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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Secondary metabolites as potential cancer therapeutic leads: Synthesis and chemical biology of Englerin A and Fusarisetin A

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

in

Chemistry

by

Eduardo J.E. Caro-Diaz

Committee in charge:

Professor Emmanuel Theodorakis, Chair
Professor Micheal Burkart
Professor William Gerwick
Professor Kyriacos C. Nicolaou
Professor Stanley Opella

2014

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The Dissertation of Eduardo J.E. Caro-Diaz is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2014

DEDICATION

I would like to dedicate my thesis and all my work throughout these years to my mother, Judith A. Diaz-Rodriguez, my father, Edward A. Caro-Lopez, my sister, Xiomara P. Caro-Diaz, and my grandmother Doña Carmen Rodriguez. They have always encouraged me to pursue a higher education, and they have been unconditionally supportive of all my decisions towards my career goals. Their love and support during good and bad times has been key to my success, and for that I will always be grateful. “Familia es familia, cariño es cariño.”

Also I would like to dedicate my work to the love of my life and the woman who has given me so much support, love, companionship, emotional effort and time. Maritxell Carrero, you are my true inspiration to continue my journey through anything that may lie ahead in my path.

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LIST OF ABBREVIATIONS

Ac	acetyl
AcOH	acetic acid
t-Bu	*tert*-butyl
Bn	beznyl
^{o} C	degrees Celsius
calcd	calculated
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CH_2Cl_2	methylene chloride
CD_3OD	deuterated methanol
CH_3OH	methanol
DCM	dichloromethane
DIPEA	diisopropylamine
DMAP	*N,N*-4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
Et_3N	triethylamine
GI_{50}	mean growth inhibition concentration
h	hours
HCl	hydrochloric acid

hυ	irradiation with light
KHMDS	potassium bis(trimethylsilyl)amide
HRMS	high-resolution mass spectrometry
IBX	*o*-iodoxybenzoic acid
IC_{50}	mean inhibition concentration
LiHMDS	lithium bis(trimethylsilyl)amide
m-CPBA	*meta*-chloroperoxybenzoic acid
Me	methyl
MeI	methyl iodide
MeOH	methanol
Mhz	megahertz
mL	milliliter
rt	room temperature
μL	microliter
μmole	micromole
mmol	millimole
NaHMDS	sodium bis(trimethylsilyl)amide
NMR	nuclear magnetic resonance
Ph	phenyl
PPh_3	triphenylphosphine
ppm	parts per million
RCM	ring-closing metathesis
R_f	retention factor
SAR	structure-activity relationship
TBAF	tetrabutylammonium fluoride

TBDPS	*tert*-butyldiphenylsilyl
TBS	*tert*-butyldimethyllsilyl
TEA	triethylamine
TES	triethylsilyl
Tf	trifluoromethanesulfonate
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl

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Tremendous thanks to Professor Theodorakis, my advisor as his doors have always been open for me. He has taught me the fundamentals of organic chemistry, and encouraged me to think critically and pursue experimental work outside the field of organic chemistry. His support has been unconditional, and for that I'm ever grateful. Thanks to Dr. Jing Xu for his help, support and training during both of my thesis projects. His training is invaluable to me and has fortified my research skills.

All of this research was supported by the UCSD Academic Senate, and the National Institue of Health (NIH). The material in Chapter 1, in full, is a reprint of the material as it appears in Enantioselective Formal Synthesis of (–)-Englerin A via a Rh-Catalyzed [4 + 3] Cycloaddition Reaction in Organic Letters 2010. Xu, J.; Caro-Diaz, E.J.E.; Theodorakis, E.A., 2010 and in Formal Synthesis of (–)-Englerin A and Cytotoxicity Studies of Truncated Englerins in Chemistry: An Asian Journal. Xu, J.; Caro-Diaz, E.J.E.; Batova, A.; Theodorakis, E.A., 2012. The dissertation author was the primary investigator and author of this material.

The material in Chapter 2, in full, is a reprint of the material as it appears in Nature-Inspired Total Synthesis of (–)-Fusarisetin A in Journal of the American Chemical Society 2012. Xu, J.; Caro-Diaz, E.J.E.; Trzoss, L., 2012, in Fusarisetin A: scalable total synthesis and related studies in Chemical Science 2012. Caro-Diaz, E.J.E.; Xu, J.; Lacoske, M.; Jamora, C.; Theodorakis, E.A., 2012 and in Fusarisetins: structure–function studies on a novel class of cell migration inhibitors in Organic Chemistry Frontiers 2013. Caro-Diaz, E.J.E.; Aung, A.; Xu, J.; Varghese, S.; Theodorakis, E.A., 2013. The dissertation author was the primary investigator and author of this material.

VITA

2008 University of Puerto Rico, Rio Piedras, San Juan, PR
B.S in Chemistry
Research Advisor: Prof. John A Soderquist

2014 University of California, San Diego, La Jolla, CA
Ph.D. in Chemistry
Research Advisor: Prof. Emmanuel A. Theodorakis

PUBLICATIONS

1. "Fusarisetins: structure–function studies on a novel class of cell migration inhibitors". Caro-Diaz, E.J.E.; Aung, A.; Xu, J.; Varghese, S.; Theodorakis, E.A. *Org. Chem. Front.* **2013**, 1, 135–139.

2. "Fusarisetin A: scalable total synthesis and related studies". Caro-Diaz, E.J.E.; Xu, J.; Lacoske, M.; Jamora, C.; Theodorakis, E.A. *Chem. Sci.* **2012**, 3, 3378–3386

3. "Nature-Inspired Total Synthesis of (–)-Fusarisetin A". Xu, J.; Caro-Diaz, E.J.E.; Trzoss, L.; Theodorakis, E.A. *J. Am. Chem. Soc.* **2012**, 134 (11), 5072–5075.

4. "Formal Synthesis of (–)-Englerin A and Cytotoxicity Studies of Truncated Englerins". Xu, J.; Caro-Diaz, E.J.E.; Batova, A.; Theodorakis, E.A. *Chem. Asian. J.* **2012**, 7 (5), 1052–1060.

5. "Enantioselective Formal Synthesis of (–)-Englerin A via a Rh-Catalyzed [4 + 3] Cycloaddition Reaction". Xu, J.; Caro-Diaz, E.J.E.; Theodorakis, E.A. *Org. Lett.* **2010**, 12 (16), 3708–3711.

AWARDS

Teaching Assistant Excellence Award	2009, 2013
AGEP Fellow	2008
AMGEN Scholar	2007
MARC-NIH Fellow	2006

ABSTRACT OF THE DISSERTATION

Secondary metabolites as potential cancer therapeutic leads: Synthesis and chemical biology of Englerin A and Fusarisetin A

by

Eduardo J.E. Caro-Diaz

Doctor of Philosophy in Chemistry

University of California, San Diego, 2014

Professor Emmanuel A. Theodorakis, Chair

Secondary metabolites generated from natural sources such as microbes, fungi, marine fauna and other microorganism have proven to represent a microcosm of chemical diversity and therefore a great source of novel phamacophoric structures. It is without question that nature in its long biological and chemical evolution has gifted us with beautiful molecular architectures with equally important biological function to

provide leads into new and potentially useful biologically active molecules. As a result, it is not surprising that drug discovery efforts, in both industry and academia, have greatly benefited from isolation and characterization of secondary metabolites. For this reason, it is still important to continue our investigative endeavors toward the discovery of novel motifs with undescribed mechanisms of action in the effort to provide new leads towards oncologic small molecule therapeutics.

As synthetic chemist secondary metabolites can also encompass lessons about complex reactivity and, with the accelerated development of more sophisticated reactions, can present new challenges to develop more efficient and elaborate strategies towards the total synthesis of complex organic structures. It is both an intricate structural architecture and unprecedented biological activity that provide a solid research project towards the development of a synthetic route of a small molecule secondary metabolite. Englerin A and fusarisetin A both represent all these important attributes and were therefore selected by the primary investigator as synthetic targets for research.

Research herein described the work surrounding the formal synthesis of englerin A, the chemical biology in terms of Structural-Activity-Relationship of englerin A, the total synthesis of fusarisetin A, and the chemical biology of fusarisetins. Chapter 1 will narrate the background and research related to synthetic studies and chemical biology of englerin A. Chapter 1 will narrate the background and research related to synthetic studies and chemical biology of fusarisetin A.

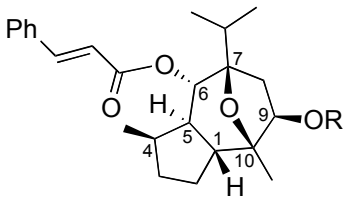
Chapter 1

Synthetic studies and chemical biology of englerin A

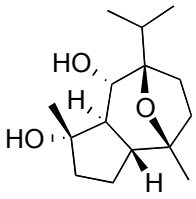
1.1 Isolation and biological characterization

The need to identify new chemical motifs as potential drug leads has spurred the screening of plant extracts used in traditional African, Ayurvedic (Indian) and Chinese medicines.[1] In particular, South Africa has a remarkable botanical diversity with over 30,000 flowering species, from which more than 3,000 are used for medicinal purposes throughout the country.[2] Among them, plants of the genus *Phyllanthus* (Euphorbiaceae, Figure 1.1.1)[3] are widely distributed and have long been used in African folk medicine to treat kidney and urinary tract infections.[4] With this in mind, the Beutler laboratory has been screening extracts of the Tanzanian plant *Phyllanthus engleri* against renal cell carcinoma (RCC) and has recently reported the isolation of two novel bioactive sesquiterpenes, named englerin A (**1**) and englerin B (**2**) (Figure 2).[5]

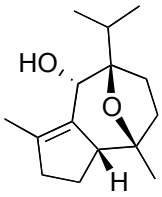
Figure 1.1.1 *Phyllanthus engleri*



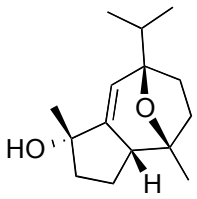
(**1**) englerin A; R = $COCH_2OH$
(**2**) englerin B; R = H
(**3**) englerin B acetate; R = Ac



orientalol E (**4**)



orientalol F (**5**)



pubinernoid B (**6**)

Figure 1.1.2 Structure of englerin A, B, and related natural products

Preliminary biological investigations[4] have shown that **1** possesses very potent growth inhibitory activity (GI_{50} = 1–87 nM, Table 1.1.1)[4] against RCC with approximately 1,000-fold tissue selectivity as compared to other carcinomas. These findings are of particular significance since RCC: (a) is among one of the ten leading cancer types in the US; [6] (b) is characterized by a lack of early warning signs, has diverse clinical manifestations and shows resistance to radiation;[7] and (c) cannot be effectively treated with current chemotherapeutic agents, leaving surgical procedures as the only treatment option.[8]

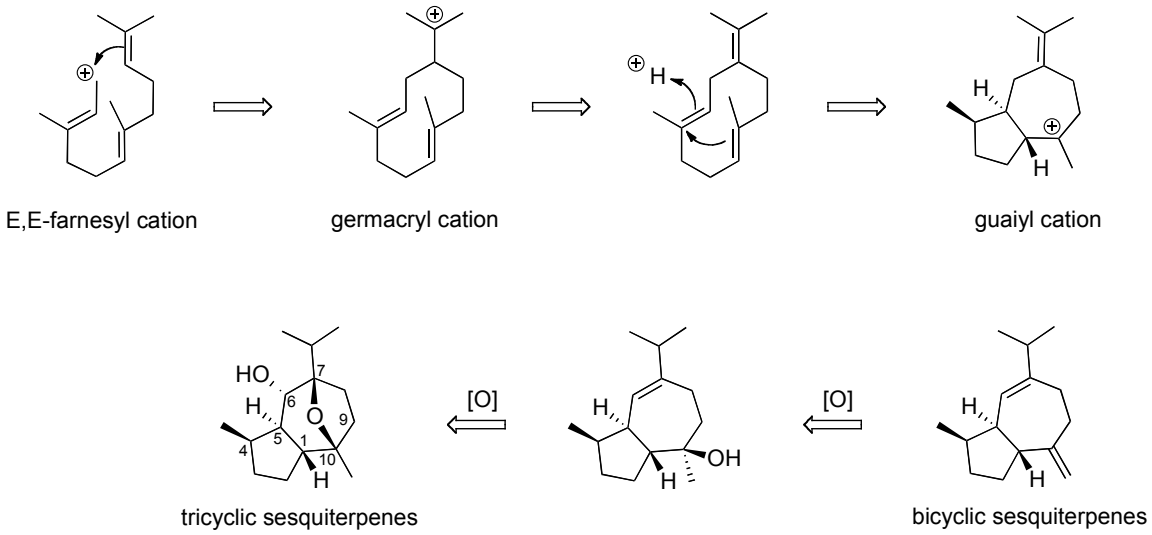
Table 1.1.1 Renal cancer cell inhibition given as GI_{50} values (in μM) for englerin A (1) compared to average values of Taxol

renal cell line	**1**	Taxol
786−0	<0.01	0.034
A498	<0.01	0.10
ACHN	<0.01	0.65
CAKI-1	15.5	0.35
RXF-393	0.011	0.041
SN12C	0.087	0.018
TK-10	15.5	0.11
UO-31	<0.01	0.45

1.2 Biosynthesis of guianine sesquiterpenes

Biogenetically, the englerins belong to the family of guaiane natural products[9] which arise originally from mutiple carbocataionic rearrangments of the E,E farnesyl cation well documented in terpene biosynthesis. The guaiyl cation can arrange and skeleton and can then undergo a sequence of oxygenations and oxo-cyclizations rearrange olefin functionalities at different regions of the common bicyclic sesquiterpene Further decoration at the periphery of this core produces the structures of **1** and **2**. In

general, the englerin family is characterized by decahydroazulene fused ring core with an oxygen bridge between C_7 and C_{10} carbon centers as well hydroxyl substitutions at the C_6 position. Synthetically, this motif represents an interesting and complex exercise to develop and investigate in the chemical laboratory.



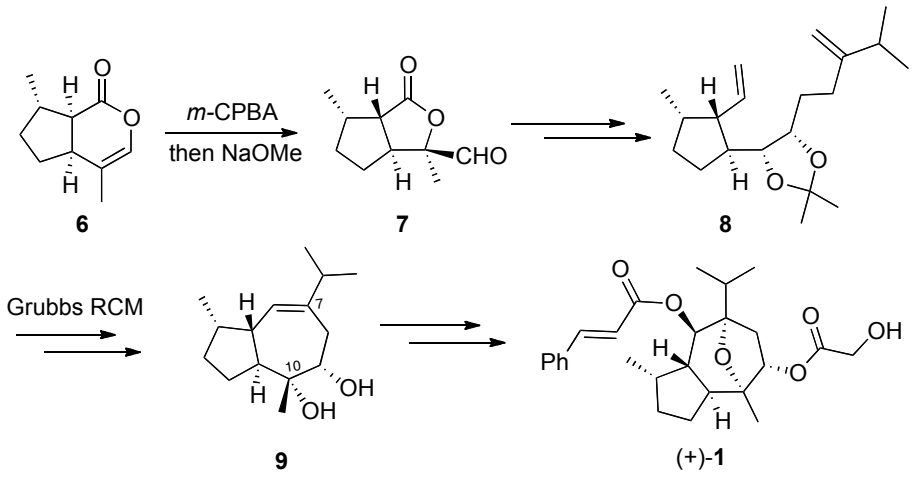
Scheme 1.1.1 Biosynthesis of guaiane sesquiterpenes.

1.3 Reported synthetic studies

Due to the combination of uncommon structural architecture, potent and selective cytotoxicity against RCC and promising pharmacology, the englerins have received enormous amount of attention from the chemical community. Since their isolation in 2008, more than half a dozen total syntheses and many strategies and studies have been reported toward these natural products. In good scientific spirit, strategies towards the synthesis of englerin A have shown to be quite dynamic and diverse in approach. The most attention has been dedicated to construction of the ABC ring system that represents the core of englerin's molecular complexity. It is worth summarizing these strategies.

1.3.1 Christmann's synthesis of (+)-englerin A

Christmann's synthesis[10] of englerin A is highlighted by the conversion of lactone **6** to pentalactone **7** via oxidative rearrangement, opening of the B ring to diolefin **8** and a Grubbs promoted ring closing metathesis to generate the decahydroazulene moiety. Finally, diol **9** can be converted to englerin A *via m*CPBA epoxidation of **9** and opening of this epoxide by C_{10} hydroxl attack to the C_7 center to generate the oxygen bridge. The final synthetic material gave the exact opposite optical rotation as the isolated natural product, therefore reassigned the absolute stereochemistry of englerin A.

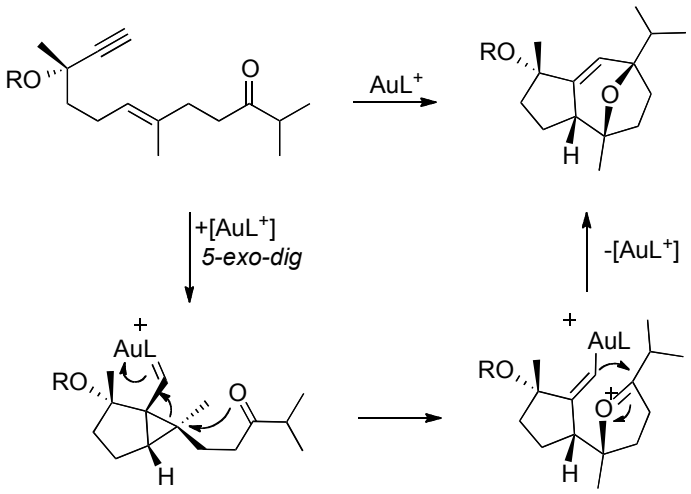


Scheme 1.3.1.1 Chirstmann's synthesis of englerin A.

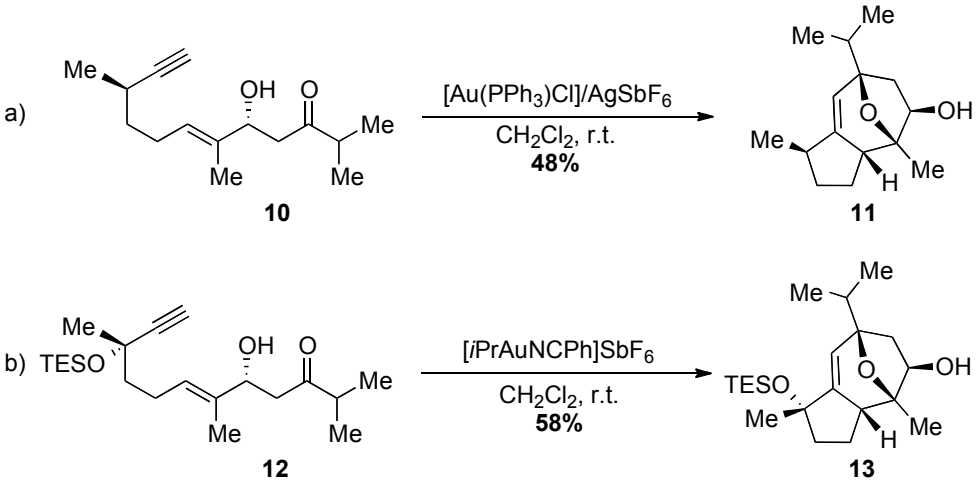
1.3.2 Echevarren's and Ma's Au-catalzyed cyclization strategy

Echevarren *et al.* have investigated the reactivity profile of intramolecular cyclizations of ketoenynes in the presence of different gold catalysts and through this strategy has successfully synthesized orientalol F (**4**) (Scheme 1.3.2.1).[11] Presumably, this pathway operates via a insertion of gold to the alkyene, attack by the alkene to generate a reactive cyclopropane intermediate that can undergo 5-exo-dig cyclization

from the ketone, seven member ring formation and reductive elimination to yield the B ring of **1**. Both Ma[12] (Scheme 1.3.2.2, a) and Echevarren[13] (Scheme 1.3.2.2, b) would use this reactivity toward the synthesis of englerin A and in this way generate the ABC core ring structure in a 1 step process. Final functionalization of the ABC core would ultimately yield (–)-englerin A.



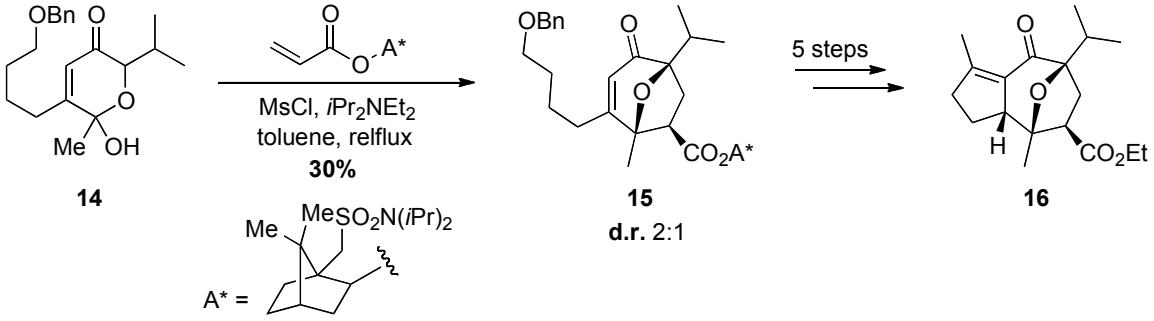
Scheme 1.3.2.1 Echevarren's Au-catalyzed cyclization strategy in the synthesis of **5**



Scheme 1.3.2.2 a) Ma's and b) Echevarren's Au-catalyzed cyclization strategy

1.3.3 Nicolaou's synthesis of (±)-englerin A and formal synthesis of (–)-engelerin A

Nicolaou *et al* took advantage of chemistry described by Wender[14] to assemble the AB ring of englerin A system *via* a [5+2] cycladdition.[15] They could then also provide a stereoselective formal synthesis by using a chiral acrylate ester to generate modest diasteremeric enrichment (2:1) (Scheme 1.3.3.1).



Scheme 1.3.3.1 Nicolaou's [5+2] annulation reaction towards the synthesis of englerin A

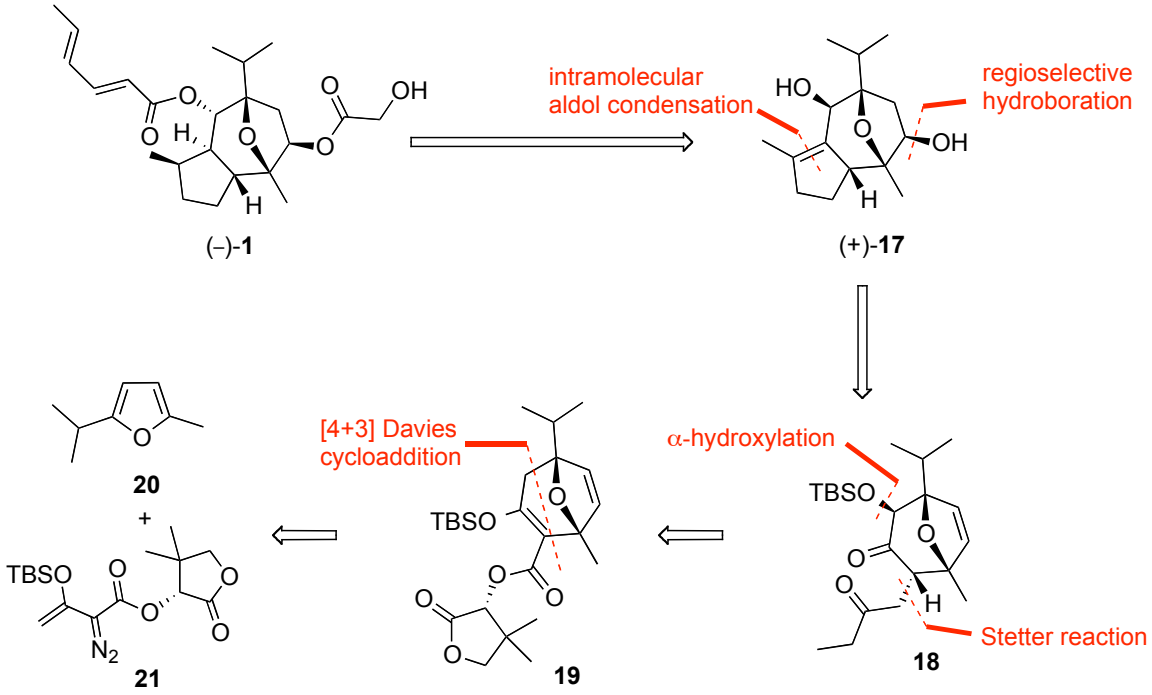
1.3.4 Chain's, Maiers', Lin's and Parkers strategies

After completion and publication of our synthetic work on englerin A, the synthetic community continued to develop new approaches towards the synthesis of **1**. Chain's presented a strategy towards the tricyclic core of englerin A through an umpolung addition of an aliphatic aldehyde moiety to a proximal α,β-unsaturated ketone promoted by SmI_2.[16] Maiers' approach encompassed a similar approach to Nicolaou's by use of a late stage [5+2] Rh-catalyzed cylcoaddition.[17] Lin's synthesis used a similar approach to Theodorakis, making us of a [4+3] cycloaddition between a furan and diene, yet differed by generating the asymmetric environment of this process using a chrial organocatalyst.[18] Most recently, Parker *et al* reported a bicylcization strategy consisting of a relay ene-yne-ene metathasis that coverts a linear substrate into the guiaine core of

englerin A.[19] Two recent reviews nicely summarize all of these approached and the current status of englerins.[20]

1.4 Formal synthesis of (–)-englerin A

Our continuous interest in exploring natural products from ethnomedicine as medicinal leads[21] prompted us to design an enantioselective strategy toward englerins.[22] Here in, we describe in detail the results of our synthetic efforts, failed strategies, and detailed mechanistic approach towards the synthesis guiaine core of ()-englerin A. [23]



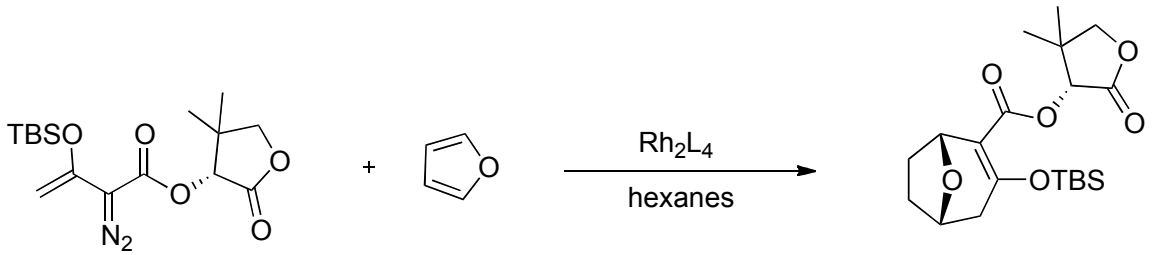
Scheme 1.4.1 Retrosynthetic analysis of (–)-englerin A

Our retrosynthetic strategy is shown in Scheme 1. We anticipated that a sequence of reactions including: inversion of the C_6 stereocenter, hydrogenation of the C_4-C_5 alkene (englerin numbering) and selective esterifications at C_6 and C_9 hydroxyl

groups would form englerin (**1**) from diol **17**. Compound **17** could be derived a regioselective hydroboration of the C_8–C_9 double bond and the cyclopentene moiety of **17** could be constructed from an intramolecular aldol condensation. An α-hydroxylation would produce the C_6 hydroxyl substitution and conjugate addition of common propanal could generate diketone **18**. Further disconnection along these lines led us to target the construction of bicyclic motif **19** possessing the BC ring scaffold of englerins. We hypothesized that compound **19** could be formed in enantiomerically pure form by exploring a asymmetric Rh(II)-catalyzed [4+3] cycloaddition between readily available furan **20** and chiral pantolactone-derived diazoester **21**.

1.4.2. Synthesis of the BC ring system

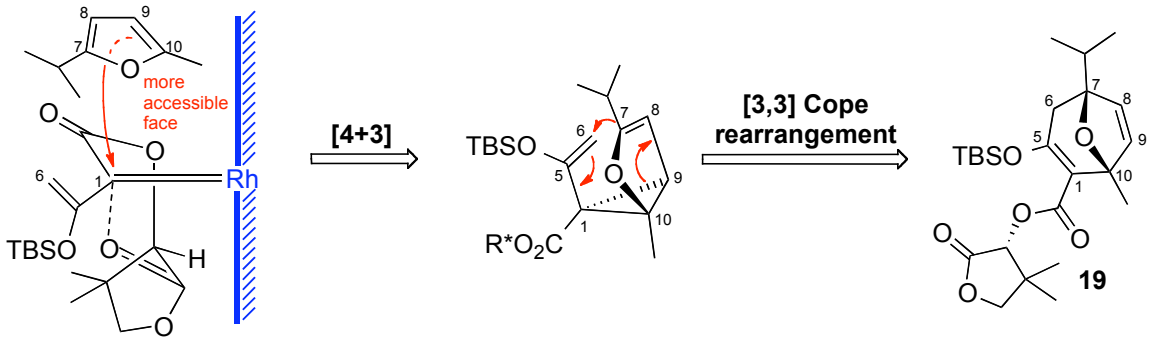
Rhodium-triggered cyclization reactions have been widely applied in synthetic chemistry.[24] In 1996, Davies *et al.* reported an elegant enantioselective Rh(II)-catalyzed [4+3] cycloaddition between furans and chiral diazoesters (Scheme 1.4.2.1).[25] Selection of the appropriate pantolactone enantiomer, as the chiral auxiliary, allows diastereo-control of the produced oxy-bridged adduct. The transfer of chirality, observed in this reaction, can be rationalized by considering that the carbonyl moiety of pantolactone interacts with the Rh-carbenoid as shown in Scheme 1.4.2.2. This interaction blocks one of the two faces of the carbene **21** leading to a selective *si*-face attack by the 2-



22 **23** **24**

Scheme 1.4.2.1 Davies [4+3] cycloaddition of furans to asymmetric chiral diazoesters

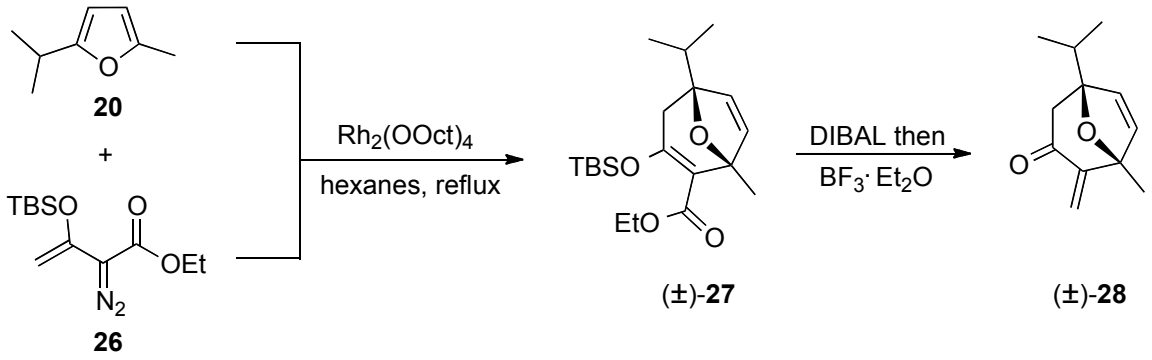
methyl-5-isopropylfuran **20**. The stereochemical outcome is consistent with a tandem cyclopropanation/Cope rearrangement. Cyclopropanation of the unsymmetric furan **20** was expected to occur in a regioselective manner at the less substituted and thus less hindered C_9-C_{10} double bond. The potential of this cycloaddition to construct bicyclic structure **19** was of great interest to our strategy, as it provided an efficient, rapid and enantioselective access to the englerin core. Notably, both of starting materials **20**[26] and **21**[19a, 27] can be simply prepared in more than 50 gram-scale from commercially available materials in 3 steps for each, respectively.



Scheme 1.4.2.2 Stereochemical rationale of Davies Rh-catalyzed [4+3] cycloaddition

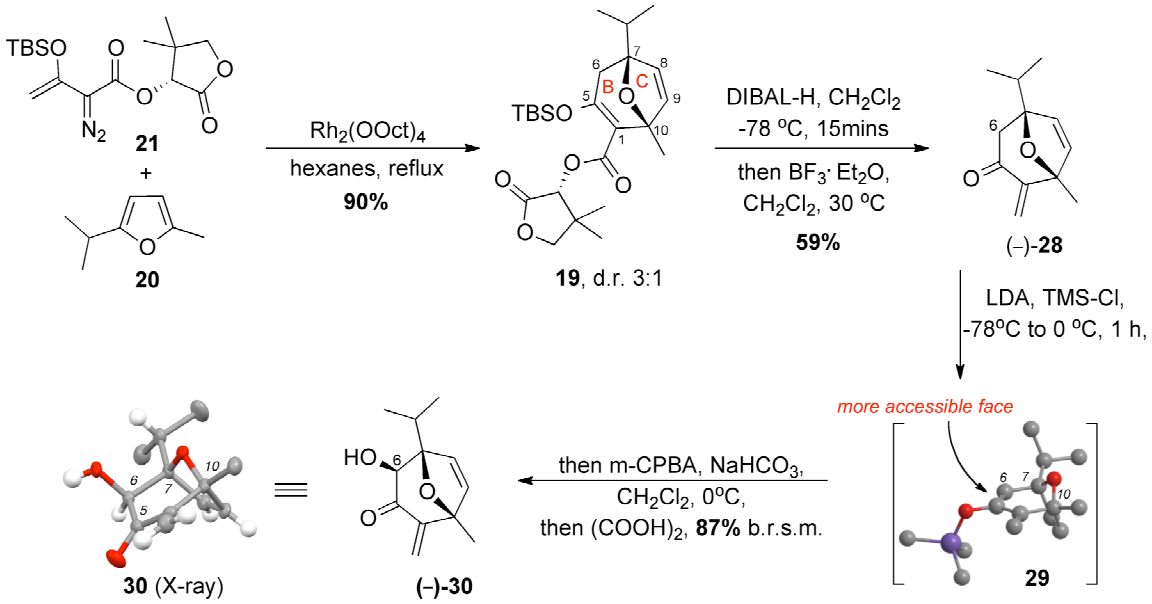
To the best of our knowledge, 2,5-disubstituted furans have not been evaluated as substrates in the Rh(II)-catalyzed [4+3] cycloaddition. With this in mind, we initially evaluated the regioselectivity of this reaction using achiral diazoester **26**[28] as a model system (Scheme 1.4.2.3). Refluxing of **20** and diazoester **26** in hexane in the presence of catalytic amounts of rhodium(II) octanoate (2 mol%), afforded oxacyclic product (±)-**28** as the only regio-isomer in excellent yields (95%). Cleavage of the ester auxiliary proved to be more challenging than anticipated. In fact, the reported $LiAlH_4$ reductive cleavage led only to decomposition. Gratifyingly, treatment of ester **27** with DIBAL-H yielded a labile β-hydroxy silyl enol ether that, upon immediate treatment with

stoichiometric amounts of BF_3•Et_2O, underwent rearrangement[29] to afford exocyclic enone (±)-**28** in good yield (81%).



Scheme 1.4.2.3 Rationale of Davies Rh-catalyzed [4+3] cycloaddition

Encouraged by these results, we synthesized chiral diazoester **21** departing from (*R*)-pantolactone.[15a,17] The Rh(II)-catalyzed [4+3] cycloaddition of **20** with **21** proceeded efficiently with $Rh_2(OOct)_4$ to yield **19** in good yield (90%) albeit in moderate diastereoselectivity (dr 3:1, calculated via ^{1}H-NMR) (Scheme 1.4.2.4). It should be noted that, under the same conditions, use of $Rh_2(OAc)_2$ led only to decomposition of the starting materials. Moreover, attempts to increase the d.r by performing this reaction at lower temperature or by using less catalyst loading led to a significant decrease in the yield without any significant enhancement of diastereoselectivity (Table 1.4.2.1). The diastereomeric mixture of **19** could be separated via a silica gel column chromatography. Our previously established reductive cleavage conditions were applied to **19** to produce optically active enone (–)-**30**, although the yield was significantly lowered (59%). This decrease of yield may be due to the presence of the two reactive carbonyl moieties in **19** that require extended reaction times and excess of DIBAL-H (7.5 equiv) as compared to reduction of (±)-**28** (2.5 equiv), leading to partial decomposition of the sensitive silyl enol ether moiety.



Scheme 1.4.2.4 Asymmetric synthesis of the BC ring of **1**

Table 1.4.2.1 Optimization of the key [4+3] cycloaddition reaction

Furan 20	Catalyst (mol%)	Temp.	Yield (dr)
10 equiv	$Rh_2(OOct)_4$ (2 mol%)	reflux	91% (3:1)
2 equiv	$Rh_2(OOct)_4$ (2 mol%)	reflux	90% (3:1)
2 equiv	$Rh_2(OOct)_4$ (1 mol%)	reflux	73% (3:1)
2 equiv	$Rh_2(OOct)_4$ (2 mol%)	–78 °C	n.r.
2 equiv	$Rh_2(OOct)_4$ (2 mol%)	–40 °C	n.r.
2 equiv	$Rh_2(OOct)_4$ (2 mol%)	0 °C	n.r.
5 equiv	$Rh_2(OOct)_4$ (2 mol%)	30 °C	31% (n/a)
5 equiv	$Rh_2(OAc)_4$ (2 mol%)	reflux	n.r.

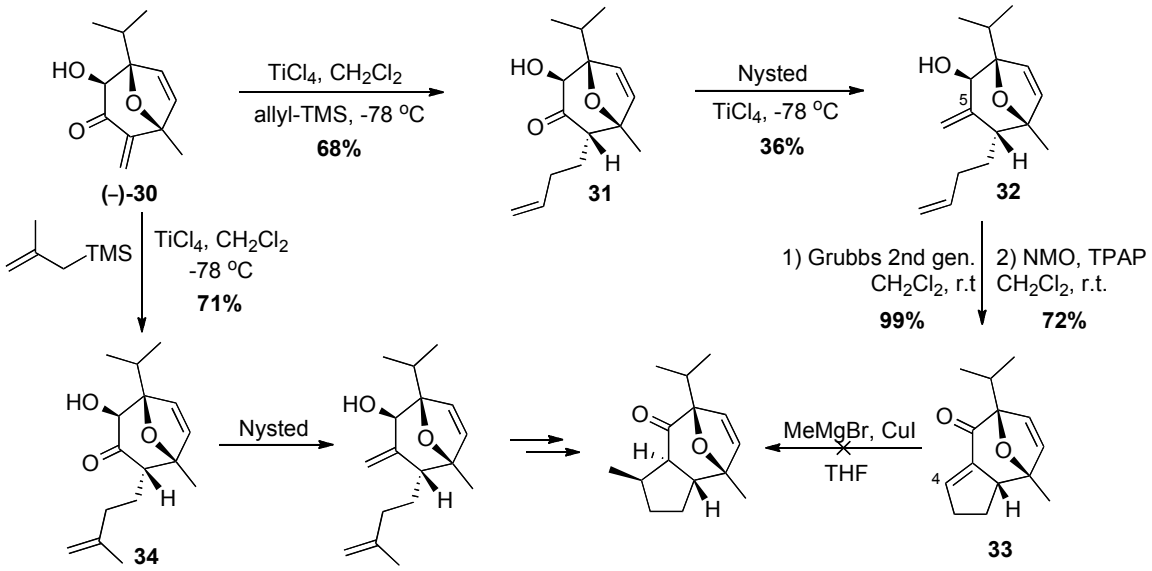
The next attempts of installing a hydroxyl group on the C_6 carbon of (–)-**28** with Davis oxaziridines[30] proved unsuccessful. Gratifyingly, Rubottom oxidation[31] provided the desired hydroxy enone **30** in good yield (87% brsm), although with the inverse

stereochemistry as compared to the englerin C_6 hydroxyl moiety. The absolute stereochemistry of hydroxyl enone **30** was established by a single crystal X-ray analysis,[32] which simultaneously confirmed the absolute stereochemistry of oxy-bridged ester **19**. We were pleased to observe that the Rubottom oxidation proceeded regioselectively at the more electronically rich TMS enol ether without affecting any other alkenes in this molecule. The stereochemical outcome of this reaction was also satisfactory since it proceeded exclusively from the top face of the intermediate TMS-enolate **29** suggesting that this face is less hindered. We predicted that this selectivity would allow us to have complete substrate control during the following steps and invert the C_6 stereochemistry at a latter stage in our synthesis.

1.4.3 Synthesis of the A ring

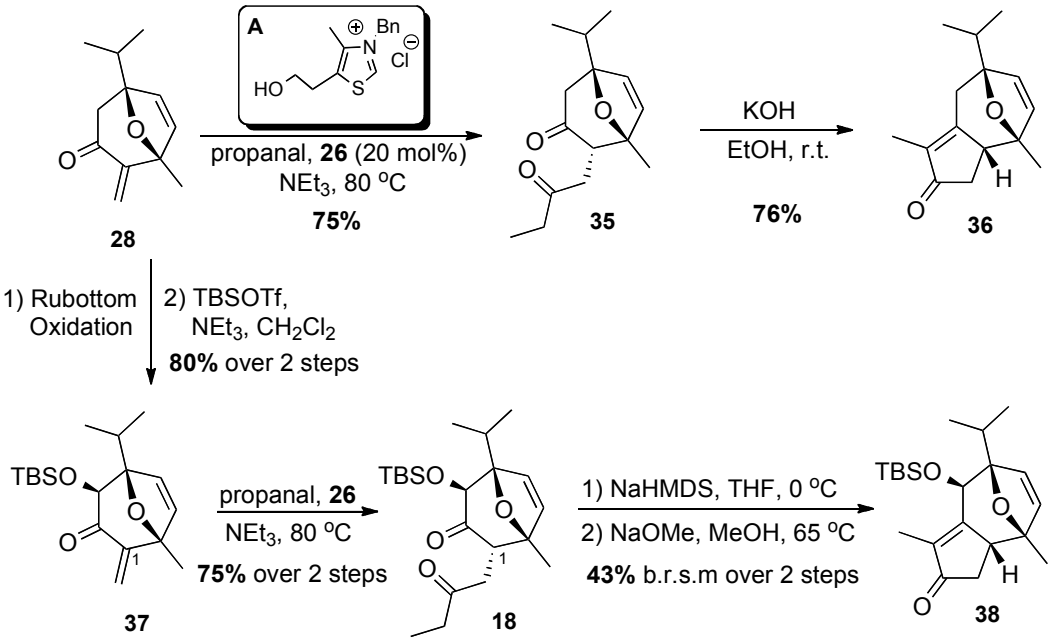
The next stage of the synthesis involved construction of the tricyclic core of englerin from compound **30**. We envisioned to accomplish this cyclization by the means of a Grubbs ring closing metathesis (RCM)[33] of precursor **32** (Scheme 1.4.3.1) that after oxidation of C_6 hydroxyl group would reveal a conjugate system for 1,4-addition of methyl nucleophile at the C_4 site. Lewis acid-promoted allylation with allyltrimethylsilane afforded the corresponding ketone **31** in moderate yield (68%) as a single diastereomer. The ensuing olefination of C_5, however, proved to be unexpectedly challenging. After attempting many different methods including Wittig reaction, Peterson olefination,[34] Petasis[35] or Tebbe olefination[36] etc., the $TiCl_4$-assisted Nysted olefination[37] gave low yields (15-36%) of trialkene **32**. Despite the inefficient formation of **32** we attempted the RCM. We were pleased to see that this reaction proceeded smoothly in almost quantitative yields to give the corresponding allylic alcohol, which after TPAP/NMO

oxidation,[38] formed α,β-unsaturated ketone **33**. Unfortunately, all efforts to add methyl nucleophiles to **33** via conjugate addition met with failure. To overcome this issue, we sought to perform the RCM on a substrate that has the challenging C_{11} methyl group preinstalled. Along these lines, we allylated **30** with methylallyltrimethyl silane. While we were successful in producing **34**, olefination of this precursor at C_5 resulted in irreproducible results. This prompted us to abandon this approach and seek an alternative strategy for the formation of the 5-membered ring from **30**.



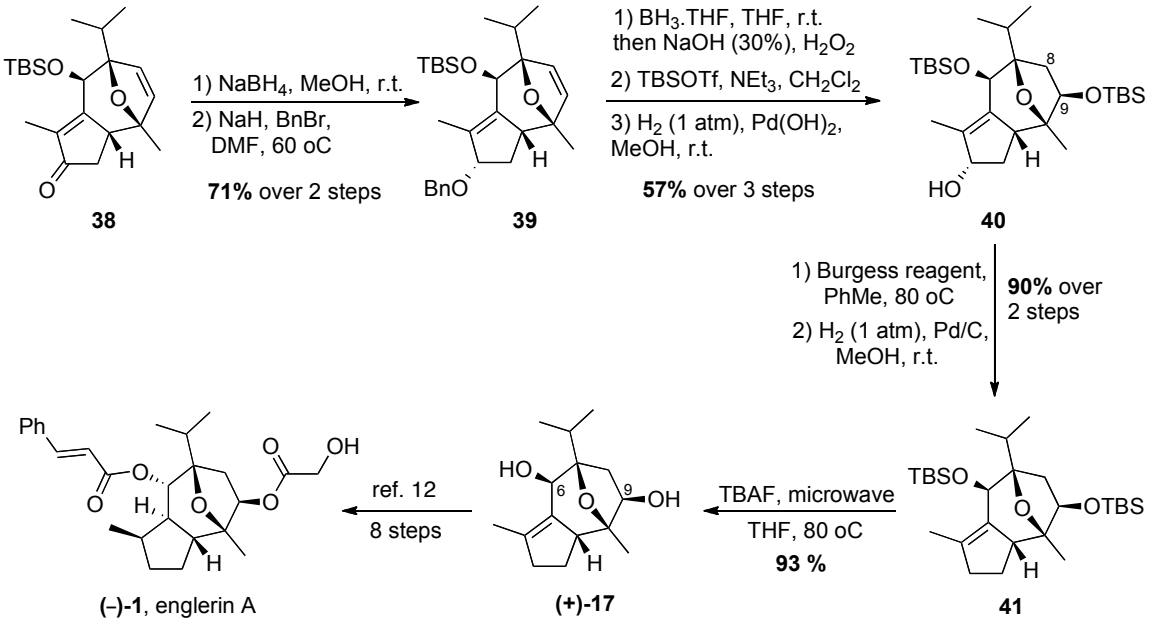
Scheme 1.4.3.1 Attempts to construct the A ring of **1** *via* a RCM reaction

Based on the above results, we pursued an alternative strategy for the construction of the tricyclic core of englerin based on an intramolecular aldol condensation. Initially, we explored the feasibility of this reaction using non hydroxylated enone **17** as a model system (Scheme 1.4.3.2). To this end, treatment of propanal with thiazolium salt (**A**) and enone **28** under Stetter conditions[39] produced diketone **35**. To our delight, the intramolecular aldol reaction of **35** proceeded under mild conditions (KOH/EtOH, rt, 24 hours) to afford **36** in good yield (76%).



Scheme 1.4.3.2 Synthesis of the A ring *via* a intramolecular aldol condesation

Motivated by this result, we converted **30** to the C_6 TBS silyl ether **37** and treated this product with propanal under the previously established Stetter conditions. This reaction proceeded efficiently under basic conditions to furnish diketone **18** in good yield (75% over two steps) as a single diastereomer (Scheme 1.4.3.2). However, application of previously successful KOH condensation only resulted in deprotection of the TBS ether with no further reaction. This prompted an extensive investigation on this intramolecular condensation, with different C_6 protecting groups (H-, MOM-, TES-, etc.), various bases (*t*-BuOK, KOH, NaOMe, LDA, etc.) and various reaction conditions. Many failed attempts of the aldol condensation to provide the tricyclic motif proved this reaction to be unexpectedly difficult. Eventually, treatment of diketone **18** with NaHMDS afforded the corresponding aldol product, which underwent sequential dehydration process (NaOMe, Δ) to produce the key tricyclic core **38** in an acceptable yield (43% brsm).



Scheme 1.4.3.3 Finalization of the formal synthesis of englerin A

1.4.4 Completion of formal synthesis

$NaBH_4$ reduction of enone **38** yielded the corresponding allylic alcohol (99%) as a single diastereomer that, without further purification, was protected as the benzyl ether to furnish **39** in good yield (71%) (Scheme 1.4.3.3). Treatment of **39** with BH_3•THF followed by H_2O_2 oxidation afforded regio- and stereoselectively alcohol C_9 (60%). That after standard TBS silylation followed by deprotection of the C_3 benzyl ether yielded **40** in 57% over 3 steps. (Scheme 7). Our efforts to hydrogenate the tetra-substituted alkene of **40** using different catalysts and H_2 pressure were unsuccessful presumably due to the steric hindrance of the double bond (C_3-C_4). This prompted us to deoxygenate the C_3 hydroxyl group under Barton-McCombie conditions[40] (40% yield). In our efforts to improve this transformation, we discovered that dehydration of **40** with Burgess

reagent[41] followed by standard hydrogenation improved significantly the yield to 90% over the two steps. Deprotection of the di-TBS ether **41** with TBAF gave poor results, however, we were pleased to find that a microwave accelerated reaction under similar conditions (TBAF/THF, 80 ºC) made diol (+)-**17** in a quick and high yielding conversion (45 min, 93%).

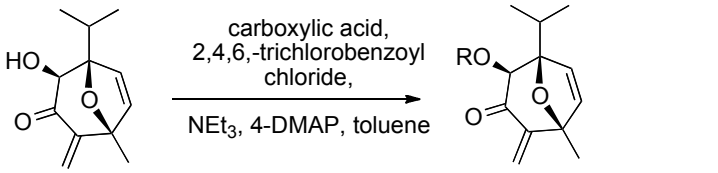
To access the natural product from diol (+)-**17**, we would need to achieve stereoselective saturation of the tetra-substituted bond followed by esterification of the C_9 hydroxyl group, inversion of C_6 stereocenter by oxidation/reduction sequence and finally esterification in the presence of cinnamic acid. Our synthetic route to (+)-**17** provides another novel and facile method for the construction of englerin A and related compounds. More significantly, the Rh-catalyzed [4+3] annulation/ intramolecular aldol condensation sequence presents a very useful, practical and general method for the preparation of the guaiane sesquiterpene core present in englerin related compounds.

1.5 Synthesis and Biology of englerin analogues

The intriguing bioactivity of englerin A has prompted several groups to perform SAR studies.[42,43] These studies have focused exclusively on modifications of the C_6 and C_9 side chains. An advantage to our strategy is that it can produce in an efficient and stereoselective manner compound **19**, which represents the BC ring system of englerins. In other words, this approach could provide information on the biological significance of the A ring of englerin. Our synthesis also allows for evaluation of different ABC core analogs parting from trycyclic ketone **38** to evaluate the SAR of the guaiane core moiety of **1**.

1.5.1 Synthesis of analogues

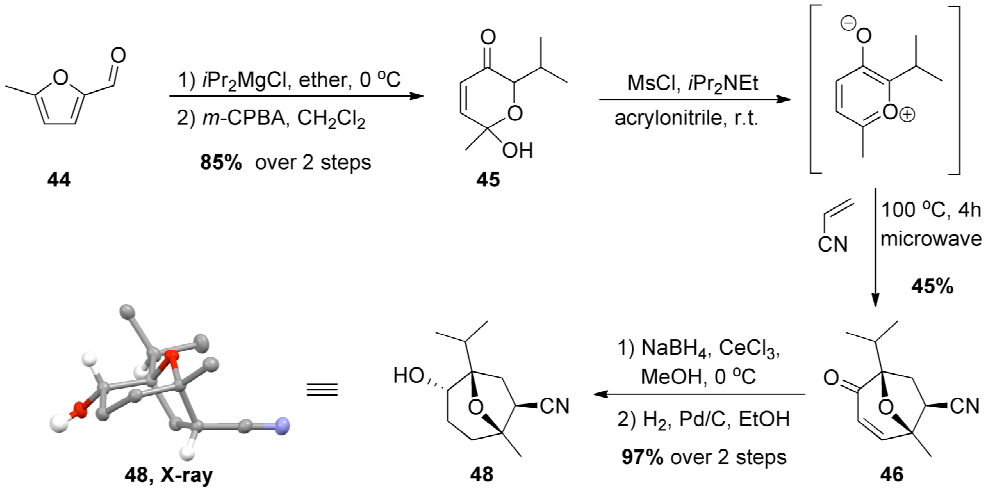
With our strategy in mind, we synthesized truncated englerins **41**, **42** and **43** (Scheme 1.5.1.1) readily from (±)-**30** in *via* 1 step conversions. To further expand such SAR studies, we designed a new approach towards a compound that could represent nor-A-ring englerin A. The synthesis of nor-A-ring **46** is highlighted in Scheme 1.5.1.2 and is inspired by Wender's pioneering [5+2] cycloaddition reactions.[14] 2-methyl-5-furfural (**44**) was first alkylated under Grignard conditions and the resulting alcohol was converted to hemiacetal **45** in 85% overall yield. Treatment of **45** with excess acrylonitrile (100 ºC, 14 h) under gave the desired bicyclic product **46** in 32% yield. This reaction could be accelerated under microwave conditions with improved yield (150 ºC, 4 h, 45%). To the best of our knowledge, this is the first example of microwave accelerated this type of [5+2] cycloaddition reaction. Luche reduction[44] followed by hydrogenation provided compound **47**[45] in almost quantitative yield and provided crystalline material to confirm the relative stereochemistry of **47**. The transformation from **47** to **50** was accomplished in a 3-step sequence that included: (a) oxidative cleavage of the cyanide group to the corresponding ketone; (b) esterification oxidative cleavage of



(±)-30

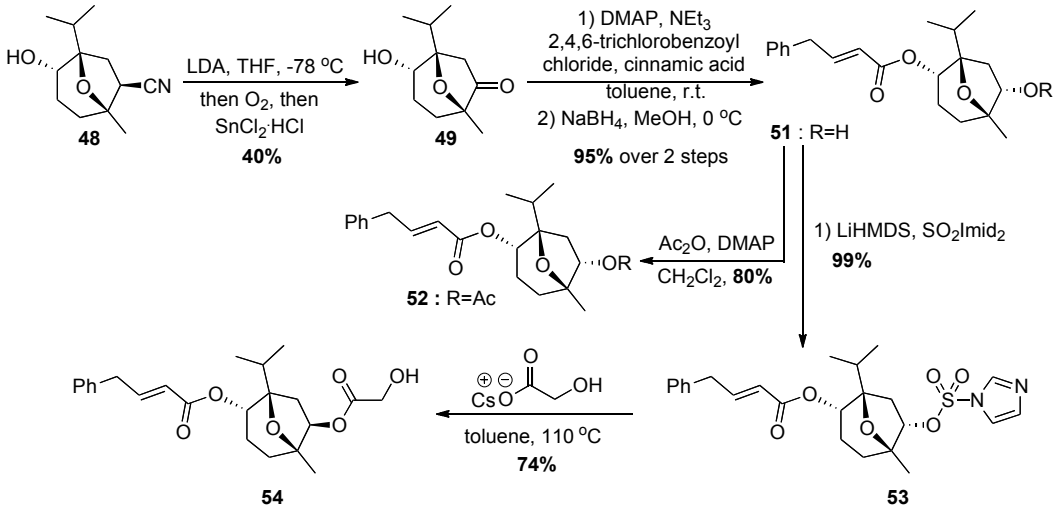
(±)-41: R = PhC(=O)-, 90%
(±)-42: R = Ac-, 95%
(±)-43: R = cinnamoyl-, 88%

Scheme 1.5.1.1 Synthesis of BC ring truncated analogues of **1**



Scheme 1.5.1.2 Syntheisis BC ring on **46** *via* a [5+2] cycloaddition

oxidative cleavage of the cyanide group to the corresponding ketone; (b) esterification with cinnamic acid under Yamaguchi condition;[46] (c) hydride reduction of C_9 carbonyl moiety. The last two steps were performed following the reported method[9b] to furnish **53**. Moreover, the acetate analogue **51** was readily prepared from acetylation of **50**.



Scheme 1.5.1.3 Synthesis of nor-A-englerin A **52** and acetate derivative **51**

1.6 Biological Evaluation

The cytotoxicity of the truncated englerins was evaluated in both A498 renal cancer cells and CEM T-cell acute lymphoblastic leukemia (T-ALL) cells using a ^{3}H-thymidine incorporation assay. In our initial study, we compared the growth inhibitory activity of englerin A to that of truncated englerins **50** and **53**. We found that englerin A inhibited the growth of A498 renal cancer cells with a GI_{50} of 45 nM which is in agreement with previous findings (Figure 2).[4,9d,9f,10e] However, compounds **50** and **53** did not have any effect on the growth of A498 cells even at concentrations of 100 nM or greater (Figure 1.6.1). These results suggest that the A ring is essential for the growth inhibitory activity of englerin A in renal cancer cells. We then evaluated the anti-proliferative activity of englerin analogues in CEM cells, a cell line in which englerin A has little activity with a reported GI_{50} of 20.4 mM.[4] Of all analogues tested, **17**, (±)-**17**,

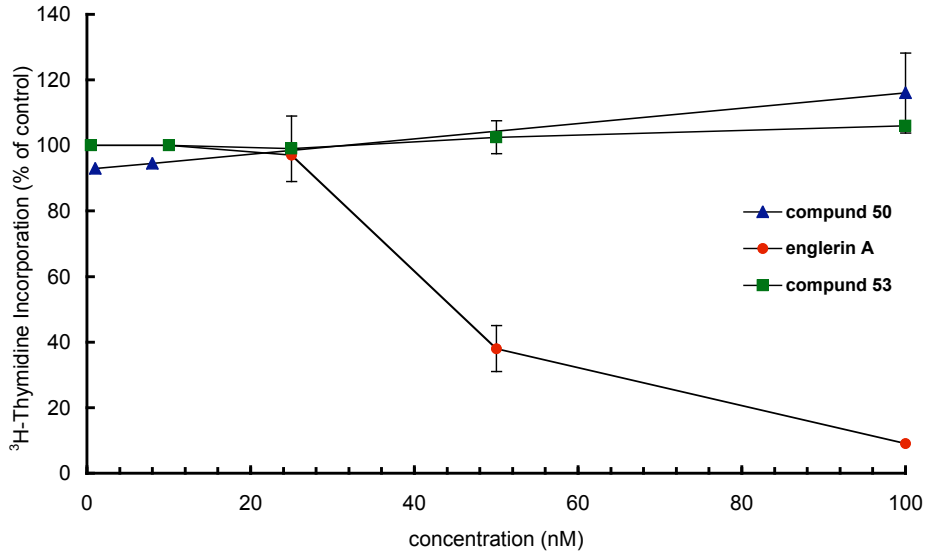


Figure 1.6.1 Effect of englerin A and analogues **50** and **53** on the proliferation in of A498 renal cancer cells

Table 1.6.1 Inhibition of cell proliferation by (–)-englerin A and analogues

Compound	% of control at 20 nM	GI_{50} (nM)
(–)-1	81.4 ± 2.5	> 20
44	74.6 ± 2.6	> 20
45	75.4 ± 4.5	> 20
46	88.7 ± 4.3	> 20
7	98.7 ± 4.0	> 20
17	0.2 ± 0	3.3
(±)-17	ND	1.8
19	0.4 ± 0.1	2.4
37	0.3 ± 0.3	ND
38	0.2 ± 0.1	ND
39	0.1 ± 0	ND

and **19** had significant cytotoxicity with GI_{50} values of 3.3, 1.8, and 2.4 mM, respectively (Figure 3, Table 2). We assume that the cytotoxicities might be due to the exocyclic enone. In contrast, englerin A and analogues containing an additional ring, such as **7**, had little or no cytotoxicity at concentrations as high as 20 mM.

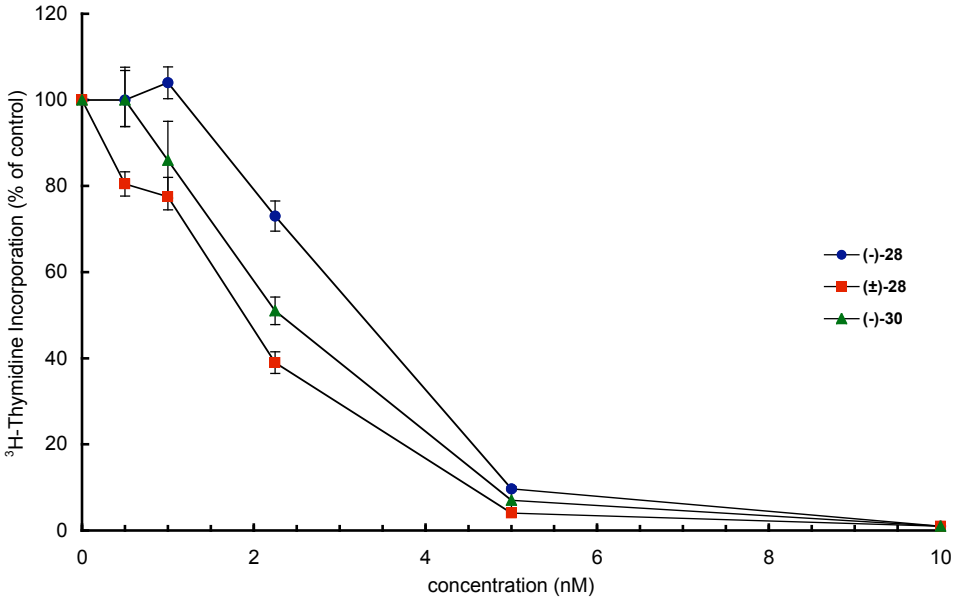


Figure 1.6.1 Effect of analogues **28** and **30** on the proliferation in of A498 renal cancer cells

1.6 Concluding remarks

We have accomplished an efficient and enantioselective synthesis of englerin A (1), a potent and selective growth inhibitor of renal cancer cells. The synthetic approach to intermediate 6 proceeds in 15 steps from readily available compounds 9 and 10 in 5% overall yield. Key to our strategy is the enantioselective formation of the BC ring of 1 via a Rh(II)-induced enantioselective [4+3] cycloaddition, followed by the construction of the A ring via an intramolecular aldol condensation. Inspired by this sequence, we have synthesized a small family of truncated englerins and have evaluated their growth inhibitory activities against certain renal cancer and leukemia cell lines. These studies suggest that the A-ring of englerin A plays an important role in its bioactivity and tissue selectivity. Interestingly, compounds (–)-17, (±)-17 and (–)-19 have shown significant growth inhibitory activity against CEM cell lines at low micromolar concentrations (GI_{50} = 1–3 mM). Consequently, these compounds may represent new lead structures for the development of small molecule therapeutics against leukaemia.

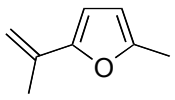
1.7 Experimental techniques and characterization data

General Techniques

All reactions were carried out under argon atmosphere. Dry tetrahydrofuran (THF), diethyl ether (Et_2O), methylene chloride (CH_2Cl_2) and dimethylformamide (DMF) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Diisopropylamine and propanaldehyde were dry distilled from flamed dried 4Å powdered molecular sieves. Dry hexanes was purchased from Sigma Aldrich and used directly. Chromatography: Flash column chromatography

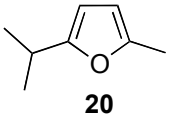
was performed on silica gel (Merck Kieselgel 60, 230-400 mesh) using Hexane-EtOAc (H-E) mixtures of increasing polarity. ^{13}C NMR and ^{1}H NMR spectra: These were recorded on 400 MHz and 500 MHz Varian instruments and 500 MHz JOEL instrument. $CDCl_3$ was treated with flamed dried K_2CO_3, chemical shifts (δH) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak ($CHCl_3$), with the abbreviations s, br s, d, t, q, hept and m denoting singlet, broad singlet, doublet, triplet, quartet, heptet and multiplet respectively. *J* = coupling constants given in Hertz (Hz) were analyzed by MestReNova software. Mass spectra**:** HRMS were recorded on a trisector WG AutoSpecQ spectrometer. Optical rotation: $[\alpha]_D$ measurements were collected on a Jasco P-1010 polarimeter using HPLC grade $CHCl_3$ (dried over molecular sieves). Microwave reactor: Microwave experiments were carried out in Biotage (model:Initiator) microwave reactor using high-pressure vessels. X-ray data were recorded on a Bruker SMART APEX 3kW Sealed Tube X-ray diffraction system.

Experimental procedure



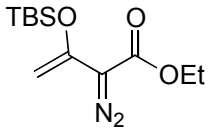
5-Methyl-2-(1-methylethenyl)furan. To a solution of 2-methylfuran (42.0 g, 0.51 mol) in ether (600 ml) *n*-BuLi (1.6 M in hexane, 300 ml, 0.48 mol) was added dropwise at –20°C under N_2. After refluxing for 4 h, the mixture was cooled to –20°C, anhydrous acetone (33.0 g, 0.58 mol) was added in dropwise, and then the mixture was refluxed for overnight. To the mixture saturated NH_4Cl solution (450 ml) was added slowly at rt, and the mixture was stirred until a clear solution was formed. The organic phase was separated and the aqueous phase was extracted with ether for 3 times. The combined organic phases were washed with brine, dried with Na_2SO_4, and evaporated to give 61.2 g of crude tertial alcohol as a red oil, which was used to the next step directly without further purification. A mixture of the crude alcohol (33 g, 240 mmol)

above, Ac_2O (33 ml, 350 mmol) and of KOAc (15 g, 150 mmol) was stirred for 30 min at 110°C under N_2. After neutralization with aqueous Na_2CO_3 solution, the organic layer was separated and the aqueous layer was extracted twice with ether. The combined organic phases were dried with Na_2SO_4 and concentrated as a brown oil. The crude was used to the next step without further purification. Analytical data were all in accordance with reported data. [P. Weyerstahl, J. Brendel, *Liebigs Ann. Chem.* **1988**, 1015–1016.]



20

5-Methyl-2-isopropylfuran (20). An ether solution (200 ml) of crude 5-Methyl-2-(l-methylethenyl)furan (~ 33 g) was added 300 mg of Pd/C (10% Pd) was added and the mixture was hydrogenated (1 bar) for 1 hour. The mixture was then filtrated through celite. Regular distillation gave **9** as a colorless oil which should be stored in freezer (–20 °C) and be consumed within 2 weeks. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.84 (s, br, 2 H), 2.90 (m, 1H), 2.28 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.3, 150.2, 105.8, 103.3, 28.0, 21.5, 13.7; HRMS (FAB) *m/z*: cacld for $C_8H_{13}O$ ($M+H^+$) 125.0961, found: 125.0962.

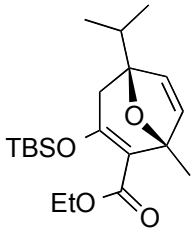


26

Ethyl 3-(TBS)oxy)-2-diazobut-3-enoate (26). To an ice-cooled, stirred mixture of ethyl acetoacetate (13.0 g, 0.10 mol) and *p*-acetamidobenzenesulfonyl azide (*p*-ABSA, 24.5 g, 0.10 mol) in anhydrous acetonitrile (750 ml) was added triethylamine (41.3 ml, 0.30 mol) in one portion. The reaction mixture is warmed to rt and stirred for 2 hours. The solvent is removed under reduced pressure, and the residue is triturated with 500 mL of a 1:1 mixture of ether/hexanes. The mixture is filtered and concentrated under reduced pressure. The crude product is purified by chromatography on silica gel (hexanes/ether, 3:1 to 1.5:1) to yield 14.7 g (94%) of ethyl diazoacetoacetate as a yellow oil. ^{1}H NMR

(500 MHz, $CDCl_3$) δ: 4.28 (q, *J* = 6.9 Hz, 2H), 2.46 (s, 3H), 1.31 (t, *J* = 6.9 Hz, 3H); To an ice-cooled solution of ethyl diazoacetoacetate (10 g, 64 mmol) in anhydrous CH_2Cl_2 (200 ml) was sequentially added triethylamine (17.9 ml, 128 mmol) and TBSOTf (19.1 ml, 83.2 mmol). This reaction was stirred for 30 min at the same temperature before get carefully quenched by 5% $NaHCO_3$ solution. This mixture was extracted with hexanes for three times and dried over Na_2SO_4 and concentrated under reduced pressure to obtain **26** as a yellow oil. This unstable TBS-enol-ether **26** was then used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.98 (d, *J* = 1.7 Hz, 1H), 4.24 (m, 3H), 1.29 (t, *J* = 5.9 Hz, 3H), 0.91 (s, 9H), 0.21 (s, 6H). HRMS (ESI) *m/z*: cacld for $C_{12}H_{22}N_2NaO_3Si$ ($M+Na^+$) 293.1292, found: 293.1295.

Oxabicyclic ethyl ester (27)

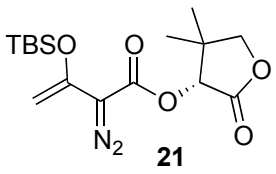


(±)-**27**

A solution of **26** (8.5 g, 31.6 mmol) in dry hexanes (750 mL) was added dropwise over 5.5 hours to a refluxing solution of **20** (7.85 g, 8.8 mL) and Rhodium(II) octanoate dimer (492 mg) in dry hexanes (750 mL). The reaction mixture was stirred an additional 30 minutes, at which time TLC showed no starting material remaining. The reaction mixture was then allowed to cooled down to room temperature, filtered and concentrated. Crude product purification using flash column chromatography afforded 11.0 g of bicyclic ester **27** as colorless thick oil (95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.40 (d, *J* = 5.7 Hz, 1H) 5.70 (d, *J* = 5.7 Hz, 1H), 4.23 (q, *J* = 5.9 Hz, 2H), 2.48 (d, *J* = 17.3 Hz, 1H), 1.88 (m, 1H), 1.82 (d, *J* = 17.3 Hz, 1H), 1.58 (s, 3H), 1.29 (t, *J* = 5.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.90 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ : 166.8, 153.2, 141.9, 126.6, 119.9, 88.6, 83.0, 60.1, 36.4,

34.3, 25.7, 20.4, 17.1, 14.5, -3.5, -3.5. HRMS (ESI) *m/z*: cacld for $C_{20}H_{34}NaO_4Si$ ($M+Na^+$) 389.2119, found: 389.2118.

Diazo pentalactone silyl enol ether (21).

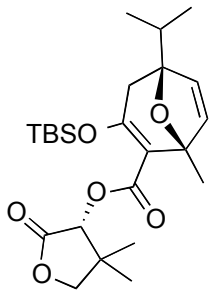


21

A solution of (*R*)-pantolactone (5.0 g, 38.4 mmol) and 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (5.5 g, 38.4 mmol) in xylene (10 ml) was placed in a round bottom flask, which was then immersed in an oil bath which was preheated to 150 ºC. This reaction was vigorously stirred at this temperature for 15 min and cooled to rt. The crude oil was then concentrated under reduced pressure to afford (*R*)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl 3-oxobutanoate as a red brown oil, which could be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.43 (s, 1H), 4.06 (m, 2H), 3.64 (m, 2H), 2.30 (s, 3H), 1.25 (s, 3H), 1.12 (s, 3H). To an ice-cooled, stirred mixture of the before mentioned red brown oil (4.3 g, 20 mmol) and *p*-acetamidobenzenesulfonyl azide (*p*-ABSA, 4.8 g, 20 mmol) in anhydrous acetonitrile (60 ml) was added triethylamine (2.95 ml, 21 mmol) in one portion. The reaction mixture is warmed to r.t. and stirred for 2 hours. The solvent is removed under reduced pressure, and the residue is triturated with 500 mL of dichloromethane. The mixture is filtered, concentrated, and the crude product was purified *via* Flash Column Chromatography on silica gel (hexanes/ethyl acetate, 5:1 to 2:1) to yield 4.6 g (96%) of (*R*)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl-2-diazo-3-oxobutanoate as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$); δ : 5.47 (s, 1H), 4.10 (m, 2H), 2.50 (s, 3H), 1.27 (s, 3H), 1.15 (s, 3H) To an ice-cooled solution of this diazooxobutanoate (4.5 g, 18.7 mmol) in anhydrous CH_2Cl_2 (50 ml) was sequentially added triethylamine (3.65 ml, 26.1 mmol) and TBSOTf (5.1 ml, 22.4 mmol). This reaction was stirred for 30 min at the same temperature before get carefully quenched by 5%

$NaHCO_3$ solution. This mixture was extracted with hexanes for three times and dried over Na_2SO_4 and concentrated under reduced pressure to obtain **21** as a yellow oil. This unstable TBS-enol-ether **21** was then used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 5.47 (s, 1H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.28 (d, *J* = 1.3 Hz, 1H), 4.07 (s, 2H), 1.24 (s, 3H), 1.11 (s, 3H), 0.93 (s, 9H), 0.25 (s, 6H) HRMS (ESI) *m/z*: cacld for $C_{16}H_{26}N_2NaO_5Si$ ($M+Na^+$) 377.1503, found: 377.1501

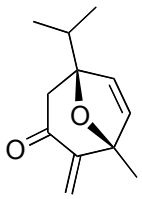
Oxabicyclic pentalactone silylether 19



19

A solution of **20** (11.2 g, 31.6 mmol) in dry hexanes (750 mL) was added dropwise over 5.5 hours to a refluxing solution of **21** (7.85 g, 8.8 mL) and Rhodium(II) octanoate dimer (492 mg) in dry hexanes (750 mL). The reaction mixture was stirred an additional 30 minutes, at which time TLC showed no starting material remaining. The reaction mixture was then allowed to cooled down to room temperature, filtered and concentrated. Crude product purification using flash column chromatography (hexanes to 10%, very slow gradient, EtOAc/hexanes) afforded 8.1 g of bicyclic ester **8** as colorless thick oil (68%). $[\alpha]_D^{23}$ = + 36.40 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 6.38 (dd, *J* = 5.7, 0.5 Hz, 1H), 5.72 (d, *J* = 5.7 Hz, 1H), 5.40 (s, 1H), 4.04 (q, *J* = 8.9 Hz, 2H), 2.41 (d, *J* = 17.4 Hz, 1H), 1.95 - 1.83 (m, 2H), 1.62 (s, 3H), 1.24 (s, 3H), 1.17 (s, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.92 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H). 13C NMR (101 MHz, CDCl3) δ : 172.4, 164.2, 157.2, 141.6, 126.8, 117.9, 88.6, 82.8, 76.3, 75.0, 40.2, 37.1, 34.3, 25.8, 23.4, 21.5, 20.6, 18.4, 17.2, 17.1, -3.4, -3.5. HRMS (FAB) *m/z*: cacld for C24H38O6SiNa (M+Na+) 473.2330, found: 473.2331.

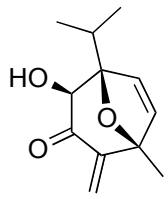
Oxabicycloexo-enone 28



(–)-**28**

To a solution of **8** (20.7 g, 45.9 mmol) in dry dichloromethane (459 mL) was added quickly dropwise via addition funnel a 1.0 M solution of DIBAL in heptanes (344.3 mmol, 344.3 mL) at -78 °C. After completion of addition, the reaction was stirred for 15 min at which time TLC showed no starting material. The reaction mixture was quenched with saturated potassium sodium tartrate solution (300 mL), was allowed to reach room temperature and was stirred for 1 hour. The reaction mixture was filtered through celite and washed with DCM until TLC showed no more crude product remaining in the filter cake. The filtered mixture was separated, extracted (2 x 150 mL), washed with brine (300 mL) dried over Na2SO4, filtered, and concentrated under vacuum to 500 mL of DCM. This solution was flushed with Argon and treated directly with $BF_3{\cdot}Et_2O$ (68.9 mmol, 8.7mL) dropwise at -30 °C. 5 min after addition TLC showed no starting material. The reaction mixture was further diluted with DCM (250 mL), quenched with saturated NaHCO3 solution (350 mL) and extracted with DCM (2 x 200 mL). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum. Crude product purification using flash column chromatography (1% to 10%, EtOAc/Hexanes) afforded 5.2 g of ketone **17** as a yellow oil (59%). $[\alpha]_D^{23}$ = – 103.01 (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, CDCl3) δ : 6.05 (d, *J* = 5.8 Hz, 1H), 5.96 (d, *J* = 0.5 Hz, 1H), 5.92 (d, *J* = 5.7 Hz, 1H), 5.24 (s, 1H), 2.56 (d, *J* = 17.7 Hz, 1H), 2.46 (d, *J* = 17.7 Hz, 1H), 2.00 - 1.89 (m, 1H), 1.61 (s, 3H), 0.99 (d, *J* = 4.9 Hz, 3H), 0.98 (d, *J* = 4.7 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ : 197.7, 147.3, 136.2, 133.4, 115.2, 89.7, 84.8, 45.8, 33.6, 19.8, 17.3. HRMS (FAB) *m/z*: cacld for $C_{12}H_{17}O_2$ (M+H+) 193.1223, found: 193.1224.

α-hydroxy oxabicyclic enone (30)

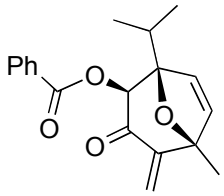


(–)-30

n-Butyllithium (77.6 mL, 124.2 mmol) was added dropwise to a solution of dry diisopropylamine (19 mL, 135.0 mmol) in dry THF (800 mL) at -78 °C. The reaction mixture was stirred for 15 minutes, and then a solution of **17** (5.2 g, 27.0 mmol) in dry THF (500 mL) was added quickly dropwise to the reaction mixture. The reaction mixture temperature was raised from -78 °C to 0 °C over 45 minutes and stirred 1 hour at 0 °C. The reaction mixture was cooled down to –78 °C and TMSCl (15.8 mL, 124.2 mmol) was added dropwise. The reaction mixture temperature was raised from –78 °C to 0 °C over 45 minutes and stirred for 1 hour at 0 °C at which time TLC showed no starting material. The reaction mixture was diluted in hexanes (650 mL) quenched with 5% NaHCO3 solution (500 mL), washed with brine (300 mL), dried over Na2SO4, filtered, and concentrated under vacuum. To a solution of the crude enol ether product in DCM (250 mL) a solution of NaHCO3 (250 mL, 10%) was added all at once at 0 °C. Then a solution of *m*-CPBA (5.2 g, 30.0 mmol) in DCM (60 mL) was added slowly while vigorous stirring of the reaction mixture. The reaction was closely monitored by TLC. When traces of enol ether (> 5%) was observed the reaction was further diluted in DCM (200 mL), quenched with saturated NaHSO3 solution (300 mL), allowed to reach room temperature, extracted with DCM (2 x 200 mL), washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under vacuum to approximately 250 mL. To this solution of crude epoxide product was added a solution of $(COOH)_2$ (15.6 g, 124.2 mmol) in MeOH (150 mL). After 30 min of stirring at room temperature TLC showed no epoxide product remaining. The reaction mixture was slowly quenched with saturated K_2CO_3 solution until neutral pH was achieved and extracted with DCM (2 x 200 mL). The combined organic layers were washed with brine (300 mL), dried over Na2SO4, filtered, and concentrated under vacuum. Crude product

purification using flash column chromatography (1% to 20%, EtOAc/Hexanes) afforded **17** (2.7 g) and 2.2 g of a-hydroxy ketone **19** as a crystalline solid (83% b.r.s.m). Recrystallization from hexanes afforded high purity crystals for X-Ray characterization. $[\alpha]_D^{23}$ = –101.00 (c = 1.3, $CHCl_3$). ^{1}H NMR (500 MHz, CDCl3) δ: 6.08 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.00 (d, *J* = 5.9 Hz, 1H), 5.97 (s, 1H), 5.28 (s, 1H), 3.81 (d, *J* = 6.4 Hz, 1H), 2.37 - 2.28 (m, 1H), 1.62 (s, 3H), 1.05 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, CDCl3) δ: 199.0, 146.9, 140.2, 130.1, 116.5, 93.4, 85.5, 73.3, 28.5, 19.4, 17.7, 17.0. HRMS (ESI) *m/z*: cacld for $C_{12}H_{16}O_3Na$ (M+Na+) 231.0992, found: 231.0993.

α-hydroxybenzoate oxabicyclic enone (41)

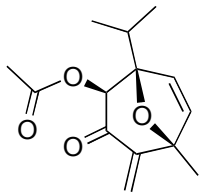


41

To a stirred mixture of benzoic acid (7.3 mg, 0.06 mol) and **30** (6.5 mg, 0.026 mol) in dry toluene (0.55 mL) were added successively NEt_3 (15 μL, 0.11mol) and 2,4,6-trichlorobenzoyl chloride (9 μL, 0.075 mol). The reaction mixture was stirred for 10 min, then catalytic amount of 4-DMAP (1 crystal) was added. After 16 h of stirring at rt, the reaction was diluted with ethyl acetate, and the organic phase was washed successively with aqueous HCl solution (1M), saturated sodium bicarbonate solution, and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuum after filtration. The residue was purified by flash chromatography to afford the benzoic keto-ester **41** (9.1 mg, 90%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ : 8.10 (m, 2H), 7.57 (m, 1H), 7.24 (m, 2H), 6.21 (dd, *J* = 5.8, 0.9 Hz, 1H), 6.09 (d, *J* = 5.8, 1H), 6.02 (s, 1H), 5.51 (s, 1H), 5.32 (s, 1H), 2.25 (m, 1H), 1.69 (s, 3H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ : 193.8, 165.5, 146.7, 141.1, 133.4, 130.1, 129.4, 128.4, 92.8,

85.6, 72.0, 28.9, 19.6, 17.4, 17.1. HRMS (ESI) *m/z*: cacld for $C_{19}H_{20}O_4Na$ ($M+Na^+$) 335.1254, found: 335.1256.

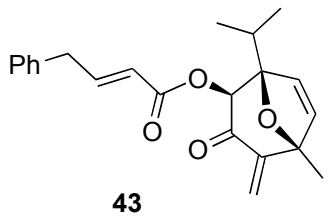
α-hydroxyacetate oxabicyclic enone (42)



42

To a solution of **30** (5 mg, 0.024 mmol) in pyridine (0.25 mL) was added 4-DMAP (0.6 mg, 0.005 mmol) and Ac_2O (0.011 mL, 0.12 mmol). The reaction was heated to 60ºC for 3 hours. The reaction was cooled down to room temperature quenched with saturated $NaHCO_3$ and extracted 3 times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, concentrated and the residue was purified by preparatory plate chromatography to yield **42** as a white foam (5.7 mg, 95%). ^1H NMR (400 MHz, $CDCl_3$) δ : 6.16 (d, *J* = 5.9 Hz, 1 H), 6.02 (d, *J* = 5.9 Hz, 1 H), 6.00 (s, 1H), 5.30 (s, 1H), 5.27 (s, 1H), 2.16 (s, 3H), 2.16 (m, 1H), 1.65 (s, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ : 194.1, 170.0, 146.6, 141.2, 129.3, 116.9, 92.6, 85.7, 71.7, 29.9, 28.8, 19.5, 17.4, 17.1. HRMS (ESI) *m/z*: cacld for $C_{14}H_{18}O_4Na$ ($M+Na^+$) 273.1097, found: 273.1098.

α-hydroxycinnamate oxabicyclic enone (43)

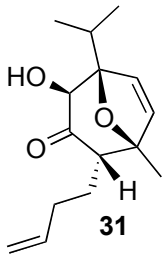


43

To a stirred mixture of cinnamic acid (8 mg, 0.053 mol) and **30** (5.5 mg, 0.026 mol) in dry toluene (0.5 mL) were added successively NEt_3 (11 _L, 0.08 mol) and 2,4,6-trichlorobenzoyl chloride (7 _L, 0.07 mol). The reaction mixture was stirred for 10 min, then catalytic amount of 4-DMAP (1 crystal) was added. After 16 h of stirring at rt, the reaction was diluted with ethyl acetate, and the organic phase was washed successively with aqueous HCl solution (1M), saturated sodium

bicarbonate solution, and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuum after filtration. The residue was purified by flash chromatography to afford the cinnamic keto-ester **43** (7.8 mg, 88%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.70 (d, *J* = 15.9 Hz, 1H), 7.52 (m, 2H), 7.39 (m, 3H), 6.52 (d, *J* = 16.0 Hz, 1H), 6.19 (d, *J* = 5.8 Hz, 1H), 6.06 (d, *J* = 5.8 Hz, 1H), 6.03 (s, 1H), 5.42 (s, 1H), 5.33 (s, 1H), 2.22 (m, 1H), 1.68 (s, 3H), 0.96 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ : 194.1, 166.0, 146.5, 141.2, 134.3, 130.7, 129.4, 129.0, 128.4, 117.2, 116.9, 92.8, 85.7, 71.7, 28.8, 19.6, 17.5, 17.1. HRMS (ESI) *m/z*: cacld for $C_{21}H_{22}O_4Na$ ($M+Na^+$) 361.1416, found: 361.1417.

β–2–propene hydroxy ketone (31)

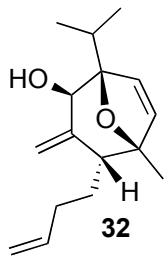


31

To a solution of **30** (180 mg, 0.86 mmol) in anhydrous CH_2Cl_2 (8 ml) was added $TiCl_4$ (1.03 ml, 1.03 mmol, 1M in CH_2Cl_2) dropwise at –78 ºC and stirred for 10 min. Then allyltrimethylsilane (0.41 ml, 2.58 mmol) was added in dropwise and the reaction was allowed to stir under the same temperature for 1 hour before get quenched by sat. $NaHCO_3$ solution. The mixture was then separated with CH_2Cl_2/brine, the aqueous phase was extracted with CH_2Cl_2 for 3 times. The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Crude product purification using flash column chromatography (hexanes/EtOAc, 50:1 to 5:1) afforded **31** (138 mg, 68%) as pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 6.08 (dd, *J* = 6.0, 1.0 Hz, 1H), 5.94 (d, *J* = 6.1 Hz, 1H), 5.81 – 5.73 (m, 1H), 5.04 – 4.98 (m, 2H), 3.79 (d, *J* = 9.6 Hz, 1H), 2.75 (dd, *J* = 8.4, 3.1 Hz, 1H), 2.61 (d, *J* = 9.7 Hz, 1H), 2.11 – 2.03 (m, 1H), 1.77 – 1.69 (m, 1H), 1.50 (s, 3H), 1.30 – 1.26 (m, 1H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ : 207.3, 138.7, 138.2, 130.1, 115.5, 93.3, 87.9, 75.1, 56.9, 33.0, 29.9, 27.8,

24.4, 21.7, 17.6, 17.1. HRMS (ESI) *m/z*: cacld for $C_{15}H_{23}O_3$ ($M+H^+$) 251.1642, found: 251.1644.

Oxybicyclic triolefin (32)

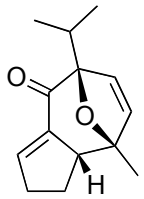


32

Due to unknown reason the following procedure was not well reproducible. To a solution of Nysted reagent (Aldrich, 4.62 mL, 2.4 mL) in THF (1.2 mL) was added $NaHCO_3$ powder (144 mg, 1.68 mmol) and $TiCl_4$ (2.4 mL, 2.4 mmol, 1M in CH_2Cl_2) at 0 ºC, followed by adding a solution of **31** (60 mg, 0.24 mmol) in THF (0.6 mL). The reaction was stirred at 0 ºC for 15 min and at rt for 45 min. Then this mixture was cooled to 0 ºC and quenched carefully with sat. $NaHCO_3$ solution, filtered through celite and washed with EtOAc. The aqueous layer was extracted with EtOAc for 3 times and the combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude product purification using flash column chromatography (hexanes/EtOAc, 50:1 to 5:1) afforded **32** (22 mg, 36%) as pale yellow oil. ^1H NMR (500 MHz, $CDCl_3$) δ : 5.89 (d, *J* = 5.7 Hz, 1H), 5.78 (d, *J* = 6.3 Hz, 1H), 5.78 (m, 1H), 5.09 (d, *J* = 1.7 Hz, 1H), 5.00 (m, 2H), 4.90 (d, *J* = 1.7 Hz, 1H), 3.94 (d, *J* = 9.6 Hz, 1H), 2.46 (m, 1H), 2.34 (d, *J* = 9.5 Hz, 1H) 2.18 (m, 2H), 2.07 (m, 1H), 1.52 (m, 1H) 1.45 (s, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H). HRMS (ESI) *m/z*: cacld for $C_{16}H_{25}O_2$ ($M+H^+$) 249.1849, found: 249.1850.

Guiaine unsaturated ketone (33)

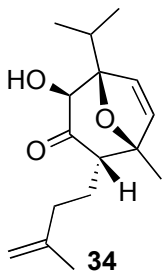
To a solution of **32** (22 mg, 0.086 mmol) in CH_2Cl_2 (8 ml) was added Grubbs (2^{nd} gen., 3.6 mg, 5 mol%). This solution was then stirred for 2 hours. Silica was added in to this solution and column chromatography (hexanes/EtOAc, 30:1 to 3:1) afforded the



33

corresponding allylic alcohol (21 mg, 99%). This alcohol (0.085 mmol) was then dissolved in CH_2Cl_2 (1 mL), TPAP (3 mg, 8.5 mmol) and NMO (30 mg, 0.25 mmol) was added in sequentially. The reaction was stirred for 1 h and filtered through celite, washed with CH_2Cl_2 and concentrated. Crude product purification using flash column chromatography (hexanes/EtOAc, 50:1 to 5:1) afforded **33** (15 mg, 72%) as an unstable, pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 6.63 (d, *J* = 5.8 Hz, 1H), 5.95 (m, 2H), 3.26 (m, 1H), 2.42 (m, 1H), 2.34 (m, 2H), 2.03 (m, 1H), 2.00 (m, 1H), 1.50 (s, 3H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H). HRMS (ESI) *m/z*: cacld for $C_{14}H_{19}O_2$ ($M+H^+$) 219.1380, found: 219.1381.

Oxybicyclic triolefin (34)

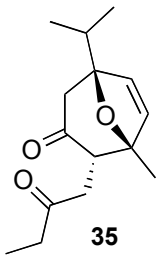


34

To a solution of **19** (208 mg, 1 mmol) in anhydrous CH_2Cl_2 (9 mL) was added $TiCl_4$ (1.1 ml, 1.1 mmol, 1M in CH_2Cl_2) dropwise at –78 ºC and stirred for 10 min. Then trimethyl(2-methylallyl))silane (0.88 ml, 5 mmol) was added in dropwise and the reaction was allowed to stir under the same temperature for 1 hour before get quenched by sat. $NaHCO_3$ solution. The mixture was then separated with CH_2Cl_2/brine, the aqueous phase was extracted with CH_2Cl_2 for 3 times. The combined organic phase was dried over Na_2SO_4, filtered and concentrated. Crude product purification using flash column chromatography (hexanes/EtOAc, 50:1 to 5:1) afforded **24** (187 mg, 71%) as pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 6.08 (dd, *J* = 6.0, 0.8 Hz, 1H), 5.94 (d, *J* = 6.1 Hz, 1H), 4.73 (s, 1H), 4.67 (d, *J* = 1.0 Hz, 1H), 3.79 (d, *J* = 9.8 Hz, 1H), 2.73 (dd, *J* = 8.3, 3.2 Hz, 1H), 2.57 (d, *J* = 9.8 Hz, 1H) 2.21 (m, 2H), 2.04 (m, 1H), 1.77 (m, 1H), 1.72 (s, 3H), 1.51 (s, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ :

207.3, 145.3, 138.8, 130.1, 110.8, 93.4, 87.9, 75.2, 56.9, 36.8, 27.8, 23.0, 21.7, 17.5, 17.1. HRMS (ESI) *m/z*: cacld for $C_{16}H_{24}NaO_3$ ($M+Na^+$) 287.1618, found: 287.1619.

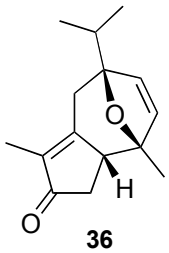
γ-keto oxybicyclicheptanone (27)



35

Propanaldehyde (75 µL, 1.04 mmol), **17** (100 mg, 0.52 mmol), catalyst **A** (57 mg, 0.21 mmol) and Et_3N (88 µL, 0.63 mmol) were heated to 85 °C in a dry conical flask under argon atmosphere for 5 h. At this time TLC showed no starting material. The reaction mixture was diluted in EtOAc (100 mL), quenched with saturated NH_4Cl solution (50 mL) and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. Crude product purification using flash column chromatography afforded 97 mg of diketone **35** (77%) as a yellow oil. (500 MHz, $CDCl_3$) δ : 6.02 (d, *J* = 5.9 Hz, 1H), 5.90 (d, *J* = 5.9 Hz, 1H), 3.25 (dd, *J* = 8.9, 3.9 Hz, 1H) 2.70 (dd, *J* = 16.6, 8.9 Hz, 1H), 2.61 (m, 1H), 2.54 (d, *J* = 15.1 Hz, 1H) 2.46 (dd, *J* = 17.6, 7.3 Hz, 1H) 2.36 (d, *J* = 15.1 Hz, 1H) 2.07 (dd, *J* = 16.7, 3.9 Hz, 1H), 1.94 (m, 1H), 1.44 (s, 3H), 1.08 (t, *J* = 5.8 Hz, 3H) 0.97 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H). HRMS (ESI) *m/z*: cacld for $C_{15}H_{22}NaO_3$ ($M+Na^+$) 273.1461, found: 273.1460.

tricyclic α,β-unsaturated ketone (36)

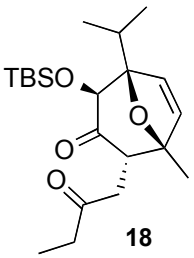


36

To a solution of **35** (9 mg, 0.036 mmol) in EtOH (1 mL) was added KOH (0.18 mL, 1.0 M in EtOH) dropwise at room temperature. The reaction mixture was stirred at this temperature for 24 hours. The reaction was mixture was concentrated under reduced pressure, dissolved in water (5 mL), acidified with 1 M HCl, extracted with diethyl ether (3x 10mL). The combined organic layers were dried over $MgSO_4$,, concentrated and the residue was purified using

preparative plate chromatography to yield 6.5 mg of **36** (76%) as a clear oil. (400 MHz, $CDCl_3$) δ : 5.94 (d, *J* = 6.1 Hz, 1H), 5.80 (d, *J* = 5.9 Hz, 1H), 4.73 (s, 1H), 2.83 (m, 1H), 2.62 (dd, *J* = 14.0, 2.6 Hz, 1H), 2.48-2.27 (m, 2H) 1.98 (m, 1H), 1.44 (s, 3H), 1.26 (s, 3H) 1.04 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 6.9 Hz, 3H). HRMS (ESI) *m/z*: cacld for $C_{15}H_{20}NaO_2$ ($M+Na^+$) 255.1361, found: 255.1359.

α -silylhydroxy diketone (18)

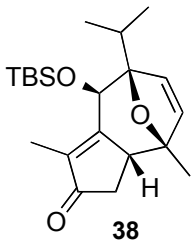


18

To a solution of **30 (**1.30 g, 6.2 mmol) in DCM (62 mL) at 0 °C was added NEt_3 (4.3 mL, 31.0 mmol). Then TBSOTf (2.9 mL, 12.4 mmol) was added dropwise to the reaction mixture. The reaction mixture was then allowed to warm up to room temperature and stirred for 1 h at which time TLC showed no starting material. The reaction mixture was diluted in hexanes (150 mL), quenched with saturated $NaHCO_3$ solution (100 mL) and extracted with hexanes (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. Crude product purification using flash column chromatography (10:1, hexanes: EtOAc) afforded 1.96 g of corresponding TBS ether **37** (98%) as a yellow solid. Propanaldehyde (1.7 mL, 24.4 mmol), TBS-ether (1.96 g, 6.1 mmol), catalyst **A** (329 mg, 1.2 mmol) and NEt_3 (1.0 mL, 7.3 mmol) were heated to 85 °C in a dry conical flask under argon atmosphere for 5 h. At this time TLC showed no starting material. The reaction mixture was diluted in EtOAc (150 mL), quenched with saturated NH_4Cl solution (100 mL) and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. Crude product purification using flash column chromatography (1% to 15%, slow gradient, EtOAc/hexanes) afforded 1.78 g of diketone **18** (77%) as a yellow oil. $[\alpha]_D^{23}$ = + 32.3 (c 0.8, $CHCl_3$). ^1H

NMR (500 MHz, $CDCl_3$) δ : 6.05 (dd, *J* = 5.9, 0.6 Hz, 1H), 5.92 (d, *J* = 6.0 Hz, 1H), 3.78 (s, 1H), 3.57 (dd, *J* = 9.5, 3.8 Hz, 1H), 2.69 - 2.55 (m, 2H), 2.52 - 2.42 (m, 1H), 2.37 - 2.29 (m, 1H), 2.10 (dd, *J* = 16.5, 3.8 Hz, 1H), 1.44 (s, 3H), 1.08 (t, *J* = 7.3 Hz, 3H), 0.94 (t, *J* = 7.1 Hz, 6H), 0.90 (s, 9H), 0.10 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 208.6, 206.8, 138.9, 131.1, 93.7, 86.6, 74.9, 74.9, 54.6, 37.6, 36.4, 27.4, 25.9, 21.4, 18.3, 17.5, 17.3, 8.0, -4.8, -5.2. HRMS (ESI) *m/z*: cacld for $C_{21}H_{36}O_4SiNa$ ($M+Na^+$) 403.2275, found: 403.2278.

tricyclic α,β-unsaturated ketone (38)

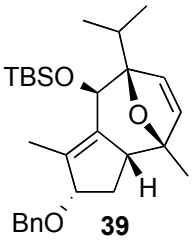


38

NaHMDS (1.0 M in THF, 11.7 mL, 5 eq.) was added dropwise to a stirred solution of **18** (890 mg, 2.34 mmol) in dry THF (3.6 mL) at 0 °C. This reaction was stirred for 2 hours at this temperature before quenched with saturated NH_4Cl solution. Then the mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (2% to 25% EtOAc/hexanes) gave 400 mg of aldol product (70% b.r.s.m.) and 250 mg recovered **30**. This aldol product was dissolved in anhydrous methanol (8 mL), and sodium methoxide (75 mg, 1.3 eq.) was quickly added in one portion. The flask was immersed in an oil bath which has pre-heated to 65 °C and stirred for 10 minutes, at which time TLC showed no starting material. This reaction was cooled to 0 °C and quenched with saturated NH_4Cl solution. Then this mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (2% to 25% EtOAc/hexanes) gave 303 mg of **38** (43% b.r.s.m. over 2 steps) as an

oil. $[\alpha]_D^{23}$ = + 188.5 (c 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ : 5.90 (d, *J* = 5.7 Hz, 1H), 5.84 (d, *J* = 5.7 Hz, 1H), 4.50 (s, 1H), 3.05 (m, 1H), 2.49 (dd, *J* = 18.9 Hz, *J* = 6.9 Hz, 1H), 2.34 (hept, *J* = 6.9 Hz, 1H), 1.71 (m, 4H), 1.44 (s, 3H), 1.00 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (s, 9H), 0.12 (s, 3H), -0.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ : 208.9, 171.0, 138.0, 135.7, 132.1, 94.4, 87.2, 64.9, 45.5, 36.2, 27.8, 25.7, 21.7, 21.0, 18.3, 17.5, 17.4, 8.6, -4.5, -4.7. HRMS (ESI) *m/z*: calcd for $C_{21}H_{34}O_3SiNa$ ($M+Na^+$) 385.2169, found: 385.2173.

Allylic benzylether (39).

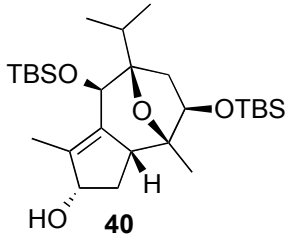


39

$NaBH_4$ (160 mg, 5 eq.) was added portion wise to a stirred solution of **38** (303 mg, 0.84 mmol) in anhydrous methanol (4 mL) at 0 °C. The ice bath was removed and this reaction was stirred for 15 min at room temperature before carefully quenched with saturated NH_4Cl solution. This mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated and dried under high vacuum to give the corresponding allylic alcohol, which was clean enough to be used directly in next step. The allylic alcohol was dissolved in anhydrous DMF (8 mL), and sodium hydride (134 mg, 4 eq., 60% in mineral oil) was added in one portion at 0 °C. The reaction was stirred at 0 °C for 5 min before benzyl bromide was added dropwise (800 µL, 8 eq.). This reaction was then heated to 60 °C for 20 minutes, at which time TLC showed no starting material. This reaction was cooled to 0 °C and quenched with saturated NH_4Cl solution. Then this mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (hexanes to 5% EtOAc/hexanes) gave about 350 mg

of the product (contaminated with dibenzylether, contains 271 mg of **32**, 71% over 2 steps based on ^{1}H NMR analysis) as a colorless oil. $[\alpha]_D^{23}$ = + 58.2 (c = 0.86, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ : 7.32 - 7.27 (m, 5H), 5.90 (d, *J* = 6.0 Hz, 1H), 5.87 (d, *J* = 6.0 Hz, 1H), 4.55 - 4.44 (m, 2H), 4.40 (t, *J* = 7.2 Hz, 1H), 4.25 (s, 1H), 2.74 (t, *J* = 8.1 Hz, 1H), 2.35 (dt, *J* = 12.5, 7.4 Hz, 1H), 2.27 (dt, *J* = 13.8, 7.0 Hz, 1H), 1.73 (m, 4H), 1.36 (s, 4H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.86 (s, 9H), 0.06 (s, 3H), -0.02 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ : 138.8, 138.4, 138.3, 137.2, 136.7, 131.6, 127.6, 94.2, 87.3, 86.2, 71.2, 64.3, 49.0, 32.4, 28.2, 25.9, 21.4, 18.4, 17.6, 17.5, 11.7, 1.12, -4.3, -4.4. HRMS (ESI) *m/z*: calcd for $C_{28}H_{42}O_3SiNa$ ($M+Na^+$) 477.2795, found: 477.2794.

Allylhydroxy disilylether 40

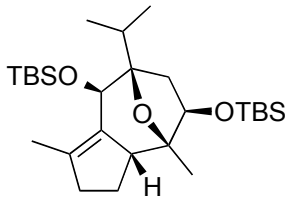


40

To a solution of **32** (150 mg, 0.33 mmol) in dry THF was added borane-THF complex (1 mL, 1.0 M in THF, 3 eq.) dropwise at 0 °C. The reaction was then stirred at room temperature for 1.5 h, at which time TLC showed no starting material. The reaction was cooled to 0°C, then was carefully added a pre-mixed solution of 3N NaOH and 30% H_2O_2 (3 mL, 1:1), the ice bath was removed and the reaction was stirred at ambient temperature for 1 h, at which time TLC showed no starting material. The reaction was quenched with saturated NH_4Cl solution, then this mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (5% to 40% EtOAc/hexanes) gave 94 mg (60%) of C-9 β-alcohol. To this alcohol (0.20 mmol) and triethylamine (110 µL, 4 eq.) was then dissolved in dry dichloromethane (2 mL), then TBS-triflate (92 µL, 2 eq.) was added dropwise at 0 °C. This reaction was then stirred at room temperature for 1 h before

quenched with saturated $NaHCO_3$ solution. The mixture was extracted with hexanes (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give crude TBS silyl ether (114 mg, 0.194 mmol) which was dissolved in methanol (2 mL) and $Pd(OH)_2$ (11 mg, 10%) was added. After hydrogenation at atmospheric pressure for 1 h, the mixture was filtered through Celite and the solvent was removed in *vacuo*. The residue was purified by column chromatography on silica gel (2% to 10% EtOAc/hexanes) to give alcohol **40** (97 mg, 100%) as a colorless oil. $[\alpha]_D^{23}$ = –2.52 (c = 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ : 4.71 - 4.64 (m, 1H), 4.06 (s, 1H), 3.98 (d, *J* = 2.0 Hz, 1H), 2.83 (t, *J* = 5.6 Hz, 1H), 2.44 - 2.37 (m, 2H), 1.75 (dd, *J* = 2.3, 1.0 Hz, 3H), 1.56 (dd, *J* = 13.5, 2.0 Hz, 1H), 1.49 - 1.44 (m, 1H), 1.19 - 1.13 (m, 4H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.88 (s, 9H), 0.86 (s, 9H), 0.82 (d, *J* = 7.0 Hz, 3H), 0.06 (s, 3H), 0.03 (d, *J* = 3.0 Hz, 6H), -0.04 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ : 137.5, 135.8, 88.1, 87.3, 79.9, 72.4, 67.4, 50.4, 39.0, 34.3, 28.1, 25.9, 25.9, 26.1, 19.5, 18.4, 18.1, 17.8, 16.2, 11.3, -4.4, -4.4, -4.5, -4.8. HRMS (ESI) *m/z*: calcd for $C_{27}H_{52}O_4Si_2Na$ ($M+Na^+$) 519.3296, found: 519.3294.

Guiaine disilylether (41)

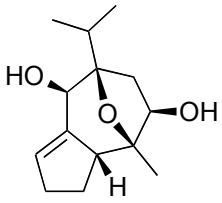


41

40 (97 mg, 0.194 mmol) was dissolved in anhydrous toluene (2 mL) and Burgess reagent (233 mg, 5 eq.) was added. This reaction was heated to 80 °C for 30 min, at which time TLC showed no starting material. Then this reaction was cooled and quenched with water, extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was dissolved directly in methanol (2 mL) and Pd/C (9 mg, 10%) was added. After hydrogenation at atmospheric pressure for 1 h, the mixture was

filtered through Celite and the solvent was removed in *vacuo*. The residue was purified by column chromatography on silica gel (hexanes to 5% EtOAc/hexanes) to give **41** (84 mg, 90% over 2 steps) as a colorless oil. $[\alpha]_D^{23}$ = +3.98 (c 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ : 4.03 (s, 1H), 3.76 (dd, *J* = 7.2, 2.0 Hz, 1H), 3.01 - 2.87 (m, 1H), 2.47 - 2.36 (m, 1H), 2.31 (m, 1H), 2.25 - 2.17 (m, 1H), 1.93 (m, 1H), 1.69 (s, 3H), 1.58 (s, 3H), 1.49 (dd, *J* = 13.4, 2.1 Hz, 1H), 1.38 (dd, *J* = 13.4, 7.2 Hz, 1H), 1.15 (s, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.87 (s, 9H), 0.85 (s, 9H), 0.80 (d, *J* = 7.0 Hz, 3H), 0.03 (s, 3H), 0.00 (s, 3H), -0.00 (s, 3H), -0.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ : 134.2, 134.1, 88.1, 87.7, 72.6, 67.6, 52.7, 39.2, 37.8, 29.9, 28.1, 26.0, 25.9, 22.9, 18.4, 18.2, 17.8, 16.4, 14.3, -4.5, -4.5, -4.6, -4.9 HRMS (ESI) *m/z*: calcd for $C_{27}H_{52}O_3Si_2Na$ ($M+Na^+$) 503.3347, found: 503.3350.

