Total Synthesis and Biological Evaluation of Hydrindane and Decalin based Natural Products: Peribysin E, Nardoaristolone B, Nootkatone, Botryosphaeridione and their Analogues

Thesis Submitted to AcSIR

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

CHEMICAL SCIENCES

By

Kishor L. Handore
(Registration Number: 10CC12J26001)

Under the guidance of
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Dedicated

To My Family

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Thesis Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Total Synthesis and Biological Evaluation of Hydrindane and Decalin based Natural Products: Peribysin E, Nardoaristolone B, Nootkatone, Botryosphaeridione and their Analogues"** submitted by **Mr. Kishor L. Handore** to Academy of Scientific and Innovative Research (AcSIR) in fulfilment of the requirements for the award of the Degree of Doctor of Philosophy, embodies original research work under my supervision. I further certify that this work has not been submitted to any other University or Institution in part or full for the award of any degree or diploma. Research material obtained from other sources has been duly acknowledged in the thesis. Any text, illustration, table etc., used in the thesis from other sources, have been duly cited and acknowledged.

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Declaration by the Candidate

I hereby declare that the original research work embodied in this thesis entitled, **"Total Synthesis and Biological Evaluation of Hydrindane and Decalin based Natural Products: Peribysin E, Nardoaristolone B, Nootkatone, Botryosphaeridione and their Analogues''** submitted to Academy of Scientific and Innovative Research for the award of degree of Doctor of Philosophy (Ph.D.) is the outcome of experimental investigations carried out by me under the supervision of **Dr. D. Srinivasa Reddy**, Senior Scientist, Organic Chemistry Division, CSIR-National Chemical Laboratory, Pune. I affirm that the work incorporated is original and has not been submitted to any other academy, university or institute for the award of any degree or diploma.

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Acknowledgement

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My family is always source of inspiration and great moral support for me in perceiving my education, I used thank god of almighty for providing me such a beautiful family. I take this opportunity to my sense of gratitude to my parents and my wife for their tons of love, sacrifice, blessings, unconditional support and encouragement.

I wish to thank the great scientific community whose achievements are constant source of inspiration for me.

Above all, I extend my prayers to God Almighty, for blessing me with this wonderful life.

Kishor L. Handore

Abbreviations

Å	angstrom
AD	Alzheimer's disease
brs	broad singlet
Bu	butyl
t-Bu	*tertiary*-butyl
brsm	based on recovered starting materials
calcd.	calculated
CD	circular dichroism
cm^{-1}	1/centimetre
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
COSY	correlation spectroscopy
CNS	central nervous system
DA	Diels-Alder
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	diisobutylaluminium hydride
DMSO	dimethylsulphoxide
DMSO-d_6	deutriated dimethylsulphoxide
dd	doublet of doublet
ddd	doublet of doublet of doublet
ddt	doublet of doublet of triplet
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ECD	electron-capture dissociation
ESI	electron spray ionization mass spectroscopy
Et	ethyl
Et_2O	diethyl ether
EtOAc	ethyl acetate
equiv.	equivalent
g	gram(s)
h	hour(s)
HRMS	high resolution mass spectrometry

Abbreviations

HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear single quantum coherence
Hz	hertz
IBX	2-iodoxybenzoic acid
In vitro	outside a living organism
In vivo	inside a living organism
IR	infrared
J	coupling constant (in NMR)
LAH	lithium aluminium hydride
LDA	lithium diisopropylamide
LPS	lipopolysaccharides
min	minute(s)
m	multiplet
m-CPBA	meta-Chloroperoxybenzoic acid
mL	milliliter(s)
mmol	millimole(s)
mp	melting point
MPLC	medium pressure liquid chromatography
m/z	mass to charge ratio
Me	methyl
MHz	megahertz
MS	molecular sieves
N	normality
NBS	*N*-bromosuccinimide
NO	nitric oxide
NMR	nuclear magnetic resonance
NMO	N-methylmorpholine N-oxide
NOE	nuclear Overhauser effect
p-TSA	para-toluenesulfonic acid
Ph	phenyl
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate

Abbreviations

PD	Parkinson's disease
ppm	parts per million
Pr	propyl
q	quartet
RCM	ring-closing metathesis
R_f	retention factor
ROS	reactive oxygen species
rt	room temperature
RM	reaction mixture
s	singlet
S_N	nucleophilic substitution
SAR	structure–activity relationship
TBS	tertiary butyl dimethylsilyl
t	triplet
tert	tertiary
TBHP (*t*-BuOOH)	*tert*-butyl hydroperoxide
TEA	triethyl amine
THF	tetrahydrofuran
TFA	trifluroacetic acid
TLC	thin layer chromatography
°C	degree celsius
μM	micromolar

General remarks

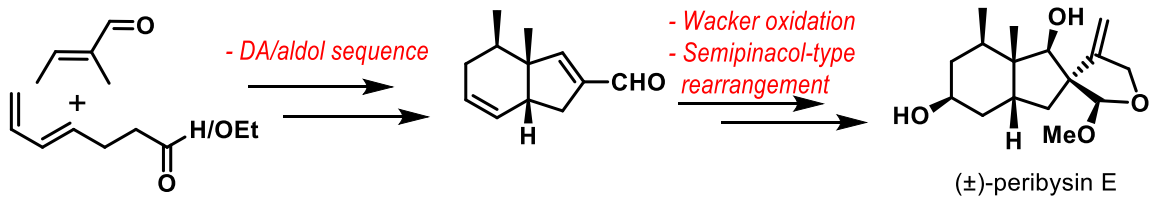
All reagents, starting materials, and solvents were obtained from commercial suppliers and used as such without further purification. Solvents were dried using standard protocols or dried using Mbraun (MB SPS-800) instrument. Reactions were carried out in oven-dried glassware under a positive pressure of argon unless otherwise mentioned with magnetic stirring. Air sensitive reagents and solutions were transferred *via* syringe or cannula and were introduced to the apparatus *via* rubber septa. The progress of reactions was monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, Iodine adsorbed on silica gel or by immersion in ethanolic solution of phosphomolybdic acid (PMA), *p*-anisaldehyde or $KMnO_4$ followed by heating with a heat gun for ~15 sec. Column chromatography was performed on silica gel (100-200 or 230-400 mesh size). All the melting points are uncorrected and were recorded using a scientific melting point apparatus (Buchi B-540). Deuterated solvents for NMR spectroscopic analyses were used as received. All ^{1}H NMR and ^{13}C NMR and 2D NMR analysis were obtained using a 200 MHz, 400 MHz or 500 MHz spectrometer. Coupling constants were measured in Hertz. All chemical shifts are quoted in ppm, relative to TMS, using the residual solvent peak as a reference standard. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. HRMS (ESI) were recorded on ORBITRAP mass analyser (Q Exactive). GC-HRMS (EI) was recorded on Agilent 7200 Accurate-mass-Q-TOF. Infrared (IR) spectra were recorded on a FT-IR spectrometer as thin films in chloroform using NaCl plates. Optical rotations were recorded on a P-2000 polarimeter at 589 nm (sodium D-line). Chemical nomenclature (IUPAC) and structures were generated using Chem Bio Draw Ultra.

Synopsis

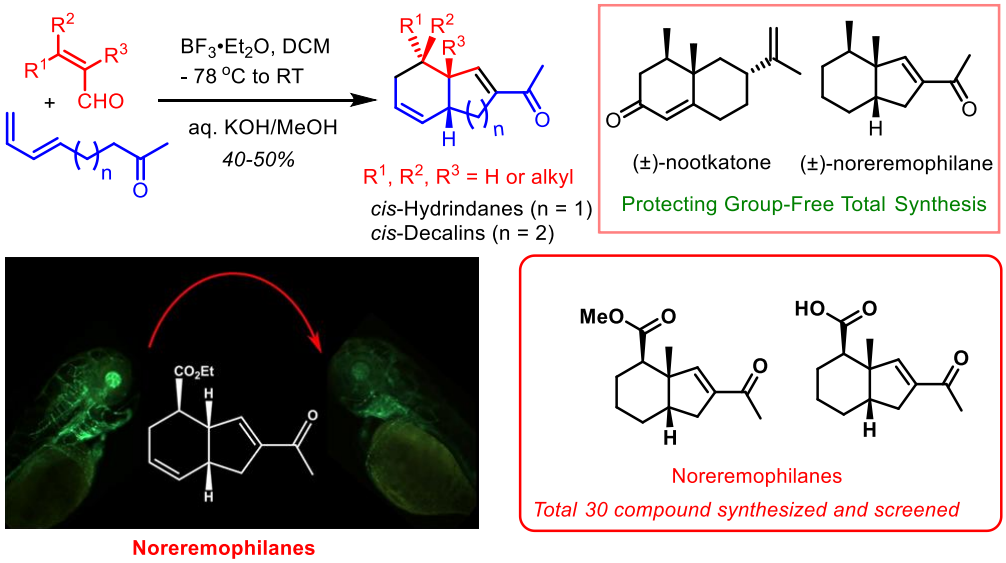
Synopsis of the Thesis to be submitted to the Academy of Scientific and Innovative Research for Award of the Degree of Doctor of Philosophy in Chemistry	
Name of the Candidate	**Kishor Laxman Handore**
Degree Enrolment No. & Date	**Ph. D in Chemical Sciences (10CC12J26001); January 2012**
Title of the Thesis	**Total Synthesis and Biological Evaluation of Hydrindane and Decalin based Natural Products: Peribysin E, Nardoaristolone B, Nootkatone, Botryosphaeridione and their Analogues**
Research Supervisor	**Dr. D. Srinivasa Reddy**

The thesis is divided into four chapters. Chapter 1 describes total synthesis and biological evaluation of peribysin E and its analogues. Chapter 2 describes a method to access *cis*-hydrindane and *cis*-decalin and application to the syntheses of nootkatone, noreremophilanes followed by their biological evaluation. Chapter 3 describes total synthesis and biological evaluation of nardoaristolone B and its analogues. The total synthesis and biological evaluation of neural anti-inflammatory agents based on natural products are included in Chapter 4.

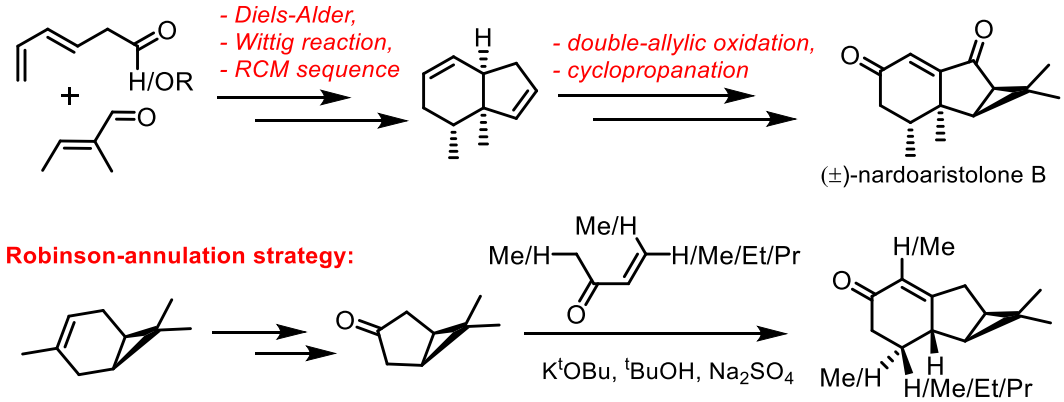
Chapter 1: Total Synthesis and Biological Evaluation of Peribysin-E and its Analogues: In 2005, isolation of natural product called peribysin E was reported from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare, *Aplysia kurodai*. Peribysin E inhibited the adhesion of human-leukemia HL-60 cells to human umbilical-vein endothelial cells (HUVEC) with IC_{50} = 11.5 μM. Due to its interesting biological activity and structural features, we have initiated a program and achieved the total synthesis of (±)-peribysin E.[1a] In addition to the protecting group free synthesis of the target molecule, we have also prepared eight novel analogues of peribysin E. All the synthesized compounds were evaluated for cell adhesion inhibitory activity and found that two of them showed better activity than natural peribysin E.[1b]



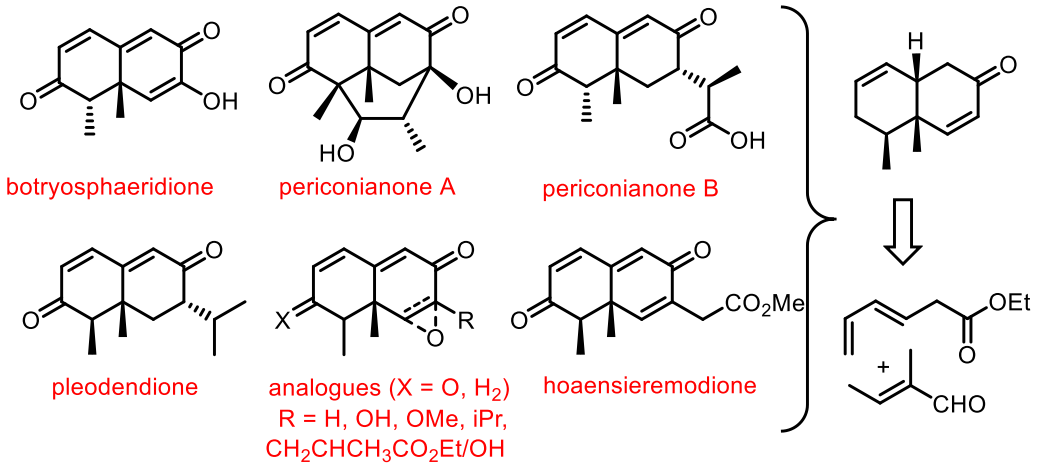
Chapter 2: Ready Access to *cis*-Hydrindane and *cis*-Decalin: Protecting Group Free Total Synthesis of Nootkatone, Noreremophilanes and their Biological Evaluation: We have designed and executed a sequential Diels-Alder/aldol method to access the functionalized *cis*-hydrindanes and *cis*-decalins in a highly diastereoselective manner. As a application, this methodology was successfully implemented to synthesis of (±)-nootkatone and (±)-noreremophilane using a protecting group free sequence.[2a,2b] We have also synthesized various derivatives of noreremophilanes and tested them using whole-organism assays in zebrafish embryos. It was found that synthesized noreremophilanes has strong anti-angiogenic effects on the developing zebrafish embryos.[5]



Chapter 3: Total Synthesis and Biological Evaluation of Nardoaristolone B and its Analogues: Nardoaristolones B, novel nor-aristolane type sesquiterpenoid with unusal 3/5/6 tricyclic ring system, was isolated from the underground parts of *Nardostachys chinensis* Batal. In a dose-dependent manner it has shown protective effects on the injury of neonatal rat cardiomyocytes. We have achieved stereoselective synthesis (±)-nardoaristolone B and its novel analogues including a compound with *exo*-cyclopropyl ring fusion.[3a] An alternate route using Robinson-annulation has been developed to access 3/5/6 tricyclic fused ring system and prepared several analogues. All the synthesized compounds were evaluated for their repellent activity against adult females of *Aedes aegypti* which is the vector for Dengue and Zika virus. Among the tested compounds, a few analogues show comparable or better activity with respect to racemic nootkatone. [3b]



Chapter 4: Total Synthesis and Biological Evaluation of Neural Anti-inflammatory Agents Based on Natural Products: Three natural products called botryosphaeridione, periconianone A and periconianone B isolated from endophytic fungus *Periconia sp.* have shown neural anti-inflammatory activity with impressive IC_{50} values of 0.23, 0.15, and 0.38 μM, respectively, in mouse microglia BV2 cells. Therefore, the compounds can be promising starting points for the development of drugs to treat CNS disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD).



We have accomplished first total syntheses of (±)-botryosphaeridione, (±)-pleodendione, (±)-hoaensieremodione, (±)-4-*epi*-periconianone B and their closely related analogues.[4] All the synthesized compounds were evaluated for their neural anti-inflammatory activity using LPS induced microglia cells (N9). Two compounds are identified as potential lead compounds for further profiling based on the initial results.

Noteworthy Findings:

- Accomplished the synthesis of (±)-peribysin E and eight novel analogues using diverted total synthesis towards identifying a potent cell adhesion inhibitor.
- Developed a facile and simple method for the constuction of *cis*-hydrindane and *cis*- decalin skeleton in a highly diastereoselective manner using Diels-Alder/aldol sequence and applied to the total synthesis of (±)-nootkatone and (±)-noreremophilanes.
- Accomplished the first stereoselective total synthesis of (±)-nardoaristolone B using a protecting-group free sequence.
- Achieved the syntheses of various neural anti-inflammatory agents such as (±)-botryosphaeridione, (±)-pleodendione, (±)-4-*epi*-periconianone B and (±)-hoaensieremodione.

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1. (a) **Handore, K. L**.; Reddy, D. S. *Org.Lett.* **2013**, *15*, 1894. (b) Reddy, D. S.; **Handore, K. L.** WO2014128723 A2, **2014**.

2. (a) **Handore, K. L**; Seetharamsingh, B.; Reddy, D. S. *J. Org. Chem.* **2013**, *78*, 8149. b) Reddy, D. S.; **Handore, K. L**.; Sen, A.; Pawar, P. V.; Joseph, M. WO 2014170915 A1, **2014**.

3. (a) **Handore, K. L**.; Reddy, D. S. *Org.Lett.* **2014**, *16*, 4252. (b) Reddy, D. S.; **Handore, K. L**. WO 2016013032 A1, **2016**.

4. **Handore, K. L**.; Jadhav, P. D.; Hazra, B.; Basu, A.; Reddy D. S. *ACS Med. Chem. Lett*., **2015**, *6*, 1117.

5. Muthukumarasamy, K. M.; **Handore, K. L**.; Kakade, D. N.; Shinde, M. V.; Ranjan, S; Kumar, N.; Sehrawat, S.; Sachidanandan, C.; Reddy, D. S. *Org. Biomol. Chem.*, **2016**, *14*, 1569.

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Chapter 1

Total Synthesis and Biological Evaluation of Peribysin E and its Analogues

1.1 Introduction to hydrindane / decalin based biologically active natural products:

The hydrindane and decalin ring systems are continuously witnessed in structurally complex and biologically active natural products. Due to the presence of multiple substitution pattern, hydrindane and decalin ring system show remarkable structural and functional diversity.[1,2] The natural products with hydrindane skeletons show interesting bioactivities, such as Aplykurodinone-1 (**1**) is a degraded steroidal natural product and various aplykurodines exhibit cytotoxic activity against a range of human cancer cell lines.[3] Peribysin E (**2**) has shown potent cell adhesion inhibitory activity,[4] Havellockate (**3**) is a polyoxygenated marine diterpene with a *cis*-fused hydrindane core and a spiro-lactone with interesting structural features [5] (Fig 1.1).

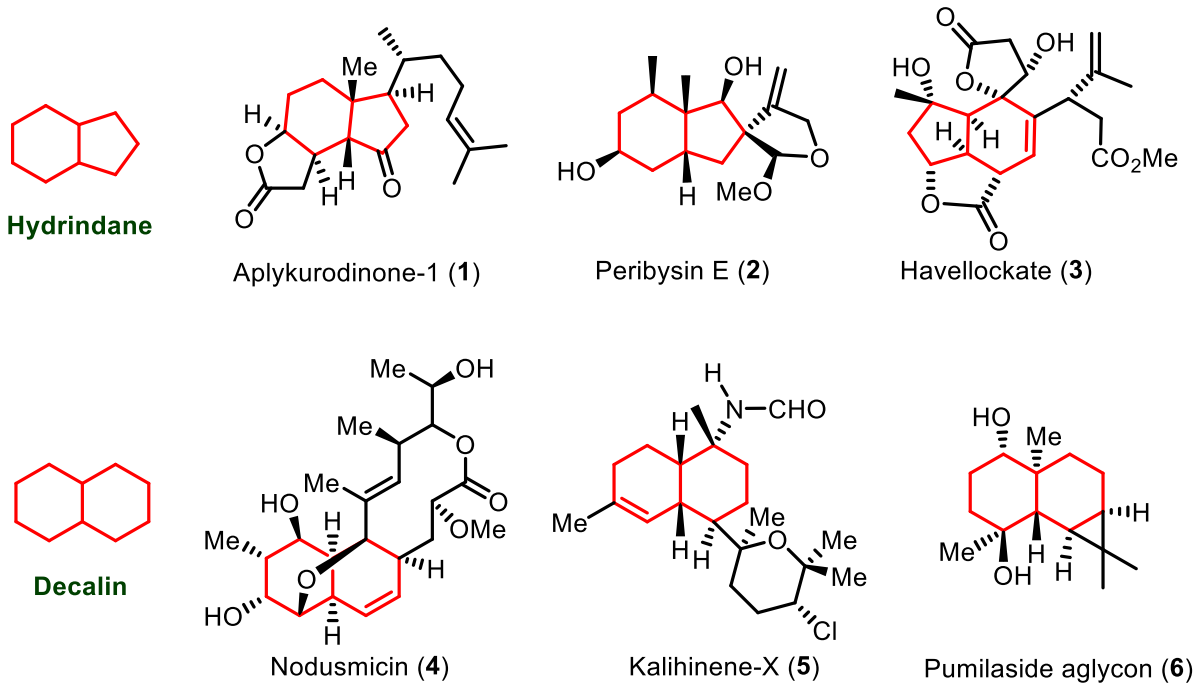


Fig 1.1: Selected natural products containing hydrindane and decalin framework

The natural product with decalin skeletons such as Nodusmicin (**4**) is active against drug-resistant bacteria.[6] Kalihinene-X (**5**) is a highly functionalized marine diterpenoid and exhibit antimicrobial activity.[7] Pumilaside aglycon (**6**) is part of glycoterpene natural

product possesses *trans*-decalin framework with antitumor and anti-inflammatory properties.[8] There are many other natural products which possess hydrindanes and decalins ring system and show remarkable biological activity such as Bakkenolide-A (**7**) displays a cytotoxicity towards carcinoma cell lines[9] and an effective insect antifeedant.[10] Nardoaristolone B (**8**) is a unusual 3/5/6 ring system nor-sesquiterpenoid which has protective effects on the injury of neonatal rat cardiomyocytes[11] (Fig 1.2). Ottelione B (**9**) which exhibits anticancer and antitubercluar activity,[12] and sesquiterpenoid glycosides, aglycon dendronobiloside A (**10**) display immunomodulatory activity.[13]

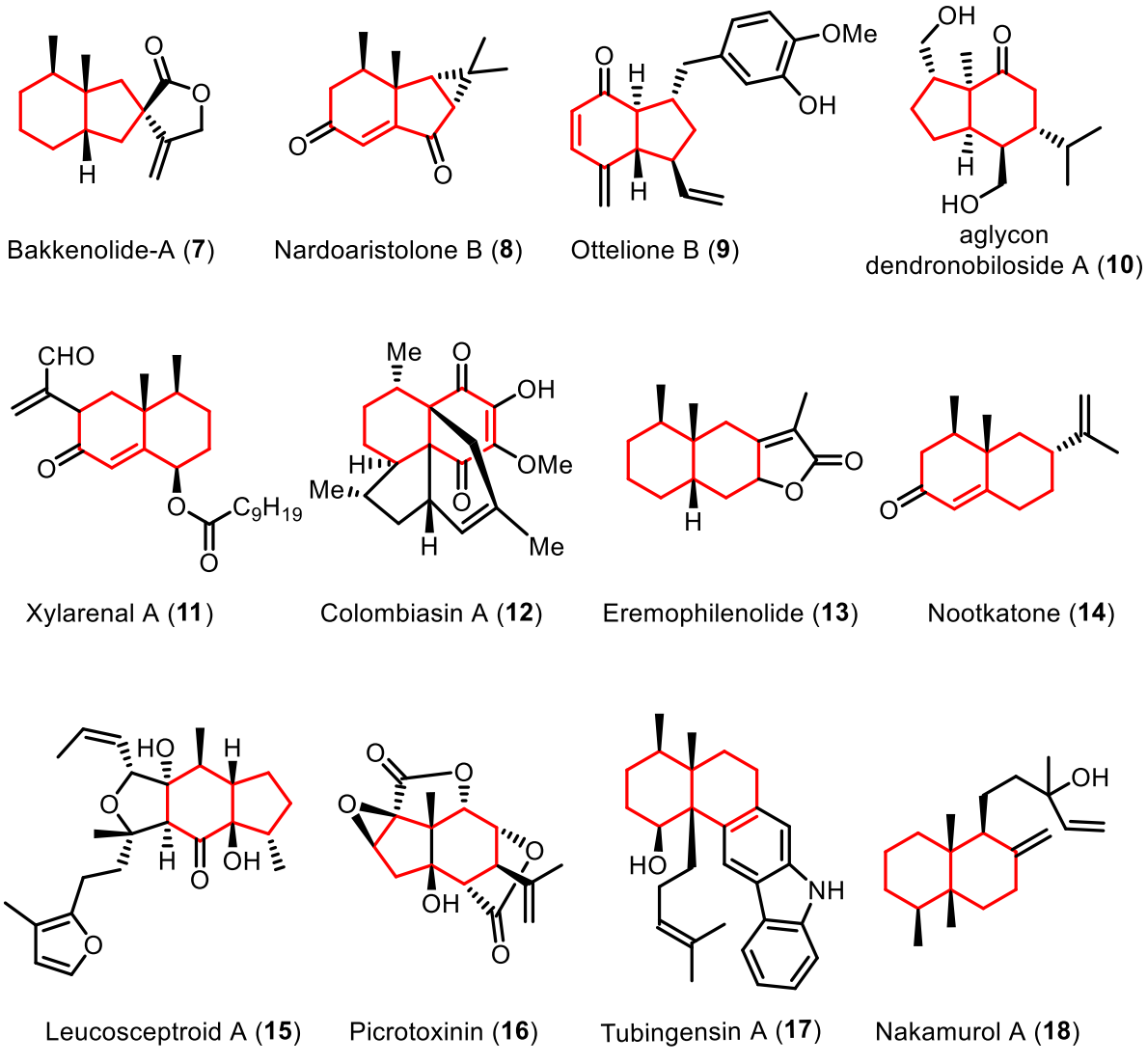


Fig 1.2: Diverse natural products containing hydrindane and decalin framework

Xylarenal A (**11**) is eremophilane type sesquiterpenoid and act as NPY-receptor antagonist.[14] Colombiasin A (**12**) is tetracyclic novel diterpene and active against *Mycobacterium tuberculosi*s H37Rv.[15] Eremophilenolide (**13**) is eremophilane type sesquiterpenoid possessing the *α,β*-unsaturated γ-lactone moiety.[16] Nootkatone (**14**) is a sesquiterpene ketone and show interesting insect repellent activity and also act as AMPK activator which is a master switch for controlling metabolic disorders.[17] Leucosceptroids A (**15**) possesses antifeedant and antifungal activities.[18] Picrotoxinin (**16**) is a potent and specific antagonist against the neurotransmitter suppressor *γ*-aminobutyric acid (GABA) and inhibits the opening of chloride ion channels *in vivo.*[19] Tubingensin A (**17**) is indole diterpenoid possesses a disubstituted carbazole that is fused to a *cis*-decalin framework and shows anti-insecticidal, antiviral and anticancer activities.[20] Nakamurol A (**18**) is marine sponge diterpenoid having *cis*-decalin framework contains four contiguous stereocenters[21] (Fig 1.2).

Many synthesized hydrindane and decalin based compounds can be important precursor in total syntheses of natural products or useful scaffolds for drug development. It is clear that many of these natural products have varying degrees of substitution and stereochemical patterns and hence, pose a considerable synthetic challenge. As a consequence of structural complexity and interesting biological activities of these hydrindane and decalin based natural products, there has been significant interest in the development of new and efficient synthetic methodologies for the construction functionalized hydrindanes and decalins. Methods such as annulations reactions,[22] Diels-Alder reactions[23] (both intramolecular and intermolecular), metal catalyzed reactions,[24] chelation-controlled cyclization,[25] and miscellaneous methods[26] like skeletal rearrangements, fragmentations are widely used for the construction of hydrindanes and decalins skeleton (Fig 1.3). Due to space limitation, details of the known methods[22-26] for the preparation of hydrindanes / decalins are not discussed here. The complex structures and diverse biological activities of hydrindanes and decalins have attracted many researchers around the world to investigate their chemical synthesis and to further study their therapeutic potential. In this context, we have identified peribysins, a unique class of marine sesquiterpenes for their synthesis and to study their biological potential.

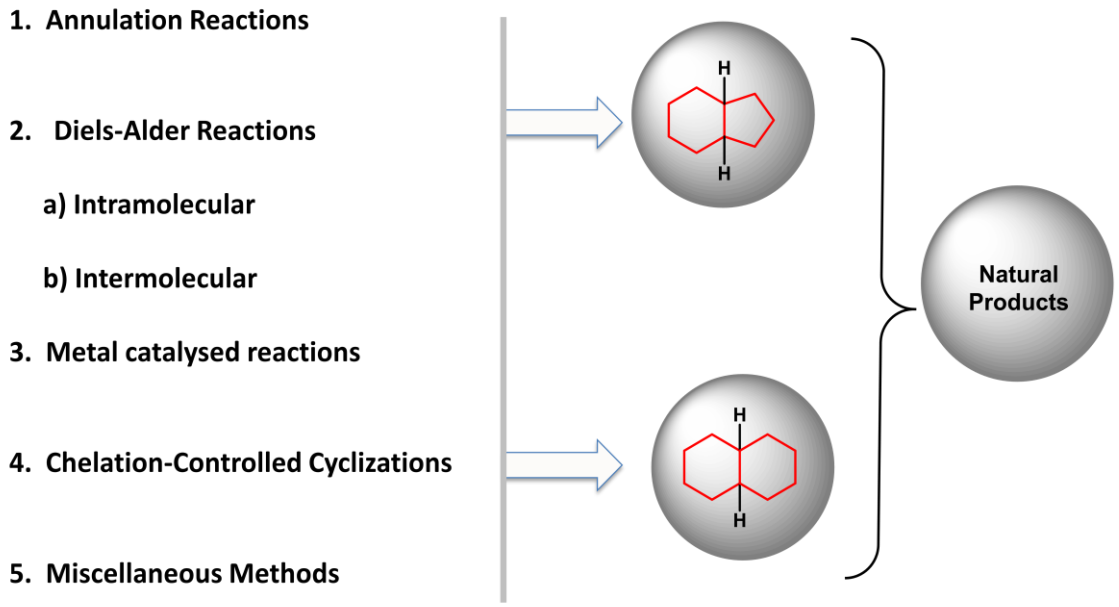


Fig 1.3: Synthetic methods for construction of hydrindanes and decalins framework

1.2 Introduction to peribysins

1.2.1 Isolation and structural elucidation of peribysins

Yamada and coworkers isolated new eremophilane type sesquiterpenoids called peribysins (A-I) from a strain of *Periconia byssoides* OUPS-N133 which was originally separated from the sea hare *Aplysia kurodai*.[27] The absolute and relative stereochemistry of peribysins was elucidated on the basis of 1D, 2D NMR techniques, CD and some chemical transformations (Fig 1.4). Initially Yamada and co-workers proposed that peribysins C and D as diastereoisomers that possess a 4,6-dihydro-1H, 3H-furo [3, 4-c] furan system but later they have reported structural revision of peribysins C (**21**) and D (**22**).[28] All the peribysins have *cis*-decalin framework as core except peribysin E (**2**) which has *cis*-hydrindane skeleton. The peribysins found to have shown potent cell adhesion inhibition and their IC_{50} values are mentioned in Fig 1.4. Their inhibitory activity was examined according to a modification of the method reported by Miki and co-workers[29] using herbimycin A as a

standard compound in the adhesion assay system using HL-60 cells and HUVEC.

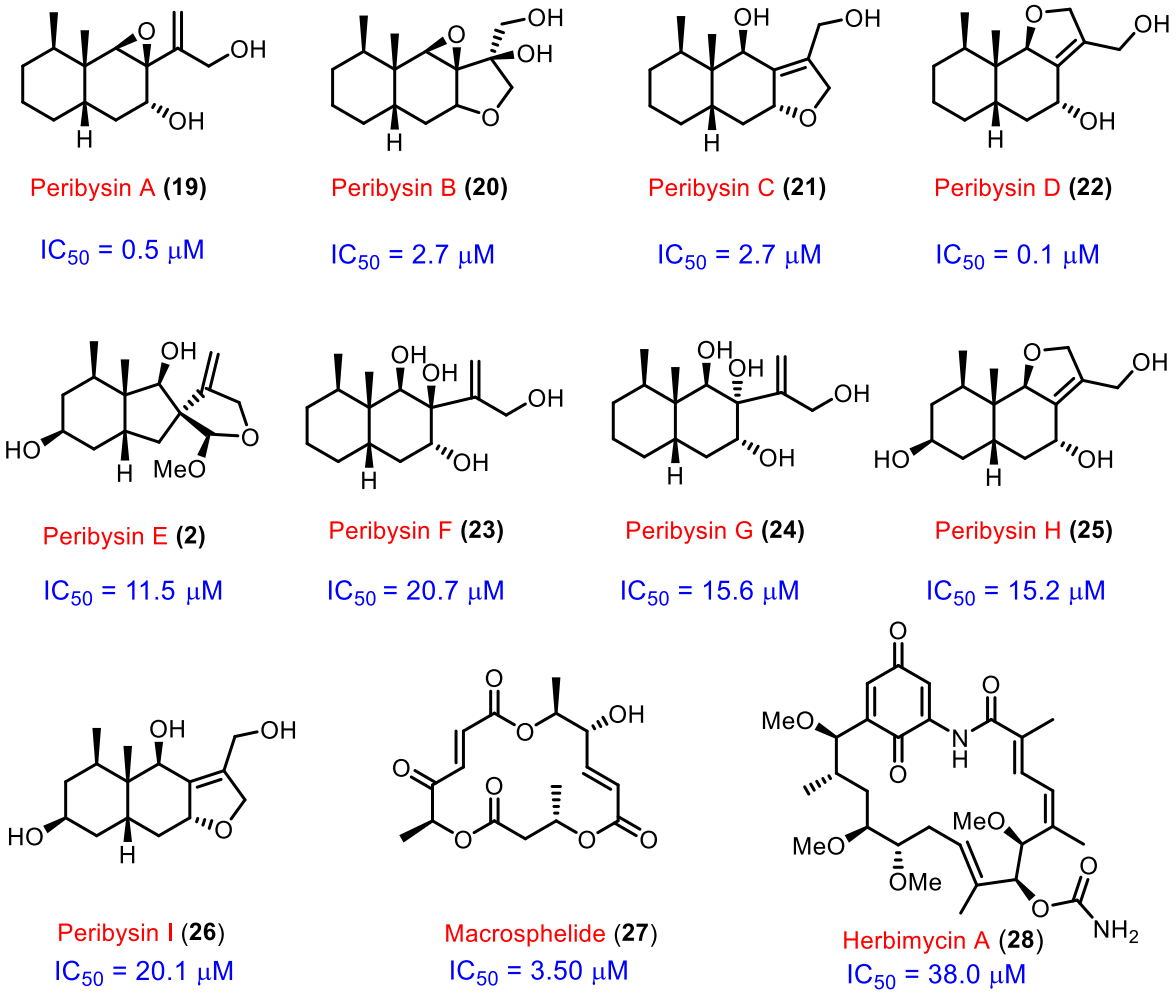


Fig 1.4: Structures of peribysins (A-I), macrosphelide, herbimycin A with cell adhesion inhibitory activity

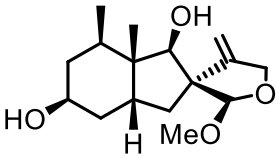
All peribysins inhibited the adhesion of HL-60 cells to HUVEC more potently than herbimycin A. Peribysin A (**19**) and D (**22**) exhibited the most potent inhibitory activity than other peribysins and were 190 to 380 times as potent compared to the standard herbimycin A (**28**). Their inhibition of cell adhesion was also more potent than that of macrosphelide (**27**), a macrocyclic structure, which was previously isolated from same species.

1.2.2 Importance of cell adhesion inhibitors

The cell adhesion is the important process for maintaining multicellular structure. Excessive cell adhesion leads to inflammatory process and plays a considerable role in cancer development and metastasis. Cancer metastasis use mechanisms of cell adhesion to establish new tumors in the body. So cell adhesive inhibitors are useful for developing anti-cancer agents and anti-inflammatory agents since, they inhibit the cell adhesion. The cell adhesive inhibitors also useful in treating the vaso-occlusive crisis associated with sickle cell anemia because of their potent cell-adhesion inhibitory activity. Sickle cell anemia is like a genetic disorder where red blood cells assume a sickle shape instead of the normal disc shape. The sickle cell patient may suffer from pain, anemia, bacterial infections or stroke at different stages of his life. The only approved drug for the causative treatment of sickle-cell anemia is hydroxyurea, but several other compounds are in clinical trials. Most of the drugs aim to prevent the frequency of vaso-occlusion and acute chest syndrome. Ultimately, peribysins can be lead compounds for developing drugs in treating inflammation, sickle cell anemia and cancers.

1.2.3 Introduction to peribysin E

Peribysin E (**2**) is a structurally complex natural product having *cis*-hydrindane skeleton which is connected to spirocyclic ring. It has five contiguous stereogenic centers with one cyclic hemiacetal (Fig 1.5). The absolute and relative stereochemistry of peribysin E (**2**) has been elucidated on the basis 1D and 2D NMR techniques like COSY, HMBC and NOESY.[20b]



Peribysin E (**2**)
cell adhesion
IC_{50} = 11.5 μM

Aplysia kurodai

Fig 1.5: Structure of peribysin E with cell adhesion inhibitory activity (picture taken from website: www.seaslugforum.net/find/15900)

Peribysin E (**2**) inhibited the adhesion of human-leukemia HL-60 cells to human umbilical-vein endothelial cells (HUVEC) with IC_{50} = 11.5 μM. The anti-adhesive property of peribysin E is enantiospecific which was demonstrated by Danishefsky's group where they have synthesized both the enantiomers and studied cell adhesion inhibition assay. More details will be discussed in next section.

1.2.4 Previous syntheses of peribysin E

The challenging structural features of peribysin E with rare spirocyclic ring, potent cell-adhesion inhibitory activity and scarcity of the material, attracted the attention of many research groups around globe including us. The first total synthesis of both the enantiomers of peribysin E starting from carvone was reported by Danishefsky and coworkers in 18 steps which helped in reassigning the absolute configuration of the natural product.[30] The synthesis highlights use of Diels-Alder reaction, Suzuki cross-coupling, nucleophilic epoxidation, semipinacol-type ring contraction reaction (Fig 1.6).

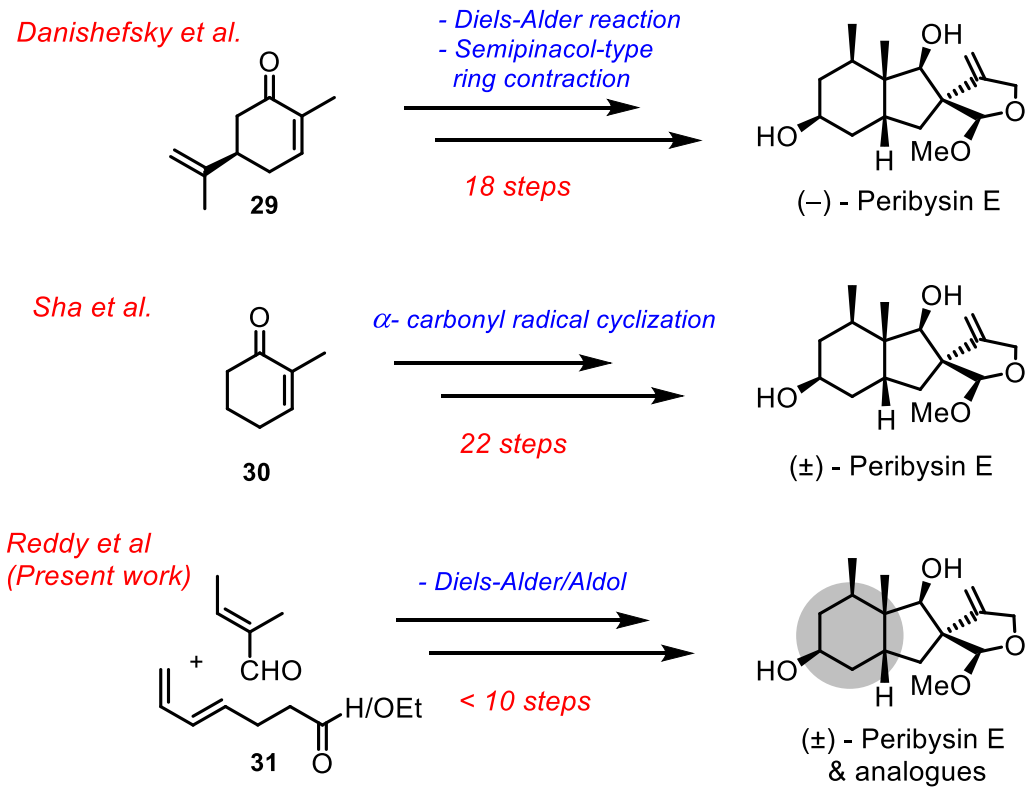
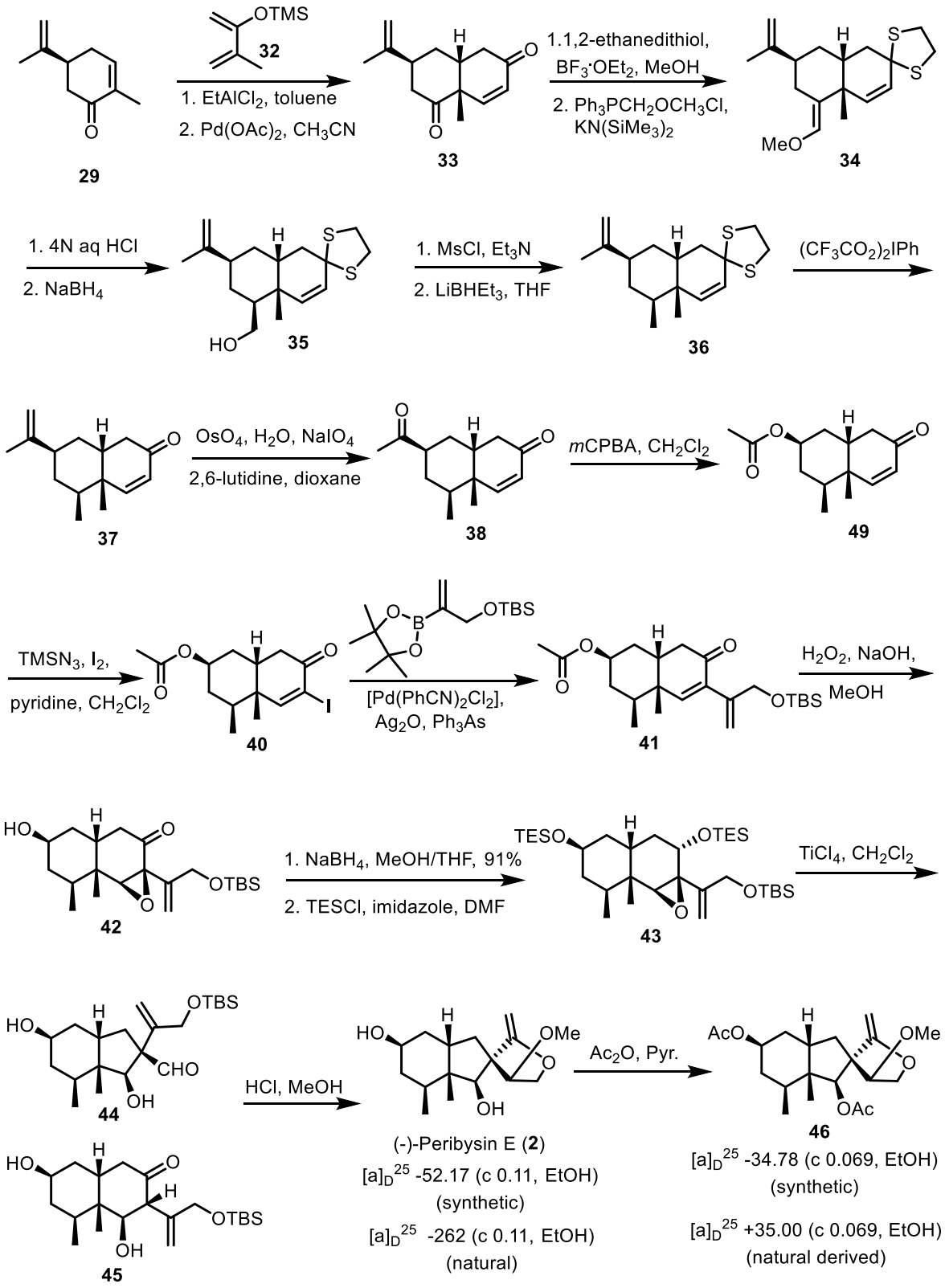


Fig 1.6: Danishefsky, Sha and Our approaches to peribysin E

Later Sha's group achieved the racemic synthesis of peribysin E by using α-carbonyl radical cyclization as a key reaction in 22 steps.[31] We have accomplished the total synthesis of the same target in 10 steps which is part of the present thesis work. Before discussing compete details of our work, we describe the syntheses by Danishefsky and Sha groups as they appeared in public domain before our publication.

1.2.4.1 Danishefsky approach to peribysin E

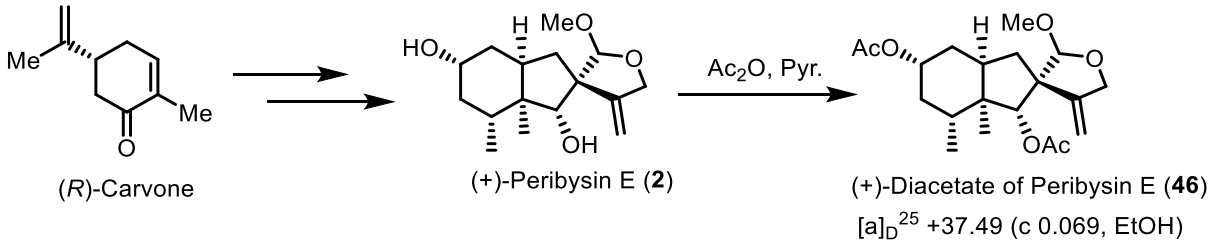
In their elegant synthesis, Danishefsky's group used (*S*)-carvone (**29**) as chiral pool starting material. The synthesis started with the Lewis acid $EtAlCl_2$ catalyzed Diels-Alder reaction of (*S*)-carvone **29** with diene **32** to give adduct which on Saegusa oxidation by $Pd(OAc)_2$ afforded enone **33.** The enone **33** was selectively protected as thioketal by 1, 2-ethanedithiol in presence of Lewis acid $BF_3{\cdot}OEt_2$ in good yield. The Wittig–Levine methoxymethenylation of thioketal afforded **34** which on hydrolysis by HCl afforded aldehyde. The aldehyde was reduced by $NaBH_4$ to give primary alcohol **35** which was protected by MsCl, Et_3N conditions. The protected compound was reduced by $LiBHEt_3$ to give **36**. Deprotection of the dithiane was achieved by $(CF_3CO_2)_2IPh$ to furnish **37** (scheme 1.1.). The compound **37** was subjected to Johnson–Lemieux oxidation of isopropenyl group to corresponding ketone to give **38**. The corresponding ketone on Baeyer–Villiger oxidation afforded acetate **39.** Next iodide group was introduced by $TMSN_3$, I_2, and pyridine at alpha position to ketone to give **40** which was subjected to cross coupling reaction with vinyl boronate to give coupled product **41**. The stereoselective epoxidation was accomplished by H_2O_2-NaOH to afford **42**. Observed selectivity was explained by axial attack of the hydrogen peroxide anion at the *β*-carbon atom of the enone to produce epoxide **42** stereospecifically. The sodium borohydride reduction of compound **42** followed by protection of corresponding alcohol using TESCl afforded trisilyl protected compound **43**. The compound **43** on titanium tetrachloride mediated ring contraction rearrangement afforded aldehyde **44** in 50% yield with required relative configuration at the quaternary carbon atom C7 of peribysin E, along with hydroxyl ketone **45** in 5% yield. Finally exposure of **44** to HCl in methanol afforded the (–)-peribysin E (**2**). Although NMR data of synthetic peribysin E was exactly matching with reported data of natural product by Yamada's group, optical rotation was found to show significant discrepancy in value with



Scheme 1.1: Total synthesis of peribysin E by Danishefsky and co-workers

the reported one. The specific rotation of synthetic natural product was s $[\alpha]_D^{25}$ -52.17 (*c* 0.11, EtOH), while the natural peribysin E had specific rotation of $[\alpha]_D^{25}$-262.2 (*c* 0.11, EtOH). Both results show the same sign of rotation, but magnitude of rotation was so large that it raises a serious concern. To clarify further, they synthesized the diacetate of synthetic peribysin E (**46**) and determined the rotation and it is found $[\alpha]_D^{25}$ -34.78 (*c* 0.069, EtOH) which has very close match with naturally derived peribysin E diacetate which has specific rotation of $[\alpha]_D^{25}$ +35.00 (*c* 0.069, EtOH). This result also suggested that the original assignment of absolute configuration for peribysin E was incorrect (scheme 1.1).

After repeating all the steps they have synthesized *ent*-peribysin E starting from (*R*)-carvone (scheme 1.2). Finally, optical rotation of the synthetic diacetate was found $[\alpha]_D^{25}$ +37.49 (*c* 0.069, EtOH), which was fully consistent with that reported naturally derived one. These results further confirmed that original assigned structure of peribysin E corresponds to *ent*-peribysin E.

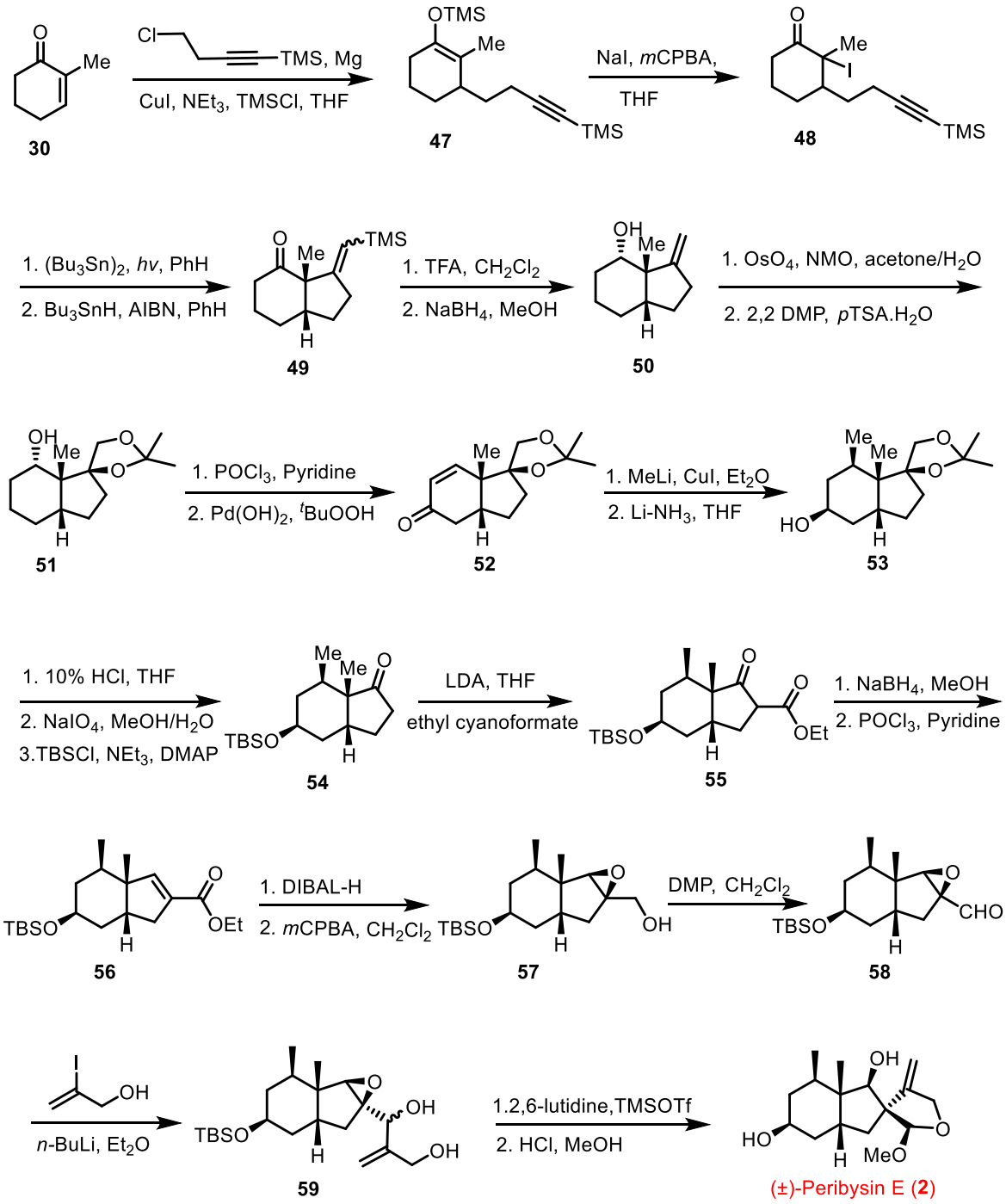


Scheme 1.2: Synthesis of (+)-peribysin E by Danishefsky and co-workers

1.2.4.2 Sha approach

Sha approach started with a CuI-mediated conjugate addition of 4-(trimethylsilyl)-3-butynylmagnesium chloride on 2-methylcyclohexen-1-one **30** to give enolate which is trapped by chlorotrimethylsilane to form TMS enol ether **47**. The resulted TMS enol ether on reaction with NaI and *m*-CPBA afforded α-iodoketone **48** which on photolysis by sunlamp in the presence of hexamethylditin, followed by reduction with tributyltin hydride, gave compound **49**. The TMS deprotection followed by reduction with $NaBH_4$ cleanly afforded alcohol **50** as a single diastereomer. The exocyclic double bond on dihydroxylation by OsO_4 and N-methylmorpholine-N-oxide gave diol which was protected by 2, 2-dimethyloxypropane, to give acetonide **51**. Dehydration of **51** with $POCl_3$ at 70 °C

followed by allylic oxidation with Pd $(OH)_2$ and *t*-BuOOH afforded enone **52**. Next 1, 4 addition of methyl lithium to enone **52** gave ketone as a single diastereomer. The resulting ketone was stereoselectively reduced with Li-NH_3 condition to give the single diastereomer **53**.



Scheme 1.3: Synthesis of (±)-peribysin E by Sha and co-workers

Next acetonide group was deprotected to give diol which on oxidative cleavage with $NaIO_4$ afforded ketone and the free alcohol group was protected with a tertbutyldimethylsilyl (TBS) group to afford the key hydrindanone **54** (scheme 1.3). The treatment of hydrindanone **54** with lithium diisopropylamide followed by quenching with ethyl cyanoformate afforded ethyl ester **55**. Reduction of ketone in compound **55** with $NaBH_4$ followed by dehydration by $POCl_3$ afforded *α,β*-unsaturated ester **56**. The ester group was reduced with DIBAL-H to give allylic alcohol which on stereoselective epoxidation by *m*-CPBA gave compound **57**. Oxidation of primary alcohol by DMP resulted in aldehyde **58**. Next the dianion generated from 2-iodopropen-1-ol by *n*-BuLi reacted with aldehyde **58** to afford epoxy-alcohols **59** as mixture of diastereomers. Finally, the epoxy-alcohols **59** was transformed to (±)-peribysin E (**2**) using semi-pinacol type rearrangement.

1.3 Present work

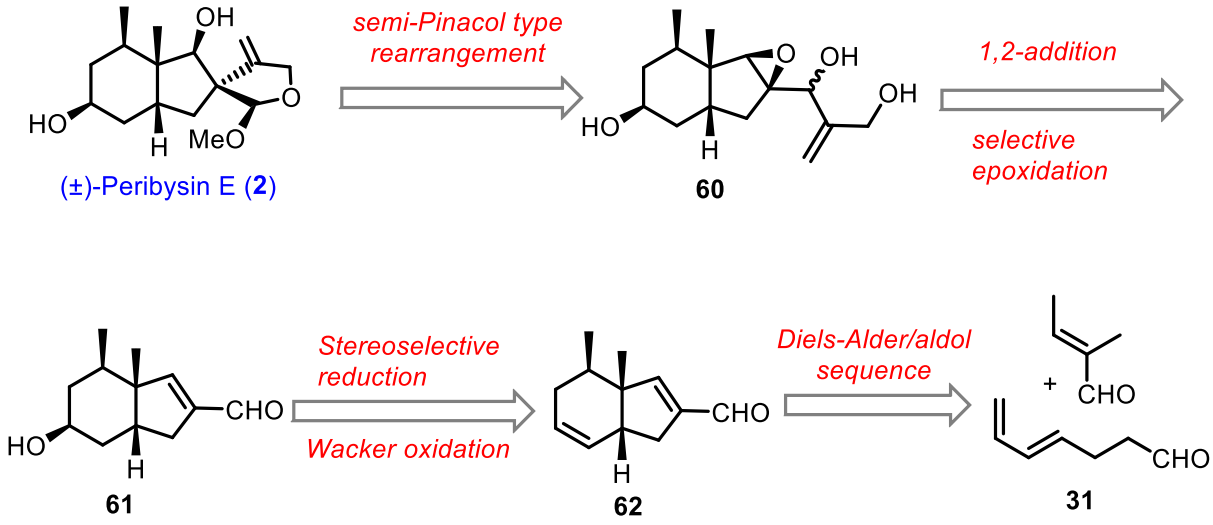
The interesting structural features of peribysin E and its potent cell-adhesion inhibitory activity prompted us to start the project to identify potent anti-cancer and anti-inflammatory agents based on peribysin E scaffold by keeping following objectives in mind.

- To develop a simple, efficient, short and scalable synthetic route to access peribysin E in sufficient quantities (better than previous approaches).
- To synthesize structurally close analogues around this scaffold.
- Biological evaluation of peribysin E and its analogues to understand SAR using cell adhesion potential towards identification of a lead compound.

1.3.1 Retrosynthesis

The retrosynthetic analysis for peribysin E (**2**) is compiled in scheme 1.4. In designing a synthetic route toward peribysin E, we planned epoxy alcohol **60** as final precursor for semi-pinacol type rearrangement to give target compound. The compound **60** could be obtained by stereoselective epoxidation followed by 1, 2-addition on compound **61**. The compound **61** could be prepared by Wacker oxidation of **62** followed by chemo-and stereoselective reduction of the resultant ketone. The required *cis*-hydrindane **62** which is a

key intermediate with chemically differentiable double bonds could be prepared from readily accessible diene **31** and commercially available tiglic aldehyde, using the Diels-Alder followed by aldol sequence, a strategy developed in our research group, a few years ago.[32]

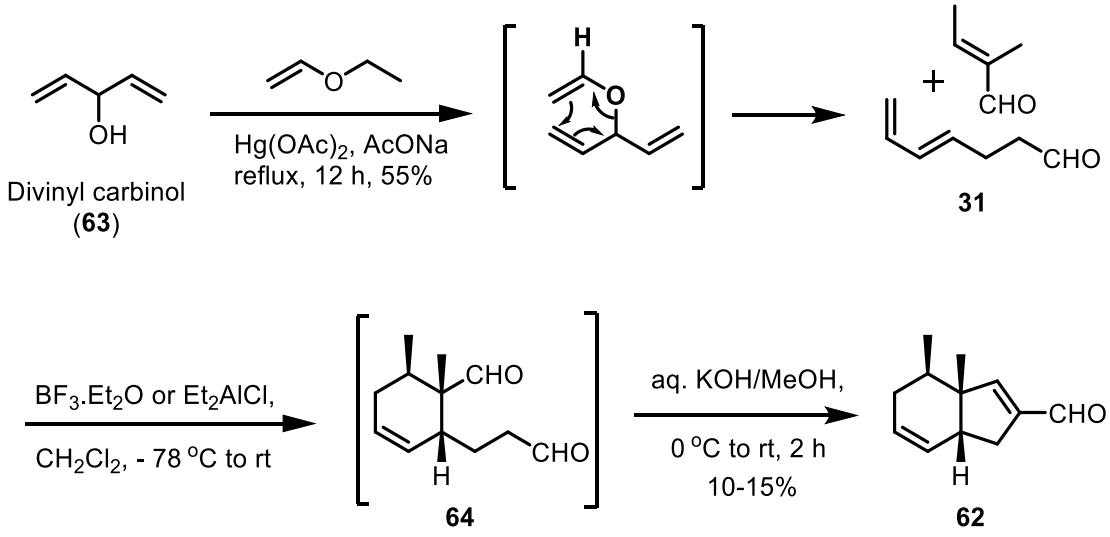


Scheme 1.4: Retrosynthetic approach for peribysin E

1.3.2 Synthesis of peribysin E

Our synthetic efforts commenced with the preparation of diene aldehyde **31** required for Diels-Alder reaction from commercially available divinyl carbinol **63**, and ethyl vinyl ether in the presence of mercuric acetate in one step via Claisen rearrangement using known literature procedure.[33] Initially, we performed the Diels-Alder reaction of tiglic aldehyde as dienophile and diene aldehyde **31** in the presence of a Lewis acid such as Et_2AlCl or $BF_3{\cdot}Et_2O$ in CH_2Cl_2 at -78 ^oC to rt for 8 to 12 h to give Diels-Alder adduct **64** which was immediately subjected for intramolecular aldol condensation in presence of 15% aq. KOH in MeOH to afford *cis*-hydrindane enal **62** in 10-15% yields (scheme 1.5). The formation of enal **62** was indicated by the presence of characteristic signals of aldehyde and olefins in ^1H NMR at 9.72 (s, 1H), 6.76 (s, 1H), 5.66–5.63 (m, 1H), 5.42–5.39 (m, 1H) ppm respectively and two methyls at 1.13 (s, 3H), 1.06 (d, J = 6.7 Hz, 3H) ppm. Similarly corresponding carbon signals at 190.3, 156.8, 145.9, 130.8, 126.0 ppm and for two methyl at 23.8, 16.6

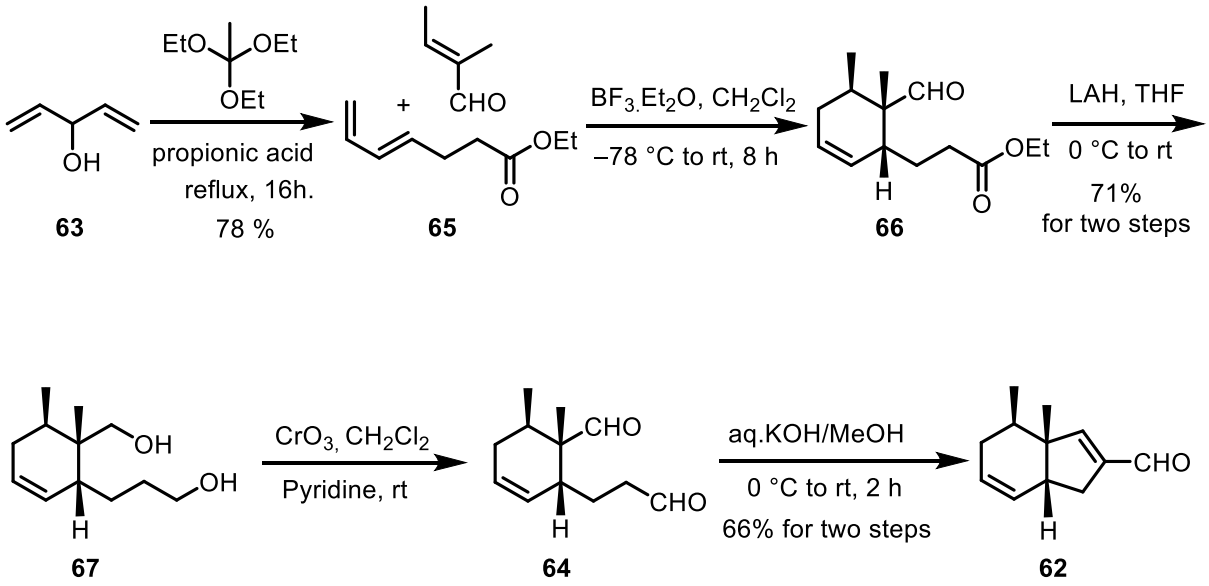
ppm in ^{13}C NMR and the assigned structure of **62** was further confirmed by HRMS which showed a peak at 177.1274 corresponding to formula $C_{12}H_{17}O$ $[M+H]^+$ with calculated value of 177.1275. The observed low yield in Diels-Alder/Aldol sequence may be due to polymerization of aldehyde or self condensation in presence of Lewis acid.



Scheme 1.5: Synthesis of enal **62**

To overcome this problem, diene aldehyde **31** was replaced with more stable diene ester **65**,[34] and the same transformation was achieved in 47% yield in a highly diastereoselective manner (scheme 1.6). The diene ester **65** was synthesized from divinyl carbinol **63**, triethyl orthoacetate in the presence of propionic acid by Claisen rearrangement by known literature procedure.[34] The Diels-Alder reaction of diene ester **65** and tiglic aldehyde in presence of Lewis acid $BF_3{\cdot}Et_2O$ at -78 °C to rt for 8 h afforded Diels-Alder adduct **66** which was immediately reduced by LAH at 0 °C in THF to afford diol **67** in 71% yield over two steps. The formation of diol **67** was indicated by presence of two sets of CH_2-OH protons in ^1H-NMR at 3.73–3.60 (m, 2H), 3.54–3.52 (m, 2H) ppm and oxygen attached carbon at 67.9, 62.2 ppm in ^{13}C NMR. The product was further confirmed by HRMS which showed a peak at 221.1512 corresponding to formula $C_{12}H_{22}O_2Na$ $[M+Na]^+$ with calculated value of 221.1510 (scheme 1.6). The diol **67** on oxidation by CrO_3 and pyridine (Collins reagent) in CH_2Cl_2 afforded dialdehyde **64** which was immediately used for next step without

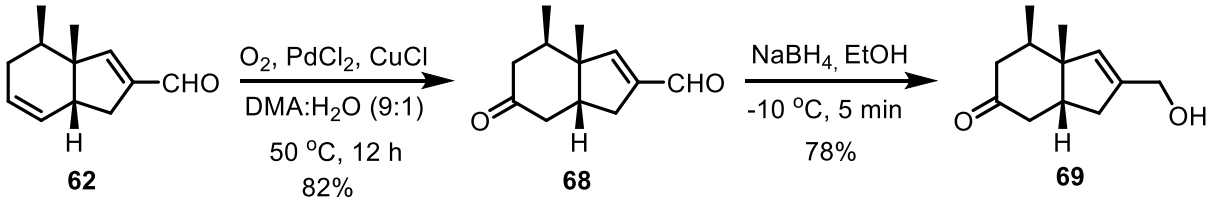
purification for intramolecular aldol condensation in presence of 15% aq. KOH in MeOH to give *cis*-hydrindane enal **62** in 66% yield for two steps. The spectral data was exactly matching with data of **62** prepared using above scheme 1.5.



Scheme 1.6: Alternative synthetic scheme for synthesis of enal **62**

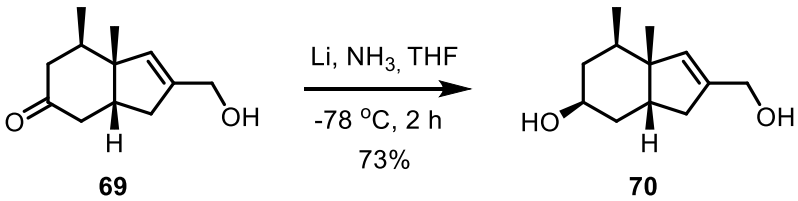
The intermolecular Diels-Alder reaction catalyzed by Lewis acid afforded the endo adduct having aldehyde functional groups in close proximity for subsequent intramolecular aldol condensation to give *cis*-hydrindane.[35] The excellent diastereoselectivity and regioselectivity in Diels-Alder reaction can be explained by secondary orbital interactions and atomic coefficient preferences.[36] Diels−Alder reaction followed by intramolecular aldol condensation sequence served to secure the stereochemistry three contiguous chiral centers of the peribysin E. The *cis*-hydrindane intermediate **62** is a functionally embellished as it possesses two chemically differentiated double bonds (conjugated and isolated) in two different rings which can be utilized for the selective functionalization of either ring. The next task was to introduce the oxygen functionality in six membered ring of compound **62**. This was achieved by Wacker oxidation[37] of isolated double in compound **62** by $PdCl_2$, CuCl in 9:1 DMA: H_2O under O_2 balloon pressure at 50 °C for 12 h to afford the desired keto-aldehyde **68** in 82% yield as single regioisomer with carbonyl group at required C-2 position (scheme 1.7). The formation of product keto-aldehyde **68** was indicated by the

presence of carbonyl signal at 211.4 ppm in ^{13}C NMR and disappearance of isolated olefin signals in the product, which was further confirmed by HRMS which showed a peak at 215.1043, corresponding to formula $C_{12}H_{16}O_2Na$ $[M+Na]^+$ with calculated value of 215.1044 (scheme 1.7).



Scheme 1.7: Synthetic scheme for keto-alcohol **69**

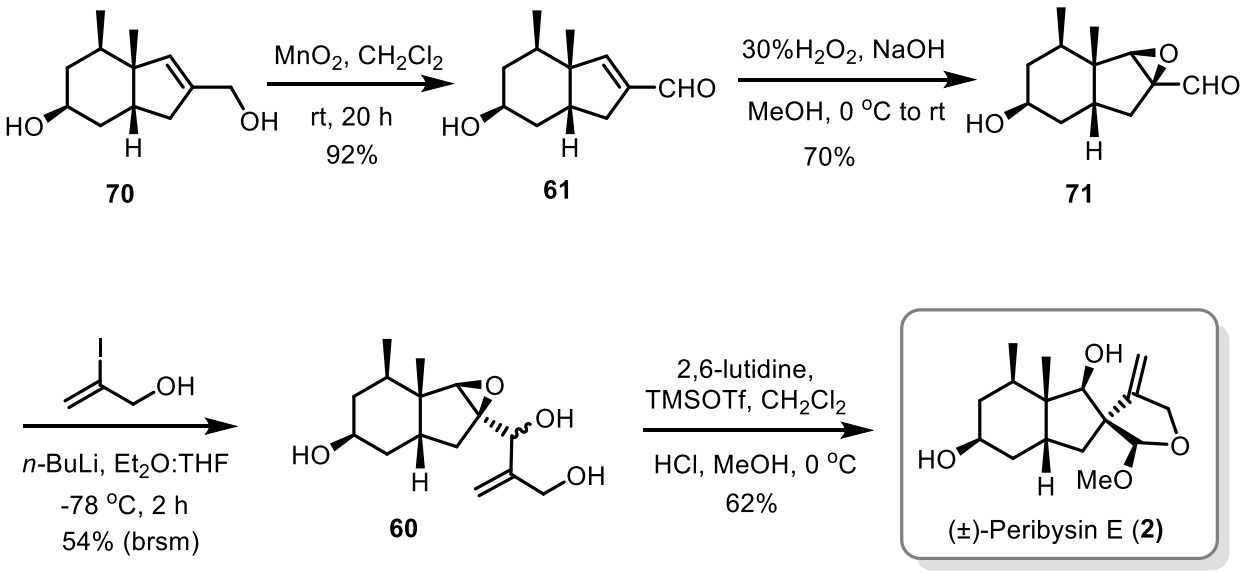
The aldehyde functionality in compound **68** was selectively reduced by $NaBH_4$ (0. 5 equiv.) in EtOH at -10 ^oC to furnish the keto-alcohol **69** in 78% yield. The formation of keto-alcohol **69** was indicated by disappearance of aldehydic proton at 9.76 (s, 1H) ppm and appearance of CH_2OH protons at 4.16 (s, 2H) ppm in ^1H-NMR and corresponding carbon signal at 62.0 ppm in ^{13}C NMR spectrum. The product formation was further confirmed by HRMS analysis which showed a peak at 217.1199 corresponding to formula $C_{12}H_{18}O_2Na$ $[M+Na]^+$ with calculated value of 217.1199. After having keto-alcohol **69** in hand, we then devoted our efforts for the stereoselective reduction of ketone to get requisite stereochemistry present in peribysin E. The desired alcohol **70** was obtained by reduction of **69** using Li-NH_3 in 73% yield (scheme 1.8).



Scheme 1.8: Synthetic scheme for alcohol **70**

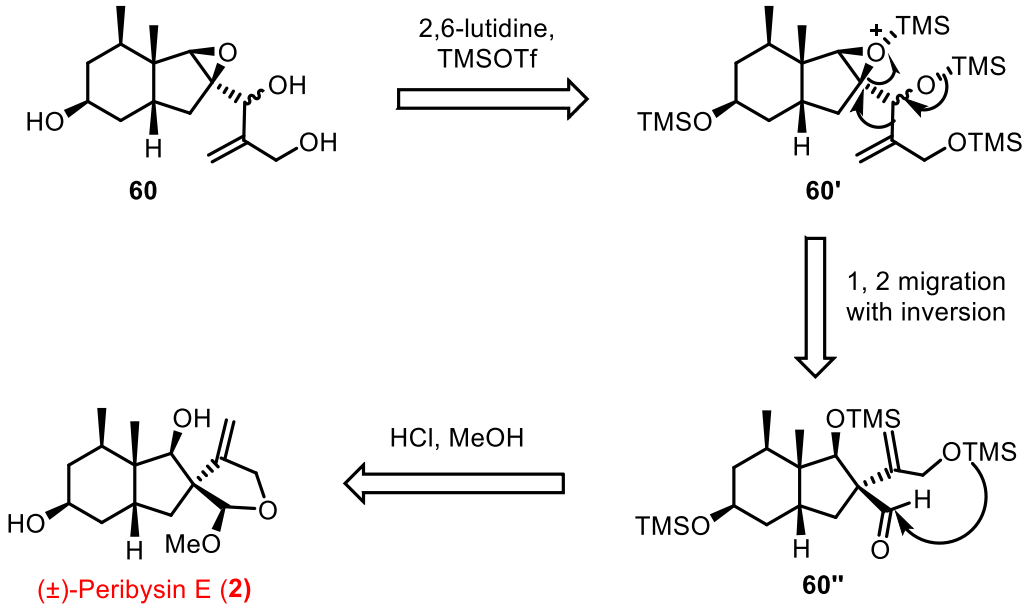
Reduction of **69** with lithium in liquid ammonia in the presence of THF affords almost exclusive equatorial product **70** since it proceeds *via* formation of a metal ketyl dimer and

transfer of a hydrogen atom from NH_4Cl take place from the α-position of one ketyl to the carbinol position of the other within the dimer to give *β*-alcohol.[38] This transformation was indicated by presence of CH-OH proton at 3.85–3.79 (m, 1H) ppm in ^{1}H NMR and signal at 68.5 ppm in ^{13}C NMR spectrum which was further confirmed by HRMS. The selective oxidation of the primary allylic alcohol was achieved by MnO_2 in CH_2Cl_2 to afford an *α,β*-unsaturated aldehyde **61** in 92% yield (scheme 1.9). The product formation was indicated by the presence of aldehydic proton at 9.68 (s, 1H) in ^{1}H-NMR and signal at 190.8 ppm in ^{13}C NMR spectrum, which was further confirmed by HRMS. The stereoselective epoxidation of the conjugated double bond in compound **61** by H_2O_2-NaOH resulted in an epoxy aldehyde **71** which was immediately used for the next step without purification. The reaction of 2-iodopropen-1-ol with *n*-BuLi at -78 ^{o}C in Et_2O resulted in dianion which was *in situ* reacted with epoxy aldehyde **71** at -78 ^{o}C for 2 h to give epoxy-alcohols **60** as diastereomeric mixture in 54% brsm (33%) which was directly used for next step after silica gel flash column chromatography purification. The mixture of epoxy alcohol **60** when treated with TMSOTf in the presence of 2, 6-lutidine at 0 ^{o}C for 30 min underwent semipinacol-type rearrangement[39] to give lactol intermediate which was immediately treated with 35% HCl in MeOH at 0 ^{o}C for 30 min to give final natural product peribysin E (**2**) in 62% yield for two steps.



Scheme 1.9: Total synthesis of (±)-peribysin E

Exposure of the epoxy-alcohols to TMSOTf resulted in chelation of TMS group to epoxide and which facilitate 1, 2 migration of alkenyl side chain to adjacent carbon to open the epoxide with inversion of stereochemistry at the migration terminus to give crude aldehyde **60''** (scheme 1.10). The resulted aldehyde on immediately exposure to 35% HCl in MeOH underwent the TMS deprotection followed by formation of cyclic hemiacetal to give peribysin E.



Scheme 1.10: Mechanism of semipinacol-type rearrangement

All the spectral data (IR, ^{1}H-NMR, ^{13}C-NMR) matched with the reported spectra.[25b,30,31] HRMS of peribysin E showed a peak at 305.1723 corresponding to formula $C_{16}H_{26}O_4$ $[M+Na]^+$ with calculated value of 305.1721. The ^{1}H and ^{13}C-NMR comparisons of natural and our synthetic (±)-peribysin E are shown in table 1.1. Thus, we have accomplished the total synthesis of the natural product peribysin E in less than 10 steps and no protecting group was used in the entire sequence. Our present approach is definitely superior when compared with other two published routes by Danishefsky and Sha groups.

Table 1.1: ^{1}H and ^{13}C-NMR comparisons of both natural and our synthetic (±)-peribysin E

No	Natural peribysin E		Our synthesized (±)-peribysin E	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
1α 1β	1.94 (dddd, *J* = 12.8, 4.8, 2.0, 1.8 Hz, 1H) 1.52 (ddd, *J* = 12.8, 10.8, 5.3 Hz, 1H)	34.2	1.94 (ddt, *J* = 13.4, 4.5, 2.4 Hz, 1H) 1.54−1.48 (m, 1H)	34.3
2	3.92 (dddd, *J* =11.8, 10.8, 4.8, 3.2 Hz, 1H)	67.1	3.94−3.88 (m, 1H)	67.2
3α 3β	1.71 (dddd, *J* = 11.8 , 10.8, 4.8, 3.2 Hz, 1H) 1.27 (q, *J* = 11.8 Hz, 1H)	40.0	1.72−1.68 (m, 1H) 1.30−1.21 (m, 1H)	40.1
4	1.57 (dqd, *J* = 11.8, 7.2, 4.0 Hz, 1H)	35.4	1.57−1.55 (m, 1H)	35.5
5	-	45.9	-	45.9
6	3.56 s	88.6	3.56 (d, *J* = 2.1 Hz, 1H)	88.7
7	-	60.8	-	60.9
8	5.09 s	105.5	5.08 (s, 1H)	105.7
9α 9β	1.76 (t, *J* = 13.8 Hz, 1H) 1.89 (dd, *J* = 13.8, 6.2 Hz, 1H)	32.9	1.78−1.75 (m, 1H) 1.88 (dd, *J* = 13.1, 5.8 Hz, 1H)	33.0
10	2.01 (m, 1H)	45.8	2.03−1.97 (m, 1H)	45.9
11	-	152.6	-	152.7
12α 12β	4.49 (dt, *J* = 12.8, 2.2 Hz, 1H) 4.40 (dt, *J* = 12.8, 2.2 Hz, 1H)	68.9	4.49 (dt, *J* = 13.1, 2.4 Hz, 1H) 4.47 (dt, *J* = 12.9, 2.4 Hz, 1H)	69.1
13A 13B	4.95 (t, *J* = 2.2 Hz, 1H) 4.99 (t, *J* = 2.2 Hz, 1H)	103.1	4.94 (t, *J* = 2.4 Hz, 1H) 4.98 (t, *J* = 1.8 Hz, 1H)	103.2
14	0.86 (d, *J* = 7.2 Hz, 3H)	16.1	0.86 (d, *J* = 6.8 Hz, 3H)	16.2
15	0.93 (s, 3H)	14.4	0.92 (s, 3H)	14.5
8–O CH_3	3.38 (s, 3H)	55.1	3.38 (s, 3H)	55.2

1.3.3 Synthesis of Peribysin E analogues

In a medicinal chemistry program aimed towards optimized lead, it is always beneficial to synthesize structurally closed analogues of natural product to identify lead molecule. So after the successful synthesis of peribysin (**2**), as per the plan, we focused our efforts to synthesize structurally close analogues of peribysin E. The previously synthesized intermediate **62** is suitable for the synthesis of diverse skeleton of peribysin E as it contain chemically differentiated double bonds in two different rings for selective functionalization of either ring (Fig 1.7).

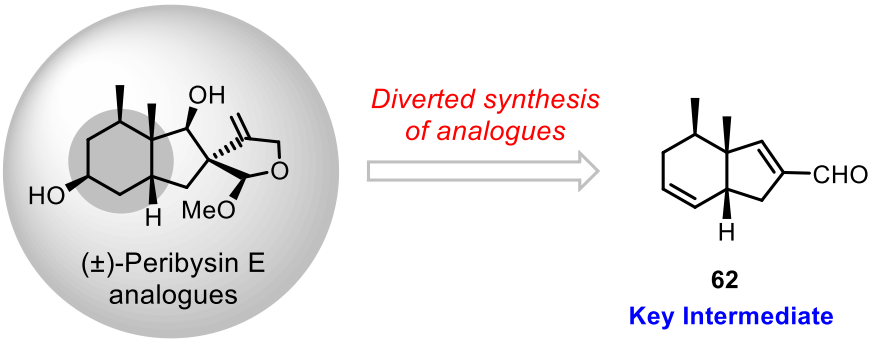
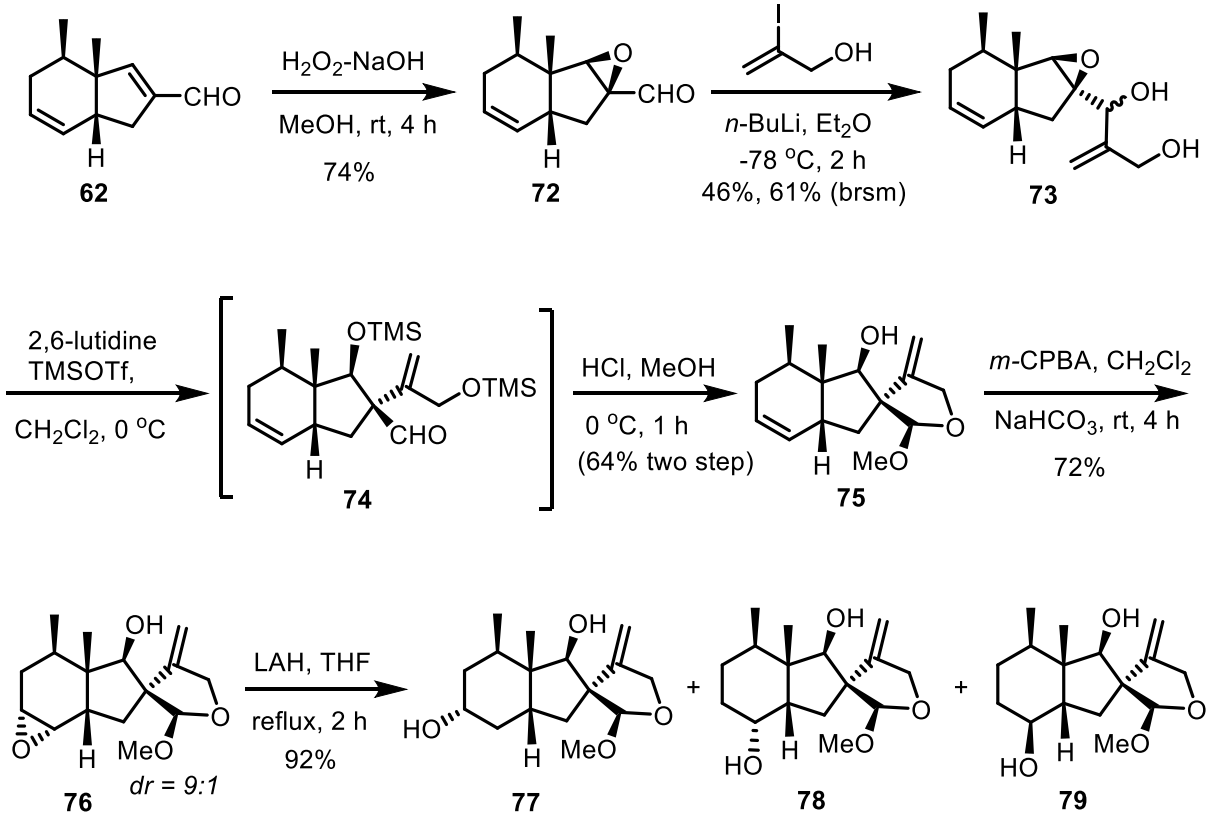


Fig 1.7: Planned analogues through diverted synthesis

Towards this effort, the conjugated double bond in enal **62** was selectively epoxidised using H_2O_2-NaOH[40] in MeOH to give **72** in 74% yield. This transformation was indicated by presence of epoxide attached proton signal at 3.71 (s, 1H) ppm in ^{1}H-NMR and carbon signals at 68.5, 67.5 ppm in ^{13}C-NMR. HRMS also showed a peak at 215.1043 corresponding to $C_{12}H_{16}O_2Na$ $[M+Na]^+$ with calculated value of 215.1041. Next the reaction of 2-iodopropen-1-ol with *n*-BuLi at -78 ^{o}C in Et_2O resulted in dianion which on treatment with epoxy aldehyde **72** at -78 ^{o}C for 2 h resulted in epoxy-alcohols **73** as ~1:1 mixture of diastereomers in 46% yield (61% brsm) as colorless oil. The mixture of epoxy alcohols **73** when treated with 2,6-lutidine followed by TMSOTf at 0 ^{o}C for 30 min underwent semipinacol-type rearrangement same as previous scheme to give lactol intermediate **74** which was immediately treated with 35% HCl in MeOH at 0 ^{o}C for 1 h to give new analogue of peribysin E **75** in 64% yield for two steps (scheme 1.11). For the

generation of more analogues, the isolated double bond in the six-membered ring in compound **75** was chemoselectively epoxidised by using *m*-CPBA in CH_2Cl_2 at 0 °C for 2 h to give epoxidised product **76** in 72% yield as ~9:1 diastereoselective mixture.[41]



Scheme 1.11: Syntheses of peribysin E analogues

The relative stereochemistry of epoxide **76** was unambiguously determined with the help of the single X-ray crystal analysis (Fig 1.8). Although attack from the *exo* face is preferred, the steric effects of the two methyl groups seem to control the product formation to obtain the *endo* product as the major isomer. The compound **76** on reduction using $LiAlH_4$ in THF at reflux condition[42] for 2 h resulted in the mixture of three alcohols (scheme 1.11). The mixture of alcohols was cleanly separated using a medium pressure liquid chromatography (Combiflash, MPLC) to give **77**, **78** and **79** in 92% yield. Interestingly, during purification we could not isolate the alcohol (out of four possible isomers) that corresponds to peribysin

E (**2**). The probable reason for this observation may be due to the minor epoxide, which was opened from one side only to give compound **77**. The assigned stereochemistry of alcohols was deduced on the basis of 2D-NMR analysis. The key NOE correlations for three compounds **77**, **78** and **79** are shown in Fig 1.8.

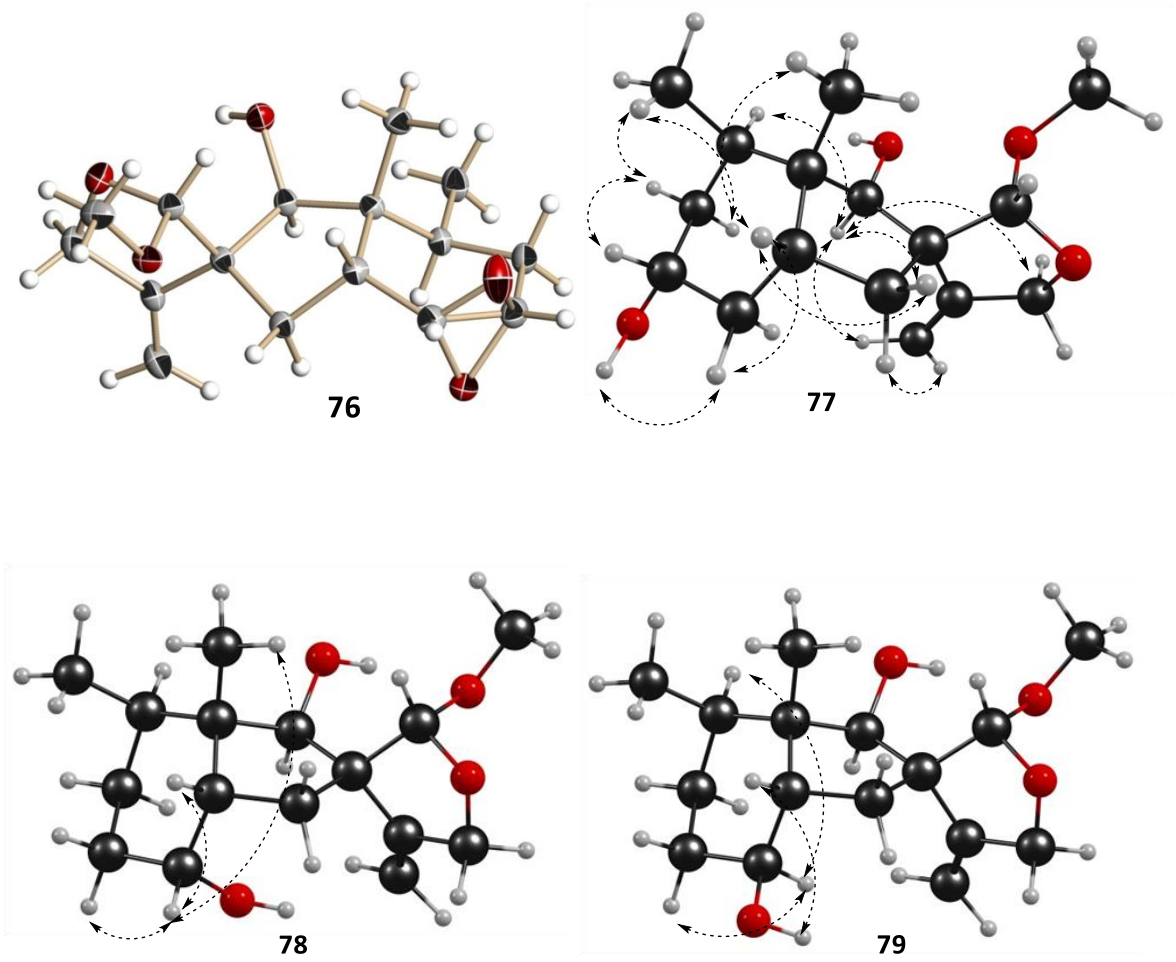
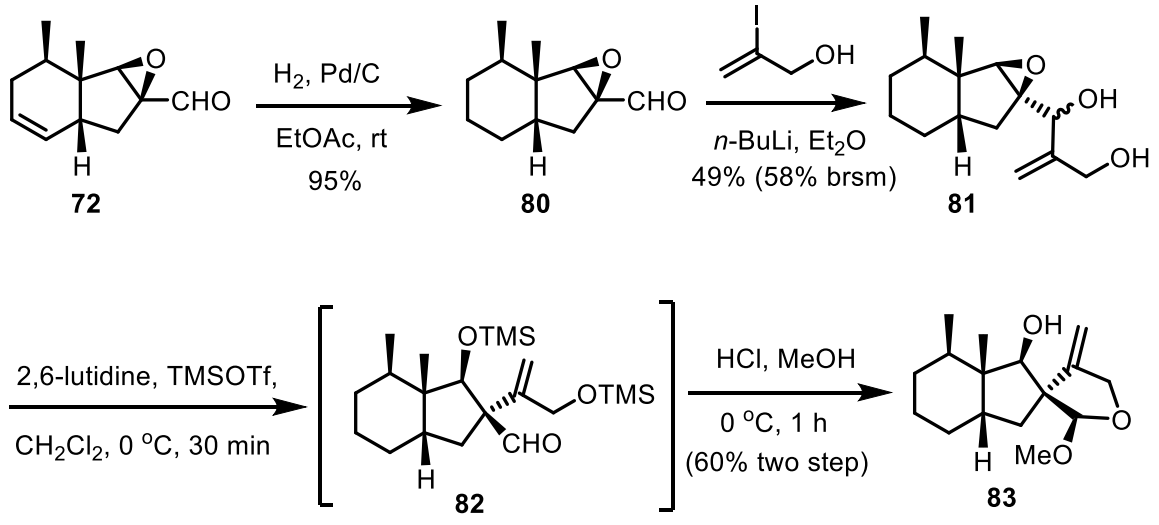


Fig 1.8: Single X-ray crystal structure of epoxide **76** and key NOE correlations on energy minimized structures of **77**, **78** and **79**

A close inspection of the ^{1}H and ^{13}C NMR spectrum of compound **77** by DEPT and ^{1}H-^{13}C COSY experiments revealed the presence of one vinylidene (C-11 and C-13), one secondary methyl (C-14), one tertiary methyl (C-15), four *sp*3- hybridized methylenes (C-1, C-3, C-9 and C-12) including one oxygen-bearing carbon (C-12), five *sp*3-methines (C-2,

C-4, C-6, C-8 and C- 10) including three oxymethines (C-2, C-6 and C-8), two quarternary *sp*3-carbons (C-5 and C-7) and one methoxyl group (8-OMe). The stereochemistry of **77** was deduced from NOESY experiments. NOE correlations from C5-CH_3 to C2β-H and from C10-H to C2β-H suggested that C2β-H is oriented *cis* to both C5- methyl group and C10-H in equatorial arrangement. The stereochemistry of **78** was deduced from NOESY experiments. NOE correlations from C1β-H and C5-CH_3, C4-CH_3 suggested that C1β-H is oriented *cis* to both C5 and C4 methyl groups in axial arrangement. In addition to NOEs from C1β-H to C10-H was indicated that both the hydrogens are *cis* to each other. In case of **79** such correlations are absent which indirectly suggested the presence of equatorial arrangement of hydrogen in structure **79**.

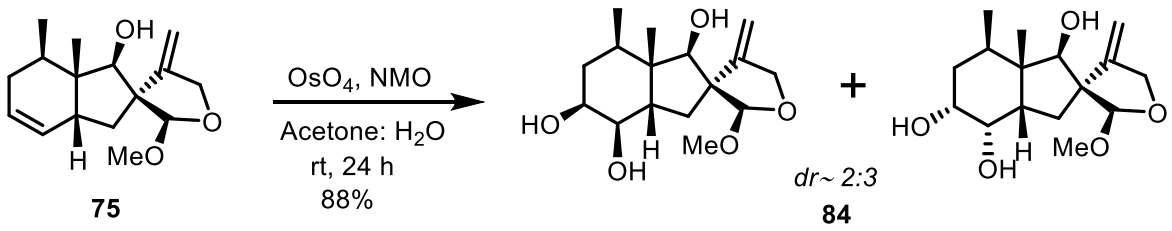
The peribysins A and D showed more potent activity than other peribysins in cell adhesion inhibition assay. As these two peribysins do not contain hydroxyl functionality in six membered ring, we have also synthesized deoxyperibysin E analogue **83** hoping that it may show better activity. Towards this, the intermediate **72** was hydrogenated using H_2, Pd/C in EtOAc solvent to give corresponding saturated hydrindane **80** in 95% yield as a colorless oil. This transformation was indicated by disappearance of olefinic protons in ^{1}H NMR and olefinic carbon in ^{13}C NMR spectra and which was further confirmed by HRMS which showed a peak at 217.1199 corresponding to formula $C_{12}H_{18}O_2Na$ $[M+Na]^+$ with calculated value of 217.1195.



Scheme 1.12: Synthesis of peribysin E analogue **83**

By following the same three-step sequence, comprising dianion addition, 2,6-lutidine followed by TMSOTf treatment (semipinacol-type rearrangement), and subsequent exposure to methanolic HCl, resulted in the deoxy-peribysin E analogue (**83**) (scheme 1.12). The product formation was confirmed by presence of terminal olefinic proton at 5.00 (t, *J* = 2.4 Hz, 1H), 4.98 (t, *J* = 1.8 Hz, 1H), CH-OH protons at 3.59 (d, *J* = 3.2 Hz, 1H), OCH_3 protons at 3.38 (s, 3H), in ^{1}H NMR spectrum and olefinic carbon at 152.2, 103.1 ppm, CH-OH carbon at 89.1 and OCH_3 carbon at 55.2 in ^{13}C NMR spectrum and which was further confirmed by HRMS which showed a peak at 289.1618.

To synthesize more diverse analogues of peribysin E, isolated double bond in six membered ring of compound **75** was selectively dihydroxylated using OsO_4-NMO in acetone: H_2O in 4:1 ratio to furnish a mixture of triols **84** in 88% yield as *3:2 dr* ratio in a highly chemoselective manner[43] (scheme1.13). Unfortunately at this stage we were unable to separate the mixture of triols using silica gel column chromatography or Combiflash MPLC. The formation of triols **84** was indicated by appearance of OHCH-CHOH protons at 4.02−3.76 (m, 2H) ppm in ^{1}H-NMR. The product formation was further confirmed by HRMS which showed a peak at 321.1723 corresponding to formula $C_{16}H_{26}O_4Na$ $[M+Na]^+$ with calculated value of 321.1720.

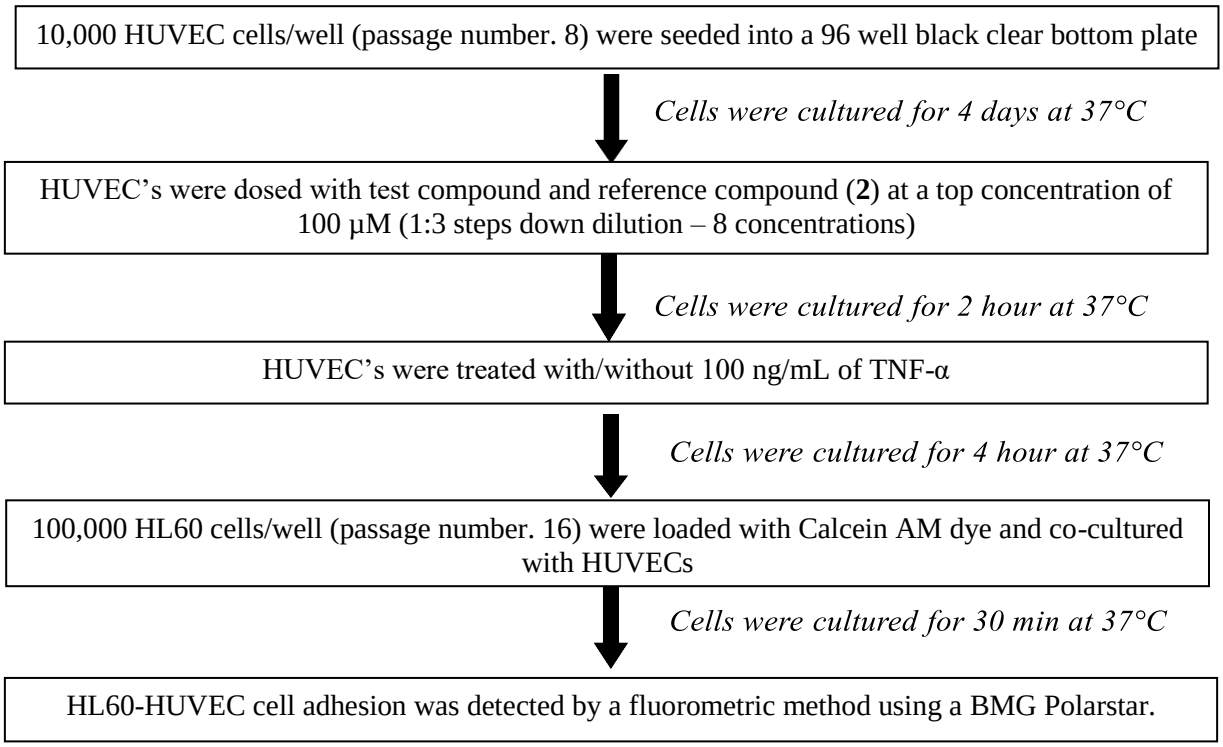


Scheme 1.13: Synthesis of peribysin E analogue **84**

After successful synthesis of peribysin E and their analogues, all the synthesized compounds were evaluated for their cell adhesion inhibition potential.

1.3.4 Biological evaluation of peribysin E and its analogues

The cell adhesion inhibition activity of synthesized analogues was determined in collaboration with GVK Biosciences Private Limited, Hyderabad, India. The main aim of this study was to determine the IC_{50} value of synthesized compounds on cell adhesion between Human promyelocytic Leukemia cells (HL60) and Human Umbilical Vein Endothelial cells (HUVEC). We have screened eight synthesized compounds including one reference compound (**2**) to identify potent inhibitors on HL60 and HUVEC cell adhesion by using a Vybrant cell adhesion assay kit with a fluorometric method for detection. An eight point dose dependent assay was carried out to assess the IC_{50}'s of the test compounds. Following flow chart for the method was adopted for the assay.



A dose-response curve indicates the relationship between increasing the concentration (or dose) of the drug and the change in response. The concentration (or dose) in these curves is represented using a semi-logarithmic plot. Accordingly, we have plotted a semi-logarithmic, the amount of drug is plotted (on the X axis) as the log of drug concentration of different compounds and response (% inhibition) is plotted (on the Y axis) using a linear scale (Fig 1.9).

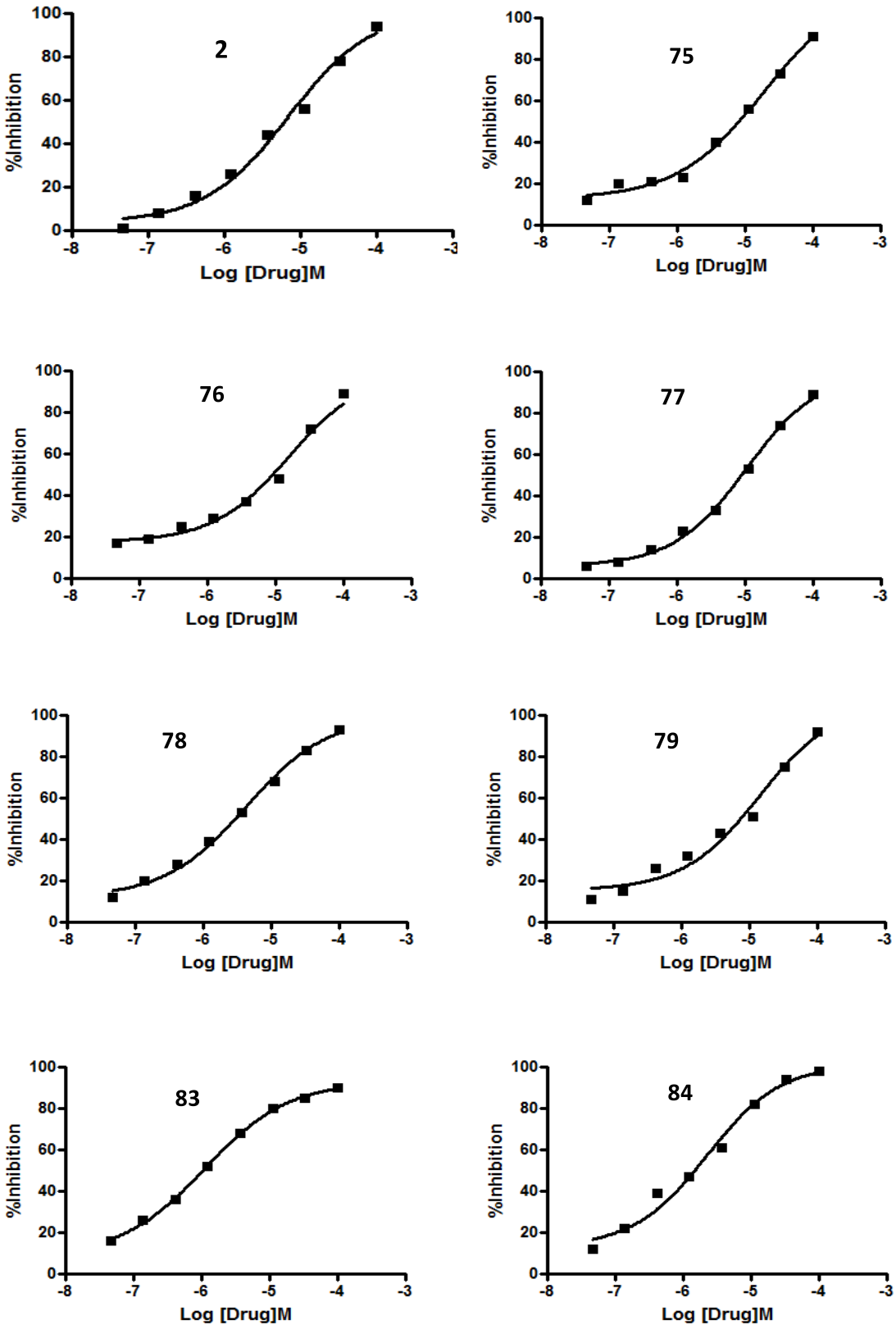


Fig 1.9: % Inhibition *vs* log of drug concentration

Table 1.2: HL60-HUVEC Cell adhesion-IC_{50} assessment of compounds

Compound No	IC_{50}	Hill Slope	R^2	Max % Binding	Max Conc µM	Min % Binding	Min Conc nM	No. of points used for the DRC
2	6.9 µM	0.8	0.98	94	100	1	45.7	8
75	18.7 µM	0.67	0.99	91	100	12	45.7	8
76	15.8 µM	0.78	0.98	89	100	17	45.7	8
77	10.4 µM	0.81	0.99	89	100	6	45.7	8
78	4.28 µM	0.72	0.99	93	100	12	45.7	8
2	12.2 µM	0.8	0.97	99	100	10	45.7	8
79	13.8 µM	0.78	0.97	92	100	11	45.7	8
83	11.0 µM	0.7	0.99	90	100	16	45.7	8
84	2.23 µM	0.8	0.98	98	100	12	45.7	8

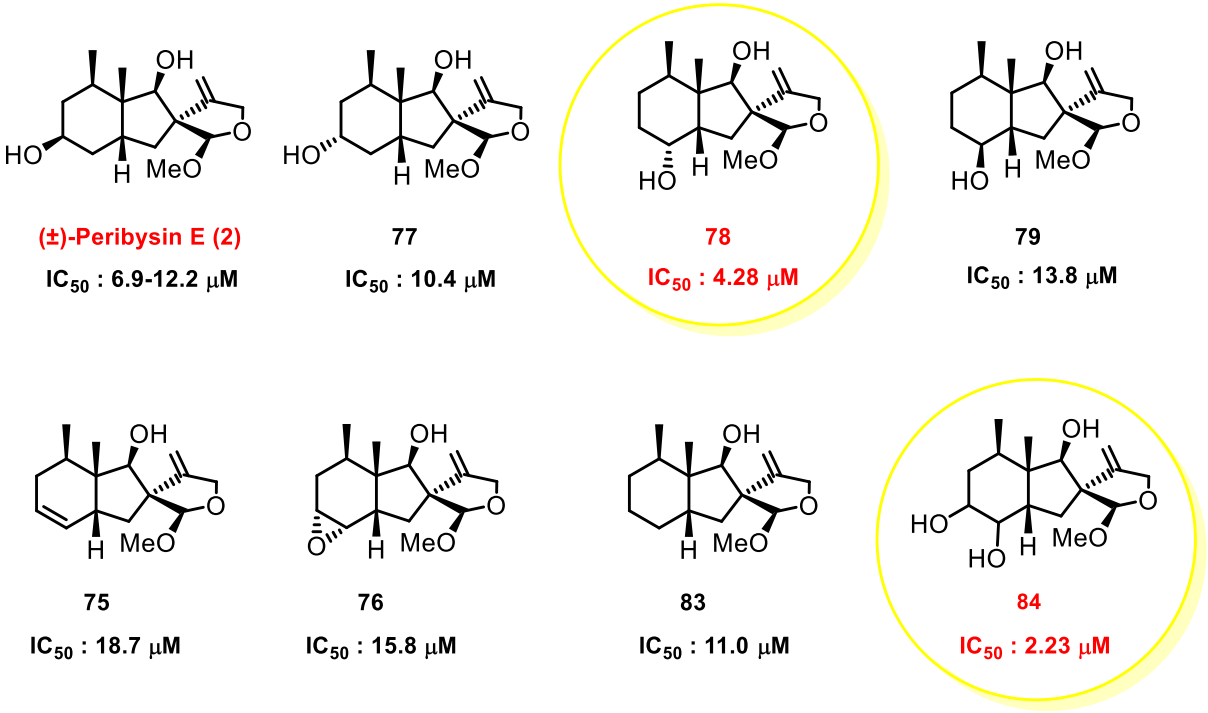


Fig 1.10: HL60-HUVEC Cell adhesion-IC_{50} assessment of compound

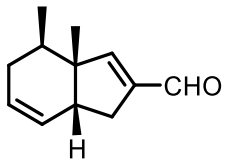
Based on initial results it is found that peribysin E **2** (reference compound) has an IC_{50} in the variable range (Plate 1: 6.9 µM and Plate 2: 12.2 µM respectively). It is worth mentioning that our synthesized Peribysin E is racemic, hence expected to show less activity when compared with enantiopure natural Peribysin E. The synthesized compounds were found to be potent inhibitors against the adhesion between HL60 and HUVEC cells, this was corroborated by their IC_{50}'s which were in the micromolar range. Their IC_{50}'s are shown in table 1.2. A couple of new analogues compound **78** and compound **84** are found to be better active than the peribysin E (Fig 1.10). Addition of one more hydroxy group in six membered ring in compound **84** increases the activity as compared to racemic peribysin E **2**. As our synthesized compounds including compound **2** are racemic, making enantiopure versions of these compounds is expected to increase potency of these molecules further.

1.4 Conclusions

We have achieved the total synthesis of peribysin E using a short, efficient and protecting group free sequence. Our synthetic route is better than previously published routes by Danishefsky and Sha groups. We have also synthesized eight new analogues of the natural product by means of "diverted total synthesis" in less than 10 steps. The present synthesis highlights the sequential Diels-Alder/aldol, regioselective Wacker oxidation, semi-pinacol type rearrangement. Cell adhesion inhibitory activity of synthesized compounds was evaluated and two new analogues were found to be better in activity than peribysin E. Efforts to synthesize other members of peribysin family is presently underway in the group.

1.5 Experimental Procedures

(3a*S*,4*R*,7a*R*)-3a,4-Dimethyl-3a,4,5,7a-tetrahydro-1H-indene-2-carbaldehyde (62):



To a solution of diene **31** (100 mg, 0.91 mmol) and (*E*)-2-methylbut- 2-enal (0.22 mL, 2.27 mmol) in dry CH_2Cl_2 (15 mL) was added $BF_3{\cdot}OEt_2$ (0.23 mL, 1.81 mmol) dropwise at –78

°C. The mixture was allowed to warm to room temperature and was stirred for 8 h at same temperature. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (3 x 10 mL) followed by H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound **64** was dissolved in methanol (5 mL), cooled to 0 °C, and 15% aq. KOH (5 mL) was added dropwise. After stirring for 1 h, diluted with 50 mL of petroleum ether, washed with water (10 mL), 1N HCl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether) to afford **62** (16 mg, 10% for two steps) as light yellow oil.

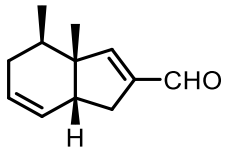
IRυ_{max} (film): 2960, 2919, 1681 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.72 (s, 1H), 6.76 (s, 1H), 5.66–5.63 (m, 1H), 5.42–5.39 (m, 1H), 2.82–2.75 (m, 1H), 2.57–2.54 (m, 1H), 2.32–2.28 (d, *J* = 16.0 Hz, 1H), 1.98–1.94 (m, 1H), 1.76–1.68 (m, 2H), 1.13 (s, 3H), 1.06 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 190.3, 156.8, 145.9, 130.8, 126.0, 52.0, 45.7, 35.2, 33.7, 31.2, 23.8, 16.6.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1275, found 177.1274.

