Synthesis of Antitumor Marine Natural Products and Structure Revision of Niphateolide A

Thèse

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Résumé

Cette recherche concerne la synthèse de métabolites antitumoraux d'éponges marines et de leurs analogues. Elle implique notamment le développement de nouvelles méthodologies et stratégies permettant la construction rapide d'hétérocycles à cinq chaînons. De plus, ce travail démontre la valeur indiscutable de la synthèse totale pour établir la structure correcte de produits naturels qui ont été mal attribués dans la littérature.

Mes travaux ont débuté avec la première synthèse de l'alcaloïde stéroïdien plakinamine G, réalisée en 5 étapes à partir de l'ergostérol. Cette synthèse concise est exemptée des groupements protecteurs, utilisant une stratégie qui utilise le groupe azido comme amine masquée à travers une série de transformations particulièrement dures impliquant des bases fortes et de l'ozone gazeux. Elle élargit également la faisabilité de la méthode de Li pour la construction de (*Z*)-γ-alkylidène-γ-lactames dans le contexte de substrats chiraux potentiellement épimérisables. De plus, nous avons développé une nouvelle voie de synthèse des γ-alkylidène-γ-lactames à partir de 2-siloxyfuranes et d'aldéhydes via des γ-alkylidène-γ-buténolides, illustrée par la synthèse d'un analogue de pulchellactame.

Mon prochain projet concerne la synthèse totale de la structure initialement attribuée au diterpénoïde niphateolide A contenant de la γ-hydroxybuténolide. Cette synthèse était particulièrement difficile car elle impliquait l'assemblage stéréocontrôlé d'une oléfine exocyclique configurée en (*Z*) sur un site stériquement encombré du noyau cyclopentane. Nous avons pu surmonter ce défi en optimisant la structure d'un intermédiaire clé nécessaire à l'oléfination de Wittig et en modifiant de manière appropriée la voie de synthèse. En fin de compte, la synthèse a été réalisée en 16 étapes linéaires à partir de la (*R*)-pulégone. Cependant, la structure initialement rapportée du niphateolide A s'est avérée incorrecte en comparant les données RMN et la rotation optique de notre échantillon synthétique avec celles du produit naturel. Après réévaluation des données RMN rapportées et des considérations biosynthétiques, nous avons proposé une structure révisée pour le niphateolide A ainsi qu'un plan de synthèse complètement différent de celui utilisé précédemment.

La structure révisée du niphateolide A a été synthétisée en 11 étapes linéaires à partir de cyclohex-2-en-1-one disponible dans le commerce. Les étapes clés comprennent: (i) une addition de Michael asymétrique/C-acylation et (ii) un couplage Kumada sp^2-sp^3 pour installer la chaîne aliphatique requise sur le noyau cyclohexène. Heureusement, notre structure révisée affiche des données RMN et optiques en parfait accord avec celles rapportées pour le niphateolide A. Après l'achèvement de cette synthèse, un article est paru dans la littérature décrivant la même structure ‘révisée’ pour un produit naturel, appelé échinohalimane B. Les auteurs ont également noté que les spectres de l'échinohalimane B étaient ‘confusément’ en parfait accord avec ceux rapportés dans la littérature pour le niphateolide A. Notre travail établit sans ambiguïté la structure correcte du niphateolide A qui, bien sûr, est identique à celle de l'échinohalimane B.

Abstract

This research concerns the synthesis of antitumor marine sponge metabolites and their analogues. In particular, it involves the development of new methodologies and strategies enabling the expedient construction of five-membered heterocycles. Moreover, this work demonstrates the indisputable value of total synthesis for establishing the correct structure of natural products that have been misassigned in the literature.

My work began with the first synthesis of the steroidal alkaloid plakinamine G, accomplished in 5 steps from ergosterol. This concise synthesis is protecting-group-free, employing a strategy that makes use of the azido group as a masked amine through a series of particularly harsh transformations involving strong bases and ozone gas. It also expands the serviceability of Li's method for constructing (*Z*)-γ-alkylidene-γ-lactams in the context of chiral, potentially epimerizable substrates. Also, we developed a new synthetic route to γ-alkylidene-γ-lactams from 2-siloxyfurans and aldehydes *via* γ-alkylidene-γ-butenolides, illustrated by the synthesis of a pulchellalactam analogue.

My next project concerns the total synthesis of the originally assigned structure of the γ-hydroxybutenolide-containing diterpenoid niphateolide A. This synthesis was particularly challenging as it entailed the stereocontrolled assemblage of a (*Z*)-configured exocyclic olefin onto a sterically hindered site of the cyclopentane core. We were able to overcome this challenge by structural optimization of a key intermediate needed for Wittig olefination and appropriate modification of the synthetic route. Ultimately, the synthesis was achieved in 16 linear steps from (*R*)-pulegone. However, the originally reported structure of niphateolide A was proven to be incorrect by comparing the NMR data and optical rotation of our synthetic sample with those of the natural product. Upon re-evaluation of the reported NMR data, and biosynthetic considerations, we proposed a revised structure for niphateolide A along with a synthetic plan that is completely different from the one employed earlier.

The revised structure of niphateolide A was synthesized in 11 linear steps from commercially available cyclohex-2-en-1-one. Key steps include: (i) an asymmetric Michael addition/C-acylation and (ii) Kumada sp^2-sp^3 coupling to install the requisite aliphatic chain onto the cyclohexene core. Pleasingly, our revised structure displayed NMR and optical data in full agreement with those reported for niphateolide A. After completion of this synthesis, an article appeared in the literature describing the same "revised" structure for a natural product, named echinohalimane B. The authors also noted that the spectra of echinohalimane B were "confusingly" in perfect agreement with those reported in the literature for niphateolide A. Our work unambiguously establishes the correct structure of niphateolide A which, of course, is identical to that of echinohalimane B.

Table of contents

List of tables

List of figures

List of schemes

List of abbreviations

AIBN: azobisisobutyronitrile
Boc: *tert*-butyloxycarbonyl
n-Bu or Bu: *n*-butyl
t-Bu: *tert*-butyl
CD: circular dichroism
COPD: chronic obstructive pulmonary disease
COSY: correlated spectroscopy
COVID-19: coronavirus disease 2019
Cp: cyclopentadienyl
CSA: camphorsulfonic acid
DBU: 1,8-diazabicyclo(5.4.0)undec-7-ene
DCM: dichloromethane
DEAD: diethyl azodicarboxylate
DIAD: diisopropyl azodicarboxylate
DIPEA: *N*,*N*-diisopropylethylamine
4-DMAB: 4-(dimethylamino)benzaldehyde
4-DMAP: *N*,*N*-4-dimethylaminopyridine
DMAPP: dimethylallyl pyrophosphate
DMDO: dimethyldioxirane
DMP: Dess-Martin periodinane
DPPA: diphenylphosphoryl azide
DMSO: dimethyl sulfoxide
E1cB: elimination unimolecular conjugate base
EA: elemental analyses
ECD: electronic circular dichroism
ELISA: enzyme-linked immunosorbent assay
FDA: Food and Drug Administration
FGI: functional group interconversion
GC: gas chromatography
GGPP: geranylgeranyl pyrophosphate
GPP: geranyl pyrophosphate
HMBC: heteronuclear multiple bond correlation
HPLC: high-pressure liquid chromatography
IPP: isopentenyl pyrophosphate
IR: infrared
LAH: lithium aluminium hydride
LDA: lithium diisopropylamide
MB: methylene blue
MNPs: marine natural products
MsCl: methanesulfonyl chloride
MTX: maitotoxin
NaHMDS: sodium *bis*(trimethylsilyl)amide
NHS: National Health Service
NIH: National Institutes of Health

NMO: *N*-methylmorpholine *N*-oxide
NMR: nuclear magnetic resonance
NOE: nuclear overhauser effect
NPs: natural products
NSAID: non-steroidal anti-inflammatory drug
PCC: pyridinium chlorochromate
Pd(dppf)Cl_2: 1,1'-Bis(diphenylphosphino)ferrocene dichloropalladium (II)
PhNTf_2: *N*-phenyltriflimide
PPTS: pyridinium *p*-toluenesulfonate
i-Pr: isopropyl
Prep GC: preparative gas chromatography
RB: rose bengal
SAR: structure-activity relationship
SFC: supercritical fluid chromatography
S_N2: substitution nucleophilic bimolecular
TBAF: *tetra*-*n*-butylammonium fluoride
TBS: *tert*-butyldimethylsilyl
TEA: trimethylamine
Teoc: 2-(trimethylsilyl)ethoxycarbonyl
TFA: trifluoroacetic acid
THF: tetrahydrofuran
TIPSOTf: triisopropylsilyltrifluoromethanesulfonate
TLC: thin layer chromatography
TM: target molecule
TMANO: trimethylamine *N*-oxide
$TMSCHN_2$: (diazomethyl)trimethylsilane
TMSCl: trimethylsilyl chloride
TPAP: tetrapropylammonium perruthenate
p-TSA: *p*-toluenesulfonic acid
TsCl: 4-toluenesulfonyl chloride
TsOH: *p*-toluenesulfonic acid
UV: ultraviolet
VAC: vinylogous aldol condensation
VAR: vinylogous aldol reaction
VMAR: vinylogous Mukaiyama aldol reaction
WHO: World Health Organization

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Introduction

I.1 Marine Natural Products

I.1.1 Natural Products from Sea Life

Natural products (NPs), as the name suggests, originate from organisms in Nature. In the broadest sense, they contain any compounds and substances produced by living creatures. In the community of chemistry, natural products are normally defined as naturally occurring organic compounds which are produced by animals, plants or microorganisms through primary or secondary metabolism. Primary metabolites, generated in the former biological pathway, display an essential role on cellular functions including growth, development, reproduction and energy production etc. They are composed of many basic building blocks of life, for example carbohydrates, lipids, amino acids, and nucleic acids.[1] Notably, primary metabolites do not exhibit any pharmacological actions. In contrast, secondary metabolites, which are not absolutely required for survival, are produced to mediate ecological interactions. Secondary metabolites possess a broad range of biological functions involving social communication with other individuals, solubilizing and transporting nutrients, and battle against competitors, prey, and predators. In other words, secondary metabolites are produced by living organisms to enhance survivability or fecundity in a complex and challenging environment.[2]

The ocean covers around 70% of the earth's surface and harbors the largest ecosystem. Sea life consists of marine animals, plants, algae, bacteria, archaea, and fungi, evolving approximately 3 billion years prior to life on land. There were more than 240,000 marine species registered by the *World Register of Marine Species* until 2016.[3] The ocean can not only continuously provide human beings with a variety of food (e.g. sea fish, crabs and lobsters), but also serves as a rich source of natural products. So far, more than 30,000 compounds have been isolated from marine species, and thousands of new compounds were reported each year since 2008.[4] Prinsep and co-workers continue reviewing newly found marine natural products (MNPs) and their biological activities.[5] The trends in new MNPs from 2016 to 2020 are shown in **Figure 1**. Fungi and bacteria are continuously prolific sources of MNPs. Besides, marine sponges and cnidarians (e.g. corals, jellyfish,

and sea anemones) are also primary sources where over 100 new MNPs were discovered every year.

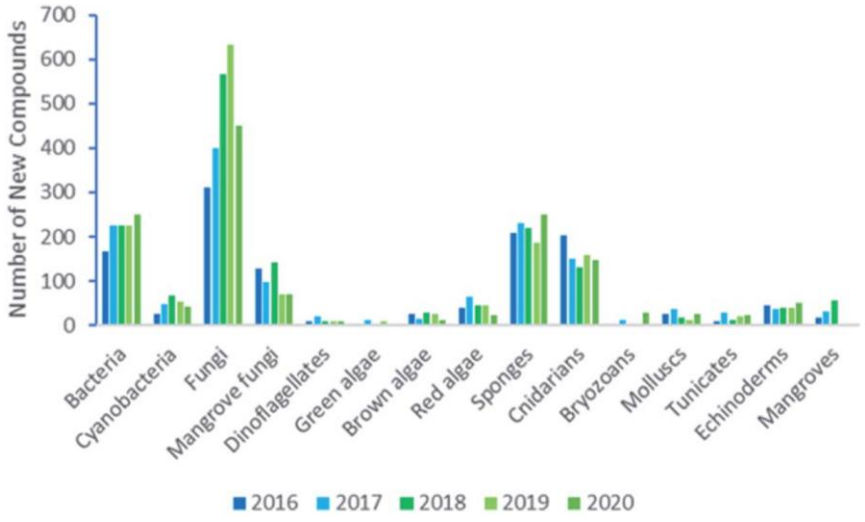


Figure 1. Trends in New Marine Natural Products[5]

The ocean provides harsh living conditions (e.g. huge ranges in temperature, pressure, light, salinity, and nutritional conditions) fundamentally different from those in terrestrial environment, urging marine life to produce specific secondary metabolites to reinforce their survivability or fecundity in the sea. Most of these metabolites possess valuable and biological properties including antitumor, antifungal, antiviral, and anti-inflammatory activities, which draw much attention from medicinal chemists and biochemists. In parallel, diversity of molecular structure, mainly composed of four groups including terpenoids, phenolics, alkaloids, and polyketides, is another hot spot of scientific research especially in the community of chemical synthesis. Artificial reproduction of these structurally complex molecules is an aspiration of many chemists, especially from those in academia.

Maitotoxin (MTX, **Figure 2**), a potent biotoxin produced by marine dinoflagellate *Gambierdiscus toxicus*,[6a] is the largest non-protein, non-polysaccharide natural molecule known thus far. This amphipathic molecule is composed of 32 ether rings, 28 hydroxyl groups, and 2 sulfuric acid esters, featuring 98 stereogenic centers and one trisubstituted

double bond. The formidable structure elucidation of maitotoxin was completed in a series of outstanding efforts by the groups of Yasumoto,[6b] Tachibana,[6c] and Kishi[6d] between 1992 and 1996. However, the stereochemistry at the J/K ring junction was questioned by Gallimore and Spencer on the basis of biosynthetic considerations.[7] To date, this controversy has not been resolved, although Nicolaou and Frederick argued about the former biosynthetic hypothesis.[8] In the absence of X-ray single crystal diffraction data, the molecular structure of maitotoxin can only be confirmed by means of total synthesis. The group of Nicolaou made important contributions to the construction of the fused ring fragments concerned.[9] Yet, the chemical synthesis of maitotoxin is still blank and also its real structure is still a mystery.

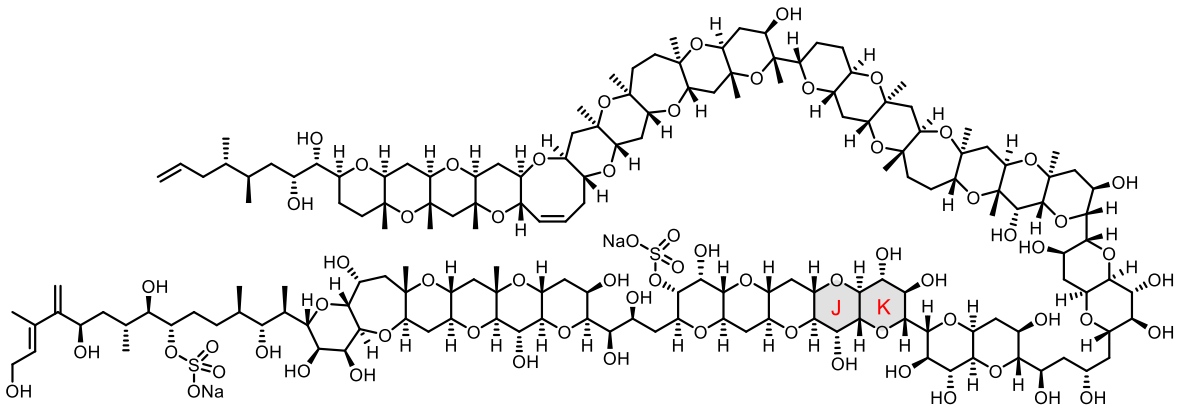


Figure 2. Molecular Structure of Maitotoxin

I.1.2 Marine Drugs

In 1967, a small symposium entitled "Drugs from the Sea" was held in Rhode Island, USA.[10] The ambitious catchphrase endured over the decades as a metaphor for drug discovery from MNPs, and an increasing number of drug exploration programs fulfilled that promise over time. The past decades have seen pronounced developments in drug discovery from marine metabolites. Three examples of marine medicines are as follows.

Ziconotide (brand name Prialt, **Figure 3**), a synthetic form of ω-conotoxin peptide derived from the toxin of the tropical marine cone snail species *Conus magus*, is the first marine-

derived drug approved by the Food and Drug Administration (FDA), notably for the treatment of chronic pain in spinal cord injury. This compound is 1000 times more powerful than morphine.[11] Plitidepsin (**Figure 3**), also known as dehydrodidemnin B, extracted from the ascidian *Aplidium albicans*, was shown to display antiviral activity against SARS-CoV-2.[12] Furthermore, in Australia, plitidepsin has been approved in combination with dexamethasone for the treatment of patients with relapsed and refractory multiple myeloma. Trabectedin (**Figure 3**), originally isolated in 1969 from the sea squirt *Ecteinascidia turbinate*, is the first marine antitumor chemotherapy medication.[13] It has been approved for usage in the treatment of advanced soft-tissue sarcoma and ovarian cancer in the European Union, the United States, Russia, and South Korea.

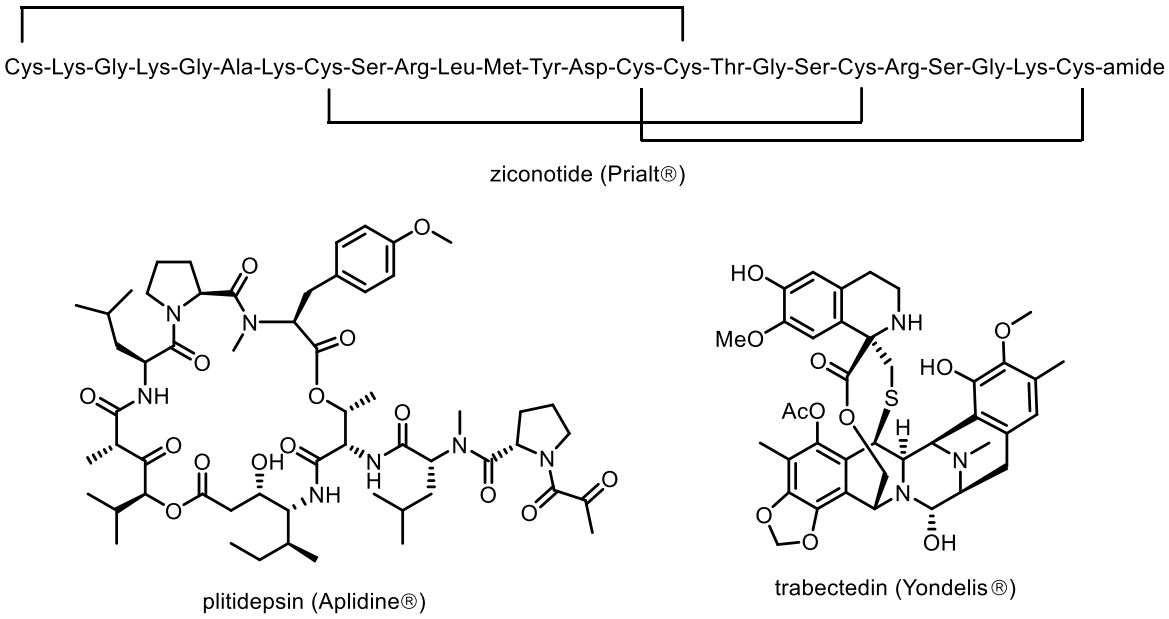


Figure 3. Examples of Marine Anticancer Drugs

In addition, a considerable number of MNP analogues have been created during the practice of chemical synthesis and structure-activity relationship (SAR) studies. Some of them display excellent pharmaceutical profiles and have been approved as drugs. For example, halichondrin B (**Figure 4**), a polyether macrolide, was originally discovered in the Japanese sponge *Halichondria okadai* by Hirata and Uemura in 1986.[14] Subsequently, a number of

marine sponges were reported to produce this macrolide, including *Axinella sp*, *Phakellia carteri*, and *Lissodendoryx sp*.[15] Halichondrin B displays exquisite activity against murine cancer cells. However, profound clinical evaluation was limited to the low concentration of the natural product in sponges (ca. ~ 1 mg/kg wet weight in *Lissodendoryx sp*.).[16] In 1992, the group of Kishi completed the first total synthesis of this structurally complex molecule, requiring approximately 90 steps.[17] In parallel, aquaculture of the sponge *Lissodendoryx sp*. was carried out by marine ecologists with government support. Yet, water temperature highly influences the survival and growth of sponges. The farmed sponges produced halichondrin B with a lower yield (~ 0.4 mg/kg) than the wild ones do. In fact, one tone of *Lissodendoryx sp*. provided only 310 mg of halichondrin B after isolation and purification.[18]

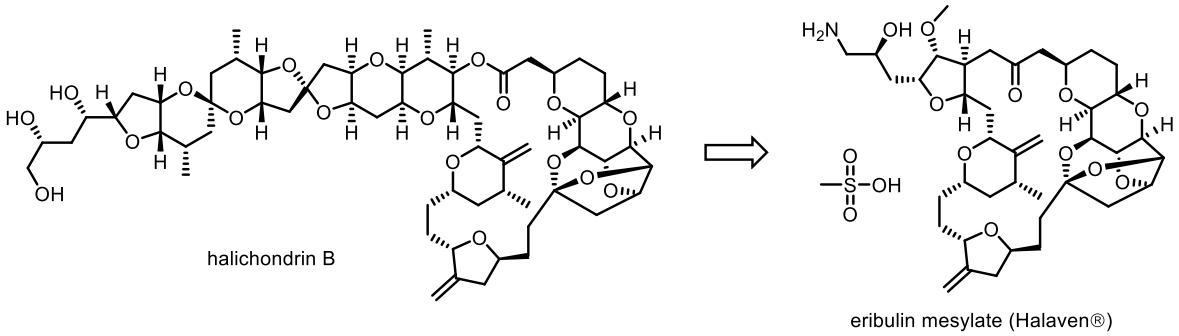


Figure 4. Structure of Halichondrin B and Eribulin Mesylate

A cheering advancement by the group of Kishi was the discovery of a halichondrin B analogue, namely eribulin mesylate (**Figure 4**), displays equipotent antitumor activity with that of the lead compound.[19] This molecule features the same macrocyclic ketone and about 70% of the molecular mass of halichondrin B. Eribulin mesylate is sold under the brand name Halaven for the treatment of breast cancer and liposarcoma.

In addition to the drugs described above, there are many other MNPs and analogues thereof that are currently undergoing preclinical or clinical trials. A number of literature reviews describe updates on the clinical advancement of marine drug candidates.[20] It is readily

apparent that the Ocean is not only a hunting ground for human food, but also a trustworthy source of medicines. However, marine pollution gradually becomes one of the international issues with the fast development of industrial civilization. Uncontrolled waste emissions (e.g. plastic and toxicants) and overfishing, threaten marine life, even destroying parts of the ecosystem. In principle, ecological protection should be implemented to keep a balance with exploitation of ocean resources.

I.2 Terpenes and Terpenoids

I.2.1 Introduction

Terpenes are a large family of naturally occurring compounds characterized by a carbon skeleton bearing single or multiple isoprene (a.k.a. 2-methylbuta-1,3-diene) units. The generic name "terpene" was initially used to label hydrocarbons discovered in turpentine. The suffix "ene" indicates the presence of olefin motifs. Although occasionally used interchangeably with terpenes, terpenoids (also known as isoprenoids) usually contain extra functional groups (e.g. oxygen and nitrogen atoms).

In 1887, the German chemist Wallach proposed the isoprene rule that declares how isoprene units are joined together.[21] Then, a Croatian-Swiss scientist, Leopold Ružička, extended this rule to terpenoids whose carbon skeleton cannot be intuitively divided into basic isoprene units, namely the *Biogenetic Isoprene Rule*.[22] In detail, isoprene which is the basic module of terpenes, can link with another one in three ways, namely head-to-head, head-to-tail, and tail-to-tail (**Figure 5**). The most common fusion is head-to-tail (1-4 link). Occasionally, the tail-to-tail coupling takes place to form unusual carbon skeletons like steroids and carotenoids. The hypothesis of head-to-head fusion has yet to be confirmed in actual natural products.

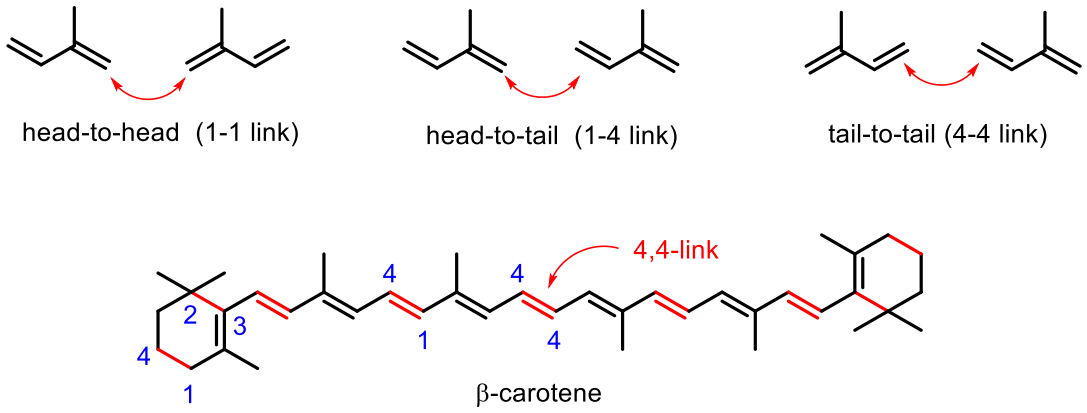


Figure 5. Links between Isoprene Molecules

Through the s-*cis* conformation of the conjugated diene group in linear C_{5n} structures, cyclization may occur. As illustrated by the symmetric molecule β-carotene shown in **Figure 5**, 2-3 links generate two cyclohexene rings. The so formed hydrocarbons may be functionalized by introducing oxygen and / or heteroatoms through chemical or biochemical process, to produce diverse terpenoids. Moreover, skeletal rearrangements or degradation reactions can occur, resulting in more complex compounds. Some of these products do not even have a simple multiple of five carbons, but are still regarded as terpenoids.

Depending on the number of isoprene units, terpenes and terpenoids can be divided into several subcategories. For example, terpenoids derived from two isoprene units (ten carbon atoms in total), are classed as monoterpenoids. **Table 1** shows all sub-divisions of the terpene and terpenoid family along with selected examples of natural products. Terpenoids, having more than eight isoprene units, are usually called polyterpenoid. Among these terpenoids, steroids are a unique branch of triterpenoids and will be discussed in a separate section.

Table 1. Classification of Terpenes and Terpenoids

Terpenes	Terpenoids	Isoprene units	Examples
isoprene	hemiterpenoids	1	prenol, DMAPP, IPP
monoterpenes	monoterpenoids	2	carvone, citral, geraniol
sesquiterpenes	sesquiterpenoids	3	farnesol, humulone
diterpenes	diterpenoids	4	vitamin A_1, paclitaxel
sesterterpenes	sesterterpenoids	5	andrastin A, manoalide
triterpenes	triterpenoids	6	limonoids, **steroids**, squalene
tetraterpenes	tetraterpenoids	8	carotenoids
polyterpenes	polyterpenoid	> 8	gutta-percha, natural rubber

I.2.2 Biologically Important Terpenoids

Terpenoids, together with the hydrocarbon terpenes, consist of approximately 80,000 compounds occupying 60% of known natural products.[23] They are produced by various natural organisms, including plants, animals and microorganisms. Being important metabolites of living organisms, terpenoids have three fundamental roles: function, defence and communication. Some biologically important terpenoids are shown in **Figure 6**.

Chlorophyll *a* is a green pigment widely existing in plant leaves. It plays a key role in the oxygenic photosynthesis through which atmospheric carbon dioxide (CO_2) is converted to glucose.[24] Warburganal, produced by plants of the genus *Warburgia*, exhibits unpleasant taste and high toxicity making plants unpalatable to insects which would otherwise eat their foliage.[25] Camphor is an allomone with a strong aroma, and protects woods from insect attack. Geraniol is found in the scent of flowers like rose, and attracts insects to achieve pollination. At the same time, the attracted insects find nectar.[26]

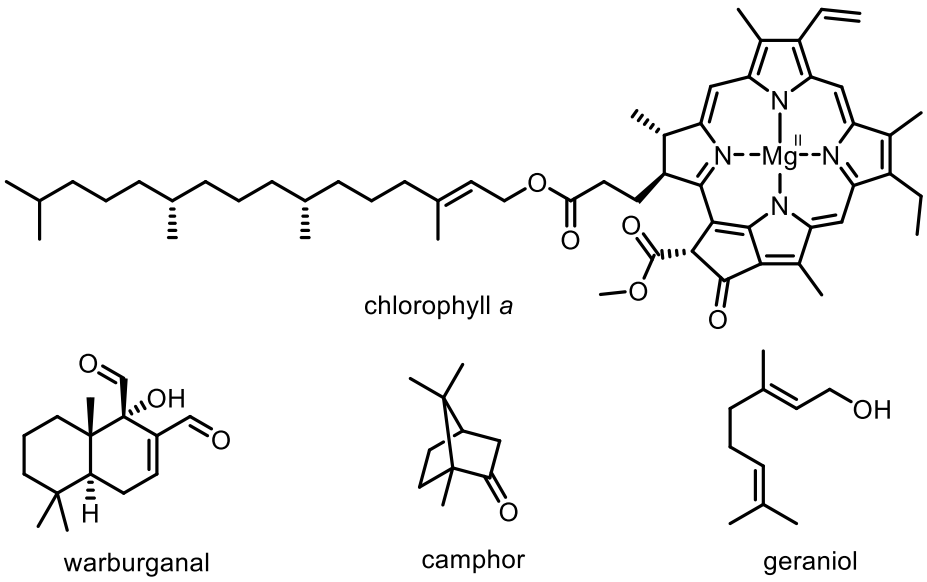


Figure 6. Examples of Biologically Functional Terpenoids

In addition, many terpenoids display potent pharmacological activity, which draw much attention from medicinal chemists. Some of terpenoids have been approved as human drugs. Two examples of terpenoid drugs (artemisinin and paclitaxel) are depicted in **Figure 7**. Artemisinin (also know as qinghaosu) is a sesquiterpene lactone discovered by the Chinese pharmaceutical chemist Youyou Tu in a traditional medicinal herb *Artemisia annua*.[27] It is strongly recommended by the World Health Organization (WHO) for the treatment of malaria due to *Plasmodium falciparum*. This powerful eastern medicine frees hundreds of millions of people from malaria-induced sickness or even death especially in developing countries. In 2015, Youyou Tu was awarded the Nobel Prize in Physiology or Medicine. Although there have been many literature reports on the total or partial synthesis of artemisinin, the plant *Artemisia annua L*. has been and still is the major source of the natural product for drug use.

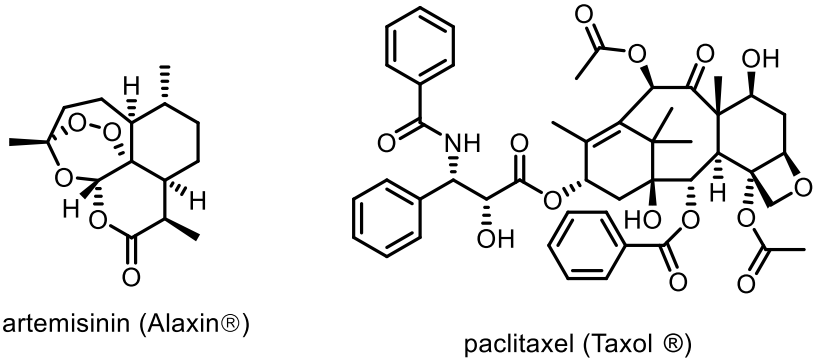


Figure 7. Examples of Terpenoid Drugs

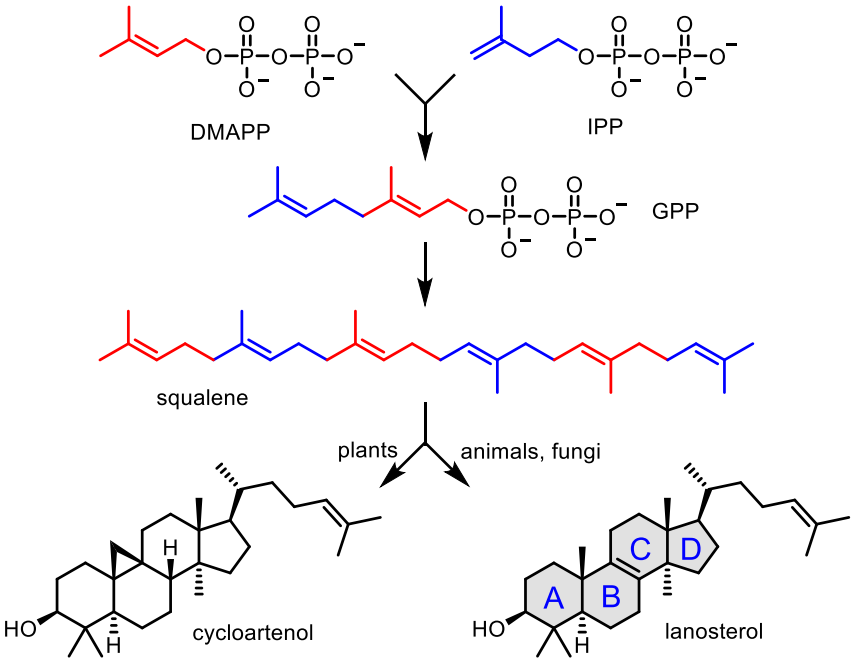
Paclitaxel (**Figure 7**), originally isolated by chemists Monroe E. Wall and Mansukh C. Wani in 1971 from the plant *Pacific yew*,[28] is a well-known anticancer drug used in the treatment of several cancers, including breast, pancreatic, ovarian, Kaposi's sarcoma and non-small-cell lung cancer.

Moreover, natural terpenes and their analogues have also been extensively used in food, agriculture, and fragrances, for example, as natural flavors, pesticides, growth regulators of crops, perfumes, and natural rubber. It can be clearly seen that terpenes are present in many aspects of human society. To some extent, these molecules also serve to illustrate the importance of chemistry to our lives.

I.2.3 Brief Introduction of Steroids

Biosynthesis of Natural Steroids

Steroids are a distinctive branch of triterpenoids, which possess a unique carbon skeleton bearing four rings A-D arranged in a specific configuration (as shown in lanosterol, **Scheme 1**). Natural steroids are found in eukaryotes, including animals, fungi and plants. These steroids own two principal biological functions: serving as crucial components of cell membranes that control the membrane fluidity, or functioning as signaling molecules.

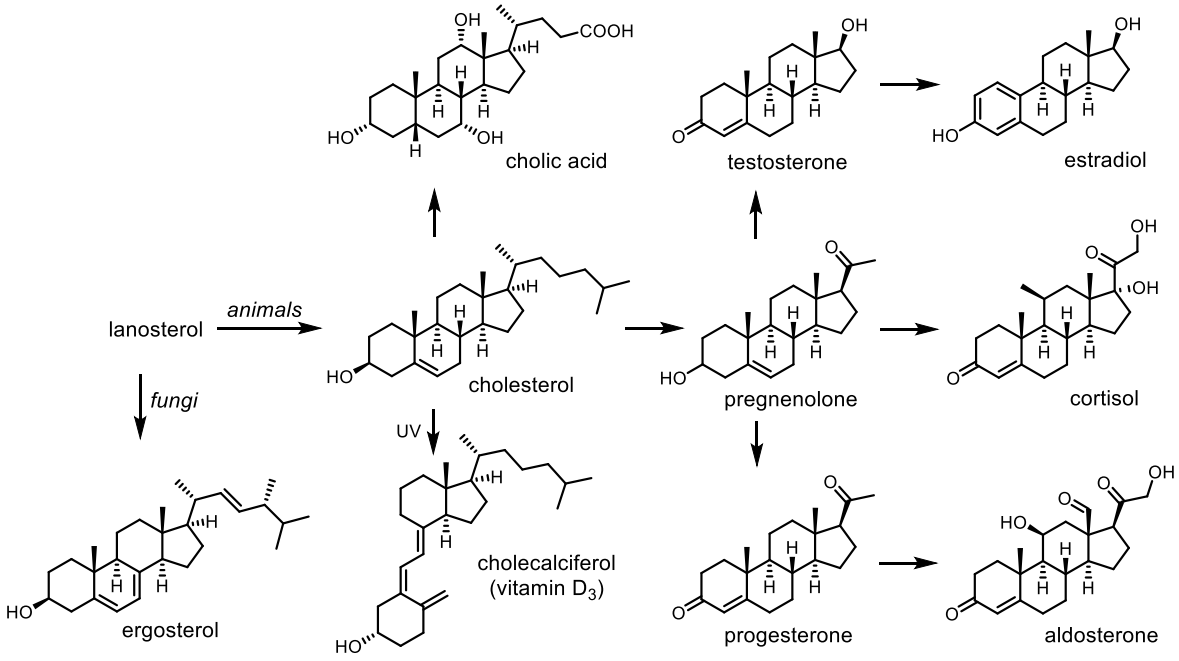


Scheme 1. Biosynthesis of Cycloartenol and Lanosterol

Steroids are a large family of terpenoids that can be obtained from living organisms or through organic synthesis. The structures of steroids vary by the chains linked to the four-ring core and / or the oxidation state of the rings, which strongly impact their biological activities. All animal and fungal steroids are derived from lanosterol that is generated from two hemiterpenoids dimethylallyl pyrophosphate (DMAPP) and isomer isopentenyl pyrophosphate (IPP) *via* biosynthetic pathway (**Scheme 1**). Then, a tail-to-tail coupling (4-4 link) occur between two geranyl pyrophosphates (GPP), to yield a triterpene namely squalene. Squalene is also a biochemical precursor to cycloartenol from which most plant steroids are derived.[29]

Hereafter, lanosterol is converted to cholesterol and ergosterol in animals and fungi, respectively (**Scheme 2**). Cholesterol was first identified in solid form in gallstone by François Poulletier de la Salle in 1769.[30] It is not only an essential component of animal cell membranes, but also serves as the crucial material for the biosynthesis of cholic acid, vitamin D_3, and steroid hormones (**Scheme 2**). Steroid hormones play an important role in

growth, development, sexual differentiation and reproduction of human beings. Cholic acid is one of the two major bile acids produced by animal livers; they act as emulsifiers to promote absorption of lipids.[31] Vitamin D_3 is biologically generated from cholesterol in the skin following UVB light exposure. Its main function is to increase calcium uptake by the intestines.[32]



Scheme 2. Pathways of Steroids Biosynthesis in Animals and Fungi

Steroid hormones mainly include pregnenolone, cortisol, progesterone, aldosterone, testosterone, and estradiol (**Scheme 2**). Among them, progesterone, testosterone, and estradiol are sex hormones. Besides, pregnenolone is found in high concentration in brain, and proved to improve cognitive and memory functions.[33] Cortisol (**Scheme 2**) has several biological functions, notably, it helps the body to regulate stress, counterbalance the effect of insulin to increase blood sugar, reduces inflammation, and helps control how the body uses fats, proteins and carbohydrates for energy.[34] Aldosterone (**Scheme 2**) plays a crucial role in the homeostatic regulation of blood pressure.[35]

Ergosterol (**Scheme 2**), a sterol discovered in cell membranes of fungi and protozoa, has similar biological functions in animal cells to those of that cholesterol in humans.[36] For instance, when exposed to ultraviolet (UV) light, ergosterol can be transformed to vitamin D_2.[37] Because ergosterol is absent in cell membranes of human beings, it is a significant target for the discovery of new antifungal and antiprotozoal drugs. In the community of organic synthesis, ergosterol is a universal starting material in many semi-syntheses of natural steroids.[38]

Steroid Medicines

Steroids exhibit multiple and significant biological functions in human beings, animals, fungi and plants, which have drawn extensive attention from scientists. Over the past decades, pharmaceutical chemists spared no effort to discover new drugs from natural steroids and their derivatives. There are 100s of approved drugs that are natural or synthetic steroids. Examples include: 1. **Anticancer drugs**: abiraterone acetate (Zytiga®), finasteride (Proscar®) and exemestane (Aromasin®); 2. **Hormones** (birth control and sex hormones): levonorgestrel (Plan B One-Step®), etonogestrel (Nexplanon®), estradiol (Estrace®), and testosterone (Androderm®); 3. **Anti-inflammatory** (Asthma, COPD, Crohn's disease etc): cortisone (Cortone Acetate®), mometasone (Nasonex®), dexamethasone (Decadron®), and budesonide (Pulmicort®); 4. **Cardiovadcular diseases**: digoxin (Lanoxin®); 5. **Antibiotics**: fusidic acid (Fucidin®); 6. **Antidepressants**: brexanolone (Zulresso®).

It is worth noting that the anti-inflammatory steroid dexamethasone (**Figure 8**) is the first drug shown to prevent deaths from the notorious COVID-19 (coronavirus disease 2019).[39] So far, there have been more than 760 million people hospitalized, and nearly 6.9 million people died from COVID-19 according to the World Health Organization. Fortunately, dexamethasone was proven in a large clinical trial, to cut deaths by one-third among critically ill COVID-19 patients.[39] Dexamethasone was recommended by the National Health Service (NHS) in 2020 in the UK and the National Institutes of Health (NIH) in the US for infected patients who need either mechanical ventilation or supplemental oxygen

(without ventilation). Notably, this commonly used steroid drug is inexpensive and available by prescription in most pharmacies.

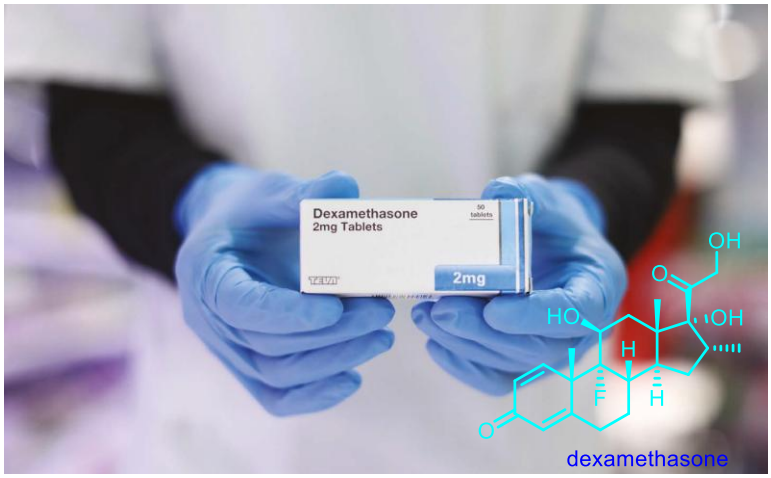


Figure 8. Dexamethasone

Table 2. Nobel Prizes Awarded for Steroid Research

Years	Scientists	Research
1927	Heinrich Otto Wieland	Constitution of bile acids and sterols and their connection to vitamins
1928	Adolf Otto Reinhold Windaus	Constitution of sterols and their connection with the vitamins
1939	Adolf Butenandt; Leopold Ružička	Studies of steroid sex hormones, and polymethylenes and higher terpenes
1950	Edward Calvin Kendall; Tadeus Reichstein; Philip Hench	Structure and biological effects of adrenal hormones
1965	Robert Burns Woodward	Synthesis of cholesterol, cortisone, and lanosterol
1969	Derek Barton; Odd Hassel	Concept of conformation in chemistry, emphasizing the steroid nucleus
1975	Vladimir Prelog	Stereochemistry of organic molecules and reactions

Owing to the importance of steroids in organic chemistry and medicine, seven Nobel Prizes have been awarded over the years for major breakthroughs in steroid research (**Table 2**). Currently, a large number of chemists and biologists focus their efforts on the biological properties, mechanism of action and synthesis of newly isolated steroid NPs, and the discovery of new steroid drugs. It is anticipated that such research will continue to have a strong impact on human health.

I.3 Isolation and Structure Elucidation of Natural Products

I.3.1 Isolation of Natural Products

Natural products have steady and ever-growing effect on human and veterinary medicine, food, agricultural, and cosmetic industries. A large number of NPs have been exhaustively researched, and exploited to improve human life. Normally, the first step is to obtain pure compound, followed by structure elucidation. However, NPs usually occur in low concentration in natural organisms, requiring much effort to obtain sufficient material for characterization and biological screening. To some extent, the development of extraction and isolation technologies exerts a profound influence on drug discovery. Also, improper isolation methods will cause loss or decomposition of new and / or desired compounds.

Extraction is the initial and coarse separation of bioactive ingredients from the raw materials, which can be broadly divided into four types: solvent extraction, distillation, pressing and sublimation. In more details, extraction methods include maceration, percolation, decoction, reflux extraction, soxhlet extraction, and pressurized liquid extraction. Besides, some modern, effective and selective extraction technologies springs out, such as, supercritical fluid extraction, ultrasound assisted extraction, microwave assisted extraction, pulsed electric field extraction, enzyme assisted extraction, hydro distillation, and steam distillation.[40c] There are many reviews describing the development of existing extraction and separation methods.[40] Actually, in order to select a proper extraction method, what should be mainly taken into consideration are solubility and temperature. Use of the appropriate solvent will decrease the volume needed affording better selectivity. Furthermore, higher temperatures can generally increase the solubility of the matrix.

However, the potential thermal decomposition of the active ingredient(s) should be taken into account.

Usually, extraction by these methods leads to a variety of natural products. For the purpose of collecting the desired bioactive fraction, further separation and purification are normally needed. Moreover, the criteria of purity are extremely important for the successful characterization of the molecule(s) concerned. Chromatography, especially column chromatography, is a traditional and convenient separation method that has been widely used to purify natural or synthetic products in both university and industry laboratories. In addition, many modern separation techniques have been developed during scientific research, and applied in some case to improve separation efficiency. These include preparative gas chromatography (Prep-GC), supercritical fluid chromatography (SFC), molecular imprinted technology, and simulated moving bed chromatography.[40d]

I.3.2 Structure Elucidation of Natural Products

With the pure natural product in hand, determination of its molecular structure is crucial and sometimes challenging, setting the stages for chemical synthesis, exploration of new synthetic analogues, and SAR studies. The exact structure elucidation process depends on the physical, chromatographic, analytical, and spectroscopic data. Physical data, such as melting (boiling) point (range) and optical rotation, can reveal the level of compound purity. Chromatographic data can also be used to assess purity, e.g. a single peak vs, multiple peaks in high-pressure liquid chromatography (HPLC) or gas chromatography (GC). On the other hand, elemental analyses (EA) together with the molecular mass allow deduction of the empirical molecular formula, by which the degree of unsaturation could be calculated.

Currently, X-ray diffraction is the most powerful method to determine molecular structure. However, this method requires the compound to be crystalline and many natural products are oils or gums. Sometimes, making a derivative of a non-crystalline NP by simple chemical transformations can give a crystalline derivative, whose X-ray analysis will indirectly provide the molecular structure of the starting NP. In practice, however, this is

rarely possible, especially when only a few mg(s) of the NP are available. Indeed, the majority of NPs are characterized solely on the basis of their spectral data (e.g. NMR, IR, MS and UV). Among them, NMR (nuclear magnetic resonance) spectroscopy is the most powerful and convenient tool for structural assignment. NMR data include 1D-NMR (e.g. ^{1}H, ^{13}C and ^{19}F NMR) and 2D-NMR (e.g. 2D COSY, NOE and HMBC). Not only can NMR data reveal the functional groups and atoms (e.g. F and P), but can also help deduce the carbon skeleton and relative stereochemistry. Infrared (IR) spectroscopy is a simple and comparatively inexpensive technique enabling chemists to identify possible functional groups present in the molecule, for example, a carbonyl (C=O, 1680-1750 cm^{-1}) or an alcohol (-OH, 3550-3200 cm^{-1}). Recently, circular dichroism (CD) spectroscopy was developed to deduce the absolute configuration of certain chiral molecules by comparison with those of appropriate model compounds and DFT calculations. The advent of CD spectroscopy has allowed the assignment of absolute stereochemistry to non-solid, optically active chiral molecules, which previously depended on chemical synthesis or X-ray diffraction analysis.[41]

Artemisinin is an example to demonstrate the isolation and structure elucidation of a new natural product.[27] Tu Youyou firstly isolated pure artemisinin from the plant *Artemisia annua*, inspired by an ancient Chinese medical book *The Handbook of Prescriptions for Emergency Treatments*. The raw material was subjected to extraction with petroleum ether or diethyl ether. The volatile solvent lowered the required temperature for evaporation, which preserved artemisinin from thermal cleavage of the weak peroxide bond. The resulting residue was purified by silica gel column chromatography, and artemisinin crystallized as colorless needles.

Then, its molecular formula was deduced as $C_{15}H_{22}O_5$ by analyzing the mass spectral data. The δ-lactone and peroxide units were confirmed by IR by the peaks at 1745, 1115, 881, and 831 cm^{-1}. The ^{1}H and ^{13}C NMR data showed the presence of three methyl-, four methylene-, five methanetriyl-, and two quaternary carbons together with a carbonyl unit. The gross structure and relative configuration of artemisinin were finally confirmed by means of X-ray diffraction.

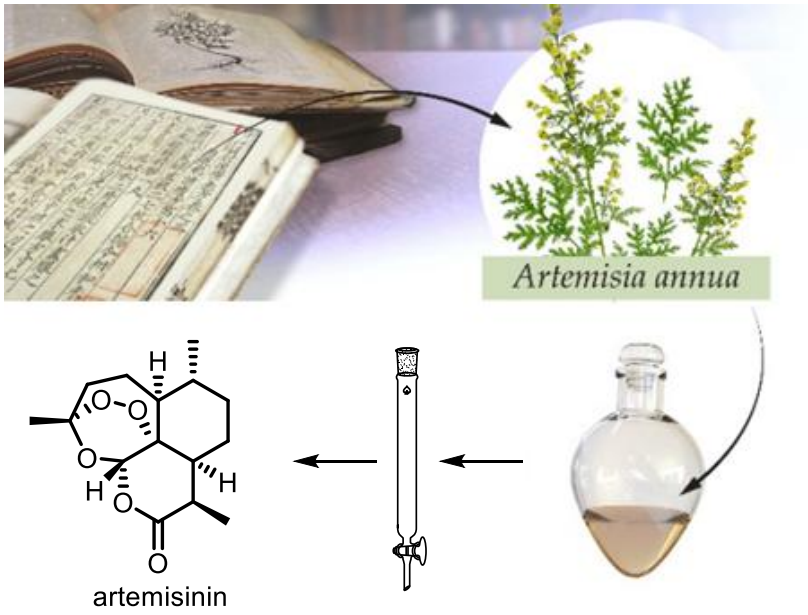


Figure 9. Discovery of Artemisinin

I.4 Natural Product Synthesis

Normally, the amount of bioactive molecules in natural organisms is extremely low, which hampers biological investigations and drug hunting. Not only can the practice of total synthesis reproduce the desired natural product in substantial amounts from easily available chemicals, but can also generate a range of new analogues that may have superior pharmacological profiles than the natural product itself. Indeed, there are countless analogues of NPs synthesized over the past decades, and some of them have been proven to be the candidates of choice for downstream development as drugs. Total synthesis endeavors involve the development of new synthetic methods and strategies, and as will be discussed below, even the discovery of the actual structure of many misassigned natural products. From a wider and more profound perspective, the practice of total synthesis challenges the very frontiers of organic chemistry.

I.4.1 Strategies in Total Synthesis

New synthetic strategies are often created to solve specific problems associated with the assembly of a single NP. Some of them gradually evolve into general approaches to a class of natural products and their analogues. Described here are three key aspects of total synthesis endeavors, namely, retrosynthetic analysis, divergent synthesis and reaction cascades.

Retrosynthetic Analysis: Retrosynthetic analysis is a powerful tool widely used in both chemical and biological synthesis. It is a common and often indispensable process during the design phase, especially for molecules featuring unique and / or complex structures. Simply put, retrosynthetic analysis involves dividing the target molecule into straightforward fragments. It can be viewed as the reverse process of a famous game Lego®. This reverse cogitation was intuitive during the history of organic synthesis. However, during the late 1980s, the American organic chemist E. J. Corey proposed a systematic approach which he coined 'retrosynthesis'. In 1990, Corey won the Nobel Prize in Chemistry for the development of the theory and methodology of organic synthesis, particularly retrosynthetic analysis.

Specifically, retrosynthetic analysis is implemented by converting a target molecule or intermediate concerned, into a structurally simpler and practically available precursor (synthon) based on a combination of established theories and logic programing. During this analytical stage, the organic chemist can also anticipate and / or control the region and stereoselectivity, if applicable. Moreover, attributes such as step-economy, environmental friendliness, and cost, could be taken into account in advance as well. As an example, Nicolaou's retrosynthetic analysis of paclitaxel is shown in **Figure 10**.[42] This diterpenoid, featuring a tetracyclic 17-atom skeleton and 11 stereocenters, was divided into five fairly simple building blocks.

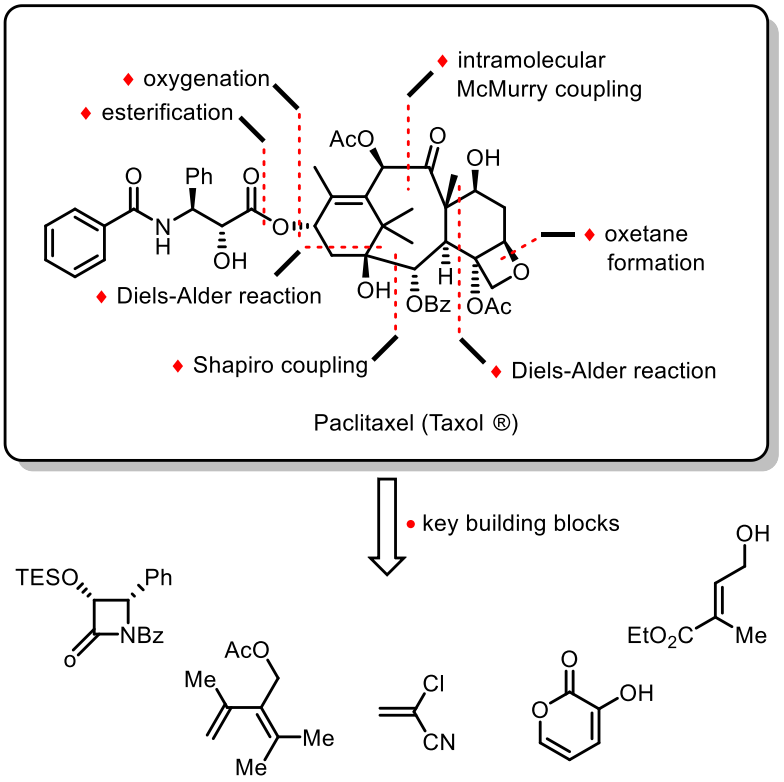
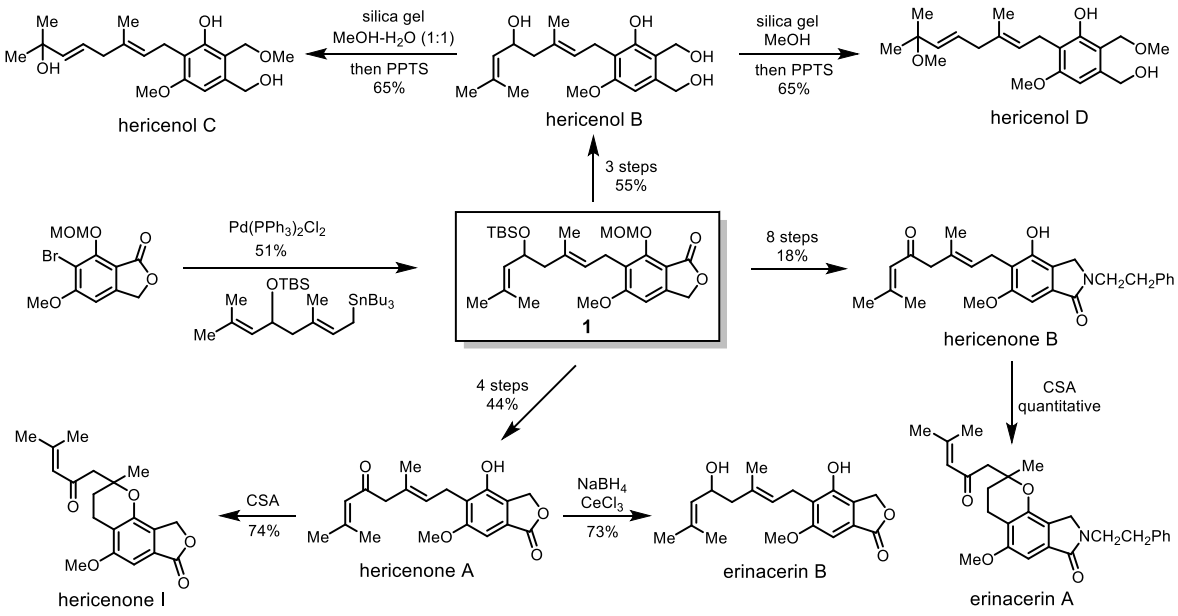


Figure 10. Nicolaou's Retrosynthetic Analysis of Paclitaxel[42b]

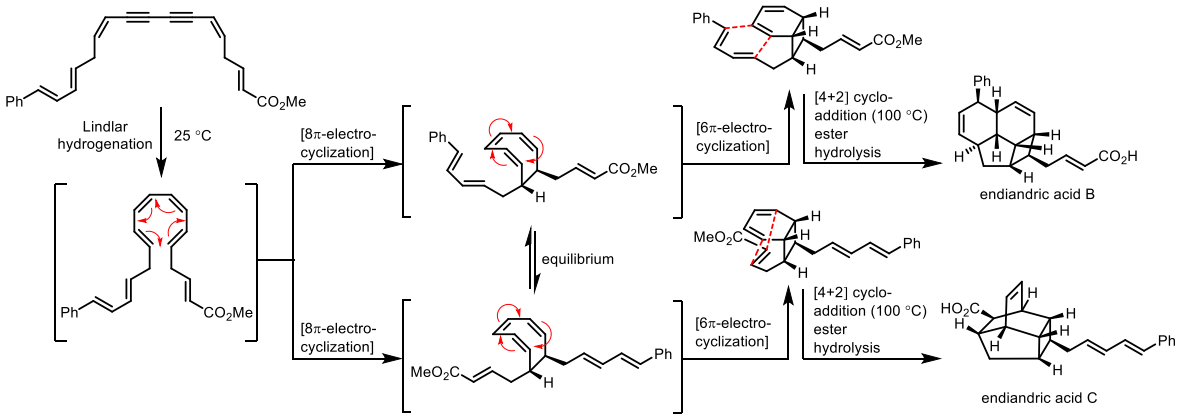
Divergent Synthesis: Originally defined by Boger in 1984, divergent synthesis is a synthesis of at least two compounds possessing a similar skeleton, from a common intermediate.[43] The shared scaffold can be a precursor of multi target molecules (TMs), or an intermediate which can be converted to TMs through separate functional group interconversions (FGIs). Compared with convergent and linear syntheses, divergent synthesis aims to improve accessibility of a range of natural products and their analogues. In recent years, this strategy has attracted growing attention by the synthetic and medicinal chemistry communities. For example, Kobayashi and co-workers accomplished the divergent total synthesis of eight natural products, namely, hericenones A, B, and I, hericenols B-D, and erinacerins A-B from common intermediate **1** (**Scheme 3**).[44]



Scheme 3. Kobayashi's Divergent Synthesis of Eight Natural Products[44b]

Cascade Reactions (**or Reaction Cascades**): Cascade reactions, also known as domino reactions or tandem reactions, allow two or more bond-forming transformations to be performed under the same conditions sequentially without the need for work-up and product isolation between successive synthesis steps. Each subsequent reaction takes place by virtue of the chemical functionality generated in the previous reaction. As such, cascade reactions achieve maximum increase in target-relevant complexity while minimizing step count, cost and chemical waste. Like one-pot procedures, cascade reactions also render the synthesis substantially "greener" as they reduce the amount of solvents used in the synthesis, work-up and product isolation, and contaminated aqueous waste generated from cleaning equipment and glassware. The first reaction cascade was described by Robinson in 1917 during his remarkable, even by today's standards, synthesis of tropinone.[45] In recent years, the group of K. C. Nicolaou have made seminal contributions on the implementation of cascade reactions in total synthesis, and have published reviews on this topic.[42b,42c] A prominent example from Nicolaou's group is the reaction cascade employed in the synthesis of endiandric acid B and C, involving sequential Lindlar hydrogenation, 8π-

electrocyclization, 6π-electro-cyclization, [4+2] cycloaddition (intramolecular Diels-Alder reaction), and ester hydrolysis (**Scheme 4**).[46]



Scheme 4. Nicolaou's Reaction Cascades to Endiandric Acids B and C[42b]

I.4.2 Total Synthesis and Structure Elucidation/Revision

The concept of molecular structure was proposed by August Kekulé and other scientists in 1857.[47] During the early period of assigning molecular structure to organic molecules (1860-1930), scientists had to spend years of effort to deduce a compound structure due to lack of capable analytical techniques, which is a dark period also called the "Stone Age" structure elucidation. At that time, structure determination mainly relied on synthetic chemistry involving degradation, derivatization, and the characteristic reactivity of different functional groups. As the well-known German chemist Reinhard W. Hoffmann wrote in his glamorous book *Classical Methods in Structure Elucidation of Natural Products*, 'this approach was compared to an attempt to learn something about a Chinese porcelain figurine in a dark room by knocking it to pieces, collecting them and to examine them later by light'.[48]

Fortunately, the advent of spectroscopy, especially NMR, and impressively powerful instrumentation has greatly reduced chemists' burden in the modern era. Unfortunately, however, heavy reliance on software simulations and / or DFT calculations has led to

numerous misassigned natural product structures, both simple and complex, continually being reported in the literature.[49] As a consequence, the traditional role of total synthesis as the ultimate proof of structure remains as valid and important as ever. In particular, it can be used to confirm, disprove and / or revise a wrongly assigned structure, and even predict potentially new NP structures which have yet to be discovered in Nature. Many publications describe the structure revision of natural products by means of total synthesis.[49]

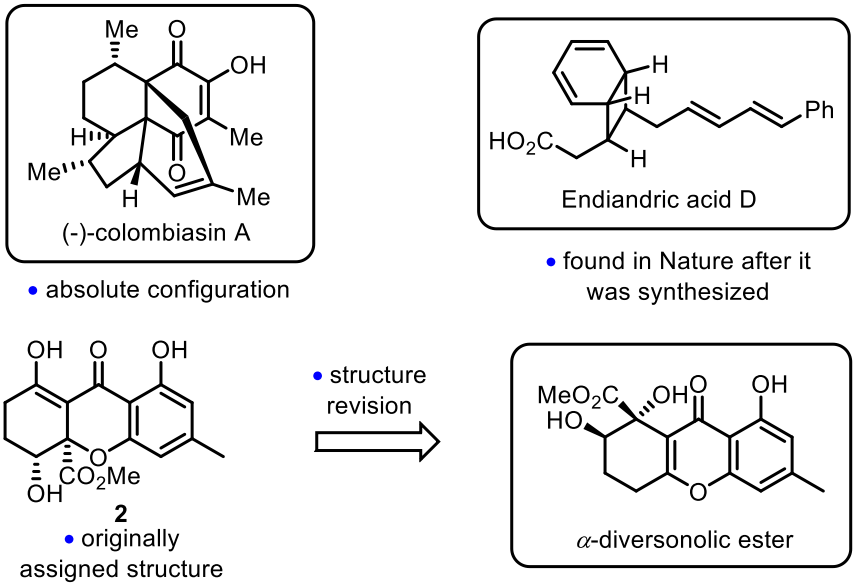


Figure 11. Examples of Structure Revisions and Predictions

Some examples of structure revision of natural products are depicted in **Figure 11**. The carbon skeleton of colombiasin A was determined by means of elegant spectroscopic studies.[50] Yet, its absolute stereochemistry was uncovered a year later by total synthesis.[51] Endiandric acid D was initially predicted as a natural product in 1980,[52a] and was synthesized along with its known family members (endiandric acids A, B, and C) by Nicolaou and co-workers in 1982.[52b] Also in that year, Black's group isolated endiandric acid D from leaves of the Australian plant *Endiandra introrsa* (*Lauraceae*).[52c] In 2008, the structure of a *Penicillium diversum* metabolite α-diversonolic ester, wrongly assigned as

molecule **2**[53b], was revised by the group of Nicolaou.[53a] The group of Boukouvalas has also made several related contributions, which will be discussed in **Chapter 3** and **4**.

I.5 Objectives of the Thesis Research

The prime objective of our research is to develop new methods and strategies enabling rapid and efficient access to natural products of biological importance. Natural products have been refined over millions of years for biological interactions, so they represent an unsurpassed source of life-saving drugs and drug leads. However, their limited availability from the natural source can severely hamper biological assessment. This is often the case with compounds isolated from marine organisms, where recollection is often problematic or impossible. In such cases, chemical synthesis is the only viable option for supplying the material needed for pharmacological investigation. A practical and flexible synthesis can also serve as a blueprint for generating custom-designed analogues of superior therapeutic performance. Importantly, target-oriented synthesis provides testing ground for existing or emerging methods, and most importantly, a powerful driving force for invention of new reactions and strategies. For a synthetic route to be viable by our group's standards, maximum brevity and overall efficiency are of paramount importance, as is virtually complete regio and stereocontrol. The chemistry should also be green and scalable!

My doctoral research focused on the synthesis of two structurally novel, bioactive marine sponge metabolites: (i) plakinamine G (**3**), an antitumor steroidal alkaloid possessing a rare (*Z*)-configured γ-alkylidene-γ-lactam motif, and (ii) niphateolide A, whose originally proposed structure **6** was proven by our synthesis to be incorrect and was ultimately revised to **7**, which in turn became a new synthesis target (**Figure 12**).

Before embarking on the actual synthesis of plakinamine G (**3**), the proposed route required the preparation of lactam **4**, which would be tested and optimized by using *i*-PrCHO as a model substrate to provide our initial target, pulchellalactam analogue **5** (**Figure 12**). An alternative pathway to **5**, employing a 2-silyloxyfuran building block instead of lactam **4**, will also be explored in order to uncover the best route to **5**, and ultimately plakinamine G

(**3**). It is anticipated that this research will establish effective and potentially general avenues, amenable to the preparation of a range of (Z)-configured γ-alkylidene-γ-lactams, including those bearing a chiral, epimerizable alkylidene substitutent, such as **3**.

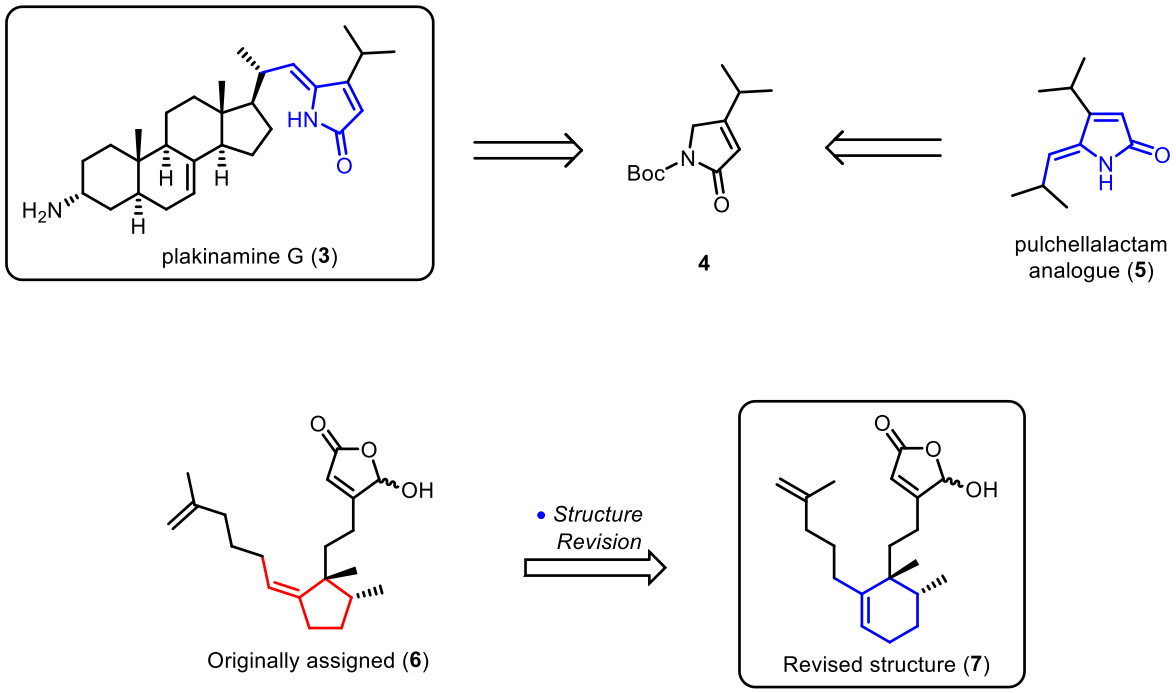


Figure 12. Our Synthesis Targets

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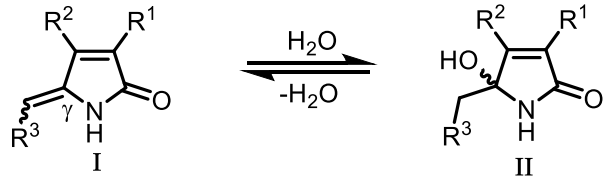
Chapter 1 Synthesis of the Marine Antitumor Metabolite Plakinamine G

1.1 Introduction: γ-Alkylidene-γ-lactams

1.1.1 Naturally Occurring γ-Alkylidene-γ-lactams

γ-Alkylidene-γ-lactams **I** (a.k.a. 5-alkylidene-pyrrolin-2-ones, **Scheme 5**) are a unique class of five-membered heterocycles that have attracted growing attention in recent years on account of their diverse biological activities. Structurally, γ-alkylidene-γ-lactams vary by the *Z* or *E* configuration of the alkylidene C=C and the nature of R^1-R^3 substituents. When R^1 is an acyl group (cf. COR), some 5-alkylidene-pyrrolin-2-ones **I** have been isolated along with their formal hydration products, namely, 5-hydroxy-5-alkyl-pyrrolin-2-ones **II** (**Scheme 5**).[1]



Scheme 5. Relation between 5-alkylidene- and 5-hydroxy-5-alkyl-pyrrolin-2-ones

Unlike their lactone counterparts, only a handful of γ-alkylidene-γ-lactams have been found to occur in Nature. Nonetheless, the comparatively few known γ-alkylidene-γ-lactam NPs display diverse structures and significant biological properties, as illustrated by the examples shown in **Figure 13**.

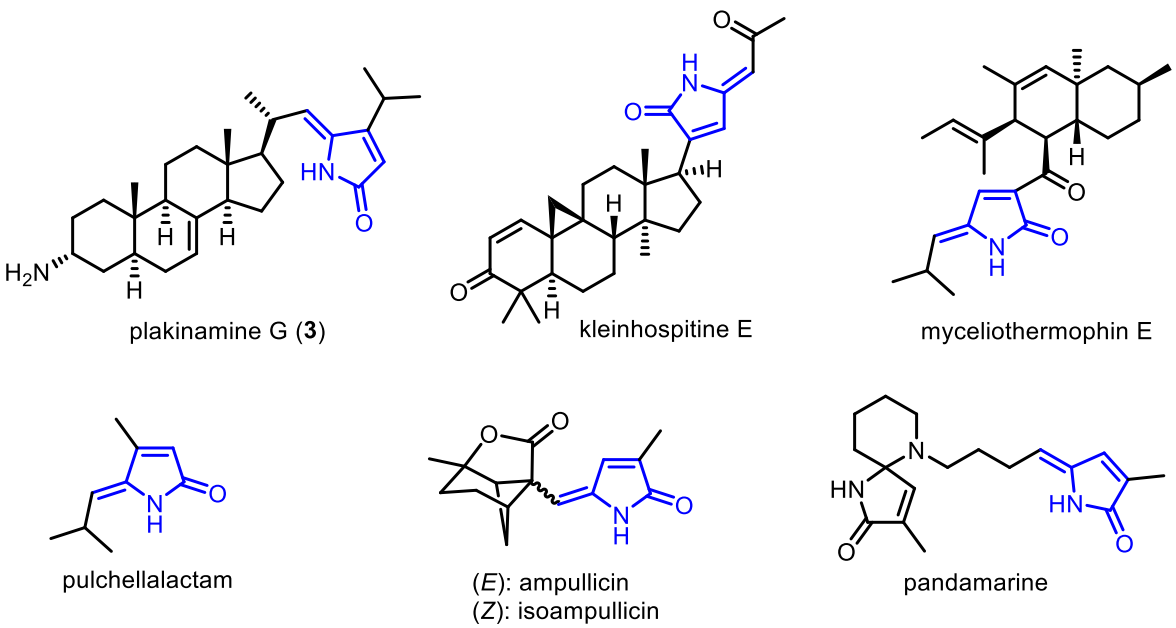


Figure 13. Naturally Occurring γ-Alkylidene-γ-lactams

Our target molecule, plakinamine G (**3**), was isolated in 2002 by Zollo and co-workers from the marine sponge *Corticium* sp. (order Homosclerophorida, family Plakinidae) collected off Porth Havannah, Vanuatu, South Pacific.[2] Structurally, it is a pregnane-type steroidal alkaloid displaying potent antiproliferative activity against rat glioma C6 cells (IC_{50} = 6.8 μg/mL).[2] In humans, glioma is the most common tumor of the brain, accounting for nearly 40% of all primary brain tumors. Furthermore, the prognosis of patients with malignant glioma is extremely poor.[3] Because only 1.1 mg of **3** were obtained from the natural source, there was not sufficient material to allow evaluation in *vivo*. Thus, a practical synthesis of **3** would open the door to more profound investigation of its antitumor properties, and would also enable access to new analogues for SAR studies.

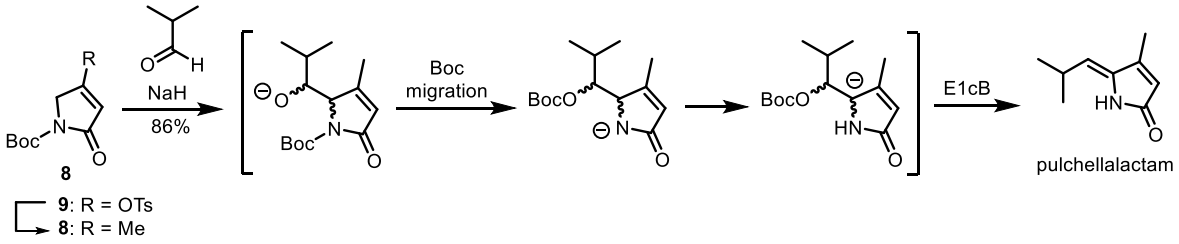
The only other steroidal γ-alkylidene lactam found in Nature is kleinhospitine E (**Figure 13**), which exhibits significant cytotoxicity against five human cancer cell lines.[4] Myceliothermophin E, a polyketide-amino acid hybrid fungal metabolite, also possesses cytotoxic properties against a number of human cancer cell lines.[5] Pulchellalactam, the simplest natural γ-alkylidene lactam, is a CD45 protein tyrosine phosphatase inhibitor.[6]

Ampullicin and isoampullicin are potent growth regulators, initially isolated from an *Ampulliferina*-like fungus obtained from a dead pine tree (*Pinus thunbergii*).[7] Pandamarine is a racemic spirocyclic alkaloid isolated by Byrne's group in 1992 from the leaves of the tropical plant *Pandanus amaryllifolius*.[8] Although there are no documented biological studies on pandamarine itself, *Pandanus amaryllifolius* leaf extracts were reported to have antihyperglycemic effects.[9]

1.1.2 Synthesis of Naturally Occurring γ-Alkylidene-γ-lactams

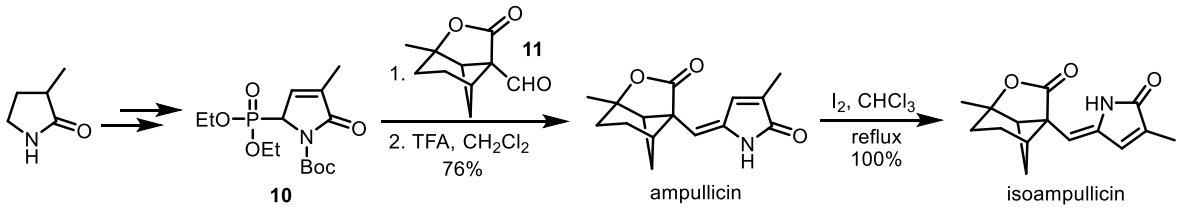
Notwithstanding the paucity of naturally occurring γ-alkylidene-γ-lactams, synthetic efforts in this area have been significant. Indeed, the majority of natural γ-alkylidene-γ-lactams known to date, e.g. pulchellalactam, ampullicin, isoampullicin, myceliothermophin E, and pandamarine have succumbed to total synthesis. As a result, several interesting methods for the de novo assembly of substituted γ-alkylidene-γ-lactams have emerged.

The first synthesis of pulchellalactam, the simplest γ-alkylidene-γ-lactam found in Nature thus far, was reported by Li's group in 2002.[10] Since then, another 11 syntheses of this molecule have bee reported in the literature.[11] Li's approach exemplifies a new method for building (*Z*)-γ-alkylidene-γ-lactams through *N*-to-*O* Boc-migration and vinylogous aldol condensation (**Scheme 6**). The requisite β-methyl-γ-lactam **8** was obtained from tosylate **9** and the *in situ* generated Gilman reagent Me_2CuLi by a conjugate addition/elimination process.[10]



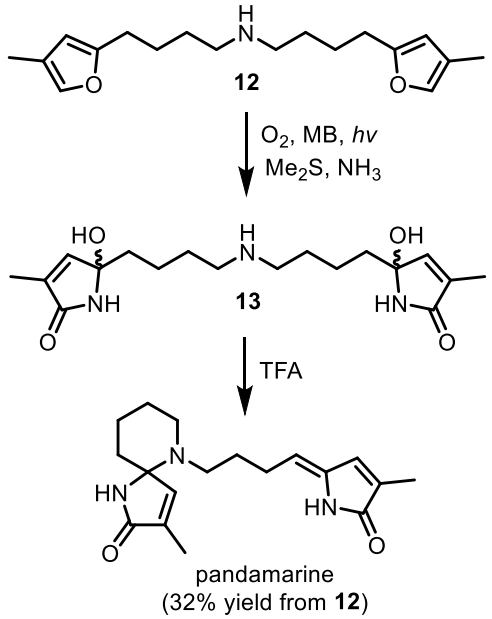
Scheme 6. Li's Vinylogous Aldol Condensation to Pulchellalactam

The total synthesis of both ampullicin and isoampullicin was accomplished by Bermejo and Rico-Ferreira (**Scheme 7**).[12] They employed a Horner-Emmons reaction to construct the (*E*)-exocyclic olefin in ampullicin. Treatment of ampullicin with iodine under reflux gave the thermodynamically more stable *Z*-isomer isoampullicin in quantitative yield.



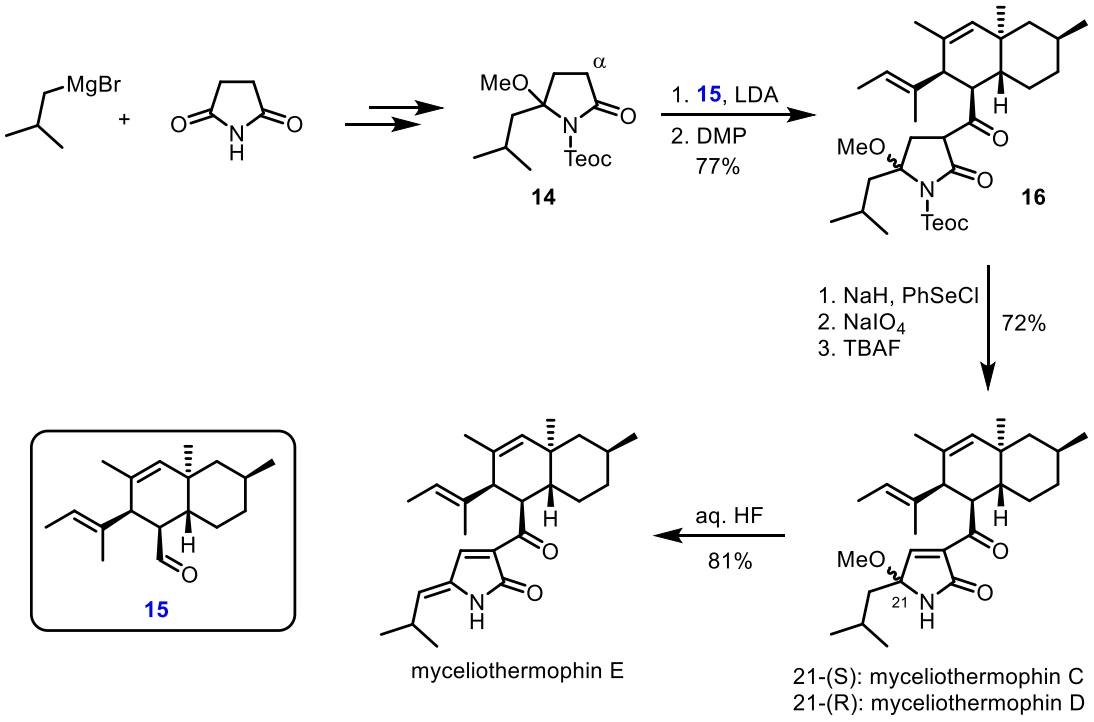
Scheme 7. Bermejo's Synthesis of Ampullicin and Isoampullicin

The total synthesis of pandamarine, a novel spirocyclic alkaloid, was achieved by the group of Vassilikogiannakis (**Scheme 8**).[13] The key step is a cascade reaction sequence initiated by photo-oxidation and aminolysis of a symmetric difuran (cf. **12**→**13**). Subsequent ketalization/dehydration of so obtained *bis*-lactam **13** afforded pandamarine (32% yield from **12**).



Scheme 8. Vassilikogiannakis's Synthetic Route to Pandamarine

The total synthesis of myceliothermophin E was reported by the group of Uchiro[14] and Nicolaou[15] in 2012 and 2014, respectively. These syntheses employ essentially the same strategy to assemble the α-substituted γ-lactam core (cf. **14** + **15** →**16**, **Scheme 9**). In Nicolaou's synthesis, the *N*-Teoc protected lactam building block **14** was prepared from succinimide and *i*-BuMgBr. Exposure of **14** to a solution of LDA, trapped by aldehyde **15** gave alcohol mixture. Oxidation of so obtained alcohol with Dess-Martin periodinane (DMP) afforded 1,3-diketone **16**, which was subjected to sequential α-selenylation, oxidation/*syn* elimination, and deprotection, to give a separable mixture of diastereomeric myceliothermophins C and D. On treatment with HF, both of these isomers were converted to (*Z*)-configured myceliothermophin E.

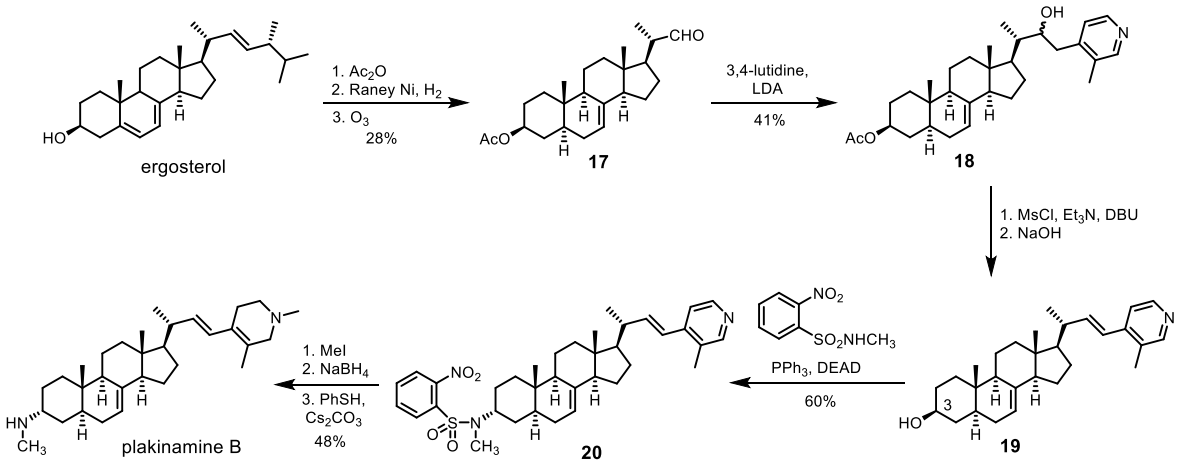


Scheme 9. Nicolaou's Synthesis of Myceliothermophins C, D and E

1.2 Previous Syntheses of Plakinamines

So far, there have been sixteen members of the plakinamine family isolated from sponges.[16] All these steroidal alkaloids are endowed with potentially useful biological properties. Yet,

only one of them, namely plakinamine B, has been synthesized to date, by Gans and Bracher (**Scheme 10**).[17] This synthesis began with the 3-step conversion of ergosterol to aldehyde **17** through acetylation, hydrogenation, and ozonolysis. Treatment of aldehyde **17** with lithiated 3,4-lutidine (generated *in situ* from 3,4-lutidine and LDA) afforded alcohol **18** as a mixture of diastereoisomers. Alcohol **18** was subjected to elimination and deprotection to give alcohol **19**, which was converted to sulfonamide **20** with inversion of stereochemistry at C-3 by a Fukuyama-Mitsunobu reaction.[18] Subsequent methylation, reduction, and cleavage of the *N*-sulfonyl bond gave plakinamine B (10 steps, ~2.2% overall yield).