Guiaine diol (17)

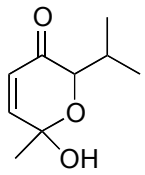


(+)-17

41 (48.1 mg, 0.1 mmol) and TBAF (1.0 M in THF, 0.3 mL, 3 eq.) was dissolved in dry THF (2 mL) in a sealed tube. This tube was then microwaved at 80 °C for 45 minutes. The reaction was then cooled to ambient temperature, quenched with pH = 7.0 buffer, Then this mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (5% to 50% EtOAc/hexanes) to give 23 mg of **17** (93%) as a colorless oil. $[\alpha]_D^{23}$ = +17.5 (c = 0.2, $CHCl_3$). $[\_]_D^{23}$ = + 70.1 (c = 0.2, CH_3OH); ^{1}H NMR (CDCl3, 500 MHz) δ : 4.07 (s, 1H), 3.90 (dd, *J* = 7.4 Hz, *J* = 1.5 Hz, 1H), 2.97 (m, 1H), 2.43 - 2.36 (m, 2H), 2.27 - 2.21 (m, 1H), 2.01 - 1.95 (m, 1H), 1.71 (br, s, 3H), 1.68 - 1.57 (m, 3H), 1.24 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (CDCl3, 126 MHz) δ : 136.8, 132.7, 88.3,

87.3, 72.5, 66.9, 52.1, 39.0, 37.5, 28.3, 23.5, 19.2, 17.8, 16.3, 13.6. HRMS (ESI) *m/z*: calcd for $C_{15}H_{24}O_3Na$ ($M+Na^+$) 275.1618, found: 275.1619.

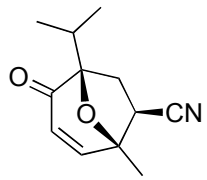
Hemiketal 45



45

To a solution of comerically available fufuraldehyde **44** (15g, 13.6 mL, 148.5 mmol) in dry ether (75mL) was added *i*PrMgCl (111.4 mL, 222.8 mmol) dropwise at -10 °C. The reaction was stirred 20 minutes at this temperature at which time TLC showed consumption of the starting material. The reaction was diluted with ether (150 mL) quenched carefully with saturated NH_4Cl solution and allowed to reach room temperature. The layers were separated; the aqueous layer was extracted with ether (2 x 100 mL), dried over Na_2SO_4 and concentrated in vacuo. The material was used crude without further purification. To a solution of crude furan (15.5 g, 124 mmol) in CH_2Cl_2 at 0 °C was added *m*-CPBA (21.5 g, 124 mmol) portion wise over 3 min. After stirring for 5 min TLC showed no starting material. The reaction mixture was filtered through a Buchner funnel and rinsed once with cold CH_2Cl_2. The filtrate was then washed with saturated $Na_2S_2O_3$ and saturated $NaHCO_3$. The combined aqueous layers were then backwashed with CH_2Cl_2. The combined organics were dried over Na_2SO_4 and concentrated *in vacuo*. Flash chromatography of the residue (15% ethyl acetate in hexanes) provided enone **45** (15.0 g, 75% over 2 steps) as an inseparable 9:1 mixture of diastereomers as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ : 6.81 (d, *J* = 10.1 Hz, 1H), 6.01 (d, *J* = 10.0 Hz, 1H), 4.35 (d, *J* = 2.9 Hz, 1H), 2.55 - 2.36 (m, 2H), 1.64 (s, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) _ 197.6, 148.4, 127.0, 92.6, 78.4, 28.8, 23.9, 19.1, 16.1. HRMS (ESI) *m/z*: calcd for $C_9H_{14}NaO_3$ ($M+Na^+$) 193.0835, found: 193.0838.

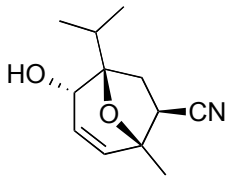
Cyano unstaturated ketone 46



46

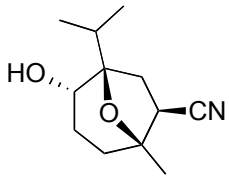
To a solution of **45** (1.6 g, 11.0 mmol) in acrylonitrile (60 mL) was added diisopropylethylamine (2.3 mL, 13 mmol), followed by methanesulfonyl chloride (1.0 mL, 13 mmol). The resulting solution was microwave heated at 150°C for 4 hours. After cooling to room temperature, the reaction mixture was filtered through a plug of Celite, and the filtrate was concentrated *in vacuo*. Flash chromatography of the residue (20% ethyl acetate in hexanes) provided the cycloadduct **46** (880 mg, 45%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 6.93 (d, *J* = 9.7 Hz, 1H), 6.01 (d, *J* = 9.8 Hz, 1H), 3.06 (dd, *J* = 9.2, 2.9 Hz, 1H), 2.52 (dd, *J* = 14.3, 2.9 Hz, 1H), 2.30 (p, *J* = 7.0 Hz, 1H), 2.19 (dd, *J* = 14.3, 9.3 Hz, 1H), 1.74 (s, 3H), 1.08 (d, *J* = 6.9 Hz, 3H), 1.04 (d, *J* = 6.9 Hz, 3H) ^{13}C NMR (126 MHz, $CDCl_3$) δ : 196.7, 151.8, 128.1, 118.8, 100.0, 90.4, 37.4, 35.2, 30.0, 21.6, 17.6, 16.5. HRMS (ESI) *m/z*: calcd for $C_{12}H_{15}NNaO_2$ ($M+Na^+$) 228.0995, found: 228.0996.

Cyanoalcohol 48



47

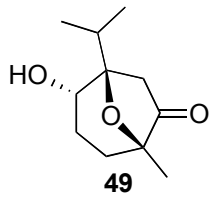
To a solution of **46** (210 mg, 1.03 mmol) in methanol (12 mL) at room temperature was added $CeCl_3{\cdot}7H_2O$ (1.1 g, 1.03 mmol). The reaction was cooled to 0 °C and $NaBH_4$ (40 mg, 1.03 mmol) was added. After 5 min, the reaction mixture was allowed to warm to room temperature at which time TLC showed no starting material remaining. The reaction was quenched with H_2O and diluted with ethyl acetate. This biphasic mixture was stirred for 5 min, and then the layers were separated. The aqueous layer was extracted once with ethyl acetate, and the combined organics were dried and concentrated *in vacuo*. Flash chromatography of the residue (20% ethyl acetate in hexanes) provided the allylic alcohol **47** (210 mg, 98%) as a white crystalline solid. ^{1}H



48

NMR (500 MHz, $CDCl_3$) δ : 5.77 (dd, *J* = 9.5, 1.5 Hz, 1H), 5.58 (dd, *J* = 9.6, 2.0 Hz, 1H), 4.65 (s, 1H), 2.94 (dd, *J* = 9.2, 1.8 Hz, 1H), 2.74 (dd, *J* = 14.0, 9.2 Hz, 1H), 2.09 - 1.96 (m, 2H), 1.53 (s, 3H), 1.09 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 133.86, 130.66, 120.11, 86.75, 80.01, 69.83, 39.79, 33.36, 32.21, 21.35, 17.64, 17.37. A suspension of allylic alcohol (210 mg, 0.07 mmol) and Pd/C (10%) in ethanol (7 mL) was stirred under an atmosphere of H_2 for 2 h. Then the reaction mixture was filtered through Celite, washed with ethyl acetate and the filtrate was concentrated *in vacuo.* The product was purified flash chromatography and recrystalized in hexanes to yield to saturated cyano alcohol **48** in quantitative yield (210 mg, 99%). ^1H NMR (400 MHz, $CDCl_3$) δ : 3.84 (dd, *J* = 10.4, 5.6 Hz, 1H), 2.90 (dd, *J* = 10.1, 4.5 Hz, 1H), 2.48 (dd, *J* = 13.8, 10.2 Hz, 1H), 2.03 (m, 2H), 1.70 (td, *J* = 13.6, 5.7 Hz, 1H), 1.60 (dd, *J* = 5.8, 1.5 Hz, 1H), 1.56 (dd, *J* = 5.9, 1.4 Hz, 1H), 1.49 (s, 3H), 1.38 (m, 1H), 1.04 (d, 3H), 1.02 (d, 3H). HRMS (ESI) *m/z*: calcd for $C_{12}H_{19}NNaO_2$ ($M+Na^+$) 232.1308, found: 232.1309.

Hydroxyketone 49

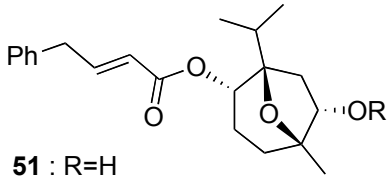


49

To a solution of diisopropylethylamine (0.31 mL, 2.2 mmol) in THF (4.7 mL) at -78 °C was added butyllithium in hexanes (1.4 mL, 2.2 mmol). The solution was warmed to 0 °C and stirred for 30 min. Then the solution was recooled to -78 °C, and a solution of the cyano alcohol **43** (210 mg, 1.0 mmol) in THF (1.4 mL) was added via cannula. After stirring for 2 min, dry oxygen (passed through KOH column) gas was bubbled into the solution for 30 min. The reaction was quenched with 28 mL of 1 M stannous chloride in 2 M HCL solution and stirred for 30 min at 0 °C. Then the reaction mixture was diluted with ethyl acetate and washed with water, 1 M NaOH and brine. The organics were dried and concentrated *in vacuo*. Flash chromatography purification yielded the corresponding

hydroxy ketone (90 mg, 40%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 4.02 (dd, *J* = 10.8, 6.0 Hz, 1H), 2.49 (d, *J* = 18.7 Hz, 1H), 2.33 (dd, *J* = 18.7, 1.2 Hz, 1H), 2.08 - 1.96 (m, 2H), 1.65 - 1.54 (m, 2H), 1.46 - 1.33 (m, 1H), 1.20 (s, 3H), 1.06 (d, *J* = 7.0 Hz, 3H), 1.04 (d, *J* = 6.9 Hz, 3H) . ^{13}C NMR (126 MHz, $CDCl_3$) δ : 218.1 (d, *J* = 2.8 Hz), 100.0, 82.8, 68.2, 40.2, 32.9, 19.6, 17.5, 16.8. HRMS (ESI) *m/z*: calcd for $C_{11}H_{18}NNaO_3$ ($M+Na^+$) 221.1148, found: 228.1150.

Cinnamic hydroxy ester (51)

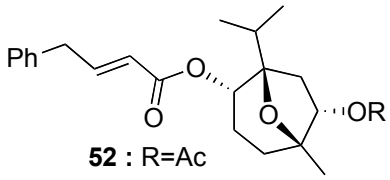


51 : R=H

To a stirred mixture of cinnamic acid (39 mg, 0.26 mol, 2.0 equiv) and hydroxy-ketone above (25 mg, 0.13 mol, 1.0 equiv) in dry toluene (1.8 mL) were added successively NEt_3 (60 μL, 0.39 mol, 3.0 equiv.) and 2,4,6-trichlorobenzoyl chloride (50 μL, 0.33 mol, 2.5 equiv). The reaction mixture was stirred for 10 min, then catalytic amount of 4-DMAP (5 mg, 40 μmol, 0.3 equiv) was added. After 16 h of stirring at rt, the reaction was diluted with ethyl acetate, and the organic phase was washed successively with aqueous HCl solution (1M), saturated sodium bicarbonate solution, and brine. The organic phase was dried over MgSO4 and concentrated in vacuo after filtration. The residue was purified by flash chromatography to afford the cinnamic keto-ester (40 mg, 95%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.66 (d, *J* = 16.0 Hz, 1H), 7.51 (m, 2H), 7.46 - 7.33 (m, 3H), 6.38 (d, *J* = 16.0 Hz, 1H), 5.26 (ddd, *J* = 10.6, 6.0, 1.1 Hz, 1H), 2.65 (d, *J* = 18.6 Hz, 1H), 2.48 (dd, *J* = 18.6, 1.2 Hz, 1H), 2.33 - 2.19 (m, 1H), 1.96 (hept, *J* = 7.0 Hz, 1H), 1.75 - 1.64 (m, 2H), 1.56 - 1.39 (m, 1H), 1.25 (s, 3H), 1.04 (d, *J* = 6.9 Hz, 3H), 1.01 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 217.3, 165.8, 145.7, 134.3, 130.7, 129.1, 128.3, 117.8, 82.3, 81.9, 69.8, 41.8, 33.7, 32.5, 25.0, 19.7, 17.6. To the solution of the cinnamic keto ester

(26 mg, 80 μmol, 1.0 equiv) in MeOH (1.5 mL) at 0 °C was added $NaBH_4$ (3.4 mg, 90 μmol, 1.1 equiv). After 30 min of stirring at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl solution and extracted twice with ethyl acetate. The combined organic phases were washed with brine, dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by flash chromatography to give **44** (26 mg, 100%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.64 (d, *J* = 16.0 Hz, 1H), 7.51 (d, *J* = 6.0 Hz, 2H), 7.42 - 7.34 (m, 3H), 6.39 (d, *J* = 16.0 Hz, 1H), 5.12 (dd, *J* = 10.0, 6.0 Hz, 1H), 4.04 (dd, *J* = 10.9, 5.1 Hz, 1H), 2.31 (ddd, *J* = 13.7, 10.9, 1.1 Hz, 1H), 2.13 (ddd, *J* = 13.3, 6.3, 1.4 Hz, 1H), 1.99 (dd, *J* = 13.7, 5.1 Hz, 1H), 1.96 - 1.77 (m, 4H), 1.60 (dt, *J* = 12.9, 6.5 Hz, 1H), 1.23 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 166.2, 145.0, 130.5, 129.0, 128.2, 118.4, 83.7, 81.3, 78.5, 71.8, 38.5, 33.2, 31.3, 24.9, 24.6, 17.6, 16.8. HRMS (ESI) *m/z*: calcd for $C_{20}H_{26}NaO_4$ ($M+Na^+$) 353.1723, found: 353.1725.

Cinnamic-aceto diester 52

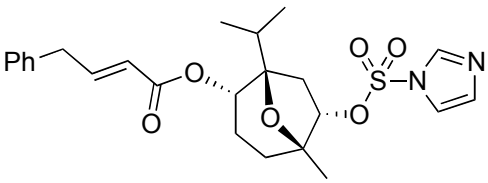


52 : R=Ac

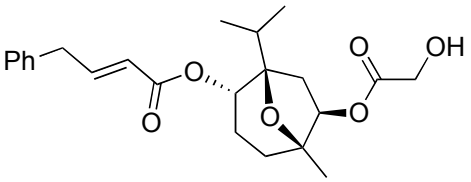
To a solution of **44** (7.5mg, 0.023 mmol) in CH_2Cl_2 (0.2 mL) was added sequentially at room temperature 4-DMAP (2 mg, 0.012 mmol), NEt_3 (15 μL, 0.92 mmol) and Ac_2O (4 mL, 0.035 mmol). The reaction was stirred at this temperature overnight. The reaction mixture was quenched with saturated $NaHCO_3$, extracted twice with CH_2Cl_2 (10 mL), dried over $MgSO_4$ and concentrated. The residue was purified through preparative plate chromatography to yield **45** (6.8 mg, 80%) as a clear oil. . ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.65 (d, *J* = 16.0 Hz, 2H), 7.41 (m, 3H), 6.47 (d, *J* = 15.9 Hz, 1H), 6.38 (dd, *J* = 16.0, 2.2 Hz, 1H) 5.27 (dd, *J* = 10.3, 5.8 Hz, 1H), 2.66 (dd, *J* = 18.5, 2.0 Hz, 1H), 2.49 (d, *J* = 18.3 Hz, 1H), 2.25 (m 1H), 2.00 (m, 1H), 1.70 (s,

3H), 1.49 (m, 1H), 1.26 (s, 3H), 1.04 (d, *J* = 6.9 Hz, 3H), 1.02 (d, *J* = 6.9 Hz, 3H). HRMS (ESI) *m/z*: calcd for $C_{22}H_{28}NaO_5$ ($M+Na^+$) 395.1829, found: 395.1831

Nor-A ring englerin 54



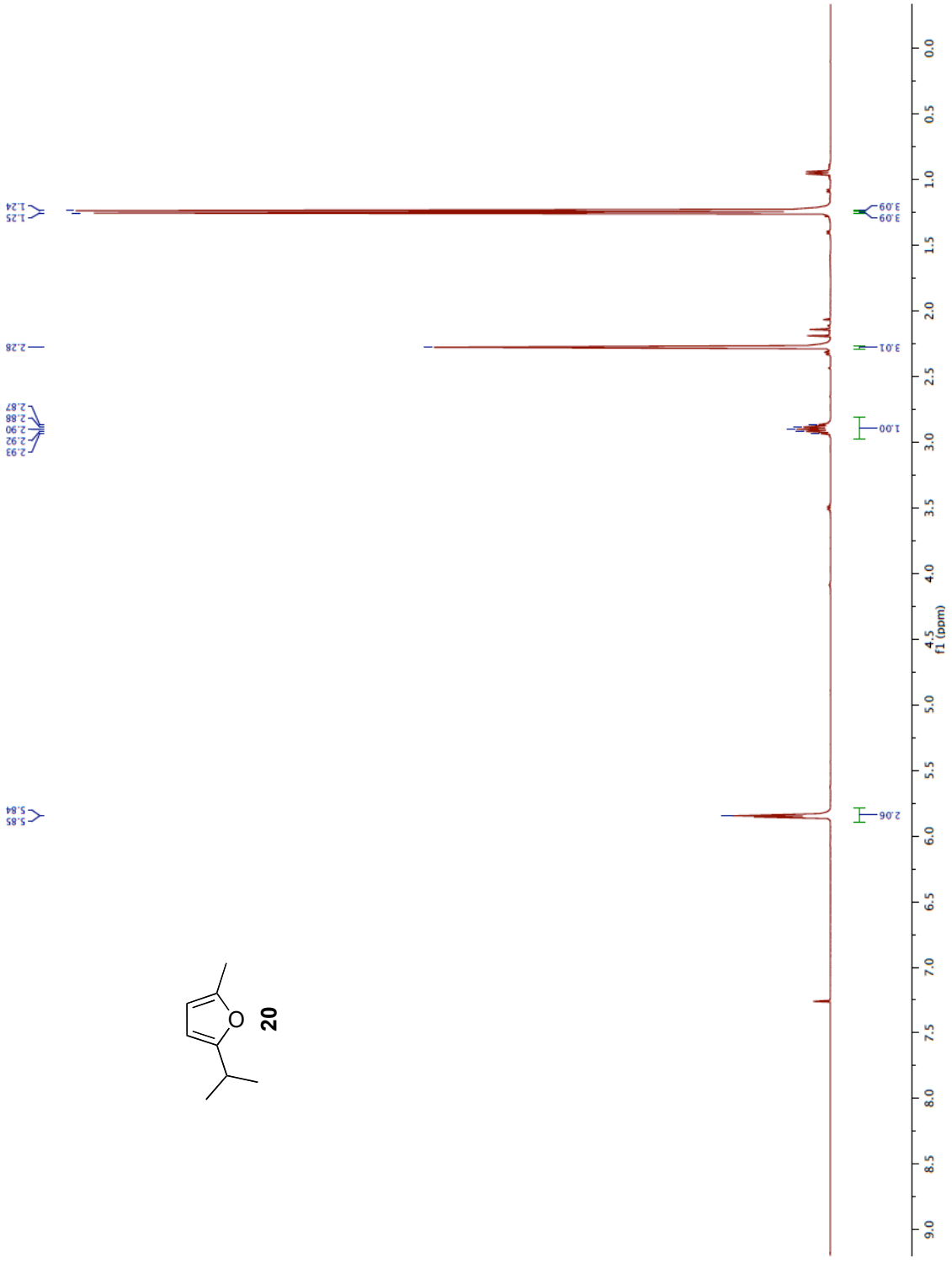
53



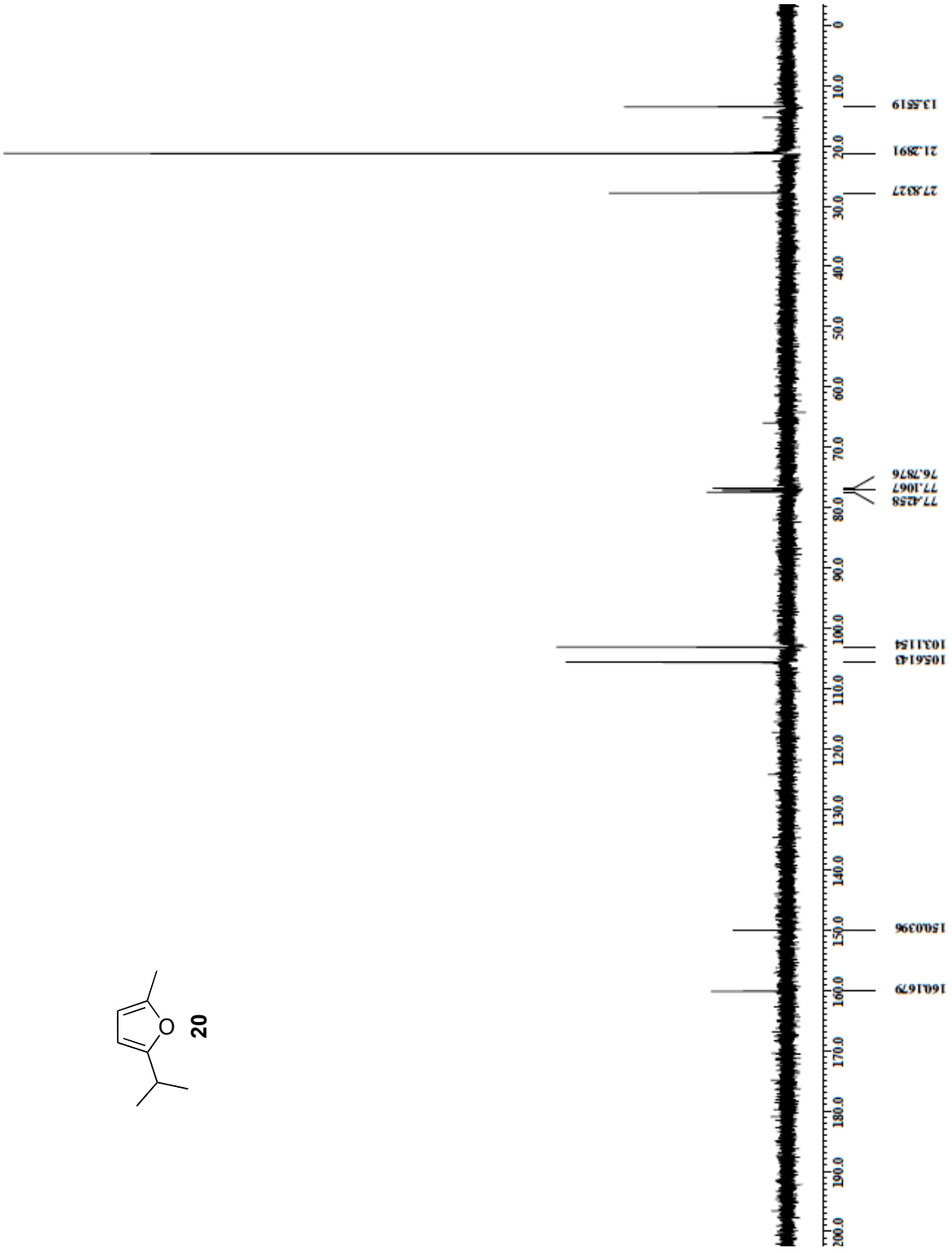
54

LiHMDS in THF (1 M, 80 μL, 80 μmol, 1.5 equiv) was added to the solution of **51** (16 mg, 50 _mol, 1.0 equiv) in dry THF (0.85 mL) at 0 °C. The solution was stirred for 30 min, then cooled to –10 °C and *N*,*N*'-sulfuryldiimidazole (16 mg, 80 μmol, 1.5 equiv) was added in one portion, and the solution was then stirred at rt overnight. The reaction was quenched with MeOH (0.2 mL). After 30 min of stirring, the solution was concentrated, diluted with EtOAc, washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified through flash chromatography to yield imidazole thiosester **53** (22 mg, 99%) as a clear oil. ^1H NMR (500 MHz, $CDCl_3$) δ : 8.04 (bs, 1H), 7.63 (d, *J* = 16.0 Hz, 2H), 7.53 (m, 2H), 7.39 (m, 4H), 7.24 (bs, 1H) 6.36 (d, *J* = 16.0 Hz, 1H), 5.08 (m, 1H), 4.50 (dd, *J* = 10.9, 4.6 Hz, 1H), 2.24 (dd, *J* = 14.5, 11.4 Hz, 1H), 2.18 (m, 1H), 2.02 (dd, *J* = 14.5, 4.7 Hz, 1H), 1.84 (m, 1H), 1.77 (m, 1H), 1.66 (m, 1H), 1.13 (s, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H). To a mixture of 18-crown-6 (11.4 mg, 43 μmol, 5.0 equiv) and cesium hydroxy acetate (9.0 mg, 43 μmol, 5.0 equiv) was added to the solution of **53** (4.0 mg, 9 μmol, 1.0 equiv) in dry toluene (0.4 mL). After 48 h of stirring at 110 °C under argon, the solution was diluted with ethyl acetate, washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography to give **54** (14 mg,

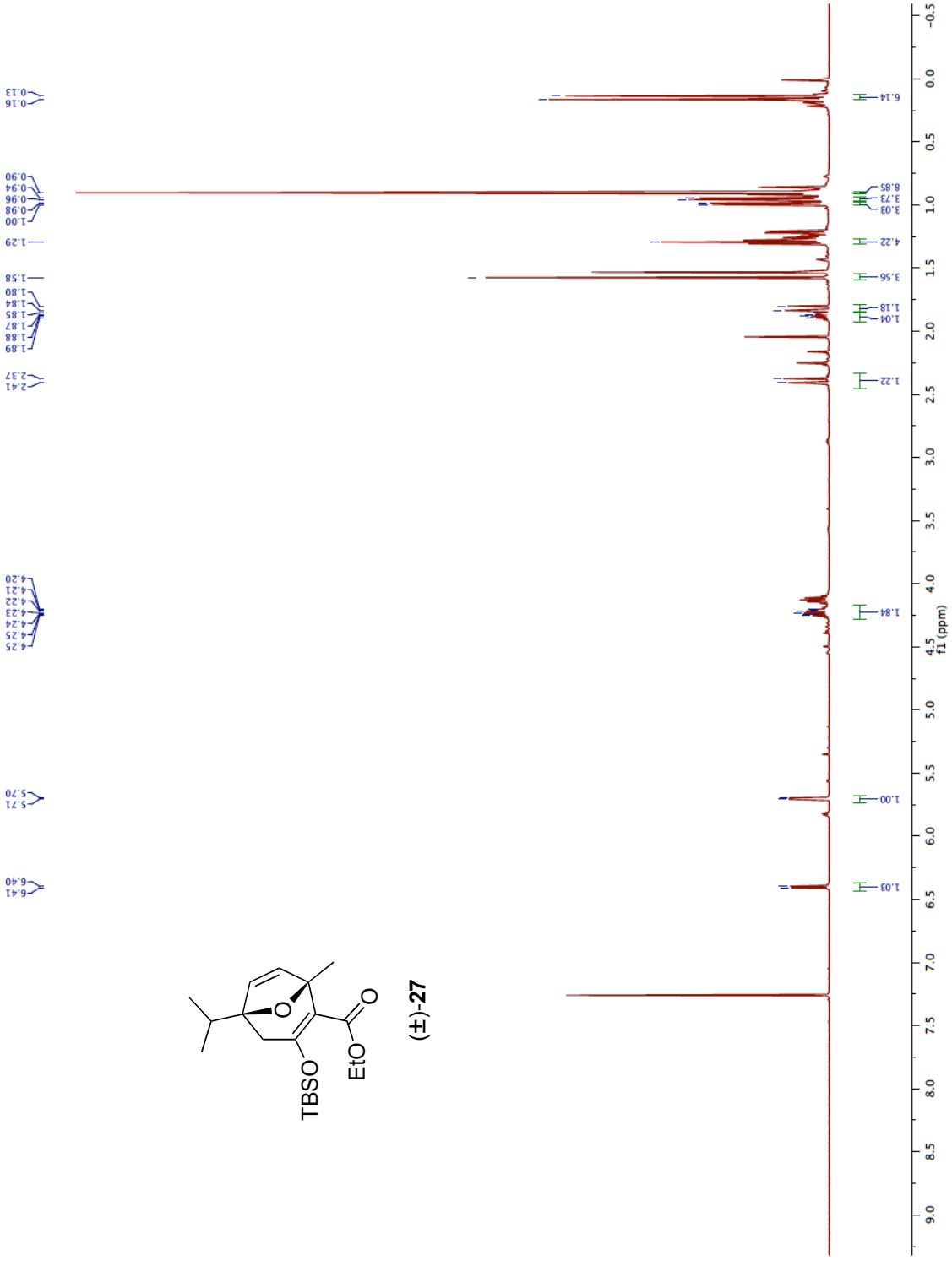
74%) as clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.65 (d, *J* = 16.0 Hz, 1H), 7.52 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.38 (d, *J* = 3.2 Hz, 3H), 6.38 (d, *J* = 16.0 Hz, 1H), 5.25 (dd, *J* = 7.8, 2.6 Hz, 1H), 5.03 (dd, *J* = 10.4, 5.8 Hz, 1H), 4.20 (d, *J* = 5.3 Hz, 2H), 2.70 (dd, *J* = 14.7, 7.9 Hz, 1H), 2.32 (t, *J* = 5.3 Hz, 1H), 2.23 - 2.16 (m, 1H), 1.89 (d, *J* = 6.9 Hz, 1H), 1.81 (dd, *J* = 14.8, 2.1 Hz, 2H), 1.74 - 1.63 (m, 2H), 1.50 - 1.43 (m, 1H), 1.20 (s, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ : 174.6, 173.0, 165.9, 145.3, 134.3, 130.5, 129.0, 128.2, 118.0, 85.3, 82.5, 79.8, 60.7, 38.9, 34.3, 33.3, 24.5, 20.4, 18.0, 17.0. HRMS (ESI) *m/z*: calcd for $C_{22}H_{28}NaO_6$ ($M+Na^+$) 411.1778, found: 411. 1779.



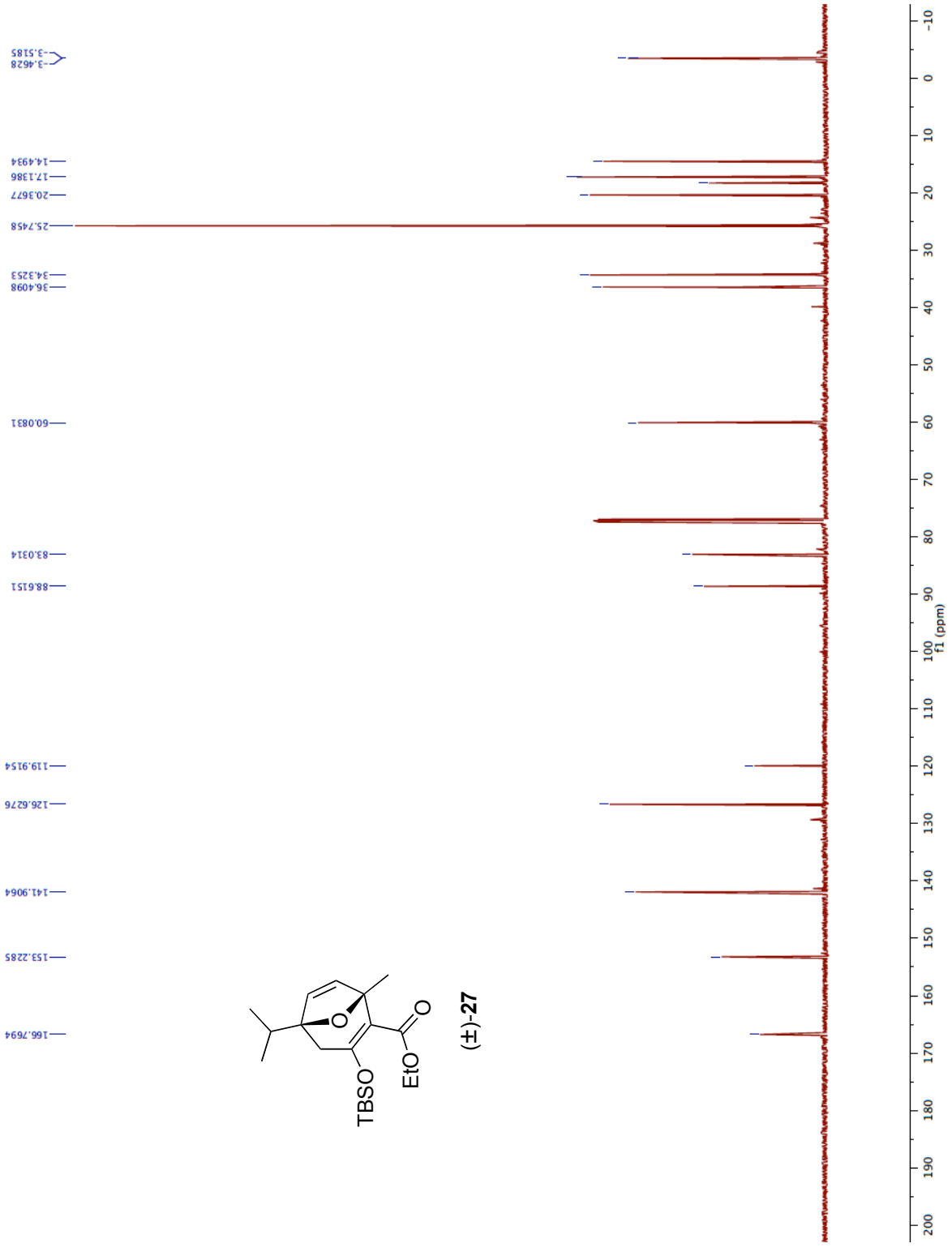
Spectrum 1.1 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20**



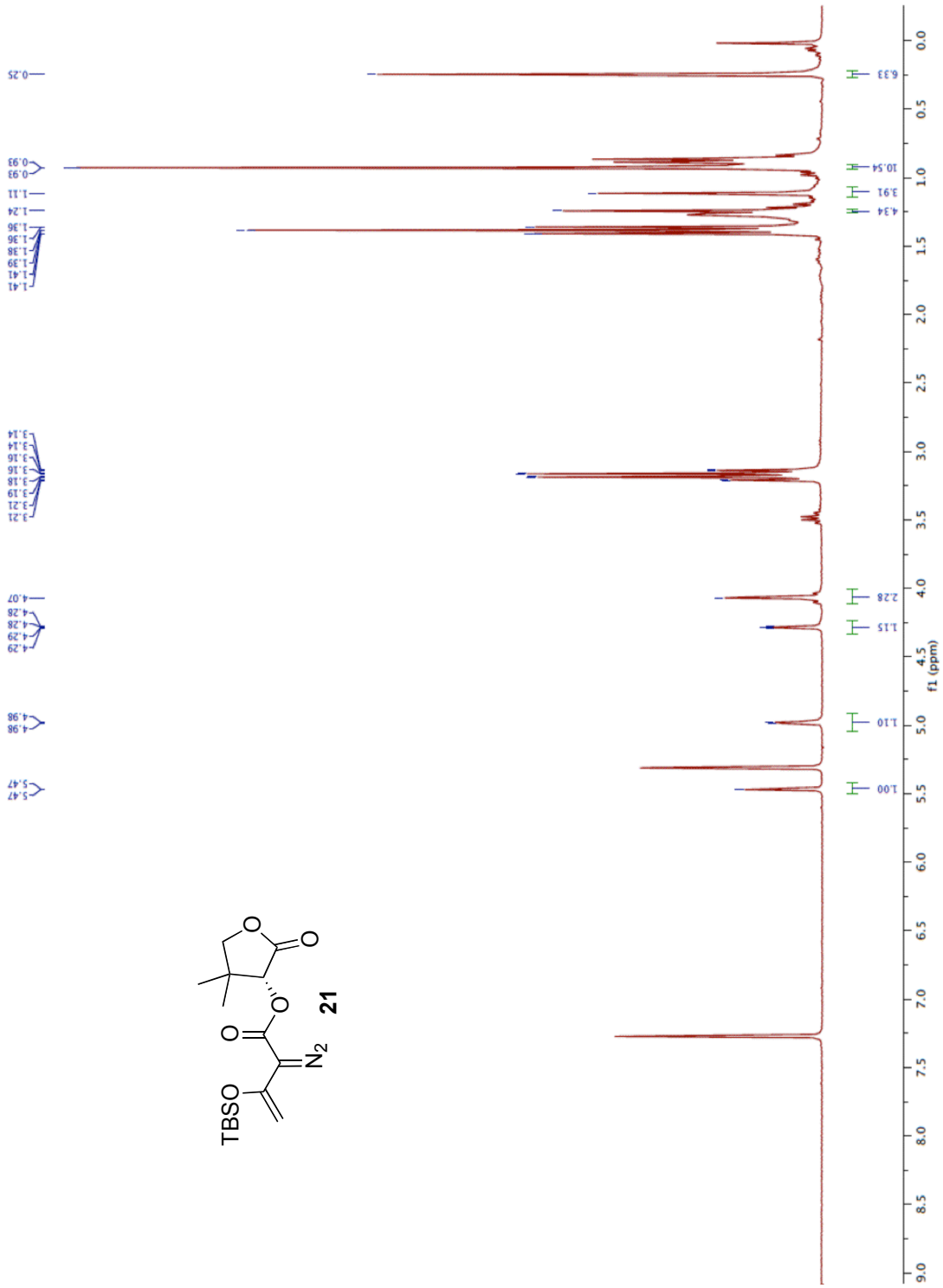
Spectrum 1.2 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20**



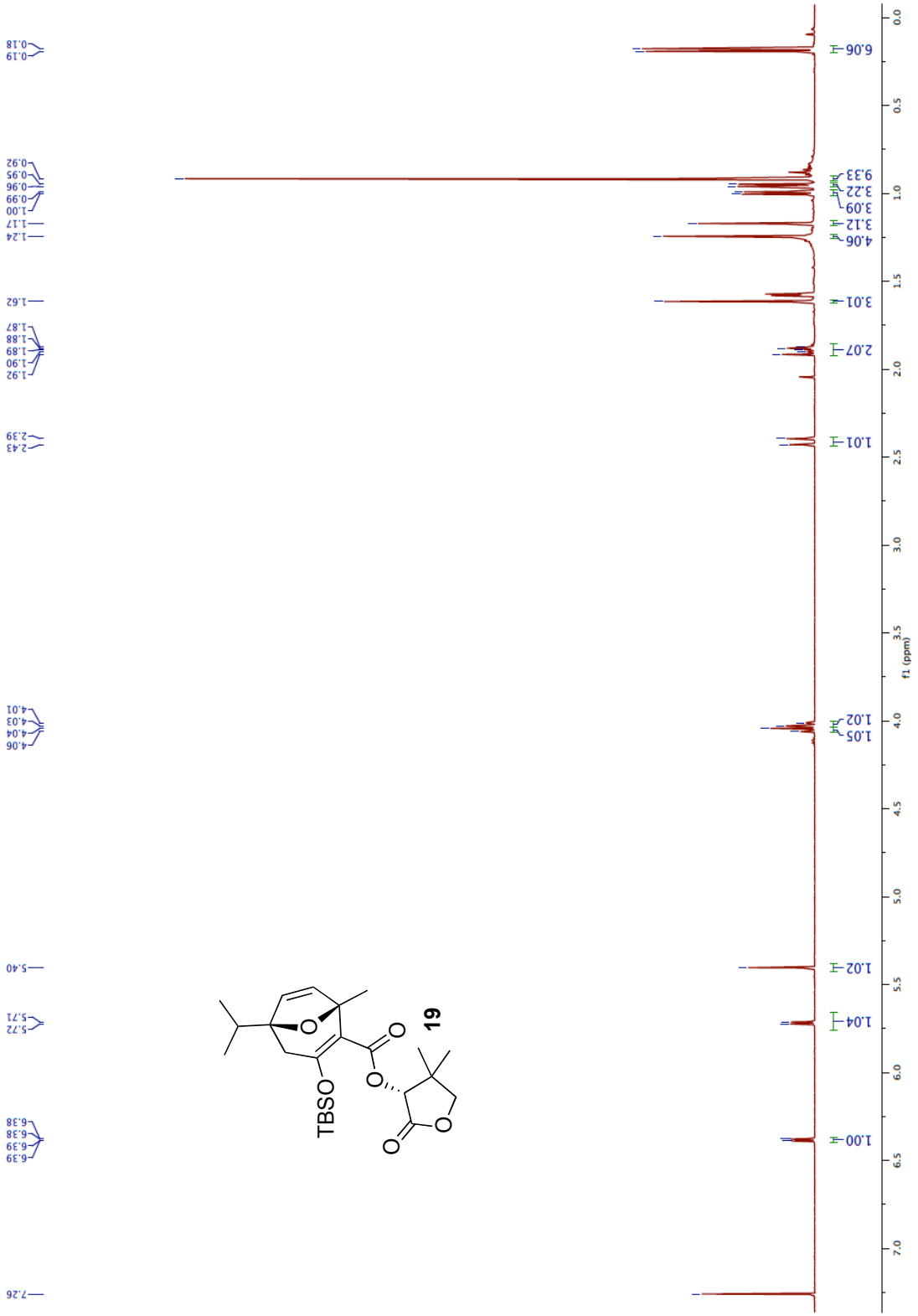
Spectrum 1.3 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **27**



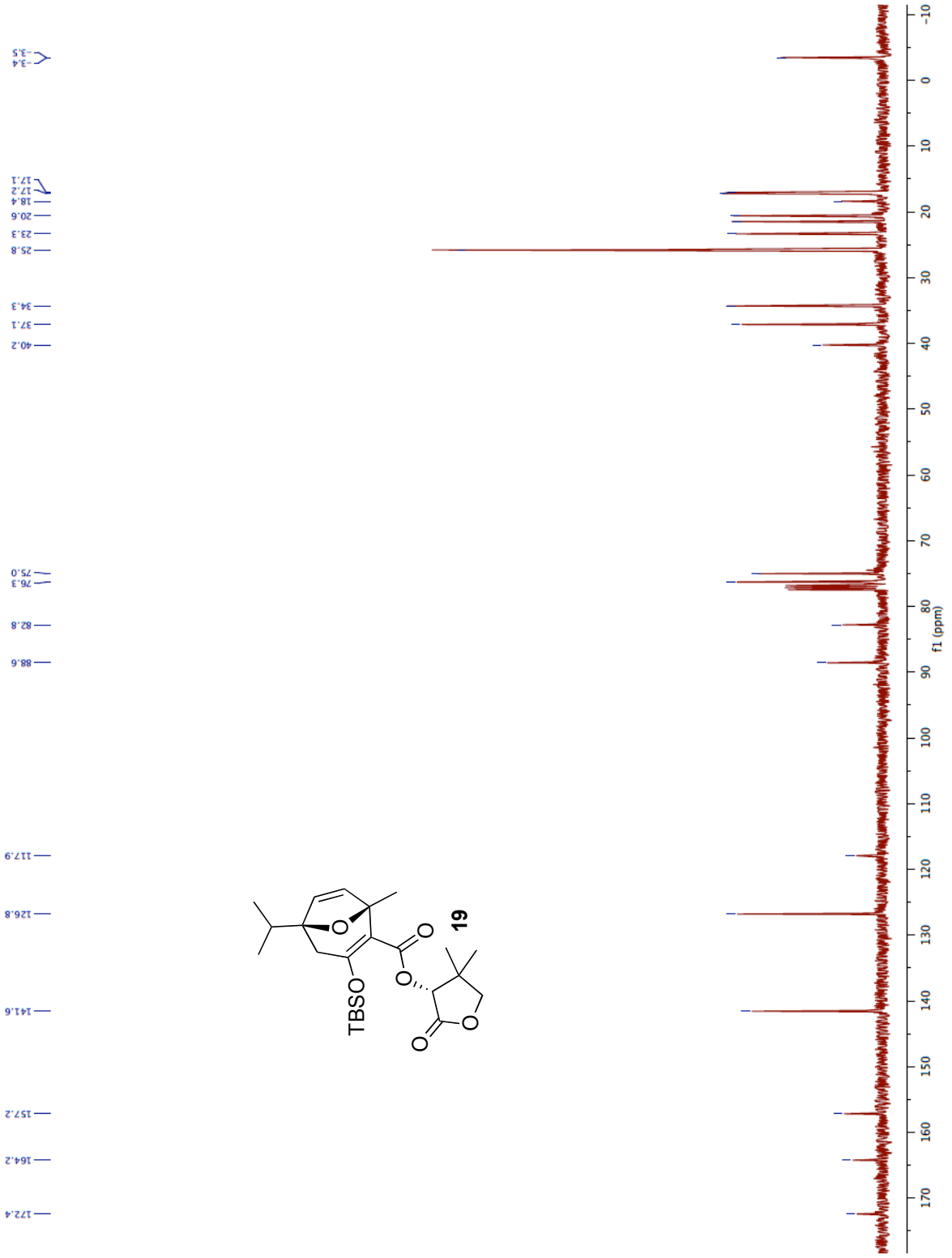
Spectrum 1.4 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **27**



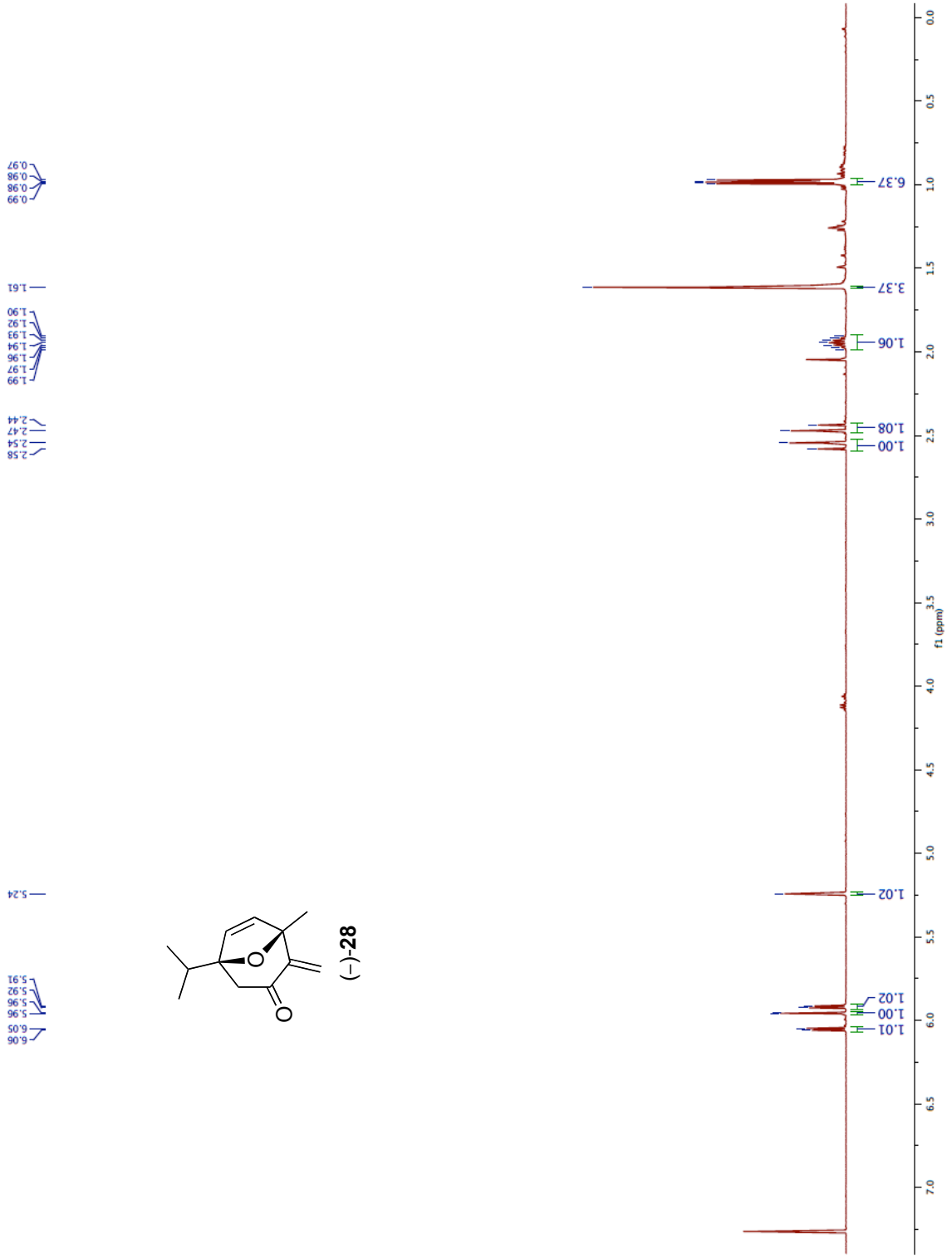
Spectrum 1.5 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **21**



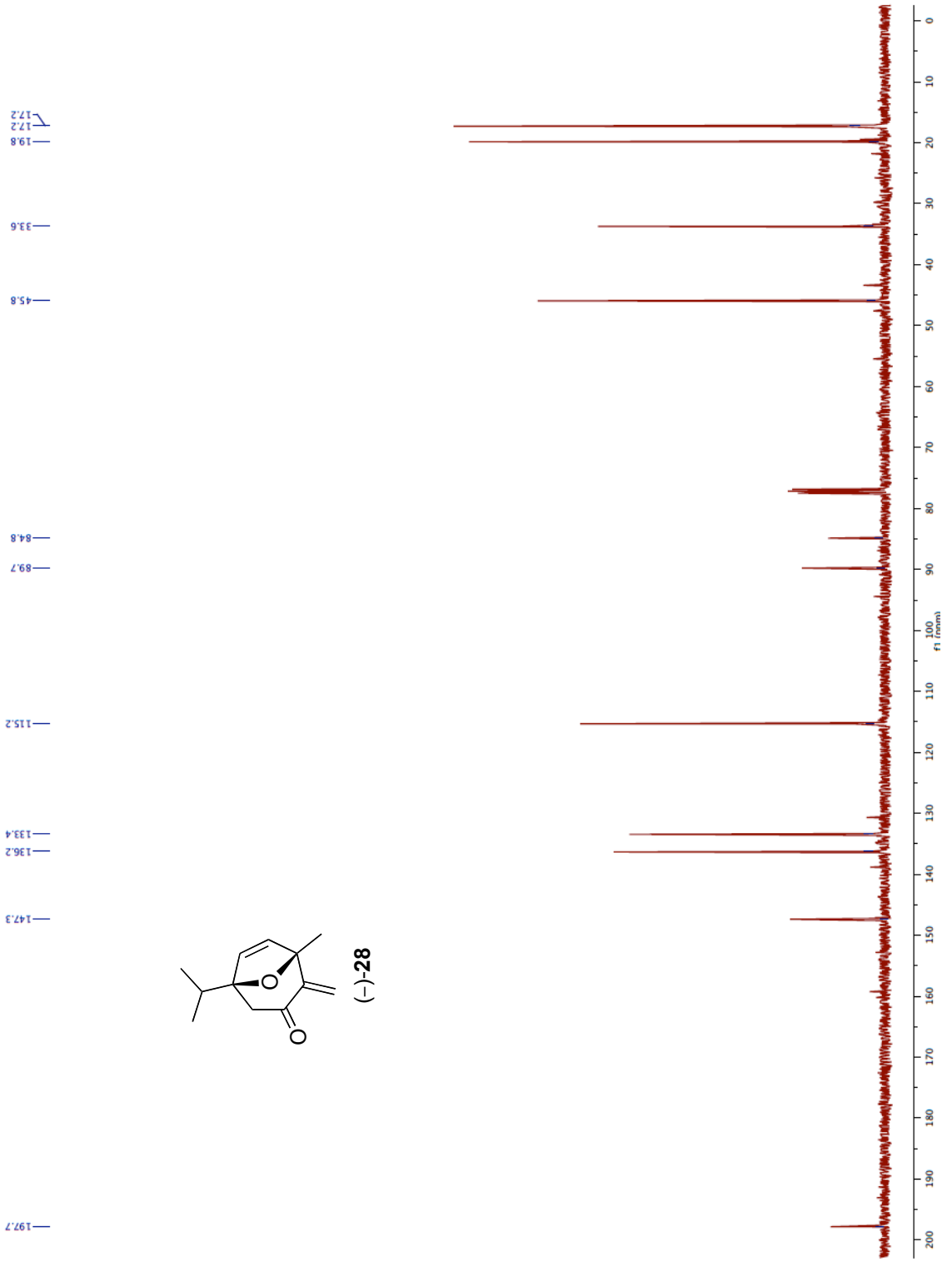
Spectrum 1.6 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **19**



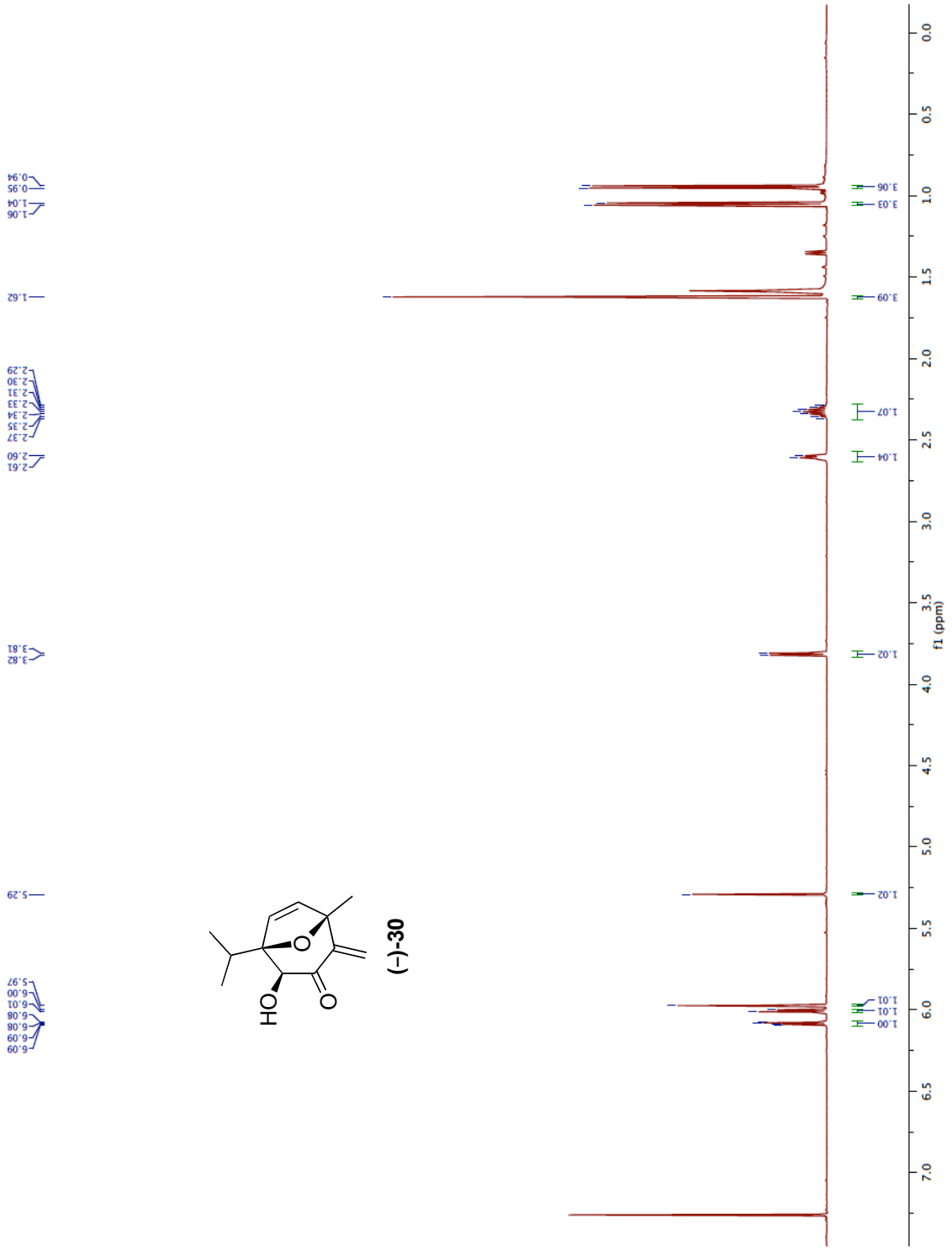
Spectrum 1.7 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **19**



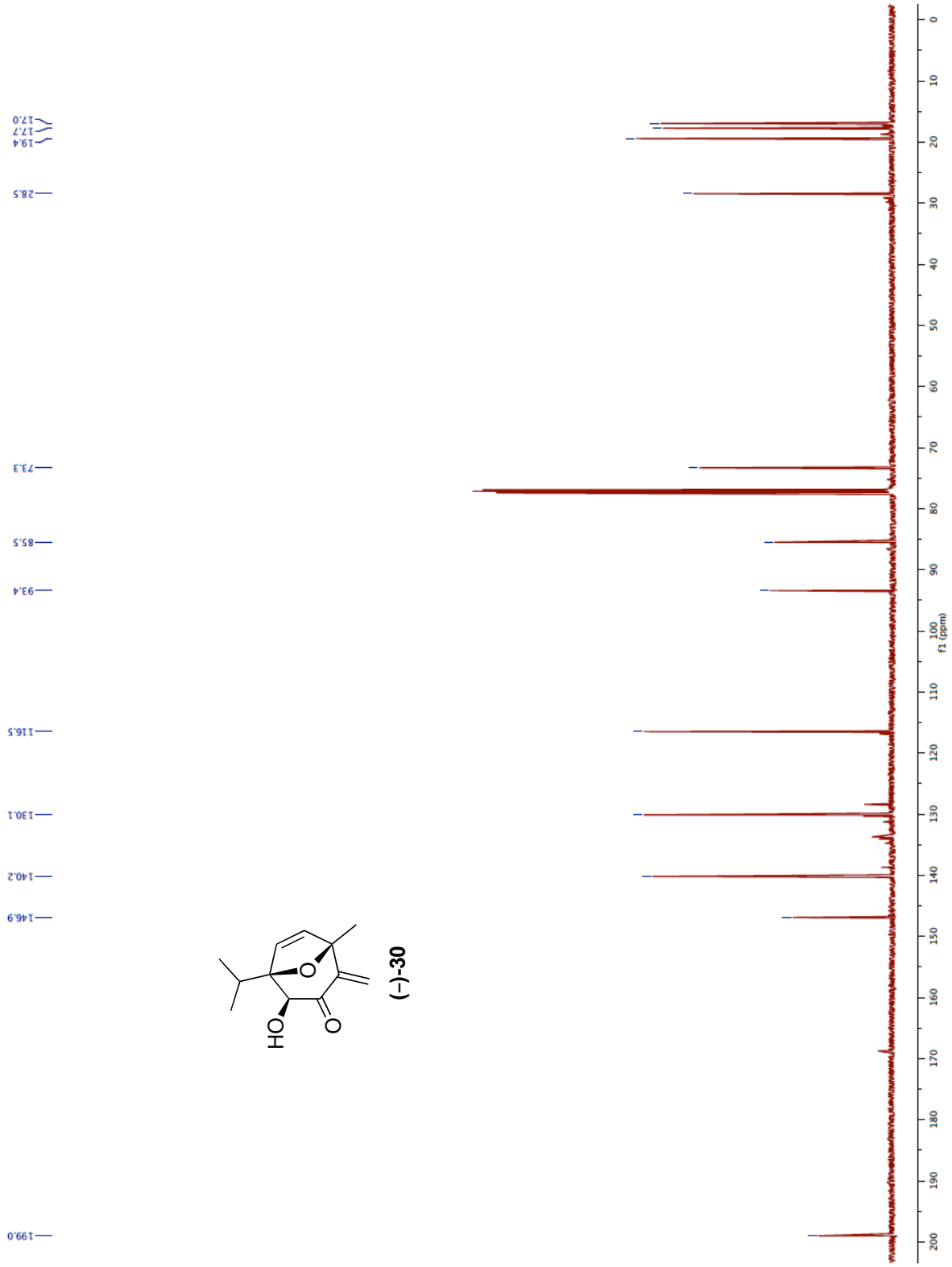
Spectrum 1.8 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **28**



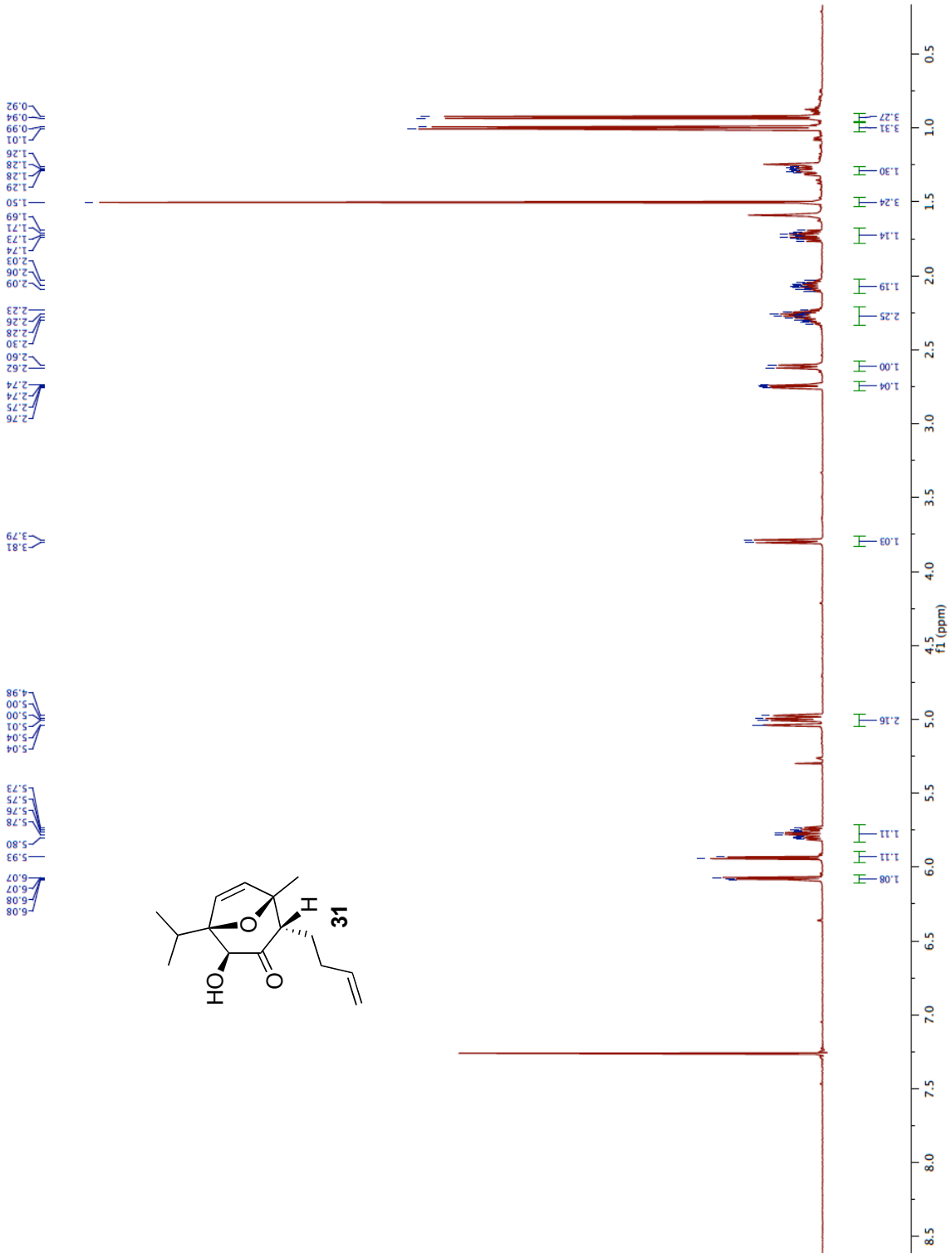
Spectrum 1.9 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **28**



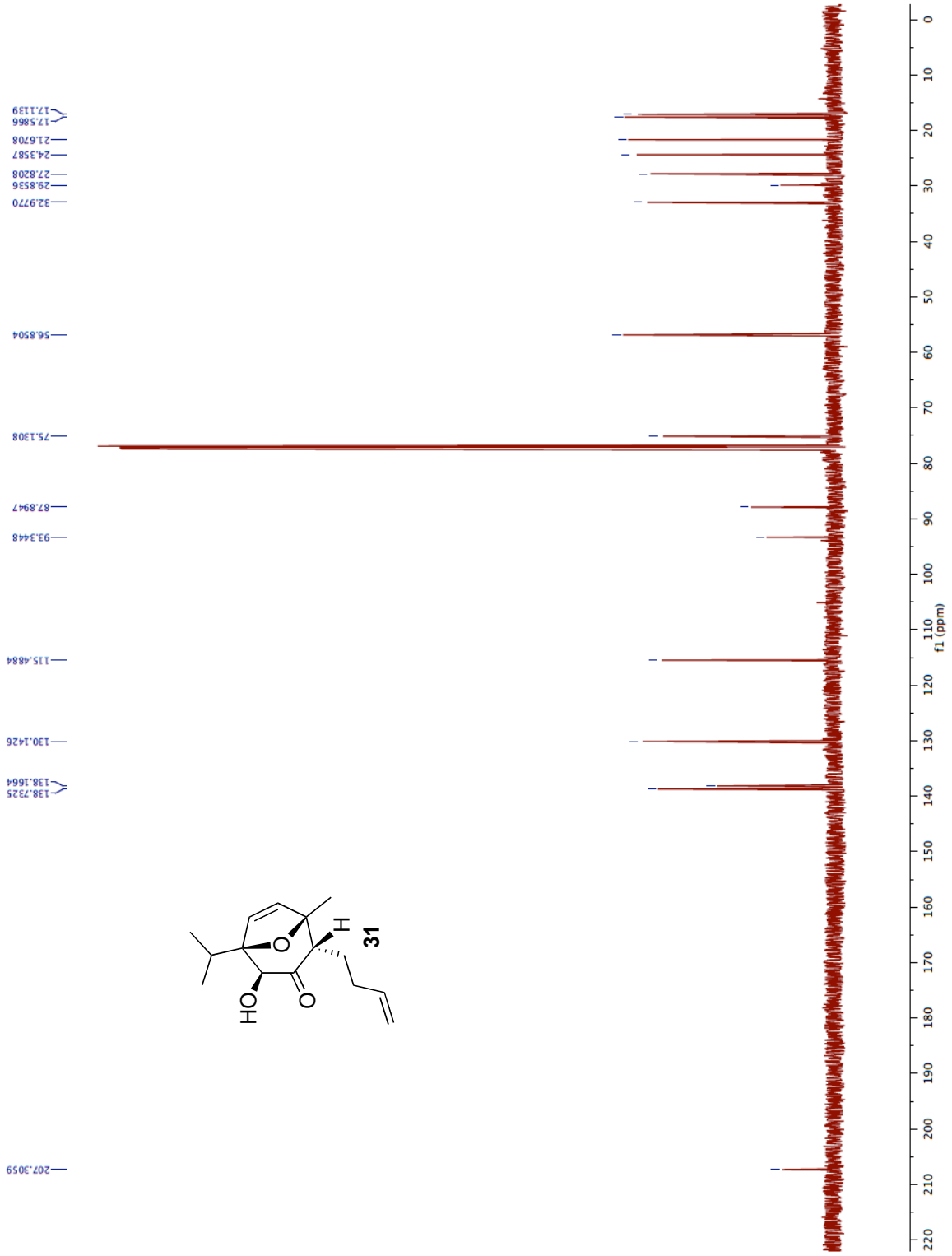
Spectrum 1.10 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **30**



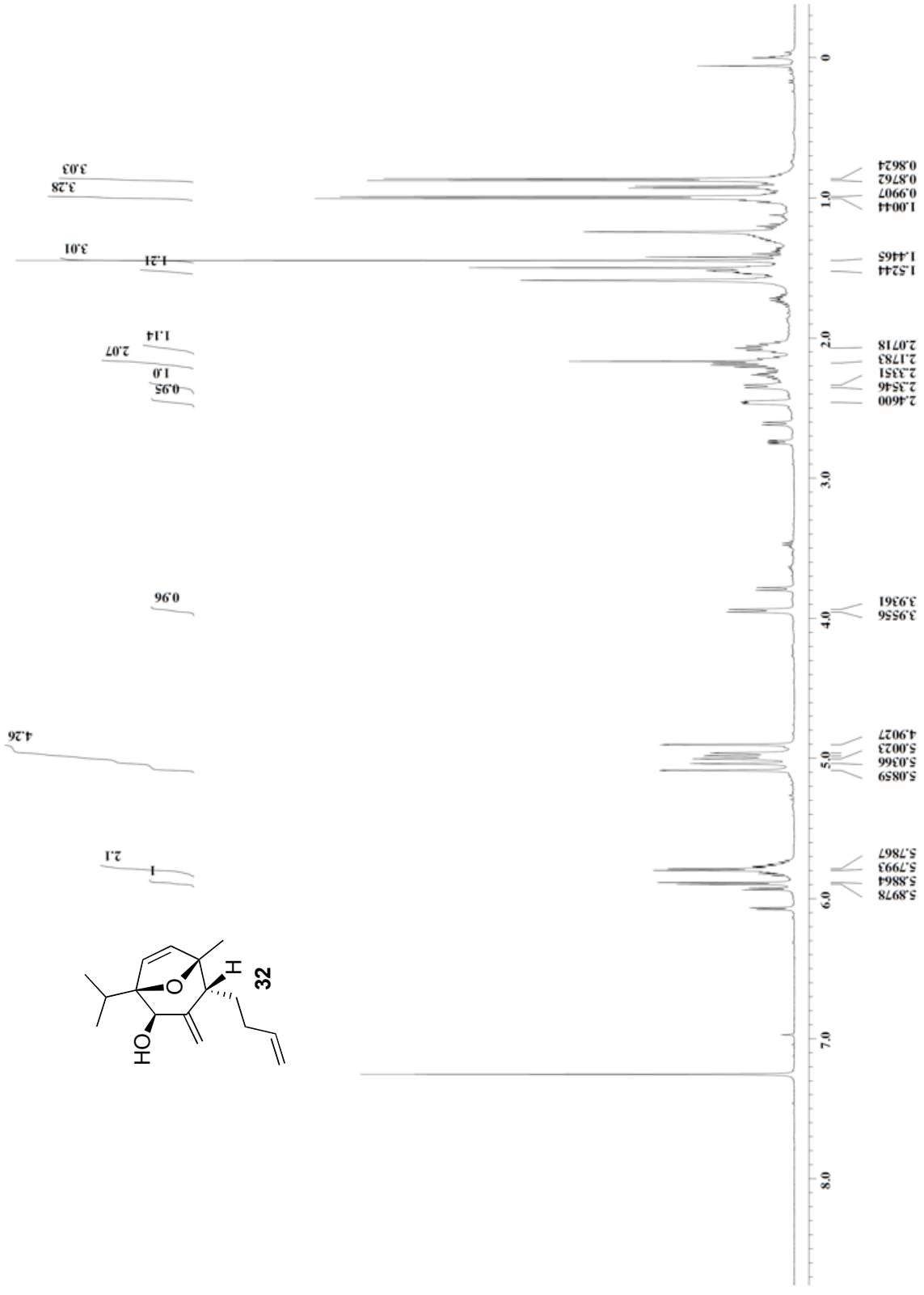
Spectrum 1.11 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **30**



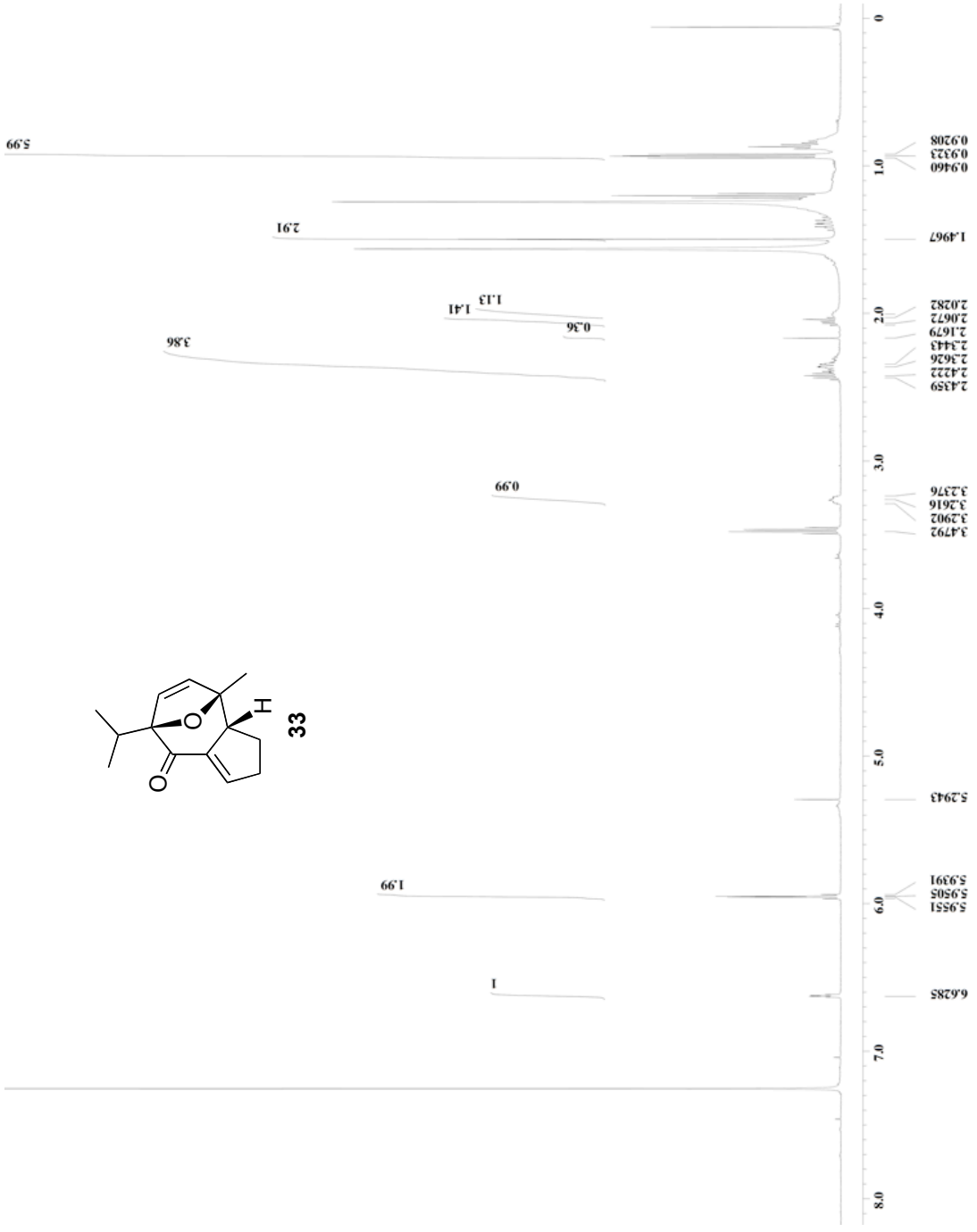
Spectrum 1.12 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **31**



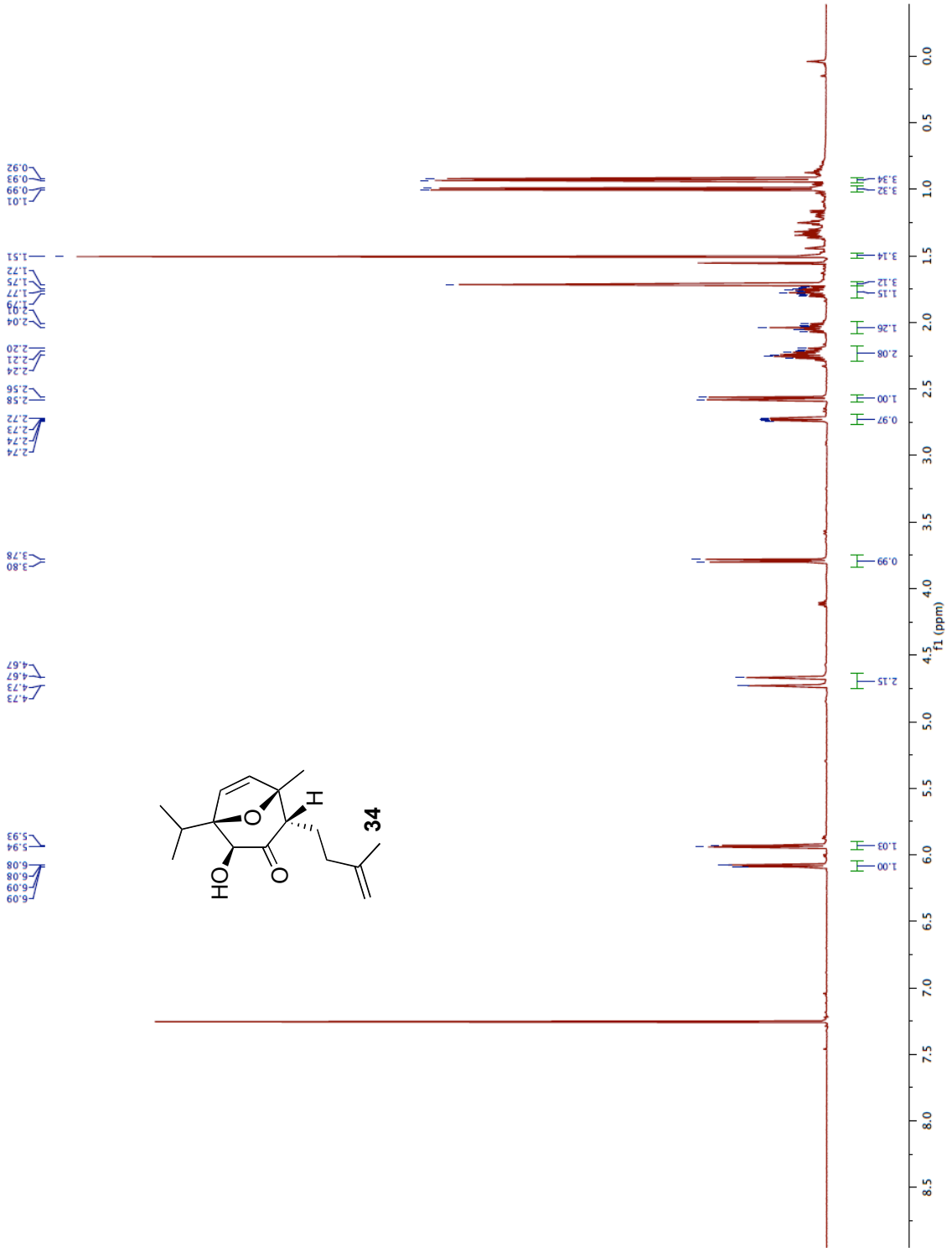
Spectrum 1.13 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **31**



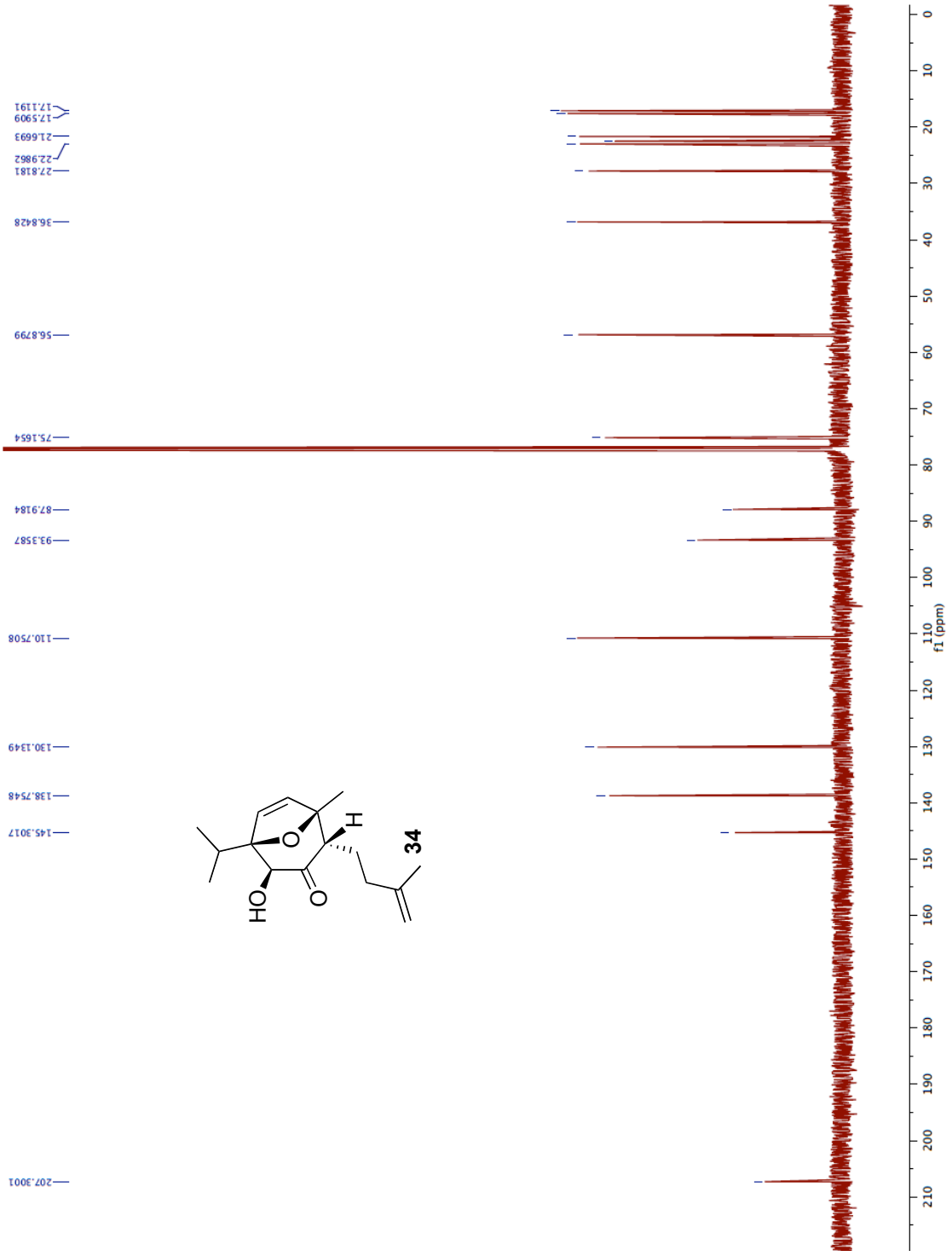
Spectrum 1.14 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **32**



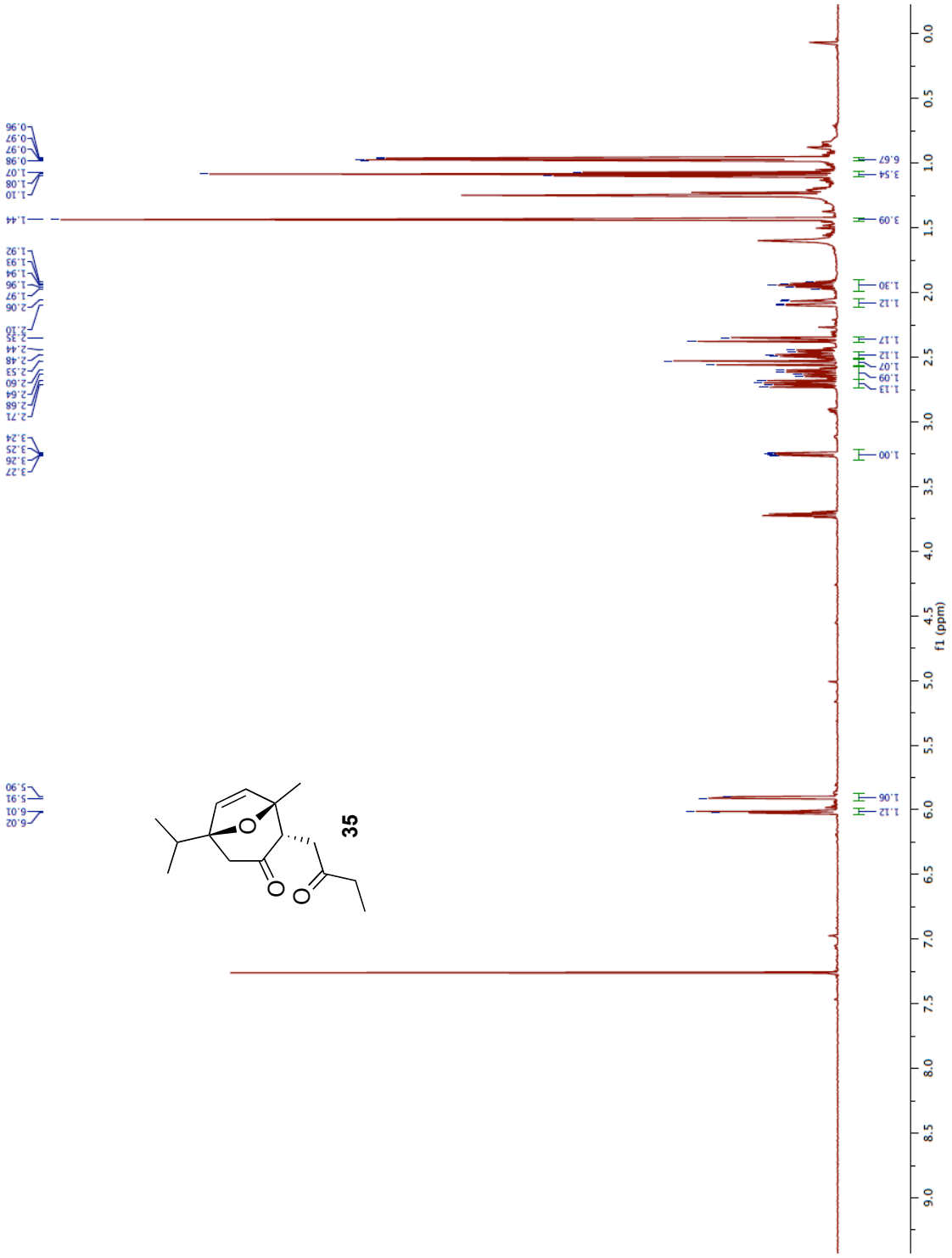
Spectrum 1.15 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **33**



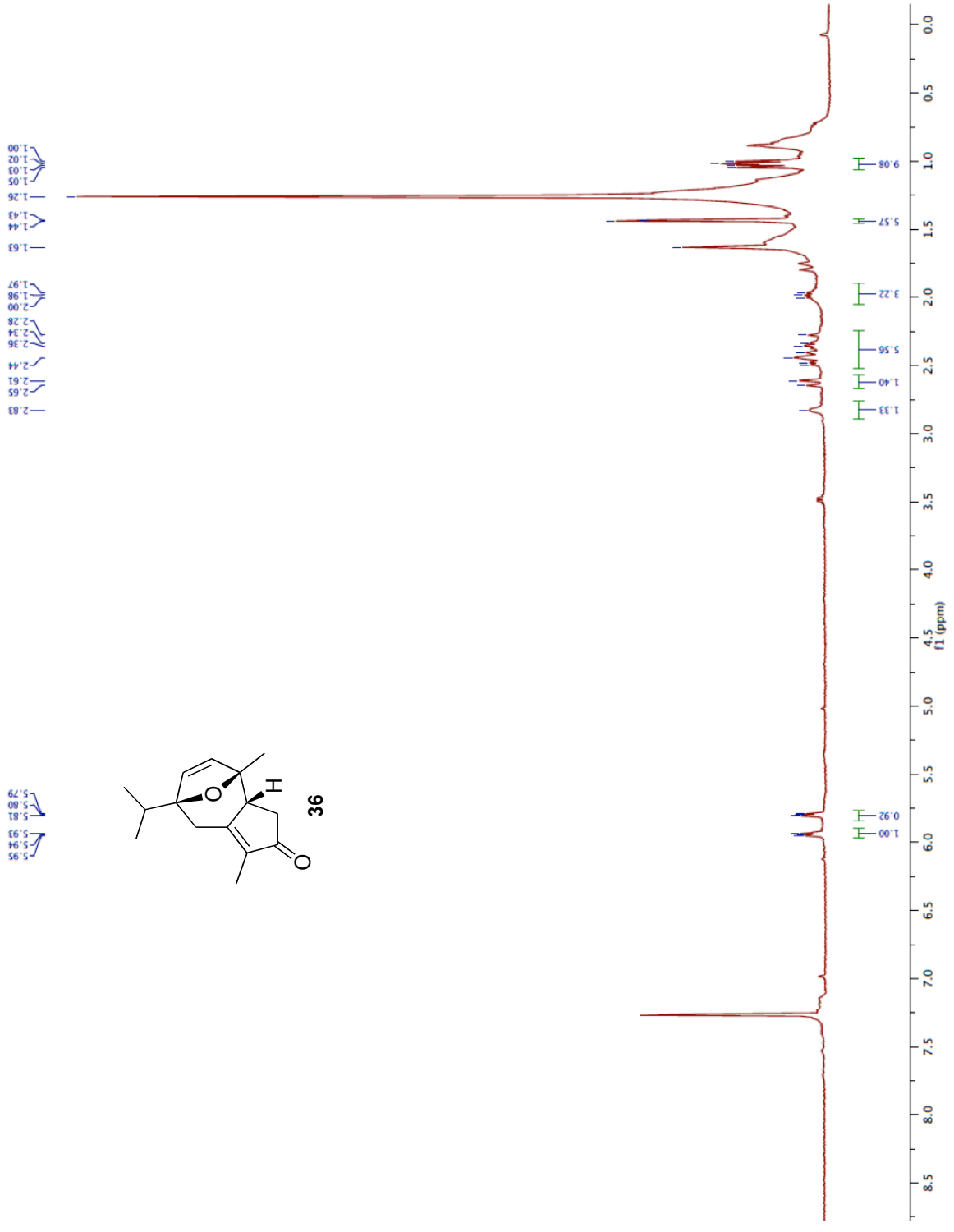
Spectrum 1.16 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **34**



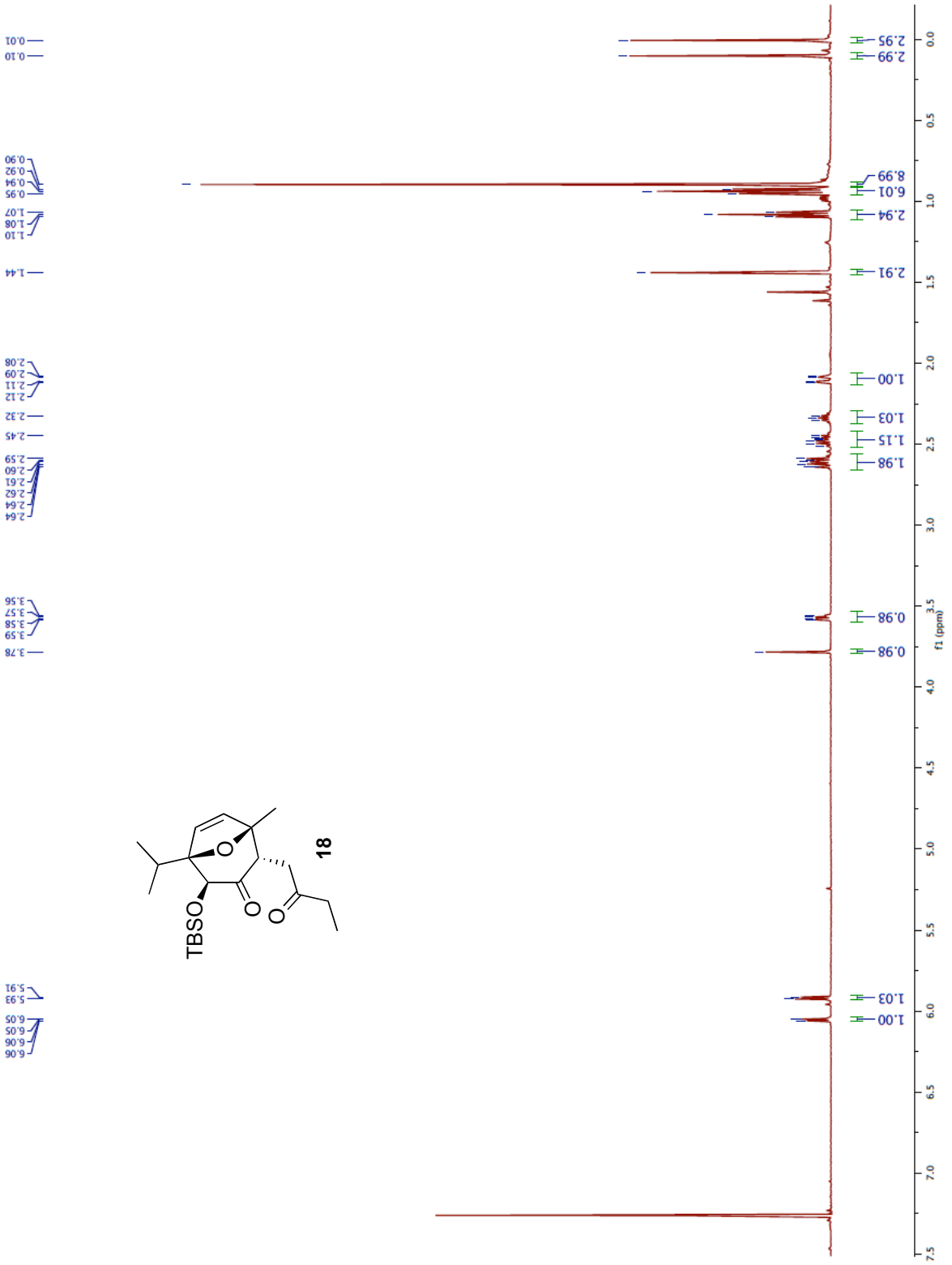
Spectrum 1.17 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **34**



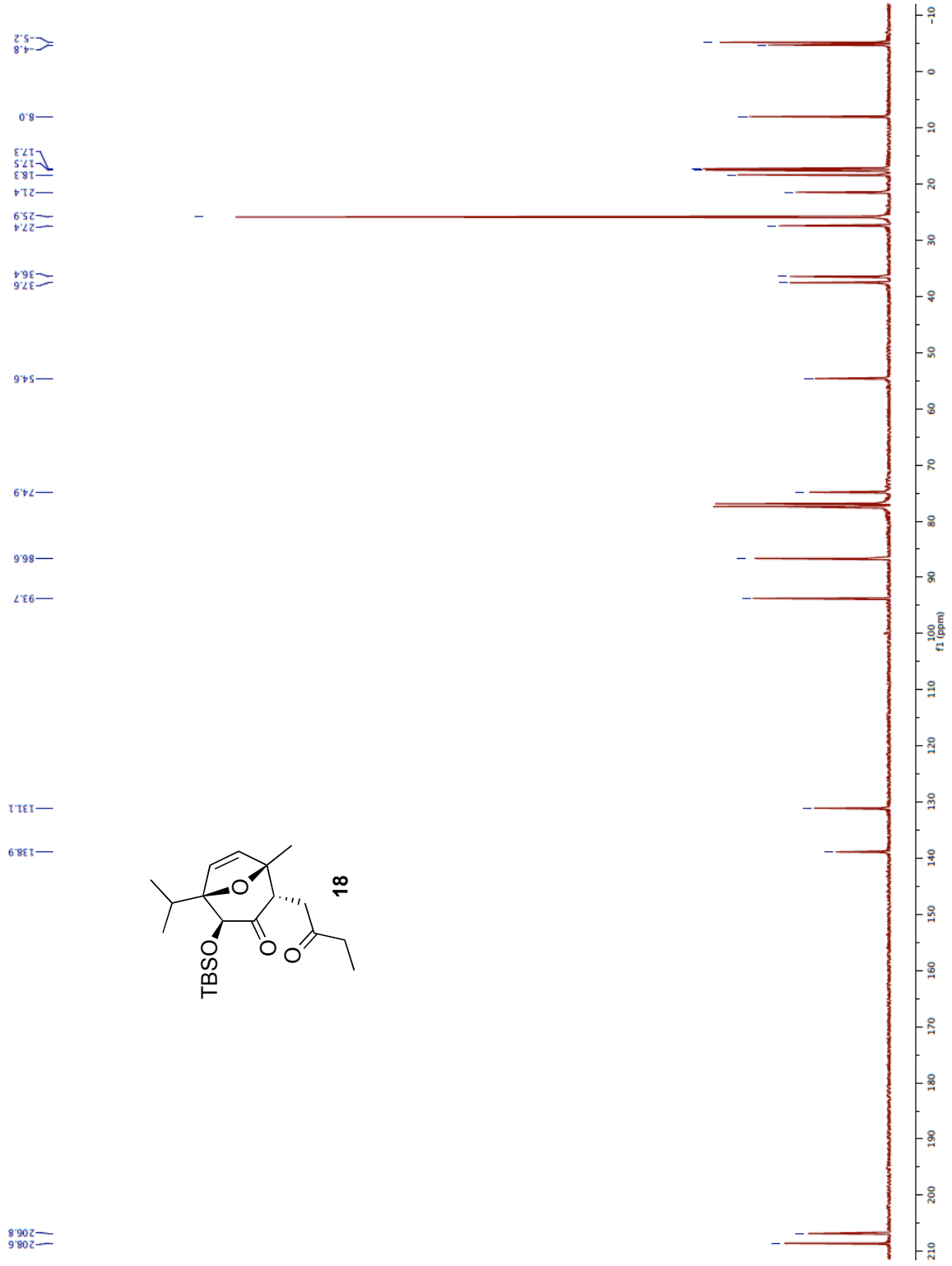
Spectrum 1.18 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **35**



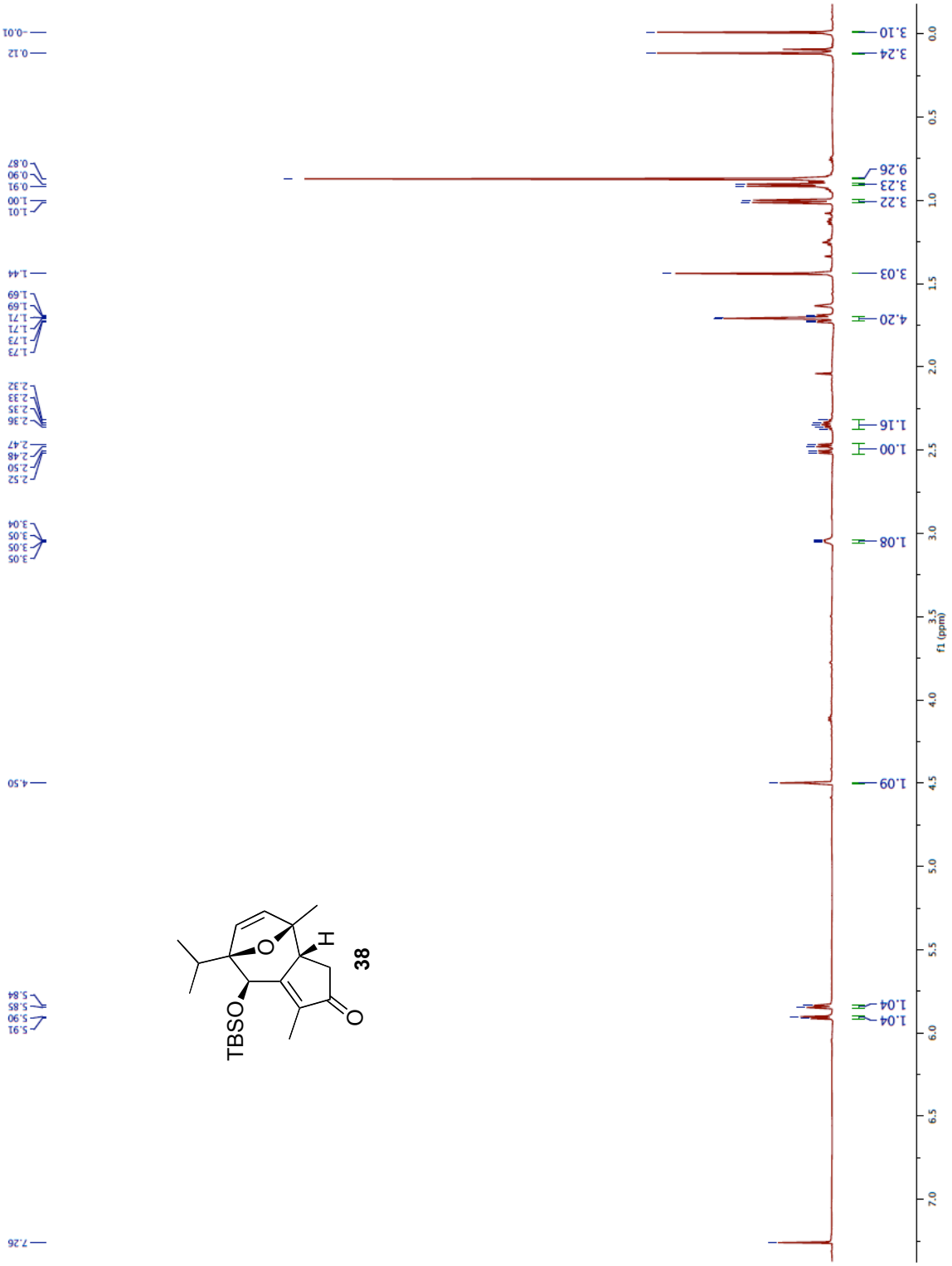
Spectrum 1.19 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **36**



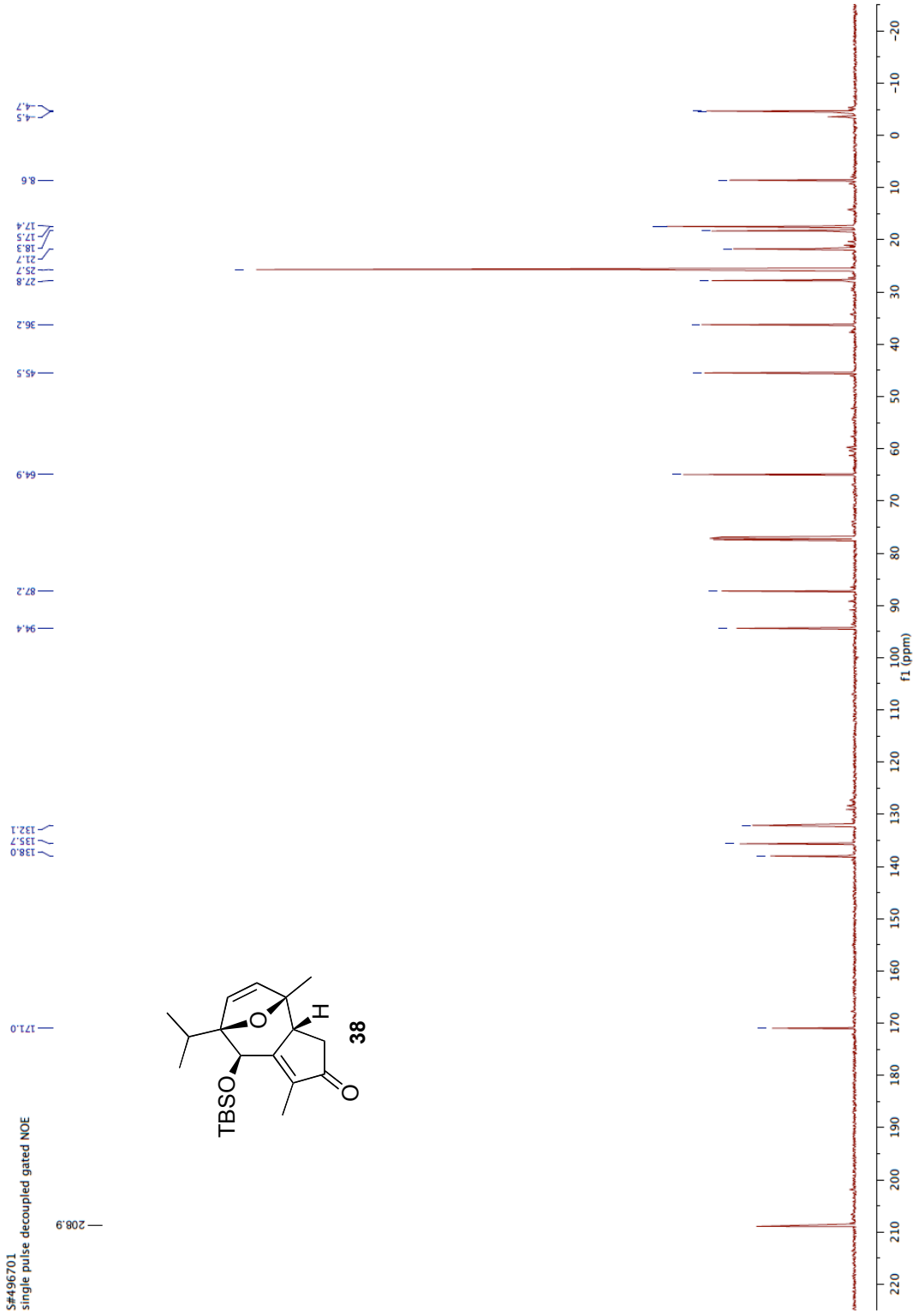
Spectrum 1.20 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **18**