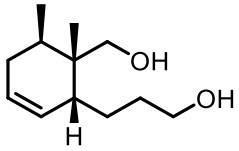
(3a*S*,4*R*,7a*R*)-3a,4-dimethyl-3a,4,5,7a-tetrahydro-1H-indene-2-carbaldehyde (62):



A solution of diene **31** (120 mg, 1.09 mmol) and (*E*)-2-methylbut- 2-enal (0.21 mL, 2.18 mmol) in 10 mL of CH_2Cl_2 was cooled to –78 °C under N_2. To this cold solution, Et_2AlCl (2M in toluene) (0.82 mL, 1.63 mmol) was added slowly, and then allowed to warm up to room temperature. After overnight stirring at room temperature, reaction mixture was poured into crushed ice and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layer was washed with water (10 mL), brine (10 mL) and dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound **64** was dissolved in methanol (5 mL), cooled to 0 °C, and 15% aq. KOH (5 mL) was added dropwise. After stirring for 1 h, diluted with

50 mL of petroleum ether, washed with water (10 mL), 1N HCl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether) to afford **62** (29 mg, 15% for two steps) as light yellow oil. Spectral data was identical with that of above compound **62.**

3-((1*R*,5*R*,6*S*)-6-(hydroxymethyl)-5,6-dimethylcyclohex-2-en-1-yl)propan-1-ol (67):



To a solution of diene **65** (6.0 g, 38.9 mmol) and (*E*)-2-methylbut- 2-enal (9.3 mL, 96.7 mmol) in dry CH_2Cl_2 (200 mL) was added $BF_3{\cdot}OEt_2$ (9.5 mL, 77.4 mmol) dropwise at –78 °C. The mixture was allowed to warm to room temperature and was stirred for 8 h at same temperature. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (3 x 100 mL) followed by H_2O (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue **66** obtained was directly used for next step without further purification. The crude material **66** in dry THF (50 mL) was added dropwise to suspension of lithium aluminum hydride (3.64 g, 95.7 mmol) in dry THF (40 mL) at 0 °C and stirred for 2 h at room temperature. The reaction was quenched with saturated Na_2SO_4 (25 mL) and ethyl acetate (50 mL) by slow addition. The resulting suspension was filtered through a pad of celite and the solvent was removed *in vacuo.* The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 4:6 ethyl acetate: petroleum ether) to afford diol **67** (5.4 g, 71% for two steps) as a white solid.

M.p: 105-108 °C.

IRυ_{max} (film): 3339, 3019, 2930, 1654 cm^{-1}.

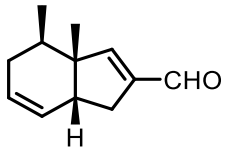
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72–5.69 (m, 1H), 5.59–5.56 (m, 1H), 3.73–3.60 (m, 2H), 3.54–3.52 (m, 2H), 2.56 (brs, 1H), 2.37 (brs, 1H), 2.06–2.00 (m, 1H), 1.89–1.86 (m, 1H), 1.78–1.67 (m, 4H), 1.56–1.46 (m, 1H), 1.21–1.13 (m, 1H), 0.88 (s, 3H), 0.78 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 129.5, 125.4, 67.9, 62.2, 40.6, 38.8, 31.8, 30.0, 29.7, 26.6,

16.8, 15.0.

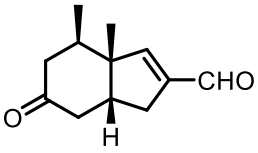
HRMS (ESI): *m/z* calcd for $C_{12}H_{22}O_2Na$ $(M+Na)^+$ 221.1510, found 221.1512.

(3a*S*,4*R*,7a*R*)-3a,4-Dimethyl-3a,4,5,7a-tetrahydro-1H-indene-2-carbaldehyde (62):



To a solution of pyridine (32.8 mL, 407 mmol) in dry CH_2Cl_2 (150 mL) was added CrO_3 (20.3 g, 203 mmol). The mixture was stirred for 30 min at room temperature. Then compound **67** (3.3 g, 16.6 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise at room temperature. After 2 h, diethyl ether (200 mL) was added and the mixture was filtered. The organic layer was washed with 1N HCl (150 mL) followed by saturated $NaHCO_3$ (150 mL), water (100 mL), and brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude compound **64** was dissolved in methanol (25 mL), cooled to 0 °C, and 15% aq. KOH (20 mL) was added dropwise. After stirring for 1 h, diluted with 150 mL of petroleum ether and washed with water (50 mL), 1N HCl (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether) to afford **62** (1.9 g, 66% for two steps) as light yellow oil. Spectral data was identical with that of above compound **62**.

(3a*S*,4*R*,7a*R*)-3a,4-Dimethyl-6-oxo-3a,4,5,6,7,7a-hexahydro-1H-indene-2-carbaldehyde (68):



A compound **62** (1.5 g, 8.52 mmol) was added to a stirred solution of $PdCl_2$ (0.15 g, 10 mol %) and CuCl (0.93 g, 9.37 mmol) in dimethylacetamide (DMA) and H_2O (40 mL, 36: 4) under oxygen atmosphere. The resulting dark brown solution was stirred vigorously for 12 h at 50 °C and then extracted with ethyl acetate (2 x 50 mL). The combined organic layer

was washed with water (25 mL) followed by brine (25 mL), dried over anhydrous Na_2SO_4 and solvent was evaporated under reduced pressure. The crude residue was purified by column chromatography (silica gel 100–200, 3:7 ethyl acetate: petroleum ether) to afford **68** (1.34 g, 82%) as a colorless oil.

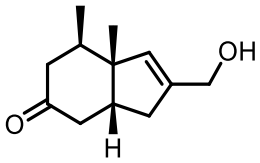
IRυ_{max} (film): 3414, 2963, 1713, 1677 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 6.77 (s, 1H), 2.89–2.83 (m, 1H), 2.42–2.37 (m, 2H), 2.36–2.34 (m, 1H), 2.29–2.24 (m, 1H), 2.23–2.22 (m, 1H), 2.21–2.20 (m, 1H), 2.15–2.09 (m, 1H), 1.10 (s, 3H), 1.03 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 211.4, 190.3, 158.2, 144.0, 50.86, 44.6, 43.8, 42.9, 34.7, 34.6, 18.8, 16.4.

HRMS (ESI): *m/z* calcd for $C_{12}H_{16}O_2Na$ $(M+Na)^+$ 215.1044, found 215.1043.

(3a*S*,4*R*,7a*R*)-2-(Hydroxymethyl)-3a,4-dimethyl-4,5,7,7a-tetrahydro-1H-inden-6(3aH)-one (69):



To a solution of **68** (1.3 g, 6.77 mmol) in EtOH (30 mL), was added $NaBH_4$ (128 mg, 3.38 mmol) in portion wise at −10 ºC. After stirring for 5 min at same temperature, reaction was quenched with saturated aqueous NH_4Cl (10 mL). The reaction mixture was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The silica gel flash column chromatography (ethyl acetate: petroleum ether, 4:6) afforded the **69** (1.0 g, 78%) as a colorless oil.

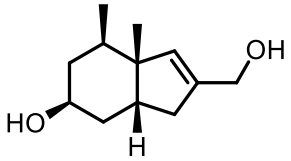
IRυ_{max} (film): 3404, 2958, 2926, 1711 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.62 (s, 1H), 4.16 (s, 2H), 2.68–2.62 (m, 1H), 2.40–2.38 (m, 2H), 2.31–2.24 (m, 1H), 2.17–2.15 (m, 2H), 2.09–2.03 (m, 2H), 1.69–1.67 (m, 1H), 1.03 (s, 3H), 0.95 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 213.2, 141.4, 133.3, 62.0, 49.3, 45.2, 44.2, 43.6, 38.9, 35.6, 19.8, 16.4.

HRMS (ESI): *m/z* calcd for $C_{12}H_{18}O_2Na$ $(M+Na)^+$ 217.1199, found 217.1199.

(3a*S*,4*R*,6*S*,7a*R*)-2-(Hydroxymethyl)-3a,4-dimethyl-3a,4,5,6,7,7a-hexahydro-1H-inden-6- ol (70):



A solution of ketone **69** (1.0 g, 5.62 mmol) in THF (30 mL) was added to liquid ammonia (30 mL) at −78 °C. Lithium (0.394 g, 56.2 mmol) was added in small pieces and reaction mixture was stirred at −78 °C for 2 h. After consumption of starting material (by TLC), solid NH_4Cl (3.0 g) was added and ammonia was allowed to evaporate at room temperature. Water (20 mL) was added and reaction mixture was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (30 mL), dried over Na_2SO_4, and concentrated. The residue obtained was subjected to silica gel flash column chromatography (ethyl acetate: petroleum ether, 4:6) to afford the diol **70** (0.73 g, 73%) as a colorless oil.

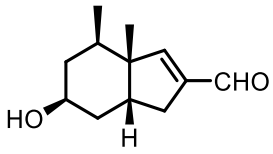
IRυ_{max} (film): 3351, 2921, 1649, 1461 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.69 (s, 1H), 4.12 (s, 2H), 3.85–3.79 (m, 1H), 2.28–2.12 (m, 3H), 1.96–1.93 (m, 1H), 1.64–1.57 (m, 3H), 1.52–1.45 (m, 2H), 1.16–1.07 (m, 1H), 0.97 (s, 3H), 0.85 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.8, 136.6, 68.5, 62.4, 48.4, 47.5, 38.9, 36.4, 36.1, 34.4, 17.7, 17.2.

HRMS (ESI): *m/z* calcd for $C_{12}H_{20}O_2Na$ $(M+Na)^+$ 219.1356, found 219.1356.

(3aS,4R,6S,7aR)-6-Hydroxy-3a,4-dimethyl-3a,4,5,6,7,7a-hexahydro-1H-indene-2-carbaldehyde (61):



To a stirred solution of **70** (0.70 g, 3.81 mmol) in CH_2Cl_2 (20 mL) was added MnO_2 (3.3 g, 38.1 mmol) at room temperature. The reaction mixture was stirred for 20 h at room temperature. After completion of reaction, filtered through a pad of celite and washed with CH_2Cl_2 and the solvent was removed *in vacuo*. The crude material obtained after the

removal of solvent was purified by column chromatography (silica gel 100–200, 4:6 ethyl acetate: petroleum ether) to afford **61** (0.64 g, 92%) as a colorless oil.

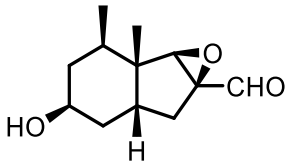
IRυ_{max} (film): 3401, 2959, 1681 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.68 (s, 1H), 6.86 (s, 1H), 3.85–3.81 (m, 1H), 2.58–2.52 (m, 1H), 2.38–2.31 (m, 1H), 2.23–2.17 (m, 1H), 2.01–1.98 (m, 1H), 1.79–1.78 (m, 1H), 1.66–1.62 (m, 1H), 1.56–1.47 (m, 2H), 1.24–1.14 (m, 1H), 1.10 (s, 3H), 0.91 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): 190.8, 162.7, 144.8, 67.8, 49.4, 47.7, 38.5, 35.6, 33.9, 32.1, 17.2, 16.8.

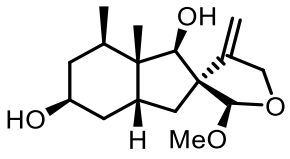
HRMS (ESI): *m/z* calcd for $C_{12}H_{19}O_2$ $[M+H]^+$ 195.1380, found 195.1382.

(1a*R*,1b*S*,2*R*,4*S*,5a*S*,6a*S*)-4-Hydroxy-1b,2-dimethyloctahydro-6aH-indeno[1,2-b] oxirene -6a-carbaldehyde (71):



A compound **61** (0.52 gm, 2.68 mmol) was dissolved in methanol (10 mL), cooled to 0 °C and added 30% aqueous H_2O_2 (0.75 mL, 6.70 mmol) and 6N NaOH (0.26 mL, 1.55 mmol). The reaction mixture was stirred at room temperature for 4 h. The mixture was diluted in diethyl ether (30 mL), washed successively with water (10 mL), brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after removal of solvent was pass through small bed of silica gel column chromatography (ethyl acetate: petroleum ether 4:6) to give the epoxide **71** (0.394 g, 70%) as colorless oil which is immediately used for next step.

(1′*R*,2*R*,2′*R*,3a′*R*,5′*S*,7′*R*,7a′*S*)-2-Methoxy-7′,7a′-dimethyl-4-methylenedecahydro-2H-spiro-[furan-3,2′-indene]-1′,5′-diol((±)-peribysin E (2):



(±)-peribysin E (**2**)

To a solution of *n*–BuLi (1.9 mL, 2.0 M solution in cyclohexane, 3.81 mmol) in Et_2O (8 mL) at −78 °C was added iodo alcohol (348 mg, 1.90 mmol) in Et_2O (8 mL) dropwise over a period of 30 min with vigorous stirring. A solution of aldehyde **71** (100 mg, 0.47 mmol) in THF (5 mL) was added slowly. The reaction mixture was then stirred for 2 h at same temperature and then quenched with saturated aqueous NH_4Cl (10 mL). The reaction mixture was extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, and concentrated. Silica gel flash column chromatography (EtOAc: petroleum ether, 7:3) afforded the diol **60** (41 mg, 33%, 54% brsm) as a diastereomeric mixture (colorless oil) which was directly used for next step. To a solution of **60** (40 mg, 0.149 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C was added 2, 6–lutidine (0.27 mL, 2.38 mmol) followed by TMSOTf (0.21 mL, 1.19 mmol). Reaction mixture was stirred for 30 min at same temperature and then quenched with saturated $NaHCO_3$ (10 mL). The reaction mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4 and concentrated to give crude product. To a solution of the crude product in MeOH (8 mL) at 0 °C was added 35% HCl (0.02 mL). The reaction mixture was stirred for 1 h and then quenched with saturated $NaHCO_3$ (10 mL). The reaction mixture was extracted with EtOAc (3 x 15 mL).The combined organic layer was washed with brine (15 mL), dried over Na_2SO_4, and concentrated which on silica gel flash column chromatography (ethyl acetate: petroleum ether, 1:2) afforded (±)-peribysin E (**2**) (26 mg, 62%) as a colorless oil.

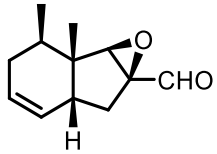
IR υ_{max} (film): 3404, 2923, 2926, 2864, 1655, 1263 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.08 (s, 1H), 4.98 (t, *J* = 1.83 Hz, 1H), 4.94 (t, *J* = 2.4 Hz, 1H), 4.49 (dt, *J* = 13.1, 2.4 Hz, 1H), 4.47 (dt, *J* = 12.9, 2.4 Hz, 1H), 3.94−3.88 (m, 1H), 3.56 (d, *J* = 2.1 Hz, 1H), 3.38 (s, 3H), 2.19 (d, *J* = 2.7 Hz, 1H), 2.03−1.97 (m, 1H), 1.94 (ddt, *J* = 13.4, 4.5, 2.4 Hz, 1H), 1.88 (dd, *J* = 13.1, 5.8 Hz, 1H), 1.78−1.75 (m, 1H), 1.72−1.68 (m, 1H), 1.64−1.63 (m, 1H), 1.57−1.55 (m, 1H), 1.54−1.48 (m, 1H), 1.30−1.21 (m, 1H), 0.92 (s, 3H), 0.86 (d, *J* =6.8 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 152.7, 105.7, 103.2, 88.7, 69.1, 67.2, 60.9, 55.2, 45.9 (2C), 40.1, 35.5, 34.3, 33.0, 16.2, 14.5.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_4Na$ $(M+Na)^+$ 305.1721, found 305.1723.

(1a*R*,1b*S*,2*R*,5a*R*,6a*S*)-1b,2-Dimethyl-1b,2,3,5a,6,6a-hexahydro-1aH-indeno[1,2-b] oxirene-6a-carbaldehyde (72):



To a stirred solution of the α,β–unsaturated aldehyde **62** (1.0 g, 5.68 mmol) in methanol (10 mL) was added 30% aqueous H_2O_2 (1.6 mL, 14.2 mmol) and 6N NaOH (0.55 mL, 3.29 mmol) at 0 °C. The reaction was stirred at room temperature for 4 h. The mixture was diluted in diethyl ether (50 mL), washed successively with water (20 mL), brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material was purified by column chromatography (silica gel 100–200, 1:9 ethyl acetate: petroleum ether) to afford epoxide **72** (780 mg, 74%) as colorless oil.

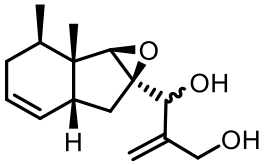
IRυ_{max} (film): 2964, 2884, 1721, 1454 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.19 (s, 1H), 5.64–5.63 (m, 2H), 3.71 (s, 1H), 2.21–2.18 (m, 1H), 1.97–1.87 (m, 3H), 1.76–1.69 (m, 1H), 1.52–1.47 (m, 1H), 1.00 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.5, 126.7, 125.9, 68.5, 67.5, 42.8, 40.8, 30.7, 30.4, 29.5, 15.5, 14.1.

HRMS (ESI): *m/z* calcd for $C_{12}H_{16}O_2Na$ $(M+Na)^+$ 215.1041, found 215.1043.

1-((1a*R*,1b*S*,2*R*,5a*R*,6a*R*)-1b,2-Dimethyl-1b,2,3,5a,6,6a-hexahydro-1aH-indeno[1,2-b]oxiren-6a-yl)-2-methylenepropane-1,3-diol (73):



To a stirred solution of *n*–BuLi (7.8 mL, 2.0 M solution in cyclohexane, 15.6 mmol) in Et_2O (15 mL) at −78 °C was added 2-iodoprop-2-en-1-ol (1.42 g, 7.81 mmol) in Et_2O (15 mL) dropwise over a period of 30 min with vigorous stirring. A solution of aldehyde **72** (0.5 g, 2.60 mmol) in Et_2O (10 mL) was added dropwise at −78 °C. The reaction mixture was stirred for 2 h at same temperature and then quenched with saturated aqueous NH_4Cl

(20 mL). The reaction mixture was extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine (30 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by column chromatography (silica gel 100–200, 4:6 ethyl acetate: petroleum ether) to afford diol **73** (0.305 g, 46%, 61% brsm) as a diastereomeric mixture (colorless oil). One diastereomer (R_f = 0.2) was isolated with silica gel flash column chromatography (ethyl acetate: petroleum ether 4:6) as eluent.

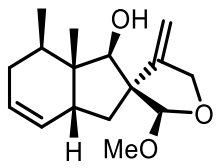
IRυ_{max} (film): 3393, 3019, 1421, 1215, cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.60–5.59 (m, 2H), 5.25 (d, *J* = 1.2 Hz, 1H), 5.16 (d, *J* = 0.9 Hz, 1H), 4.50 (s, 1H), 4.25 (d, *J* = 13.5 Hz, 1H), 4.12 (d, *J* = 14.1 Hz, 1H), 3.47 (s, 1H), 2.89 (brs, 1H), 2.76 (brs, 1H), 2.13–2.07 (m, 1H), 1.93–1.86 (m, 2H), 1.75−1.68 (m, 1H), 1.51−1.44 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.93 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 146.2, 127.4, 125.7, 115.4, 73.2, 68.7, 66.9, 63.7, 42.3, 41.5, 33.4, 30.5, 30.0, 15.5, 13.6.

HRMS (ESI): *m/z* calcd for $C_{15}H_{22}O_3Na$ $(M+Na)^+$ 273.1464, found 273.1461.

(2*R*,2'*R*,3'*R*,3a'*S*,4'*R*,7a'*R*)-2-Methoxy-3a',4'-dimethyl-4-methylene-1',3',3a',4,4',5,5',7a'- octahydro-2H-spiro[furan-3,2'-inden]-3'-ol (75):



To a stirred solution of **73** (160 mg, 0.64 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C was added 2,6–lutidine (1.1 mL, 8.96 mmol) followed by TMSOTf (0.89 mL, 4.48 mmol). Reaction mixture was stirred for 30 min at same temperature and then quenched with saturated $NaHCO_3$ (20 mL). The reaction mixture was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layer was washed with brine (15 mL) and dried over Na_2SO_4, filtered and concentrated *in vacuo* to give crude product **74**. To a solution of the crude product **74** in MeOH (20 mL) was added 35% HCl (0.05 mL). The reaction mixture was stirred for 1 h and then quenched with saturated $NaHCO_3$ (15 mL). The reaction mixture was extracted with EtOAc (3 x 30 mL).The combined organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Silica gel flash column

chromatography (ethyl acetate: petroleum ether 2:8) afforded **75** (108 mg, 64%) as a colorless oil.

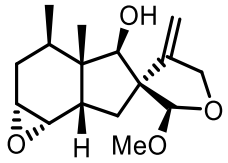
IRυ_{max} (film): 3478, 2927, 1660, 1452, 1042 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71–5.62 (m, 2H), 5.12 (s, 1H), 5.00 (t, *J* = 2.0 Hz, 1H), 4.96 (t, *J* = 2.4 Hz, 1H), 4.49 (dt, *J* = 11.3, 2.4 Hz, 1H), 4.41 (dt, *J* = 11.2, 2.7 Hz, 1H), 3.60 (d, *J* = 3.4 Hz, 1H), 3.40 (s, 3H), 2.15 (d, *J* = 3.9 Hz, 1H), 2.10–2.06 (m, 2H), 1.98–1.92 (m, 1H), 1.85–1.82 (m, 1H), 1.56– 1.51 (m, 1H), 1.44–1.39 (m, 1H), 0.93 (d , *J* = 6.7 Hz, 3H), 0.87 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.1, 128.7, 126.0, 105.6, 103.4, 90.2, 69.0, 60.4, 55.1, 46.8, 44.4, 35.6, 34.7, 30.8, 15.2 (2C).

HRMS (ESI): *m/z* calcd for $C_{16}H_{24}O_3Na$ $(M+Na)^+$ 287.1609, found 287.1618.

(1a'*R*,2*R*,3*R*,3'*R*,3a'*S*,4'*R*,6a'*S*,6b'*S*)-2-Methoxy-3',3a'-dimethyl-4-methylenedeca hydro- 2H-spiro[furan-3,5'-indeno[4,5-b]oxiren]-4'-ol (76):



To a stirred solution of **75** (170 mg, 0.643 mmol) in CH_2Cl_2 (20 mL) and 15% $NaHCO_3$ (1.8 mL, 3.21 mmol) at 0 °C was added *m*-CPBA (132 mg, 0.77 mmol) in portions. The mixture was stirred at 0 °C for 2 h and filtered. The filtrate was washed successively with saturated aqueous $NaHCO_3$ (10 mL), H_2O (10 mL) then with brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a solid, which was purified by Silica gel flash column chromatography (ethyl acetate: petroleum ether 2:8) afforded **76** (128 mg, 72%) as a white solid.

M.p: 82–84 °C.

IRυ_{max} (film): 3435, 2928, 1661, 1035 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.05 (s, 1H), 5.01 (m, 2H), 4.49 (dt, *J* = 13.2, 2.1 Hz, 1H), 4.42 (dt, *J* = 13.1, 2.4 Hz, 1H), 3.45 (d, *J* = 3.2 Hz, 1H), 3.40 (s, 3H), 3.27–3.26 (m, 1H), 3.17–3.15 (m, 1H), 2.14 (d, *J* = 3.9 Hz, 1H), 2.50−2.00 (m, 1H), 1.93–1.88 (m, 2H), 1.77–1.71 (m, 1H), 1.65–1.63 (m, 2H), 0.88–0.83 (m, 6H).

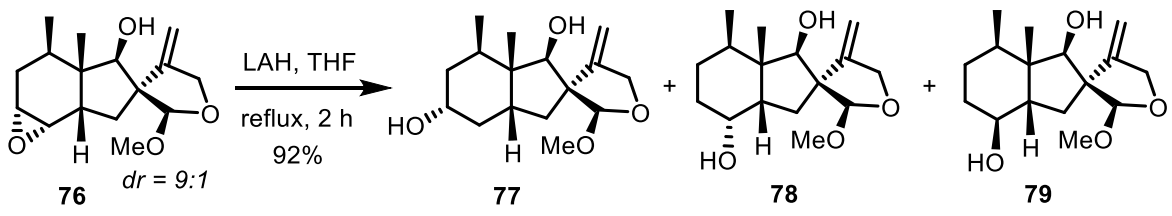
^{13}C NMR (100 MHz, $CDCl_3$): δ 151.4, 105.4, 103.8, 90.8, 69.3, 59.4, 55.2, 53.4, 53.2, 44.7, 42.7, 32.5, 30.2, 29.52, 16.1, 14.9.

HRMS (ESI): *m/z* calcd for $C_{16}H_{24}O_4Na$ $(M+Na)^+$ 303.1564, found 303.1567.

A single crystal of compound 76: A single crystal of compound **76** was obtained from ethyl acetate-hexane mixture. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at room temperature 296(2) K. The X-ray generator was operated at 50 kV and 30 mA. Diffraction data were collected with a ω scan width of 0.5° and at different settings of φ and 2θ. The sample-to-detector distance was fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX II program suite. All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX II program package. The structure was solved by direct method and refined by full matrix least squares, based on *F*, using SHELX-97. Molecular diagrams were generated using XSHELL program integrated in SHELXTL package. All the H-atoms were placed in geometrically idealized position and constrained to ride on their parent atoms.

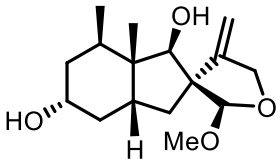
Crystallographic data for 76 ($C_{16}H_{24}O_4$): M = 280.35, Crystal dimensions 0.62 x 0.41 x 0.16 mm^3, triclinic, space group P -1, a = 6.9099(6), b = 18.7473(16), c = 11.4333(10) Å, α = 90.0 (2), β = 98.401 (2), γ =90.0 (2)°, V = 1465.2(2) Å^3, Z = 4, ρ_{calcd} = 1.271 gcm^{-3}, μ (Mo-Kα) = 0.090 mm^{-1}, *F(000)* = 608, $2\theta_{max}$ = 56.76°, T = 296(2) K, 14390 reflections collected, 3586 unique, 3084 observed ($I > 2\sigma(I)$) reflections, 176 refined parameters, R value 0.04796, $wR2$ = 0.1437, S = 1.028, minimum and maximum transmission 0.951 and 0.987; maximum and minimum residual electron densities +0.30 and –0.18 eÅ^{-3}.

LiAlH$_4$ reduction of (1a'*R*,2*R*,3*R*,3'*R*,3a'*S*,4'*R*,6a'*S*,6b'*S*)-2-Methoxy-3',3a'-dimethyl-4-methylenedecahydro-2H-spiro[furan-3,5'-indeno[4,5-b]oxiren]-4'-ol (76):



To a stirred suspension of $LiAlH_4$ (135 mg, 3.57 mmol) in dry THF (10 mL) was slowly added a solution of epoxide **76** (125 mg, 0.44 mmol) in dry THF (10 mL) at room temperature. The resulting suspension was heated at reflux for 2 h, and then allowed to cool to room temperature. EtOAc (20 mL) was added followed by saturated aqueous NH_4Cl (10 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by MPLC (combiflash *rf*) column chromatography (ethyl acetate: petroleum ether, 3:7) afforded corresponding three diols **77** (45 mg, 36%), **78** (54 mg, 43%) and **79** (16 mg, 13%) as a colorless oil.

(1'*R*,2*R*,2'*R*,3a'*R*,5'*R*,7'*R*,7a'*S*)-2-Methoxy-7',7a'-dimethyl-4-methylenedecahydro-2Hspiro[furan-3,2'-indene]-1',5'-diol (77):



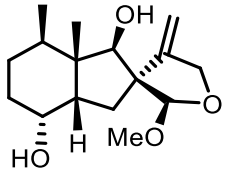
IRυ_{max} (film): 3401, 2926, 1661, 1459, 1044 $cm^{-1.}$

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.10 (s, 1H), 5.01 (m, 2H), 4.49 (dt, *J* = 13.0, 2.2 Hz, 1H), 4.40 (dt, *J* = 13.2, 2.2 Hz, 1H), 3.95−3.90 (m, 1H), 3.59 (d, *J* = 2.2 Hz, 1H), 3.36 (s, 3H), 2.21–2.15 (m, 2H), 2.02−1.97 (m, 1H), 1.87 (m, 1H), 1.78–1.74 (m, 1H), 1.63–1.60 (m, 2H), 1.58–1.50 (m, 1H), 1.46–1.41 (m, 1H), 1.31–1.27 (m, 1H), 0.92 (s, 3H), 0.79 (d, *J* = 6.3 Hz, 3H).

^{13}CNMR (100 MHz, $CDCl_3$): δ 152.8, 105.8, 103.4, 88.2, 69.4, 69.0, 60.5, 55.3, 51.3, 48.9, 34.6, 30.0, 29.2, 27.0, 15.8, 14.9.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_4Na$ $(M+Na)^+$ 305.1720, found 305.1723.

(1'*R*,2*R*,2'*R*,3a'*S*,4'*R*,7'*R*,7a'*S*)-2-Methoxy-7',7a'-dimethyl-4-methylenedecahydro-2H-spiro[furan-3,2'-indene]-1',4'-diol (78):



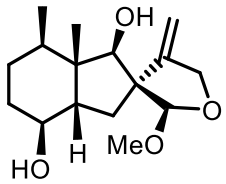
IRυ_{max} (film): 3411, 2926, 1661, 1459, 1045 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.09 (s, 1H), 5.04 (t, *J* = 2.4 Hz, 1H), 4.99 (t, *J* = 2.1 Hz, 1H), 4.48 (dt, *J* = 13.0, 2.3 Hz, 1H), 4.39 (dt, *J* = 13.2, 2.4 Hz, 1H), 4.03−4.01 (m, 1H), 3.68 (d, *J* = 2.2 Hz, 1H), 3.38 (s, 3H), 2.21 (d, *J* = 2.7 Hz, 1H), 2.08−2.03 (m, 1H), 1.96–1.90 (m, 2H), 1.87–1.74 (m, 2H), 1.61–1.54 (m, 3H), 1.40 (m, 1H), 0.89 (m, 6H).

^{13}CNMR (100 MHz, $CDCl_3$): δ 152.9, 106.0, 103.6, 88.9, 69.0, 66.9, 60.9, 55.2, 46.0, 42.9, 37.6, 35.2, 34.5, 31.6, 16.3, 15.7.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_4Na$ $(M+Na)^+$ 305.1722, found 305.1723.

(1'*R*,2*R*,2'*R*,3a'*S*,4'*S*,7'*R*,7a'*S*)-2-Methoxy-7',7a'-dimethyl-4-methylenedecahydro-2H-spiro[furan-3,2'-indene]-1',4'-diol (79):



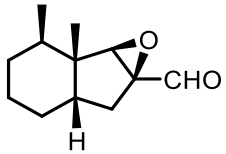
IRυ_{max} (film): 3401, 2926, 1661, 1459, 1044, cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.11 (s, 1H), 5.0 (m, 2H), 4.50 (dt, *J* = 13.1, 1.9 Hz, 1H), 4.41 (dt, *J* = 13.3, 2.2 Hz, 1H), 3.86−3.82 (m, 1H), 3.62 (d, *J* = 2.1 Hz, 1H), 3.39 (s, 3H), 2.17 (d, *J* = 2.2 Hz, 1H), 2.09−2.04 (m, 1H), 1.80–1.76 (m, 2H), 1.68–1.62 (m, 1H), 1.55–1.53 (m, 2H), 1.45–1.41 (m, 1H), 1.29–1.25 (m, 2H), 1.09 (s, 3H), 0.88 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.9, 106.0, 103.5, 89.2, 70.6, 69.1, 59.9, 55.3, 51.7, 46.9, 36.1, 32.2, 29.7, 25.7, 17.4, 16.3.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_4Na$ $(M+Na)^+$ 305.1720, found 305.1723.

(1a*R*,1b*S*,2*R*,5a*S*,6a*S*)-1b,2-dimethyloctahydro-1aH-indeno[1,2-b]oxirene-6a-carbaldehyde (80):



To a solution of **72** (100 mg, 0.52 mmol) in EtOAc (10 mL) was added Pd/C (10 mg) and the mixture was stirred under hydrogen balloon pressure. After 1 h catalyst was filtered off and concentrated to afford **80** (96 mg, 95%) as colorless oil.

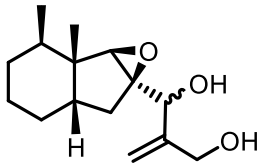
IRυ_{max} (film): 2960, 2928, 2857, 1721 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.20 (s, 1H), 3.62 (s, 1H), 2.20–2.14 (m, 1H), 1.82–1.76 (m, 1H), 1.64–1.60 (m, 1H), 1.54–1.47 (m, 3H), 1.39–1.31 (m, 2H), 1.29–1.18 (m, 2H), 1.04 (s , 3H), 0.88 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.5, 68.6, 67.7, 42.8, 37.9, 32.9, 29.9, 26.5, 22.9, 21.4, 17.0, 14.0.

HRMS (ESI): *m/z* calcd for $C_{12}H_{18}O_2Na$ $(M+Na)^+$ 217.1195, found 217.1199.

1-((1a*R*,1b*S*,2*R*,5a*S*,6a*R*)-1b,2-dimethyloctahydro-1aH-indeno[1,2-b]oxiren-6a-yl)-2-methylenepropane-1,3-diol (81):



Compound **81** was synthesized from **80** using the procedure similar to preparation of **73**.

Yield: 49%, 58% brsm.

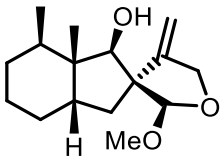
IRυ_{max} (film): 3368, 2925, 1649, 1458, 1034 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.27 (d, *J* = 1.2 Hz, 1H), 5.18 (d, *J* = 0.9 Hz, 1H), 4.55 (s, 1H), 4.29 (d, *J* = 13.5 Hz, 1H), 4.14 (d, *J* = 14.1 Hz, 1H), 3.39 (s, 1H), 1.79–1.71 (m, 2H), 1.69–1.63 (m, 2H), 1.48–1.45 (m, 3H), 1.37–1.33 (m, 1H), 1.23–1.20 (m, 1H), 1.08–1.02 (m, 1H), 0.98 (s, 3H), 0.89 (d, *J* = 7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 146.5, 115.1, 73.6, 68.1, 67.6, 63.9, 42.3, 38.5, 33.5, 30.0, 23.2, 23.1, 21.7, 17.0, 14.0.

HRMS (ESI): *m/z* calcd for $C_{15}H_{24}O_3Na$ $(M+Na)^+$ 275.1464, found 275.1461.

(1′*R*,2*R*,2′*R*,3a′*S*,7′*R*,7a′*S*)-2-methoxy-7′,7a′-dimethyl-4-methylenedecahydro-2H spiro-[furan-3,2′-inden]-1′-ol (83):



Compound **83** was synthesized from **81** using the procedure similar to preparation of **75**.

Yield: 60%.

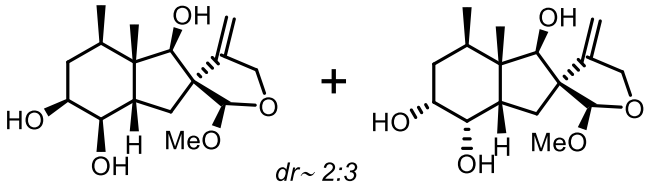
IRυ_{max} (film): 3401, 2926, 1661, 1038 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.09 (s, 1H), 5.00 (t, *J* = 2.4 Hz, 1H), 4.98 (t, *J* = 1.8 Hz, 1H), 4.49 (dt, *J* = 10.7, 2.4 Hz, 1H), 4.41 (dt, *J* = 10.7, 2.1 Hz, 1H), 3.59 (d, *J* = 3.2 Hz, 1H), 3.38 (s, 3H), 2.12 (d, *J* = 2.1 Hz, 1H), 1.87–1.82 (m, 2H), 1.80–1.79 (m, 1H), 1.61–1.57 (m, 2H), 1.52–1.41 (m, 4H), 1.21–1.18 (m, 1H), 0.90 (s, 3H), 0.80 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.2, 106.1, 103.1, 89.1, 69.1, 60.6, 55.2, 46.6, 44.8, 35.8, 32.2, 30.5, 25.1, 21.3, 16.5, 14.9.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_3Na$ $(M+Na)^+$ 289.1609, found 289.1618.

(1'*R*,2*R*,2'*R*,3a'*S*,7'*R*,7a'*S*)-2-Methoxy-7',7a'-dimethyl-4-methylenedecahydro-2H spiro[furan-3,2'-indene]-1',4',5'-triol (84):



To a solution of alkene **75** (60 mg, 0.227 mmol) in a 4:1 acetone: water mixtures (12 mL) were added NMO.H_2O (110 mg, 0.91 mmol) and a 2.5% solution of OsO_4 in *t*-BuOH (0.1 mL, 0.01 mmol). The mixture was stirred at room temperature for 24 h, followed by addition of $NaHSO_3$ (10 mg) and further stirring for 30 min. The acetone was removed under reduced pressure, brine was added and the aqueous layer was extracted with EtOAc (3 x 30 mL).The combined organic layer was dried over Na_2SO_4, and concentrated *in vacuo*. Silica gel flash column chromatography of the residue using ethyl acetate: petroleum ether, 7:3 afforded **84** as inseparable mixture (58 mg, 88%) as colorless oil.

IRυ_{max} (film): 3418, 2927, 1645, 1040 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.10 (s, 1H), 5.04−4.93 (m, 2H), 4.50−4.38 (m, 1H), 4.40 (dt, *J* = 13.2, 2.2 Hz, 1H), 4.02−3.76 (m, 2H), 3.63 (s, 1H), 3.39 (s, 3H), 2.21–2.14 (m, 2H),

2.08−2.04 (m, 1H), 1.98−1.93 (m, 1H), 1.77–1.71 (m, 1H), 1.61–1.45 (m, 2H), 1.05 (s, 2H), 0.90−0.82 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 152.3, 105.4, 103.7, 90.0, 71.6, 70.1, 69.6, 60.5, 55.3, 51.7, 45.3, 37.3, 31.0, 29.8 16.6, 16.0.

HRMS (ESI): *m/z* calcd for $C_{16}H_{26}O_4Na$ $(M+Na)^+$ 321.1720, found 321.1723.

1.6 References

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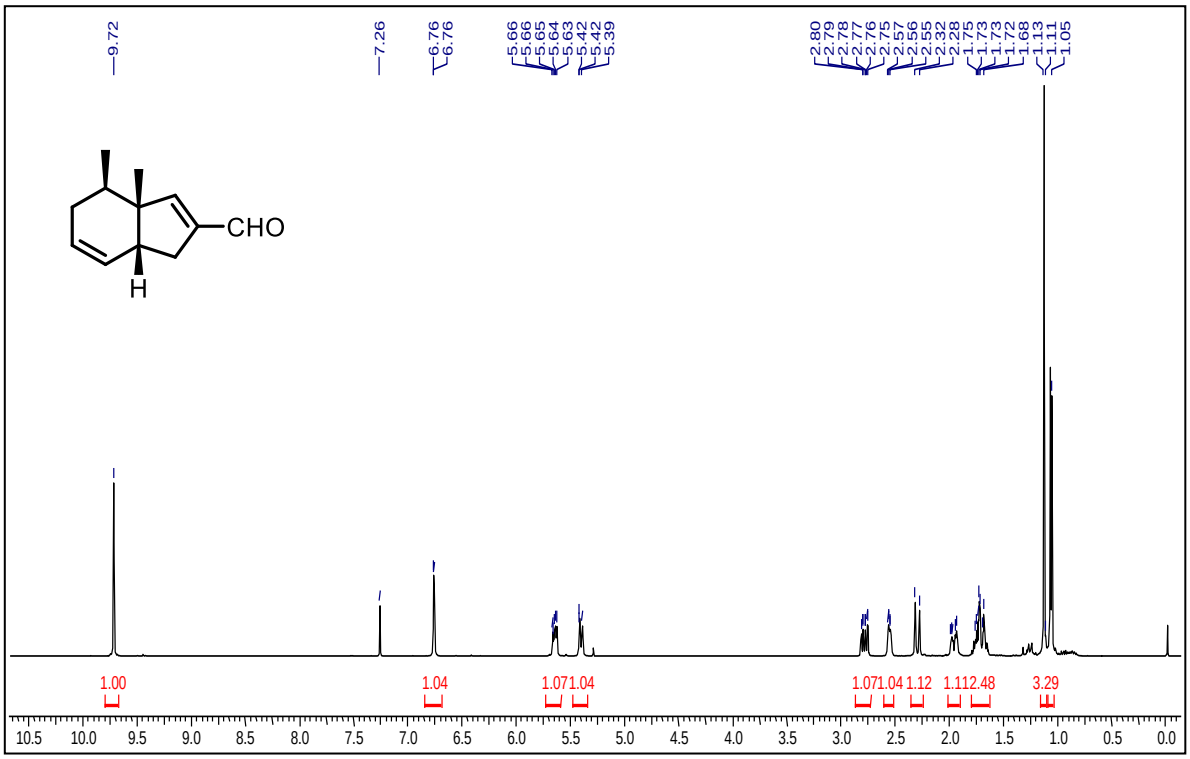
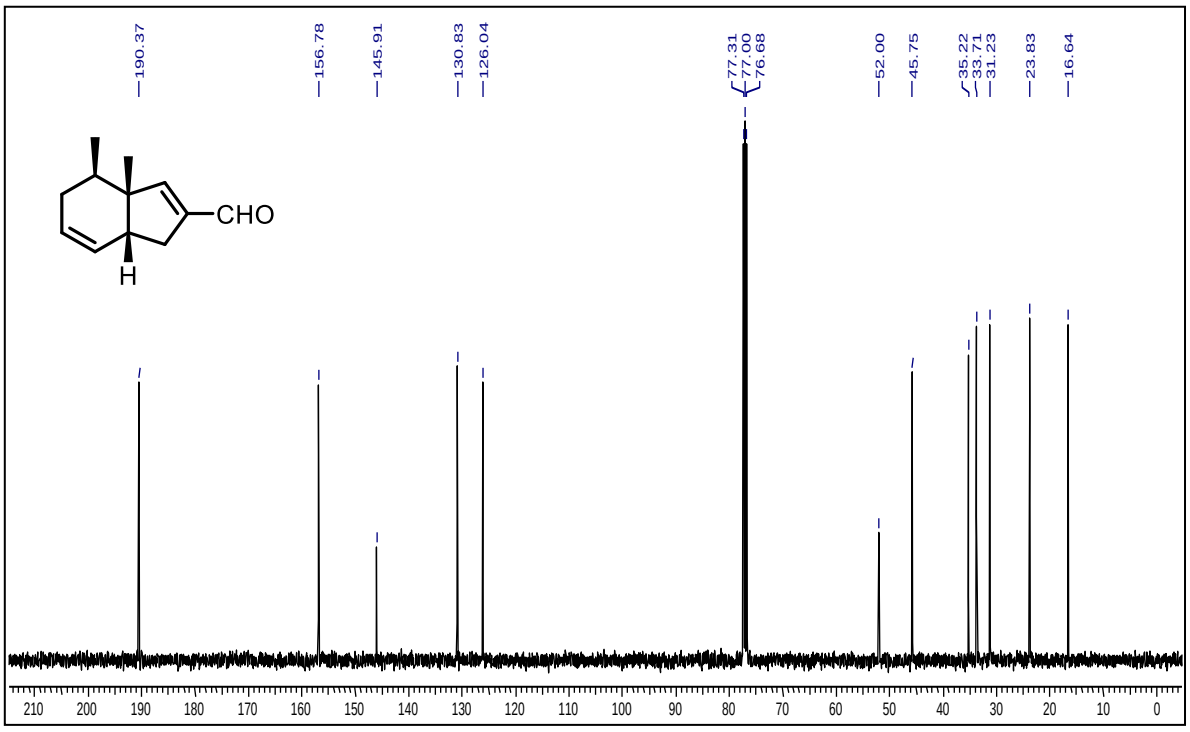
1.7 Selected copies of NMR spectra

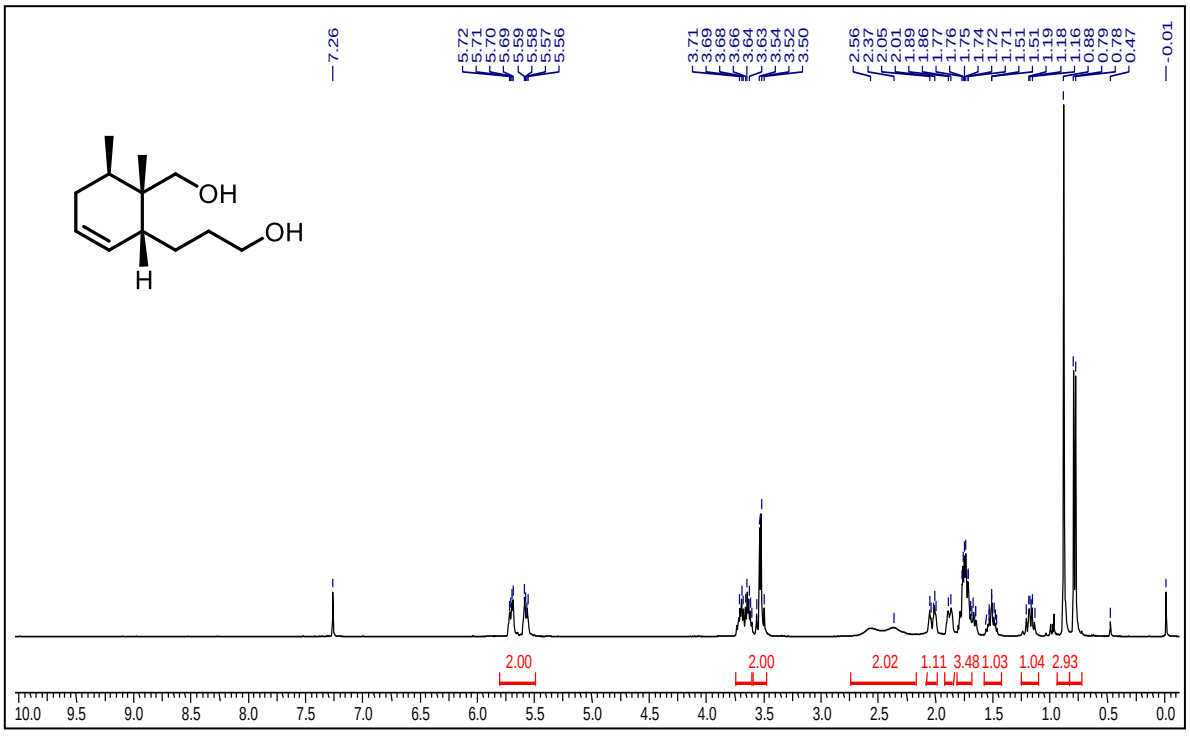
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 62



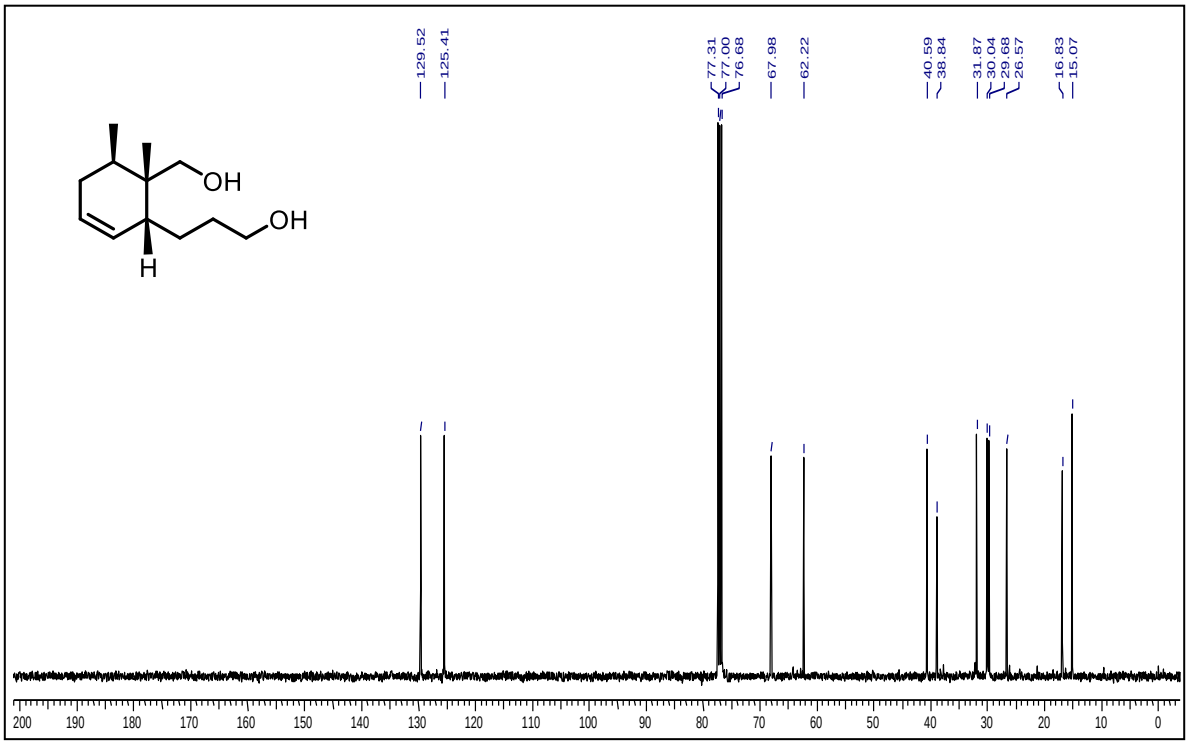
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 62



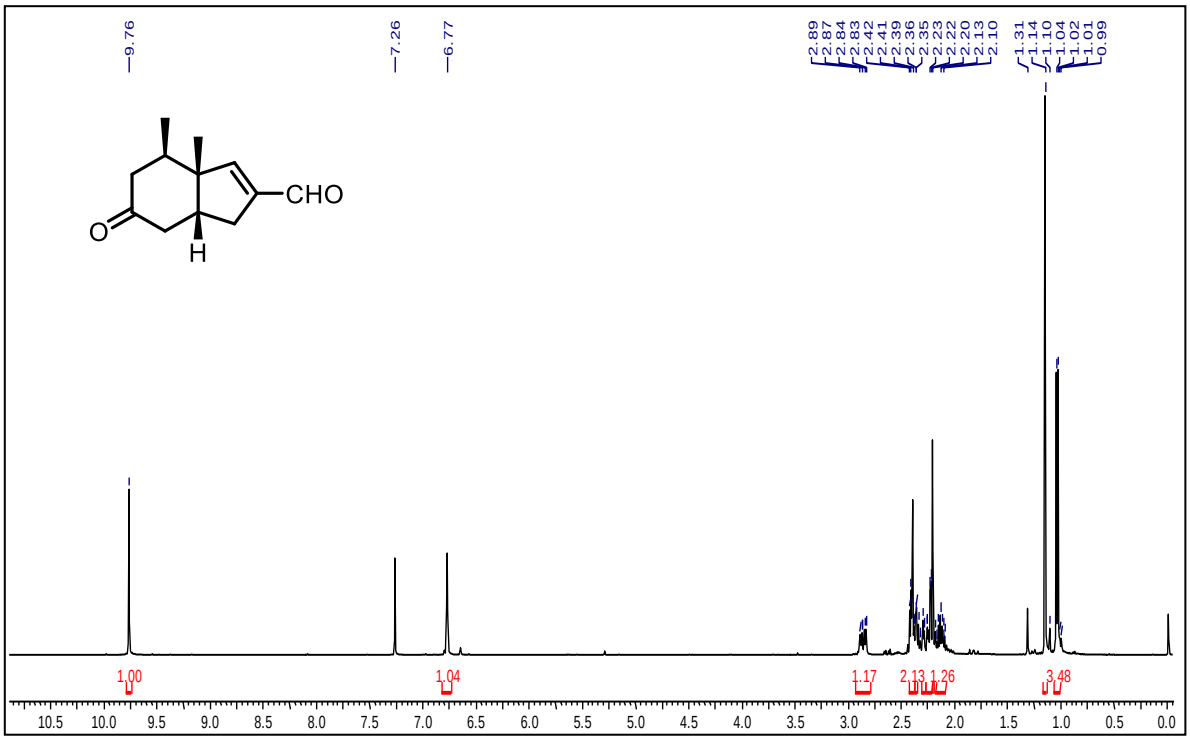
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 67



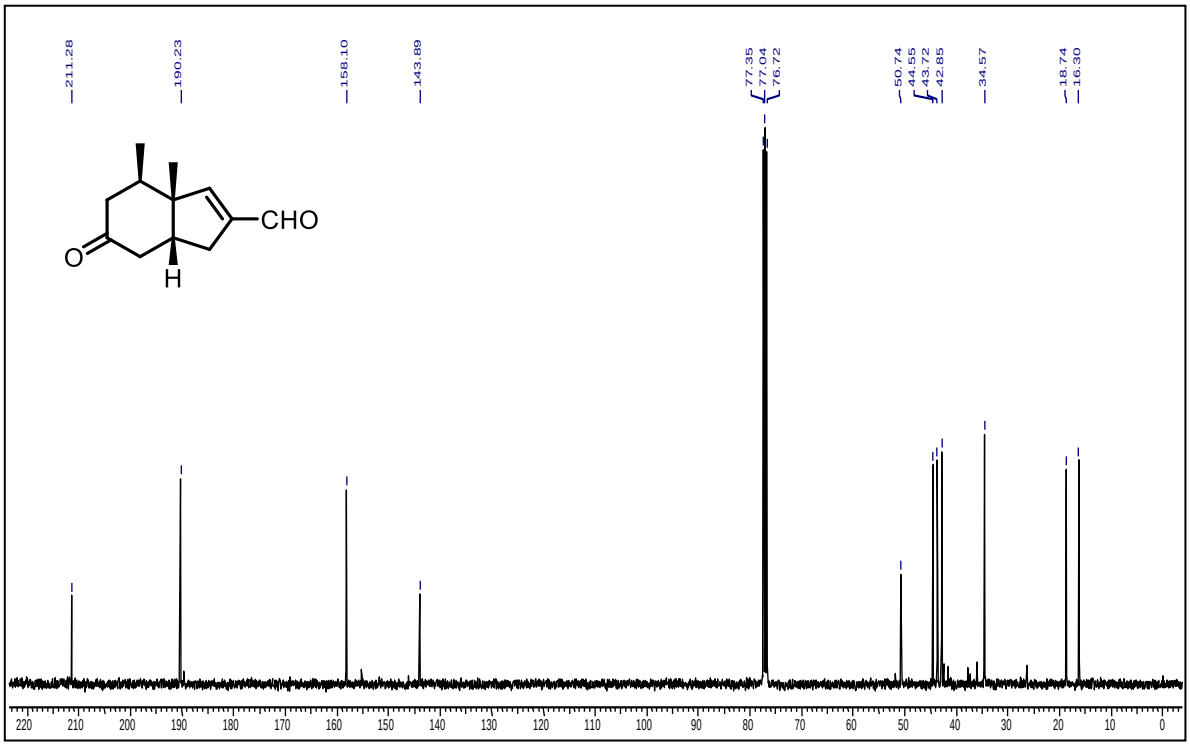
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 67



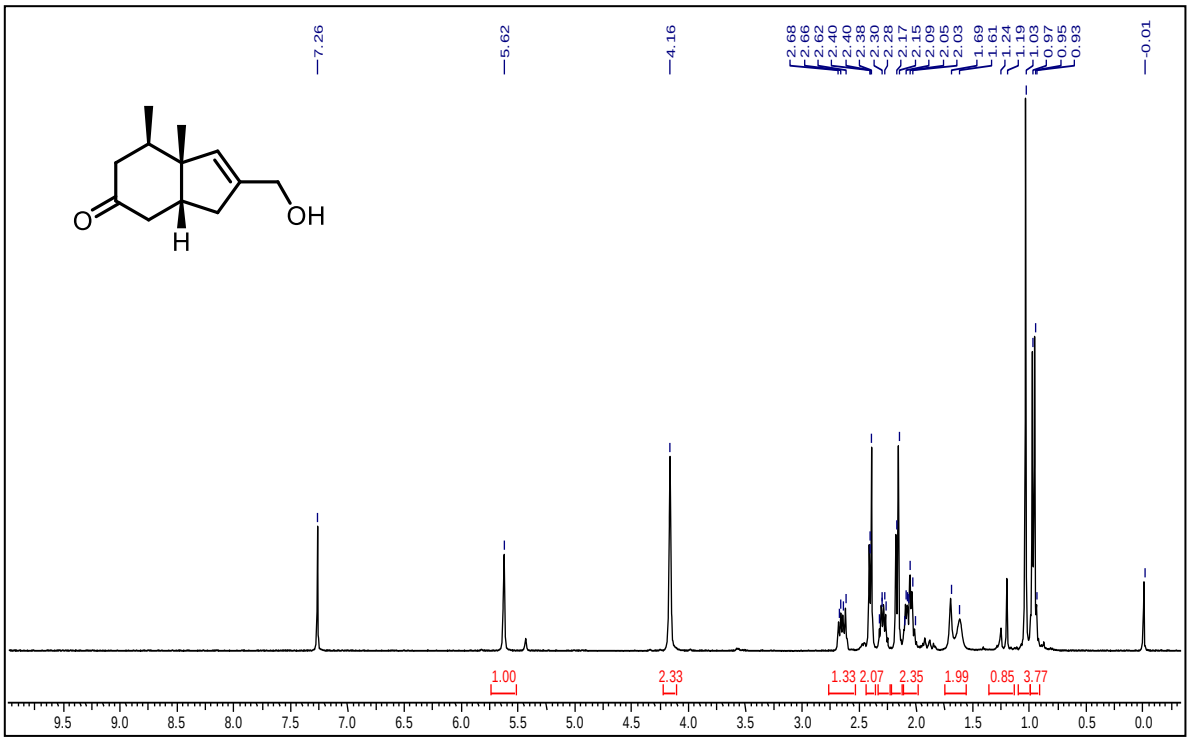
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 68



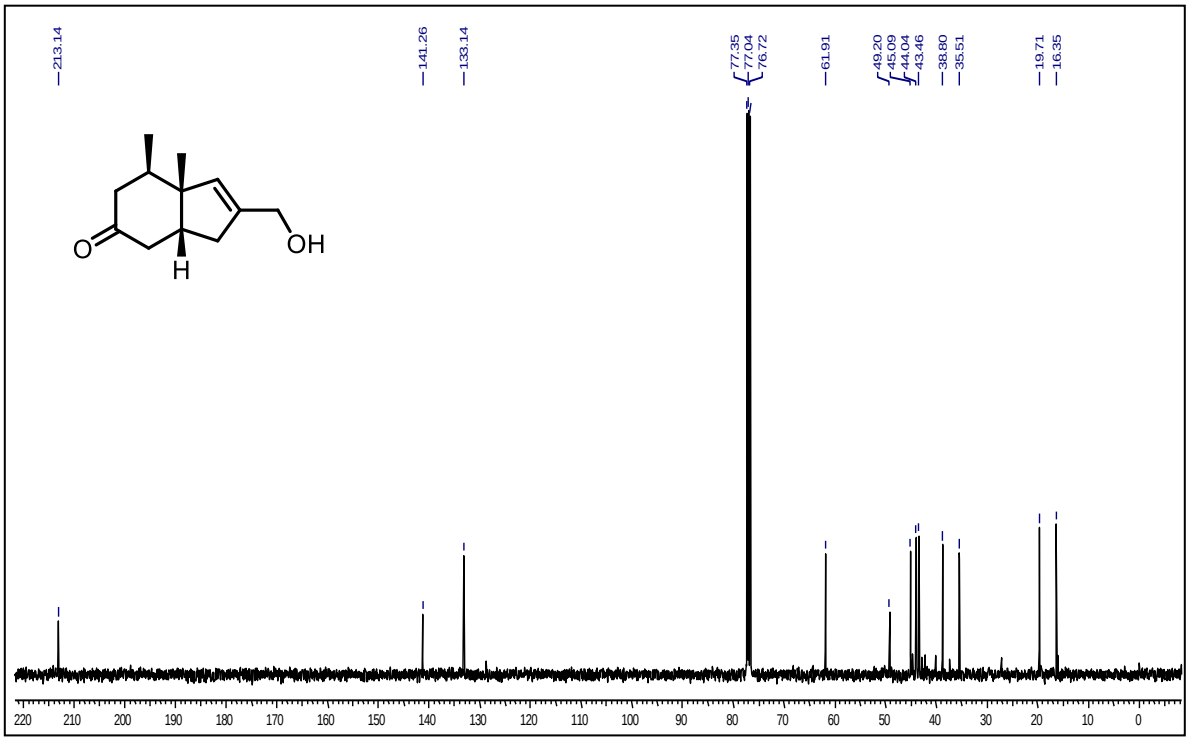
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 68



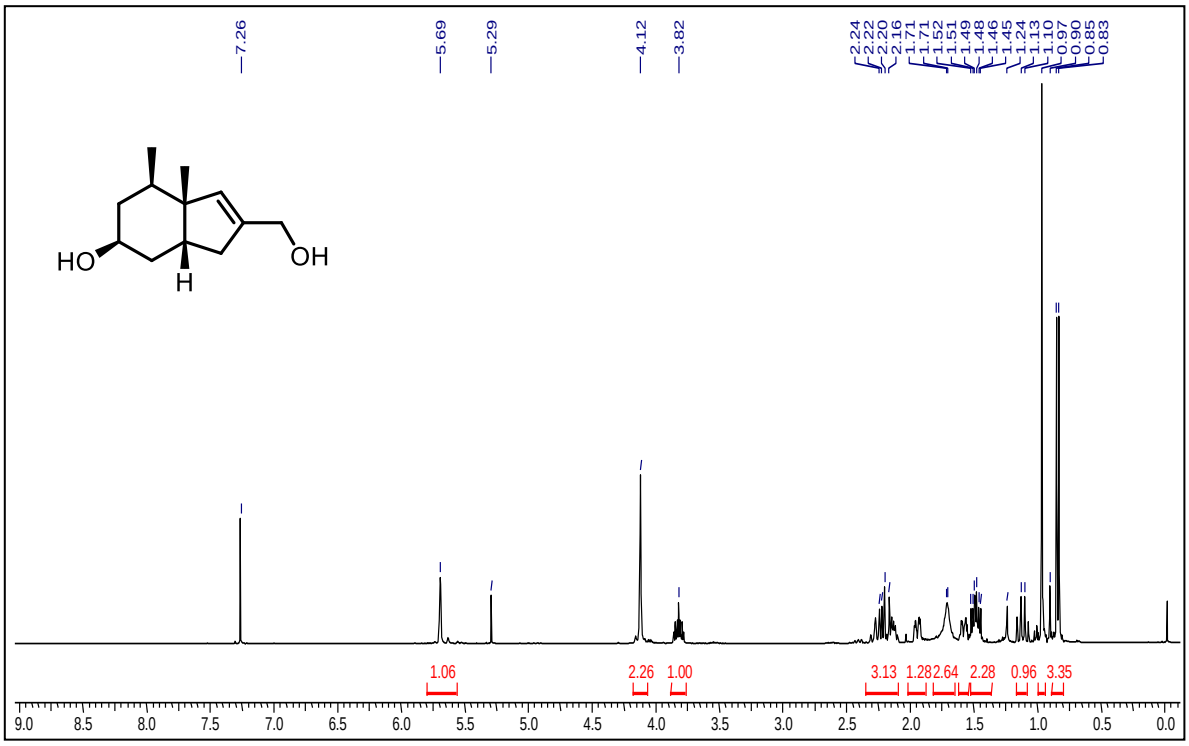
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 69



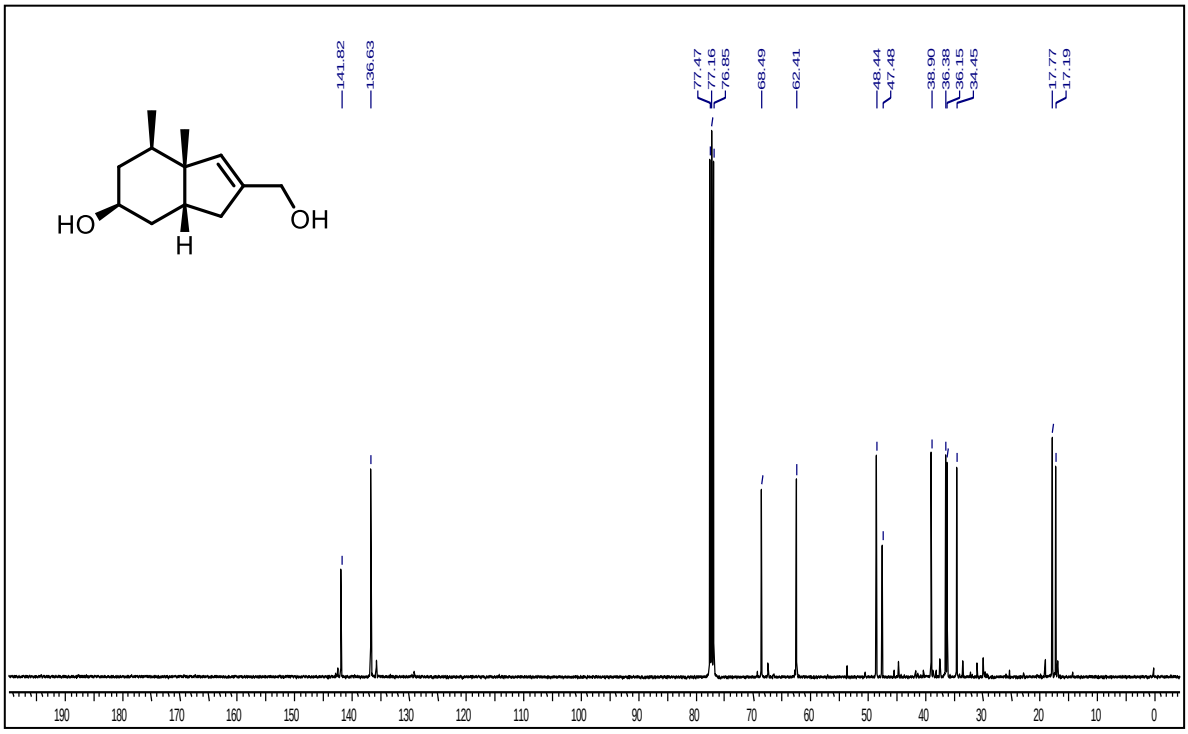
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 69



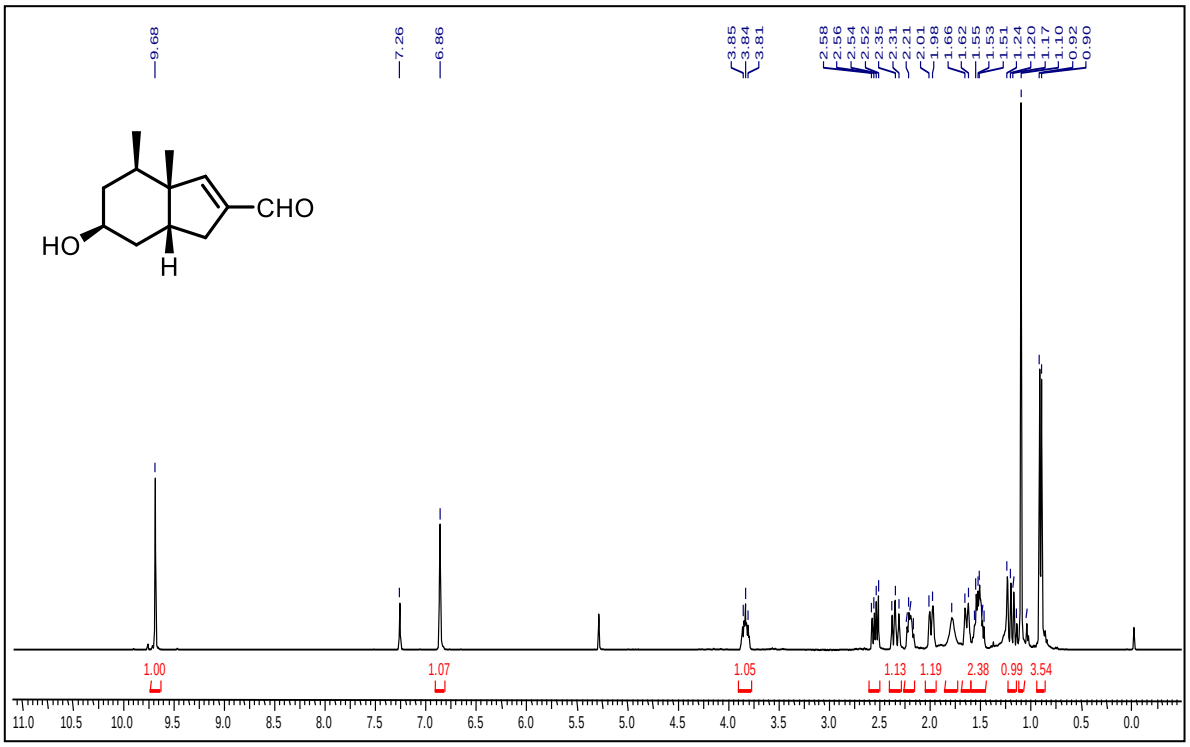
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 70



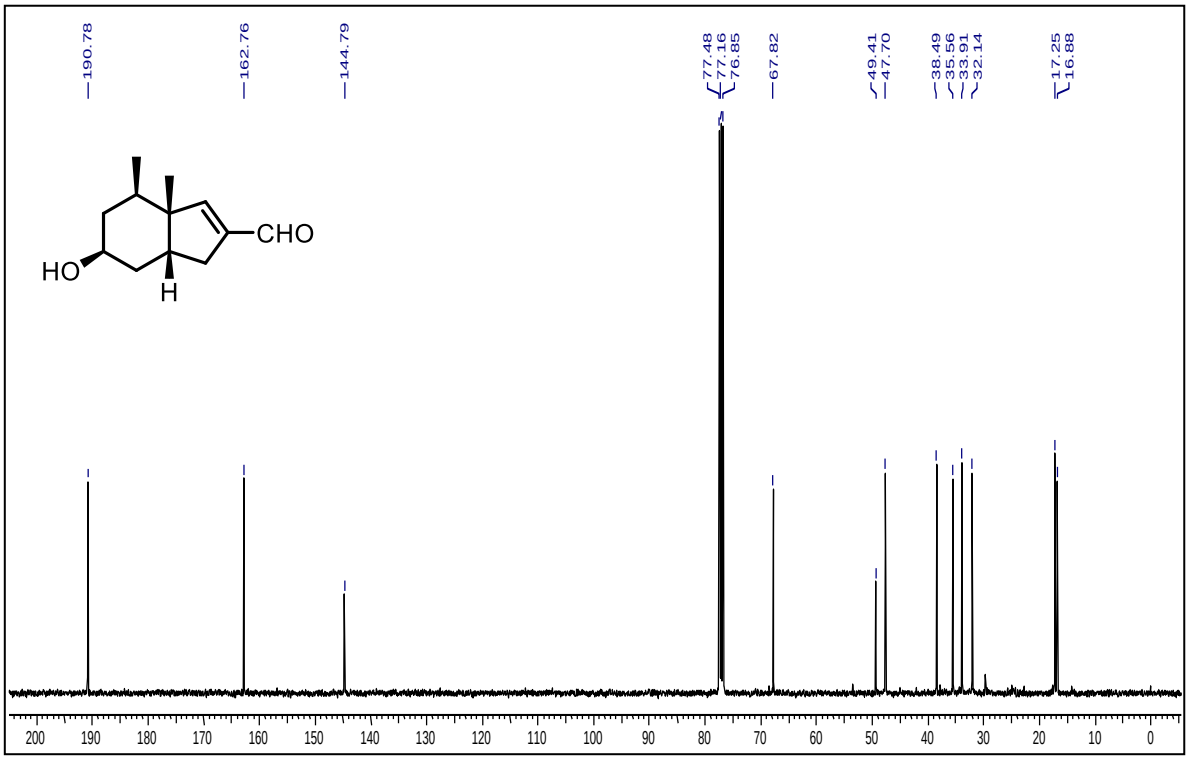
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 70



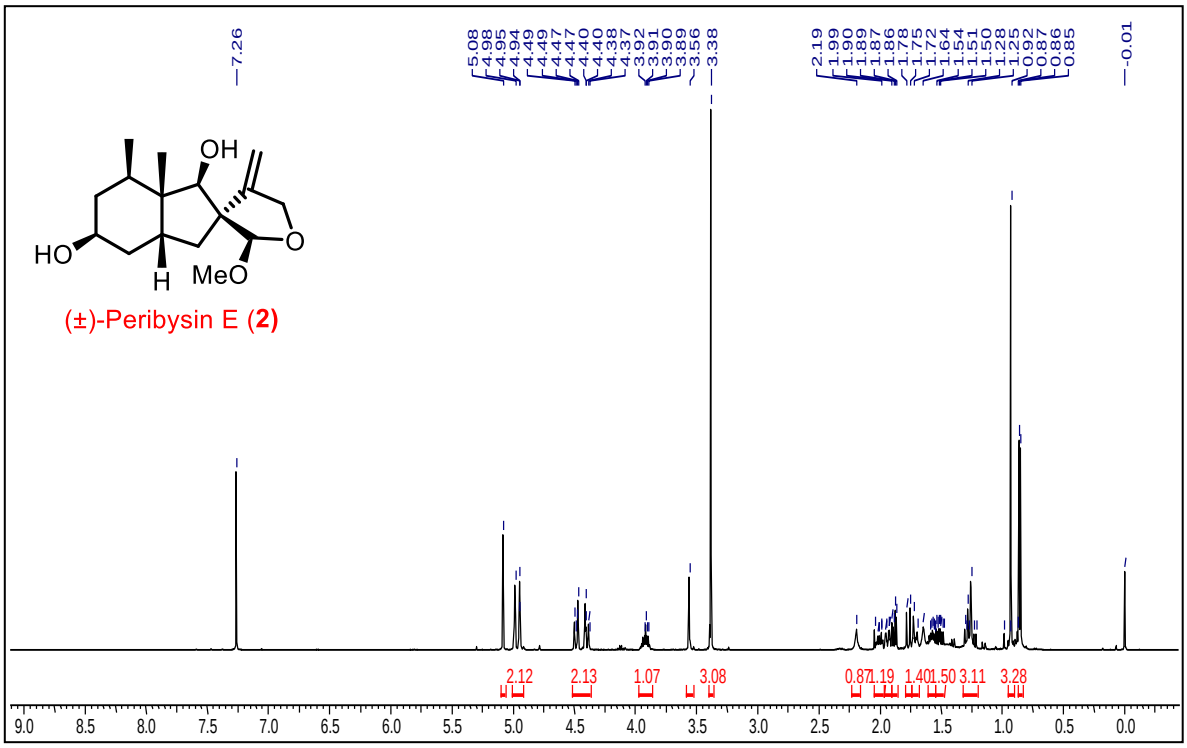
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 61



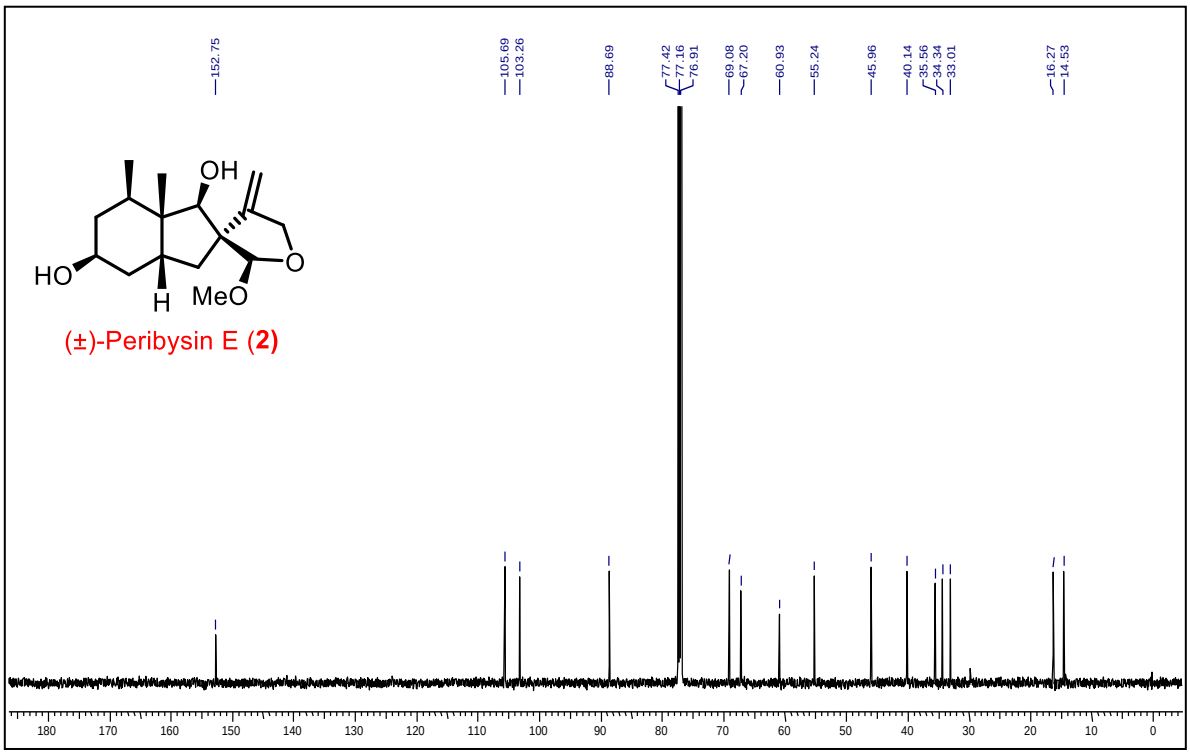
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 61



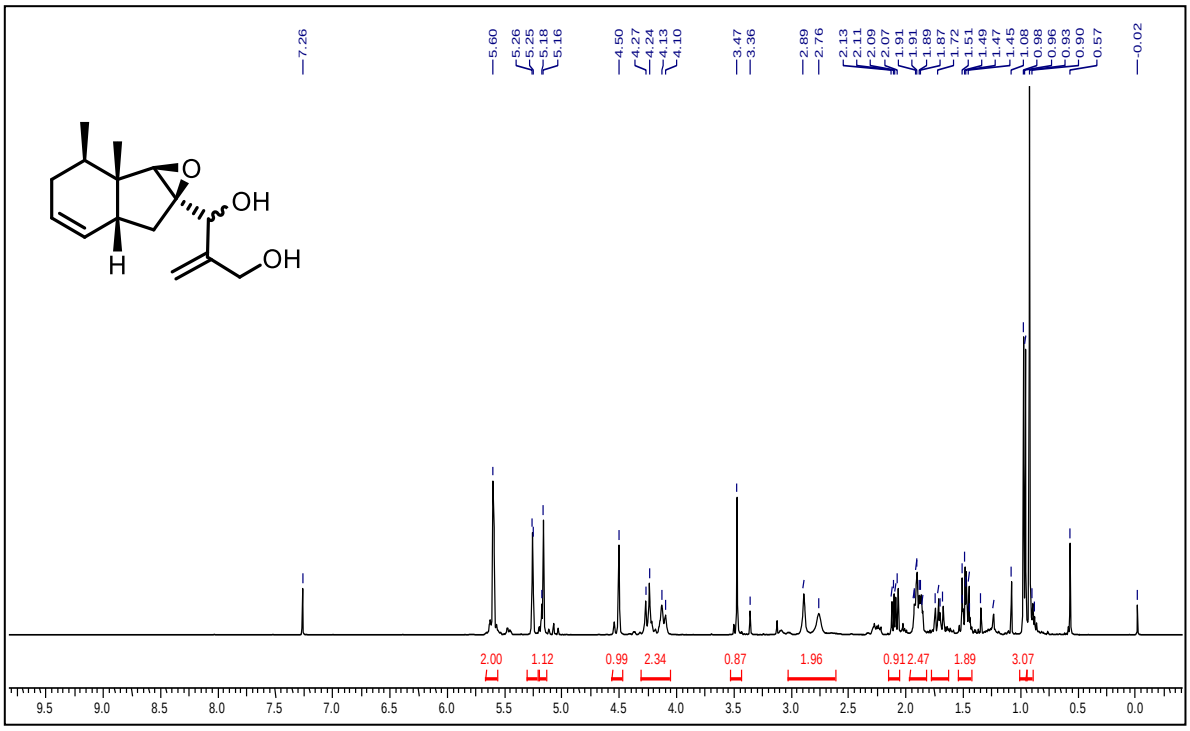
^{1}H NMR ($CDCl_3$, 500 MHz) of (±)-Peribysin E (2)



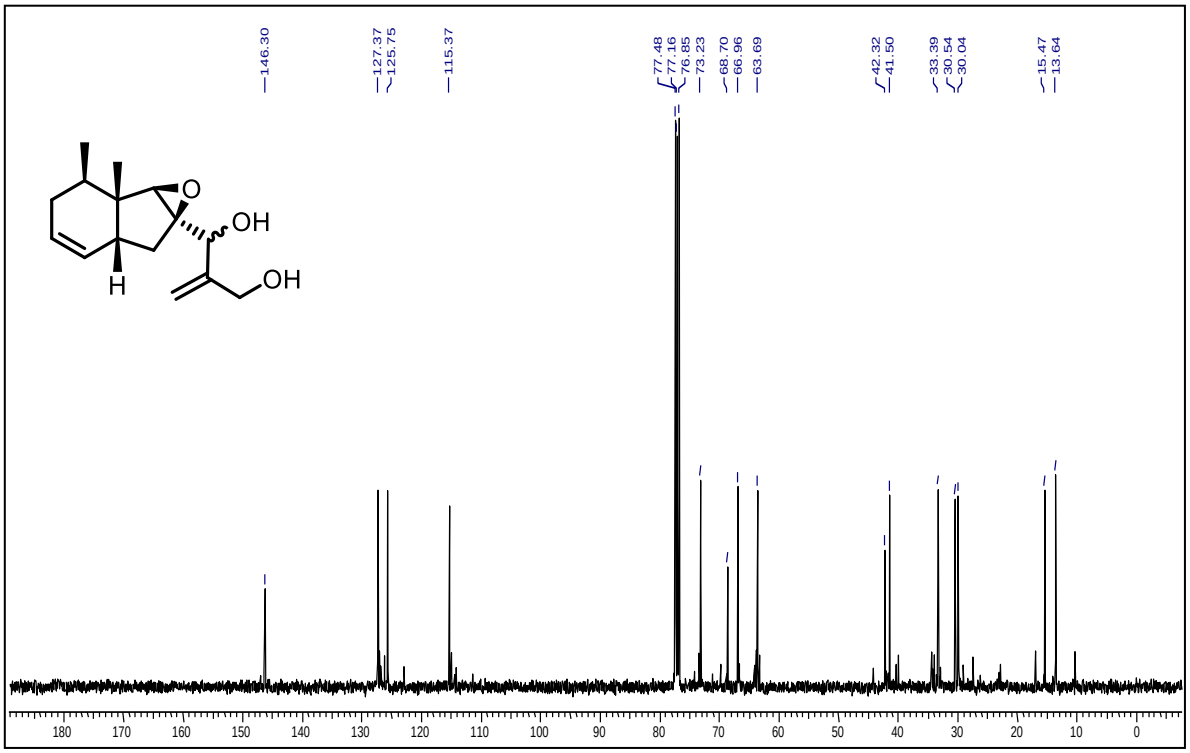
^{13}C NMR ($CDCl_3$, 125 MHz) of (±)-Peribysin E (2)



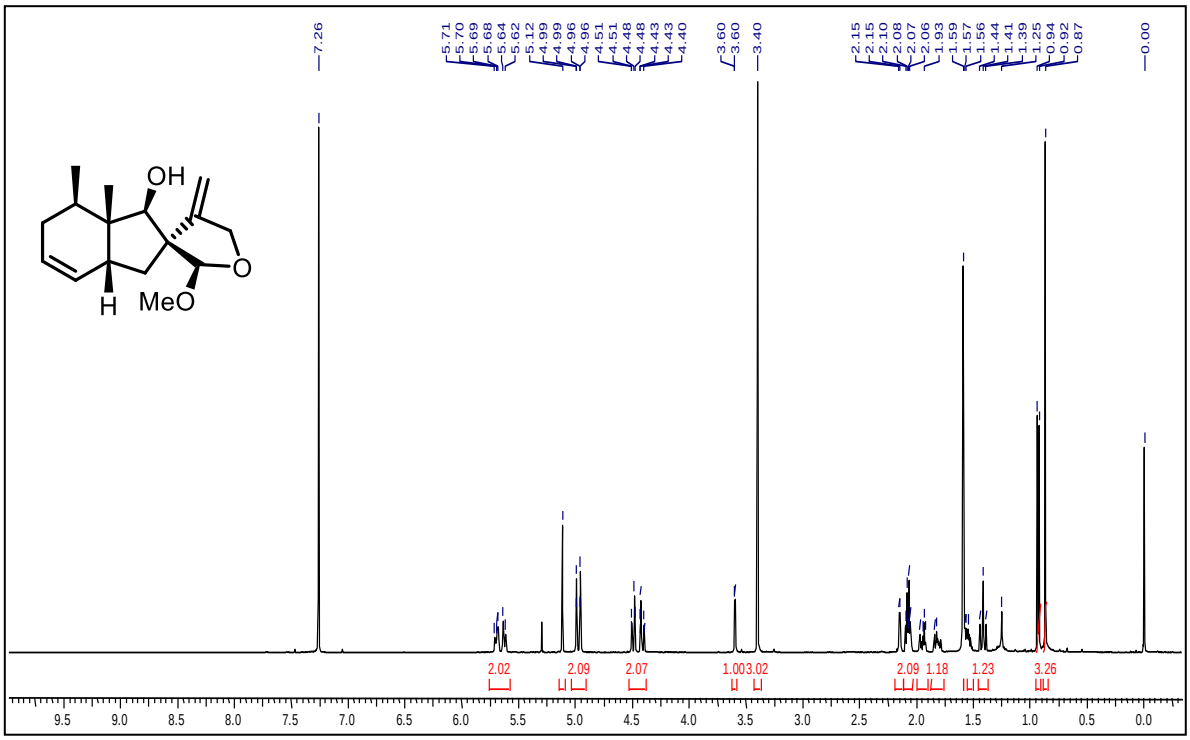
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 73



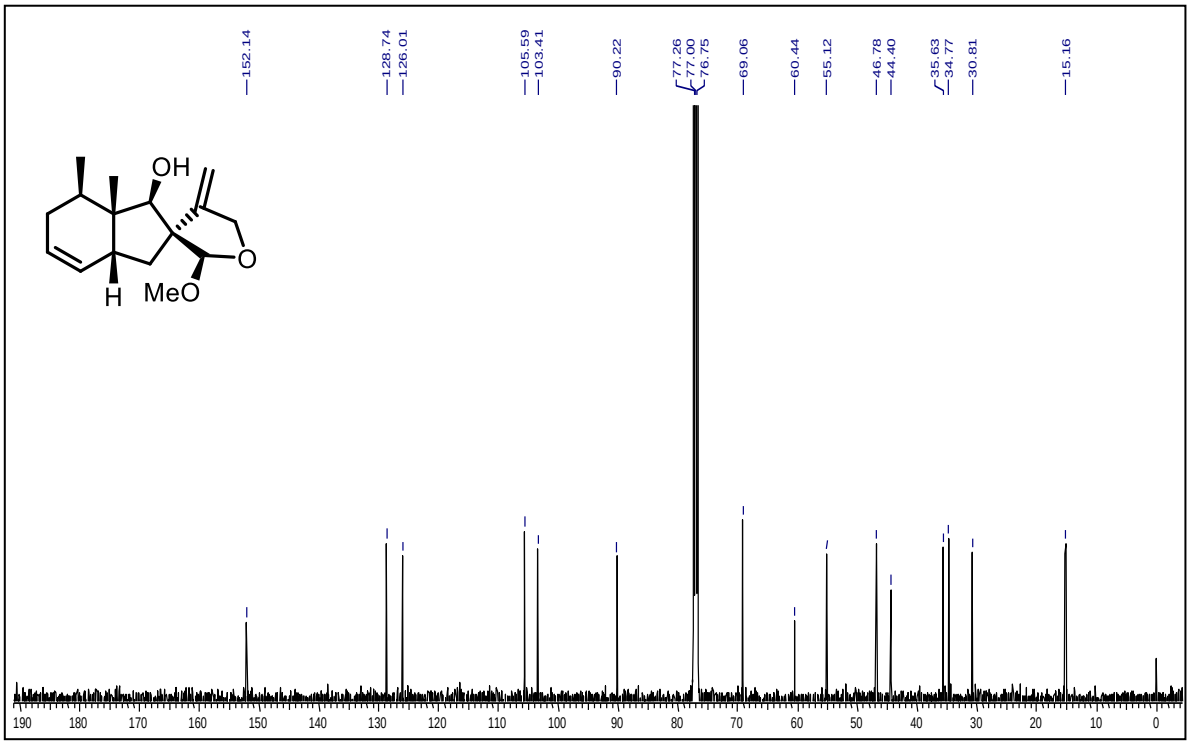
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 73



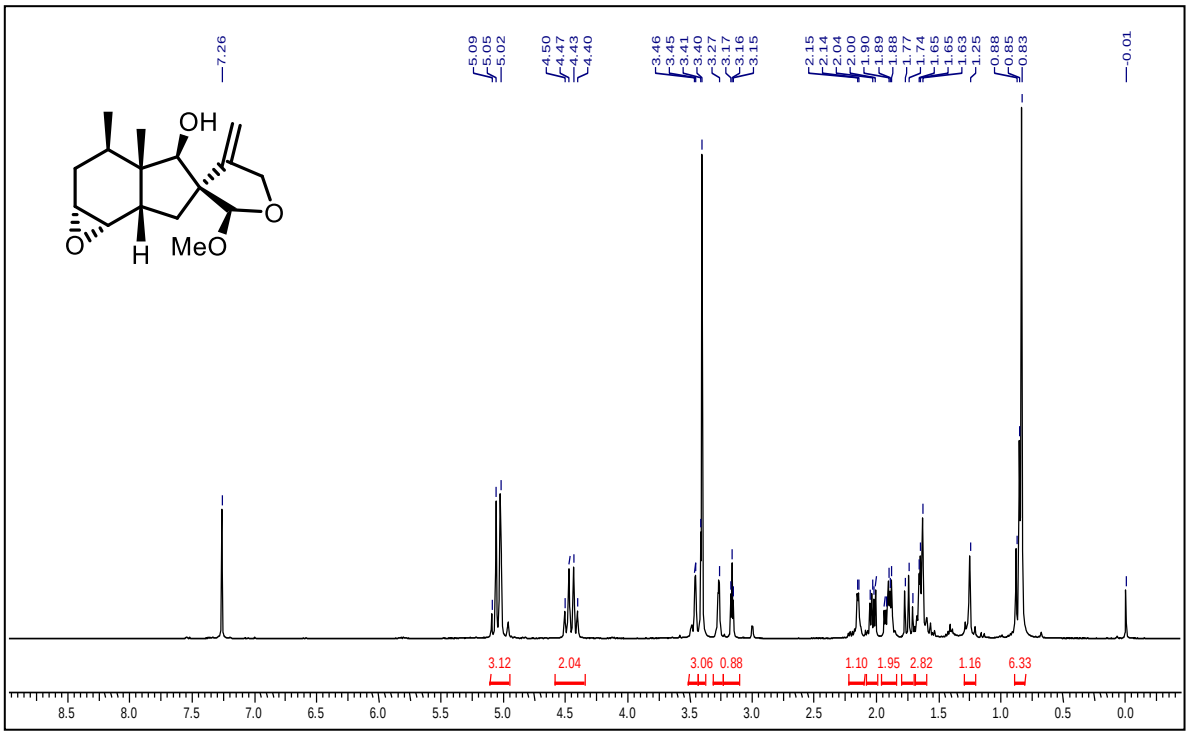
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 75



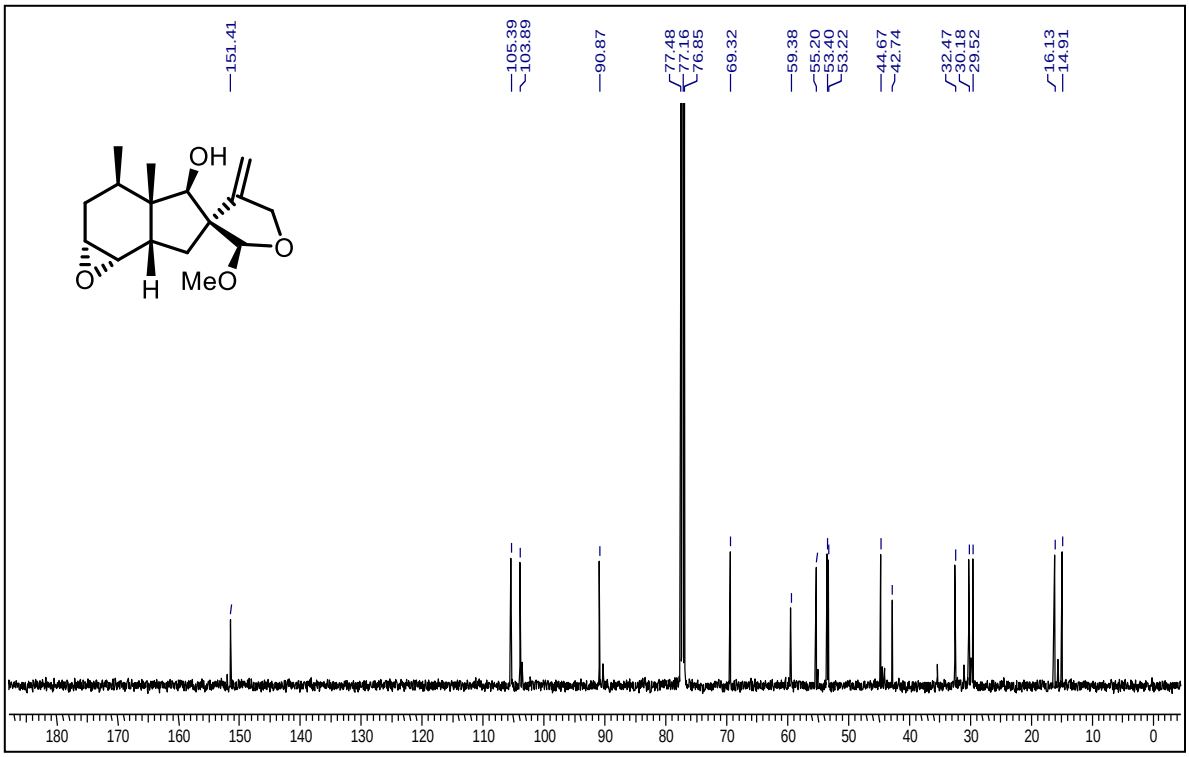
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 75



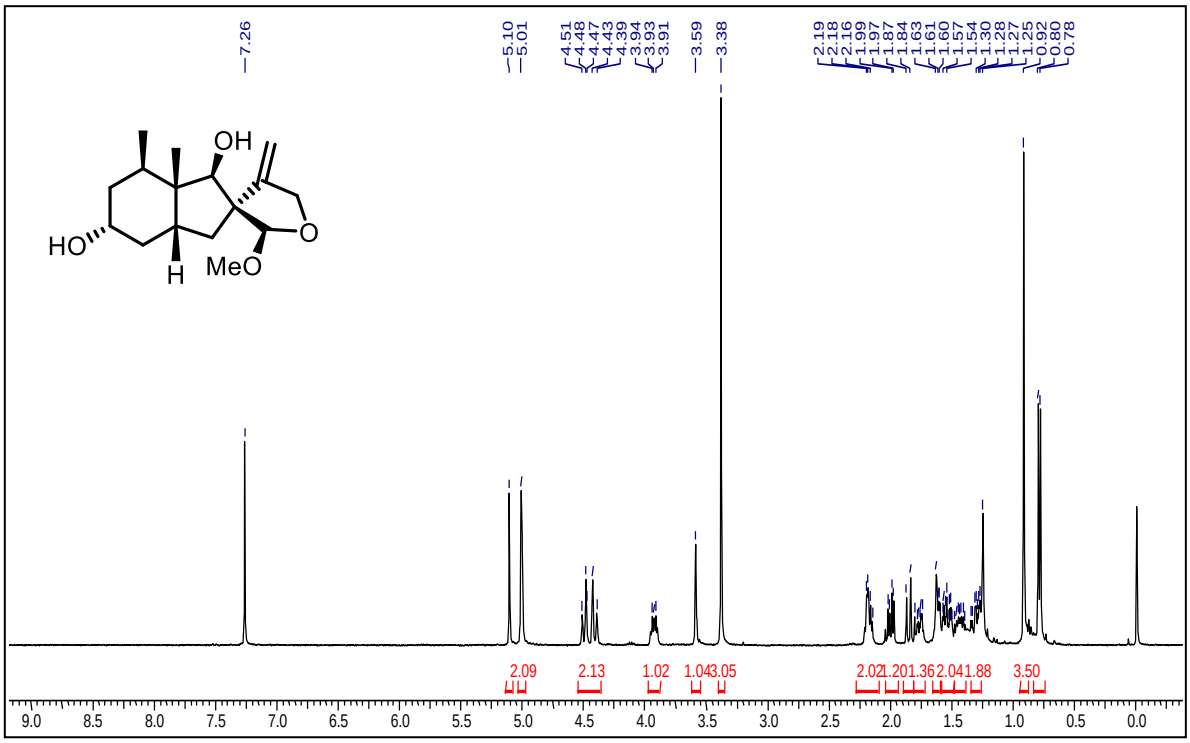
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 76



^{13}C NMR ($CDCl_3$, 100 MHz) of compound 76



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 77



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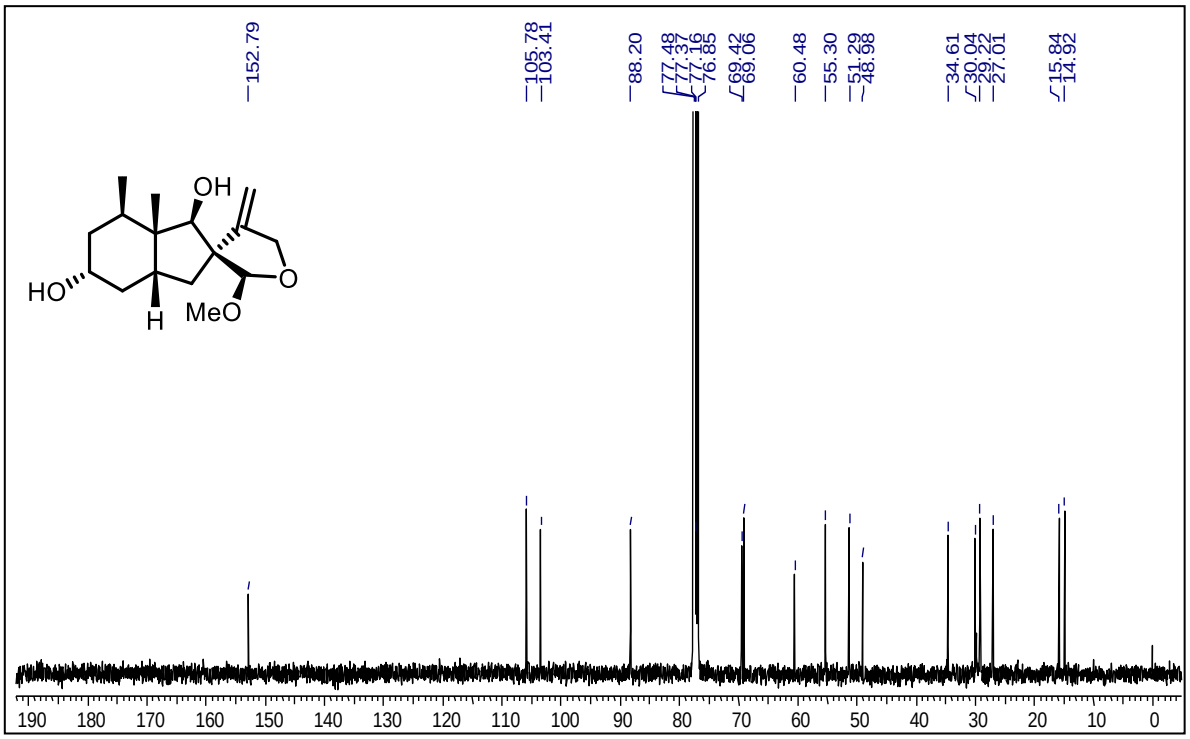
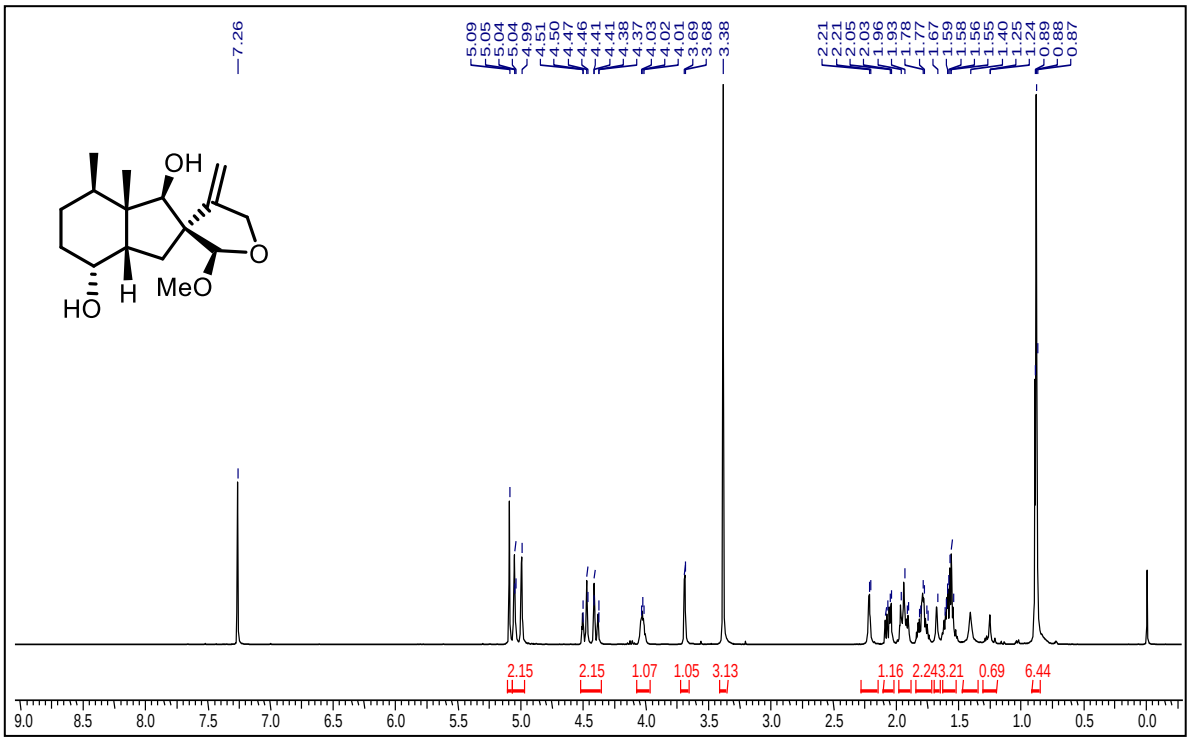
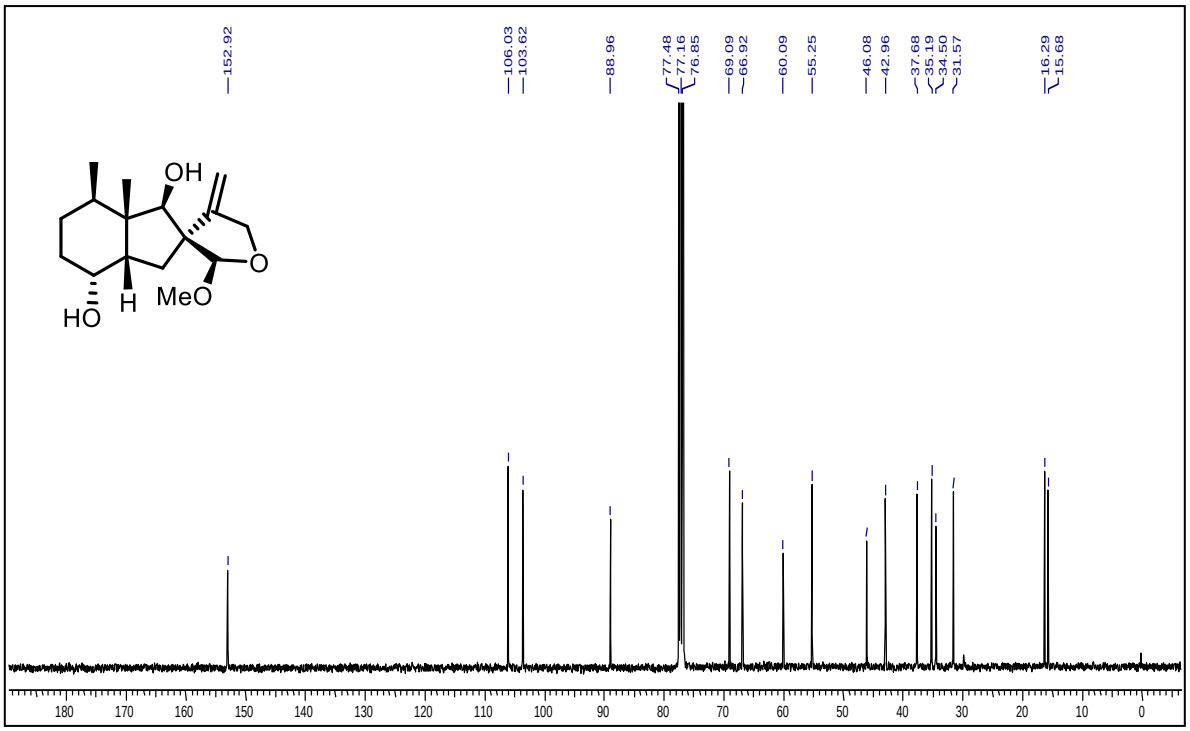


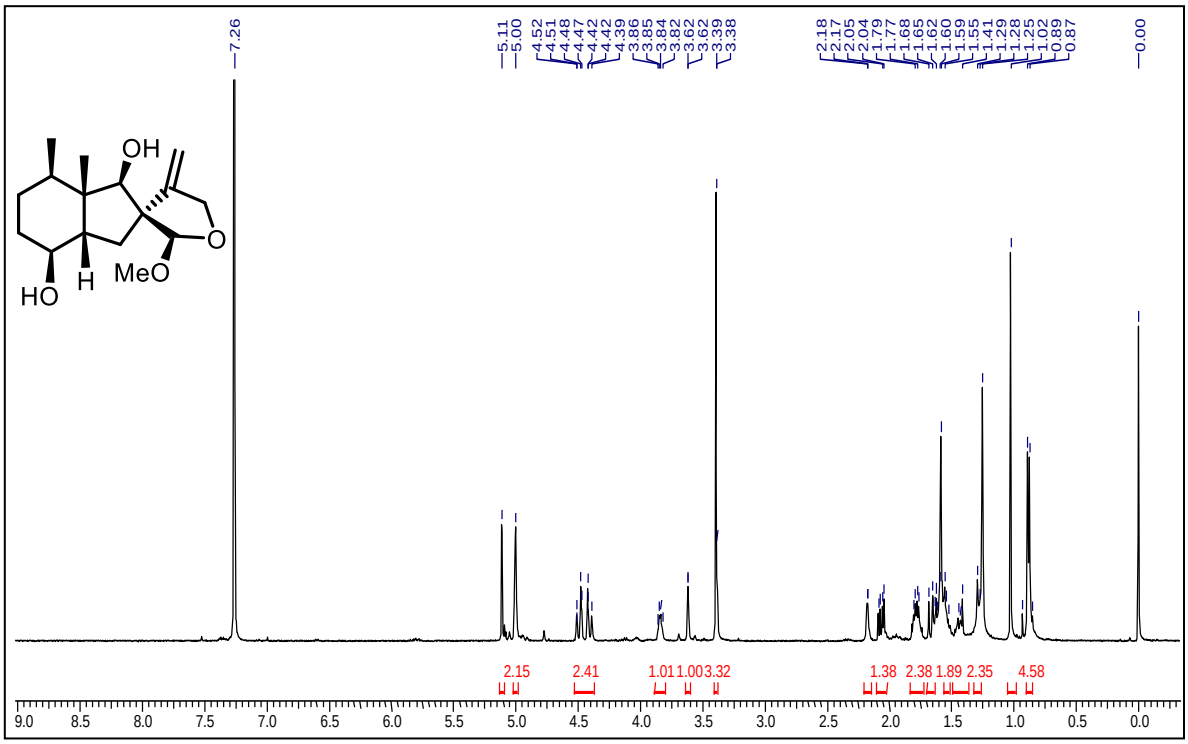
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 78



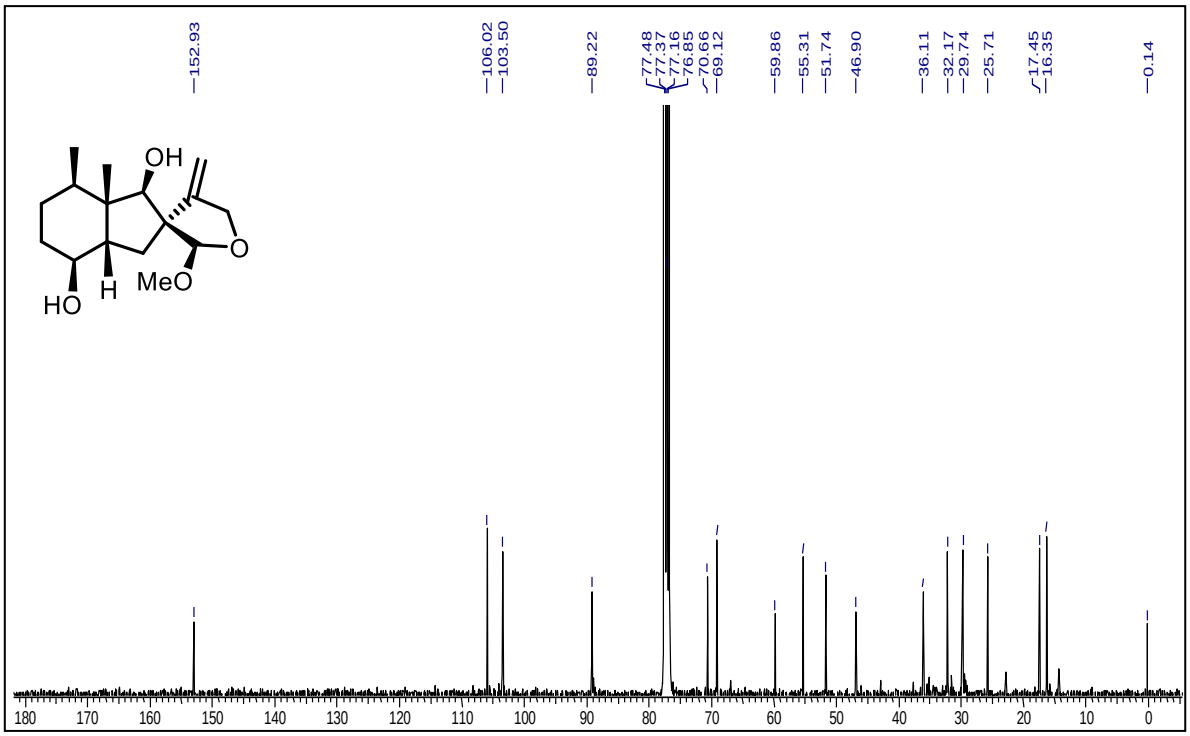
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 78