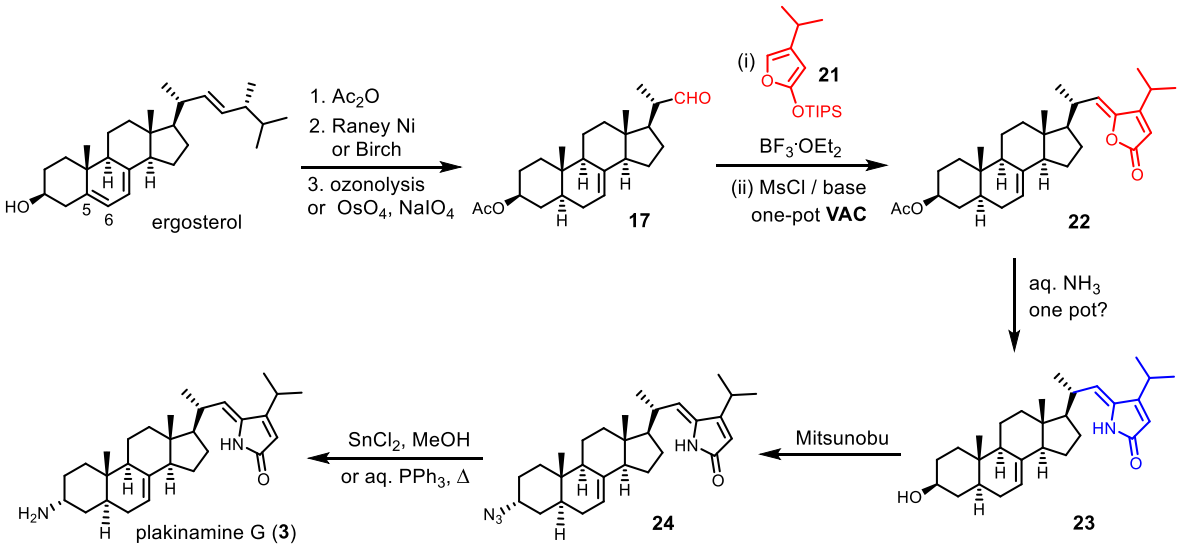
Scheme 10. Bracher's Semi-syntheses of Plakinamine B

1.3 Our Synthesis of Plakinamine G

1.3.1 Initial Synthetic Plan for Plakinamine G

Our initial synthetic approach to plakinamine G (**3**) is outlined in **Scheme 11**. The most important transformation is the appendage of the (*Z*)-configured γ-alkylidene-γ-lactam onto aldehyde **17**. We envisioned accomplishing this by utilizing new methodology *via* (*Z*)-γ-alkylidenebutenolide **22**. The requisite (*Z*)-configured γ-lactam **23** could be directly or indirectly obtained from butenolide **22** *via* aminolysis using either ammonium hydroxide or

liquid ammonia.[19] A more detailed plan for each transformation will be described with the aid of a separate scheme further below.



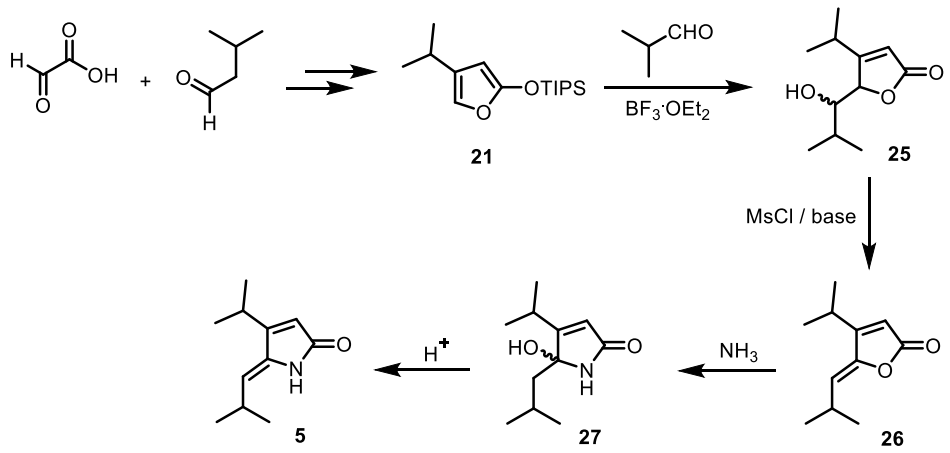
Scheme 11. Initial Synthetic Route to Plakinamine G

Access to steroidal aldehyde **17** from ergosterol could be gained by using literature precedent (cf. **Scheme 10**).[17] Alternatively, Birch reduction can be used to selectively saturate only the C5-C6 double bond of the conjugated diene.[20] Likewise, regioselective oxidative cleavage of the side-chain disubstituted C=C double bond into the desired aldehyde, is also accessible by sequential dihydroxylation with OsO_4 and oxidation.[21] Removal of the acetate should occur during the ammonolysis step, thereby accomplishing both the conversion of butenolide to lactam and deprotection of the masked alcohol group (**22→23**, **Scheme 11**).

At this point, alcohol **23** would be transformed to (*R*)-azide **24** by means of Mitsunobu chemistry (**Scheme 11**).[22] Finally, the conversion of **24** to plakinamine G (**3**) should be achievable through chemoselective reduction of the azido group in **24** under exceptionally mild conditions (e.g. $SnCl_2$, MeOH, 0 °C), as recently demonstrated by our group in the inaugural total synthesis of the antitumor antibiotic basidalin.[23]

Of course, the success of the overall synthesis plan would largely depend upon the serviceability of the proposed methodology for transforming aldehyde **17** to (*Z*)-γ-alkylidene-γ-lactam **23** *via* (*Z*)-γ-alkylidenebutenolide **22** (**Scheme 11**).

Accordingly, the first task was to establish the viability of this chemistry using a model aldehyde, e.g. isobutyraldehyde (**Scheme 12**). The appropriate building block, 2-silyloxyfuran **21** would be prepared from glyoxylic acid and isovaleraldehyde using a literature method.[24] Vinylogous Mukaiyama aldol reaction (VMAR) of 2-silyloxyfuran **21** with isobutyraldehyde should provide the corresponding aldol adduct **25**, whose dehydration to γ-alkylidene-γ-butenolide **26** was anticipated to occur upon conversion to the corresponding mesylate with methanesulfonyl chloride in the presence of a weak organic base such as triethylamine or pyridine.[25]

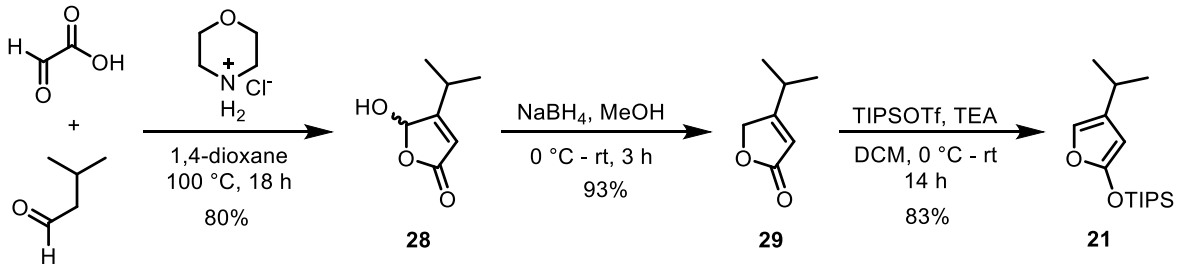


Scheme 12. Plan for the Synthesis of a Pulchellalactam Analogue **5**

Ammonolysis of butenolide **26** should afford γ-hydroxylactam **27**, which in turn, would undergo dehydration in the presence of a strong acid, hopefully in one-pot fashion, to deliver the desired (*Z*)-γ-alkylidene-γ-lactam **5** (**Scheme 12**).

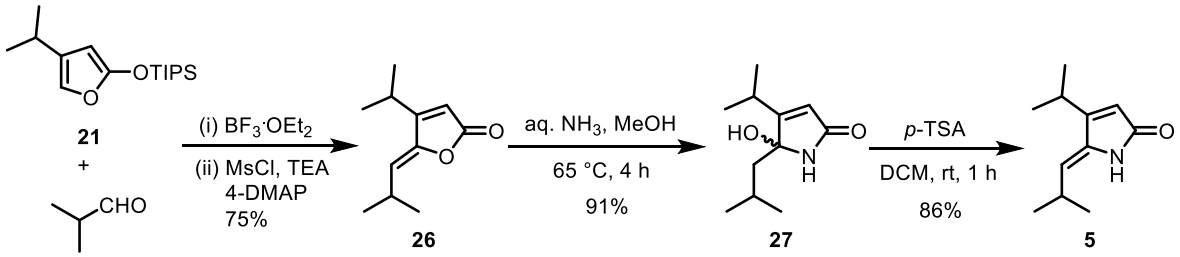
1.3.2 Synthesis of a Pulchellalactam Analogue (5)

The synthesis of the requisite 2-silyloxyfuran **21**, is shown in **Scheme 13**. Using a literature procedure,[24] reaction of glyoxylic acid with isovaleraldehyde in the presence of morpholinium chloride in 1,4-dioxane at 100 °C for 18 h furnished β-isopropyl-γ-hydroxybutenolide **28** in 80% yield. Next, reduction of **28** with sodium borohydride provided the corresponding butenolide **29** in excellent yield (93%). Using an established procedure, silylation of **29** with TIPSOTf and triethylamine in anhydrous dichloromethane overnight (~ 14 h), delivered 2-silyoxyfuran **21** in 83% yield. Because **21** is acid-sensitive, it was not subjected to purification by column chromatography but only to a standard work-up, before the next step, namely, its vinylogous Mukaiyama aldol reaction with isobutyraldehyde using boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) as a Lewis acid (**Scheme 14**).



Scheme 13. Synthesis of 2-Silyloxyfuran **21**

Again, the resulting crude *syn* and / or *anti* aldol adduct(s) were not chromatographed, but directly subjected to the action of methanesulfonyl chloride in the presence of triethylamine and *N*,*N*-4-dimethylaminopyridine (4-DMAP) to furnish (*Z*)-γ-alkylidene-γ-butenolide **26** as the only detectable isomer in 75% yield after flash column chromatography.

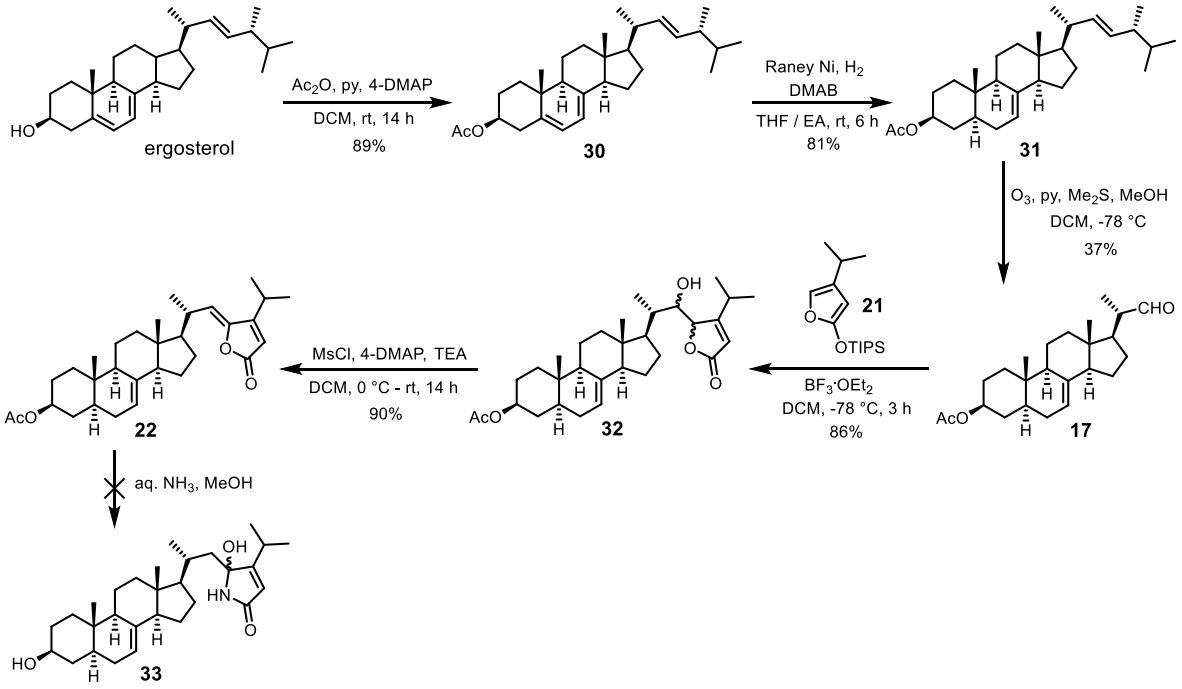


Scheme 14. Synthesis of a Pulchellalactam Analogue **5**

Exposure of the so obtained butenolide **26** to ammonia in water/MeOH at 65 °C for 4 h led to γ-hydroxylactam **27** in excellent yield (91%, **Scheme 14**). Treatment of **27** with a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) in anhydrous methylene dichloride for 1 h at room temperature gave pulchellalactam analogue **5** as the sole isomer in a yield of 86% after chromatography.

1.3.3 Attempted Synthesis of Plakinamine G

The synthesis of plakinamine G (**3**) commenced with the acetylation of commercially available ergosterol (**Scheme 15**). Thus, reaction of ergosterol with acetic anhydride in the presence of 4-DMAP and pyridine afforded ester **30** in 89% yield. To our disappointment, the selective hydrogenation of the desired C=C double bond in **30** using commercial Raney nickel did not take place smoothly, probably due to poor quality of the Raney catalyst. Therefore, we prepared Raney nickel from nickel aluminum powder and carried out the reaction under a hydrogen gas atmosphere at room temperature for 6 hours. Also, 4-(dimethylamino) benzaldehyde (4-DMAB) was included in the reaction as an additive to prevent hydrogenation of the other C=C double bonds. Under these conditions, we were able to obtain compound **31** in a respectable yield of 81%.

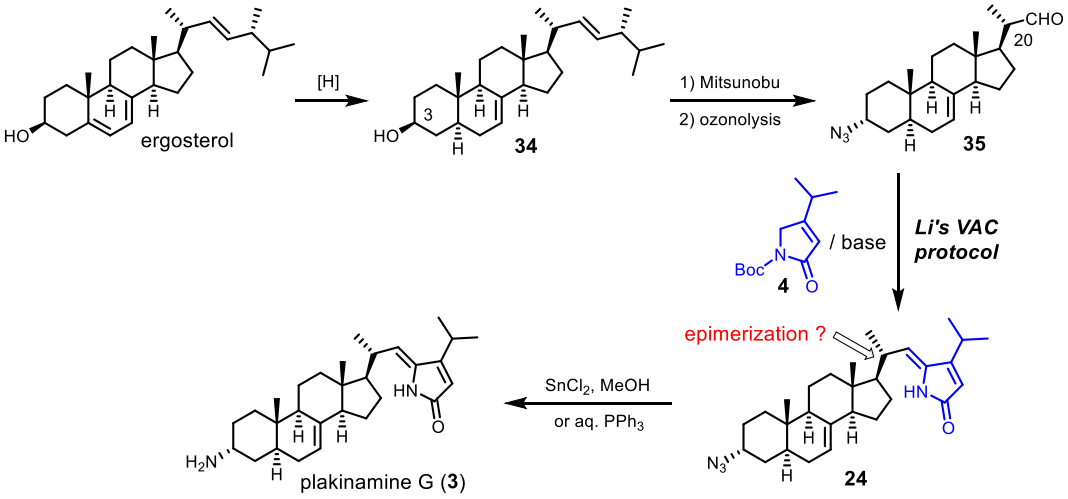


Scheme 15. Attempted Synthesis of Plakinamine G

Ozonolysis of **31** provided aldehyde **17** in a moderate 37% yield, which is comparable to that reported in the literature (32-40%) for the same transformation (**Scheme 15**). The 2-step conversion of **17** to (*Z*)-γ-alkylidenebutenolide **22** proceeded uneventfully under the conditions described for the synthesis of pulchellalactam analogue **5** (cf. Section 2.3.2). At this point, the ammonolysis of **22** was tried using ammonia hydroxide solution in water/MeOH. Unfortunately, however, the NMR spectrum of the crude product mixture revealed the absence of the expected lactam **33**. A possible explanation for the failure of the ammonolysis reaction is the poor solubility of our steroid substrate **22** in water or methanol. We also tried to use a less polar, water-miscible solvent such as THF. However, when MeOH was replaced by THF, the alkylidenebutenolide moiety in **22** remained intact. Thus, in spite of the success of this chemistry with a model compound (Section 2.3.2), ammonolysis of alkylidenebutenolide **22** into the actual plakinamine precursor, lactam **33**, turned out to be problematic. Hence, we decided to explore a completely different route to plakinamine G from alcohol **34** that does not involve a protection-deprotection cycle thereby making the synthesis shorter (**Scheme 16**).

1.3.4 New, **Protecting-Group-Free Approach**

Our new, protecting-group-free approach to plakinamine G (**3**) is outlined in **Scheme 16**. Considering the vulnerable amino-group in **3**, we planned to introduce early on in the synthesis an azido group, which would serve as a masked amine. Advantage would be taken of the remarkable stability of azides over a wide range of conditions, including hard Lewis acids and strong bases. The azido group would be installed with concomitant inversion of stereochemistry at C3 by means of a Mitsunobu reaction (cf. **34**→**35**).[22]



Scheme 16. New, Protecting-Group-Free Approach to Plakinamine G

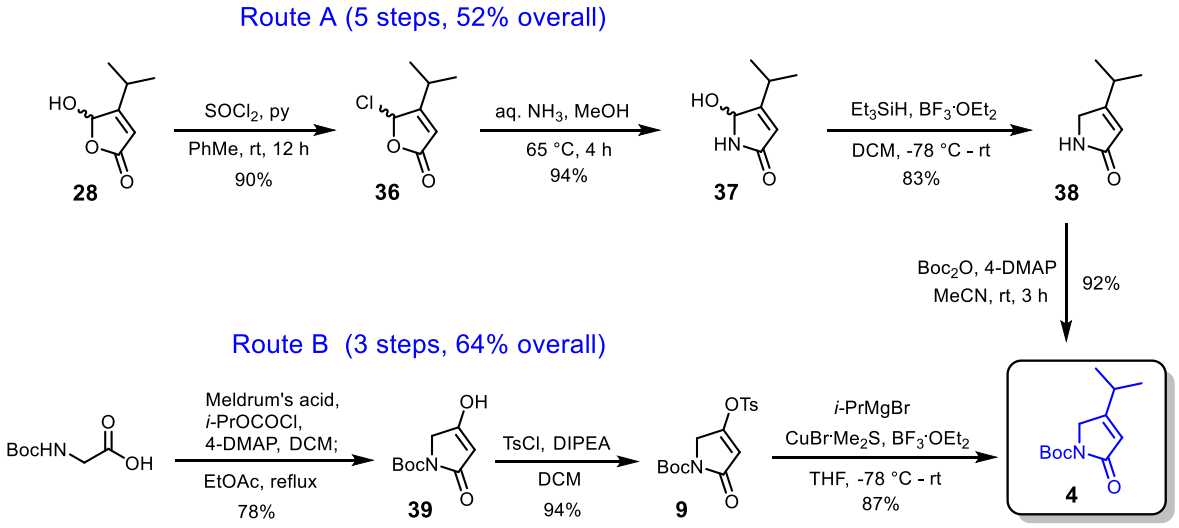
Assemblage of the γ-alkylidene-γ-lactam moiety (cf. **35**→**24**) would be carried out by adaptation of Li's one-pot method.[10] As far as we are aware, Li's method has only been employed on an achiral substrate. An obvious concern was whether the use of the required strong base (cf. LDA) would cause epimerization of aldehyde **35** and/or lactam **24**, as both bear an acidic proton attached to C20.

1.3.5 Synthesis of *N*-Boc-γ-Lactam 4

As outlined in Scheme **17**, we established two synthetic routes to requisite building block **4**. Initially, access to **4** was gained from γ-hydroxybutenolide **28** (***route* A**). We already had

gram quantities of **28** in our lab, as this compound was prepared earlier in one step for the synthesis silyloxyfuran **21** (cf. **Scheme 13**).

Treatment of **28** with thionyl chloride in the presence of pyridine furnished γ-chloro butenolide **36** in 90% yield. Ammonolysis of **36** with ammonia in water/MeOH, afforded γ-hydroxy-γ-lactam **37** in excellent yield (94%).[27] Reduction of **37** with triethylsilane (Et_3SiH) in the presence of $BF_3{\cdot}OEt_2$,[28] provided γ-lactam **38** which was smoothly transformed to the requisite *N*-Boc lactam **4** (5 steps, 52% overall yield).

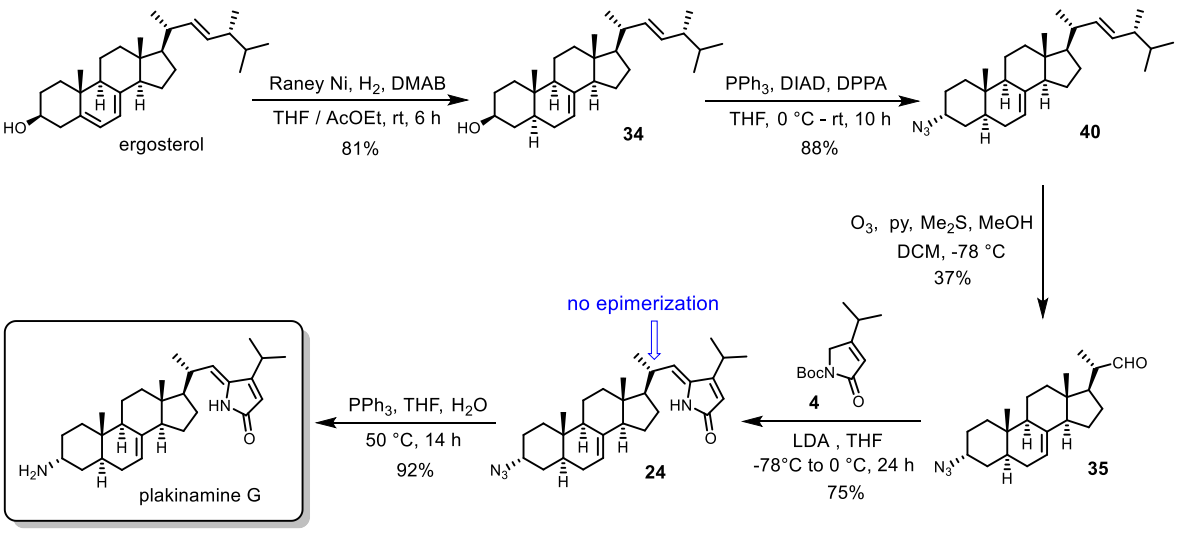


Scheme 17. Synthesis of *N*-Boc-γ-Lactam **4**

In order to cut-off steps, we also explored ***route B***, where the isopropyl is introduced at a late stage (cf. **9→4**, **Scheme 17**). Lactam **9** was easily prepared on a gram-scale in 2 steps from Boc-Gly-OH using literature methods.[10] Michael addition of the *in situ* generated isopropylcopper reagent (*i*-PrCu) to **9** in the presence of acid $BF_3{\cdot}OEt_2$ and elimination of the tosylate afforded **4** in excellent yield (87%). **Route B** is shorter and more efficient than route A, requiring only 3 steps from commercial chemicals.

1.3.6 First Synthesis of Plakinamine G

With supplies of lactam **4** in hand, our new synthetic plan could now be tested (**Scheme 18**). Hydrogenation of ergosterol under the same conditions described earlier, smoothly gave stellasterol **34** in 81% yield. Next, **34** was subjected to Mitsunobu reaction with diphenylphosphoryl azide (DPPA) using a literature procedure, to afford 3α-azido steroid **40** in 88% yield.[26] In line with previous experience (Section 2.3.3), the ozonolysis of **40** provided aldehyde **35** in a moderate yield (37%).



Scheme 18. First Synthesis of Plakinamine G

Crucially, stereocontrolled assembly of the key (*Z*)-configured γ-alkylidene-γ-lactam moiety could be achieved in a single operation by adapting Li's method (cf. **35→24**, **Scheme 18**). In accordance with Li's procedure,[10] sodium hydride was used as base, but the desired lactam **24** was obtained in only ca. 25% yield according to the proton NMR spectrum of the crude reaction product mixture. A slight excess of reagent **4** and base increased the yield to 57%. Considering the vague amount of sticky sodium hydride 60% dispersion in mineral oil, we decided to use commercial LDA in THF instead. After some experimentation, we found that the optimal procedure consisted in using LDA in THF and a slight excess of *N*-Boc lactam **4** (1.5 equiv), affording key intermediate **24** in a respectable

yield (75%). Importantly, no epimerization at C20 was observed in the product after chromatography.

Finally, chemoselective reduction of the azido group in **24**, using Staudinger chemistry,[29] afforded plakinamine G (**3**) in excellent yield (92%). The NMR spectra and specific rotation value {$[\alpha]_D^{22}$ -22.2° (*c* 0.09, MeOH), lit.[2] $[\alpha]_D^{22}$ -24.4° (*c* 0.09, MeOH)} of our synthetic plakinamine G were in good agreement with those reported for the natural product.[2]

1.4 Conclusion

The first synthesis of plakinamine G has been accomplished from ergosterol (5 linear steps, 18.2% overall yield) in protecting-group free, stereocontrolled fashion, by exploiting the azido group as a masked amine. The synthesis further demonstrates for the first time the serviceability of Li's method in the context of chiral, epimerizable substrates. Moreover, as a result of our model study, we developed a new route to γ-alkylidene-γ-lactams from 2-siloxyfurans and aldehydes which should be useful for making a range of new pulchellalactam analogues and related targets. Collectively, these syntheses are anticipated to facilitate more profound biological studies of both natural and unnatural members of this class of bioactive compounds.

1.5 References

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Chapter 2 Total Synthesis of the Originally Proposed Structure of Niphateolide A

2.1 Introduction

2.1.1 Biologically Important Butenolides

The butenolide or 2(5*H*)-furanone ring is a key structural feature of numerous biologically important natural products and natural product-inspired molecules. As a class, butenolides display diverse and significant biological properties including anticancer, antiepileptic, anticoagulant, analgesic, antibacterial, anti-fungal and anti-inflammatory activities among others.[1] As a consequence, there is continuing interest in the synthesis, chemistry and biological action of such compounds. Several of them have been approved as medicines for the treatment of human or animal diseases. Others have found commercial use in crop protection and serve as lead compounds in the search of new insecticides, acaricides, herbicides and plant growth regulators.[2]

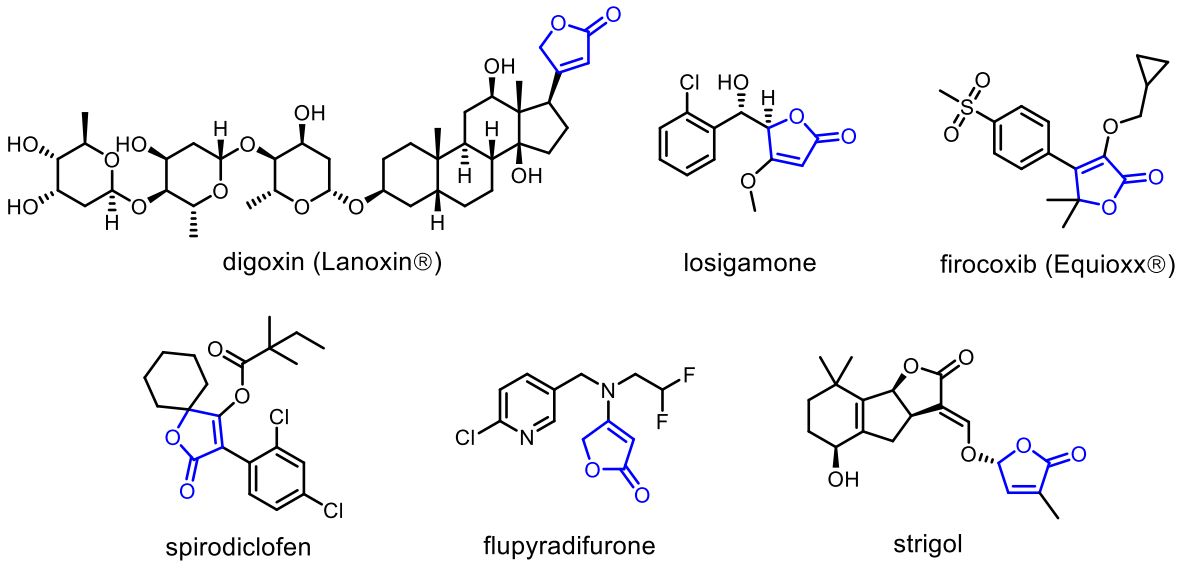


Figure 14. Some Biologically Important Butenolides

Some bioactive butenolides are shown in **Figure 14**. Digoxin, a steroidal butenolide isolated from the common foxglove *digitalis purpurea*,[3] is currently sold under the brand

name Lanoxin® and has become an essential medication used to treat a series of heart conditions. It is frequently used for atrial flutter, atrial fibrillation and heart failure. Losigamone (**Figure 14**), a chlorine-containing tetralonate, is an experimental drug that has been advanced to phase III clinical trials for the treatment of epilepsy.[4] Firocoxib (**Figure 14**), a non-steroidal anti-inflammatory drug (NSAID), was found to inhibit prostaglandin synthesis and impart analgesic, antipyretic and anti-inflammatory properties. It has been marketed for use in veterinary medicine, notably horses (Equioxx®) and dogs (Previcox®).[5]

Besides their value to medicine, butenolides have been also found use in agriculture. In fact, several commercial insecticides were inspired from the structures of natural products. Spirodiclofen (**Figure 14**), developed by Bayer CropScience, can cause damage to mites and pests and has been marketed as insecticide and acaricide.[6] Flupyradifurone (Sivanto®, Bayer), a fluorine-containing butenolide is a new Integrated Pest Management (IPM) insecticide controlling agricultural and horticultural pests.[7] Besides, strigol (**Figure 14**) is a member of the strigolactone family of naturally occurring growth regulators that stimulate germination and prevent plant branching.[8]

2.1.2 Niphateolide A

Niphateolide A (**6**, **Figure 15**), a butenolide-containing diterpenoid, was isolated in 2015 by Tsukamoto and co-workers from the Indonesian marine sponge *Niphates olemda*.[9] This natural product was shown to be a rare inhibitor of the p53-Hdm2 interaction which is a prime target for new anticancer drugs. Niphateolide A inhibited this interaction with an IC_{50} value of 16 μM in a biological assay using ELISA. It was assigned structure **6** by means of NMR and ECD at the vacuum-ultraviolet region with a theoretical calculation.[9] This molecule displays an unprecedented and unusual carbon skeleton bearing a (*Z*)-configured olefin appendage and a β-substituted-γ-hydroxybutenolide. To date, this new skeleton has neither been found in Nature nor synthesized. These attributes did not escape the attention of our group, promptly elaborating a synthesis plan that will be detailed in a new section of this thesis.

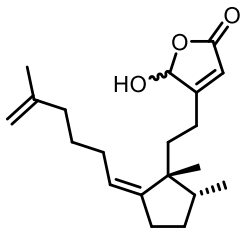


Figure 15. Originally Proposed Structure of Niphateolide A

2.2 3D-Structure of 6 is Reminiscent to Dysidiolide

The research group of Tsukamoto proposed a 3D-structure of **6** (**Figure 16**) based on calculations with B3LYP/6-31G*.[9] The two parallel chains that bear a terminal isopropene unit and a β-substituted-γ-hydroxybutenolide moiety respectively, promoted us to recall the prominnent natural sesterterpenoid dysidiolide (**Figure 16**), which is a novel cdc25A inhibitor isolated from the Caribbean sponge *Dysidea etheria* de Laubenfels.[10] Given its highly sought after biological activity and unique structure, over 12 publications described the total synthesis of dysidiolide.[11]

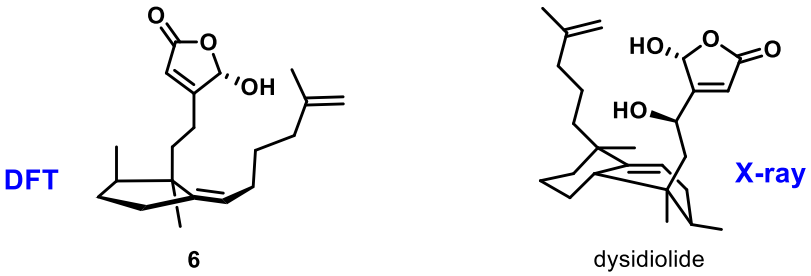
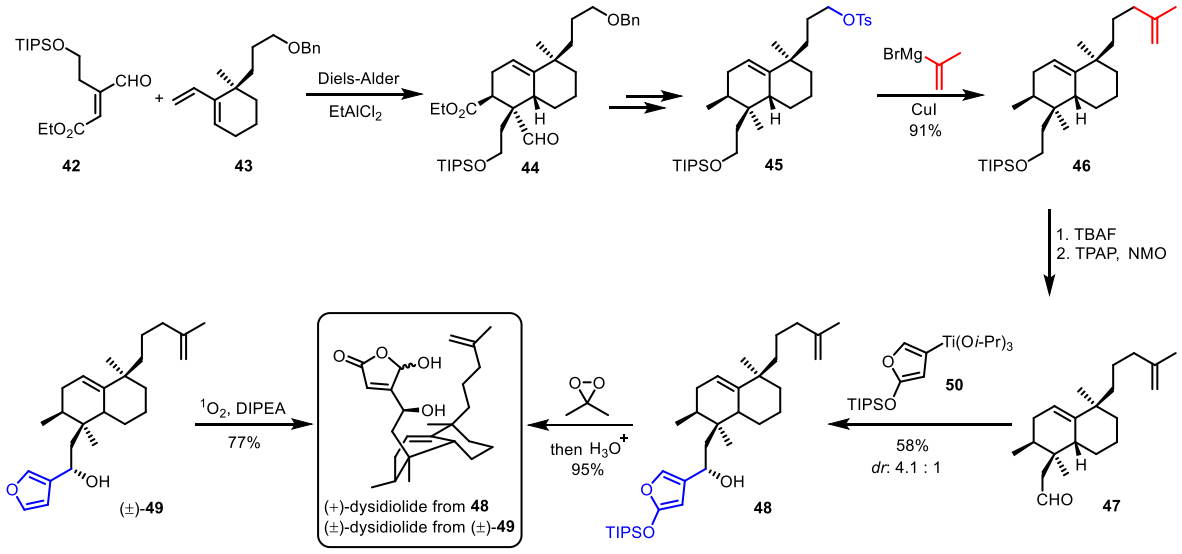


Figure 16. 3D-Structures of **6** and Dysidiolide

Among the twelve total syntheses of dysidiolide, the first three were published within a month's time by the groups of Corey,[12] Boukouvalas,[13] and Danishefsky.[14] Described here is the Boukouvalas enantioselective synthesis of (+)-dysidiolide (**Scheme 19**). The ethylaluminum dichloride catalysed Diels-Alder reaction of dienophile **42** with diene **43**,

built the decalin framework. The terminal isopropene unit was installed by cross-coupling of tosylate **45** with isopropenylmagnesium bromide in the presence of copper iodide (CuI). Desilylation of **46**, followed by TPAP-oxidation delivered aldehyde **47**. The (siloxyfuranyl)titanium reagent **50**, serving as a masked β-substituted-γ-hydroxybutenolide, was added to aldehyde **47** to generate precursor **48**. Regiospecific oxidation of silyloxyfurane **48** with dimethyldioxirane (DMDO), followed by treatment with Amberlyst-15/H_2O afforded enantiopure (+)-dysidiolide.[13]



Scheme 19. Total Synthesis of (+)-Dysidiolide by the Boukouvalas Group

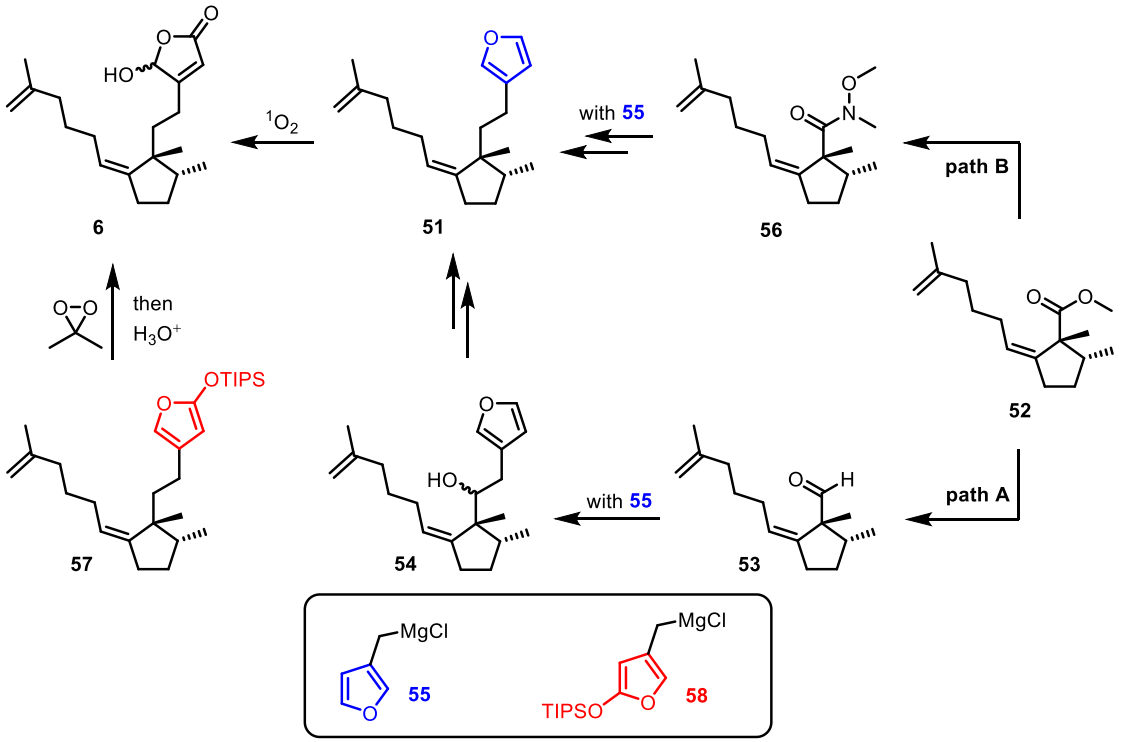
Alternatively, in the Danishefsky's route,[14] 3-furyllithium was used instead of **50** yielding racemic 3-alkyl furan **49** (**Scheme 19**). Then, (±)-**49** was subjected to one-pot photo-sensitized oxygenation / regionselective Kornblum-DeLaMare rearrangement (Faulkner method) to afford racemic dysidiolide.

2.3 Total Synthesis of the Alleged Structure of Niphateolide A

2.3.1 Initial Synthetic Approach

Compound **6** (**Scheme 20**) displays an unprecedented and unusual carbon skeleton and some serious synthetic challenges. One of them concerns the installation of the exocyclic

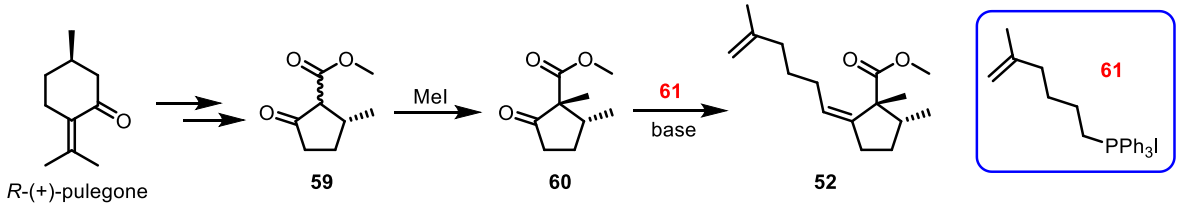
(*Z*)-configured olefin moiety. Our initial synthetic approach to **6** is outlined in **Scheme 20**. The proposed route to key intermediate **52** is shown in **Scheme 21**.



Scheme 20. Our Initial Approach to **6**

As shown in **Scheme 20**, we envisioned two different pathways to advanced intermediate **51** that serves as the precursor of target structure **6**. Both pathways start from carboxylic ester **52**. Path **A**: Ester **52** is transformed to aldehyde **53** through sequential reduction and oxidation, which is then converted to alcohol **54** by reaction with Grignard reagent **55**. Deoxygenation of alcohol **54** would provide furan **51**.[15] Alternatively (Path **B**), the precursor **51** is generated from Weinreb amide **56**.[16] Conversion of 3-alkyl furan **51** to **6** should be accessible using Faulkner method. In principle, Grignard reagent **58** can be used instead of **55**, although the acid-sensitive 2-silyloxyfuran may prove challenging to maintain during some of the steps.[17] This variation would generate 2-silyloxyfuran **57**, which should easily deliver **6** *via* DMDO-oxidation (cf. **57**→**6**).

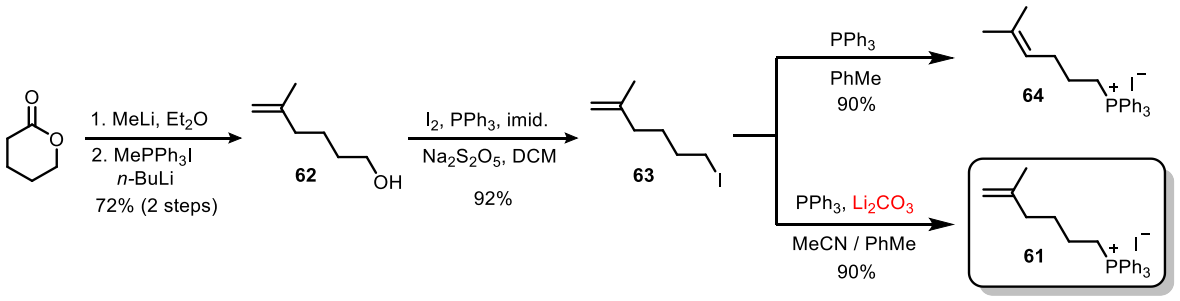
The starting point of our synthesis is the fairly inexpensive (*R*)-pulegone (**Scheme 21**), whose conversion to **59** was easily performed on a 10+ gram-scale by the Marx-Norman procedure.[18] After diastereoselective methylation of **59**, Wittig reaction should, in principle, install the (*Z*)-configured olefin appendage on **60** to generate key intermediate **52**.



Scheme 21. Synthetic Plan for Key Intermediate **52**

2.3.2 Synthesis of Wittig Salt

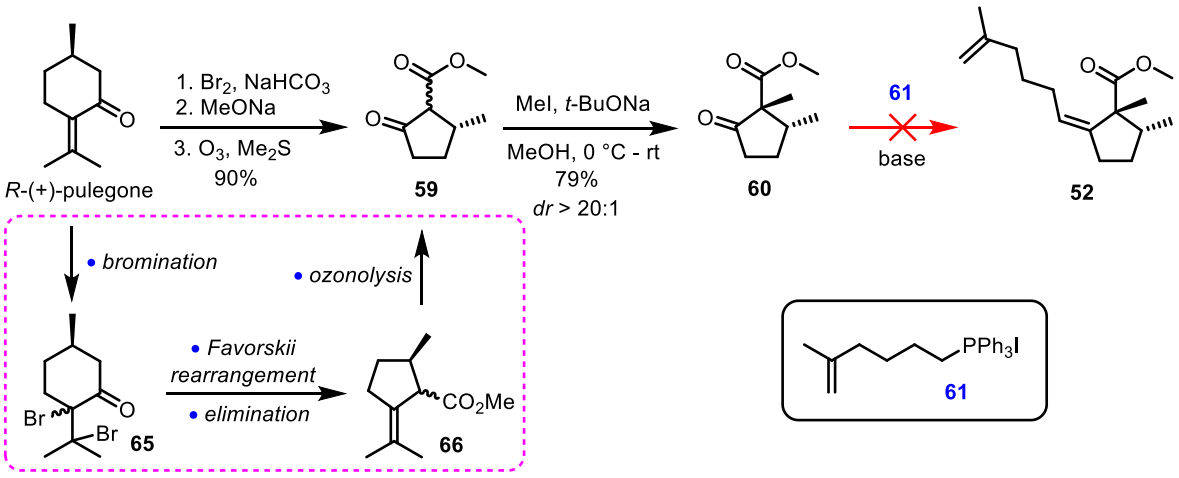
The synthesis of phosphonium salt **61** commenced with the conversion of δ-valerolactone to alcohol **62** through 2 steps, including ring-opening of lactone using methyllithium, and Wittig methyleneation (**Scheme 22**).[19] Converting alcohol **62** into iodide **63** was readily achieved (92% yield) using the Garegg-Samuelsson reaction.[20] Unfortunately, treatment of **63** with triphenylphosphine under reflux in toluene led to complete C=C double-bond isomerization to give **64** instead of the desired salt **61** (cf. **63**→**64**). To remedy this, we added excess of a weak base such as lithium carbonate (Li_2CO_3).[21] Gratifyingly, **61** was generated in 90% yield under these conditions. No isomerized product could be observed after recrystallization.



Scheme 22. Synthesis of Phosphonium Salt **61**

2.3.3 Attempted Synthesis of an Early Intermediate

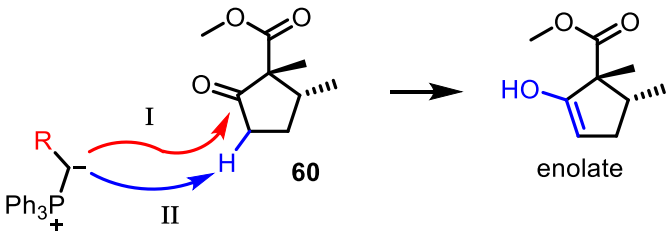
Our synthesis began with the preparation of the chiral building block **60** (**Scheme 23**). The first intermediate, β-keto ester **59**, was obtained from (*R*)-pulegone using the Marx-Norman procedure in 90% yield. Specifically, pulegone was first subjected to bromination with liquid bromine in the presence of sodium bicarbonate to yield dibromide **65**.[18a] To the resulting reaction mixture containing α-bromo cyclohexanone **65** was added a solution of sodium methoxide (MeONa) which was freshly prepared from sodium metal and methanol. The so obtained suspension was heated to reflux to give the methyl pulegenate **66** *via* Favorskii rearrangement[22] and elimination. Oxidative cleavage of the olefin linkage in **66**, was achieved by ozonolysis to afford β-keto ester **59**.[18a] Alkylation of **59** with iodomethane was originally performed using cesium carbonate (Cs_2CO_3) as the base, to deliver **60** in 70% yield (*dr*: ~2.2 : 1). Interestingly, when an organic base was used instead, cf. sodium *tert*-butoxide, the diastereoselectivity was substantially improved (*dr*: > 20:1). The group of Herzon also reported the same methylation in the total synthesis of pleuromutilins.[23]



Scheme 23. Attempt Synthesis of Key Intermediate **52**

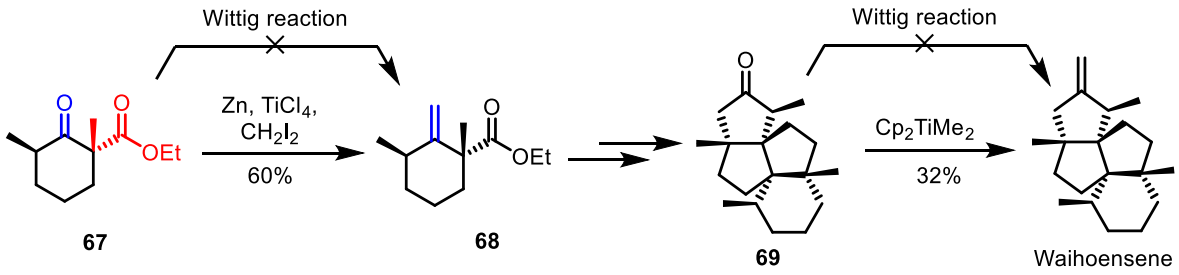
Having aquired ketone **60**, we next addressed the challenging installation of the olefin appendage. Previously, Amalyn Nain-Perez, a visiting PhD student in our group from Brazil, attempted the Wittig reaction of synthetic substrate **60** with phosphonium salt **61**

under a variety of conditions involving different bases and reaction temperatures. Unfortunately, none of the desired olefination product **52** was formed, and most of the starting material **60** could be recovered. Even the simplest methylenation of **60** with ylide CH_2=PPh_3 was unsuccessful. It appears that because the ketone group in **60** is pretty hindered, α-hydrogen atom abstraction occurs to form an enolate (path **II**, **Scheme 24**). In other words, enolization makes the carbonyl group unavailable for nucleophilic attack by the ylide. We also tried to carry out the olefination using the Peterson method.[24] However, no olefination was observed, and ketone **60** was recovered again.



Scheme 24. Possible Problem in Wittig Olefination

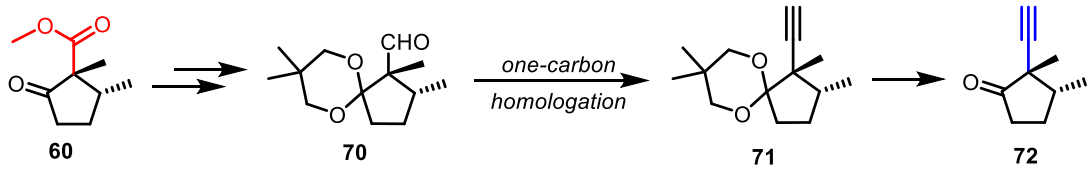
Related problems with the Wittig reaction have been frequently documented in the literature.[25] For example, a similar failure was reported by Lee and co-workers in the total synthesis of (±)-waihoensene (**Scheme 25**).[25a] The synthesis started with the olefination of a β-keto ester **67** whose carbonyl unit is also placed in a crowded environment bearing the carboxyl ester and dimethyl units. Then, the conversion of the ketone in **67** to exocyclic olefin in **68** was achieved using Lombardo-Takai methylenation that is considered to tolerate steric hindrance around concerned carbonyl moiety. Besides, the Lombardo-Takai olefination features a neutral nature which prevents epimerization at the chiral center bearing the methyl group in **67**. Interestingly, the authors encountered a similar problem again in the final step concerning methylenation of **69**. The steric congestion surrounding the ketone group in **69** blocked Wittig olefination. Eventually, they were able to install the exocyclic olefin by using the Petasis reagent, albeit in a rather modest 32% yield.



Scheme 25. Olefination Problems in Lee's Synthetic Route to (±)-Waihoensene

2.3.4 Revised Synthesis Plan

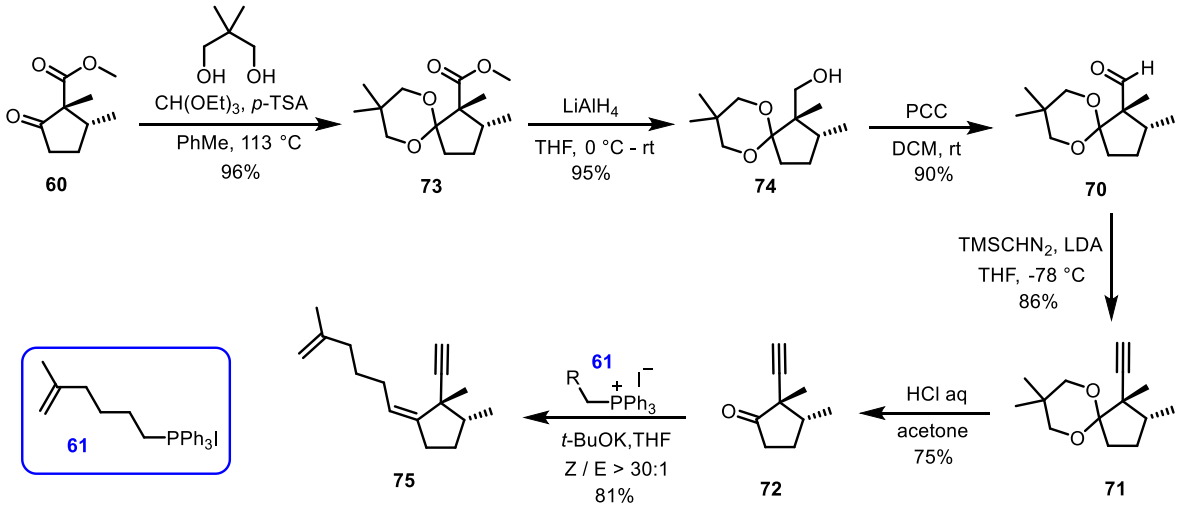
In an effort to reduce the steric hinderance surrounding the keto group in **60** (**Scheme 26**), we opted to explore the less hindered analogue **79**, which bears a slender acetylene substituent instead of an ester, as key intermediate. Give the versatility of the terminal alkyne group, it was further anticipated that the conversion of **72** to **6**-precursor **51** should be achieveable with little addition effort. Access to **72** was to be gained from ester **60** through one-carbon homologation (cf. **70**→**71**)[26] and simple functional group interconversions.



Scheme 26. Proposed Route to a Newly Designed Olefination Substrate

2.3.5 Total Synthesis of the Alleged Structure of Niphateolide A

The synthesis of new olefination substrate **72** is shown in **Scheme 27**. Reaction of ketone **60** with neopentyl glycol, using triethyl orthoformate as a "water scavenger", and a catalytic amount of *p*-toluenesulfonic acid, gave acetal **73** in 96% yield. Reduction of **73** with $LiAlH_4$ provided alcohol **74**, which was treated with pyridinium chlorochromate (PCC) in dry dichloromethane to yield aldehyde **70** in excellent yield (90%).

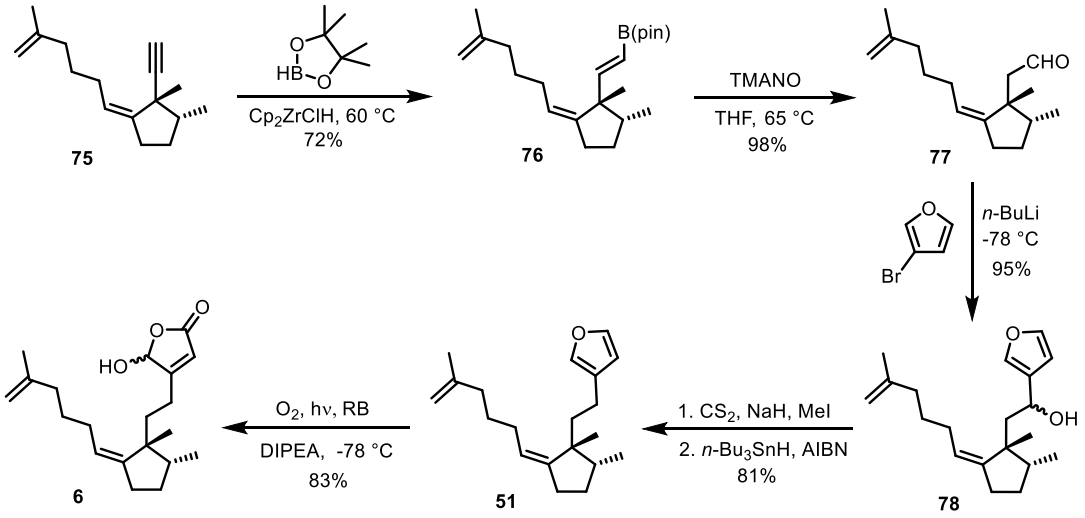


Scheme 27. Synthesis of Key Intermediated **75**

Conversion of **70** to acetylene **71** (**Scheme 27**) was originally performed by means of Seyferth-Gilbert homologation.[27] However, there was no desired product observed, which could result from the cramped environment of carbonyl unit in **70**. Then, we used the reagent (diazomethyl)trimethylsilane ($TMSCHN_2$),[28] and fortunately, acetylene **71** was furnished in 86% yield. Deprotection of acetal **71** using hydrochloric acid and acetone, generated the requisite ketone **72** in 75% yield. It is worth noting that ketone **72** is volatile, leading to an inevitable decrease in yield. Next, we carried out the olefination of our new ketone **72** with phosphonium salt **61** using *t*-BuOK as base. To our delight, the exocyclic (*Z*)-configured alkene **75** was smoothly obtained in 81% yield and excellent stereoselectivity (*Z* / *E* > 30:1).

With the crucial olefination product **75** in hand, we then focused our attention on the assemblage of the 3-alkyl furan unit in (**Scheme 28**). Hydroboration of alkyne **75** with pinacolborane, catalyzed by Cp_2ZrHCl[29] gave the *trans*-configured vinyl pinacol boronate **76** as the sole isomer in 72% yield. The boronate **76** was subjected to the oxidation with trimethylamine *N*-oxide (TMANO), to smoothly afford aldehyde **77** in nearly quantitative yield (98%).[30] Treatment of **77** with 3-furyllithium, generated *in situ* by reaction of 3-bromofuran with *n*-BuLi at -78 °C, provided the corresponding alcohol mixture **78** in 95%

yield.[31] This epimer mixture was directly used in the next step, namely, deoxygenation using the Barton-McCombie method (cf. **78**→**51**, **Scheme 28**).[15b].



Scheme 28. Total Synthesis of Compound **6**

Specifically, the alcohol group in **78** was first esterified with carbon disulfide and iodomethane to generate the xanthate.[15] Then, the xanthate segment was removed through a radical reduction with *n*-Bu_3SnH using AIBN as an initiator. Under these conditions, we were able to obtain precursor **51** in 81% yield over two steps. We also tried the one-step deoxygenation of **78** involving excess $NaCNBH_3$ (8.0 equiv) and ZnI_2.[32] A small amount of **51** was observed in the crude ^1H NMR (ca. 16% yield), and most starting material remained intact. Also, extra reaction times decreased the yield.

Conversion of the furan into γ-hydroxybutenolide was accomplished following regioselective photo-oxidation involving singlet oxygen ($^1O_2$) and the bulky Hünig's base *N*,*N*-diisopropylethylamine (cf. **51**→**6**, **Scheme 28**).[33] Compound **6** was obtained as a sole isomer in 83% yield after chromatography. Hence, the total synthesis of **6** was achieved from (*R*)-pulegone in 16 linear steps and an overall yield of 13.8%.

2.4 Spectroscopic Comparison of Natural and Synthetic Niphateolide A

The β-substituted-γ-hydroxybutenolide and isopropene units in **6** were unequivocally confirmed by direct comparison of its NMR spectra with those of the well-known sesterterpenoid dysidiolide.[13] Furthermore, the NOE correlation between C5-H and C13-H in olefination product **75** (**Figure 17**) confirmed the (*Z*)-configuration of the exocyclic olefin unit. The NOE correlation between C10-H and C18-H in **75** shows the *trans*-18,19-dimethyl configuration.

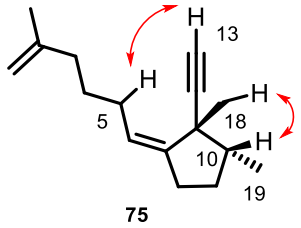


Figure 17. Key NOE Correlations Observed in **75**

However, we are surprised that neither the ^{1}H nor the ^{13}C NMR data of the cyclopentane core in synthetic **6** matched those of natural niphateolide A reported by Tsukamoto (**Table 3**).[9] Given the big differences in the chemical shifts of the C6-11 and C18-19 fragments, and the optical rotation {$[\alpha]_D^{22}$ -39.2° (*c* 0.05, MeOH); lit.[9]: $[\alpha]_D^{20}$ +13° (*c* 0.46, MeOH)}, it is evident that the originally assigned structure of niphateolide A is incorrect.

Table 3. Comparison of Natural and Synthetic Niphateolide A by ^{1}H and ^{13}C NMR

Position	^{1}H NMR {δ ppm, multipl., *J* (Hz)} $CDCl_3$, 500 Hz		^{13}C NMR {δ (ppm)} $CDCl_3$, 125 Hz		
	Natural	Synthetic	Natural	Synthetic	Difference
1	1.70, s	1.72, s	22.43/22.45	22.47	< 0.1
2			145.95/145.96	145.96	0
3	2.01, br t (7.3)	2.02, br t (8.1)	37.91/37.93	37.53	-0.4
4	1.39, m; 1.63, m	1.49, m	27.30/27.34	27.86	+0.6
5	1.86, m; 1.92, m	2.19, m; 2.40, m	30.73/30.75	31.14	+0.3
6	5.48, br t	5.30, m	122.17/122.25	123.47	+1.2
7			141.8	147.11	+5.3
8	1.97, m; 2.06, m	1.73, m; 2.37, m	23.83/23.88	33.23	+6.0
9	1.54 m	1.29, m; 1.80, m	27.16/27.19	28.36	+1.17
10	1.60 m	2.05, m	37.68/37.76	46.31	+8.6
11			39.7	47.19	+7.5
12	1.55, m; 1.69, m	1.62, m	32.52/32.55	31.95	-0.6
13	2.29, m; 2.39, m	2.35, m	24.0	24.49	+0.5
14			169.90	170.96	+1.06
15	5.82, s	5.85, s	117.12/117.17	117.12	0
16			169.90/170.58	170.12	< 0.1
17	5.96, s	6.02, s	98.43/98.51	99.09	+0.58
18	1.07, s	1.22, s	26.46/26.47	25.18	-1.29
19	0.93, d (6.7)	0.96, d (7.0)	16.01/16.03	13.61	-2.42
20	4.65, s; 4.69, s	4.72, s; 4.68, s	109.85/109.86	109.82	< 0.1

2.5 Conclusion

We have completed the first total synthesis of the alleged structure of niphateolide A in 16 linear steps and an overall yield of 13.8%, starting from commercially available (*R*)-pulegone. The exocyclic (*Z*)-configured olefin appendage was ultimately installed through successful modification of the structure of the ketone substrate, thereby reducing the steric hindrance surrounding the keto group. Finally, the β-alkyl-γ-hydroxybutenolide moiety was constructed in highly regioselective fashion using Faulkner method. Our synthesis has proven that the structure of niphateolide A proposed by Tsukamoto et al (cf. **6**)[9] is incorrect.

The actual structure of niphateolide A will be revealed in the next chapter along with irrefutable proof by total synthesis.

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Chapter 3 Revision of Structure and Total Synthesis of Niphateolide A

3.1 Total Synthesis Endeavors Leading to the Correction of Structure of Misassigned Natural Products

Since the birth of the concept of 'molecular structure', chemical synthesis has been a reliable tool to probe the geometric features of unknown compounds. Nowadays, although the science of assigning molecular structure has advanced considerably due to the impressive developments of modern spectroscopic methods and X-ray crystallography, it is somewhat surprising that total synthesis still plays a crucial role in the field. Much like in the early days, it remains the ultimate proof of structure and plays a pivotal role in the elucidation of incompletely assigned structures, and in disproving and revising molecular structures that are misassigned (many of which appear frequently in today's literature).[1]

In our laboratory, over 40 natural products have been synthesized so far. In addition to confirming the originally assigned structures, our group has established the hitherto unknown stereochemistry of (+)-zerumin B (**Figure 18**).[2] Zerumin B was first isolated in 1996 from the Chinese medicinal plant *Alpinia zerumbet*,[3] which exhibits cytotoxic activity against a panel of human tumor cell lines.[4] The gross skeleton and absolute configuration around the decalin core of zerumin B were determined by NMR studies. Yet, the stereochemistry at C-12 in zerumin B was unknown before its first synthesis in our lab. The synthesis of (+)-zerumin B was accomplished from (+)-sclareolide (five steps, 48% overall yield),[2] and the stereochemistry of C-12 was established as depicted in **Figure 18**.[5]

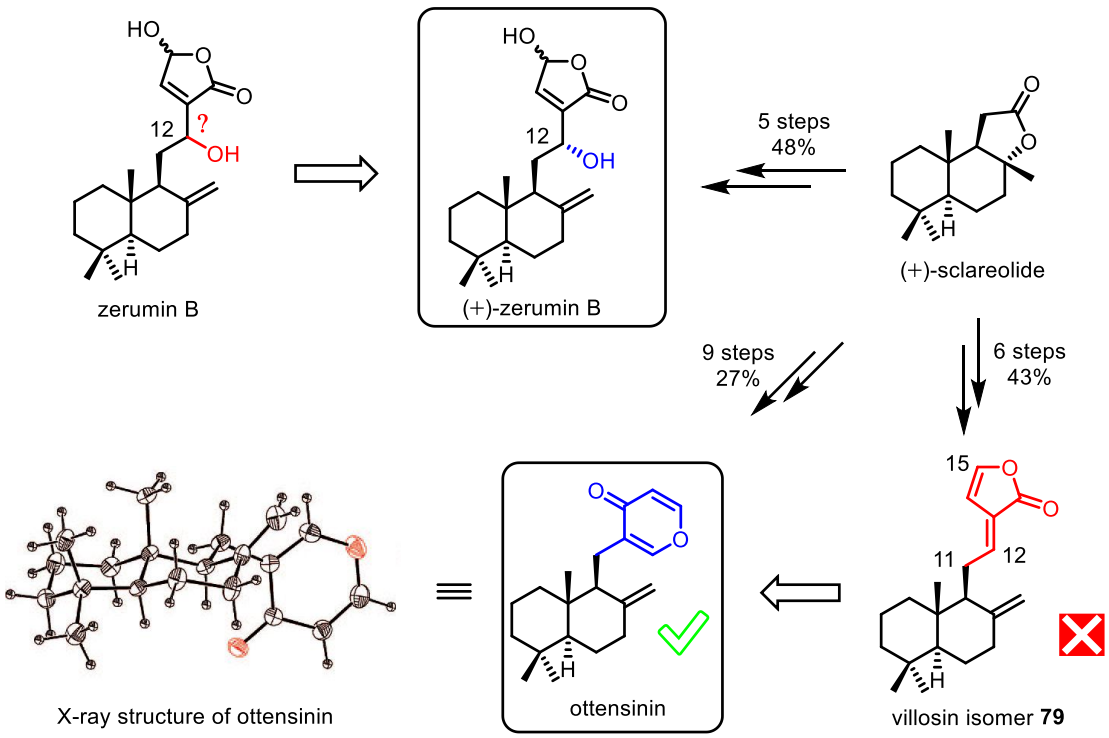


Figure 18. Examples of Structure Revision from the Group of Boukouvalas

In 2006, Kikuzaki and co-workers isolated a new diterpenoid from the rhizome of *Zingiber ottensii* Val. (Zingiberaceae).They assigned structure **79** to this molecule[6] (**Figure 18**), which represents an isomer of the known diterpenoid villosin. Interestingly, **79** features a rare (*E*)-3-alkylidene-2(3*H*)-furanone moiety and the same decalin core as zerumin B displays. Shortly after Kikuzaki's publication, our group completed the synthesis of **79** from (+)-sclareolide (six steps, 43% overall yield).[7] However, the irreconcilable differences in the chemical shifts (^{1}H and ^{13}C) of the C11-16 fragment, disapproved the previously assigned structure **79**.

To uncover the actual structure of the Kikuzaki's natural product, our group focused on the segment (C11-16) that differs with the synthetic compound **79** in the chemical shifts. The distinctly downfield protons (δ 7.67 and 7.52) that were originally assigned as C12-H and C14-H in **79**, are contradictive to the shifts of α-alkylidenebutenolide protons, which appear below 7 ppm. In addition, neither γ-ylidenebutenolides nor 2-ylidenefuran-3-ones

have protons obove δ 7.2. Based on these observations and the NMR data, a completely different heterocyclic ring structure was proposed, namely, 4*H*-pyran-4-one fragment (**80**, **Figure 19**) which settles the two downfield protons. Thus, a new structure was proposed along with the name "ottensinin" for this originally misassigned natural product (**Figure 18**).[8]

A literature survey revealed only two other natural products possessing such a 3-alkyl 4*H*-pyran-4-one structure: the fungal metabolite xylaric acid and its congener **81** (**Figure 19**).[9] Significantly, both the ^{1}H and ^{13}C NMR data of the pyranone core in **81** matched well with those of ottensinin's C12-16 fragment, which endorsed the above speculation. Finally, the revised structure was validated by the synthesis of the newly proposed structure of ottensinin from (+)-sclareolide (nine steps, 27% overall yield) and X-ray diffraction analysis of the synthetic sample (**Figure 18**).[8] Unlike the naturally derived sample which was reported to be an oil, which contained small amounts of other compounds, the highly pure and crystalline synthetic sample enabled unambiguous confirmation of its structure by X-ray analysis.

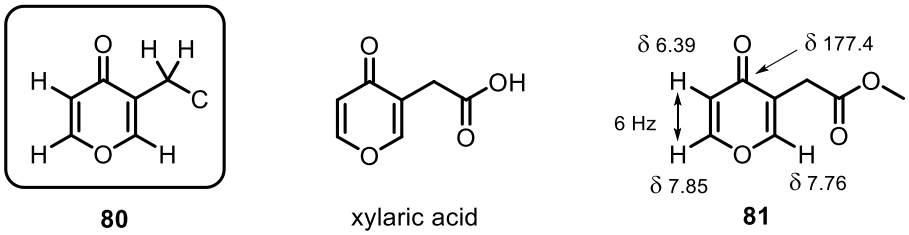


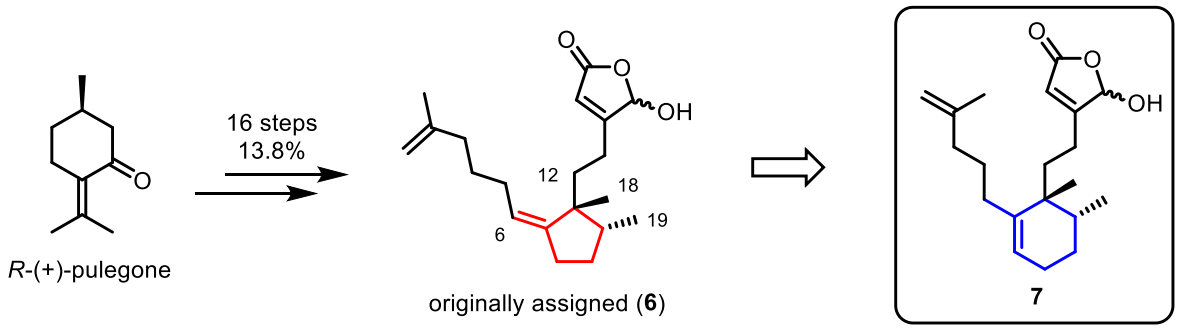
Figure 19. Known Compounds with Substructure **80**[8]

3.2 Structure Revision of Niphateolide A

3.2.1 Newly Proposed Structure

As described in **Chapter 3**, our synthesis revealed that the originally proposed structure **6** (**Figure 20**) for niphateolide A by Tsukamoto et al[10] is incorrect. Structure revision is (usually) an unexpected but important mission of total synthesis endeavors, which has

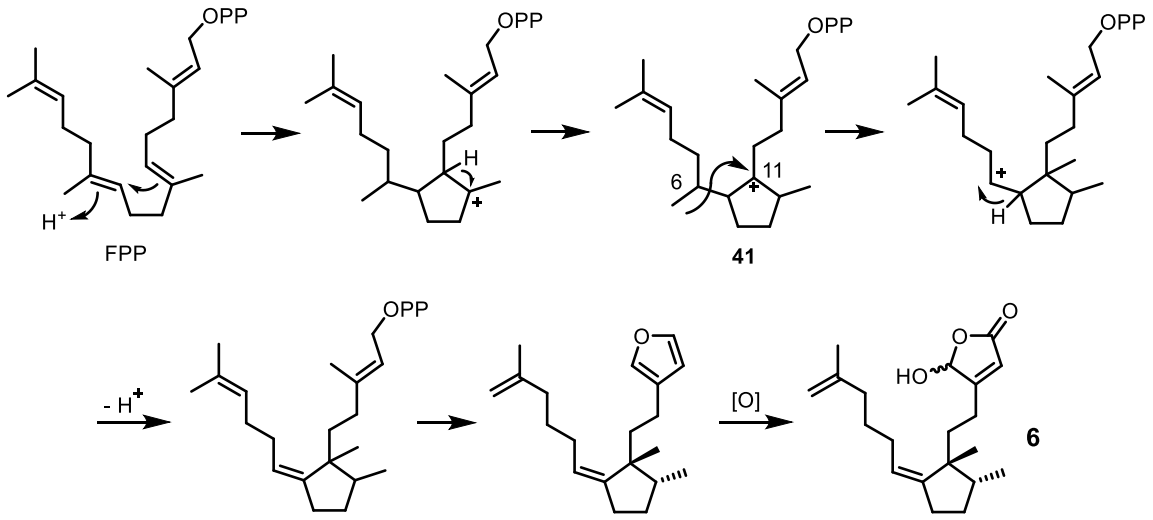
presented itself on numerous occasions in our group. It is both a challenge and scientific duty to uncover the correct structure of novel bioactive molecules such as niphateolide A.



Caption: **6** (originally assigned structure of niphateolide A[10]), **7** (Correct structure of niphateolide A)

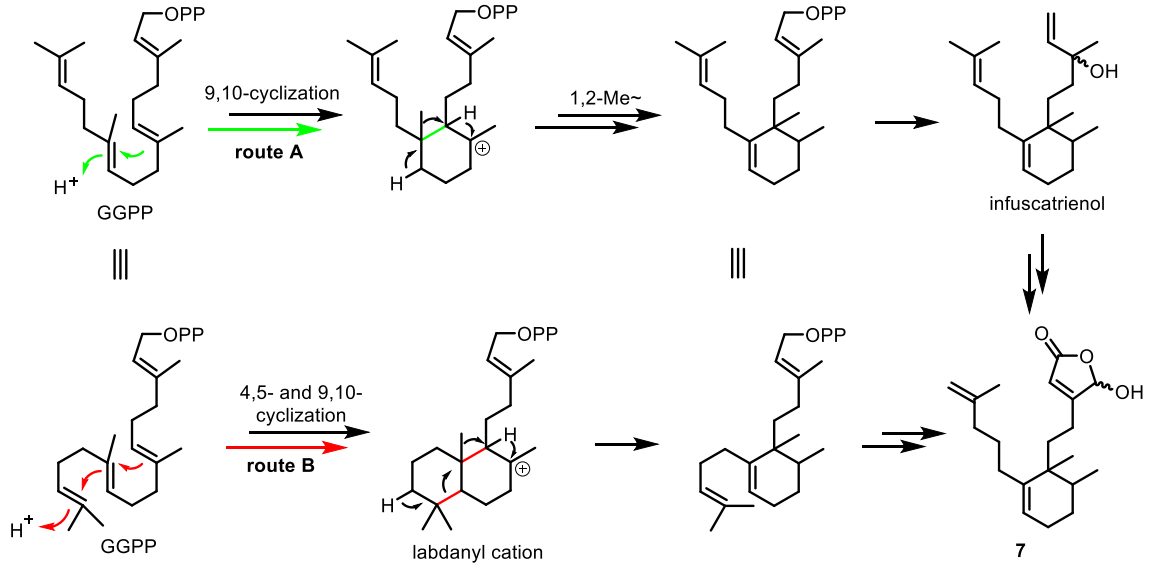
Figure 20. Proposed New Structure

The task at hand began with a thorough assessment of all available data. First, both the β-substituted-γ-hydroxybutenolide and terminal isopropene fragments in **6** were unequivocally confirmed by comparison of their NMR data with those of the same moieties in the prominent natural product dysidiolide whose structure was confirmed by chemical synthesis and X-ray diffraction analysis.[11] Therefore, our attention was directed at the central cycloalkane/C=C dond postion of the molecule. We were intrigued by the difference in the chemical shifts of the C6-11 fragment and the dimethyl unit (C-18 and C-19). At the same time, we thoroughly examined the biosynthesis of **6** proposed by Tsukamoto et al[10] which involves a rather unusual 1,3-shift of a methyl group, accompanied by the transformation of a tertiary cation to a less stable secondary cation (**Scheme 29**). A literature survey failed to uncover any precedent for such a contra thermodynamic transformation in natural product biosynthesis. Having questioned the validity of Tsukamoto's pathway, we proposed a more realistic and indeed likely biosynthesis of niphateolide A leading to a central cyclohexene core, shown in **Scheme 30**. Indeed, a closely related biosynthetic route has been proposed by Asakawa and co-workers for infuscatrienol that possess a similarly substituted cyclohexene core.[12] These considerations led as to propose structure **7** for niphateolide A.



Scheme 29. Proposed Biosynthesis of **6** by Tsukamoto et al[10]

In particular, GGPP could undergo a 9,10-cyclization, followed by deprotonation, methyl and proton shifts to generate unprecedented monocyclic skeleton (**route A**, **Scheme 30**).[12] Thus, it can be inferred that compound **7** could also be generated from GGPP through the route A or directly from infuscatrienol *via* oxidation, cyclization, and olefin migration process. Alternatively, **7** might be obtained from the labdane-type cation *via* a rearrangement of the decalin core (**route B**).



Scheme 30. Proposed Biosynthesis of **7**

Indeed, a literature survey revealed that the 3,4-dimethyl cyclohexene ring is frequently found in natural terpenes (**Figure 21**). It became apparent from reported NMR data that the chemical shift value of C6-H (δ 5.48) of niphateolide A is due to an endocyclic C=C-H rather than the exocyclic olefin in **6**. All that remained at this point was to clarify whether the vicinal methyl substituents of the cyclohexene ring were *cis* or *trans*. This task entailed further comparison of the NMR data of niphateolide A with those of several natural products containing a *cis* or *trans vic*-dimethyl substituted cyclohexene core.

Pleasingly, we were able to find an article by Gavagnin and co-workers who summarized the ^{13}C NMR data of the dimethyl unit in 3,4-dimethyl cyclohexene fragment (**Figure 21**).[13] The reported ^{13}C chemical shifts of C-10 (δ 37.7), Me-18 (δ 26.5) and Me-19 (δ 16.0) match well with those of *trans*-**82** respectively (δ 37.6-37.8, 26.3-26.6 and 15.9-16.0). In addition, ^{13}C chemical shifts of Me-18 (δ 20.9-21.1) and C-10 (33.1-33.3) in *cis*-**82** are apparently different from those of *trans*-**82**, suggesting the *trans*-configuration of the dimethyl unit.

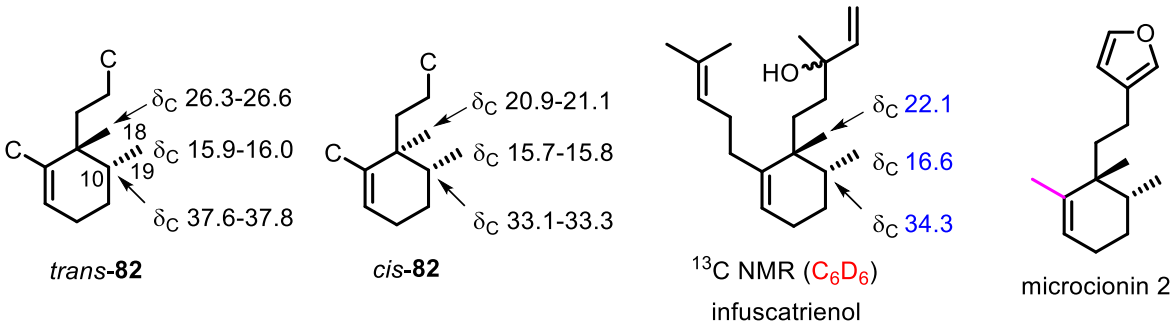


Figure 21. Examples of Compounds with Substructure *trans*-**82**

Of course, the closest structure to **7** is that of infuscatrienol (**Figure 21**) featuring both the *trans*-3,4-dimethyl cyclohexene core, and two appendages. Infuscatrienol was isolated by Asakawa and co-workers in 1997 from a leafy liverwort *Jungermannia infusca*.[12] Yet, the authors provided the NMR data of infuscatrienol using C_6D_6 as solvent instead of $CDCl_3$,

which probably brings about a slight difference in the ^{13}C chemical shifts of C-10, C-18, and C-19.

Then, we compared the reported NMR data of niphateolide A (assumed to possess structure **7**) with those of (-)-mcrocionin 2 which has a methyl group attached on the olefin unit (**Figure 21**).[14] The negligible difference (**Table 4**) in the NMR data of dimethyl cyclohexene core supported our hypothesis, and encouraged us to confirm **7** by total synthesis.

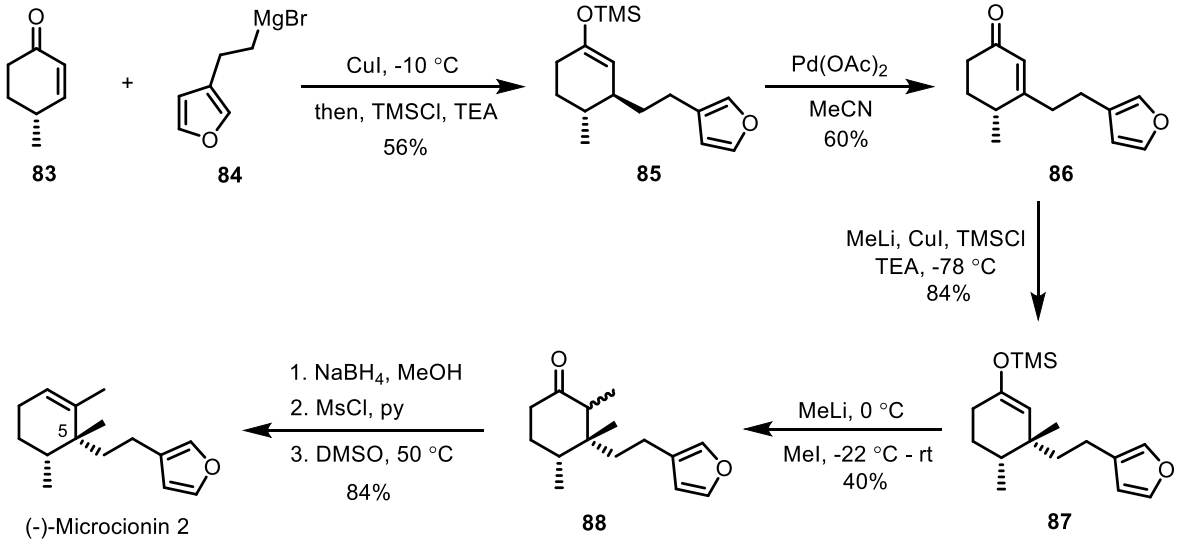
Table 4. ^{1}H and ^{13}C NMR Data for Fragments of **7** and Mcrocionin 2

Position	^{1}H NMR (δ ppm, $CDCl_3$)		^{13}C NMR (δ ppm, $CDCl_3$)	
	Isolation	Mcrocionin 2	Isolation	Mcrocionin 2
6			141.8	142.49
7	5.48	5.43	122.17	122.83
10	1.60	1.68-1.41	37.68	37.53
11			39.7	39.50
18	1.07	1.07	26.46	26.15
19	0.93	0.97	16.01	15.85

3.2.2 Total Synthesis of (-)-Microcionin 2

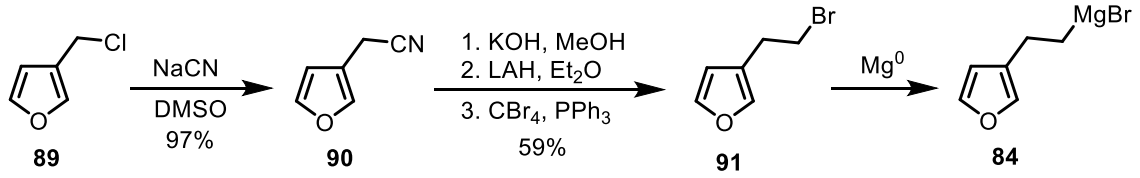
The only reported total synthesis of (-)-microcionin 2 was carried out at Université Laval by Canonne and Potvin in 1996 (**Scheme 31**).[15] The alkylfuran appendage was installed through conjugate addition of *in situ* generated 2-(3-furyl)ethyl cuprate to homochiral cyclohexanone **83** followed by trapping the enolate with trimethylsilyl chloride (TMSCl). The resulting silyl enol ether **85** was subjected to oxidation using stoichiometric palladium (II) acetate to furnish α,β-unsaturated ketone **86**. Treatment of **86** with a Gilman reagent (Me_2CuLi) followed with TMSCl, afforded silyl enol ether **87** as major isomer. On exposure to methyllithium at low temperature, silyl enol ether **87** was transformed to the corresponding llithium enolate which underwent alkylation with iodomethane (cf. **87**→**88**).

Reduction of **88** with $NaBH_4$ followed by mesylation and elimination gave (-)-microcionin 2.