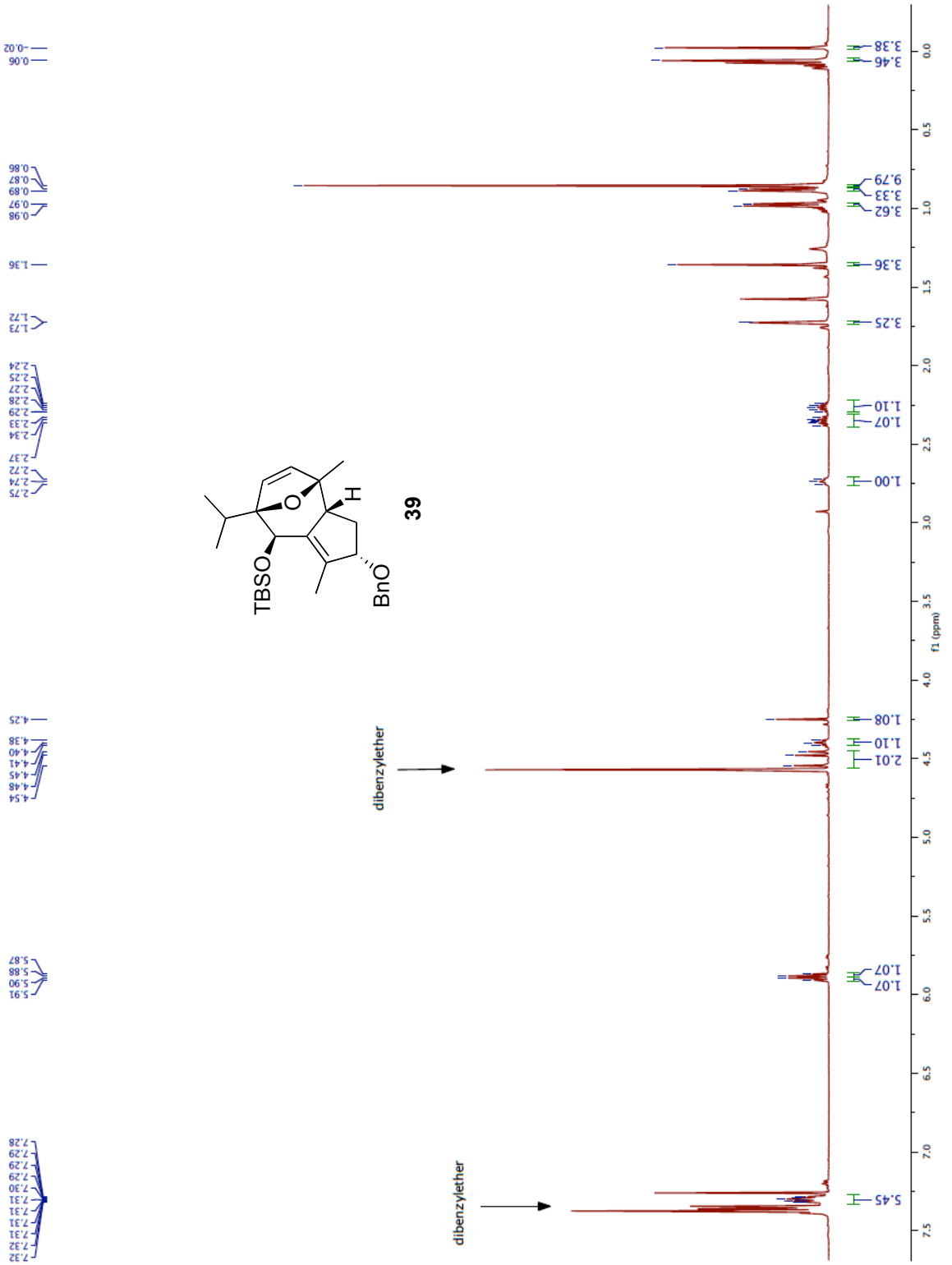
Spectrum 1.21 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **18**



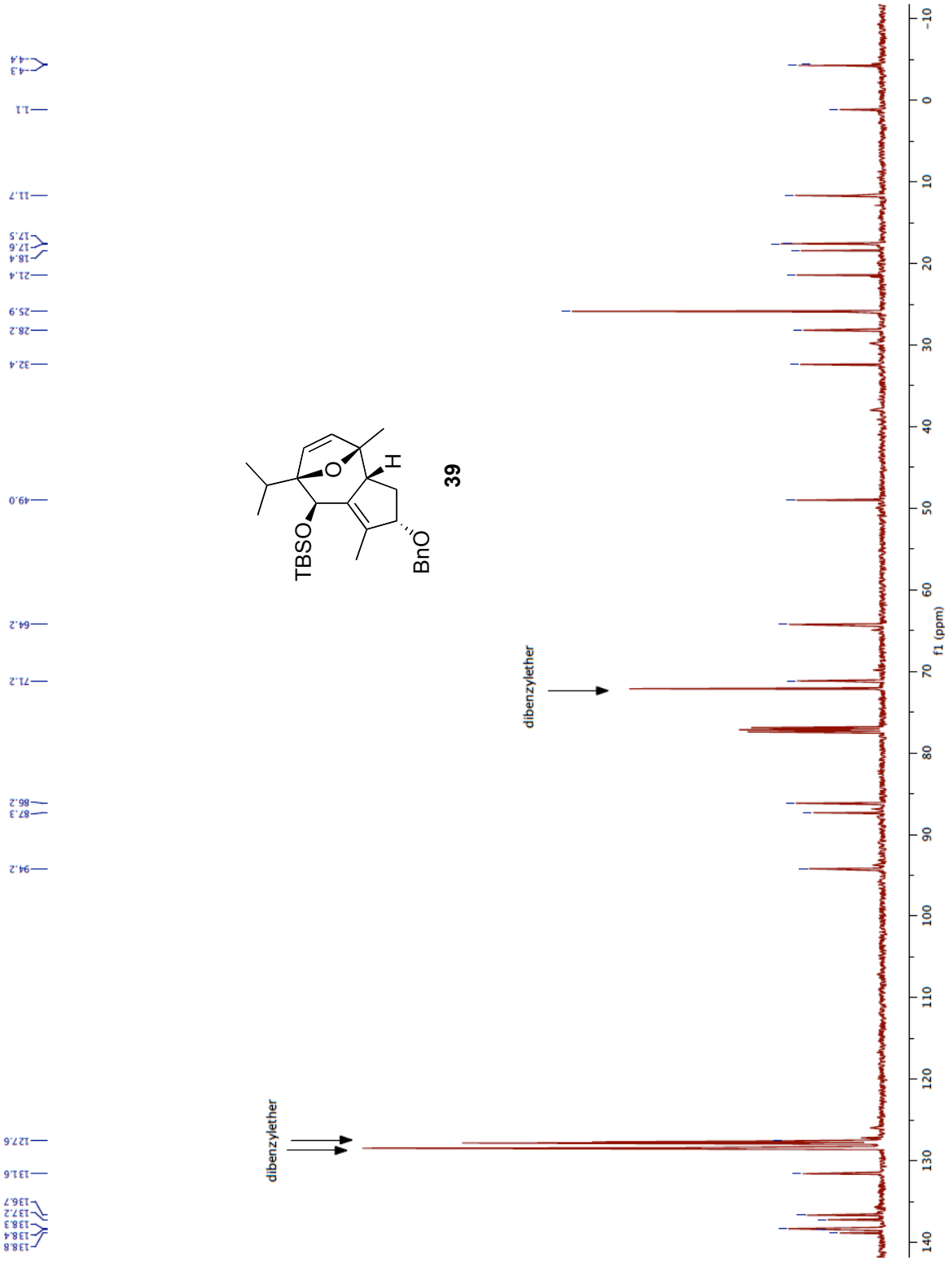
Spectrum 1.22 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **38**



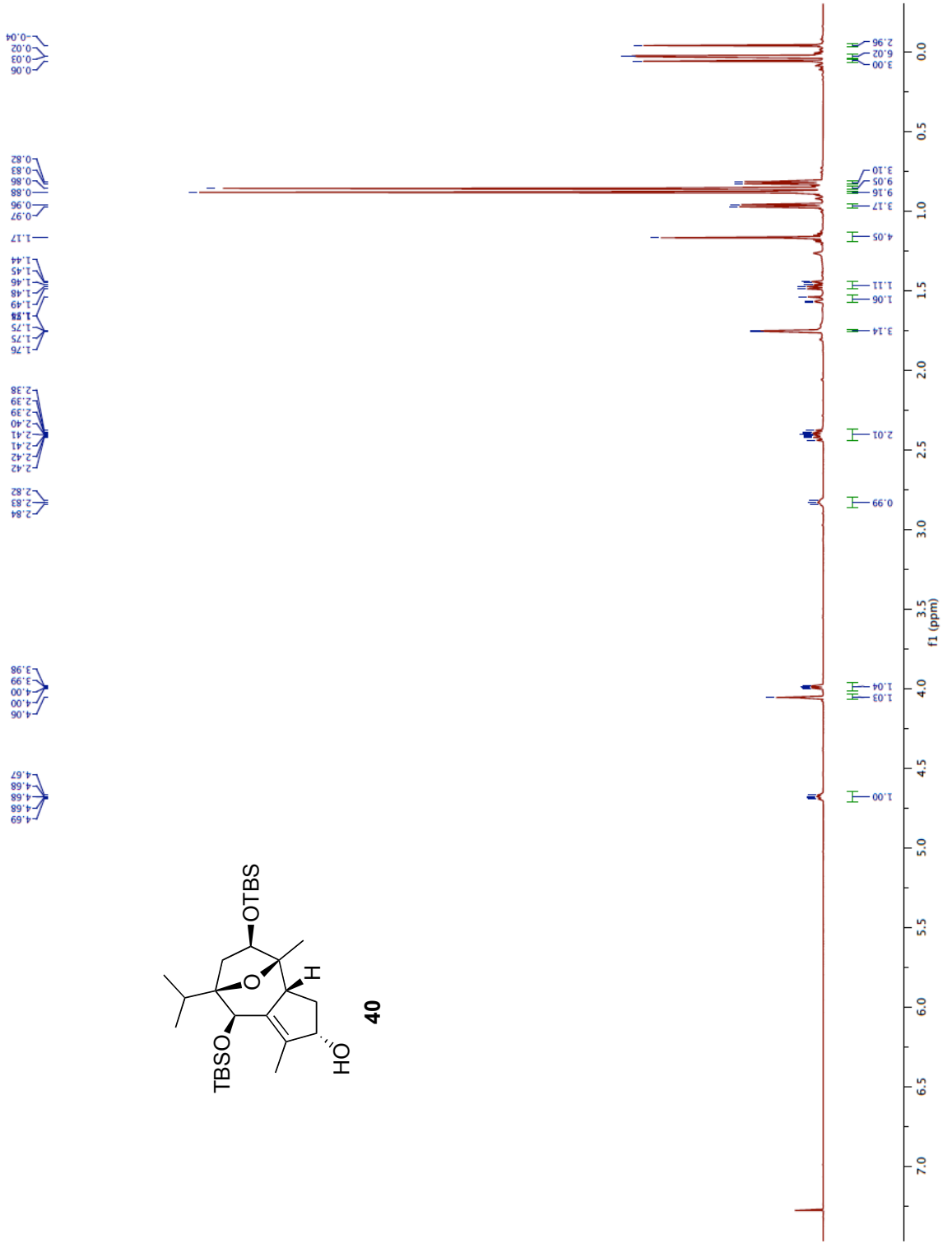
Spectrum 1.23 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **38**



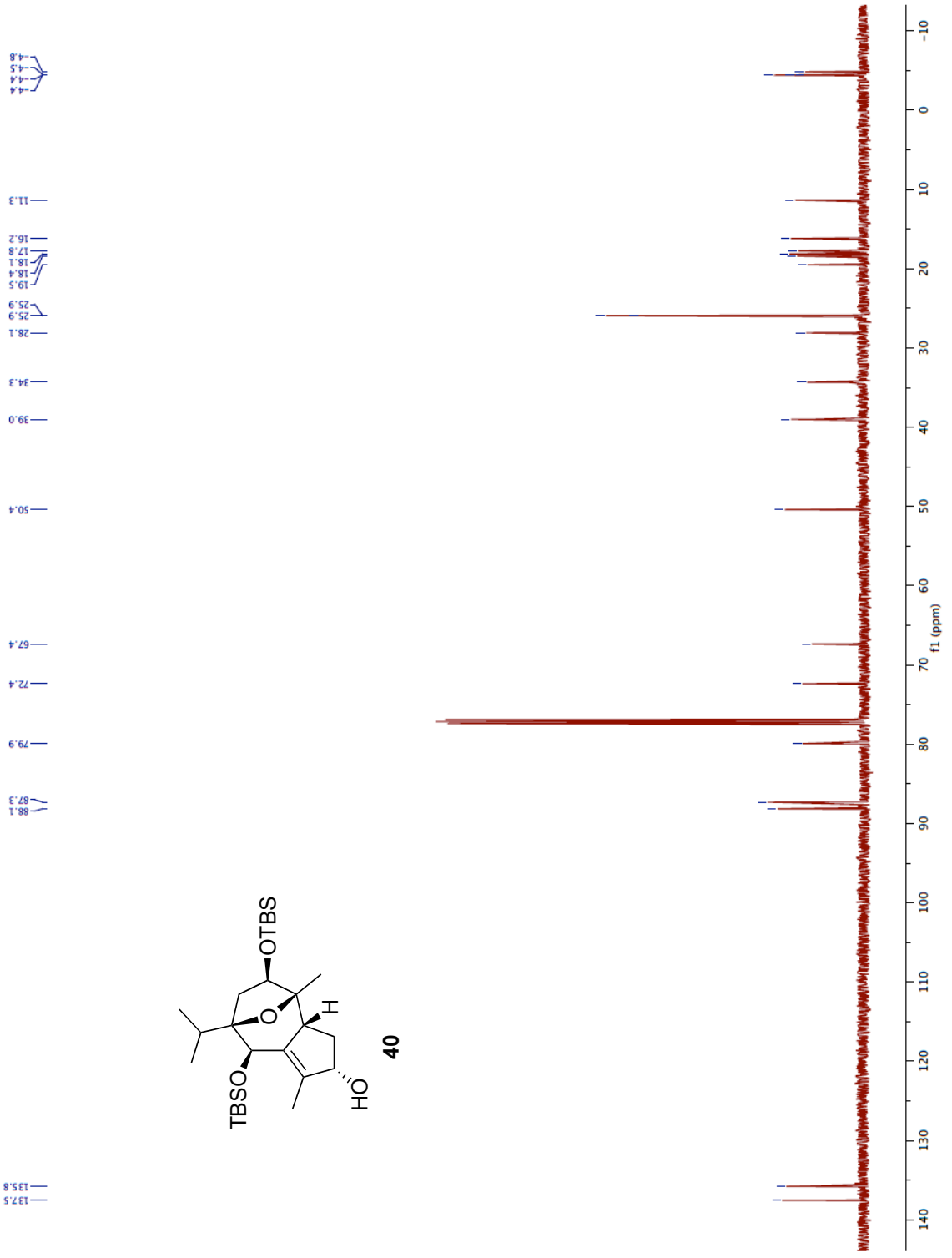
Spectrum 1.24 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **39**



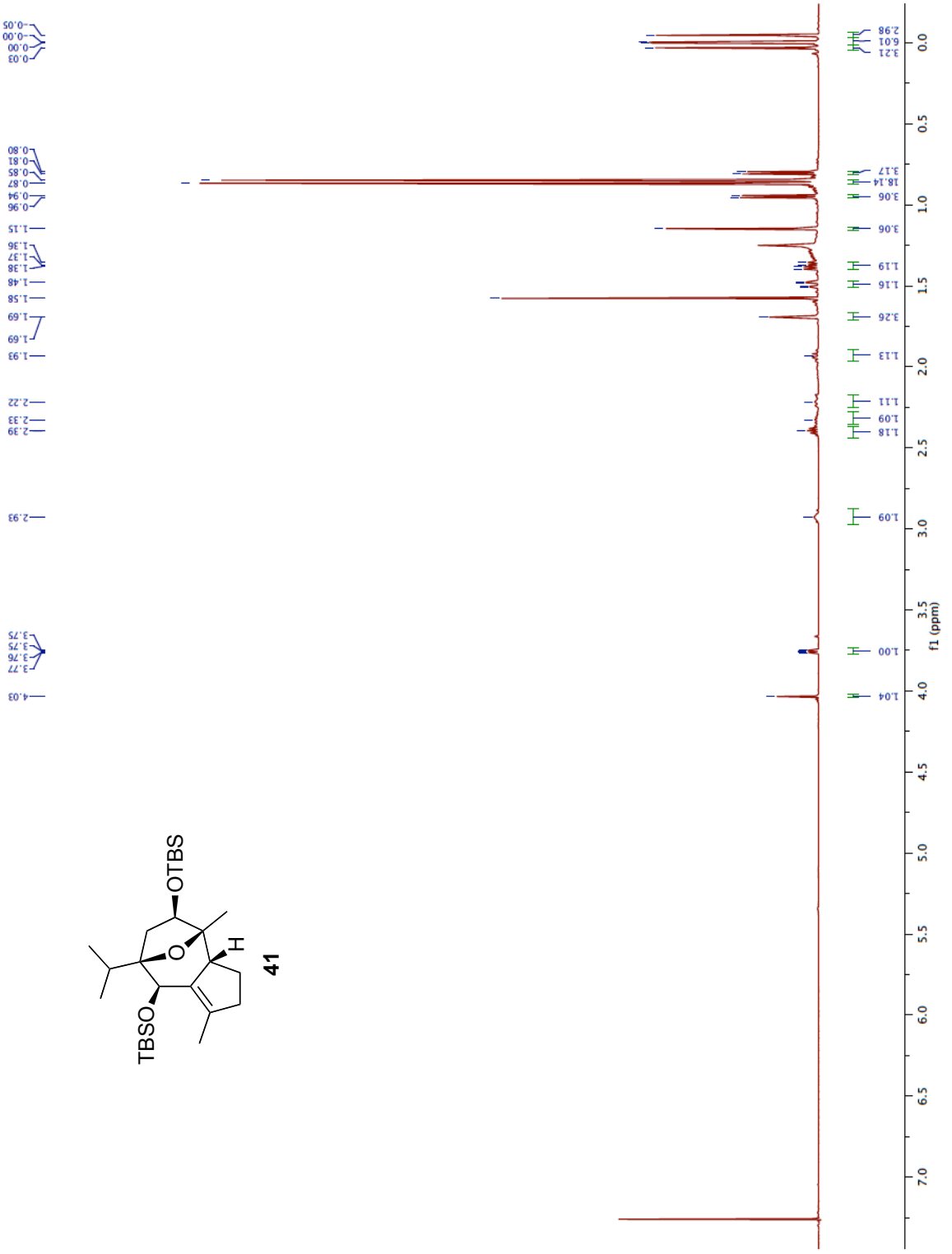
Spectrum 1.25 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **39**



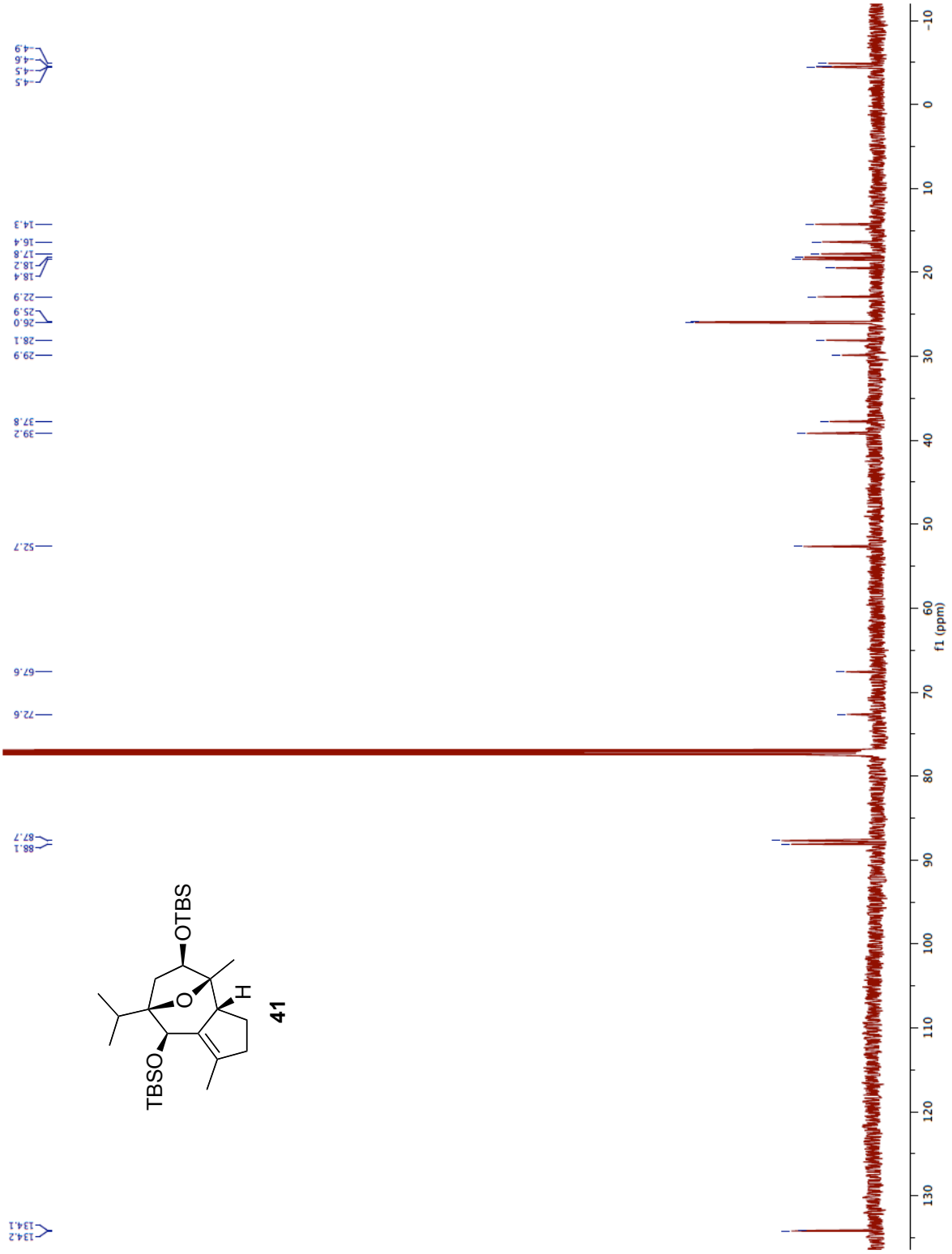
Spectrum 1.26 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **40**



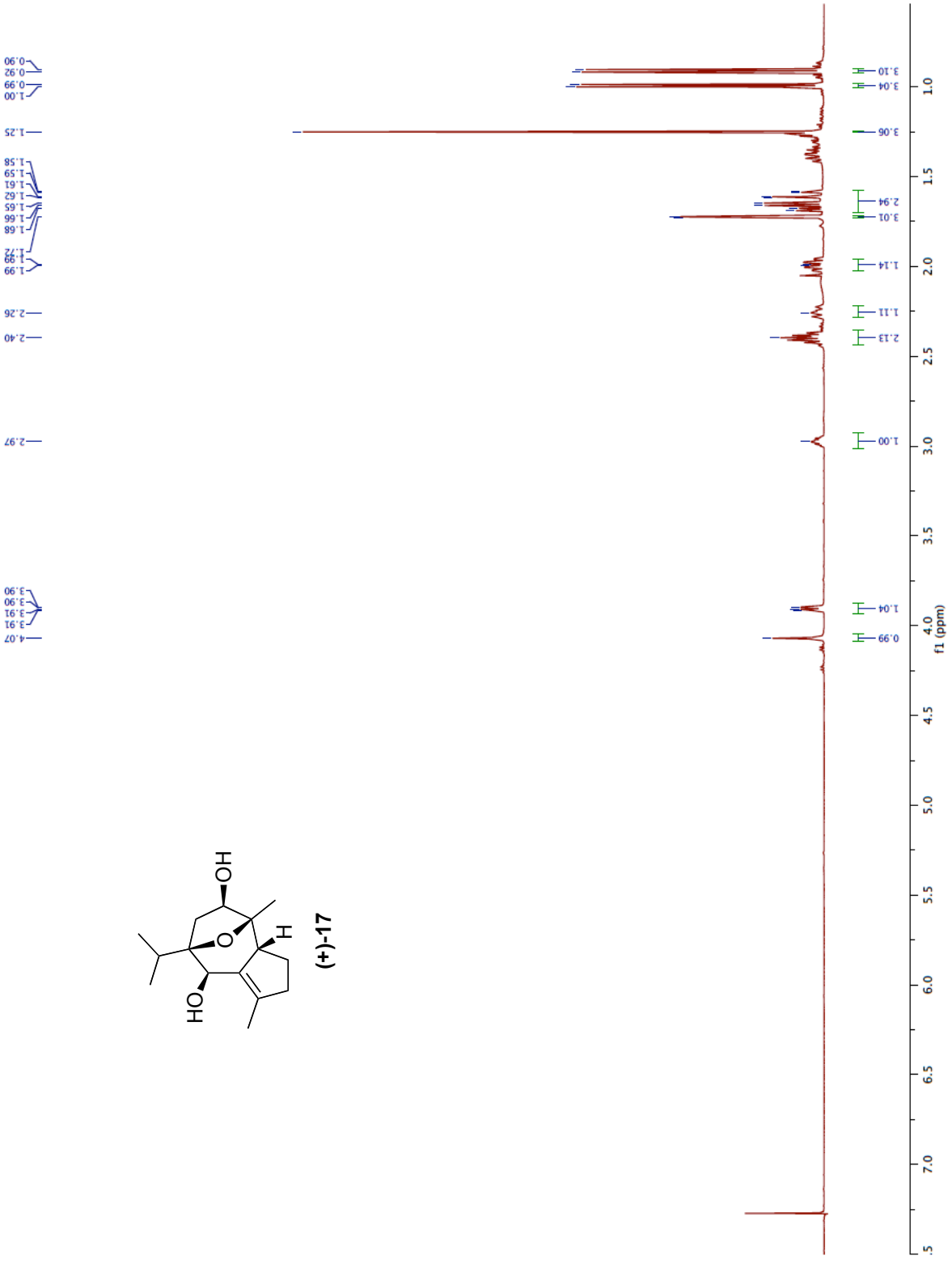
Spectrum 1.27 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **40**



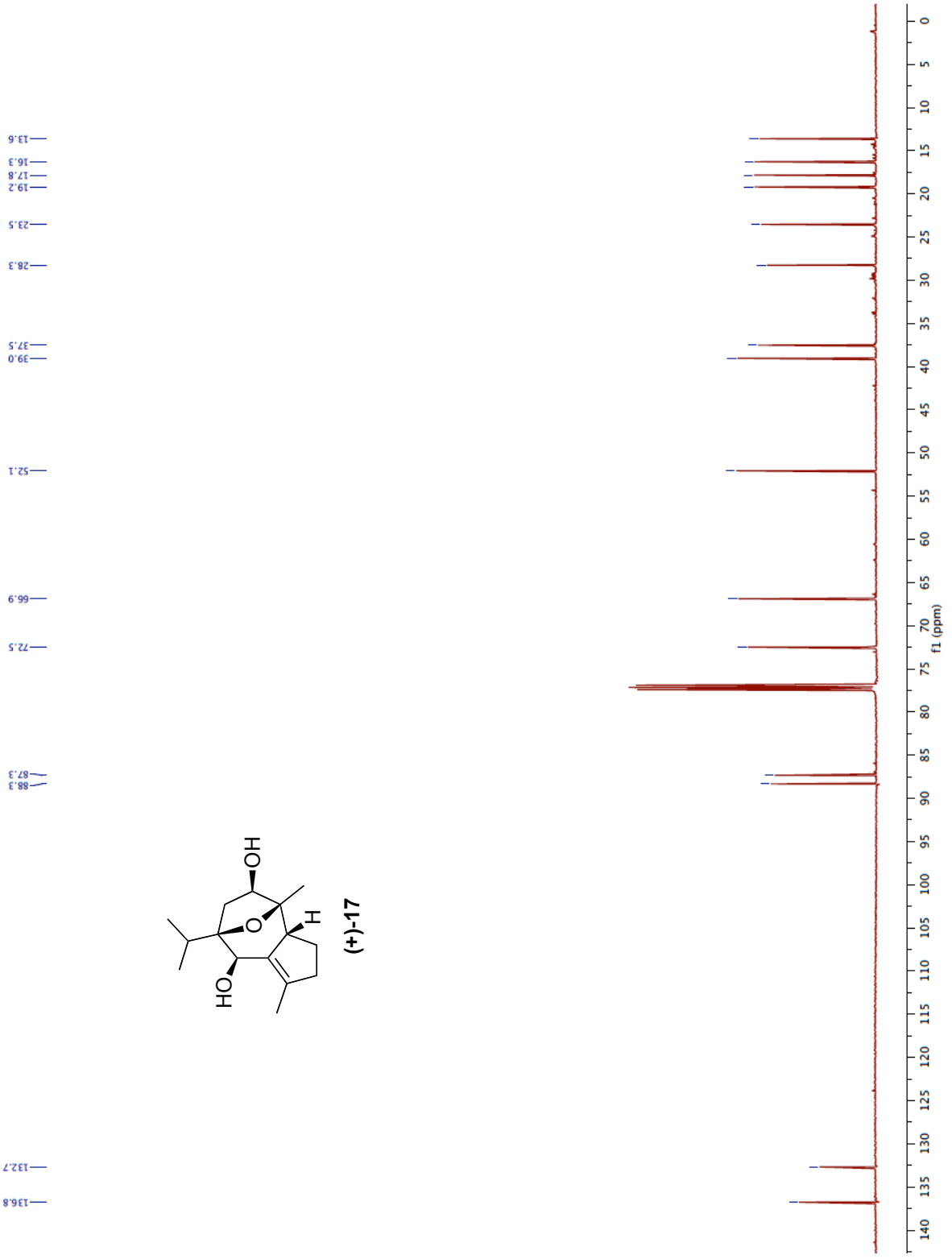
Spectrum 1.28 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **41**



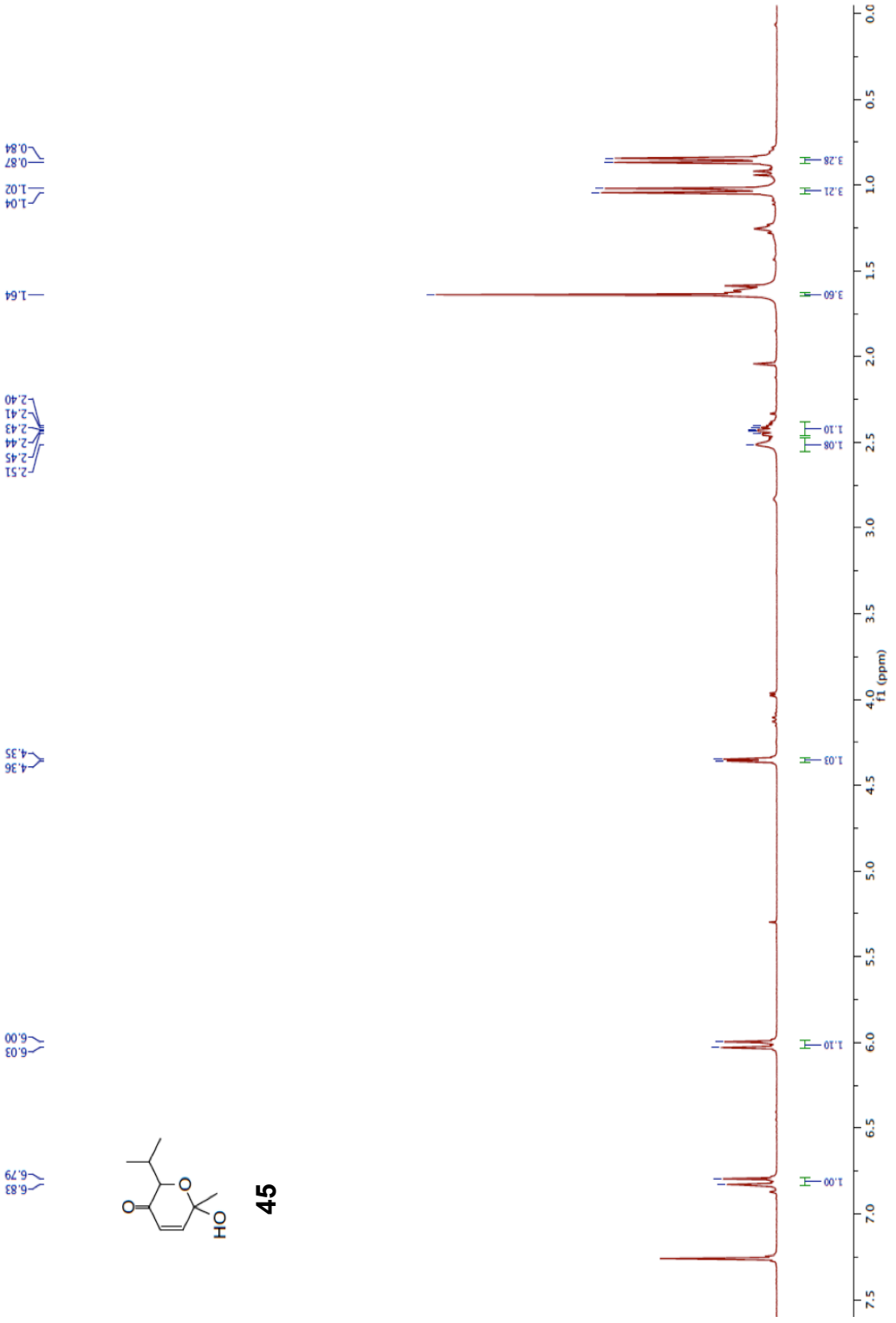
Spectrum 1.29 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **41**



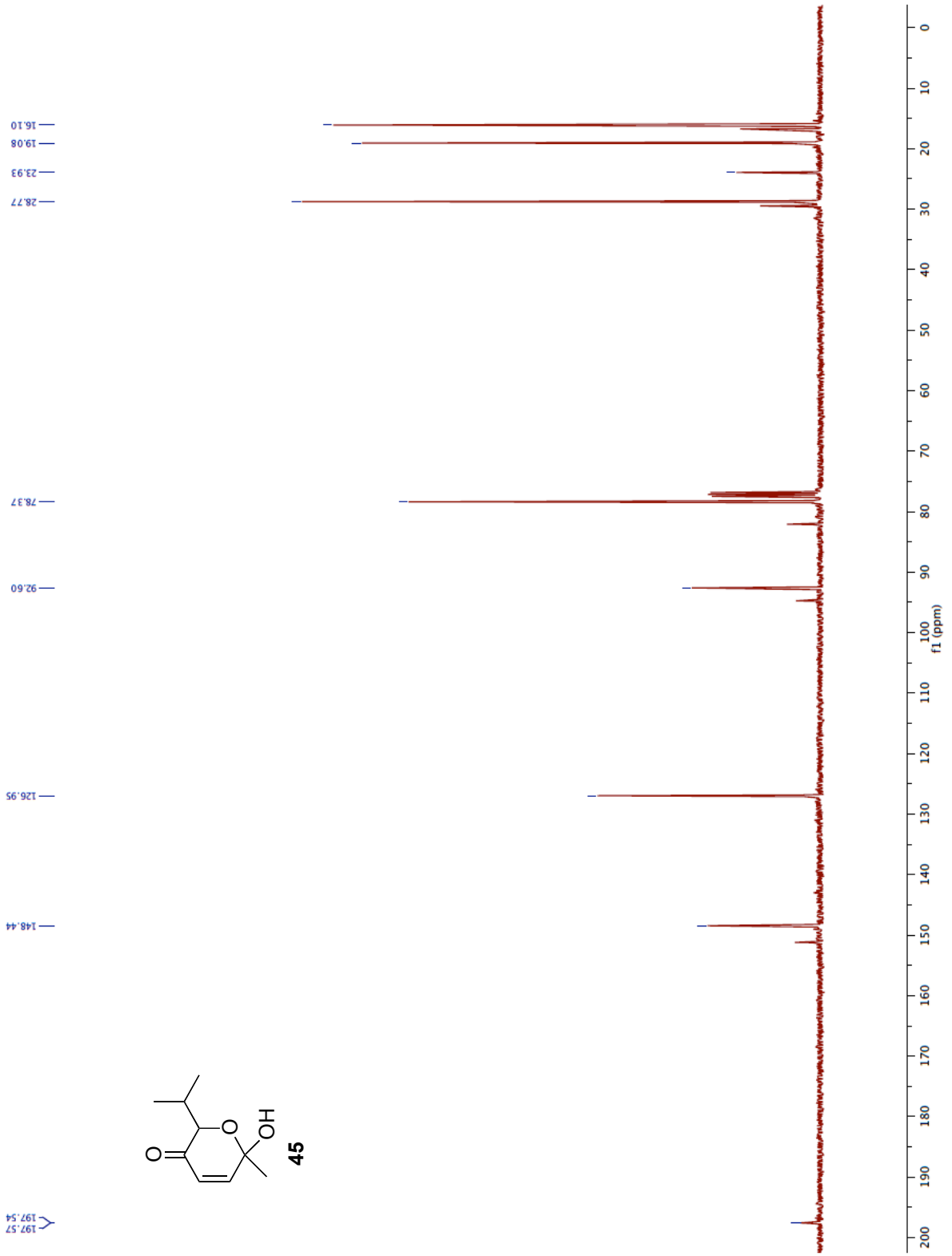
Spectrum 1.30 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **17**



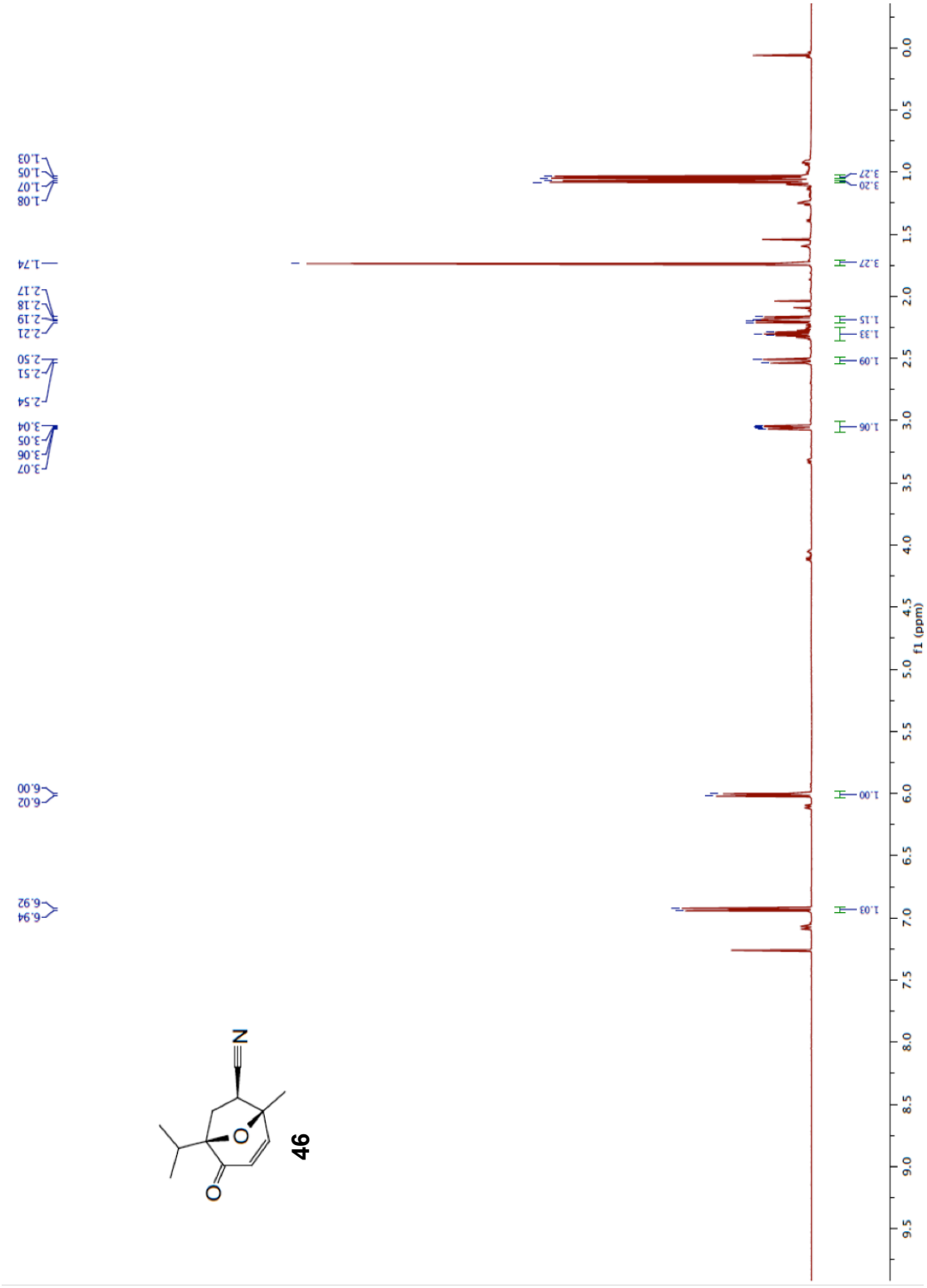
Spectrum 1.31 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **17**



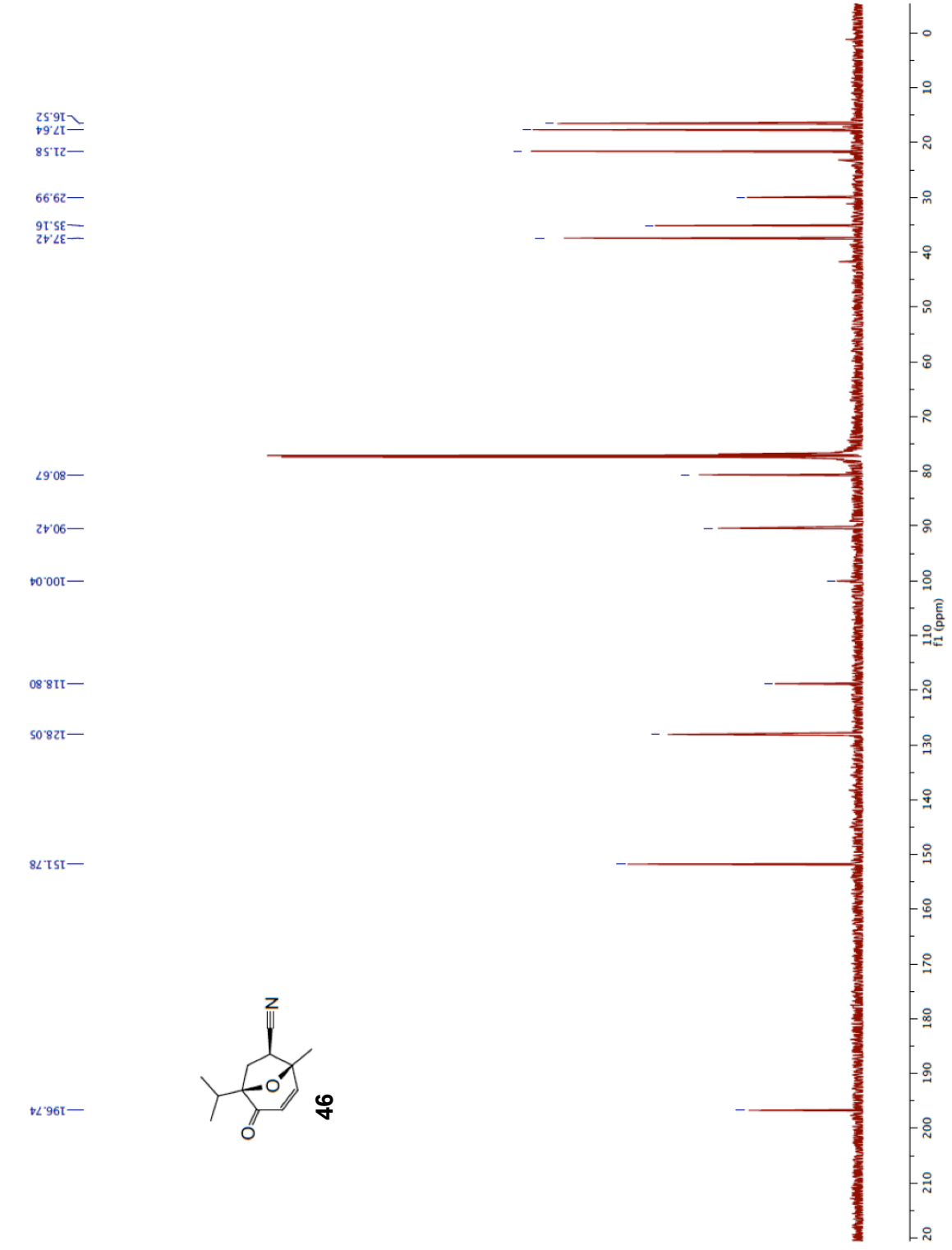
Spectrum 1.32 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **45**



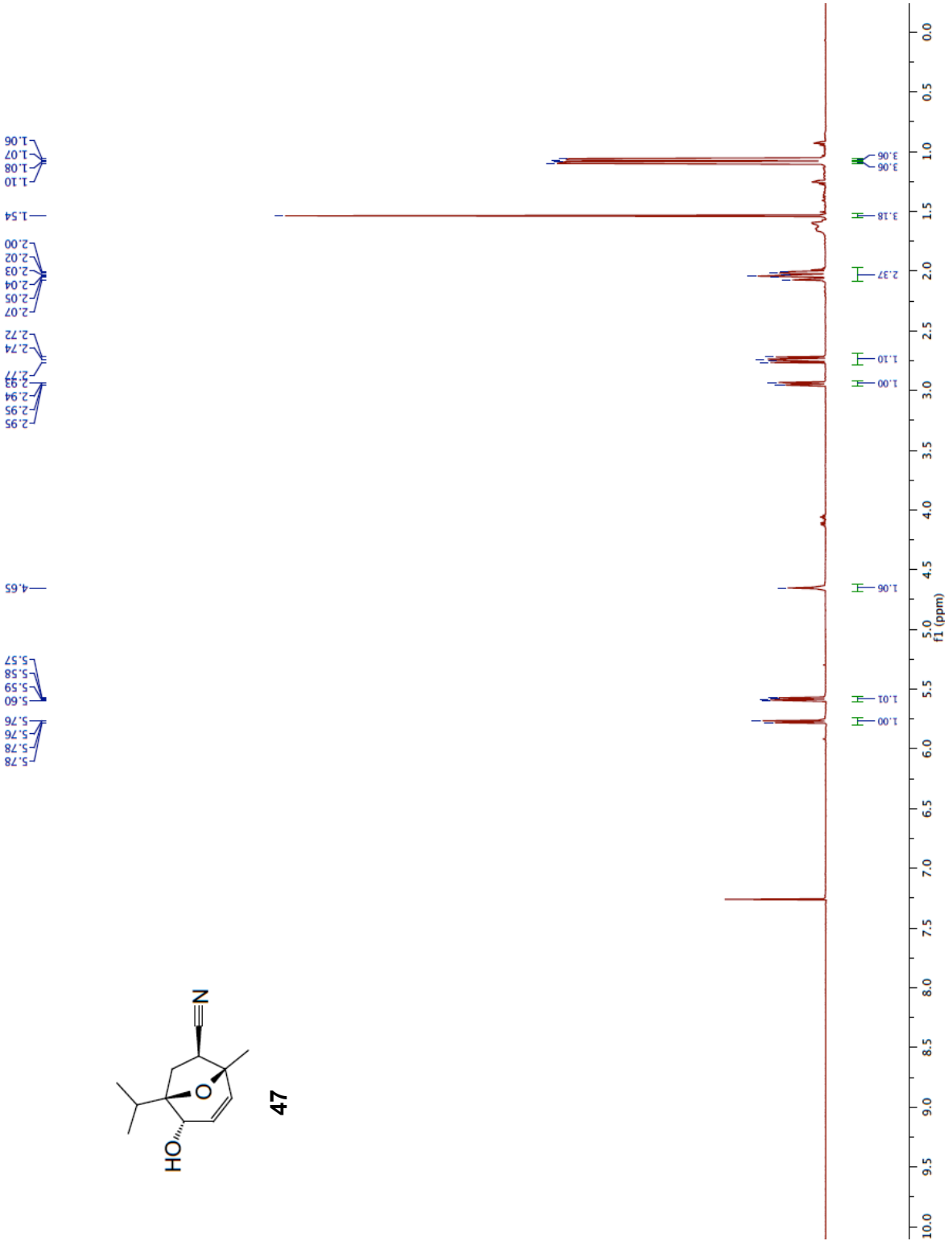
Spectrum 1.33 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **45**



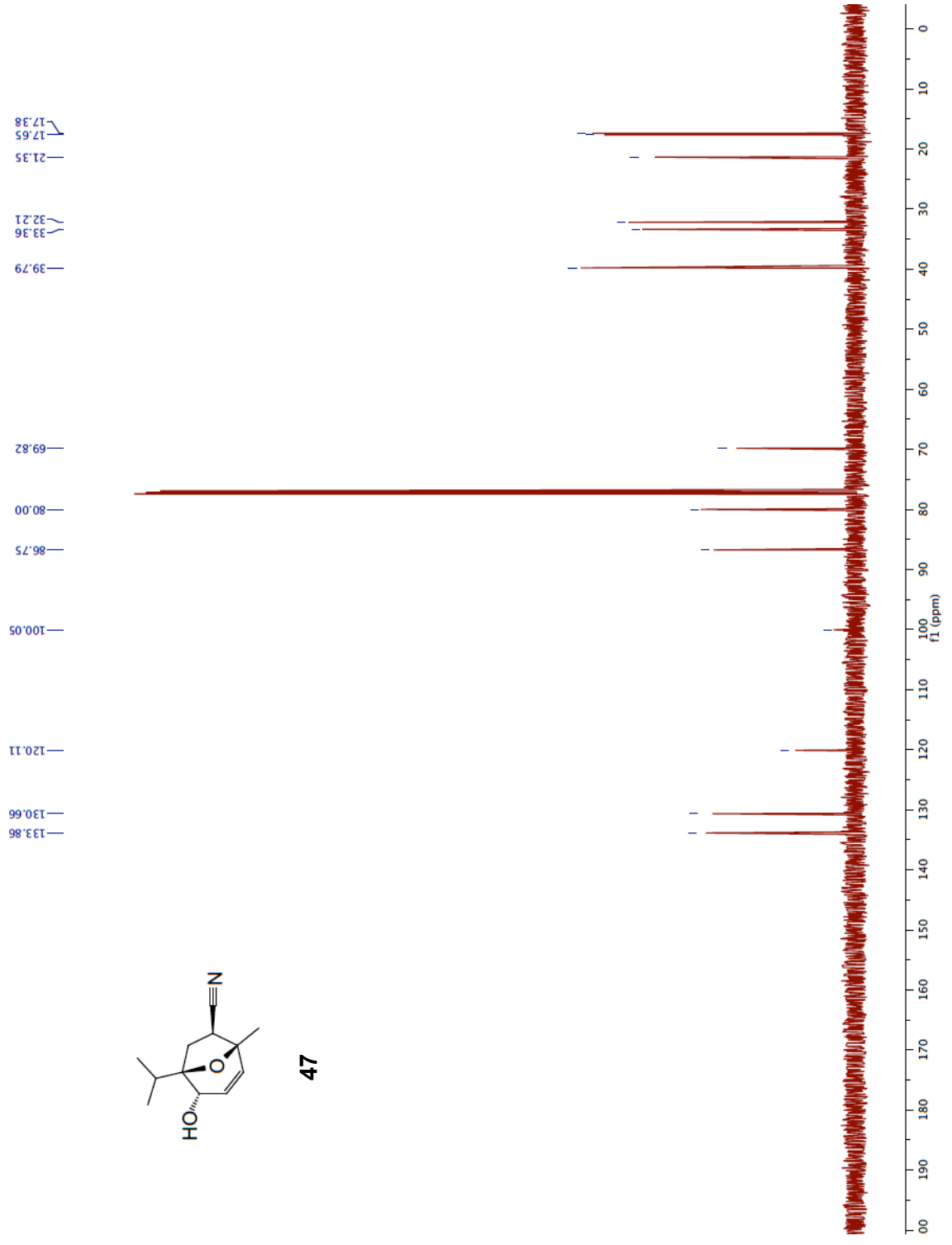
Spectrum 1.34 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **46**



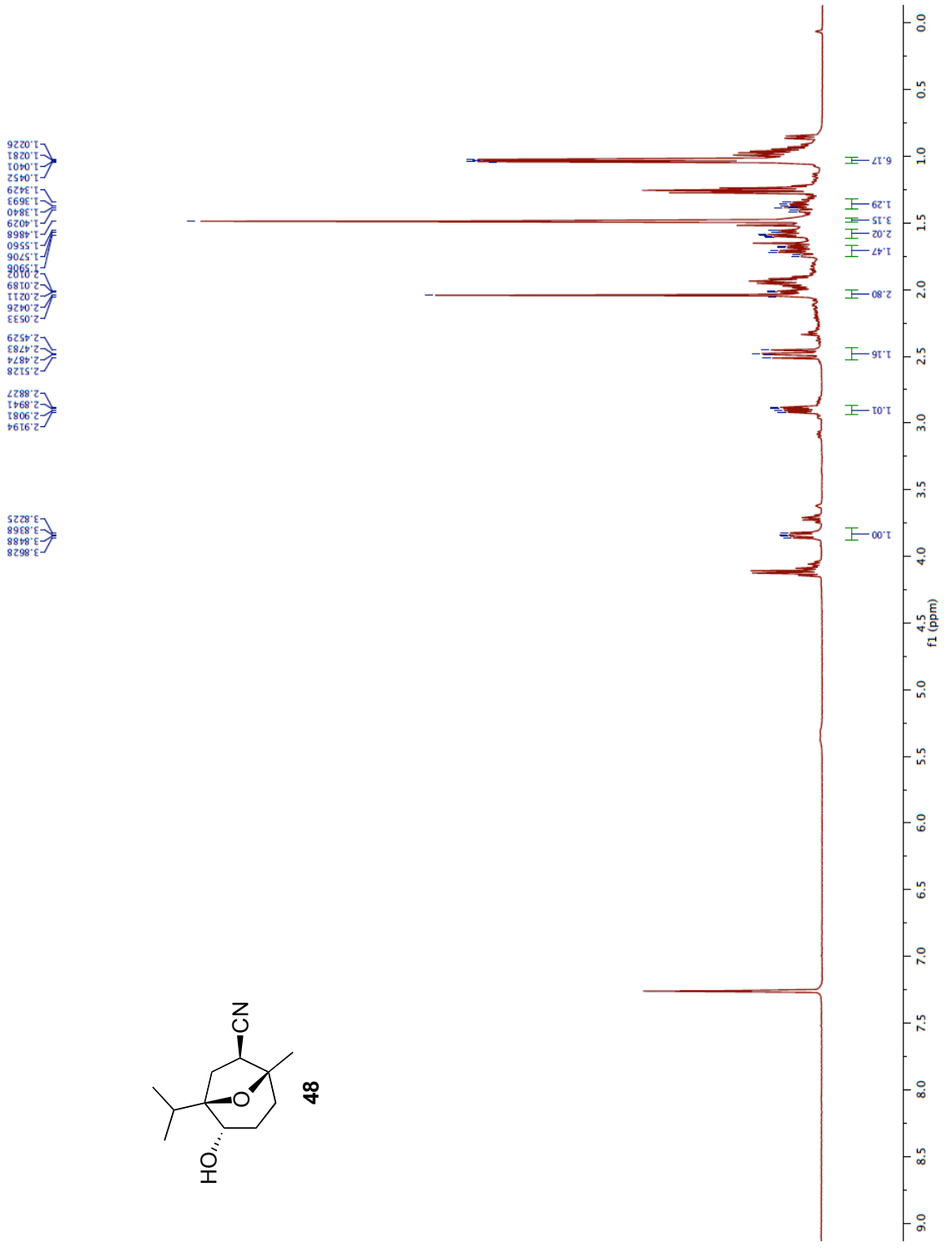
Spectrum 1.35 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **46**



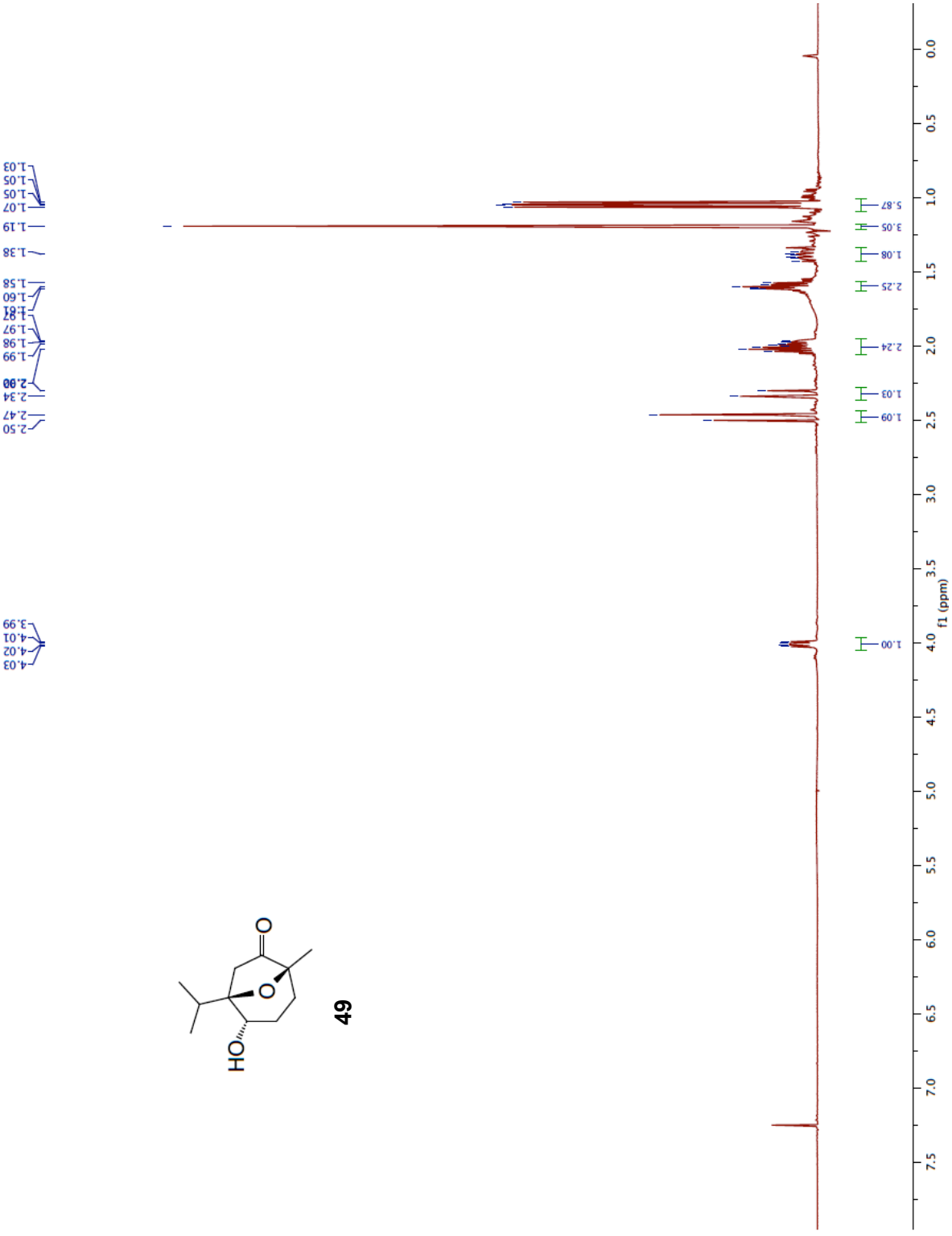
Spectrum 1.36 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **47**



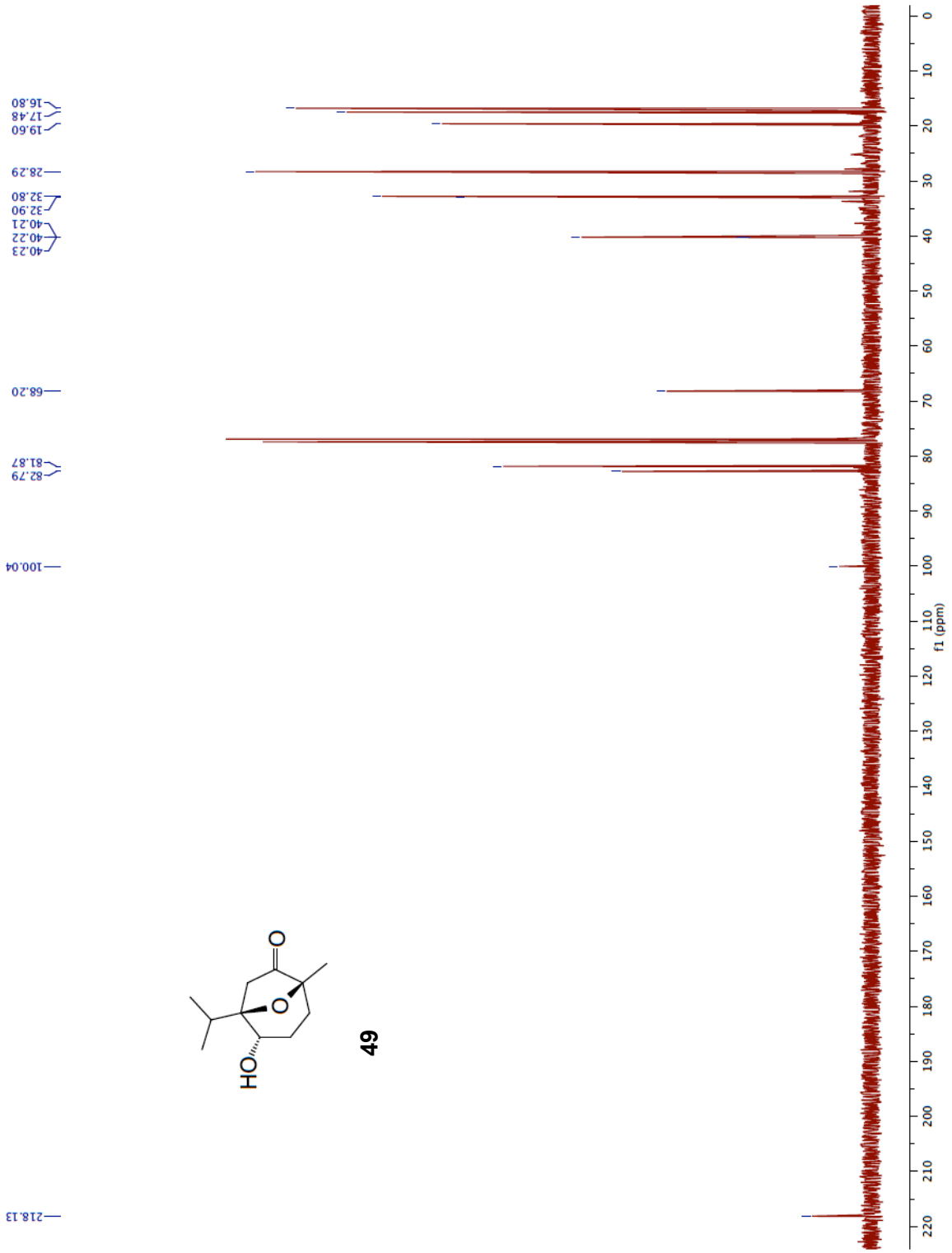
Spectrum 1.37 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **47**



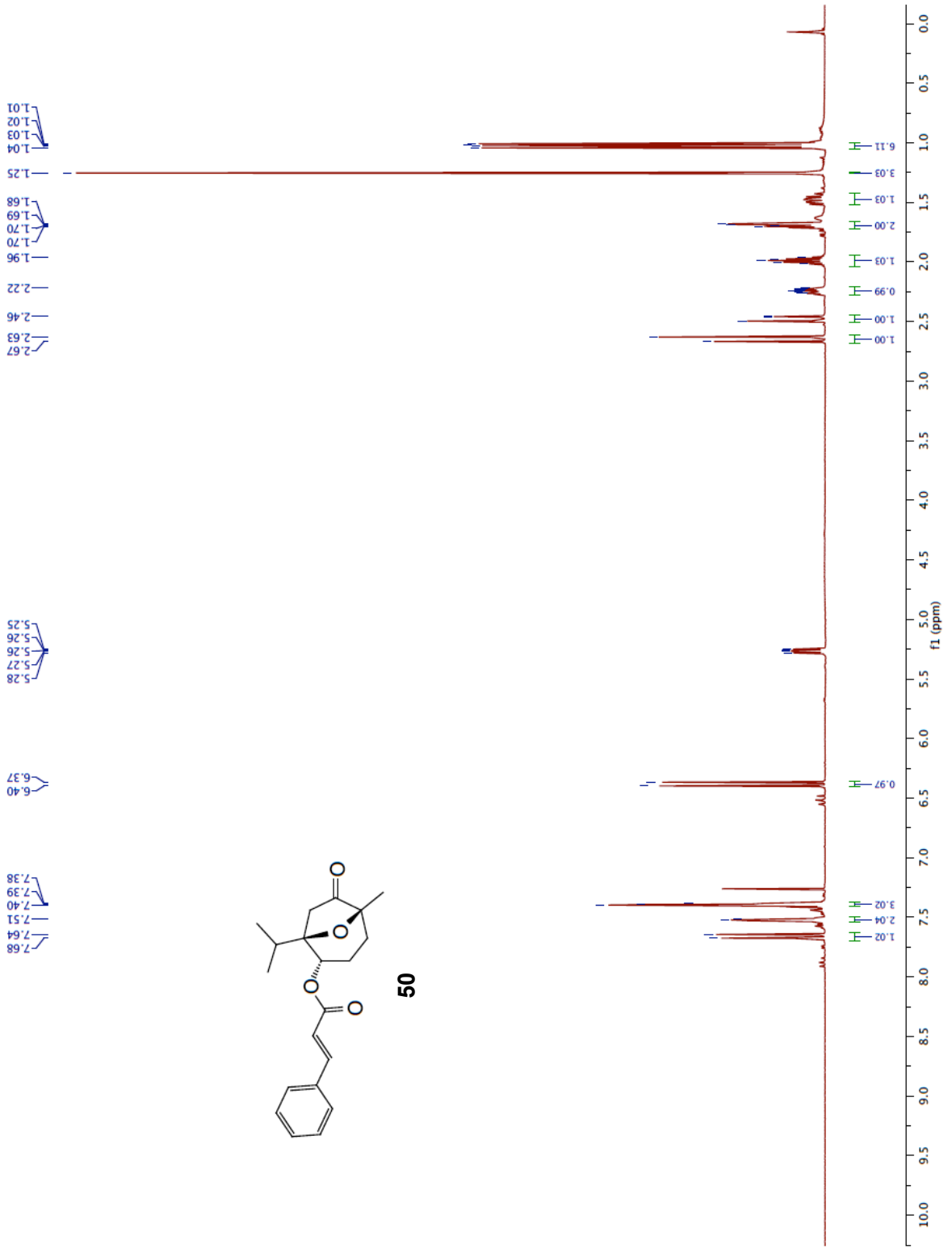
Spectrum 1.38 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **48**



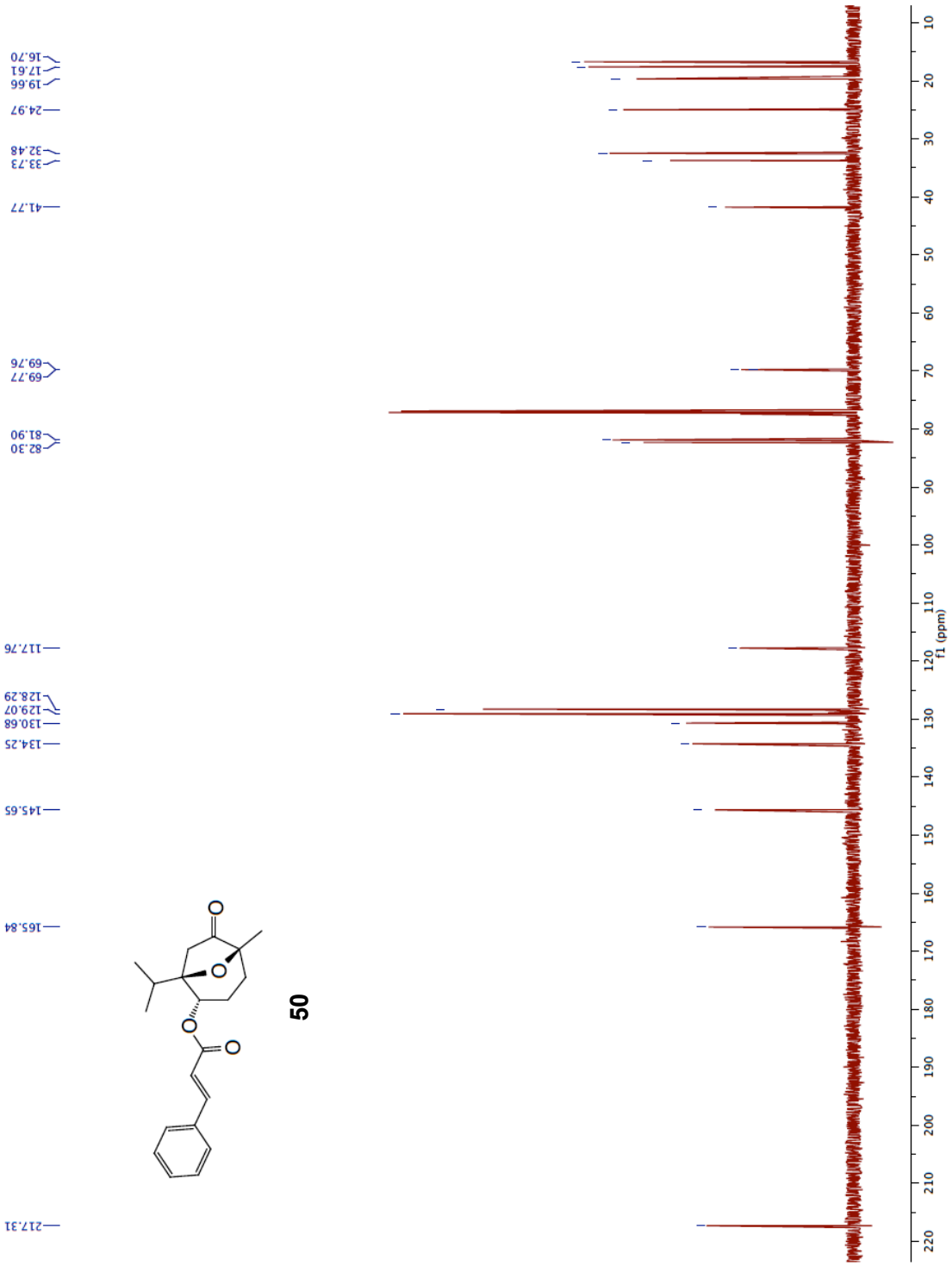
Spectrum 1.39 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **49**



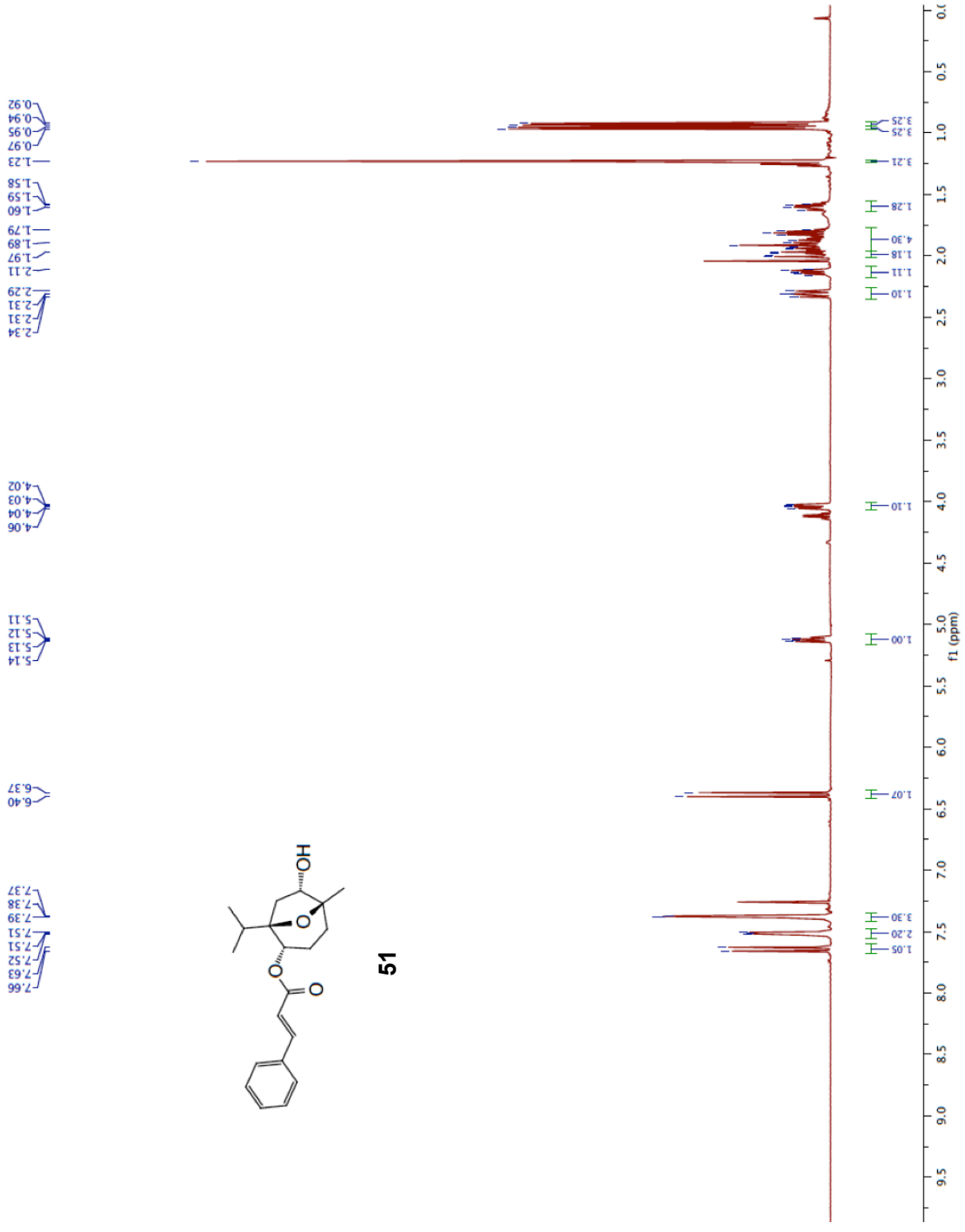
Spectrum 1.40 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **49**



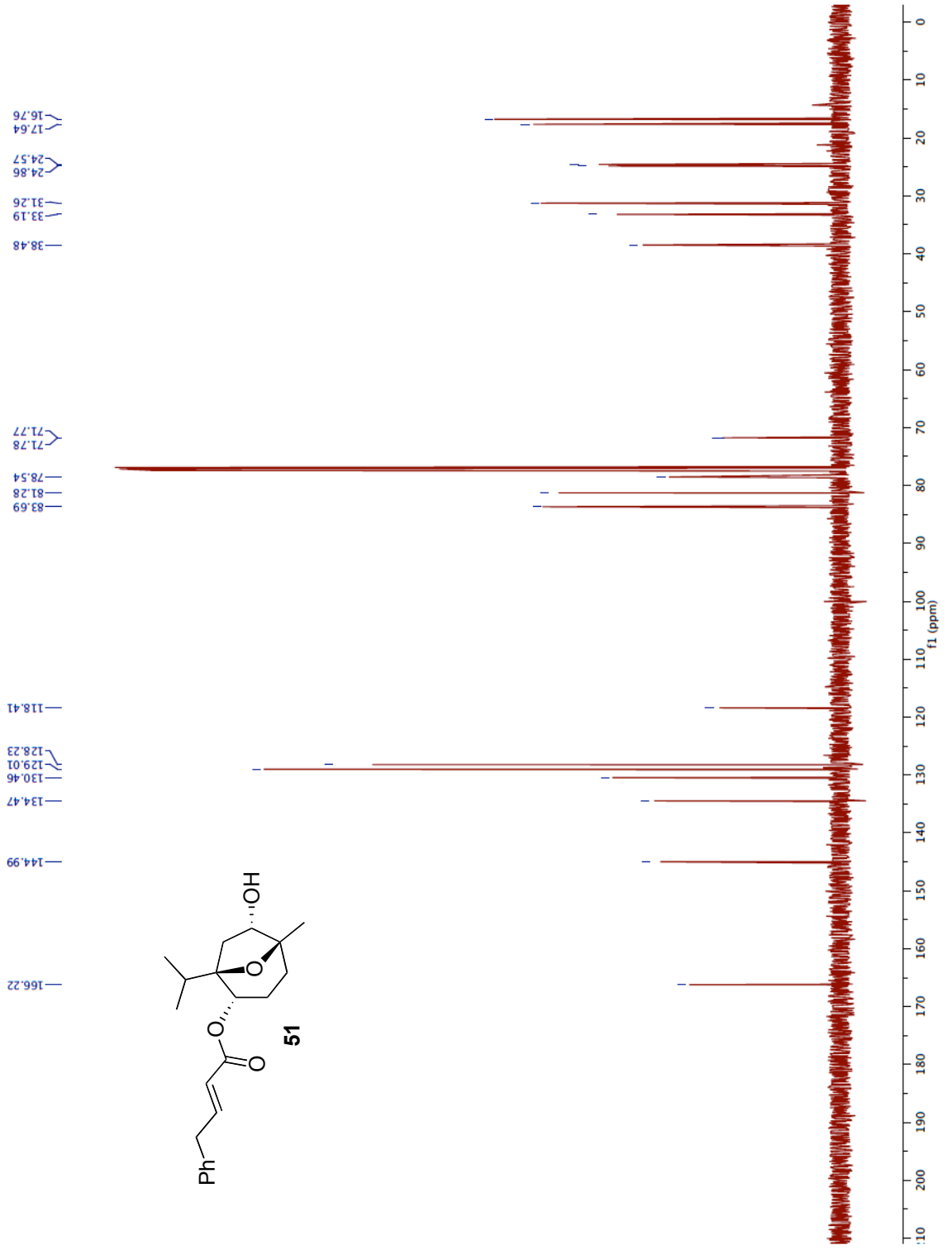
Spectrum 1.41 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **50**



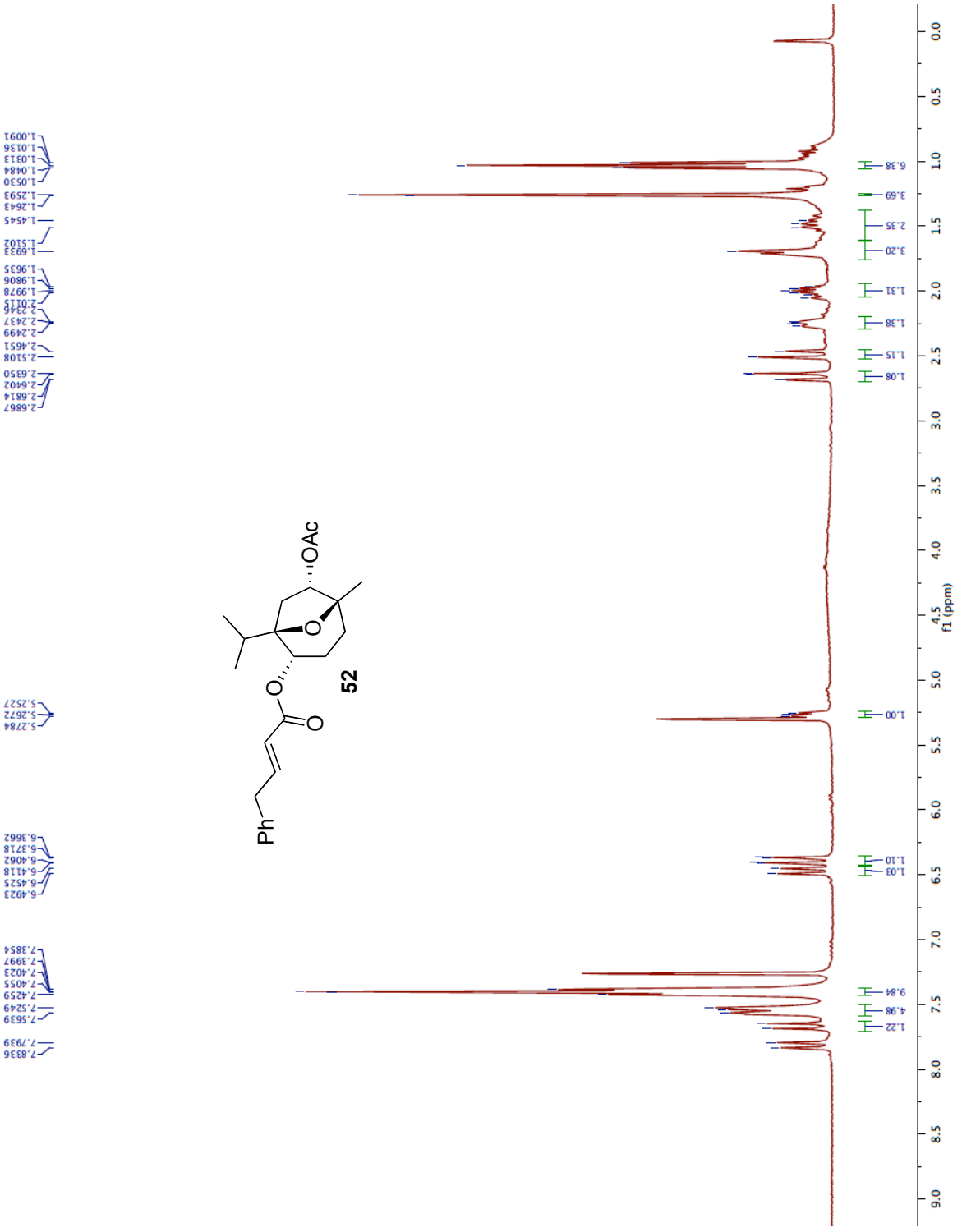
Spectrum 1.42 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **50**



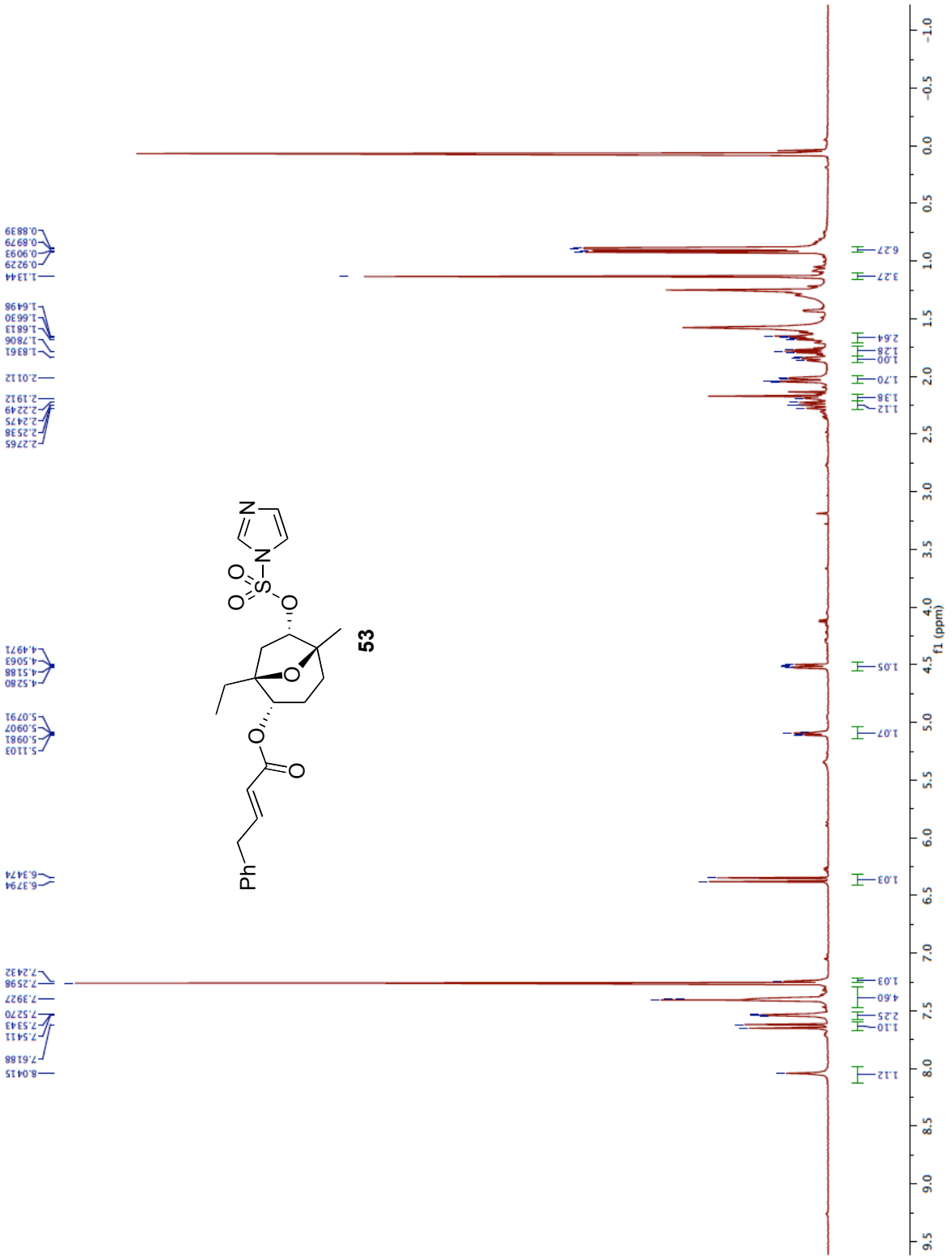
Spectrum 1.43 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **51**



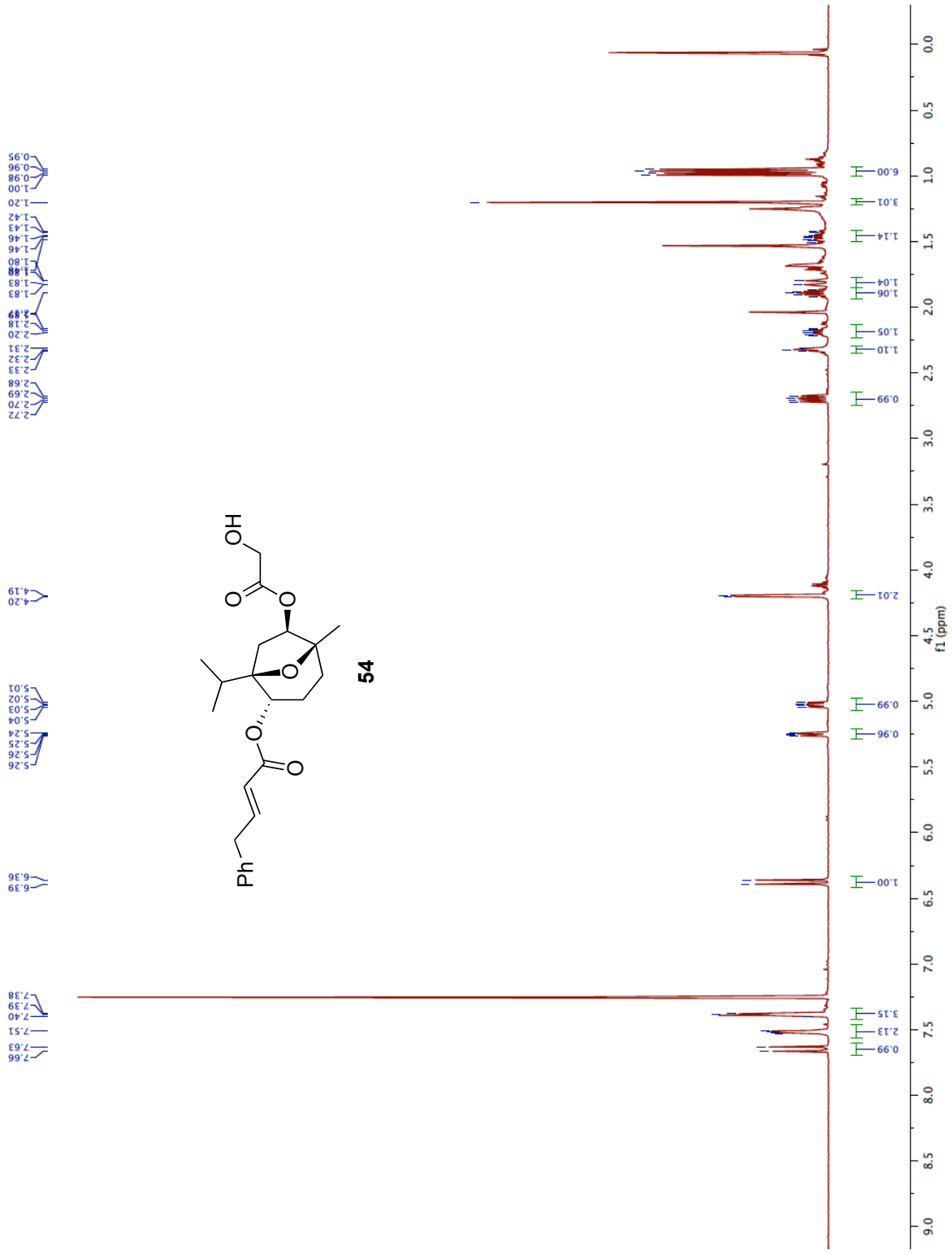
Spectrum 1.44 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **51**



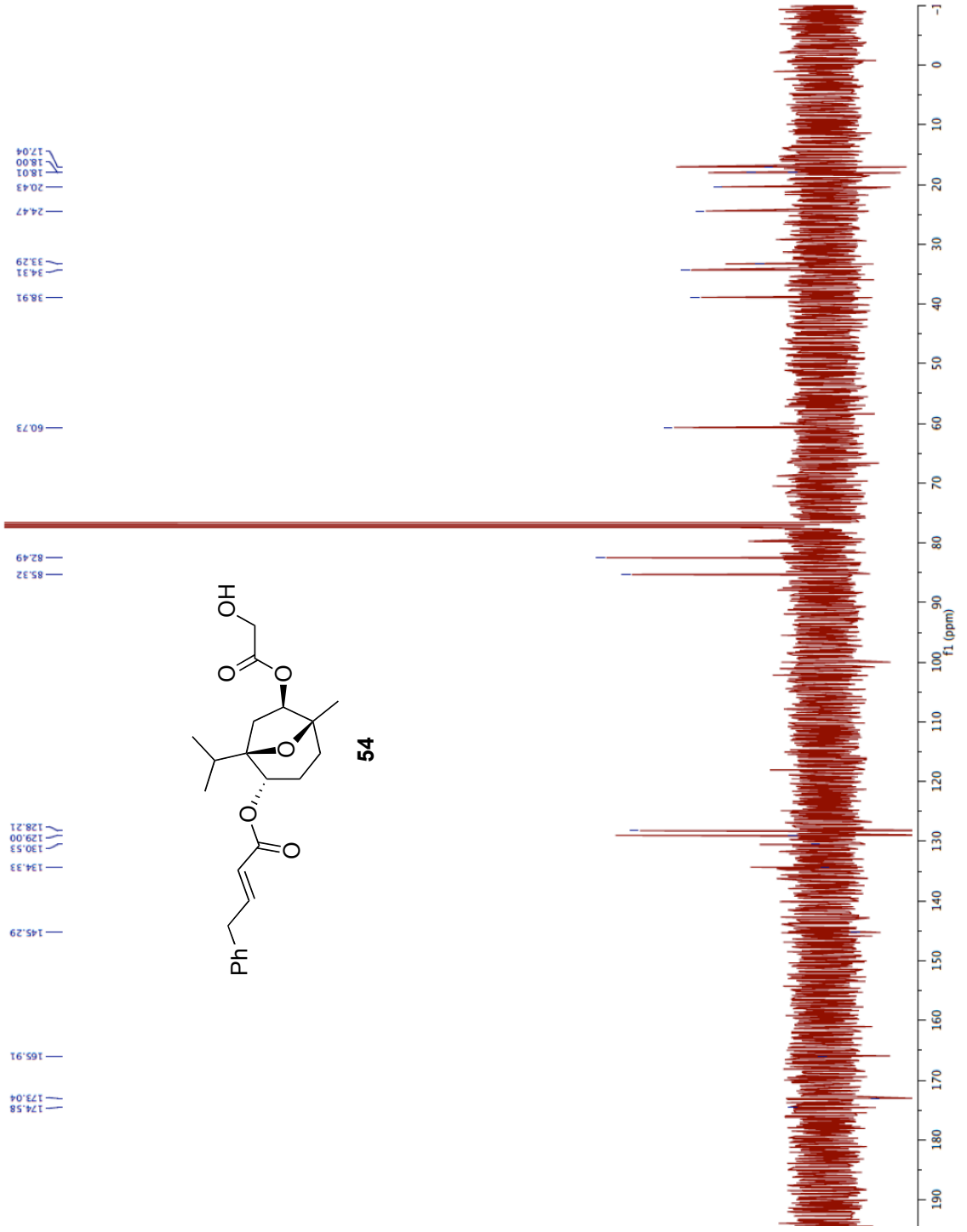
Spectrum 1.45 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **52**



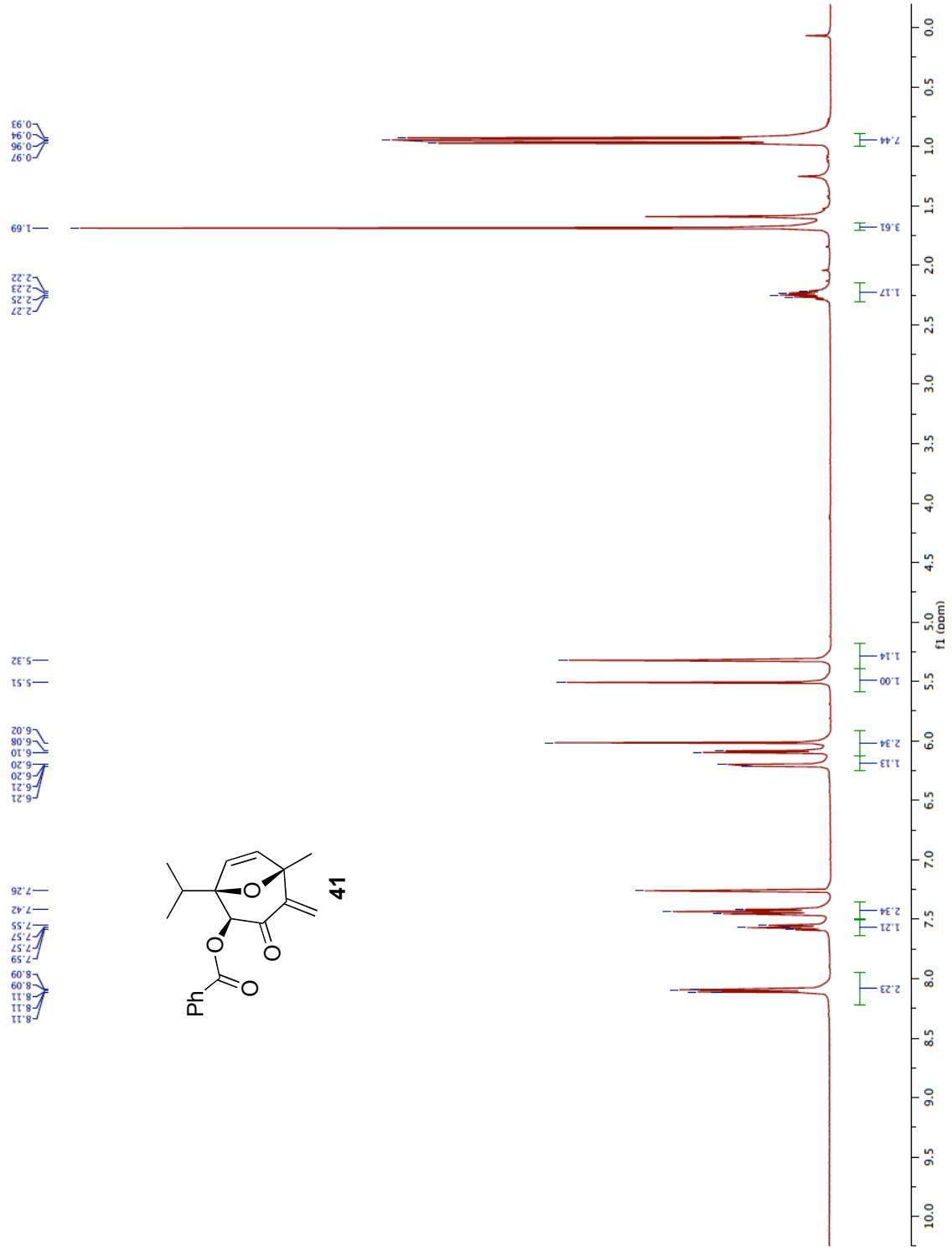
Spectrum 1.46 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **53**



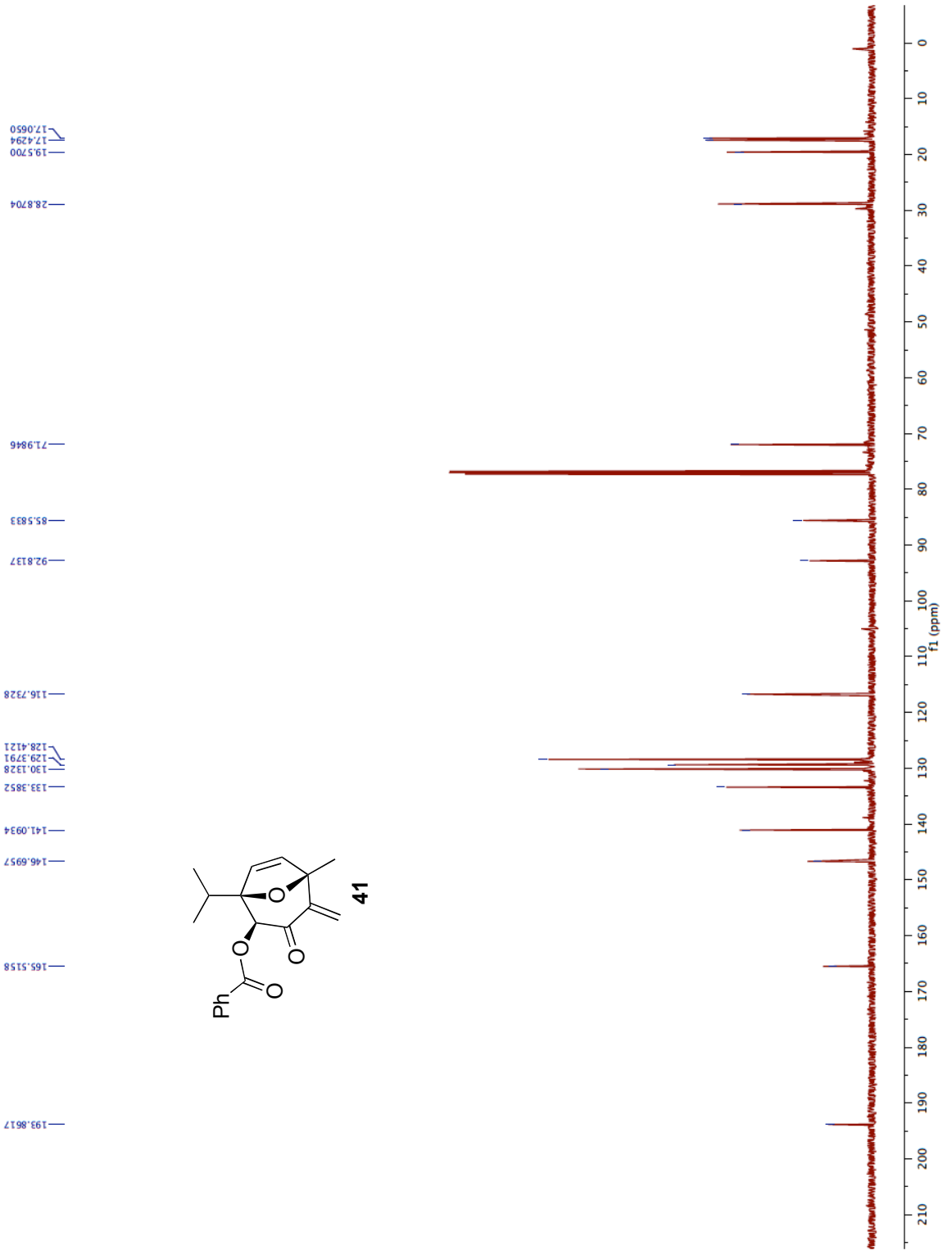
Spectrum 1.47 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **54**



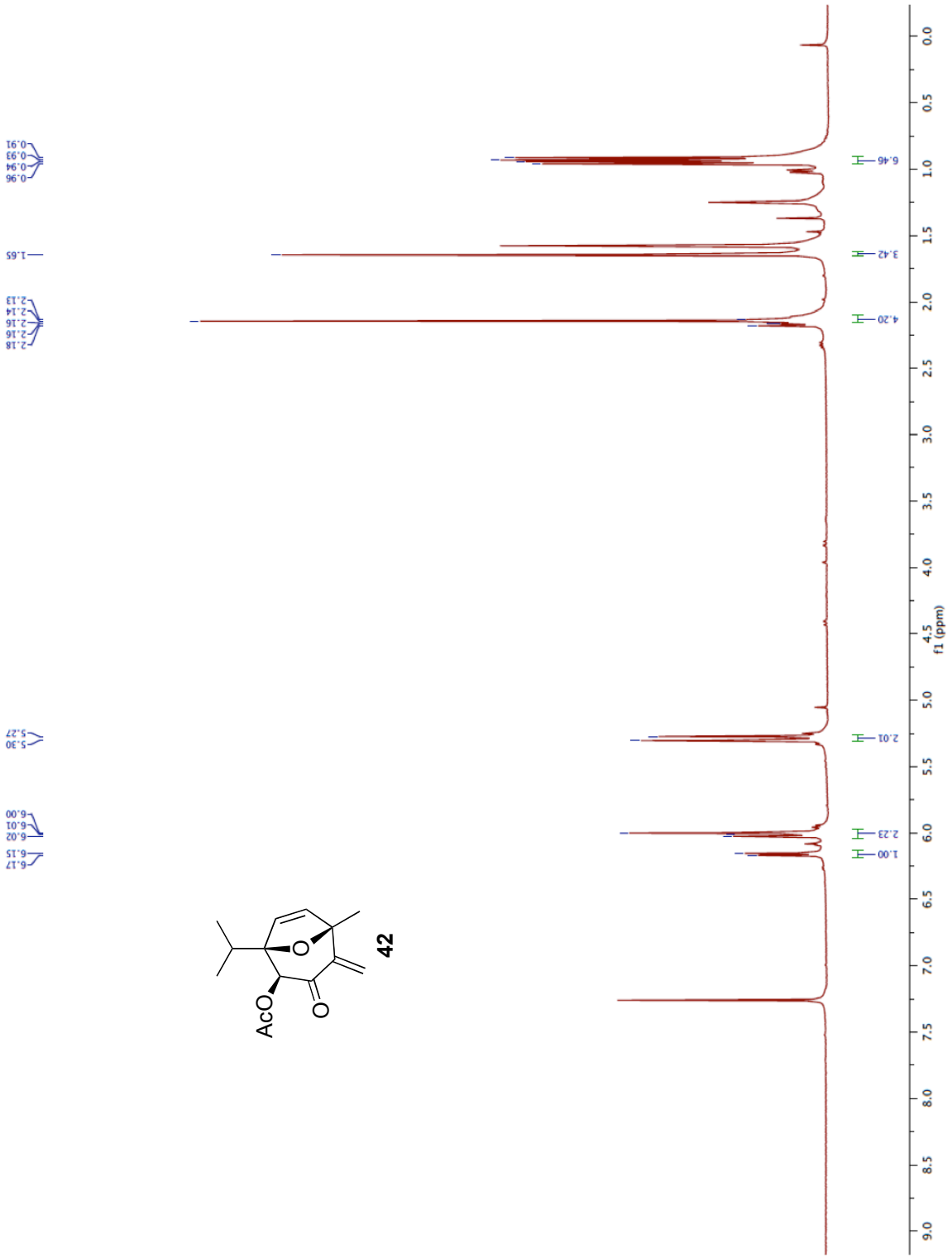
Spectrum 1.48 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **54**



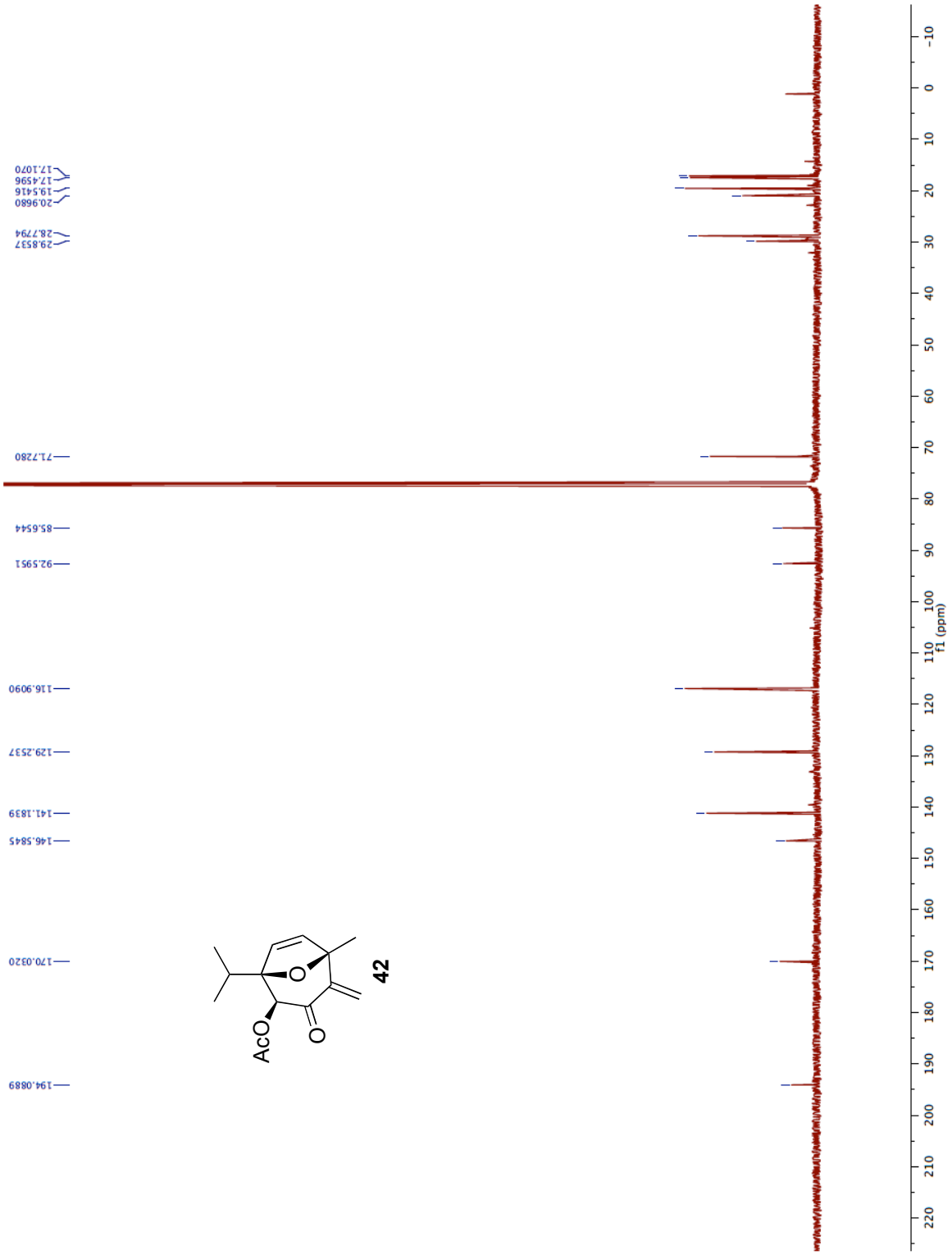
Spectrum 1.49 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **41**



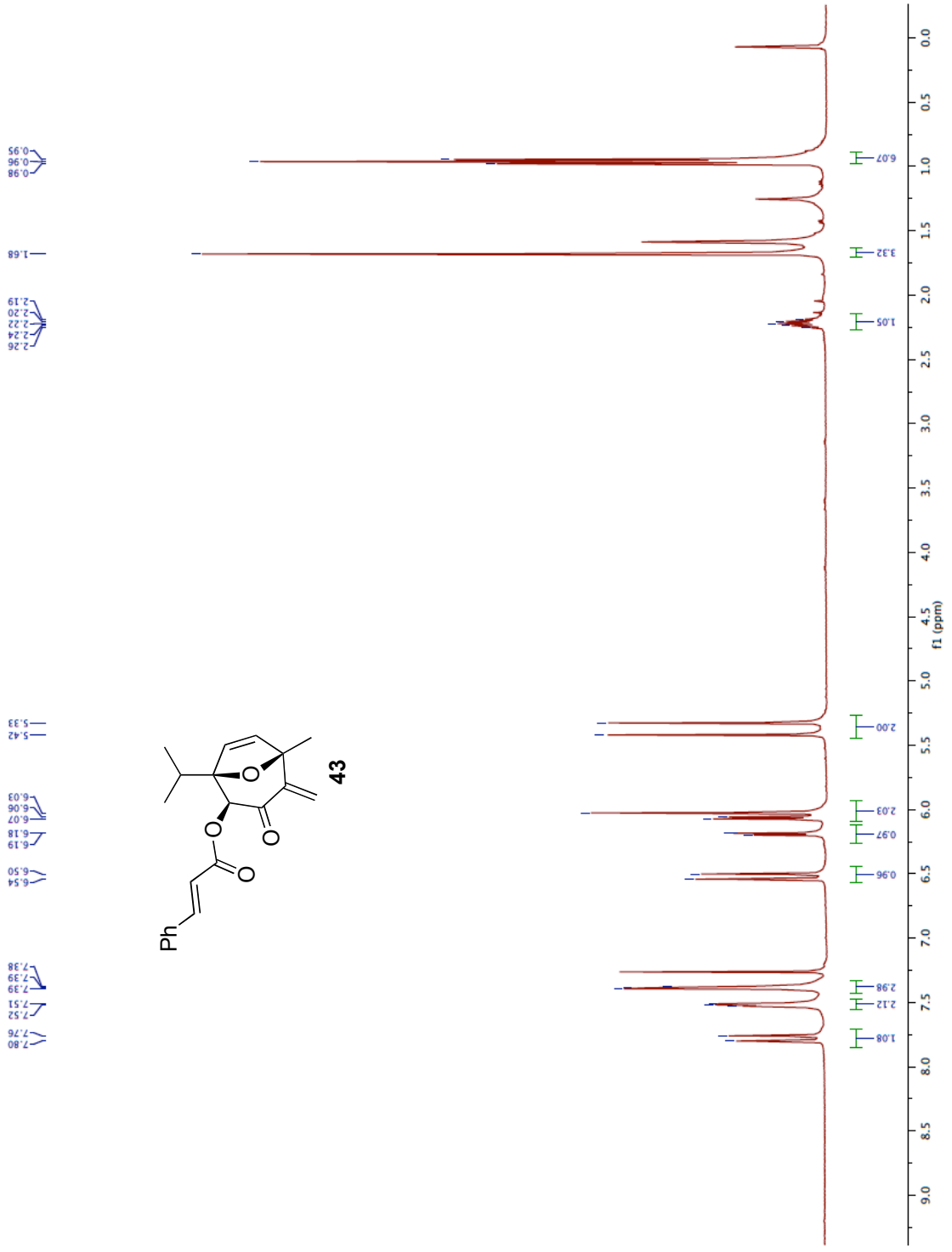
Spectrum 1.50 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **41**



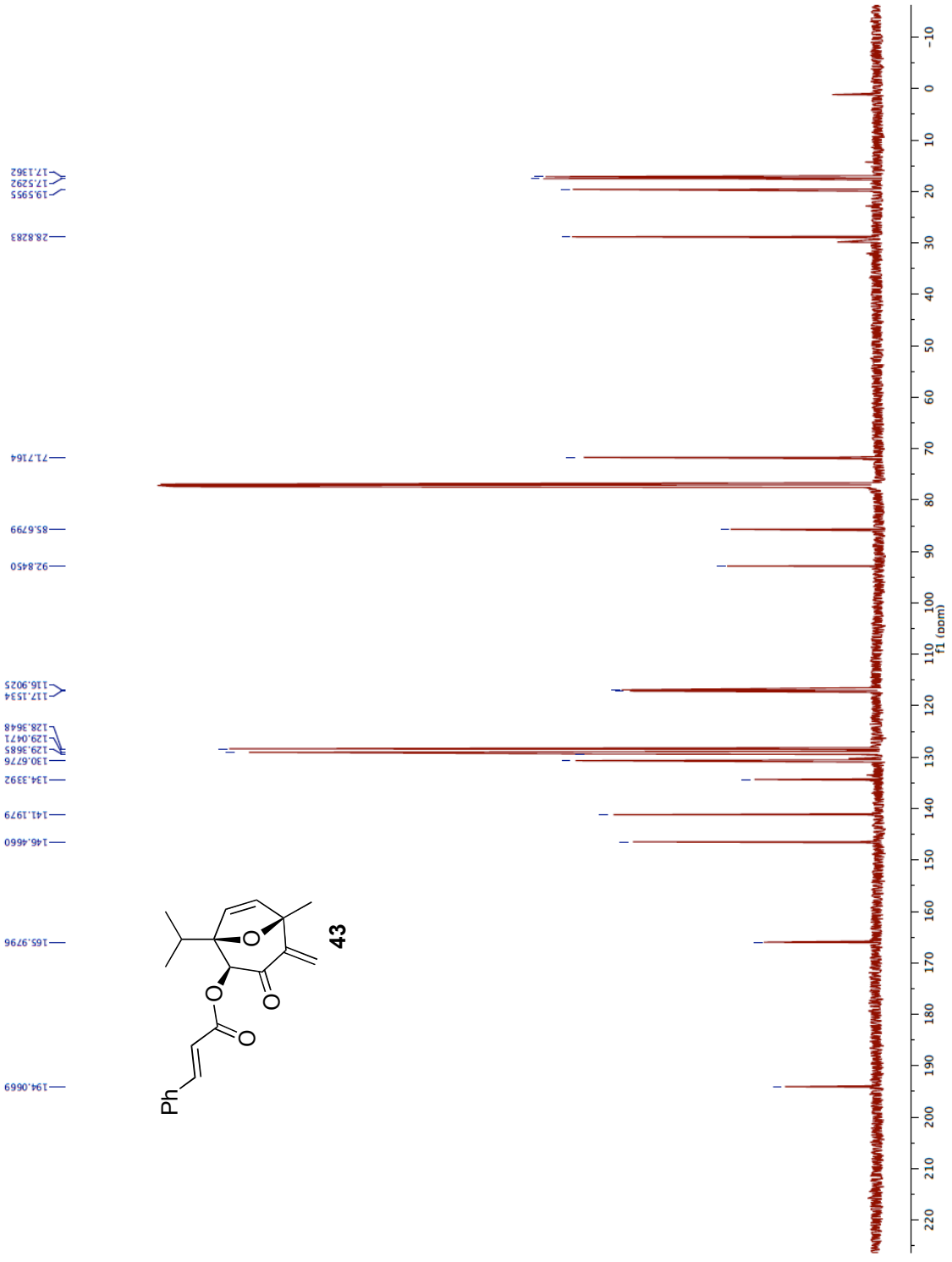
Spectrum 1.51 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **42**



Spectrum 1.52 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **42**



Spectrum 1.53 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **43**



Spectrum 1.54 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **43**

Table 1.8.1 Crystal data and structure refinement for compound (-)-**30**.