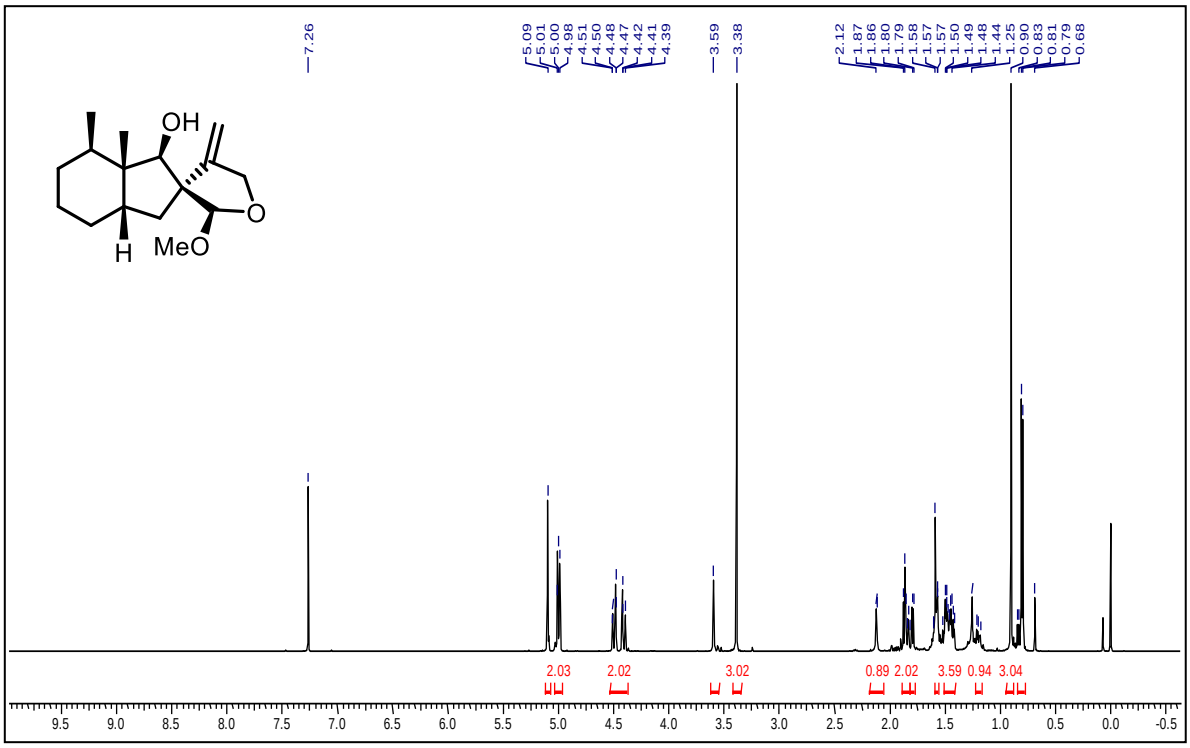
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 79



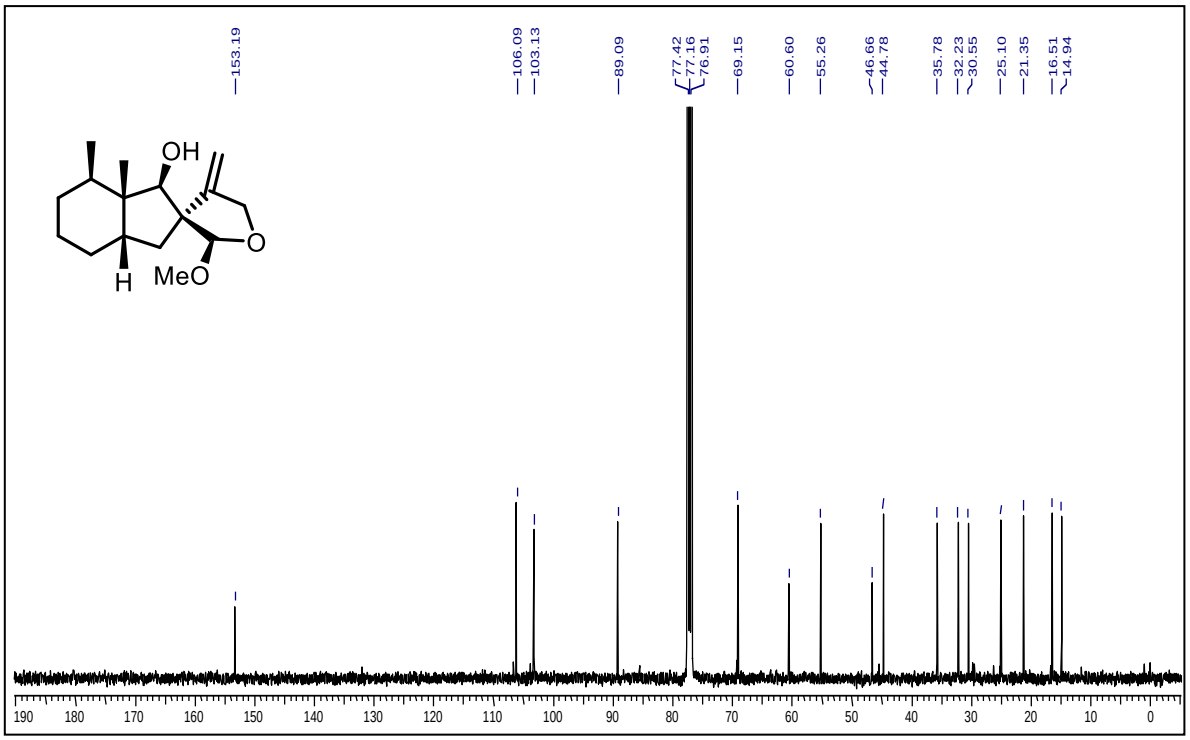
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 79



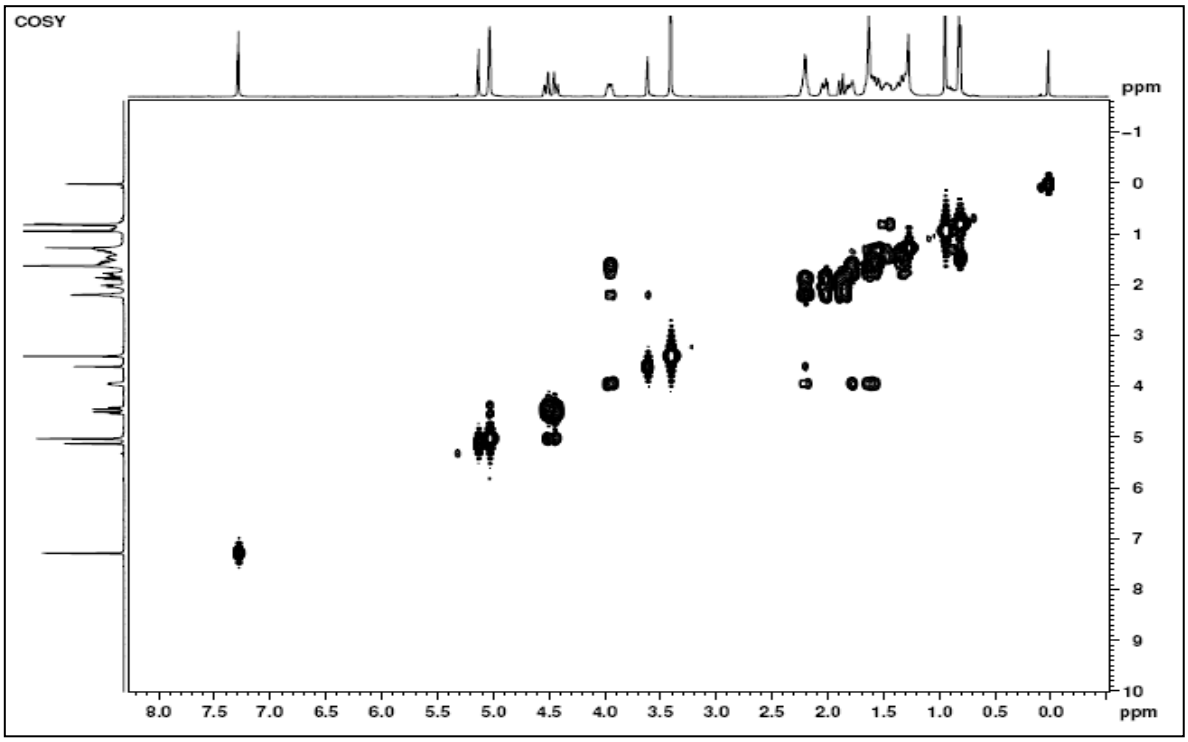
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 83



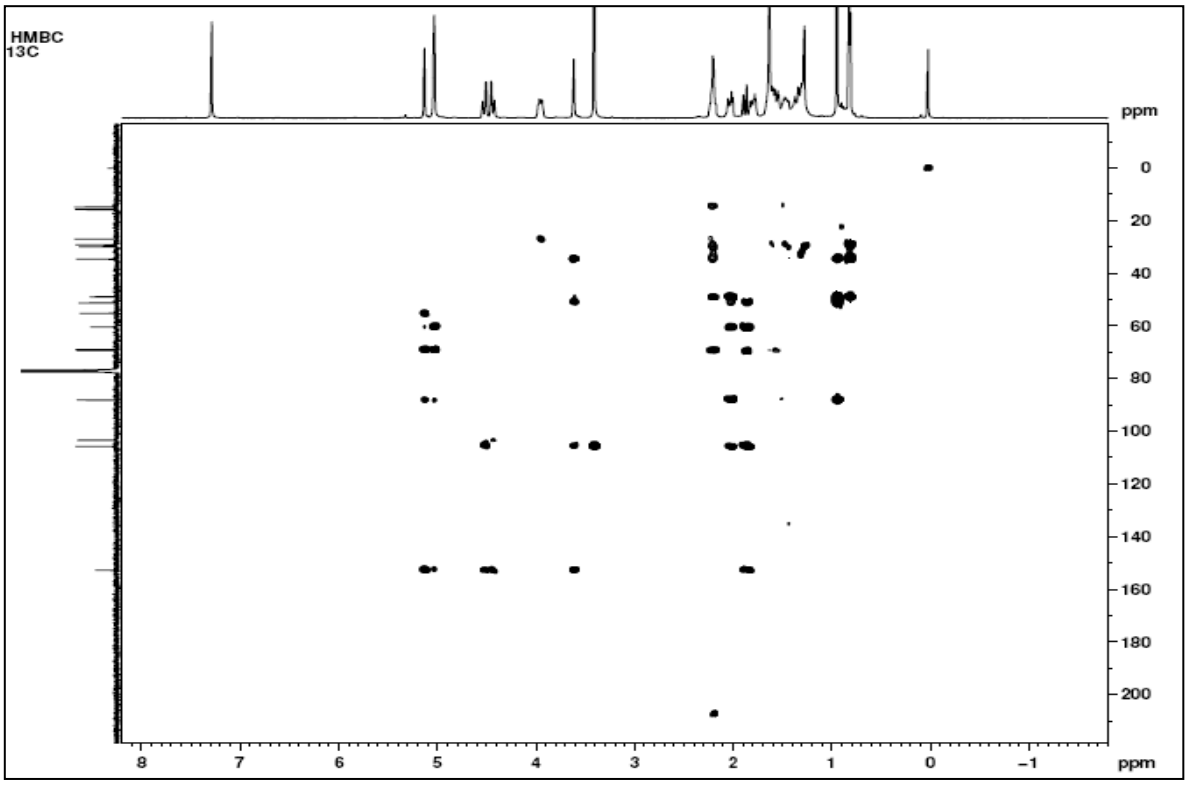
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 83



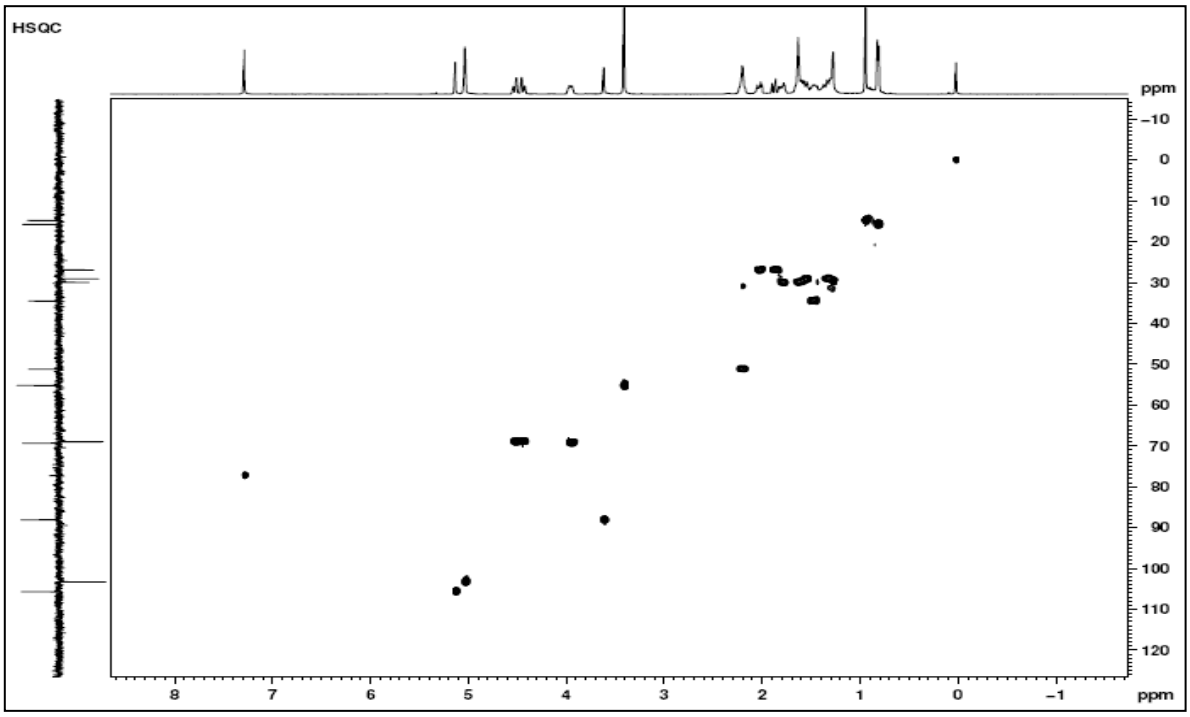
^{1}H-^{1}H COSY spectrum (400 MHz) of 77 in $CDCl_3$



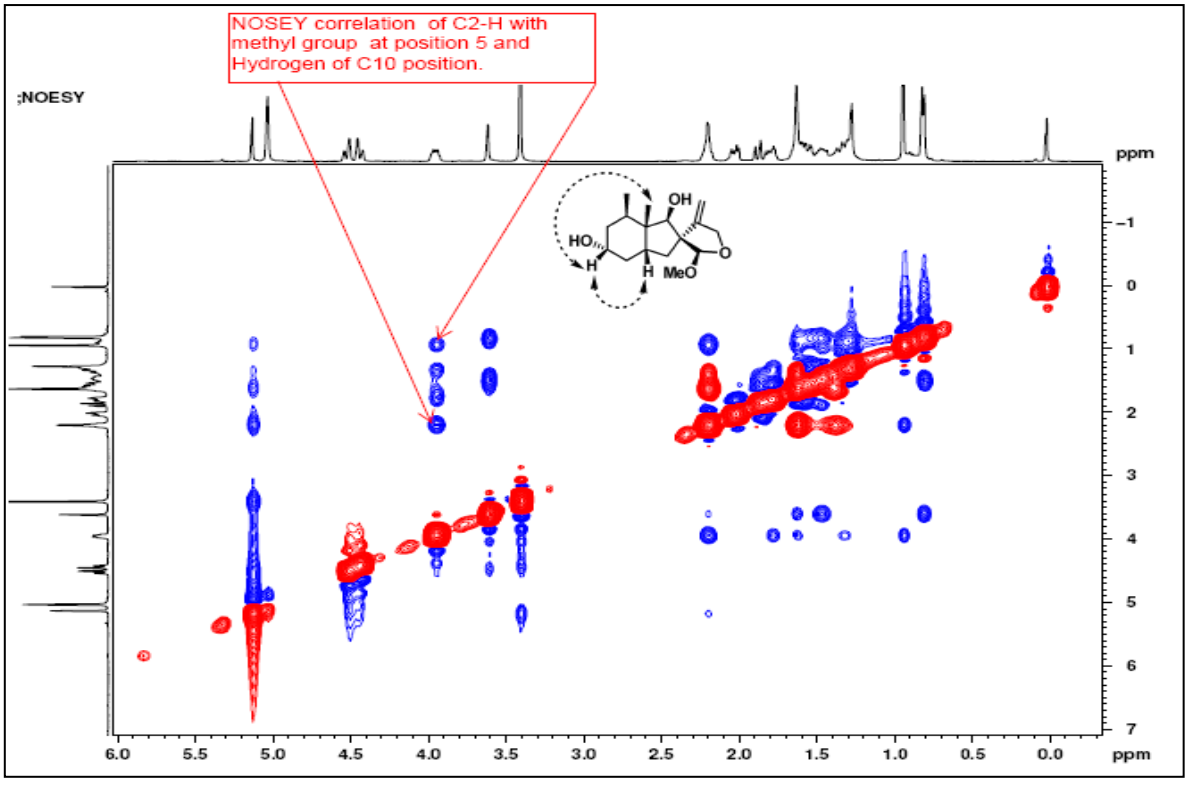
HMBC Spectrum (400 MHz) of 77 in $CDCl_3$



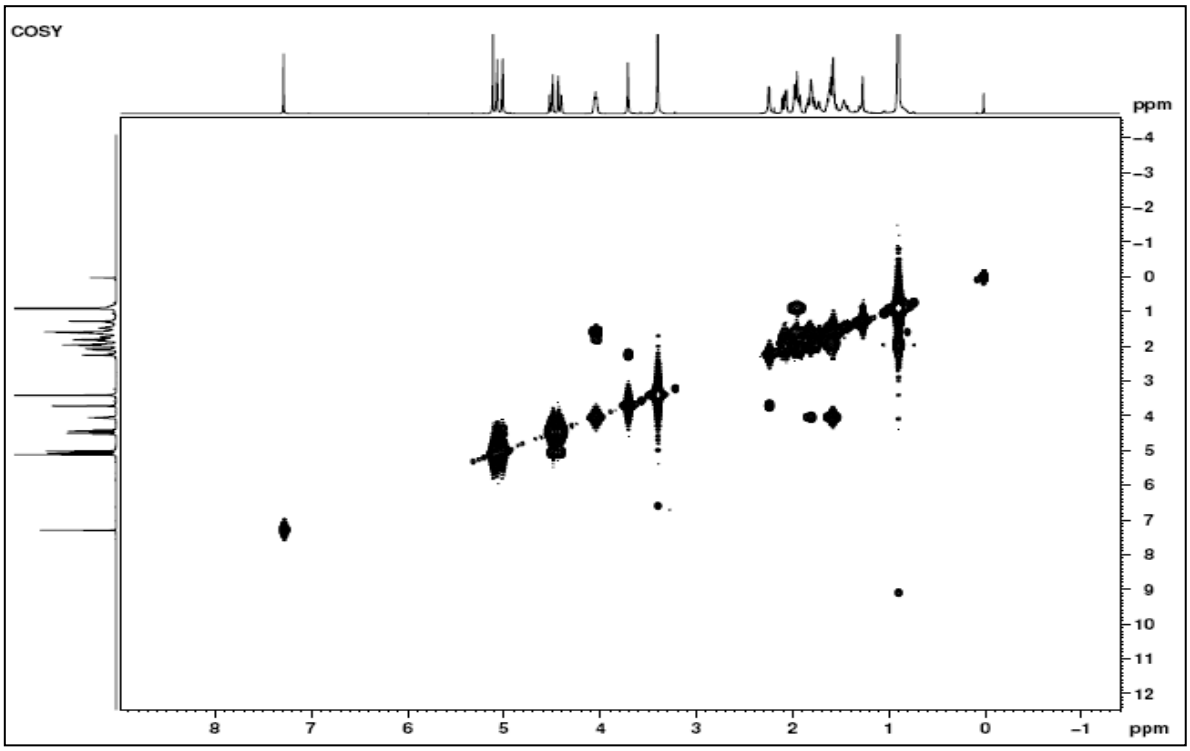
HSQC Spectrum (400 MHz) of 77 in $CDCl_3$



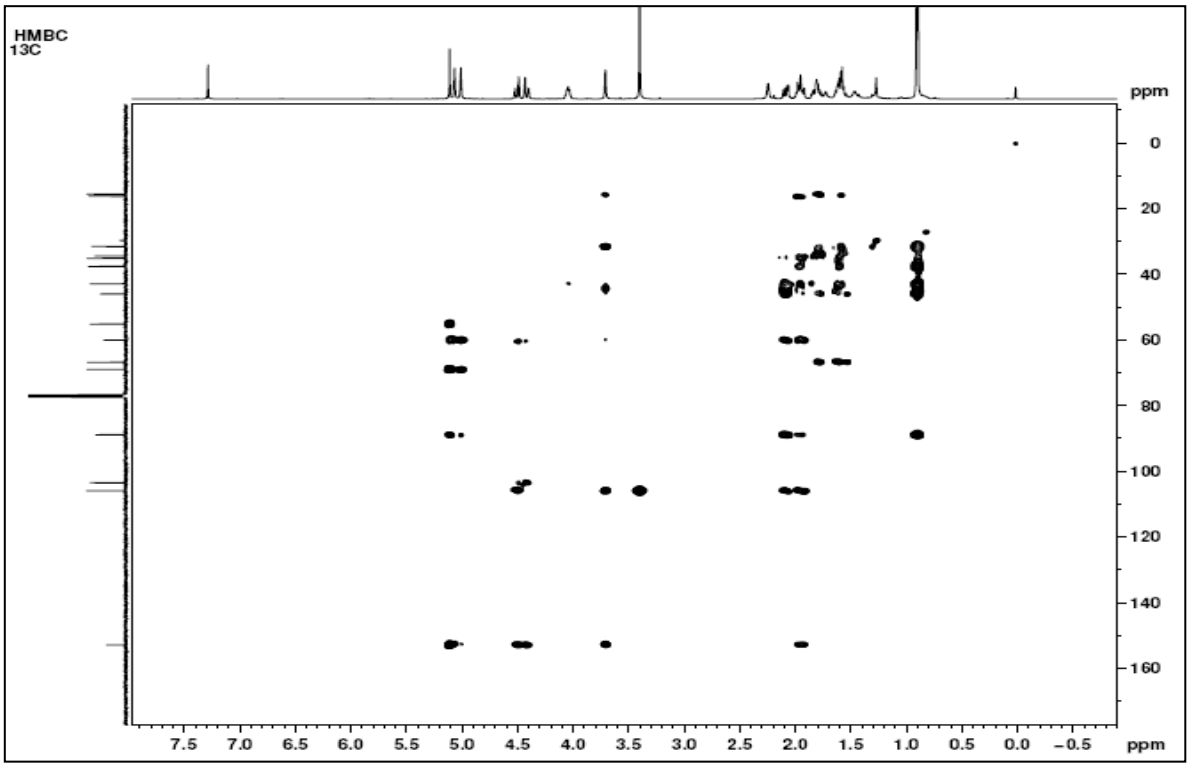
NOESY Spectrum (400 MHz) of 77 in $CDCl_3$



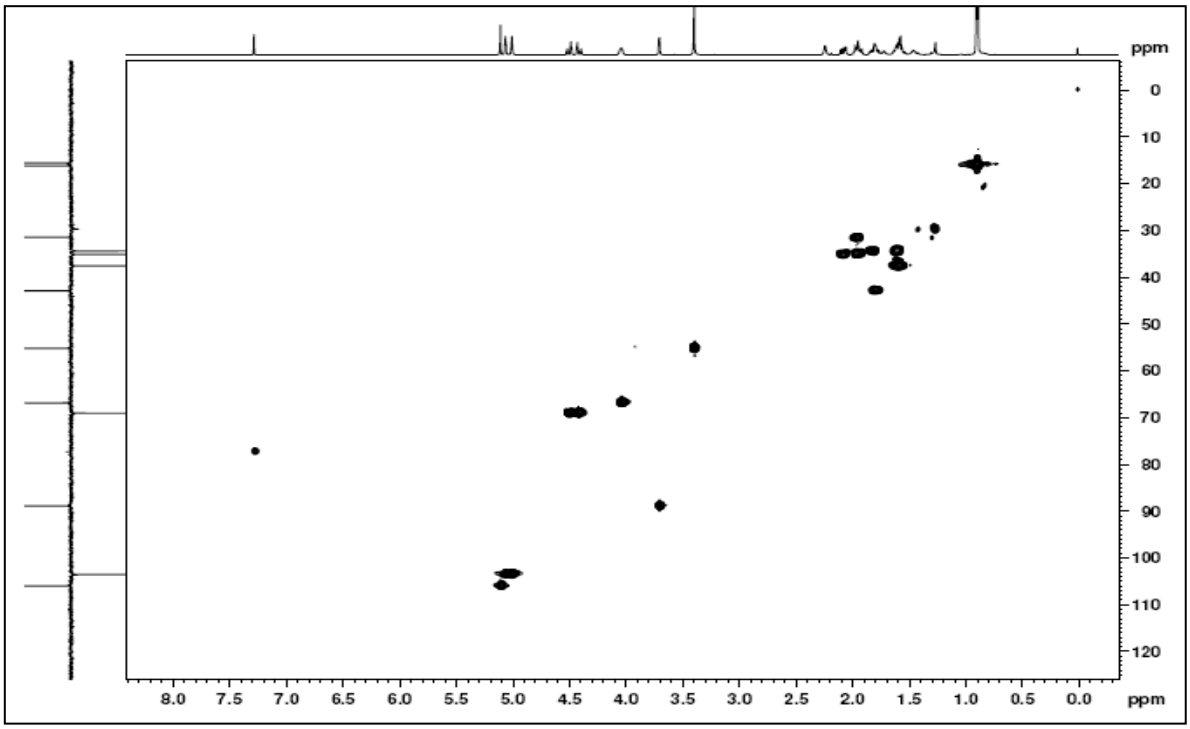
` **^{1}H-^{1}H COSY spectrum (400 MHz) of 78 in $CDCl_3$**



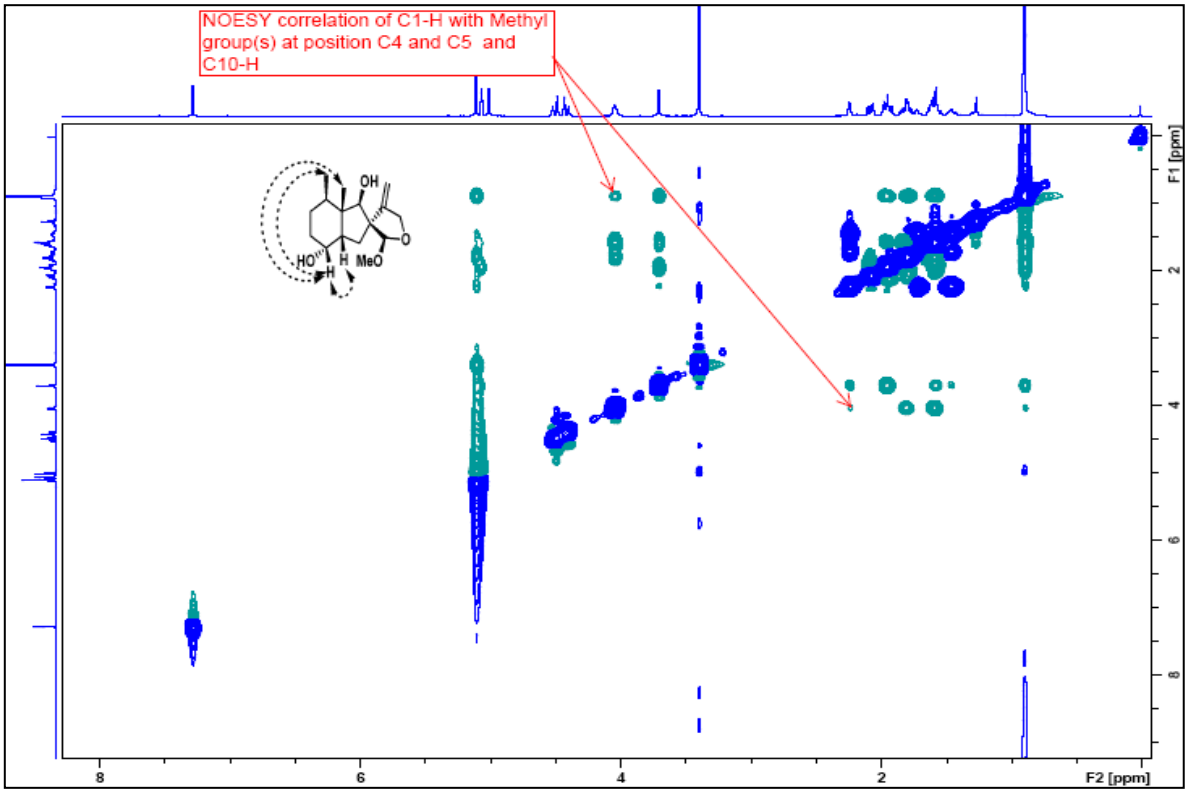
HMBC Spectrum (400 MHz) of 78 in $CDCl_3$



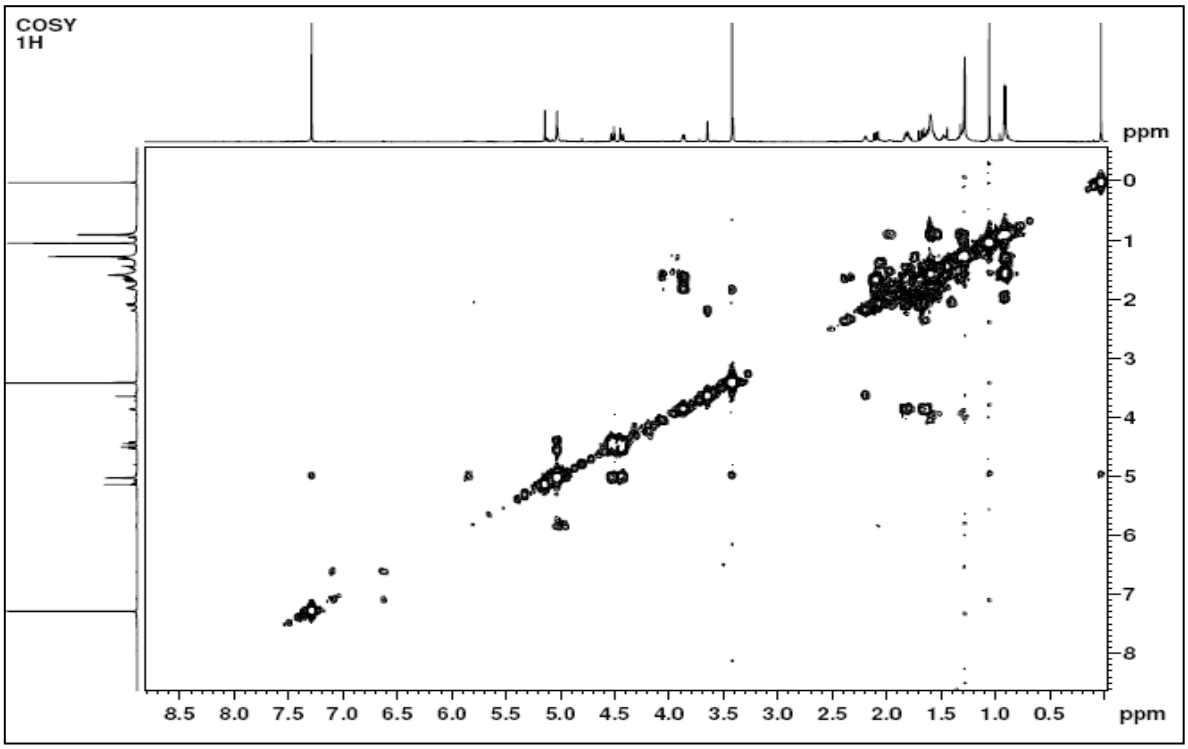
HSQC Spectrum (400 MHz) of 78 in $CDCl_3$



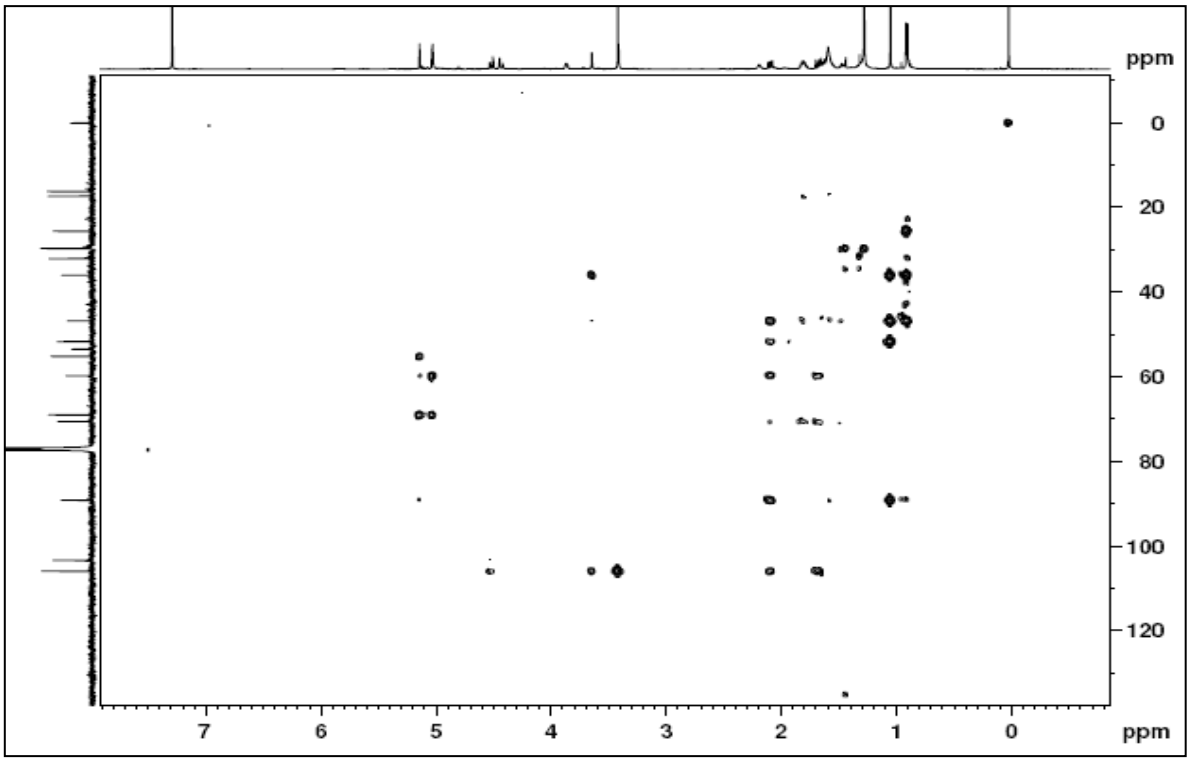
NOESY Spectrum (400 MHz) of 78 in $CDCl_3$



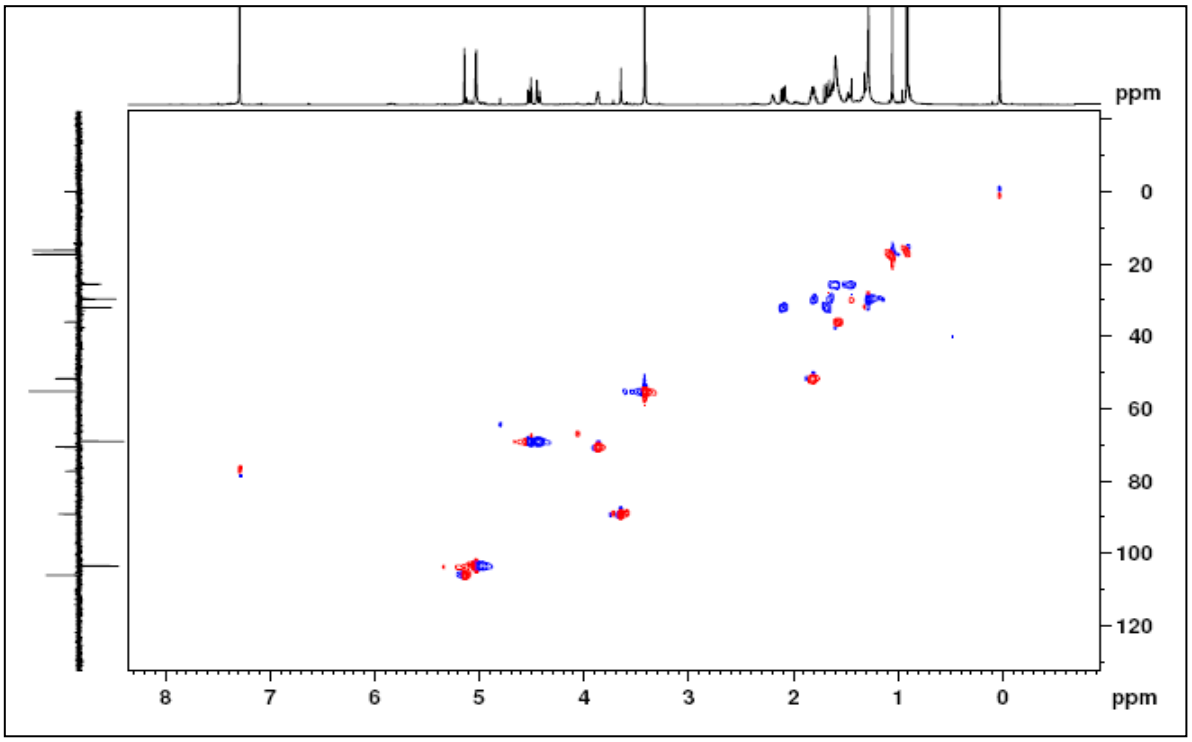
^{1}H-^{1}H COSY spectrum (400 MHz) of 79 in $CDCl_3$



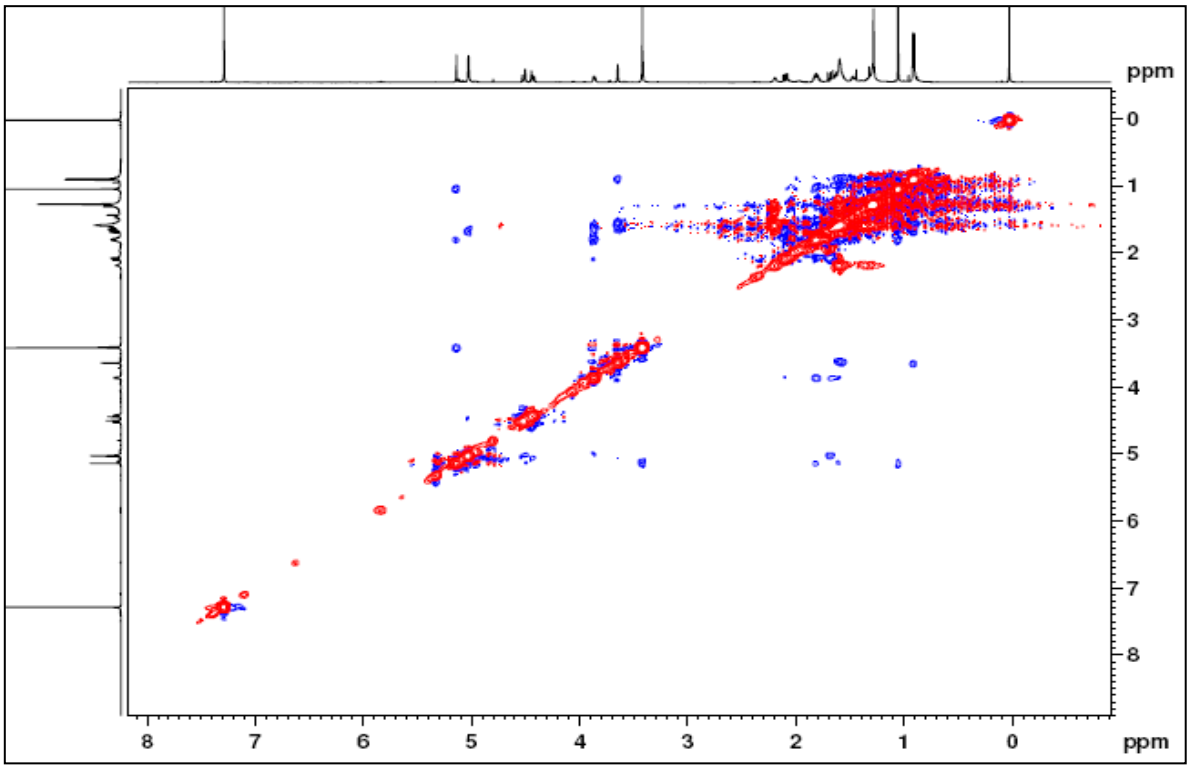
HMBC Spectrum (400 MHz) of 79 in $CDCl_3$



HSQC Spectrum (400 MHz) of 79 in $CDCl_3$



NOESY Spectrum (400 MHz) of 79 in $CDCl_3$



Chapter 2

Ready Access to *cis*-Hydrindane and *cis*-Decalin: Protecting Group Free Total Synthesis of Nootkatone, Noreremophilanes and their Biological Evaluation

2.1 Introduction to *cis*-hydrindane & *cis*-decalin based natural products

The *cis*-hydrindane & *cis*-decalin skeletons are present in various classes of natural products such as Bakkenolide **1**, Peribysin E **2**, Picrotoxinin **3**, Nakamurol A **4**, Kalihinene-X **5** and Eremophilenolide **6** (Fig 2.1).[1] Many of these *cis*-hydrindane & *cis*-decalin based natural products exhibit diverse range of remarkable biological activities and are always endowed with various functionalities and stereochemical patterns. These scaffolds have received the attention of many researchers around the globe to explore new methods and applied to the synthesis of a variety of natural products.[2,3] Accessing the functionally embellished natural products containing this complex framework remains a challenge for the synthetic organic community and developing new strategies for their syntheses is always rewarding.

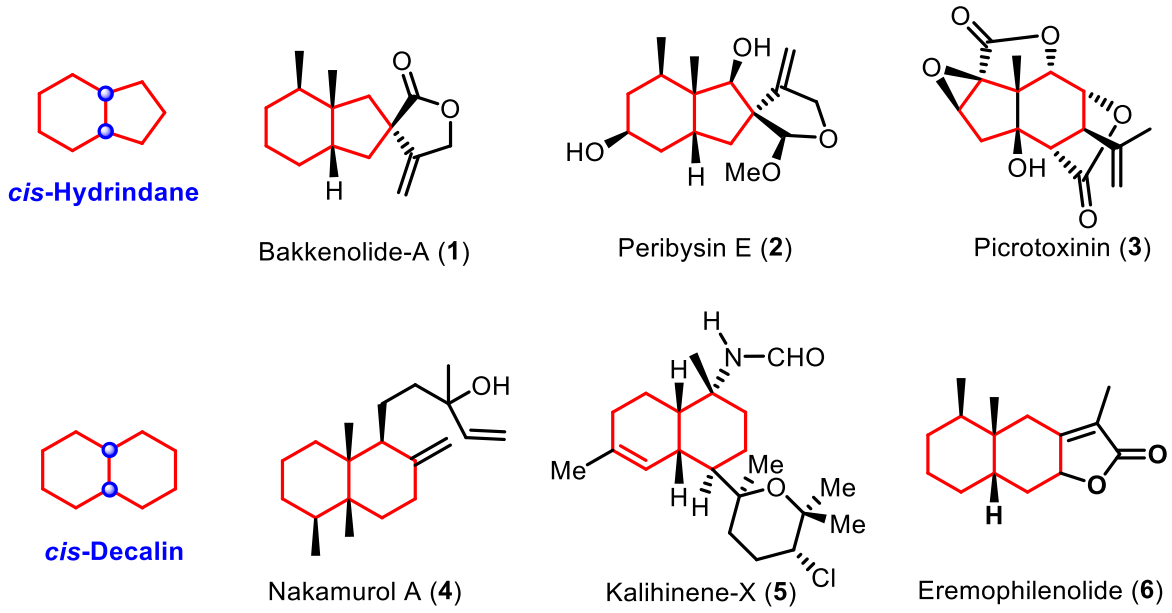


Fig 2.1: Selected natural products with *cis*-hydrindane and *cis*-decalin frameworks

2.2 Background of Diels-Alder/aldol sequence

In 2004, Reddy independently reported a simple and efficient approach to the family of Bakkanes[4-6] (Fig 2.2) by developing a simple method to access *cis*-hydrindane skeleton using a Diels-Alder/aldol sequence in a highly diastereoselective manner and applied to a

short synthesis of bakkenolide-A (**1**),[7] an prominent member of bakkane class of compounds. Bakkane family comprises of large number of natural compounds with hydrindane skeleton with rich functional group decorations on them. Selected members of this family are shown in following figure 2.2.

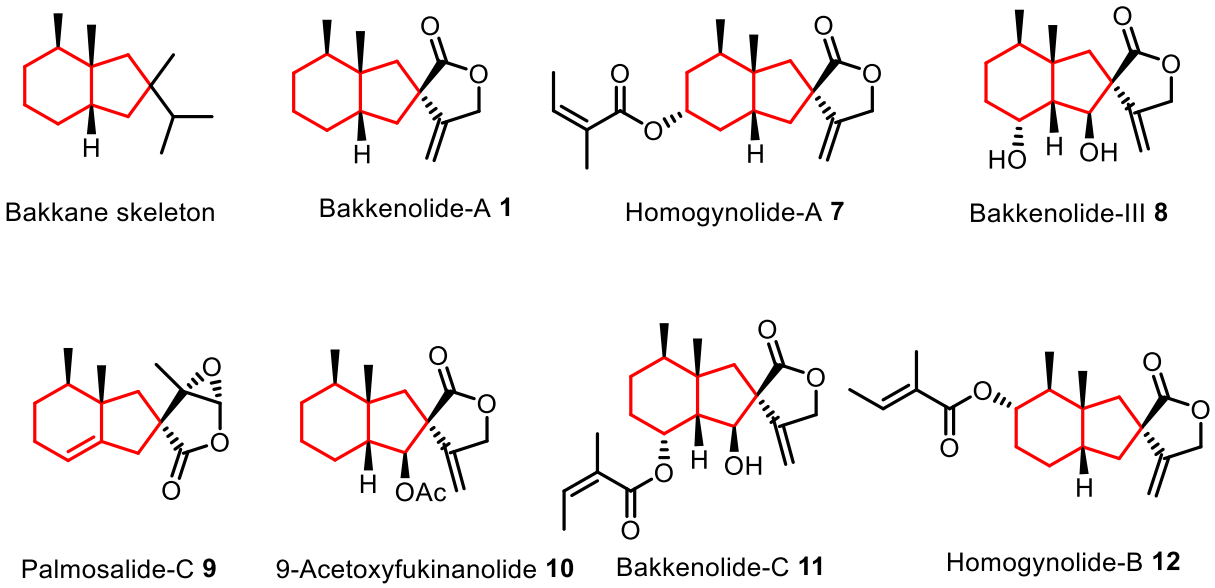
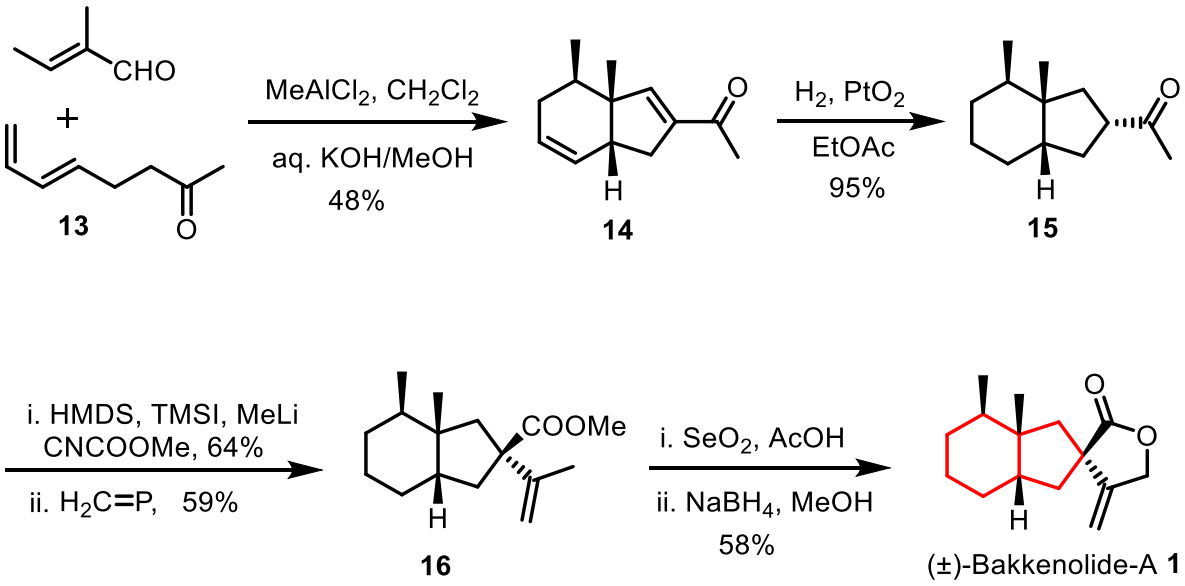


Fig 2.2: Selected natural products of bakkane family

Reddy's approach for bakkenolide-A **1** commenced with intermolecular Diels-Alder reaction of diene **3** with tiglic aldehyde in the presence of a Lewis acid $MeAlCl_2$ to give Diels-Alder adduct which on treatment with aq. KOH in MeOH furnished enone **14** in a highly diastereoselective fashion (scheme 2.1). Next, compound **14** on hydrogenation by H_2, PtO_2 afforded saturated ketone **15** in a highly stereoselective manner (>9:1). The reaction of **15** with methyl cyanoformate (Mander's reagent) affords keto-ester followed by single carbon Wittig olefination resulted in compound **16**. Finally, the compound **16** on allylic oxidation with SeO_2 followed by $NaBH_4$ reduction furnished bakkenolide A **1** in 58% yield over two steps.



Scheme 2.1: Reddy's approach to the synthesis of bakkenolide A **1**

2.3 Present work

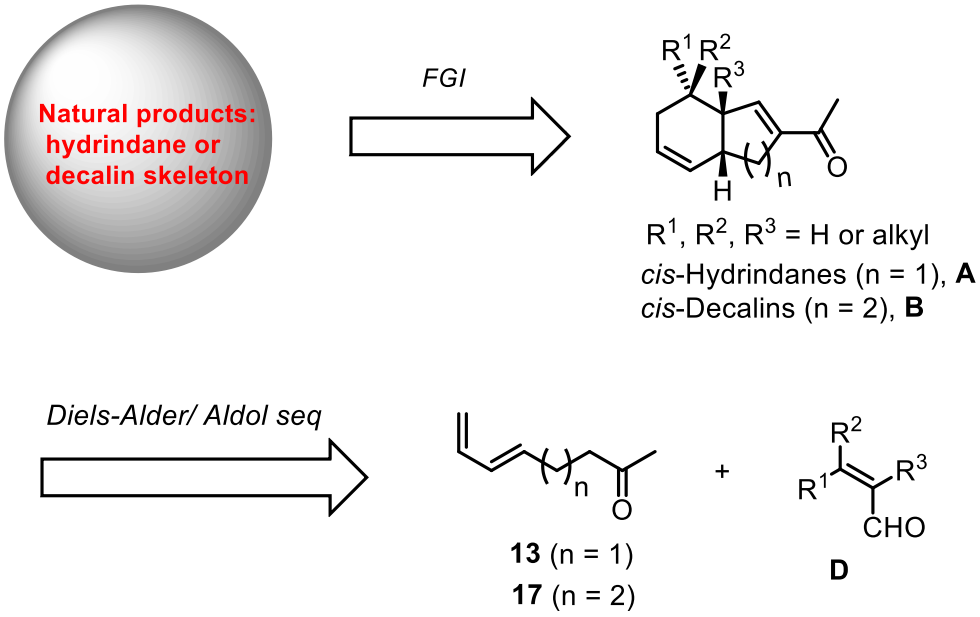
The Diels-Alder/aldol sequence is highly efficient and diastereoselective for the construction of *cis*-hydrindane and it was proved based on previous work on bakkenolide and peribysin E (previous chapter of this thesis). As a fresh extension of this sequence, we have initiated a project with the following aims.

- To generalize the method to access several new *cis*-hydrindanes / *cis*-decalins.
- To implement developed method for the synthesis of different natural products.
- To generate a library of analogues around natural products scaffold and study their biological properties.

2.3.1 Our retrosynthetic approach

Our general retrosynthetic approach is shown in scheme 2.2. As per the plan, *cis*-hydrindane (**A**) or *cis*-decalin (**B**) which possesses chemically differentiated double bonds (isolated and conjugated) could be used for the synthesis of various natural products by

some functional group transformations. The key intermediates *cis*-hydrindane (**A**) or *cis*-decalin (**B**) could be constructed by differently substituted dienophiles **D** with dienes **1** and **2**, respectively, using Diels-Alder/aldol sequence. The starting components diene **13**, **17** and various dienophiles **D** are commercially available or can be prepared using known literature procedures.



Scheme 2.2: Our Retrosynthetic Approach

2.3.2 Scope of Diels-Alder/aldol sequence

To explore the scope of this method, we have chosen different dienes and different substituted dienophiles. The following substituted dienophiles were selected for the Diels-Alder reaction (Fig 2.3) and some of them are prepared using known literature procedures.[8]

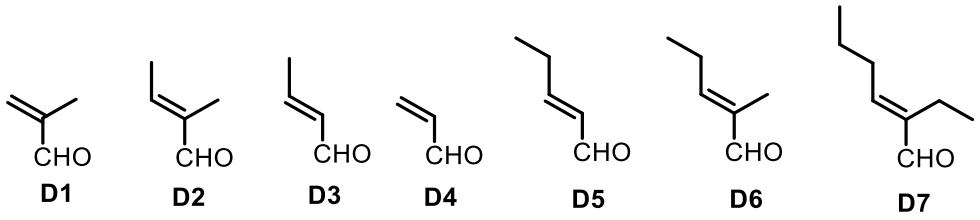
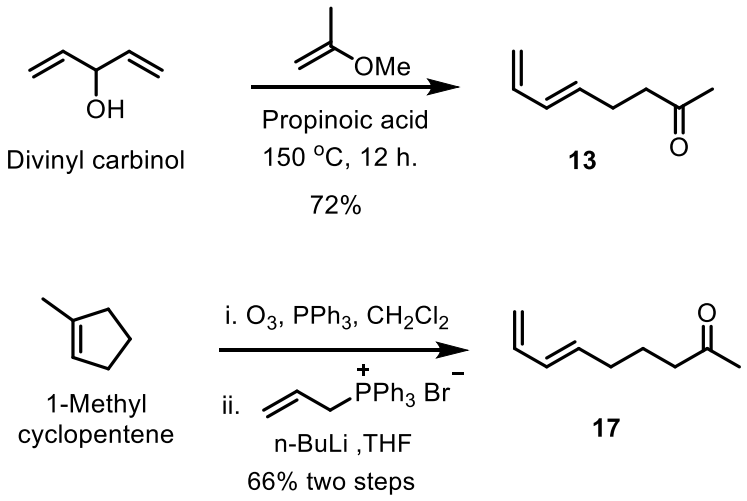


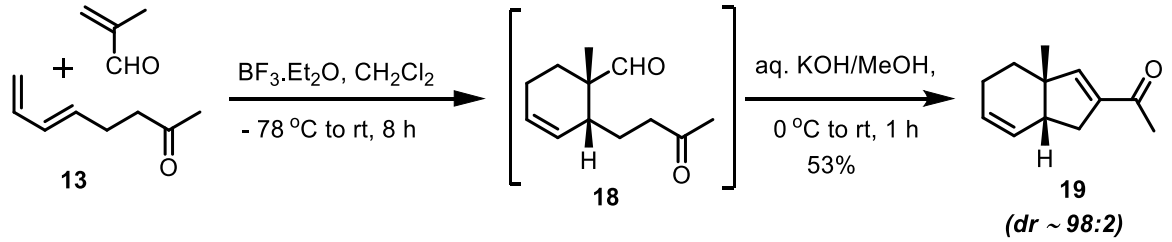
Fig 2.3: Different dienophiles **D**

Different dienes were chosen and prepared them using known literature procedures. The diene **13** was prepared from divinyl carbinol**,** 2-methoxy propene in the presence of propionic acid in one step via Claisen rearrangement[9] in 72% yield whereas diene **17**[10a] was prepared from ozonolysis of 1-methyl cyclopentene to give keto-aldehyde[10b] followed by Wittig reaction with allyltriphenylphosphonium bromide in 66% yield over two steps (scheme 2.3).



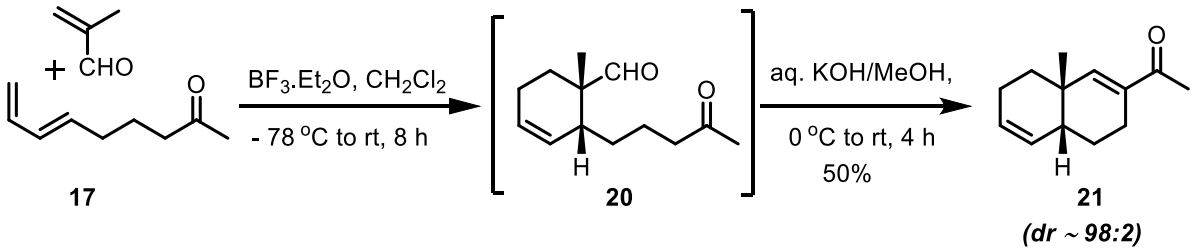
Scheme 2.3: Synthesis of diene **13** and **17**

For intermolecular Diels-Alder reaction, we have selected $BF_3{\cdot}Et_2O$ as Lewis acid because of its ready availability and safety than $MeAlCl_2$. We commenced our fresh exploration with Lewis acid $BF_3{\cdot}Et_2O$ mediated intermolecular Diels-Alder reaction[11-13] between diene **13** and 2.5 eq. of dienophile methacrolein in CH_2Cl_2 at -78 °C to rt to afford crude keto-aldehyde adduct **18** which was immediately subjected to intramolecular aldol condensation reaction with 15% aq. KOH in MeOH to furnish *cis*-hydrindane **19** in a excellent diastereoselective fashion (*dr* ~98:2) with a moderate yield of 53% (scheme 2.4). The formation of *cis*-hydrindane **19** was indicated presence of acetyl group at 2.29 (s, 3H) ppm, olefinic proton signals at 6.49 (s, 1H), 5.73−5.61 (m, 2H) ppm and angular methyl signal at 1.11 (s, 3H) ppm in ^{1}H NMR spectrum. Similarly corresponding olefinic carbon signals at 153.1, 143.2, 129.8, 126.0 ppm and ketone carbonyl at 197.6 ppm in ^{13}C NMR, which was supported by the absorption at 1663 cm^{-1} in the IR spectrum. The assigned structure of **19** was further confirmed by HRMS which showed a peak at 177.1274 corresponding to formula $C_{12}H_{17}O$ $[M+H]^+$ with calculated value of 177.1274.



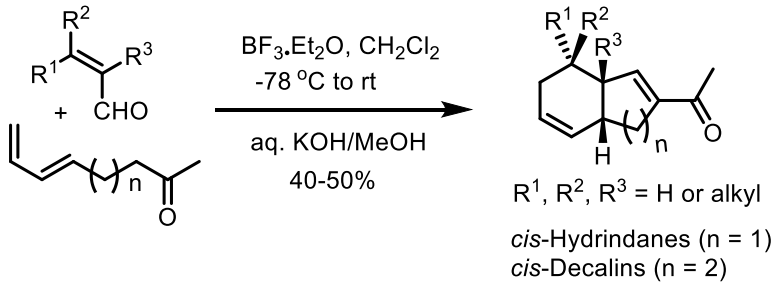
Scheme 2.4: Synthesis of *cis*-hydrindane **19**

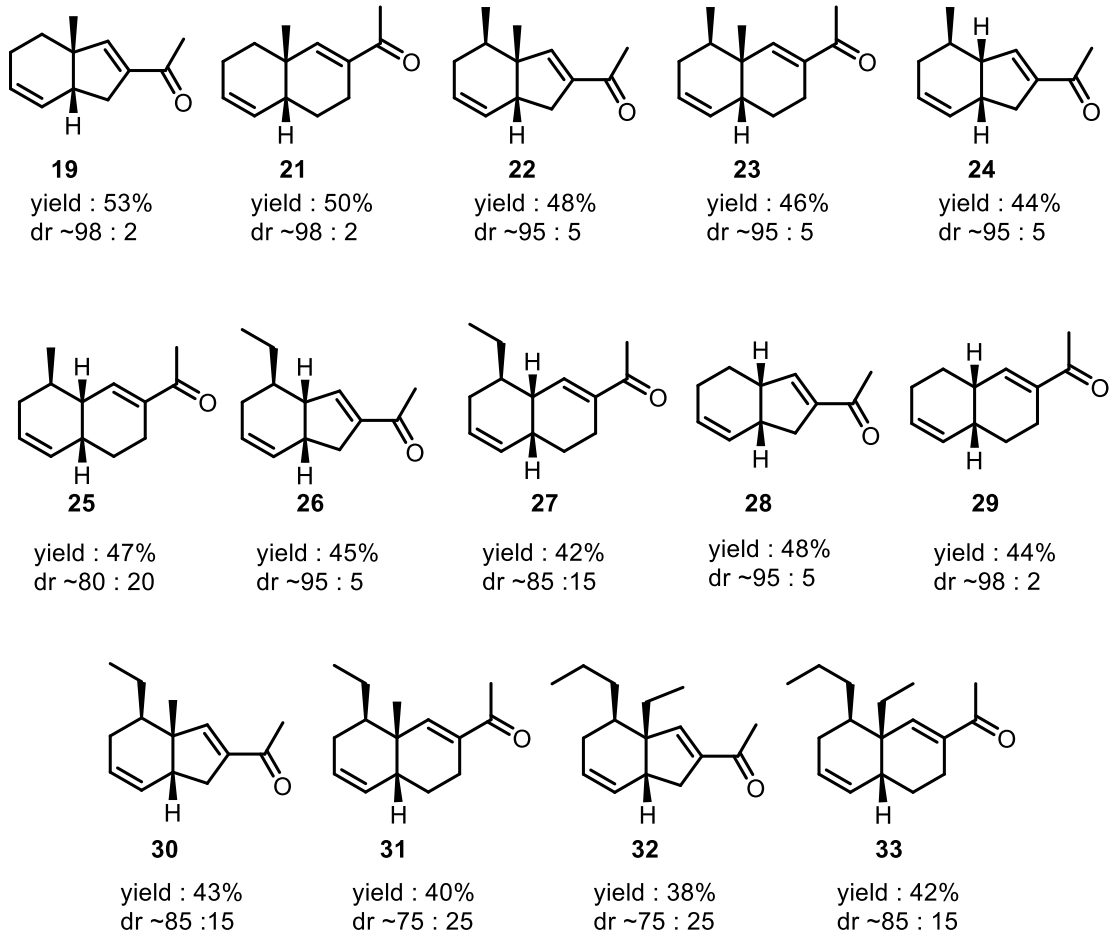
Similarly, we performed the intermolecular Diels-Alder reaction between diene **17** and methacrolein followed by base treatment to afford *cis*-decalin **21** in 50% yields with *dr* ~ 98:2 ratios (scheme 2.5). The product **21** was characterized the presence of acetyl group at 2.29 (s, 3H) ppm, olefinic proton signals at 6.44 (s, 1H), 5.69−5.64 (m, 1H), 5.49−5.45 (m, 1H) ppm and angular methyl signal at 1.10 (s, 3H) ppm in ^{1}H NMR spectrum. Similarly corresponding olefinic carbon signals at 149.0, 138.3, 130.3, 126.7 ppm and ketone carbonyl at 199.8 ppm in ^{13}C NMR which was further supported by the absorption at 1669 cm^{-1} in the IR spectrum. The compound **21** showed a peak at 191.1430 corresponding to formula $C_{13}H_{19}O$ $[M+H]^{+}$ with calculated value of 191.1430 in HRMS which further confirmed the assigned structure.



Scheme 2.5: Synthesis of *cis*-decalin **21**

By using tiglic aldehyde as dienophile we have prepared the *cis*-hydrindane **22** and *cis*-decalin **23** in moderate yield and the diastereomeric ratio was found to be ~95:5 in both cases. By applying similar reaction condition using different dienes and dienophiles, this methodology was successfully implemented for the synthesis of various substituted *cis*-hydrindanes & *cis*-decalins in moderate yield (scheme 2.6). Diastereomeric ratio was determined in NMR detectable limits.

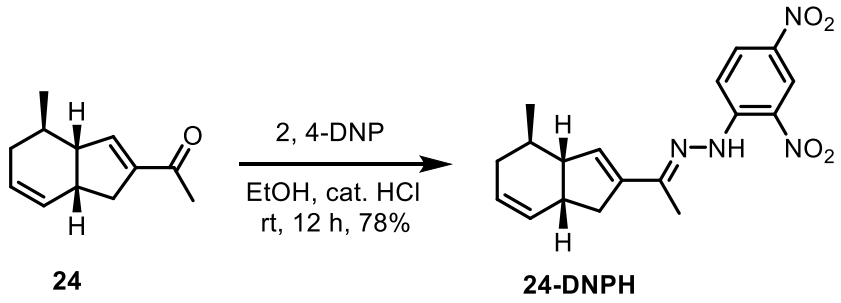




Scheme 2.6: Scope of method

Although we are confident about the assignment of stereochemistry, we wanted to make sure the stereochemistry without any ambiguity, particularly, where angular methyl group is absent. This is because presence of acidic proton next to aldehyde may result in possible epimerization under basic conditions. It was also pointed out by one of the reviewer when

we submitted this work for publication. For this purpose, we treated compound **24** with 2, 4-dinitrophenylhydrazine (2, 4-DNP) in EtOH to provide its 2, 4 DNP derivatives **24-DNPH** (scheme 2.7) as a crystalline solid. The compound **24-DNPH** was recrystallized using ethyl acetate-hexane solvent mixture. The assigned *cis*-stereochemistry of the ring junction in compound **24** was unambiguously determined with the help of the single X-ray crystal analysis (Fig 2.4) of its 2,4 DNP derivative **24-DNPH** and accordingly, the stereochemistry was assigned to major isomers of all the hydrindanes and decalins as shown in scheme 2.6.



Scheme 2.7: Synthesis of **24-DNPH**

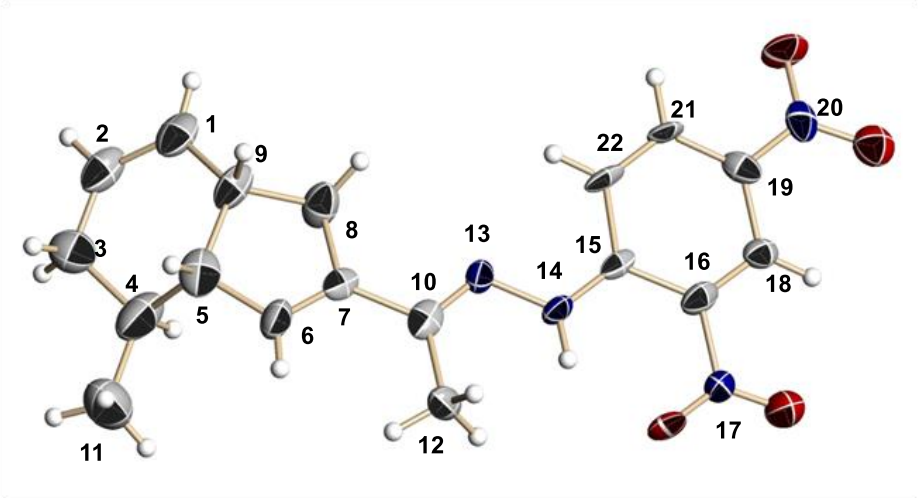


Fig 2.4: ORTEP of **24-DNPH**.

The assigned *cis*-stereochemistry of the ring junction in compounds **24**, **25**, **26**, and **27** was further confirmed by 2D-NMR analysis to exclude any possibility of epimerization before the aldol condensation. In case of compound **24**, the ^{13}C NMR spectrum indicated the presence of a carbonyl group at 197.2 ppm, which was supported by the absorption at 1669

cm^{-1} in the IR spectrum. An acetyl group was detected at 2.30 (s, 3H) in the ^1H NMR spectrum. The ^1H NMR spectrum showed the presence of a secondary methyl group at 1.02 (d, J = 6.4 Hz, 3H) as well as one proton absorption at 6.82 (d, J = 3.2 Hz, 1H) assignable to an olefinic proton. The ^1H NMR and DEPT spectrum also showed the presence of internal two olefinic protons. The HMBC spectrum indicated correlations from the methyl group at C-12 into C-1, C-2, and C-9. The sequence of protons of H-1, H-2, H-3, H- 4, H-5 and H-9 were indicated by the COSY spectrum. The NOE spectrum indicated that H-9 is having a positive NOE correlation with methyl at C-1 position. The H-5 is also showing a positive NOE correlation methyl at C-1 position. The 2β-H also shows a positive NOE correlation to H-9 and to H-5. There is also seen a positive NOE correlation between H-9 and H-5 (Fig 2.5). Thus it indicated hydrindane **24** was *cis*-fused. In case of compound **26**, NOE spectrum indicated that H-9 is having a positive NOE correlation with 12β-H. The H-5 is also showing a positive NOE correlation with 12β-H. The 2β-H also shows a positive NOE correlation to H-9 and to H-5. In case of **26**, positive NOE correlation between H-9 and H-5 was present which indicated that hydrindane **26** was indeed *cis*-fused junction.

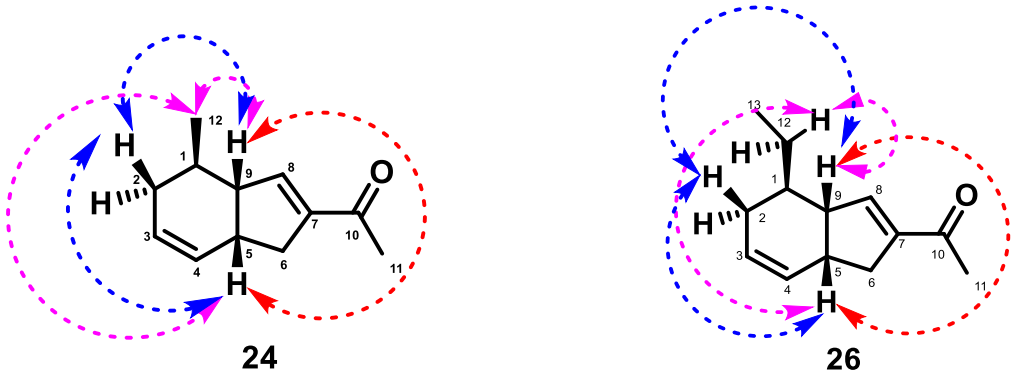


Fig 2.5: NOE correlation of compound **24** and **26**

In case of compound **25**, the NOE spectrum indicated that H-10 is having a positive NOE correlation with methyl at C-1 position. The H-5 is also showing a positive NOE correlation methyl at C-1 position. The 2β-H also shows a positive NOE correlation to H-10 and to H-5. There is also seen a positive NOE correlation between H-10 and H-5. Thus it indicated decalin **25** was *cis*-fused. In case of compound **27**, the NOE spectrum indicated that H-10 is having a positive NOE correlation with 13β-H. The H-5 is also showing a positive NOE correlation with 13β-H. H-10 also having a correlation with methyl at 14

position (Fig 2.6). Thus it was indicated decalin **27** is having *cis*-fused ring junction.

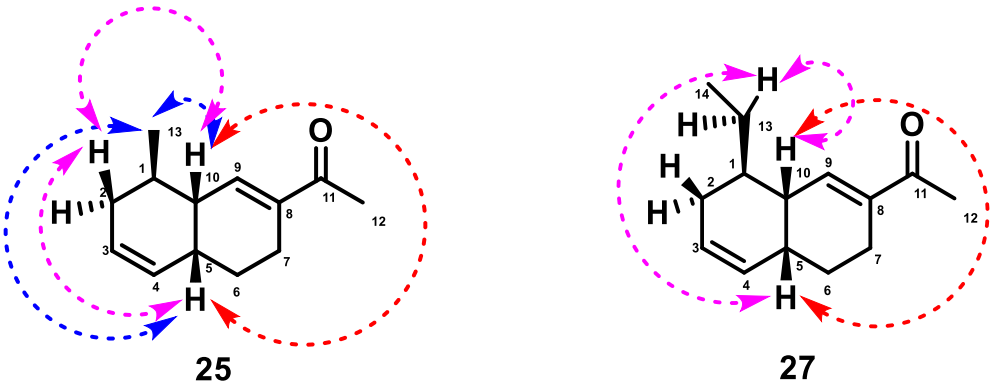


Fig 2.6: NOE correlation of compound **25** and **27**

We observed that majority of hydrindanes show high diastereoselectivity as compared with that of decalins.[14] The presence of bulky substitution at α- and *β*-positions of the dienophile lowers the diastereoselectivity in the case of hydrindanes **30**, **32** and decalins **31**, **33**. So after successful synthesis of different *cis*-hydrindanes / *cis*-decalins using Diels-Alder/aldol sequence, we have applied this methodology for the synthesis of natural products such as nootkatone and noreremophilanes.

2.3.3 Nootkatone

2.3.3.1 Introduction of Nootkatone

The eremophilane type sesquiterpene called (+)-nootkatone **34** belongs to the valencane **36** family (Fig 2.7). It was first isolated from the heartwood of Alaskan yellow cedar (*Chamaecyparis nootkatensis*), was later found in trace amounts of grapefruit (*Citrus paradise*), pummelo (*Citrus grandis*) and vetiver oil (*Vetiveria zizanioides*).[15] It has wide application in flavor and fragrance arenas since it is an important flavoring component of grapefruit and commercially used to flavour soft drinks and other beverages and in perfumes. (+)-Nootkatone **34** possesses very impressive insect repellent and/or insecticidal activity against various ticks, mosquitos, termites, bed bugs, etc.[16] Recently, using limited structure activity relationship studies, two close derivatives of (+)-nootkatone (**34**), tetrahydronootkatone (THN) (**35)** and 1,10-dihydronootkatone (**35**') were observed to act

as potent insecticidal and repellent activities against several arthropods including termites, ants, cockroaches and ticks.

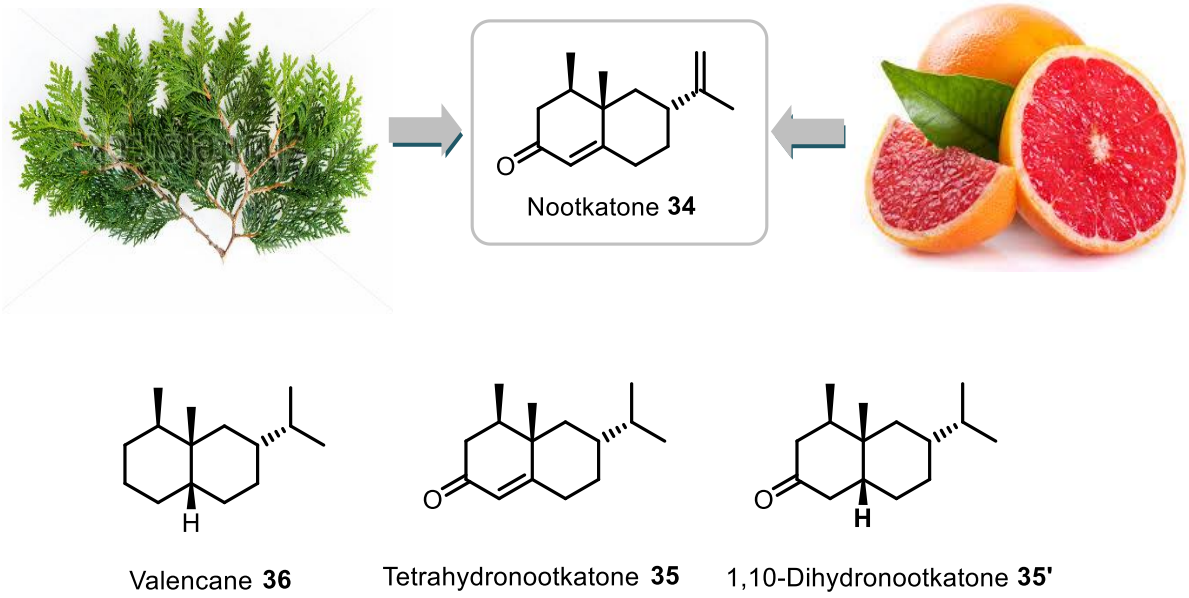
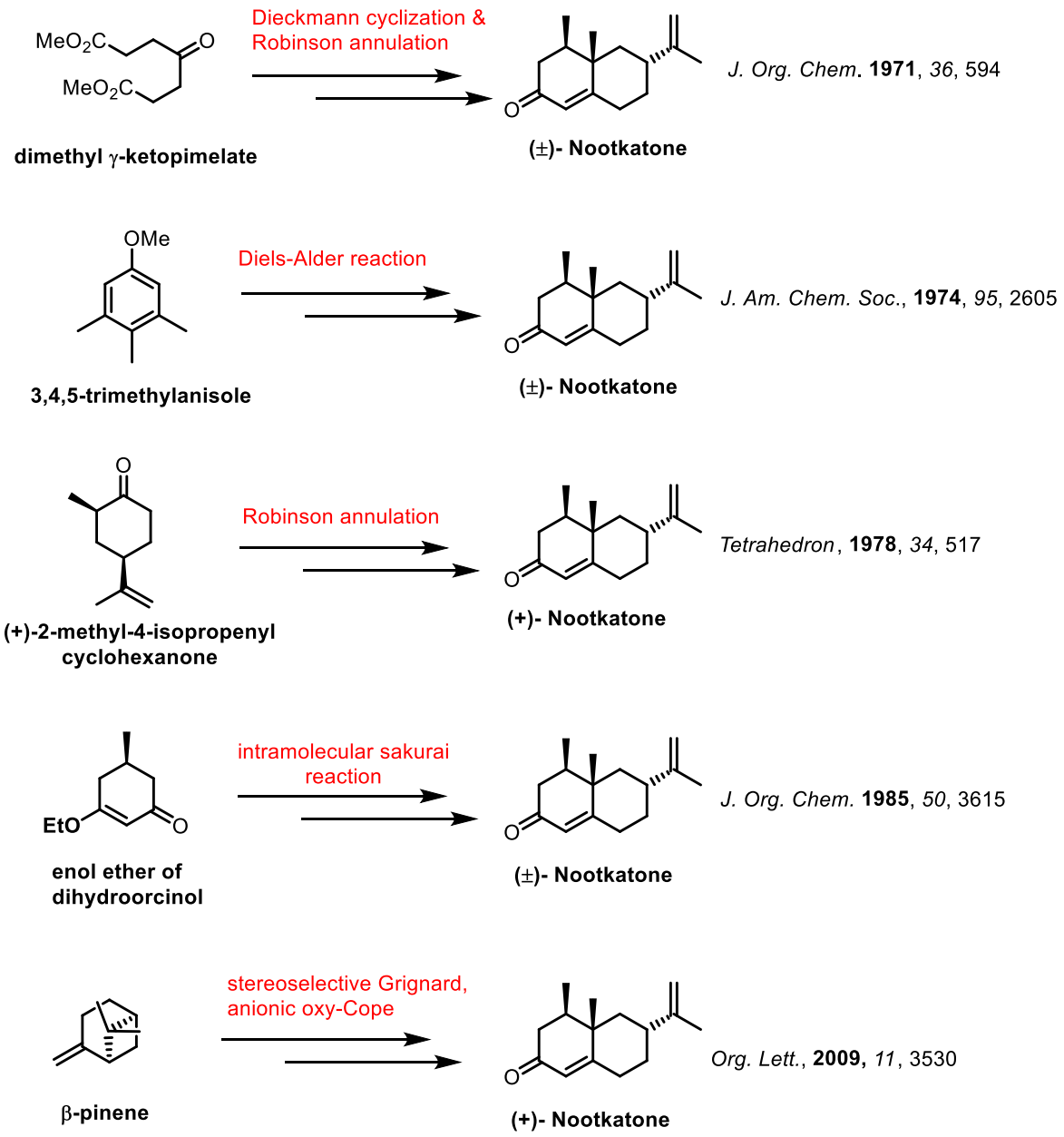


Fig 2.7: Structure of natural products and their derivatives
(Pictures of plant (*Chamaecyparis nootkatensis*) and fruit (*Citrus paradise*) taken from the internet)

Tetrahydronootkatone **35** shows very high repellency and toxicity to *C. formosanus* (Formosan subterranean termite), nine times effective than nootkatone. It is believed that mode of action of nootkatone is by blocking the octopamine receptor and the neurotransmitter octopamine is found only in insects.[17] Therefore compound like nootkatone are safe for human beings, as humans and animals do not have these receptors as like insects. It was also discovered that nootkatone acts as AMPK (AMP activated protein kinase activator), that is serine/threonine kinase and concerned in the control of energy metabolism and is known to be a molecular target for the treatment of metabolic syndrome. Nootkatone induced the phosphorylation of AMPK and the downstream target acetyl-CoA carboxylase (ACC), and enhanced AMPK activities *in vitro* and *in vivo*.[18] As a result, there is a continuing interest in the development of efficient methods for the synthesis of (+)-nootkatone **34** among synthetic organic communities.

2.3.3.2 Previous syntheses of nootkatone

There are several syntheses of nootkatone reported in the literature and some of them are summarized in scheme 2.8. Most synthetic approaches to the valencane skeletal framework have relied on Robinson annulation reactions. Marshalla and coworker reported the stereoselective total synthesis of racemic nootkatone **34** using Dieckmann cyclization followed by Robinson annulation as key reaction to construct the nootkatone skeleton.[19]

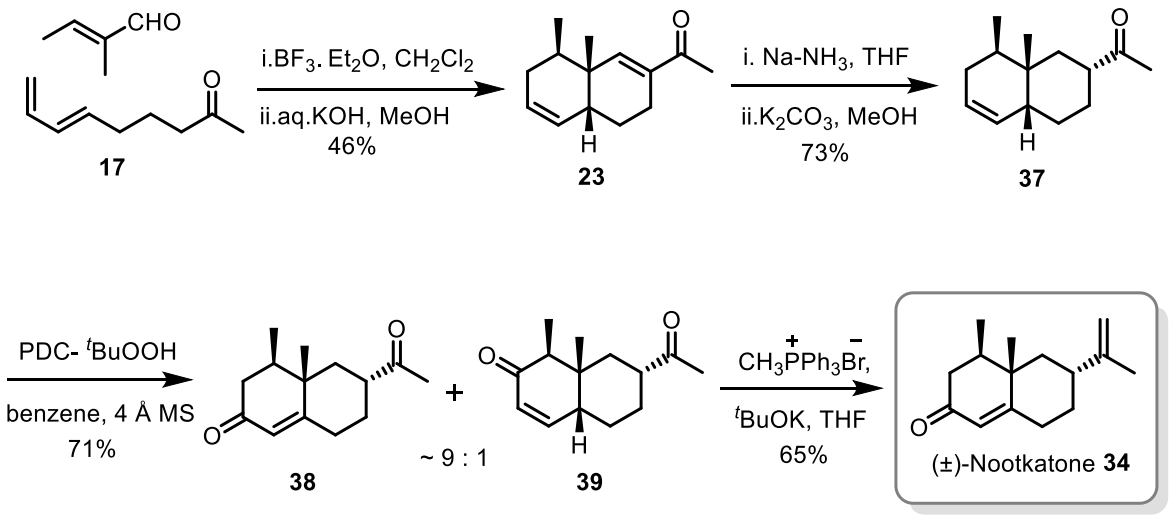


Scheme 2.8: Previous syntheses of nootkatone

Dastur utilized the Diels-Alder reaction to construct eremophilane sesquiterpene skeleton and synthesized the racemic nootkatone **34** starting from 3, 4, 5-trimethylanisole[20] (scheme 2.8). Takagi et al. synthesized the (+)-nootkatone **34** from (+)-2-methyl-4-isopropenyl cyclohexanone via Robinson annulation strategy in six steps.[21] Majetich and coworkers reported stereoselective synthesis of (±)-nootkatone and (±)-valencene via an intramolecular sakurai reaction.[22] Recently Laine's group achieved efficient and economical synthesis of (+)-nootkatone **34** starting from β-pinene through conjunctive stereoselective Grignard/anionic oxy-Cope (AOC) reactions and it is capable of meeting industrial needs.[23]

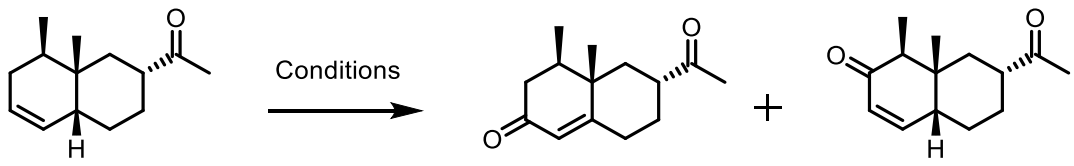
2.3.3.3 Our synthesis of nootkatone

Although several syntheses are documented in the literature, an implementation of our developed method and interesting biological features of nootkatone attracted our attention to choose this target for synthesis. Our synthetic effort towards nootkatone commenced with Diels-Alder/ aldol sequence to give *cis*-decalin **23** in 42% yield. The formation of *cis*-decalin **23** was characterized by the presence of acetyl group at 2.28 (s, 3H) ppm, olefinic protons signals at 6.64 (s, 1H), 5.60−5.56 (m, 1H), 5.53−5.48 (m, 1H) ppm and two methyl signals at 1.00 (s, 3H), 0.96 (d, J = 6.4 Hz, 3H) ppm in ^{1}H NMR spectrum. Similarly corresponding olefinic carbon signals at 149.3, 137.8, 130.1, 125.5 ppm and ketone carbon at 198.0 ppm in ^{13}C NMR spectrum, which was further supported by IR absorption at 1665 cm^{-1} and the assigned structure of **23** was further confirmed by HRMS. The compound **23** possess chemically differentiated double bonds in two different rings, the enone double bond present in **23** was chemoselectively reduced using Na-liq.NH_3 at −78 ^{o}C in THF conditions to give ~1:1 mixture of diastereomers. The resulting mixture on epimerization by exposure to K_2CO_3 in MeOH furnished the single diastereomer **37** in 73% isolated yield (scheme 2.9). The base-promoted epimerization of ketone furnish the thermodynamically favorable isomer **37**. The formation of product **37** was indicated by disappearance of one olefinic proton at 6.64 (s, 1H) ppm in compound **23** and appearance of C-H proton adjacent to carbonyl group at 2.46−2.40 (m, 1H) ppm in ^{1}H-NMR and corresponding carbon signal at 46.4 ppm in ^{13}C NMR spectrum. The structure of **37** was further confirmed by HRMS corresponding to $C_{14}H_{23}O$ $[M+H]^{+}$.



Scheme 2.9: Synthesis of nootkatone

The next task was to introduce the enone functionality in six membered ring. This was achieved by allylic oxidation of isolated double bond in **37** by PDC-^{t}BuOOH condition[24] to afford the major product **38** with enone moiety in right place as that of target molecule. The formation of product **38** indicated by presence of only one enone proton at 5.77 (s, 1H) ppm in ^{1}H-NMR and presence of carbonyl carbon signal at 210.7, 199.4 ppm and olefinic carbon at 168.7, 125.3 ppm in ^{13}C NMR spectrum. The product formation was further confirmed by HRMS which showed a peak at 221.1534 corresponding to formula $C_{14}H_{21}O_2$ $[M+H]^+$ with calculated value of 221.1536. In addition to the desired compound **38**, we have also isolated an undesired enone **39** in minor amount (10% with respect to **38**). Both the compounds **38** and **39** were well-separated on a TLC plate and cleanly separated using silica gel column chromatography in 20% EtOAc in petroleum ether. All the spectral data including HRMS support the assigned structure to compound **39**. To improve the yield, we have also attempted a few conditions to achieve the desired allylic oxidation by using Pd/C-^{t}BuOOH, K_2CO_3 and $NaClO_2$-^{t}BuOOH conditions[25] but always obtained a mixture of compounds (**38** and **39**) (scheme 2.10). Finally, compound **38** was subjected to a single carbon Wittig olefination in presence of potassium *tert*-butoxide to have the target compound (±)-nootkatone **34** in 65% isolated yield. All the spectral data (IR, ^{1}H and ^{13}C NMR) are found to be identical to those reported in the literature.[15] The ^{1}H-NMR and ^{13}C-NMR comparisons of Laine's group synthesized (+)-nootkatone **34** and our synthetic (±)-nootkatone **34** are shown in table 2.1 and 2.2.



37 38 39

Sr.No	Conditions	% yield	Ratio of **38** and **39**
1	**PDC-^{t}BuOOH, benzene, rt, 12 h**	**71%**	**9:1**
2	Pd/C-^{t}BuOOH, K_2CO_3, CH_2Cl_2, rt, 12 h	66%	7:3
3	$NaClO_2$, ^{t}BuOOH, CH_3CN: H_2O, 45 ^oC, 16 h	74%	1:1

Scheme 2.10: Optimization conditions of allylic oxidation

Table 2.1: ^1H comparisons of Laine's group (+)-nootkatone **34** and our synthetic (±)-nootkatone **34**

No	Laine's synthesized (+)-nootkatone **34**	Our synthesized (±)-nootkatone **34**
1	5.77 (s, 1H)	5.77 (s, 1H)
2	4.74 (s, 1H)	4.74 (s, 1H)
3	4.72 (s, 1H)	4.72 (s, 1H)
4	Not available (NA)	2.50 (ddt, *J* = 15.3, 5.0, 1.8 Hz, 1H)
5	NA	2.40–2.24 (m, 4H)
6	NA	2.04–1.89 (m, 3H)
7	1.74 (s, 3H)	1.74 (s, 3H)
8	NA	1.40−1.29 (m, 2H)
9	1.13 (s, 3H)	1.13 (s, 3H)
10	0.97 (d, *J* = 6.7 Hz, 3H)	0.96 (d, *J* = 6.7 Hz, 3H)

Table 2.2: ^{13}C-NMR comparisons of Laine's group (+)-nootkatone **34** and our synthetic (±)-nootkatone **34**

No	Laine's synthesized (+)-nootkatone	Our synthesized (±)-nootkatone
1	199.6	199.9
2	170.5	170.7
3	149.0	149.3
4	124.7	124.8
5	109.3	109.4
6	43.9	44.0
7	42.1	42.2
8	40.5	40.6
9	40.3	40.5
10	39.3	39.5
11	33.0	33.2
12	31.6	31.7
13	20.8	21.0
14	16.8	17.0
15	14.9	15.0

Thus, we have accomplished the total synthesis of (±)-nootkatone in five steps. Interestingly, no protecting group was used in the entire synthetic sequence while achieving target nootkatone.

2.3.4 Noreremophilanes

2.3.4.1 Introduction of Noreremophilanes

Noreremophilanes are isolated from genus *Ligularia* herbaceous plants and a rare class of *cis*-hydrindanes which shows interesting biological activities such as antibacterial and cytotoxicity activities. The new noreremophilane-types sesquiterpenes **40**, **41** and **42** (Fig 2.8) was isolated from the roots of *Ligularia macrophylla, Ligularia przewalskii* and

Ligularia virgaurea respectively.[26-28] As application of developed method further, we have accomplished the first synthesis of a (±)-noreremophilane **40**.

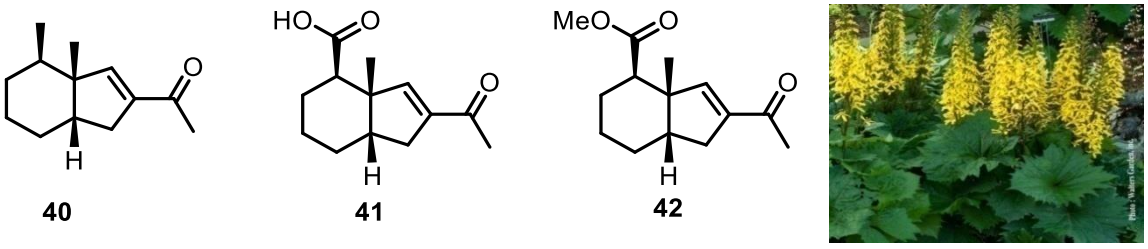
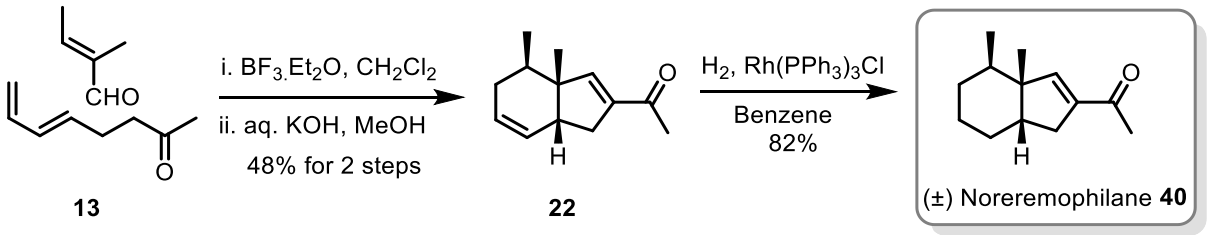


Fig 2.8: Selected noreremophilanes **40**, **41**, **42**

(Picture of plant taken from website: www.pinterest.com/pin/433472151366517 94)

2.3.4.2 Synthesis of noreremophilane 40

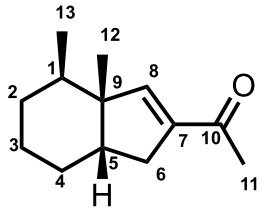
Our effort began with synthesis of *cis*-hydrindane intermediate **22** through Diels-Alder/aldol sequence (scheme 2.11)**.** The formation of product was indicated by presence of two sets olefinic protons in ^{1}H-NMR at 6.68 (s, 1H), 5.67−5.66 (m, 2H) ppm and two methyl at 0.98 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H) ppm.



Scheme 2.11: Synthesis of (±)-noreremophilane **40**

The isolated double bond in compound **22** was chemoselectively reduced using 15 mol % Wilkinson catalyst [$RhCl(PPh_3)_3$] under hydrogen balloon pressure to give target natural product (±)-noreremophilane **40** in 82% yield.[29] All the spectral data (IR, ^{1}H and ^{13}C NMR) were found to be identical to those reported in the literature.[26] The ^{1}H and ^{13}C-NMR comparisons of natural and synthetic (±)-noreremophilane **40** are shown in table 2.3.

Table 2.3: ^{1}H and ^{13}C-NMR comparisons of natural and synthetic (±)-noreremophilane **40**

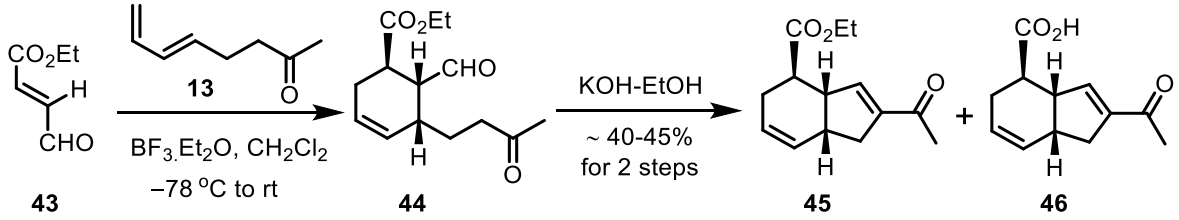


No	Natural noreremophilane **40**		Synthetic (±)-noreremophilane **40**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
13	0.67 (d, *J* = 6.6 Hz, 3H)	17.4	0.67 (d, *J* = 6.8 Hz, 3H)	17.4
12	0.84 (s, 3H)	17.3	0.84 (s, 3H)	17.3
2	0.86 (m, 1H)	29.3	0.86-0.85 (m, 1H)	29.3
1	1.09 (m, 1H)	37.0	1.10-108 (m, 1H)	36.9
2	1.11 (m, 1H)	29.3	1.13-1.11 (m, 1H)	29.3
3	1.18 (m, 1H)	22.3	1.22-1.17 (m, 1H)	22.2
3	1.31 (m, 1H)	22.3	1.34-1.31 (m, 1H)	22.2
4	1.34 (m, 1H)	24.5	1.38-1.34 (m, 1H)	24.4
4	1.42 (m, 1H)	24.5	1.44-1.41 (m, 1H)	24.4
5	1.75 (m, 1H)	46.6	1.78-1.73 (m, 1H)	46.5
11	1.97 (s, 3H)	26.0	1.97 (s, 3H)	25.9
6	2.43 (ddd, *J* = 16.0, 11.3, 2.4 Hz, 1H)	33.7	2.43 (ddd, *J* = 16.1, 11.3, 2.3 Hz, 1H)	33.6
6	2.57 (dd, *J* = 16.0, 8.1 Hz, 1H)	33.7	2.57 (dd, *J* = 16.1, 8.3 Hz, 1H)	33.6
8	6.30 (d, *J* = 2.4 Hz, 1H)	153.0	6.30 (d, *J* = 2.0 Hz, 1H)	153.1
9	-	50.0	-	49.6
7	-	144.0	-	143.9
10	-	195.6	-	195.6

Thus, we have accomplished the first total synthesis of a (±)-noreremophilane in shortest route, which also further confirmed the previously assigned structure based on NMR.

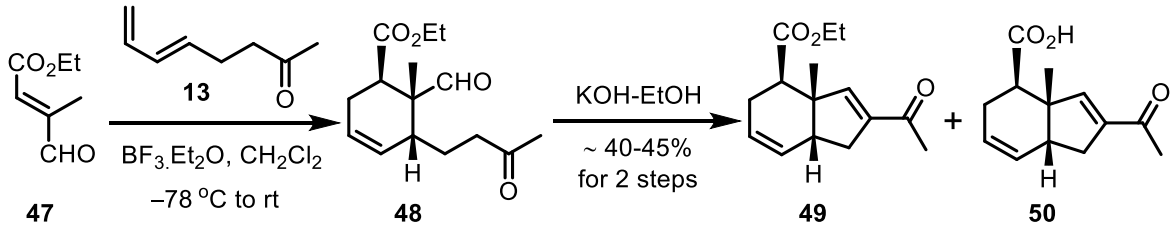
2.3.4.3 Synthesis of noreremophilane 41 and 42

Further utility of the method, we have planned to synthesize various *cis*-hydrindanes based on naturally occurring noreremophilane scaffold and screen them for multiple biological activities using high-content zebrafish embryonic development assays. For the synthesis of noreremophilane **41** and **42**, we used similar Diels-Alder/aldol sequence to construct *cis*-hydrindanes. The required dienophiles **43** and **47** in present purpose were prepared using known procedures.[30] The Diels-Alder reaction of diene **13** and dienophile **43** in presence of $BF_3.Et_2O$ in CH_2Cl_2 gave Diels-Alder keto-aldehyde adduct **44**. The **44** was subjected to intramolecular aldol condensation reaction by exposing it to 15% KOH in ethanol to furnish **45** along with varying amounts of corresponding carboxylic acid **46** in ~ 40-45% yields[30] (scheme 2.12).



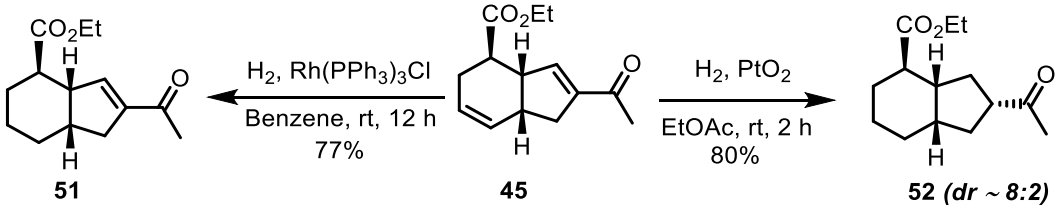
Scheme 2.12: Synthesis of *cis*-hydrindanes **45** and **46**

The formation of *cis*-hydrindane **45** was indicated by the presence of olefinic protons signals ^{1}H NMR at 6.70 (s, 1H), 5.74–5.73 (m, 2H) ppm and ester attached protons O=C-O-CH_2 at 4.19–4.14 (m, 2H) ppm. Similarly the corresponding olefinic carbon signals at 145.6, 145.5, 129.4, 124.7, ppm and ketone and ester carbon at 197.0, 174.8 ppm in ^{13}C NMR spectrum, which supported by IR absorption at 1670, 1730 cm^{-1}. The assigned structure of **45** was further confirmed by HRMS which showed a peak at 257.1147 corresponding to formula $C_{14}H_{18}O_3$ $[M+Na]^+$ with calculated value of 257.1148 and for acid **46**, HRMS showed a peak at 229.0835 corresponding to formula $C_{12}H_{14}O_3$ $[M+Na]^+$ with calculated value of 229.0835. By following the same protocol, compounds **49** and **50** were prepared using dienophile **47** in which angular hydrogen atom was replaced with a methyl group (scheme 2.13). Both compounds **49** and **50** were well characterized by IR, ^{1}H-NMR, ^{13}C-NMR, HRMS.



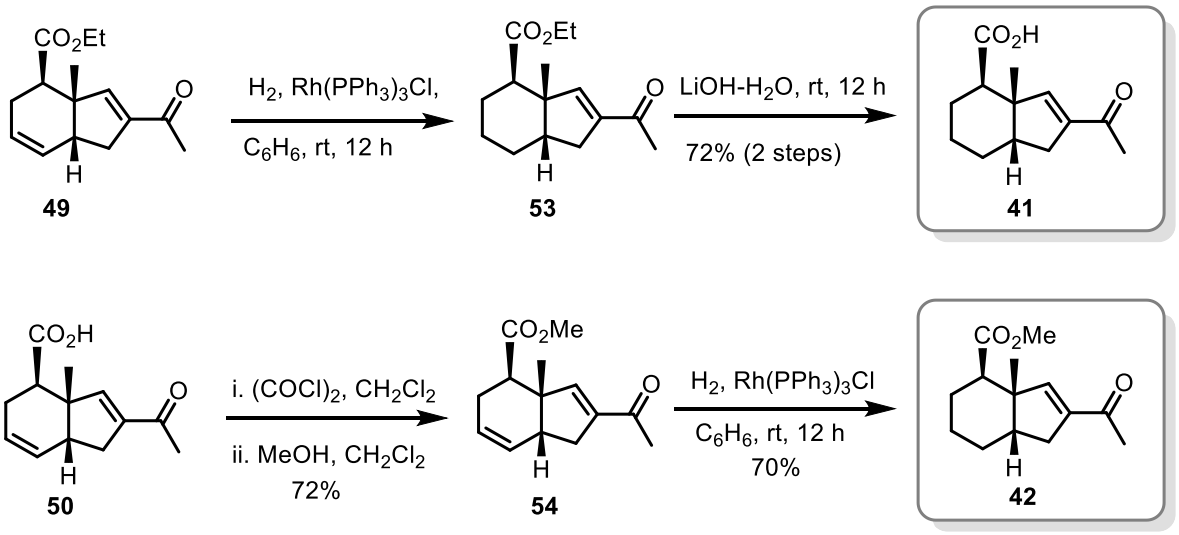
Scheme 2.13: Synthesis of *cis*-hydrindanes **49** and **50**

The isolated double bond in compound **45** on partial chemoselective reduction using 20 mol % Wilkinson's catalyst [$RhCl(PPh_3)_3$] under hydrogen atmosphere resulted in compound **51** in 77% yield whereas compound **45** on complete reduction using H_2, PtO_2 conditions gave saturated hydrindane **52** in 80% isolated yield with ~8:2 diastereoselective ratios (scheme 2.14). Both the compound **51** and **52** were well characterized by IR, ^{1}H-NMR, ^{13}C-NMR, HRMS.



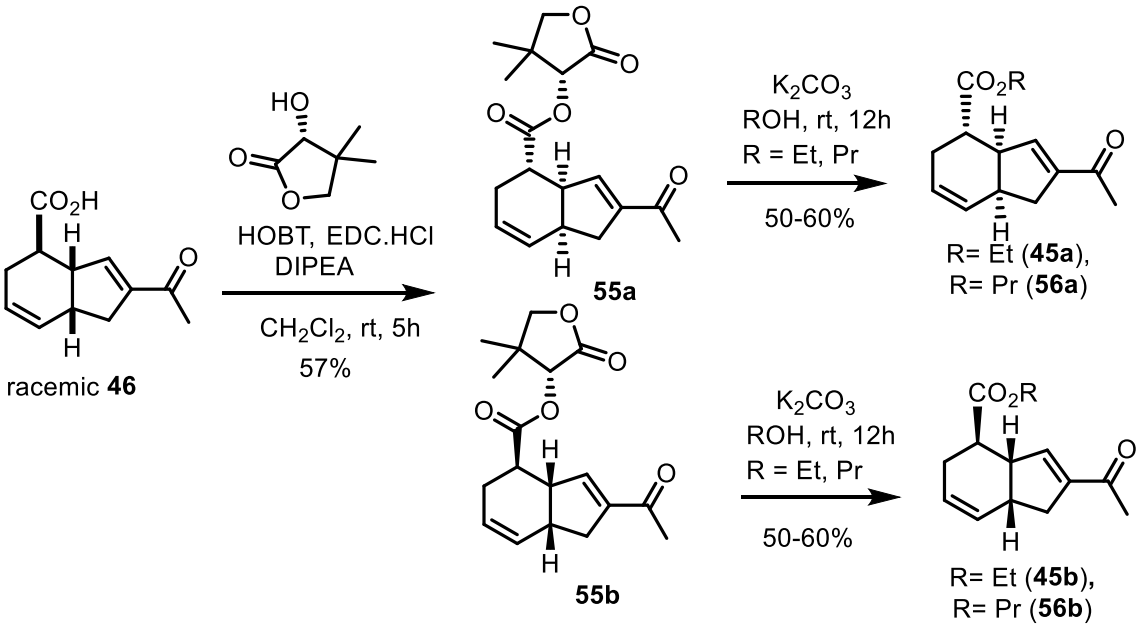
Scheme 2.14: Synthesis of *cis*-hydrindanes **51** and **52**

Similarly, isolated double bond in compound **49** on chemoselective reduction using 20 mol % Wilkinson's catalyst [$RhCl(PPh_3)_3$] under hydrogen atmosphere resulted in compound **53**. The **53** on ester hydrolysis using LiOH-H_2O in ethanol furnished the target noreremophilane **41** in 72% isolated yield for two steps (scheme 2.15). All the spectral data (IR, ^{1}H and ^{13}C NMR) for compound **41** found to be identical to those reported in the literature.[27] The compound **50** on esterification with MeOH using standard procedure afforded compound **54** which on reduction of the isolated double bond using 15 mol % Wilkinson's catalyst [$RhCl(PPh_3)_3$] gave natural product noreremophilane **42** in 70% yield (scheme 2.15). All the spectral data (IR, ^{1}H and ^{13}C NMR) for compound **42** was found to be identical to those reported in the literature.[28]



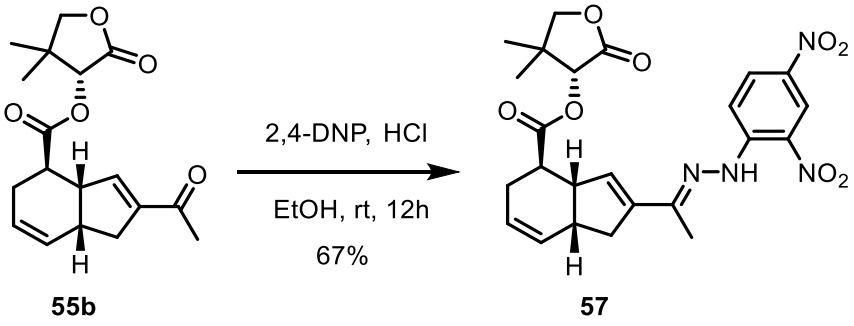
Scheme 2.15: Synthesis of noreremophilane **41** and **42**

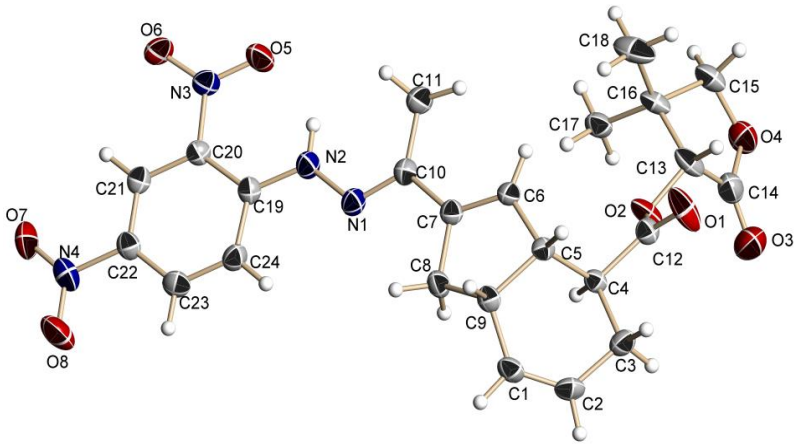
After successful synthesis of natural products **41** and **42,** we decided to make some compounds in enantiopure form to understand the role of stereochemistry on activity. For this purpose, racemic compound **46** was chosen. The compound acid **46** was coupled with D-(-)-pantolactone using HOBT, EDC.HCl, DIPEA conditions[31] to obtain a mixture of diastereomers **55a** and **55b** which were cleanly separated by silica gel column chromatography.



Scheme 2.16: Synthesis of enantiopure hydrindanes

Both the compounds were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, HRMS. The compound **55a** and **55b** individually subjected for trans-esterification using EtOH/PrOH, K_2CO_3 conditions[32] gave corresponding two enantiomers **45a/56a** and **45b/56b** (scheme 2.16). At this stage, we become interested in establishing the relative and absolute configuration of the synthesized enantiopure noreremophilane derivatives. Towards this effort, one of the pure enantiomer **55b** was treated with 2, 4 DNP in EtOH to provide its 2, 4 DNP derivative **57** as a crystalline solid. The compound **57** was recrystallized using ethyl acetate-hexane. Analysis of single crystal X-ray of **57** established the relative and absolute configurations as drawn (scheme 2.17). Accordingly, all other enantiopure hydrindanes configurations were derived as drawn in scheme 2.16.





Scheme 2.17: Synthesis of **57** and ORTEP of **57**

To understand systematic SAR of this class of compounds, we proceeded towards the generation of library of compounds around natural products and noreremophilane **45**. The carboxylic acid **46** was coupled with appropriate alcohols and amines which afforded the corresponding analogues.