Scheme 31. Canonne's Synthesis of (-)-Microcionin 2

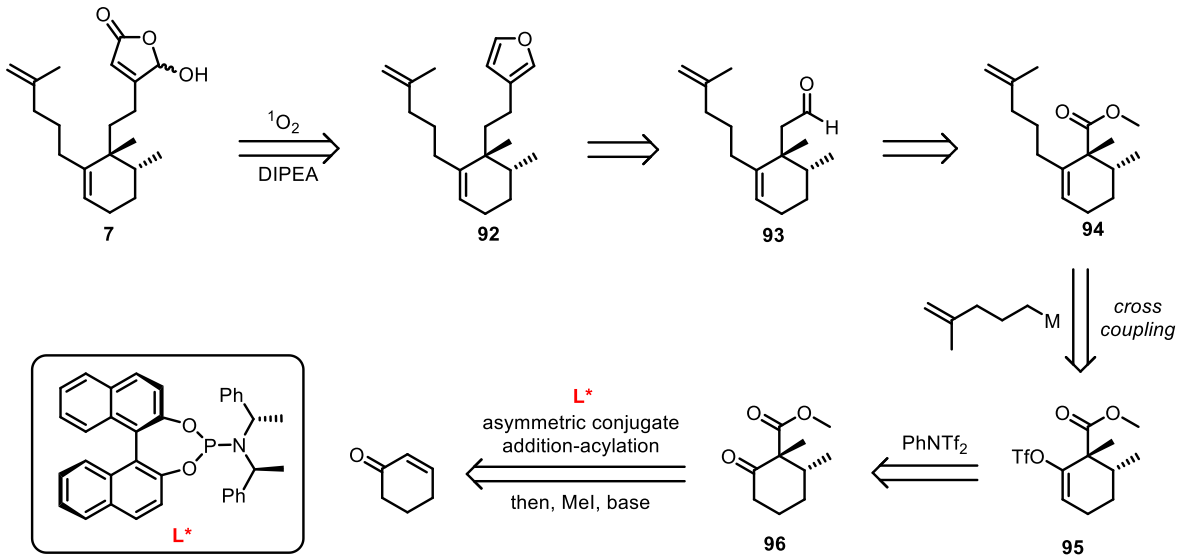
Canonne's synthesis is based on two conjugate additions for the construction of the requisite chiral quaternary center at C5 of Microcionin 2 (**Scheme 31**). However, the first conjugate addition (cf. **83→85**) was modestly efficient (56% yield). Furthermore, the requisite Grignard reagent **84** was prepared from 3-furylmethyl chloride **89** through five linear five steps **(Scheme 32**).[15]



Scheme 32. Canonne's Preparation of Grignard Reagent **84**[15]

3.3 Total Synthesis of Newly Proposed Structure 7 of Niphateolide A

3.3.1 Retrosynthetic Analysis

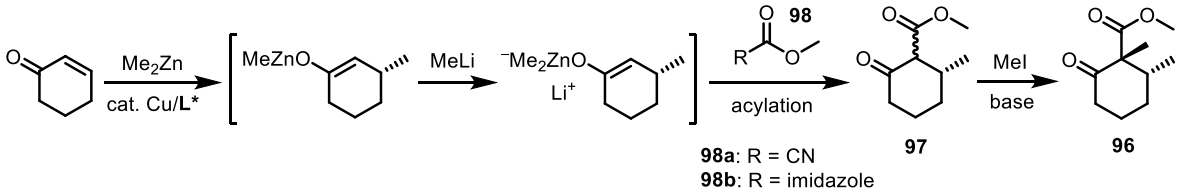


Scheme 33. Retrosynthetic Analysis of **7**

The retrosynthetic analysis of **7** is shown in **Scheme 33**. Given the presence of the same β-alkyl-γ-hydroxybutenolide fragment in both niphateolide A structures **6** and **7**, we opted to use a similar strategy that described for the synthesis of **6** (cf. **Scheme 28**). In the present instance, the requisite precursor is aldehyde **93**, which will be transformed into furan **92** and ultimately **7** by means of the Faulkner method (cf. **93**→**92**→**7**).[16] Aldehyde **93** would arise from carboxylic ester **94** by reduction, oxidation, and Wittig-type homologation[17]. Ester **94** would in turn arise from vinyl triflate **95** through Kumada[18] or Negishi coupling[19] (cf. **95**→**94**). Triflate **95** should be easily accessible by a standard procedure[19] from β-keto ester **96**.

The requisite homochiral building block **96** has been synthesized by Herzon and co-workers[20] by asymmetric conjugate addition/C-acylation of cyclohexanone and subsequent methylation (**Scheme 33**). Specifically, this sequence involves copper-catalyzed enantioselective 1,4-addition of dimethylzinc to cyclohex-2-ene-1-one, followed by *in situ*

reaction of resulting zinc enolate with methyllithium, and C-acylation with Mander's reagent methylcyanoformate **98a**[21] or Heller-Sarpong reagent *N*-(carbomethoxy)-imidazole **98b**[22] (**Scheme 34**). The so obtained β-keto ester **97** is subjected to stereoselective methylation to furnish *trans*-dimethyl cyclohexanone **96**. This method has been used in total synthesis of natural products, such as (+)-pleuromutilin[23] and (+)-12-*epi*-mutilins.[24]

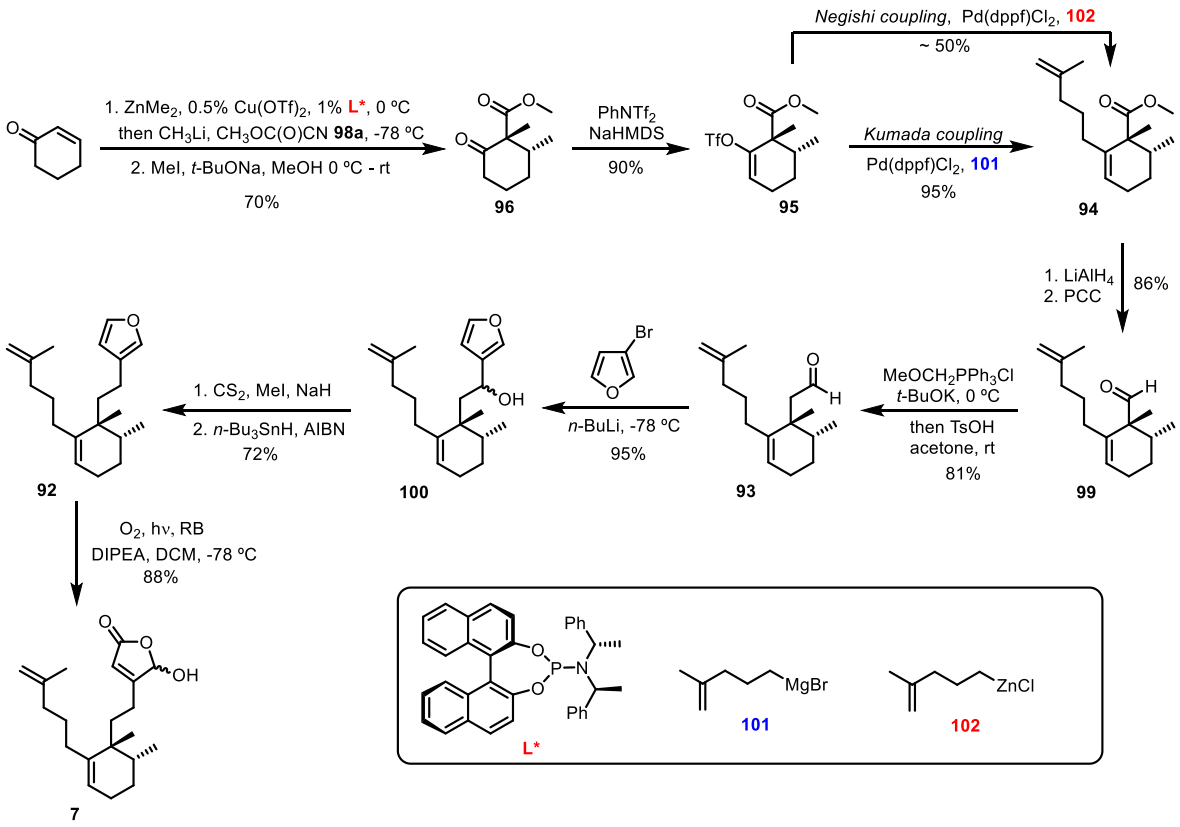


Scheme 34. Herzon's Asymmetric Michael Addition/C-acylation/methylation sequence

3.3.2 Total Synthesis of 7-the Actual Structure of Niphateolide A

Our synthesis of **7** began with the copper-catalyzed asymmetric Michael addition of cyclohex-2-ene-1-one, followed by *in situ* acylation using methylcyanoformate **98a** (**Scheme 35**). The resulting mixture, composed of β-keto ester and tautomer enolate, was directly subjected to α-methylation using iodomethane and sodium *tert*-butoxide to give **96** in 70% yield over two steps. Exposure of **96** to a solution of sodium bis(trimethylsilyl)amide (NaHMDS) in THF, and trapping of the resulting sodium enolate with *N*-phenyltriflimide ($PhNTf_2$) provided vinyl triflate **95** in 90% yield.

With triflate **95** in hand, the installation of the aliphatic chain was addressed (**95→94**). We first tried Negishi coupling with alkylzinc reagent **102**,[25] which gave **94** in a moderate yield (ca. 50%). Much to our delight, Kumada coupling with Grignard reagent **101** proved a better option affording **94** in an excellent yield (95%).[26] The preparation to the requisite reagents, **101** and **102**, is shown in **Scheme 36**.



Scheme 35. Total Synthesis of **7**

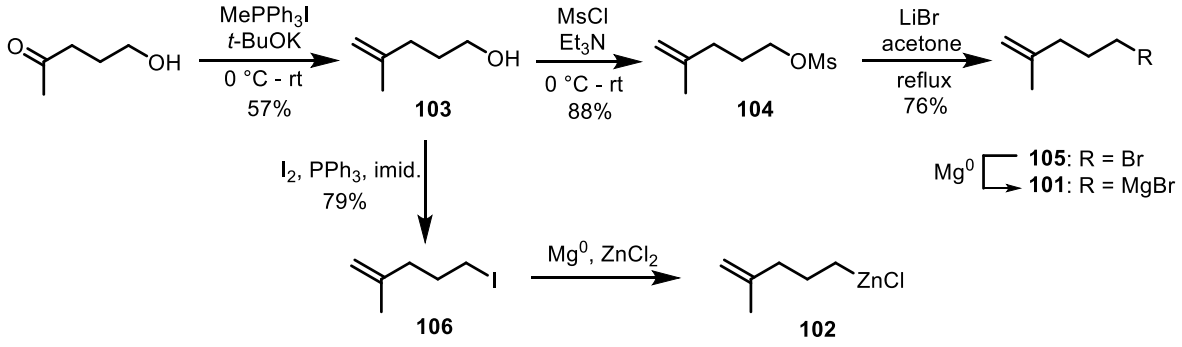
Reduction of the ester group in **94** with $LiAlH_4$ in THF and oxidation of resulting alcohol with PCC provided aldehyde **99** in 86% yield over two steps. Aldehyde **99** was subjected to Wittig olefination with (methoxymethyl)triphenylphosphonium chloride ($MeOCH_2PPh_3Cl$) to give a mixture of *Z*/*E*-enol ethers. The crude mixture was directly converted to the homologous aldehyde **93**, through an acid-induced (*p*-TSA) hydrolysis in acetone in 81% yield.

The conversion of aldehyde **93** to **7** was performed in essentially the same manner as previously described for the synthesis of **6** (**Chapter 3**). Treatment of **93** with 3-furyllithium furnished alcohol mixture **100** in 95% yield (*dr* = 3:1). Barton-McCombie deoxygenation uneventfully delivered furan **92** which was transformed to **7** in high yield

using the Faulkner method.[16] Thus, the first synthesis of **7** was achieved in **11** linear steps and 25.1% overall yield.

3.3.3 Synthesis of Organometallic Reagents 101 and 102

The synthesis of Grignard reagent **101** commenced with the Wittig methylenation of 5-hydroxy-2-pentanone to give the volatile alcohol **103** in 57% yield (**Scheme 36**). To convert alcohol **103** to bromide **105**, we initially used the Appel reaction which provided bromide **105** that is known to be volatile as well.[27] Although this reaction worked well according to TLC analysis, most of the bromide **105** evaporated under low pressure vacuum when removing the solvent (dichloromethane) and purifying the residue. In order to improve the overall yield, we utilized a two-step route to **105** *via* mesylate **104**. Thus, the alcohol **103** was converted to mesylate **104** which is not volatile under vacuum. Then, heating mesylate **104** with lithium bromide in acetone provided bromide **105** in 76% yield after chromatography. Importantly, the solvents (acetone and hexanes) were removed under mild pressure vacuum at ca. 10 °C.



Scheme 36. Synthesis of Requisite Reagents

As shown in **Scheme 36**, the organozinc reagent **102** for Negishi coupling was made from **103**. Conversion of **103** to iodide **106** was achieved by means of Garegg-Samuelsson reaction involving I_2, PPh_3, and imidazole.[28] Then, **102** was generated *in situ* from **106** using magnesium-zinc transmetalation.[29]

3.4 Confirmation of Proposed Structure 7 for Niphateolide A

Both the ^{1}H and ^{13}C NMR spectrum of the synthetic compound **7** matched well those reported by Tsukamoto for niphateolide A,[10] which proves that **7** is indeed the correct structure of the natural product. Furthermore, the sign of optical rotation of synthetic niphateolide A is the same as that of the natural product {synthetic **7**: $[\alpha]_D^{22}$ +35.7°, *c* 0.06, MeOH}, natural niphateolide A { $[\alpha]_D^{20}$ +13°, *c* 0.46, MeOH}[10]. Therefore, both the structure and absolute stereochemistry of niphateolide A are established to be as depicted in **7.**

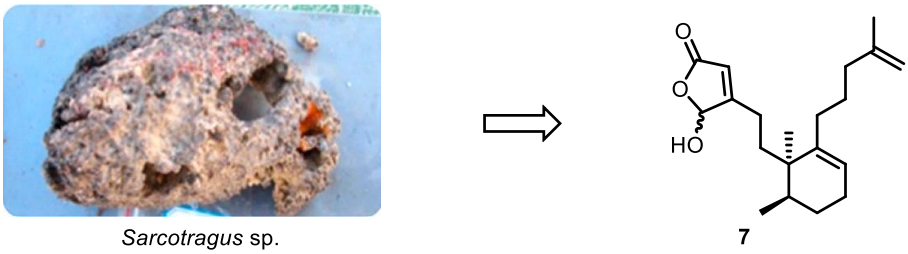


Figure 22. Isolation of **7** from *Sarcotragus* sp.

Interestingly, after completion of our work, a compound possessing **7** was isolated by Li and co-workers from the sponge *Sarcotragus* sp. collected in the South China Sea (**Figure 22**).[30] The authors named this compound echinohalimane B and made the comment "Confusingly, the spectroscopic data and physical properties of **8** (Echinohalimane B) agreed perfectly with those reported for Niphateolide A". The specific rotation value of echinohalimane B was reported as $[\alpha]_D^{25}$ +37° (*c* 0.20, MeOH)[30] that is also the same in terms of sign and very close in magnitude to that of our synthetic niphateolide A (a.k.a. echinohalimane B).

3.5 Conclusion

In summary, the originally reported structure of niphateolide A (**6**), was proven to be incorrect by total synthesis and was subsequently revised to **7** on the basis of biosynthetic considerations and re-analysis of the NMR data. Our synthesis of **7** is concise and efficient

(11 steps, 25.1% overall yield) and should readily allow access to new analogues for future biological studies.

3.6 References

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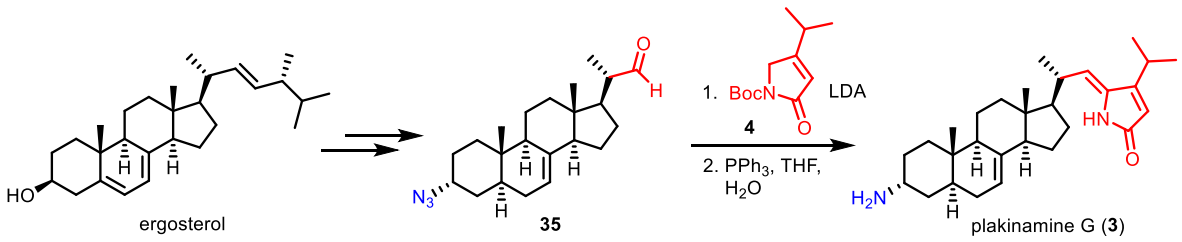
Conclusion and Perspectives

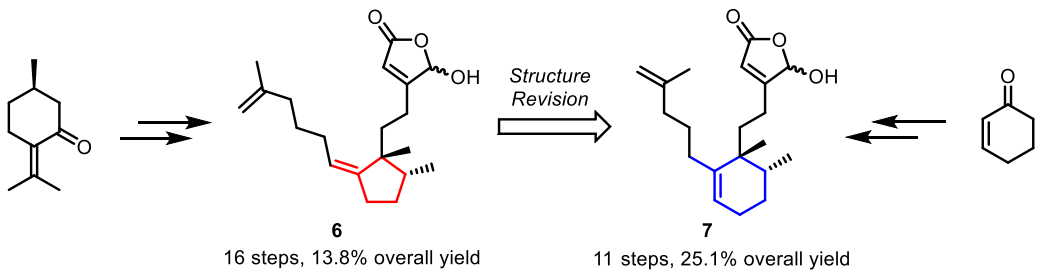
C.1 Accomplishments and Perspectives

We have achieved the first synthesis of the marine antitumor steroidal alkaloid plakinamine G (**3**) from commercially available ergosterol (**Scheme 37**). The synthesis demonstrates the serviceability of Li's method[1] for installing the (*Z*)-γ-alkylidene-γ-lactam motif (cf. **35**→**3**) onto chiral, readily epimerizable aldehyde substrates. Previously, this method had only been utilized in the synthesis of Pulchellalactam, a simple achiral molecule assembled from isobutyraldehyde. We also employed a new, more sterically demanding lactam reagent (cf. **4**) which bears a β-isopropyl substituent instead of a methyl group featuring in Li's work. We therefore anticipate that our synthesis will promote wider application of this methodology. It is also worth noting that our synthesis of **3** was performed in protecting-group-free fashion. In doing so, the azido group served as a masked amine, and was shown to be robust under various reagents and conditions, for example, during ozonolysis and the presence of strong bases.



Scheme 37. Synthesis of Plakinamine G

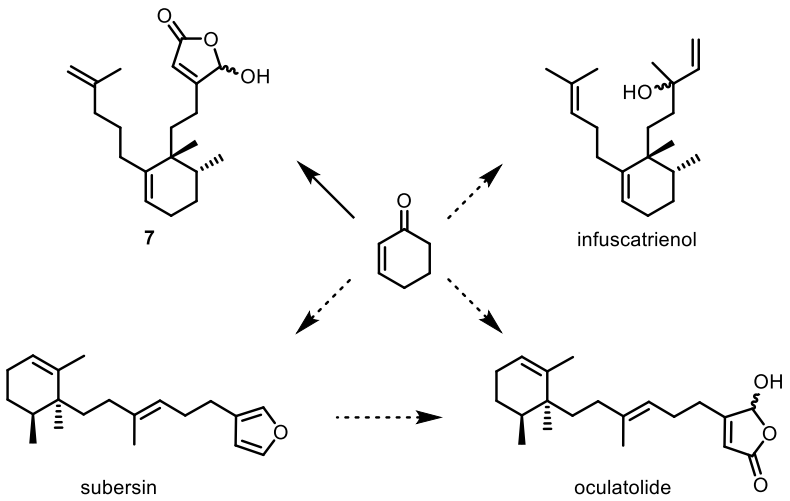
In addition, the first total synthesis of niphateolide A (**6**), initially proposed by Tsukamoto[2] as the molecular structure of a marine sponge diterpenoid, was accomplished from fairly inexpensive (*R*)-pulegone (**Scheme 38**). However, the huge difference of spectroscopy data revealed that molecule **6** is not the real structure of the natural product found in the sponge *Niphates olemda*. Re-analyzing the reported ^{1}H and ^{13}C NMR data, we put forward a new structure **7** which was finally confirmed by an enantioselective synthesis from cyclohex-2-en-1-one.



Caption : **6** (originally assigned structure of Niphateolide A), **7** (Correct structure of Niphateolide A)

Scheme 38. Structure Revision and Enantioselective Total Synthesis of (+)-Niphateolide A

Access to sufficient quantities of (+)-niphateolide A by total synthesis would permit more profound biological studies on this molecule. Furthermore, our concise enantioselective route may serve as template for the expedient synthesis of infuscatrienol, that shares the same carbon skeleton, and related diterpenoids (e.g. subersin and oculatolide) that possess a similar *trans* dimethyl-substituted cyclohexene core (**Scheme 39**).



Scheme 39. Monocyclic Diterpenoids Targeted by Our Group

C.2 References

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Experimental Part

E.1 General Methods:

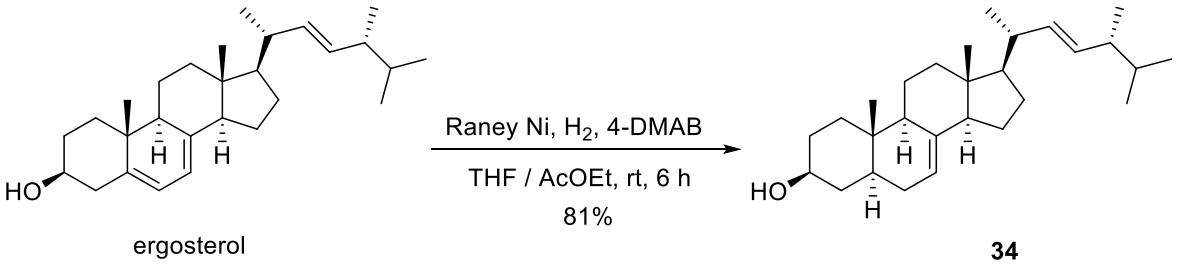
All air and/or moisture sensitive reactions were carried out under argon or nitrogen gas atmosphere in flame-dried glassware using freshly distilled or commercially available anhydrous solvents. Dichloromethane was distilled from calcium hydride, acetone and toluene from calcium chloride, and THF from sodium/acetophenone. Ozone gas was generated using A2Z MP-1000 Multi-Purpose Ozone Generator. A 500 W lamp was used as the light source of Faulkner method. Hydrogenation was carried out with a balloon full of H_2 gas. Unless otherwise indicated, commercial reagents were used as received. Disposable syringes and stainless steel needles were used to transfer air- and moisture-sensitive liquids/solutions. Sealed tube reactions were performed in Kimble Kimax® thick-walled screw capped tubes measuring 13 x 100 mm. Reactions were monitored by thin layer chromatography (TLC) carried out using Merck 0.2 mm silica gel 60 F254 aluminum-backed plates. These plates were visualized by UV light and either a potassium permanganate solution or *p*-anisaldehyde stain was used as developing agent. Flash column chromatography was performed using Silicycle silica gel 60 (230-400 mesh).

NMR spectra were recorded on an Agilent DDR spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C nuclei, or a Varian Inova spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei, or Bruker AC spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C nuclei; ^{1}H and ^{13}C spectra are reported in parts per million (ppm) from tetramethylsilane with the solvent resonance as the internal standard (^{1}H NMR: δ_H 7.26 in $CDCl_3$, 3.31 in methanol-d_4; ^{13}C NMR: δ_C 77.13 in $CDCl_3$, 49.00 in methanol-d_4). Coupling constants *J* values are given in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Electrospray ionization (ESI) high-resolution mass spectra were recorded on an Agilent 6210 TOF LC/MS instrument. Infra-red spectra were recorded on a Bomem Arid Zone MB-series FTIR instrument, with samples prepared on single NaCl plates, as either a pure film if in liquid form, or a neat layer if solid. Melting points were recorded on a

Barnstead/Thermolyne MelTemp® Electrothermal Apparatus. All optical rotations were measured using a Jasco DIP-360 Digital Polarimeter.

E.2 Chapter 1: Synthesis of Plakinamine G

5,6-Dihydro-ergosterol **34**:



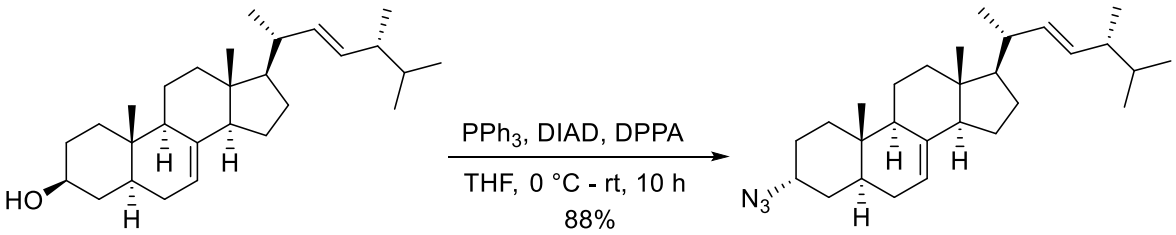
ergosterol **34**

To a solution of NaOH (3.75 g) in water (15 ml) was added portion-wise nickel-aluminium powder (3.00 g) at 0 °C. Then the reaction mixture was allowed to warm to room temperature over one hour, and stirred at 70 °C for 2 h. The grey suspension was allowed to come back to room temperature, and the obtained catalyst was washed with water, EtOH and EtOAc in sequence.

To a stirred suspension of Raney nickel catalyst prepared above in EtOAc (18 ml) and THF (18 ml), was added ergosterol (1.00 g, 2.52 mmol, 1.0 equiv) and 4-dimethylaminobenzaldehyde (4-DMAB, 0.19 g, 1.26 mmol, 0.5 equiv). The hydrogenation of the reaction mixture was carried out at room temperature and at atmospheric pressure (H_2 balloon) for 6 h. Then, the reaction mixture was decanted, filtered over celite. The resulting filtrate was concentrated under vacuum, and recrystallised from $CHCl_3$/MeOH to afford **34** (0.82 g, 81%) as white solid. NMR data were in agreement with those previously reported.[1] mp 172-173 °C. lit.[2] 172.5-175 °C; $[\alpha]_D^{22}$ -22.8° (*c* 0.10, $CHCl_3$), lit.[3] $[\alpha]_D^{20}$ -20° (*c* 1, $CHCl_3$); IR (NaCl, film) *v* 3436, 2953, 2869, 1664, 1469, 1457, 1446, 1382, 1368, 1048, 1039, 966, 869 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.24-5.13 (m, 3H), 3.63-3.55 (m, 1H), 2.06-1.96 (m, 2H), 1.88-1.19 (m, 19H), 1.12-1.08 (m, 1H), 1.01 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.83 (t, *J* = 6.4 Hz, 6H), 0.79 (s, 3H), 0.54 (s, 3H); ^{13}C NMR (100

MHz, $CDCl_3$) δ 139.5, 135.7, 131.9, 117.4, 71.0, 55.9, 55.1, 49.4, 43.3, 42.8, 40.5, 40.3, 39.4, 38.0, 37.1, 34.2, 33.1, 31.5, 29.6, 28.1, 22.9, 21.5, 21.1, 19.9, 19.6, 17.6, 13.0, 12.1; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{28}H_{47}O$ 399.3627, found 399.3612.

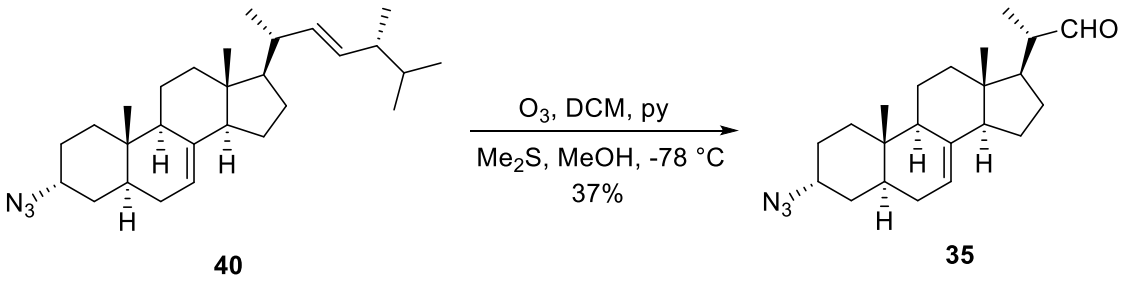
3α-Azido-5,6-Dihydro-ergosterol **40**:



34 **40**

To the solution of PPh_3 (94.3 mg, 0.36 mmol, 1.3 equiv) in 1.4 mL of dry THF was slowly added DIAD (83 μL, 0.42 mmol, 1.5 equiv) at 0 °C under N_2 atmosphere. The resulting suspension was stirred for 10 min. To the reaction mixture was added a solution of alcohol **34** (0.11 g, 0.28 mmol, 1.0 equiv) in 0.8 mL of dry THF. It was stirred for another 10 min before DPPA (80 μL, 0.37 mmol, 1.3 equiv) was added dropwise. The reaction mixture was allowed to warm to room temperature, and stirred for 10 h. Then the solvent was evaporated and the residue was purified by flash chromatography (2 % EtOAc in hexanes) to afford **40** (0.10 g, 88%) as white solid. ^{1}H NMR Spectroscopic data were in agreement with those previously reported.[4] mp 96-97 °C; $[\alpha]_D^{22}$ +0.37° (*c* 0.10, $CHCl_3$); IR (NaCl, film) *v* 2947, 2923, 2877, 2853, 2825, 2099, 1455, 1382, 1367, 1320, 1277, 1263, 966, 760 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.25-5.15 (m, 3H), 3.94-3.90 (m, 1H), 2.06-1.98 (m, 2H), 1.90-1.81 (m, 2H), 1.79-1.22 (m, 19H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H), 0.84 (t, *J* = 7.0 Hz, 6H), 0.80 (s, 3H), 0.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.5, 135.7, 131.8, 117.3, 57.8, 55.9, 55.1, 49.3, 43.3, 42.8, 40.5, 39.4, 35.4, 34.6, 33.1, 32.7, 32.0, 29.2, 28.1, 25.6, 22.9, 21.2, 21.1, 20.0, 19.7, 17.6, 12.4, 12.1; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{28}H_{46}N_3$ 424.3692, found 424.3671.

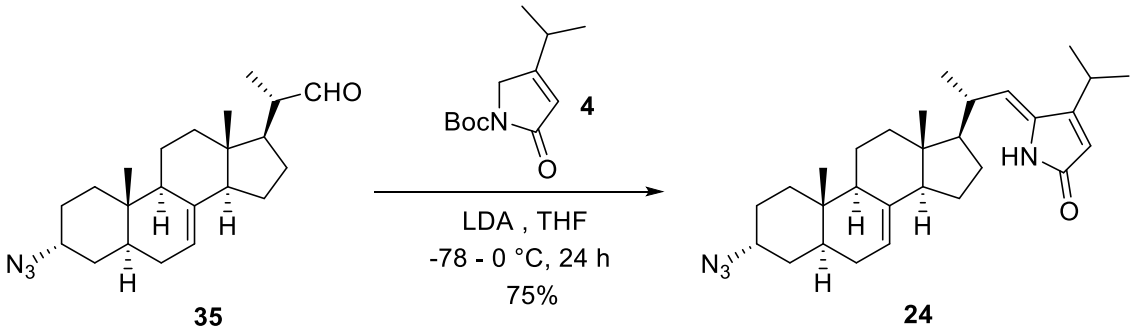
3α-Azido-steroidal aldehyde **35**:



40 **35**

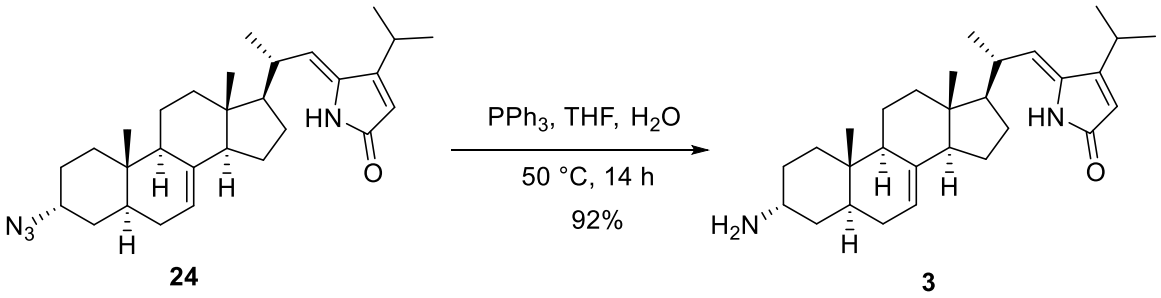
To a stirred solution of azide **40** (0.20 g, 0.47 mmol, 1.0 equiv) and pyridine (0.25 ml, 3.10 mmol, 6.6 equiv) in dichloromethane (45 mL) was bubbled ozone at -78 °C for 3 min. After further 2 min, MeOH (2.5 ml) and dimethyl sulfide (0.12 ml, 1.65 mmol, 3.5 equiv) were added. The reaction mixture was stirred for 30 min at -78 °C, and then allowed to warm to room temperature. The solvent was removed under vacuum, and the residue was purified by flash chromatography (hexanes to recover the starting material, and then 5% EtOAc in hexane) to afford **35** (61.7 mg, 37%) as white solid: mp 114-115 °C; $[\alpha]_D^{22}$ +10.0° (*c* 0.10, $CHCl_3$); IR (NaCl, film) *v* 3423, 2935, 2874, 2704, 2100, 1723, 1664, 1455, 1445, 1381, 1368, 1278, 1262, 1238, 1192, 1094, 985, 886, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.59 (d, *J* = 3.0 Hz, 1H), 5.20 (s, 1H), 3.95-3.90 (m, 1H), 2.41-2.33 (m, 1H), 2.01-1.83 (m, 3H), 1.82-1.29 (m, 17H), 1.15 (d, *J* = 6.5 Hz, 3H), 0.80 (s, 3H), 0.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.0, 138.6, 118.0, 57.8, 54.3, 50.9, 49.8, 49.2, 43.9, 39.2, 35.4, 34.5, 32.7, 31.9, 29.2, 26.7, 25.6, 23.2, 21.1, 13.6, 12.4, 12.3; HRMS (ESI-TOF) *m*/*z* $[M + NH_4]^+$ calcd for $C_{22}H_{37}N_4O$ 373.2967, found 373.2949.

Steroidal lactam **24**:



To the solution of lactam **4** (67.5 mg, 0.30 mmol, 1.5 equiv) in 2 mL of dry THF was dropwise added LDA (0.26 mL, 0.26 mmol, 1.0 M in THF, 1.3 equiv) at -78 °C under N_2 atmosphere. After 5 min, the solution of **35** (71.0 mg, 0.20 mmol, 1.0 equiv) in 1 mL of dry THF was added dropwise, and the reaction mixture was stirred for 30 min at the same temperature. It was allowed to warm up to room temperature, and stirred for 24 h. Then it was quenched with water, and extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc in hexanes) to obtain **24** (69.3 mg, 75%) as white solid: mp 234-235 °C; $[\alpha]_D^{22}$ -58.8° (*c* 0.10, $CHCl_3$); IR (NaCl, film) *v* 3169, 3036, 2962, 2930, 2871, 2850, 2099, 1689, 1455, 1443, 1446, 1382, 1368, 1279, 1261, 1045, 964, 845, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (s, 1H), 5.84 (s, 1H), 5.20-5.15 (m, 2H), 3.94-3.90 (m, 1H), 2.82-2.72 (m, 1H), 2.50-2.40 (m, 1H), 2.06-2.00 (m, 1H), 1.94-1.81 (m, 2H), 1.80-1.24 (m, 17H), 1.20 (dd, *J* = 6.5, 8.5 Hz, 6H), 1.14 (d, *J* = 6.5 Hz, 3H), 0.80 (s, 3H), 0.61 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 159.8, 138.9, 135.5, 118.7, 117.8, 117.4, 57.8, 56.2, 54.9, 49.2, 43.6, 39.3, 35.8, 35.4, 34.6, 32.7, 31.9, 29.2, 27.5, 25.6 (1), 25.6 (0), 23.3, 22.9, 22.5, 21.2, 20.5, 12.4, 12.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{29}H_{43}N_4O$ 463.3437, found 463.3414.

Plakinamine G (**3**):



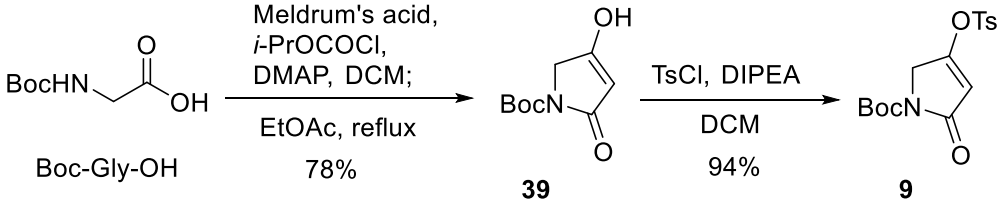
To the solution of **24** (0.15 g, 0.33 mmol, 1.0 equiv) in THF (13 mL) and H_2O (0.8 mL) was added PPh_3 (0.17 g, 0.66 mmol, 2.0 equiv). The reaction mixture was heated to reflux for 14 h. The solvent was evaporated under vacuum and The residue was purified by column chromatography (10% MeOH in DCM, then 1% TEA in MeOH) to obtain plakinamine G (0.13 g, 92%) as colorless gum; $[\alpha]_D^{22}$ -22.2° (*c* 0.09, MeOH), lit.[5] $[\alpha]_D^{22}$ -24.4° (*c* 0.09, MeOH); IR (NaCl, film) *v* 3398, 2952, 2922, 2871, 2849, 1688, 1558, 1457, 1446, 1383, 1368, 1135, 845, 617 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.82 (s, 1H), 5.36 (d, *J* = 10.5 Hz, 1H), 5.27-5.17 (m, 1H), 3.29 (s, 1H), 2.90-2.81 (m, 1H), 2.65-2.73 (m, 1H), 2.11-2.07 (m, 1H), 1.93-1.89 (m, 1H), 1.83-1.59 (m, 10H), 1.55-1.31 (m, 8H), 1.22 (t, *J* = 7.0 Hz, 6H), 1.14 (d, *J* = 6.5 Hz, 3H), 0.85 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 174.5, 162.0, 140.4, 137.2, 122.1, 119.0, 117.7, 57.5, 56.2, 50.8, 46.9, 44.8, 40.7, 36.7, 35.9 (9), 35.9 (8), 35.7, 32.9, 30.8, 29.2, 28.6, 26.6, 24.0, 23.7, 23.0, 22.3, 20.8, 12.7, 12.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{29}H_{45}N_2O$ 437.3532, found 437.3513.

Table 5. Comparison of Natural and Synthetic Plakinamine G by ^{1}H and ^{13}C NMR[a]

position	^{1}H NMR {δ ppm, multipl., *J* (Hz)}		^{13}C NMR {δ (ppm)}	
	Natural[5]	Synthetic	Natural[5]	Synthetic
1	1.63, 1.38	1.63, 1.36	32.8	32.8
2	1.73, 1.30	1.74, 1.33	30.6	30.8
3	3.25 br s	3.26 br s	47.6	46.9
4	1.66, 1.45	1.67, 1.47	35.1	35.7
5	1.65	1.66	35.6	35.9
6	1.73	1.74	30.3	29.2
7	5.22	5.21	118.8	119.0
8			140.4	140.4
9	1.83	1.83	50.7	50.8
10			35.9	35.9
11	1.65, 1.51	1.65, 1.51	22.3	22.3
12	2.08 br d (11.4),	2.09 br d (11.9)	40.7	40.7
13			44.4	44.8
14	1.90	1.91	56.0	56.2
15	1.57, 1.45	1.57, 1.47	23.9	23.7
16	1.87, 1.58	1.86, 1.60	28.5	28.6
17	1.50	1.51	57.7	57.5
18	0.66 s	0.67 s	12.3	12.6
19	0.83 s	0.85 s	12.7	12.7
20	2.68 m	2.69 m	36.7	36.7
21	1.13 d (6.6)	1.14 d (6.6)	20.9	20.8
22	5.36 d (10.7)	5.37 d (10.6)	122.3	122.1
23			137.1	137.2
24			162.1	162.0
25	2.85 m	2.86 m	26.6	26.6
26	1.20 d[b]	1.20 d[b]	24.0	24.1
27	1.22 d[c]	1.22 d[c]	22.8	23.0
28	5.82 s	5.83 s	117.8	117.7
29			174.5	174.5

[a] ^{1}H and ^{13}C NMR data recorded at 500 MHz and 125 MHz respectively. [b] Overlapped with CH_3-27. [c] Overlapped with CH_3-26.

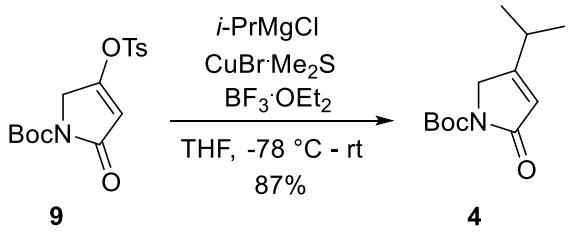
Tosylate lactam **9**:



To a stirred solution of Boc-Gly-OH (1.50 g, 8.57 mmol, 1.0 equiv) in dry DCM (18 mL) were added Meldrum's acid (1.48 g, 10.28 mmol, 1.2 equiv) and 4-DMAP (2.61 g, 21.42 mmol, 2.5 equiv) at 0 °C under argon. Then, a solution of isopropyl chloroformate in toluene (13 mL, 1.0 M, 12.86 mmol, 1.5 equiv) was added dropwise. The reaction mixture was stirred for 3 h at the same temperature. It was washed twice with 15% $KHSO_4$ (aq., 15 mL), dried over $MgSO_4$. The solvent was evaporated under vacuum to afford the crude acylated Meldrum's acid. This material was refluxed in ethyl acetate (170 mL) for 1 h. Then, ethyl acetate was evaporated under vacuum. The resulting crude tetramic acid **39** was directly used in the following step.

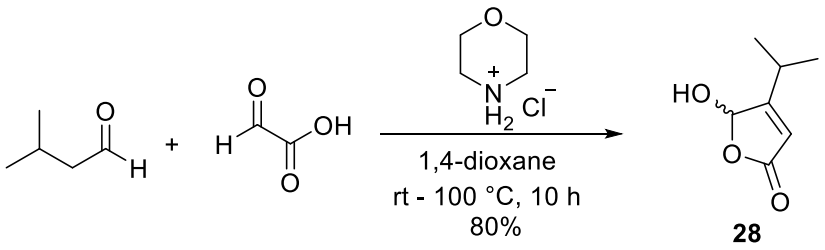
To the solution of crude *N*-Boc-tetramic acid **39** obtained above (normally 6.68 mmol, 1.0 equiv) and *p*-toluenesulfonyl chloride (1.28 g, 6.68 mmol, 1.0 equiv) in dry DCM (60 mL) was added DIPEA (2.33 mL, 13.37 mmol, 2.0 equiv) dropwise at ambient temperature under argon. Then, it was stirred for 6 h at the same temperature. The reaction mixture was washed by 5% HCl aq., 5% $NaHCO_3$ aq., and brine in sequence. The organic layer was dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc in hexanes) to obtain **9** (2.22g, 94%) as a pale yellow solid. NMR data were in agreement with those previously reported.[6] mp 106-108 °C, lit.[6] 115-117 °C; IR (NaCl, film) *v* 2976, 2923, 1786, 1747, 1715, 1633, 1391, 1163, 1084, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.34-7.32 (m, 2H), 5.66 (t, *J* = 1.6 Hz, 1H), 4.13 (d, *J* = 1.2 Hz, 2H), 2.41 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 162.1, 148.2, 146.5, 130.4, 129.8, 127.9, 107.0, 82.9, 48.8, 27.4, 21.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{16}H_{20}NO_6S$ 354.1011, found 354.1017.

Lactam **4**:



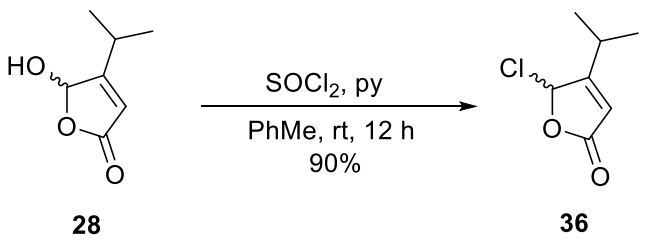
To a suspension of $CuBr{\cdot}Me_2S$ (49.4 mg, 0.24 mmol, 1.2 equiv) in dry THF (0.5 mL) was added dropwise isopropylmagnesium chloride solution (0.24 mL, 2.0 M in THF, 0.48 mmol, 2.4 equiv) at -20 °C under argon atmosphere, and the mixture was stirred for 10 min at the same temperature. Then, it was cooled to -78 °C. The resulting mixture was slowly added to a solution of tosylate lactam **9** (70.6 mg, 0.20 mmol, 1.0 equiv) and $BF_3{\cdot}Et_2O$ (50 μL, 0.40 mmol, 2.0 equiv) in THF (1 mL) at -78 °C, and the stirring was continued for 1 h. Then, it was allowed to warm to 0 °C and stirred for 2 h. The reaction mixture was stirred for another 15 h at room temperature. The reaction mixture was quenched with water, and extracted with EtOAc, and the combined organic layers were then washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc in hexanes) to afford **4** (39.2 mg, 87%) as a pale yellow solid: mp 94-95 °C; IR (NaCl, film) *v* 2969, 2934, 2876, 1777, 1737, 1711, 1625, 1457, 1444, 1362, 1349, 1327, 1289, 1159, 1069, 847, 772 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.82 (d, *J* = 1.0 Hz, 1H), 4.24 (d, *J* = 1.0 Hz, 2H), 2.69-2.61 (m, 1H), 1.55 (s, 9H), 1.20 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.6, 168.1, 149.7, 120.1, 82.7, 51.8, 29.2, 28.1, 21.1; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{20}NO_3$ 226.1443, found 226.1434.

γ-Hydroxyl butenolide **28**:



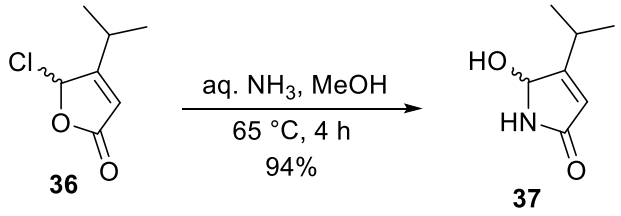
To a solution of glyoxylic acid monohydrate (2.96 g, 20.00 mmol, 50 w/w% in H_2O, 1.0 equiv) in 20 mL of 1,4-dioxane was added morpholine hydrochloride (2.47 g, 20.00 mmol, 1.0 equiv) at room temperature. A solution of isovaleraldehyde (2.19 mL, 20.00 mmol, 1.0 equiv) in 5 mL of 1,4-dioxane was added dropwise *via* syringe, and the resulting solution was stirred at room temperature for 2 h. Then it was heated to 100 °C, and stirred for 10 h. The solvent was evaporated under vacuum. The residue was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with the saturated $NaHCO_3$ solution and brine in sequence, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (50% EtOAc and 1% MeOH in hexanes) to obtain **28** (2.27 g, 80%) as a white solid. NMR data were in agreement with those previously reported.[7] mp 73-75 °C, lit.[8] 80.0 °C; IR (NaCl, film) *v* 3352, 2970, 2938, 2878, 2736, 1743, 1642, 1469, 1389, 1370, 1180, 1137, 1120, 953, 864, 742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.10 (s, 1H), 5.75 (s, 1H), 2.75-2.66 (m, 1H), 1.16 (d, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 172.8, 115.7, 99.0, 27.4, 20.4; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_7H_{11}O_3$ 143.0708, found 143.0704.

γ-Chloro-butenolide **36**:



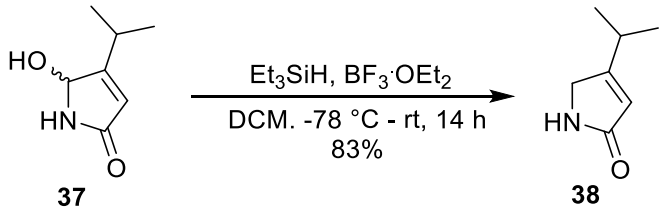
To a solution of **28** (0.30 g, 2.10 mmol, 1.0 equiv) in 10 mL of toluene was added pyridine (1.1 mL, 13.65 mmol, 6.5 equiv) at room temperature. A solution of $SOCl_2$ (0.38 mL, 5.25 mmol, 2.5 equiv) in 2.4 mL of toluene was added dropwise *via* syringe. The reaction mixture was stirred for 12 h, and then the solvent was removed under vacuum. The residue was purified by column chromatography (30% EtOAc in hexanes) to afford **36** (0.30 g, 90%) as a pale yellow oil; IR (NaCl, film) *v* 3111, 2973, 2938, 2879, 1800, 1764, 1634, 1468, 1386, 1371, 1231, 1155, 1017, 879, 866, 716 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.48 (d, *J* = 1.0 Hz, 1H), 5.83 (dd, *J* = 1.0, 1.5 Hz, 1H), 2.74-2.65 (m, 1H), 1.18 (d, *J* = 6.5 Hz, 3H), 1.07 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 169.5, 115.1, 87.3, 27.3, 20.7, 19.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_7H_{10}ClO_2$ 161.0369, found 161.0345.

γ-Hydroxyl lactam **37**:



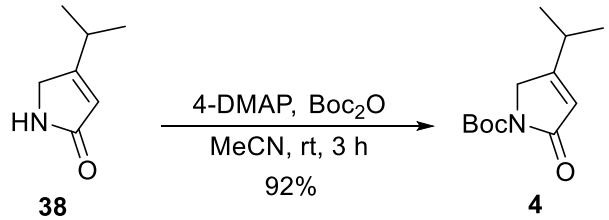
To a solution of **36** (0.50 g, 3.10 mmol, 1.0 equiv) in 2 mL of MeOH was added ammonium hydroxide (6 mL, 28 w/w% in H_2O) at room temperature. The reaction mixture was stirred for 4 h at 65 °C. The solvent was removed under vacuum. The residue was extracted with EtOAc (5 x 10 mL), and the combined organic layers were dried over $MgSO_4$, and concentrated to give **37** (0.41 g, 94%) as a white solid: mp 119-121 °C; IR (NaCl, film) *v* 3284, 3222, 2964, 2934, 2874, 1697, 1632, 1413, 1265, 1183, 1079, 871, 733 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 5.70-5.69 (m, 1H), 5.51 (s, 1H), 2.78-2.65 (m, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.19 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 173.9, 171.8, 118.3, 80.5, 26.8, 20.7, 19.2. HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_7H_{12}NO_2$ 142.0868, found 142.0851.

Lactam **38**:



To a solution of **37** (0.12 g, 0.85 mmol, 1.0 equiv) in 8 mL of dry DCM was added Et_3SiH (0.42 mL, 2.55 mmol, 3.0 equiv) and boron trifluoride etherate (0.21 mL, 1.70 mmol, 2.0 equiv) at -78 °C under N_2 atmosphere. The reaction mixture was stirred at the same temperature for 2 h. Then, it was allowed to warm to room temperature, and stirred for 14 h. The mixture was poured into the saturated $NaHCO_3$ solution, and extracted with DCM (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by column chromatography (EtOAc) to afford **38** (88.2 mg, 83%) as a white solid: mp 45-47 °C; IR (NaCl, film) *v* 3288, 2971, 2937, 2878, 2734, 1721, 1673, 1469, 1448, 1388, 1366, 1288, 1205, 1184, 1153, 861, 722 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30 (s, br, 1H), 5.81 (s, 1H), 3.96 (s, 2H), 2.64 (heptd, *J* = 6.9, 1.4 Hz, 1H), 1.17 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 169.1, 119.9, 49.0, 29.1, 21.5; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_7H_{12}NO$ 126.0919, found 126.0907.

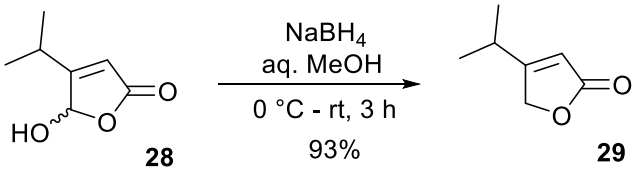
Lactam **4**:



To a solution of **38** (0.40 g, 3.20 mmol, 1.0 equiv) and Boc_2O (1.05 g, 4.80 mmol, 1.5 equiv) in 15 mL of anhydrous acetonitrile was added dropwise a solution of 4-DMAP (39.0 mg, 0.32 mmol, 0.1 equiv) in 4 mL of acetonitrile at room temperature

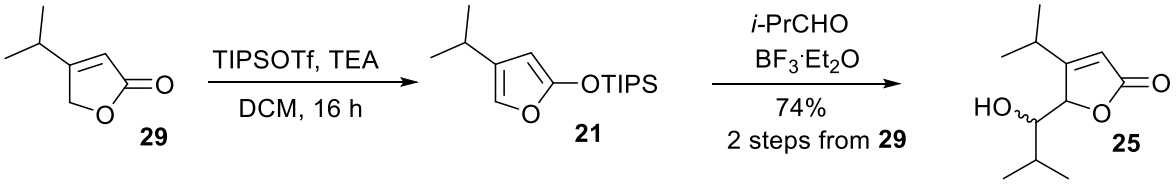
under N_2 atmosphere. The reaction mixture was stirred for 3 h. The solvent was removed under vacuum. The obtained residue was purified by column chromatography (10% EtOAc in hexanes) to afford **4** (0.66 g, 92%).

Butenolide **29**:



To a solution of **28** (1.42 g, 10.00 mmol, 1.0 equiv) in dry MeOH (20 mL) was added $NaBH_4$ (1.15 g, 30.00 mmol, 3.0 equiv) in small portions at 0 °C. The resulting mixture was allowed to warm to room temperature, and stirred for 1 h. The solvent was evaporated under vacuum, and to the obtained residue was added the solution of hydrochloric acid (10%). It was extracted with ethyl acetate (3 x 40 mL), and the combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc in hexanes) to obtain **29** (1.17 g, 93%) as a colorless yellow oil. NMR data were in agreement with those previously reported.[9] IR (NaCl, film) *v* 2970, 2936, 2877, 1785, 1750, 1634, 1470, 1447, 1264, 1160, 1021, 890, 858; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.78 (ht, *J* = 3.2, 1.3 Hz, 1H), 4.81-4.75 (m, 2H), 2.73-2.64 (m, 1H), 1.20 (dq, *J* = 6.9, 2.2 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.3, 174.2, 113.7, 71.9, 28.5, 20.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_7H_{11}O_2$ 127.0759, found 127.0771.

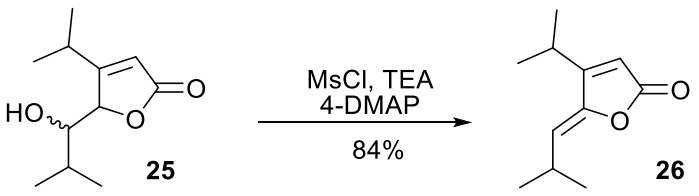
Butenolide **25**:



To a solution of **29** (0.10 g, 0.80 mmol, 1.0 equiv) in dry DCM (1 mL) was added trimethylamine (0.56 mL, 4.00 mmol, 5.0 equiv) at 0 °C under argon. It was stirred for 15 min before the dropwise addition of TIPSOTf (0.27 mL, 0.96 mmol, 1.2 equiv). The mixture was allowed to warm to room temperature, and stirred for 24 h. It was quenched with the saturated $NaHCO_3$ solution, and extracted with DCM (3 x 15 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The obtained residue **21** was directly used in the following step.

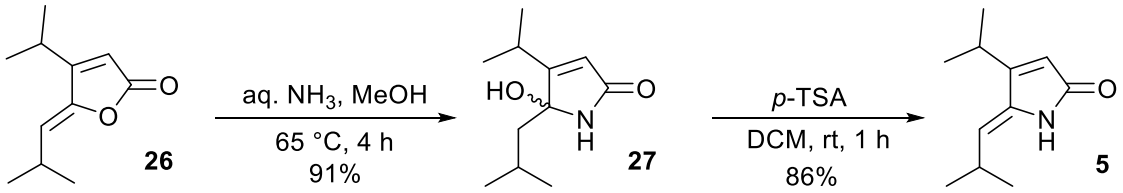
To the obtained residue **21** in a round-flask was added dry DCM (2.5 mL) and isobutyraldehyde (0.15 mL, 1.60 mmol, 2.0 equiv) at room temperature under argon. It was cooled to -78 °C, and stirred for 5 min before the dropwise addition of boron trifluoride etherate (50 μL, 0.40 mmol, 0.5 equiv). The resulting mixture was stirred for 3 h at the same temperature. It was quenched with the saturated $NaHCO_3$ solution before being allowed to warm to room temperature, and extracted with DCM (3 x 15 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc and 1% MeOH in hexanes) to obtain **25** (0.12 g, 74% over two steps) as a white solid. mp 71-74 °C; IR (NaCl, film) *v* 3307, 2967, 2935, 2875, 1761, 1635, 1468, 1407, 1079, 1035, 965, 860; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.65 (t, *J* = 1.6 Hz, 1H), 5.00 (t, *J* = 1.6 Hz, 1H), 3.32 (t, *J* = 7.9 Hz, 1H), 2.93-2.87 (m, 1H), 2.55 (heptd, *J* = 6.9, 1.5 Hz, 1H), 1.91 (dp, *J* = 8.8, 6.7 Hz, 1H), 1.14 (d, *J* = 6.7 Hz, 3H), 1.06 (d, *J* = 7.1 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.3, 174.1, 114.4, 83.4, 75.1, 32.0, 27.5, 21.6, 20.1, 19.6, 19.1; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{11}H_{19}O_3$ 199.1334, found 199.1335.

Butenolide **26**:



To a solution of **25** (0.10 g, 0.50 mmol, 1.0 equiv) in dry DCM (2 mL) was added trimethylamine (0.18 mL, 1.25 mmol, 2.5 equiv) and 4-DMAP (0.13 g, 1.00 mmol, 2.0 equiv). It was cooled to 0 °C, and stirred for 5 min before the dropwise addition of methanesulfonyl chloride (0.1 mL, 1.25 mmol, 2.5 equiv) under argon. It was stirred for 3 h at the same temperature. Another 0.18 mL of trimethylamine was added to the solution, and it was stirred for 14 h at room temperature. The resulting dark brow mixture was quenched with water and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc in hexanes) to obtain **26** (75.6 mg, 84%) as a colorless oil. IR (NaCl, film) *v* 2970, 2937, 2877, 1775, 1468, 1389, 1224, 999, 931, 859; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (t, *J* = 0.9 Hz, 1H), 5.20 (dd, *J* = 9.6, 0.7 Hz, 1H), 3.02 (dp, *J* = 9.6, 6.7 Hz, 1H), 2.77 (pd, *J* = 6.9, 1.1 Hz, 1H), 1.24 (d, *J* = 6.9 Hz, 6H), 1.10 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 165.9, 147.7, 119.8, 112.9, 26.2, 25.8, 22.6, 22.4; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{11}H_{17}O_2$ 181.1229, found 181.1222.

Lactam **5**:

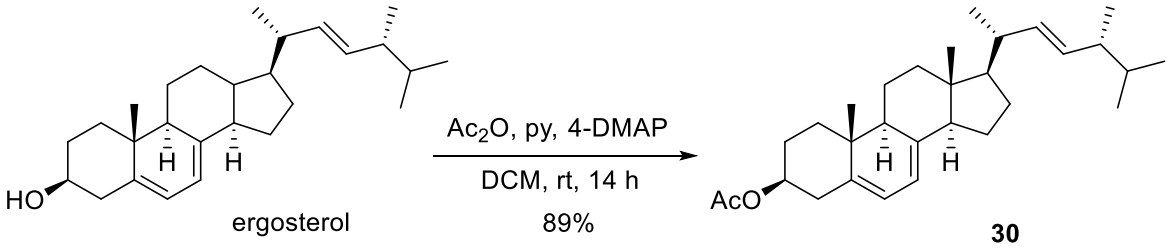


To a solution of **26** (72.0 mg, 0.40 mmol, 1.0 equiv) in MeOH (2 mL) was added ammonia solution (6 mL, 28%). The reaction mixture was heated to 65 °C, and stirred for 4 h. The solution of hydrochloric acid was added to the mixture until the pH < 7. It was extracted with ethyl acetate (4 x 10 mL), and the combined organic layers were dried over magnesium sulfate, and concentrated under vacuum to give **27** (72.1 mg, 91%) as a white solid.

To a solution of **27** (39.4 mg, 0.20 mmol, 1.0 equiv) in dry DCM (2 mL) was added *p*-toluenesulfonic acid monohydrate (7.6 mg, 0.04 mmol, 0.2 equiv) at room temperature

under argon. It was stirred for 1 h at the same temperature before the saturated $NaHCO_3$ solution was added. The solvent was evaporated under vacuum, and DCM was added to the obtained residue. It was washed with brine, and the organic layer was dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOaC in hexanes) to obtain **5** (30.8 mg, 86%) as a white solid. mp 155-157 °C; IR (NaCl, film) *v* 3168, 3037, 2964, 2925, 2866, 1676, 1464, 1360, 1050, 842, 814, 768, 675; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H), 5.85 (dt, *J* = 1.6, 0.8 Hz, 1H), 5.18 (d, *J* = 9.8 Hz, 1H), 2.84-2.68 (m, 2H), 1.18 (d, *J* = 6.8 Hz, 6H), 1.09 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 159.7, 136.2, 120.5, 117.4, 27.4, 25.4, 23.0, 22.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{11}H_{18}NO$ 180.1388, found 180.1392.

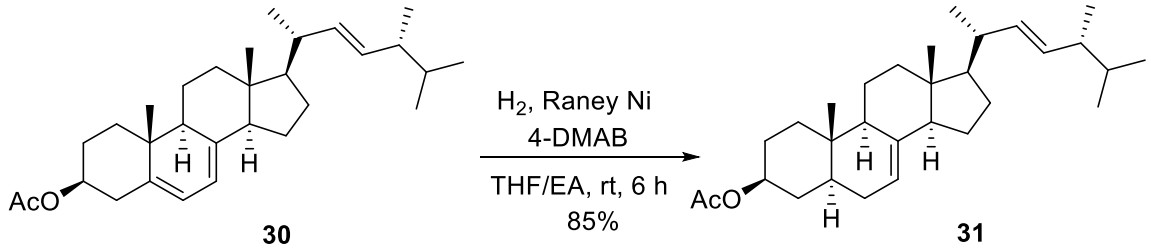
Ergosterol acetate **30**:



To a solution of ergosterol (2.00 g, 5.04 mmol, 1.0 equiv) in DCM (30 mL) were added acetic anhydride (0.95 mL, 10.08 mmol, 2.0 equiv), pyridine (0.8 mL, 10.08 mmol, 2.0 equiv) and 4-DMAP (0.12 g, 1.01 mmol, 0.2 equiv) in sequence. The reaction mixture was stirred for 14 h at room temperature. It was quenched with water, and extracted with DCM (3 x 20 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (2% EtOAc in hexanes) to obtain **30** (1.91 g, 89%) as a white solid. NMR data were in agreement with those previously reported.[10] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.56 (dd, *J* = 5.8, 2.5 Hz, 1H), 5.38 (dt, *J* = 5.5, 2.8 Hz, 1H), 5.27-5.11 (m, 2H), 4.76-4.64 (m, 1H), 2.50 (ddd, *J* = 14.4, 5.1, 2.4 Hz, 1H), 2.41-2.30 (m, 1H), 2.08-1.17 (m, 21H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.95 (s, 3H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.83 (dd, *J* = 6.6 Hz, 6H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 141.5, 138.5, 135.5, 131.9, 120.2, 116.3, 72.8, 55.7, 54.5, 46.0,

42.8, 40.4, 39.0, 37.9, 37.1, 36.6, 33.1, 28.3, 28.1, 23.0, 21.4, 21.1, 21.0, 19.9, 19.6, 17.6, 16.2, 12.0.

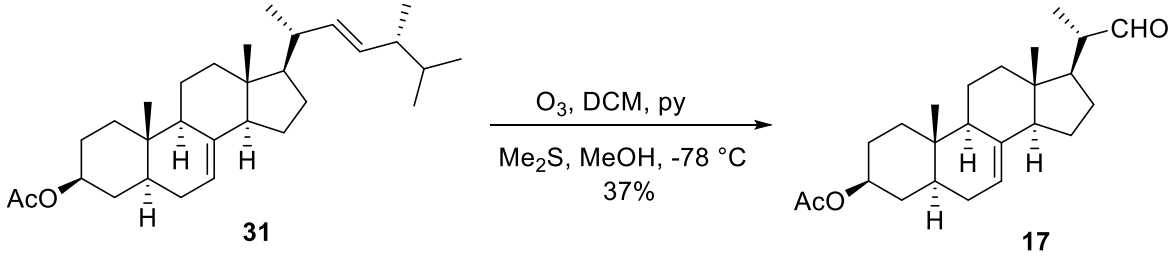
Steroid **31**:



To a solution of NaOH (3.75 g) in water (15 ml) was added portion-wise nickel-aluminium powder (3.0 g) at 0 °C. Then the reaction mixture was allowed to warm to room temperature over one hour, and stirred at 70 °C for 2 h. The reaction mixture was allowed to come back to room temperature, and the catalyst was washed with water, EtOH and EtOAc in sequence.

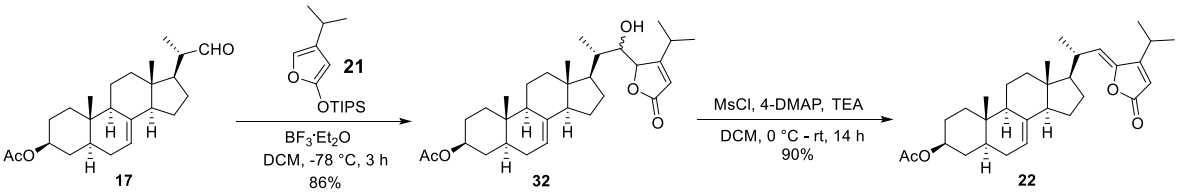
To a stirred suspension of Raney nickel catalyst (obtained above) in EtOAc (18 ml) and THF (18 ml), was added ergosterol acetate (**30**, 1.00 g, 2.35 mmol, 1.0 equiv) and 4-dimethylaminobenzaldehyde (4-DMAB, 0.14 g, 1.18 mmol, 0.5 equiv). The hydrogenation of the reaction mixture was carried out at room temperature and at atmospheric pressure (H_2 balloon) for 6 h. After hydrogenation, the reaction mixture was decanted, filtered over celite. The resulting filtrate was concentrated under vacuum, and recrystallized from $CHCl_3$/MeOH to afford **31** (0.88 g, 85%) as a white solid. NMR data were in agreement with those previously reported.[11] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26-5.11 (m, 3H), 4.73-4.60 (m, 1H), 2.04-1.96 (m, 5H), 1.86-1.64 (m, 9H), 1.56 (s, 2H), 1.49-1.32 (m, 6H), 1.29-1.22 (m, 4H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.85-0.80 (m, 9H), 0.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 139.5, 135.6, 131.9, 117.3, 110.0, 73.5, 55.9, 55.0, 49.2, 43.3, 42.8, 40.5, 40.1, 39.4, 36.8, 34.2, 33.8, 33.1, 29.5, 28.1, 27.5, 22.9, 21.5, 21.1, 19.9, 19.6, 17.6, 12.9, 12.1.

Steroidal aldehyde **17**:



To a stirred solution of **31** (0.20 g, 0.45 mmol, 1.0 equiv) and pyridine (0.24 ml, 2.97 mmol, 6.6 equiv) in dichloromethane (45 mL) was bubbled ozone at -78 °C for 3 min. After further 2 min, MeOH (2.4 ml) and dimethyl sulfide (0.12 ml, 1.58 mmol, 3.5 equiv) were added. The reaction mixture was stirred for 30 min at -78 °C and then was allowed to warm to room temperature. The solvent was removed under vacuum and the residue was purified by flash chromatography (hexanes to recover the starting material, and then 5% EtOAc in hexanes) to afford **17** (61.9 mg, 37%) as a white solid. NMR data were in agreement with those previously reported.[11] ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.58 (d, *J* = 3.2 Hz, 1H), 5.17 (s, 1H), 4.76-4.63 (m, 1H), 2.36 (dqd, *J* = 10.1, 6.8, 3.2 Hz, 1H), 2.03 (s, 3H), 1.99-1.56 (m, 13H), 1.49-1.28 (m, 7H), 1.13 (d, *J* = 6.9 Hz, 3H), 0.81 (s, 3H), 0.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.0, 170.7, 138.6, 118.0, 73.4, 54.2, 51.0, 49.8, 49.2, 43.9, 40.0, 39.2, 36.8, 34.2, 33.7, 29.5, 27.5, 26.7, 23.3, 21.4, 21.4, 13.6, 12.9, 12.3.

Steroidal butenolide **22**:



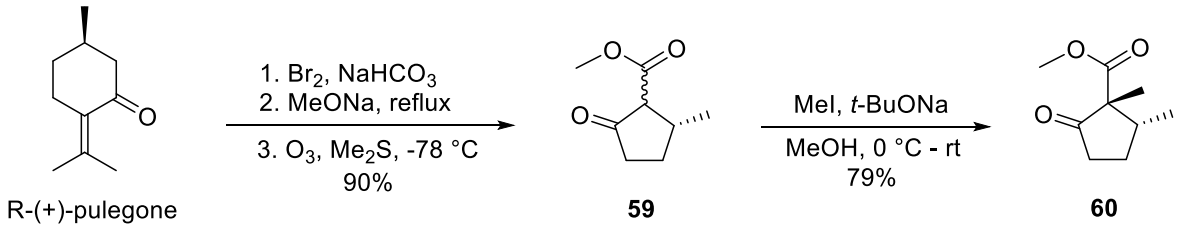
A solution of **17** (0.15 g, 0.54 mmol, 2.0 equiv) and **21** (0.10 g, 0.27 mmol, 1.0 equiv) in dry DCM (2 mL) was cooled to -78 °C, and stirred for 5 min before the dropwise addition

of boron trifluoride etherate (20 μL, 0.14 mmol, 0.5 equiv). The resulting mixture was stirred for 3 h at the same temperature. It was quenched with the saturated $NaHCO_3$ solution, and extracted with DCM (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc and 1% MeOH in hexanes) to obtain mixture **32** (0.12 g, 86%) as white solid.

To a solution of **32** (0.12 g, 0.24 mmol, 1.0 equiv) in dry DCM (2 mL) was added trimethylamine (0.1 mL, 0.60 mmol, 2.5 equiv) and 4-DMAP (58.6 mg, 0.48 mmol, 2.0 equiv). The resulting solution was cooled to 0 °C, and stirred for 5 min before the dropwise addition of methanesulfonyl chloride (50 μL, 0.60 mmol, 2.5 equiv) under argon. It was stirred for 3 h at the same temperature. Another 0.18 mL of trimethylamine was added to the reaction mixture, and it was stirred for 14 h at room temperature. The resulting dark brow mixture was quenched with water and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc in hexanes) to obtain **22** (0.10 g, 90%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85-5.84 (m, 1H), 5.17 (d, *J* = 10.4 Hz, 1H), 5.15-5.12 (m, 1H), 4.68 (tt, *J* = 11.1, 4.6 Hz, 1H), 2.97- 2.85 (m, 1H), 2.76 (qd, *J* = 6.8, 1.1 Hz, 1H), 2.06-1.98 (m, 5H), 1.88-1.18 (m, 24H), 1.10 (d, *J* = 6.7 Hz, 3H), 0.80 (s, 3H), 0.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 169.9, 165.8, 147.4, 139.0, 119.0, 117.7, 112.7, 73.4, 55.9, 54.9, 49.2, 43.7, 40.0, 39.3, 36.8, 34.2, 34.1, 33.8, 29.5, 27.5, 27.4, 25.9, 22.9, 22.6, 22.0, 21.4, 21.4, 20.2, 12.9, 12.2.

E.3 Chapter 2: Synthesis of 6

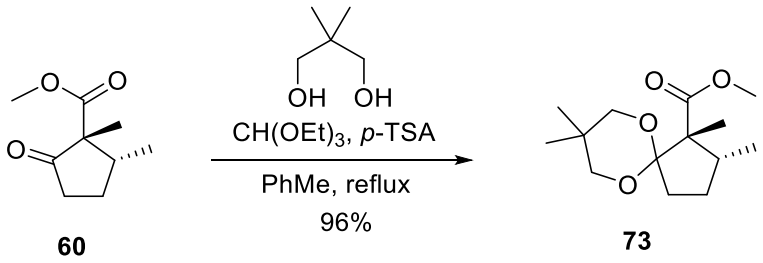
β-Keto ester **60**:



R-(+)-pulegone **59** **60**

Compound **59** was obtained from *R*-(+)-pulegone by the Marx-Norman procedure.[12] To a solution of **59** (1.00 g, 6.41 mmol, 1.0 equiv) and iodomethane (2.00 mL, 32.05 mmol, 5.0 equiv) in MeOH (13 mL) was added sodium *tert*-butoxide (1.23 g, 12.82 mmol, 2.0 equiv) at 0 °C under argon atmosphere. Then, the resulting mixture was allowed to warm to room temperature and stirred for 12 h. The solvent was evaporated under vacuum, and the obtained residue was treated with the saturated aqueous ammonium chloride. It was extracted with diethyl ether (3 x 50 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (1% EtOAc in hexanes) to obtain **60** (0.86 g, 79%) as a pale yellow oil. ^{1}H NMR data of **60** matches well with literature.[13] $[\alpha]_D^{22}$ +94.1° (*c* 0.19, $CHCl_3$); IR (NaCl, film) *v* 2961, 2937, 2881, 1751, 1733, 1459, 1243, 1167, 1125, 1064, 984; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (s, 3H), 2.59-2.46 (m, 1H), 2.22 (ddd, *J* = 19.2, 11.0, 8.6 Hz, 1H), 2.08-1.94 (m, 2H), 1.86-1.68 (m, 1H), 1.22 (s, 3H), 1.01 (d, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 216.6, 171.2, 59.4, 51.7, 43.9, 37.8, 28.0, 18.2, 15.4; HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_9H_{15}O_3$ 171.1021, found 171.1011.

Acetal **73**:

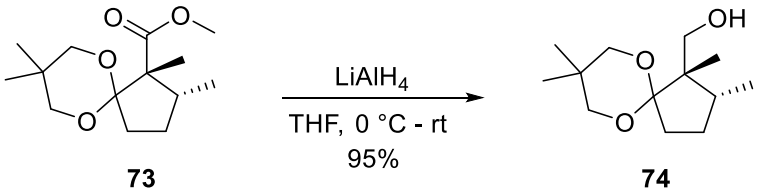


60 **73**

To a solution of **60** (1.30 g, 7.60 mmol, 1.0 equiv), *p*-toluenesulfonic acid monohydrate (0.36 g, 1.89 mmol, 0.25 equiv) and 2,2-dimethyl-1,3-propanediol (7.90 g, 76.0 mmol, 10.0 equiv) in toluene (60 mL) was added triethyl orthoformate (3 mL, 18.24 mmol, 2.4 equiv). The resulting mixture was heated to reflux for 2 h. Then, another 1.5 mL of triethyl orthoformate was added to the mixture. The reflux was maintained for 1 h before cooling to room temperature. The reaction mixture was diluted with diethyl ether, washed with the saturated aqueous $NaHCO_3$ and brine. The organic layers were dried over magnesium

sulfate, and concentrated under vacuum. The residue was purified by column chromatography (5% EtOAc in hexanes) to obtain **73** (1.87 g, 96%) as a colorless oil. $[\alpha]_D^{22}$ -28.9° (*c* 0.44, $CHCl_3$); IR (NaCl, film) *v* 2980, 2953, 2873, 1729, 1463, 1311, 1250, 1136, 1094, 996, 912, 851, 783; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (s, 3H), 3.47-3.26 (m, 4H), 2.38-2.28 (m, 1H), 2.11-1.89 (m, 2H), 1.86-1.59 (m, 2H), 1.22 (s, 3H), 1.13 (s, 3H), 0.84 (d, *J* = 6.9, 3H), 0.67 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 109.5, 72.5, 70.5, 61.4, 51.1, 41.0, 30.2, 29.2, 27.5, 22.7, 22.0, 15.0, 14.8; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{14}H_{25}O_4$ 257.1753, found 257.1749.

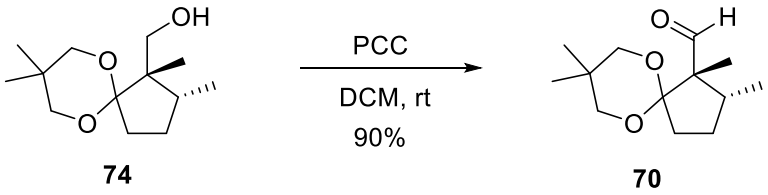
Alcohol **74**:



73 **74**

To a solution of **73** (0.48 g, 1.88 mmol, 1.0 equiv) in dry THF (15 mL) was added dropwise lithium aluminium hydride solution (1.88 mL, 2.0 M in THF, 3.76 mmol, 2.0 equiv) at 0 °C under argon atmosphere. The reaction mixture was allowed to warm to room temperature and the stirring was maintained for 2 h. It was cooled to 0 °C, and carefully quenched with the saturated aqueous ammonium chloride. The mixture was extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc in hexanes) to obtain **74** (0.41 g, 95%) as a colorless oil. $[\alpha]_D^{22}$ +13.9° (*c* 0.46, $CHCl_3$); IR (NaCl, film) *v* 3383, 2957, 2938, 2877, 1729, 1459, 1375, 1037, 992; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (d, *J* = 12 Hz, 1H), 3.63 (d, *J* = 8 Hz, 1H), 3.54 (d, *J* = 12 Hz, 1H), 3.44-3.32 (m, 3H), 2.33-2.26 (m, 1H), 1.97-1.71 (m, 3H), 1.42-1.27 (m, 1H), 1.18 (s, 3H), 1.10 (s, 3H), 0.86 (d, *J* = 7.1, 3H), 0.72 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.4, 72.2, 70.4, 65.2, 50.3, 39.6, 30.3, 28.4, 26.5, 22.7, 22.0, 15.3, 13.9; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{25}O_3$ 229.1804, found 229.1793.

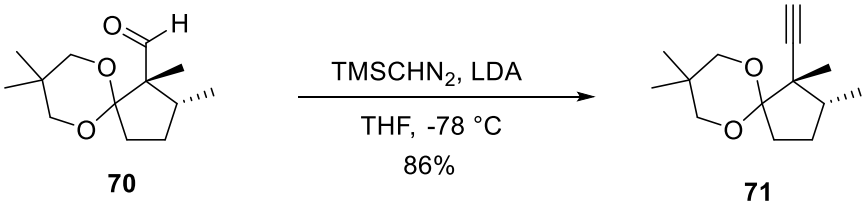
Aldehyde **70**:



74 **70**

To a vigorously stirred solution of **74** (0.70 g, 3.07 mmol, 1.0 equiv) in dry DCM (25 mL) was added pyridinium chlorochromate (1.66 g, 7.68 mmol, 2.5 equiv) at room temperature under argon atmosphere. Stirring was maintained for 3 h at the same temperature. The resulting mixture was subjected to filtration through pad of silica, eluted with DCM. The solvent was evaporated under vacuum, and the obtained residue was purified by column chromatography (5% EtOAc in hexanes) to obtain **70** (0.62 g, 90%) as a colorless oil. $[\alpha]_D^{22}$ -47.3° (*c* 0.15, $CHCl_3$); IR (NaCl, film) *v* 2953, 2893, 2862, 2752, 1718, 1475, 1452, 1304, 1125, 1091, 988, 923, 741; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 3.65 (d, *J* = 12 Hz, 1H), 3.47 (d, *J* = 8 Hz, 1H), 3.41-3.30 (m, 2H), 2.50-2.43 (m, 1H), 2.10-1.95 (m, 3H), 1.71-1.58 (m, 1H), 1.13 (s, 3H), 1.09 (s, 3H), 0.88 (d, *J* = 8 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.4, 108.9, 72.4, 70.5, 63.8, 40.6, 30.3, 29.2, 28.1, 22.5, 22.1, 14.7, 11.8. HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{13}H_{23}O_3$ 227.1647, found 227.1648.

Acetylene **71**:

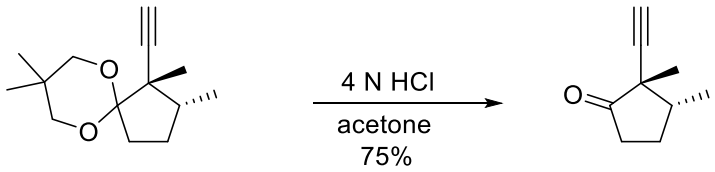


70 **71**

To a flame dried flask was added dry THF (14 mL) and diisopropylamine (0.45 mL, 3.18 mmol, 1.5 equiv) under argon atmosphere. It was cooled to -78 °C. The *n*-BuLi solution (1.2 mL, 2.5 M in hexanes, 2.97 mmol, 1.4 equiv) was added to the mixture dropwise. Then,

it was stirred for 30 min. The 5.0 mL of trimethylsilyldiazomethane (10% w/w in hexanes, 0.6 M, 2.97 mmol, 1.4 equiv) solution was added slowly to the reaction mixture. The mixture was stirred for 30 min before the solution of aldehyde **70** (0.48 g, 2.12 mmol, 1.0 equiv) in dry THF (5 mL) was dropwise added. After being stirred for 1 h at -78 °C, the reaction mixture was allowed to warm to room temperature. The stirring was continued for 30 min. The reaction mixture was quenched with ice water, and underwent extraction with diethyl ether (3 x 10 mL). The combined organic layers was dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (2% EtOAc in hexanes) to obtain **71** (0.41 g, 86%) as a pale yellow oil. $[\alpha]_D^{22}$ -47.5° (*c* 0.09, $CHCl_3$); IR (NaCl, film) *v* 3311, 3280, 2953, 2934, 2870, 2854, 1459, 1394, 1303, 1136, 1091, 992, 627; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.63 (d, *J* = 12 Hz, 1H), 3.53 (s, 2H), 3.38 (d, *J* = 12 Hz, 1H), 2.32-2.25 (m, 1H), 2.22 (s, 1H), 1.98-1.79 (m, 2H), 1.79-1.66 (m, 1H), 1.56-1.38 (m, 1H), 1.26 (s, 3H), 1.18 (s, 3H), 1.03 (d, *J* = 8 Hz, 3H), 0.71 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 108.4, 86.0, 72.9, 72.0, 70.9, 50.8, 39.9, 30.3, 28.2, 26.4, 22.6, 22.0, 17.9, 14.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{14}H_{23}O_2$ 223.1698, found 223.1700.

Acetylene **72**:

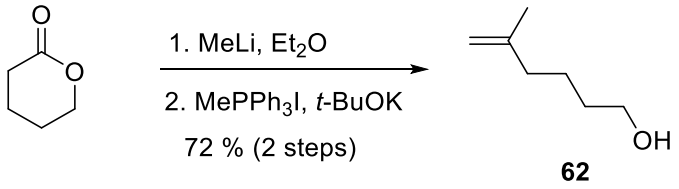


71 **72**

To a solution of **71** (0.25 g, 1.13 mmol, 1.0 equiv) in acetone (13 mL) was added HCl (4.0 N, 1.1 mL). It was stirred for 1 h at room temperature. The solvent was evaporated under vacuum in cooling bath (ca. 10 °C, the product **72** was volatile). The obtained residue was treated with diethyl ether, washed with the saturated $NaHCO_3$ solution. The organic layer was dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (3% Et_2O in hexanes) to obtain **72** (0.12 g, 75%) as a colorless oil. $[\alpha]_D^{22}$ -24.5° (*c* 0.06, $CHCl_3$); IR (NaCl, film) *v* 3292, 2962, 2932, 2874, 1750,

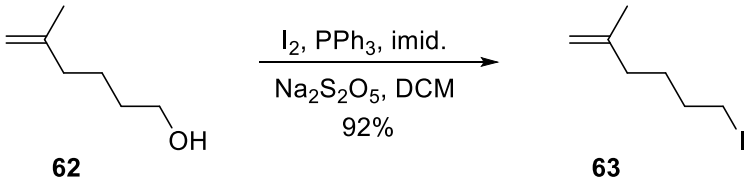
1457, 1406, 1380, 1288, 1193, 1134, 1094, 1033, 990, 803, 636; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.59-2.49 (m, 1H), 2.23 (s, 1H), 2.21-2.13 (m, 1H), 2.02-1.93 (m, 1H), 1.86-1.65 (m, 2H), 1.27 (s, 3H), 1.18 (d, *J* = 8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 214.5, 81.2, 73.6, 49.3, 42.91, 35.9, 27.3, 20.4, 15.0; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_9H_{13}O$ 137.0966, found 137.0964.

Alcohol **62**:



The alcohol **62** was obtained from δ-valerolactone through two steps using reported procedure[14] in 72% yield over two steps. The NMR data of compound **62** matched well with the reported data. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.68 (s, 1H), 4.65 (s, 1H), 3.64-3.59 (m, 2H), 2.01 (t, *J* = 7.7 Hz, 2H), 1.69 (s, 3H), 1.58-1.43 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.7, 109.9, 62.7, 37.5, 32.3, 23.7, 22.3.

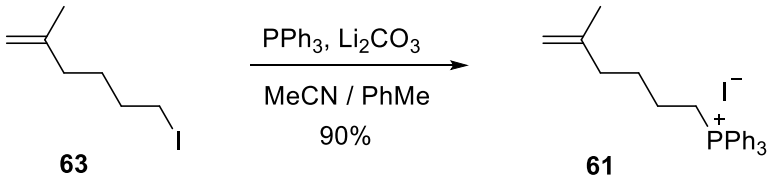
Iodide **63**:



To a solution of PPh_3 (0.31 g, 1.20 mmol, 1.2 equiv) and imidazole (88.5 mg, 1.30 mmol, 1.3 equiv) in dry DCM (1.5 mL) was added I_2 (0.31 g, 1.20 mmol, 1.2 equiv) at 0 °C under argon atmosphere. It was stirred for 15 min at the same temperature. To the resulting mixture was added dropwise the solution of **62** (0.11 g, 1.00 mmol, 1.0 equiv) in dry DCM (0.5 mL). It was allowed to warm to room temperature, and stirred for 16 h. The reaction

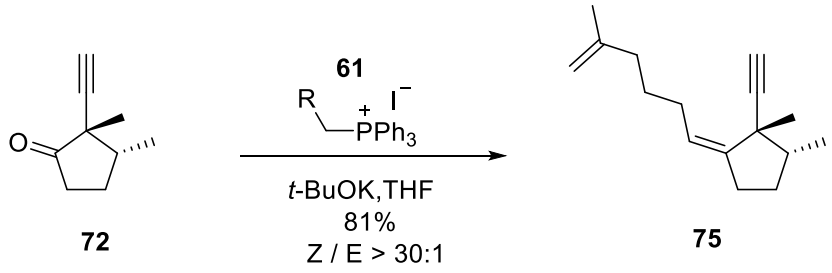
mixture was quenched with the saturated $Na_2S_2O_3$ solution, and underwent extraction with DCM (2 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (hexanes) to obtain **63** (0.21 g, 92%) as a colorless oil. The spectroscopic data of compound **63** matched with the reported data.[15] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71 (q, *J* = 1.8 Hz, 1H), 4.67 (dq, *J* = 2.4, 1.2 Hz, 1H), 3.19 (td, *J* = 6.9, 1.6 Hz, 2H), 2.03 (t, *J* = 7.6 Hz, 2H), 1.86-1.75 (m, 2H), 1.72 (s, 3H), 1.54 (td, *J* = 7.6, 1.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.1, 110.4, 36.6, 33.0, 28.3, 22.3, 7.0.