Identification code	Compound **13**	
Empirical formula	C12 H16 O3	
Formula weight	208.25	
Temperature	100(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 6.8430(5) Å	α= 90°
	b = 10.1285(7) Å	β= 90°
	c = 17.0834(12) Å	γ = 90°
Volume	1184.04(15) $Å^3$	
Z	4	
Density (calculated)	1.168 g/cm^3	
Absorption coefficient	0.676 mm^{-1}	
F(000)	448	
Crystal size	0.33 x 0.22 x 0.11 mm^3	
Theta range for data collection	5.08 to 62.57°	
Index ranges	-7<=h<=7, -11<=k<=11, -19<=l<=18	
Reflections collected	3417	
Independent reflections	1500 [R(int) = 0.0364]	
Completeness to theta = 55.00°	95.1 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1500 / 0 / 136	
Goodness-of-fit on F^2	1.055	
Final R indices [I>2sigma(I)]	R1 = 0.0393, wR2 = 0.0967	
R indices (all data)	R1 = 0.0446, wR2 = 0.0993	
Absolute structure parameter	0.0(3)	
Largest diff. peak and hole	0.130 and -0.183 e $Å^{-3}$	

Table 1.8.2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^{2}x 10^3) for Compound (-)-**30**.
U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	7673(2)	4447(2)	9449(1)	22(1)
O(2)	5174(2)	2432(2)	9972(1)	26(1)
O(3)	3774(3)	2274(2)	8288(1)	38(1)
C(1)	4514(3)	3447(2)	9461(1)	21(1)
C(2)	5664(4)	4716(2)	9649(1)	21(1)
C(3)	5117(4)	5748(2)	9043(1)	26(1)
C(4)	6328(4)	5655(2)	8446(1)	26(1)
C(5)	7772(4)	4559(2)	8606(1)	23(1)
C(6)	6920(4)	3274(2)	8288(1)	23(1)
C(7)	4958(4)	2961(2)	8630(1)	24(1)
C(8)	5562(4)	5116(2)	10508(1)	29(1)
C(9)	6812(5)	6338(3)	10659(2)	46(1)
C(10)	3455(5)	5354(3)	10771(2)	36(1)
C(11)	9864(4)	4832(3)	8369(2)	33(1)
C(12)	7758(4)	2465(3)	7790(2)	34(1)

Table 1.8.3. Bond lengths [Å] and angles [°] for Compound **30**.

O(1)-C(5)	1.446(3)
O(1)-C(2)	1.442(3)
O(2)-C(1)	1.423(3)
O(3)-C(7)	1.217(3)
C(1)-C(7)	1.533(3)
C(1)-C(2)	1.540(3)
C(2)-C(8)	1.524(3)
C(2)-C(3)	1.518(3)
C(3)-C(4)	1.318(4)
C(4)-C(5)	1.511(3)
C(5)-C(11)	1.514(4)

Table 1.8.3 (cont.) Bond lengths [Å] and angles [°] for Compound (-)-**30**.

C(5)-C(6)	1.526(3)
C(6)-C(12)	1.314(4)
C(6)-C(7)	1.498(4)
C(8)-C(10)	1.529(4)
C(8)-C(9)	1.527(4)
C(5)-O(1)-C(2)	105.34(17)
O(2)-C(1)-C(7)	105.86(17)
O(2)-C(1)-C(2)	108.21(17)
C(7)-C(1)-C(2)	111.07(19)
O(1)-C(2)-C(8)	108.72(19)
O(1)-C(2)-C(3)	101.78(18)
C(8)-C(2)-C(3)	117.53(19)
O(1)-C(2)-C(1)	106.28(17)
C(8)-C(2)-C(1)	113.51(19)
C(3)-C(2)-C(1)	107.84(18)
C(4)-C(3)-C(2)	108.9(2)
C(3)-C(4)-C(5)	108.8(2)
O(1)-C(5)-C(4)	102.02(18)
O(1)-C(5)-C(11)	109.0(2)
C(4)-C(5)-C(11)	115.8(2)
O(1)-C(5)-C(6)	105.65(18)
C(4)-C(5)-C(6)	108.2(2)
C(11)-C(5)-C(6)	114.9(2)
C(12)-C(6)-C(7)	120.8(2)
C(12)-C(6)-C(5)	126.6(2)
C(7)-C(6)-C(5)	112.6(2)
O(3)-C(7)-C(6)	122.1(2)
O(3)-C(7)-C(1)	119.7(2)
C(6)-C(7)-C(1)	118.1(2)
C(2)-C(8)-C(10)	111.6(2)
C(2)-C(8)-C(9)	110.6(2)
C(10)-C(8)-C(9)	110.5(2)

Table 1.8.4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for Compound (-)-**30**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2} U^{11} + ... + 2 h k a^* b^* U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	24(1)	18(1)	25(1)	4(1)	-3(1)	-1(1)
O(2)	26(1)	17(1)	34(1)	8(1)	-2(1)	-3(1)
O(3)	31(1)	41(1)	41(1)	-16(1)	3(1)	-10(1)
C(1)	22(1)	16(1)	24(1)	2(1)	-1(1)	-1(1)
C(2)	24(1)	13(1)	27(1)	1(1)	0(1)	-2(1)
C(3)	28(1)	16(1)	33(1)	2(1)	-3(1)	2(1)
C(4)	30(1)	16(1)	32(1)	9(1)	-4(1)	2(1)
C(5)	23(1)	19(1)	26(1)	6(1)	-1(1)	1(1)
C(6)	27(1)	20(1)	23(1)	2(1)	-2(1)	4(1)
C(7)	29(1)	17(1)	27(1)	0(1)	-3(1)	-1(1)
C(8)	40(2)	18(1)	29(1)	0(1)	-1(1)	-5(1)
C(9)	61(2)	35(2)	42(2)	-14(1)	3(1)	-17(1)
C(10)	48(2)	28(1)	33(1)	-4(1)	9(1)	3(1)
C(11)	30(1)	32(1)	38(1)	8(1)	3(1)	-2(1)
C(12)	35(1)	32(1)	35(1)	-1(1)	6(1)	4(1)

Table 1.8.5 Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2\ x\ 10^3$) for Compound (-)-**30**.

	x	y	z	U(eq)
H(2A)	4230	1942	10093	39
H(1A)	3079	3598	9528	25
H(3A)	4072	6361	9087	31
H(4A)	6299	6189	7989	31
H(8A)	6105	4374	10827	34
H(9A)	8154	6170	10484	69
H(9B)	6812	6539	11220	69

Table 1.8.5 (cont.) Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for Compound (-)-**30**.

	x	y	z	U(eq)
H(9C)	6274	7089	10368	69
H(10A)	2674	4561	10669	55
H(10B)	2908	6101	10479	55
H(10C)	3435	5552	11332	55
H(11A)	10290	5672	8598	50
H(11B)	9950	4884	7797	50
H(11C)	10707	4118	8559	50
H(12A)	7120	1670	7642	41
H(12B)	9003	2675	7577	41

Table 1.8.6 Crystal data and structure refinement for **47**

Identification code	Compound **47**	
Empirical formula	C12 H19 N O2	
Formula weight	209.28	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 8.582(3) Å	a= 90°.
	b = 13.251(4) Å	b= 90°.
	c = 20.272(6) Å	g = 90°.
Volume	2305.5(12) $Å^3$	
Z	8	
Density (calculated)	1.206 Mg/m^3	
Absorption coefficient	0.081 mm^{-1}	
F(000)	912	
Crystal size	0.27 x 0.24 x 0.11 mm^3	
Crystal color, habit	Colorless Plate	
Theta range for data collection	1.84 to 28.39°.	

Index ranges	-11<=h<=11, -17<=k<=17, -27<=l<=25
Reflections collected	24152
Independent reflections	5339 [R(int) = 0.0479]
Completeness to theta = 25.00°	100.0 %
Absorption correction	Multi-scan
Max. and min. transmission	0.9911 and 0.9784
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5339 / 0 / 279
Goodness-of-fit on F^2	1.054
Final R indices [I>2sigma(I)]	R1 = 0.0437, wR2 = 0.0869
R indices (all data)	R1 = 0.0540, wR2 = 0.0928
Absolute structure parameter	0.0(9)
Largest diff. peak and hole	0.255 and -0.225 e.$Å^{-3}$

Table 1.8.7 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **47**.
U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	-3984(1)	-7783(1)	-1616(1)	18(1)
O(2)	-3001(1)	-10061(1)	-720(1)	23(1)
N(1)	-3871(2)	-5257(1)	-428(1)	33(1)
C(1)	-3346(2)	-6008(1)	-585(1)	25(1)
C(2)	-2655(2)	-6985(1)	-748(1)	20(1)
C(3)	-3642(2)	-7864(1)	-471(1)	21(1)
C(4)	-4079(2)	-8491(1)	-1080(1)	17(1)
C(5)	-2841(2)	-9298(1)	-1210(1)	18(1)
C(6)	-1220(2)	-8838(1)	-1216(1)	20(1)
C(7)	-1183(2)	-7889(1)	-1643(1)	20(1)
C(8)	-2584(2)	-7212(1)	-1505(1)	19(1)
C(9)	-2622(2)	-6299(1)	-1951(1)	23(1)
C(10)	-5736(2)	-8917(1)	-1054(1)	22(1)
C(11)	-6954(2)	-8078(2)	-1089(1)	30(1)

Table 1.8.7 (cont.) Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **47**.

	x	y	z	U(eq)
C(12)	-6057(2)	-9689(1)	-1595(1)	27(1)
O(1')	-9013(1)	-3033(1)	-1676(1)	18(1)
O(2')	-10265(1)	-1172(1)	-476(1)	23(1)
N(1')	-9128(2)	-5873(1)	-1001(1)	30(1)
C(1')	-8805(2)	-5057(1)	-888(1)	23(1)
C(2')	-8449(2)	-4000(1)	-740(1)	20(1)
C(3')	-9985(2)	-3410(1)	-620(1)	21(1)
C(4')	-10021(2)	-2624(1)	-1175(1)	18(1)
C(5')	-9245(2)	-1645(1)	-940(1)	19(1)
C(6')	-7655(2)	-1858(1)	-644(1)	21(1)
C(7')	-6706(2)	-2562(1)	-1083(1)	20(1)
C(8')	-7688(2)	-3442(1)	-1332(1)	19(1)
C(9')	-6791(2)	-4103(1)	-1805(1)	24(1)
C(10')	-11637(2)	-2456(1)	-1483(1)	22(1)
C(11')	-11636(2)	-1645(1)	-2018(1)	26(1)
C(12')	-12311(2)	-3428(2)	-1768(1)	32(1)

Table 1.8.8 Bond lengths [Å] and angles [°] for **47**.

O(1)-C(8)	1.437(2)	C(2)-H(2)	1.0000
O(1)-C(4)	1.439(2)	C(3)-C(4)	1.535(2)
O(2)-C(5)	1.425(2)	C(3)-H(3A)	0.9900
O(2)-H(2O)	0.8400	C(3)-H(3B)	0.9900
N(1)-C(1)	1.137(2)	C(4)-C(5)	1.530(2)
C(1)-C(2)	1.463(2)	C(4)-C(10)	1.531(2)
C(2)-C(3)	1.546(2)	C(5)-C(6)	1.519(2)
C(2)-C(8)	1.564(2)	C(5)-H(5)	1.0000

Table 1.8.8 (cont.) Bond lengths [Å] and angles [°] for **47**.

C(6)-C(7)	1.527(2)	C(7)-H(7A)	0.9900
C(6)-H(6A)	0.9900	C(7)-H(7B)	0.9900
C(6)-H(6B)	0.9900	C(8)-C(9)	1.511(2)
C(7)-C(8)	1.526(2)	C(9)-H(9A)	0.980
C(9)-H(9B)	0.9800	C(6')-C(7')	1.526(2)
C(9)-H(9C)	0.9800	C(6')-H(6A')	0.9900
C(10)-C(12)	1.524(3)	C(6')-H(6B')	0.9900
C(10)-C(11)	1.528(3)	C(7')-C(8')	1.524(2)
C(10)-H(10)	1.0000	C(7')-H(7A')	0.9900
C(11)-H(11A)	0.9800	C(7')-H(7B')	0.9900
C(11)-H(11B)	0.9800	C(8')-C(9')	1.510(2)
C(11)-H(11C)	0.9800	C(9')-H(9A')	0.9800
C(12)-H(12A)	0.9800	C(9')-H(9B')	0.9800
C(12)-H(12B)	0.9800	C(9')-H(9C')	0.9800
C(12)-H(12C)	0.9800	C(10')-C(12')	1.525(2)
O(1')-C(8')	1.439(2)	C(10')-C(11')	1.528(3)
O(1')-C(4')	1.4390(19)	C(10')-H(10A)	1.0000
O(2')-C(5')	1.431(2)	C(11')-H(11D)	0.9800
O(2')-H(2O')	0.8400	C(11')-H(11E)	0.9800
N(1')-C(1')	1.140(2)	C(11')-H(11F)	0.9800
C(1')-C(2')	1.464(2)	C(12')-H(12D)	0.9800
C(2')-C(3')	1.553(2)	C(12')-H(12E)	0.9800
C(2')-C(8')	1.553(2)	C(12')-H(12F)	0.9800
C(2')-H(2')	1.0000	C(8)-O(1)-C(4)	105.72(12)
C(3')-C(4')	1.534(2)	C(5)-O(2)-H(2O)	109.5
C(3')-H(3A')	0.9900	N(1)-C(1)-C(2)	176.9(2)
C(3')-H(3B')	0.9900	C(1)-C(2)-C(3)	111.26(14)
C(4')-C(5')	1.534(2)	C(1)-C(2)-C(8)	114.15(14)
C(4')-C(10')	1.537(2)	C(3)-C(2)-C(8)	103.48(13)
C(5')-C(6')	1.518(2)	C(1)-C(2)-H(2)	109.3
C(5')-H(5')	1.0000	C(3)-C(2)-H(2)	109.3

Table 1.8.8 (cont.) Bond lengths [Å] and angles [°] for **47**.

C(8)-C(2)-H(2)	109.3	C(4)-C(3)-H(3B)	110.9
C(4)-C(3)-C(2)	104.44(13)	C(2)-C(3)-H(3B)	110.9
C(4)-C(3)-H(3A)	110.9	H(3A)-C(3)-H(3B)	108.9
C(2)-C(3)-H(3A)	110.9	O(1)-C(4)-C(5)	106.59(13)
O(1)-C(4)-C(10)	108.59(13)	C(9)-C(8)-C(2)	115.58(14)
C(5)-C(4)-C(10)	113.17(14)	C(7)-C(8)-C(2)	108.93(14)
O(1)-C(4)-C(3)	103.93(13)	C(8)-C(9)-H(9A)	109.5
C(5)-C(4)-C(3)	110.38(14)	C(8)-C(9)-H(9B)	109.5
C(10)-C(4)-C(3)	113.52(14)	H(9A)-C(9)-H(9B)	109.5
O(2)-C(5)-C(6)	112.20(14)	C(8)-C(9)-H(9C)	109.5
O(2)-C(5)-C(4)	107.96(13)	H(9A)-C(9)-H(9C)	109.5
C(6)-C(5)-C(4)	110.89(14)	H(9B)-C(9)-H(9C)	109.5
O(2)-C(5)-H(5)	108.6	C(12)-C(10)-C(11)	109.31(15)
C(6)-C(5)-H(5)	108.6	C(12)-C(10)-C(4)	112.98(15)
C(4)-C(5)-H(5)	108.6	C(11)-C(10)-C(4)	111.45(15)
C(5)-C(6)-C(7)	110.69(14)	C(12)-C(10)-H(10)	107.6
C(5)-C(6)-H(6A)	109.5	C(11)-C(10)-H(10)	107.6
C(7)-C(6)-H(6A)	109.5	C(4)-C(10)-H(10)	107.6
C(5)-C(6)-H(6B)	109.5	C(10)-C(11)-H(11A)	109.5
C(7)-C(6)-H(6B)	109.5	C(10)-C(11)-H(11B)	109.5
H(6A)-C(6)-H(6B)	108.1	H(11A)-C(11)-H(11B)	109.5
C(8)-C(7)-C(6)	111.26(14)	C(10)-C(11)-H(11C)	109.5
C(8)-C(7)-H(7A)	109.4	H(11A)-C(11)-H(11C)	109.5
C(6)-C(7)-H(7A)	109.4	H(11B)-C(11)-H(11C)	109.5
C(8)-C(7)-H(7B)	109.4	C(10)-C(12)-H(12A)	109.5
C(6)-C(7)-H(7B)	109.4	C(10)-C(12)-H(12B)	109.5
H(7A)-C(7)-H(7B)	108.0	H(12A)-C(12)-H(12B)	109.5
O(1)-C(8)-C(9)	107.99(14)	C(10)-C(12)-H(12C)	109.5
O(1)-C(8)-C(7)	108.70(13)	H(12A)-C(12)-H(12C)	109.5
C(9)-C(8)-C(7)	112.15(14)	H(12B)-C(12)-H(12C)	109.5
O(1)-C(8)-C(2)	102.90(13)	C(8')-O(1')-C(4')	105.99(12)

Table 1.8.8 (cont.) Bond lengths [Å] and angles [°] for **47**.

C(5')-O(2')-H(2O')	109.5	C(3')-C(2')-C(8')	103.77(13)
N(1')-C(1')-C(2')	178.0(2)	C(1')-C(2')-H(2')	110.2
C(1')-C(2')-C(3')	109.68(14)	C(3')-C(2')-H(2')	110.2
C(1')-C(2')-C(8')	112.64(15)	C(8')-C(2')-H(2')	110.2
C(4')-C(3')-C(2')	104.10(13)	C(8')-C(7')-H(7B')	109.3
C(4')-C(3')-H(3A')	110.9	C(6')-C(7')-H(7B')	109.3
C(2')-C(3')-H(3A')	110.9	H(7A')-C(7')-H(7B')	108.0
C(4')-C(3')-H(3B')	110.9	O(1')-C(8')-C(9')	108.26(14)
C(2')-C(3')-H(3B')	110.9	O(1')-C(8')-C(7')	108.06(13)
H(3A')-C(3')-H(3B')	109.0	C(9')-C(8')-C(7')	111.79(15)
O(1')-C(4')-C(5')	106.05(13)	O(1')-C(8')-C(2')	102.78(13)
O(1')-C(4')-C(3')	104.46(13)	C(9')-C(8')-C(2')	115.37(14)
C(5')-C(4')-C(3')	109.70(14)	C(7')-C(8')-C(2')	109.96(14)
O(1')-C(4')-C(10')	108.14(13)	C(8')-C(9')-H(9A')	109.5
C(5')-C(4')-C(10')	113.29(14)	C(8')-C(9')-H(9B')	109.5
C(3')-C(4')-C(10')	114.45(14)	H(9A')-C(9')-H(9B')	109.5
O(2')-C(5')-C(6')	111.75(14)	C(8')-C(9')-H(9C')	109.5
O(2')-C(5')-C(4')	108.02(13)	H(9A')-C(9')-H(9C')	109.5
C(6')-C(5')-C(4')	110.88(14)	H(9B')-C(9')-H(9C')	109.5
O(2')-C(5')-H(5')	108.7	C(12')-C(10')-C(11')	108.95(15)
C(6')-C(5')-H(5')	108.7	C(12')-C(10')-C(4')	111.95(15)
C(4')-C(5')-H(5')	108.7	C(11')-C(10')-C(4')	112.92(15)
C(5')-C(6')-C(7')	111.23(14)	C(12')-C(10')-H(10A)	107.6
C(5')-C(6')-H(6A')	109.4	C(11')-C(10')-H(10A)	107.6
C(7')-C(6')-H(6A')	109.4	C(4')-C(10')-H(10A)	107.6
C(5')-C(6')-H(6B')	109.4	C(10')-C(11')-H(11D)	109.5
C(7')-C(6')-H(6B')	109.4	C(10')-C(11')-H(11E)	109.5
H(6A')-C(6')-H(6B')	108.0	H(11D)-C(11')-H(11E)	109.5
C(8')-C(7')-C(6')	111.43(14)	C(10')-C(11')-H(11F)	109.5
C(8')-C(7')-H(7A')	109.3	H(11D)-C(11')-H(11F)	109.5
C(6')-C(7')-H(7A')	109.3	H(11E)-C(11')-H(11F)	109.5

C(10')-C(12')-H(12D)	109.5	C(10')-C(12')-H(12F)	109.5
C(10')-C(12')-H(12E)	109.5	H(12D)-C(12')-H(12F)	109.5
H(12D)-C(12')-H(12E)	109.5	H(12E)-C(12')-H(12F)	109.5

Table 1.8.9 Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **47**. The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
O(1)	18(1)	19(1)	18(1)	1(1)	0(1)	-2(1)
O(2)	23(1)	20(1)	25(1)	6(1)	2(1)	2(1)
N(1)	42(1)	25(1)	31(1)	-6(1)	5(1)	2(1)
C(1)	29(1)	22(1)	22(1)	-1(1)	0(1)	-3(1)
C(2)	22(1)	18(1)	21(1)	0(1)	0(1)	0(1)
C(3)	24(1)	20(1)	19(1)	-2(1)	2(1)	-1(1)
C(4)	18(1)	18(1)	16(1)	2(1)	0(1)	0(1)
C(5)	19(1)	18(1)	18(1)	2(1)	0(1)	0(1)
C(6)	17(1)	20(1)	23(1)	0(1)	1(1)	2(1)
C(7)	18(1)	21(1)	21(1)	1(1)	2(1)	-1(1)
C(8)	19(1)	17(1)	19(1)	-1(1)	0(1)	0(1)
C(9)	28(1)	19(1)	23(1)	1(1)	0(1)	-2(1)
C(10)	19(1)	24(1)	23(1)	2(1)	1(1)	-1(1)
C(11)	16(1)	35(1)	38(1)	2(1)	3(1)	3(1)
C(12)	23(1)	27(1)	32(1)	0(1)	-2(1)	-8(1)
O(1')	17(1)	22(1)	15(1)	0(1)	0(1)	3(1)
O(2')	23(1)	21(1)	27(1)	-6(1)	-3(1)	2(1)
N(1')	32(1)	24(1)	36(1)	1(1)	-4(1)	-1(1)
C(1')	22(1)	24(1)	24(1)	4(1)	-1(1)	1(1)
C(2')	22(1)	19(1)	20(1)	2(1)	-1(1)	0(1)
C(3')	20(1)	21(1)	22(1)	0(1)	2(1)	-2(1)
C(4')	16(1)	18(1)	19(1)	-2(1)	1(1)	1(1)
C(5')	19(1)	18(1)	20(1)	-2(1)	0(1)	0(1)
C(6')	20(1)	21(1)	22(1)	-1(1)	-4(1)	-2(1)
C(7')	17(1)	21(1)	23(1)	2(1)	-2(1)	-2(1)

Table 1.8.9 (cont.) Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **47**. The anisotropic displacement factor exponent takes the form: $-2p^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(8')	19(1)	20(1)	19(1)	2(1)	-3(1)	1(1)
C(9')	22(1)	27(1)	24(1)	-1(1)	1(1)	4(1)
C(10')	17(1)	24(1)	24(1)	-5(1)	-2(1)	1(1)
C(11')	25(1)	26(1)	26(1)	-4(1)	-6(1)	4(1)
C(12')	26(1)	28(1)	40(1)	-5(1)	-8(1)	-4(1)

Table 1.8.10 Hydrogen coordinates ($x\ 10^4$) and isotropic displacement parameters ($Å^2 x\ 10^3$) for **47**.

	x	y	z	U(eq)
H(2O)	-2180	-10406	-704	34
H(2)	-1580	-7022	-560	24
H(3A)	-3030	-8271	-154	25
H(3B)	-4586	-7607	-247	25
H(5)	-3045	-9606	-1653	22
H(6A)	-465	-9336	-1389	24
H(6B)	-909	-8664	-759	24
H(7A)	-211	-7510	-1554	24
H(7B)	-1183	-8085	-2115	24
H(9A)	-3579	-5915	-1870	35
H(9B)	-2596	-6519	-2412	35
H(9C)	-1715	-5872	-1860	35
H(10)	-5864	-9266	-620	27
H(11A)	-7985	-8357	-990	45
H(11B)	-6960	-7787	-1533	45
H(11C)	-6698	-7552	-767	45
H(12A)	-5828	-9389	-2026	41
H(12B)	-7155	-9891	-1579	41

Table 1.8.10 (cont.) Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **47**.

	x	y	z	U(eq)
H(12C)	-5395	-10282	-1527	41
H(2O')	-9747	-789	-229	35
H(2')	-7761	-3956	-343	24
H(3A')	-10898	-3864	-646	25
H(3B')	-9975	-3078	-182	25
H(5')	-9111	-1186	-1328	23
H(6A')	-7786	-2170	-203	25
H(6B')	-7084	-1215	-586	25
H(7A')	-6296	-2179	-1464	24
H(7B')	-5807	-2827	-831	24
H(9A')	-6355	-3686	-2159	36
H(9B')	-7492	-4610	-1994	36
H(9C')	-5943	-4441	-1569	36
H(10A)	-12352	-2225	-1124	26
H(11D)	-12694	-1562	-2192	38
H(11E)	-10935	-1847	-2376	38
H(11F)	-11279	-1004	-1830	38
H(12D)	-11613	-3687	-2111	47
H(12E)	-13337	-3289	-1960	47
H(12F)	-12415	-3931	-1417	47

Table 1.8.11 Hydrogen bonds for **47** [Å and °]. Symmetry transformations used to generate equivalent atoms: #1 x+1,y-1,z #2 x-1/2,-y-1/2,-z

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2O)...O(2')#1	0.84	1.99	2.8152(18)	168.7
O(2')-H(2O')...N(1)#2	0.84	2.06	2.894(2)	169.4

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Chapter 2

Synthetic studies and chemical biology of fusarisetin A

2.1 Introduction

Despite the tremendous advances, cancer still represents an enormous medical challenge since, only in America, it is responsible for more than half a millions of deaths per year.[1] About 90% of these deaths are attributed to cancer metastasis, which is the ability of tumor cells to migrate from their tissue of origin and colonize elsewhere in the body.[2] When cancer is detected at a premetastatic stage, it can often be treated successfully either by local therapy (surgery, radiation) or by systemic therapy (chemotherapy, targeted therapy, hormonal therapy).[3] However, when it is detected after it has metastasized, such treatments are much less successful. Along these lines, metastasis is considered as the "last frontier" in cancer management for which, to-date, there is no effective treatment.[4,5]

In principle, it is possible to halt (or retard) cancer metastasis with the help of small molecules that inhibit cell migration.[6] Recent advances in high-throughput screening and high content imaging techniques permit the identification of new cancer metastasis inhibitors from libraries of natural products or small molecules.[7,8] On the other hand, the availability of complex natural products via improved isolation techniques and streamlined synthetic strategies (or genetic engineering) allows evaluation of their effect in cell migration assays. Sceptrin[9] and migrastatin[10] represent a few recent examples of such efforts. Importantly, the development of scalable syntheses of these compounds[11,12] have resulted in the development of new chemical tools for the study of proteins involved in cancer metastasis and the discovery of potent cell migration inhibitors for further preclinical studies.[11b,12c,d]

2.2 Isolation and Biological Characterization

Recent efforts to identify potent inhibitors of cancer metastasis have led to the isolation of novel structure from from the soil fungus *Fusarium* sp. FN080326 (Figure 1.2.1).[13] Fusarisetin A (**1**) (Figure 1.2.2) has attracted considerable attention due to its unprecedented complex molecular architecture and remarkable bioactivity. This compound was found to inhibit cancer metastasis in MDA-MB-231 cells, a particularly aggressive breast cancer cell line. Specifically, **1** was found to inhibit acinar morphogenesis (IC_{50} ca 77 mM), cell migration (IC_{50} ca 7.7 mM) and cell invasion (IC_{50} ca 26 mM) in these cell lines without any significant cytotoxicity in concentrations up to 77 mM. Interestingly, the proteomic profiling of **1** was found to be significantly different to those of other reference compounds. Moreover, **1** did not inhibit the phosphorylation of ERK1/2, AKT, c-Jun and p38 kinases in response to EGF treatment, as it is commonly observed with compounds that inhibit cancer metastasis by altering protein kinases.[6]

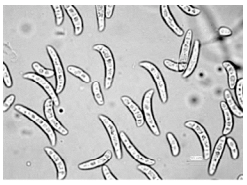


Figure 2.2.1 *Fusarium* sp. FN080326 and the structure of fusarisetin A

These findings suggest that the molecular target of fusarisetin A is different from those of known compounds and thus, its identification could produce new fundamental knowledge in the pathways related to cancer metastasis. Structurally, fusarisetin A possesses an unprecedented pentacyclic ring system of which the CDE rings contain various polar functionalities (ketone, lactam, hemiketal and primary alcohol).

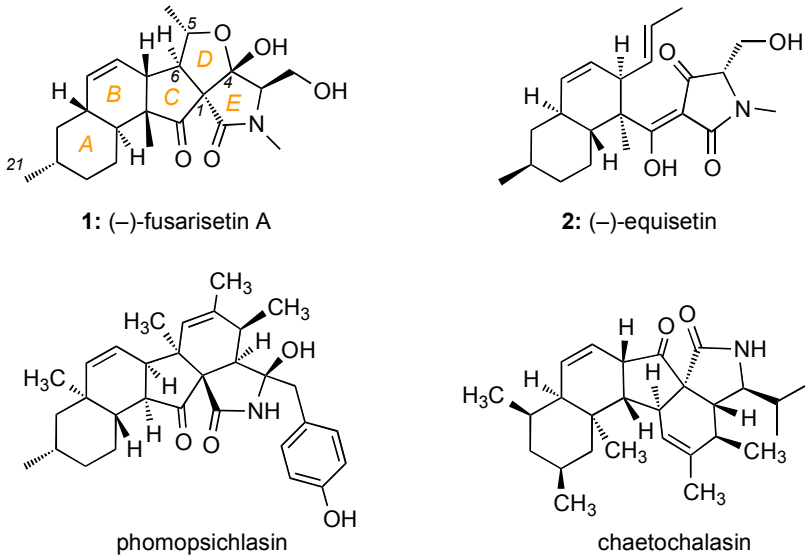
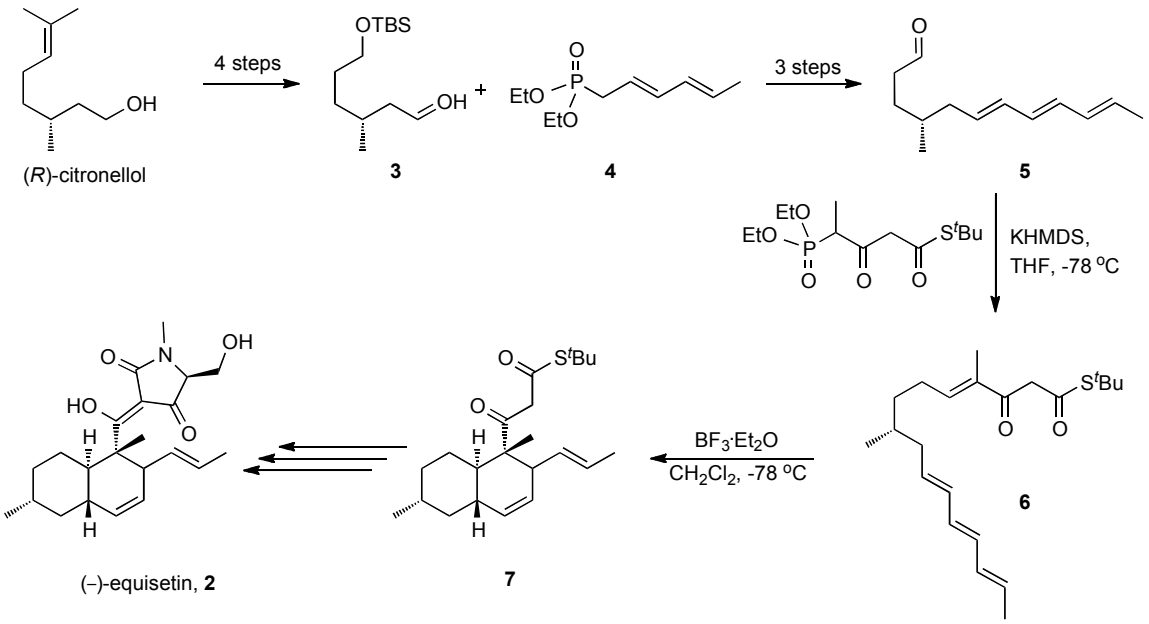


Figure 2.2.2 Structure of fusarisetin A and structurally related secondary metabolites

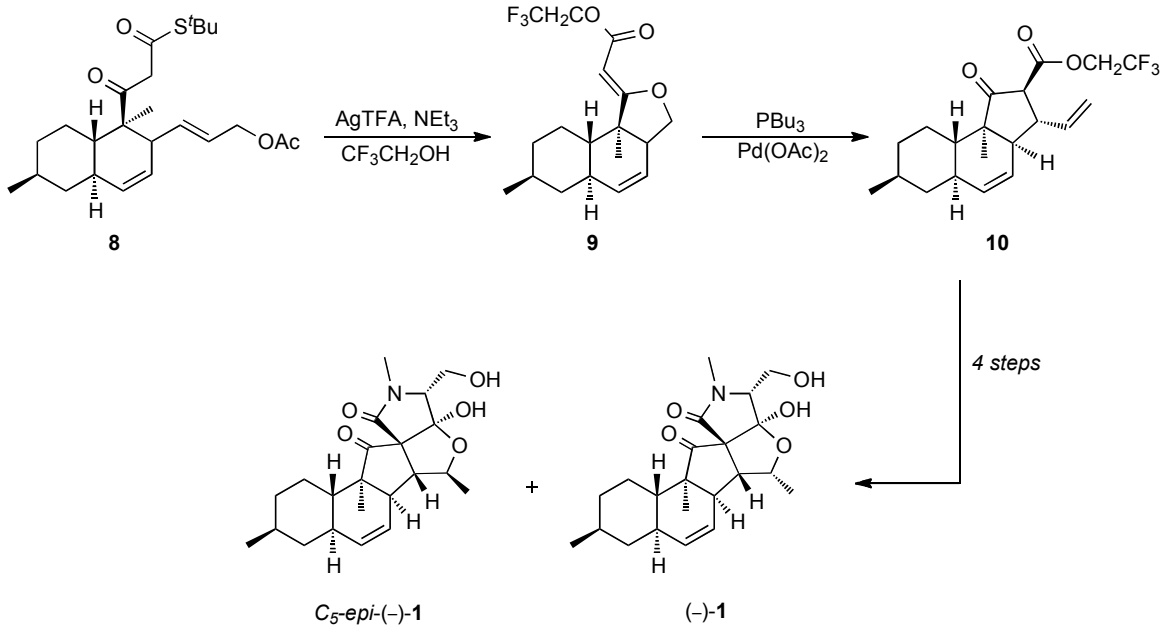
2.3 Previous synthetic work

The combination of impressive chemical structure and potent bioactivity drew great attention from the synthetic community. This is evidenced by the production of multiple publications describing various syntheses of **1** in about one year after its structure became known in the literature. Several researchers took advantage of chemistry previously described by Ley[14] to describe the synthesis of the AB decalin ring of fusarisetin A in which sequential Horner-Wadsworth-Emmons olefinations[15] yield triene **4** (Scheme 1.3.1) which can undergo Lewis Acid promoted Intramolecular Diels-Alder Reaction (IMDA) to form decalin β-ketothioester **6**. This decalin moiety can be further functionalized to yield (–)-equisetin (**2**)[16], a cytotoxic secondary metabolite known to be a potent HIV-1 integrase inhibitor.[17]



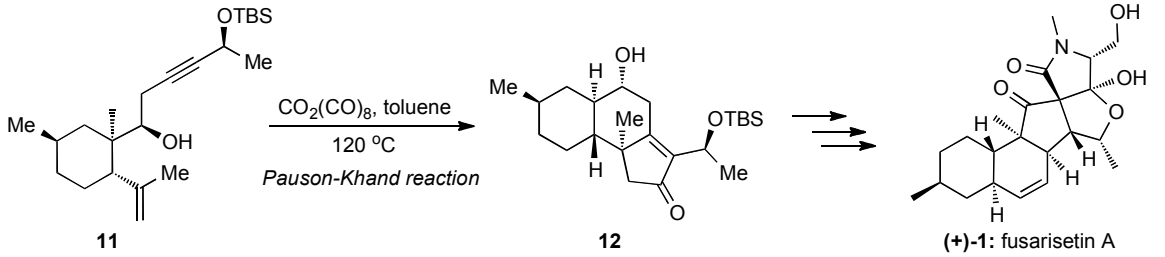
Scheme 2.3.1 Ley's enantioselective synthesis of (–)-equisetin

Not surprisingly, Li and co-workers[18] would use Ley's chemistry to develop a synthetic strategy toward's the synthesis of (–)-fusarisetin A. Parting from β-ketothioester **7**, which was generated *via* Ley's protocol starting from (*S*)-(–)-citronellal, Li was able to construct the C ring through a Pd-catalyzed O-C allylic rearrangement (Scheme 1.3.2). Peptide coupling of the serine moiety, followed by Wacker oxidation and finally $NaBH_4$ reduction/Dieckmann condensation sequence yielded fusarisetin A and it's C_5 epimer. The synthetic material gave the exact opposite optical rotation as the isolated natural product, therefore reassigning the absolute stereochemistry of fusarisetin A and providing insight to the biosynthetic relationship of (–)-equisetin and (+)-**1**.



Scheme 2.3.2 Li's synthesis of (–)-fusarisetin A and its C_5 epimer

After our synthetic work towards the synthesis of fusarisetin A was published,[19] Yang and co-workers[20] reported an asymmetric total synthesis of (+)-**1**. Yang devised a different strategy in which the generated the ABC ring system of fusarisetin A *via* a Pauson-Khand reaction.[21] Final fictionalization along with the well-described Dieckmann condensation yielded the natural isomer of fusarisetin A.



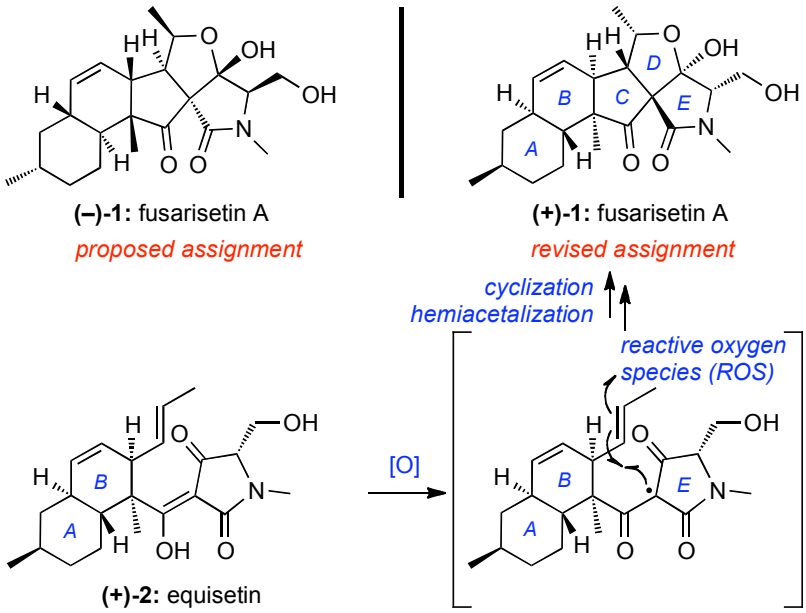
Scheme 2.3.3 Yang's synthesis of (+)-fusarisetin A

2.4 Synthesis of (–)-Fusarisetin A

Intrigued by this molecule, we devised a project focused on the development of a scalable chemical synthesis that may allow the study of its chemical biology. The developed strategy is short, efficient and stereoselective and is highlighted by the use of a key oxidative radical cyclization (ORC) reaction that allows conversion generation of the CD ring system of fusarisetin A (**1**) following a likely bioinspired pathway. Moreover, we able to generate a more robust and scalable approach in a 2nd generation synthesis *via* equisetin that represent a biomimmetic approach to fusarisetin A and related analogues. Herein we report a detailed account on the synthesis of fusarisetin A.

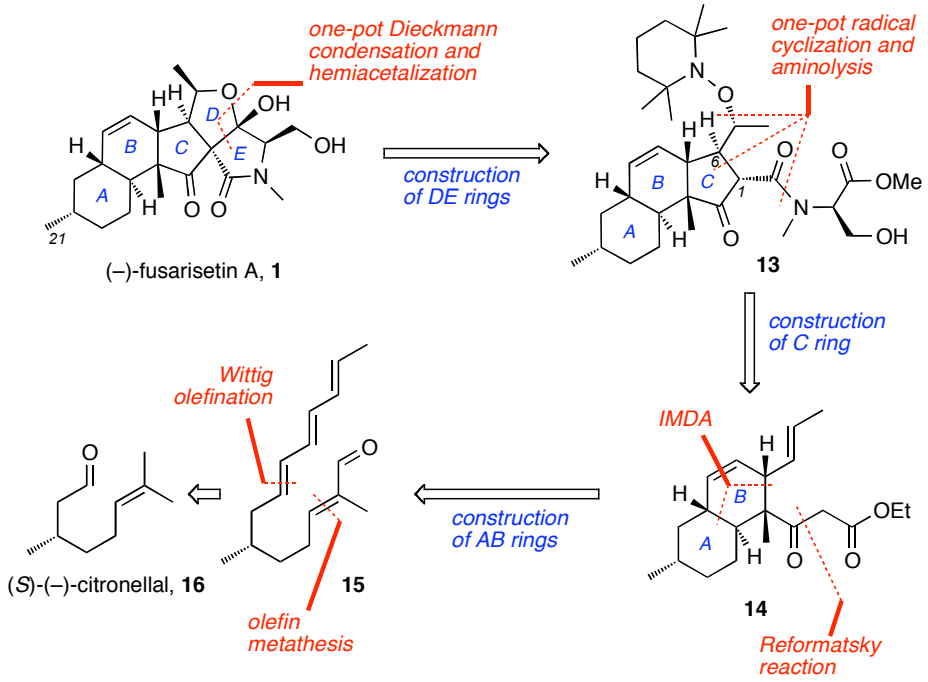
2.4.1 Retrosynthetic analysis

Close inspection of the fusarisetin framework, specifically after reassigment of absolute stereochemistry, reveals the fusion of a *trans*-decalin unit (AB ring system) with a tetramic acid moiety (E ring). These rings can also be found in the structure of equisetin (**2**),[16,22] another secondary metabolite produced by a *Fusarium* species, suggesting that both molecules may arise from a common biosynthetic pathway (Scheme 2.4.1).[23] Along these lines, we hypothesized that **1** derives biogenetically from oxidation of **2** upon exposure to reactive oxygen species (ROS).[24] This biosynthetic scenario could account for the formation of stabilized radical **12** that, upon cyclization at the pendant alkene followed by trapping by ROS and hemiketalization, would produce the CD ring system and furnish **1**.



Scheme 2.4.1.1 Biosynthetic hypothesis of the relationship of equisetin to fusarisetin A

Translating this proposal to a synthetic plan, we had envisioned that (–)-fusarisetin A could arise from a one-pot Dieckmann condesation and hemiketalization to from de DE ring. A β-ketoamide intermidiate could undergo a 5-*exo*-*trig* oxidative radical cyclization (ORC)[25] to form the C_1-C_6 bond. The resulting C_5 radical could then be trapped by ROS like TEMPO. Further bond disconnection suggested that **1** could be produced from b-ketoester **4**, which would arise from a Reformasky reaction with ethylbromoacetate and the correponding aldehyde. The *trans*-decalin motif of which (AB ring system) could be made via an Lewis acid induced intramolecular Diels-Alder reaction (IMDA).[26] This scenario led to consider polyene **5** as the potential precursor of the IMDA. In turn, **5** could be synthesized through an olefin metathesis and Wittig olifenation from commercially available citronellal (**6**) whose motif contains the C_{21} methyl group with the desired stereochemistry.[22]

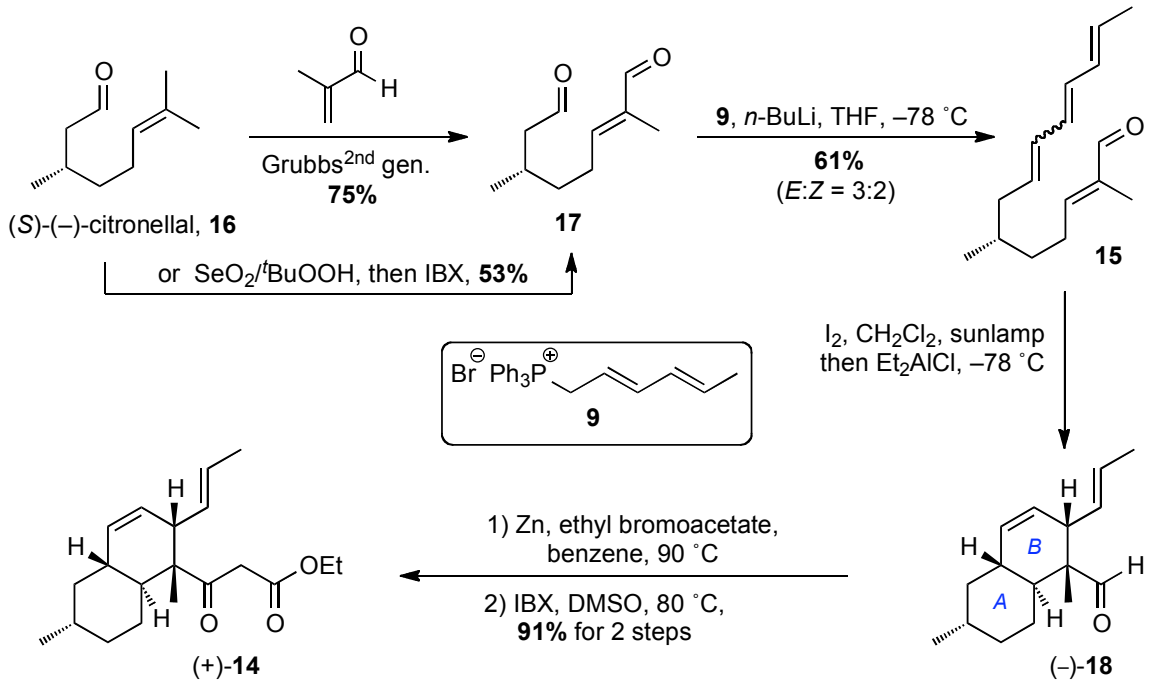


Scheme 2.4.1.2 Retrosynthetic analysis of (–)-fusarisetin A

2.4.2 Synthesis of the AB decalin ring system

Guided by the original assignment of fusarisetin A,[13] we started our synthesis with commercially available (*S*)-(–)-citronellal (***ent*-16**) (Scheme 1).[27] Inspiration for our studies came from previously reported syntheses of equisetin by the Danishefsky, Dixon and Shishido groups.[14,28] With an eye toward step-economy,[29] we sought to develop an alternative synthesis of this compound. To this end, ***ent*-17** was synthesized from ***ent*-16** via cross-metathesis with methacrolein using a Ru-carbene catalyst (Grubbs 2nd generation, 5 mol%)[30] (75% yield). Alternatively, allylic oxidation of ***ent*-16** with SeO_2/IBX can also form ***ent*-17** in 65% yield. The two chemically differentiable carbonyl groups of this compound provide the possibility to install the polyene motif in a regioselective

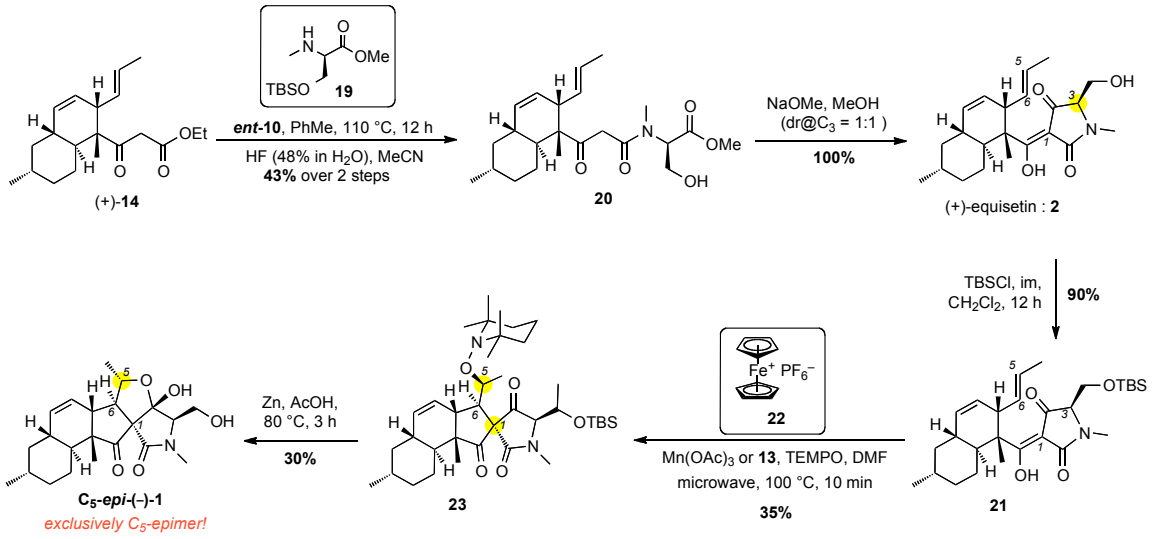
manner. Initial olefination studies of **17** under HWE or Julia[31] conditions proved to be unsatisfactory.[24b,c] However, slow addition of the Wittig ylide, generated upon deprotonation of phosphonium salt **9**,[32] to **17** afforded polyene **15** in 61% overall yield as a mixture of *E/Z* isomers (*E*:*Z* = ca 3:2). Photo-induced isomerisation of this mixture with catalytic amount of iodine[33] produced exclusively the *trans* polyene. Without purification, this compound was subjected to a Et_2AlCl-promoted IMDA reaction, that stereoselectively produced the desired *trans*-decalin aldehyde (–)-**18** (dr > 10:1, 82% yield). It is noted that the rapid construction of this *trans*-decalin motif could grant access to other biologically interesting natural products.[34] Treatment of (–)-**18** with ethyl bromoacetate under Reformatsky conditions followed by IBX oxidation yielded b-keto ester (+)-**14** in 91% combined yield.



Scheme 2.4.2.1 Synthesis of the AB ring system *via* an IMDA reaction

2.4.3 Synthesis of the CDE ring system

In our initial efforts to synthesize the CDE ring of fusarisetin A, me invisioned to target equisetin (**2**) as a key synthetic precursor. To those effects, aminolysis of (+)-**14** with (*D*)-serine derivative **19**, followed by deprotection of the TBS group, produced β-ketoamide **20** (43% yield overall). Dieckmann condensation of **20** produced a mixture of (+)-equisetin together with its C_3-epimer (*ent*-C_3-*epi*-**2**) (100% yield, dr = 1:1).[35] It should be noted that the tendency of equisetin to epimerize at the C_3 center under basic conditions has been previously reported[36] and has been observed consistently in our studies. Low temperature ^{1}H NMR experiments have also confirmed that equisetin exists exclusively in the enol form. The structures were also confirmed by comparison with the known data.[14,16,22,28]

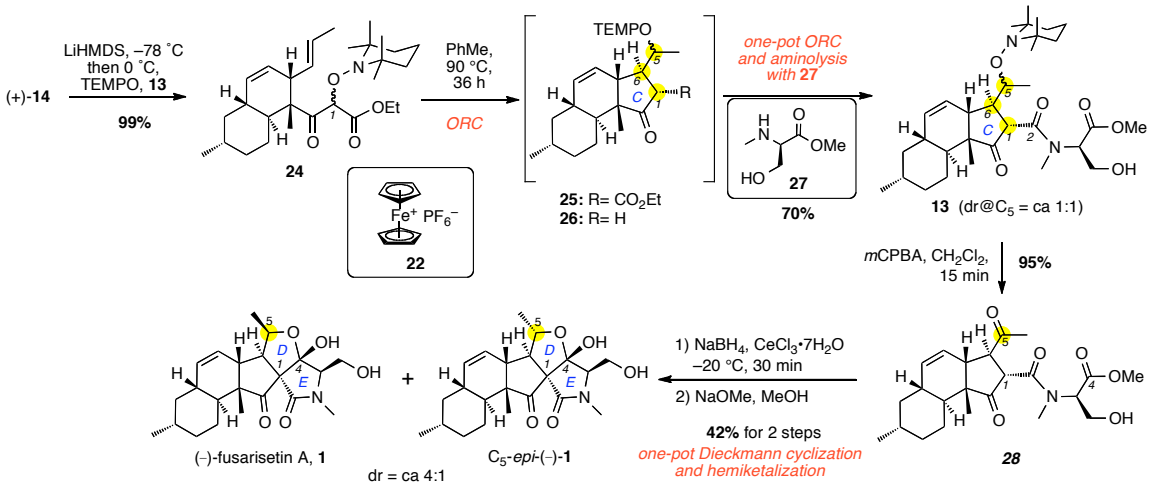


Scheme 2.4.3.1 Conversion of (+)-equisetin to C_5-*epi*-(–)-**1** through an ORC reaction

With *ent*-**2** in hand, we sought to explore ORC processes for the formation of the C ring of fusarisetin. It is worth noting that although radical reactions have often been used in natural products synthesis for the construction of C-C bonds,[37] their application to the formation of C-O bonds remains limited.[38] A report by Jahn *et al* on the construction of 5-membered rings, using 1,3-dicarbonyl groups and alkenes under TEMPO conditions, provided a possible way for the desired transformation.[39] However, our initial studies with ***ent*-2** gave unsatisfactory results, presumably due to the sensitivity of its C_3 hydroxymethyl group. To overcome this issue, we protected equisetin as its TBS ether **21** (90% yield). Gratifyingly this compound underwent the desired ORC, using ferrocenium hexafluorophosphate (**13**) or Mn(III) acetate as the oxidants,[40] to afford cyclized TEMPO-product **23** albeit in moderate yield (35%). Mechanistically, this reaction proceeds via a heat-promoted homolytic cleavage of the TEMPO-C_1 bond.[41] The resulting stabilized radical at C_1 reacts with the pendant C_5-C_6 alkene to generate the C_5-radical that can subsequently be trapped by the available TEMPO.[42] Reduction of the alkoxylamine bond of **23** under Zn/AcOH conditions[43] liberated the C_5-alcohol that underwent the desired hemiketalization, along with concomitant deprotection of the TBS group, to form a compound that was spectroscopically identified as the C_5-epimer of (–)-fusarisetin A (**1**).[18]

The results of this study allowed us to draw several conclusions related to the TEMPO-mediated ORC reaction. As predicted, the TEMPO can indeed act as an ROS synthetic alternative and could form the C ring of **23** albeit in low yield. Gratifyingly, the stereochemistry of the C_1-C_6 bond was efficiently cotrolled by the structure of the decalin ring. Unfortunately, the stereochemistry of the C_5 center was not the desired one. Moreover, we encountered difficulties applying this reaction to a non-protected equisetin (***ent*-2**). These considerations prompted us to apply the TEMPO-mediated ORC on a

less functionalized substrate. β-Keto ester (+)-**14** appeared to be an attractive substrate for the TEMPO-mediated ORC, since it is less functionalized than equisetin and also contains an easily oxidizable C_1 center. With this in mind, **14** was treated with LiHMDS and the resulting C_1 enolate was *in situ* oxidized with **13** to afford, after quenching of the C_1 radical with TEMPO, compound ***ent*-15**. As expected, under these conditions (5 min, 0 °C) the ORC did not occur and ***ent*-15** was isolated and fully characterized as a mixture of C_1-isomers (ca 2.5:1) in 99% yield. Heating this isomeric mixture at 90 °C over a period of 36 h gave rise to the tricyclic motif of ***ent*-16** via the desired 5-*exo*-*trig* cyclization. Similarly with the above study, the formation of the C_1-C_6 bond proceeded with excellent stereocontrol, presumably due to the stereochemical bias of the decalin motif. Interestingly however, in this case we obtained a mixture of stereoisomers at C_5 (ca 1:1). It is worth mentioning that attempts to decrease the reaction time by raising the temperature proved to be problematic since they led to significant amounts of decarboxylated product ***ent*-17.**



Scheme 2.4.3.2 Total synthesis of (–)-fusarisetin A (**1**) via a TEMPO-mediated ORC

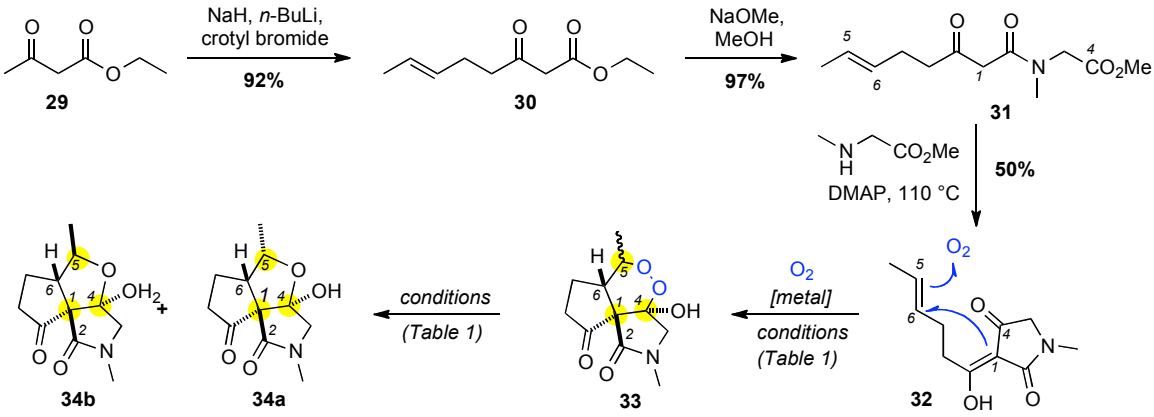
To further enhance the overall efficiency, we also examined the one-pot ORC and aminolysis sequence in presence of serine derivative ***ent*-18**. To our delight, this one-pot reaction gave rise to compound ***ent*-19** (C_5 dr = ca 1:1) in 70% overall yield. To avoid the difficult separation of these diastereomers, ***ent*-19** was directly treated with *m*-CPBA[44] to oxidatively cleave the *N*-*O* bond producing ***ent*-20** in 95% yield. Regio- and stereo-selective reduction of this compound under Luche conditions[45,18] followed by a one-pot Dieckmann condensation/hemiketalization yielded (–)-fusarisetin A (***ent*-1**) together with its C_5-epimer (dr = ca 4:1, 42% over 2 steps). Synthetic (–)-fusarisetin A was identical in all aspects with naturally occurring fusarisetin A (^{1}H-NMR, ^{13}C-NMR and HR-MS), except for the optical rotation (synthetic: $[a]_D^{23}$ = –86.2 (c = 0.065 in MeOH); natural: $[a]_D^{25}$ = +84.6 (c = 0.2 in MeOH)[13], reported synthetic (–)-**1**: $[a]_D^{27}$ = –88.0 (c = 0.15 in MeOH).[18] The structure of ***ent*-C_5-*epi*-1** was confirmed by comparison to the literature data.[18]

2.5. Synthesis of (+)-fusarisetin A

After successful completion of the total synthesis of (–)-**1** our interest turned to make the natural isomer of fusarisetin A to begin the exploration into the chemical biology of these novel phamacophores. For this reason, we proposed that the biosynthetic relationship of equisetin and fusarisetin could be approached in a synthetic study. Here in, we report the synthesis of (+)-fusarisetin A *via* a biomimmetic strategy that would prove to not only be efficient but also scalable. Our strategy allowed for large amounts of natural fusarisetin A to be prepared and for this reason facilitate multiple biological studies that begin to describe the chemical biology of (+)-**1**.

2.5.1 Biomimetic approach and model studies

Upon completion of the first generation synthesis of fusarisetin A, we focused our efforts on the evaluation of metal-promoted ORC reactions. We had correctly hypothesized that equisetin and fusarisetin A were related based on stereochemistry of the ABD ring structure as well as the reactivity profile in the presence of ROS. The feasibility of this proposal was initially evaluated in model system **32** that contains all key carbons needed for the proposed cyclization (Scheme 3). This compound was prepared via a sequence of 3 steps that included: (a) kinetic alkylation[46] of ethyl acetoacetate (**29**) with crotyl bromide to form **30**; (b) aminolysis of the ester group with *N*-methyl glycine methyl ester to produce **31**; and (c) Dieckmann condensation (formation of the C_1-C_4 bond) to yield **32** (45% yield overall). Tetramic acid **32** was then subjected to various reagents and conditions in order to perform the desired ORC (Table 2.5.1.1). Scarce literature reports[38] indicate the feasibility of this transformation that, nonetheless, has never been applied to intramolecular systems or any natural product synthesis.



Scheme 2.5.1 Model studies of metal-promoted ORC reactions

Oxidant	Solvent	Temp (°C)	Time	Yield of **25**	Reductant	Yield of **26**
$Co(OAc)_2$	AcOH	70	5 min	20%	CuCl	80%
$Co(OAc)_2$	AcOH	70	5 min	20%	thiourea	n.r. [c]
$Co(OAc)_2$	AcOH	25	4 h	10%	CuCl	79%
$Co(OAc)_2$	^{i}PrOH	25	12 h	n.r.	-	-
$CoCl_2$	AcOH	25	12 h	n.r.	-	-
$Mn(OAc)_3$	AcOH	25	12 h	5%	-	-
$CeCl_3$	AcOH	25	12 h	trace	-	-
Fe(III)[d]	AcOH	25	12 h	15%	CuCl	81%
CAN	AcOH	25	3 h	57%	CuCl	82%
CAN	AcOH	–20	18 h	57%	CuCl	79%
CAN	AcOH	70	5 min	20%	-	-
CAN[e]	AcOH	25	3 h	40%	CuCl	80%
CAN[f]	AcOH	70	3 h	30%	CuCl	81%

[a] For a detailed screening study see SI. [b] all the reactions were performed under 1 bar of oxygen and 1 equiv of the oxidant unless otherwise noted. [c] no reaction occurred. [d] ferrocenium hexafluorophosphate. [e] 0.1 equiv of CAN was used. [f] reaction opened to air. CAN = cerium(IV) ammonium nitrate.

Table 2.5.1 Conversion of **32** to **33** and **34** via a metal-promoted ORC reaction.[a],[b]

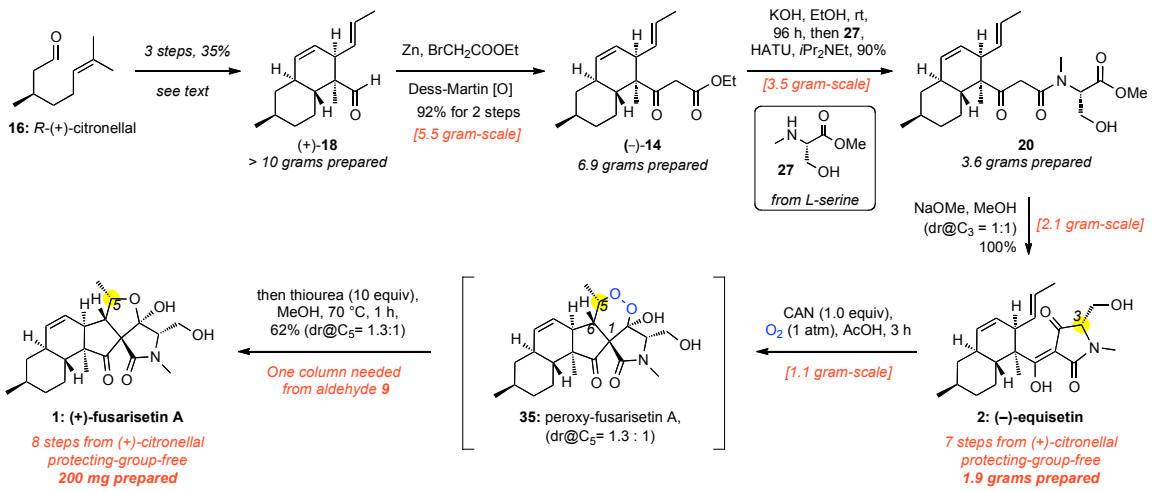
It is known that certain high oxidation state metals, such as Mn(III), Co(II), Ag(II), Pd(II), Pb(IV) and Ce(III), can promote the addition of carbon radicals derived from ketones to alkenes.[42] On the other hand, molecular oxygen exists as a persistent triplet diradical in its ground state and as such it can react rapidly with carbon-centered radicals.[43] With this in mind, we treated **24** with various metals in the presence of O_2 and, in certain cases, were able to isolate peroxyhemiketal **25** (as C_5 isomers). Reduction of the peroxide motif of **25** then produced **26** (as C_5 isomers) representing the tricyclic core of fusarisetin A.

2.5.2 Scalable synthesis of (–)-equisetin and (+)-fusarisetin A

Encouraged with these results, we proceeded to implement the optimized ORC conditions to the synthesis of (+)-fusarisetin A, the natural occuring isomer, using (–)-equisetin, also the natural occuring stereoisomer, as the key synthetic intermediate (Scheme 4). It is worth noting that during the course of our research, Gao and co-workers[47,] instated our working hypothesis to convert (–)-equisetin to (+)-fusarisetin A using a metal oxidant and O_2 gas as the ROS. Even so, our unique approach to the decalin system combined with our optimized ORC reaction represented an extremely efficient, short and scalable route to access (+)-**1**.

Along tbese lines, decalin aldehyde **18** was rapidly and stereoselectively constructed from (*R*)-citronellal (**16**) in decagram-scale (35% over 3 steps) following the above procedure. Conversion of **18** to 1**4** proceeded under Reformatsky conditions followed by oxidation of the resulting alcohol with Dess-Martin periodinane (2 steps, 92% overall yield, ca 7 grams prepared). It is worth noting that the DMP oxidation protocol is highly dependent on the quality and freshness of the DMP oxidant. Also, IBX oxidation when heated to 80 ^{o}C produced significant amounts of decarboxylated material. We can circumvent this problem by mild heating (45 ^{o}C) in which case no decarboxilation is observed and more reproducible high yields are obtained. Aminolysis of ester **4** under various conditions, such as DMAP[18,19] and NHC-based reagents,[48,] with (L)-*N*-methyl serine methylester (**27**)[49,] afforded **11** in low yield. However, mild hydrolysis of **14** with ethanolic KOH (rt, 96 h) quantitatively produced the corresponding carboxylic acid that, upon coupling with **18** under HATU conditions afforded the desired amide **20** in 90% yield. Dieckmann condensation of **20** quantitatively produced a mixture of (–)-equisetin

(**2**) and C_3-*epi*-equisetin (**C_3-*epi*-2**) (100%, dr = ca 1:1).[14,16,36,47,50,] Gratifyingly, the previously defined ORC conditions were successfully applied for the conversion of (–)-equisetin (**2**) to (+)-fusarisetin A (**1**). Specifically, oxidation of **2** under CAN/AcOH/O_2 conditions produced an inseparable mixture of peroxy-fusarisetin A (**35**) and its C_5 epimer (**C_5-*epi*-35**) (dr = 1.3:1). This mixture was further reduced with thiourea (CuCl reduction interestingly failed) to afford (+)-fusarisetin A (**1**) together with its C_5 epimer (**C_5-*epi*-1**) (62% overall, dr = 1.3:1). The structures of both C_5 epimers of **35** and **1** have been confirmed by ^{1}H-NMR, ^{13}C NMR and HR-MS analysis.[13,18,47] As observed previously, the stereoselectivity of this ORC reaction is substrate-controlled and affords the desired stereochemistry at the C_1 and C_6 centers. It is worth noting that after our synthetic work had finished, a patent filed by the isolation authors[51,] became available to the public. It revealed that the C_5 epimer of fusarisetin A is in fact another natural product (from now on fusarisetin B) that was isolated from the same *Fusarium* strain.



Scheme 2.5.2.1 Scalable total synthesis of (–)-equisetin and (+)-fusarisetin A

We were also able to use the mixture of equisetin (**2**) and C_3-*epi*-equisetin (C_3-*epi*-**2**) in the ORC reaction. In fact, 1.1 grams of this mixture (dr = 1:1) were treated under CAN/AcOH/O_2 conditions and the resulting crude mixture of peroxy-fusarisetins was reduced with excess thiourea. Purification of this mixture produced 200 mg of (+)-fusarisetin A (**1**). Notably, the whole synthetic process from decalin (+)-**18** to (+)-fusarisetin A and B was performed on gram-scale and requires only one purification via column chromatography. In summary, the syntheses of both **1**, **2** and are scalable, redox-/step-economic and protecting-group free. [52,]

2.6 Chemical biology of fusarisetin A

Even though extensive synthetic investigations had been done, no further reports had been published on the chemical biology of fusarisetin A. For this reason, our chemical investigation geared into a more biological approach and led us to experiment with MDA-MB-231 cell lines to better understand the biological significance of **1**. Here in, we report our research towards understanding the chemical biology of (+)-**1**.

2.6.1 Cell migration assays

To confirm and expand upon the previously reported findings, we evaluated the biological activity of (+)-fusarisetin A (**1**) in a scratch-wound assay and in a Boyden Chamber Transwell assay. The first assay (Figure 2.7.1.1) involves inflicting a scratch wound in a confluent cell monolayer and measuring the migration of cells.[53] We were pleased to find that synthetic **1** inhibited migration of these cells at concentrations as low as 1 μg/mL (Figure 2.7.1.1, C) as compared to vehicle control (B). Importantly, removing

1 from the cells followed by incubation with fresh growth media allowed cells to migrate in a similar fashion to the control experiment (D). This observation demonstrates that the effect of **1** on these cells is reversible, in turn suggesting that (+)-fusarisetin A has little to no cytotoxicity at 1 μg/mL concentration.

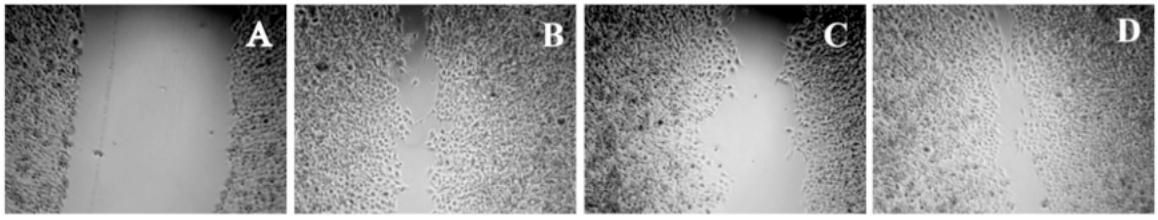


Figure 2.6.1.1 (+)-Fusarisetin A (**1**) reversibly inhibits the migration of MDA-MB-231 breast cancer cells in an *in vitro* scratch-wound assay.

We then performed Transwell migration assays using increasing concentrations of **1** normalized with the appropriate DMSO controls. This assay measures the capacity of cells to migrate across a porous membrane using serum-rich media as a chemo-attractant. [54] As seen in Figure 3, cell migration was significantly inhibited at 3.0 and 6.0 μg/mL, while almost complete inhibition is observed at 12.0 μg/mL. Even at this concentration we did not observe any changes in the cell morphology, suggesting that **1** exhibits low cytotoxicity. The results of these two assays confirm the reported biological activity of fusarisetin A *in vitro*. Encouraged by these findings, we then evaluated (+)-fusarisetin A in an *ex vivo* assay measuring migration of cells from a 5 mm mouse skin biopsy (Figure 2.7.1.3).[55] We observed that both keratinocyte and fibroblast migration is inhibited upon exposure to **1** (10 μg/mL). Specifically, a substantial amount of cell migration is observed 5 days after plating the skin explant (Figure 2.6.1.3, A), as compared to the initial time of plating (B). In contrast, when explants were exposed to 10 μg/mL of **1** (C), there was no detectable migration of keratinocytes from the explants,

while fibroblast migration was reduced by approximately 80%. The observed ability of fusarisetin A to inhibit cell migration from skin explants is particularly exciting. The migration of fibroblasts is an example of mesenchymal cell migration whereas keratinocytes move via collective cell migration.[56] Interestingly, compounds that target one type of migration have had disappointing results in clinical studies as the cancer cells are able to adapt and switch between different modes of migration.[57] Since fusarisetin A can significantly inhibit both types of migratory behavior, it could provide a powerful tool to circumvent the ability of cancer cells to alter their mode of motility if one pathway is inhibited.

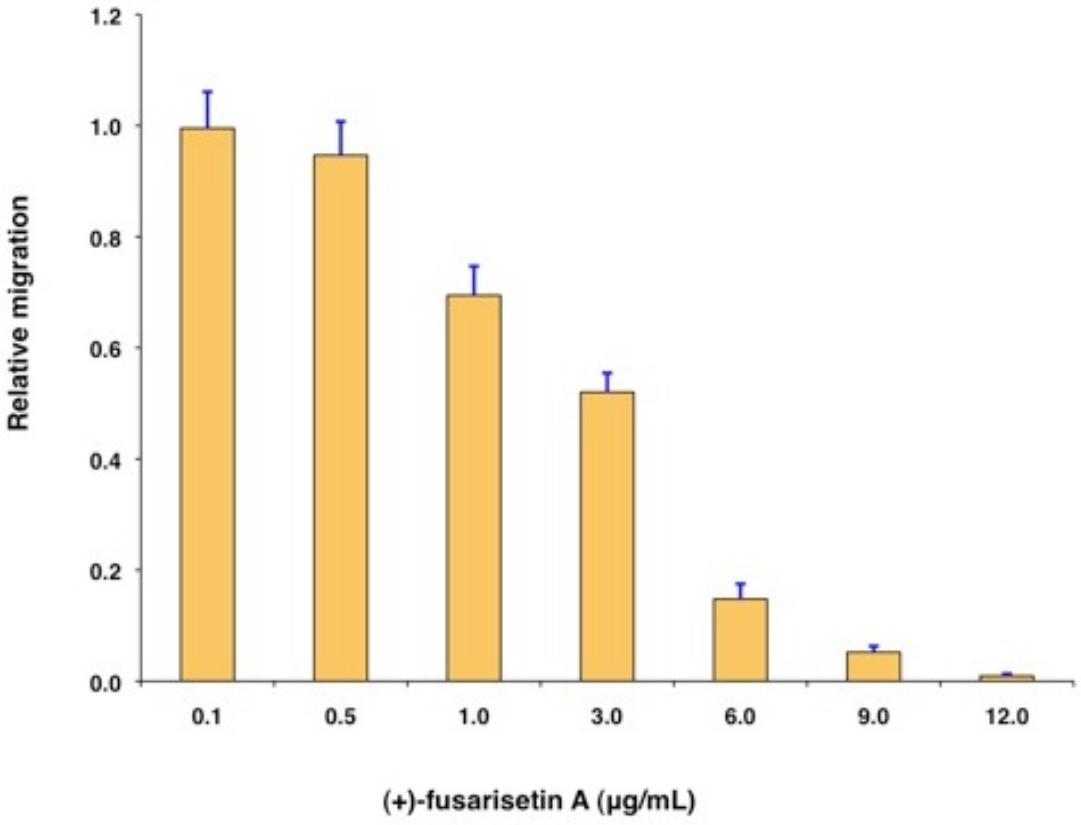


Figure 2.6.1.2 (+)-Fusarisetin A (**1**) inhibits the migration of MDA-MB-231 breast cancer cells in an *in vitro* Transwell migration assay

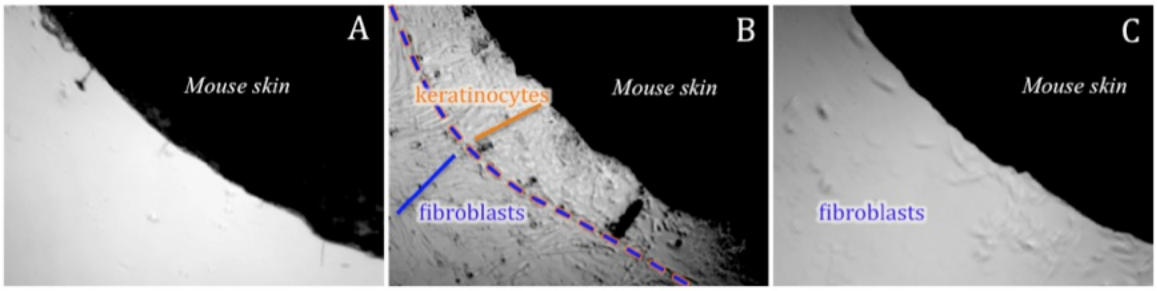


Figure 2.6.1.3 (+)-Fusarisetin A (1) inhibits cell migration in an *ex vivo* mouse skin assay.

Having demonstrated the cell-migration inhibitory properties of **1** both *in vitro* and *ex vivo* we then screened selected compounds containing the fusarisetin framework using the scratch-wound assay. We observed that natural (–)-equisetin (**2**), its enantiomer ***ent*-2**, and *ent*-fusarisetin A (***ent*-1**) do not exhibit any activity at the concentrations tested (Figure 2.6.1.4). However, the C_5 epimer of natural fusarisetin A (**C_5-*epi*-1**) was found to display similar activity to that of (+)-**1**. These initial findings attest to the importance of the CDE ring structure and suggest that only the naturally occurring enantiomer of fusarisetin A could be used as a motif for the identification of new inhibitors of cell migration.

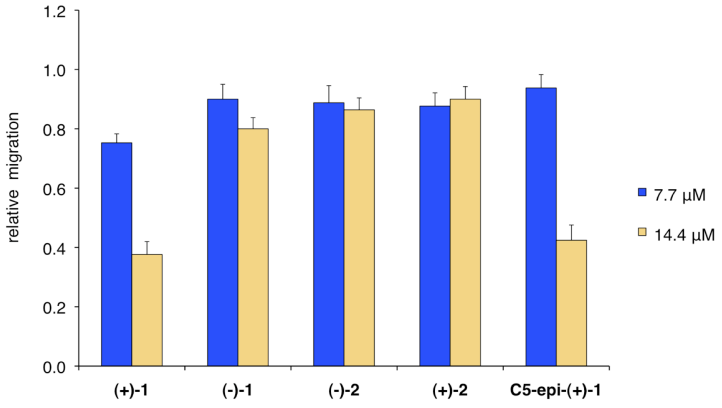
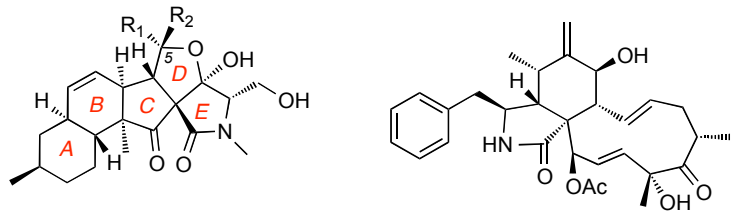


Figure 2.6.1.4 Screening of fusarisetin A (**1**), equisetin (**2**) and their stereoisomers

2.6.2 Fusarisetin A and actin networks

At the onset of this investigation, we evaluated the effects of **1** on the cell morphology and function. In general, motility inhibitors are known to interfere with microtubules,[58] actin[59] and/or cell adhesion processes.[60] For instance cytochalasin D (**3**), a natural product that binds to actin filaments and induces actin depolymerization, is a well-known cell motility inhibitor.[61] Intrigued by the observation that **3** is structurally and biogenetically related to **1**,[62] we sought to compare their effects *in vitro*.



1: R_1 = Me, R_2 = H: (+)-fusarisetin A
36: R_1 = H, R_2 = Me: (+)-fusarisetin B

37: cytochalasin D

Figure 2.6.2.1 Structures of fusarisetin A, fusarisetin B and cytochalasin D

Incubation of cells with **3** induced the expected actin depolymerization as shown by intense staining of monomeric actin and lack of actin fibers (Figure 2.7.2.1, column A). This effect was reversible, since removal of **3**, by washing the cells with PBS and re-incubation with vehicle control, led to recovery of the actin network (Figure 2, column B). Removal of **3** followed by re-incubation with growth media containing **1**, led to reconstitution of actin filaments (Figure 2, column C), indicating that fusarisetin A does not affect actin polymerization. Similar observations were made upon sole treatment of cells with **1** (Figure 2, column D). Moreover, in all cases we observed healthy microtubules morphology (Figure 2). Based on these findings we can conclude that

fusarisetin A does not affect actin nor microtubules dynamics. These results, parallel previous findings[13] and further support the notion that fusarisetin A acts via a novel mechanism of action that is distinctly different from those of known anti-migration agents.

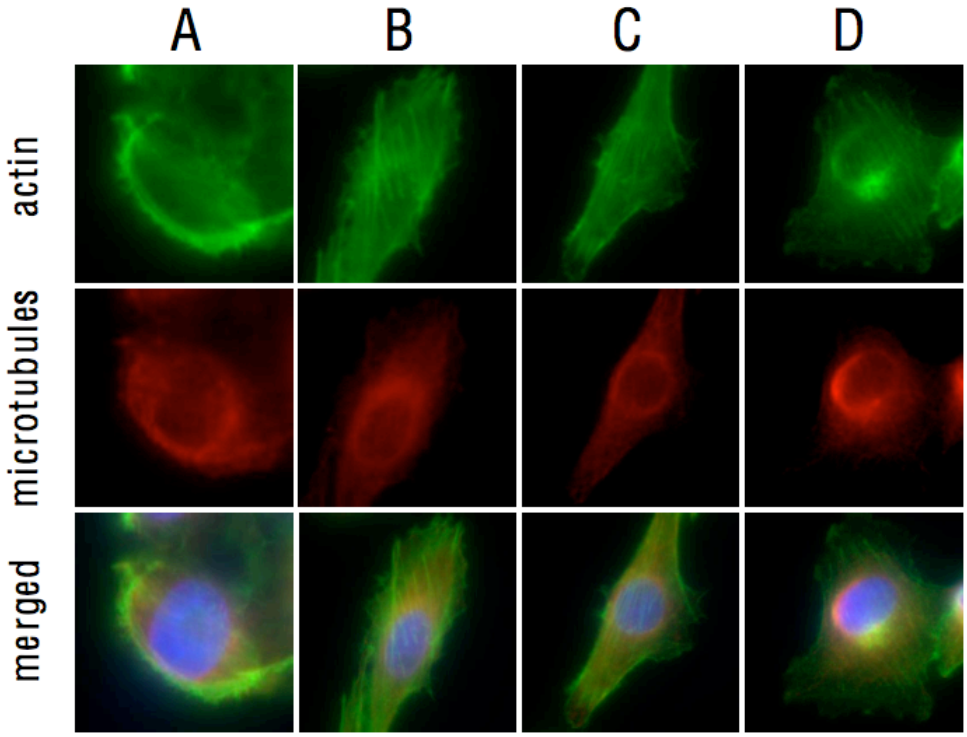


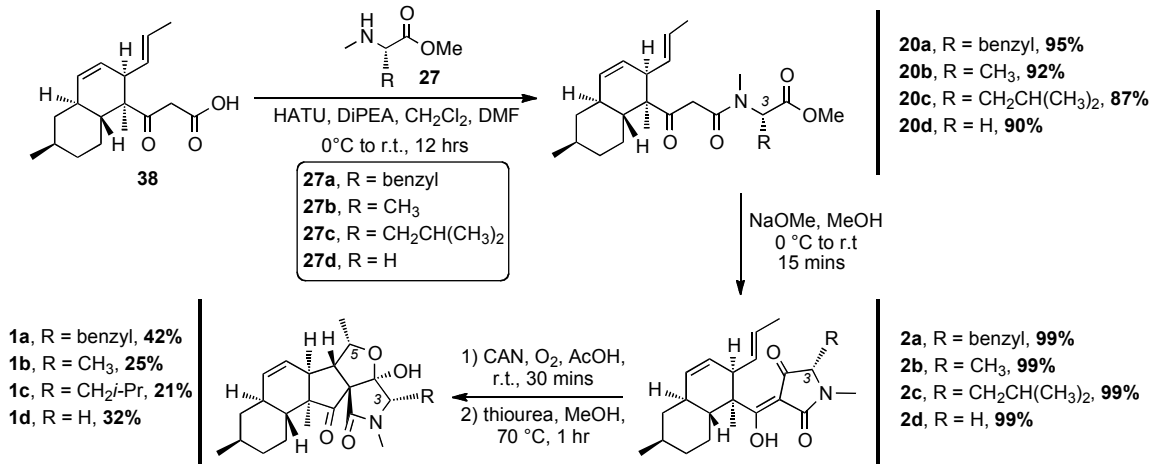
Figure 2.6.2.2 Effect of cytochalasin D and fusarisetin A on actin and microtubule dynamics.

2.6.3 Synthesis of analogues and biological evaluation

At present, the key structural features of fusarisetins that account for their anti-motility properties are unknown. To address this issue, we sought to construct a library of analogs in which key reactive sites of **1** were systematically evaluated for their

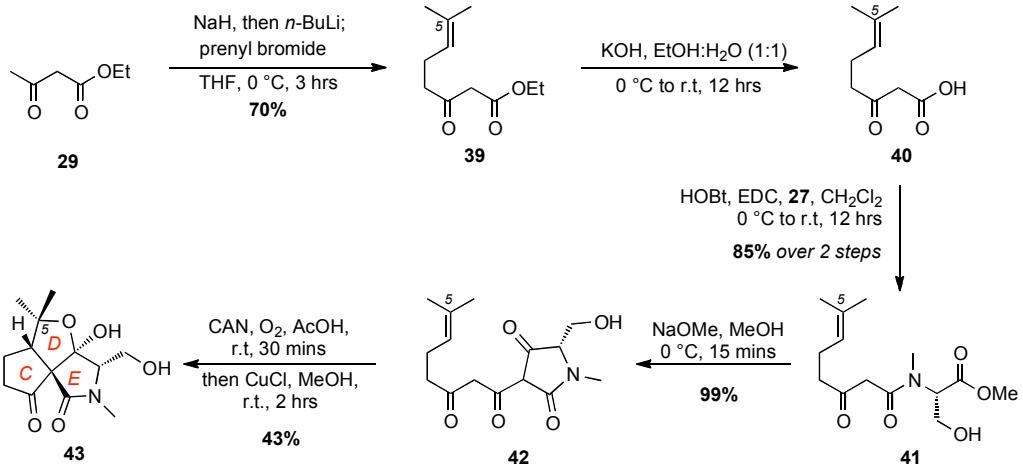
bioactivity. Interestingly, incorporation of various amino acids has been shown to occur in biosynthetically related natural products that derive from the same gene cluster of *Fusarium* species.[62] We began this study by exploring the biological significance of the *N*-methyl serine moiety in fusarisetin A by varying the amino acid motif (C3 modification) (Scheme 1). Our synthetic strategy towards **1** allows coupling of *N*-methyl serine with β-keto acid **38**, and conversion of the resulting amide to fusarisetin A via a key oxidative radical cyclization (ORC) reaction cascade. Using this strategy, β-keto acid **38** was coupled with the *N*-methyl amino methyl esters of phenyl alanine (**27a**), alanine (**27b**), isoleucine (**27c**) and glycine (**27d**) to produce compounds **20a**-**20d** respectively. Treatment of these adducts with NaOMe led to tetramic acid analogs **2a** to **2d** (86-94% over 2 steps). It is worth noting that during this Dieckmann condensation we observed only minimal epimerization at the C3 center. On the other hand, significant racemization at the C3 center was observed when a serine analog was cyclized under the same conditions en route to the synthesis of **1**.[15] This difference is attributed to the methyl hydroxy group of serine that inductively increases the acidity of the C3 proton.[63] Exposure to cerium ammonium nitrate in acetic acid under oxygen atmosphere followed by reduction of the resulting endoperoxides,[64] produced fusarisetin analogs **1a-1d**.

The AB decalin ring system of fusarisetins is also present in a wide array of natural products.[65] It was hypothesized that the biological properties of **1** arise from the unique architectural motif of its CDE ring system. We also speculated that the stereochemistry at the C5 center is not critical to the fusarisetin bioactivity since both fusarisetin A (**1**) and B (**36**) are equipotent.[51] With this in mind, we developed a synthesis of truncated analog **12** containing a C5 dimethylated center that, in turn, simplifies the stereoisomeric ratio obtained during the ORC reaction (Scheme 2.6.3.2). Compound **43** was synthesized *via* the following sequence: (a) kinetic alkylation of ethylacetoacetate



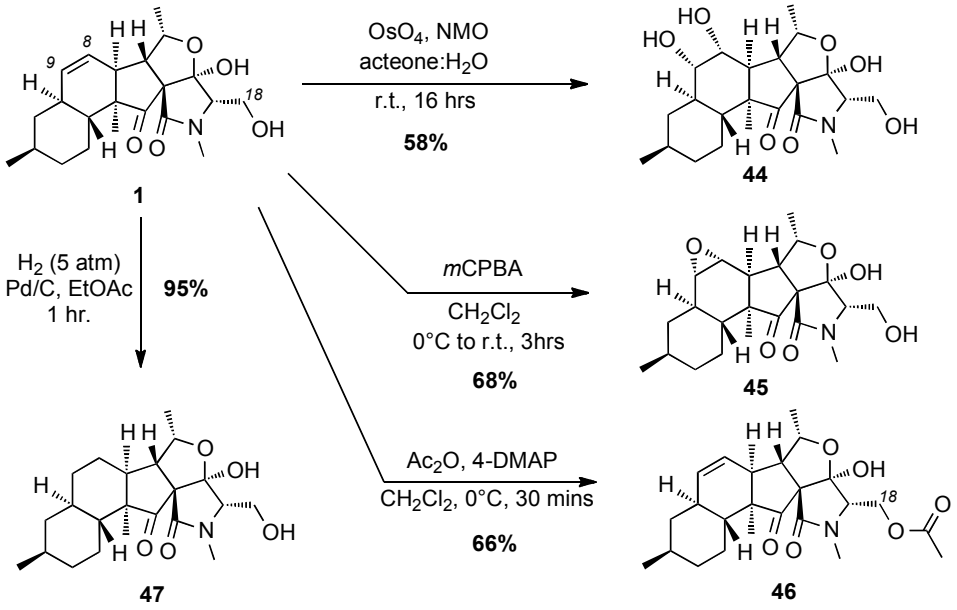
Scheme 2.6.3.1 Synthesis of C3 analogs via a peptide coupling

(**29**) with prenyl bromide to form **39**; (b) saponification of the ethyl ester followed by coupling of the resulting carboxylic acid (**40**) with **27** to produce **41** (85% yield over 2 steps); (c) Dieckmann condensation (NaOMe/MeOH); and (d) ORC reaction (CAN, O_2) followed by endoperoxide reduction (CuCl) to form **12** (34% yield over 2 steps).



Scheme 2.6.3.2 Synthesis of CDE core fusarisetin analog **43**

We then explored the structure/function relationship of the decalin system of **1** by functionalizing the C_8-C_9 alkene (Scheme 3). OsO_4-catalyzed dihydroxylation produced diol **13** (58% yield). The stereoselectivity of this reaction has been unambiguously confirmed by X-ray structure analysis.[13] Similarly, treatment of **1** with *m*CPBA selectively afforded epoxide **14** (68% yield). Reduction of the C8-C9 alkene proceeded under Pd-catalyzed hydrogenation conditions to afford saturated analog **15** (95% yield). On the other hand, acetylation of the C18 hydroxyl group produced acetate **16** (66% yield).



Scheme 2.6.3.3 Synthesis of derivatives of fusarisetin A

Initial cell-based evaluation of all fusarisetins (synthetic material,[15] 1-100μM) was performed using a well-described scratch wound assay.[53] MDA-MB-231 cells were grown as a confluent monolayer, scratched and treated with analogs over a 24 hour period. Compounds **8a-8d** proved to be inactive up to 100 μM concentrations suggesting that the hydroxy-methyl group of serine plays a significant role in the biological activity of

1. Truncated fusarisetin **12**, representing the CDE motif, was also inactive in the scratch wound assay even at high μM concentrations (Table 1). Among the synthetic fusarisetin derivatives (compounds **13**-**16**) neither epoxide **14** nor reduced analog **15** showed any activity *in vitro.* However, dihydroxylated derivative **13** (Figure 2.5.1.3, C) and acetate **16** (Figure 2.5.1.3, D) demonstrated inhibition of wound healing as compared to the original wound (A) and DMSO control (B) shown in Figure 2.5.1.3. These compounds were then subjected to a Boyden chamber Transwell assay to quantitatively determine their anti-motility activity (Figure 4). The IC_{50} values of fusarisetin analogs are shown in Table 1.

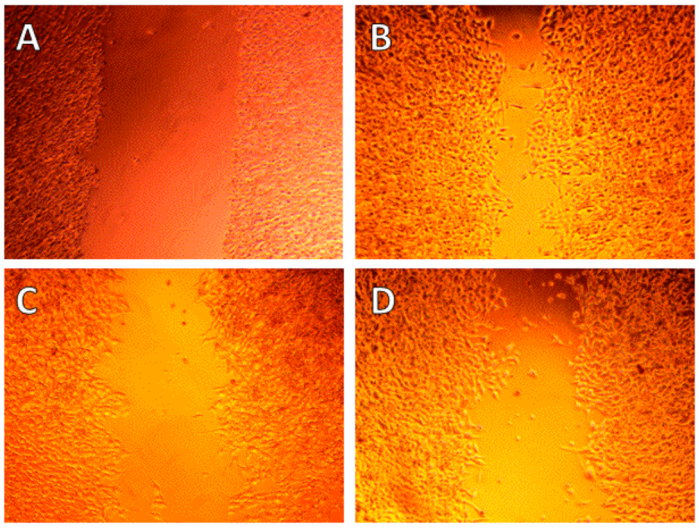


Figure 2.6.3.1 Evaluation of synthetic fusarisetins in a scratch wound assay

Figure 2.6.1.5 summarizes the observed structure-function relationship of fusarisetins. Comparison of the bioactivity data indicates that: (a) both the AB decalin motif and the serine amino acid are critical to the biological profile of fusarisetins; (b) the C5 stereochemistry is insignificant to the activity; and (c) although the B ring alkene is significant to bioactivity, its dihydroxylation can lead to analogs that maintain the

bioactivity albeit at a higher concentration. Similarly, acetylation of the C18 oxygen produces a compound that inhibits cell migration at high μMolar concentration.

