In contrast, there is no clear dependence of activity upon chain

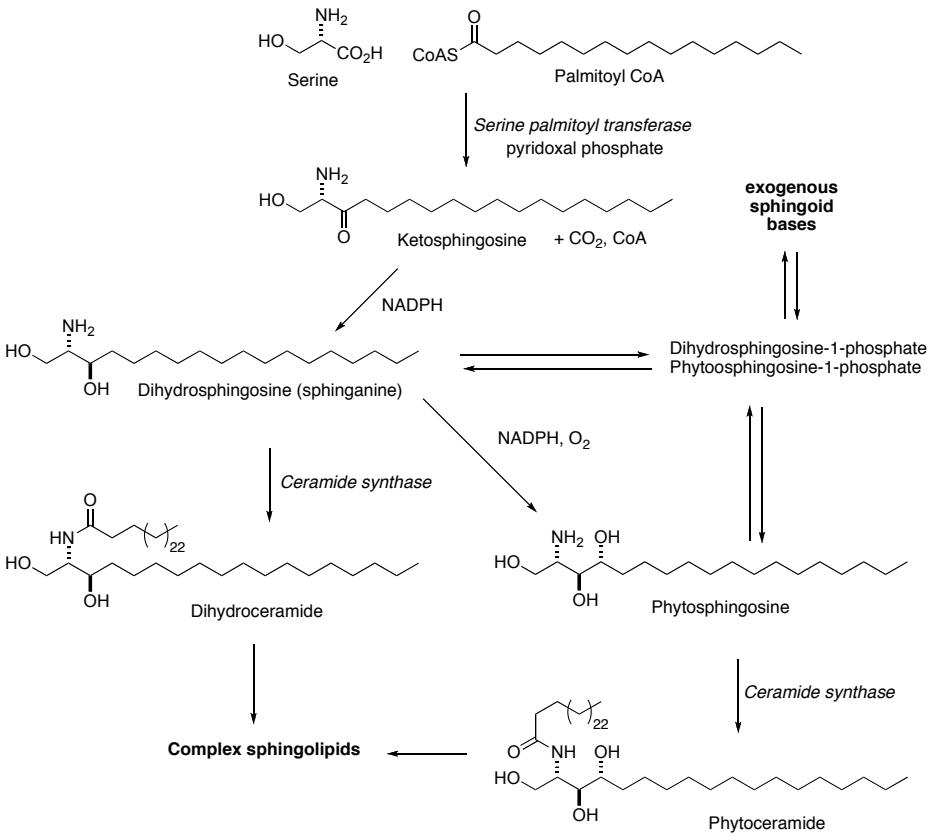
length as compounds (−)-**3.48** and (−)-**3.49** display activity comparable to (−)-**3.2.**

Given the well-documented reactivity of the 2*H*-azirine ring toward nucleophiles and electrophiles it is likely that α,β-unsaturated azirines (such as dysidazirine) may be potent Michael acceptors. At first glance the activity observed for (−)-**3.2** and (−)-**3.3** might be interpreted as a result of non-specific toxicity associated with alkylation of fungal cell proteins, however the present data suggests a more subtle mechanism of action. The binding pocket of the as yet unidentified target protein must accommodate both the 2*H*-azirine terminus and the lipid chain. Binding of the latter is subject to tight steric restraints as terminal branching (as in **3.41**) diminishes activity. The requirement for C4-C5 unsaturation for optimal activity suggests that the α,β-unsaturated azirine ring does function as an electrophilic Michael acceptor, possibly binding the target protein irreversibly. The enhanced activity of (−)-**3.3** (with a *Z*-olefin) over (−)-**3.2** also seems to support this hypothesis. Taken together, these considerations point to a 2-point binding motif wherein the lipid chain binds in tight a hydrophobic pocket while the 2*H*-azirine terminus binds (and possibly reacts) at a distal site.

As Molinski and Ireland noted, (−)-**3.2** and (−)-**3.3** share the same chain length (C18) as sphingosine.[3] It has been well documented that many sphingosine-like marine natural products display anti-fungal activity.[23] In fact, sphingosine itself has antifungal activity (MIC_{50} 30 μg/mL, *C. glabrata*).[24]

Sphingosine is a 2-amino-1,3-diol that forms the backbone of many sphingolipids common to mammalian cells such as ceramides and cerebrosides. Phytosphingosine and dihydrosphingosine (sphinganine) are the fungal cell homologues with phytosphingosine by far the most abundant. Sphingolipids function as components of the fungal cell wall but also play a role as signaling molecules. Studies with *Saccharomyces cerevisiae* have shown that phytosphingosine activates certain kinases of the AGC family that help control cell growth, stress resistance and cell wall integrity.[25]

Yeast produce phytosphingosine *via* a *de novo* biosynthetic pathway (summarized in Scheme 3.16). The first step of the pathway involves decarboxylative condensation of serine with palmitoyl CoA catalyzed by serine-palmitoyl transferase. The reaction requires pyridoxal phosphate as co-factor. The resulting ketosphingosine is then reduced by an NADPH-dependant reductase to give dihydrosphingosine (sphinganine). Acylation of the amine with a C26 fatty acyl chain then gives dihydroceramide. Alternatively, oxidation of dihydrosphingosine gives phytosphingosine, which can also be acylated to give phytoceramide. This entire process prior to formation of ceramide takes place in the endoplasmic reticulum. The products are shuttled to the Golgi apparatus where they are further modified to produce more complex sphingolipids.



Scheme 3.16 The *de novo* synthetic pathway by which yest produce dihydrosphingosine, phytosphingosine and ceramides.

The structural similarity between several antifungal long-chain amino alcohols of marine origin and the sphingoid bases described above is striking. Searle and Molinski described the isolation of three isomeric compounds **3.67-69** from an ascidian (*Didemnum* sp., collected from the Great Barrier Reef) in 1992.[26] Compounds **3.67-69** (trifluoroacetate salt form) showed moderate activity against *C. albicans* (9 mm zone of inhibition at 50 μg/disk in a disk diffusion assay), while the free base of **3.67** was slightly more active (11 mm at 50 μg/disk). Scheuer *et al.* reported the isolation of diastereomeric amino alcohols **3.70** and **3.71** from the sponge *Xestospongia* sp. collected in Papua

New Guinea.[27] These compounds proved difficult to separate by HPLC, however 19 μg of a mixture of **3.70** and **3.71** gave rise to an 8 mm zone of inhibition in a disk diffusion assay against *C. albicans*. Crucigasterins 277 and 275 (**3.72** and **3.73**), this time from a Mediterranean tunicate *Pseudodistoma crucigaster*, were isolated by the group of Rinehart.[28] These highly unsaturated amino alcohols proved unstable in concentrated form, but were potently antifungal in the disk diffusion assay: 9 mm (**3.72**) and 12 mm (**3.73**) zones of inhibition against *S. cerevisiae* at 5 μg/disk.

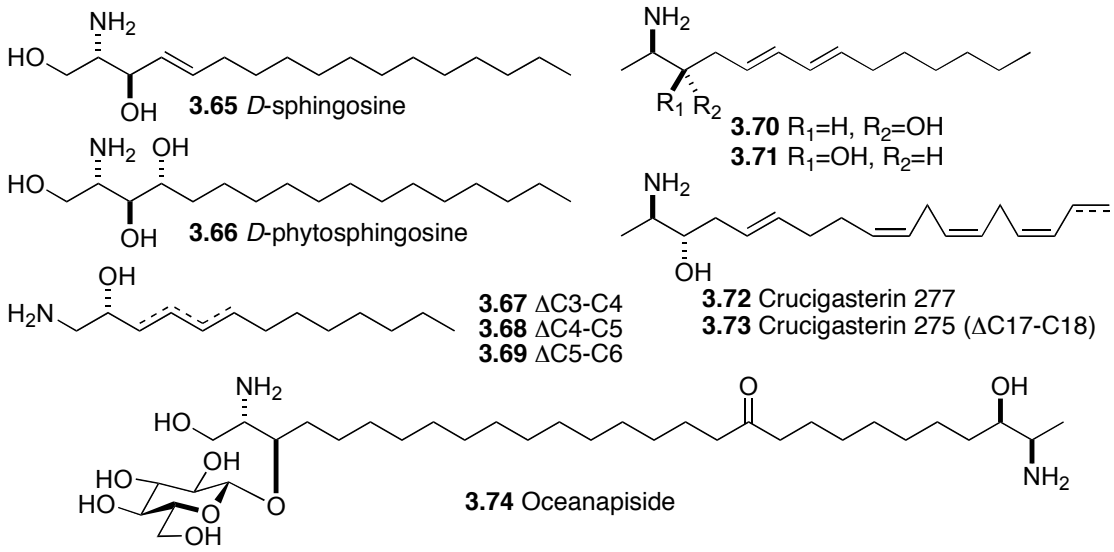


Figure 3.10 Structures of sphingosine, phytosphingosine and several antifungal long-chain amino-aclohols isolated from marine sponges.

More recently, Molinski *et al.* isolated the dimeric glycosphingolipid oceanapiside A (**3.74**) from the sponge *Oceanapia phillipensis*.[29] This compound exhibited selective activity against the Fluconazole-resistant *C.*

glabrata (MIC 10 μg/mL). Interestingly, the aglycone of **3.74** was even more active (MIC 3 μg/mL). Oceanapiside was inactive against *C. albicans* and *C. krusei*. There is strong evidence to suggest that oceanapiside (**3.74**) is an inhibitor of sphingolipid biosynthesis in *C. glabrata* leading to arrest of cell growth.[30] This is a significant finding because no currently used antifungal drug acts by this mechanism. It is possible that (*E*)- and (*Z*)-dysidazirine also act as 'anti-metabolites' and disrupt sphingolipid metabolism, however further studies at the molecular biology level are required to validate this hypothesis.

3.7 Conclusion

In summary, the total synthesis of (−)-(*Z*)-dysidazirine ((−)-**3.3**) was completed in five steps. The flexible synthetic approach allowed for the preparation of a group of analogues, each of which represented an alteration to a specific functional group in the parent dysidazirine structure: the lipid chain terminus (compound (−)-**3.46**), the lipid chain length ((−)-**3.48** and (−)-**3.49**) and the C4-C5 olefin ((−)-**3.2**, (−)-**3.40** and (−)-**3.47**). All synthetic compounds were tested against a panel of clinically-relevant fungal pathogens. The data clearly shows that both (−)-**3.2** and (−)-**3.3** are potently antifungal against essentially all cells tested. These results are in line with the reported activity of natural (−)-(*E*)-dysidazirine. The following generalizations regarding antifungal activity of long chain 2*H*-azirines can be made:

1) Substitution and/or branching at the lipid chain terminus diminishes activity.

2) A C4-C5 olefin or alkyne is required for high activity. (−)-(*Z*)-dysidazirine is slightly more active than (−)-(*E*)-dysidazirine, supporting the hypothesis that the conjugated 2*H*-azirine acts as a Michael acceptor.
3) Chain length does not greatly affect activity, however the dysidazirines (C18) were generally the most active compounds tested.
4) Configuration at C2 does not have a significant effect on activity, although (±)-**3.3** was slightly more active than (−)-**3.3** against some cells.

Chapter 3 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592-2597. The dissertation author was the primary researcher/author on this paper.

Chapter 3 is, in part, a reproduction of the material as it appears in the following publication: Skepper, C. K.; Dalisay, D. S.; Molinski, T. F. "Synthesis and Antifungal Activity of (–)-*Z*-Dysidazirine", *Org. Lett.* **2008**, *10*, 5269-5271. The dissertation author was the primary researcher/author on this paper.

Chapter 3 is, in part, currently being prepared for submission for publication of the material. Skepper, C. K.; Dalisay, D. S.; Molinski, T. F. "Antifungal Structure-Activity-Relationships of Long Chain 2*H*-Azirine Carboxylates". The dissertation author was the primary researcher/author of this material.

3.8 Experimental Section

Isolation of 3.4-3.8

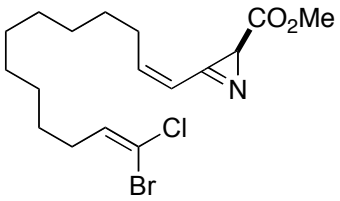
Animal Material. *Dysidea fragilis* (01-18-154) was collected using scuba at Arrow Wall, Pohnpei, Federated States of Micronesia in September 2001. The sample was frozen (652.3 g wet weight) and stored at −20 ˚C until extraction. A voucher sample of the sponge is kept at UC San Diego.

Extraction and Isolation. Freeze dried sponge (182.5 g dry weight) was soaked in MeOH (1.2 L) for 4 hours. The methanol extract was filtered off, fresh MeOH (1.2 L) added and the sponge allowed to soak overnight. The combined MeOH extracts were concentrated under reduced pressure to ~500 mL. Water (75 mL) was added, and the aqueous mixture was partitioned against hexanes (500 mL). The aqueous MeOH layer was separated and diluted with a further 75 mL of water, then partitioned against $CHCl_3$ (500 mL). After separation the aqueous MeOH layer was diluted with water (100 mL) and re-extracted with $CHCl_2$. *n*-BuOH (20 mL) was added to the aqueous MeOH layer, and the MeOH was removed under reduced pressure. The remaining aqueous layer was partitioned twice against *n*-butanol (250 mL).

The hexanes-soluble fraction was evaporated to dryness giving a black oil (2.24 g). A portion of this fraction (1.67 g) was subjected to gradient flash chromatography (10–100% EtOAc/hexanes). The second collected fraction eluting at 1:9 EtOAc/hexanes (468 mg, orange oil) was purified by passage through a reversed phase cartridge (C_{18}, 20g, 5:95 H_2O/MeOH) to give a

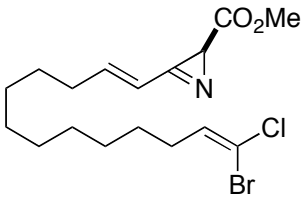
mixture of compounds (+)-**3.6**, (+)-**3.7**, (−)-**3.8**, (*E*)-antazirine ((+)-**3.4**) and (*Z*)-antazirine ((+)-**3.5**).[4] This mixture was subjected to silica HPLC (Dynamax Microsorb, 1:19 EtOAc/hexanes, 15 mL/min, λ 254 nm) yielding three fractions. Reversed phase HPLC separation (Dynamax Microsorb C18, 17.5:82.5 H_2O/MeOH, 15 mL/min, λ 254 nm) of the three fractions yielded (+)-**3.4** (22 mg, 0.016%), (+)-**3.5** (136 mg, 0.10 %), (+)-**3.6** (50 mg, 0.037%), (+)-**3.7** (24 mg, 0.018%) and (−)-**3.8** (27 mg, 0.02%), each as a clear, colorless oil.

(+)-3.6



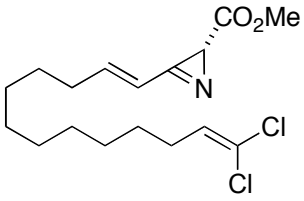
$[\alpha]^{24}$ +96.9 (*c* 1.09, *n*-hexane); UV (*n*-hexane) 210 nm (ε 11 767); IR (thin film) ν_{max} 2925, 2855, 1759, 1732, 1611, 1433, 1336, 1200, 1180, 1025, 846, 799 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.55 (dt, 1H, *J* = 10.5, 8.0 Hz, H5), 6.41 (dt, 1H, *J* = 10.5, 1.2 Hz, H4) 6.06 (t, 1H, *J* = 7.5 Hz, H15), 2.71 (s, 3H, OMe), 2.61 (s, 1H, H2), 2.48 (m, 2H, H6), 2.09 (q, 2H, *J* = 7.5 Hz, H14), 1.43-1.35 (br m, 2H), 1.25 (br s, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 172.0 (C, C1), 154.0 (C, C3), 152.4 (CH, C5), 132.8 (CH, C15), 111.1 (CH, C4), 106.2 (C, C16), 52.3 (CH_3, OMe), 31.9 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.38 (CH_2), 29.35 (CH_2), 29.32 (CH, C2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.0 (CH_2); LRESIMS *m/z* 392.04 $[M+H]^+$; HREIMS *m/z* 389.0754 $[M]^+$ (calc. for $C_{17}H_{25}BrClNO_2$, 389.0752).

(+)-3.7



$[\alpha]^{24}$ +8.9 (*c* 0.51, *n*-hexane); UV (*n*-hexane) 210 nm (ε 15 660); IR (thin film) ν_{max} 2925, 2846, 1767, 1728, 1468, 1433, 1336, 1262, 1200, 1184 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (dt, 1H, *J* = 15.4, 6.8 Hz, H5), 6.53 (dt, 1H, *J* = 15.4, 1.4 Hz Hz, H4), 6.06 (t, 1H, *J* = 7.3 Hz, H15), 2.71 (s, 3H, OMe), 2.56 (s, 1H, H2), 2.35 (br q, 2H, *J* = 6.8 Hz, H6), 2.10 (q, 2H, *J* = 7.3 Hz, H14), 1.53-1.46 (br m, 2H), 1.42-1.35 (br m, 2H), 1.26 (br s, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1 (C, C1), 156.6 (C, C3), 155.6 (CH, C5), 132.9 (CH, C15), 112.9 (CH, C4), 106.3 (C, C16), 52.2 (CH_3, OMe), 32.2 (CH_2), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.3 (CH, C2), 27.9 (CH_2), 27.8 (CH_2); LRESIMS *m/z* 392.01 $[M+H]^+$; HREIMS *m/z* 389.0755 $[M]^+$ (calc. for $C_{17}H_{25}BrClNO_2$, 389.0752).

(−)-3.8



$[\alpha]^{24}$ −4.1 (*c* 0.62, *n*-hexane); UV (*n*-hexane) 218 nm (ε 14 235); IR (thin film) ν_{max} 2925, 2846, 1774, 1735, 1623, 1434, 1341, 1264, 1200, 1035, 972, 875, 793, 725 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (dt, 1H, *J* = 15.3, 7.0 Hz, H5), 6.53 (dt, 1H, *J* = 15.3, 1.5 Hz, H4), 5.83 (t, 1H, *J* = 7.4 Hz, H15), 2.71 (s, 3H, OMe), 2.56 (s, 1H, H2), 2.35 (br q, 2H, *J* = 7.0 Hz, H6), 2.14 (q, 2H, *J* = 7.4 Hz, H14), 1.52-1.46 (br m, 2H), 1.41-1.35 (br m, 2H), 1.27 (br s, 10H); ^{13}C

NMR (100 MHz, $CDCl_3$) δ 172.2 (C, C1), 156.6 (C, C3), 155.7 (CH, C5), 130.0 (CH, C15), 119.7 (C, C16), 112.9 (CH, C4), 52.2 (CH_3, OMe), 32.2 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.24 (CH_2), 29.22 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 28.3 (CH, C2), 28.1 (CH_2), 27.8 (CH_2); HREIMS *m/z* 345.1255 $[M]^+$ (calc. for $C_{17}H_{25}Cl_2NO_2$, 345.1257).

(+)-(*E*)-(*S*)-antazirine ((+)-3.4) $[\alpha]^{24}$ +16.7 (*c* 0.58, *n*-hexane), lit. +10.3 (*c* 0.39, $CHCl_3$);[4] LRESIMS *m/z* 435.98 $[M+H]^+$. See Table 3.1 for enantiomeric composition.

(+)-(*Z*)-(*S*)-antazirine ((+)-3.5) $[\alpha]^{24}$ +98.9 (*c* 2.33, *n*-hexane); LRESIMS *m/z* 435.99 $[M+H]^+$. See Table 3.1 for enantiomeric composition.

Preparation of 1-bromo-1-chloro heptenes (3.12 and 3.13)

Compounds **3.12** and **3.13** were prepared according to the method of Masuda et al.[9] 1-Heptyne was halogenated (NBS/$AgNO_3$) to give 1-bromoheptyne (**3.9**), or treated with *n*-BuLi/NCS to give 1-chloroheptyne (**3.10**). Alkynes **3.9** and **3.10** were then subjected to hydroboration with di-*sec*-butylborane followed by halogenation with either CuCl or $CuBr_2$ to give **3.12** and **3.13**, respectively. The products were separately purified after extractive work up with pentane by HPLC (silica, pentane, 3 mL/min, followed by C_{18} reversed phase HPLC, MeOH, 2.5 mL/min, refractive index detection).

(*Z*)-1-bromo-1-chloro-1-heptene (**3.12**)

IR (neat) ν 2917, 2851, 1476, 1464, 866, 726 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.07 (t, 1H, 7.2 Hz), 2.10 (q, 2H, *J* = 7.2 Hz), 1.40 (m, 2H), 1.34-1.25 (m, 4H), 0.88 (t, 3H, 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.0 (CH), 106.2 (C), 31.9 (CH_2), 31.2 (CH_2), 27.7 (CH_2), 22.4 (CH_2), 14.0 (CH_3); HREIMS *m/z* 209.9808 $[M]^+$ (calc. for $C_7H_{12}BrCl$, 209.9805).

(*E*)-1-bromo-1-chloro-1-heptene (**3.13**)

IR (neat) ν 2958, 2933, 2859, 1608, 1456, 827 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.07 (t, 1H, *J* = 7.4 Hz), 2.12 (q, 2H, *J* = 7.4 Hz), 1.39 (m, 2H), 1.35-1.24 (m, 4H), 0.88 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.2 (CH), 102.7 (C), 31.2 (CH_2), 30.5 (CH_2), 27.7 (CH_2), 22.4 (CH_2), 14.0 (CH_3); HREIMS *m/z* 209.9805 $[M]^+$ (calc. for $C_7H_{12}BrCl$, 209.9805).

Chiral HPLC analysis of natural 2*R*-(*E*)-dysidazirine ((−)-**3.2**)

The original purified sample of natural (*E*)-dysidazirine ((−)-**3.2**), isolated from *Dysidea fragilis*, collected in Fiji, 1987[3] and stored at –20 ˚C since 1988) was verified as unchanged by ^1H NMR (500 MHz, $CDCl_3$). The sample was analyzed by chiral HPLC (Chiralpak AD, 1:9 *i*-PrOH/hexanes, 0.5 mL/min). Integration of peaks corresponding to *S* and *R* enantiomers indicated 22 %*ee*: $[\alpha]^{22}$ –32.5 (*c* 1.29, MeOH) (lit.[3] $[\alpha]_D$ –165 (*c* 0.5, MeOH)).

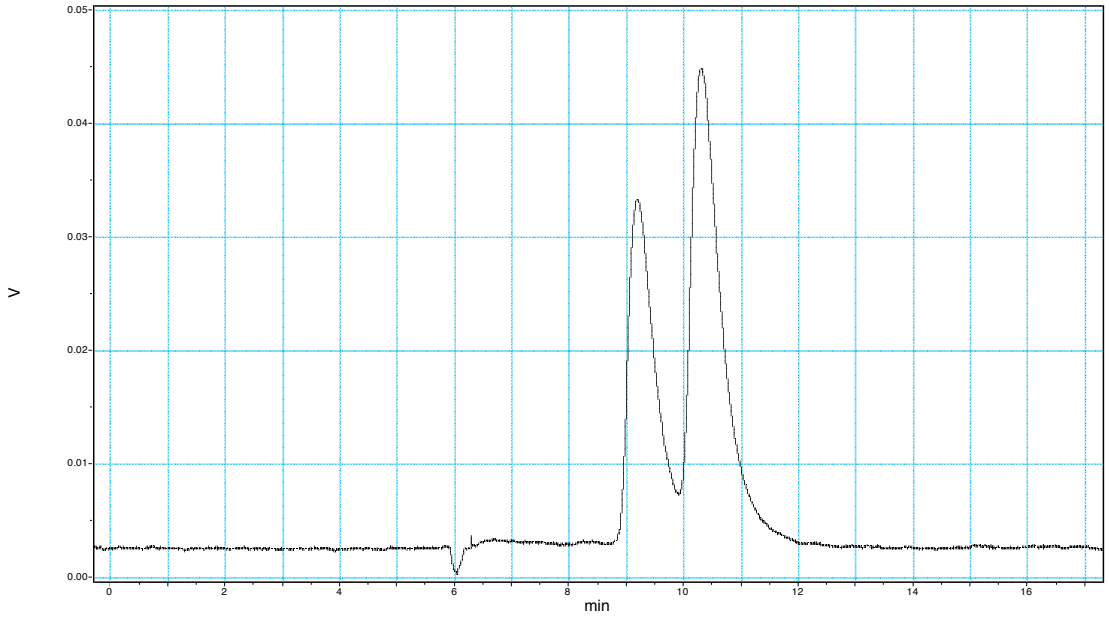


Figure 3.11 Chiral HPLC chromatogram of natural 2*R*-*E*-dysidazirine. *ee* = 22 %, $[a]^{22}$ – 32.5 (*c* 1.29, MeOH).

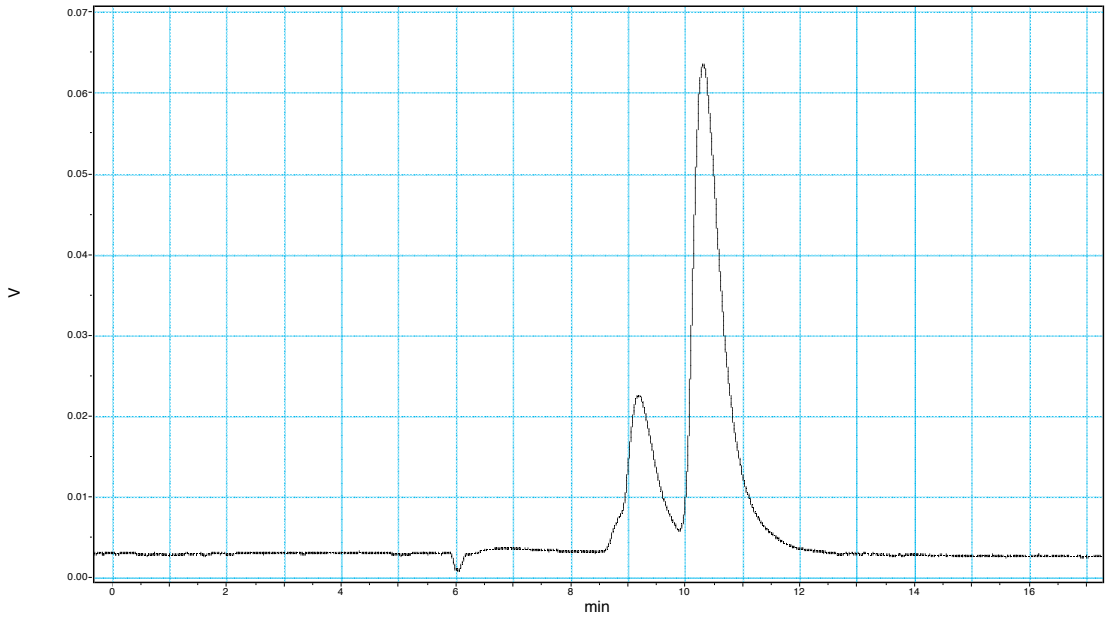
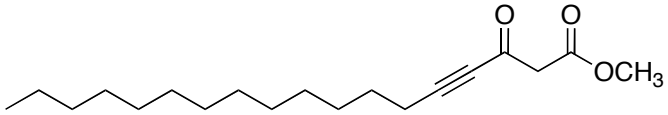


Figure 3.12 Chiral HPLC chromatogram of synthetic 2*R*-*E*-dysidazirine, *ee* = 53 %, $[\alpha]^{21}$ –85.2 (*c* 0.88, $CHCl_3$).

Total Synthesis of 2*R*-(*Z*)-dysidazirine and Analogues

General Procedures. IR spectra were recorded on an FTIR 550 spectrometer. ^{1}H NMR (400 or 500 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are expressed as δ ppm and are referenced to residual solvent signal (δ 7.24 ppm for ^{1}H and δ 77.0 for ^{13}C). Optical rotations were recorded on a digital polarimeter. All anhydrous reactions were conducted under an atmosphere of nitrogen using oven-dried glassware. Solvents for dry reactions were filtered through 2x alumina columns. Methyl malonyl chloride was distilled under reduced pressure prior to use. All other chemicals were reagent grade and used as received.

methyl 3-oxooctadec-4-ynoate (3.42)

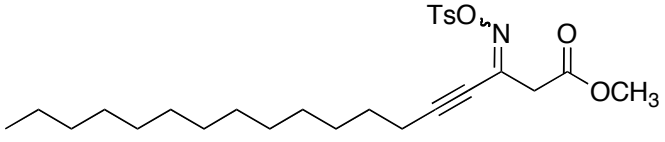


EtMgBr (1.20 mmol, 380 µL of a 2.13 M solution in Et_2O) was added dropwise to a solution of pentadecyne (250 mg, 1.20 mmol) in THF (2.5 mL) at 0 ˚C. Reaction was warmed to ambient temperature and stirred for 2.5 h, then cooled back to 0 ˚C. Methyl malonyl chloride (82 mg, 0.6 mmol) was added dropwise as a solution in THF (0.5 mL) and the resulting yellow mixture was stirred for 1 hour at 0 ˚C. Reaction was quenched by addition of saturated $NH_4Cl_{(aq)}$ (10 mL) and water (10 mL). Layers were separated and the aqueous layer extracted with Et_2O (2 × 20 mL). Combined organic extracts were

washed with brine (25 mL). Combined aquoues layers were extracted once with Et_2O (10 mL), then combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography (5:95 EtOAc/hexanes) gave **3.38** as a clear, colorless oil (130 mg, 70 %) along with recovered pentadecyne (142 mg).

IR (neat) ν 2923, 2854, 2216, 1754, 1677, 1648, 1608, 1448, 1383, 1324, 1251, 1171 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) keto tautomer: δ 2.73 (s, 3H), 2.55 (s, 2H), 2.35 (t, 2H, *J* = 7.2 Hz), 1.55 (m, 2H), 1.36 (m, 2H), 1.23 (s, 18H), 0.85 (t, 3H, *J* = 6.8 Hz); enol tautomer: δ 11.79 (s, 1H), 5.26 (s, 1H), 2.72 (s, 3H), 2.34 (t, 2H, *J* = 7.2 Hz), 1.55 (m, 2H), 1.36 (m, 2H), 1.23 (s, 18H), 0.85 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) keto tautomer: δ 178.7 (C), 166.6 (C), 97.1 (C), 80.3 (C), 52.4 (CH_3), 51.1 (CH_2), 31.9 (CH_2), 29.62 (CH_2), 29.60 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.97 (CH_2), 28.79 (CH_2), 27.5 (CH_2), 22.6 (CH_2), 19.0 (CH_2), 14.1 (CH_3); enol tautomer: δ 172.6 (C), 155.9 (C), 96.4 (C), 95.8 (CH), 75.4 (C), 51.4 (CH_3), 31.9 (CH_2), 29.62 (CH_2), 29.60 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 28.81 (CH_2), 27.8 (CH_2), 22.6 (CH_2), 19.2 (CH_2), 14.1 (CH_3); HRFABMS m/*z* 309.2420 $[M+H]^+$ (calc. for $C_{19}H_{33}O_3$ 309.2424).

(*E*,*Z*)-methyl 3-(tosyloxyimino)octadec-4-ynoate (3.41)



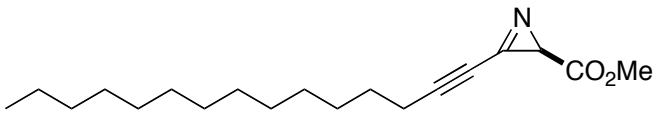
To a solution of methyl 3-oxooctadec-4-ynoate **3.42**

(110 mg, 0.36 mmol) in ethanol (1.8 mL) was added $NH_2OH.HCl$ (27 mg, 0.39 mmol) and pyridine (32 μL, 0.39 mmol). The mixture was heated to 55 ºC with stirring for 30 minutes, then evaporated to dryness under reduced pressure. The residue was partitioned between water, brine and EtOAc (3 mL each), and the aqueous layer extracted with EtOAc (2 × 3 mL). The organic layers were combined, dried (Na_2SO_4) and evaporated under reduced pressure. The crude oxime was then taken up in anhydrous CH_2Cl_2 (1.8 mL) in a 20 mL scintillation vial; *p*-toluenesulfonic anhydride (128 mg, 0.39 mmol) was added followed by pyridine (32 μL, 0.39 mmol) and dimethylaminopyridine (1 crystal). After 30 minutes at ambient temperature, a further 58 mg of *p*-toluenesulfonic anhydride and 15 μL of pyridine were added. The resulting mixture was stirred for 1 hour then diluted with CH_2Cl_2 (10 mL). Saturated $NH_4Cl_{(aq)}$ (20 mL) was added, and layers separated. The aquoues layer was extracted with CH_2Cl_2 (2 × 10 mL), then combined organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Silica gel chromatography (2:3 CH_2Cl_2/hexanes then 7:3 CH_2Cl_2/hexanes) gave **3.41** (122 mg, 72 %) as a clear yellow oil.

IR (neat) ν 2934, 2864, 2226, 1746, 1597, 1396, 1195, 1175, 819, 680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) major isomer: δ 7.82 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.2 Hz), 2.65 (s, 3H), 2.32 (s, 2H), 2.41 (s, 3H), 2.39 (t, 2H, *J* = 7.2 Hz), 1.54 (m, 2H), 1.35 (m, 2H), 1.23 (s, 18H), 0.85 (t, 3H, *J* = 6.8 Hz); minor isomer: δ 7.82 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.2 Hz), 2.67 (s, 3H), 2.49

(s, 2H), 2.41 (s, 3H), 2.29 (t, 2H, *J* = 7.2 Hz), 1.54 (m, 2H), 1.35 (m, 2H), 1.23 (s, 18H), 0.85 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: δ 167.6 (C), 145.1 (C), 144.6 (C), 132.4 (C), 129.5 (CH), 128.8 (CH), 107.9 (C), 70.7 (C), 52.3 (CH_3), 40.0 (CH_2), 31.9 (CH_2), 29.59 (CH_2), 29.56 (CH_2), 29.4 (CH_2), 29.3(CH_2), 29.0 (CH_2), 28.7 (CH_2), 27.6 (CH_2), 22.6 (CH_2), 21.6 (CH_3), 19.7 (CH_2), 14.0 (CH_3); HRFABMS m/*z* 478.2629 $[M+H]^+$ (calc. for $C_{26}H_{40}O_5NS$ 478.2622).

(*R*)-methyl 3-(pentadec-1-ynyl)-2*H*-azirine-2-carboxylate (3.40)

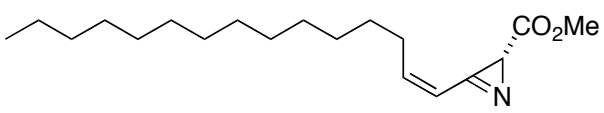


3.41 (60 mg, 0.12 mmol) was added dropwise as a solution in toluene (10 mL) to a mixture of quinidine (122 mg, 0.38 mmol) in toluene (30 mL) at 0 ˚C. The resulting mixture was stirred for 48 hours at 0 ºC, then HCl (0.05 M, 20 mL) was added. The biphasic mixture was stirred at 0 ºC for 15 minutes then extracted with hexanes (2 × 20 mL). Organic layers were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (1:19 EtOAc/hexanes) gave **3.40** (32.4 mg, 84%) as a clear oil.

$[\alpha]^{23}$ –111.2 (*c* 1.57, $CHCl_3$); IR (neat) ν 2934, 2855, 2226, 1750, 1734, 1466, 1437, 1344, 1274, 1204, 1029 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, 3H), 2.69 (s, 1H), 2.56 (t, 2H, *J* = 7.2 Hz), 1.63 (p, 2H, *J* = 7.4 Hz), 1.40 (m, 2H), 1.23 (s, 20H), 0.85 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ

171.2 (C), 149.6 (C), 118.5 (C), 64.9 (C), 52.7 (CH_3), 32.1 (C), 31.8 (CH), 29.89 (CH_2), 29.87 (CH_2), 29.86 (CH_2), 29.81 (CH_2), 29.64 (CH_2), 29.58 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.7 (CH_2), 22.9 (CH_2), 20.6 (CH_2), 14.4 (CH_3); HREIMS m/*z* 305.2354 $[M]^+$ (calc. for $C_{19}H_{31}NO_2$ 305.2349).

(−)-(*Z*)-dysidazirine ((−)-3.3)

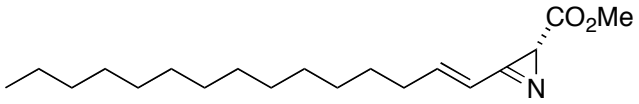


A suspension of Lindlar's catalyst ($Pd/CaCO_3$ poisoned with lead, 6.6 mg, 2.1 × 10^{-6} mol palladium, 0.05 eq) and quinoline (108 µL of a stock solution prepared from 40 µL quinoline in 20 mL hexanes) in hexanes (4.8 mL) was evacuated and purged with H_2 five times then cooled to 0 ˚C. Alkyne **3.40** (19 mg, 6.22 × 10^{-5} mol) was added as a solution in hexanes (2 mL) and the mixture stirred for 20 minutes at 0 ˚C. A further 0.05 eq of Lindlar catalyst was added, the reaction was stirred for 20 minutes at 0 ˚C then warmed to ambient temperature for 20 minutes. The mixture was then filtered immediately through celite. Solvent was removed under reduced pressure, and the crude material chromatographed on silica gel (5:95 Et_2O/hexanes) then further purified by Si HPLC (1.5:98.5 EtOAc/hexanes) to give (*Z*)-dysidazirine (9.9 mg, 52%). *ee* = 59% determined by chiral HPLC (Chiralpak AD, 1:9 *i*-PrOH/hexanes, 0.75 mL/min).

$[\alpha]^{22}$ –92.5 (*c* 0.16, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.56 (dt, 1H, *J* = 10.3, 8.0 Hz), 6.41 (d, 1H, *J* = 10.3 Hz), 2.72 (s, 3H), 2.62 (s, 1H), 2.48 (m,

2H), 1.41 (m, 2H), 1.23 (s, 20H), 0.86 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ 172.2 (C), 154.2 (C), 152.7 (CH), 111.2 (CH), 52.2 (CH_3), 31.9 (CH_2), 29.7 (CH_2), 29.63 (CH_2), 29.60 (CH_2), 29.5 (CH_2), 29.35 (CH_2), 29.34 (CH), 29.26 (CH_2), 29.2 (CH_2), 28.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HREIMS m/*z* 307.2508 $[M]^+$ (calc. for $C_{19}H_{33}O_2N$ 307.2506).

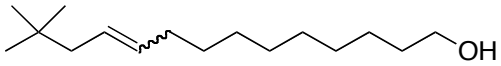
(−)-(*E*)-dysidazirine, ((−)-3.2)



(*Z*)-dysidazirine (6 mg, 0.02 mmol, *ee*=59%) was combined with iodine (0.49 mg, 0.002 mmol) in CH_2Cl_2 (600 µL) in a 2.5 mL vial. The vial was capped and cooled to 0 ˚C in an ice-water bath. The stirred mixture was irradiated with a 275 W sun lamp for 12.5 hours. During the first 10 hours the ice-water bath was replenished as necessary to maintain the reaction temperature at ≤ 25 ˚C. During the final 2.5 hours the ice-water bath was not refreshed (final temperature ~ 35 ˚C). Reaction was monitored periodically by NMR. After 12.5 hours saturated sodium thiosulfate (1 mL) was added to the reaction mixture. Layers were mixed then separated, and the aqueous layer extracted with CH_2Cl_2 (3 × 1 mL). Combined organic layers were dried (Na_2SO_4) and evaporated. Crude material was purified by silica column chromatography (1:19 EtOAc/hexanes) then silica HPLC (3:97 EtOAc/hexanes) to give (*E*)-dysidazirine (−)-**15** (1.5 mg, 25 %). Chiral HPLC (Chiralpak AD, 1:9 *i*-PrOH/hexanes, 1.0 mL/min) gave *ee* = 59%.

$[\alpha]^{21}$ –85.2 ($CHCl_3$, *c* 0.88); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (dt, 1H, *J* = 15.3, 6.6 Hz), 6.53 (d, 1H, *J* = 15.3 Hz), 2.71 (s, 3H), 2.56 (s, 1H), 2.35 (q, 2H, *J* = 7.2 Hz), 1.49 (m, 2H), 1.24 (br s, 20H), 0.86 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (C), 156.6 (C), 155.9 (CH), 112.9 (CH), 52.2 (CH_3), 32.2 (CH_2), 31.9 (CH_2), 29.66 (CH_2), 29.63 (CH_2), 29.59 (CH_2), 29.5 (CH_2), 29.34 (CH_2), 29.33 (CH_2), 29.2 (CH_2), 28.3 (CH), 27.8 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HREIMS m/*z* 307.2509 $[M]^+$ (calc. for $C_{19}H_{33}O_2N$ 307.2506).

13,13-dimethyltetradec-10-en-1-ol, 3.51

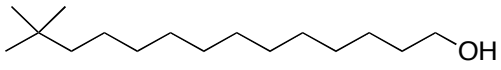


Phosphonium bromide **3.50** (2.87 g, 7.08 mmol) was azeotropically dried from anhydrous toluene five times. THF (35 mL) was added and the resulting cloudy suspension was cooled to 0 ˚C. *n*-BuLi (15 mmol, 5.95 mL of a 2.5 M solution in hexanes) was added dropwise, the bright red mixture was warmed to ambient temperature. After 2 hours, an additional 25 mL of THF was added, and the mixture stirred for a further 2 hours. Additional *n*-BuLi was added (1.5 mmol, 0.6 mL of 2.5 M solution) and the mixture stirred for 15 minutes before being cooled to – 78 ˚C. 3,3-dimethylbutanal was added neat, dropwise, producing a pale yellow mixture that was allowed to warm to room temperature over 14 hours. Water (100 mL) was added and the mixture extracted with diethyl ether (4 × 50 mL). Combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4)

and evaporated. Silica chromatography (1:9 – 1:4 EtOAc/hexanes) gave **3.51** (1.17 g, 69 %).

IR (neat) ν 3336, 2943, 2847, 1470, 1368, 1243, 1199, 1056, 971, 777, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) major isomer: δ 5.45-5.33 (m, 2H), 2.60 (t, 2H, *J* = 6.6 Hz), 1.98 (m, 2H), 1.89 (d, 2H, *J* = 6.4 Hz), 1.53 (m, 2H), 1.25 (br s, 13H), 0.86 (s, 9H); minor isomer: δ 5.45-5.33 (m, 2H), 2.60 (t, 2H, *J* = 6.6 Hz), 1.98 (m, 2H), 1.82 (d, 2H, *J* = 6.0 Hz), 1.53 (m, 2H), 1.25 (br s, 13H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: δ 131.3 (CH), 126.5 (CH), 62.9 (CH_2), 41.1 (CH_2), 32.7 (CH_2), 31.2 (C), 29.7 (CH_2), 29.54 (CH_2), 29.45 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.23 (CH_3), 27.2 (CH_2), 25.7 (CH_2); minor isomer: δ 132.6 (CH), 127.2 (CH), 62.9 (CH_2), 47.1 (CH_2), 32.6 (CH_2), 30.8 (C), 29.7 (CH_2), 29.54 (CH_2), 29.45 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.21 (CH_3), 27.2 (CH_2), 25.7 (CH_2); HREIMS 240.2445 $[M]^+$ (calc. for $C_{16}H_{32}O$ 240.2448).

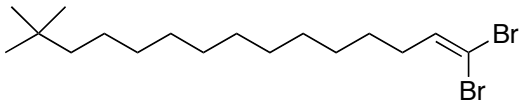
13,13-dimethyltetradecan-1-ol, 3.52



3.51 (1.27 g, 5.28 mmol) was dissolved in ethyl acetate (26.4 mL) and Pd/C (1.12 g, 1.06 mmol) was added. Reaction vessel was purged with hydrogen, then stirred for 1.5 hour at room temperature. The mixture was vacuum filtered through a short pad of celite, and solvent was evaporated. Silica chromatography (1:9 EtOAc/hexanes) gave **3.52** (1.15 g, 90%).

IR (neat) ν 3336, 2934, 2855, 1470, 1365, 1056 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.60 (t, 2H, *J* = 6.8 Hz), 1.53 (m, 2H), 1.35-1.10 (br m, 21H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.0 (CH_2), 44.3 (CH_2), 32.8 (CH_2), 30.6 (CH_2), 30.2 (C), 29.73 (CH_2), 29.68 (CH_2), 29.66 (CH_2), 29.60 (CH_2), 29.58 (CH_2), 29.42 (CH_2), 29.40 (CH_3), 25.7 (CH_2), 24.5 (CH_2); HREIMS m/*z* 242.2607 $[M]^+$ (calc. for $C_{16}H_{34}O$ 242.2604).

1,1-dibromo-14,14-dimethylpentadec-1-ene, 3.53



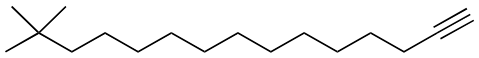
To a solution of oxalyl chloride (1.05 g, 8.15 mmol) in CH_2Cl_2 (20 mL) at −78 ˚C was added dropwise DMSO (1.27 g, 16 mmol) as a solution in CH_2Cl_2 (5 mL). The mixture was stirred for 30 minutes, then **3.47** (0.99 g, 4.08 mmol) was added dropwise as a solution in CH_2Cl_2 (2 mL) with a 2 mL wash. After 30 minutes, triethylamine (2.48 g, 24 mmol) was added dropwise. Mixture was allowed to warm to room temperature over 2 hours, then poured into water (100 mL). Layers were separated, and aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). Combined organic extracts were washed successively with 1% HCl (3 × 50 mL), 5 % $NaHCO_3$ (1 × 50 mL), water (1 × 50 mL) and brine (1 × 50 mL). Combined aqueous washes were back extracted with CH_2Cl_2 (50 mL). Solvent was evaporated.

Separately a solution of triphenylphosphine (4.28 g, 16.3 mmol) in CH_2Cl_2 (15 mL) at 0 ˚C was treated with carbon tetrabromide (2.71 g, 8.16

mmol). The bright red mixture was stirred 15 minutes, then crude aldehyde was added as a solution in CH_2Cl_2 (5 mL). Mixture was stirred for 1 hour, then hexanes (200 mL) was added and the mixture filtered through celite followed by a cotton plug. Solvent was evaporated under reduced pressure, and the crude material chromatographed on silica (hexane) to give **3.53** (1.39 g, 86 %).

IR (neat) ν 2943, 2855, 1467, 1365, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (t, 1H, *J* = 7.2 Hz), 2.07 (q, 2H, *J* = 7.3 Hz), 1.40 (br m, 2H), 1.30-1.10 (br m, 18H), 0.84 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9 (CH), 88.4 (C), 44.3 (CH_2), 32.0 (CH_2), 30.6 (CH_2), 30.3 (C), 29.74 (CH_2), 29.68 (CH_2), 29.64 (CH_2), 29.52 (CH_2), 29.44 (CH_3), 29.35 (CH_2), 29.05 (CH_2), 27.8 (CH_2), 24.6 (CH_2); HREIMS m/*z* 394.0859 $[M]^+$ (calc. for $C_{17}H_{32}Br_2$ 394.0865).

14,14-dimethylpentadec-1-yne, 3.54

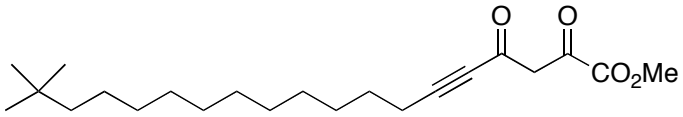


To a solution of **3.53** (1.31 g, 2.31 mmol) in THF (17 mL) at -78 ˚C was added *n*-BuLi (7.27 mmol, 2.91 mL of a 2.5 M solution in hexanes) dropwise. The resulting pale red solution was warmed to room temperature over 1 hour, then poured into water (100 mL). Hexanes (10 mL) was added, and the layers separated. The aqueous layer was extracted with hexanes (2 × 50 mL), then combined organic extracts were dried (Na_2SO_4) and solvent was evaporated

under reduced pressure. Silica gel chromatography (hexanes) gave alkyne **3.54** (782 mg, quant.).

IR (neat) 3318, 2943, 2855, 2121, 1466, 1370, 1256, 627 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (dt, 2H, *J* = 7.2, 2.7 Hz), 1.91 (t, 1H, *J* = 2.4 Hz), 1.50 (m, 2H), 1.36 (m, 2H), 1.25 (br s, 14H), 1.14 (m, 2H), 0.84 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 84.8 (C), 68.0 (C), 44.3 (CH_2), 30.6 (CH_2), 30.27 (C), 29.73 (CH_2), 29.67 (CH_2), 29.62 (CH_2), 29.5 (CH_2), 29.4 (CH_3), 29.1 (CH_2), 28.8 (CH_2), 28.5 (CH_2), 24.6 (CH_2), 18.4 (CH_2); HREIMS m/*z* 236.2493 $[M]^+$ (calc. for $C_{17}H_{32}$ 236.2499).

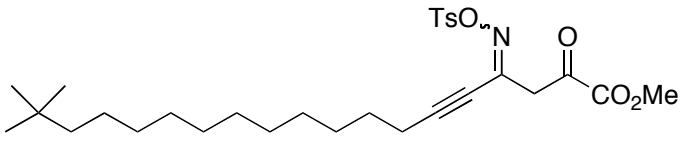
methyl 17,17-dimethyl-3-oxooctadec-4-ynoate, 3.55



To a solution of **3.54** (308 mg, 1.30 mmol) in THF (5 mL) at 0 ˚C was added *n*-BuLi (1.30 mmol, 0.52 mL of a 2.5 M solution in hexanes) dropwise. The resulting mixture was stirred for 1 hour, then cooled to – 78 ˚C. Methyl malonyl chloride (89 mg, 0.65 mmol) was added dropwise as a solution in THF (4 mL, 1 mL rinse), and the yellow mixture was warmed to room temperature over 2.25 hours at which time saturated $NH_4Cl_{(aq)}$ (20 mL) was added. Mixture was extracted with hexanes (3 × 25 mL), then combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4) and solvent was removed under reduced pressure. Silica chromatography (1:19 EtOAc/hexanes) gave **3.55** (99 mg, 45 %).

IR (neat) 2943, 2855, 2217, 1755, 1685, 1650, 1612, 1445, 1390, 1359, 1324, 1250, 1177, 808 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) keto tautomer: δ 2.73 (s, 3H), 2.55 (2H, s), 2.35 (t, 2H, *J* = 7.0 Hz), 1.55 (m, 2H), 1.36 (m, 2H), 1.24 (br s, 14H), 1.13 (m, 2H), 0.83 (s, 9H); enol tautomer: δ 11.79 (s, 1H), 5.26 (s, 1H), 2.72 (s, 3H), 2.34 (t, 2H, *J* = 7.0 Hz), 1.55 (m, 2H), 1.36 (m, 2H), 1.24 (br s, 14H), 1.13 (m, 2H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) keto form: δ 178.7 (C), 166.6 (C), 97.1 (C), 80.3 (C), 52.4 (CH_3), 51.1 (CH_2), 44.2 (CH_2), 30.6 (CH_2), 30.2 (C), 29.7 (CH_2), 29.6 (CH_2), 29.56 (CH_2), 29.4 (CH_3), 28.98 (CH_2), 28.79 (CH_2), 27.5 (CH_2), 24.5 (CH_2), 19.0 (CH_2); enol form: δ 172.6 (C), 155.8 (C), 96.4 (C), 95.8 (CH), 75.4 (C), 51.4 (CH_3), 44.2 (CH_2), 30.6 (CH_2), 30.2 (C), 29.7 (CH_2), 29.6 (CH_2), 29.56 (CH_2), 29.4 (CH_3), 29.01 (CH_2), 28.82 (CH_2), 27.8 (CH_2), 24.5 (CH_2), 19.2 (CH_2); HREIMS m/*z* 336.2660 $[M]^+$ (calc. for $C_{21}H_{36}O_3$ 336.2659).

methyl 17,17-dimethyl-3-(tosyloxyimino)octadec-4-ynoate, 3.56



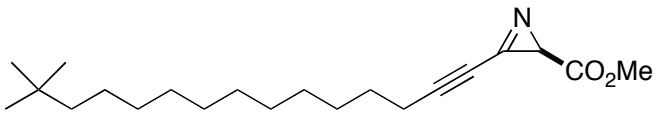
To a solution of **3.55** (250 mg, 0.74 mmol) in EtOH (2.71 mL) was added pyridine (66 µL, 0.82 mmol) then hydroxylamine hydrochloride (57 mg, 0.82 mmol). The mixture was heated to 55 ˚C for 45 minutes, at which time it was cooled and solvent removed under reduced pressure. The residue was suspended in water (5 mL) and extracted with EtOAc (4 × 5 mL). Combined

organic extracts were dried (Na_2SO_4) and solvent removed under reduced pressure. Crude oxime was dissolved in anhydrous CH_2Cl_2 (2.71 mL) and to this solution was added pyridine (0.12 mL, 1.49 mmol), *p*-toluenesulfonic anhydride (480 mg, 1.49 mmol) and a catalytic amount of DMAP. The mixture was stirred for 45 minutes. Saturated $NH_4Cl_{(aq)}$ (5 mL) was added, and layers were separated. Aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and combined extracts were washed with water (5 mL), dried (Na_2SO_4) and evaporated under reduced pressure. Silica chromatography (2:3 CH_2Cl_2/hexanes) gave **3.56** (267 mg, 71 %) as a clear, yellow oil.

IR (neat) 2925, 2847, 2366, 2331,2226, 1751, 1604, 1472, 1437, 1386, 1200, 1181, 1095, 823, 664, 551 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) major isomer: δ 7.83 (d, 2H, *J* = 8.2 Hz), 7.31 (d, 2H, *J* = 8.2 Hz), 2.66 (s, 3H), 2.33 (s, 2H), 2.42 (s, 3H), 2.40 (t, 2H, *J* = 7.2 Hz), 1.58-1.46 (m, 2H), 1.40-1.17 (br m, 16 H), 1.12 (m, 2H), 0.83 (s, 9H); minor isomer: δ 7.83 (d, 2H, *J* = 8.2 Hz), 7.32 (d, 2H, *J* = 8.2 Hz), 2.67 (s, 3H), 2.50 (s, 2H), 2.42 (s, 3H), 2.30 (t, 2H, *J* = 7.2 Hz), 1.58-1.46 (m, 2H), 1.40-1.17 (br m, 16 H), 1.12 (m, 2H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer δ 167.7 (C), 145.13 (C), 144.6 (C), 132.3 (C), 129.59 (CH), 128.89 (CH), 107.9 (C), 70.7 (C), 52.4 (CH_3), 44.3 (CH_2), 40.1 (CH_2), 37.4 (CH_2), 30.6 (CH_2), 30.3 (C), 29.72 (CH_2), 29.66 (CH_2), 29.61 (CH_2), 29.44 (CH_2), 29.41 (CH_3), 29.0 (CH_2), 28.7 (CH_2), 27.69 (CH_2), 24.5 (CH_2), 21.69 (CH_3), 19.7 (CH_2); minor isomer δ 166.7 (C), 147.4 (C), 145.39 (C), 132.0 (C), 129.68 (CH), 128.96 (CH), 100.1 (C), 72.8 (C), 52.6

(CH_3), 44.3 (CH_2), 40.1 (CH_2), 37.4 (CH_2), 30.6 (CH_2), 30.3 (C), 29.69 (CH_2), 29.64 (CH_2), 29.58 (CH_2), 29.44 (CH_2), 29.41 (CH_3), 29.0 (CH_2), 28.8 (CH_2), 27.71 (CH_2), 24.5 (CH_2), 21.72 (CH_3), 19.5 (CH_2); HREIMS m/*z* 505.2865 $[M]^+$ (calc. for $C_{28}H_{43}O_5NS$ 505.2856).

methyl 3-(14,14-dimethylpentadec-1-ynyl)-2*H*-azirine-2-carboxylate (3.57)

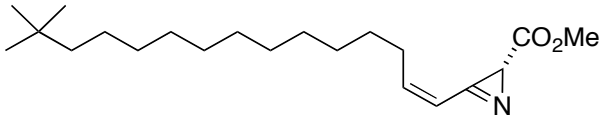


To a suspension of quinidine (289 mg, 0.89 mmol) in toluene (60 mL) at 0 ˚C was added **3.56** (150 mg, 0.29 mmol) as a solution in toluene (10 mL). The cloudy mixture was stirred at 0 ˚C for 24 hours, at which time a further 1 equivalent (22 mg) of quinidine was added, and the mixture stirred at 0 ˚C for 14 hours, then warmed to 10 ˚C for 2.5 hours before being poured into ice-cold HCl (0.05 M, 50 mL). The mixture was stirred vigorously for 5 minutes, then the layers were separated and aqueous layer extracted with hexanes (3 × 25 mL). Combined organic layers were washed with water (25 mL), brine (50 mL) and then dried (Na_2SO_4) before solvent was removed under reduced pressure. Silica chromatography (1:19 EtOAc/hexanes) gave **3.57** (79 mg, 80 %).

$[\alpha]^{21}$ −110.6 ($CHCl_3$, *c* 2.16); IR (neat) 2943, 2855, 2235, 1754, 1476, 1441, 1344, 1270, 1204 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, 3H), 2.69 (s, 1H), 2.56 (t, 2H, *J* = 7.2 Hz), 1.63 (m, 2H), 1.40 (m, 2H), 1.32-1.16 (br s,

14H), 1.13 (m, 2H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9 (C), 149.4 (C), 118.3 (C), 64.6 (C), 52.4 (CH_3), 44.2 (CH_2), 31.6 (CH), 30.6 (CH_2), 30.2 (C), 29.7 (CH_2), 29.63 (CH_2), 29.56 (CH_2), 29.40 (CH_3), 29.38 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 27.4 (CH_2), 24.5 (CH_2), 20.3 (CH_2); HREIMS m/*z* 332.2658 $[M]^+$ (calc. for $C_{21}H_{35}O_2N$ 332.2662).

(*R*,*Z*)-methyl 3-(14,14-dimethylpentadec-1-enyl)-2*H*-azirine-2-carboxylate ((−)-3.46)

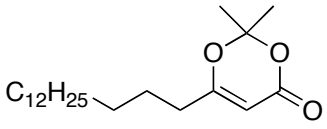


To a stirred suspension of Lindlar's catalyst (32 mg, 0.015 mmol) in hexanes (10 mL) at 0 ˚C under an atmosphere of H_2 was added **3.57** (50 mg, 0.15 mmol) as a solution in hexanes (2 mL). After stirring for 20 minutes, the mixture was filtered through a plug of celite, followed by a C18 sep pak. Final purification by C18 HPLC (6:94 H_2O/MeOH) gave **(−)-3.46** (29 mg, 58%). *ee* = 60 %, determined by chiral HPLC (Chiralpak AD, 1:9 IPA/hexanes, 0.75 mL/min).

$[\alpha]^{23}$ –90.7 ($CHCl_3$, *c* 1.43); IR 2934, 2855, 1771, 1736, 1476, 1437, 1367, 1340, 1270, 1196, 1025 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 (dt, 1H, *J* = 10.8, 7.9 Hz), 6.40 (dt, 1H, *J* = 10.8, 1.3Hz), 2.71 (s, 3H), 2.61 (s, 1H), 2.48 (2H), 1.41 (m, 2H), 1.23 (br s, 16H), 1.12 (m, 2H), 0.83 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (C), 154.2 (C), 152.7 (CH), 111.2 (CH), 52.2 (CH_3), 44.2 (CH_2), 30.6 (CH_2), 30.2 (C), 29.7 (CH_2), 29.64 (CH_2), 29.59 (CH_2), 29.5

(CH_2), 29.4 (CH_3), 29.3 (CH_2), 29.22 (CH), 29.17 (CH_2), 28.8 (CH_2), 24.5 (CH_2); HREIMS m/*z* 335.2818 $[M]^+$ (calc. for $C_{21}H_{37}O_2N$ 335.2819).

2,2-dimethyl-6-pentadecyl-4*H*-1,3-dioxin-4-one (3.60)

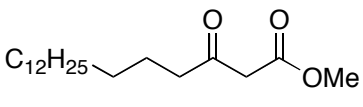


To a solution of diisopropylamine (99 µL, 0.70 mmol) in anhydrous THF (1.5 mL) at 0 ˚C was added *n*-BuLi (281 µL of a 2.5M solution in hexanes, 0.70 mmol) dropwise. The mixture was stirred for 15 minutes at 0 ˚C then HMPA (0.61 mL) was added, followed by stirring for a further 15 minutes. 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one **3.58** (100 mg, 0.70 mmol) was then added, and the solution was stirred for 20 minutes before being cooled to – 40 ˚C. Bromotetradecane (0.58 g, 2.11 mmol) was added dropwise as a solution in THF (0.5 mL), and the solution was allowed to warm slowly to room temperature over the course of three hours, then heated to 50 ˚C for 1 hour. Reaction was worked up by addition of 1 M HCl (5 mL) and extraction with Et_2O (3 × 10 mL). Combined organic extracts were washed with brine (20 mL), dried (sodium sulfate) and evaporated to dryness. Silica gel chromatography (1:9 Et_2O/hexane) gave the desired product **3.60** (42 mg, 18 %) and the undesired α-alkylation product (38 mg, 16 %).

IR (neat) ν 2919, 2848, 1736, 1633, 1469, 1396, 1251, 1205, 1014, 902, 808 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.19 (s, 1H), 2.17 (t, 2H, *J* = 7.4 Hz), 1.65 (s, 6H), 1.51 (m, 2H), 1.25 (s, 24H), 0.85 (s, 3H, 6.8 Hz); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 171.8 (C), 161.2 (C), 106.1 (C), 92.0 (CH), 32.7 (CH_2), 31.9 (CH_2), 29.8 (CH_2), 29.73 (CH_2), 29.69(CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 25.8 (CH_2), 25.1 (CH_3), 22.8 (CH_2), 14.2 (CH_2); HREIMS m/z 338.2820 $[M]^+$ (calc. for $C_{21}H_{38}O_3$ 338.2815).

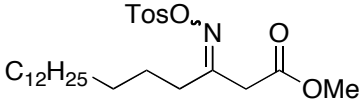
methyl 3-oxooctadecanoate (3.61)



A solution of **3.60** (42 mg, 0.12 mmol) in MeOH (1 mL) was heated by microwave to 120 ˚C for 20 minutes. Solvent was evaporated under reduced pressure, and the crude material was chromatographed on silica gel (1:9 Et_2O/hexane) to give **3.61** (27 mg, 70%).

IR (neat) ν 2927, 2856, 1754, 1715, 1472, 1408, 1275, 1163 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.72 (s, 3H), 2.42 (s, 2H), 2.50 (t, 2H, *J* = 7.6 Hz), 1.57 (m, 2H), 1.23 (s, 22H), 0.86 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9 (C), 167.7 (C), 52.3 (CH_3), 48.9 (CH_2), 42.1 (CH_2), 31.9 (CH_2), 29.67 (CH_2), 29.66 (CH_2), 29.64 (CH_2), 29.63 (CH_2), 29.628 (CH_2), 29.616 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 22.4 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HREIMS m/z 312.2655 $[M]^+$ (calc. for $C_{19}H_{36}O_3$ 312.2659).

methyl 3-(tosyloxyimino)octadecanoate (3.62)



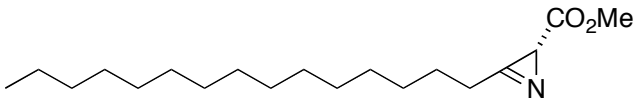
To a solution of **3.61** (19 mg, 0.06 mmol) in EtOH (0.64 mL) was added hydroxylamine

hydrochloride (4.9 mg, 0.07 mmol), followed by pyridine (6 µL, 0.07 mmol). The mixture was heated to 50 ˚C for 45 minutes, at which time solvent was evaporated under reduced pressure. The crude material was suspended in water (1 mL) then extracted with ethyl acetate (3 × 1 mL). Combined organic extracts were dried (Na_2SO_4) and evaporated. The crude oxime was then dissolved in anhydrous CH_2Cl_2 (320 µL). Pyridine (12 µL, 0.14 mmol) was added followed by *p*-toluenesulfonic anhydride (46 mg, 0.14 mmol). The mixture was stirred at room temperature 1 hour, then saturated aqueous NH_4Cl (1 mL) was added. Mixture was extracted with CH_2Cl_2 (3 × 1 mL), then the aqueous extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Silica gel chromatography (2:3 CH_2Cl_2/hexane) gave **3.61** (24 mg, 82 %) as a clear yellow oil.

IR (neat) ν 2919, 2864, 1749, 1599, 1467, 1381, 1261, 1194, 1180, 1096, 1018, 811cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) major isomer: δ 7.81 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.2 Hz), 2.66 (s, 3H), 2.70 (s, 2H), 2.42 (s, 3H), 2.28 (t, 2H, *J* = 7.4 Hz), 1.41 (m, 2H), 1.23 (s, 22H), 0.85 (t, 3H, *J* = 6.8 Hz); minor isomer: δ7.81 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.2 Hz), 2.64 (s, 3H), 2.22 (s, 2H), 2.42 (s, 3H), 2.44 (t, 2H, *J* = 7.4 Hz), 1.41 (m, 2H), 1.23 (s, 22H), 0.85 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: δ 167.1 (C), 162.6 (C), 144.7 (C), 132.5 (C), 129.3 (CH), 128.7 (CH), 52.5 (CH_3), 38.9 (CH_2), 34.9 (CH_2), 32.0 (CH_2), 29.79 (CH_2), 29.75 (CH_2), 29.70 (CH_2), 29.67 (CH_2), 29.55 (CH_2), 29.52 (CH_2), 29.45 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 25.3

(CH_2), 22.8 (CH_2), 21.8 (CH_3), 14.2 (CH_3); minor isomer: δ 168.2 (C), 164.8 (C), 144.7 (C), 132.5 (C), 129.4 (CH), 128.6 (CH), 52.4 (CH_3), 38.9 (CH_2), 34.9 (CH_2), 32.0 (CH_2), 29.79 (CH_2), 29.75 (CH_2), 29.70 (CH_2), 29.67 (CH_2), 29.55 (CH_2), 29.52 (CH_2), 29.45 (CH_2), 29.3 (CH_2), 28.9 (CH_2), 25.5 (CH_2), 22.8 (CH_2), 21.8 (CH_3), 14.2 (CH_3); HREIMS m/z 481.2864 $[M]^+$ (calc. for $C_{26}H_{43}NO_5S$ 481.2856).

(*R*)-methyl 3-pentadecyl-2*H*-azirine-2-carboxylate ((−)-3.47)

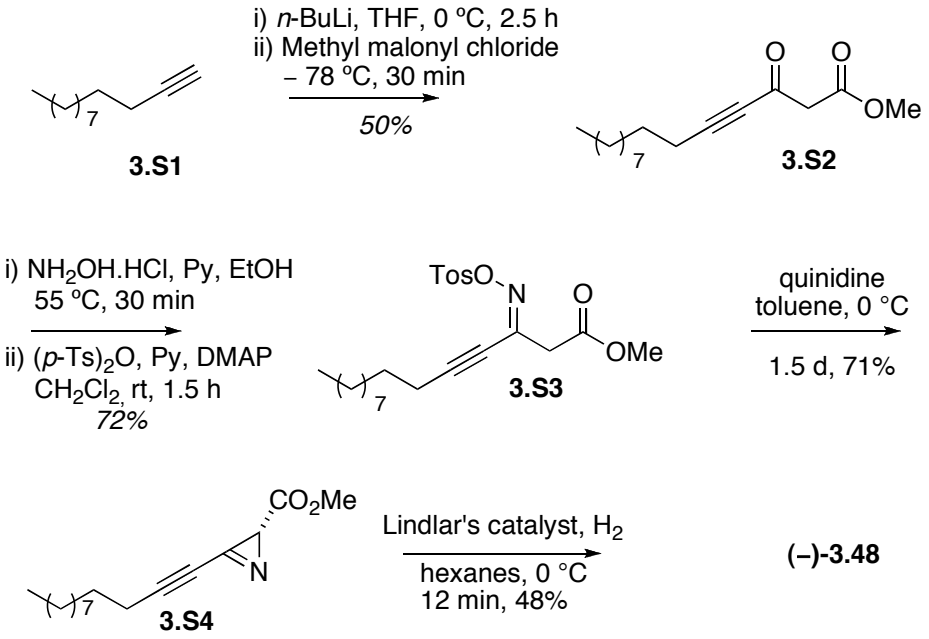


To a solution of quinidine (30 mg, 0.09 mmol) in anhydrous toluene (4.5 mL) at 0 ˚C was added **3.62** (15 mg, 0.03 mmol) as a solution in toluene (3 mL). The mixture was stirred for 24 hours at 0 ˚C, then worked up by addition of 0.05 M HCl (10 mL). Layers were separated, and aqueous portion was extracted with hexanes (2 × 4 mL). Combined organic extracts were washed with brine (4 mL) then dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (2.5:97.5 EtOAc/hexane) gave **(−)-3.47** (8.9 mg, 91%). *ee* = 71% determined by chiral HPLC (Chiralpak AD, 15:85 isopropanol/hexanes, 0.5 mL/min, ELSD detection).

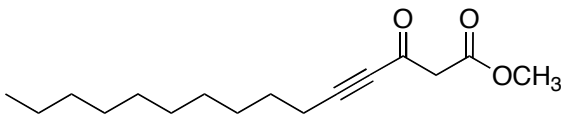
$[\alpha]_D^{23}$ −32.8 (*c* 0.69, $CHCl_3$); IR (neat) ν 2935, 2848, 1794, 1736, 1466, 1436, 1345, 1269, 1196, 1035 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), δ 2.69 (s, 3H), 2.79 (t, 2H, *J* = 7.2 Hz), 2.42 (s, 1H), 1.73 (p, 2H, *J* = 7.8 Hz), 1.40 (m, 2H), 1.23 (s, 22H), 0.86 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4 (C),

161.8 (C), 52.2 (CH_3), 32.0 (CH_2), 29.79 (CH_2), 29.78 (CH_2), 29.75 (CH_2), 29.72 (CH_2), 29.65 (CH_2), 29.48 (CH_2), 29.46 (CH_2), 29.23 (CH_2), 29.18 (CH_2), 28.7 (CH), 26.9 (CH_2), 24.3 (CH_2), 22.8 (CH_2), 14.3 (CH_3); HREIMS m/z 309.2663 $[M]^+$ (calc. for $C19H_{35}NO_2$ 309.2662).

Preparation of (−)-3.48



methyl 3-oxopentadec-4-ynoate (3.S2)

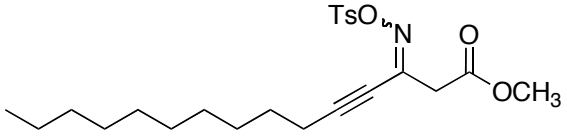


n-BuLi (4.37 mmol, 1.75 mL of a 2.5 M solution in hexanes) was added dropwise to a solution of dodecyne (726 mg, 4.37 mmol) in THF (22 mL) at 0 ˚C. The mixture was stirred for 2.5 hours, then cooled to −78 ˚C. Methyl malonyl chloride (298 mg, 2.18 mmol) was added dropwise as a

solution in THF (5 mL followed by a 1 mL rinse). After stirring 30 minutes the reaction was quenched by addition of saturated $NH_4Cl_{(aq)}$ (30 mL), warmed to rt, diluted with water (20 mL) and extracted with EtOAc (3 × 50 mL). Combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (1:19 EtOAc/hexanes) gave **3.S2** (290 mg, 50%) as a clear oil.

^{1}H NMR (400 MHz, $CDCl_3$) keto tautomer: δ 2.73 (s, 3H), 2.55 (s, 2H), 2.35 (t, 2H, *J* = 7.2 Hz), 1.55 (p, 2H, *J* = 7.8 Hz), 1.36 (m, 2H), 1.24 (s, 14 H), 0.85 (t, 3H, *J* = 6.4 Hz); enol tautomer: δ 11.80 (s, 1H), 5.72 (s, 1H), 2.72 (s, 3H), 2.34 (t, 2H, *J* = 7.2 Hz), 1.54 (p, 2H, *J* = 7.4 Hz), 1.36 (m, 2H), 1.24 (s, 14 H), 0.85 (t, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) keto tautomer: δ 178.9 (C), 166.8 (C), 97.4 (C), 80.6 (C), 52.7 (CH_3), 51.4 (CH_2), 32.1 (CH_2), 29.8 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 29.05 (CH_2), 27.8 (CH_2), 22.9 (CH_2), 19.3 (CH_2),14.3 (CH_3); enol tautomer: 172.9 (C), 156.1 (C), 96.7 (C), 96.1 (CH), 75.7 (C), 51.7 (CH_3), 32.1 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 28.1 (CH_2), 22.9 (CH_2), 19.5 (CH_2), 14.3 (CH_3); LRESIMS m/*z* 266.93 $[M+H]^+$; HREIMS 266.1877 $[M]^+$ (calc. for $C_{16}H_{26}O_3$ 266.1876)

methyl 3-(tosyloxyimino)pentadec-4-ynoate (3.S3)



A mixture of hydroxylamine hydrochloride (27 mg, 0.39 mmol),

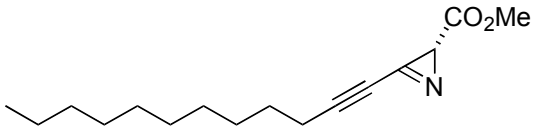
pyridine (32 μL, 0.39 mmol) and **3.S2** (110 mg, 0.36 mmol) in EtOH (1.8 mL) was heated at 55 ˚C for 30 minutes, then concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with 50% saturated NaCl (6 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL) then combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure.

The crude oxime was dissolved in anhydrous CH_2Cl_2 (1.8 mL) and *p*-toluenesulfonic anhydride (128 mg, 0.39 mmol), pyridine (32 μL, 0.39 mmol) and a small crystal of DMAP were added. The bright orange mixture was stirred for 30 minutes at which time a further 58 mg of *p*-toluenesulfonic anhydride and 15 μL of pyridine was added. After stirring for 1 hour the mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated $NH_4Cl_{(aq)}$ (20 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), then the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (2:3 → 7:3 CH_2Cl_2/hexanes) gave **3.S3** (122 mg, 72%) as a clear yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) major isomer: δ 7.91 (d, 2H, *J* = 8.2 Hz), 7.39 (d, 2H, *J* = 8.2 Hz), 2.71 (s, 3H), 2.34 (s, 2H), 2.47 (s, 3H), 2.42 (t, 2H, *J* = 7.2 Hz), 1.53 (p, 2H, *J* = 7.2 Hz), 1.35 (m, 2H), 1.24 (s, 12H), 0.85 (t, 3H, *J* = 7.2 Hz); minor isomer: δ 7.91 (d, 2H, *J* = 8.2 Hz), 7.39 (d, 2H, *J* = 8.2 Hz), 2.71 (s, 3H), 2.47 (s, 2H), 2.47 (s, 3H), 2.31 (t, 2H, *J* = 7.2 Hz), 1.53 (p, 2H, 7.2 Hz), 1.35 (m, 2H), 1.24 (s, 12H), 0.85 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 167.5 (C), 144.9 (C), 144.4 (C), 132.1 (C), 129.5 (CH), 128.8 (CH), 107.9 (C), 70.6 (C), 52.5 (CH_3), 40.1 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 28.7 (CH_2), 27.7 (CH_2), 22.7 (CH_2), 21.7 (CH_3), 19.8 (CH_2), 14.2 (CH_3).

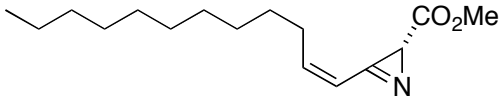
(*R*)-methyl 3-(dodec-1-ynyl)-2*H*-azirine-2-carboxylate (3.S4)



3.S3 (100 mg, 0.23 mmol) was added as a soution in toluene (2 mL) to a mixture of quinidine (223 mg, 0.69 mmol) in toluene (50 mL) at 0 ˚C. After stirring 1.5 days 0.05 M HCl (10 mL) was added and the mixture extracted with EtOAc (3 × 10 mL). Combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (1:9 EtOAc/hexanes) gave **3.S4** (43 mg, 71%) as a clear, colorless oil.

$[\alpha]^{23}$ –51.4 (*c* 0.18, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.73 (s, 3H), 2.70 (s, 1H), 2.56 (t, 2H, *J* = 7.5 Hz), 1.63 (p, 2H, *J* = 7.5 Hz), 1.41 (m, 2H), 1.25 (s, 12H), 0.86 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9 (C), 149.4 (C), 118.2 (C), 64.7 (C), 52.4 (CH_3), 31.8 (CH_2), 31.5 (CH), 29.5 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 27.4 (CH_2), 22.6 (CH_2), 20.3 (CH_2), 14.0 (CH_3).

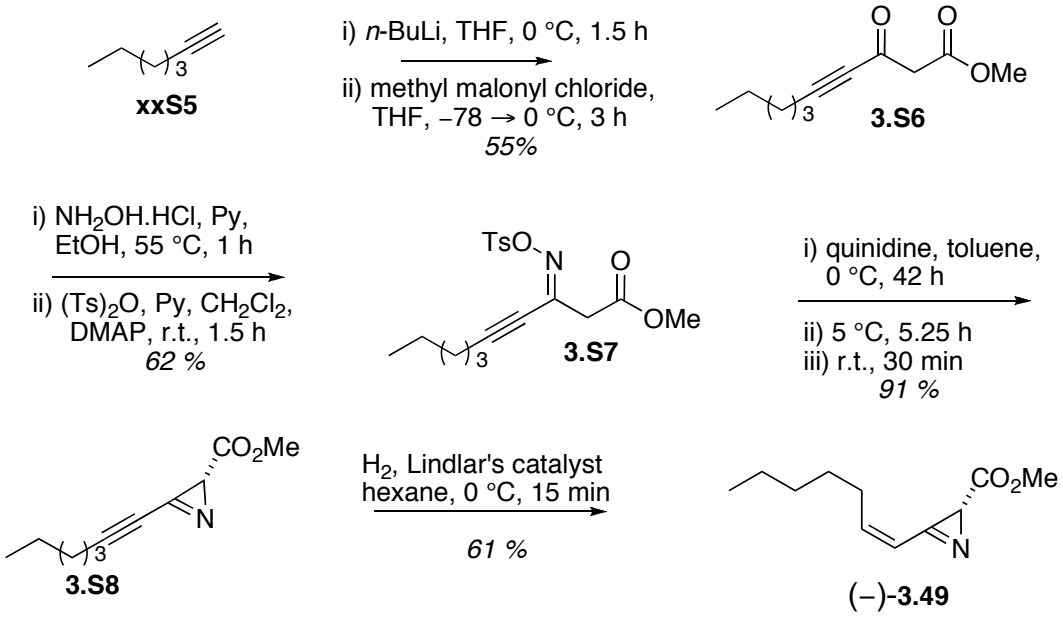
(*R*,*Z*)-methyl 3-(dodec-1-enyl)-2*H*-azirine-2-carboxylate ((−)-3.48)



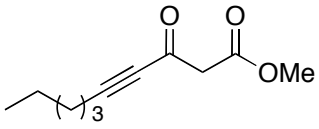
A mixture of Lindlar's catalyst (1.05 mg, 0.0099 mmol) and quinoline (146 µL of a stock solution prepared from 40 µL quinoline in 20 mL hexanes) in hexanes (2.6 mL) was evacuated and purged with H_2 then cooled to 0 ˚C. **3.S4** (26 mg, 0.099 mmol) was added as a solution in hexanes (2 mL followed by a 2 mL rinse). The mixture was stirred vigorously for 12 minutes then filtered through a plug of celite and concentrated under reduced pressure. Silica gel pencil column (1:19 EtOAc/hexanes) followed by SiO_2 HPLC (1.5:98.5 EtOAc/hexanes gave **(−)-3.48** (12.6 mg, 48%) as a clear, colorless oil.

$[\alpha]^{22}$ −132.8 (*c* 1.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.56 (dt, 1H, *J* = 10.5, 8 Hz), 6.41 (d, 1H, *J* = 10.5 Hz), 2.71 (s, 3H), 2.62 (s, 1H), 2.48 (m, 2H), 1.42 (m, 2H), 1.23 (s, 12H), 0.86 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2 (C), 154.2 (C), 152.7 (CH), 111.2 (CH), 52.2 (CH_3), 31.9 (CH_2), 29.7 (CH_2), 29.53 (CH_2), 29.50 (CH_2), 29.3 (CH_2), 29.28 (CH_2), 29.24 (CH), 29.18 (CH_2), 28.9 (CH_2), 22.7 (CH_2), 14.1 (CH_3); HREIMS m/*z* 265.2033 $[M]^+$ (calc. for $C_{16}H_{27}O_2N$ 265.2036).

Preparation of (−)-3.49



methyl 3-oxodec-4-ynoate (3.S6)

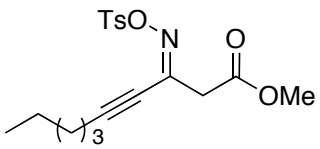


n-BuLi (21 mmol, 8.32 mL of a 2.5 M solution in hexanes) was added dropwise to a mixture of 1-heptyne (2.0 g, 21 mmol) in THF (100 mL) at 0 ˚C. After stirring 1.5 hours the mixture was cooled to −78 ˚C and methyl malonyl chloride was added slowly as a solution in THF (30 mL followed by a 10 mL). The resulting pale yellow mixture was warmed to 0 ˚C over 3 hours then quenched by addition of saturated $NH_4Cl_{(aq)}$ (50 mL). Layers were separated and the aqueous layer was extracted with Et_2O (3 × 100 mL). Combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure.

Silica gel chromatography (1:9 EtOAc/hexanes) gave **3.S6** (1.13 g, 55%) as a clear, colorless oil.

IR (neat) ν 2960, 2855, 2226, 1755, 1678, 1612, 1448, 1386, 1331, 1254, 1166, 1020, 815 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) Keto tautomer: δ 2.71 (s, 3H), 2.53 (s, 2H), 2.33 (t, 2H, *J* = 7.0 Hz), 1.54 (p, 2H, *J* = 7.6 Hz), 1.36-1.25 (m, 4H), 0.86 (t, 3H, *J* = 7.0 Hz); Enol form: δ 11.77 (s, 1H), 5.24 (s, 1H), 2.70 (s, 3H), 2.32 (t, 2H, *J* = 7.2 Hz), 1.54 (p, 2H, *J* = 7.6 Hz), 1.36-1.25 (m, 4H), 0.86 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) Keto form: δ 178.7 (C), 166.6 (C), 97.1 (C), 80.2 (C), 52.4 (CH_3), 51.1 (CH_2), 30.9 (CH_2), 27.1 (CH_2), 22.0 (CH_2), 18.9 (CH_2), 12.8 (CH_3); Enol form: δ 172.6 (C), 155.8 (C), 96.4 (C), 95.8 (CH), 75.3 (C), 51.4 (CH_3), 30.9 (CH_2), 27.5 (CH_2), 22.0 (CH_2), 19.1 (CH_2), 12.8 (CH_3); HR-ESI-TOF-MS 197.1170 $[M+H]^+$ (calc. for $C_{11}H_{17}O_3$ 197.1178).

(*Z*)-methyl 3-(tosyloxyimino)dec-4-ynoate (3.S7)



A mixture of **3.S6** (1.0 g, 5.1 mmol), hydroxylamine hydrochloride (389 mg, 5.60 mmol) and pyridine (453 μL, 5.60 mmol) in EtOH (25 mL) was heated to 50 ˚C for 1 hour, then concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL) and washed with water (100 mL). The aqeous layer was extracted with EtOAc (3 × 25 mL) then combined

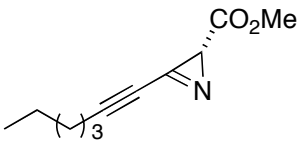
organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure.

Crude oxime was dissolved in anhydrous CH_2Cl_2 (25 mL) and *p*-toluenesulfonic anhydride (1.83 g, 5.60 mmol), pyridine (798 μL, 5.60 mmol) and a small crystal of DMAP were added. After 30 minutes a further 415 mg of *p*-toluenesulfonic anhydride and 100 μL of pyridine were added. After stirring 1 hour the mnixture was poured into saturated $NH_4Cl_{(aq)}$ (50 mL). Layers were separated and the aqueous layer extracted with EtOAc (3 × 25 mL). Combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Two rounds of silica flash chromatography (70:25:5 hexanes/CH_2Cl_2/Et_2O then 1:9 EtOAc/hexanes) gave **3.S7** (1.16 g, 62%) as a thick yellow oil.

IR (neat) 2960, 2931, 2865, 2227, 1748, 1600, 1445, 1390, 1196, 1177, 1103, 1021, 955, 885, 823, 668, 548 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) Major isomer: δ 7.81 (m, 2H), 7.32-7.29 (m, 2H), 2.65 (s, 3H), 2.32 (s, 2H), 2.41 (s, 3H), 2.39 (t, 2H, *J* = 7.2 Hz), 1.58-1.48 (m, 2H), 1.38-1.22 (m, 4H), 0.88 (t, 3H, *J* = 7.0 Hz); Minor isomer: δ 7.81 (m, 2H), 7.32-7.29 (m, 2H), 2.66 (s, 3H), 2.49 (s, 2H), 2.41 (s, 3H), 2.30 (t, 2H, *J* = 7.0 Hz), 1.58-1.48 (m, 2H), 1.38-1.22 (m, 4H), 0.88 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) Major: δ 167.6 (C), 145.1 (C), 144.6 (C), 132.2 (C), 129.6 (CH), 128.8 (CH), 107.8 (C), 70.7 (C), 52.4 (CH_3), 40.0 (CH_2), 30.8 (CH_2), 27.3 (CH_2), 22.0 (CH_2), 21.6 (CH_3), 19.6 (CH_2), 12.8 (CH_3); Minor isomer: δ 166.7 (C), 147.4 (C), 145.4 (C), 131.9 (C), 129.7 (CH), 128.9 (CH), 100.1 (C), 72.8 (C), 52.5 (CH_3), 37.4 (CH_2), 30.9

(CH_2), 27.3 (CH_2), 22.0 (CH_2), 21.7 (CH_3), 19.4 (CH_2), 12.8 (CH_3); HR-ESI-TOF-MS 366.1373 $[M+H]^+$ (calc. for $C_{18}H_{24}NO_5S$ 366.1375).

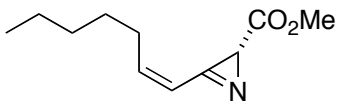
(*R*)-methyl 3-(hept-1-ynyl)-2*H*-azirine-2-carboxylate (3.S8)



3.7 (500 mg, 1.37 mmol) was added as a solution in toluene (10 mL followed by a 5 mL rinse) to a mixture of quinidine (1.33 g, 4.10 mmol) in toluene (230 mL) at 0 ˚C. The mixture was stirred at this temperature for 42 hours, then at 5 ˚C for 5.25 hours then room temperature for 30 minutes. The mixture was then poured into 0.05 M HCl (200 mL) and extracted with Et_2O (3 × 100 mL). Combined organic layers were washed with water (100 mL) and brine (100 mL) then dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (1:9 Et_2O/hexanes) gave **3.S8** (241 mg, 91%) as a clear, colorless oil.

$[\alpha]^{22}$ −192.5 (*c* 1.55, $CHCl_3$); IR (neat) ν 2955, 2935, 2865, 2230, 1740, 1464, 1441, 1348, 1274, 1196, 1037, 1002, 796, 715, 536, 485 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.71 (s, 3H), 2.68 (s, 1H), 2.55 (t, 2H, *J* = 7.2 Hz), 1.63 (p, 2H, *J* = 7.4 Hz), 1.40-1.26 (m, 4H), 0.88 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (C), 149.3 (C), 118.2 (C), 64.6 (C), 52.4 (CH_3), 31.5 (CH), 30.9 (CH_2), 27.1 (CH_2), 22.0 (CH_2), 20.2 (CH_2), 12.8 (CH_3); HR-ESI-TOF-MS 194.1180 $[M+H]^+$ (calc. for $C_{11}H_{16}NO_2$ 194.1181).

(*R*,*Z*)-methyl 3-(hept-1-enyl)-2*H*-azirine-2-carboxylate ((−)-3.49)



A mixture of Lindlar's catalyst (8.3 mg, 0.078 mmol) and quinoline (2.71 mL of a stock solution prepared from 40 µL quinoline in 20 mL hexanes) in hexanes (50 mL) was evacuated and purged with H_2 five times then cooled to 0 ˚C. **3.S8** (150 mg, 0.78 mmol) was added as a solution in hexanes (6.2 mL). After stirring 15 minutes at 0 ˚C the mixture was filtered through celite and concentrated under reduced pressure. Silica flash chromatography (15:85 Et_2O/hexanes) followed by SiO_2 HPLC (8:92 EtOAc/hexanes, 10 mL/min) gave (−)-**3.49** (96 mg) in 95% purity (61%) as a clear colorless oil. The compound was further purified by RP C18 HPLC (18:82 MeOH/H_2O, 8 mL/min) prior to characterization and fungal assay. Chiral HPLC (Chiralpak AD, 1:9 *i*-PrOH/hexanes) showed *ee* = 61%.

$[\alpha]^{22}$ −182.1 (*c* 1.69, $CHCl_3$); IR (neat) ν 2958, 2929, 2861, 1763, 1732, 1623, 1441, 1340, 1266, 1196, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.54 (dt, 1H, *J* = 10.6, 7.9 Hz), 6.38 (dt, 1H, *J* = 10.6, 1.3 Hz), 2.69 (s, 3H), 2.59 (s, 1H), 2.46 (m, 2H), 1.41 (m, 2H), 1.30-1.18 (m, 4H), 0.84 (t, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1 (C), 154.1 (C), 152.6 (CH), 111.1 (CH), 52.1 (CH_3), 31.2 (CH_2), 29.5 (CH_2), 29.2 ($CH_)$, 28.4 (CH_2), 22.3 (CH_2), 12.9 (CH_3); HR-ESI-TOF-MS 196.1336 $[M+H]^+$ (calc. for $C_{11}H_{18}NO_2$ 196.1338).

References

(1) 1*H*- and 2*H*-azirine: Mayer, P. M.; Taylor, M. S.; Wong, M. W.; Radom, L. *J. Phys. Chem. A* **1998**, *102*, 7074-7080. Aziridine: Nielsen, I. M. B. *J. Phys. Chem.* **1998**, *102*, 3193-3201. Oxirane: Benson, S. W.; Cruickshank, D. M.; Golden, D. M.; Haugen, G.R.; O'Neal, H. E.; Rodgers, A. S.; Shaw, R.; Walsh, R. *Chem. Rev.* **1969**, 279-324.

(2) Miller, T. W.; Tristram, E. W.; Wolf, F. J. *J. Antibiot.* **1971**, *24*, 48-50.

(3) Molinski, T. F.; Ireland, C. M. *J. Org. Chem.* **1988**, *53*, 2103-2105.

(4) Salomon, C. E.; Williams, D. H.; Faulkner, D. J. *J. Nat. Prod.* **1995**, *58*, 1463-1466.

(5) Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592-2597.

(6) The only other reported 1-bromo-1-chloro-1-alkenes from natural sources are simple aliphatic ketones, alcohols and carboxylic acids from marine algae that may derive from haphazard electrophilic α-halogenation-elimination reactions of small molecular weight carbonyl compounds. (a) McConnell, O. J.; Fenical, W. *Tetrahedron Lett*. **1977**, *22*, 1851-1854, (b) (a) McConnell, O. J.; Fenical, W. *Phytochemistry* **1980**, *19*, 233-247. (c) Woolard, F. X.; Moore, R. E. *Tetrahedron* **1976**, *32*, 2843-2846. (d) Burreson, B. J.; Moore, R. E.; Roller, P. P. *J. Agric. Food Chem.* **1976**, 24, 856-861.

(7) Natural (*E*)-dysidazirine isolated in 1987 and stored at −20 °C until 2007. Compound was confirmed as intact and unchanged ^{1}H NMR. Optical rotation ($[\alpha]^{22}$ −32.5) was also significantly lower than the original report (−165, Ref. 3). The optical purity of natural (−)-**3.2** was estimated to be 89% following total synthesis of (−)-**3.2** (*ee* ≥ 95%) by Davis *et al.* (Ref. 17).

(8) Zweifel, G. Lewis, W.; On, H. P. *J. Am. Chem. Soc.* **1979**, *101*, 5101-5102.

(9) Masuda, Y.; Suyama, T.; Murata, T.; Watanabe, S.; *J. Chem. Soc. Perkin Trans. 1.* **1995**, 2995

(10) Padwa, A. *Acct. Chem. Res.* **1976**, *9*, 371-378.

(11) a) Palacios, F.; de Retana, A. M. O.; de Marigorta, E. M.; de los Santos, J. M. *Org. Prep. Proced. Int.* **2002**, *34*, 219-269. b) Padwa, A.; Woolhouse, A. D. *In Comprehensive Heterocyclic Chemistry*; Katritzky, A. R.; Rees, C. W., Eds.; Pergamom Press: New York, 1984; Vol. 7, Chapter 5, p. 47.

(12) Neber, P. W.; Burgard, A. *Justus Liebigs Ann. Chem.* **1932**, 281-294.

(13) Cram, D. J.; Hatch, M. J. *J. Am. Chem. Soc.* **1953**, *75*, 33-38.

(14) Corkins, H. G.; Storace, L.; Osgood, E. *J. Org. Chem.* **1980**, *45*, 3156-3159.

(15) Piskunova, I. P.; Eremeev, A. V.; Mishnev, A. F.; Vosekalnaa, I. A. *Tetrahedron* **1993**, *49*, 4671-4676.

(16) Verstappen, M. M. H.; Ariaans, G. J. A.; Zwannenburg, B. *J. Am. Chem. Soc.* **1996**, *118*, 8491-8492.

(17) a) Palacios, F.; de Retana, A. M. O.; Gil, J. I.; Ezpeleta, J. M. *J. Org. Chem.* **2000**, *65*, 3213-3217. b) Palacios, F.; de Retana, A. M. O.; Gil, J. I. *Tetrahedron Lett.* **2000**, *41*, 5363-5366.

(18) Gentilucci, L.; Grijzen, Y.; Thijs, L.; Zwanenburg, B. *Tetrahedron Lett.* **1995**, *36*, 4665-4668.

(19) a) Davis, F. A.; Reddy, G. V.; Liu, H. *J. Am. Chem. Soc.* **1995**, *117*, 3651-3652. b) Davis, F. A.; Liu, H.; Liang, C. –H.; Reddy, G. V.; Zhang, Y.; Fang, T.; Titus, D. D. *J. Org. Chem.* **1999**, *64*, 8929-8935.

(20) Sakai, T.; Kawabata, I.; Kishimoto, T.; Ema, T.; Utaka, M. *J. Org. Chem.* **1997**, *62*, 4906-4907.

(21) Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, *36*, 3769-3772.

(22) Performed by Dr. Doralyn S. Dalisay. The *in vitro* susceptibility of each compound was determined by the broth micro dilution method according to the guidelines of thev NCCLS: National Committee for Clinical Laboratory Standards. 2002 . Reference method for broth dilution antifungal susceptibility testing of yeast, 2nd ed. Approved standard M27-A2. National Committee for Clinical Laboratory Standards, Wayne, PA.

(23) Molinski, T. F. *Curr. Med. Chem. – Anti-Infective Agents* **2004**, *3*, 197-220.

(24) Nicholas, G. N.; Li, R.; MacMillan, J. B.; Molinski, T. F. *Biorg. Med. Chem. Lett.* **2002**, *12*, 2159-2162.

(25) Reviews on sphingolipid metabolism: a) Sims, K. J.; Spassieva, S. D.; Voit, E. O.; Obeid, L. M. *Biochem. Cell Biol.* **2004**, *82*, 45-61. b) Dickson, R. C.; Lester, R. L. *In Sphingolipid Biology* Hirabayashi, Y.; Igarashi, Y.; Merrill, Jr., A. H. Eds. Springer: New York, 2006, Part 2, Chapter 9, p141. c) Funato, K.; Vallée, B.; Riezman, H. *Biochemistry* **2002**, *41*, 15105-15114.

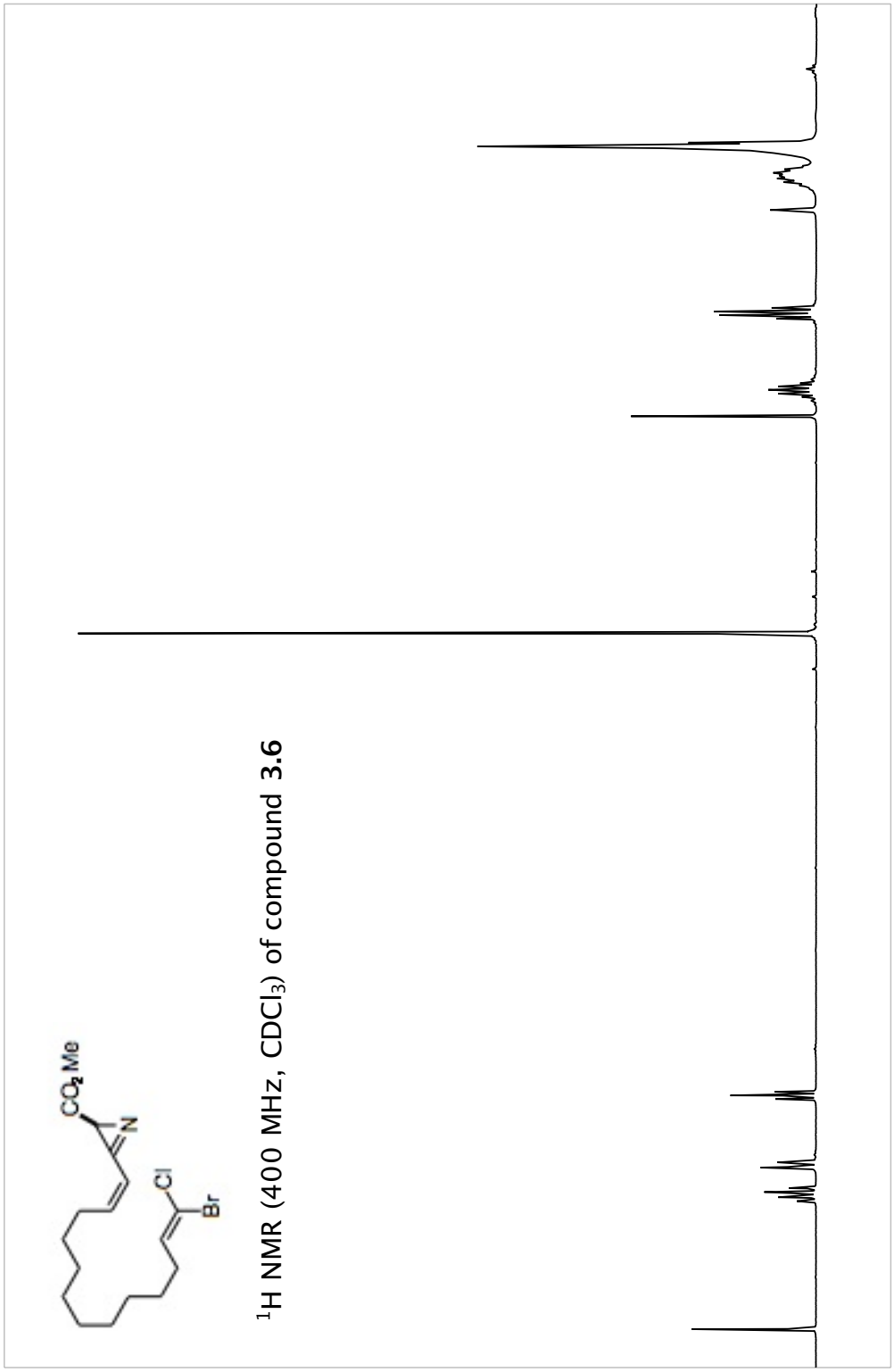
(26) Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1993**, *58*, 7578-7580.

(27) Gulavita, N. K.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 366-369.

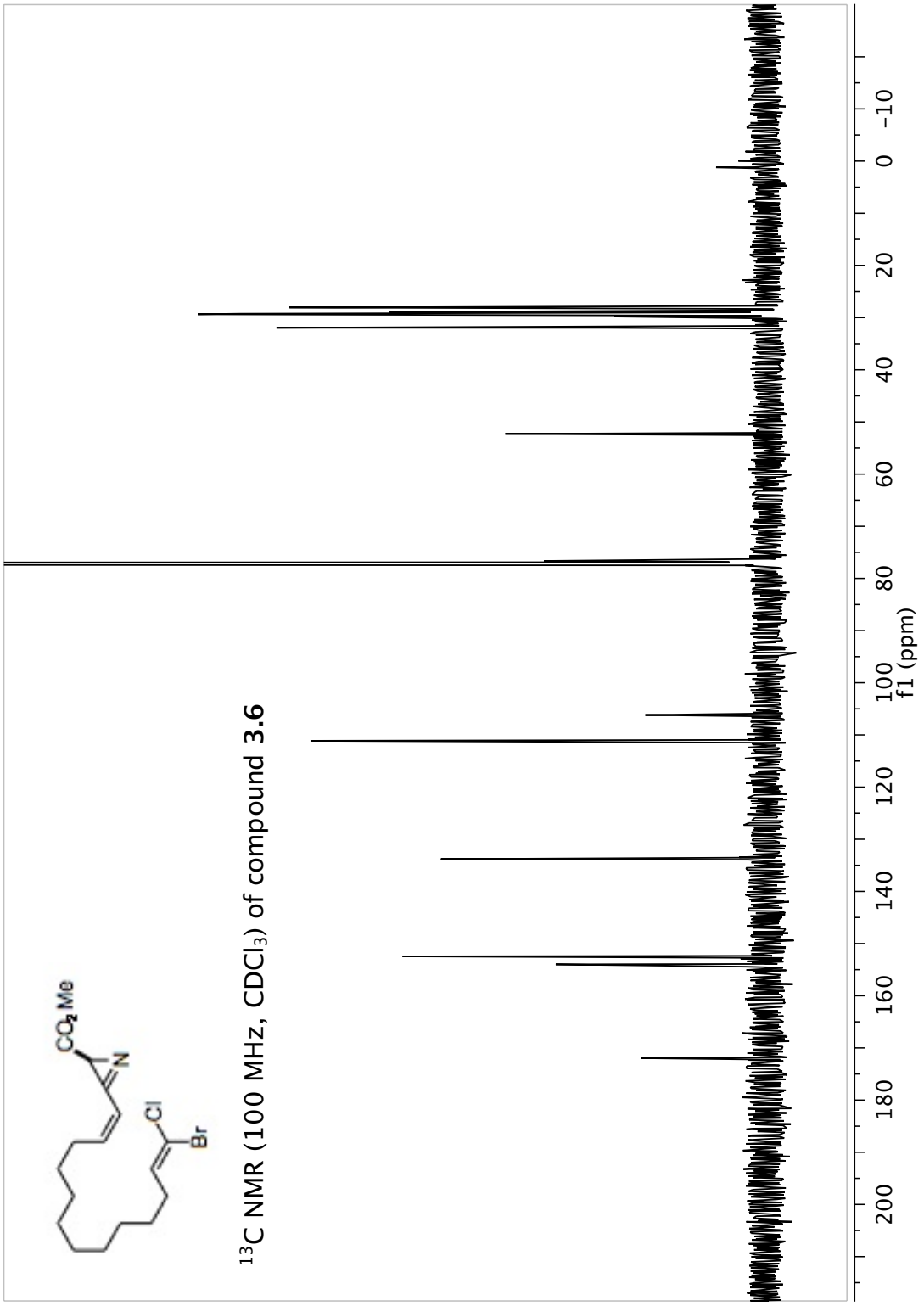
(28) Jares-Erijman, E. A.; Bapat, C. P.; Lithgow-Bertelloni, A.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 5732-5737.

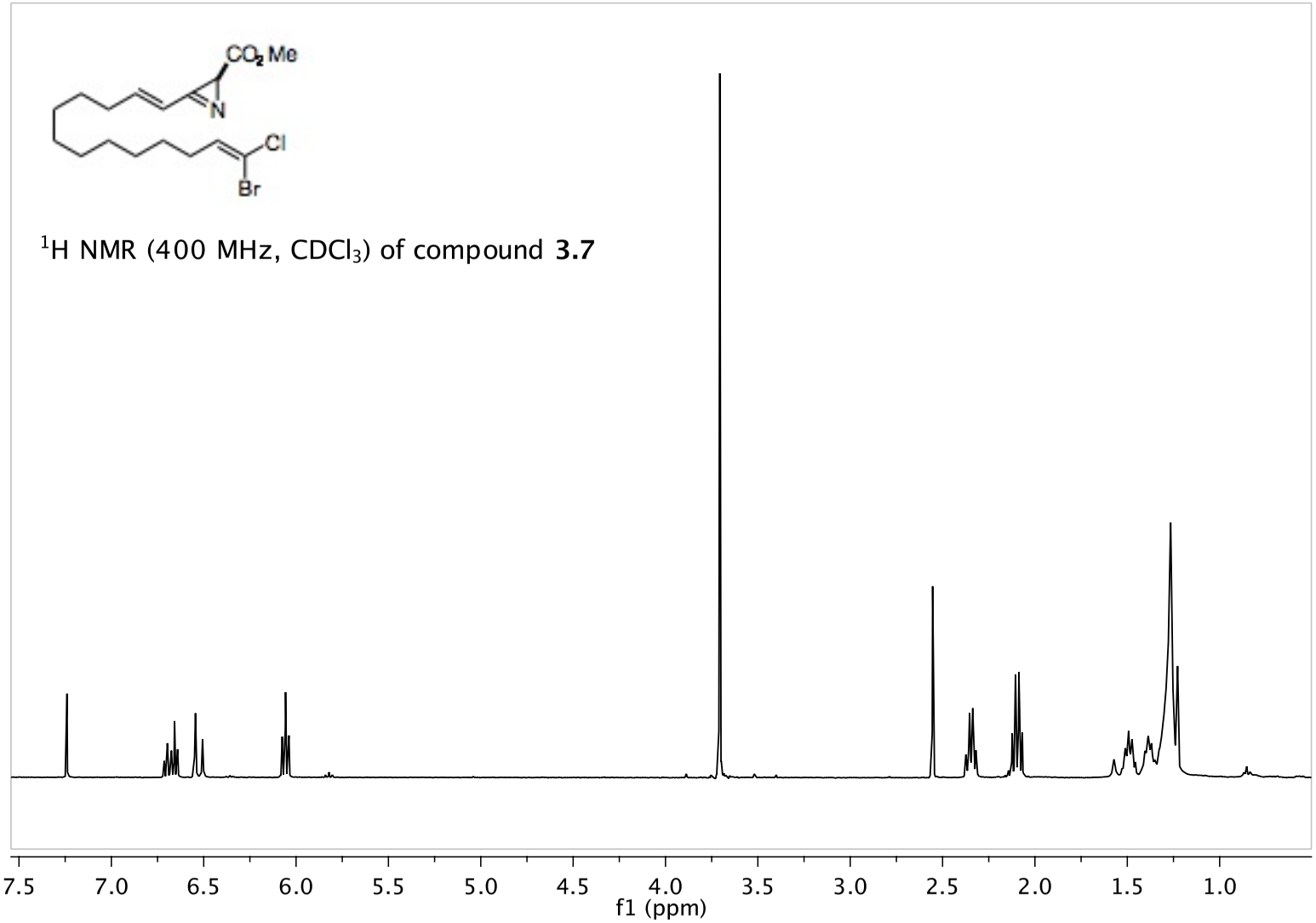
(29) Nicholas, G. M.; Hong, T. W.; Molinski, T. F.; Lerch, M. L.; Cacnilla, M. T.; Lebrilla, C. B. *J. Nat. Prod.* **1999**, *62*, 1678-1681.

(30) Dalisay, D. S.; Molinski, T.F. unpublished results.