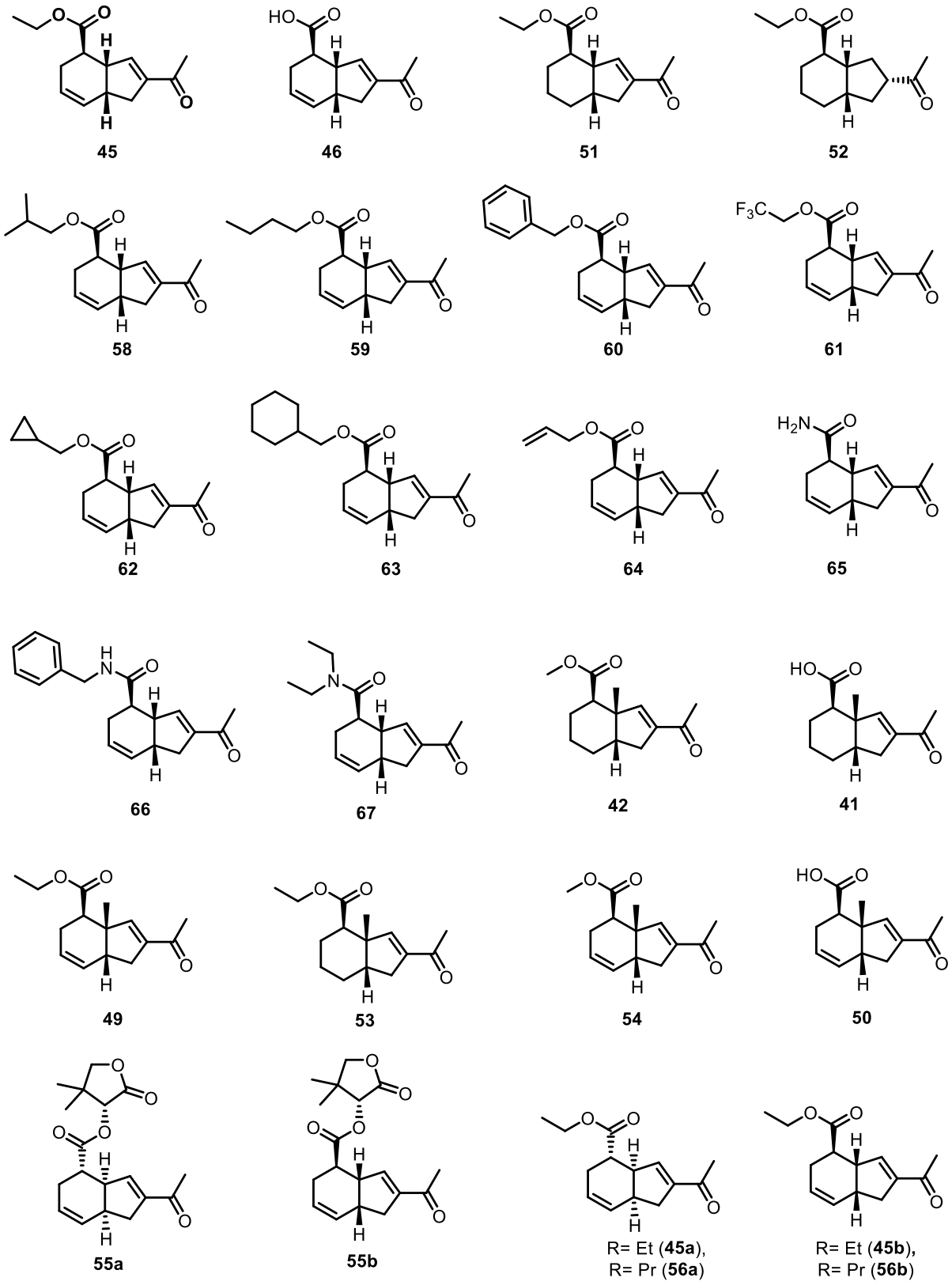


Fig 2.9: Library of synthesized compounds around natural products scaffolds

All the synthesized compounds are compiled in Fig 2.9 for ready reference and all were well characterized by different analytical tools such as IR, ^{1}H-NMR, ^{13}C-NMR, HRMS. Next we screened all these compounds and the details are discussed in following sections.

2.3.4.4 Biological evaluations of noreremophilane in zebrafish

Zebrafish has emerged as an ideal model organism to have high throughput assays while being complex enough to model the vertebrate biology.[33-36] Flow chart for the method is shown in Fig. 2.10. In zebrafish assay, there is collection of large number of embryos after mating of zebrafish. Next array all collected embryos individually into each well of a 96-well plate. Each plate was then treated with chemicals at different concentration and effects on phenotypes were studied.

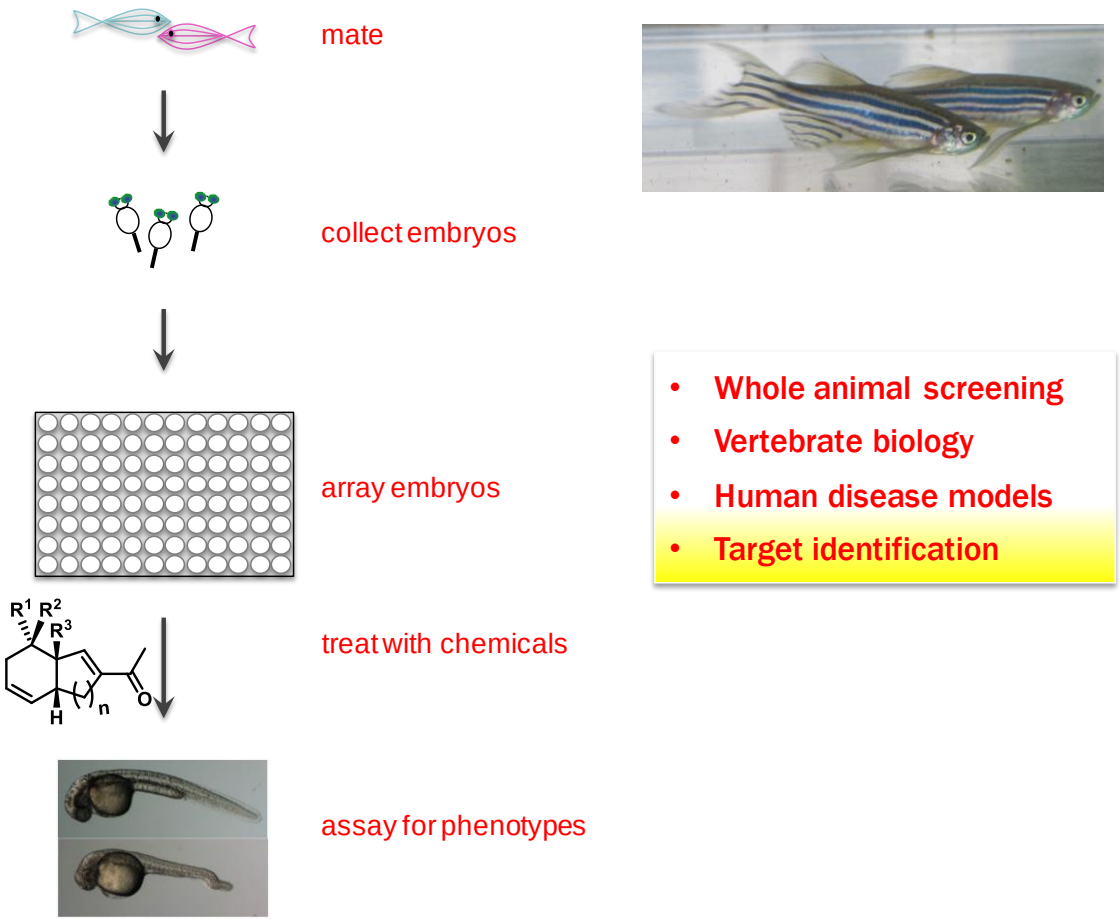


Fig 2.10: Small molecule screens in zebrafish

There are recent reports which used zebrafish embryo as a tool to study tumour angiogenesis.[37] Angiogenesis is the formation of new blood vessels from the pre-existing ones and is a process essential for both physiological and pathological events. Many human diseases such as tumor growth, retinopathy and inflammation are due excessive angiogenesis. All our compounds were profiled in these assays to identify potent angiogenesis inhibitor and it was done in collaboration with Dr. Chetana Sachidanandan's group, at a CSIR-Institute of Genomics & Integrative Biology (CSIR-IGIB), New Delhi.
For this purpose, two separate assays were performed: (1) for anti-angiogenesis activity and (2) for teratogenic activity. Angiogenesis was assayed in 2–3-day-old embryos while teratogenicity was assayed in 1-day/6-hour-old old embryos.

1) Angiogenesis assay: In anti-angiogenic activity assay 2-day-old embryos exposed to different compounds and the formation of subintestinal vessels (SIVs) assayed at 3 days. The percentage of embryos with complete or partial inhibition of angiogenesis is quantified and plotted, n = 25 for each compound. The dotted line represents the 50% mark. Embryos were exposed to 50 μM concentration of the compound generally, but where this concentration was lethal, the highest non-lethal concentrations were used such as 25 μM, 12.5 μM, and 6.25 μM. The size of the circle is proportional to the concentration of the compound used for the assay. The circles are coloured according to the potency of the compounds to block angiogenesis: yellow > green > blue > pink > grey. The x-axis shows the compound number codes (Fig 2.11). In the angiogenesis assay, for compounds that were lethal at 50 μM, a titration was performed and the percentage embryos that showed inhibition of angiogenesis at the highest non-lethal concentrations were quantified and plotted for comparison (Fig 2.11). The two enantiomers **45a** and **45b** showed more potent anti-angiogenic activity as compared to racemic compound **45**. Both enantiomers **45a** and **45b** elicited a similar percentage inhibition of angiogenesis at 25 μM as compared to **45** which did at 50 μM (Fig 2.11). In case of enantiopure esters with the pantolactone moiety **55a** & **55b**, we observed that there is no difference in activity between the enantiomers, both showed complete inhibition of angiogenesis. However, in case of enantiomers **56a** & **56b,** we observed a clear difference in activity with **56a** showing potent anti-angiogenic activity at lower concentrations compared to **56b**.

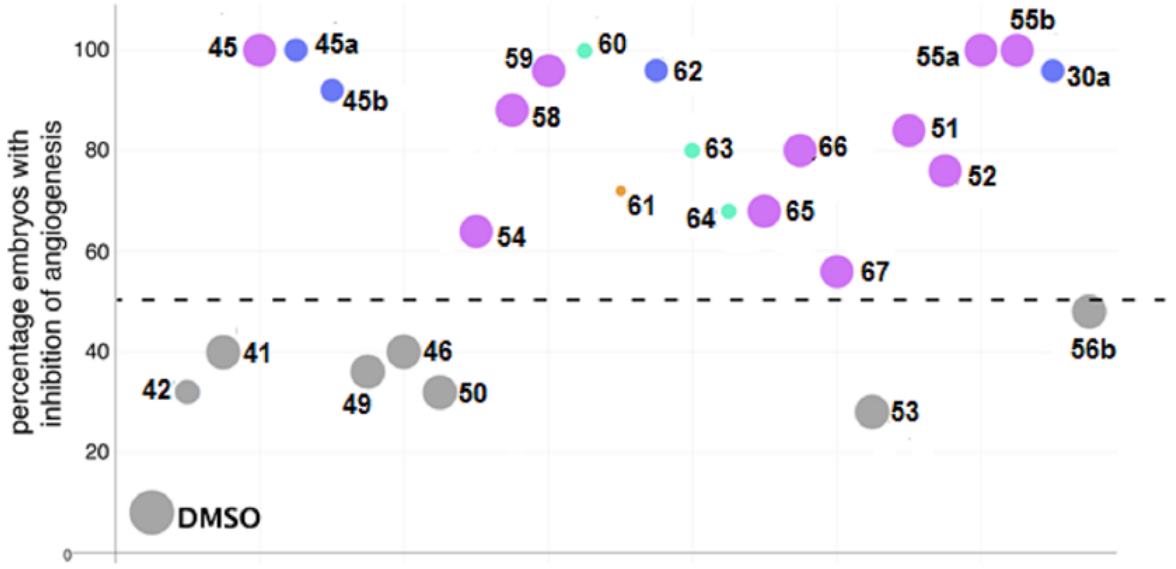


Fig. 2.11: Angiogenesis assay in zebrafish embryos.

We also observed that anti-angiogenic activity were inferior in case of compounds with an angular methyl group such as **41, 42, 49, 50, 53** and **54** with respect to the corresponding compounds with no angular methyl group. We found that many potent anti-angiogenic compounds were also toxic at higher concentrations (**45a**, **45b**, **56a**, **60**, **61**, **62**, **63**, **64**), but not all (**45**, **51**, **52**, **54**, **55a**, **55b**, **58**, **59**, **65**, **66**, **67**). During angiogenesis assay, we observed that ester compounds with angular hydrogen such as **45**, **58**, **60**, **61**, and **62** are all potent anti-angiogenic agents.

2) Teratogenicity assay: In teratogenicity assay, 6-hour-old zebrafish embryos were exposed to 50 μM compounds and the percentage of embryos with either abnormal development or death at 1 day was quantified and plotted, n = 25. The dotted line represents the 50% mark. The colours of the circles show their potency in inhibiting angiogenesis (from a) for comparison. The x-axis shows the compound number codes (fig 2.16). In our teratogenicity assay (Fig 2.12) we found that there were clearly two groups of compounds, (a) where most embryos (more than 60%) were abnormal and (b) where most embryos (less than 50%) were normal. Based on this screening, we found that compounds containing an ester moiety with angular hydrogen such as **45**, **58**, **59**, **60**, **61**, **62**, and **64** showed strong bioactivity.

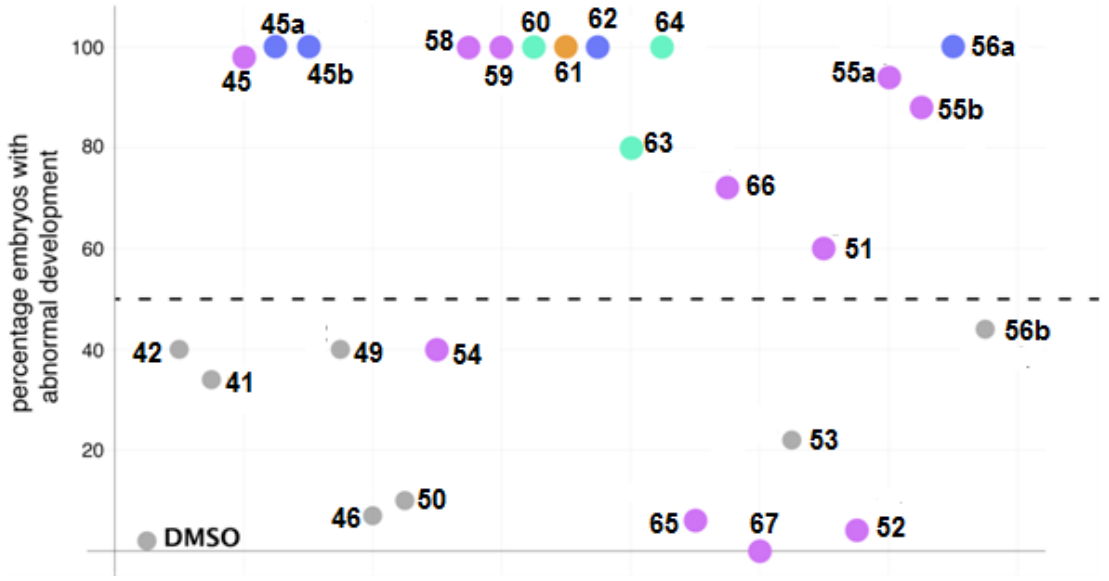


Fig 2.12: Teratogenicity assay in zebrafish embryos.

During SAR we also found that, partially saturated compound with angular hydrogen (**51**) has more activity than the ones partially saturated with an angular methyl group (**41**, **42**, and **53**). The fully saturated hydrindane (**52**) has no bioactivity indicating that both double bonds are essential for activity. The enantiopure compounds with ethyl ester moiety **45a**, **45b** and pantolactone moiety **55a**, **55b** were also found to have more adverse effects on embryo survival, with no difference between the enantiomers. However, as in the case of angiogenesis, **56a** and **56b** shows a clear difference in activity. The compound **56a** is more bioactive than compound **56b**. Further SAR examination found that the corresponding acids (**46**, **50**) and amides (**65**, **67**) did not have any considerable activity. According to these results, it may conclude that esters are readily absorbed by the zebrafish skin rather than the corresponding carboxylic acids. However, the corresponding benzyl amide (**66**) seems to have more bioactivity. Based on this result it can be concluded that, noreremophilanes derivatives could be arranged in a decreasing order of bioactivity on the embryo as: esters > acids = amides (Fig. 2.12). After studying SAR analysis of noreremophilanes, we discovered a noreremophilanes based on the natural *cis*-hydrindane backbone that is a potent angiogenesis inhibitor. The noreremophilane **45** appears to inhibit

angiogenesis in normal zebrafish embryos more potently than other derivatives (Fig 2.13).

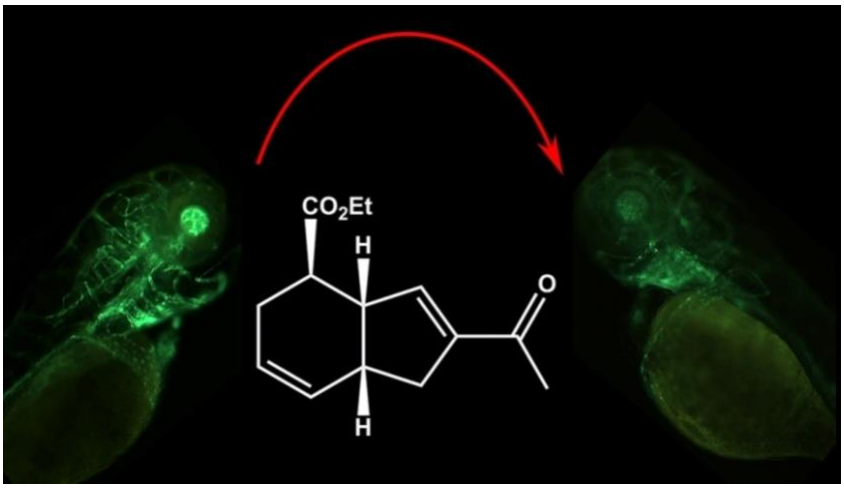


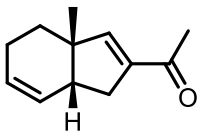
Fig 2.13: Noreremophilane **45** inhibit angiogenesis zebrafish embryos

2.4. Conclusions

Developed a simple and efficient method for the construction of *cis*-decalins and *cis*-hydrindanes in a highly diastereoselective manner using the Diels−Alder/ aldol sequence. The synthesized hydrindanes and decalins with loaded orthogonal functional groups on them can also serve as key intermediates in the synthesis of complex molecules. As a direct application of method, we have synthesized the natural products nootkatone and noreremophilanes in short routes and in *protecting group-free* manner. Zebrafish assays were carried out in collaboration with IGIB to have SAR analysis and finally to identify structural requirements which are critical for the desired anti-angiogenic potential.

2.5 Experimental Procedures

1-((3a*S*,7a*R*)-3a-Methyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethan-1-one (19):



To a solution of diene **13** (200 mg, 1.61 mmol) and methacrolein (0.34 mL, 4.03 mmol) in dry CH_2Cl_2 (10 mL) was added $BF_3{\cdot}OEt_2$ (0.39 mL, 3.22 mmol) dropwise at −78 °C. The mixture was allowed to warm at room temperature and was stirred for 8 h at the same temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (3 x 5.0 mL), followed by H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The crude material **18** obtained after the removal of solvent was dissolved in methanol (5.0 mL), cooled to 0 °C, and treated with 15% aqueous KOH (5.0 mL). After stirring for 1 h at room temperature, the reaction mass was diluted with petroleum ether (30 mL), washed with water (10 mL), 1 N HCl (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Purification by flash chromatography over silica gel (0.5:9.5; EtOAc−petroleum ether) afforded dienone **19** (150 mg, 53%) as a light yellow oil.

IR υ_{max} (film): 1663, 1637, 1216 cm^{-1}.

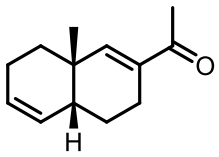
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (s, 1H), 5.73−5.61 (m, 2H), 2.87−2.81 (m, 1H), 2.35−2.32 (m, 1H), 2.29 (s, 3H), 2.27−2.21 (m, 1H), 2.01−1.97 (m, 2H), 1.54−1.51 (m, 2H), 1.11 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.6, 153.1, 143.2, 129.8, 126.0, 47.2, 44.4, 36.6, 30.7, 26.5, 24.6, 22.0.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1274.

Compounds **22**, **24**, **26**, **28**, **30**, and **32** were prepared using the similar experimental procedure as described above compound **19.**

1-((4a*R*,8a*S*)-8a-Methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)ethan-1-one (21):



To a solution of diene **17** (200 mg, 1.44 mmol) and methacrolein (0.30 mL, 3.62 mmol) in dry CH_2Cl_2 (10 mL) was added $BF_3{\cdot}OEt_2$ (0.35 mL, 2.89 mmol) dropwise at −78 °C. The mixture was allowed to warm to room temperature and was stirred for 8 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (3 x 5.0 mL), followed by H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Crude material **20** obtained after the removal of solvent was dissolved in methanol (5.0 mL), cooled to 0 °C, and treated with 15% aqueous KOH (5.0 mL). After stirring for 4 h at room temperature, the reaction mass was diluted with petroleum ether (30 mL), washed with water (10 mL), 1 N HCl (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Purification by flash chromatography over silica gel (0.5:9.5; EtOAc−petroleum ether) afforded dienone **21** (137 mg, 50%) as a light yellow oil.

IR υ_{max} (film): 1669, 1640, 1452, 1236 cm^{-1}.

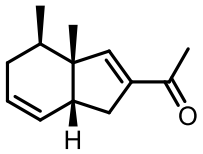
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (s, 1H), 5.69−5.64 (m, 1H), 5.49−5.45 (m, 1H), 2.29 (s, 3H), 2.26−2.20 (m, 1H), 2.16−2.10 (m, 1H), 2.02−1.96 (m, 3H), 1.84−1.79 (m, 1H), 1.61−1.41 (m, 3H), 1.10 (s, 3H)

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.8, 149.0, 138.3, 130.3, 126.7, 39.9, 34.9, 32.6, 26.6, 25.6, 25.4, 22.6, 21.3.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O$ $[M+H]^+$ 191.1430, found 191.1430.

Compounds **23**, **25**, **27**, **29**, **31** and **33** were prepared using the similar experimental procedure as described above compound **21**.

1-((3a*S*,4*R*,7a*R*)-3a,4-Dimethyl-3a,4,5,7a-tetrahydro-1H inden- 2-yl)ethan-1-one (22):



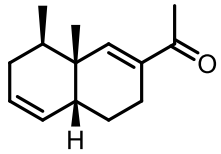
Yield: 48%.

IRυ_{max} (film): 1669, 1637, 1452, 1237 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.68 (s, 1H), 5.67−5.66 (m, 2H), 2.78 (dd, *J* = 15.5, 8.2 Hz, 1H), 2.33−2.30 (m, 1H), 2.28 (s, 3H), 2.24−2.17 (m, 1H), 1.96−1.89 (m, 1H), 1.79−1.61 (m, 2H), 0.98 (s, 3H), 0.91 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 197.5, 153.4, 143.3, 128.4, 125.9, 49.8, 46.9, 36.6, 33.2, 30.6, 26.5, 18.1, 15.9.

1-((**4a*R*,8*R*,8a*S*)-8,8a-Dimethyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)ethanone (23):**



Yield: 46%.

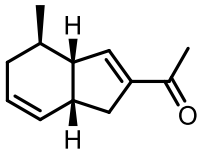
IRυ_{max} (film): 1665, 1637, 1452, 1237 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.64 (s, 1H), 5.60−5.56 (m, 1H), 5.53−5.48 (m, 1H), 2.28 (s, 3H), 2.12−2.00 (m, 2H), 1.93−1.88 (m, 2H), 1.90−1.63 (m, 3H), 1.43 (ddd, *J* = 18.9, 9.15, 5.49 Hz, 1H), 1.00 (s, 3H), 0.96 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 198.0, 149.3, 137.8, 130.1, 125.5, 40.4, 37.3, 34.2, 31.6, 25.6, 25.5, 22.6, 21.1, 15.1.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O$ $[M+H]^+$ 205.1587, found 205.1586.

1-((3a*S*,4*R*,7a*R*)-4-Methyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethan-1-one (24):



Yield: 44%.

IRυ_{max} (film): 2962, 1669, 1461 cm^{-1}.

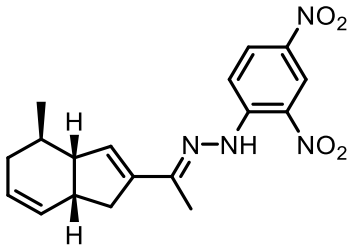
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.82 (d, *J* = 3.2 Hz, 1H), 5.71−5.67 (m, 2H), 2.81−2.72 (m, 2H), 2.51−2.49 (m, 1H), 2.30 (s, 3H), 2.25−2.20 (m, 1H), 2.06−2.01 (m, 1H), 1.71−1.66

(m, 1H), 1.59−1.53 (m, 1H), 1.02 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 197.2, 147.5, 145.2, 129.3, 126.1, 51.6, 38.1, 36.5, 32.5, 31.0, 26.6, 19.7.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1274.

(*E*)-1-(2,4-Dinitrophenyl)-2-(1-((3a*S*,4*R*,7a*R*)-4-methyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethylidene) hydrazine (24-DNPH):



To a solution of ketone **24** (30 mg, 0.170 mmol) in EtOH (2.0 mL) was added 2,4-dinitrophenylhydrazine (57 mg, 0.289 mmol) followed by 3 drops of conc. HCl at room temperature. The mixture was allowed to stir at room temperature for overnight. Solvent was removed *in vacuo*. Ether (5 mL) was added followed by 10% $NaHCO_3$ (2 mL). The aqueous layer was extracted by ether (3 x 5 mL) and dried over anhydrous Na_2SO_4. Purification by flash chromatography (0.5: 9.5% EtOAc in hexane) gave **24-DNPH** (47 mg, 78%) as orange colored solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.20 (s, 1H), 9.14 (d, *J* = 2.75 Hz, 1H), 8.31 (dd, *J* = 9.46, 2.44 Hz, 1H), 7.97 (d, *J* = 9.46 Hz, 1H), 6.47 (m, 1H), 5.80–5.74 (m, 2H), 3.01–2.96 (m, 1H), 2.89–2.84 (m, 1H), 2.54–2.50 (m, 1H), 2.45–2.41 (m, 1H), 2.23 (s, 3H), 2.09–2.04 (m, 1H), 1.75–1.69 (m, 1H), 1.60–1.57 (m, 1H), 1.04 (d, *J* = 6.71 Hz, 3H).

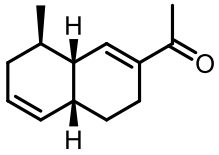
^{13}C NMR (125 MHz, $CDCl_3$): δ 151.1, 145.0, 142.9, 139.5, 138.1, 130.1, 129.7, 129.6, 126.3, 123.6, 116.8, 51.4, 38.2, 37.7, 32.5, 31.5, 19.8, 13.0.

HRMS (ESI): *m/z* calcd for $C_{18}H_{21}O_4N_4$ $(M+H)^+$ 357.1557, found 357.1555.

Crystal Data: X-ray intensity data measurements of all the compounds **24-DNPH** was carried out on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (MoK_α= 0.71073Å) radiation at room temperature 297(2) K. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from reflections harvested from three sets of 36

frames. Data were collected with a frame time of 10 sec keeping the sample-to-detector distance fixed at 4.00 cm. A total of 2042 frames were collected. The X-ray data collection was monitored by APEX2 program (Bruker, 2006). All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs (Apex2, Bruker, 2006). SHELX-97 was used for structure solution and full matrix least-squares refinement on *F*. All the hydrogen atoms were placed in geometrically idealized position and constrained to ride on their parent atoms. crystals of **24-DNPH** were orange color grown by slow evaporation from ethyl acetate-hexane mixture. $C_{18}H_{20}N_4O_4$,0.5($CH_3COOCH_2CH_3$), M = 400.43, orange color needle, 0.62 x 0.15 x 0.12 mm^3, monoclinic, space group *P*-1, *a* = 7.360(11), *b* = 14.74(2), *c* = 19.75(3) Å, α = 68.53(3)°, β = 89.15(3)°, γ = 89.81(3)°, *V* = 1994(5) $Å^3$, Z = 4, *T* = 297(2) K, $2\theta_{max}$ = 50.00°, D_{calc} (g cm^{-3}) = 1.334, *F*(*000*) = 848, μ (mm^{-1}) = 0.097, 23073 reflections collected, 6931 unique reflections (R_{int} = 0.2972), 2069 observed ($I > 2\sigma$ (*I*)) reflections, multi-scan absorption correction, T_{min} = 0.942, T_{max} = 0.988, 530 refined parameters, *S* = 1.162, *R*1 = 0.1974, *wR*2 = 0.4412 (all data *R* = 0.3713, *wR*2 = 0.5380), maximum and minimum residual electron densities; $\Delta\rho_{max}$ = 0.90, $\Delta\rho_{min}$= -0.65 $eÅ^{-3}$. Crystals of compound **24-DNPH** were fine fibrous needle which did not diffract to high angle. Because of the poor diffracting power of the crystal, diffraction was limited to the resolution of 1.45 Å. This is the main reason behind high *R* and *wR*-values.

1-((4a*R*,8*R*,8a*S*)-8-Methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)ethan-1-one (25):



Yield: 47%.

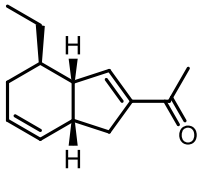
IRυ_{max} (film): 2962, 1667, 1461, cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.88 (d, *J* = 3.6 Hz, 1H), 5.64−5.52 (m, 2H), 2.35−2.27 (m, 1H), 2.29 (s, 3H), 2.26−2.20 (m, 2H), 2.14−2.06 (m, 2H), 1.77−1.68 (m, 3H), 1.47−1.41 (m, 1H), 1.09 (d, *J* = 6.3 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 199.5, 143.7, 139.8, 130.3, 126.3, 41.2, 33.0, 32.8, 31.1, 26.3, 25.5, 22.6, 19.3.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O$ $[M+H]^+$ 191.1430, found 191.1431.

1-((3a*S*,4*R*,7a*R*)-4-Ethyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethan-1-one (26):



Yield: 45%.

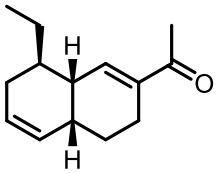
IRυ_{max} (film): 2962, 1669, 1461, 1373 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.80 (d, *J* = 3.3 Hz, 1H), 5.69−5.65 (m, 2H), 2.78−2.72 (m, 2H), 2.63−2.63 (m, 1H), 2.30 (s, 3H), 2.27−2.23 (m, 1H), 2.15−2.09 (m, 1H), 1.76−1.64 (m, 1H), 1.59−1.54 (m, 1H), 1.47−1.42 (m, 1H), 1.29−1.26 (m, 1H), 0.91 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 197.2, 147.9, 145.1, 129.4, 125.9, 49.7, 37.8, 37.2, 36.5, 28.5, 26.6, 26.4, 11.5.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O$ $[M+H]^+$ 191.1430, found 191.1430.

1-((4a*R*,8*R*,8a*S*)-8-Ethyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)ethan-1-one (27):



Yield: 42%.

IRυ_{max} (film): 2962, 1667, 1461, 1373 cm^{-1}.

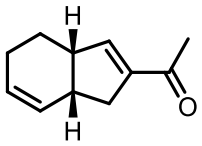
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.80 (d, *J* = 3.7 Hz, 1H), 5.64− 5.44 (m, 2H), 2.38−2.35 (m, 1H), 2.29−2.28 (m, 1H), 2.27 (s, 3H), 2.26−2.15 (m, 2H), 2.13−2.05 (m, 1H), 1.78−1.72 (m, 1H), 1.67−1.52 (m, 4H), 1.41−1.33 (m, 1H), 0.94 (t, *J* = 7.2 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 199.5, 144.3, 139.8, 129.9, 126.4, 39.1, 38.0, 31.9, 28.7,

26.3, 25.6, 25.5, 21.8, 11.7.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O$ $[M+H]^+$ 205.1587, found 205.1586.

1-((3a*S*,7a*R*)-3a,4,5,7a-Tetrahydro-1H-inden-2-yl)ethan-1-one (28):



Yield: 48%.

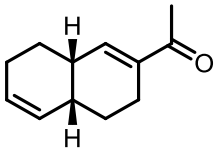
IRυ_{max} (film): 2962, 1669, 1461, 1380 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75−6.70 (m, 1H), 5.70−5.53 (m, 2H), 2.55−2.46 (m, 1H), 2.30 (s, 3H), 2.24−2.16 (m, 1H), 2.06−2.00 (m, 2H), 1.95−1.86 (m, 1H), 1.79−1.68 (m, 1H), 1.56−1.28 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6, 145.0, 143.3, 131.2, 127.0, 39.9, 33.3, 28.6, 25.8, 25.4, 22.1.

HRMS (ESI): *m/z* calcd for $C_{11}H_{15}O$ $[M+H]^+$ 163.1117, found 163.1117.

1-((4a*R*,8a*S*)-3,4,4a,7,8,8a-Hexahydronaphthalen-2-yl)- ethan-1-one (29):



Yield: 44%.

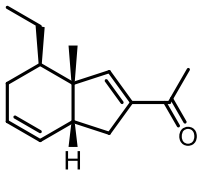
IRυ_{max} (film): 2962, 1668, 1461 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75−6.71 (m, 1H), 5.73−5.56 (m, 2H), 2.52− 2.46 (m, 1H), 2.38−2.31 (m, 1H), 2.30 (s, 3H), 2.24−2.18 (m, 1H), 2.14−2.08 (m, 1H), 2.06−2.01 (m, 1H), 1.93−1.89 (m, 1H), 1.81−1.70 (m, 1H), 1.53−1.27 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.8, 145.2, 139.6, 131.5, 127.3, 40.1, 33.5, 28.6, 26.0, 25.6, 24.5, 22.3.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1273.

1-((3a*S*,4*R*,7a*R*)-4-Ethyl-3a-methyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethan-1-one (30):



Yield: 43%.

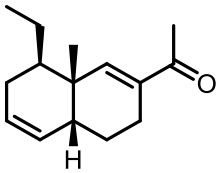
IRυ_{max} (film): 2962, 1669, 1461 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.72−5.65 (m, 2H), 2.82−2.78 (m, 1H), 2.30 (s, 3H), 2.25−2.13 (m, 2H), 1.67−1.60 (m, 2H), 1.49−1.35 (m, 2H), 1.26−1.23 (m, 1H), 1.00 (s, 3H), 0.90 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.6, 153.6, 143.3, 128.2, 125.8, 49.9, 47.6, 40.6, 36.7, 27.2, 26.6, 23.3, 18.7, 12.8.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O$ $[M+H]^+$ 205.1587, found 205.1587.

1-((4a*R*,8*R*,8a*S*)-8-Ethyl-8a-methyl-3,4,4a,7,8,8a-hexahydronaphthalen- 2-yl)ethan-1-one (31):



Yield: 40%.

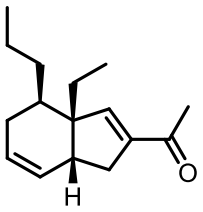
IRυ_{max} (film): 2962, 1669, 1233 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (s, 1H), 5.63−5.50 (m, 2H), 2.36−2.32 (m, 1H), 2.29 (s, 3H), 2.19−2.04 (m, 2H), 1.87−1.84 (m, 1H), 1.81−1.68 (m, 2H), 1.63−1.58 (m, 1H), 1.51−1.39 (m, 2H), 1.22−1.16 (m, 1H), 1.00 (s, 3H), 0.90 (t, *J* = 7.32 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.9, 149.6, 137.6, 130.2, 125.4, 41.4, 40.9, 37.6, 27.6, 25.6, 25.5, 22.6, 21.5, 21.4, 12.8.

HRMS (ESI): *m/z* calcd for $C_{15}H_{23}O$ $[M+H]^+$ 219.1743, found 219.1743.

1-((3a*S*,4*R*,7a*R*)-3a-Ethyl-4-propyl-3a,4,5,7a-tetrahydro-1H-inden-2-yl)ethan-1-one (32):



Yield: 38%.

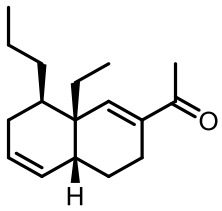
IRυ_{max} (film): 2962, 1667, 1461 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (s, 1H), 5.68−5.67 (m, 2H), 2.88−2.75 (m, 1H), 2.60−2.52 (m, 1H), 2.31 (s, 3H), 2.26−2.17 (m, 1H), 2.12−2.04 (m, 1H), 1.80−1.62 (m, 2H), 1.42−1.38 (m, 1H), 1.38−1.23 (m, 3H), 0.93−0.85 (m, 5H), 0.77 (t, *J* = 7.32 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3, 152.8, 149.1, 131.5, 125.5, 55.5, 41.2, 36.5, 32.3, 28.2, 27.3, 26.6, 21.1, 14.5, 9.2, 8.9.

HRMS (ESI): *m/z* calcd for $C_{16}H_{25}O$ $[M+H]^+$ 233.1900, found 233.1899.

1-((4a*R*,8*R*,8a*S*)-8a-Ethyl-8-propyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)ethan-1-one (33):



Yield: 42%.

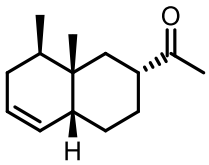
IRυ_{max} (film): 2962, 1669, 1461 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.71 (s, 1H), 5.61−5.47 (m, 2H), 2.34−2.33 (m, 1H), 2.30 (s, 3H), 2.16−2.13 (m, 1H), 2.09−2.04 (m, 3H), 1.83−1.78 (m, 1H), 1.74−1.71 (m, 1H), 1.60−1.49 (m, 2H), 1.47−1.41 (m, 2H), 1.21−1.16 (m, 1H), 0.96−0.85 (m, 5H), 0.76 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.6, 148.9, 139.3, 130.2, 125.7, 43.9, 40.6, 38.2, 36.3, 30.6, 27.6, 25.6, 25.5, 21.4, 14.6, 8.3 (2C).

HRMS (ESI): *m/z* calcd for $C_{17}H_{27}O$ $[M+H]^+$ 247.2056, found 247.2054.

1-((2*R*,4a*R*,8*R*,8a*S*)-8,8a-Dimethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-2-yl)ethan-1-one (37):



A solution of *α, β*- unsaturated ketone **23** (0.5 g, 2.45 mmol) in THF (20 mL) was added to liquid ammonia (20 mL) at −78 °C. Sodium (0.67 g, 29.4 mmol) was added in small pieces, and the reaction mixture was stirred at −78 °C for 1 h. After consumption of starting material (by TLC), solid NH_4Cl (1.0 g) was added and ammonia was allowed to evaporate at room temperature. Water (10 mL) was added, and the reaction mixture was extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with brine (20 mL) and dried over Na_2SO_4, and the solvent was concentrated to afford the ketone as a 1:1 mixture of diastereomers. The crude material was treated with K_2CO_3 (1.35 g, 9.80 mmol) in MeOH (20 mL) and refluxed for 2 h. The solvent was removed under *vacuo*, and the crude residue was diluted with water (10 mL) and extracted with ether (2 x 30 mL). The combined organic layer was washed with brine (20 mL) and dried over anhydrous Na_2SO_4. Purification by flash chromatography over silica gel (0.5:9.5; EtOAc−hexanes) afforded **37** (368 mg, 73%) as a colorless oil.

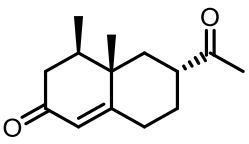
IR υ_{max} (film): 1709, 1453, 1352 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.59−5.48 (m, 2H), 2.46−2.40 (m, 1H), 2.13 (s, 3H), 2.02−1.97 (m, 2H), 1.88−1.84 (m, 1H), 1.78−1.72 (m, 2H), 1.63−1.53 (m, 1H), 1.34−1.23 (m, 2H), 1.06−1.00 (m, 2H), 0.83−0.81 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 212.5, 131.1, 125.2, 46.4, 44.1, 38.2, 34.6, 32.6, 29.9, 28.7, 28.1, 27.2, 21.6, 14.6.

HRMS (ESI): *m/z* calcd for $C_{14}H_{23}O$ $[M+H]^+$ 207.1743, found 207.1743.

(4*R*,4a*S*,6*R*)-6-Acetyl-4,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one (38):



To a solution of the ketone **37** (250 mg, 1.21 mmol) in benzene (20 mL) at 15 °C were added PDC (3.6 g, 9.70 mmol) and *t*-BuOOH (2.5 mL). After the reaction mixture stirred

for 15 min, it was brought to ambient temperature and further stirred for 12 h. The reaction mixture was diluted with ether (30 mL), filtered through a celite bed, and washed with ethyl acetate (2 x 10 mL). The filtrate was concentrated in *vacuo*. Purification by flash chromatography over silica gel (2:8; EtOAc: Petroleum ether) afforded **38** (170 mg, 63%) and **39** (20 mg, 8%) as colorless oils.

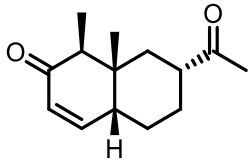
IRυ_{max} (film): 1708, 1665, 1354 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (s, 1H), 2.77−2.71 (m, 1H), 2.52−2.38 (m, 2H), 2.28−2.25 (m, 2H), 2.19 (s, 3H), 2.10−2.01 (m, 3H), 1.49−1.38 (m, 1H), 1.25−1.21 (m, 1H), 1.10 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 210.7, 199.4, 168.7, 125.3, 48.8, 42.1, 40.3, 40.0, 38.9, 32.1, 28.6, 28.3, 16.8, 15.0.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O_2$ $[M+H]^+$ 221.1536, found 221.1534.

(1*S*,4a*R*,7*R*,8a*R*)-7-Acetyl-1,8a-dimethyl-4a,5,6,7,8,8ahexahydronaphthalen-2(1H)-one (39):



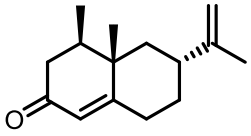
IRυ_{max} (film): 1708, 1675, 1451, 1354 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (dd, *J* = 5.7 Hz, 10.0 Hz, 1H), 5.91 (d, *J* = 10.3 Hz, 1H), 2.71 (m, 1H), 2.46 (tt, *J* = 12.3 Hz, 2.7 Hz, 1H), 2.16 (s, 3H), 2.05−1.97 (m, 2H), 1.94−1.86 (m, 2H), 1.50−1.35 (m, 2H), 1.17−1.08 (m, 1H), 1.04 (d, *J* = 6.7 Hz, 3H), 0.89 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 211.1, 201.6, 150.6, 127.8, 46.5, 45.0, 43.3, 38.8, 38.2, 28.3, 28.0, 27.2, 23.2, 6.7.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O_2$ $[M+H]^+$ 221.1536, found 221.1534.

(4*R*,4a*S*,6*R*)-4,4a-Dimethyl-6-(prop-1-en-2-yl)-4,4a,5,6,7,8-hexahydronaphthaen-2(3H)-one ((±)-Nootkatone 34):



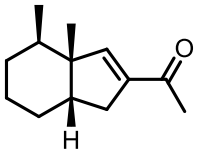
To a suspension of methyl triphenylphosphonium bromide (148 mg, 0.40 mmol) in dry THF (5.0 mL) was added potassium *tert*-butoxide (40 mg, 0.34 mmol) at 0 °C. After 5 min, the solution became canary yellow color, and to that, diketone compound **38** (30 mg, 0.136 mmol) in THF (5.0 mL) was added and allowed to stirred at 0 °C for 1 h. The reaction was quenched with H_2O (5.0 mL) and extracted with ether (2 x 25 mL). The combined organic layer was washed with water (5.0 mL) and brine (5.0 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Purification by flash chromatography over silica gel (1:9; EtOAc−hexanes) afforded (±)-nootkatone **34** (19 mg, 65%).

IR υ_{max} (film): 2923, 1668, 1606, 1459 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (s, 1H), 4.74 (s, 1H), 4.72 (s, 1H), 2.50 (ddt, *J* = 15.3, 5.0, 1.8 Hz, 1H), 2.40−2.24 (m, 4H), 2.04−1.89 (m, 3H), 1.74 (s, 3H), 1.40−1.29 (m, 2H), 1.11 (s, 3H), 0.96 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.9, 170.7, 149.3, 124.8, 109.4, 44.0, 42.2, 40.6, 40.5, 39.5, 33.2, 31.7, 21.0, 17.0, 15.0.

1-((3a*S*,4*R*,7a*S*)-3a,4-Dimethyl-3a,4,5,6,7,7a-hexahydro-1H-inden-2-yl)ethan-1-one ((±)-Noreremophilane 40):



The compound **22** (30 mg, 0.16 mmol) and Wilkinson's catalyst [$(PPh_3)_3RhCl$] (29 mg, 0.03 mmol) were placed in an oven-dried round-bottom flask. Dry benzene (5.0 mL) was added via syringe, the flask was then flushed with hydrogen gas to expel the argon. The reaction was allowed to proceed at room temperature under hydrogen balloon pressure for 12 h. Upon completion of reaction (monitored by TLC), the mixture was passed through an alumina column and concentrated. Purification by flash chromatography over silica gel (0.5:9.5; EtOAc−petroleum ether) afforded (±)-noreremophilane **40** (25 mg, 82%) as a colorless liquid.

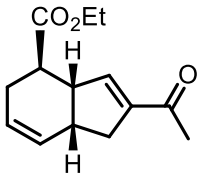
IRυ_{max} (film): 1668, 1606, 1367 cm^{-1}.

^{1}H NMR (400 MHz, C_6D_6): δ 6.30 (d, *J* = 2.0 Hz, 1H), 2.57 (dd, *J* = 16.1, 8.3 Hz, 1H), 2.43 (ddd, *J* = 16.1, 11.3, 2.26 Hz, 1H), 1.97 (s, 3H), 1.78−1.73 (m, 1H), 1.44−1.41 (m, 1H), 1.38−1.35 (m, 1H), 1.34−1.31 (m, 1H), 1.22−1.17 (m, 1H), 1.13−1.11 (m, 1H), 1.10−1.08 (m, 1H), 0.86−0.85 (m, 1H), 0.84 (s, 3H), 0.67 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, C_6D_6): δ 195.6, 153.1, 143.9, 49.6, 46.5, 36.9, 33.6, 29.3, 25.9, 24.4, 22.2, 17.3 (2C).

HRMS (ESI): *m/z* calcd for $C_{13}H_{21}O$ $[M+H]^+$ 193.1587, found 193.1589.

Ethyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (45):



To a solution of diene **13** (1.0 g, 8.06 mmol) and ethyl (*E*)-4-oxobut-2-enoate **43** (2.1 g, 16.12 mmol) in dry CH_2Cl_2 (50 mL) was added $BF_3{\cdot}OEt_2$ (1.5 mL, 12.09 mmol) dropwise at –78 °C. The mixture was allowed to warm up to room temperature and was stirred for 4 h at room temperature. The CH_2Cl_2 layer was washed with saturated aqueous $NaHCO_3$ (3 x 15 mL) followed by H_2O (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*. The crude material **44** was passed through small pad of silica gel using 30% EtOAc in petroleum ether as eluent. The eluent was concentrated and dissolved in EtOH (20 mL), cooled to 0 °C, and treated with 15% ethanolic KOH (10 mL). After stirring at room temperature for 1 h, reaction mass was evaporated. The water (5 mL) was added and extracted with ethyl acetate (3 x 15 mL). The aqueous layer was acidified by 1N HCl and extracted by ethyl acetate (3 x 15 mL). The combined organic layers was washed by brine (20 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*. Purification by flash chromatography over silica gel (1.5:8.5; EtOAc–Petroleum Ether) afforded **45** (0.68 g, 36%) as light yellow oil and (4:6; EtOAc–Petroleum Ether) afforded **46** (0.17 g, 10%) as white solid.

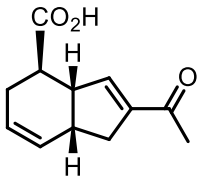
IRυ_{max} (film): 2931, 1730, 1670, 1179 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.70 (s, 1H), 5.74–5.73 (m, 2H), 4.19–4.14 (m, 2H), 3.13–3.09 (m, 1H), 2.91–2.77 (m, 2H), 2.39–2.32 (m, 1H), 2.29 (s, 3H), 2.27–2.17 (m, 3H), 1.26 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.0, 174.8, 145.6, 145.5, 129.4, 124.7, 60.7, 46.1, 42.0, 37.6, 36.5, 27.2, 26.6, 14.4.

HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_3Na$ $[M+Na]^+$ 257.1148, found 257.1147.

***(*3a*S*,4*R*,7a*R*)*-2-Acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylic acid (46):**



IRυ_{max} (film): 3146, 2934, 1646 cm^{-1}.

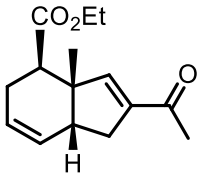
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77 (s, 1H), 5.77–5.76 (m, 2H), 3.18–3.14 (m, 1H), 2.95–2.91 (m, 1H), 2.87–2.80 (m, 1H), 2.47–2.44 (m, 1H), 2.38–2.34 (m, 1H), 2.33 (s, 3H), 2.29–2.22 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3, 180.7, 145.8, 145.4, 129.6, 124.4, 45.8, 41.7, 37.5, 36.6, 26.8, 26.7.

HRMS (ESI): *m/z* calcd for $C_{12}H_{14}O_3Na$ $[M+Na]^+$ found 229.0835, found 229.0835.

Compound **49** and **50** was synthesized using the procedure similar to preparation of **45** and **46**.

Ethyl(3a*S*,4*R*,7a*R*)-2-acetyl-3a-methyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (49):



IRυ_{max} (film): 2931, 1730, 1670, 1179 cm^{-1}.

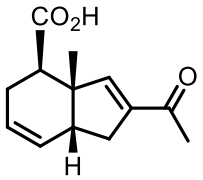
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (s, 1H), 5.76–5.69 (m, 2H), 4.21–4.11 (m, 2H), 2.85 (dd, *J* = 8.2, 15.8 Hz, 1H), 2.49–2.46 (m, 1H), 2.39–2.35 (m, 1H), 2.31 (s, 3H), 2.31–2.29

(m, 1H), 2.24–2.16 (m, 2H), 1.28 (t, *J* = 7.3 Hz, 3H), 1.11 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.4, 173.8, 152.4, 142.8, 128.0, 124.3, 60.5, 48.3, 47.4, 44.8, 36.6, 26.5, 24.5, 19.8, 14.5.

HRMS (ESI): *m/z* calcd for $C_{15}H_{20}O_3Na$ $[M+Na]^+$ 271.1305, found 271.1302.

(3a*S*,4*R*,7a*R*)-2-Acetyl-3a-methyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylic-acid (50):



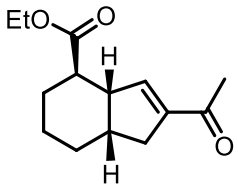
IRυ_{max} (film): 3146, 2934, 1646 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (s, 1H), 5.80–5.71 (m, 2H), 2.88 (dd, *J* = 8.3, 16.1 Hz, 1H), 2.58–2.54 (m, 1H), 2.44–2.39 (m, 1H), 2.34 (s, 3H), 2.32–2.30 (m, 1H), 2.29–2.18 (m, 2H), 1.18 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.6, 179.6, 151.9, 143.1, 128.2, 124.0, 48.2, 47.5, 44.7, 36.7, 26.6, 24.5, 19.9.

HRMS (ESI): *m/z* calcd for $C_{13}H_{16}O_3Na$ $[M+Na]^+$ 243.0992, found 243.0992.

Ethyl (3a*S*,4*R*,7a*S*)-2-acetyl-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylate (51):



To the solution of compound **45** (30 mg, 0.128 mmol) in dry benzene (5.0 mL) was added Wilkinson's catalyst $[(PPh_3)_3RhCl]$ (24 mg, 0.025 mmol). The reaction mixture was degassed by piercing argon for 5 min and the flask was then flushed with hydrogen gas to expel the argon. The reaction was allowed to proceed at room temperature under hydrogen balloon pressure for 12 h. Upon completion of reaction (monitored by TLC), the mixture was concentrated and purified by flash chromatography over silica gel (0.5:9.5; EtOAc–Petroleum Ether) afforded **51** (23 mg, 77%) as colorless oil.

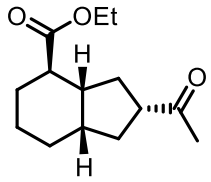
IRυ_{max} (film): 2933, 1732, 1670 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (s, 1H), 4.18–4.13 (m, 2H), 2.95–2.91 (m, 1H), 2.53–2.42 (m, 2H), 2.30 (s, 3H), 2.20–2.14 (m, 2H), 1.86–1.77 (m, 2H), 1.61–1.55 (m, 2H), 1.43–1.38 (m, 2H), 1.27 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.5, 175.3, 147.7, 145.8, 60.6, 46.5, 45.0, 37.3, 33.8, 27.1, 26.5, 26.3, 20.9, 14.3.

HRMS (ESI): *m/z* calcd for $C_{14}H_{20}O_3Na$ $[M+Na]^+$ 259.1412, found 259.1412.

Ethyl (2*R*,3a*R*,4*R*,7a*S*)-2-acetyloctahydro-1H-indene-4-carboxylate (52):



To a solution of **45** (50 mg, 0.213 mmol) in EtOAc (5.0 mL) was added PtO_2 (~ 5 mg) and the mixture was stirred under hydrogen balloon pressure. After 2 h catalyst was filtered off and concentrated to afford saturated keto-ester **52** (40 mg, 80% *dr-8:2*) as colourless oil.

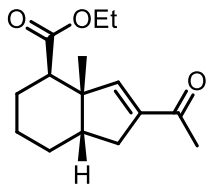
IRυ_{max} (film): 2931, 1730, 1711 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.15–4.08 (m, 2H), 3.13–2.92 (m, 1H), 2.20–2.17 (m, 2H), 2.15 (s, 3H), 1.99–1.92 (m, 1H), 1.89–1.82 (m, 1H), 1.80–1.68 (m, 4H), 1.54–1.52 (m, 2H), 1.48–1.38 (m, 3H), 1.26–1.22 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 210.8, 176.2, 60.3, 50.4, 43.8, 40.5, 38.6, 32.4, 31.4, 29.2, 27.9, 26.4, 20.5, 14.4.

HRMS (ESI): *m/z* calcd for $C_{14}H_{22}O_3Na$ $[M+Na]^+$ 261.1461, found 261.1459.

Ethyl-(3a*S*,4*R*,7a*S*)-2-acetyl-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylate (53): Compound **53** was synthesized from **49** using the procedure similar to preparation of **51**.



Yield: 75%.

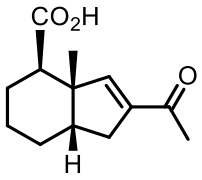
IRυ_{max} (film): 2933, 1732, 1670 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (s, 1H), 4.20–4.09 (m, 2H), 2.49 (dd, *J* = 8.1, 15.8 Hz, 1H), 2.43–2.36 (m, 1H), 2.31 (s, 3H), 2.30–2.27 (m, 1H), 2.05–1.99 (m, 1H), 1.73–1.63 (m, 3H), 1.60–1.53 (m, 1H), 1.50–1.36 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.12 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.9, 174.5, 154.6, 142.7, 60.3, 48.9, 48.6, 46.6, 33.2, 26.4, 24.0, 23.1, 20.9, 19.3, 14.5.

HRMS (ESI): *m/z* calcd for $C_{15}H_{22}O_3Na$ $[M+Na]^+$ 273.1461, found 273.1460.

(3a*S*,4*R*,7a*S*)-2-Acetyl-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylic acid (41):



To a solution of **53** (35 mg, 0.14 mmol) in EtOH (2 mL) and water (2 mL) was added lithium hydroxide monohydrate (12 mg, 0.28 mmol) at 0 °C. The mixture was warmed up to room temperature and stirred for 12 h. The mixture was acidified to pH 2 with 1N HCl. The volatiles were evaporated and the residue was extracted with EtOAc (2 x 5 mL). The combined organic layer was washed by brine (3 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*. Purification by flash chromatography over silica gel (3.0:7.0; EtOAc–Petroleum Ether) afforded **41** (30 mg, 96%) as white solid.

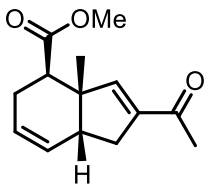
IRυ_{max} (film): 3144, 2936, 1646 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (s, 1H), 2.52 (dd, *J* = 8.2, 16.0 Hz, 1H), 2.45–2.36 (m, 2H), 2.34 (s, 3H), 2.09–2.05 (m, 1H), 1.78–1.76 (m, 1H), 1.69–1.66 (m, 2H), 1.62–1.57 (m, 1H), 1.50–1.42 (m, 2H), 1.20 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.2, 180.7, 154.3, 143.0, 48.7, 48.5, 46.6, 33.2, 26.5, 23.9, 22.9, 20.7, 19.3.

HRMS (ESI): *m/z* calcd for $C_{13}H_{18}O_3Na$ $[M+Na]^+$ 245.1148, found 245.1146.

Methyl-(3a*S*,4*R*,7a*R*)-2-acetyl-3a-methyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (54):



To a solution of acid **50** (100 mg, 0.454 mmol) in dry CH_2Cl_2 (10 mL) was added $(COCl)_2$ (0.08 mL, 0.909 mmol) followed by 1 drop of DMF at 0 °C. The mixture was allowed stir for 2 h at same temperature. The solution was concentrated in *vacuo*, to give yellow oil. The crude was dissolved in CH_2Cl_2 (10 mL), cooled to 0 °C and treated with MeOH (1 mL). The reaction mixture was stirred at room temperature for 2 h and then concentrated. The crude was purified by flash chromatography over silica gel (1.5:8.5; EtOAc–Petroleum Ether) afforded **54** (76 mg, 72%) as colorless oil.

Yield: 72%.

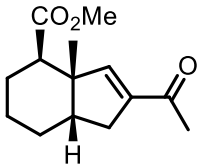
IRυ_{max} (film): 2930, 1732, 1671 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (s, 1H), 5.76–5.69 (m, 2H), 3.70 (s, 3H), 2.86 (dd, *J* = 8.2, 15.9 Hz, 1H), 2.52–2.49 (m, 1H), 2.40–2.36 (m, 1H), 2.32 (s, 3H), 2.30–2.17 (m, 3H), 1.12 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.5, 174.3, 152.1, 143.0, 128.1, 124.2, 57.7, 48.3, 47.4, 44.8, 36.6, 26.6, 24.7, 19.9.

HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_3Na$ $[M+Na]^+$ 257.1148, found 257.1147.

Methyl(3a*S*,4*R*,7a*S*)-2-acetyl-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-indene-4-carboxylate (42): Compound **42** was synthesized from **54** using the procedure similar to preparation of **51**.



Yield: 70%.

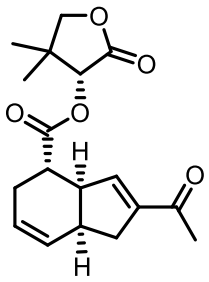
IRυ_{max} (film): 1720, 1668 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (s, 1H), 3.70 (s, 3H), 2.51 (dd, *J* = 8.1, 16.1 Hz, 1H), 2.44–2.37 (m, 1H), 2.32 (s, 3H), 2.06–2.00 (m, 1H), 1.76–1.72 (m, 1H), 1.66–1.64 (m, 2H), 1.57–1.54 (m, 2H), 1.52–1.49 (m, 1H), 1.45–1.41 (m, 1H), 1.12 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.9, 175.0, 154.3, 142.9, 51.6, 48.9, 48.7, 46.6, 33.4, 26.5, 24.0, 23.2, 20.9, 19.5.

Synthesis of Enantiopure Noreremophilane Derivatives: To a solution of **rac-46** (300 mg, 1.46 mmol) in dry CH_2Cl_2 (20 mL) under nitrogen atmosphere was added D (-) Pantolactone (190 mg, 1.46 mmol), HOBT (296 mg, 2.19 mmol), EDC.HCl (420 mg, 2.19 mmol) and DIPEA (0.4 mL, 2.19 mmol) at room temperature. The reaction mixture was allowed to stir at room temperature for 12 h. The mixture was washed with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4, concentrated in *vacuo*. Purification by flash chromatography over silica gel (2.0:8.0; EtOAc–Petroleum Ether) afforded **53a** (143 mg, 31%) and **53b** (120 mg, 26%) as white solid.

(*R*)-4,4-Dimethyl-2-oxotetrahydrofuran-3-yl(3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetra hydro-1H-indene-4-carboxylate (55a):



IRυ_{max} (film): 1720, 1668 cm^{-1}.

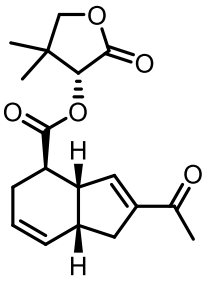
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (s, 1H), 5.82–5.78 (m, 2H), 5.44 (s, 1H), 4.09–4.04 (m, 2H), 3.38–3.33 (m, 1H), 2.98–2.92 (m, 1H), 2.88–2.82 (m, 1H), 2.58–2.52 (m, 1H), 2.34 (s, 3H), 2.32–2.31 (m, 2H), 2.29–2.22 (m, 1H), 1.21 (s, 3H), 1.12 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.4, 174.1, 172.4, 145.7, 145.5, 129.7, 124.2, 76.4, 75.3, 46.8, 41.8, 40.3, 37.8, 36.4, 26.8, 26.6, 23.1, 20.1.

HRMS (ESI): *m/z* calcd for $C_{18}H_{22}O_5Na$ $[M+Na]^+$ 341.1359, found 341.1357.

Specific rotation: $[\alpha]_D^{25}$ + 9.70 (c = 1.30, $CHCl_3$).

(*R*)-4,4-Dimethyl-2-oxotetrahydrofuran-3-yl(3a*R*,4*S*,7a*S*)-2-acetyl-3a,4,5,7a-tetra hydro-1H-indene-4-carboxylate (55b):



IRυ_{max} (film): 1720,1668 cm^{-1}.

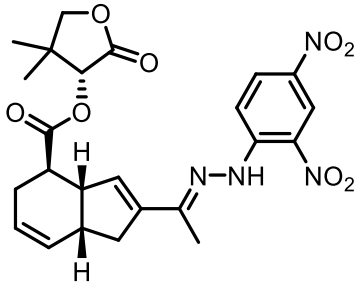
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.78 (s, 1H), 5.78 (s, 2H), 5.40 (s, 1H), 4.09–4.03 (d, *J* = 3.2 Hz, 2H), 3.22–3.18 (m, 1H), 2.96–2.81 (m, 2H), 2.57–2.51 (m, 1H), 2.44–2.38 (m, 1H), 2.31 (s, 3H), 2.30–2.23 (m, 2H), 1.23 (s, 3H), 1.13 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.0, 173.5, 172.2, 145.9, 145.0, 129.5, 124.5, 76.3, 75.3, 46.0, 42.2, 40.2, 37.7, 36.5, 27.3, 26.7, 23.2, 20.1.

HRMS (ESI): *m/z* calcd for $C_{18}H_{22}O_5Na$ $[M+Na]^+$ 341.1359, found 341.1354.

Specific rotation: $[\alpha]_D^{25}$–11.05 (c = 2.90, $CHCl_3$).

(*R*)-4,4-Dimethyl-2-oxotetrahydrofuran-3-yl-(3a*S*,4*R*,7a*R*)-2-((*E*)-1-(2-(2,4-dinitrophe -nyl) hydrazono) ethyl)-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (57):



To a solution of **55b** (20 mg, 0.062 mmol) in EtOH (3.0 mL) was added 2,4-dinitrophenylhydrazine (21 mg, 0.106 mmol) followed by 1 drops of conc. HCl at room temperature. The mixture was allowed to stir at room temperature for overnight. Solvent was removed *in vacuo*. Ether (5 mL) was added followed by 10% $NaHCO_3$ (2 mL). The aqueous layer was extracted by ether (2 x 5 mL) and dried over anhydrous Na_2SO_4. Purification by flash chromatography (2.0:8.0% EtOAc: Petroleum Ether) to afford **57** (21 mg, 67%) as orange colored solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 11.2 (s, 1H), 9.11 (d, *J* = 2.4 Hz, 1H), 8.30 (dd, *J* = 9.5, 2.4 Hz, 1H), 7.95 (d, *J* = 9.5 Hz, 1H), 6.54 (s, 1H), 5.88–5.78 (m, 2H), 5.45 (s, 1H), 4.07 (s, 2H), 3.16–3.01 (m, 3H), 2.56–2.52 (m, 1H), 2.47–2.41 (m, 1H), 2.34–2.32 (m, 2H), 2.23 (s, 3H), 1.22 (s, 3H), 1.11 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 174.3, 172.5, 151.0, 144.9, 143.6, 138.2, 137.5, 130.1, 130.0, 129.7, 124.3, 123.6, 116.8, 76.4, 75.2, 46.4, 42.5, 40.3, 37.9, 37.5, 27.0, 23.1, 20.1, 12.9.

Crystal data of 57: Single crystal of compound **57** was obtained from ethyl acetate-hexane mixture. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at room temperature 296(2) K. The X-ray generator was operated at 50 kV and 30 mA. Diffraction data were collected with a ω scan width of 0.5° and at different settings of φ and 2θ. The sample-to-detector distance was fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX II program suite. All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX II program package. The structures were solved by direct method and refined by full matrix least squares, based on *F*, using SHELX-97. Molecular diagrams were generated using XSHELL program integrated in SHELXTL package. All the H-atoms (except H-atom bound to N atom) were placed in geometrically idealized position (C-H = 0.93 Å for the phenyl H-atom, C-H = 0.97 Å for the methylene H-atom, C-H = 0.98 Å for the methine H-atom and C-H = 0.96 Å for the methyl H-atom) and constrained to ride on their parent atoms [U_{iso}(H) = 1.2U_{eq}(C) for the phenyl, methylene and methine group and U_{iso}(H) = 1.5 U_{eq}(C) for the methyl group]. The H-atom attached to N-atom in **57** is located in difference Fourier and refined isotropically.

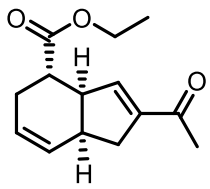
Crystallographic data for 57 ($C_{24}H_{26}N_4O_8$): *M* = 498.49, Crystal dimensions 0.53 x 0.49 x 0.30 mm^3, monoclinic, space group *P* 2_1,*a* = 5.9435(4), *b* = 12.4041(8), *c* = 16.5108(11) Å, β= 92.519(4)°, *V* = 1216.06(14) Å^3, *Z* = 2, ρ_{calcd} = 1.361 gcm^{-3}, μ (Mo-K$_\alpha$) = 0.104 mm^{-1}, *F(000)* = 524, $2\theta_{max}$ = 56.64°, *T* = 296(2) K, 23510 reflections collected, 5326 unique, 4403 observed ($I > 2\sigma(I)$) reflections, 332 refined parameters, *R* value 0.0545, *wR*2 = 0.1114, (all data *R* = 0.0702, *wR*2 = 0.1181), *S* = 1.123, minimum and maximum

transmission 0.947 and 0.970; maximum and minimum residual electron densities +0.20 and –0.21 e $Å^{-3}$.

General procedure for trans-esterification of 55a and 55b:

To a stirred solutions of **55a/55b** (0.157 mmol) in EtOH/PrOH (10 mL) was added K_2CO_3 (0.314 mmol) at room temperature. The mixture was allowed to stir at room temperature for 12 h and then concentrated. The crude was purified by flash chromatography over silica gel (1.5:8.5; EtOAc–Petroleum Ether) to afford **45a/56a** and **45b/56b** in 50-60% yield as colorless oils.

Ethyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (45a):



Yield: 59%.

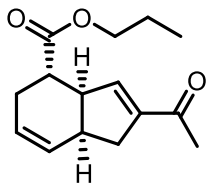
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.78–5.76 (m, 2H), 4.21–4.16 (m, 2H), 3.15–3.11 (m, 1H), 2.94–2.79 (m, 2H), 2.41–2.35 (m, 1H), 2.31 (s, 3H), 2.27–2.17 (m, 3H), 1.28 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.8, 145.7, 145.6, 129.5, 124.7, 60.8, 46.1, 42.1, 37.7, 36.5, 27.2, 26.7, 14.1.

HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_3Na$ $[M+Na]^+$ 257.1148, found 257.1147.

Specific rotation: $[\alpha]_D^{25.4}$ + 20.87 (c = 1.0, $CHCl_3$).

Propyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (56a):



Yield: 55%.

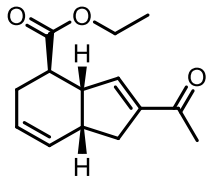
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.77 (m, 2H), 4.09 (t, *J* = 6.8 Hz, 2H), 3.16–3.11 (m, 1H), 2.90–2.79 (m, 2H), 2.42–2.33 (m, 2H), 2.31 (s, 3H), 2.29–2.18 (m, 2H), 1.72–1.63 (m, 2H), 0.96 (t, *J* = 7.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 144.7, 145.5, 129.5, 124.7, 66.4, 46.1, 42.1, 37.7, 36.5, 27.3, 26.7, 22.2, 10.6.

HRMS (ESI): *m/z* calcd for $C_{15}H_{20}O_3Na$ $[M+Na]^+$ 271.1305, found 271.1306.

Specific rotation: $[\alpha]_D^{25}$ +18.42 (c = 0.9, $CHCl_3$).

Ethyl (3a*R*,4*S*,7a*S*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (45b):



Yield: 56%.

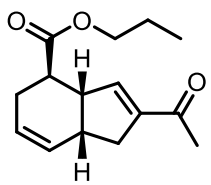
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.78–5.76 (m, 2H), 4.21–4.16 (m, 2H), 3.15–3.11 (m, 1H), 2.94–2.79 (m, 2H), 2.41–2.35 (m, 1H), 2.31 (s, 3H), 2.27–2.17 (m, 3H), 1.28 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.8, 145.7, 145.6, 129.5, 124.7, 60.8, 46.1, 42.1, 37.7, 36.5, 27.2, 26.7, 14.1.

HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_3Na$ $[M+Na]^+$ 257.1148, found 257.1146.

Specific rotation: $[\alpha]_D^{25}$ –21.92 (c = 1.0, $CHCl_3$).

Propyl (3a*R*,4*S*,7a*S*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (56b):



Yield: 50%.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.77 (m, 2H), 4.09 (t, *J* = 6.8 Hz, 2H), 3.16–3.11 (m, 1H), 2.90–2.79 (m, 2H), 2.42–2.33 (m, 2H), 2.31 (s, 3H), 2.29–2.18 (m, 2H),

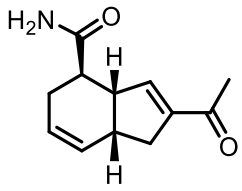
1.72–1.63 (m, 2H), 0.96 (t, *J* = 7.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 144.7, 145.5, 129.5, 124.7, 66.4, 46.1, 42.1, 37.7, 36.5, 27.3, 26.7, 22.2, 10.6.

HRMS (ESI): *m/z* calcd for $C_{15}H_{20}O_3Na$ $[M+Na]^+$ 271.1305, found 271.1305.

Specific rotation: $[\alpha]_D^{25}$ – 15.37 (c = 0.5, $CHCl_3$).

(3a*S*,4*R*,7a*R*)-2-Acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxamide (65):



To a solution of acid **46** (100 mg, 0.485 mmol) in dry CH_2Cl_2 (10 mL) was added $(COCl)_2$ (0.08 mL, 0.970 mmol) followed by 1 drop of DMF at 0 °C. The mixture was allowed stir for 2 h at same temperature.The solution was concentrated in *vacuo*, to give yellow oil. The crude was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C and treated with NH_4OH (25% aqueous solution, 1 mL), the mixture was stirred at room temperature for 2 h and then concentrated. The crude was purified by flash chromatography over silica gel (0.5:9.5; MeOH– CH_2Cl_2) afforded **65** (72 mg, 72%) as white solid.

IRυ_{max} (film): 3470, 1675 cm^{-1}.

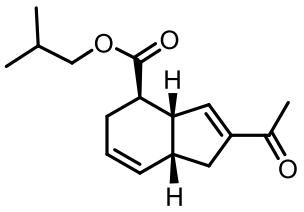
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.76 (s, 1H), 5.83–5.77 (m, 2H), 5.67–5.61 (m, 2H), 3.15–3.11 (m, 1H), 2.92–2.79 (m, 2H), 2.31 (s, 3H), 2.26–2.15 (m, 4H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.3, 176.9, 145.7, 145.6, 129.5, 124.8, 46.4, 43.5, 37.8, 36.5, 28.2, 26.7.

HRMS (ESI): *m/z* calcd for $C_{12}H_{15}O_2NNa$ $[M+Na]^+$ 228.0995, found 228.0994.

Compound **58**, **59**, **60**, **61**, **62**, **63**, **64**, **66** and **67** were synthesized using the procedure similar to compound **65**.

Isobutyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4 carboxylate (58):



Yield: 73%.

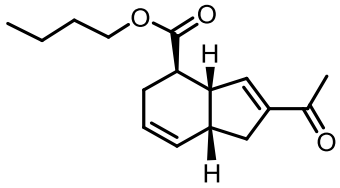
IRυ_{max} (film): 2931, 1732, 1671 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (s, 1H), 5.76–5.75 (m, 2H), 3.93–3.89 (m, 2H), 3.16–3.12 (m, 1H), 2.92–2.79 (m, 2H), 2.43–2.37 (m, 1H), 2.31 (s, 3H), 2.25–2.22 (m, 3H), 1.98–1.92 (m, 1H), 0.95 (d, *J* = 6.8 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 145.8, 145.6, 129.5, 124.7, 70.9, 46.1, 42.1, 37.7, 36.5, 27.9, 27.3, 26.6, 19.2 (2C).

HRMS (ESI): *m/z* calcd for $C_{16}H_{22}O_3Na$ $[M+Na]^+$ 285.1461, found 285.1461.

Butyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (59):



Yield: 71%.

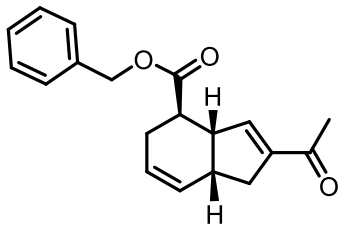
IRυ_{max} (film): 2931, 1730, 1670, 1179 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.71 (s, 1H), 5.76–5.75 (m, 2H), 4.15–4.11 (m, 2H), 3.14–3.10 (m, 1H), 2.93–2.79 (m, 2H), 2.41–2.37 (m, 1H), 2.30 (s, 3H), 2.23–2.19 (m, 2H), 1.64–1.61 (m, 3H), 1.42–1.36 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 145.8, 145.6, 129.4, 124.7, 64.7, 46.1, 42.1, 37.7, 36.5, 30.8, 27.2, 26.6, 19.3, 13.8.

HRMS (ESI): *m/z* calcd for $C_{16}H_{22}O_3Na$ $[M+Na]^+$ 285.1461, found 285.1460.

Benzyl (3a*S*,4*R*,7a*R*)-2-Acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (60):



Yield: 76%.

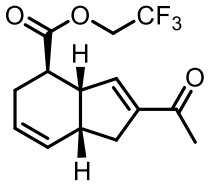
IRυ_{max} (film): 2931, 1730, 1670, 1179 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.33 (m, 5H), 6.61 (s, 1H), 5.78–5.76 (m, 2H), 5.23–5.12 (m, 2H), 3.15–3.10 (m, 1H), 2.93–2.77 (m, 2H), 2.46–2.40 (m, 1H), 2.36–2.30 (m, 1H), 2.26–2.25 (m, 1H), 2.24 (s, 3H), 2.21–2.18 (m, 1H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.6, 145.6 (2C), 136.0, 129.4, 128.8 (2C), 128.6, 128.3 (2C), 124.6, 66.5, 46.2, 42.1, 37.7, 36.4, 27.1, 26.6.

HRMS (ESI): *m/z* calcd for $C_{19}H_{20}O_3Na$ $[M+Na]^+$ 319.1305, found 319.1304.

2,2,2-Trifluoroethyl(3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (61):



Yield: 71%.

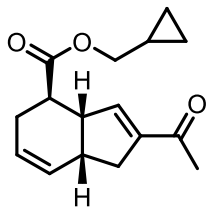
IRυ_{max} (film): 1728, 1668 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.68 (s, 1H), 5.80–5.73 (m, 2H), 4.61–4.43 (m, 2H), 3.16–3.12, (m, 1H), 2.96–2.81 (m, 2H), 2.54–2.48 (m, 1H), 2.38–2.32 (m, 1H), 2.30 (s, 3H), 2.28–2.22 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.0, 173.3, 146.0, 144.6, 129.5, 124.2, 121.7, 61.4 (q, 1C, J = 36.2 Hz), 46.1, 41.7, 37.7, 36.4, 26.8, 26.6.

HRMS (ESI): *m/z* calcd for $C_{14}H_{15}O_3F_3Na$ $[M+Na]^+$ 311.0866, found 311.0862.

Cyclopropyl-methyl-(3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (62):



Yield: 64%.

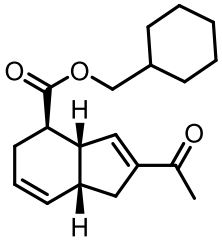
IRυ_{max} (film): 1730, 1663 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.78–5.72 (m, 2H), 4.00–3.90 (m, 2H), 3.13–3.09, (m, 1H), 2.90–2.78 (m, 2H), 2.41–2.35 (m, 1H), 2.33–2.28 (m, 1H), 2.30 (s, 3H), 2.26–2.19 (m, 2H), 1.16–1.11 (m, 1H), 0.59–0.54 (m, 2H), 0.30–0.26 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 145.7, 145.5, 129.3, 124.7, 69.4, 46.2, 42.1, 37.7, 36.4, 27.2, 26.6, 9.9, 3.3 (2C).

HRMS (ESI): *m/z* calcd for $C_{16}H_{20}O_3Na$ $[M+Na]^+$ 283.1305, found 283.1303.

Cyclohexyl-methyl-(3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (63):



Yield: 68%.

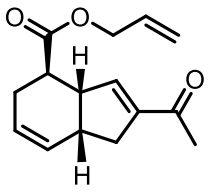
IRυ_{max} (film): 2931, 1732, 1671 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.71 (s, 1H), 5.75 (m, 2H), 3.93 (d, *J* = 6.4 Hz, 2H), 3.44–3.42 (m, 1H), 3.14–3.09 (m, 1H), 2.90–2.78 (m, 2H), 2.40–2.34 (m, 1H), 2.30 (s, 3H), 2.32–2.25 (m, 1H), 2.24–2.17 (m, 2H), 1.74–1.71 (m, 4H), 1.24–1.20 (m, 4H), 1.02–.093 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.9, 145.8, 145.5, 129.4, 124.7, 70.0 68.9, 46.2, 42.1, 40.6, 37.7, 37.3, 36.5, 29.8, 27.2, 26.4, 25.9, 25.7.

HRMS (ESI): *m/z* calcd for $C_{19}H_{26}O_3Na$ $[M+Na]^+$ 325.1774, found 325.1772.

Allyl (3a*S*,4*R*,7a*R*)-2-acetyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxylate (64):



Yield: 66%.

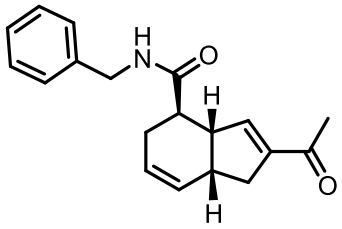
IRυ_{max} (film): 2930, 1730, 1669 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.71 (s, 1H), 5.96–5.89 (m, 1H), 5.76 (m, 2H), 5.33 (dd, *J* = 1.2, 17.1 Hz, 1H), 5.25 (dd, *J* =1.2, 10.5 Hz, 1H), 4.63 (d, *J* = 5.1 Hz, 2H), 3.16–3.12 (m, 1H), 2.92–2.79 (m, 2H), 2.45–2.39 (m, 1H), 2.35–2.29 (m, 1H), 2.30 (s, 3H), 2.27–2.21 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.1, 174.5, 145.6, 145.6, 132.2, 129.5, 124.6, 118.5, 65.4, 46.1, 42.0, 37.7, 36.5, 27.2, 26.6.

HRMS (ESI): *m/z* calcd for $C_{15}H_{18}O_3Na$ $[M+Na]^+$ 269.1148, found 269.1147.

(3a*S*,4*R*,7a*R*)-2-Acetyl-N-benzyl-3a,4,5,7a-tetrahydro-1H-indene-4-carboxamide (66):



Yield: 80%.

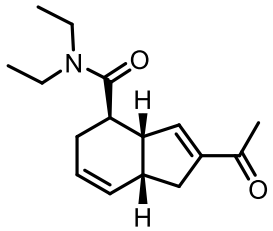
IRυ_{max} (film): 2931, 1670, 1179 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.28 (m, 5H), 6.65 (s, 1H), 6.04 (bs, 1H), 5.77–5.76 (m, 2H), 4.57–4.52 (m, 1H), 4.40–4.35 (m, 1H), 3.16–3.12 (m, 1H), 2.89–2.75 (m, 2H), 2.28–2.25 (m, 1H), 2.20 (s, 3H), 2.17–2.04 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.2, 174.3, 146.0, 145.5, 138.5, 129.2, 128.9 (2C), 127.8 (2C), 127.7, 125.1, 46.6, 44.3, 43.6, 37.9, 36.4, 28.2, 26.5.

HRMS (ESI): *m/z* calcd for $C_{19}H_{21}O_2NNa$ $[M+Na]^+$ 318.1465, found 318.1462.

(3a*S*,4*R*,7a*R*)-2-Acetyl-N,N-diethyl-3a,4,5,7a-tetrahydro-1H-indene-4carboxamide (67):



Yield: 74%.

IRυ_{max} (film): 2931, 1670, 1179 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.66 (s, 1H), 5.83–5.79 (m, 2H), 3.50–3.45 (m, 1H), 3.36–3.18 (m, 4H), 2.90–2.80 (m, 2H), 2.42–2.35 (m, 1H), 2.26 (s, 3H), 2.24–2.17 (m, 2H), 2.11–2.06 (m, 1H), 1.14–1.06 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 197.2, 173.7, 146.6, 145.3, 129.1, 125.4, 47.0, 42.0, 40.6, 39.2, 38.0, 36.4, 28.7, 26.6, 15.2, 13.3.

HRMS (ESI): *m/z* calcd for $C_{16}H_{23}O_2NNa$ $[M+Na]^+$ 284.1621, found 284.1620.

2.6 References

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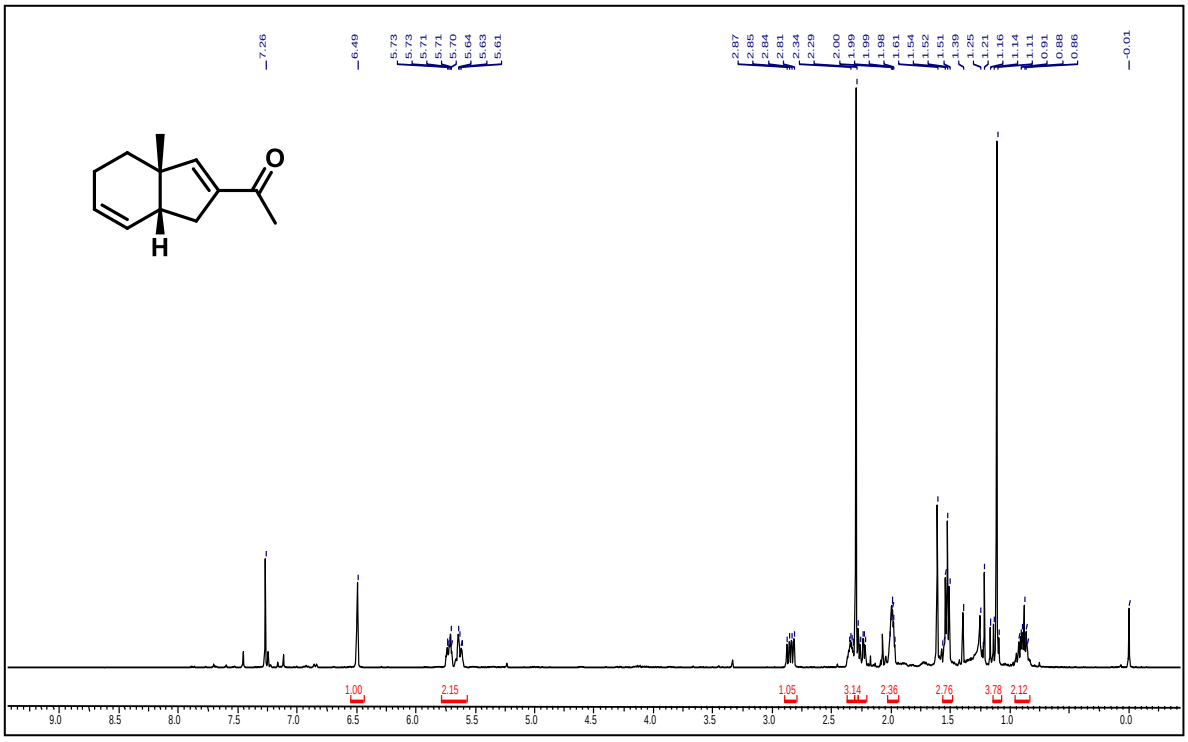
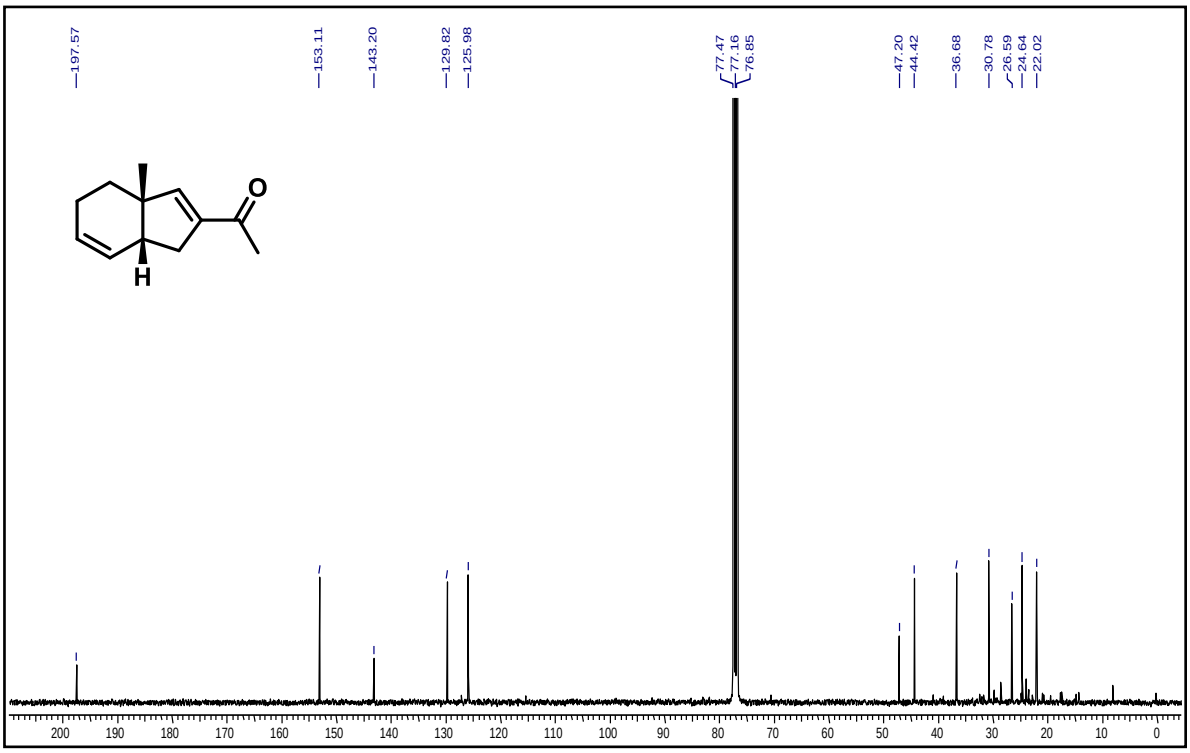
2.7 Selected copies of NMR Spectra

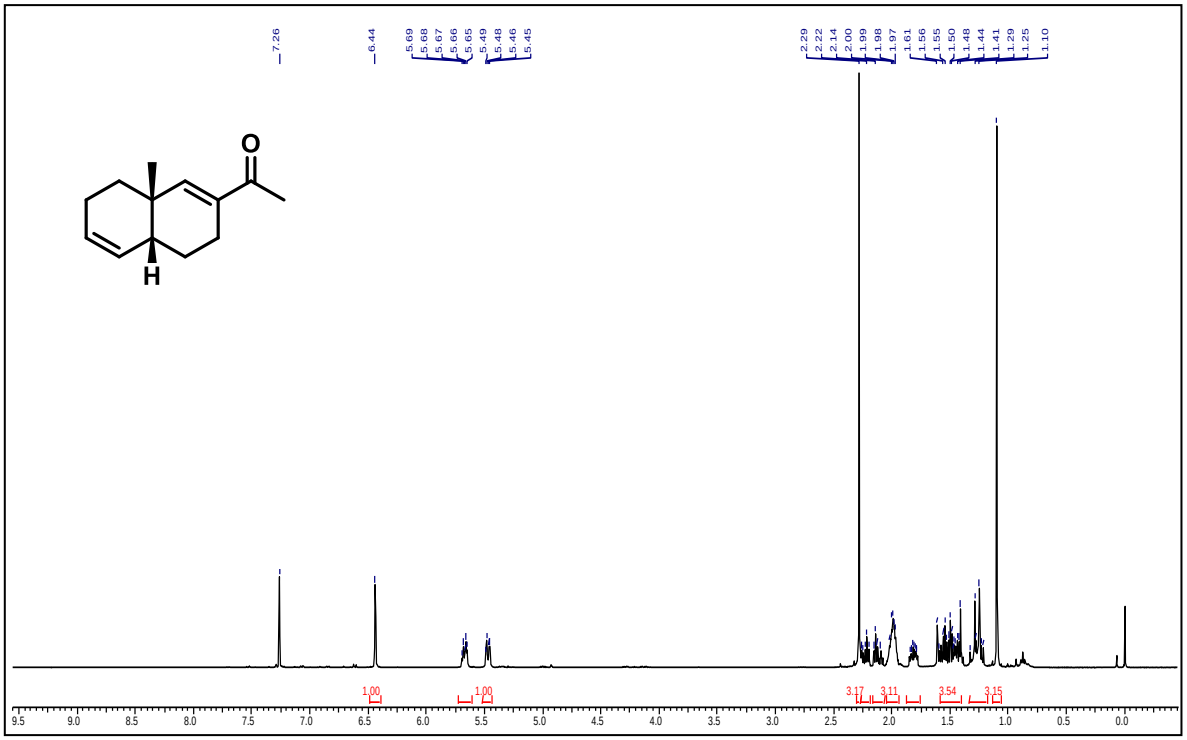
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 19



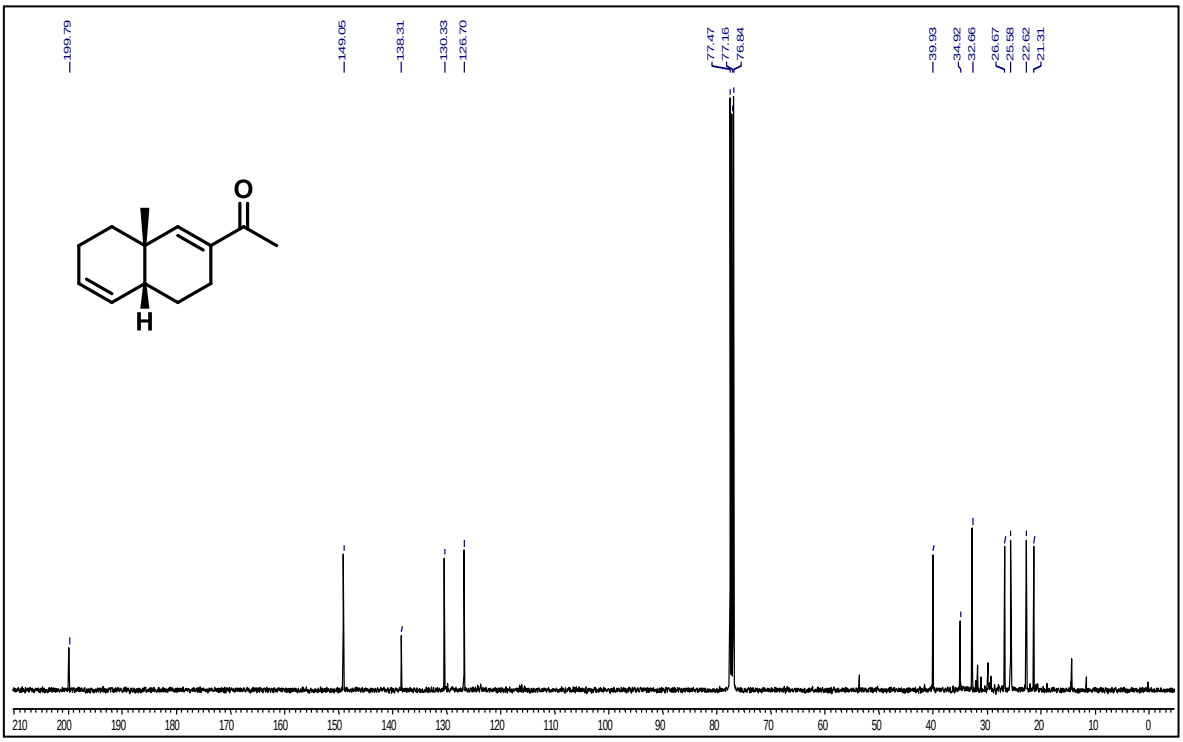
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 19



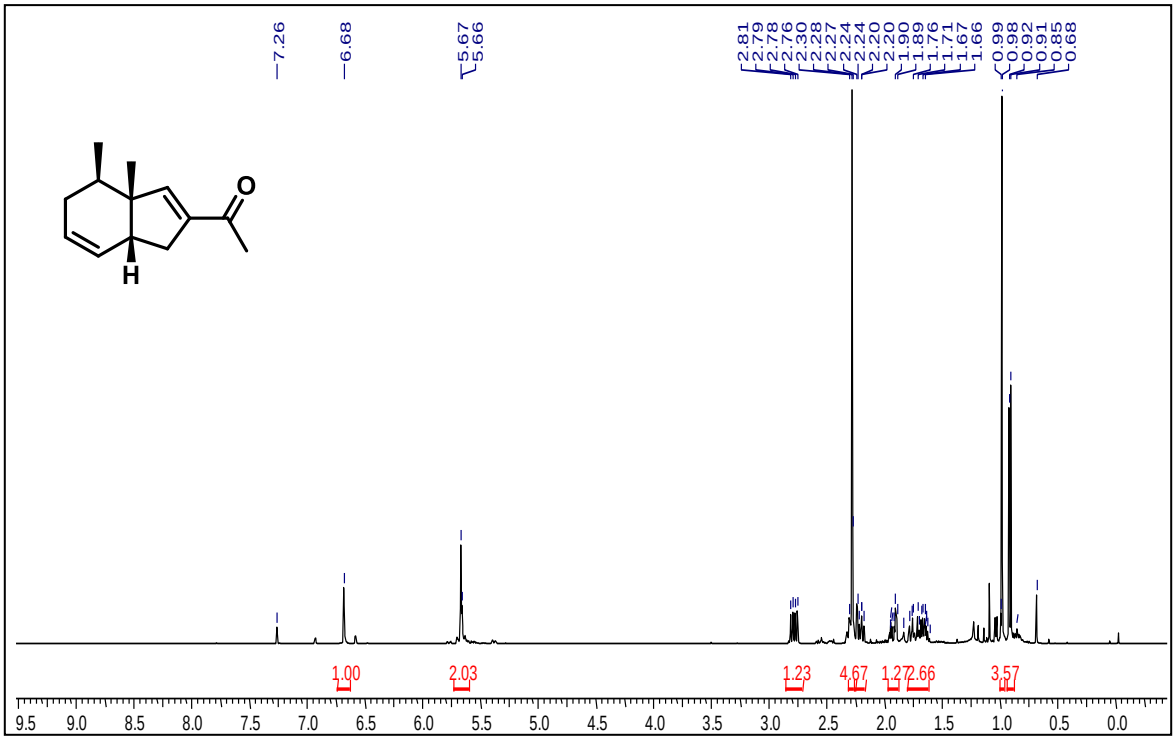
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 21



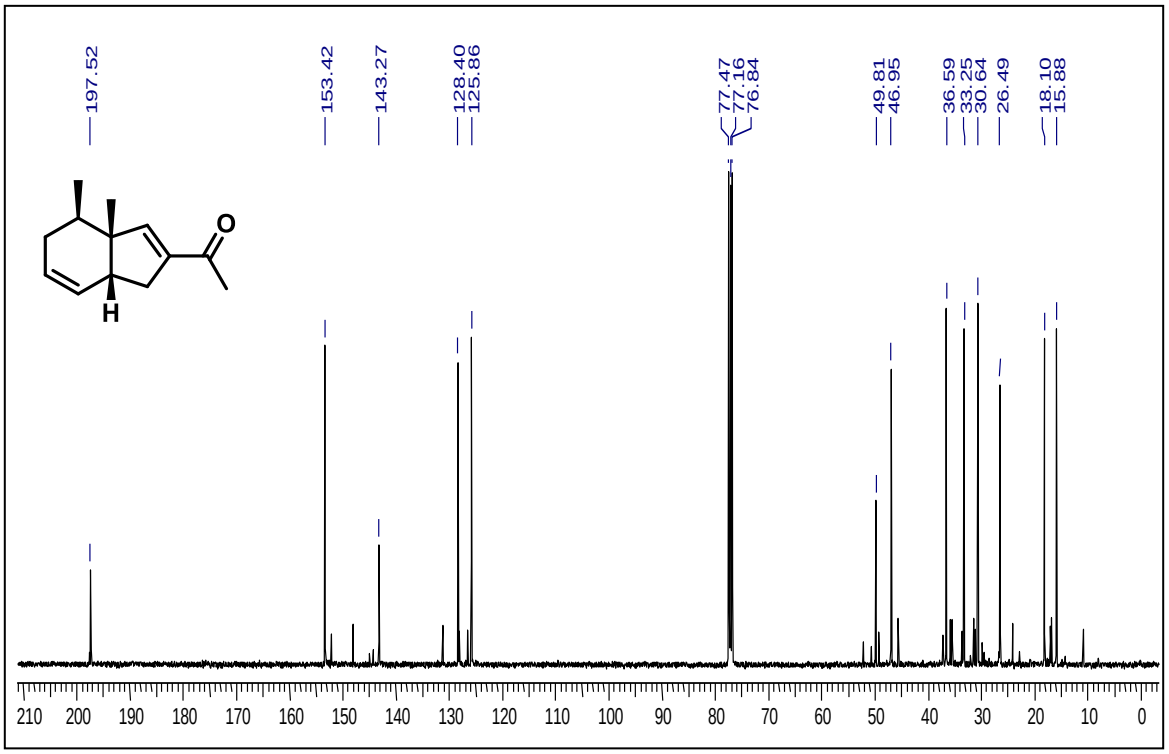
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 21



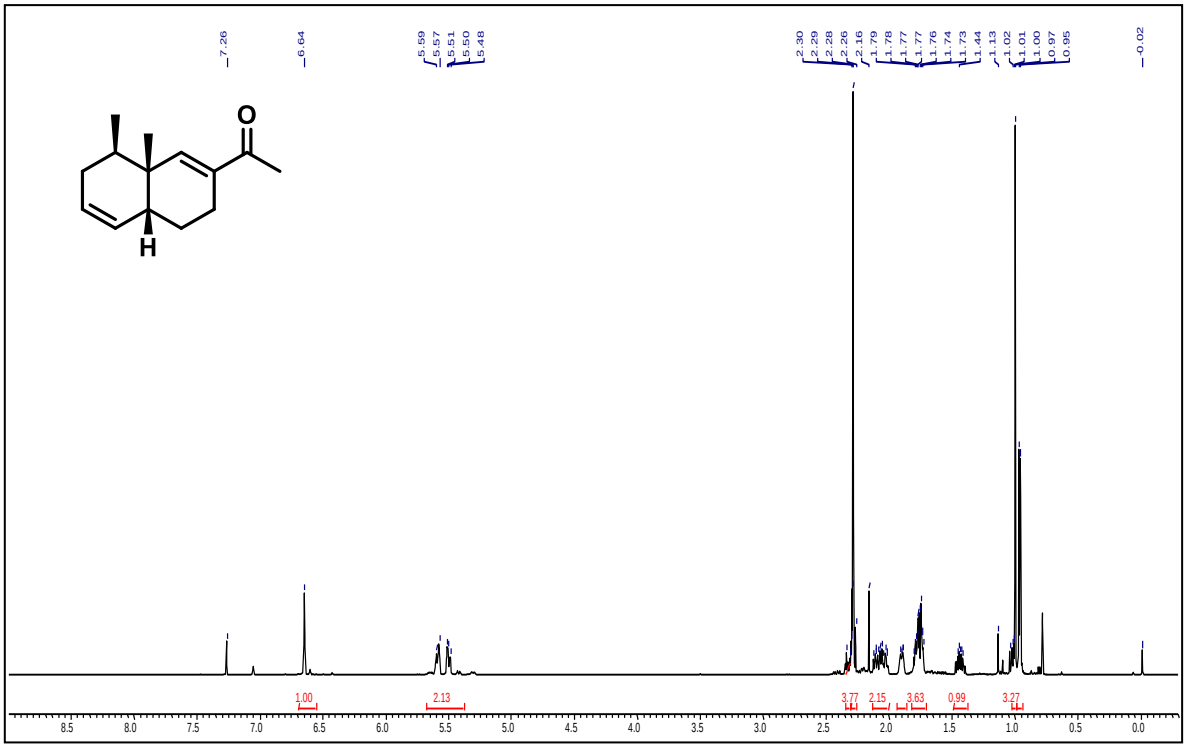
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 22



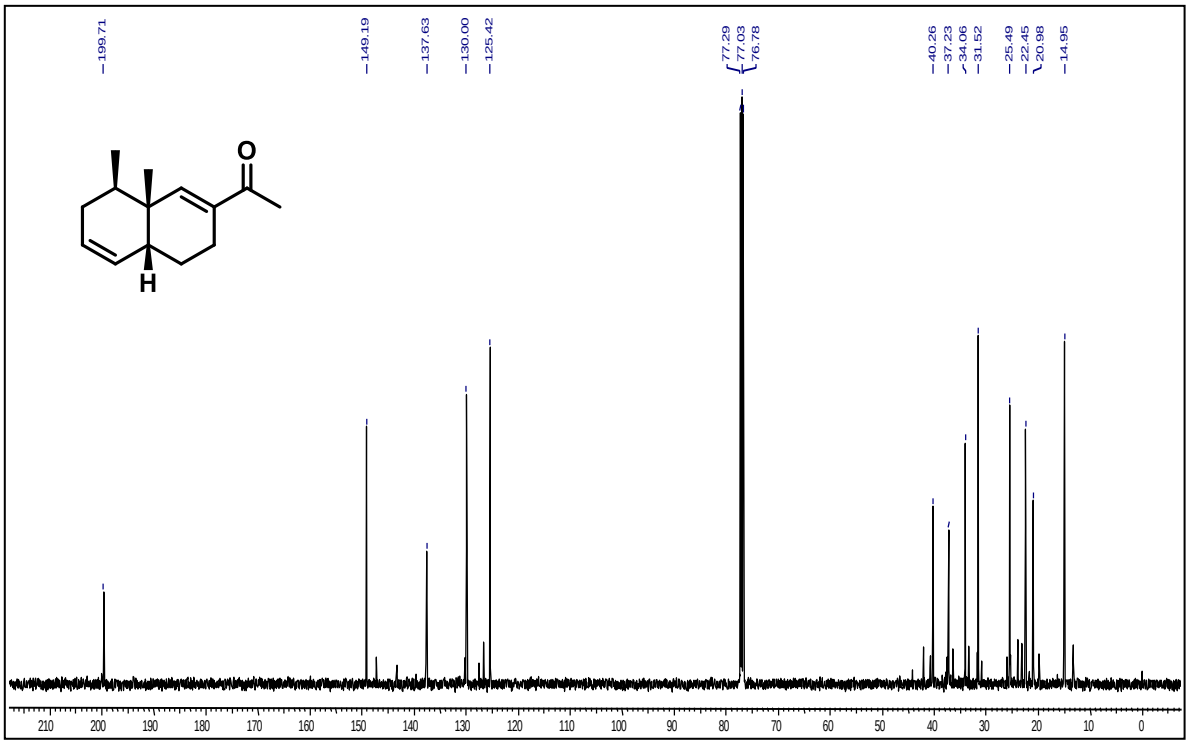
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 22



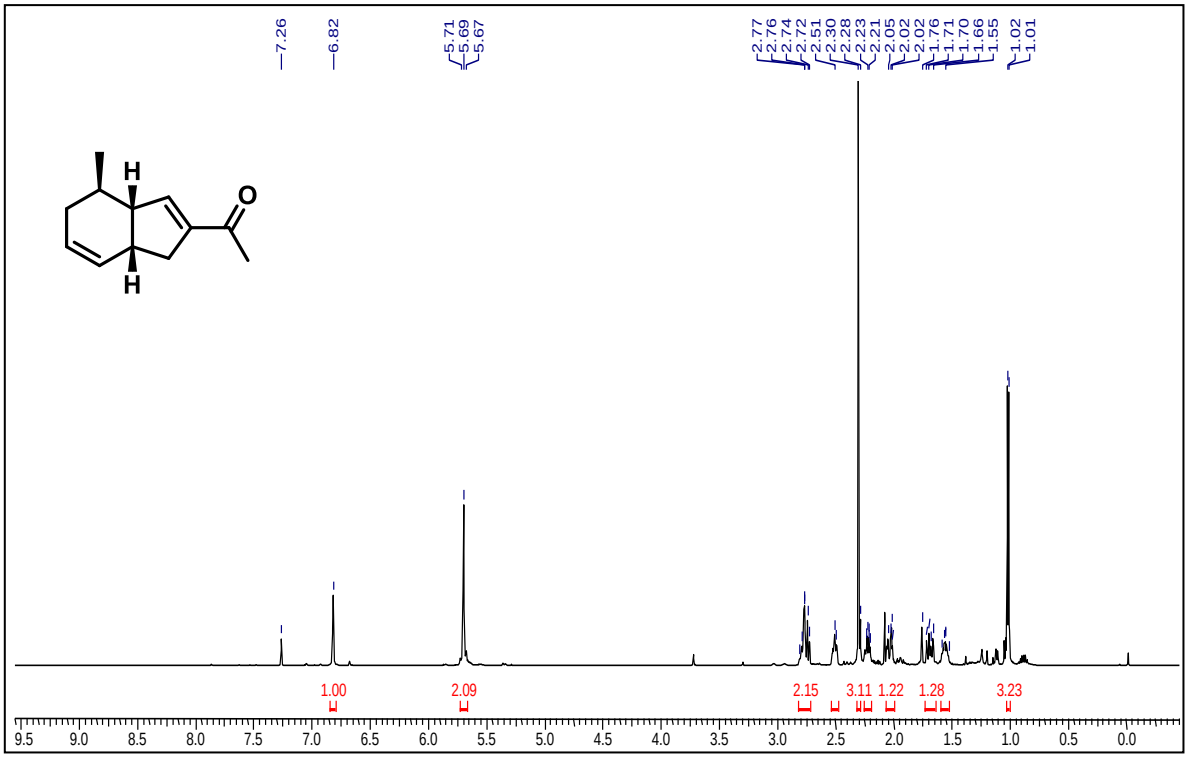
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 23



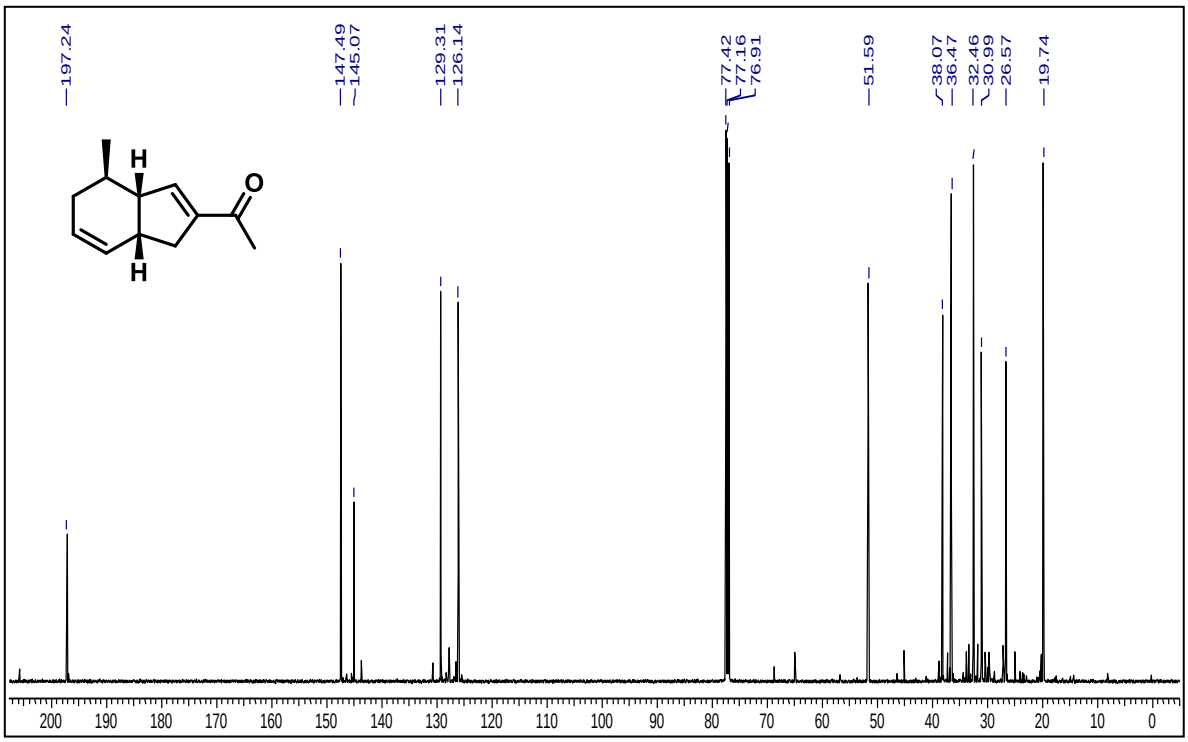
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 23