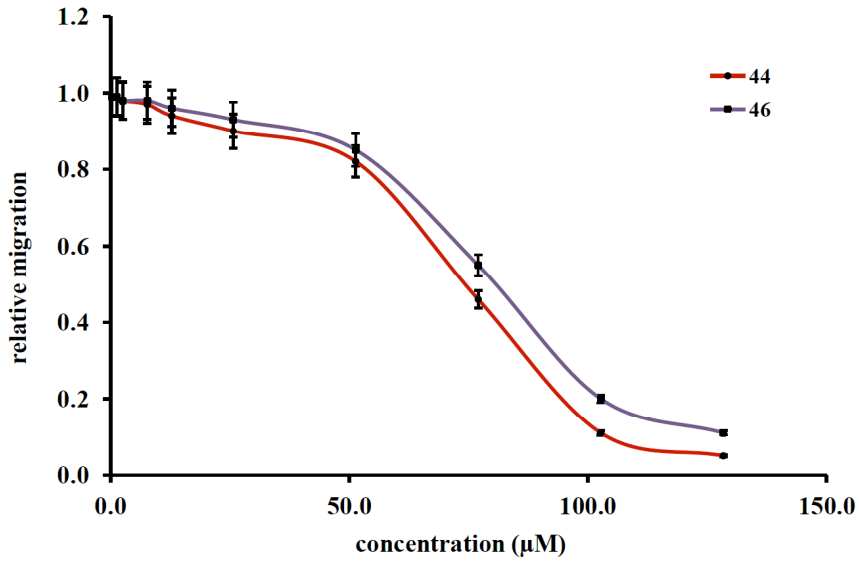


Figure 2.6.3.2 Dose-dependent inhibition of Transwell cell migration by **44** and **46**

Table 2.6.3.1 IC_{50} values of fusarisetin analogs

Compound	IC50 (μM)
1	7.7[15]
2	7.7[b]
1a	> 100
1b	> 100
1c	> 100
1d	> 100
43	> 100[c]
44	> 100
45	74.5 ± 3.2
46	> 100
47	85.3 ± 3.8

*[a]Compounds with no significant activity in the scratch wound assay (up to 100 μM) were not submitted to the Transwell assay. IC_{50} values of compounds **13** and **16** were determined by the Transwell assay. [b]Value obtained from references 10 and 15. [c]Compound **12** was tested as the racemate.*

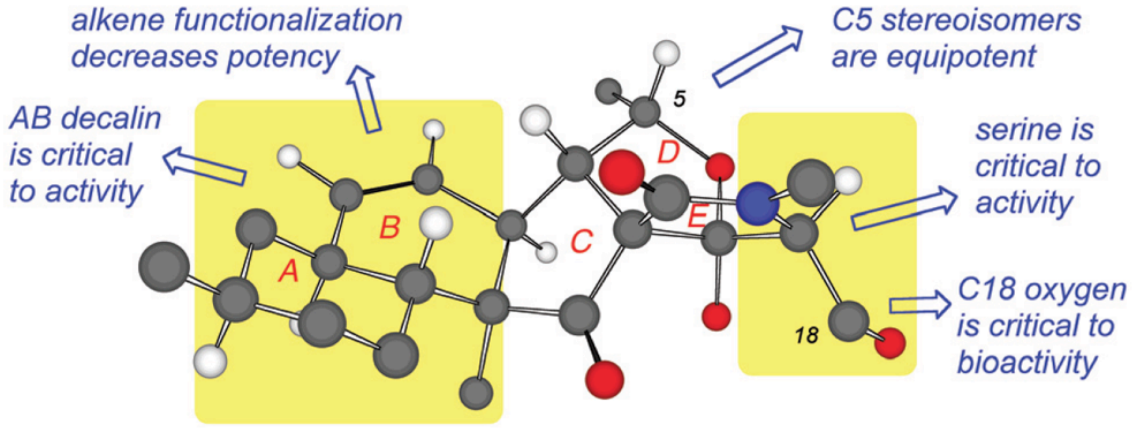


Figure 2.6.3.3 Structure-function relationship map of fusarisetins

2.7 Concluding remarks

We report here a concise, efficient, and protecting group-free synthesis of fusarisetin A (**1**). Key to our synthetic strategy is the implementation of a bioinspired oxidative radical cyclization (ORC) reaction that forms the C ring of **1** via stereoselective construction of the C_1-C_6 bond. Subsequent oxidation at the C_5 center allows formation of the D ring of **1** ultimately converting equisetin (**2**) to fusarisetin A (**1**). The TEMPO-mediated ORC reaction could be successfully applied for the conversion of β-ketoester **14** to a tricyclic motif **28** that, upon Dieckmann condensation/ hemiketalization, formed fusarisetin (**1**) together with its C_5 epimer (***C_5-epi*-1**). However, treatment of equisetin under these conditions produced exclusively the C_5 epimer of fusarisetin A (***C_5-epi*-1**). On the other hand, metal-mediated ORC reactions, such as $Co(OAc)_2$, $Mn(OAc)_3$, ferrocenium- and cerium(IV)-salts, could be successfully applied in a model system for the construction of the CDE ring of **1**. Moreover, the conversion of **2** to **1** was best

achieved using CAN/AcOH/O_2. Overall, the optimized synthesis of (+)-fusarisetin A proceeds in 8 steps from commercially available *R*-(+)-citronellal.

We have also confirmed that (+)-fusarisetin A exhibits potent inhibitory activities against cancer metastasis *in vitro* and demonstrated its capability to inhibit different types of cell migration in mice skin. Interestingly, equisetin (either enantiomer) and (–)-fusarisetin A were found to be inactive in these assays, while ***C*$_5$*-epi*-1** displayed comparable activities to that of the natural product. In turn, this suggests that the motif of (+)-fusarisetin A could lead to new potent cancer metastasis inhibitors.

The scalable synthetic strategy presented has paved the way for more detailed structure-activity relationship and chemical biology studies. Fusarisetin A, the archetype of this family, inhibits cell motility without directly targeting actin or microtubule networks. Empowered by our robust synthetic strategy, we have produced several analogs that were used to interrogate the biological significance of the fusarisetin framework. We found that structural modifications of this rigid scaffold, such as deletion of the AB ring system or replacement of the E-ring serine with other amino acids, result in acute loss of potency. However, subtle changes at the periphery of the fusarisetin motif, such as shuffling of the stereochemistry at the C5 center, result in retention of activity. Moreover, acetylation of the C18 hydroxyl group or dihydroxylation of the C8-C9 alkene produces compounds that maintain biological function albeit at higher concentration, suggesting that these functionalities could be used as tethering sites for further functionalization. Our observations strongly support the notion that fusarisetins operate via an unexplored mechanism of action associated with cell motility. Importantly, these findings attest to the uncharted and highly promising potential of fusarisetins as novel leads for the development of cancer metastasis inhibitors.

2.8 Experimental techniques and characterization data

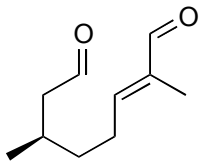
General Techniques

Unless indicated, all commercially available reagents and anhydrous solvents were purchased at the highest commercial quality and were used as received without further purification. All non-aqueous reactions were carried out under argon atmosphere using dry glassware that had been flame-dried under a stream of argon unless otherwise noted. Anhydrous tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh) using hexanes-EtOAc or CH_2Cl_2-MeOH mixtures of increasing polarity. The progress of all the reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel-60 F_{254} to a thickness of 0.5 mm (Merck), and compounds were visualized by irradiation with UV light and/or by treatment with a solution of ninhydrin stain or Ceric Ammonium Molybdate (CAM) stain followed by heating. ^{13}C NMR and ^{1}H NMR spectra were recorded on a 400 MHz, 500 MHz, 800 MHz Varian instrument or a 500 MHz JEOL instrument. $CDCl_3$ was treated with flame dried K_2CO_3, chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak reference ($CDCl_3$ or CD_3OD), with the abbreviations s, br s, d, t, q, m, td, dt and qd denoting singlet, broad singlet, doublet, triplet, quartet, multiplet, quartet of doublets, triplet of doublets, doublet of triplets and quartet of doublets, respectively. *J* = coupling constants given in Hertz (Hz). High resolution Mass spectra (HRMS) were recorded on a trisector WG AutoSpecQ spectrometer. Optical rotation data were collected on a Jasco P-1010 polarimeter using HPLC grade anhydrous $CHCl_3$ or anhydrous MeOH. Microwave experiments were

carried out in Biotage (model:Initiator) microwave reactor using high pressure vessels. Cell cultures were incubated in NABCO CO_2 6000 incubator and biological assays were performed in 24-well Falcon Multiwell (3047) cell dishes. Micrographs were processed with ImageJ software

Experimental procedure

Di-aldehyde 17 (Method 1): To a solution of (*R*)-(+)-citronellal (**6**, 1.45 ml, 1.23 g, 8.0 mmol, purchased from TCI America) in CH_2Cl_2 (150 ml) was added methacrolein (1.32 ml, 16.0 mmol) and Grubbs catalyst (2nd generation, 340 mg, 0.4 mmol). The reaction mixture was refluxed for 24 hours under argon atmosphere. The reaction was allowed to cool to room temperature and concentrated. The residue was purified via silica column chromatography (hexanes:EtOAc, 100:1 to 10:1) to recover the (*R*)-(+)-citronellal (205 mg, 17%) and yield the di-aldehyde **17** (1.01 g, 75 %, 90% brsm) as a pale yellow oil. R_f = 0.5 (silica gel, hexanes:EtOAc, 2:1); $[\alpha]_D^{23}$ = +13.7 (*c* = 1.0 , $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.77 (d, *J* = 1.7 Hz, 1H), 9.39 (s, 1H), 6.46 (t, *J* = 5.8 Hz, 1H) 2.42-2.33 (m, 4H), 2.34 (m, 1H), 1.74 (s, 3H), 1.57 (m, 1H), 1.42 (m, 1H), 1.01 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 202.5, 195.4, 154.3, 139.7, 51.0, 35.4, 27.9, 26.7, 19.9, 9.4; HRMS (ESI) m/e 191.1042 [M+Na^+] calcd for $C_{10}H_{16}O_2Na^+$: 191.1043.

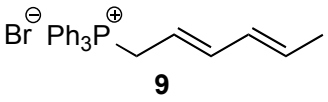


17

Di-aldehyde 17 (Method 2): To a solution of SeO_2 (416 mg, 3.7 mmol) and salicylic acid (1.99 g, 12.4 mmol) in CH_2Cl_2 (40 ml) was added *t*-butyl hydrogenperoxide slowly (70% in H_2O, 71.0 ml, 496 mmol). The mixture was stirred for 15 min then (*R*)-(+)-citronellal (**6**,

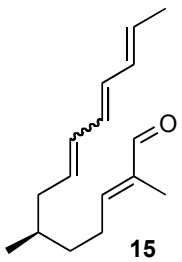
18.8 g, 122 mmol) was added. The reaction was stirred at room temperature for 96 hours. The reaction was diluted with benzene (100 ml) and concentrated. The residue was diluted with ether (400 ml) and washed with 10% NaOH (2 x 130 ml) and brine (120 ml). The organic layer was dried over $MgSO_4$, filtered, concentrated and purified through silica column chromatography (hexanes:EtOAc, 200:1 to 5:1) to recover the (*R*)-(+)-citronellal (1.0 g, 5%) and yield the di-aldehyde **7** (4 g, 20%) and corresponding allylic alcohol (10.2 g, 49%) as a clear oil. To a solution of this allylic alcohol (10.2 g, 60 mmol) in DMSO (220 ml) was added IBX (24 g, 85.7 mmol) in one portion at 0 ºC. The reaction was stirred for 1.5 hours at rt, then was diluted with water (500 ml) and filtered through Celite® to remove the precipitate and washed thoroughly with ether. The filtrate separated, and the aqueous phase was extracted with ether (5 x 500 ml). The combined organic layers were washed with brine (1000 ml) and 10% NaOH (2 x 500 ml), dried over $MgSO_4$, filtered and concentrated to yield **17** (9.16 g, 90 %) as a pale yellow oil. The analytical data was identical with the one obtained from method 1.

(2*E*,4*E*)-Hexa-2,4-dien-1-yltriphenylphosphonium bromide (**9**): To a stirred solution of (2*E*,4*E*)-hexadien-l-o1 (9.80 g, 100 mmol) in CH_2Cl_2 (20 ml) at –10 ºC was slowly added a solution of phosphorus tribromide (9.20 g, 34.0 mmol) in CH_2Cl_2 (20 ml) dropwise via an additional funnel. After all the phosphorous tribromide was added, the reaction mixture was stirred for 3 hours before it was diluted with ether (150 ml) and quenched with a saturated $NaHCO_3$ (100 ml) solution. The mixture was separated with diethyl ether with the aid of brine. The aqueous phase was extracted with ether (2 x 100 ml). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford crude (2*E*,4*E*)-hexadienylbromide (10.4 g, 65%) as a brown



9

oil. The crude (2*E*,4*E*)-hexadienylbromide was then dissolved in anhydrous toluene (90 ml), followed by the addition of triphenyl phosphine (18.9 g, 72.0 mmol). This reaction was then stirred for 72 hours at room temperature, and the resulting crystalline product was collected by suction filtration, rinsing the solids with a small amount of toluene. After pumping under high vacuum at room temperature for 12 hours, the phosphonium salt **8** were obtained (27.2 g, 99%, 64% from (2*E*,4*E*)-hexadien-l-ol). mp: 159-160 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 15H), 6.36 (m, 1H), 5.89 (m, 1H), 5.67 (m, 1H), 5.28 (m, 1H), 4.83 (dd, *J* = 15.5 Hz, 7.5 Hz, 2H), 1.68 (br d, *J* = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 140.7, 140.6, 135.0, 135.0, 133.9, 133.8, 132.6, 132.6, 130.4, 130.3, 130.0, 129.9, 118.3, 117.6, 113.2 (d, *J* = 45.9 Hz), 28.2 (d, *J* = 195.8 Hz), 18.2; HRMS (ESI) m/e 343.1613 [M–Br^-] calcd for $C_{24}H_{24}P^+$: 343.1610.

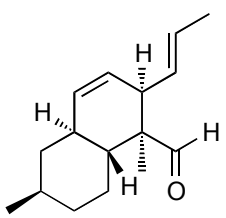


15

Polyene 15: To a suspension of (2*E*,4*E*)-hexa-2,4-dien-1-yltriphenylphosphonium bromide **9** (41.2 g, 97.4 mmol) in THF (500 ml) was added dropwise *n*-BuLi (60.8 ml, 97.4 mmol, 1.6 M in hexane) via addition funnel at –78 ºC. The mixture was stirred for 1 h at –60 ºC then re-cooled to –78 ºC and transferred via cannula, slowly dropwise to a solution of the di-aldehyde **7** (16.4 g, 97.4 mmol) in THF (500 ml) at –78 ºC over 6 hours. After completion of addition the reaction mixture was stirred at this temperature for 10 min, quenched with saturated NH_4Cl solution (500 ml), diluted with ethyl ether (500 ml) and allowed to reach room temperature. The layers were separated and the aqueous layer was extracted with ether (2 x 500 ml). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated and purified through neutralized (Et_3N, 1%) silica column chromatography (pure hexanes, then hexanes:EtOAc, 500:1 to 150:1) to yield polyene **15** (13.7 g, 62%) as a pale yellow oil as an inseparable *E*/*Z*

isomeric mixture (*E*:*Z* = ca. 3:2). R_f = 0.5 (silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) (*E*:*Z* = ca. 3:2) δ: 9.38 (s, 1H), 6.47 (t, *J* = 6.1 Hz, 1H), 6.36-6.01 (m, 4H), 5.75-5.36 (m, 2H), 2.35 (m, 2H), 2.22-1.96 (m, 2H), 1.77 and 1.76 (d, d, *J* = 13.8 Hz, 13.8 Hz, 3H), 1.74 (s, 3H), 1.56-1.49 (m, 2H), 1.35-1.28 (m, 1H), 0.93 and 0.91 (d, d, *J* = 6.9 Hz, 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (*E*:*Z* = ca. 3:2) δ: 209.0, 208.2, 130.9, 130.8, 129.5, 128.5, 128.5, 126.8, 126.6, 125.7, 49.0, 42.9, 41.7, 41.7, 39.9, 39.2, 38.8, 37.3, 37.2, 35.5, 35.4, 35.4, 33.4, 33.2, 27.1, 27.0, 22.5, 18.0, 17.9, 13.9, 13.8; HRMS (ESI) m/e 255.1720 [M+Na^+] calcd for $C_{16}H_{24}ONa^+$: 255.1719.

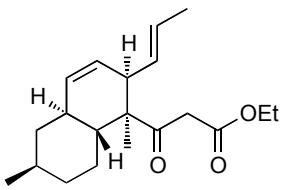
Decalin aldehyde 18: To a solution of polyene **15** (13.7 g, 58.96 mmol) in CH_2Cl_2 (700 ml) was added dropwise a solution of I_2 (752 mg, 2.95 mmol) in CH_2Cl_2 (5 ml). The reaction mixture was irradiated with visible light (sunlamp, visible light) for 5 minutes. (caution: keep the flask in a certain distance away from the light source to avoid the heat-induced IMDA reaction.) The mixture was then cooled down to –78 ºC, at which time Et_2AlCl (65.5 ml, 58.96 mmol, 0.9 M in toluene) was added dropwise. The reaction mixture was stirred for 24 hours at this temperature. The reaction was quenched with saturated $Na_2S_2O_3$/$NaHCO_3$ solution (500 ml, 1:1) and allowed to reach room temperature. The mixture was filtered through a Celite plug and the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 300 ml). The combined organic layers were washed with brine (300 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure to obtain the *trans*-decalin aldehyde **9** as a clear viscous oil (11.28 g, 82%). This material can be used directly to the next step without further purification. Large scale purification can be achieved with a silica plug (100% Hexanes). An analytical sample of **18** was purified through preparative TLC (silica gel,



(+)-**18**

hexanes:EtOAc, 20:1). R_f = 0.6 (silica gel, hexanes:EtOAc, 10:1); $[\alpha]_D^{23}$= +283.7 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.47 (s, 1H), 5.47-5.43 (m, 4H), 2.53 (m, 1H), 1.82-1.73 (m, 3H), 1.66 (m, 1H), 1.65 (d, *J* = 5.2 Hz, 3H), 1.48 (m, 1H), 1.38-1.35 (m, 1H), 1.12-1.02 (m, 2H) 1.00 (s, 3H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.87 (q, *J* = 12.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 209.1, 131.0, 129.5, 128.6, 126.8, 50.3, 49.1, 41.7, 38.8, 37.4, 35.4, 33.2, 27.1, 22.6, 18.0, 13.9. HRMS (ESI) m/e 255.1717 [M+Na^+] calcd for $C_{16}H_{24}ONa^+$: 255.1719.

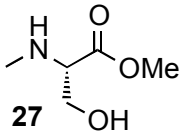
β-ketoester 4: To a solution of **9** (5.5 g, 23.7 mmol) in benzene (200 ml) was added ethyl bromoacetate (7.89 ml, 71.1 mmol) and activated zinc dust (7.76 g, 118.5 mmol). The reaction mixture was then refluxed for 45 minutes. The reaction mixture was allowed to cool to room temperature, acidified with 1 N HCl and extracted with EtOAc (3 x 300 ml). The combined organic layers were washed with saturated $NaHCO_3$ (300 ml), brine (300 ml), dried over $MgSO_4$ and concentrated to afford the corresponding isomeric alcohol mixture. The crude alcohols were then dissolved in CH_2Cl_2 (200 ml) and Dess-Martin periodinane (20.0 g, 47.2 mmol) was added portionwise at room temperature. The reaction mixture was then stirred for 2 hours. The reaction was quenched with with saturated $Na_2S_2O_3$/$NaHCO_3$ solution (500 ml, 1:1), filtered through Celite® (washed with 500 ml of ethyl ether) and the filtrate was extracted with ethyl ether (3 x 300 ml). The combined organic layers were washed with brine (300 ml), dried over $MgSO_4$ and concentrated to yield crude b-ketoester **4** (6.85 g, 92%) as a viscous yellow oil. This material can be used directly to the next step without further purification. An analytical sample of **4** was purified through preparative TLC (silica gel, hexanes:EtOAc, 10:1). R_f = 0.4 (silica gel, hexanes:EtOAc, 10:1); $[\alpha]_D^{23}$ = –146.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500



(–)-**14**

MHz, $CDCl_3$) (with minor amount of enol-form) δ: 5.42-5.35 (m, 3H), 5.16-5.07 (m, 1H), 4.19-4.14 (m, 2H), 3.49 (d, *J* = 15.8 Hz, 1H), 3.33 (d, *J* = 15.8 Hz, 1H), 2.54 (m, 1H), 1.80-1.62 (m, 5H), 1.60 (d, *J* = 6.2 Hz, 3H), 1.59 (m, 1H), 1.47 (m, 1H), 1.25 (t, *J* = 7.6 Hz, 3H), 1.17 (s, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (q, *J* = 12.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 205.7, 167.9, 130.7, 130.5, 127.1, 126.4, 61.1, 53.5, 49.5, 46.6, 42.0, 39.6, 38.4, 35.6, 33.4, 27.2, 22.5, 17.9, 17.0, 14.2. HRMS (ESI) m/e 341.2088 [$M+Na^+$] calcd for $C_{20}H_{30}O_3Na^+$: 341.2087.

***N*-Methyl-L-serine methyl ester 27** : L-serine methyl ester hydrochloride (5.0 g, 32 mmol, Sigma Aldrich) was dissolved in a minimum amount of methanol and stirred with Amberlite IRA-410 for 2 h and filtered and rinsed with methanol. The collected solution of free amine was concentrated and concentrated in *vacuo*. The neutral amine was dissolved in MeOH (300 ml). To this solution, fresh distilled benzaldehyde (3.42 ml, 34 mmol) was added in one portion. After 3 hours of stirring at room temperature, $NaBH_3CN$ (2.12 g, 34 mmol) was added. After stirring for 18 hours, solid powder paraformaldehyde ($(CH_2O)_n$, 3.02 g, 32 mmol (for MW = 90.1)) was added and allowed to dissolve within 8 hours. Following full dissolution an additional portion of $NaBH_3CN$ (2.12 g, 34 mmol) was added and the reaction was allowed to stir at room temperature for 18 hours. The reaction mixture was concentrated in *vacuo*. Ethyl acetate was added and the resulting slurry was filtered though Celite® and concentrated in *vacuo*. Flash column chromatography (hexanes:Et_2O, 2:1) on neutralized silica gel afforded the *N*-methyl-*N*-benzyl-L-serine methyl ester (6.5 g, 90%) as a clear oil. R_f = 0.3 (silica gel, hexanes:EtOAc, 2:1); $[\alpha]_D^{24}$ = –77.0, (*c* = 1.0, MeOH); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.35-7.25 (m, 5H), 3.86 (d, *J* = 13.2 Hz, 1H), 3.80-3.72 (m, 3H), 3.76 (s, 3H), 3.53 (dd, *J* = 9.3 Hz, 5.9 Hz, 1H), 2.33 (s,

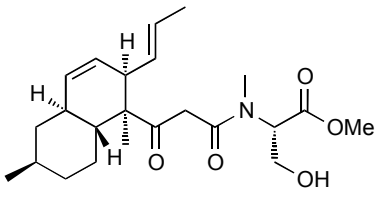


27

3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.1, 138.4, 128.9, 128.5, 127.4, 126.9, 65.7, 59.2, 58.8, 51.4, 37.4; HRMS (ESI) m/e 224.1282 [M+H$^+$] calcd for $C_{12}H_{18}NO_3^+$: 224.1281.

A high pressure steel autoclave equipped with magnetic stir bar was filled with *N*-methyl-*N*-benzyl-D-serine methyl ester prepared as described above (4.3 g, 19 mmol), $Pd(OH)_2$ (20% on activated charcoal, 2.2 g) and MeOH (200 ml). The autoclave was pressurized to 60 atm with H_2 and the suspension was vigorously stirred at room temperature for 12 hours. The pressure was released slowly and the mixture was filtered through a Celite® pad. The filter pad was washed with MeOH (5 x 200 ml), and the combined filtrates were concentrated to afford *N*-methyl-D-serine methyl ester **27** (2.0 g, 80%) as a colorless oil. $[a]_D^{24}$ = –12.1, (*c* = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ: 3.75 (m, 2H), 3.74 (s, 3H), 3.29 (t, *J* = 4.9 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ: 174.3, 65.7, 63.3, 52.4, 34.4; HRMS (ESI) m/e 134.0811 [M+H$^+$] calcd for $C_5H_{12}NO_3^+$: 134.0812. (Note: the amine **27** should be prepared freshly and used for the next step.)

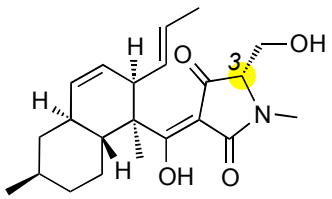
Ketoamide 11: To a solution of β-keto ester **14** (3.5 g, 11.2 mmol) in anhydrous EtOH (6.6 ml) was added the ethanolic KOH solution (3.5 g KOH in 35 ml EtOH), the reaction was then stirred at rt for 96 hrs. Alternatively, to a solution of β-keto ester **14** (700 mg, 2.2 mmol) in EtOH (11.7 mL) was added dropwise KOH (1M, 5.8 mL) and stirred for 9 hrs. In both protocols, the reaction mixture was acidified with 2M HCl solution to pH = 2. The mixture was extracted with ether (3 x 200 ml), the combined organic layer was dried over $MgSO_4$ and concentrated in *vacuo*. This afforded acid was that can be used directly without further purification. This β-ketoacid can be purified *via* a short silica column chromatography (Hex:CH_2Cl_2, then



20

100% CH_2Cl_2, then 40:1-CH_2Cl_2:Acetone, R_f: 0.12). The β-ketoacid was dissolved in CH_2Cl_2 (20 ml) and transferred to a round bottom flask which contains the freshly prepared amine **27** (1.79 g, 13.4 mmol). To this solution was added DMF (4.4 ml), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU, 4.68 g, 12.3 mmol) and cooled to 0 °C, followed by adding in the diisopropylethylamine (DIPEA, 5.93 ml, 33.6 mmol) dropwise. The reaction was stirred at rt for 2 hrs before it was acidified with 2M HCl solution to pH = 2. The mixture was then diluted with EtOAc (500 ml), sequentially washed with 2M HCl solution (3 x 200 ml), $NaHCO_3$ (100 ml) and brine (2 x 200 ml). The organic layer was dried over $MgSO_4$ and concentrated in *vacuo* to afford the desired β-ketoamide **20** as a yellow oil (90%, 4.0 g). This β-ketoamide **20** was used directly to the next step without further purification. Large scale silica chromotography purification can be perfomed using a CH_2Cl_2:MeOH solvent system (100% CH_2Cl_2 to 25:1). An analytical sample of **20** was purified with preparative TLC (EtOAc : Hexanes, 1:1 x 3). The ^{1}H NMR and ^{13}C NMR were complicated by the enol-keto tautomers and the amide rotamers. R_f = 0.4 (silica gel, CH_2Cl_2:MeOH, 20:1); $[\alpha]_D^{23}$ = –192.3 (*c* = 0.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.43-5.32 (m, 3H), 5.15 (m, 1H), 4.79 (m, minor), 4.07-3.99 (m, 2H), 3.74 (m, 3H), 3.54-3.47 (m, 1H), 2.96 (m, 3H), 2.57-2.51 (br m, 1H), 1.80-1.65 (m, 6H), 1.59 (m, 3H), 1.57-1.40 (m, 1H), 1.23 (s, 3H), 1.05 (m, 1H), 0.90 (m, 1H), 0.88 (d, *J* = 8.2 Hz, 3H) 0.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 207.9, 169.8, 168.7, 130.9, 130.5, 126.9, 126.4, 61.0, 60.0, 53.5, 52.3, 49.8, 46.3, 41.9, 39.8, 38.7, 38.4, 35.4, 33.4, 27.2, 22.4, 17.9, 17.3. HRMS (ESI) m/e 405.2519 [M+H$^+$] calcd for $C_{23}H_{35}O_5N^+$: 405.2517.

(–)-Equisetin (2) and C_3-*epi*-equisetin (C_3-*epi*-2): To a solution of β-ketoamide **20** (2.1 g, 5.11 mmol) in methanol (840 ml) was added methanolic sodium methoxide solution

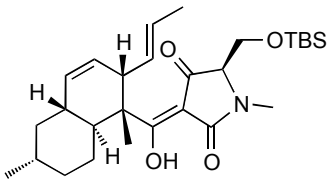


2: (–)-equisetin

(51.1 ml, 0.5 M, 25.6 mmol) via syringe at rt. After 10 min the reaction was quenched with 1 N HCl (200 ml). To the mixture was added water (500 ml) and CH_2Cl_2 (1000 ml), the aqueous phase was extracted with CH_2Cl_2 (5 x 1000 ml). The combined organic phase was dried over $MgSO_4$ and concentrated in *vacuo* to give the (–)-equisetin **2** and C_3-*epi*-equisetin (C_3-*epi*-**2**) as a pale red oil (1.9 g, dr = 1:1, 100% overall). This crude material was used directly to the next step without further purification. Analytical samples of **2** and C_3-*epi*-**2** were purified with preparative TLC (CH_2Cl_2:MeOH:AcOH, 50:1:0.1, 3 times).

(–)-equisetin **2**: R_f = 0.45 (silica gel, CH_2Cl_2:MeOH:AcOH, 50:1:0.5, 2 times); $[\alpha]_D^{26}$ = –240.0 (*c* = 1.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.40 (m, 2H), 5.30-5.10 (m, 2H), 4.03 (dd, *J* = 11.5, 3.4 Hz, 1H) 3.88 (m, 1H), 3.63 (t, *J* = 4.6 Hz, 1H), 3.34 (br, 1H), 3.05 (s, 3H), 1.97 (m, 1H), 1.90-1.70 (m, 4H), 1.55 (d, *J* = 4.6 Hz, 3H), 1.60-1.40 (m, 3H), 1.17-1.00 (m, 3H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.88 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 199.2, 190.6, 177.2, 131.0, 130.1, 127.1, 126,7, 100.0, 66.8, 60.5, 48.8, 45.1, 42.3, 40.0, 38.7, 35.8, 33.6, 28.3, 27.4, 22.5, 18.0, 14.0; HRMS (ESI) m/e 396.2141 [M+Na$^+$] calcd for $C_{22}H_{31}O_4NNa^+$: 396.2151.

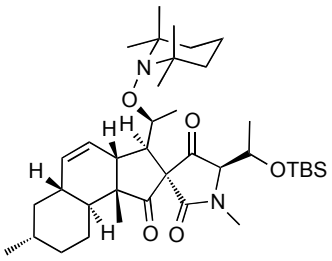
C_3-*epi*-2: R_f = 0.5 (silica gel, CH_2Cl_2:MeOH:AcOH, 50:1:0.5, 2 times); $[\alpha]_D^{27}$ = –126.0 (*c* = 2.17, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.39 (m, 2H), 5.27-5.10 (m, 2H), 4.04 (m, 1H), 3.84 (dd, *J* = 4.6, 11.5 Hz, 1H), 3.66 (m, 1H), 3.34 (br, 1H), 3.04 (s, 3H), 1.95 (m, 1H), 1.85-1.70 (m, 4H), 1.52 (d, *J* = 5.5 Hz, 3H), 1.66-1.40 (m, 3H), 1.16-1.00 (m, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 199.1, 190.7, 177.1, 130.9, 130.0, 127.2, 126.7, 100.4, 66.4, 60.3, 48.9, 45.0, 42.4, 40.0, 38.6, 35.8, 33.6, 28.4, 27.3, 22.6, 18.0, 14.2; HRMS (ESI) m/e 396.2150 [M+Na$^+$] calcd for $C_{22}H_{31}O_4NNa^+$: 396.2151.



21

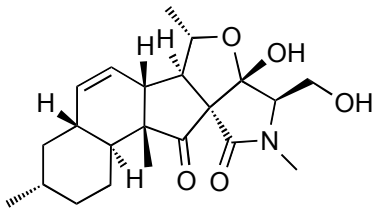
TBS-(+)-equisetin 21 (*synthesized from (S)-(–)-citronellal*): To a solution of (+)-equisetin (*ent*-**2**, 37 mg, 0.1 mmol) in CH_2Cl_2 (1 ml) was added imidazole (13.6 mg, 0.2 mmol) and TBS-Cl (23 mg, 0.15 mmol). This reaction was stirred for 12 h before it was quenched with saturated NH_4Cl solution (5 ml). The mixture was extracted with CH_2Cl_2 (3 x 10 ml), dried over and concentrated. Purification *via* silica column chromatography (hexanes:EtOAc, 100:1 to 20:1) afforded **21** as pale red oil (44 mg, 90%). R_f = 0.7 (silica gel, hexanes:EtOAc, 5:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.39 (m, 2H), 5.30-5.15 (m, 2H), 3.92 (m, 2H), 3.57 (m, 1H), 3.31 (br, 1H), 3.03 (s, 3H), 1.94 (m, 1H), 1.85-1.70 (m, 4H), 1.54 (d, *J* = 5.4 Hz, 3H), 1.42 (br s, 3H), 1.11 (m, 1H), 1.02 (m, 1H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (m, 1H), 0.81 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H); HRMS (ESI) m/e 510.3016 [M+Na^+] calcd for $C_{28}H_{45}NO_4SiNa^+$: 510.3010.

TEMPO ether 23: A solution in a sealed tube contains *ent*-**12** (24 mg, 50 mmol), TEMPO (23 mg, 0.15 mmol), ferrocenium hexafluorophosphate (**13**, 33 mg, 0.1 mmol) or Mn(III) acetate (27 mg, 0.1 mmol) in DMF (0.71 ml) was heated in a microwave at 100 °C for 10 min. The residue was directly purified via preparative TLC (hexanes:EtOAc, 20:1 x 2) to afford *ent*-**14** as a colorless oil (11 mg, 35%). R_f = 0.7 (silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.82 (ddd, *J* = 11.9, 5.0, 2.4 Hz, 1H), 5.52 (d, *J* = 12.5 Hz, 1H), 4.25 (m, 2H), 4.04 (m, 1H), 3.96 (m, 1H), 3.13 (s, 3H), 2.89 (t, *J* = 11.9 Hz, 1H), 2.43 (dd, *J* = 14.4, 6.3 Hz, 1H), 1.81-1.65 (m, 4H), 1.50 (m, 2H), 1.45 (d, *J* = 7.6 Hz, 3H), 1.38 (m, 6H), 1.07 (m, 1H), 1.02 (m, 1H), 1.00 (s, 6H), 0.97 (s, 3H), 0.94 (s, 3H), 0.91 (s,



23

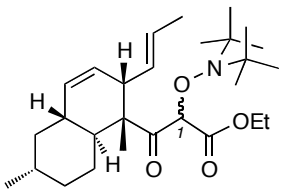
9H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.88 (s, 3H), 0.10 (s, 6H); HRMS (ESI) m/e 665.4318 [M+Na^+] calcd for $C_{37}H_{62}N_2O_5SiNa^+$: 665.4320.



(–)-fusarisetin B

(–)-fusarisetin B (*ent*-C_5-*epi*-1): To a solution of **23** (9.2 mg, 14.6 mmol) in THF (100 ml) and water (100 ml) was added acetic acid (300 ml) and activated zinc dust (95 mg, 1.46 mmol). The mixture was heated at 80 ºC for 3 hours and cooled to room temperature. To this mixture saturated solution of $NaHCO_3$ (1 ml) was slowly dropped in to neutralize the solution. The mixture was then diluted with ethyl acetate (100 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified through preparative TLC (silica gel, hexanes:EtOAc, 1:1 x 5) to afford (–)-fusarisetin B (*ent*-C_5-*epi*-**1**) as a white powder (1.7 mg, 30%). For the analytical data, see page 173.

α-TEMPO-β-ketoester *ent*-15: To a solution of HMDS (0.57 ml, 2.70 mmol) in 1,2-dimethoxyethane (30 ml) at –78 ºC was added *n*-BuLi (1.6 ml, 2.60 mmol, 1.6 M in hexane) dropwise. The mixture was stirred at this temperature for 30 min. Then a solution of b-ketoester **4** (550 mg, 1.73 mmol) in 1,2-dimethoxyethane (30 ml) was added dropwise to the reaction mixture and the mixture was warmed up to –60 ºC and stirred for 30 min. The reaction was then raised to 0 ºC, TEMPO (283 mg, 1.80 mmol) was added in one portion, stirred for 5 min at this temperature, followed by addition of ferrocenium hexafluorophophate (850 mg, 2.6 mmol) in one portion. The dark blue mixture was stirred for 5 min at 0 ºC and quenched with 20 drops of saturated NH_4Cl solution. The reaction mixture was diluted with ether (120 ml) and filtered through a short



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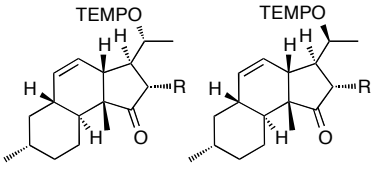
silica pad. The filtrate was concentrated and purified through silica column chromatography (hexanes:EtOAc, 200:1 to 50:1) to yield α-TEMPO ester *ent*-**15** (785 mg, as an inseparable C-1 isomeric mixture, 99%) as a clear oil. R_f = 0.5 (silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) (C-1 isomeric mixture, ca 3:1) δ: 5.42-5.25 (m, 3H), 5.25-5.10 (m, 2H), 4.28-4.07 (m, 2H), 2.56 and 2.47 (t, *J* = 5.9 Hz, 1H in total), 1.76-1.65 (m, 4H), 1.60 (d, *J* = 5.2 Hz, 1H), 1.58-1.53 (m, 5H), 1.40 (s, 3H), 1.38 (m, 1H), 1.31 (t, *J* = 7.4 Hz, 3H), 1.30-1.25 (m, 2H), 1.22 and 1.20 (s, 3H in total), 1.17 (s, 3H), 1.16-1.05 (m, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.88 (d, *J* = 6.4 Hz, 3H), 0.84-0.80 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) (C-1 isomeric mixture) δ: 204.1, 203.7* (minor isomer), 167.4, 167.2*, 131.1*, 130.5, 130.1, 130.0*, 127.3*, 126.9, 126.4, 125.8*, 91.1, 89.8*, 61.3, 61.2*, 60.8, 59.7*, 59.6, 53.7, 53.4*, 49.2*, 48.2, 41.7, 40.8*, 40.4, 40.4*, 40.2, 40.2, 38.9*, 38.4, 38.2*, 35.6, 35.5*, 33.8*, 33.3*, 33.3, 33.1, 33.1, 32.9*, 26.9, 22.5, 20.5, 20.3, 18.6*, 18.2, 17.9*, 17.1, 16.4, 15.3*, 14.1, 14.0*; HRMS (ESI) m/e 474.3577 [M+H$^+$] calcd for $C_{29}H_{48}NO_4^+$: 474.3578.

tricyclic TEMPO ketone 25 and 26: A solution of **24** (47 mg, 0.1 mmol) in toluene (1 ml) was heated at 90 °C for 3 h. The reaction was concentrated and purified via column chromatography (hexanes:EtOAc, 200:1 to 50:1) to yield the cyclized ester **25a** (21 mg, 45%) and **25b** (21 mg, 45%) along with the decarboxylated product **26a** and **26b** (inseparable C_5 isomeric mixtures, ca 1:1, 2 mg, 5%) all as clear oil.

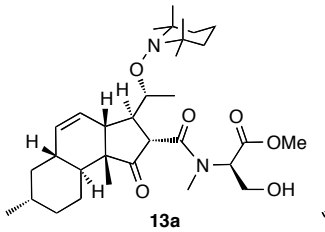


25a: R= CO_2Et
26a: R= H
25b: R= CO_2Et
26b: R= H

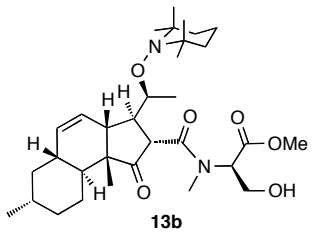
25a: R_f = 0.68 (more polar, silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.80 (ddd, *J* = 10.4, 7.5, 2.9 Hz, 1H), 5.51 (d, *J* = 10.4 Hz, 1H), 4.17 (m, 3H), 3.23 (d, *J* = 10.3 Hz, 1H), 2.84 (td, *J* = 10.9, 4.6 Hz, 1H), 2.08 (dd, *J* = 11.5, 4.6 Hz, 1H), 1.83 (m, 2H), 1.72 (m, 1H), 1.52-1.39 (m, 8H), 1.29 (m, 2H), 1.26 (m,

7H), 1.13 (s, 3H), 1.11 (s, 3H), 1.10 (s, 3H), 0.98 (s, 3H), 0.96 (s, 3H), 0.90 (d, *J* = 6.3 Hz, 3H), 0.86 (m, 1H); HRMS (ESI) m/e 496.3394 [M+Na^+] calcd for $C_{29}H_{47}NO_4Na^+$: 496.3397. **25b**: R_f = 0.72 (less polar, silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.86 (ddd, *J* = 9.8, 6.5, 2.3 Hz, 1H), 5.52 (d, *J* = 9.8 Hz, 1H), 4.18 (m, 3H), 3.49 (d, *J* = 10.3 Hz, 1H), 2.60 (td, *J* = 10.3, 2.9 Hz, 1H), 2.46 (dd, *J* = 11.5, 5.2 Hz, 1H), 1.83 (m, 2H), 1.72 (m, 1H), 1.50-1.38 (m, 8H), 1.29 (m, 2H), 1.25 (m, 7H), 1.16 (s, 3H), 1.13 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.84 (m, 1H); HRMS (ESI) m/e 496.3398 [M+Na^+] calcd for $C_{29}H_{47}NO_4Na^+$: 496.3397. **26a** and **26b** (inseparable C_5 isomeric mixtures, ca 1:1): R_f = 0.8 (less polar, silica gel, hexanes:EtOAc, 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.95 (m, 1H), 5.87 (m, 1H), 5.49 (d, *J* = 10.3 Hz, 1H), 5.45 (d, *J* = 10.3 Hz, 1H), 4.19 (m, 1H), 4.02 (m, 1H), 2.54 (m, 2H), 2.49 (m, 2H), 2.21 (m, 1H), 2.15 (m, 1H), 2.09 (m, 2H), 1.82 (m, 4H), 1.75 (m, 2H), 1.50-1.35 (m, 12H), 1.30 (m, 6H), 1.24 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 1.21(s, 3H), 1.20 (s, 3H), 1.18 (s, 3H), 1.08 (m, 4H), 1.06 (s, 3H), 1.05 (m, 3H), 1.02 (s, 3H), 0.98 (m, 2H), 0.96 (s, 3H), 0.93 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.88 (d, *J* = 6.3 Hz, 3H), 0.85 (m, 2H); HRMS (ESI) m/e 424.3188 [M+Na^+] calcd for $C_{26}H_{43}NO_2Na^+$: 424.3186.

Tricyclic β-ketoamide 13a and 13b: To a solution of **12** (20.0 mg, 42.2 mmol) in toluene (0.5 ml) was added 4-DMAP (10.3 mg, 84.4 mmol), freshly prepared amine **18** (28.1 mg, 0.21 mmol) and 4Å molecule seives (50 mg). The mixture was heated at 90°C for 36 hours and then was allowed to cool to room temperature, concentrated and purified through preparative TLC (CH_2Cl_2:MeOH, 120:1 x 5) to yield tricyclic TEMPO adducts *ent*-**19a** (8.2 mg, 34%) and its C_5-epimer (*ent*-**19b**,



13a

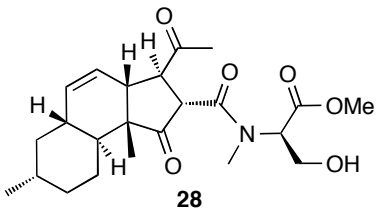


13b

8.7 mg, 36%) as colorless oils. **13a**: R_f = 0.2 (slightly less polar, silica gel, hexanes:EtOAc, 2:1); $[\alpha]_D^{23}$ = –59.2, (*c* = 0.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.87 (m, 1H), 5.48 (d, *J* = 10.3 Hz, 1H), 4.60 (t, *J* = 6.6 Hz, 1H), 4.13 (m, 2H), 3.77 (m, 1H), 3.72 (d, *J* = 11.5 Hz, 1H), 3.67 (s, 3H), 3.28 (s, 3H), 3.05 (td, *J* = 10.9 Hz, 4.6 Hz, 1H), 2.68 (t, *J* = 6.9 Hz, 1H), 2.23 (dd, *J* = 10.9 Hz, 4.6 Hz, 1H), 1.81-1.76 (m, 2H), 1.67-1.63 (m, 2H), 1.48-1.27 (m, 8H), 1.23 (s, 3H), 1.13 (br s, 6H), 1.10 (s, 3H), 1.04 (m, 1H), 1.01 (s, 3H), 0.95 (s, 3H), 0.87 (d, *J* = 6.3 Hz, 3H), 0.85-0.80 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 215.0, 170.9, 169.3, 131.9, 126.1, 78.6, 61.8, 61.1, 60.5, 59.0, 54.2, 52.8, 52.4, 48.8, 46.4, 41.6, 40.5, 40.2, 37.7, 36.8, 35.9, 35.3, 35.1, 34.8, 34.5, 29.9, 25.4, 22.8, 22.4, 21.2, 17.6, 15.3; HRMS (ESI) m/e 561.3896 [M+H$^+$] calcd for $C_{32}H_{53}N_2O_6^+$: 561.3898.

13b: R_f = 0.2 (slightly more polar, silica gel, hexanes:EtOAc, 2:1); $[\alpha]_D^{23}$ = –39.8, (*c* = 0.42, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.86 (m, 1H), 5.51 (d, *J* = 9.8 Hz, 1H), 4.74 (t, *J* = 6.9 Hz, 1H), 4.25 (m, 1H), 4.11 (m, 1H), 4.04 (d, *J* = 9.7 Hz, 1H), 3.83 (m, 1H), 3.67 (s, 3H), 3.29 (s, 3H), 2.87 (td, *J* = 9.2 Hz, 2.3 Hz, 1H), 2.58 (m, 2H, overlapped with OH), 1.84-1.77 (m, 2H), 1.68-1.64 (m, 2H), 1.50-1.30 (m, 8H), 1.19 (s, 3H), 1.14 (br s, 6H), 1.07 (s, 3H), 1.04 (s, 3H), 1.02 (m, 1H), 0.98 (s, 3H), 0.86 (d, *J* = 6.9 Hz, 3H), 0.85-0.80 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 215.0, 170.8, 169.9, 132.5, 125.2, 74.6, 61.0, 60.5, 59.0, 52.4, 52.2, 52.1, 50.3, 45.0, 41.6, 40.6, 40.3, 37.5, 36.8, 35.3, 35.2, 34.7, 34.4, 33.0, 29.8, 25.2, 22.4, 20.9, 19.8, 17.3, 15.3, 14.2; HRMS (ESI) m/e 561.3895 [M+H$^+$] calcd for $C_{32}H_{53}N_2O_6^+$: 561.3898.

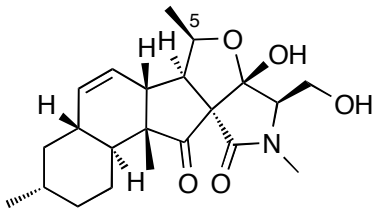
Tricyclic di-ketoamide 28: To a mixture of **13a** and **13b** (50 mg, 89 mmol) in CH_2Cl_2 (1 ml) at 0 °C was added a solution of 3-chloroperoxybenzoic acid (19 mg, 107 mmol) in



28

CH_2Cl_2 (200 ml) dropwise. The reaction mixture was stirred for 15 min at the same temperature and then quenched with saturated $Na_2S_2O_3$ solution (0.5 ml) followed by adding saturated $NaHCO_3$ solution (0.5 ml). The mixture was vigorously stirred for 5 min at room temperature and was diluted with 200 ml of EtOAc, washed with NaOH solution (10%) and brine, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. Purification of the crude product by silica column chromatography (hexane:ethyl acetate, 10:1 to 2:1) afforded ketone **28** (35 mg, 95%) as a colorless oil. R_f = 0.3 (silica gel, hexanes:EtOAc, 1:1); $[\alpha]_D^{24}$ = –27.5, (*c* = 0.33, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.74 (m, 1 H), 5.60 (d, *J* = 10.3 Hz, 1 H), 4.90 (t, *J* = 5.9 Hz, 1 H), 4.10-4.05 (m, 2 H), 3.90-3.81 (m, 2 H), 3.70 (s, 3 H), 3.21 (s, 3 H), 2.37 (m, 1 H), 2.27 (s, 3 H), 1.86-1.81 (m, 2 H), 1.72-1.69 (m, 1H), 1.48-1.40 (m, 3H), 1.08 (m, 1 H), 0.98 (s, 3 H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.85-0.80 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 212.6, 209.5, 170.2, 168.7, 133.8, 123.7, 60.8, 60.5, 56.3, 55.7, 52.6, 52.1, 47.4, 41.6, 38.3, 37.0, 35.3, 34.7, 33.1, 32.0, 25.2, 22.4, 15.4; HRMS (ESI) m/e 420.2380 [M+H$^+$] calcd for $C_{23}H_{34}NO_6^+$: 420.2381.

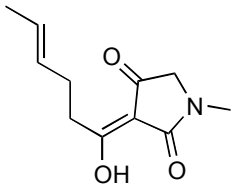
(–)-Fusarisetin A: To a solution of tricyclic di-ketoamide **28** (35 mg, 85 mmol) in MeOH (850 ml) was added $CeCl_3$•$7H_2O$ (48 mg, 128 mmol) and stirred at rt for 10 min. The reaction was then cooled to –20 °C and to this solution $NaBH_4$ (3.5 mg, 94 mmol) was added. This reaction was allowed to stir for 30 min at this temperature, then saturated NH_4Cl solution (0.5 ml) was added in. The mixture was warmed up to room temperature and extracted with EtOAc (3 x 20 ml), the combined organic phase was washed with brine, dried over



(–)-fusarisetin A, **1**

anhydrous $MgSO_4$ and concentrated in *vacuo*. The residue was purified by flash column chromatography (EtOAc:hexanes, 1:1 to 3:1) to give a ca. 4:1 mixture of tricyclic alcohols as a white foam. The obtained alcohol was dissolved in anhydrous MeOH (2 ml) and a solution of sodium methoxide (850 ml, 425 mmol, 0.5 M in MeOH) was dropped in at 0 ºC. This reaction was allowed to warm up to room temperature and stirred for 10 min and quenched by saturated NH_4Cl solution (1 ml). The mixture was then diluted with ethyl acetate (200 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified through preparative TLC (EtOAc:hexanes, 1:1 x 5) to afford (–)-fusarisetin A (*ent*-**1**) as a white foam (11.2 mg, 34% from *ent*-**20**) and its C_5-epimer (*ent*-C_5-*epi*-**1**, 2.8 mg, 8%). *ent*-**1**: $[\alpha]_D^{23}$ = –86.3, *c* = 0.065, MeOH; natural: $[\alpha]_D^{23}$ = +84.6, *c* = 0.2, MeOH). For other analytical data, see page 173 and Table 2.8.2 (pg. 174).

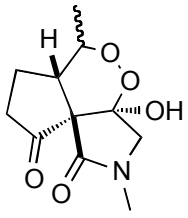
Tetramic acid 32: To a suspension of sodium hydride (6.05 g, 60% in mineral oil, 151 mmol) in THF (500 ml) was added ethyl acetoacetate **29** (17.55 ml, 137 mmol) dropwise at 0 °C and stirred for 10 min. Then *n*-BuLi (99.5 ml, 1.45 M in hexanes, 144 mmol) was added in dropwise at the same temperature. Upon completion of addition, this solution was stirred for another 10 min. Then a solution of crotyl bromide (15.1 ml, 147.6 mmol) in THF (200 ml) was slowly dropped into the previous solution over 15 min. The reaction was then allowed to warm up to rt and stirred for 2 hrs before it was quenched carefully with conc. HCl/H_2O (20 ml/200 ml). The mixture was extracted with ether (3 x 500 ml), and the combined organic phase was washed with water until the solution become neutral. Silica column chromatography (hexanes:EtOAc, 200:1 to 10:1) afforded the corresponding ketoester **30** (23.3 g, 92%). The ketoester **30** (7.5 g, 40.7 mmol), *N*-



32

methyl glycine methyl ester hydrochloride (11.3 g, 81.4 mmol), 4-DMAP (9.95 g, 81.4 mmol) and Et_3N (17.0 ml, 122.1 mmol) was refluxed in toluene (80 ml) for 12 hrs. The reaction was then cooled down and absorbed with silica gel. Following silica column chromatography (CH_2Cl_2:MeOH, 200:1 to 20:1) afforded the corresponding ketoamide **31** (4.9 g, 50%). The obtained ketoamide **31** (1.7 g, 7.0 mmol) was then dissolved in MeOH (210 ml) and the sodium methoxide solution (14 ml, 0.5 M in methanol) was added in. The reaction was stirred at rt for 2 hrs before it was quenched with 1N HCl (300 ml). The mixture was diluted with water (500 ml) and CH_2Cl_2 (500 ml) and separated, the aqueous layer was further extracted with CH_2Cl_2 (500 ml x 5). The combined organic phase was dried over $MgSO_4$ and concentrated in *vacuo* to yield the tetramic acid **32** as a dark red oil (1.43 g, 97%). R_f = 0.3 (CH_2Cl_2:MeOH, 20:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.50-5.35 (m, 2H), 3.68 (s, 2H), 2.98 (s, 3H), 2.83 (t, *J* = 7.4 Hz, 2H), 2.30 (m, 2H), 1.59 (d, *J* = 5.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 191.4, 186.8, 173.5, 128.8, 126.6, 101.8, 57.7, 32.8, 28.7, 28.5, 17.9; HRMS (ESI) m/e 208.0979 [M–H$^-$] calcd for $C_{11}H_{14}O_3N^-$: 208.0980.

endoperoxy β-ketoamide 33: solution of tetramic acid **32** (20.9 mg, 0.1 mmol) and ceric ammonium nitrate (CAN, 54.8 mg, 0.1 mmol) in acetic acid (0.5 ml) was stirred under oxygen atmosphere (1 atm) for 3 hrs. The reaction mixture was then diluted with CH_2Cl_2 (5 ml), passed through a short silica pad, washed with CH_2Cl_2/MeOH (20:1, 20 ml) and concentrated in *vacuo*. An analytical sample of **33** could be isolated as an inseparable C_5 diastereomeric mixture via preparative TLC (CH_2Cl_2:MeOH:AcOH, 30:1:0.15, 2 times).

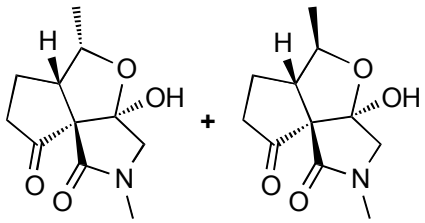


33

33 (ca 2:1, as inseparable mixture): R_f = 0.25 (CH_2Cl_2:MeOH:AcOH, 50:1:0.5, 2 times); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.43 (major, qd, *J* = 6.9, 2.9 Hz, 1H), 4.11* (minor, qd, *J* =

6.9, 1.2 Hz, 1H), 3.71 (d, *J* = 10.9 Hz, 1H), 3.69* (d, *J* = 10.9 Hz, 1H), 3.18* (d, *J* = 10.9 Hz, 1H), 3.07 (d, *J* = 10.9 Hz, 1H), 2.94* (s, 3H), 2.92 (s, 3H), 2.80 (ddd, *J* = 9.7, 6.9, 2.9 Hz, 1H), 2.73* (ddd, *J* = 9.7, 6.9, 1.2 Hz, 1H), 2.57* (m, 1H), 2.53 (m, 1H), 2.37 and 2.37* (overlapped, 2H), 2.22 (m, 1H), 2.13* (m, 2H), 2.07 (m, 1H), 1.37* (d, *J* = 6.9 Hz, 3H), 1.20 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 211.9* (minor), 211.5 (major), 170.4*, 169.5, 101.0, 100.9*, 77.6*, 75.1, 62.3, 59.3*, 56.3*, 55.9, 39.0, 38.3*, 29.9, 29.8*, 25.1, 22.8*, 19.7, 18.5*, 14.8, 14.2*; HRMS (ESI) m/e 240.0873 [M–H$^-$] calcd for $C_{11}H_{14}O_5N^-$: 240.0877.

tricyclic β-ketoamide 34: A crude residue of **33** was dissolved in anhydrous MeCN (0.5 ml), followed by the addition of CuCl (99 mg, 1 mmol). This reaction was stirred at rt for 2 hr, and was then concentrated in *vacuo* and purified via preparative TLC (CH_2Cl_2:MeOH: AcOH, 30:1:0.1, 3 times) to afford the tricyclic compound **34a** (5.1 mg, 32%) and **34b** (3.9 mg, 25%). **34a** : R_f = 0.2 (slighly less polar than **34b**, CH_2Cl_2:MeOH:AcOH, 50:1:0.5, 2 times); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.35 (qd, *J* = 6.3, 6.3 Hz, 1H), 3.58 (d, *J* = 9.8 Hz, 1H), 3.50 (d, *J* = 10.3 Hz, 1H), 2.91 (s, 3H), 2.91 (m, 1H), 2.54 (m, 2H), 2.27 (m 1H), 1.80 (m, 1H), 1.33 (d, *J* = 5.8 Hz, 3H); ^{13}C NMR (200 MHz, $CDCl_3$) δ: 210.9, 170.2 107.5, 84.0, 73.5, 59.5, 53.3, 39.8, 32.1, 20.0, 14.3; HRMS (ESI) m/e 248.0894 [M+Na$^+$] calcd for $C_{11}H_{15}NO_4Na^+$: 248.0893.



34a **34b**

34b: R_f = 0.2 (slighly more polar than **34a**, CH_2Cl_2:MeOH:AcOH, 50:1:0.5, 2 times); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.31 (qd, *J* = 5.5, 5.5 Hz, 1H), 3.62 (d, *J* = 10.9 Hz, 1H), 3.51 (d, *J* = 10.3 Hz, 1H), 3.17 (m, 1H), 2.91 (s, 3H), 2.49 (m, 2H), 2.03 (m, 2H), 1.36 (d, *J* =

6.3 Hz, 3H) ^{13}C NMR (125 MHz, $CDCl_3$) δ: 211.0, 169.6, 107.5, 78.8, 73.7, 60.9, 49.9, 40.5, 29.8, 20.3, 16.0; HRMS (ESI) m/e 248.0892 [M+Na^+] calcd for $C_{11}H_{15}NO_4Na^+$: 248.0893

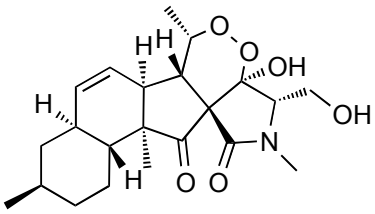
Table 2.8.1 Studies of the oxidative radical cyclization (ORC) of **32**

Oxidant	**Equiv.**	**Solvent**	**Temp.**	**O_2**	**Time**	**Yield of 25**	**Reductant**	**Yield of 26**
$Co(OAc)_2$	1.0	AcOH	70 °C	1 bar	5 min	20%	CuCl	80%
$Co(OAc)_2$	1.0	AcOH	70 °C	1 bar	5 min	20%	thiourea	n.r.
$Co(OAc)_2$	1.0	AcOH	25 °C	1 bar	4 h	10%	CuCl	79%
$Co(OAc)_2$	1.0	*i*-PrOH	25 °C	1 bar	12 h	n.r.	-	-
$Co(OAc)_2$	1.0	*i*-PrOH	50 °C	1 bar	12 h	decomp.	-	-
$CoCl_2$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$CeCl_3$	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
$CeSO_4$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$Mn(OAc)_3$	1.0	AcOH	25 °C	1 bar	12 h	5%	-	-
MnO_2	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$Mn(OAc)_2$ (0.2 eq) $Co(OAc)_2$ (0.1 eq)	1.0	AcOH	25 °C	1 bar	12 h	7%	-	-
$CrCl_2$	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
$BiO(NO)_3$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
VCl_3	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
$V(acac)_3$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$PhI(OAc)_2$	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-

Table 2.8.1 (cont.) Studies of the oxidative radical cyclization (ORC) of **32**

Oxidant	Equiv.	Solvent	Temp.	O_2	Time	Yield of 25	Reductant	Yield of 26
$CuCl_2$	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
PbO_2	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
$Pb(NO_3)_2$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$Pd(OAc)_2$	1.0	AcOH	25 °C	1 bar	12 h	trace	-	-
$Pd(OAc)_2$/BQ	0.1	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$InCl_3$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$[Fe(C_5H_5)_2]PF_6$	1.0	AcOH	25 °C	1 bar	12 h	15%	CuCl	81%
$Fe(acac)_3$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
Fe(*S*,*S*-PDP)	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
AgO	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
$AgNO_3$	1.0	AcOH	25 °C	1 bar	12 h	n.r.	-	-
CAN	1.0	AcOH	25 °C	1 bar	3 h	57%	CuCl	82%
CAN	1.0	MeCN	25 °C	1 bar	3 h	56%	CuCl	80%
CAN	1.0	AcOH	–20 °C	1 bar	18 h	57%	CuCl	79%
CAN	1.0	AcOH	70 °C	1 bar	5 min	20%	-	-
CAN	0.1	AcOH	25 °C	1 bar	6 h	40%	CuCl	80%
CAN	1.0	AcOH	70 °C	air	1 h	30%	CuCl	81%

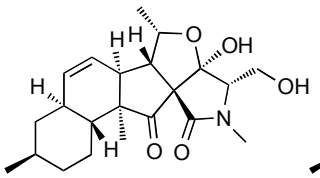
Peroxy-fusarisetin A (35) and A solution of crude equisetin (**2**, 1.1 g, ~50% purity, 2.95 mmol) in acetic acid (14 ml) was degassed with an oxygen balloon for 5 minutes, ceric ammonium nitrate (CAN, 1.62 g, 2.95 mmol) was added in and this reaction was stirred

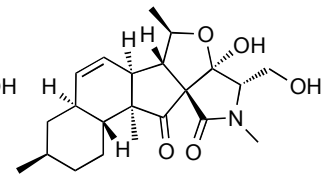


35: peroxy-fusarisetin A

at rt under oxygen atmosphere (1 atm, balloon) for 3 hrs. The reaction was diluted with CH_2Cl_2 (200 ml), passed through a short silica pad, washed with CH_2Cl_2/MeOH (20:1, 500 ml) and concentrated in *vacuo*. Analytical amount of peroxy-fusarisetin A **35** and C_5-*epi*-peroxy-fusarisetin A (C_5-*epi*-**35**) can be isolated as an inseparable mixture via preparative TLC (hexanes:EtOAc, 1:1 x 3). An analytical sample of 0.7:1 mixture of **27** and C_5-*epi*-**27** was used for characterization; R_f = 0.25 (silica gel, hexanes:EtOAc, 1:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ:: 5.82* (**27**, minor, m, 1H), 5.77 (C_5-*epi*-**27**, major, m, 1H), 5.59 (d, *J* = 9.8 Hz, 1H), 5.57* (d, *J* = 10.4 Hz, 1H), 4.47* (qd, *J* = 4.6, 2.3 Hz, 1H), 4.31 (qd, *J* = 6.9, 0.6 Hz, 1H), 4.27* (dd, *J* = 10.3, 9.2 Hz, 1H), 4.20 (dd, *J* = 10.9, 9.2 Hz, 1H), 3.99 and 3.99* (m, 2H, overlapped), 3.26 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.15* (dd, *J* = 9.2, 3.5 Hz, 1H), 2.97 (s, 3H), 2.95* (s, 3H), 2.75 and 2.75* (m, 2H, overlapped), 2.66* (dd, *J* = 12.1, 4.6 Hz, 1H), 2.49 (d, *J* = 12.0 Hz, 1H), 1.88-1.79 (m, 4H, overlapped), 1.70-1.40 (m, 6H, overlapped), 1.37 (d, *J* = 6.9 Hz, 3H), 1.35* (d, *J* = 6.9 Hz, 3H), 1.10 (m, 2H, overlapped), 1.02 and 1.02* (d, *J* = 5.2 Hz, 6H, overlapped), 0.90 (m, 2H, overlapped), 0.90 (m, 2H, overlapped), 0.88 (d, *J* = 6.3 Hz, 6H), 0.83 (m, 2H, overlapped); ^{13}C NMR (125 MHz, $CDCl_3$) δ:: 213.9, 213.4*, 169.6, 168.7*, 133.7, 133.3*, 126.1*, 123.6, 102.3*, 101.8, 76.0*, 74.6, 65.7, 65.5*, 63.7, 61.1*, 60.4*, 59.5, 52.4*, 51.3, 46.3, 44.7*, 44.1, 43.5*, 41.7, 41.6*, 38.3*, 38.2, 37.0*, 37.0, 35.3*, 35.3, 32.9, 32.9*, 29.3*, 29.2, 25.1*, 25.0, 22.4, 19.1*, 17.2*, 15.1, 14.7, 14.2*; HRMS (ESI) m/e 428.2047 [M+Na$^+$] calcd for $C_{22}H_{31}NO_6Na^+$: 428.2044.

(+)-Fusarisetin A (1) and B (36): The crude residue was then dissolved in anhydrous MeOH (30 ml), followed by the addition of thiourea (2.2 g, 29.5 mmol). This reaction was

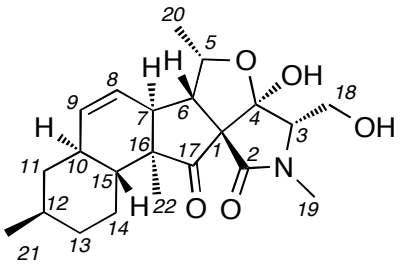

1: (+)-fusarisetin A


36: (+)-fusarisetin B

refluxed for 1 hr, and then was allowed to cool to rt and was concentrated in *vacuo*. The crude product was purified via silica column chromatography (slow gradient, hexanes:EtOAc, 50:1 to 1:1) to afford (+)-fusarisetin A (**1**, 201 mg, 35%) as white foam (+)-fusarisetin B (**36**, 155 mg, 27%, contaminated with ca 15% minor isomers, analytical sample of C_5-*epi*-**1** was purified via preparative TLC (hexanes:EtOAc, 3:1 x 8) as a white foam. **1**: R_f = 0.2 (silica gel, hexanes:EtOAc, 1:2); $[a]_D^{25}$ = +85.3, *c* = 0.2, MeOH; natural: $[a]_D^{23}$ = +84.6, *c* = 0.2, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ: 5.80 (ddd, *J* = 10.1, 4.8, 2.6 Hz, 1H), 5.56 (d, *J* = 10.1 Hz, 1H), 4.35 (dq, *J* = 6.4, 6.4 Hz, 1H), 3.86 (dd, *J* = 12.0, 5.6 Hz, 1H), 3.82 (dd, *J* = 12.0, 5.6 Hz, 1H), 3.59 (t, *J* = 5.2 Hz, 1H), 2.97 (s, 3H), 2.85 (dd, *J* = 11.2, 5.8 Hz, 1H), 2.68 (dd, *J* = 11.2, 4.9 Hz, 1H), 1.89 (m, 2H), 1.75 (br d, *J* = 12.7 Hz, 1H), 1.56-1.48 (m, 3H), 1.44 (d, *J* = 6.5 Hz, 3H), 1.11 (qd, *J* = 12.2, 3.2 Hz, 1H), 0.99 (m, 1H), 0.96 (s, 3H), 0.92 (d, *J* = 6.6 Hz, 3H), 0.83 (q, *J* = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD): δ: 214.1, 172.1, 133.6, 126.9, 109.6, 79.7, 76.5, 71.7, 61.9, 56.4, 55.3, 44.7, 43.3, 39.0, 38.0, 36.5, 34.3, 30.0, 26.6, 23.0, 17.8, 14.4; HRMS (ESI) m/e 412.2092 [M+Na^+] calcd for $C_{22}H_{31}NO_5Na^+$: 412.2094.

36: R_f = 0.25 (silica gel, hexanes:EtOAc, 1:2); $[a]_D^{22}$ = +51.2 (*c* = 0.15, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.72 (m, 1H), 5.52 (d, *J* = 10.4 Hz, 1H), 4.56 (qd, *J* = 6.3, 3.5 Hz, 1H), 3.97 (m, 2H), 3.52 (dd, *J* = 6.9, 2.9 Hz, 1H), 2.92 (s, 3H), 2.73 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.37 (dd, *J* = 9.8, 4.6 Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d, *J* = 6.4 Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 213.1, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI) m/e 412.2093 [M+Na^+] calcd for $C_{22}H_{31}O_5NNa^+$: 412.2094.

Table 2.8.2. ^{1}H and ^{13}C NMR datum comparison of synthetic and natural (+)-**1**



1: (+)-fusarisetin A

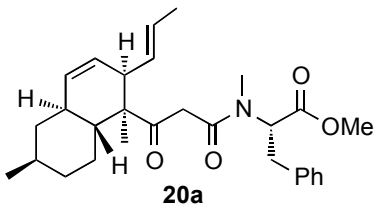
Pos	δ ^{1}H natural (CD_3OD)	δ ^{1}H synthetic (CD_3OD)	Δ	δ ^{13}C natural	δ ^{13}C synthetic	Δ
1				75.3	76.3	1.0
2				170.9	171.9	1.0
3	3.60, dd, 5.0, 2.5	3.57, t, 5.2	0.03	70.5	71.6	1.1
4				108.5	109.5	1.0
5	4.37, q, 6.3	4.34, q, 6.4	0.03	78.5	79.5	1.0
6	2.87, dd, 11.0, 5.8	2.85, dd, 11.2, 5.8	0.02	54.2	55.2	1.0
7	2.69, dd, 11.0, 4.8	2.66, dd, 11.2, 4.9	0.03	43.5	44.5	1.0
8	5.83, ddd, 2.5, 4.8, 10.0	5.80, ddd, 2.6, 4.8, 10.1	0.03	125.7	126.8	1.1
9	5.58, d, 10.0	5.55, d, 10.1	0.03	132.5	133.5	1.0
10	1.90, m	1.89, m	0.01	36.8	37.9	1.2
11	1.87, m; 0.85, q, 12.8	1.86, m; 0.83, q, 12.5	0.01 0.02	42.1	43.2	1.1
12	1.51, m	1.48, m	0.03	33.1	34.2	1.1
13	1.76, br d, 12.8; 0.99, m	1.75, br d, 12.7 0.99, m	0.01 0.00	35.3	36.4	1.1
14	1.56, m; 1.13, ddd, 12.8, 9.6, 3.2	1.54, m 1.10, qd, 12.2, 3.2	0.02 0.02	25.4	26.4	1.0
15	1.53, m	1.52, m	0.01	37.9	38.9	1.0
16				55.2	56.3	1.1
17				212.9	214.0	1.1
18	3.89, dd, 12.0, 5.0 3.84, dd, 12.0, 5.0	3.86, dd, 12.0, 5.6 3.82, dd, 12.0, 5.6	0.03 0.02	60.6	61.7	1.1

Table 2.8.2 (cont.) ^{1}H and ^{13}C NMR datum comparison of synthetic and natural (+)-**1**

Pos	δ ^{1}H natural (CD_3OD)	δ ^{1}H synthetic (CD_3OD)	Δ	δ ^{13}C natural	δ ^{13}C synthetic	Δ
19	2.97, s	2.95, s	0.02	28.8	29.8	1.0
20	1.47, d, 6.5	1.44, d, 6.5	0.03	16.6	17.7	1.1
21	0.94, d, 6.5	0.91, d, 6.6	0.03	21.7	22.8	1.1
22	0.98, s	0.96, s	0.02	13.2	14.3	1.1

Amino esters 5a-5d: All amine coupling partners are commercially available from Sigma Aldrich (CDSO15644, PH006931) and FCH Group (FCH1120224, FCH1241743). They also can be readily prepared using standard reductive amination strategies.

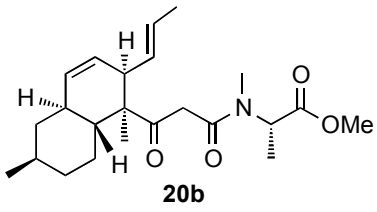
C_3-benzyl β-ketoamide 20a : To a solution β-ketoacid **38** (42 mg, 0.144 mmol) was transferred to a round bottom flask which contained *N*-methyl phenyl alanine methyl ester (33 mg, 0.173 mmol) in CH_2Cl_2 (0.6 mL). To this solution was added, DMF (0.1 mL), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyl-uroniumhexafluorophosphate (HATU, 54 mg, 0.144 mmol) and cooled to 0 °C, followed by adding in the diisopropylethylamine (DIPEA, 0.16 ml, 0.36 mmol) dropwise. The reaction was stirred at rt for 2 hrs before it was acidified with 2M HCl solution to pH = 2. The mixture was then diluted with EtOAc , sequentially washed with 2M HCl solution, $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in *vacuo* to afford the desired ketoamide **6a** as a yellow oil (95%, 64 mg). This ketoamide was used directly to the next step without further purification. The ^{1}H NMR and ^{13}C NMR can be complicated due to enol-keto tautomers and amide rotamers. $[\alpha]^{25}_{D}$ = -80.4 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$, complicated by enol-keto tautomer and amide rotamers) δ: 7.24 (m, 5H), 5.41-5.30 (m, 2H), 5.19 (m, 2H), 3.71 (m, 3H), 3, 3.56 (d, *J* =



20a

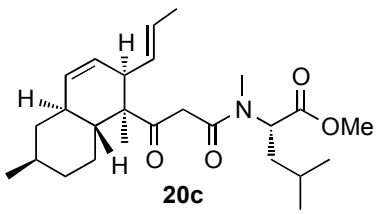
15.6 Hz, 1H), 3.35 (dd, *J* = 14.1, 5.5 Hz, 1H), 3.22 (d, *J* = 16.0 Hz, 1H), 3.04 (m, 1H), 2.79 (m, 3H), 2.47 (br m, 1H), 1.80-1.65 (m, 6H), 1.56 (d, *J* = 6 Hz, 3H), 1.57- 1.40 (m, 1H), 1.08 (s, 3H), 1.05 (m, 1H), 0.90 (m, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.84 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 206.5, 171.2, 168.3, 137.3, 131.4, 130.7, 129.1, 128.6, 126.8, 126.5, 126.3, 58.7, 53.5, 52.4, 49.5, 45.6, 42.0, 39.8, 38.5, 35.6, 34.9, 33.8, 33.5, 29.8, 27.2, 22.6, 17.9, 17.1. HRMS (ESI) m/e 466.2952 [M+H$^+$] calcd for $C_{29}H_{40}NO_4^+$: 466.2950.

C_3-methyl β-ketoamide 20b : Same procedure as **6a** yielded **6b** (35 mg, 92%) as pale yellow oil. $[\alpha]^{25}_D$ = –104.9 (c = 1.0, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$, complicated by enol-keto tautomer and amide rotamers) δ: 5.40 (m, 2H), 5.16 (m, 2H), 3.71 (m, 3H), 3.44 (m, 2H), 2.86 (m, 3H), 2.54 (br m, 1H), 1.80-1.62 (m, 6H), 1.59 (d, *J* = 8.0 Hz, 3H), 1.52- 1.38 (m, 4H), 1.24 (d, *J* = 5.2 Hz, 3H), 1.22 (s, 3H), 1.20 – 0.96 (m, 1H), 0.90 (m, 1H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.84 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 207.1, 172.4, 168.1, 130.6, 129.4, 126.6, 126.0, 68.4, 52.6, 52.2, 42.0, 40.0, 38.5, 35.6, 33.5, 31.1, 29.8, 28.9, 27.3, 22.7, 17.9, 17.3, 14.6. HRMS (ESI) m/e 390.2639 [M+H$^+$] calcd for $C_{23}H_{36}NO_4^+$: 390.2637.



20b

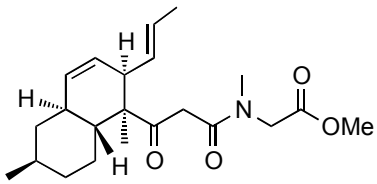
C_3-isobutyl β-ketoamide 20c: Same procedure as **20a** yielded **6c** (31 mg, 87%) as pale yellow oil $[\alpha]^{25}_D$ = –134.3 (c = 1.0, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$, complicated by enol-keto tautomer and amide rotamers) δ: 5.40 (m, 2H), 5.17 (m, 2H), 3.69 (s, 3H), 3.48 – 3.30 (m, 2H), 2.93 (m, 1H), 2.85 (s, 3H), 2.56 (br m, 1H), 1.80-1.62 (m, 6H), 1.59 (d, *J* = 6.0 Hz, 3H), 1.52- 1.38 (m, 4H), 1.25 (s, 3H),



20c

1.21 (s, 3H), 1.20 – 0.96 (m, 1H), 0.98 – 0.84 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 206.9, 171.9, 168.7, 131.1, 130.7, 126.9, 126.6, 59.9, 53.6 , 51.9, 49.7, 46.4, 42.0, 39.9, 35.6, 33.5, 31.9, 29.8, 27.3, 24.8, 22.6, 18.0, 17.3, 15.7. HRMS (ESI) m/e 432.3108 [M+H$^+$] calcd for $C_{26}H_{42}NO_4^+$: 432.3110

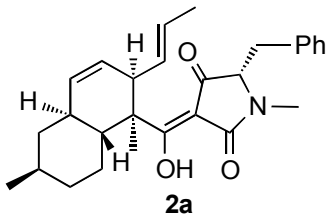
C_3-dihydro β-ketoamide 20d: Same procedure as **20a** yielded **6b** (52 mg, 90%) as



20d

pale yellow oil. $[\alpha]^{25}_D$ = –127.0 (*c* = 4.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, complicated by enol-keto tautomer and amide rotamers) δ: 5.36-5.31 (m, 2H), 5.22-5.12 (m, 2H), 4.20-4.06 (m, 2H), 3.76-3.70 (s, 3H), 3.46-3.33 (m, 1H), 3.03-2.95 (s, 3H), 2.55-2.38 (m, 1H), 1.78-1.56 (m, 9H), 1.25-1.21 (s, 3H), 1.12-0.92 (m, 3H), 0.85 (d, *J* = 6.4 Hz, 3H), 0.83 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): _ 206.8, 169.7, 168.2, 131.1, 130.5, 126.6, 126.5, 53.5, 52.2, 49.6, 49.3, 45.7, 42.0, 40.0, 38.4, 37.3, 35.6, 33.4, 27.2, 22.5, 17.9, 17.3; HRMS (ESI) m/e 398.2303 [M+Na]$^+$ calcd for $C_{22}H_{33}NO_4Na$: 398.2302.

C_3-phenyl tetramic acid 2a: β-ketoamide **20a** (45 mg, 0.097 mmol) was dissolved in

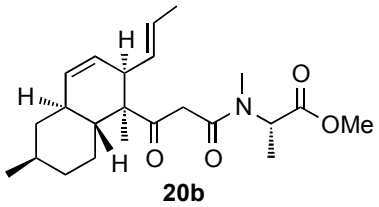


2a

MeOH (5.3 ml) and then NaOMe solution (0.97 mL, 0.5 M in MeOH) was added dropwise. The reaction was stirred at rt for 30 mins before it was quenched with 1M HCl. The mixture was diluted with water and CH_2Cl_2 and separated, the aqueous layer was further extracted with CH_2Cl_2 (5x). The combined organic phase was dried over $MgSO_4$ and concentrated to yield tetramic acid **2a** (40 mg, 95%) as a red-brown oil. $[\alpha]^{25}_D$ = –124.1 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.22 (m, 3H), 7.12 (m, 2H), 5.39 (br s, 2H), 5.24 (m, 1H), 5.16 (m, 1H), 3.83 (t, *J* = 4.5 Hz, 1H), 3.74

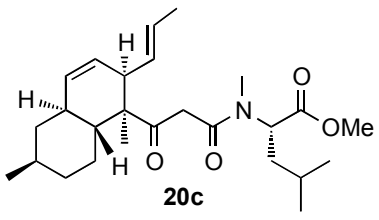
(m, 1H), 3.36 (br s, 1H), 2.91 (br s, 4H), 1.84 -1.58 (m, 4H), 1.50-1.40 (m, 2H), 1.53 (d, *J* = 4.2 Hz, 3H), 1.33 (s, 3H), 1.15-0.95 (m, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 198.1, 191.1, 176.7, 135.4, 131.1, 130.0, 129.5, 128.6, 127.1, 126.8, 124.4, 100.4, 67.0, 53.6, 48.7, 45.2, 42.4, 39.9, 38.7, 35.8, 33.6, 29.8, 28.9, 22.6, 18.1, 13.8; HRMS (ESI) m/e 456.2509 [M+Na$^+$] calcd for $C_{28}H_{35}NO_3Na^+$: 456.2510

C_3-methyl tetramic acid 2b: Same procedure as **2a** afforded **2b** (32 mg, 99%) as red-brown oil. $[\alpha]^{25}_D$ = –148.9 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ : 5.39 (br s, 2H), 5.27 (m, 1H), 5.19 (m, 1H), 3.70 (m, 1H), 3.42 (br s, 1H), 2.98 (s, 3H), 1.97 (m, 1H), 1.86-1.58 (m, 4H), 1.56 (m, 3H), 1.46 (d, *J* = 5.0 Hz, 3H), 1.40 (m, 3H), 1.19-1.05 (m, 3H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.86 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) : 198.7, 192.3, 176.0, 131.2, 130.5, 130.0, 126,9, 100.3, 57.4, 48.6, 45.2, 42.4, 40.1, 38.5, 35.8, 33.7, 29.7, 28.4, 26.9, 22.5, 18.0, 15.2; HRMS (ESI) m/e 380.2196 [M+Na$^+$] calcd for $C_{22}H_{31}O_4NNa^+$: 380.2194



20b

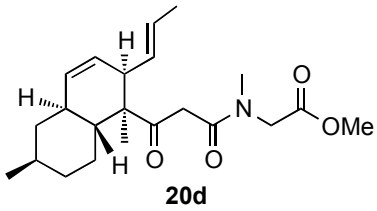
C_3-isobutyl tetramic acid 2c: Same procedure as **2a** afforded **2c** (28 mg, 99%) as red-brown oil. $[\alpha]^{25}_D$ = –178.3 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ : 5.40 (br s, 2H), 5.24 (m, 2H), 3.59 (m, 1H), 3.36 (br s, 1H), 2.98 (s, 3H), 1.97 (m, 1H), 1.81 (m, 3H), 1.68 (m, 1H), 1.46 (d, *J* = 5.0 Hz, 3H), 1.34 (d, *J* = 6.9 Hz, 3H), 1.13-1.04 (m, 3H), 0.91 (d, *J* = 6.3 Hz, 3H), 0.84 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) : 204.5, 191.8, 176.6, 131.5, 131.1, 130.0, 127.1, 101.1, 65.0, 48.5, 44.9,



20c

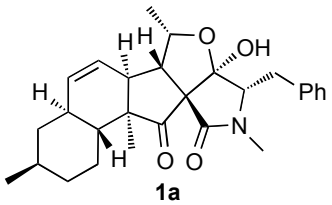
42.4, 40.0, 38.7, 35.9, 33.7, 29.8, 28.5, 27.2, 24.9, 22.6, 18.1, 17.8, 14.3, 12.4; HRMS (ESI) m/e 422.2666 [M+Na$^+$] calcd for $C_{25}H_{37}O_3NNa^+$ 422.2663

C_3-dihydro tetramic acid 2d: Same procedure as **2a** afforded **2d** (47 mg, 99%) as red-brown oil. $[\alpha]^{25}_D$ = –189.6 (*c* = 2.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ : 5.39 (br s, 2H), 5.10 (m, 2H), 3.62 (m, 2H), 3.32 (br s, 1H), 3.02 (s, 3H), 1.95 (m, 1H), 1.86-1.58 (m, 4H), 1.53 (d, *J* = 5.2 Hz, 3H), 1.50-1.40 (m, 3H), 1.15-0.95 (m, 3H), 0.90 (d, *J* = 6.6 Hz, 3H), 0.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 198.1, 188.7, 176.8, 131.0, 129.9, 127.1, 126.8, 100.6, 57.0, 48.6, 45.1, 42.3, 40.1, 38.7, 35.8, 33.6, 28.9, 28.4, 22.6, 18.0, 14.2; HRMS (ESI) m/e 366.2039 [M+Na]$^+$ calcd for $C_{21}H_{29}NO_3Na$: 366.2040.



20d

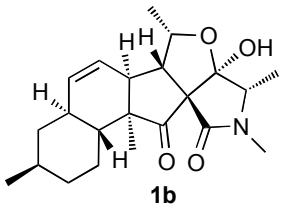
C_3-phenyl fusarisetin 1a : A solution of tetramic acid (**2a**, 12 mg, 0.027 mmol) in acetic acid (0.1 ml) was added ceric ammonium nitrate (CAN, 16 mg, 0.027 mmol). The mixture was stirred at rt under oxygen atmosphere (1 atm, balloon) for 30 mins. The reaction was diluted with CH_2Cl_2, passed through a short silica pad, washed with CH2Cl2/MeOH (20:1) and concentrated in *vacuo*. The residue obtained above was then dissolved in anhydrous MeOH (0.5 ml), followed by the addition of thiourea (22 mg, 0.27 mmol). This reaction was heated in a sealed microwave vial at 70°C for 1 hr. The reaction was allowed to cool to rt and was concentrated in *vacuo*. The crude product was purified purified *via* preparative TLC to afford **1a** (5.2 mg, 42%) as a semi-solid oil. $[\alpha]^{25}_D$ = +58.5 (*c* = 0.53, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ : 7.29 (m, 4H), 7.22 (m, 1H), 5.72 (m, 1H), 5.52 (d, *J* = 9.7 Hz, 1H), 4.45 (m, 1H), 3.81 (m, 1H),



1a

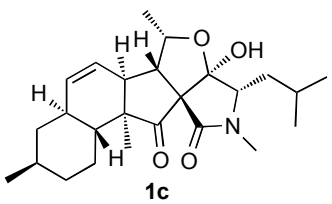
3.15 (m, 1H), 3.08 (m, 1H), 3.02 (dd, *J* = 10.8, 6.3 Hz, 1H), 2.73 (s, 3H), 2.60 (dd, *J* = 10.9, 4.6 Hz, 1H), 2.49 (s, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.42 (d, *J* = 6.3 Hz, 3H), 1.05 (m, 1H), 1.00 (s, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 211.7, 169.3, 138.3, 133.0, 129.5, 129.2, 126.9, 125.4, 109.1, 79.8, 75.1, 70.4, 54.5, 54.2, 43.5, 41.9, 37.5, 36.9, 36.8, 35.2, 33.2, 30.0, 29.9, 25.5, 22.6, 17.9, 14.3. HRMS (ESI) m/e 472.2458 $[M+Na]^+$ calcd for $C_{28}H_{35}O_4NNa^+$: 472.2459.

C_3-methyl tetramic acid 8b: Same procedure as **1a** afforded **1b** (6.2 mg, 25%) as a semi-solid oil. $[\alpha]^{25}_D$ = +46.1 (*c* = 0.62, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.72 (m, 1H), 5.52 (d, *J* = 10.4 Hz, 1H), 4.56 (qd, *J* = 6.3, 3.5 Hz, 1H), 3.97 (m, 2H), 3.52 (dd, *J* = 6.9, 2.9 Hz, 1H), 2.92 (s, 3H), 2.73 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.37 (dd, *J* = 9.8, 4.6 Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d, *J* = 6.4 Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) _: 213.1, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI) m/e 396.2145 $[M+Na]^+$ calcd for $C_{25}H_{37}NO_4Na$: 396.2143.



1b

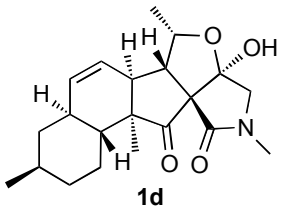
1c : Same procedure as **1a** afforded **1c** (4.0 mg, 21%) as a semi-solid oil. $[\alpha]^{25}_D$ = +34.7 (c = 0.4, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.71 (ddd, *J* = 10.3, 4.9, 2.5 Hz, H), 5.53 (d, *J* = 9.8 Hz, 1H), 4.42 (quint, *J* = 6.4, 1H), 3.97 (m, 2H), 3.52 (dd, *J* = 6.9, 2.9 Hz, 1H), 2.92 (s, 3H), 2.73 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.37 (dd, *J* = 9.8, 4.6 Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d, *J* = 6.4 Hz,



1c

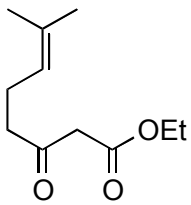
3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) _: 213.1, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI) m/e 438.2615 $[M+Na]^+$ calcd for $C_{25}H_{37}NO_4Na$: 438.2617.

1d: Same procedure as **1a** afforded **1d** (7.1 mg, 32%) as a semi-solid oil. $[\alpha]^{25}_D$ = +44.6 (*c* = 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.71 (ddd *J* = 10.3, 5.2, 2.9 Hz, 1H), 5.53 (d, *J* = 10.3 Hz, 1H), 4.31 (qd, *J* = 6.3, 6.3 Hz, 1H), 3.62 (d, *J* = 10.3 Hz, 1H), 3.52 (d, *J* = 10.3 Hz, 1H), 3.04 (br s, 1H), 2.92 (dd, *J* = 10.9, 5.7 Hz, 1H), 2.90 (s, 3H), 2.48 (dd, *J* = 10.3, 4.6 Hz, 1H), 1.82 (m, 2H), 1.73 (m, 1H), 1.63 (m, 1H), 1.54 (m, 2H), 1.45 (d, *J* = 6.9 Hz, 3H), 1.09 (qd, *J* = 13.2, 3.5 Hz, 1H), 1.01 (qd, *J* = 11.5, 3.4 Hz, 1H), 0.97 (s, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.85 (q, *J* = 12.6 Hz, 1H); ^{13}C NMR (200 MHz, $CDCl_3$): _ 213.0, 169.4, 132.8, 125.2, 107.2, 77.9, 74.3, 61.1, 54.5, 54.4, 43.4, 41.7, 37.2, 36.5, 35.2, 33.0, 29.8, 25.5, 22.4, 16.8, 14.0; HRMS (ESI) m/e 382.1990 $[M+Na]^+$ calcd for $C_{21}H_{29}NO_4Na$: 382.1989.



1d

Synthesis of CDE ring core analog

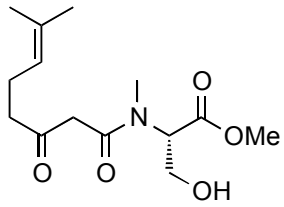


39

β-keto ester 39: To a solution of ethyl acetoacetate (1.0 g, 0.98 mL, 7.68 mmol) in THF at 0 °C was added all at once NaH (60% in mineral oil, 340 mg, 8.45 mmol). The reaction was allowed to stir for 30 mins and then n-BuLi (1.6 M in Hexanes, 5.8 mL, 8.07 mmol) was added drop wise to form a bright blood-orange solution. The mixture was stirred at this temperature for 30 mins, followed by rapid addition of 3,3-dimethylallyl bromide. The reaction was stirred at 0 °C for 3 hours, then allowed to reach room

temperature and stirred for an additional 4 hours when TLC showed consumption of starting material. The reaction mixture was then quenched with saturated NH_4Cl solution and extracted with diethyl ether (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica flash column chromatography to yield β-keto ester **39** (1.1 g, 73 %) as a clear oil. R_f: 0.3 (10:1, Hex:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.05 (t, *J* = 8.8 Hz, 1H), 4.19 (q, *J* = 8.8 Hz, 1H), 3.43 (s, 2H), 2.56 (t, *J* = 9.4 Hz, 2H), 2.27 (q, *J* = 8.8 Hz, 1H), 1.67 (s, 3H), 1.61 (s, 3H), 1.28 (t, *J* = 8.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 203.1, 169.6, 134.7, 119.7, 61.2, 59.7, 29.0, 26.9, 25.7, 17.7, 14.0; HRMS (ESI) m/e 198.1256 $[M+Na]^+$ calcd for $C_{11}H_{18}O_3Na^+$: 198.1257.

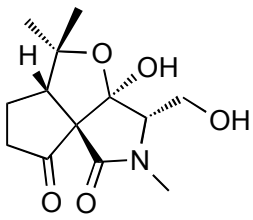
β-ketoamide 41: To a solution of β-keto ester **39** (110 mg, 0.56 mmol) in EtOH (2 mL) was added KOH (1M in H_2O, 1 mL) at room temperature. The reaction mixture was stirred for 10 hours at which time the reaction was diluted with CH_2Cl_2 and was quenched with 1M HCl. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was used without further purification. To a solution of crude β-keto acid (40 mg, 0.24 mmol) and freshly prepared *N*-methyl serine methyl ester (45 mg, 0.34 mmol) in CH_2Cl_2 (0.8 mL) was added consecutively HOBt (70.4 mg, 0.46 mmol) and EDC (71.4 mg, 0.45 mmol) at room temperature. The reaction was stirred overnight at this temperature and was then diluted with EtOAc and quenched with 1M HCl. The aqueous layers was extracted with EtOAc (3x), washed with brine, dried over Na_2SO_4 and concentrated. The crude residue was used was purified via silica flash column chromatography (CH_2Cl_2:MeOH, 200:1 to 20:1) to yield **41** (65 mg,



41

96%) as a pale yellow oil. R_f : 0.44 (15:1, CH_2Cl_2:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$, complicated by enol-keto tautomer and amide rotamers) δ: 5.11 - 5.05 (m, 1H), 4.83 (dd, *J* = 7.3, 5.4 Hz, 1H), 4.54 (dd, minor), 4.06 (dd, *J* = 11.6, 4.9 Hz, 1H), 4.00 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.78 (s, minor), 3.76 (s, 3H), 3.67 (s, minor), 3.62 (s, 2H), 3.04 (s, minor), 3.02 (s, 3H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.27 (q, *J* = 7.5 Hz, 2H), 1.66 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 204.5, 169.9, 168.1, 133.4, 122.2, 61.0, 60.4, 52.6, 49.7, 43.1, 35.6, 25.8, 22.4, 17.9; HRMS (ESI) m/e 308.1468 $[M+Na]^+$ calcd for $C_{14}H_{23}O_5NNa^+$: 308.1470.

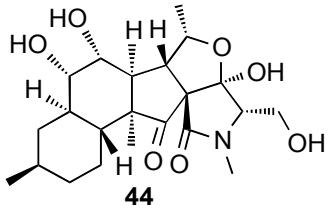
CDE ring analog 10: β-ketoamide **11** (45 mg, 0.16 mmol) was dissolved in MeOH (5.3 ml) and then NaOMe solution (1.6 ml, 0.5 M in MeOH) was added dropwise. The reaction was stirred at rt for 30 mins before it was quenched with 1M HCl. The mixture was diluted with water and CH_2Cl_2, separated, and the aqueous layer was further extracted with CH_2Cl_2 (5x). The combined organic phase was dried over $MgSO_4$ and concentrated to yield the corresponding tetramic acid (40 mg, 98%) as a dark red oil. This tetramic acid was dissolved in acetic acid (0.8 mL) and ceric ammonium nitrate (CAN, 88 mg, 0.16 mmol) was added. The reaction was stirred under oxygen atmosphere (1 atm) for 30 mins and then diluted with CH2Cl2 (5 ml), passed through a short silica pad, washed with CH2Cl2/MeOH (20:1, 20 ml) and concentrated in *vacuo*. The crude residue above was dissolved in anhydrous MeCN (0.8 ml), followed by the addition of CuCl (160 mg, 1.6 mmol). This reaction was stirred at rt for 2 hr, and was then concentrated in *vacuo* and purified via preparative TLC (CH2Cl2:MeOH 25:1, 4 times) to afford tricyclic core analog **10** (6.2 mg, 14 %) as a clear oil. R_f : 0.22 (15:1, CH_2Cl_2:MeOH); ^{1}H NMR (500 MHz, CDCl3) δ : 3.92 (m, 2H), 3.53 (t, *J* = 4.0 Hz, 1H), 3.1



43

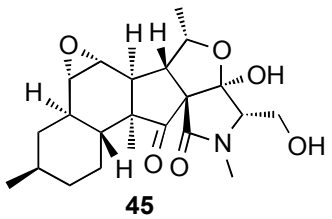
(t, *J* = 9.2 Hz, 1H), 2.94 (s, 3H), 2.49 (m, 2H), 2.14 (m, 2H), 1.45 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ : 211.9, 170.7, 108.9, 87.2, 68.8, 59.6, 55.1, 41.2, 30.6, 29.9, 29.2, 25.2, 22.7; HRMS (ESI) m/e 269.1263 [M+Na^+] calcd for $C_{13}H_{19}O_5NNa^+$: 269.1261

dihydroxy fusarisetin 44 : To a solution of (+)-**1** (4 mg, 0.01 mmol) in acetone:H_2O (9:1, 0.5 mL) was added sequentially NMO (1.4 mg, 0.01 mmol) and OsO_4 (4% w/w in H_2O, 13 μL) at room temperature. The reaction mixture was stirred for 16 hours was then diluted with EtOAc and water, extrated with EtOAc (3x), washed with brine, dried over Na_2SO_4 and concentrated. The residue was purfied by prep TLC (20:1 CH_2Cl_2:MeOH 3x) to yield **44** (2.6 mg, 62%) as a white solid. $[\alpha]^{25}_D$ = +65.6 (*c* = 0.26, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.39 (quint, *J* = 6.9 Hz, 1H), 4.00-3.90 (m, 3H), 3.56 (dd, *J* = 4.6, 2.3 Hz, 1H), 3.25 (dd, *J* = 12.1, 6.3 Hz, 1H), 2.94 (s, 3H), 2.25 (m, 1H), 1.76-1.63 (m, 4H), 1.55-1.45 (m, 2H), 1.39 (d, *J* = 6.4 Hz, 3H), 1.04 (s, 3H), 0.98-0.91 (m, 2H), 0.84 (d, *J* = 6.3 Hz, 3H), 0.80 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) d: 211.4, 169.4, 108.6, 83.8, 78.7, 74.7, 73.3, 69.5, 69.1, 67.2, 59.0, 54.2, 47.6, 47.3, 38.3, 37.0, 36.5, 34.5, 32.0, 29.0, 25.4, 22.6, 17.1; HRMS (ESI) m/e 446.2149 [M+Na]$^+$ calcd for $C_{22}H_{33}O_7NNa^+$: 446.2150.



44

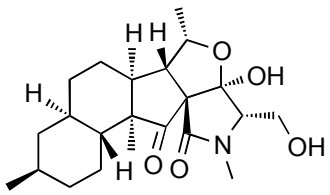
epoxy fusarisetin 45 : To a solution of **1** (3 mg, 0.0077 mmol) in CH_2Cl_2 (0.13 mL) at 0°C was added *m*-CPBA (70% w/w, 2.3 mg). The reaction was allowed to reach room temperature and stirred for 3 hours. The reaction mixture was diluted with CH_2Cl_2,



45

quenched with saturated aq. $Na_2S_2O_3$ and saturated aq. $NaHCO_3$ (1:1) and stirred for 15 mins. The aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were washed with saturated aq. $NaHCO_3$, dried over Na_2SO_4 and concentrated. The crude residue was purified by prep TLC (30:1, CH_2Cl_2:MeOH, 4x) to yield **45** (2.4 mg, 77%) as a white powder. $[\alpha]^{25}_D$ = +72.8 (*c* = 0.24, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.72 (m, 1H), 5.52 (d, *J* = 10.4 Hz, 1H), 4.56 (qd, *J* = 6.3, 3.5 Hz, 1H), 3.97 (m, 2H), 3.52 (dd, *J* = 6.9, 2.9 Hz, 1H), 2.92 (s, 3H), 2.73 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.37 (dd, *J* = 9.8, 4.6 Hz, 1H), 1.89-1.78 (m, 2H), 1.75-1.65 (m, 2H), 1.49-1.30 (m, 2H), 1.26 (d, *J* = 6.4 Hz, 3H), 1.05 (m, 2H), 0.96 (s, 3H), 0.89 (d, *J* = 6.3 Hz, 3H), 0.85 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) d: 213.2, 169.3, 109.0, 78.8, 73.8, 68.9, 59.2, 57.6, 51.1, 48.8, 41.0, 35.6, 34.7, 33.2, 33.0, 32.1, 28.7, 25.2, 22.9, 14.3; HRMS (ESI) m/e 428.2044 $[M+Na]^+$ calcd for $C_{22}H_{31}O_6NNa^+$: 428.2041.

Saturated decalin fusarisetin 47: To a solution of **1** (3 mg, 0.0077 mmol) in EtOAc (0.1 mL) was added Pd/C (0.5 mg). The mixture was stirred under an atmosphere of H_2 (5 atm) for 1 hour, at which time the mixture was filtered through a plug of celite, rinsed with EtOAc and concentrated to yield analog **47** (2.5 mg, 95%) as a clear oil. $[\alpha]^{25}_D$ = +79.4 (*c* = 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.56 (quint, *J* = 6.4 Hz, 1H), 4.05-3.91 (m, 3H), 3.55 (dd, *J* = 4.9, 2.5 Hz, 1H), 3.25 (dd, *J* = 11.7, 5.9, 1H), 2.94 (s, 3H), 2.23 (m, 1H), 1.73-1.59 (m, 4H), 1.57-1.42 (m, 3H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.31 (m, 2H), 1.04 (s, 3H), 0.98-0.91 (m, 2H), 0.84 (d, *J* = 6.9 Hz, 3H), 0.78 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) d: 212.2, 169.9, 132.5, 124.6, 109.3, 83.7, 74.8, 67.4, 59.2, 57.7, 55.4, 50.3, 41.7, 36.8, 36.5, 35.2, 33.0, 29.1, 25.4, 22.5, 22.4, 14.2; HRMS (ESI) m/e 414.2251 $[M+Na]^+$ calcd for $C_{22}H_{33}O_5NNa^+$: 414.2254.



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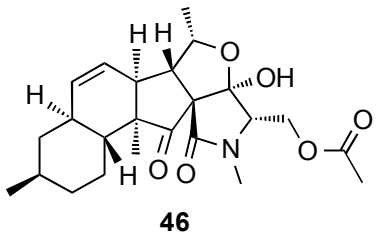
Aceto-fusarisetin 16 : To a solution of **1** (4 mg, 0.01 mmol) in CH_2Cl_2 (0.3 mL) at 0°C was added sequentially 4-DMAP (1.3 mg, 0.01) and Ac_2O (0.1 mL, 0.1 M in CH_2Cl_2). The reaction mixture was raised to r.t. temperature a stirred for 30 mins diluted with CH_2Cl_2 then quenched with saturated NH_4Cl. The mixture was extracted with CH_2Cl_2 (3x), the combined organic layers washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered and concentrated. The residue was purified by prep TLC (hexanes:EtOAc, 20:1, 3x) to yield **47** (3.4 mg, 78%) as a pale yellow oil. $[\alpha]^{25}_D$ = +52.1 (*c* = 0.34, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.72 (ddd, *J* = 10.3, 5.2, 2.9 Hz, 1H), 5.53 (d, *J* = 9.7 Hz, 1H), 4.46 (dd, *J* = 12.0, 6.3 Hz, 1H), 4.39 (m, 1H), 3.63 (dd, *J* = 6.9, 4.0 Hz, 1H), 2.97 (dd, *J* = 11.5, 5.7 Hz, 1H), 2.94 (s, 3H), 2.58 (dd, *J* = 9.5, 4.6 Hz, 1H), 2.14 (s, 3H), 1.87-1.78 (m, 3H), 1.75-1.67 (m, 2H), 1.60-1.51 (m, 2H), 1.46 (d, *J* = 6.3 Hz, 3H), 1.11-1.05 (m, 2H), 0.98 (s, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.83 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 211.8, 170.7, 169.4, 133.1, 125.3, 107.9, 78.9, 74.6, 67.9, 61.6, 54.5, 43.5, 41.8, 37.5, 36.7, 33.1, 29.2, 25.5, 22.9, 22.5, 21.0, 17.4, 14.3; HRMS (ESI) m/e 454.2200 $[M+Na]^+$ calcd for $C_{24}H_{33}O_6NNa^+$: 454.2201.



46

Biological Experiments

Scratch wound assay: MDA-MB-231 cells (5 x10^5 cells/24-well plate) were plated in dishes, and after 24 h of incubation at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), the confluent monolayer of cells was scratched with a pipette tip twice (in the shape of a cross, forming 4 quarter-

circle quadrants) to create a cell-free zone in each well. The medium was aspirated and each well washed with 10% Phosphate-buffered Saline (PBS) solution to remove any detached cells. The PBS was aspirated and replaced with fresh DMEM medium (500 μL) in the presence or absence of appropriate concentrations compound. The scratch-wounds (cell-free zones) were photographed (4 pictures per well; top, bottom, right and left) under a microscope (10x magnification). After 48 h, migrated cells were photographed (4 pictures per well; top, bottom, right and left) under a microscope (10x magnification). Values are the means ± SD for quadruplicate (n = 4) samples of the ratio (original wound area/area occupied by migrated cells) standardized to DMSO controls for each concentration.

Boyden-Chamber Transwell assay: Transwell cell migration assays were carried out using Transwell membrane filter inserts (BioExpress Transwell PC well insert, 6.5mm diameter) in a 24-well tissue-culture plate. The Transwell filter has 8 μm pore-size membranes. MDA-MB-231 cells ($5x10^5$ cells/well) suspended in serum free DMEM medium with diverse concentrations of fusarisetin A were added to the upper chambers, and DMEM medium containing 10% FBS was placed in the lower well, then incubated for 24 h at 37 °C in 5% CO_2. Non-invading cells on the upper surface of the membrane were removed by wiping them out with a cotton swab, and migrated cells on the lower surface were fixed with 4% formaldehyde solution and stained with Crystal Violet staining solution. The number of invaded cells per membrane was counted under a light microscope at 10x magnification. Values are the means ± SD for triplicate samples as a ratio of migrated cells with (+)-**1** : migrated with corresponding concentration of DMSO.