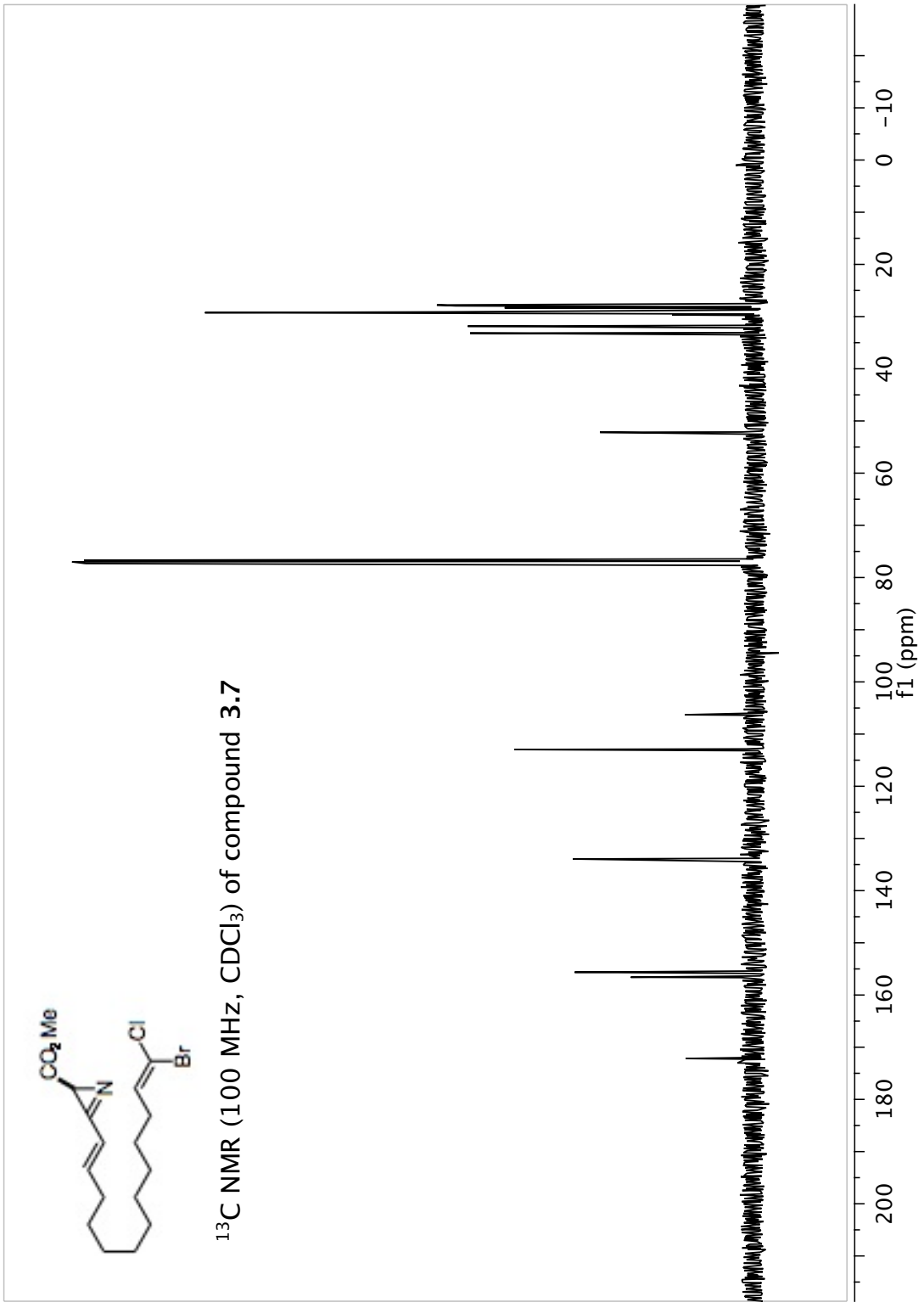


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.6**

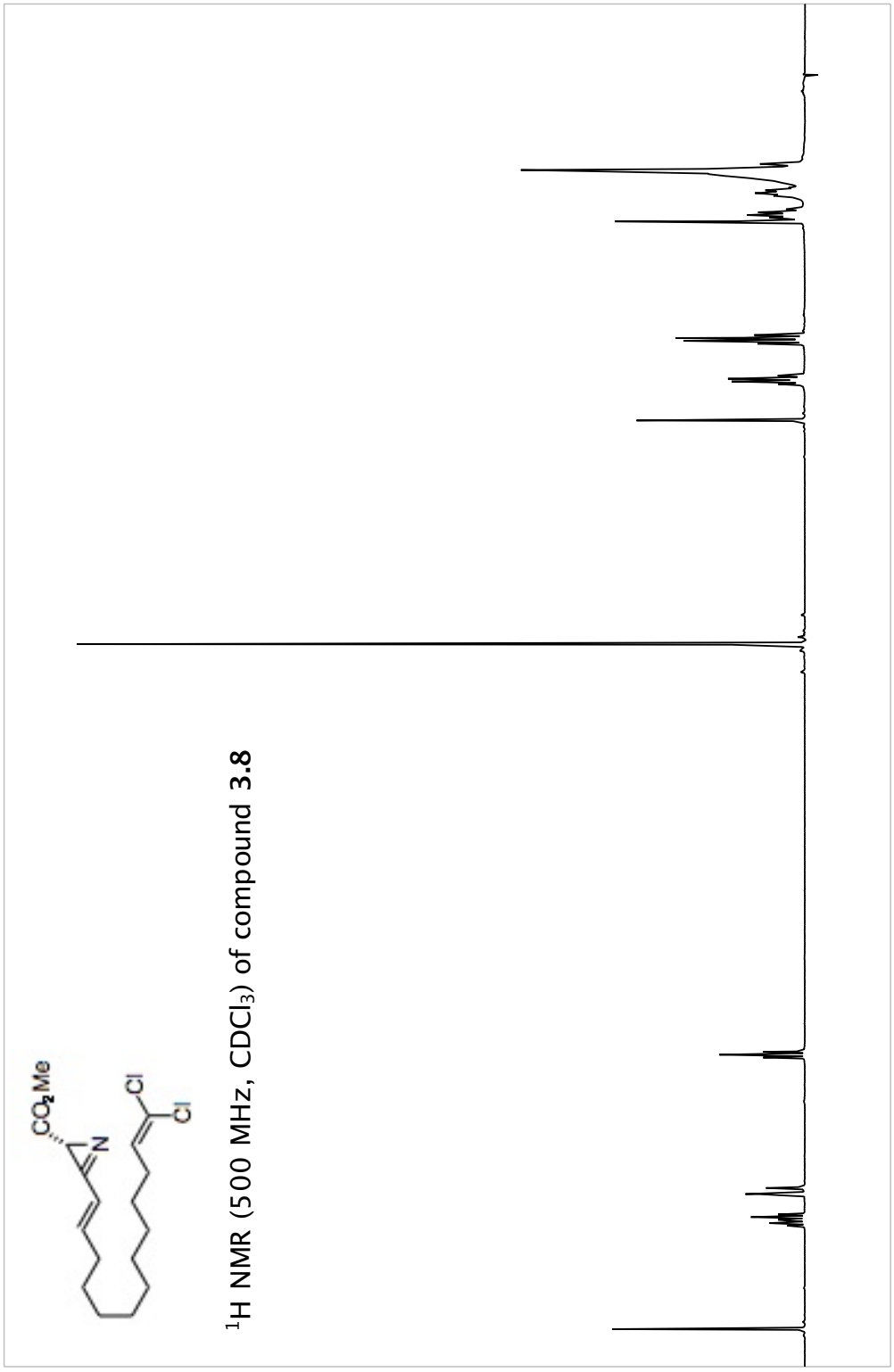

^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.6**
CO_2Me
N
Cl
Br
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



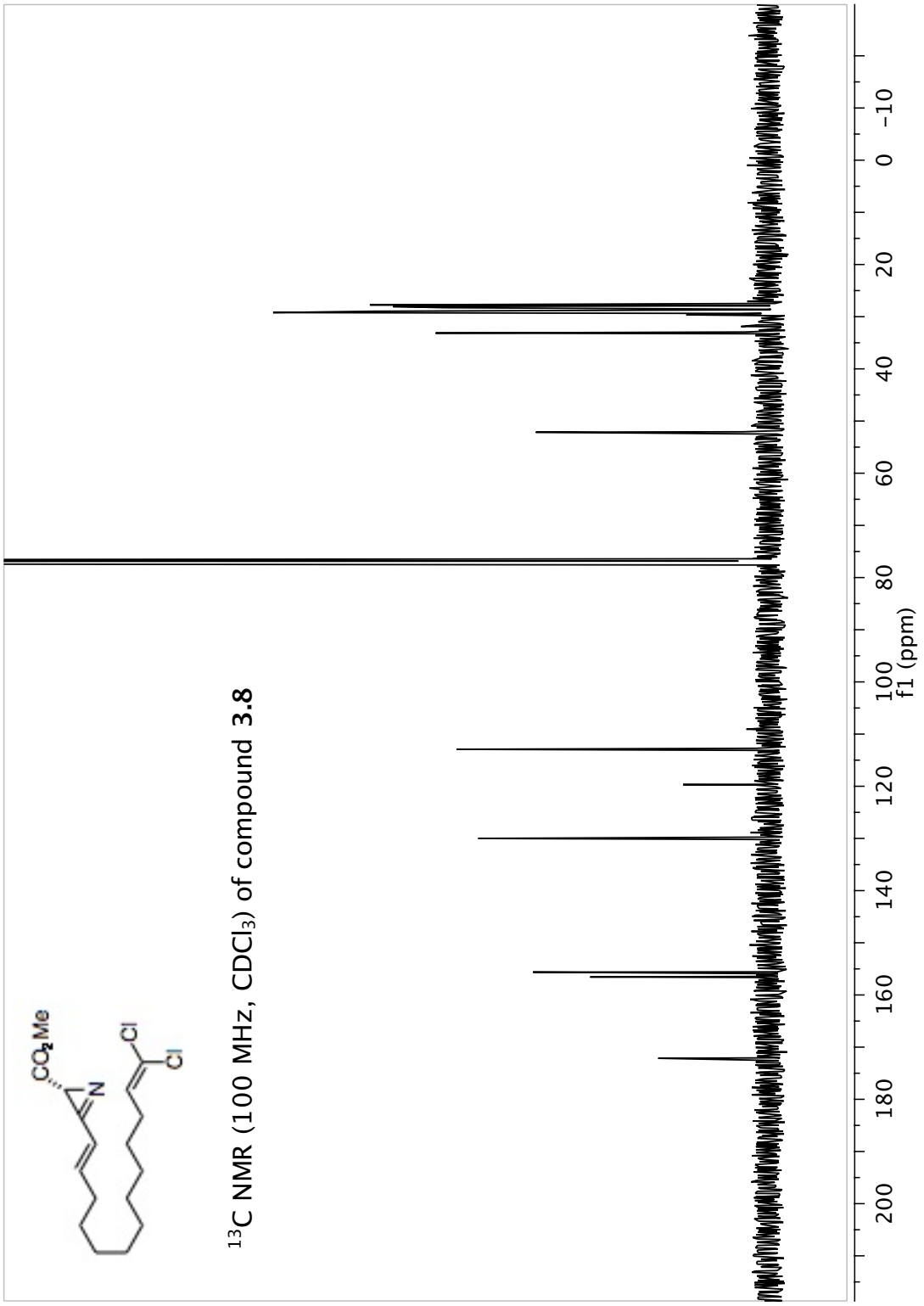
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.7**



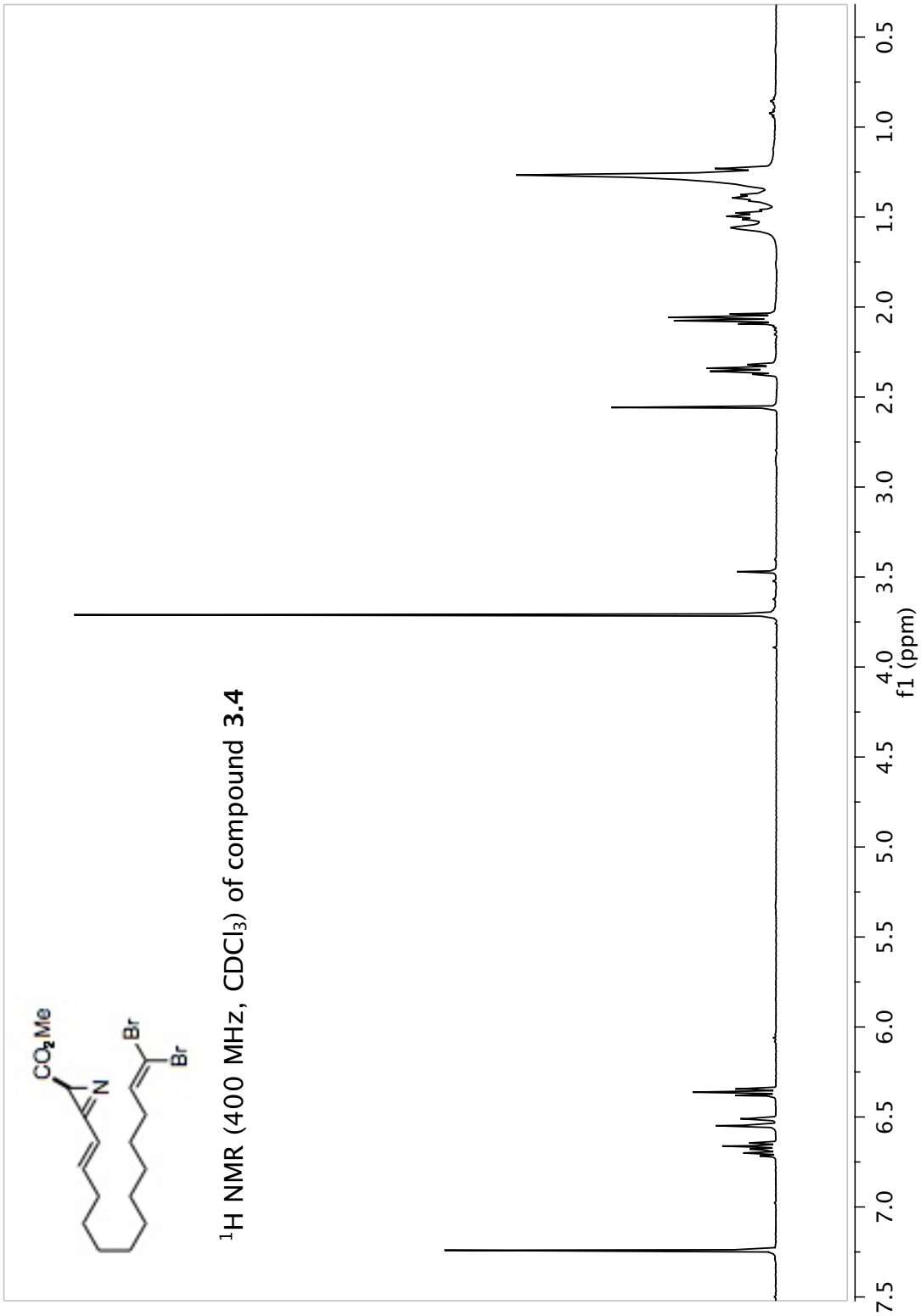
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.7**



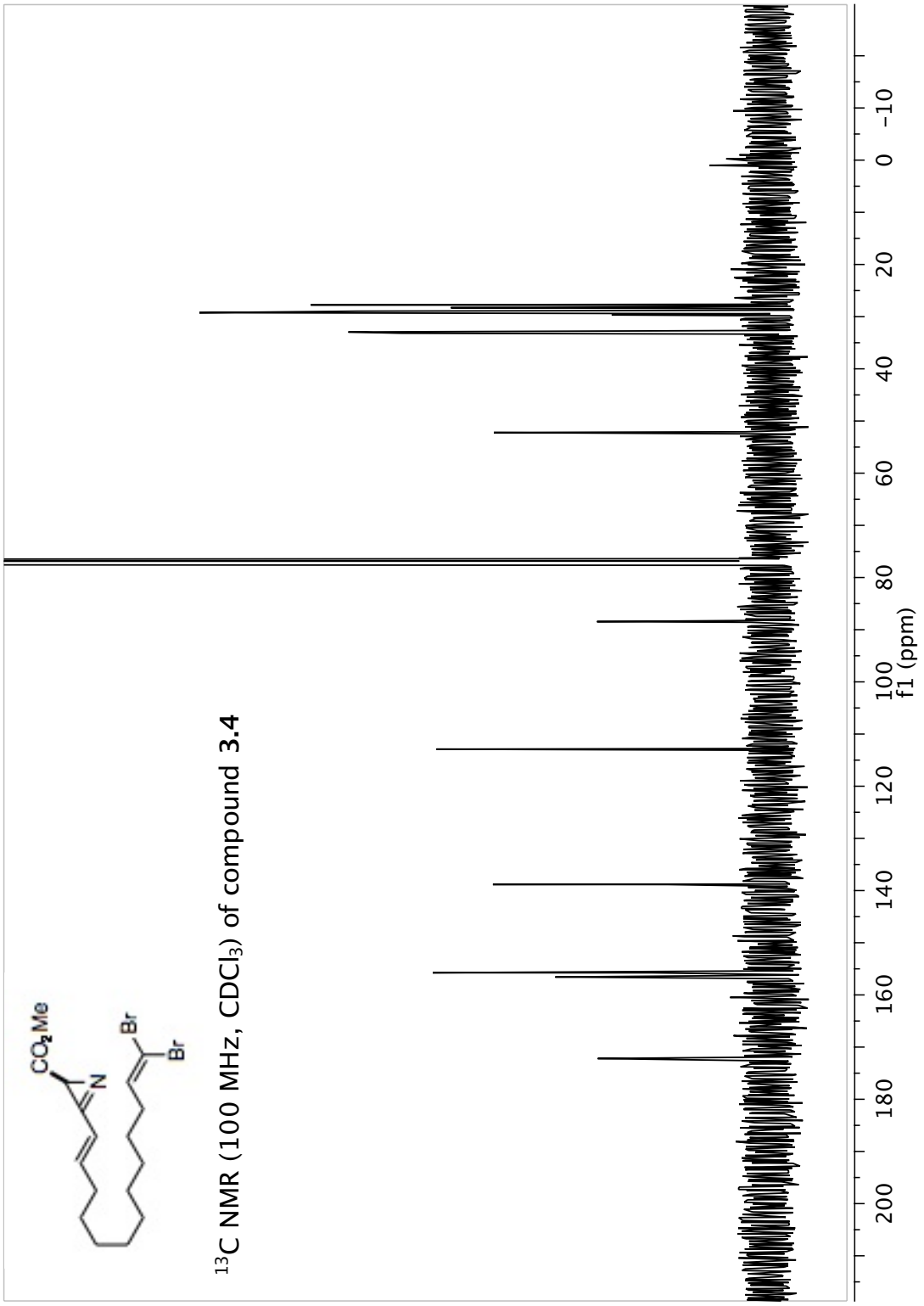
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3.8**



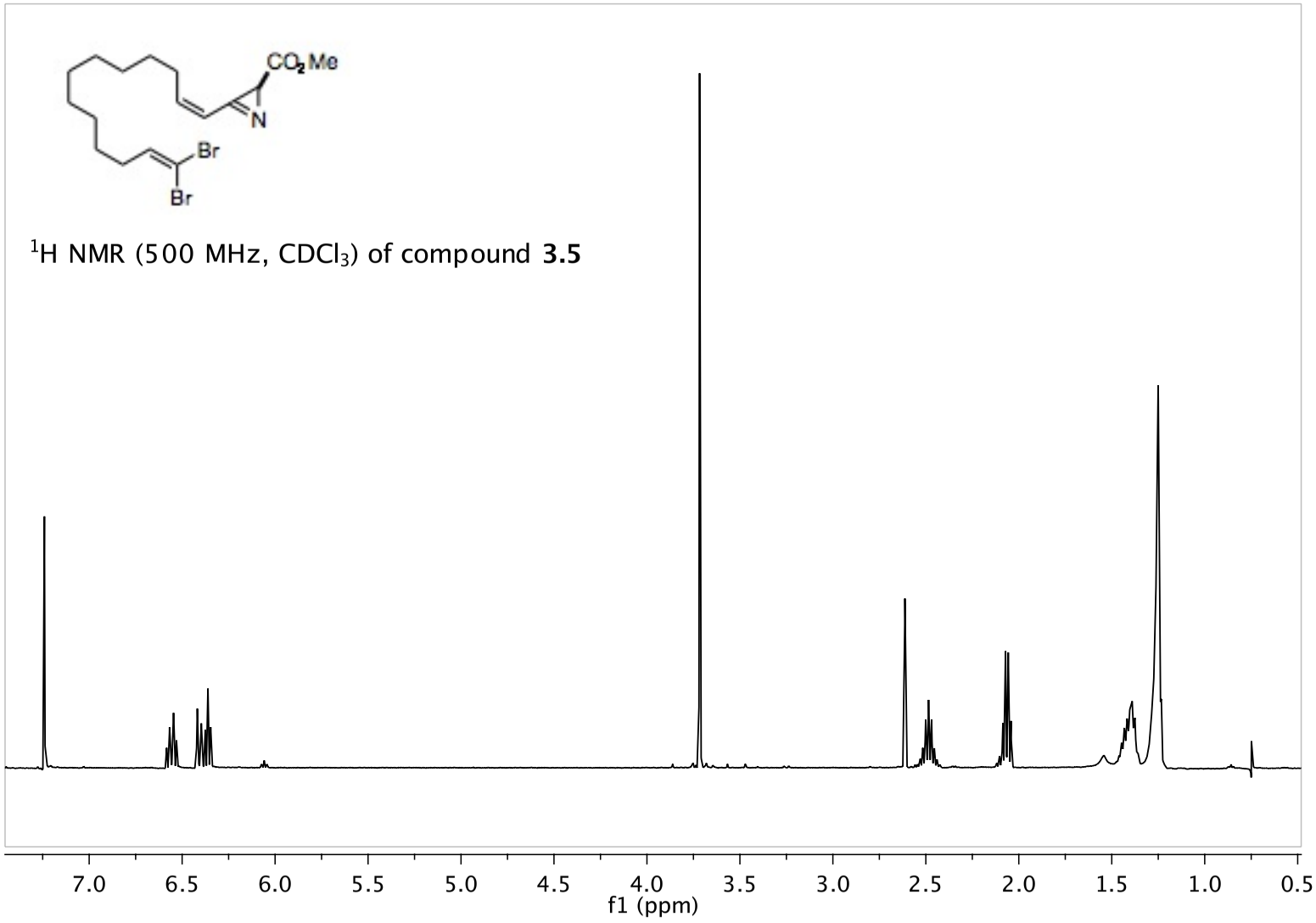
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.8**

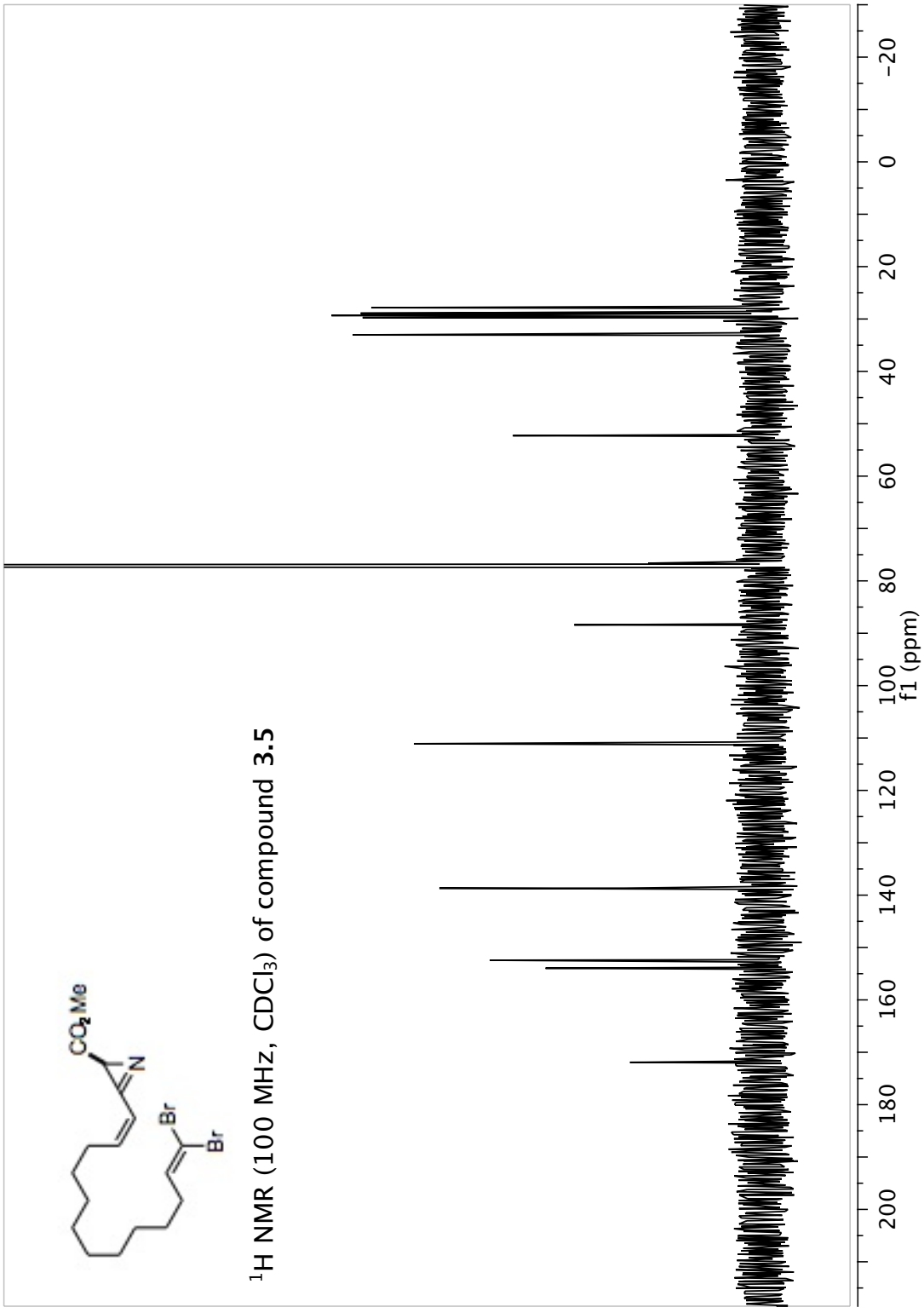


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.4**

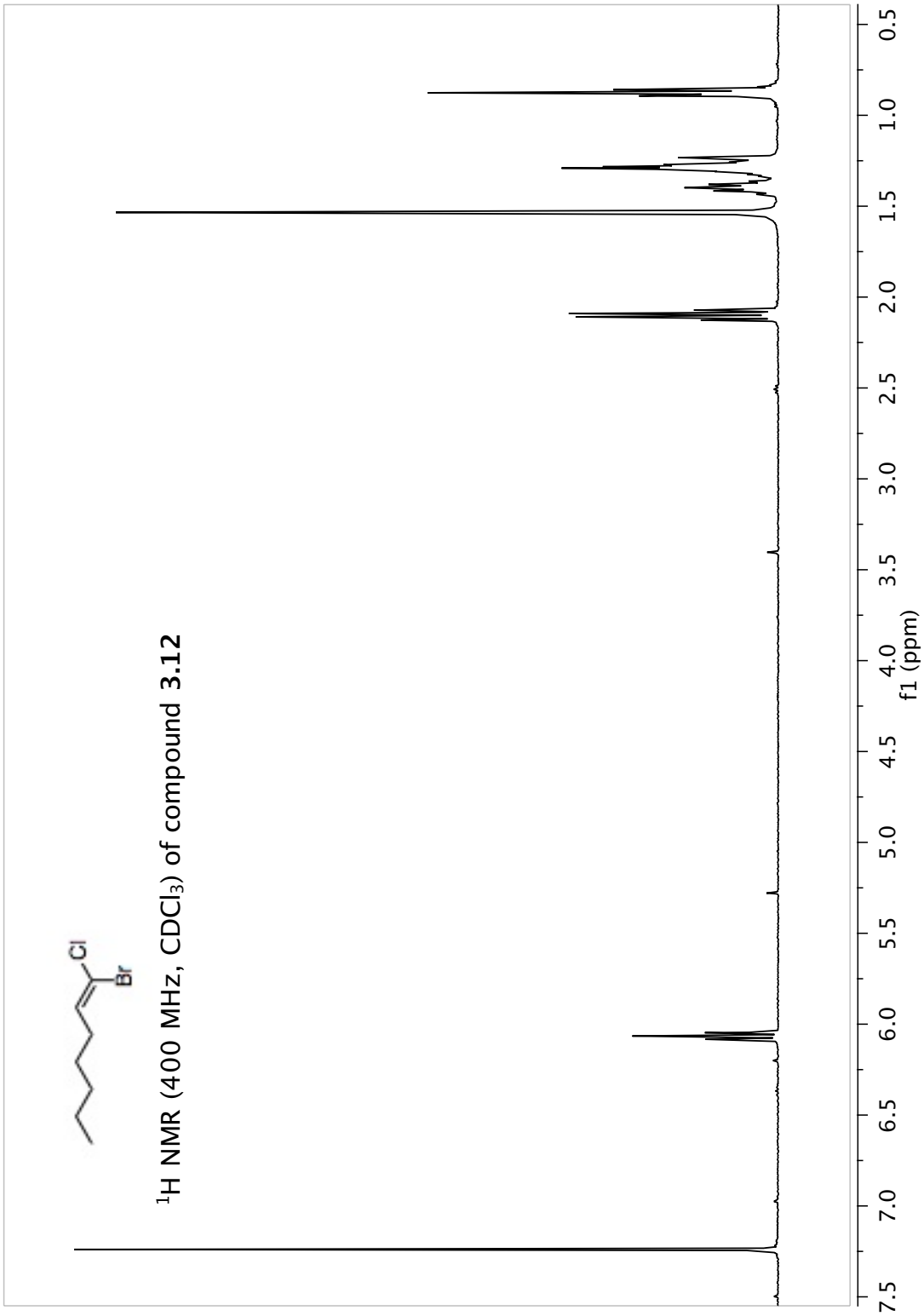


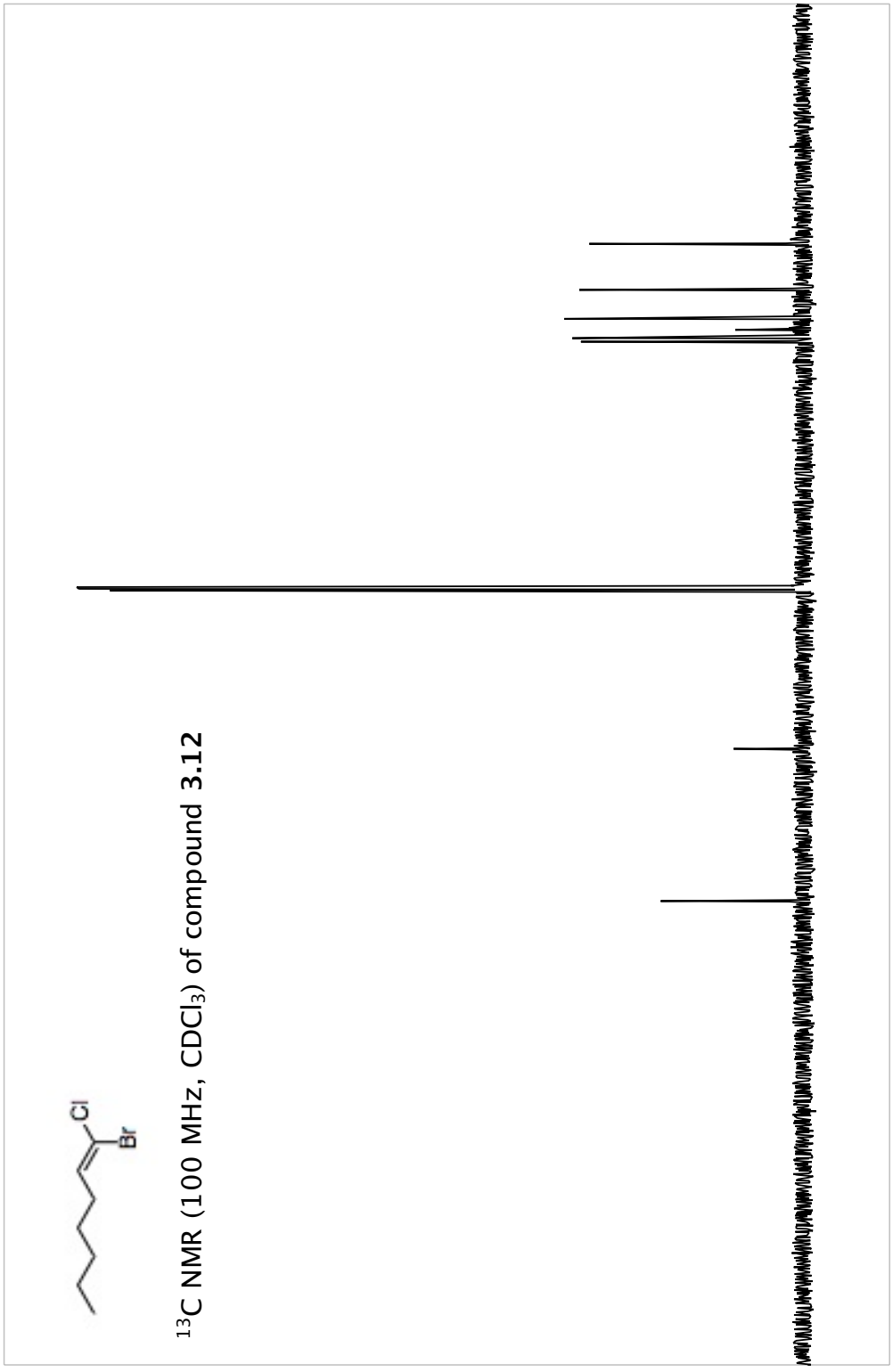
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.4**

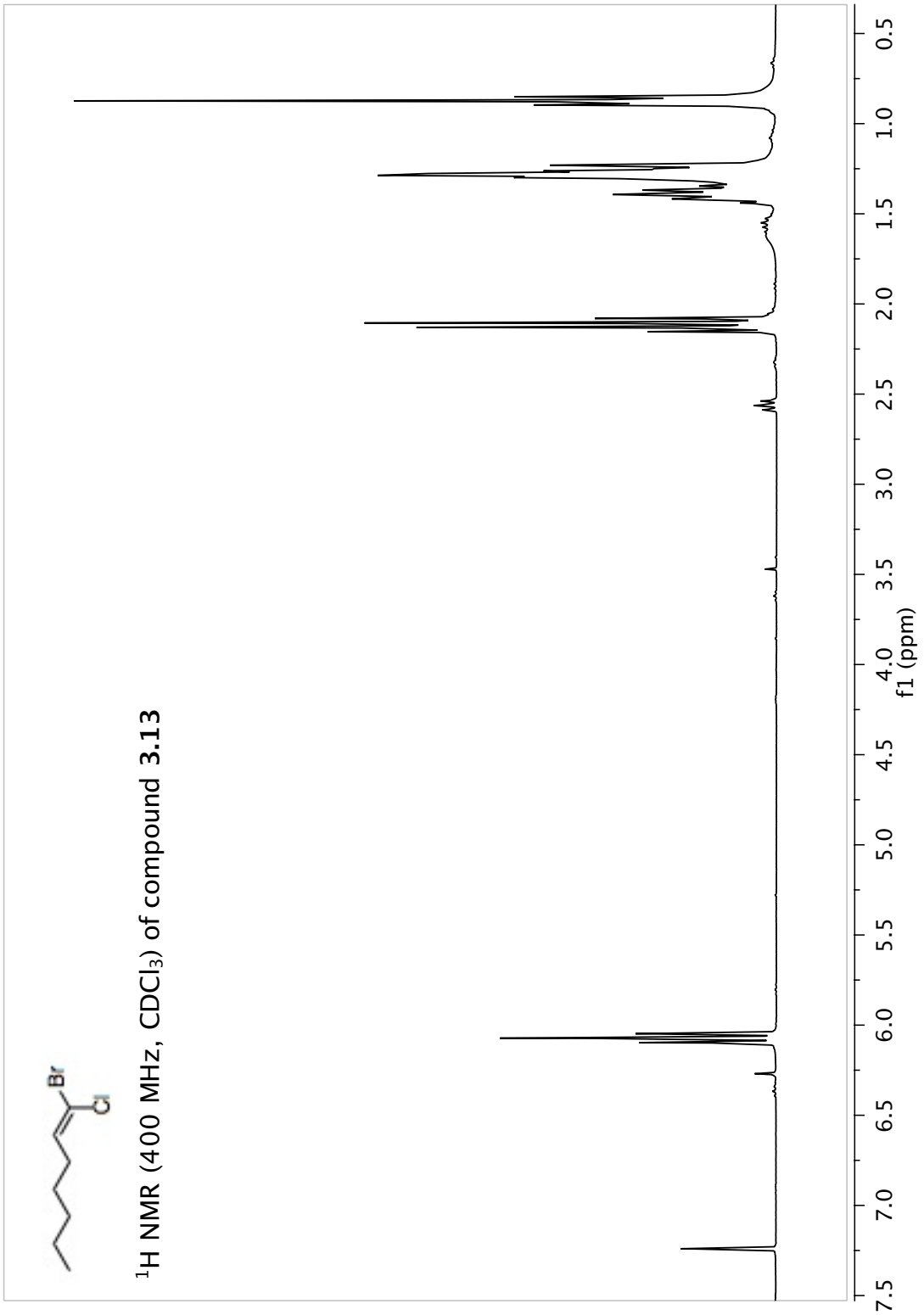

CO2Me
N
Br
Br
1H NMR (500 MHz, CDCl3) of compound 3.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

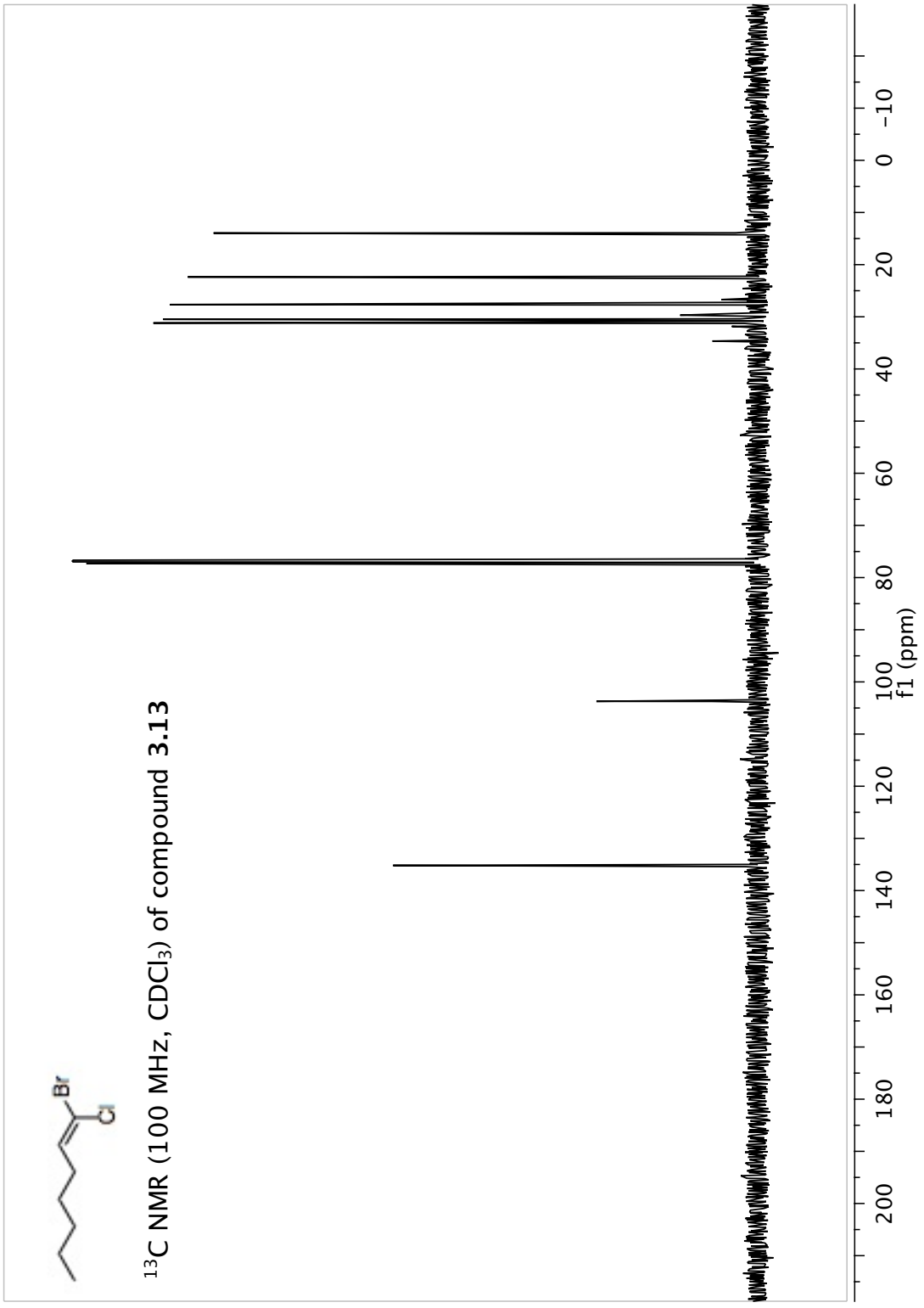


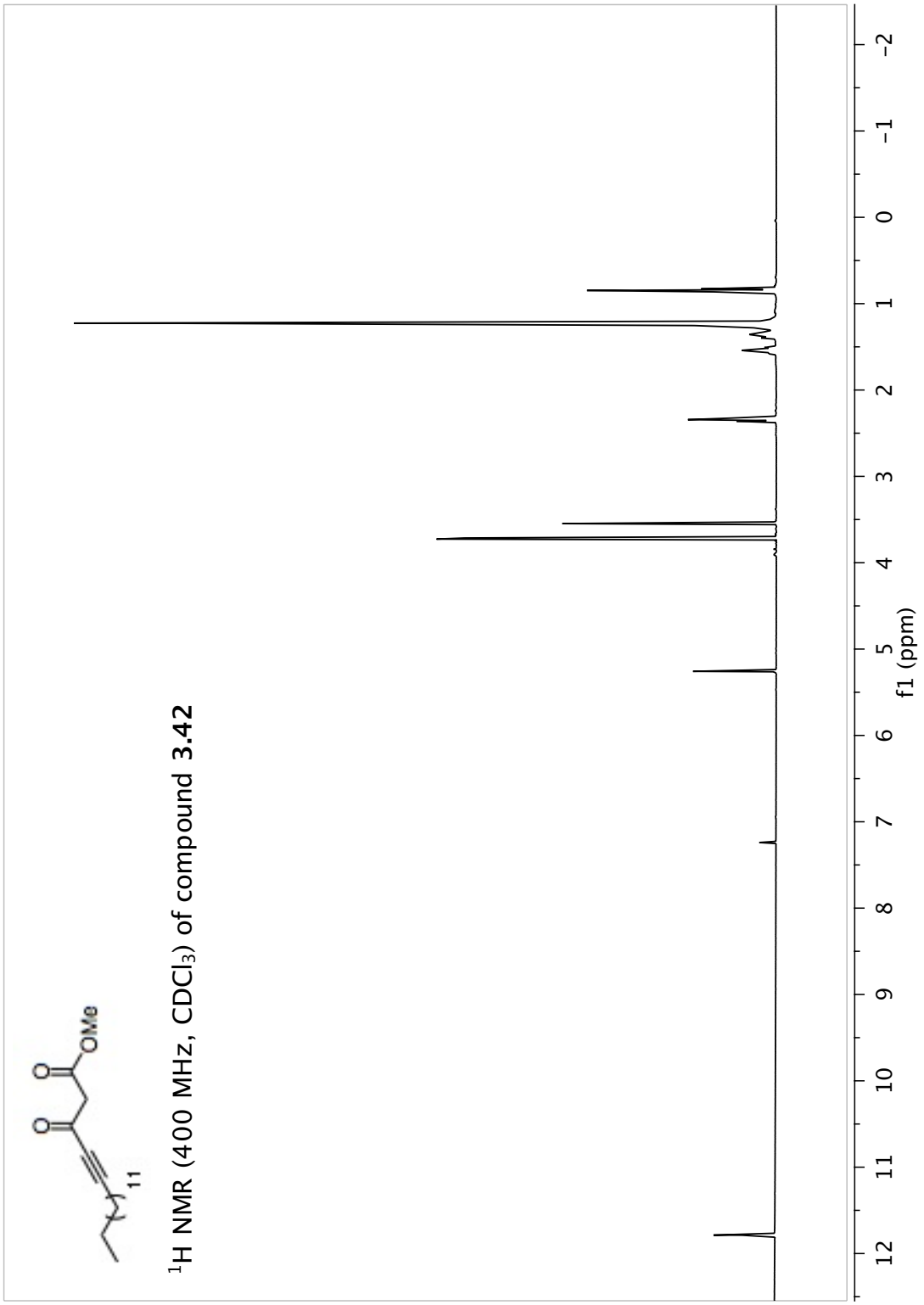
^{1}H NMR (100 MHz, $CDCl_3$) of compound **3.5**


Cl
Br
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.12**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

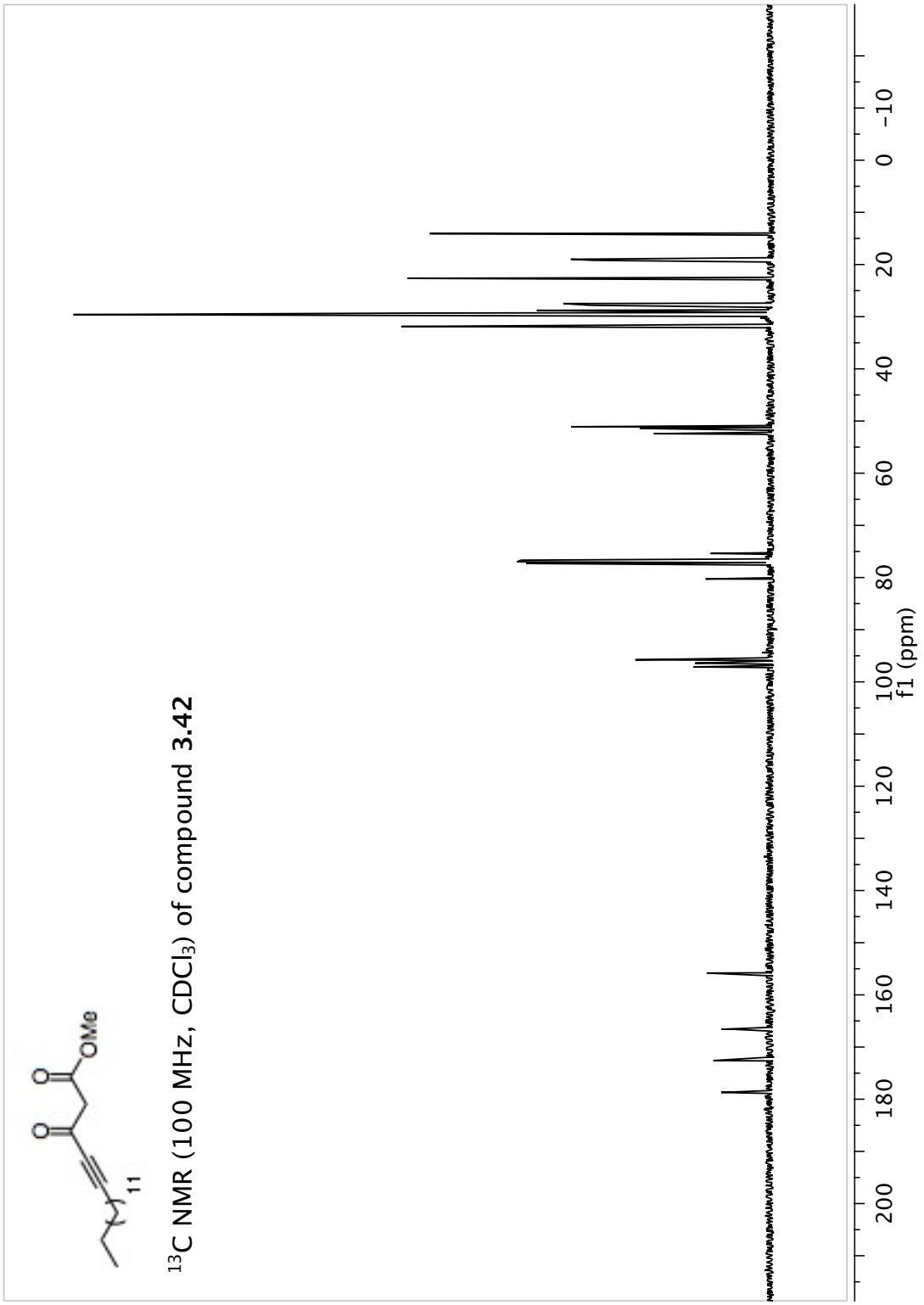

Cl
Br
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.12**
200
180
160
140
120
100
80
60
40
20
0
–10
f1 (ppm)


Br
Cl
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.13**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

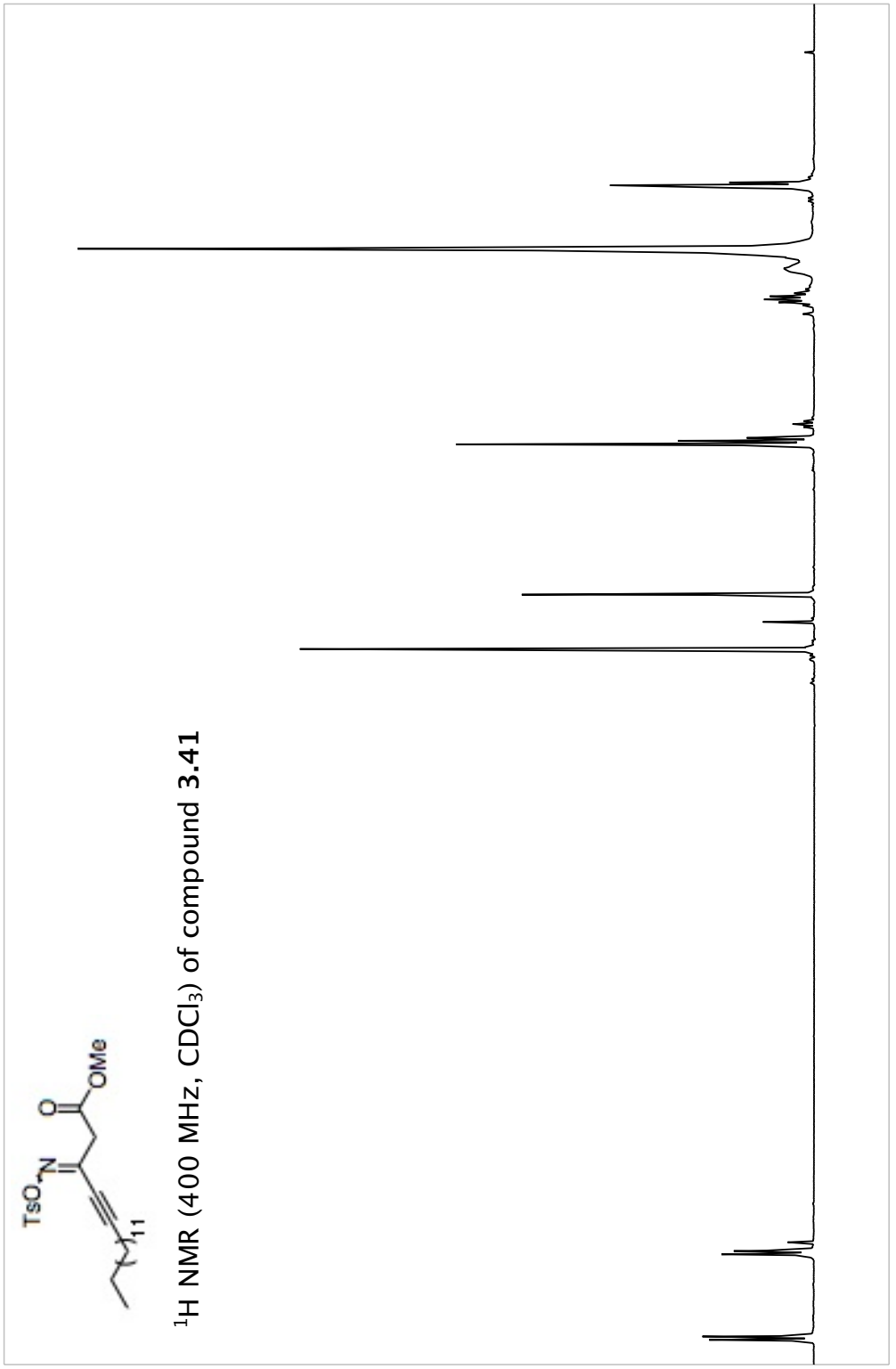

Br
Cl
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.13**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



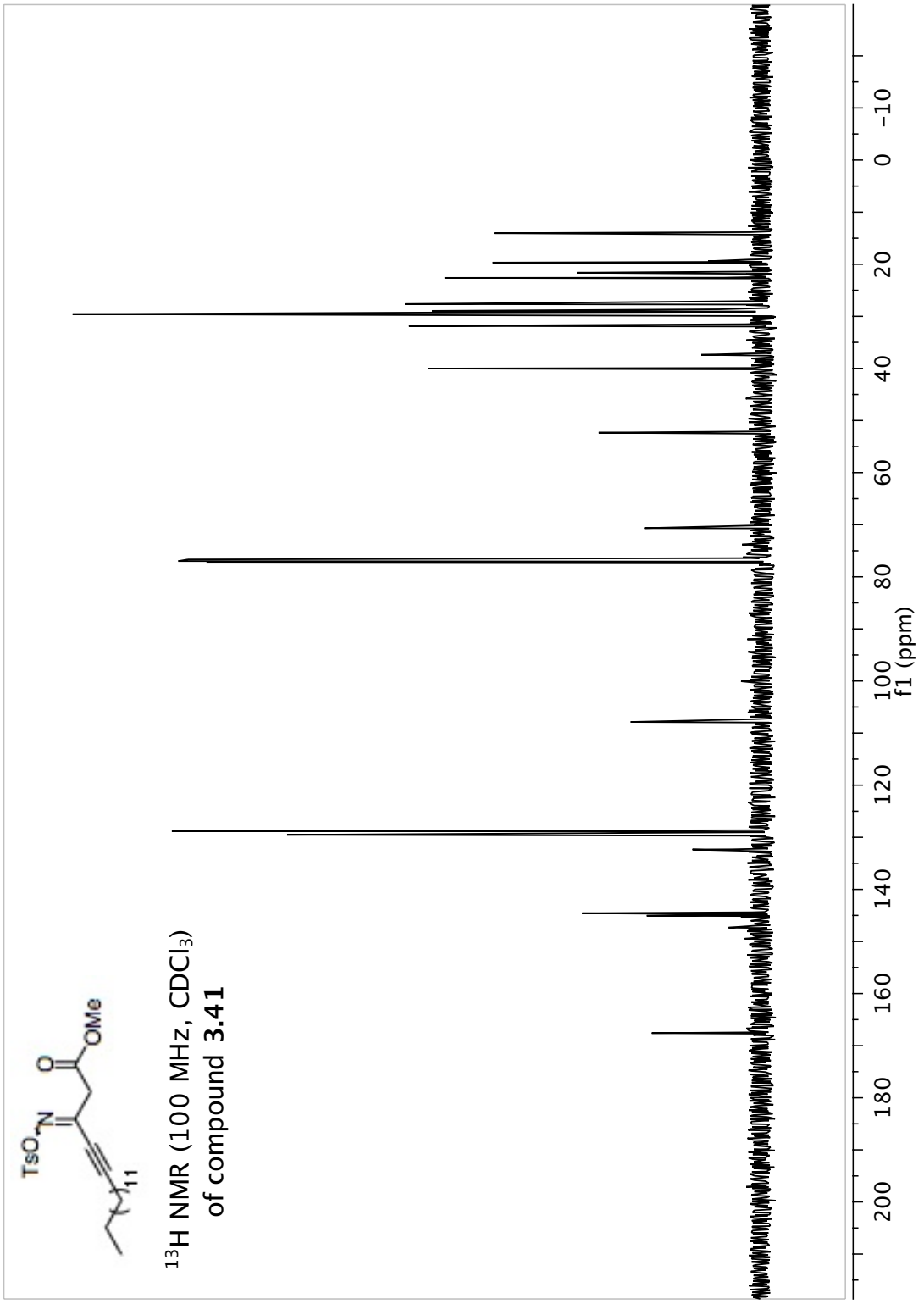
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.42**



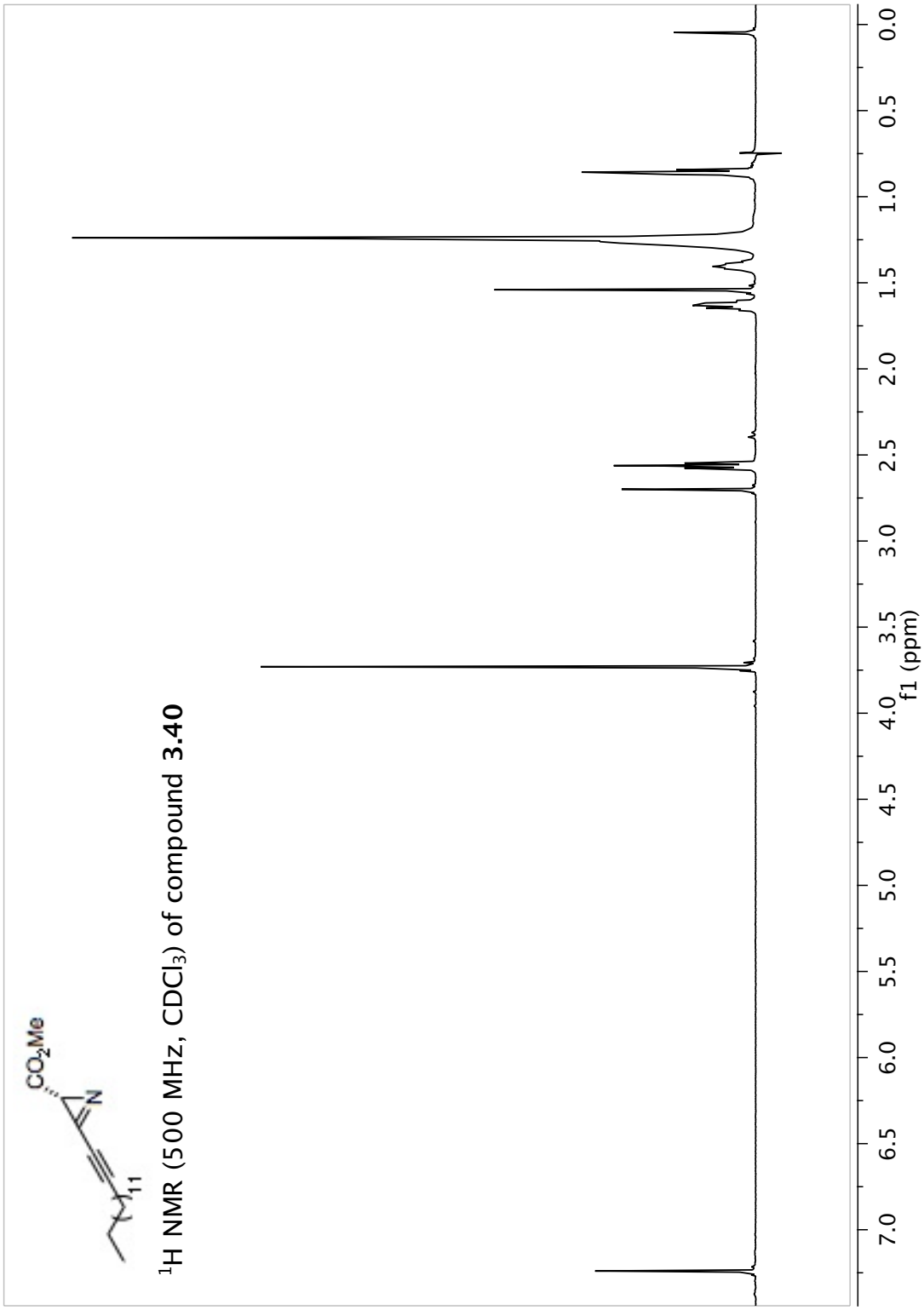
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.42**



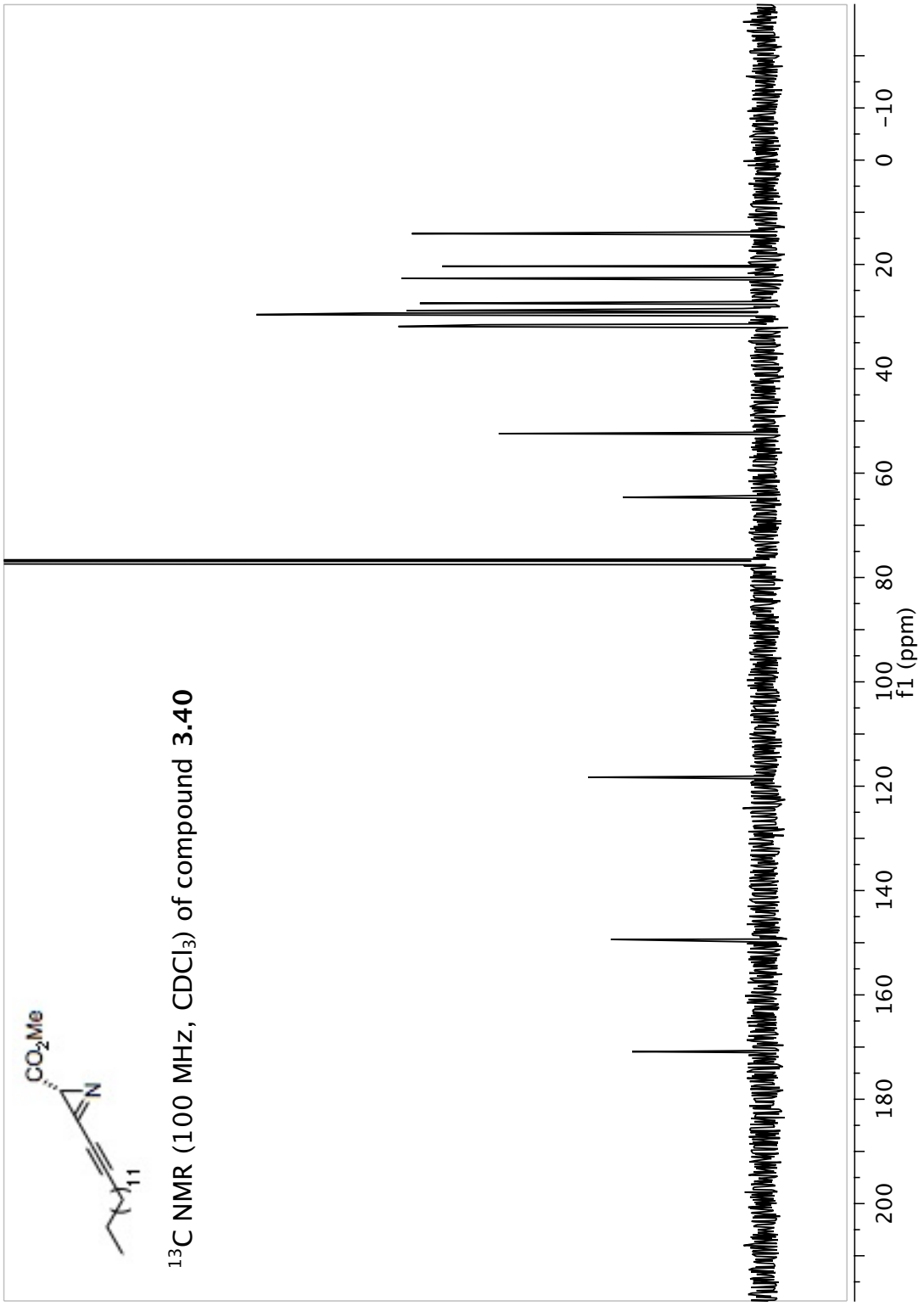
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.41**

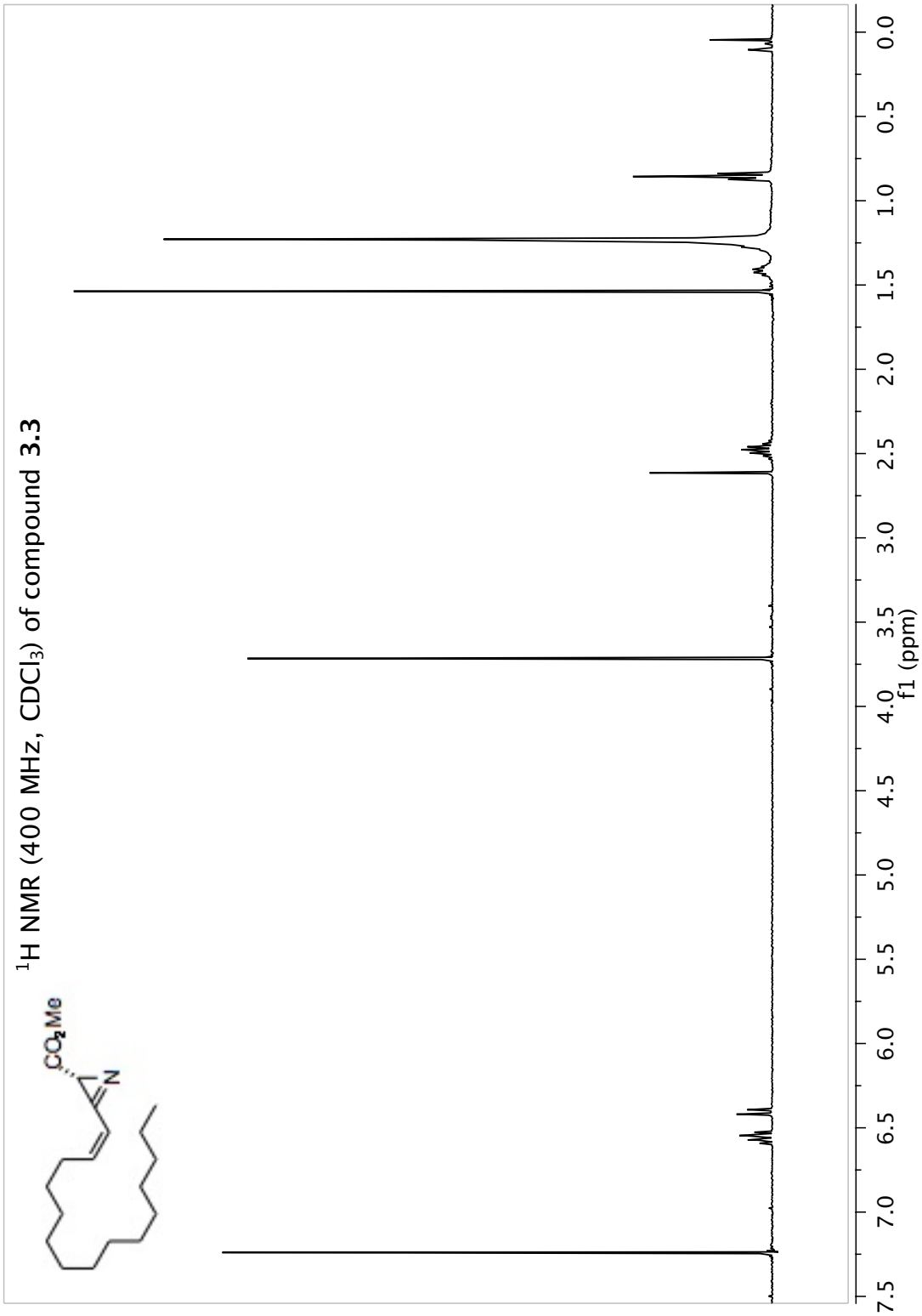


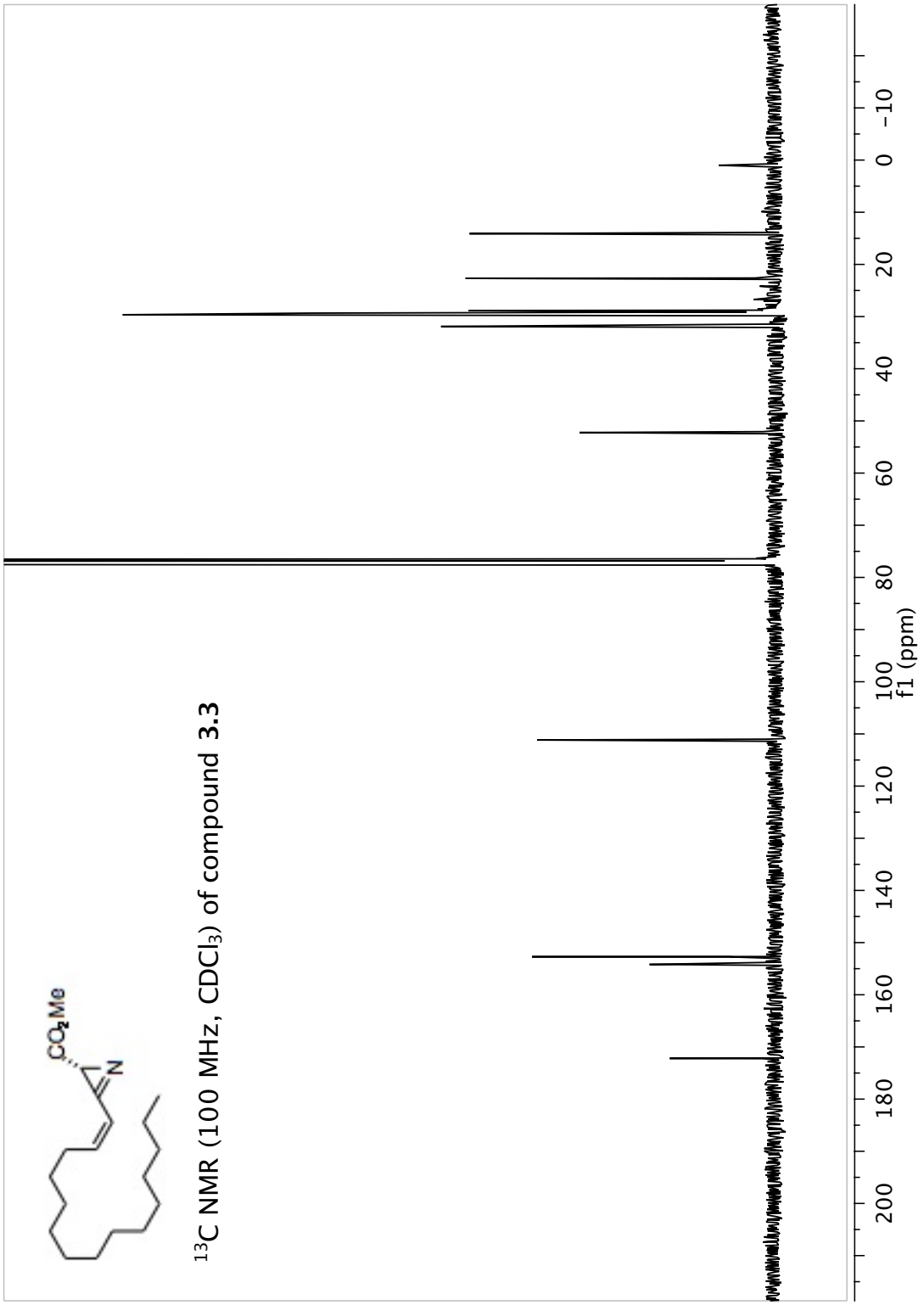
^{13}H NMR (100 MHz, $CDCl_3$)
of compound **3.41**

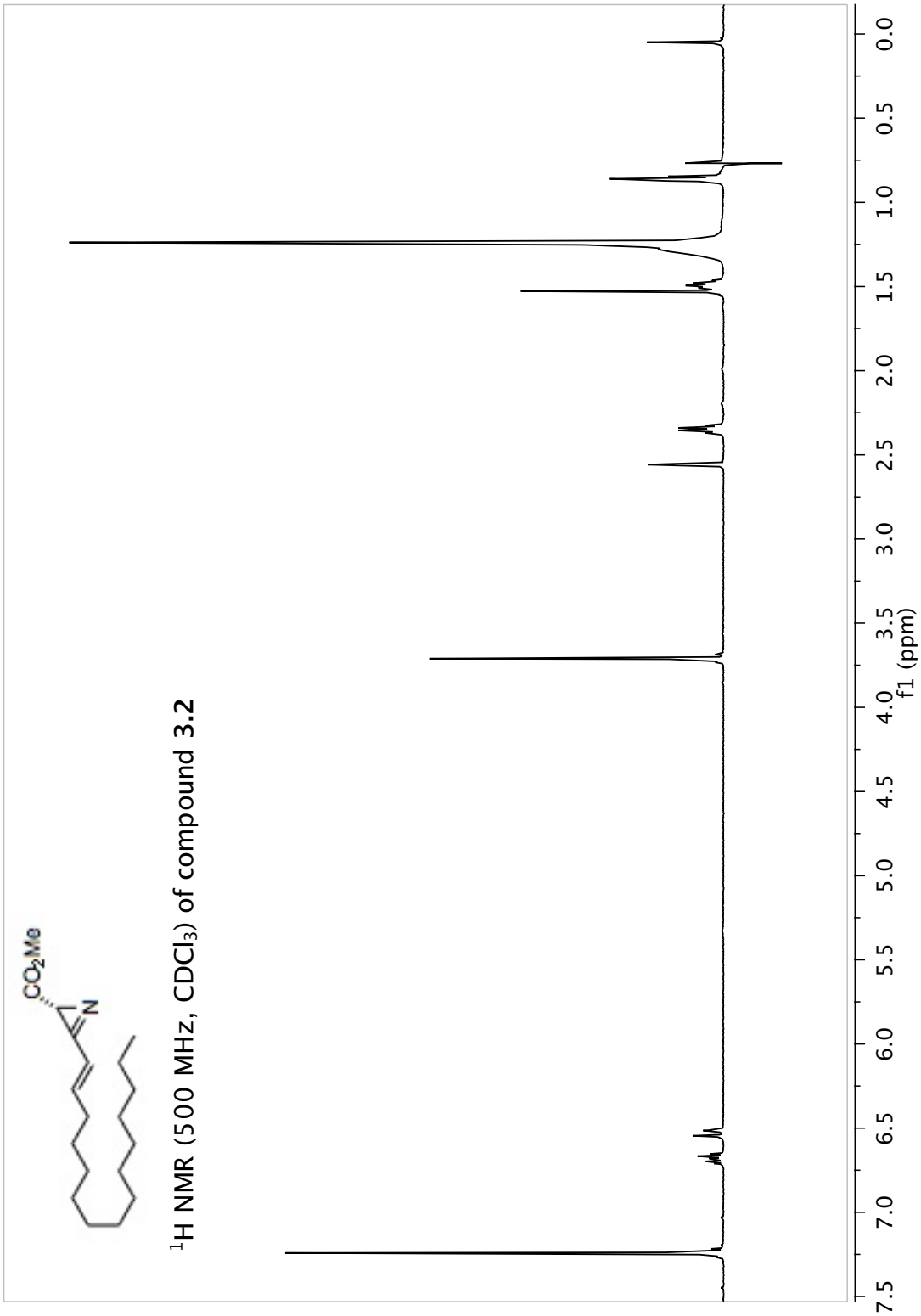


^{1}H NMR (500 MHz, $CDCl_3$) of compound **3.40**

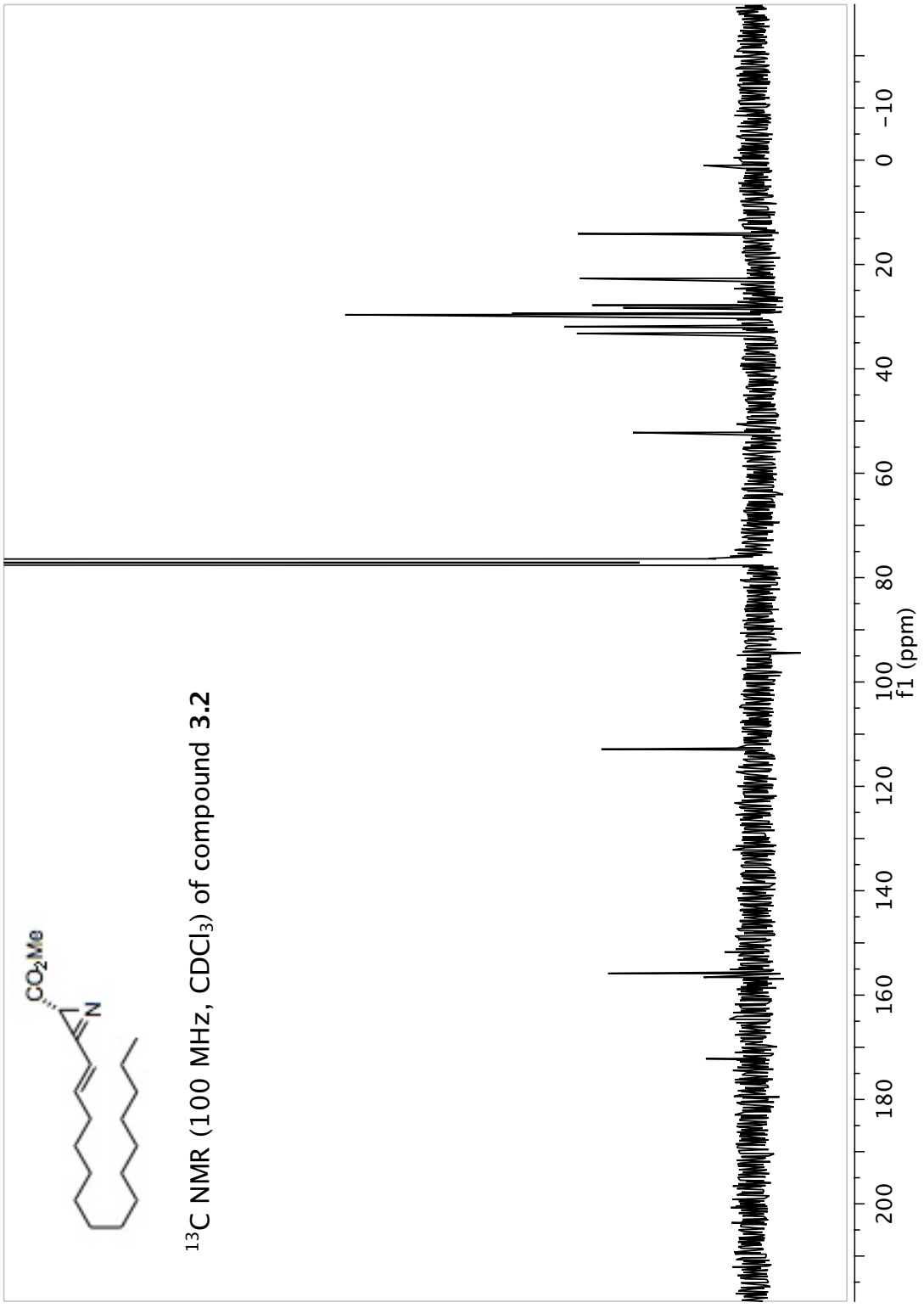

^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.40**
CO_2Me
N
11
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.3**
CO_2Me
N
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

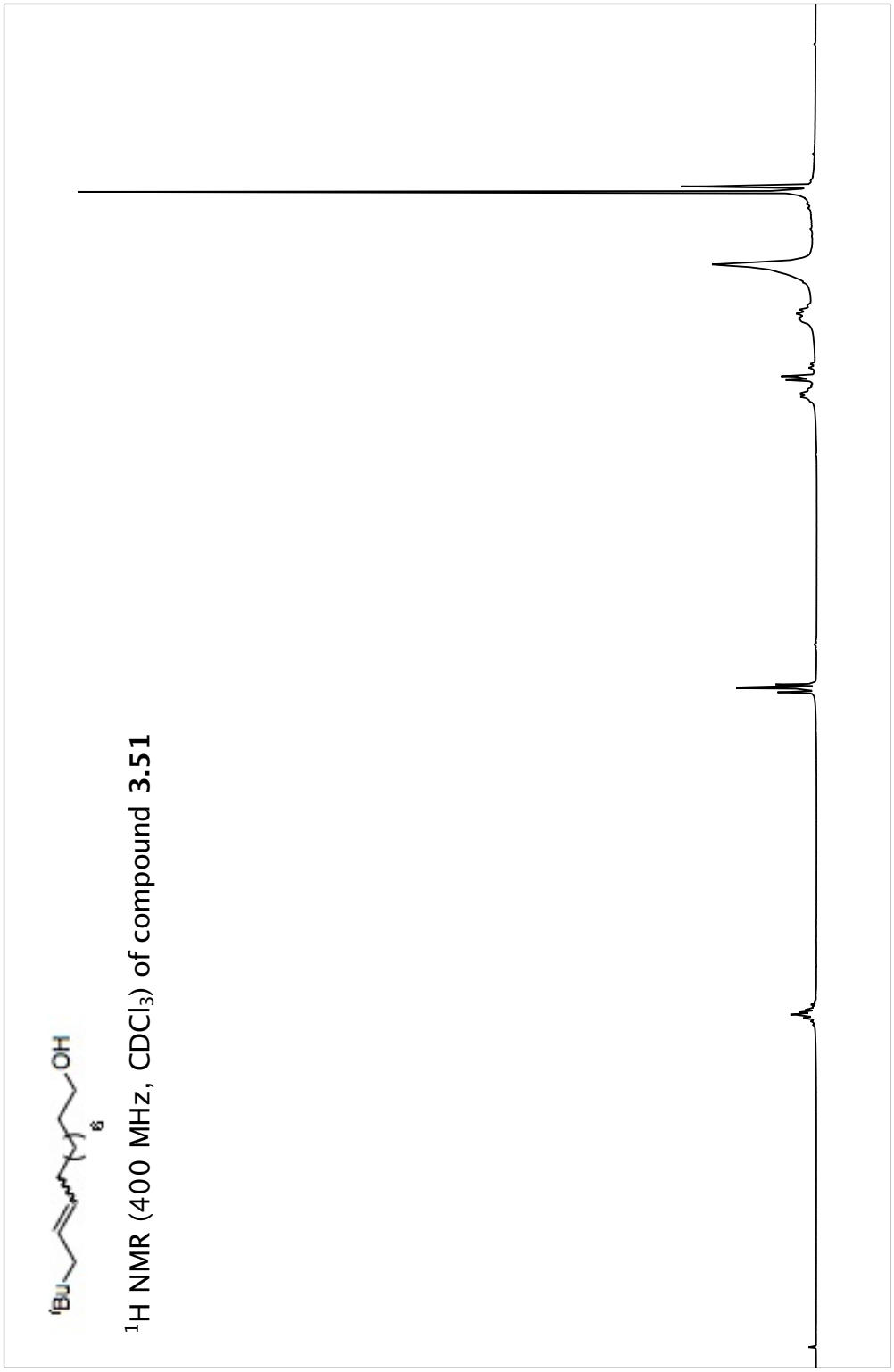

^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.3**
CO_2Me
N
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



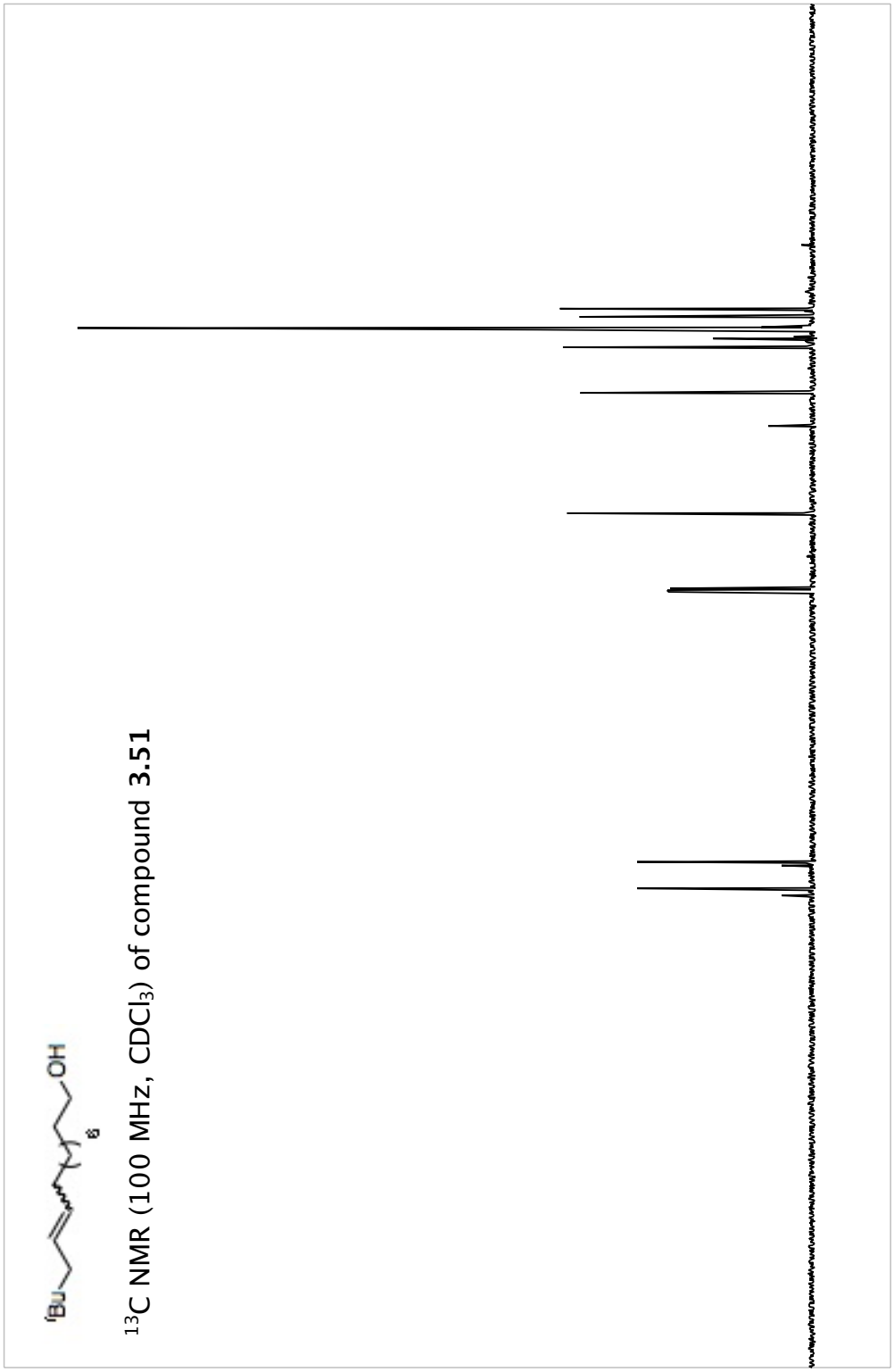
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3.2**



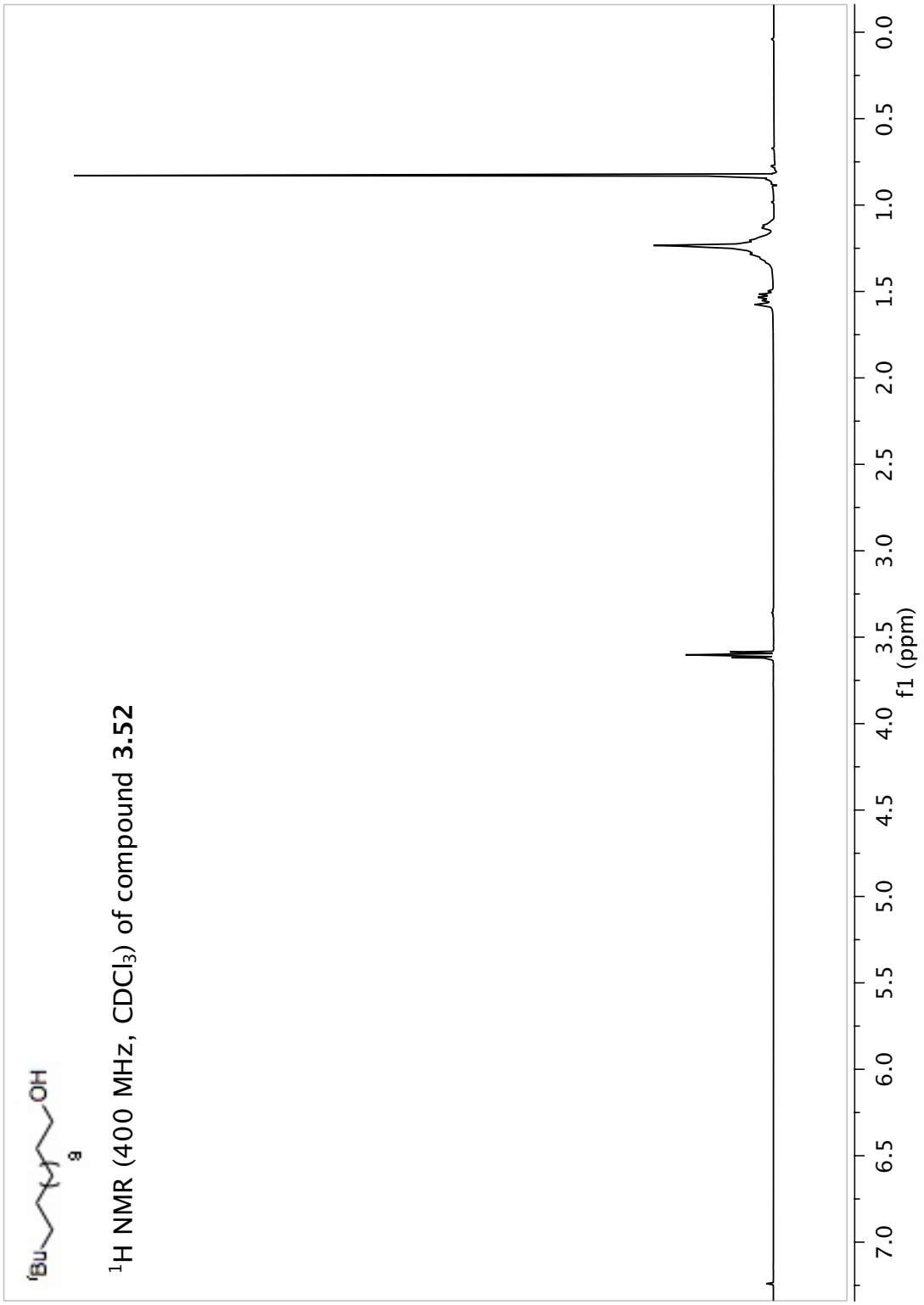
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.2**



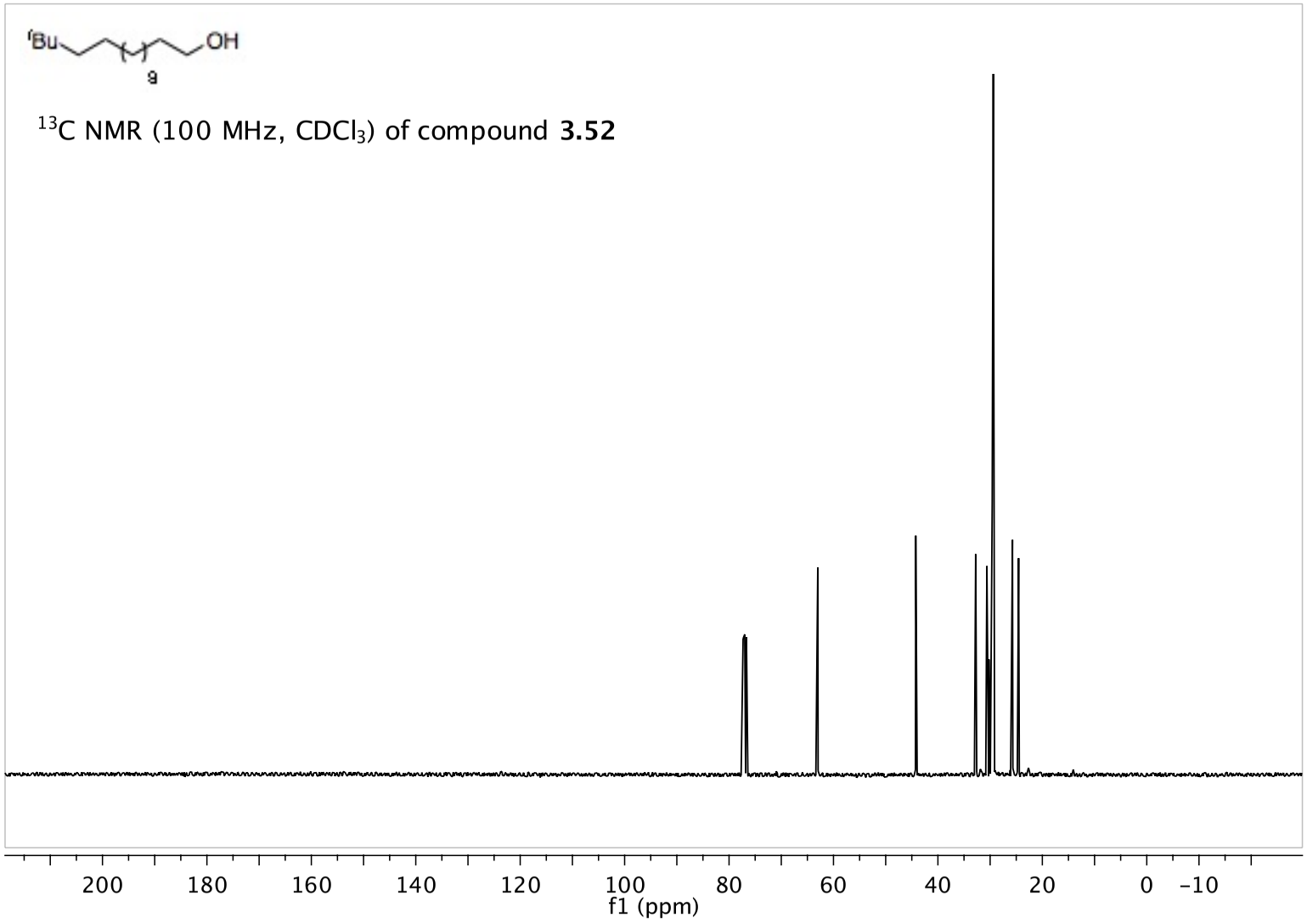
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.51**

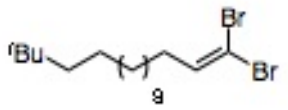


^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.51**

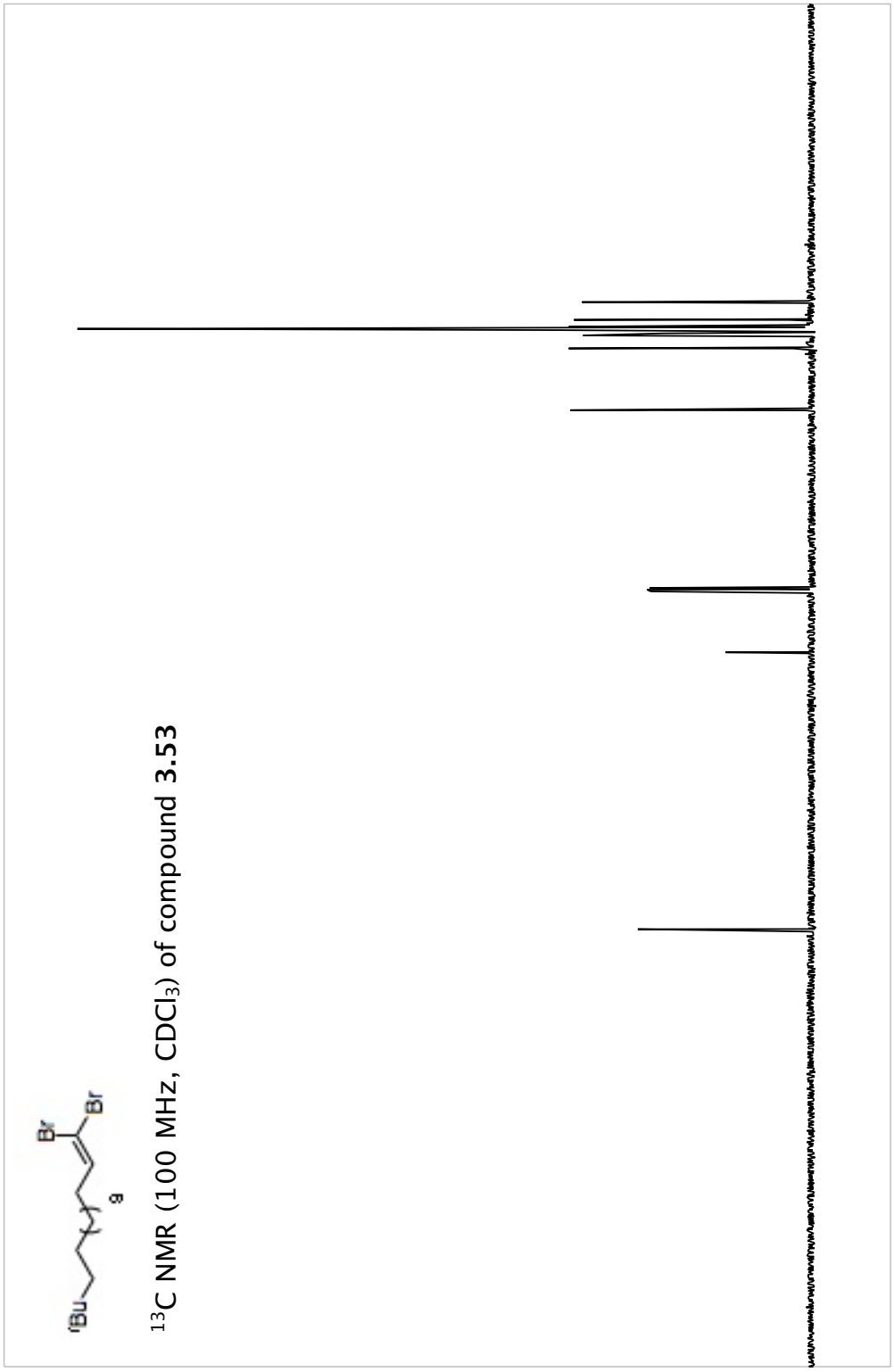


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.52**

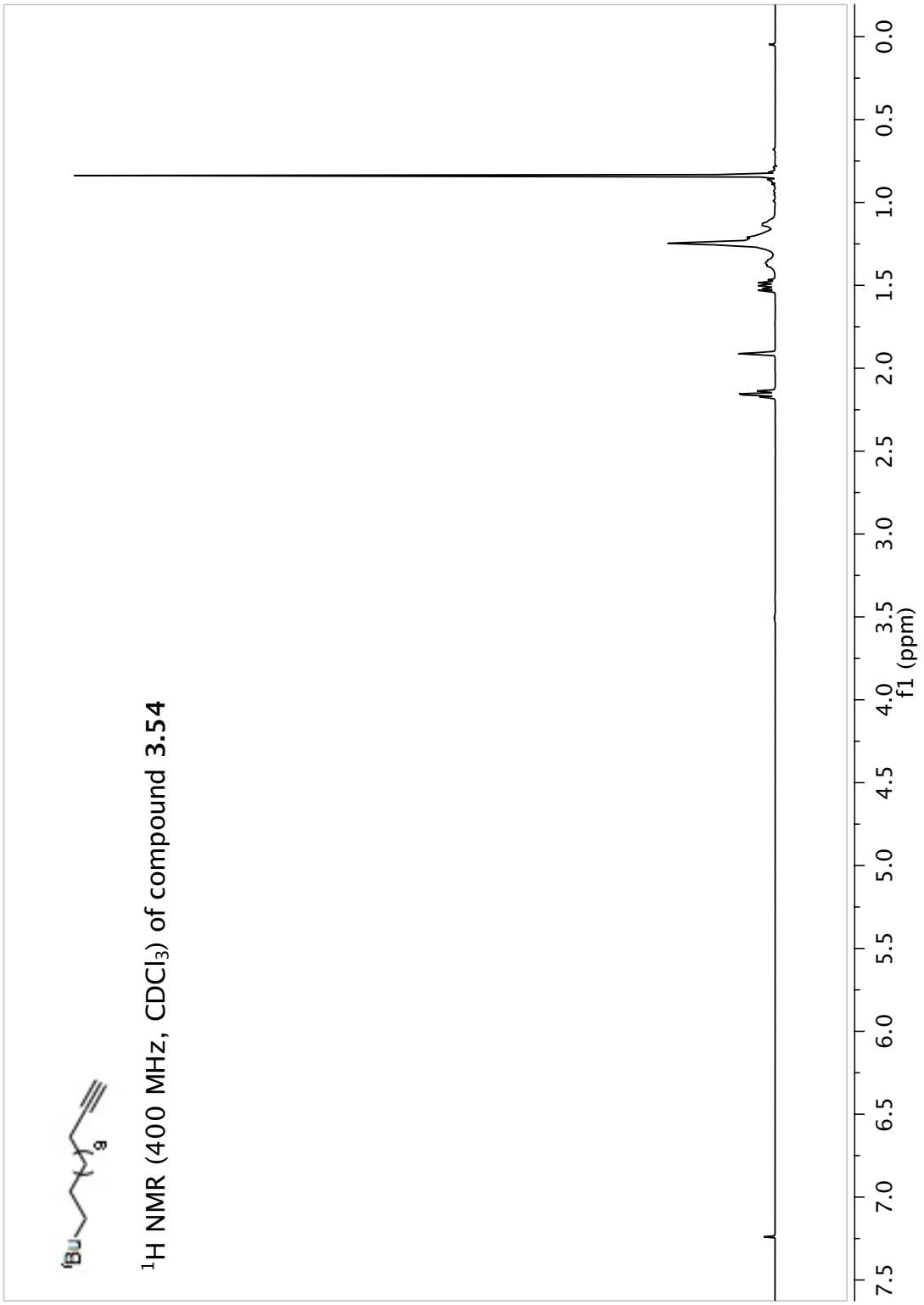

tBu
OH
9
13C NMR (100 MHz, CDCl3) of compound 3.52
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



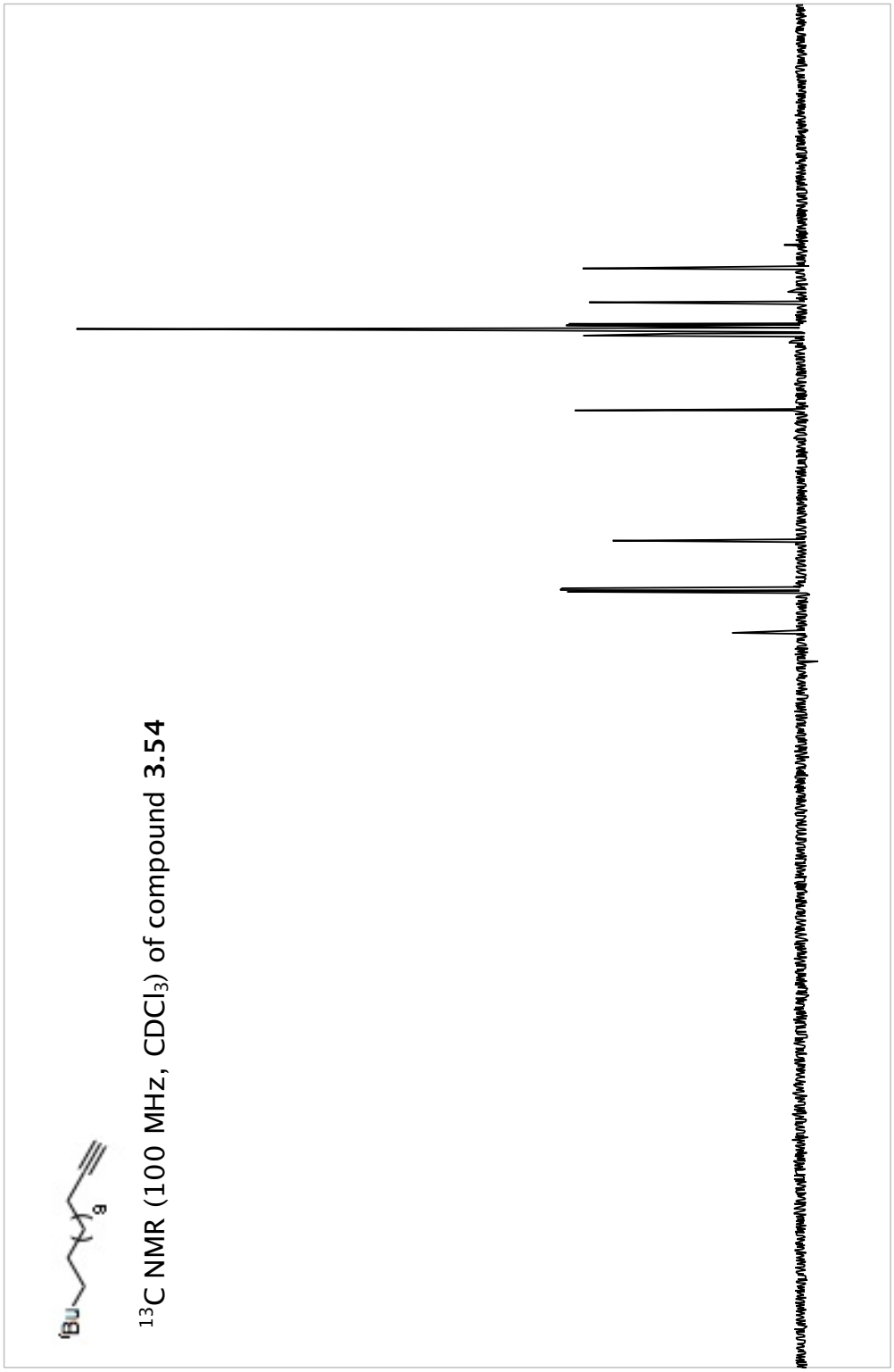
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.53**



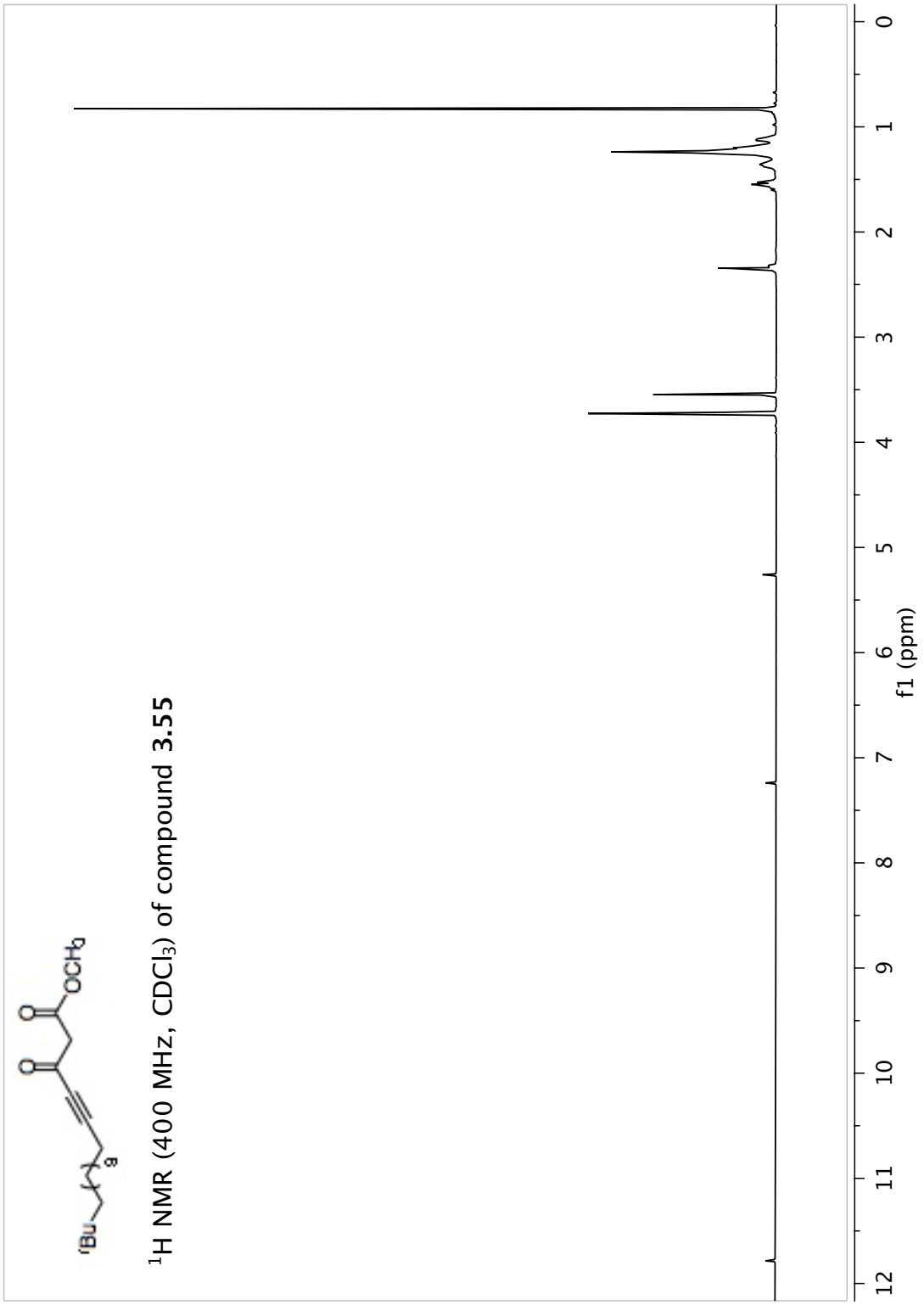
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.53**



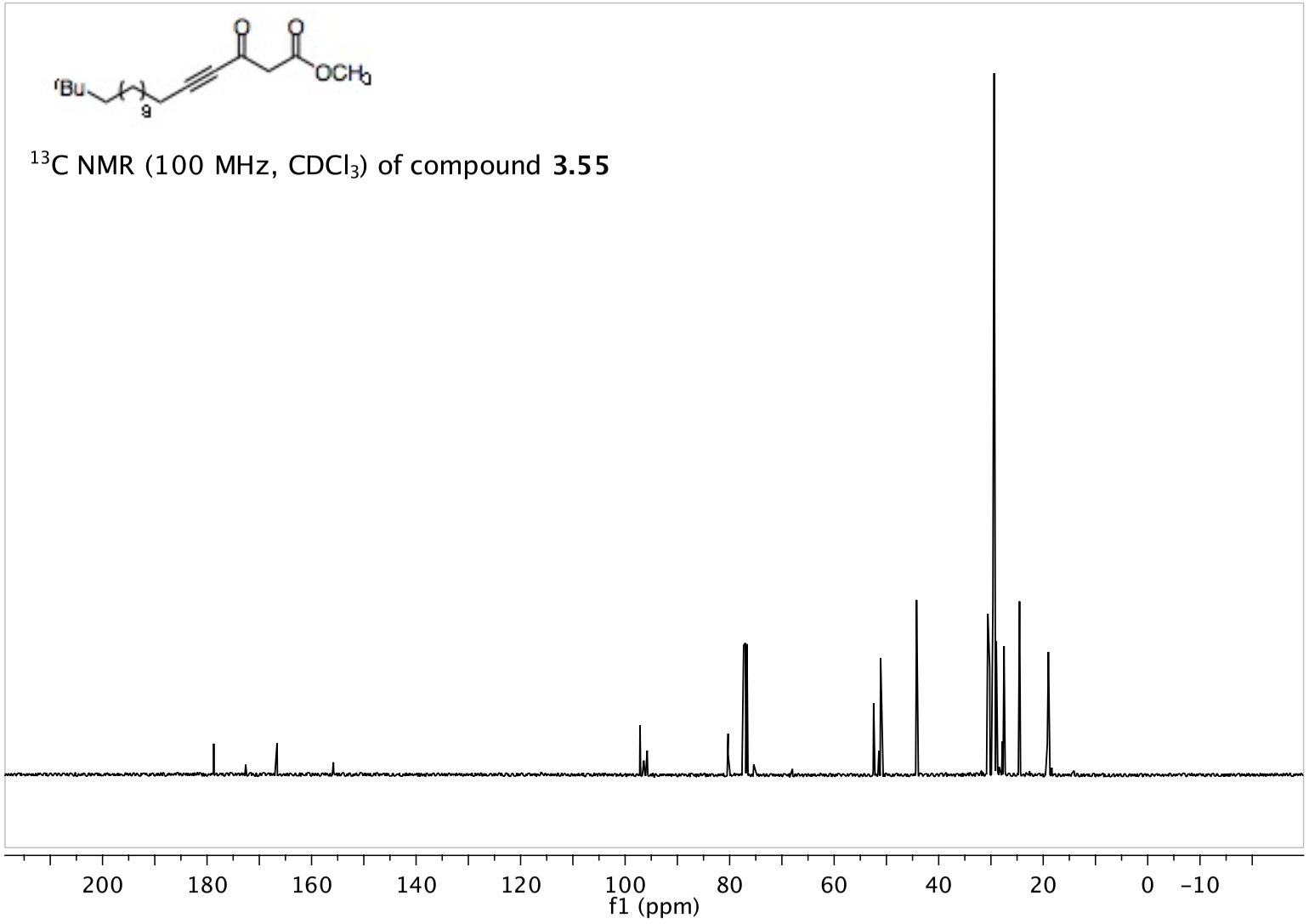
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.54**