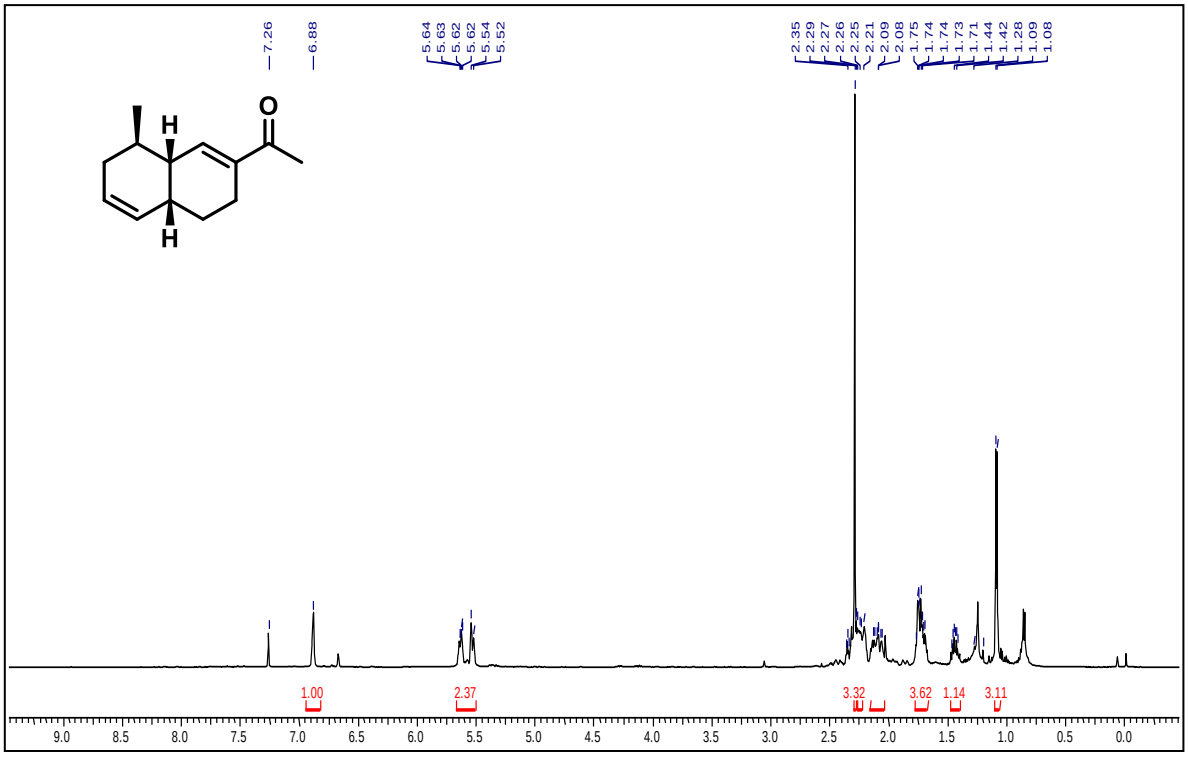
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 24



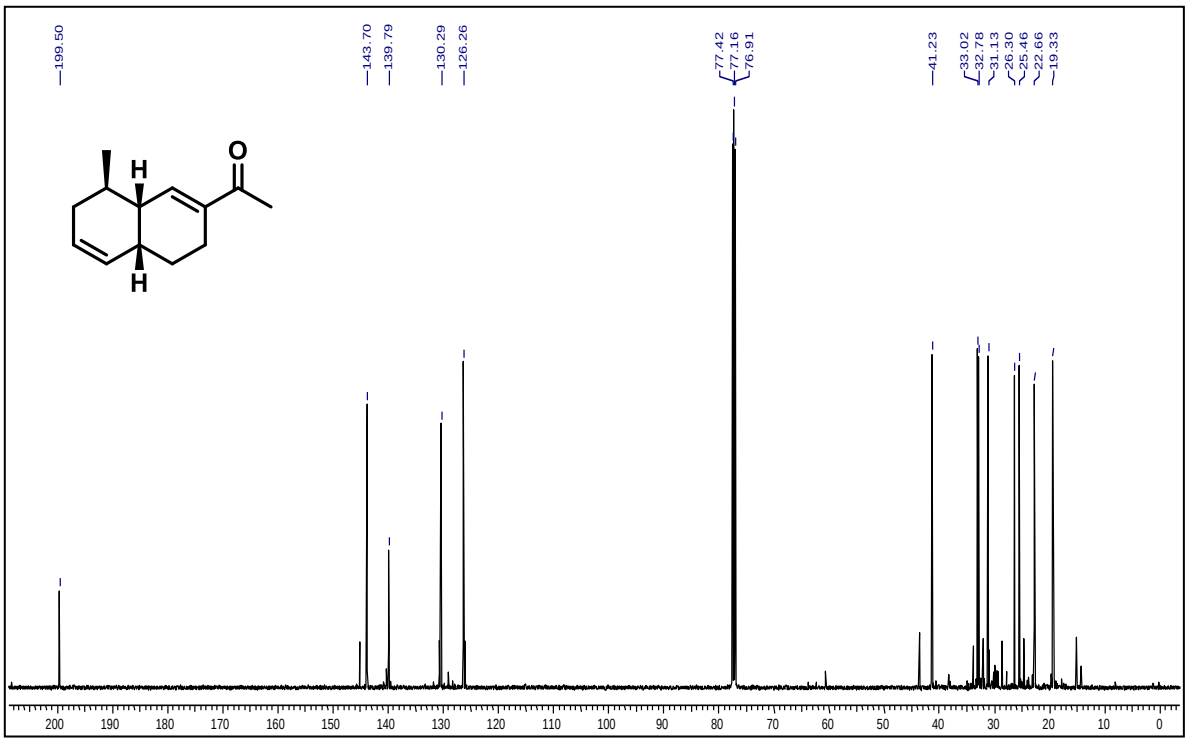
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 24



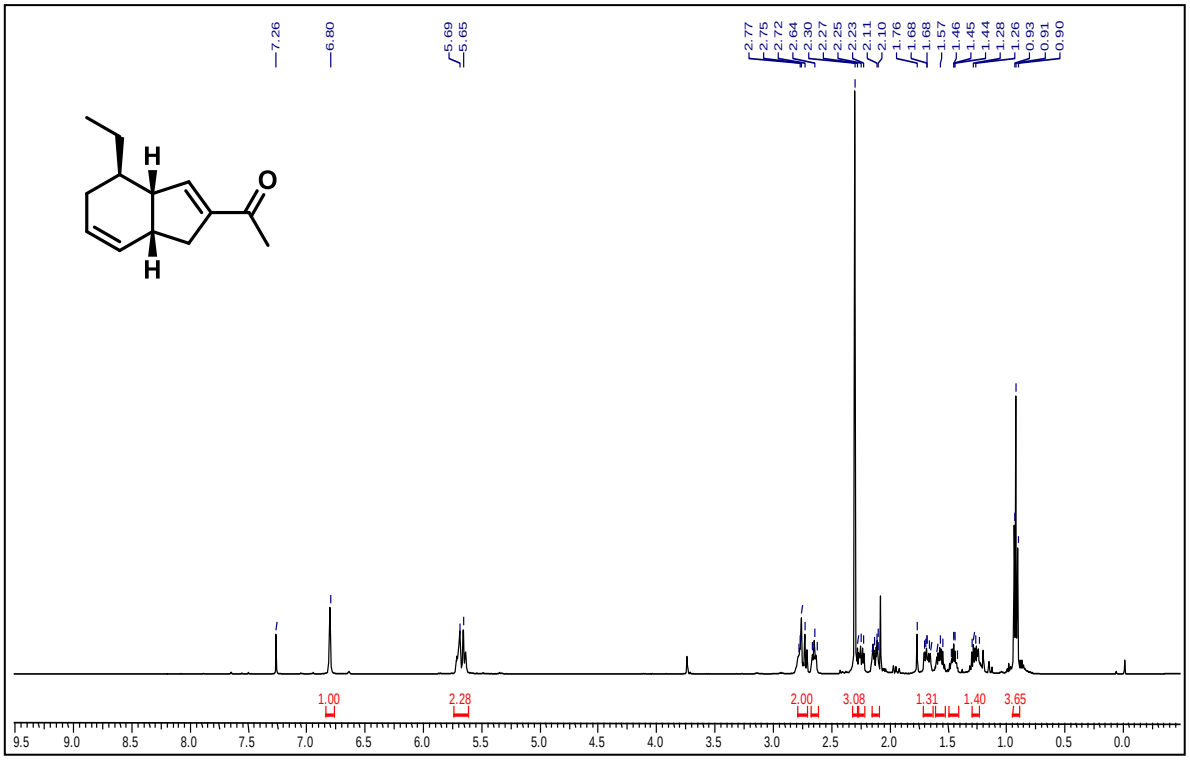
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 25



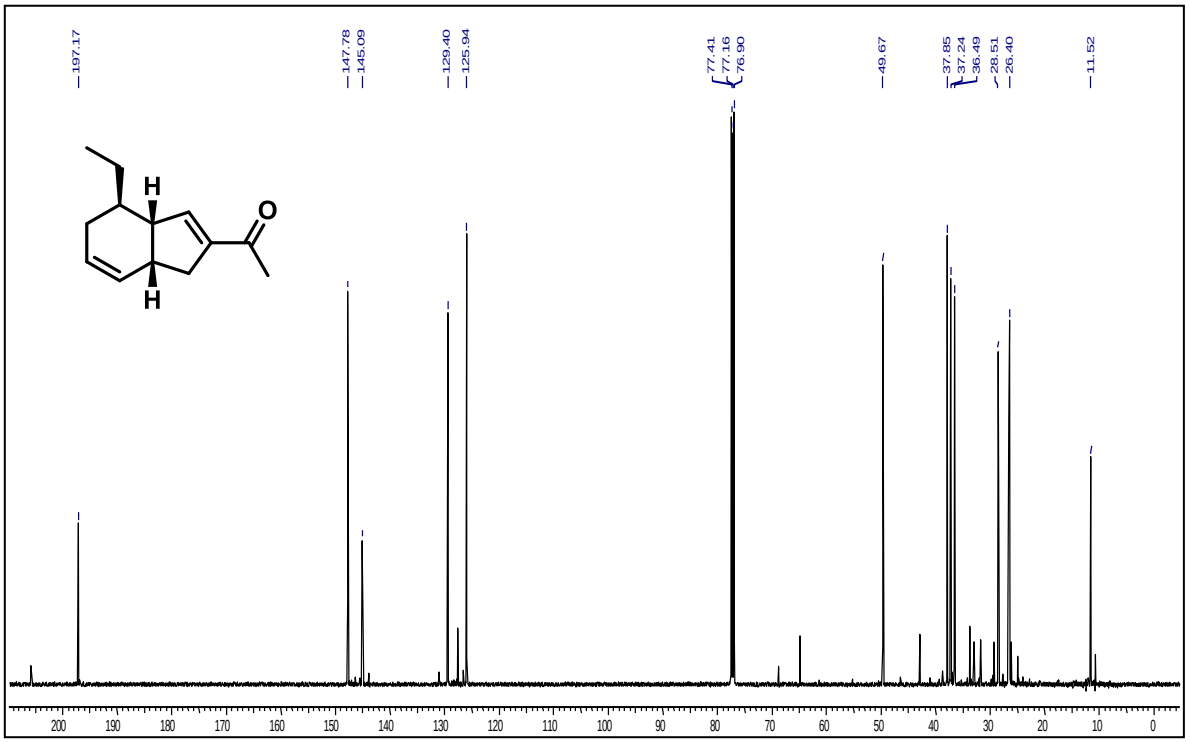
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 25



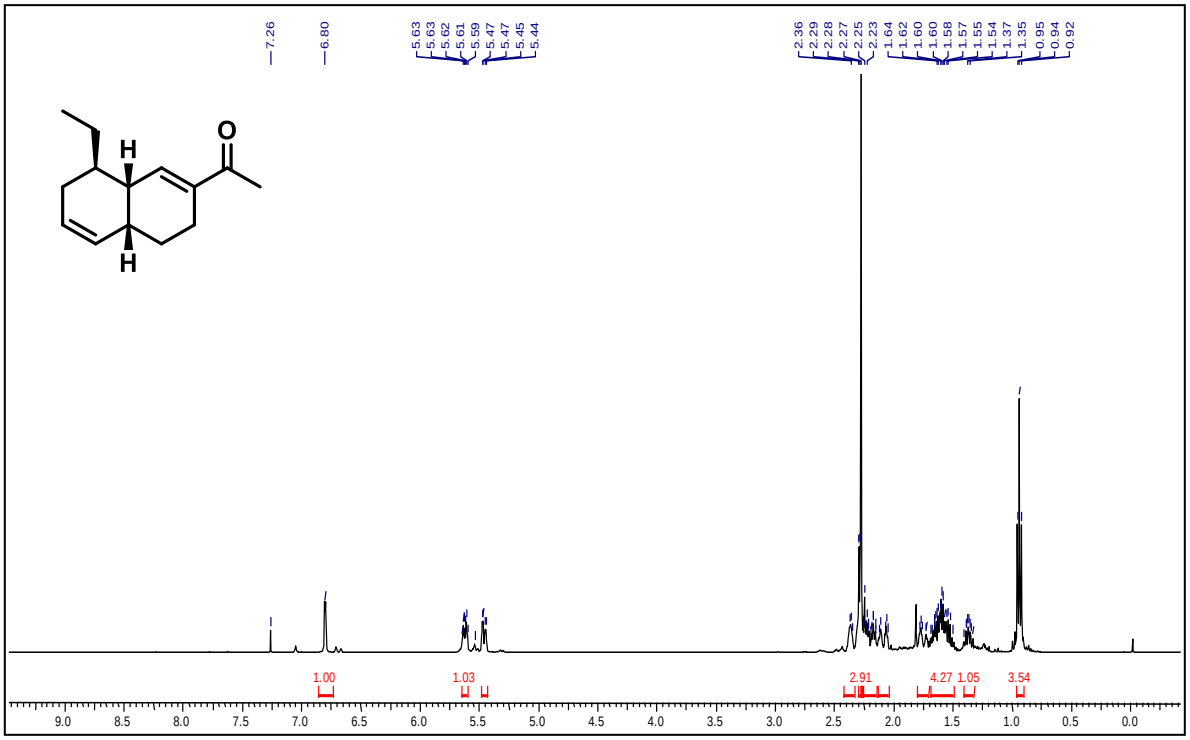
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 26



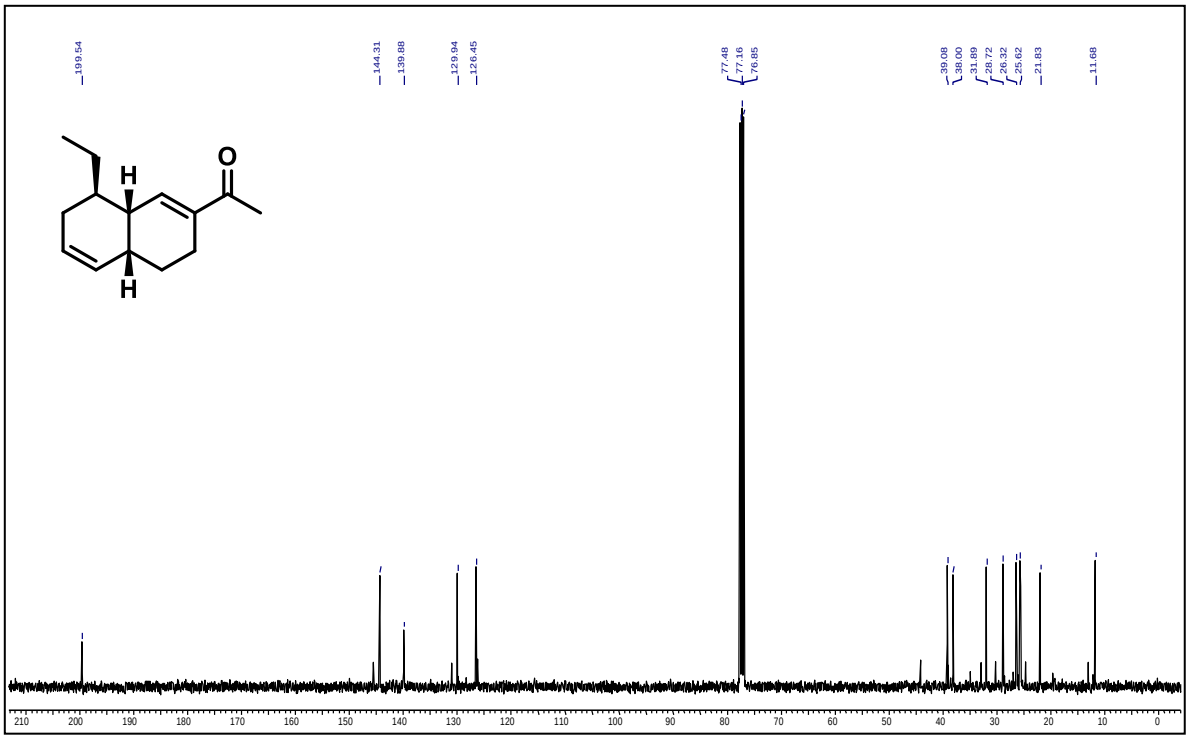
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 26



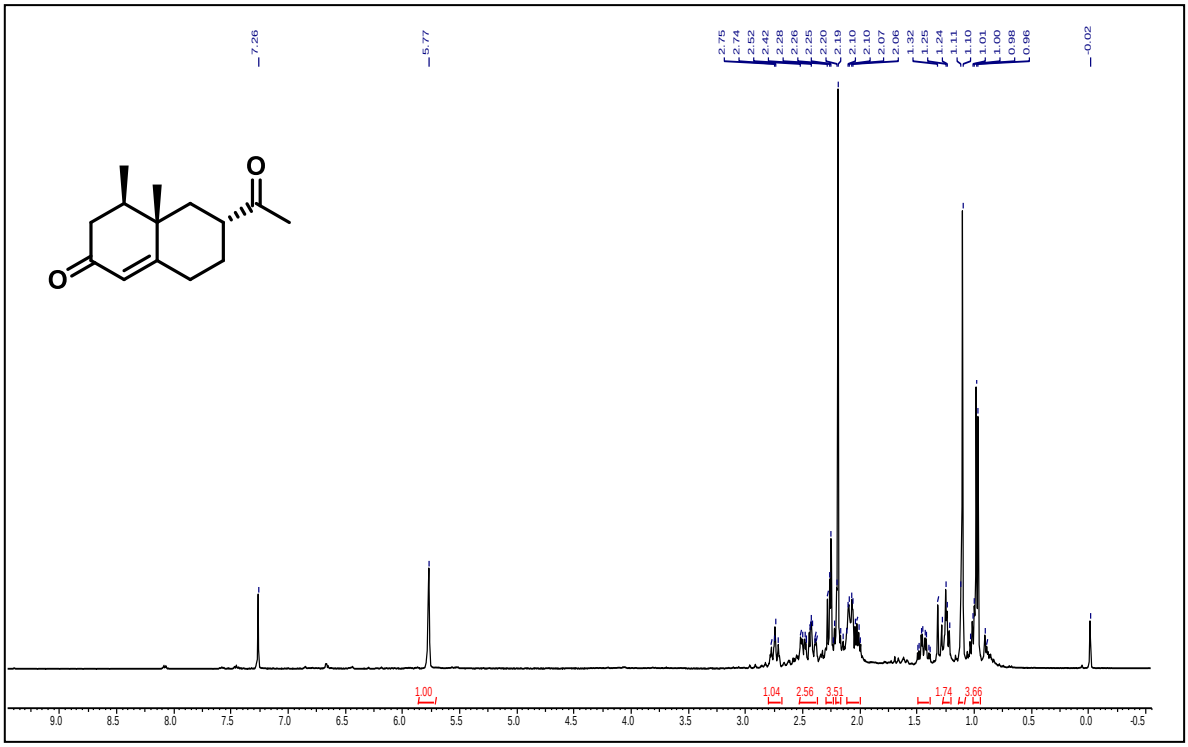
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 27



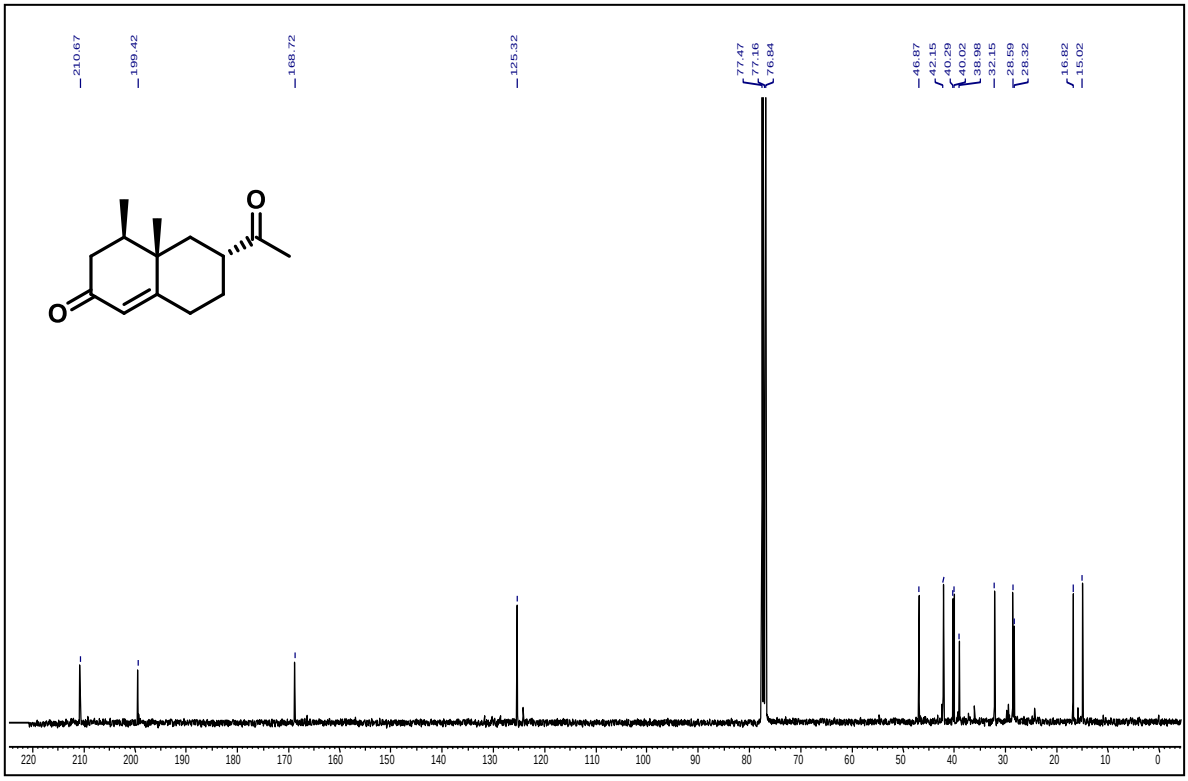
^{13}C NMR ($CDCl_3$, 500 MHz) of compound 27



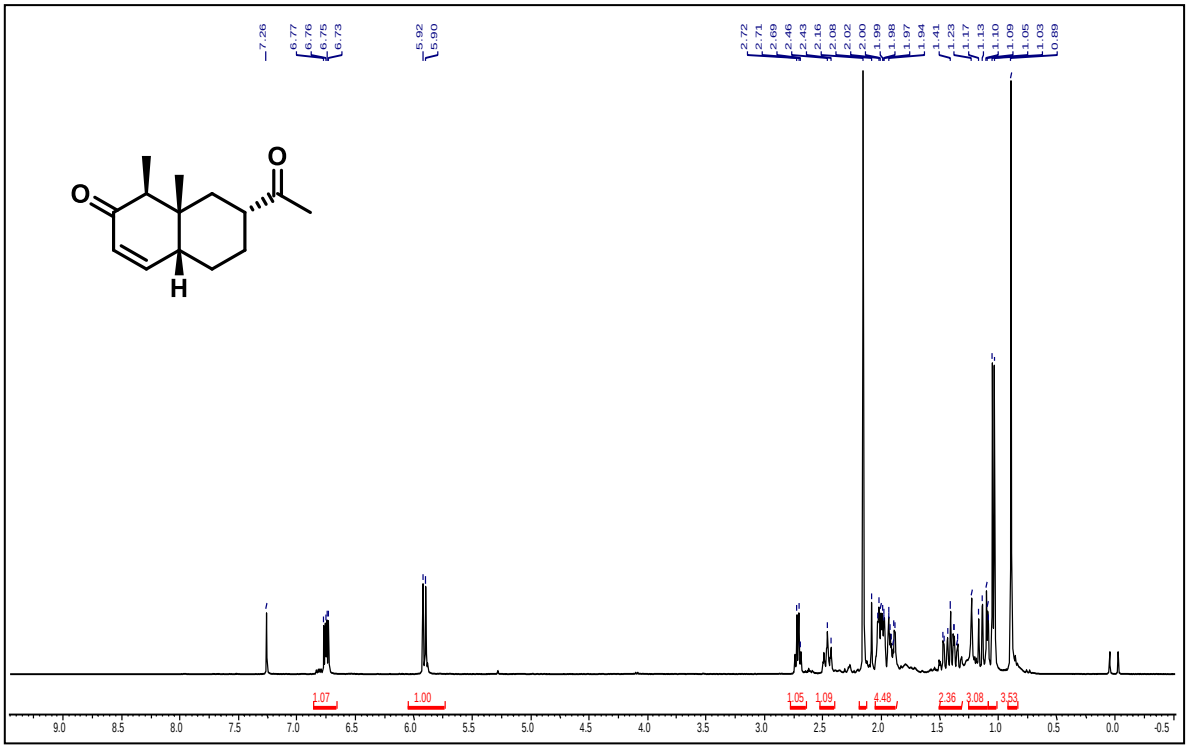
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 38



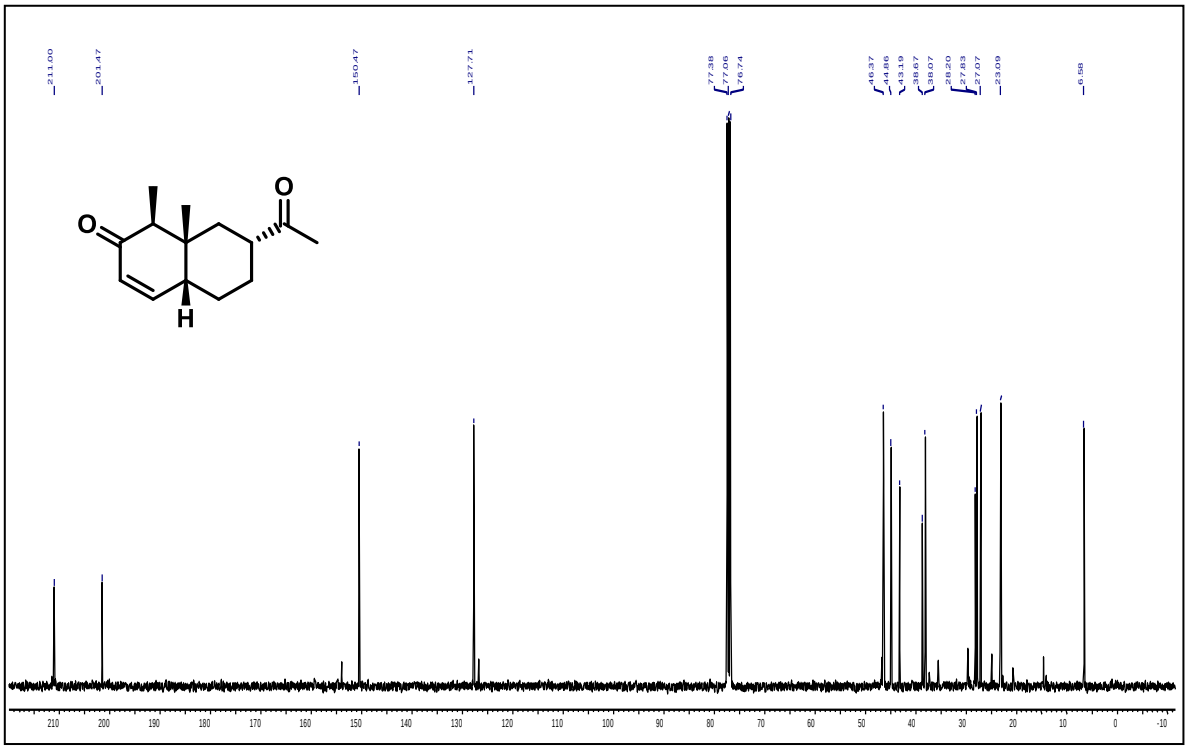
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 38



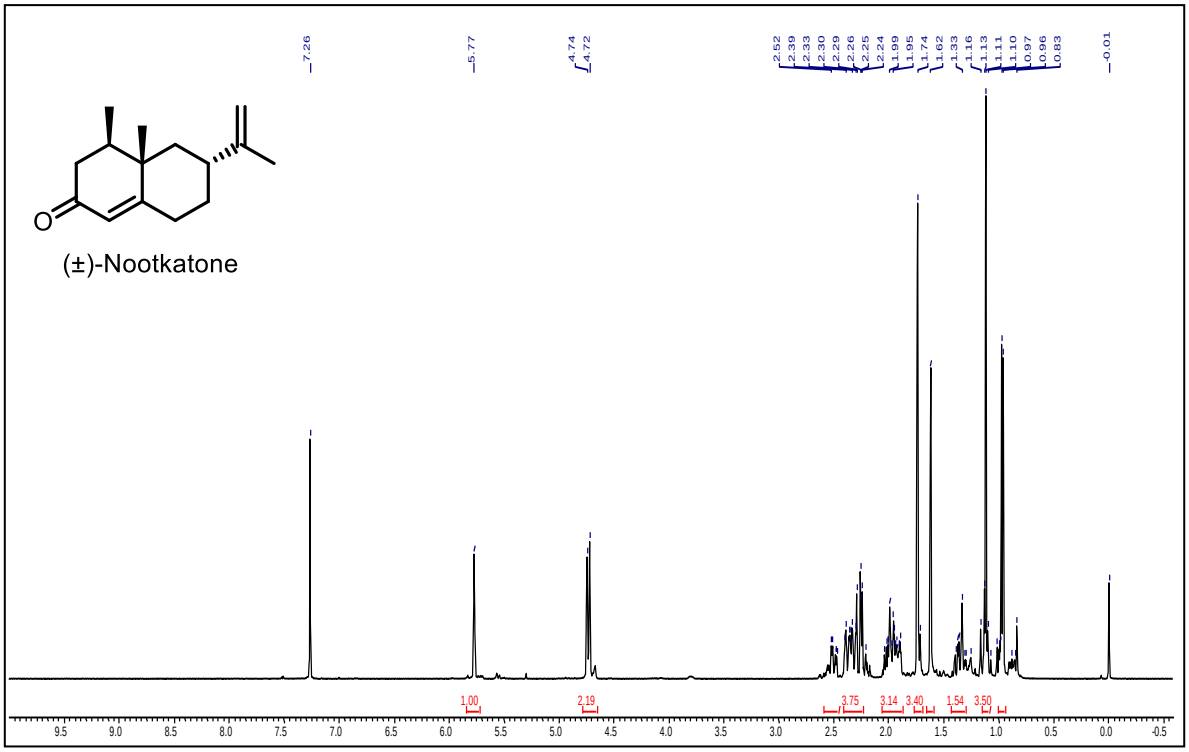
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 39



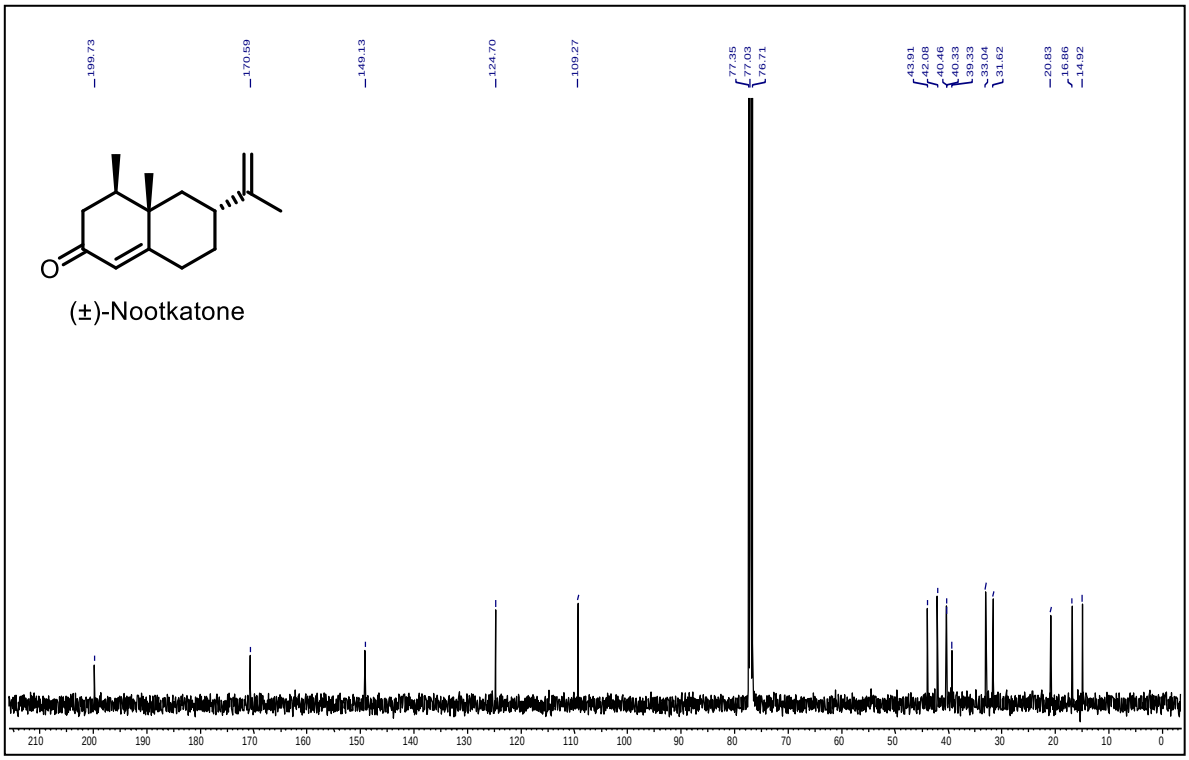
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 39



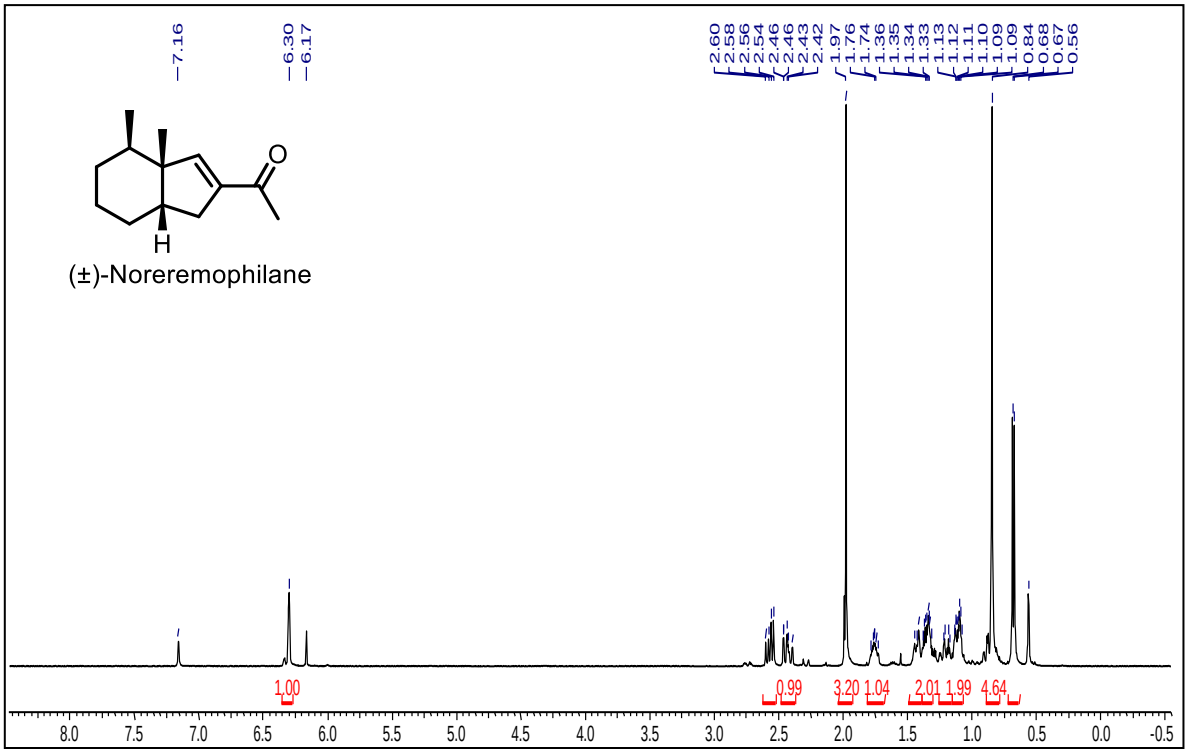
^{1}H NMR ($CDCl_3$, 400 MHz) of (±)-Nootkatone 34



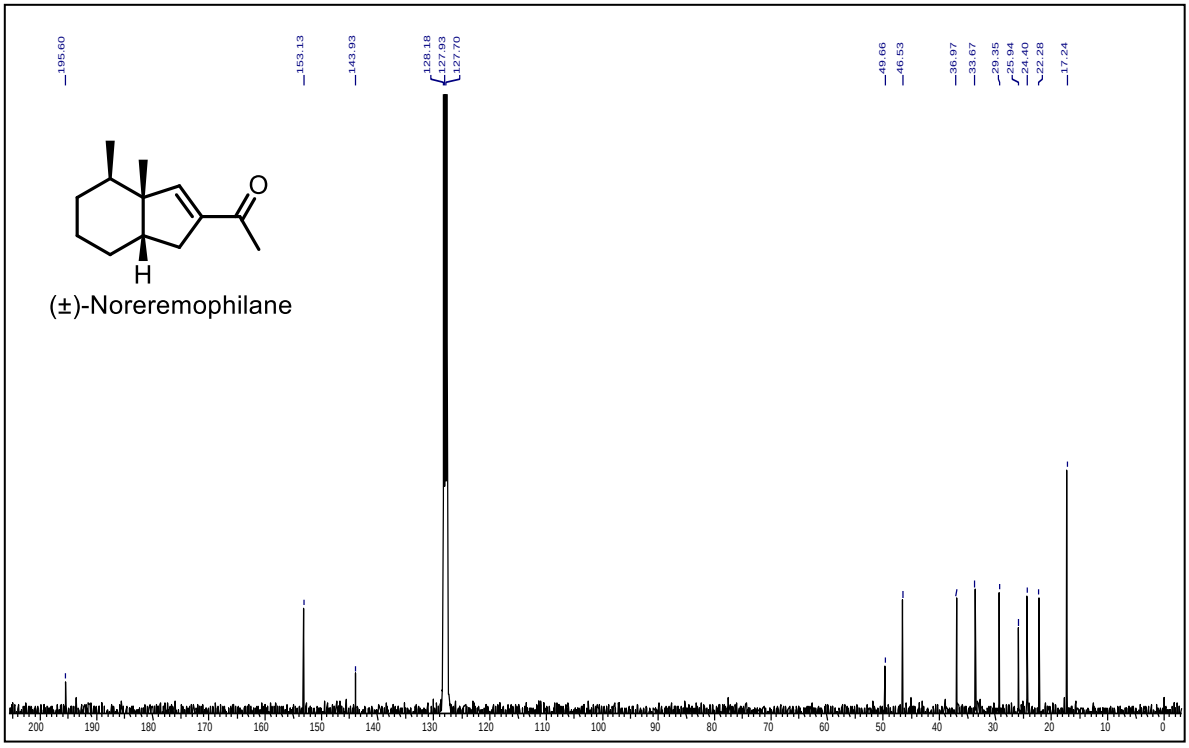
^{13}C NMR ($CDCl_3$, 100 MHz) of (±)-Nootkatone 34



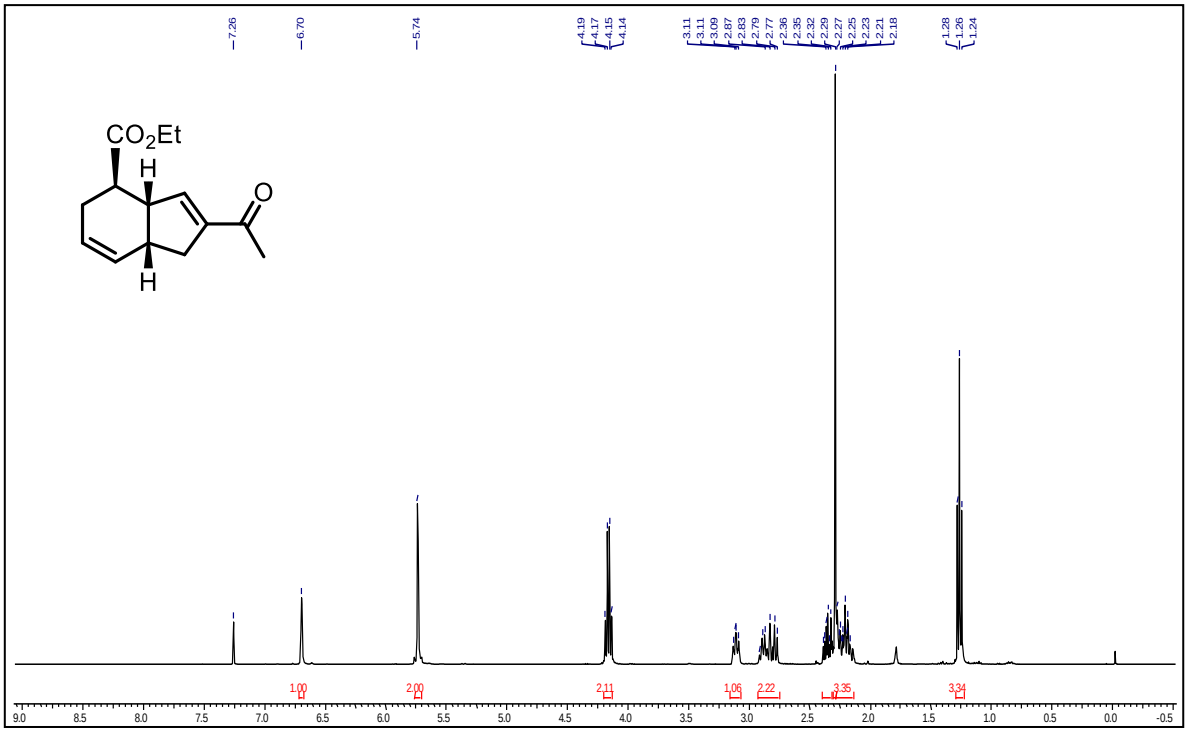
^{1}H NMR (C_6D_6, 400 MHz) of (±)-Noreremophilane 40



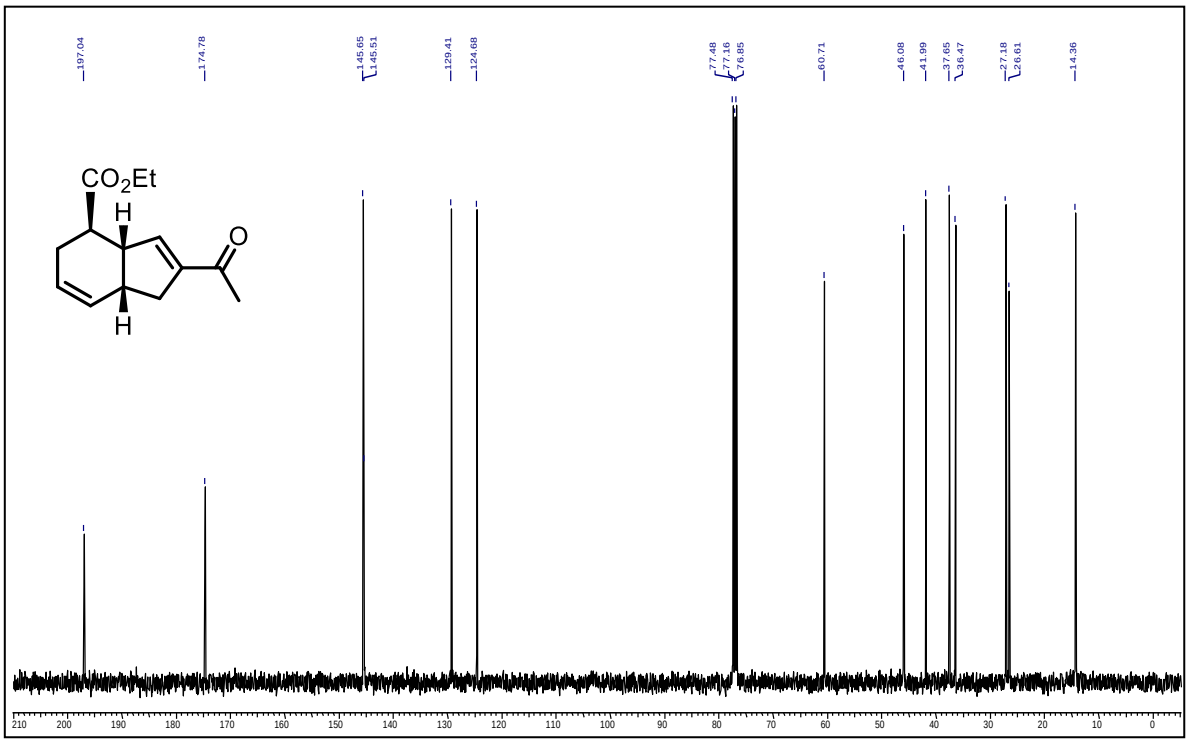
^{13}C NMR (C_6D_6, 100 MHz) of (±)-Noreremophilane 40



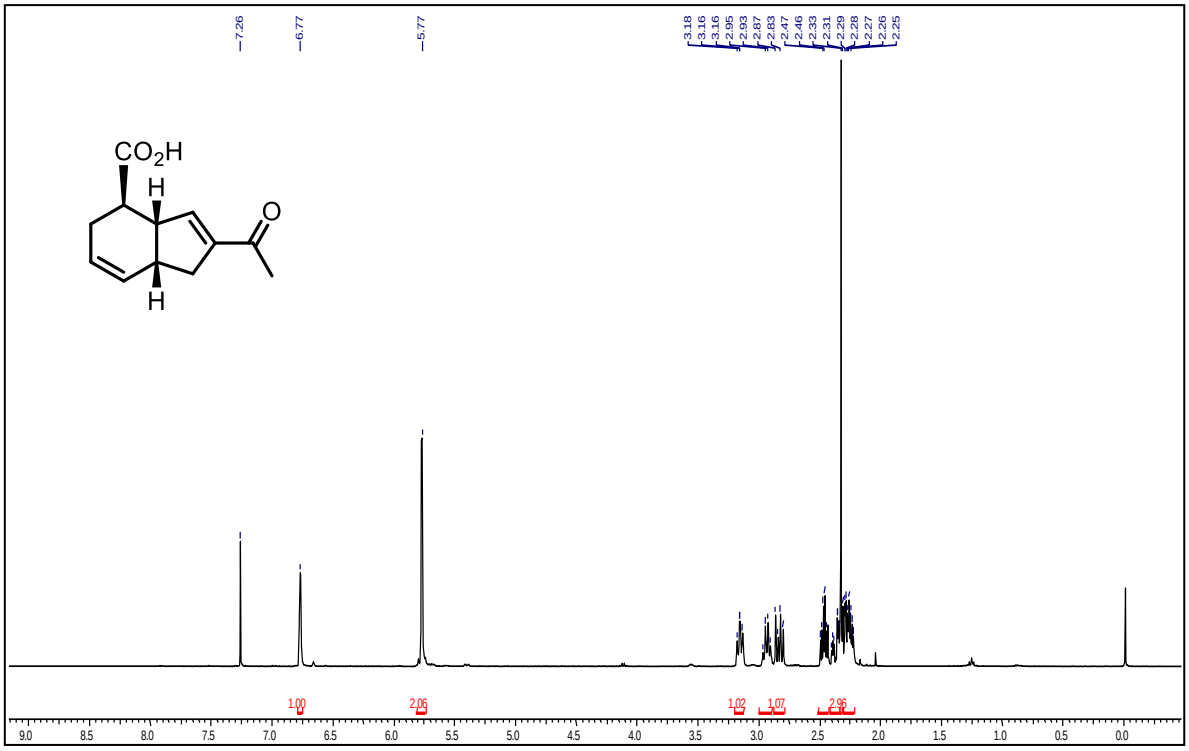
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 45



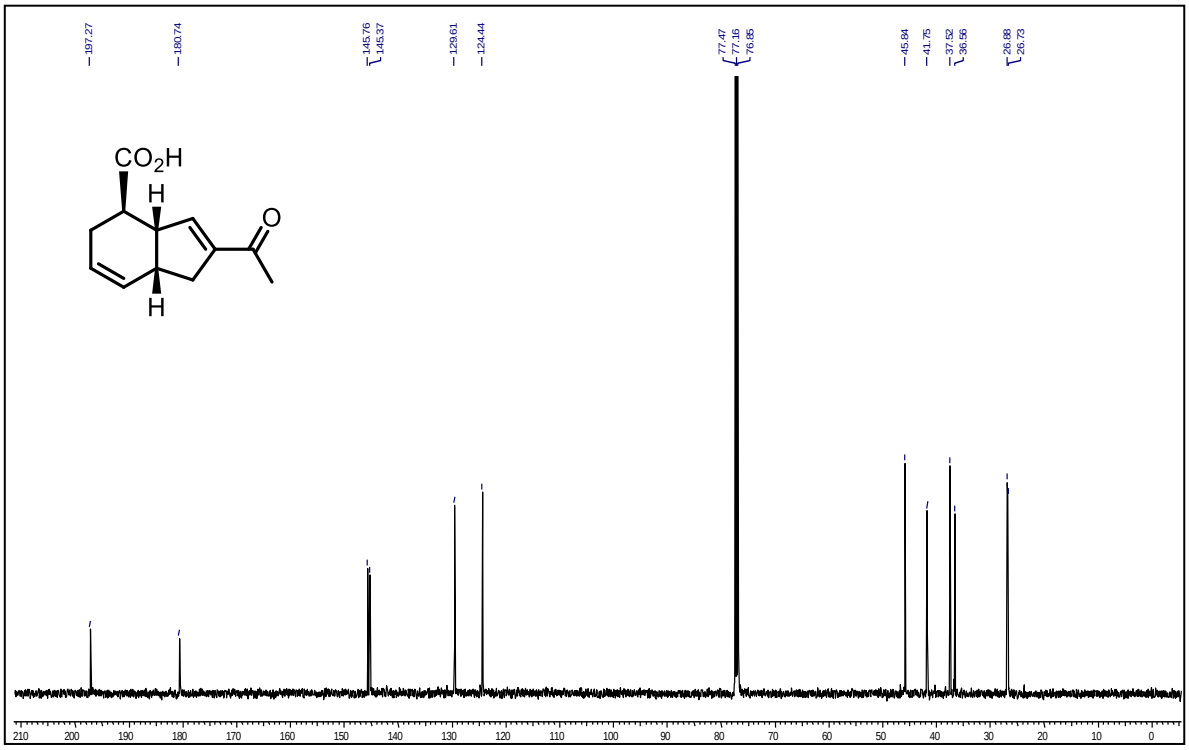
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 45



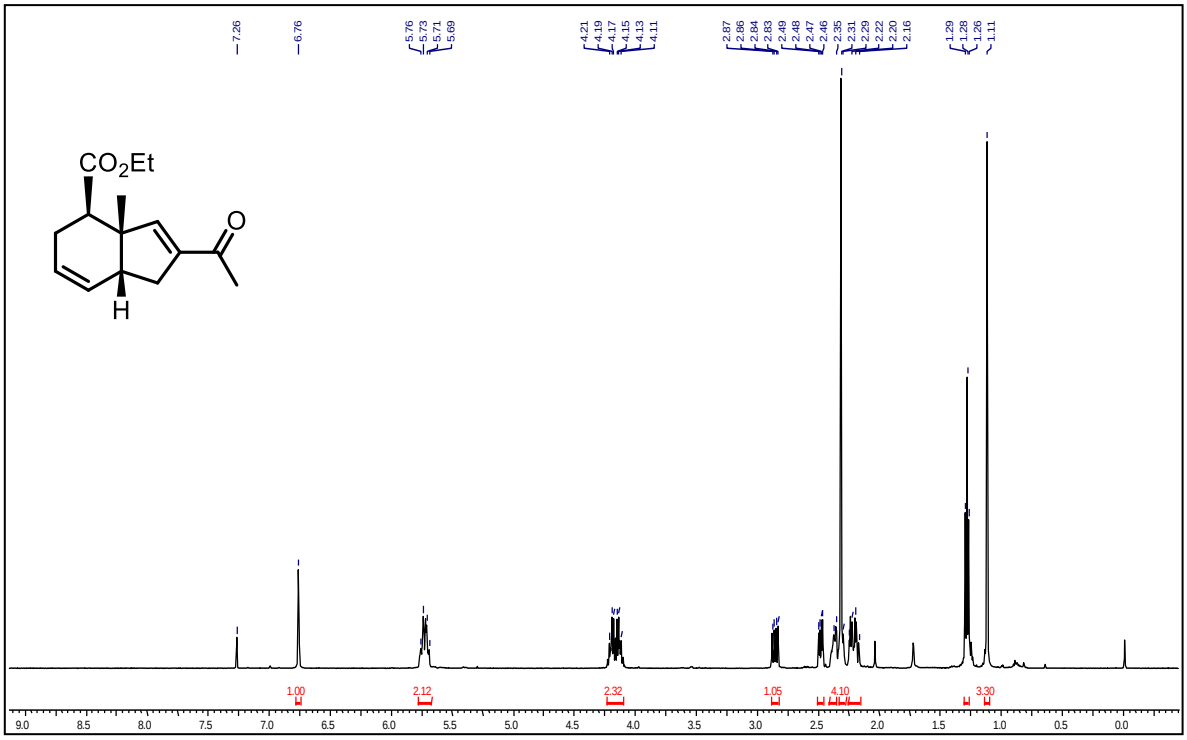
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 46



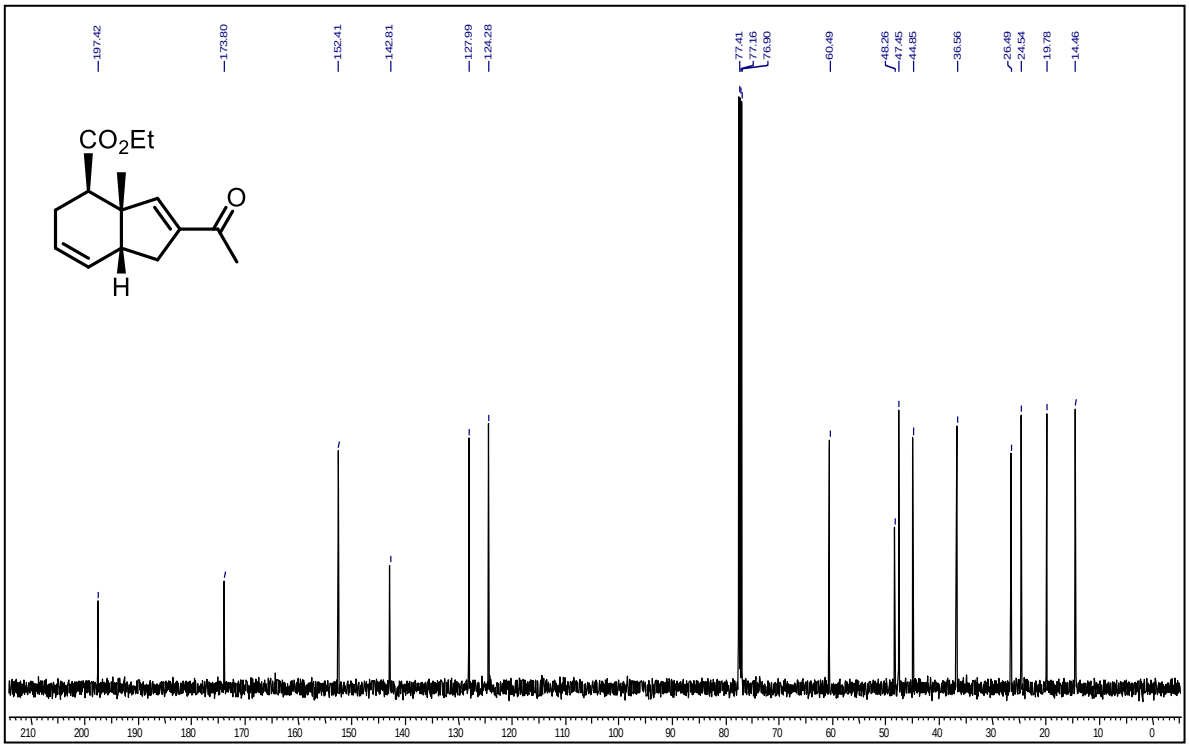
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 46



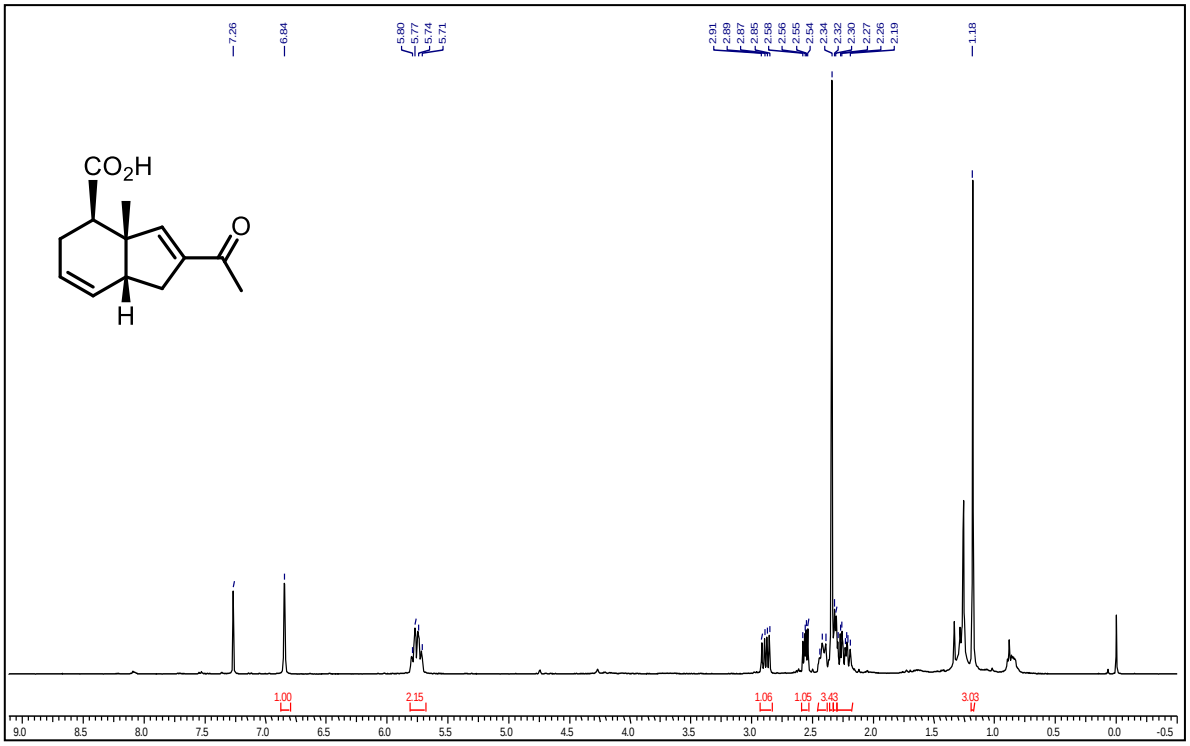
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 49



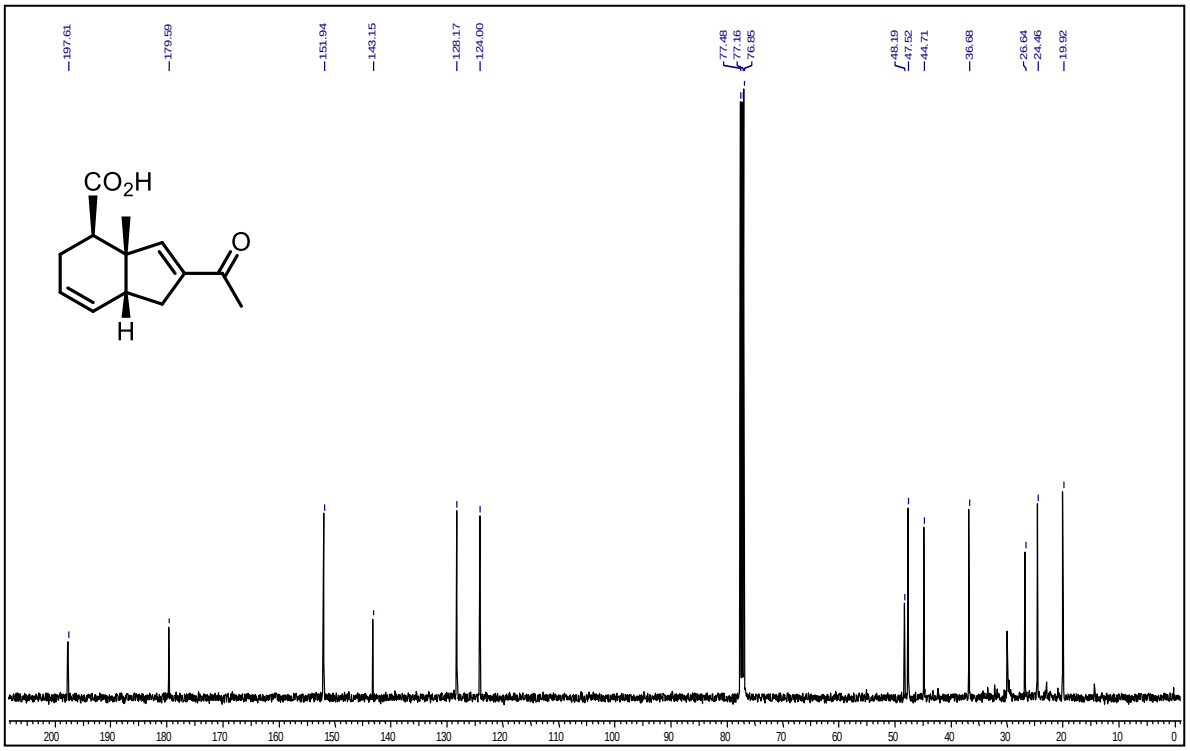
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 49



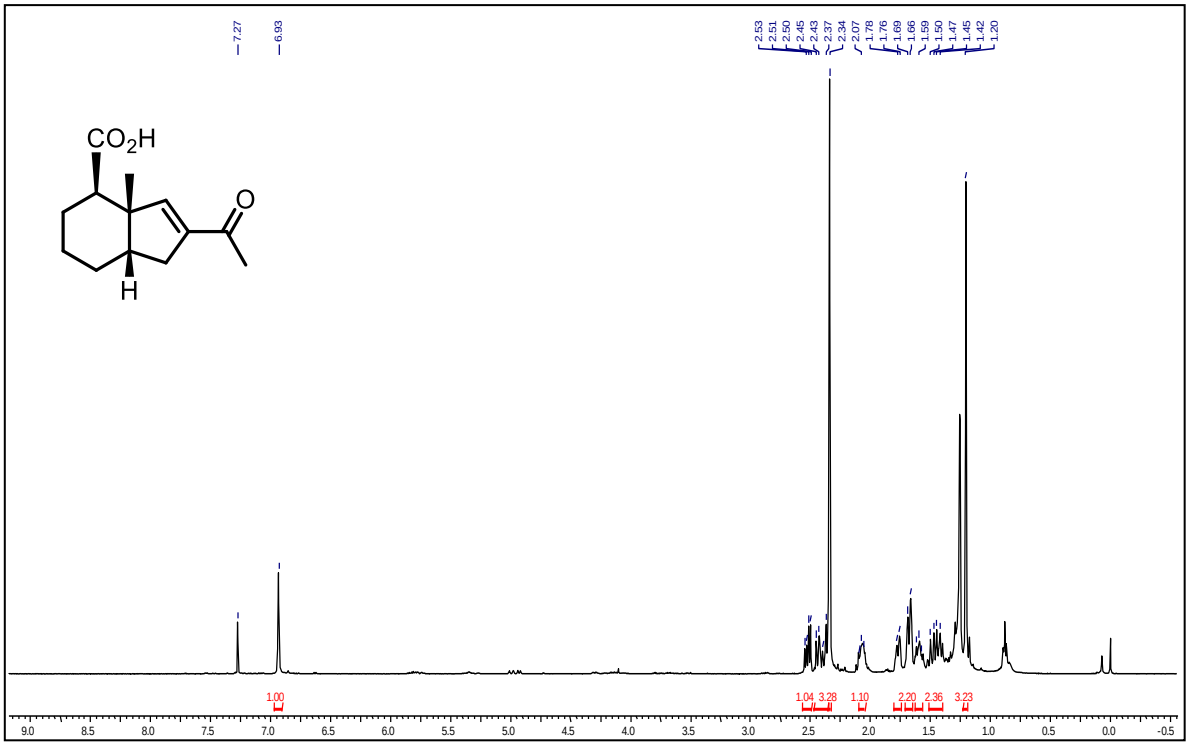
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 50



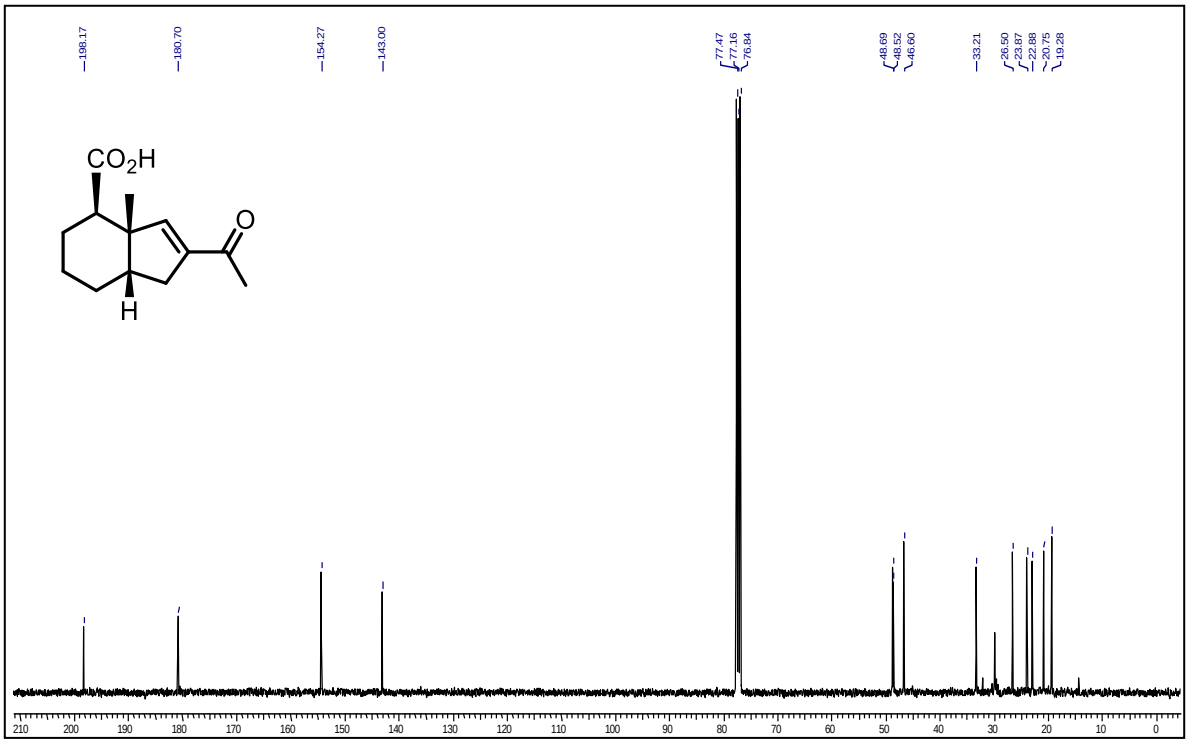
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 50



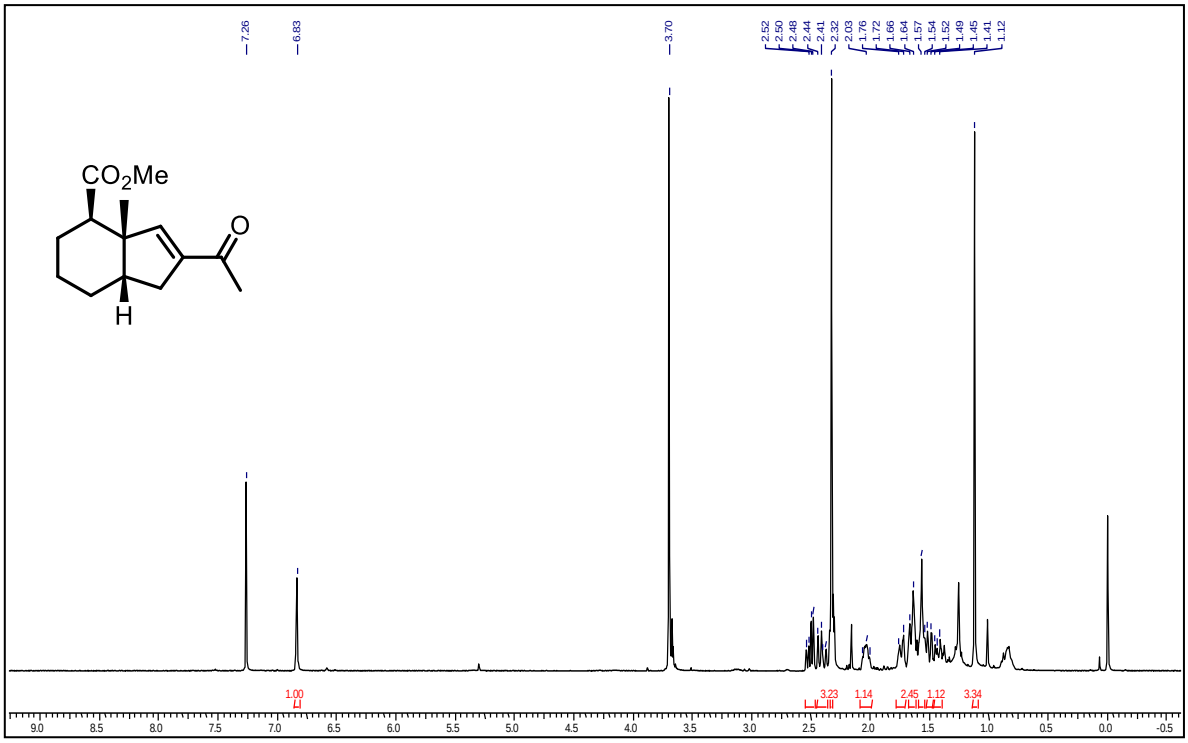
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 41



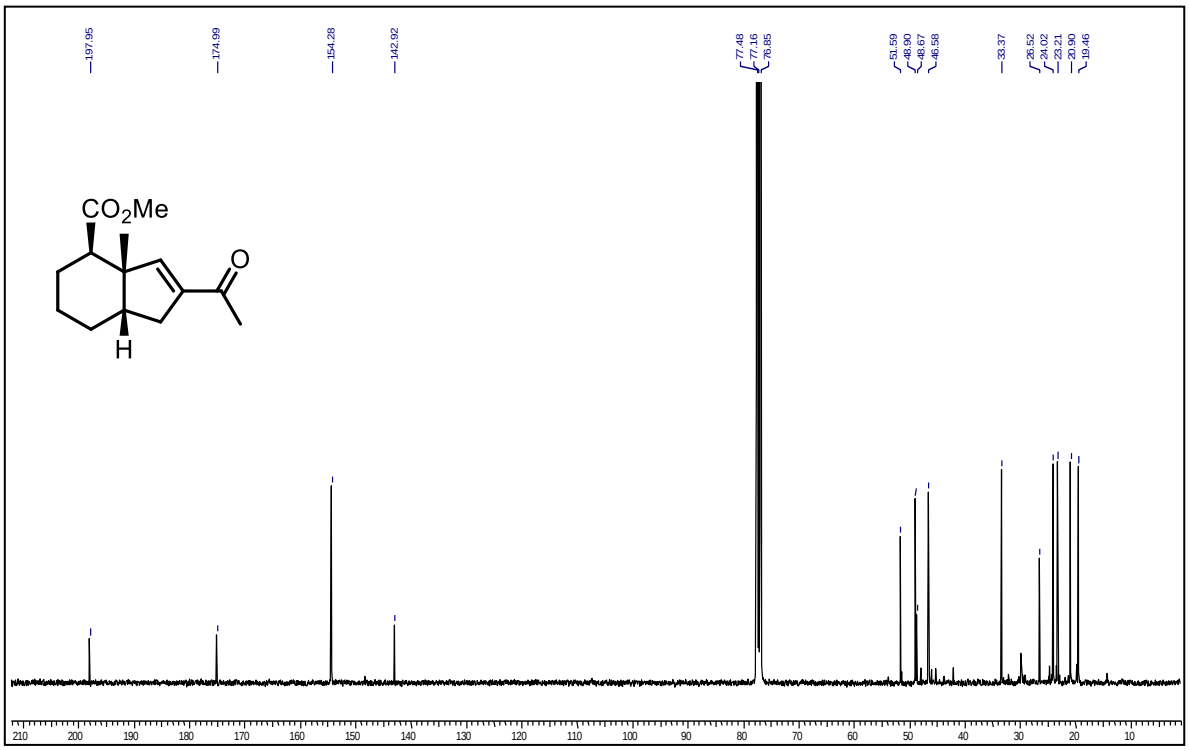
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 41



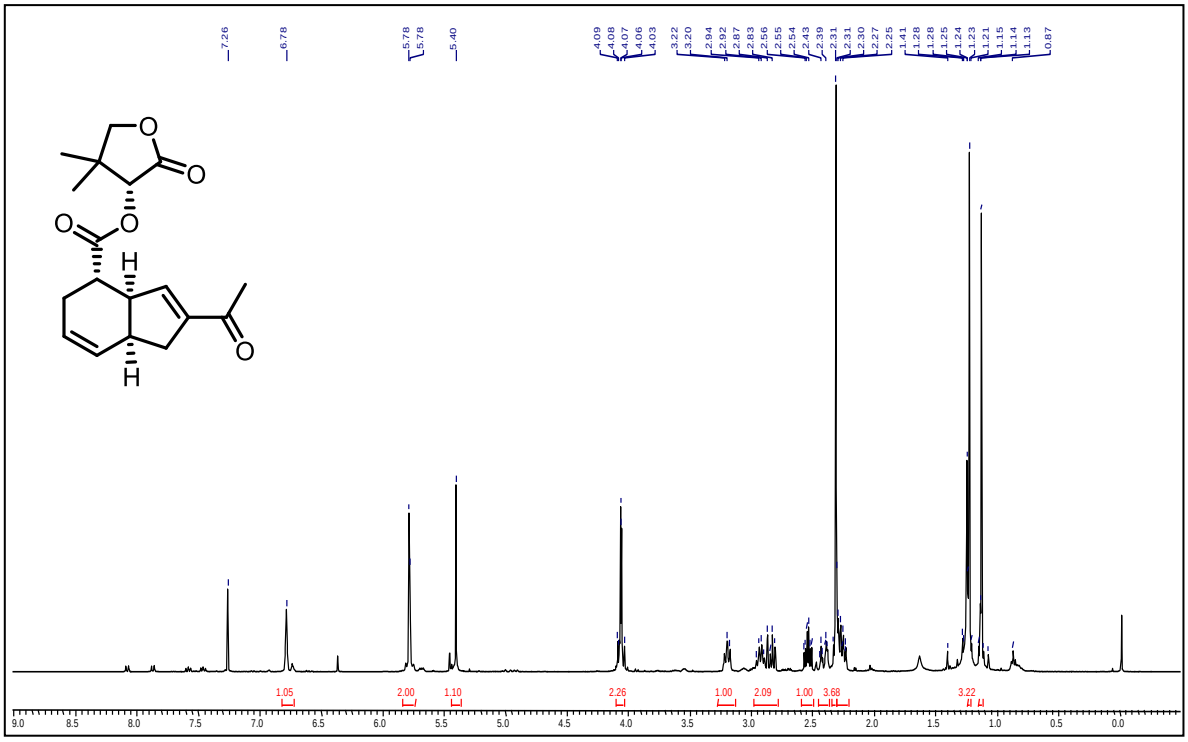
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 42



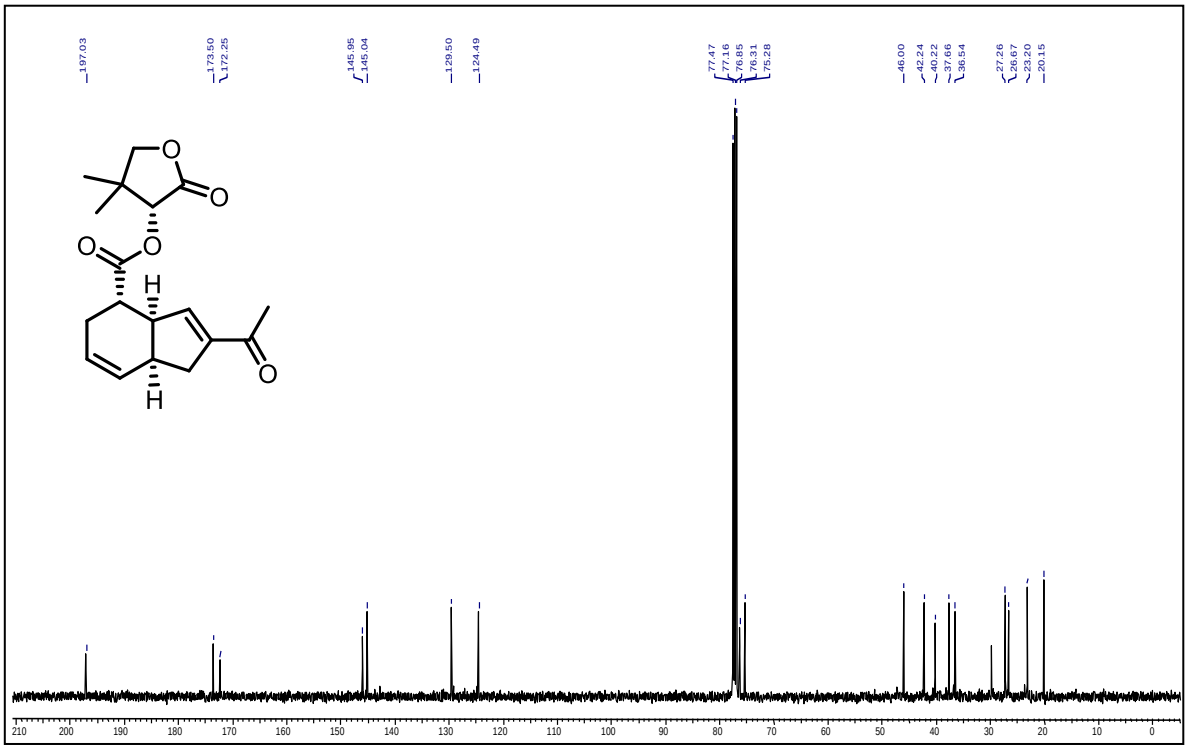
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 42



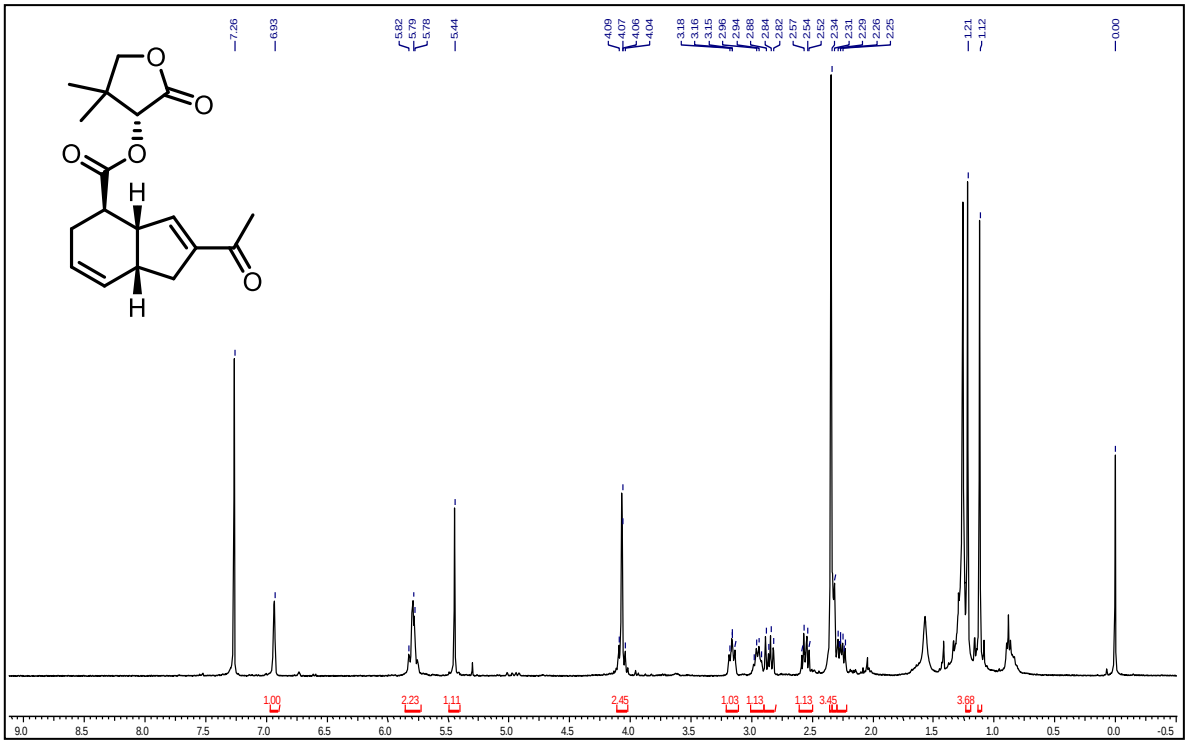
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 55a



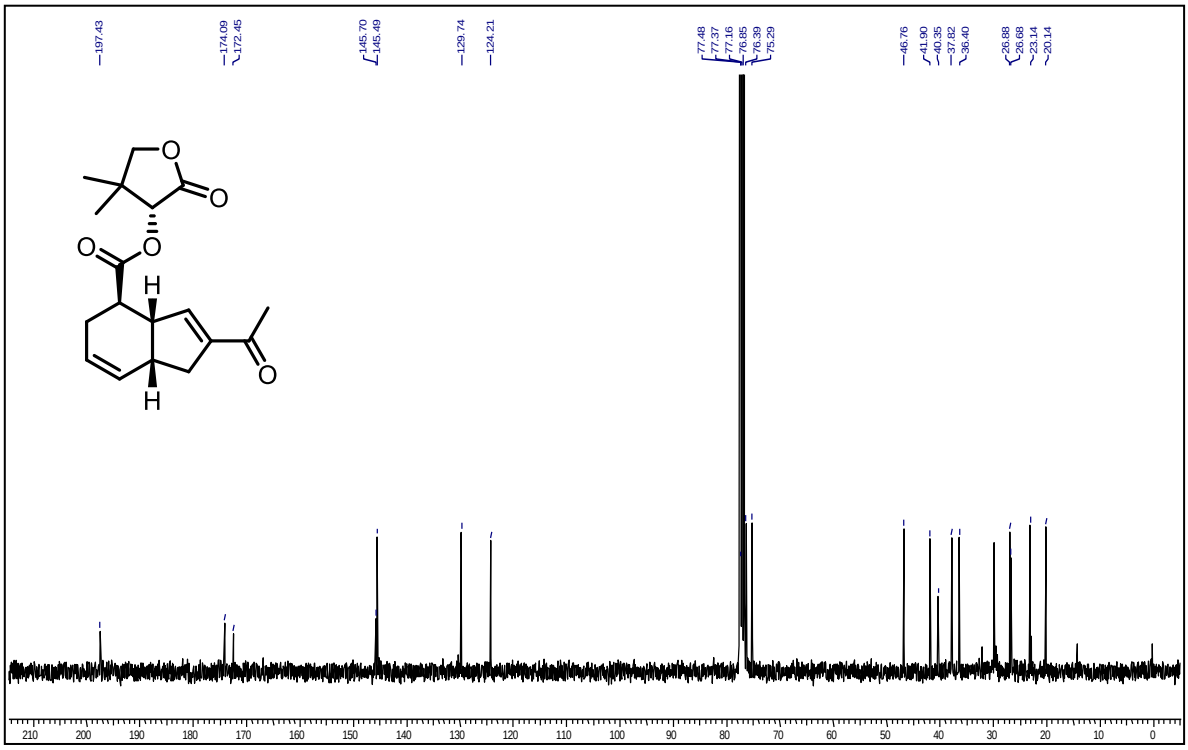
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 55a



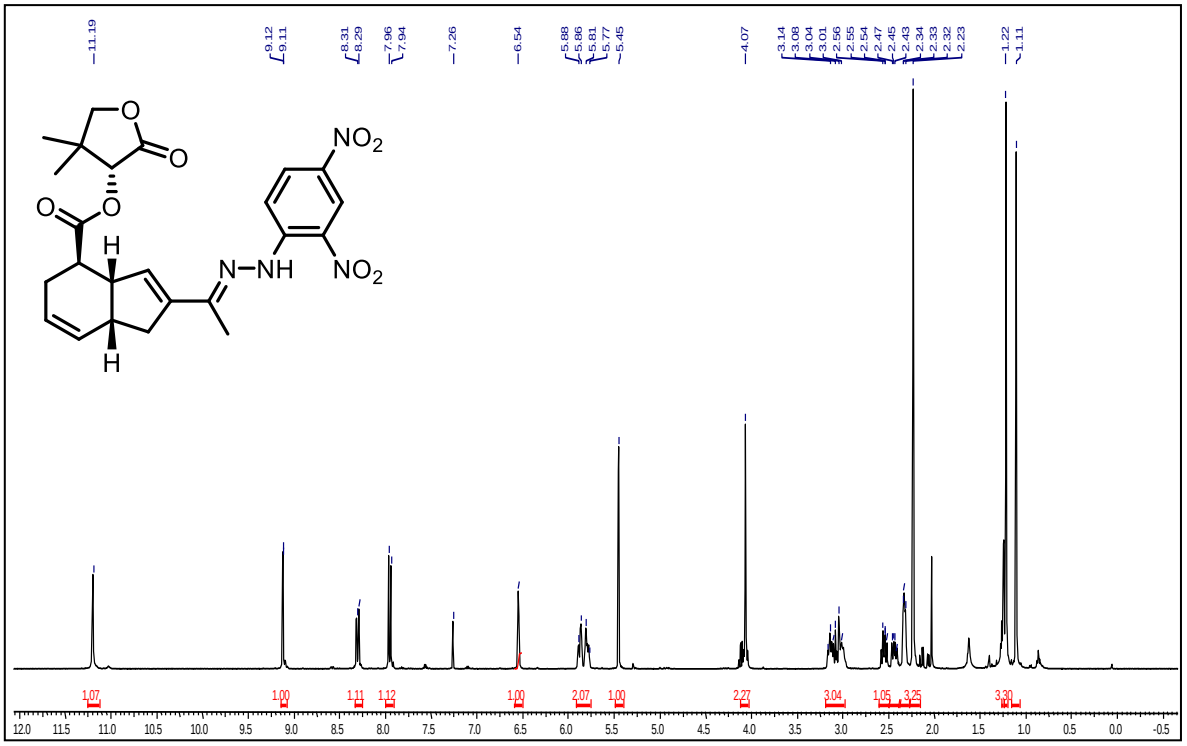
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 55b



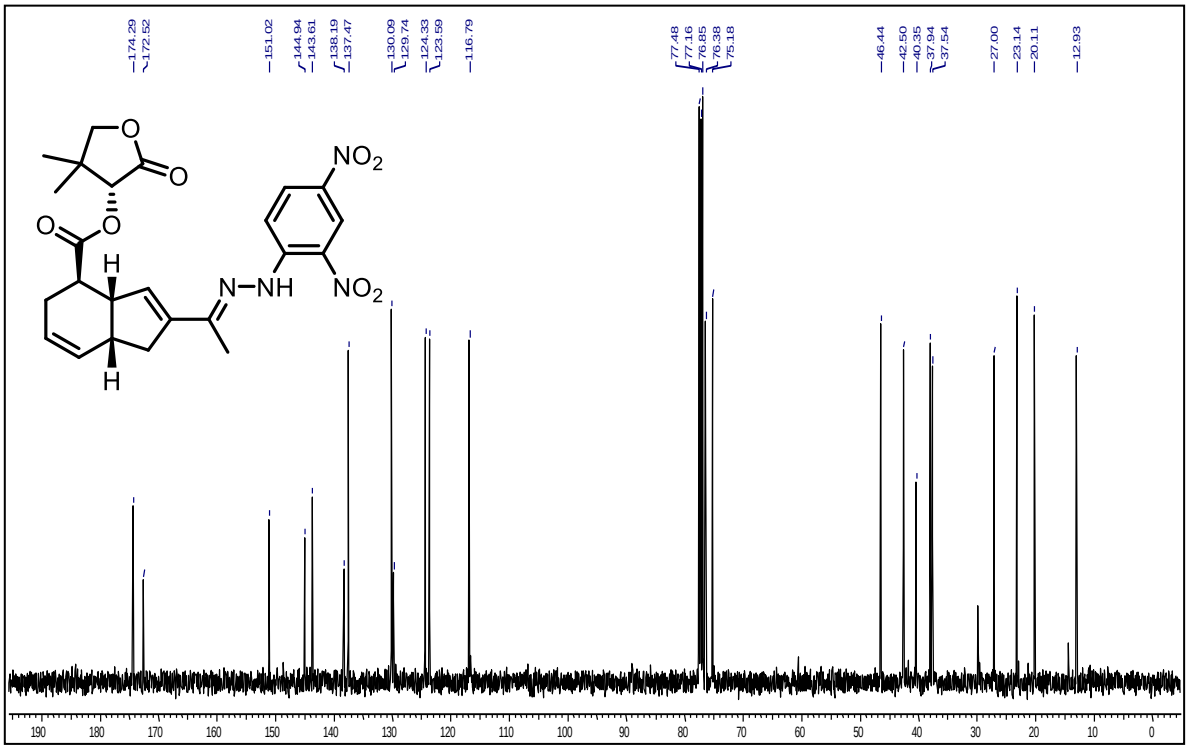
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 55b



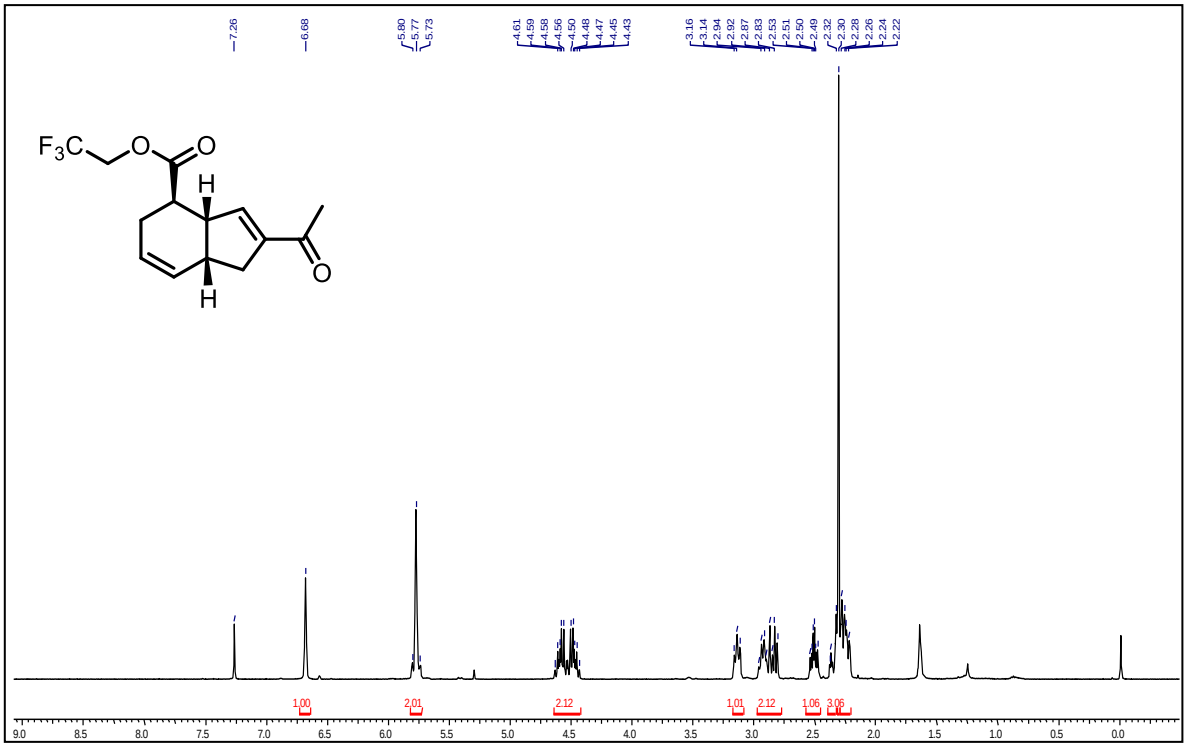
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 57



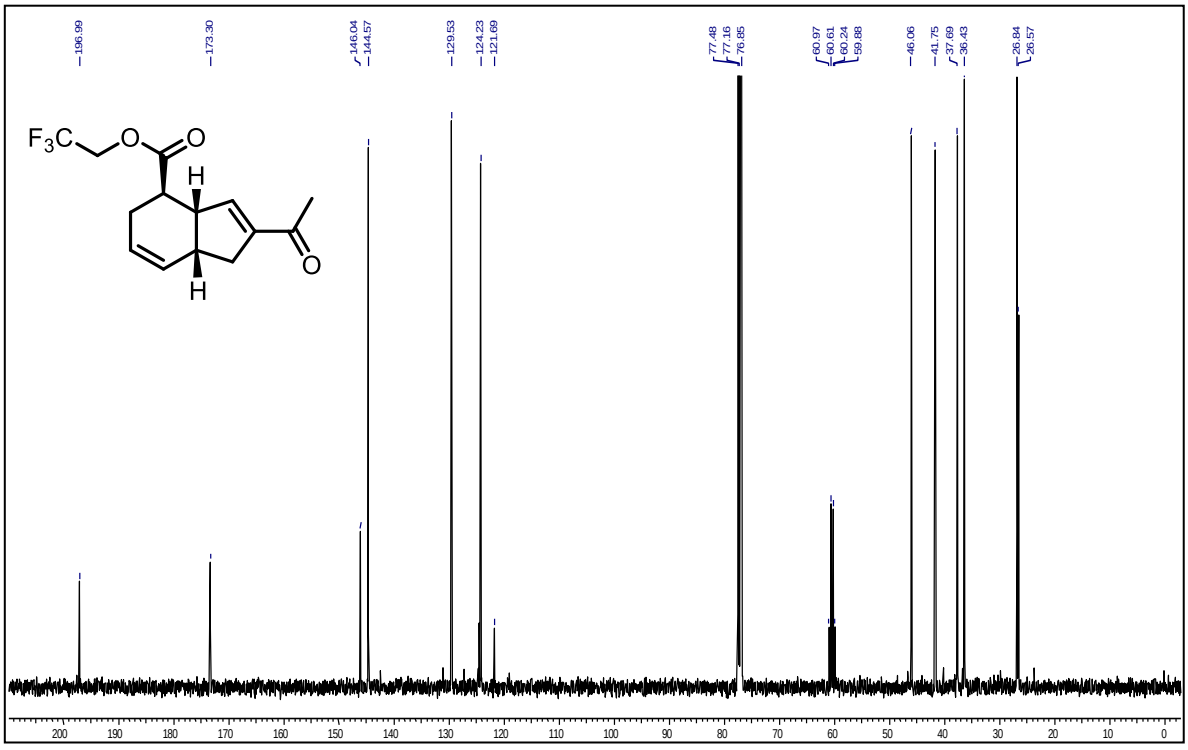
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 57



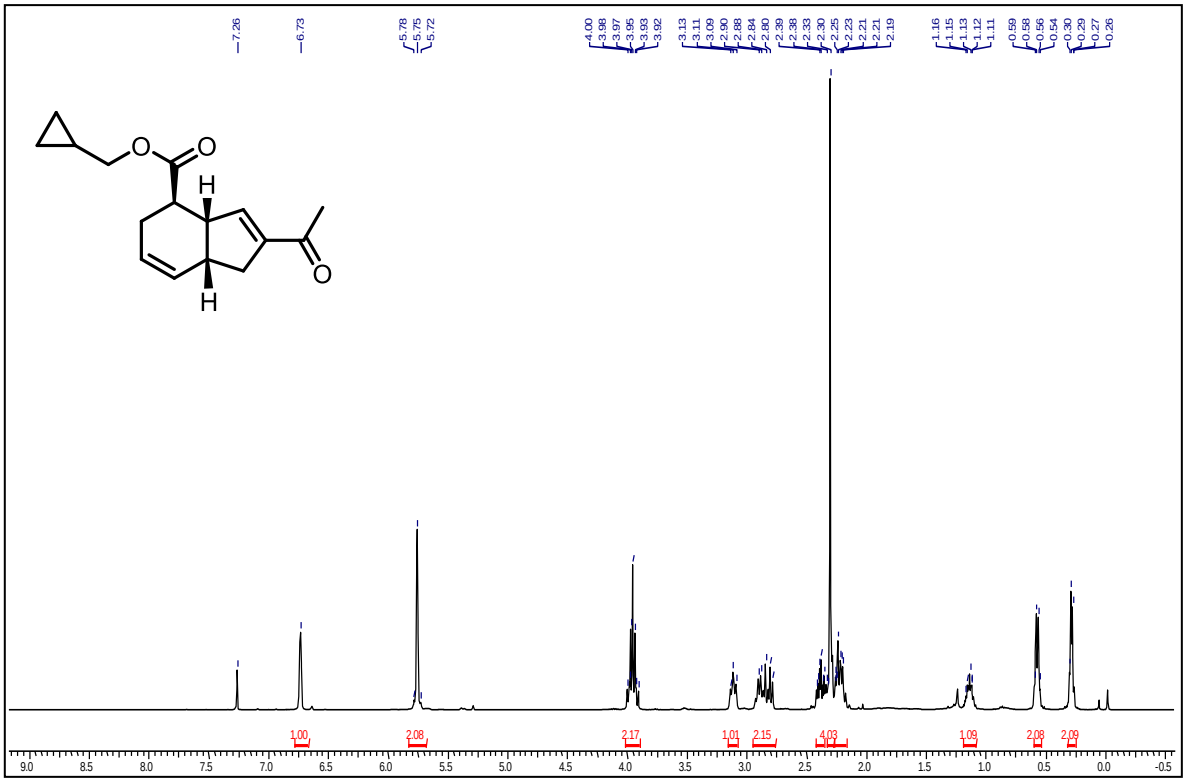
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 61



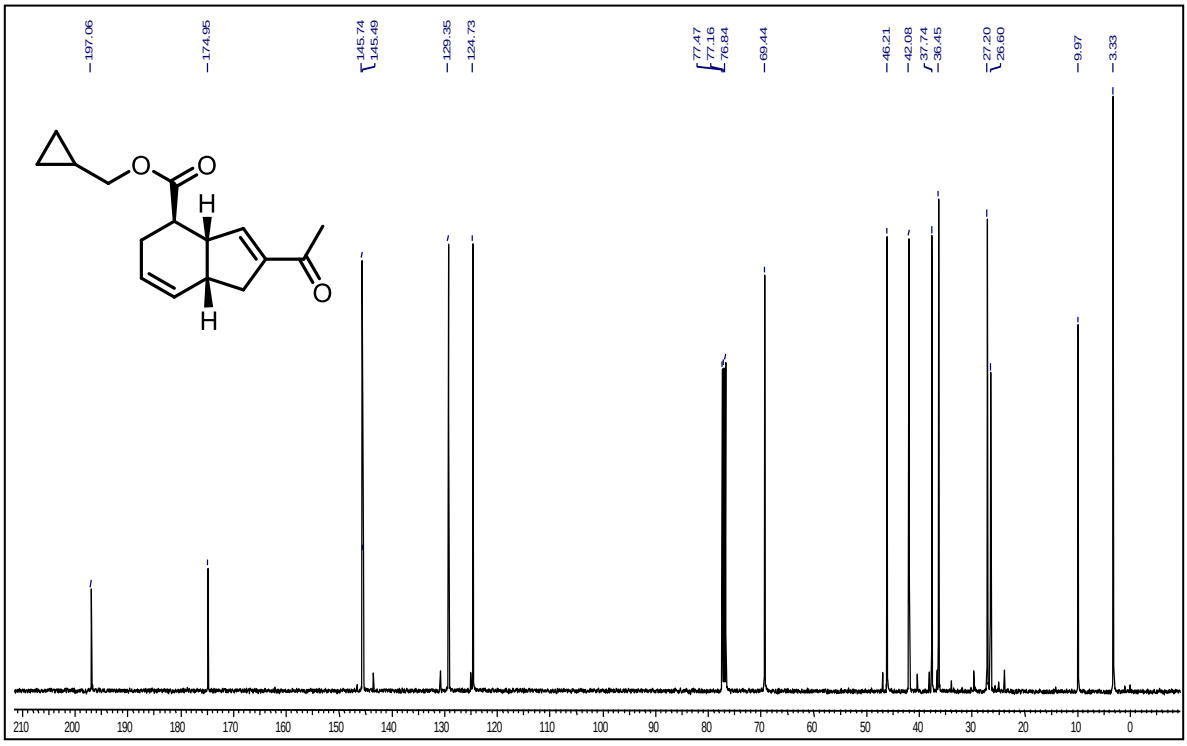
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 61



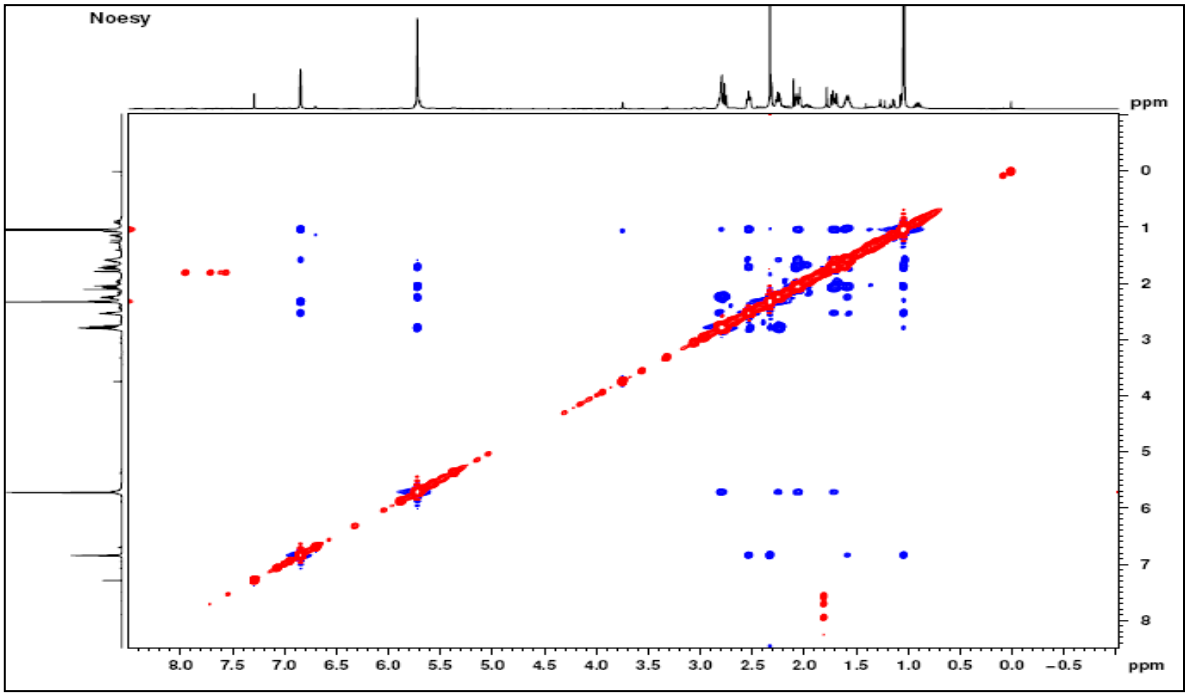
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 62



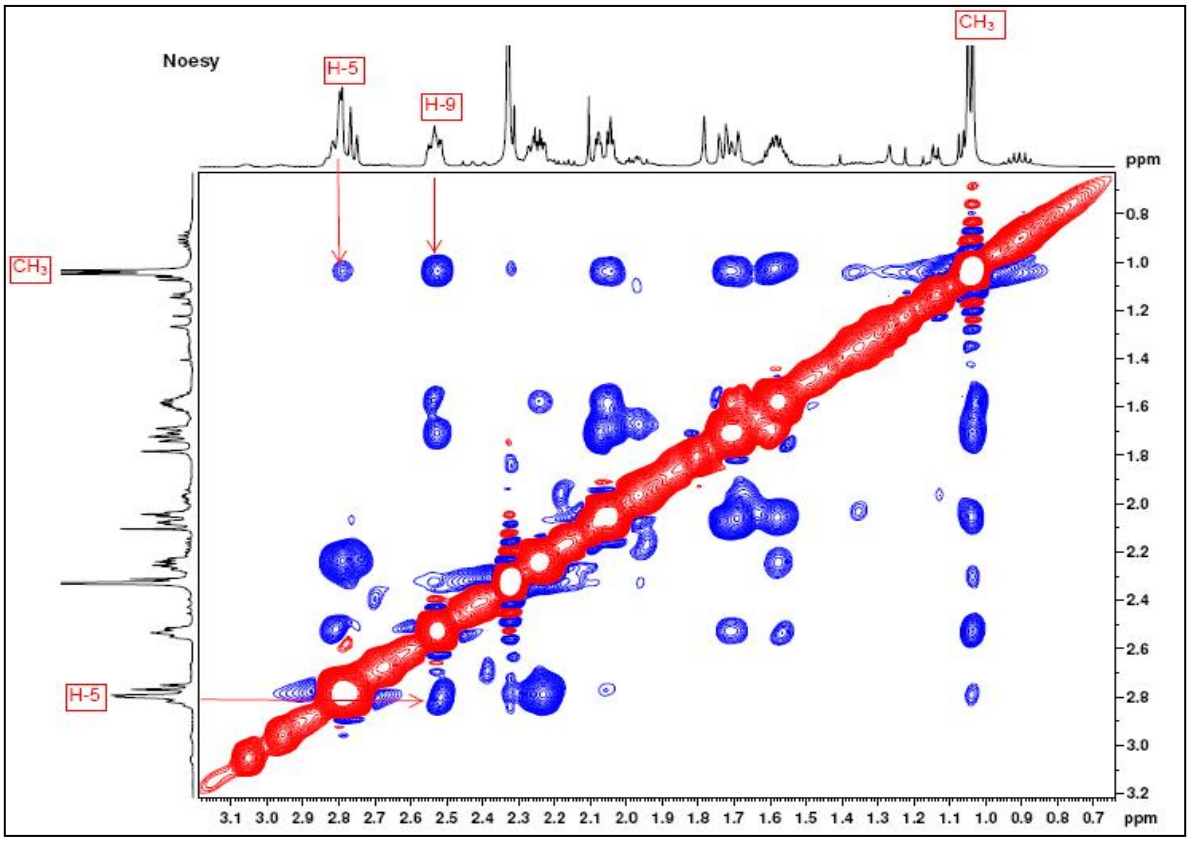
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 62



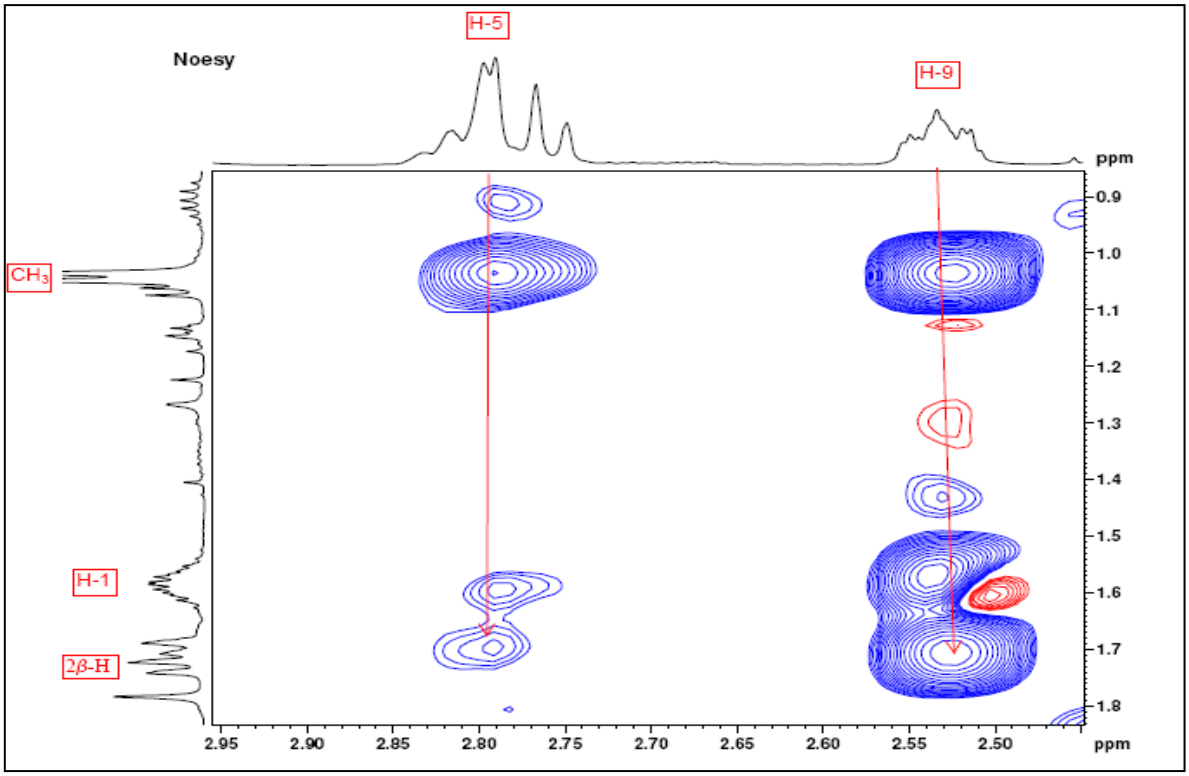
NOESY Spectrum (500 MHz) of 24 in $CDCl_3$



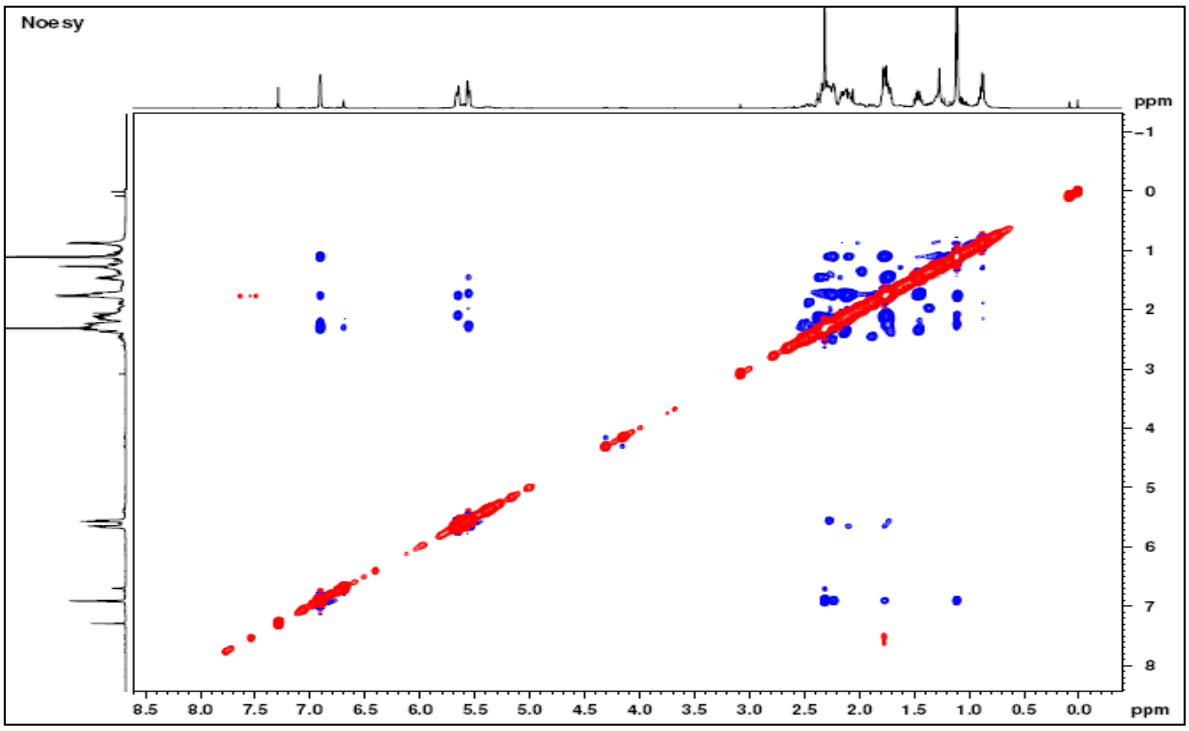
Enlarged NOESY Spectrum (500 MHz) of 24 in $CDCl_3$