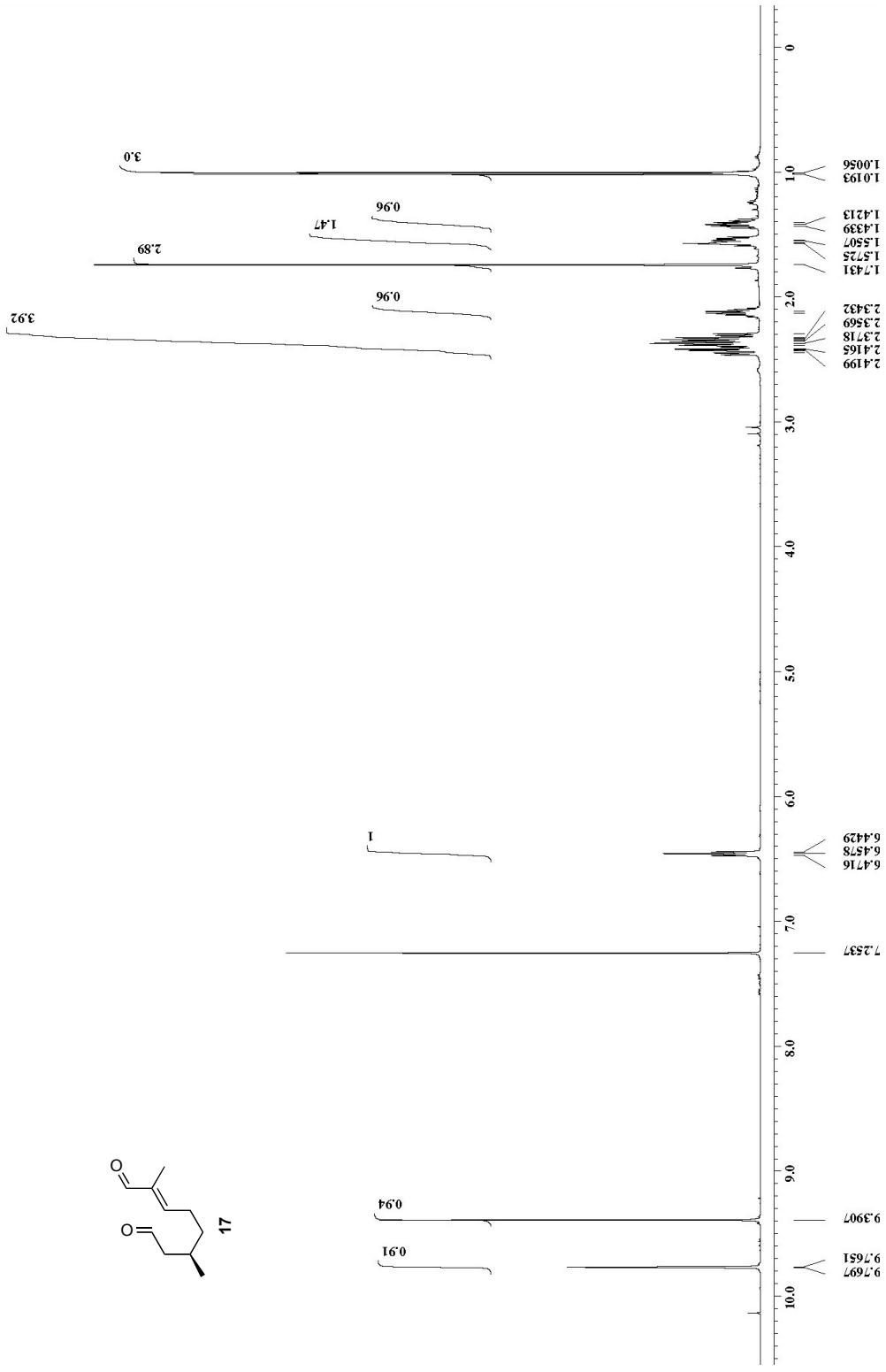
***ex-vivo* Mice skin assay**: Mice skin explants (5mm) were plated in a tissue culture dish

and incubated (37°C at 5% CO_2 atmosphere) with DMEM growth media containing 10 μg/ml of (+)-**1** or corresponding amount of DMSO. After 5 days of incubation the explants were photographed under a microscope (10x).

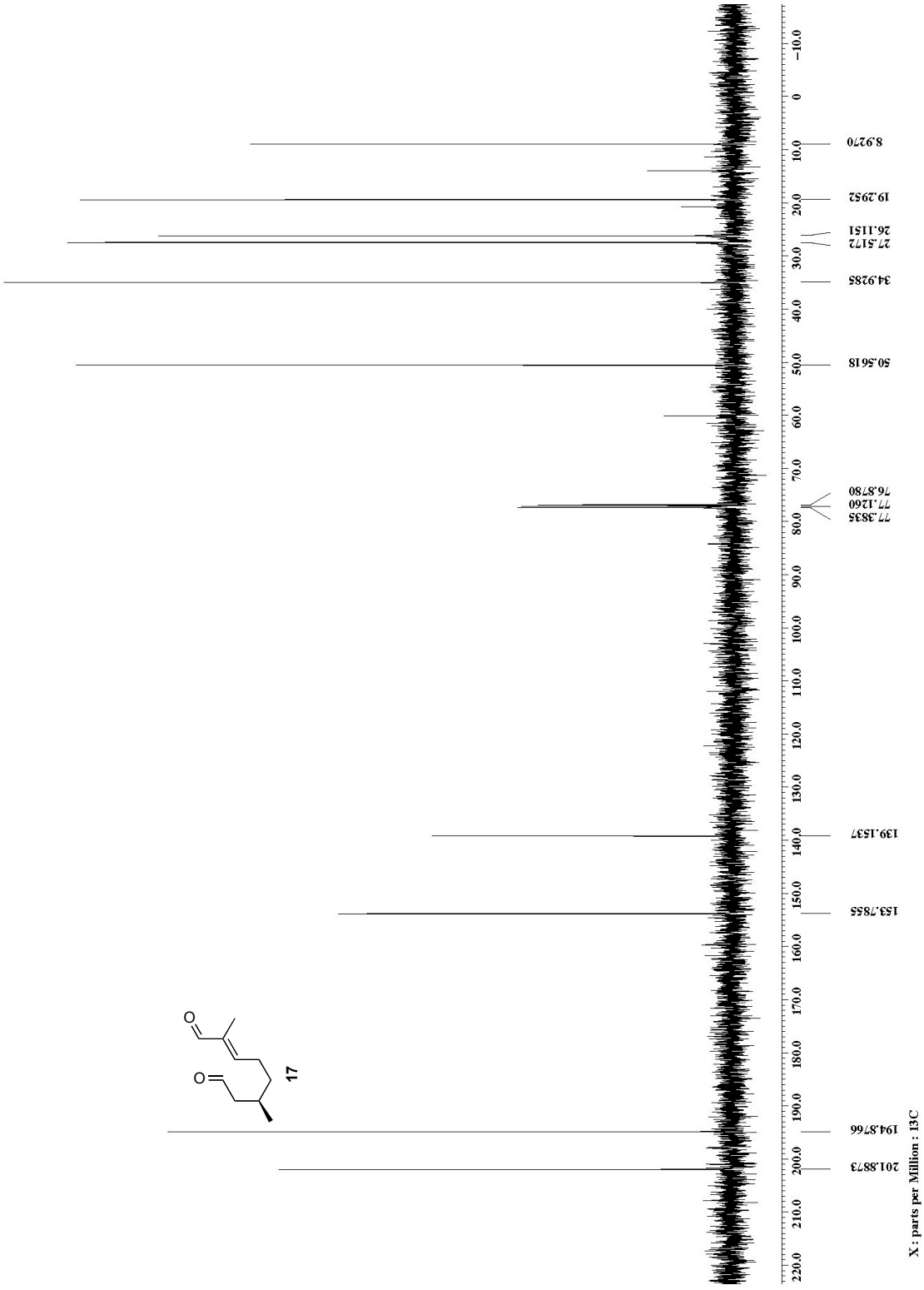
Immunofluorescence Assay: *Cell culturing:* MDA-MB-231 cells (1 x 10^4 cells/24-well plate) were plated in 12-well cell culture dishes (with circular cover slips installed inside the wells) and incubated for 24 hrs at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS). The wells were then aspirated and given DMEM (10% FBS) containing 10 μg/mL of Cytochalasin D (**2**) (Sigma Aldrich, C8273), 10 μg/mL of Fusarisetin A or appropriate amount of DMSO (vehicle control) then incubated for 4 hrs (37 °C, 5% CO_2). At t = 4hrs, select coverslips were removed from wells and washed for fixing. Media was then removed from remaining wells containing Cytochalsin D by aspiration and wells were washed with 10% Phosphate-buffered Saline (PBS, 3x), given DMEM (10% FBS) containing 10 μg/mL of Fusarisetin A, or appropriate amount of DMSO (vehicle control) and incubated for 4 hrs (37 °C, 5% CO_2). At t = 8hrs, coverslips were removed and washed for fixing. The well containing Fusarisetin A (since t = 0) was allowed to incubate for an additional 16 hrs (37 °C, 5% CO_2) at which time the coverslip was removed and washed (PBS 3x) for fixing.

Fixing and Staining Protocol*:* All removed cover slips were washed with PBS (3x) then treated with paraformaldehyde (4% in PBS) for 10 mins. The formaldehyde solution was aspirated and the slips were washed with PBS (3x). At this time, they were treated with blocking buffer [0.1% Triton X, 3% bovine serum albumin (BSA) both in PBS] for 30 mins. Then the slips were treated with FITC-conjugated Phalloidin (Sigma Aldrich, P5282) and Rhodamine-conjugated anti-tubulin antibody (Cytoskeleton Inc., TL590M)

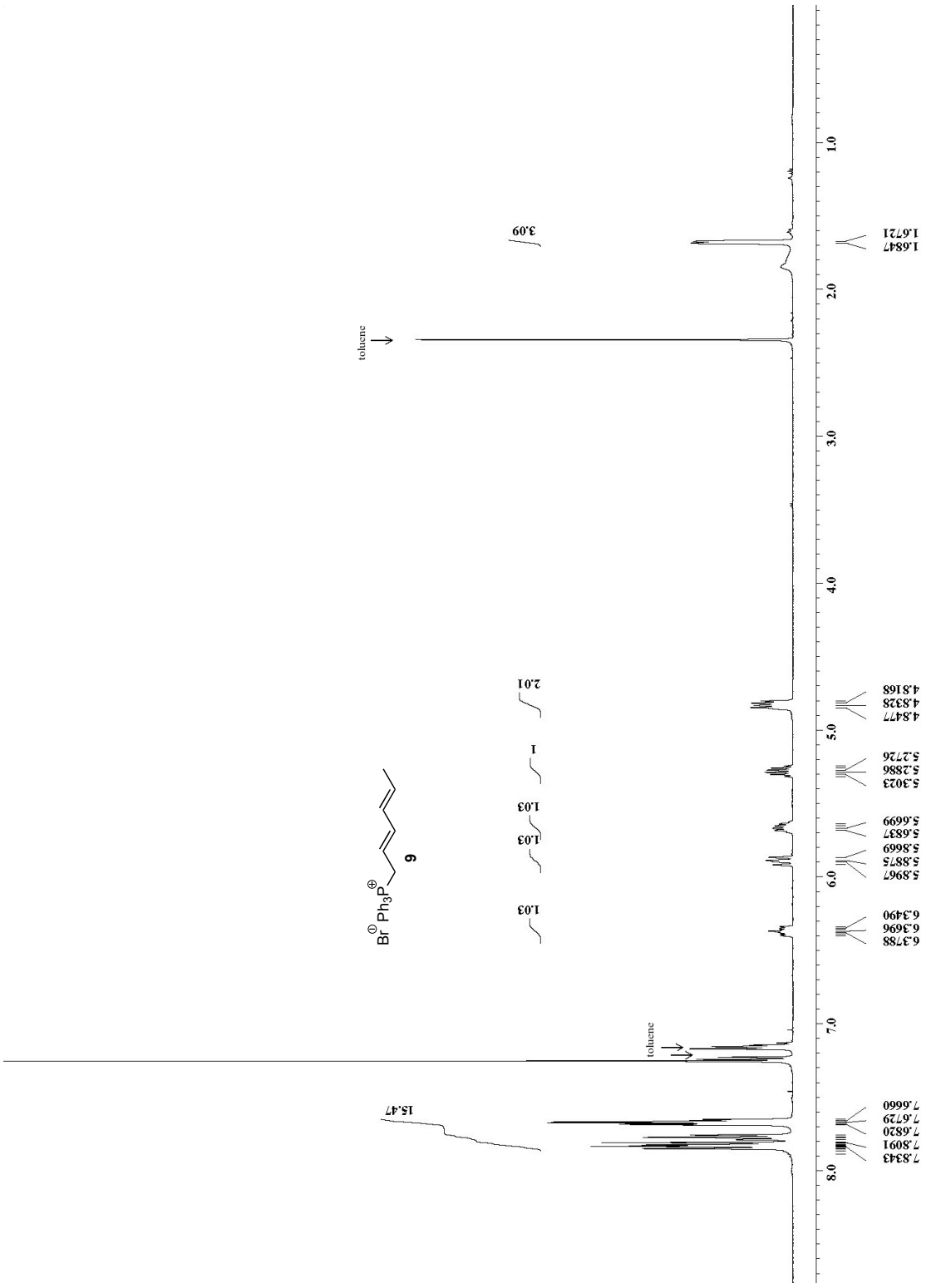
both at 1:100 dilution in blocking buffer for 1hr, then washed with PBS (3x). The coverslips were finally mounted onto microscope slides with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Life Technologies, D1306) solution. Fluorescent images were taken at 40x magnification.



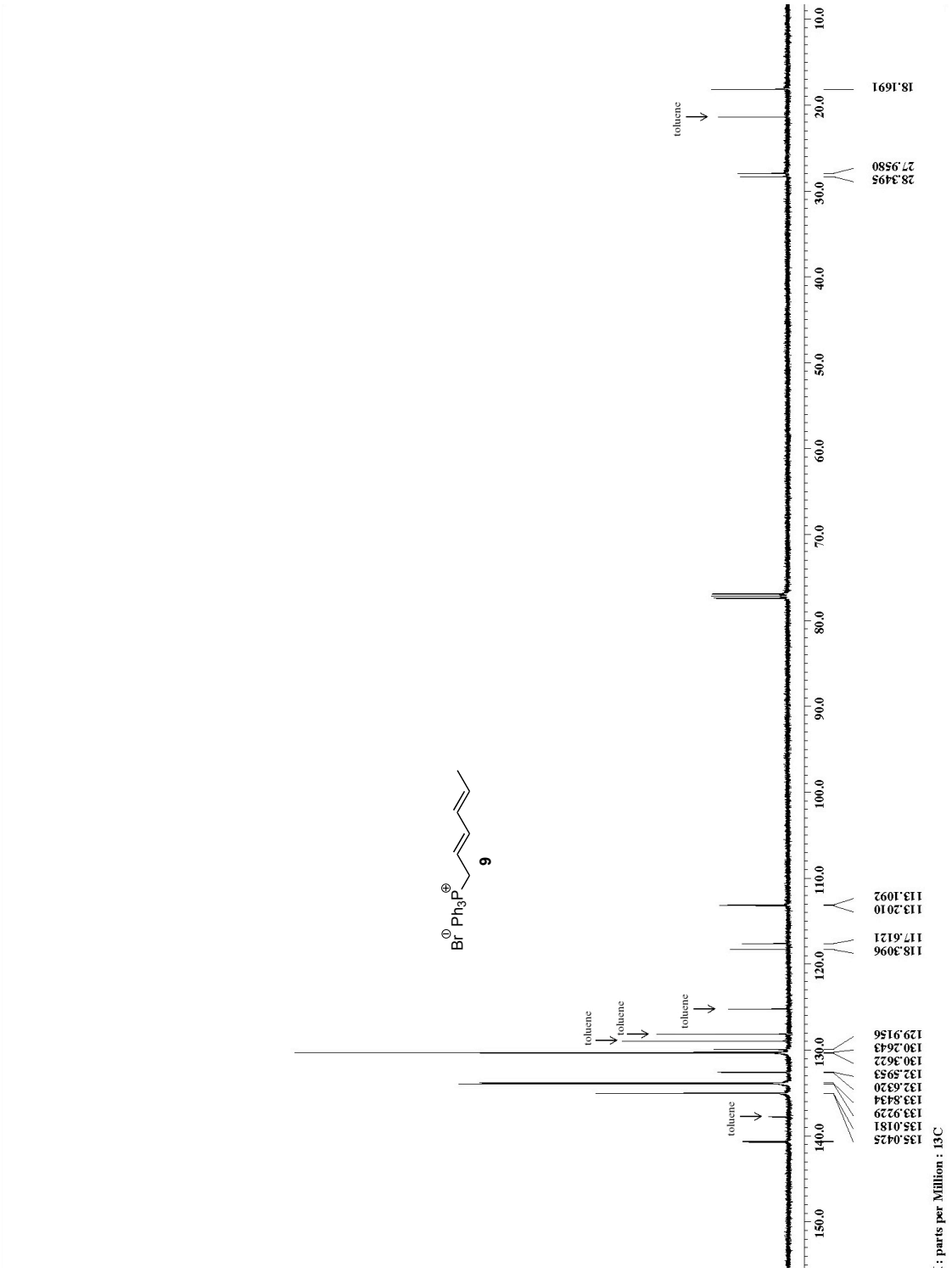
Spectrum 2.1 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **17**



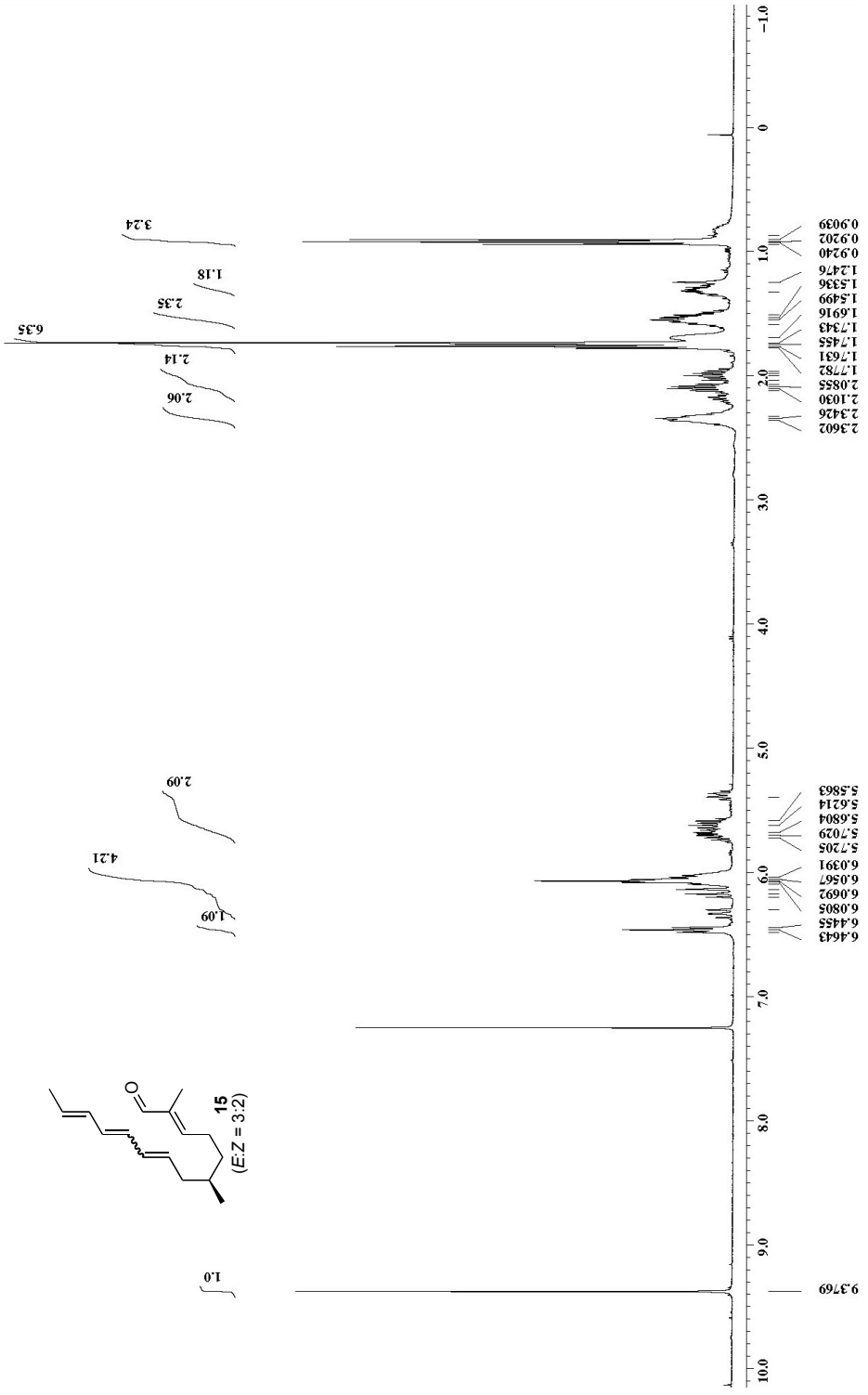
Spectrum 2.2 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **17**



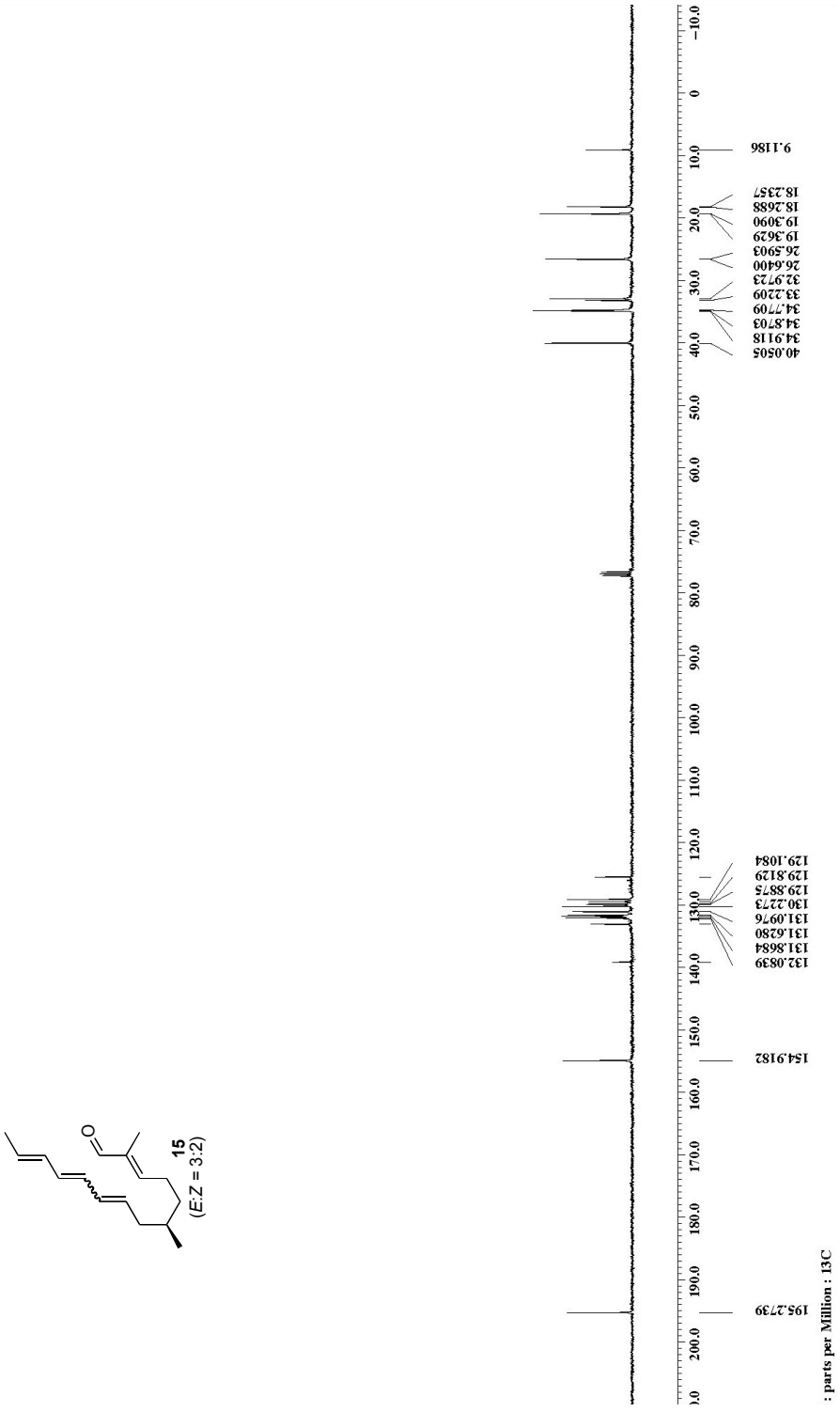
Spectrum 2.3 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **9**



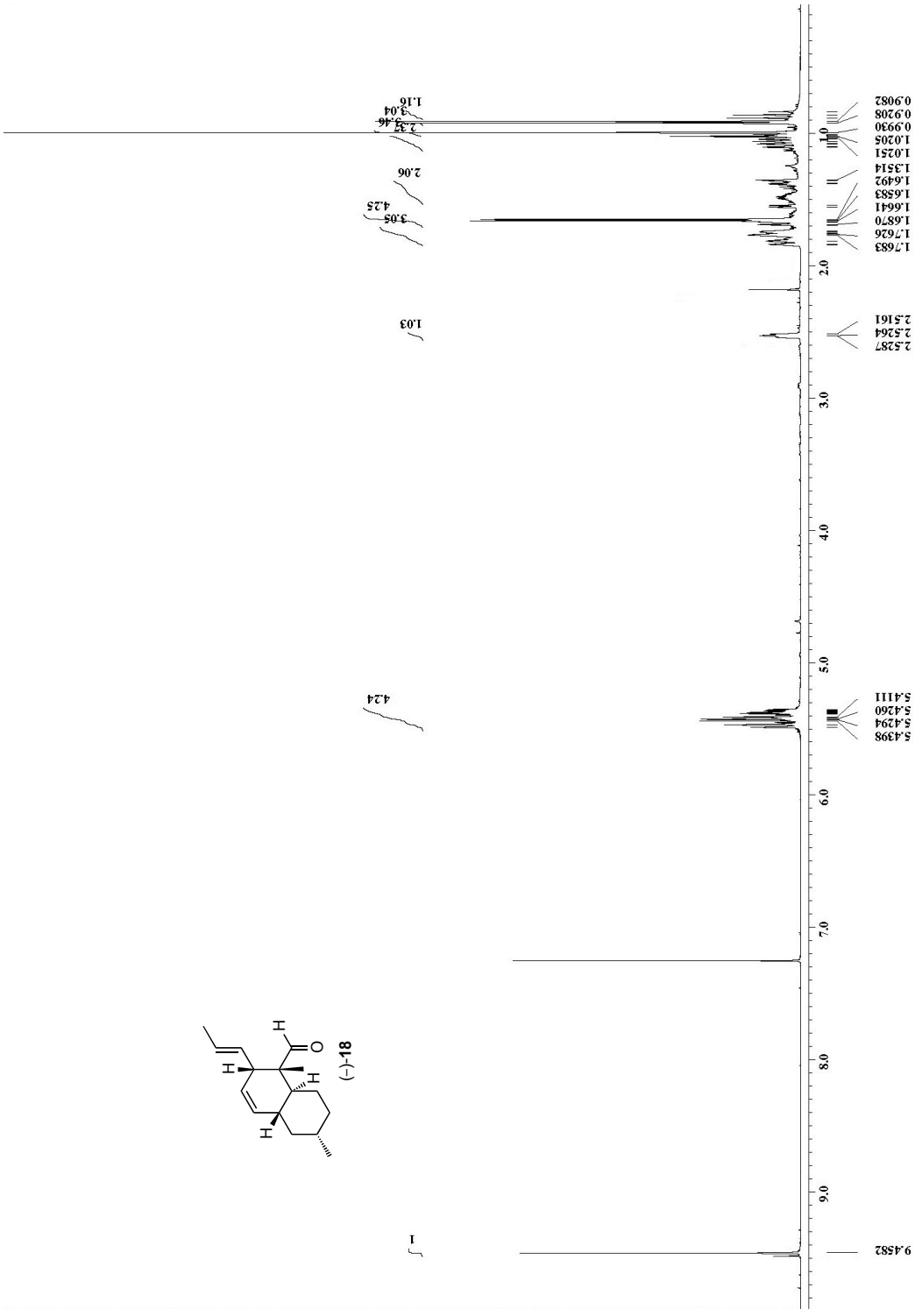
Spectrum 2.4 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **9**



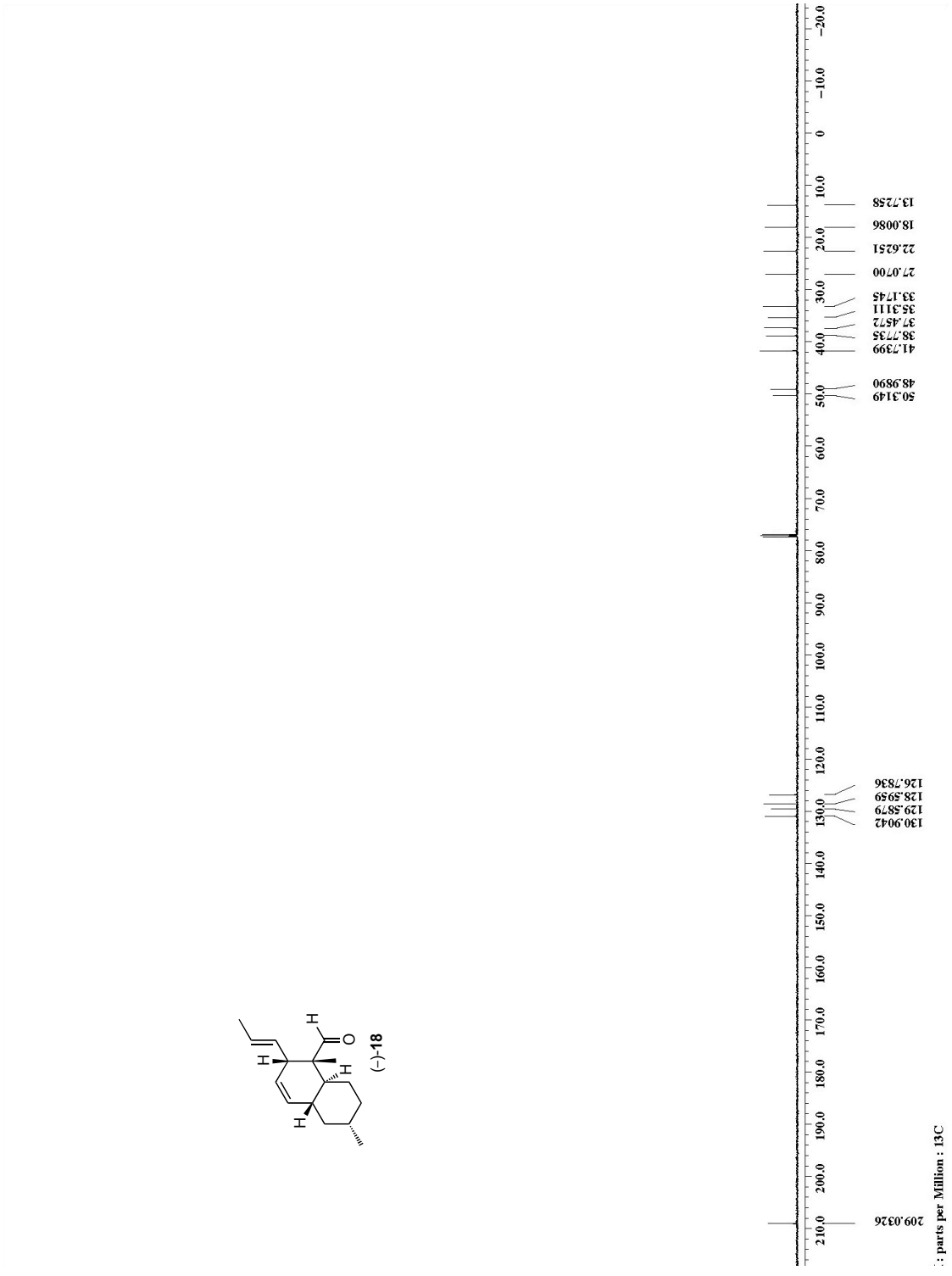
Spectrum 2.5 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **15**



Spectrum 2.6 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **15**

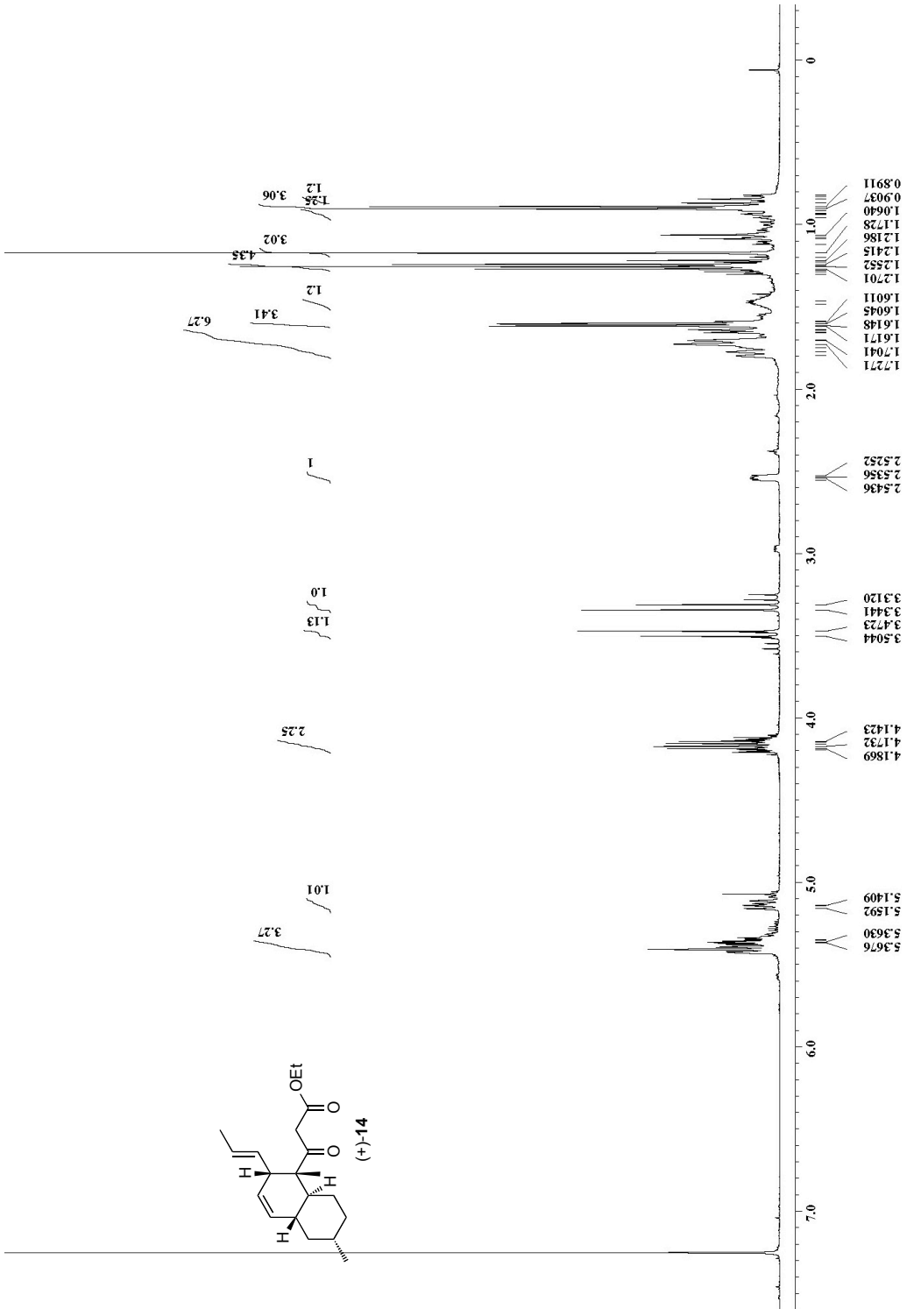


Spectrum 2.7 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **18**

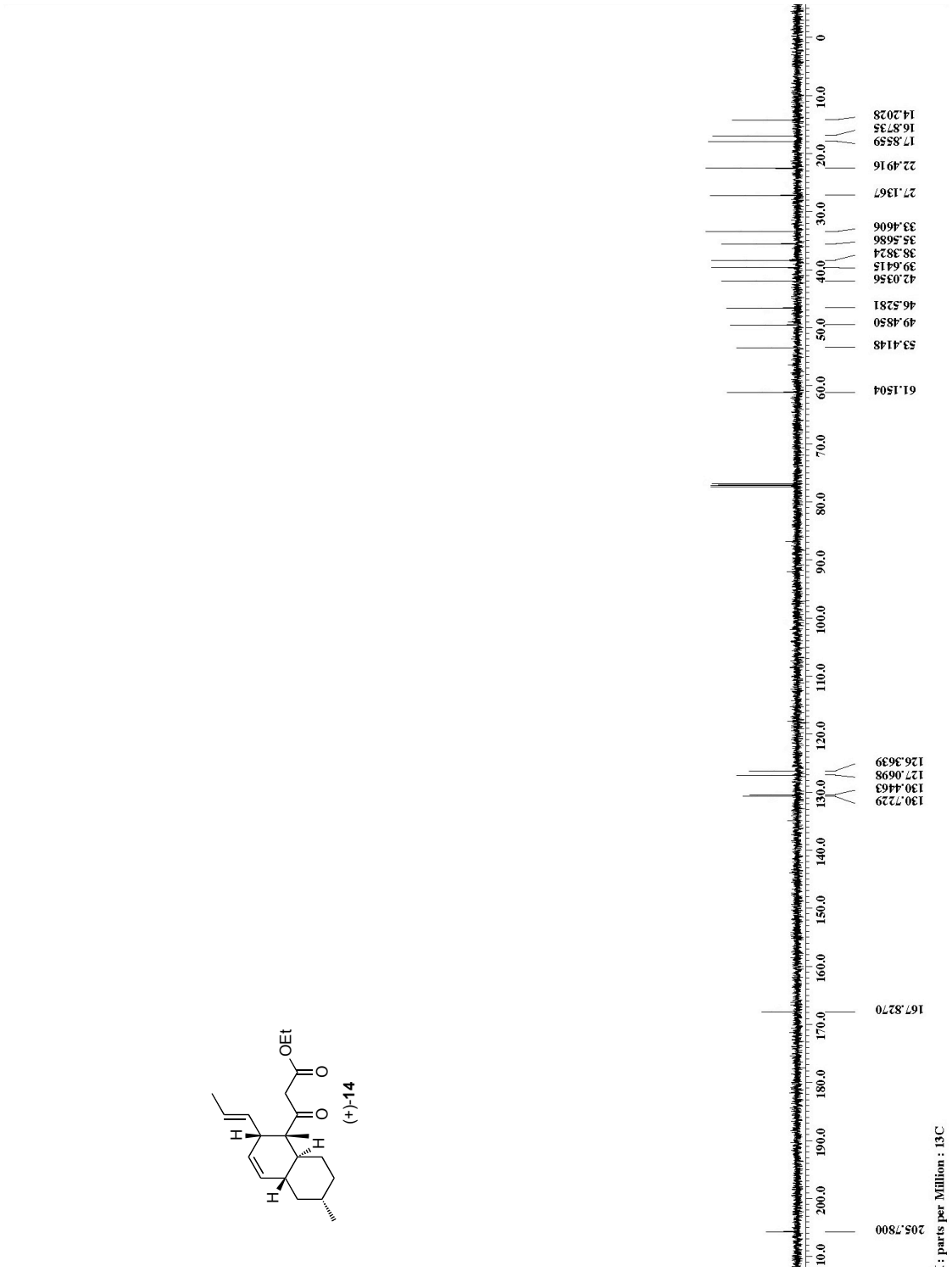


(−)-**18**

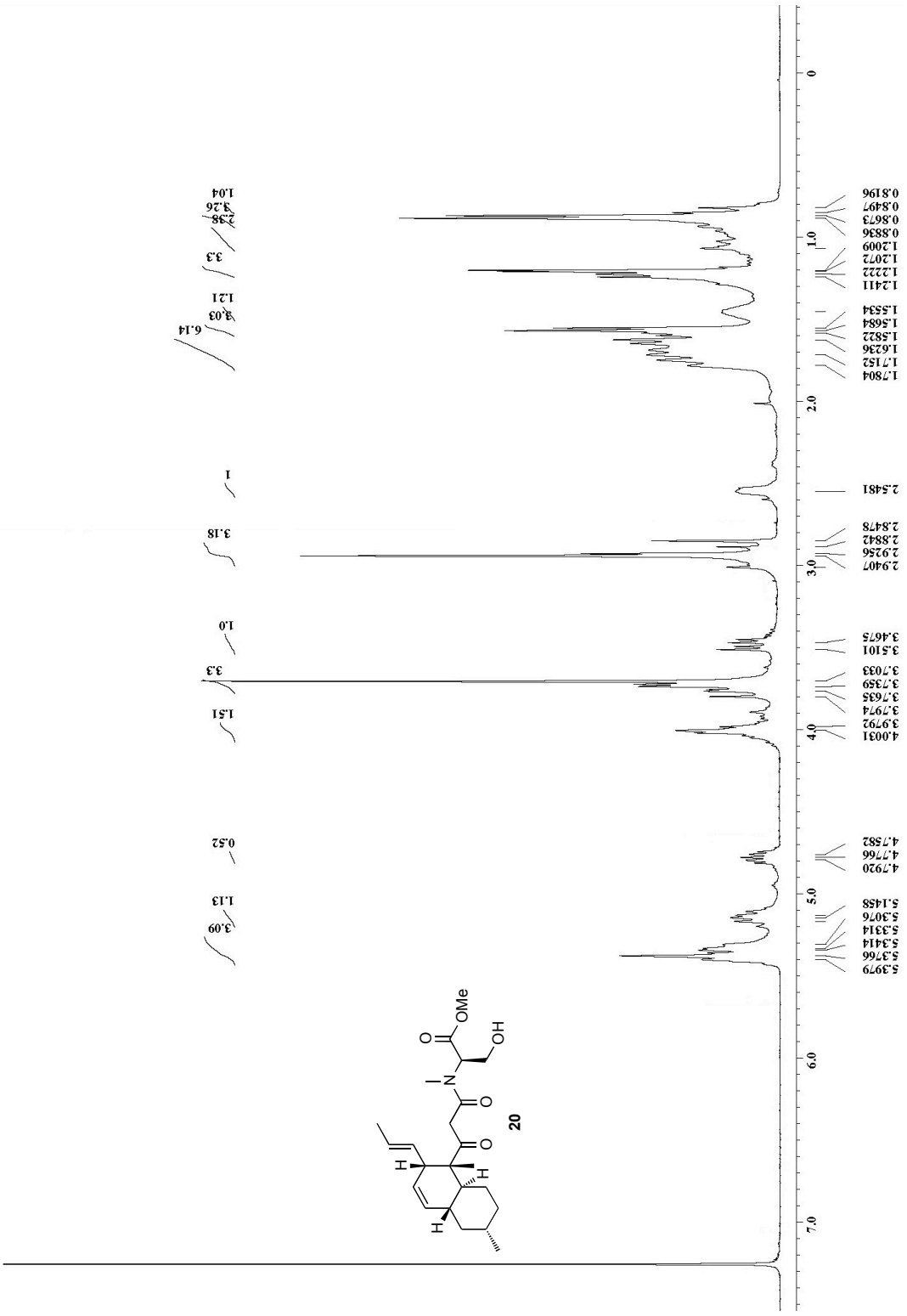
Spectrum 2.8 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **18**



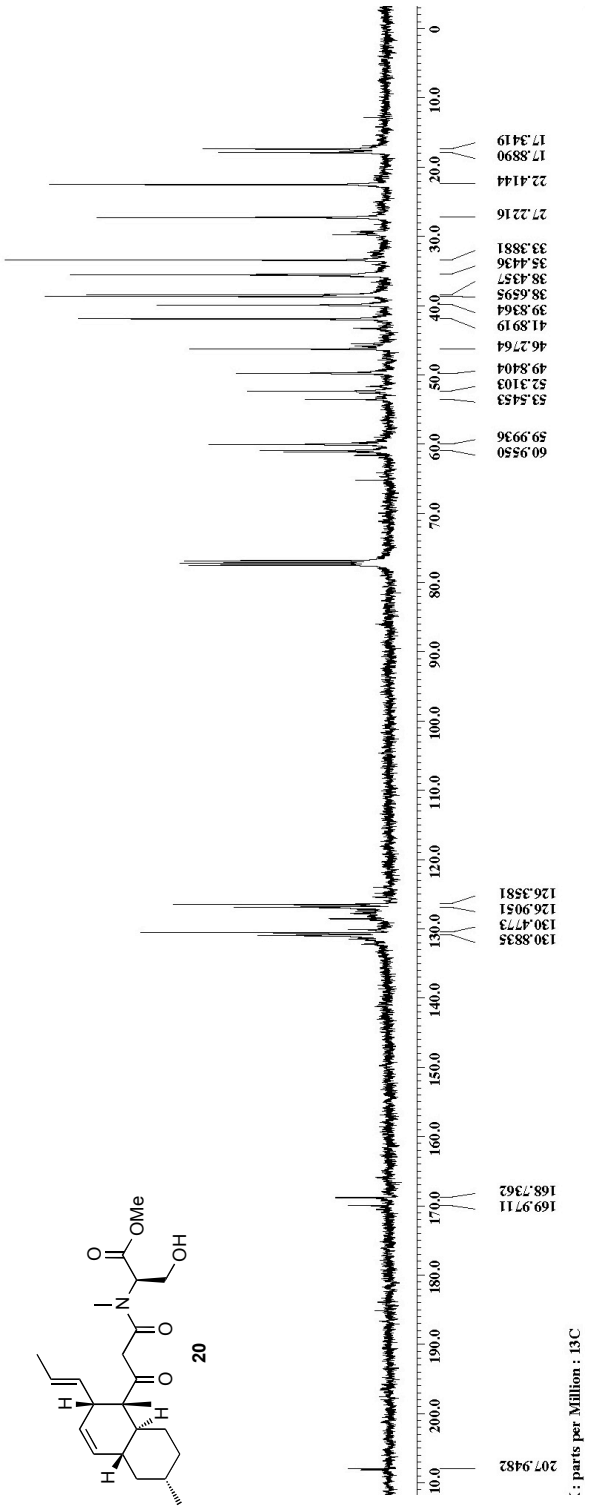
Spectrum 2.9 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **14**



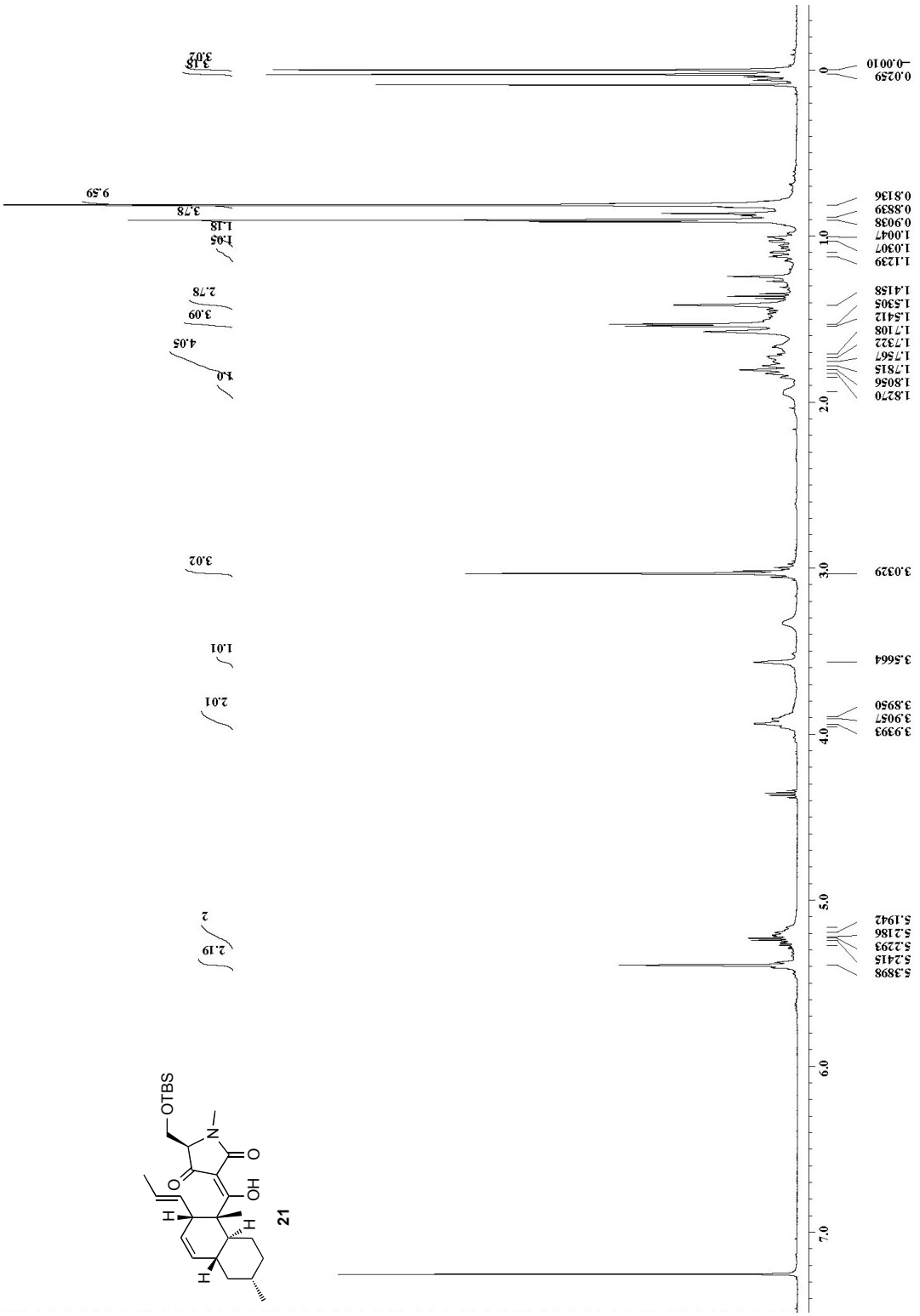
Spectrum 2.10 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **14**



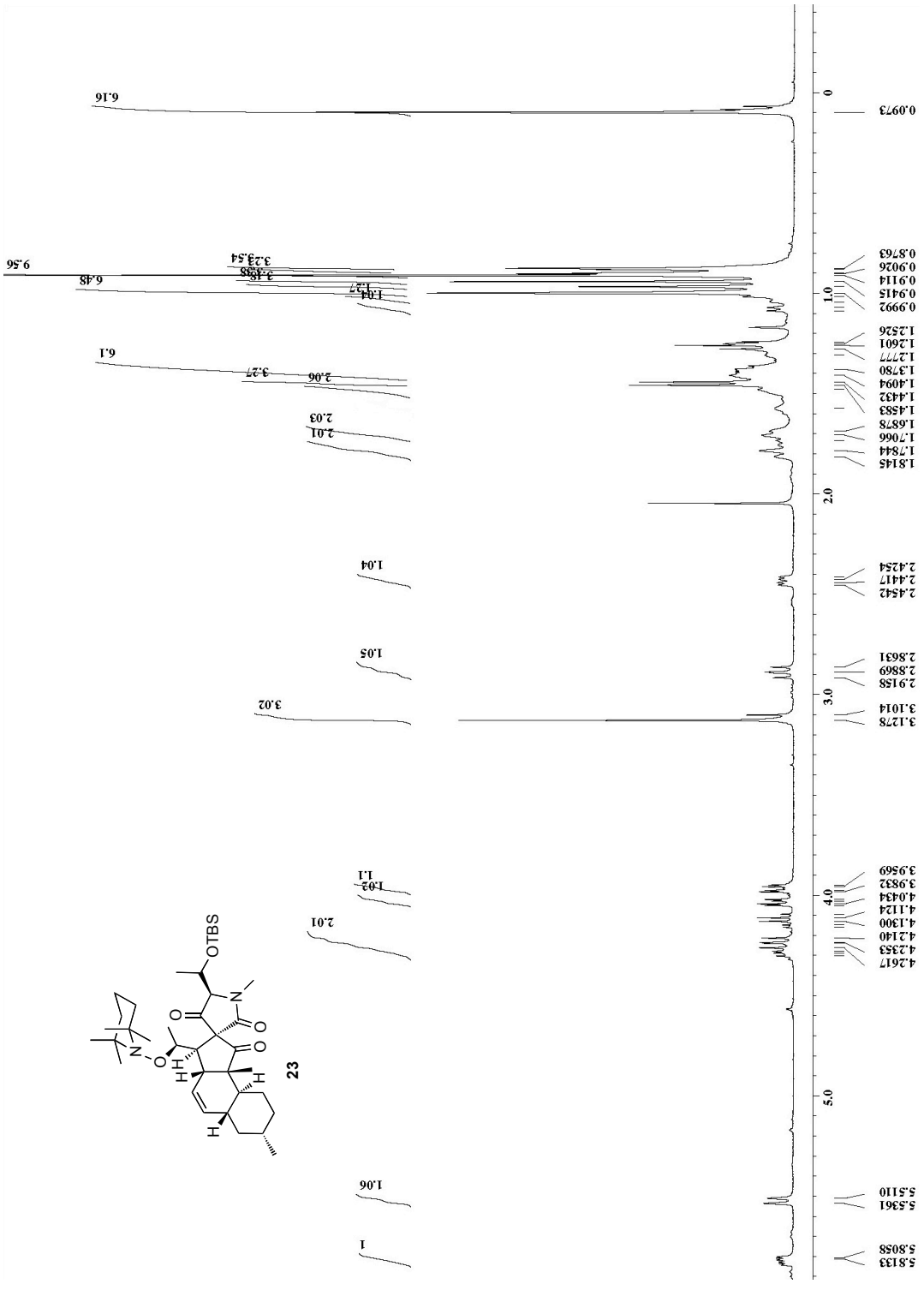
Spectrum 2.11 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20**



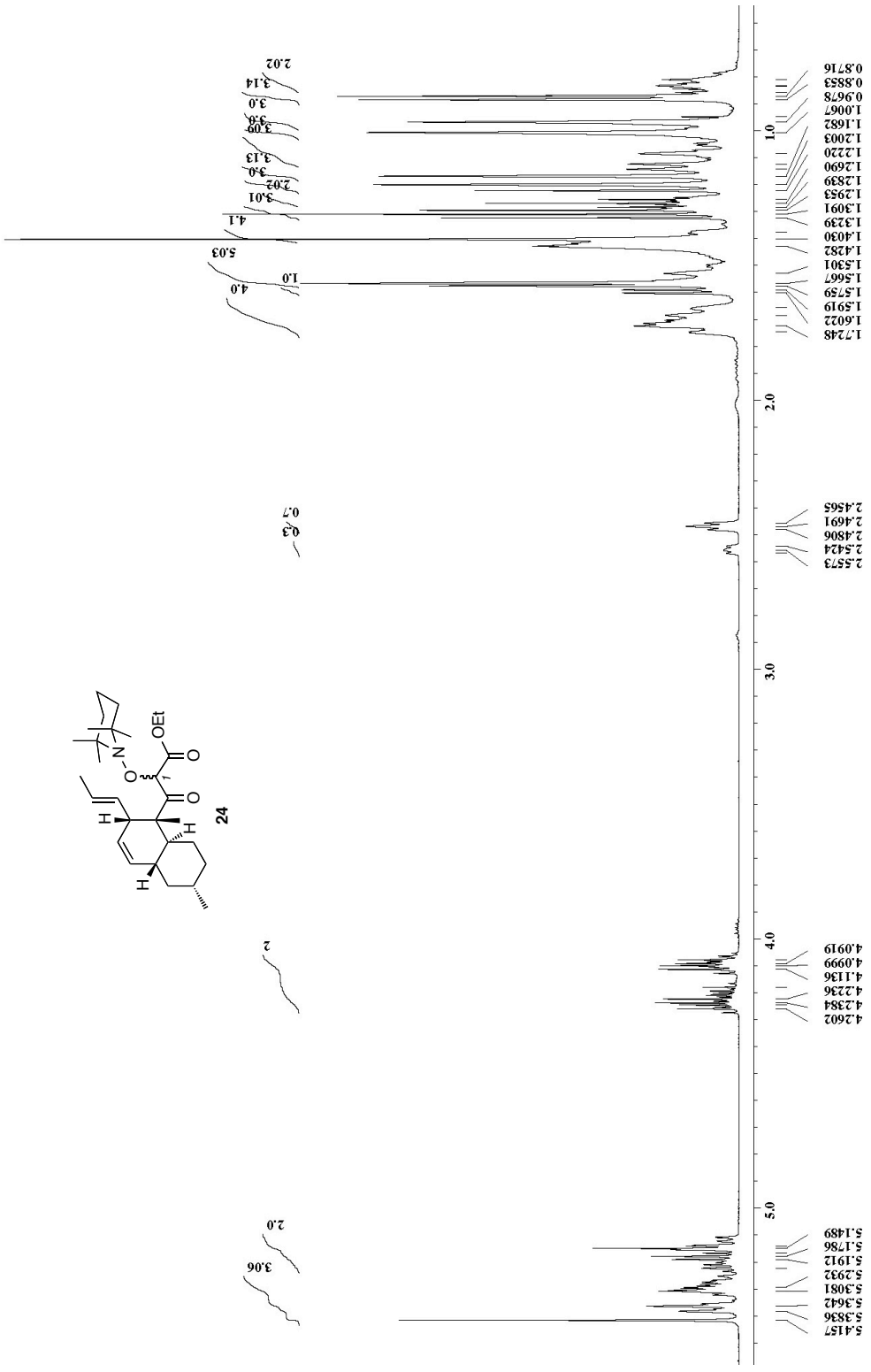
Spectrum 2.12 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20**



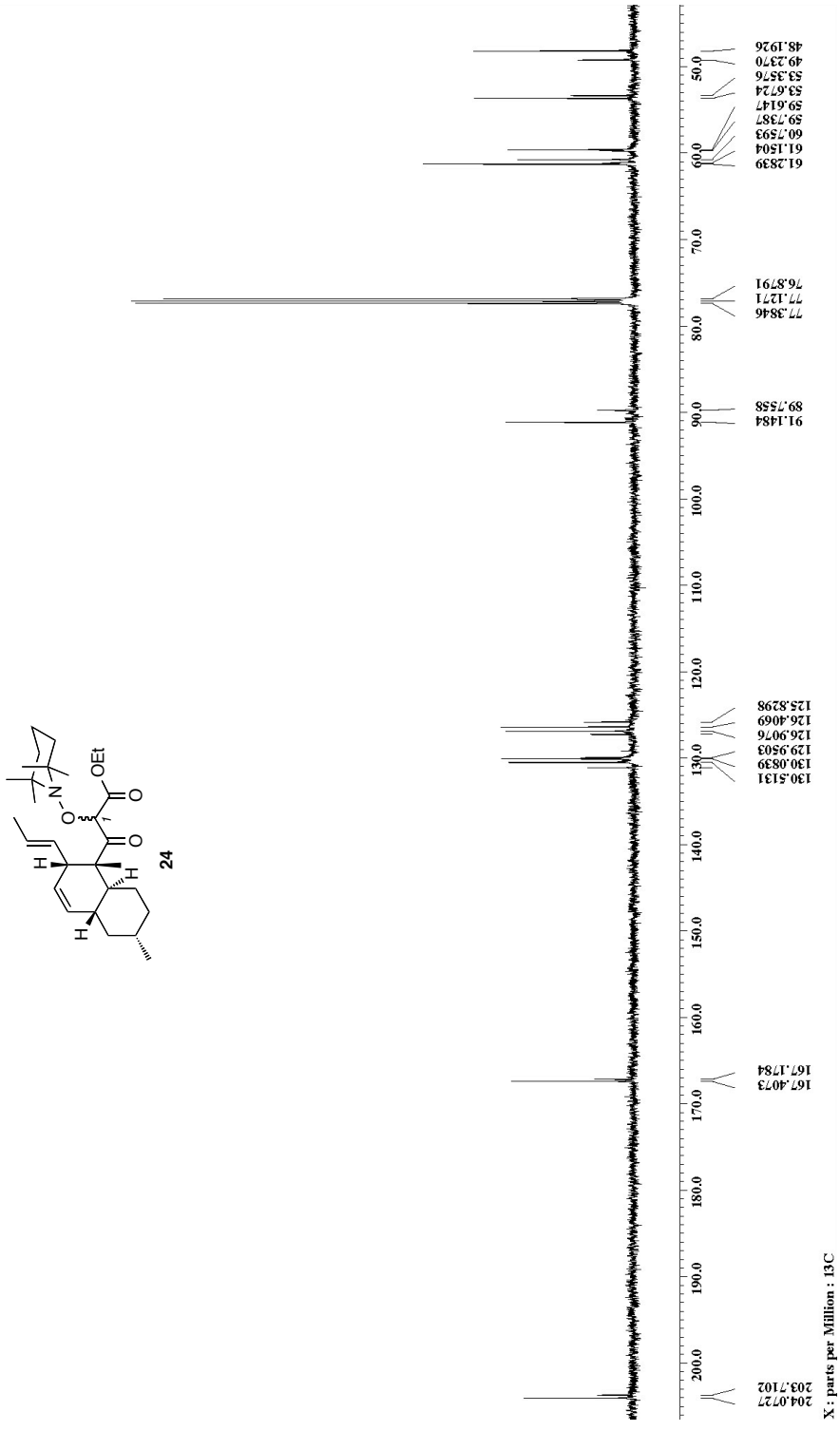
Spectrum 2.13 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **21**



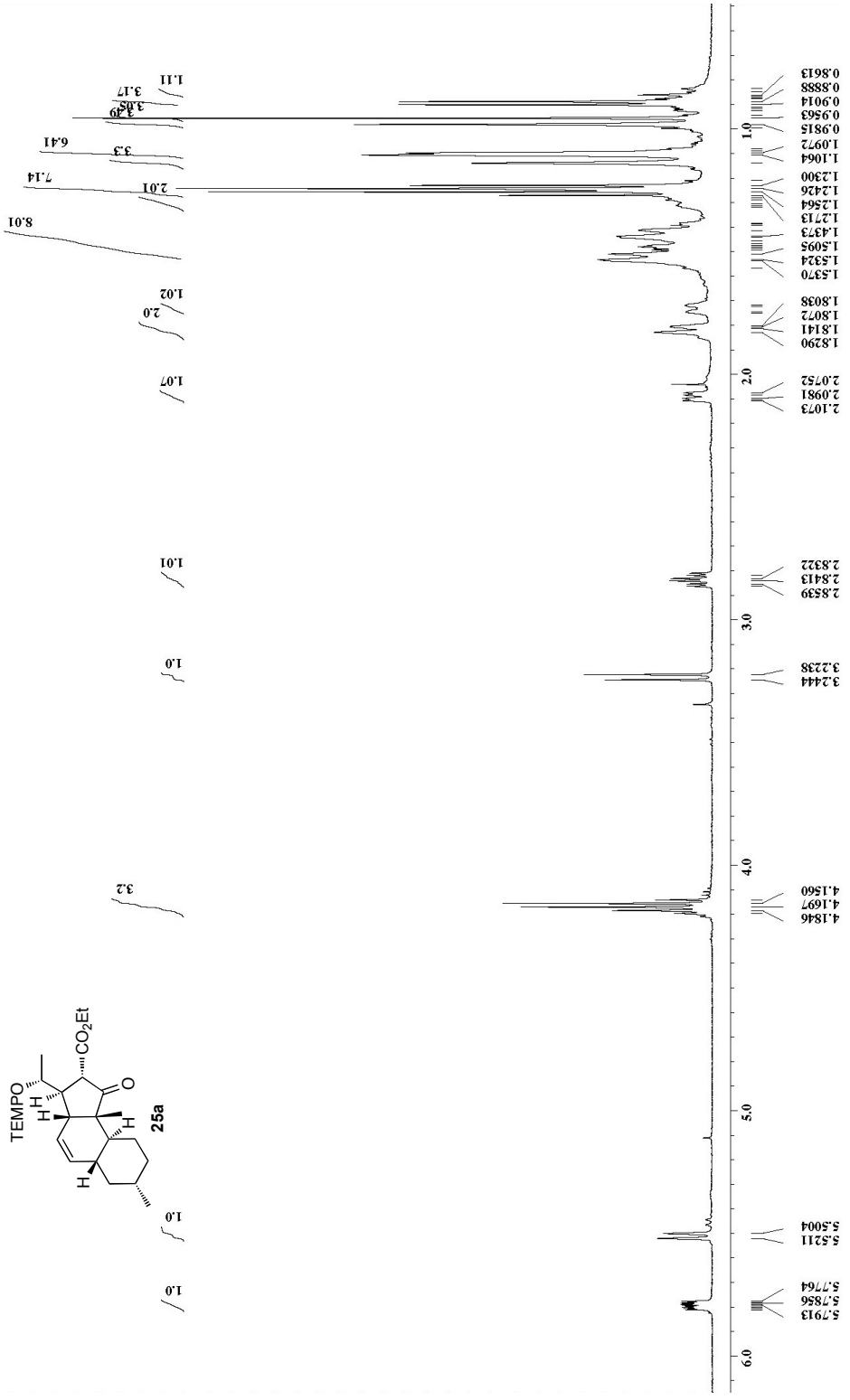
Spectrum 2.14 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **23**



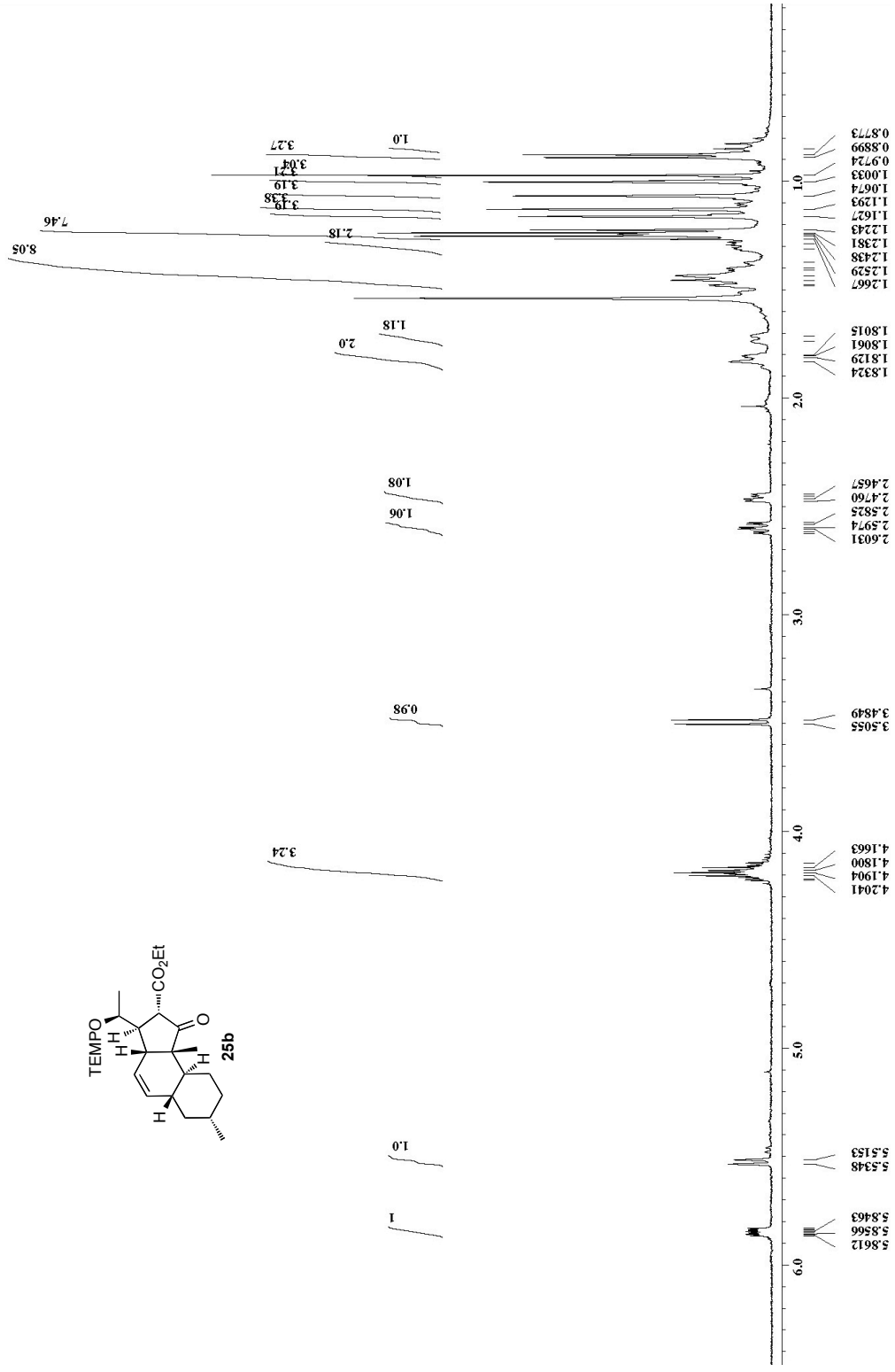
Spectrum 2.15 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **24**



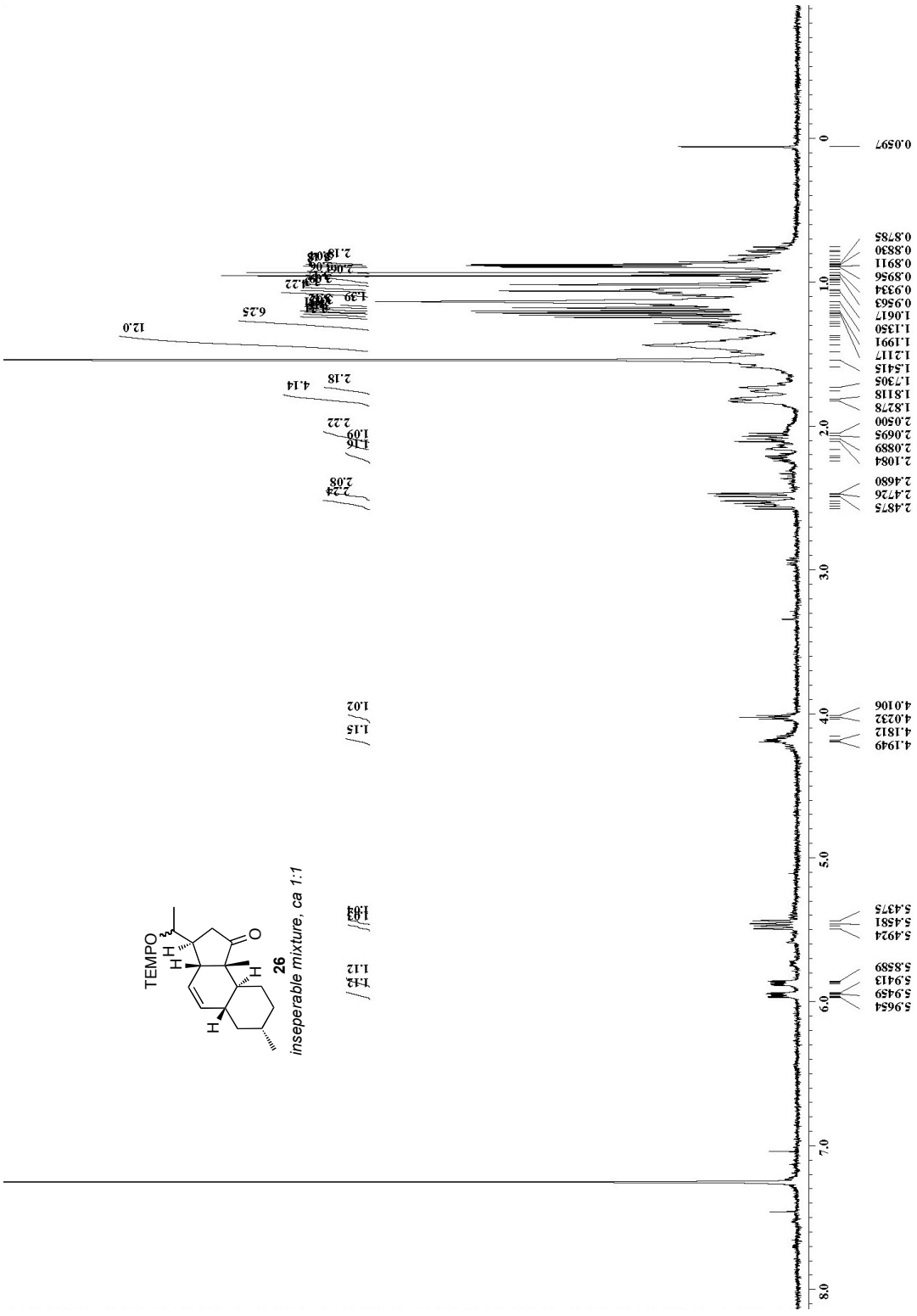
Spectrum 2.16 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **24**



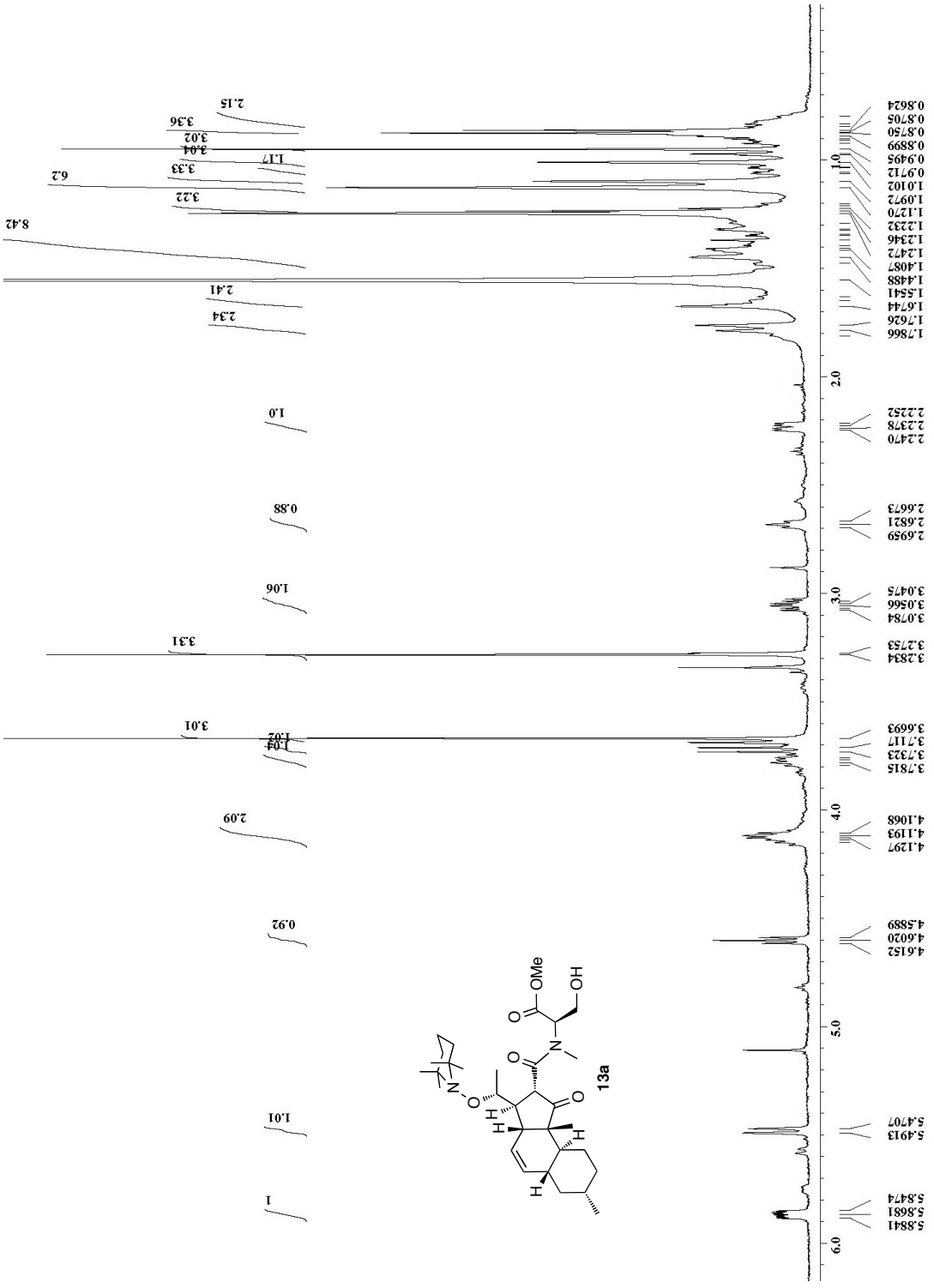
Spectrum 2.17 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **25a**



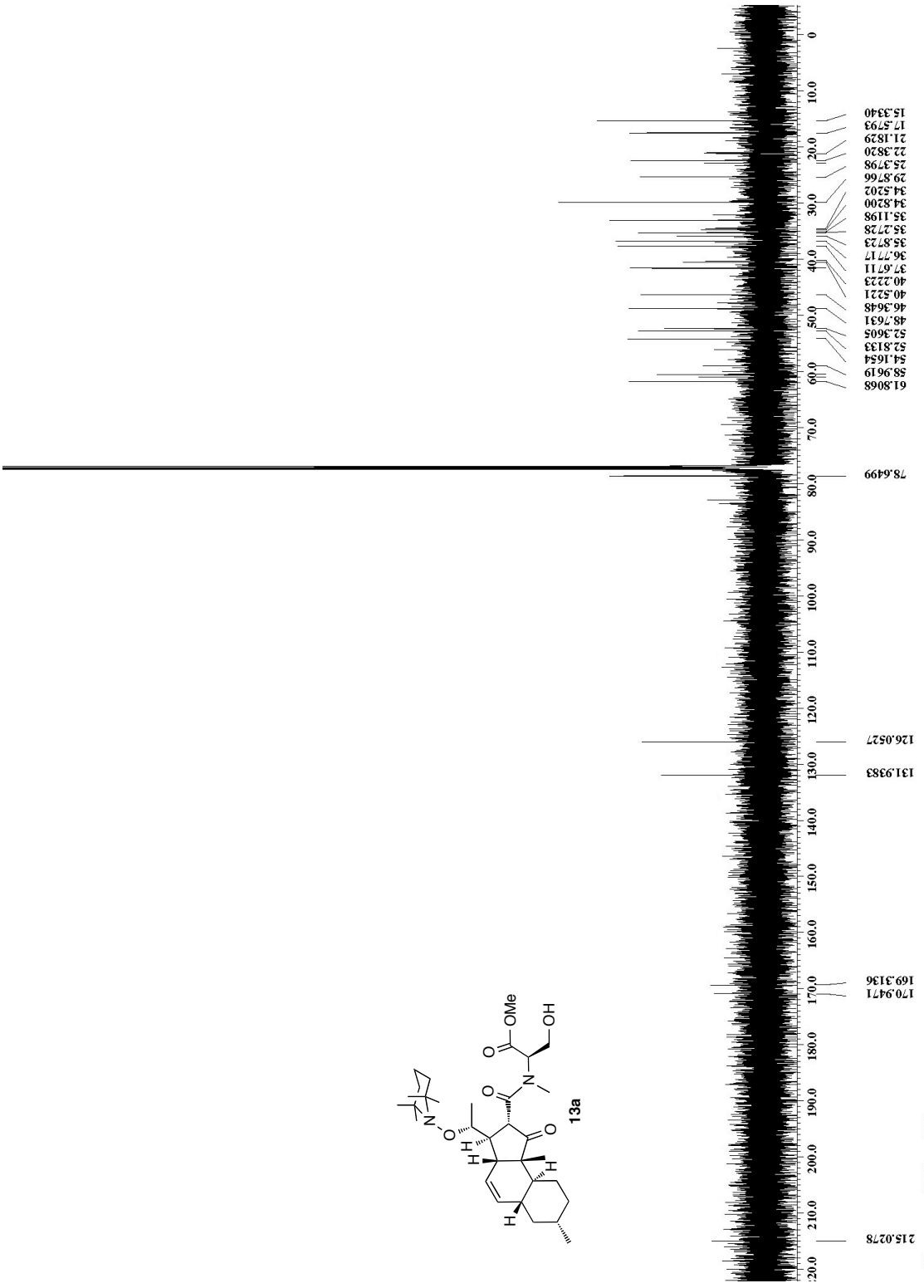
Spectrum 2.18 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **25b**



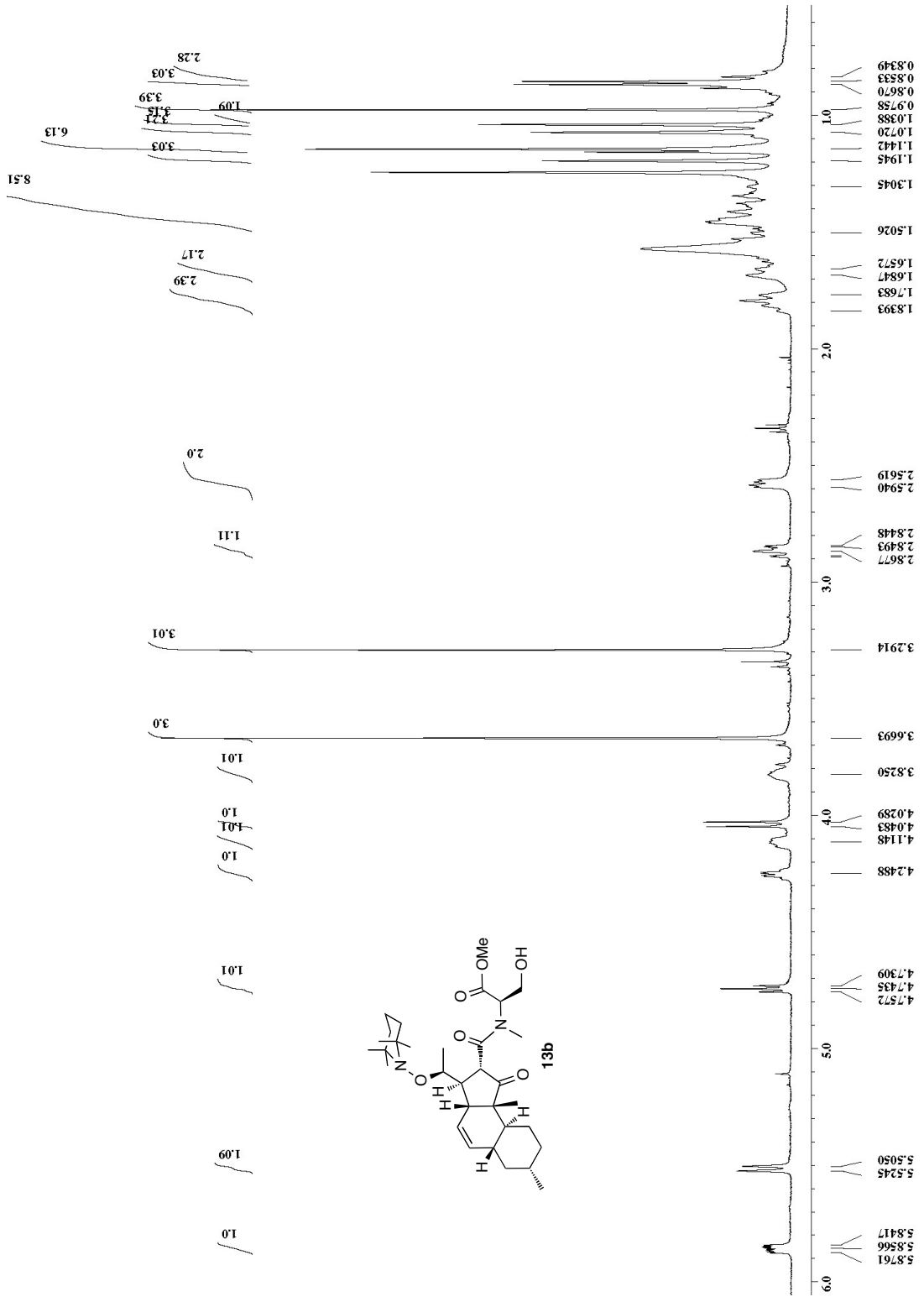
Spectrum 2.19 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **26**



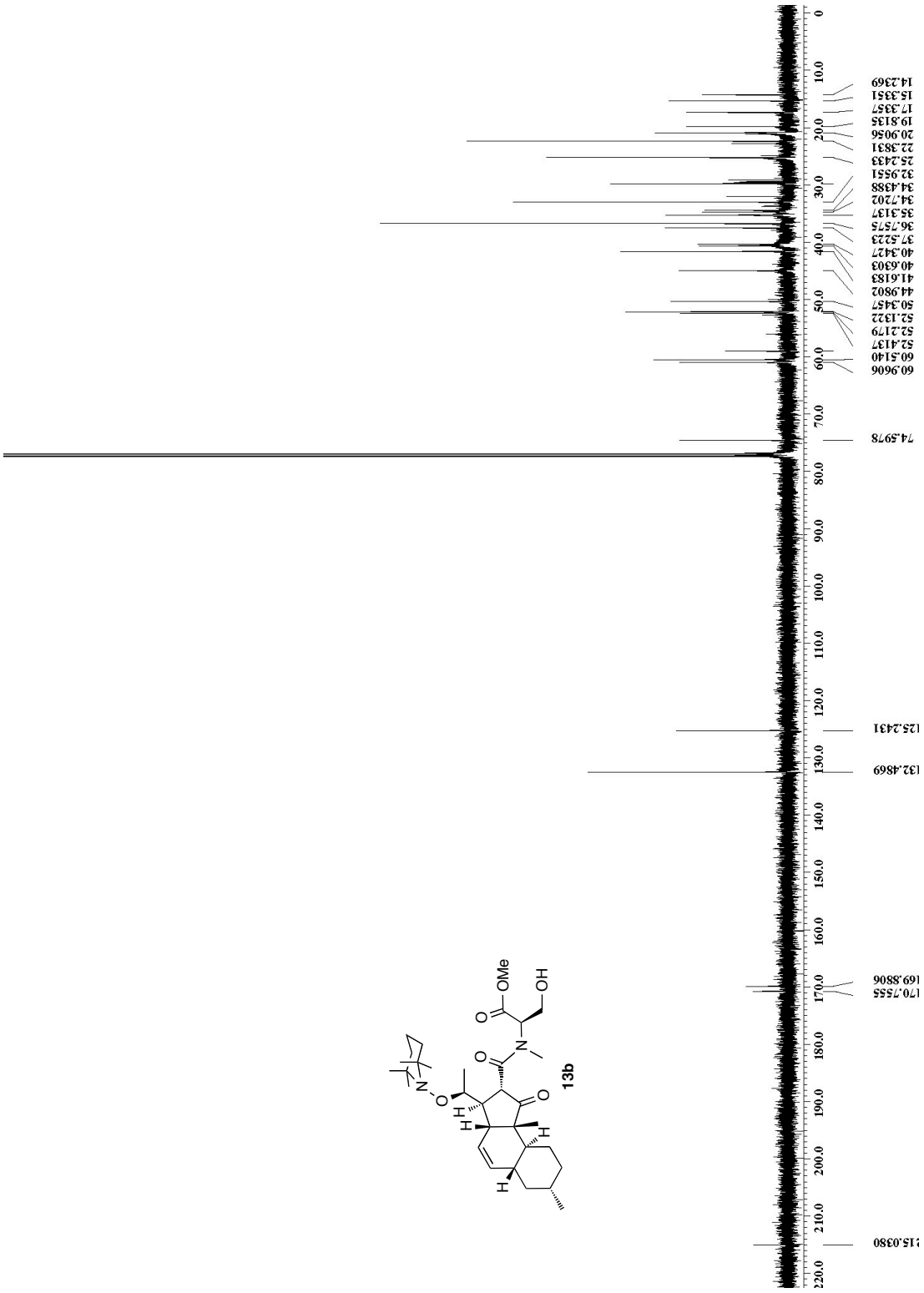
Spectrum 2.20 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **13a**



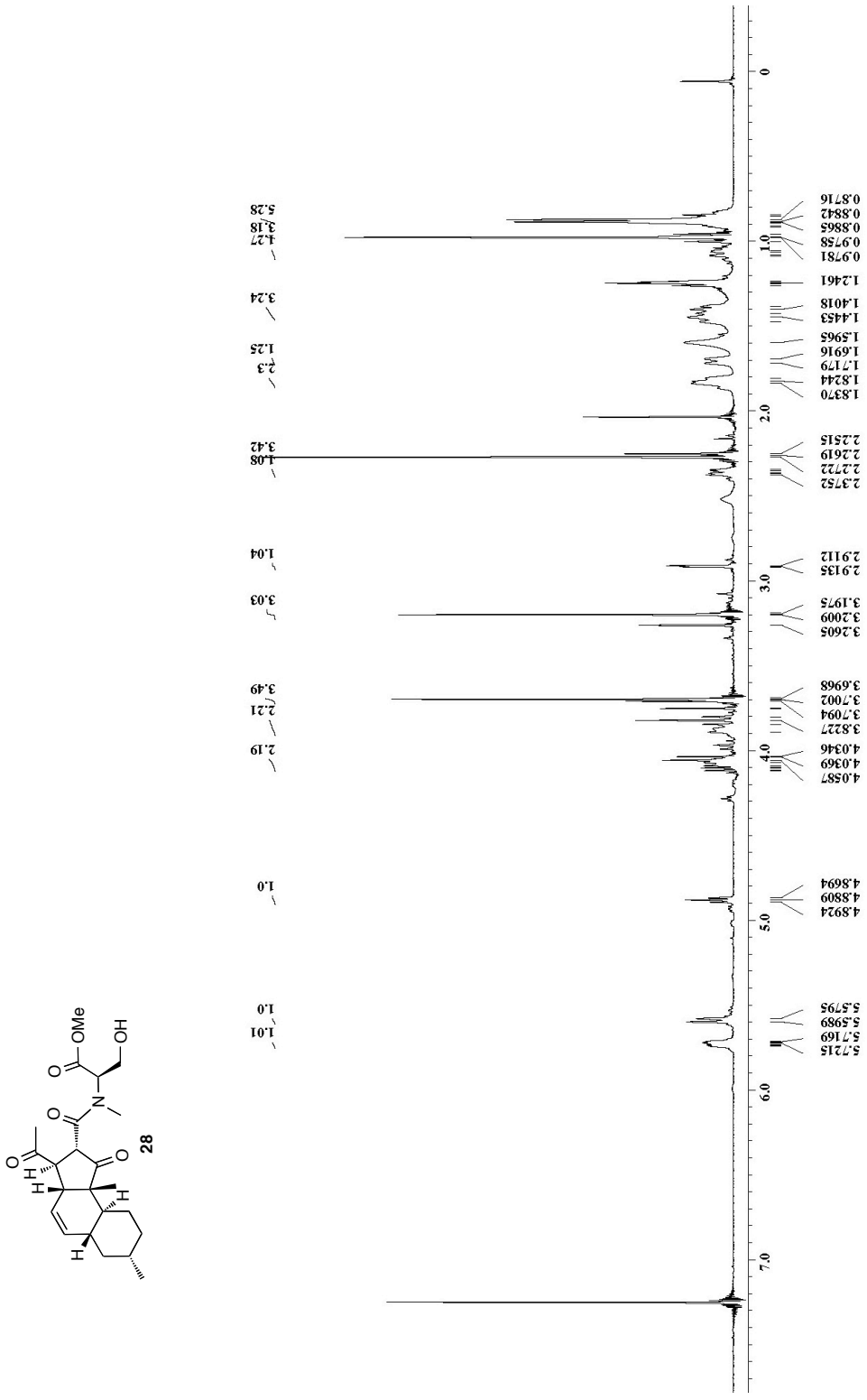
Spectrum 2.21 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **13a**



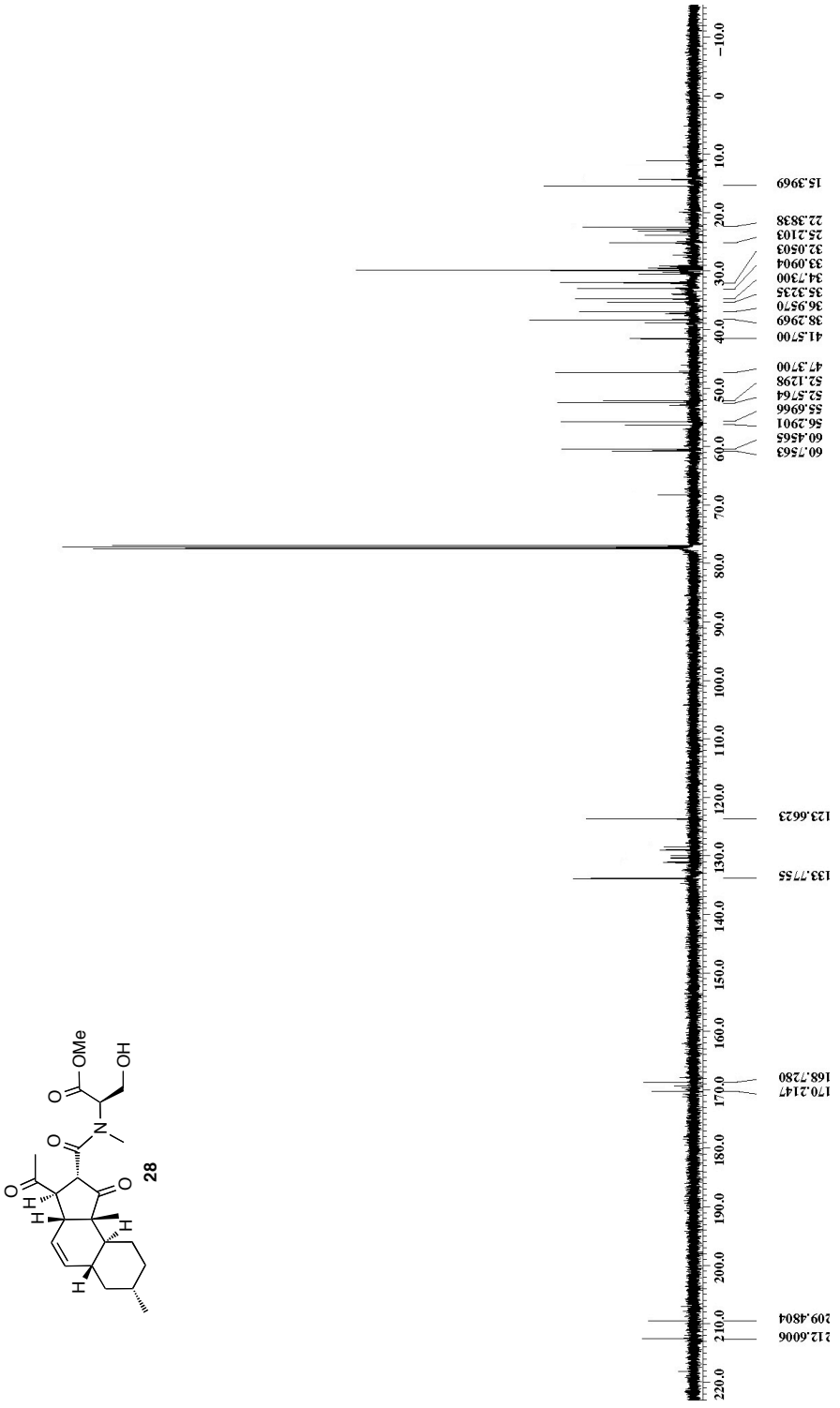
Spectrum 2.22 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **13b**



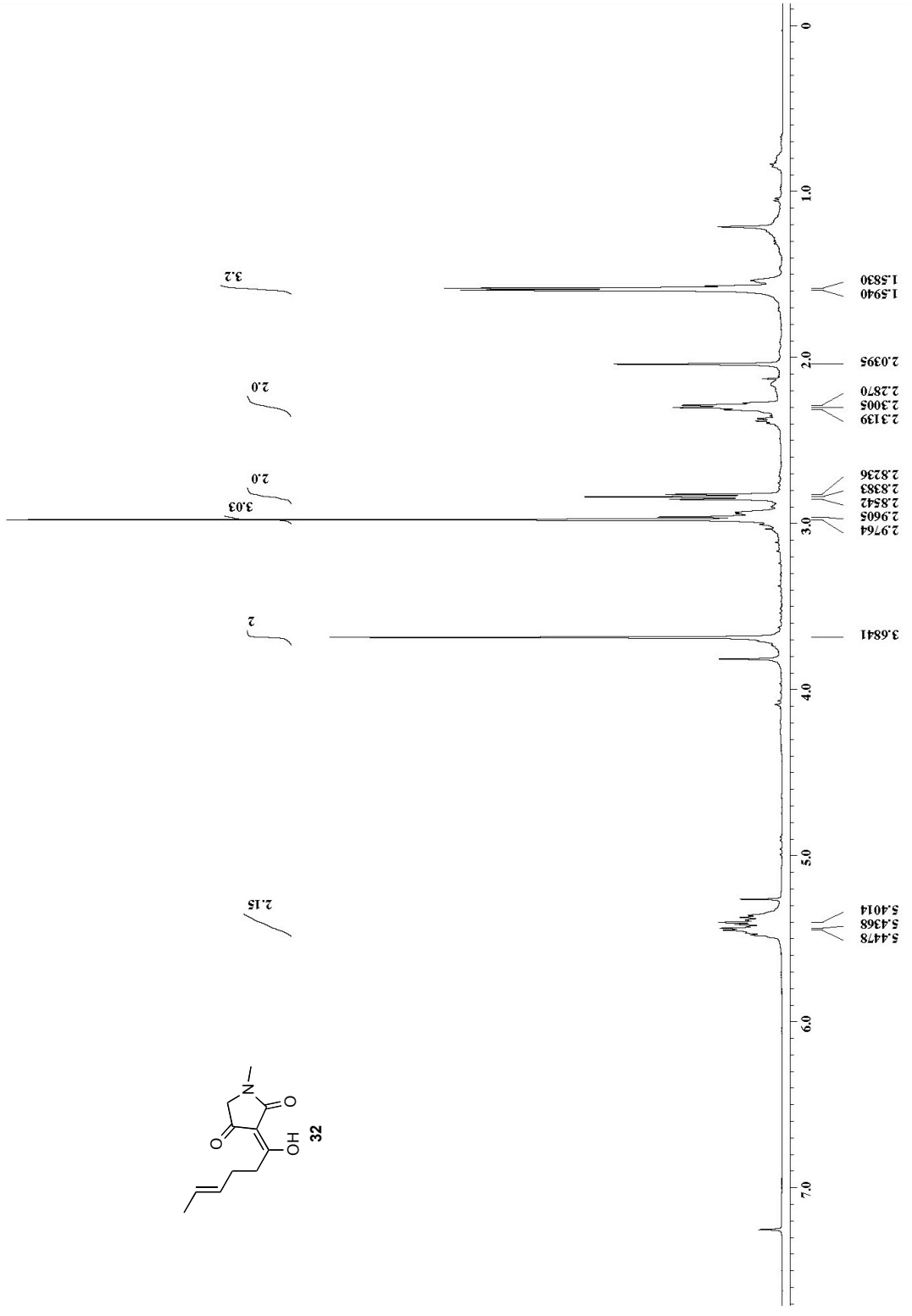
Spectrum 2.23 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **13b**



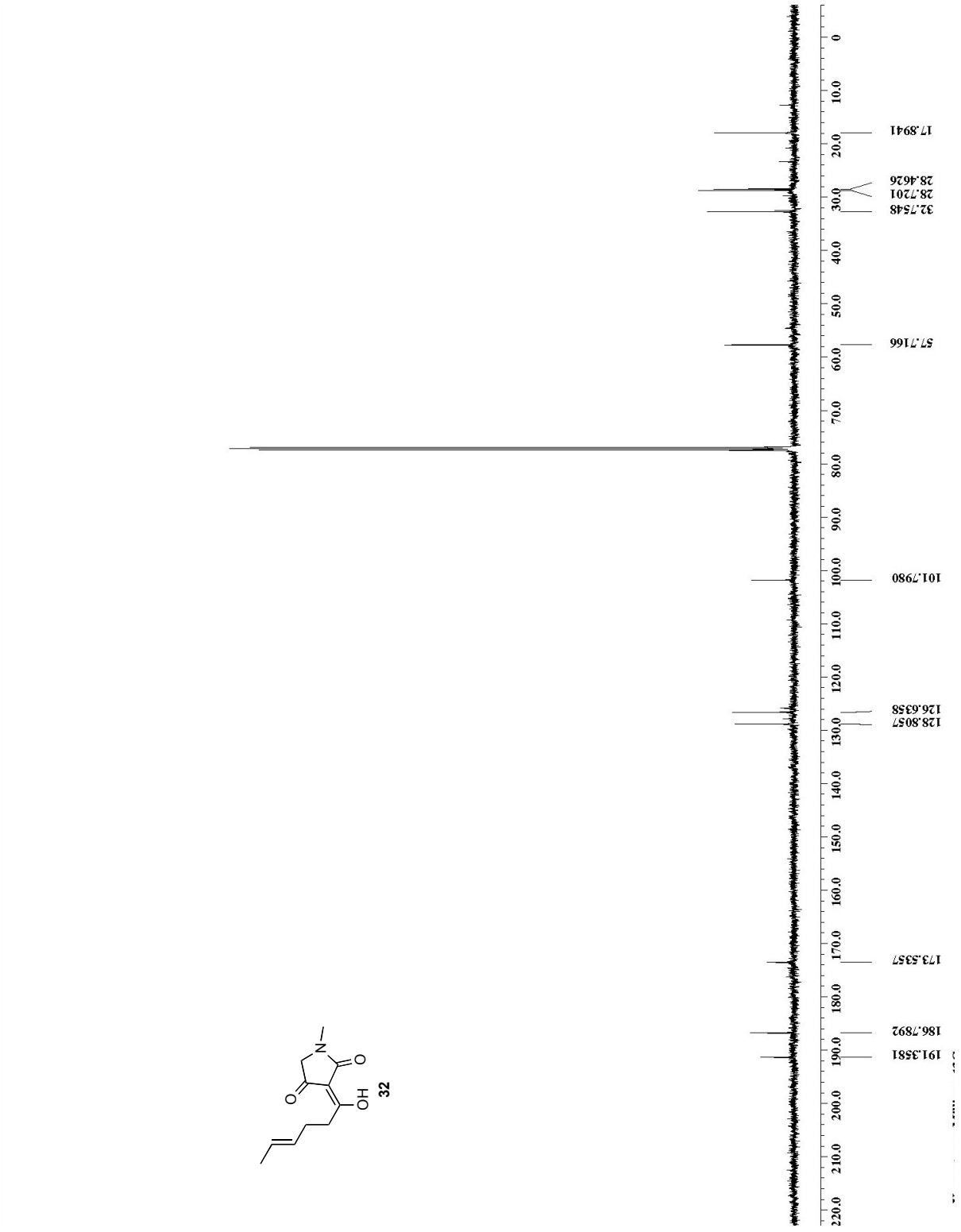
Spectrum 2.24 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **28**



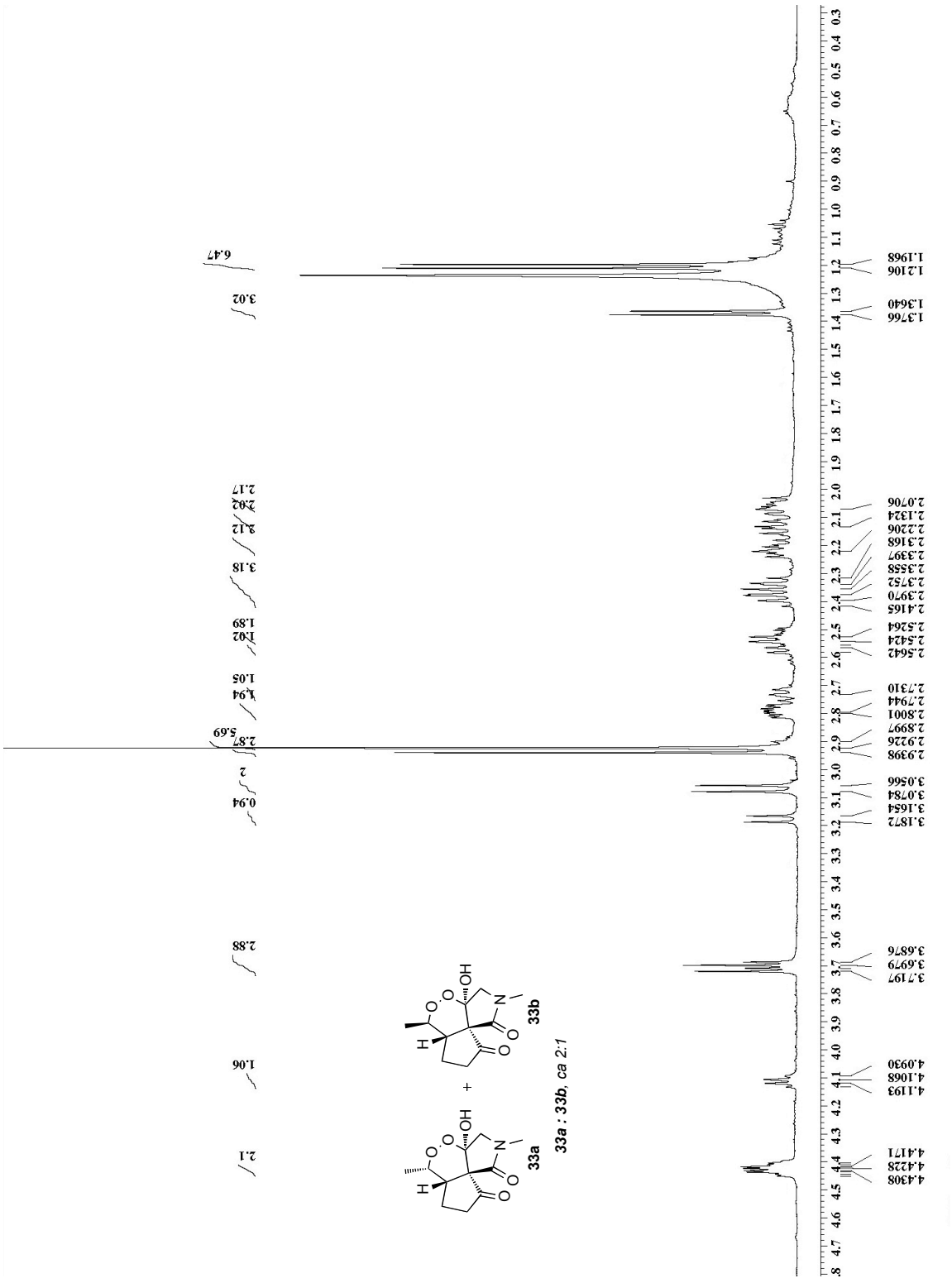
Spectrum 2.25 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **28**