Salt **61**:



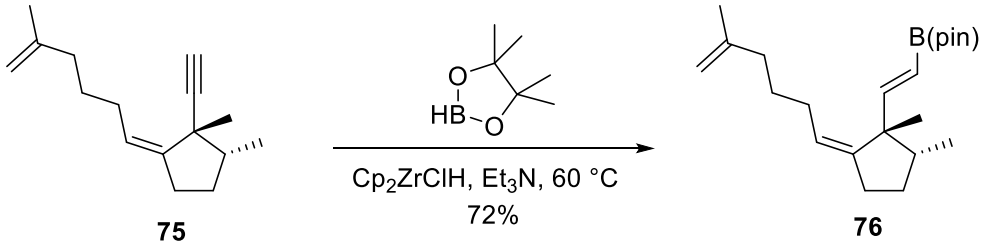
To a flame-dried flask were added PPh_3 (0.13 g, 0.50 mmol, 1.0 equiv) and Li_2CO_3 (0.37 g, 5.00 mmol, 10 equiv) under argon atmosphere. A solution of **61** (0.11 g, 0.50 mmol, 1.0 equiv) in MeCN/toluene (4:1, 2 mL) was added, and the resulting suspension was stirred at 70 °C for 18 h. The resulting mixture was subjected to filtration through pad of silica, eluted with MeCN. The solvent was evaporated under vacuum, and the obtained residue was purified by recrystallization (MeCN/Et_2O) to salt **61** (0.22 g, 90%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (ddd, *J* = 13.7, 7.3, 3.6 Hz, 9H), 7.70 (td, *J* = 8.0, 3.6 Hz, 6H), 4.62 (t, *J* = 1.8 Hz, 1H), 4.56 (s, 1H), 3.70 (d, *J* = 13.8 Hz, 2H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.81 (p, *J* = 7.4 Hz, 2H), 1.64 (d, *J* = 9.5 Hz, 5H).

Compound **75**:



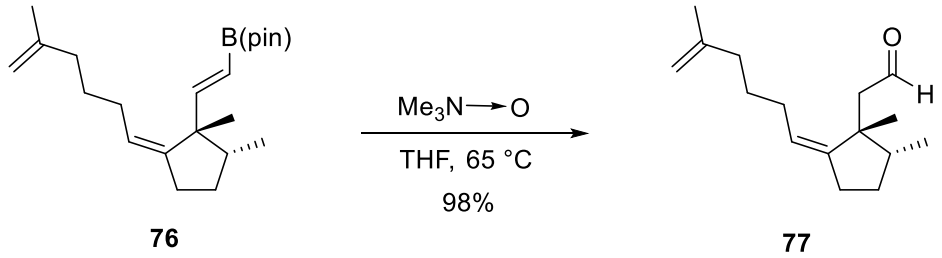
To a flame-dried flask were added **61** (0.19 g, 0.39 mmol, 1.3 equiv) and THF (1 mL) under argon atmosphere. It was cooled to 0 °C, and the *t*-BuOK solution (1.0 M in THF, 0.39 mL, 0.39 mmol, 1.3 equiv) was added to the suspension dropwise. It was stirred for 30 min, and the solution of **72** (40.8 mg, 0.30 mmol, 1.0 equiv) in THF (0.5 mL) was added dropwise to the orange solution. The reaction mixture was stirred at 65 °C for 6 h. It was quenched with the saturated aqueous ammonium chloride, and extracted with diethyl ether (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (hexanes) to obtain **75** (52.5 mg, 81%) as a pale yellow oil. $[\alpha]_D^{22}$ -88.9° (*c* 0.12, $CHCl_3$); IR (NaCl, film) *v* 3303, 3075, 2968, 2934, 2877, 1649, 1452, 1376, 1224, 1034, 889, 623; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (ddt, *J* = 9.4, 7.4, 1.7 Hz, 1H), 4.72-4.67 (m, 2H), 2.44-2.19 (m, 3H), 2.17 (s, 1H), 2.05 (t, *J* = 7.7 Hz, 2H), 1.79-1.65 (m, 5H), 1.64-1.42 (m, 2H), 1.41-1.28 (m, 5H), 1.11 (d, *J* = 8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 145.9, 124.5, 109.7, 87.6, 70.1, 47.6, 42.8, 37.7, 33.4, 32.3, 27.9 (4), 27.9 (0), 26.8, 22.5, 15.3; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{25}$ 217.1956, found 217.1950.

Compound **76**:



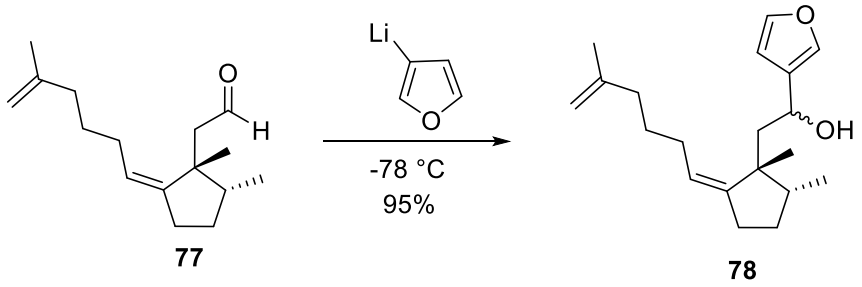
The acetylene **75** (0.16 g, 0.74 mmol, 1.0 equiv) was added to a 10 mL vial, followed by pinacolborane and Schwartz's reagent Cp_2ZrClH (19.1 mg, 0.074 mmol, 0.1 equiv). To the suspension was added TEA (11 μL, 0.074 mmol, 0.1 equiv) before being degassed with argon. It was heated to 60 °C, and stirred for 18 h. The reaction mixture was directly transferred to silica column, eluted with 10% EtOAc in hexanes to obtain **76** (0.18 g, 72%) as a pale yellow oil. $[\alpha]_D^{22}$ -62.2° (*c* 0.10, $CHCl_3$); IR (NaCl, film) *v* 2976, 2957, 2930, 2870, 1630, 1456, 1376, 1345, 1319, 1224, 1148, 1003, 972, 885, 851; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (d, *J* = 18.3 Hz, 1H), 5.34 (d, *J* = 18.3 Hz, 1H), 5.27-5.21 (m, 1H), 4.62 (dt, *J* = 9.8, 1.2 Hz, 2H), 2.38-2.23 (m, 2H), 1.9641.84 (m, 4H), 1.77-1.58 (m, 5H), 1.42-1.19 (m, 18H), 0.81 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1, 147.2, 146.0, 124.0, 109.5, 82.8, 50.8, 48.6, 37.6, 34.0, 31.8, 27.9, 27.7, 24.8, 24.6, 23.2, 22.4, 14.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{38}BO_2$ 345.2965, found 345.2976.

Aldehyde **77**:



To a solution of **76** (79.1 mg, 0.23 mmol, 1.0 equiv) in dry THF (5 mL) was added trimethylamine *N*-oxide (69.8 mg, 0.93 mmol, 4.0 equiv) under argon atmosphere. The resulting suspension was heated to 65 °C, and stirred for 2 h. The reaction mixture was poured into water, and underwent extraction with diethyl ether (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (3% EtOAc in hexanes) to obtain **77** (52.7 mg, 98%) as a colorless oil. $[\alpha]_D^{22}$ -28.6° (*c* 0.14, $CHCl_3$); IR (NaCl, film) *v* 2957, 2934, 2877, 2733, 1720, 1649, 1463,1376, 1279, 1052, 889; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (t, *J* = 3.3 Hz, 1H), 5.33-5.27 (m, 1H), 4.71-4.64 (m, 2H), 2.41-2.34 (m, 4H), 2.23-2.12 (m, 1H), 2.09-1.96 (m, 3H), 1.85-1.69 (m, 5H), 1.48 (p, *J* = 7.6 Hz, 2H), 1.30 (s, 3H), 1.27-1.14 (m, 1H), 0.92 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.3, 146.8, 145.7, 123.7, 109.9, 49.2, 47.4, 45.6, 37.4, 33.1, 31.1, 28.1, 27.9, 25.6, 22.4, 14.0; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{27}O$ 235.2062, found 235.2055.

Alcohol **77**:

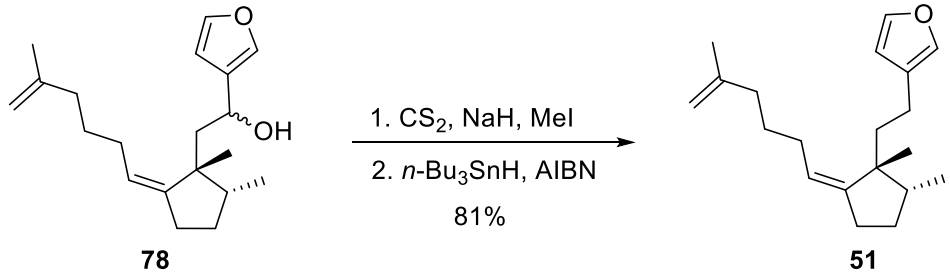


77 **78**

To a solution of 3-bromofuran (90 μL, 1.00 mmol, 10.0 equiv) in dry THF (0.8 mL) was dropwise added *n*-BuLi (0.4 mL, 2.5 M in hexanes, 1.00 mmol, 10.0 equiv) at -78 °C under argon atmosphere. It was stirred for 30 min at the same temperature. Then, a solution of **77** (23.4 mg, 0.10 mmol, 1.0 equiv) in THF (1 mL) was added to the mixture dropwise. The resulting solution was stirred for 20 min. The reaction was quenched with the saturated aqueous ammonium chloride at -78 °C. The mixture was extracted with diethyl ether (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc in

hexanes) to obtain diastereomers mixture **78** (28.7 mg, 95%) as a colorless oil. IR (NaCl, film) *v* 3455, 2934, 2874, 1649, 1593, 1456, 1371, 1159, 1022, 855, 874, 791; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.35 (m, 2H), 6.39 (ddd, *J* = 7.1, 1.6, 0.9 Hz, 1H), 5.46-5.32 (m, 1H), 5.07-4.86 (m, 1H), 4.75-4.68 (m, 2H), 2.59-2.16 (m, 4H), 2.06 (t, *J* = 7.6 Hz, 2H), 1.92-1.41 (m, 11H), 1.37-1.24 (m, 3H), 0.95 (dd, *J* = 50.8, 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.7, 149.1, 145.9, 145.7, 143.1 (2), 143.1 (0), 138.5, 138.3, 130.5, 129.9, 124.7, 124.6, 110.0, 109.9, 108.6, 108.4, 65.9, 65.0, 48.0, 47.3, 46.5, 45.9, 43.2, 42.7, 37.6, 37.5, 33.5, 32.2, 31.6, 30.3, 29.1, 28.4, 28.2, 28.0, 27.8, 26.9, 23.2, 22.5, 22.4, 13.4, 13.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{20}H_{31}O_2$ 303.2324, found 303.2346.

Compound **51**:



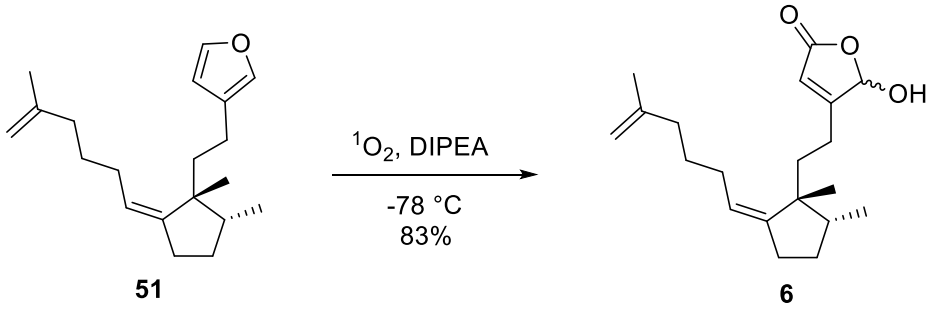
78 **51**

To a solution of **78** (22.0 mg, 0.073 mmol, 1.0 equiv) and CS_2 (27 μL 0.44 mmol, 6.0 equiv) in dry THF (1 mL) was added NaH (17.5 mg, 60% dispersion in mineral oil, 6.0 equiv) at 0 °C under argon atmosphere. It was stirred for 30 min at the same temperature. Iodomethane (27 μL, 0.44 mmol, 6.0 equiv) was added to the suspension, and the resulting mixture was stirred for another 30 min. It was quenched with the saturated aqueous ammonium chloride at 0 °C. The mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (3 % EtOAc in hexanes) to obtain crude xanthate mixture (24.3 mg, 85%) as a yellow oil.

To a solution of xanthate mixture (24.3 mg, 0.062 mmol, 0.1 equiv) in toluene was added AIBN (5.1 mg, 0.031 mmol, 0.5 equiv) under argon, followed by *n*-Bu_3Sn-H (0.13 mL,

0.50 mmol, 8.0 equiv). It was bubbled with argon for 10 min, and heated to 80 °C for 2 h. The solvent was removed under vacuum. The residue was purified by chromatography (hexanes) to obtain **51** (17.7 mg, 81% from **78**) as a colorless oil. $[\alpha]_D^{22}$ -47.7° (*c* 0.12, $CHCl_3$); IR (NaCl, film) *v* 2953, 2938, 2877, 1653, 1505, 1459, 1380, 1163, 1030, 889, 874, 783; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (t, *J* = 1.7 Hz, 1H), 7.21 (dd, *J* = 1.6, 0.9 Hz, 1H), 6.27 (dd, *J* = 1.8, 0.9 Hz, 1H), 5.28 (tt, *J* = 7.5, 2.1 Hz, 1H), 4.72-4.66 (m, 2H), 2.45-2.31 (m, 4H), 2.26-2.17 (m, 1H), 2.14-2.06 (m, 1H), 2.06-2.01 (m, 2H), 1.82-1.75 (m, 1H), 1.75-1.68 (m, 4H), 1.64-1.58 (m, 3H), 1.52-1.47 (m, 1H), 1.39-1.26 (m, 1H), 1.22 (s, 3H), 0.97 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 147.9, 146.0, 142.6, 138.4, 126.1, 122.8, 111.0, 109.7, 47.4, 46.6, 37.6, 35.7, 33.4, 31.2, 28.5, 27.9, 25.3, 22.4, 21.4, 13.6; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}O$ 287.2375, found 287.2363.

Synthetic Niphateolide A (**6**):



To a solution of precursor **51** (17.7 mg, 0.06 mmol, 1.0 equiv) and DIPEA (0.1 mL, 0.60 mmol, 10 equiv) in dry DCM (5 mL) was added Rose Bengal (3.0 mg) at room temperature. Anhydrous oxygen was bubbled in for 10 min. It was placed at -78 °C under O_2, and irradiated with a 500 W lamp for 6 h. Then, it was allowed to warm to room temperature, and the saturated oxalic acid solution was added. It was stirred for 30 min. The resulting mixture was extracted with DCM (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20 % EtOAc in hexanes) to obtain **6** (15.8 mg, 83%) as a coloress oil. $[\alpha]_D^{22}$ -39.2° (*c* 0.05, MeOH), lit.[16] $[\alpha]_D^{20}$ +13° (*c* 0.46, MeOH); IR (NaCl, film) *v* 3345, 2954, 2925, 2871, 2854, 1741, 1648, 1458, 1375, 1286, 1137, 951, 887, 860, 722; ^{1}H NMR

(500 MHz, $CDCl_3$) δ 6.02 (s, 1H), 5.85 (td, *J* = 1.8, 0.9 Hz, 1H), 5.32-5.27 (m, 1H), 4.73-4.66 (m, 2H), 2.45-2.31 (m, 4H), 2.24-2.15 (m, 1H), 2.08-2.00 (m, 3H), 1.85-1.70 (m, 5H), 1.65-1.58 (m, 2H), 1.53-1.44 (m, 2H), 1.34-1.26 (m, 1H), 1.22 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.96, 170.12, 147.11, 145.96, 123.47, 117.12, 109.82, 99.09, 47.19, 46.31, 37.53, 33.23, 31.95, 31.14, 28.36, 27.86, 25.18, 24.49, 22.47, 13.61; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{31}O_3$ 319.2273, found 319.2251.

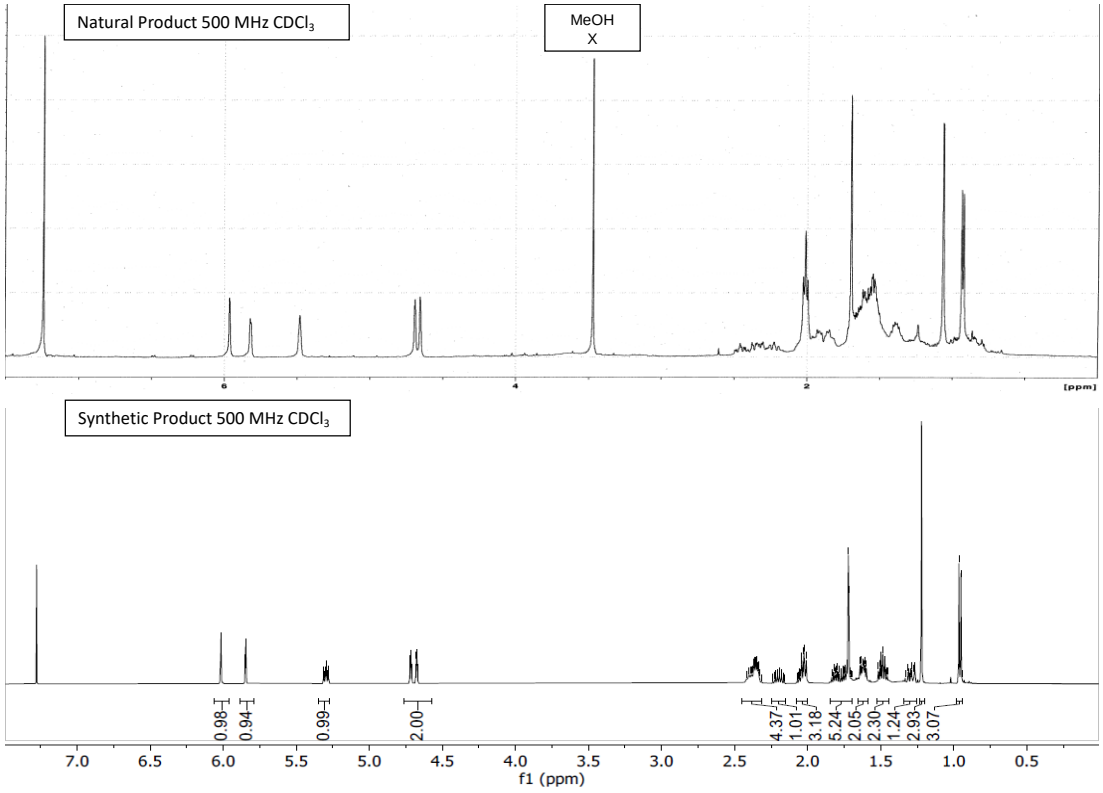


Figure 23. Spectroscopic Comparison of **6** and Natural Niphateolide A by ^1H NMR

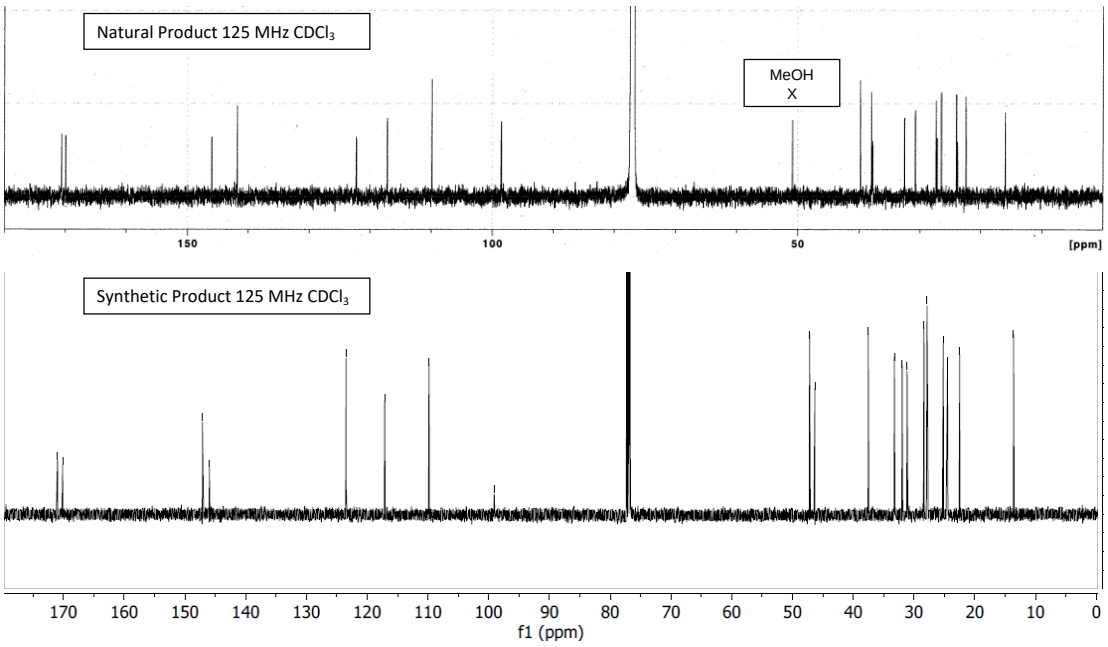
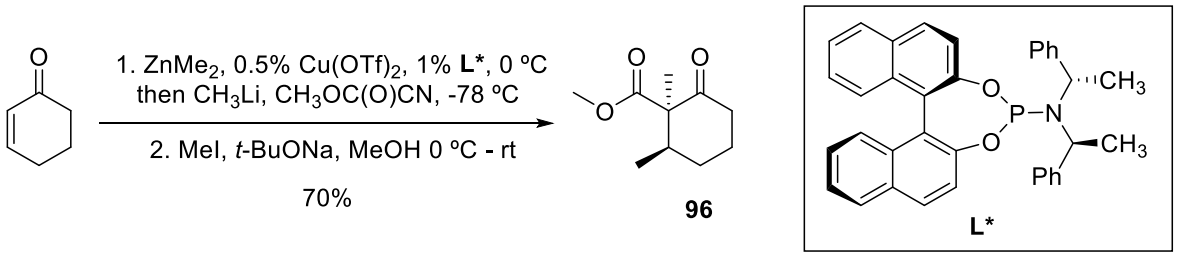


Figure 24. Spectroscopic Comparison of **6** and Natural Niphateolide A by ^{13}C NMR

E.4 Chapter 3: Synthesis of 7

β-Keto ester **96**:

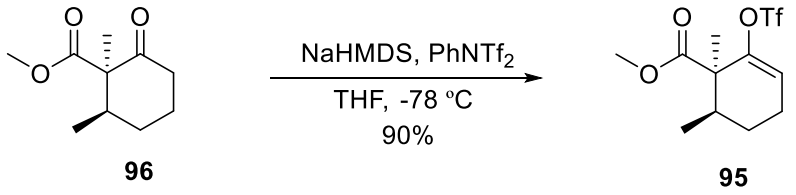


To a flame dried flask was added $Cu(OTf)_2$ (20.7 mg, 57 μmol, 0.50 mol %), **L*** (61.8 mg, 114 μmol, 1.00 mol %) and anhydrous toluene (16 mL) at room temperature under argon atmosphere. The suspension was stirred for 30 min. The reaction mixture was cooled to 0 °C using ice bath, and stirred for 20 min before adding cyclohex-2-ene-1-one (1.1 mL, 11.40 mmol, 1.0 equiv). To the resulting solution was added dimethylzinc (1.2 M in toluene, 10 mL, 12.54 mmol, 1.1 equiv) over 20 min, and it was stirred for 30 min. Then, the

mixture was cooled to -78 °C using dry ice in acetone bath, and stirred for 20 min. A solution of methyllithium in ether (1.6 M, 7.5 mL, 12.54 mmol, 1.1 equiv) was added dropwise over 5 min before methyl cyanoformate (1.1 mL, 13.68 mmol, 1.2 equiv) was added. The resulting mixture was stirred for 2 h, and then allowed to warm to 0 °C over 30 min. It was quenched with the saturated aqueous ammonium chloride, and diluted with water. The resulting mixture was allowed to warm to room temperature, and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine, dried over magnesium sulfate. The solution was filtered and concentrated under vacuum to obtain the residue, which was directly used in the following methylation.

To the obtained residue in MeOH (23 mL) was added iodomethane (3.6 mL, 57.00 mmol, 5.0 equiv) and sodium *tert*-butoxide (2.55 g, 22.80 mmol, 2.0 equiv) in sequence at 0 °C under argon atmosphere. The reaction mixture was allowed to warm to room temperature, and stirred over 12 h. The solvent was removed under vacuum, and the saturated aqueous ammonium chloride was added to the residue. It was extracted with diethyl ether (3 x 20 mL). The combined organic layers were washed with brine, dried over magnesium sulfate. The solution was filtered, and concentrated under vacuum. The residue was purified by column chromatography (5% ether in hexanes) to obtain **96** as a colorless oil (1.47 g, 70%). NMR data were in agreement with those previously reported.[17] $[\alpha]_D^{22}$ -102.6° (*c* 0.47, CH_2Cl_2), lit.[17] $[\alpha]_D^{22}$ -100.2° (*c* 1.0, CH_2Cl_2); IR (NaCl, film) *v* 2942, 2870, 1742, 1714, 1452, 1250, 1227, 1201, 1163, 1072, 1091; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.69 (s, 3H), 2.78-2.66 (m, 1H), 2.43 (dddd, *J* = 14.1, 4.4, 2.6, 1.4 Hz, 1H), 2.07-1.98 (m, 1H), 1.96-1.84 (m, 1H), 1.73-1.58 (m, 3H), 1.34 (s, 3H), 1.14 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.4, 171.8, 60.8, 51.9, 43.8, 39.8, 30.2, 25.4, 18.8, 17.0; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{10}H_{17}O_3$ 185.1178, found 185.1173.

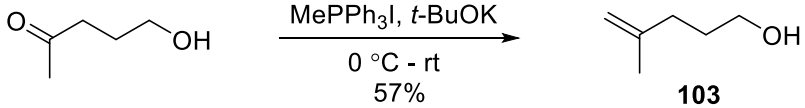
Triflate enol **95**:



96 **95**

A solution of ketone **96** (0.13 g, 0.70 mmol, 1.0 equiv) and $PhNTf_2$ (0.30 g, 0.84 mmol, 1.2 equiv) in dry THF (4.5 mL) was stirred for 5 min at -78 °C under argon atmosphere. To the mixture was added dropwise the solution of NaHMDS (1.0 M, 1.05 mL, 1.05 mmol, 1.5 equiv) in THF over 10 min. The resulting solution was stirred at the same temperature for 1 h before the saturated aqueous ammonium chloride was added. It was allowed to warm to room temperature, and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with brine, dried over magnesium sulfate. The solution was filtered and concentrated under vacuum. The obtained residue was purified by column chromatography (5% ether in hexanes) to obtain vinyl triflate **95** as a colorless oil (0.20 g, 90%). NMR data were in agreement with those previously reported.[18] $[\alpha]_D^{22}$ +73.0° (*c* 0.32, CH_2Cl_2); IR (NaCl, film) *v* 2995, 2957, 2885, 2847, 1742, 1414, 1250, 1213, 1144, 1041, 901, 814, 604; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.93 (dd, *J* = 5.4, 3.0 Hz, 1H), 3.72 (d, *J* = 0.4 Hz, 3H), 2.35-2.18 (m, 2H), 1.82-1.70 (m, 2H), 1.60-1.56 (m, *J* = 3.0 Hz, 1H), 1.43 (s, 3H), 0.97 (d, *J* = 6.6, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 149.9, 119.2, 52.1, 50.6, 40.1, 26.1, 23.2, 20.6, 16.7; HRMS (ESI-TOF) *m*/*z* $[M + NH_4]^+$ calcd for $C_{11}H_{19}F_3NO_5S$ 334.0936, found 334.0932.

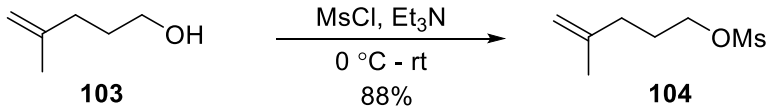
Alcohol **103**:



103

To a suspension of methyltriphenylphosphonium iodide (3.92 g, 9.75 mmol, 1.3 equiv) in dry THF (24 mL) was added dropwise the solution of *t*-BuOK (1.0 M, 9.7 mL, 9.75 mmol, 1.3 equiv) in THF at 0 °C under argon atmosphere. The resulting orange solution was stirred for 30 min at room temperature. The mixture was cooled to 0 °C, and a solution of 5-hydroxy-2-pentanone (0.77 mL, 7.50 mmol, 1.0 equiv) in THF (5 mL) was added dropwise. It was stirred for 18 h at room temperature before the saturated aqueous ammonium chloride was added. It was extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered and concentrated at 10 °C under vacuum. The obtained residue was purified by column chromatography (50% ether in hexanes) to obtain alcohol **103** as a colorless volatile oil (0.43 g, 57%). NMR data were in agreement with those previously reported.[19] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.70 (dtd, *J* = 5.9, 2.5, 1.4 Hz, 2H), 3.63 (td, *J* = 6.5, 1.0 Hz, 2H), 2.08 (t, *J* = 7.7 Hz, 2H), 1.94-1.83 (m, 1H), 1.74-1.64 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.5, 110.1, 62.6, 34.1, 30.5, 22.3.

Mesylate **104**:

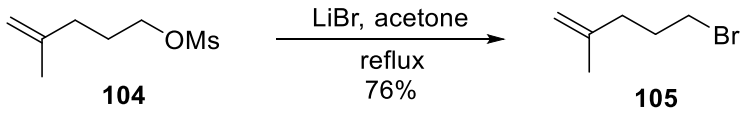


103 **104**

To a solution of alcohol **103** obtained (0.20 g, 2.00 mmol, 1.0 equiv) above and Et_3N (0.84 mL, 6.00 mmol, 3.0 equiv) in dry DCM was dropwise added methanesulfonyl chloride (MsCl, 0.23 mL, 3.00 mmol, 1.5 equiv) at 0 °C under argon atmosphere. The resulting mixture was allowed to warm to room temperature, and stirred for 5 h. It was quenched with water, extracted with DCM (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered, and concentrated under vacuum. The obtained residue was purified by column chromatography (10% EtOAc in hexanes) to obtain mesylate **104** as a colorless volatile oil (0.31 g, 88%). NMR data were in agreement with those previously reported.[19] ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.78-4.69 (m, 2H), 4.22 (t,

J = 6.6 Hz, 2H), 3.00 (s, 3H), 2.12 (t, *J* = 7.5 Hz, 2H), 1.93-1.85 (m, 2H), 1.74-1.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8, 111.1, 69.6, 37.3, 33.3, 26.3, 22.3.

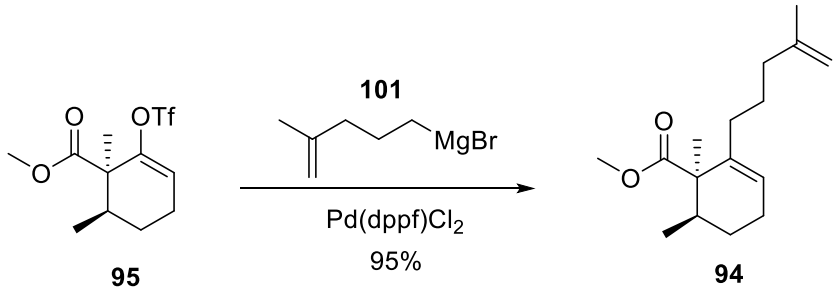
Bromide **105**:



104 **105**

To a flame dried LiBr (0.15 g, 1.76 mmol, 1.0 equiv) in flask was added acetone (3.5 mL) and mesylate **104** (0.31 g, 1.76 mmol, 1.0 equiv). It was refluxed over 14 h. Then, the reaction mixture was cooled to room temperature, and the saturated aqueous sodium bicarbonate solution was added to the suspension. It was extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered, and concentrated under vacuum using a cooling bath. The obtained residue was purified by column chromatography (hexanes) to obtain **105** as a colorless volatile oil (0.22 g, 76%). NMR data were in agreement with those previously reported.[20] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78-4.70 (m, 2H), 3.41 (t, *J* = 6.7 Hz, 2H), 2.16 (dd, *J* = 8.1, 6.6 Hz, 2H), 2.05-1.95 (m, 2H), 1.73 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.9, 111.0, 36.0, 33.3, 30.6, 22.3.

Compound **94**:

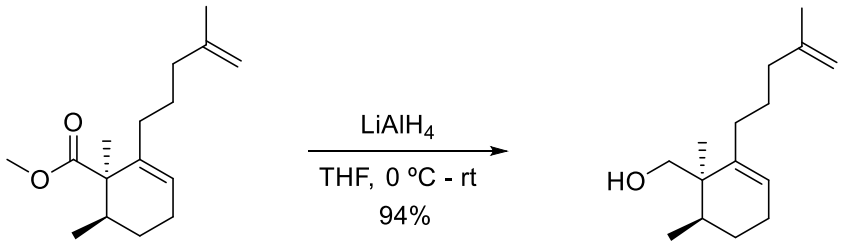


95 **94**

To a suspension of magnesium turnings (72.0 mg, 3.00 mmol, 3.0 equiv) in THF (3 mL) was added I_2 (a crystal) under argon atmosphere. Then some bromide **105** was added, and the mixture was refluxed for 30 min. The other bromide **105** (0.49 g in total, 3.00 mmol, 3.0 equiv) was added dropwise at reflux, keeping it boiling. Then it was refluxed for 1 h.

To a solution of **95** (0.32 g, 1.00 mmol, 1.0 equiv) and $Pd(dppf)Cl_2$ (0.15 g, 0.20 mmol, 0.2 equiv) in dry THF (10 mL) was dropwise added Grignard reagent **101** prepared above. It was stirred for 1 h at the same temperature before it was cooled to room temperature. The saturated aqueous sodium bicarbonate was added to the mixture. It was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered and concentrated under vacuum. The obtained residue was purified by column chromatography (2% EtOAc in hexanes) to obtain **94** as a colorless oil (0.24 g, 95%). $[\alpha]_D^{22}$ +170.8° (*c* 0.24, $CHCl_3$); IR (NaCl, film) *v* 2937, 2878, 2839, 1730, 1649, 1456, 1433, 1375, 1227, 1194, 1163, 1113, 983, 885; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.63 (dq, *J* = 5.3, 1.5 Hz, 1H), 4.71-4.65 (m, 2H), 3.65 (s, 3H), 2.16-1.96 (m, 4H), 1.95-1.89 (m, 1H), 1.87-1.80 (m, 1H), 1.75-1.66 (m, 4H), 1.61-1.46 (m, 4H), 1.29 (s, 3H), 0.90 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 145.8, 138.9, 123.3, 109.8, 51.4, 50.5, 39.3, 37.7, 32.7, 27.0, 26.4, 24.9, 22.4, 22.4, 17.4; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{27}O_2$ 251.2011, found 251.1991.

Alcohol **107**:

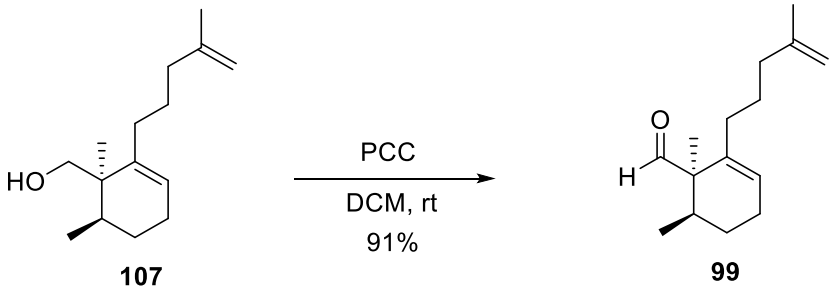


94 **107**

To a solution **94** (90.0 mg, 0.36 mmol, 1.0 equiv) in dry THF (3 mL) was dropwise added the solution of $LiAlH_4$ (2.0 M, 0.36 mL, 0.72 mmol, 2.0 equiv) in THF at 0 °C under argon

atmosphere. The reaction mixture was stirred at room temperature for 2 h. It was quenched with the saturated aqueous ammonium chloride solution at 0 °C, and extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered and concentrated under vacuum. The obtained residue was purified by column chromatography (10% EtOAc in hexanes) to obtain **107** as a colorless oil (75.1 mg, 94%). $[\alpha]_D^{22}$ +43.1° (*c* 0.11, CH_2Cl_2); IR (NaCl, film) *v* 3391, 3074, 2968, 2933, 2881, 2839, 1649, 1456, 1375, 1030, 885; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.73 (ddd, *J* = 4.4, 3.2, 1.5 Hz, 1H), 4.74-4.67 (m, 2H), 3.58-3.42 (m, 2H), 2.09-1.86 (m, 6H), 1.75-1.52 (m, 8H), 1.36-1.18 (m, 1H), 1.04 (d, *J* = 6.7 Hz, 3H), 0.97 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.9, 139.6, 125.6, 109.9, 65.6, 42.4, 38.0, 37.9, 30.3, 27.9, 27.5, 24.7, 22.4, 21.9, 16.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{15}H_{27}O$ 223.2062, found 223.2047.

Aldehyde **99**:

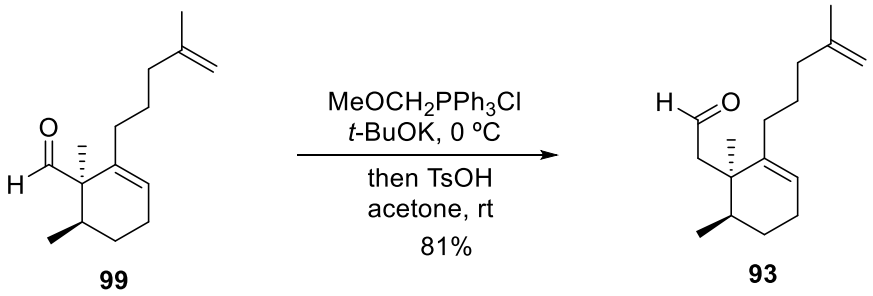


107 **99**

To a vigorously stirred solution of **107** (51.1 mg, 0.23 mmol, 1.0 equiv) in dry DCM (4 mL) was added pyridinium chlorochromate (0.13 g, 0.58 mmol, 2.5 equiv) at room temperature under argon atmosphere. Stirring was maintained for 3 h at the same temperature. The resulting mixture was subjected to filtration through pad of silica, eluted with DCM. The solvent was evaporated under vacuum, and the obtained residue was purified by column chromatography (5% EtOAc in hexanes) to obtain **99** (46.0 mg, 91%) as a colorless oil. $[\alpha]_D^{22}$ +396.9° (*c* 0.080, $CHCl_3$); IR (NaCl, film) *v* 3078, 2933, 2873, 2839, 2706, 1722, 1649, 1460, 1437, 1375, 885, 795; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 5.84 (ddt, *J* = 4.7, 2.9, 1.4 Hz, 1H), 4.71-4.63 (m, 2H), 2.28-2.12 (m, 2H), 2.04-1.93 (m, 2H), 1.87 (ddddd, *J* = 14.0, 9.8, 5.6, 2.6, 1.3 Hz, 1H), 1.79-1.62 (m, 7H), 1.57-1.40 (m, 2H), 1.18 (s,

3H), 0.98 (d, J = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.9, 145.7, 135.9, 126.7, 110.0, 54.1, 38.2, 37.7, 31.8, 27.6, 26.6, 25.2, 22.4, 17.7, 16.9; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{15}H_{25}O$ 221.1905, found 221.1878.

Aldehyde **93**:

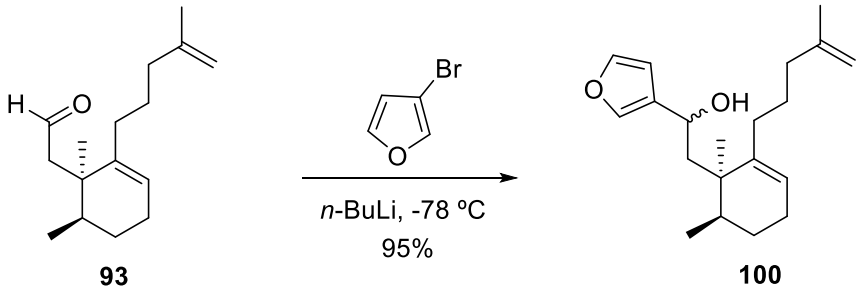


99 **93**

To a suspension of (methoxymethyl)triphenylphosphonium chloride (0.17 g, 0.48 mmol, 2.5 equiv) in dry THF was dropwise added the solution of *t*-BuOK (1.0 M, 0.48 mL, 0.48 mmol, 2.5 equiv) in THF at 0 °C under argon atmosphere. It was stirred at the same temperature for 1 h. A solution of **99** (41.8 mg, 0.19 mmol, 1.0 equiv) in dry THF (0.5 mL) was added to the cherry-red solution dropwise. It was stirred at room temperature for 14 h, and quenched with water. The mixture was extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate. The solution was filtered and concentrated under vacuum to obtain residue. Acetone (10 mL) was added to the flask equipped with the residue. *p*-Toluenesulfonic acid monohydrate (57.0 mg, 0.30 mmol, 1.6 equiv) was added to the solution at 0 °C under argon atmosphere. The reaction mixture was stirred at room temperature for 2 h. The solvent was removed under vacuum, and ethyl acetate was added. It was washed with saturated aqueous sodium bicarbonate. The organic layer was dried over magnesium sulfate. The solution was filtered and concentrated under vacuum. The obtained residue was purified by column chromatography (2% EtOAc in hexanes) to obtain **93** as a colorless oil (36.0 mg, 81%). $[\alpha]_D^{22}$ +10.32° (*c* 0.07, CH_2Cl_2); IR (NaCl, film) ν 3070, 2968, 2930, 2878, 2839, 2729, 1718, 1649, 1456, 1440, 1375, 1045, 885; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.81 (t, J = 3.4 Hz, 1H), 5.54 (ddt, J = 4.5, 2.9, 1.4 Hz, 1H), 4.81-4.62 (m, 2H), 2.37 (d, J = 3.4 Hz, 2H), 2.09-1.88 (m, 5H), 1.74 (s, 3H), 1.72-1.51

(m, 5H), 1.34-1.24 (m, 1H), 1.21 (s, 3H), 0.98 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.5, 145.8, 141.0, 122.4, 110.0, 49.1, 40.8, 38.8, 37.9, 30.9, 27.2, 27.0, 26.0, 24.1, 22.4, 16.5; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for $C_{16}H_{27}O$ 235.2062, found 235.2056.

Alcohol **100**:

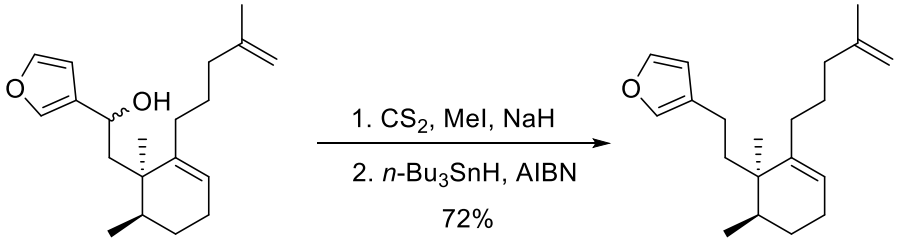


93 **100**

To a solution of 3-bromofuran (0.32 mL, 3.50 mmol, 10.0 equiv) in dry THF (1.2 mL) was dropwise added *n*-BuLi (1.4 mL, 2.5 M, 3.50 mmol, 10.0 equiv) in hexanes at -78 °C under argon atmosphere. It was stirred for 30 min at the same temperature. Then, a solution of **93** (81.9 mg, 0.35 mmol, 1.0 equiv) in THF (1 mL) was added to the mixture dropwise. The resulting solution was stirred for 20 min. The reaction was quenched with saturated aqueous ammonium chloride at -78 °C, and allowed to warm to room temperature. The mixture was extracted with diethyl ether (3 x 20 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc in hexanes) to obtain diastereomers **100a** and **100b** (*dr* = 3:1, 0.10 g, 95%) as colorless oil. **Isomer 100a**: $[\alpha]_D^{22}$ +74.4° (*c* 0.040, CH_2Cl_2); IR (NaCl, film) *v* 3470, 3074, 2961, 2934, 2873, 1649, 1504, 1456, 1371, 1159, 1065, 1022, 885, 874, 791; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.37 (m, 2H), 6.40 (dd, J = 1.8, 1.0 Hz, 1H), 5.68 (dd, J = 3.3, 1.8 Hz, 1H), 5.08 (dt, J = 10.6, 2.2 Hz, 1H), 4.74-4.70 (m, 2H), 2.82 (d, J = 1.8 Hz, 1H), 2.21-2.03 (m, 6H), 1.91 (ddd, J = 14.9, 10.6, 0.7 Hz, 1H), 1.78-1.73 (m, 4H), 1.72-1.55 (m, 5H), 1.09-1.05 (d, J = 6.4 Hz, 3H), 1.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.9, 145.0, 143.1, 138.4, 129.8, 123.6, 109.9, 108.5, 65.6, 43.4, 39.6, 39.5, 38.0, 30.5, 28.2, 27.6, 27.3, 25.3, 22.4, 16.7; HRMS (ESI-TOF) m/z [M + H]$^+$ calcd for

$C_{20}H_{31}O_2$ 303.2324, found 303.2316; **Isomer 100b**: $[\alpha]_D^{22}$ +14.2° (*c* 0.025, CH_2Cl_2); IR (NaCl, film) *v* 3431, 3074, 2964, 2931, 2878, 2839, 1649, 1504, 1456, 1375, 1159, 1061, 1026, 885, 874, 791; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.35 (m, 2H), 6.41 (dd, *J* = 1.8, 0.9 Hz, 1H), 5.46 (t, *J* = 3.8 Hz, 1H), 4.86 (dt, *J* = 8.6, 3.0 Hz, 1H), 4.72-4.67 (m, 2H), 2.11-1.86 (m, 6H), 1.96-1.46 (m, 10H), 1.19 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.0, 143.3, 143.1, 138.4, 130.9, 121.8, 109.8, 108.5, 64.4, 44.0, 39.8, 38.5, 38.0, 31.4, 27.4, 26.4, 26.0, 24.1, 22.4, 16.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{20}H_{31}O_2$ 303.2324, found 303.2313.

Compound **92**:



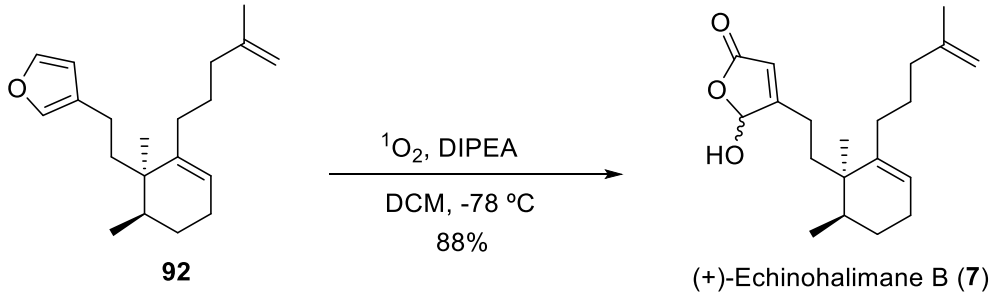
100 **92**

To a solution of alcohol mixture **100** (0.10 g, 0.33 mmol, 1.0 equiv) and CS_2 (0.12 mL, 1.98 mmol, 6.0 equiv) in dry THF (5 mL) was added NaH (79.2 mg, 60% dispersion in mineral oil, 1.98 mmol, 6.0 equiv) at 0 °C under argon atmosphere. It was stirred for 30 min at the same temperature. Iodomethane (0.13 mL, 1.98 mmol, 6.0 equiv) was added to the suspension, and the resulting mixture was stirred for another 30 min. It was quenched with the saturated aqueous ammonium chloride at 0 °C. The mixture was allowed to warm to room temperature, and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (3% EtOAc in hexanes) to obtain crude diastereomers mixture as a yellow oil.

To a solution of xanthate mixture obtained above in toluene (5 mL) was added AIBN (26.2 mg, 0.16 mmol, 0.5 equiv) under argon, followed by *n*-Bu_3Sn-H (0.72 mL, 2.64 mmol, 8.0

equiv). It was degassed three times with argon, and heated to 80 °C for 2 h. After being cooled to room temperature, the resulting mixture was directly purified by chromatography (hexanes) to obtain **92** (68.2 mg, 72% from **100**) as a colorless oil. $[\alpha]_D^{22}$ +43.6° (*c* 0.25, CH_2Cl_2); IR (NaCl, film) *v* 3074, 2932, 2874, 2840, 1653, 1508, 1456, 1375, 1162, 1065, 1026, 886, 873, 779,725; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (t, *J* = 1.7 Hz, 1H), 7.22 (dq, *J* = 2.0, 1.0 Hz, 1H), 6.28 (dd, *J* = 1.8, 0.9 Hz, 1H), 5.48 (td, *J* = 3.4, 1.9 Hz, 1H), 4.73-4.69 (m, 2H), 2.47-2.29 (m, 2H), 2.11-1.88 (m, 5H), 1.74-1.44 (m, 11H), 1.10 (s, 3H), 1.00 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.1, 142.8, 142.6, 138.4, 126.1, 121.3, 110.9, 109.7, 39.9, 38.0, 37.7, 36.3, 30.9, 27.4, 27.2, 26.6, 23.9, 22.5, 20.9, 16.0; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{20}H_{31}O$ 287.2375, found 287.2366.

(+)-Echinohalimane B (**7**)



92 (+)-Echinohalimane B (**7**)

To a solution of precursor **92** (17.2 mg, 0.06 mmol) and DIPEA (0.1 mL, 0.60 mmol, 10 equiv) in dry DCM (5 mL) was added Rose Bengal (3.0 mg) at room temperature. Anhydrous oxygen was bubbled in for 10 min. It was placed under O_2 at -78 °C, and irradiated with a 500 W lamp for 6 h. Then, it was allowed to warm to room temperature, and the saturated oxalic acid solution was added. It was stirred for 30 min. The resulting mixture was extracted with DCM (3 x 10 mL). The combined organic layers were dried over magnesium sulfate, and concentrated under vacuum. The residue was purified by column chromatography (20 % EtOAc in hexanes) to obtain **7** (16.8 mg, 88%) as a coloress oil. NMR data were in agreement with those previously reported.[16] $[\alpha]_D^{22}$ +35.7° (*c* 0.06, MeOH), lit.[21] $[\alpha]_D^{25}$ +37° (*c* 0.20, MeOH); IR (NaCl, film) *v* 3361, 2961, 2933, 2878, 2840, 2728, 1740, 1648, 1455, 1374, 1336, 1295, 1192, 1133, 1007, 952, 887, 724; ^{1}H NMR (500

MHz, $CDCl_3$) δ 6.01 (s, 1H), 5.82 (s, 1H), 5.48 (t, br, 1H), 4.69 (s, 1H), 4.66 (s, 1H), 2.43-2.23 (m, 2H), 2.10-1.78 (m, 6H), 1.70 (s, 3H), 1.69-1.50 (m, 6H), 1.42-1.37 (m, 1H), 1.06 (s, 3H), 0.93 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.88, 170.07/170.06, 146.00/145.99, 141.75, 122.22/122.15, 117.15/117.09, 109.87/109.86, 98.64/98.55, 39.73, 37.94/37.91, 37.73/37.65, 32.52/32.49, 30.74/30.71, 27.32/27.28, 27.19/27.16, 26.50/26.48, 24.02, 23.88/23.83, 22.48/22.46, 16.04/16.02; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{20}H_{31}O_3$ 319.2273, found 319.2265.

Table 6. Comparison of **7** and Natural Niphateolide A by ^{1}H and ^{13}C NMR

Position	^{1}H NMR {δ ppm, multipl., *J* (Hz)} $CDCl_3$, 500 Hz		^{13}C NMR {δ (ppm)} $CDCl_3$, 125 Hz	
	Natural[16]	**7**	Natural[16]	**7**
1	1.70, s	1.70, s	22.43/22.45	22.46/22.48
2			145.95/145.96	145.99/146.00
3	2.01, br t (7.3)	2.01, br t (7.7)	37.91/37.93	37.91/37.94
4	1.39, m; 1.63, m	1.39, m; 1.63, m	27.30/27.34	27.28/27.32
5	1.86, m; 1.92, m	1.86, m; 1.92, m	30.73/30.75	30.71/30.74
6	5.48, br t	5.48, br t	122.17/122.25	122.15/122.22
7			141.8	141.8
8	1.97, m; 2.06, m	1.97, m; 2.06, m	23.83/23.88	23.83/23.88
9	1.54 m	1.54 m	27.16/27.19	27.16/27.19
10	1.60 m	1.60 m	37.68/37.76	37.65/37.73
11			39.7	39.7
12	1.55, m; 1.69, m	1.56, m; 1.67, m	32.52/32.55	32.49/32.52
13	2.29, m; 2.39, m	2.30, m; 2.39, m	24.0	24.0
14			169.90	170.07
15	5.82, s	5.82, s	117.12/117.17	117.09/117.15
16			169.90/170.58	170.88
17	5.96, s	6.01, s	98.43/98.51	98.55/98.64
18	1.07, s	1.06, s	26.46/26.47	26.48/26.50
19	0.93, d (6.7)	0.93, d (7.0)	16.01/16.03	16.02/16.04
20	4.65, s; 4.69, s	4.66, s; 4.69, s	109.85/109.86	109.86/109.87