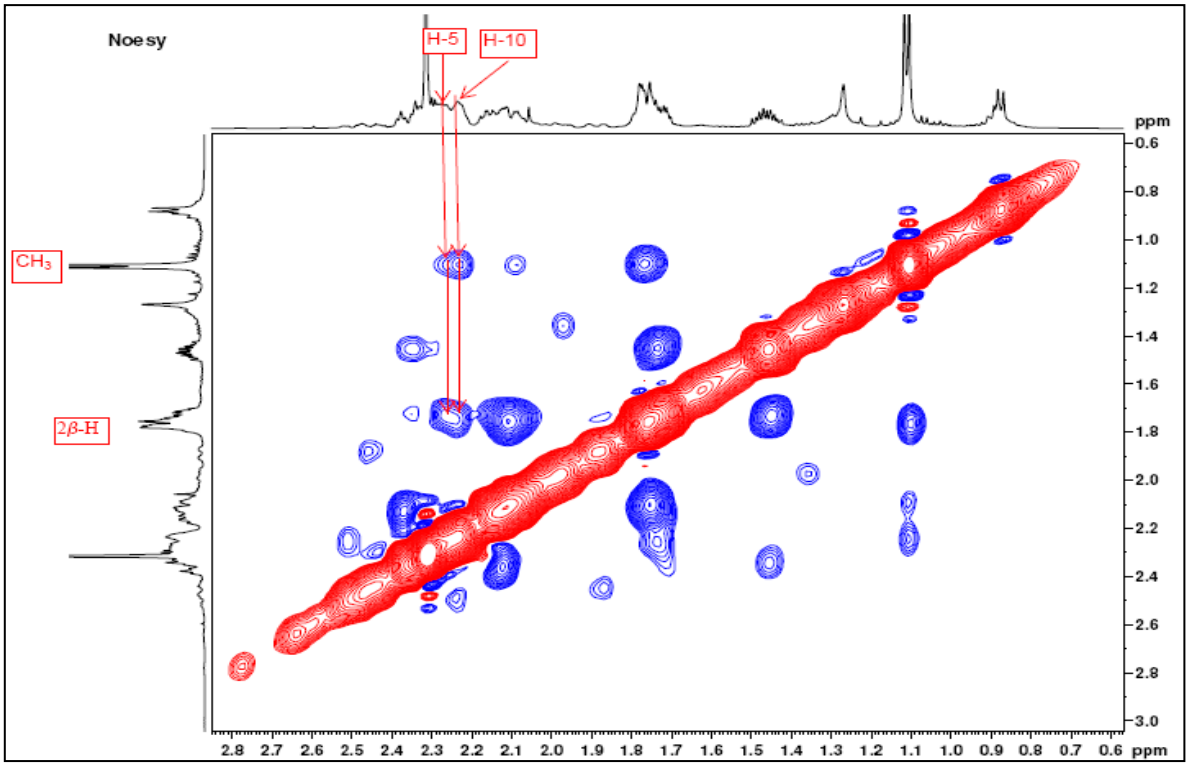
Enlarged NOESY Spectrum (500 MHz) of 24 in $CDCl_3$



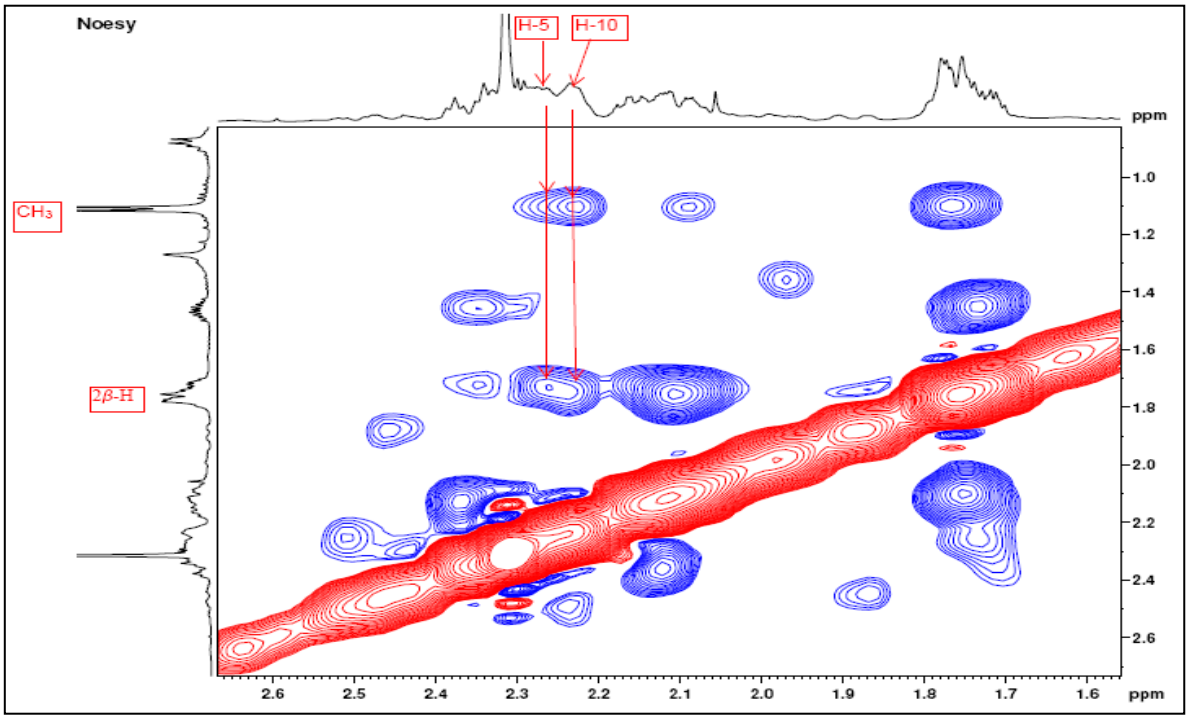
NOESY Spectrum (500 MHz) of 25 in $CDCl_3$



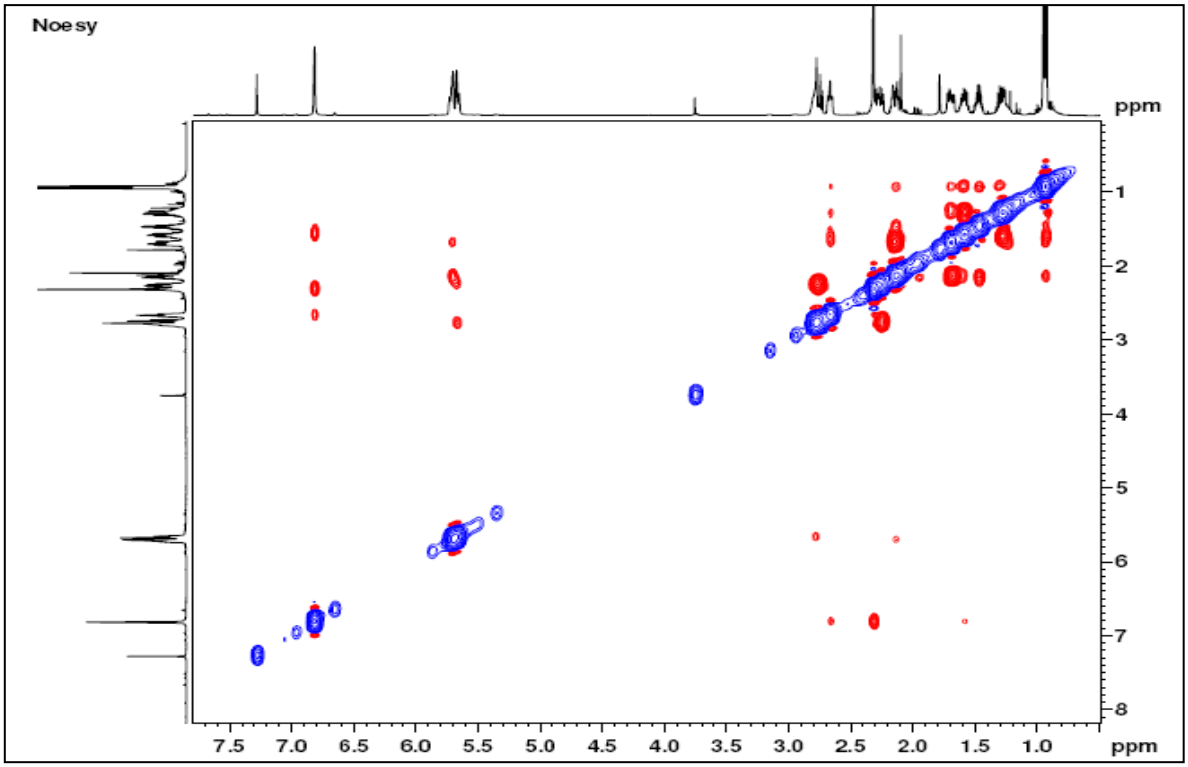
Enlarged NOESY Spectrum (500 MHz) of 25 in $CDCl_3$



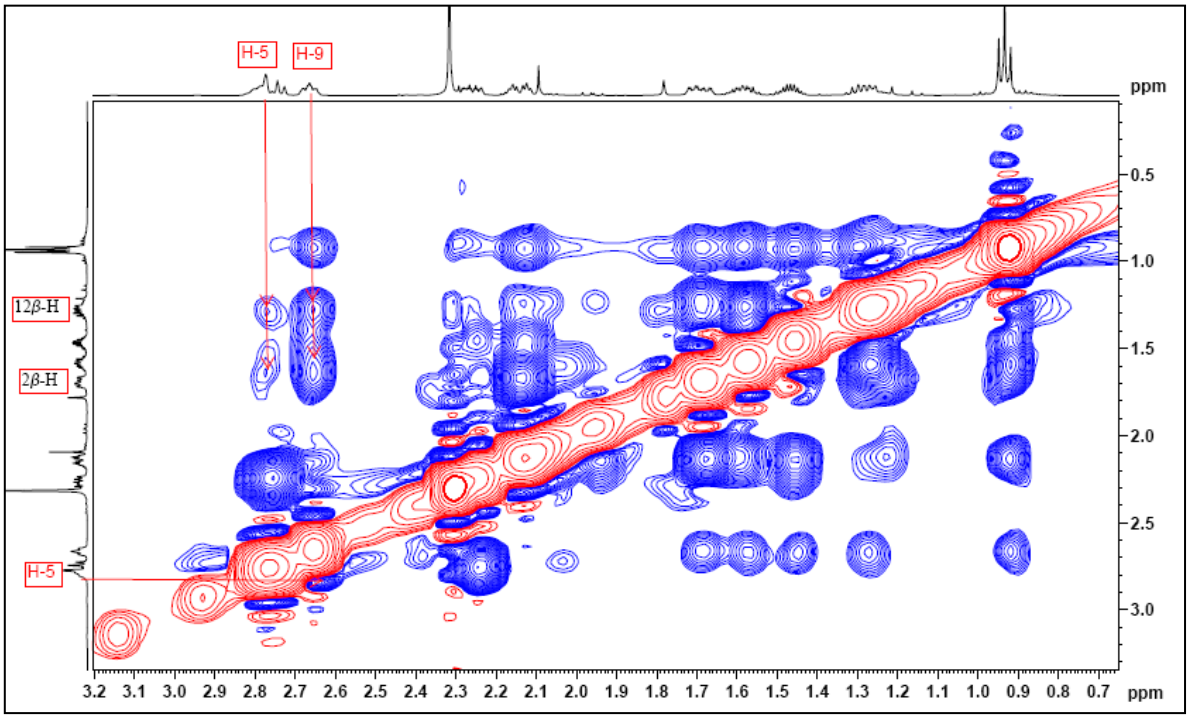
Enlarged NOESY Spectrum (500 MHz) of 25 in $CDCl_3$



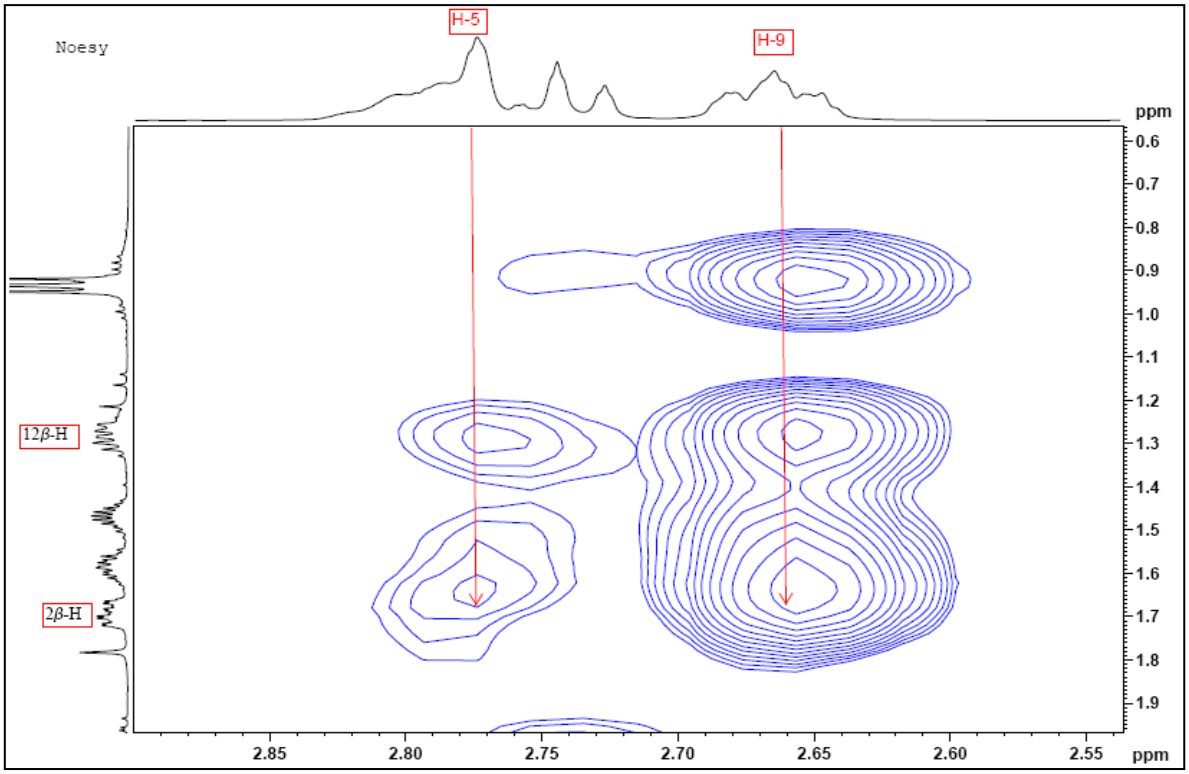
NOESY Spectrum (500 MHz) of 26 in $CDCl_3$



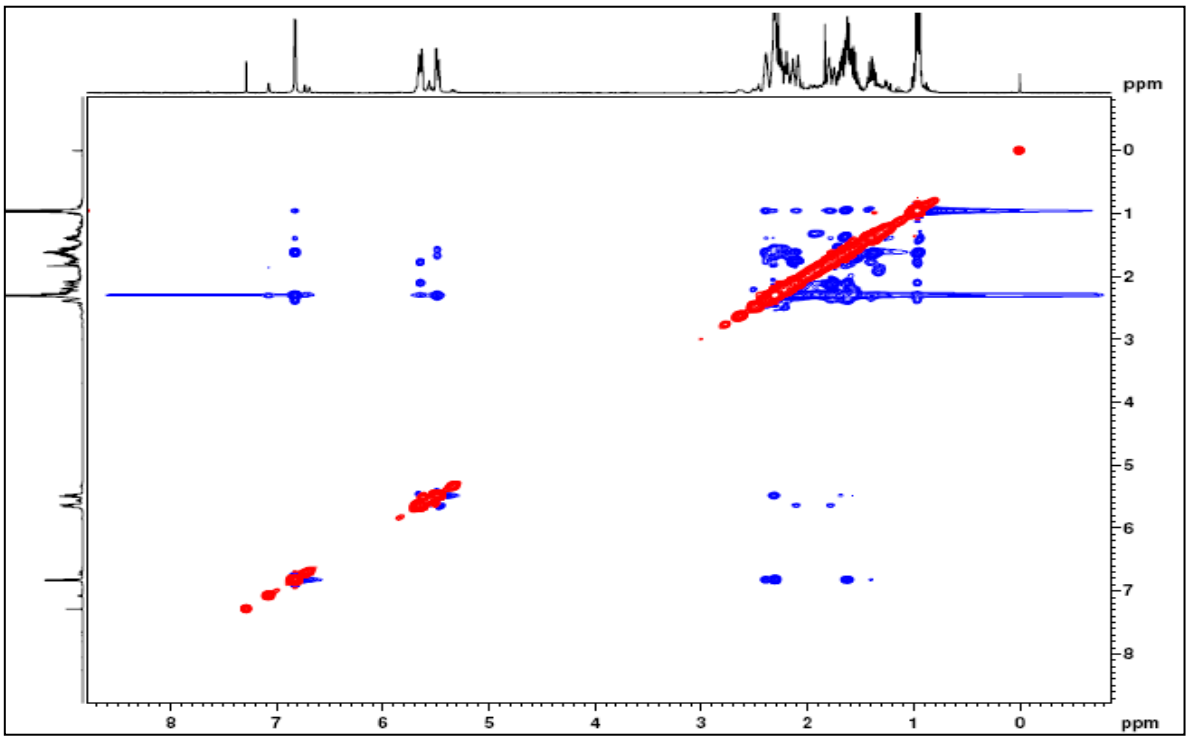
Enlarged NOESY Spectrum (500 MHz) of 26 in $CDCl_3$



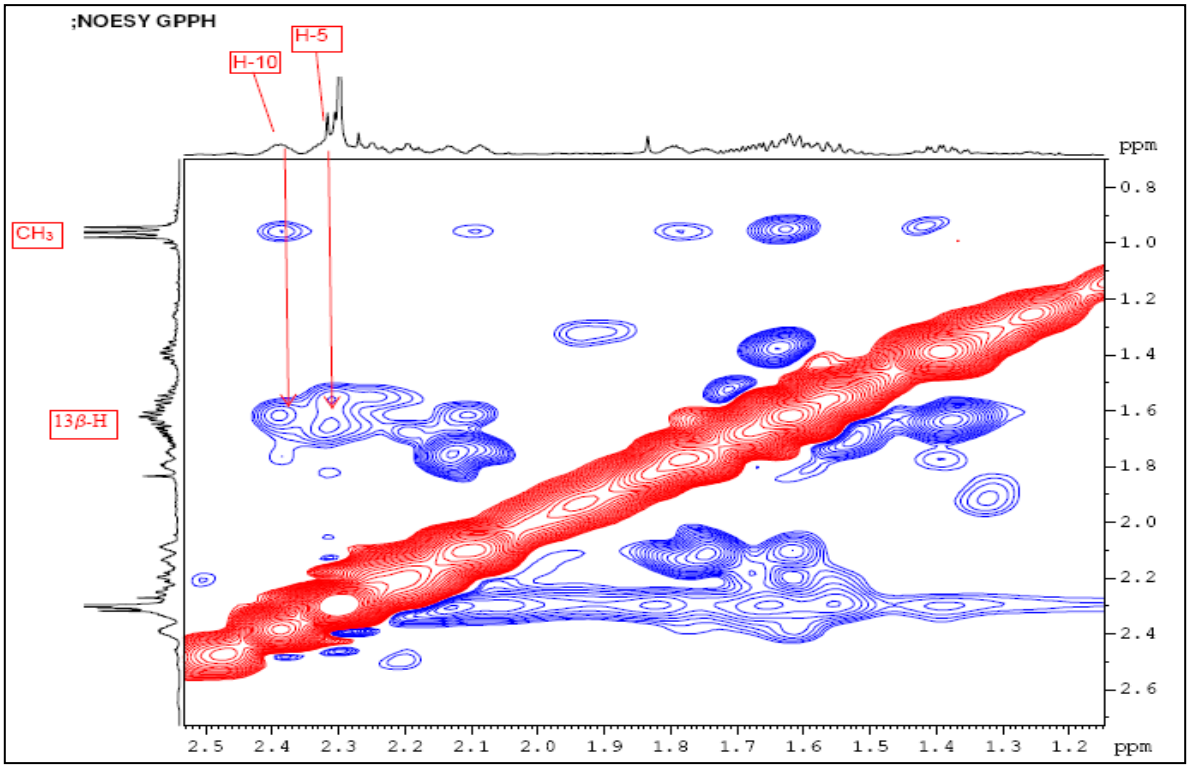
Enlarged NOESY Spectrum (500 MHz) of 26 in $CDCl_3$



NOESY Spectrum (500 MHz) of 27 in $CDCl_3$



Enlarged NOESY Spectrum (500 MHz) of 27 in $CDCl_3$



Chapter 3

Total Synthesis and Biological Evaluation of Nardoaristolone B and its Analogues

3.1 Introduction to 3/5/6 skeleton based natural products

Nature has been a rich and diverse source of cyclopropane-containing secondary metabolites. As the cyclopropyl containing 3/5/6 tricyclic fused ring system is unusual only limited natural products are present in nature with this rare skeleton. The examples of natural products with 3/5/6 tricyclic fused ring system include Lindenene (**1**), a furansesquiterpene which possesses a lindenane skeleton, was isolated from the dried root of *Lindera strychnifolia Vill* in 1960 by Takeda[1] (Fig 3.1); Chloranthalactone A (**2**) also named shizukanolide B, was first isolated from the *Chloranthus* genus in 1978 and shows moderate antifungal activity;[2] Oxyonoseriolide (**3**) was isolated from *Hedyosmum angustifolium* showed anti-leishmanial activity[3] and Chloraholide A (**4**) was isolated by Yue and co-workers from South China *Chloranthus holostegius* has shown potent and selective inhibition on the delayed rectifier (I_K) K^+ current (selective blocker of the potassium channel) with an IC_{50} values of 10.9 μM.[4] These interesting natural products with unusual skeletons pose significant challenges to the synthetic chemists.

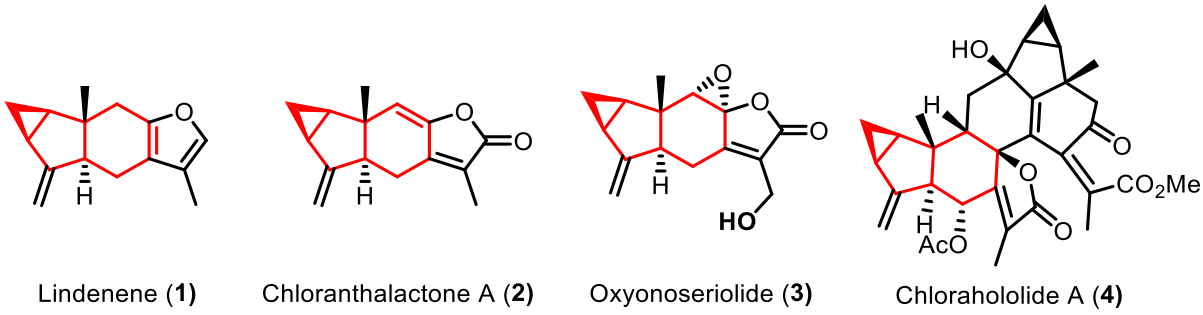
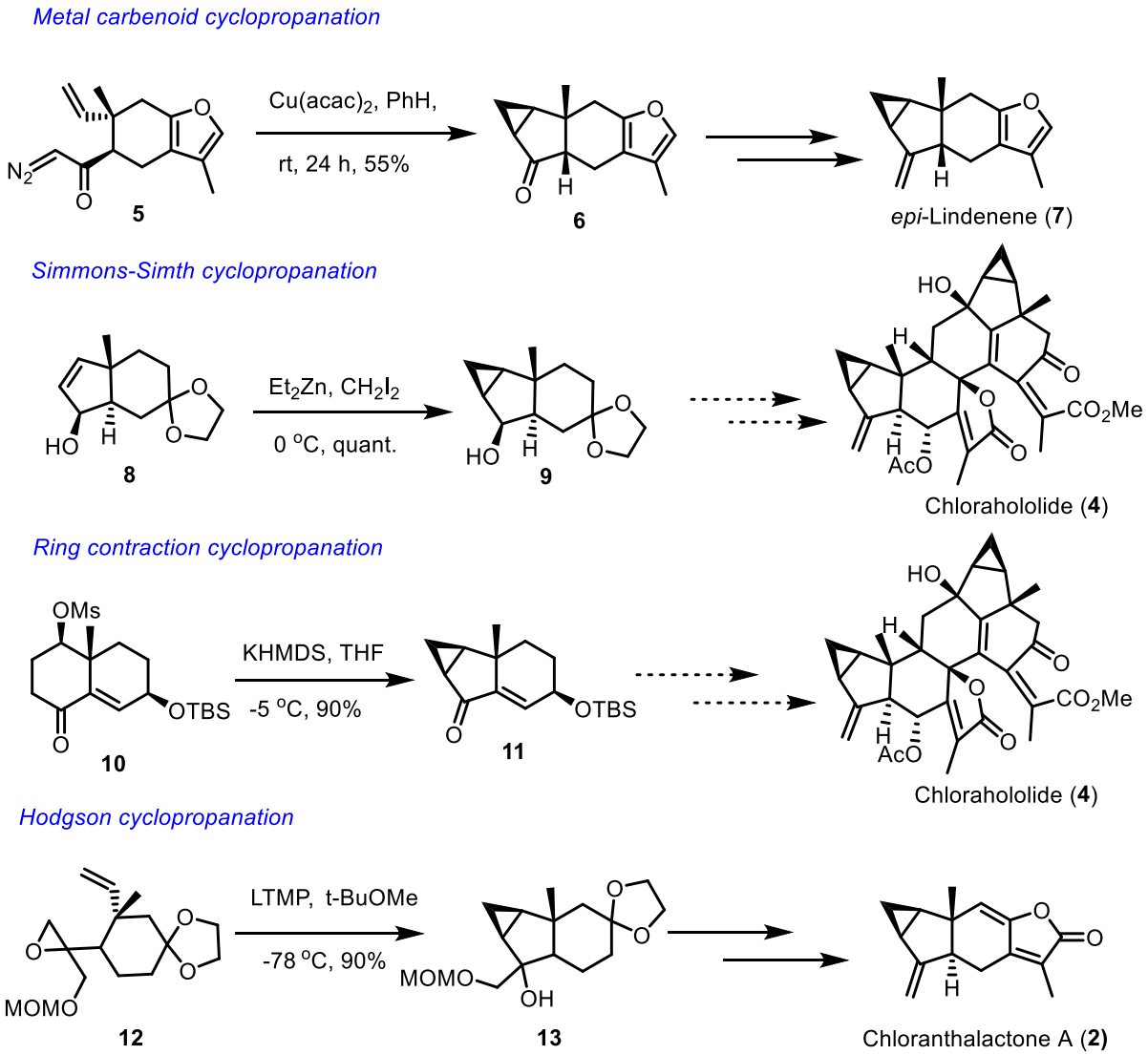


Fig 3.1: Selected natural products with 3/5/6 skeleton

There are a few methods developed for the construction of 3/5/6 fused ring system which help in making the natural products. Selected methods are discussed here. Baldwin and co-workers reported that diazoketone **5** on treatment with a catalytic amount of copper (II) 2,4-pentanedionate in benzene at room temperature underwent an intramolecular cyclopropanation to afford the compound **6** (20:1 ratio) with 3/5/6 skeleton and later this product was elaborated to (±)-*epi*-lindenene **7**.[5] Zhao et al. reported that allylic alcohol **8** on

modified Simmons–Smith reaction with Et_2Zn and CH_2I_2 in CH_2Cl_2 at 0 °C furnished the cyclopropane derivative **9** (scheme 3.1) in quantitative yield and with excellent diastereoselectivity.[6] In an effort towards chlorahololide A (**4**), Peng and co-workers reported that treatment of enone **10** with KHMDS resulted in ring contraction to have cyclopropyl enone **11** exclusively via an S_N2-type intramolecular nucleophilic substitution and the same was converted to heptacyclic core of **4**.[7] Liu et al. reported that treatment of **12** with lithium 2,2,6,6-tetramethylpiperidide (LTMP) smoothly generated the 3/5/6 tricyclic compound **13** and it was later transformed to chloranthalactone A (**2**).[8]



Scheme 3.1: Selected literature reports for 3/5/6 containing natural products

Recently, Echavarren's group developed gold (I)-catalyzed oxidative cyclization method for the construction of 3/5/6 tricyclic compounds and applied to the synthesis of nardoaristolone B.[9] More details Echavarren's group will be discussed in next section. In our work, we have identified nardoaristolone B a novel class sesquiterpenoid with 3/5/6 fused ring system. We became interested in making this natural product utilizing some of the chemistry developed in our group.[10] Details are presented in the following sections.

3.2 Nardoaristolone B

In the year 2013, Yao and coworkers isolated a novel terpenoid nardoaristolone B **14** derived from the nor-aristolane type sesquiterpenoid from the underground parts of *Nardostachy chinensis* plants[11] which has been used as sedative and analgesic agents in Chinese traditional medicine for centuries.[12] Nardoaristolone B **14** possesses an unusual 3/5/6 tricyclic ring system with two vicinal methyl groups oriented on the same side of the ring system (Fig 3.2).

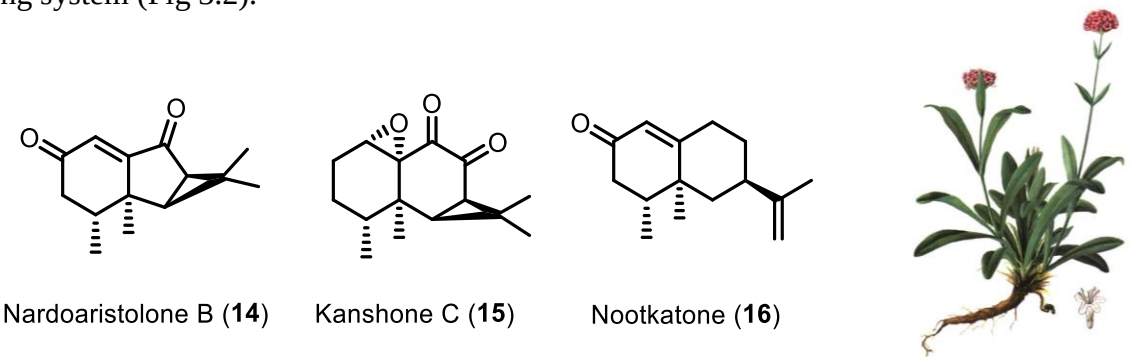
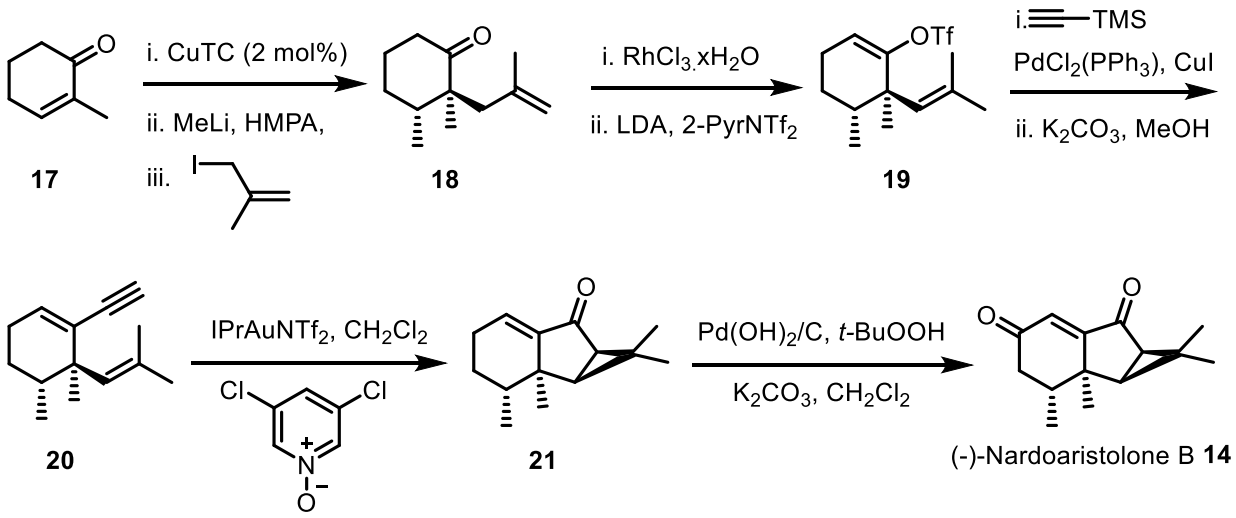


Fig 3.2: Selected natural products (plant picture taken from internet)

The structure elucidation of **14** was carried out using various spectral and X-ray diffraction methods. Nardoaristolone B **14** can be a cardio protective agent as it was shown relevant biological assay. It was evaluated for their protective effects on H_2O_2-induced myocardial injury using the MTT method and salvianolic acid B as the positive control and it has shown protective effects on the injury of neonatal rat cardiomyocytes in a dose-dependent manner.[11] It was proposed that nardoaristolone B **14** is biogenetically derived from Kanshone C **15** through series of reaction such as ring-opening reaction, benzilic acid rearrangement, decarboxylation, dehydration, and oxidation.[11] It is also important to

highlight that structure of nardoaristolone B **14** is close to that of nootkatone **16**, an interesting and well known natural sesquiterpene with effective insect repellent/insecticidal activity[13] and AMPK (Adenosine Monophosphate Kinase) activity.[14] Since the structural features are similar to that of nootkatone, nardoaristolone B **14** and its analogues are expected to show interesting biological activities.[15] Therefore we became interested in working on this project. After our report, Echavarren's group achieved enantioselective total synthesis of (−)-nardoaristolone B using gold (I)-catalyzed oxidative cyclization in seven steps.[9] Although, this work appeared much later than our publication, as it is only relevant work on the target (nardoaristolone B), details are presented here.



Scheme 3.2: Synthesis of (–)-nardoaristolone B by Echavarren's group

Echavarren's approach started with CuI-mediated conjugate methylation of 2-methyl-2-cyclohexenone **17** followed by α-alkylation using methallyl bromide afforded compound **18** with moderate yield. The isomerization of exo-olefin in compound **18** was achieved by $RhCl_3.xH_2O$ (5 mol %) in ethanol at 75 °C to give desired endo-olefin which was converted to enol triflate **19** by LDA and 2-$PyrNTf_2$. Sonogashira cross-coupling of **19** with trimethylsilyl acetylene using Pd $(PPh_3)_2Cl_2$ and CuI followed by methanolysis of the TMS group led to 1, 5-enyne **20.** The 1, 5-enyne **20** on oxidative cyclization in the presence of only 5 mol % of $IPrAuNTf_2$ and 3, 5-dichloropyridine N-oxide afforded compound **21**. Finally allylic oxidation was achieved by Pearlman's catalyst ($Pd(OH)_2/C$) and *t*-BuOOH to

give (–)-nardoaristolone B **14** in excellent yield.

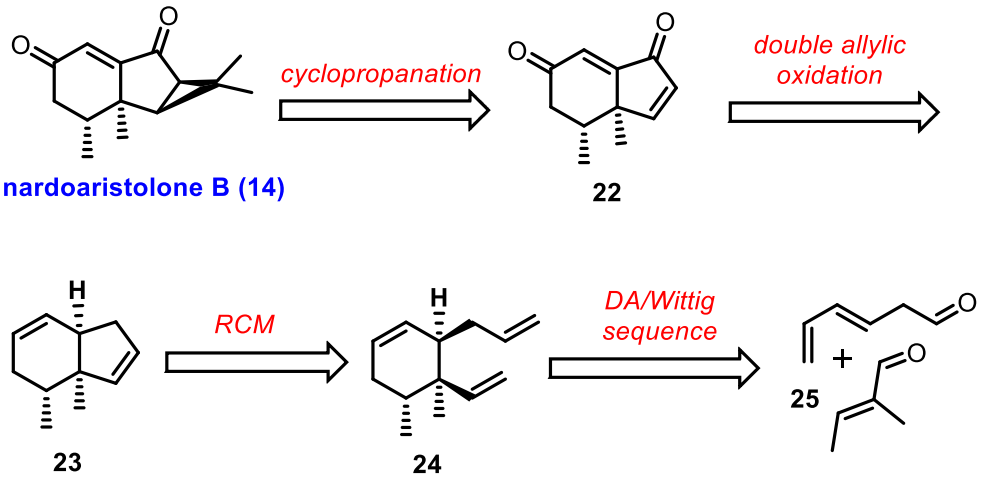
3.3 Present Work

A rare skeleton 3/5/6 tricyclic fused ring system of nardoaristolone B, cardioprotective effect and structural similarities with well-known and important natural product nootkatone attracted our attention to initiate the program on nardoaristolone B. Following were the aims of this project when initiated the program.

- Develop a simple and efficient synthetic route to access nardoaristolone B to provide the compound in sufficient quantities.
- Synthesize structurally close analogues around 3/5/6 scaffold towards optimization and structural simplification of the scaffold.
- Biological evaluation of nardoaristolone B and its analogues to identify lead compound and explore new activities.

3.3.1. Our approach

Our retrosynthetic analysis is shown in scheme 3.3. The target compound nardoaristolone B **14** could be prepared by stereoselective cyclopropanation of dienone **22** where we wanted to take advantage of methyl groups present on the skeleton.

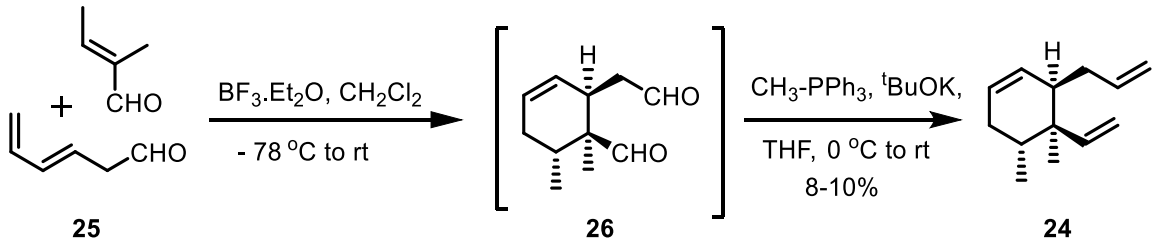


Scheme 3.3: Our retrosynthetic approach

The dienone **22** could be prepared from *cis*-hydrindane diene **23** by using double allylic oxidation (scheme 3.3). The requisite *cis*-hydrindane **23** could be constructed through ring closing metathesis of triene **24**. The triene **24** could be constructed from tiglic aldehyde and the diene **25** through DA (Diels-Alder) followed by Wittig sequence. Related stereoselective DA reaction was used in the previous sections of this thesis.

3.3.2 Total synthesis of nardoaristolone B

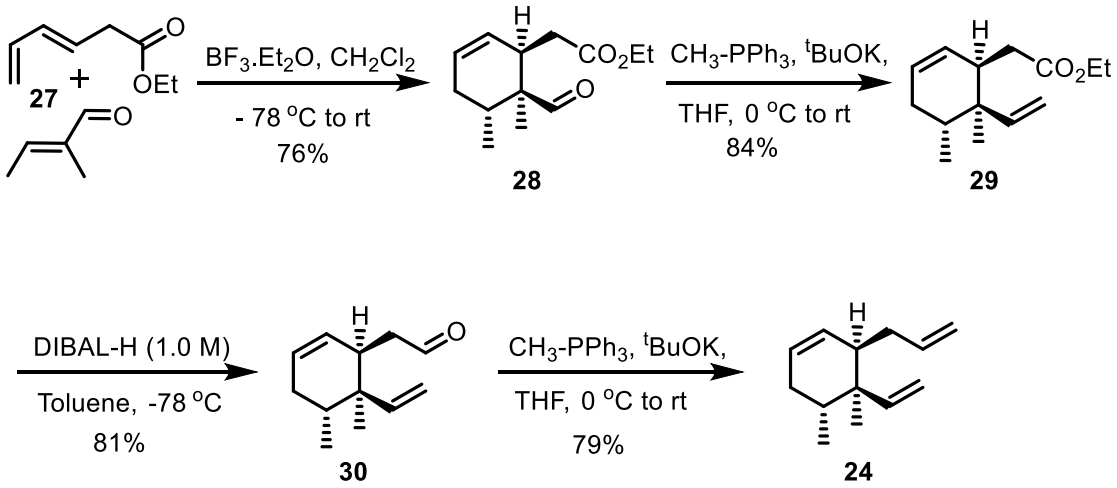
Our synthetic effort towards nardoaristolone B commenced with a Lewis acid mediated intermolecular Diels-Alder reaction[16-17] between dienophile tiglic aldehyde and diene aldehyde **25**[18] in CH_2Cl_2 at –78 ^oC to rt to afford crude Diels-Alder adduct **26** which was immediately subjected to one carbon Wittig reaction in presence of potassium *tert*-butoxide in THF at 0 ^oC give the triene **24** in low yield (8-10%) but with very high diastereoselectivity (>9:1) (scheme 3.4). The observed low yield in Diels-Alder/ Wittig sequence could probably be due to polymerization or inter/intra-molecular condensation of both starting aldehydes. The formation of triene **24** was indicated by the presence of eight olefinic signals in ^1H NMR at 6.01–5.94 (m, 1H), 5.86–5.77 (m, 1H), 5.62–5.61 (m, 2H), 5.05–4.99 (m, 4H) ppm and two methyl group signals at 1.03 (s, 3H), 0.86 (d, J = 6.8 Hz, 3H) ppm, respectively. Similarly corresponding carbon signals at 146.1, 138.3, 129.1, 125.1, 115.7, 112.3 ppm and for two methyls at 19.3, 16.4 ppm in ^{13}C NMR were observed. The assigned structure of **24** was further confirmed by HRMS. The Lewis acid mediated intermolecular Diels-Alder reaction produces the *endo*-adduct having both aldehydes on the same side. The diastereo- and regioselectivity can be explained in a similar fashion to that of previous cases, on the basis of secondary orbital interactions and atomic coefficient preferences, respectively.[19-20]



25 26 24

Scheme 3.4: Synthesis of triene **24** *via* Diels-Alder/ Wittig sequence

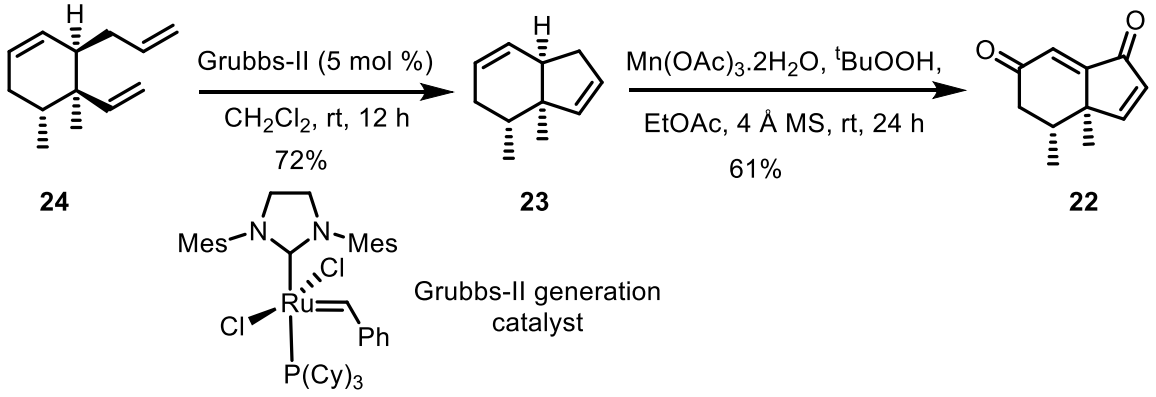
To improve the yield, we have replaced aldehyde diene **25** with more stable ester diene **27**.[21] The Diels-Alder reaction of diene ester **27** and tiglic aldehyde in presence of Lewis acid $BF_3.Et_2O$ at –78 °C to rt afforded adduct **28** in 76% yield which on single carbon Wittig reaction in presence of potassium *tert*-butoxide in THF at 0 °C give the **29** in 84% yield. The product **29** was characterized by presence of internal olefinic protons at 5.65–5.53 (m, 2H) ppm and terminal olefinic protons at 5.86 (dd, J = 11.0 Hz, 18.3 Hz, 1H), 5.06–5.05 (m, 1H), 5.03–5.02 (m, 1H) ppm in ^{1}H-NMR spectrum. The corresponding olefinic carbon signals appeared at 128.6, 126.2 ppm and at 145.2, 113.3 ppm in ^{13}C-NMR spectrum. The product **29** was further confirmed by HRMS which showed a peak at 245.1508 corresponding to formula $C_{14}H_{22}O_2$ $[M+Na]^+$ with calculated value of 245.1512.



Scheme 3.5: Improved synthesis of triene **24**

The ester group in compound **29** was reduced using DIBAL-H (1.0 M in toluene) in presence of toluene at –78 °C to give aldehyde **30** in 81% yield as colorless oil which was immediately used for next step. The one carbon Wittig reaction of **30** in presence of potassium *tert*-butoxide in THF at 0 °C afforded **24** in 79% yields. Spectral data was identical with that of above compound **24** synthesized via Diels-Alder/Wittig sequence. The overall transformation of **24** from **27** was achieved in ~ 41% overall yield (scheme 3.5). Next, compound triene **24** was subjected to ring-closing metathesis (RCM) using 5 mol % Grubbs' second-generation catalyst to give the *cis*-hydrindane **23** in 72% yield.[22] The formation of *cis*-hydrindane **23** indicated by presence two sets of internal olefinic proton at

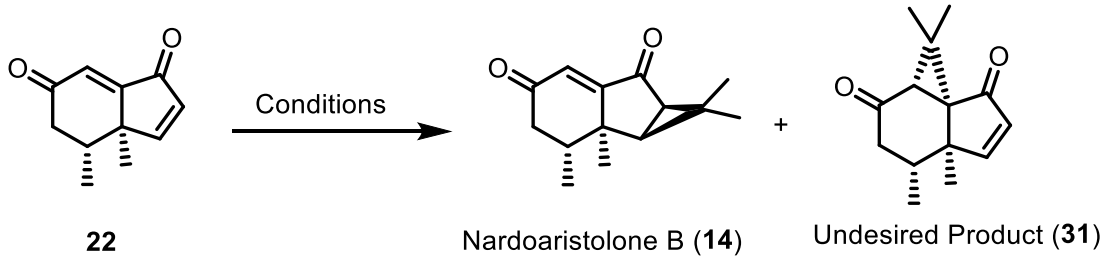
5.79–5.72 (m, 2H), 5.69–5.66 (m, 2H) ppm in ^{1}H NMR spectrum and corresponding carbon signal at 141.7, 129.3, 128.5, 125.9 ppm in ^{13}C NMR spectrum which was further confirmed by GC-HRMS which showed a peak at 148.1252 corresponding to formula $C_{11}H_{16}$.

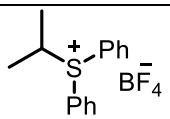
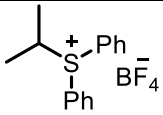
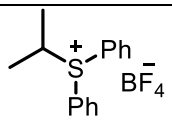


Scheme 3.6: Synthesis of dienone **22**

To introduce the oxygen functionality in both the rings, we planned double allylic oxidation in a single operation. To perform this task on compound **23**, we have used the combination of $Mn(OAc)_3.2H_2O$-^{t}BuOOH[23] in presence of EtOAc at rt for 24 h to obtained dienone compound **22** in 61% yield (scheme 3.6). In addition to the desired dienone **22**, we have also isolated mono-oxidized products mixture by purification. The mixture of mono-oxidized products was again converted to **22** under the same reactions conditions. The reported 61% yield represent overall isolated yield of **22** starting from **23**. This transformation was indicated by presence of olefinic proton at 7.70 (d, J = 5.9 Hz, 1H), 6.35 (d, J = 5.9 Hz, 1H), 6.27 (s, 1H) ppm in ^{1}H NMR and corresponding carbon signal at 165.0, 160.4, 133.6, 121.8 ppm and two carbonyl carbon at 199.7, 195.8 ppm in ^{13}C NMR spectrum. The product **22** was further confirmed by HRMS which showed a peak at 177.0909 corresponding to formula $C_{11}H_{13}O_2$ $[M+H]^+$ with calculated value of 177.0910. The final task was stereoselective insertion of *gem*-dimethyl cyclopropyl group on five membered ring enone to get the target compound; towards that effort, initially we have tried some conditions but with no success. Finally, *gem*-dimethyl cyclopropanation[24] on dienone **22** was achieved by the treatment of diphenyl isopropylsulfonium

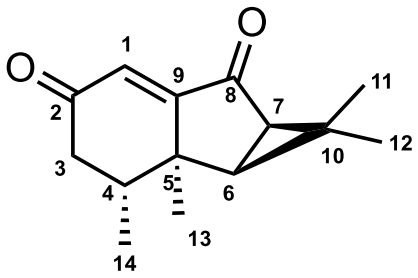
tetrafluoroborate[25] with ^{t}BuLi in THF at –78 ^{o}C to – 30 ^{o}C to give the target compound nardoaristolone B (**14**) in 32% yield along with minor amounts of **31** (scheme 3.7). All the spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) were found to be identical to those reported in the isolation paper.[11] Spectral data (^{1}H-NMR and ^{13}C-NMR) comparison of natural nardoaristolone B and our synthesized (±)-nardoaristolone B are shown in table 3.1. Initially, we have assigned the structure to compound **31** where cyclopropane ring and two methyl groups opposite to each other but it was later confirmed by x-ray structure of enantiomeric series as drawn in Scheme 3.7 which is not part of this thesis.[26] Thus, we have accomplished the total synthesis of (±)-nardoaristolone B for the first time. To improve the yield in final steps, we have attempted a few other conditions but the results were inferior. The three different conditions tried for cyclopropanation are captured below in scheme 3.7.



Sr. No	Conditions	Observation and % yield
1	 LDA, DME, -78 ^{o}C, 2 h	only starting material (**22**) observed
2	 ^{t}BuLi, THF, -78 ^{o}C, 2 h	Major **22** (~ 70%) and minor amount of nardoaristolone B **14** (~5%) and undesired product **31** (~5-8%)
3	 ^{t}BuLi, THF, -78 ^{o}C to -30 ^{o}C, 1 h	Nardoaristolone B **14** (32%) and undesired product **31** (~10%)

Scheme 3.7 : Conditions tried for cyclopropanation

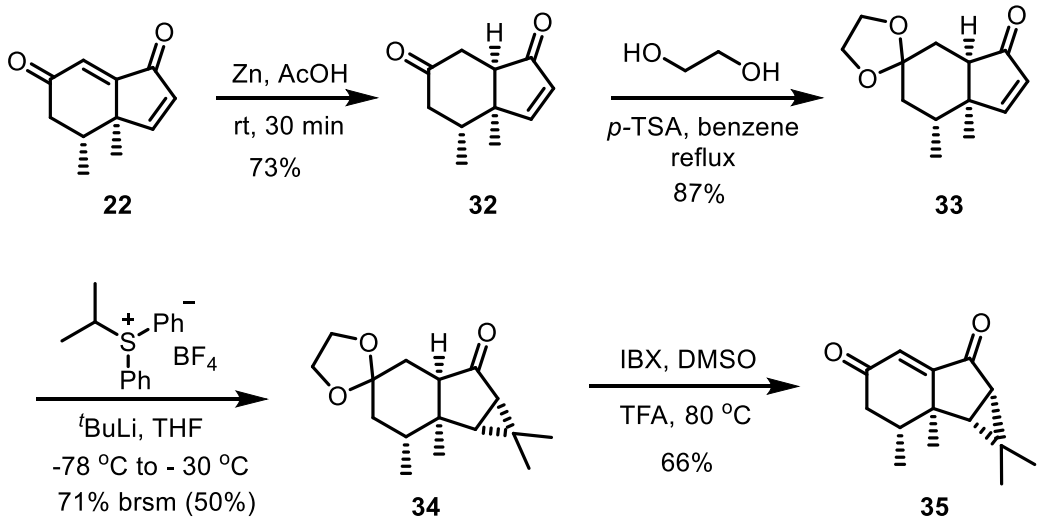
Table 3.1: Spectral data comparison of natural nardoaristolone B **14** and our synthesized (±)-nardoaristolone B **14**



No	Natural nardoaristolone B		Synthesized (±)-nardoaristolone B	
	^{1}H	^{13}C	^{1}H	^{13}C
1	6.16 (s, 1H)	123.4	6.18 (s, 1H)	123.6
2	-	200.0	-	200.0
3	α: 2.23 (dd, *J* = 13.5, 18.0 Hz, 1H) β: 2.35 (dd, *J* = 4.7, 18.0 Hz, 1H)	42.1	α: 2.24 (dd, *J* = 13.7, 18.0 Hz, 1H) β: 2.38 (dd, *J* = 4.8, 18.3 Hz, 1H)	42.2
4	2.34 (m, 1H)	35.4	2.34 (m, 1H)	35.5
5	-	44.2	-	44.3
6	1.79 (d, *J* = 5.5 Hz, 1H)	42.3	1.77 (d, *J* = 5.6 Hz, 1H)	42.3
7	1.94 (d, *J* = 5.5 Hz, 1H)	40.2	1.94 (d, *J* = 5.6 Hz, 1H)	40.3
8	-	201.6	-	201.5
9	-	165.1	-	165.1
10	-	32.2	-	32.1
11	1.09 (s, 3H)	28.7	1.09 (s, 3H)	28.8
12	1.18 (s, 3H)	17.7	1.17 (s, 3H)	17.8
13	1.13 (s, 3H)	20.8	1.13 (s, 3H)	20.8
14	1.07 (d, *J* = 6.5 Hz, 3H)	15.8	1.07 (d, *J* = 6.6 Hz, 3H)	15.8

3.3.3 Synthesis of nardoaristolone B analogues

As the nardoaristolone B scaffold is novel with 3/5/6 rings fusion and also to understand SAR around the scaffold, we have planned to make a few close analogues around the target. In this context, double bond between two carbonyl group in dienone compound **22** was selectively reduced with zinc dust in presence of acetic acid[27] to yield *cis*-hydrindane **32** in 73% yield in a highly chemo and stereoselective manner. The chemoselective reduction proceeded via enolate of **22** and resulted enolate undergoes stereoselective protonation at ring junction from the convex side of the bicyclic fused ring system, thus able to produced functionalized *cis*-hydrindane **32** (scheme 3.8). The formation of product **32** was indicated by disappearance of one olefinic proton corresponding to the ring junction at 6.27 (s, 1H) ppm and appearance of ring junction proton at 2.67–2.62 (m, 1H) in ^{1}H NMR spectrum.



Scheme 3.8: Synthesis of *exo*-cyclopropyl compound **35**

To perform the cyclopropanation reaction on enone in compound **32**, it is necessary to protect isolated ketone to avoid side reactions. The isolated ketone present in six membered ring of compound **32** was selectively protected as ketal using ethylene glycol in cat. *p*-TSA, reflux condition to give **33** in 87% yield. Compound **33** was characterized by different spectral data. Having compound **33** in hand, the *gem*-dimethyl cyclopropanation was

achieved by the treatment of **33** with diphenyl isopropylsulfonium tetrafluoroborate with ^{t}BuLi in THF at –78 ^{o}C to – 30 ^{o}C to afford the compound **34** with *exo*-cyclopropyl ring in 50% (71% brsm) yield (scheme 3.8). Initially the assigned structure of compound **34** was confirmed by NMR spectral data, in particular, presence of four methyl signal in ^{1}H-NMR at 1.34 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 0.97 (d, J = 6.8 Hz, 3H) ppm. The assigned stereochemistry of compound **34** was unambiguously confirmed by its single crystal X-ray analysis (crystallized using ethyl acetate-hexane solvents) where cyclopropane ring and two methyl groups were situated on the same side of the plane (Fig 3.3).

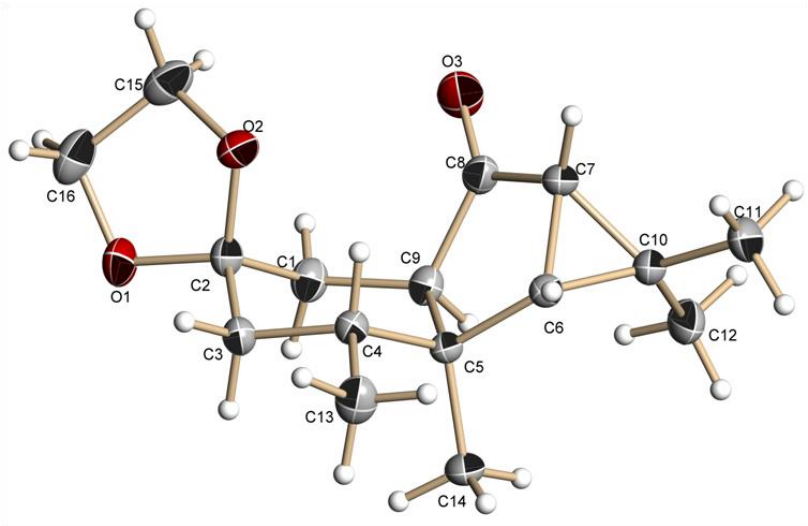


Fig 3.3: Single X-ray crystal structure of compound **34**

The next task was deprotection of ketal group on six-membered ring followed by dehydrogenation to get the desired *exo*-cyclopropyl compound. This transformation was achieved in a single pot by the treatment of compound **34** with Nicolaou's protocol[28] IBX/DMSO in presence of catalytic TFA at 80 °C to give the desired compound **35** in 66% overall yields. The structural transformation was indicated by presence of one olefinic proton at 6.12 (s, 1H) ppm in ^{1}H NMR spectrum and corresponding carbon signal at 165.0, 122.1 ppm and two carbonyl carbon at 201.6, 200.1 ppm in ^{13}C NMR spectrum. The product was further confirmed by HRMS which showed a peak at 219.1378 corresponding to formula $C_{14}H_{19}O_2$ $[M+H]^+$ with calculated value of 219.1380. The assigned structure of compound **35** was further confirmed by additional 2D NMR spectroscopic data. Key NOE

correlations on energy minimized structure **35** are shown in Fig 3.4. The ^{13}C NMR and DEPT spectra exhibited 14 carbon signals, corresponding to two carbonyl carbons, three quaternary carbons (one olefinic carbon), four methines (one olefinic carbon), one methylene, and four methyls. The ^{1}H-^{1}H COSY spectrum provided connectivities for two spin systems: H-3, H-4, CH_3-14 and H-6, H-7. A dimethyl cyclopropane unit was deduced due to the HMBC correlations from CH_3-11 and CH_3-12 to C-10, C-6, and C-7.

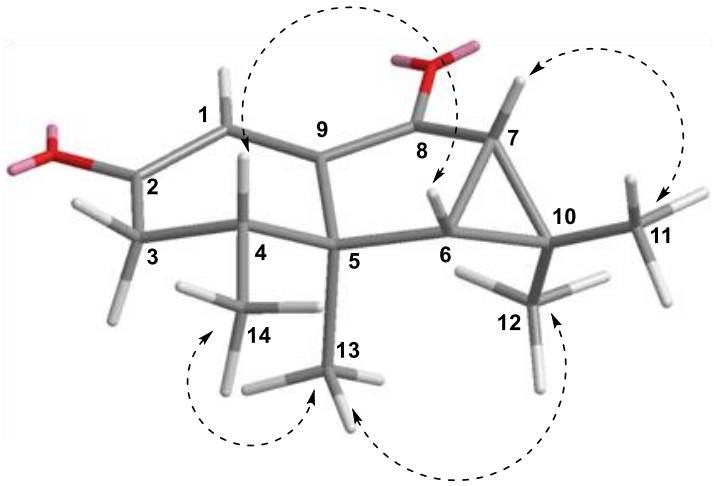


Fig 3.4: Key NOE correlations on energy minimized structure **35**.

Further, the stereochemistry of **35** was derived from NOESY experiments. NOE correlations from methyl at C-4 and methyl at C-5 suggested that both CH_3 are *cis* oriented. CH_3-13 having correlation with CH_3-12 suggests that both CH_3 are placed on same side of plane. There is strong NOE from C4-H to C6-H which implies both hydrogens are axial to each other. C7-H having NOE with methyl at C11 position which suggest that C7-H and CH_3-11 are also on same side. This indicated that C7-H, C6-H and C4-H are situated on same side and confirmed the assigned structure of compound **35**.

During cyclopropanation reaction we have observed the opposite stereoselectivities in compounds **22** and **33** to give *endo*-cyclopropyl compound **14** and *exo*-cyclopropyl compound **35** respectively. The opposite stereoselectivities could be explained simply by considering the shape of these compounds (**22** and **33**) as shown in Fig 3.5. The compound **22** has nearly planar structure which will favor the reagent sulfur ylide to approach from the *endo* side (opposite side of vicinal methyl groups) to give compound **14** and the compound

33 has convex-shape which will favor the approach from *exo* side (same side of vicinal methyl groups) to give compound **35** after deprotection and dehydrogenation.

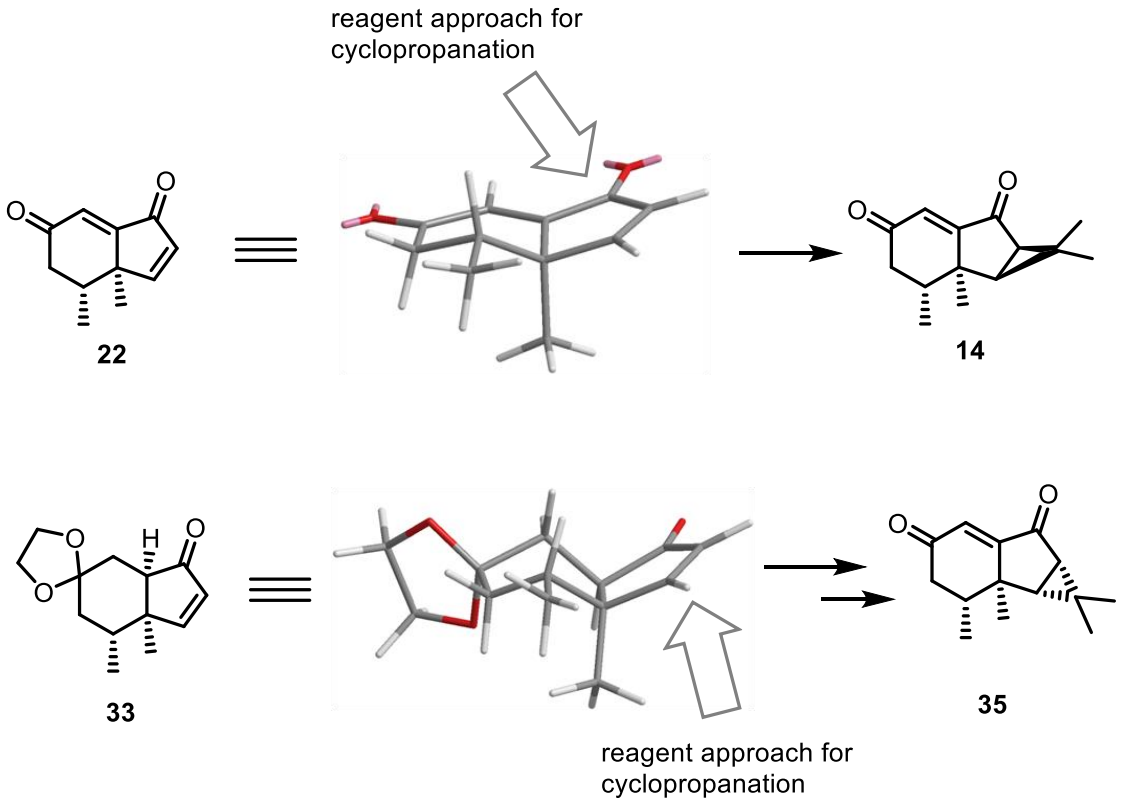
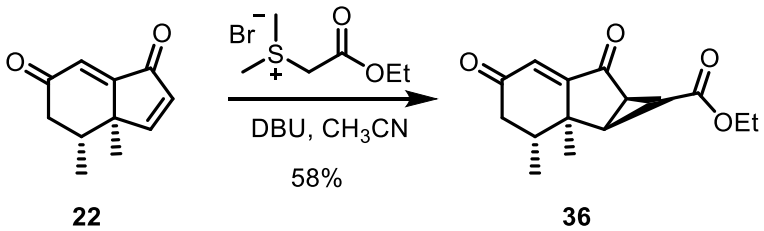


Fig 3.5: A reasonable explanation for the observed stereoselectivity for cyclopropanation

It is possible to produce diverse cyclopropyl containing compounds using the intermediates dienone **22** and enone **33** with various cyclopropanating reagents. To expand the potential, of dienone intermediate **22** to generate library of molecules, **22** was treated with carboethoxymethyl-dimethyl sulfonium bromide ($Me_2S^+CH_2CO_2Et\ Br^-$)[29] in the presence of DBU, CH_3CN to afford compound **36** in 58% yield (scheme 3.8).

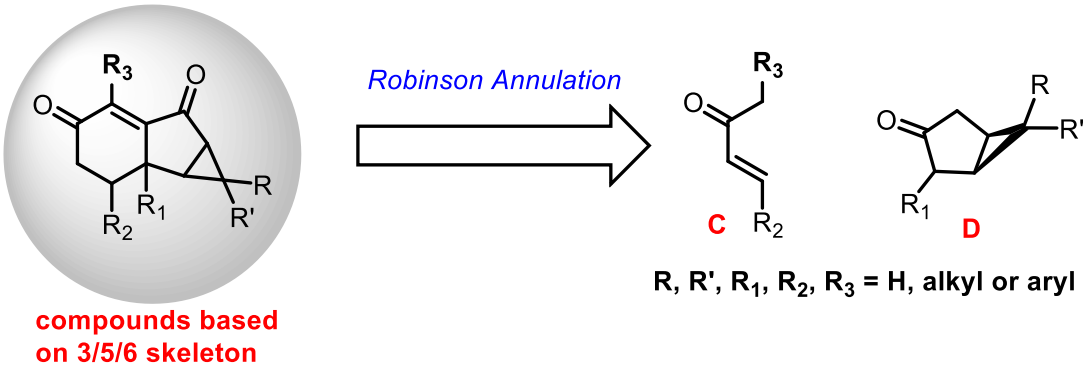


Scheme 3.8: Synthesis of ester **36**

The formation of product **36** was indicated by presence one olefinic proton at 6.22 (s, 1H) ppm and ester attached protons at 4.09–4.02 (m, 2H), 1.19 (t, J = 7.0 Hz, 3H) ppm in ^{1}H NMR spectrum and corresponding olefinic carbon signal at 161.6, 124.2 ppm and two carbonyl carbons at 199.9, 199.6 and ester carbon at 168.8 ppm in ^{13}C NMR spectrum. The product was further confirmed by HRMS which showed a peak at 285.1094 which corresponds to the formula $C_{15}H_{18}O_4Na$ $[M+Na]^+$ with calculated value of 285.1097. Although, we are able to produce a couple of new analogues of nardoaristolone B and the route has potential to make more analogues using intermediate **22**, it is not robust or economic towards generation of library of compounds. Therefore, we have planned a new strategy, which will be discussed in next section.

3.3.4 New strategy to access 3/5/6 skeleton by Robinson annulation

To develop a simple and efficient method to access compounds based on 3/5/6 skeleton, we have visualized Robinson annulation approach (scheme 3.9). Robinson annulation involves the Michael addition of an enolate (Michael donor) on a conjugated ketone (Michael acceptor) and subsequent intramolecular aldol reaction and elimination of an alcohol to give a conjugated ketone. We have chosen appropriately substituted enones **C** (Michael acceptors) and cyclic ketones **D** (Michael donors) for the present purpose to construct of 3/5/6 tricyclic fused system (scheme 3.9).



Scheme 3.9: Robinson annulation strategy to access 3/5/6 skeleton based compounds

We have chosen the following substituted enones **C** for Robinson annulation and some of them are prepared using known literature procedures[30] (Fig 3.6).

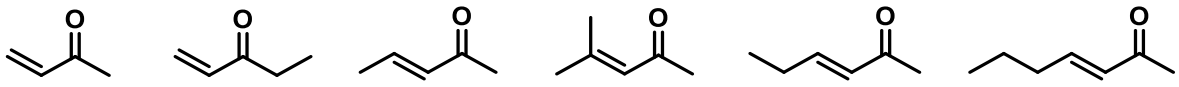
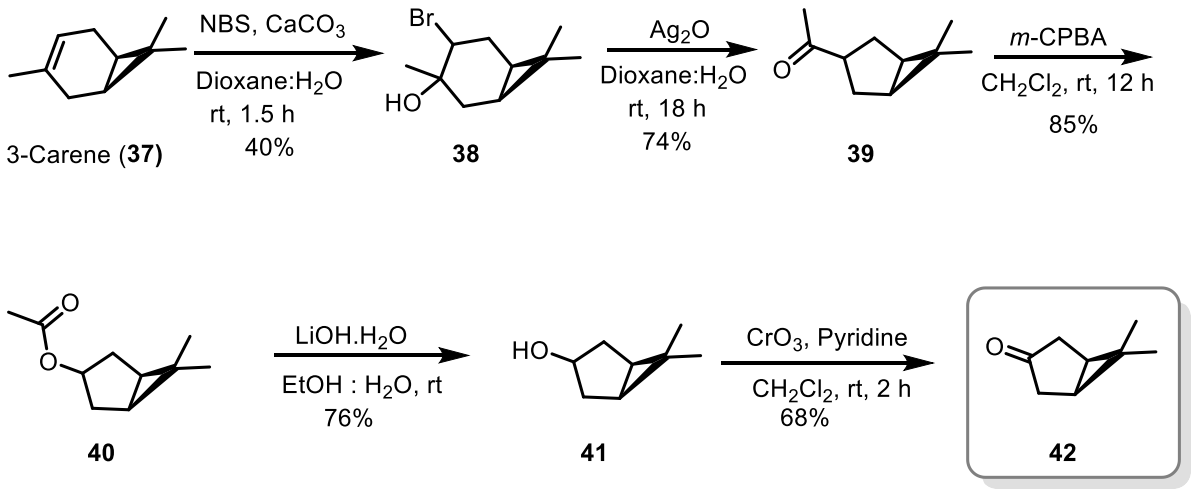


Fig 3.6: Different enones selected for Robinson annulation

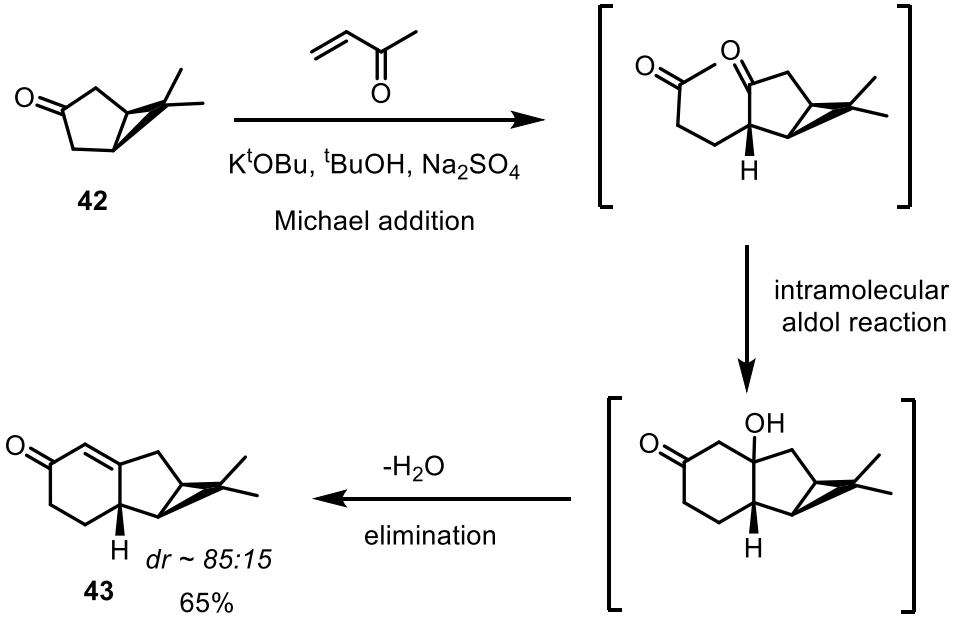
The required symmetric ketone **42** was prepared from 3-carene **37** using known literature procedure.[31] The reaction of 3-carene **37** with NBS, $CaCO_3$ afforded bromohydrin **38** which undergoes semi-pinacol rearrangement catalyzed by Ag_2O to give ketone **39**. The Baeyer-Villiger oxidation of ketone **39** by *m*-CPBA afforded ester **40** followed by hydrolysis using LiOH gave alcohol **41**. The secondary alcohol was oxidized by CrO_3 and pyridine (Collins reagent) to give symmetrical ketone **42** in 68% isolated yield (scheme 3.10).



Scheme 3.10: Synthesis of symmetric ketone **42** from 3-carene

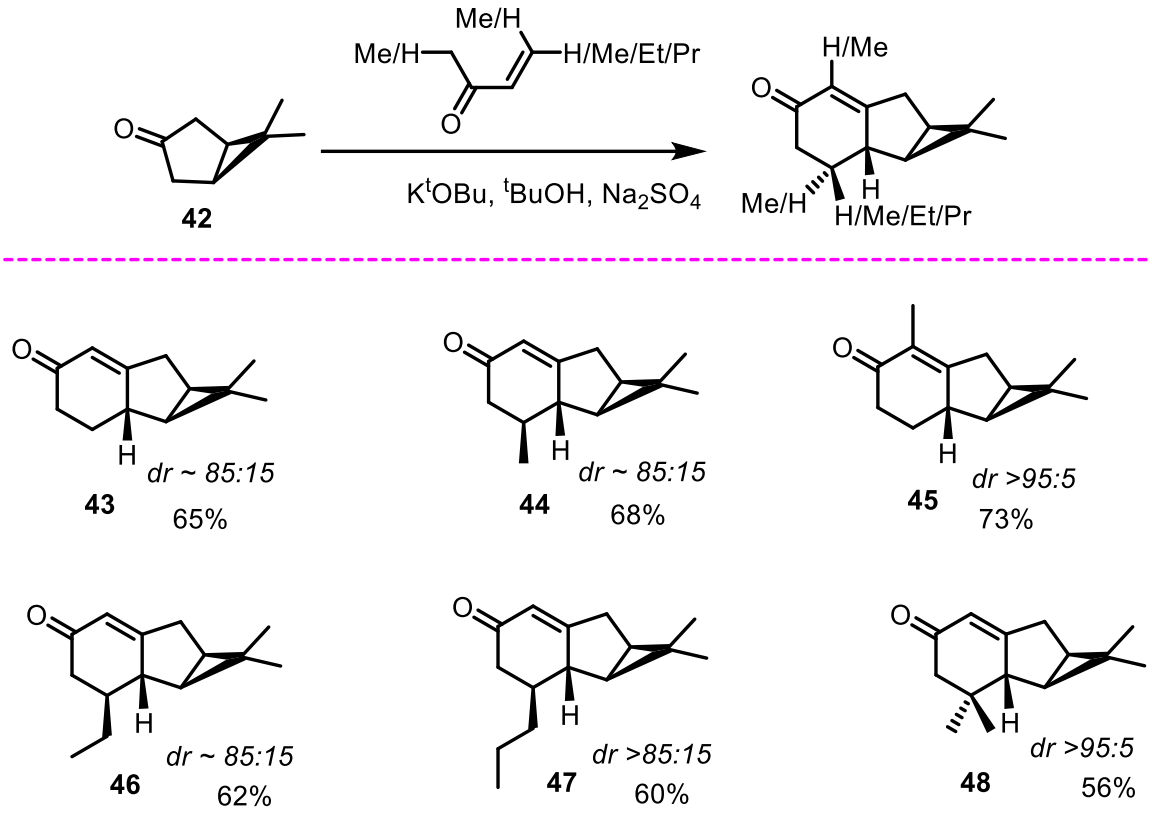
To demonstrate the planned strategy, the symmetric ketone **42** was treated with 10 mol % of potassium *tert*-butoxide in *t*-butanol followed by methyl vinyl ketone to undergo Robinson annulation[32] which afforded the desired enone **43** with 3/5/6 tricyclic fused

system in just one step (scheme 3.11). In this case, we observed ~85:15 diastereoselective ratios and it was determined in NMR detectable limits. The formation of product **43** was indicated by presence of olefinic proton at 5.70 (s, 1H) ppm in ^{1}H NMR spectrum and corresponding olefinic carbon signal at 181.1, 120.9 ppm and carbonyl carbon at 199.6 ppm in ^{13}C NMR spectrum. The product **43** was further confirmed by HRMS which showed a peak at 177.1268 corresponding to formula $C_{12}H_{17}O$ $[M+H]^{+}$ with calculated value of 177.1274.



Scheme 3.11: Synthesis of enone **43** *via* Robinson annulation

By applying similar reaction condition using ketone **42** and different substituted enones, this methodology was successfully implemented for the synthesis of various 3/5/6 tricyclic fused ring systems in moderate to good yields (scheme 3.12). The high stereoselectivity of the newly generated chiral centers can be explained by the rigid convex shape of the starting ketone **42**. All the synthesized 3/5/6 tricyclic fused ring systems were fully characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS and the assigned stereochemistry was supported by 2D NMR experiments on two compounds **45** and **46**.



Scheme 3.12: New strategy to access 3/5/6 skeleton by Robinson annulation

In case of **45** ^{13}C NMR spectrum indicated the presence of a carbonyl group (198.9), which was supported by the absorption at 1663 cm^{-1} in the IR spectrum. The ^{1}H NMR spectrum show the presence one allylic methyl protons absorption at δ 1.65 (s, 3 H).

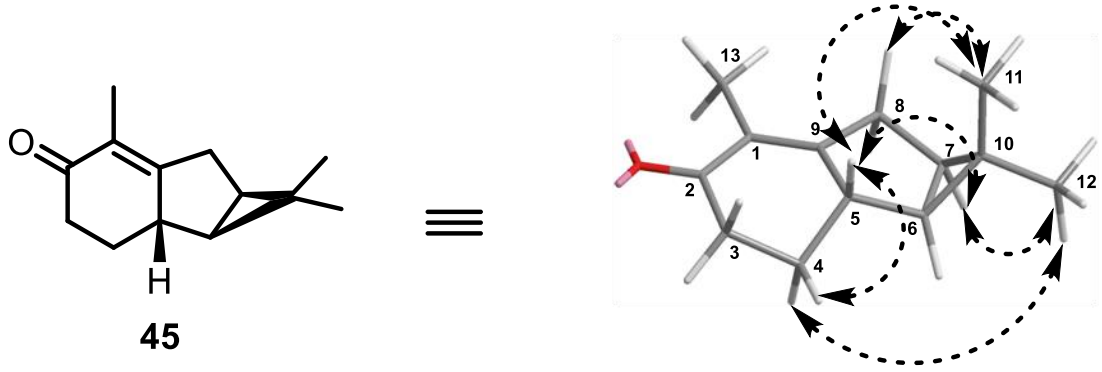


Fig 3.7: Key NOE correlations on energy minimized structure of **45**

The sequence of protons of H-3, H-4, H-5 were indicated by the COSY spectrum. The NOE spectrum indicated that H-5 has a positive NOE correlation with CH_3-11 position. There is also positive NOE correlation from CH_3-11 with Ha-8 position. The H-7 showed a positive NOE correlation with Hb-8 and CH_3-12 position. Thus it indicated that in compound **45**, methyl at 11 position, H-5 and Ha-8 were situated on the same side (Fig 3.7).

In case of **46** ^{13}C NMR spectrum indicated the presence of a carbonyl group (200.2), which was supported by the absorption at 1667 cm^{-1} in the IR spectrum. The ^{1}H NMR spectrum showed the presence of one olefinic proton absorption at δ 6.82 (s, 1 H). The sequence of protons of H-3, H-4, H-5 were indicated by the COSY spectrum. The HMBC spectrum indicated correlations from the hydrogen at C-5 into C-4 and C-9. The NOE spectrum indicated that H-5 is having a positive NOE correlation with CH_3-11, Ha-13, and Ha-3 position. There is also positive NOE correlation from CH_3-11 with Ha-8 position. The Hb-8 is also showing a positive NOE correlation H-7 position. The H-4 and H-6 have shown NOE correlations proving that they are in same phase. Thus it indicated in compound **46** CH_3-11, H-5 and Ha-13 were situated on the same side and it also suggests that C5-H and C4-H are *trans* to each other (Fig 3.8).

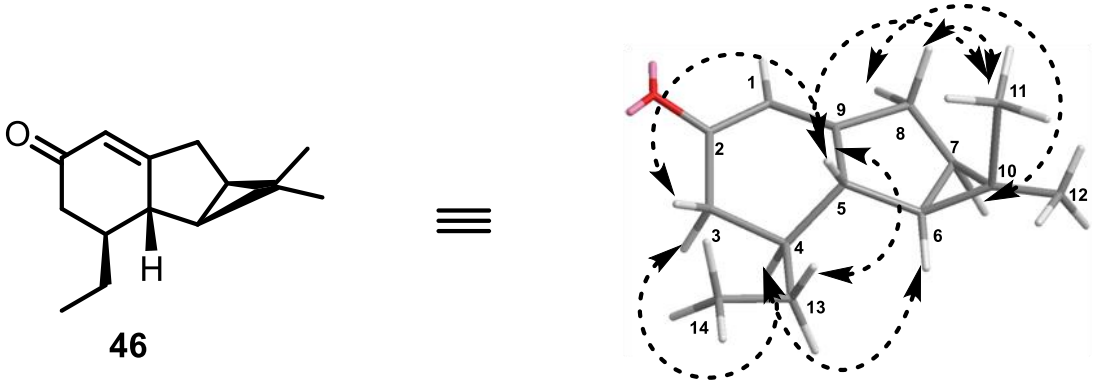


Fig 3.8: Key NOE correlations on energy minimized structure **46**

After successful implementation of Robinson annulation strategy to access 3/5/6 skeleton, all the synthesized compounds were evaluated for their repellent activity against adult females of *Aedes aegypti*.

3.3.5 Biological evaluation

3.3.5.1 Introduction

The several infectious diseases mainly transmitted by mosquito bites remain as one of the main causes of concern for human health worldwide. Successful population control of such insect vectors is the key to prevent transmission and epidemics of infectious diseases. Such control heavily relies on use of personal protective measures such as insecticides/ insect repellent.[33] One popular class of insecticides called pyrethroid are widely used to control population because of its high and rapid toxic activity toward insects and low toxicity to mammals.[34] However, resistance to commonly used insecticides has been reported[35-37] resulting in the need to increase concentrations of the active ingredients to improve efficacy. Several compounds with repellent activity are now commercially available and include DEET (**49**), Metofluthrin (**50**), Picaridin (**51**), IR3535 (**52**) (structurally similar to β-alanine), and oil of eucalyptus (*p*-menthane-3, 8-diol **53**) (Fig. 3.9).

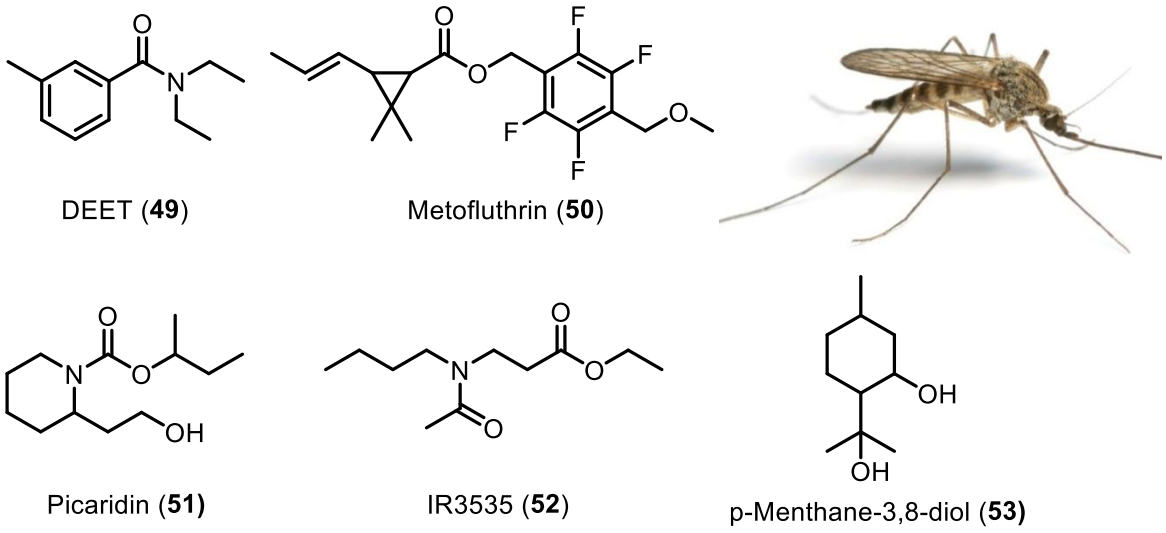


Fig 3.9: Selected insect repellents and mosquito (picture taken from internet)

Aedes aegypti also called the yellow fever mosquito is major vector responsible for spreading of diseases like dengue fever, chikungunya, Zika fever, and yellow fever viruses, and other diseases (Fig 3.10).

Fig 3.10: *Aedes aegypti* vector for dengue, chikungunya, Zika (picture taken from http://www.shutterstock.com/pic-377862691)

Only the female bites for blood, which she needs to mature her eggs. *A. aegypti* developed resistance to most widely used insecticides for mosquito control. There is urgent requirement to find safe and effective insect repellent to control such infectious diseases. In this context, we have hypothesized nardoaristolone B **14** natural product to show insecticidal activity as it has structural similarity to that of naturally occurring nootkatone (**16**),[38] (Fig 3.11) a well known natural product possesses very impressive insect repellent and/or insecticidal activity against various ticks, mosquitoes, termites, bed bugs, fleas etc.[39] with a mean LC_{50} range of 0.0029 – 0.0083% against *A.aegypti*, *Ixodes scapularis* and *Xenopsylla cheopis*. While the mode of action of nootkatone has not yet been established, it has been shown that it does not inhibit the target sites that confer resistance to organophosphates, carbamates, permethrins and dieldrin. This unique characteristic makes it an ideal candidate for vector control against insects that have developed resistance to currently used pesticides. Therefore, it is expected that compound **14** and its analogues show range of biological activities as the structural features are similar to that of nootkatone **16**. We have tested the repellent activity of nardoaristolone B and its analogues against adult females of *Aedes aegypti*, a vector of yellow fever, dengue fever and Zika fever for the first time.

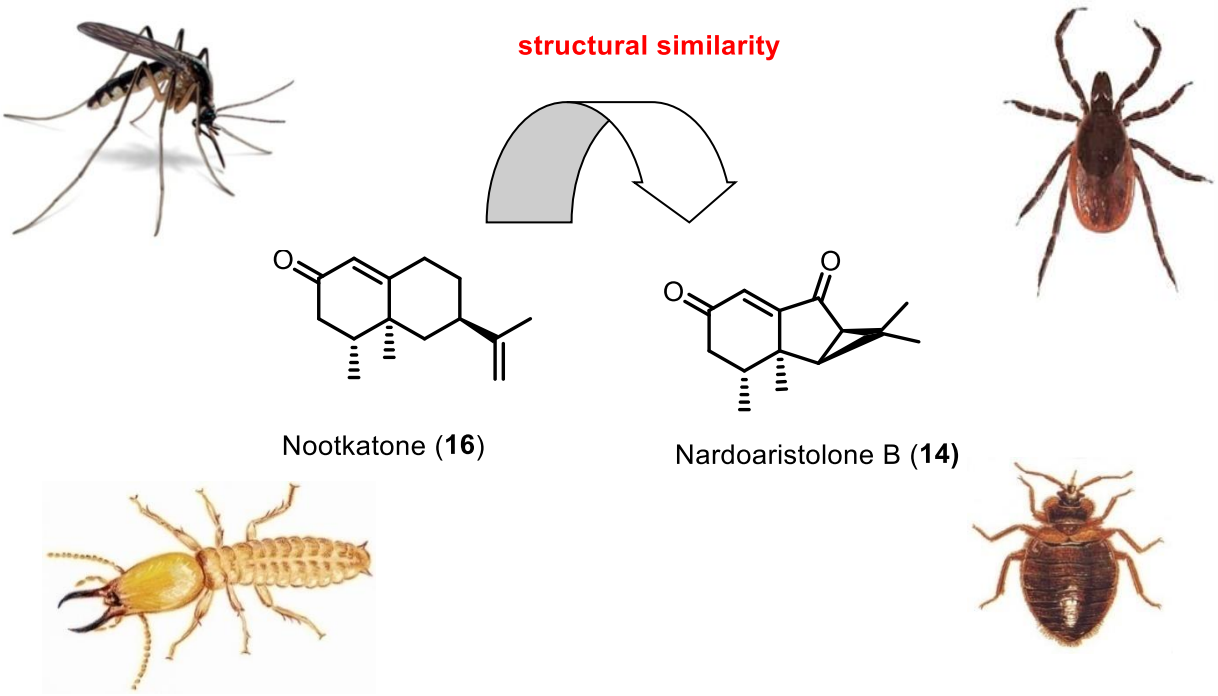


Fig 3.11: Selected natural products and insects mosquito, ticks, termite and bed bug (pictures taken from internet)

3.3.5.2 Mosquito repellence bioassay results

The list of compounds tested in mosquito repellent assays and protection time (min) offered by compounds are compiled in Fig 3.12. It was done in collaboration with Dr. Sen's group, entomology department at CSIR-NCL. Mosquito repellence activity was assessed on the basis of protection period (min) offered by nootkatone and various analogues of nardoaristolone B against adult females of *A.aegypti*. At the lower concentration of 0.25 mg/cm^2, the mean protection time for the positive control (+)-nootkatone **16** was 325 min of protection time. At 0.5 mg/cm^2, the mean of protection time (433 min) was significantly higher as compared to lower concentration. Under the same assay conditions, *rac*-nootkatone **16** provided 187 and 300 min protection from mosquitoes with 0.25 mg/cm^2 and 0.5 mg/cm^2, respectively. Nardoaristolone B **14** showed significant repellent activity with good dose response, in fact compound **14** showed superior activities when compared with *rac*-nootkatone **16**, it provided 217 and 368 min protection from mosquitoes with 0.25 mg/cm^2 and 0.5 mg/cm^2, respectively. Having these initial results in hand, we subjected all other analogues synthesized based on nardoaristolone natural products and we have found very interesting results. Out of eight compounds tested based on this skeleton, half of them

showed comparable or more potency than the racemic nootkatone. Two compounds, **44** and **45** resulted in about 360 min (6 h) or more protection from mosquitoes at 0.5 mg/cm^2 concentration (Fig 3.13). This protection period is more than racemic nootkatone and slightly lower than enantiopure (+)-nootkatone. As all our compounds are racemic compounds, it is appropriate for us to compare the data with racemic nootkatone. It is expected that chirally pure versions (atleast one of the enantiomers) of compounds **44** and **45** are expected to have better protection than natural (+)-nootkatone.

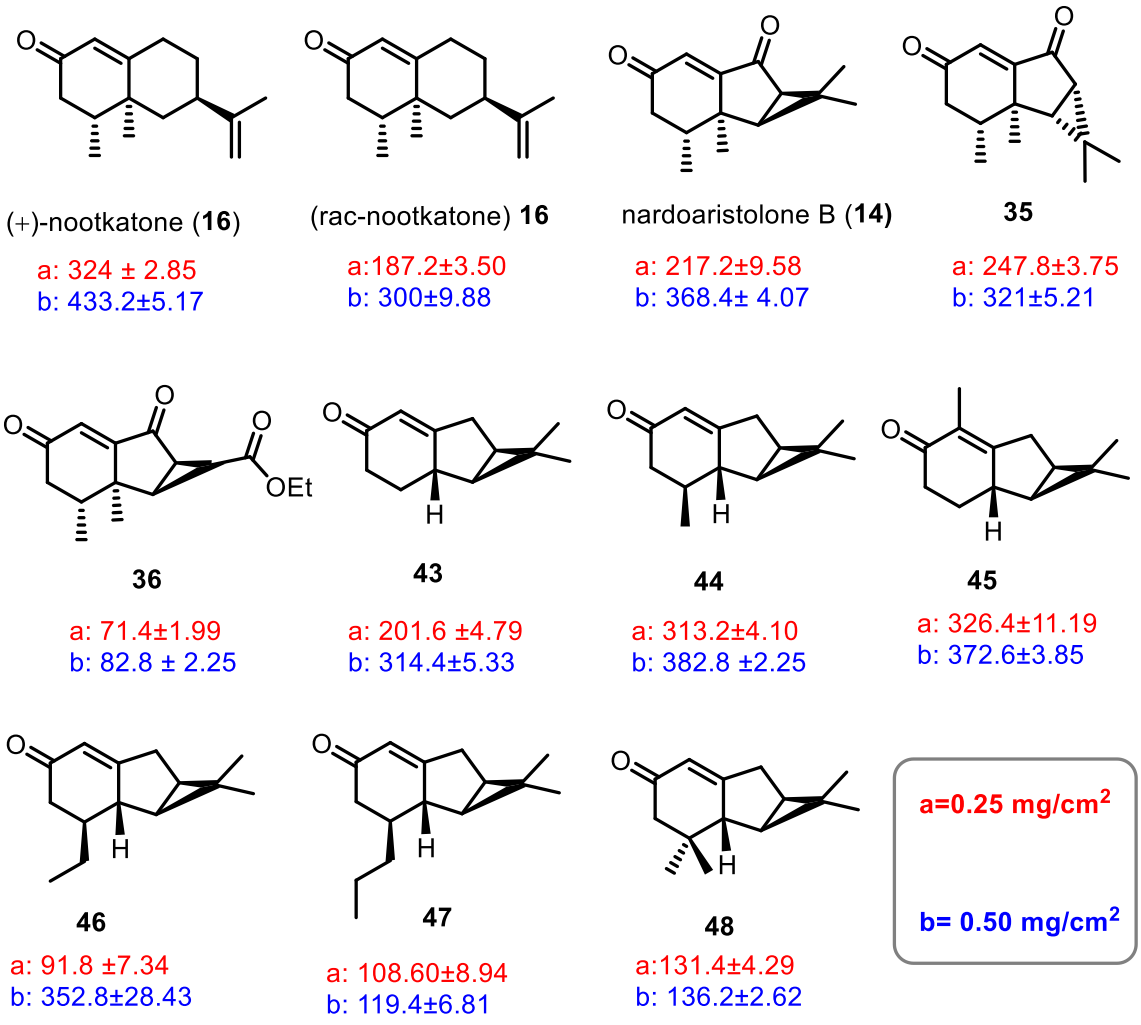


Fig 3.12: Repellent activity of all synthesized compounds from adult females of *A. aegypti* at two different concentration (a=0.25 mg/cm^2 and b= 0.50 mg/cm^2).

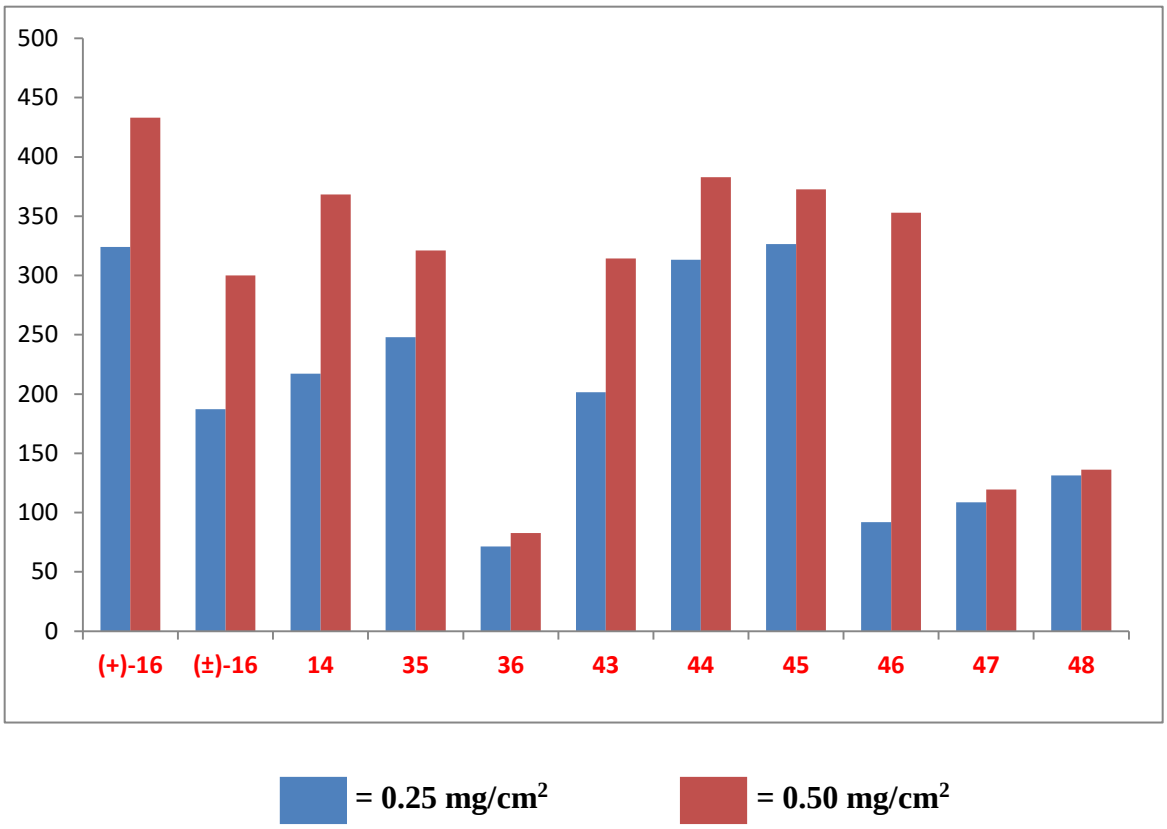


Fig 3.13: Protection period in min @ different concentration

Limited SAR analysis suggest that increase in chain length of alkyl substitution on six-membered ring, placing of *gem*-dimethyl group on the six membered ring or introduction of carboxylic ester moiety on the cyclopropyl ring significantly reduces the activity. To our knowledge, this is the first mosquito repellent study on this scaffold. Efforts along these lines are underway in our group to prepare enantiopure compounds and also to expand the scope of identified compounds as insect repellent and/or insecticidal activities against various ticks, mosquitoes, bed bugs, ants, fleas, flies, termites, etc.

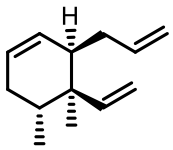
3.4 Conclusions

The first stereoselective synthesis of (±)-nardoaristolone B was accomplished using a very short and protecting-group free sequence. Our synthesis highlights use of sequential Diels-Alder/Wittig/RCM reactions, double allylic oxidation and stereoselective

cyclopropanations. We have synthesized a few novel analogues of nardoaristolone B including *exo*-cyclopropyl containing compound. We have also designed and demonstrated another simple strategy based on Robinson-annulation to access library of molecules of 3/5/6 tricyclic fused ring system. Considering the structural similarities between the nardoaristolone B and nootkatone, we have tested the repellent activity of all the synthesized analogues based on nardoaristolone B against adult females of *Aedes aegypti*, a vector for spreading dengue and Zika virus. Among them, two analogues (**44** and **45**) showed better activity compared to *rac*-nootkatone.

3.5 Experimental Procedures

(3*R*,4*S*,5*R*)-3-Allyl-4,5-dimethyl-4-vinylcyclohex-1-ene (24):



To a solution of diene **25** (100 mg, 1.04 mmol) and (*E*)-2-methylbut-2-enal (0.20 mL, 2.08 mmol) in dry CH_2Cl_2 (20 mL) was added $BF_3{\cdot}OEt_2$ (0.19 mL, 1.56 mmol) dropwise at –78 °C. The mixture was allowed to warm at room temperature and was stirred for 10 h at same temperature. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (3 x 10 mL) followed by H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material **26** obtained after the removal of solvent was immediately used for next step. To a suspension of methyl triphenylphosphonium bromide (743 mg, 2.08 mmol) in dry THF (10 mL) was added potassium *tert*-butoxide (233 mg, 2.08 mmol) at 0 ^oC. After 30 minutes, the solution became canary yellow color, to that above crude *bis*-aldehyde **26** in THF (10 mL) was added and allowed to stir at 0 ^oC for 1 h. The reaction was quenched with NH_4Cl (5.0 ml) and extracted with petroleum ether (2 x 30 mL). Combined organic layer was washed with water (15 mL), brine (15 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, petroleum ether as eluent) afforded **24** (18 mg, 10%) as colorless oil.

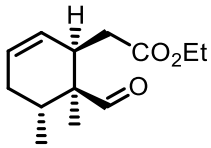
IRυ_{max} (film): 3054, 2986, 1604, 896 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01–5.94 (m, 1H), 5.86–5.77 (m, 1H), 5.62–5.61 (m, 2H), 5.05–4.99 (m, 4H), 2.31–2.19 (m, 2H), 1.91–1.84 (m, 2H), 1.72–1.64 (m, 2H), 1.03 (s, 3H), 0.86 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 146.1, 138.3, 129.1, 125.1, 115.7, 112.3, 43.9, 40.9, 35.8, 35.1, 31.6, 19.3, 16.4.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}$ [M-H] 175.1481, found 175.1480.

Ethyl 2-((1*R*,5*R*,6*S*)-6-formyl-5,6-dimethylcyclohex-2-en-1-yl)acetate (28):



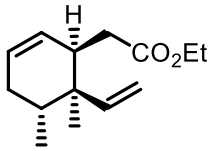
To a solution of diene **27** (6.0 g, 0.042 mol) and (*E*)-2-methylbut-2-enal (10.3 mL, 0.107 mol) in dry CH_2Cl_2 (200 mL) was added $BF_3{\cdot}OEt_2$ (10.6 mL, 0.085 mol) dropwise at –78 °C. The mixture was allowed to warm to room temperature and was stirred for 12 h at same temperature. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (3 x 50 mL) followed by H_2O (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 1.0:9.0 ethyl acetate: petroleum ether) to afford **28** (7.3 g, 76%) as light yellow oil.

IRυ_{max} (film): 2978, 1732, 1174 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 5.71–5.66 (m, 1H), 5.64–5.97 (m, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.65–2.59 (m, 1H), 2.45 (dd, *J* = 5.4 Hz, 15.8 Hz, 1H), 2.37–2.30 (m, 1H), 2.25–2.18 (m, 1H), 2.17–2.05 (m, 1H), 1.79–1.72 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.08 (s, 3H), 0.93 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 172.6, 127.4, 126.1, 60.4, 49.6, 37.6, 35.6, 30.6, 29.5, 15.7, 15.6, 13.9.

Ethyl 2-((1*R*,5*R*,6*S*)-5,6-dimethyl-6-vinylcyclohex-2-en-1-yl)acetate (29):



To a suspension of methyl triphenylphosphonium bromide (26.8 g, 0.075 mol) in dry THF (60 mL) was added potassium *tert*-butoxide (7.6 g, 0.068 mol) at 0 °C. After 30 minutes, the solution became canary yellow color, to that aldehyde **28** (5.1 g, 0.022 mol) in THF (30 mL) was added and allowed to stir at 0 °C for 1 h. The reaction was quenched with brine (30 ml) and extracted with ethyl acetate (2 x 50 mL). Combined organic layer was washed with water (30 mL), brine (30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether) afforded **29** (4.2 g, 84%) as light brown oil.

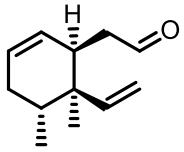
IR υ_{max} (film): 1725, 1521, 1417, 1215 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (dd, *J* = 11.0 Hz, 18.3 Hz, 1H), 5.65–5.53 (m, 2H), 5.06–5.05 (m, 1H), 5.03–5.02 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.48–2.39 (m, 2H), 2.23–2.18 (m, 1H), 2.12–2.07 (m, 1H), 1.73–1.62 (m, 2H), 1.25 (t, *J* = 7.3 Hz, 3H), 1.02 (s, 3H), 0.87 (d, *J* = 6.4 Hz, 3H);

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 145.2, 128.6, 126.2, 113.3, 60.4, 41.2, 40.5, 36.7, 34.4, 31.5, 18.8, 16.4, 14.4.

HRMS (ESI): *m/z* calcd for $C_{14}H_{22}O_2Na$ $[M+Na]^+$ 245.1512, found 245.1508.

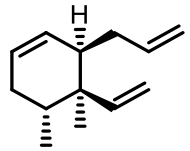
2-((1*R*,5*R*,6*S*)-5,6-Dimethyl-6-vinylcyclohex-2-en-1-yl)acetaldehyde (30):



To a solution of ester **29** (0.5 g, 2.27 mmol) in dry distilled toluene (15 mL) was added DIBAL-H (1.0 M in toluene, 1.36 mL, 1.36 mmol) at -78 °C dropwise. Stirred at this temperature for 10 min and then added DIBAL-H (1.0 M in toluene, 1.13 mL, 1.13 mmol) at -78 °C dropwise. After stirring at the same temperature for 0.5 h, the reaction was quenched with methanol (3 mL), diluted with Et_2O (20 mL) and sat. Na/K tartrate (15 mL). The solution was stirred at room temperature for 2 h. Extracted the solution with ethyl

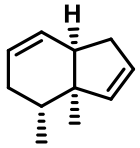
acetate (3 x 20 mL) and the combined extracts were dried over Na_2SO_4. The crude mixture was passed through small bed of silica gel and eluted with 20% ethyl acetate: petroleum ether. The eluent was concentrated *in vacuo* to give the aldehyde **30** (0.33 g, 81%) as a colorless oil which was immediately used for next step.

(3*R*,4*S*,5*R*)-3-Allyl-4,5-dimethyl-4-vinylcyclohex-1-ene (24) :



To a suspension of methyl triphenylphosphonium bromide (14.5 g, 0.040 mol) in dry THF (40 mL) was added potassium *tert*-butoxide (4.1 g, 0.037 mol) at 0 ^oC. After 30 minutes, the solution became canary yellow color, to that aldehyde **30** (2.2 g, 0.012 mol) in THF (20 mL) was added and allowed to stir at 0 ^oC for 1 h. The reaction was quenched with NH_4Cl (20 ml) and extracted with petroleum ether (2 x 60 mL). Combined organic layer was washed with water (20 mL), brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, petroleum ether as eluent) afforded **24** (1.7 g, 79%) as colorless oil. Spectral data was identical with that of above compound **24.**

(3a*R*,4*R*,7a*R*)-3a,4-Dimethyl-3a,4,5,7a-tetrahydro-1H-indene (23):



To a solution of **24** (2.0 g, 0.011 mol) in dry CH_2Cl_2 (100 mL) was added Grubbs' second-generation catalyst (480 mg, 5 mol %) at room temperature. After stirring for 24 h, reaction mixture was filtered through celite and filtrate was concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, petroleum ether as eluent) to afford **23** (1.2 g, 72%) as colorless oil.

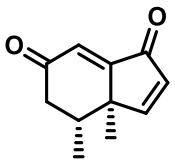
IRυ_{max} (film): 3054, 2987, 1667, 986 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.79–5.72 (m, 2H), 5.69–5.66 (m, 2H), 2.56–2.49 (m, 1H), 2.25–2.20 (m, 1H), 2.11–2.04 (m, 1H), 1.93–1.87 (m, 1H), 1.78–1.69 (m, 1H), 1.63–1.56 (m, 1H), 0.92 (s, 3H), 0.90 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 141.7, 129.3, 128.5, 125.9, 48.0, 47.7, 38.9, 34.1, 31.0, 18.7, 15.8.

GC-HRMS (EI): *m/z* calcd for $C_{11}H_{16}$, 148.1252 found 148.1252.

((3aR,4R)-3a,4-Dimethyl-1,6-dioxo-3a,4,5,6-tetrahydro-1H-inden-2-ylium) (22):



To a solution of diene **23** (1 g, 6.75 mmol) in EtOAc (100 mL) were added 4 Å molecular sieves (2 g) and ^{t}-BuOOH (5.0 M in decane, 6.7 mL, 33.7 mmol) at room temperature. The reaction mixture was stirred for 30 min before $Mn(OAc)_3.2H_2O$ (905 mg, 3.37 mmol) was added at room temperature. The resulting mixture was stirred in nitrogen atmosphere for 24 h before it was filtered through celite, eluted with EtOAc (30 mL) and concentrated in *vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 2:8 ethyl acetate: petroleum ether) to afford **22** and mono oxidised mixtures which was again oxidised using similar reaction condition to give **22** (725 mg, 61% recycled yield)) as yellow solid.

M.p.: 70–72 °C.

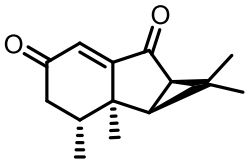
IRυ_{max} (film): 2975, 2893, 1720, 1664, 1218 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70 (d, *J* = 5.9 Hz, 1H), 6.35 (d, *J* = 5.9 Hz, 1H), 6.27 (s, 1H), 2.50–2.46 (m, 2H), 2.27–2.21 (m, 1H), 1.22 (s, 3H), 1.13 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.7, 195.8, 165.0, 160.4, 133.6, 121.8, 46.5, 42.8, 36.3, 18.4, 15.0.

HRMS (ESI): *m/z* calcd for $C_{11}H_{13}O_2$ $[M+H]^+$ 177.0910, found 177.0909.

(1a*S*,1b*R*,2*R*,6a*R*)-1,1,1b,2-Tetramethyl-1,1a,1b,2,3,6a-hexahydrocyclopropa[a] indene-4,6-dione (14):



(±)-Nardoaristolone B **14**

A 1.6 M solution of ^{t}-BuLi in pentane (0.54 mL, 0.852 mmol) was added dropwise to a suspension of isopropyl diphenylsulfonium tetrafluoroborate (296 mg, 0.937 mmol) in THF (4 mL) at -78 °C. After 30 min, **22** (50 mg, 0.284 mmol) was added as a solution in THF (2 mL) at -78 °C. The resulting mixture was maintained at -30 °C for 1 h, quenched with saturated aqueous NH_4Cl (2 mL), and allowed to warm to room temperature. The reaction mixture was then extracted with ethyl acetate (3 x 5 mL). Combined organic layer was washed with water (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.5:8.5 ethyl acetate: petroleum ether) afforded (±)-Nardoaristolone B **14** (20 mg, 32%) as yellow solid.

M.p.: 60-62 ºC.

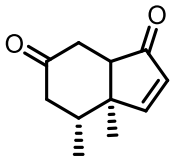
IRυ_{max} (film): 1706, 1440, 1219 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.18 (s, 1H), 2.40–2.23 (m, 3H), 1.94 (d, *J* = 5.6 Hz, 1H), 1.77 (d, *J* = 5.6 Hz, 1H), 1.17 (s, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 1.06 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 201.5, 200.0, 165.1, 123.6, 44.3, 42.3, 42.2, 40.3, 35.5, 32.1, 28.8, 20.8, 17.8, 15.8.

HRMS (ESI): *m/z* calcd for $C_{14}H_{19}O_2$ $[M+H]^+$ 219.1380, found 219.1377.

(3a*R*,4*R*)-3a,4-Dimethyl-4,5,7,7a-tetrahydro-1H-indene-1,6(3aH)-dione (32):



To a stirred solution of dienedione **22** (200 mg, 1.13 mmol) in CH_3COOH (10 mL) at room temperature was added zinc dust (436 mg, 6.81 mmol). The resulting mixture was stirred for 30 min before being filtered on a short pad of celite and washed with EtOAc (20 mL). The resulting organic layer was concentrated *in vacuo* and the crude obtained was purified on column chromatography (silica gel 100–200, 2.5:7.5 ethyl acetate: petroleum ether) to afford enone **32** (147 mg, 73%) as white solid.