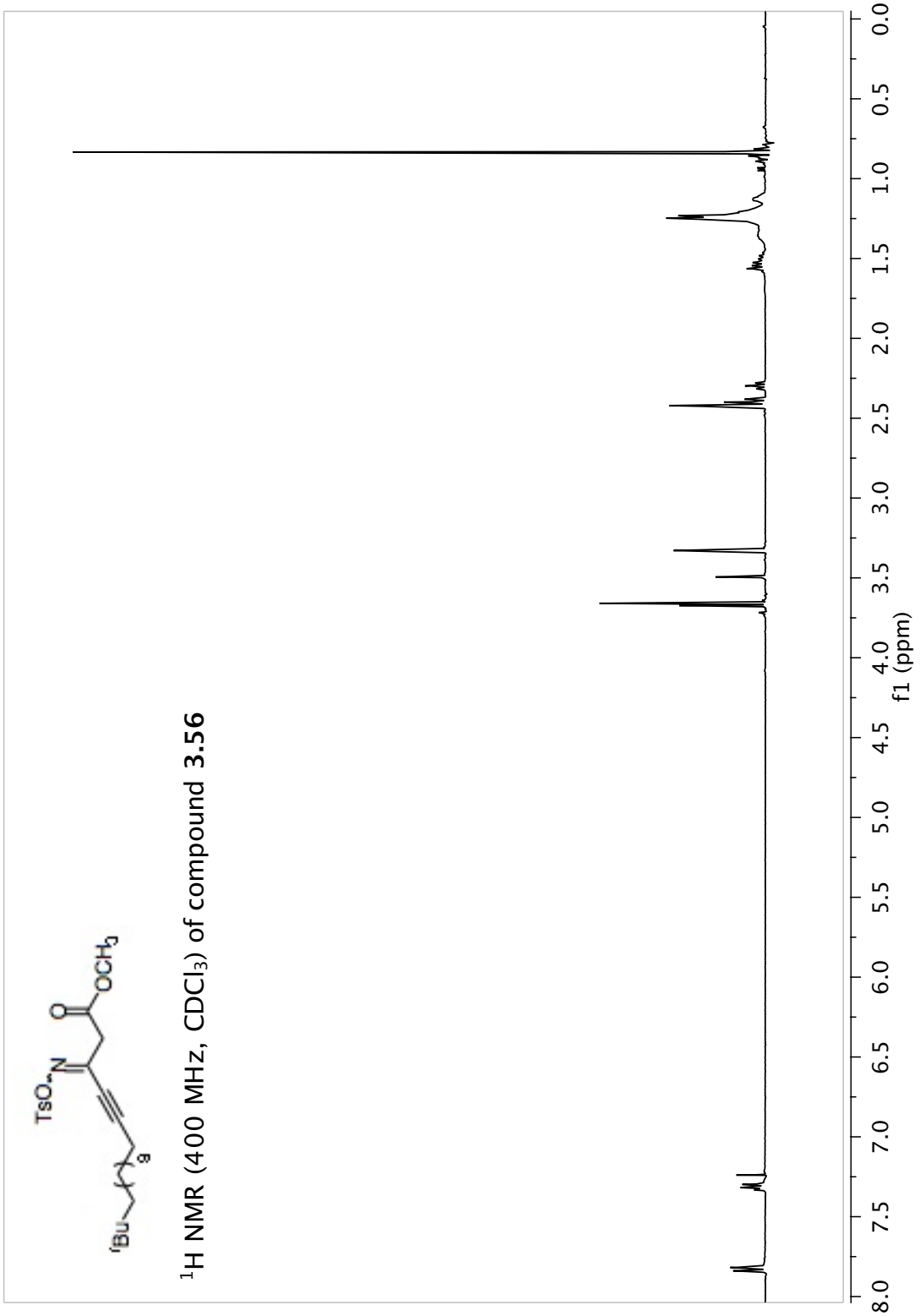


^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.54**

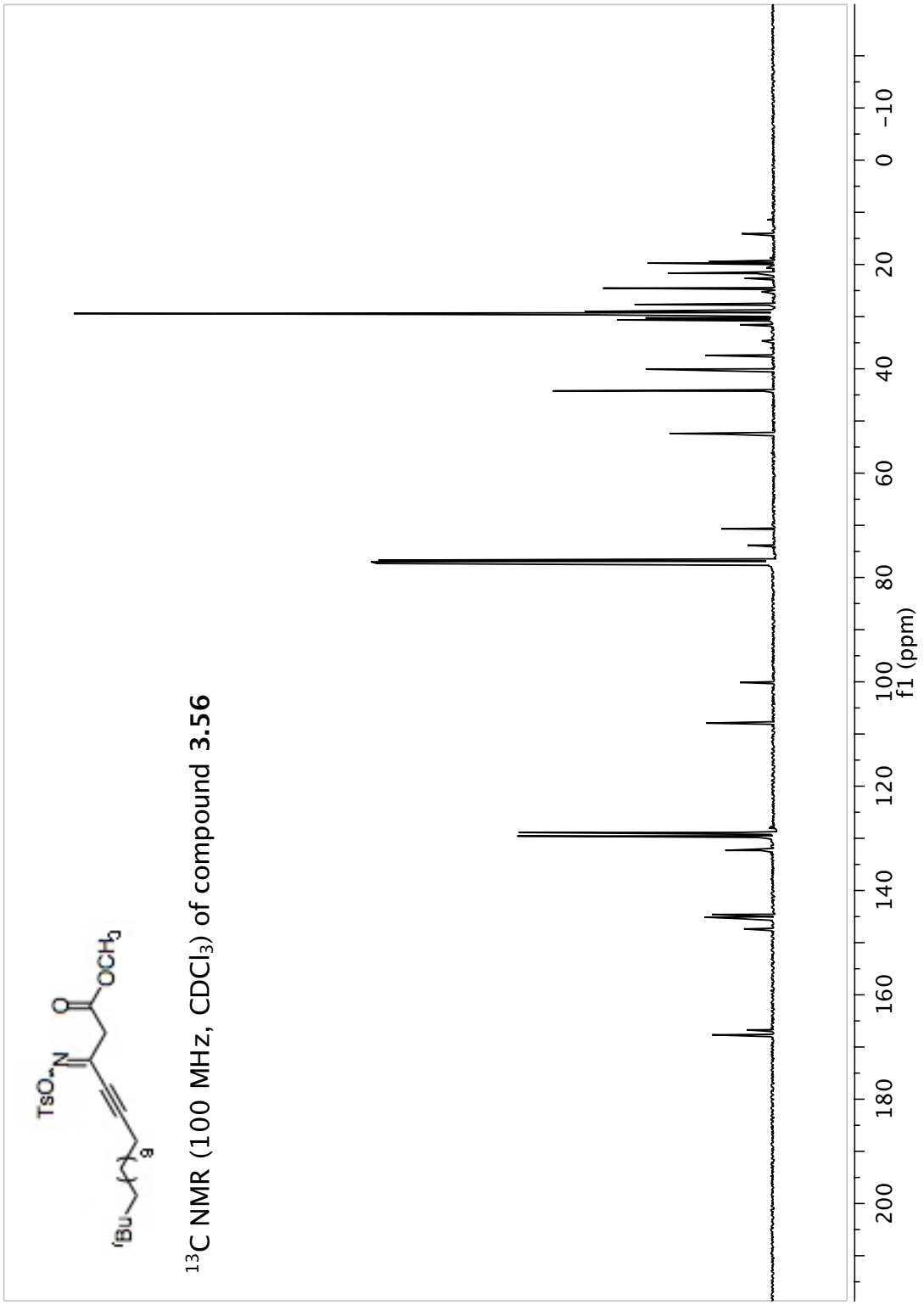


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.55**

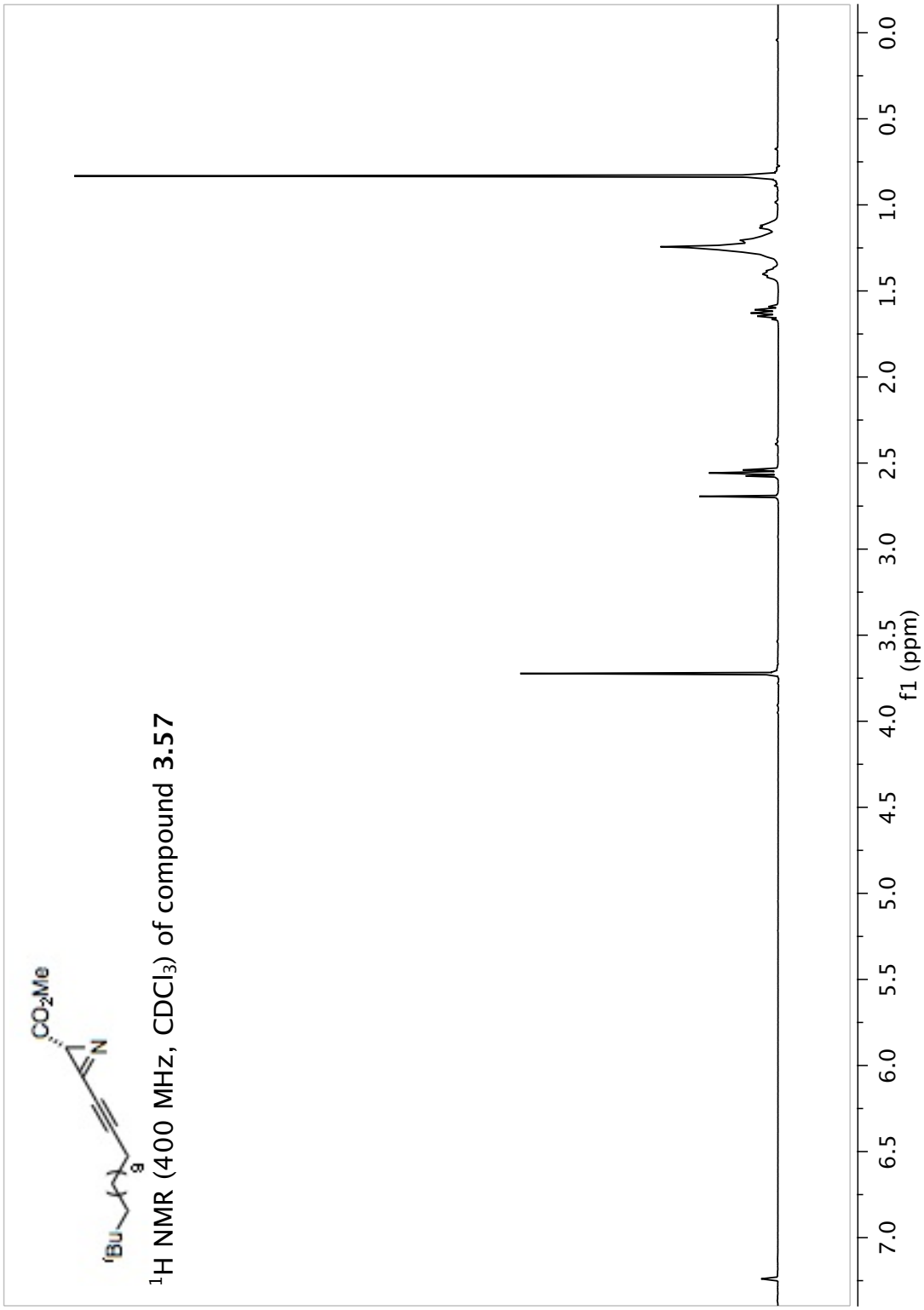

tBu
9
OCH3
13C NMR (100 MHz, CDCl3) of compound 3.55
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



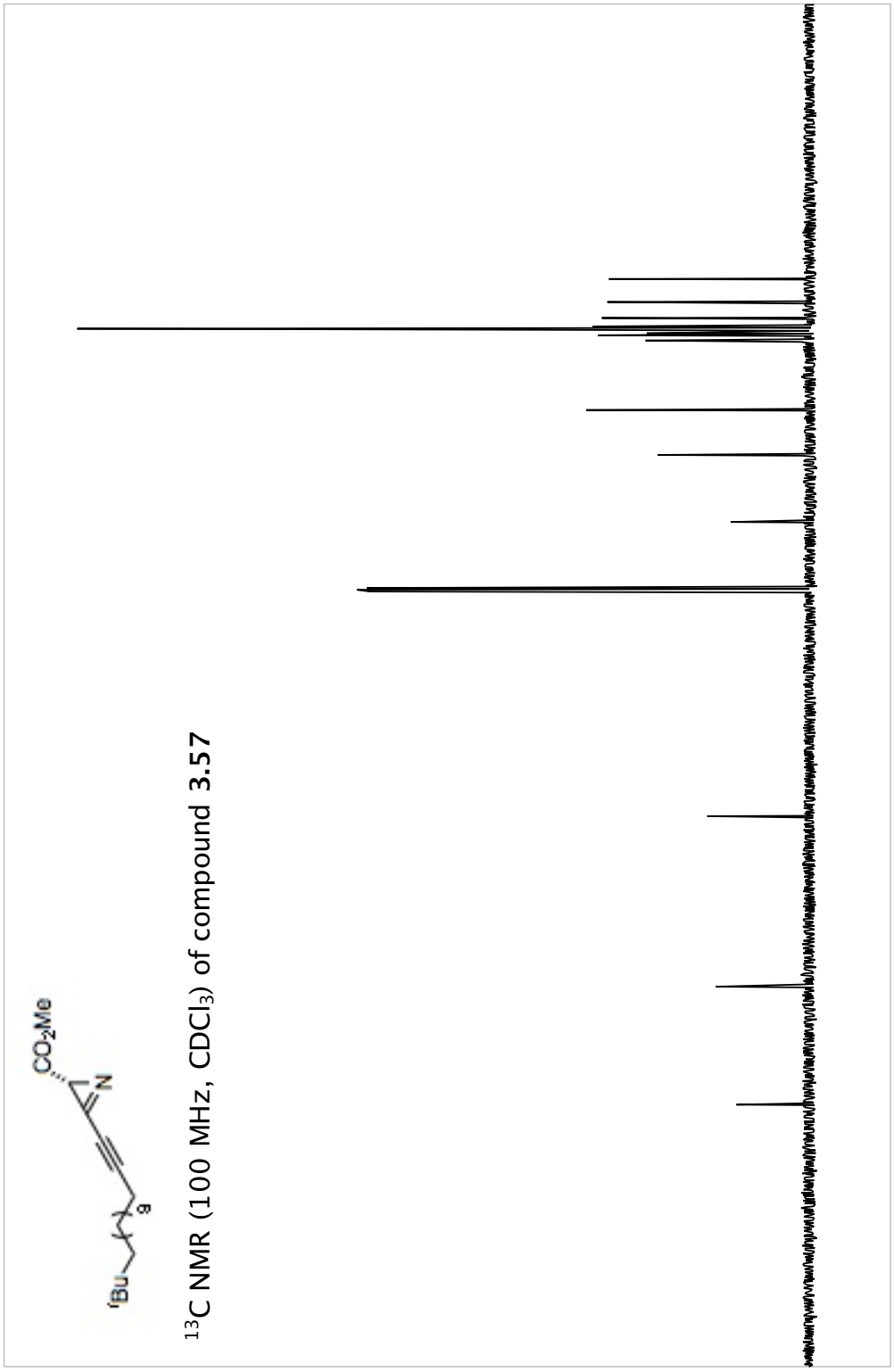
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.56**



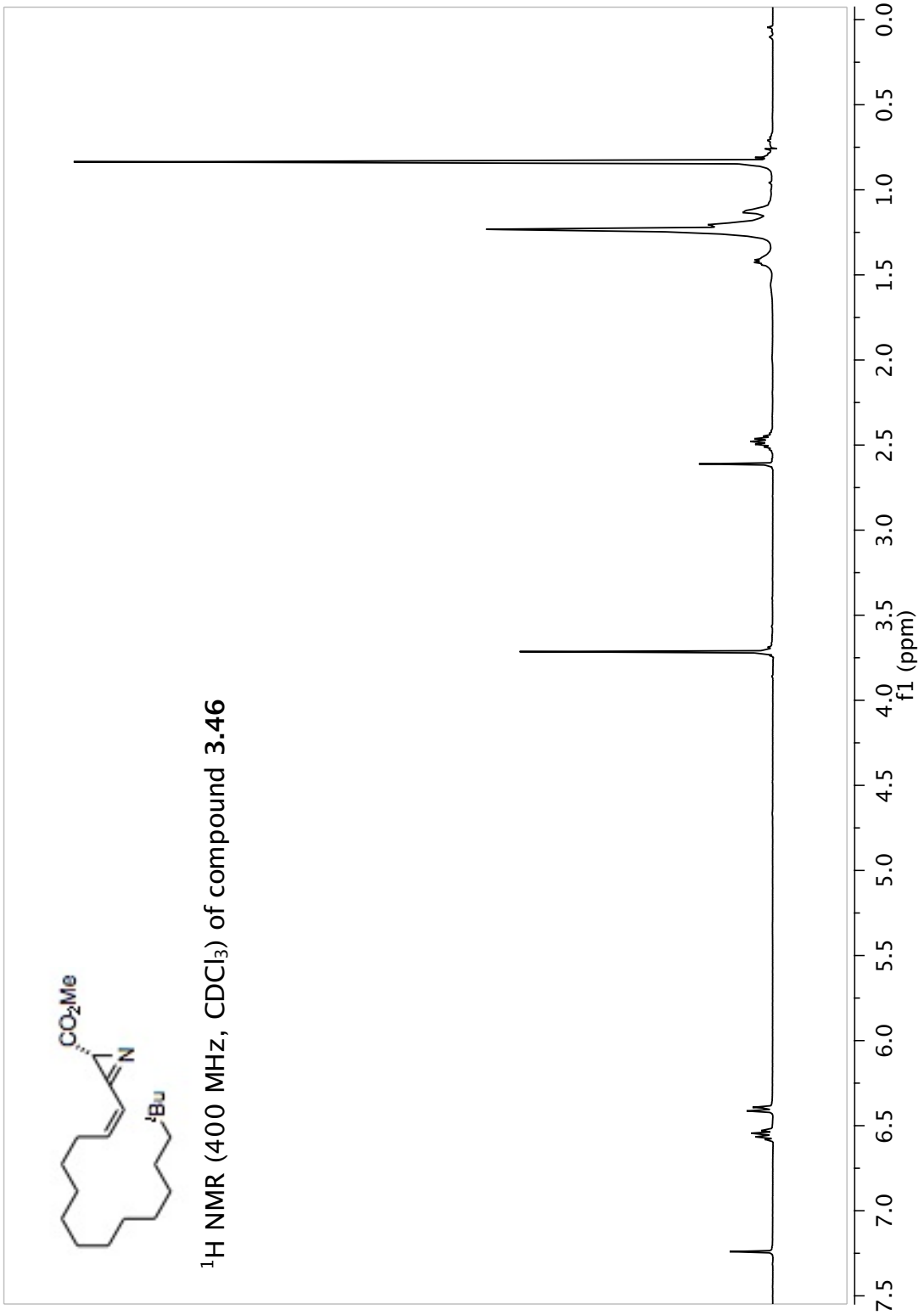
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.56**



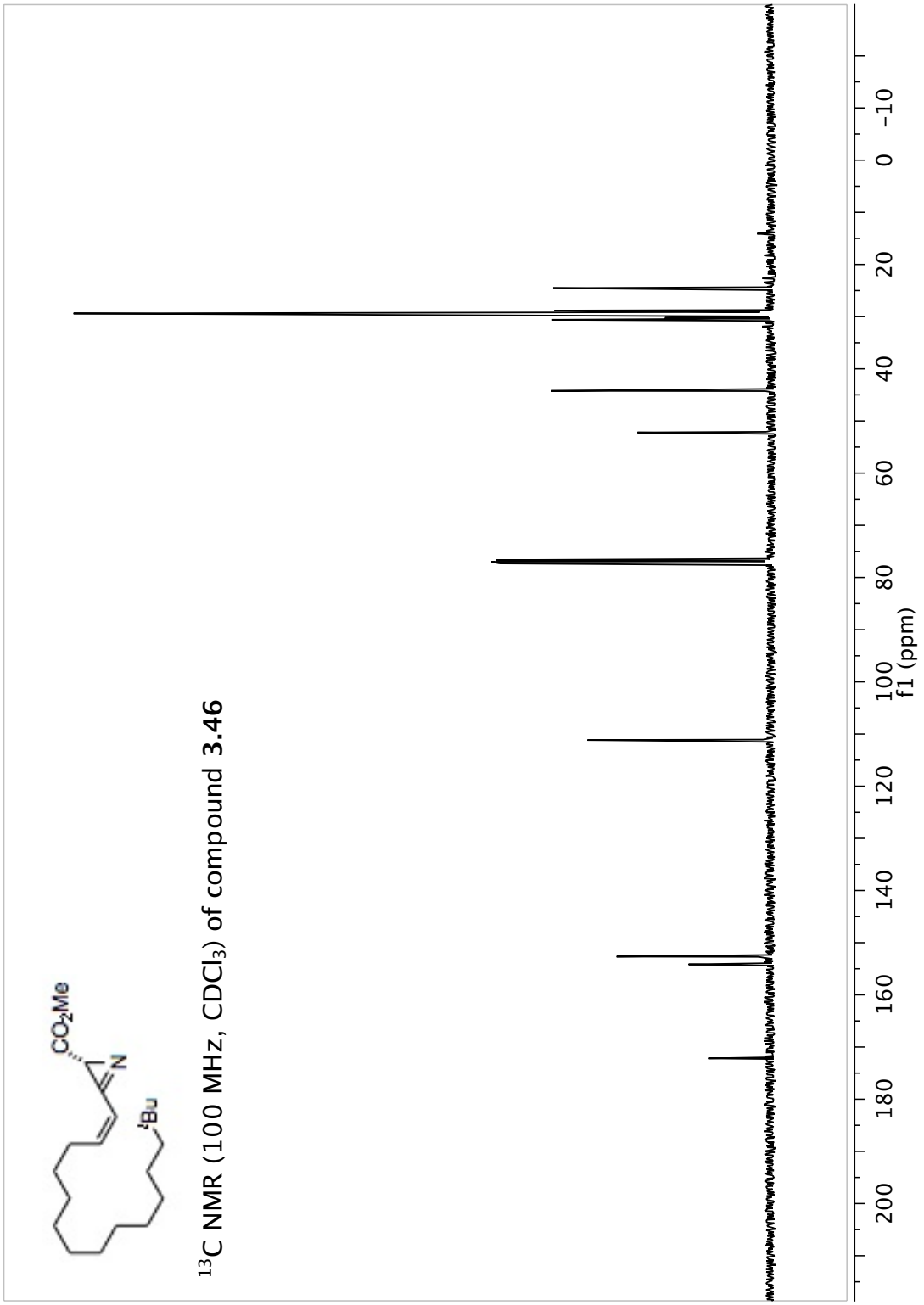
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.57**



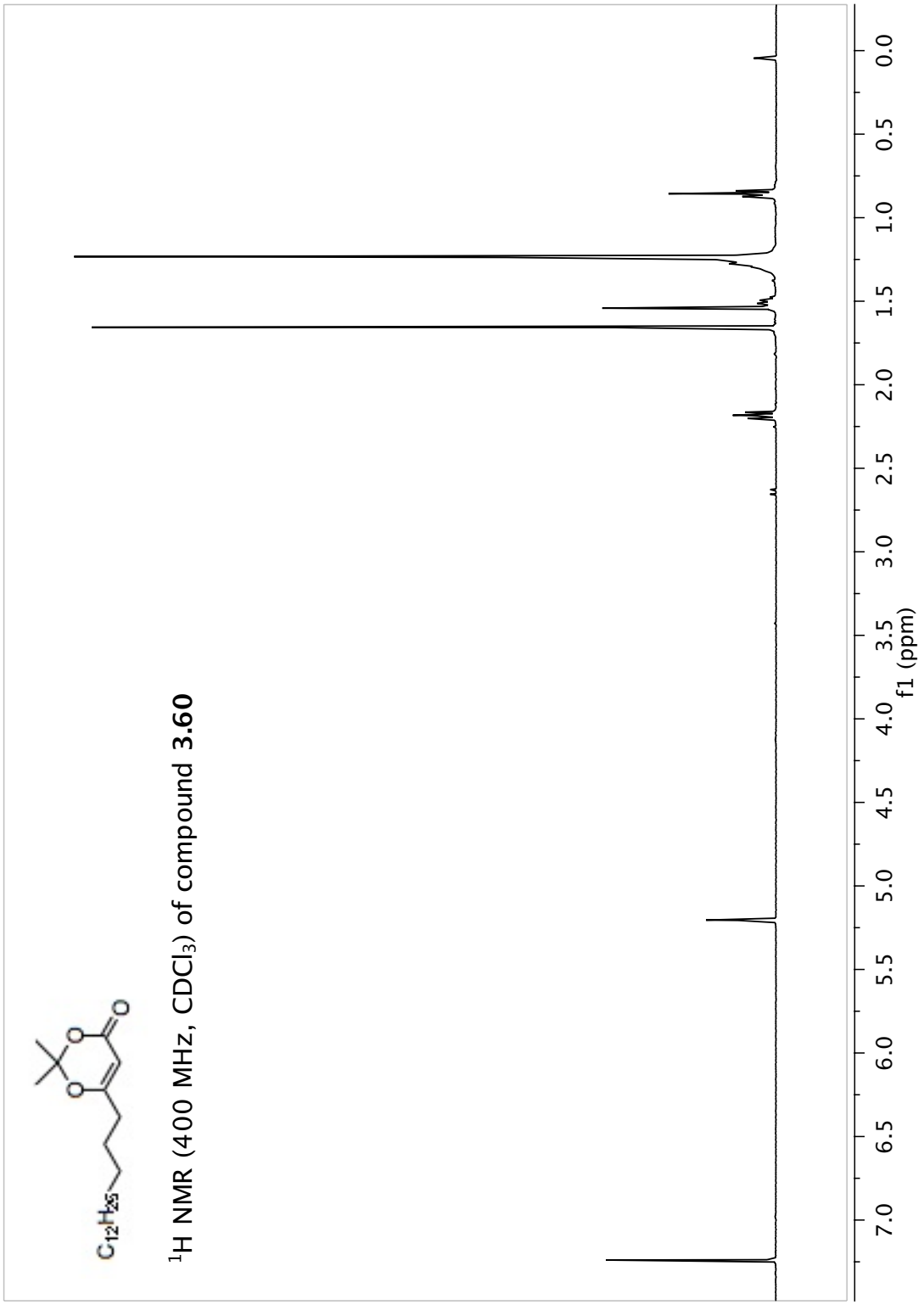
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.57**



^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.46**

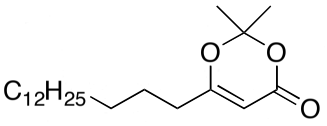


^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.46**

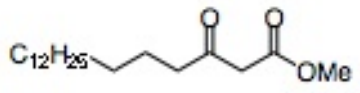

$C_{12}H_{25}$
O
O
O
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.60**
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

cs.06.2.278_C13

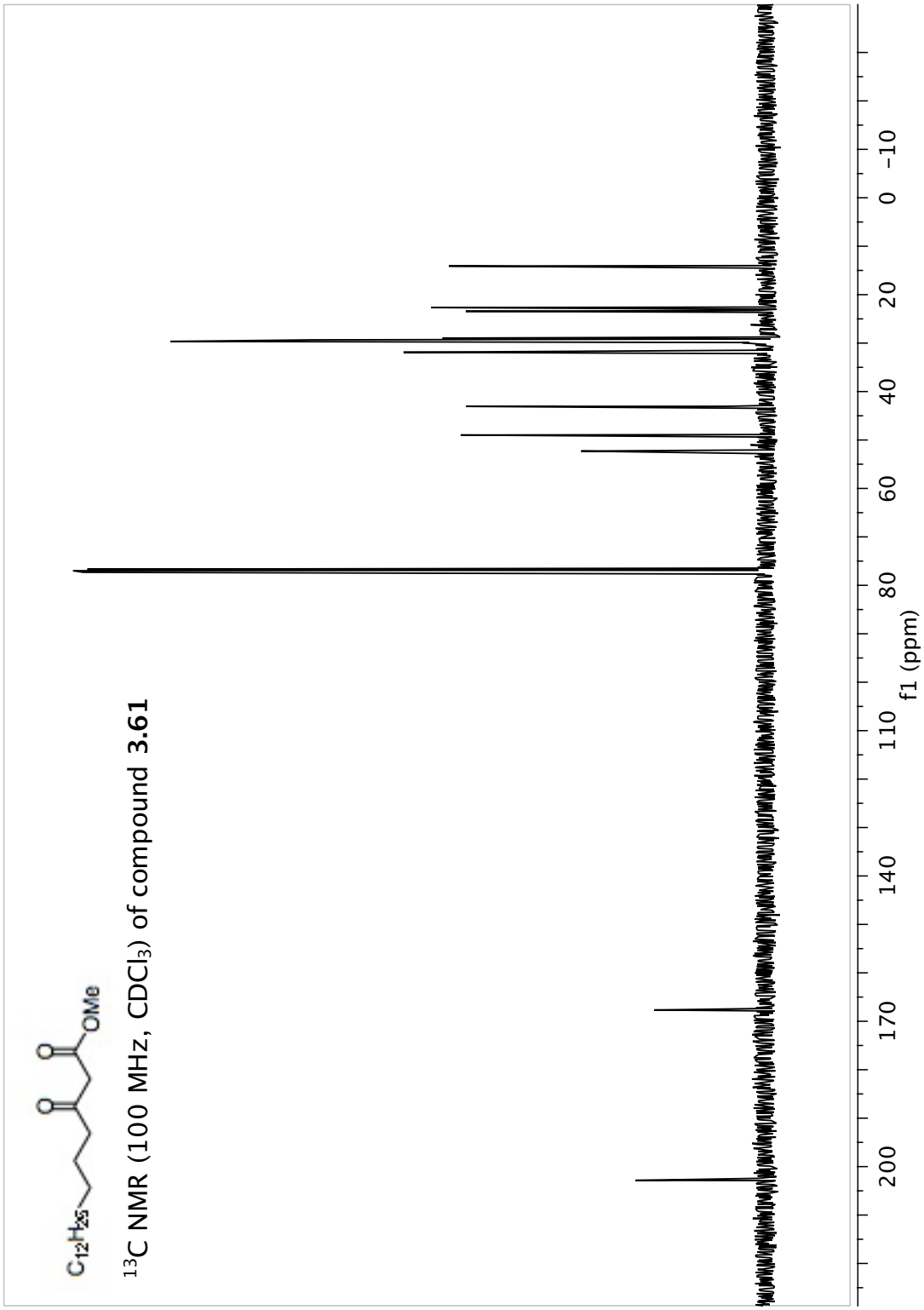
```
13C OBSERVE

exp2  std13c

      SAMPLE
date   Jul 26 2006
solvent      CDCl3
file           exp
   ACQUISITION
sfrq        100.603
tn              C13
at              1.500
np              75008
sw           25000.0
fb              13800
bs                 16
tpwr               57
pw                6.0
d1              1.700
tof                0
nt                600
ct                480
alock               n
gain        not used
      FLAGS
il                  n
in                  n
dp                  y
     DISPLAY
sp           -3018.8
wp           25000.0
vs               162
sc                 0
wc               250
hzmm          100.00
is            500.00
rfl          10764.5
rfp           7745.7
th                20
ins           100.000
nm  no  ph

      DEC. & VT
dfrq         400.056
dn                H1
dpwr              43
dof                0
dm               yyy
dmm                w
dmf             9300
     PROCESSING
lb               1.00
wtfile
proc               ft
fn          not used
werr
wexp
wbs
wnt
```

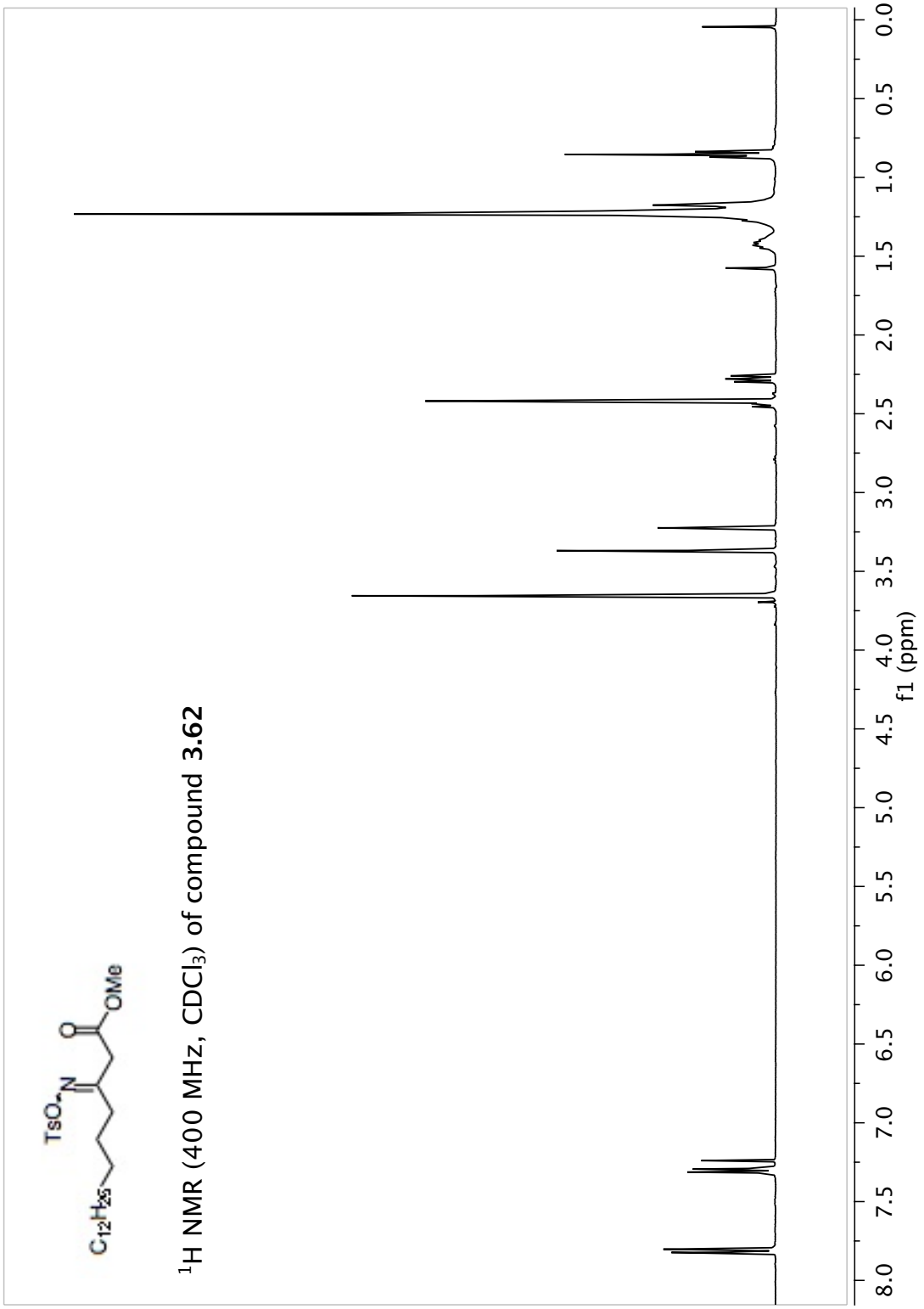


^{13}C NMR (100 MHz, $CDCl_3$)
of compound **3.60**

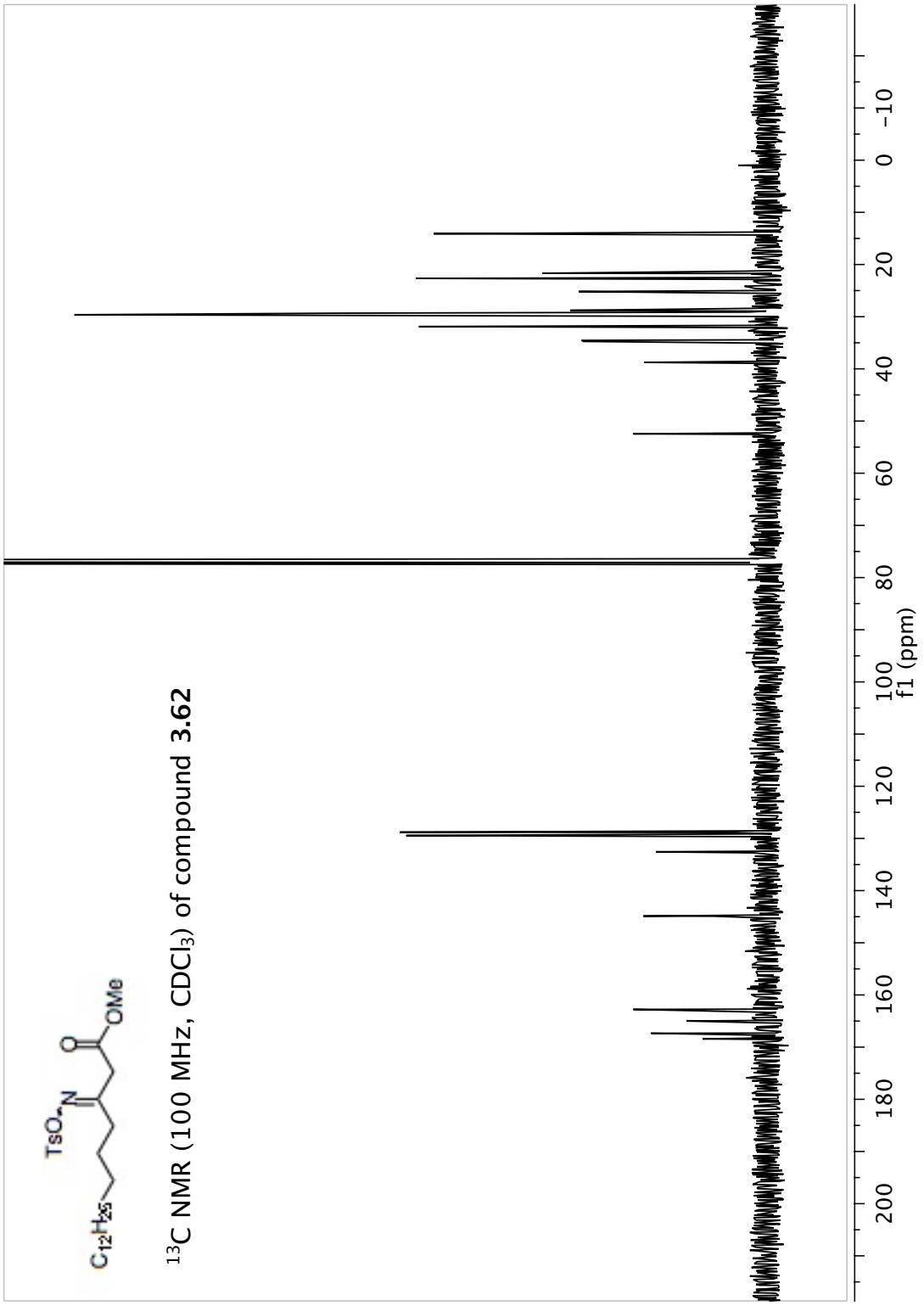


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.61**

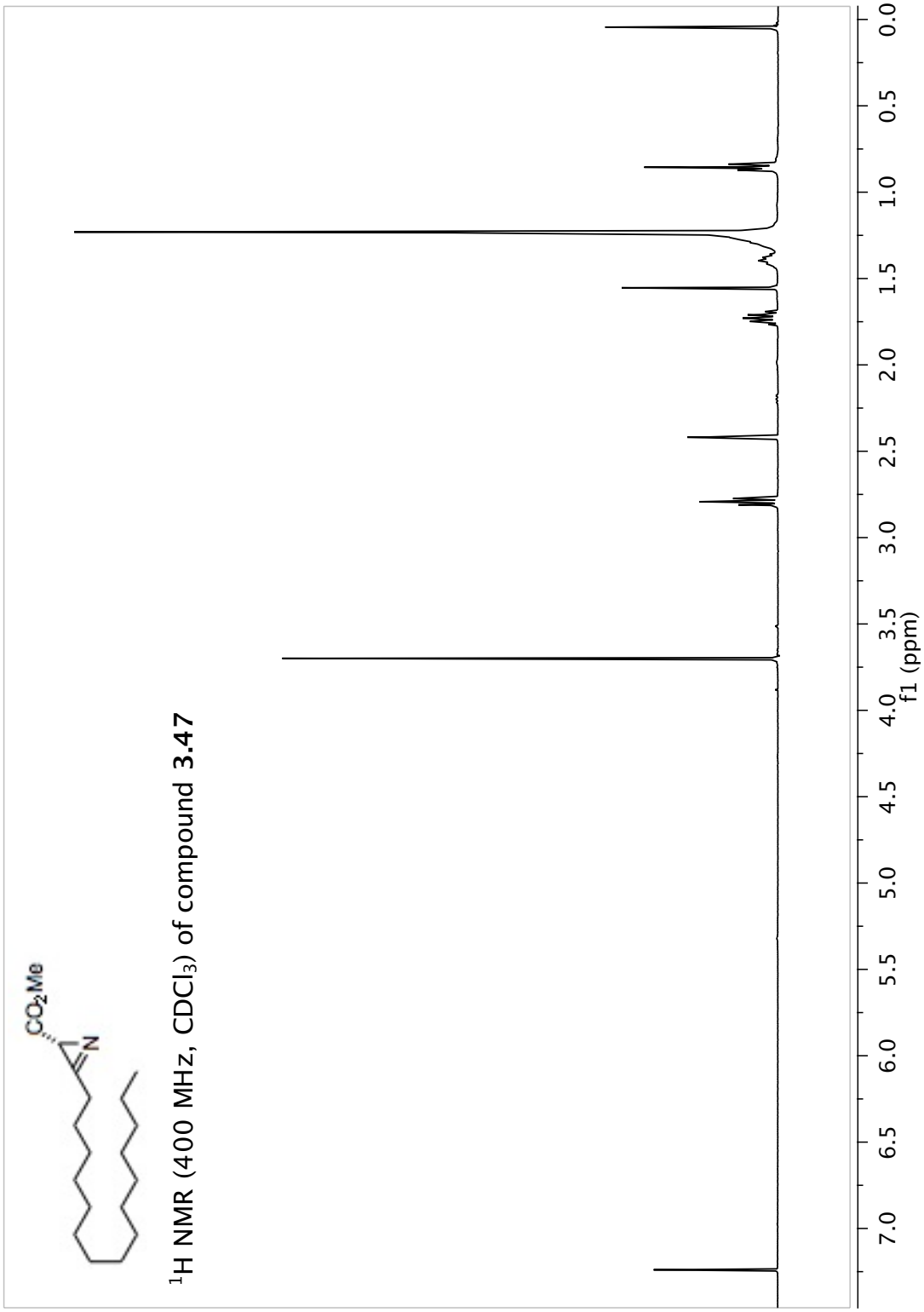

$C_{12}H_{25}$
OMe
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.61**
200
170
140
110
80
60
40
20
0
-10
f1 (ppm)



^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.62**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.62**



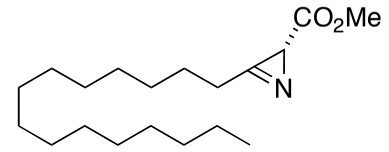
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.47**

CS.06.2.286_C13

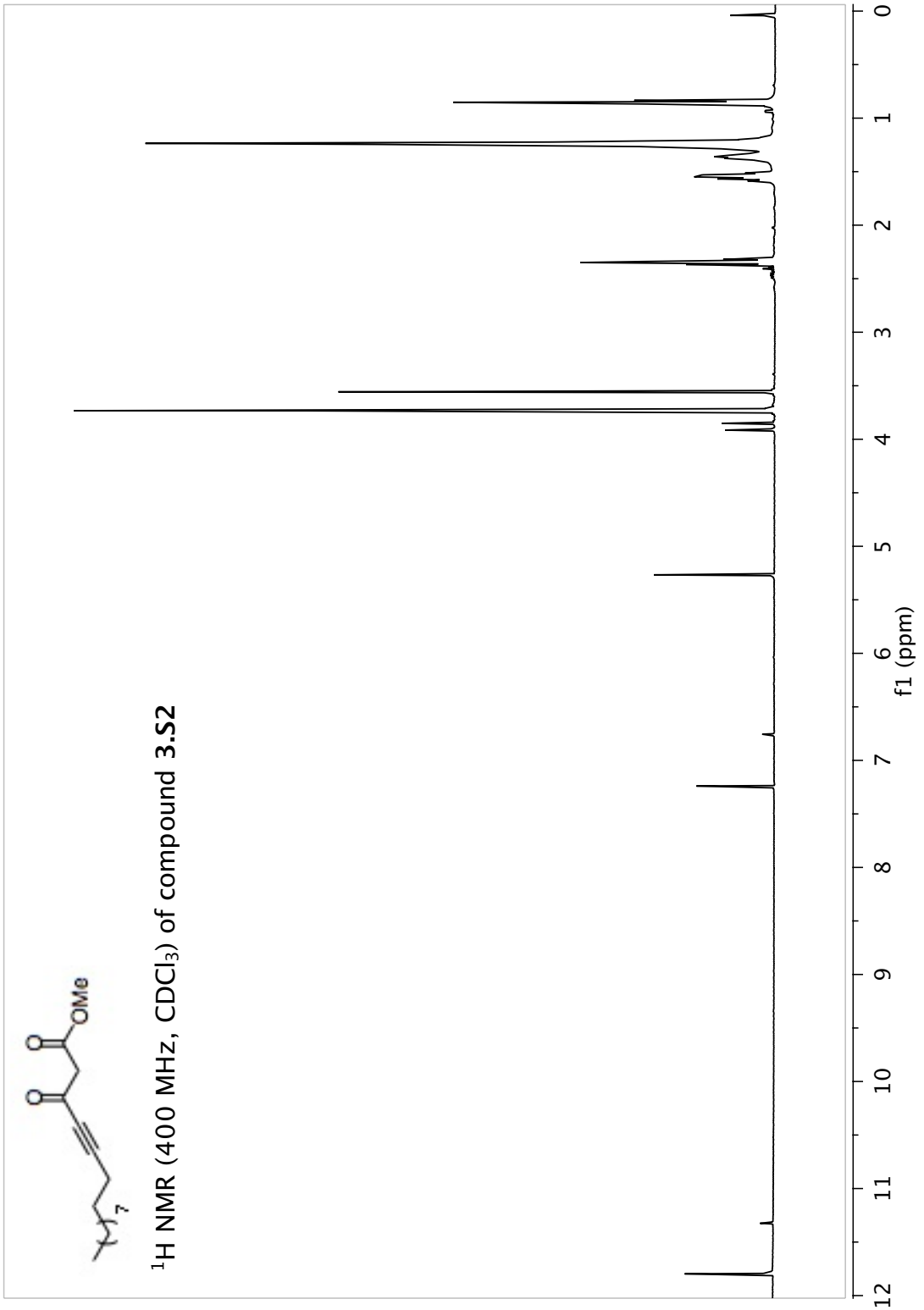
13C OBSERVE

exp2 std13c

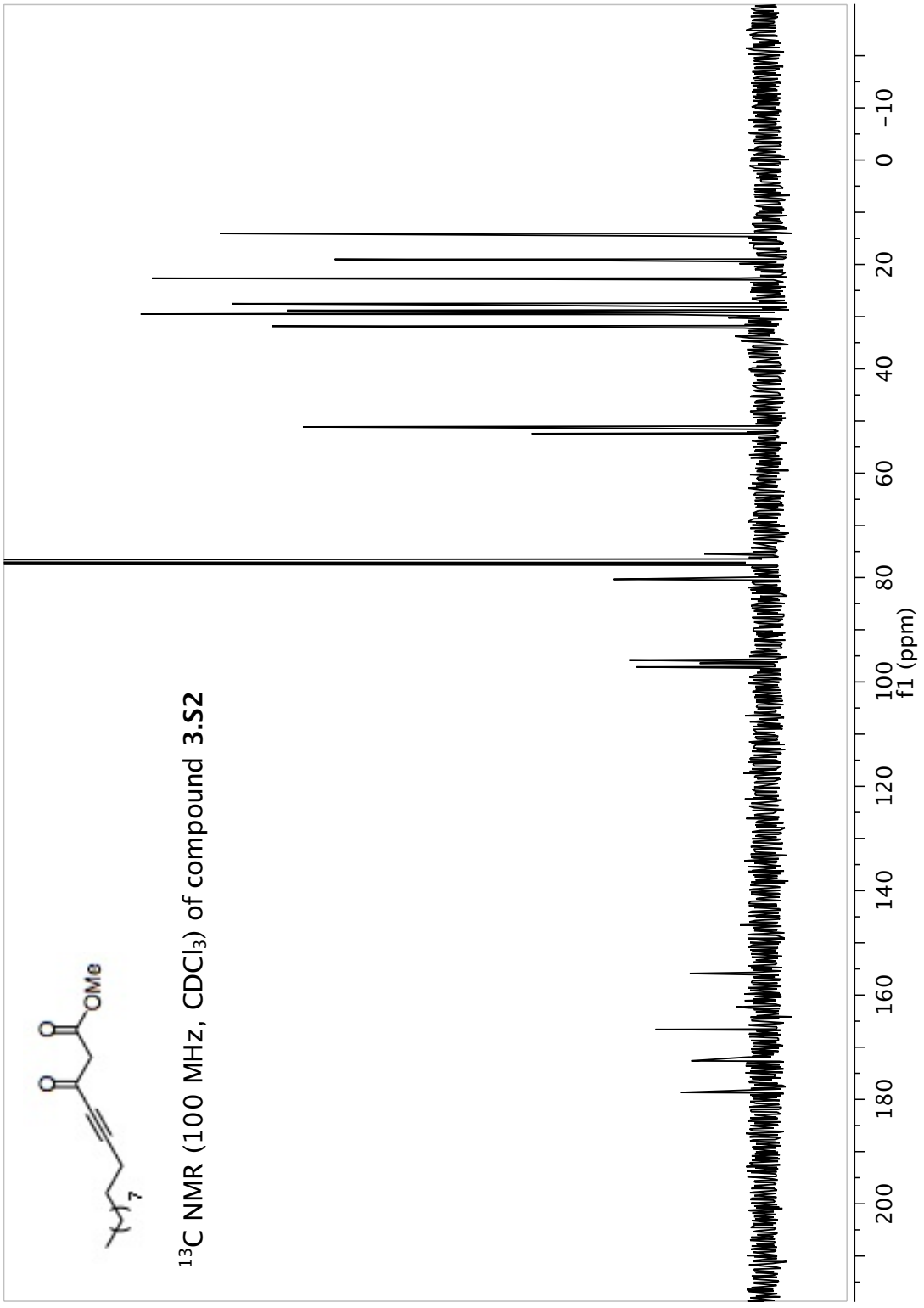
SAMPLE		DEC. & VT	
date	Aug 3 2006	dfrq	400.056
solvent	CDCl3	dn	H1
file	exp	dpwr	43
ACQUISITION		dof	0
sfrq	100.603	dm	yyy
tn	C13	dmm	w
at	1.500	dmf	9300
np	75008	PROCESSING	
sw	25000.0	lb	1.00
fb	13800	wtfile	
bs	16	proc	ft
tpwr	57	fn	not used
pw	7.0		
d1	1.800	werr	
tof	0	wexp	
nt	10000	wbs	
ct	10000	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-3016.5		
wp	25000.0		
vs	162		
sc	0		
wc	250		
hzmm	100.00		
is	500.00		
rfl	10762.2		
rfp	7745.7		
th	20		
ins	100.000		
nm no ph			



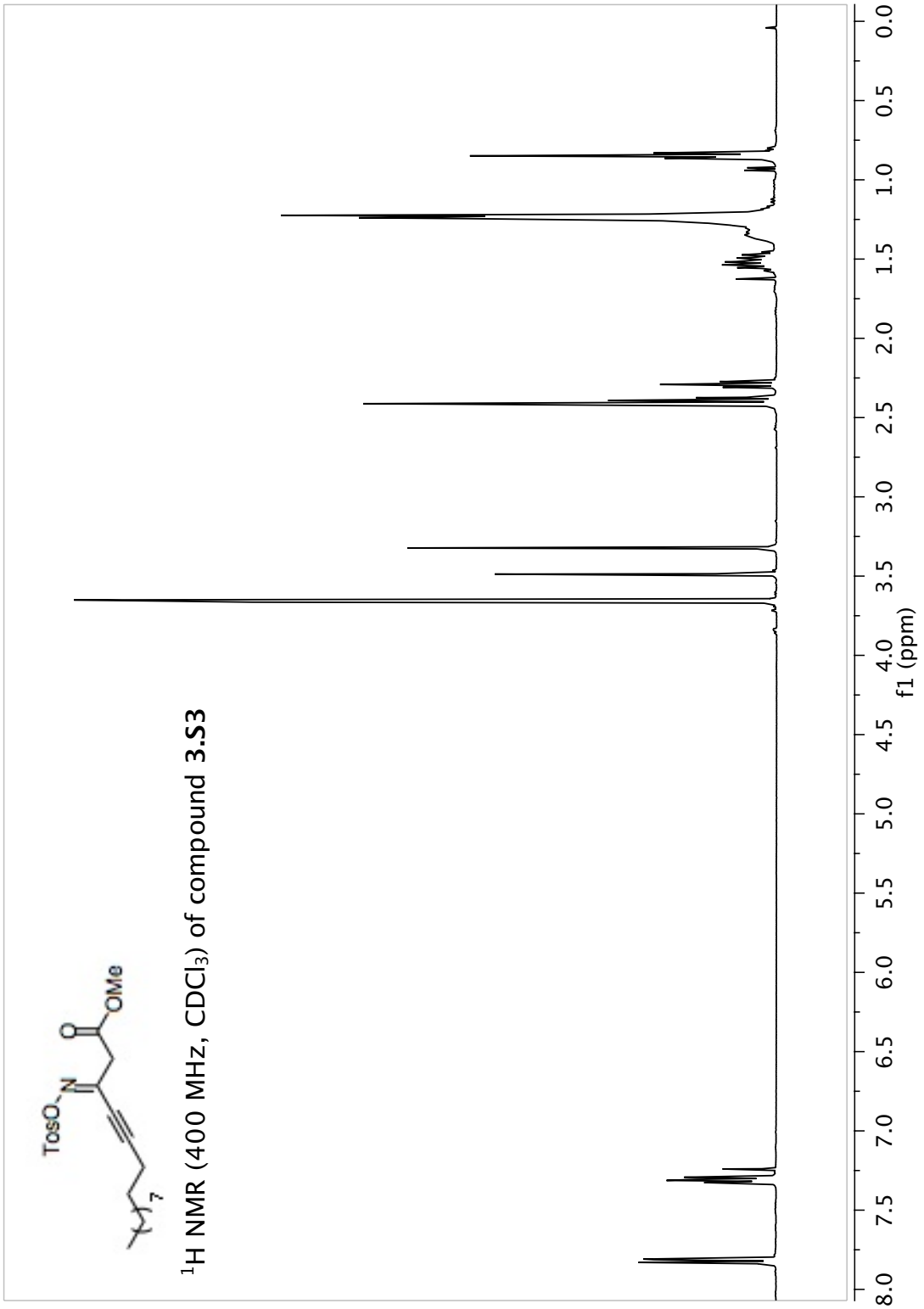
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **3.47**



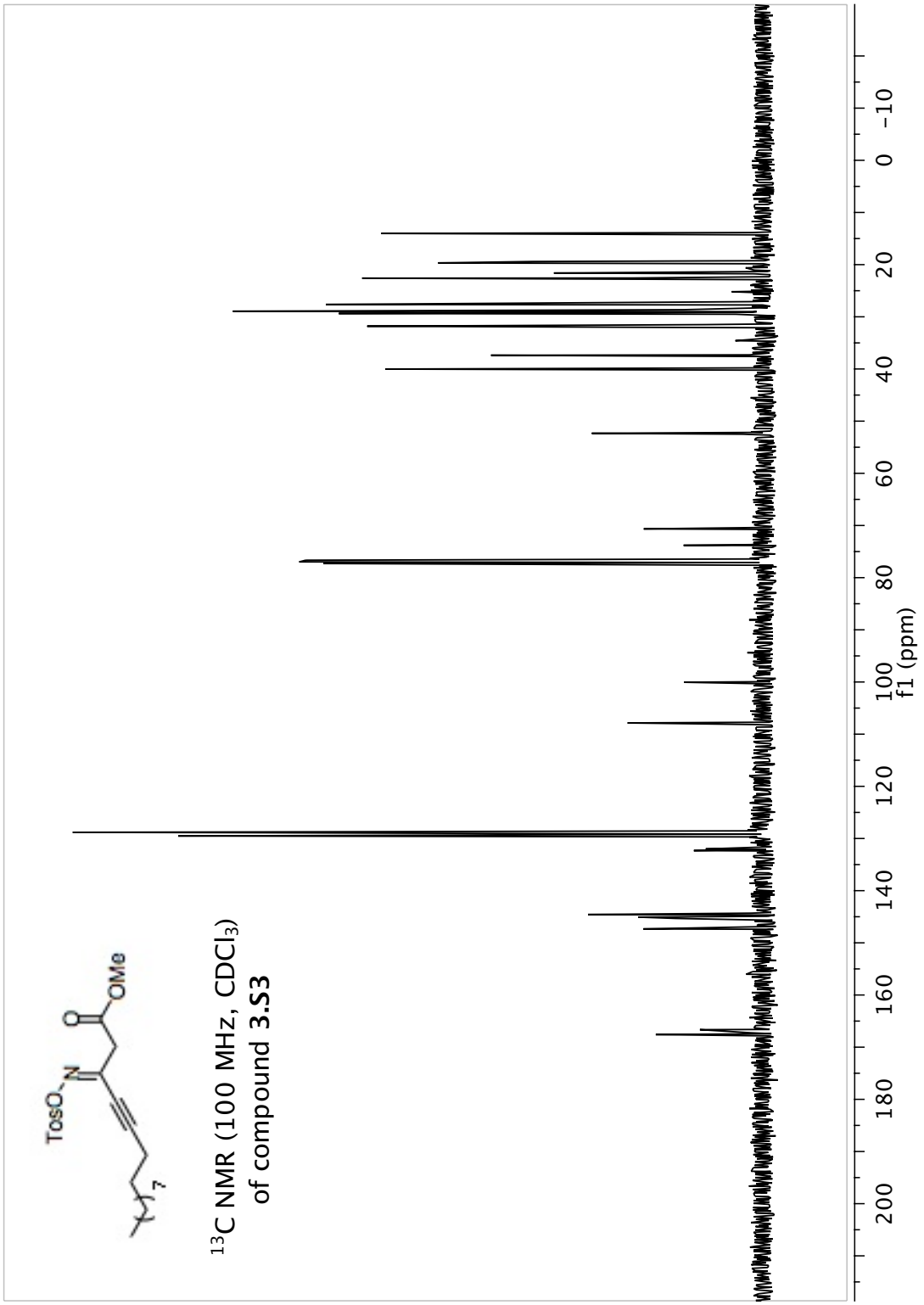
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.S2**



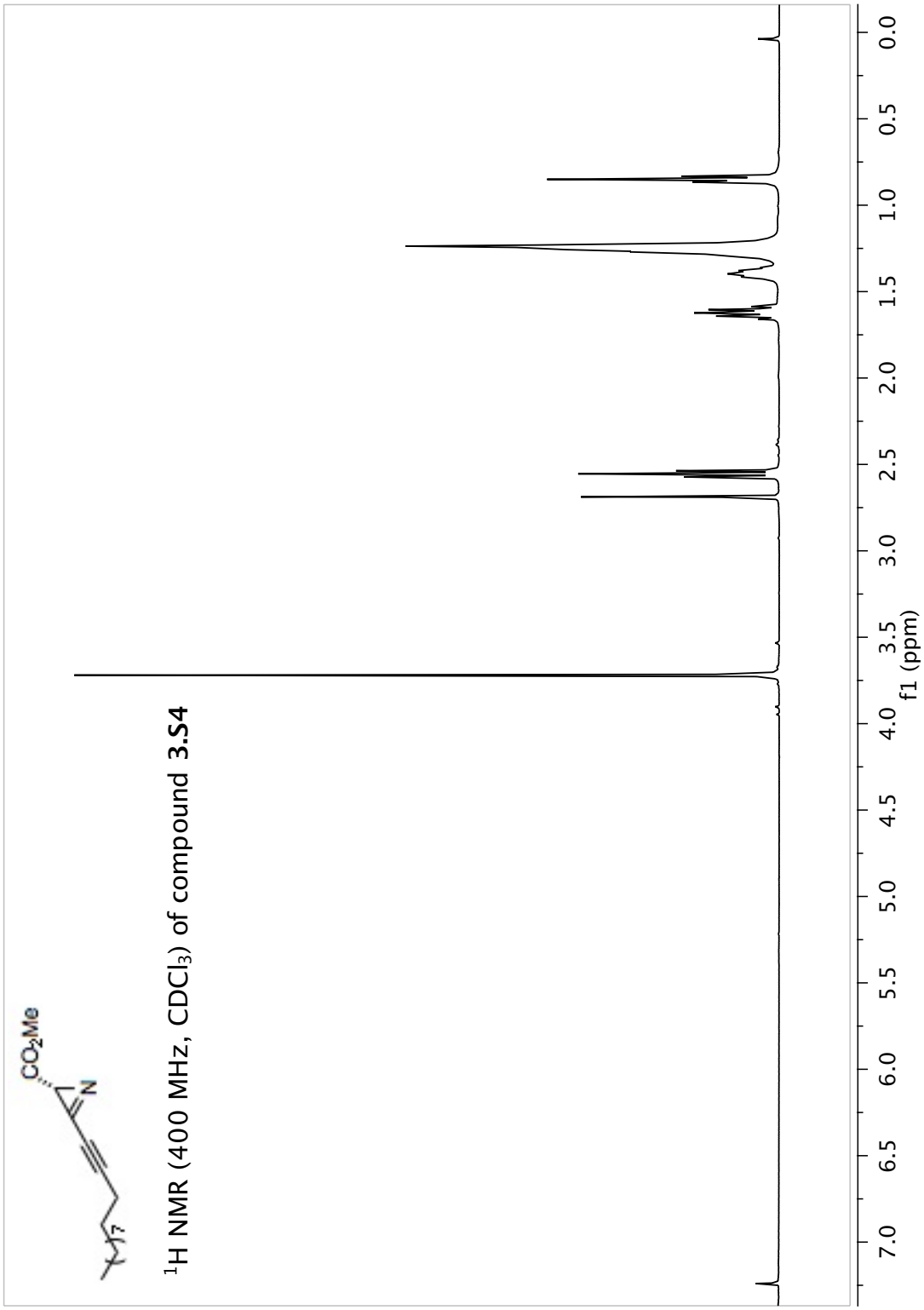
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.S2**



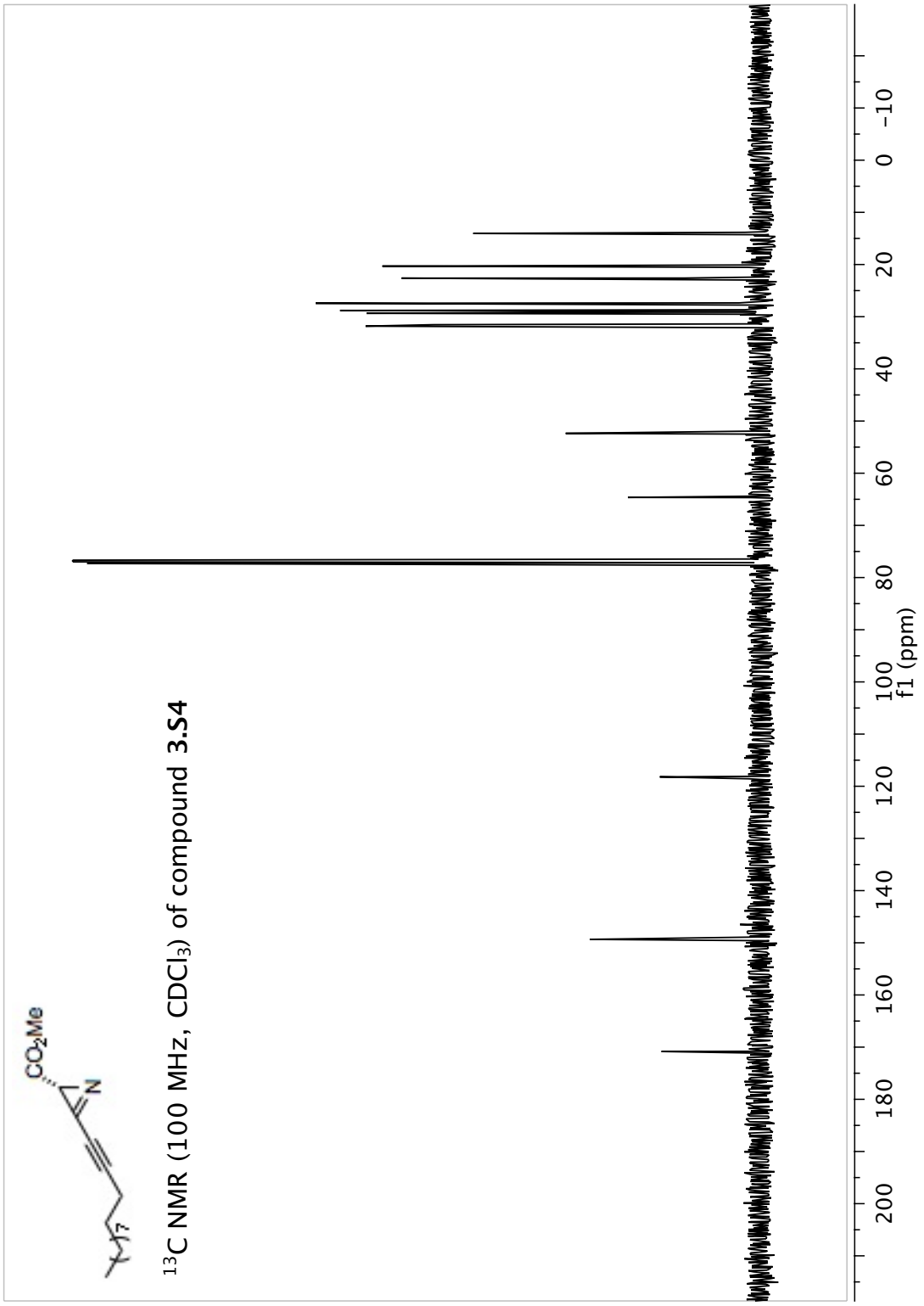
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.S3**



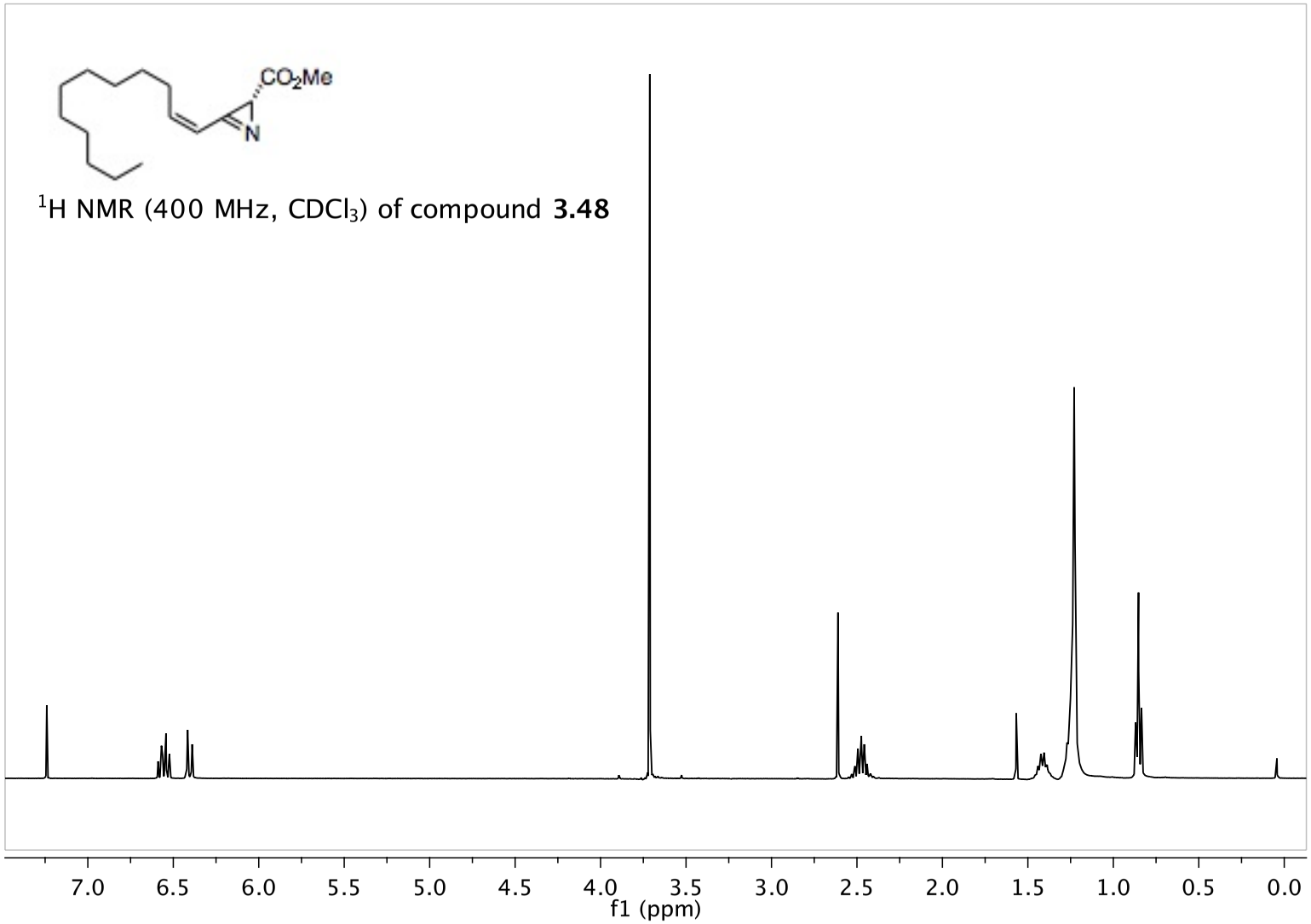
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **3.S3**

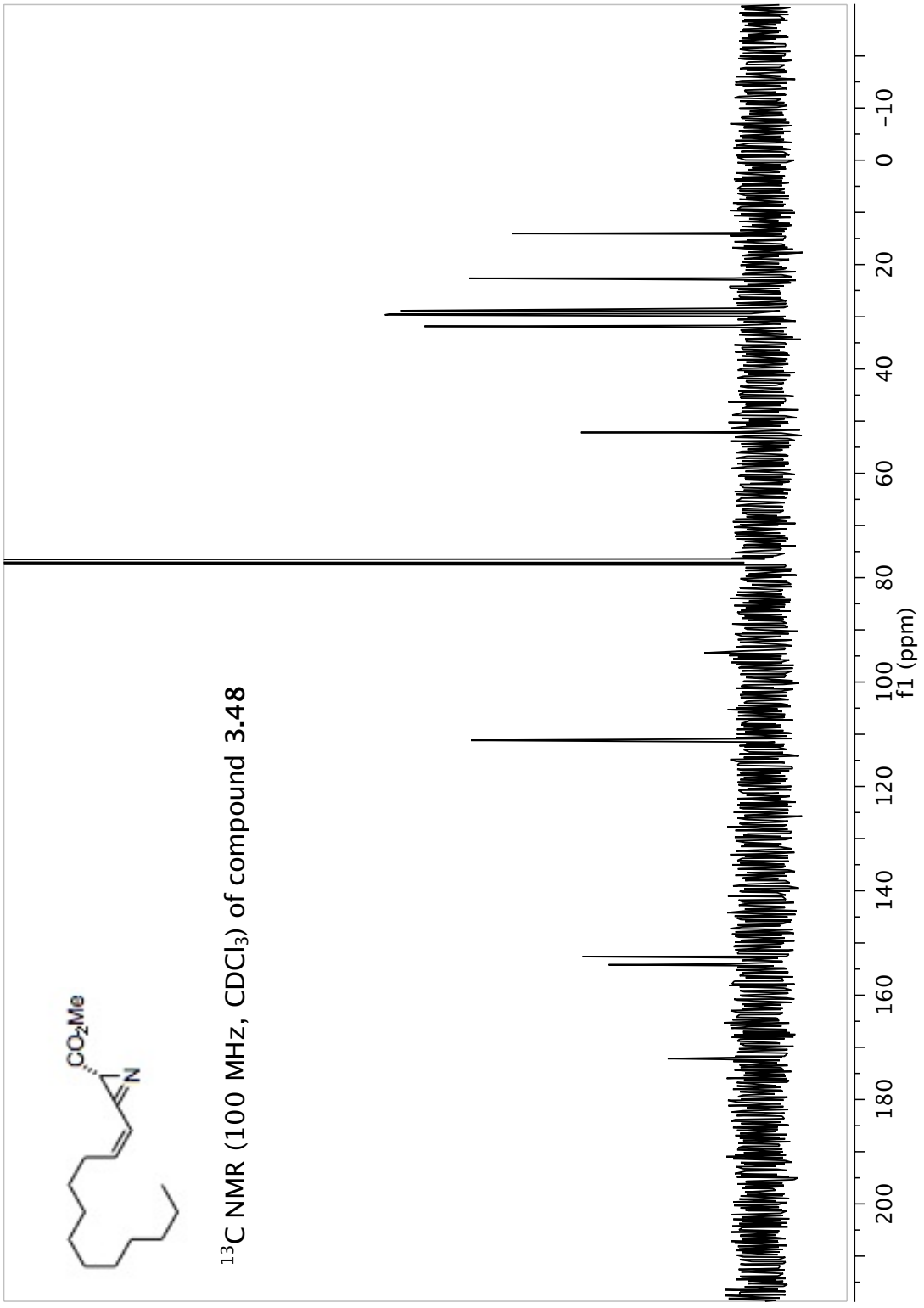


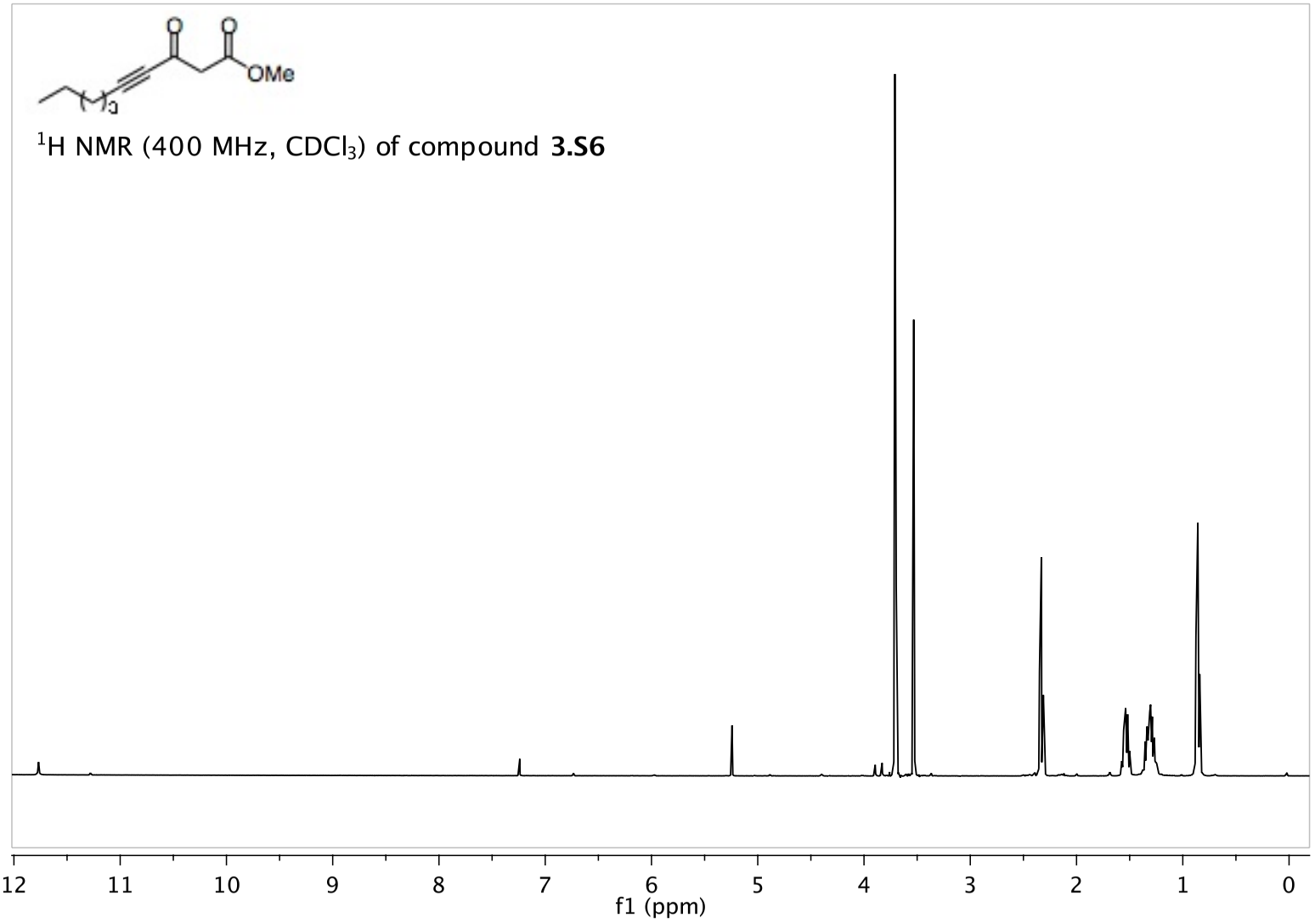
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.S4**

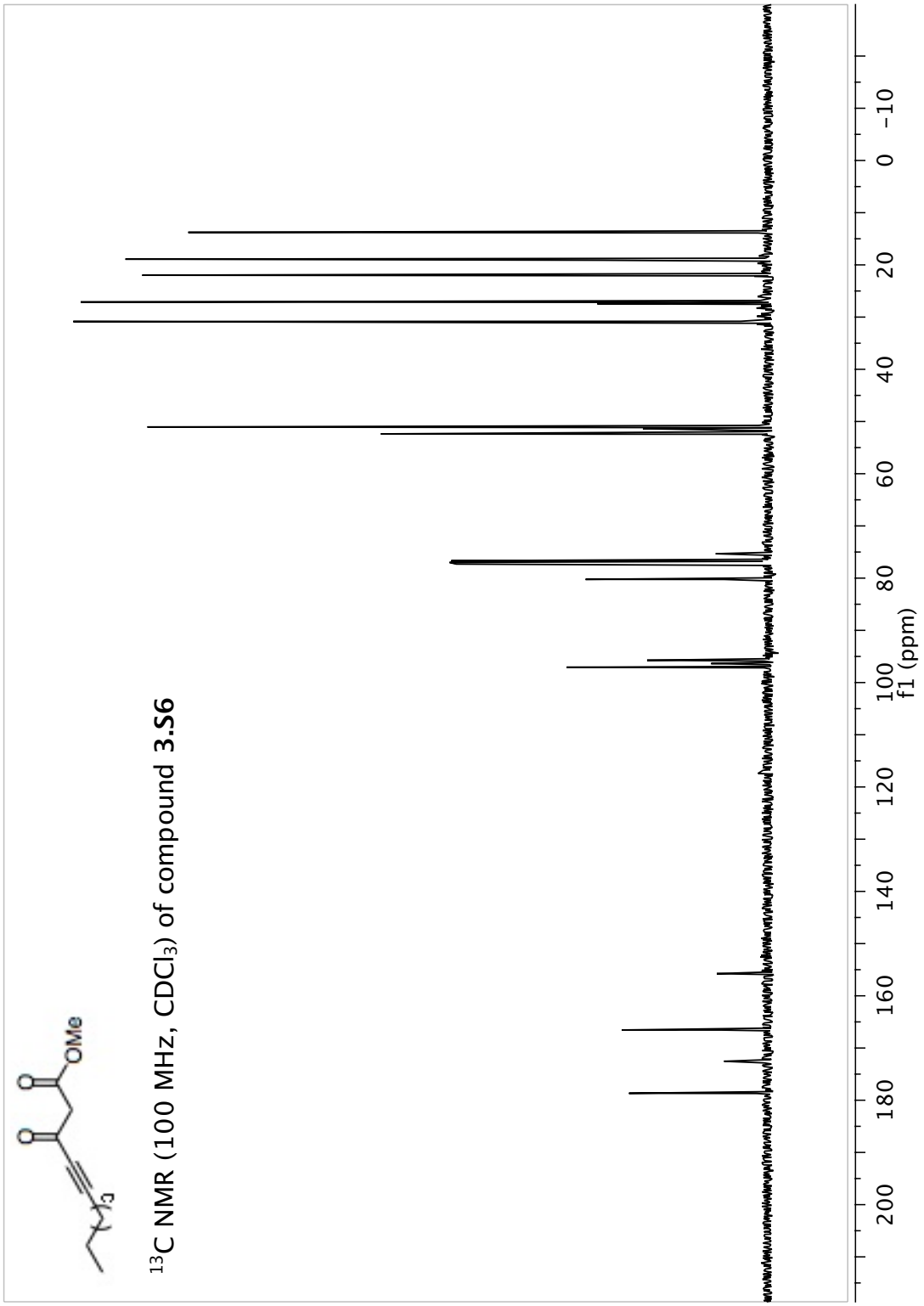


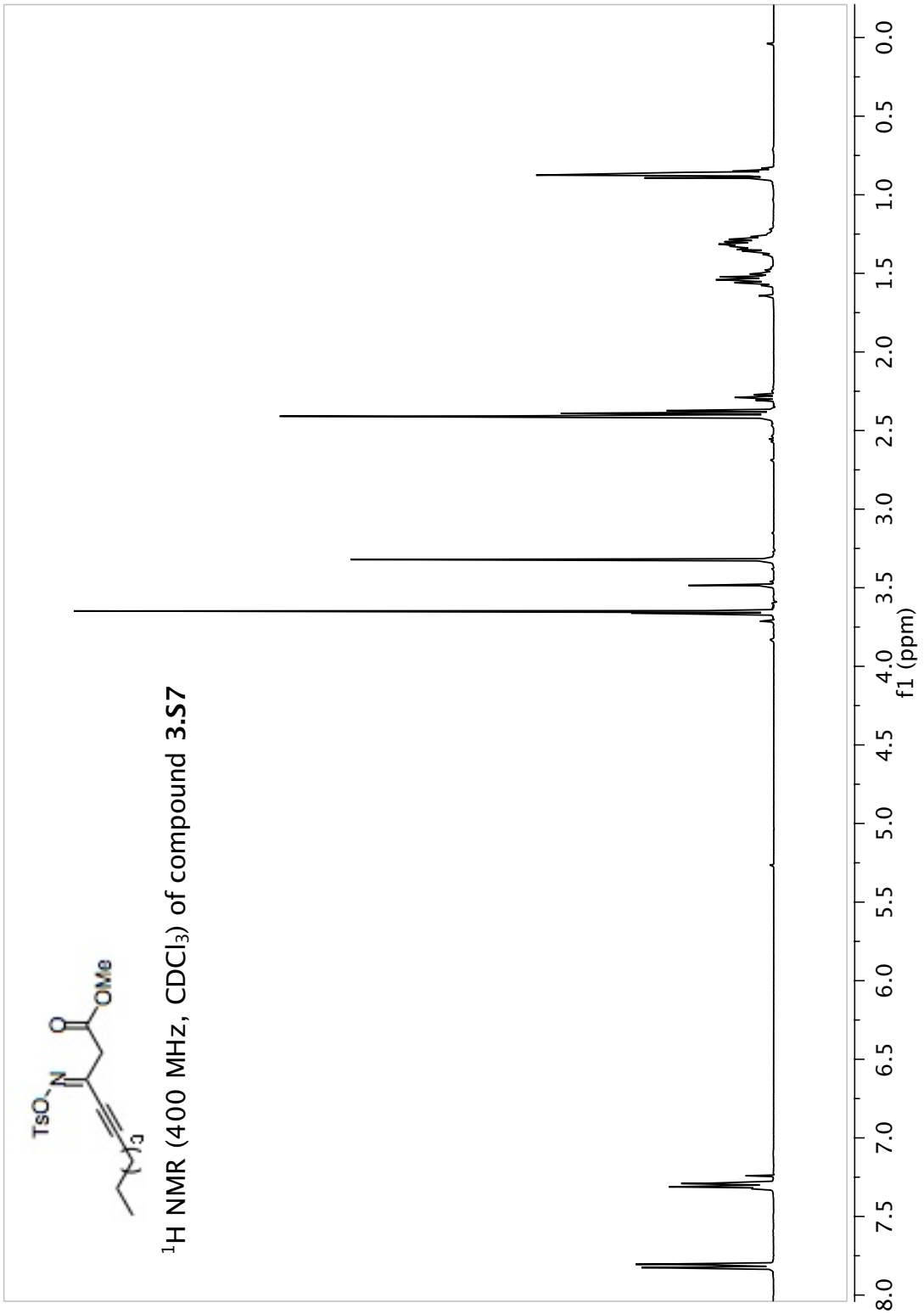
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.S4**


CO2Me
N
1H NMR (400 MHz, CDCl3) of compound 3.48
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

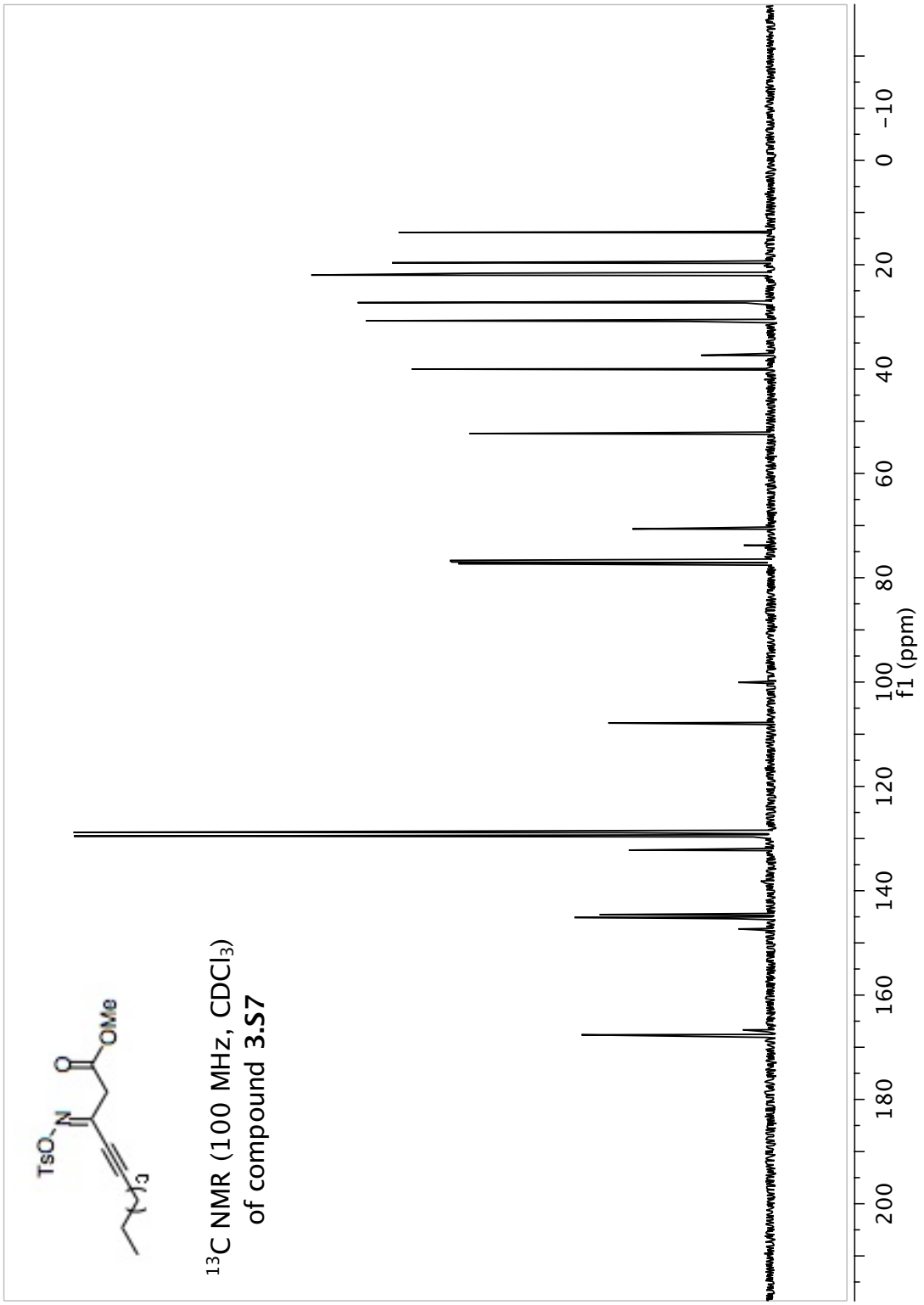

CO2Me
N
13C NMR (100 MHz, CDCl3) of compound 3.48
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

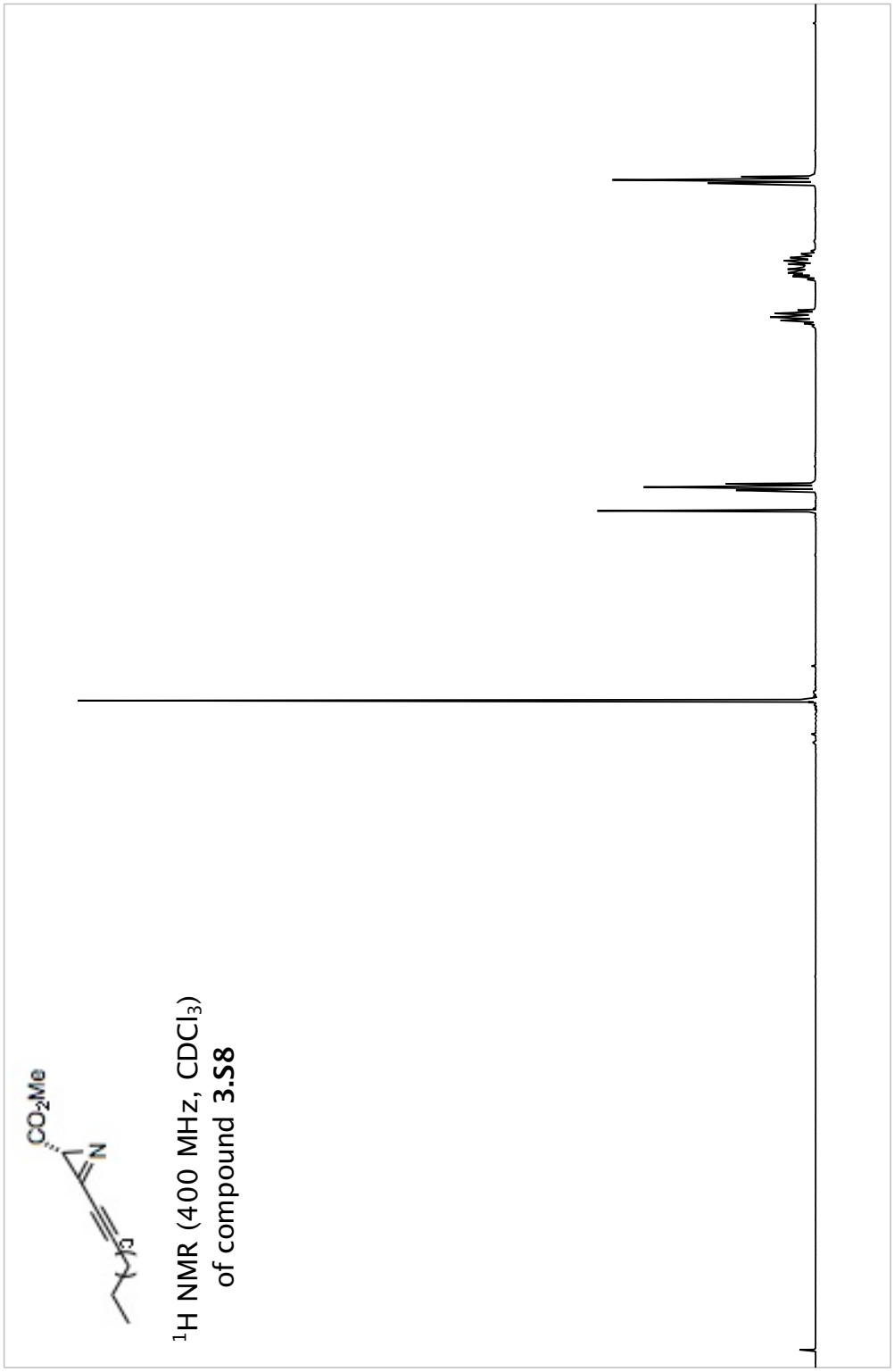

OMe
^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.S6**
12
11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


OMe
^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.S6**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

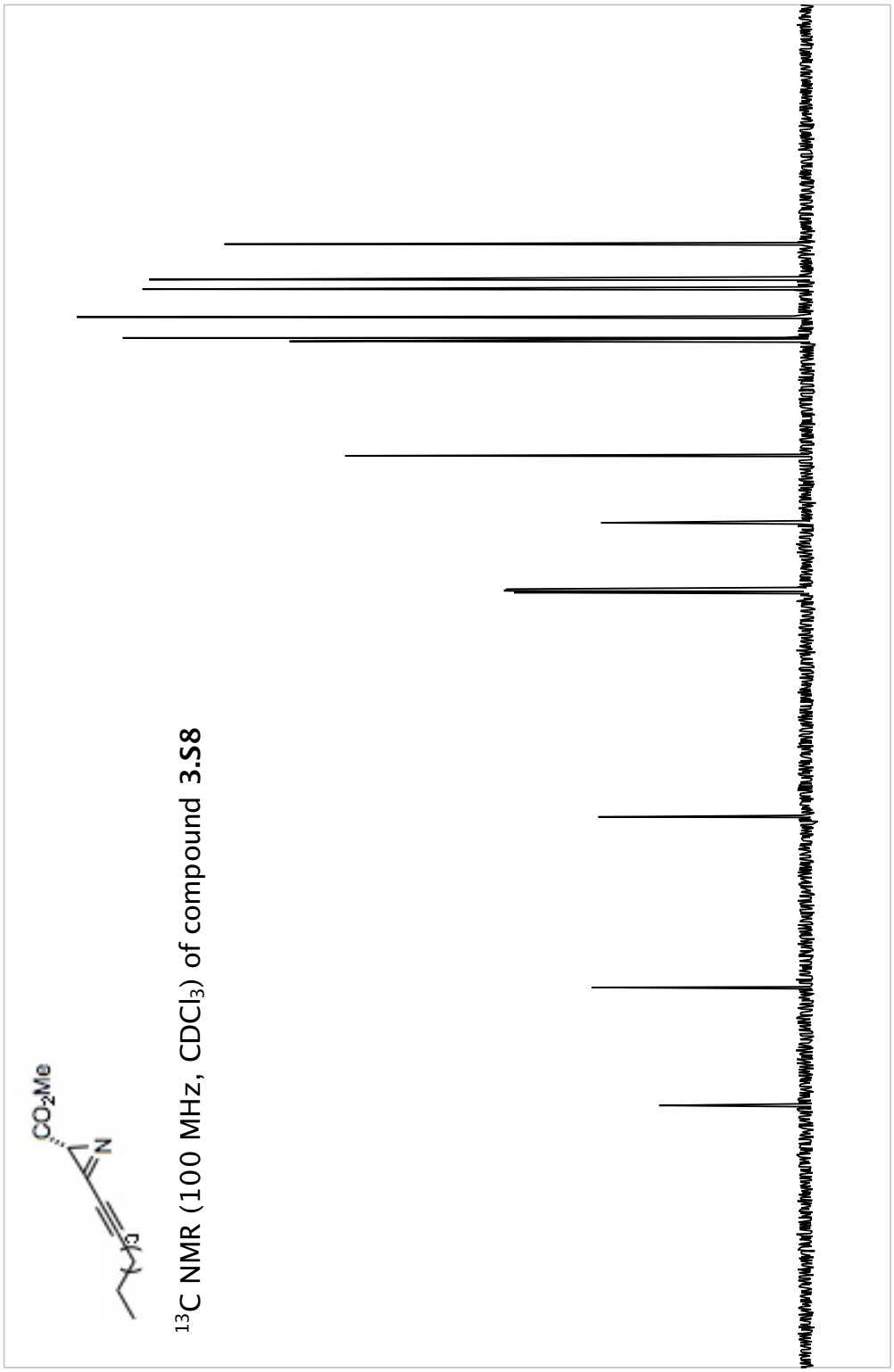


^{1}H NMR (400 MHz, $CDCl_3$) of compound **3.S7**

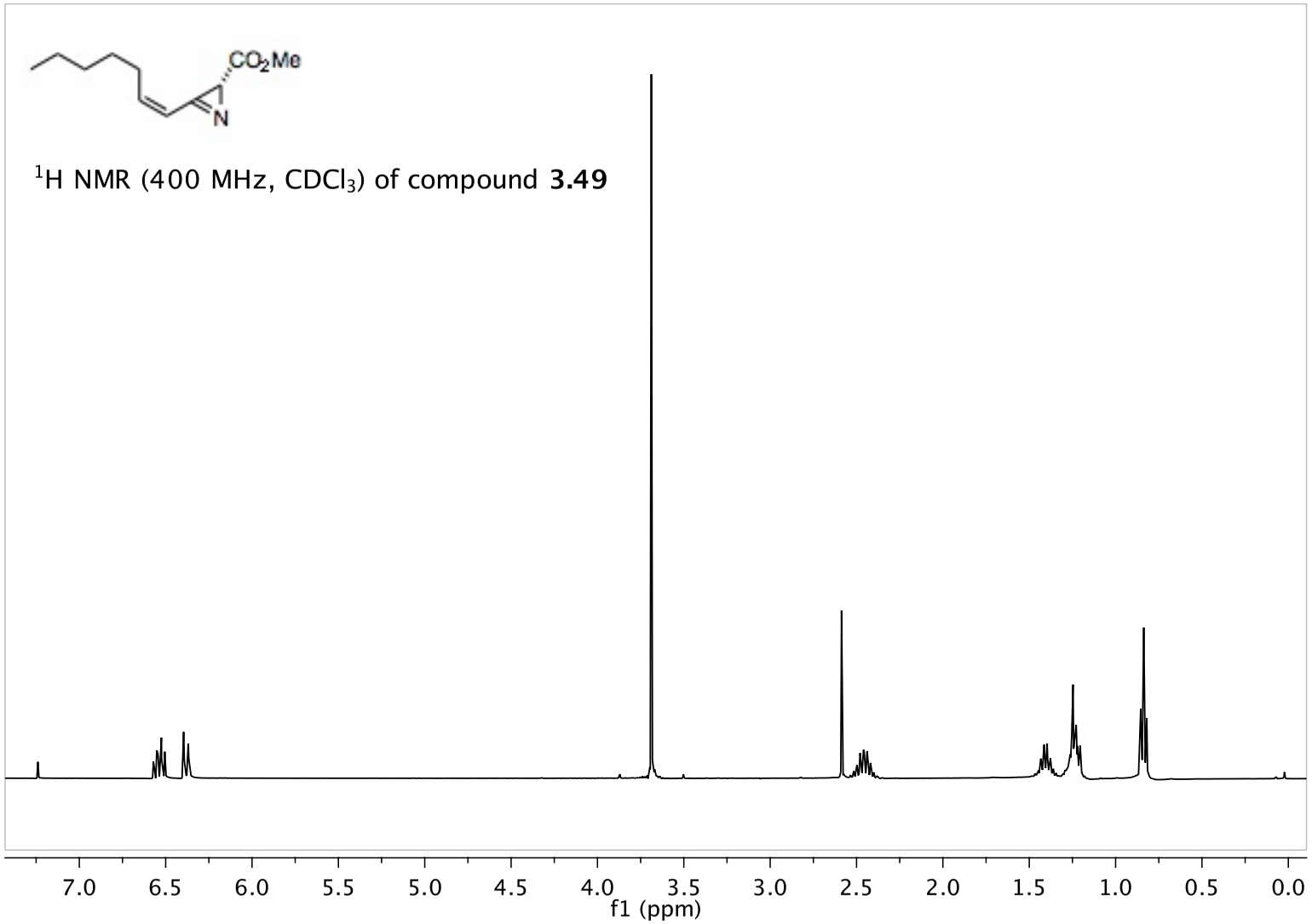

TsO
N
O
OMe
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **3.S7**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

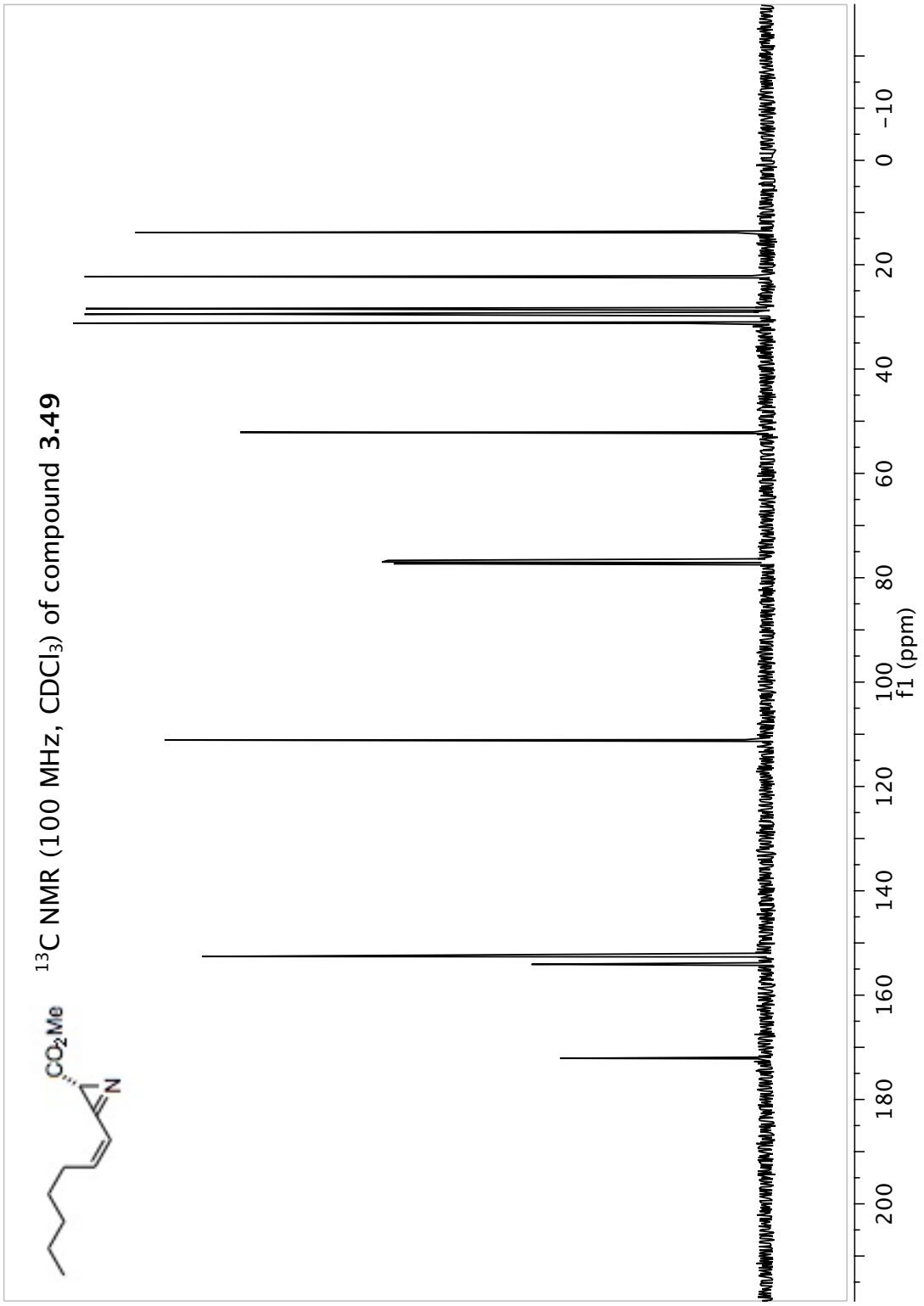


^{1}H NMR (400 MHz, $CDCl_3$)
of compound **3.58**



^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.S8**


CO2Me
N
1H NMR (400 MHz, CDCl3) of compound 3.49
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


^{13}C NMR (100 MHz, $CDCl_3$) of compound **3.49**
CO_2Me
N
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

CHAPTER 4

TOTAL SYNTHESIS OF ENIGMAZOLE A

4.1 Introduction – Enigmazoles A and B

As briefly outlined in Chapter 1, traditional cancer chemotherapeutic agents have been based on cytotoxins that display little selectivity for cancerous cells over healthy cells. More recently, cancer drug development has become focused on the discovery of compounds with subcellular targets unique to cancer cells. Many cancers arise from aberrations in the networks of signaling enzymes that control cell growth, proliferation and apoptosis.[1] Kinase- and phosphatase-dependent signal transduction has become a major target area for the pharmaceutical industry; Gleevec, for example, is a highly successful anticancer drug that inhibits the kinases Bcr-Abl, c-Kit and several others.[2]

c-Kit is a Type III transmembrane protein tyrosine kinase that shares structural homology with platelet-derived growth factor receptor (PDGFR) and macrophage colony-stimulating-factor receptor (CSF-1). c-Kit is responsible for mediating numerous signaling pathways critical for control of gametogenesis, embryogenesis, hematopoiesis, mast cell development and function, melanogenesis and development of the Interstitial Cells of Cajal (a type of cell found in smooth muscle that triggers gut contraction in the GI tract).[3,4,5,6,7] The molecular structure of c-Kit comprises an extracellular ligand-binding domain comprised of five immunoglobulin-like motifs, a single

transmembrane α-helix and an intracellular domain (Figure 4.1A).[3,5,8] The intracellular domain consists of a juxtamembrane (JM) region (residues 544-581) and a kinase domain which is made up of two lobes: a small N-terminal lobe (residues 582-671) that binds ATP and a large C-terminal lobe (residues 678-953) that catalyzes phosphorylation. The active site for phosphorylation is located in the cleft formed between the two lobes, which interconvert between an open conformation (for binding ATP) and a closed conformation (for positioning substrate residues in the active site) during catalysis.[3]

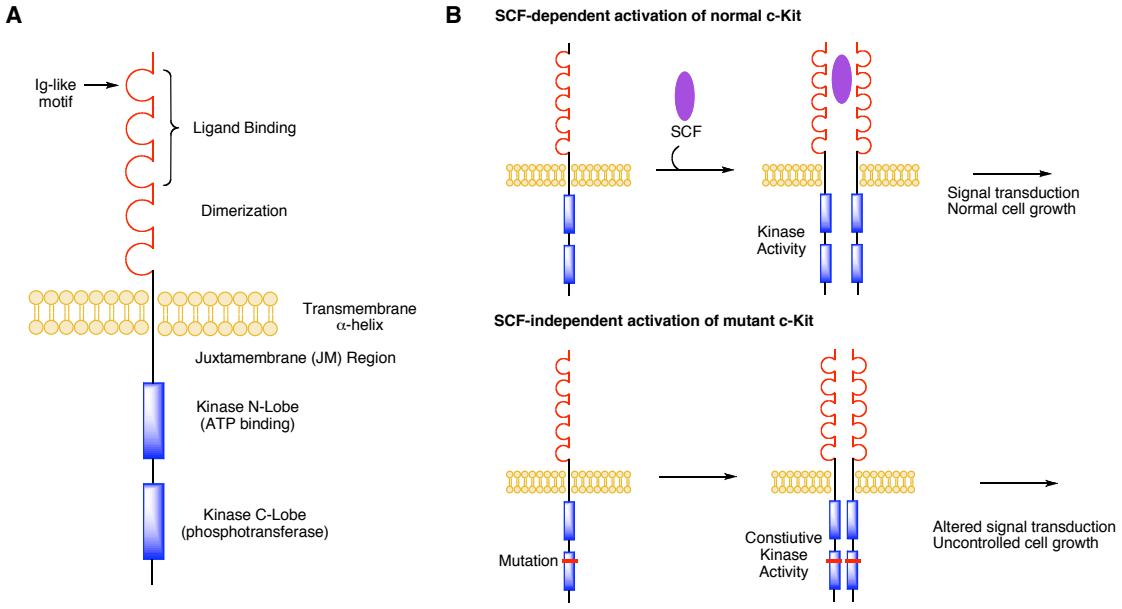


Figure 4.1 Structure and function of the transmembrane kinase c-Kit.

c-Kit is activated by Stem Cell Factor (SCF), alternatively known as Steel Factor and Mast Cell Growth Factor.[3-7,9,10,11] In healthy cells, SCF binds to the extracellular domain of c-Kit, inducing receptor dimerization (Figure 4.1B). Two tyrosine residues in the auto-inhibitory JM region (568 and 570)

are autophosphorylated, releasing the kinase N- and C-lobes. In addition, residue 823 of the A-Loop is phosphorylated, stabilizing the active, extended conformation.[12] c-Kit is now catalytically active and binds to a variety of adaptor proteins and enzymes such as Src family kinases, APS, Shp1, Shp2, Shc, Crk, Grb2, Grb7, Chk, phosphotidylinositol 3-kinase and phospholipase Cγ. Interaction with these adaptor proteins leads to activation of further downstream signaling components such as Akt, the Ras/mitogen-activated protein kinases and the Jak/STAT pathways.[3] In the case of human mast cells, the SCF/c-Kit interaction is believed to be critical for cell proliferation, suppression of apoptosis, differentiation (in the case of bone marrow-derived mast cells), adhesion to fibronectin, chemotaxis, cytoskeletal rearrangement and enhancement of mediator release.

Mutations in c-Kit that confer constitutive, ligand-independent kinase activity have been implicated in a variety of cancers including systemic mastocytosis (SM), acute myelogenous leukemia (AML), gastrointestinal stromal cell tumors (GISTs) and sinonasal natural killer/ T-cell lymphomas. Mutations occur most commonly in either the JM region or the kinase domain. Such mutations destabilize the inactive form of the enzyme, leading to c-Kit that phosphorylates substrate proteins independent of activation by SCF. Signaling pathways that promote cell growth and proliferation are thus permanently ‘switched on’ and contribute to tumorogenesis. Furthermore, it has been shown that the nature of the c-Kit mutation and the cell type affected

correlates with the type of cancer observed. For example, mutations in the JM region (most commonly at residues 557-559) are commonly associated with gastro-intestinal stromal cell tumors (GISTs), while replacement of Asp816 in the kinase C-lobe with valine, tyrosine, histidine or asparagine is common in mastocytosis, mast cell leukemia, acute myeloid leukemia (AML) and germ cell tumors.[3,5,7,8,13]

Over the past decade significant efforts have been directed toward the discovery of selective inhibitors of mutant c-Kit. To date several inhibitors of c-Kit have received FDA approval. The most widely used of these is Imatinib Mesylate (Gleevec®, Novartis, Basel, Switzerland).[Error! Bookmark not defined.] Gleevec was approved by the FDA for treatment of chronic myelogenous leukemia (CML) in May 2001, a remarkably short 2.5 months after NDA filing. In 2002 Gleevec received FDA approval for treatment of GISTs.[14] While Gleevec has proven highly effective against cancers that feature c-Kit JM domain mutations (i.e. CML and GISTs) it is ineffective against those that involve kinase domain mutatations (e.g. AML, mast cell leukemia, mastocytosis).[5,8,15] Furthermore, it has been found recently that many patients treated with Gleevec for GIST acquire drug resistance due to secondary mutations in the c-Kit kinase domain.[16] Therefore, drugs leads which affect tumor cells *via* these alternative c-Kit domains are of interest.

Gustafon *et al.* recently conducted a screening of >100,000 natural product extracts in the search for selective inhibitors of mutant c-Kit. Each

extract was tested against two lines of murine IC2 mast cells – one expressing wild type c-Kit and one expressing c-Kit with an activating kinase domain mutation. A remarkably sparse “hit-rate” was observed with less than 6 extracts showing differential cytotoxicity. One of these extracts from the marine sponge *Cinachyrella enigmatica* was purified to yield a new family of macrolides that includes enigmazoles A and B (**4.1** and **4.2**).[17] Enigmazole B displayed 10-fold enhanced activity against the mutant cells (IC_{50} 0.17 μg/mL vs mutant cells, 1.9 μg/mL vs. WT) while enigmazole A showed potent but unselective cytotoxicity (IC_{50} 0.3 μg/mL). Interestingly, enigmazole B was tested against a panel of phosphatases and kinases but showed no inhibitory activity. This suggests that **4.2** elicits a highly unusual phenotypic response from the mutant mast cells independent of kinase activity. The cellular target of **4.1** and **4.2** has not yet been identified.

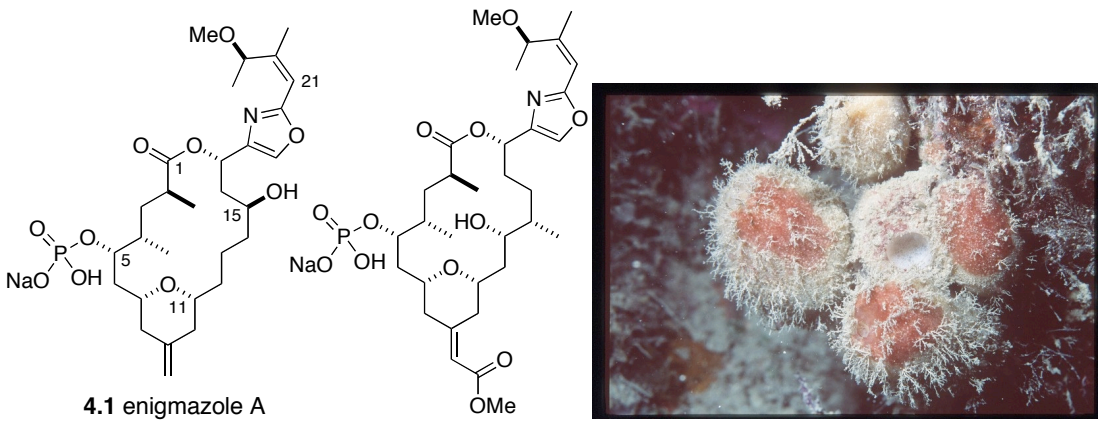
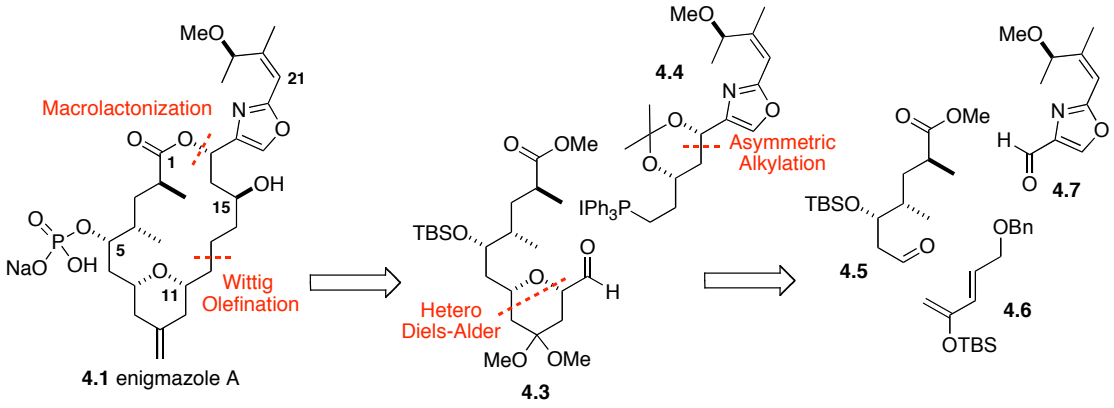


Figure 4.2 Structurures of enigmazoles A and B (**4.1** and **4.2**) and photograph of the sponge *Cinachyrella enigmatica* (courtesy of CCRF).

The limited availability of enigmazoles from the natural source has thus far impeded more extensive biological investigations. Our aim was to design and implement a total synthesis of **4.1** and **4.2** that could facilitate further evaluation of the natural products and provide a platform for synthesis of analogues. Hybrid analogues of **4.1** and **4.2** would be of great value in determining the structural features that confer selective cytotoxicity on **4.2** but not **4.1**. Carefully designed synthetic analogues will also be required to identify the protein or enzyme target of enigmazoles. Synthesis of appropriate affinity-tagged analogues would facilitate target pull-down experiments, while fluorescent tagged analogues would be useful for cell uptake and subcellular localization studies. In addition, enigmazoles were judged to be a good platform for deployment of new methodology for the construction of 2,4-disubstituted oxazoles.

Enigmazoles A and B are characterized by an 18-membered macrolactone with an embedded 2,6-disubstituted pyran ring. Both compounds feature a rare phosphate ester attached at the C5 alcohol and an identical, densely functionalized oxazole side chain appended at C17. Although only enigmazole B (**4.2**) shows selective cytotoxicity we determined that an initial synthesis of the less-complex congener enigmazole A (**4.1**) would inform a later synthesis of **4.2**. We therefore designed a synthetic route toward enigmazole A incorporating strategies and transformations that could be applied to enigmazole B. Implementation of this route has resutled in the first total synthesis of enimgazole A.