E.5 References

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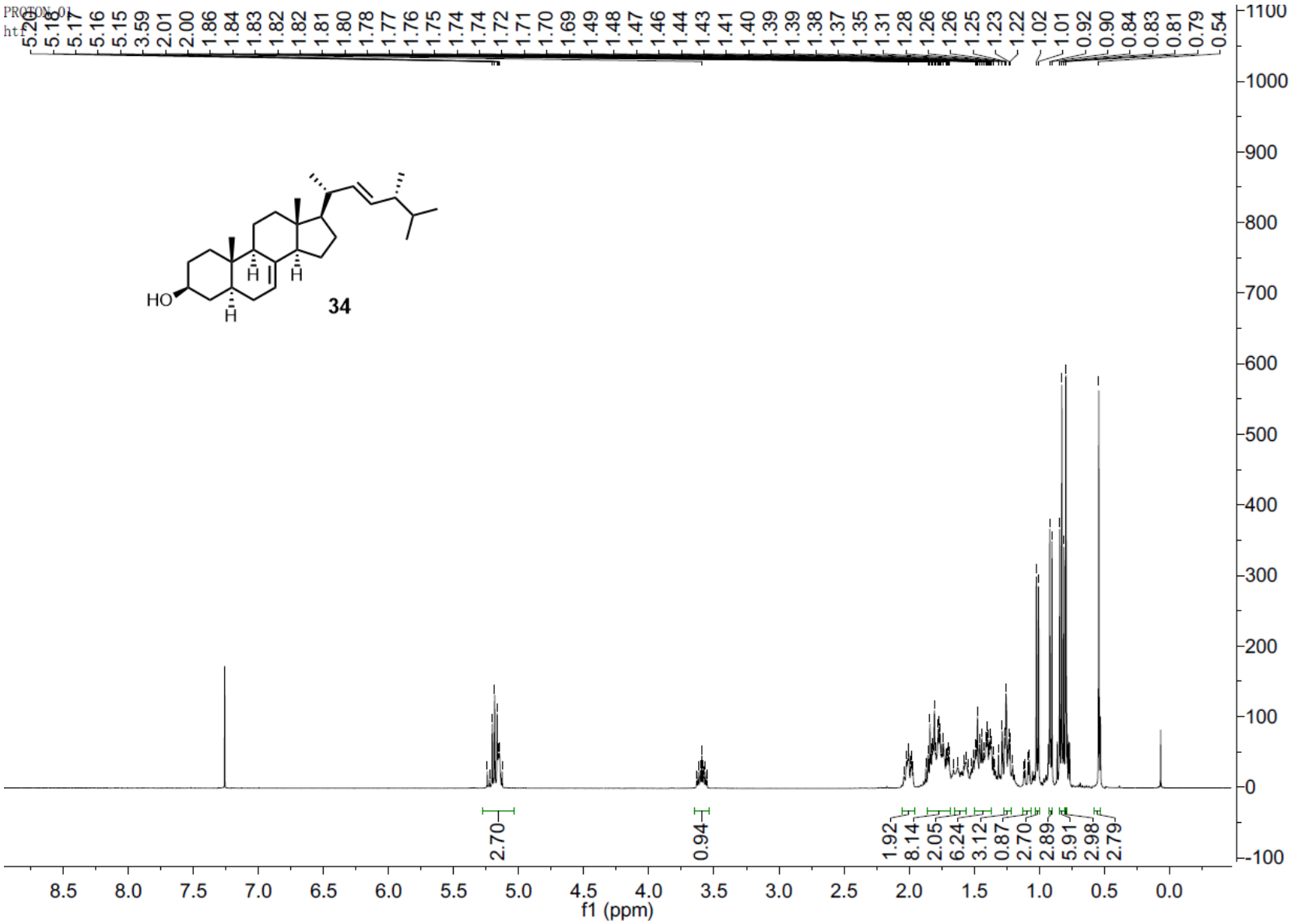
Annexe NMR Spectrum of Synthesized Compounds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34
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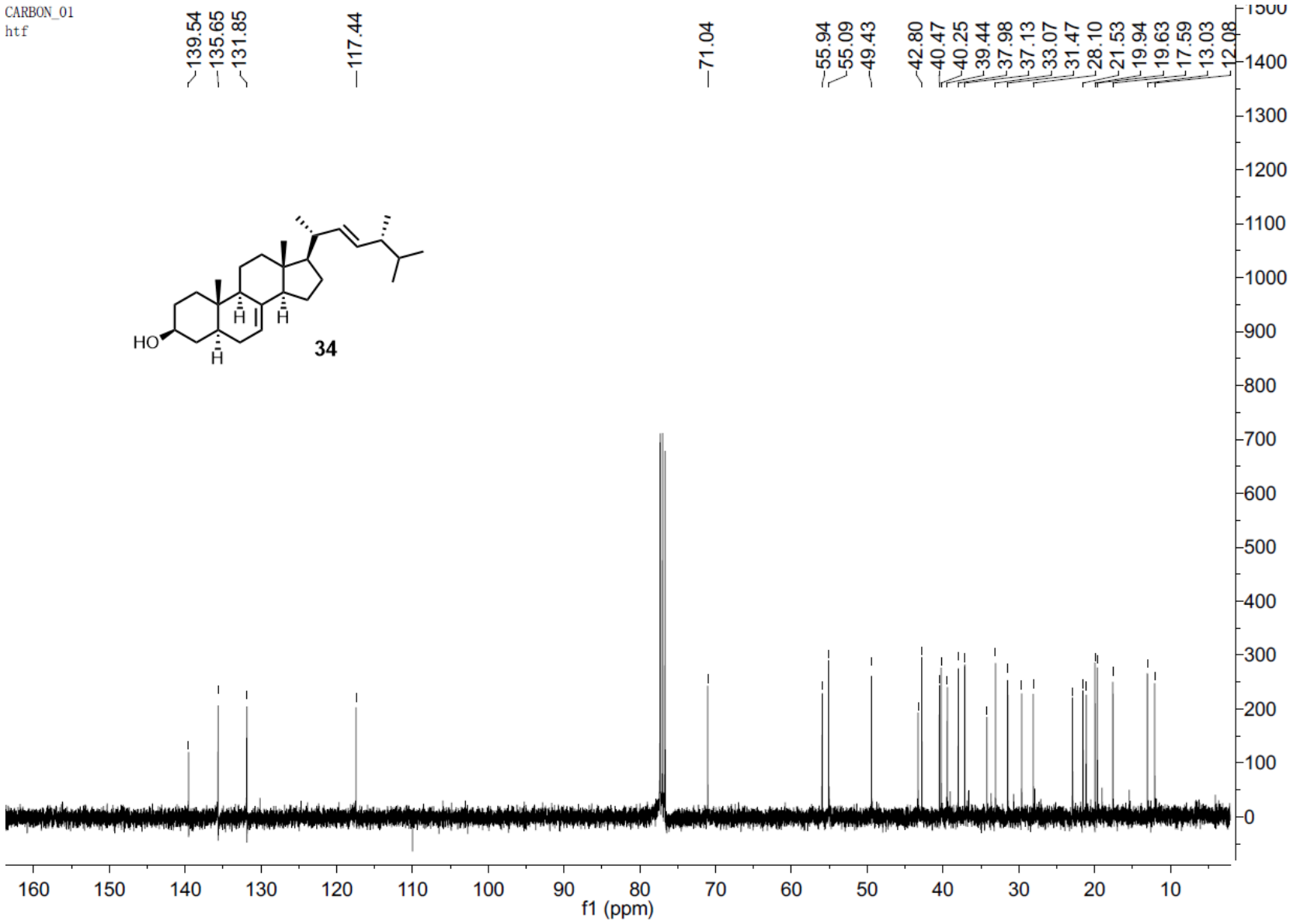
1.92 8.14 2.05 6.24 3.12 0.87 2.70 2.89 5.91 2.98 2.79
f1 (ppm)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39.44
37.98
37.13
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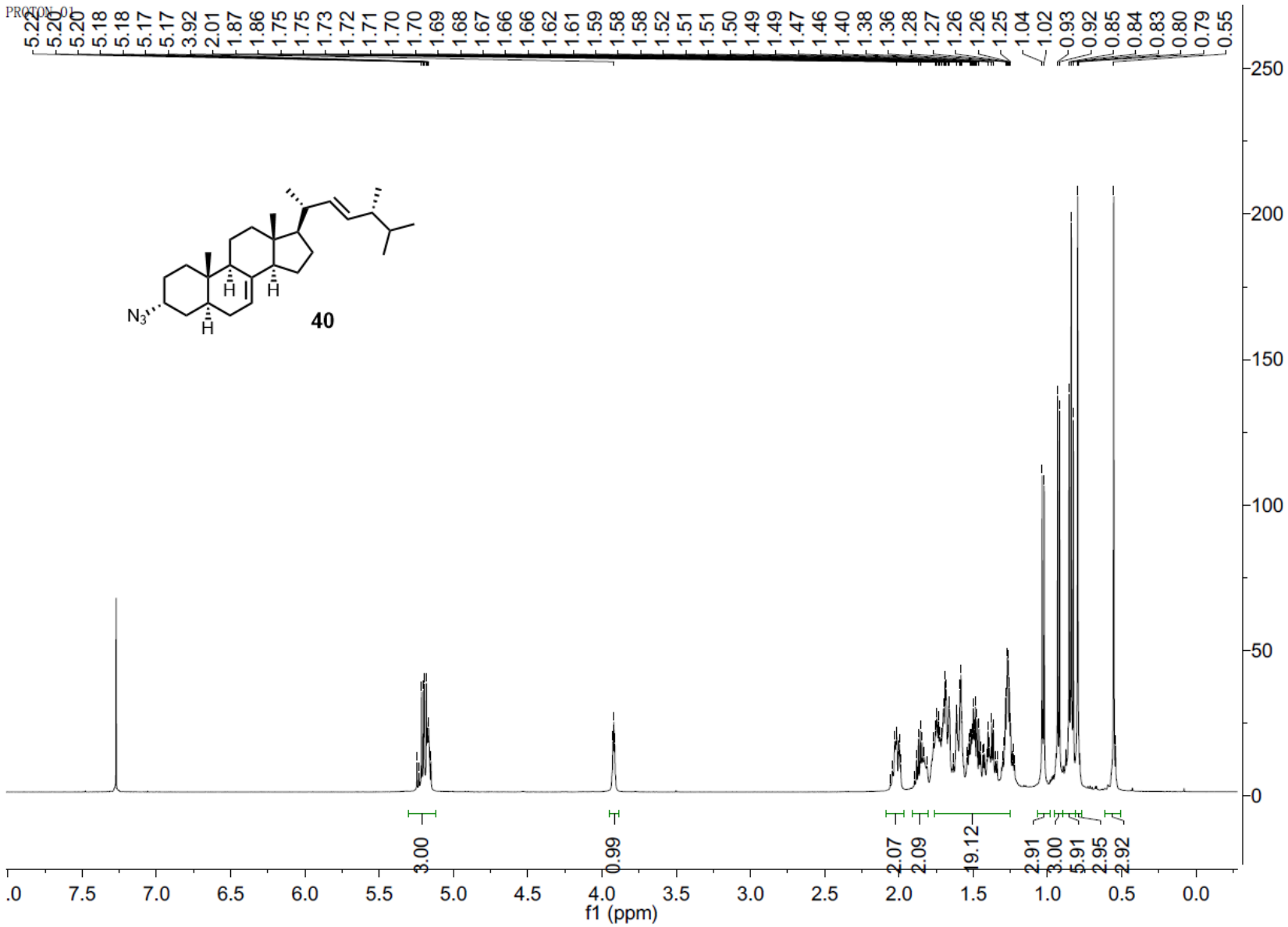
13.03
12.08
HO
34
f1 (ppm)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N3
H
H
H
40
250
200
150
100
50
0
3.00
0.99
2.07
2.09
19.12
2.91
3.00
5.91
2.95
2.92
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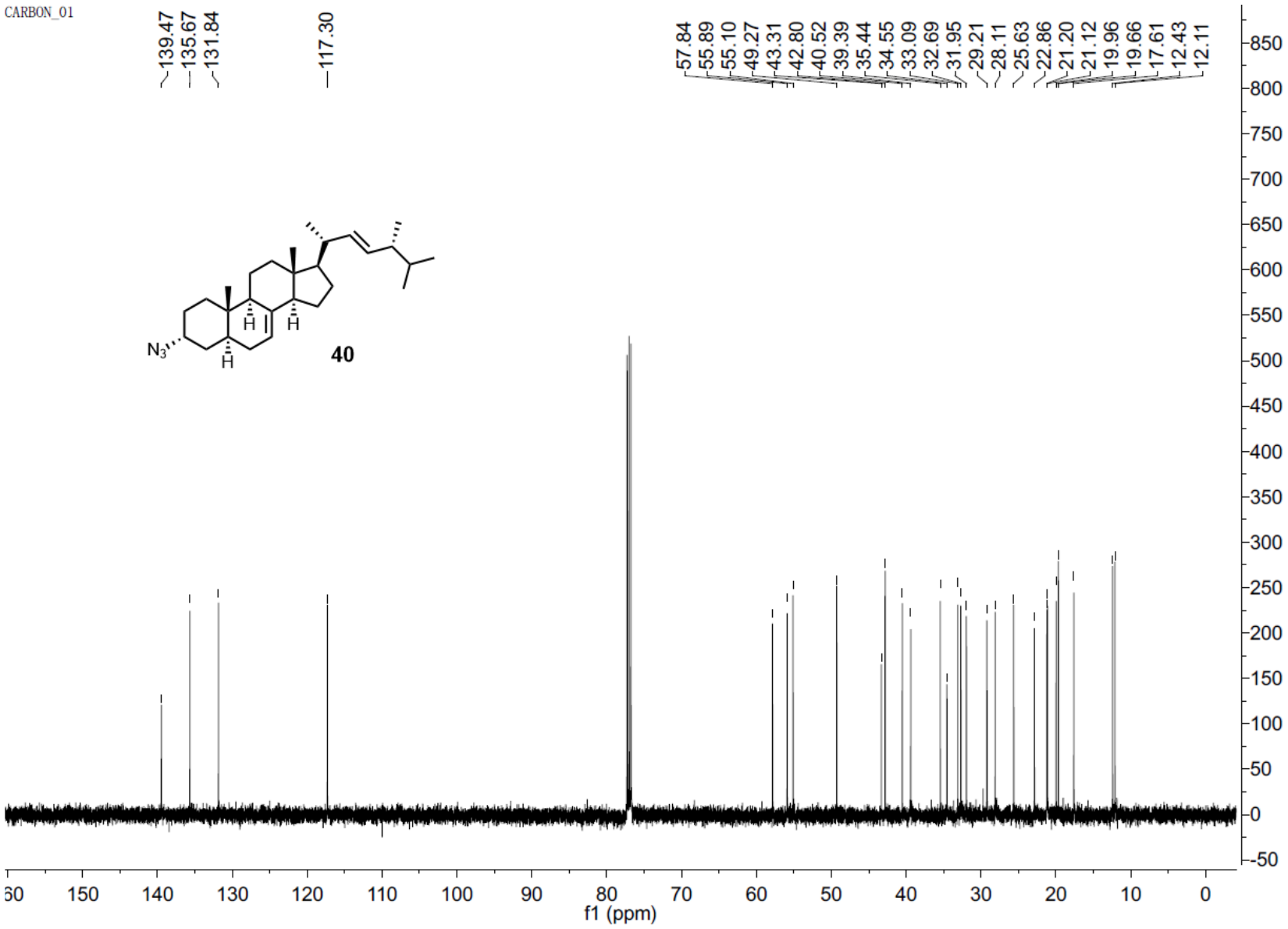
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39.39
35.44
34.55
33.09
32.69
31.95
29.21
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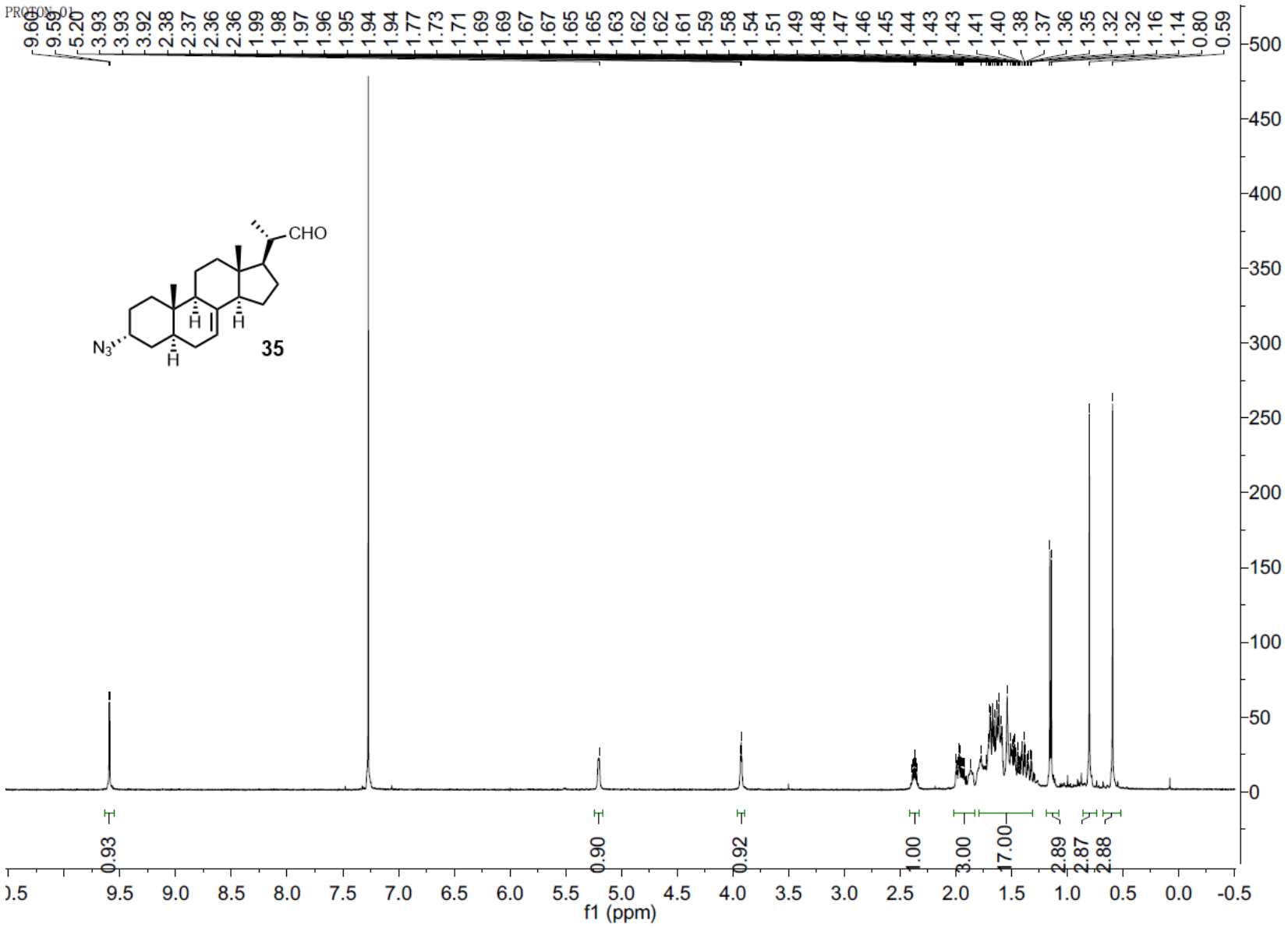
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CHO
H
H
H
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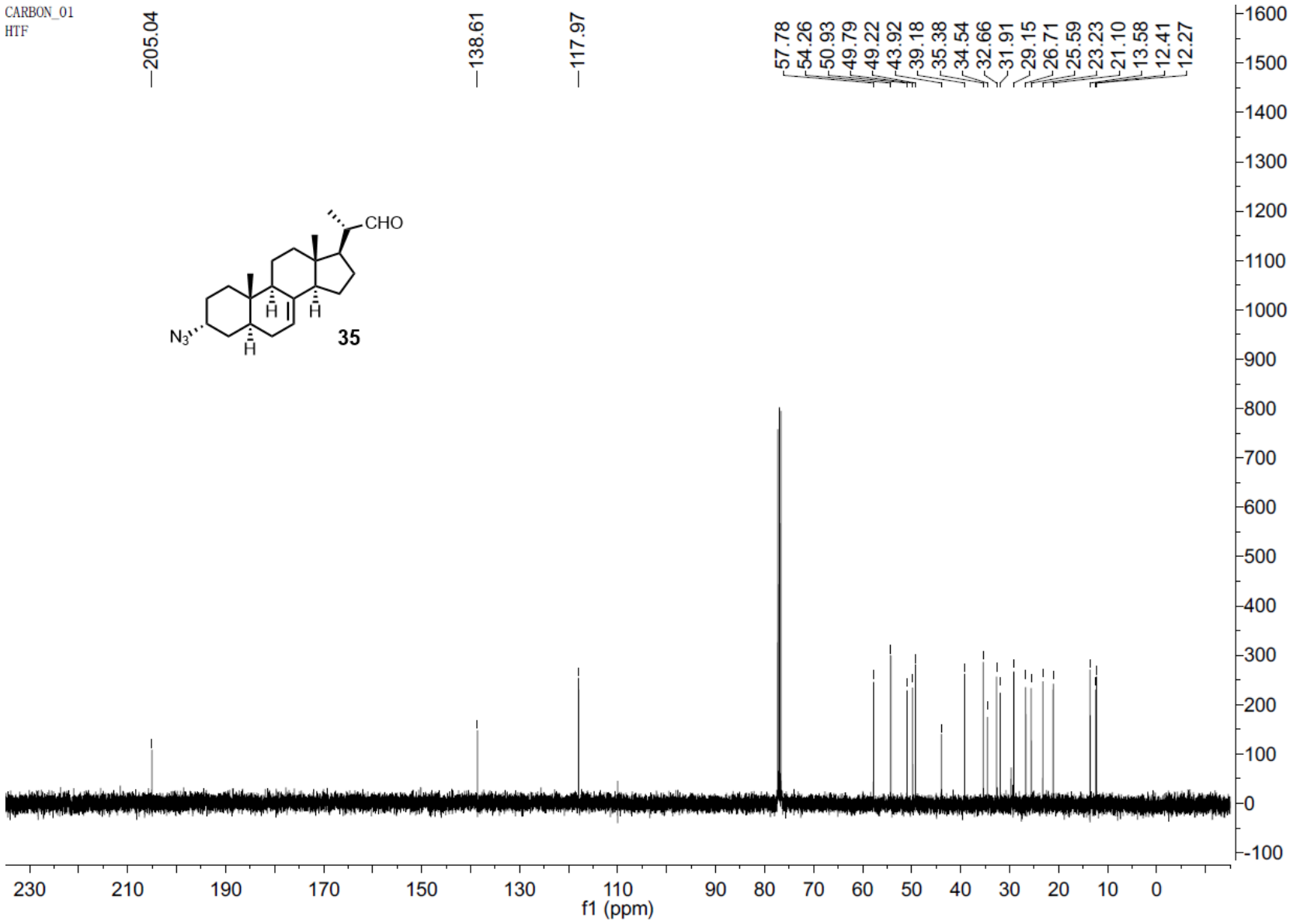
f1 (ppm)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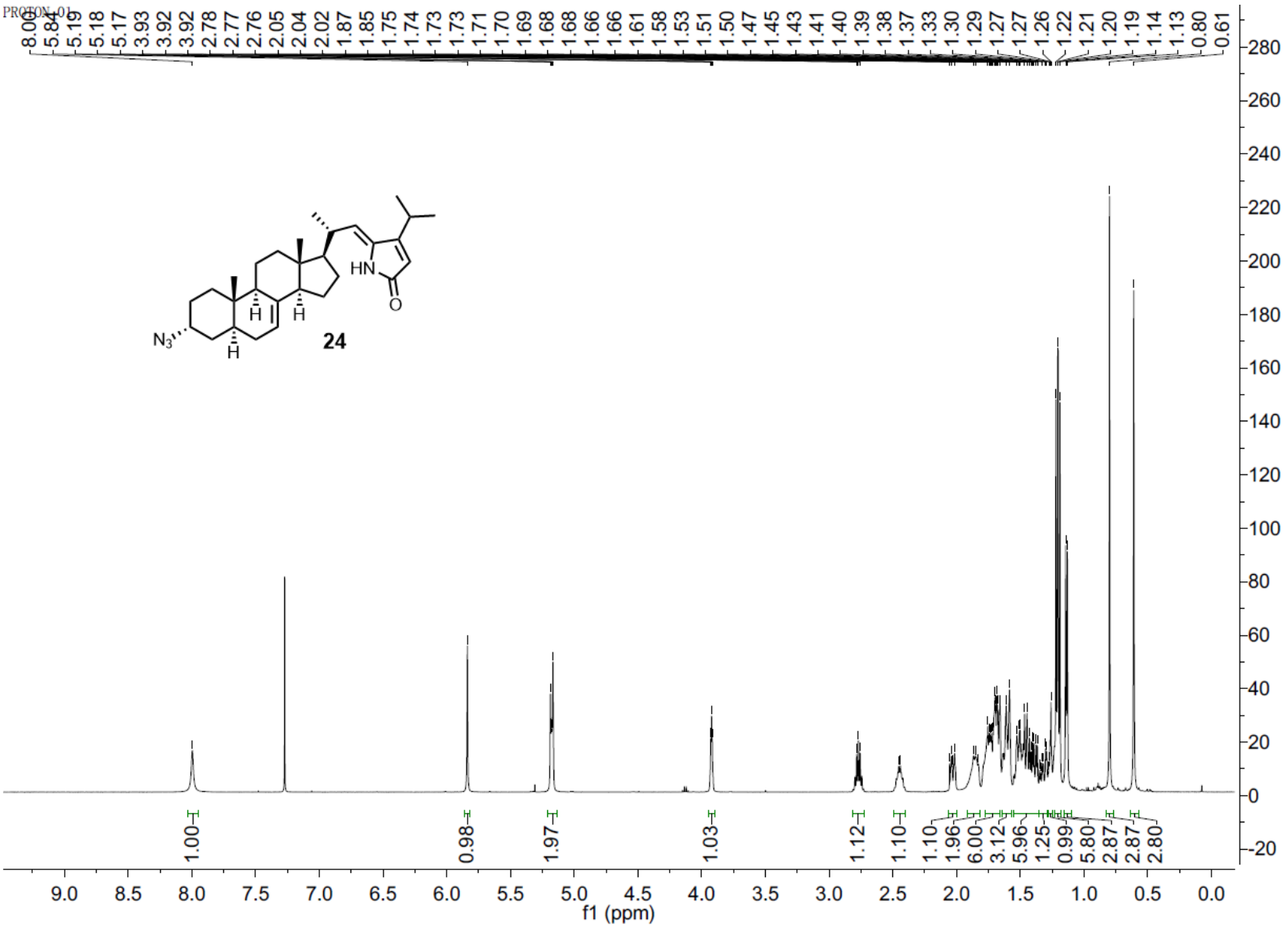
f1 (ppm)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HN
O
H
H
H
N3
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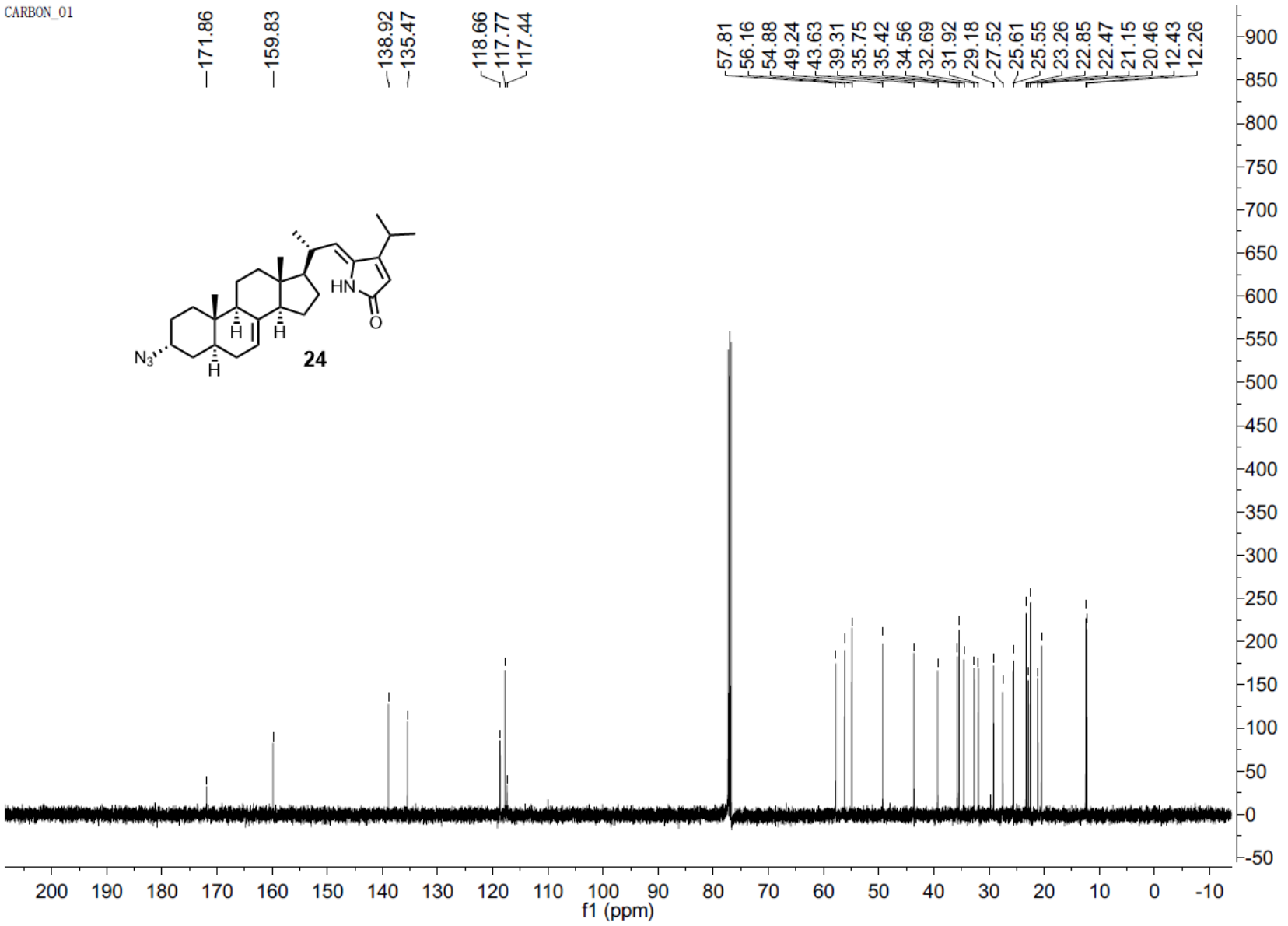
f1 (ppm)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43.63
39.31
35.75
35.42
34.56
32.69
31.92
29.18
27.52
25.61
25.55
23.26
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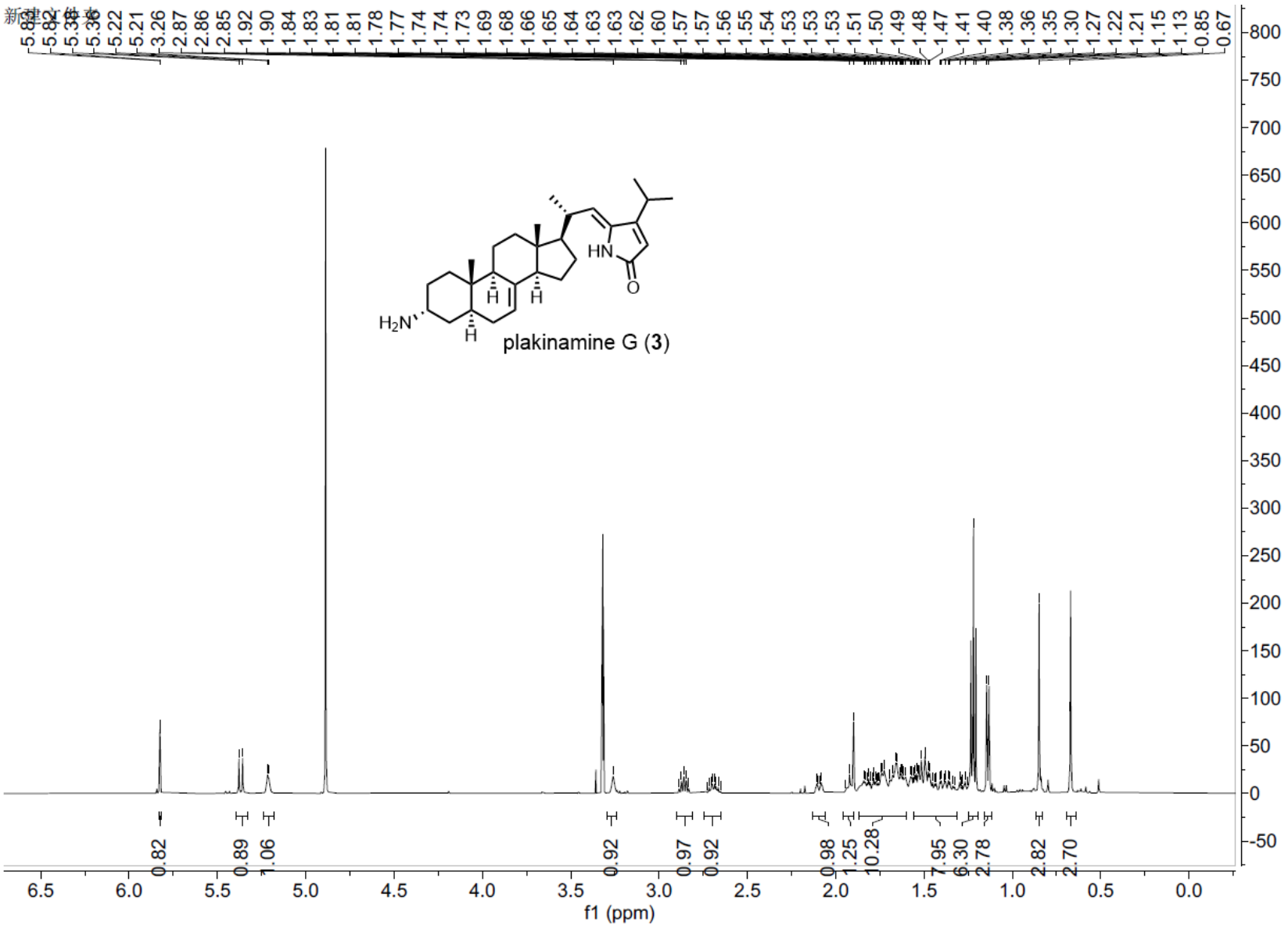
O
H
H
H
N3
24
f1 (ppm)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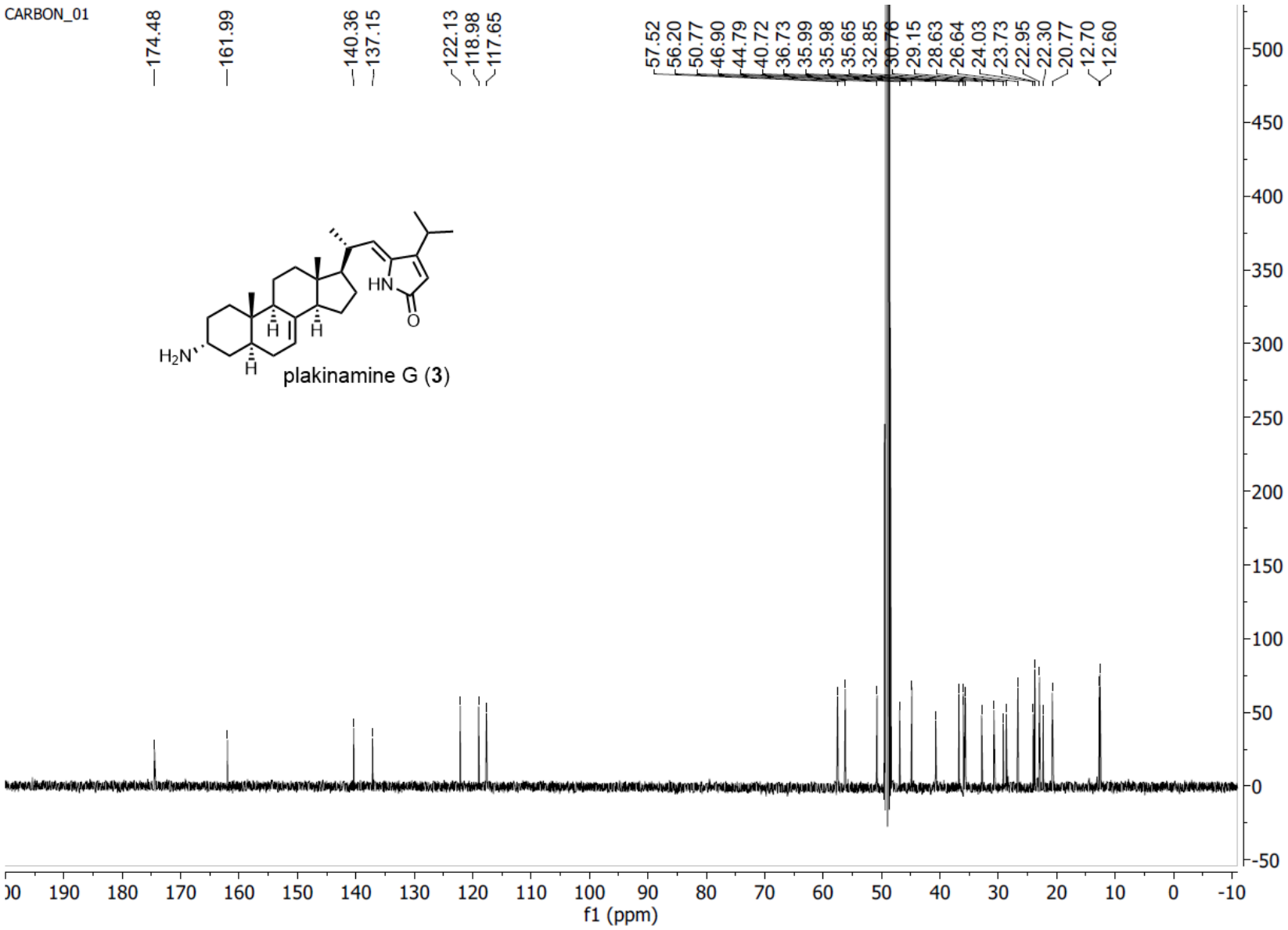
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44.79
40.72
36.73
35.99
35.98
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32.85
30.76
29.15
28.63
26.64
24.03
23.73
22.95
22.30
20.77
12.70
12.60
HN
O
H
H
H
H2N
plakinamine G (3)
500
450
400
350
300
250
200
150
100
50
0
-50
190
180
170
160
150
140
130
120
110
100
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80
70
60
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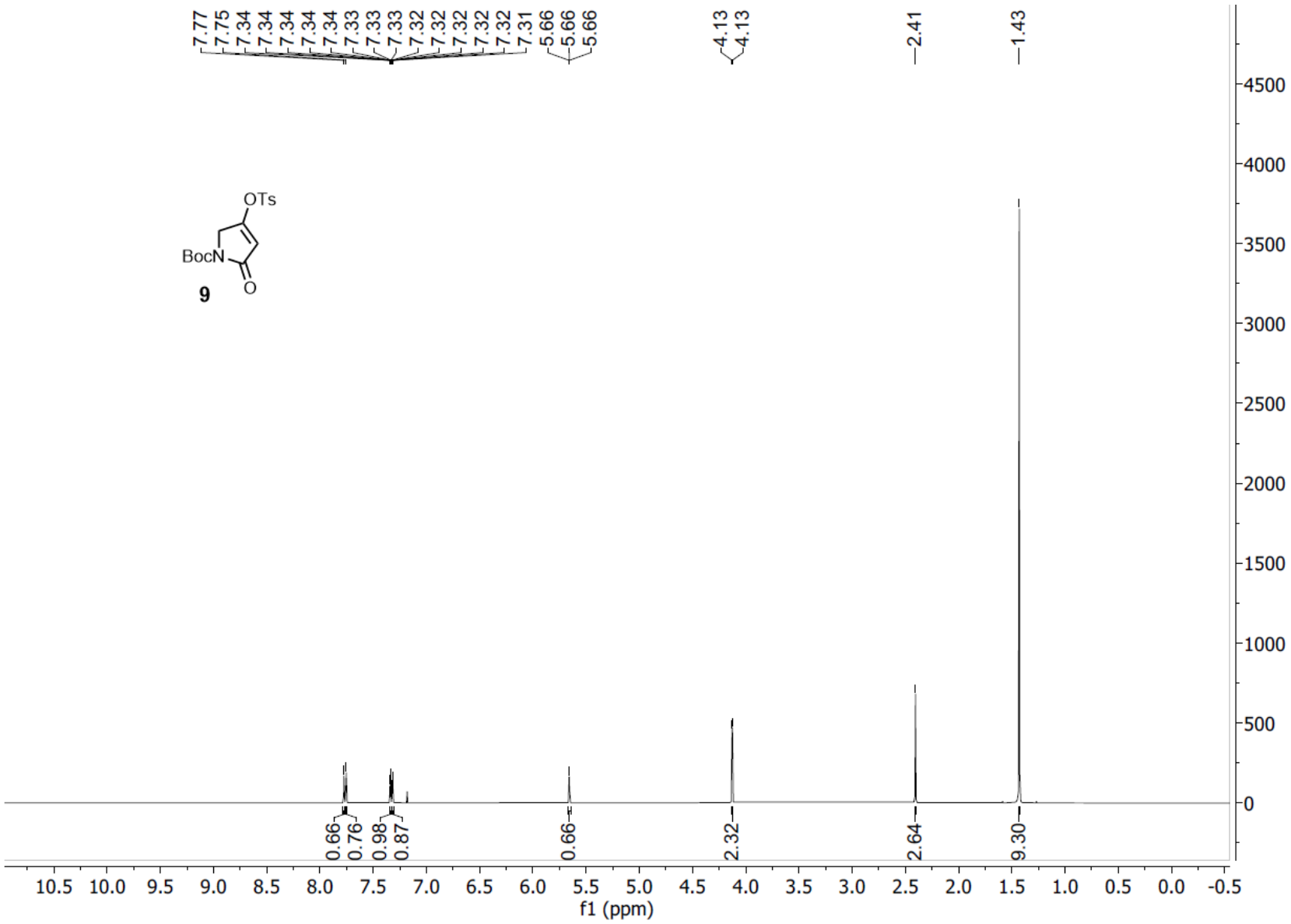
40
30
20
10
0
-10
f1 (ppm)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5.66
5.66
4.13
4.13
2.41
1.43
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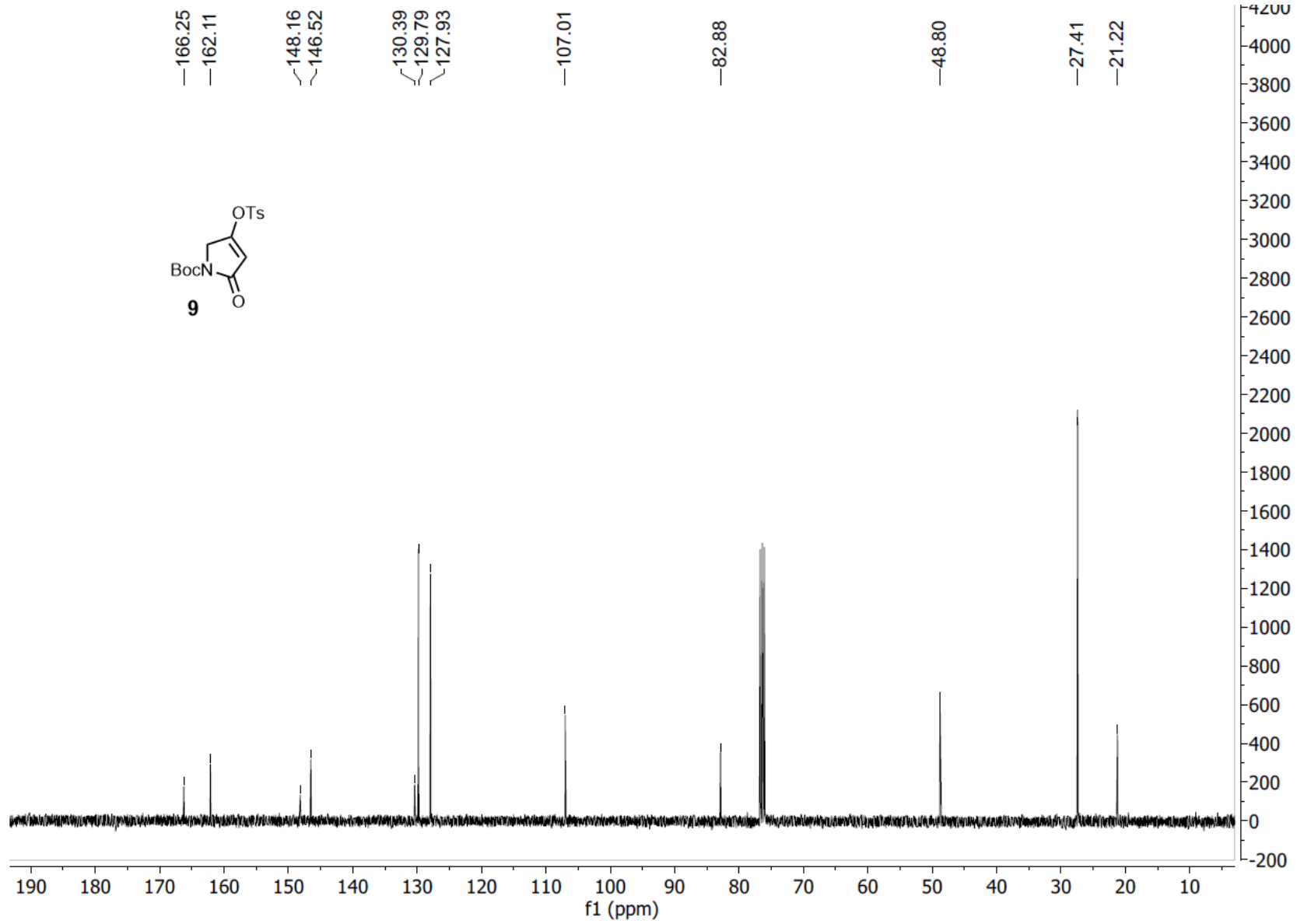
f1 (ppm)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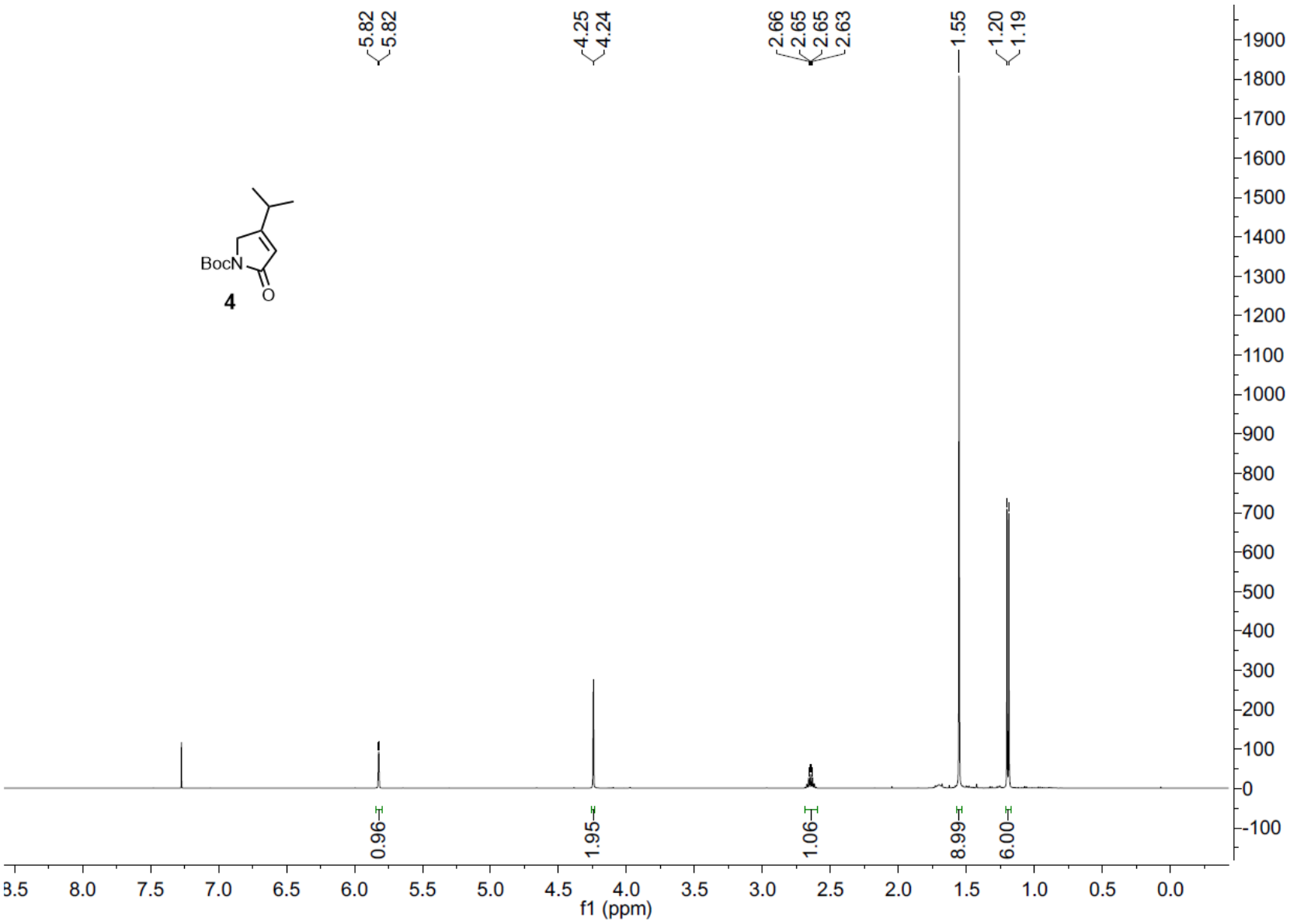
O
9
f1 (ppm)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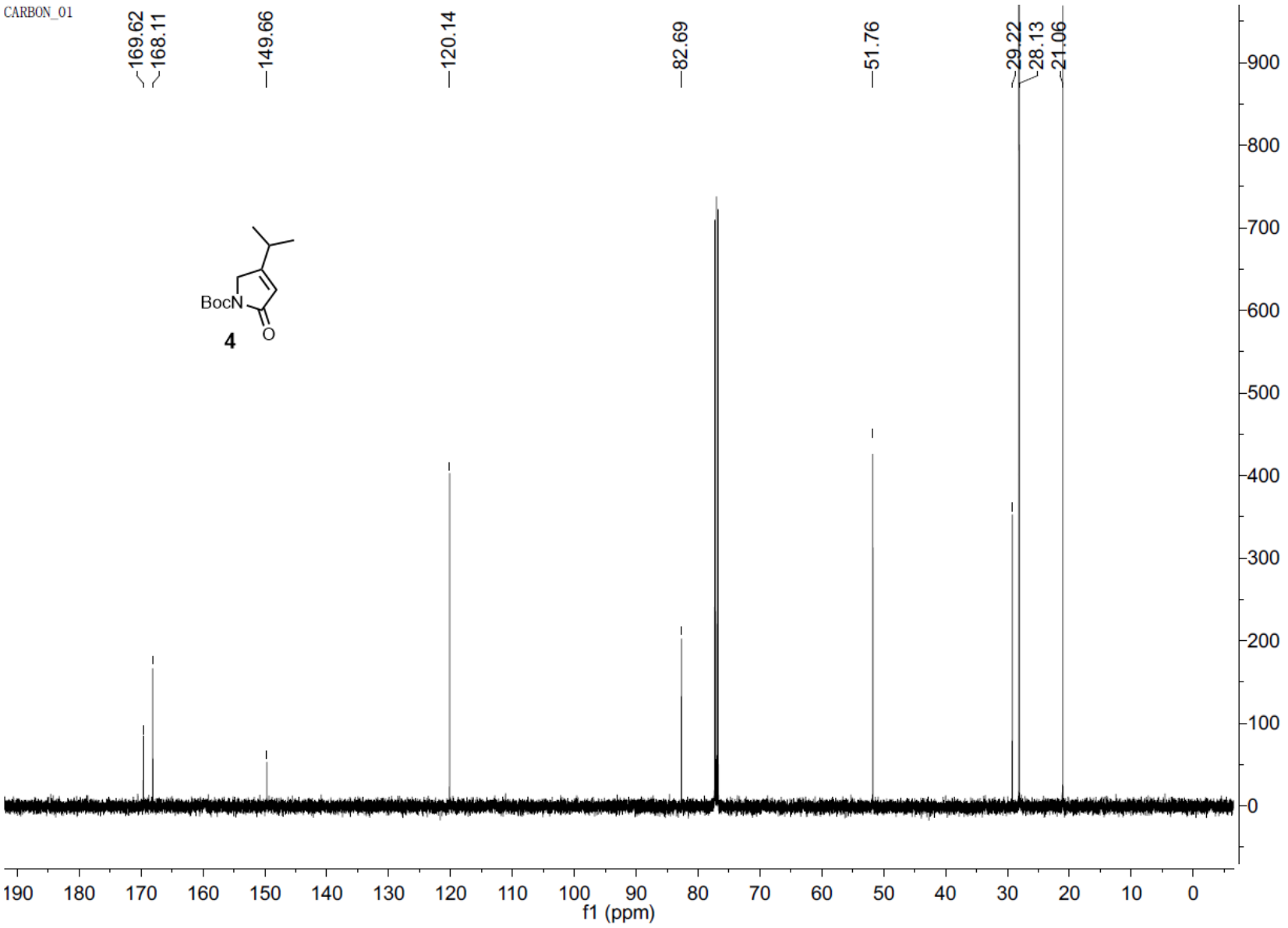
6.00
f1 (ppm)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21.06
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O
4
900
800
700
600
500
400
300
200
100
0
190
180
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90
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50
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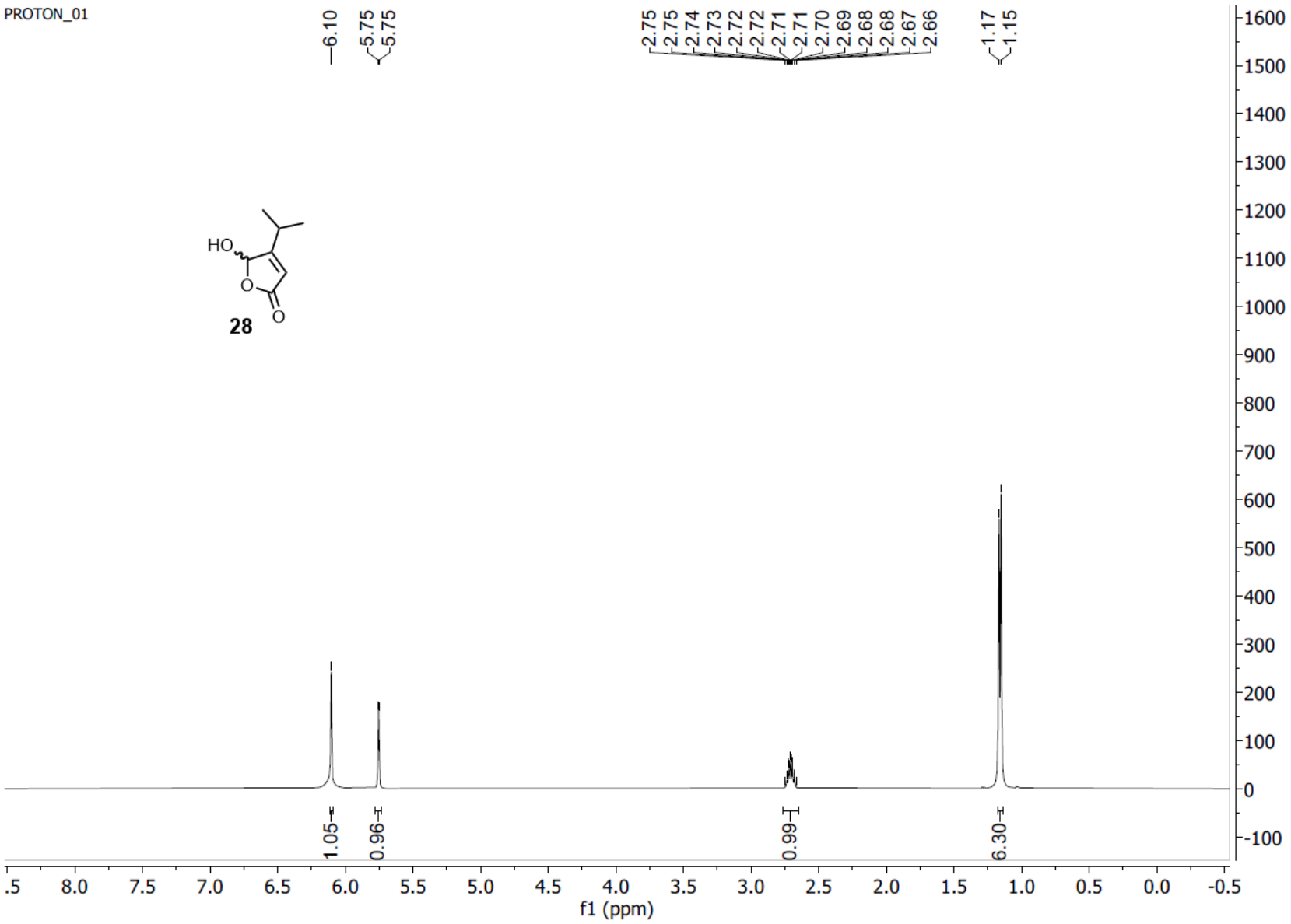
20
10
0
f1 (ppm)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2.72
2.71
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2.70
2.69
2.68
2.68
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1.15
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O
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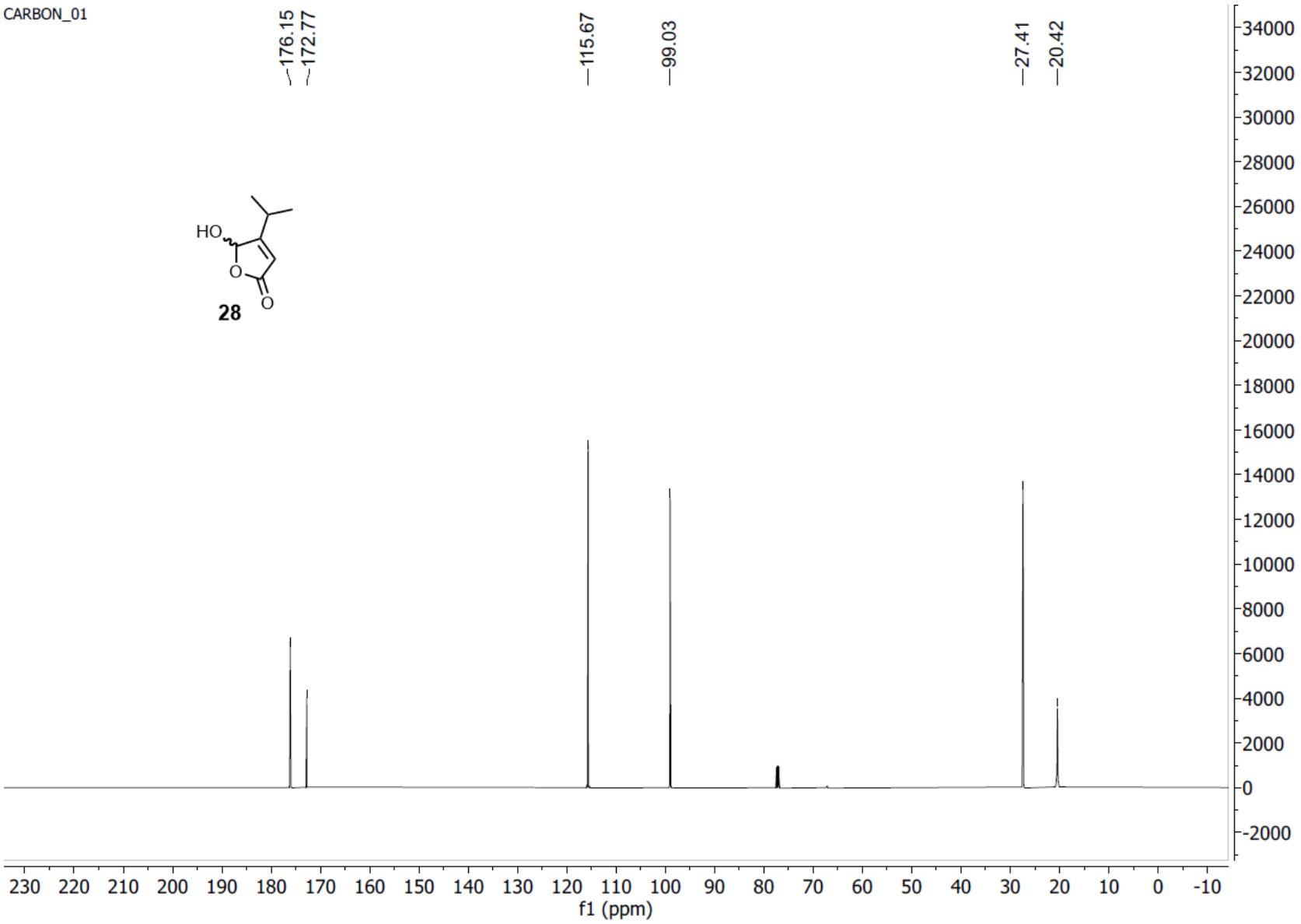
6.30
f1 (ppm)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20.42
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O
O
28
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32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
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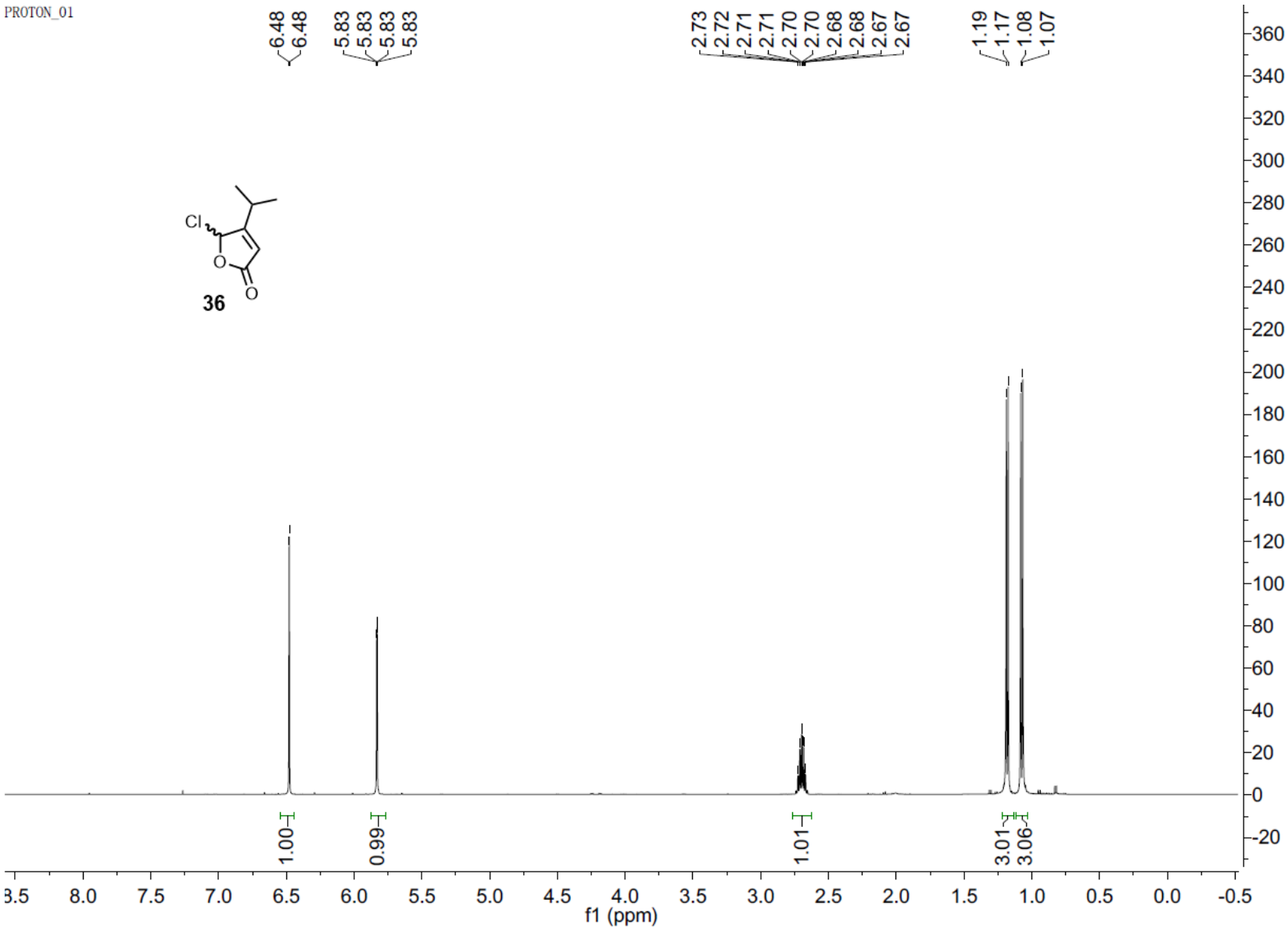
f1 (ppm)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5.83
5.83
5.83
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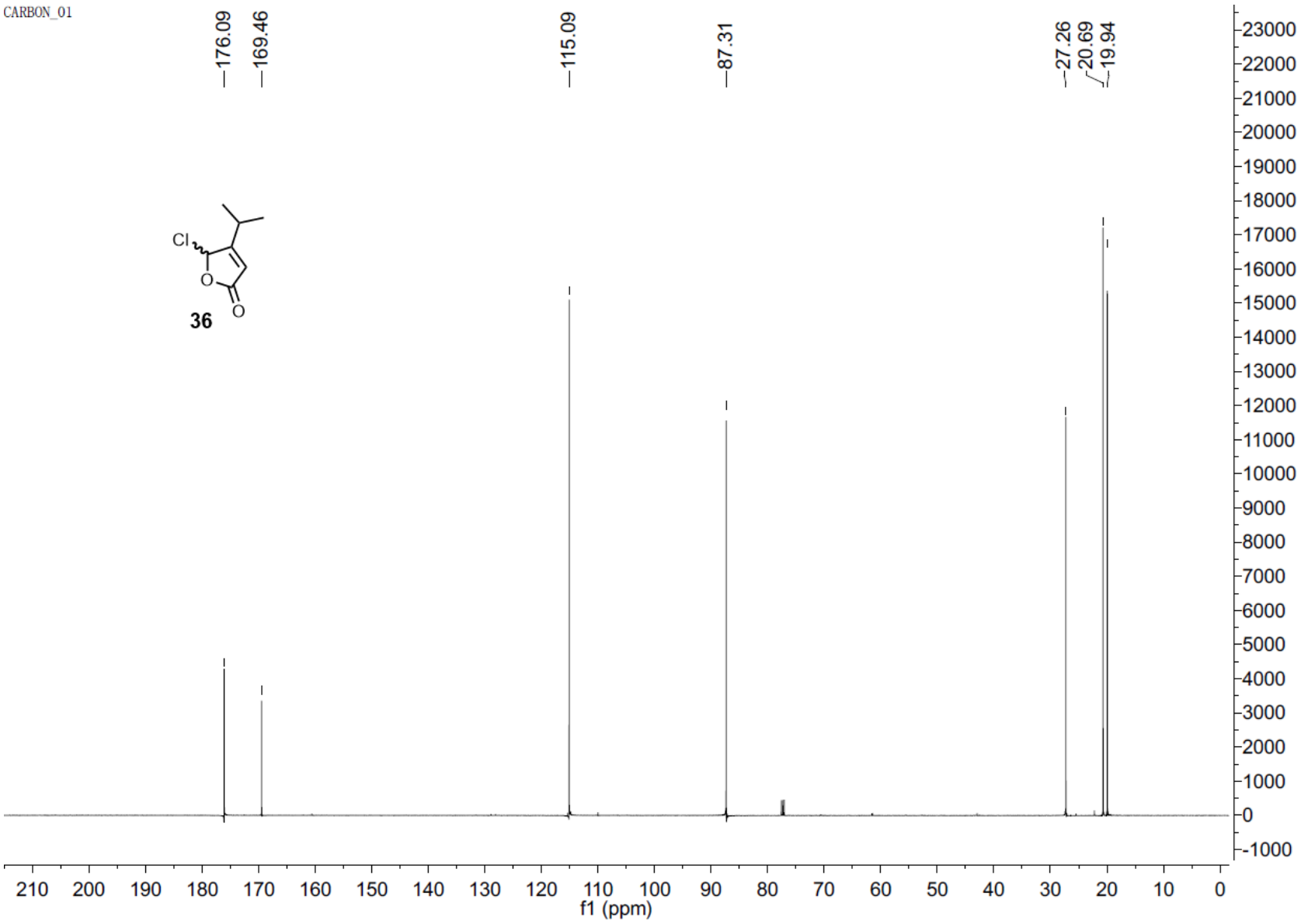
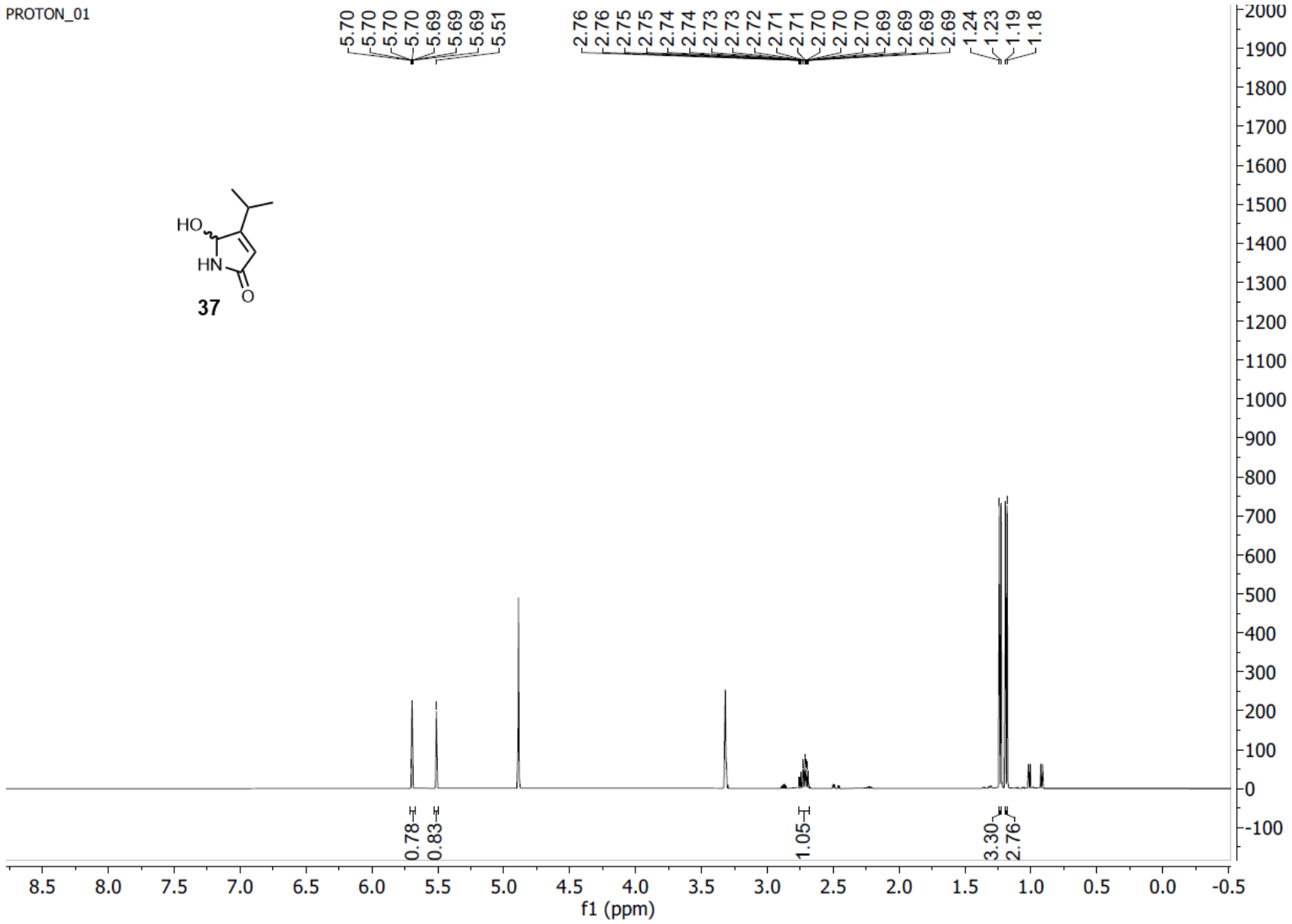
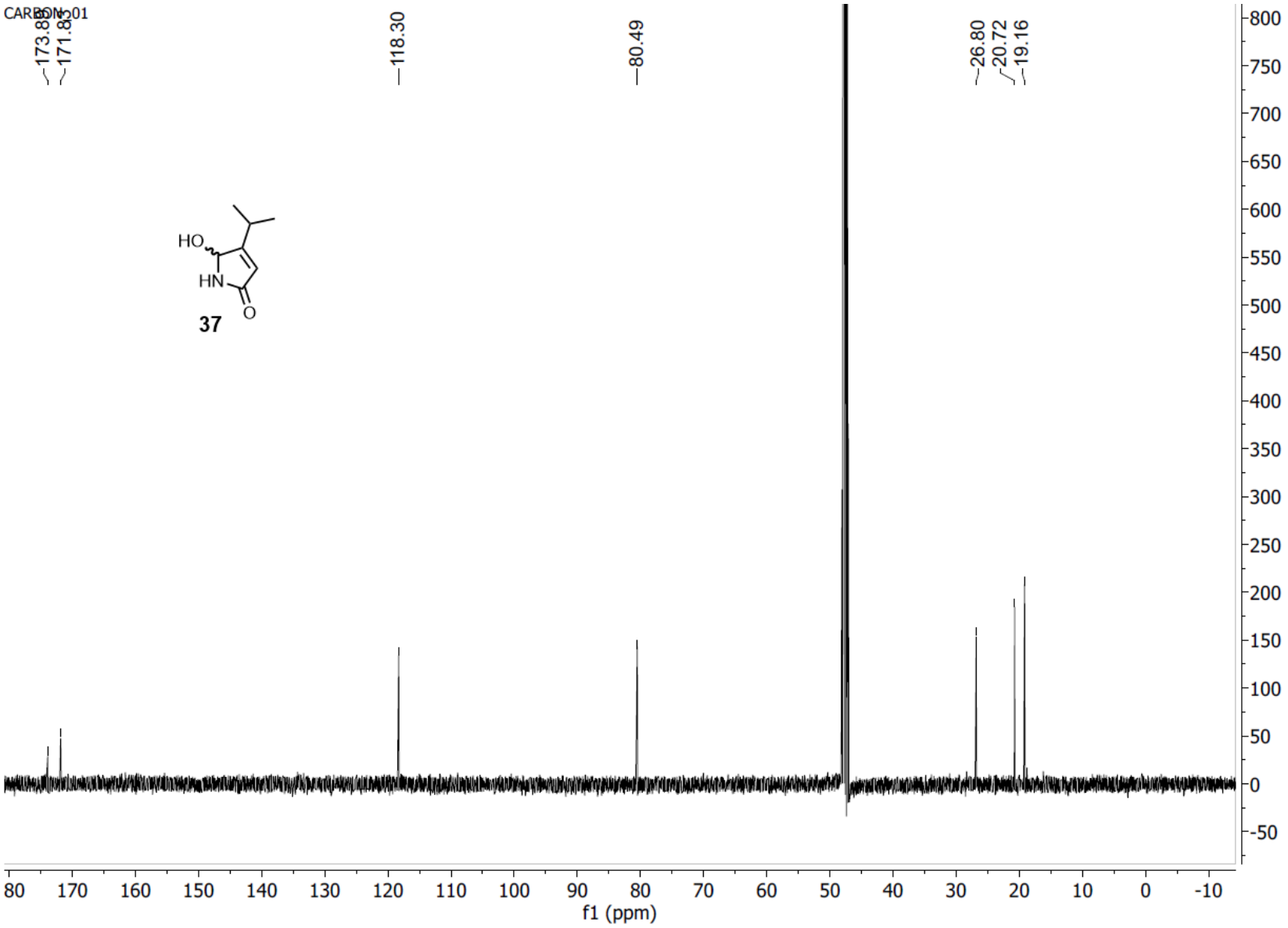
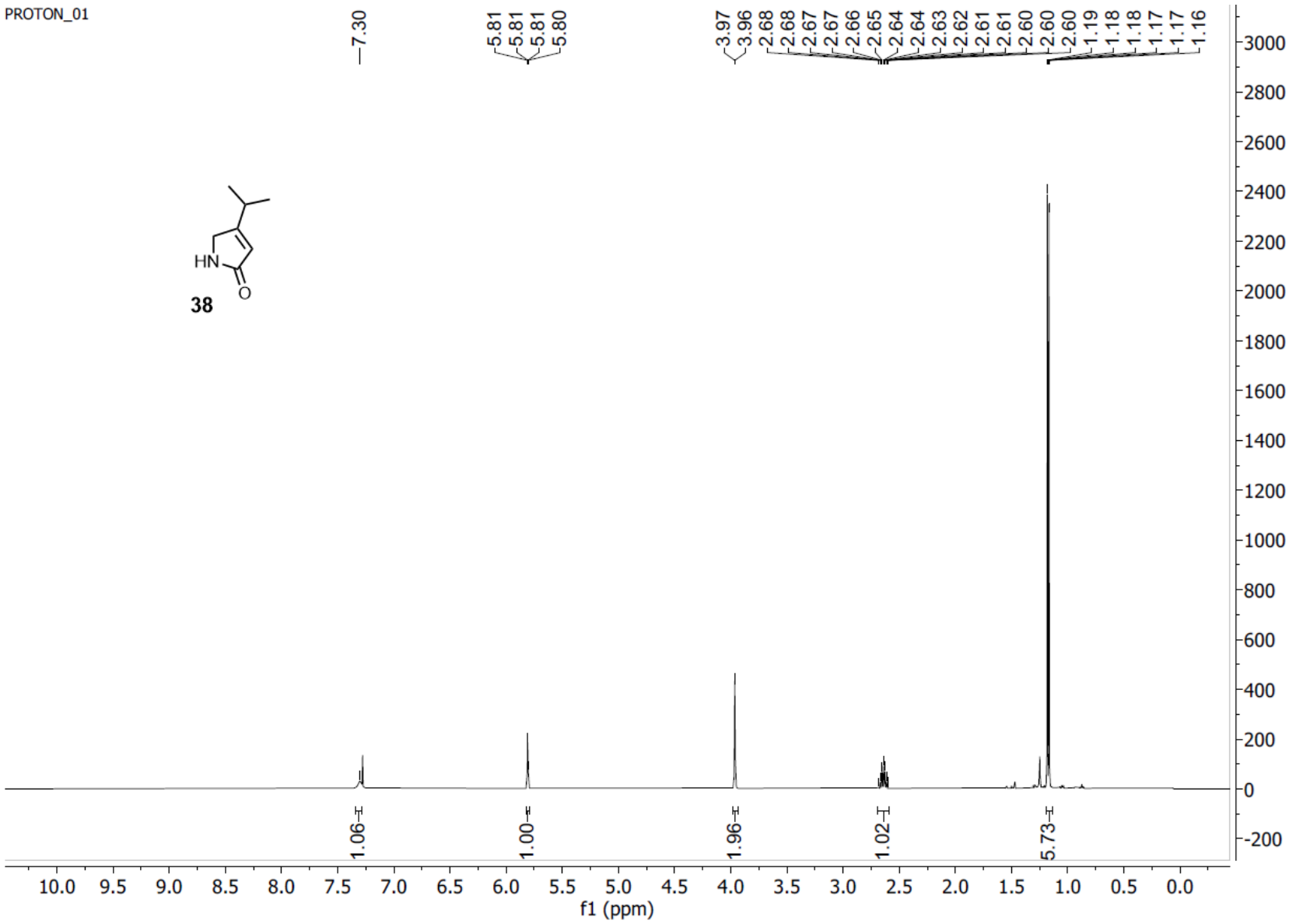
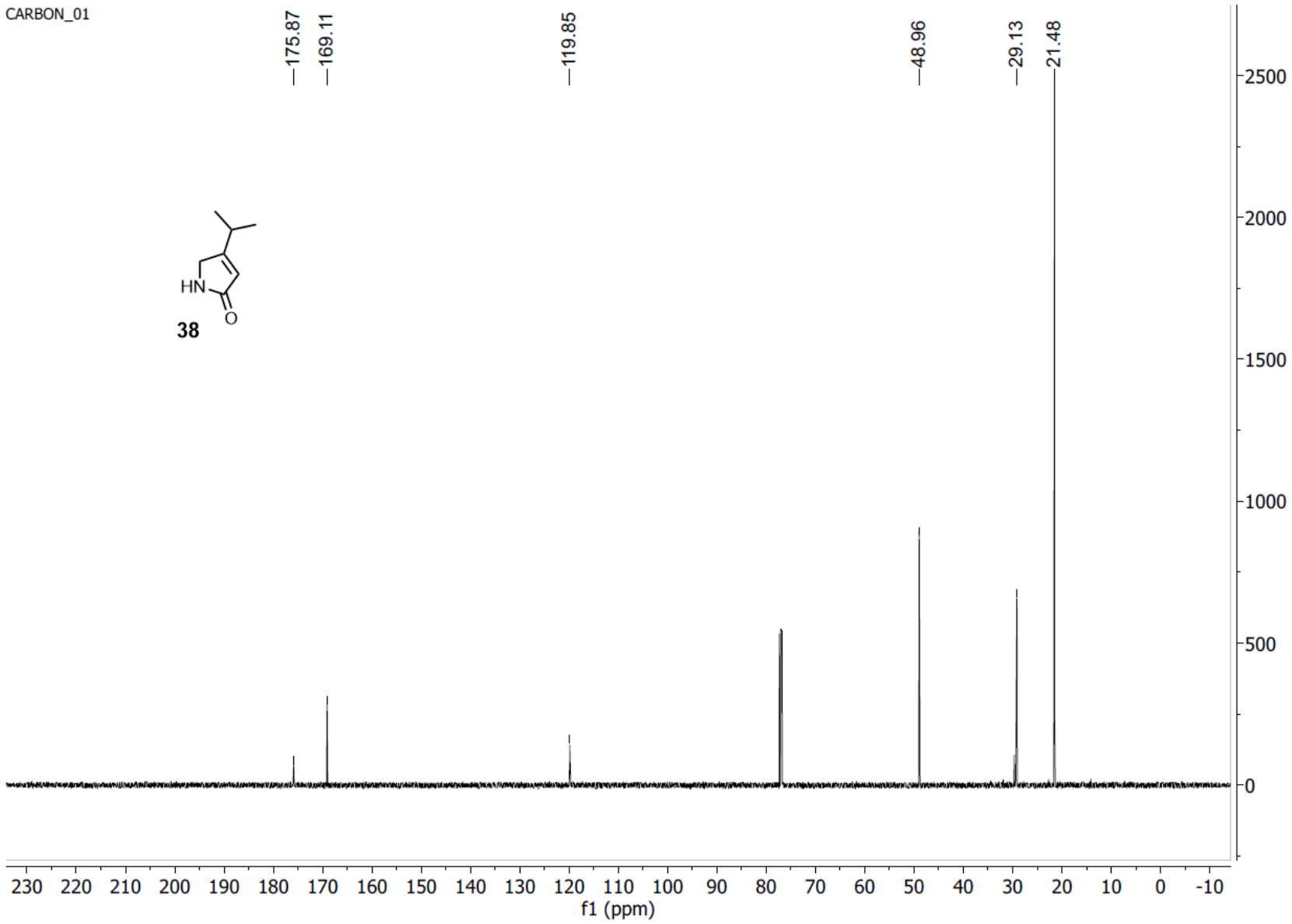
2.72
2.71
2.71
2.70
2.70
2.68
2.68
2.67
2.67
1.19
1.17
1.08
1.07
Cl
O
O
O
36
1.00
0.99
1.01
3.01
3.06
f1 (ppm)

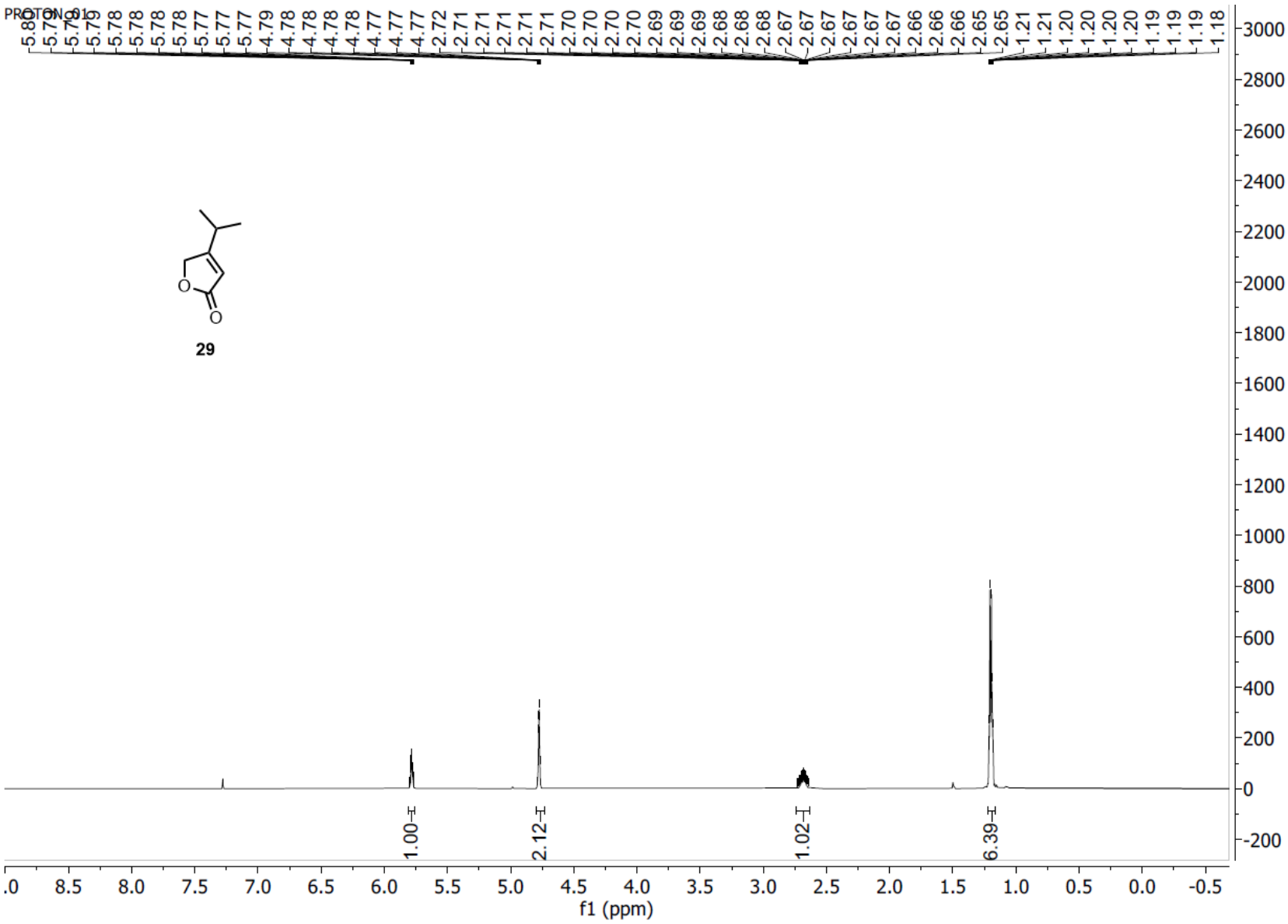

CARBON_01
176.09
169.46
115.09
87.31
27.26
20.69
19.94
Cl
O
O
36
f1 (ppm)

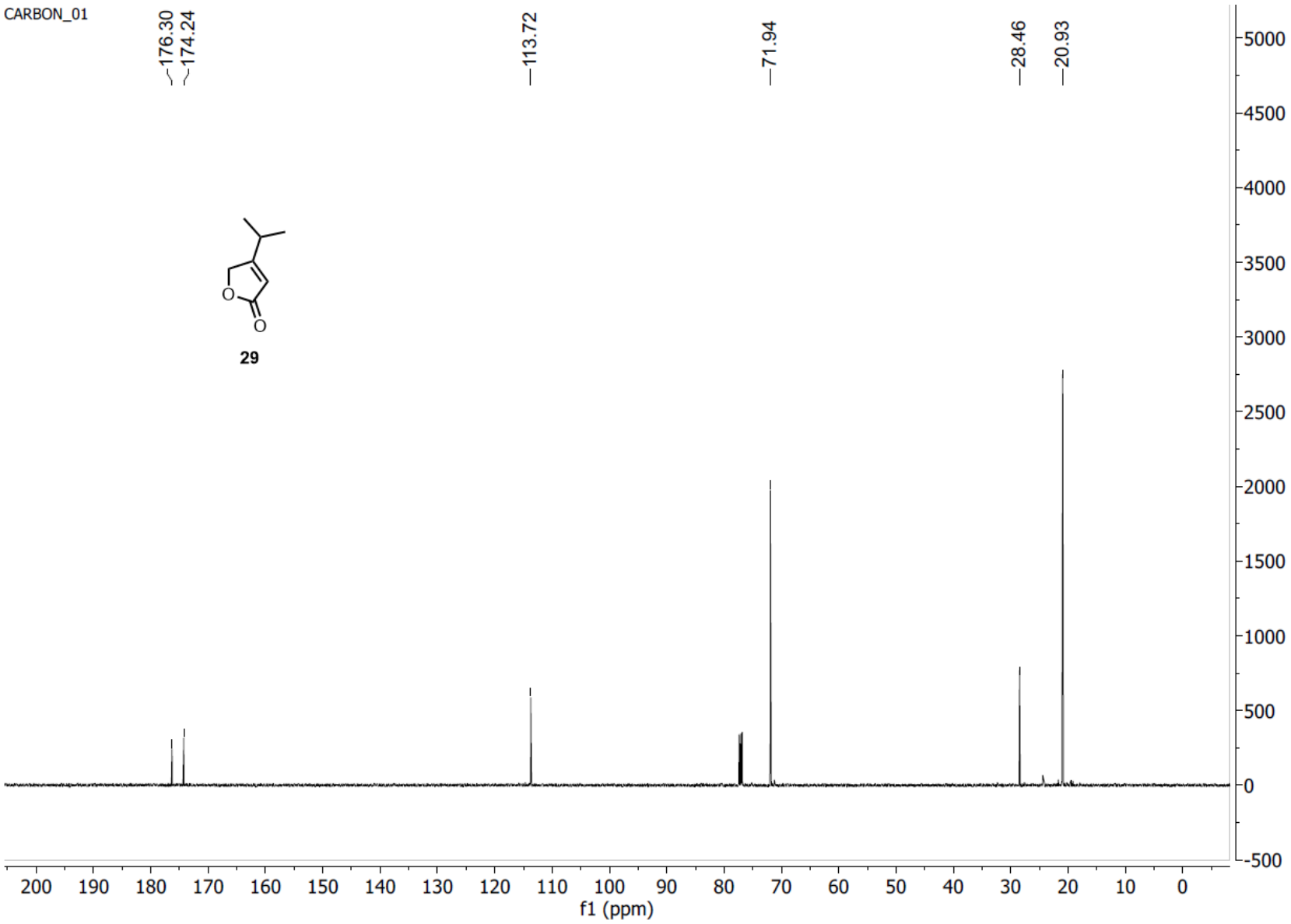

PROTON_01
5.70
5.70
5.70
5.70
5.69
5.69
5.69
5.51
2.76
2.76
2.75
2.75
2.74
2.74
2.73
2.73
2.72
2.71
2.71
2.70
2.70
2.70
2.69
2.69
2.69
2.69
1.24
1.23
1.19
1.18
HO
HN
O
37
0.78
0.83
1.05
3.30
2.76
f1 (ppm)

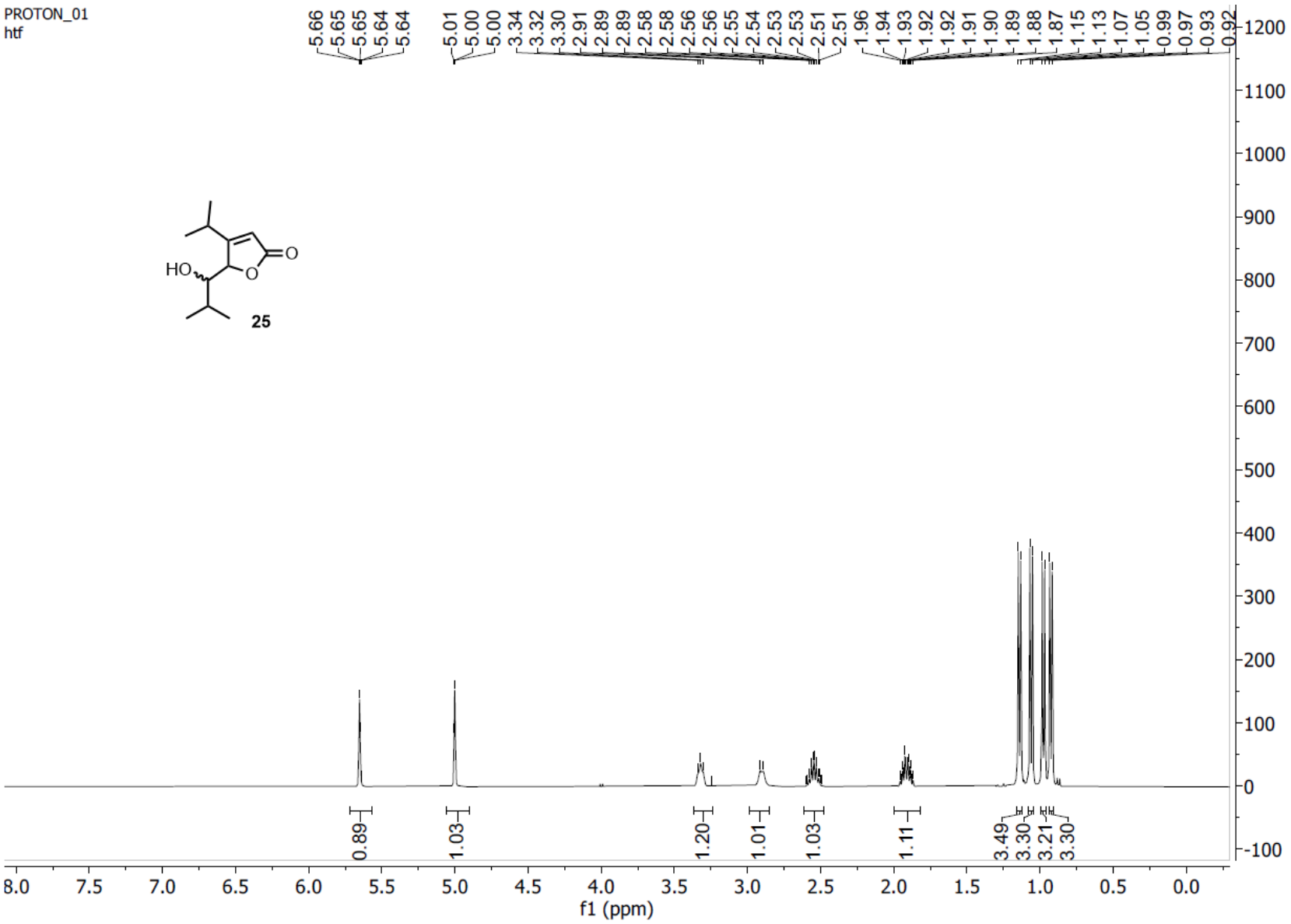

CARBON-01
173.80
171.83
118.30
80.49
26.80
20.72
19.16
HO
HN
O
37
f1 (ppm)

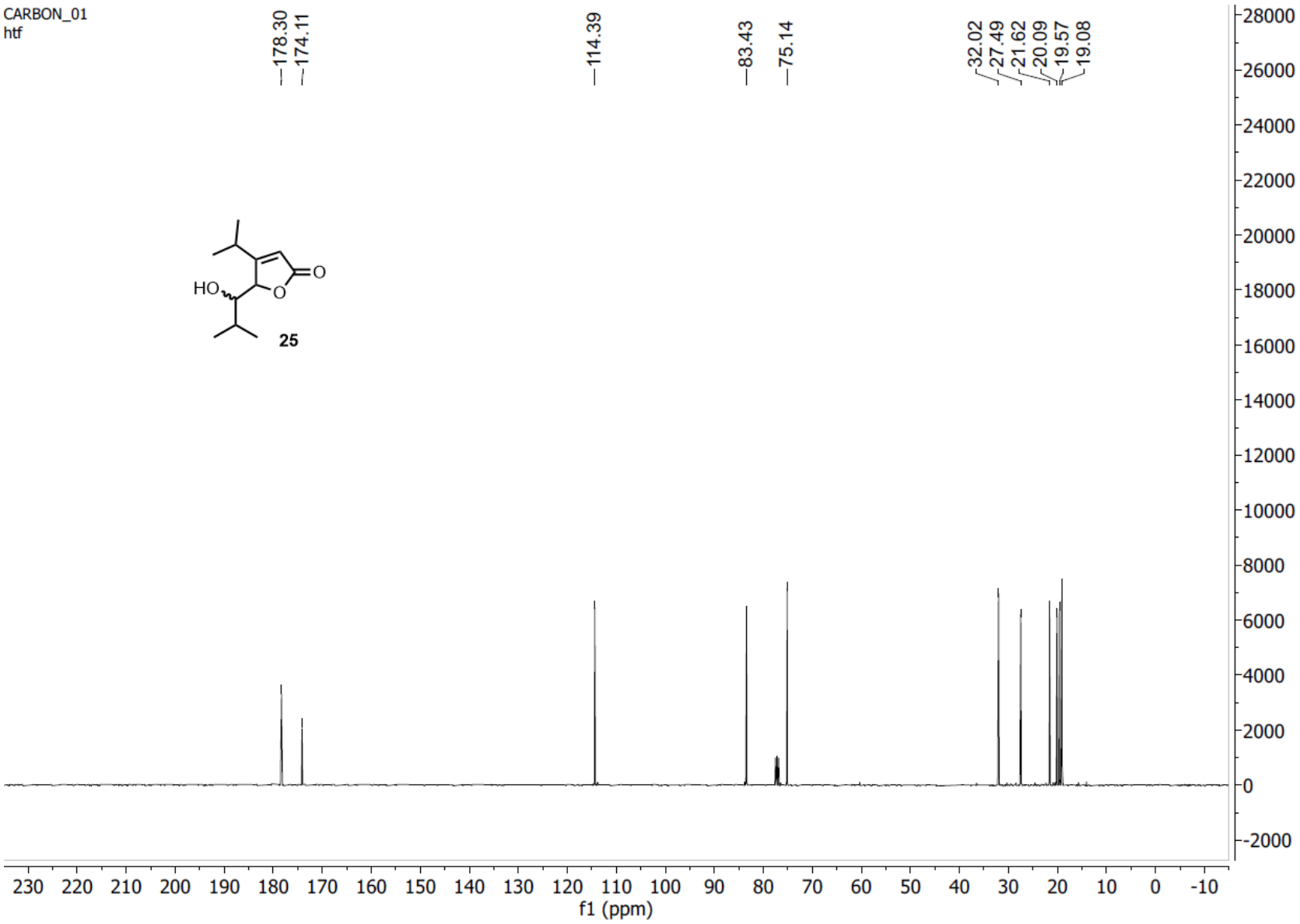

PROTON_01
7.30
5.81
5.81
5.81
5.80
3.97
3.96
2.68
2.68
2.67
2.67
2.66
2.65
2.64
2.64
2.63
2.62
2.61
2.61
2.60
2.60
2.60
1.19
1.18
1.18
1.17
1.17
1.16
HN
O
38
1.06
1.00
1.96
1.02
5.73
f1 (ppm)

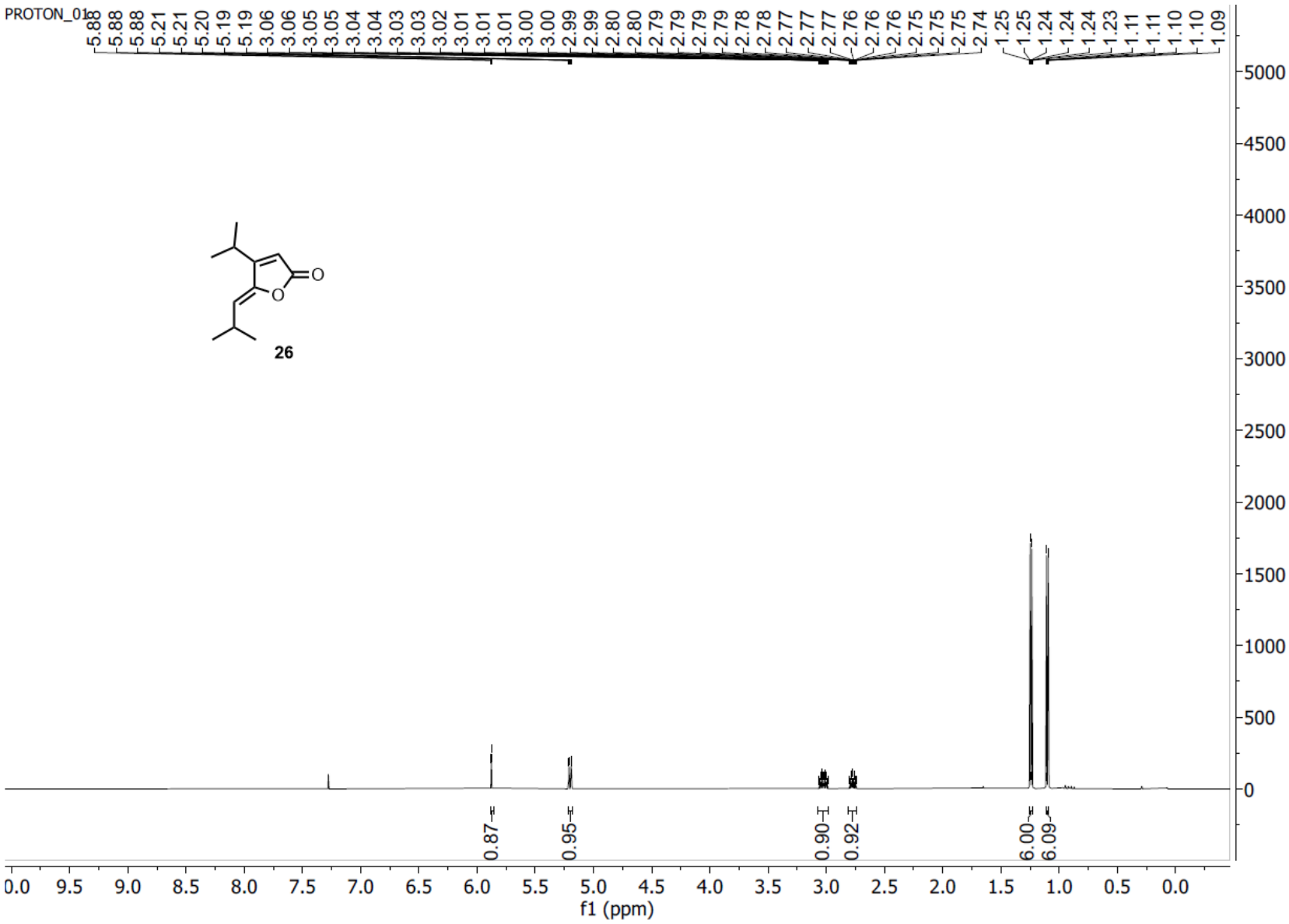

CARBON_01
175.87
169.11
119.85
48.96
29.13
21.48
HN
O
38
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

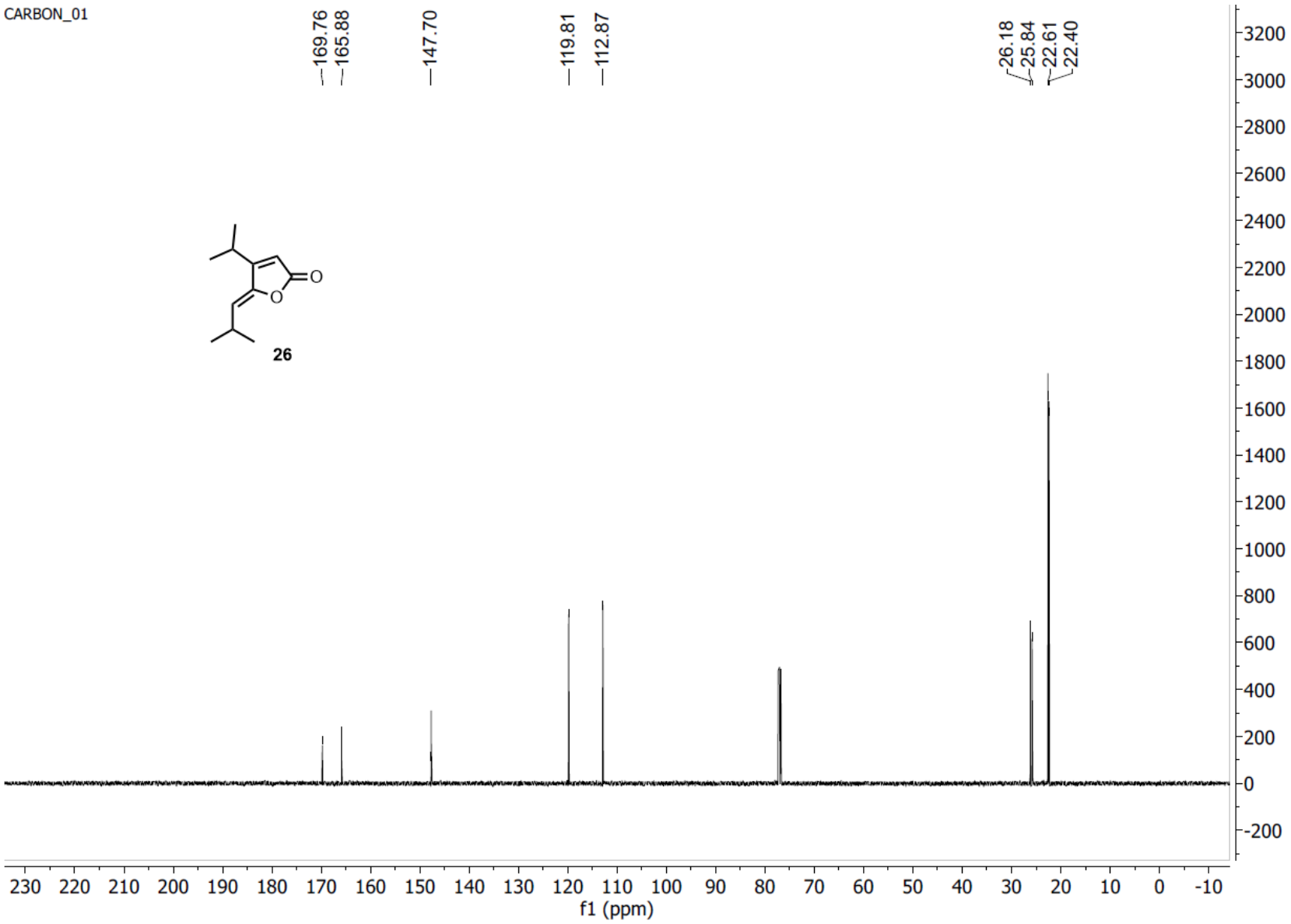

PROTON-01
29
1.00
2.12
1.02
6.39
f1 (ppm)

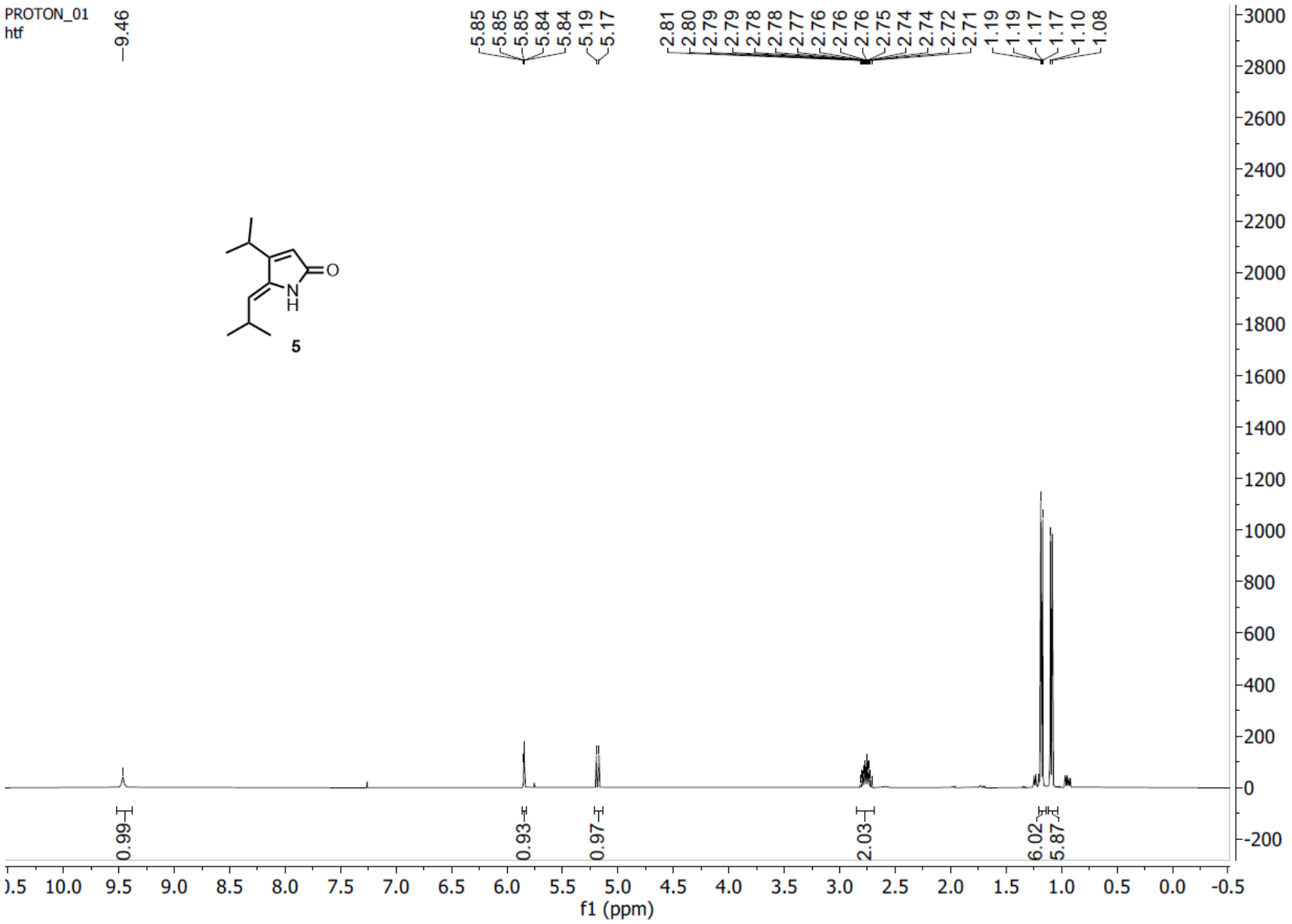

CARBON_01
176.30
174.24
113.72
71.94
28.46
20.93
O
O
29
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

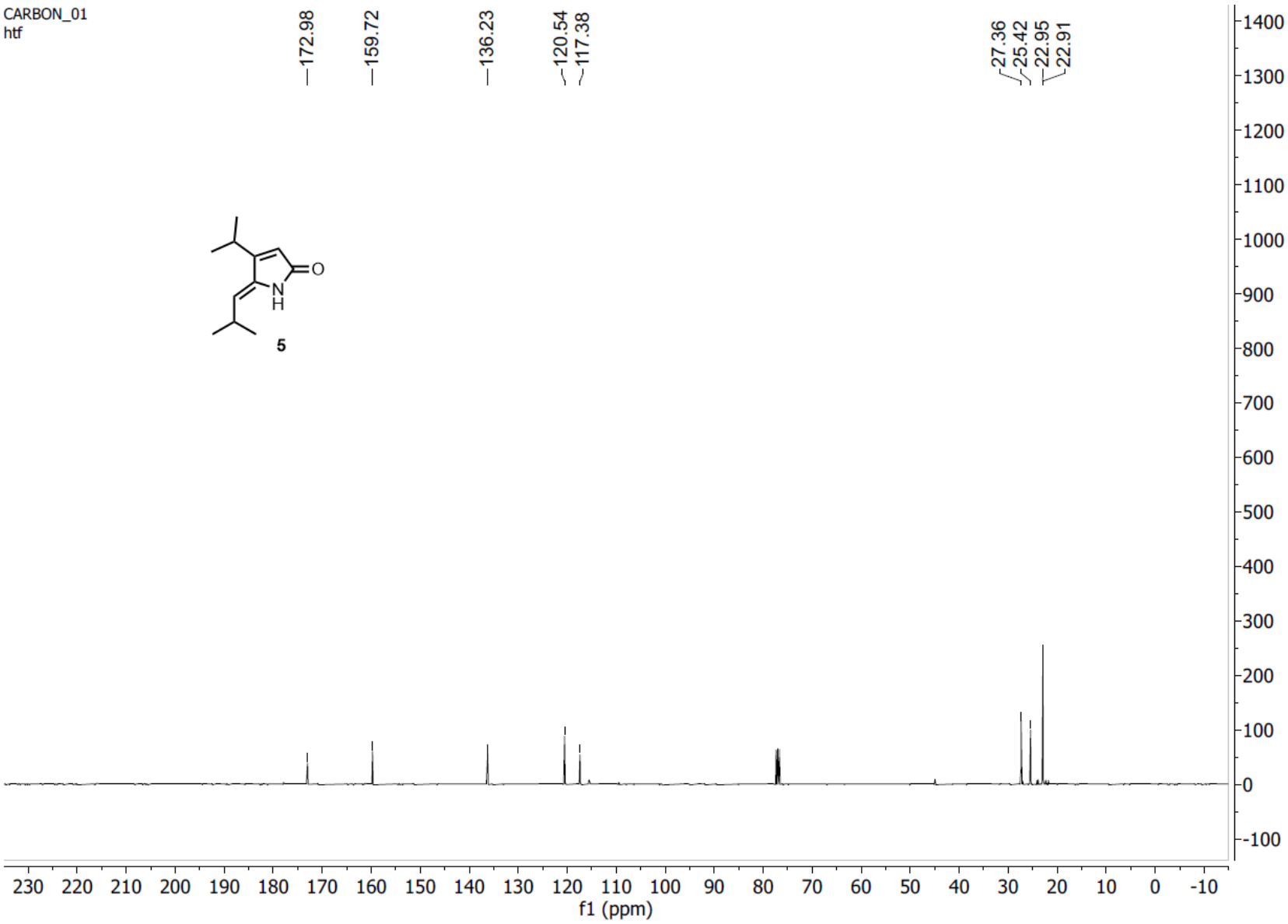

PROTON_01
htf
5.66 5.65 5.65 5.64 5.64
5.01 5.00 5.00
3.34 3.32 3.30 2.91 2.89 2.89 2.58 2.58 2.56 2.56 2.55 2.54 2.53 2.53 2.51 2.51
1.96 1.94 1.93 1.92 1.92 1.91 1.90 1.89 1.88 1.87 1.15 1.13 1.07 1.05 0.99 0.97 0.93 0.92
HO
O
O
25
0.89 1.03 1.20 1.01 1.03 1.11 3.49 3.30 3.21 3.30
f1 (ppm)

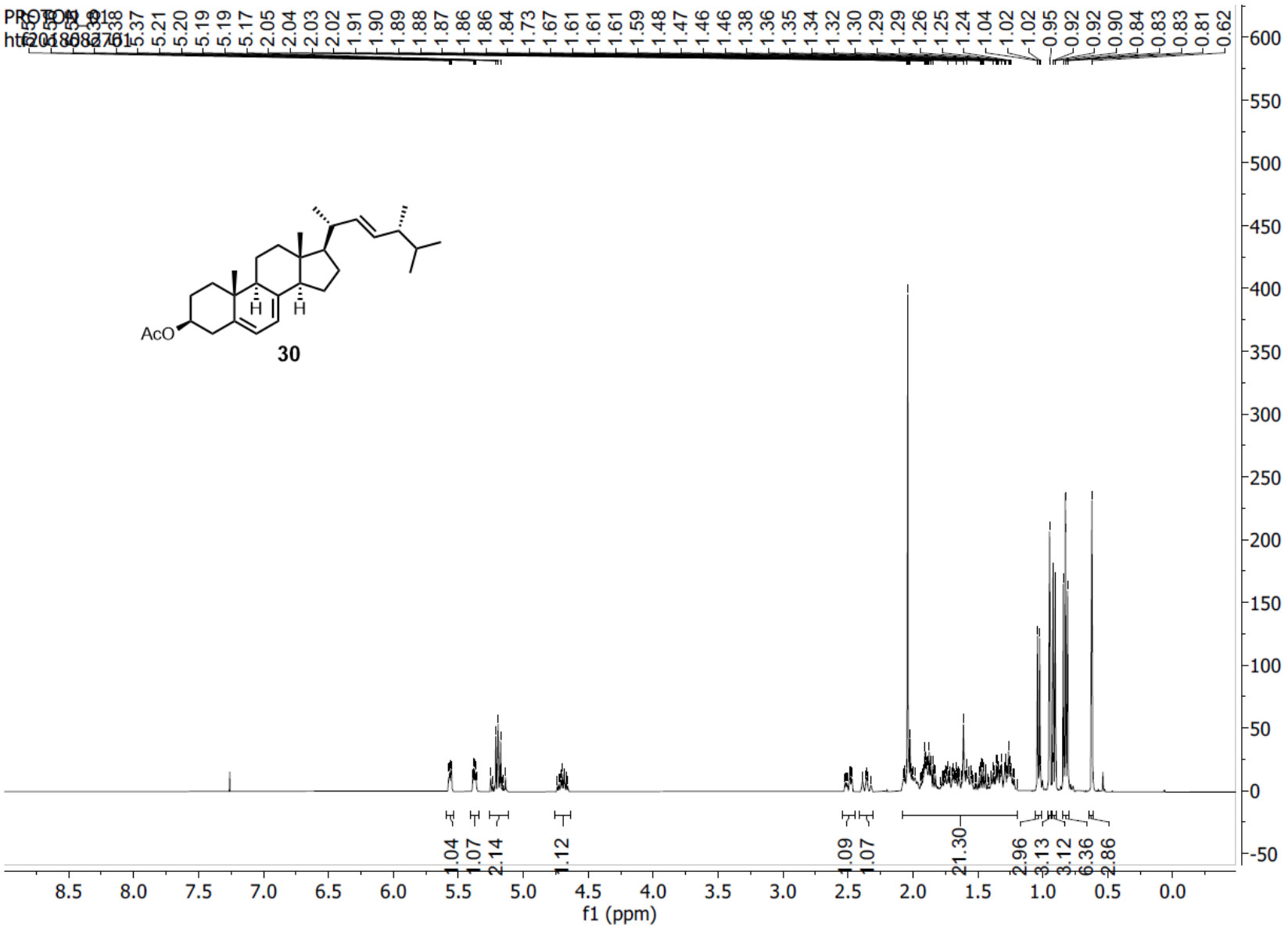

CARBON_01
htf
178.30
174.11
114.39
83.43
75.14
32.02
27.49
21.62
20.09
19.57
19.08
HO
O
O
25
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