M.p.: 68–70 °C.

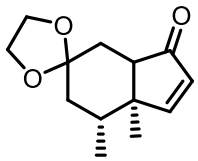
IRυ_{max} (film): 3020, 2975, 1702, 1602 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, *J* = 5.8 Hz, 1H), 6.17 (d, *J* = 5.8 Hz, 1H), 2.67–2.62 (m, 1H), 2.53–2.48 (m, 1H), 2.32–2.26 (m, 2H), 2.20–2.14 (m, 1H), 2.07–2.04 (m, 1H), 1.16 (s, 3H), 1.07 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 210.0, 209.5, 170.5, 131.2, 51.5, 47.0, 43.1, 38.7, 35.1, 19.8, 15.5.

HRMS (ESI): *m/z* calcd for $C_{11}H_{14}O_2Na$ $[M+Na]^+$ 201.0886, found 201.0885.

(7*R*,7a*R*)-7,7a-Dimethyl-3a,6,7,7a-tetrahydrospiro[indene-5,2'-[1,3]dioxolan]-3(4H)-one (33):



To a solution of the **32** (120 mg, 0.674 mmol) in benzene (10 mL) was added ethylene glycol (86 mg, 1.34 mmol) and *p*-TSA (12 mg, 0.067 mmol) at room temperature. The reaction was refluxed for 1 h and then quenched by saturated $NaHCO_3$ (3 mL) and extracted with ethyl acetate (3 x 5 mL). Combined organic layer was washed with water (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 2.0:8.0 ethyl acetate: petroleum ether) afforded **33** (130 mg, 87%) as white solid.

M.p.: 62–64 °C.

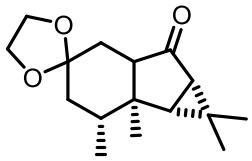
IRυ_{max} (film): 2973, 1712, 1425, 928 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.65 (d, *J* = 5.8 Hz, 1H), 6.07 (d, *J* = 5.8 Hz, 1H), 3.98–3.87 (m, 4H), 2.21–2.12 (m, 2H), 1.81–1.77 (m, 1H), 1.74–1.70 (m, 1H), 1.66–1.56 (m, 2H), 1.15 (s, 3H), 0.96 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 209.4, 171.1, 130.5, 108.8, 64.4, 64.1, 54.6, 46.6, 39.5, 37.3, 30.1, 18.0, 16.4.

HRMS (ESI): *m/z* calcd for $C_{13}H_{18}O_3Na$ $[M+Na]^+$ 245.1148, found 245.1143.

(1a*R*,1b*R*,2*R*,6a*S*)-1,1,1b,2-Tetramethyloctahydro-6H-spiro[cyclopropa[a]indene-4,2'-[1,3]dioxolan]-6-one (34):



A 1.6 M solution of ^{t}BuLi in pentane (0.84 mL, 1.34 mmol) was added dropwise to a suspension of isopropyl diphenylsulfonium tetrafluoroborate (465 mg, 1.47 mmol) in THF (6 mL) at -78 °C. After 30 min, **33** (100 mg, 0.446 mmol) was added as a solution in THF (4 mL). The resulting mixture was maintained at -30 °C for 1 h, quenched with saturated aqueous NH_4Cl (3 mL), and allowed to warm to room temperature. The reaction mixture was then extracted with ethyl acetate (3 x 10 mL). Combined organic layer was washed with water (10 mL), brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.5:8.5 ethyl acetate: petroleum ether as eluent) afforded **34** (59 mg, 50%, 71% brsm) as white solid.

IRυ_{max} (film): 1712, 1522, 1425, 1216 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.99–3.83 (m, 4H), 2.32–2.28 (m, 1H), 2.19–2.17 (m, 1H), 1.94–1.89 (m, 1H), 1.76 (d, *J* = 5.8 Hz, 1H), 1.68 (d, *J* = 5.8 Hz, 1H), 1.50−1.42, (m, 3H), 1.34 (s, 3H), 1.12 (s, 3H), 1.09 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 211.8, 107.8, 64.3, 63.9, 53.4, 43.7, 40.5, 40.2, 38.7, 37.2, 29.9, 28.9, 28.2, 18.0, 16.9, 16.4.

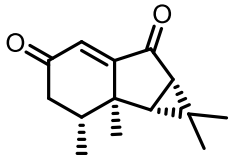
HRMS (ESI): *m/z* calcd for $C_{16}H_{24}O_3Na$ $[M+Na]^+$ 287.1618, found 287.1614.

Single X-ray Crystal Structure of 34: A single crystal of compound **34** was obtained from ethyl acetate-hexane mixture. X-ray intensity data were collected on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (Mo Kα=0.71073 Å) radiation at room temperature 296(2) K. The X-ray generator was operated at 50 kV and 30 mA. Diffraction data were collected with a ω scan width of 0.5° and at different settings of φ and 2θ. The sample-to-detector distance was fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX II program suite.[40] All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX II program package.[40] The structure was solved by direct

method and refined by full matrix least squares, based on *F*, using SHELX-97.[41] Molecular diagrams were generated using XSHELL program integrated in SHELXTL package. All the H-atoms were placed in geometrically idealized position and constrained to ride on their parent atoms.

Crystallographic data for 34 ($C_{16}H_{24}O_3$): M = 264.35, Crystal dimensions 0.62 x 0.41 x 0.16 mm^3, triclinic, space group P -1, a = 6.6062(3), b = 9.7077(4), c = 11.6325(5) Å, α = 95.271(2), β = 103.669(2), γ =93.602(2)°, V = 719.05(5) $Å^3$, Z = 2, ρ_{calcd} = 1.221 gcm^{-3}, μ (Mo-Kα) = 0.082 mm^{-1}, *F(000)* = 288, $2\theta_{max}$ = 56.76°, T = 296(2) K, 14390 reflections collected, 3586 unique, 3084 observed ($I > 2\sigma(I)$) reflections, 176 refined parameters, R value 0.0470, $wR2$ = 0.1323, (all data R = 0.0538, $wR2$ = 0.1387), S = 1.058, minimum and maximum transmission 0.951 and 0.987; maximum and minimum residual electron densities +0.30 and –0.18 $eÅ^{-3}$.

(1a*R*,1b*R*,2*R*,6a*S*)-1,1,1b,2-Tetramethyl-1,1a,1b,2,3,6a-hexahydrocyclopropa[a] indene-4,6-dione (35):



To a solution of ketone **34** (40 mg, 0.151 mmol) in DMSO (5 mL) were added IBX (128 mg, 0.454 mmol) and TFA (~1 drop) at room temperature. After being stirred at 80 °C for 24 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (2 mL). The aqueous layer was extracted with EtOAc (3 x 5 mL) and combined organic layer was washed with saturated aqueous $NaHCO_3$ (5 mL), water (5 mL), brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.5:8.5 ethyl acetate: petroleum ether as eluent) afforded **35** (22 mg, 66%) as yellow solid.

M.p.: 58–60 °C.

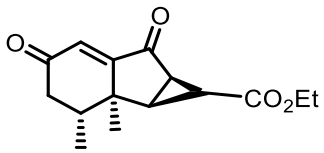
IRυ_{max} (film): 1706, 1440, 1219 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.12 (s, 1H), 2.46–2.31 (m, 2H), 2.19−2.14 (m, 1H), 2.01 (d, J = 5.6 Hz, 1H), 1.94 (d, J = 5.6 Hz, 1H), 1.33 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H), 1.15 (d, J = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 201.6, 200.1, 165.0, 122.1, 42.5, 41.8, 41.5, 41.4, 40.0, 31.5, 29.1, 20.6, 16.4, 15.0.

HRMS (ESI): *m/z* calcd for $C_{14}H_{19}O_2$ $[M+H]^+$ 219.1380, found 219.1378.

Ethyl (1*R*,1a*S*,1b*R*,2*R*,6a*R*)-1b,2-dimethyl-4,6-dioxo-1,1a,1b,2,3,4,6,6a-octahydrocyclopropa[a]indene-1-carboxylate (36):



DBU (0.06 mL, 0.426 mmol) was added to a suspension of (ethoxycarbonylmethyl)-dimethylsulfonium bromide (97 mg, 0.426 mmol) in $CHCl_3$ (5.0 mL) at room temperature. After 30 min, compound **22** (50 mg, 0.284 mmol) in $CHCl_3$ (2.0 mL) was added and the solution was allowed to stir for 16 h at room temperature. The organic solvents were evaporated and then partitioned between CH_2Cl_2 (15 mL) and water (5 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel column chromatography (silica gel 100–200, 2.5:7.5 ethyl acetate: petroleum ether as eluent) afforded **36** (43 mg, 58%) as colorless oil.

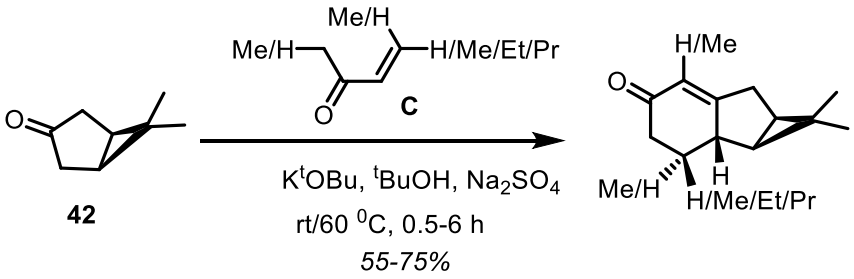
IRυ_{max} (film): 1724, 1664, 1421, 1265 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.22 (s, 1H), 4.09–4.02 (m, 2H), 2.52–2.48 (m, 1H), 2.42–2.31 (m, 4H), 2.28–2.24 (m, 1H), 1.26 (s, 3H), 1.19 (t, *J* = 7.0 Hz, 3H), 1.15 (d, *J* = 6.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.9, 199.6, 168.8, 161.6, 124.2, 61.9, 43.5, 42.2, 35.4, 35.1, 31.5, 31.0, 21.3, 15.5, 14.0.

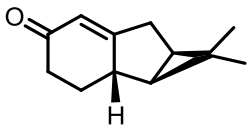
HRMS (ESI): *m/z* calcd for $C_{15}H_{18}O_4Na$ $[M+Na]^+$ 285.1097, found 285.1094.

General Optimization Procedure for D:



To a stirred solution of compound **42** (0.806 mmol, 1 eq) and Na_2SO_4 (400 mg) in dry *tert* butanol (5 mL) at room temperature was added catalytic amount of KO^{t}Bu (0.080 mmol, 0.1 eq) and then **C** (0.967 mmol, 1.2 eq) slowly in dropwise manner. Stirring was continued for 0.5 h–6 h at room temperature or 60 ^{o}C. Reaction was quenched by the addition of saturated aq. NH_4Cl (2 mL). Organic solvent was evaporated, water (5 mL) and ethyl acetate (10 mL) were added, organic solvent was separated and aqueous layer was extracted with ethyl acetate (2 x 10 mL), combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Compound was purified by column chromatography (silica gel 100-200) using ethyl acetate and petroleum ether as an eluent to afford enones.

(1a*R*,1b*S*,6a*R*)-1,1-Dimethyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (43):



dr ~ 85:15

Yield: 65%.

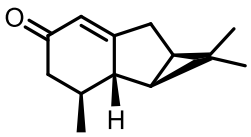
IRυ_{max} (film): 1663, 1367, 1247 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$): δ 5.70 (s, 1H), 2.71–2.64 (m, 1H), 2.46−2.35 (m, 4H), 2.31−2.26 (m, 1H), 2.23−2.18 (m, 1H), 1.81−1.77 (m, 1H), 1.34−1.30 (m, 1H), 1.05 (s, 3H), 1.00 (s, 3H).

^{13}C NMR (100 MHz, CDCl$_3$): δ 199.6, 181.1, 120.9, 40.9, 37.9, 34.5, 32.4, 30.4, 29.1, 27.2, 22.8, 14.3.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1268.

(1a*R*,1b*S*,6a*R*)-1,1,2-Trimethyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (44):



dr ~ 85:15

Yield: 68%.

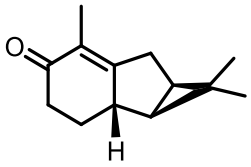
IRυ_{max} (film): 1667, 1373, 1236 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (s, 1H), 2.70–2.64 (m, 1H), 2.42−2.34 (m, 2H), 2.03−1.96 (m, 2H), 1.29−1.25 (m, 1H), 1.14−1.12 (m, 1H), 1.11 (d, *J* = 5.9 Hz, 3H), 1.08−1.06 (m, 1H), 1.06 (s, 3H), 0.98 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.0, 180.3, 121.2, 48.1, 46.3, 37.0, 32.8, 32.3, 28.6, 27.2, 22.8, 20.7, 14.4.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O$ $[M+H]^+$ 191.1430, found 191.1429.

(1a*R*,1b*S*,6a*R*)-1,1,5-Trimethyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (45):



dr > 95:5

Yield: 73%.

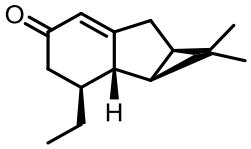
IRυ_{max} (film): 1663, 1216 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.78–2.71 (m, 1H), 2.51−2.42 (m, 2H), 2.35−2.25 (m, 2H), 2.15−2.11 (m, 1H), 1.82−1.72 (m, 1H), 1.65 (s, 3H), 1.32−1.28 (m, 1H), 1.04 (s, 3H), 1.04−1.02 (m, 1H), 0.97 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.9, 174.2, 126.7, 41.4, 38.4, 34.9, 31.4, 30.3, 29.3, 27.2, 22.1, 14.3, 11.6.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O$ $[M+H]^+$ 191.1430, found 191.1427.

(1a*R*,1b*S*,6a*R*)-2-Ethyl-1,1-dimethyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (46):



dr ~ 85:15

Yield: 62%.

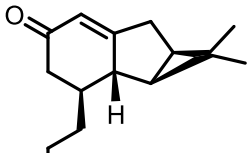
IRυ_{max} (film): 1668, 1368, 1248 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.71 (s, 1H), 2.70–2.64 (m, 1H), 2.53−2.49 (m, 1H), 2.38−2.34 (m, 1H), 2.11−2.08 (m, 1H), 1.97−1.90 (m, 1H), 1.87−1.82 (m, 1H), 1.77−1.71 (m, 1H), 1.36−1.25 (m, 2H), 1.15−1.13 (m, 1H), 1.06 (s, 3H), 0.98 (s, 3H), 0.92 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.2, 180.6, 121.0, 46.1, 43.1, 42.8, 32.7, 32.3, 28.7, 27.4, 27.3, 22.9, 14.4, 10.6.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O$ $[M+H]^+$ 205.1587, found 205.1584.

(1a*R*,1b*S*,6a*R*)-1,1-Dimethyl-2-propyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (47):



dr ~ 85:15

Yield: 60%.

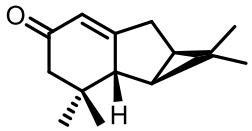
IRυ_{max} (film): 1669, 1351, 1233 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.67 (s, 1H), 2.65–2.60 (m, 1H), 2.49−2.47 (m, 1H), 2.35−2.30 (m, 1H), 2.17−2.10 (m, 1H), 2.06−2.04 (m, 1H), 1.91−1.86 (m, 2H), 1.63−1.59 (m, 1H), 1.43−1.35 (m, 1H), 1.25−1.19 (m, 2H), 1.12−1.10 (m, 1H), 1.03 (s, 3H), 0.95 (s, 3H), 0.89 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.1, 180.5, 121.0, 46.5, 43.4, 41.5, 37.2, 32.8, 32.3, 28.6, 27.3, 22.9, 19.4, 14.4, 14.3.

HRMS (ESI): *m/z* calcd for $C_{15}H_{23}O$ $[M+H]^+$ 219.1743, found 219.1739.

(1a*R*,1b*R*,6a*R*)-1,1,2,2-Tetramethyl-1a,1b,2,3,6,6a-hexahydrocyclopropa[a]inden-4(1H)-one (48):



dr > 95:5

Yield: 56%.

IRυ_{max} (film): 1668, 1367, 1246 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (s, 1H), 2.71–2.64 (m, 1H), 2.44−2.36 (m, 2H), 2.18 (s, 2H), 1.28−1.26 (m, 1H), 1.13 (s, 3H), 1.11−1.09 (m, 1H), 1.07 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.7, 178.3, 121.4, 53.1, 51.6, 38.5, 32.6, 30.4, 30.0, 28.7, 27.3, 21.8, 19.9, 14.3.

HRMS (ESI): *m/z* calcd for $C_{14}H_{21}O$ $[M+H]^+$ 205.1587, found 205.1583.

Mosquito repellence bioassays

The protection period was measured on the basis of the concept "time until the first bite".[43] Repellence tests were carried out between 09.00 – 17.00 h against 3-5 days old, disease free, blood starved but sucrose fed (0.5 M solution) *A.aegypti* female mosquitoes drawn from an established laboratory colony maintained at 27±1 ^{o}C, 70 ± 5% RH and a 12:12 h light: day cycle. The light intensity was regulated at 300-500 lux. A human volunteer's hand washed with unscented soap and subsequently rinsed with 70% ethanol was air-dried and covered with polythene disposable gloves and introduced in a cage (40 x 40 x 40 cm) containing about 200 hungry mosquitoes. A control arm was used to estimate the readiness of mosquitoes to take a bite. Mosquitoes were allowed to bite on the back of the hand through muslin screen stuck over a small window (2 x 2 cm) cutout in the polythene glove. Various analogues of nardoaristolone B were loaded on the muslin cloth screen instead of direct skin application so as to avoid the potential risk involved in the evaluation of natural products of unknown mammalian toxicity. A 1% stock solution of each compound was prepared using Analar grade acetone. 100 μL and 200 μL of this stock solution were spread evenly on the muslin cloth screen (thereby yielding a dose of 0.25 mg/cm^2 and 0.50

mg/cm^2, respectively) and the solvent was allowed to evaporate. A similar polyethylene glove with muslin cloth screen and treated with solvent alone served as control. After introduction of the hand covered with the polythene glove with the treated muslin screen into the mosquito cage, the numbers of mosquito bites received in the subsequent 5 min were counted. In the event of no bites during the initial 5 min exposure, the test hand was exposed repeatedly after every consecutive ½ hr for 5 min test till the time a confirmed bite was received. The number of minutes before the receipt of a confirmed bite represented the protection period offered by the test compound. In control, the rate of mosquito bite was 10-12 bites/min with the first bite occurring within 10 secs. The above test was repeated with both male (3) and female (2) human volunteers using a new batch of mosquitoes for each test. The two-way ANOVA indicated strong differences between the different analogues ($F=11.43$, df 25, $p<0.0001$), concentration ($F=43.33$; df 25, $p<0.0001$) and a significant interaction between compound/concentration ($F=41.43$; df 25, $p<0.0001$). The analysis of contrasts by the Tukey's method indicated differences between the individual analogues and concentration.

3.6 References

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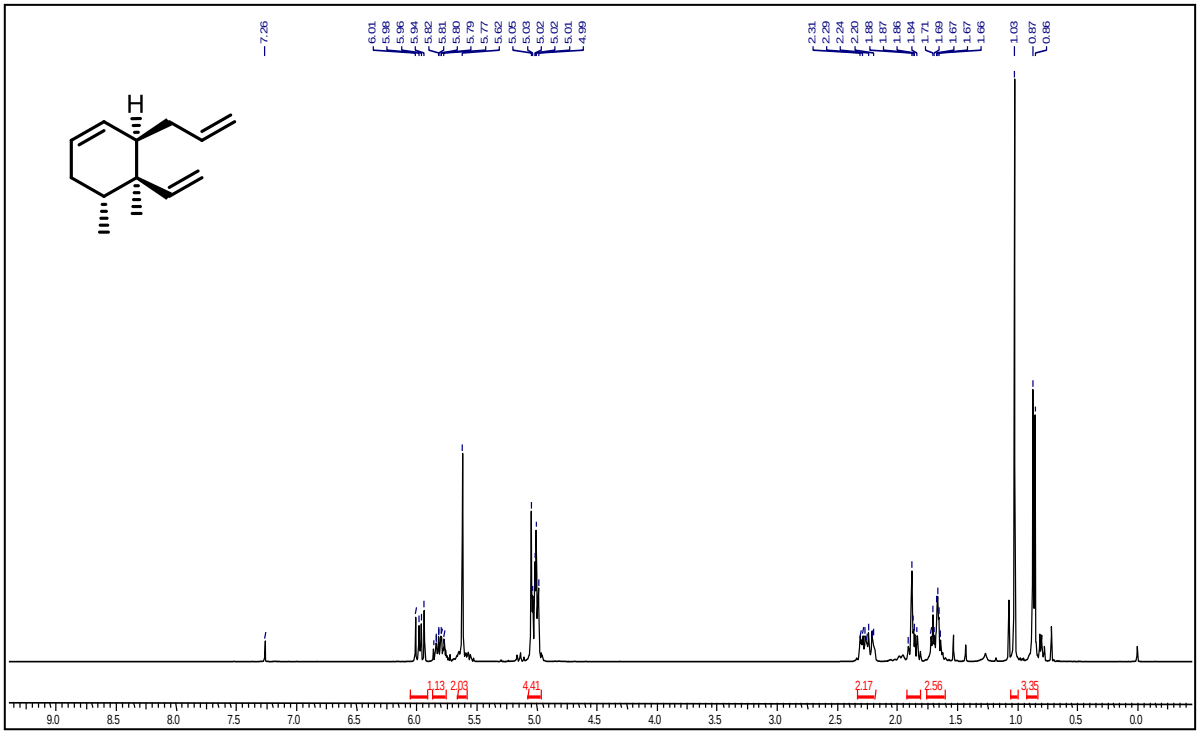
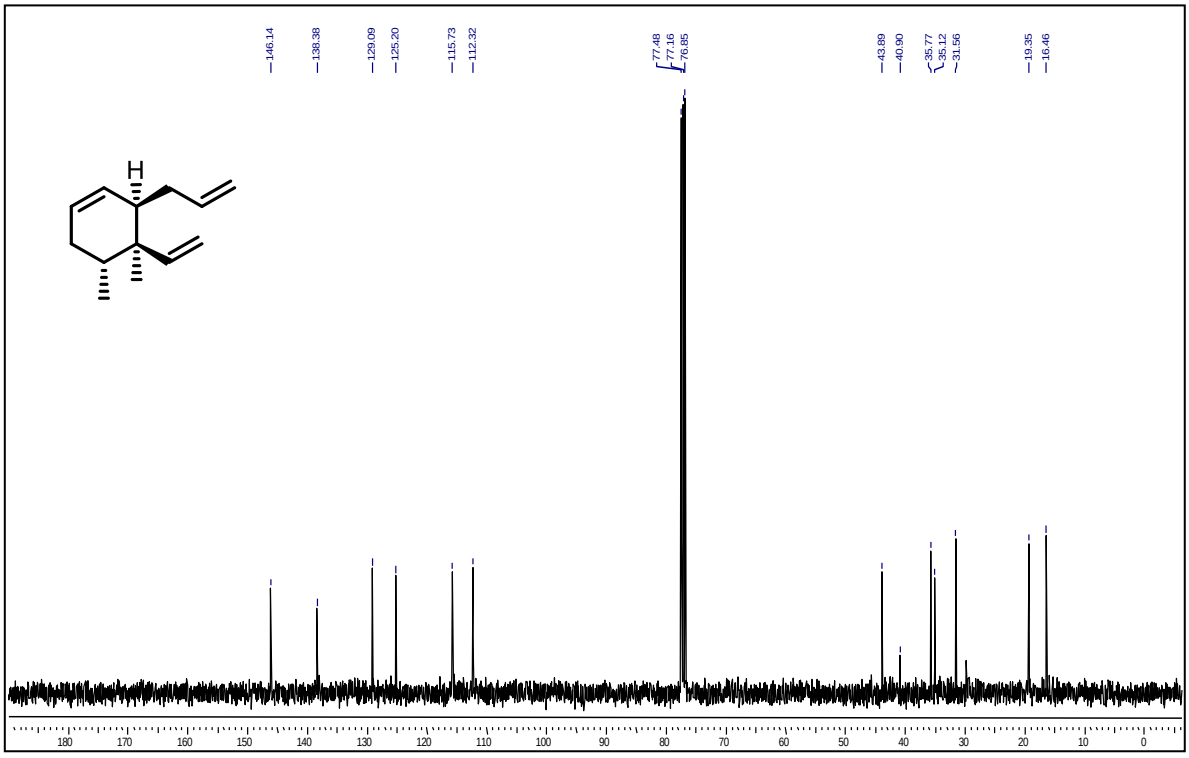
3.7 Selected copies of NMR Spectra

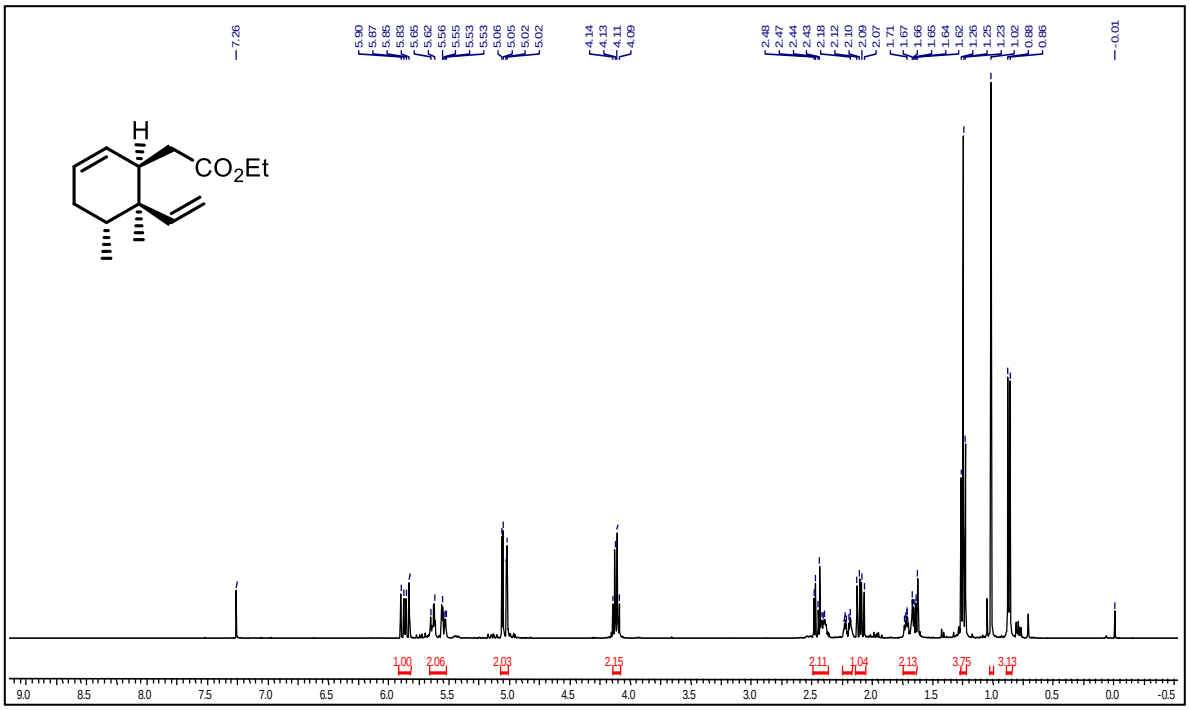
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 24



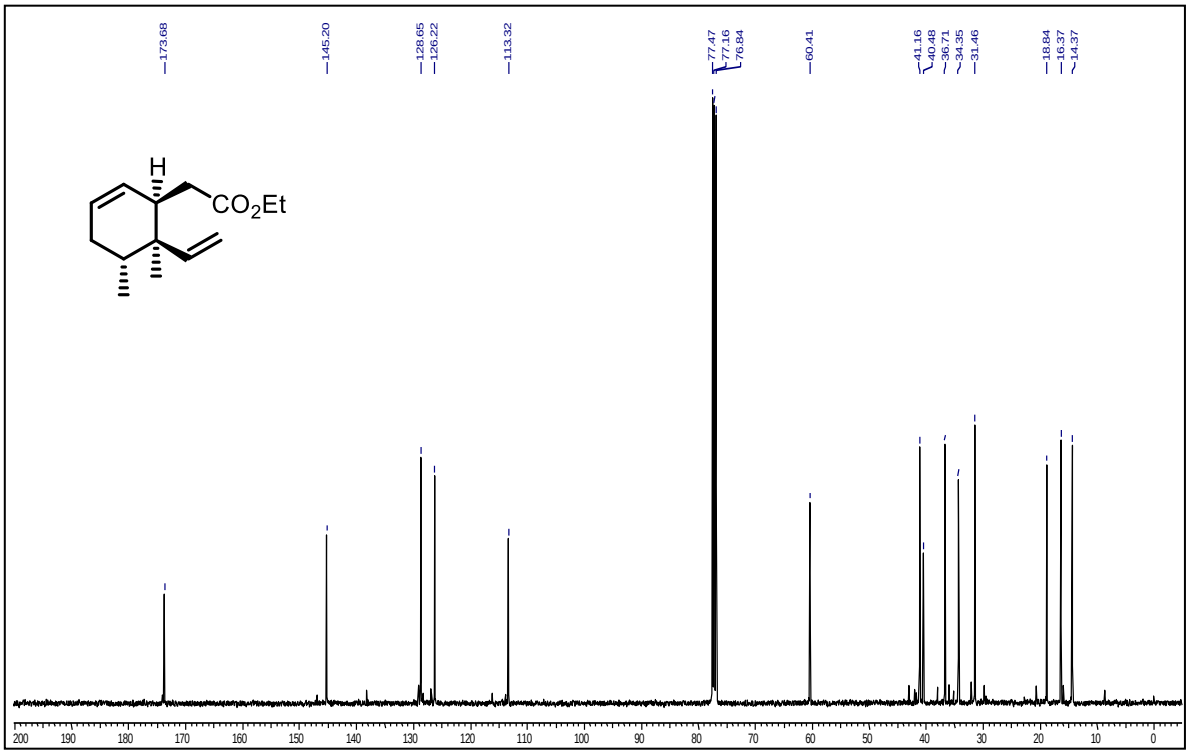
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 24



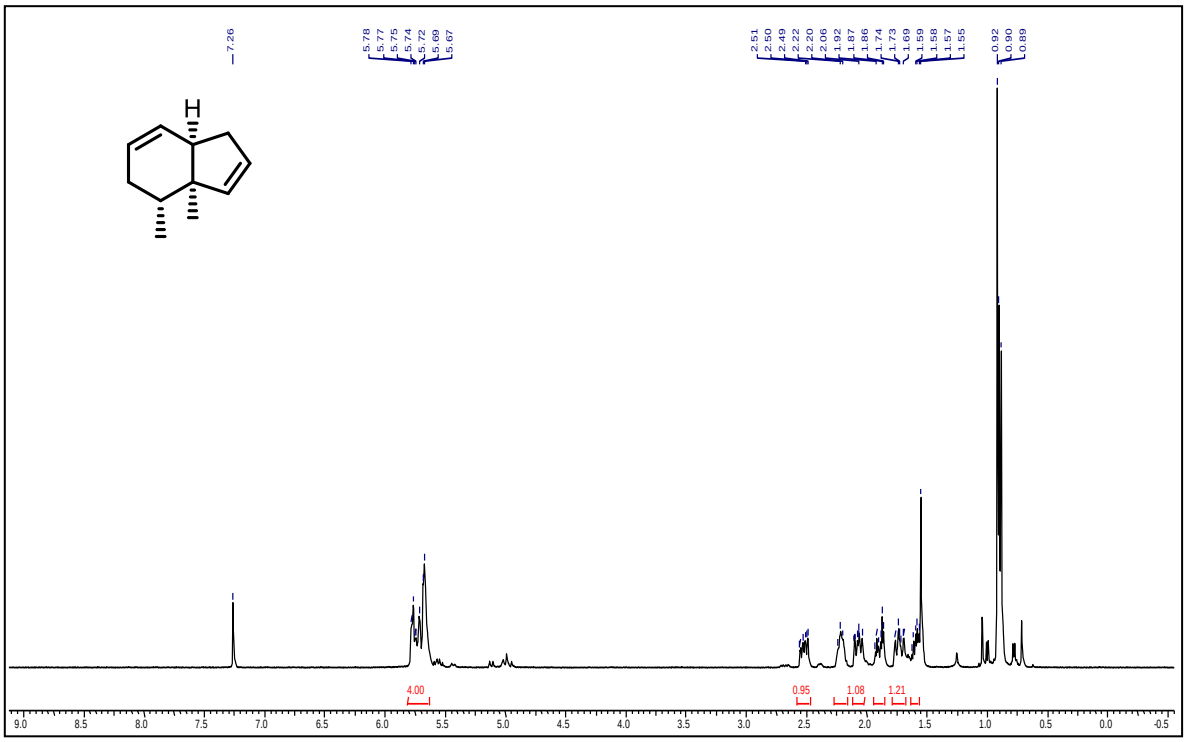
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 29



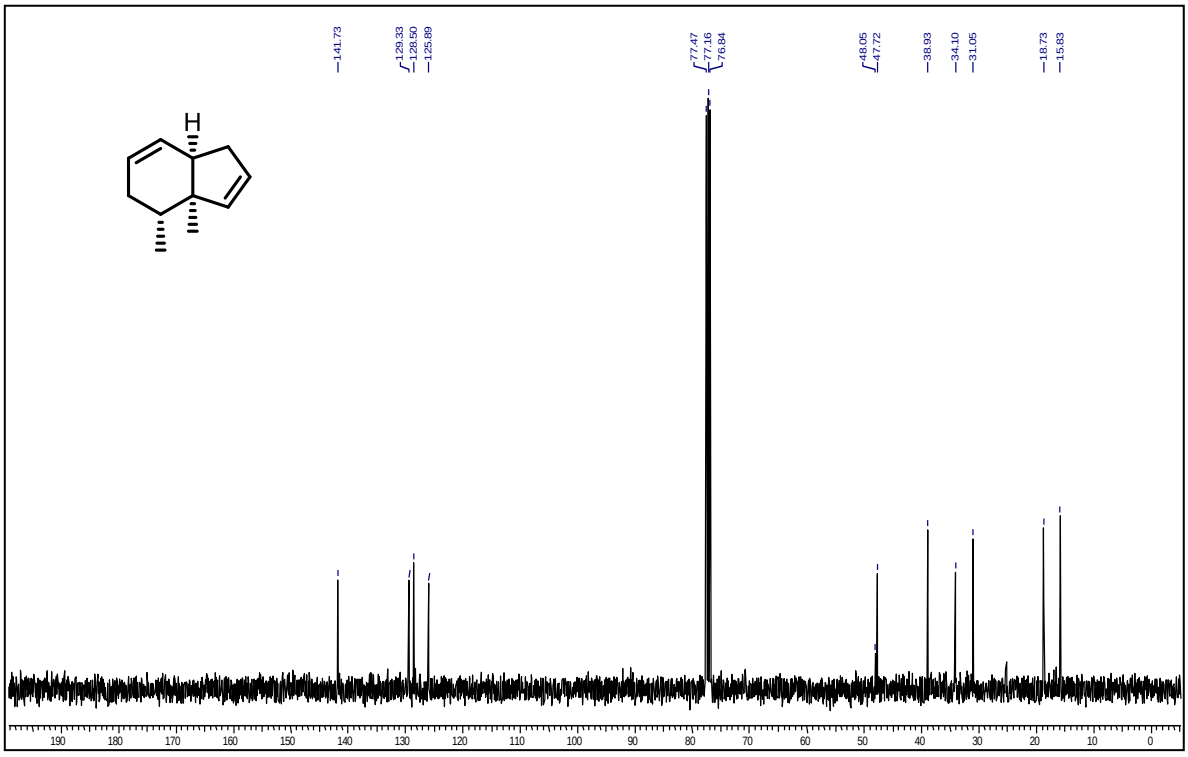
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 29



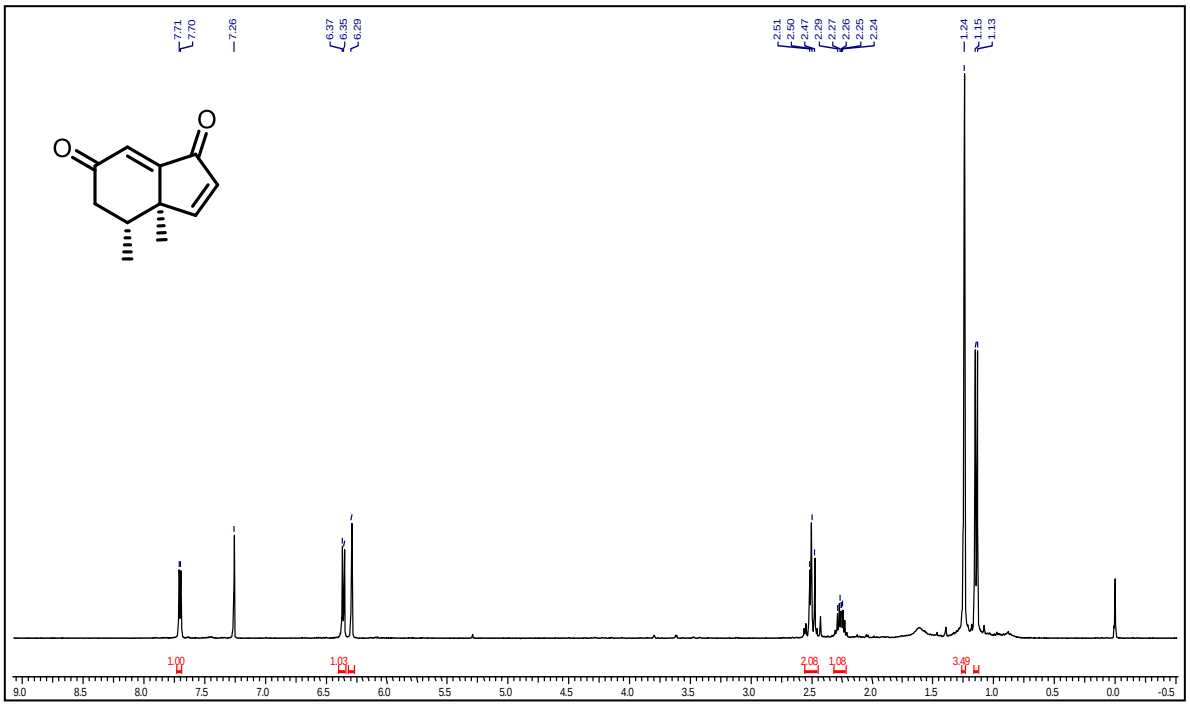
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 23



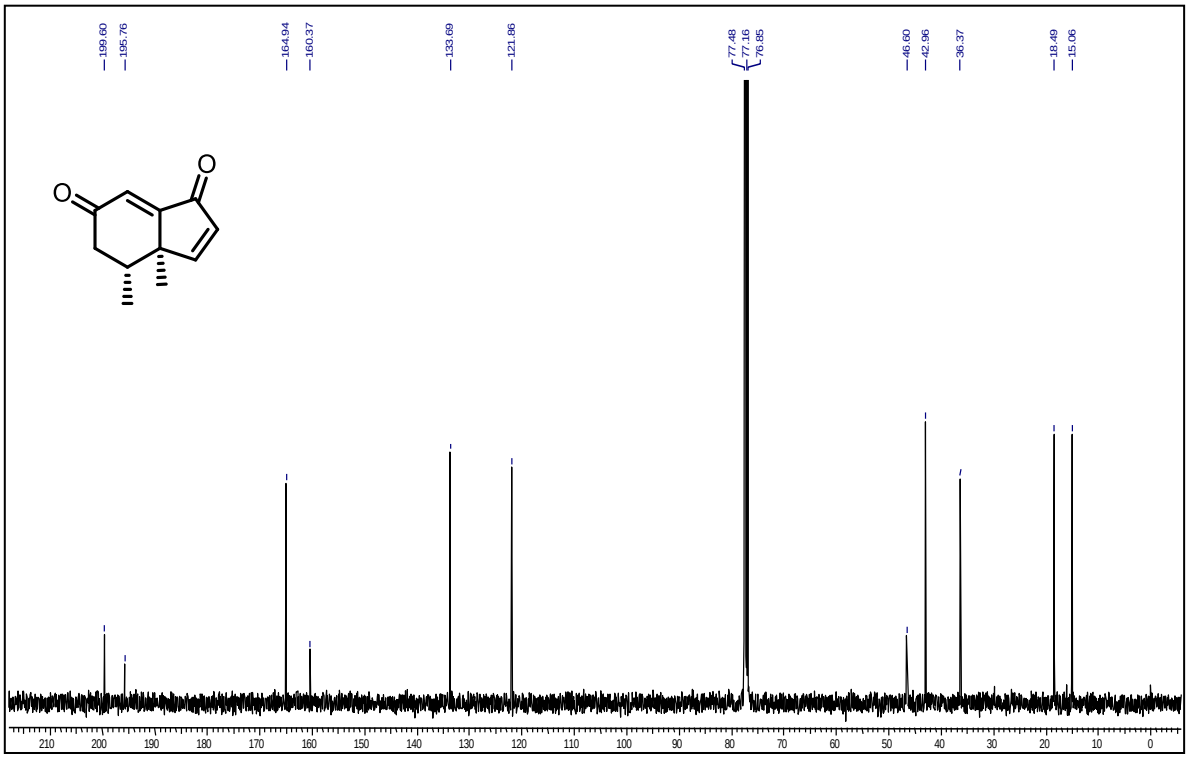
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 23



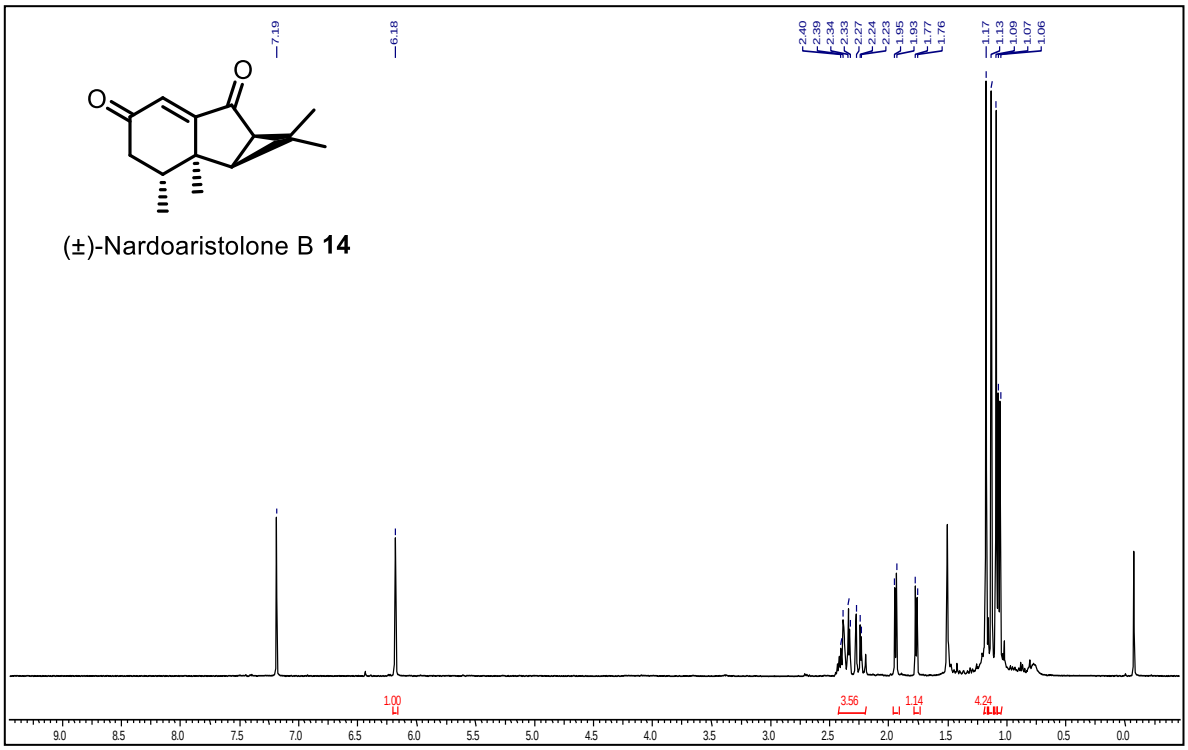
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 22



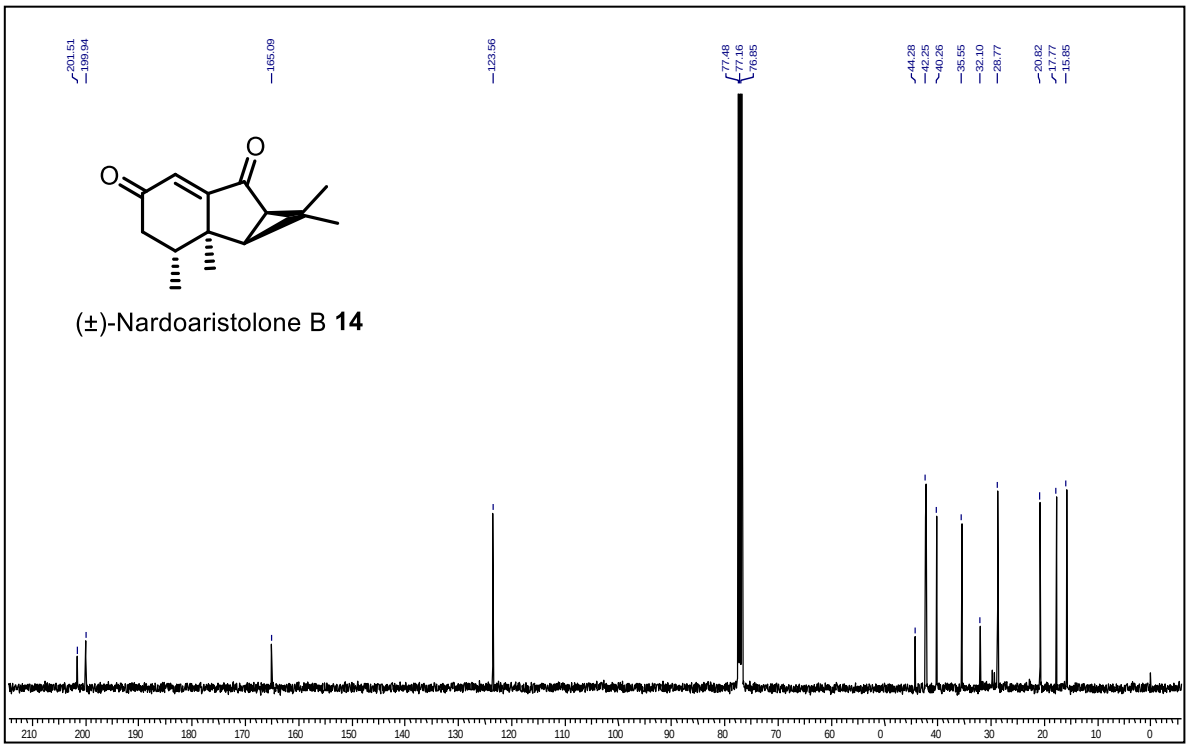
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 22



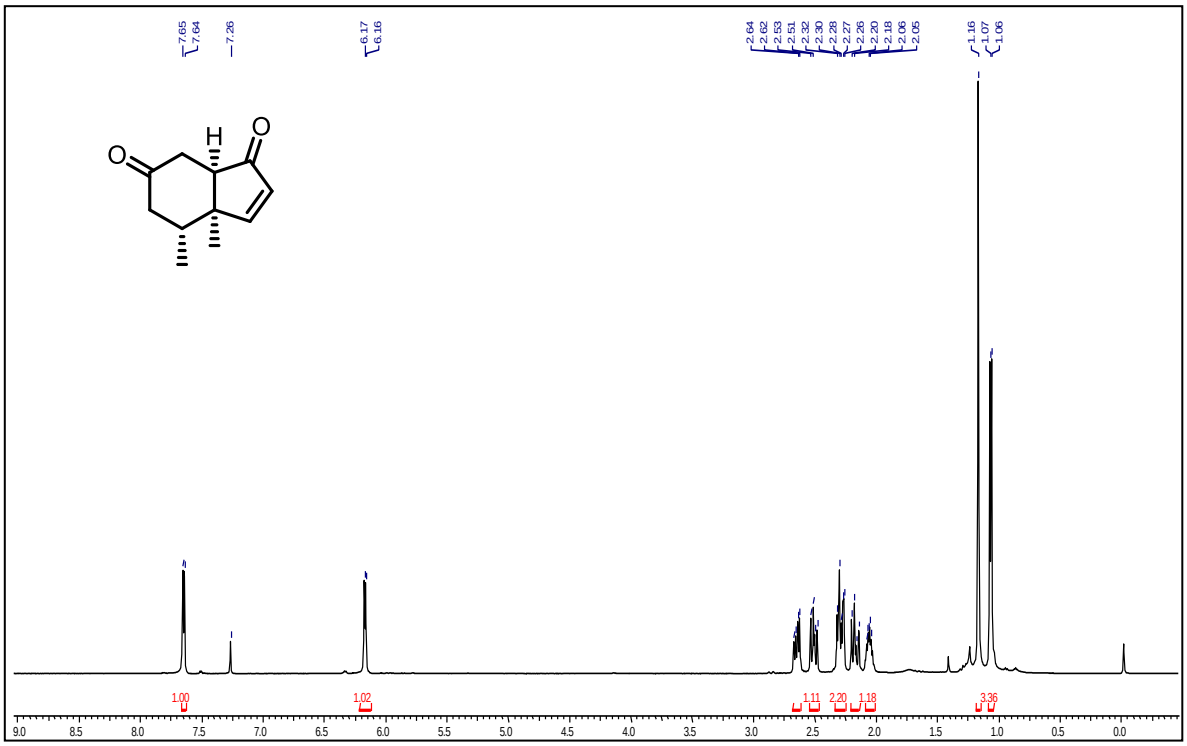
^{1}H NMR ($CDCl_3$, 400 MHz) of (±)-Nardoaristolone B 14



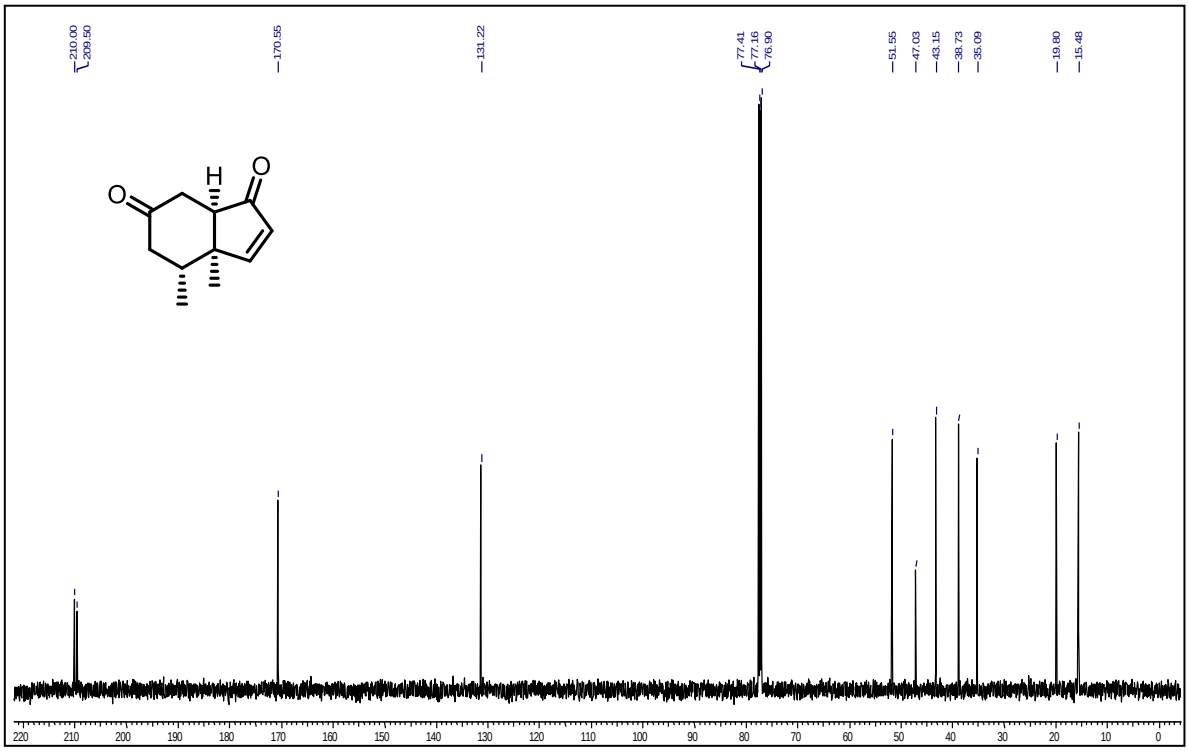
^{13}C NMR ($CDCl_3$, 100 MHz) of (±)-Nardoaristolone B 14



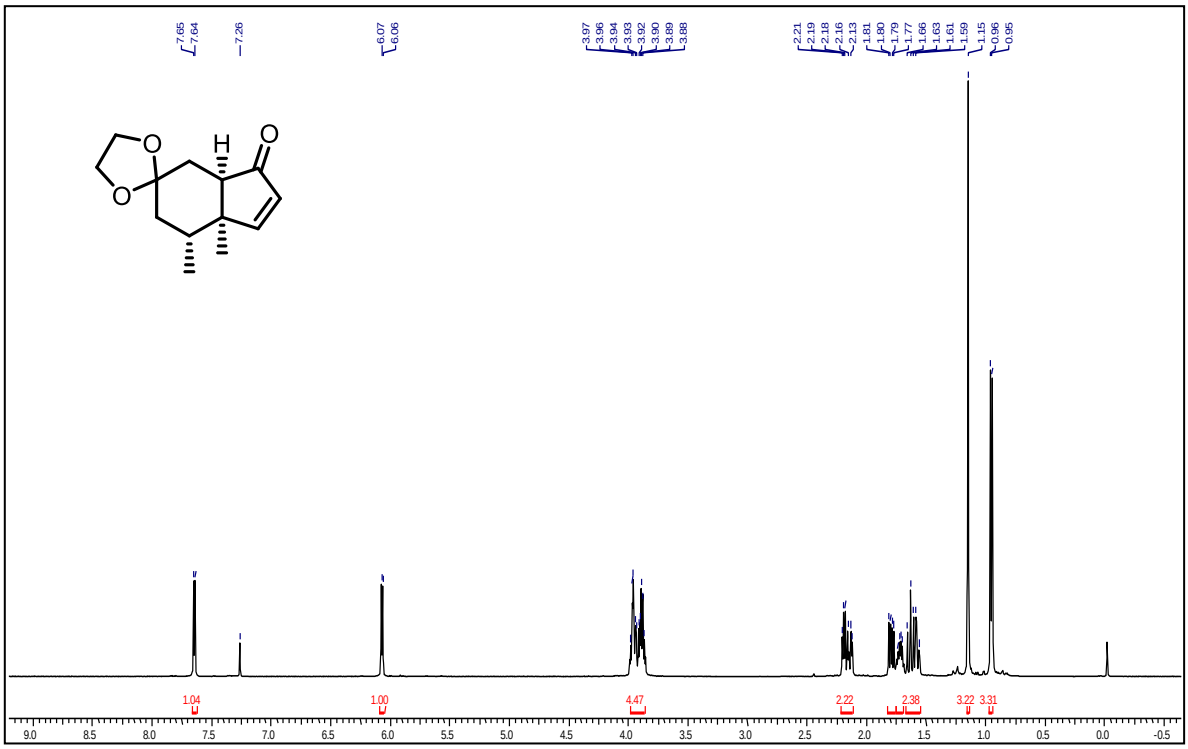
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 32



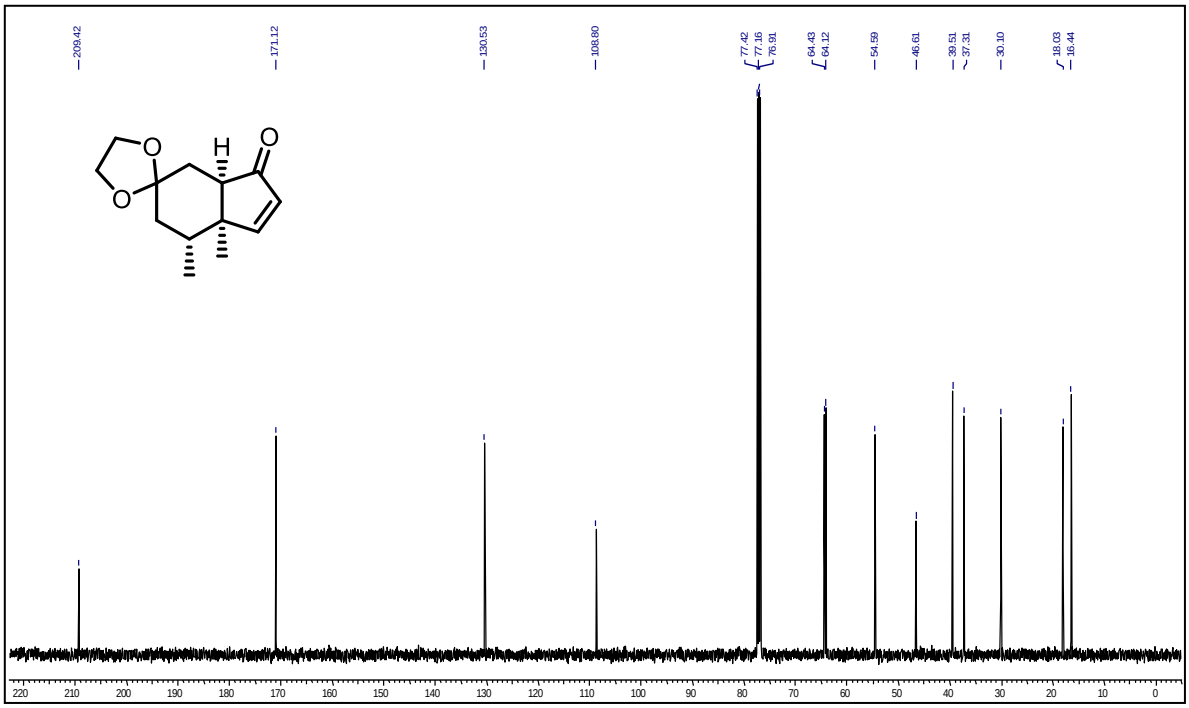
^{13}C NMR ($CDCl_3$, 125 MHz) of compound 32



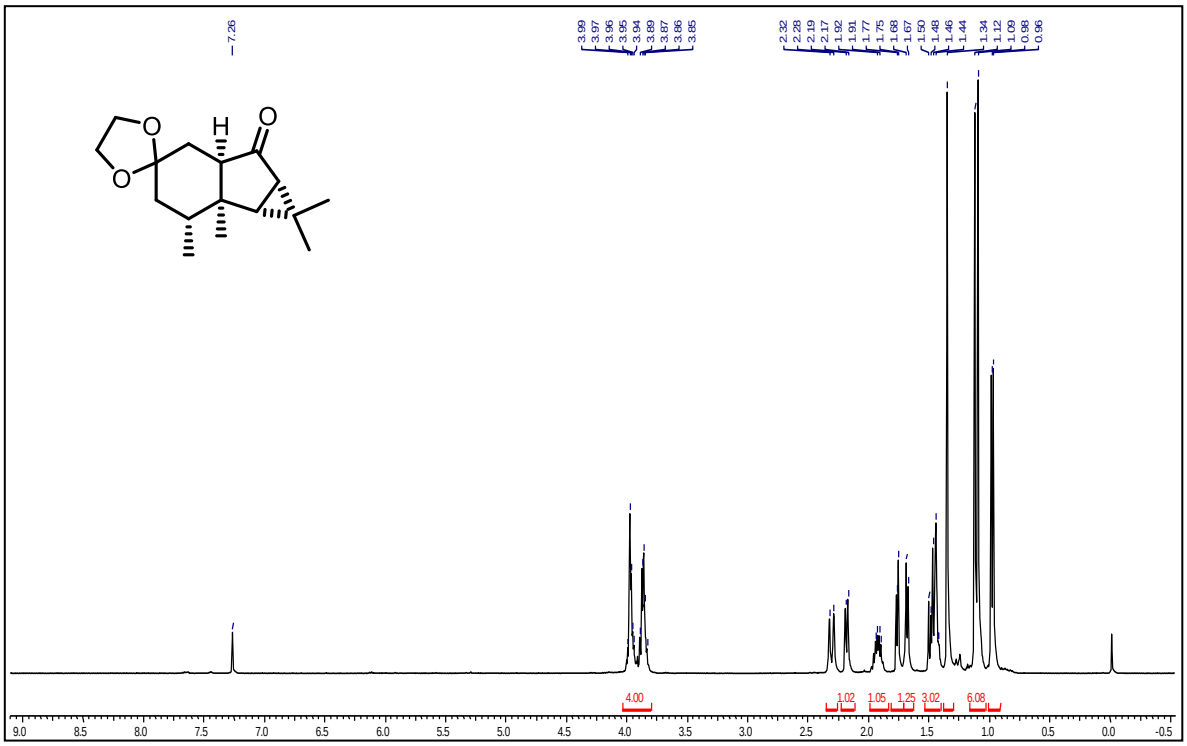
^{1}H NMR ($CDCl_3$, 500 MHz) of compound 33



^{13}C NMR ($CDCl_3$, 125 MHz) of compound 33



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 34



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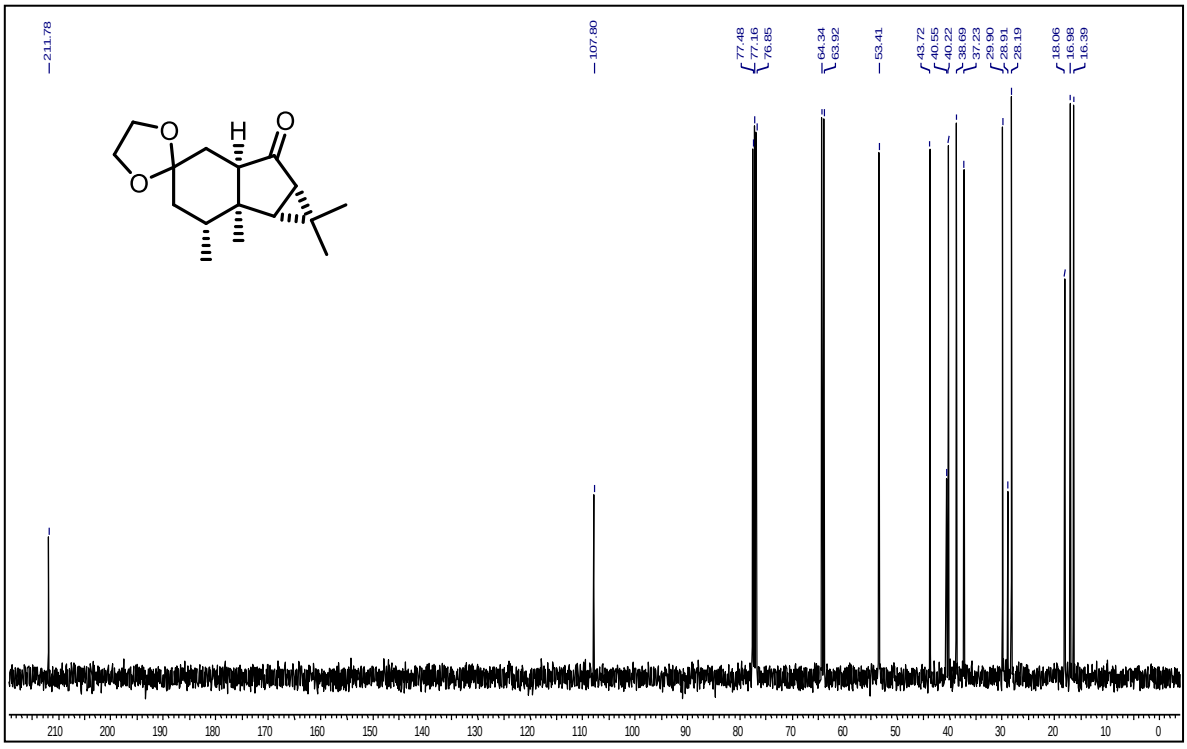

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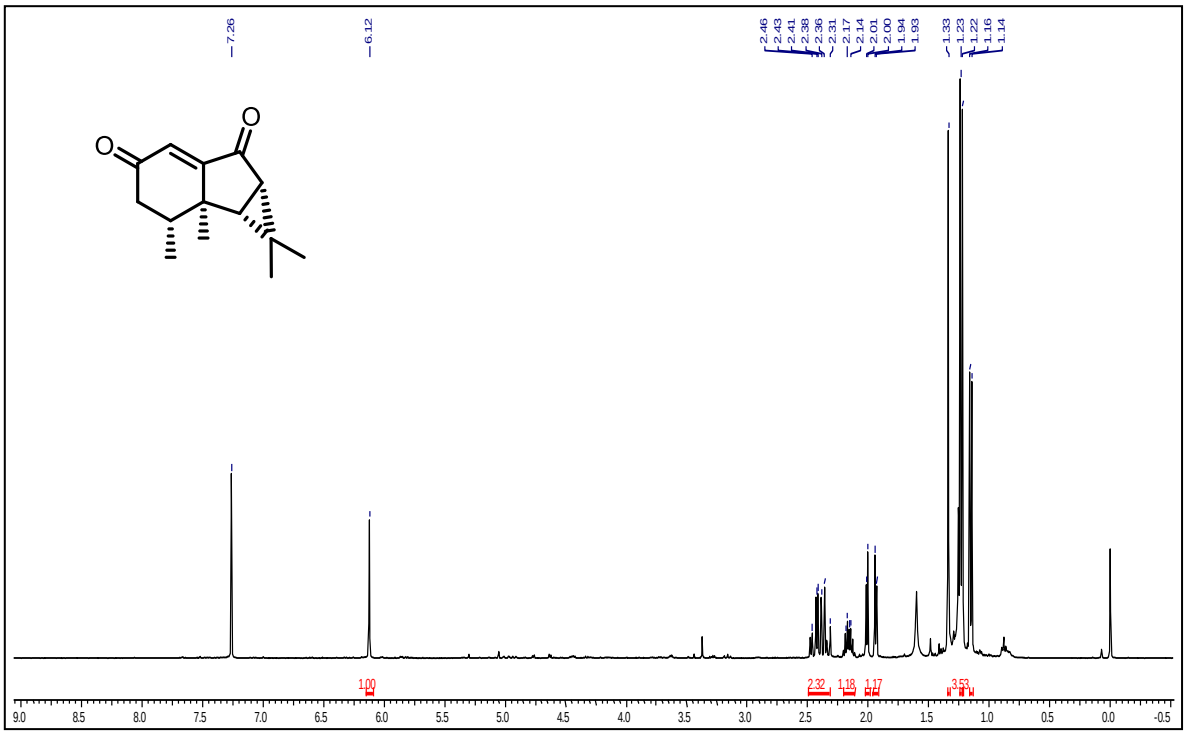

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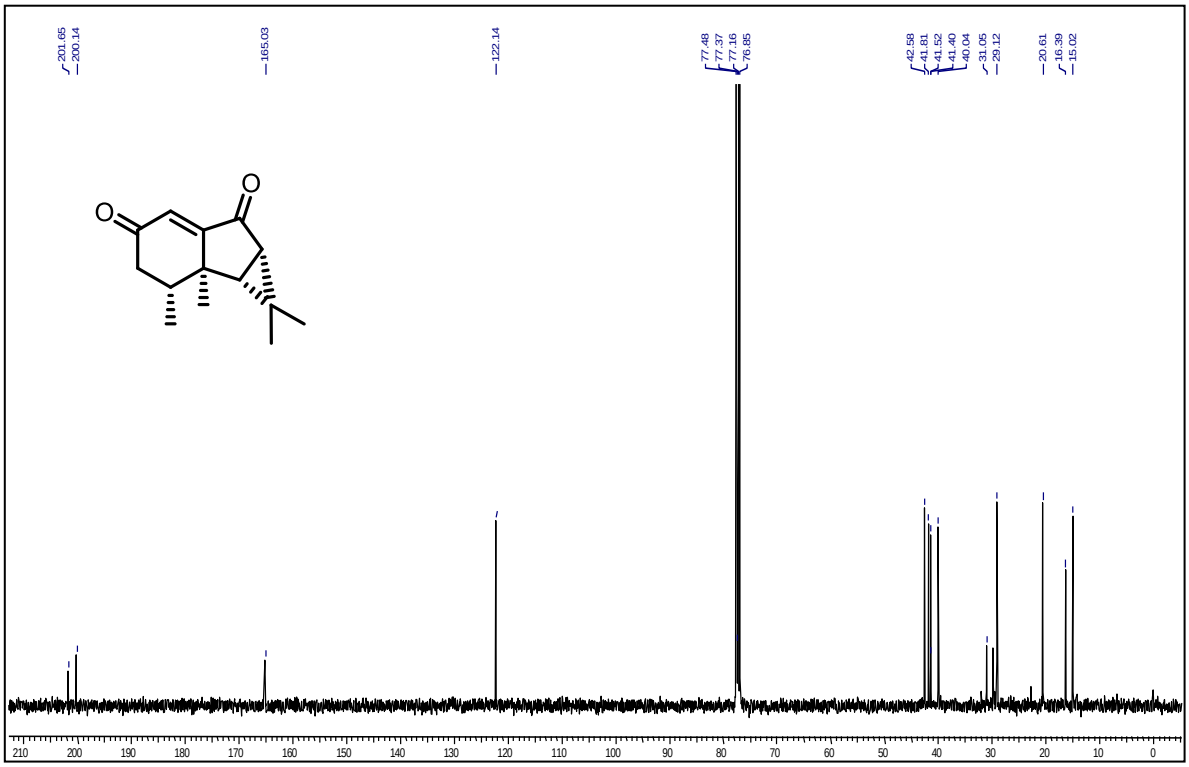

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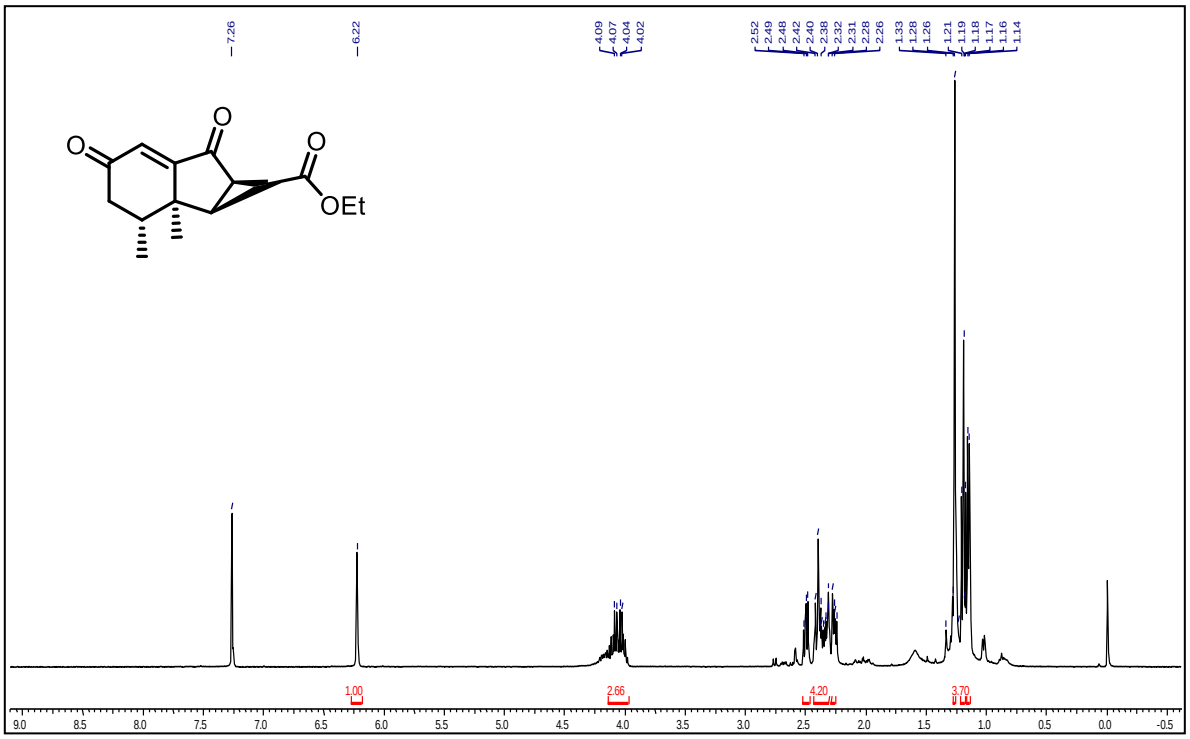
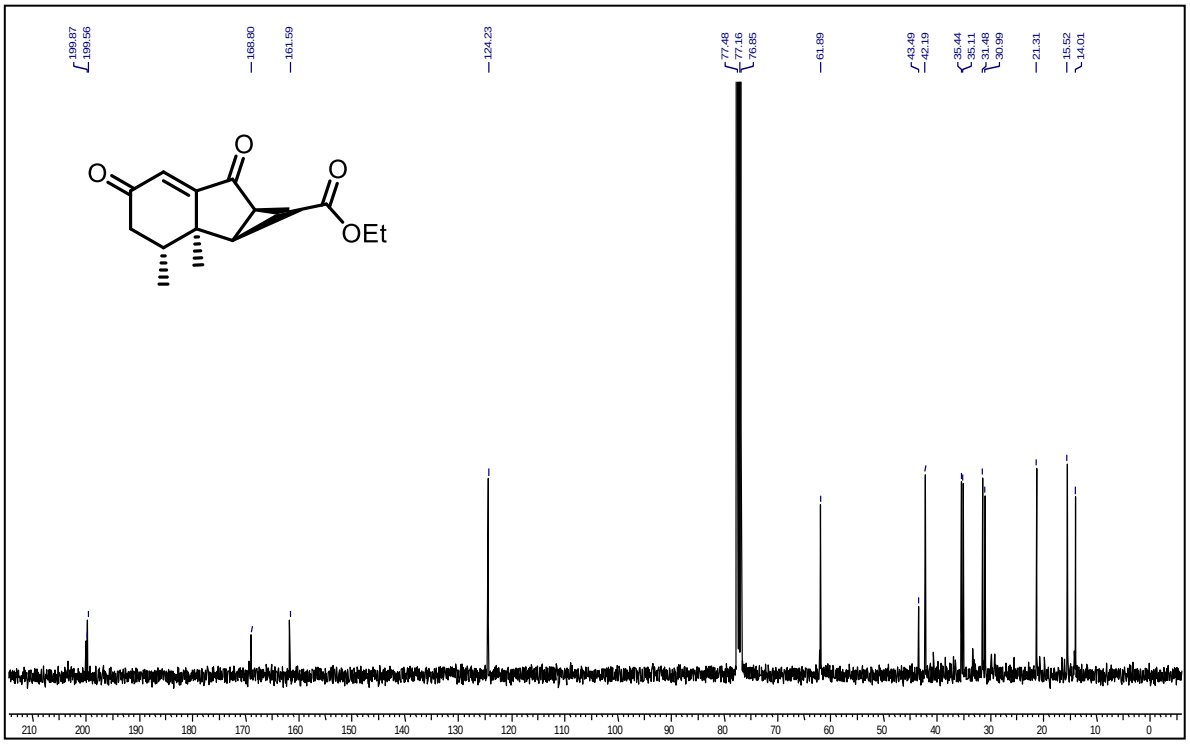


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 36



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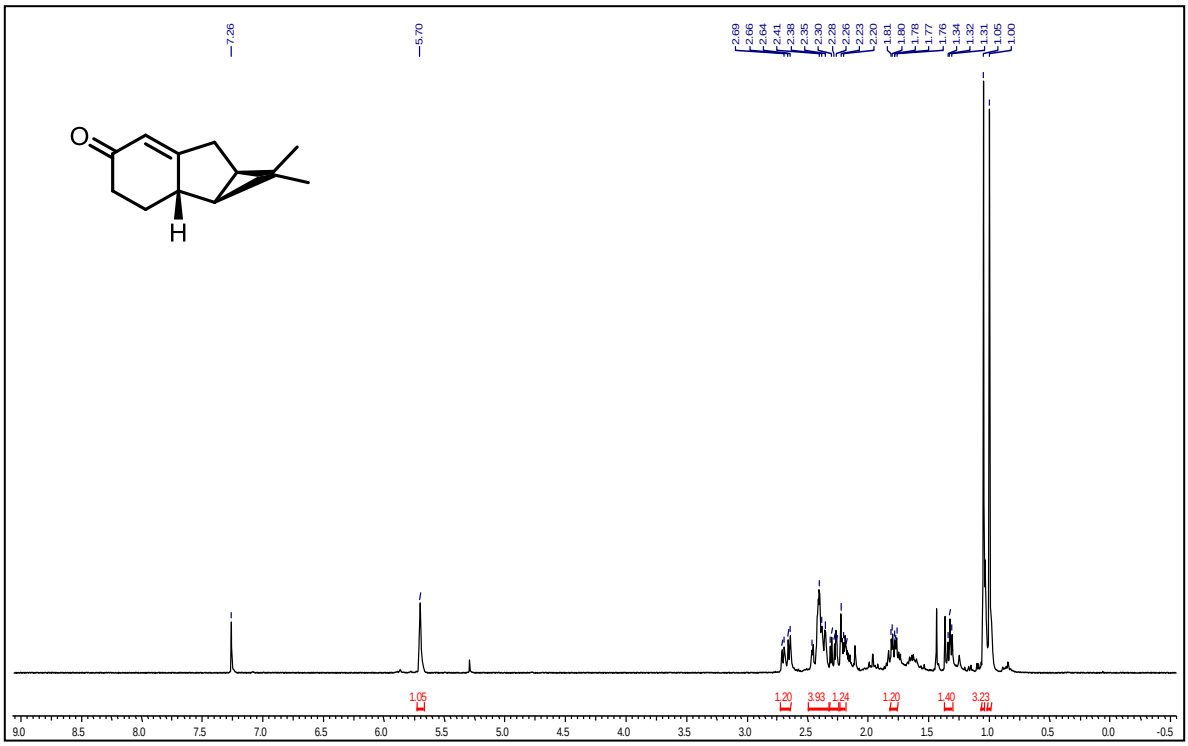

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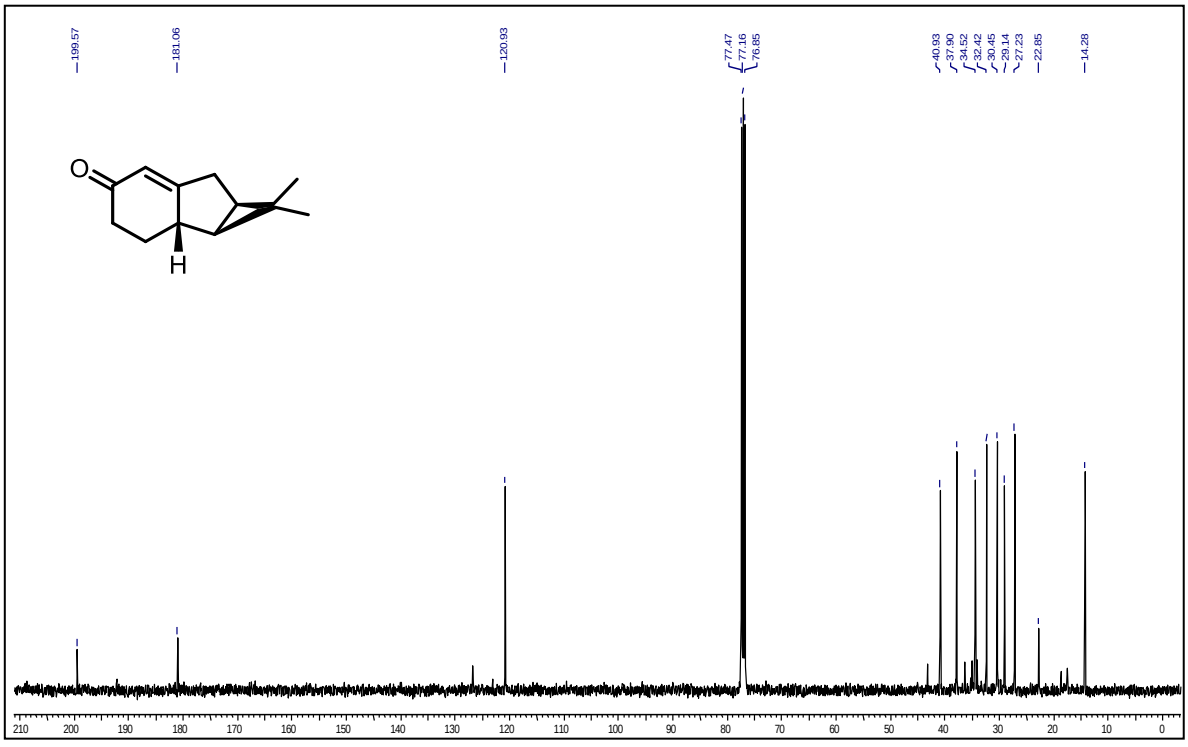
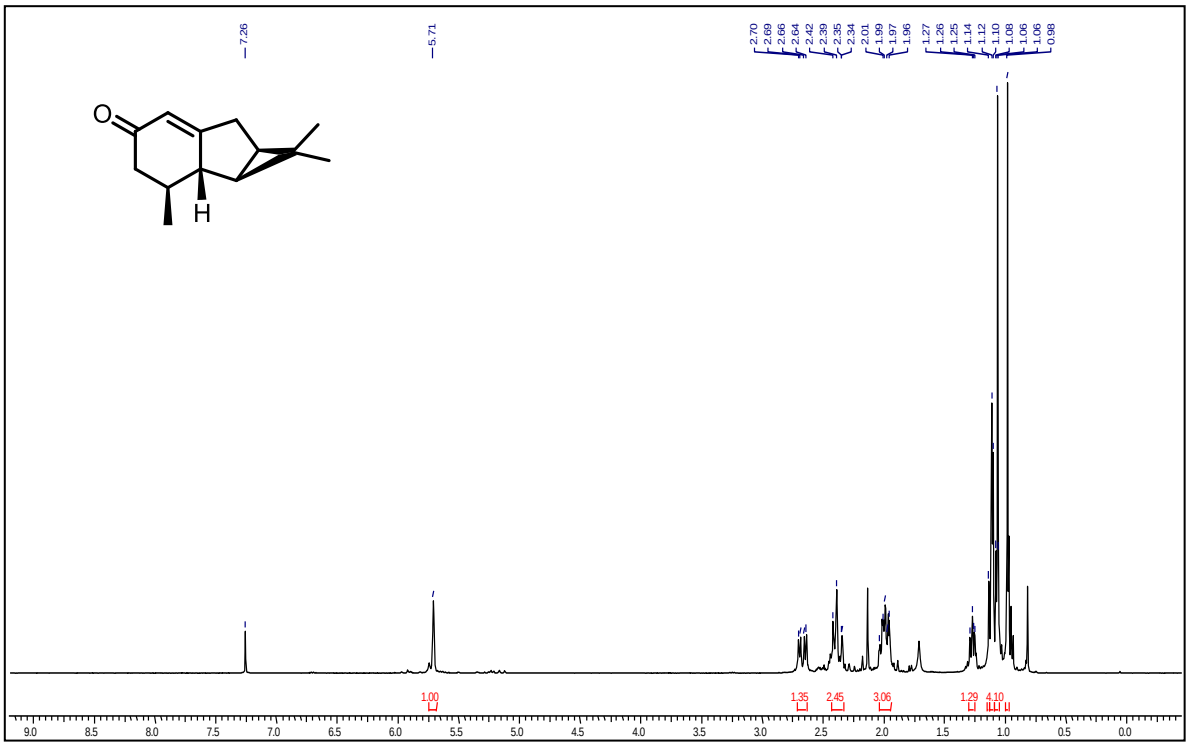


^{1}H NMR ($CDCl_3$, 400 MHz) of compound 44



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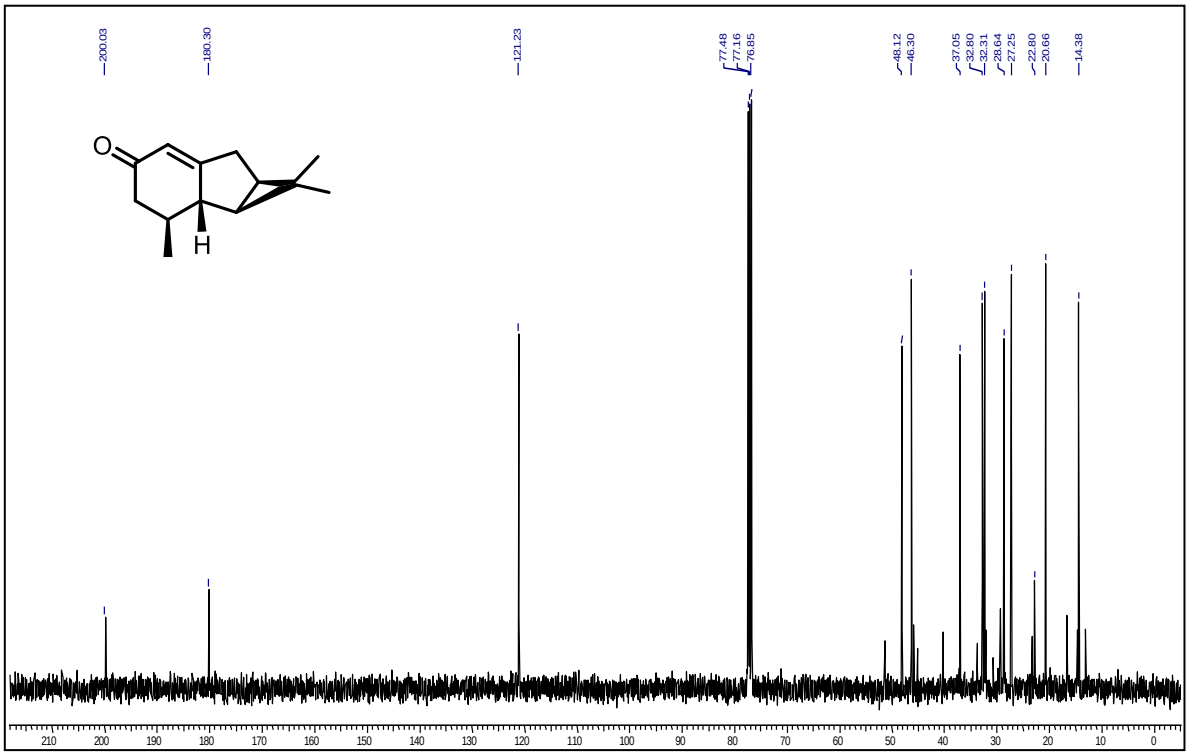

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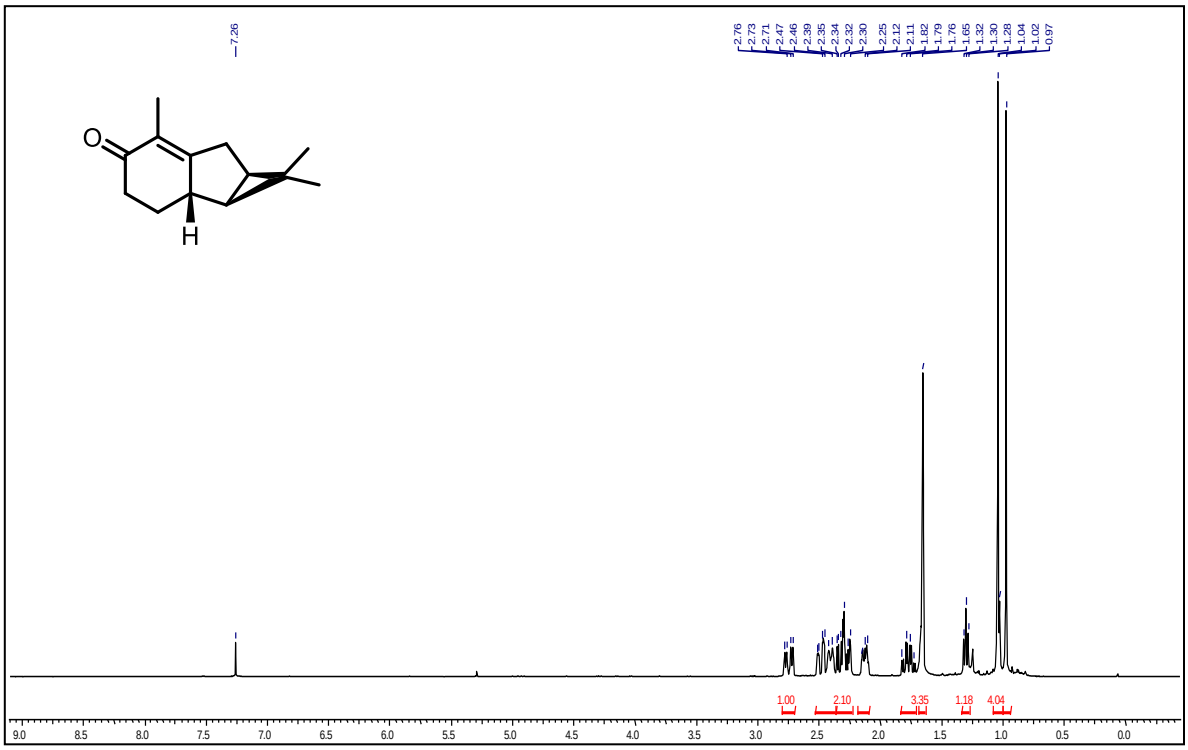

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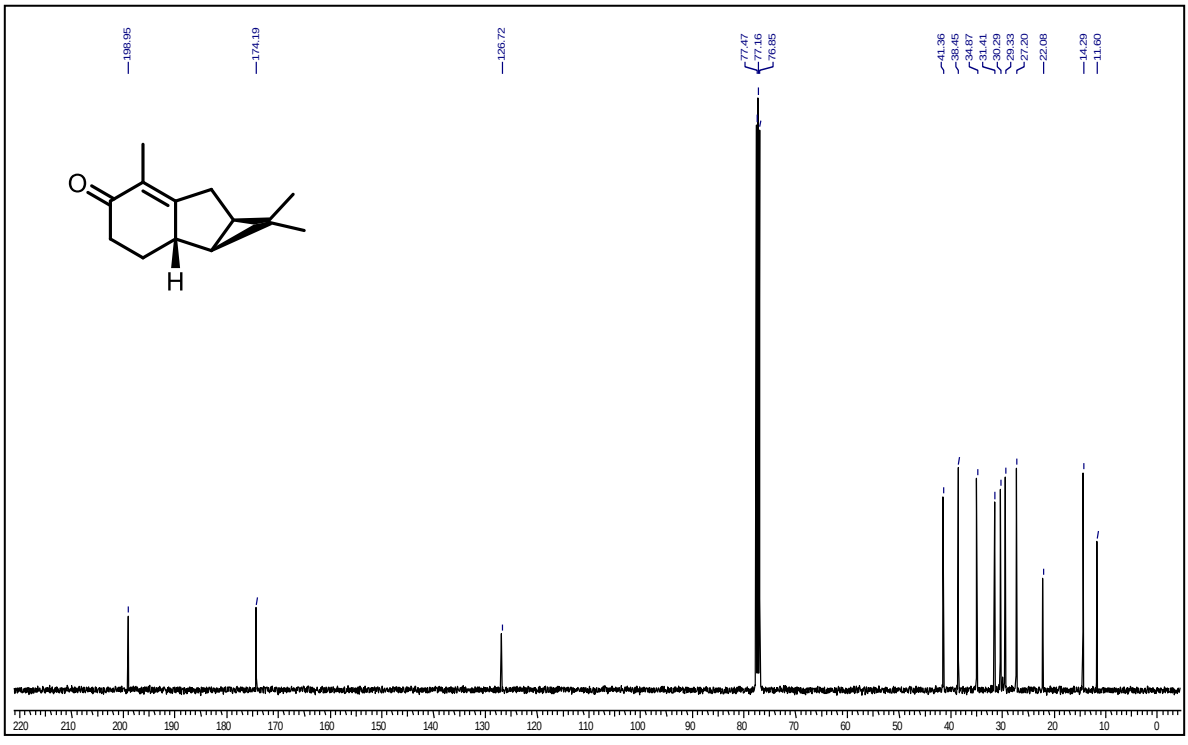

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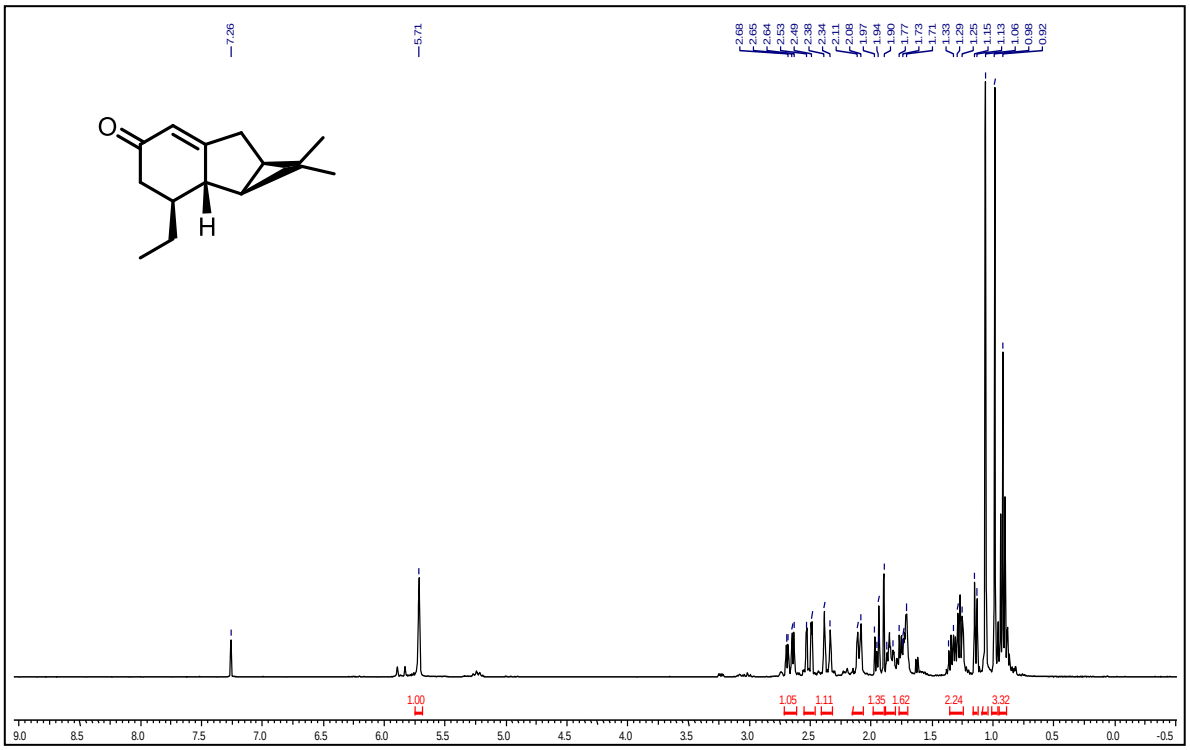

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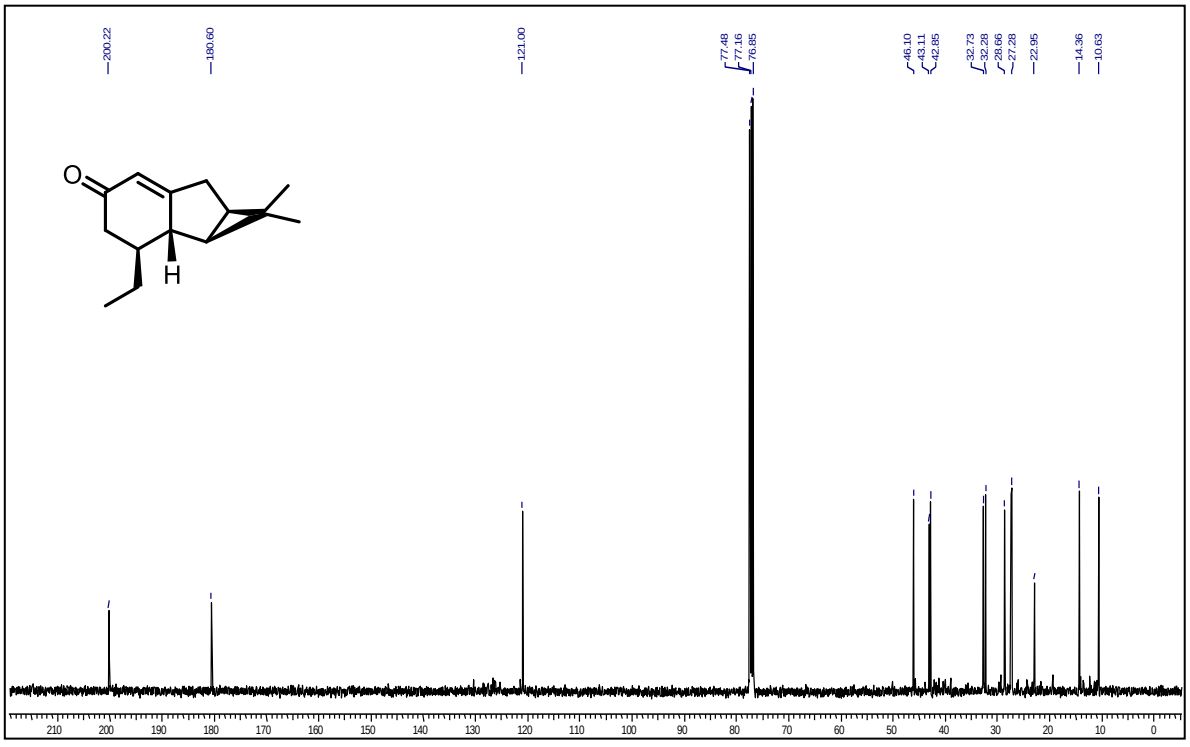

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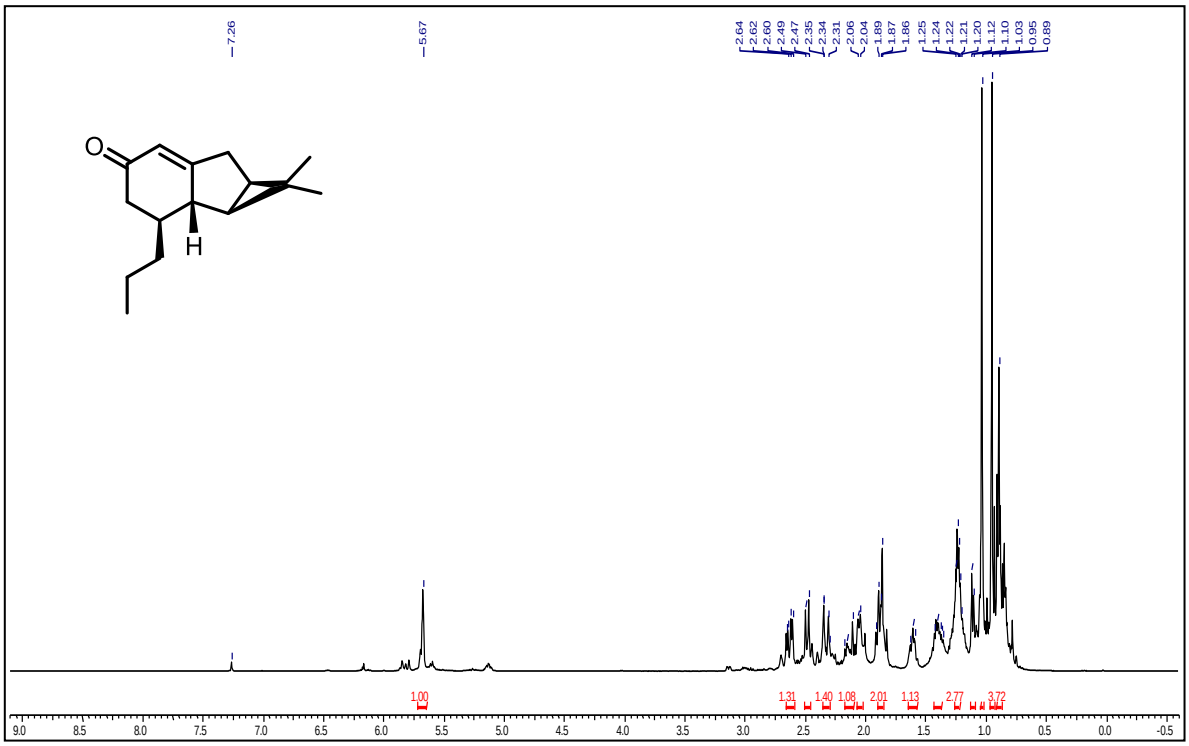

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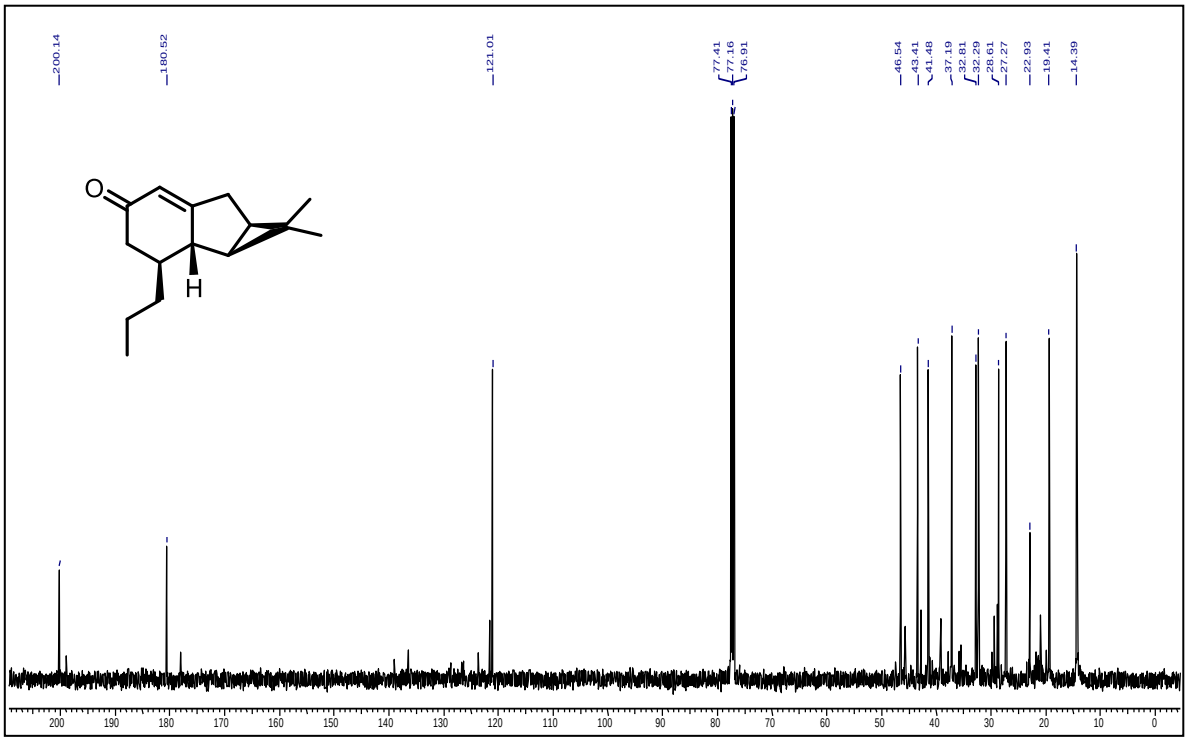

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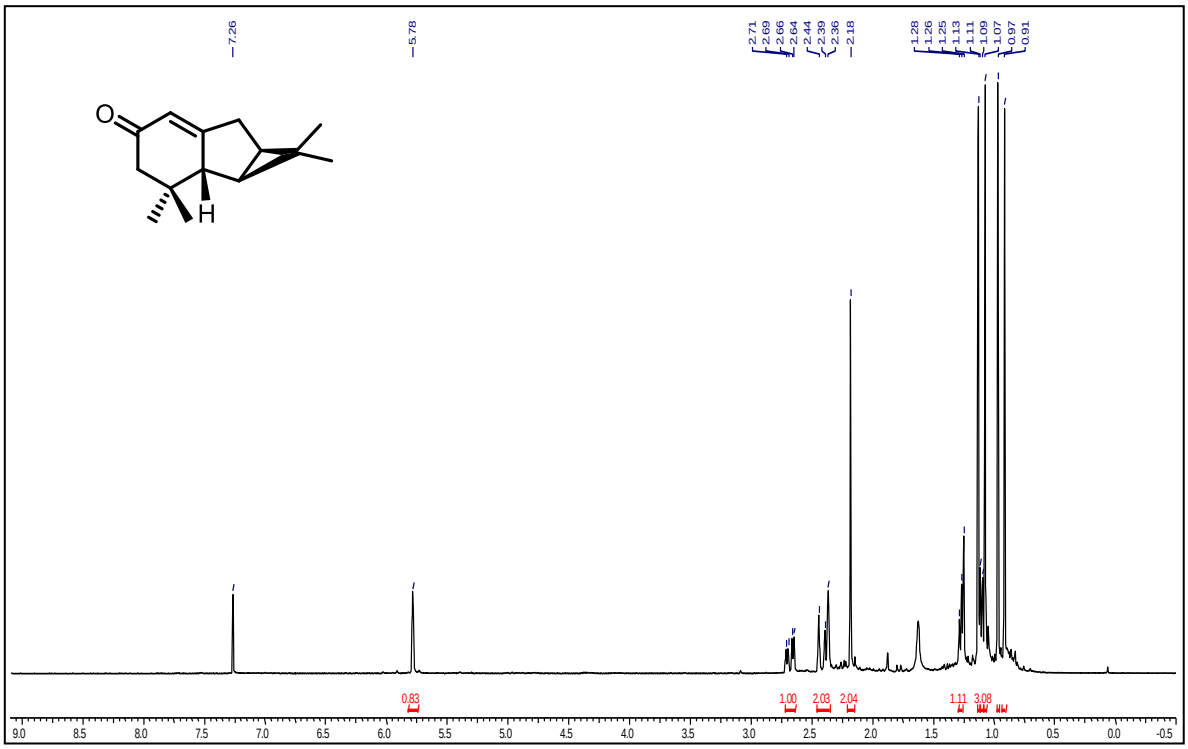
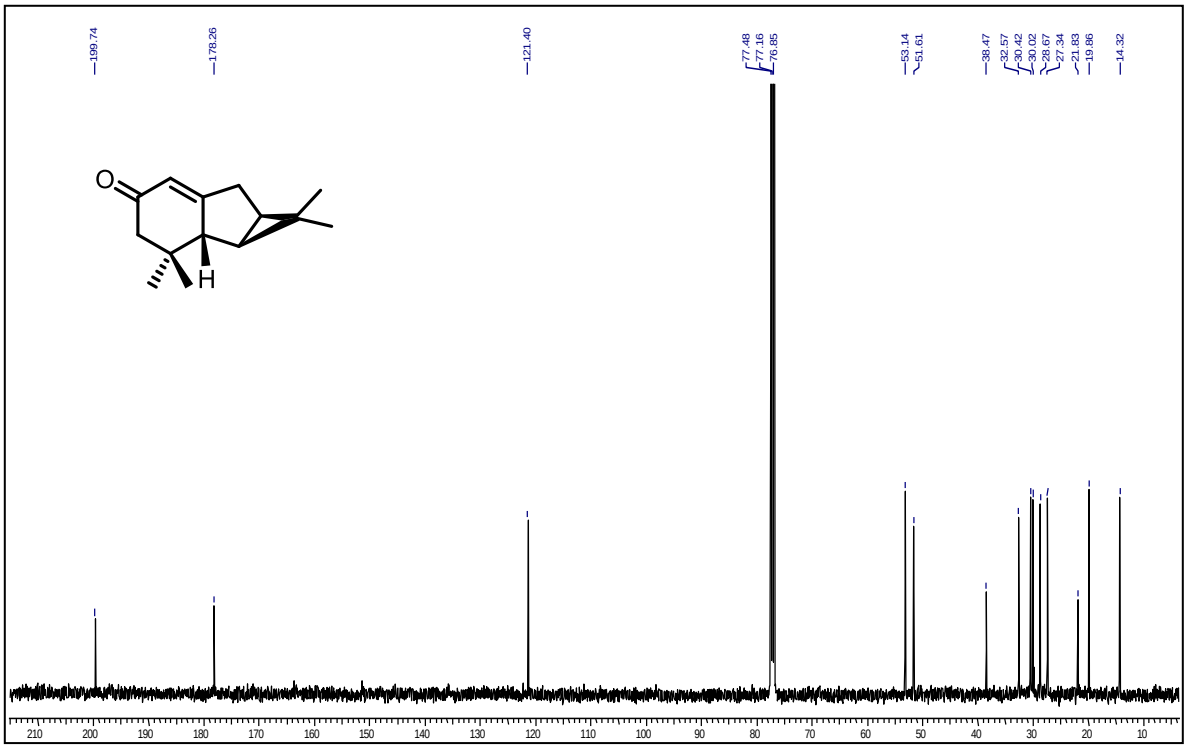
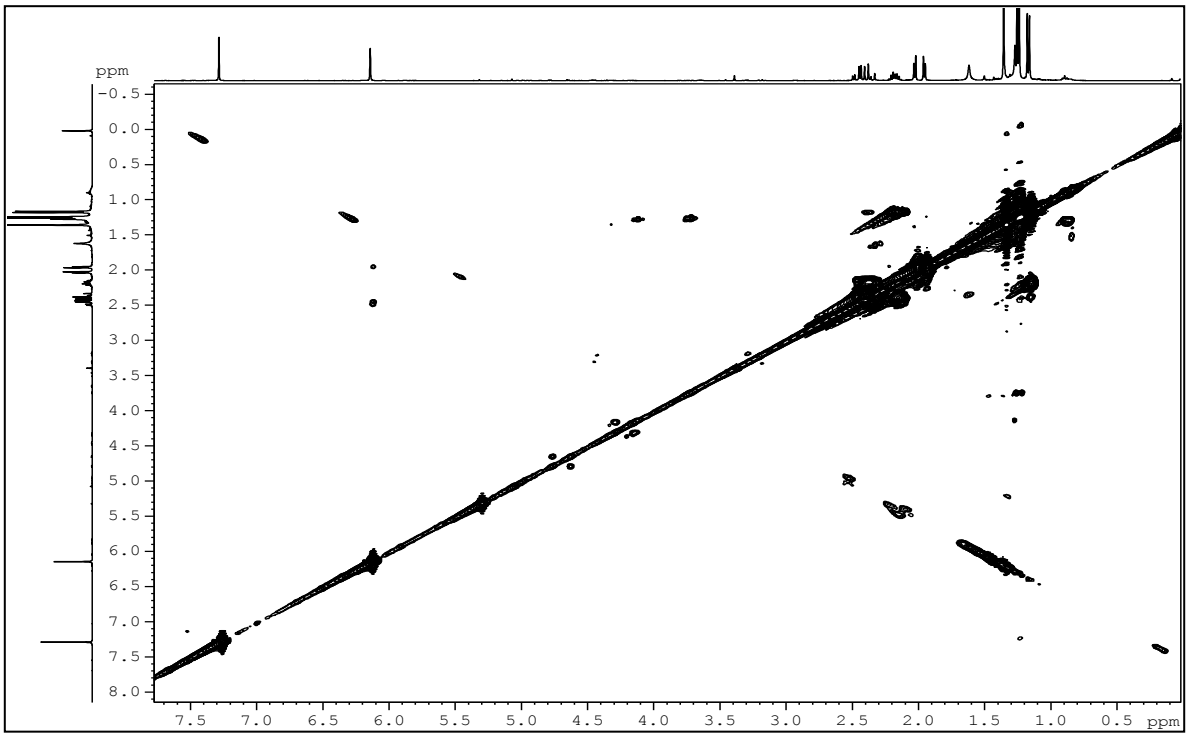