Scheme 4.1 Retrosynthetic analysis of enigmazole A (**4.1**)

Retrosynthetic analysis of enigmazole A suggested a logical disconnection at the ester bond and at the C12-C13 bond to give aldehyde **4.3** and phosphonium salt **4.4** that we planned to unite by a late-stage Wittig reaction. Western hemisphere **4.3** in turn could be derived from a hetero-Diels-Alder reaction between aldehyde **4.5** and diene **4.6**. Further disconnection of

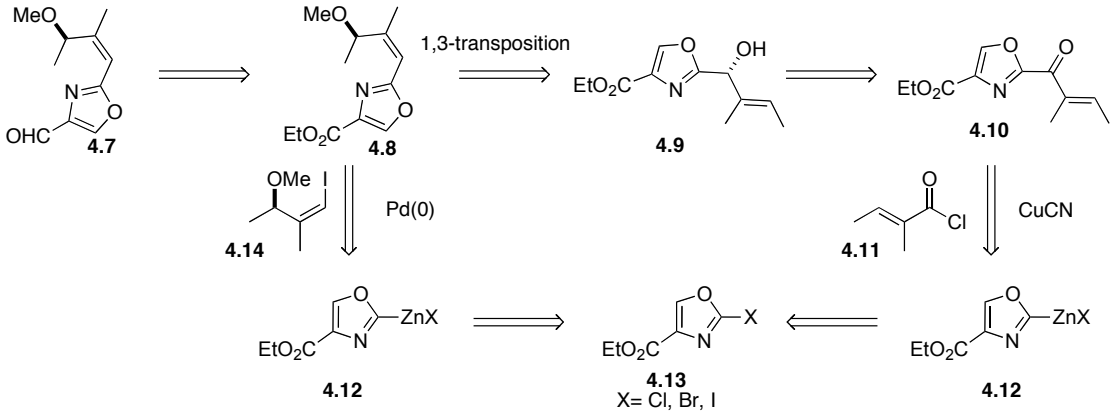
the eastern hemisphere **4.4** at the C16-C17 bond leads oxazole-4-carboxaldehyde **4.7** which is primed for an asymmetric alkylation to set the C17 stereocenter.

4.2 Synthesis of the Eastern Hemisphere

Numerous methods have been reported for the construction of 2,4-disubstituted oxazoles (see Chapter 1). In the context of natural product synthesis, the most popular method is the cyclodehydration of *N*-acylserine amide precursors which usually requires 2 steps – a cyclodehydration step to form an oxazoline which is subsequently oxidized to the corresponding oxazole.[18] While largely successful, implementation of this methodology in complex precursors with sensitive functionality can be risky and low yielding, as demonstrated by Forsyth in their synthesis of phorboxazole A.[19] The α-center adjacent to the oxazole C2 position is prone to epimerization, as demonstrated in Ley's synthesis of bengazole A.[20]

The side chain of enigmazole A contains a *Z*-olefin conjugated to an oxazole ring with an isolated stereocenter at the allylic position (C23). In designing a route to the eastern hemisphere of **4.1** the major consideration was installation of the oxazole ring without epimerization at C23 and with retention of the *Z*-olefin geometry. Consequently we planned to introduce the oxazole ring early in the synthetic sequnce as an intact unit without relying on a cylodehydration/oxidation strategy.

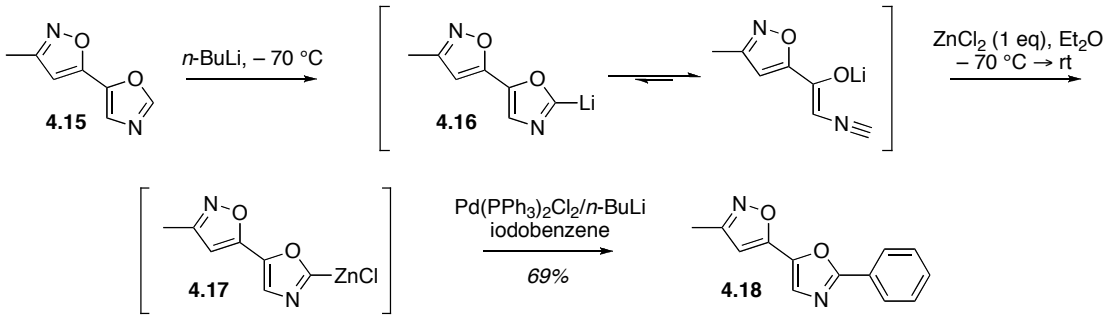
Aldehyde **4.7** was expected to arise from reduction of the corresponding ethyl ester **4.8** (Scheme 4.2). Ester **4.8** could then be derived from one of two sequences. In the first, the C21 stereocenter could be set by a [1,3] sigmatropic rearrangement of optically pure alcohol **4.9** obtained by stereoselective reduction of ketone **4.10**. Ketone **4.10** was envisioned to arise from copper catalyzed acylation of oxazol-2-yl zinc reagent **4.12** with tigloyl chloride (**4.11**). Alternatively, **4.8** could be obtained directly by Negishi coupling[21] of oxazol-2-yl zinc reagent **4.12** with vinyl iodide **4.14**. In either case, zincate **4.12** would be obtained *via* the direct zinc insertion reaction of a 2-halooxazole such as **4.13** (X = Br, I).



Scheme 4.2 Retrosynthetic analysis of oxazole fragment **4.7**

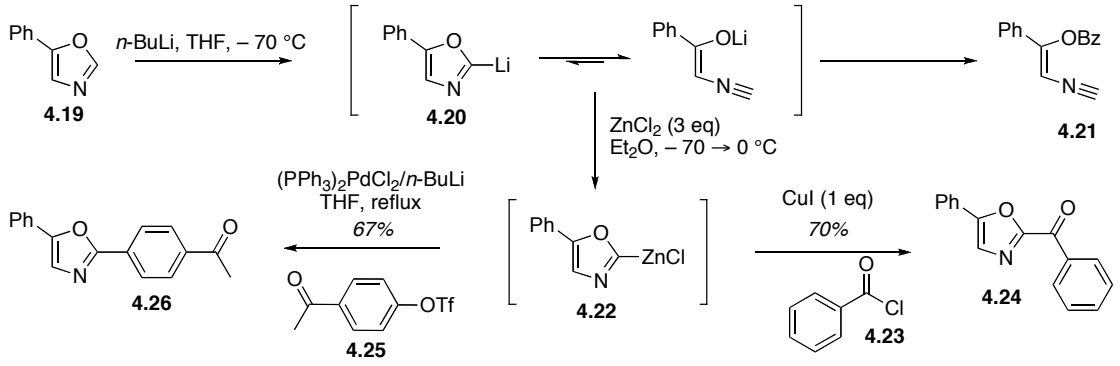
C2-Metalated oxazoles have not been widely exploited in natural product synthesis. This is most likely due to the difficulty of controlling the reactivity of 2-lithiooxazoles, the most easily accessed metalated oxazole. 2-Lithiooxazoles undergo facile ring opening,[22] however the corresponding oxazol-2-yl zincates strongly favor the ring closed form.

Hughes *et al.* showed that 2-lithiooxazole **4.16** (derived from **4.15**) can be transmetalated with $ZnCl_2$ in Et_2O to provide the corresponding oxazol-2-yl zincate (**4.17**, Scheme 4.3).[23] Quenching with D_2O and ^1H NMR analysis indicated >85% deuterium incorporation at C2. Zincate **4.17** was found to undergo palladium catalyzed cross coupling with aryl and vinyl iodides in reasonable yield to give C2-coupled products, for example **4.18**.



Scheme 4.3 Hughes' prepartion of oxazol-2-yl zinc chlorides

Anderson further showed that oxozol-2-yl zinc chlorides such as **4.22** (Scheme 4.4) undergo acylation in the presence of CuI to give ketones, for example, **4.24**.[24] In contrast, treatment of 2-lithiooxazole **4.20** with benzoyl chloride leads predominantly to the *O*-acylated product **4.21**. Oxazol-2-yl zinc chloride **4.22** also underwent Negishi-type coupling with aryl and vinyl triflates and aryl iodides.[25]

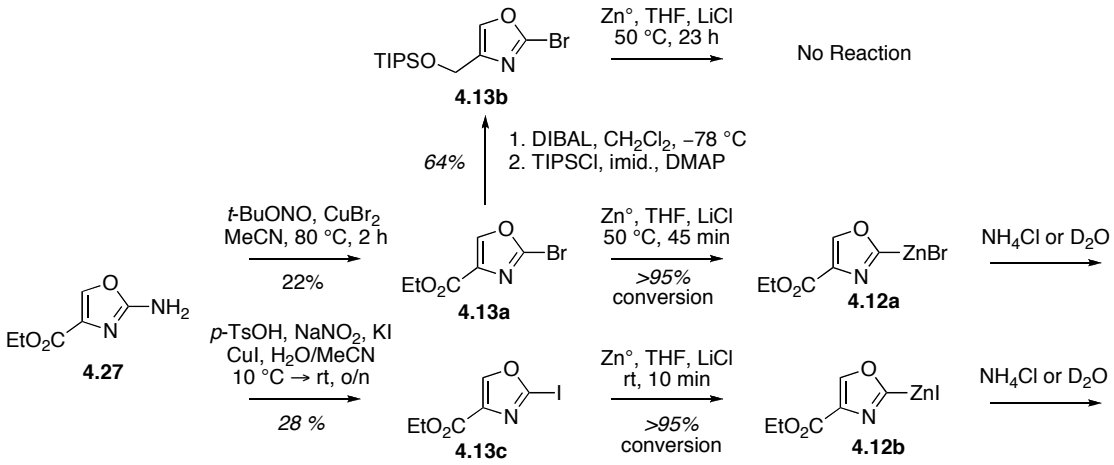


Scheme 4.4 Anderson's synthesis of oxazol-2-ylzinc chlorides and subsequent acylation and Negishi coupling reactions.

Reeder *et al.* reported that formation of oxazol-2-yl zinc chlorides is improved by transmetalating 2-lithiooxazoles with solid $ZnCl_2$ (as opposed to solutions of $ZnCl_2$ in ether solvents).[26] The zincates obtained in this manner were found to be somewhat more reactive in subsequent palladium(0) catalyzed cross coupling reactions. Recently, Mongin *et al.* described a method for preparation of oxazole-2-yl zinc chlrorides involving deprotonation of the parent oxazole using a mixture of $ZnCl_2$•TMEDA and lithium 2,2,6,6-tetramethylpiperidide in THF.[27]

In each of the above cases the oxazol-2-ylzinc reagent is prepared by deprotonation of the unsubstituted parent oxazole using a strong base. Because fragment **4.7**, substituted at C4 with a carboxylate group, is incompatible with typical alkyl lithium reagents we sought to form the necessary zincate by direct zinc insertion from a 2-halooxazole, **4.13**. Since a search of the literature revealed no report of an oxazol-2-yl zinc reagent formed by direct zinc insertion we embarked on development of this strategy.

The known 2-aminooxazole **4.27**,[28] prepared by condensation of urea and ethyl bromopyruvate, was transformed into ethyl 2-bromooxazole-4-carboxylate (**4.13a**) according to the procedure reported by Hodgetts *et al*.[29] (Scheme 4.5). Reduction of the ethyl ester (DIBAL) and protection of the primary alcohol (TIPSCl, imidazole) gave derivative **4.13b**. The novel ethyl 2-iodooxazole-4-carboxylate (**4.13c**) was prepared by diazotization/iodination.[30,31] It was found that addition of catalytic CuI increased the rate of reaction (as judged by TLC) and led to slightly improved yields of **4.13c**. Although the yield of **4.13c** is modest overall, the product is easily purified by a short silica column and can be prepared on a gram scale routinely.



Scheme 4.5 Preparation of oxazol-2-yl zinc reagents **4.12a** and **4.12b**

With halooxazoles **4.13a-c** in hand the formation of oxazol-2-yl zincates was examined using conditions previously described by Knochel for formation of arylzincates.[32] The relatively electron-rich 2-bromooxazole derivative **4.13b**

proved to be inert when treated with metallic zinc dust and LiCl in THF (50 ˚C, 23 h), however **4.13a** underwent smooth insertion at the same temperature over 45 minutes to give **4.12a** with a distinctive red-brown color. Remarkably, 2-iodooxazole **4.13c** underwent complete zinc insertion in only 10 minutes at room temperature to give **4.12b**. Unlike **4.12a**, solutions of **4.12b** were a pale yellow color.

The yields of zinc insertion were quantitated by quenching experiments (see Figure 4.3). Two aliquots of zincate **4.12b** were quenched separately with saturated $NH_4Cl_{(aq)}$ and D_2O. Examination of the product reulting from $NH_4Cl_{(aq)}$ quench by ^1H NMR revealed only ethyl oxazole-4-carboxylate **4.28** (Figure 4.3b), indicating complete consumption of **4.13c**. The product from D_2O quench (Figure 4.3c) revealed 90-95% incorporation of deuterium at C2 indicating high titers of the desired zincate **4.12b**. These results support previous findings that oxazol-2-yl zincates favor the ring-closed tautomer and react with electrophiles primarily at C2.

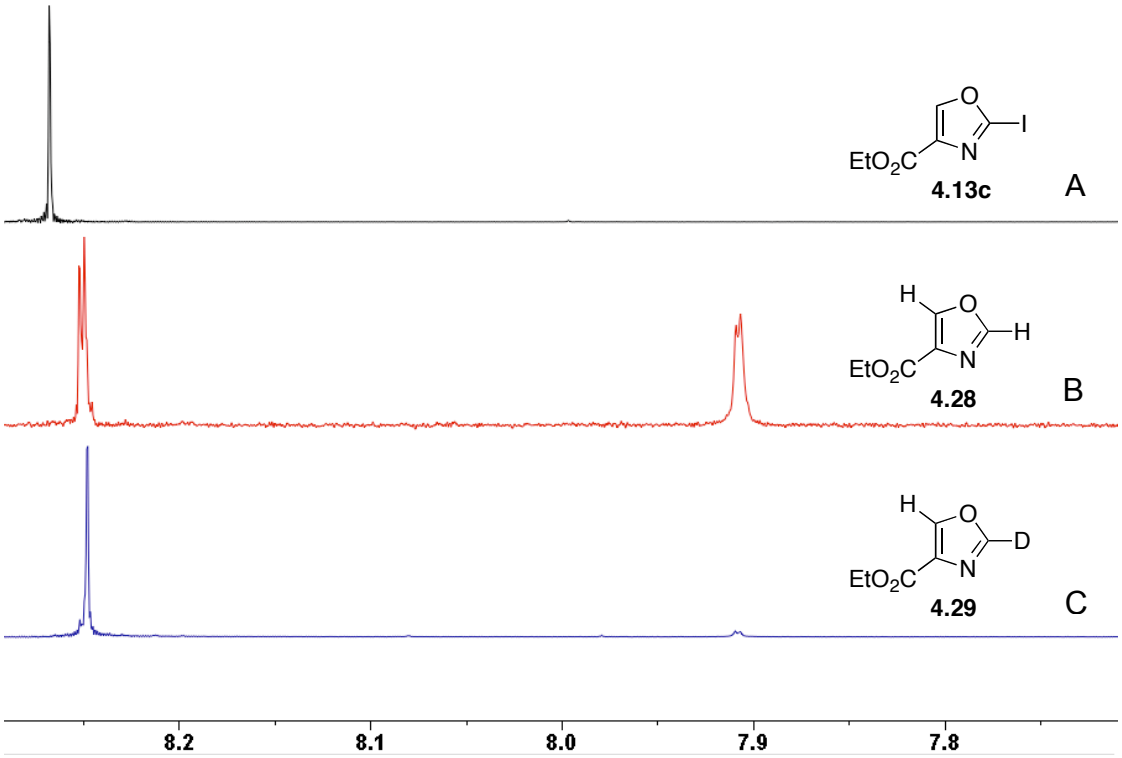
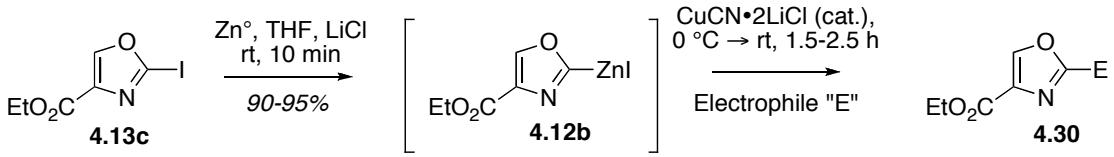
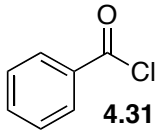
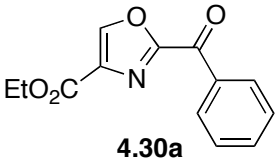
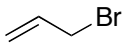
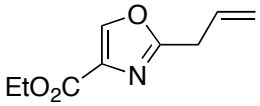
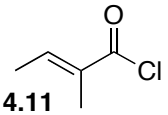
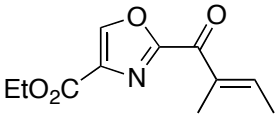


Figure 4.3 ^{1}H NMR (400 MHz, $CDCl_3$) of a) ethyl 2-iodooxazole-4-carboxylate (**4.13c**); b) Ethyl oxazole-4-carboxylate (**4.28**) derived from quench of zincate **4.12b** with NH_4Cl; c) ethyl 2-deuteriooxazole-4-carboxylate (**4.29**) derived from quench of **4.12b** with D_2O.

The superior reactivity of **4.13c** lead to the selection of **4.12b** for use in all subsequent experiments. Zincate **4.12b** was found to undergo acylation reactions in the presence of a catalytic amount of CuCN•2LiCl, yielding **4.30a** and **4.30b** with benzoyl chloride (**4.31**) and allyl bromide (**4.32**) respectively.[32] Importantly, reaction with tigloyl chloride (**4.11**) provided the desired ketone **4.10** in good yield.



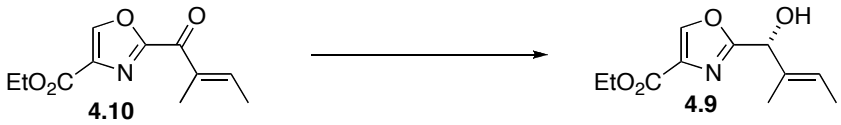
4.13c **4.12b** **4.30**

	Electrophile	Product	Yield
1	 **4.31**	 **4.30a**	72%
2	 **4.32**	 **4.30b**	50%
3	 **4.11**	 **4.10**	77%

Scheme 4.6 Acylation of oxazol-2-yl zinc reagent **4.12c**

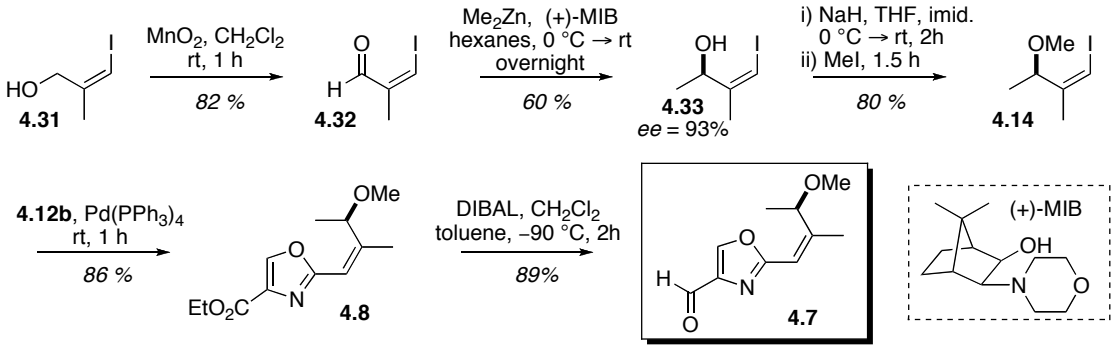
Unfortunately all attempts to reduce ketone **4.10** stereoselectively with Corey's Me-CBS catalyst[33] led to decomposition (Table 4.1, entry 1-4). Interestingly, reduction of **4.10** using Luche conditions[34] (Table 4.1, entry 5) resulted in cleavage of the acyl group from the oxazole ring and isolation of ethyl oxazole-4-carboxylate (**4.28**). Thus it appeared from these experiments that **4.10** was simply too unstable to withstand even mild reduction conditions.

Table 4.1 Attempted reduction of ketone **4.10**



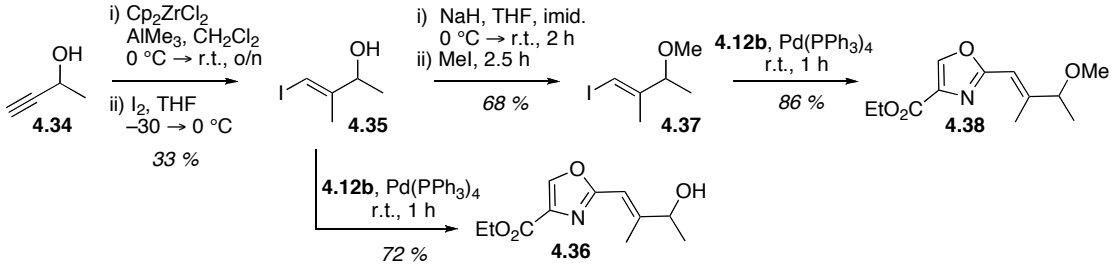
	Reductant	Catalyst	Temperature (Time)	Solvent	Result
1	BH_3·THF	*R*-Me-CBS	0 ˚C → rt (2.25 h)	THF	Decomposition
2	BH_3·THF	*S*-Me-CBS	0 ˚C → rt (2.25 h)	THF	Decomposition
3	BH_3·DMS	*R*-Me-CBS	−30 ˚C (45 min)	THF	Decomposition
4	catechol·BH_3	*R*-Me-CBS	−78 ˚C (26 h) then rt (14 h)	toluene	Decomposition
5	$NaBH_4$	$CeCl_3.7H_2O$	0 ˚C → r.t (4.75 h)	MeOH	Isolated **4.28**

An alternative route to oxazolyl carbinols involving Negishi-type coupling between **4.12c** and vinyl iodide **4.14** was examined. Oxidation of known vinyl iodide **4.31**[35] gave the geometrically unstable aldehyde **4.32** which was used immediately in the next step (Scheme 4.7). Addition of Me_2Zn in the presence of (+)-3-*exo*-(morpholino)isoborneol ((+)-MIB)[36] gave alcohol **4.33** in reasonable yield and high enantiomeric excess (*ee* = 93%, determined by Mosher's ester analysis[37]). Methylation of alcohol **4.33** using standard conditions provided **4.14** in 80% yield. Gratifyingly, the crucial Negishi coupling between zincate **4.12c** and **4.14** proceeded smoothly and in high yield to give **4.8** which was subsequently reduced with DIBAL at low temperature to provide aldehyde **4.7** in 89% yield.



Scheme 4.7 Synthesis of key oxazole fragment **4.7**.

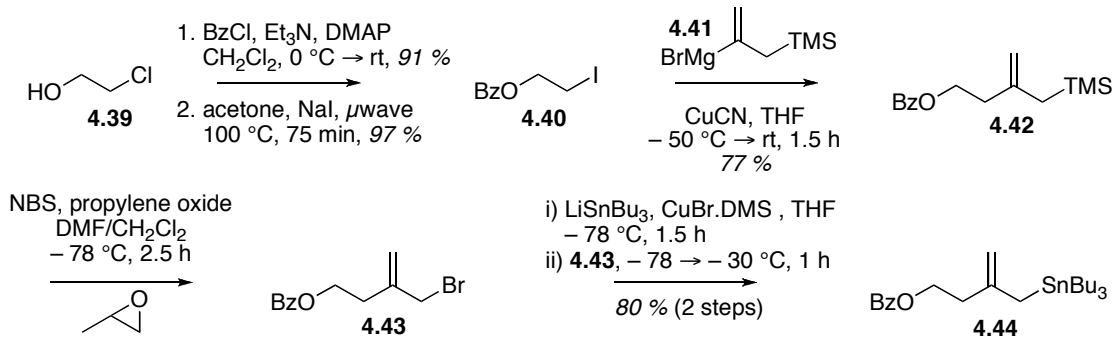
The Negishi coupling was equally successful with vinyl iodide **4.37** (the geometric isomer of **4.14**) which was prepared in 2 steps from 2-butyn-3-ol (**4.34**) (Scheme 4.8).[38] Furthermore, successful coupling (albeit in slightly reduced yield) was observed with the free alcohol **4.35** highlighting the versatility and low basicity of organozinc reagent **4.12c**.



Scheme 4.8 Negishi coupling between **4.12b** and vinyl iodides **4.35** and **4.37**

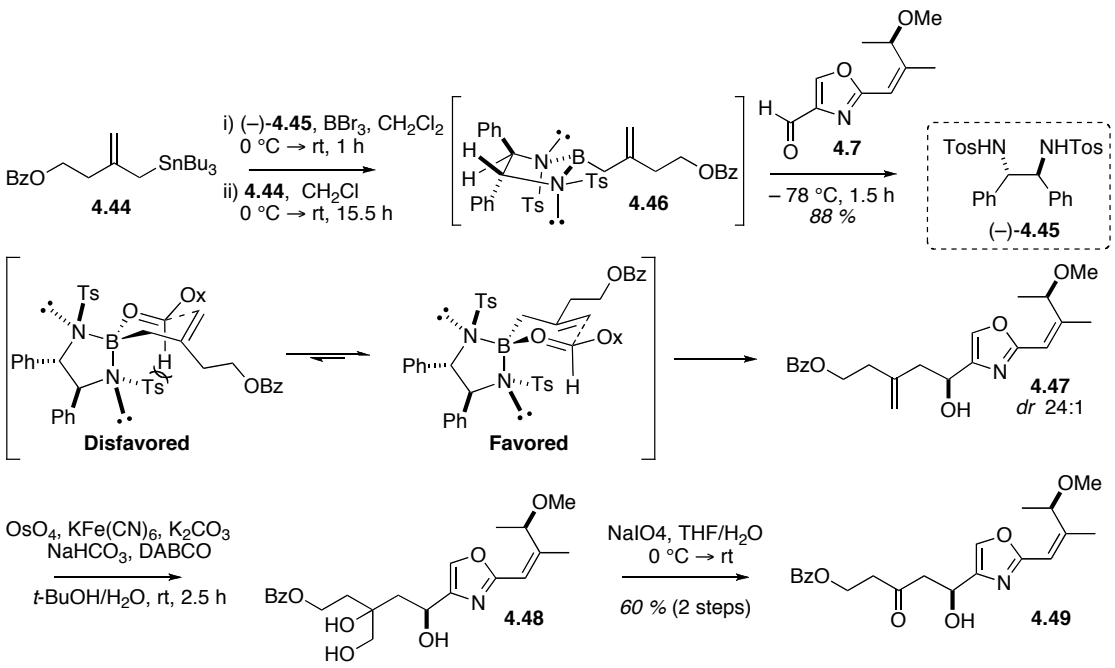
In preparation for asymmetric alkylation of **4.7** allyl stannane **4.44** was prepared starting with 2-chloroethanol **4.39** (Scheme 4.9). Benzoylation of the free hydroxyl (91%) and displacement of chloride with iodide under microwave conditions (97%) gave **4.40**.[39] Addition of Grignard reagent **4.41**[40] (CuCN, THF, −50 ˚C → rt) gave allyl silane **4.42**. Two-step conversion to the

corresponding stannane **4.44** proceeded in 80% yield according to the method developed by Williams *et al*.[41]



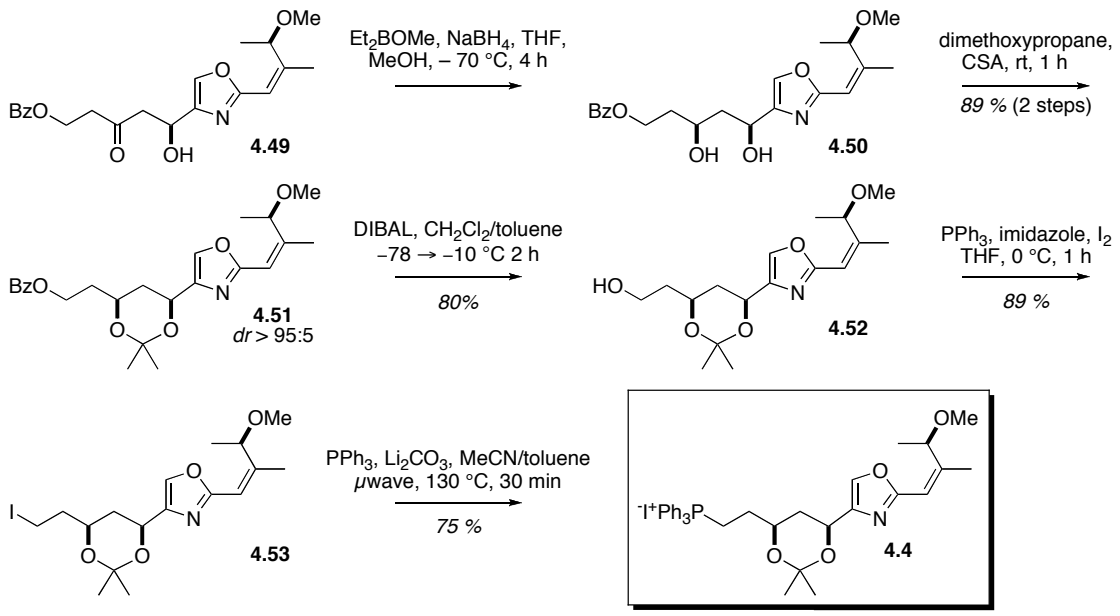
Scheme 4.9 synthesis of allyl stannane **4.44**

Williams and coworkers have previously demonstrated the utility of asymmetric sulfonamide catalyst **4.45** (originally developed by Corey[42]) in allylation reactions involving complex aldehydes and allyl stannanes. In particular, allylations with **4.45** were successfully applied in syntheses of phorboxazole A, amphidinolide K, hennoxazole and leucascandrolide.[43] In this case, reaction of (−)-**4.45** with BBr_3 produced the active catalyst *in situ* that underwent transmetalation with stannane **4.43** to give intermediate **4.46** (Scheme 4.10). Addition of **4.46** to oxazole aldehyde **4.7** proceeds through a chair-like transition state to provide **4.47** in high yield (89%) and with good selectivity (*dr* = 24:1). The configuration of the newly-formed stereocenter was confirmed by Mosher ester analysis. Catalytic dihydroxylation (OsO_4, NMO) of the vinylidene group of **4.47** followed by oxidative cleavage ($NaIO_4$) provided β-hydroxyketone **4.49** in 60% yield over 2 steps.



Scheme 4.10 Asymmetric allylation of oxazole aldehyde **4.7**

Asymmetric reduction of **4.49** (Et_2BOMe, $NaBH_4$, *dr* > 95:5) furnished the desired 1,3-*syn* diol **4.50** which was protected immediately as the acetonide **4.51** (Scheme 4.11).[44] Analysis of the acetonide ^{13}C NMR chemical shifts using the Evans-Rychnovsky model confirmed the *syn* relationship of the 1,3-diol (δ_{13C} Me_{axial} = 19.6 ppm, $Me_{equatorial}$ = 30.0 ppm).[45] Reductive removal of the benzoate protecting group (DIBAL) proceeded without incident and the resulting primary alcohol was then converted to the iodide **4.53** (PPh_3, I_2). Finally, microwave heating of **4.53** in the presence of PPh_3 and Li_2CO_3 provided key phosphonium salt **4.4** in good yield. It should be noted that in the absence of Li_2CO_3 traces of HI catalyzed acetonide cleavage and elimination of the benzylic hydroxyl group.[46]

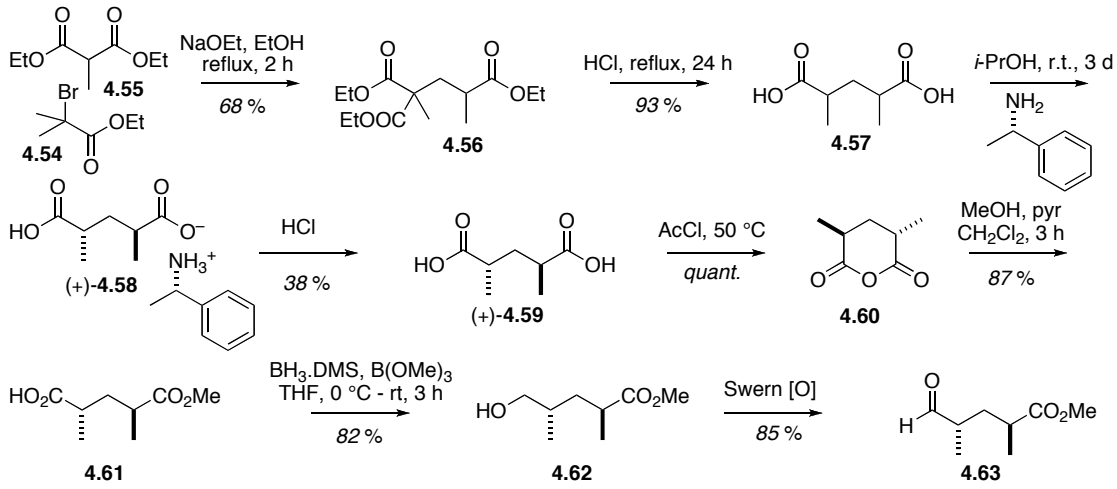


Scheme 4.11 Synthesis of phosphonium salt **4.4**, the 'eastern hemisphere' of enigmazole A

4.3 Synthesis of the Western Hemisphere

Construction of the western hemisphere of enigmazole A began with condensation of diethyl methylmalonate (**4.55**) and ethyl α-bromoisobutyrate (**4.54**) under basic conditions (NaOEt, EtOH) to give **4.56** (Scheme 4.12).[47,48] Decarboxylation in concentrated HCl gave a mixture of (±)- and *meso*- isomers of 2,4-dimethylglutaric acid (**4.57**) which was resolved by fractional crystallization of the salt derived from *S*-α-methylbenzylamine.[49] The free acid (+)-**4.59** (*ee* > 95%) was recovered by treating the diastereomerically pure salt (+)-**4.58** with HCl. Heating diacid (+)-**4.59** briefly in the presence of acetyl chloride provided the C_2-anhydride **4.60**[49] which was opened with MeOH in the presence of pyridine to give monoester **4.61** without epimerization of the α-

methyl stereocenter.[50] Reduction of the remaining carboxylic acid (BH_3·DMS, $B(OMe)_3$)[51] and oxidation of the resulting alcohol (Swern conditions) furnished aldehyde **4.63**.[52]

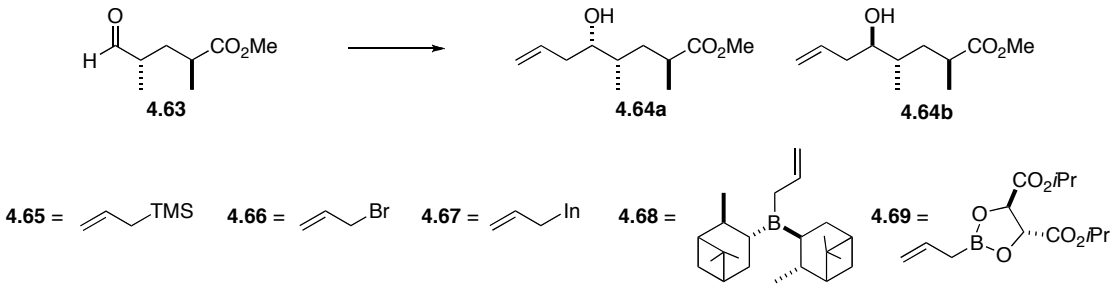


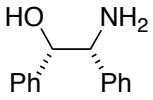
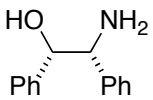
Scheme 4.12 Synthesis of 'western hemisphere' intermediate **4.63**

A variety of conditions were explored for the diastereoselective allylation of aldehyde **4.63** (Table 4.2). Sakurai allylation (Table 4.2, entries 1-4) gave good yields of **4.64a** and **4.64b** but poor selectivity (1:1 → 1.6:1) that could not be improved by varying the Lewis acid ($TiCl_4$, BF_3·OEt_2, $SnCl_4$).[53] Nozaki-Hiyama allylation (allyl bromide, $CrCl_2$) gave similar results (entry 5).[54] The recently reported Barbier reaction by Singaram (entries 6,7) using stoichiometric (1*S*,2*R*)-2-amino-1,2-diphenylethanol gave high yields of product but still modest selectivity (3.9:1).[55] Brown allylation (entry 8) gave **4.64a** with >10:1 selectivity, however isolation of the product was hampered by extensive side product formation during oxidative workup.[56] Finally, Roush

allylation (entry 9) produced **4.64a** cleanly, in high yield and with good selectivity (9:1).[57]

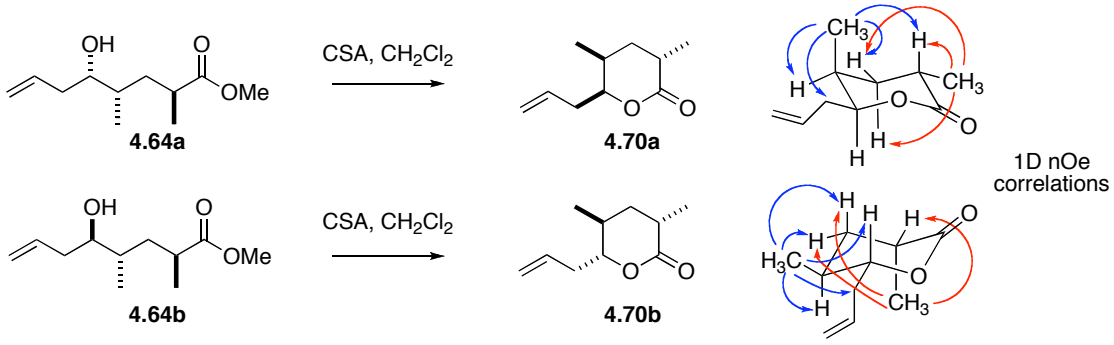
Table 4.2 Optimization of allylation conditions for preparation of **4.64a/b**



	Allyl donor	Lewis Acid	Additive	Temp (˚C)	Solvent	Time (h)	Yield (%)	Ratio 4.65a: 4.65b
1[a]	**4.65**	$TiCl_4$	N/A	– 40 → r.t.	CH_2Cl_2	1	70	1.6:1
2[a]	**4.65**	$TiCl_4$	N/A	– 78	CH_2Cl_2	3	66	1.7:1
3	**4.65**	$BF_3{\cdot}OEt_2$	N/A	– 78 → 0	CH_2Cl_2	2.5	59	1:1
4[a]	**4.65**	$SnCl_4$	N/A	– 78 → rt	CH_2Cl_2	5	ND	1.3:1
5	**4.66**	$CrCl_2$	N/A	0 → r.t.	THF	2	66	1.2:1
6	**4.67**	N/A		– 78	THF	1.5	81	3.9:1
7	**4.67**	N/A		– 95	THF	1.5	83	3.9:1
8	**4.68**	N/A	N/A	– 95	Et_2O	0.5	29	≥10:1
9	**4.69**	N/A	powdered 4Å MS	– 78	toluene	2	85	9:1

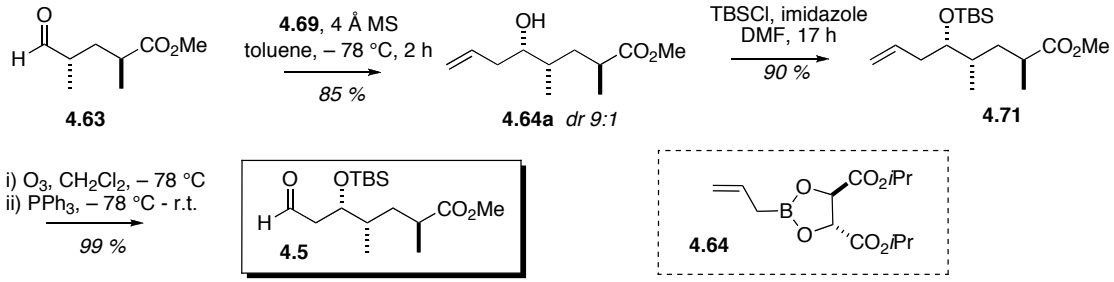
[a] **4.70a** and **4.70b** were isolated directly following aqueous workup (see Scheme 4.13).

The stereochemical outcome of the allylation reaction was easily evaluated by converting the allylic alcohols to the δ-lactones **4.70a** and **4.70b** (Scheme 4.13) and assignment of relative configuration by 1D nOe NMR. Consequently the C5 (enigmazole numbering) configuration of allylic alcohols **4.64a** and **4.64b** was also assigned.



Scheme 4.13 Confirmation of relative configuration of **4.64a** and **4.64b**

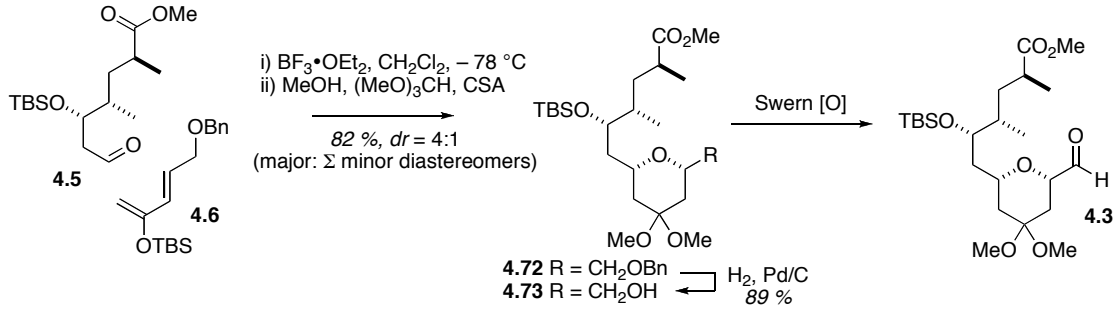
Protection of alcohol **4.64a** (TBSCl, imidazole) gave silyl ether **4.71** which was then ozonolyzed to yield the western aldehyde fragment **4.5** in nearly quantitative yield (Scheme 4.14).



Scheme 4.14 Completion of fragment **4.5**, the 'western hemisphere of enigmazole A

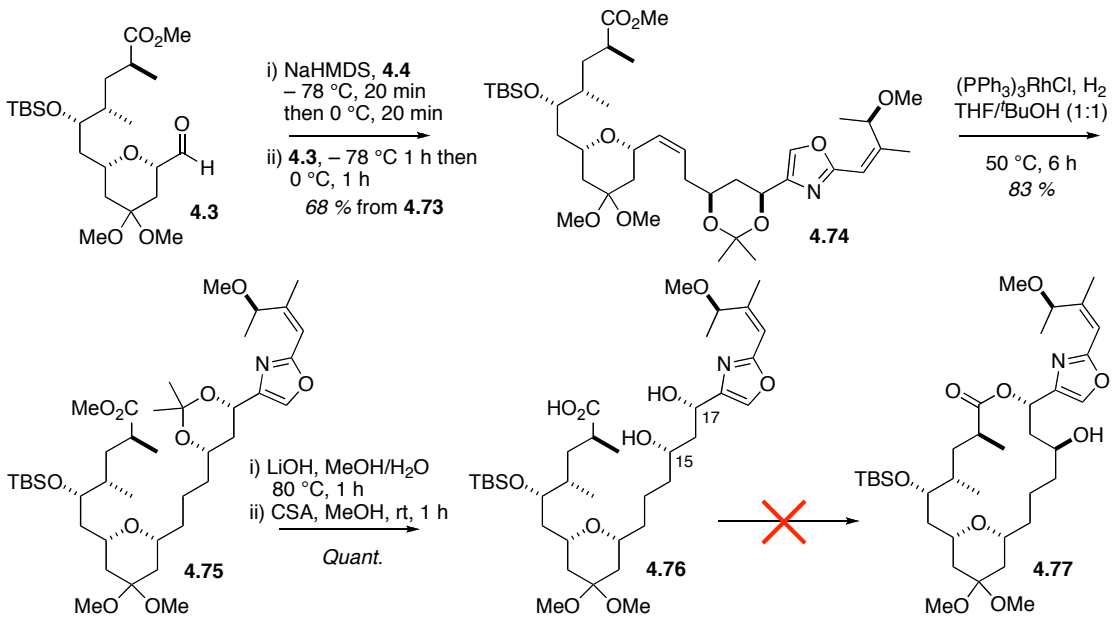
4.4 Fragment Coupling and Final Elaboration

The 3-step conversion of **4.5** to fragment **4.3** was optimized and carried out by Dr. Tim Quach. Hetero-Diels-Alder reaction between **4.7** and **4.6** was effected with $BF_3{\cdot}OEt_2$ to give pyran **4.72** (Scheme 4.15). Removal of the benzyl protecting group and Swern oxidation yielded aldehyde **4.3**.[52]



Scheme 4.15 Preparation of pyran intermediate **4.3**

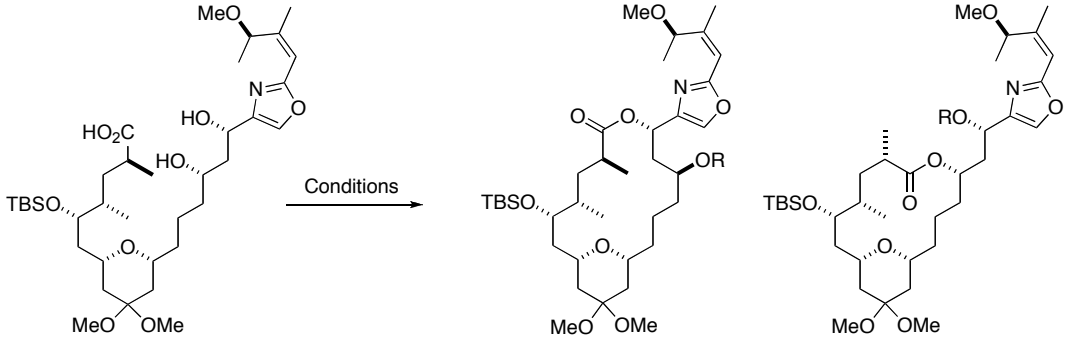
The Wittig reaction between **4.3** and the ylide derived from **4.4** was found to be highly oxygen-sensitive, however taking the precaution of using degassed solvent provided *Z*-olefin **4.74** in 68% overall yield from **4.73** (Scheme 4.16). Selective homogeneous hydrogenation of the C12-C13 olefin was carried out with Wilkinson's catalyst [$(PPh_3)_3RhCl$] at 50 °C.[58] Initial trials for this reaction were carried out with toluene as solvent, however the reaction was sluggish and generally could not be forced to completion. Using the Zhu modification (1:1 THF/^{t}BuOH) provided an increase in reaction rate and yield, giving **4.75** in 83% yield.[59]



Scheme 4.16 Fragment coupling and attempted macrolactonization.

Saponification of the methyl ester and removal of the acetonide group was uneventful, furnishing dihydroxy acid **4.76** in essentially quantitative yield. At this point it was necessary to close the macrolide ring with selective reaction at the C17 hydroxyl. Unfortunately, all attempts to induce a selective macrolactonization were unsuccessful. Yamaguchi (both standard[60] and Yonemitsu[61] variations) and Shiina conditions[62] led to intractable mixtures of products. Mukaiyama macrolactonization[63] gave a low yield of product that favored the smaller 16-membered macrolide ring (1:2.5 **4.77a/b**). Keck conditions[64] gave the most promising results (Table 4.3), however in almost every case formation of the smaller ring was observed. The only exception was Entry 6, however purification of the products proved difficult.

Table 4.3 Attempted Keck macrolactonization of **4.76**

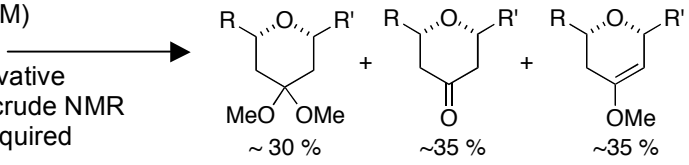


4.76 **4.77a** **4.77b**

	Solvent	Conditions	Temp.	Addition Time	R	Ratio 4.77a:4.77b	Yield (%)
1	$Cl(CH_2)_2Cl$	A	83 ˚C	16 h	Ac	0:100	~100 %[a]
2	$Cl(CH_2)_2Cl$	A	83 ˚C	16 h	H	0:100	47[b]
3	$Cl(CH_2)_2Cl$	A	60 ˚C	16 h	H	ND	0[c]
4	THF	A	66 ˚C	16 h	H	ND	0[c]
5	toluene	A	110 ˚C	16 h	H	1:3	31
6	$CHCl_3$	A	61 ˚C	16 h	H	4:1	6 (**8**)[d] 22 (**9**)[d]
7	$CHCl_3$	A	61 ˚C	2 h	H	ND	0[c]
8	$CHCl_3$	B	61 ˚C	16 h	H	0:100	50
9	$CHCl_3$	C	61 ˚C	16 h	H	~ 1:10	30
10	CH_2Cl_2	A	40 ˚C	16 h	H	ND	0[c]

Conditions A: Slow addition of **4.76** to a refluxing solution of DCC (25 eq) and DMAP (22 eq), DMAP.HCl (24 eq) in the indicated solvent (*c* = 0.3 mM); **B**: Slow addition of **4.76** to a refluxing solution of DCC (20 eq), pyridine (100 eq) and PPTS (20 eq) in the indicated solvent (*c* = 0.3 mM); **C**: Slow addition of **4.76** to a refluxing solution of EDC.HCl (5 eq), and DMAP (5 eq) in the indicated solvent (*c* = 0.3 mM)

[a] Reaction gave three products:



[b] Reaction conducted on C9 keto derivative

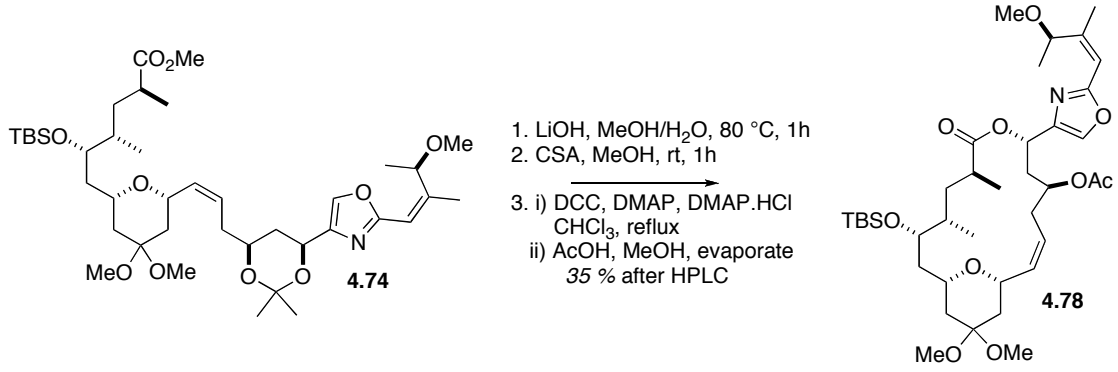
[c] Neither **4.77a** or **4.77b** observed in crude NMR

[d] Extensive side-product formation, required HPLC purification

The success of any given macrolactonization event is strongly dependent on the configuration and preferred conformation of the substrate.[65] These results suggested that compound **4.76** exists in a conformation that strongly favors kinetic acylation at the undesired C15 hydroxyl. Paterson *et al.* encountered a similar problem in their syntheses of aplyronine and scytophicin.[66] In both cases, Yamaguchi macrolactonization of a 1,3-diol

carboxylic acid favored acylation of the undesired alcohol. Fortunately it was found that treating the unwanted isomer with Ti(O*i*Pr)$_4$ induced 1,3-acyl migration and formation of the desired macrolide. In this case, however, treating **4.77b** with Ti(O*i*Pr)$_4$ (CH_2Cl_2, rt, 23 h) failed to induce any acyl migration, returning only starting material.

In an attempt to re-direct the macrolactonization to the C17 hydroxyl, Wittig product **4.74** was deprotected without hydrogenating the C12-C13 olefin (Scheme 4.17). In this case, Keck macrolactonization provided the desired 18-membered macrolide **4.78** in 35% as the *only* product. It was found that addition of acetic acid prior to workup of the Keck reaction (intended to deompose excess DCC) led to convenient protection of the remaining C15 hydroxyl group as an acetate ester.



Scheme 4.17 Successful macrolactonization from unsaturated precursor **4.74**

Analysis of a simple ball-and-stick molecular model provides some insight into the outcome of the macrolactonization decribed above. In the case of **4.76**, assuming the C12-C17 carbon chain adopts a roughly staggered

conformation the C15 hydroxyl is clearly projected toward the reactive carbonyl group and the C17 hydroxyl is directed away (Figure 4.4). Placing a *Z*-double bond between C12 and C13, as in **4.74**, forces the C12-C17 chain to re-orient thereby directing the C17 hydroxyl toward the reactive carbonyl and favoring formation of the larger 18-membered macrolide.

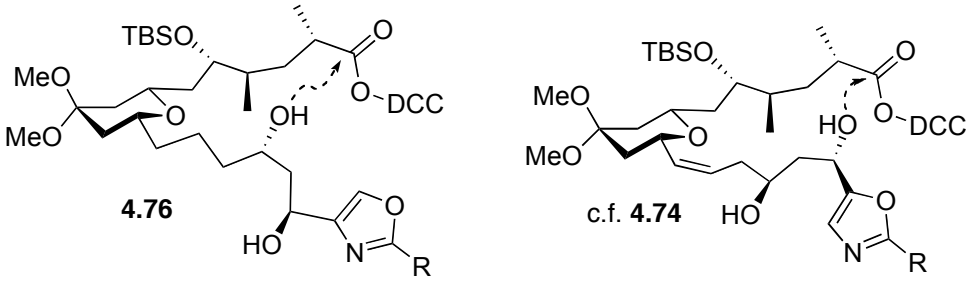
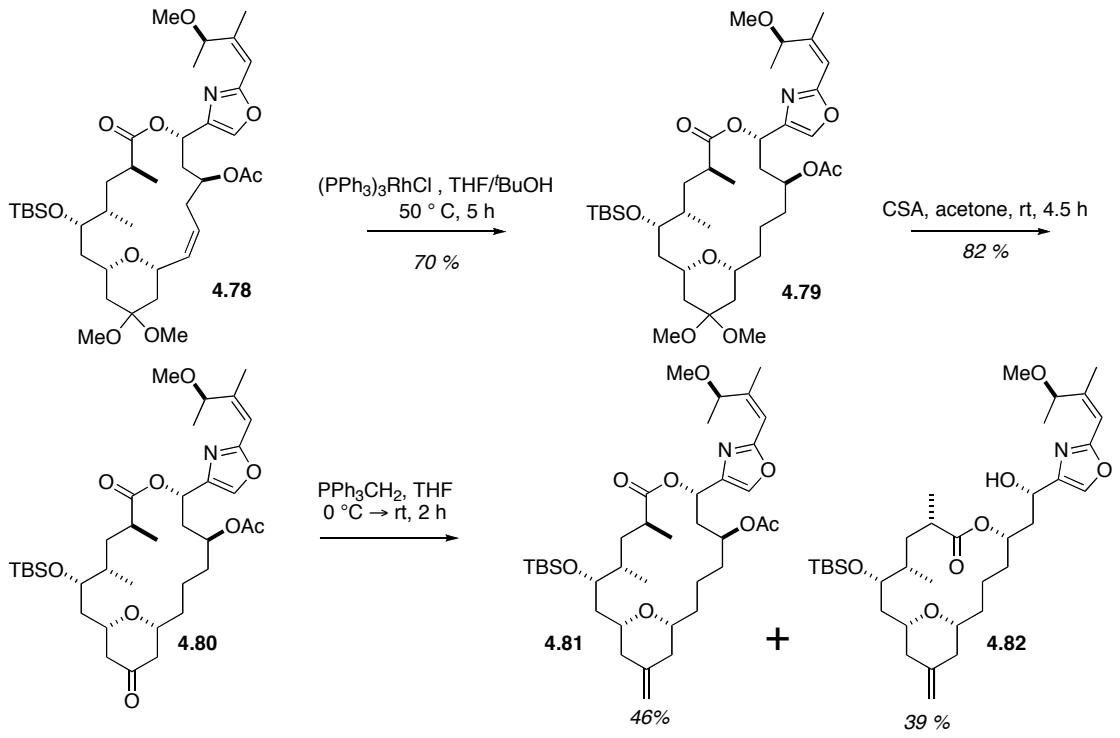


Figure 4.4 Conformational analysis of macrolactonization precursors.

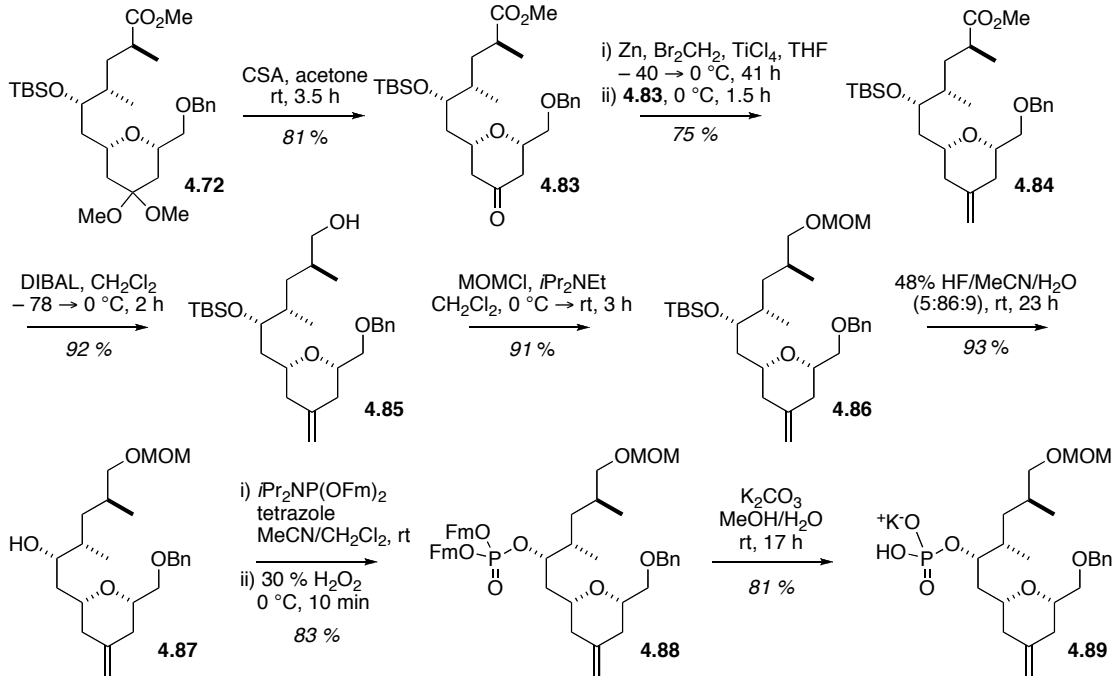
Selective hydrogenation of the C12-C13 olefin in **4.78** occurred smoothly to give **4.79** which was treated with acid (CSA, acetone) to cleave the dimethylacetal and reveal the C9 ketone **4.80** (Scheme 4.18). Installation of the pyran *exo*-methylene was expected to be facile, however exposure of **4.80** to the ylide derived from methyltriphenylphosphonium bromide resulting in partial cleavage of the C17 acetate group and 1,3-acyl migration to yield a substantial quantity of the 16-membered macrolide **4.82**. Evidently the appreciable basicity of the Wittig reagent tended to induce rearrangement of the kinetically-formed 18-membered macrocycle to the thermodynamically-favored 16-membered ring. Running the reaction at lower temperature (−40 ˚C) suppressed this side reaction but delivered low yields of **4.81**.



Scheme 4.18 Attempted formation of pyran *exo*-methylene by Wittig reaction

At this juncture a model system was sought that would allow the exploration of alternative olefination procedures as well as the final steps of the synthesis of enigmazole A. Toward this end **4.72** was treated with acid to reveal ketone **4.83** (Scheme 4.19). Olefination of **4.83**, this time with the non-basic Lombardo reagent ($TiCl_4$, CH_2Br_2, Zn) proceeded smoothly and very cleanly to give alkene **4.84**.[67] The Lombardo olefination protocol has previously been used successfully with base-sensitive substrates, so we anticipated that these conditions might be amenable to use with advanced ketone **4.80**. Reduction of the methyl ester to the primary alcohol and protection as the corresponding MOM ether occurred in high yield to give **4.86**.

Removal of the TBS group with HF revealed the C5 (enigmazole numbering) primary alcohol that was phosphorylated using phosphoramidite chemistry as originally described by Watanabe *et al.* and more recently applied by Waldman and Boger in syntheses of cytostatin.[68] Protected phosphate ester **4.83** was subsequently isolated in 83% yield.



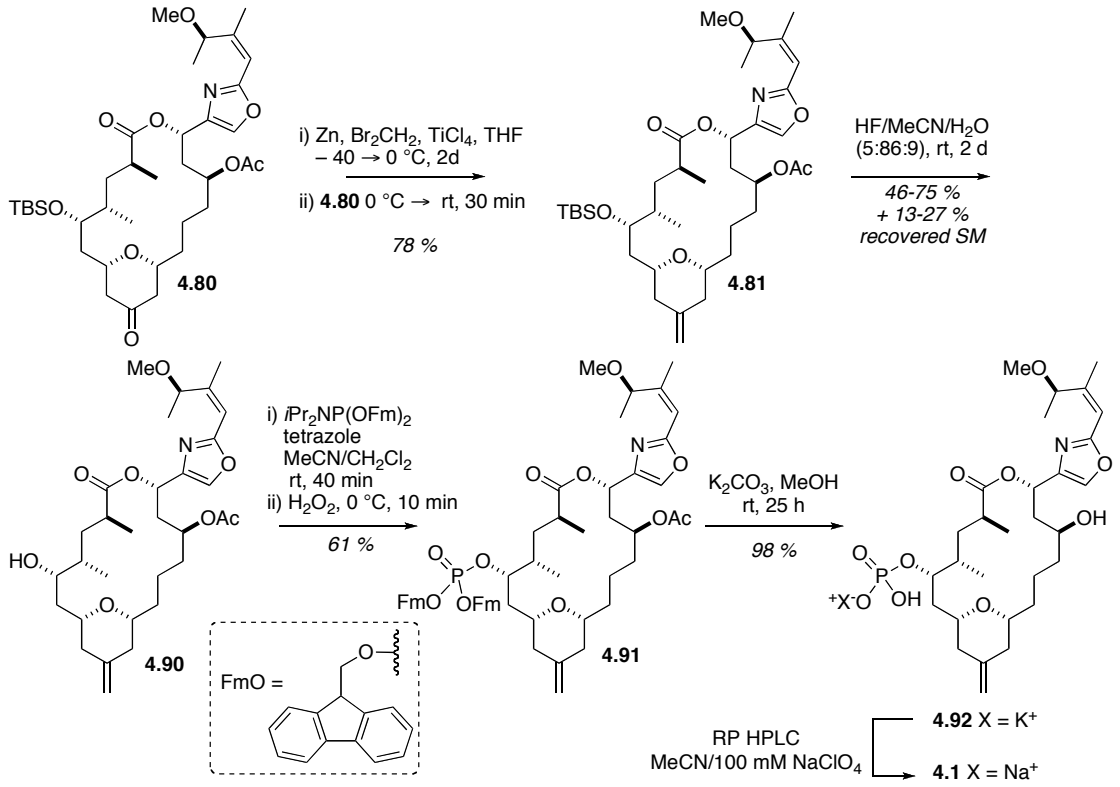
Scheme 4.19 Model study for completion of enigmazole A

The 9-fluorenylmethyl (Fm) protecting group was selected for the phosphate ester based on its reported ease of removal (*via* β-elimination) under mildly basic conditions (e.g. Et_3N in MeCN).[68] For enigmazole A we envisaged a one-pot deprotection step that would remove the 9-fluorenymethyl groups as well as the C15 acetate. The most obvious conditions to achieve this end appeared to be either NH_3/MeOH or

K_2CO_3/MeOH. Preliminary test reactions with compound **4.79** indicated that the C15 acetate was cleaved easily with K_2CO_3/MeOH but was completely inert to NH_3/MeOH at room temperature. Final deprotection of **4.88** was therefore effected using the former conditions (K_2CO_3/MeOH) and was found to proceed cleanly. One equivalent of 9-fluorenylmethylene was eliminated within 30 minutes (as judged by TLC), while the remaining fluorenylmethyl group and the C15 acetate were removed more slowly over the course of 17 hours, providing model compound **4.89**.

Having successfully demonstrated the endgame strategy in model system **4.89**, we turned to the completion of enigmazole A. Application of the Lombardo olefination procedure to complex ketone **4.80** resulted in clean formation of the desired product **4.81** free from any transesterification products (Scheme 4.20). Removal of the TBS group proved to be much more sluggish in this system compared with model **4.86**. Deprotection occurred steadily but slowly over 2 days, at which time a side product began to form (observed as a lower-running spot by TLC). At this point the reaction was terminated and recovered starting material was recycled to provide a 72% yield (averaged over 3 runs) of **4.90**. Phosphorylation of the C5 alcohol was relatively uneventful, although a large excess of $i\text{Pr}_2\text{NP(OFm)}_2$ had to be employed to ensure complete reaction. Final deprotection (K_2CO_3, MeOH) then provided the K^+ salt of enigmazole A. Ion exchange was facilitated by reversed phase

HPLC purification using a buffered solvent system consisting of 28→53% MeCN in 100 mM $NaClO_4$ to give enigmazole A (**4.1**).



Scheme 4.20 Completion of the synthesis of enigmazole A

Synthetic enigmazole A was found to be identical in all respects to the natural compound by ^{1}H and ^{13}C NMR. Natural and synthetic **4.1** gave almost superimposable CD spectra and a sample of synthetic **4.1** co-eluted with natural **4.1** by HPLC. ^{1}H and ^{13}C NMR data for natural and synthetic enigmazole A are tabulated in the Experimental Section (Tables 4.4 and 4.5). Table 4.4 contains ^{1}H and ^{13}C NMR data for natural enigmazole A as reported by N. Oku *et al.*[69] while Table 4.5 contains ^{13}C NMR data for natural enigmazole A provided by Dr. Kirk Gustafson (original FID data files from Dr.

Gustafson were processed using MestreNova software). The two sets of ^{13}C NMR data for natural **4.1** are very similar yet differ significantly in the chemical shift of H5. The proximity of H5 to the phosphate ester suggests that this difference may be due to differences in concentration between the samples used to obtain the data.

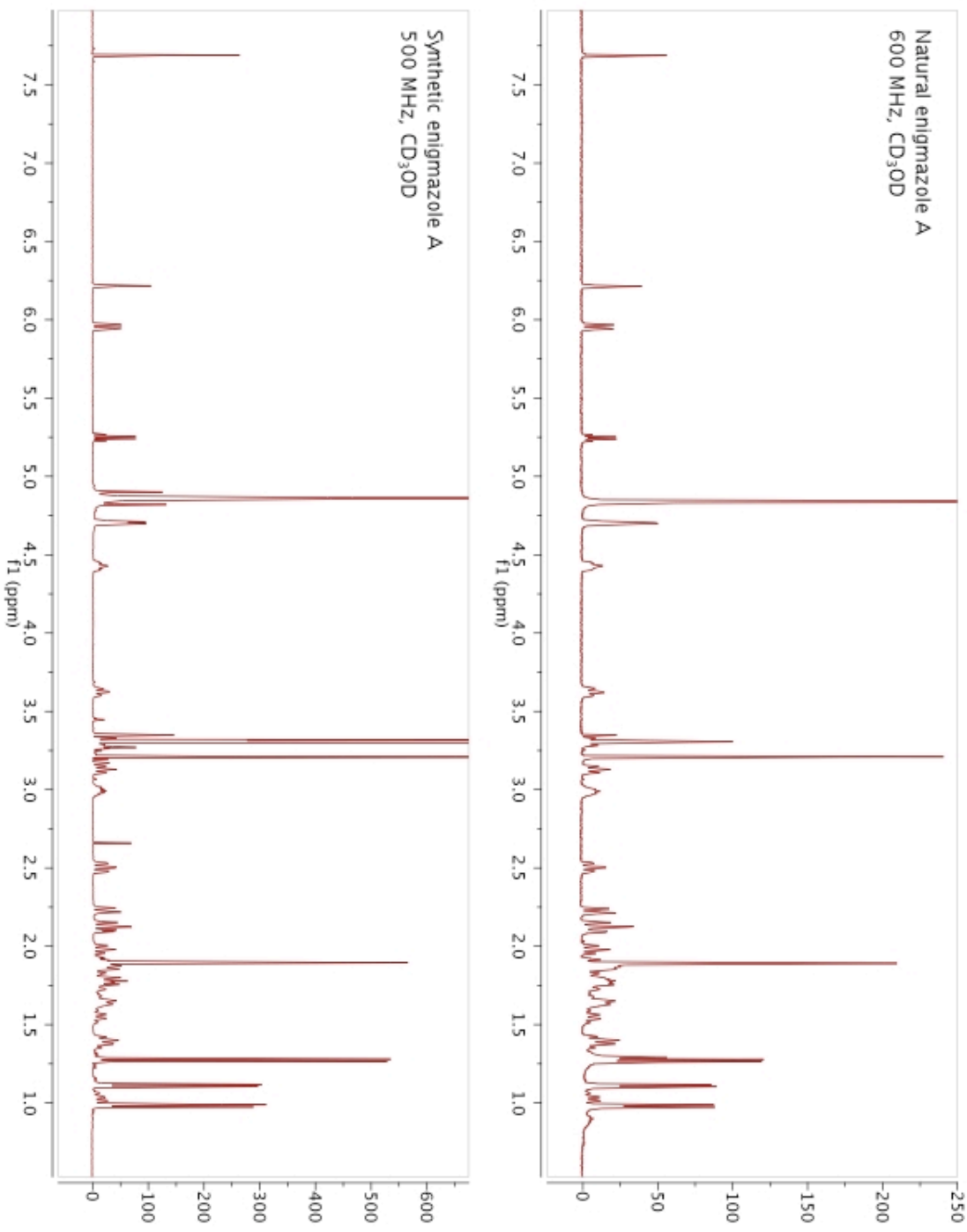


Figure 4.5 ^{1}H NMR (CD_3OD) of natural and synthetic enigmazole A.

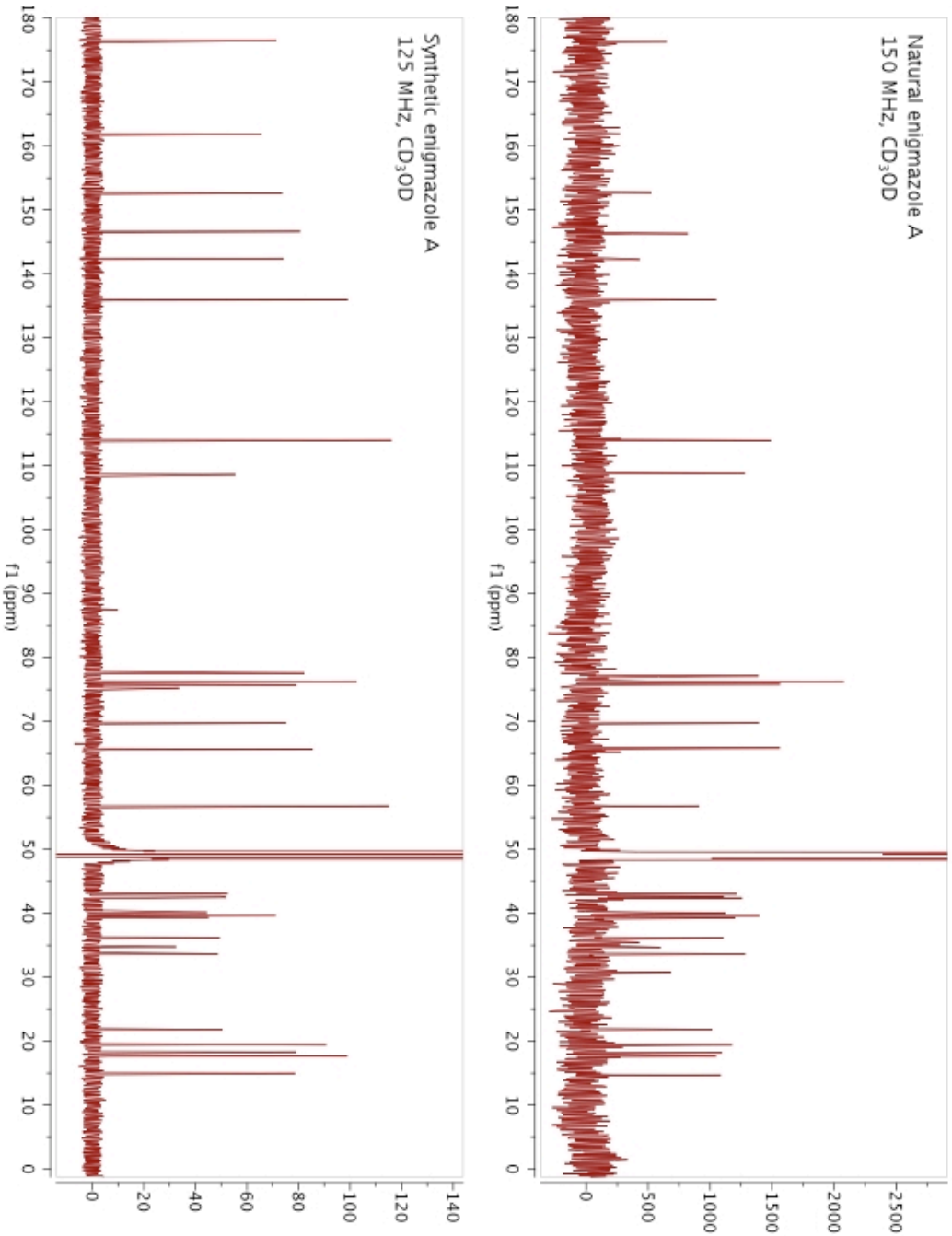


Figure 4.6 ^{13}C NMR (CD_3OD) of natural and synthetic enigmazole A.

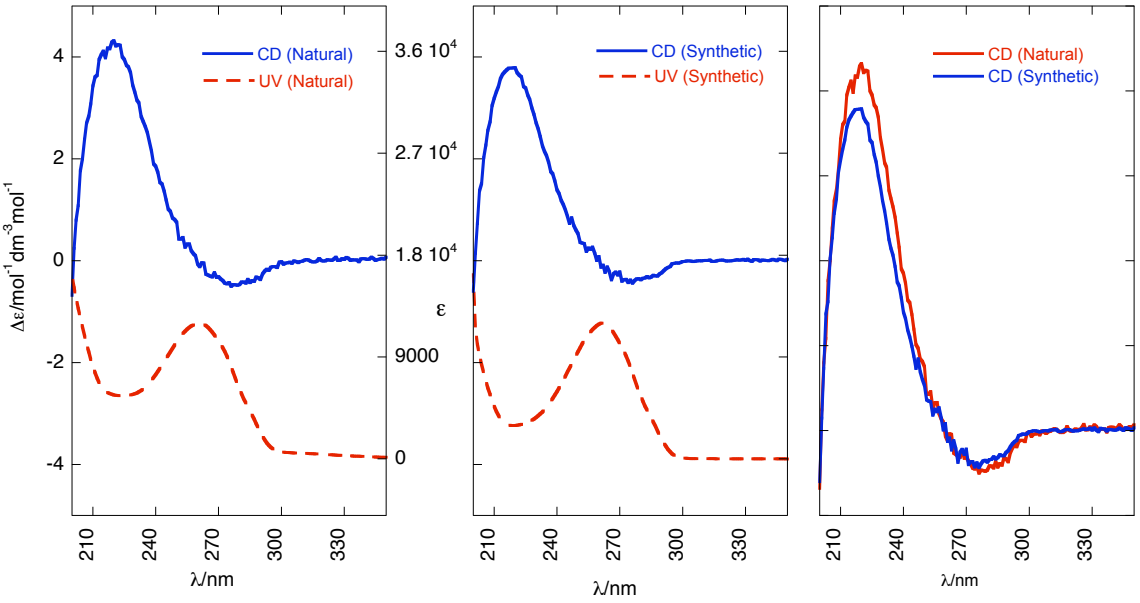


Figure 4.7 CD and UV spectra (MeOH, 25 ˚) of natural **4.1** and synthetic **4.1**

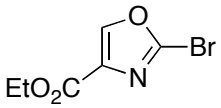
4.5 Conclusions

The synthesis of enigmazole A was completed in 22 steps and 0.41% overall yield from known aldehyde **4.32**. The 2,4-disubstituted oxazole was constructed using an efficient Negishi coupling between newly-developed oxazol-2-ylzinc reagent **4.12b** and vinyl iodide **4.14**. This demonstrates the first preparation of an oxazol-2-yl zinc reagent by direct insertion of zinc metal into the carbon-iodine bond of the parent 2-iodooxazole **4.13c**. It is anticipated that this methodology will find application in the synthesis of enigmazole B and structural analogues that will help to define the minimum pharmacophore and mechanism of action of these unique marine macrolides.

Chapter 4 is, in part, currently being prepared for submission for publication of the material. Quach, T.; Skepper, C. K.; Molinski, T. F. “Synthesis of C1-C16 of Enigmazole A: A Hetero-Diels-Alder Approach” and Skepper, C. K.; Molinski, T. F. “Total Synthesis of Enigmazole A Using an Oxazole Grafting Approach”. The dissertation author was the primary researcher and anuthor of this material.

4.6 Experimental Section

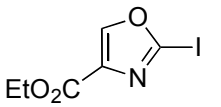
ethyl 2-bromooxazole-4-carboxylate (4.13a)



Reaction was conducted as described by Hodgetts *et al.* Yield = 22%. Spectroscopic data matched literature values.[29]

ethyl 2-iodooxazole-4-carboxylate (4.13c)

A modification of the procedure for aprotic diazotization/iodination or aromatic and heteroaromatic amines published by Knochel et al.[30]



Ethyl 2-aminooxazole-4-carboxylate **4.27** (10 g, 64 mmol) was added to a mixture of $TsOH.H_2O$ (36.55 g, 192 mmol) and CuI (1.22 g, 6.4 mmol) in MeCN (256 mL) at 10 ˚C. A solution of $NaNO_2$ (8.84 g, 128 mmo) and KI (26.58 g, 160 mmol) in H_2O (40 m L) was added slowly *via* dropping funnel to the mechanically stirred mixture. Internal reaction temperature was monitored carefully and maintained at 5→10 ˚C over the course of the addition. Upon completion, the mixture was stirred at 10 ˚C for 10 minutes then allowed to warm to room temperature and stirred for 19

hours. The mixture was diluted with 5% $NaHCO_3/H_2O$/saturated $Na_2S_2O_3$ (4:4:1, 900 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a green-brown solid that was purified by SiO_2 flash chromatography (column dimensions: 7.5cm high, 10 cm wide) eluting with 3:7 Et_2O/hexanes to give **4.13c** as a slightly off-white solid (4.77 g, 28%).

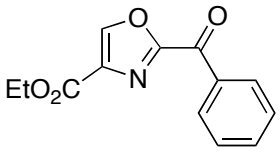
m.p. 113-116 ˚C; IR (neat) ν 3169, 3124, 2982, 2960, 2926, 2854, 1719, 1582, 1474, 1446, 1370, 1311, 1281, 1170, 1112, 1076, 1021, 980, 922, 830, 769, 671 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 4.37 (q, 2H, *J* = 7.2 Hz), 1.36 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8 (C), 148.9 (CH), 136.5 (C), 102.3 (C), 61.6 (CH_2), 14.2 (CH_3); HRMS 267.9457 (calc. for $C_6H_7NO_3I$ 267.9465).

Preparation of 4.12b for copper catalyzed acylation reactions:

LiCl (40 mg, 0.94 mmol) was heated to 160 ˚C under high vacuum for 20 min. Zinc dust (< 10 μm, 92 mg mg, 1.4 mmol) was added and the solid mixture heated to 160 ˚C under high vacuum for a further 20 min. After cooling to room temperature the flask was evacuated and purged with N_2 three times. THF (1 mL) was added followed by dibromoethane (4.0 μL, 0.047 mmol) and TMSCl (1.2 μL, 9.4 μmol). 2-iodooxazole **4.13c** (250 mg, 0.94 mmol) was added as a solution in THF (0.75 mL followed by 0.25 mL rinse). The resulting mixture was stirred for 10 minutes then centrifuged briefly to settle excess zinc

dust. NMR analysis of an aliquot quenched with D_2O revealed a 91 % conversion to the zincate.

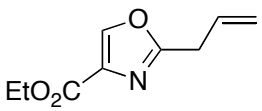
ethyl 2-benzoyloxazole-4-carboxylate (4.30a)



Benzoyl chloride (52 mg mg, 0.37 mmol) was added neat to a solution of oxazole zincate **4.12c** (0.7 mL of the mixture prepared above, ~ 0.31 mmol,) in THF (~ 0.7 mL) at 0 ˚C. A catalytic amount of CuCN•2LiCl (2 drops, 0.5 M in THF) was added and the mixture was allowed to warm to room temperature After 1.5 h the reaction was quenched with saturated $NH_4Cl_{(aq)}$ (5 mL) and extracted with Et_2O (3×3 mL). Combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (2:3 CH_2Cl_2/hexanes) gave **4.30a** (59.1 mg, 78%) as a colorless crystalline solid.

IR (neat) ν 3102, 2985, 1759, 1734, 1650, 1600, 1452, 1371, 1281, 1235, 1169, 1103, 1034, 997, 892, 843, 759, 704, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 8.70 (s, 1H), 8.20 (m, 2H), 7.69 (m, 1H), 7.53 (m, 2H), 4.34 (q, 2H, *J* = 7.2 Hz), 1.36 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (C), 160.8 (C), 160.1 (C), 145.9 (CH), 135.3 (CH), 130.8 (CH), 129.1 (CH), 126.3 (C), 62.6 (CH_2), 14.1 (CH_3); HRMS *m*/*z* 142.0495 $[M-PhCO+H]^+$.

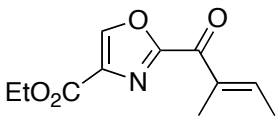
ethyl 2-allyloxazole-4-carboxylate (4.30b)



Allyl bromide (25.5 mg, 0.21 mmol) was added neat to a solution of oxazole zincate **4.12c** (~ 0.23 mmol, 0.45 mL of the zincate mixture prepared above) at 0 ˚C. CuCN.2LiCl (0.012 mmol, 23.4 μL of a 0.5 M solution in THF) was added and the mixture was warmed to room temperature and stirred there for 1 hour. The reaction was quenched with 2 mL saturated $NH_4Cl_{(aq)}$ and extracted with Et_2O (3 × 1 mL). Combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (2:1 hexanes/Et_2O) gave **4.30b** (19.2 mg, 50 %) as a clear colorless oil.

IR (neat) ν 3087, 2983, 2938, 2907, 2875, 2851, 1740, 1719, 1644, 1584, 1466, 1447, 1425, 1393, 1371, 1316, 1285, 1260, 1233, 1182, 1141, 1107, 1023, 984, 924, 892, 834, 764, 687, 677 cm $^{-1}$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 5.93 (ddt, 1H, *J* = 17.0, 10.2, 6.6 Hz), 5.18 (dq, 1 H, *J* = 17.0, 1.4 Hz), 5.16 (dq, 1H, *J* = 10.2, 1.4 Hz), 4.33 (q, 2H, *J* = 7.2 Hz), 3.55 (dt, 1H, *J* = 6.6, 1.4 Hz), 1.32 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4 (C), 161.2 (C), 143.8 (CH), 133.5 (C), 130.4 (CH), 118.9 (CH_2), 61.1 (CH_2), 32.5 (CH_2), 14.2 (CH_3); HRMS *m*/*z* 182.0815 $[M+H]^+$ (calc. for $C_9H_{12}NO_3$ 182.0812).

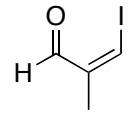
(*E*)-ethyl 2-(2-methylbut-2-enoyl)oxazole-4-carboxylate (4.10)



Tigloyl chloride (43 mg, 0.36 mmol) was added neat to a solution of oxazole zincate **4.12c** (0.63 mL of the zincate mixture prepared above, ~ 0.3 mmol) at 0 ˚C causing the color to fade slightly. A catalytic amount of CuCN•2LiCl (10 μL of a 0.5 M solution in THF) was added. The reaction was allowed to wardm to room temperature and stirred for 2 hours. Saturated $NH_4Cl_{(aq)}$ (4 mL) was added and the mixture extracted with Et_2O (3 × 2 mL). Combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Two rounds of silica gel chromatography (1:1 CH_2Cl_2/hexanes) gave **4.10** (57 mg, 85%) as a clear colorless oil.

IR (neat) ν 3101, 2985, 2938, 2112, 2873, 2124, 1734, 1643, 1445, 1371, 1319, 1280, 1245, 1230, 1171, 1122, 1097, 1063, 1032, 1005, 927, 886, 860, 839, 759, 716 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.29 (m, 1H), 4.31 (q, 2H, *J* = 7.1 Hz), 1.92 (m, 3H), 1.91 (dq, 3H, *J* = 10.4, 1.2 Hz), 1.33 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6 (C), 161.5 (C), 160.3 (C), 146.2 (CH), 144.5 (CH), 125.8 (C), 62.4 (CH_2), 15.1 (CH_3), 14.0 (CH_3), 11.8 (CH_3); HRMS *m*/*z* 224.0909 $[M+H]^+$ (calc. for $C_9H_{12}NO_3$ 224.0917).

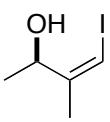
(*Z*)-3-iodo-2-methylacrylaldehyde (4.32)



Activated MnO_2 (4.39 g, 51 mmol) was added to a solution of **4.31** (3.3 g, 16.7 mmol) in CH_2Cl_2 (250 mL). The mixture was stirred for 1 hour in the absence of light, then filtered and concentrated under reduced pressure to give **4.32** (2.7 g, 82 %) as a yellow solid. The aldehyde was used *immediately* in the following reaction due to geometric instability.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.43 (d, 1H, J = 1.6 Hz), 1.87 (d, 3H, J = 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4, 141.9, 96.6, 18.4.

(*R*,*Z*)-4-iodo-3-methylbut-3-en-2-ol (4.33)

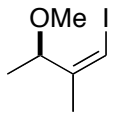


Dimethylzinc (27.6 mmol, 23 mL of a 1.2 M solution in toluene) was added dropwise to a solution of (+)-MIB (330 mg, 1.38 mmol) in anhyhdrous hexanes (46 mL) at 0˚C. Aldehyde **4.32** (2.7 g, 13.8 mmol) was added as a solution in anhydrous hexanes (34 mL) and the resulting cloudy yellow mixture was warmed to room temperature for 10.5 hours. The mixture was cooled to 0˚C and quenched by addition of ice-cold saturated NH_4Cl (100 mL). After stirring 10 minutes the layers were separated and the aqueous layer extracted with Et_2O (2 × 100 mL). Combined organic extracts were washed with brine (200 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (4:1 hexanes/Et_2O) gave **4.33** (1.45 g) along with a mixed fraction that was re-purified to give a further 310 mg of product (1.76 g

total, 60 %) as a clear colorless oil. Mosher's ester analysis revealed *ee* = 93 %.

$[\alpha]^{21}$ +12.9 (*c* = 2.65, $CHCl_3$); IR (neat) ν 3317, 3051, 2972, 2916, 2869, 2848, 1613, 1433, 1367, 1328, 1277, 1143, 1099, 1070, 1037, 1020, 974, 901, 768 cm^{-1}; 1H NMR (300 MHz, $CDCl_3$) δ 5.87 (m, 1H), 4.76 (dq, 1H, *J* = 6.4, 3.9 Hz), 1.87 (d, 3H, *J* = 1.8 Hz), 1.59 (br m, 1H), 1.24 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.1 (C), 73.4 (CH), 72.2 (CH), 20.1 (CH_3), 18.2 (CH_3).

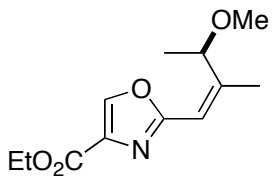
(*R*,*Z*)-1-iodo-3-methoxy-2-methylbut-1-ene (4.14)



Imidazole (58 mg, 0.85 mmol) was added to a suspension of NaH (17 mmol, 407 mg of a 60 % dispersion in mineral oil) in anhydrous THF (32 mL). The mixture was cooled to 0 ˚C and **4.33** (1.80 g, 8.49 mmol) was added dropwise as a solution in THF (8 mL followed by a 2 mL rinse). The pale yellow mixture was warmed to room temperature and stirred for 2 hours at which time methyl iodide (4.82 g, 34 mmol) was added dropwise. The mixture was stirred for 2 hours then poured into H_2O (200 mL) and extracted with pentane (3 × 50 mL). Combined pentane extracts were washed with brine (100 mL), dried ($MgSO_4$) and concentrated carefully under reduced pressure (caution: volatile!). Silica flash chromatography (2.5:97.5 Et_2O/pentane) gave **4.14** (1.53 g, 80 %) as a colorless liquid.

$[\alpha]^{22}$ +3.4 (*c* = 2.55, $CHCl_3$); IR (neat) ν 30512978, 2952, 2922, 2884, 2819, 1613, 1460, 1441, 1369, 1339, 1320, 1280, 1205, 1145, 1114, 1094, 1065, 1030, 968, 866, 773, 655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.00 (d, 1H, *J* = 1.6 Hz), 4.24 (q, 1H, *J* = 6.6 Hz), 3.19 (s, 3H), 1.77 (d, 3H, *J* = 1.6 Hz), 1.17 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.2 (C), 80.7 (CH), 75.5 (CH), 56.2 (CH_3), 18.4 (CH_3), 17.8 (CH_3); HRMS *m*/*z* 225.9849 $[M]^+$ (calc. for $C_6H_{11}IO$ 225.9849).

(*R*,*Z*)-ethyl 2-(3-methoxy-2-methylbut-1-enyl)oxazole-4-carboxylate (4.8)

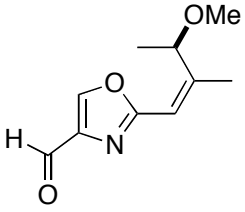


LiCl (349 mg, 8.24 mmol) was heated to 160 ˚C under high vacuum for 20 min. Zinc dust (< 10 μm, 807 mg, 12.4 mmol) was added and the solid mixture heated to 160 ˚C under high vacuum for a further 20 min. After cooling to room temperature the flask was evacuated and purged with N_2 three times. THF (8.25 mL) was added followed by dibromoethane (35 μL, 0.41 mmol) and TMSCl (11 μL, 0.082 mmol). 2-iodooxazole **4.13c** (2.20 g, 8.24 mmol) was added as a solution in THF (5 mL followed by 1 mL rinse). The resulting mixture was stirred for 10 minutes then the excess zinc dust was allowed to settle. NMR analysis of an aliquot quenched with D_2O revealed a 92 % conversion to the zincate. The zincate solution was then transferred *via* syringe to a flask containing $Pd(PPh_3)_4$ (952 mg, 0.82 mmol). Vinyl iodide **4.14**

(1.49 g, 6.59 mmol) was then added neat, followed by THF (1 mL) to rinse. After stirring 1.5 hours the reaction mixture was poured into saturated NH_4Cl (100 mL) and extracted with Et_2O (3 × 50 mL). Combined extracts were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Crude product was triturated with Et_2O, then again with 30 % Et_2O in hexanes. The supernatant from the second trituration was loaded onto a silica gel column and eluted with 30 % Et_2O/hexanes to afford **4.8**. A final trituration with Et_2O afforded pure **4.8** (1.35 g, 86 %) as a yellow oil.

$[\alpha]^{23}$ + 39.4 (*c* 1.76, $CHCl_3$); IR (neat) ν 3153, 3113, 2980, 2933, 2821, 1741, 1719, 1653, 1575, 1562, 1525, 1446, 1370, 1332, 1315, 1279, 1217, 1206, 1176, 1134, 1109, 1069, 1024, 985, 971, 947, 927, 834, 770, 724 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 6.24 (t, 1H, *J* = 1.2 Hz), 5.12 (q, 1H, *J* = 6.2 Hz), 4.37 (q, 2H, *J* = 7.2 Hz), 3.21 (s, 3H), 1.90 (d, 3H, *J* = 1.6 Hz), 1.36 (t, 3H, *J* = 7.2 Hz), 1.30 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2 (C), 160.8 (C), 153.0 (C), 142.6 (CH), 134.0 (C), 112.5 (CH), 74.6 (CH), 61.0 (CH_2), 56.4 (CH_3), 19.2 (CH_3), 17.7 (CH_3), 14.2 (CH_3); HRMS *m*/*z* 240.1240 $[M+H]^+$ (calc. for $C_{12}H_{18}NO_4$ 240.1236).

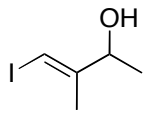
(*R*,*Z*)-2-(3-methoxy-2-methylbut-1-enyl)oxazole-4-carbaldehyde (4.7)



DIBAL (5.27 mmol, 3.51 mL of a 1.5M solution in toluene) was added dropwise to a solution of **4.8** (630 mg, 2.63 mmol) in CH_2Cl_2 (17.6 mL) at − 90 ˚C. After stirring 2 hours at this temperature MeOH (1.3 mL) was added dropwise followed by 13 mL saturated sodium/potassium tartrate. Mixture was warmed to room temperature and diluted with CH_2Cl_2 and H_2O (80 mL each) and stirred vigorously for 30 minutes. Layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 50 mL). Combined CH_2Cl_2 extracts were washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (15% EtOAc/hexanes) gave **4.7** (457 mg, 89 %) as a colorless solid.

mp 56.5 – 59.5 ˚C; $[\alpha]^{24}$ + 46.9 (*c* 2.18, $CHCl_3$); IR (neat) ν 3144, 3088, 2980, 2932, 2822, 2753, 1698, 1652, 1563, 1447, 1393, 1368, 1337, 1206, 1177, 1149, 1114, 1095, 1068, 1034, 1002, 971, 948, 938, 833, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 8.15 (s, 1H), 6.19 (t, 1H, *J* = 1.2 Hz), 5.20 (q, 1H, *J* = 6.4 Hz), 3.20 (s, 3H), 1.90 (d, 3H, *J* = 1.2 Hz), 1.28 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5 (CH), 161.2 (C), 154.5 (C), 142.9 (CH), 141.4 (C), 111.9 (CH), 74.7 (CH), 56.5 (CH_3), 19.1 (CH_3), 17.8 (CH_3); HRMS *m*/*z* 196.0977 $[M+H]^+$ (calc. for $C_{10}H_{14}NO_3$ 196.0974).

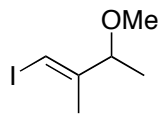
(*E*)-4-iodo-3-methylbut-3-en-2-ol (4.35)[38]



Trimethylaluminum (0.77 g, 10.7 mmol) was added as a solution in CH_2Cl_2 (5 mL) to a suspension of zirconocene dichloride (Cp_2ZrCl_2, 1.04 g, 3.57 mmol) in CH_2Cl_2 (2 mL) at 0 ˚C. 3-Butyn-2-ol (250 mg, 3.57 mmol) was added dropwise to the yellow mixture as a solution in CH_2Cl_2 (2.5 mL). Reaction was warmed to room temperature and stirred 15 hours, then cooled to – 30 ˚C. Iodine (1.09 g, 4.28 mmol) was added as a solution in THF (5 mL). Mixture was warmed to 0 ˚C for 10 minutes, then quenched by addition of saturated sodium potassium tartrate (10 mL). The resulting slurry was poured into 1:1 H_2O/Et_2O (100 mL) and stirred vigorously for 10 minutes. Layers were separated and the aqueous layer was extracted with Et_2O (2 × 50 mL). Combined organic extracts we re washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (15:85 EtOAc/hexanes) gave **4.35** (253 mg, 33 %) as a clear, colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.26 (t, 1 H, *J* = 1.4 Hz), 4.33 (m, 1H), 2.04 (d, 1H, *J* = 3.2 Hz), 1.80 (d, 3H, *J* = 1.2 Hz), 1.25 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.0, 77.6, 72.4, 21.6, 19.9.

(*E*)-1-iodo-3-methoxy-2-methylbut-1-ene (4.37)

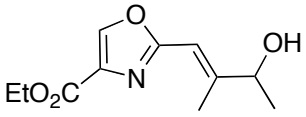


A single crystal of imidazole was added to a suspension of sodium hydride (1.89 mmol, 75 mg of a 60 % dispersion in

mineral oil) in anhydrous THF (3.7 mL). The mixture was cooled to 0 ˚C and **4.35** was added dropwise as a solution in THF (1 mL). The yellow mixture was warmed to room temperature and stirred for 2 hours, at which time methyl iodide (536 mg, 3.77 mmol) was added dropwise. Stirring was continued for a further 2.5 hours then the mixture was poured into H_2O (10 mL) and extracted with pentane (3 × 5 mL). Combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated carefully under reduced pressure (product is extremely volatile!). Silica flash chromatography (2.5:97.5 Et_2O/pentane) gave **4.37** (146 mg, 68 %) as a clear, colorless oil.

IR (neat) ν 2979, 2931, 2865, 2819, 1617, 1450, 1372, 1269, 1204, 1156, 1112, 1091, 1069, 1035, 961, 865, 788, 663 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.19 (dt, 1H, *J* = 1.6, 1.2 Hz), 3.80 (q, 1H, *J* = 6.5 Hz), 3.17 (s, 3H), 1.74 (d, 3H, *J* = 0.8 Hz), 1.21 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.0 (C), 81.5 (CH), 78.3 (CH), 56.2 (CH_3), 20.0 (CH_3), 18.5 (CH_3).

(*E*)-ethyl 2-(3-hydroxy-2-methylbut-1-enyl)oxazole-4-carboxylate (4.36)



Lithium chloride (27 mg, 0.64 mmol) was heated to 160 ˚C under hi-vac in a septum-sealed 3.5 mL vial for 15 minutes. Zinc dust (< 10 μm, 62 mg, 0.96 mmol) was added, and the vial heated to 160 ˚C under hi-vac for 50 minutes. Upon cooling, anhydrous THF (0.6 mL) was added, followed by 1,2-dibromoethane (2.7 μL, 0.03 mmol)

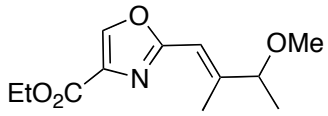
and chlorotrimethylsilane (0.8 μL, 0.006 mmol). Ethyl-2-iodooxazole-4-carboxylate (**4.13c**, 170 mg, 0.64 mmol) was added as a solution in THF (0.5 mL) and the mixture stirred for 10 minutes then centrifuged briefly to settle excess zinc dust. Quenching of a 10 μL aliquot with D_2O revealed 90 % conversion to the zincate as determined by ^1H NMR.

The oxazol-2-yl zincate solution was transferred to a clean, dry 3.5 mL vial containing $Pd(PPh_3)_4$ (74 mg, 0.064 mmol). Vinyl iodide **4.35** (108 mg, 0.51 mmol) was added as a solution in 0.5 mL THF and the resulting mixture stirred for 1 hour at which time TLC showed complete consumption of **4.35**. The reaction mixture was diluted with saturated NH_4Cl (5 mL) and extracted with Et_2O (3 × 10 mL). Combined Et_2O extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified twice by silica flash chromatography (4:1 CH_2Cl_2/Et_2O) to give **4.36** (82 mg, 72 %) as a colorless oil.

IR (neat) ν 3397 (br), 3163, 2979, 2934, 2905, 2874, 1727, 1660, 1577, 1566, 1553, 1526, 1446, 1371, 1317, 1278, 1242, 1218, 1177, 1144, 1111, 1077, 1023, 1077, 1023, 988, 943, 907, 866, 836, 769, 727 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 6.46 (s, 1H), 4.37 (q, 2H, *J* = 7.2 Hz), 4.36 (m, 1H), 2.19 (d, 3H, *J* = 1.2 Hz), 1.69 (br d, 1H, *J* = 2.8 Hz), 1.37 (t, 3H, *J* = 7.2 Hz), 1.34 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1 (C), 161.3 (C), 154.3 (C), 142.6 (CH), 133.8 (C), 108.7 (CH), 72.0 (CH), 61.1

(CH_3), 21.7 (CH_3), 15.3 (CH_3), 14.2 (CH_3); HRESIMS *m*/*z* 226.1074 $[M+H]^+$ (calc. for $C_{11}H_{16}NO_4$ 226.1079).

(*E*)-ethyl 2-(3-methoxy-2-methylbut-1-enyl)oxazole-4-carboxylate (4.38)



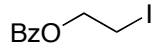
Lithium chloride (24 mg, 0.56 mmol) was heated to 160 ˚C under hi-vac in a septum-sealed 3.5 mL vial for 20 minutes. Zinc dust (< 10 μm, 55 mg, 0.84 mmol) was added, and the vial heated to 160 ˚C under hi-vac for 20 minutes. Upon cooling, anhydrous THF (0.6 mL) was added, followed by 1,2-dibromoethane (2.4 μL, 0.028 mmol) and chlorotrimethylsilane (0.7 μL, 0.0056 mmol). Ethyl-2-iodooxazole-4-carboxylate (**4.13c**, 150 mg, 0.56 mmol) was added as a solution in THF (0.4 mL) and the mixture stirred for 10 minutes then centrifuged briefly to settle excess zinc dust. Quenching of a 10 μL aliquot with D_2O revealed 94 % conversion to the zincate as determined by ^1H NMR.

The oxazol-2-yl zincate solution was transferred to a clean, dry 3.5 mL vial containing $Pd(PPh_3)_4$ (65 mg, 0.056 mmol) and the original vial was rinsed with 0.2 mL THF. Vinyl iodide **4.37** (102 mg, 0.45 mmol) was added as a solution in 0.4 mL THF and the resulting mixture stirred for 1 hour at which time TLC showed complete consumption of **4.37**. The reaction mixture was diluted with saturated NH_4Cl (5 mL) and extracted with Et_2O (3 × 3 mL). Combined Et_2O extracts were dried ($MgSO_4$) and concentrated under reduced

pressure. Crude product was triturated with hexanes, and the supernatant loaded onto a silica column and eluted with 7:3 hexanes/Et_2O to give **4.38** (92 mg, 86 %) as a yellow oil.

IR (neat) ν 3154, 3112, 2979, 2933, 2822, 1740, 1719, 1659, 1575, 1563, 1553, 1529, 1447, 1370, 1314, 1277, 1222, 1202, 1176, 1137, 1109, 1072, 1024, 985, 939, 869, 835, 768, 725 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 4.32 (q, 2H, *J* = 7.2 Hz), 3.73 (q, 1H, *J* = 6.4 Hz), 3.18 (s, 3H), 2.09 (d, 3H, *J* = 1.2 Hz), 1.31 (t, 3H, *J* = 7.2 Hz), 1.22 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7 (C), 161.3 (C), 151.5 (C), 142.6 (CH), 133.9 (C), 110.9 (CH), 81.9 (CH), 61.0 (CH_2), 56.4 (CH_3), 20.0 (CH_3), 14.2 (CH_3), 14.1 (CH_3); HRESIMS *m*/*z* 240.1235 $[M+H]^+$ (calc. for $C_{12}H_{18}NO_4$ 240.1236).

2-iodoethylbenzoate (4.40)



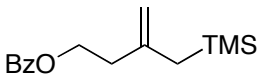
Et_3N (17.3 mL, 124 mmol) and 2-chloroethanol (5.0 g, 62 mmol) were added to a mixture of 4-dimethylaminopyridine (1 large crystal) in anhydrous CH_2Cl_2 (124 mL) at 0 ˚C. Benzoyl chloride (13.09 g, 93 mmol) was added dropwise producing a colorless precipitate. After stirring 5 minutes at 0 ˚C the mixture was warmed to room temperature and stirred 1 hour then quenched with saturated $NH_4Cl_{(aq)}$ (100 mL). Layers were separated and the aqueous layer extracted with CH_2Cl_2 (100 mL). Combined CH_2Cl_2 extracts were washed with H_2O (100 mL) and brine (100 mL) then dried ($MgSO_4$) and concentrated under reduced pressure. Crude material was distilled (82-86 ˚C,

~ 0.2 mmHg) giving 2-chloroethylbenzoate (10.45 g, 91 %) as a colorless liquid. Spectroscopic data matched literature values.[39]

A mixture of 2-chloroethylbenzoate (7.0 g, 38 mmol) and sodium iodide (11.37 g, 76 mmol) in acetone (30 mL) was heated to 100 ˚C for 75 minutes in a CEM Discover microwave reactor. Upon cooling the mixture was filtered through celite, washing the filter cake thoroughly with acetone. The filtrate was diluted with H_2O (300 mL) and extracted with hexanes (3 × 100 mL). Combined hexanes extracts were washed with brine (100 mL) containing 25 mL of saturated $Na_2S_2O_3$ (25 mL), then dried ($MgSO_4$) and concentrated under reduced pressure to give 2-iodoethylbenzoate (**4.40**) as a yellow liquid that was used without further purification (10.14 g, 97%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (m, 2H), 7.57 (m, 1H), 7.44 (m, 2H), 4.56 (t, 2H, *J* = 6.7 Hz), 3.42 (t, 2H, *J* = 6.7 Hz).

3-((trimethylsilyl)methyl)but-3-enyl benzoate (4.42)

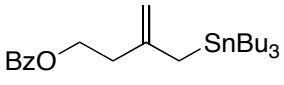


3-Trimethylsilyl-2-propenyl magnesium bromide (**4.41**)[40] (2.35 mmol, 7.13 mL of a 0.33 M solution in THF) was added to a mixture of CuCN (32 mg, 0.36 mmol) in THF (2 mL) at − 50 ˚C. Iodide **4.40** (500 mg, 1.81 mmol) was added dropwise to the resulting yellow mixture, which was then allowed to warm to room temperature over 1 hour. After 30 minutes at room temperature the reaction was quenched with saturated $NH_4Cl_{(aq)}$ (10 mL) followed by H_2O and stirred 5 minutes. Mixture

was extracted with Et_2O (3 × 10 mL), then combined Et_2O extracts were washed with brine (10 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (3.5 % Et_2O/hexanes) gave **4.42** (368 mg, 77 %) as a clear, colorless oil.

IR (neat) ν 3073, 3034, 2954, 2897, 1718, 1634, 1603, 1584, 1492, 1469, 1451, 1418, 1380, 1314, 1269, 1248, 1175, 1157, 1110, 1069, 1027, 984, 963, 849, 837, 769, 708, 689, 665, 657, 620 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (m, 2H), 7.53 (tt, 1H, *J* = 7.6, 1.6 Hz), 7.42 (t, 2H, *J* = 7.6 Hz), 4.70 (q, 1H, *J* = 1.6 Hz), 4.63 (s, 1H), 4.42 (t, 2H, *J* = 7.0 Hz), 2.41 (t, 2H, *J* = 7.0 Hz), 1.58 (s, 2H), 0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5 (C), 143.2 (C), 132.8 (CH), 130..4 (C), 129.5 (CH), 128.3 (CH), 109.2 (CH_2), 63.3 (CH_2), 37.1 (CH_2), 26.9 (CH_2), -1.42 (CH_3); HR-ESI-MS *m*/*z* 263.1471 $[M+H]^+$ (calc. for $C_{15}H_{23}O_2Si$ 263.1462).

3-((tributylstannyl)methyl)but-3-enyl benzoate (4.44)



Propylene oxide (4.43 g, 76.2 mmol) and *N*-bromosuccinimide (5.43 g, 30.5 mmol) were added to a mixture of **4.42** (2 g, 7.62 mmol) in DMF (24 mL) and CH_2Cl_2 (16 mL) at − 78 ˚C. The resulting mixture was stirred 2.5 hours at − 78 ˚C then quenched with 10 % $NaHSO_{3(aq)}$ (40 mL) and warmed to room temperature. The mixture was partitioned between Et_2O (300 mL) and H_2O (150 mL). The Et_2O layer was washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced

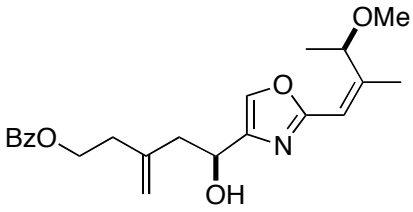
pressure. The resulting crude allyl bromide was concentrated three times from toluene, giving 2.18 g of **4.43** as a yellow oil that was used immediately in the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (m, 2H), 7.55 (tt, 1H, *J* = 7.6, 1.3 Hz), 7.42 (t, 2H, *J* = 7.6 Hz), 5.27 (s, 1H), 5.08 (s, 1H), 4.47 (t, 2H, *J* = 6.6 Hz), 4.03 (s, 2H), 2.69 (t, 2H, *J* = 6.6 Hz).

n-BuLi (24.4 mmol, 9.76 mL of a 2.5 M solution in hexanes) was added dropwise to a mixture of diisopropylamine (3.42 mL, 24.4 mmol) in THF (24.4 mL) at − 78 ˚C. The light yellow mixture was stirred 15 minutes at − 78 ˚C, then 30 minutes at 0 ˚C. Tributyltin hydride (6.65 g, 22.9 mmol) was added dropwise, giving a dark yellow solution that was stirred 45 minutes at 0 ˚C then added *via* syringe to a suspension of CuBr•DMS (4.70 g, 22.9 mmol) in THF (22.9 mL) at − 78 ˚C. After 1.5 hours at − 78 ˚C, **4.43** (2.18 g) was added added as solution in THF (5 mL). The dark, almost black mixture was warmed slowly to − 20 ˚C over 1.25 hours then quenched with saturated $NH_4Cl_{(aq)}$ (100 mL) and warmed to room temperature. The mixture was diluted with H_2O (150 mL) and stirred vigorously to dissolve solids then extracted with hexanes (200 mL). The hexanes extract was washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (3:96:1 Et_2O/hexanes/Et_3N) gave **4.44** (2.93 g, 80 %) as a clear colorless oil.

IR (neat) ν 3071, 3033, 2954, 2923, 2871, 2851, 1720, 1628, 1603, 1584, 1452, 1418, 1377, 1314, 1269, 1175, 1111, 1098, 1069, 1026, 1000, 983, 960, 863, 838, 776, 734, 709, 687, 676, 663, 648, 624, 603 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 2H, *J* = 7.2 Hz), 7.53 (tt, 1H, *J* = 7.6, 1.6 Hz), 7.41 (t, 2H, *J* = 7.8 Hz), 4.62 (s, 1H), 4.56 (s, 1H), 4.43 (t, 2H, *J* = 6.8 Hz), 1.83 (s, 2H), 1.55-1.36 (m, 6H), 1.34-1.22 (m, 6H), 0.87 (t, 15H, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5 (C), 145.8 (C), 132.8 (CH), 130.4 (C), 129.6 (CH), 128.3 (CH), 106.7 (CH_2), 63.5 (CH_2), 37.1 (CH_2), 29.1 (CH_2), 27.3 (CH_2), 19.1 (CH_2), 13.7 (CH_3), 9.44 (CH_2); HR-ESI-MS *m*/*z* 481.2137 $[M+H]^+$ (calc. for $C_{24}H_{41}O_2Sn$ 481.2123).

(*S*)-5-hydroxy-5-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-3-methylenepentyl benzoate (4.47)



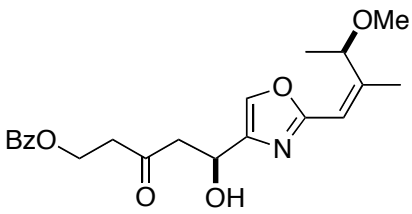
(*S*,*S*)-1,2-diphenyl-1,2-ethylenediamine bis(toluenesulfonamide)[33] (−)-**4.45** was concentrated 4 times from a mixture of anhydrous toluene/CH_2Cl_2, then dried under high vacuum overnight (room temperature), followed by 2 hours at 85 ˚C. Upon cooling, the catalyst (1.82 g, 3.50 mmol) was dissolved in anhydrous CH_2Cl_2 and cooled to 0 ˚C. BBr_3 (3.50 mmol, 3.5 mL of a 1.0 M solution in CH_2Cl_2) was added dropwise. The clear mixture was stirred 10 minutes, then warmed to room temperature for 1 hour. Solvent and HBr were removed under

high vacuum; the resulting off-white solid was re-dissolved in CH_2Cl_2 (20 mL) and concentrated once more. This process was repeated twice more, after which the catalyst was dissolved in CH_2Cl_2 (25 mL) and cooled to 0 ˚C. Stannane **4.44** (1.68 g, 3.50 mmol) was added dropwise as a solution in CH_2Cl_2 (5 mL) followed by a 5 mL rinse. Mixture was stirred overnight, warming slowly to room temperature. By the following morning, the reaction mixture was clear and bright pink/red. After cooling to − 78 ˚C, aldehyde **4.7** (455 mg, 2.33 mmol) was added as a solution in CH_2Cl_2 (3 mL), rinsed in with a further 2 mL. After stirring 2 hours pH 7.0 buffer (50 mL) was added. After warming to room temperature the layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 25 mL). Combined organic extracts were washed with brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (30:69:1 EtOAc/hexanes/Et_3N) gave **4.47** (804 mg, 90 %) as a yellow oil.