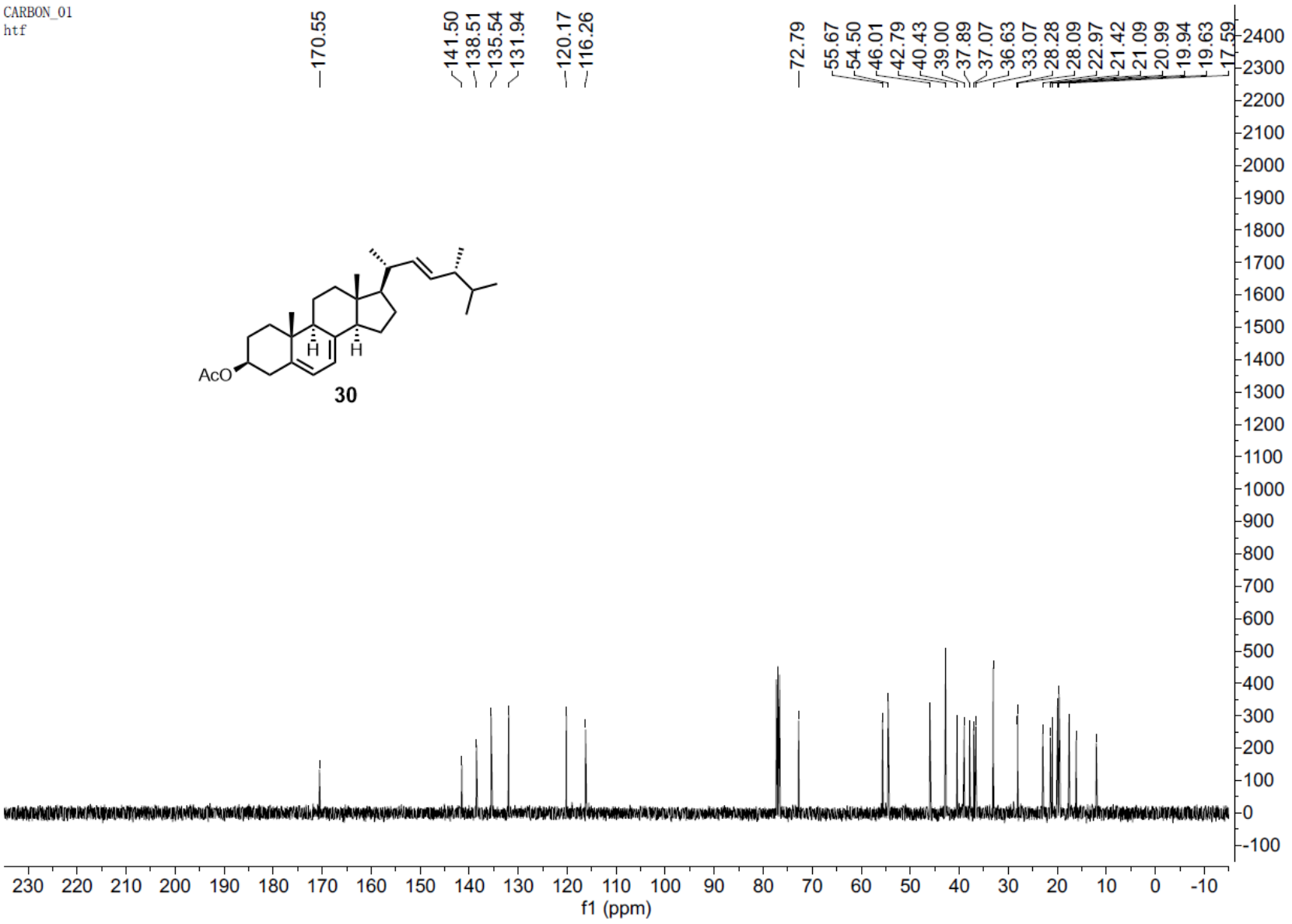

PROTON_01
5.88 5.88 5.88 5.21 5.21 5.20 5.19 5.19 3.06 3.06 3.05 3.05 3.04 3.04 3.03 3.03 3.02 3.01 3.01 3.01 3.00 3.00 2.99 2.99 2.80 2.80 2.79 2.79 2.79 2.79 2.78 2.78 2.77 2.77 2.77 2.76 2.76 2.76 2.75 2.75 2.75 2.74 1.25 1.25 1.24 1.24 1.24 1.23 1.11 1.11 1.10 1.10 1.09
O
O
26
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0.87
0.95
0.90
0.92
6.00
6.09
0.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

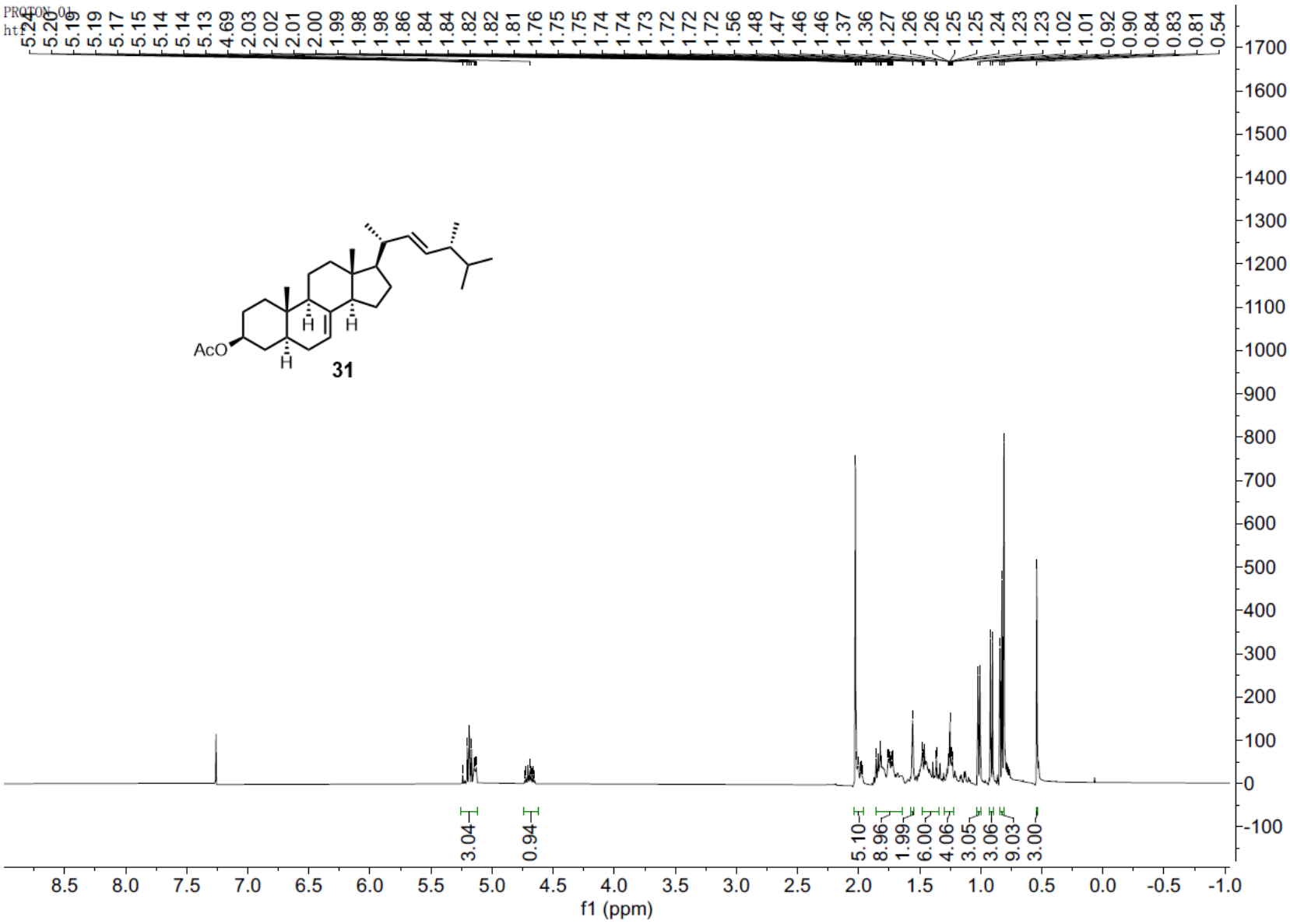

CARBON_01
169.76
165.88
147.70
119.81
112.87
26.18
25.84
22.61
22.40
O
O
26
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

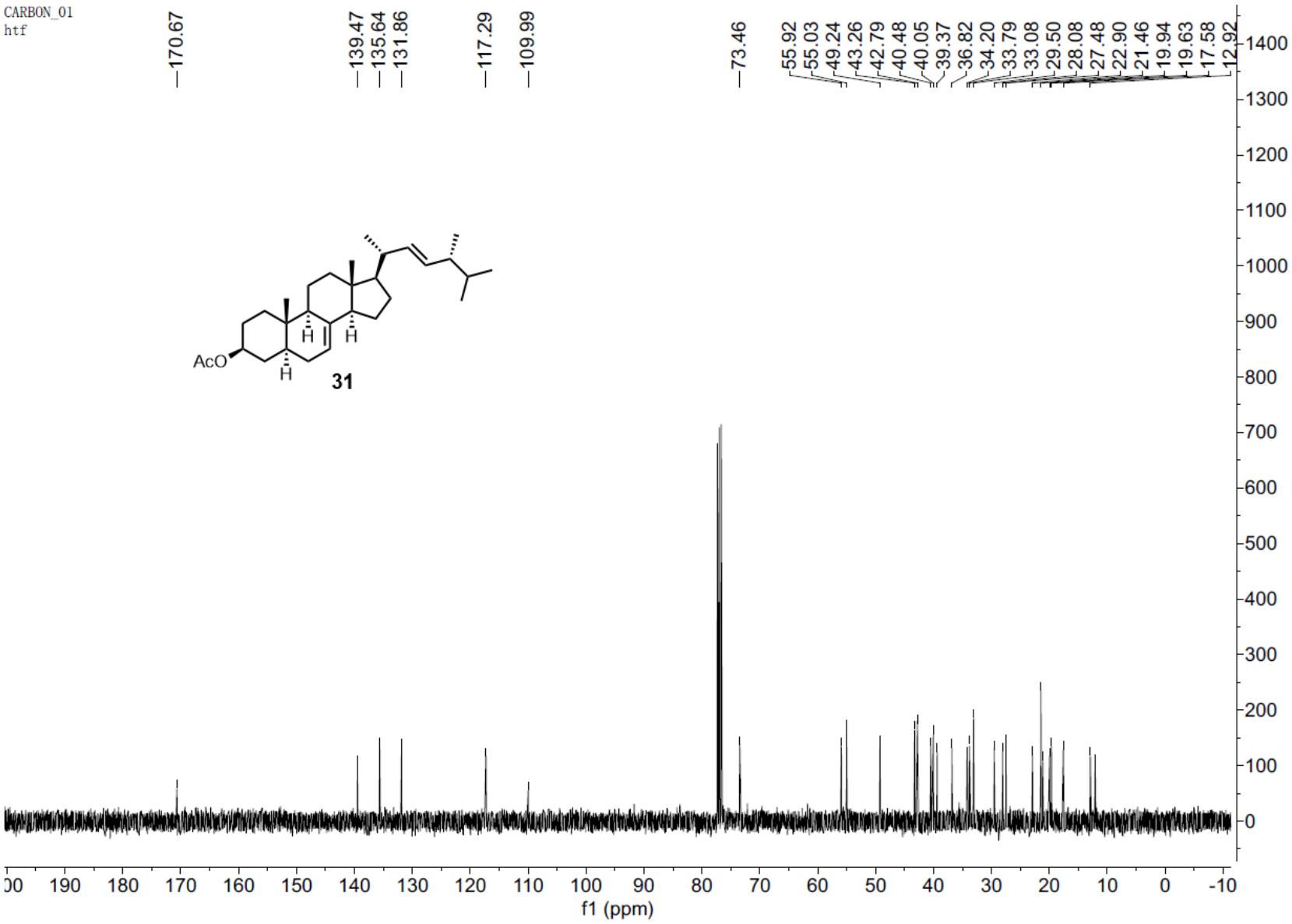

PROTON_01
htf
9.46
5.85
5.85
5.85
5.84
5.84
5.19
5.17
2.81
2.80
2.79
2.79
2.78
2.78
2.77
2.76
2.76
2.76
2.75
2.74
2.74
2.72
2.71
1.19
1.19
1.17
1.17
1.10
1.08
O
N
H
5
0.99
0.93
0.97
2.03
6.02
5.87
f1 (ppm)

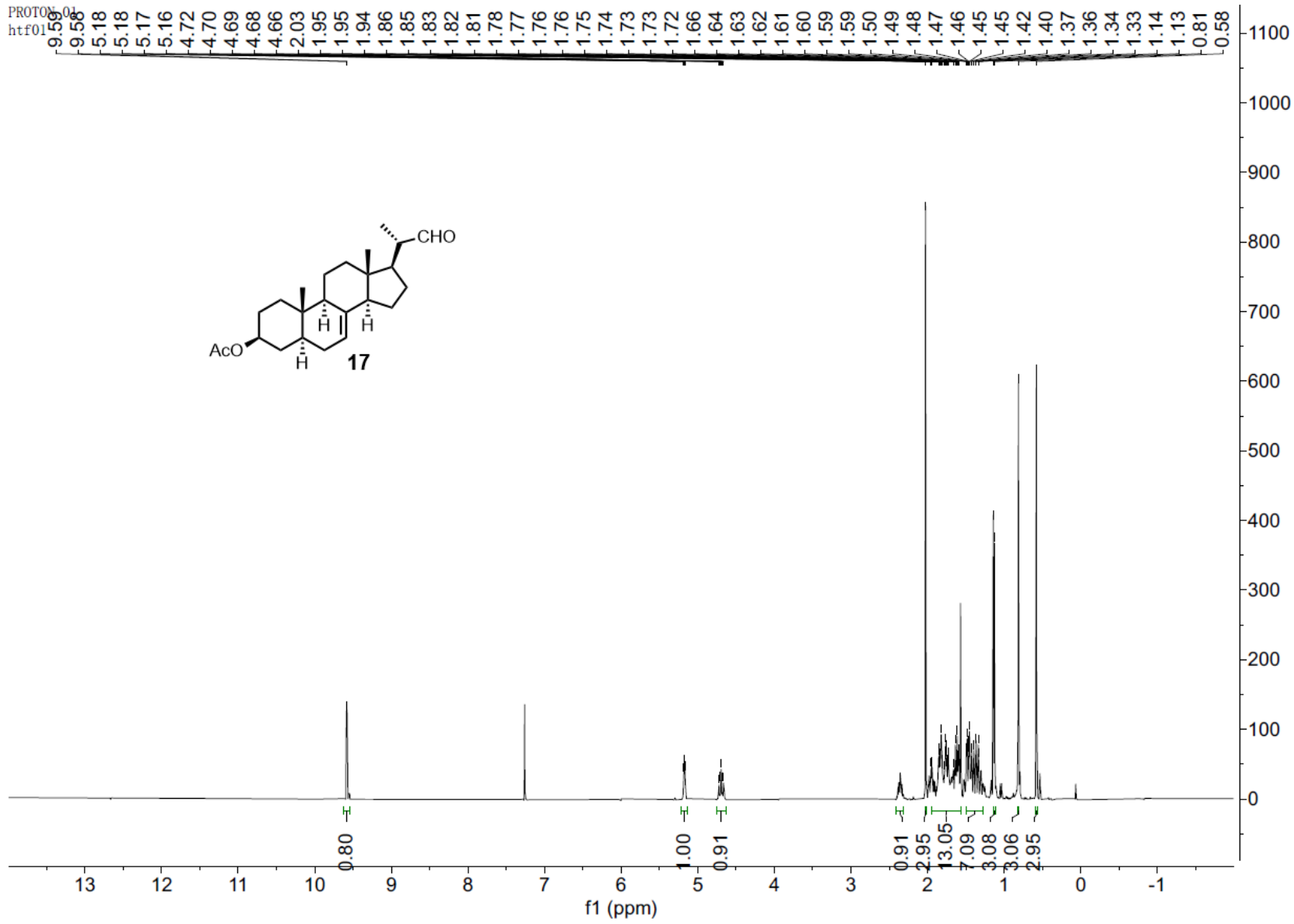

CARBON_01
htf
172.98
159.72
136.23
120.54
117.38
27.36
25.42
22.95
22.91
O
N
H
5
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

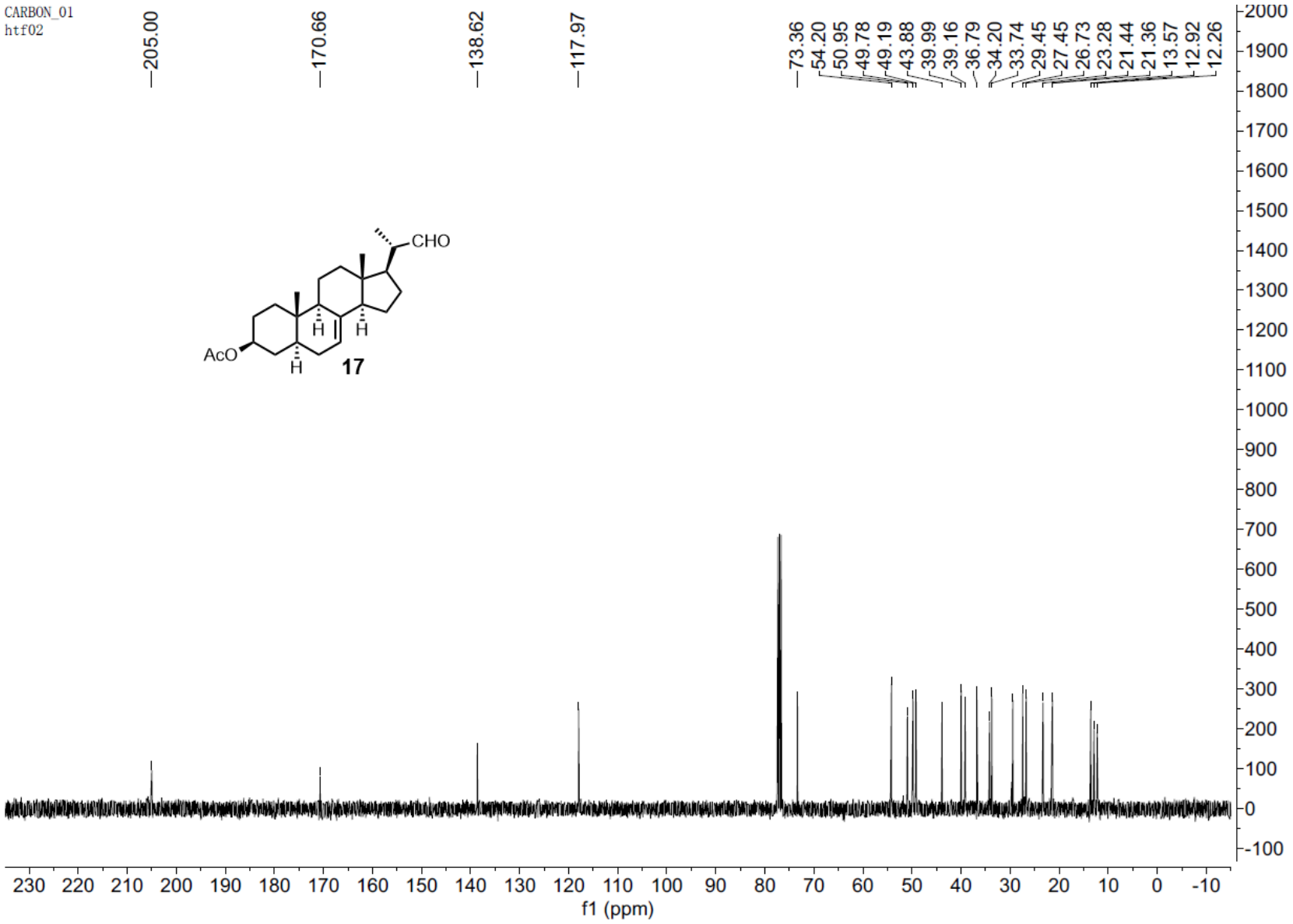

AcO
30
f1 (ppm)

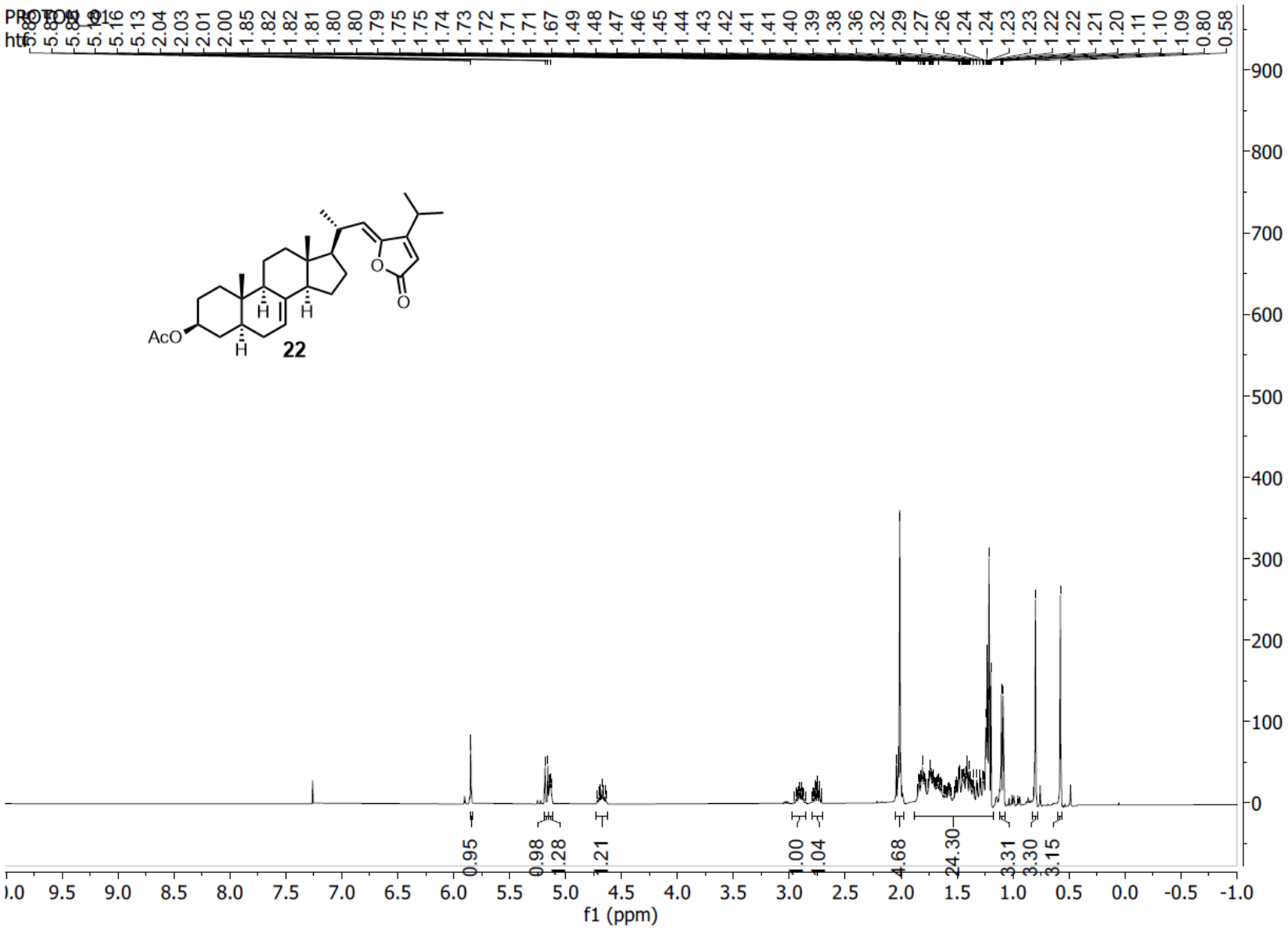

CARBON_01
htf
170.55
141.50
138.51
135.54
131.94
120.17
116.26
72.79
55.67
54.50
46.01
42.79
40.43
39.00
37.89
37.07
36.63
33.07
28.28
28.09
22.97
21.42
21.09
20.99
19.94
19.63
17.59
AcO
30
f1 (ppm)

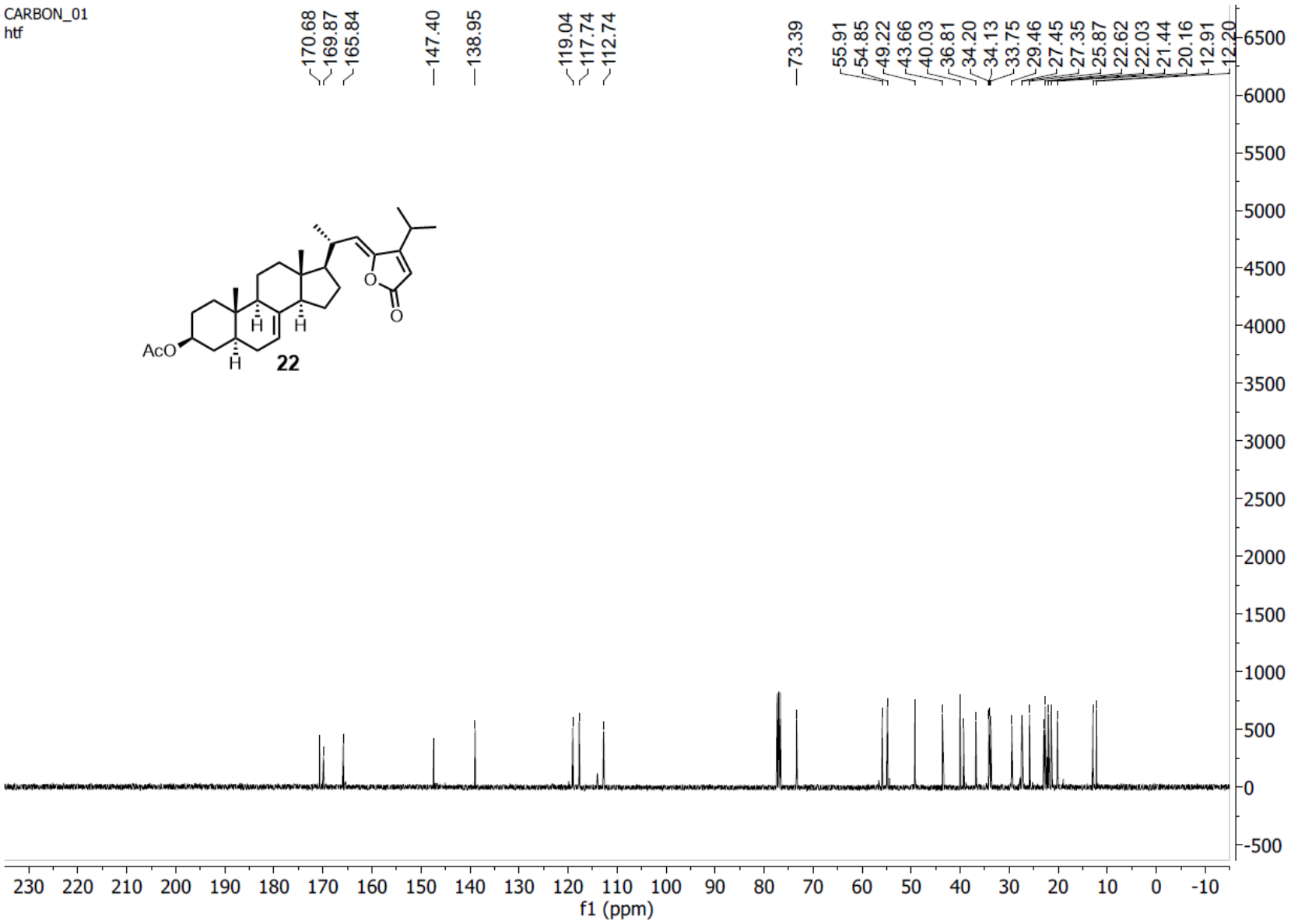

AcO
H
H
H
31
5.24 5.20 5.19 5.19 5.17 5.15 5.14 5.14 5.13 4.69 2.03 2.02 2.01 2.00 1.99 1.98 1.98 1.86 1.84 1.84 1.82 1.82 1.81 1.76 1.75 1.75 1.74 1.74 1.73 1.72 1.72 1.72 1.56 1.48 1.47 1.46 1.46 1.37 1.36 1.27 1.26 1.26 1.25 1.25 1.24 1.23 1.23 1.02 1.01 0.92 0.90 0.84 0.83 0.81 0.54
3.04
0.94
5.10
8.96
1.99
6.00
4.06
3.05
3.06
9.03
3.00
f1 (ppm)

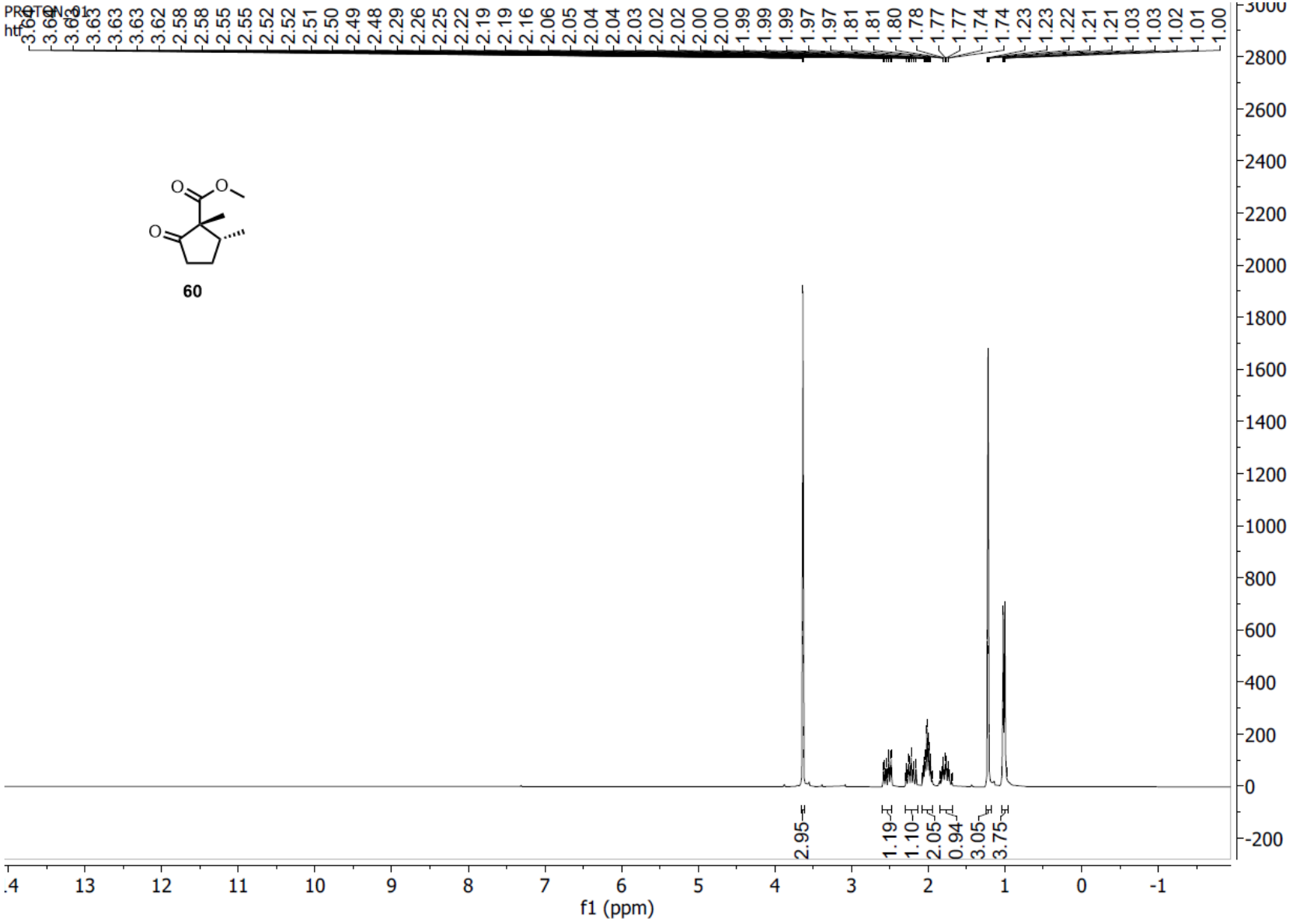

CARBON_01
htf
170.67
139.47
135.64
131.86
117.29
109.99
73.46
55.92
55.03
49.24
43.26
42.79
40.48
40.05
39.37
36.82
34.20
33.79
33.08
29.50
28.08
27.48
22.90
21.46
19.94
19.63
17.58
12.92
AcO
H
H
H
31
f1 (ppm)

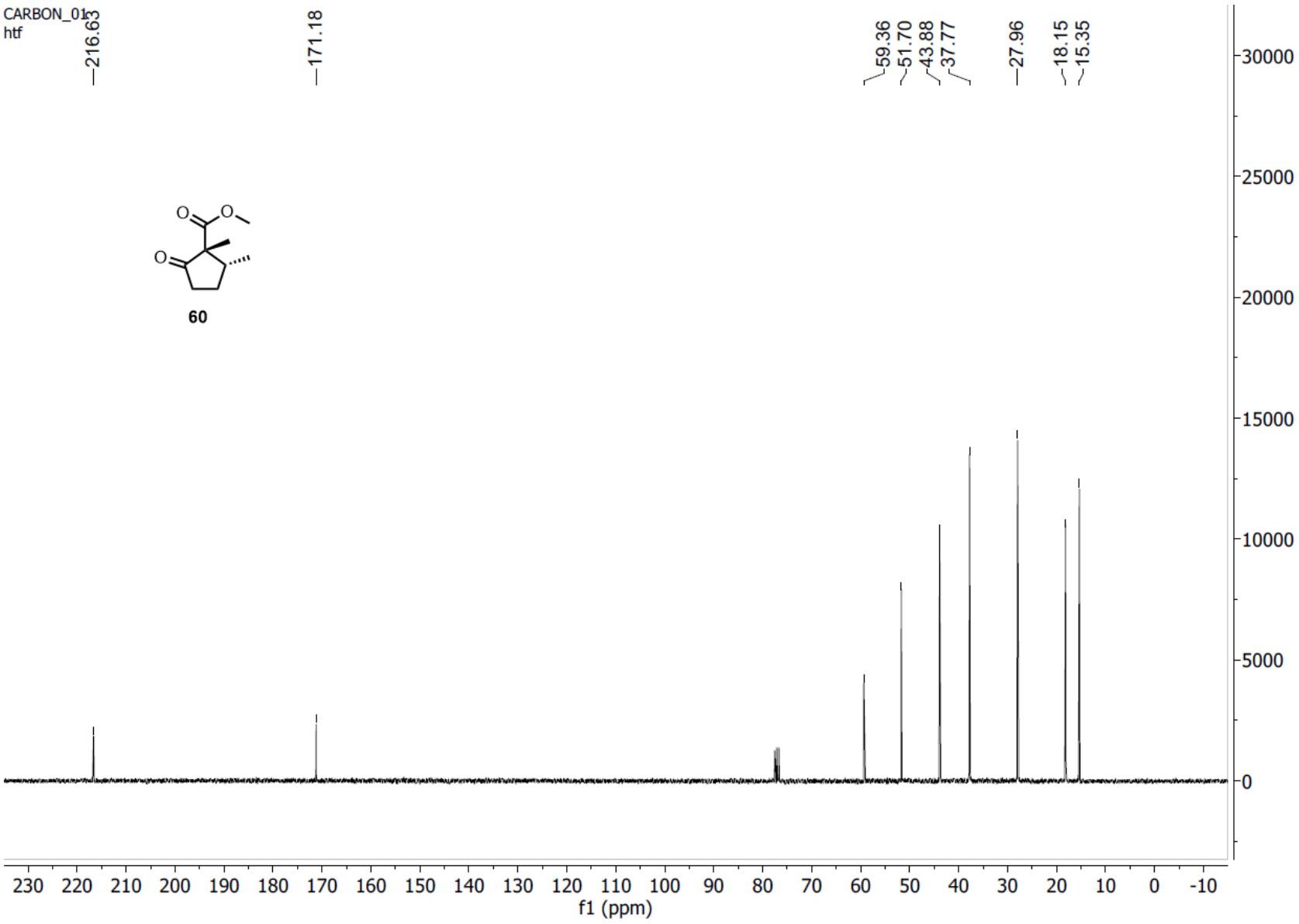

PROTON_01
htf01
9.59 9.58 5.18 5.18 5.17 5.16 4.72 4.70 4.69 4.68 4.66 2.03 1.95 1.95 1.94 1.86 1.85 1.83 1.82 1.81 1.78 1.77 1.76 1.76 1.75 1.74 1.73 1.73 1.72 1.66 1.64 1.63 1.62 1.61 1.60 1.59 1.59 1.50 1.49 1.48 1.47 1.46 1.45 1.45 1.42 1.40 1.37 1.36 1.34 1.33 1.14 1.13 0.81 0.58
CHO
AcO
H
H
H
17
0.80
1.00
0.91
0.91
2.95
13.05
7.09
3.08
3.06
2.95
f1 (ppm)

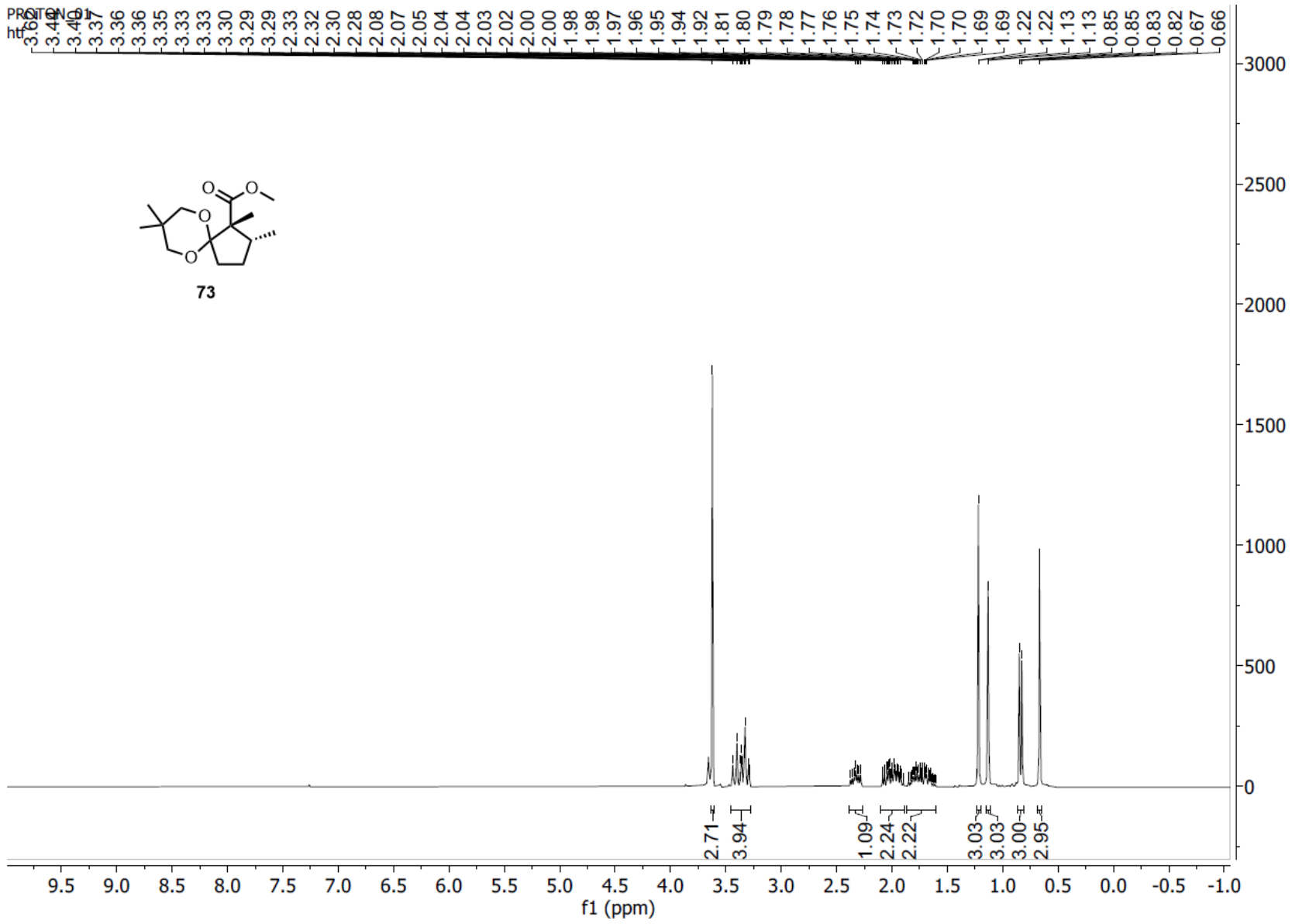

CARBON_01
htf02
205.00
170.66
138.62
117.97
73.36
54.20
50.95
49.78
49.19
43.88
39.99
39.16
36.79
34.20
33.74
29.45
27.45
26.73
23.28
21.44
21.36
13.57
12.92
12.26
CHO
AcO
H
H
H
17
f1 (ppm)

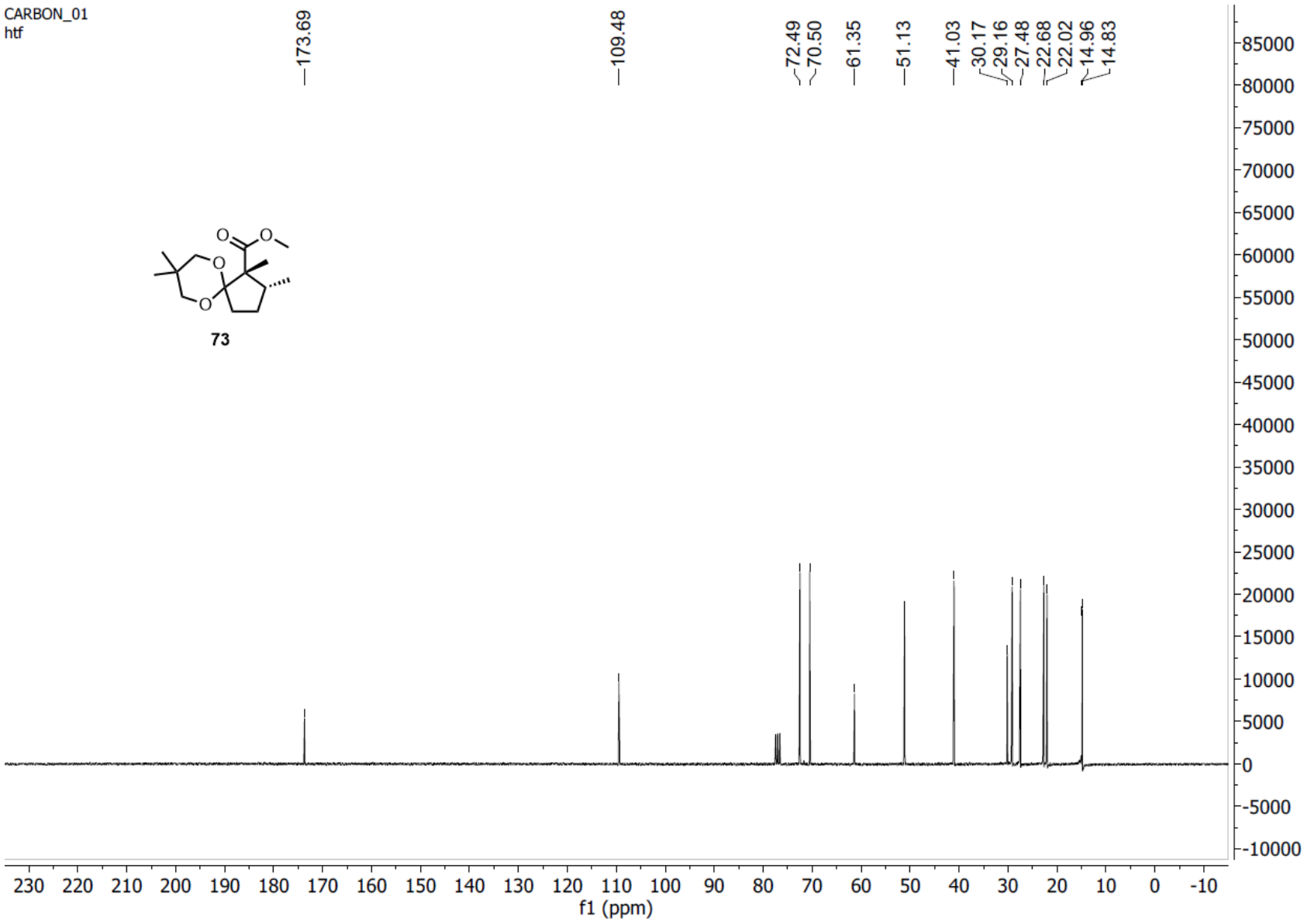

AcO
22
f1 (ppm)

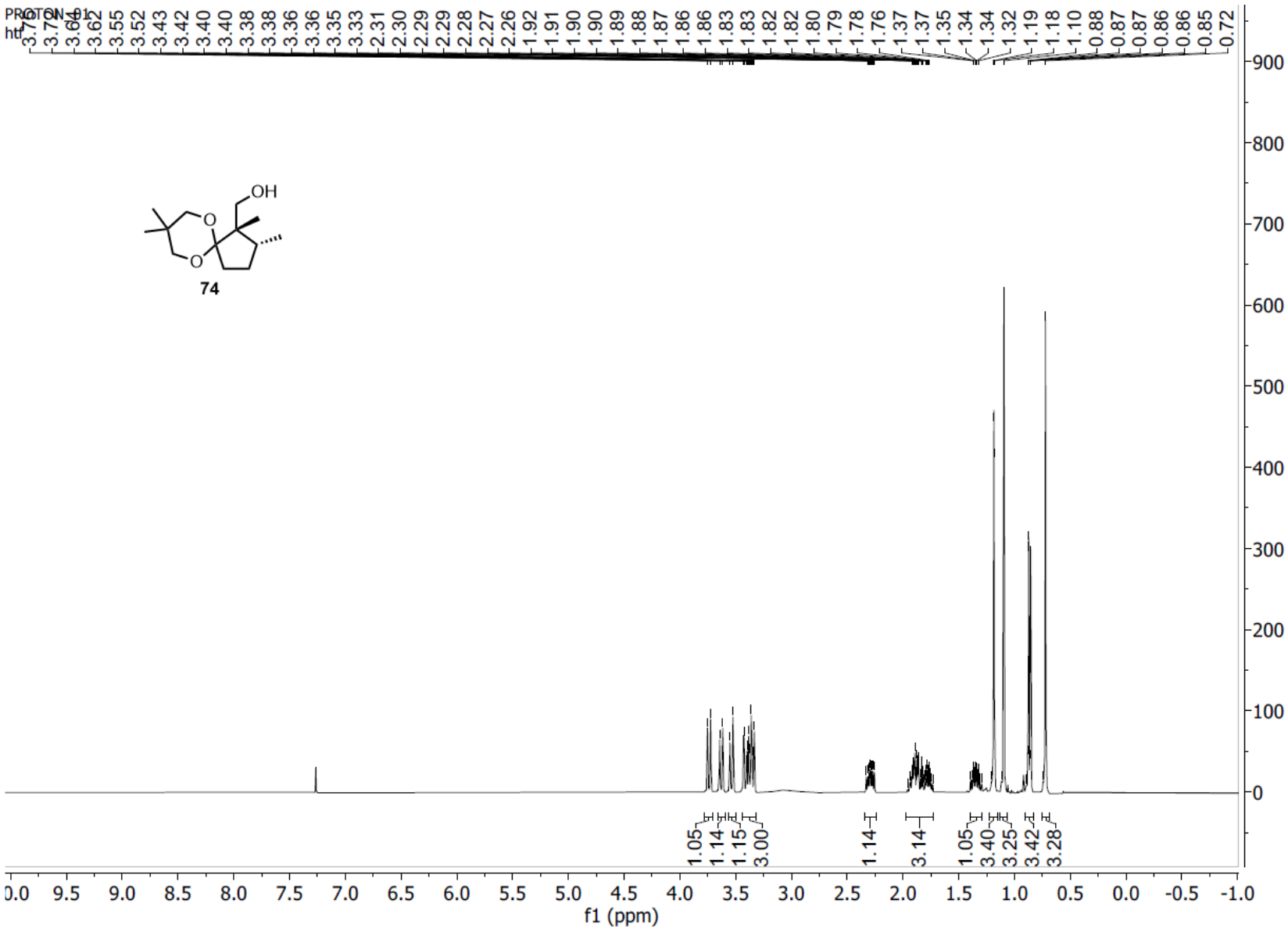

CARBON_01
htf
170.68
169.87
165.84
147.40
138.95
119.04
117.74
112.74
73.39
55.91
54.85
49.22
43.66
40.03
36.81
34.20
34.13
33.75
29.46
27.45
27.35
25.87
22.62
22.03
21.44
20.16
12.91
12.20
AcO
H
H
H
O
O
22
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

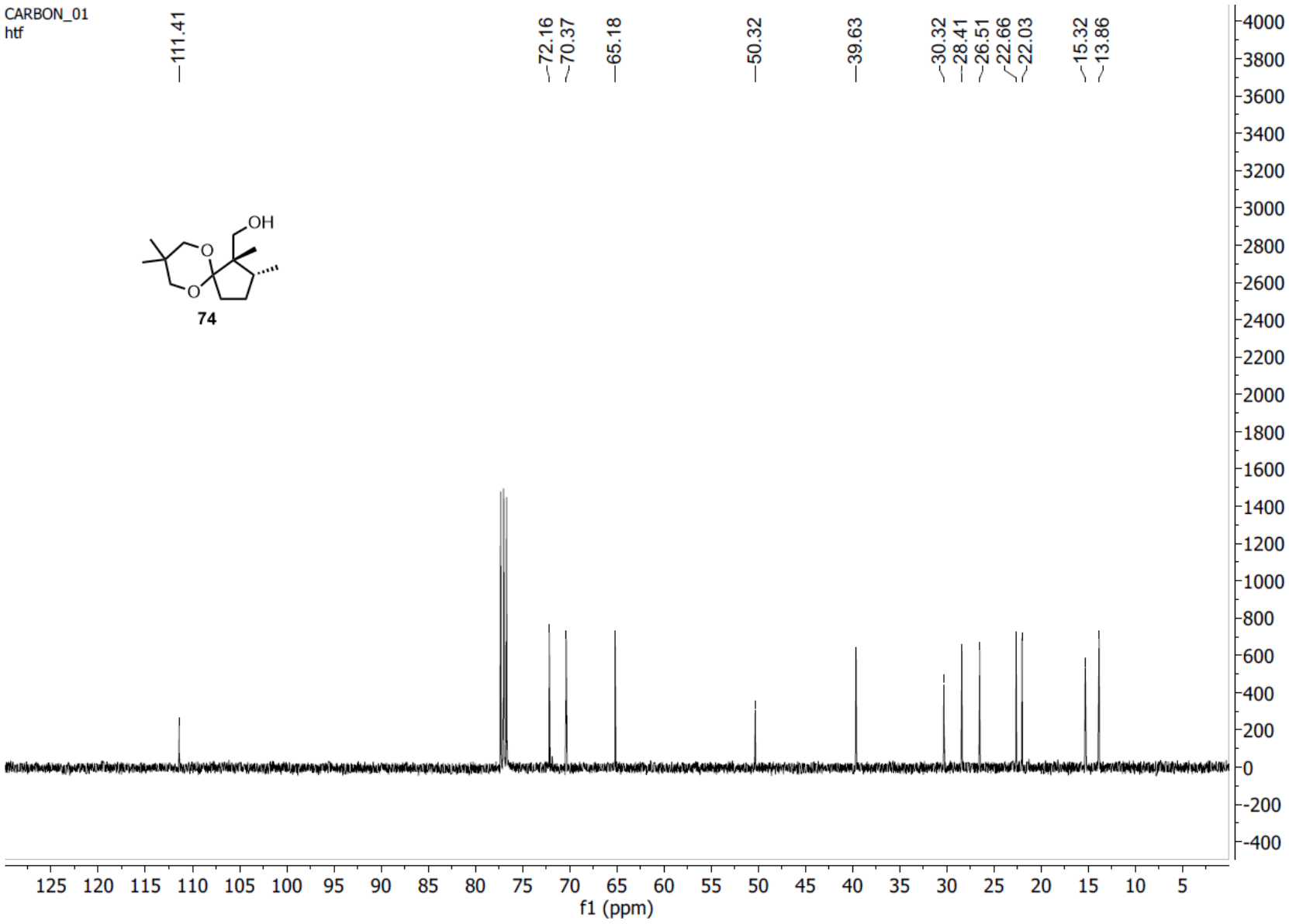

PROTON-01
3.64 3.64 3.63 3.63 3.63 3.63 3.62 2.58 2.58 2.55 2.55 2.52 2.52 2.51 2.50 2.49 2.48 2.29 2.26 2.25 2.22 2.19 2.19 2.16 2.06 2.05 2.04 2.04 2.03 2.02 2.02 2.00 2.00 1.99 1.99 1.99 1.97 1.97 1.81 1.81 1.80 1.78 1.77 1.77 1.74 1.74 1.23 1.23 1.22 1.21 1.21 1.03 1.03 1.02 1.01 1.00
60
2.95
1.19
1.10
2.05
0.94
3.05
3.75
f1 (ppm)

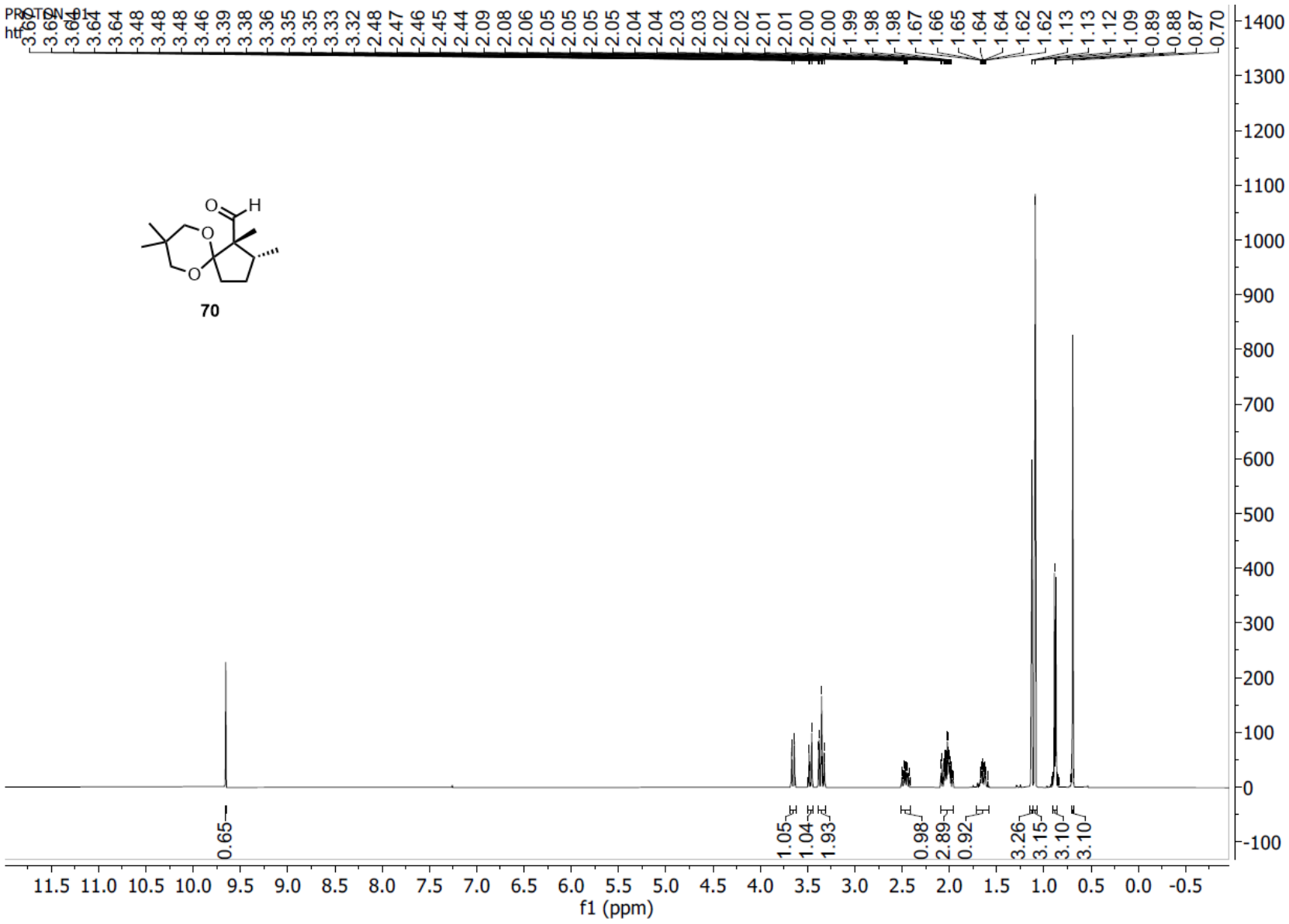

CARBON_01
htf
216.63
171.18
59.36
51.70
43.88
37.77
27.96
18.15
15.35
60
30000
25000
20000
15000
10000
5000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

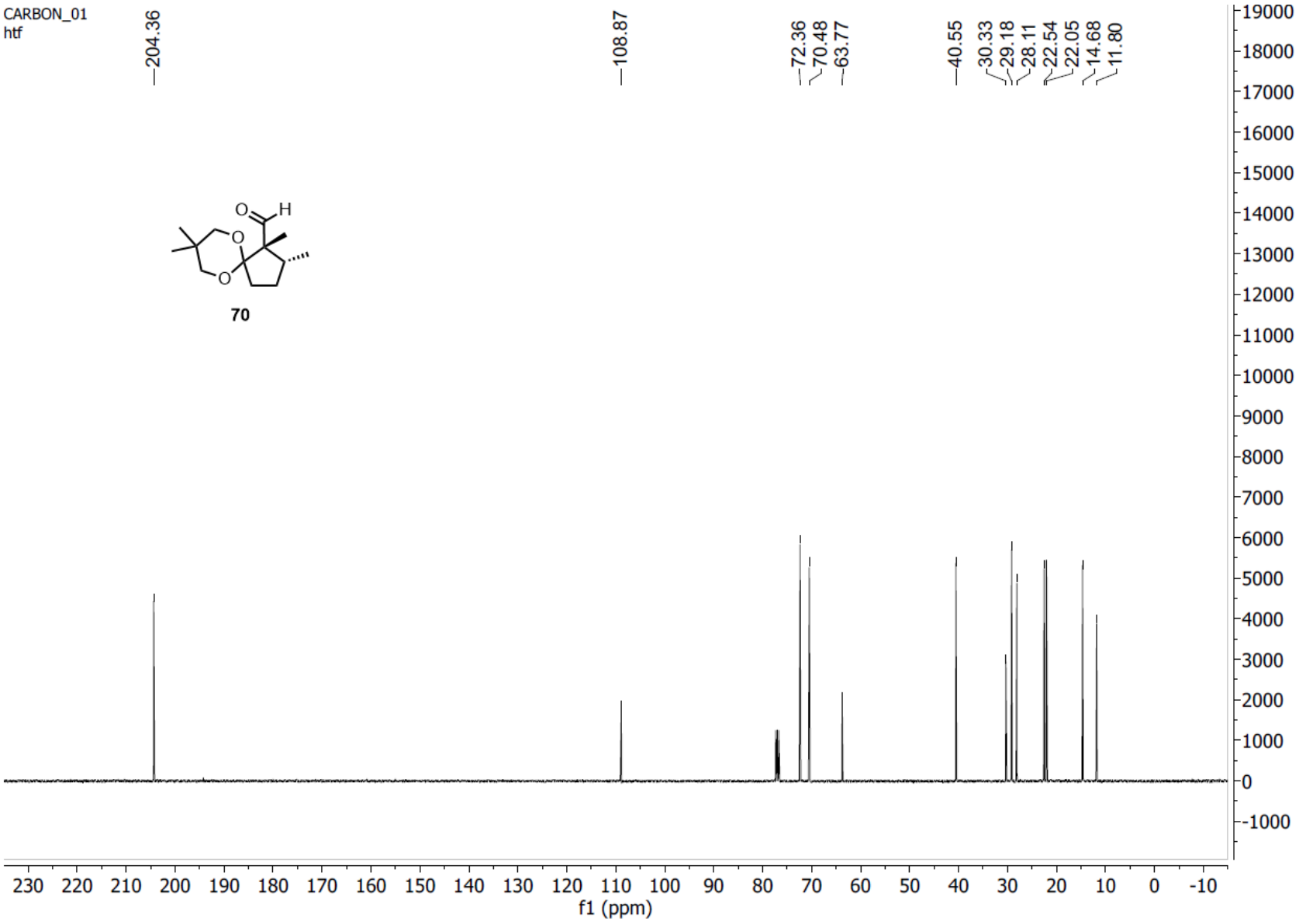

73
f1 (ppm)

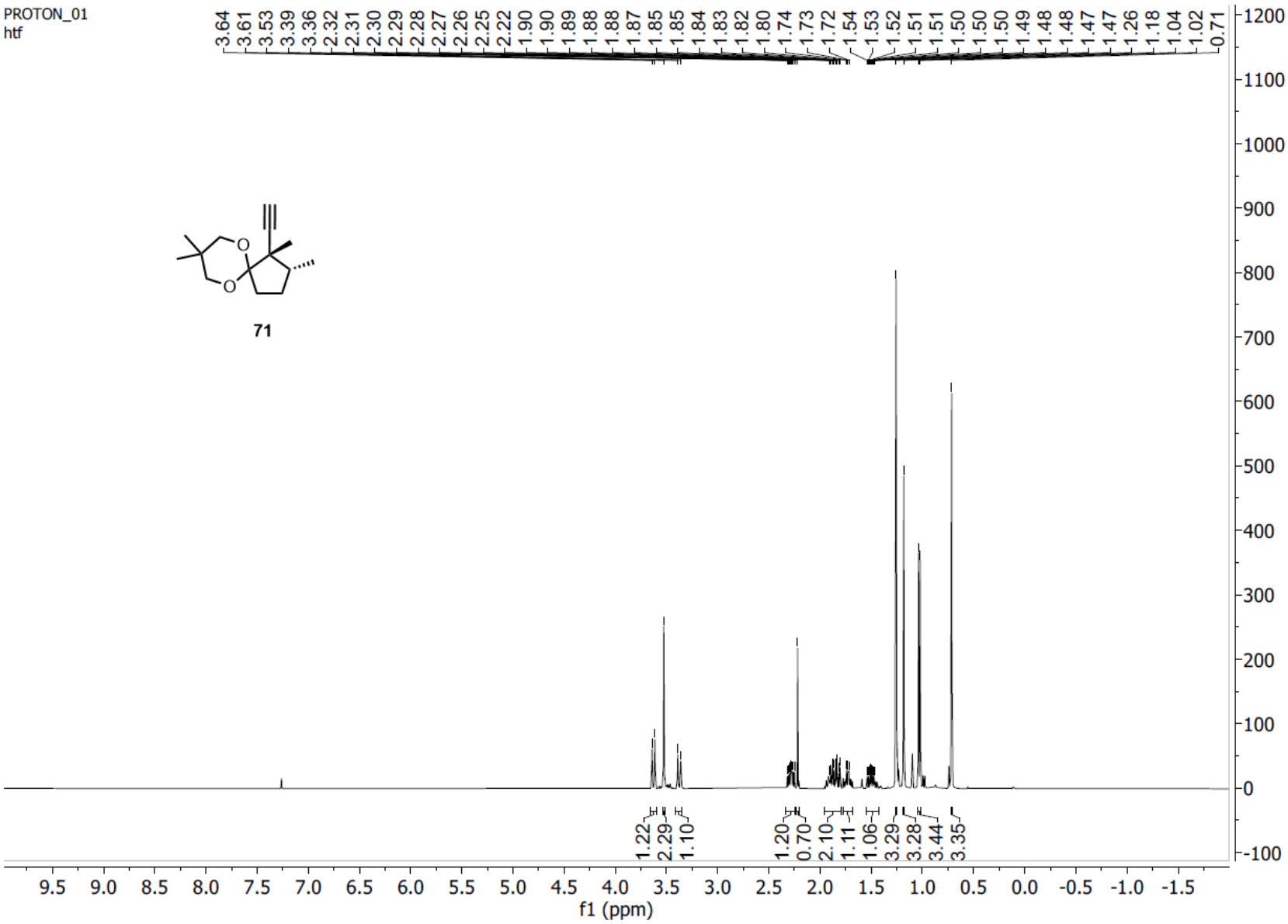

CARBON_01
htf
173.69
109.48
72.49
70.50
61.35
51.13
41.03
30.17
29.16
27.48
22.68
22.02
14.96
14.83
73
f1 (ppm)

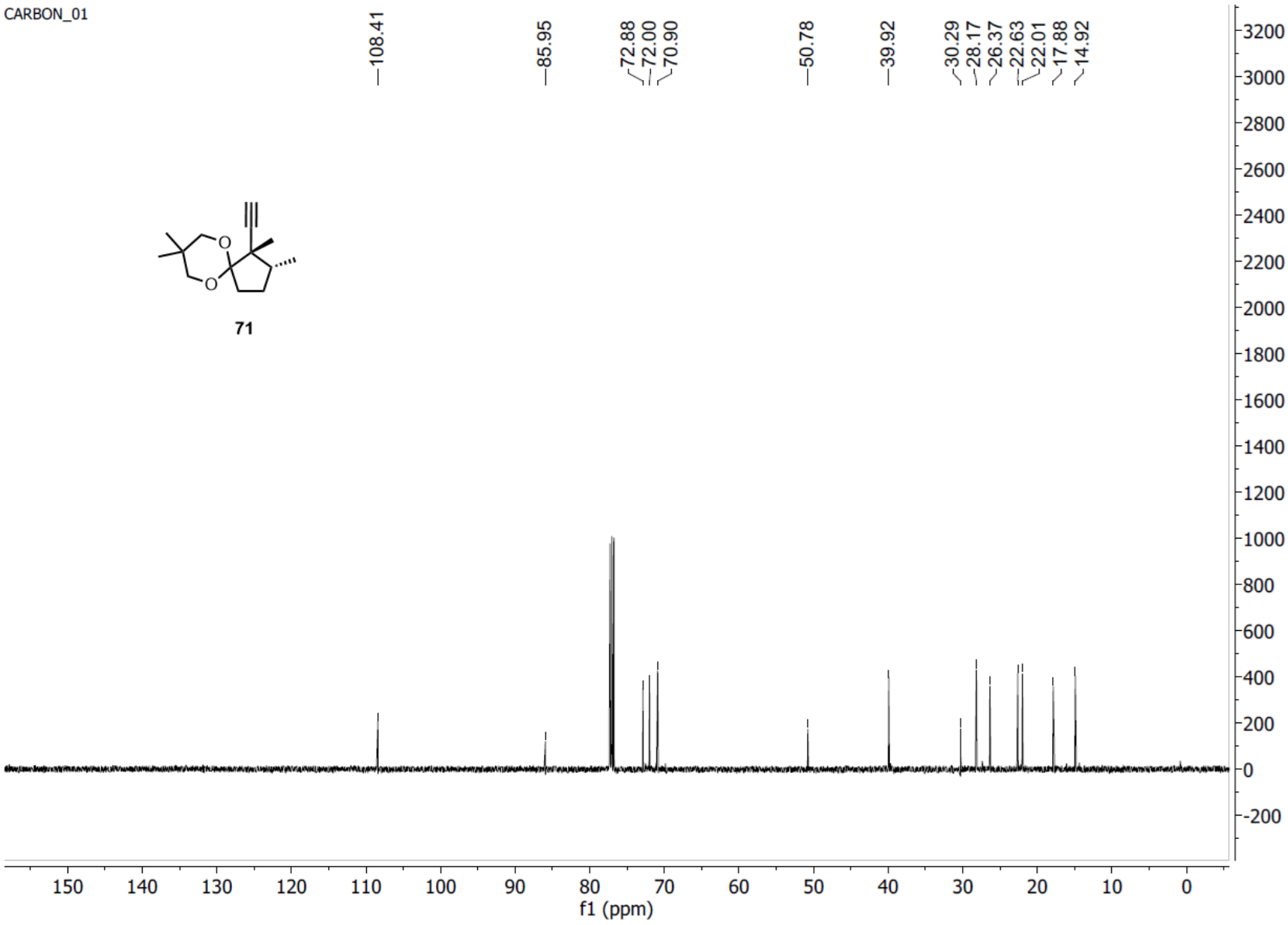

PROTON-01
3.76 3.72 3.64 3.62 3.55 3.52 3.43 3.42 3.40 3.40 3.38 3.38 3.36 3.36 3.35 3.33 2.31 2.30 2.29 2.29 2.28 2.27 2.26 1.92 1.91 1.90 1.90 1.89 1.88 1.87 1.86 1.86 1.83 1.83 1.82 1.82 1.80 1.79 1.78 1.76 1.37 1.37 1.35 1.34 1.34 1.32 1.19 1.18 1.10 0.88 0.87 0.87 0.86 0.86 0.85 0.72
OH
O
O
74
1.05 1.14 1.15 3.00 1.14 3.14 1.05 3.40 3.25 3.42 3.28
f1 (ppm)

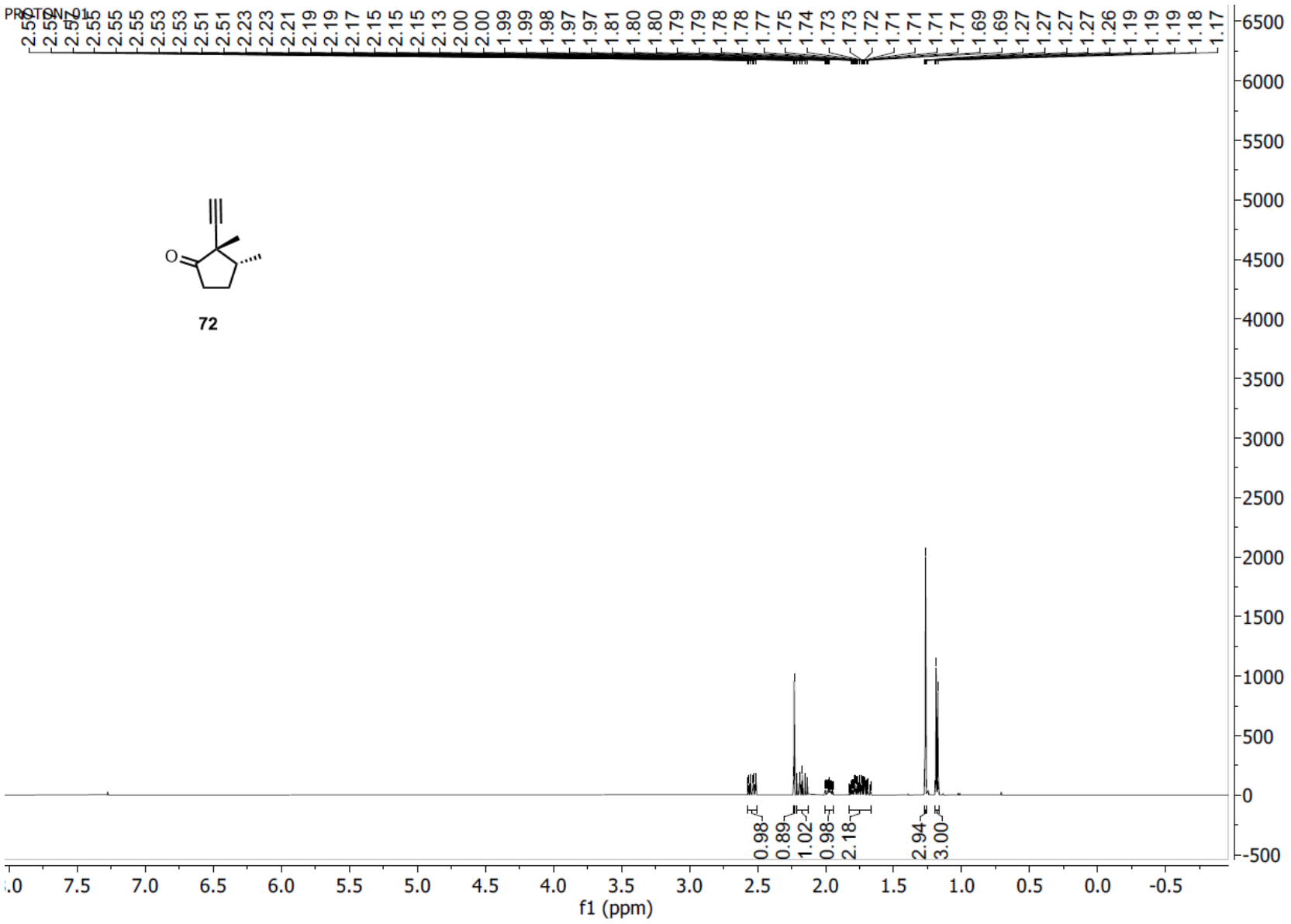

CARBON_01
htf
111.41
72.16
70.37
65.18
50.32
39.63
30.32
28.41
26.51
22.66
22.03
15.32
13.86
OH
O
O
74
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
-400
125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
f1 (ppm)

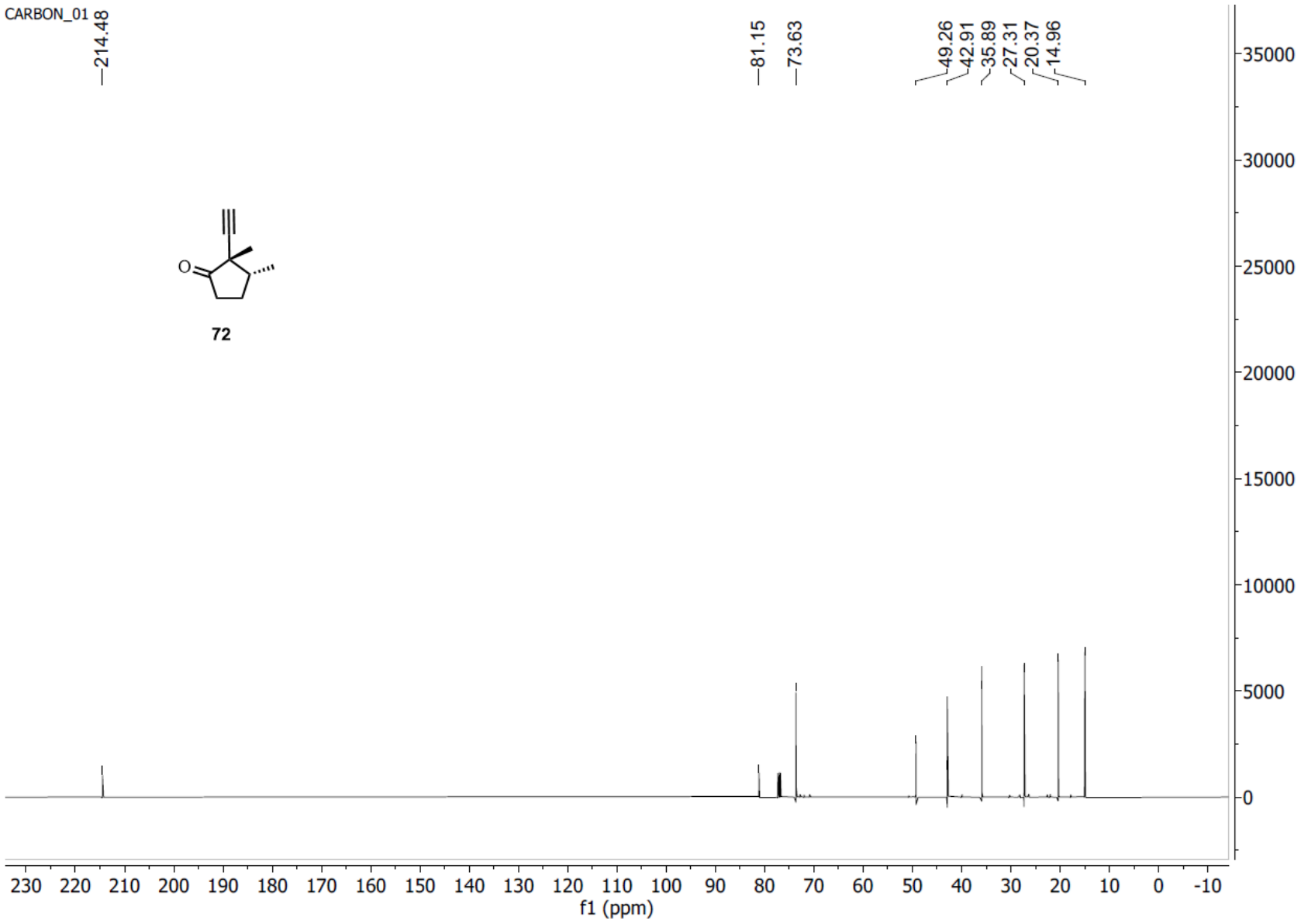

PROTON_01
ht
3.67 3.64 3.64 3.64 3.64 3.48 3.48 3.48 3.46 3.39 3.38 3.36 3.35 3.35 3.33 3.32 2.48 2.47 2.46 2.45 2.44 2.09 2.08 2.06 2.05 2.05 2.05 2.05 2.04 2.04 2.03 2.03 2.02 2.02 2.01 2.01 2.00 2.00 1.99 1.98 1.98 1.67 1.66 1.65 1.64 1.64 1.62 1.62 1.13 1.13 1.12 1.09 0.89 0.88 0.87 0.70
O
H
O
O
70
0.65
1.05
1.04
1.93
0.98
2.89
0.92
3.26
3.15
3.10
3.10
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0 -100

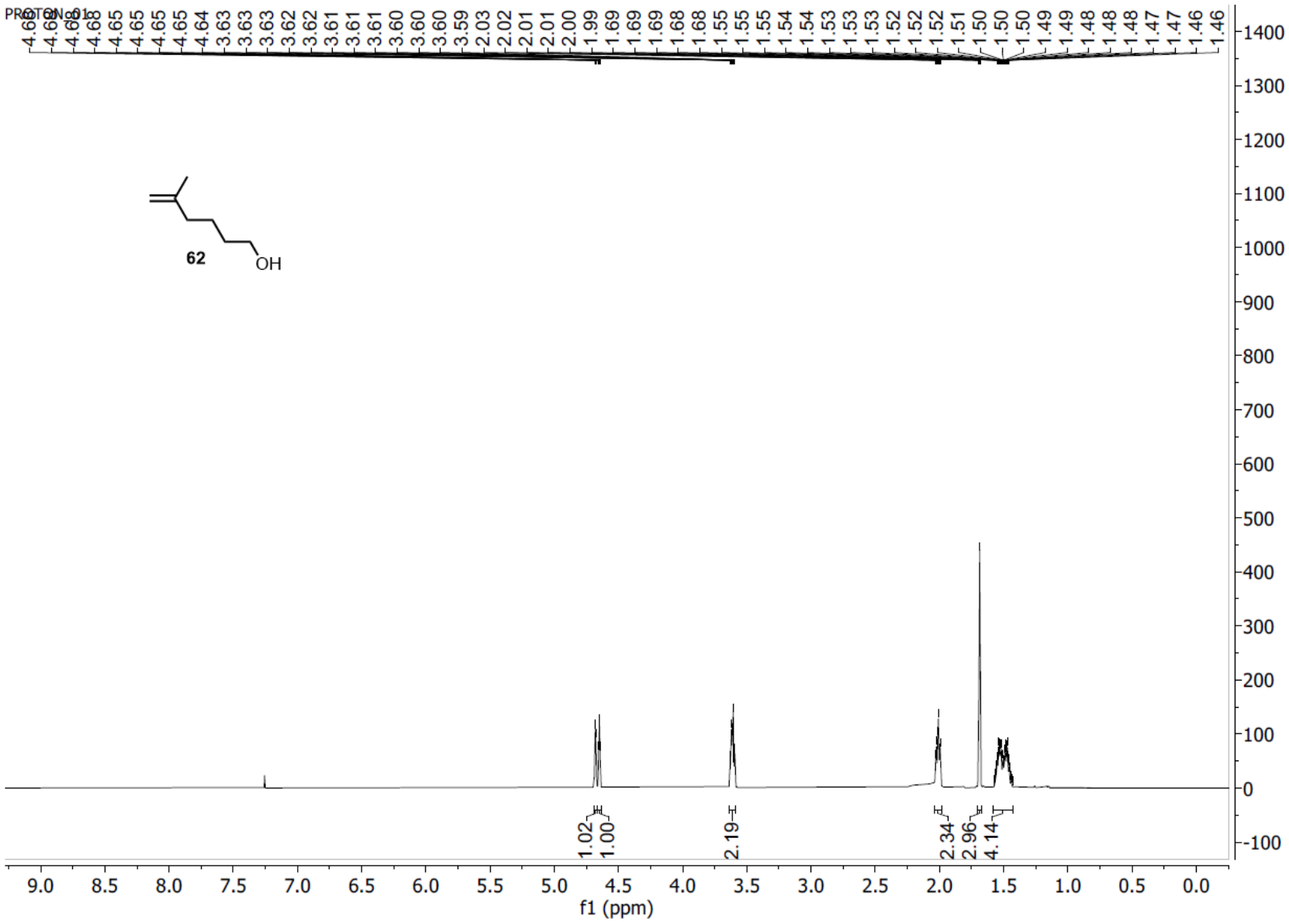

CARBON_01
htf
204.36
108.87
72.36
70.48
63.77
40.55
30.33
29.18
28.11
22.54
22.05
14.68
11.80
O
H
O
O
70
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

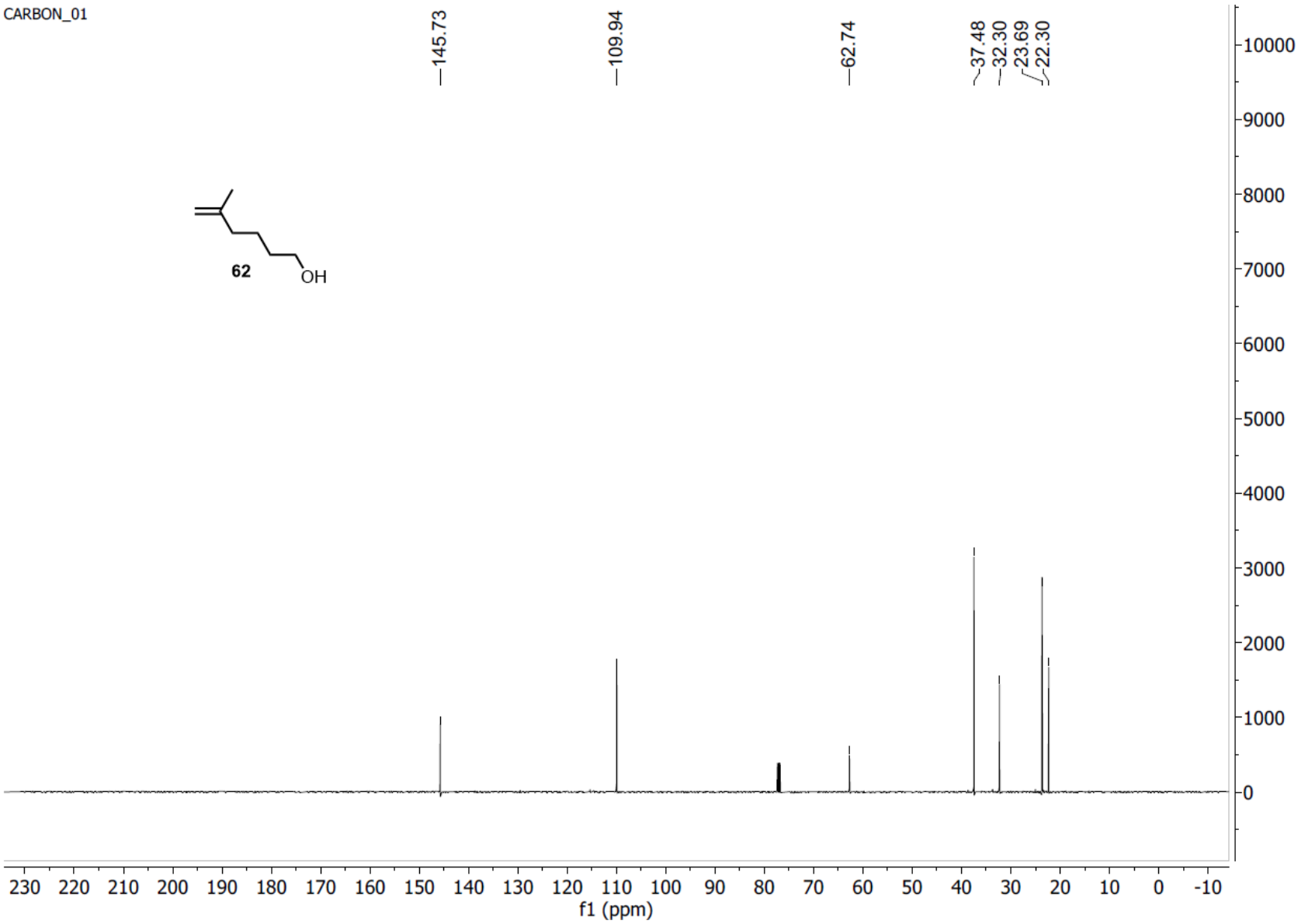

PROTON_01
htf
3.64 3.61 3.53 3.39 3.36 2.32 2.31 2.30 2.29 2.28 2.27 2.26 2.25 2.22 1.90 1.90 1.89 1.88 1.88 1.87 1.85 1.85 1.84 1.83 1.82 1.80 1.74 1.73 1.72 1.54 1.53 1.52 1.51 1.51 1.50 1.50 1.50 1.49 1.48 1.48 1.47 1.47 1.26 1.18 1.04 1.02 0.71
71
1.22 2.29 1.10 1.20 0.70 2.10 1.11 1.06 3.29 3.28 3.44 3.35
f1 (ppm)