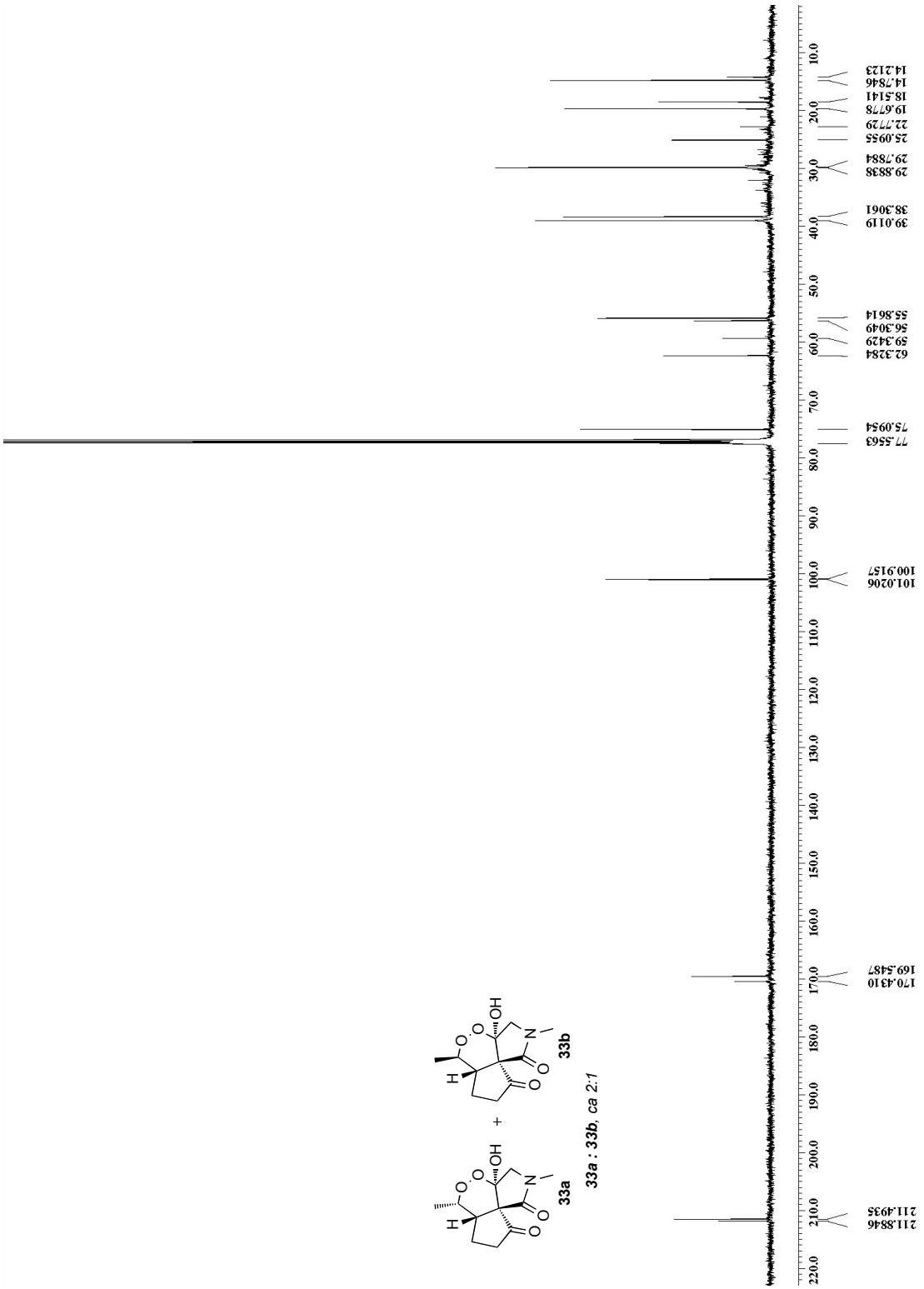
Spectrum 2.26 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **32**



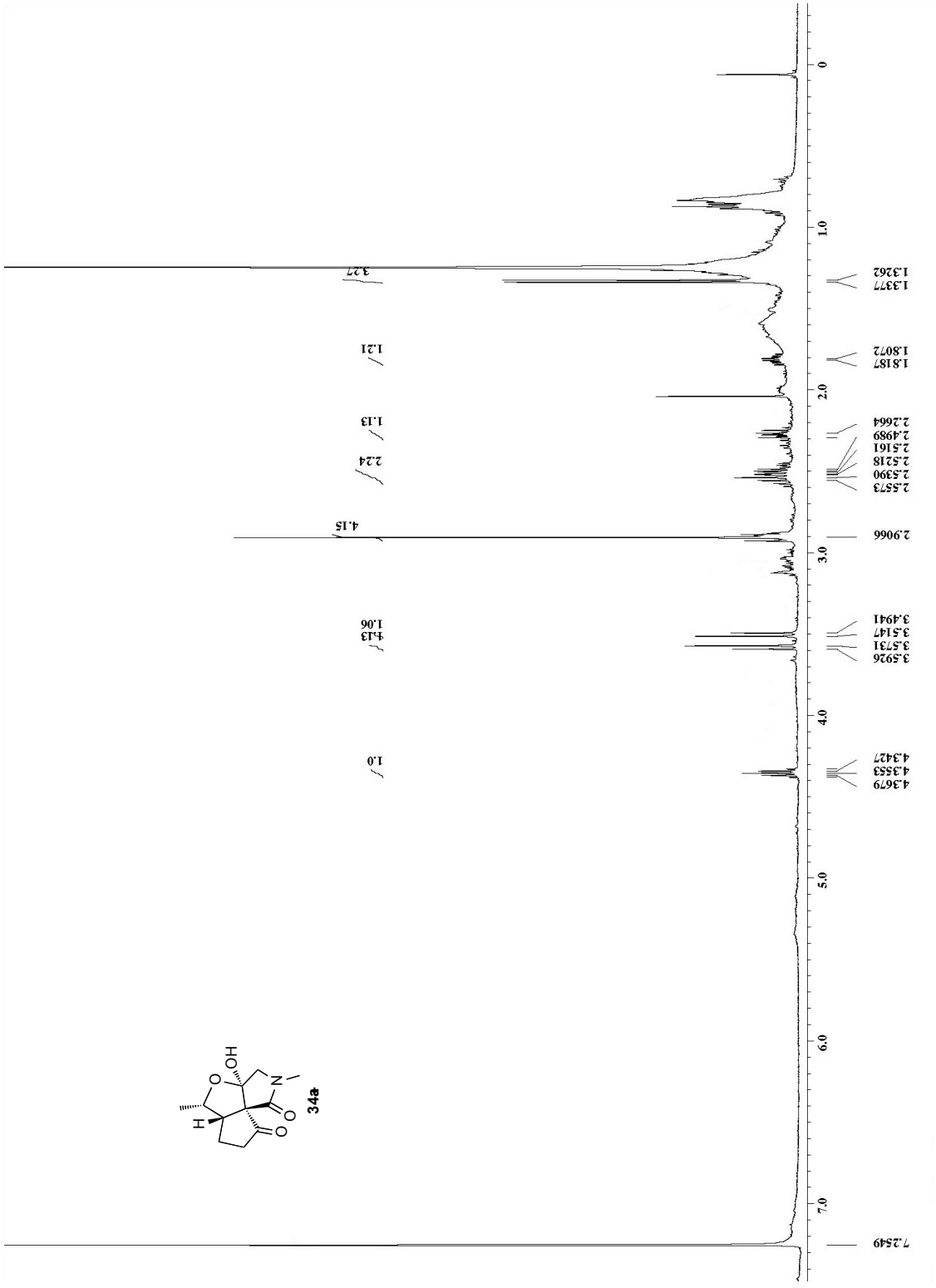
Spectrum 2.27 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **32**



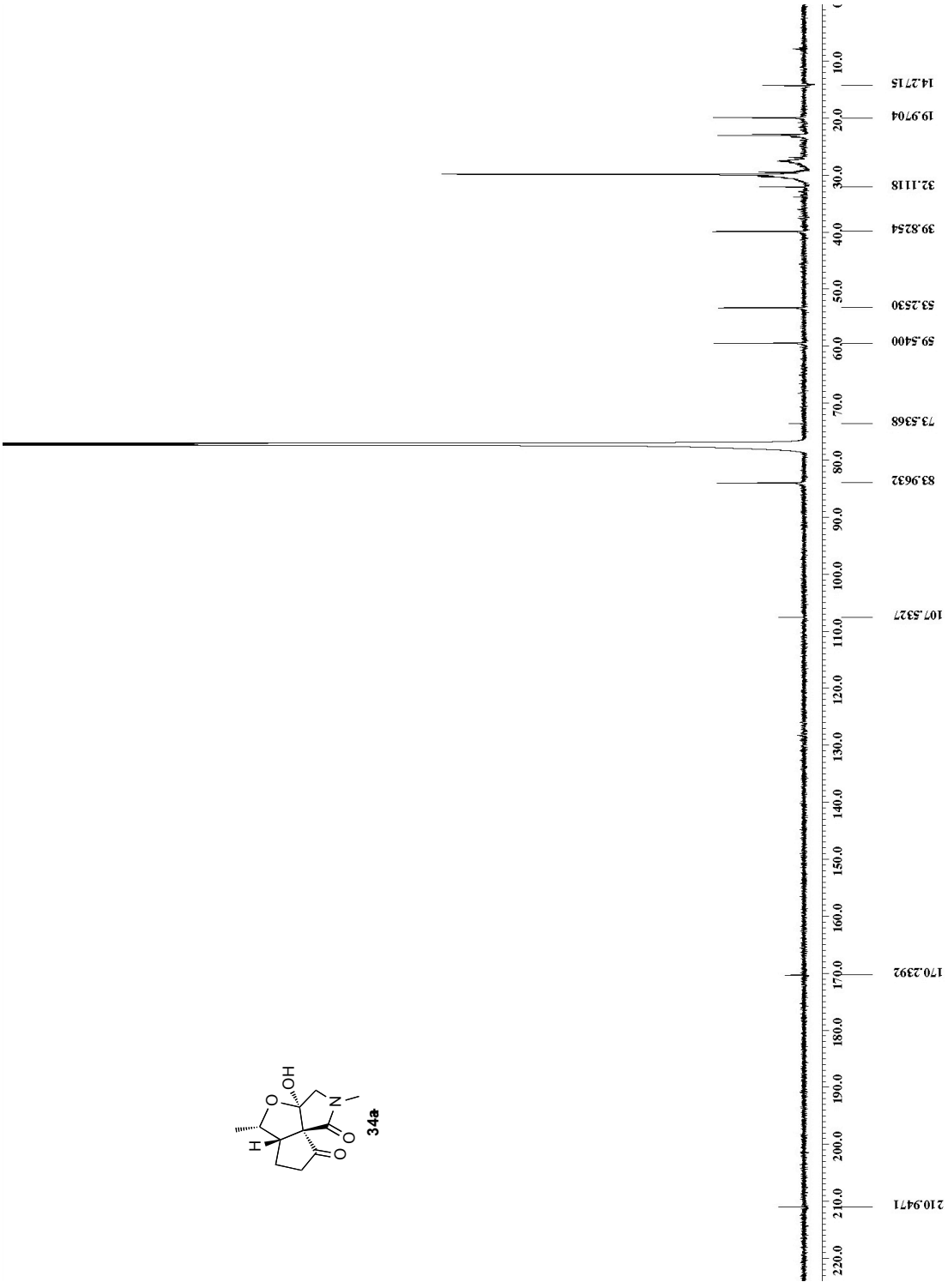
Spectrum 2.28 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **33**



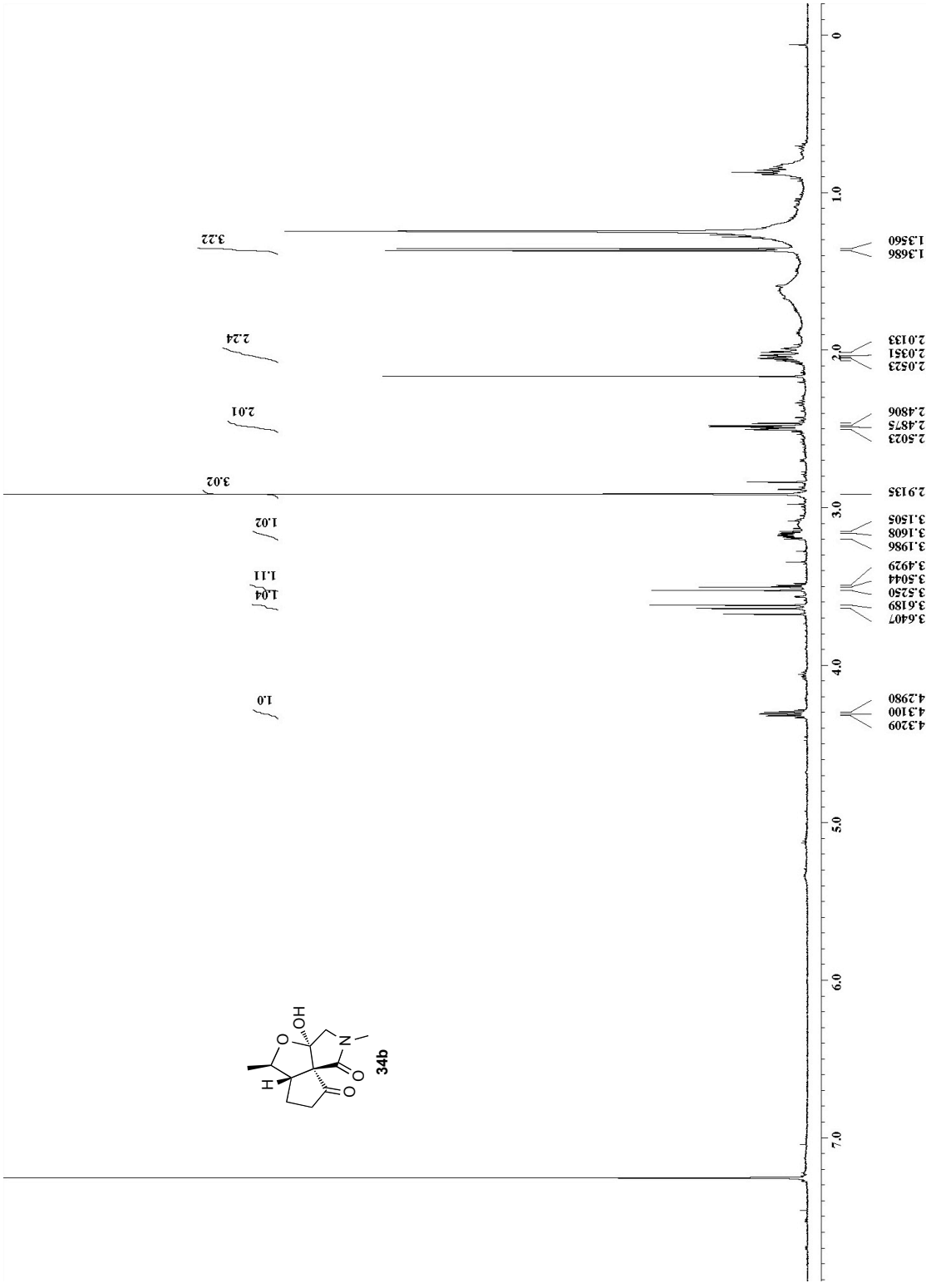
Spectrum 2.29 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **33**



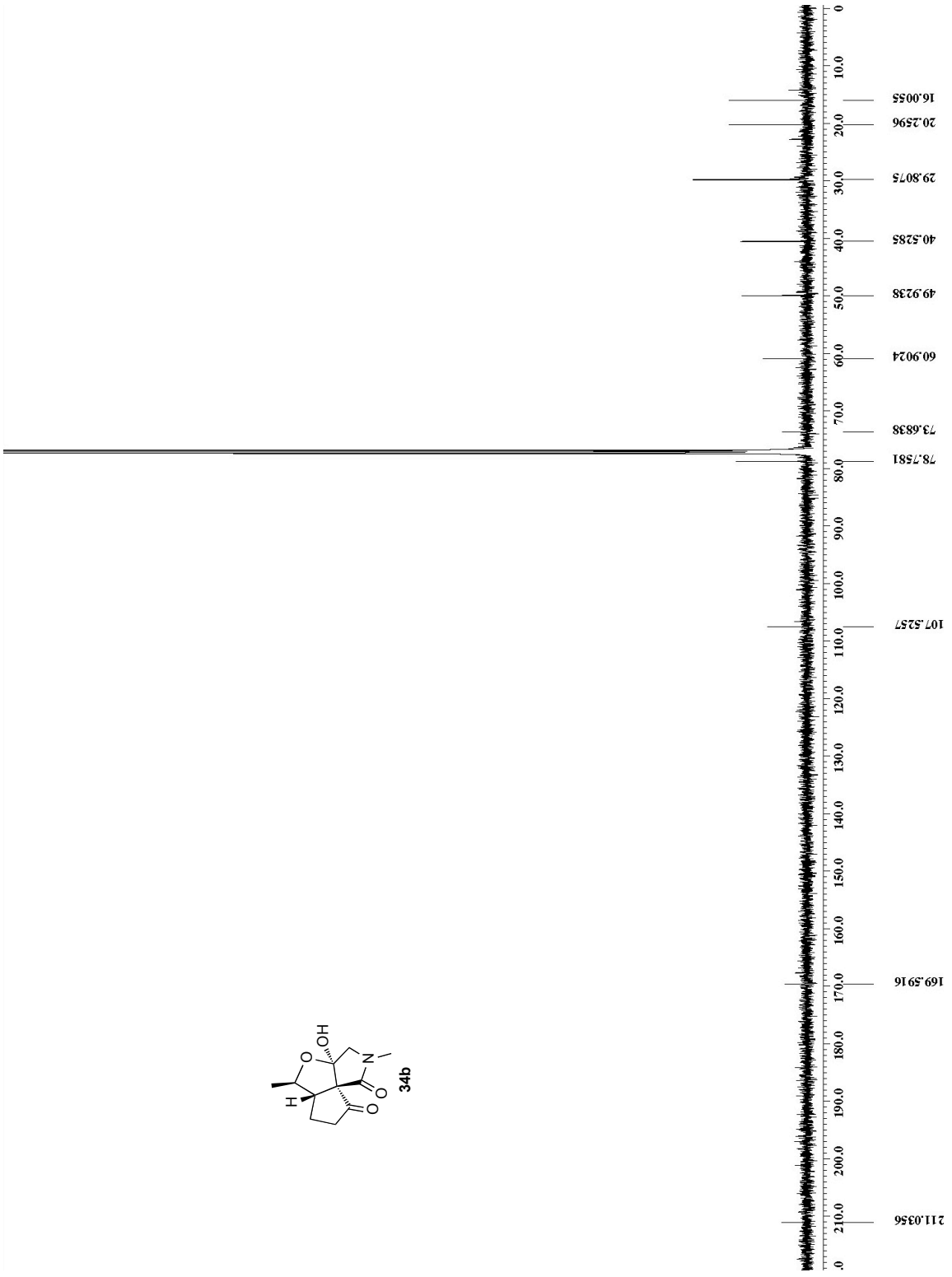
Spectrum 2.30 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **34a**



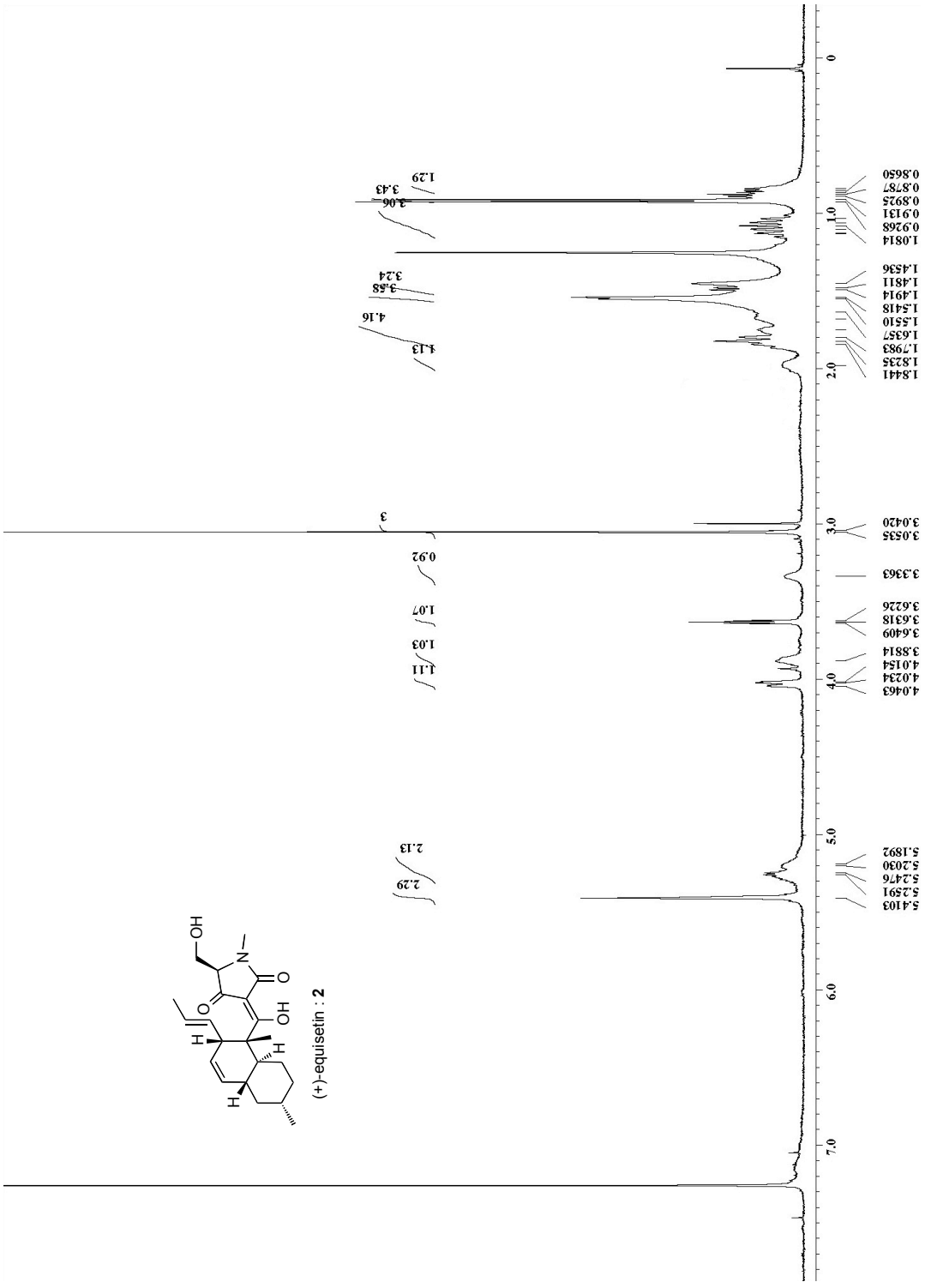
Spectrum 2.31 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **34a**



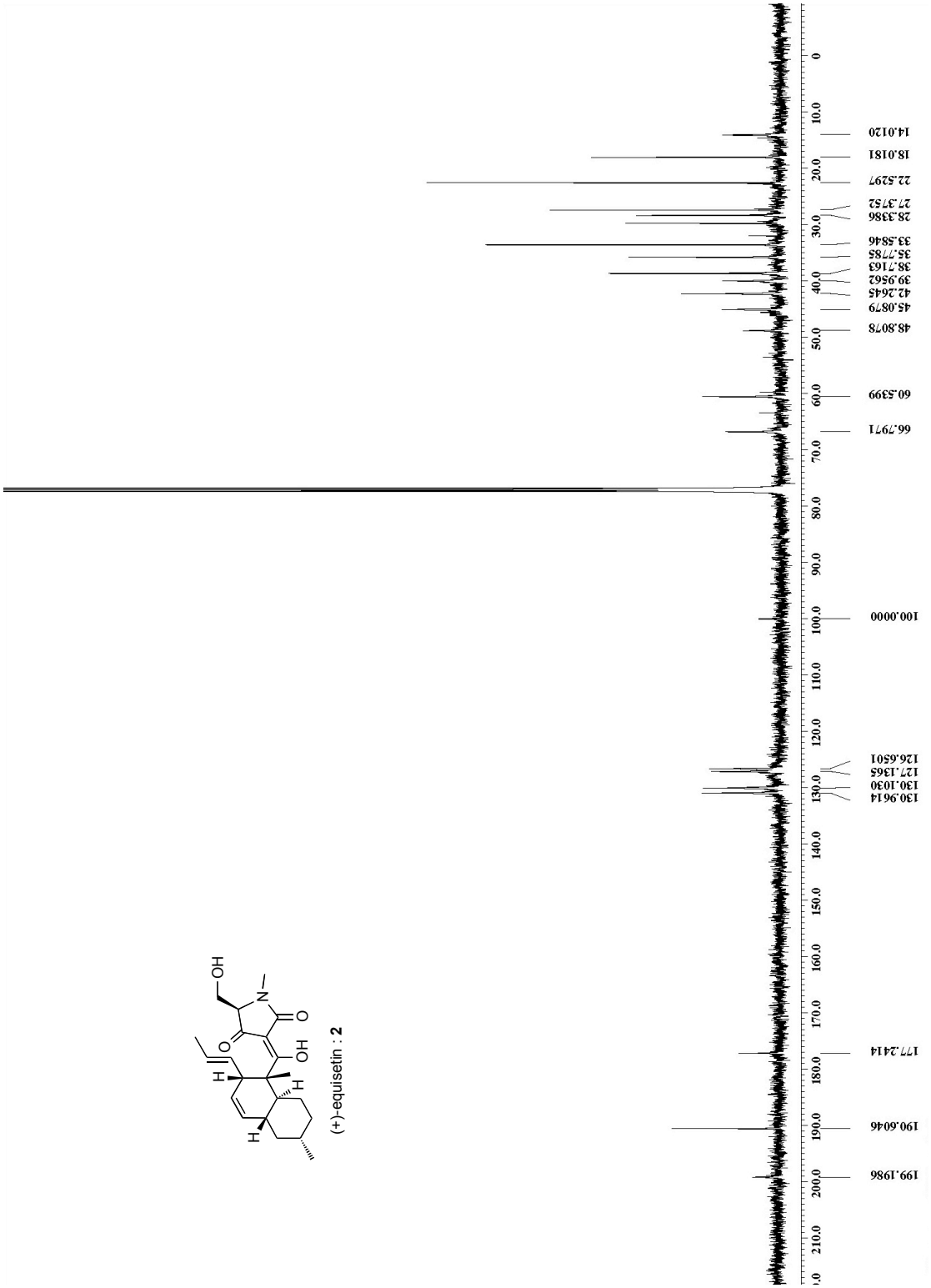
Spectrum 2.32 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **34b**



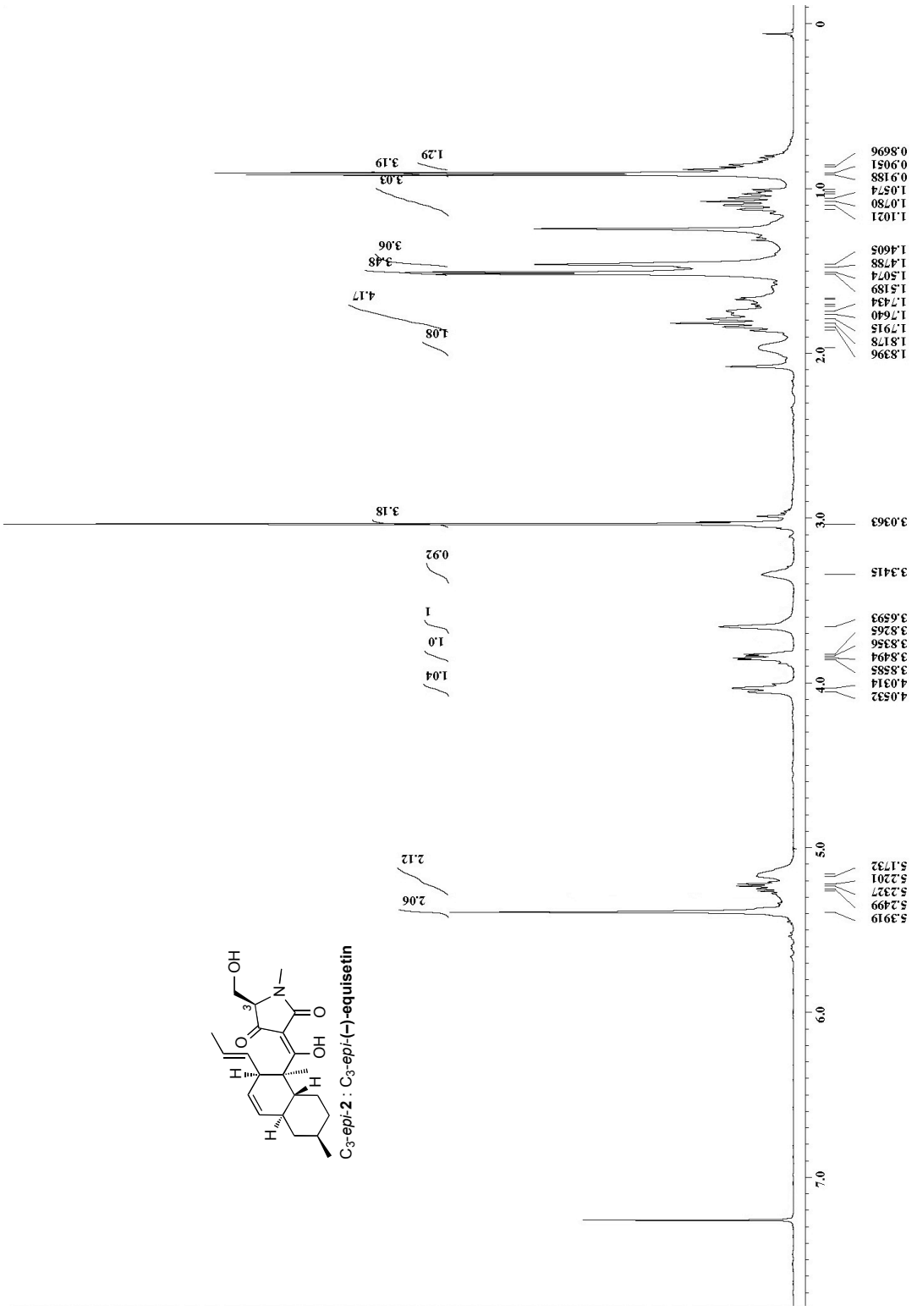
Spectrum 2.33 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **34b**



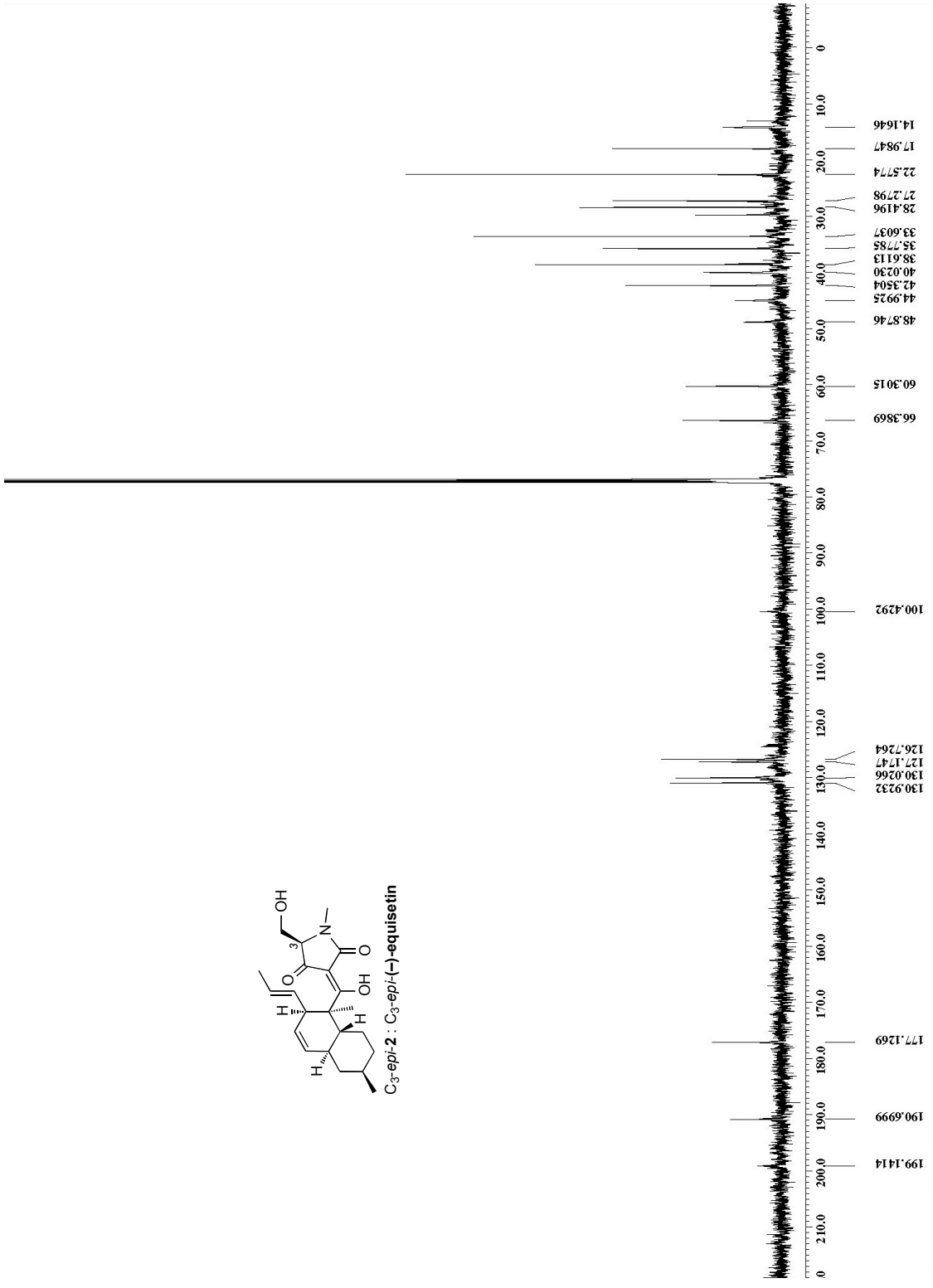
Spectrum 2.34 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **2**



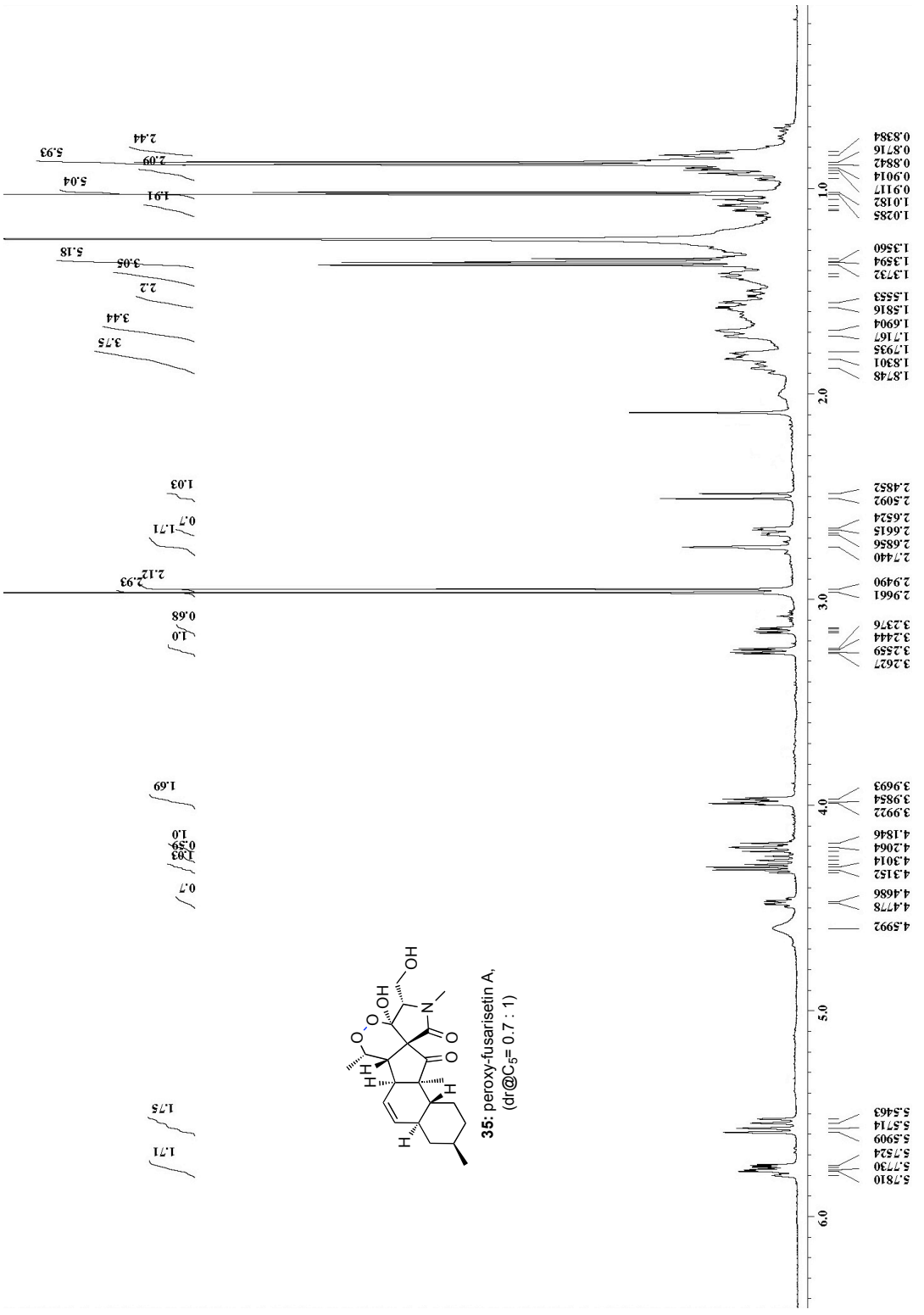
Spectrum 2.35 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **2**



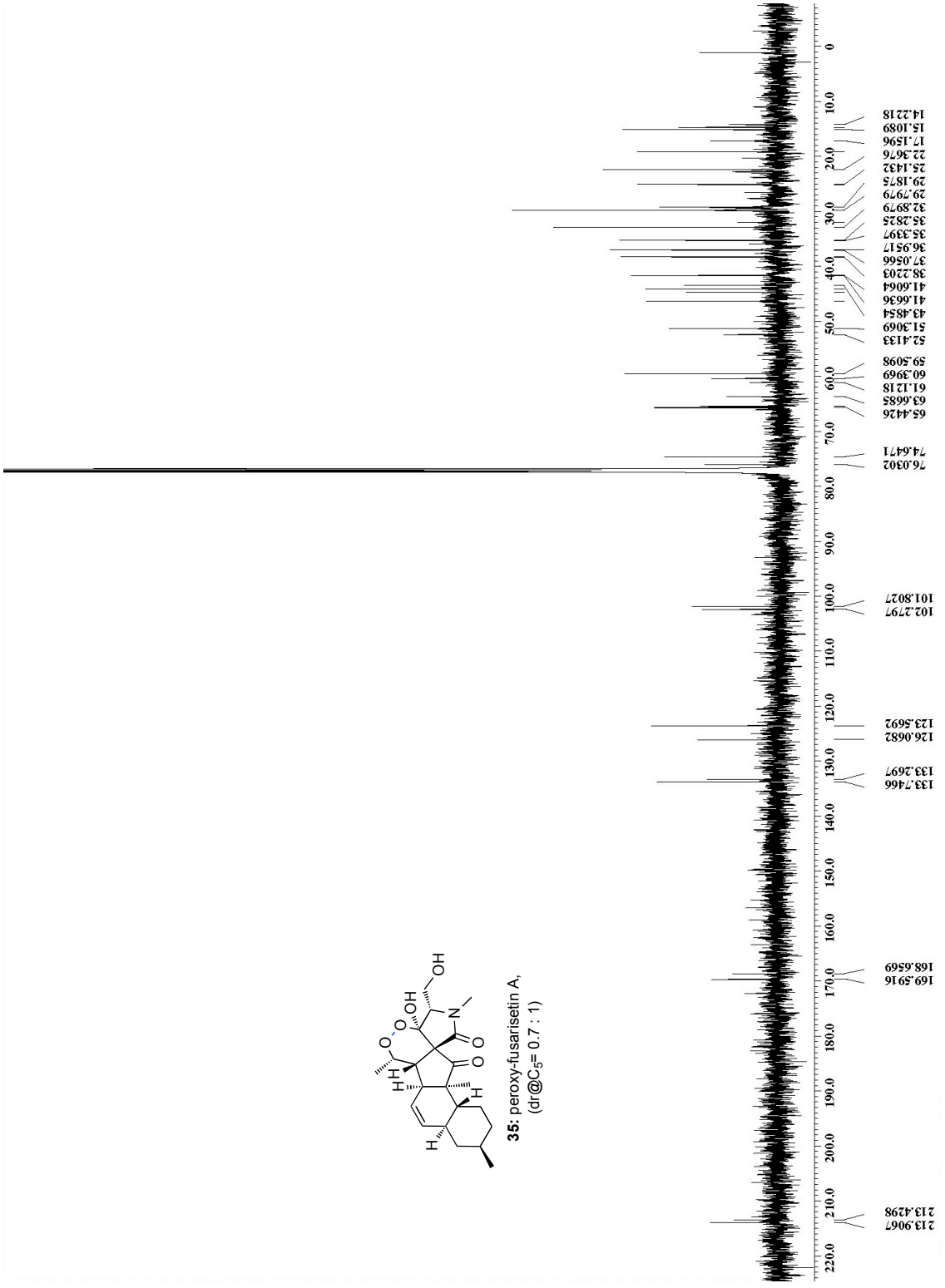
Spectrum 2.36 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound C_3-epi **2**



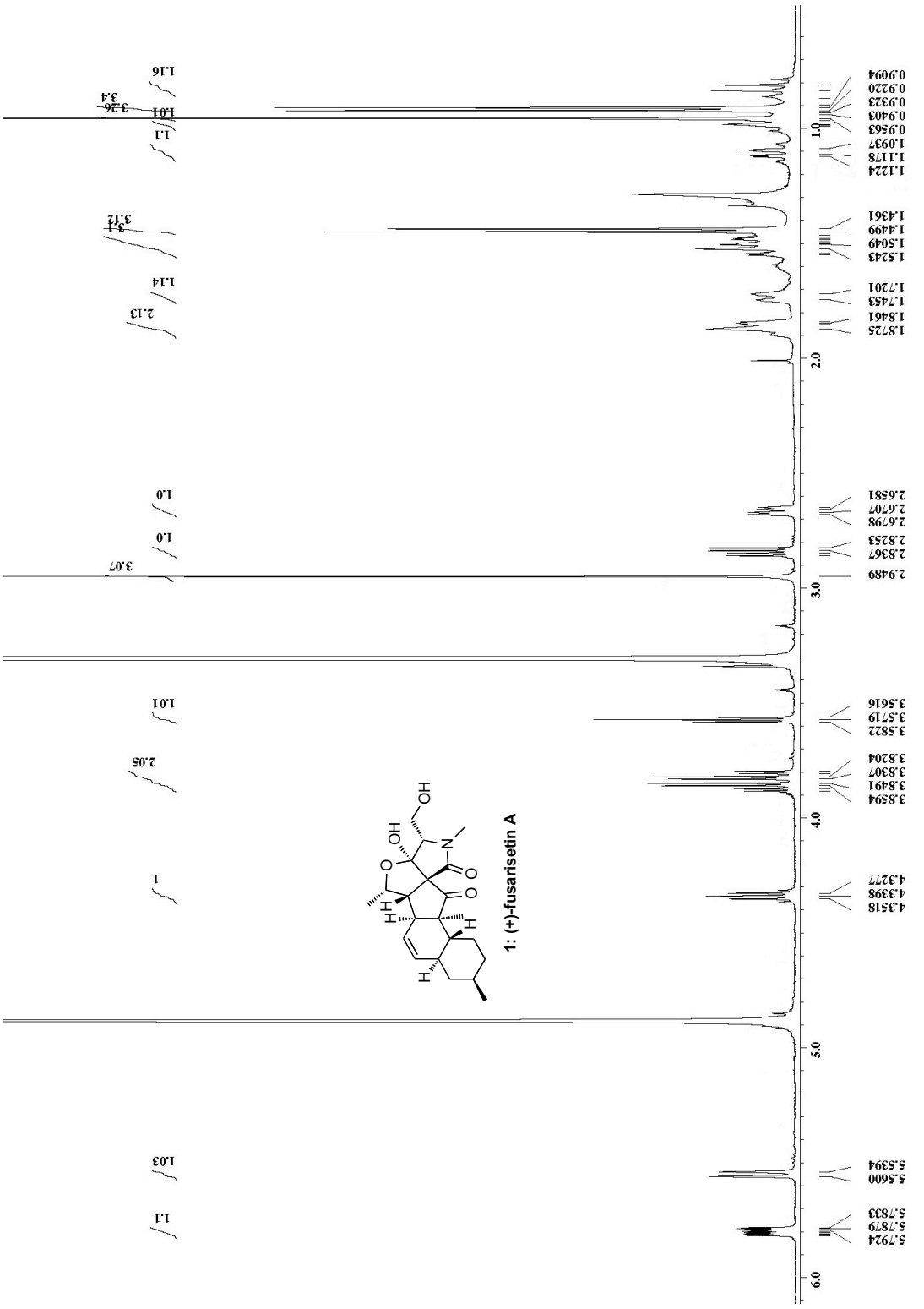
Spectrum 2.36 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound C_3-epi **2**



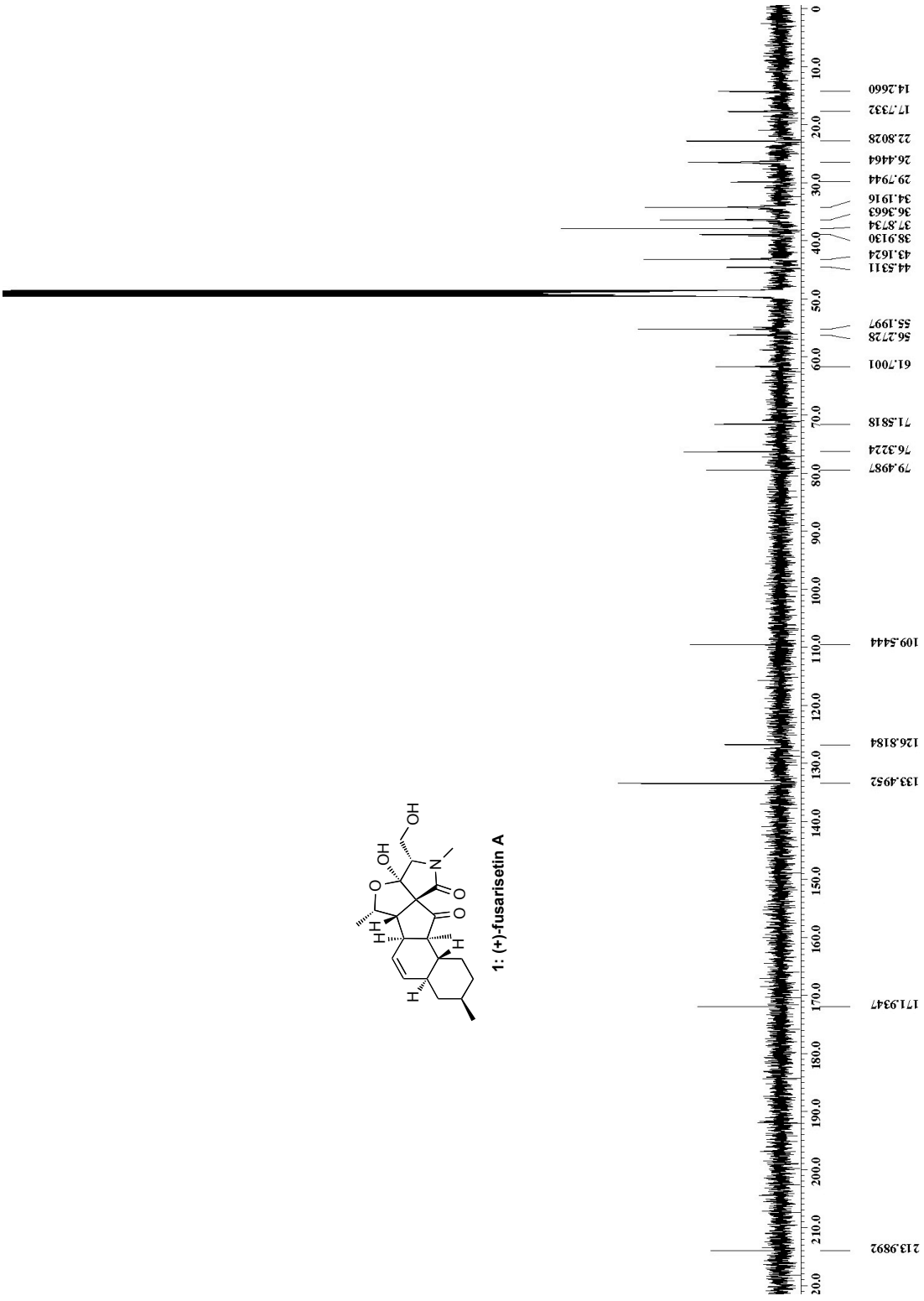
Spectrum 2.37 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **35**



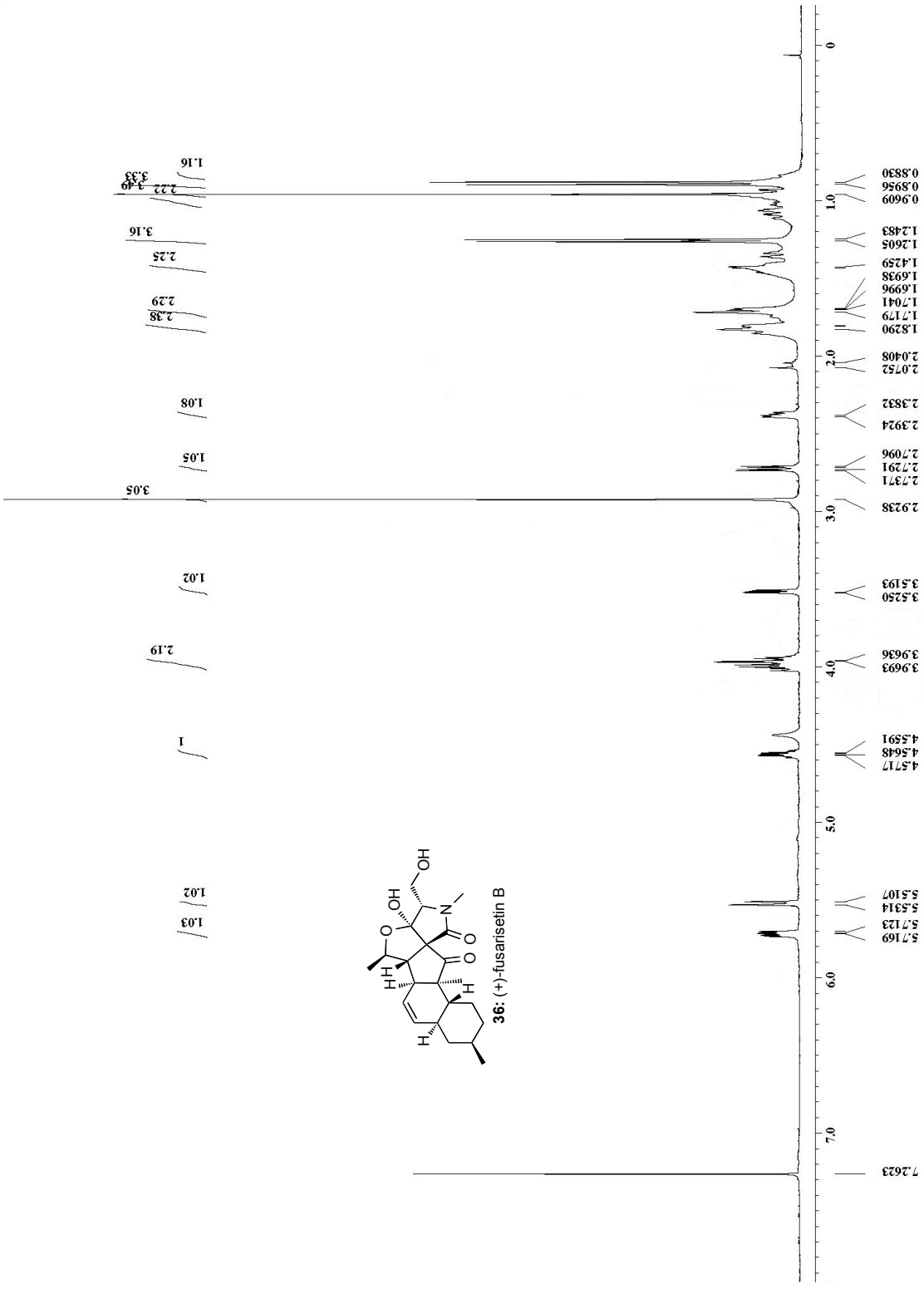
Spectrum 2.38 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **35**



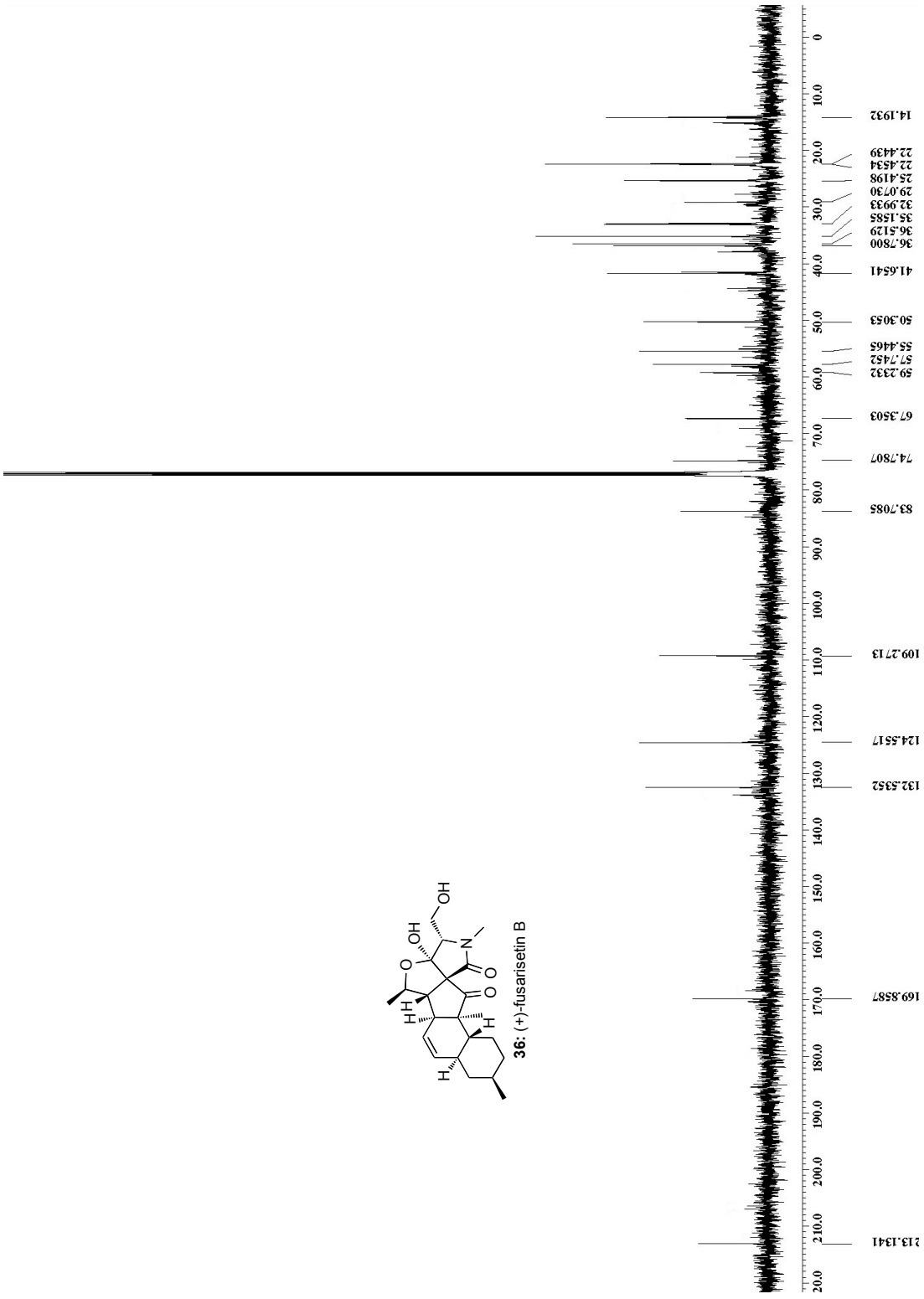
Spectrum 2.39 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **1**



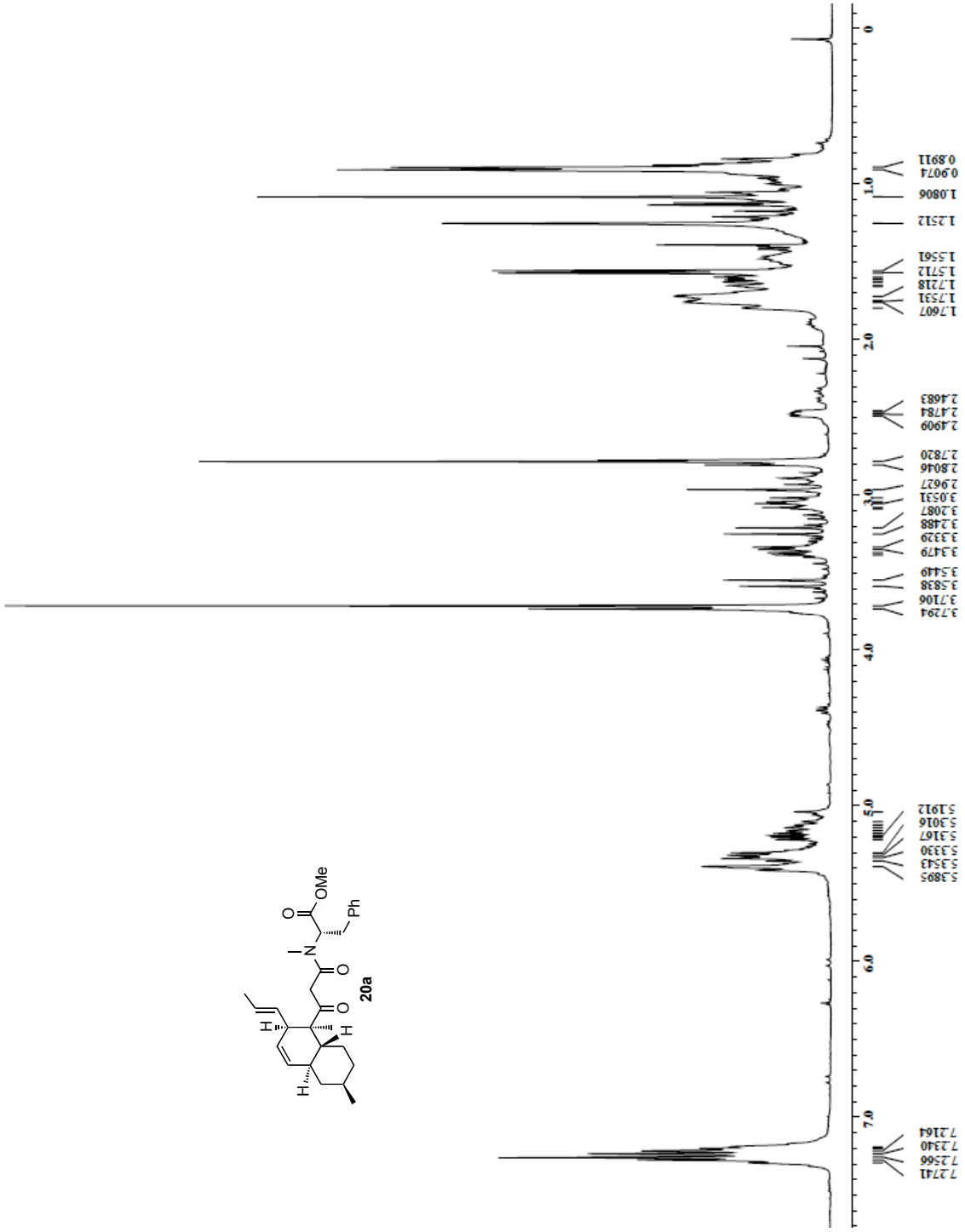
Spectrum 2.40 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **1**



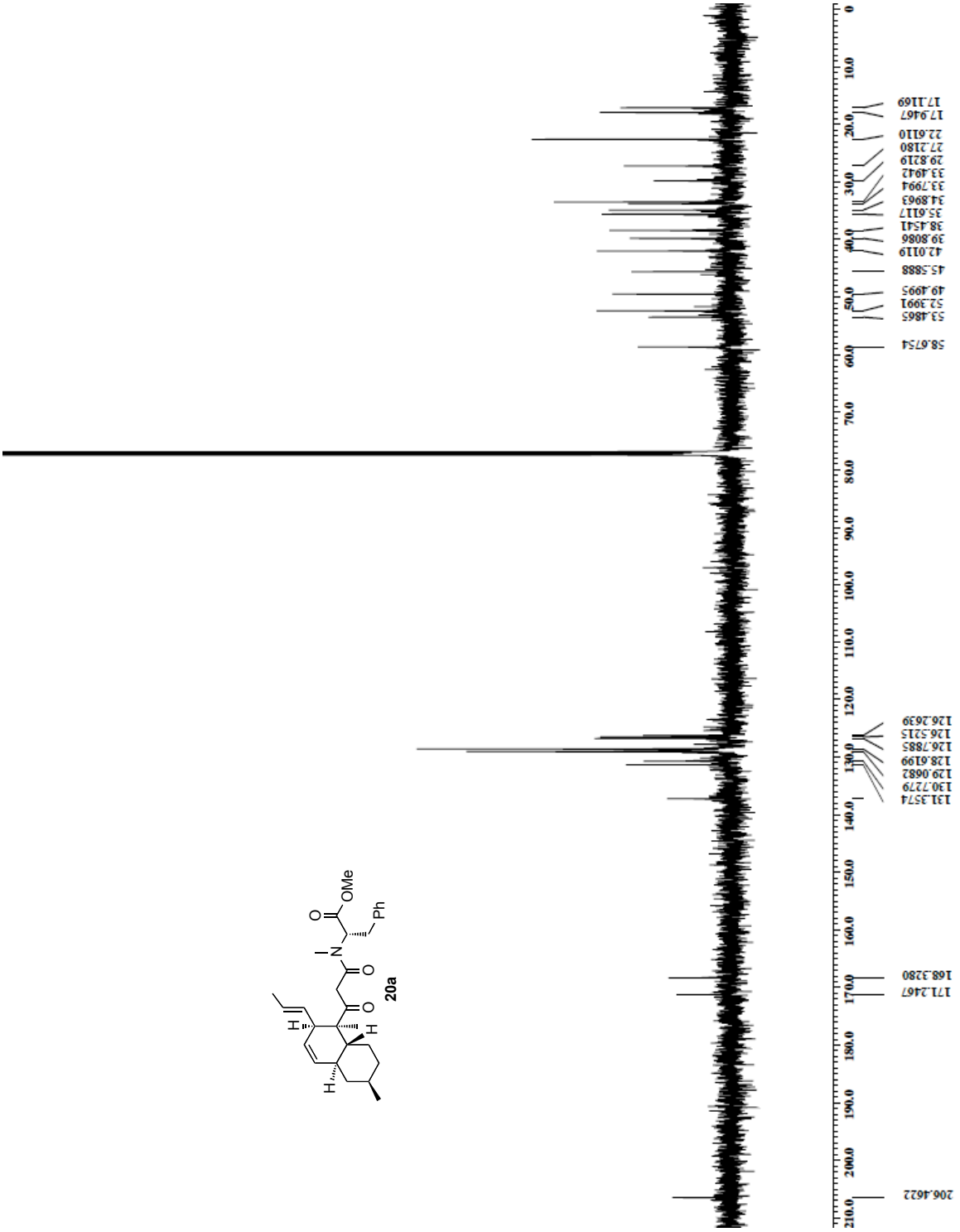
Spectrum 2.41 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **36**



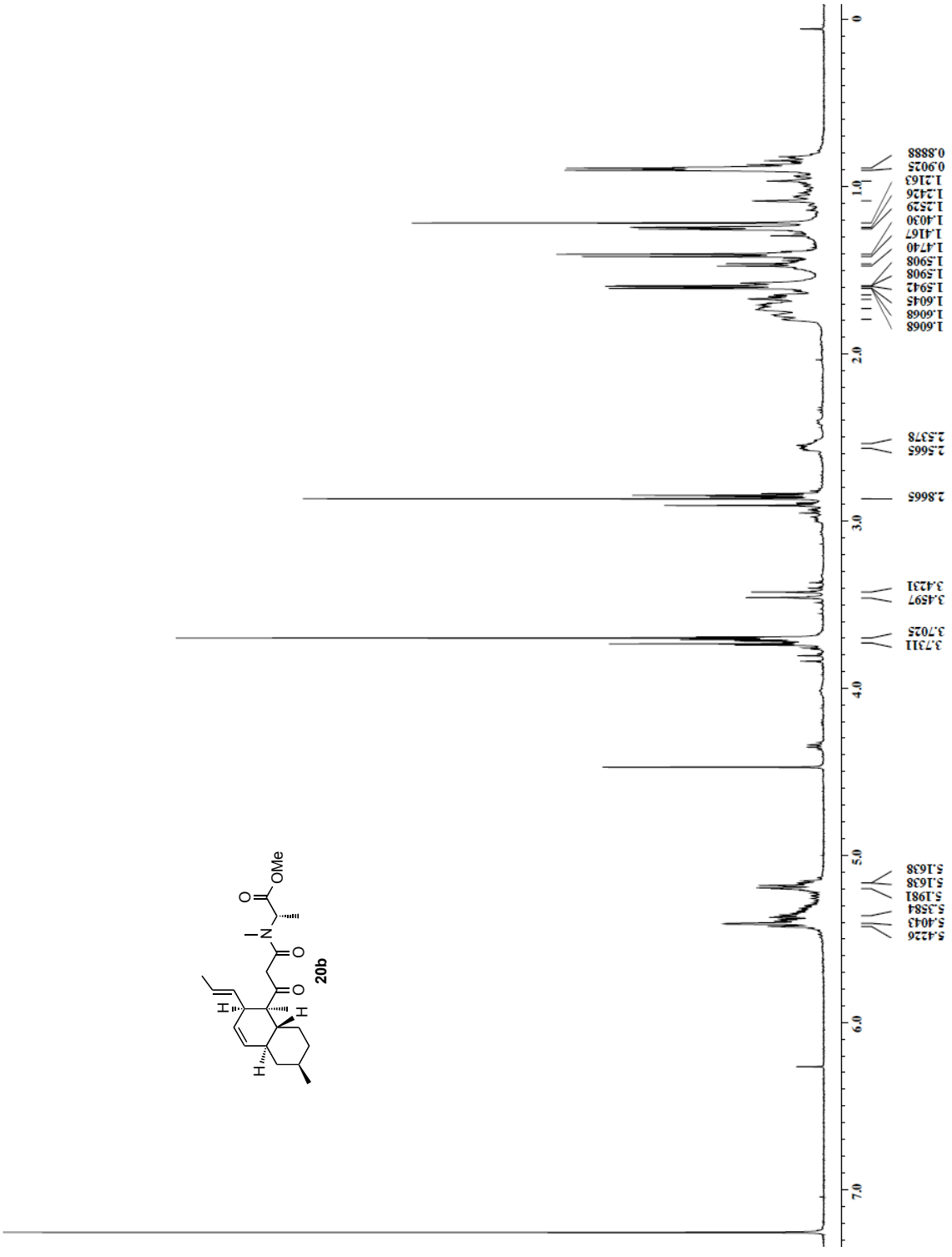
Spectrum 2.42 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **36**



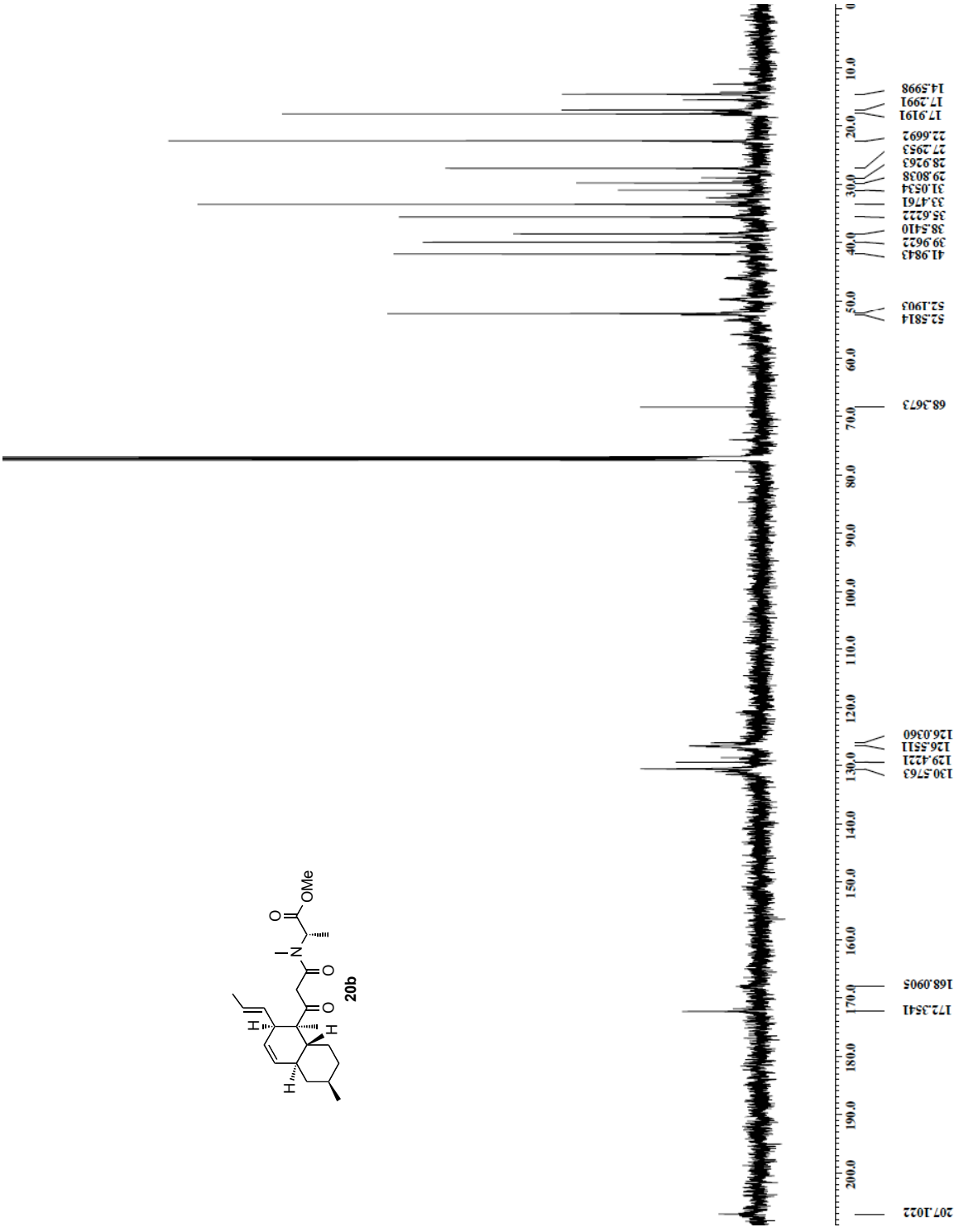
Spectrum 2.43 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20a**



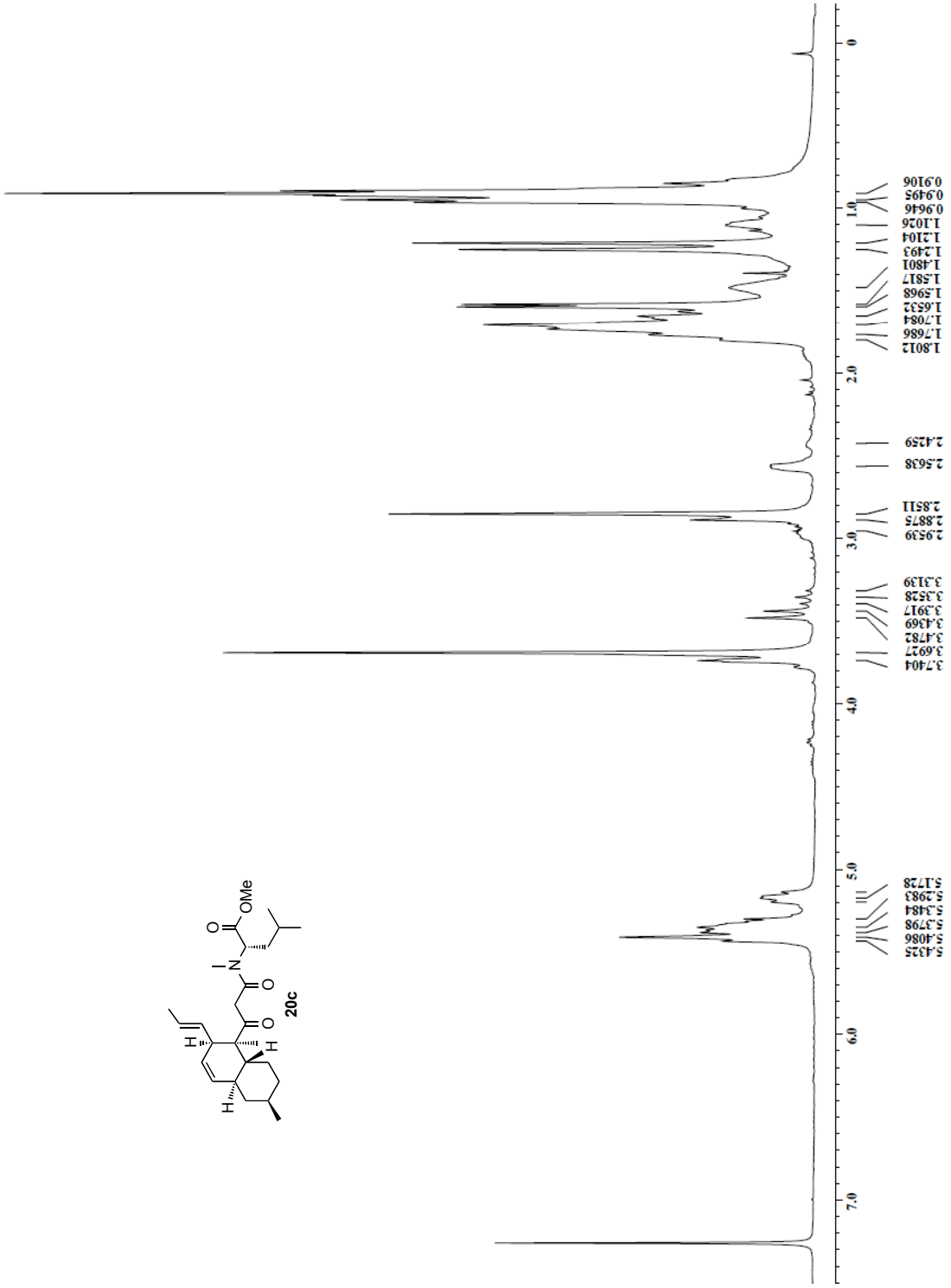
Spectrum 2.44 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20a**



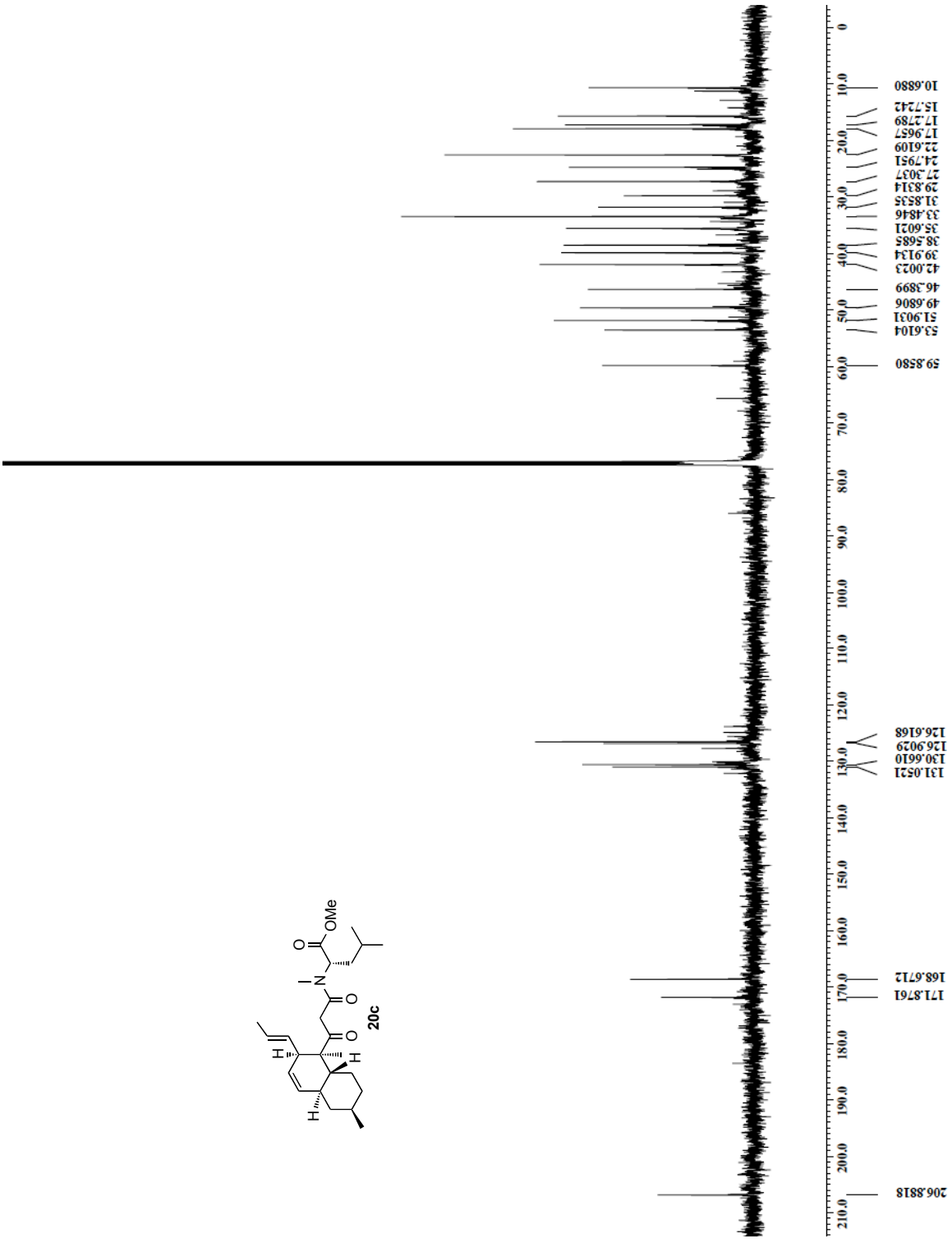
Spectrum 2.45 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20b**



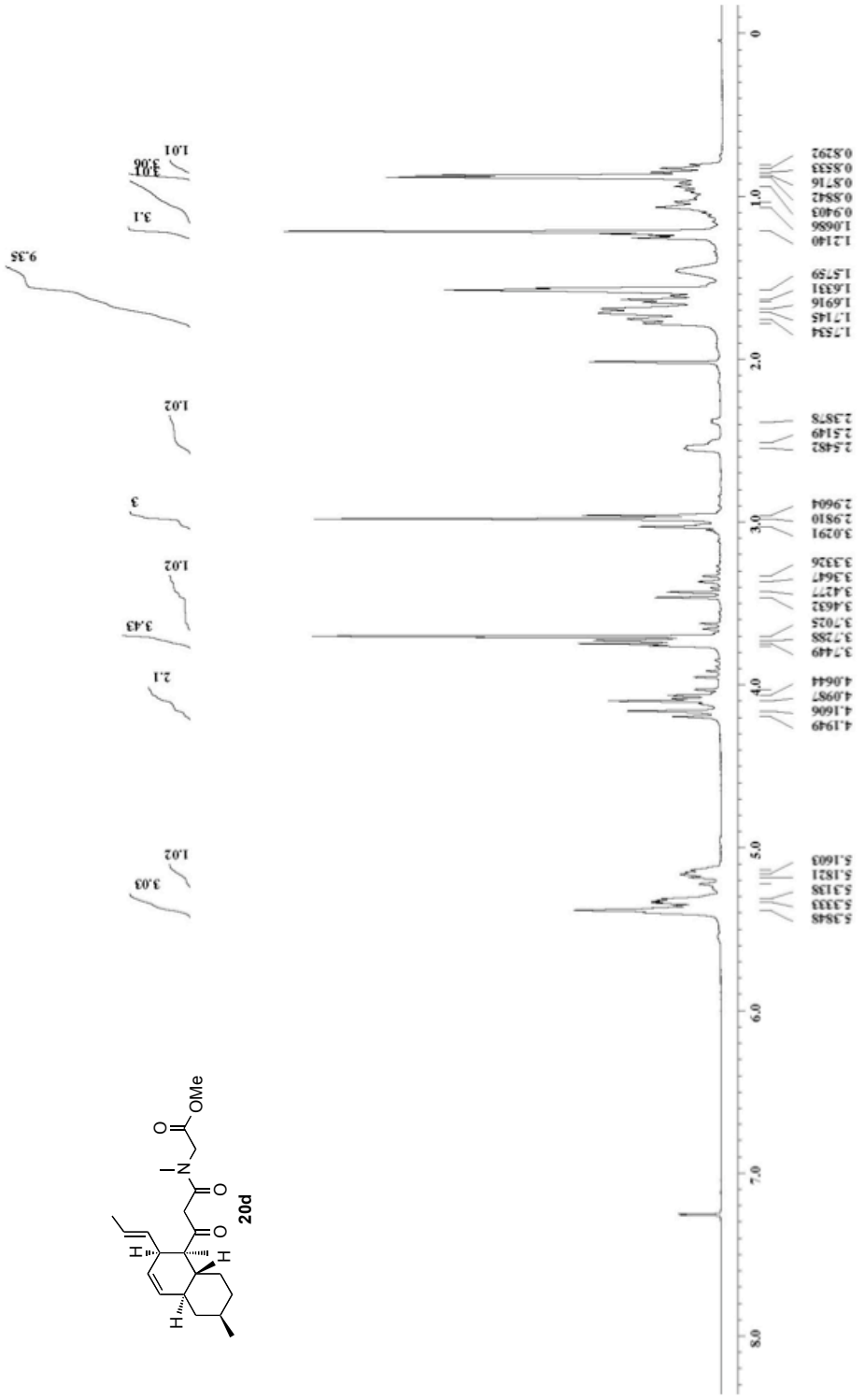
Spectrum 2.46 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20b**



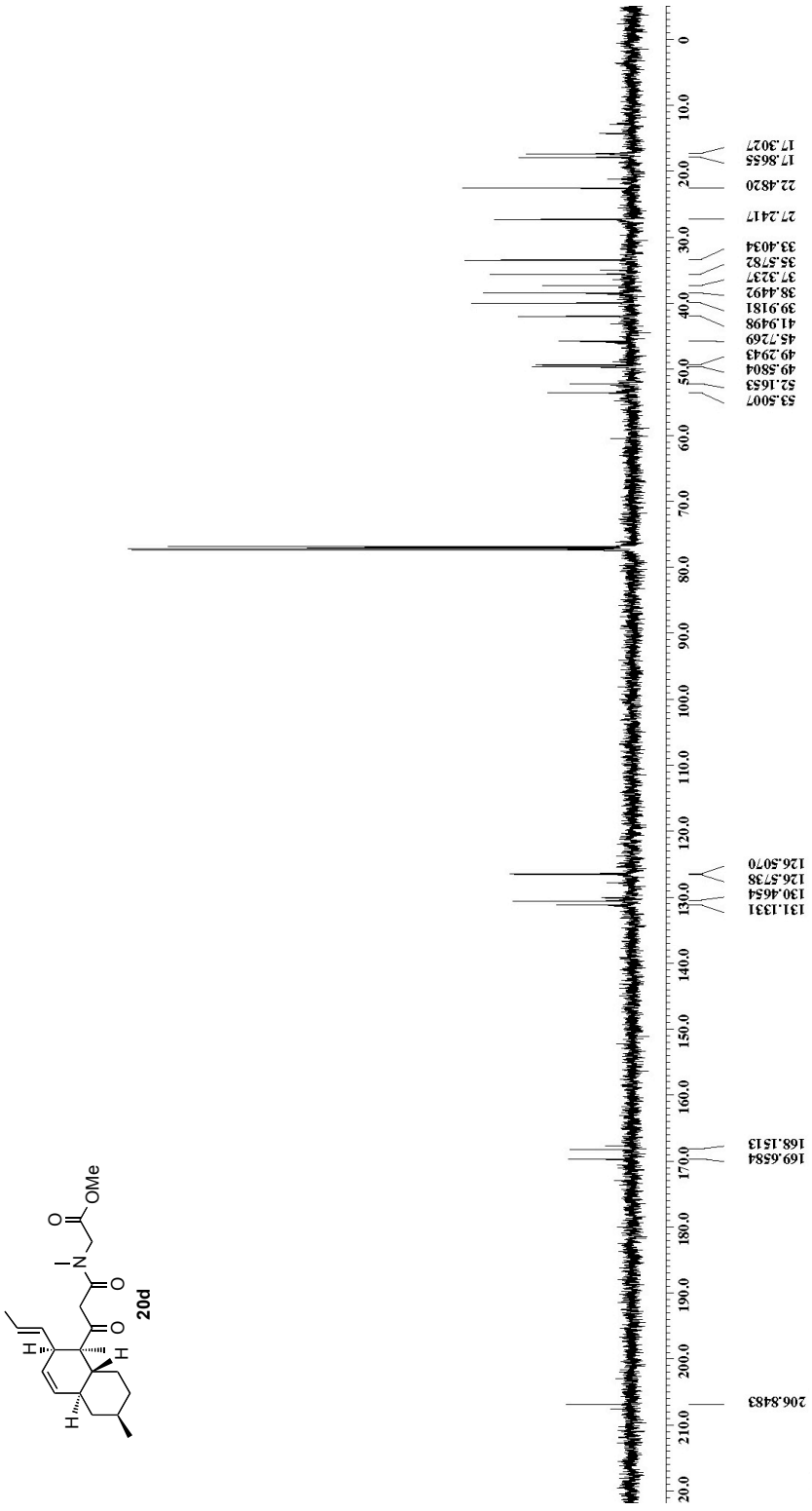
Spectrum 2.47 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20c**



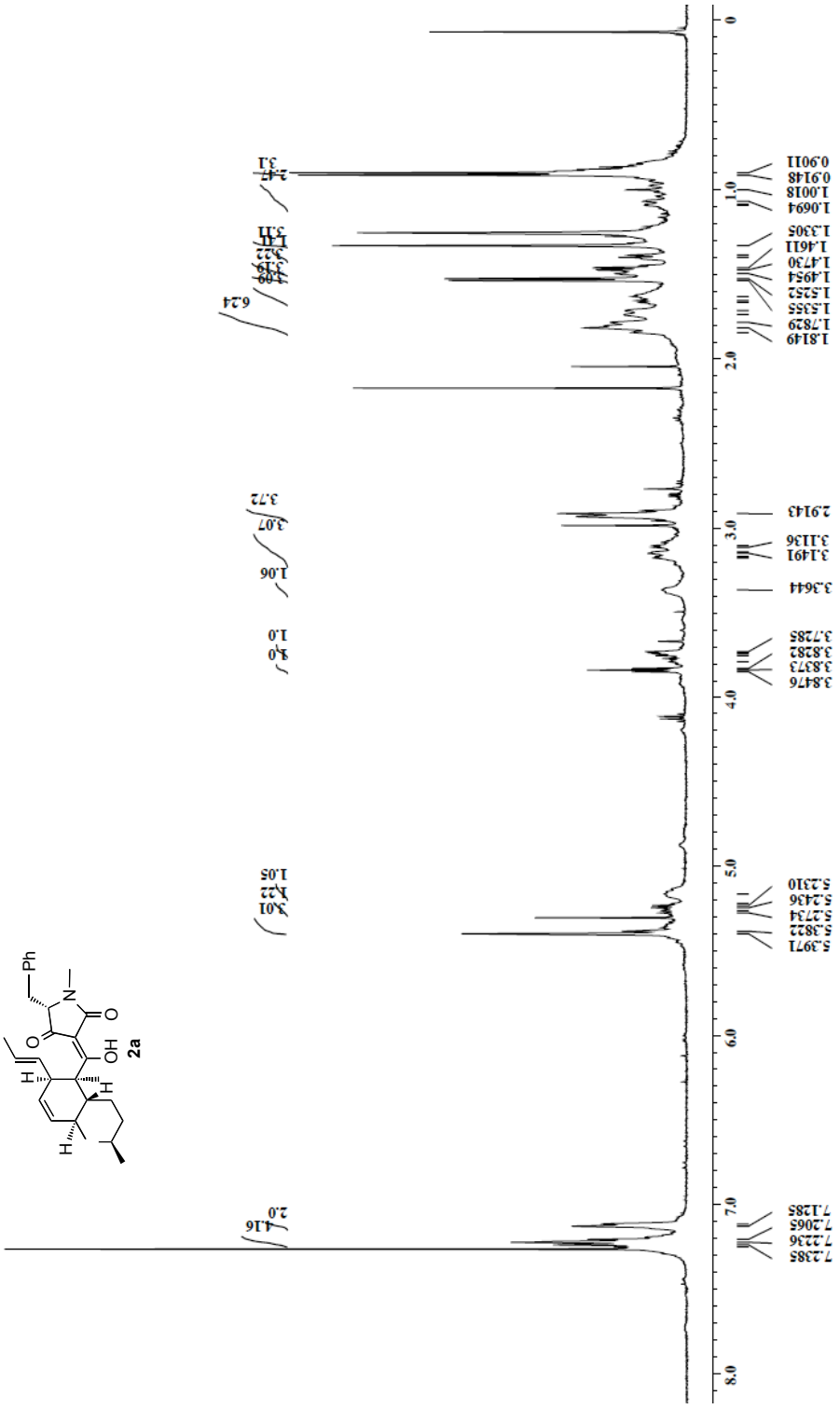
Spectrum 2.48 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20c**



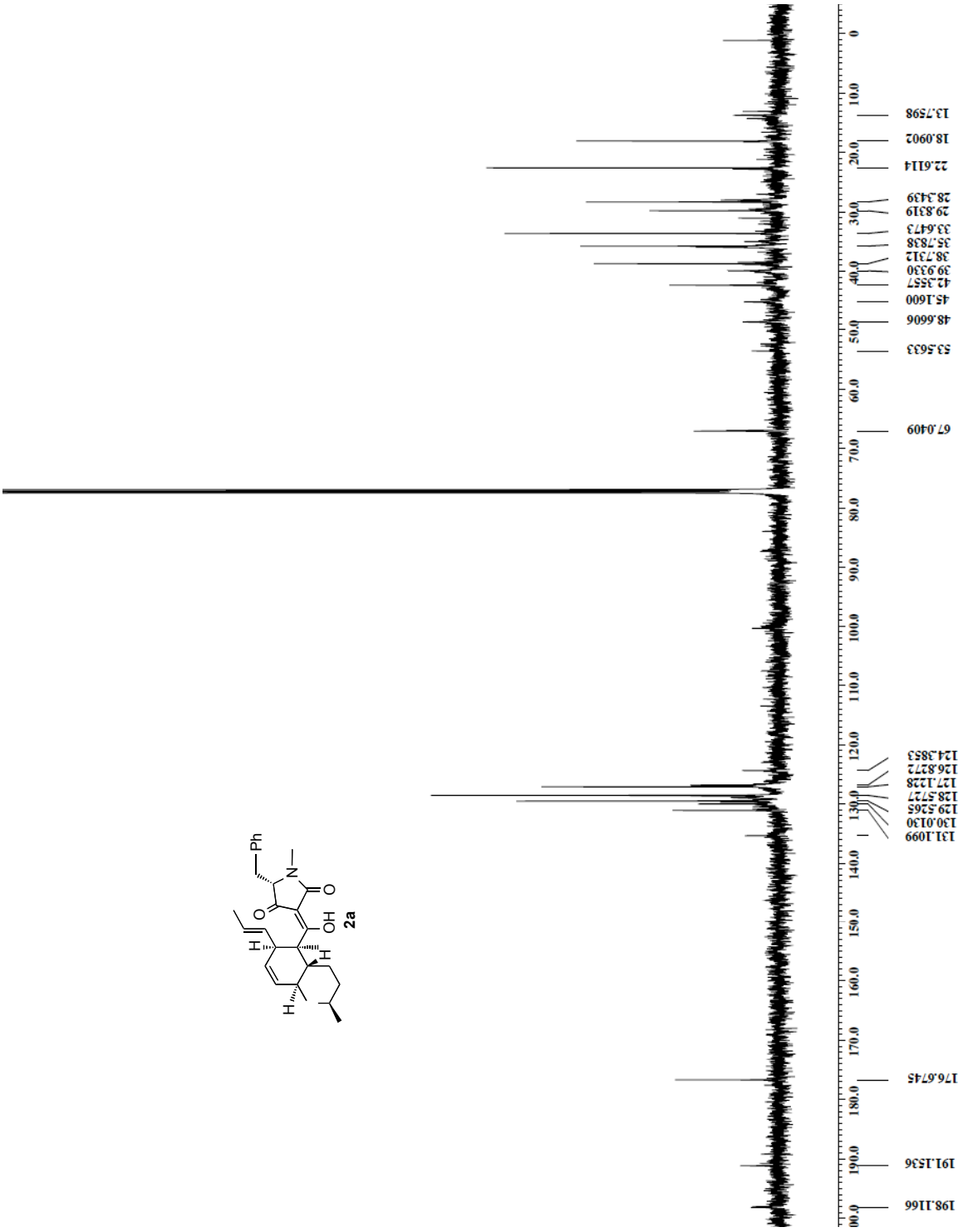
Spectrum 2.49 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **20d**



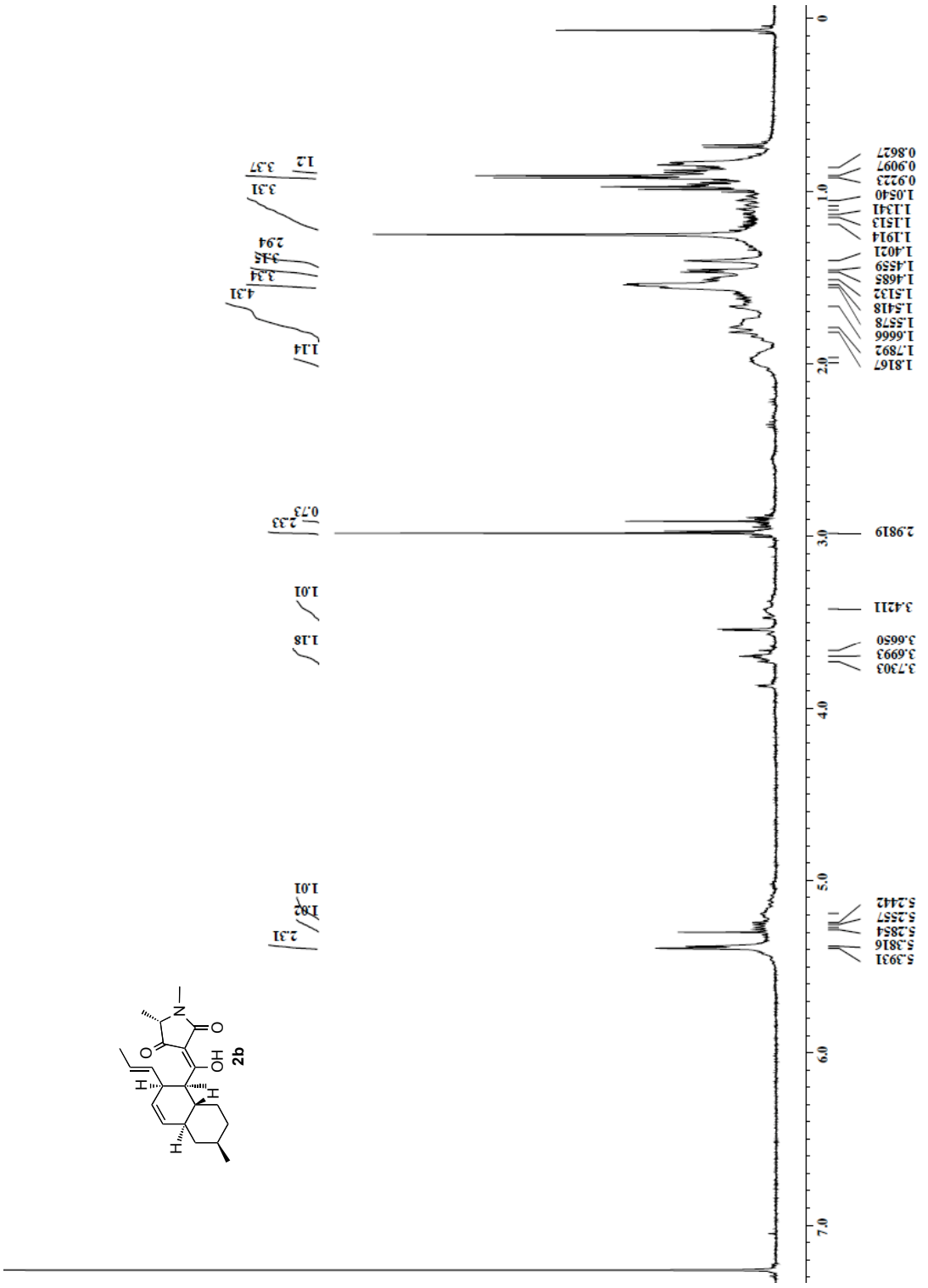
Spectrum 2.50 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **20d**



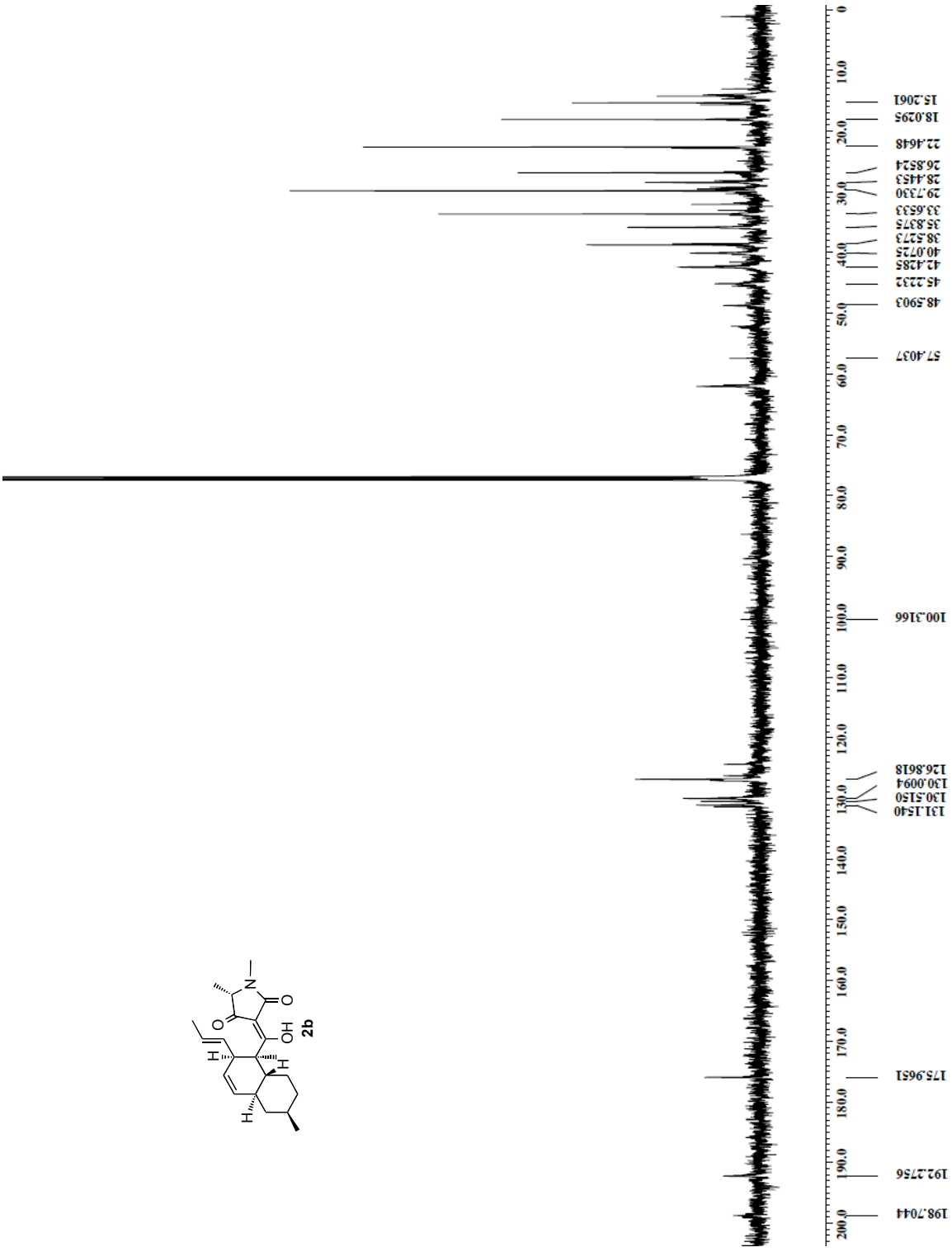
Spectrum 2.51 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **2a**



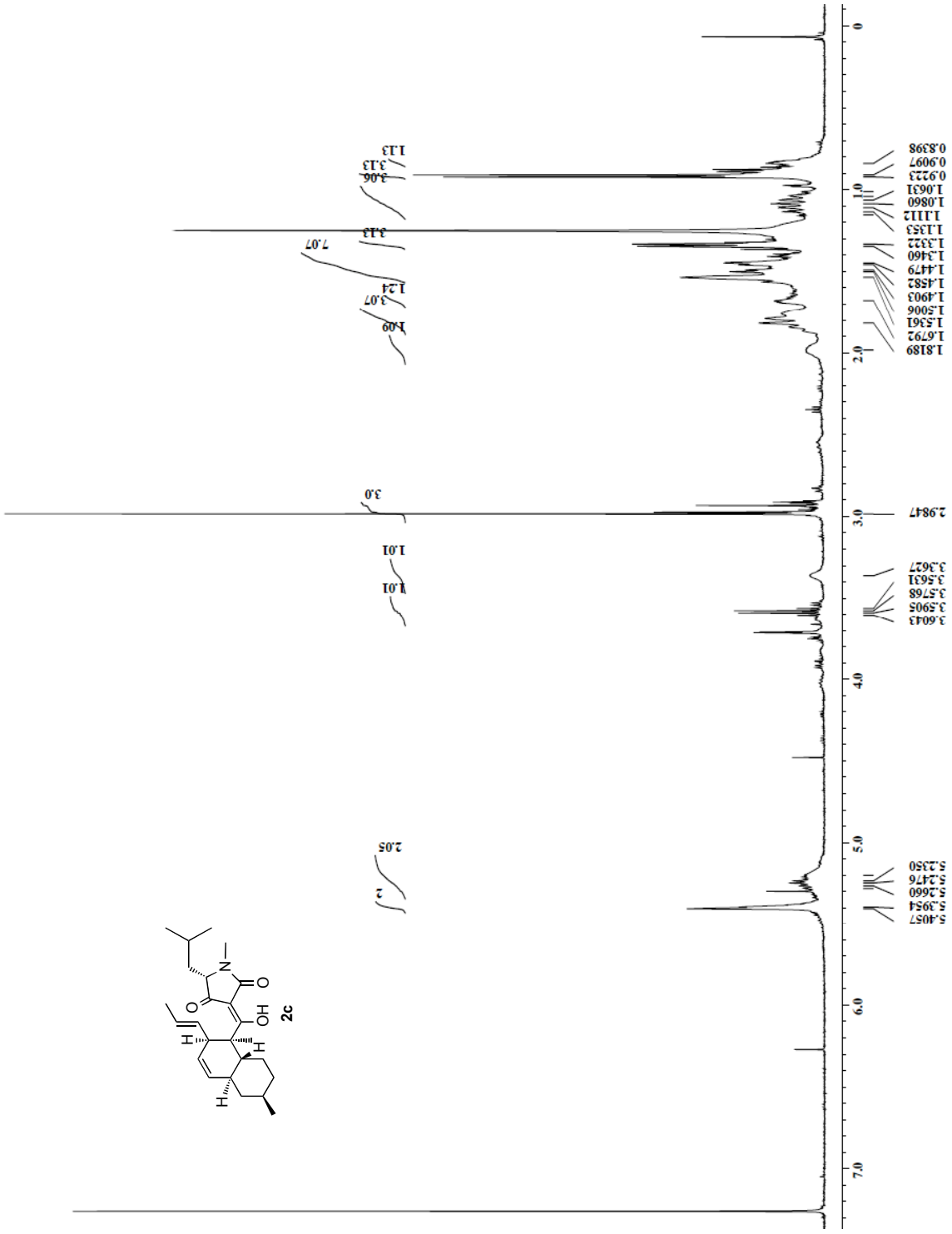
Spectrum 2.52 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **2a**