$[\alpha]^{22}$ + 23.6 (*c* 4.10, $CHCl_3$); IR (neat) ν 3435 (br), 3073, 2979, 2930, 2820, 1718, 1650, 1602, 1584, 1543, 1517, 1450, 1381, 1315, 1273, 1206, 1177, 1152, 1113, 1097, 1070, 1027, 992, 973, 938, 902, 869, 822, 805, 757, 712, 687, 675, 666, 656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 2H), 7.54 (tt, 1H, *J* = 7.2, 1.5 Hz), 7.46 (d, 1H, *J* = 0.8 Hz), 7.41 (t, 2H, *J* = 7.8 Hz), 6.18 (s, 1H), 5.15 (q, 1H, *J* = 6.4 Hz), 5.03 (d, 2H, *J* = 7.6 Hz), 4.85 (m, 1H), 4.46 (m, 2H), 3.20 (s, 3H), 2.69 (dd, 1H, *J* = 14.4, 4.4 Hz), 2.58-2.52 (m, 3H), 2.43 (d, 1H, 4.0 Hz), 1.87 (d, 3H, *J* = 1.6 Hz), 1.28 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 166.5 (C), 160.4 (C), 150.7 (C), 143.9 (C), 141.6 (C), 133.1 (CH), 132.9 (CH), 130.1 (C), 129.5 (CH), 128.3 (CH), 115.1 (CH_2), 113.3 (CH), 74.7 (CH), 65.3 (CH), 62.9 (CH_2), 56.4 (CH_3), 43.2 (CH_2), 35.0 (CH_2), 19.2 (CH_3), 17.5 (CH_3); HR-ESI-MS *m*/*z* 386.1959 $[M+H]^+$ (calc. for $C_{22}H_{28}NO_5$ 386.1962).

(*S*)-5-hydroxy-5-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-3-oxopentyl benzoate (4.49)



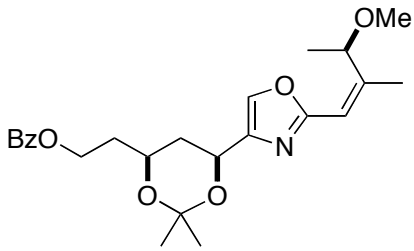
$K_3Fe(CN)_6$ (1.08 g, 1.10 mmol), K_2CO_3 (455 mg, 1.10 mmol), $NaHCO_3$ (277 mg, 1.10 mmol), DABCO (25 mg, 0.22 mmol) and OsO_4 (0.055 mmol, 0.69 mL of a 2.5 wt % solution in *t*-BuOH) were dissolved in *t*-BuOH (20 mL) and H_2O (10 mL). The resulting solution was added to a mixture of **4.47** (423 mg, 1.10 mmol) in *t*-BuOH (10 mL) and stirred for 2.5 hours. Solid Na_2SO_3 (2 g) was added and the mixture stirred vigorously for 45 minutes. H_2O (50 mL) and EtOAc (50 mL) were added and stirring continued for 3.5 hours. Layers were separated and the aqueous layer extracted with EtOAc (2 × 50 mL). Combined organic extracts were washed with pH 7.0 buffer (50 mL) and brine (50 mL) then dried (Na_2SO_4) and concentrated under reduced pressure to give the crude triol **4.48** as a yellow oil.

$NaIO_4$ (939 mg, 4.39 mmol) was added to a mixture of the triol obtained above in THF (15 mL) and H_2O (15 mL) at 0 ˚C. After several minutes the

reaction mixture was warmed to room temperature and stirred 30 minutes then partitioned between H_2O (15 mL) and EtOAc (15 mL). The aqueous layer was extracted with EtOAc (2 × 20 mL), then combined EtOAc extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (2:3 EtOAc/hexanes) gave **4.49** (253 mg, 60 % over 2 steps) as a thick, colorless oil.

$[\alpha]^{22}$ + 5.6 (*c* 2.64, $CHCl_3$); IR (neat) ν 3425 (br), 2978, 2930, 2821, 1716, 1656, 1602, 1584, 1541, 1517, 1451, 1383, 1370, 1315, 1274, 1205, 1177, 1152, 1110, 1096, 1070, 1027, 972, 948, 940, 860, 766, 757, 749, 713, 688, 676, 657, 647, 607; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (m, 2H), 7.52 (tt, 1H, *J* = 7.6, 1.2 Hz), 7.49 (d, 1H, *J* = 0.8 Hz), 7.39 (t, 2H, *J* = 7.8 Hz), 6.14 (s, 1H), 5.15 (br m, 1H), 5.11 (q, 1H, *J* = 6.4 Hz), 4.57 (t, 2H, *J* = 6.2 Hz), 3.51 (br s, 1H), 3.18 (s, 3H), 3.05 (dd, 1H, *J* = 17.6, 4.6 Hz), 2.99 (dd, 1H, *J* = 17.6, 7.8 Hz), 2.93 (t, 2H, *J* = 6.2 Hz), 1.85 (d, 3H, *J* = 1.2 Hz), 1.26 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.7 (C), 166.3 (C), 160.5 (C), 150.9 (C), 142.9 (C), 133.5 (CH), 133.1 (CH), 129.7 (C), 129.5 (CH), 128.3 (CH), 113.2 (CH), 74.7 (CH), 63.8 (CH), 59.3 (CH_2), 56.4 (CH_3), 48.4 (CH_2), 42.3 (CH_2), 19.2 (CH_3), 17.6 (CH_3); HR-ESI-MS *m*/*z* 388.1758 $[M+H]^+$ (calc. for $C_{21}H_{26}NO_6$ 388.1755).

2-((4*S*,6*S*)-6-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-2,2-dimethyl-1,3-dioxan-4-yl)ethyl benzoate (4.51)



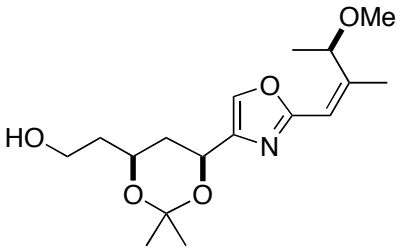
Et_2BOMe (55 mg, 0.55 mmol) was added to a mixture of **4.49** (193 mg, 0.50 mmol) in THF (3.8 mL) and MeOH (0.96 mL). After 15 minutes, $NaBH_4$ (21 mg, 0.55 mmol) was added. After stirring at − 78 ˚C for 4 hours the reaction was quenched with glacial acetic acid (0.5 mL). EtOAc (5 mL) was added and the mixture warmed to room temperature. After diluting with a further 20 mL of EtOAc the mixture was washed with saturated $NaHCO_3$ (25 mL), brine (25 mL) then dried ($MgSO_4$) and concentrated under reduced pressure to give the 1,3-diol **4.50** as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 2H), 7.54 (tt, 1H, *J* = 7.2, 1.5 Hz), 7.46 (d, 1H, *J* = 0.8 Hz), 7.41 (t, 2H, *J* = 7.8 Hz), 6.14 (s, 1H), 5.09 (q, 1H, *J* = 6.3 Hz), 4.93 (dd, 1H, *J* = 9.2, 3.0 Hz), 4.62 (ddd, 1H, *J* = 11.3, 9.0, 4.8 Hz), 4.36 (ddd, 1H, *J* = 11.3, 5.5 Hz), 4.08 (br s, 2H), 3.99 (br s, 1H), 3.17 (s, 3H), 2.01 (dt, 1H, *J* = 14.8, 3.0 Hz), 1.98-1.86 (m, 3H), 1.85 (d, 3H, *J* = 1.2 Hz), 1.25 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 160.4, 150.8, 144.2, 133.1, 133.0, 129.8, 129.6, 128.4, 113.2, 74.7, 68.6, 68.1, 61.5, 56.4, 42.6, 37.0, 19.2, 17.6.

Camphorsulfonic acid (11.6 mg, 0.05 mmol) was added to a mixture of the 1,3-diol **4.50** obtained above in 2,2-dimethyoxypropane (5 mL). The mixture was stirred 1 hour, then quenched with saturated $NaHCO_3$ (10 mL) and H_2O (25 mL). The mixture was extracted with Et_2O (3 × 25 mL). Combined Et_2O extracts were washed with brine (25 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (15 % EtOAc/hexanes) gave **4.51** (194 mg, 89 %) as a clear, colorless oil. Diasteromeric ratio was ≥ 95:5 based on ^{1}H NMR.

$[\alpha]^{23}$ +18.9 (*c* 1.23, $CHCl_3$); IR (neat) ν 2980, 2923, 2878, 2819, 1718, 1655, 1601, 1584, 1540, 1518, 1451, 1381, 1366, 1314, 1272, 1199, 1167, 1107, 1096, 1069, 1027, 997, 957, 942, 917, 872, 818, 806, 762, 712, 687, 676, 656, 618 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (m, 2H), 7.54 (tt, 1H, *J* = 7.2, 1.5 Hz), 7.48 (s, 1H), 7.42 (t, 2H, *J* = 7.6 Hz), 6.18 (s, 1H), 5.10 (q, 1H, *J* = 6.6 Hz), 4.95 (dd, 1H, *J* = 12.0, 1.8 Hz), 4.43 (m, 2H), 4.18 (ddt, 1H, *J* = 11.8, 6.0, 2.2 Hz), 1.95 (q, 2H, *J* = 6.3 Hz), 1.89 (dt, 1H, *J* = 12.4, 2.4 Hz), 1.85 (d, 3H, *J* = 1.6 Hz), 1.62 (q, 1H, *J* = 12.4 Hz), 1.51 (s, 3H), 1.42 (s, 3H), 1.26 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5 (C), 160.4 (C), 150.4 (C), 142.6 (C), 133.7 (CH), 132.9 (CH), 130.2 (C), 129.5 (CH), 128.3 (CH), 113.5 (CH), 99.2 (C), 74.7 (CH), 65.8 (CH), 65.5 (CH), 61.2 (CH_2), 56.4 (CH_3), 36.3 (CH_2), 35.3 (CH_2), 30.0 (CH_3), 19.6 (CH_3), 19.3 (CH_3), 17.6 (CH_3); HR-ESI-MS *m*/*z* 430.2216 $[M+H]^+$ (calc. for $C_{24}H_{32}NO_6$ 430.2224).

2-((4*S*,6*S*)-6-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-2,2-dimethyl-1,3-dioxan-4-yl)ethanol (4.52)

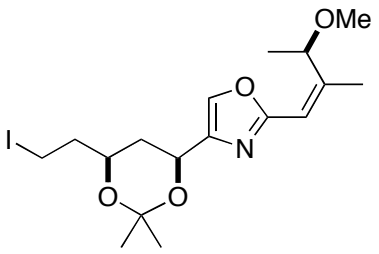


DIBAL (1.28 mmol, 0.85 mL of a 1.5 M solution in toluene) was added dropwise to a mixture of **4.51** (183 mg, 0.43 mmol) in CH_2Cl_2 (2.1 mL) at − 78 ˚C. The mixture was allowed to warm slowly to − 10 ˚C over 2 hours, then re-cooled to − 78 ˚C and quenched with MeOH (360 µL) followed by saturated potassium sodium tartrate (3.6 mL) and warmed to room temperature. H_2O (10 mL) and CH_2Cl_2 (5 mL) were added and the mixture stirred vigorously for 45 minutes. Layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 3 mL). Combined CH_2Cl_2 extracts were washed with brine (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (1:1 EtOAc/hexanes) gave **4.52** (111 mg, 80 %) as a clear, colorless oil.

$[\alpha]^{23}$ +7.47 (*c* 2.33, $CHCl_3$); IR (neat) ν 3420, 2989, 2936, 2879, 2820, 1656, 1602, 1540, 1518, 1447, 1381, 1367, 1337, 1259, 1200, 1164, 1113, 1093, 1066, 1021, 1009, 961, 951, 909, 876, 866, 810 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 6.16 (q, 1H, *J* = 0.8 Hz), 5.08 (q, 1H, *J* = 6.4 Hz), 4.93 (dd, 1H, *J* = 11.8, 2.2 Hz), 4.21 (dddd, 1H, *J* = 9.4, 9.4, 4.8, 2.4 Hz), 3.75 (m, 2H), 3.17 (s, 3H), 2.58 (br s, 1H), 1.84 (d, 3H, *J* = 1.2 Hz), 1.82 (dt, 1H, *J* = 11.0, 2.4 Hz), 1.74 (m, 2H), 1.62 (q, 1H, *J* = 11.9 Hz), 1.53 (s, 3H), 1.42 (s, 3H), 1.25 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.4 (C), 150.4

(C), 142.6 (C), 133.7 (CH), 113.5 (CH), 99.1 (C), 74.7 (CH), 68.7 (CH), 65.5 (CH), 60.3 (CH_2), 56.4 (CH_3), 38.1 (CH_2), 36.1 (CH_2), 30.1 (CH_3), 19.7 (CH_3), 19.2 (CH_3), 17.5 (CH_3); HR-ESI-MS *m*/*z* 326.1969 $[M+H]^+$ (calc. for $C_{17}H_{28}NO_5$ 326.1967).

4-((4*S*,6*R*)-6-(2-iodoethyl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazole (4.53)

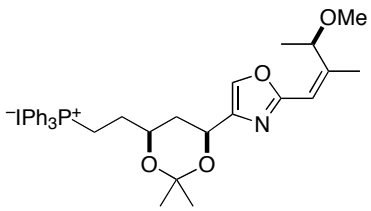


Imidazole (65 mg, 0.96 mmol), PPh_3 (126 mg, 0.48 mmol) and I_2 (122 mg, 0.48 mmol) were added, in that order, to a mixture of **4.52** (104 mg, 0.32 mmol) in THF (4.70 mL) at 0 ˚C. The mixture was stirred for 1 hour at 0 ˚C at which point Et_2O (5 mL) and saturated $Na_2S_2O_3$ (5 mL) were added. Mixture was stirred until 2 clear, colorless layers were apparent, then further diluted with H_2O (25 mL). Layers were separated and the aqueous layer extracted with Et_2O (2 × 20 mL). Combined Et_2O extracts were washed with brine (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. Silica flash chromatography (15 % EtOAc/hexanes) gave **4.53** (125 mg, 89 %) as a clear, colorless oil.

$[\alpha]^{23}$ +4.62 (*c* 1.58, $CHCl_3$); IR (neat) ν 2988, 2932, 2893, 2818, 1654, 1602, 1541, 1519, 1447, 1381, 1365, 1256, 1202, 1175, 1168, 1145, 1113, 1096, 1069, 1050, 1036, 1015, 971, 942, 877, 857, 816 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 6.16 (s, 1H), 5.09 (q, 1H, *J* = 6.4 Hz), 4.94 (dd,

1H, *J* = 11.8, 2.6 Hz), 4.08 (dddd, 1H, *J* = 11.4, 7.7, 4.0, 3.2 Hz), 3.31-3.21 (m, 2H), 3.17 (s, 3H), 2.00-1.88 (m, 2H), 1.84 (d, 3H, *J* = 1.6 Hz), 1.82 (dt, 1H, *J* = 15.2, 2.6 Hz), 1.57 (q, 1H, *J* = 12.0 Hz), 1.53 (s, 3H), 1.41 (s, 3H), 1.25 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.3 (C), 150.4 (C), 142.6 (C), 133.6 (CH), 113.4 (CH), 99.2 (C), 74.7 (CH), 68.3 (CH), 65.5 (CH), 56.4 (CH_3), 39.3 (CH_2), 35.6 (CH_2), 29.9 (CH_3), 19.7 (CH_3), 19.2 (CH_3), 17.5 (CH_3), 2.29 (CH_2); HR-ESI-MS *m*/*z* 436.0982 $[M+H]^+$ (calc. for $C_{17}H_{27}NO_4I$ 436.0985).

(2-((4*R*,6*S*)-6-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-2,2-dimethyl-1,3-dioxan-4-yl)ethyl)triphenylphosphonium iodide (4.4)

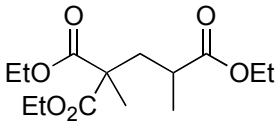


A mixture of **4.53** (125 mg, 0.29 mmol), PPh_3 (450 mg, 1.72 mmol) and Li_2CO_3 (423 mg, 5.72 mmol) in acetonitrile (11.4 mL) and toluene (2.9 mL) was heated to 130 ˚C in a CEM Discover microwave reactor for 30 minutes. Upon cooling the reaction mixture was filtered through a cotton plug and washed with hexanes (8 × 50 mL) to remove excess PPh_3. The remaning acetonitrile layer was concentrated under reduced pressure to give **4.4** (149 mg, 75 %) as a yellow foam that was used without further purification.

IR (neat) ν 3077, 3053, 2987, 2930, 2930, 2873, 2819, 1653, 1587, 1540, 1519, 1484, 1437, 1382, 1366, 1338, 1321, 1257, 1199, 1172, 1153, 1110, 1030, 1011, 996, 968, 947, 909, 874, 857 cm^{-1}; ^{1}H NMR (500 MHz,

$CDCl_3$) δ 7.79-7.69 (m, 9H), 7.67-7.62 (m, 6H), 7.50 (s, 1H), 6.11 (s, 1H), 5.00 (q, 1H, *J* = 6.3 Hz), 4.93 (dd, 1H, *J* = 11.2, 2.2 Hz), 4.42 (m, 1H), 3.91 (m, 1H), 3.44 (m, 1H), 3.13 (s, 3H), 1.98 (br s, 1H), 1.90 (dt, 1H, *J* = 12.9, 2.5 Hz), 1.78 (d, 3H, *J* = 1.6 Hz), 1.66 (m, 1H), 1.55 (s, 3H), 1.52 (q, 1H, *J* = 12.0 Hz), 1.38 (s, 3H), 1.19 (d, 1H, *J* = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.2 (C), 149.9 (C), 141.4 (C), 134.9 (CH, d, 2.6 Hz), 134.2 (CH), 133.3 (CH, d, 10.3 Hz), 130.3 (CH, d, 12.5 Hz), 117.6 (C, d, 86.1 Hz), 113.3 (CH), 99.4 (C), 74.4 (CH), 67.5 (CH, d, 15.2 Hz), 64.2 (CH), 56.1 (CH_3), 34.8 (CH_2), 29.6 (CH_3), 28.8 (CH_2), 20.1 (CH_3), 18.9 (CH_3), 18.3 (CH_2, d, 53.2 Hz), 17.3 (CH_3); HR-ESI-MS *m*/*z* 570.2766 $[M]^+$ (calc. for $C_{35}H_{41}NO_4P$ 570.2773).

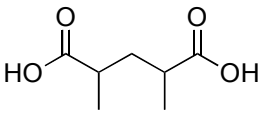
triethyl pentane-2,2,4-tricarboxylate[47] (**4.56**)



Sodium metal (57 g) was dissolved in EtOH (1100 mL) by adding pieces over the course of 30 minutes. The excess EtOH was distilled under N_2 into a 2 L 3-neck round bottom flask which was subsequently fitted with a reflux condensor. Sodium (39.6 g, 1.72 mol) was added to the anhydrous EtOH portionwise over 30 minutes. Upon complete dissolution the mixture was heated to reflux and diethyl methylmalonate (300 g, 1.72 mol) was added *via* a dropping funnel. After 5 minutes ethyl 2-bromoisobutyrate (335.5 g, 1.72 mol) was added *via* a dropping funnel. The mixture was refluxed for 2 hours then filtered and concentrated under reduced pressure to remove most of the EtOH. H_2O (1 L)

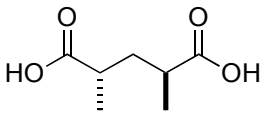
was added and the mixture was extracted with Et_2O (3 × 250 mL). Combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give ~500 g of a yellow liquid. Distillation afforded 338 g of **4.56** (b.p. 90-105 ˚C, ~0.1 mmHg) as a clear, colorless liquid.

2,4-dimethylglutaric acid (4.57)



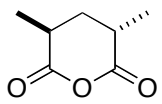
A modification of the procedure described by Lautens et al.[51] A mixture of compound **4.56** (60 g, 0.21 mol) and concentrated HCl (145.7 mL) was heated to reflux for 21 hours. The mixture was transferred to a beaker and boiled until the volume was reduced by ~50%. Upon cooling to room temperature **4.57** crystallized and was collected by filtration. The crystals were re-dissolved in Et_2O, dried (Na_2SO_4) and concentrated under reduced pressure to give **4.57** as a colorless, crystalline solid (30 g). The aqueous mother liquor from the initial crystallization was concentrated under reduced pressure and the colorless solid thus obtained was re-crystallized from EtOAc/hexanes to give a further 1.17 g of **4.57** (31.17 g total, 93%).

(2*S*,4*S*)-2,4-dimethylglutaric acid ((+)-4.59)



2,4-dimethylglutaric acid was resolved according to the procedure of Stanton *et al.*[49] $[\alpha]^{21}$ +34.6 (*c* 4.37, EtOH). Lit.[49] for (+)-**4.59** $[\alpha]_D$ −35.8 (*c* 2, EtOH).

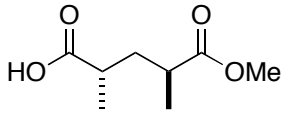
(2*S*,4*S*)-2,4-dimethylglutaric anhydride 4.60[49]



A mixture of (2*S*,4*S*)-2,4-dimethylglutaric acid (+)-**4.59** (7 g, 0.044 mol) in acetyl chloride (15.5 mL, 0.22 mol) was stirred at 50 ˚C for 30 minutes, then volatiles were removed under reduced pressure to give **4.60** (6.37 g, 100 %) as a low-melting colorless solid. Compound **4.60** was azeotrope-dried from toluene prior to use in the next reaction.

$[\alpha]^{23}$ −54.0 (*c* 4.59, $CHCl_3$), lit. for *ent*-**60** + 56.5 ˚ (*c* 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.87 (sextet, 2H, 6.9 Hz), 1.85 (t, 2H, 6.9 Hz), 1.36 (d, 6H, 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0 (C), 33.2 (CH), 31.3 (CH_2), 16.3 (CH_3).

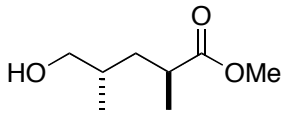
(2*S*,4*S*)-5-methoxy-2,4-dimethyl-5-oxopentanoic acid 4.61[50]



To a solution of **4.60** (6.28 g, 44 mmol) in anhydrous CH_2Cl_2 (12.6 mL) was added anhydrous pyridine (7.15 mL, 88 mmol) followed immediately by anhydrous methanol (3.84 mL). The mixture was stirred for 3 hours at room temperature, then cooled to 0 ˚C. 5 M HCl (10 mL) was added dropwise. After 5 minutes at 0 ˚C the mixture was diluted in Et_2O (50 mL) and H_2O (25 mL). Layers were separated and the Et_2O layer was washed with 1M HCl (3 × 30 mL), H_2O (30 mL) and brine (30 mL). Combined aqueous layers were extracted with Et_2O (25 mL), then combined Et_2O extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give **4.61** (6.69 g, 87 %) as a clear, colorless oil.

$[\alpha]^{21}$ +20.4 (*c* 5.34, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.65 (s, 3H), 2.53 (sextet, 1H, *J* = 7.2 Hz), 2.47 (sextet, 1H, *J* = 7.2 Hz), 1.74 (t, 2H, *J* = 7.2 Hz), 1.17 (d, 3H, *J* = 7.2 Hz), 1.14 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.5 (C), 176.7 (C), 51.7 (CH_3), 37.54 (CH), 37.49 (CH), 37.2 (CH_2), 17.7 (CH_3), 17.6 (CH_3).

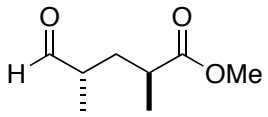
(2*S*,4*S*)-methyl 5-hydroxy-2,4-dimethylpentanoate 4.62[50]



According to the procedure described by Lautens et al. for the reduction of (2S,4R)-5-methoxy-2,4-dimethyl-5-oxopentanoic acid.[51] To a solution of **4.61** (6.5 g, 37 mmol) in THF (93 mL) at 0 ˚C was added $B(OMe)_3$ (12.4 mL, 112 mmol). After 10 minutes, BH_3•DMS (45 mmol, 22.4 mL of a 2 M solution in THF) was added. The mixture was allowed to warm to room temperature and stirred there for 5 hours at which time the mixture was cooled to 0 ˚C and diluted with Et_2O (25 mL). A solution of H_2O/glycerine (3:1, 80 mL) was added and the mixture stirred at room temperature for 30 minutes before being diluted in 1:1 Et_2O/H_2O (200 mL). Layers were separated and the aqueous layer extracted with Et_2O (3 × 50 mL). Combined Et_2O extracts were washed with H_2O (100 mL) and brine (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica chromatography (1:4 EtOAc/hexanes) gave **4.62** as a clear, colorless oil (4.88 g, 82 %).

$[\alpha]^{21}$ −26.2 (*c* 0.29, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (s, 3H), 3.44 (m, 2H), 2.54 (sextet, 1H, *J* = 7.0 Hz), 1.65 (m, 1H), 1.62 (br s, 1H), 1.50 (m, 2H), 1.12 (d, 3H, *J* = 7.0 Hz), 0.88 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ177.6 (C), 67.8 (CH_2), 51.6 (CH_3), 36.98 (CH_2), 36.93 (CH), 33.5 (CH), 17.2 (CH_3), 16.4 (CH_3).

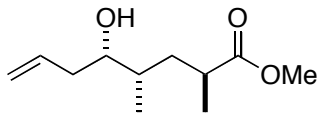
(2*S*,4*S*)-methyl 2,4-dimethyl-5-oxopentanoate 4.63



To a mixture of oxalyl chloride (3.88 mL, 43.7 mmol) in CH_2Cl_2 (82 mL) at − 78 ˚C was added DMSO (6.2 mL, 87 mmol) dropwise. After 30 minutes, **4.62** (3.5 g, 21.8 mmol) was added dropwise as a solution in CH_2Cl_2 (9 mL). After a further 30 minutes Et_3N (18.3 mL, 131 mmol) was added dropwise. The mixture was allowed to warm to 0 ˚C over 2 hours and then poured into H_2O (200 mL). Layers were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). Combined organic extracts were washed with 1 % HCl (3 × 100 mL), 5 % $NaHCO_3$ (100 mL), H_2O (100 mL) and brine (200 mL) then dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (7:3 hexanes/Et_2O) gave 4.**63** (2.95 g, 85 %) as a light yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.60 (d, 1H, *J* = 1.6 Hz), 3.65 (s, 3H), 2.54 (sextet, 1H, *J* = 7.2 Hz), 2.39 (m, 1H), 1.86-1.63 (m, 2H), 1.16 (d, 3H, *J* = 7.2 Hz), 1.09 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.0 (CH), 176.6 (C), 51.9 (CH_3), 44.3 (CH), 37.0 (CH), 34.1 (CH_2), 17.4 (CH_3), 13.7 (CH_3).

(2*S*,4*S*,5*S*)-methyl 5-hydroxy-2,4-dimethyloct-7-enoate, 4.64a



Aldehyde **4.63** (400 mg, 2.53 mmol), precooled to – 78 ˚C, was added as a solution in toluene (1.9 mL) to a mixture of (3*R*,4*R*)-diisopropyl 1-allylborolane-3,4-dicarboxylate (**4.69**, 1.42 g, 5.06 mmol) and powdered molecular sieves (380 mg) in toluene (16.4 mL) at – 78 ˚C. The resulting mixture was stirred for 2 hours at – 78 ˚C, then filtered through a plug of cotton wool. Solvents were removed under reduced pressure and the crude product chromatographed on silica gel (3:7 Et_2O/hexanes) to give **4.4.64a** (431 mg, 85 %) as a clear colorless oil (*dr* 9:1 based on ^1H NMR integrations).

$[\alpha]^{22}$ +8.19 (*c* 1.88, $CHCl_3$); IR (neat) ν 3459, 3076, 2973, 2951, 2937, 2878, 1733, 1718, 1641, 1460, 1435, 1378, 1335, 1259, 1195, 1171, 155, 1106, 1090, 1032, 979, 912, 867, 830, 764 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.77 (m, 1H), 5.10 (m, 2H) 3.63 (s, 3H), 3.54 (br m, 1H), 2.52 (m, 1H), 2.22 (m, 1H), 2.13 (dt, 1H, *J* = 14.0, 8.3 Hz), 1.62-1.51 (m, 4H), 1.11 (d, 3H, *J* = 7.2 Hz), 0.85 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.5 (C), 135.2 (CH), 117.9 (CH_2), 73.1 (CH), 51.5 (CH_3), 39.0 (CH_2), 37.0 (CH), 36.9 (CH_2), 35.3 (CH), 16.9 (CH_3), 13.5 (CH_3); HRESIMS *m*/*z* 223.1302 $[M+Na]^+$ (calc. for $C_{11}H_{20}O_3Na$ 223.1305).

Exemplary procedure for Singaram Barbier-type allylation of 4.63.

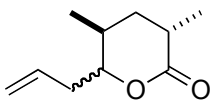
(1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol (135 mg, 0.63 mmol) and indium powder (73 mg, 0.63 mmol) were suspended in anhydrous THF.

Pyridine (51 µL, 0.63 mmol) and allyl bromide (76 mg, 0.63 mmol) were added and the resulting mixture stirred vigorously for 30 minutes. Hexane (0.64 mL) was added, the mixture cooled to – 78 ˚C and aldehyde **4.63** (50 mg, 0.32 mmol) was added. After stirring 1.5 h at –78 ˚C the reaction was quenched with H_2O (2 mL), warmed to room temperature and extracted with Et_2O (3 × 2 mL). Combined Et_2O extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (40 % Et_2O/hexanes) gave **4.64** (51 mg, 81%) as a mixture of diastereomers (3:1 **64a:4.64b** based on ^1H NMR integration).

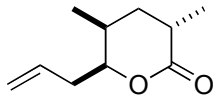
Exemplary procedure for Brown $(Ipc)_2BAll$ allylation of 4.63.

Freshly prepared $(Ipc)_2BAll$[56] (~ 0.31 mmol) was dissolved in anhydrous Et_2O and cooled to – 95 ˚C. Aldehyde **4.63** (50 mg, 0.31 mmol) was added as a solution in Et_2O (0.3 mL). After 30 minutes at – 95 ˚C, MeOH (25 µL) was added and the mixture warmed to room temperature. THF/H_2O (1:1, 2 mL) was added followed by sodium perborate tetrahydrate (97 mg, 0.63 mmol). The resulting mixture stirred for 2 hours, diluted in H_2O (5 mL) and extracted with Et_2O (2 × 2 mL). Combined Et_2O extracts were washed with H_2O and brine, dried (Na_2SO_4) and concentrated under reduced pressure. Separation of **4.64** from isopinocampheol proved difficult: silica flash chromatography (15 % EtOAc/hexanes) gave **4.64a** (18.6 mg, est. 80 % pure, ~ 29 %).

(3*S*,5*S*)-6-allyl-3,5-dimethyltetrahydro-2*H*-pyran-2-one (4.70a and 4.70b)

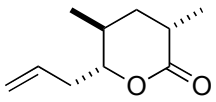


A mixture of alcohols **4.64a** and **4.64b** (5 mg, 0.025 mmol, *dr* = 9:1) was combined with camphor sulfonic acid (1.1 mg, 0.005 mmol) in CH_2Cl_2 (0.125 mL) and stirred for 20 minutes. Solvent was removed under reduced pressure and the residue was redissolved in CH_2Cl_2 and stirred for a further 10 minutes. At this time the solvent was removed once again under reduced pressure and the crude residue chromatographed on silica gel (2:3 Et_2O/hexanes) to give lactones **4.70a** and **4.70b** (3.3 mg, 78%). The diastereomers could be separated by SiO_2 HPLC (1:9 EtOAc/hexanes) to provide pure **4.70a** and **4.70b**.



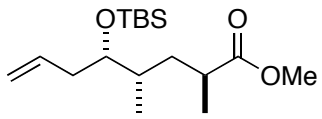
$[\alpha]^{21}$ −52.95 (*c* 1.46, $CHCl_3$); IR (neat) 3079, 2971, 2935, 2877, 1733, 1645, 1462, 1357, 1322, 1204, 1165, 1108, 1060, 990, 736, 645, 579, 518 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.77 (dddd, 1H, *J* = 17.0, 10.9, 7.8, 6.4 Hz), 5.14 (dq, 1H, *J* = 17.0, 1.6 Hz), 5.09 (dq, 1H, *J* = 10.4, 1.3 Hz), 4.35 (dt, 1H, *J* = 7.2, 2.8 Hz), 2.60 (ddq, 1H, *J* = 11.8, 7.2, 7.1 Hz), 2.46 (m, 1H), 2.28-2.21 (m, 1H), 2.03 (ddq, 1H, *J* = 7.1, 7.1, 3.8 Hz), 1.90 (ddd, 1H, *J* = 13.6, 7.1, 4.0), 1.67 (ddd, 1H, *J* = 13.6, 11.8, 4.0), 1.27 (d, 3H, *J* = 7.0 Hz), 0.99 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3 (C), 132.9 (CH), 118.1 (CH_2),

83.1 (CH), 37.0 (CH_2), 35.9 (CH_2), 31.3 (CH), 29.2 (CH), 17.8 (CH_3), 11.1 (CH_3).



^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (dddd, 1H, *J* = 17.0, 10.0, 7.0, 7.0 Hz), 5.15-5.10 (m, 2H), 3.97 (ddd, 1H, *J* = 10.0, 6.5, 3.6 Hz), 2.61 (m, 1H), 2.52-2.47 (m, 1H), 2.31 (dt, 1H, *J* = 15.5, 7.1 Hz), 1.89 (m, 1H), 1.72-1.61 (m, 2H), 1.19 (d, 3H, *J* = 6.5 Hz), 0.99 (d, 3H, *J* = 6.0 Hz).

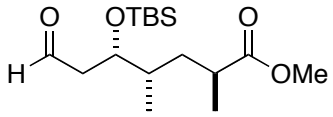
(2*S*,4*S*,5*S*)-methyl 5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyloct-7-enoate (4.71)



Imidazole (2.21 g, 32.5 mmol) was added to a solution of **4.64a** (2.6 g, 13 mmol) in DMF (16.7 mL). TBSCl (3.92 g, 26 mmol) was added and the resulting mixture was stirred for 3 hours at which time a further 980 mg of TBSCl and 442 mg of imidazole were added. After stirring for a further 2 hours, 25 mL of water was added and the aqueous mixture stirred for 5 minutes before being diluted in a further 25 mL H_2O and extracted with Et_2O (3 × 25 mL). The combined Et_2O extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (19:1 hexanes/Et_2O) gave **4.71** (3.67 g, 90 %) as a clear, colorless oil.

$[\alpha]^{22}$ +2.35 (*c* 1.87, $CHCl_3$); IR (neat) ν 3077, 2952, 2930, 2884, 2857, 2804, 1734, 1641, 1471, 1462, 1435, 1379, 1361, 1252, 1193, 1158, 1051, 1019, 1004, 938, 911, 854, 833, 809, 772, 670 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.71 (ddt, 1H, *J* = 16.6, 10.0, 7.2 Hz), 4.99 (m, 2H), 3.63 (s, 3H), 3.58 (dt, 1H, *J* = 6.4, 2.8 Hz), 2.45 (m, 1H), 2.16 (m, 2H), 1.60-1.40 (m 3H), 1.08 (d, 3H, *J* = 6.6 Hz), 0.85 (s, 9H), 0.79 (d, 3H, *J* = 6.6 Hz), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6 (C), 135.5 (CH), 116.5 (CH_2), 74.7 (CH), 51.4 (CH_3), 38.7 (CH_2), 37.1 (CH), 36.6 (CH_2), 35.0 (CH), 25.9 (CH_3), 18.1 (C), 16.9 (CH_3), 13.8 (CH_3), -4.1 (CH_3), -4.6 (CH_3); LRESIMS *m*/*z* 337.19 $[M+Na]^+$; HREIMS m/*z* 314.2277 (calc. for $C_{17}H_{34}O_3Si$ 314.2272).

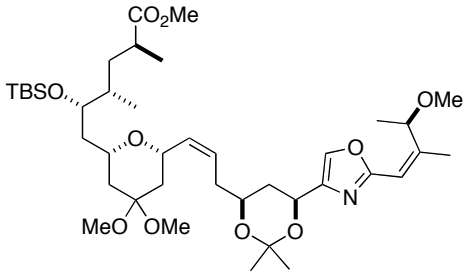
(2*S*,4*S*,5*S*)-methyl 5-(*tert*-butyldimethylsilyloxy)-2,4-dimethyl-7-oxoheptanoate (4.5)



Ozone was bubbled through a solution of alkene **4.71** (305 mg, 0.97 mmol) in CH_2Cl_2 (4.8 mL) at – 78 ˚C until a permanent blue color was observed. The mixture was purged with N_2 until the blue color disappeared, then treated with a solution of triphenylphosphine (500 mg, 1.91 mmol) in CH_2Cl_2 (1 mL). The mixture was warmed to room temperature over 1 hour then stirred there for 30 minutes before being reduced under reduced pressure. Silica flash chromatography gave **4.5** (305 mg, 99 %) as a clear, colorless oil.

$[\alpha]^{22}$ −19.7 (*c* 1.66, $CHCl_3$); IR ν 2953, 2930, 2884, 2857, 2720, 1731, 1463, 1435, 1379, 1362, 1253, 1195, 1160, 1137, 1086, 1057, 1016, 1007, 987, 939, 836, 775, 677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.75 (dd, 1H, *J* = 2.8, 2.0 Hz), 4.14 (ddd, 1H, *J* = 7.6, 4.5, 3.2 Hz), 3.64 (s, 3H), 2.52 (dd, 1H, *J* = 15.6, 7.4, 2.8 HZ), 2.46 (m, 1H), 2.42 (dd, 1H, *J* = 16.0, 4.0, 1.9 Hz), 1.60 (m, 2H), 1.45 (m, 1H), 1.10 (d, 3H, *J* = 6.8 Hz), 0.84 (s, 3H), 0.81 (d, 3H, *J* = 6.8 Hz), 0.03 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 201.9 (CH), 177.4 (C), 70.9 (CH), 51.6 (CH_3), 47.5 (CH_2), 37.2 (CH), 36.8 (CH), 35.3 (CH_2), 25.7 (CH_3), 17.9 (C), 16.8 (CH_3), 14.7 (CH_3), -4.50 (CH_3), -4.64 (CH_3); HRESIMS *m*/*z* 339.1968 $[M + Na]^+$ (calc. for $C_{16}H_{32}O_4SiNa$ 339.1962).

(2*S*,4*S*,5*S*)-methyl 5-(*tert*-butyldimethylsilyloxy)-6-((2*S*,6*S*)-4,4-dimethoxy-6-((*Z*)-3-((4*S*,6*S*)-6-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-2,2-dimethyl-1,3-dioxan-4-yl)prop-1-enyl)tetrahydro-2*H*-pyran-2-yl)-2,4-dimethylhexanoate (4.74)



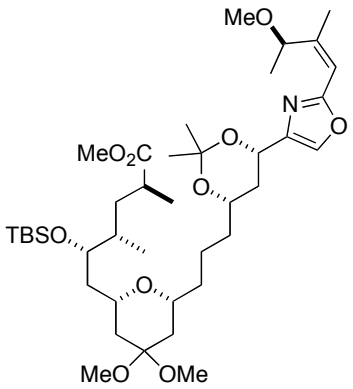
Phosphonium salt **4.4** (75 mg, 0.11 mmol) was concentrated three times from a 2:1 mixture of anhydrous toluene/CH_2Cl_2 then dried under high vacuum for 2 hours. Anhydrous THF (1 mL) was added and the mixture subjected to three freeze-pump-thaw cycles to remove O_2 then cooled to −78 ˚C. LiHMDS (0.11 mmol, 110 µL of a 1.0 M solution in hexanes) was

added dropwise. The bright orange mixture was stirred 20 minutes at −78 ˚C then 20 minutes at 0 ˚C and was then re-cooled to −78 ˚C. Aldehyde **4.3** (~0.10 mmol) was added slowly down the inside of the vial as a solution in degassed THF (0.3 mL, risnsed in with a further 0.3 mL). The orange color rapidly dissipated to give a pale yellow mixture which was stirred at −78 ˚C for 1 hour and then warmed to 0 ˚C for 1 hour. The reaction was quenched with saturated NH_4Cl (3 mL) and warmed to room temperature. Sufficient H_2O was added to dissolve all solids and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 1 mL). Combined organic extracts were washed with brine (2 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (5:95 *i*-PrOH/hexanes) gave **4.74** (51.7 mg, 71 %) as a viscous, colorless oil (68 % yield over 2 steps from **4.73**).

$[\alpha]^{22}$ −11.9 (*c* 1.93, $CHCl_3$); FTIR (neat) ν 2953, 2927, 2855, 1738, 1462, 1434, 1380, 1361, 1255, 1197, 1163, 1135, 1108, 1072, 1052, 1007, 973, 948, 927, 861, 836, 774, 661 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1H), 6.17 (s, 1H), 5.45 (m, 2H), 5.08 (q, 1H, *J* = 6.5 Hz), 4.90 (dd, 1H, *J* = 12.0, 2.6 Hz), 4.22 (m, 1H), 3.95 (m, 1H), 3.80 (m, 1H), 3.62 (s, 3H), 3.55 (m, 1H), 3.18 (s, 3H), 3.15 (s, 3H), 3.12 (s, 3H), 2.40 (m, 1H), 2.27 (m, 2H), 1.84 (s, 3H), 1.88-1.80 (m, 3H), 1.58 (m, 2H), 1.50 (s, 3H), 1.49 (m, 1H), 1.45-1.38 (m, 2H), 1.43 (s, 3H), 1.35-1.16 (m, 3H), 1.26 (d, 3H, *J* = 6.8 Hz), 1.06 (d, 3H, *J* = 6.8 Hz), 0.84 (s, 9H), 0.75 (d, 3H, *J* = 6.8 Hz), -0.022 (s, 3H), -0.033 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C), 160.4 (C), 150.3 (C), 142.8 (C),

133.6 (CH), 132.6 (CH), 125.9 (CH), 113.5 (CH), 99.1 (C), 98.7 (C), 74.7 (CH), 71.5 (CH), 70.6 (CH), 69.8 (CH), 68.6 (CH), 65.6 (CH), 56.4 (CH_3), 51.5 (CH_3), 47.6 (CH_3), 47.3 (CH_3), 38.9 (CH_2), 38.71 (CH_2), 38.68 (CH_2), 37.2 (CH), 36.4 (CH), 36.2 (CH_2), 34.8 (CH_2), 34.7 (CH_2), 30.1 (CH_3), 25.9 (CH_3), 19.7 (CH_3), 19.3 (CH_3), 18.0 (C), 17.6 (CH_3), 16.6 (CH_3), 14.8 (CH_3), -4.2 (CH_3), -4.8 (CH_3); HREIMS *m*/*z* 774.4584 $[M+Na]^+$ (calc. for $C_{40}H_{69}NNaO_{10}Si$ 774.4588).

(2*S*,4*S*,5*S*)-methyl 5-(*tert*-butyldimethylsilyloxy)-6-((2*S*,6*R*)-4,4-dimethoxy-6-(3-((4*S*,6*S*)-6-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-2,2-dimethyl-1,3-dioxan-4-yl)propyl)tetrahydro-2*H*-pyran-2-yl)-2,4-dimethylhexanoate (4.75)

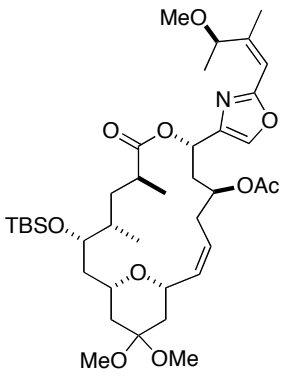


Wilkinson's catalyst (10.3 mg, 0.011 mmol) and **4.74** (20 mg, 0.0027 mmol) were combined in degassed THF/^{t}BuOH (1:1, 0.5 mL) and heated to 50 ˚C under H_2 (1 atm). After 6 hours the reaction mixture was cooled, diluted with Et_2O and filtered through a short plug of celite, eluting with Et_2O. Filtrate was concentrated under reduced pressure. Silica flash chromatography (1:4 EtOAc/hexanes) gave **4.75** (16.6 mg, 83 %) as a clear, colorless oil.

$[\alpha]^{23}$ −3.31 (*c* 1.36, $CHCl_3$); FTIR (neat) ν 2954, 2929, 2882, 2857, 2828, 1738, 1655, 1600, 1541, 1519, 1462, 1434, 1380, 1365, 1332, 1256,

1230, 1198, 1165, 1135, 1102, 1071, 1053, 1006, 966, 941, 854, 836, 807, 774, 661 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H), 6.19 (s, 1H), 5.10 (q, 1H, *J* = 6.4 Hz), 4.92 (dd, 1H, *J* = 12.0, 2.0 Hz), 3.93 (m, 1H), 3.84 (dt, 1H, *J* = 9.2, 2.4 Hz), 3.64 (s, 3H), 3.52 (m, 1H), 3.40 (m, 1H), 3.19 (s, 3H), 3.18 (s, 3H), 3.12 (s, 3H), 2.43 (m, 1H), 1.91 (m, 1H), 1.86 (d, 3H, *J* = 1.6 Hz), 1.84 (m, 1H), 1.63-1.30 (unresolved m, 11H), 1.52 (s, 3H), 1.43 (s, 3H), 1.27 (d, 3H, *J* = 6.8 Hz), 1.24-1.14 (m, 4H), 1.09 (d, 3H, *J* = 7.2 Hz), 0.87 (s, 9H), 0.78 (d, 3H, *J* = 6.8 Hz), 0.027 (s, 3H), 0.016 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C), 160.4 (C), 150.3 (C), 143.0 (C), 133.7 (CH), 113.6 (CH), 99.1 (C), 99.0 (C), 74.7 (CH), 73.6 (CH), 71.9 (CH), 69.9 (CH), 68.7 (CH), 65.7 (CH), 56.4 (CH_3), 51.5 (CH_3), 47.6 (CH_3), 47.3 (CH_3), 39.1 (CH_2), 39.0 (2 × CH_2), 37.3 (CH), 36.6 (CH), 36.5 (CH_2), 36.4 (CH_2), 35.9 (CH_2), 34.8 (CH_2), 30.2 (CH_3), 25.9 (CH_3), 21.2 (CH_2), 19.7 (CH_3), 19.3 (CH_3), 18.1 (C), 17.6 (CH_3), 16.6 (CH_3), 14.8 (CH_3), −4.09 (CH_3), −4.62 (CH_3); HRMS *m*/*z* 754.4909 $[M+H]^+$ (calc. for $C_{40}H_{72}NO_{10}Si$ 754.4920).

(1*S*,5*S*,7*S*,10*S*,12*S*,13*S*,15*S*,*Z*)-13-(*tert*-butyldimethylsilyloxy)-17,17-dimethoxy-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-9-oxo-8,19-dioxabicyclo[13.3.1]nonadec-2-en-5-yl acetate (4.78)



$LiOH.H_2O$ (1.70 mmol, 0.42 mL of a 4M solution in H_2O) was added to a solution of compound **4.74** (31.9 mg, 0.042 mmol) in MeOH (3.2 mL). The vial was sealed and heated to 80 ˚C for 1 hour. The mixture was cooled to room temperature, acidified with 1M HCl (2 mL) and extracted immediately with EtOAc (4 × 2mL). Combined organic extracts were washed with brine (3 mL), dried (Na_2SO_4) and concentrated to give a quantitative yield of the carboxylic acid as a clear, colorless oil.

Camphorsulfonic acid (0.0085 mmol, 197 µL of a 10 mg/mL solution in MeOH) was added to a solution of the carboxylic acid in MeOH (3.2 mL). The mixture was stirred for 1 hour then diluted with saturated $NH_4Cl_{(aq)}$ (5 mL), 2 mL H_2O and extracted with EtOAc (4 × 2 mL). Combined organic extracts were washed with brine (3 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give a quantitative yield of the 1,3-diol as a clear, colorless oil.

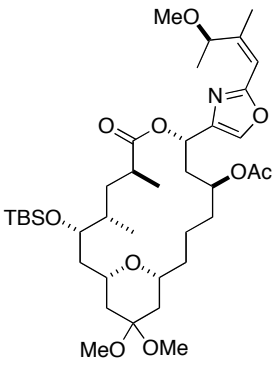
The dihydroxy acid obtained above was added *via* syringe pump (0.013 mL/min) as a solution in ethanol-free chloroform (10 mL) to a refluxing mixture

of dicyclohexylcarbodiimide (228 mg, 1.11 mmol), DMAP (119 mg, 0.97 mmol) and DMAP.HCl (169 mg, 1.11 mmol) in ethanol-free chloroform (126 mL) (addition time ~ 16 h). Upon completion of the addition the syringe was charged with ethanol-free chloroform (3 mL) which was added to the refluxing mixture *via* syringe pump (0.04 mL/min). Upon completion of this final addition, the mixture was refluxed for a further 1 hour, then cooled to room temperature. AcOH (127 μL, 2.21 mmol) and MeOH (537 μL, 13.3 mmol) were added, and the solvent removed under reduced pressure. The resulting solid was suspended in Et_2O, filtered and concentrated under reduced pressure to a volume of ~ 1 mL. The mixture was diluted with an equal volume of hexanes and filtered. The solids were washed thoroughly with 1:1 Et_2O/hexanes, and the filtrate cocnetrated under reduced pressure. Silica flash chromatography (1:4 EtOAc/hexanes) followed by SiO_2 HPLC (15:85 EtOAc/hexanes, 4 mL/min) gave macrolide **4.78** (10.7 mg, 35 % overall) as a clear, colorless oil.

$[\alpha]^{21}$ −23.2 (*c* 2.17, $CHCl_3$); IR (neat) ν 2956, 2931, 2856, 2829, 1739, 1658, 1547, 1461, 1371, 1318, 1289, 1249, 1235, 1204, 1180, 1151, 1137, 1103, 1068, 1052, 1032, 1009, 975, 921, 903, 852, 836, 818, 799, 773, 738, 719, 673, 654 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41 (s, 1H), 6.15 (s, 1H), 5.94 (dd, 1H, *J* = 9.4, 4.2 Hz), 5.49 (dt, 1H, *J* = 10.4, 5.3 Hz), 5.41 (dd, 1H, *J* = 9.8, 9.8 Hz), 5.18 (q, 1H, *J* = 6.5 Hz), 4.77 (m, 1H), 4.12 (m, 1H), 3.88 (dd, 1H, *J* = 10.4, 4.4 Hz), 3.36 (t, 1H, *J* = 9.8 Hz), 3.21 (s, 3H), 3.188 (s, 3H), 3.184 (s, 3H), 2.61 (m, 2H), 2.37 (ddd, 1H, *J* = 14.4, 9.2, 4.9 Hz), 2.21 (ddd, 1H, *J* =

14.0, 9.3, 4.9 Hz), 2.13 (m, 1H), 2.00 (s, 3H), 1.88 (dt, 1H, *J* = 13.2, 2.5 Hz), 1.87 (d, 3H, *J* = 1.2 Hz), 1.82-1.73 (m, 2H), 1.62 (m, 1H), 1.53 (m, 2H), 1.42 (dd, 1H, *J* = 14.2, 4.7 Hz), 1.32 (m, 2H), 1.27 (d, 3H, *J* = 6.4 Hz), 1.11 (d, 3H, *J* = 6.8 Hz), 0.84 (s, 9H), 0.81 (d, 3H, *J* = 6.0 Hz), 0.008 (s, 3H), − 0.019 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1 (C), 170.0 (C), 160.4 (C), 151.3 (C), 140.5 (C), 133.9 (CH), 132.4 (CH), 127.8 (CH), 113.0 (CH), 98.7 (C), 74.7 (CH), 71.3 (CH), 71.0 (CH), 70.9 (CH), 68.9 (CH), 65.0 (CH), 56.5 (CH_3), 47.8 (CH_3), 47.3 (CH_3), 41.2 (CH_2), 39.7 (CH_2), 38.8 (CH), 38.4 (CH_2), 37.8 (CH_2), 37.6 (CH_2), 34.4 (CH), 31.5 (CH_2), 25.8 (CH_3), 21.1 (CH_3), 19.1 (CH_3), 18.2 (CH_3), 18.1 (C), 17.6 (CH_3), 13.5 (CH_3), − 3.86 (CH_3), − 4.87 (CH_3); HRESIMS *m*/*z* 722.4297 $[M+H]^+$ (calc. for $C_{38}H_{64}NO_{10}Si$ 722.4294).

(1*R*,5*S*,7*S*,10*S*,12*S*,13*S*,15*S*)-13-(*tert*-butyldimethylsilyloxy)-17,17-dimethoxy-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-9-oxo-8,19-dioxabicyclo[13.3.1]nonadecan-5-yl acetate (4.79)

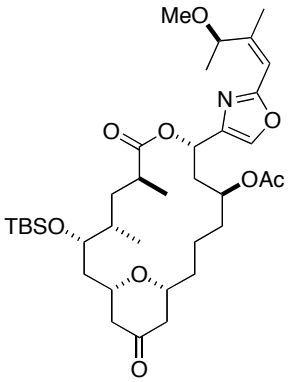


Alkene **4.78** (10.7 mg, 0.015 mmol) was combined with Wilkinson's catalyst (5.5 mg, 0.0059 mmol) in degassed THF/^{t}BuOH (1:1, 0.3 mL). The vial was evacuated and purged with H_2 5 times then heated to 50 ˚C for 5.5 hours. The mixture was diluted with Et_2O (2 mL), filtered through a short plug of celite and concentrated

under reduced pressure. Silica gel chromatography (1:4 EtOAc/hexanes) gave the desired product **4.79** (7.6 mg, 71 %) as a clear colorless oil.

$[\alpha]^{21}$ − 4.53 (*c* 1.61, $CHCl_3$); IR (neat) ν 2956, 2930, 2856, 2829, 2829, 1736, 1655, 1543, 1455, 1379, 1370, 1332, 1307, 1278, 1249, 1237, 1202, 1181, 1151, 1141, 1111, 1098, 1064, 1045, 1008, 997, 970, 930, 897, 851, 836, 811, 774, 754, 742, 700, 672, 656 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.39 (s, 1H), 6.14 (s, 1H), 5.97 (dd, 1H, *J* = 12.5, 2.7 Hz), 5.16 (q, 1H, *J* = 6.5 Hz), 4.94 (m, 1H), 3.92 (dd, 1H, *J* = 10.8, 4.4 Hz), 3.43 (t, 1H, *J* = 11.5 Hz), 3.29 (t, 1H, *J* = 9.5 Hz), 3.21 (s, 3H), 3.18 (s, 3H), 3.17 (s, 3H), 2.60 (m, 1H), 2.46 (dt, 1H, *J* = 13.1, 3.9 Hz), 2.02 (s, 3H), 1.91 (m, 1H), 1.87 (d, 3H, *J* = 1.5 Hz), 1.86-1.76 (m, 4H), 1.64-1.58 (m, 2H), 1.55-1.38 (unresolved m, 6H), 1.44-1.38 (m, 2H), 1.33-1.25 (m, 2H), 1.27 (d, 3H, *J* = 6.4 Hz), 1.16 (m, 1H), 1.13 (d, 3H, *J* = 6.4 Hz), 0.90 (d, 3H, *J* = 6.4 Hz), 0.85 (s, 9H), 0.012 (s, 3H), − 0.018 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8 (C), 170.3 (C), 160.4 (C), 151.1 (C), 141.0 (C), 133.6 (CH), 113.1 (CH), 98.9 (C), 74.7 (CH), 71.4 (CH), 71.3 (CH), 70.8 (CH), 69.8 (CH), 64.7 (CH), 56.5 (CH_3), 47.8 (CH_3), 47.3 (CH_3), 41.2 (CH_2), 40.2 (CH_2), 39.3 (CH_2), 38.7 (CH), 38.1 (CH_2), 38.0 (CH_2), 34.4 (CH_2), 34.2 (CH), 30.0 (CH_2), 25.8 (CH_3), 21.1 (CH_3), 20.2 (CH_2), 19.2 (CH_3), 18.1 (C), 17.9 (CH_3), 17.7 (CH_3), 13.8 (CH_3), − 3.82 (CH_3), − 4.86 (CH_3); HRESIMS *m*/*z* 724.4446 $[M+H]^+$ (calc. for $C_{38}H_{66}NO_{10}Si$ 724.4456).

(1*R*,5*S*,7*S*,10*S*,12*S*,13*S*,15*S*)-13-(*tert*-butyldimethylsilyloxy)-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-9,17-dioxo-8,19-dioxabicyclo[13.3.1]nonadecan-5-yl acetate (4.80)

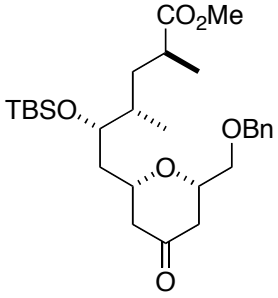


Compound **4.79** (7.2 mg, 0.0099 mmol) was combined with camphorsulfonic acid (0.46 mg, 0.002 mmol, delivered as 46 µL of a 10 mg/mL solution in CH_2Cl_2) in acetone (1.4 mL). After 3.25 h a further 1 mL of acetone was added and the reaction stirred for a further 45 minutes. The mixture was diluted with H_2O (3 mL) and extracted with EtOAc (3 × 2 mL). Combined EtOAc extracts were washed with brine (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica gel chromatography (1:4 EtOAc/hexanes) gave ketone **4.80** (6.1 mg, 91%) as a clear, colorless oil.

$[\alpha]^{21}$ −13.0 (*c* 1.50, $CHCl_3$); IR (neat) ν 2953, 2930, 2855, 1731, 1655, 1541, 1472, 1455, 1433, 1380, 1370, 1328, 1296, 1277, 1249, 1201, 1146, 1094, 1074, 1060, 1031, 1008, 971, 935, 924, 906, 897, 889, 851, 838, 816, 800, 773, 759, 735, 724, 682, 671, 661 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 7.39 (s, 1H), 6.14 (s, 1H), 5.98 (dd, 1H, *J* = 12.7, 2.5 Hz), 5.16 (1H, q, *J* = 6.4 Hz), 4.92 (m, 1H), 3.94 (dd, 1H, *J* = 11.2, 3.9 Hz), 3.61 (m, 1H), 3.43 (m, 1H), 3.20 (s, 3H), 2.59 (m, 1H), 2.47 (dt, 1H, *J* = 13.1, 3.4 Hz), 2.32-2.23 (m, 3H), 2.15 (dd, 1H, *J* = 14.7, 11.2 Hz), 2.04 (s, 3H), 1.99-1.94 (m, 1H), 1.90 (ddd, 1H, *J* = 13.8, 10.9, 2.9 Hz), 1.87 (d, 3H, *J* = 1.5 Hz), 1.80 (m, 1H), 1.71 (m, 1H), 1.65-

1.61 (m, 1H), 1.56 (m, 1H), 1.52-1.45 (m, 4H), 1.35-1.26 (m, 2H), 1.26 (d, 3H, *J* = 6.4 Hz), 1.13 (d, 3H, *J* = 6.4 Hz), 0.94 (d, 3H, *J* = 6.9 Hz), 0.85 (s, 9H), 0.02 (s, 3H), − 0.018 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 207.0 (C), 174.9 (C), 170.3 (C), 160.5 (C), 151.3 (C), 140.7 (C), 133.6 (CH), 113.0 (CH), 74.7 (CH), 74.3 (CH), 73.0 (CH), 71.1 (CH), 70.3 (CH), 64.5 (CH), 56.5 (CH_3), 49.0 (CH_2), 48.2 (CH_2), 41.3 (CH_2), 38.7 (CH), 38.0 (CH_2), 37.9 (CH_2), 35.1 (CH_2), 34.0 (CH), 30.0 (CH_2), 25.8 (CH_3), 21.1 (CH_3), 20.3 (CH_2), 19.2 (CH_3), 18.1 (C), 17.65 (CH_3), 17.64 (CH_3), 13.5 (CH_3), − 3.92 (CH_3), −4.89 (CH_3); HRESIMS *m*/*z* 678.4035 $[M+H]^+$ (calc. for $C_{36}H_{59}NO_9Si$ 678.4032).

(2*S*,4*S*,5*S*)-methyl 6-((2*S*,6*S*)-6-(benzyloxymethyl)-4-oxotetrahydro-2*H*-pyran-2-yl)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethylhexanoate (4.83)

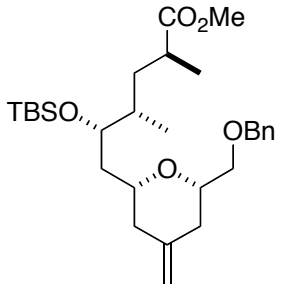


Camphorsulfonic acid (7.8 mg, 0.034 mmol) was added to a solution of dimethyl acetal **4.72** (92.6 mg, 0.17 mmol) in acetone (1.68 mL). The mixture was stirred for 90 minutes, at which time a further 1.7 mL of acetone was added. After a further 30 minutes, camphorsulfonic acid (7.8 mg, 0.034 mmol) was added. After 90 minutes the reaction mixture was concentrated to dryness and re-dissolved in acetone (3.4 mL). After 5 minutes the mixture was diluted in H_2O (25 mL) and extracted with Et_2O (3 × 10 mL). Combined Et_2O extracts were washed with 20 mL each of saturated $NaHCO_{3(aq)}$ and brine then dried ($MgSO_4$) and concentrated. Silica flash

chromatography (1:4 EtOAc/hexanes) gave ketone **4.83** (68.6 mg, 81 %) as a clear colorless oil.

$[\alpha]_D^{21}$ − 23.4 (*c* 1.17, $CHCl_3$); IR (neat) ν 2953, 2929, 2882, 2857, 1732, 1462, 1435, 1382, 1362, 1333, 1276, 1255, 1196, 1162, 1140, 1116, 1096, 1074, 1031, 1006, 937, 927, 837, 801, 775, 740, 698, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34-7.24 (m, 5H), 4.60 (d, 1H, *J* = 12.6 Hz), 4.55 (d, 1H, *J* = 12.6 Hz), 3.90 (dt, 1H, *J* = 10.0, 2.6 Hz), 3.74 (m, 1H), 3.68 (m, 1H), 3.64 (s, 3H), 3.55 (m, 2H), 2.43 (m, 2H), 2.36-2.27 (m, 2H), 2.22 (dd, 1H, *J* = 14.4, 11.2 Hz), 1.63 (m, 3H), 1.42 (m, 2H), 1.09 (d, 3H, *J* = 7.2 Hz), 0.84 (s, 9H), 0.81 (d, 3H, *J* = 6.8 Hz), 0.00 (s, 3H), −0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.1 (C), 177.6 (C), 137.9 (C), 128.3 (CH), 127.6 (CH), 127.5 (CH), 75.8 (CH), 73.5 (CH), 73.3 (CH_2), 72.0 (CH_2), 71.4 (CH), 51.5 (CH_3), 48.2 (CH_2), 44.2 (CH_2), 39.0 (CH_2), 37.3 (CH), 36.5 (CH), 34.3 (CH_2), 25.8 (CH_3), 17.9 (C), 16.6 (CH_3), 15.0 (CH_3), −4.24 (CH_3), −4.85 (CH_3); HR-ESI-FT-MS *m*/*z* 529.2960 $[M+Na]^+$ (calc. for $C_{28}H_{46}O_6SiNa$ 529.2956).

(2*S*,4*S*,5*S*)-methyl 6-((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethylhexanoate (4.84)



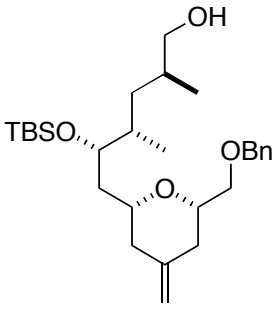
Zinc dust (<10 μm, 571 mg, 8.73 mmol) was briefly flame dried under vacuum. Upon cooling, THF (7.1 mL) was added followed by 1,2-dibromoethane (38 μL, 0.44 mmol) and TMSCl (11 μL, 0.087 mmol). After stirring 10 minutes dibromomethane (494 mg, 2.84 mmol) was added and the mixture cooled to − 40 ˚C. $TiCl_4$ (385 mg, 2.03 mmol) was added dropwise and the resulting mixture warmed slowly to 0 ˚C and stirred 45 hours.

At this time an aliquot of the Lombardo reagent (235 μL, 0.068 mmol) was added to a mixture of **4.83** (23 mg, 0.045 mmol, azeotrope-dried 3 × from toluene prior to use) in CH_2Cl_2 (0.45 mL) at 0 ˚C. After stirring 1 hour at 0 ˚C a further 78 μL of the Lombardo reagent was added. After stirring a further 15 minutes the reaction was quenched by addition of saturated $NaHCO_3$. The mixture was diluted with H_2O (4 mL) and extracted with Et_2O (2 × 2mL). Combined Et_2O extracts were washed with H_2O (4 mL) and brine (4 mL) then dried (Na_2SO_4 and $NaHCO_3$) and concentrated under reduced pressure. Silica flash chromatography (1:4 Et_2O/hexanes) gave **4.84** (17.1 mg, 75%) as a clear colorless oil.

$[\alpha]_D^{21}$ − 27.0 (*c* 1.94, $CHCl_3$); IR (neat) ν 3070, 3030, 2951, 2929, 2887, 2856, 1737, 1653, 1497, 1471, 1462, 1455, 1434, 1380, 1361, 1329, 1309,

1253, 1194, 1172, 1159, 1099, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 4H), 7.27 (m, 1H), 4.71 (br s, 2H), 4.58 (d, 1H, *J* = 12.6 Hz), 4.55 (d, 1H, *J* = 12.6 Hz), 3.87 (dt, 1H, *J* = 9.2, 2.8 Hz), 3.64 (s, 3H), 3.55-3.43 (m, 3H), 3.34 (m, 1H), 2.43 (m, 1H), 2.24 (br d, 1H, *J* = 12.8 Hz), 2.13 (br d, 1H, *J* = 13.2 Hz), 2.02 (br t, 1H, *J* = 11.6 Hz), 1.92 (br t, 1H, *J* = 12.2 Hz), 1.62 (m, 2H), 1.53 (dd, 1H, *J* = 9.6, 2.8 Hz), 1.47-1.37 (m, 2H), 1.09 (d, 3H, *J* = 6.8 Hz), 0.85 (s, 9H), 0.80 (d, 3H, *J* = 6.4 Hz), −0.004 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C), 144.4 (C), 138.5 (C), 128.3 (CH), 127.52 (CH), 127.49 (CH), 108.7 (CH_2), 77.2 (CH), 74.8 (CH), 73.3 (CH_2), 73.1 (CH_2), 71.7 (CH), 51.5 (CH_3), 41.4 (CH_2), 39.1 (CH_2), 37.5 (CH_2), 37.3 (CH), 36.6 (CH), 34.8 (CH_2), 25.9 (CH_3), 18.1 (C), 16.7 (CH_3), 14.9 (CH_3), −4.26 (CH_3), −4.67 (CH_3); HR-ESI-FT-MS *m*/*z* 527.3164 $[M+Na]^+$ (calc. for $C_{29}H_{48}O_5SiNa$ 527.3163).

(2*S*,4*S*,5*S*)-6-((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-5-(*tert*-butyldimethylsilyloxy)-2,4-dimethylhexan-1-ol (4.85)

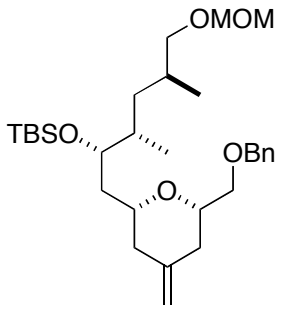


DIBAL (0.11 mmol, 71 μL of a 1.5 M solution in toluene) was added dropwise to a mixture of **4.84** (18 mg, 0.036 mmol) in CH_2Cl_2 (0.36 mL) at − 78 ˚C. Mixture was warmed slowly to 0 ˚C over 2.25 hours, then returned to − 78 ˚C and quenched with MeOH (30 μL) followed by saturated sodium potassium tartrate (0.3 mL). The mixture was warmed to room temperature, diluted with CH_2Cl_2 and H_2O (2 mL each) and stirred

vigorously for 20 minutes to give 2 clear layers. Layers were separated and aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL). Combined CH_2Cl_2 extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure. Silica flash chromatography (15:85 EtOAc/hexanes) gave **4.85** (15.7 mg, 92 %) as a clear, colorless oil.

$[\alpha]_D^{22}$ − 43.2 (*c* 1.79, $CHCl_3$); IR (neat) ν 3387 (br), 3071,3029, 2953, 2928, 2891, 2857, 1654, 1471, 1462, 1430, 1385, 1362, 1329, 1310, 1254, 1207, 1177, 1101, 1072, 1037, 1007, 984, 965, 938, 929, 891, 836, 801, 774, 747, 735, 716, 698, 686, 676, 665 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 4H), 7.27 (m, 1H), 4.71 (s, 2H), 4.59 (d, 1H, *J* = 12.6 Hz), 4.56 (d, 1H, *J* = 12.6 Hz), 3.88 (dt, 1H, *J* = 9.6, 2.6 Hz), 3.56-3.34 (m, 6H), 2.24 (br d, 1H, *J* = 13.6 Hz), 2.13 (br d, 1H, *J* = 13.2 Hz), 2.01 (br t, 1H, *J* = 11.6 Hz), 1.92 (br t, 1H, *J* = 12.4 Hz), 1.73-1.61 (m, 2H), 1.54 (ddd, 1H, *J* = 14.4, 9.8, 2.4 Hz), 1.40 (ddd, 1H, *J* = 14.0, 10.0, 2.6 Hz), 1.35 (br s, 1H), 1.27 (ddd, 1H, *J* = 13.6, 10.2, 3.1 Hz), 1.12 (ddd, 1H, *J* = 12.8, 10.6, 3.6 Hz), 0.86 (s, 9H), 0.85 (d, 3H, *J* = 7.6 Hz), 0.81 (d, 3H, *J* = 6.8 Hz), 0.007 (s, 3H), −0.003 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5 (C), 138.5 (C), 128.3 (CH), 127.53 (CH), 127.50 (CH), 108.7 (CH_2), 77.2 (CH), 74.9 (CH), 73.3 (CH_2), 73.1 (CH_2), 72.2 (CH), 69.4 (CH_2), 41.5 (CH_2), 39.0 (CH_2), 37.5 (CH_2), 35.9 (CH), 34.0 (CH_2), 33.2 (CH), 26.0 (CH_3), 18.1 (C), 16.0 (CH_3), 15.2 (CH_3), −4.16 (CH_3), −4.71 (CH_3); HR-ESI-FT-MS *m*/*z* 499.3216 $[M+Na]^+$ (calc. for $C_{28}H_{48}O_4SiNa$ 499.3214).

(6*S*,8*S*,9*S*)-9-(((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)methyl)-6,8,11,11,12,12-hexamethyl-2,4,10-trioxa-11-silatridecane (4.86)

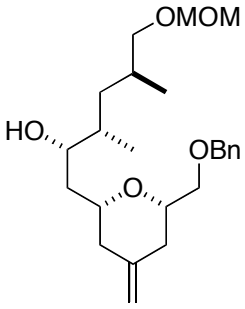


Chloromethyl methyl ether (12.4 mg, 0.13 mmol) was added to a mixture of **4.85** (12.5 mg, 0.026 mmol) and *i*Pr_2NEt (27.1 mg, 0.21 mmol) and CH_2Cl_2 (0.26 mL) at 0 ˚C. The mixture was allowed to warm to room temperature and stirred for 3 hours then quenched with saturated NH_4Cl and extracted with Et_2O (3 × 2 mL). Combined Et_2O extracts were washed with H_2O and brine (2 mL each) and combined aqueous layers were re-extracted with Et_2O (2 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Silica pencil column (1:9 Et_2O/hexanes) gave **4.86** (12.4 mg, 91 %) as a clear, colorless oil.

$[\alpha]_D^{21}$ −38.9 (*c* 1.14, $CHCl_3$); IR (neat) ν 2953, 2928, 2886, 2856, 1653, 1471, 1462, 1386, 1329, 1310, 1255, 1211, 1147, 1110, 1071, 1048, 1007, 985, 975, 967, 951, 922, 890, 853, 836, 803, 774, 745, 737, 723, 696, 677, 666, 658 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 4H), 7.26 (m, 1H), 4.71 (br s, 2H), 4.60 (s, 2H), 4.57 (s, 2H), 3.86 (dt, 1H, *J* = 9.6, 2.8 Hz), 3.55-3.45 (m, 3H), 3.39-3.27 (m, 3H), 3.34 (s, 3H), 2.24 (br d, 1H, *J* = 13.2 Hz), 2.13 (br d, 1H, *J* = 13.2 Hz), 2.02 (br t, 1H, *J* = 11.2 Hz), 1.92 (br t, 1H, *J* = 12.2 Hz), 1.78-1.66 (m, 2H), 1.54 (ddd, 1H, *J* = 14.0, 9.2, 2.7 Hz), 1.40 (ddd, 1H, *J* = 13.6, 10.0, 3.2 Hz), 1.26 (ddd, 1H, *J* = 13.2, 10.6, 2.9 Hz), 1.15 (ddd, 1H, *J* =

12.8, 11.0, 3.9 Hz), 0.86 (d, 3H, *J* = 7.2 Hz), 0.85 (s, 9H), 0.80 (d, 3H, *J* = 7.2 Hz), 0.003 (s, 3H), −0.007 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5 (C), 138.5 (C), 128.3 (CH), 127.52 (CH), 127.48 (CH), 108.6 (CH_2), 96.6 (CH_2), 77.2 (CH), 74.9 (CH), 74.4 (CH_2), 73.3 (CH_2), 73.1 (CH_2), 72.3 (CH), 55.1 (CH_3), 41.5 (CH_2), 39.1 (CH_2), 37.5 (CH_2), 35.9 (CH), 34.4 (CH_2), 30.9 (CH), 26.0 (CH_3), 18.1 (C), 16.4 (CH_3), 15.0 (CH_3), −4.17 (CH_3), −4.69 (CH_3); HR-ESI-FT-MS *m*/*z* 543.3480 $[M+Na]^+$ (calc. for $C_{30}H_{52}O_5SiNa$ 543.3476).

(2*S*,3*S*,5*S*)-1-((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-6-(methoxymethoxy)-3,5-dimethylhexan-2-ol (4.87)

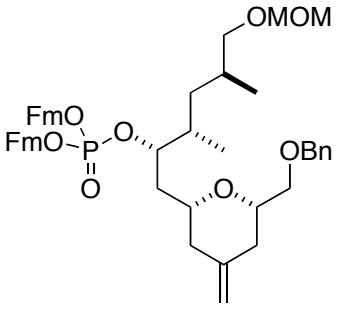


A mixture of 48%HF/MeCN/H_2O (5:86:9, 3 mL) was added to a mixture of compound **4.86** (19.2 mg, 0.0037 mmol) in MeCN (0.75 mL). The mixture was stirred for 23 hours then made basic (pH ~ 8) with saturated $NaHCO_3$ and extracted with Et_2O (3 × 5mL). Combined Et_2O extracts were washed with H_2O and brine (5 mL each), dried (Na_2SO_4) and concentrated under reduced pressure. Silica pencil column (1:4 EtOAc/hexanes) gave **4.87** (14 mg, 93%) as a clear colorless oil.

$[\alpha]_D^{23}$ − 18.6 (*c* 0.80, $CHCl_3$); IR (neat) ν 3450 (br), 3070, 3031, 2927, 2887, 1651, 1462, 1454, 1384, 1370, 1330, 1310, 2110, 1145, 1109, 1075, 1043, 967, 952, 941, 922, 893, 858, 810, 735, 716, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 4H), 7.27 (m, 1H), 4.73 (br s, 2H), 4.60 (s, 2H), 4.56

(d, 1H, *J* = 12.7 Hz), 4.53 (d, 1H, *J* = 12.7 Hz), 3.76 (ddd, 1H, *J* = 9.2, 4.6, 2.2 Hz), 3.60 (dddd, 1H, *J* = 10.4, 7.0, 3.5, 3.5 Hz), 3.56-3.43 (m, 3H), 3.34 (m, 1H), 3.34 (s, 3H), 3.30 (dd, 1H, *J* = 9.2, 6.0 Hz), 2.54 (br s, 1H), 2.21 (br d, 1H, *J* = 12.8 Hz), 2.17-2.07 (m, 2H), 2.00 (br t, 1H, *J* = 12.4 Hz), 1.81 (m, 1H), 1.72 (ddd, 1H, *J* = 14.8, 9.4, 3.6 Hz), 1.64-1.56 (m, 2H), 1.28 (ddd, 1H, *J* = 13.6, 9.8, 4.2 Hz), 1.15 (ddd, 1H, *J* = 13.6, 9.6, 3.9), 0.89 (d, 3H, *J* = 6.4 Hz), 0.88 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8 (C), 138.2 (C), 128.4 (CH), 127.63 (CH), 127.61 (CH), 109.2 (CH_2), 96.6 (CH_2), 77.7 (CH), 76.4 (CH), 74.1 (CH_2), 73.4 (CH_2), 73.2 (CH_2), 72.2 (CH), 55.1 (CH_3), 40.3 (CH_2), 39.3 (CH_2), 37.2 (CH_2), 36.8 (CH_2), 35.7 (CH), 30.8 (CH), 16.5 (CH_3), 14.0 (CH_3); HR-ESI-FT-MS *m*/*z* 429.2615 $[M+Na]^+$ (calc. for $C_{24}H_{38}O_5Na$ 429.2611).

bis((9*H*-fluoren-9-yl)methyl) (2*S*,3*S*,5*S*)-1-((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-6-(methoxymethoxy)-3,5-dimethylhexan-2-yl phosphate (4.88)



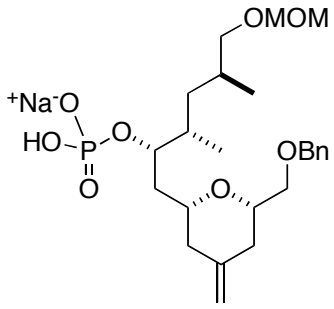
i$Pr_2NP(OFm)_2$ (43.1 mg, 0.0083 mmol) was added as a solution in CH_2Cl_2 (0.41 mL) to a mixture of **4.87** (8.4 mg, 0.0021 mmol) and tetrazole (4.34 mg, 0.0062 mmol, 183 μL of a 3 wt% solution in MeCN) in MeCN (0.23 mL) under N_2. The mixture was stirred 40 minutes, then cooled to 0 ˚C and treated with 30% H_2O_2 (94 μL,

0.083 mmol). After 10 minutes the reaction was quenched with saturated $NaHCO_3$ (2 mL) and extracted with CH_2Cl_2 (3 × 2 mL). Combined CH_2Cl_2 extracts were washed with brine (5 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica pencil column (1:19→1:9 Et_2O/CH_2Cl_2) gave **4.88** (14.5 mg, 83%) as a colorless, slightly cloudy oil.

$[\alpha]_D^{23}$ − 12.8 (*c* 0.83, $CHCl_3$); IR (neat) ν 3065, 3039, 2951, 2927, 2889, 1652, 1477, 1449, 1384, 1322, 1266, 1212, 1149, 1105, 1075, 1040, 1006, 988, 943, 914, 898, 869, 839, 824, 802, 793, 757, 740, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71-7.64 (m, 4H), 7.50 (br t, 2H, *J* = 8.2 Hz), 7.43 (br t, 2H, *J* = 6.4 Hz), 7.35 (br t, 2H, *J* = 7.4 Hz), 7.32-7.16 (m, 11H), 4.68 (br d, 2H, *J* = 6.0 Hz), 4.55 (m, 1H), 4.51 (s, 2H), 4.48 (d, 1H, *J* = 8.6 Hz), 4.45 (d, 1H, *J* = 8.6 Hz), 4.24 (t, 2H, *J* = 6.4Hz), 4.16 (m, 1H), 4.13-4.04 (m, 3H), 3.47-3.38 (m, 4H), 3.28 (s, 3H), 3.22 (d, 2H, *J* = 6.8 Hz), 2.16 (br d, 1H, *J* = 13.2 Hz), 2.11 (br d, 1H, *J* = 13.2 Hz), 2.02 (m, 1H), 1.89 (m, 2H), 1.71-1.60 (m, 3H), 1.22 (m, 1H), 1.14 (ddd, 1H, *J* = 13.2, 10.0, 3.4 Hz), 0.80 (d, 3H, *J* = 6.8 Hz), 0.75 (d, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8 (C), 143.27 (C), 143.21 (C), 143.18 (C), 143.12 (C), 141.3 (C), 141.29 (C), 141.25 (C), 138.4 (C), 128.3 (CH), 127.8 (CH), 127.6 (CH), 127.5 (CH), 127.0 (CH), 125.3 (CH), 125.2 (CH), 125.1 (CH), 119.95 (CH, d, 2.6 Hz), 119.89 (CH, d, 2.3 Hz), 109.1 (CH_2), 96.5 (CH_2), 81.1 (CH, d, 6.4 Hz), 77.2 (CH), 74.7 (CH), 73.9 (CH_2), 73.2 (CH_2), 73.0 (CH_2), 69.2 (CH_2, d, 5.6 Hz), 68.9 (CH_2, d, 5.7 Hz), 55.0 (CH_3), 47.95 (CH, d, 7.6 Hz), 47.87 (CH, d, 7.6 Hz), 41.0 (CH_2), 37.95 (CH_2, d, 5.3

Hz), 37.2 (CH_2), 35.1 (CH_2), 34.48 (CH, d, 3.0 Hz), 30.6 (CH), 16.3 (CH_3), 14.3 (CH_3); HR-ESI-FT-MS *m*/*z* 865.3846 $[M+Na]^+$ (calc. for $C_{52}H_{59}O_8PNa$ 865.3840).

potassium (2*S*,3*S*,5*S*)-1-((2*R*,6*S*)-6-(benzyloxymethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-6-(methoxymethoxy)-3,5-dimethylhexan-2-yl hydrogenphosphate (4.89)



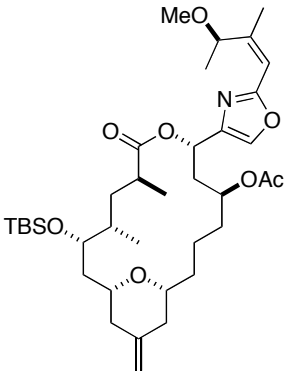
K_2CO_3 (20.5 mg, 0.015 mmol) was added as a solution in H_2O (205 µL) to a mixture of **4.88** (12.5 mg, 0.0015 mmol) in MeOH (1.25 mL). The mixture was stirred for 17 hours, then diluted with H_2O and extracted with Et_2O (3 × 1 mL). The aqueous layer was concentrated under reduced pressure to remove all MeOH (leaving a solution of **4.89** in H_2O) then loaded onto a 200 mg C18 sep pak. K_2CO_3 was eluted with 100% H_2O, then the product was eluted with 1:4 H_2O/MeOH, giving 7.1 mg of the desired compound. A second C18 sep pak purification (2:3 H_2O MeOH) gave **4.89** (6.3 mg, 81 %) as a clear colorless oil.

Compound **4.89** was converted to the sodium salt by passing through a short column of Dowex 50X2-400 (50W-hydrogen) strongly acidic resin with 1:1 MeOH/H_2O (the resin was washed with H_2O, 5% $NaHCO_3$, H_2O and 1:1 MeOH/H_2O prior to use). Finally, **4.89** was purified by RP C18 HPLC prior to

characterization (Microsorb 100-C5 C18 column, 250 × 10 mm, 3:7 H_2O/MeOH, 3 mL/min, UV detection at 220 nm).

$[\alpha]_D^{23}$ − 25.5 (*c* 1.45, MeOH); IR (neat) ν 3391 (br), 2951, 2931, 2881, 1652, 1455, 1386, 1371, 1150, 1107, 1074, 1042, 936, 925, 899, 866, 856, 847 819, 760, 739, 714, 699, 678, 666, 659 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.37-7.31 (m, 4H), 7.27 (tt, 1H, *J* = 7.0, 2.1 Hz), 4.73 (br m, 2H), 4.58 (m, 4H), 4.34 (br s, 1H), 3.60 (m, 1H), 3.53-3.48 (m, 3H), 3.37 (dd, 1H, *J* = 9.5, 6.0 Hz), 3.33 (s, 3H), 3.30 (m, 1H), 2.23 (br t, 2H, *J* = 14.3 Hz), 2.07 (br m, 1H), 2.01 (br t, 1H, *J* = 12.5 Hz), 1.94 (br t, 1H, *J* = 12.3 Hz), 1.81 (m, 1H), 1.70 (m 2H), 1.40 (m, 1H), 1.26 (m, 1H), 0.91 (d, 3H, *J* = 6.5 Hz), 0.89 (d, 3H, *J* = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0 (C), 139.7 (C), 129.4 (CH), 128.9 (CH), 128.6 (CH), 109.2 (CH_2), 97.6 (CH_2), 78.4 (CH), 78.0 (br, CH), 77.0 (CH), 75.3 (CH_2), 74.4 (CH_2), 74.2 (CH_2), 55.4 (CH_3), 42.3 (CH_2), 39.6 (CH_2), 38.3 (CH_2), 36.5 (CH_2), 35.5 (CH), 32.1 (CH), 17.1 (CH_3), 15.0 (CH_3); HR-ESI-FT-MS *m*/*z* 509.2273 $[M+Na]^+$ (calc. for $C_{24}H_{39}O_8PNa$ 509.2275).

(1*R*,5*S*,7*S*,10*S*,12*S*,13*S*,15*R*)-13-(*tert*-butyldimethylsilyloxy)-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-17-methylene-9-oxo-8,19-dioxabicyclo[13.3.1]nonadecan-5-yl acetate (4.81)



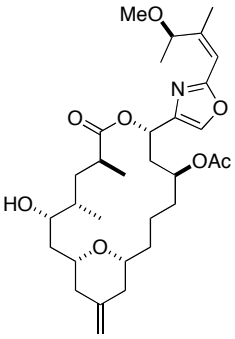
Zinc dust (571 mg, 8.73 mmol) was briefly flame dried under high vacuum in a 20 mL vial fitted with a septum cap. Upon cooling, the vial was flushed with N_2 and anhydrous THF was added (7.1 mL), followed by 1,2-dibromoethane (82 mg, 0.44 mmol) and TMSCl (9.5 mg, 0.087 mmol). After stirring 10 min, dibromomethane (494 mg, 2.84 mmol) was added. The mixture was cooled to −40 ˚C and $TiCl_4$ (385 mg, 2.03 mol) was added slowly, dropwise over 5-10 minutes. The thick, dark gray mixture was warmed to 0 ˚C over 45 minutes then stirred at that temperature for 2 days. After 20 hours a further 1 mL of anhydrous THF was added to assist with stirring.

An aliquot of the Lombardo reagent thus-obtained (106 µL, 0.027 mmol, 4 eq) was added to a mixture of ketone **4.80** (4.5 mg, 0.0066 mmol, azeotrope-dried 3 times from toluene) in CH_2Cl_2 (600 µL) at 0 ˚C. The mixture was warmed to room temperature and stirred for 30 minutes then diluted with Et_2O (2 mL) and quenched with saturated $NaHCO_3$ (2 mL). The resulting biphasic mixture was stirred vigorously for 5 minutes, then the layers were separated and the aqueous layer extracted with Et_2O (2 × 2 mL). Combined organic extracts were washed with brine (2 mL), dried (Na_2SO_4) and

concentrated under reduced pressure. Silica pencil column (1:9 EtOAc/hexanes) gave **4.81** (3.5 mg, 78%) as a clear, colorless oil.

$[\alpha]^{24}$ − 18.0 (*c* 0.88, $CHCl_3$); IR (neat) ν 2931, 2903, 2855, 1736, 1652, 1542, 1471, 1455, 1436, 1379, 1368, 1325, 1279, 1248, 1233, 1202, 1151, 1096, 1063, 1033, 1005, 968, 937, 896, 851, 836, 813, 773, 671, 656 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.38 (d, 1H, *J* = 0.5 Hz), 6.14 (br m, 1H), 5.95 (dd, 1H, *J* = 11.5, 2.5 Hz), 5.16 (q, 1H, *J* = 6.5 Hz), 4.93 (m, 1H), 4.67 (br m, 2H), 3.91 (dd, 1H, *J* = 11.0, 3.8 Hz), 3.26 (m, 1H), 3.20 (s, 3H), 3.09 (ddd, 1H, *J* = 11.0, 8.5, 2.5 Hz), 2.60 (m, 1H), 2.47 (dt, 1H, *J* = 13.1, 3.8 Hz), 2.09-2.05 (m, 2H), 2.02 (s, 3H), 1.99 (m, 1H), 1.91-1.82 (m, 2H), 1.86 (d, 3H, *J* = 1.5 Hz), 1.79 (tt, 1H, *J* = 13.0, 2.5 Hz), 1.66-1.56 (m, 3H), 1.52-1.37 (m, 5H), 1.32-1.23 (m, 2H), 1.26 (d, 3H, *J* = 6.5 Hz), 1.13 (d, 3H, *J* = 7.0 Hz), 0.92 (d, 3H, *J* = 6.5 Hz), 0.85 (s, 9H), 0.02 (s, 3H), −0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9 (C), 170.3 (C), 160.4 (C), 151.2 (C), 144.9 (C), 140.9 (C), 133.6 (CH), 113.1 (CH), 108.4 (CH_2), 75.7 (CH), 74.7 (CH), 74.1 (CH), 71.4 (CH), 70.8 (CH), 64.7 (CH), 56.5 (CH_3), 42.3 (CH_2), 41.4 (CH_2), 41.3 (CH_2), 38.8 (CH), 38.1 (CH_2), 38.0 (CH_2), 34.7 (CH_2), 34.1 (CH), 30.1 (CH_2), 25.8 (CH_3), 21.1 (CH_3), 20.2 (CH_2), 19.2 (CH_3), 18.1 (C), 17.8 (CH_3), 17.6 (CH_3), 13.7 (CH_3), −3.86 (CH_3), −4.86 (CH_3); HR-ESI-TOF-MS *m*/*z* 676.4238 $[M+H]^+$ (calc. for $C_{37}H_{61}NO_8Si$ 676.4239).

(1*R*,5*S*,7*S*,10*S*,12*S*,13*S*,15*R*)-13-hydroxy-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-17-methylene-9-oxo-8,19-dioxabicyclo[13.3.1]nonadecan-5-yl acetate (4.90)

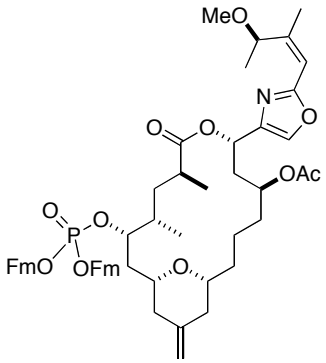


A mixture of 48%HF/MeCN/H_2O (5:86:9, 0.5 mL) was added to a solution of compound **4.81** (2.6 mg, 0.0039 mmol) in MeCN (150 µL) in a 0.25 oz polypropylene bottle. The mixture was stirred for 2 days, then made basic with saturated $NaHCO_3$ (pH ~ 8) and extracted with Et_2O (3 × 2 mL). Combined Et_2O extracts were washed with brine (~ 4 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica pencil column (1:4→3:7 EtOAc/hexanes) gave **4.90** (1.0 mg, 57 % BORSM) and recovered **4.81** (0.5 mg, 19 %). Two other runs delivered **4.90** in 75% and 86% yield (BORSM).

$[\alpha]^{24}$ − 17.6 (*c* 1.06, $CHCl_3$); IR (neat) ν 3462, 2929, 2853, 1733, 1653, 1542, 1455, 1438, 1369, 1325, 1281, 1244, 1202, 1150, 1112, 1094, 1086, 1053, 1025, 1008, 957, 925, 896, 859, 811, 779, 751, 669, 661 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.44 (s, 1H), 6.15 (s, 1H), 5.86 (dd, 1H, *J* = 11.5, 3.3 Hz), 5.14 (q, 1H, *J* = 6.5 Hz), 5.01 (tt, 1H, *J* = 10.3, 3.5 Hz), 4.67 (s, 2H), 3.81 (t, 1H, *J* = 6.5 Hz), 3.27 (m, 2H), 3.20 (s, 3H), 2.69 (m, 1H), 2.51 (ddd, 1H, *J* = 14.0, 12.0, 3.9 Hz), 2.12-2.10 (m, 2H), 2.01 (s, 3H), 1.98 (m, 1H), 1.94 (ddd, 1H, *J* = 14.0, 10.3, 3.4 Hz), 1.89-1.84 (m, 1H), 1.86 (d, 3H, *J* = 1.5 Hz), 1.81 (m, 1H), 1.77 (tt, 1H, *J* = 12.5, 3.4 Hz), 1.72-1.50 (m, 6H), 1.46-1.43 (m, 1H),

1.39-1.34 (m, 1H), 1.31-1.27 (m, 1H), 1.27 (d, 3H, *J* = 6.5 Hz), 1.22 (d, 3H, *J* = 6.5 Hz), 0.91 (d, 3H, 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8 (C), 170.3 (C), 160.4 (C), 151.2 (C), 144.7 (C), 140.4 (C), 134.2 (CH), 113.1 (CH), 108.5 (CH_2), 75.9 (CH), 74.7 (2 × CH), 71.2 (CH), 68.9 (CH), 65.4 (CH), 56.5 (CH_3), 41.8 (CH_2), 41.5 (CH_2), 41.4 (CH_2), 37.9 (CH), 37.7 (CH_2), 36.4 (CH_2), 34.9 (CH_2), 33.8 (CH), 30.5 (CH_2), 21.1 (CH_3), 20.7 (CH_2), 19.2 (CH_3), 17.6 (CH_3), 16.7 (CH_3), 13.3 (CH_3); HR-ESI-TOF-MS *m*/*z* 562.3375 $[M+H]^+$ (calc. for $C_{31}H_{47}NO_8$ 662.3374).

(1*R*,5*S*,7*S*,10*S*,12*S*,13*S*,15*R*)-13-(bis((9*H*-fluoren-9-yl)methoxy)phosphoryloxy)-7-(2-((*R*,*Z*)-3-methoxy-2-methylbut-1-enyl)oxazol-4-yl)-10,12-dimethyl-17-methylene-9-oxo-8,19-dioxabicyclo[13.3.1]nonadecan-5-yl acetate (4.91)



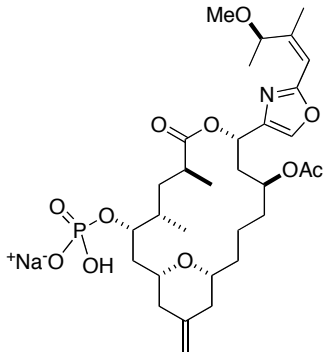
Tetrazole (2.06 mg, 0.029 mmol) was added as a solution in MeCN (87 µL) to compound **4.90** (1.1 mg, 0.002 mmol) in a conical-bottom vial fitted with a septum cap under N_2. *i*$Pr_2NP(OFm)_2$ (20.4 mg, 0.039 mmol) was added as a solution in CH_2Cl_2 (50 µL). The mixture was stirred vigorously, becoming cloudy. After 40 minutes, the mixture was cooled to 0 ˚C and 30% H_2O_2 (44 µL, 0.39 mmol) was added. After 10 minutes at 0 ˚C the reaction was quenched with saturated $NaHCO_3$ (1 ml) and partitioned between H_2O and CH_2Cl_2 (2 mL

each). Layers were separated and the aqueous layer extracted with CH_2Cl_2 (3 × 2mL). Combined CH_2Cl_2 extracts were washed with brine (2 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Silica pencil column (5→10% Et_2O/CH_2Cl_2) gave **4.91** (1.7 mg) which was further purified first by SiO_2 HPLC (Phenomenex Luna 3 μm SiO_2, 250 × 4.6 mm, 3:7 EtOAc/hexanes, 15 min then 3:7→6:4 EtOAc/hexanes linear gradient over 10 min, 1 mL/min, λ = 254 nm) then RP HPLC (Phenomenex Luna 3 μm C18, 250 × 4.6 mm, 2.5:97.5 H_2O/MeOH, 1 mL/min, λ = 254 nm) to give **4.91** (1.2 mg, 61%) as a slightly cloudy oil.

$[\alpha]^{24}$ −0.63 (*c* 0.64, $CHCl_3$); IR (neat) ν 3067, 3045, 3018, 2973, 2936, 2843, 2821, 1732, 1652, 1541, 1478, 1450, 1370, 1324, 1246, 1204, 1151, 1099, 1074, 1004, 991, 940, 911, 896, 880, 868, 842, 830, 819, 791, 780, 758, 741, 726, 713, 703, 675, 660 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.73-7.68 (m, 4H), 7.53-7.49 (m, 3H), 7.45 (dd, 1H, *J* = 7.5, 1.0 Hz), 7.39-7.36 (m, 3H), 7.34-7.31 (m, 2H), 7.28-7.25 (m, 2H), 7.24-7.20 (m, 2H), 6.13 (br s, 1H), 5.97 (dd, 1H, *J* = 12.5, 2.3 Hz), 5.15 (q, 1H, *J* = 6.5 Hz), 4.87 (m, 1H), 4.70 (dd, 2H, *J* = 7.0, 1.5 Hz), 4.61 (m, 1H), 4.28-4.14 (m, 4H), 4.11 (q, 2H, *J* = 6.3 Hz), 3.26 (m, 1H), 3.20 (s, 3H), 3.05 (m, 1H), 2.72 (m, 1H), 2.40 (dt, 1H, *J* = 13.0, 3.7 Hz), 2.08 (d, 1H, *J* = 13.5 Hz), 2.02 (m, 1H), 2.02 (s, 3H), 1.93-1.87 (m, 3H), 1.86 (d, 3H, *J* = 1.5 Hz), 1.84 (m, 1H), 1.77 (m, 1H), 1.74 (dd, 1H, *J* = 14.0, 4.8 Hz), 1.65-1.55 (m, 4H), 1.43-1.38 (m, 2H), 1.32-1.25 (m, 2H), 1.26 (d, 3H, *J* = 6.5 Hz), 1.00 (d, 3H, *J* = 7.0 Hz), 0.93 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (125 MHz,

$CDCl_3$) δ 174.3 (C), 170.4 (C), 160.4 (C), 151.3 (C), 144.3 (C), 143.12 (C), 143.09 (C), 143.06 (C), 143.05 (C), 141.34 (2 × C), 141.31 (C), 140.9 (C), 133.6 (C), 127.90 (CH), 127.88 (CH), 127.87 (CH), 127.85 (CH), 127.2 (3 × CH), 127.13 (CH), 127.08 (CH), 125.2 (CH), 125.0 (2 × CH), 124.9 (CH), 120.09 (CH), 120.03 (CH), 120.02 (CH), 119.99 (CH), 113.1 (CH), 108.7 (CH_2), 78.5 (CH, d, 6.88 Hz), 74.9 (CH), 74.7 (CH), 74.1 (CH), 71.2 (CH), 69.2 (CH_2,d, 6.0 Hz), 68.9 (CH_2, d, 6.0 Hz), 64.5 (CH), 56.5 (CH_3), 47.93 (CH, d, 7.88 Hz), 47.90 (CH, d, 8.25 Hz), 41.7 (CH_2), 41.2 (CH_2), 38.7 (CH_2), 38.2 (CH_2), 38.1 (CH), 37.8 (CH_2), 34.6 (CH_2), 33.51 (CH, d, 6.50 Hz), 30.1 (CH_2), 21.1 (CH_3), 20.3 (CH_2), 19.2 (CH_3), 17.6 (CH_3), 13.7 (CH_3); HR-ESI-TOF-MS *m*/*z* 998.4607 $[M+H]^+$ (calc. for $C_{59}H_{68}NO_{11}P$ 998.4603).

Enigmazole A (4.1)



K_2CO_3 (1.94 mg, 0.014 mmol) was added as a solution in H_2O (19.4 µL) to a mixture of **4.91** (0.7 mg, 0.0007 mmol) in MeOH (200 µL). The mixture was stirred for 23 hours, at which time C18 TLC (1:4 H_2O/MeOH) indicated a single product spot. The mixture was diluted with H_2O (0.4 mL) and loaded onto a C18 sep pak (1000 mg Strata 55 µm, 70 Å, C18). Excess K_2CO_3 was eluted with H_2O, then enigmazole A was eluted with 2:3 H_2O/MeOH giving **1** (0.44 mg, 98 %) as it's potassium salt **4.92**. Final purification was achieved by

RP HPLC (Phenomenex Luna 3 μm C18, 250 × 4.6 mm, 28→53% MeCN in 100 mM $NaClO_4$ linear gradient over 30 min, then 100% MeCN, 15 min, 0.75 mL/min, λ = 254 nm) to give enigmazole A sodium salt (**4.1**) as a colorless film.

IR (neat) ν 3300 (br), 2988, 2949, 2838, 1651 (br), 1449, 1407, 1112, 1013, 660 (br) cm^{-1}; HR-ESI-TOF-MS *m*/*z* 600.2935 $[M+H]^+$ (calc. for $C_{29}H_{47}NO_{10}P$ 600.2938).

Table 4.4 ^{1}H and ^{13}C NMR data for natural and synthetic enigmazole A.

Position	δ ^{1}H Natural[a]	δ ^{13}C Natural[a]	δ ^{1}H Synthetic[b]	δ ^{13}C Synthetic[c]
1		176.4		176.5
2	2.98	39.3	2.99, m	39.7
2-CH_3	1.10, d, 6.4 Hz	18.0	1.11, d, 6.5 Hz	18.3
3	1.38, t, 10.8 Hz	39.0	1.39, m	39.3
	1.88 m		1.89, m	
4	1.62	36.5	1.63	34.7, d, 6.1 Hz
4-CH_3	0.97, d, 6.4 Hz	14.6	0.98, d, 6.5 Hz	15.0
5	4.42, m	75.0	4.43, m	75.2, d, 6.1 Hz
6	1.87, m	39.9	1.88, m	40.1
	2.10, m		2.11, m	
7	3.12, dd, 10.3, 9.8 Hz	77.3	3.13, m	77.6
8	1.97, dd, 12.3, 12.8 Hz	42.8	1.98, t, 12.3 Hz	43.0
	2.21, d, 12.8 Hz		2.23, d, 13.0 Hz	
9		146.4		146.6
9-CH_2	4.69, d, 1.5 Hz	108.4	4.70, br s	108.6
	4.70, d, 1.5 Hz		4.71, br s	
10	1.84	42.2	1.86	42.5
	2.13, d, 12.8 Hz		2.14, d, 14.0 Hz	
11	3.29	75.3	3.30	75.7
12	1.37, t, 11.3 Hz	35.9	1.38, m	36.2
	1.64		1.66	
13	1.54, q, 12.4 Hz	21.4	1.55, q, 12.5 Hz	21.8
	1.72		1.73	
14	1.02, dt, 3.4, 12.0 Hz	33.3	1.04, dt, 12.0, 3.2	33.6
	1.76		Hz	
			1.79	
15	3.62, dt, 11.1, 4.3 Hz	69.4	3.63, m	69.8
16	1.77	42.4	1.78	42.7
	2.50, dt, 13.2, 3.4 Hz		2.51, dt, 13.3, 3.8	
			Hz	
17	5.95, dd, 12.8, 2.5 Hz	65.3	5.96, dd, 12.5, 2.5	65.6
			Hz	
18		142.1		142.4
19	7.68, s	135.7	7.69, s	135.9
20		161.7		161.9
21	6.21, s	113.1	6.22, br s	114.0
22		152.4		152.7
22-CH_3	1.89, s	17.3	1.89, d, 1.5 Hz	17.7
23	5.24, q, 6.5	75.9	5.25, q, 6.3 Hz	76.2
23-OCH_3	3.20, s	56.4	3.21, s	56.8
24	1.26, d, 6.4 Hz	19.1	1.27, d, 6.5 Hz	19.4

[a] ^{1}H and ^{13}C NMR data (CD_3OD) as reported by N. Oku *et al.*[69] [b] 500 MHz, CD_3OD. [c] 125 MHz, CD_3OD.

Table 4.5 ^{13}C NMR data for natural and synthetic enigmazole A.

Position	δ ^{13}C Natural[a]	δ ^{13}C Synthetic[b]
1	176.4	176.5
2	39.6	39.7
2-CH_3	18.2	18.3
3	39.3	39.3
4	34.7	34.7, d, 6.1 Hz
4-CH_3	14.7	15.0
5	77.0	75.2, d, 6.1 Hz
6	40.1	40.1
7	77.1	77.6
8	43.0	43.0
9	146.3	146.6
9-CH_2	108.8	108.6
10	42.4	42.5
11	75.8	75.7
12	36.2	36.2
13	21.8	21.8
14	33.6	33.6
15	69.8	69.8
16	42.6	42.7
17	65.9	65.6
18	142.3	142.4
19	136.0	135.9
20	N/O	161.9
21	113.9	114.0
22	152.7	152.7
22-CH_3	17.7	17.7
23	76.2	76.2
23-OCH_3	56.8	56.8
24	19.4	19.4

[a] Raw ^{13}C NMR data (CD_3OD) provided by Dr. Kirk Gustafson as an FID file and processed using MestreNova. [b] 125 MHz, CD_3OD.

References

(1) Hanahan, D.; Weinberg, R. A. *Cell* **2005**, *100*, 50-70. b) Blume-Jensen, P.; Hunter, T. *Nature* **2001**, *411*, 355-365.

(2) Capdeville, R.; Buchdunger, E.; Zimmermann, J.; Matter, A. *Nat. Rev. Drug Discovery* **2002**, *1*, 493-502. b) Müller, B. A. *Curr. Pharm. Des.* **2009**, *15*, 120-133.

(3) Roskoski, R. Jr. *Biochem. Biophys. Res. Commun.* **2005**, *338*, 1307-1315.

(4) Ronnstrand, L. *Cell Mol. Life Sci.* **2004**, *61*, 2535-2548.

(5) Sattler, M.; Salgia, R. *Leuk. Res.* **2004**, *28 (Suppl. 1)*, 11-20.

(6) Lennartsson, J.; Jelacic, T.; Linnekin, D.; Shivakrupa, R. *Stem Cells* **2005**, *23*, 16-43.

(7) Taylor, M. L.; Metcalfe, D. D. *Hematol. Oncol. Clin. North Am.* **2000**, *14*, 517-535.

(8) Tibes, R.; Trent, J.; Kurzrock, R. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 357-384.

(9) Gibson, P.; Cooper, K. *Adv. Anat. Pathol.* **2002**, *9*, 65-69.

(10) Yarden, Y.; Kuang, W. J.; Yang-Feng, T.; Coussens, L.; Munemitsu, S.; Dull, T. J.; Chen, E.; Schlessinger, J.; Francke, U.; Ullrich, A. *EMBO J* **1987**, *6*, 3341-3351.

(11) Qui, F.; Brown, K.; Barker, P.; Jhanwar, S.; Ruddle, F.; Besmer, P. *EMBO J* **1988**, *7*, 1003-1011.

(12) Mol, C. D.; Dougan, D. R.; Schneider, T. R.; Skene, R. J.; Kraus, M. L.; Scheibe, D. N.; Snell, G. P.; Zou, H.; Sang, B. –C.; Wilson, K. P. *J. Biol. Chem.* **2004**, *279*, 31655-31663.

(13) Henrich, C. J.; Goncharova, E. I.; Wilson, J. A.; Gardella, R. S.; Johnson, T. R.; McMahon, J. B.; Takada, K.; Bokesch, H. R.; Gustafson, K. R. *Chem. Biol. Drug. Des.* **2007**, *69*, 321-330.

(14) http://www.gleevec.com

(15) Heinrich, M. C.; Blanke, C. D.; Druker, B. J.; Corless, C. L. *J. Clin. Oncol.* **2002**, *20*, 1692-1703.

(16) a) Boyar, M. S.; Taub, R. N. *Cancer Investigation* **2007**, *25*, 328-335. b) Heinrich, M. C.; Corless, C. L.; Blanke, C. D.; Demetri, G. D.; Joensuu, H.; Roberts, P. J.; Eisenberg, B. L.; von Mehren, M.; Fletcher, C. D. M.; Sandau, K.; McDougell, K.; Ou, W. –B.; Chen, C. –J.; Fletcher, J. A. *J. Clin. Oncol.* **2006**, *24*, 4764-4774.; c) Antonescu, C. R.; Besmer, P.; Guo, T.; Arkun, K.; Hom, G.; Korytowski, B.; Leversha, M. A.; Jeffrey, P. D.; Desantis, D.; Singer, S.; Brennan, M. F.; Maki, R. G.; DeMatteo, R. P.; *Clin. Cancer Res.* **2005**, *11*, 4182-4190. d) Debiec-Rychter, M.; Cools, J.; Dumez, H.; Sciot, R.; Stul, M.; Mentens, N.; Vrankx, H.; Wasag, B.; Prenen, H.; Roesel, J.; Hagemeijer, A.; Van Oosterom, A.; Marynen, P. *Gastroenterology* **2005**, *128*, 270-279 and references therein.

(17) Takada, K *47th Annual Meeting of the American Society of Pharmacognosy* **2006**, Arlington, VA.

(18) Yeh, V. S. C. *Tetrahedron* **2004**, *60*, 11995-21042.

(19) Forsyth, C. J.; Ahmed, F.; Cink, R. D.; Lee, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 5597-5598.

(20) a) Bull, J. A.; Balskus, E. P.; Horan, R. A. J.; Langner, M.; Ley, S. V. *Angew. Chem. Int. Ed.* **2006**, *45*, 6714-6718. b) Bull, J. A.; Balskus, E. P.; Horan, R. A. J.; Langner, M.; Ley, S. V. *Chem. Eur. J.* **2007**, *13*, 5515-5538.

(21) Negishi, E.; Van Horn, D. E.; King, A. O.; Okukado, N. *Synthesis*, **1979**, 501-502.

(22) a) Schröder, R.; Schöllkopf, U.; Blume, E.; Hoppe, I. *Justus Liebigs Ann. Chem.* **1975**, 533-546. b) Jacobi, P. A.; Ueng, S.; Carr, D. *J. Org. Chem.* **1979**, *44*, 2042-2044. c) Hodges, J. C.; Patt, W. C.; Connolly, C. J. *J. Org. Chem.* **1991**, *56*, 449-452.

(23) Crowe, E.; Hossner, F.; Hughes, M. J. *Tetrahedron* **1995**, *51*, 8889-8900.

(24) Harn, N. K.; Gramer, C. J.; Anderson, B. A. *Tetrahedron Lett.* **1995**, *36*, 9453-9456.

(25) Anderson, B. A.; Harn, N. K. *Synthesis* **1996**, 583-585.

(26) Reeder, M. R.; Gleaves, H. E.; Hoover, S. A.; Imbordino, R. J.; Pangborn, J. J. *Org. Proc. Res. Dev.* **2003**, *7*, 696-699.

(27) L'Helgoual'ch, J. –M.; Seggio, A.; Chevallier, F.; Yonehara, M.; Jeanneau, E.; Uchiyama, M.; Mongin, F. *J. Org. Chem.* **2008**, *73*, 177-183.

(28) Crank, G.; Foulis, M. J. *J. Med. Chem.* **1971**, *14*, 1075.

(29) Hodgetts, K. J.; Kershaw, M. T.; *Org. Lett.* **2002**, *4*, 2905-2907.

(30) Krasnokutskaya, E. A.; Semenischeva, N. I.; Filimonov, V. D.; Knochel, P. *Synthesis*, **2007**, *1*, 81-84.

(31) Ethyl 2-iodooxazole-4-carboxylate (**4.13c**) has appeared only once before in the chemical literature. Its preparation was detailed in a Patent application lodged by a medicinal chemistry group at Bayer Healthcare.

Nell, P.; Hübsch, W.; Albrecht-Küpper, B.; Keldenich, J.; Vakalopoulis, A.; Süss-Meier, F.; Zimmermann, K.; Lang, D.; Meibom, D. Substituted Aryloxazoles and the Use Thereof. International Patent WO 2009/015776 A1, February 5, 2009.

(32) Krasovskiy, A.; Malakhov, V.; Gavryushin, A.; Knochel, P. *Angew. Chem. Int. Ed.* **2006**, *45*, 6040-6044.

(33) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C. –P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925-7926. See also the following review and references therein: Corey, E. J.; Helal, C. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1986-2012.