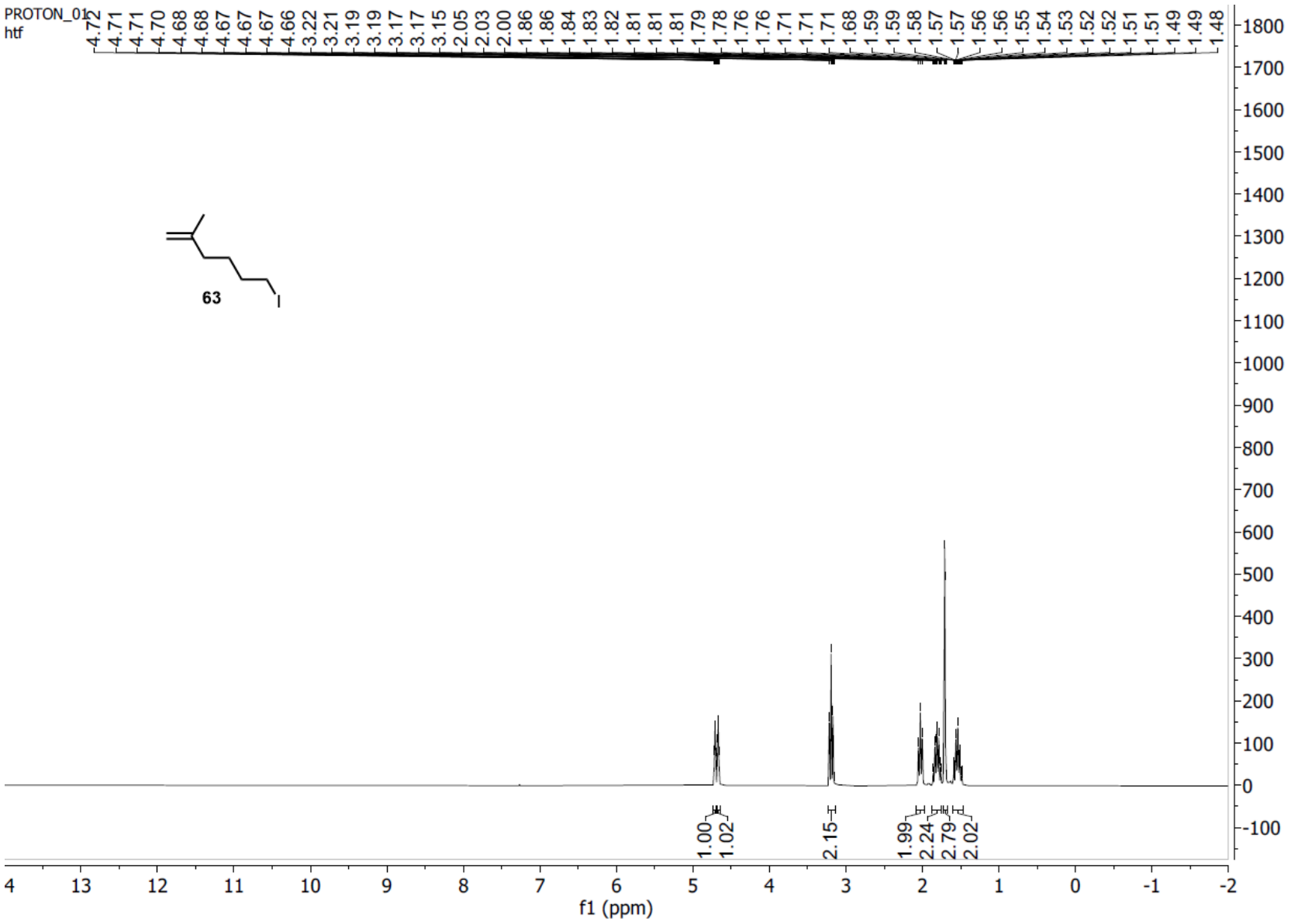

CARBON_01
108.41
85.95
72.88
72.00
70.90
50.78
39.92
30.29
28.17
26.37
22.63
22.01
17.88
14.92
71
f1 (ppm)

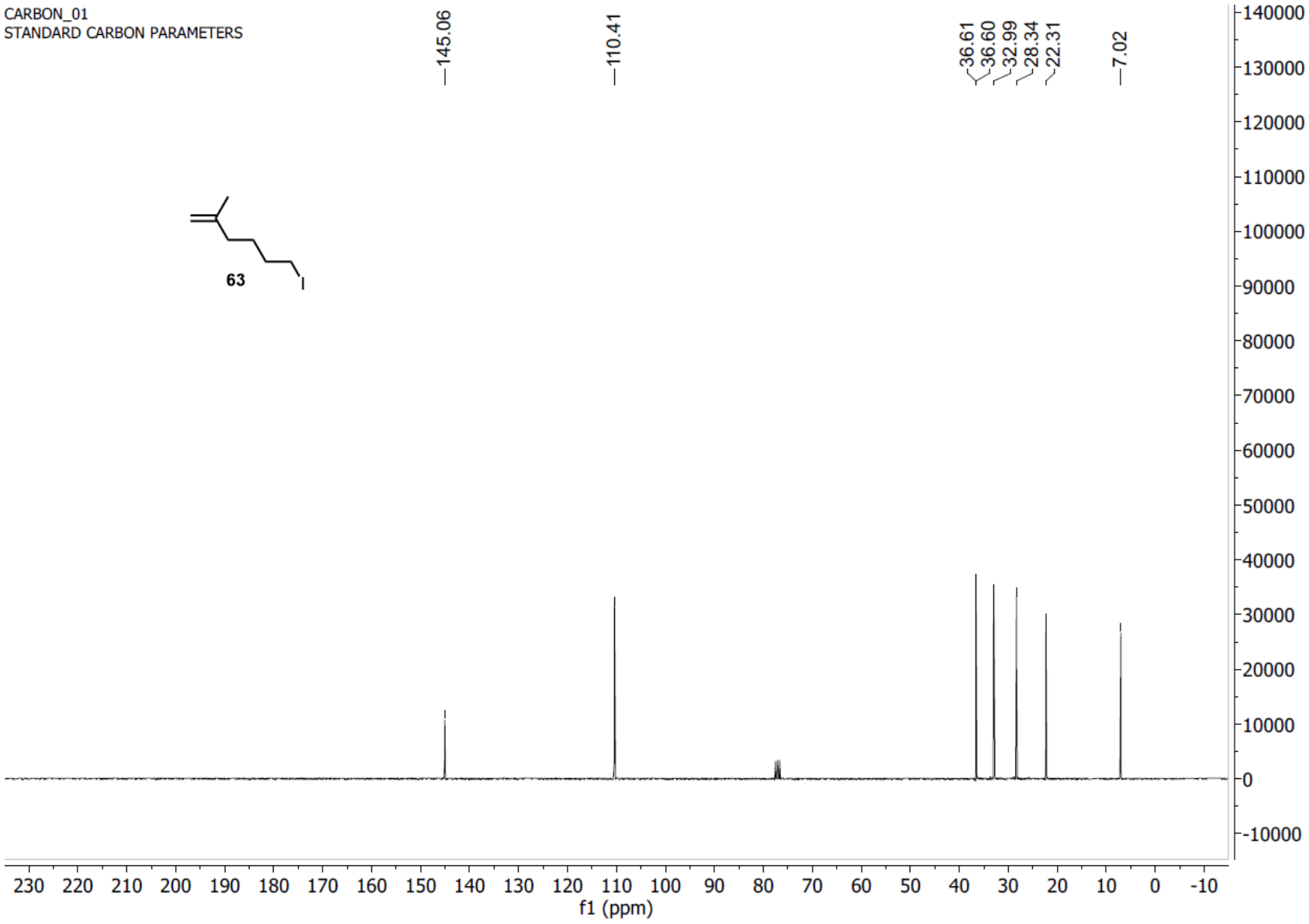

PROTON_01
2.59 2.57 2.55 2.55 2.55 2.55 2.53 2.53 2.51 2.51 2.23 2.23 2.21 2.19 2.19 2.17 2.15 2.15 2.15 2.13 2.00 2.00 1.99 1.99 1.98 1.97 1.97 1.81 1.80 1.80 1.79 1.79 1.78 1.78 1.77 1.75 1.74 1.73 1.73 1.72 1.71 1.71 1.71 1.71 1.69 1.69 1.27 1.27 1.27 1.27 1.26 1.19 1.19 1.19 1.18 1.17
O
72
0.98 0.89 1.02 0.98 2.18 2.94 3.00
f1 (ppm)

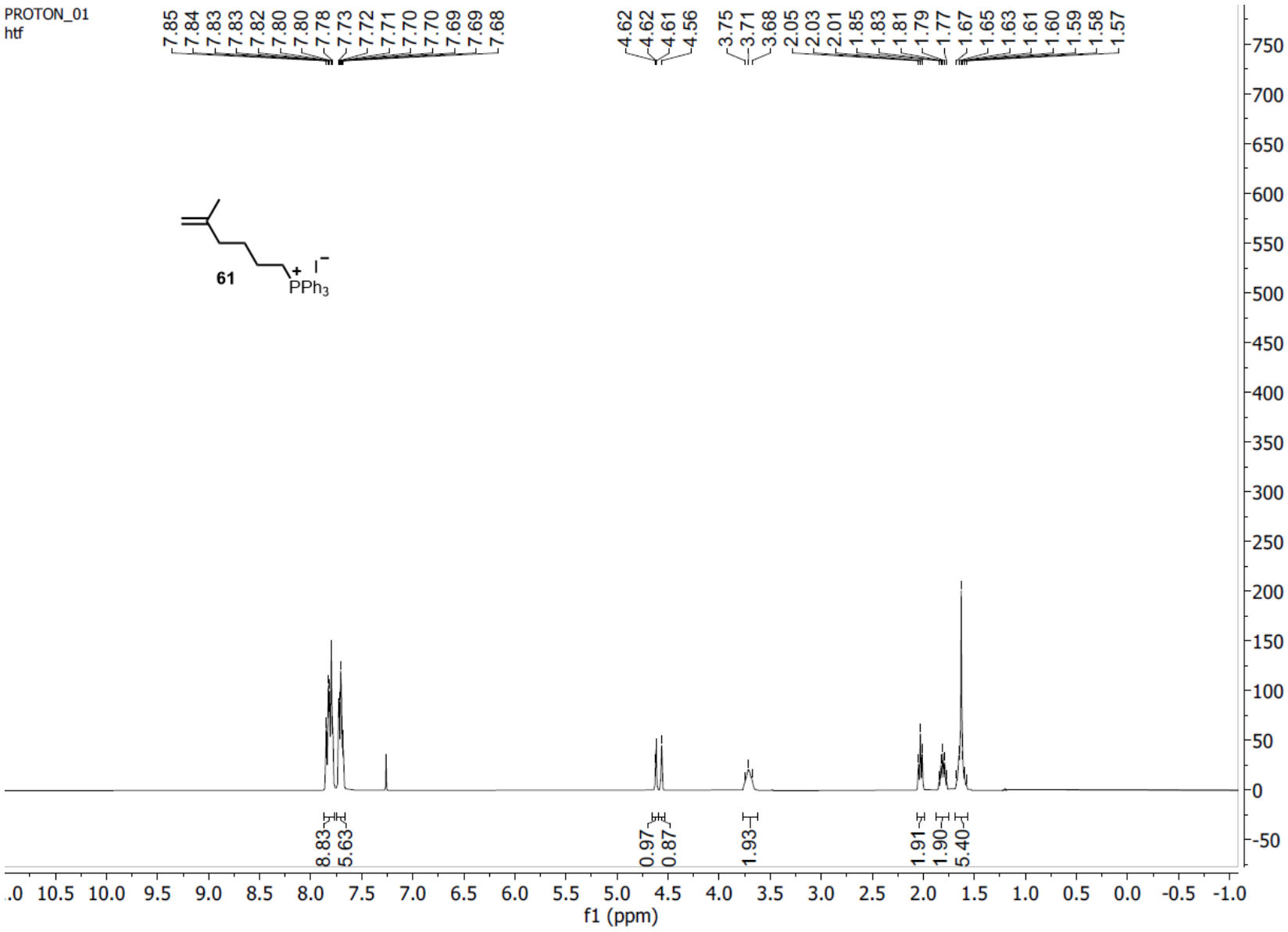

CARBON_01
214.48
81.15
73.63
49.26
42.91
35.89
27.31
20.37
14.96
O
72
35000
30000
25000
20000
15000
10000
5000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

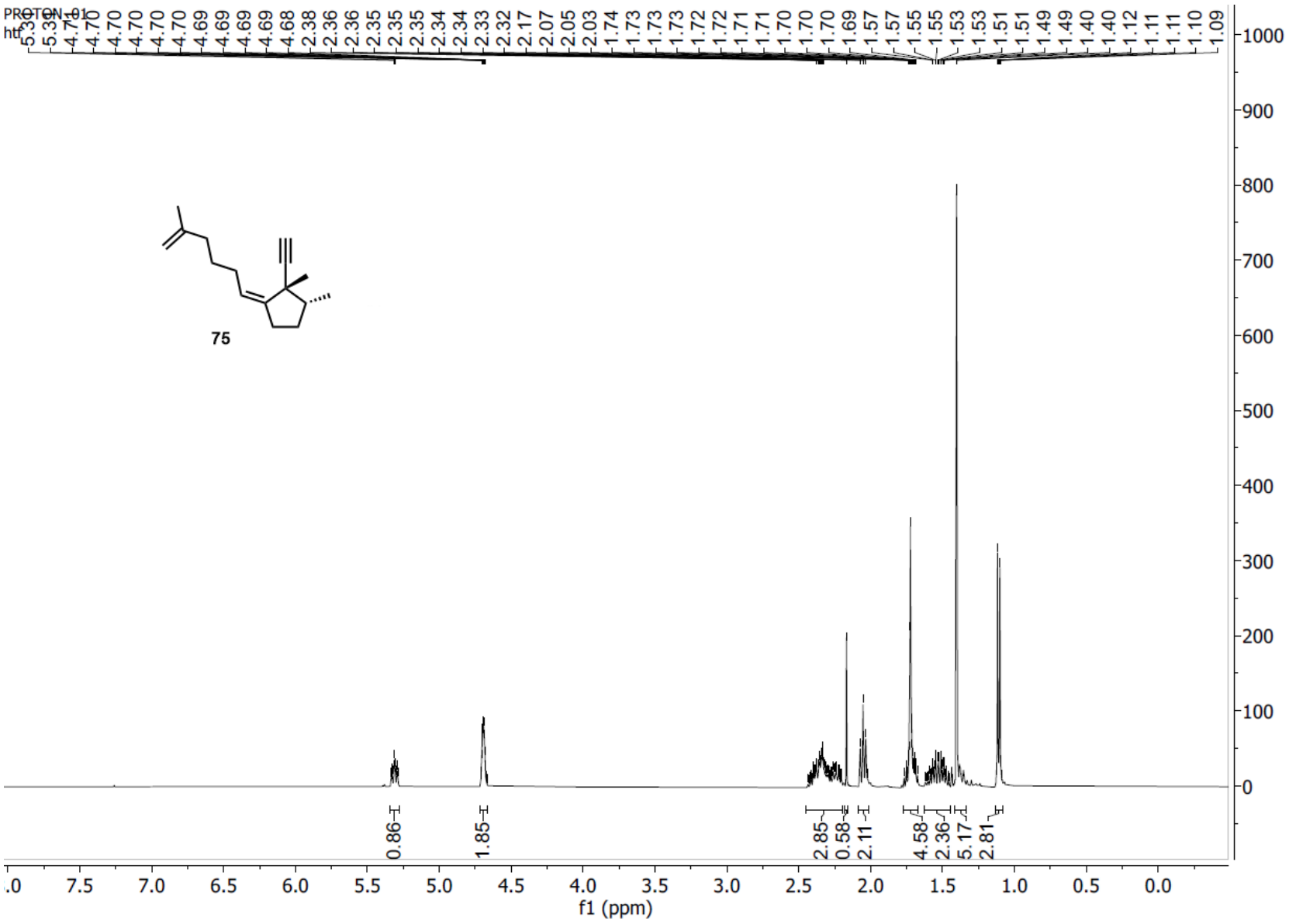

PROTON_01
62
OH
1.02
1.00
2.19
2.34
2.96
4.14
f1 (ppm)

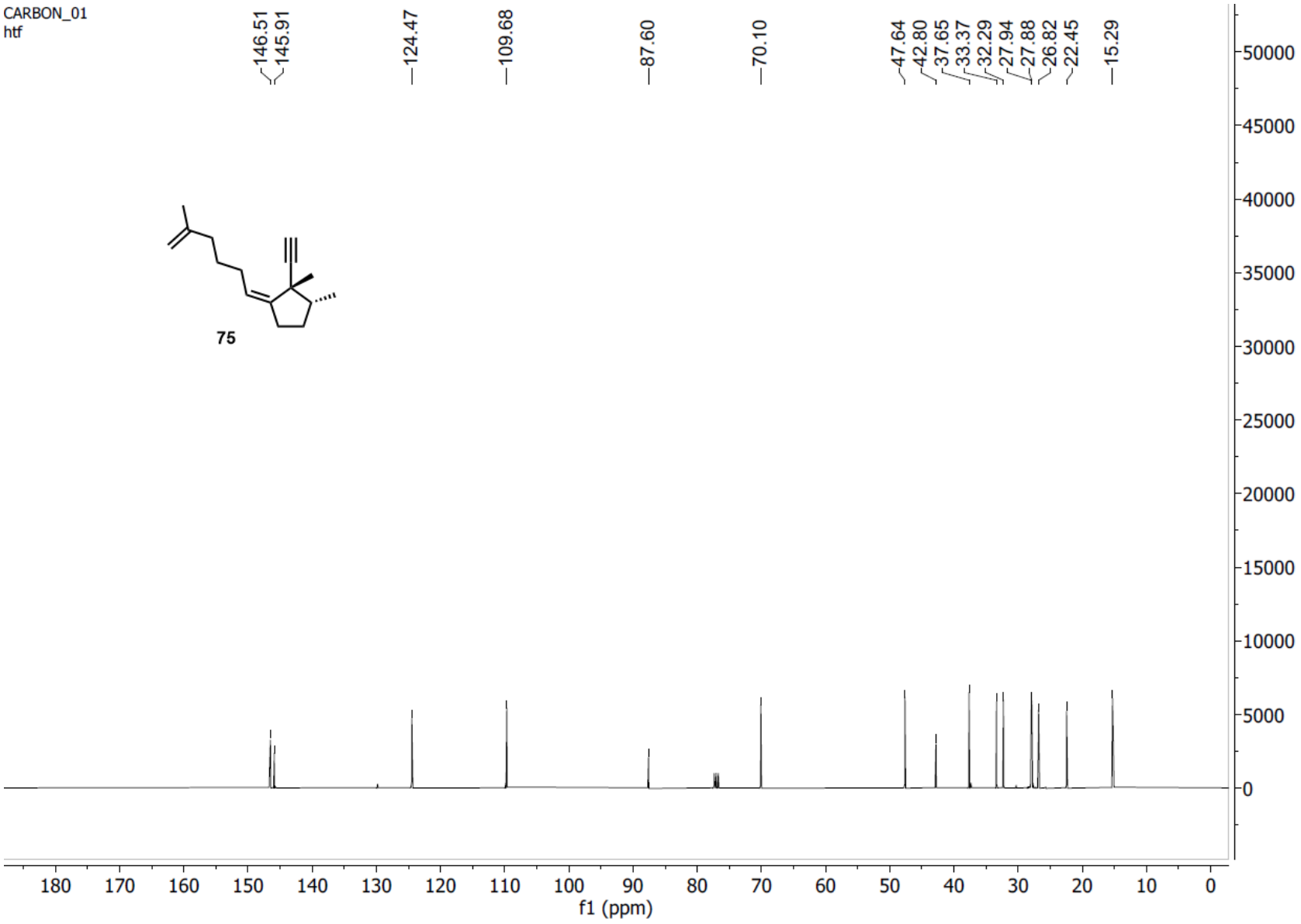

CARBON_01
145.73
109.94
62.74
37.48
32.30
23.69
22.30
62
OH
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

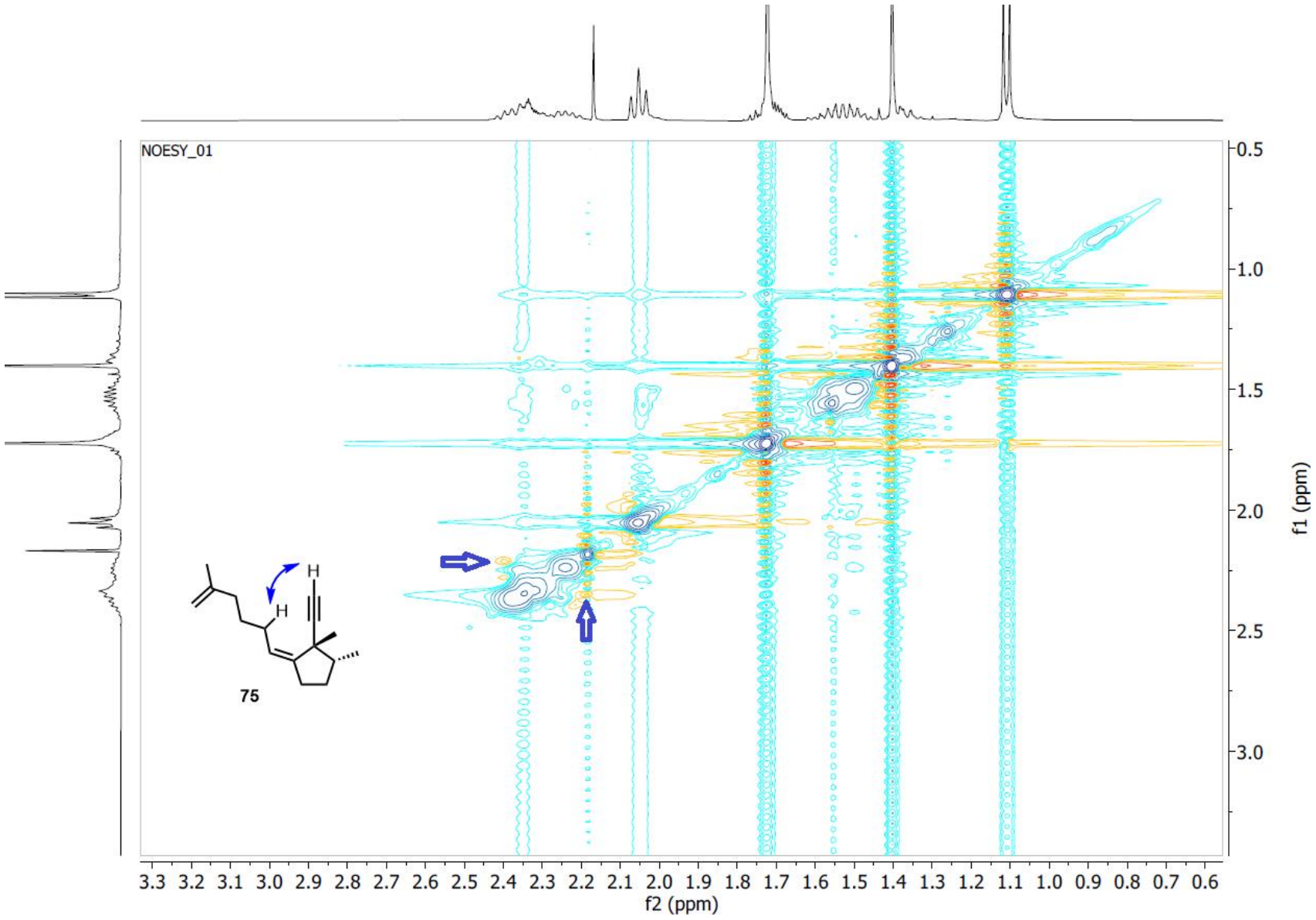

PROTON_01
htf
4.72 4.71 4.71 4.70 4.68 4.68 4.67 4.67 4.67 4.66 3.22 3.21 3.19 3.19 3.17 3.17 3.15 2.05 2.03 2.00 1.86 1.86 1.84 1.83 1.82 1.81 1.81 1.81 1.79 1.78 1.76 1.76 1.71 1.71 1.71 1.68 1.59 1.59 1.58 1.57 1.57 1.56 1.56 1.55 1.54 1.53 1.52 1.52 1.51 1.51 1.49 1.49 1.48
63
I
1.00 1.02 2.15 1.99 2.24 2.79 2.02
f1 (ppm)

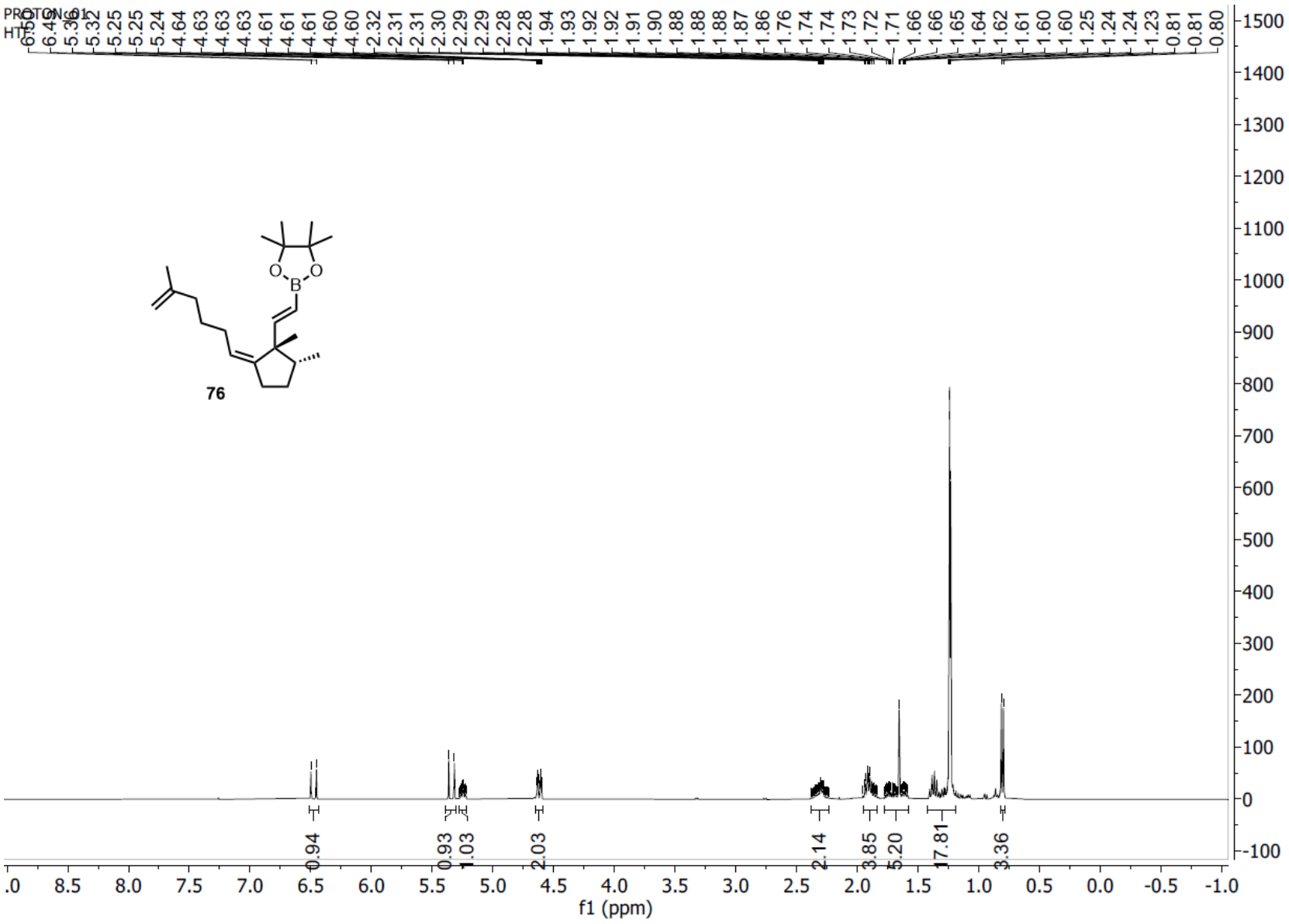

CARBON_01
STANDARD CARBON PARAMETERS
145.06
110.41
36.61
36.60
32.99
28.34
22.31
7.02
63
I
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

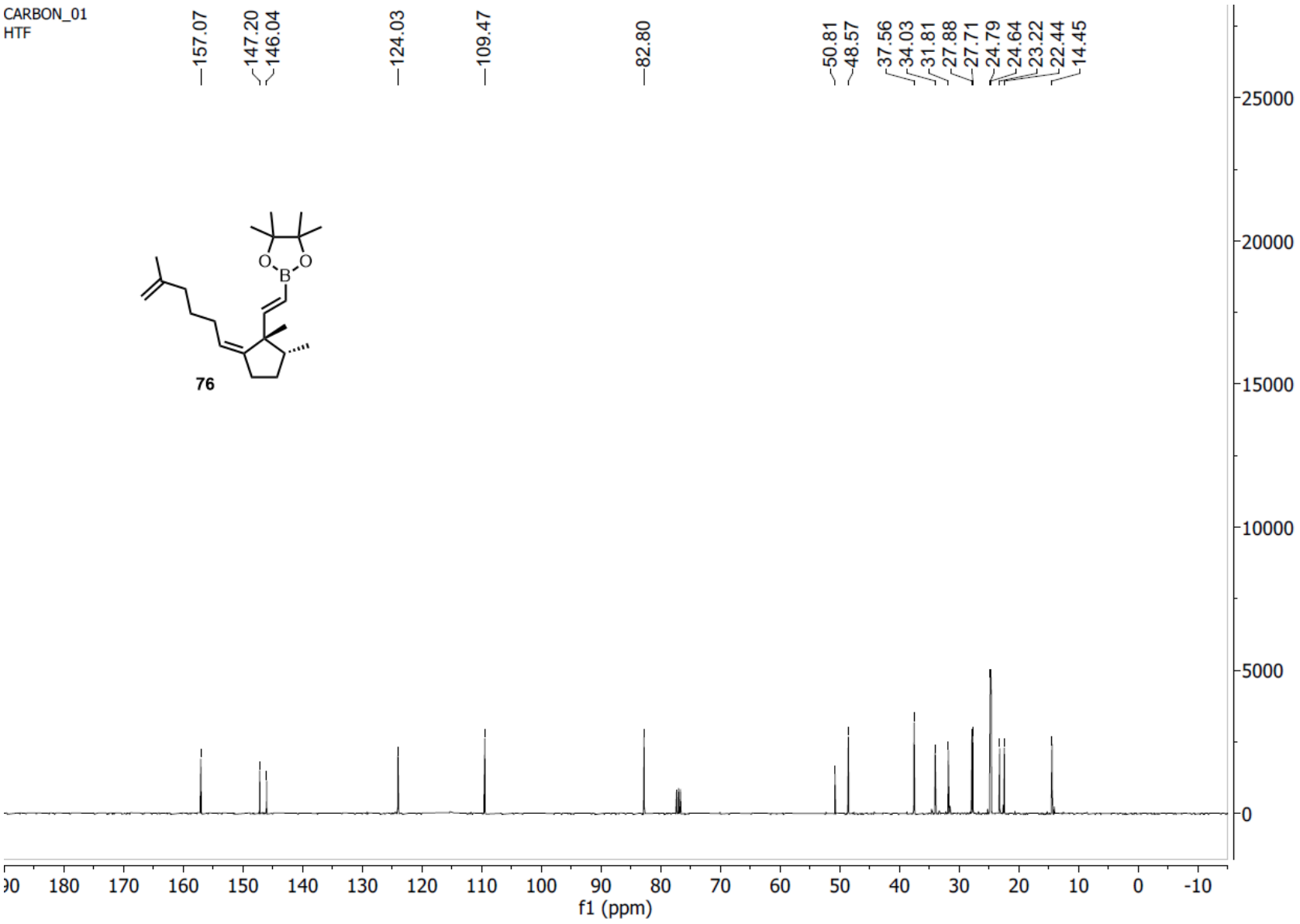

PROTON_01
htf
7.85 7.84 7.83 7.83 7.82 7.80 7.80 7.78 7.73 7.72 7.71 7.70 7.70 7.69 7.69 7.68
4.62 4.62 4.61 4.56
3.75 3.71 3.68
2.05 2.03 2.01 1.85 1.83 1.81 1.79 1.77 1.67 1.65 1.63 1.61 1.60 1.59 1.58 1.57
61
PPh3
8.83
5.63
0.97
0.87
1.93
1.91
1.90
5.40
f1 (ppm)

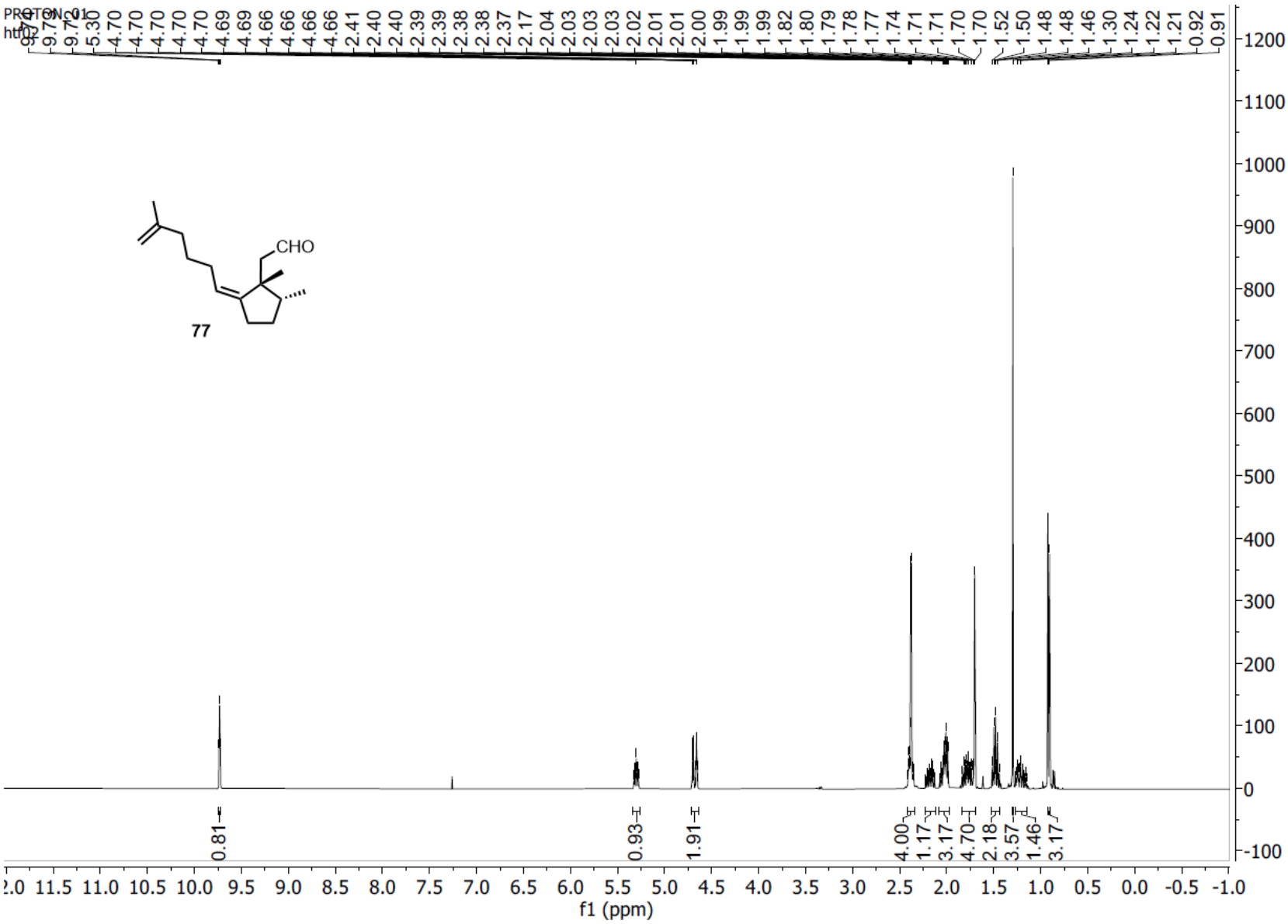

PROTON-01
5.39
4.71
4.70
4.70
4.70
4.70
4.70
4.69
4.69
4.69
4.69
4.68
2.38
2.36
2.36
2.35
2.35
2.35
2.34
2.34
2.33
2.32
2.17
2.07
2.05
2.03
1.74
1.73
1.73
1.73
1.72
1.72
1.71
1.71
1.70
1.70
1.70
1.69
1.57
1.57
1.55
1.55
1.53
1.53
1.51
1.51
1.49
1.49
1.40
1.40
1.12
1.11
1.11
1.10
1.09
75
0.86
1.85
2.85
0.58
2.11
4.58
2.36
5.17
2.81
f1 (ppm)

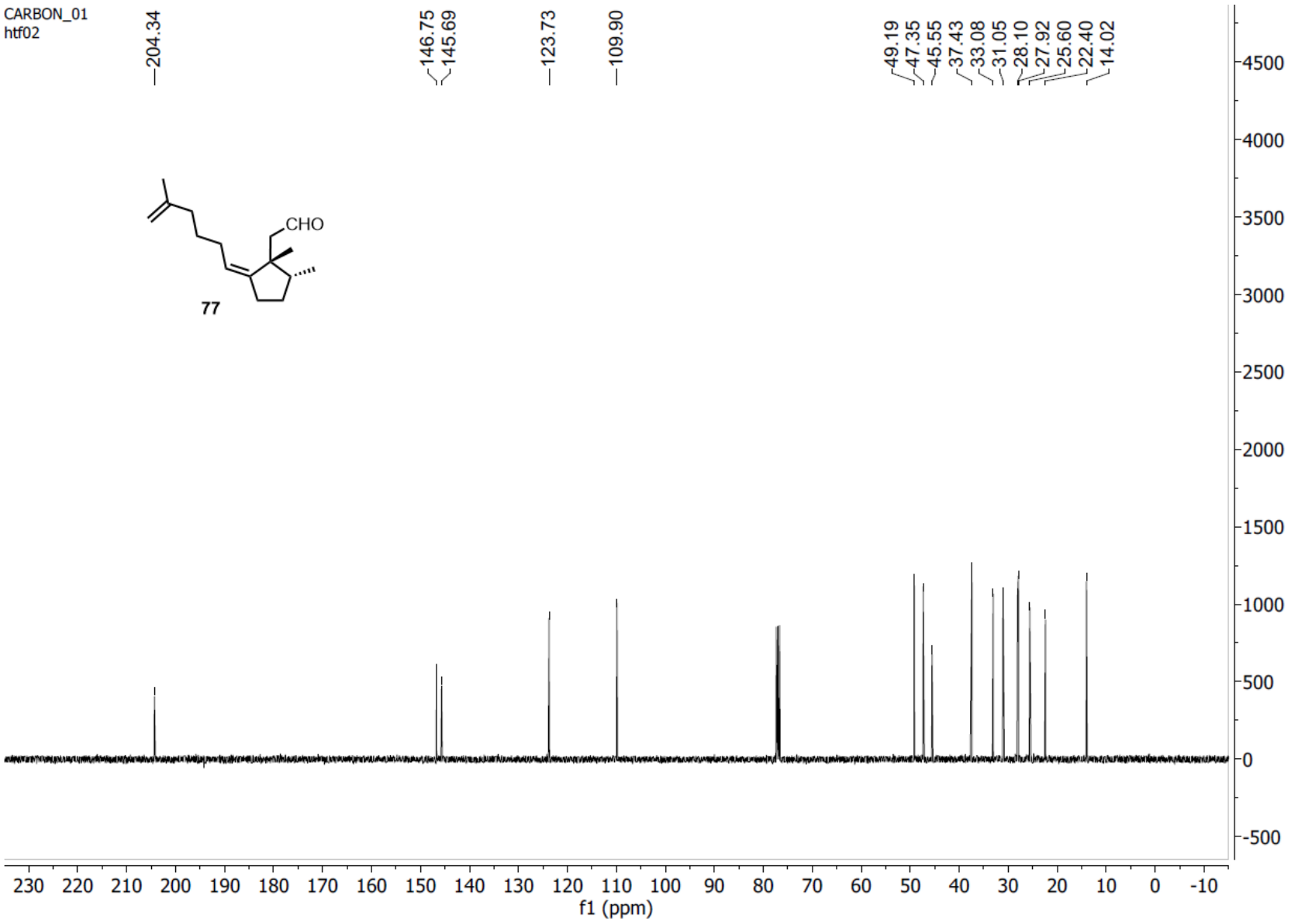

CARBON_01
htf
146.51
145.91
124.47
109.68
87.60
70.10
47.64
42.80
37.65
33.37
32.29
27.94
27.88
26.82
22.45
15.29
75
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

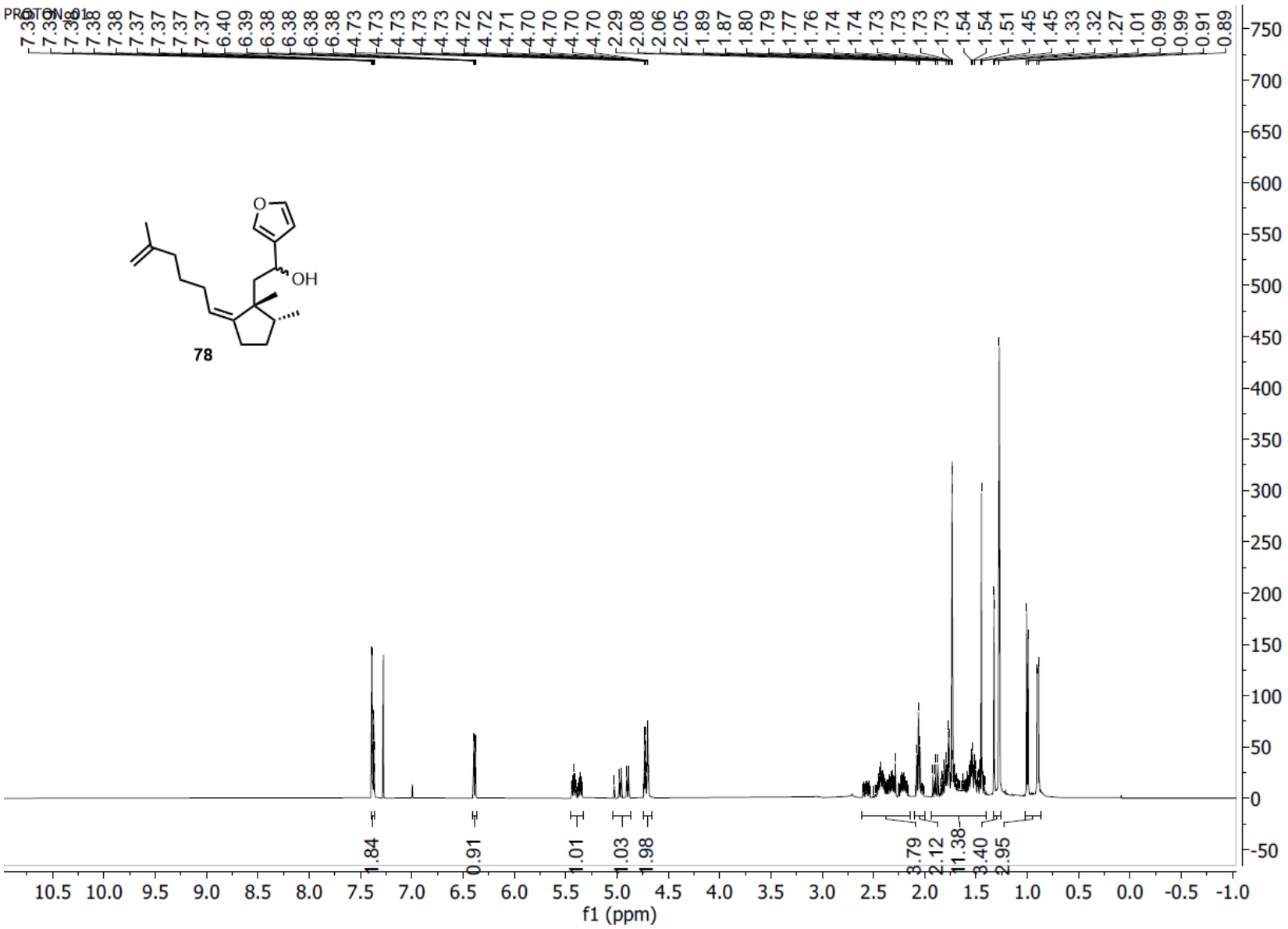

NOESY_01
H
H
75
0.5
1.0
1.5
2.0
2.5
3.0
f1 (ppm)
3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 0.6
f2 (ppm)

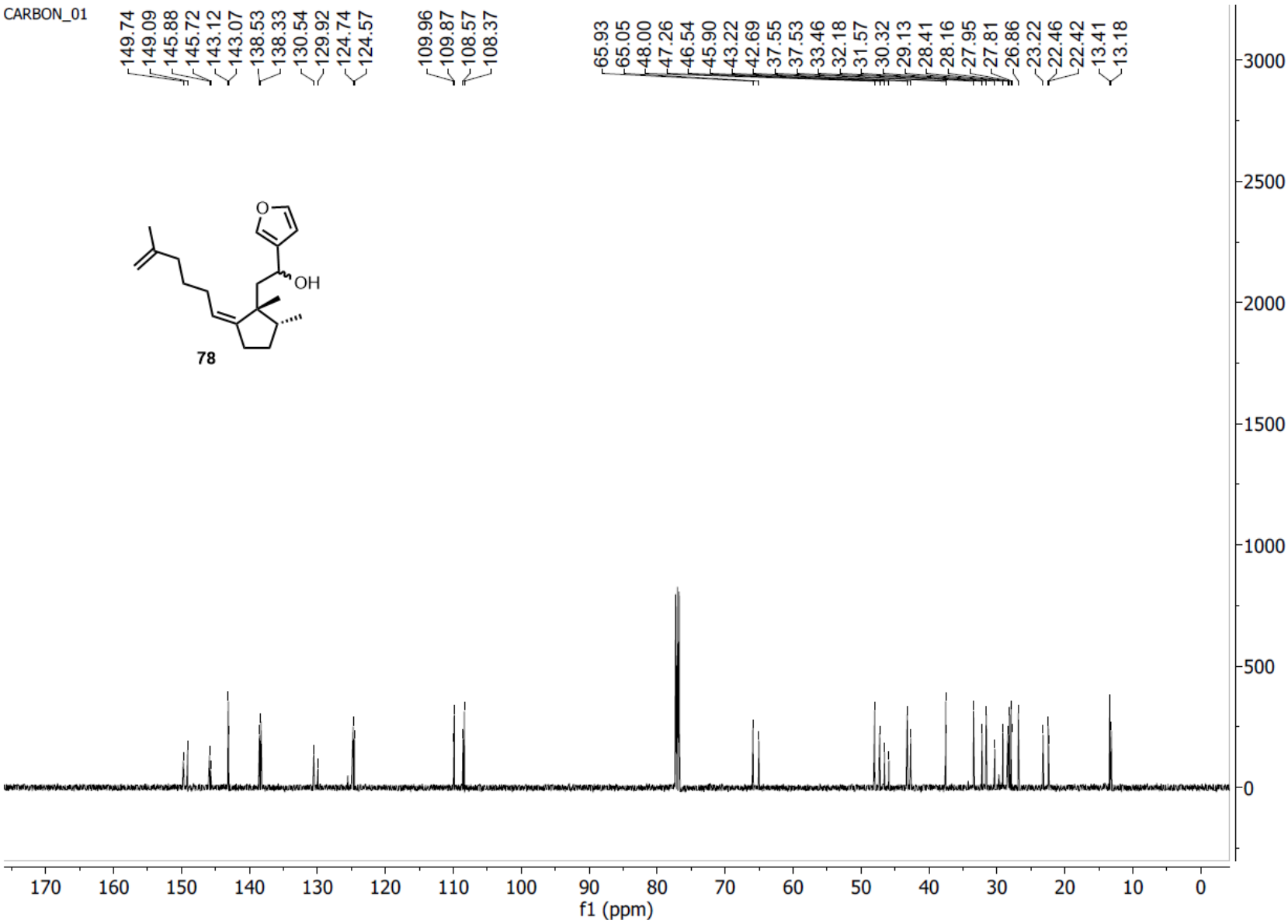

76
0.94
0.93
1.03
2.03
2.14
3.85
5.20
17.81
3.36
f1 (ppm)

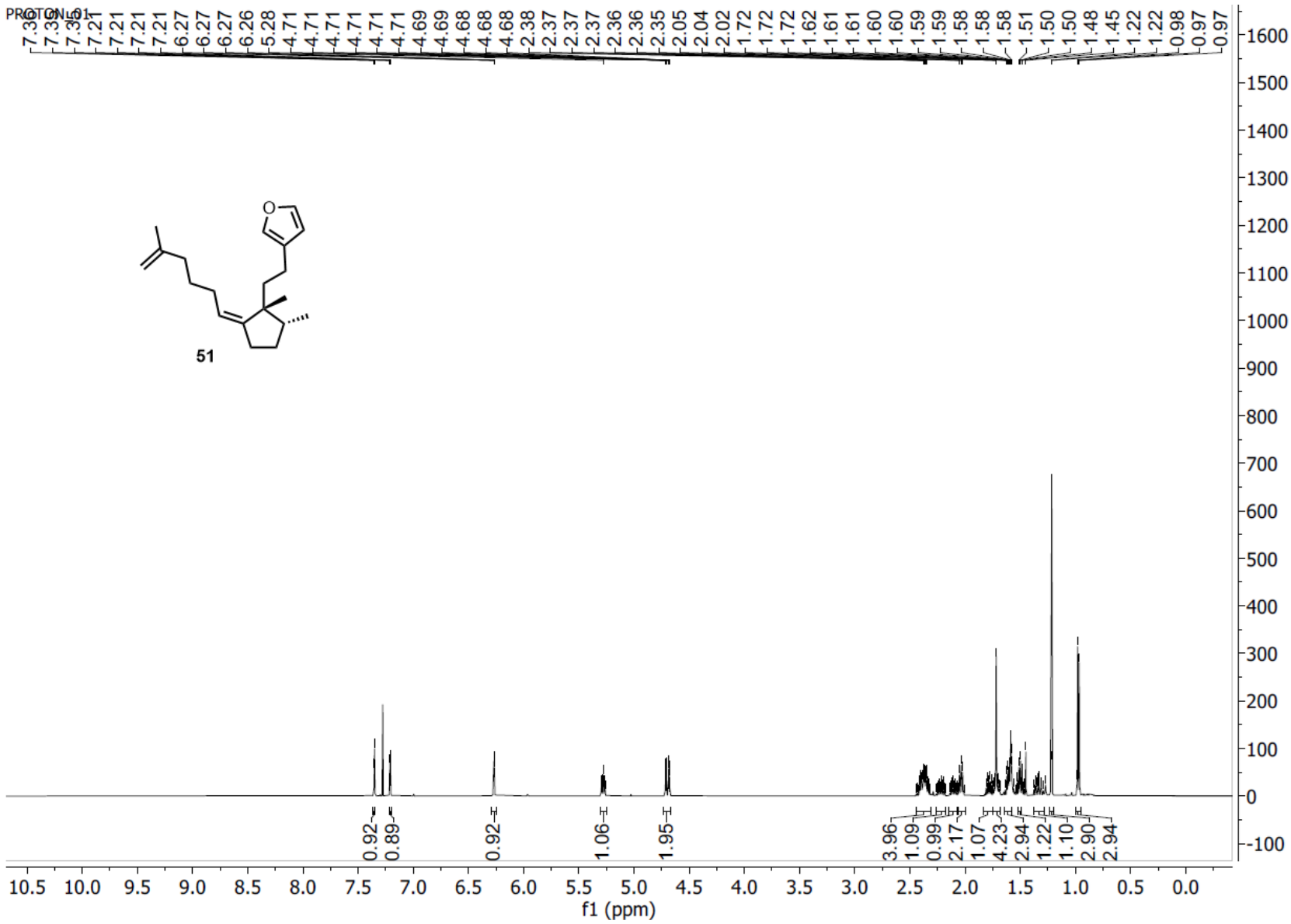

CARBON_01
HTF
157.07
147.20
146.04
124.03
109.47
82.80
50.81
48.57
37.56
34.03
31.81
27.88
27.71
24.79
24.64
23.22
22.44
14.45
O
B
O
76
25000
20000
15000
10000
5000
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

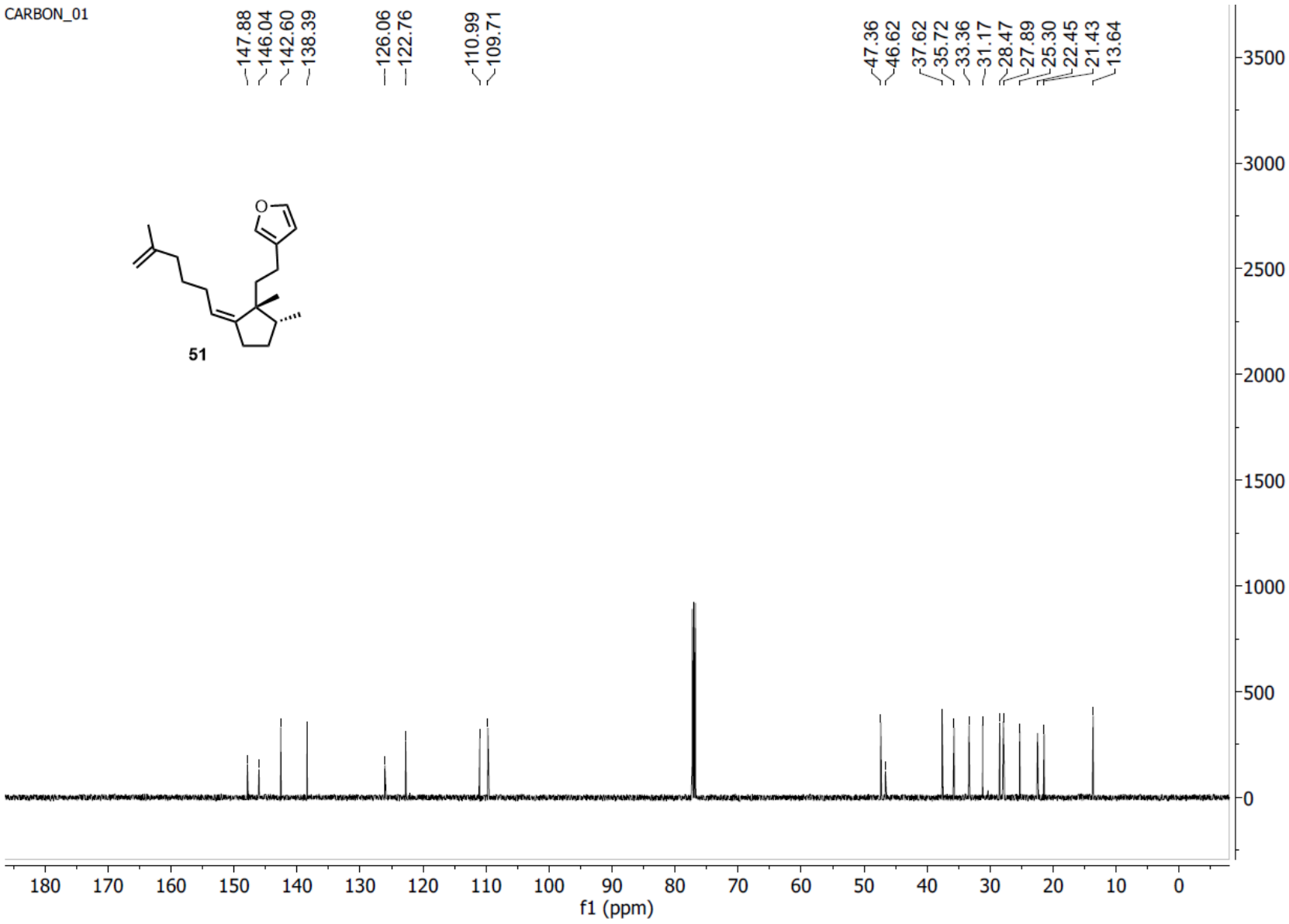

CHO
77
f1 (ppm)

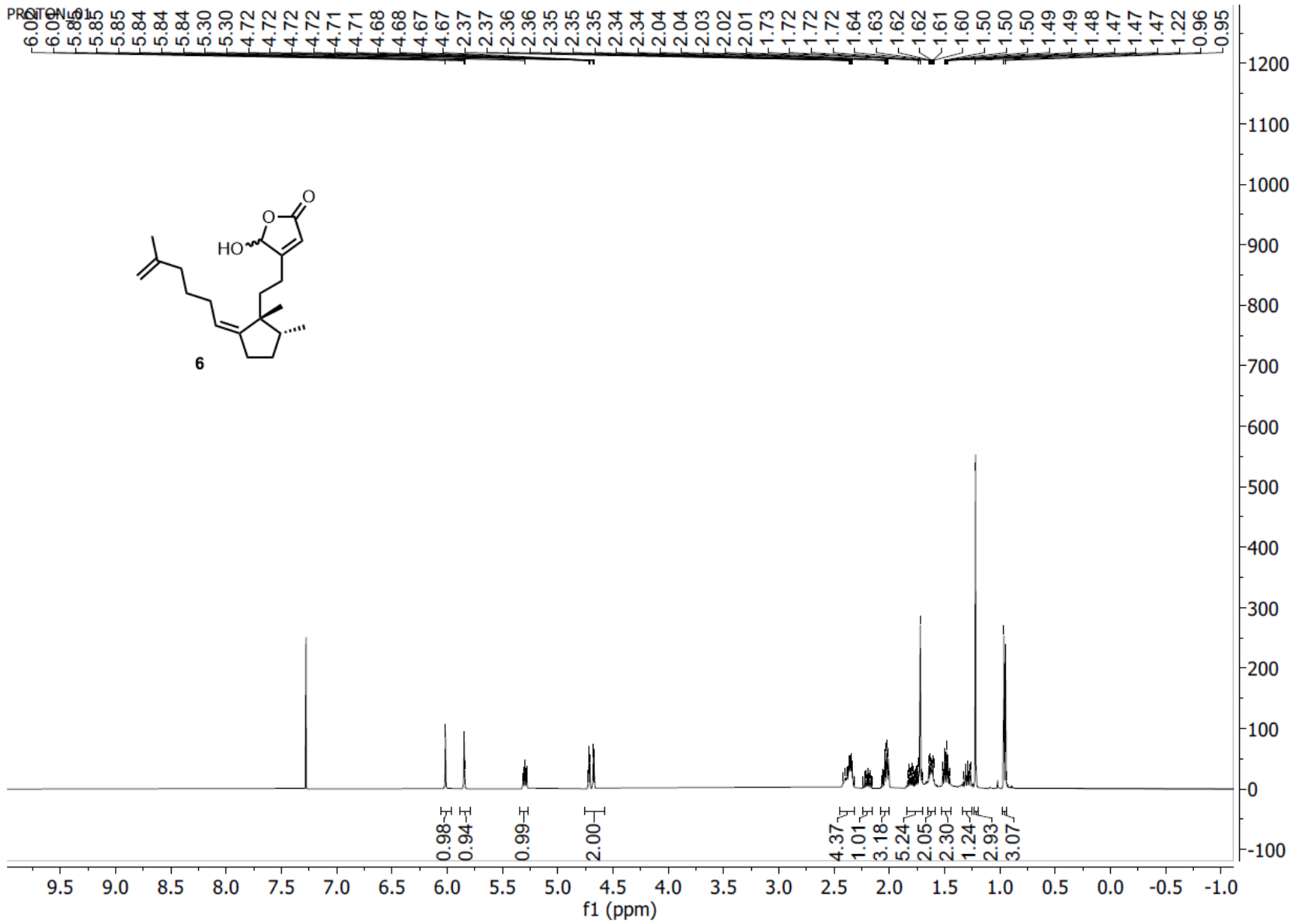

CARBON_01
htf02
204.34
146.75
145.69
123.73
109.90
49.19
47.35
45.55
37.43
33.08
31.05
28.10
27.92
25.60
22.40
14.02
CHO
77
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

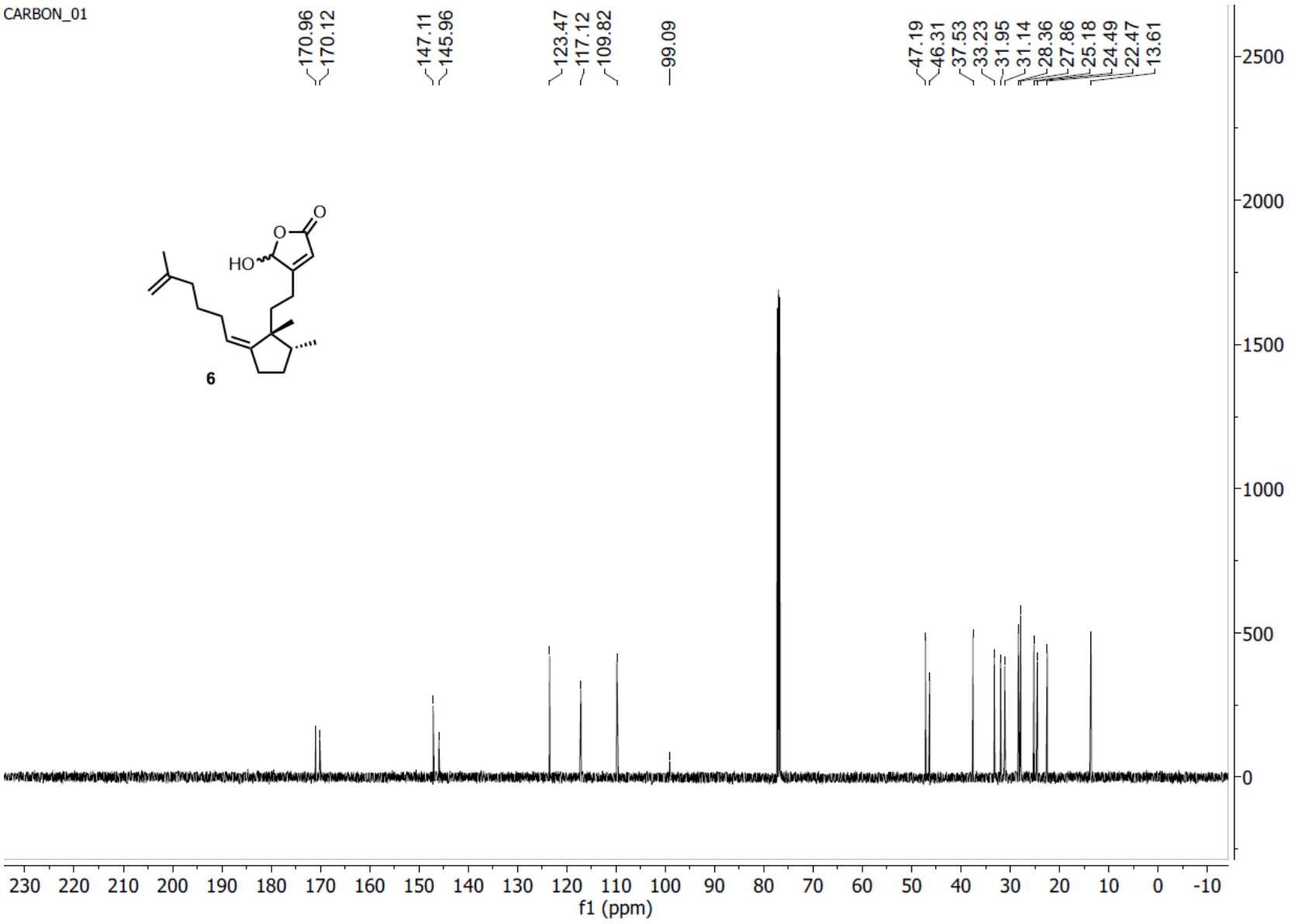

PROTON-01
OH
78
1.84
0.91
1.01
1.03
1.98
3.79
2.12
11.38
3.40
2.95
f1 (ppm)

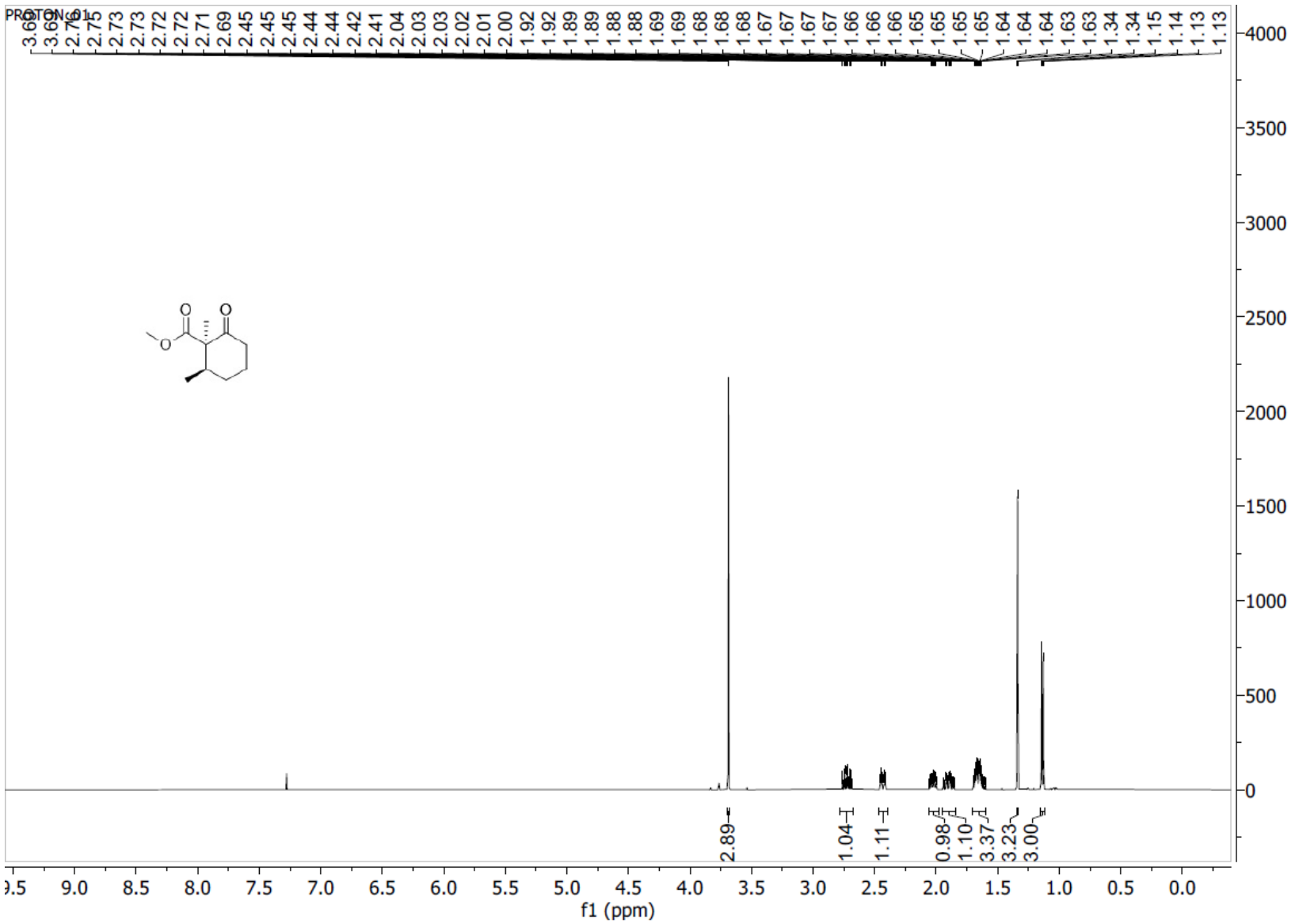

CARBON_01
149.74
149.09
145.88
145.72
143.12
143.07
138.53
138.33
130.54
129.92
124.74
124.57
109.96
109.87
108.57
108.37
65.93
65.05
48.00
47.26
46.54
45.90
43.22
42.69
37.55
37.53
33.46
32.18
31.57
30.32
29.13
28.41
28.16
27.95
27.81
26.86
23.22
22.46
22.42
13.41
13.18
O
OH
78
3000
2500
2000
1500
1000
500
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

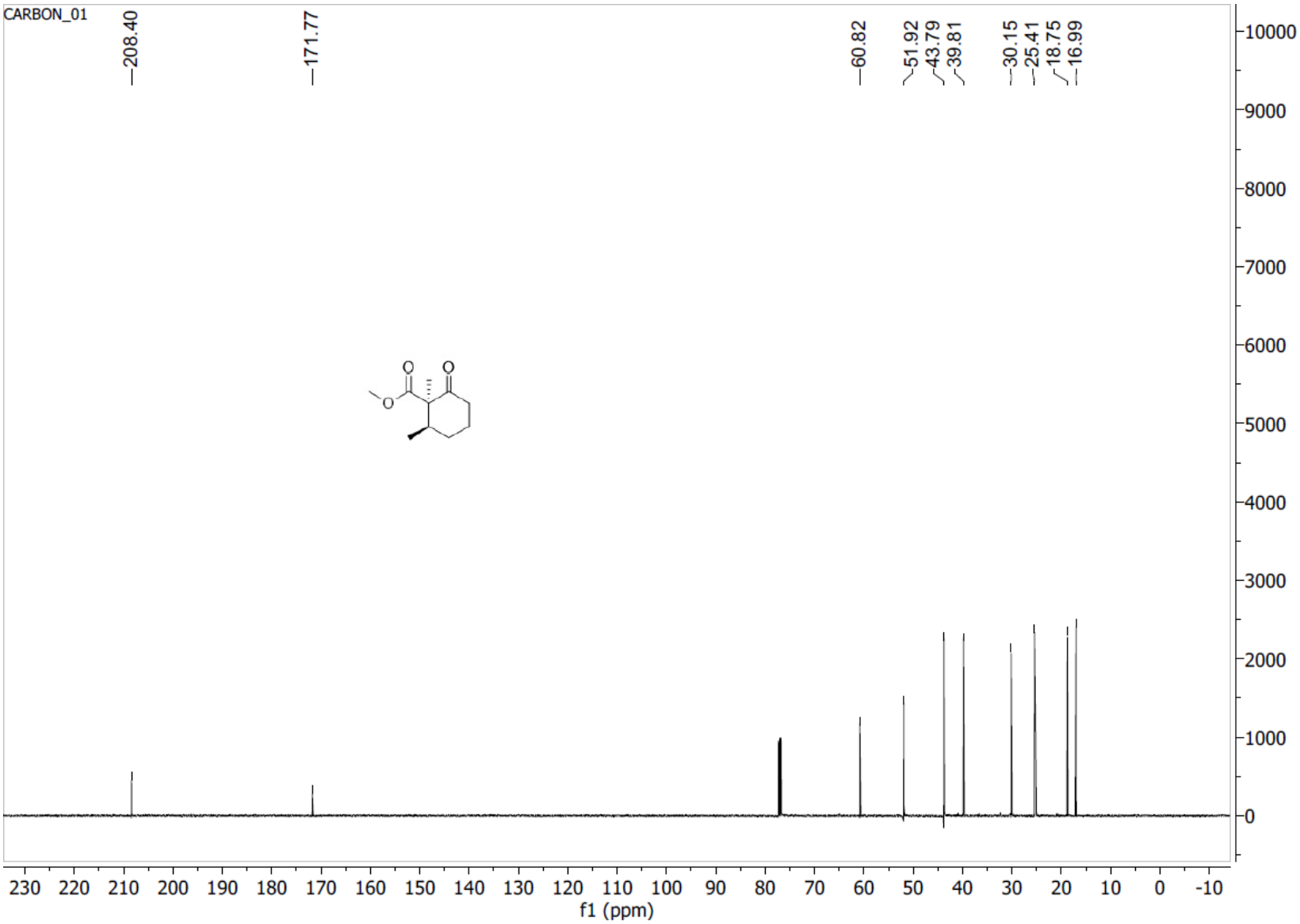

PROTON_01
51
f1 (ppm)

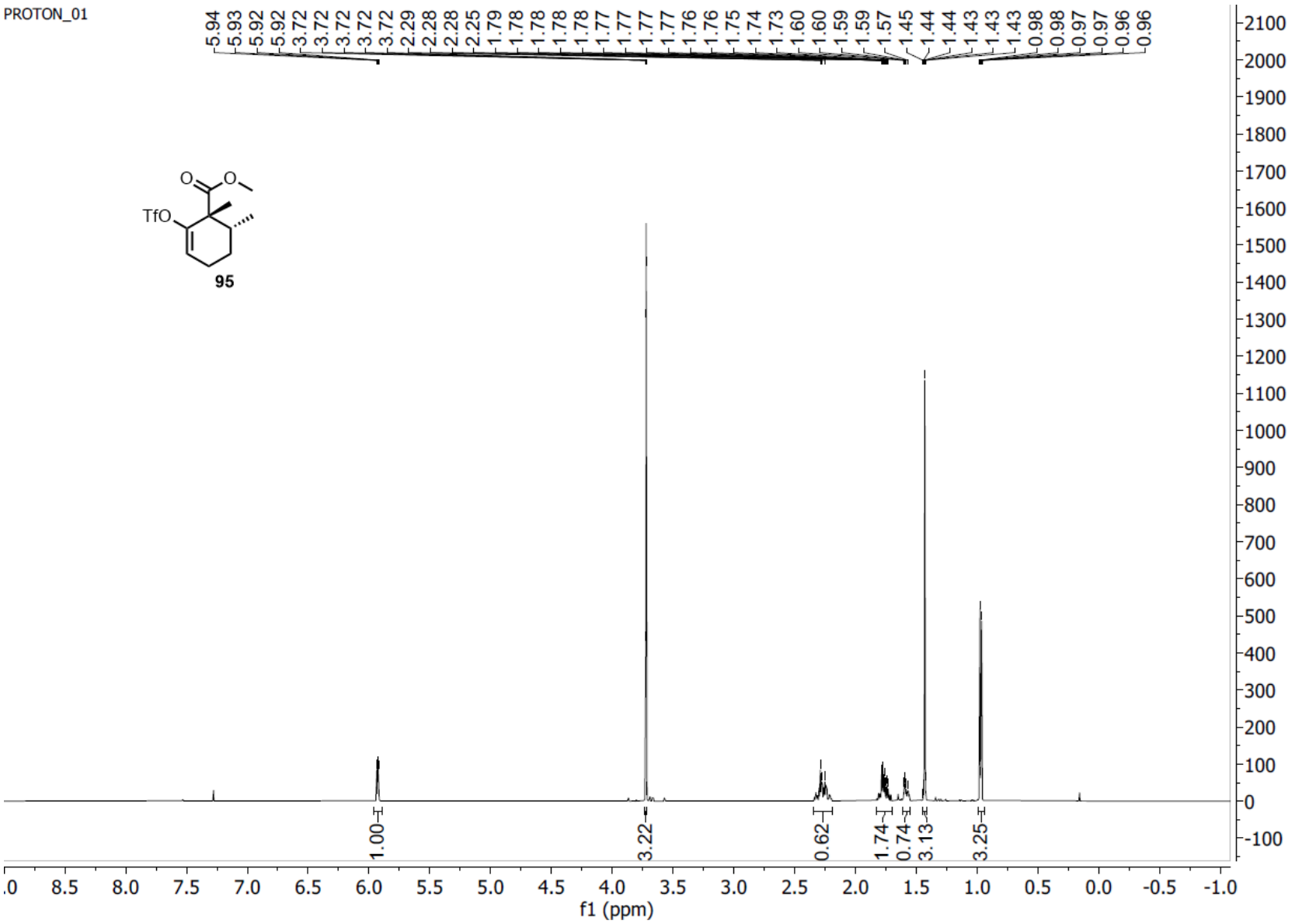

CARBON_01
147.88
146.04
142.60
138.39
126.06
122.76
110.99
109.71
47.36
46.62
37.62
35.72
33.36
31.17
28.47
27.89
25.30
22.45
21.43
13.64
O
51
3500
3000
2500
2000
1500
1000
500
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

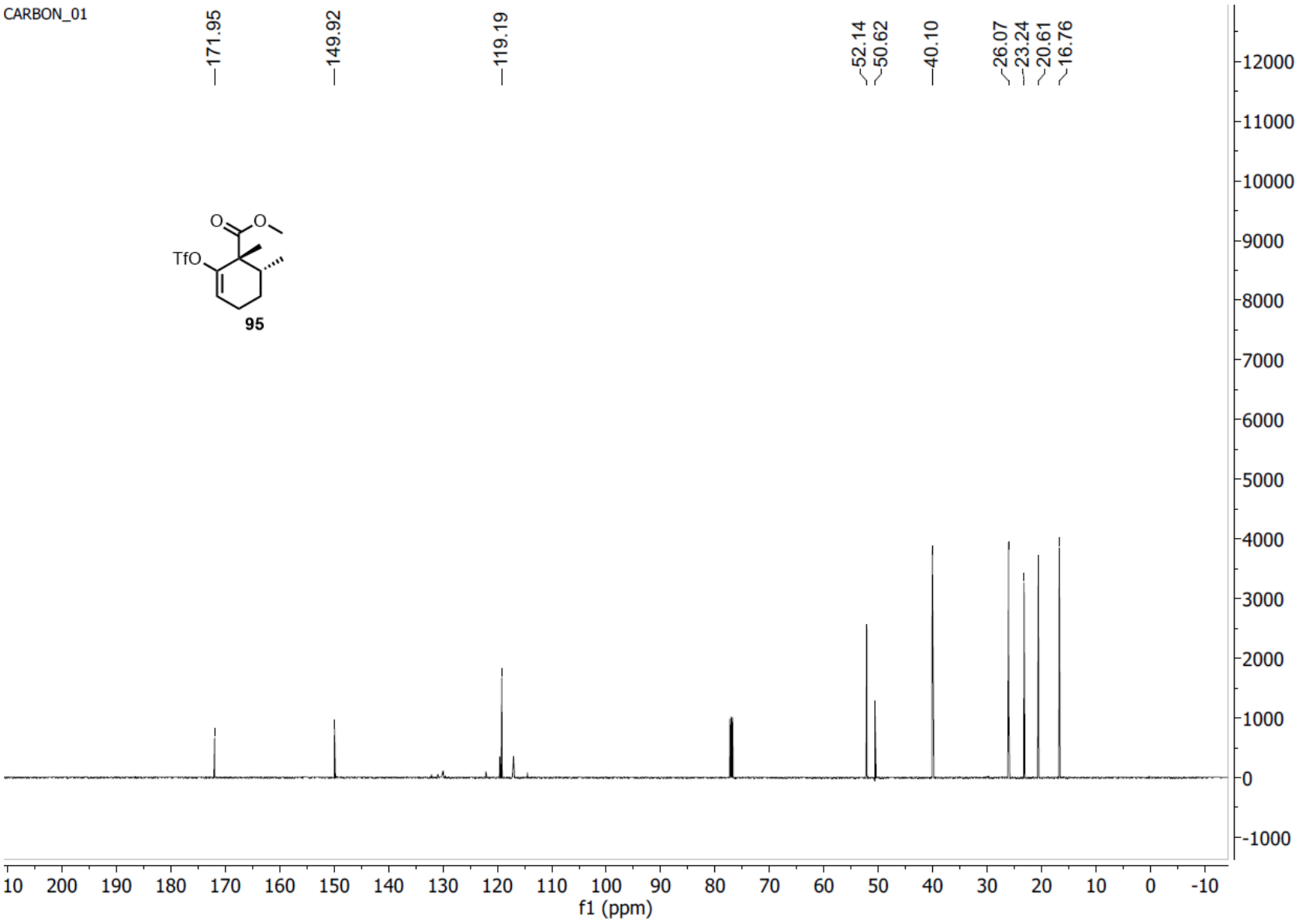

PROTON_01
HO
O
O
6
0.98
0.94
0.99
2.00
4.37
1.01
3.18
5.24
2.05
2.30
1.24
2.93
3.07
f1 (ppm)

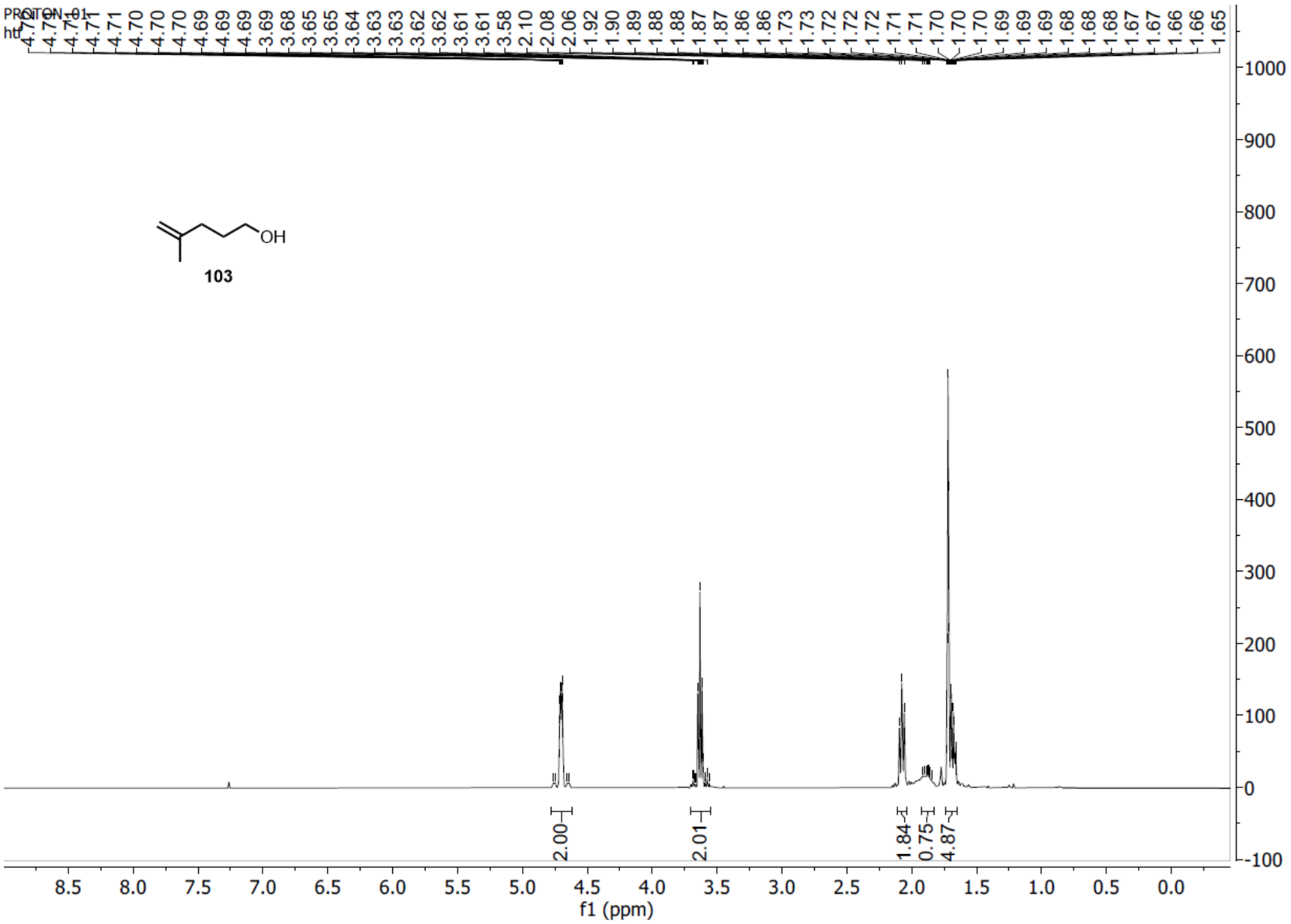

CARBON_01
170.96
170.12
147.11
145.96
123.47
117.12
109.82
99.09
47.19
46.31
37.53
33.23
31.95
31.14
28.36
27.86
25.18
24.49
22.47
13.61
HO
O
O
6
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