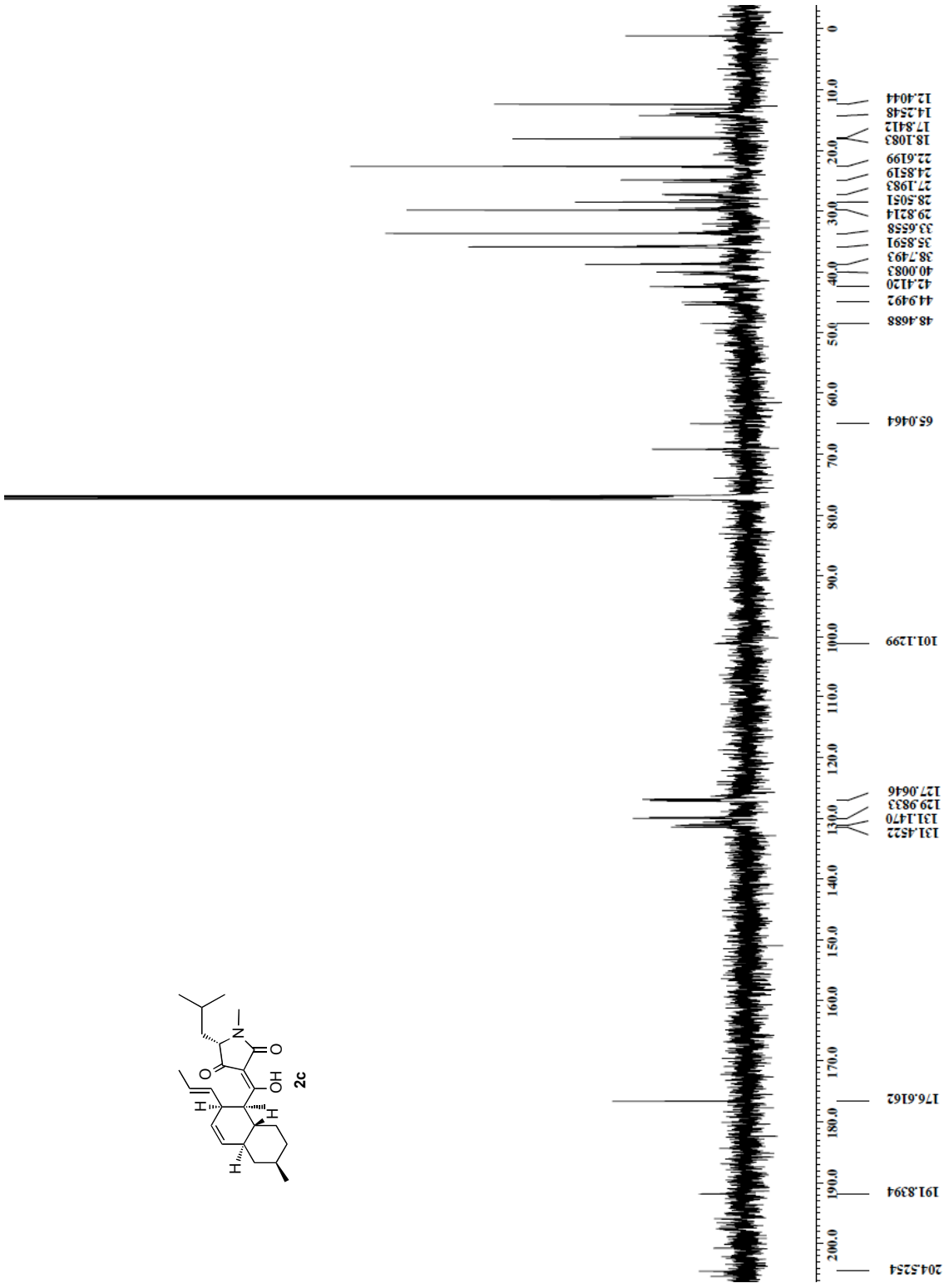
Spectrum 2.53 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **2b**



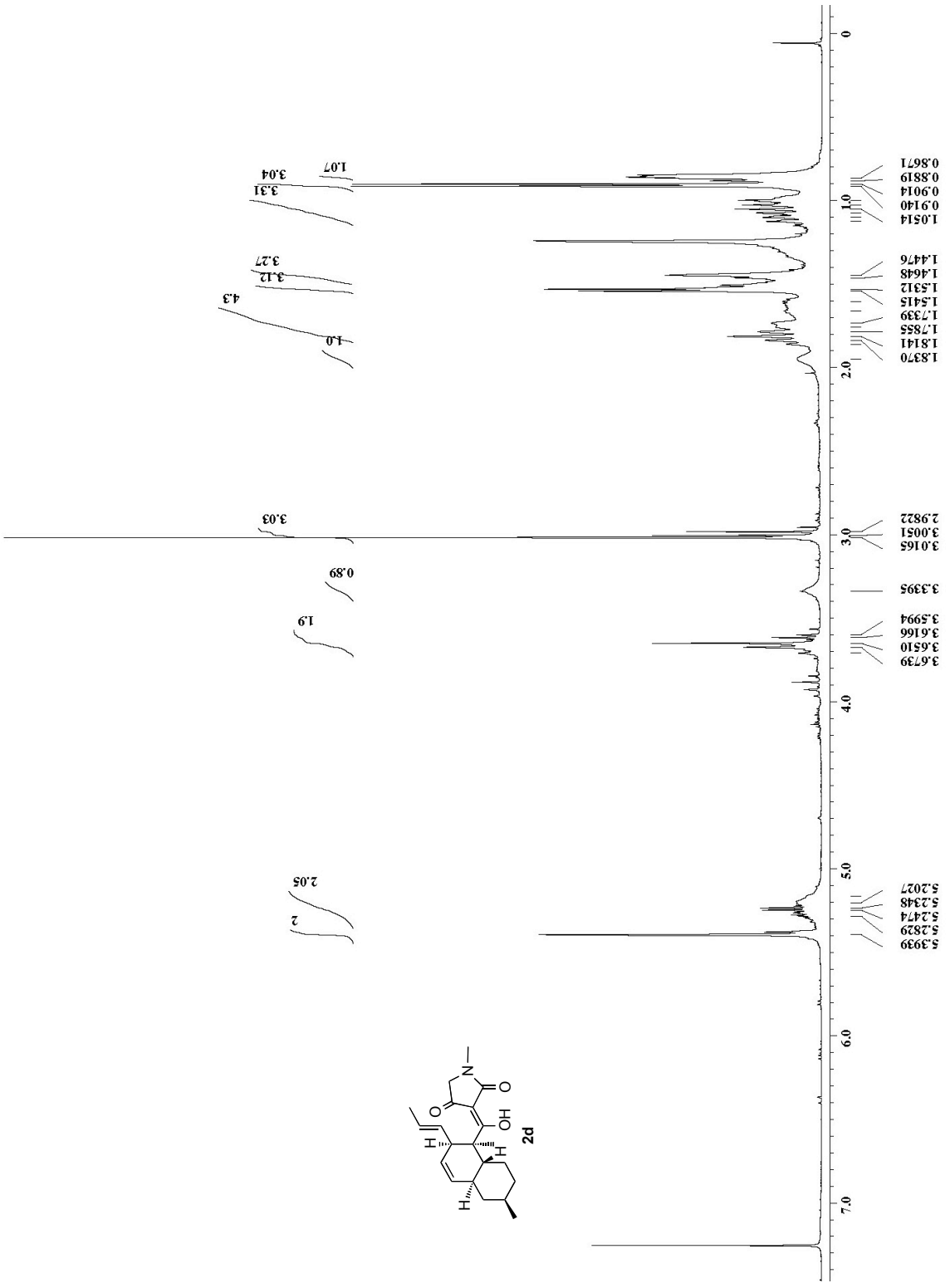
Spectrum 2.54 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **2b**



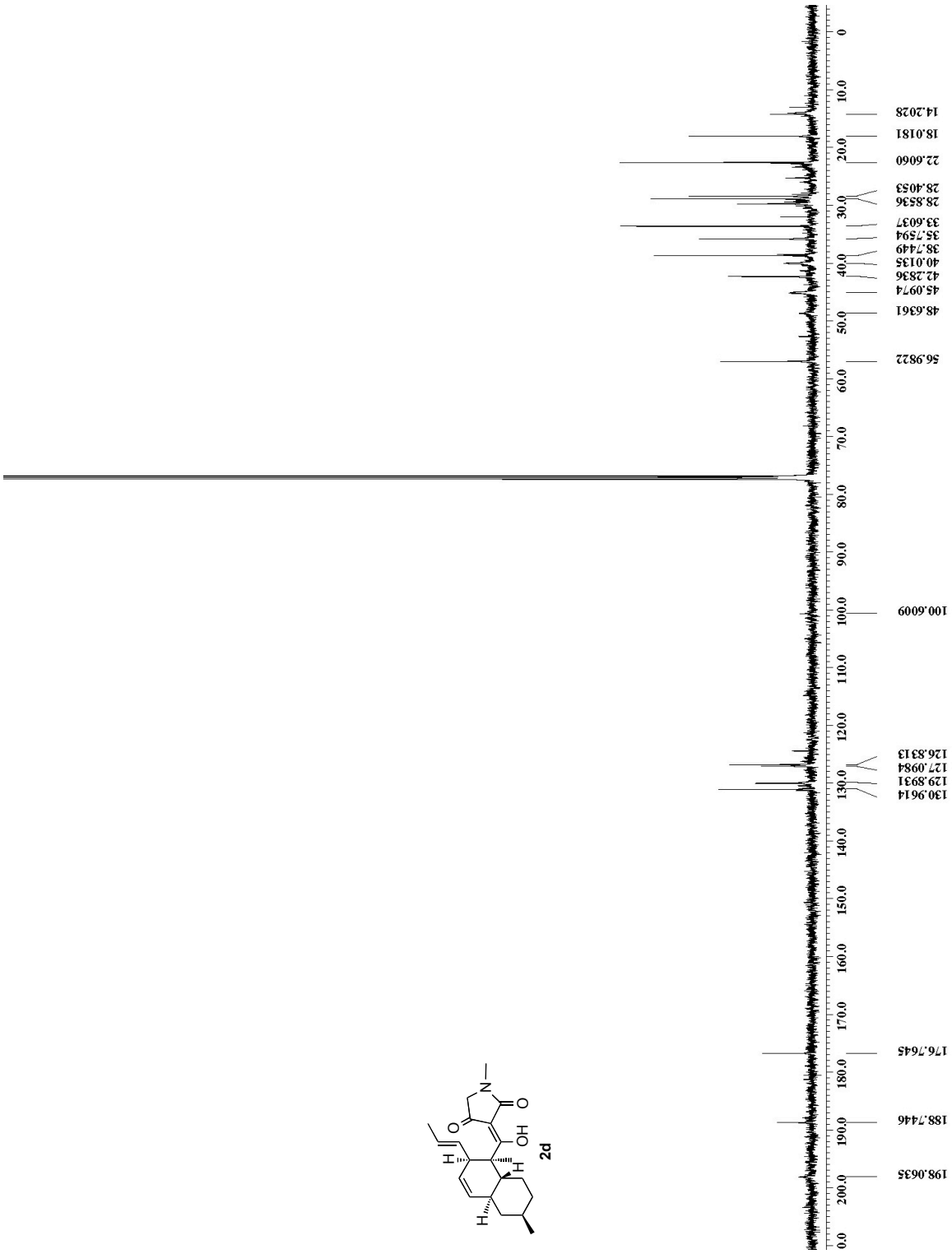
Spectrum 2.55 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **2c**



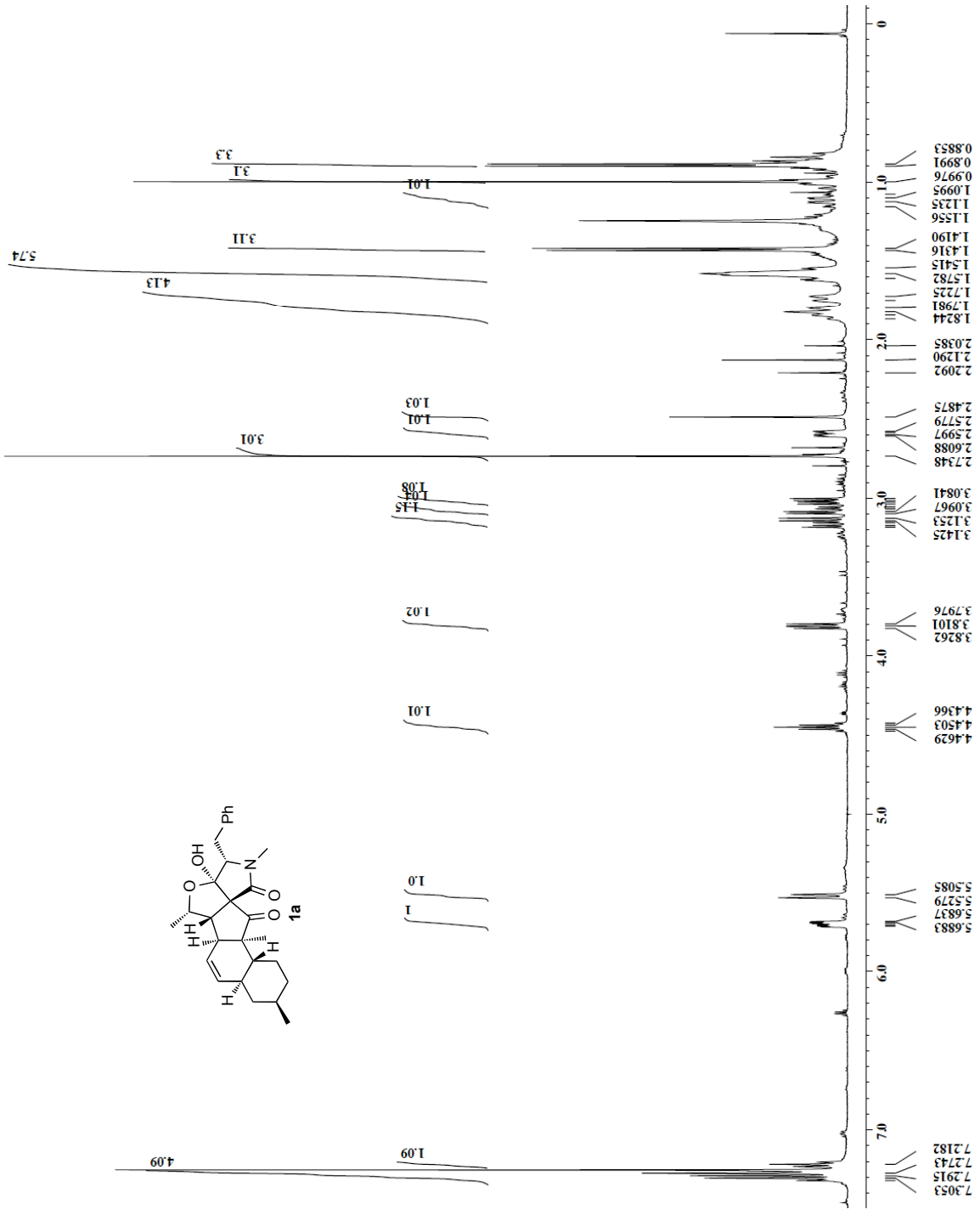
Spectrum 2.56 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **2c**



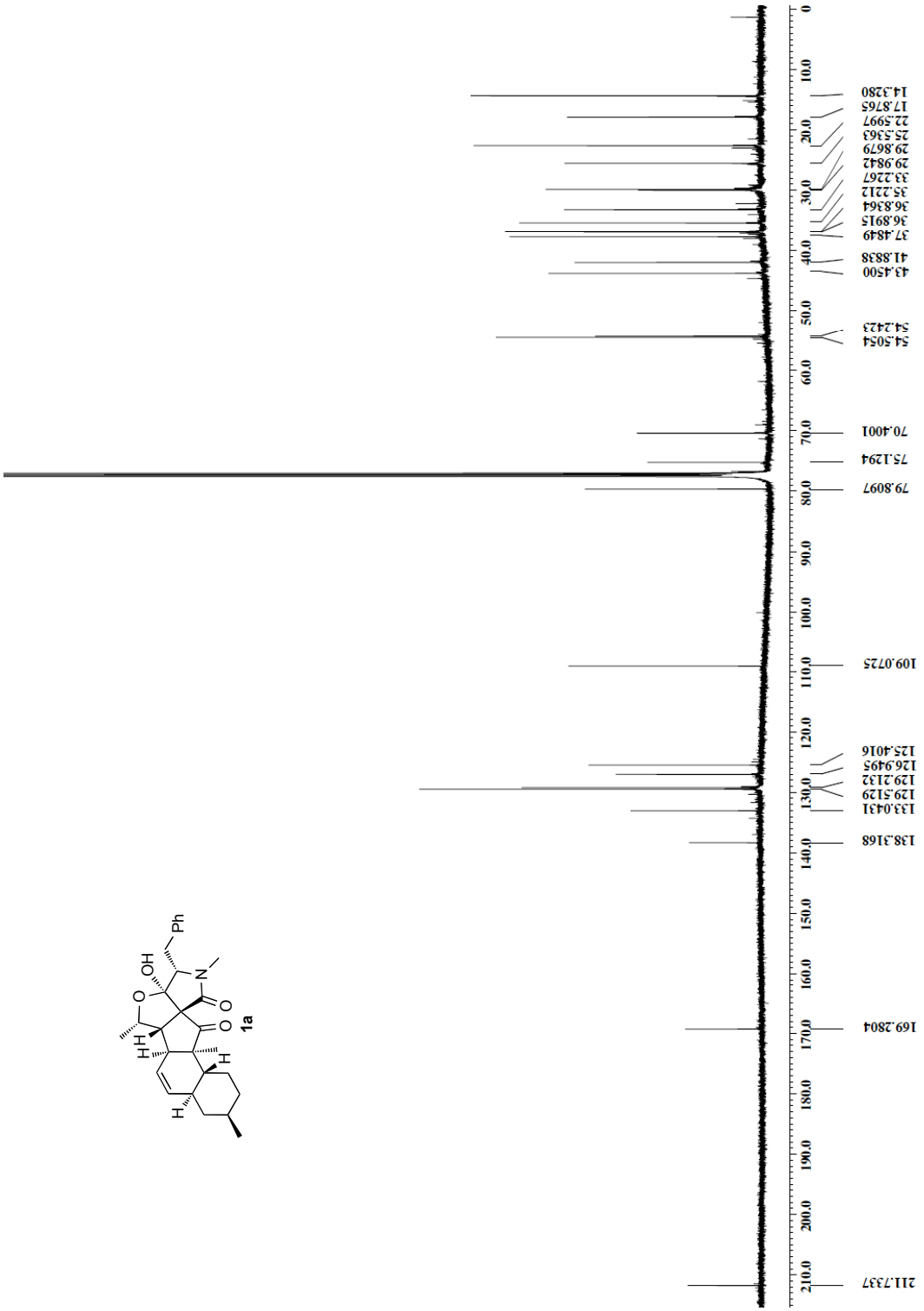
Spectrum 2.57 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **2d**



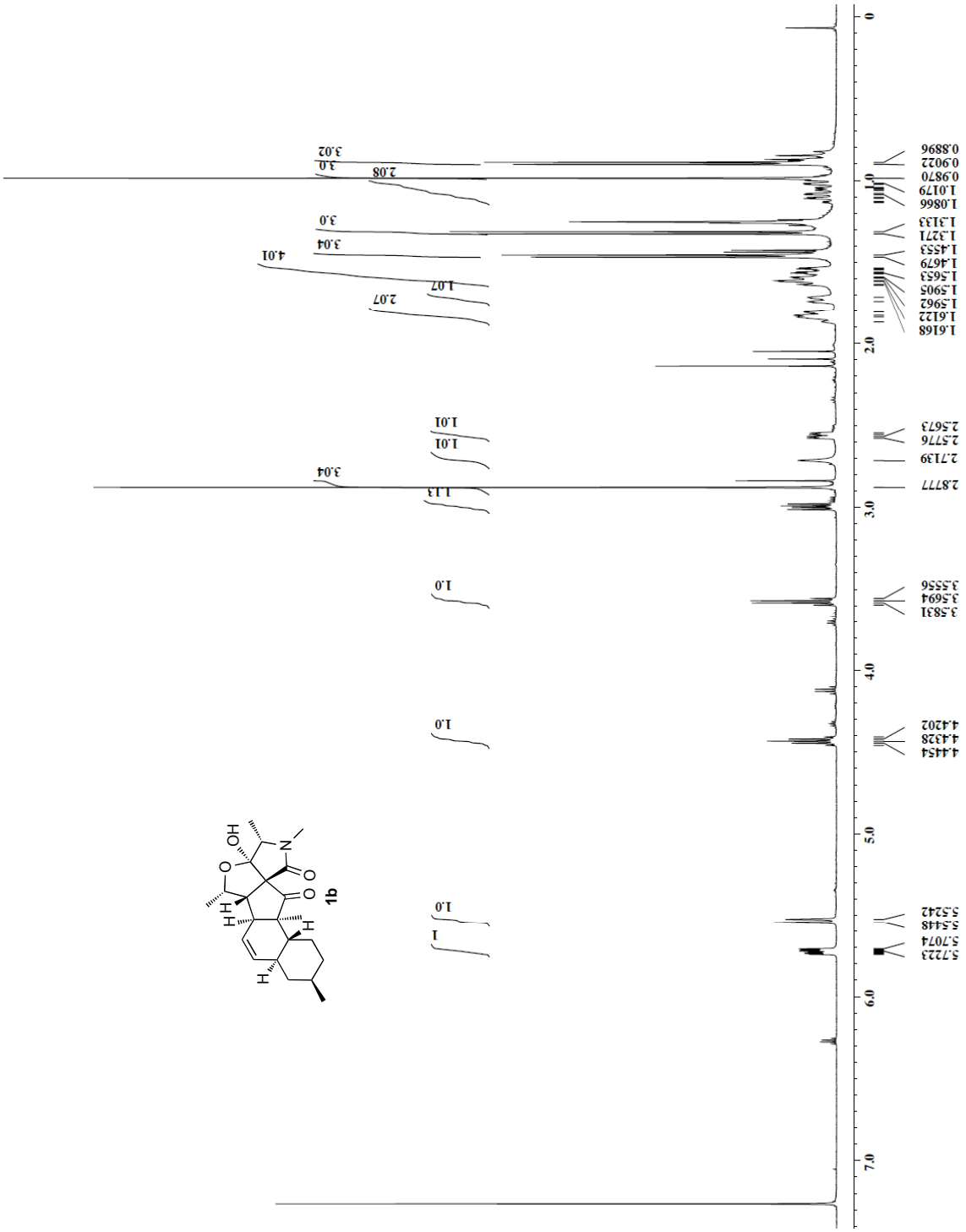
Spectrum 2.58 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **2d**



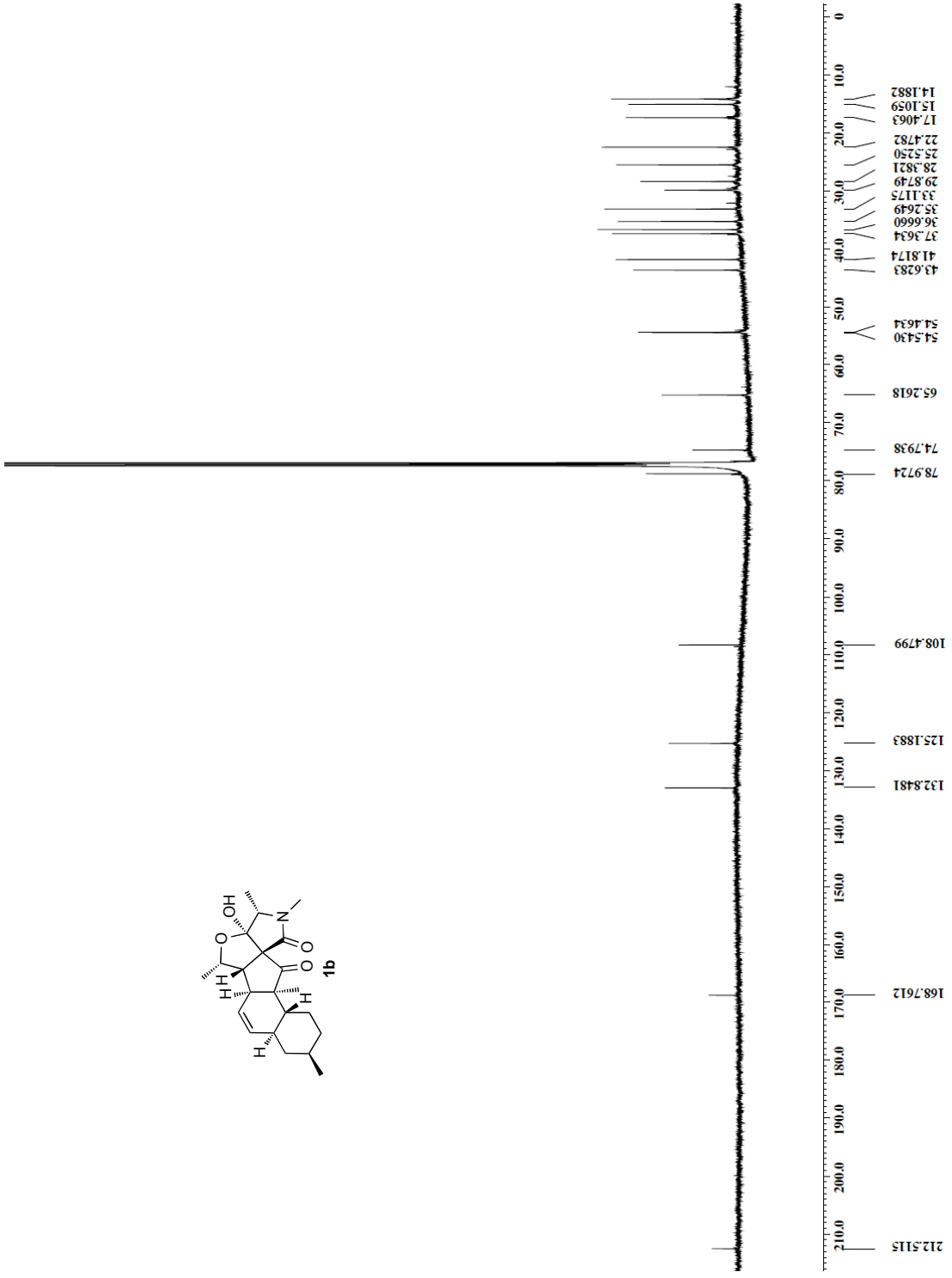
Spectrum 2.59 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **1a**



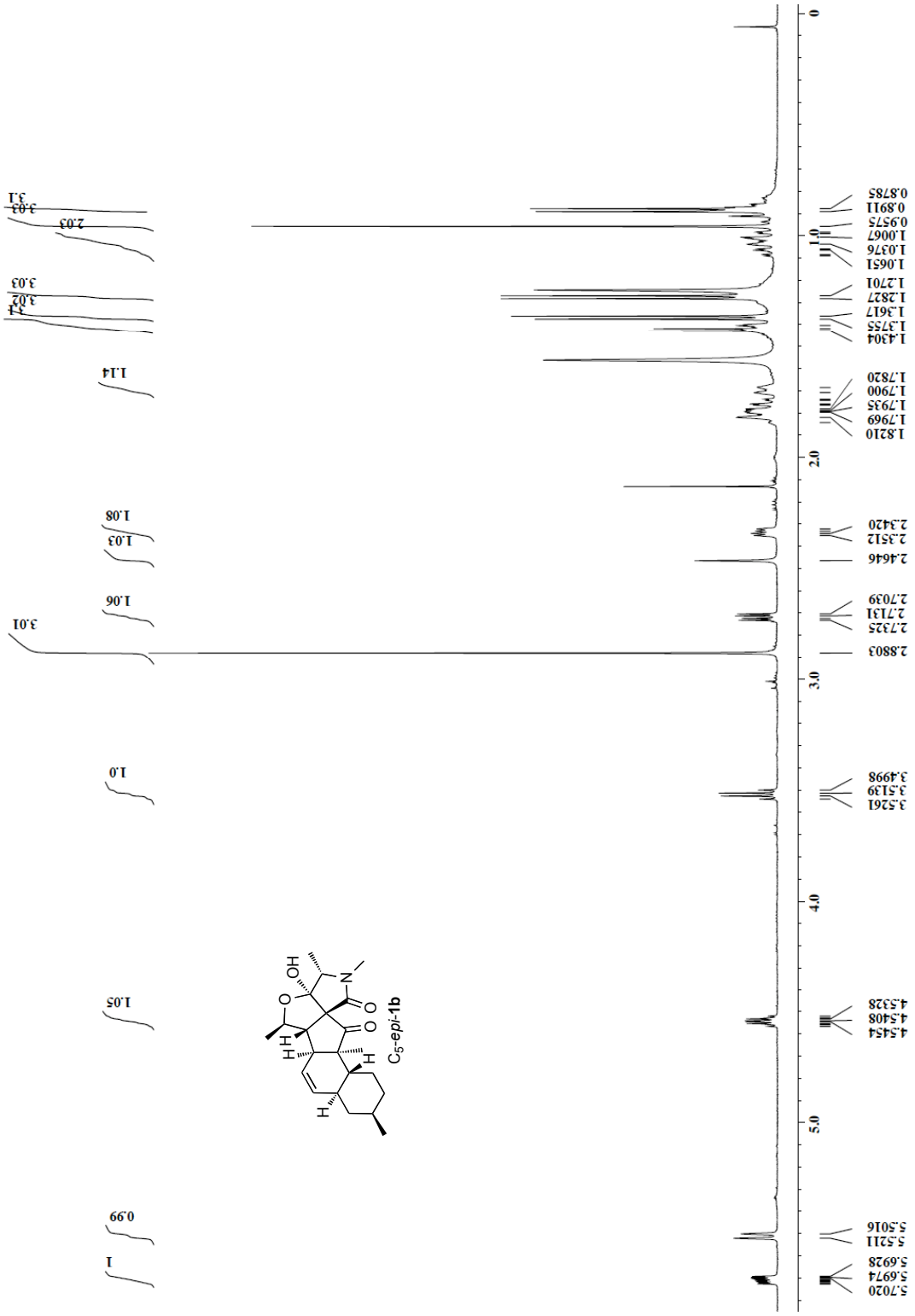
Spectrum 2.60 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **1a**



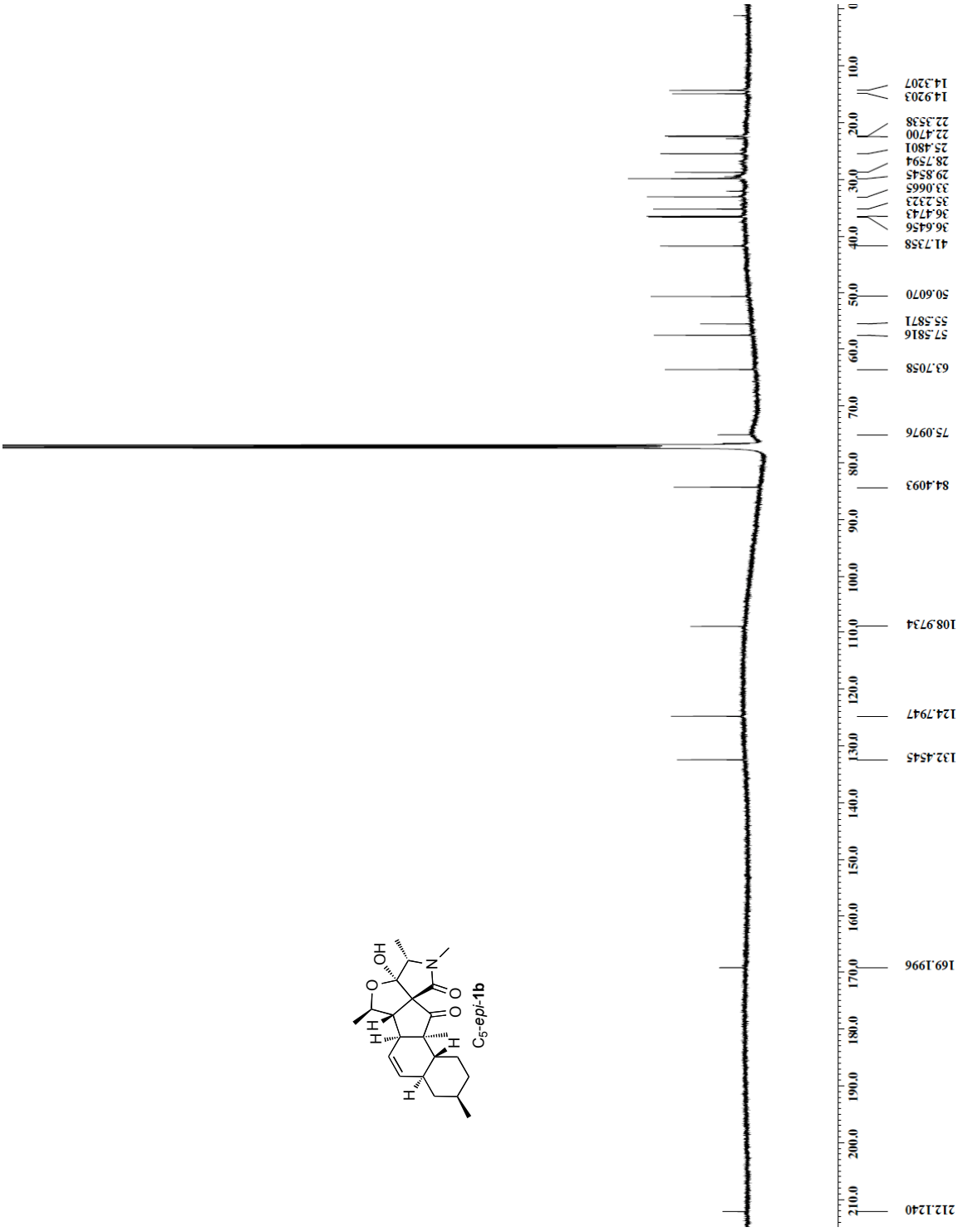
Spectrum 2.61 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **1b**



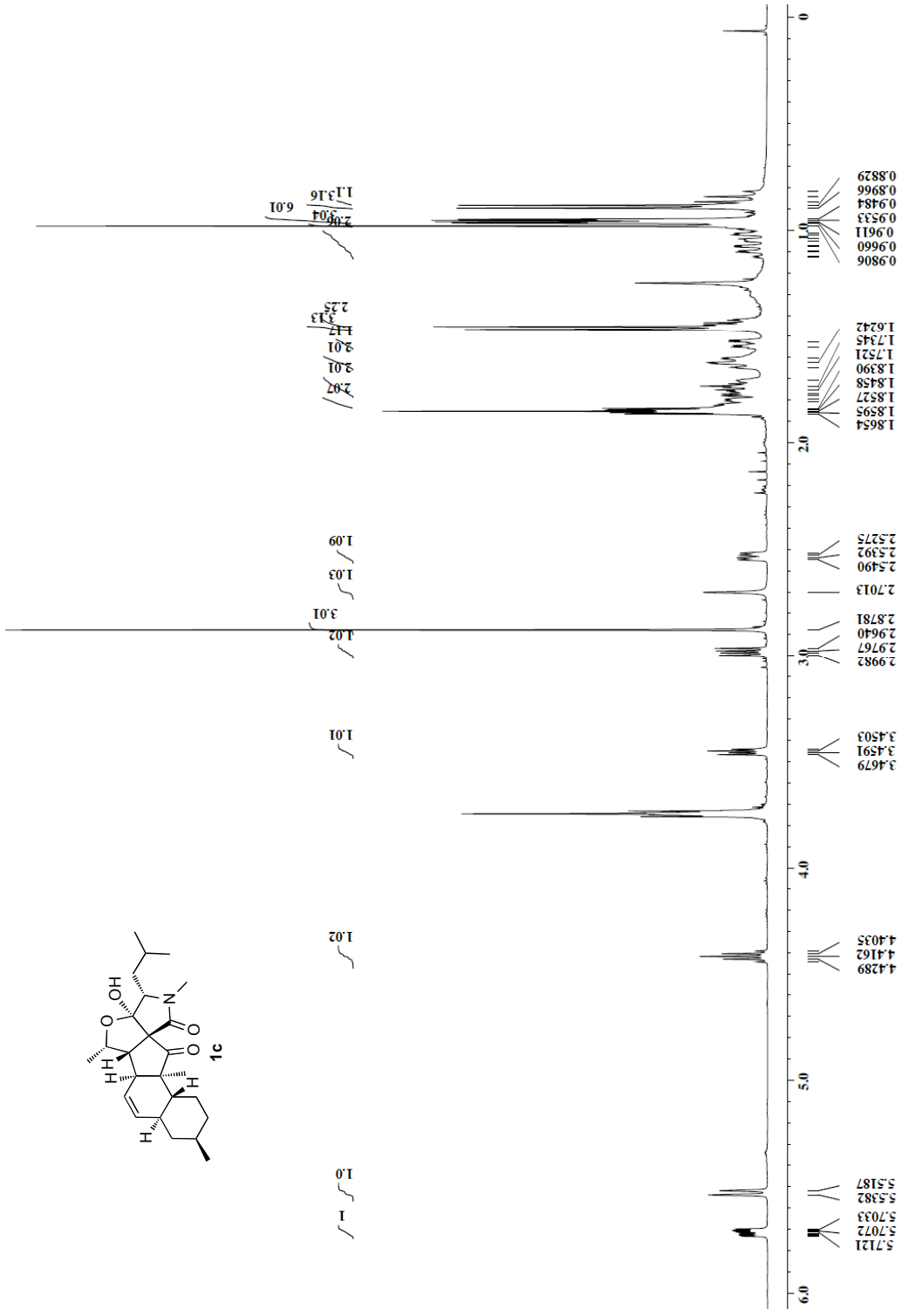
Spectrum 2.62 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **1b**



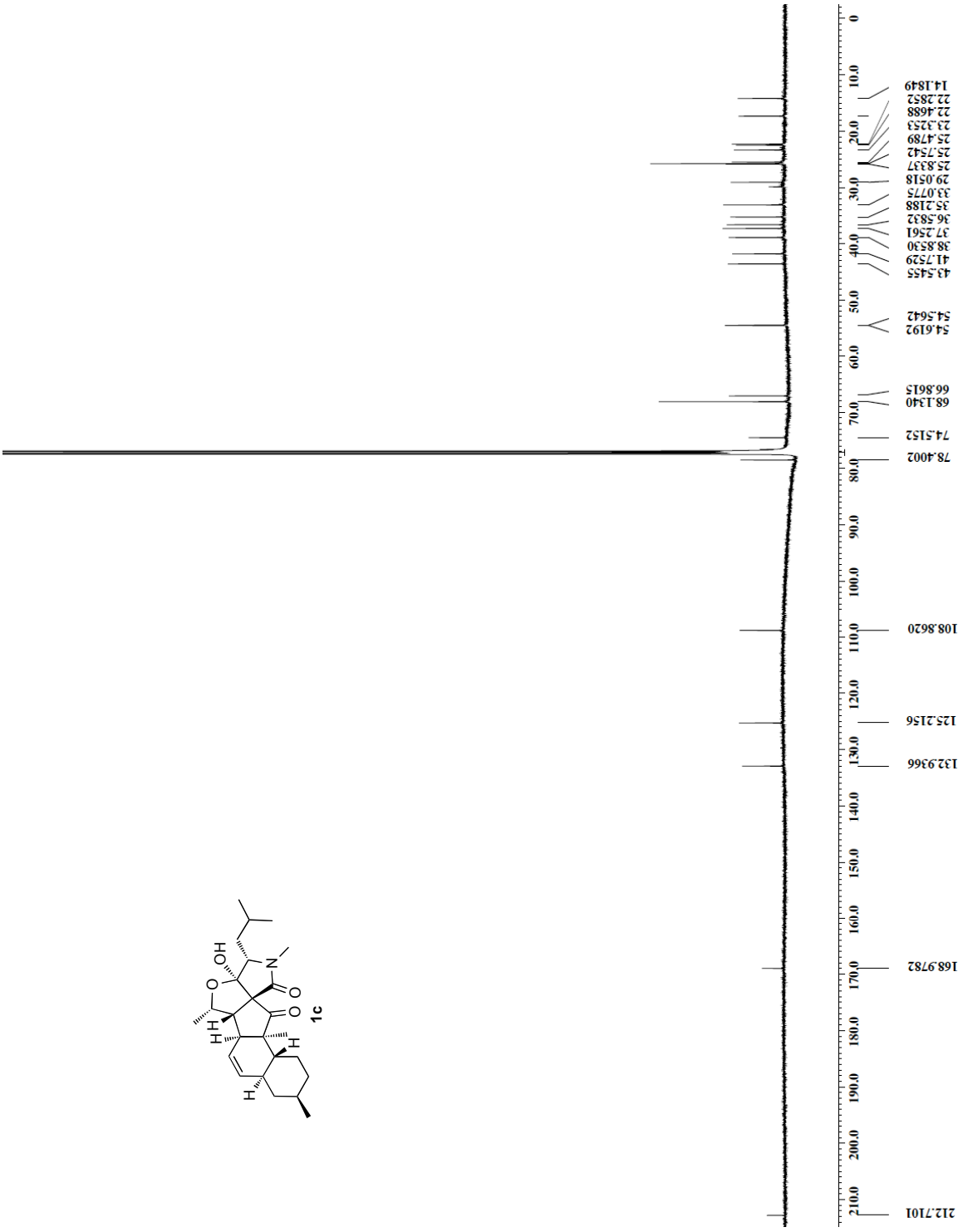
Spectrum 2.63 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound C_5-*epi*-**1b**



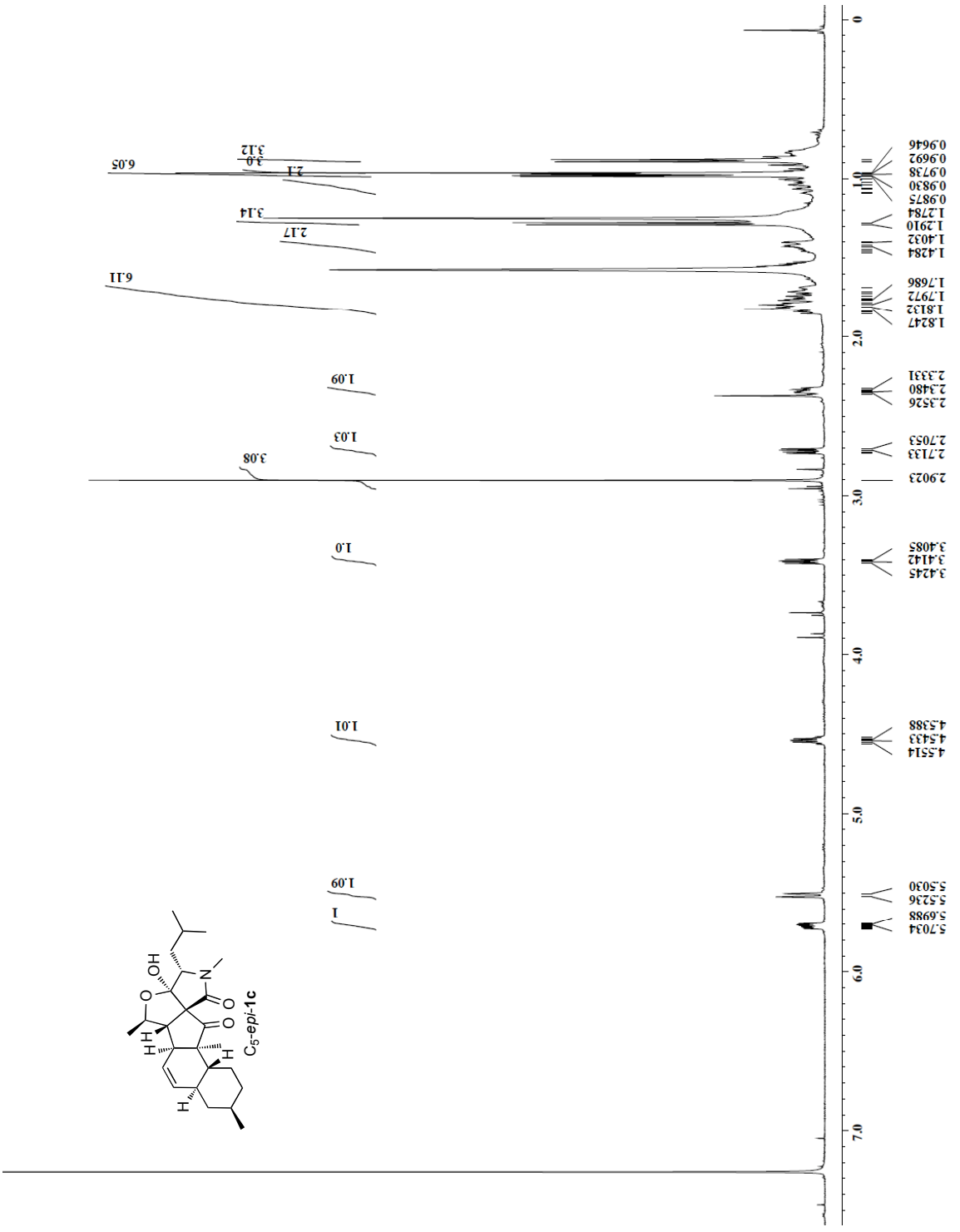
Spectrum 2.64 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound C_5-*epi*-**1b**



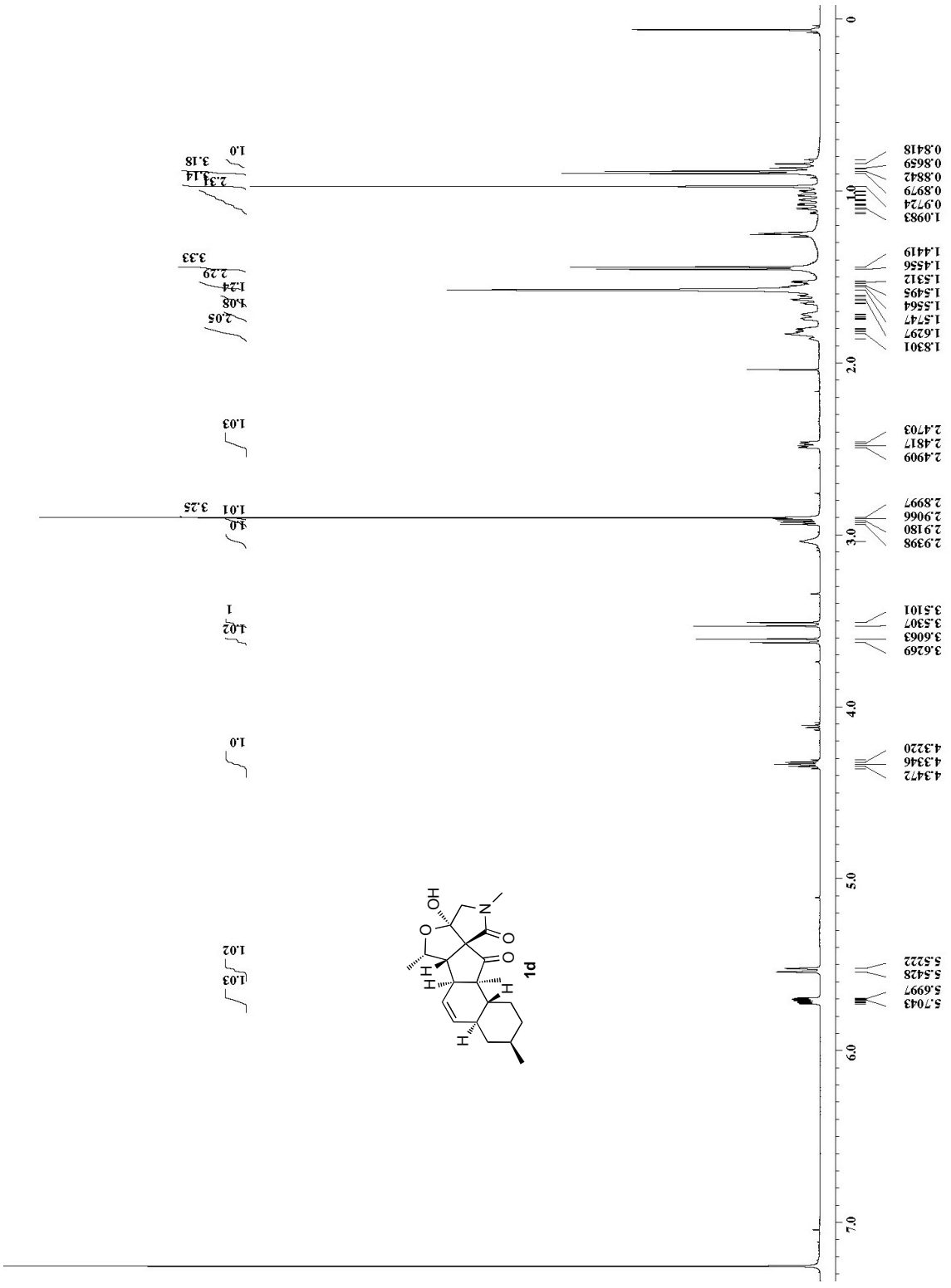
Spectrum 2.65 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **1c**



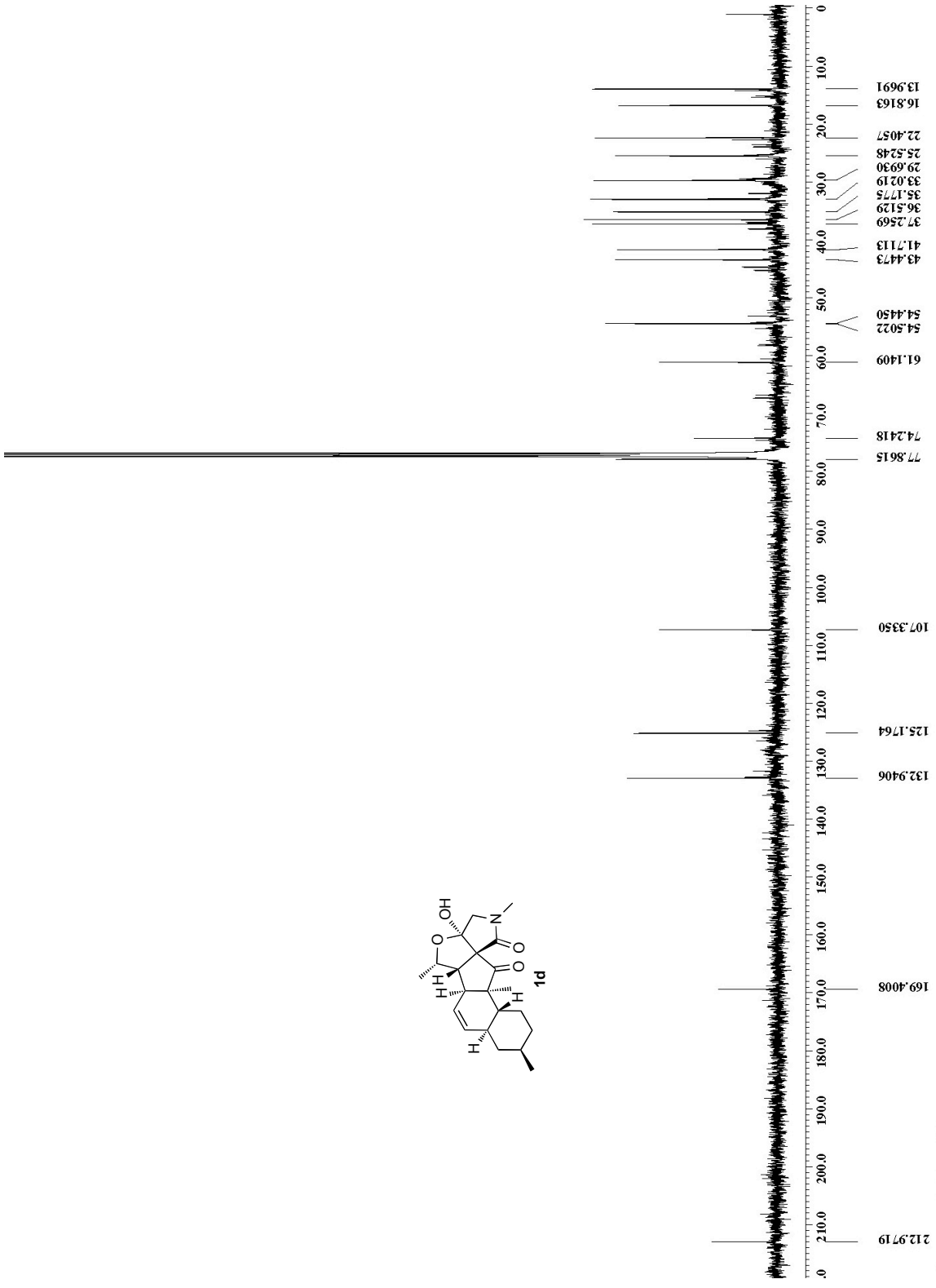
Spectrum 2.66 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **1c**



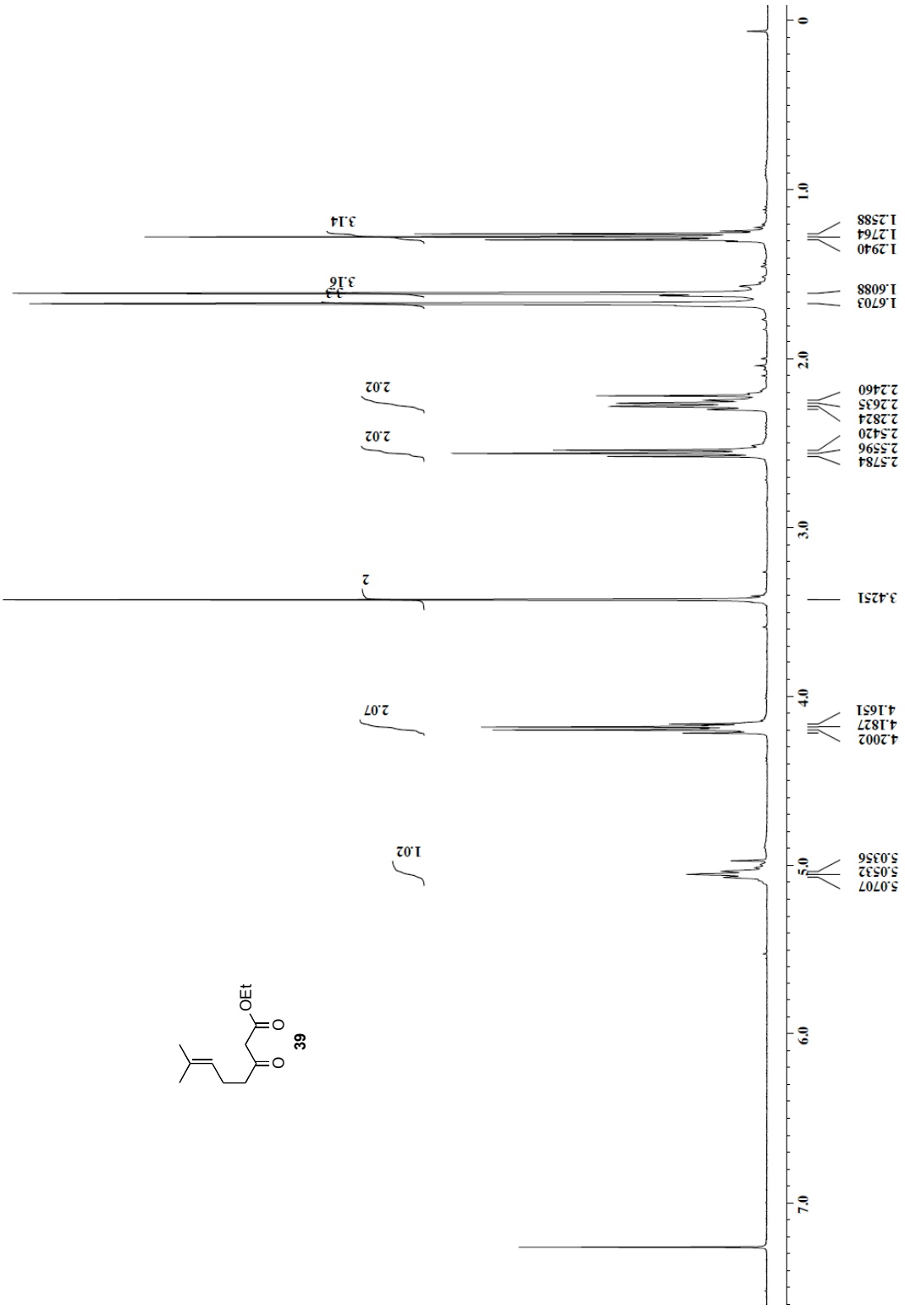
Spectrum 2.67 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound C_5-*epi*-**1c**



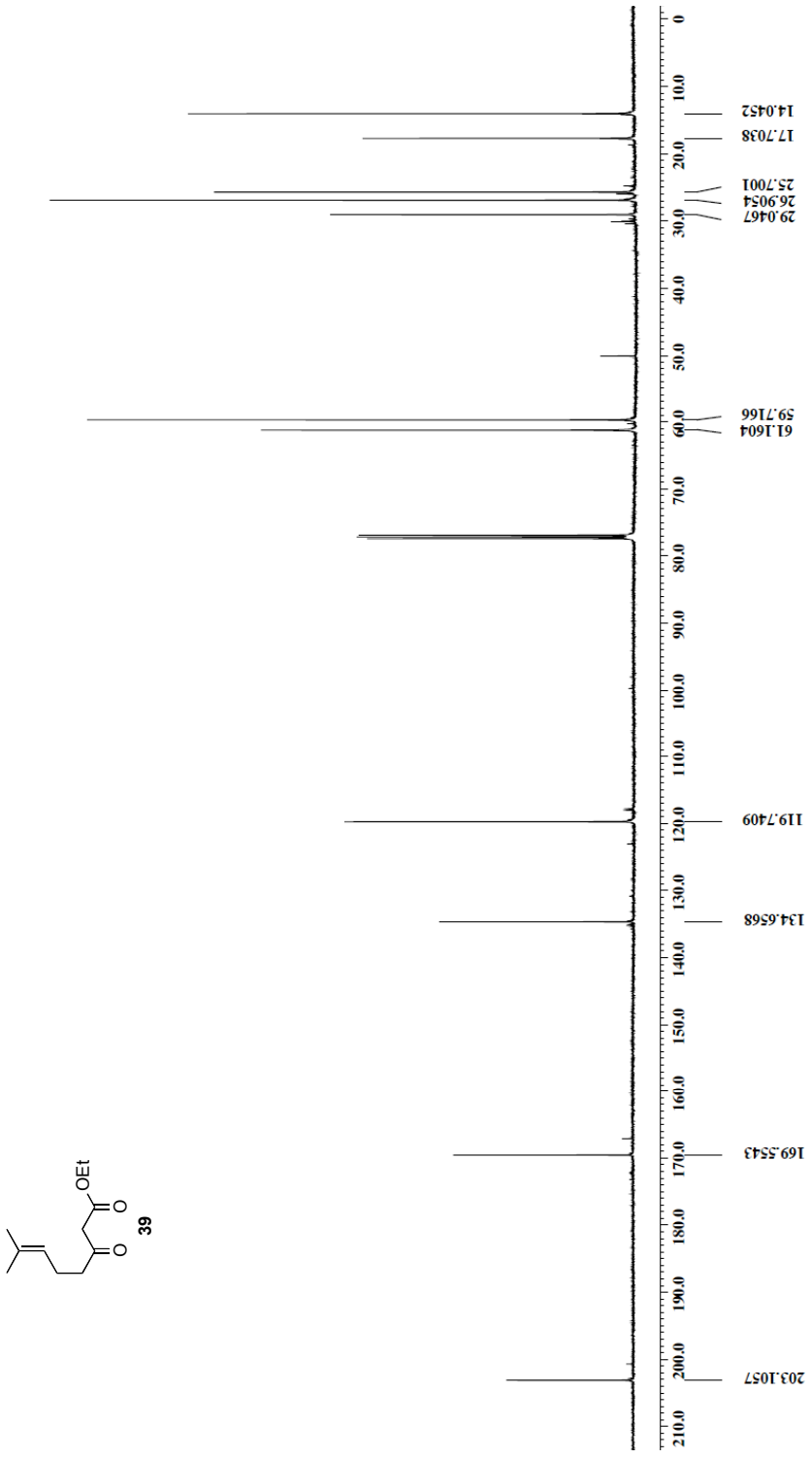
Spectrum 2.68 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **1d**



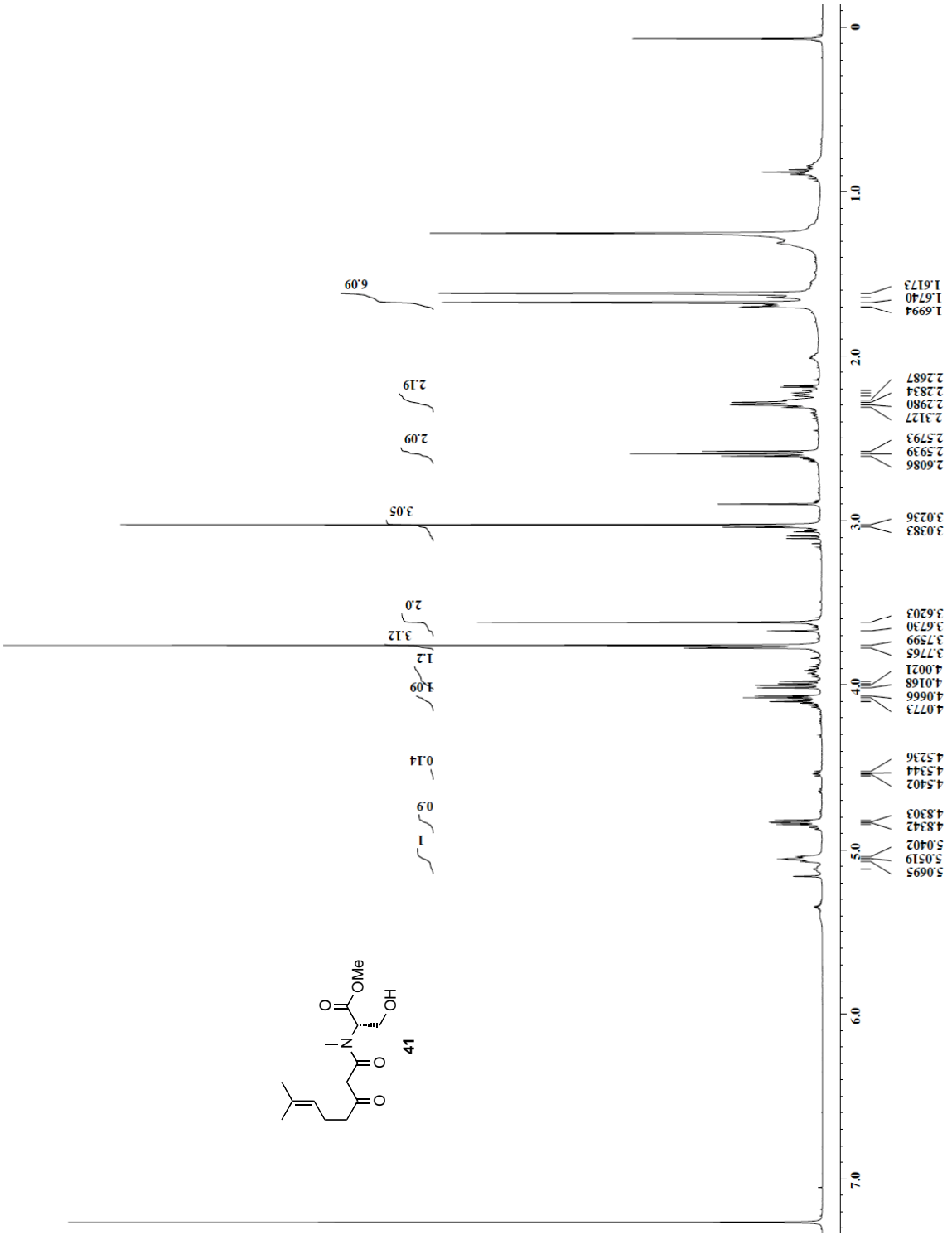
Spectrum 2.69 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **1d**



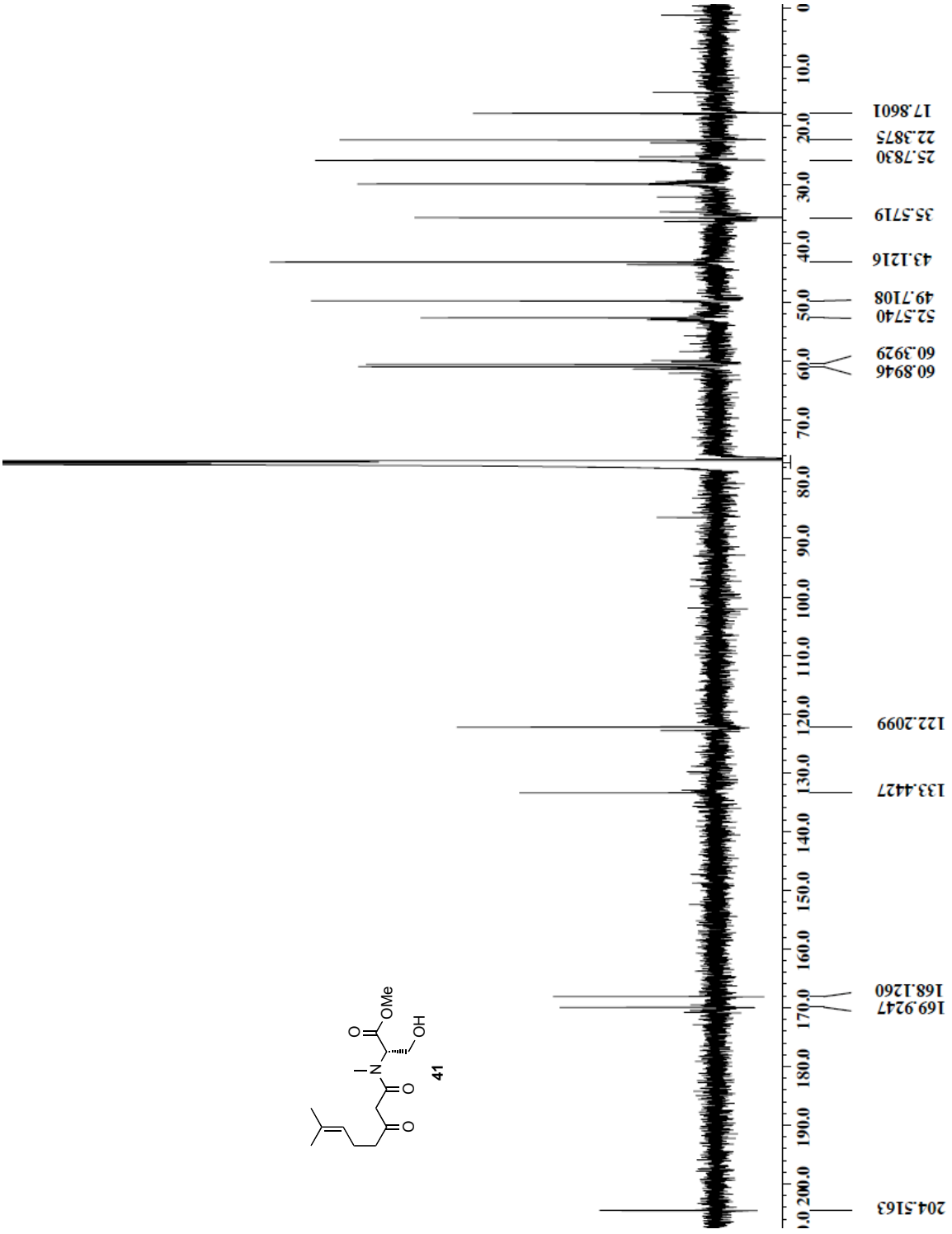
Spectrum 2.70 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **39**



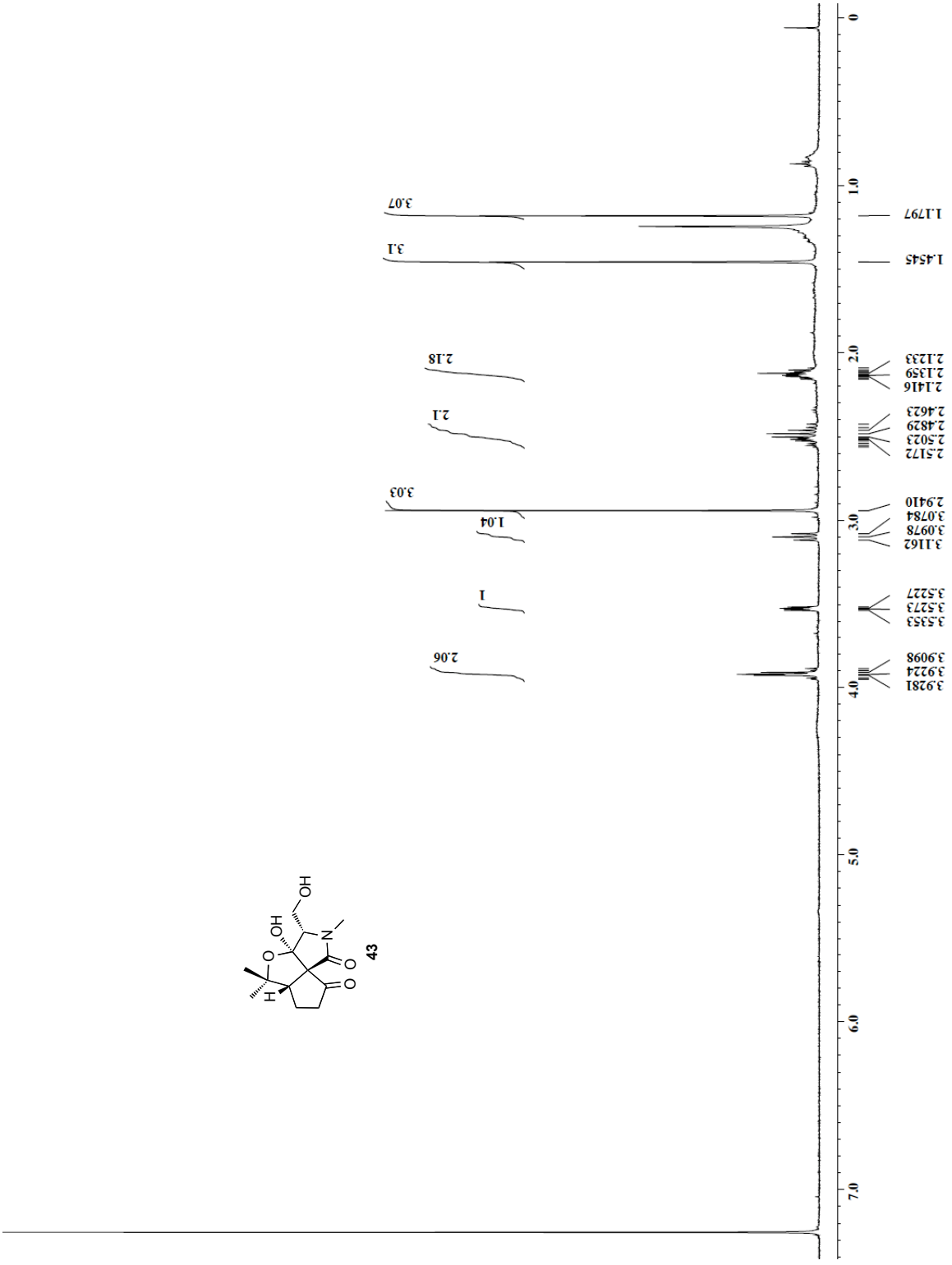
Spectrum 2.71 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **39**



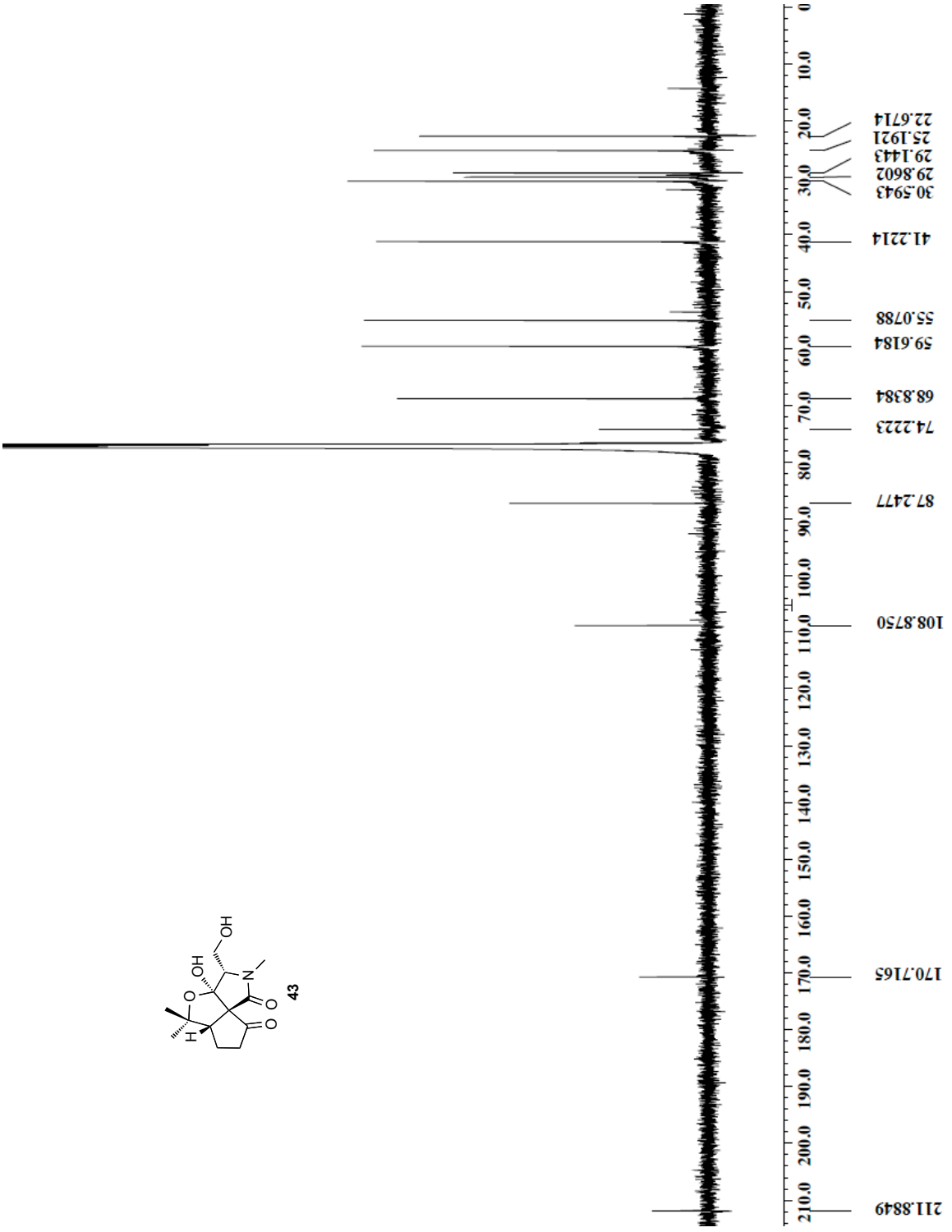
Spectrum 2.72 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **41**



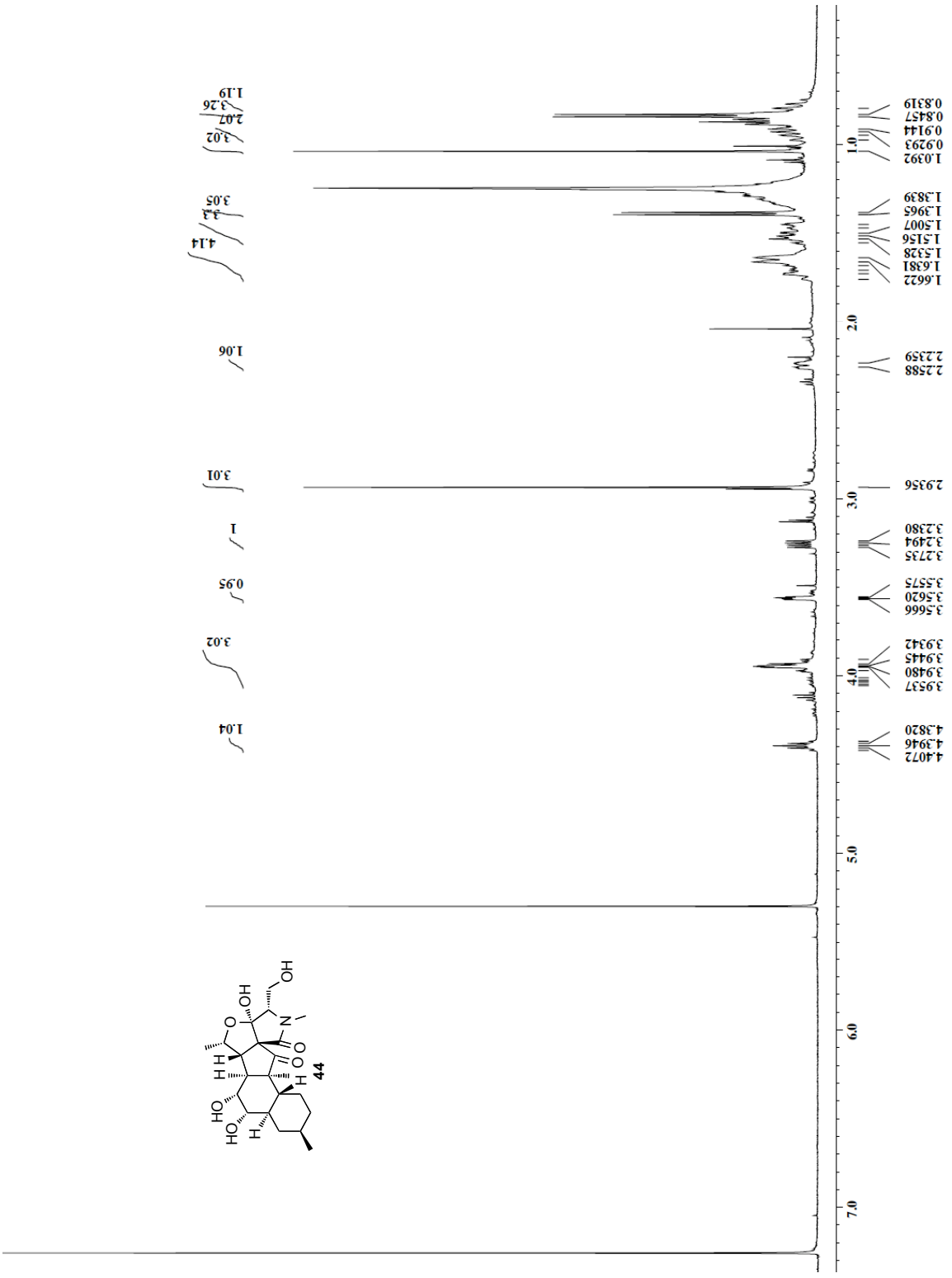
Spectrum 2.73 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **41**



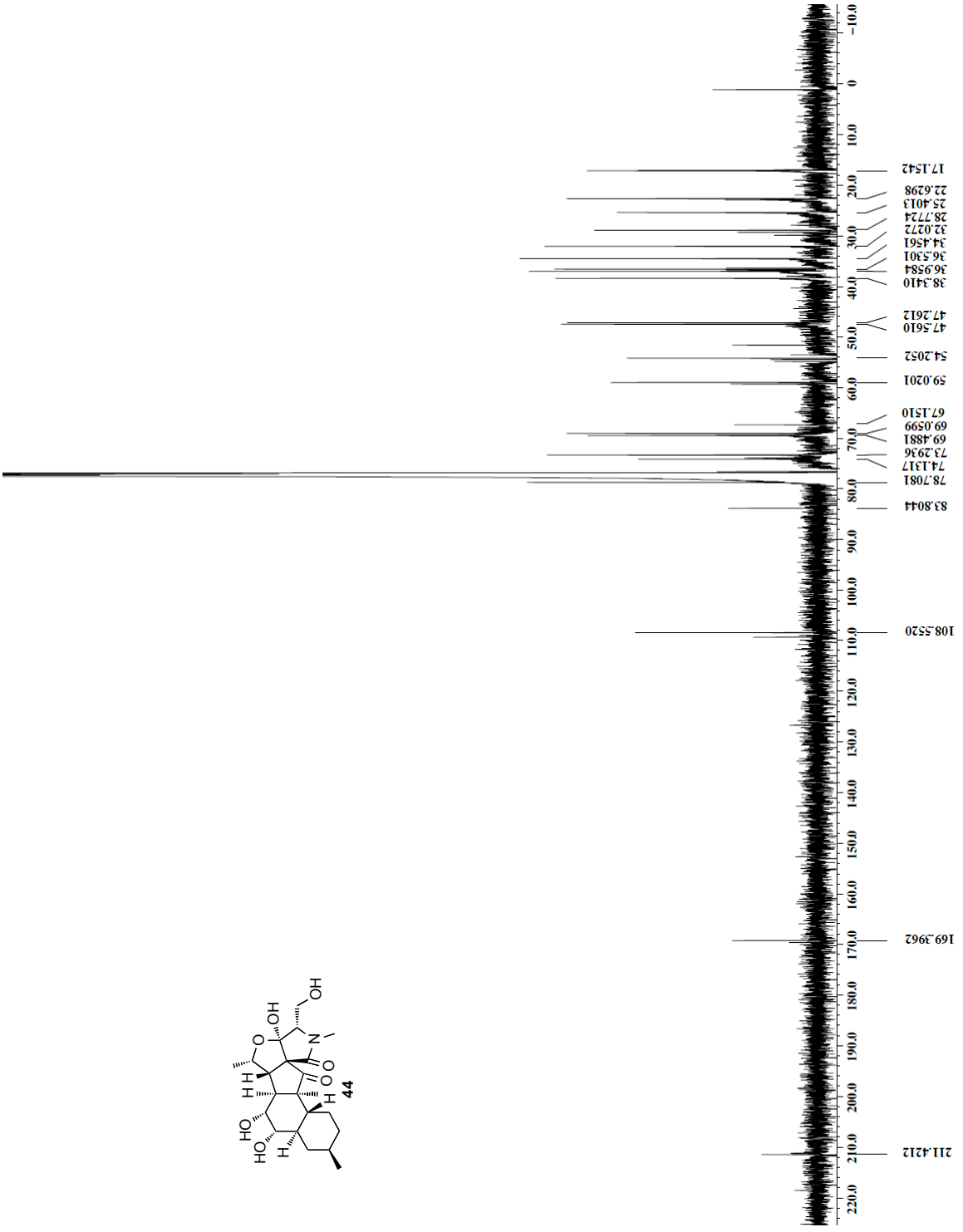
Spectrum 2.74 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **43**



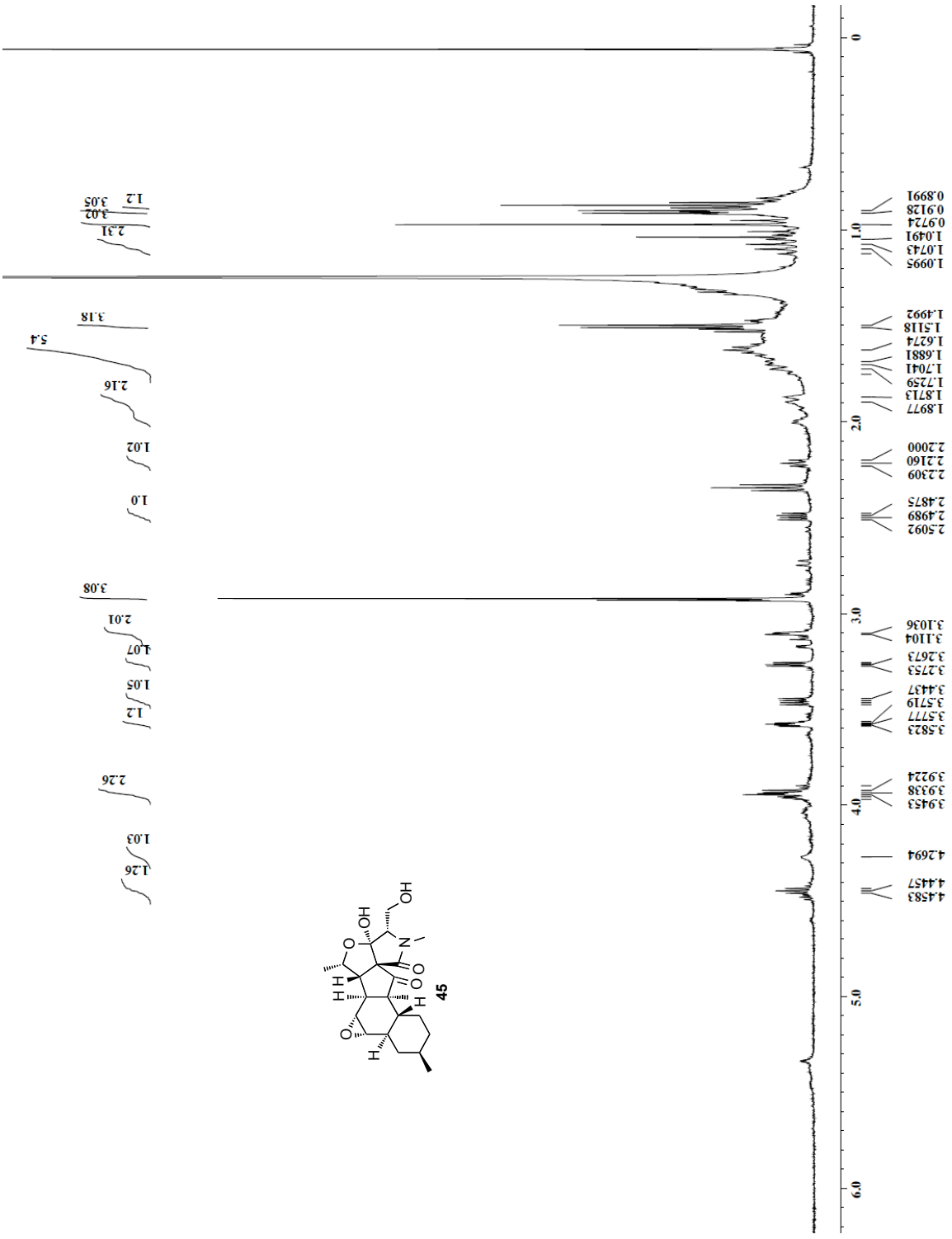
Spectrum 2.76 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **43**



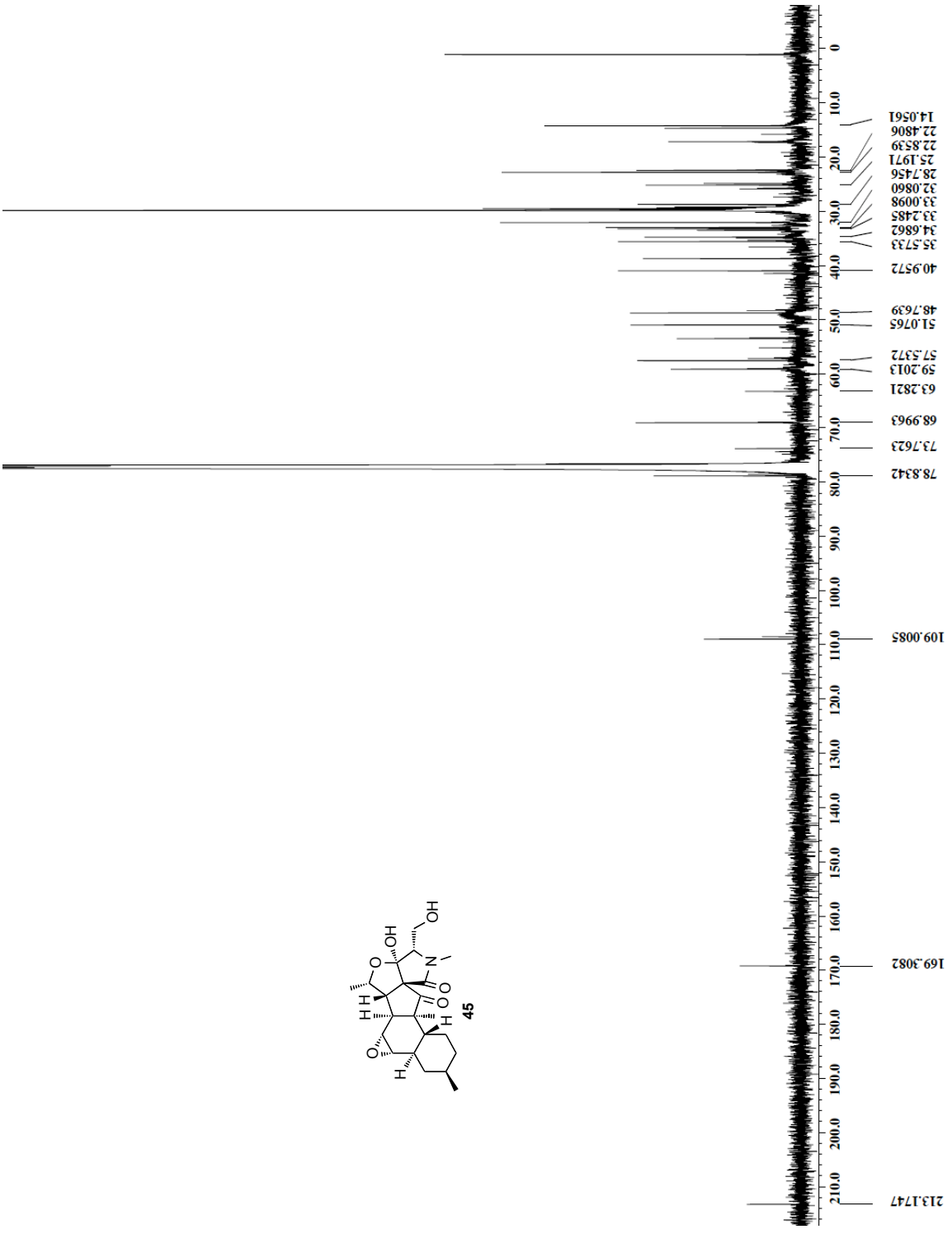
Spectrum 2.77 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **44**



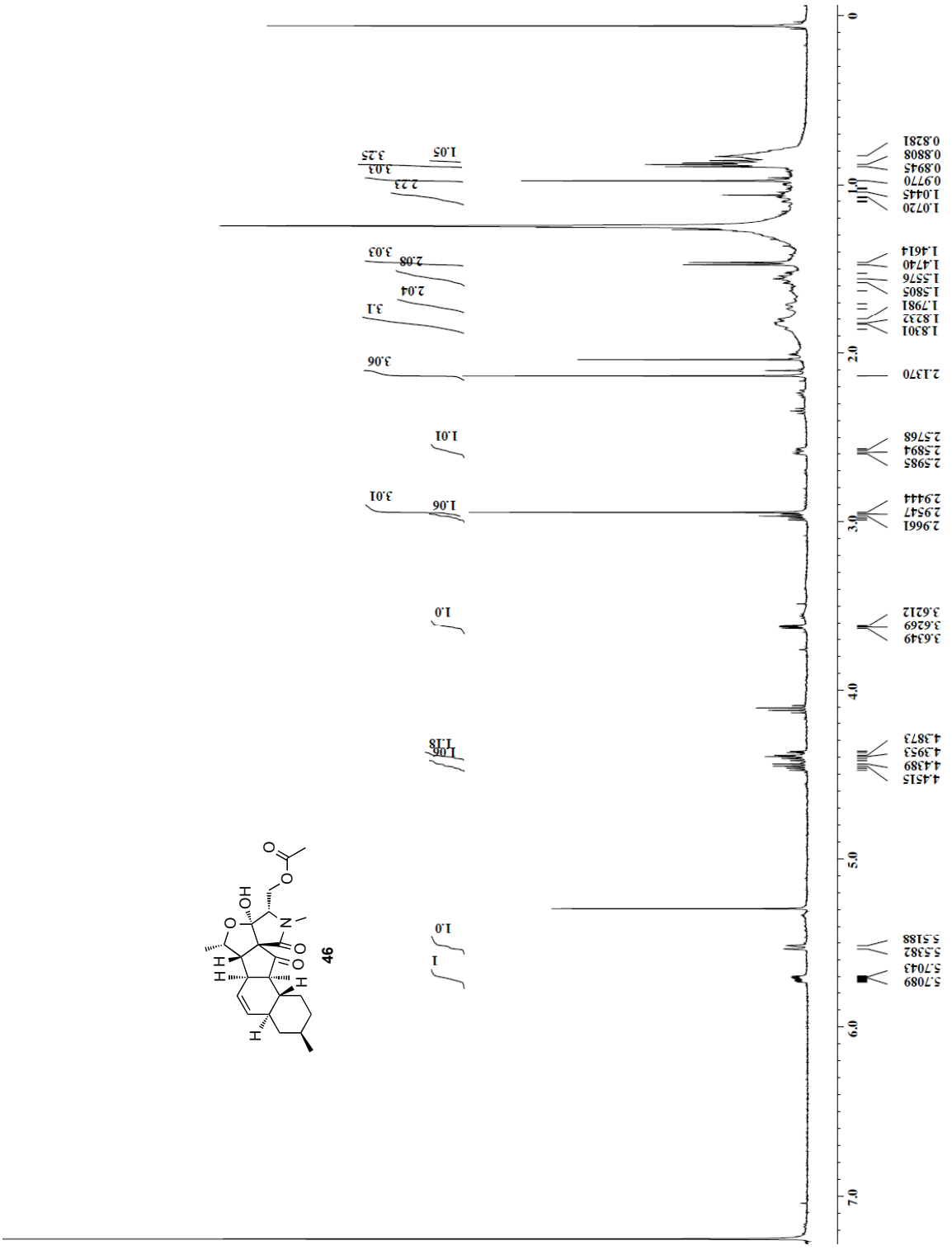
Spectrum 2.78 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **44**



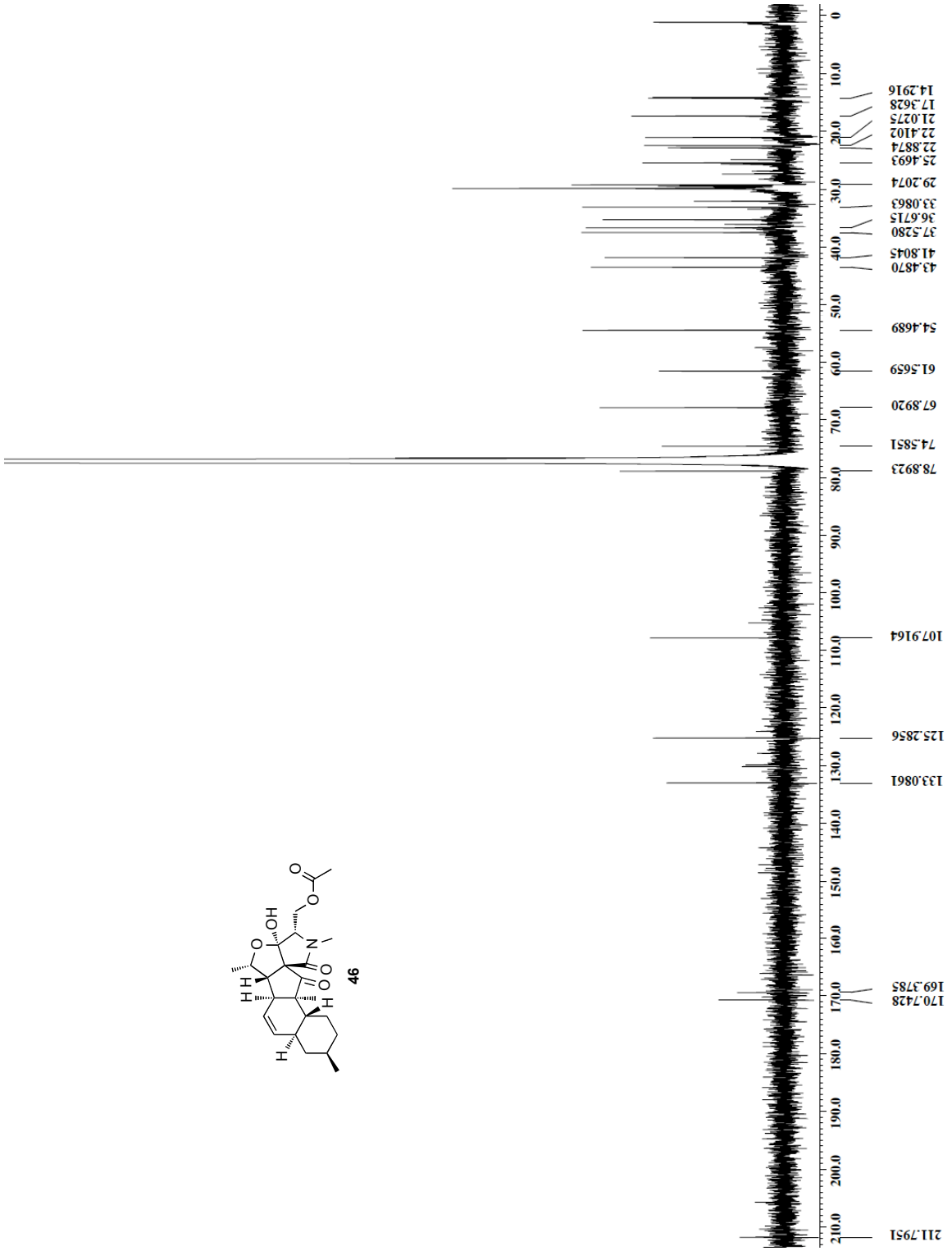
Spectrum 2.79 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **45**



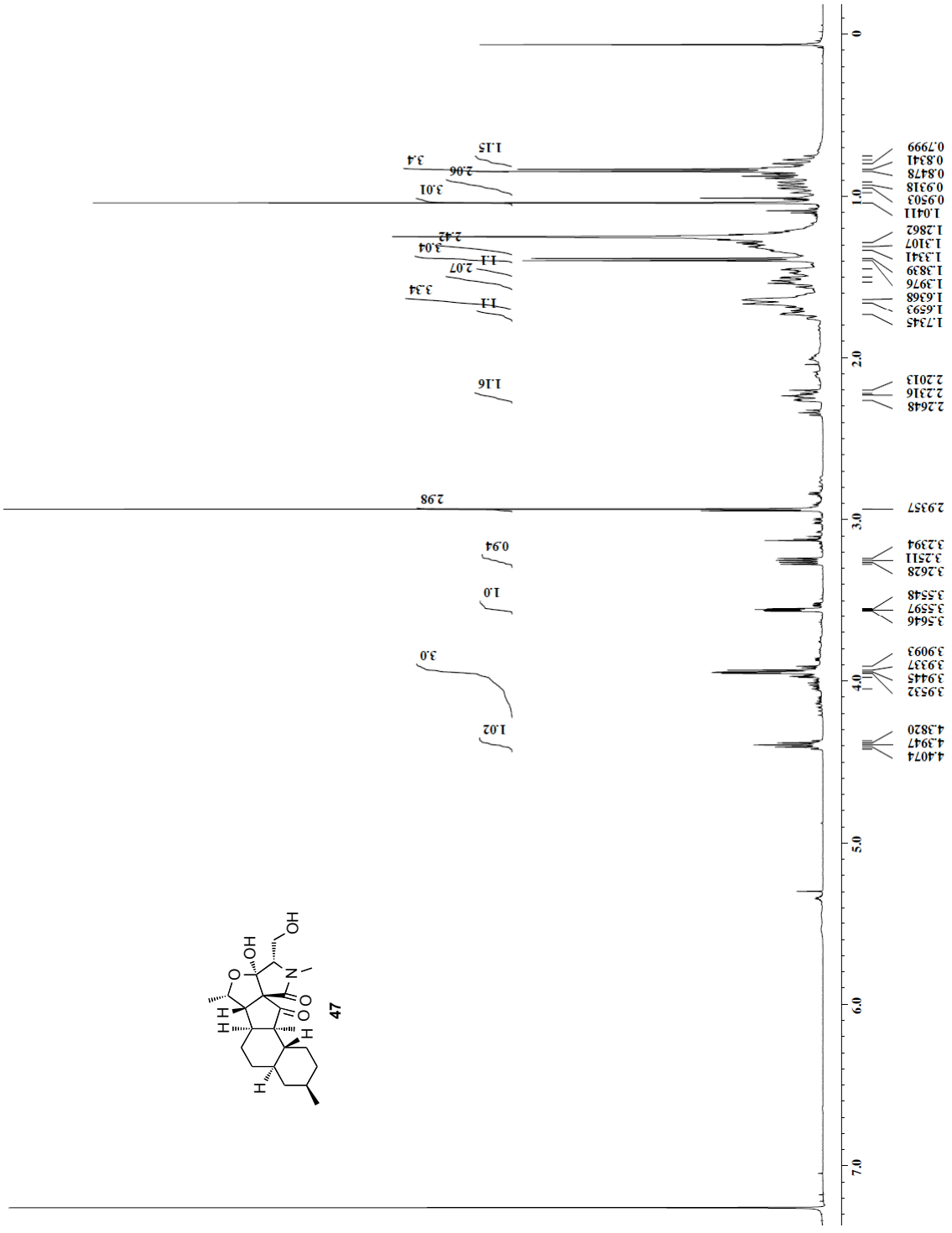
Spectrum 2.80 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **45**



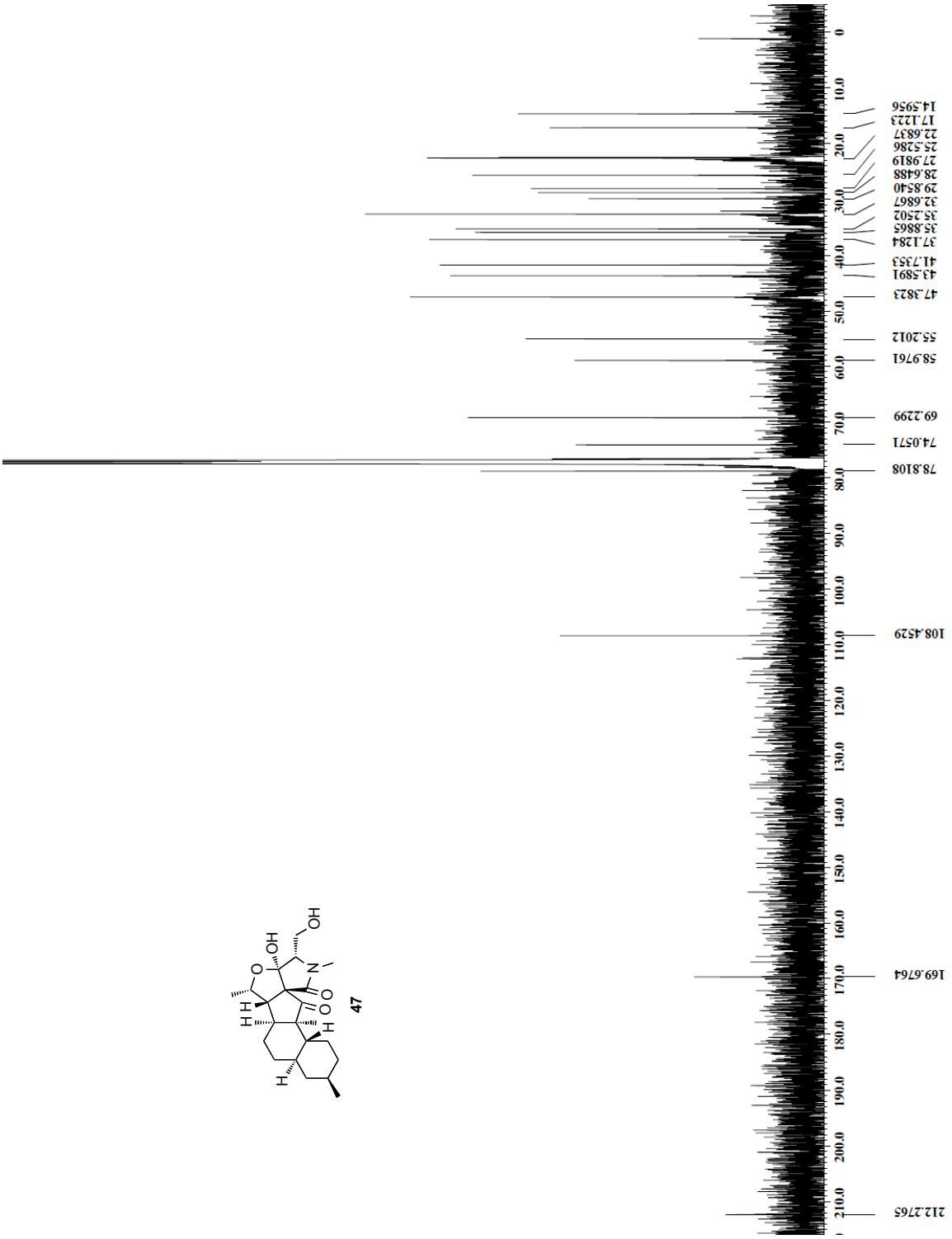
Spectrum 2.81 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **46**



Spectrum 2.82 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **46**



Spectrum 2.83 ^{1}H NMR ($CDCl_3$, 500 MHz) of compound **47**



Spectrum 2.84 ^{13}C NMR ($CDCl_3$, 100 MHz) of compound **47**

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2.10 Acknowledgements

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