(34) Luche, J. L.; Gemal, A. L. *J. Am. Chem. Soc.* **1979**, *101*, 5848-5849.

(35) a) Duboudin, J. G.; Jousseaume, B.; Bonakdar, A. *J. Organomet. Chem.* **1979**, *168*, 227-232; b) Wiemer, D. F.; Han, Q. *J. Am. Chem. Soc.* **1992**, *114*, 7692-7697; c) Larock, R. C.; Doty, M. J.; Han, X. *J. Org. Chem.* **1999**, *64*, 8770-8779; d) Onyango, E. O.; Tsurumoto, J.; Imai, N.; Takahashi, K.; Ishihara, J.; Hatakeyama, S. *Angew. Chem. Int. Ed.* **2007**, *46*, 6703-6705.

(36) a) Nugent, W. A. *J. Chem Soc., Chem. Commun.* **1997**, 1369-1370; b) Chen, Y. K.; Jeon, S. –J.; Walsh, P. J.; Nugent, W. A. *Org. Synth.* **2005**, *82*, 87; c) Kim, H. Y.; Lurain, A. E.; Gacia-Garcia, P.; Carroll, P. J.; Walsh, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 13138-13139.

(37) Ohtani, I.; Kusuki, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**,*113*, 4092-4096.

(38) a) Negishi, E.; Van Horn, D. E.; King, A. O.; Okukado, N. *Synthesis*, **1979**, 501-502; b) Rand, C. L.; Van Horn, D. E.; Moore, M. W.; Negishi, E. *J. Org. Chem.* **1981**, *46*, 4096-4097; c) Negishi, E.; Van Horn, D. E.; Yoshida, T. *J. Am. Chem. Soc.* **1985**, *107*, 6639-6647; d) Alvarez, R.; Herrero, M.; López, S.; de Lera, A. R. *Tetrahedron* **1998**, *54*, 6793-6810.

(39) a) Ford-Moore, A. H. *Org. Synth.* **1950**, *30*, 10; b) Guo, Q.; Miyaji, T.; Hara, R.; Shen, B.; Takahashi, T. *Tetrahedron* **2002**, *58*, 7327-7334.

(40) Trost, B. M.; Grese, T. A.; Chan, D. M. T. *J. Am. Chem. Soc.* **1991**, *113*, 7350-7362.

(41) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Proc. Natl. Acad. Sci.* **2004**, *101*, 12058-12063.

(42) Corey, E. J.; Imwinkelried, R.; Pikul, S.; Xiang, Y. B. *J. Am. Chem. Soc.* **1989**, *111*, 5493-5494.

(43) a) Catalyst development: Williams, D. R.; Brooks, D. A.; Meyer, K. G.; Clark, M. P. *Tetrahedron Lett.* **1998**, *39*, 7251-7254; b) Williams, D. R.; Meyer, K. G.; Shamim, K.; Patnaik, S. *Can. J. Chem.* **2005**, *82*, 120-130. Phorboxazole A: a) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Proc. Natl. Acad. Sci.* **2004**, *101*, 12058-12063. b) Williams, D. R.; Kiryanov, A. A.; Emde, U.; Clark, M. P.; Berliner, M. A.; Reeves, J. T. *Angew. Chem. Int. Ed.* **2003**, *42*, 1258-1262. c) Williams, D. R.; Clark, M. P.; Emde, U.; Berliner, M. A. *Org. Lett.* **2000**, *2*, 3023-3026. Amphidinolide K: Williams, D. R.; Meyer, K. G. *J. Am. Chem. Soc.* **2001**, *123*, 765-766. Hennoxazole: Williams, D. R.; Brooks, D. A.; Berliner, M. A. *J. Am. Chem. Soc.* **1999**, *121*, 4924-4925. Leucascandrolide: a) Williams, D. R.; Plummer, S. V.; Patnaik, S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3934-3938. b) Williams, D. R.; Patnaik, S.; Plummer, S. V. *Org. Lett.* **2003**, *5*, 5035-5038.

(44) Chen, K. -M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155-158.

(45) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511-3515.

(46) For another example of Li_2CO_3 in formation of a phosphonium salt see: Smith, A. B. III; Zhu, W.; Shirakami, S.; Sfouggatakis, C.; Doughty, V. A.; Bennett, C. S.; Sakamoto, Y. *Org. Lett.* **2003**, *5*, 761-764.

(47) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V. Jr.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. *J. Am. Chem. Soc.* **1957**, *79*, 6062-6070.

(48) Allinger, N. L. *J. Am. Chem. Soc.* **1959**, *81*, 232-235.

(49) Gruenfeld, N.; Stanton, J. L.; Yuan, A. M.; Ebetino, F. H.; Browne, L. J.; Gude, C.; Huebner, C. F. *J. Med. Chem.* **1983**, *26,* 1277-1282.

(50) Hoffman, R. W.; Ditrich, K.; Koster, G.; Sturmer, R. *Chem. Ber.* **1989**, *122,* 1783-1789.

(51) Lautens, M.; Colucci, J. T.; Hiebert, S.; Smith, N. D.; Bouchain, G. *Org. Lett.* **2002**, *4,* 1879-1882.

(52) Leopold, E. J. *Org. Synth.* **1986**, *64,* 164-171.

(53) Sakurai, H.; Hosomi, A.; Hayashi, J. *Org. Synth.* **1984**, *62*, 86

(54) Hiyama, T.; Kimura, K.; Nozaki, H. *Tetrahedron Lett.* **1981**, *22*, 1037-1040.

(55) Hirayama, L. C.; Gamsey, S.; Knueppel, D.; Steiner, D.; DeLaTorre, K.; Singaram, B. *Tetrahedron Lett.* **2005**, *46*, 2315-2318.

(56) Racherla, U. S.; Brown, H. C. *J. Org. Chem.* **1991**, *56*, 401-404.

(57) a) Roush, W. R.; Walts, A. E.; Hoong, L. K. *J. Am. Chem. Soc.* **1985**, *107*, 8186-8190; b) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348-6359.

(58) Osborn, J. A.; Jardine, F. H.; Young, J. F.; Wilkinson, G. *J. Chem. Soc. (A)* **1966**, 1711-1732.

(59) Jourdant, A.; Gonzlez-Zamora, E.; Zhu, J. *J. Org. Chem.* **2002**, *67*, 3163-3164.

(60) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989

(61) a) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *J. Org. Chem.* **1990**, *55*, 7-9; b) Tone, H; Nishi, T.; Oikawa, Y.; Hikota, M. Yonemitsu, O. *Tetrahedron Lett.* **1987**, *28*, 4569-4572; c) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367-6370

(62) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822-1830.

(63) Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.***1976**, 49.

(64) Boden, E.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394-2395.

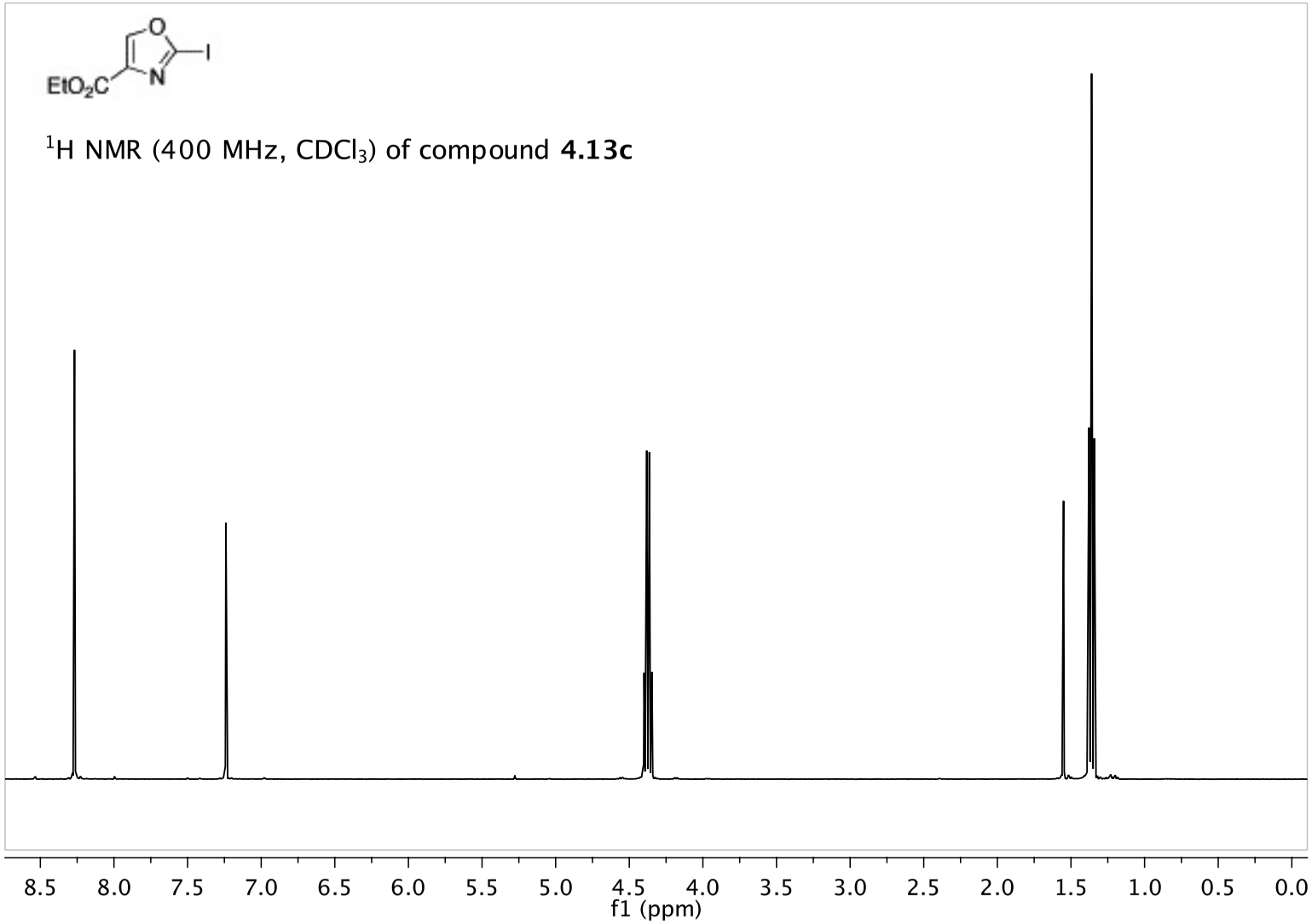
(65) Parenty, A.; Moreau, X.; Campagne, J. –M. *Chem. Rev.* **2006**, *106*, 911-939.

(66) a) Paterson, I.; Watson, C.; Yeung, K. –S. Ward, R. A.; Wallace, P. A. *Tetrahedron* **1998**, *54*, 11955-11970. b) Paterson, I.; Woodrow, M. D.; Cowden, C. J. *Tetrahedron Lett.* **1998**, *39*, 6041-6044.

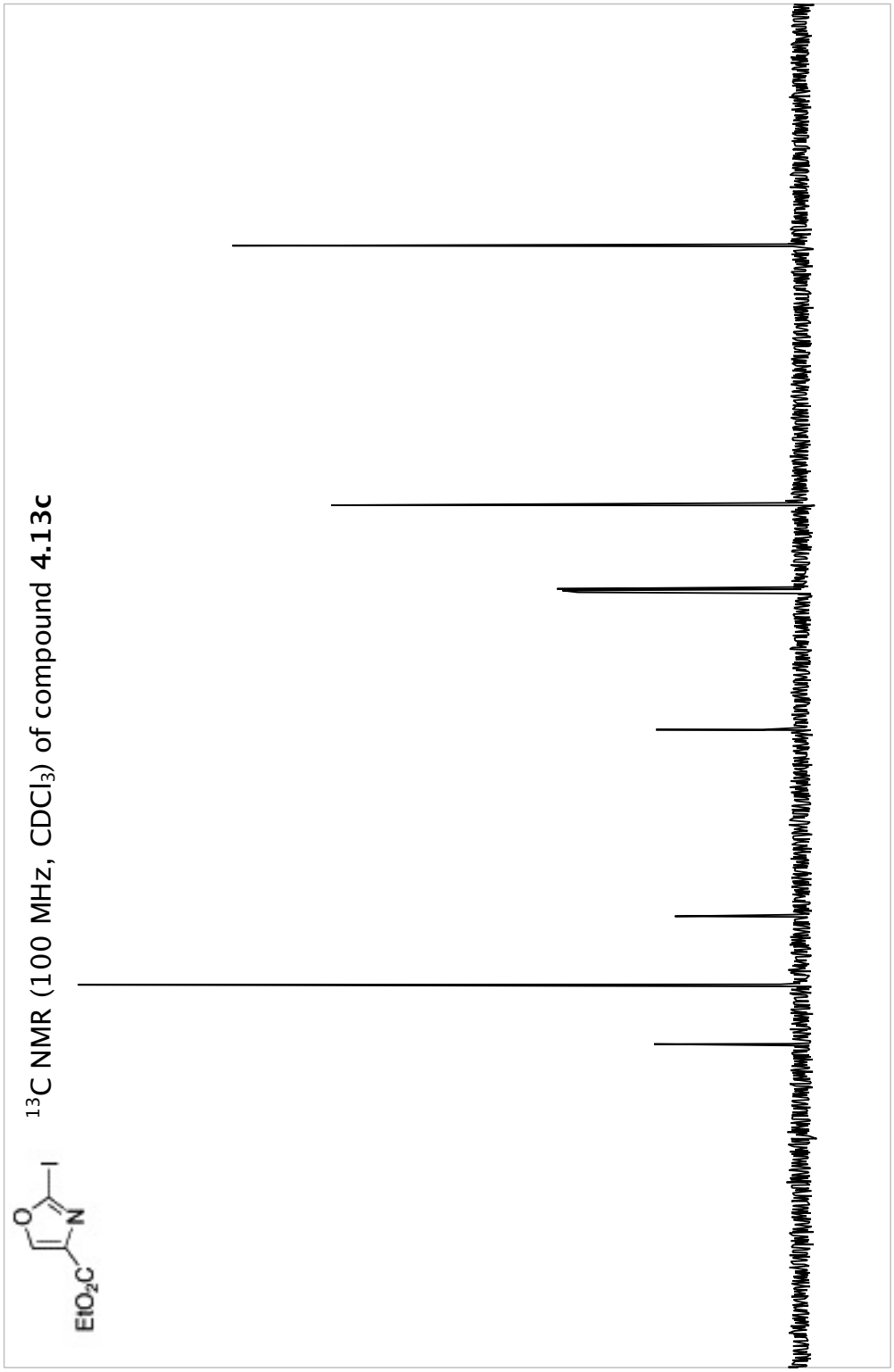
(67) Lombardo, L. *Org. Synth.* **1987**, *65*, 81-85.

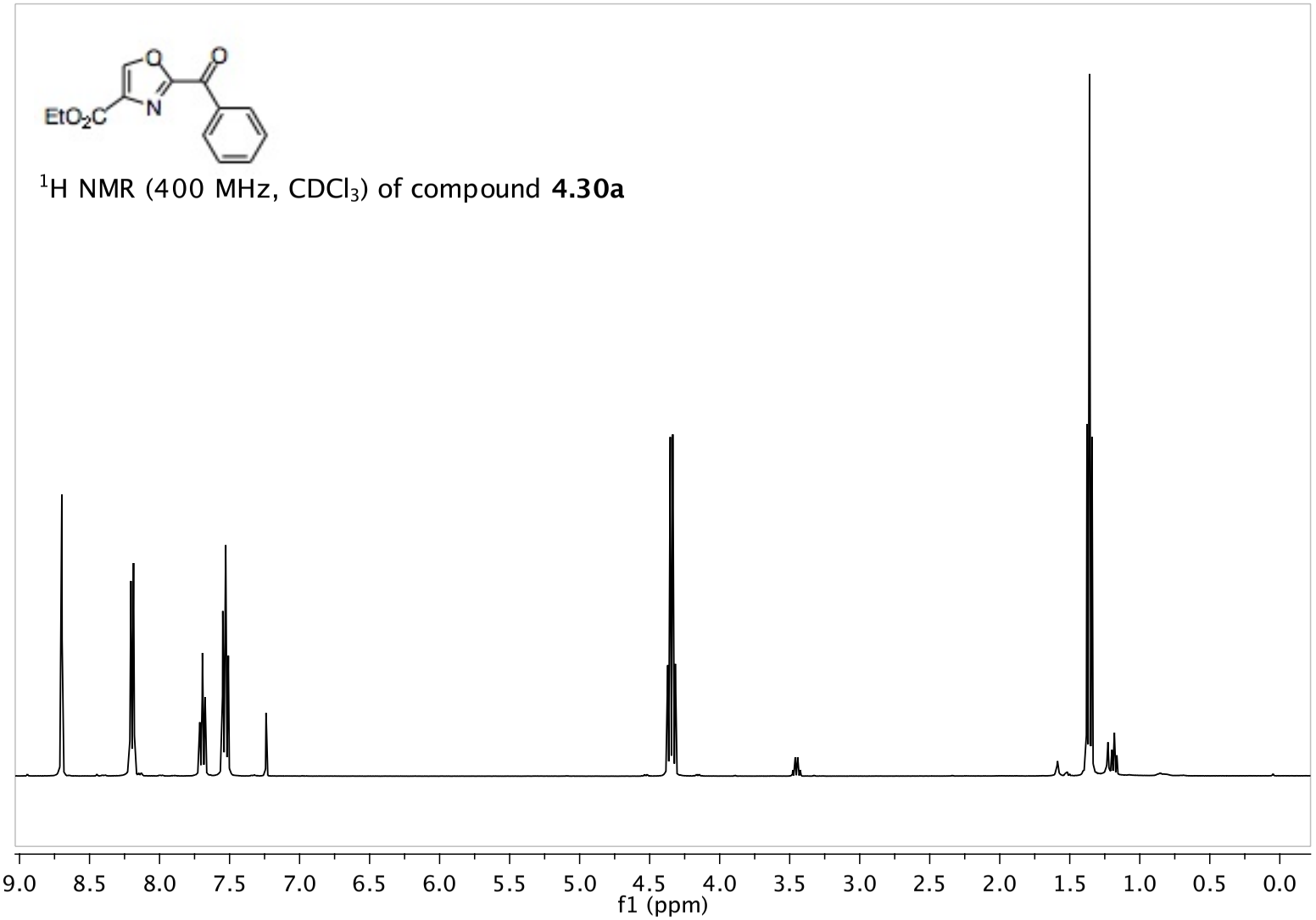
(68) a) Watanabe, Y.; Nakamura, T.; Mitsumoto, H. *Tetrahedron Lett.* **1997**, *38*, 7407-7410; b) Bialy, L.; Waldmann, H. *Chem. Eur. J.* **2004**, *10*, 2759-2780; c) Lawhorn, B. G.; Boga, S. B.; Wolkenberg, S. E.; Colby, D. A.; Gauss, C. –M.; Swingle, M. R.; Amable, L.; Honkanen, R. E.; Boger, D. L. *J. Am. Chem. Soc.* **2006**, *128*, 16720-16732.

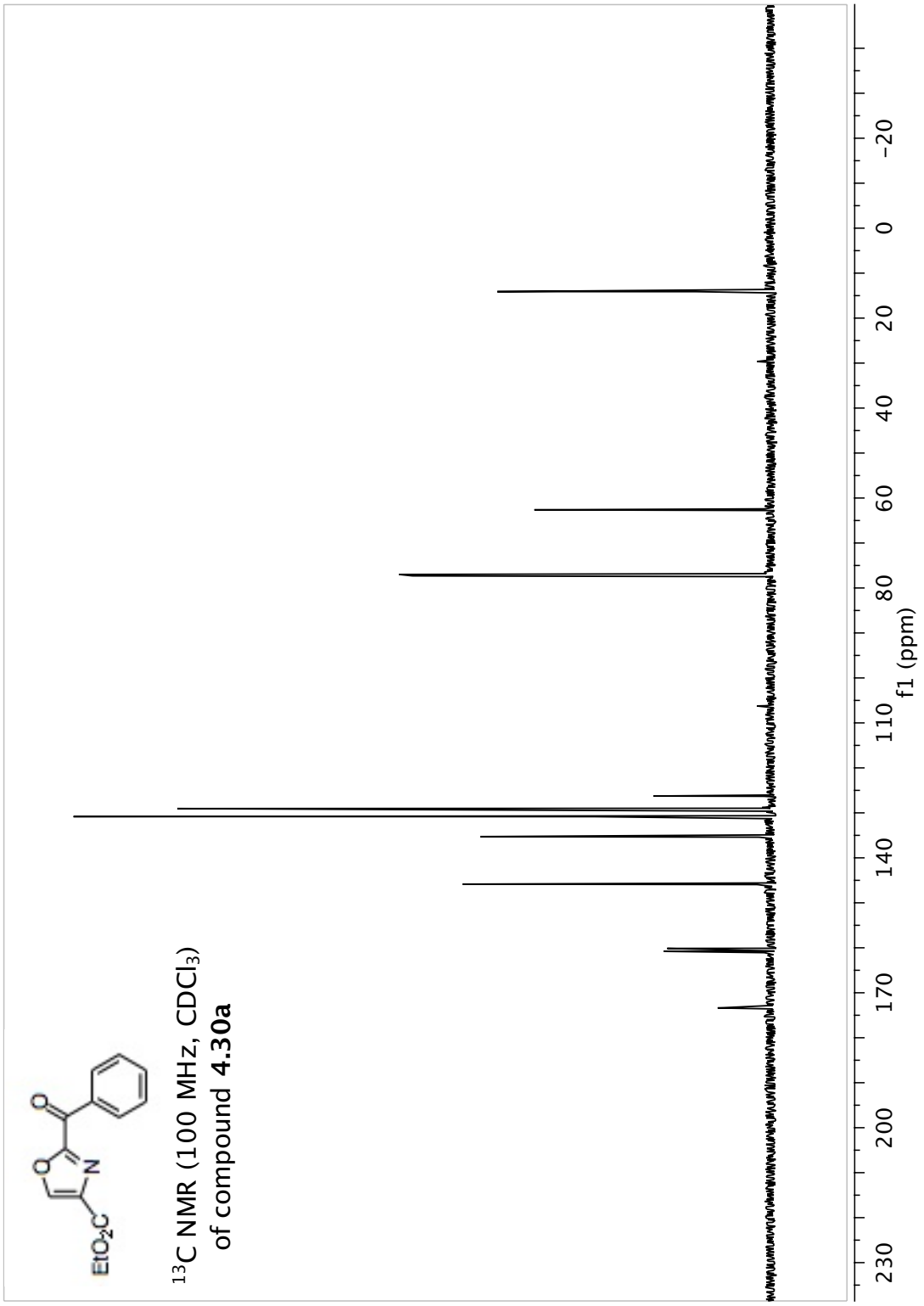
(69) Oku, N.; Gustafson, K. R.; Fuller, R. W.; Wilson, J. A.; Pannell, L. K.; McMahon, J. B. *International Symposium on the Chemistry of Natural Products* **2006**, Japan. (http://ci.nii.ac.jp/naid/110006682633/en)

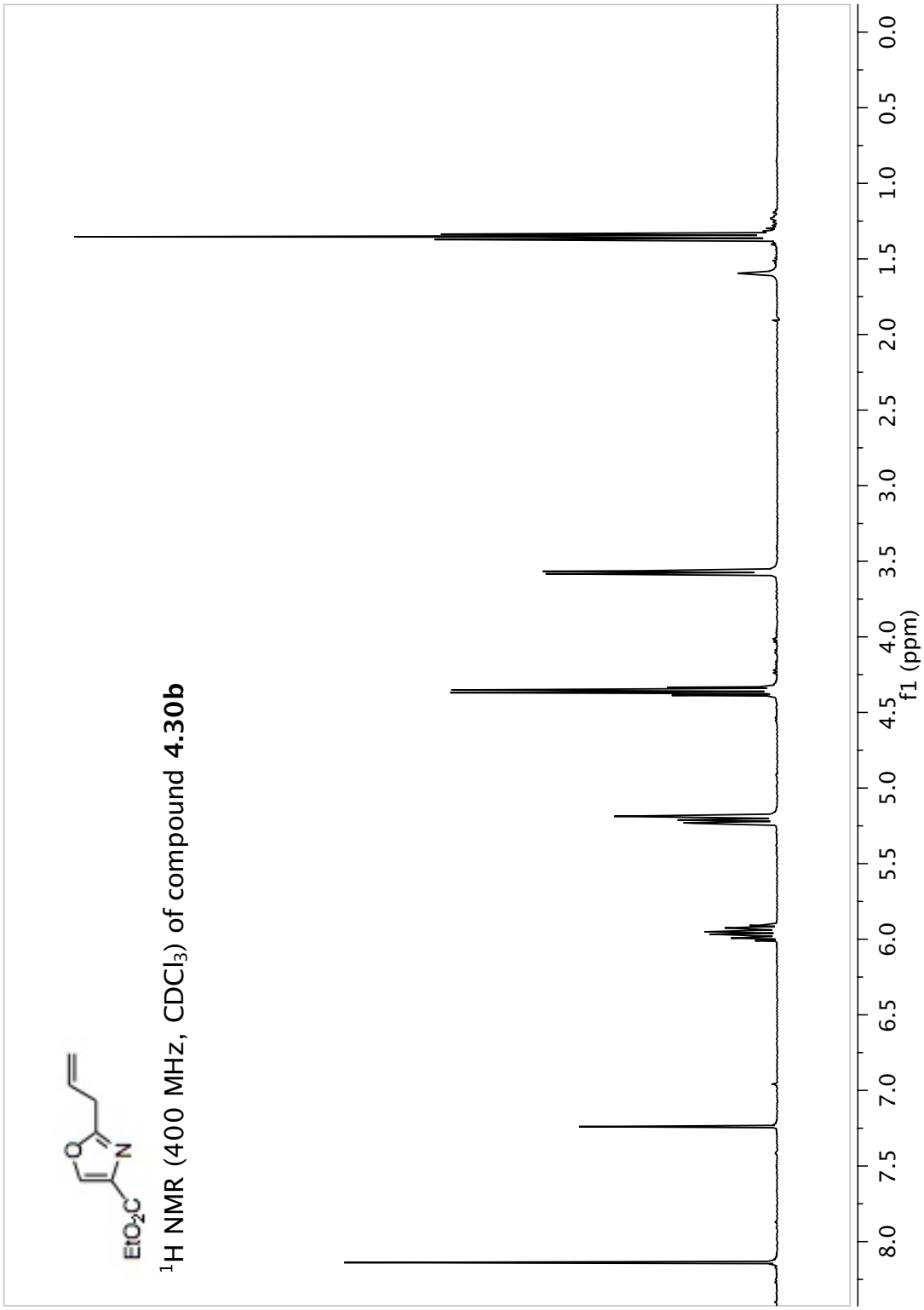


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.13c**

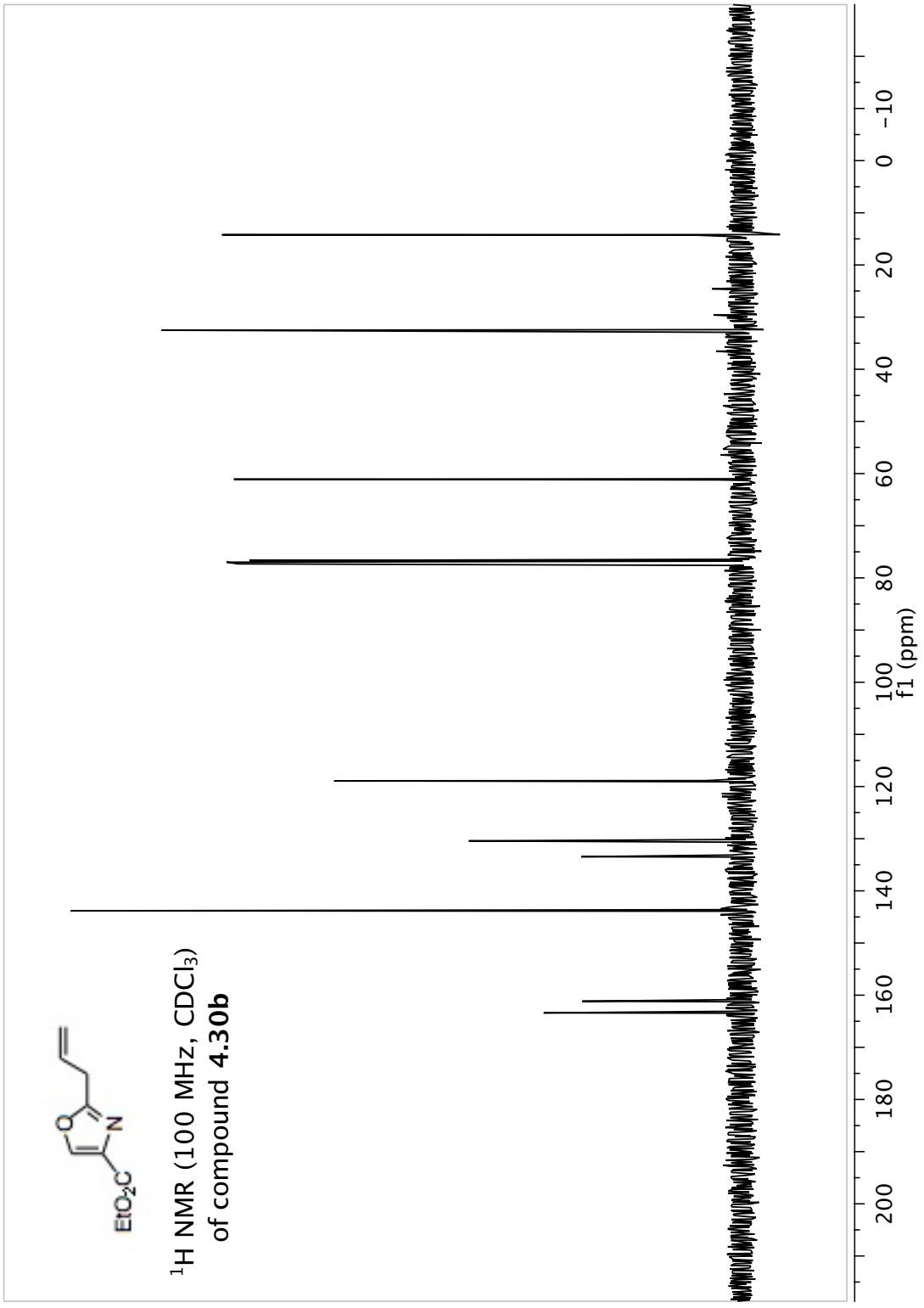

^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.13c**
EtO2C
I
O
N
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)


EtO2C
O
N
O
1H NMR (400 MHz, CDCl3) of compound 4.30a
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

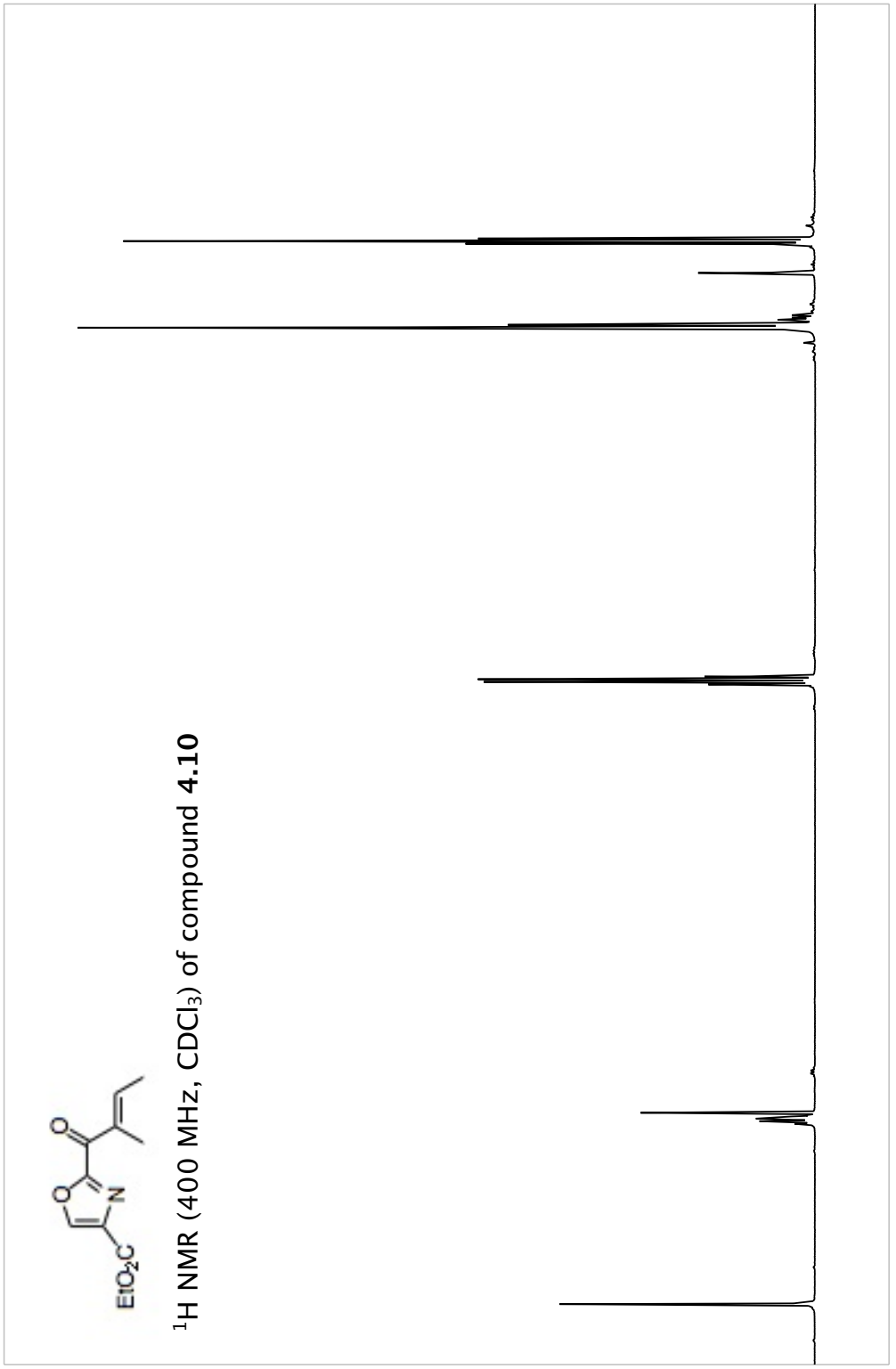

^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.30a**
EtO_2C
O
O
N
230
200
170
140
110
80
60
40
20
0
−20
f1 (ppm)



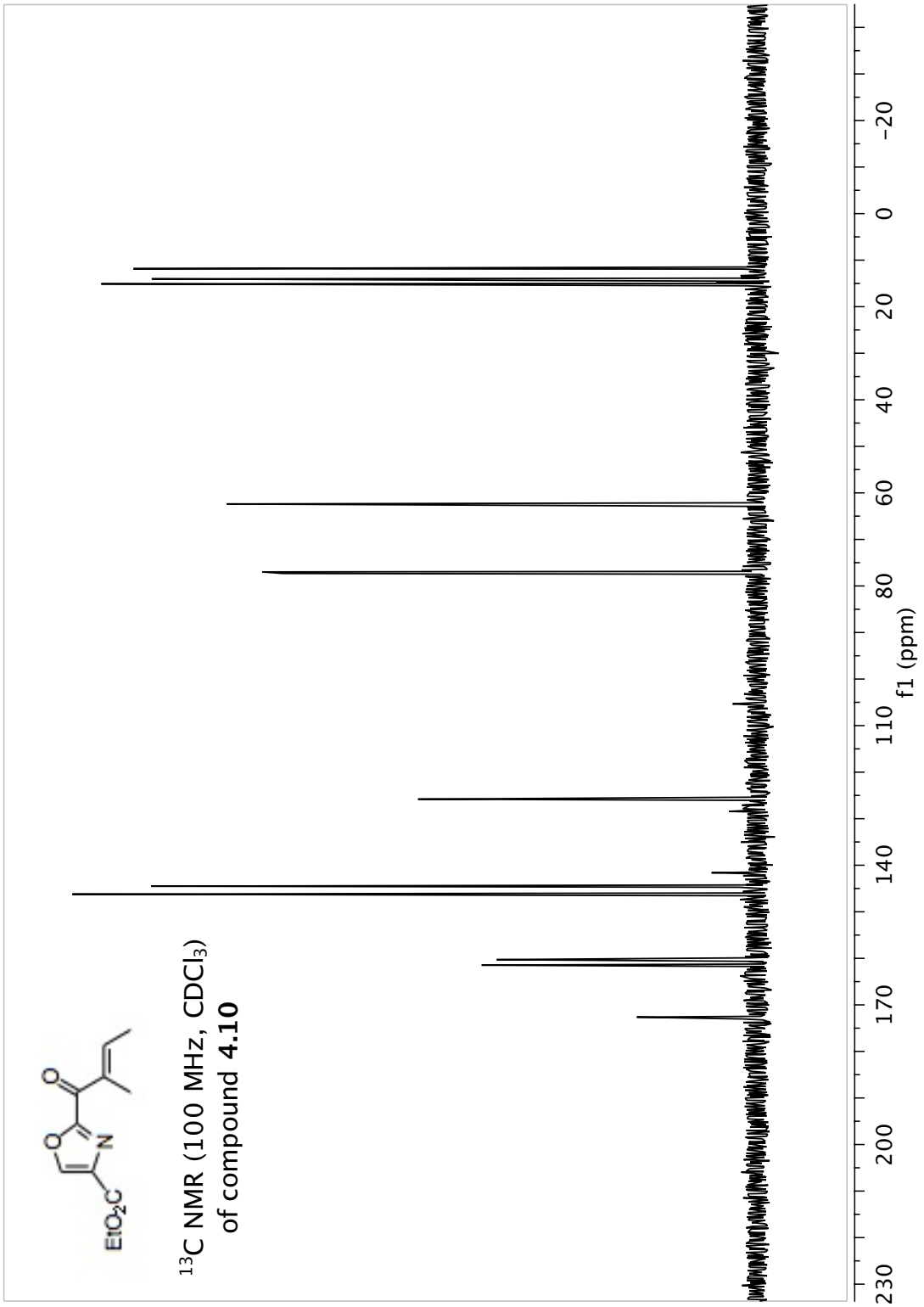
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.30b**

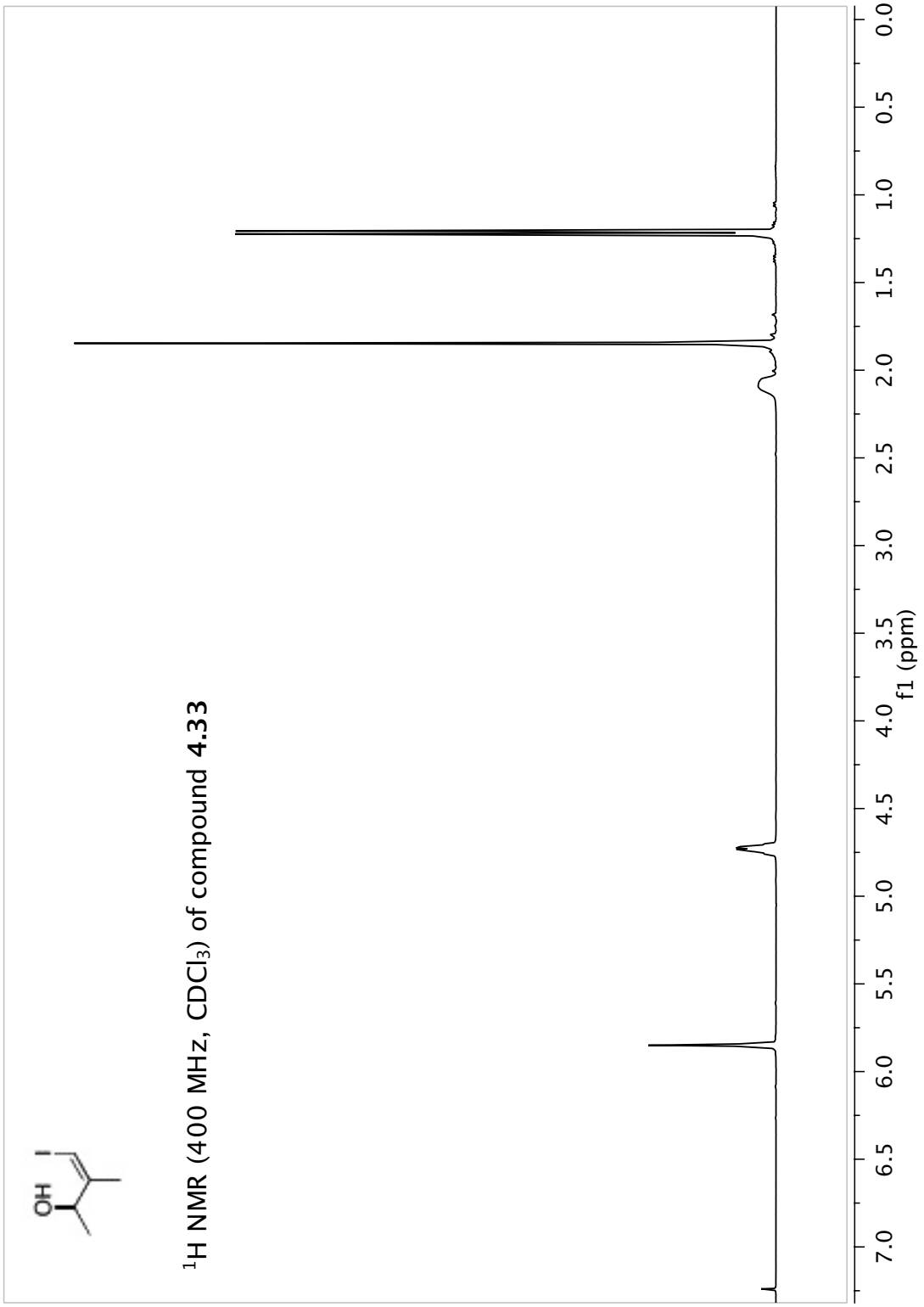


^{1}H NMR (100 MHz, $CDCl_3$)
of compound **4.30b**

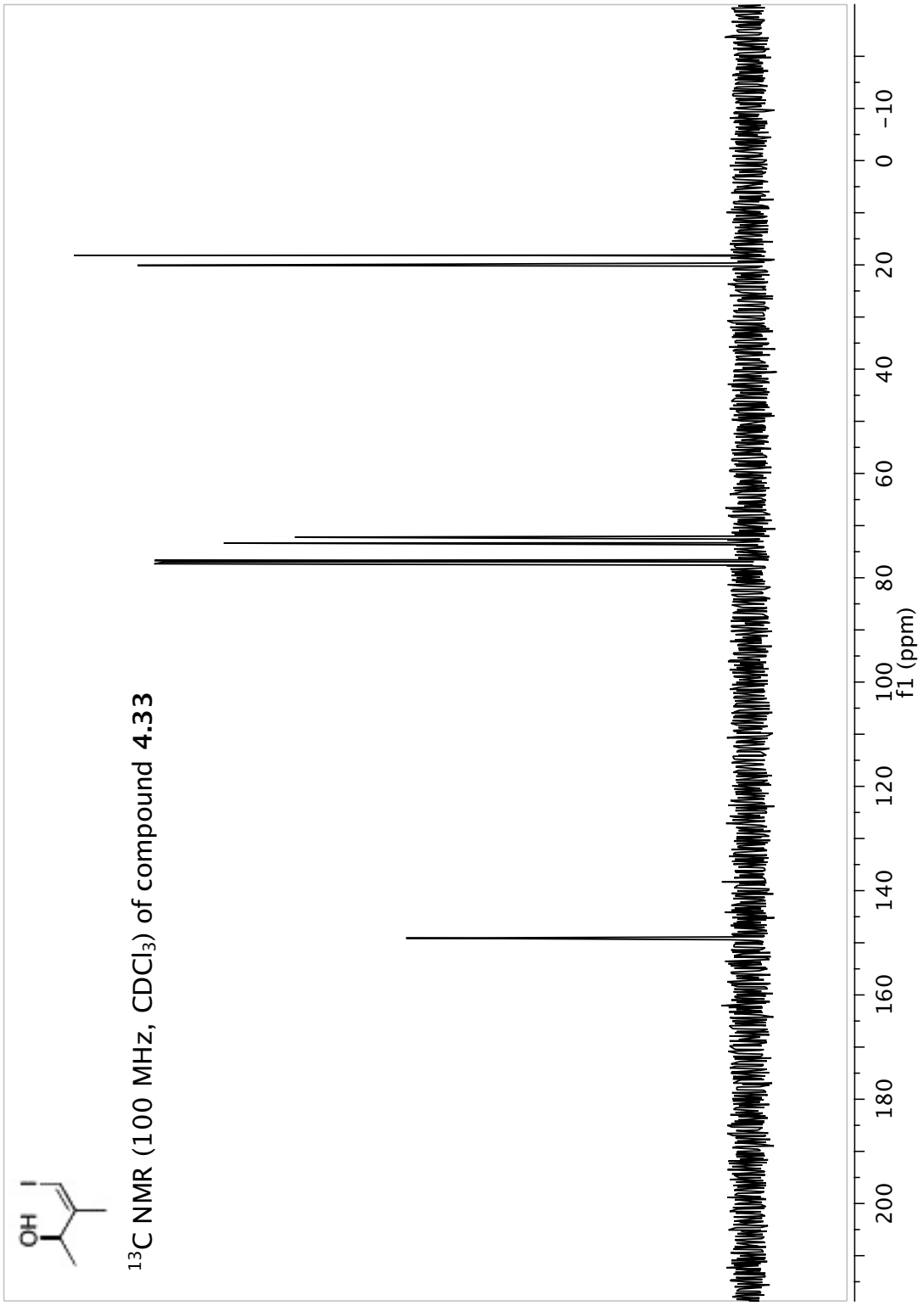


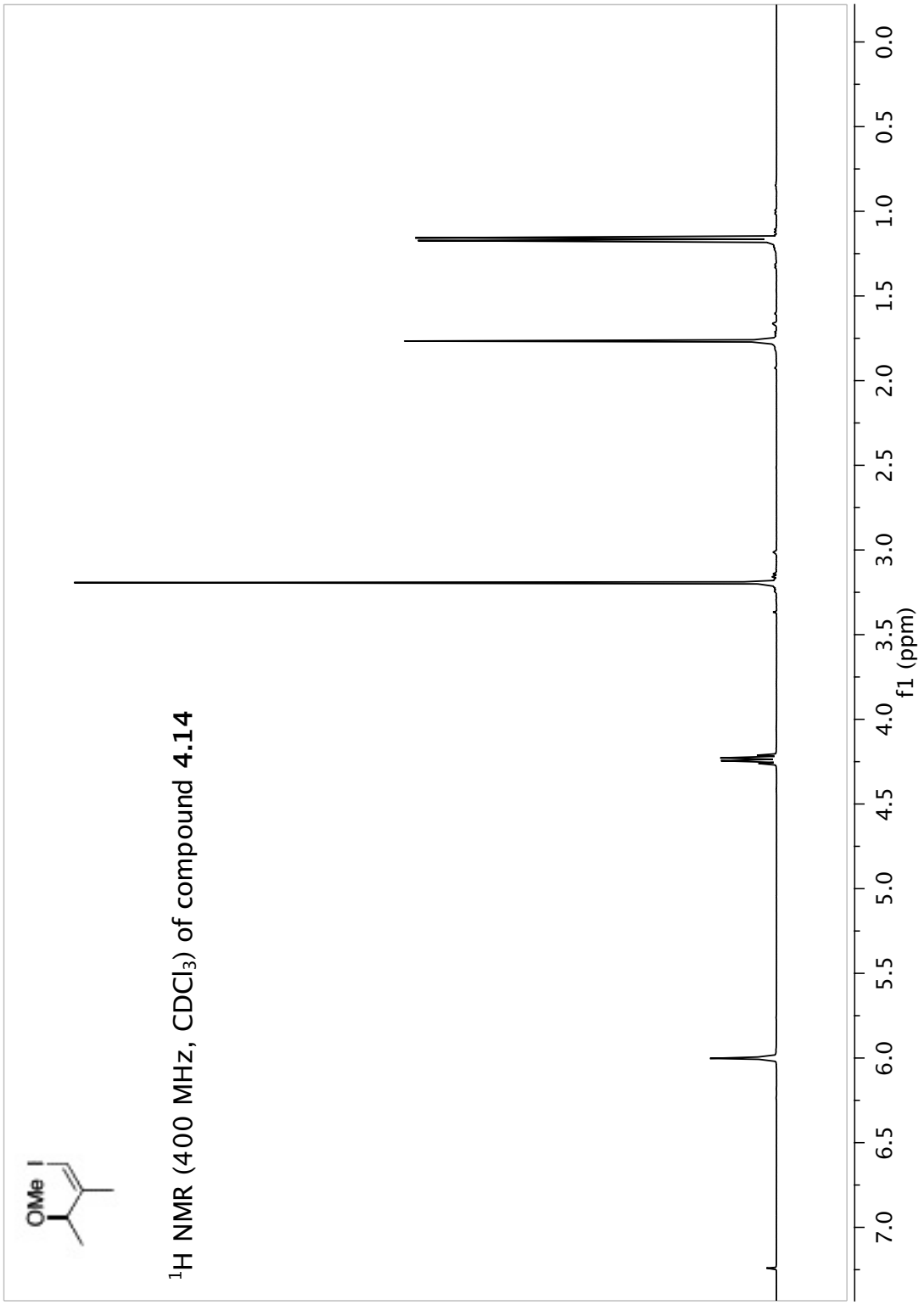
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.10**


^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.10**
EtO_2C
O
O
N
230
200
170
140
110
80
60
40
20
0
−20
f1 (ppm)

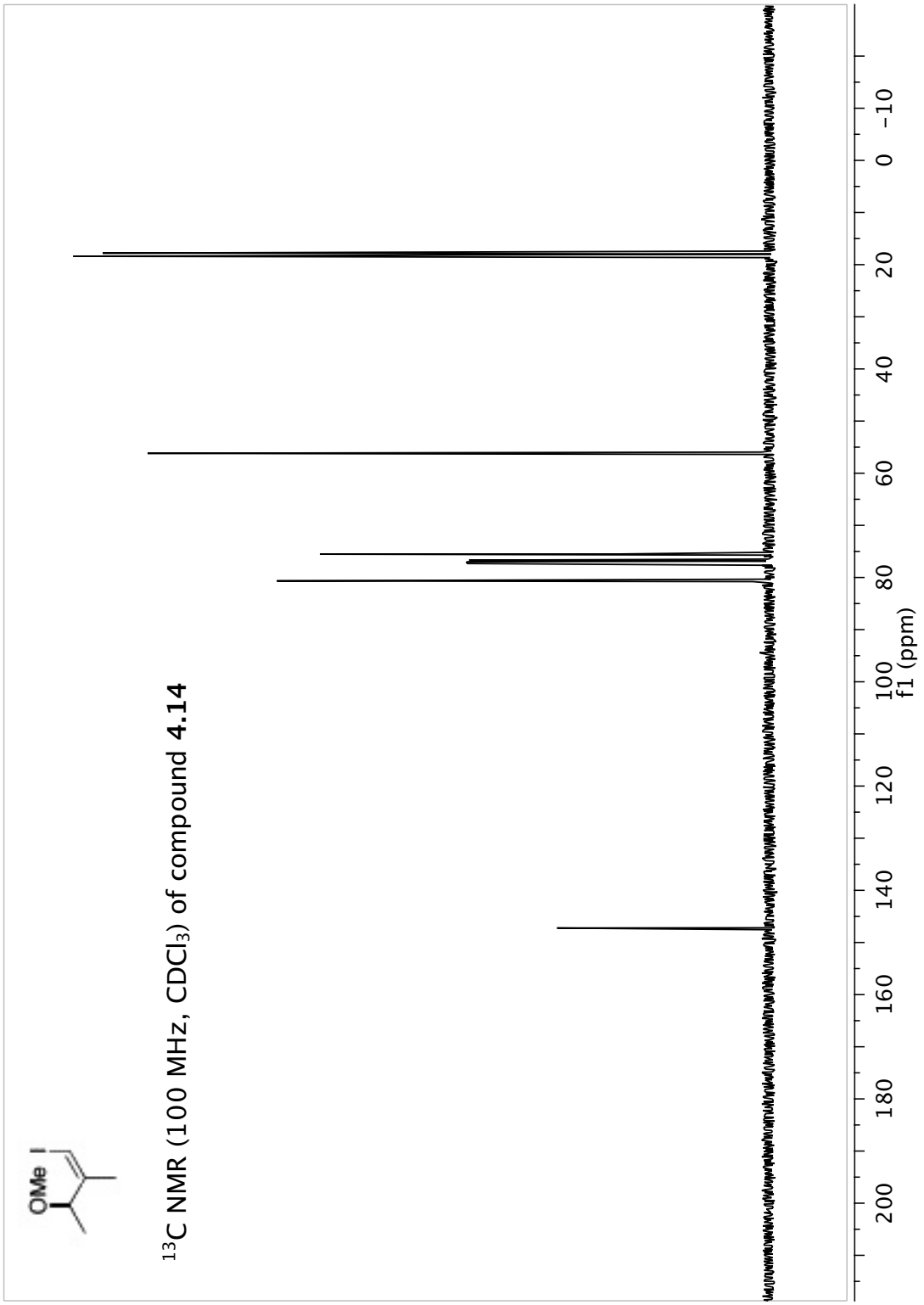


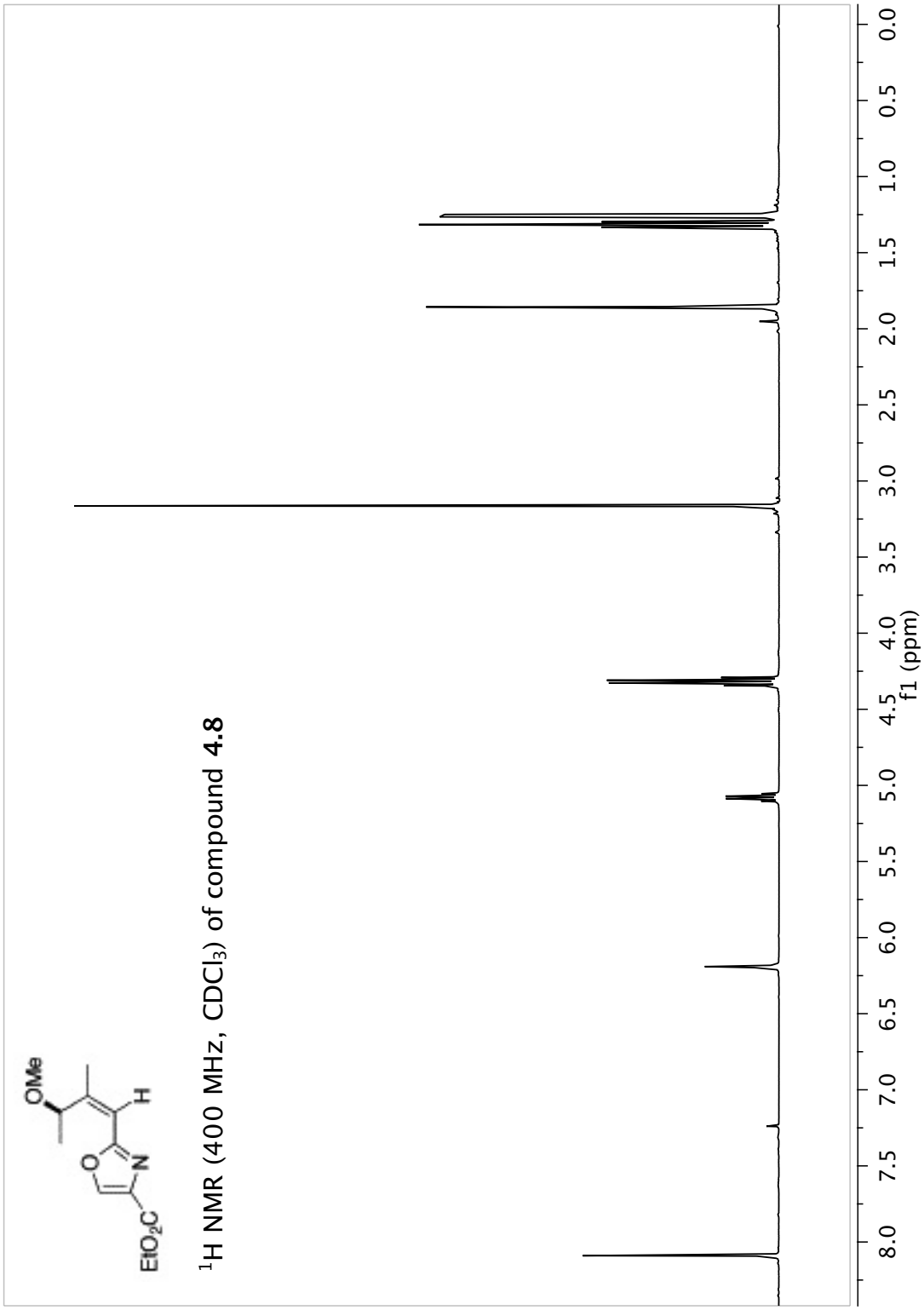
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.33**


OH
I
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.33**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

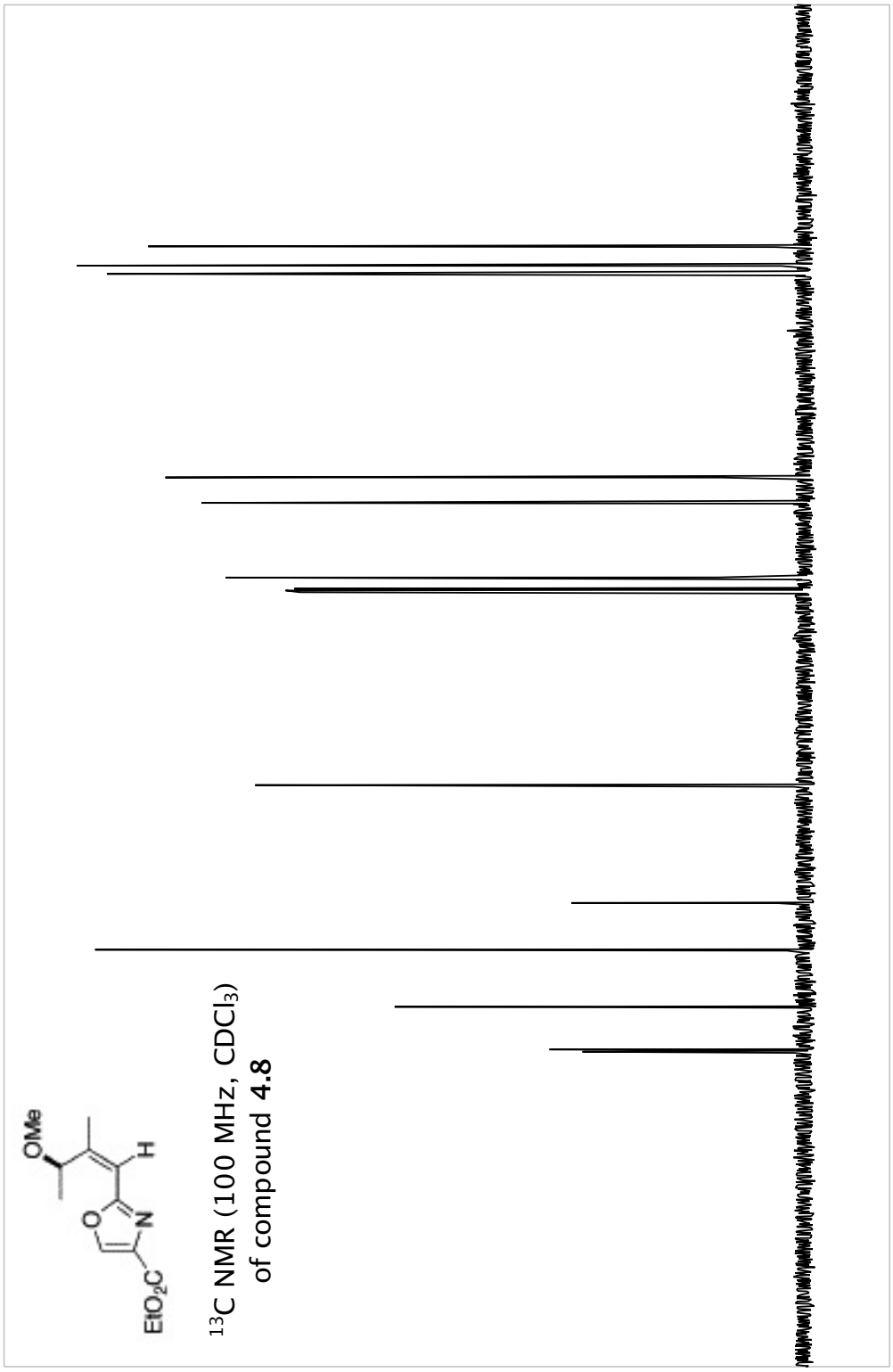


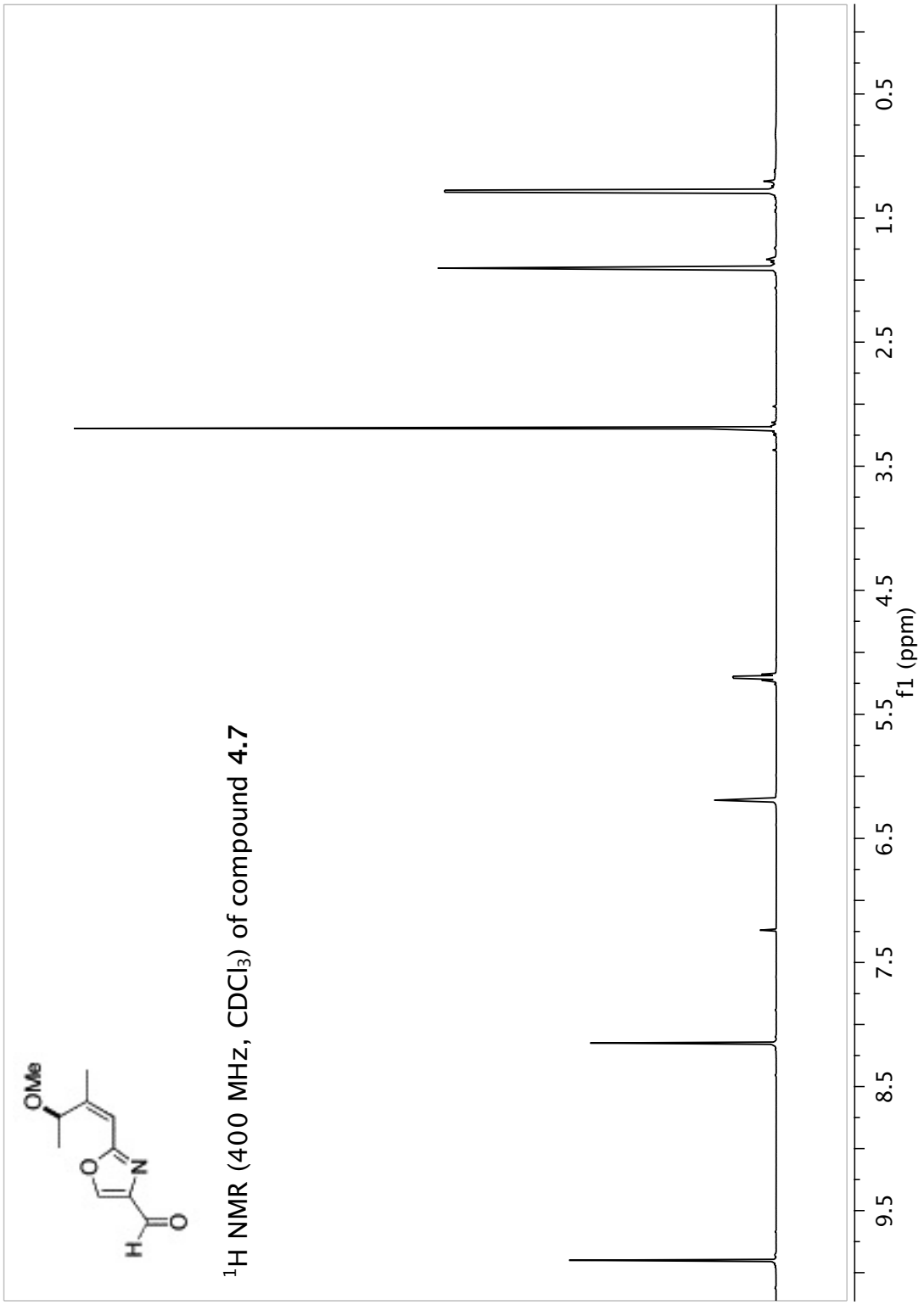
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.14**


OMe
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.14**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

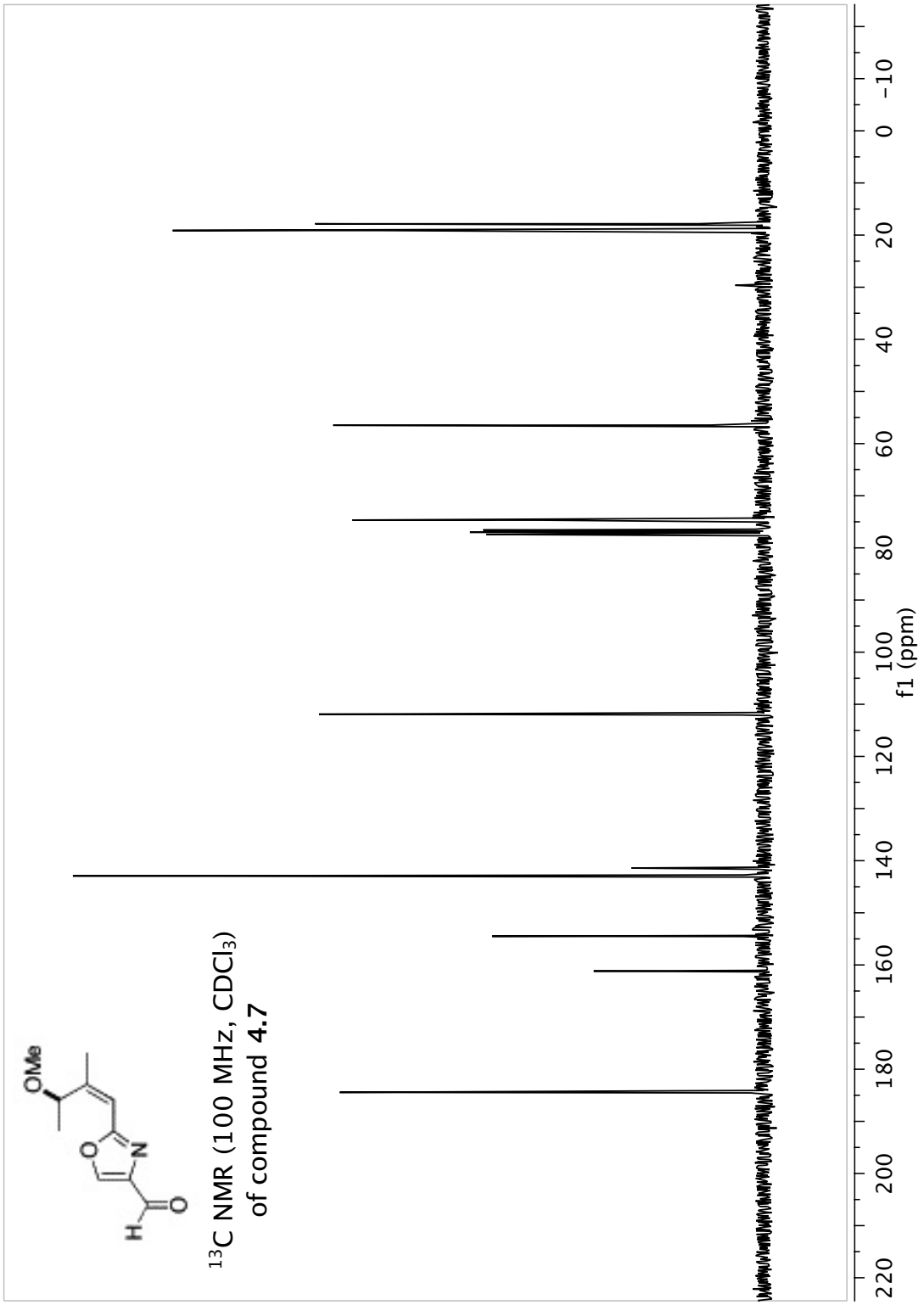


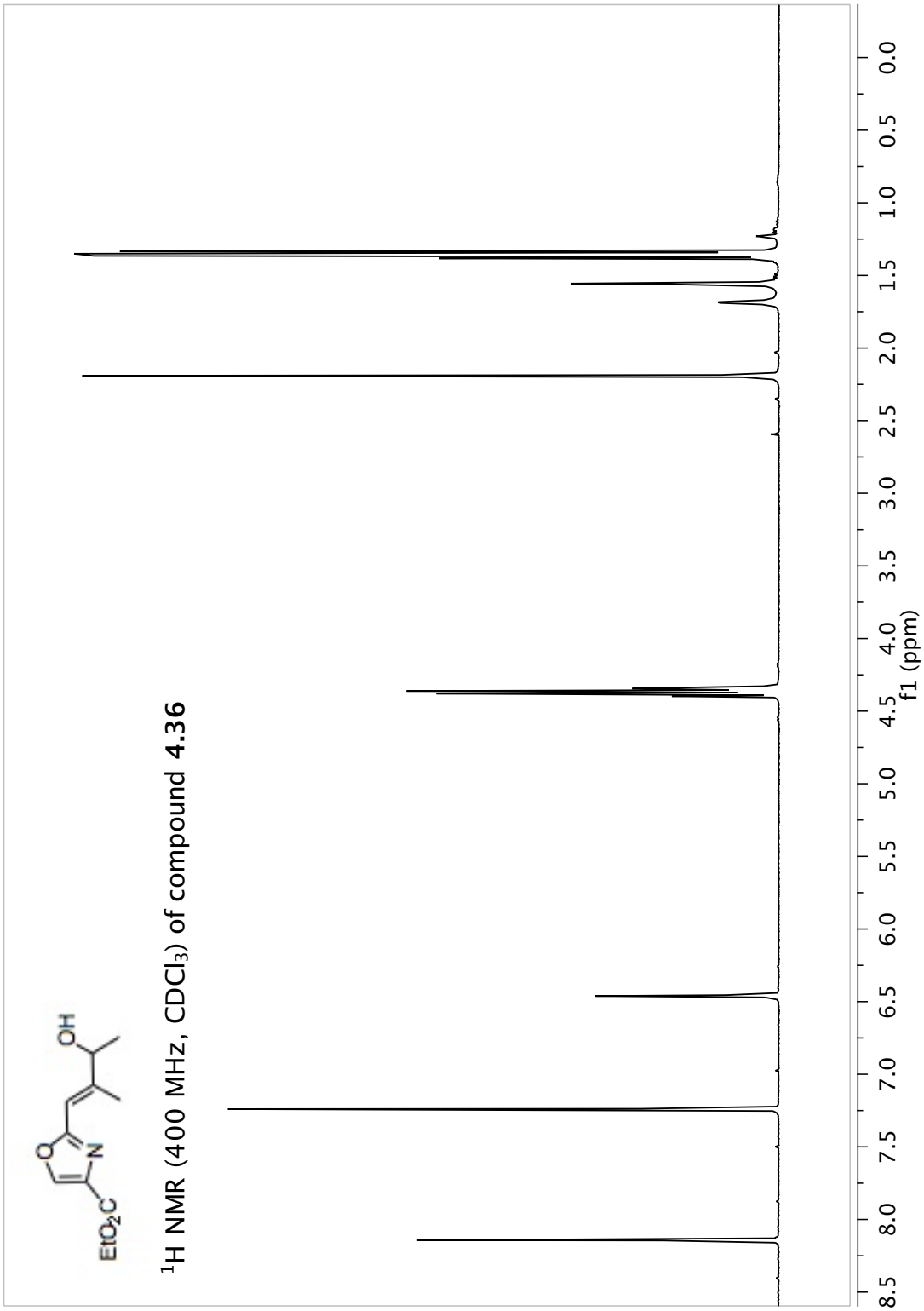
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.8**


OMe
H
O
N
EtO2C
13C NMR (100 MHz, CDCl3)
of compound 4.8
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

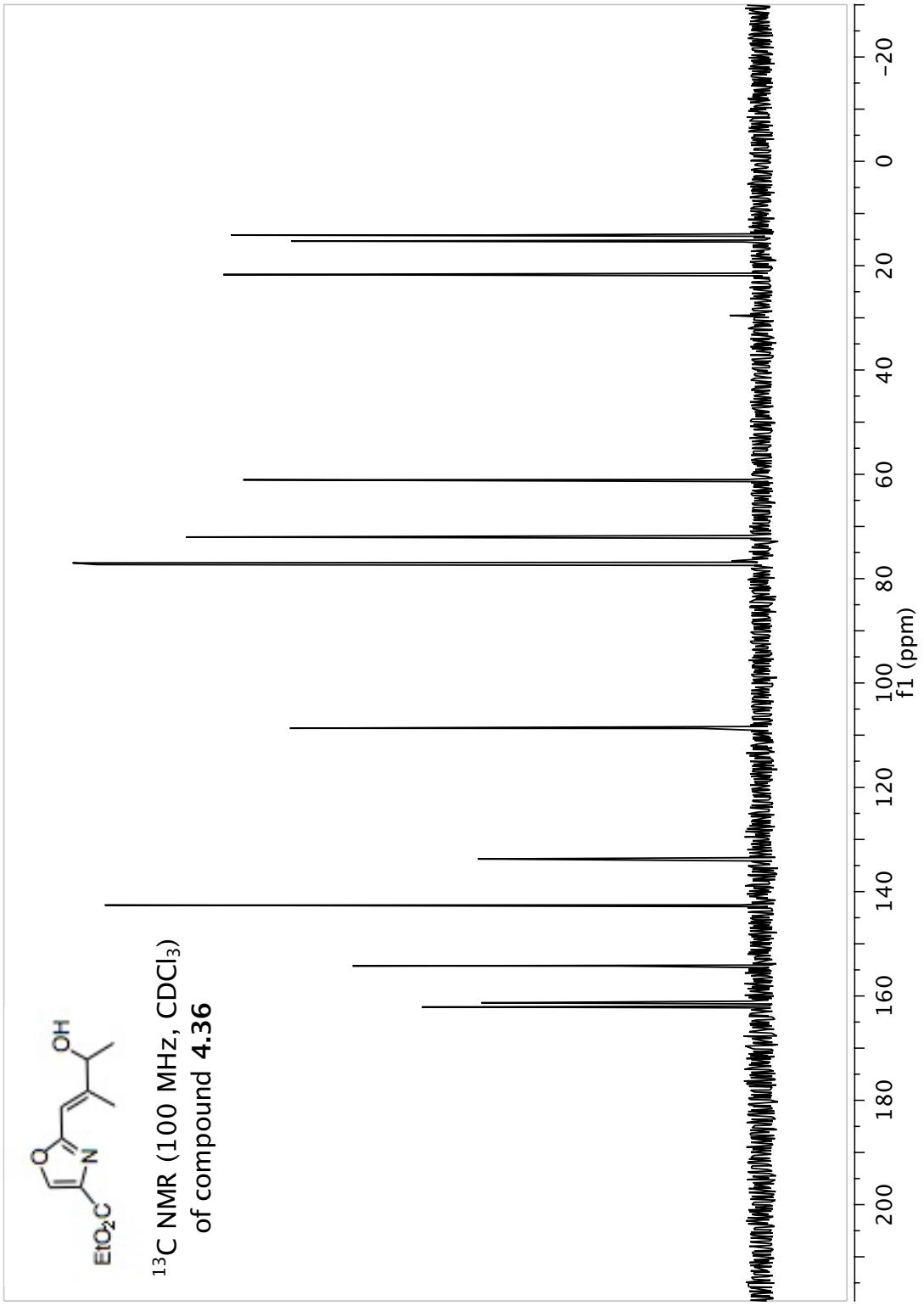


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.7**

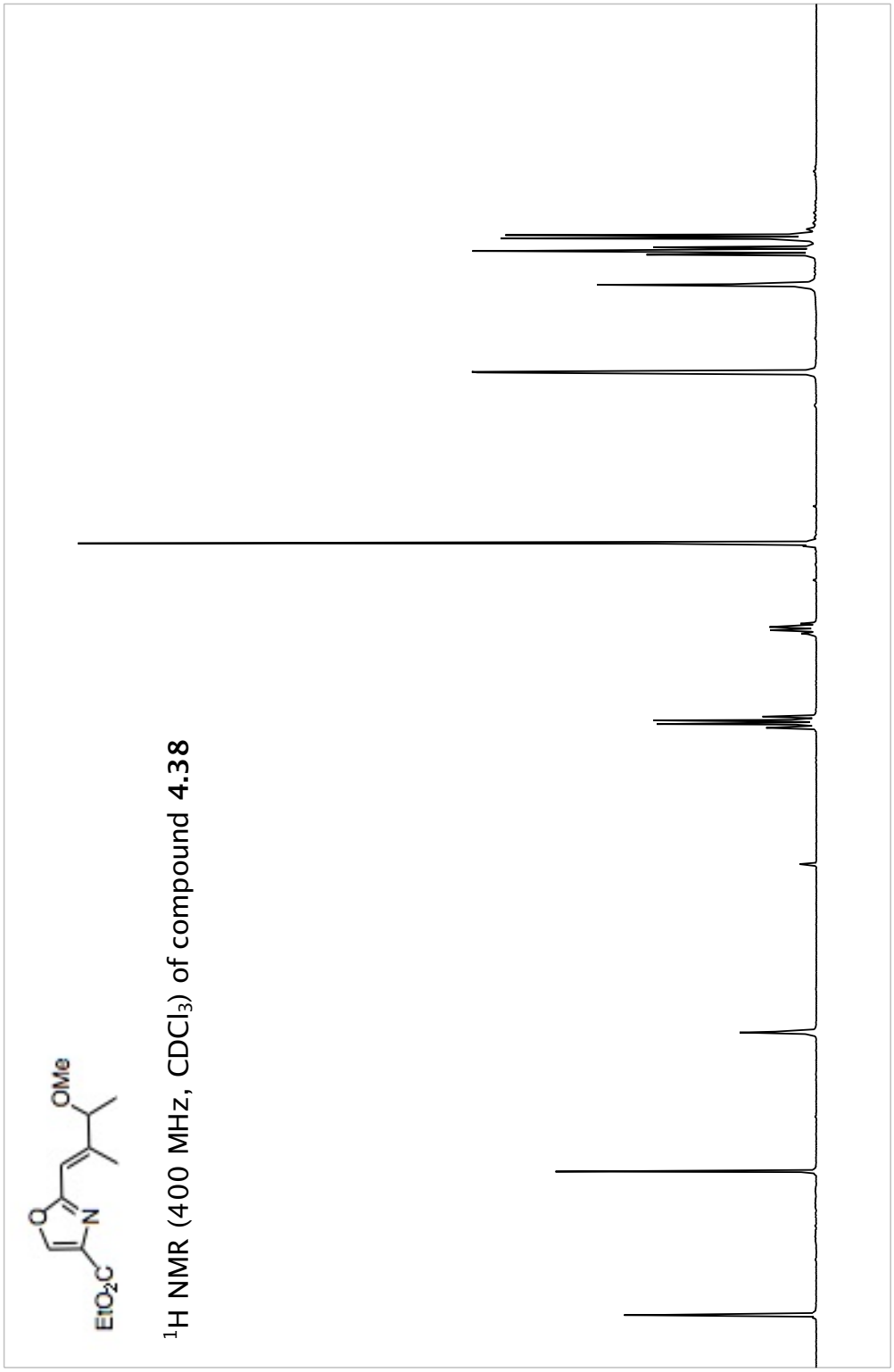

OMe
O
N
H
O
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.7**
220
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



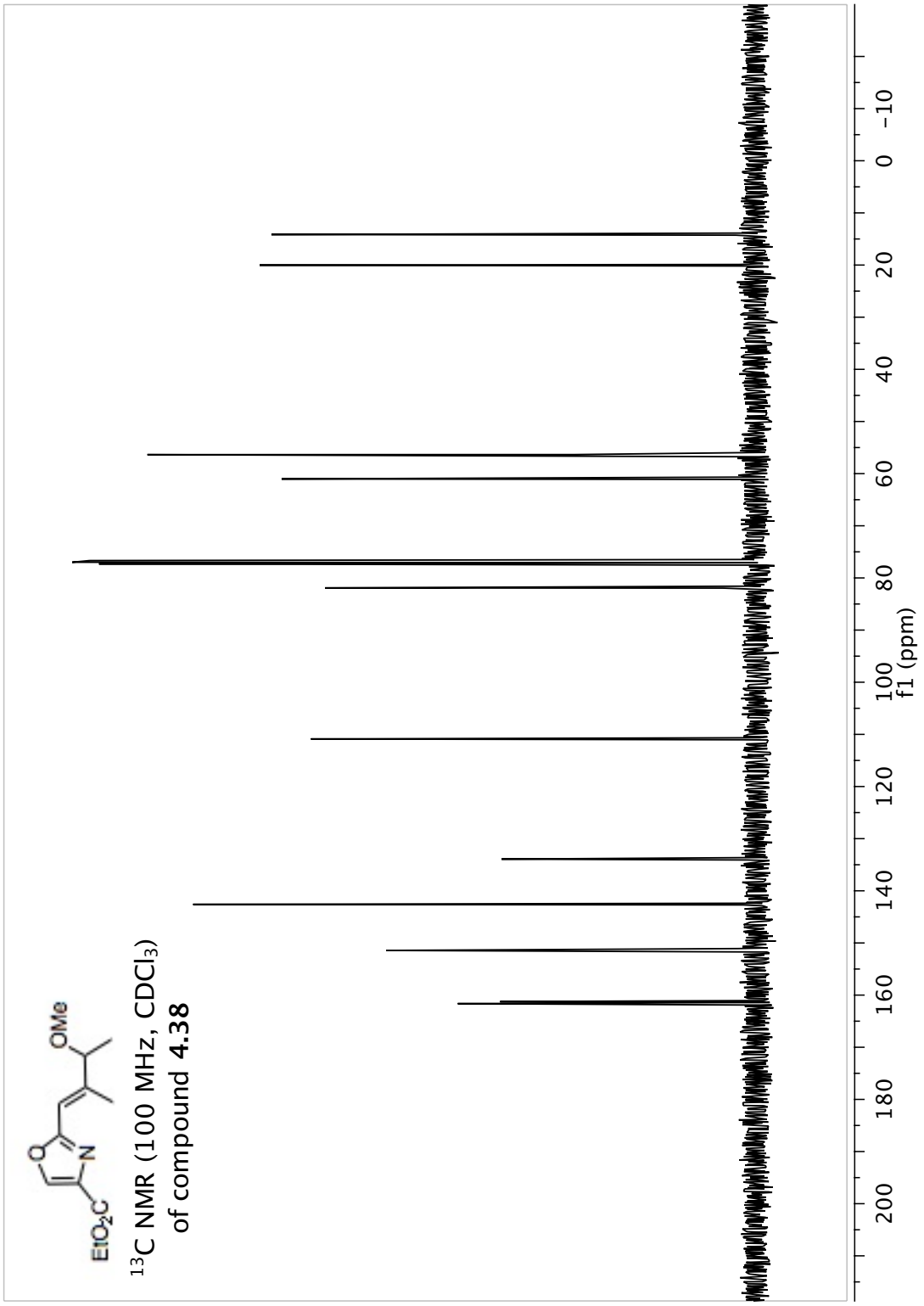
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.36**



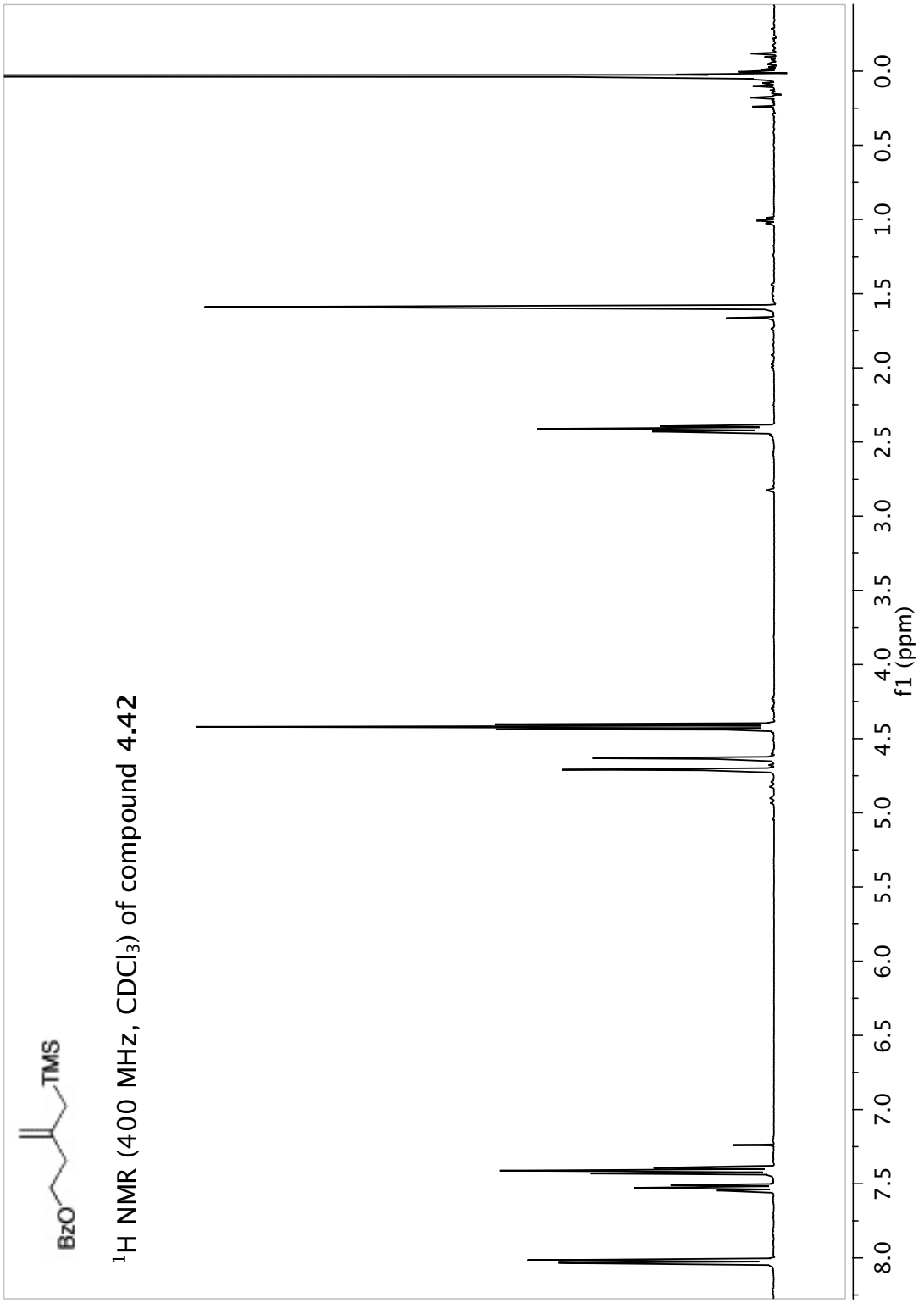
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.36**



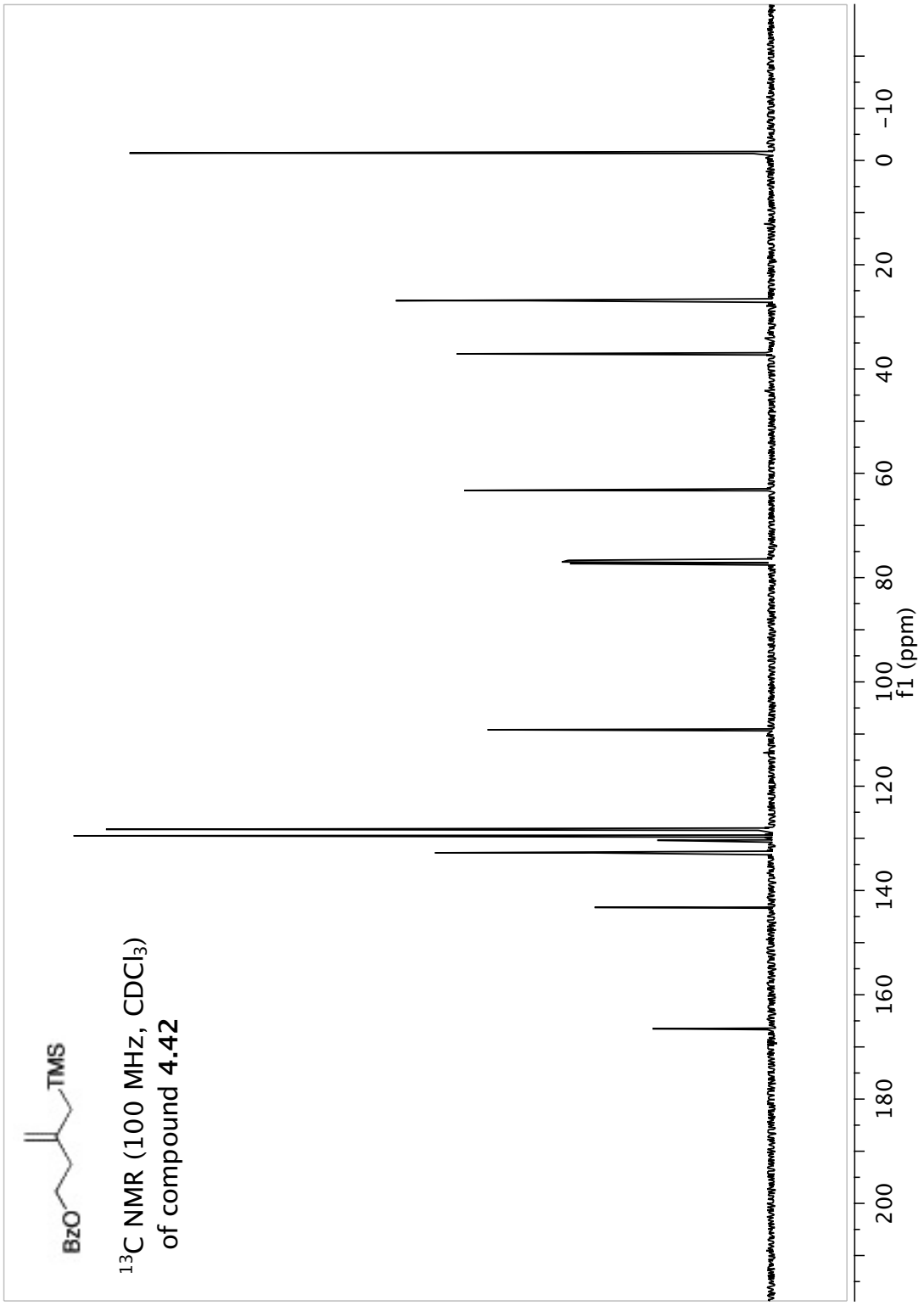
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.38**

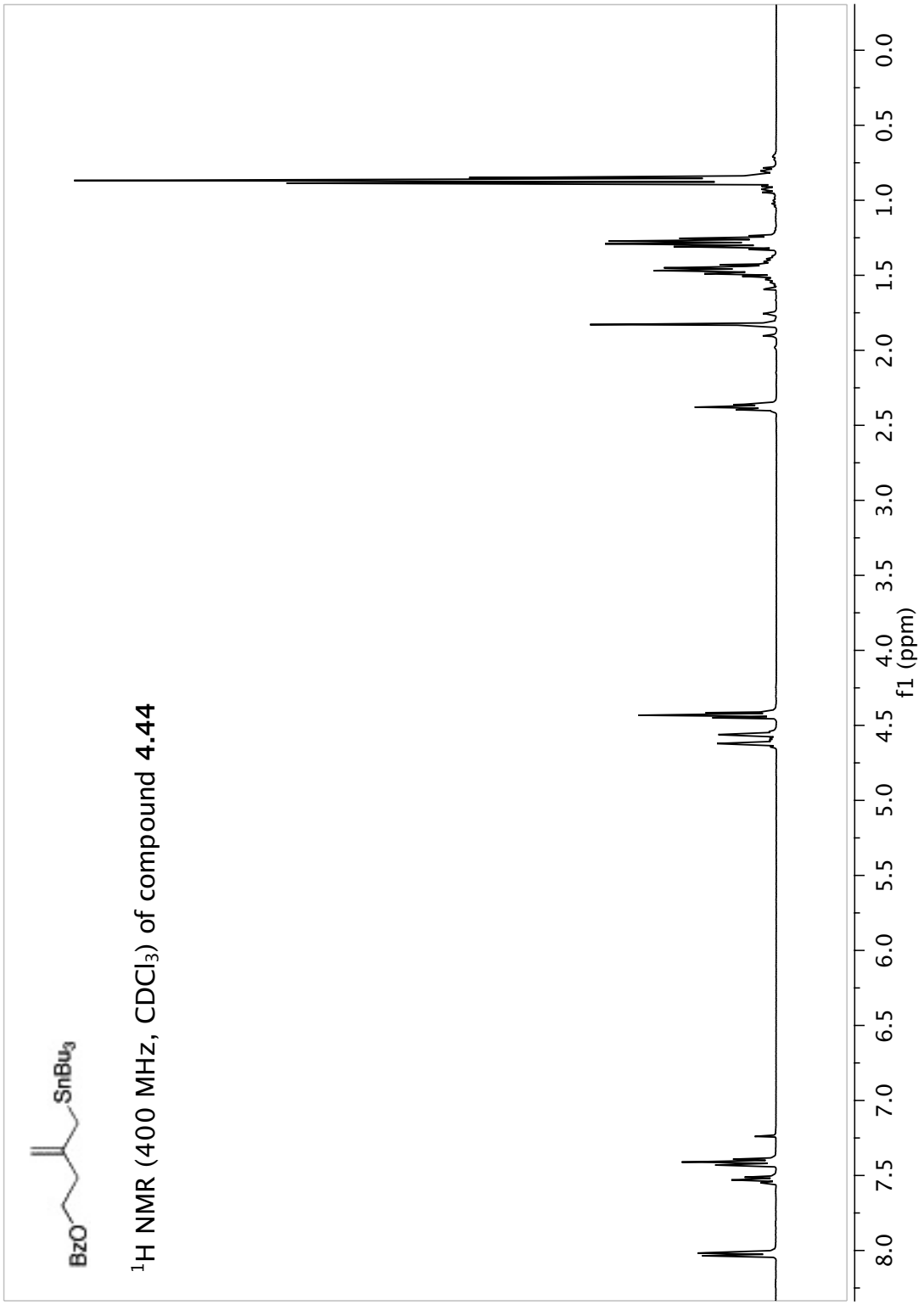


^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.38**

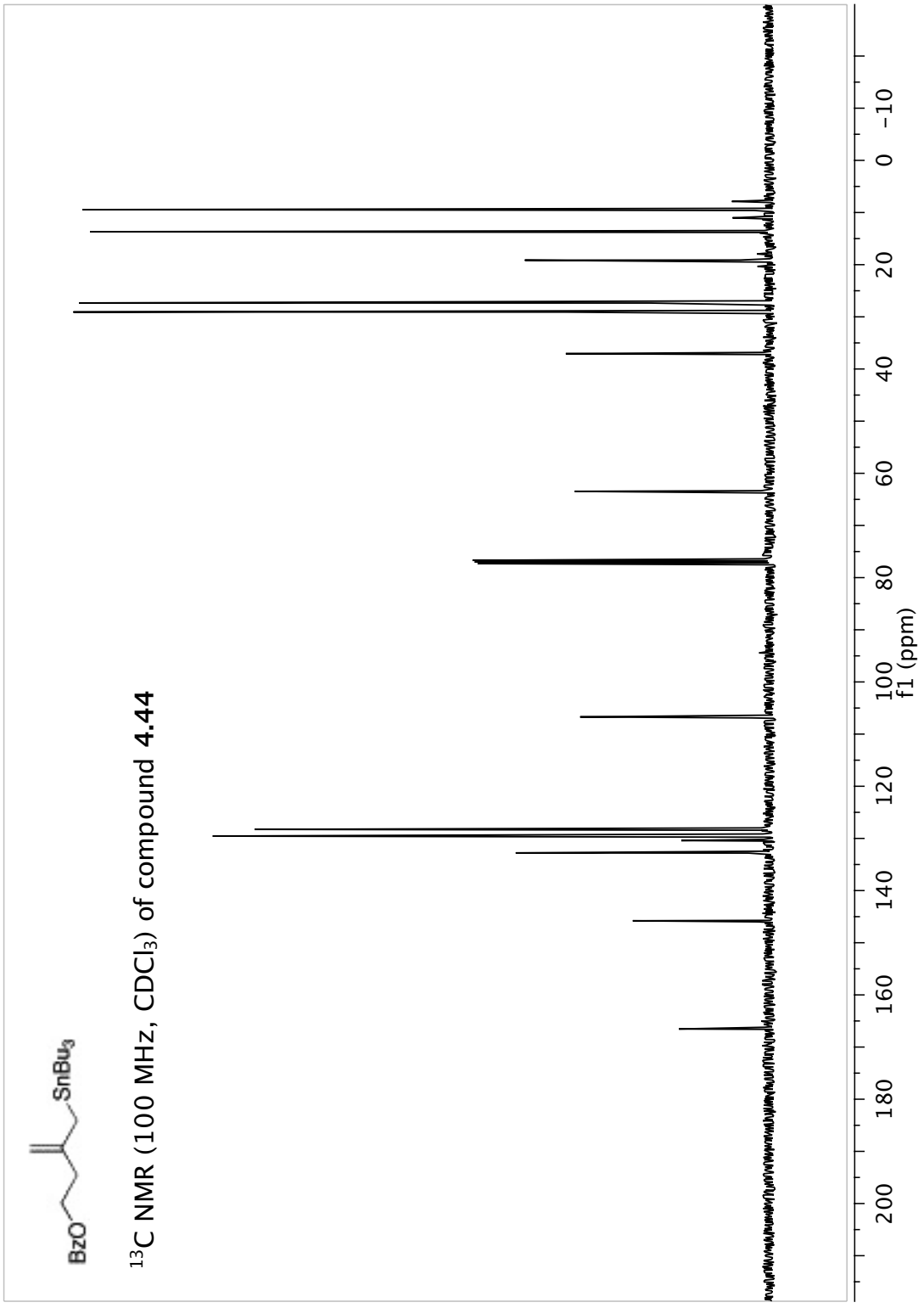


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.42**

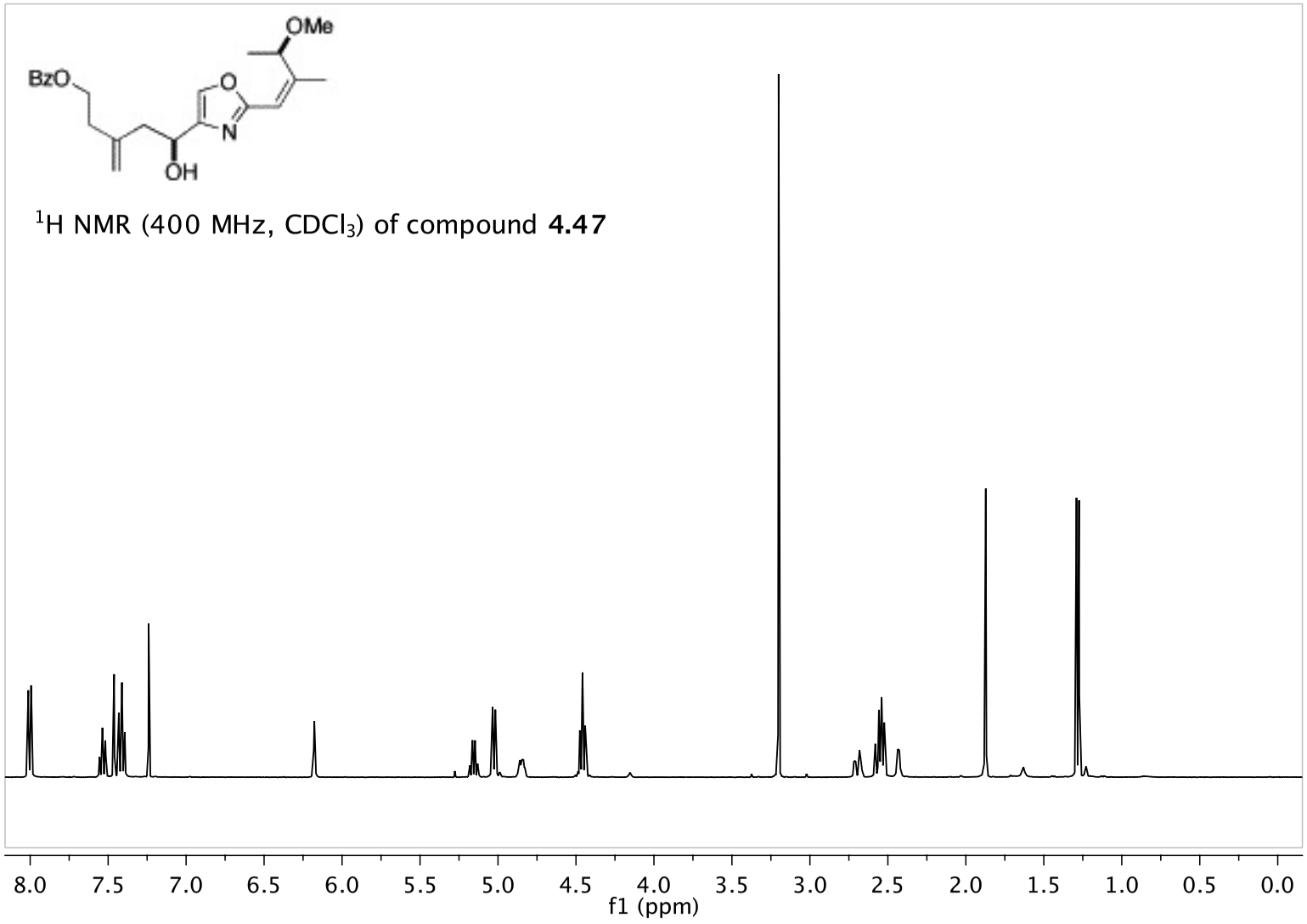

BzO
TMS
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.42**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



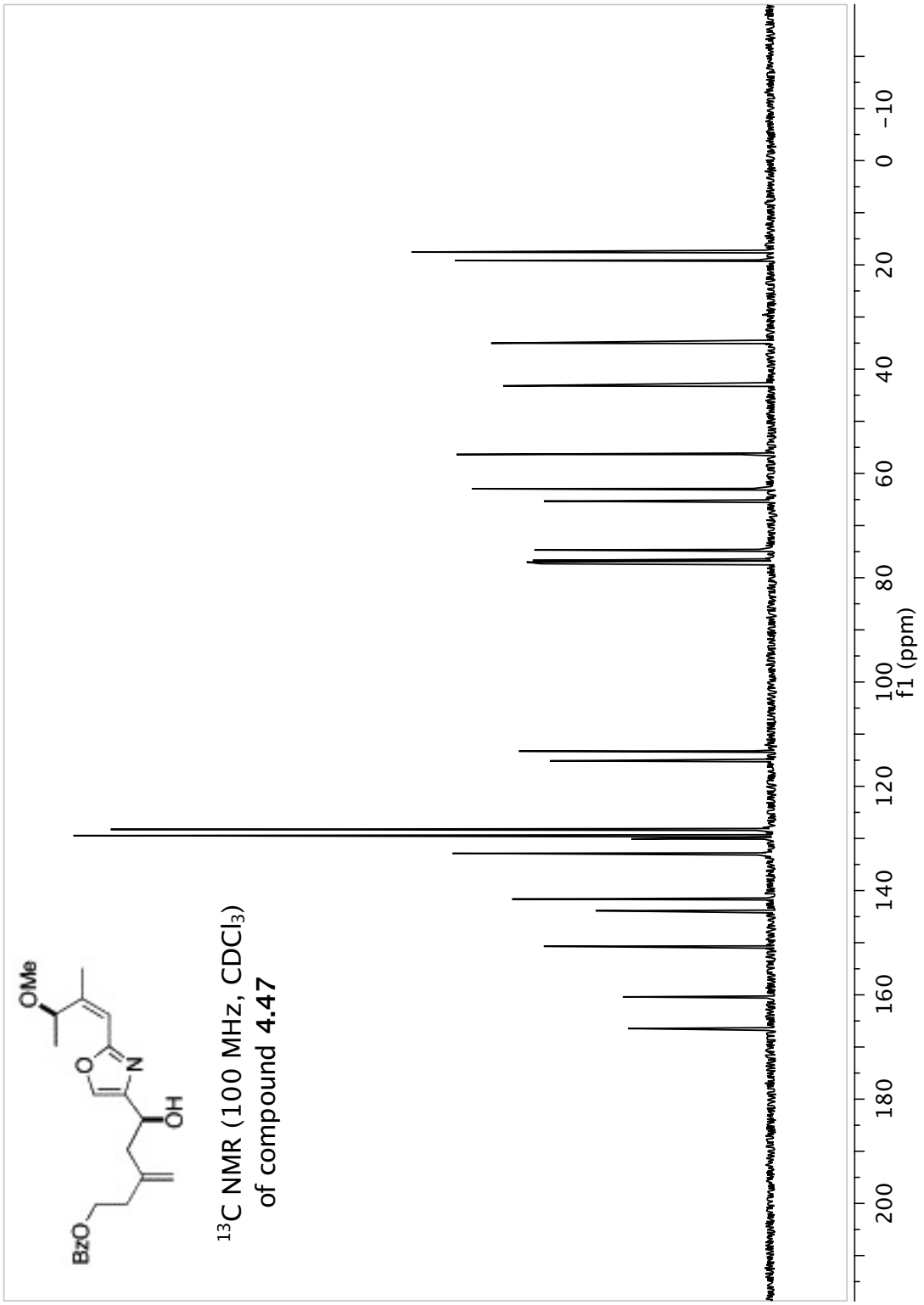
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.44**

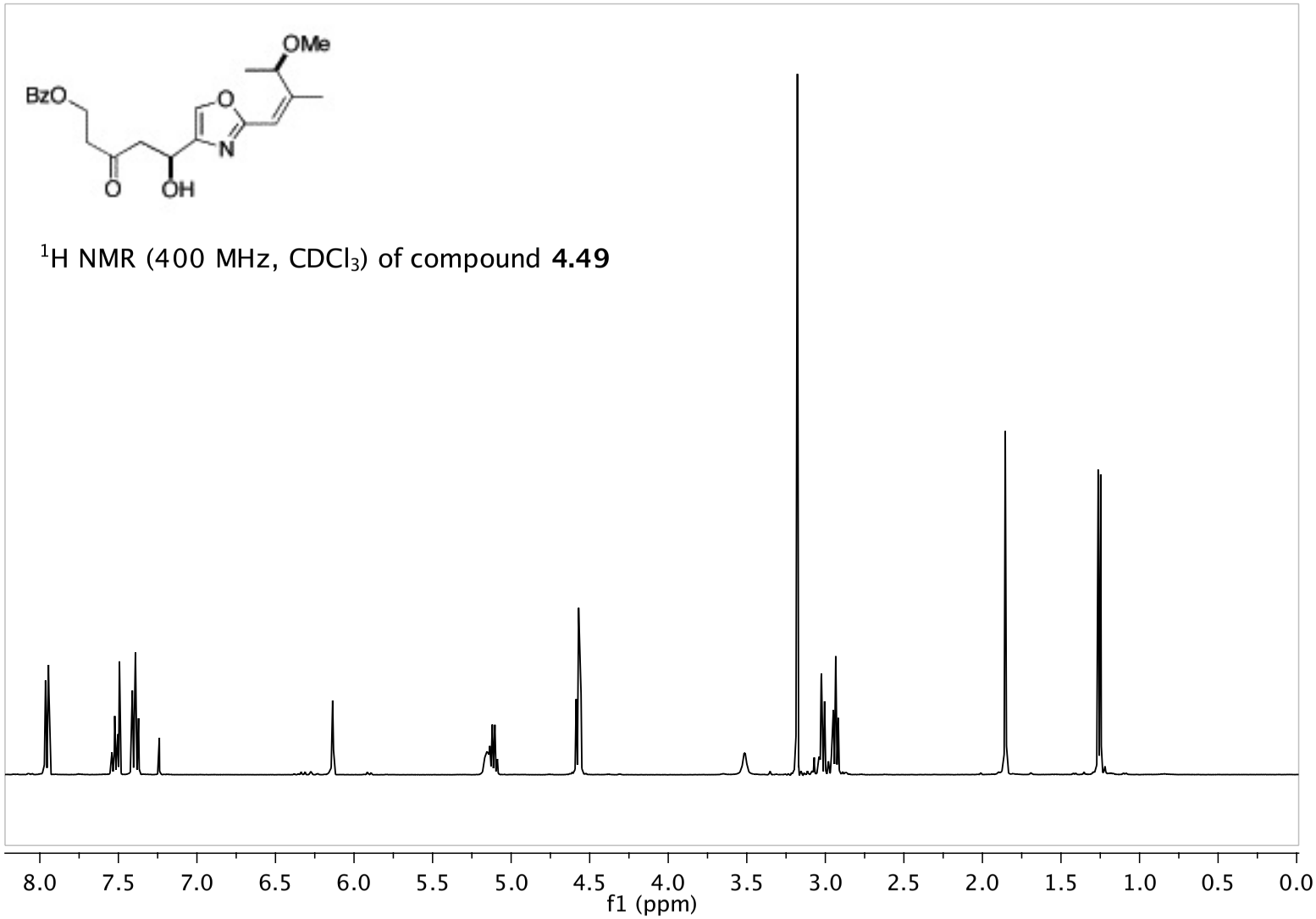


^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.44**

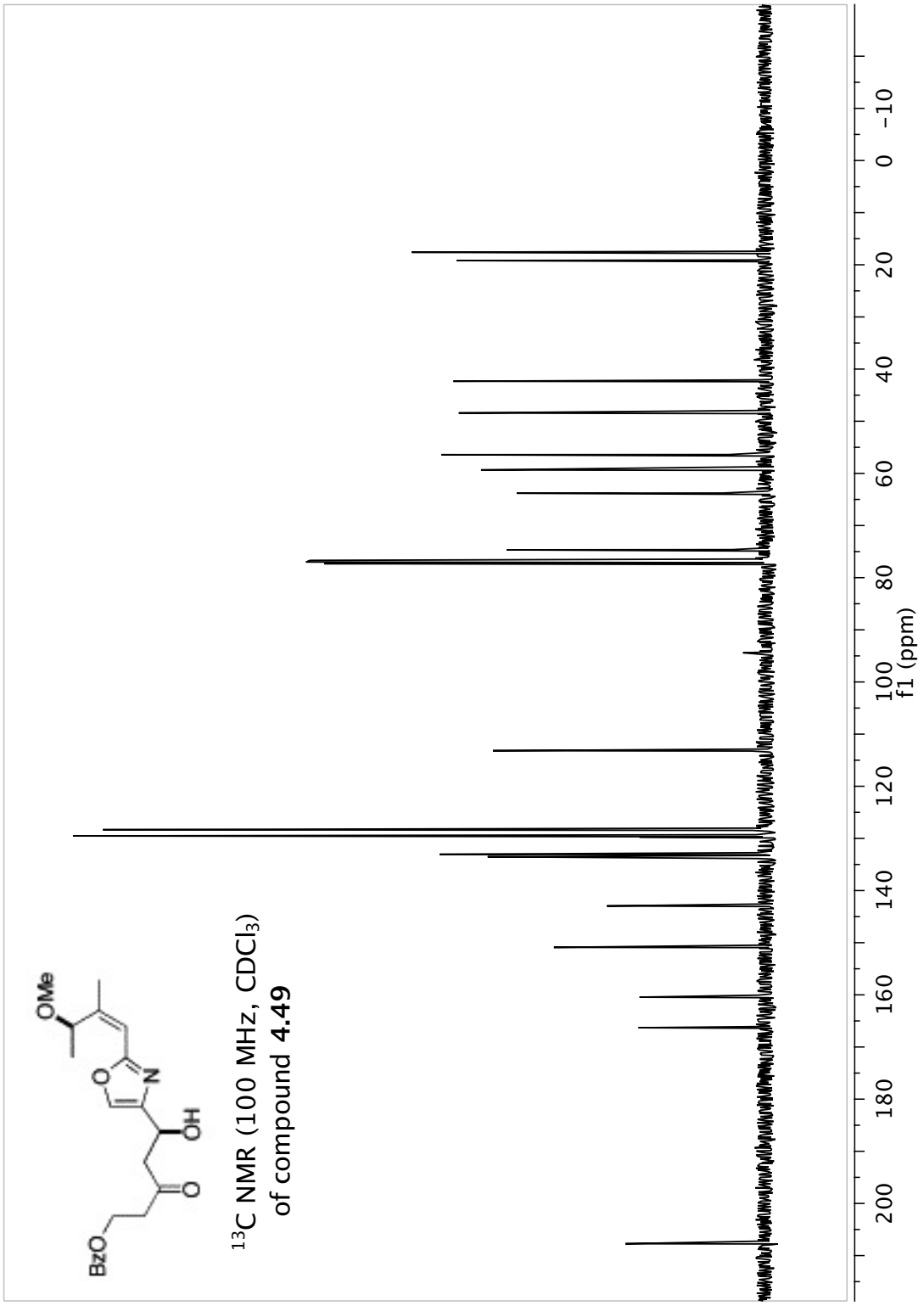


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.47**

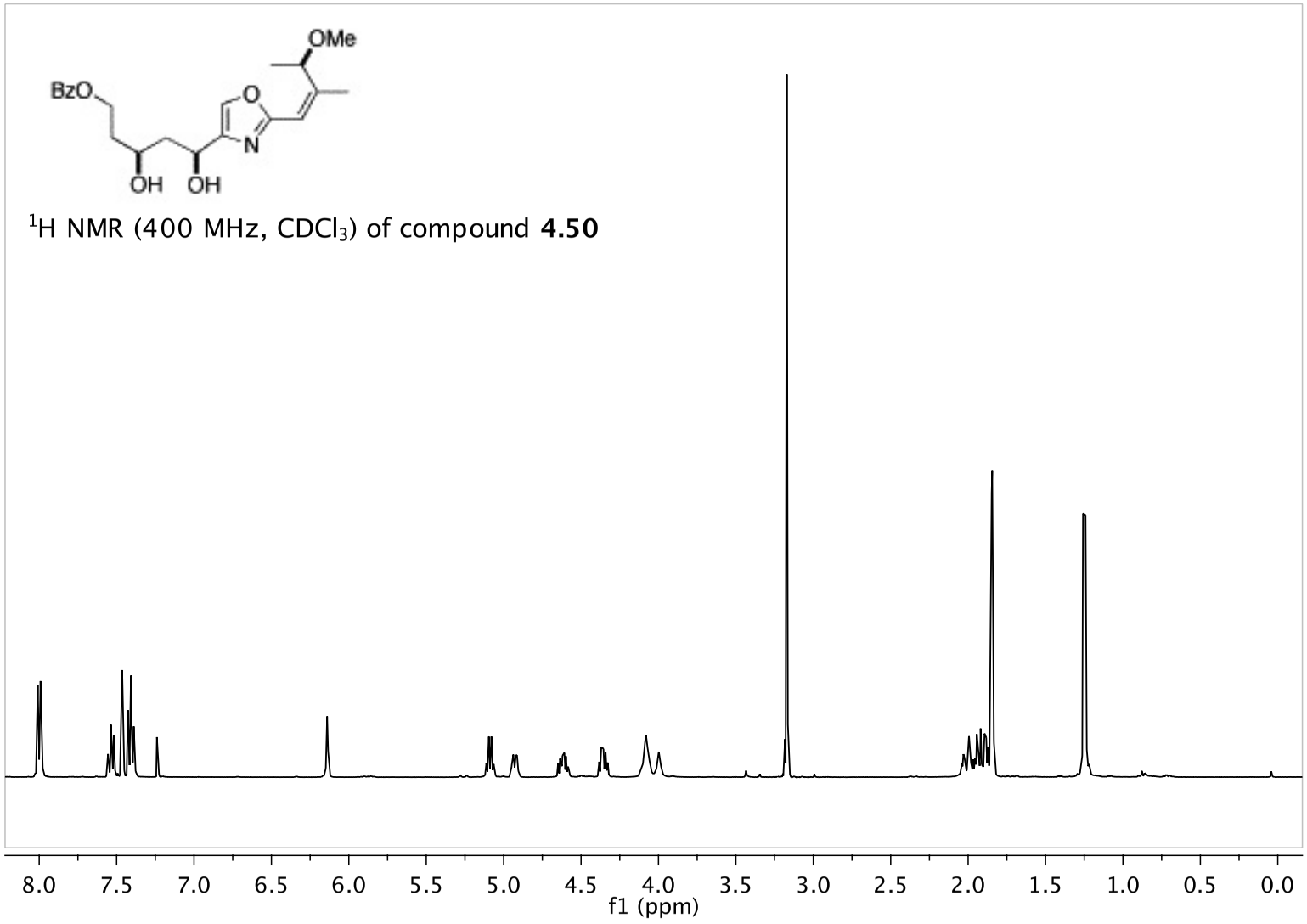

OMe
O
N
OH
BzO
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.47**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