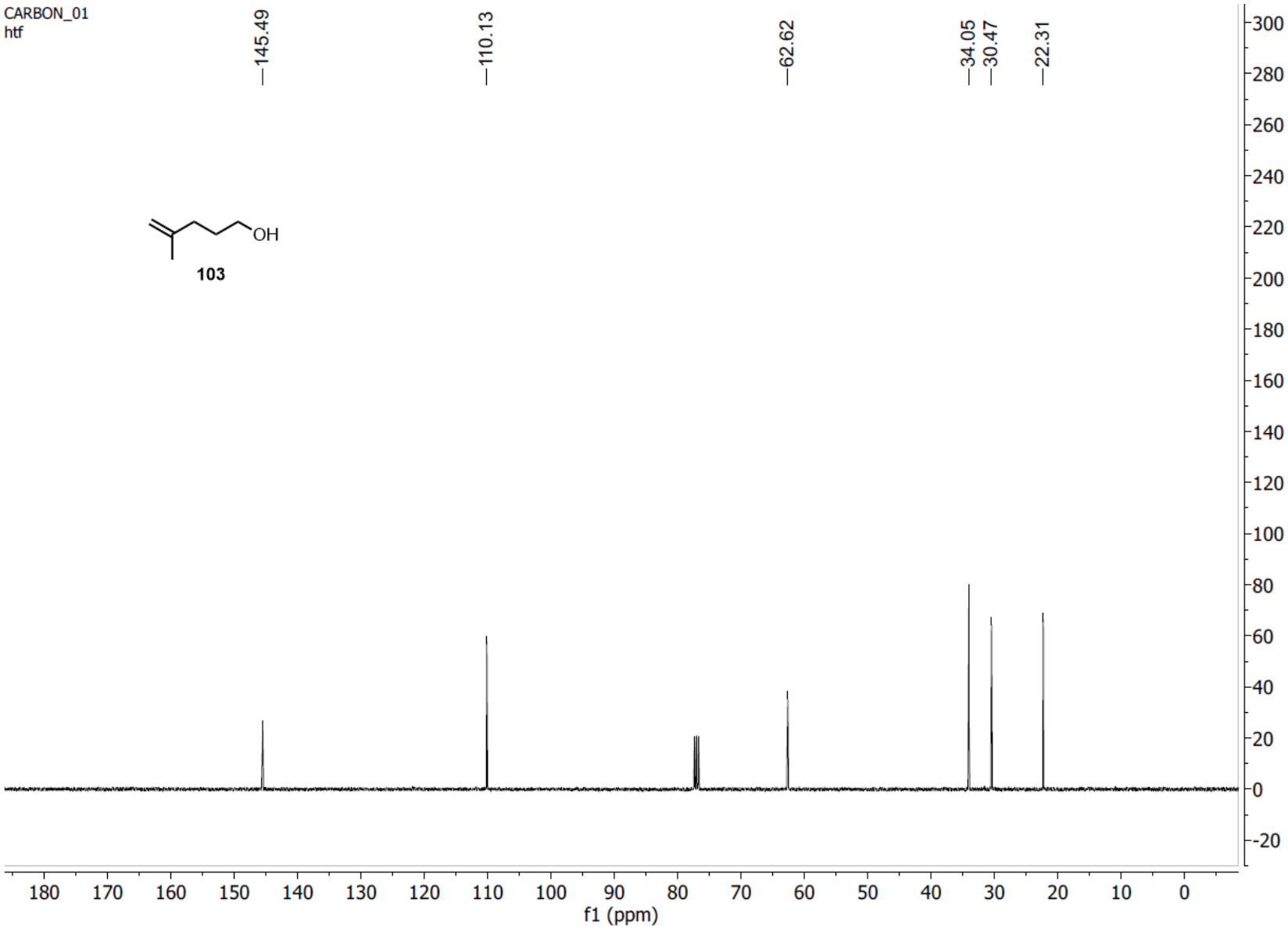

PROTON-01
3.69 3.69 2.76 2.75 2.73 2.73 2.72 2.72 2.71 2.69 2.45 2.45 2.45 2.44 2.44 2.42 2.41 2.04 2.03 2.03 2.02 2.01 2.00 1.92 1.92 1.89 1.89 1.88 1.88 1.69 1.69 1.68 1.68 1.68 1.67 1.67 1.67 1.67 1.66 1.66 1.66 1.65 1.65 1.65 1.65 1.64 1.64 1.64 1.63 1.63 1.34 1.34 1.15 1.14 1.13 1.13
O
O
O
2.89
1.04
1.11
0.98
1.10
3.37
3.23
3.00
4000
3500
3000
2500
2000
1500
1000
500
0
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

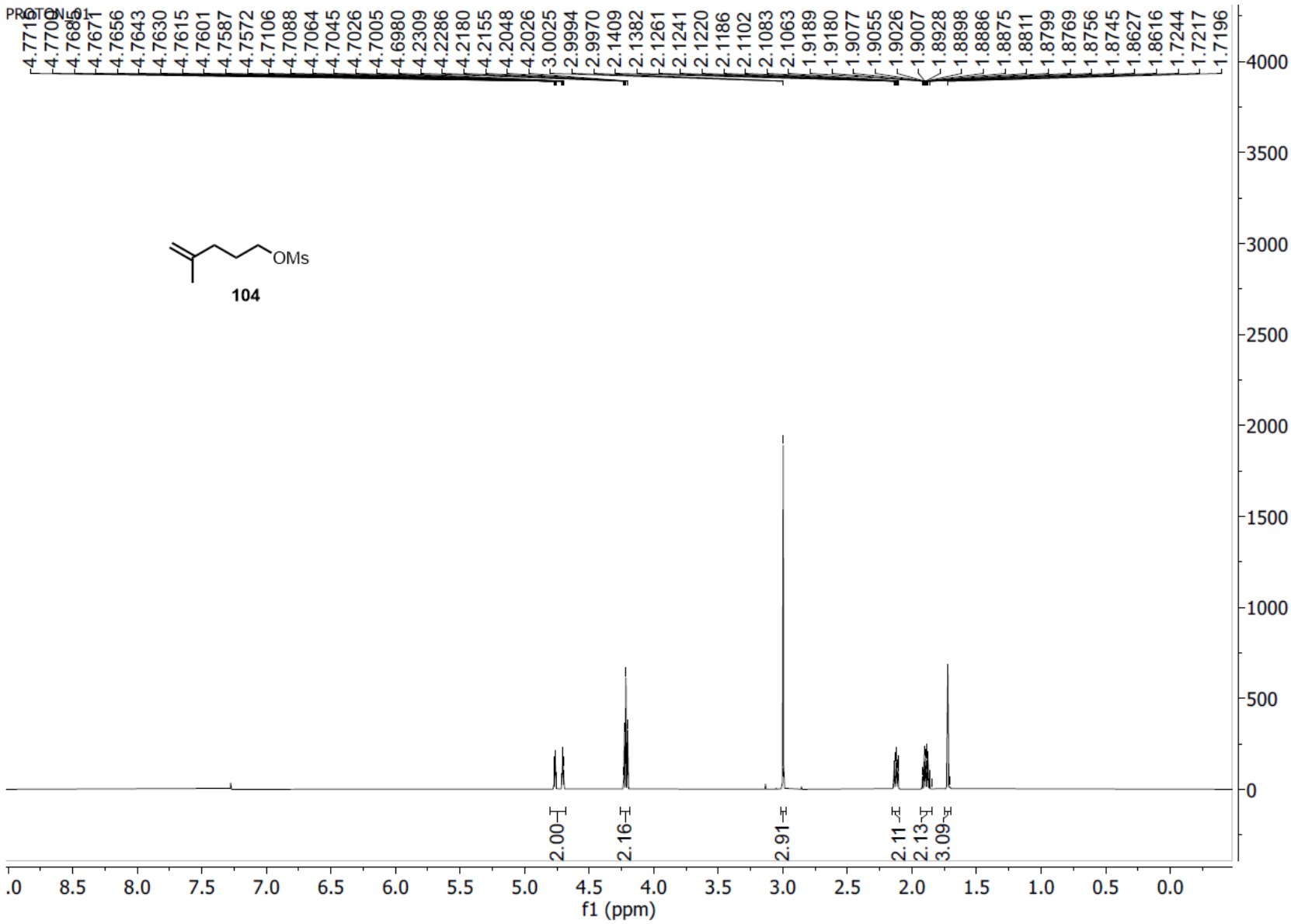

CARBON_01
208.40
171.77
60.82
51.92
43.79
39.81
30.15
25.41
18.75
16.99
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

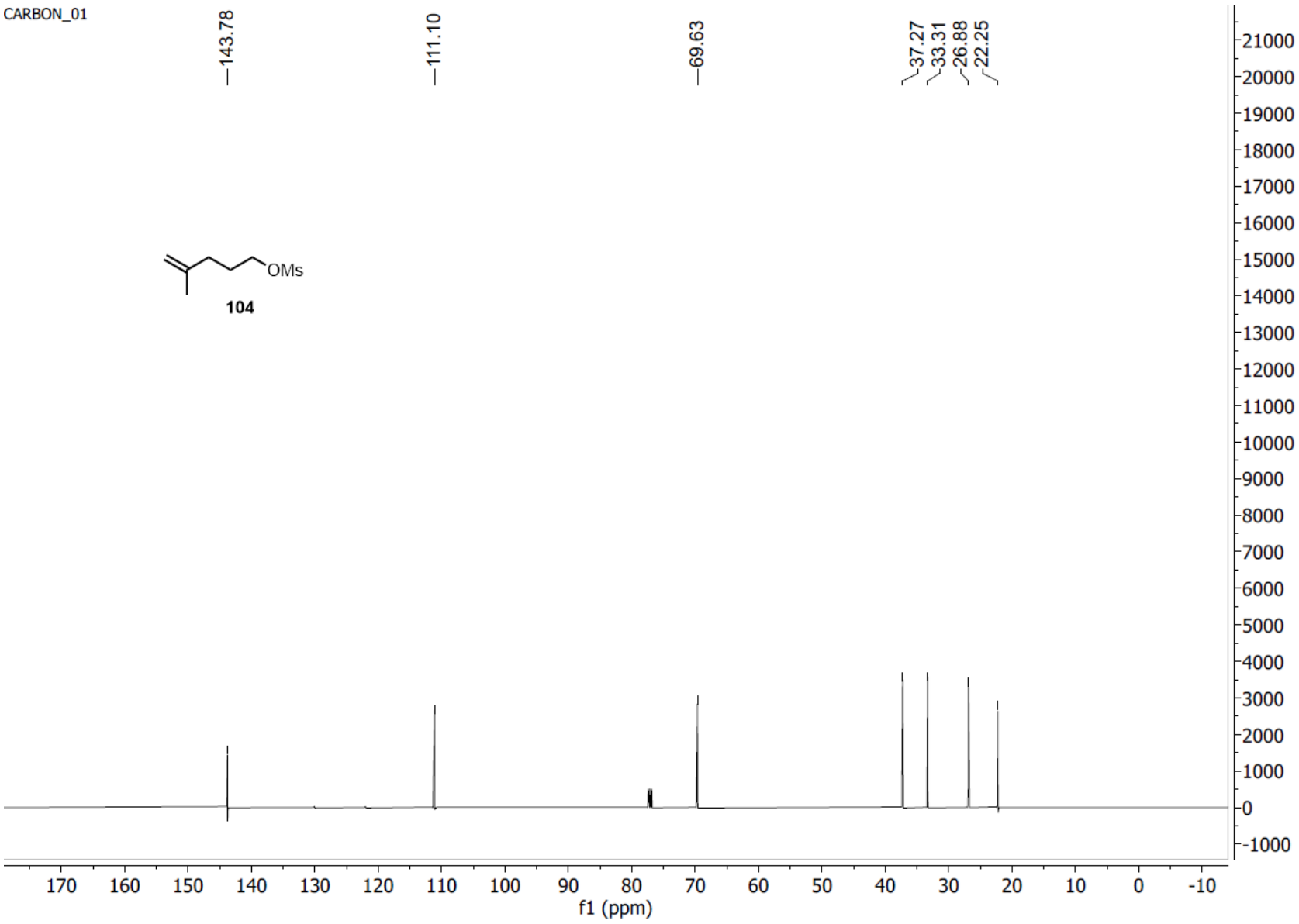

PROTON_01
5.94 5.93 5.92 5.92 3.72 3.72 3.72 3.72 3.72 2.29 2.28 2.28 2.25 1.79 1.78 1.78 1.78 1.78 1.77 1.77 1.77 1.77 1.76 1.76 1.75 1.74 1.73 1.60 1.60 1.59 1.59 1.57 1.45 1.44 1.44 1.43 1.43 1.43 0.98 0.98 0.97 0.97 0.96 0.96
TfO
95
1.00 3.22 0.62 1.74 0.74 3.13 3.25
f1 (ppm)

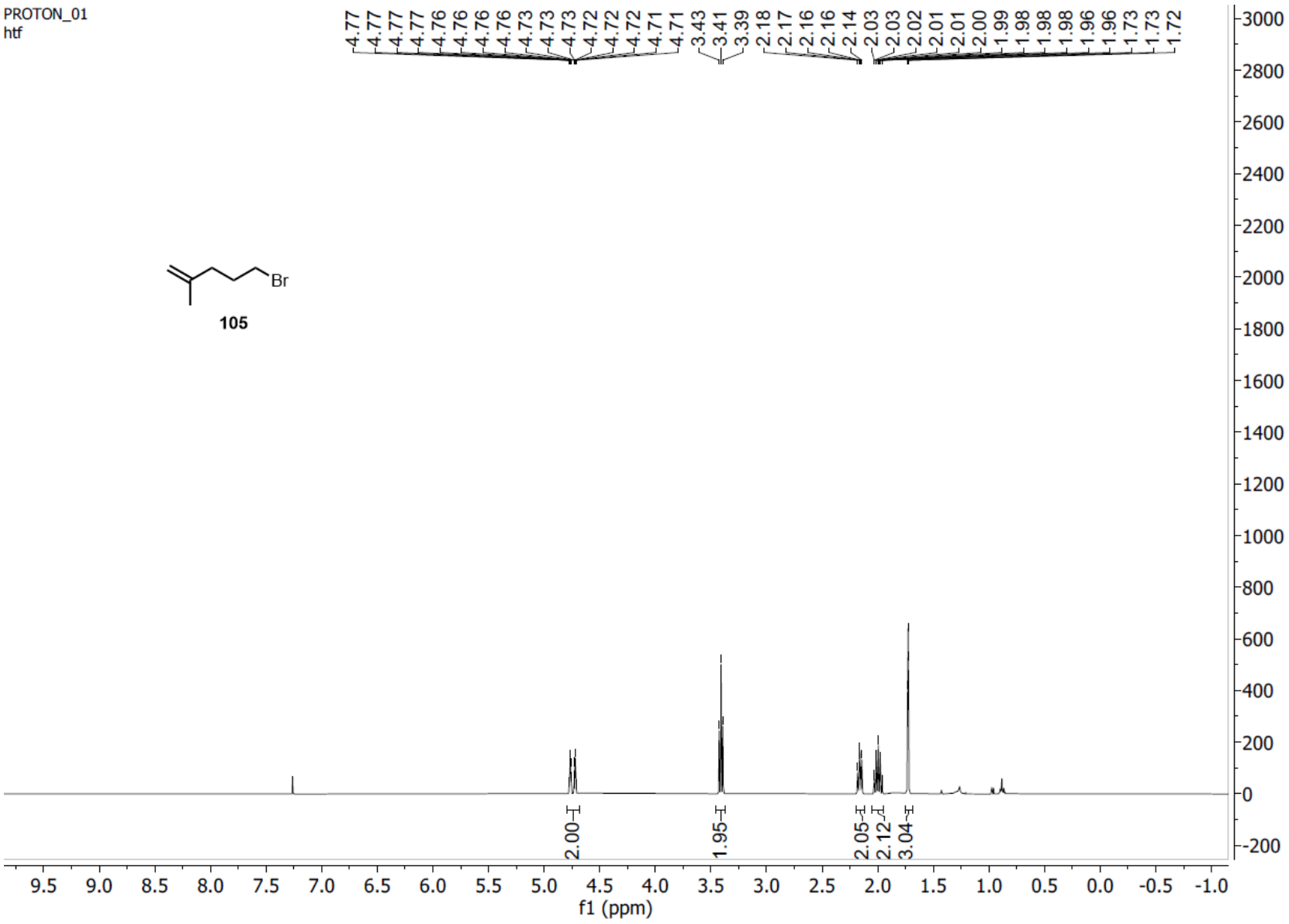

CARBON_01
171.95
149.92
119.19
52.14
50.62
40.10
26.07
23.24
20.61
16.76
TfO
95
f1 (ppm)

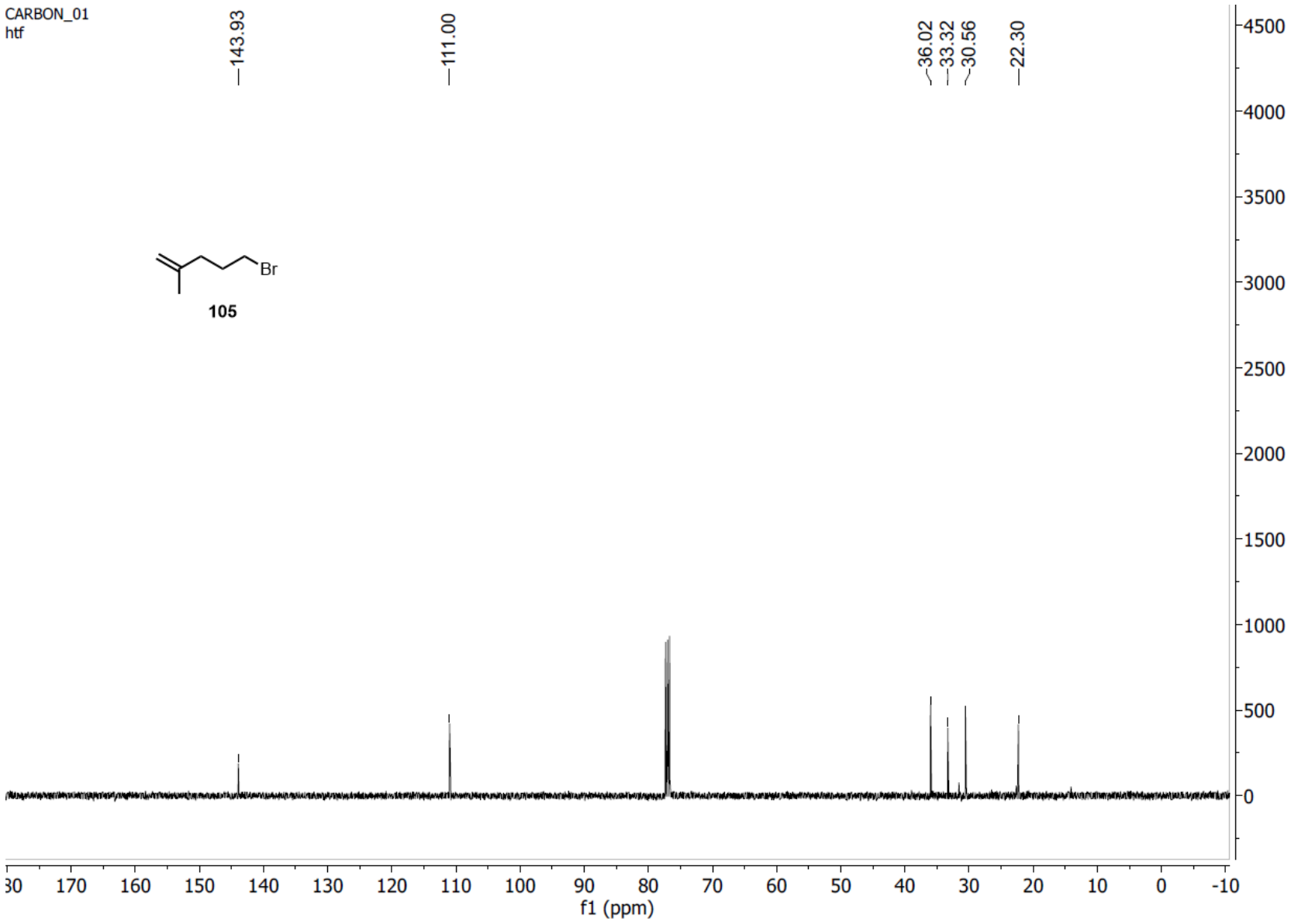

OH
103
2.00
2.01
1.84
0.75
4.87
f1 (ppm)

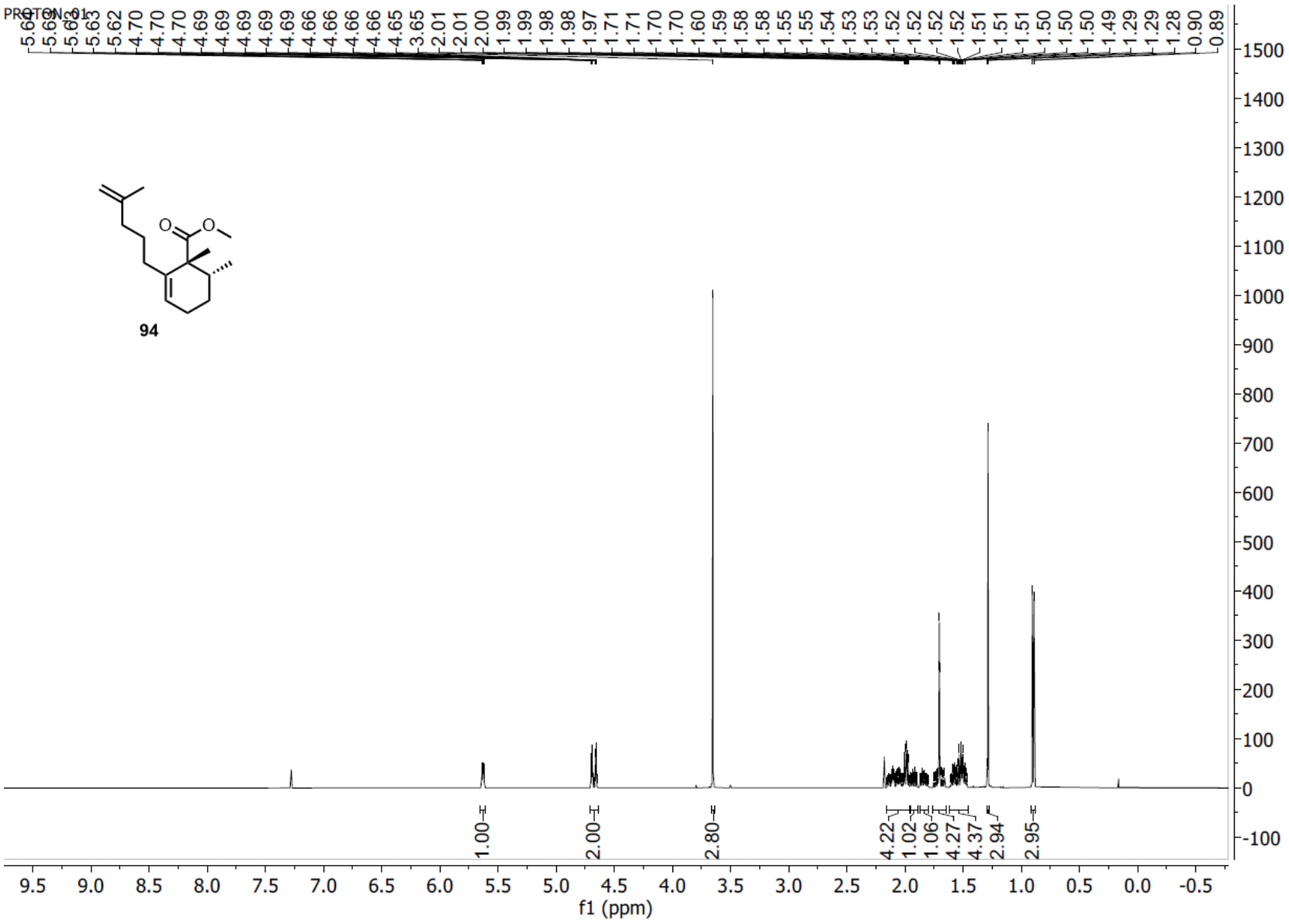

CARBON_01
htf
145.49
110.13
62.62
34.05
30.47
22.31
OH
103
f1 (ppm)

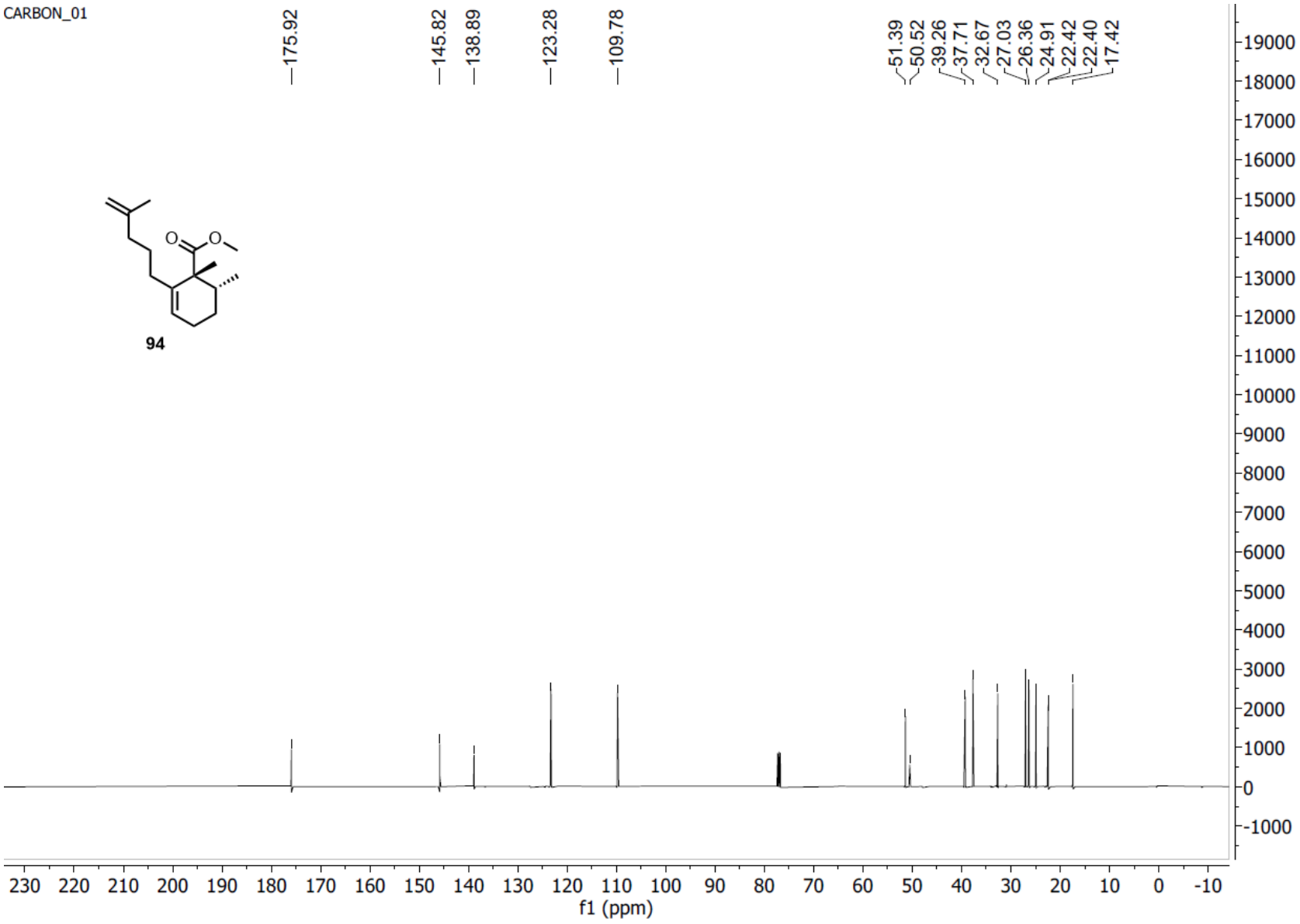

PROTON_01
4.7710
4.7700
4.7685
4.7671
4.7656
4.7643
4.7630
4.7615
4.7601
4.7587
4.7572
4.7106
4.7088
4.7064
4.7045
4.7026
4.7005
4.6980
4.2309
4.2286
4.2180
4.2155
4.2048
4.2026
3.0025
2.9994
2.9970
2.1409
2.1382
2.1261
2.1241
2.1220
2.1186
2.1102
2.1083
2.1063
1.9189
1.9180
1.9077
1.9055
1.9026
1.9007
1.8928
1.8898
1.8886
1.8875
1.8811
1.8799
1.8769
1.8756
1.8745
1.8627
1.8616
1.7244
1.7217
1.7196
OMs
104
2.00
2.16
2.91
2.11
2.13
3.09
f1 (ppm)

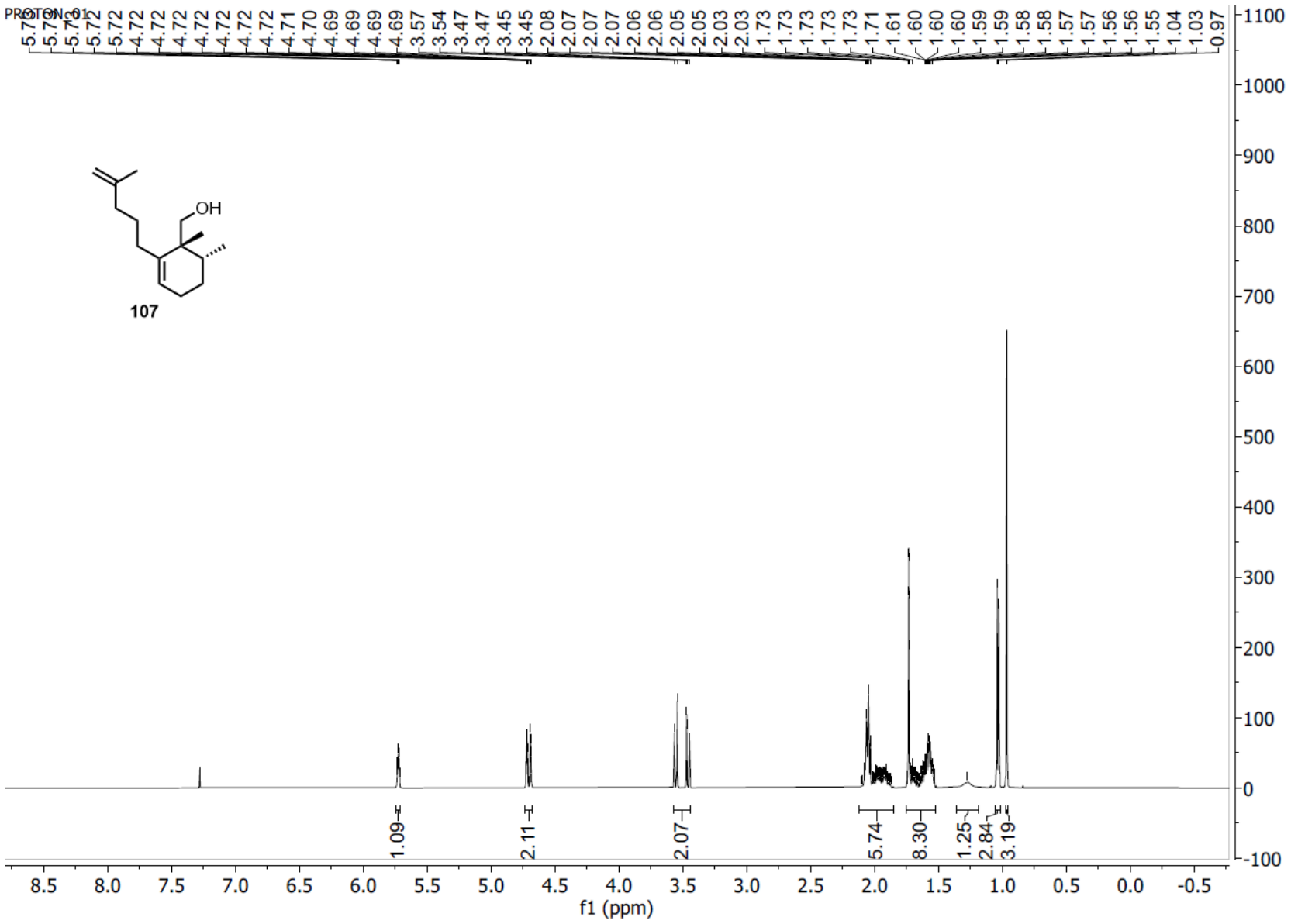

CARBON_01
143.78
111.10
69.63
37.27
33.31
26.88
22.25
OMs
104
21000
20000
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

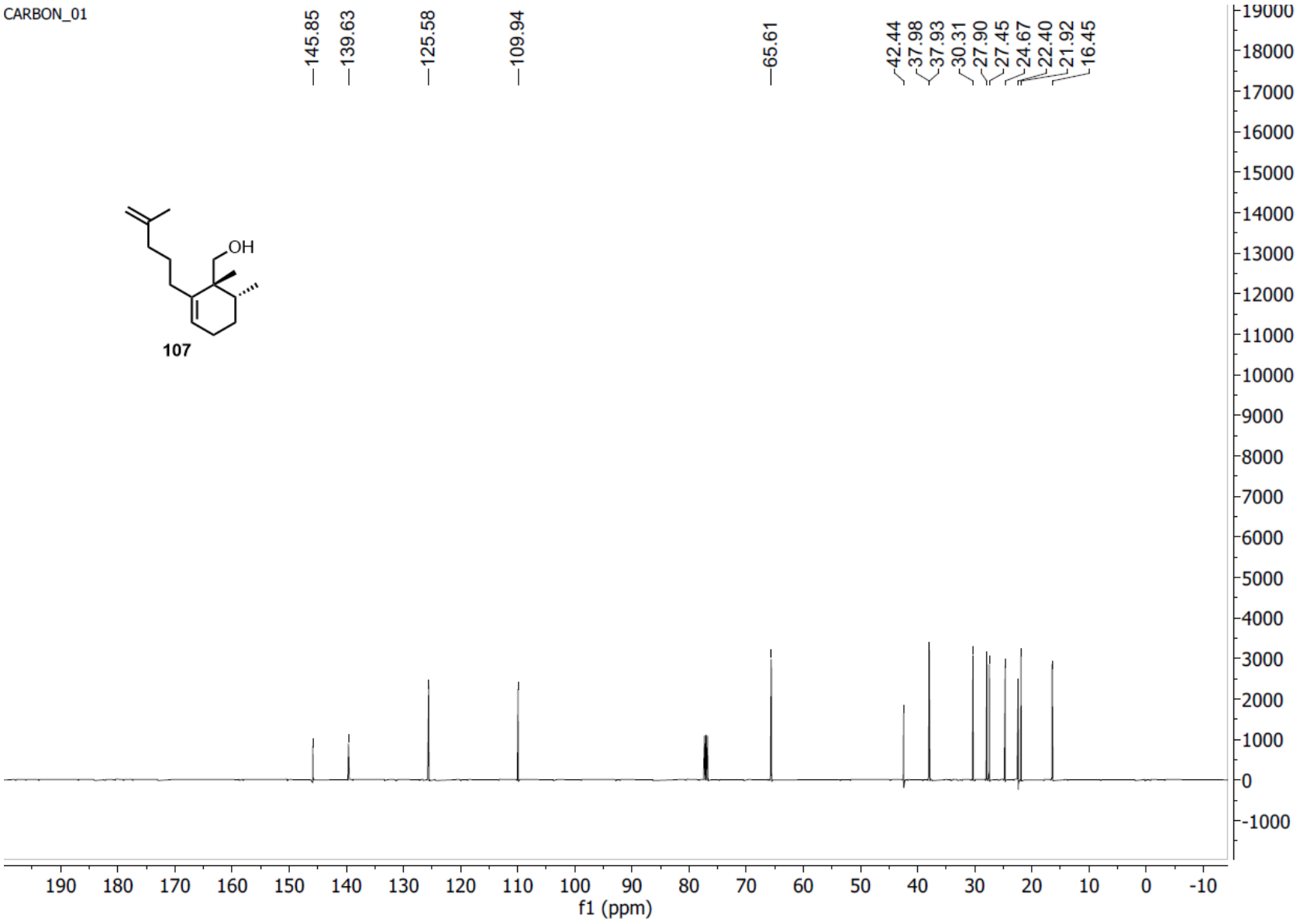

PROTON_01
htf
4.77 4.77 4.77 4.77 4.76 4.76 4.76 4.76 4.73 4.73 4.73 4.72 4.72 4.72 4.71 4.71
3.43 3.41 3.39
2.18 2.17 2.16 2.16 2.14 2.03 2.03 2.02 2.01 2.01 2.00 1.99 1.98 1.98 1.98 1.96 1.96 1.73 1.73 1.72
Br
105
2.00
1.95
2.05
2.12
3.04
f1 (ppm)

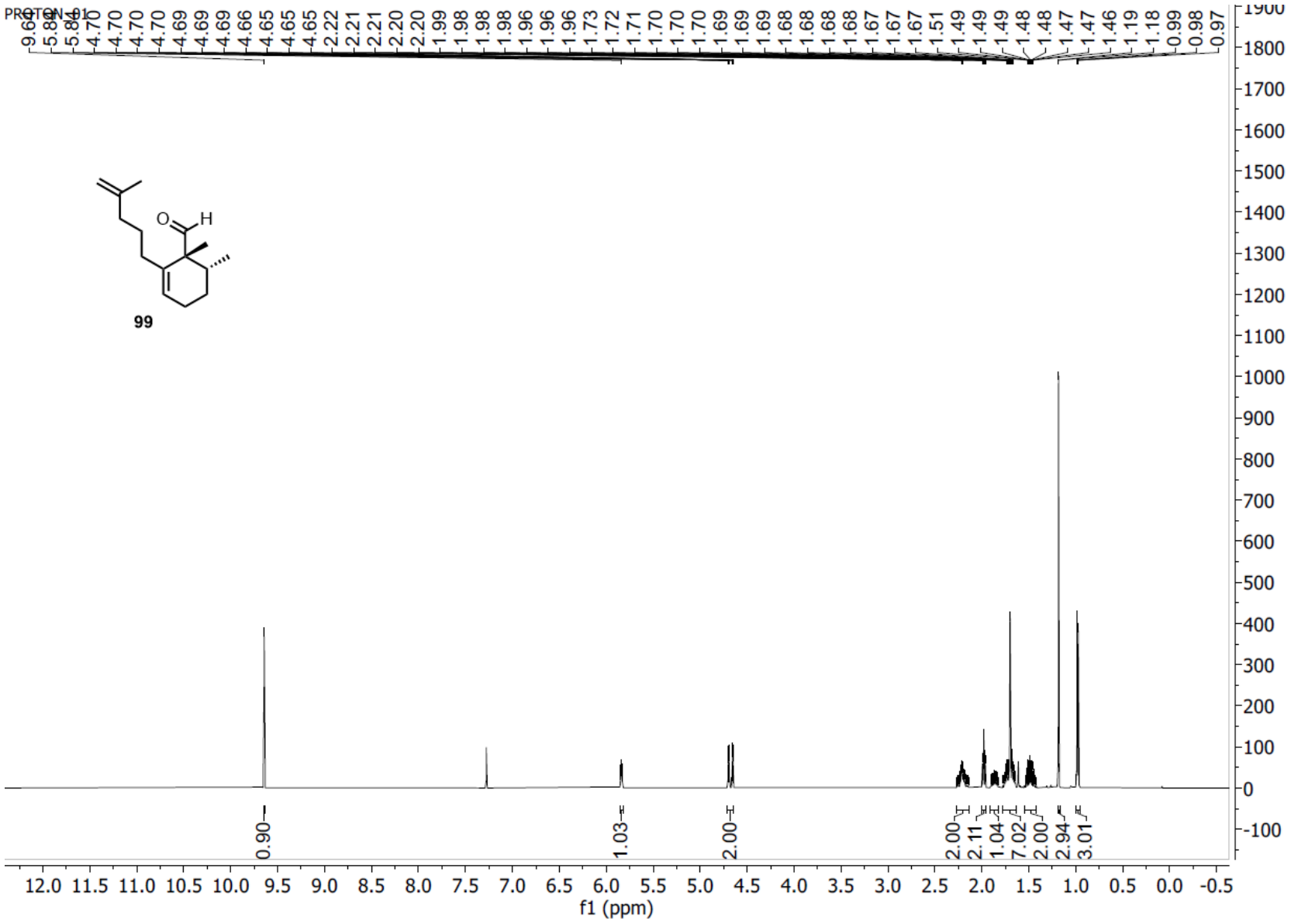

CARBON_01
htf
143.93
111.00
36.02
33.32
30.56
22.30
Br
105
4500
4000
3500
3000
2500
2000
1500
1000
500
0
f1 (ppm)

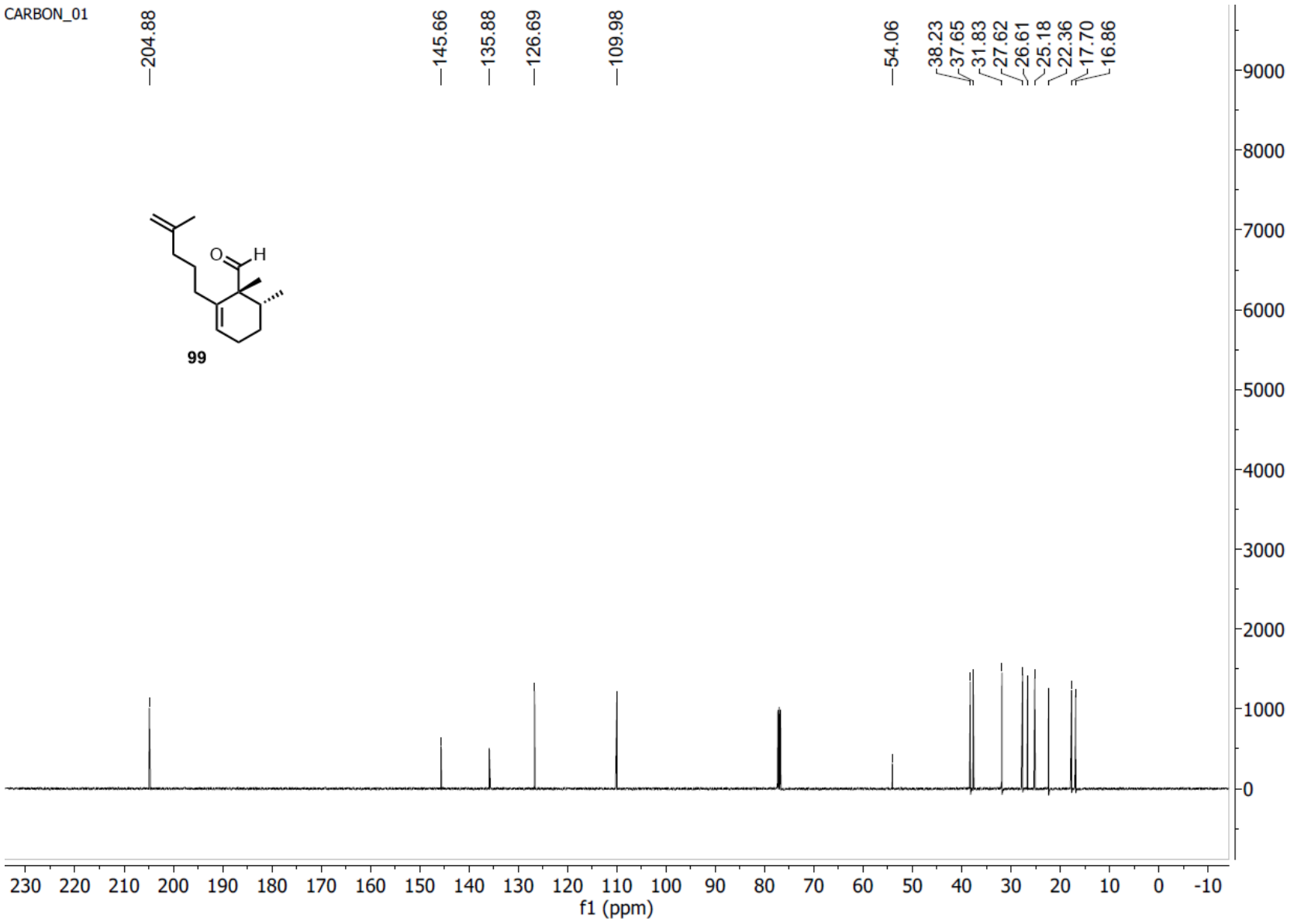

PROTON_01
94
1.00
2.00
2.80
4.22
1.02
1.06
4.27
4.37
2.94
2.95
f1 (ppm)

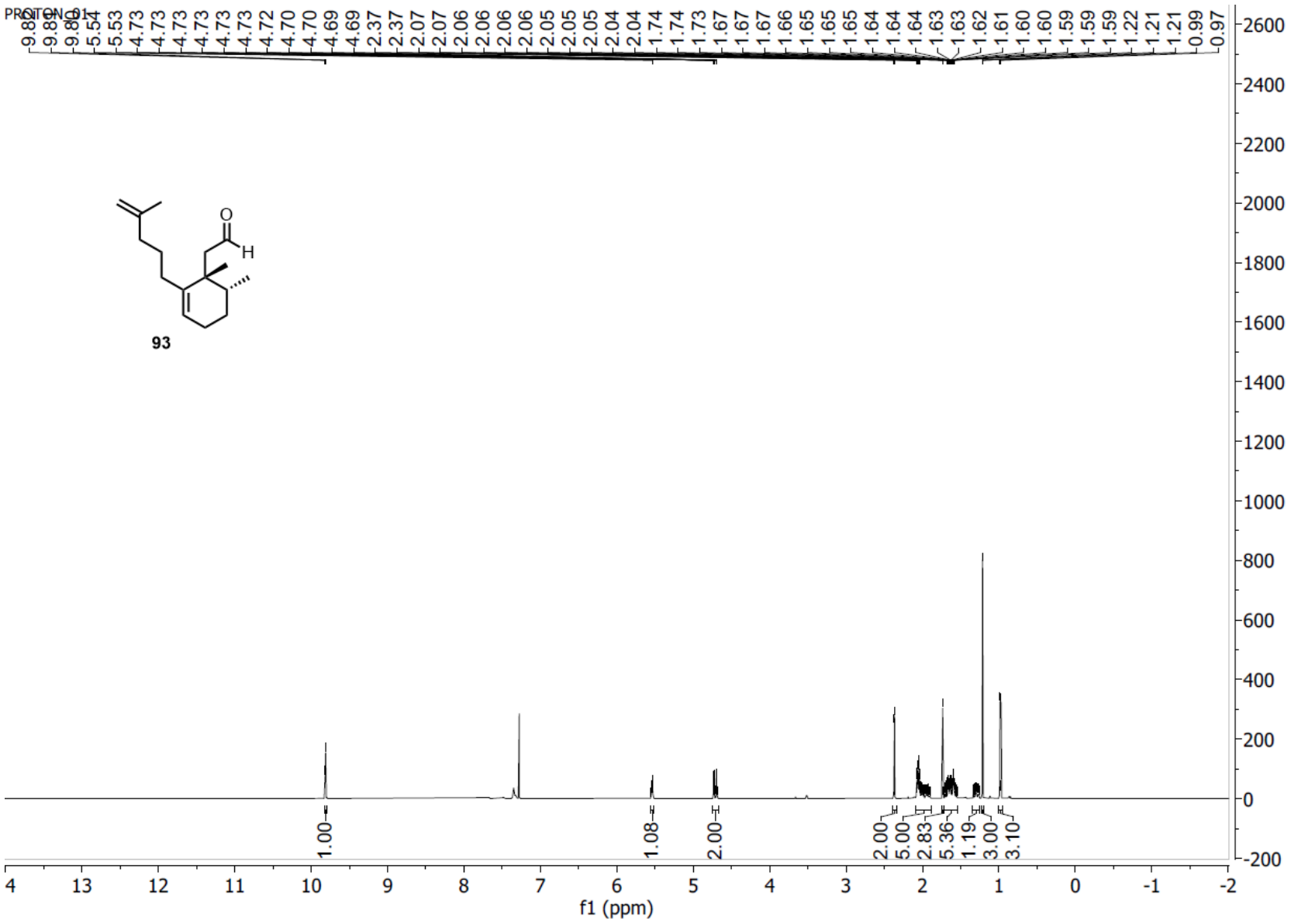

CARBON_01
175.92
145.82
138.89
123.28
109.78
51.39
50.52
39.26
37.71
32.67
27.03
26.36
24.91
22.42
22.40
17.42
O
O
94
19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

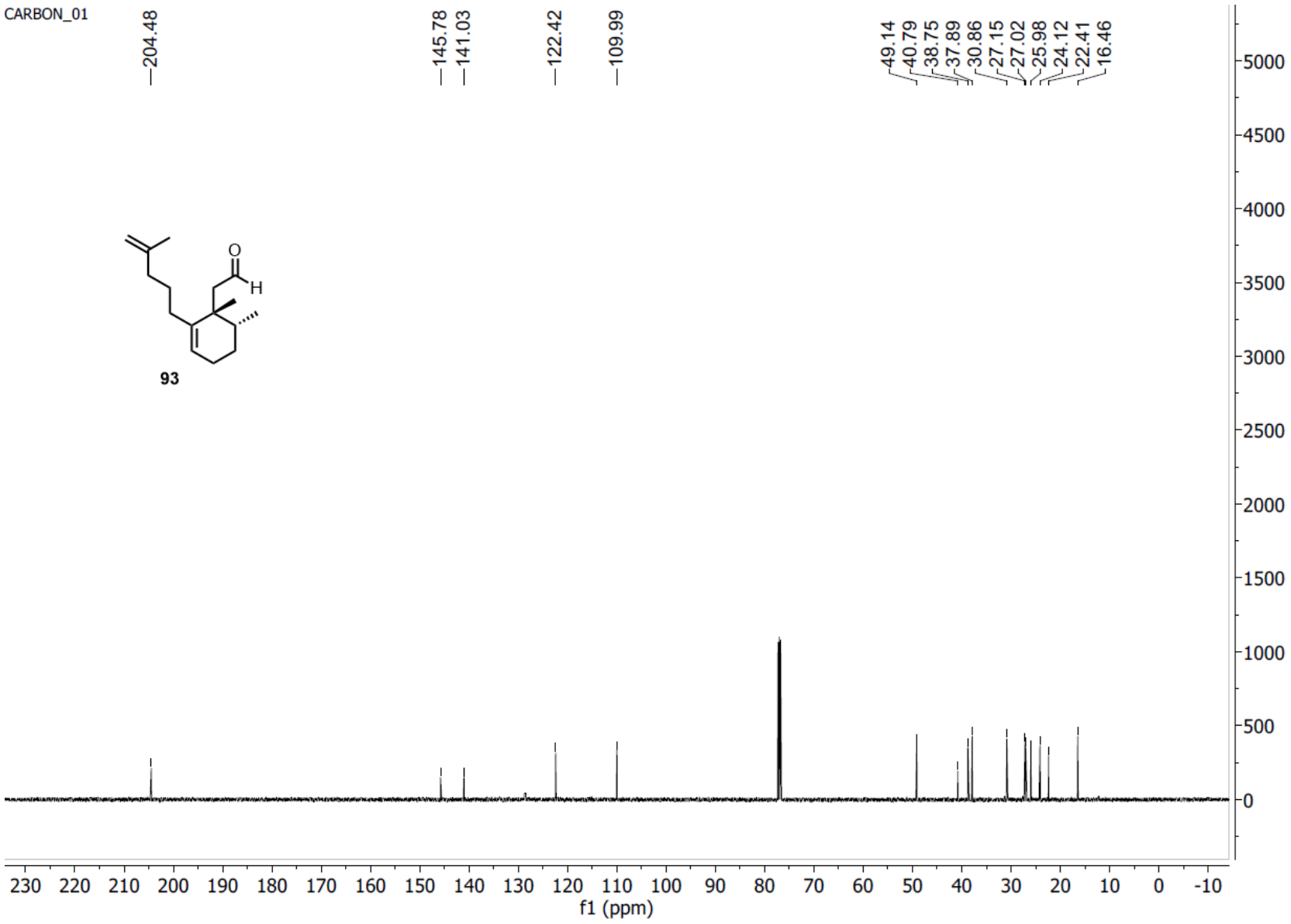

PROTON_01
OH
107
1.09
2.11
2.07
5.74
8.30
1.25
2.84
3.19
f1 (ppm)

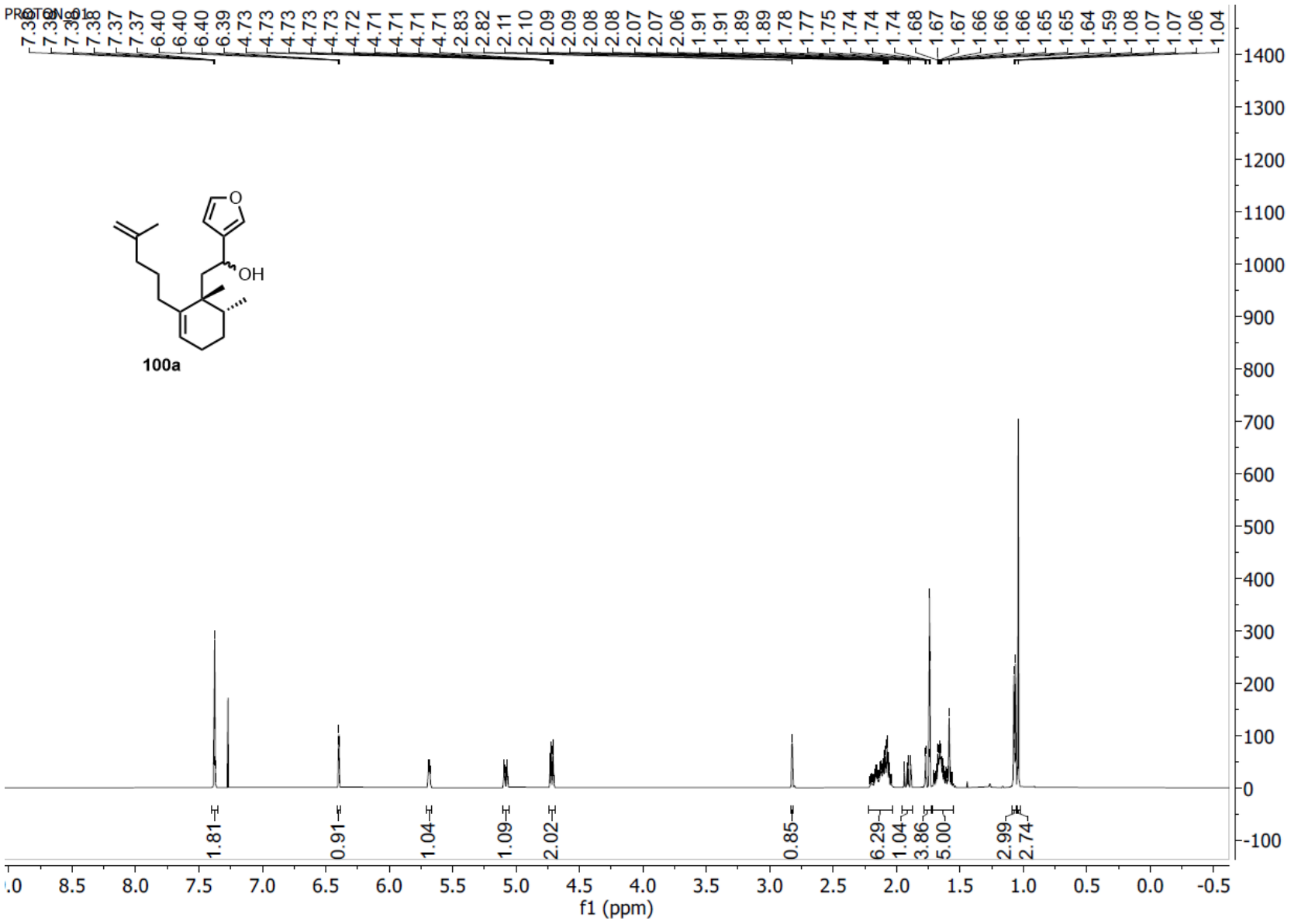

CARBON_01
145.85
139.63
125.58
109.94
65.61
42.44
37.98
37.93
30.31
27.90
27.45
24.67
22.40
21.92
16.45
OH
107
f1 (ppm)

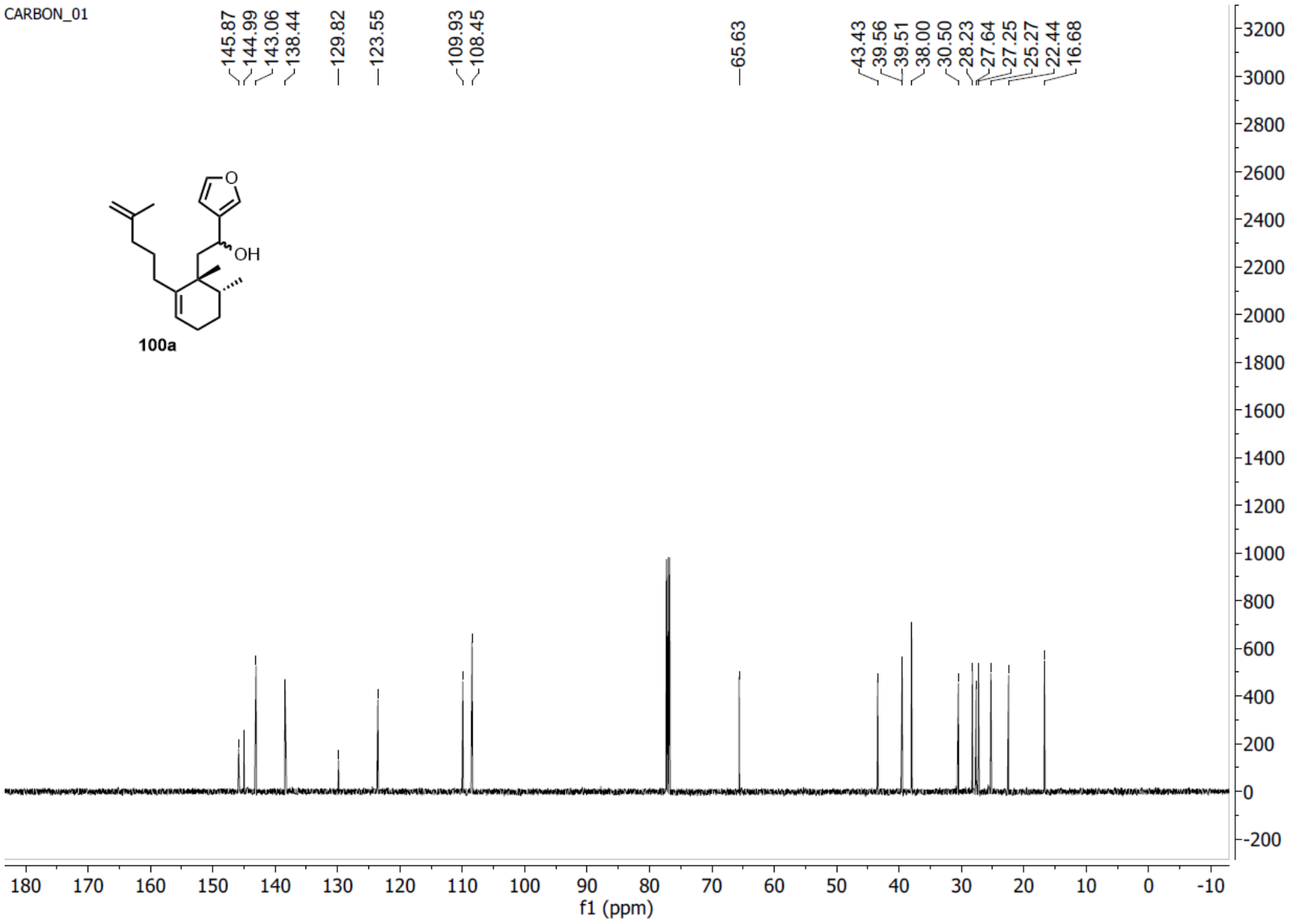

PROTON-01
O
H
99
0.90
1.03
2.00
2.00
2.11
1.04
7.02
2.00
2.94
3.01
f1 (ppm)

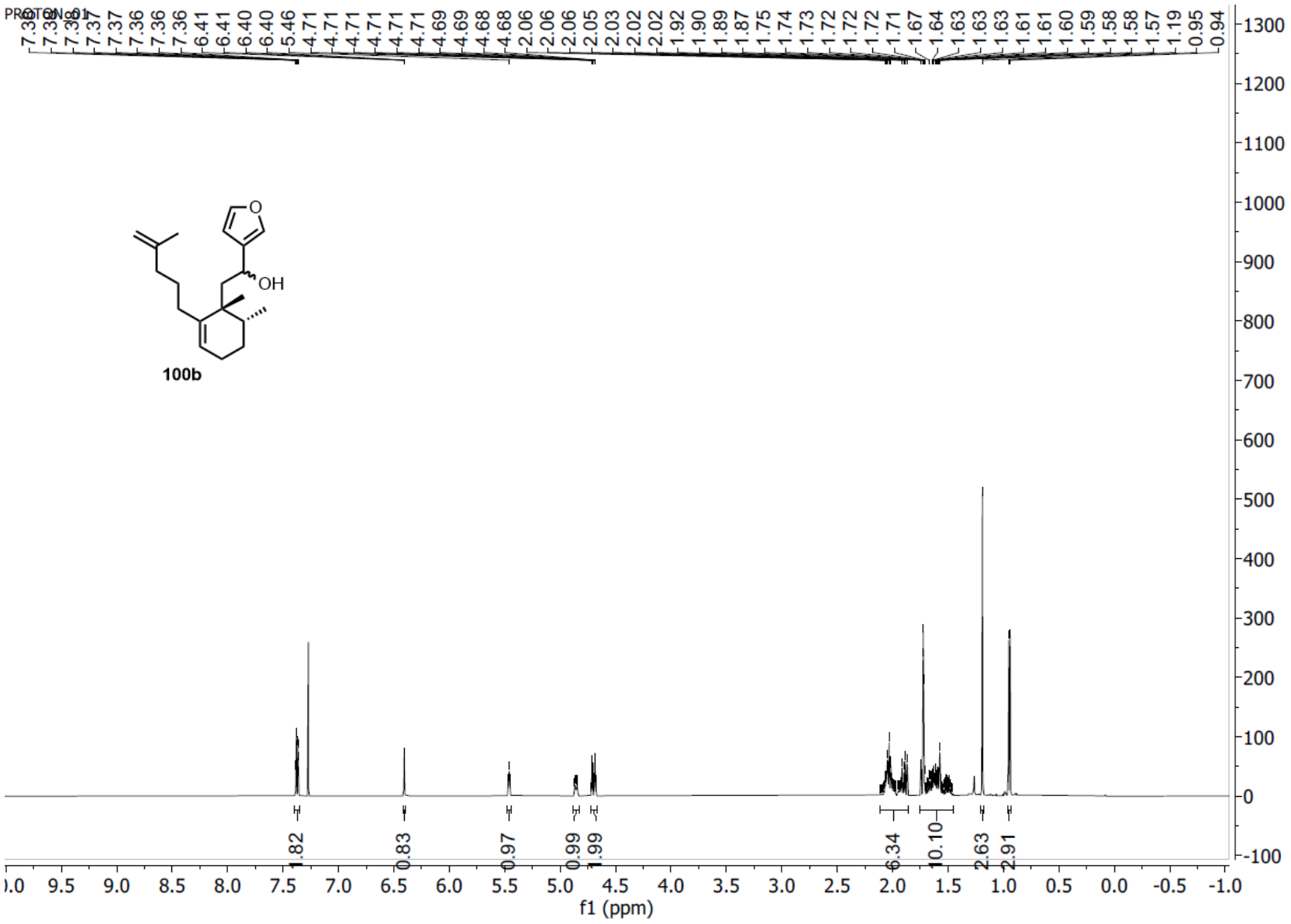

CARBON_01
204.88
145.66
135.88
126.69
109.98
54.06
38.23
37.65
31.83
27.62
26.61
25.18
22.36
17.70
16.86
O
H
99
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

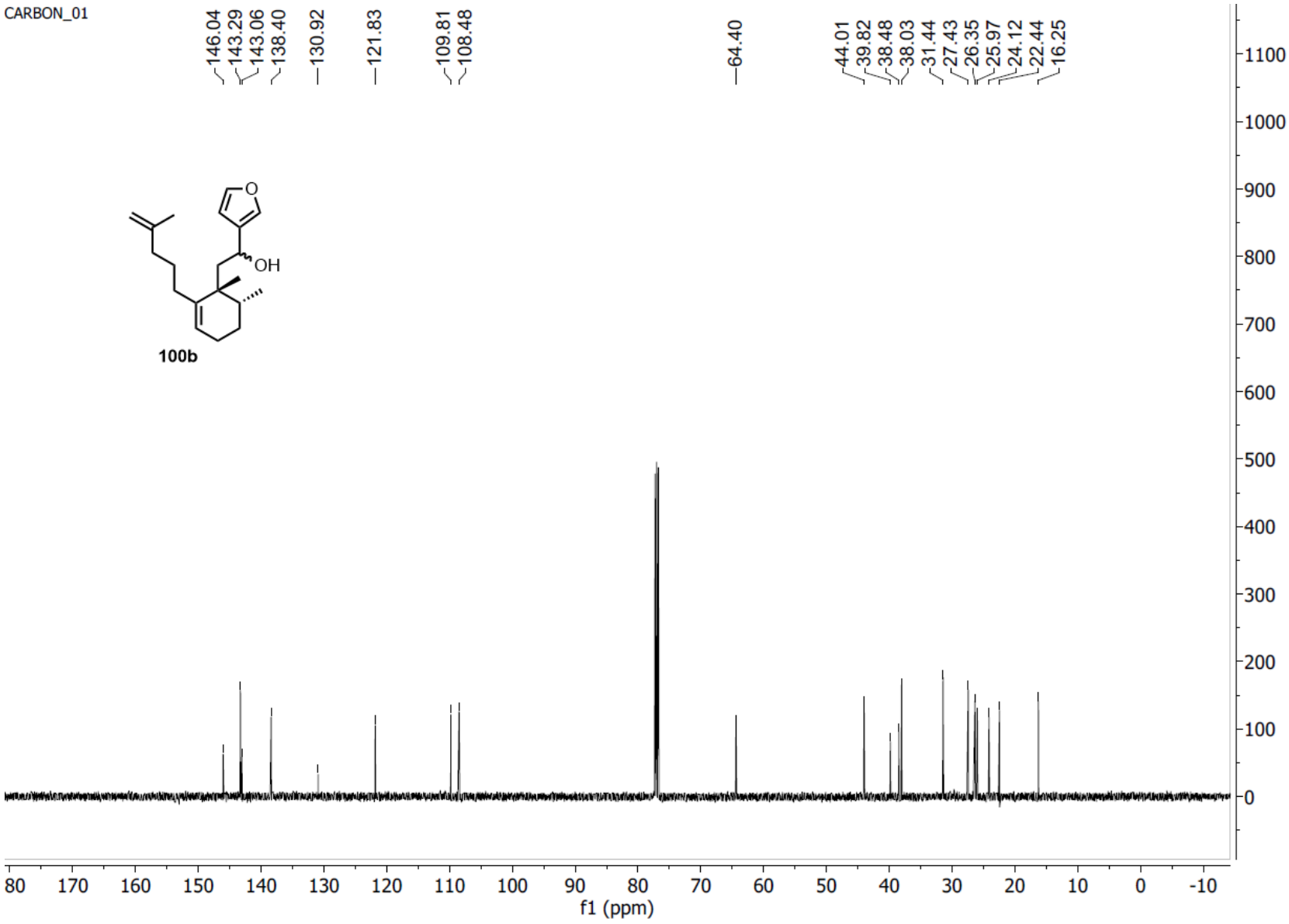

PROTON_01
9.82
9.81
9.80
5.54
5.53
4.73
4.73
4.73
4.73
4.73
4.73
4.72
4.70
4.70
4.69
4.69
2.37
2.37
2.07
2.07
2.06
2.06
2.06
2.06
2.05
2.05
2.05
2.04
2.04
1.74
1.74
1.73
1.67
1.67
1.67
1.66
1.65
1.65
1.65
1.64
1.64
1.64
1.63
1.63
1.62
1.61
1.60
1.60
1.59
1.59
1.59
1.22
1.21
1.21
0.99
0.97
O
H
93
1.00
1.08
2.00
2.00
5.00
2.83
5.36
1.19
3.00
3.10
f1 (ppm)

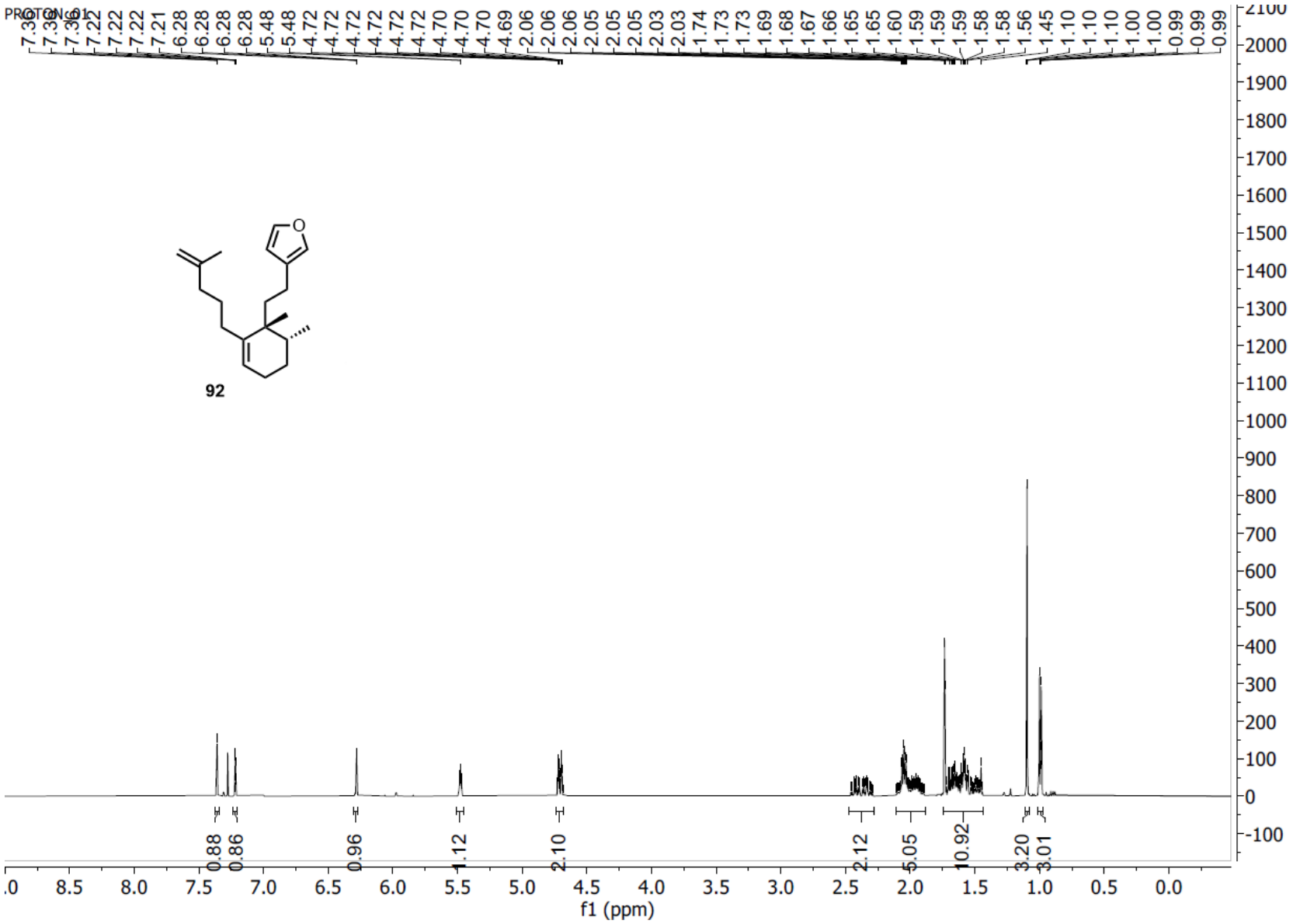

CARBON_01
204.48
145.78
141.03
122.42
109.99
49.14
40.79
38.75
37.89
30.86
27.15
27.02
25.98
24.12
22.41
16.46
O
H
93
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

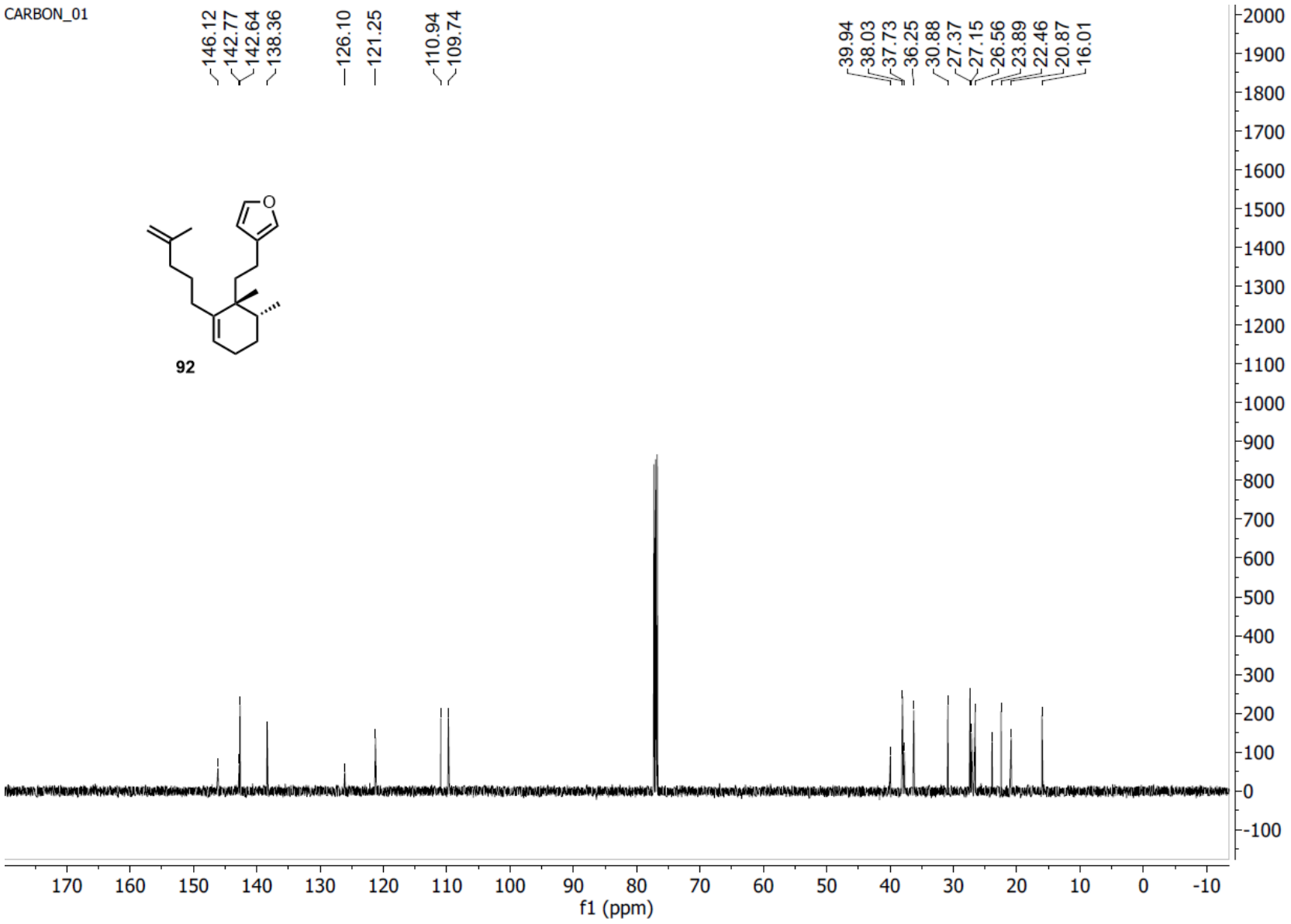

PROTON_01
7.38 7.38 7.38 7.37 7.37 6.40 6.40 6.40 6.39 4.73 4.73 4.73 4.73 4.73 4.72 4.71 4.71 4.71 4.71 2.83 2.82 2.11 2.10 2.09 2.09 2.08 2.08 2.07 2.07 2.06 1.91 1.91 1.89 1.89 1.78 1.77 1.75 1.74 1.74 1.74 1.68 1.67 1.67 1.66 1.66 1.66 1.65 1.65 1.64 1.59 1.08 1.07 1.07 1.06 1.04
O
OH
100a
1.81 0.91 1.04 1.09 2.02 0.85 6.29 1.04 3.86 5.00 2.99 2.74
f1 (ppm)

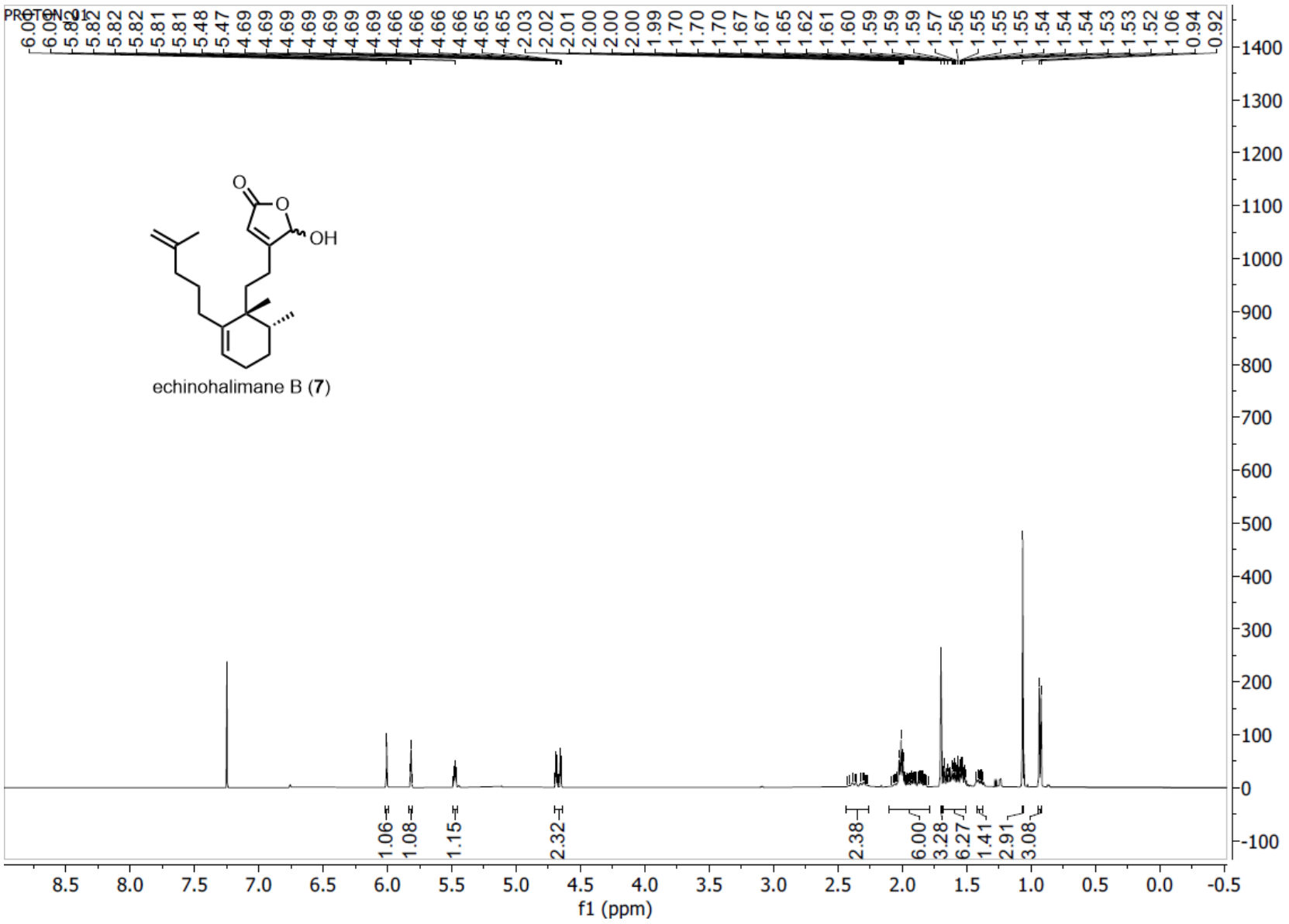

CARBON_01
145.87
144.99
143.06
138.44
129.82
123.55
109.93
108.45
65.63
43.43
39.56
39.51
38.00
30.50
28.23
27.64
27.25
25.27
22.44
16.68
O
OH
100a
f1 (ppm)

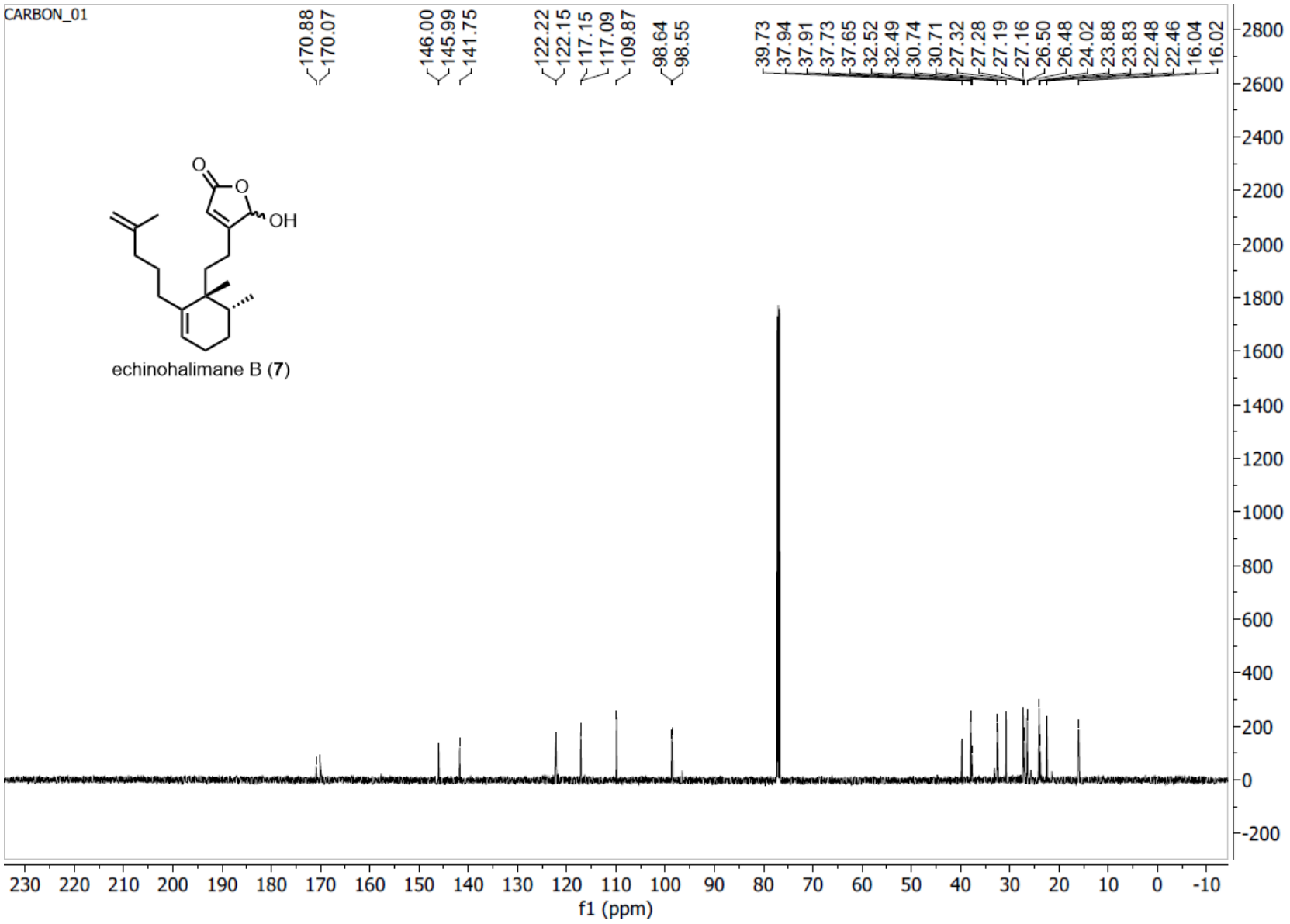

PROTON_01
7.38 7.38 7.38 7.37 7.37 7.36 7.36 7.36 6.41 6.41 6.40 6.40 5.46 4.71 4.71 4.71 4.71 4.71 4.71 4.69 4.69 4.68 4.68 2.06 2.06 2.06 2.05 2.03 2.02 2.02 1.92 1.90 1.89 1.87 1.75 1.74 1.73 1.72 1.72 1.72 1.71 1.67 1.64 1.63 1.63 1.63 1.61 1.61 1.60 1.59 1.58 1.58 1.57 1.19 0.95 0.94
O
OH
100b
1.82
0.83
0.97
0.99
1.99
6.34
10.10
2.63
2.91
f1 (ppm)


CARBON_01
146.04
143.29
143.06
138.40
130.92
121.83
109.81
108.48
64.40
44.01
39.82
38.48
38.03
31.44
27.43
26.35
25.97
24.12
22.44
16.25
O
OH
100b
f1 (ppm)


PROTON-01
92
0.88
0.86
0.96
1.12
2.10
2.12
5.05
10.92
3.20
3.01
f1 (ppm)


CARBON_01
146.12
142.77
142.64
138.36
126.10
121.25
110.94
109.74
39.94
38.03
37.73
36.25
30.88
27.37
27.15
26.56
23.89
22.46
20.87
16.01
O
92
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


PROTON-01
6.00 6.00 5.83 5.82 5.82 5.82 5.81 5.81 5.48 5.47 4.69 4.69 4.69 4.69 4.69 4.69 4.66 4.66 4.66 4.66 4.65 4.65 2.03 2.02 2.01 2.00 2.00 2.00 1.99 1.70 1.70 1.67 1.67 1.65 1.62 1.61 1.60 1.59 1.59 1.59 1.57 1.56 1.55 1.55 1.55 1.54 1.54 1.54 1.53 1.53 1.52 1.06 0.94 0.92
O
O
OH
echinohalimane B (7)
1.06 1.08 1.15 2.32 2.38 6.00 3.28 6.27 1.41 2.91 3.08
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100 0 -100


CARBON_01
170.88
170.07
146.00
145.99
141.75
122.22
122.15
117.15
117.09
109.87
98.64
98.55
39.73
37.94
37.91
37.73
37.65
32.52
32.49
30.74
30.71
27.32
27.28
27.19
27.16
26.50
26.48
24.02
23.88
23.83
22.48
22.46
16.04
16.02
O
O
OH
echinohalimane B (7)
f1 (ppm)