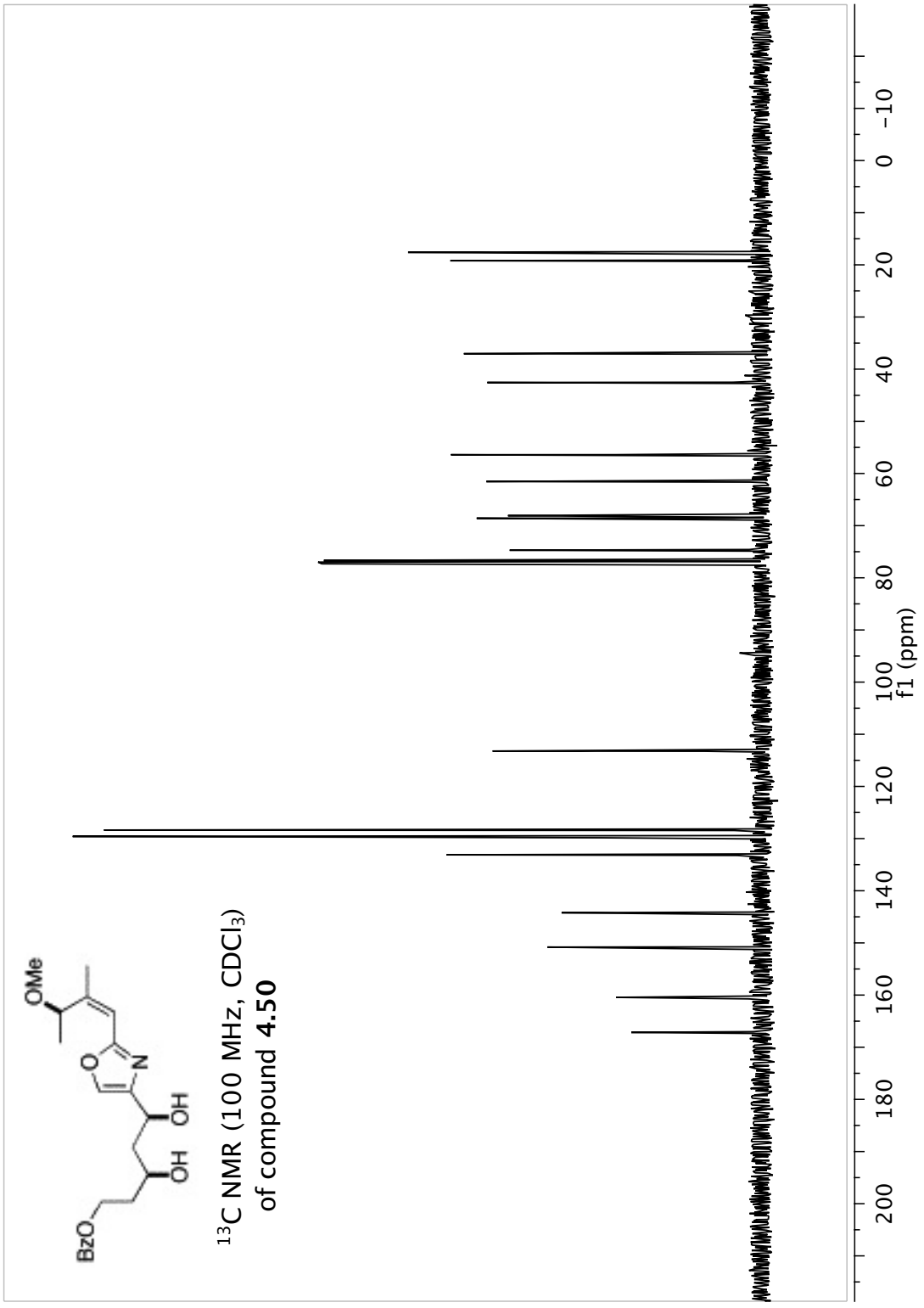
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.49**



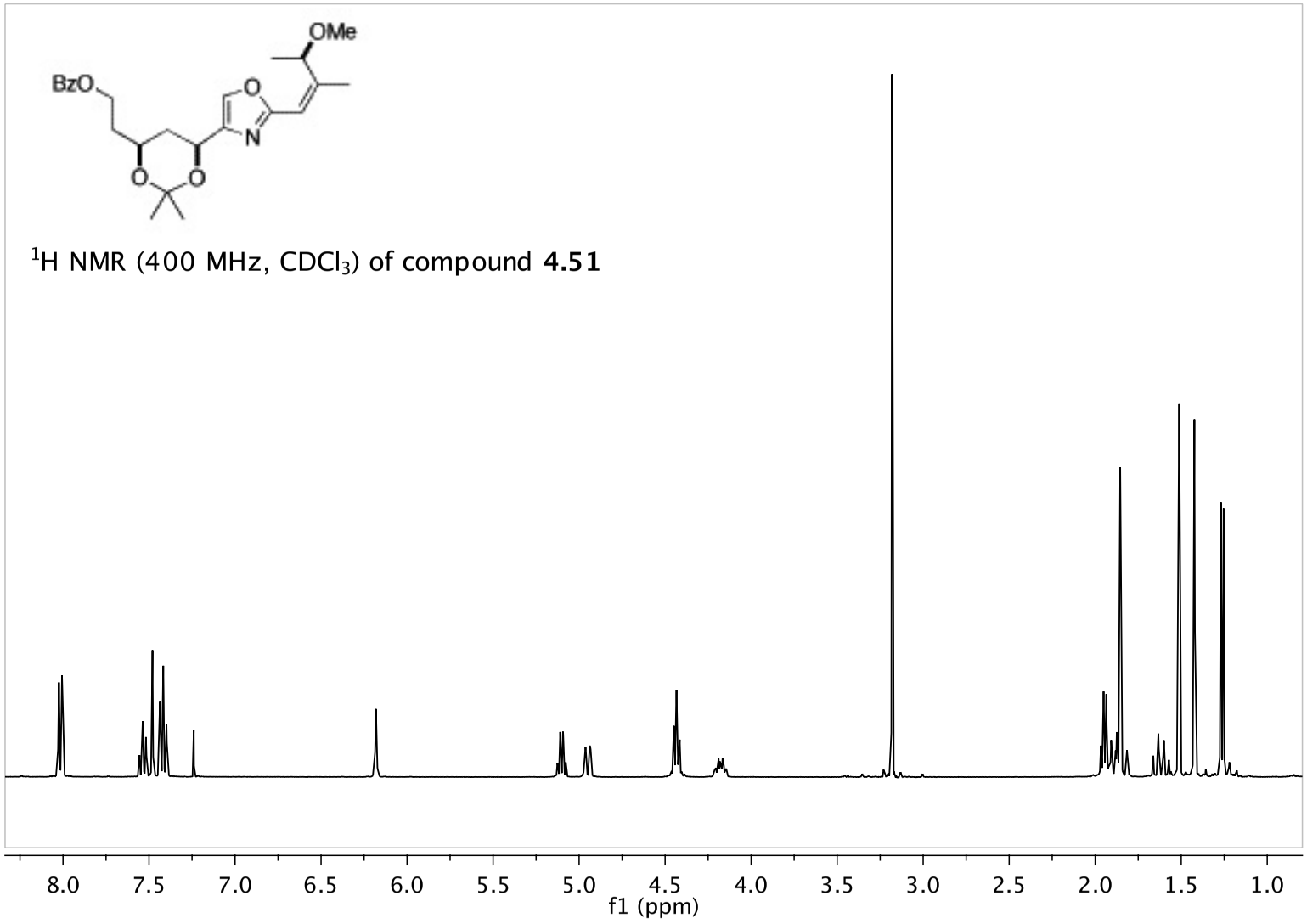
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.49**



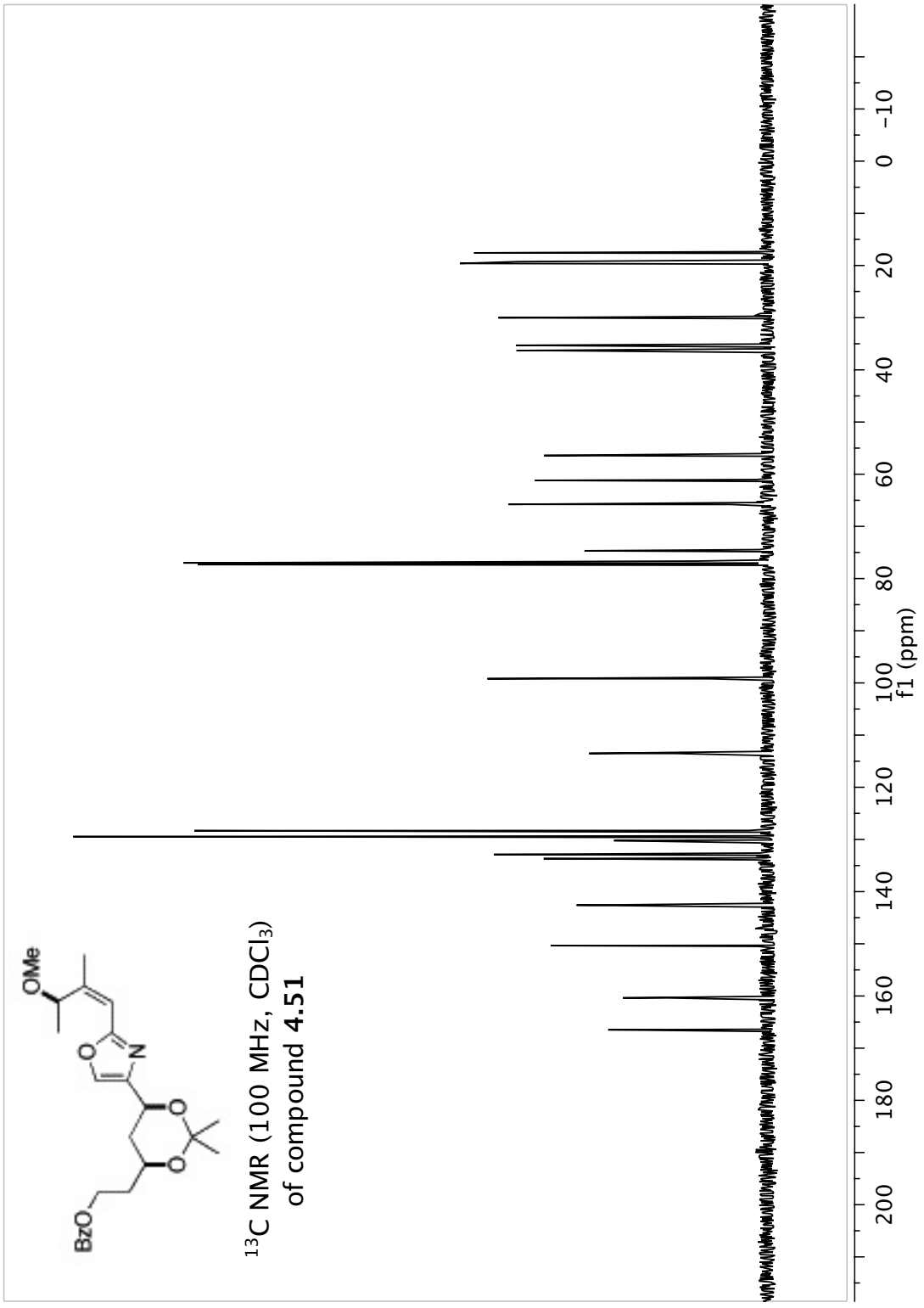
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.50**



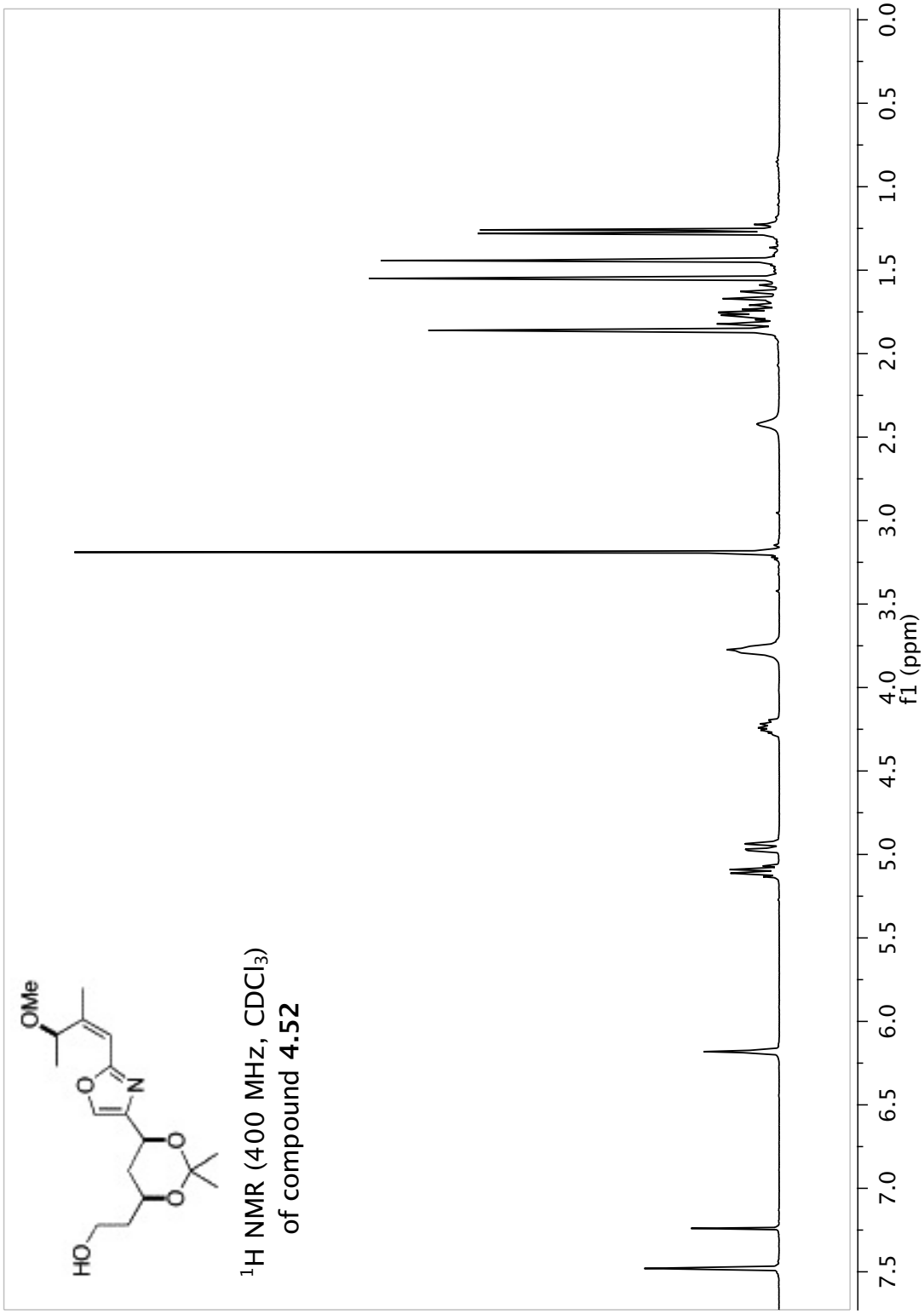
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.50**



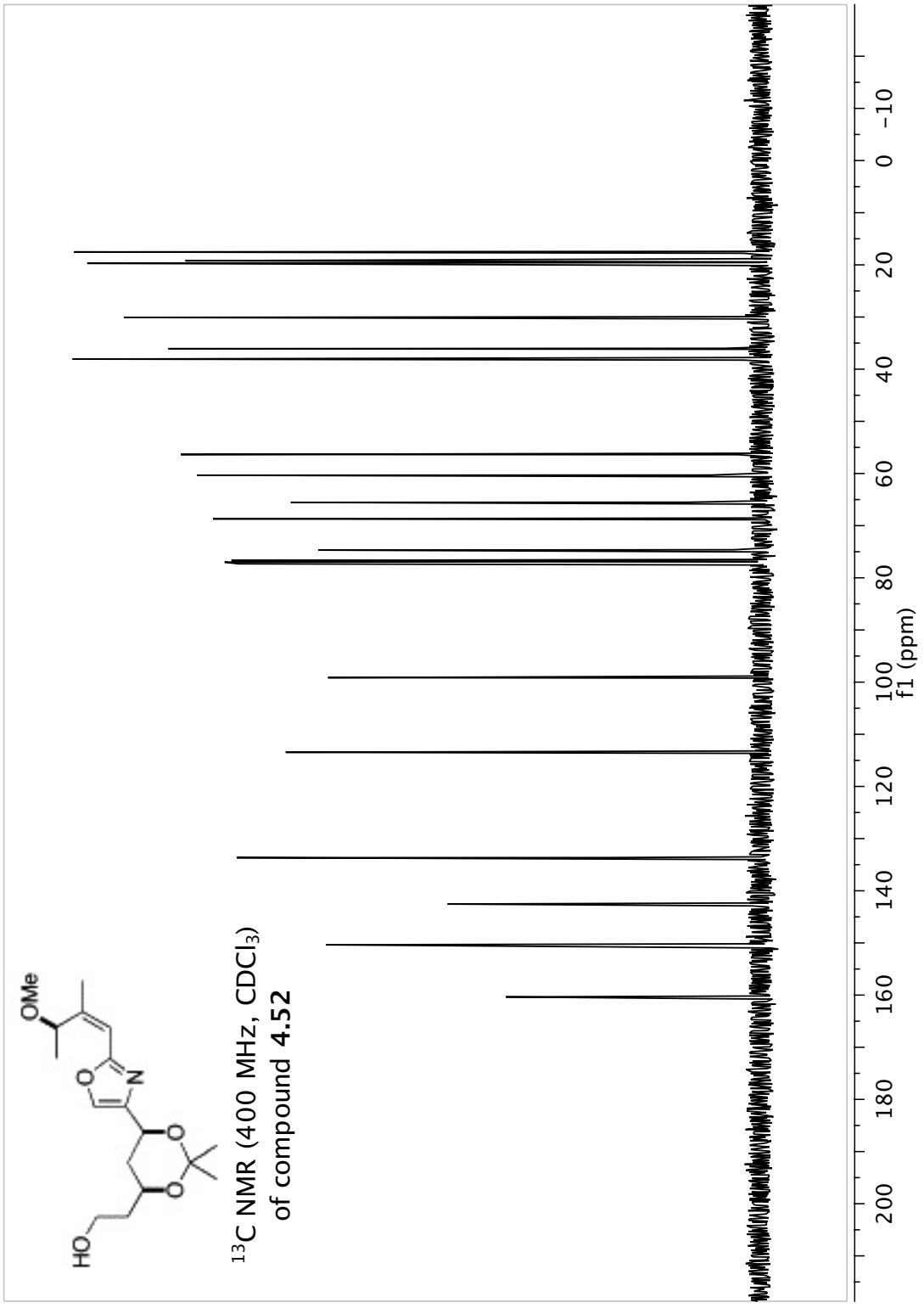
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.51**



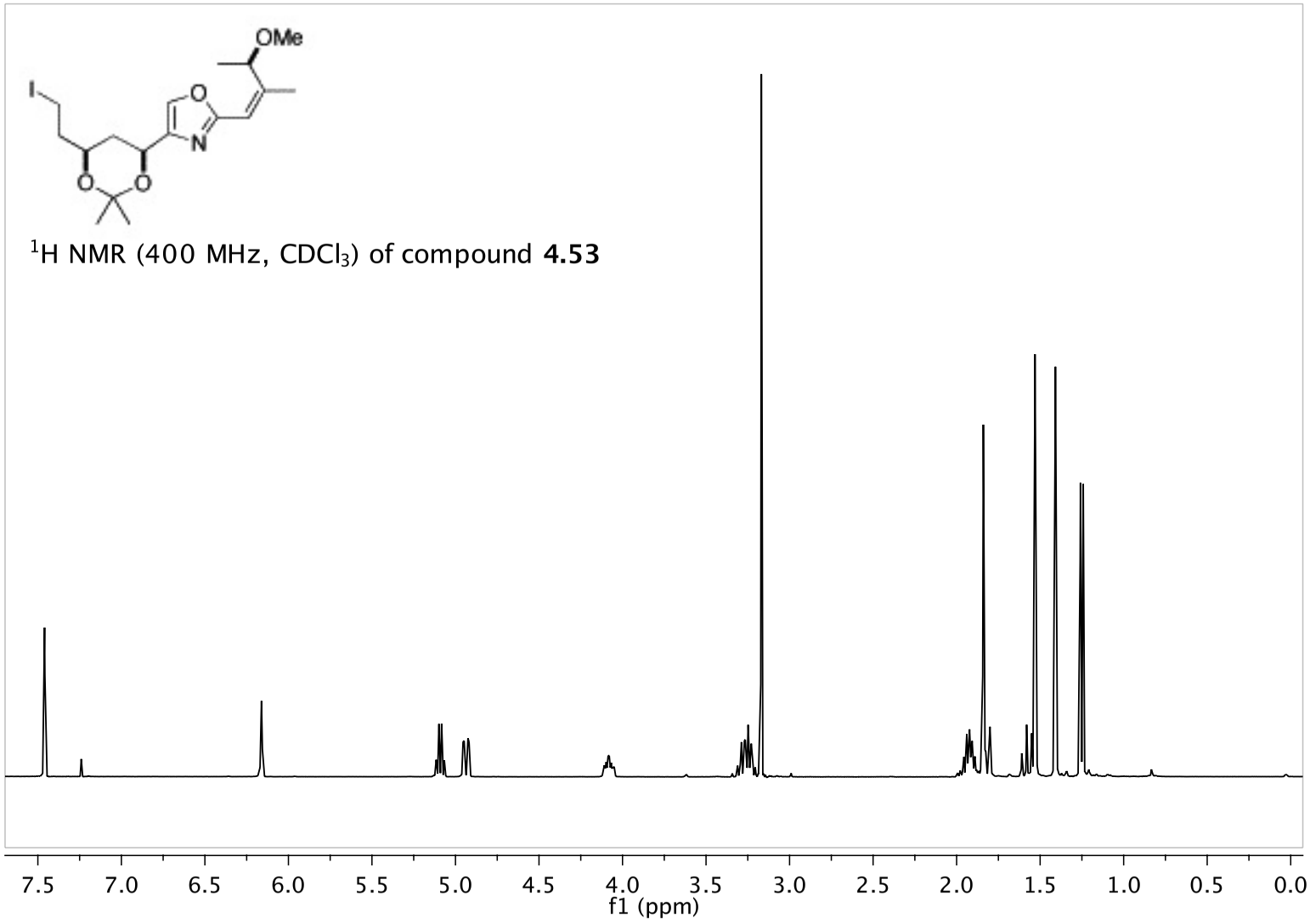
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.51**

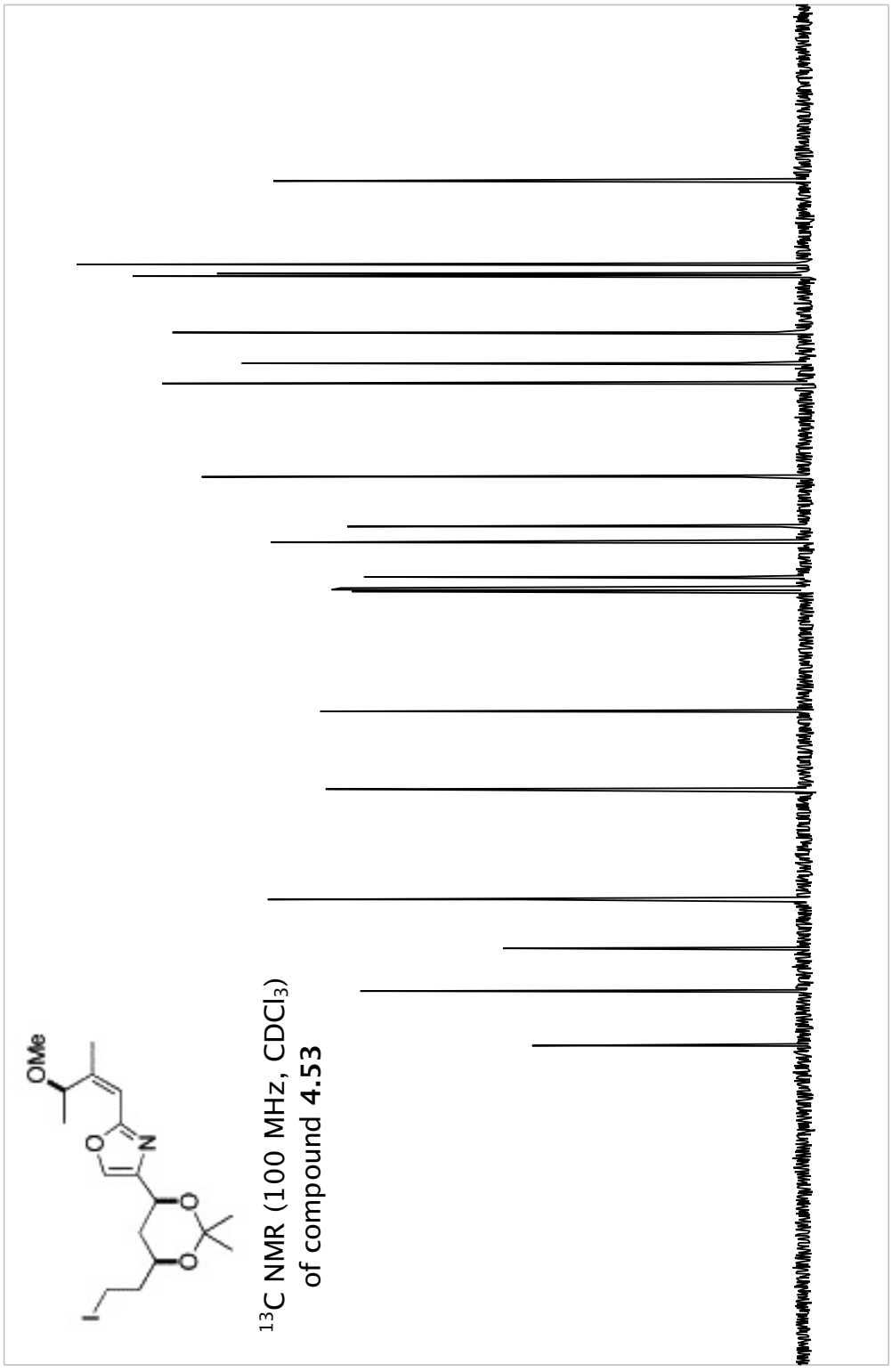


^{1}H NMR (400 MHz, $CDCl_3$)
of compound **4.52**

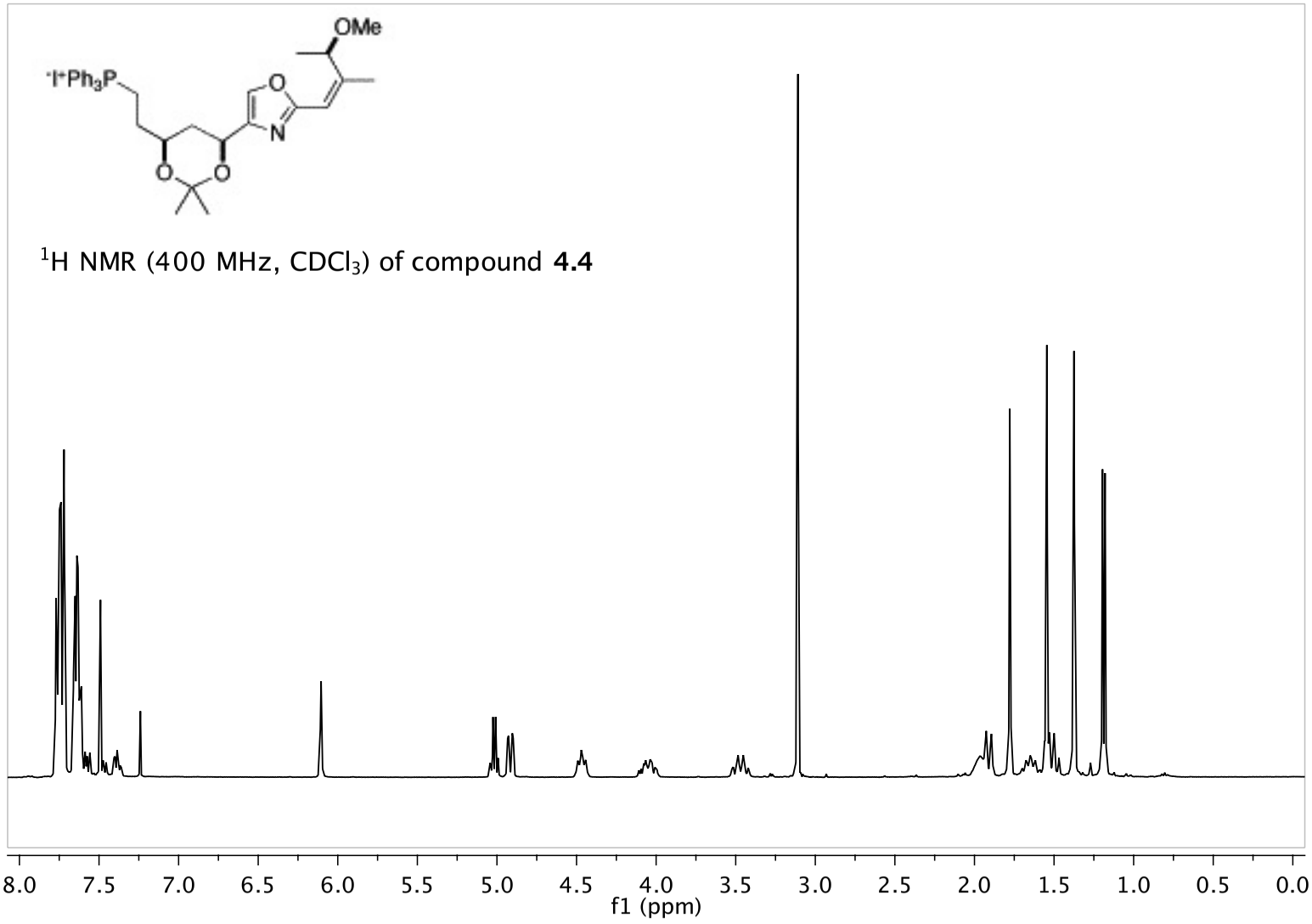


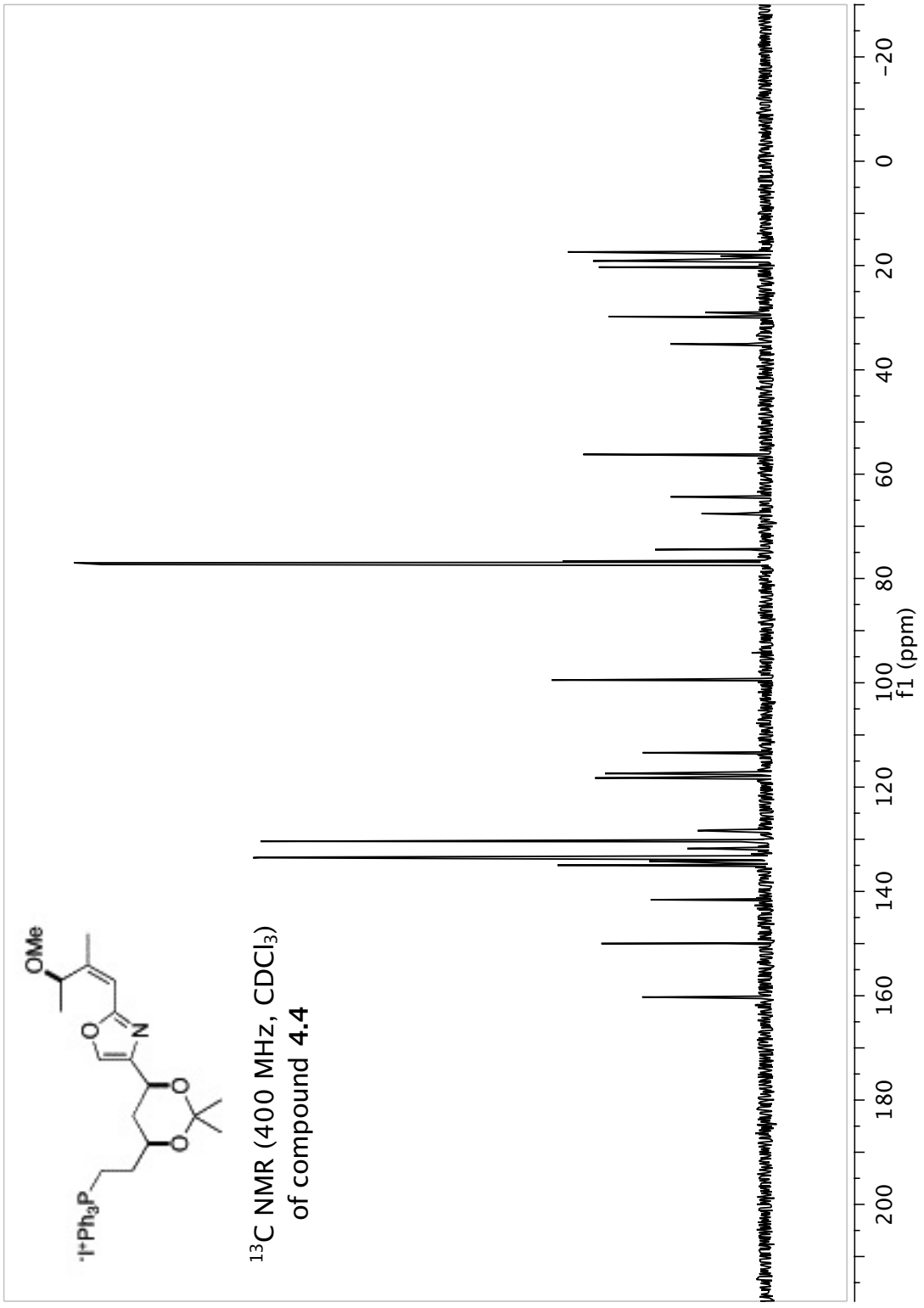
^{13}C NMR (400 MHz, $CDCl_3$)
of compound **4.52**


OMe
I
O
N
O
O
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.53**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

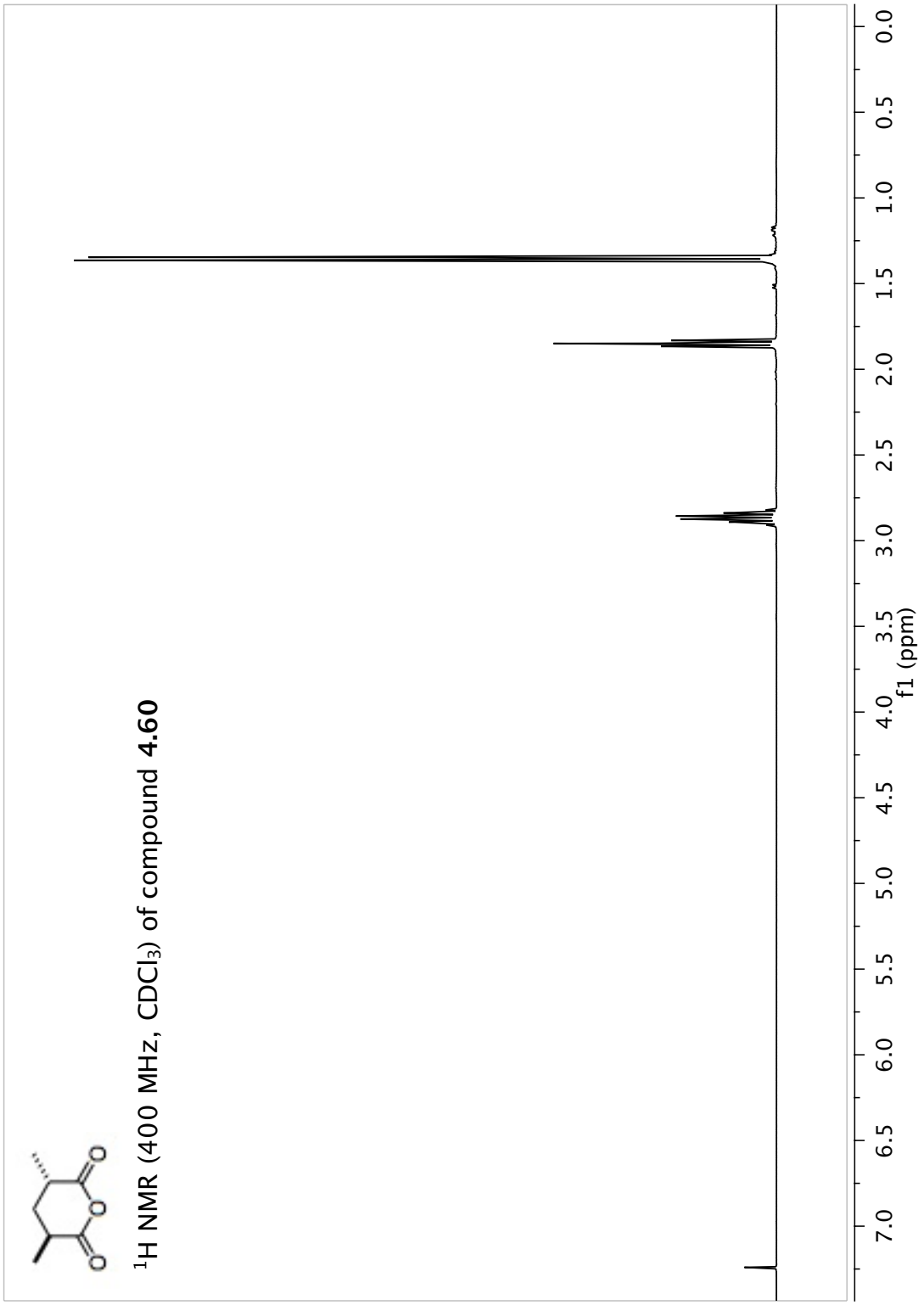


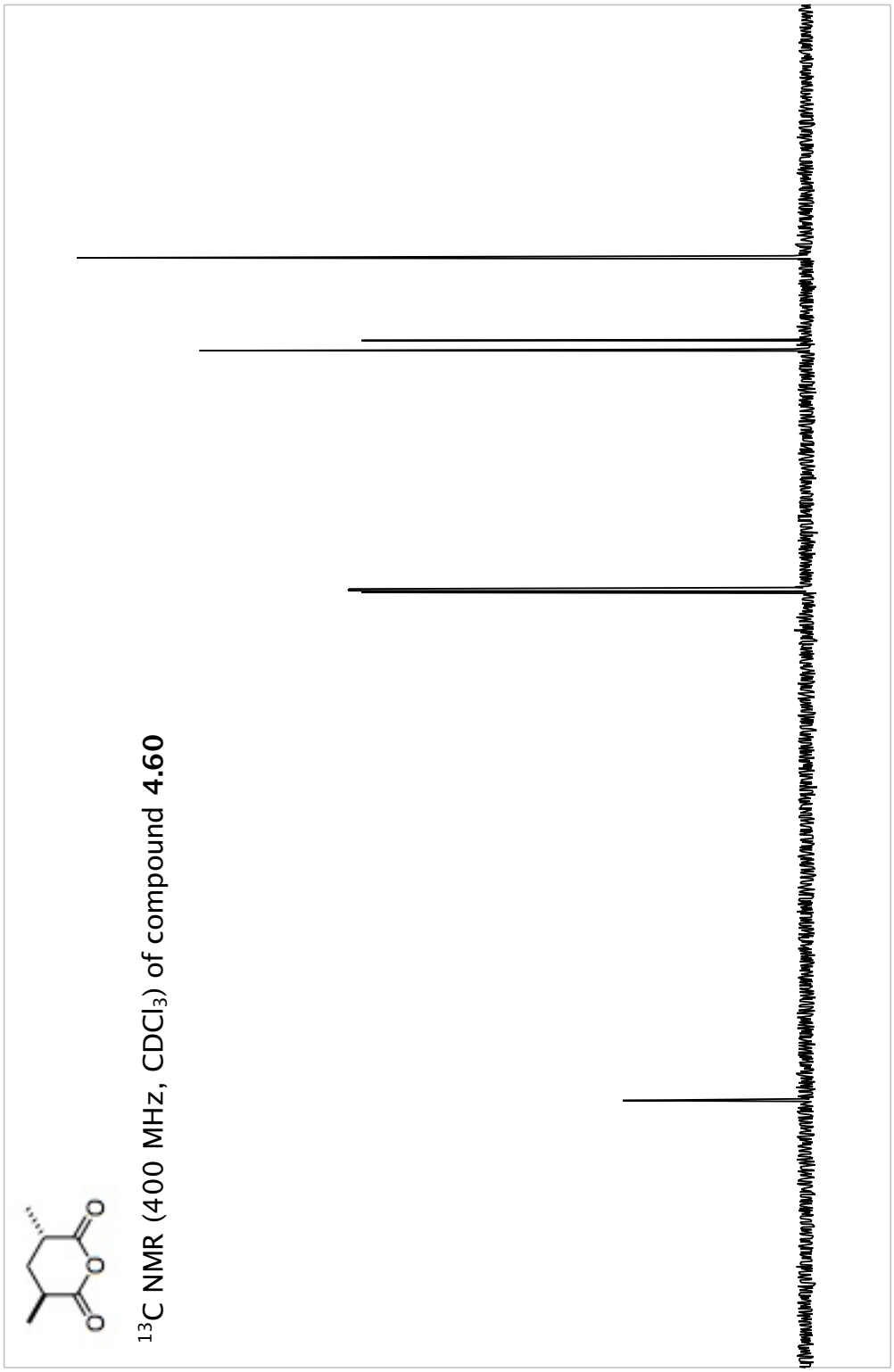
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.53**

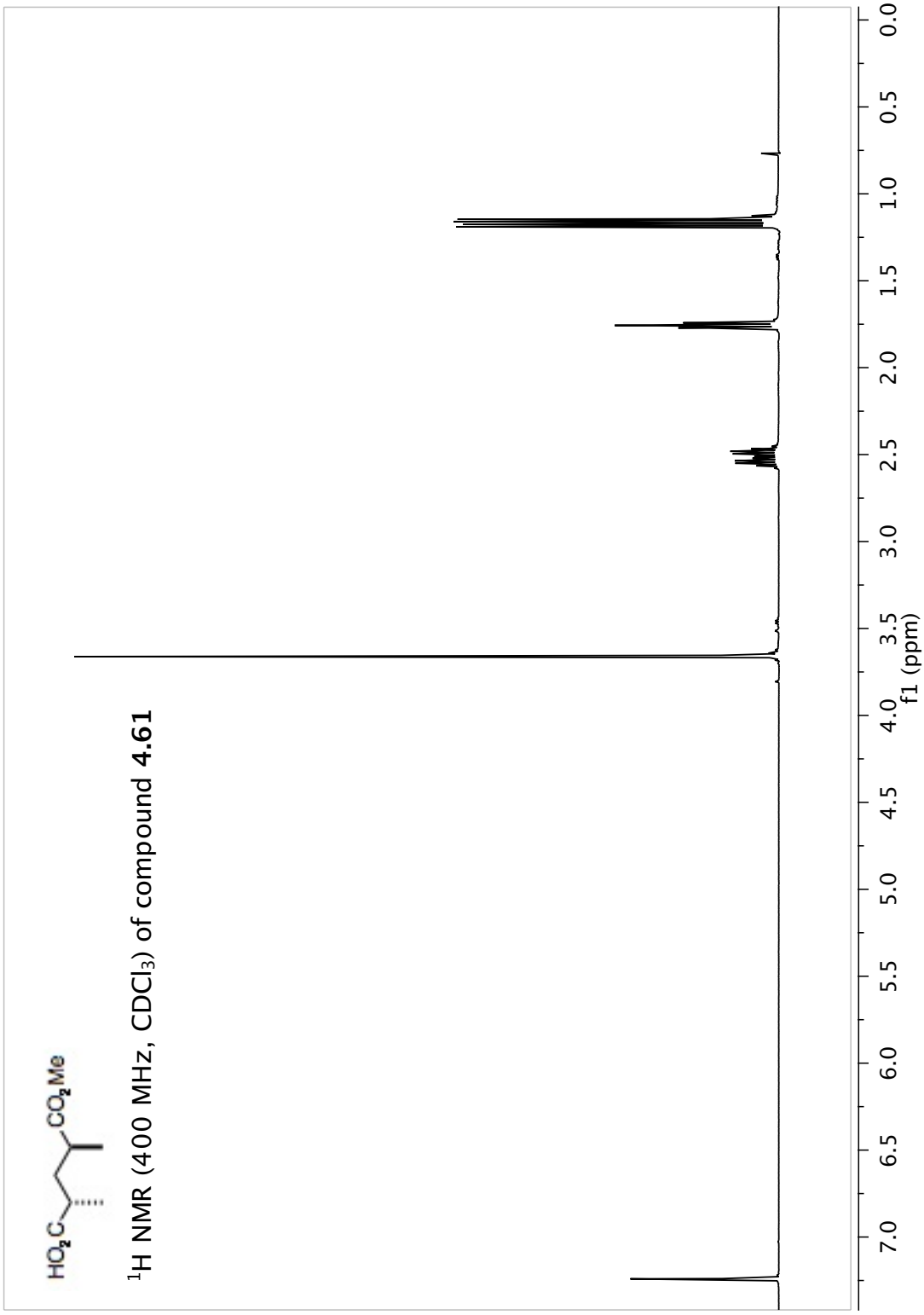

OMe
I⁻Ph3P
O
N
O
O
1H NMR (400 MHz, CDCl3) of compound 4.4
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



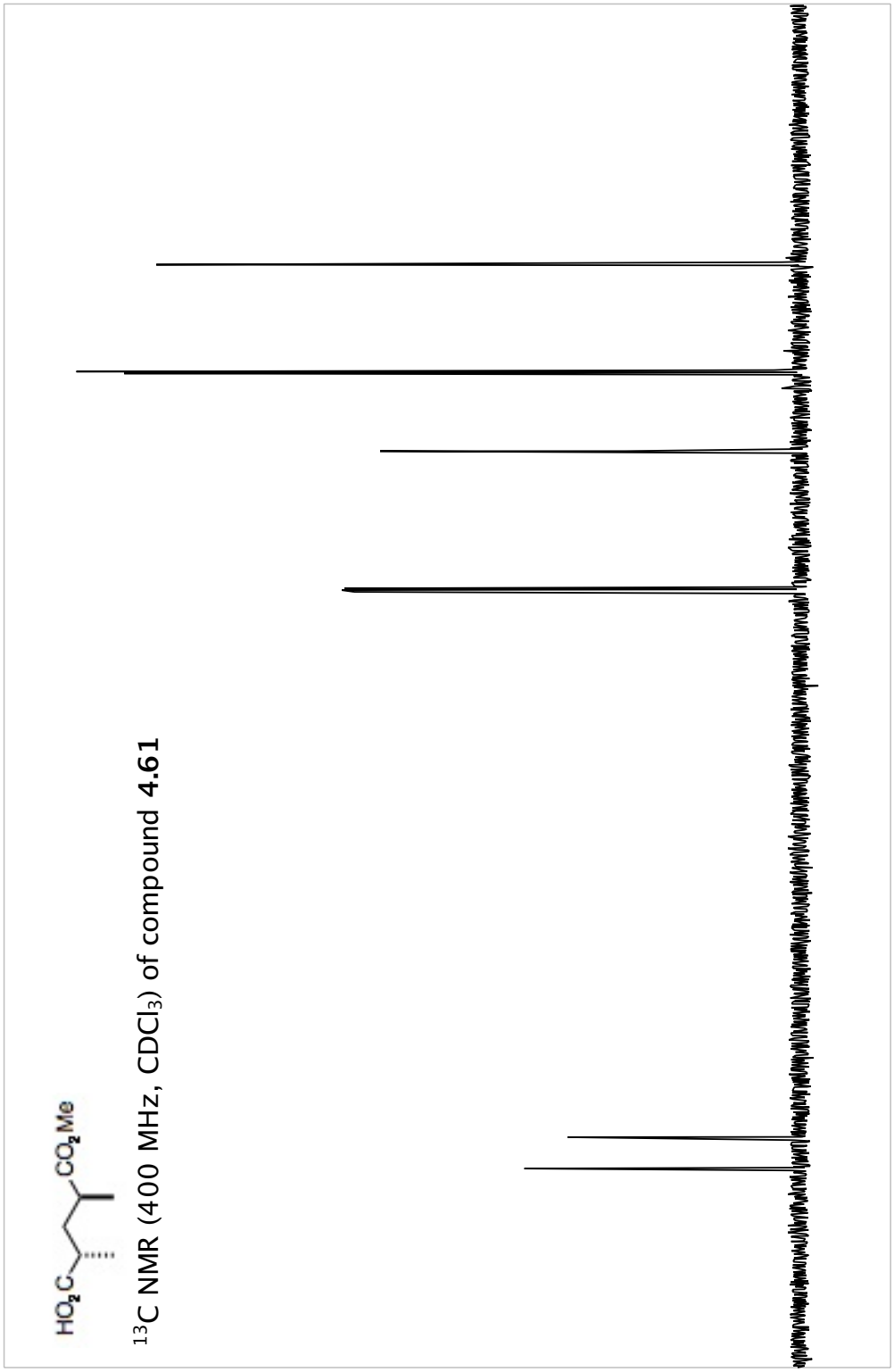
^{13}C NMR (400 MHz, $CDCl_3$)
of compound **4.4**


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.60**
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

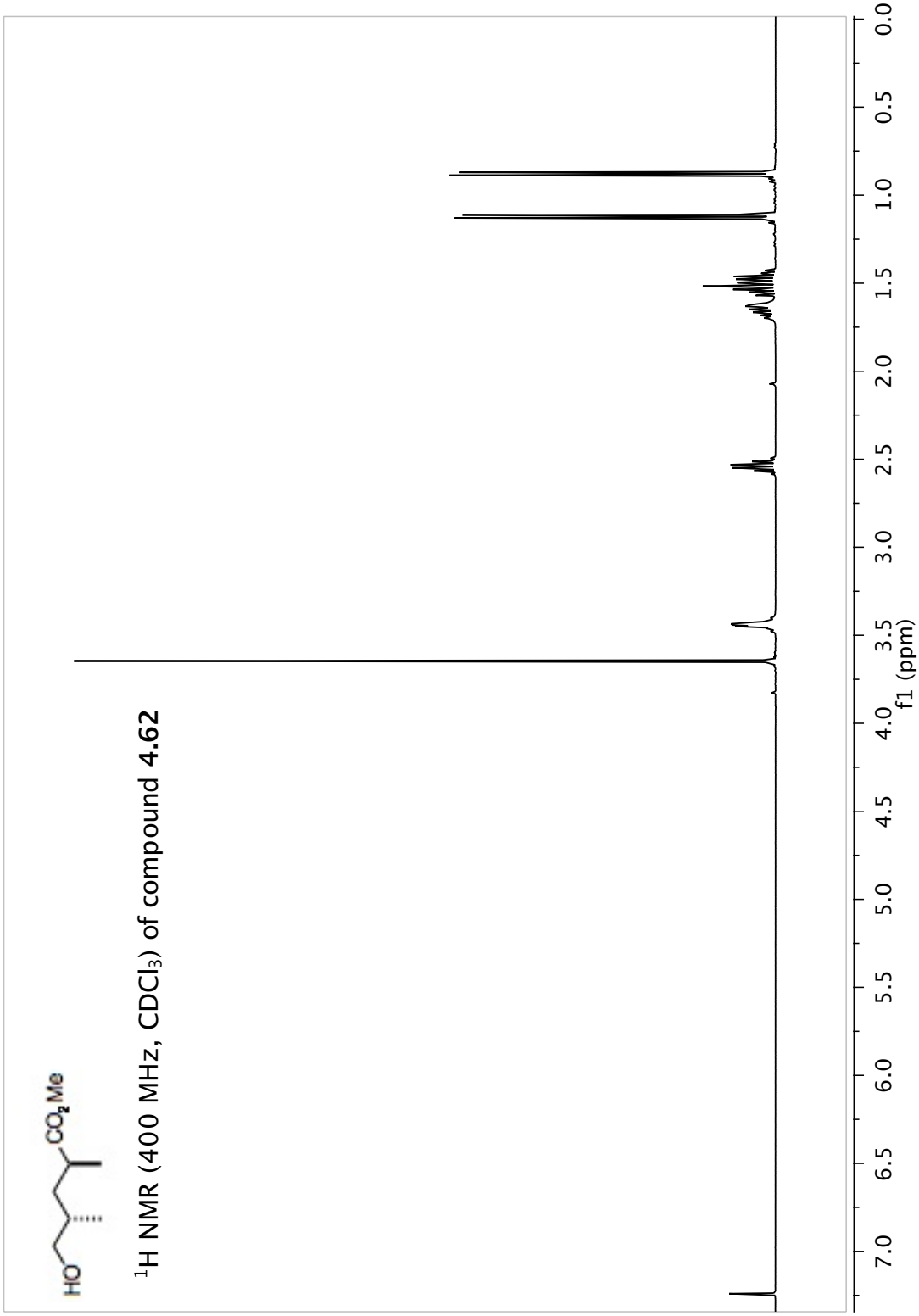

^{13}C NMR (400 MHz, $CDCl_3$) of compound **4.60**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



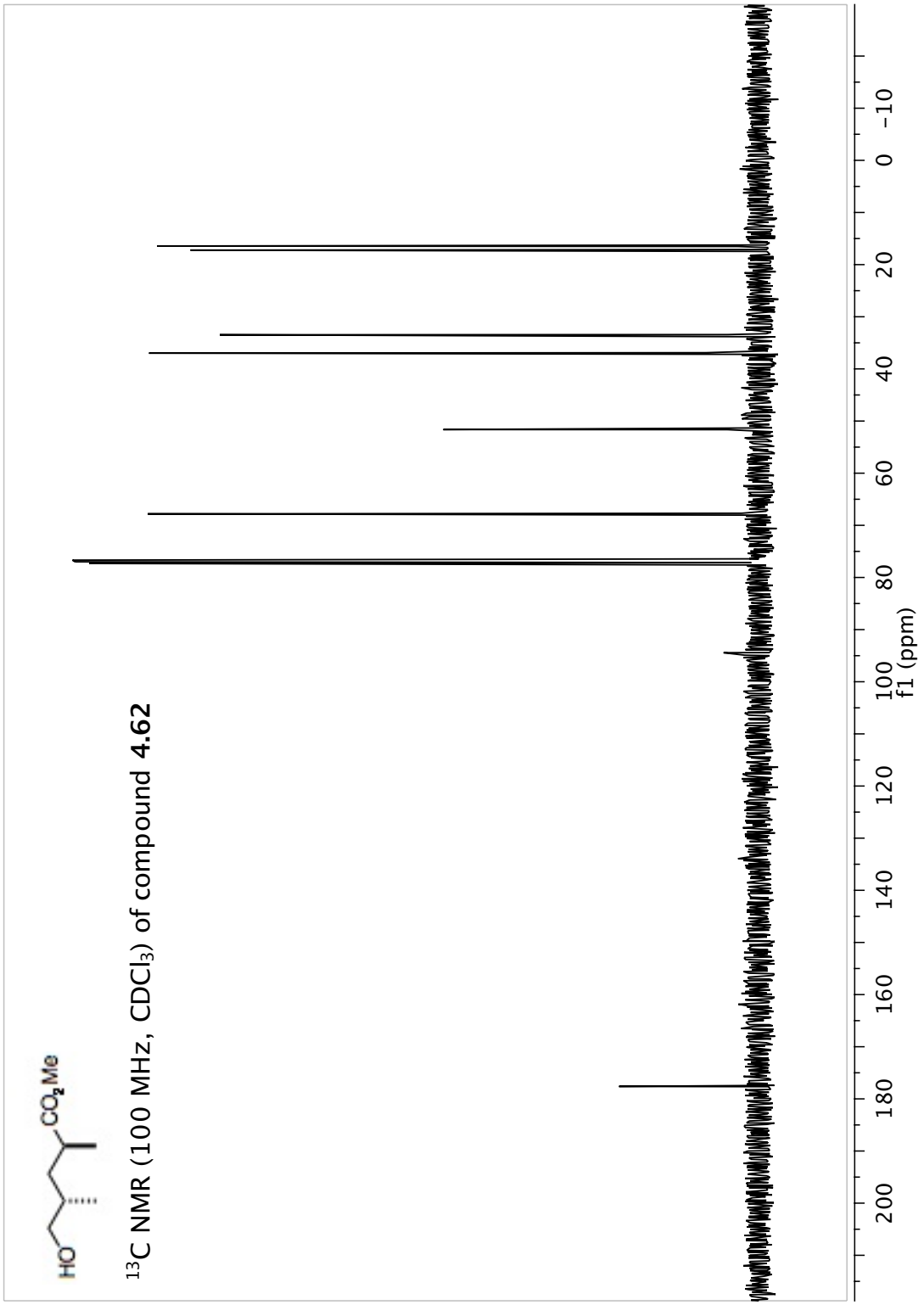
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.61**



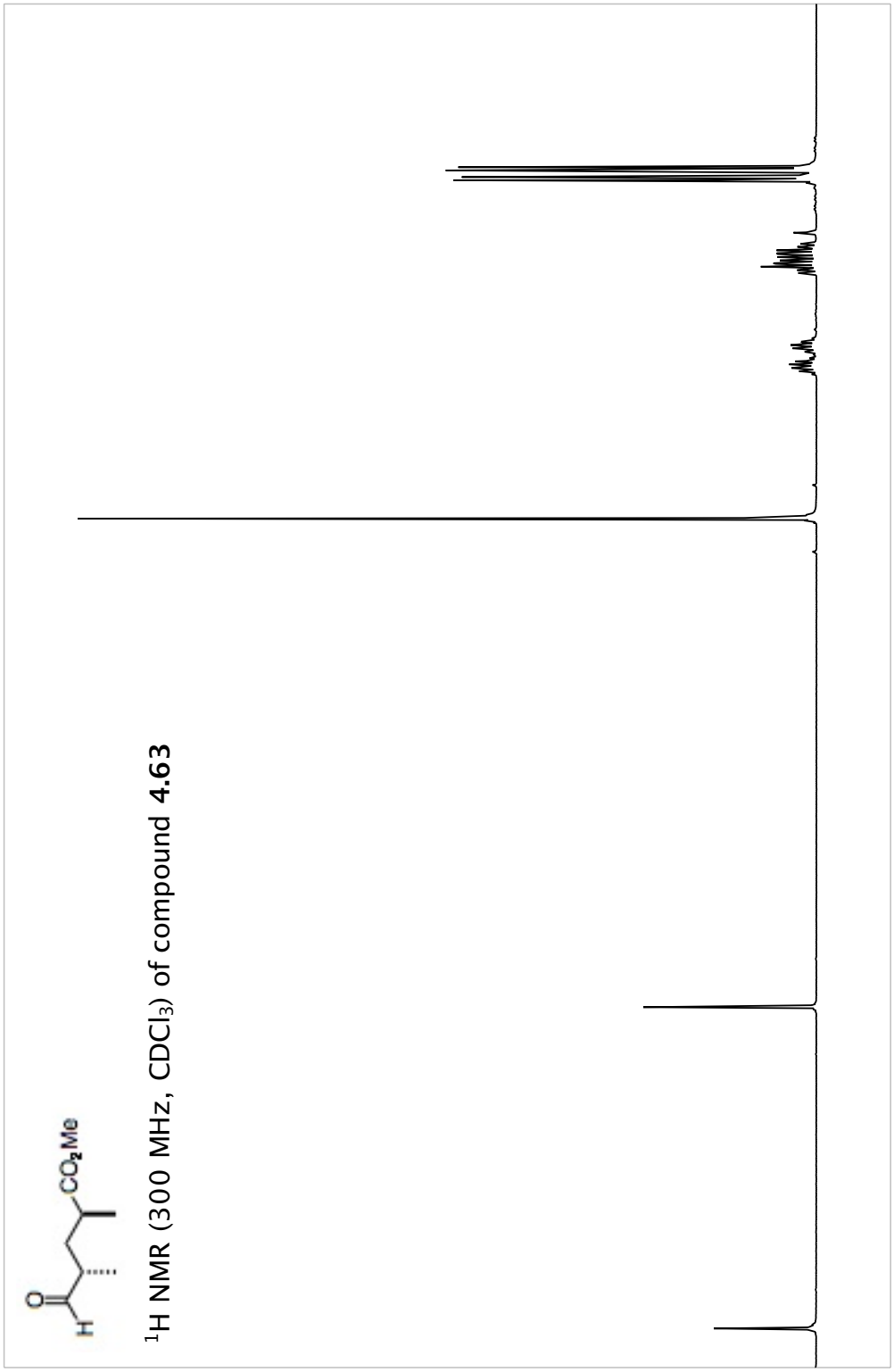
^{13}C NMR (400 MHz, $CDCl_3$) of compound **4.61**



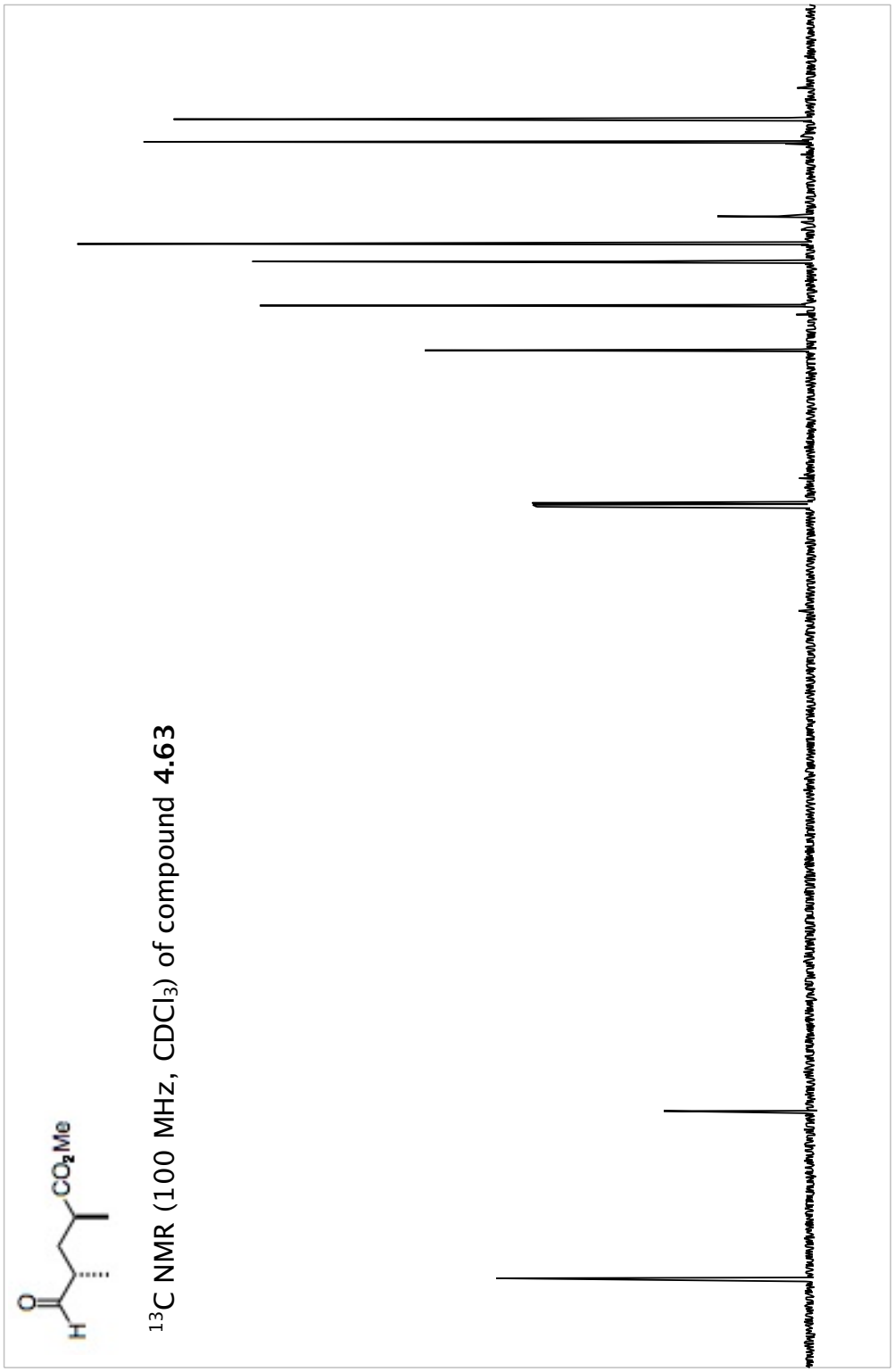
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.62**



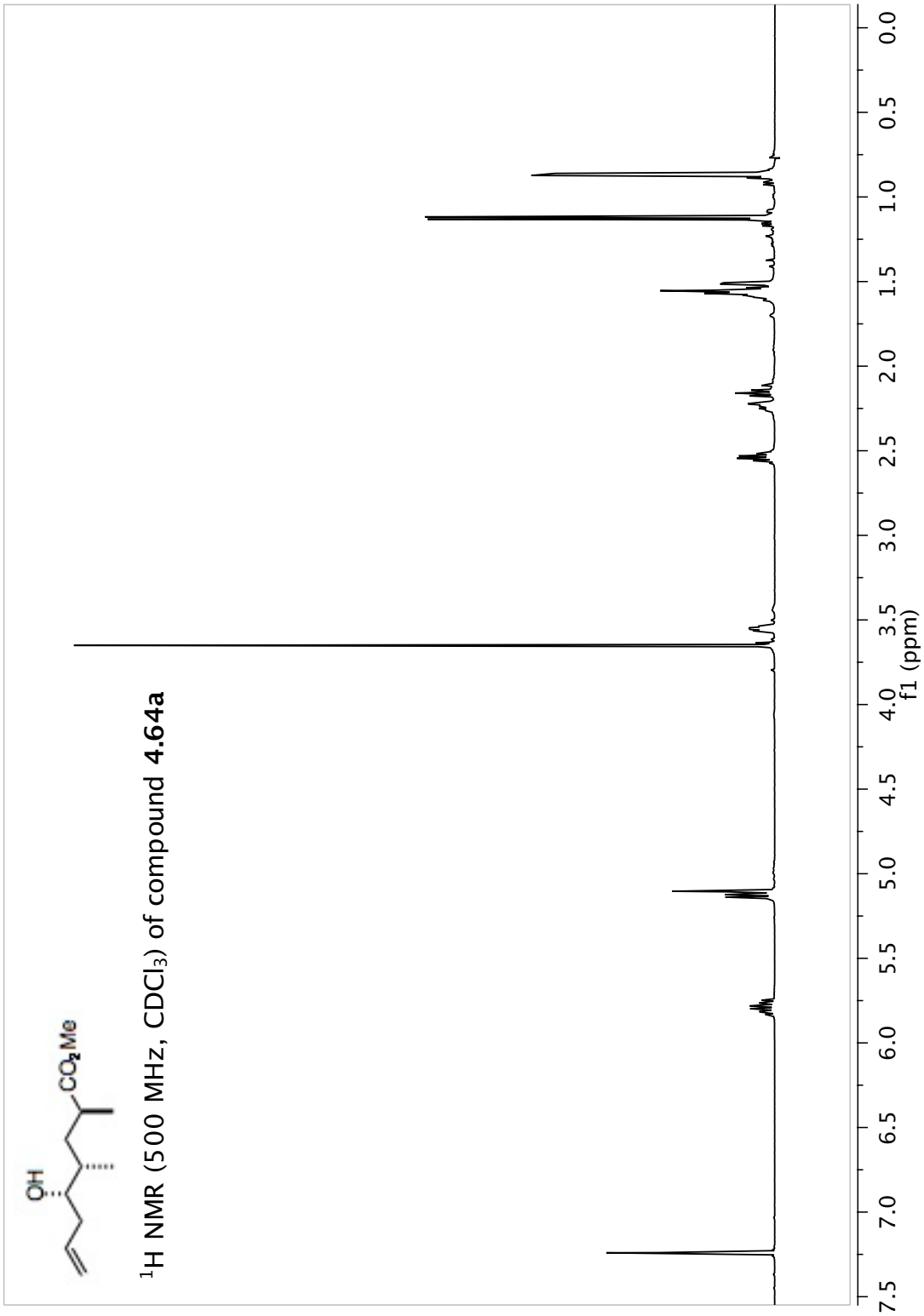
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.62**



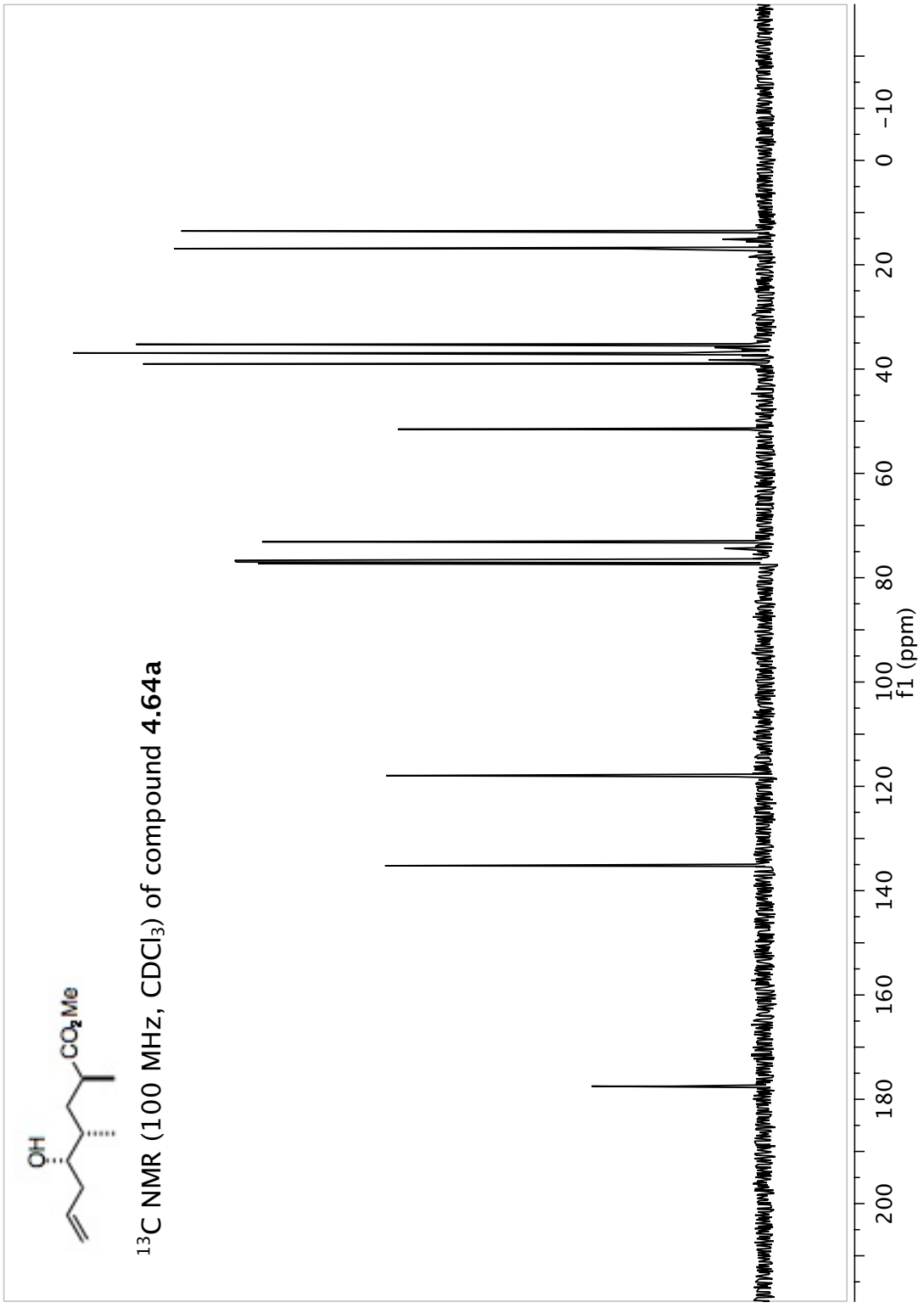
^{1}H NMR (300 MHz, $CDCl_3$) of compound **4.63**



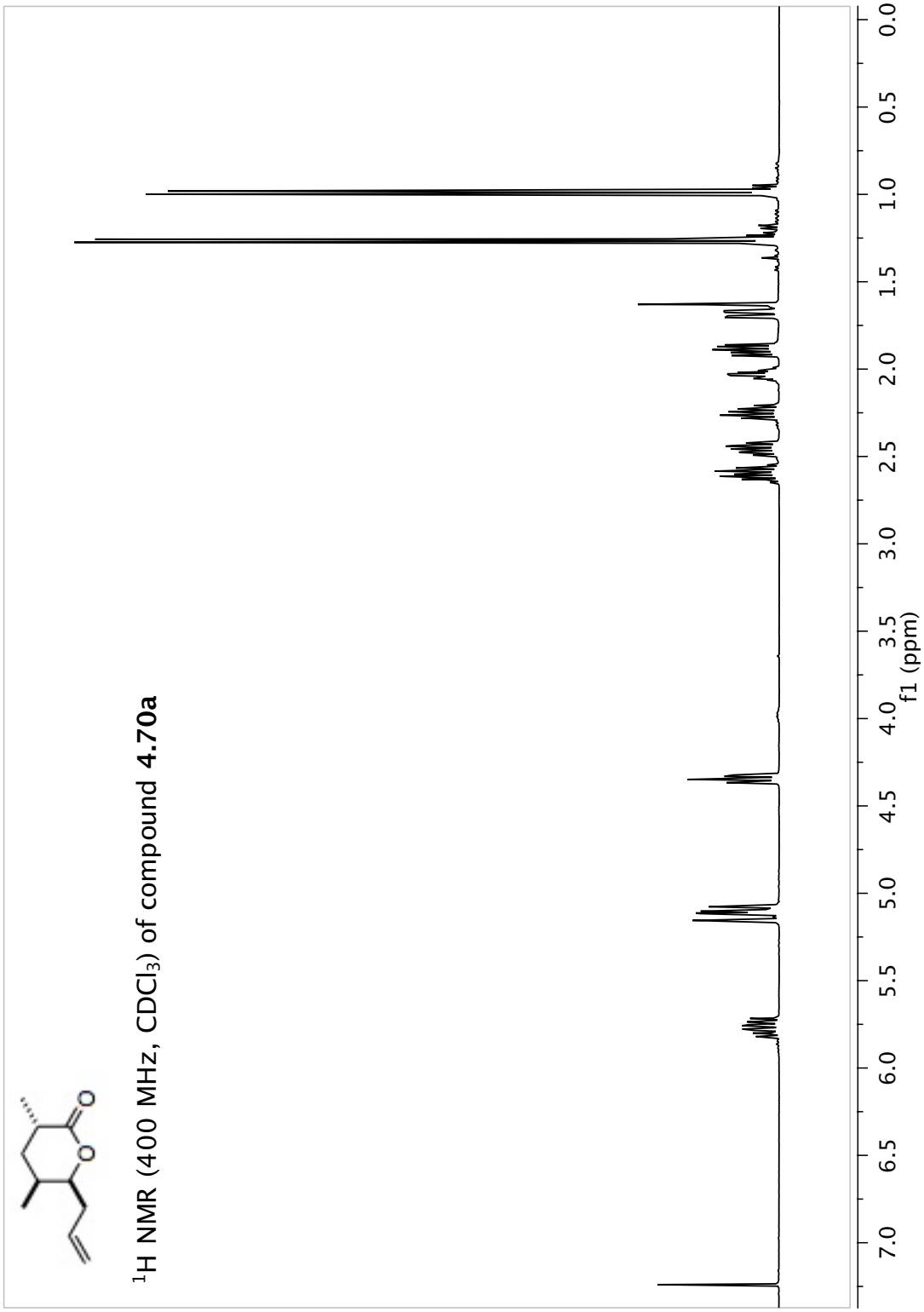
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.63**



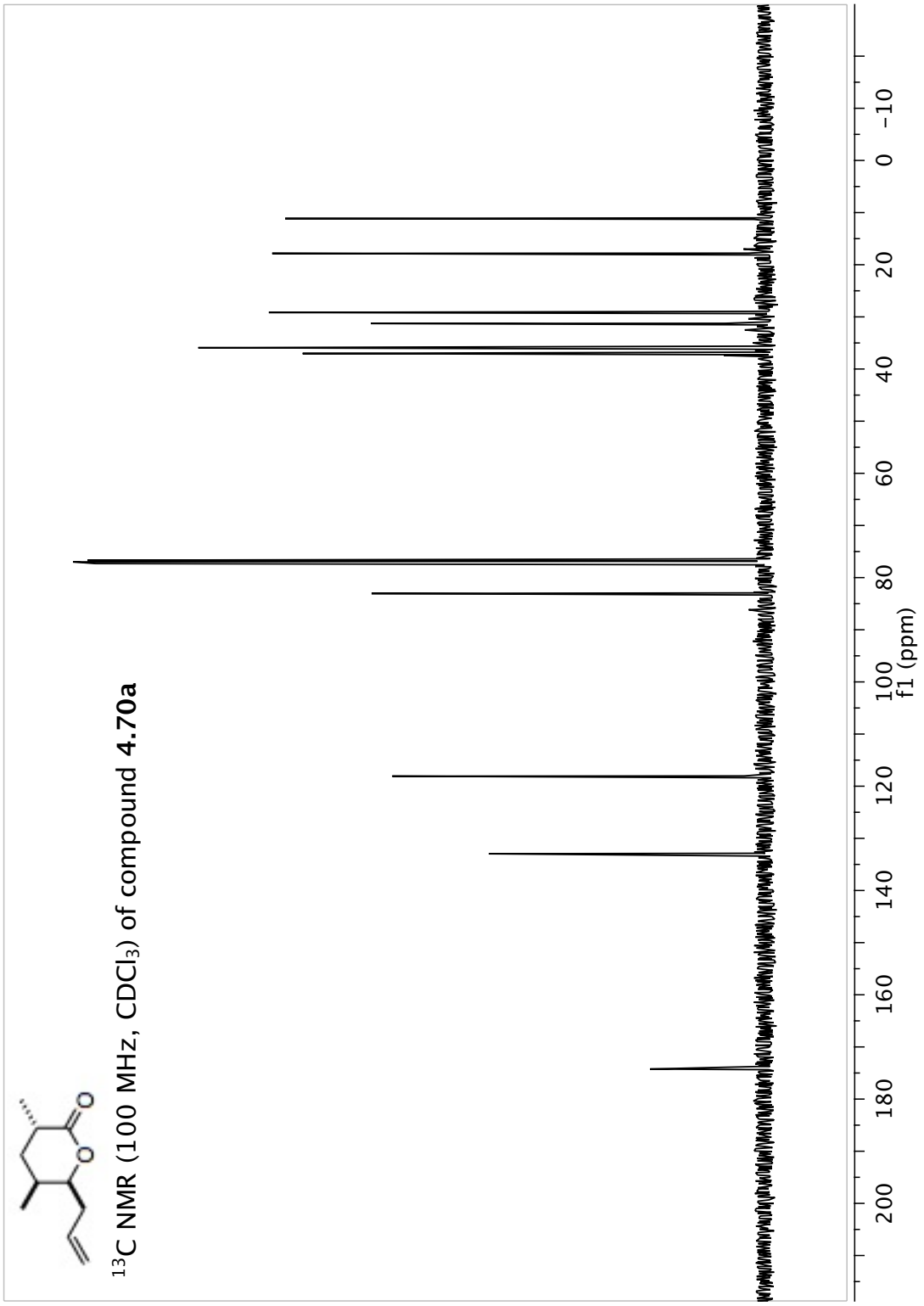
^{1}H NMR (500 MHz, $CDCl_3$) of compound **4.64a**

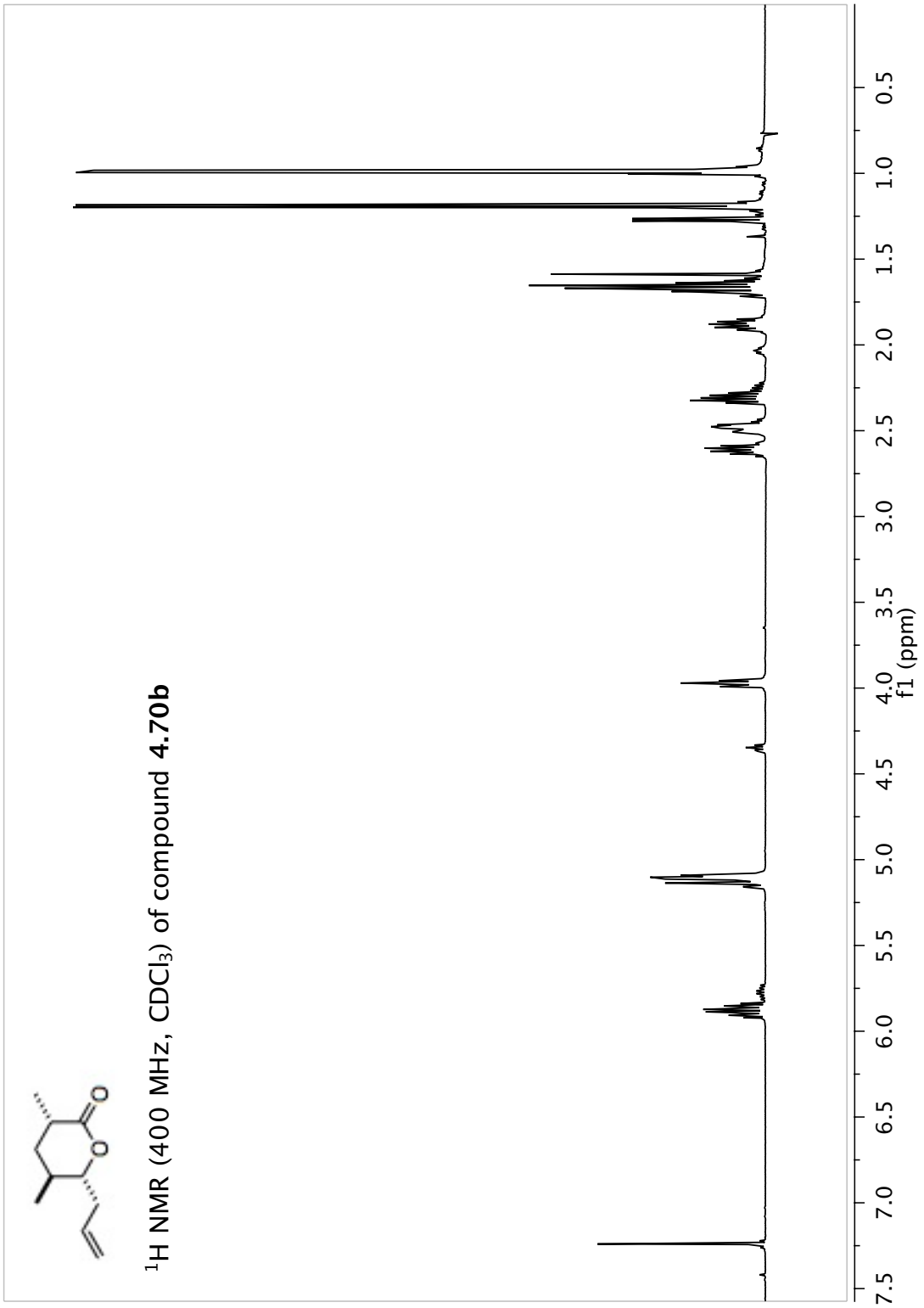


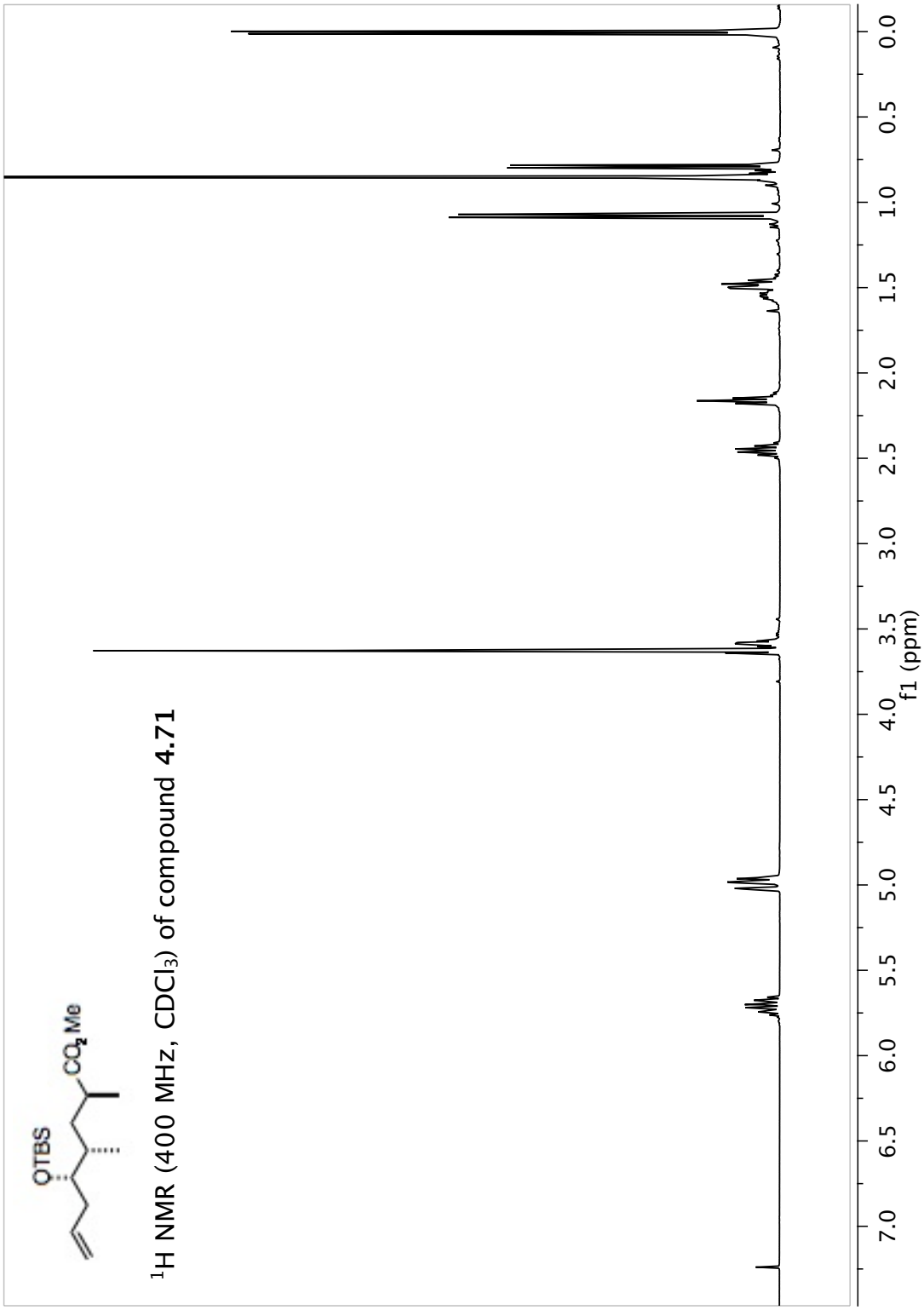
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.64a**



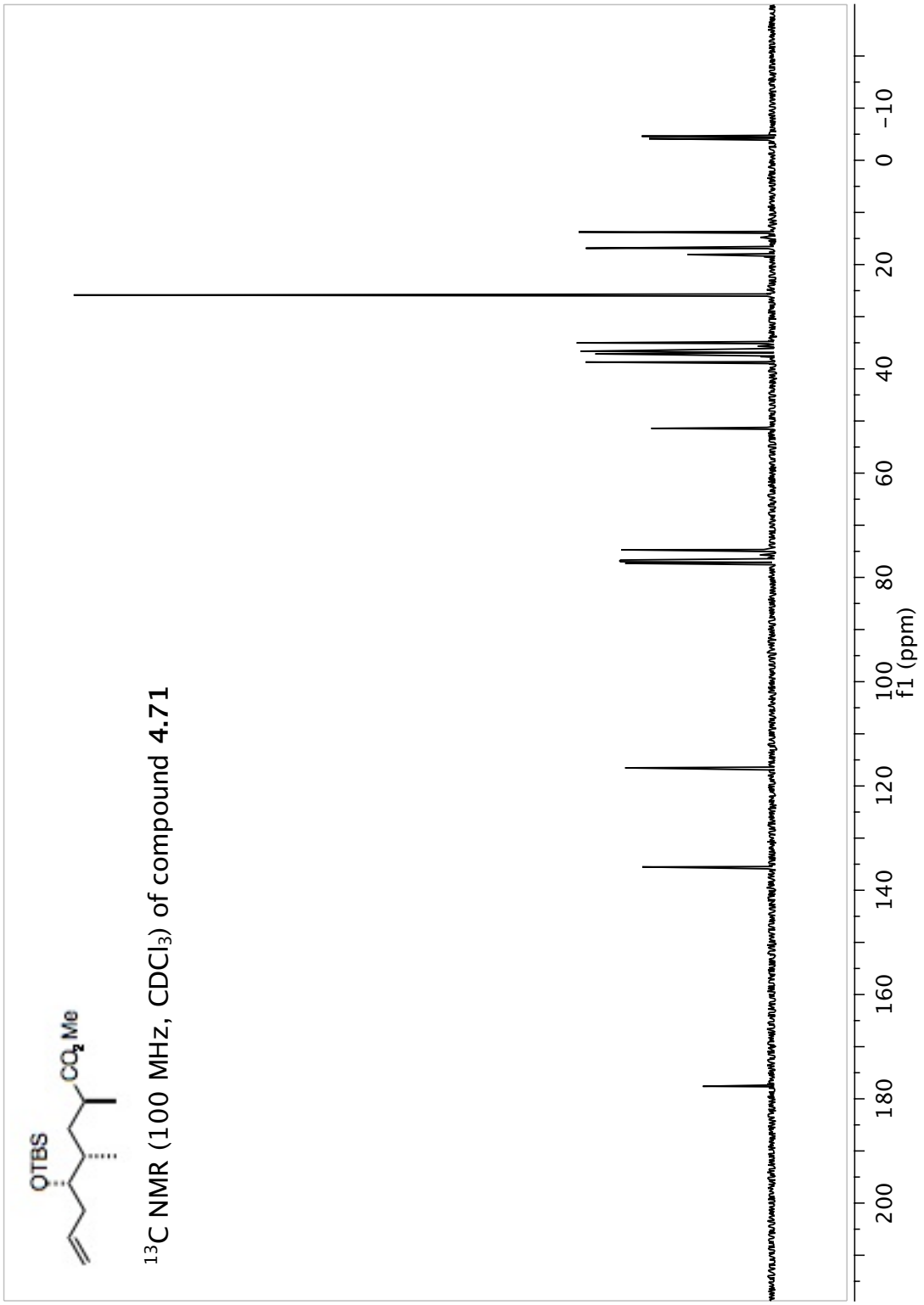
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.70a**


^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.70a**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

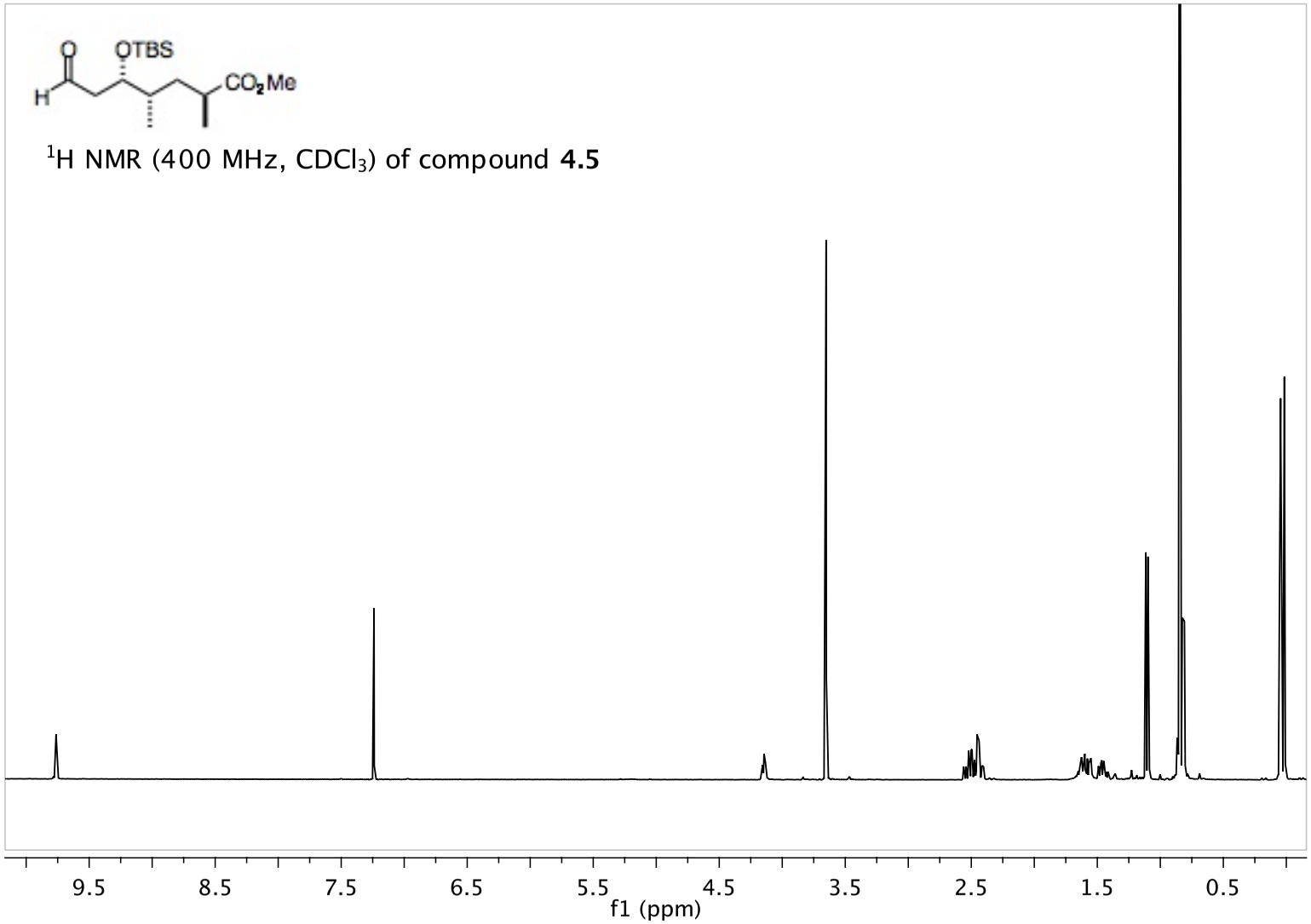

^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.70b**
O
O
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

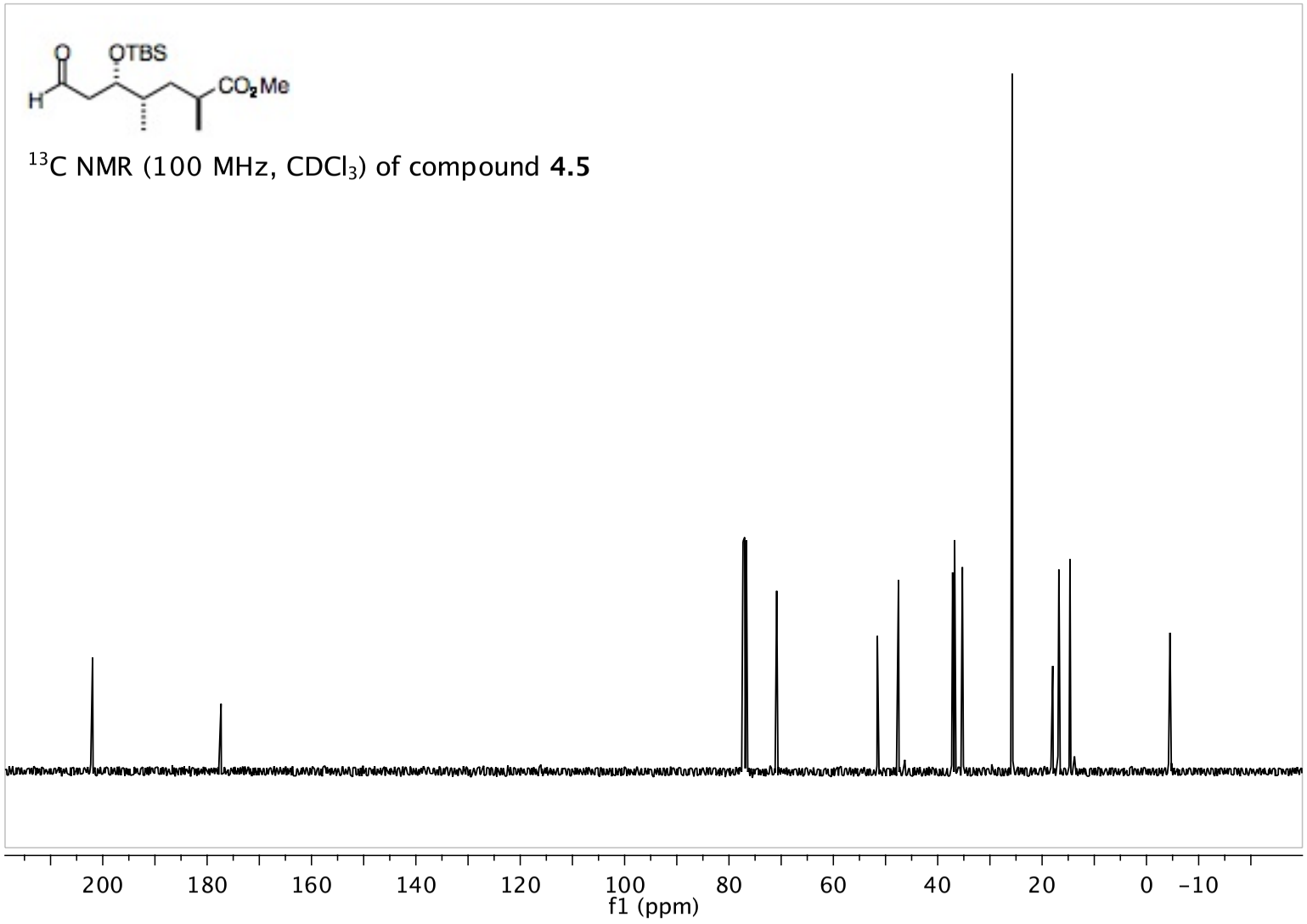


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.71**

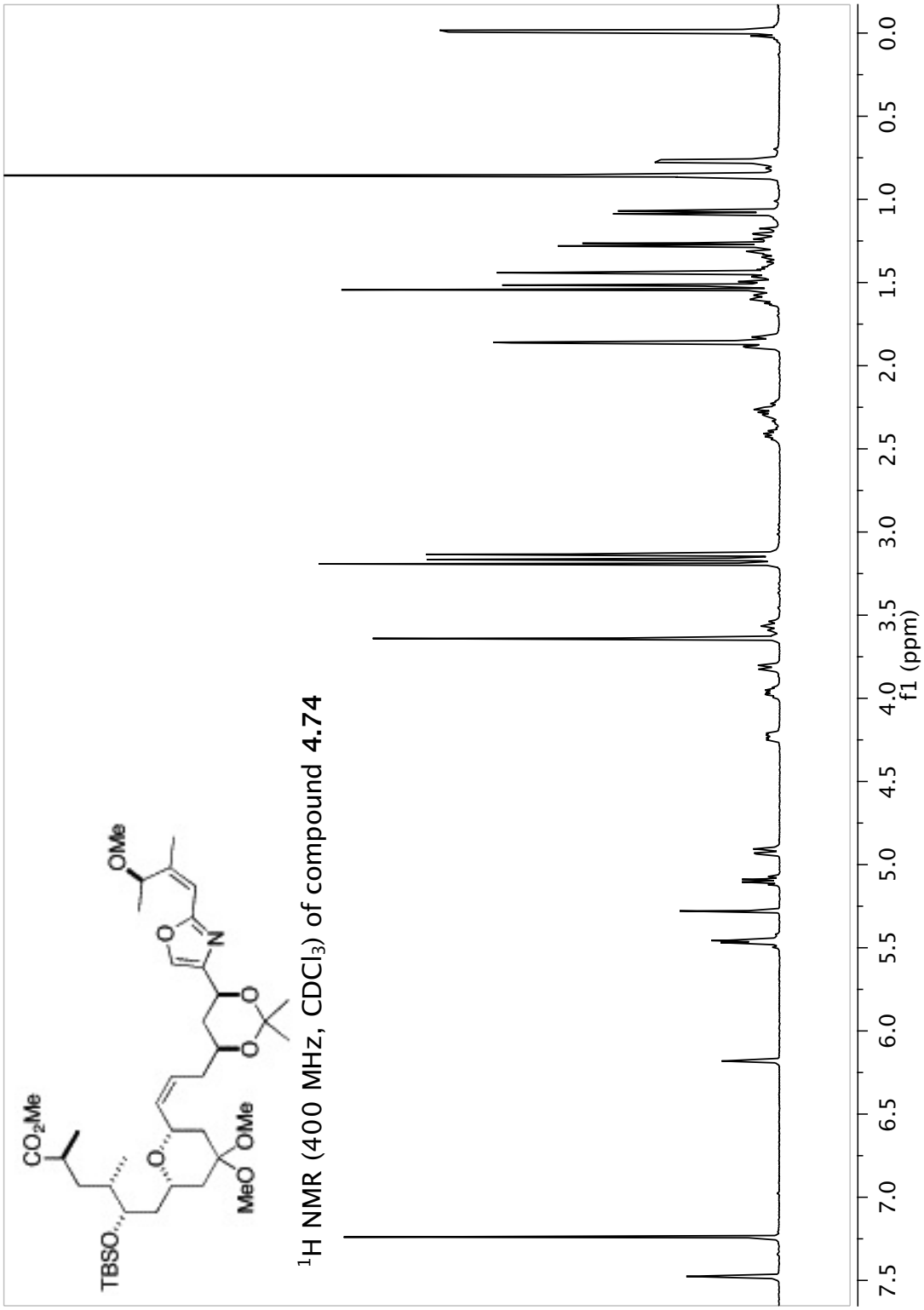


^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.71**

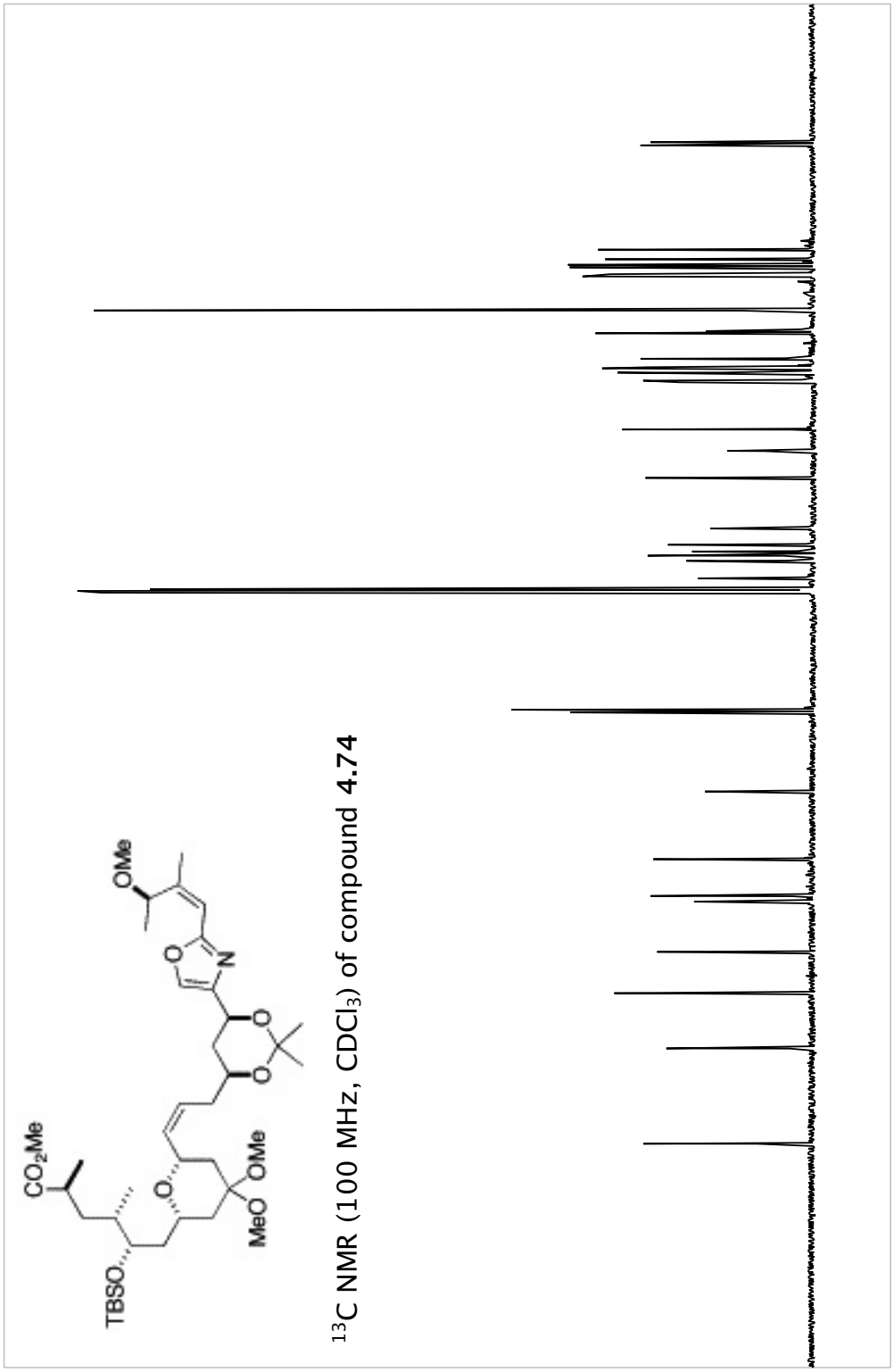

O
OTBS
H
CO2Me
1H NMR (400 MHz, CDCl3) of compound 4.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)



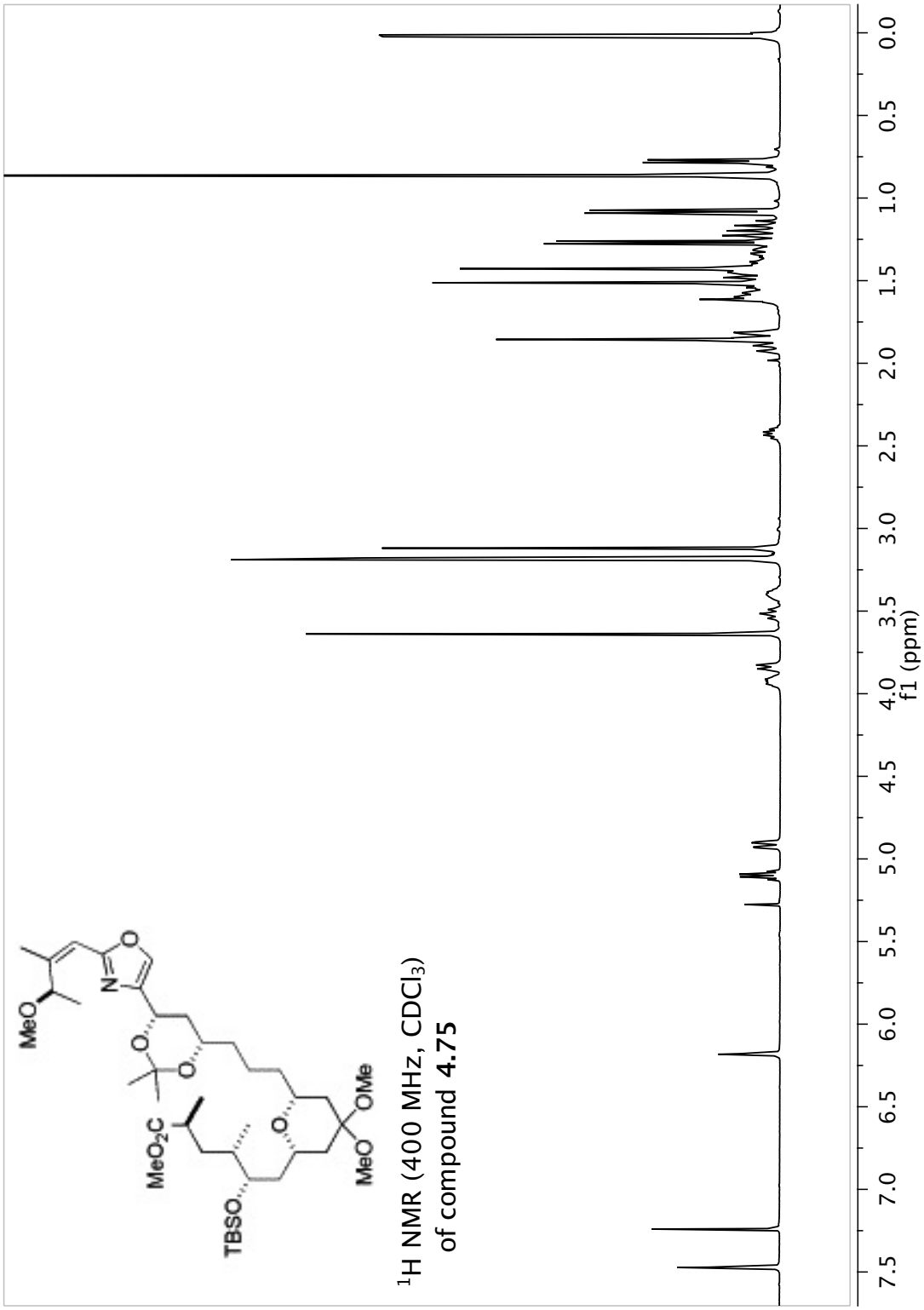
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.5**



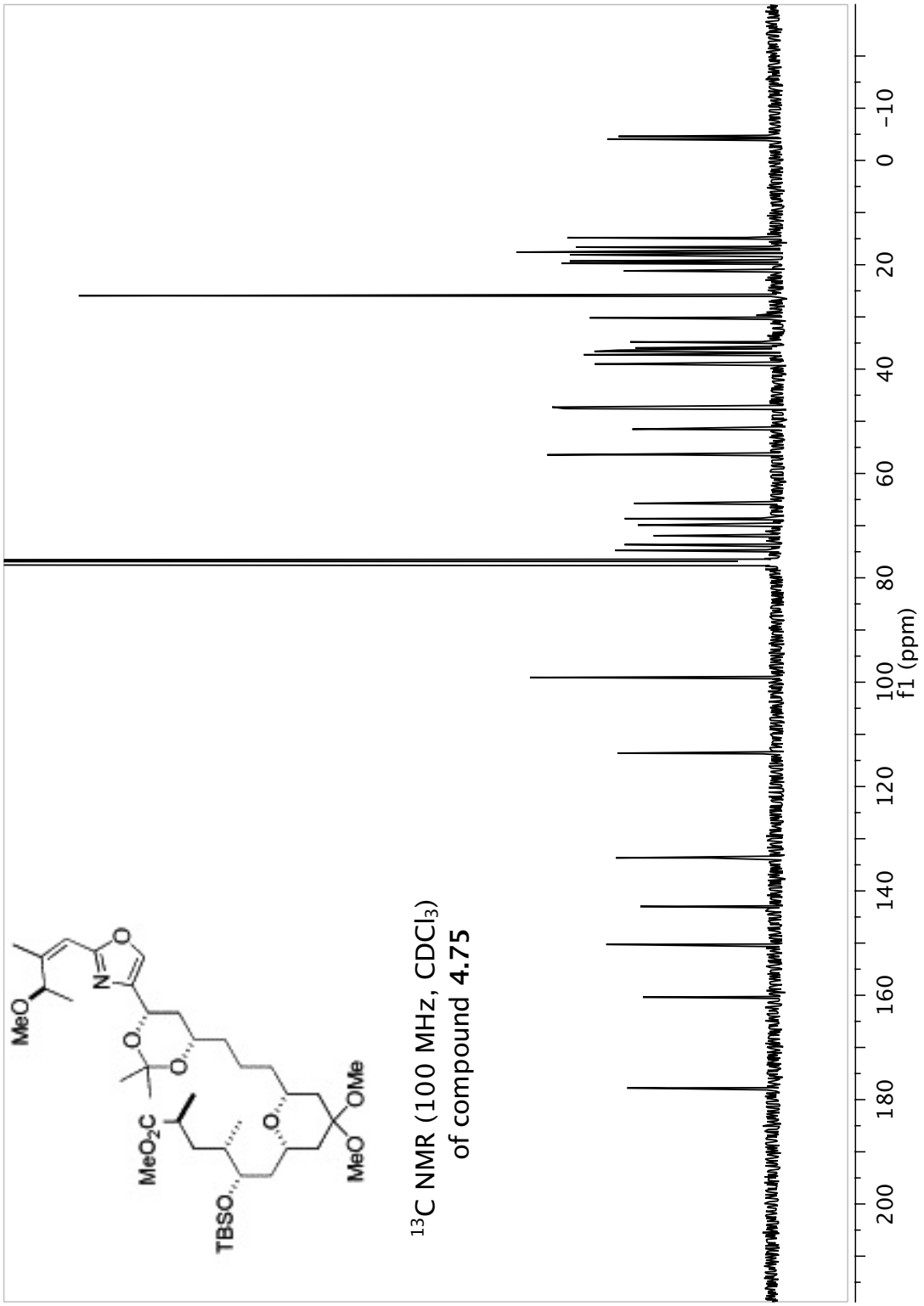
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.74**



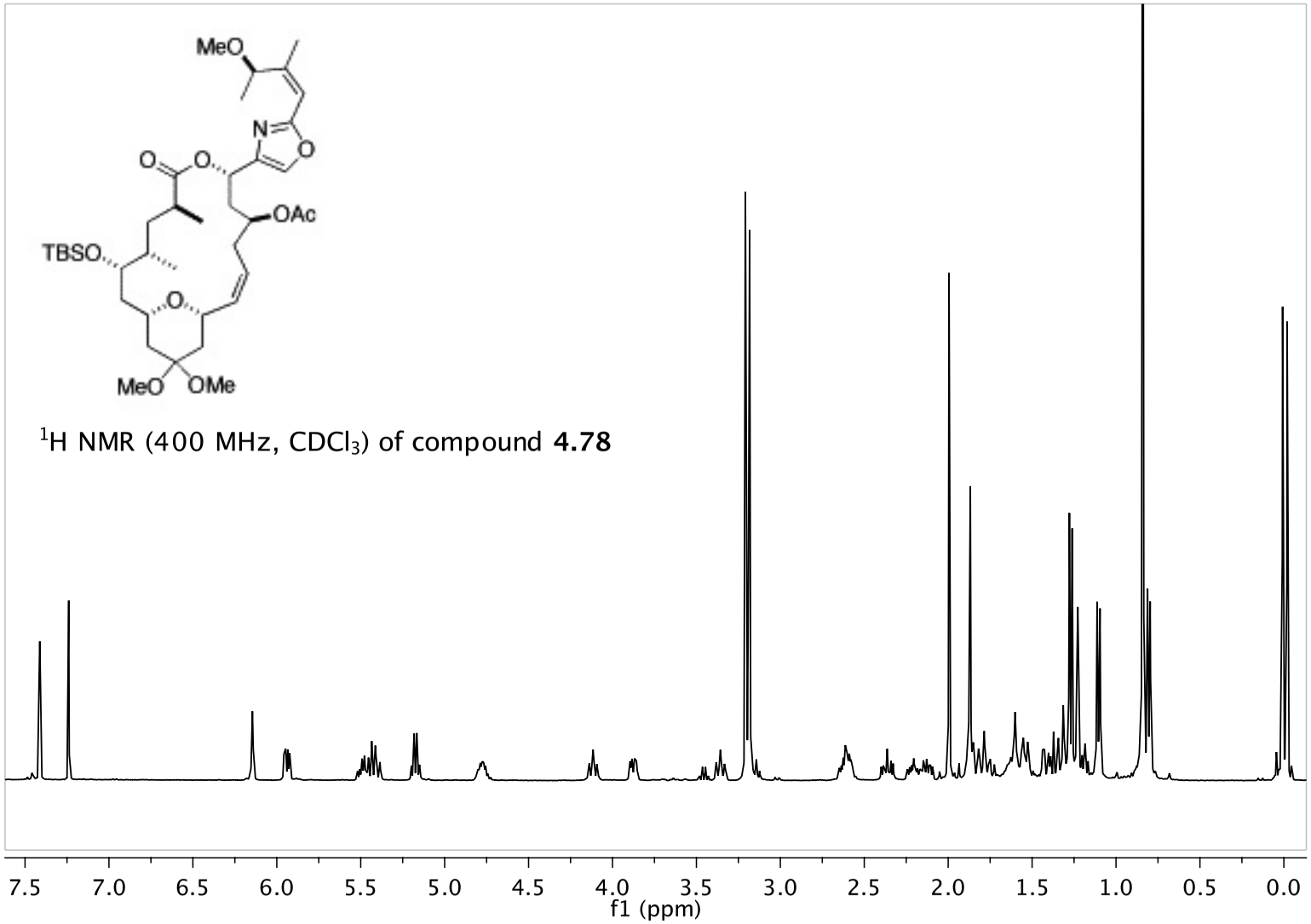
^{13}C NMR (100 MHz, $CDCl_3$) of compound **4.74**



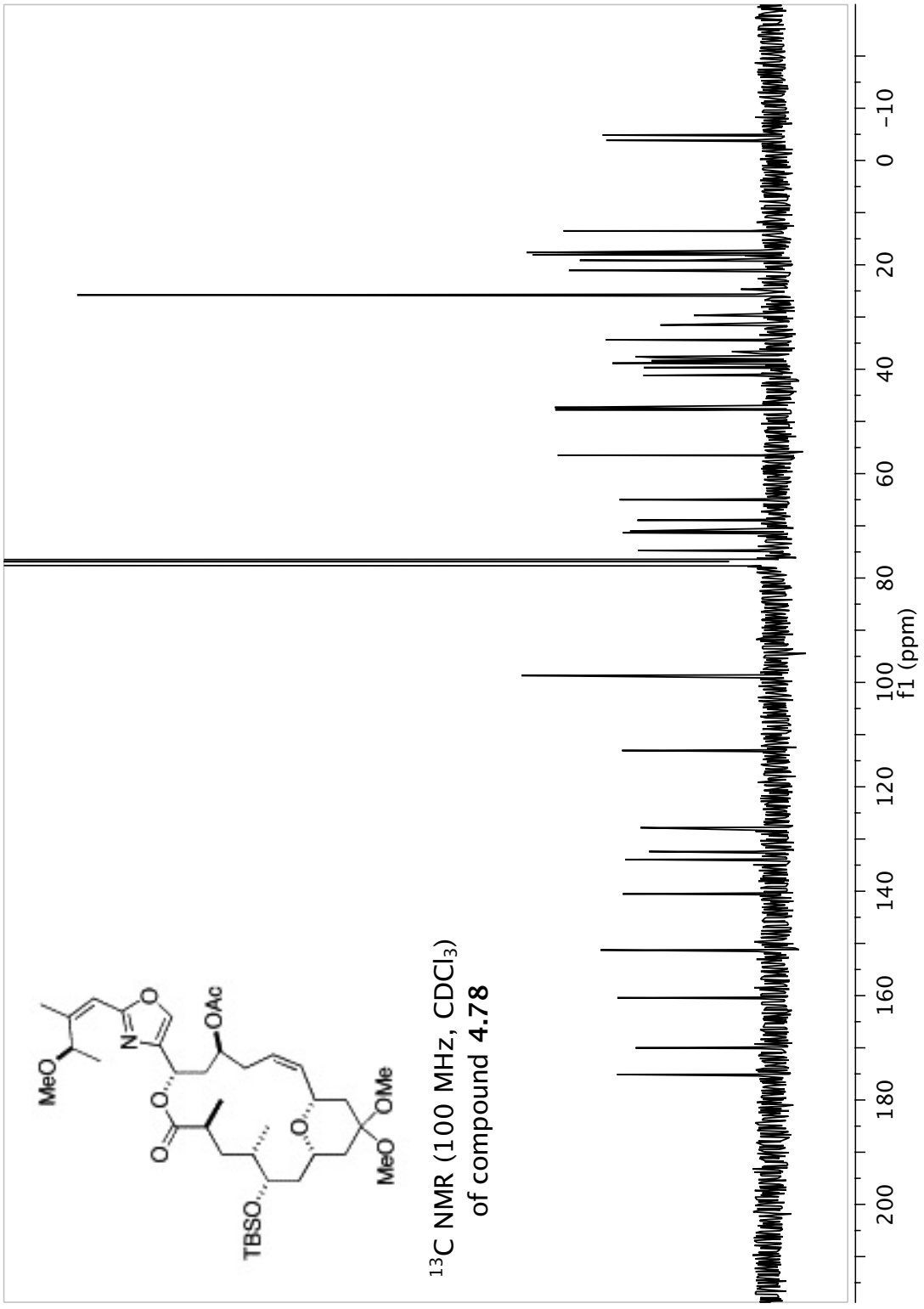
^{1}H NMR (400 MHz, $CDCl_3$)
of compound **4.75**



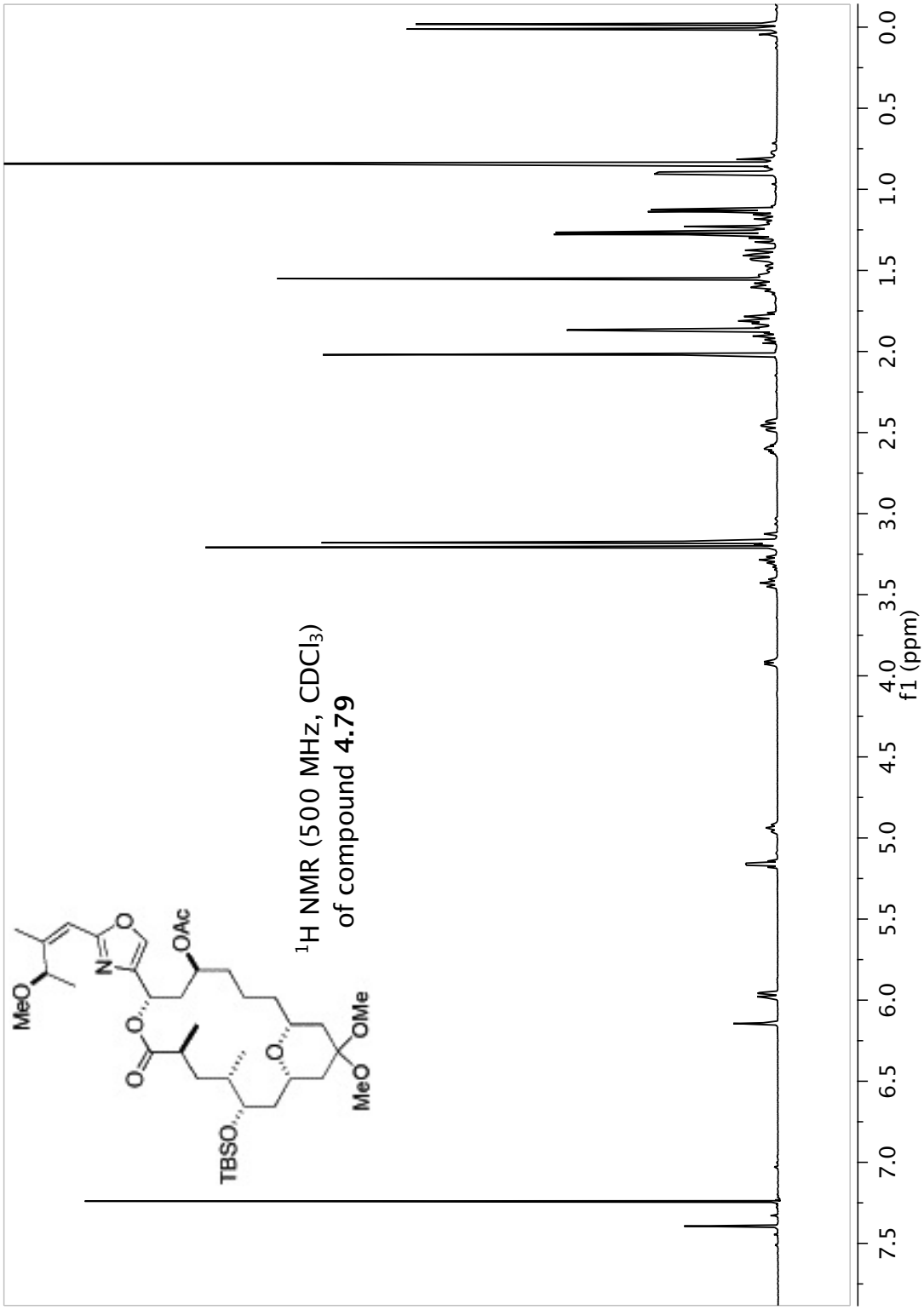
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.75**

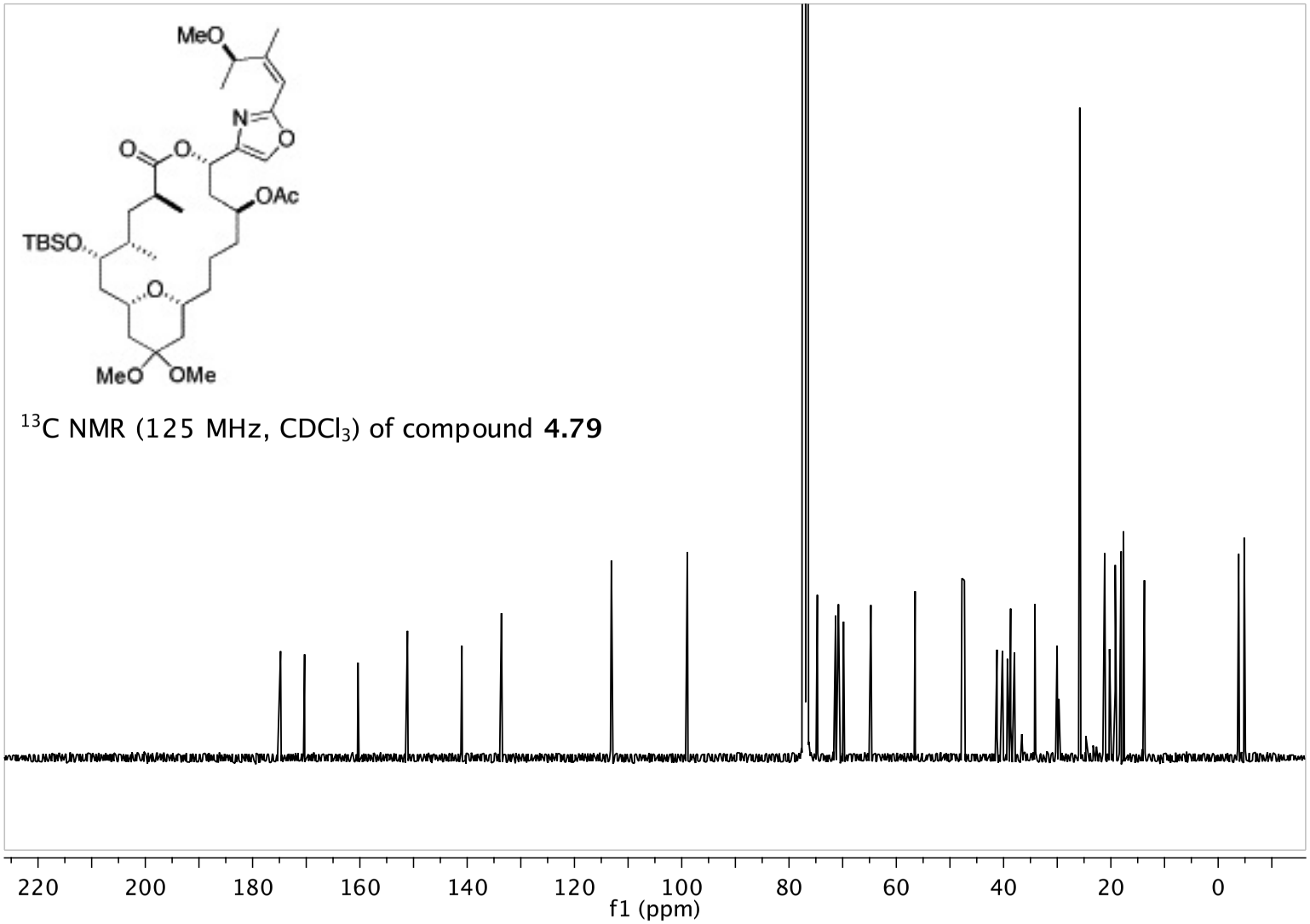


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.78**

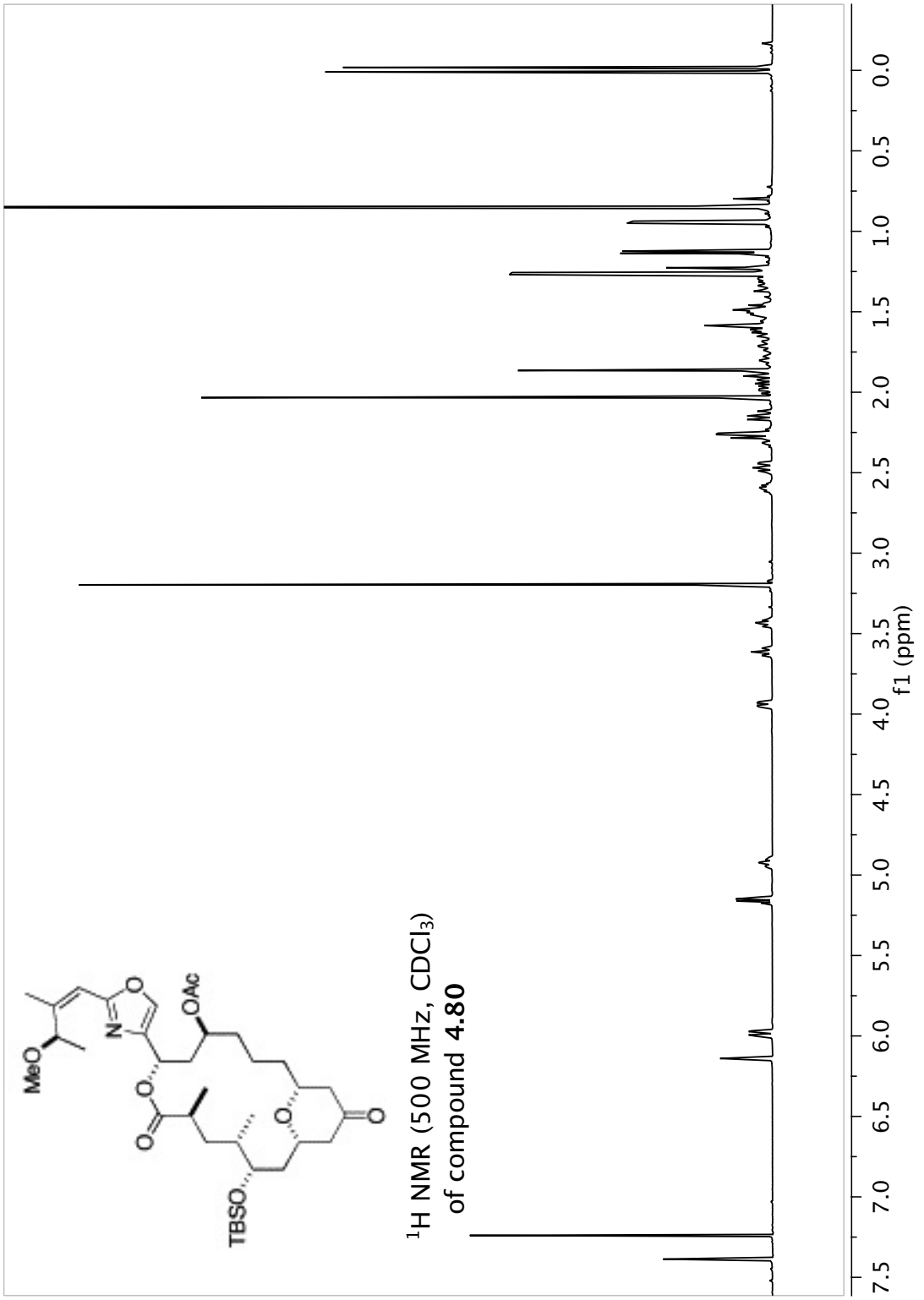


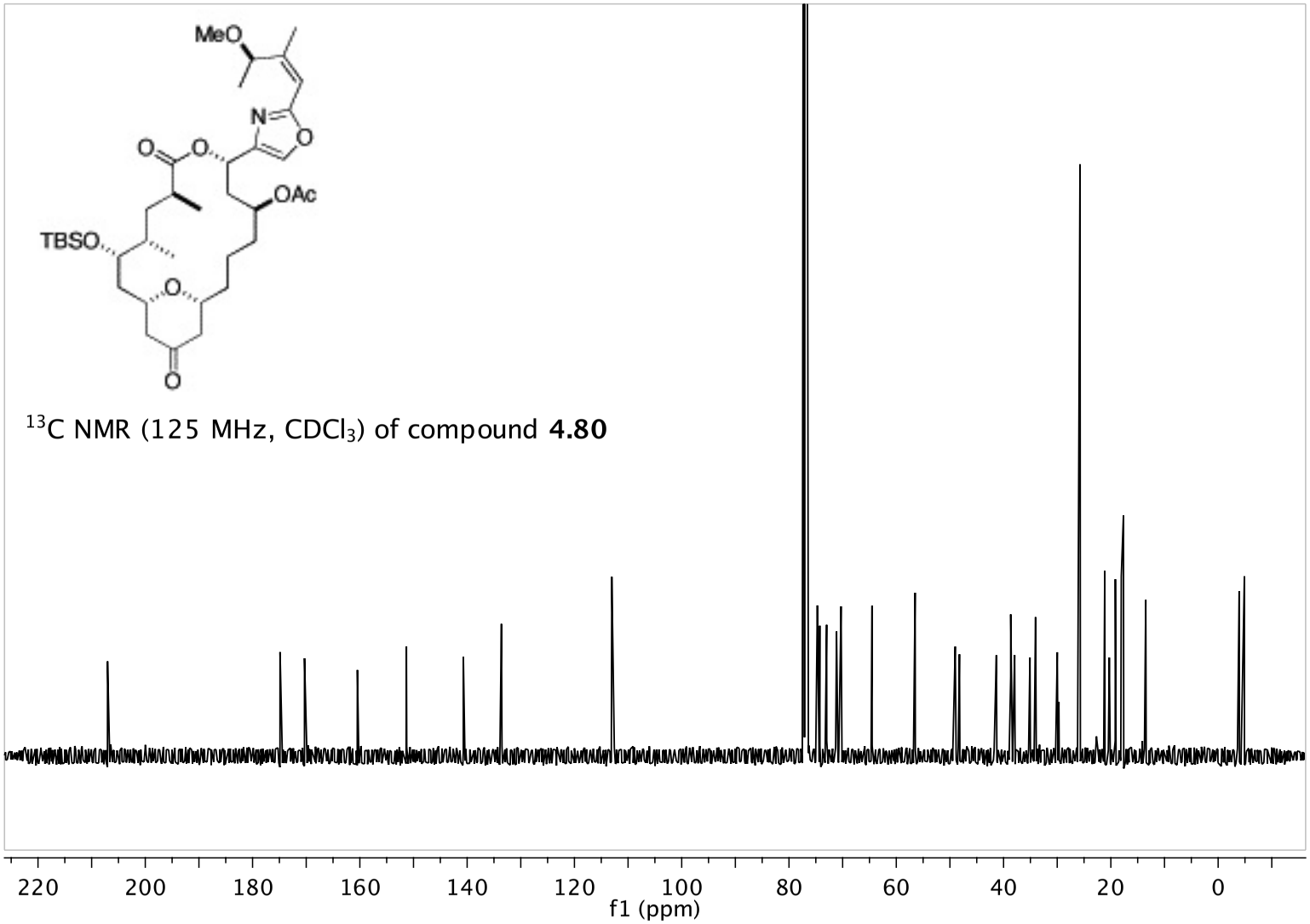
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.78**


^{1}H NMR (500 MHz, $CDCl_3$)
of compound **4.79**
MeO
OAc
OMe
MeO
TBSO
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

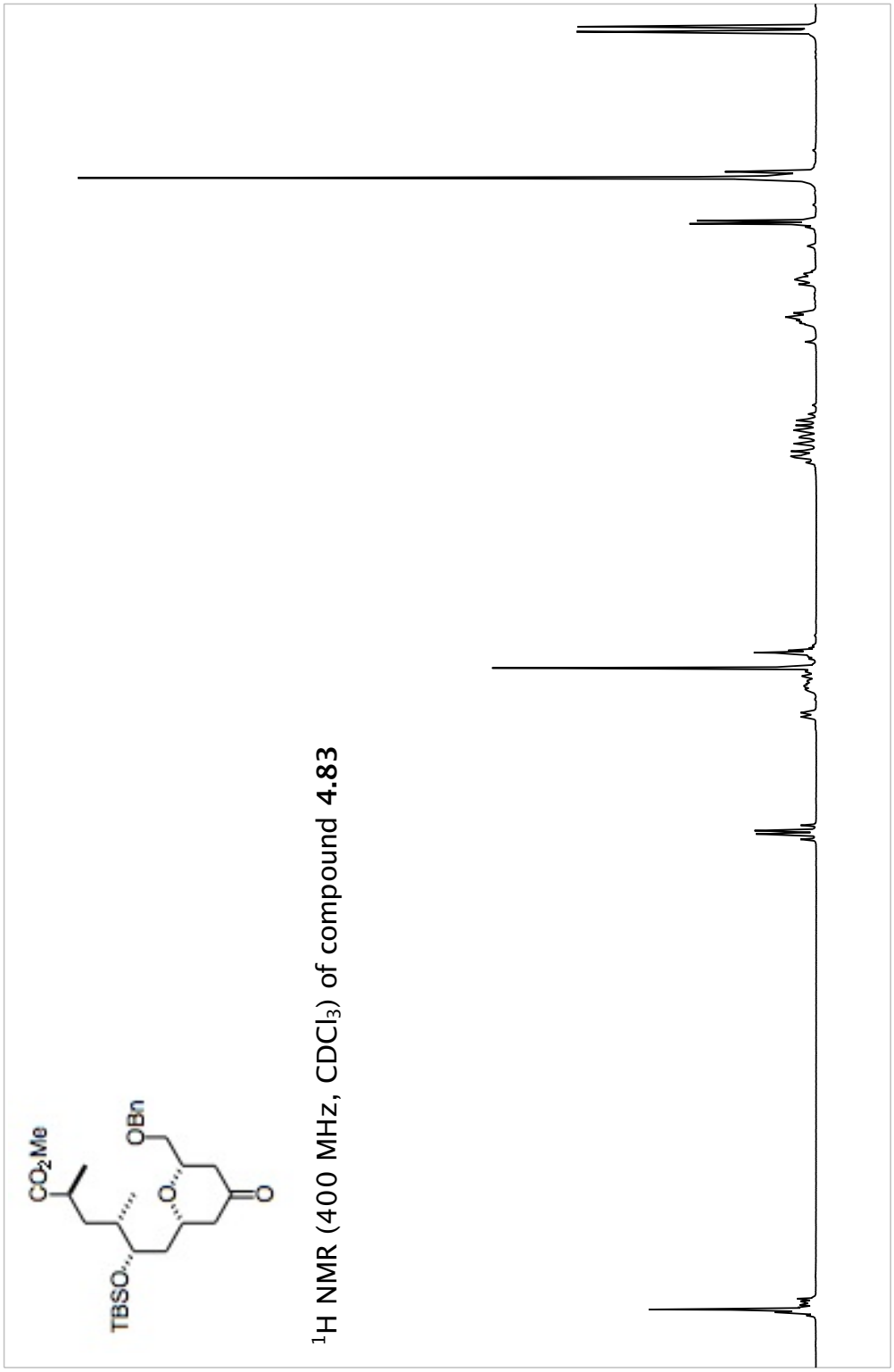


^{13}C NMR (125 MHz, $CDCl_3$) of compound **4.79**

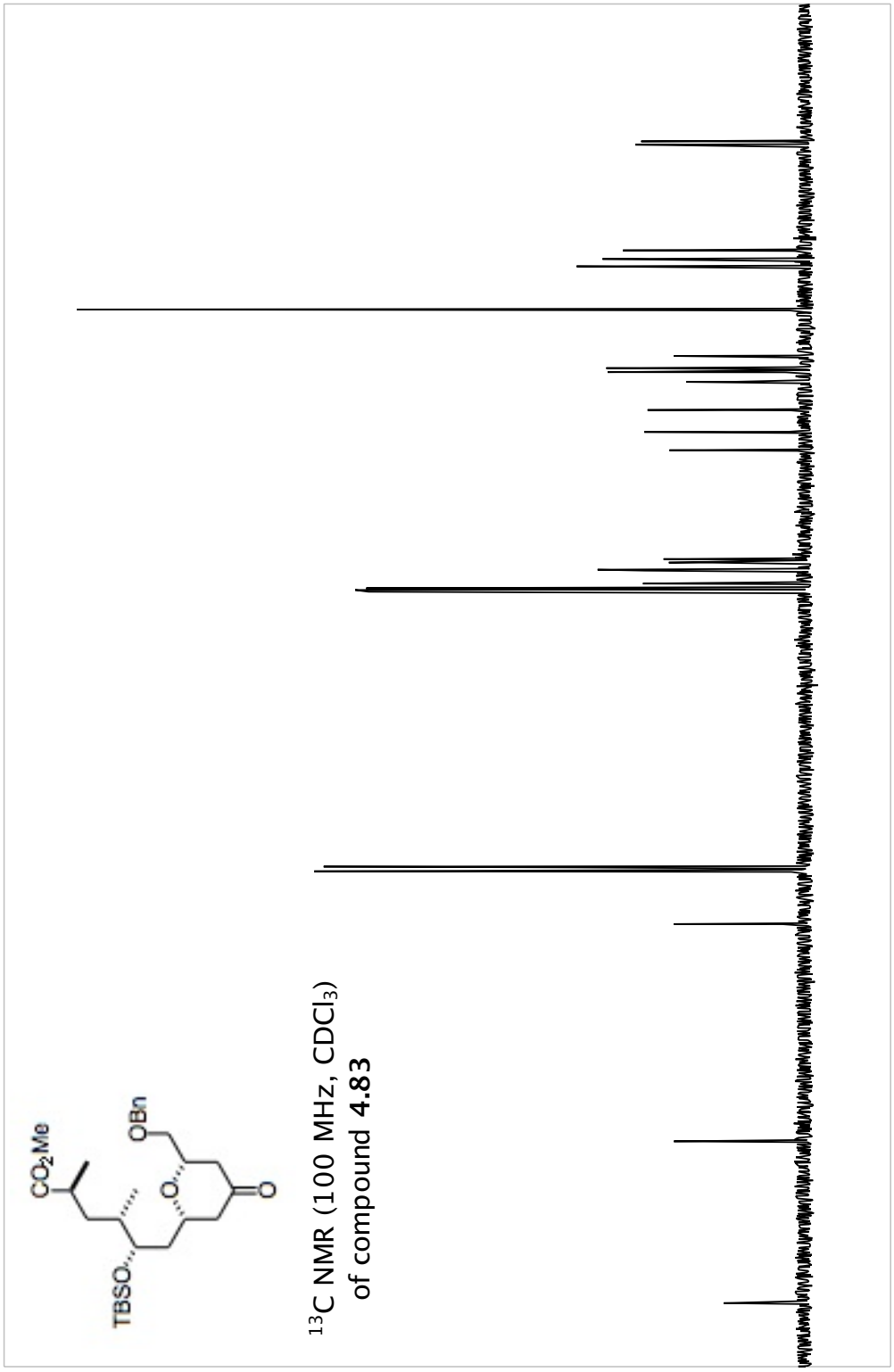

MeO
OAc
TBSO
^{1}H NMR (500 MHz, $CDCl_3$)
of compound **4.80**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

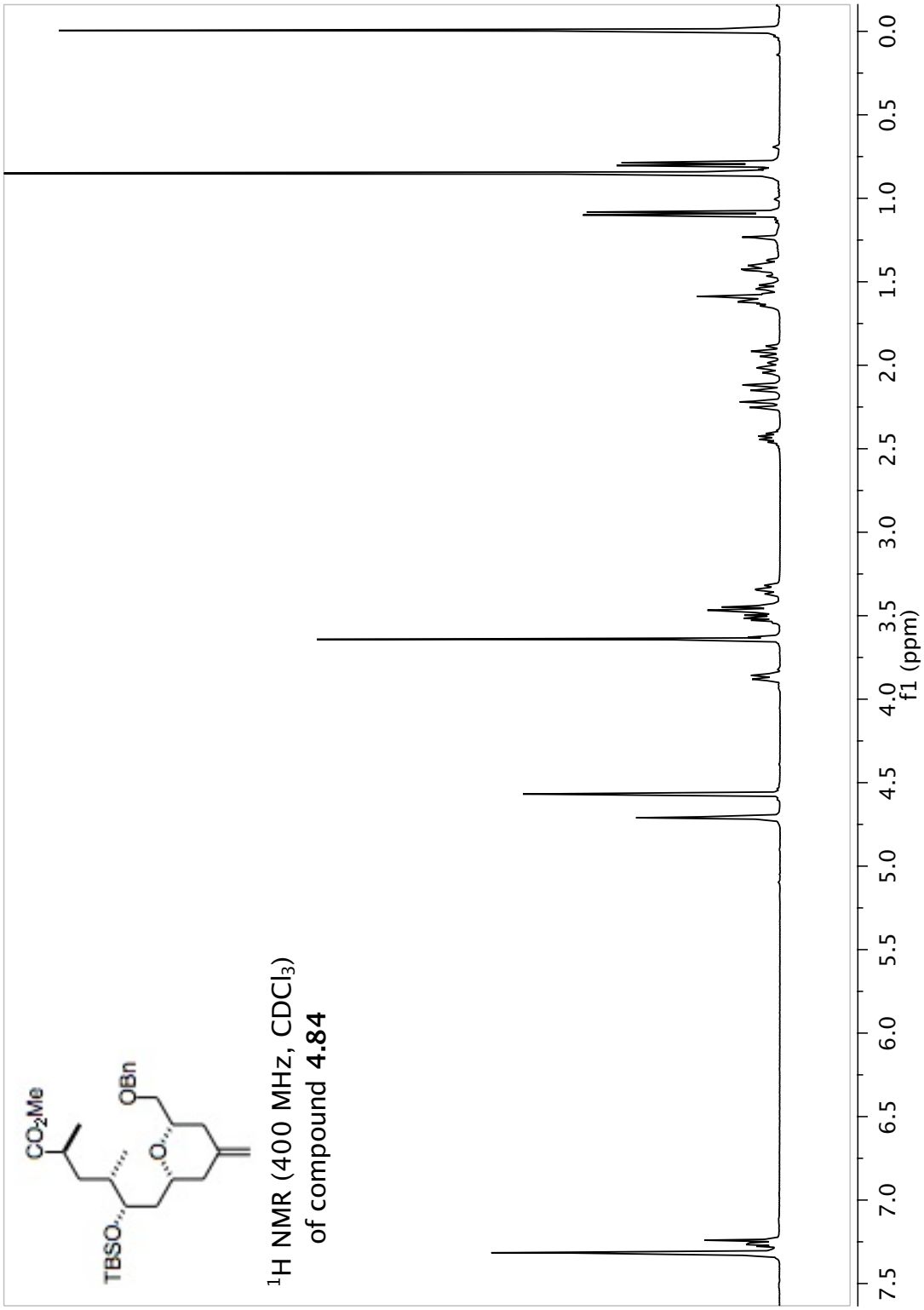


^{13}C NMR (125 MHz, $CDCl_3$) of compound **4.80**

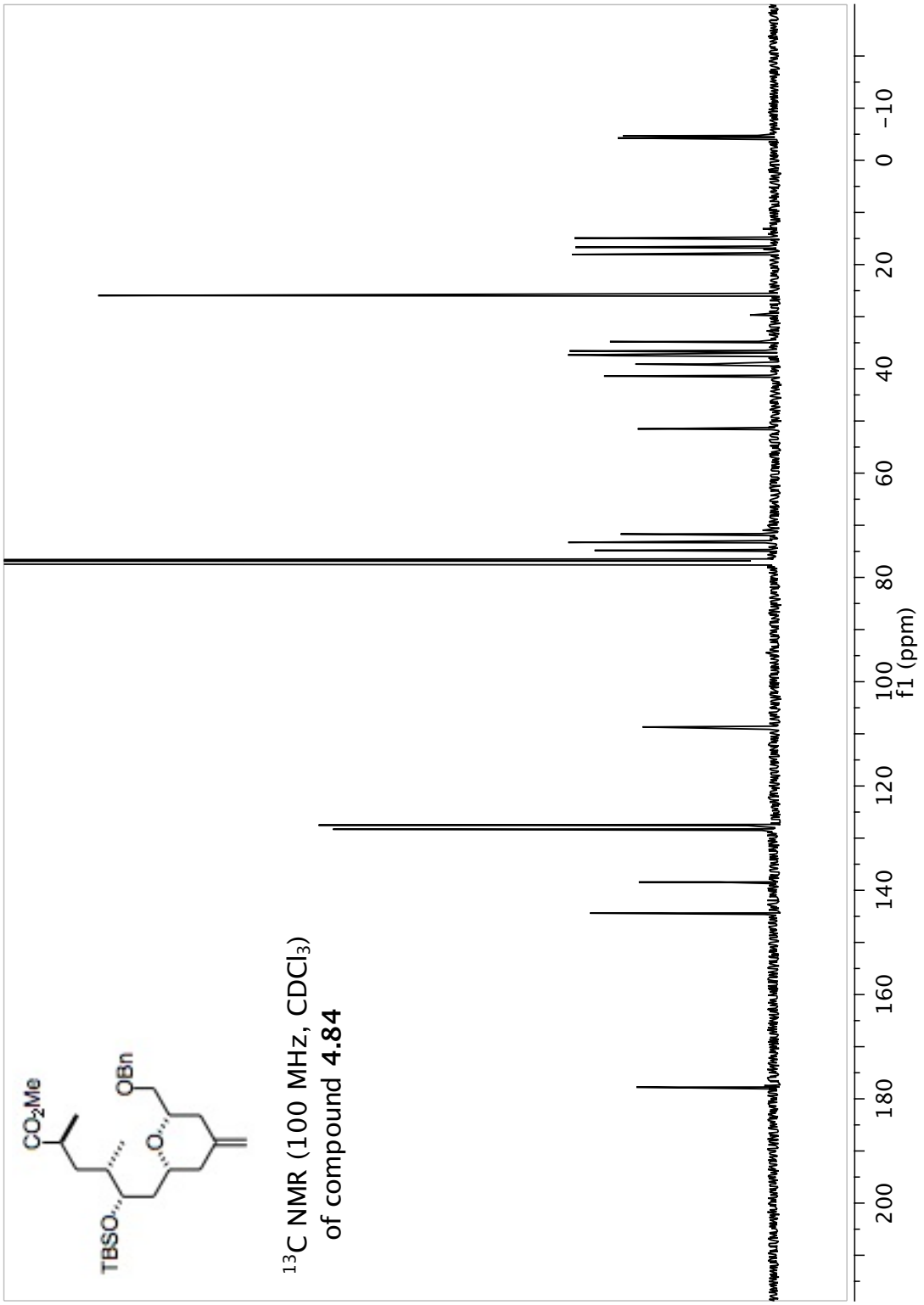


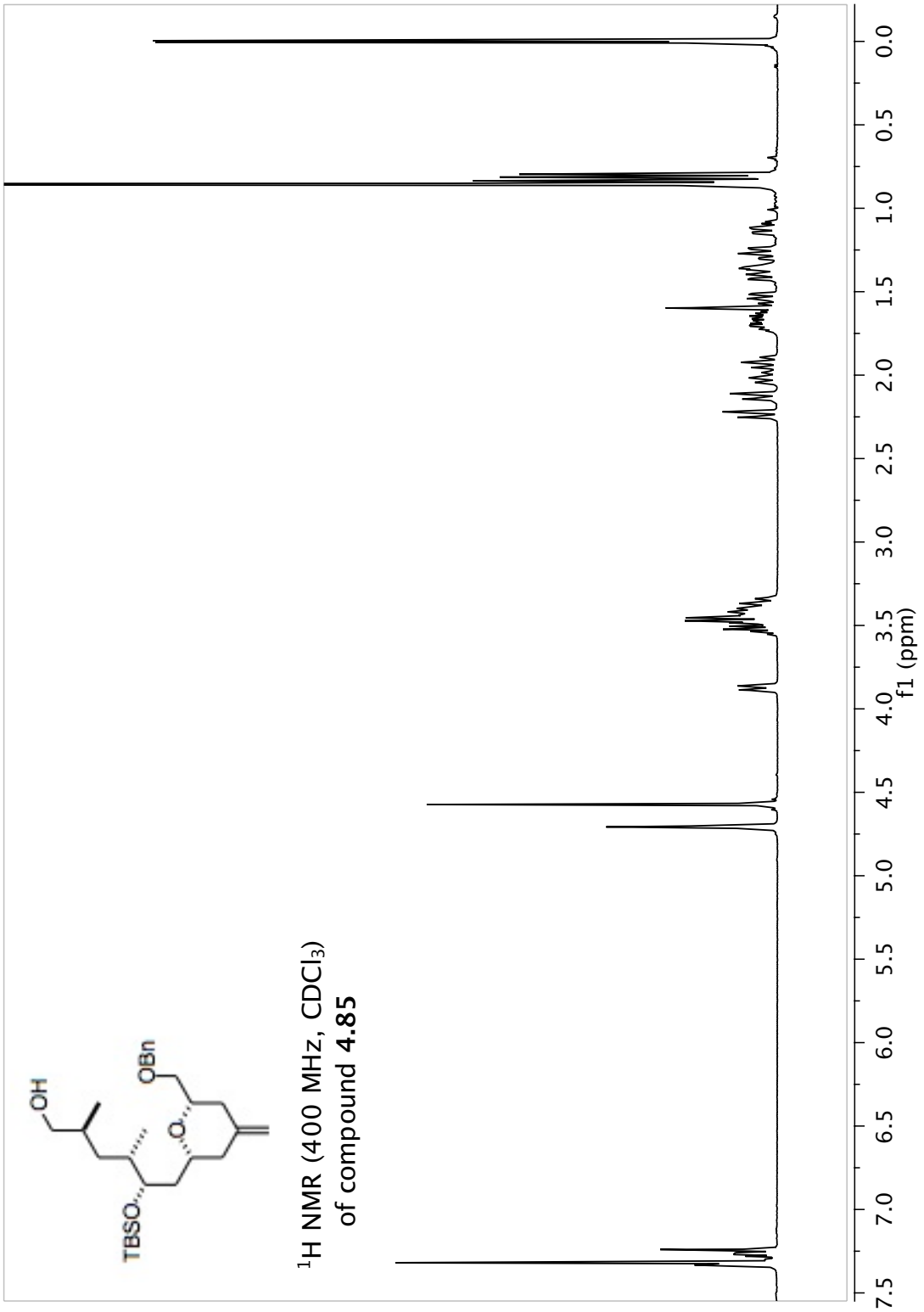
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.83**

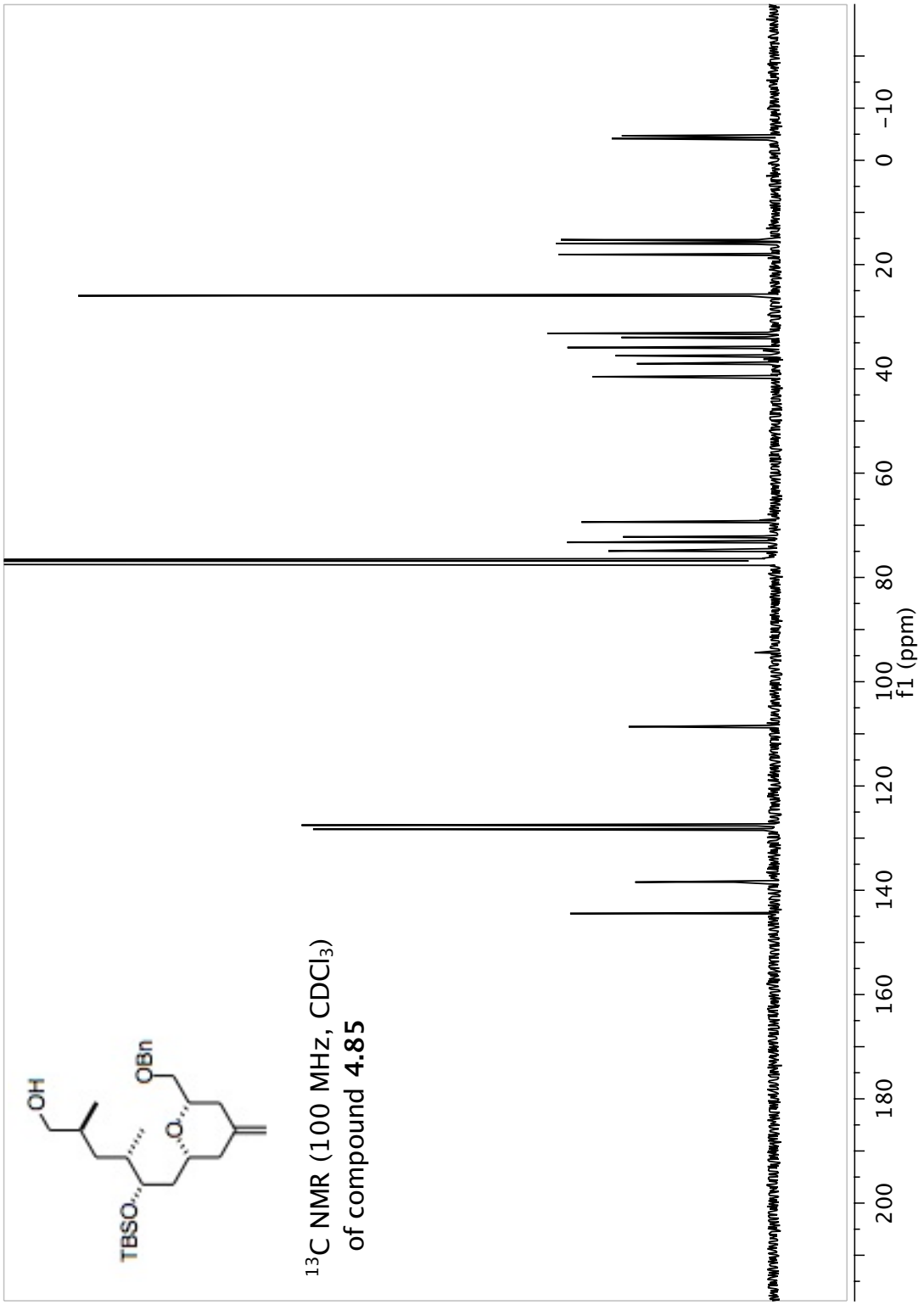

CO2Me
OBn
TBSO
O
O
13C NMR (100 MHz, CDCl3)
of compound 4.83
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

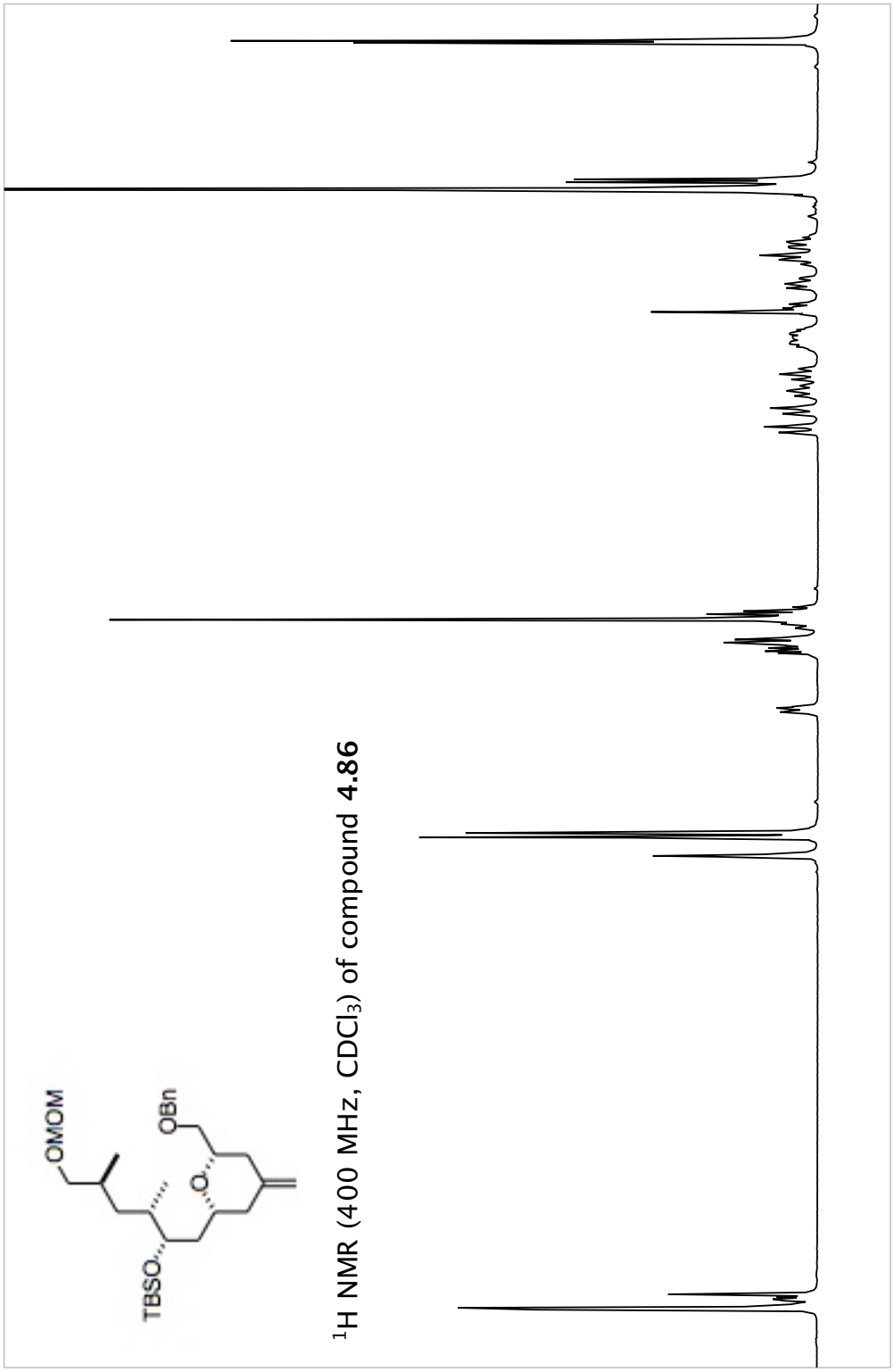


^{1}H NMR (400 MHz, $CDCl_3$)
of compound **4.84**

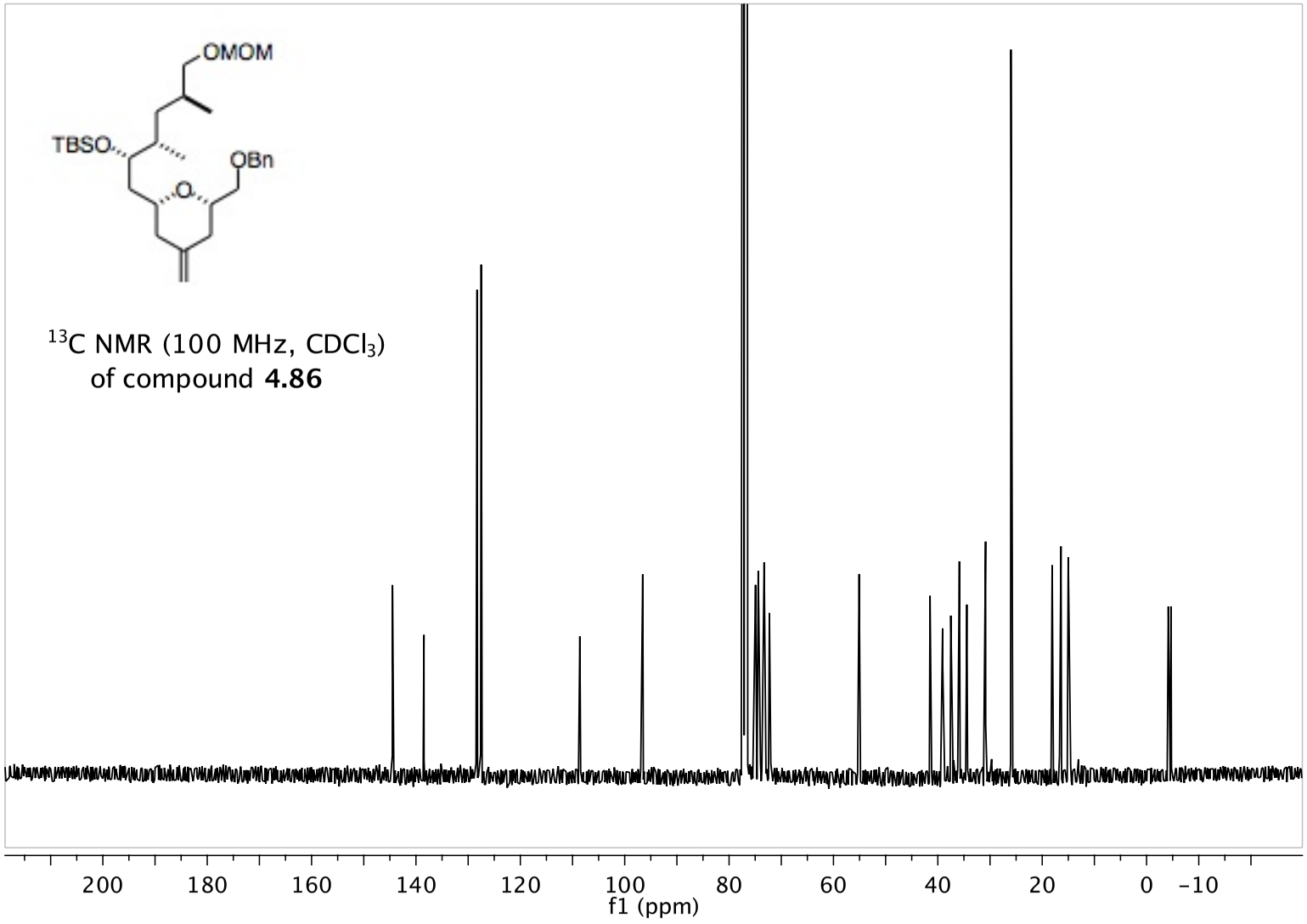

CO2Me
OBn
TBSO
O
13C NMR (100 MHz, CDCl3)
of compound 4.84
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

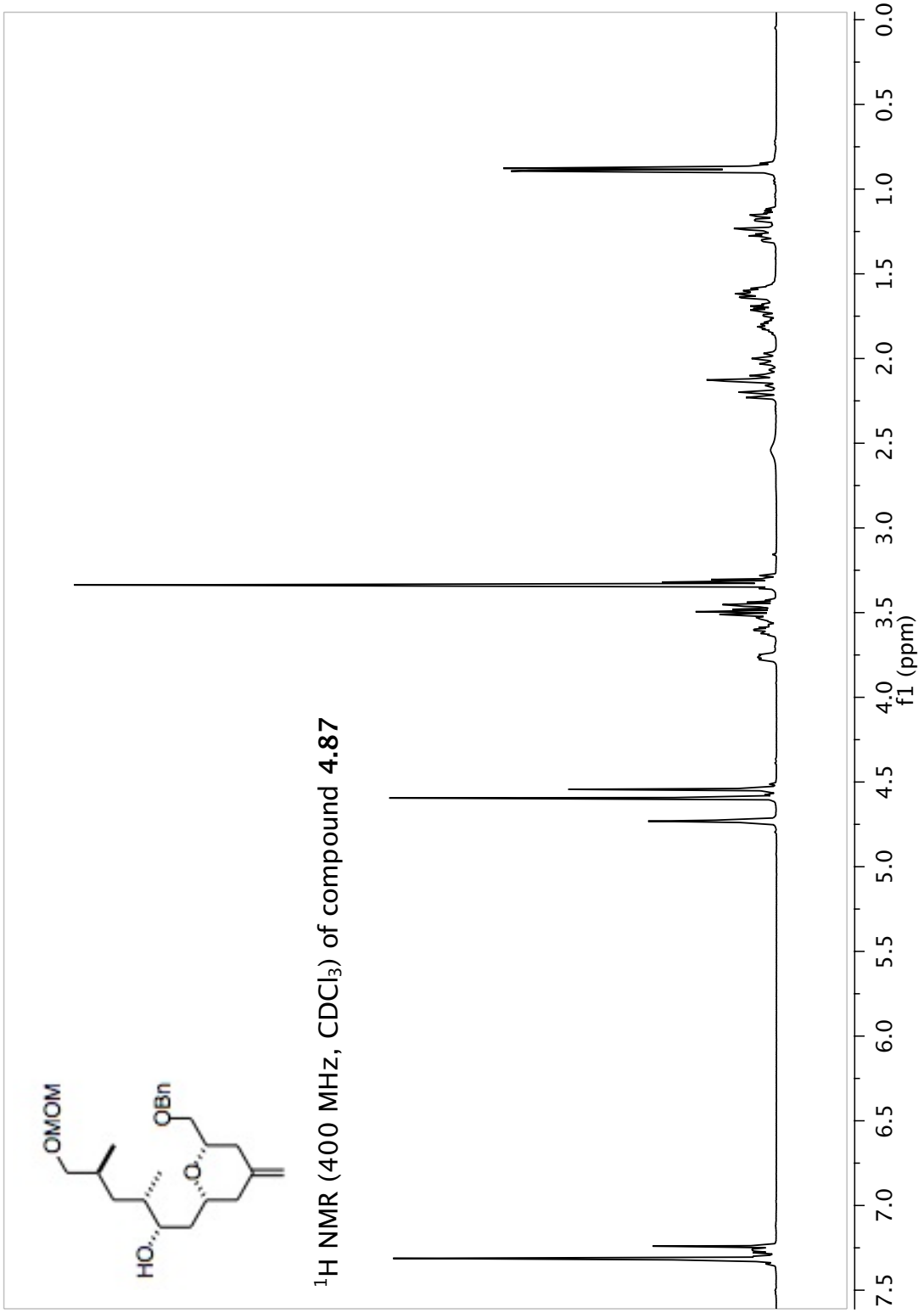

OH
OBn
O
TBSO
^{1}H NMR (400 MHz, $CDCl_3$)
of compound **4.85**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


OH
OBn
O
TBSO
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.85**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)

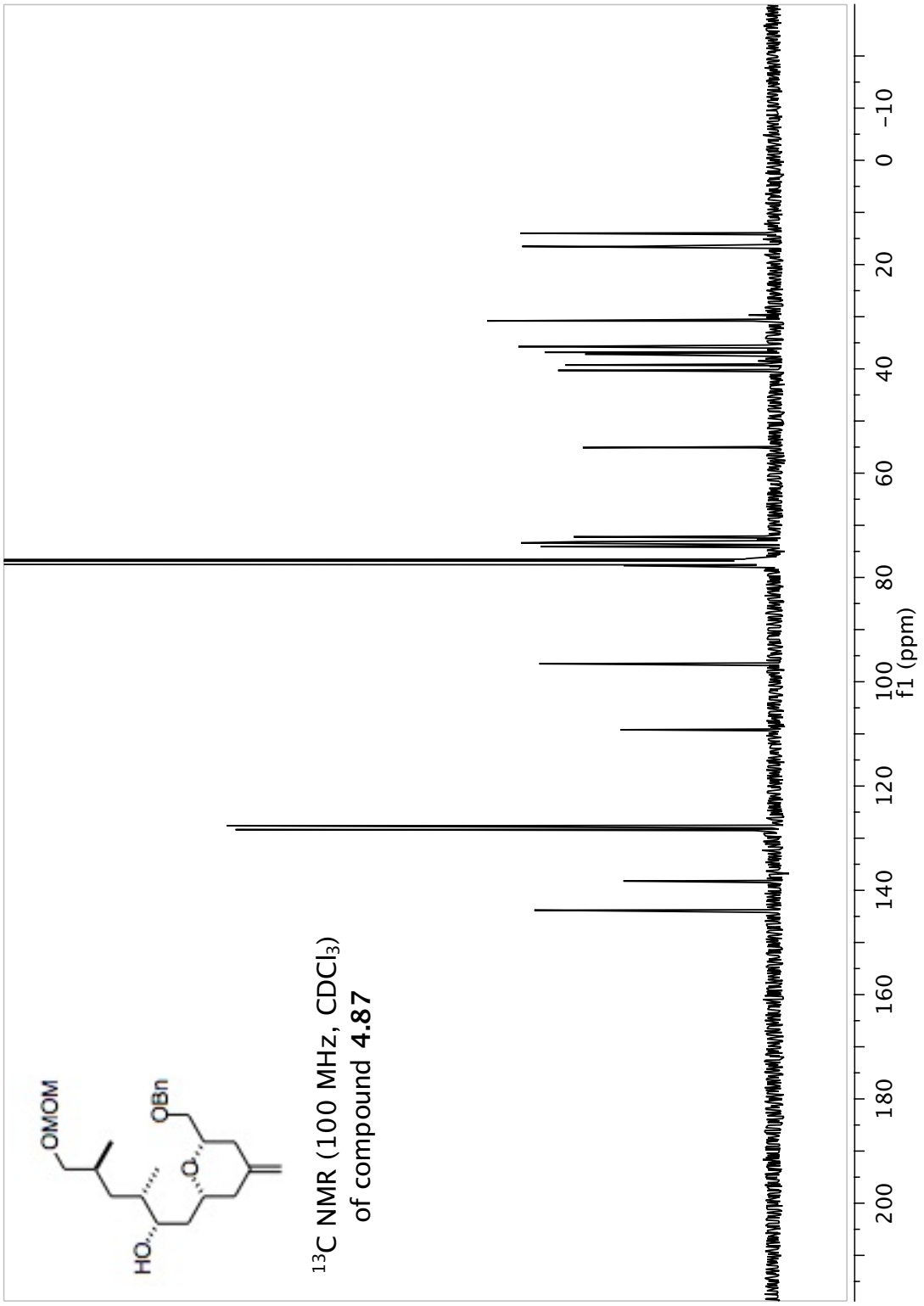


^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.86**

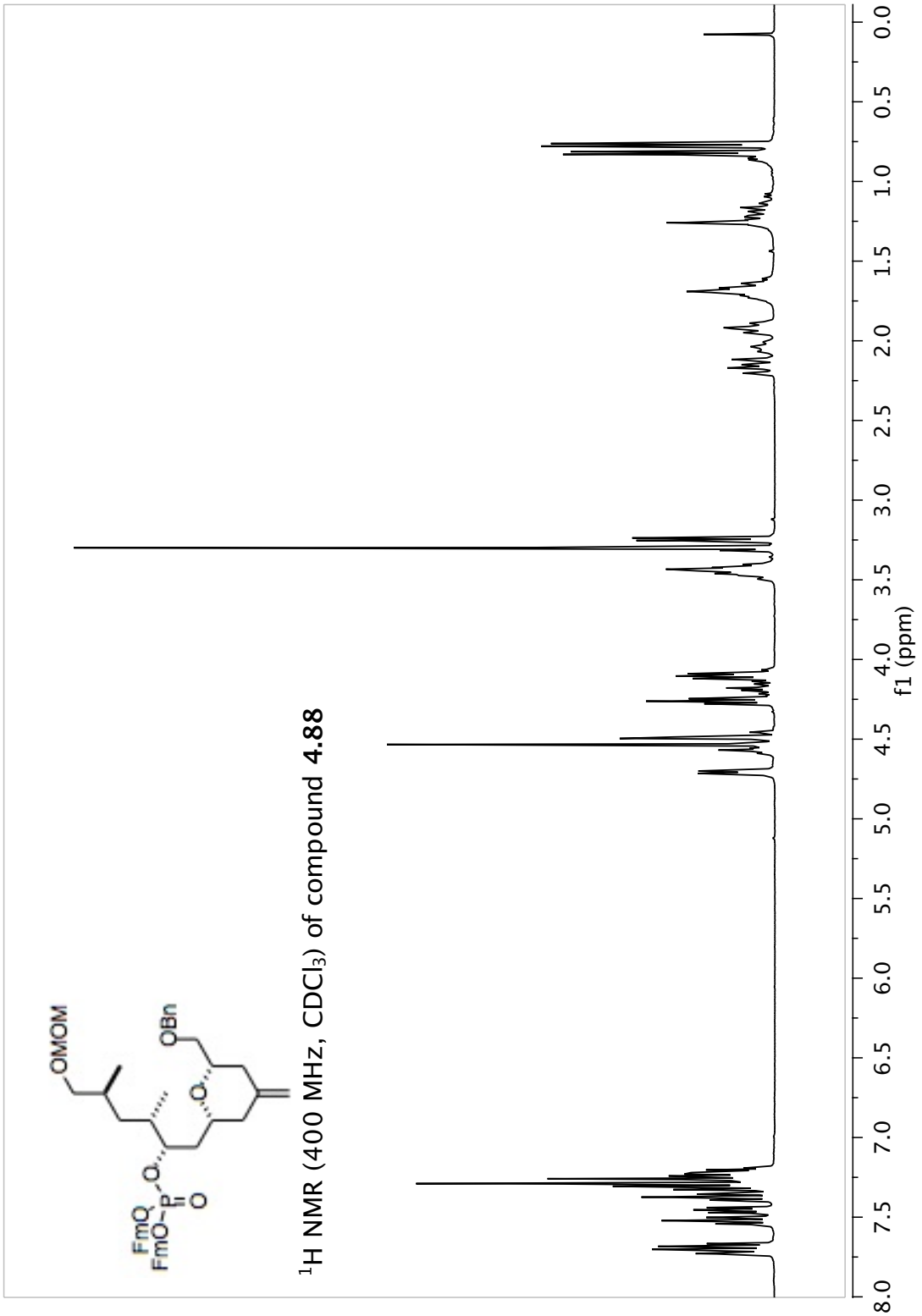

OMOM
TBSO
OBn
O
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.86**
200
180
160
140
120
100
80
60
40
20
0
−10
f1 (ppm)



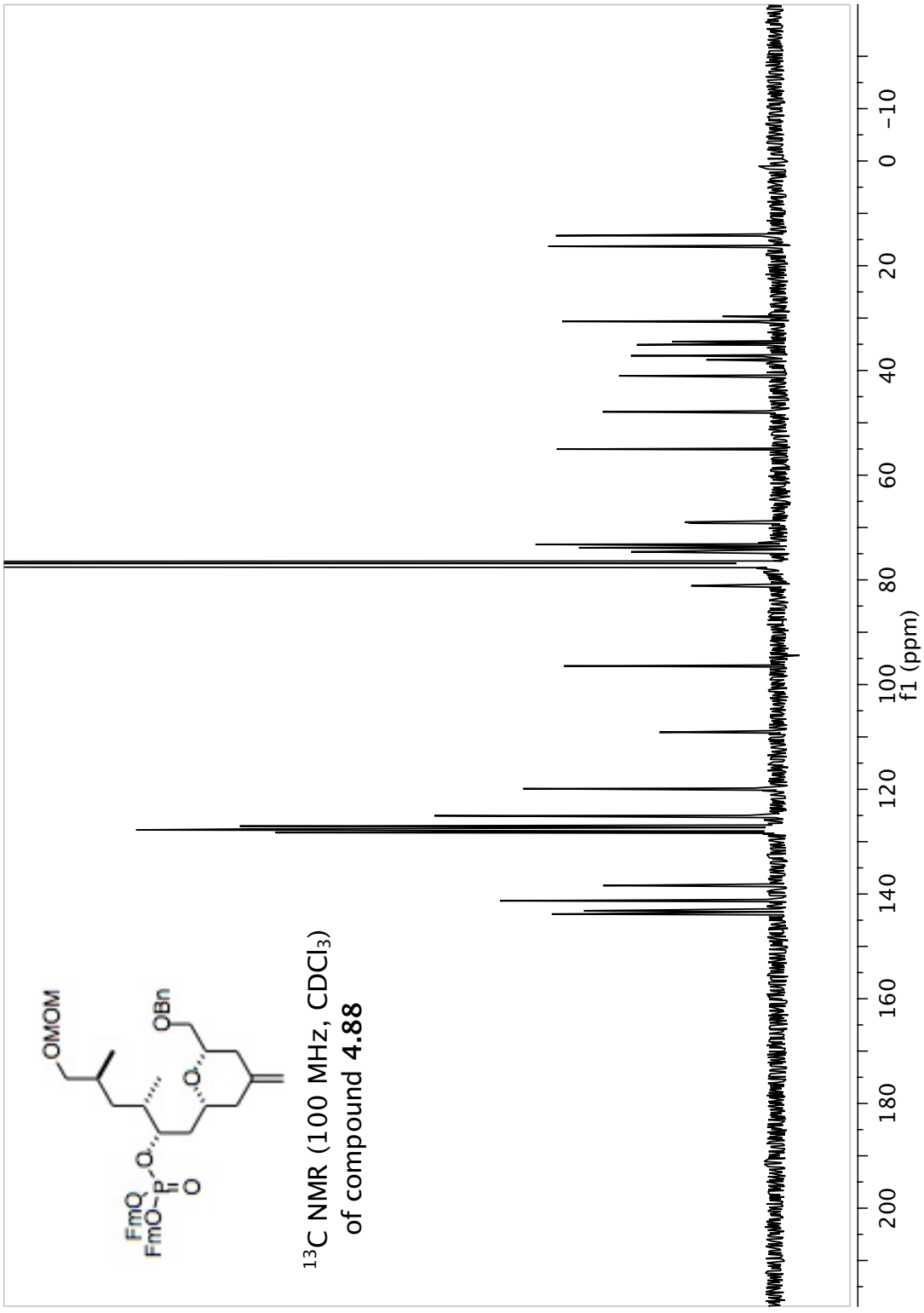
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.87**



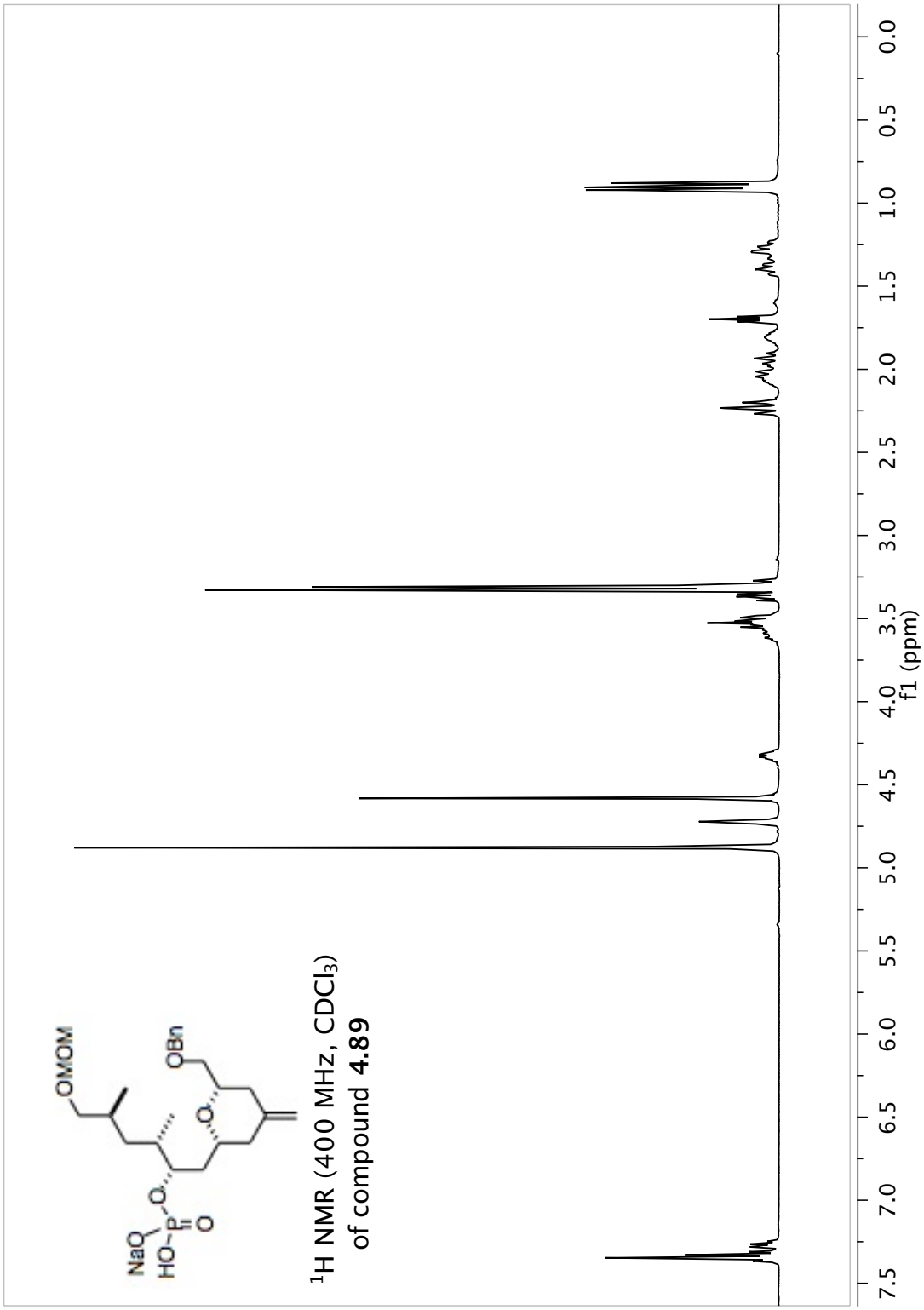
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.87**



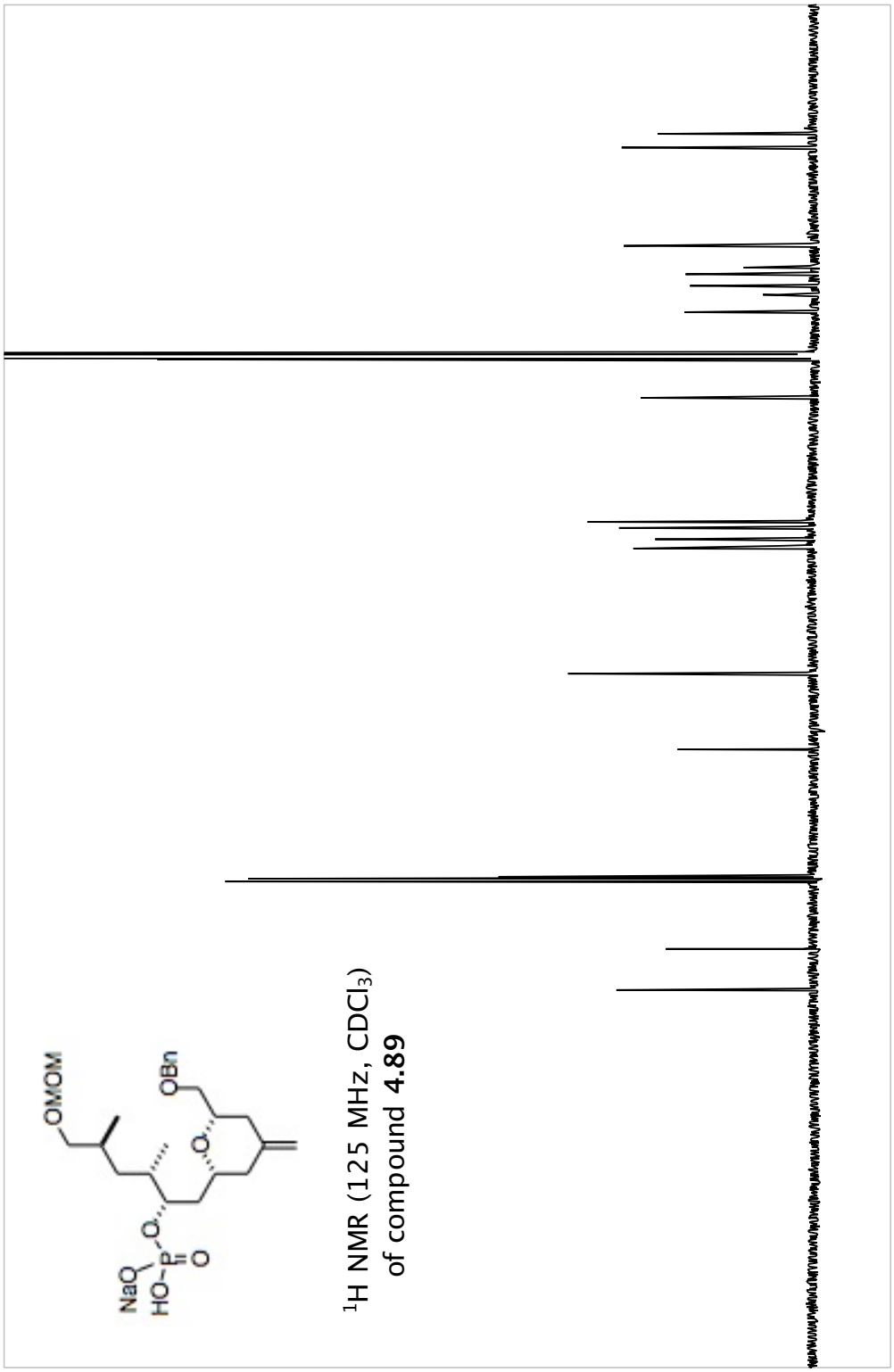
^{1}H NMR (400 MHz, $CDCl_3$) of compound **4.88**



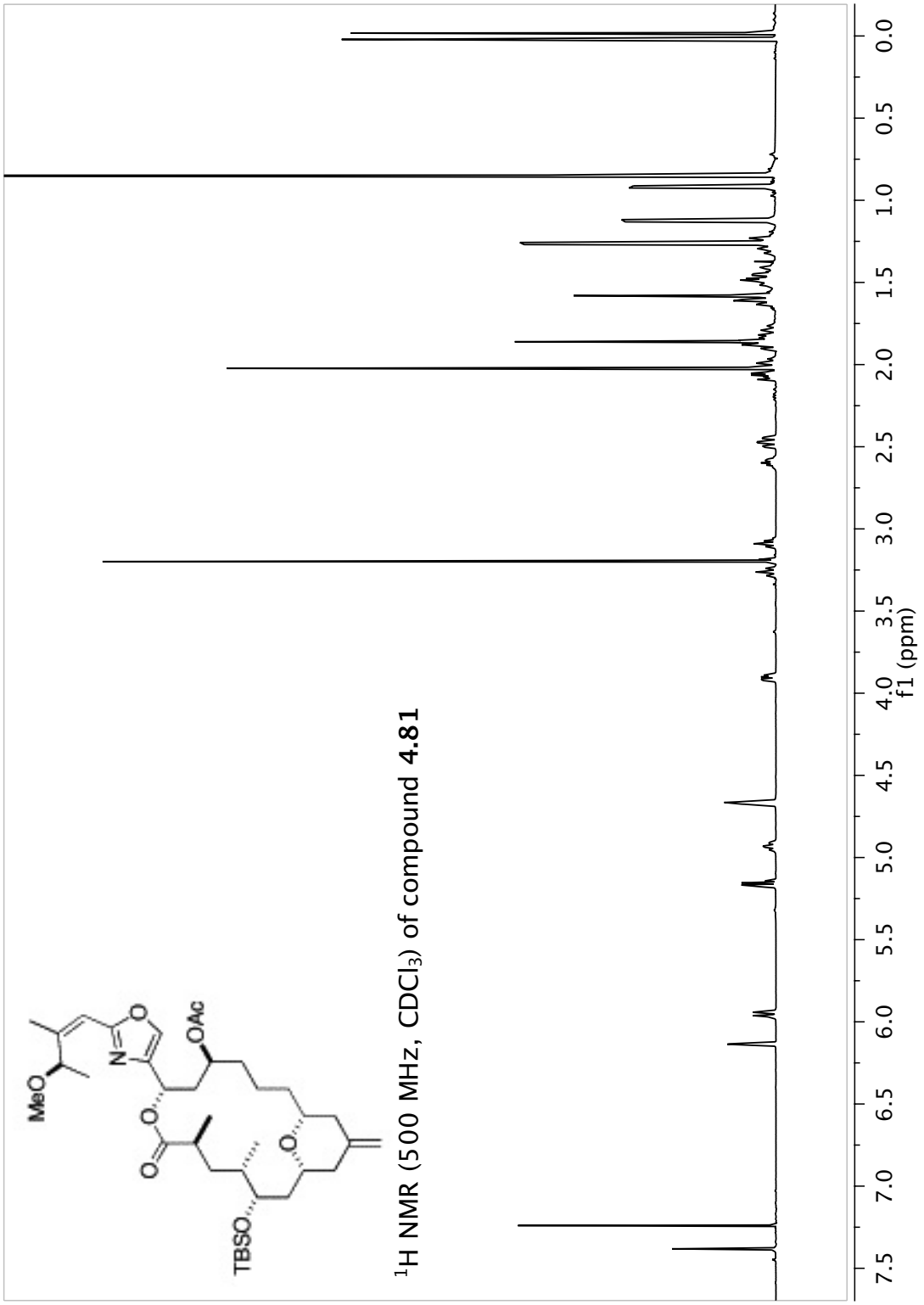
^{13}C NMR (100 MHz, $CDCl_3$)
of compound **4.88**



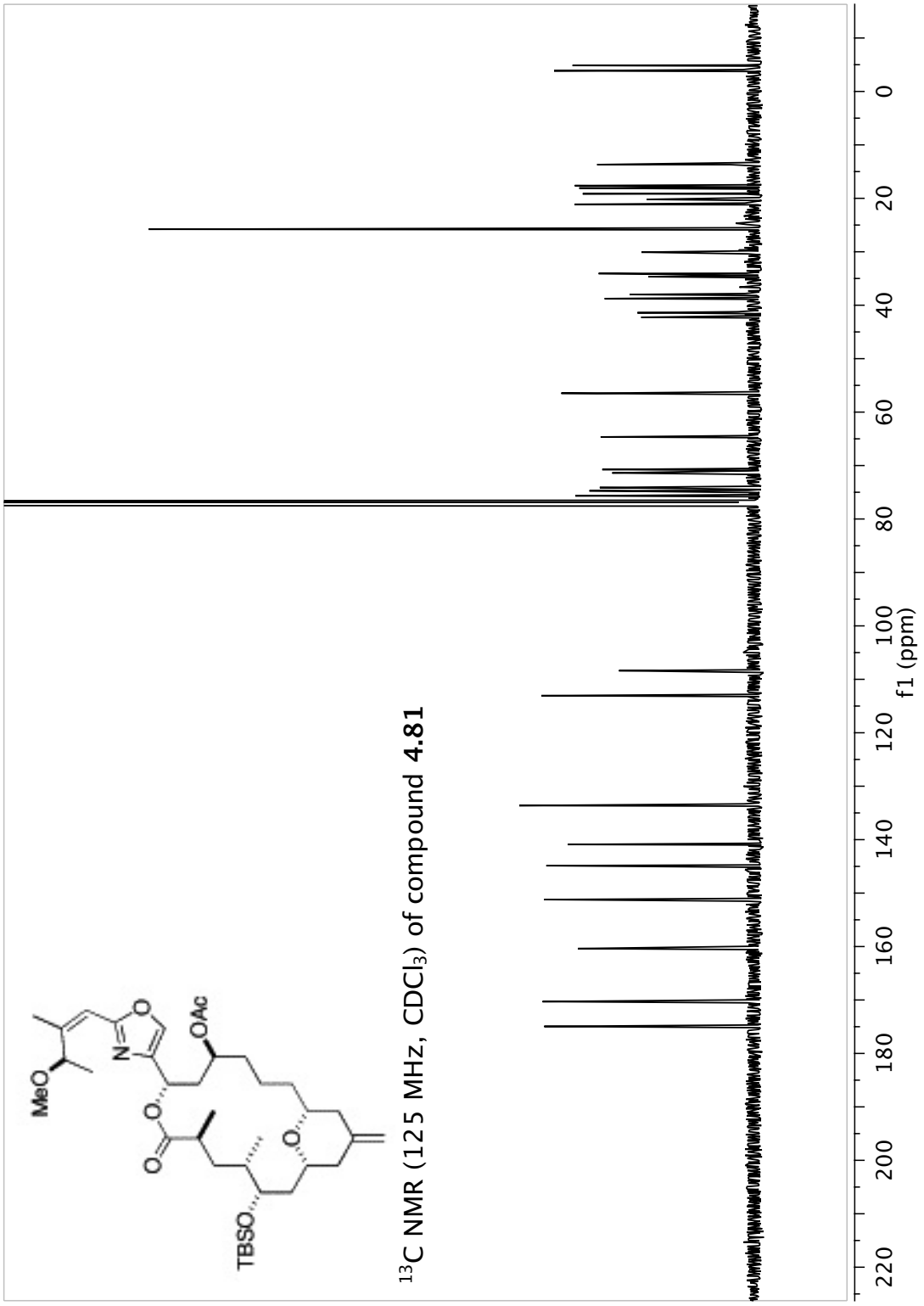
^{1}H NMR (400 MHz, $CDCl_3$)
of compound **4.89**



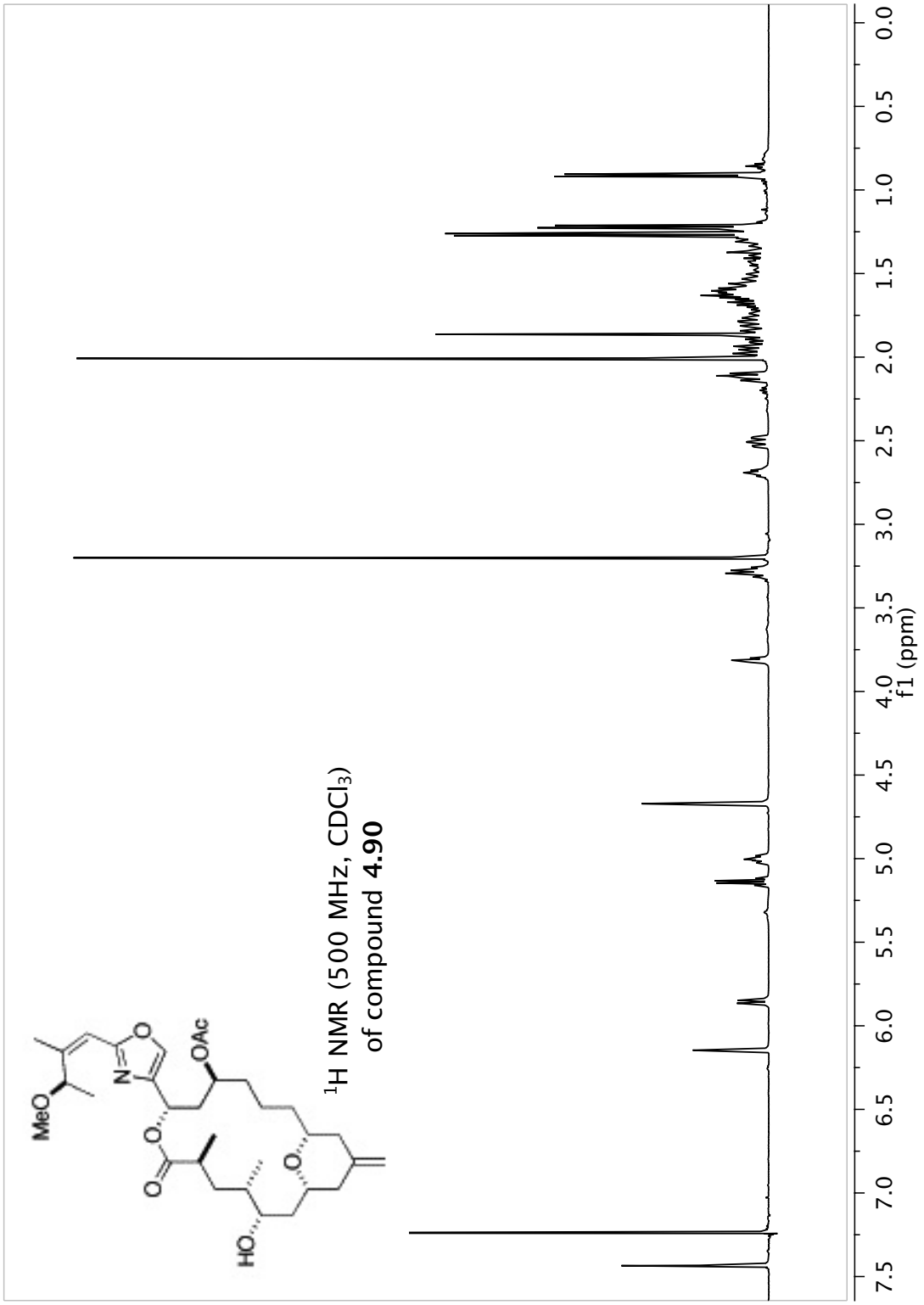
^{1}H NMR (125 MHz, $CDCl_3$)
of compound **4.89**

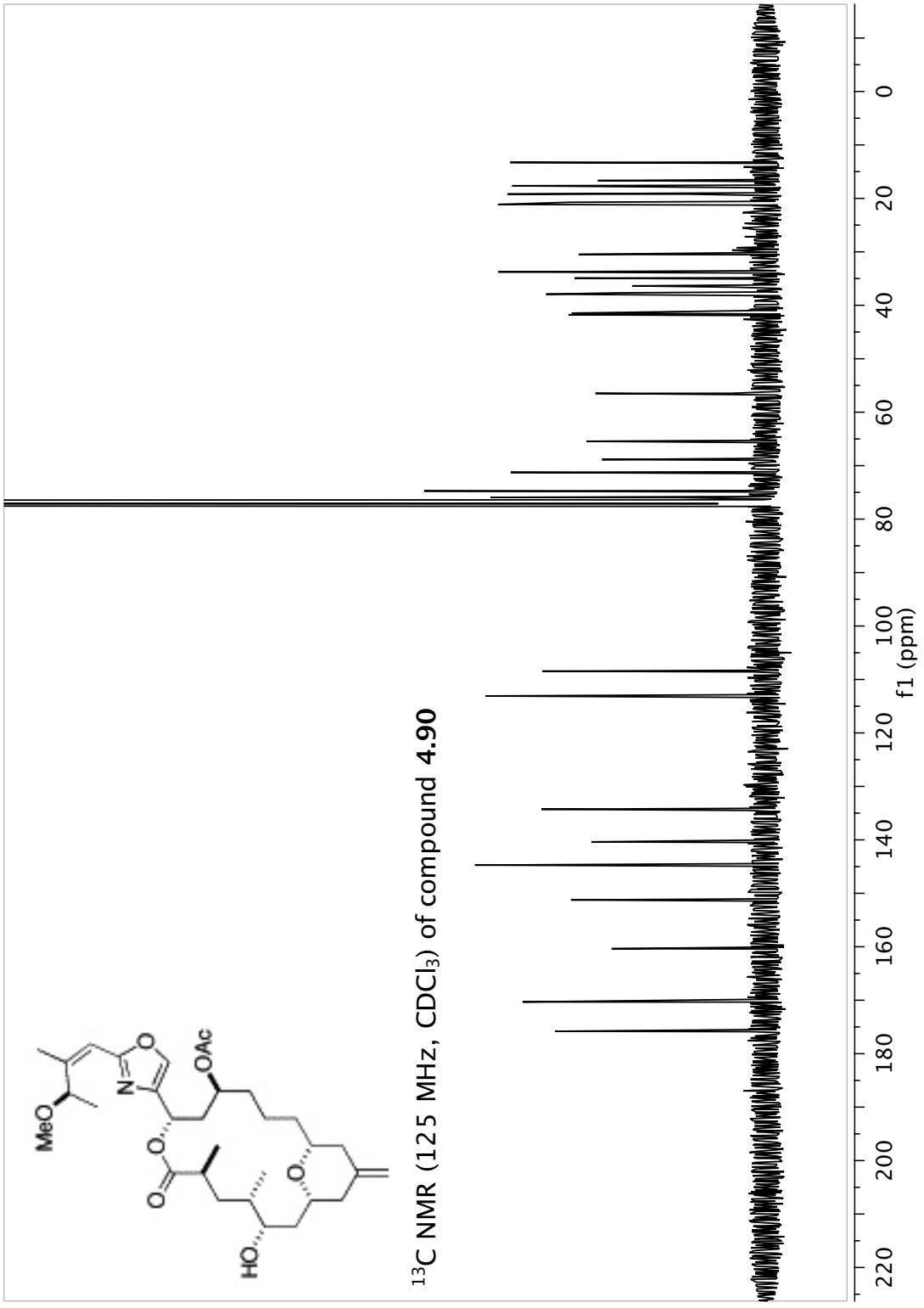


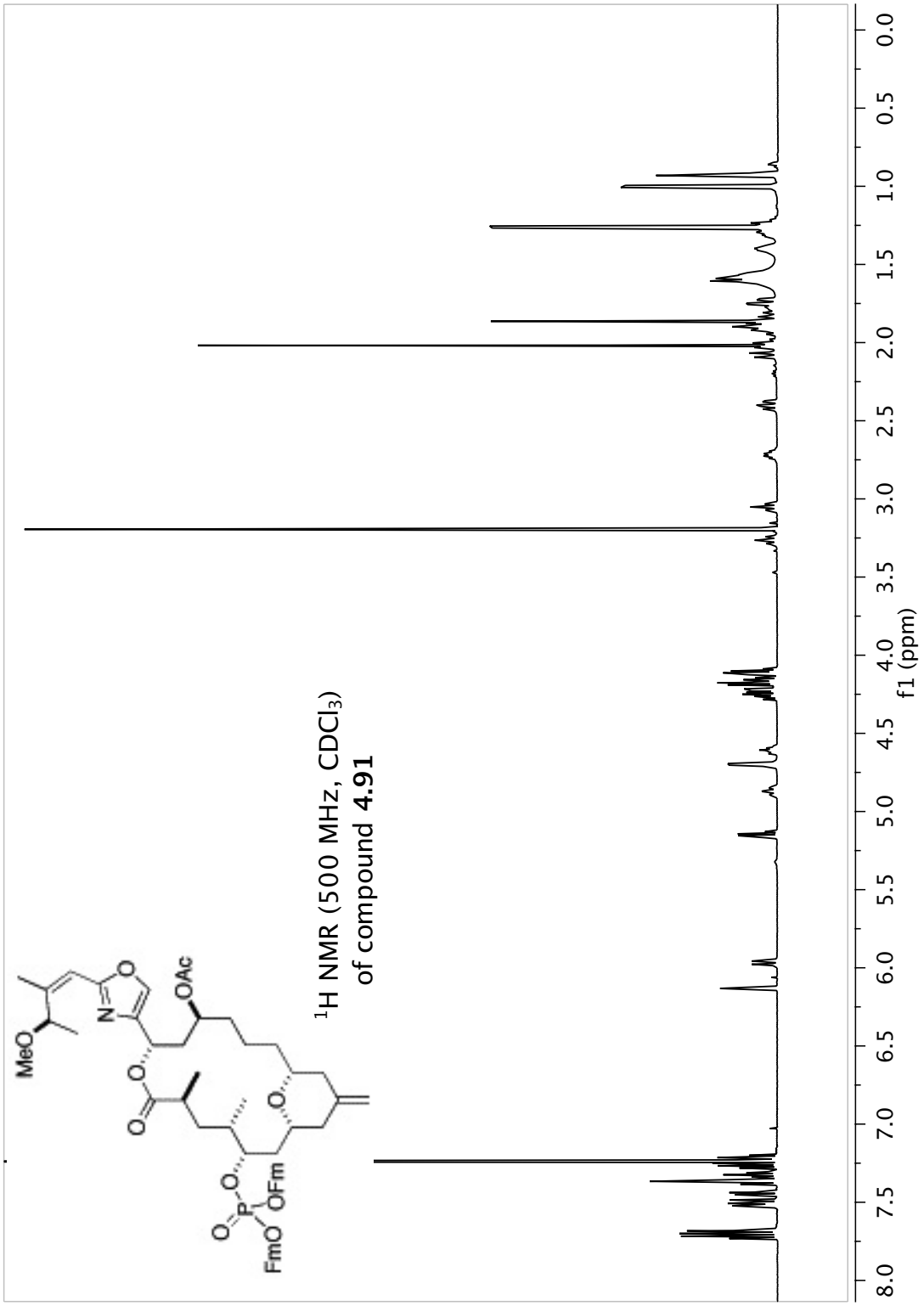
^{1}H NMR (500 MHz, $CDCl_3$) of compound **4.81**

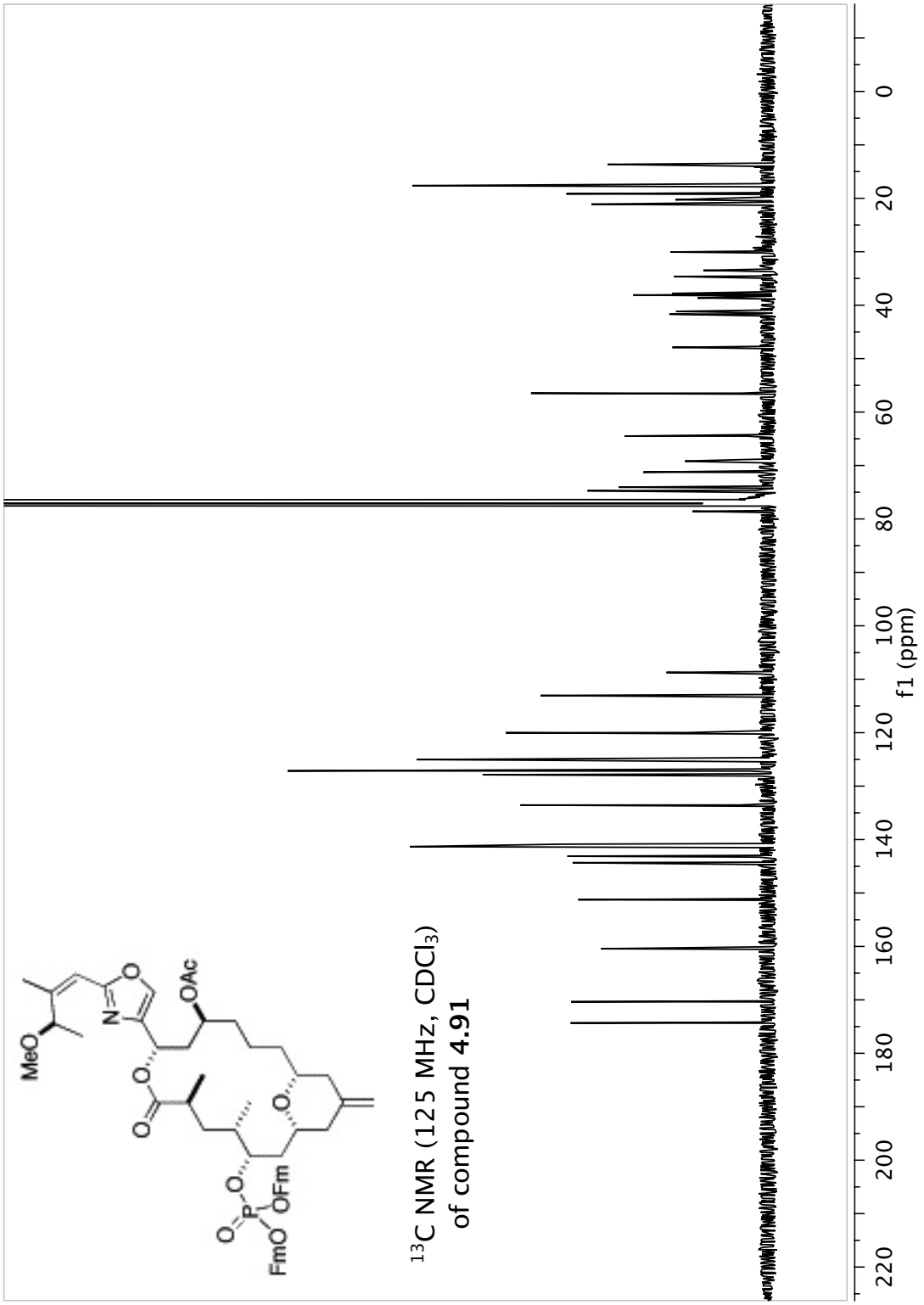


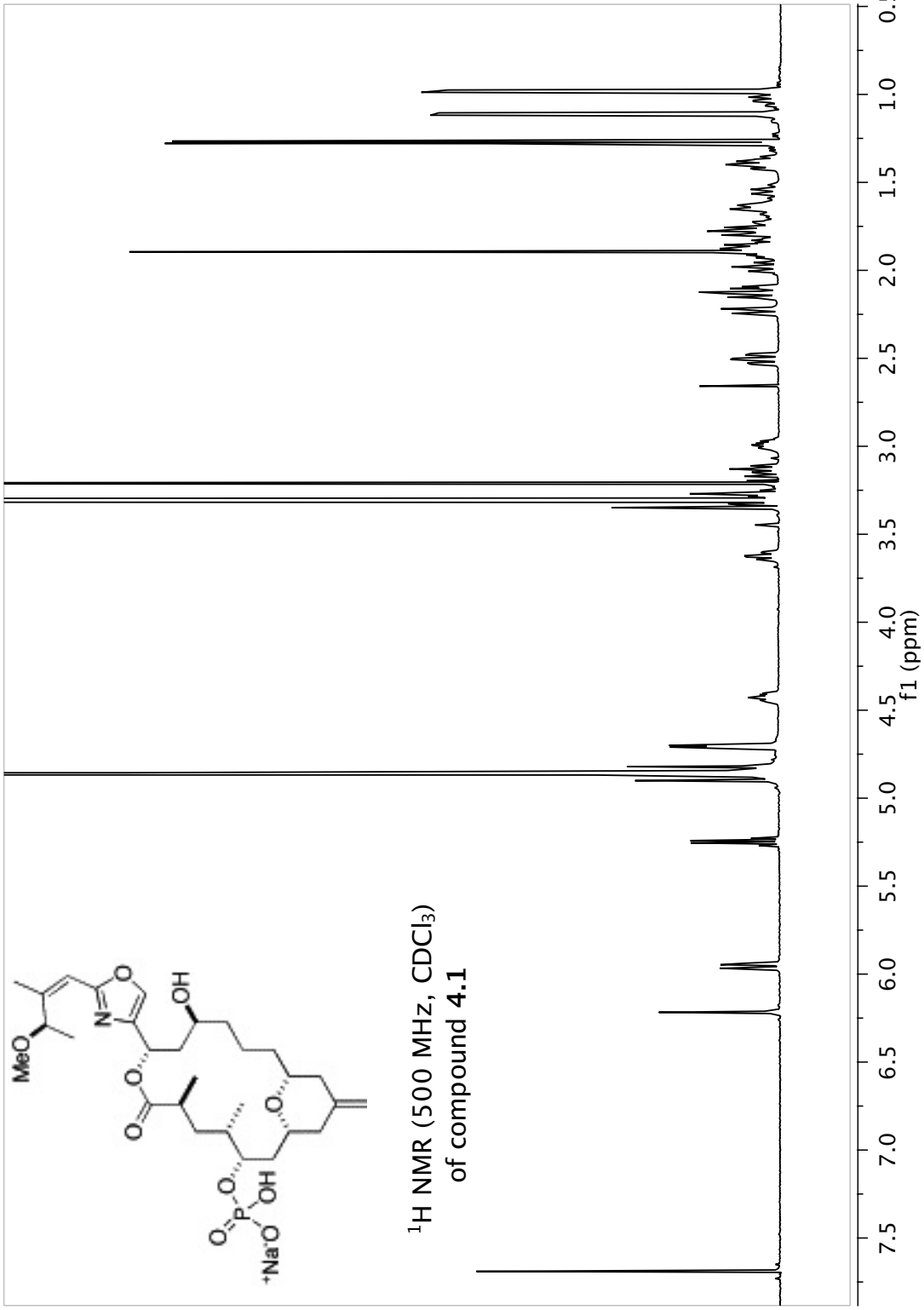
^{13}C NMR (125 MHz, $CDCl_3$) of compound **4.81**


MeO
N
O
OAc
O
O
O
HO
^{1}H NMR (500 MHz, $CDCl_3$)
of compound **4.90**
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

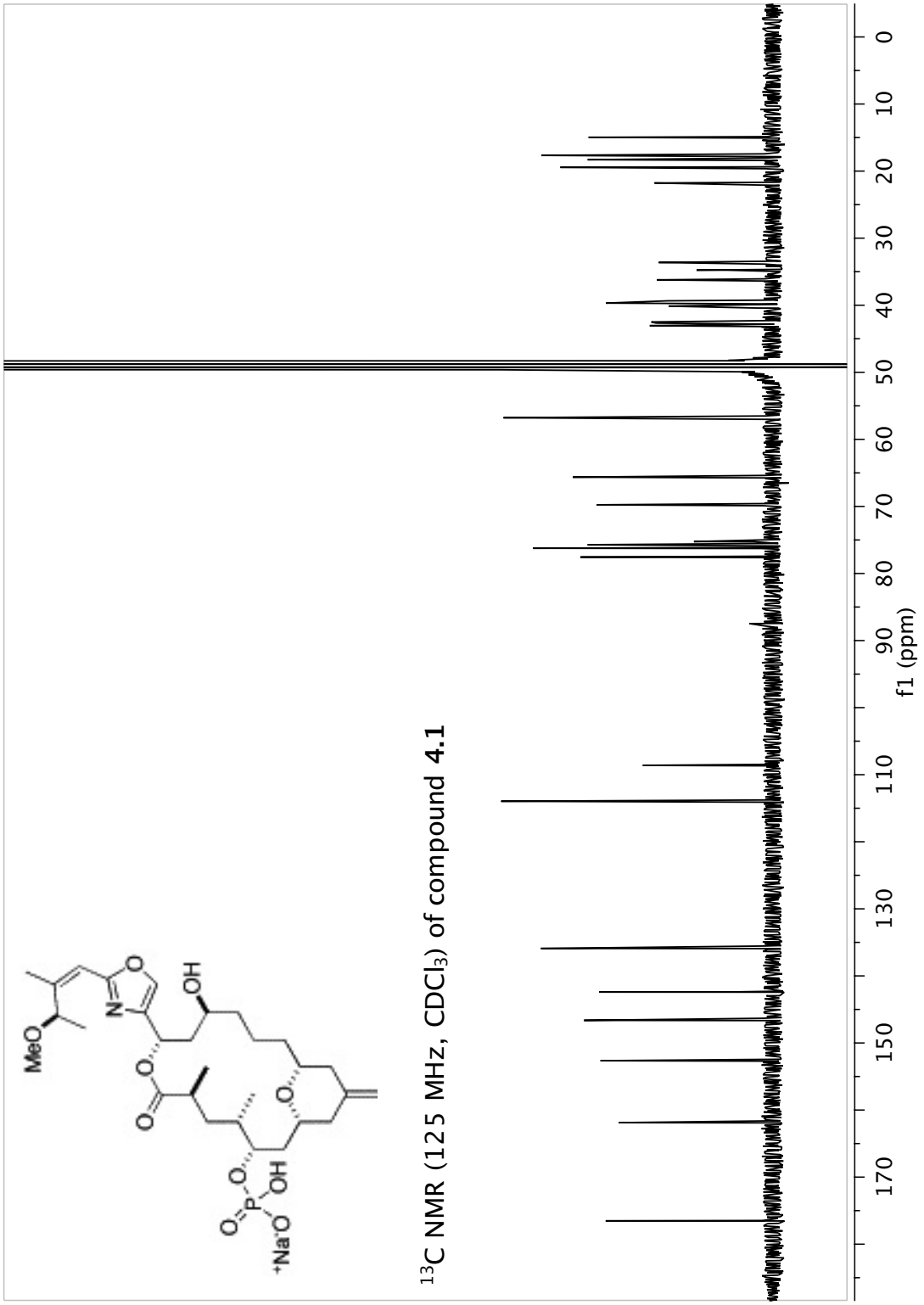

MeO
N
O
OAc
O
O
O
HO
^{13}C NMR (125 MHz, $CDCl_3$) of compound **4.90**
220
200
180
160
140
120
100
80
60
40
20
0
f1 (ppm)


MeO
N
O
OAc
O
O
O
O
O
P
O
FmO
OFm
^{1}H NMR (500 MHz, $CDCl_3$)
of compound **4.91**
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


MeO
N
O
OAc
O
O
O
O
P
O
FmO
OFm
^{13}C NMR (125 MHz, $CDCl_3$)
of compound **4.91**
220
200
180
160
140
120
100
80
60
40
20
0
f1 (ppm)



^{1}H NMR (500 MHz, $CDCl_3$)
of compound **4.1**



^{13}C NMR (125 MHz, $CDCl_3$) of compound **4.1**