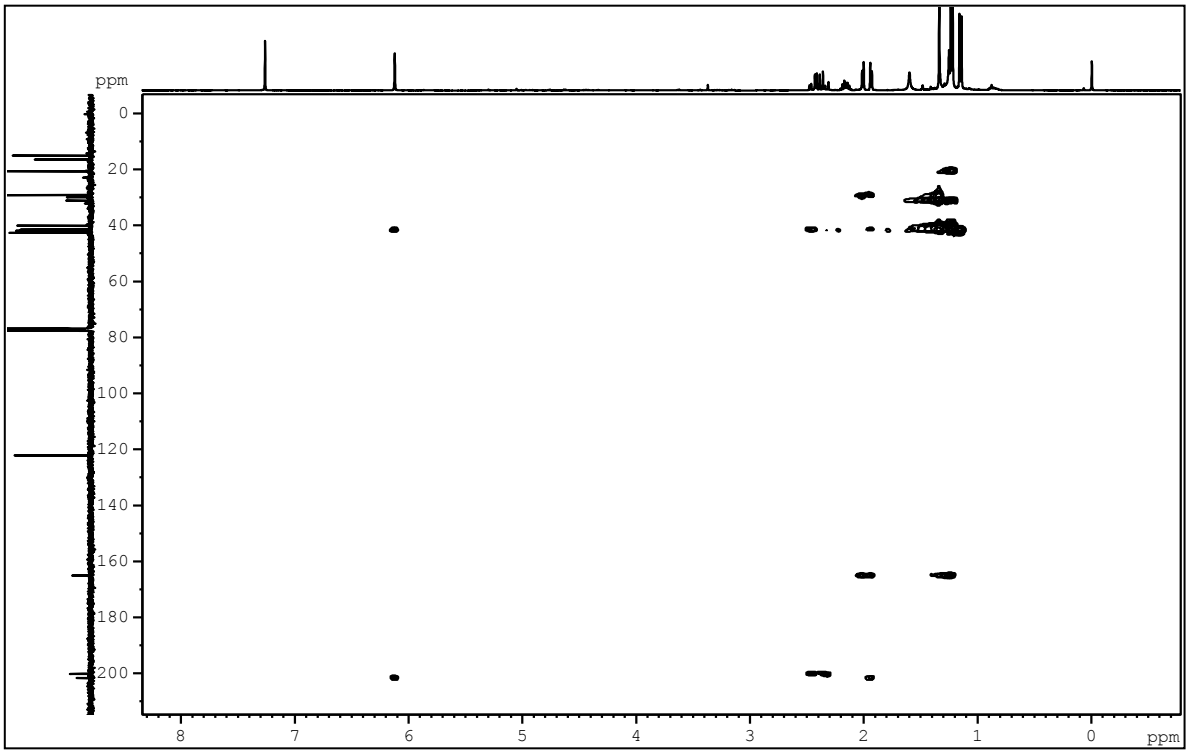
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 48



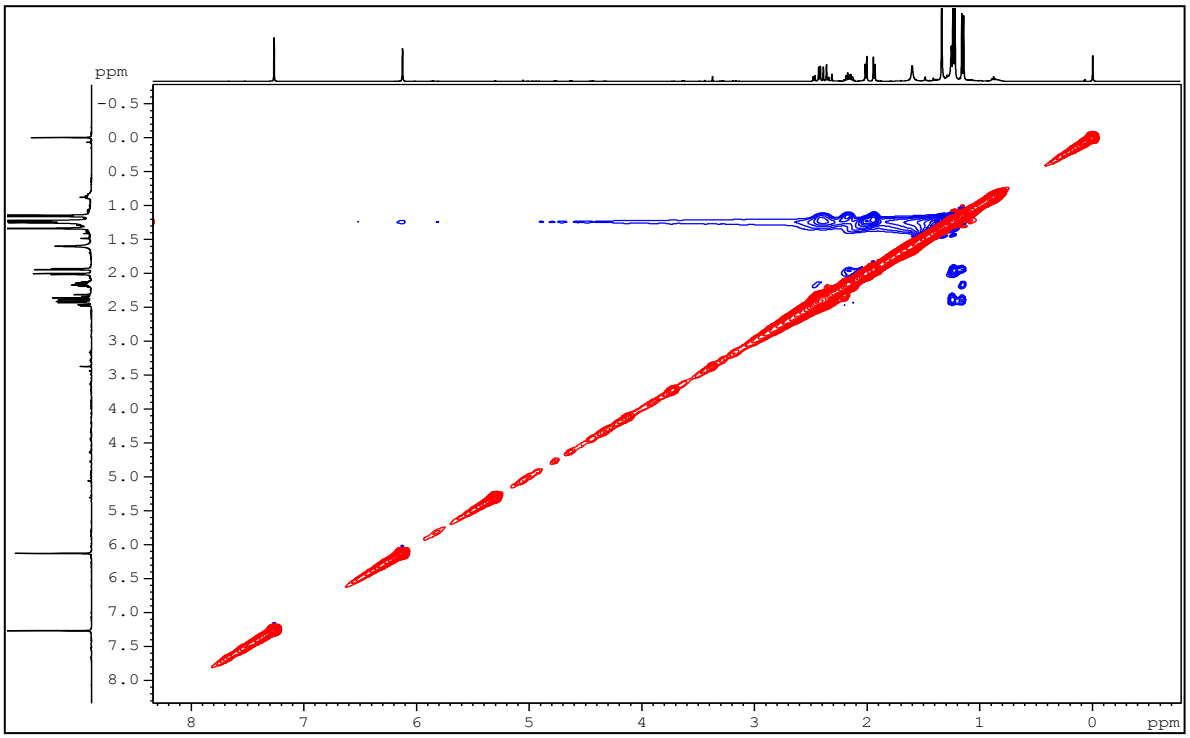
^{1}H-^{1}H COSY spectrum (400 MHz) of 35 in $CDCl_3$



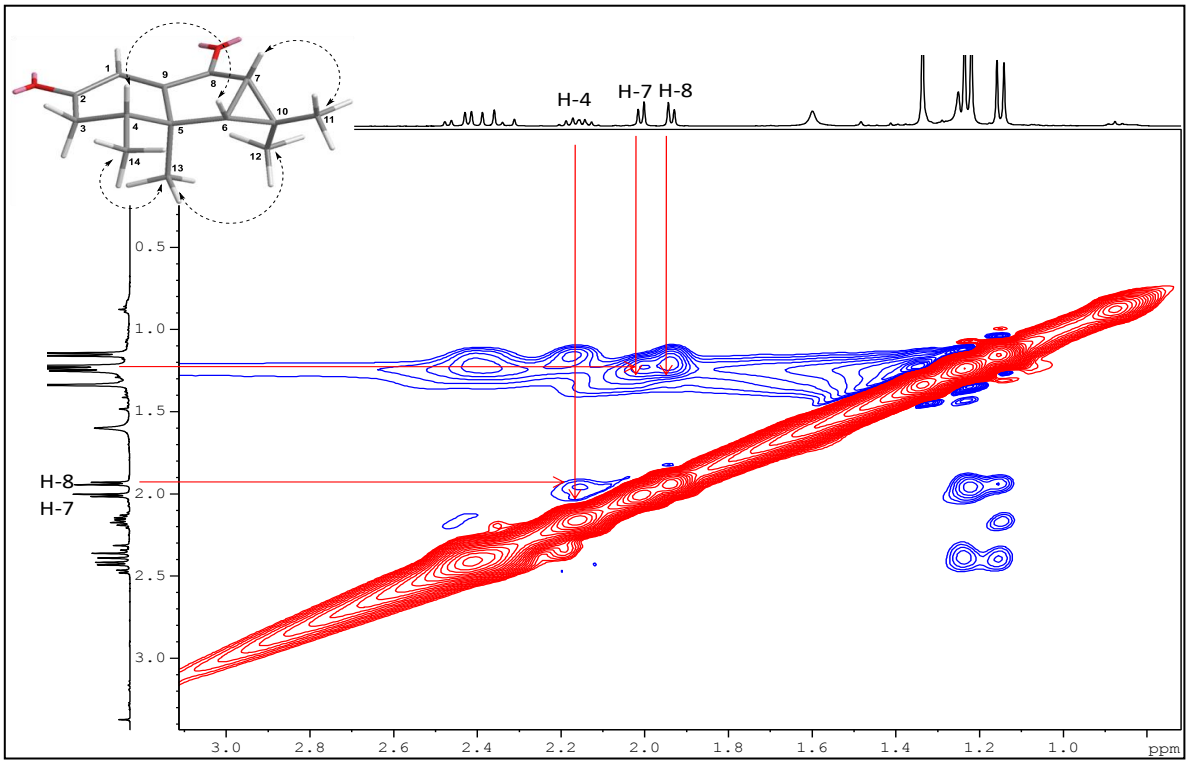
HMBC Spectrum (400 MHz) of 35 in $CDCl_3$



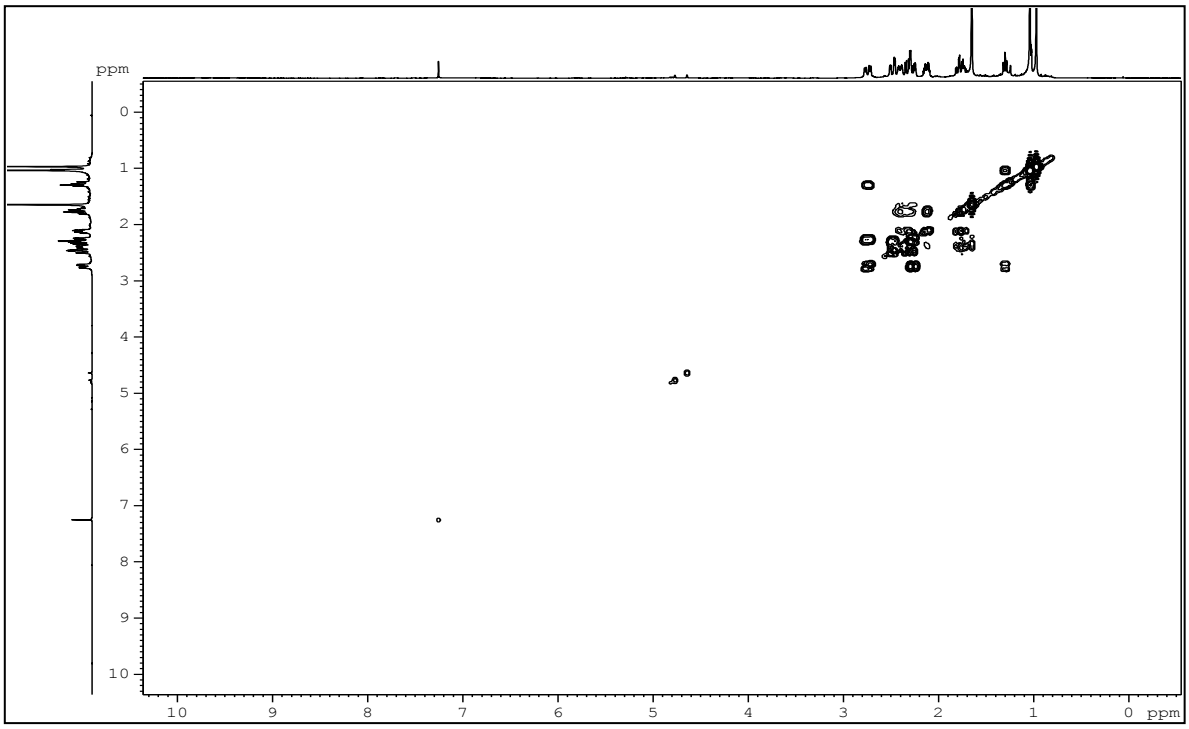
NOESY Spectrum (400 MHz) of 35 in $CDCl_3$



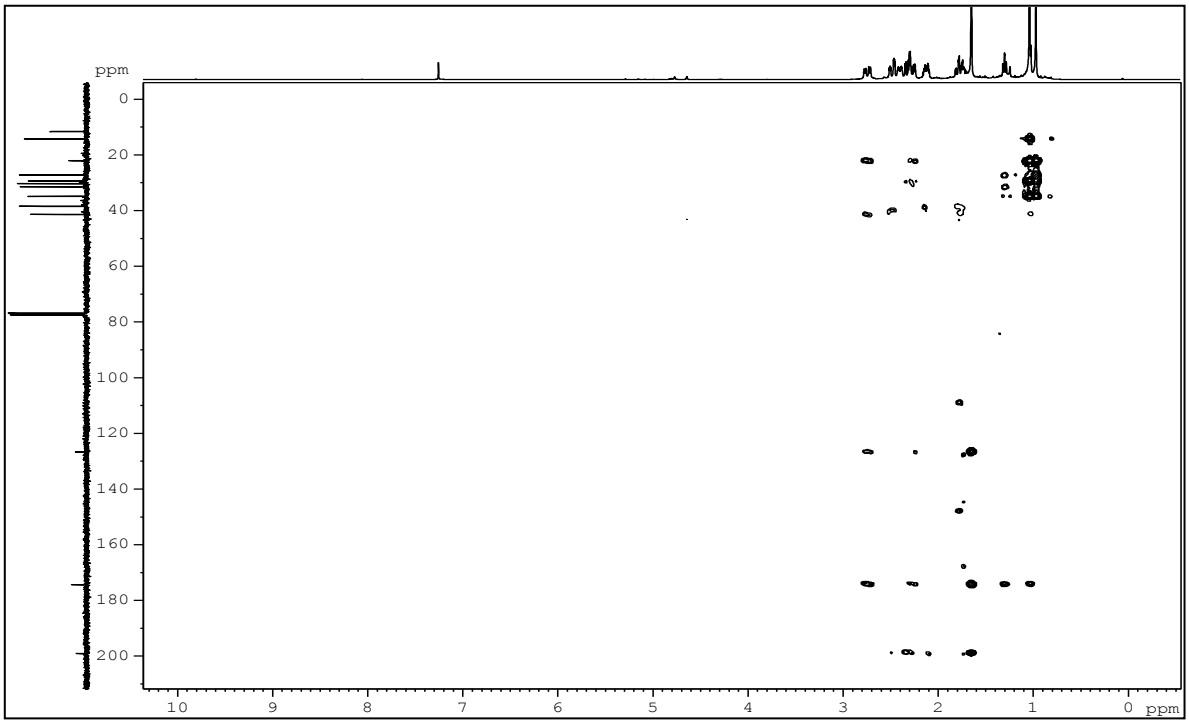
Enlarged NOESY Spectrum (400 MHz) of 35 in $CDCl_3$



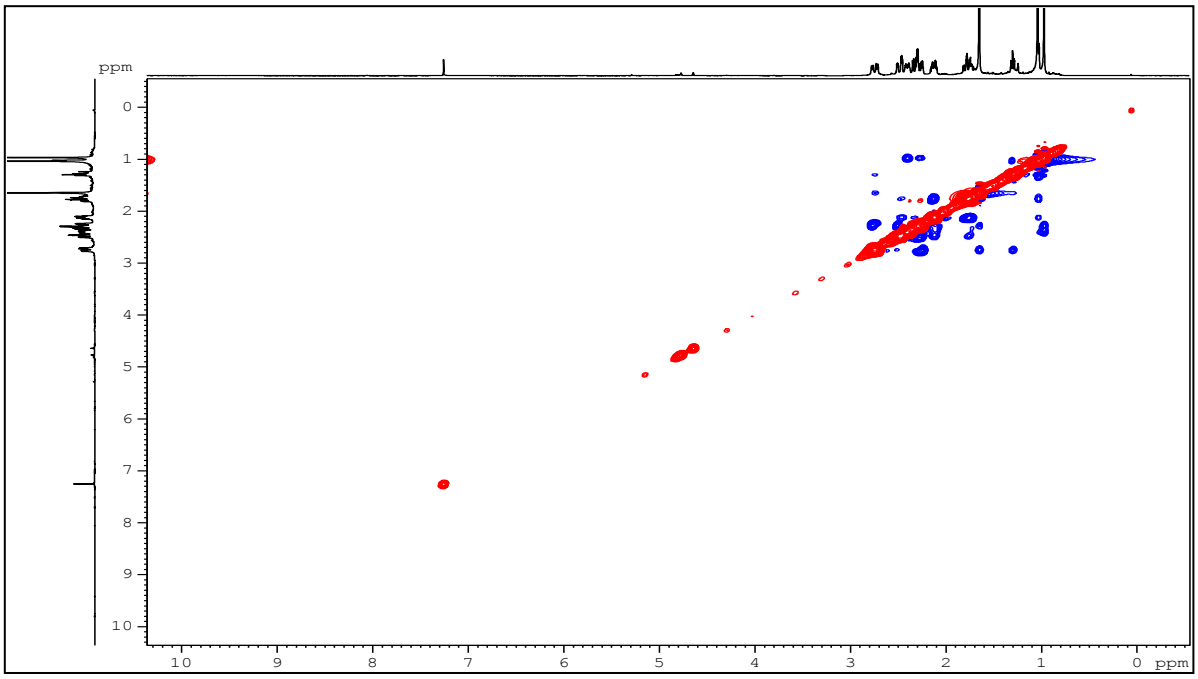
^{1}H-^{1}H COSY spectrum (400 MHz) of 45 in $CDCl_3$



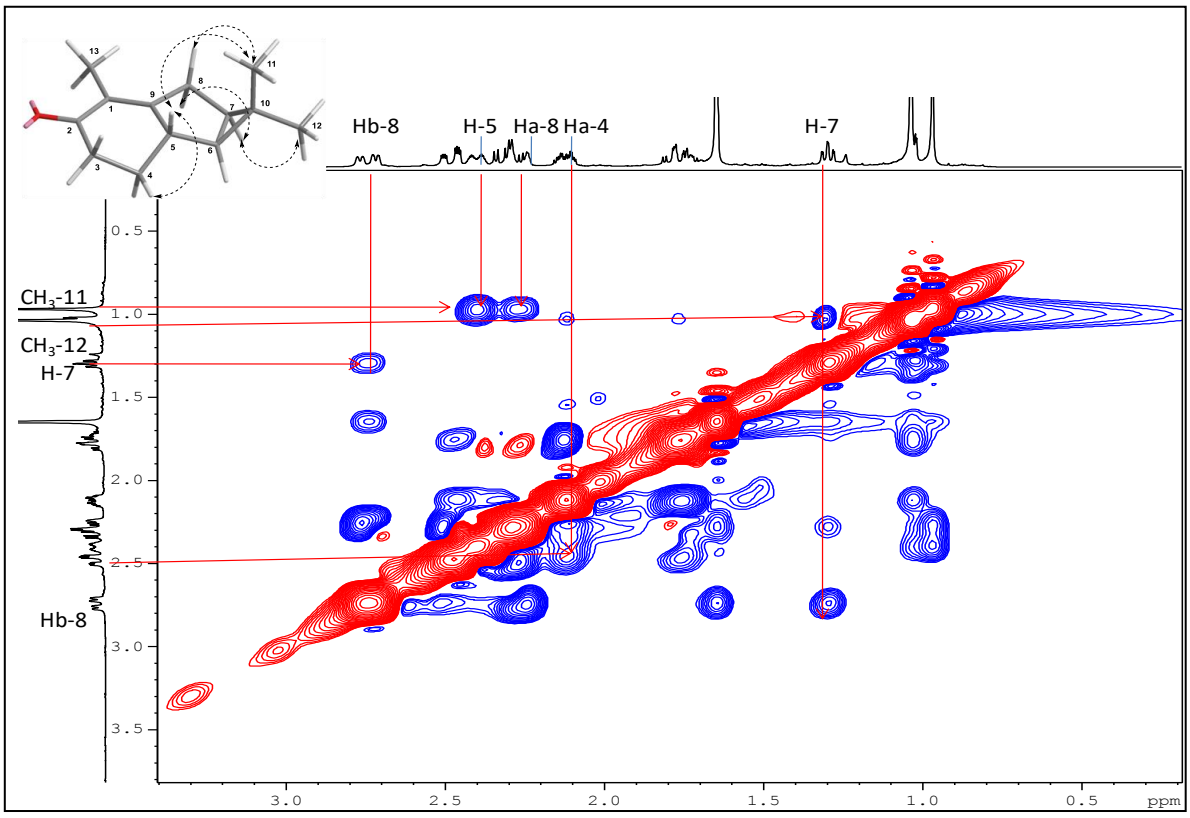
HMBC Spectrum (400 MHz) of 45 in $CDCl_3$



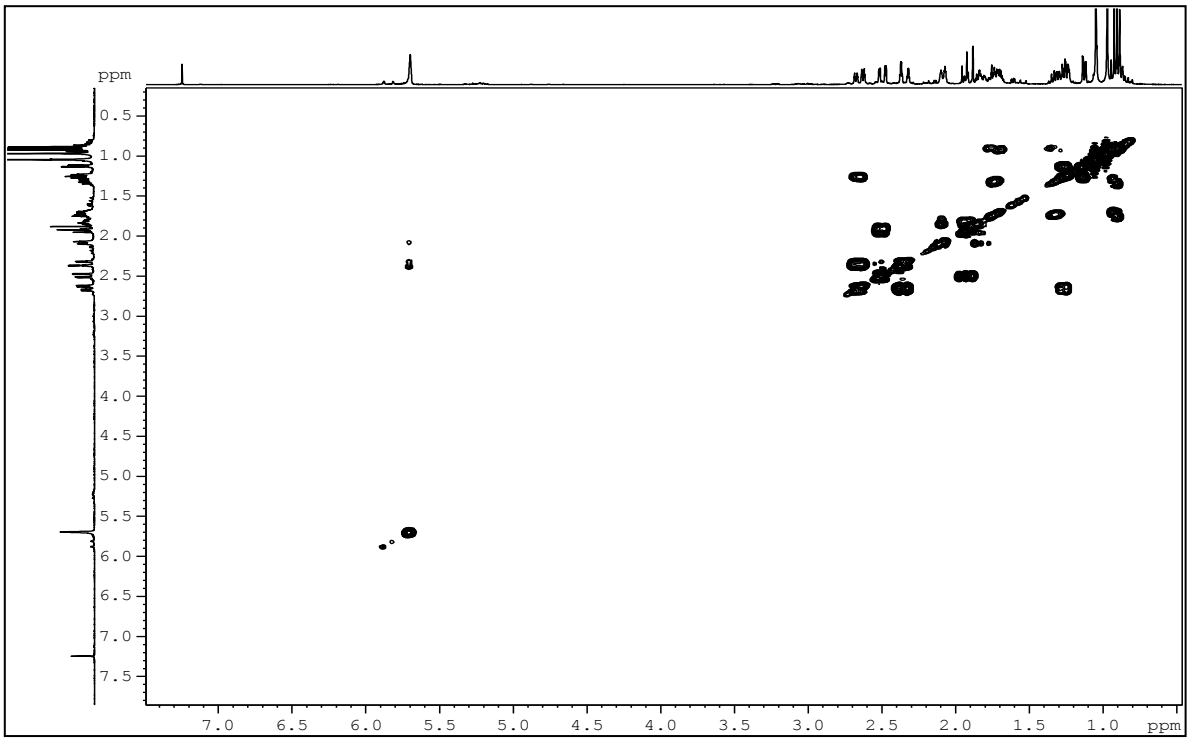
NOESY Spectrum (400 MHz) of 45 in $CDCl_3$



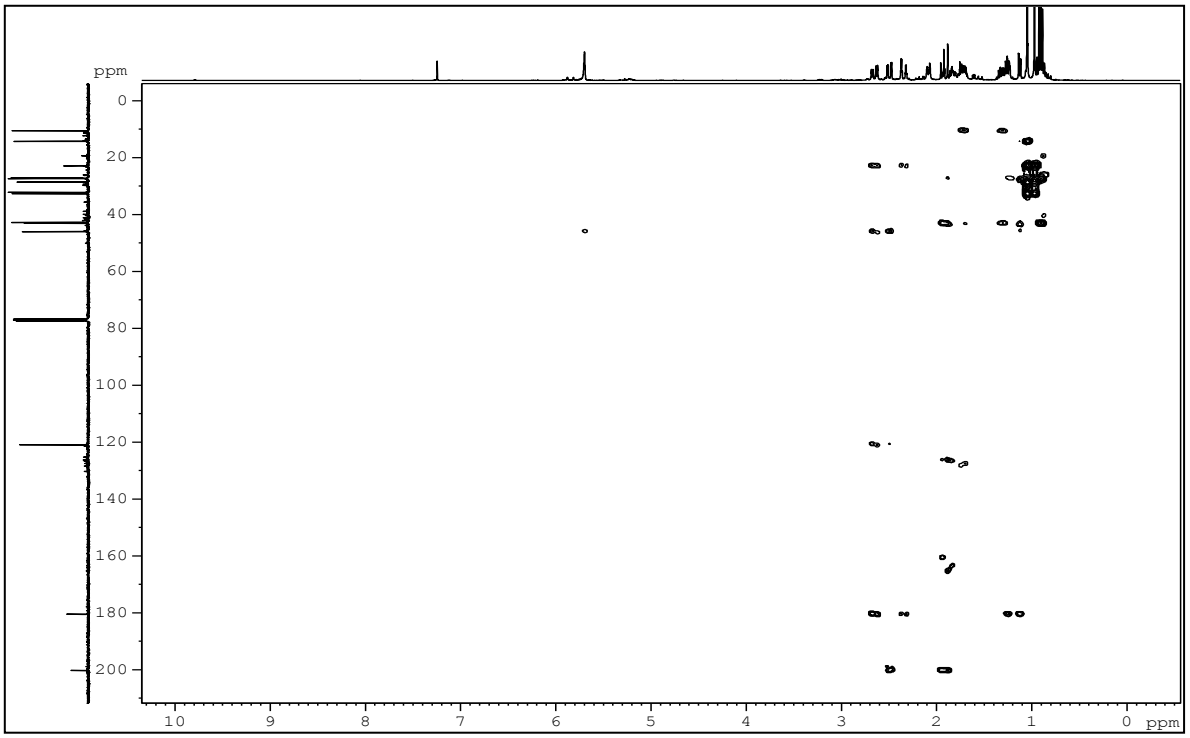
Enlarged NOESY Spectrum (400 MHz) of 45 in $CDCl_3$



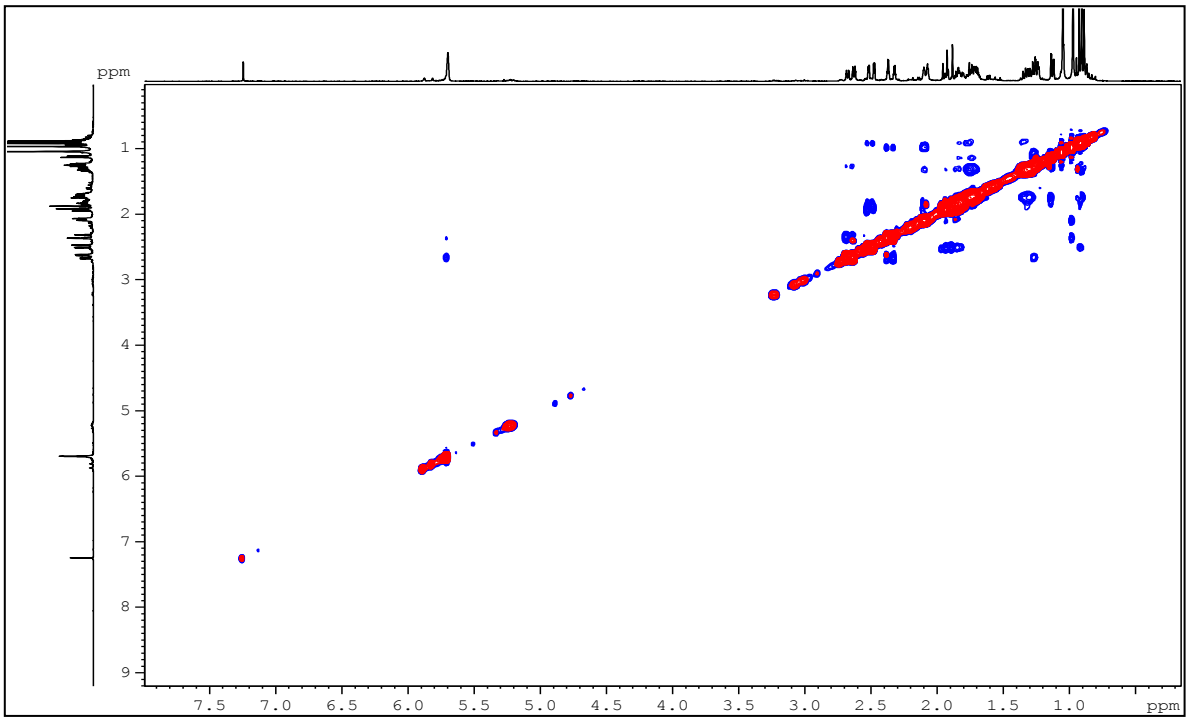
^{1}H-^{1}H COSY spectrum (400 MHz) of 46 in $CDCl_3$



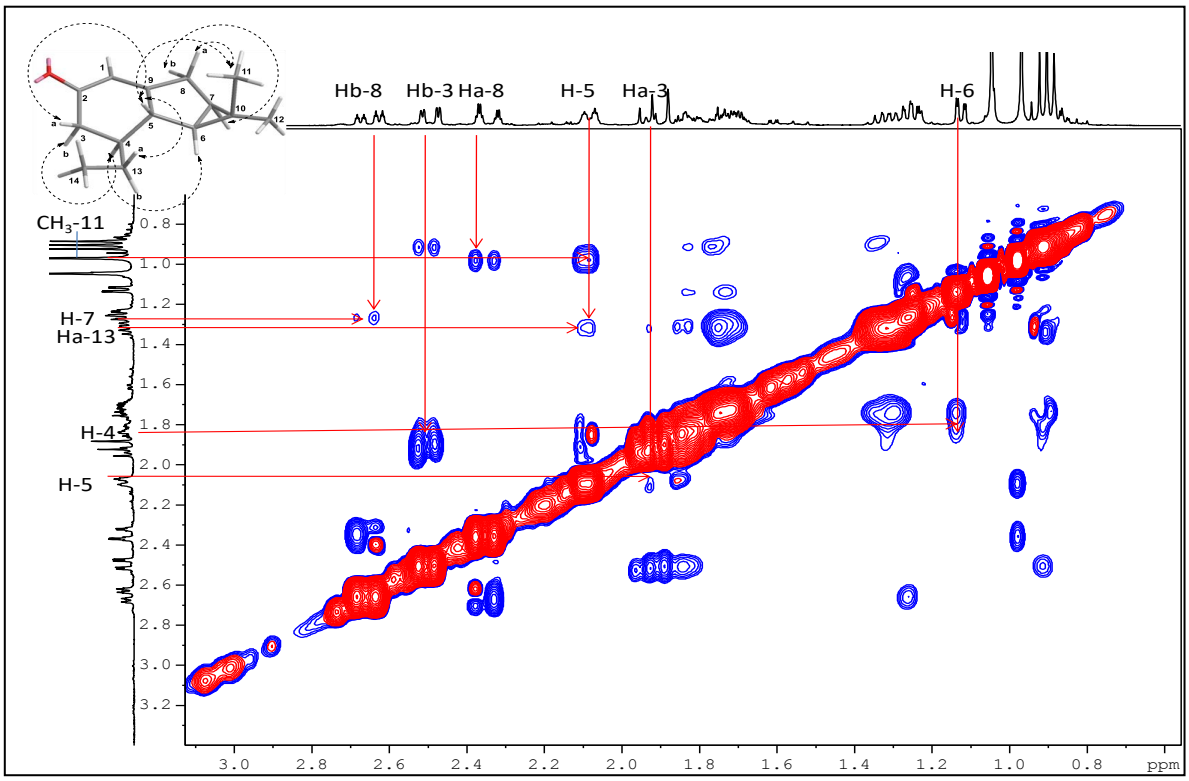
HMBC Spectrum (400 MHz) of 46 in $CDCl_3$



NOESY Spectrum (400 MHz) of 46 in $CDCl_3$



Enlarged NOESY Spectrum (400 MHz) of 46 in $CDCl_3$



Chapter 4

Total Synthesis and Biological Evaluation of Neural Anti-inflammatory Agents Based on Natural Products

4.1 Introduction to neuroinflammation

Neuroinflammation is related to nervous tissue in central nervous system (CNS), which can be triggered by due to various kinds of infections, traumatic brain injury, toxic metabolites (toxins) and autoimmunity.[1] In response to inflammatory signals, microglia cells, the resident macrophage-like immune cells resulted in the production of various pro-inflammatory cytokines such as nitric oxide (NO), tumor necrosis factor a (TNF-α), and interleukin 1β (IL-1β), which are responsible to the development of neurodegenerative diseases.[2-3] Neuroinflammation can result in neurodegenerative disorders[4] such as including Alzheimer's disease (AD), Parkinson's disease (PD). Both the diseases AD and PD are neurodegenerative diseases which damage the brain cells (neurons) and are progressive, so get worse over the time and typically begin late in life. In the late stages of both diseases, the neurodegeneration can eventually leads to a severe impairment in memory. Therefore any effort towards development of new, effective, and safe treatments for neurodegenerative disorders is worth. We became interested in decalin based natural products scaffold and the details are discussed in next sections.

4.2 Introduction to neural anti-inflammatory agents

As part of early drug discovery efforts in our group based on natural products, we have identified periconianones and related natural products a unique class of eremophilane-type sesquiterpene to treat neurodegenerative disorders as they show neural anti-inflammatory properties. Endophytic fungus *Periconia sp.* F-31 which was derived from the medicinal plant *Annonsa muricata* was resulted in the isolation of three natural products called botryosphaeridione (**1**), periconianone A (**2**) and periconianone B (**3)** (Fig 4.1).[5] It was proposed that compounds **1-3** biogenetically derived from eremophilane-type sesquiterpenoid through series of oxidations, hydroxylations, decarboxylations and intramolecular aldol condensation. All three compounds **1-3** were reported to inhibit the nitric oxide (NO) production induced by lipopolysaccharide (LPS) in mouse microglia BV2 cells using curcumin **4** as a positive control and shown impressive IC_{50} values of 0.23, 0.15, and 0.38 μM, respectively. This results suggest that compound based on dihydro-, tetrahydro-naphthalene-2,6-dione scaffolds can be promising lead structures for the treatment of CNS disorders such as PD and AD as microglia are endogenous immune cells

in the CNS that play critical roles in neurodegenerative disorders.[6-7]

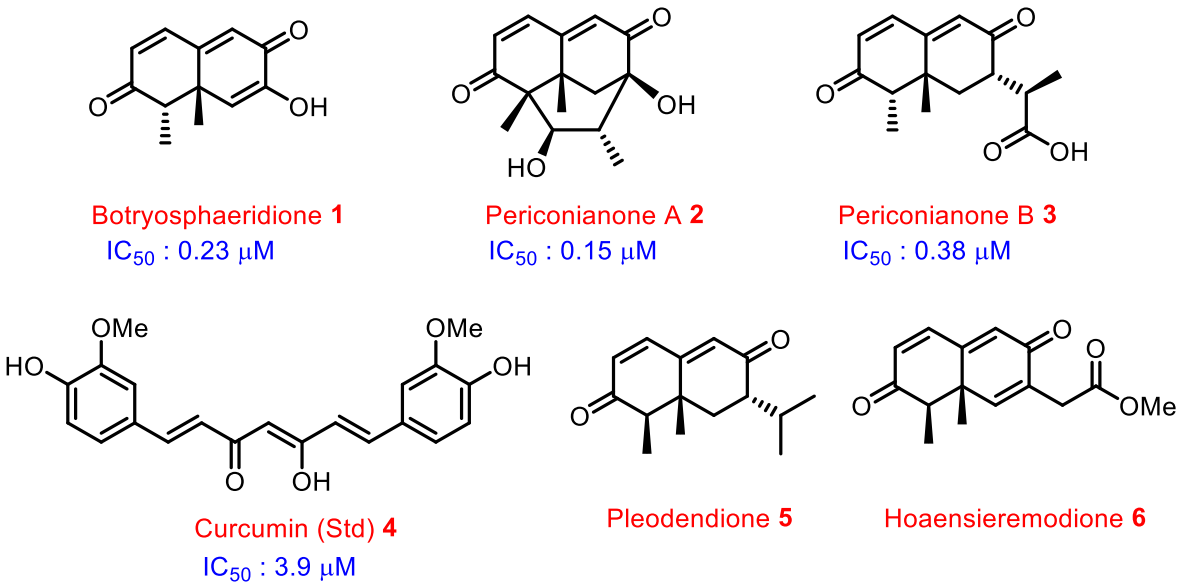


Fig 4.1: Structures of natural products based on dihydro-, tetrahydro-naphthalene-2, 6-dione scaffolds and Curcumin.

Related compounds pleodendione **5**, hoaensieremodione **6** and botryosphaeridione **1** were isolated previously.[8-10] The structures and absolute configurations of all compounds shown in Fig 4.1 were established by extensive spectroscopic analysis, ECD calculations, and single-crystal X-ray diffraction methods.

4.3 Present work

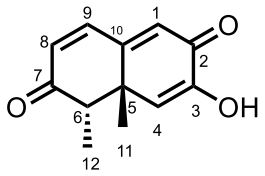
The interesting structural features of periconianones and related natural products and neural anti-inflammatory activity; we became interested in this novel chemotype to synthesize several compounds and to test their neural anti-inflammatory potential with keeping following objectives in mind.

- Total synthesis of target natural products in sufficient quantities for further biological profiling.
- Synthesis of structurally close analogues around dihydro-, tetrahydro-naphthalene-2, 6-dione scaffold.

- Biological evaluation of synthesized compounds towards identification lead compound.

4.3.1 Botryosphaeridione

The botryosphaeridione **1** also known as a trinorsesquiterpenic diketo-alcohol was first isolated from the endophytic fungus *Botryosphaeria rhodina* in 2009.[10] Its relative configuration was determined based on the DFT analysis of chemical shifts in ^{13}C-NMR and NOESY correlations. The absolute configuration of compound **1** was unambiguously established by experimental and calculated CD spectra as well as by X-ray diffraction. The bioassay showed that **1** inhibited the seed germination of lettuce. It possesses dihydronaphthalene-2, 6-dione skeleton with *trans*-configuration of the two methyls at C-5 and C-6 positions (Fig 4.2). Interestingly it contains OH group on alpha carbon (C-3) of an enone (C-2) and only a few methods exist in the literature for the introduction of hydroxy functionality on alpha carbon of enone moiety.[11-14]



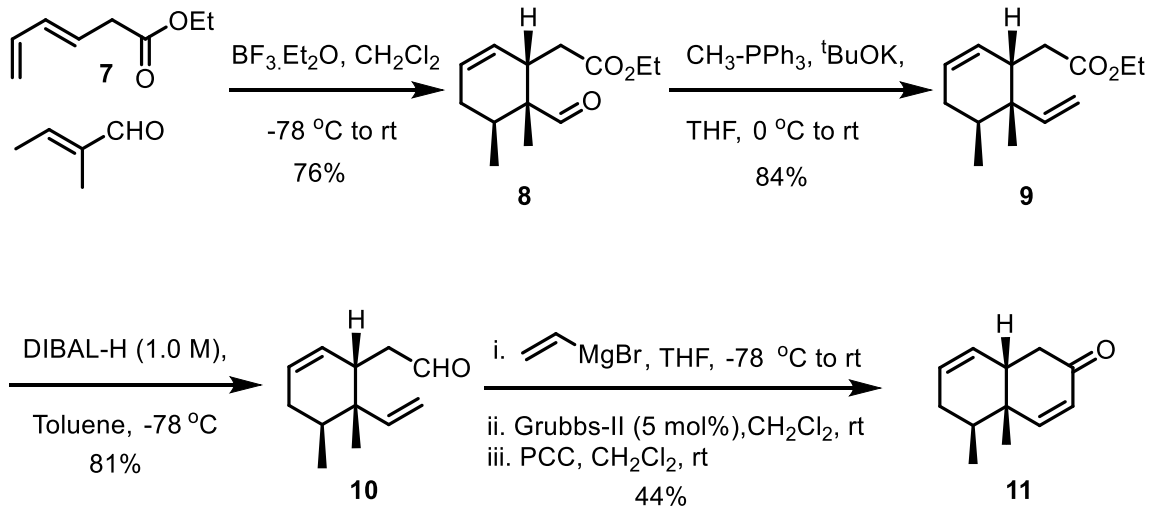
Botryosphaeridione **1**

Fig 4.2: Structure of botryosphaeridione **1**

4.3.1.1 Synthesis of botryosphaeridione (1)

Our synthetic efforts commenced with Diels-Alder reaction of diene ester **7** and tiglic aldehyde in presence of Lewis acid BF_3•Et_2O at -78 ^oC to rt afforded **8** in 76% yield which on single carbon Wittig reaction in presence potassium *tert*-butoxide at 0 ^oC give the **9** in 84% yield. The ester group in compound **9** was reduced using DIBAL-H (1.0 M in toluene) in presence of toluene at -78 ^oC to give aldehyde **10**[15] in 81% yield as colorless oil. This transformation was characterized by the presence of aldehydic proton at 9.74 (s, 1H) in ^{1}H-NMR spectrum which was further reassured by absorption at 1734 cm^{-1} in IR spectrum. The aldehyde **10** on Grignard reaction using vinylmagnesium bromide gave alcohol which

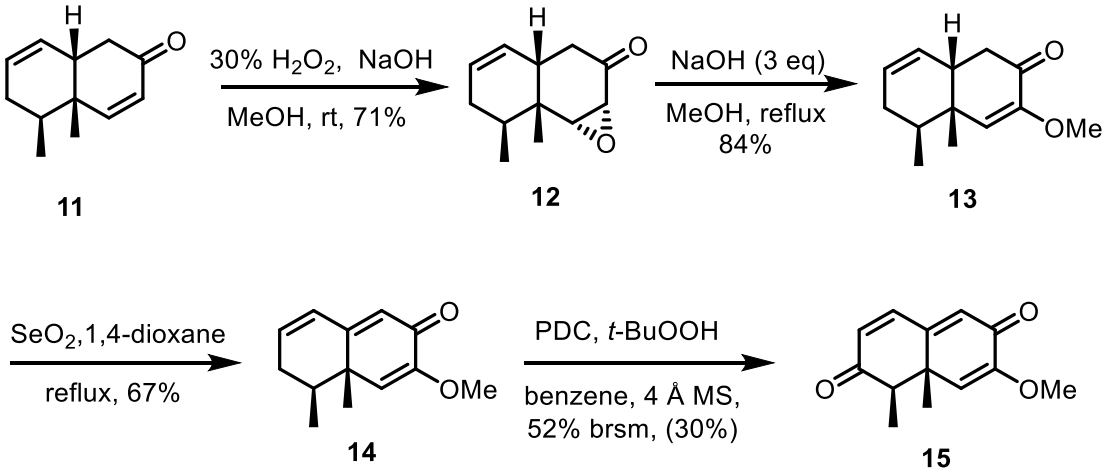
was subjected to ring-closing metathesis (RCM) reactions[16] to obtain the *cis*-fused decalin which was further oxidized by PCC[17] to the desired enone **11** in overall 44% yield from **10** (scheme 4.1). The formation of enone **11** was indicated by the presence of two distinguishable olefins in ^{1}H NMR at 6.71 (d, J = 10.3 Hz, 1H), 5.86 (d, J = 10.3 Hz, 1H), 5.64–5.47 (m, 2H) ppm and two vicinal methyl groups at 1.05 (s, 3H), 0.94 (d, J = 6.8 Hz, 3H) ppm. Similarly corresponding carbon signals at 159.1, 128.8, 127.1, 126.3 ppm for olefins, for at 20.4, 14.9 ppm two methyl groups and carbonyl carbon at 199.4 ppm in ^{13}C NMR. The assigned structure of **11** was also further supported by HRMS which showed a peak at 177.1274 corresponding to formula $C_{12}H_{17}O$ $[M+H]^+$ with calculated value of 177.1274.



Scheme 4.1: Synthesis of key intermediate enone **11**

The conjugated double bond in enone **11** was subjected to selective epoxidation using 30% H_2O_2-NaOH in MeOH to produce compound **12** in 71% yields.[18] The assigned stereochemistry of the epoxide in compound **12** was established by 2D-NMR analysis. The ^{13}C NMR and DEPT spectra of epoxide **12** exhibited 12 carbon signals, corresponding to one carbonyl carbons, one quaternary carbons, two olefinic carbon, two oxygen attached carbon and two methyl groups. The ^{1}H-^{1}H COSY spectrum provided connectivities for H-6 and H-7. The stereochemistry of epoxide **12** was deduced from NOESY experiments. H-6

having correlation with CH_3-12 and CH_3-11. This suggested that H-6, H-7, CH_3-12 and CH_3-11 are situated on same side making the epoxide in *α*-orientation. The key NOE correlations are shown in Fig 4.3.



Scheme 4.2: Synthesis of **15**

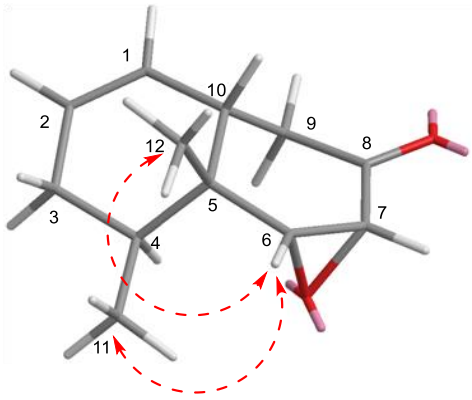
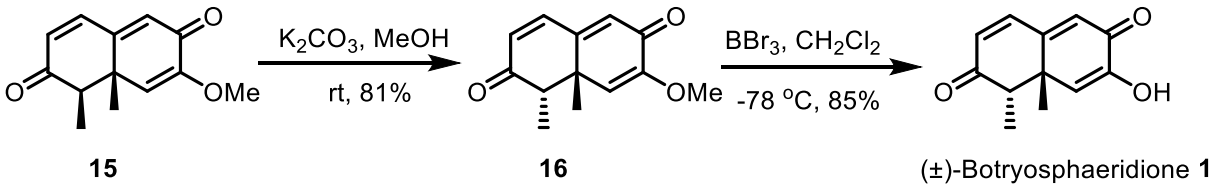


Fig 4.3: Key NOE correlation of epoxide **12**

Next epoxide **12** was treated with NaOH in MeOH at reflux conditions[19] for 30 min, TLC indicated clean conversion with the formation of a slightly polar spot which was isolated by column chromatography to give methoxy enone **13** in 84% yields. The formation of the methoxy enone **13** was indicated by the presence of two distinguishable olefins in ^{1}H NMR at 5.66–5.61 (m, 1H), 5.60 (s, 1H), 5.51–5.49 (m, 1H) ppm and signal at 3.58 (s, 3H) ppm corresponds to OMe protons. The assigned structure of **13** was also further confirmed by

HRMS. The methoxy enone **13** on treatment with selenium dioxide (SeO_2) in 1,4 dioxane at reflux conditions[20] undergoes allylic oxidation followed by elimination in same pot to produce compound **14** in 67% yields. During this reaction, we expected that further allylic oxidation might take place in the same pot to have the desired compound **15** but we did not observe that transformation. The desired allylic oxidation was achieved using additional step under *t*-BuOOH-PDC conditions[21] to have compound **15** in 52% brsm yields (scheme 4.2). The formation of **15** was indicated by the presence of olefins protons in ^{1}H NMR at 7.21 (d, J = 9.8 Hz, 1H), 6.45 (s, 1H), 6.28 (d, J = 9.8 Hz, 1H), 5.99 (s, 1H) ppm and OMe at 3.72 (s, 3H) ppm. Similarly corresponding carbon signals at 158.0, 151.5, 142.0, 131.5, 128.4, 121.0 ppm and two carbonyl carbon at 198.6, 180.4 ppm in ^{13}C NMR and peak at *m/z* 219.1014 in the HRMS analysis corresponding to $[M+H]^+$ with molecular formula $C_{13}H_{15}O_3$ confirmed the formation of oxidized product **15**. To achieve the final target, epimerization of the methyl group at C-4 position in compound **15** was required and it was accomplished by treating compound **15** to K_2CO_3 in methanol[22] to give epimerized product **16** in 81% yield. The significant shift of methyl signal (doublet) in ^{1}H NMR spectrum from 1.34 ppm to 0.92 ppm indicated the epimerization. The thermodynamic stability of compound **16** as compared to **15**, favors the equilibrium to shift its side to give more stable conformation of **16** where two methyl groups are *trans* to each other and same conformation also exist in natural product botryosphaeridione **1**.

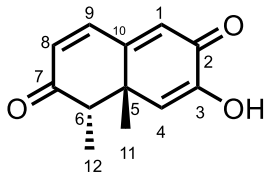


Scheme 4.3: Synthesis of (±)-botryosphaeridione **1**

After having the compound **16** in hand, final deprotection of enol group was achieved using BBr_3[23] in CH_2Cl_2 at -78 °C to have the target compound (±)-botryosphaeridione (**1**) in 85% isolated yield (scheme 4.3). All the spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) were found to be identical to the values reported in the isolation paper.[10] The ^{1}H and ^{13}C-NMR comparisons of both natural and synthetic (±)-botryosphaeridione are shown in table 4.1.

Thus, we have accomplished the first total synthesis of a (±)-botryosphaeridione **1**.

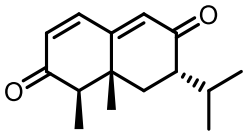
Table 4.1: ^{1}H and ^{13}C-NMR comparisons of both natural and synthetic **(±)-**botryosphaeridione **1**



No	Natural Botryosphaeridione		Synthesized (±)-Botryosphaeridione	
	^{1}H-NMR	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C-NMR**
1	6.64 (s, 1H)	128.1	6.63 (s, 1H)	128.1
2	-	181.4	-	181.4
3-OH	6.41 (s, 1H)	148.2	6.42 (s, 1H)	148.2
4	6.06 (s, 1H)	123.7	6.05 (s, 1H)	123.8
5	-	45.7	-	45.7
6	2.64 (q, *J* = 7.2 Hz, 1H)	53.4	2.64 (q, *J* = 7.2 Hz, 1H)	53.4
7	-	201.0	-	201.5
8	6.20 (d, *J* =10.2 Hz, 1H)	129.7	6.20 (d, *J* = 10.2 Hz, 1H)	129.7
9	7.22 (d, *J* =10.2 Hz, 1H)	141.5	7.21 (d, *J* = 10.2 Hz, 1H)	141.5
10	-	158.2	-	158.2
11	1.39 (s, 3H)	29.4	1.38 (s, 3H)	29.4
12	0.91 (d, *J* = 7.2 Hz, 3H)	15.6	0.91 (d, *J* = 7.2 Hz, 3H)	15.6

4.3.2 Pleodendione

An eremophilane-type sesquiterpene, pleodendione **5** was isolated from tree *Pleodendron costaricense* and it has tetrahydronaphthalene-2, 6-dione skeleton with presence of isopropyl group (Fig 4.4).[8] Pleodendione has shown weak antifungal activity and the amount of **5** was not sufficient to carry out any further biological profiling.[8]

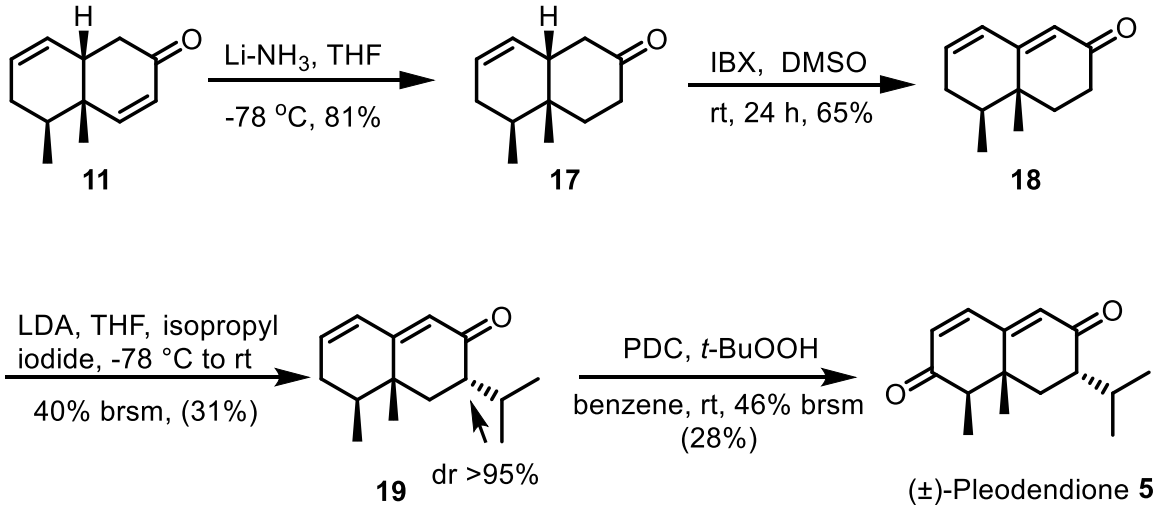


Pleodendione **5**

Fig 4.4: Structure of pleodendione **5**

4.3.2.1 Synthesis of pleodendione 5

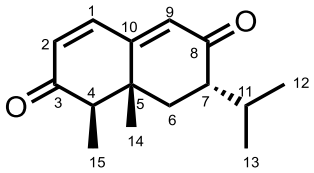
After the successful synthesis of botryosphaeridione **1**, we diverted our effort to synthesize pleodendione **5** from intermediate enone **11**. Synthesis commenced with chemoselective reduction of enone double bond present in compound **11** using Li-liq.NH_3 conditions[24] to give the corresponding saturated ketone **17** in 81% yield which was immediately used for next step without purification. The compound **17** on treatment with IBX/ DMSO[25] undergoes dehydrogenation to recreate the double bond other side of ring to give dienone **18**. Probably, this can be explained by extended conjugation which installed double bond in a highly regioselective manner (scheme 4.4). The product formation of **18** was indicated by the presence of three olefin protons in ^{1}H NMR at 6.23–6.11 (m, 2H), 5.68 (s, 1H) ppm.



Scheme 4.4: Syntheses of (±)-pleodendione **5**

Stereoselective alkylation of the lithium enolate of enone **18** generated by lithium diisopropylamide (LDA) with isopropyl iodide in presence of THF gave alkylated product **19** in low yields (40% brsm) but high diastereoselectivity (>95%).[26] The alkylated product **19** was indicated by presence of characteristic isopropyl protons at 0.97 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H) and 2.65–2.57 (m, 1H) ppm in ^{1}H-NMR spectrum. Finally, compound **19** on allylic oxidation using *t*-BuOOH-PDC conditions same as used in the synthesis of **1** to afford (±)-pleodendione **5** in 46% brsm yields. All the spectral data (^{1}H NMR, ^{13}C NMR and MS) were found to be identical with the literature values.[8] The ^{1}H and ^{13}C-NMR comparisons of natural and synthetic (±)-pleodendione **5** are shown in table 4.2

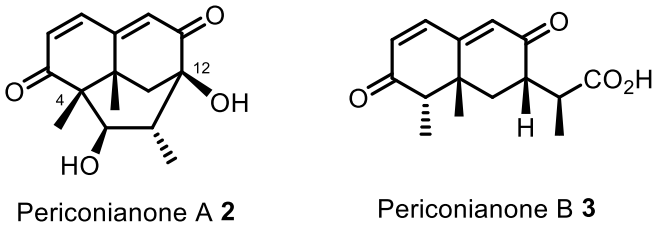
Table 4.2: ^{1}H and ^{13}C-NMR comparisons of both natural and synthetic (±)-pleodendione **5**



No	Natural Pleodendione		Synthesized (±)-Pleodendione	
	^{1}H-NMR	**^{13}C-NMR**	**^{1}H-NMR**	**^{13}C-NMR**
1	6.96 (d, *J* = 9.8 Hz, 1H)	142.3	6.96 (d, *J* = 9.8 Hz, 1H)	142.3
2	6.19 (d, *J* = 9.8 Hz, 1H)	132.2	6.19 (d, *J* = 9.8 Hz, 1H)	132.1
3	-	200.0 or 200.2	-	200.1 or 200.2
4	2.56 (q, *J* = 6.8 Hz, 1H)	52.6	2.56 (q, *J* = 6.8 Hz, 1H)	52.5
5	-	40.4	-	40.3
6	1.97 (dd, *J* = 12.9, 4.7 Hz, 1H) 1.74 (t, *J* = 14.0 Hz, 1H)	34.6	1.97 (dd, *J* = 12.9, 4.7 Hz, 1H) 1.74 (t, *J* = 13.8 Hz, 1H)	34.4
7	2.33 (ddd, *J* = 14.1, 4.6, 3.2 Hz, 1H)	47.5	2.36-2.31 (m, 1H)	47.4
8	-	200.0 or 200.2	-	200.1 or 200.2
9	6.00 (s, 1H)	129.8	6.00 (s, 1H)	129.7
10	-	158.4	-	158.3
11	2.63-2.55 (m, 1H)	26.2	2.63-2.54 (m, 1H)	26.0
12	0.82 (d, *J* = 7.0 Hz, 3H)	17.9 or 20.4	0.81 (d, *J* = 6.8 Hz, 3H)	17.7 or 20.3
13	0.97 (d, *J* = 7.0 Hz, 3H)	17.9 or 20.4	0.97 (d, *J* = 6.8 Hz, 3H)	17.7 or 20.3
14	1.12 (s, 3H)	18.6	1.12 (s, 3H)	18.6
15	1.14 (d, *J* = 7.0 Hz, 3H)	7.2	1.14 (d, *J* = 6.8 Hz, 3H)	7.2

4.3.3 Periconianones A and B

Periconianone A (**2**) polyoxygenated sesquiterpenoid possesses rigid 6/6/6 carbocyclic skeleton. It consisting one new six-membered carbonic ring formed by the connection of C-4 and C-12.[5] It has tetrahydronaphthalene-2, 6-dione skeleton. Periconianone B (**3**) is an eremophilane-type sesquiterpenoid possesses tetrahydro- naphthalene-2, 6-dione skeleton and having acid moiety in side chain[5] (Fig 4.5).



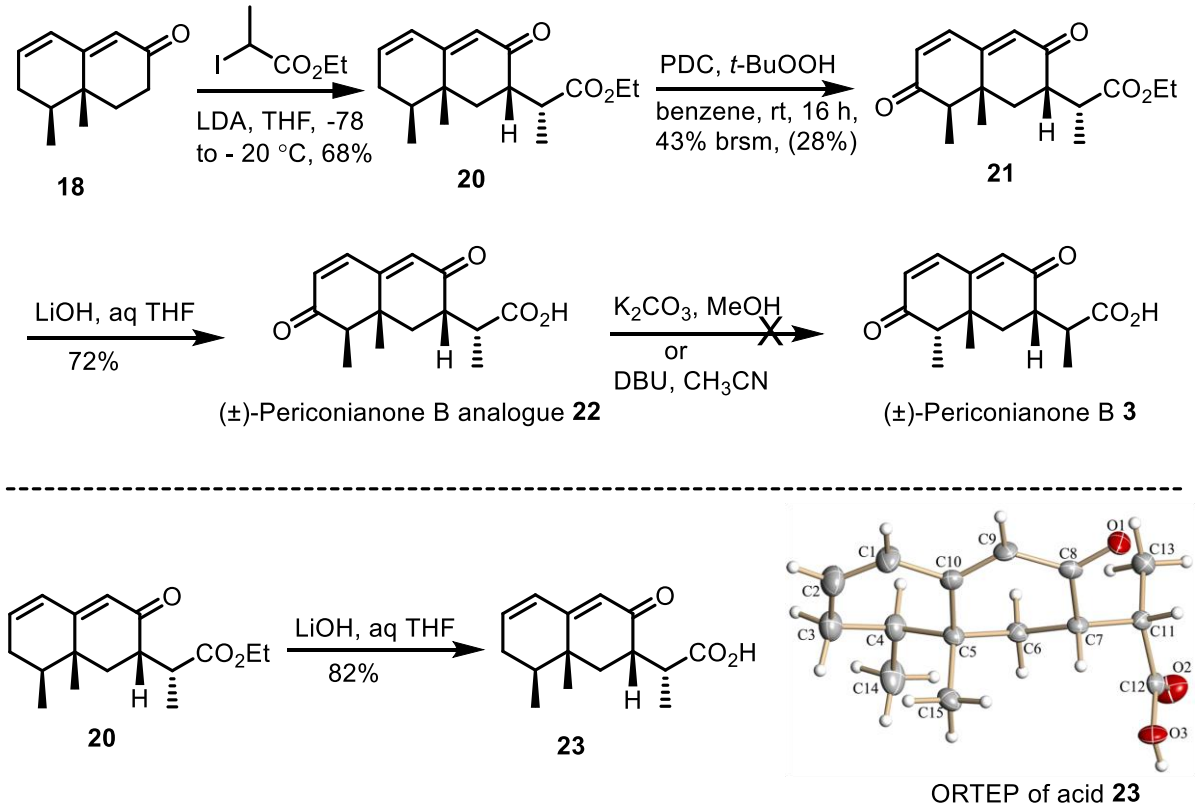
Periconianone A **2** Periconianone B **3**

Fig 4.5: Structure of periconianone A (**2)** and periconianone B (**3**).

4.3.3.1 Attempts towards Periconianone B

For the synthesis of periconianone B **3**, enone **18** on alkylation with ethyl-2-iodopropanoate using lithium diisopropylamide (LDA) in THF at -78 ^{o}C afforded desired ketoester **20** as a major diastereomer (>85%) with 68% isolated yield.[27] This result suggest that methyl group epimerized in the reaction due to basic medium to give more stable orientation of methyl group. To confirm the stereochemistry of newly generated chiral centers in compound **20**, we have synthesized its corresponding carboxylic acid **23** as a crystalline solid. The acid **23** was recrystallized using ethyl acetate-hexane mixture. The assigned stereochemistry ketoester **20** was unambiguously determined with the help of the single X-ray crystal analysis of compound **23** (scheme 4.5). The compound **20** was subjected to allylic oxidation using *t*-BuOOH-PDC conditions to give compound **21** in 43% brsm yields. Ester group in compound **21** was hydrolysis using LiOH in aqueous THF[28] to give periconianone B analogue **22** in 72% yields. The assigned structure of **22** was supported by a thorough 2D-NMR analysis and it suggested that the methyl group at C-4 position was situated at β-orientation and it not epimerized under basic conditions. The stereochemistry of **22** was deduced from NOESY experiments. NOE correlations from CH_3-15 with CH_3-14, H-6β

and H-7 indicated their β-orientations. There is strong NOE from H-4 to H-6α indicated their α-orientations (Fig 4.6).



Scheme 4.5: Synthetic attempts towards periconianone-B **3**

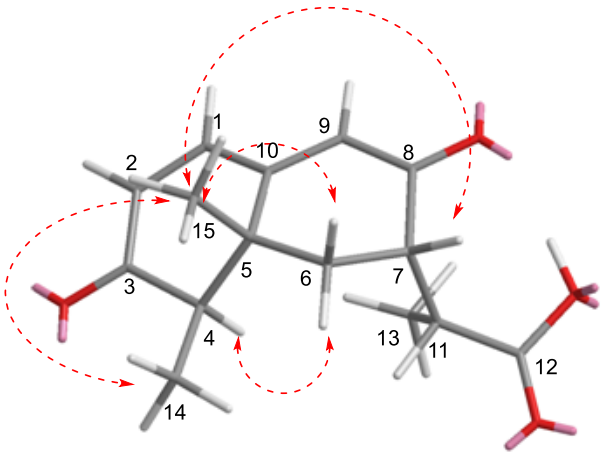


Fig 4.6: Key NOE correlations of compound **22**

Next, we have attempted some conditions such as K_2CO_3/MeOH and DBU/CH_3CN[29] to give epimerization of the methyl group at C-4 position but the desired epimerization at C-4 methyl group in the present case was not successful in our hands towards periconianone-B **3**. In case compound **15,** we succeed in epimerization of the methyl group at C-4 position which can be explained by the formation of enolate with extended conjugation, such extended conjugation is not possible in the case of compound **22**. While comparing the ^{1}H-NMR spectra, we found some similarity in coupling constant for compound **22** and **3**, so after careful analysis of coupling constants of H-7 with H-11 and H-6 in natural periconianone B **3** and synthesized periconianone B analogue **22**, it is observed that they are identical (ddd 14.4, 4.8, 4.8 Hz), this result prompt us make further analysis. Next we compared the coupling constant for compounds **20**, **21**, **23** and found that they were almost the same for a series of compounds **20**, **21**, **23** as listed in Fig 4.7.

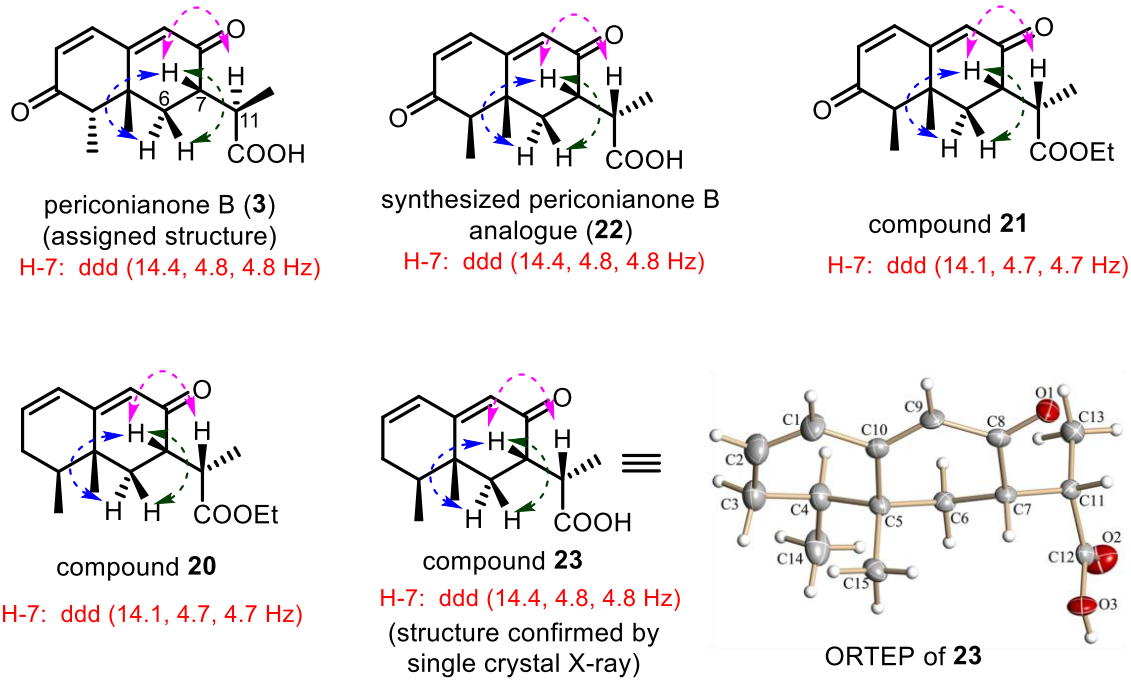
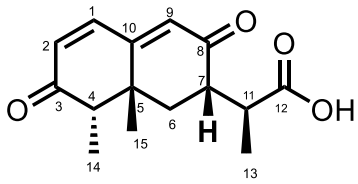


Fig 4.7: Comparisons of coupling constants between H-7, H-11 and H-6

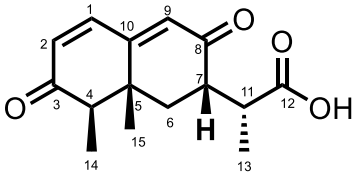
Based on single X-ray crystal structure analysis of compound **23**, we know that H-7 and H-11 are *cis* to each other. The very close match of coupling constants of H-7 with H-11 and H-6 suggests that the stereochemistry of C13 methyl group in periconianone B **3** is in

question which needs further investigation. The spectral data comparisons of ^{1}H and ^{13}C-NMR of natural periconianone B **3** and (±)-synthetic periconianone B analogue **22** are shown in table 4.3.

Table 4.3: ^{1}H and ^{13}C-NMR comparisons of natural periconianone B **3** and (±)-synthetic periconianone B analogue **22**



Natural Periconianone B

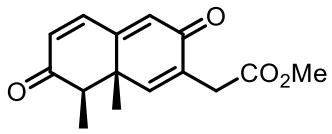


Synthesized Periconianone B analogue

No	**Natural Periconianone B**		**(±)-Synthesized Periconianone B analogue**	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
1	7.28 (d, *J* = 9.6 Hz, 1H)	142.6	7.27 (d, *J* = 9.8 Hz, 1H)	142.3
2	6.12 (d, *J* = 9.6 Hz, 1H)	129.1	6.23 (d, *J* = 9.8 Hz, 1H)	128.3
3	-	202.5	-	199.7
4	2.37 (q, *J* = 7.2 Hz, 1H)	52.4	2.73 (q, *J* = 6.7 Hz, 1H)	51.3
5	-	39.2	-	merged with DMSO-d_6
6	2.11 (dd, *J* = 14.4, 13.2 Hz, 1H) 1.52 (dd, *J* = 13.2, 4.8 Hz, 1H)	32.5	1.91-1.89 (m, 2H)	35.7
7	3.04 (ddd, *J* = 14.4, 4.8, 4.8 Hz, 1H)	43.6	2.97-2.95 (m, 1H)	43.8
8	-	198.6	-	198.5
9	6.25 (s, 1H)	130.6	6.16 (s, 1H)	131.7
10	-	155.9	-	158.8
11	2.91 (dq, *J* = 4.8, 7.2 Hz, 1H)	37.6	2.93-2.91 (m, 1H)	37.6
12	-	176.4	-	176.4
13	1.02 (d, *J* = 7.2 Hz, 3H)	13.0	1.02 (d, *J* = 7.0 Hz, 3H)	13.0
14	0.96 (d, *J* = 7.2 Hz, 3H)	14.4	0.99 (d, *J* = 6.7 Hz, 3H)	6.94
15	1.28 (s, 3H)	24.6	1.10 (s, 3H)	18.0

4.3.4 Hoaensieremodione

An eremophilane sesquiterpene, another closely related natural product called hoaensieremodione **6** was isolated from roots of *Drypetes hoaensis*. Its structure was established on the basis of spectroscopic analysis and it has dihydronaphthalene-2, 6-dione skeleton[9] (Fig 4.8).

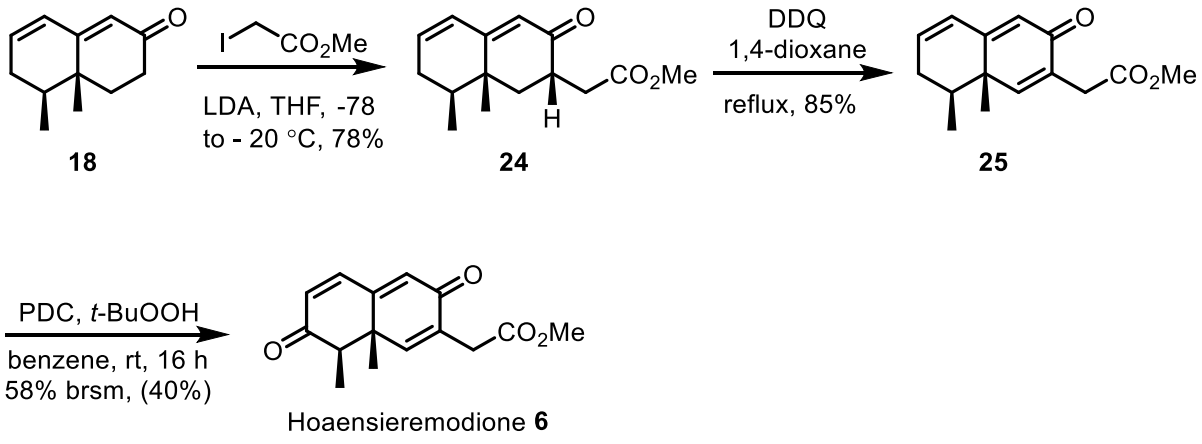


Hoaensieremodione **6**

Fig 4.8: Structure of hoaensieremodione **6**

4.3.4.1 Synthesis of hoaensieremodione 6

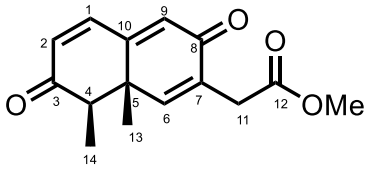
To expand the potential, we have also attempted the synthesis of hoaensieremodione by following similar chemistry as mentioned for above natural products synthesis. Previously synthesized intermediate **18** was alkylated using LDA in THF at -78 ^{o}C with methyl iodoacetate to give compound **24** in 78% yields with very high diastereoselectivity.[27] The alkylated product **24** was confirmed by the presence of olefins in ^{1}H NMR at 6.22–6.10 (m, 2H), 5.68 (s, 1H) ppm and OMe at 3.70 (s, 3H) ppm. Ester carbonyl at 173.3 ppm in ^{13}C NMR spectrum and HRMS analysis also revealed a mass peak at 249.1485 corresponding to $C_{15}H_{21}O_3$ $[M+H]^+$ matching with the structure **24**.



Scheme 4.6: Synthesis of (±)-hoaensieremodione **6**

The compound **24** was subjected to dehydrogenation using DDQ in 1, 4 dioxane at reflux conditions[30] to produced the compound **25** in 85% yield (scheme 4.6). This transformation was characterized by presence of olefinic protons at 6.95 (s, 1H), 6.26–6.23 (m, 1H), 6.13–6.08 (m, 1H), 6.01 (s, 1H) ppm, methyl ester proton at 3.67 (s, 3H) ppm in ^{1}H-NMR and further confirmed by HRMS. Finally allylic oxidation of compound **25** was achieved using PDC/*t*-BuOOH conditions to furnish the target compound (±)-hoaensieremodione **6** in 58% brsm yields. All the spectral data is in complete agreement with the reported data.[9]

Table 4.4: ^{1}H and ^{13}C-NMR comparisons of both natural and synthetic (±)-hoaensieremodione 6

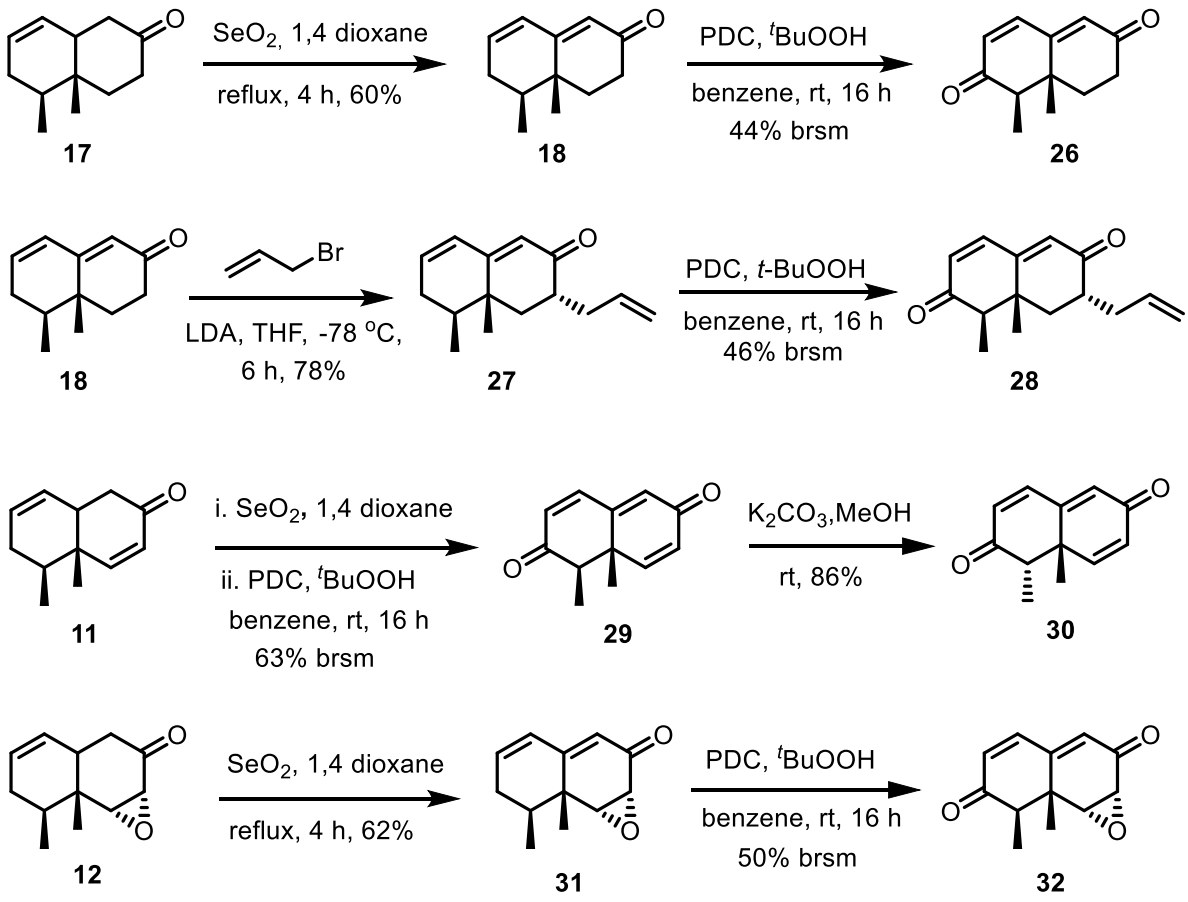


No	Natural Hoaensieremodione		Synthesized (±)-Hoaensieremodione	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	7.21 (d, *J* = 9.9 Hz, 1H)	142.1	7.20 (d, *J* = 9.8 Hz, 1H)	142.1
2	6.28 (d, *J* = 9.9 Hz, 1H)	131.3	6.27 (d, *J* = 9.8 Hz, 1H)	131.2
3	-	198.1	-	198.2
4	2.64 (q, *J* = 6.8 Hz, 1H)	49.6	2.63 (q, *J* = 6.8 Hz, 1H)	49.5
5	-	44.4	-	44.3
6	7.03 (s, 1H)	151.2	7.02 (s, 1H)	151.3
7	-	132.7	-	132.6
8	-	184.4	-	184.1
9	6.45 (s, 1H)	128.3	6.44 (s, 1H)	128.3
10	-	157.5	-	157.4
11	3.37 (d, *J* = 16.5 Hz, 1H) 3.42 (d, *J* = 16.5 Hz, 1H)	34.6	3.35 (d, *J* = 16.5 Hz, 1H) 3.41 (d, *J* = 16.5 Hz, 1H)	34.7
12	-	171.1	-	171.1
13	1.25 (s, 3H)	22.3	1.24 (s, 3H)	22.3
14	1.33 (d, *J* = 6.8 Hz, 3H)	7.8	1.33 (d, *J* = 6.8 Hz, 3H)	7.8
12-OMe	3.71 (s, 3H)	52.0	3.71 (s, 3H)	52.0

The spectral data comparisons of ^{1}H and ^{13}C-NMR of natural hoaensieremodione **6** and synthetic (±)-hoaensieremodione **6** are shown in table 4.4. Thus, we have accomplished the first total synthesis of a (±)-hoaensieremodione **6**.

4.3.5 Synthesis of Analogues

Towards the generation of more analogues around the working scaffolds, the ketone **17** was dehydrogenated using SeO_2 in 1, 4- dioxane at reflux conditions followed by allylic oxidation using PDC/*t*-BuOOH conditions gave the compound **26** in 44% brsm yield. The enone **18** was treated with lithium diisopropylamide (LDA) and allyl bromide in THF to afford alkylated product **27** in 78% yield and the pleasing outcome was high diastereoselectivity (>95%).[31]



Scheme 4.7: Synthesis of analogues

The alkylated product **27** shows characteristics terminal protons at 5.83–5.73 (m, 1H), 5.08–5.02 (m, 2H) in ^{1}H-NMR spectrum and further confirmed by HRMS. The compound **27** was subjected to allylic oxidation using *t*-BuOOH-PDC conditions to give compound **28** in 46% yields (scheme 4.7). Next the compound **11** was dehydrogenated using SeO_2 in 1, 4- dioxane at reflux conditions to give trienone-I which on allylic oxidation using PDC/*t*-BuOOH conditions gave the compound **29**. The formation of compound **29** was indicated by the presence of olefins in ^{1}H NMR at 7.19 (d, J = 10.0 Hz, 1H), 7.11 (d, J = 10.0 Hz, 1H), 6.41 (s, 1H), 6.37 (dd, J = 10.1, 1.8 Hz, 1H), 6.27 (d, J = 10.0 Hz, 1H) ppm and the assigned structure of **29** was further confirmed by HRMS which showed a peak at 189.0910 corresponding to formula $C_{12}H_{13}O_2$ $[M+H]^+$. Final epimerization of the methyl group at C-4 position in compound **29** was accomplished by treating compound **29** to K_2CO_3 in methanol to give epimerized product **30** in 86% yield (scheme 4.7). By using similar reactions sequence compound **32** was prepared from compound **12** (scheme 4.7). All the synthesized compounds were well characterized by using different analytical tools such as IR, ^{1}H-NMR, ^{13}C-NMR and HRMS.

4.3.6 Biological Evaluation

As per the plan, after synthesizing all the natural compounds and their analogues, they were screened for their anti-neuroinflammatory potential in the presence of LPS-induced inflammation using mouse microglia N9 cells. In neuroinflammation, large amount of NO is produced and in turn exaggerate the inflammatory response. Accordingly all the synthesized compounds were evaluated for their inhibition of NO production by following protocols.[32] The primary results indicated that almost of the compounds decreased NO level in LPS treated cells as shown in table 4.5. Meanwhile, the cytotoxicity of these compounds in N9 cells were also assessed by MTT assay.[33] Cytotoxicity has been performed by taking conc. range of 0-100 μM. The anti-inflammatory activity by measuring nitrite in mouse microglial cell (N9) has been carried out by taking 0-10 μM conc. range for compounds and standard curcumin. Based on the results, the IC_{50} have been determined and shown in table 4.5. All the biological experiments were carried out in Dr. Anirban Basu's laboratory at NBRC, Haryana, India.

Table 4.5: Cytotoxicity and inhibition of compounds on LPS induced NO generation in N9 cells

	Cytotoxicity (A)		NO inhibition (B)		
Compound	IC_{50} (µM)	95% confidence interval (µM)	IC_{50} (µM)	95% confidence interval (µM)	Selectivity index (A/B)
1	479.5	311.-648.1	3.3	2-4.5	145.3
5	132.4	110.4-154.3	20.6	11.5-29.7	6.4
14	107.2	79.5-135	34.5	18.8-50.2	3.1
15	8.1	7.7-8.6	2.4	1.1-3.7	3.3
16	67.3	69-105.7	6.4	2.3-10.5	10.5
18	678.6	404.1-953.2	61.7	46.6-74	10.9
20	60.9	43.3-78.5	58.9	41.5-76.4	1.1
23	785.8	664.8-906.7	177	131-223.2	4.4
21	299.1	351-247.2	18	13.6-22.5	16.6
22	284.2	260.7-307.8	26.1	18-34.2	10.9
29	570.7	402.1-739.3	4.1	2.9-5.1	139.2
30	416.2	335.2-497	17.6	13.9-21.4	23.6
32	5.3	4.2-6.3	3.7	2.8-4.8	1.4
28	183.5	163.8-203.2	12.3	8.5-16	14.9
26	125.8	76.8-174.9	34.1	25.1-43.2	3.7

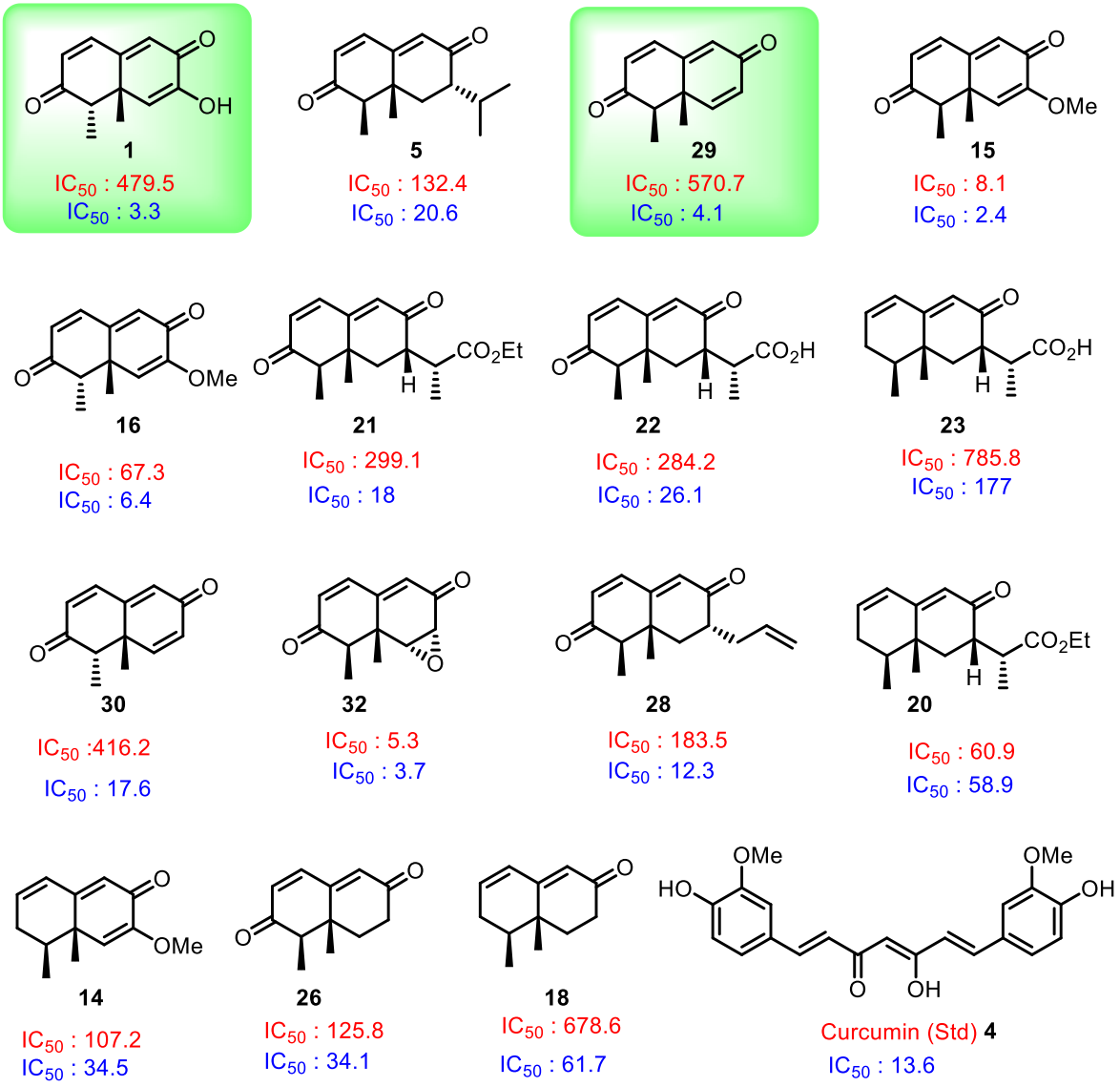


Fig 4.9: Structures of screened compounds with cytotoxicity and inhibition NO generation with IC_{50} in µM.

Structure–activity relationships (SAR):

Following conclusions were made based on anti-inflammatory potential (Fig 4.10). The compounds with dihydronaphthalene-2, 6-dione skeleton such as **1**, **15**, **16**, **29** showing better inhibition than tetrahydronaphthalene-2,6-dione. Esters (**20**, **21**) showing better activity than corresponding acids (**23**, **22**) (Fig 4.9). Introduction of oxygen at α position to carbonyl in compounds **15**, **16**, **32** increases the inhibition. 2, 6-dione functionality (**15**, **16**,

29 and **30**) is essential for activity as compared to enone functionality (**14**, **18**, **20** and **23**). The α-methyl at C-4 position increases the activity.

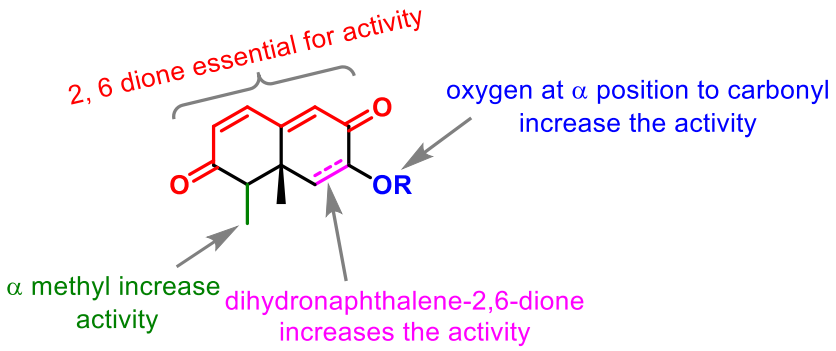


Fig 4.10: Structure–activity relationship (SAR)

The compounds **1** (IC_{50} 3.3 μM) and **29** (IC_{50} 4.1 μM) displayed better NO inhibition than the well-known standard curcumin (IC_{50}, 13.6 μM) as shown in Fig 4.11. Both the compounds **1** and **29** show effective NO inhibitory activity with low cytotoxicity than other compounds. Compound **1** and **29** were further profiled based on the selectivity index (cytotoxicity IC_{50}/NO inhibition IC_{50}) since they exhibited superior selectivity indices (>100) among all the derivatives.

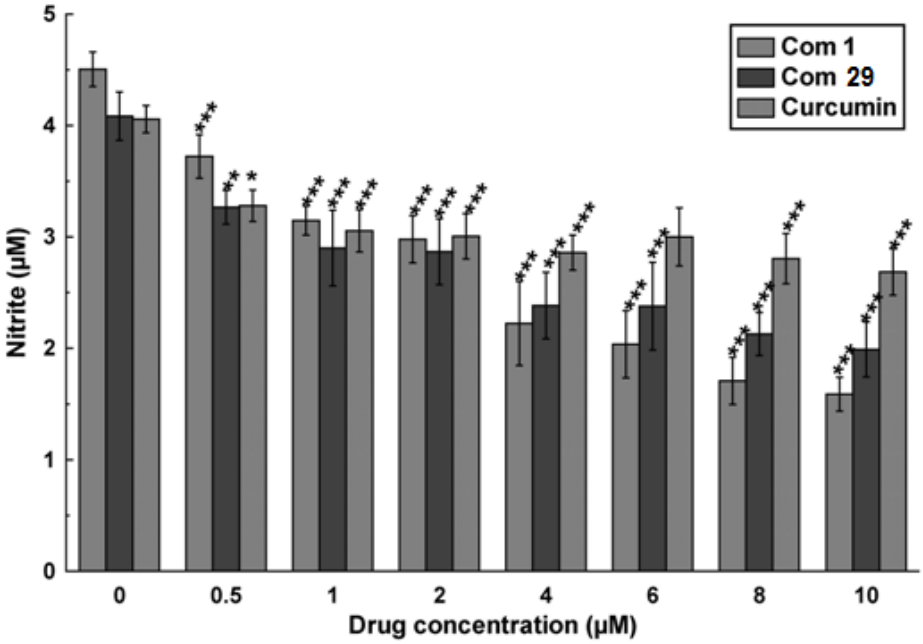


Fig 4.11: NO inhibition *vs* different concentration of **1**, **29**, curcumin (std.)

To study further, the anti-inflammatory potency of compounds **1** and **29** were profiled in additional assays. The reactive oxygen species (ROS) measurement in LPS treated N9 cells was carried out.[34] Reactive oxygen species (ROS) play a significant role in the development of inflammatory disorders since they are key signaling molecules. Generation of ROS in microglia is a key marker of neuro-inflammation. The treatment of N9 cells with LPS increased the ROS by more than two-fold as analyzed by measuring the mean fluorescence intensity (MFI) by Fluorescence Activated Cell Sorting (FACS). After the treatment, both compounds **1** and **29** resulted intense reduction in ROS levels as compared to LPS treated cell in a dose-dependent manner (5 and 10 µM) (Fig 4.12).

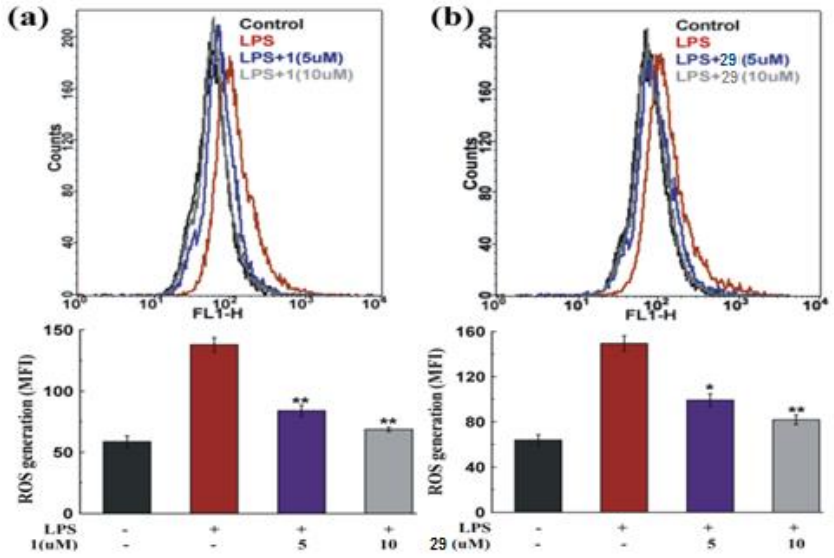


Fig 4.12: Compound effects on ROS level in LPS stimulated N9 cells. The graph and subsequent plot at below represent the MFI of ROS generation in the presence of compound **1** (a) and **29** (b). Data represent mean ± SD of three separate experiments. *p<0.05 and **p<0.01 as compared to LPS-treated values**.**

LPS treatment in N9 cells can induce neuroinflammation and result in the extreme production of numerous pro-inflammatory mediators such as TNF-α, IFN-γ, MCP-1 and IL-6.[35] Hence, these markers were measured using compounds **1** and **29** in LPS treated N9 cells by cytokine bead array.[36] Results showed that both compounds **1** and **29** significantly suppressed the inflammatory mediators dose-dependently with respect to LPS-treated

conditions. Dose-dependent addition of compounds **1** and **29** revealed substantial decrease in the levels of different inflammatory markers TNF-α (a), IL-6 (b), IFN-γ(c) and MCP-1 (d) compared to LPS treated cells as shown in Fig 4.13.

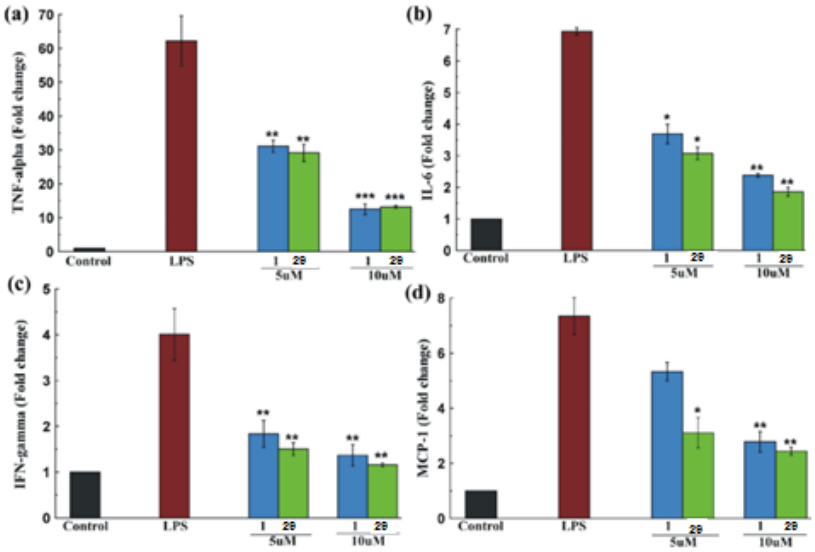


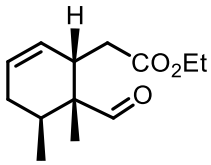
Fig 4.13: CBA (Cytometric Bead Array) analysis of protein extract isolated from N9 cells treated with LPS along with compounds. Data represent mean ± SD of three separate experiments. *p<0.05, **p<0.01 and ***p<0.001 in comparison to LPS treated values.

4.4 Conclusions

First syntheses of botryosphaeridione, pleodendione, 4-*epi*-periconianone B and hoaensieremodione was achieved. The present synthesis highlights the Diels-Alder, base mediated epoxide opening, stereoselective alkylation, and allylic oxidation reactions. Synthesized compounds were screened for their anti-neuroinflammatory potential in the presence of LPS-induced inflammation using mouse microglia cells. Most of the screened compounds decreased NO level in LPS treated cells. On the basis of their potency, selectivity index and modulation of different inflammatory markers, two compounds (**1** and **29**) were identified as potential molecules for further profiling.

4.5 Experimental Procedures

Ethyl 2-((1*R*,5*R*,6*S*)-6-formyl-5,6-dimethylcyclohex-2-en-1-yl) acetate (8):



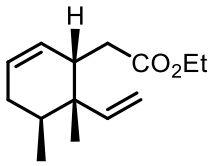
To a solution of diene **7** (6.0 g, 0.042 mol) and (*E*)-2-methylbut-2-enal (10.3 mL, 0.107 mol) in dry CH_2Cl_2 (200 mL) was added $BF_3{\cdot}OEt_2$ (10.6 mL, 0.085 mol) dropwise at –78 °C. The mixture was allowed to warm to room temperature and was stirred for 12 h at same temperature. The CH_2Cl_2 layer was washed with saturated $NaHCO_3$ (3 x 50 mL) followed by H_2O (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 1.0:9.0 ethyl acetate: petroleum ether) to afford **8** (7.3 g, 76%) as light yellow oil.

IRυ_{max} (film): 2978, 1732, 1174 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 5.71–5.66 (m, 1H), 5.64–5.57 (m, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.65–2.59 (m, 1H), 2.45 (dd, *J* = 15.8, 5.4 Hz, 1H), 2.37–2.30 (m, 1H), 2.25–2.18 (m, 1H), 2.17–2.05 (m, 1H), 1.79–1.72 (m, 1H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.08 (s, 3H), 0.93 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 206.6, 172.6, 127.4, 126.1, 60.4, 49.6, 37.6, 35.6, 30.6, 29.5, 15.7, 15.6, 13.9.

Ethyl 2-((1*R*,5*R*,6*S*)-5,6-dimethyl-6-vinylcyclohex-2-en-1-yl) acetate (9):



To a suspension of methyl triphenylphosphonium bromide (26.8 g, 0.075 mol) in dry THF (100 mL) was added potassium *tert*-butoxide (7.6 g, 0.068 mol) at 0 °C. After 30 minutes, the solution became canary yellow color, to that aldehyde **8** (5.1 g, 0.022 mol) in THF (50 mL) was added and allowed to stir at 0 °C for 1 h. The reaction was quenched with brine (30 mL) and extracted with ethyl acetate (3 x 100 mL). Combined organic layer was

washed with water (40 mL), brine (40 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether) afforded **9** (4.2 g, 84%) as light brown oil.

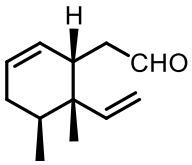
IRυ_{max} (film): 1725, 1521, 1417, 1215 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (dd, *J* = 18.3, 11.0 Hz, 1H), 5.65–5.53 (m, 2H), 5.06–5.05 (m, 1H), 5.03–5.02 (m, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.48–2.39 (m, 2H), 2.23–2.18 (m, 1H), 2.12–2.07 (m, 1H), 1.73–1.62 (m, 2H), 1.25 (t, *J* = 7.3 Hz, 3H), 1.02 (s, 3H), 0.87 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 173.7, 145.2, 128.6, 126.2, 113.3, 60.4, 41.2, 40.5, 36.7, 34.4, 31.5, 18.8, 16.4, 14.4.

HRMS (ESI): *m/z* calcd for $C_{14}H_{22}O_2Na$ $[M+Na]^+$ 245.1512, found 245.1508.

2-((1*S*,5*S*,6*R*)-5,6-Dimethyl-6-vinylcyclohex-2-en-1-yl) acetaldehyde (10):



To a solution of ester **9** (0.5 g, 2.25 mmol) in dry distilled toluene (15 mL) was added DIBAL-H (1.0 M in toluene, 1.36 mL, 1.36 mmol) at –78 ^oC dropwise. Stirred at this temperature for 10 min and then added DIBAL-H (1.0 M in toluene, 1.13 mL, 1.13 mmol) at –78 ^oC dropwise. After stirring at the same temperature for 0.5 h, the reaction was quenched with methanol (3 mL), diluted with Et_2O (20 mL) and saturated Na/K tartrate (15 mL). The solution was stirred at room temperature for 2 h. Extracted the solution with ethyl acetate (3 x 20 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The crude mixture was passed through small bed of silica gel and eluted with 20% ethyl acetate: petroleum ether. The eluent was concentrated *in vacuo* to give the aldehyde **10** (0.33 g, 81%) as a colorless oil which was immediately used for next step.

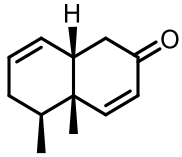
IRυ_{max} (film): 2978, 1734, 1710 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1H), 5.86–5.79 (m, 1H), 5.67–5.63 (m, 1H), 5.54–5.50 (m, 1H), 5.08–5.04 (m, 2H), 2.59–2.47 (m, 2H), 2.31–2.18 (m, 2H), 1.75–1.63 (m, 2H), 1.01 (s, 3H), 0.85 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 202.8, 145.4, 128.6, 126.4, 113.8, 46.2, 40.4, 39.4, 34.1, 31.3, 18.7, 16.3.

HRMS (ESI): *m/z* calcd for $C_{12}H_{19}O$ $[M+H]^+$ 179.1430, found 179.1431.

(4a*S*,5*S*,8a*S*)-4a,5-Dimethyl-4a,5,6,8a-tetrahydronaphthalen-2(1H)-one (11):



To a stirred solution of **10** (0.80 g, 4.49 mmol) in dry THF (20 mL) was added a solution of vinylmagnesium bromide (1M in THF, 6.7 mL, 6.74 mmol) at –78 °C, and the mixture was stirred at 0 °C for 1 h. The reaction was quenched with saturated aqueous ammonium chloride solution, and the mixture was extracted with ethyl acetate (3 x 20 mL). Combined organic layer was washed with water (15 mL), brine (15 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.5:8.5 ethyl acetate: petroleum ether as eluent) afforded alcohol as a mixture of diastereomers (0.70 g, 76%). This colorless oil was dissolved in dry CH_2Cl_2 (40 mL) and treated with Grubbs' second-generation catalyst (144 mg, 5 mol %) at room temperature. After stirring for 24 h, reaction mixture was filtered through celite pad and filtrate was concentrated *in vacuo*. The crude material obtained after the removal of solvent was purified by column chromatography (silica gel 100–200, 1.5:8.5 ethyl acetate: petroleum ether as eluent) to afford allylic alcohol as a mixture of diastereomers (435 mg, 72%).

To a solution of above allylic alcohols (435 mg, 2.44 mmol) in CH_2Cl_2 (20 mL) at 0 ^oC was added PCC (788 mg, 3.66 mmol) portion wise. The resulting mixture was stirred for 3 h at room temperature. The reaction mixture was diluted with diethyl ether (30 mL), filtered through a celite bed, and washed with diethyl ether (2 x 10 mL). The filtrate was concentrated in *vacuo*. Purification by column chromatography (silica gel 100–200, 1.0:9.0 ethyl acetate: petroleum ether as eluent) afforded enone **11** (344 mg, 80%) as colorless oil.

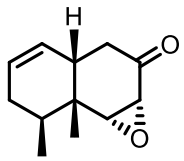
IRυ_{max} (film): 2964, 1683 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.71 (d, *J* = 10.3 Hz, 1H), 5.86 (d, *J* = 10.3 Hz, 1H), 5.64–5.47 (m, 2H), 2.54–2.49 (m, 1H), 2.38–2.37 (m, 1H), 2.31–2.25 (m, 1H), 2.11–2.06 (m, 1H), 1.90–1.83 (m, 2H), 1.05 (s, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4, 159.1, 128.8, 127.1, 126.3, 40.8, 40.7, 37.6, 33.0, 31.7, 20.4, 14.9.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1274.

(1a*R*,3a*S*,7*S*,7a*R*,7b*R*)-7,7a-Dimethyl-3,3a,6,7,7a,7b-hexahydronaphtho[1,2-b] oxiren-2(1aH)-one (12):



To a solution of the *α,β*–unsaturated ketone **11** (3.0 g, 0.017 mol) in 30 mL of methanol was added 30% aqueous H_2O_2 (5.8 mL, 0.051 mol) and 6N NaOH (1.7 mL, 0.010 mol) at 0 °C. The reaction was stirred at room temperature for 5 h. The mixture was diluted with diethyl ether (80 mL), washed successively with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.0:9.0 ethyl acetate: petroleum ether as eluent) afforded epoxide **12** (2.3 g, 71%) as colorless oil.

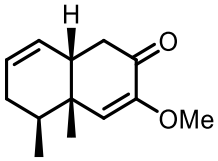
IRυ_{max} (film): 2968, 1714, 1030 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.55–5.38 (m, 2H), 3.28 (d, *J* = 3.9 Hz, 1H), 3.16 (d, *J* = 3.9 Hz, 1H), 2.63–2.57 (m, 1H), 2.29–2.24 (m, 1H), 2.08–2.03 (m, 1H), 1.92–1.86 (m, 1H), 1.75–1.63 (m, 2H), 1.10 (s, 3H), 0.91 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 205.8, 128.6, 125.7, 64.0, 56.1, 39.7, 36.8, 34.9, 31.1, 30.9, 18.1, 15.2.

HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O_2$ $[M+H]^+$ 193.1223, found 193.1227.

(4a*R*,5*S*,8a*S*)-3-Methoxy-4a,5-dimethyl-4a,5,6,8a-tetrahydronaphthalen-2(1H)-one (13):



To a solution of **12** (2.1 g, 0.010 mol) in MeOH (25 mL) was added NaOH (1.32 g, 0.033 mol) at room temperature. The resulting reaction mixture was heated to reflux for 30 min. The mixture was diluted with diethyl ether (60 mL), washed successively with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 2.0:8.0 ethyl acetate: petroleum ether as eluent) afforded **13** (1.9 g, 84%) as colorless oil.

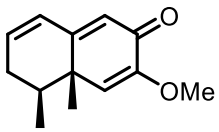
IRυ_{max} (film): 3018, 1687, 1215, 764 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.66–5.61 (m, 1H), 5.60 (s, 1H), 5.51–5.49 (m, 1H), 3.58 (s, 3H), 2.64–2.58 (m, 1H), 2.45–2.40 (m, 2H), 2.15–2.10 (m, 1H), 1.93–1.88 (m, 1H), 1.83–1.77 (m, 1H), 1.08 (s, 3H), 0.97 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 193.9, 149.3, 128.5, 126.6, 125.4, 54.9, 41.3, 40.7, 37.4, 33.4, 31.8, 21.8, 15.1.

HRMS (ESI): *m/z* calcd for $C_{13}H_{19}O_2$ $[M+H]^+$ 207.1380, found 207.1379.

(4a*S*, 5*S*)-3-Methoxy-4a, 5-dimethyl-5, -dihydronaphthalen-2(4aH)-one (14):



To a stirred solution of **13** (1.7 g, 8.25 mmol) in 1, 4 dioxane (25 mL) at room temperature was added SeO_2 (2.7 g, 24.7 mmol) and the resulting mixture was refluxed for 4 h. After completion (by TLC), reaction mixture was allowed to cool at room temperature and diluted with EtOAc (60 mL) and washed with saturated aqueous $NaHCO_3$ (15 mL), water (15 mL), brine (15 mL). The resulting organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* and the crude obtained was purified on column chromatography (silica gel 100–200, 4.0:6.0 ethyl acetate: petroleum ether) to afford **14** (1.1 g, 67%) as reddish oil.

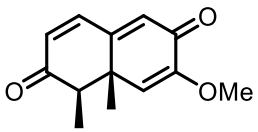
IRυ_{max} (film): 2927, 1665, 1210 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25–6.23 (m, 1H), 6.14–6.10 (m, 1H), 6.03 (s, 1H), 5.90 (s, 1H), 3.66 (s, 3H), 2.29–2.22 (m, 1H), 2.08–2.01 (m, 1H), 1.89–1.81 (m, 1H), 1.11 (d, *J* = 7.0 Hz, 3H), 1.10 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 181.5, 162.6, 151.1, 136.1, 127.4, 123.4, 119.9, 54.9, 41.2, 36.4, 32.7, 20.6, 15.9.

HRMS (ESI): *m/z* calcd for $C_{13}H_{17}O_2$ $[M+H]^+$ 205.1223, found 205.1222.

(1*R*,8a*R*)-7-Methoxy-1,8a-dimethyl-1,8a-dihydronaphthalene-2,6-dione (15):



To a stirred solution of **14** (1.1 g, 5.39 mmol) in benzene (60 mL) were added *t*-BuOOH (5M in decane), (5.4 mL, 26.9 mmol) and 4 Å MS (1 g) at room temperature. After 5 min, PDC (10.1 g, 26.9 mmol) was added and reaction mixture was stirred for 16 h. The reaction mixture was diluted with EtOAc (60 mL), filtered through a celite bed, and washed with EtOAc (2 x 20 mL). The filtrate was concentrated in *vacuo*. Purification by column chromatography (silica gel 100–200, 4.5:5.5 ethyl acetate: petroleum ether as eluent) afforded **15** (332 mg, 52% brsm, 30%) as light yellow solid.

M.p.: 110–112 °C.

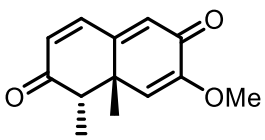
IRυ_{max} (film): 2967, 1654, 1619, 1207 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 9.8 Hz, 1H), 6.45 (s, 1H), 6.28 (d, *J* = 9.8 Hz, 1H), 5.99 (s, 1H), 3.72 (s, 3H), 2.56 (q, *J* = 6.6 Hz, 1H), 1.34 (d, *J* = 6.8 Hz, 3H), 1.25 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.6, 180.4, 158.0, 151.5, 142.0, 131.5, 128.4, 121.0, 55.2, 51.0, 44.9, 23.8, 8.3.

HRMS (ESI): *m/z* calcd for $C_{13}H_{15}O_3$ $[M+H]^+$ 219.1016, found 219.1014.

(1*S*,8a*R*)-7-Methoxy-1,8a-dimethyl-1,8a-dihydronaphthalene-2,6-dione (16):



Compound **15** (60 mg, 0.275 mmol) was treated with K_2CO_3 (190 mg, 1.376 mmol) in MeOH (10 mL) at room temperature and stirred for 24 h. Solvent was removed under *vacuo* and crude residue was diluted with water (5 mL), and extracted with EtOAc (3 x 15 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 4.5:5.5 ethyl acetate: petroleum ether as eluent) afforded **16** (49 mg, 81%) as light yellow solid.

M.p.: 108–110 °C.

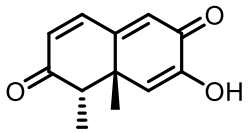
IR υ_{max} (film): 2967, 1654, 1619, 1207 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, *J* = 9.8 Hz, 1H), 6.55 (s, 1H), 6.17 (d, *J* = 9.8 Hz, 1H), 5.68 (s, 1H), 3.74 (s, 3H), 2.62 (q, *J* = 6.6 Hz, 1H), 1.39 (s, 3H), 0.92 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 201.5, 180.9, 155.2, 152.5, 141.9, 130.5, 129.3, 121.9, 55.2, 53.4, 45.4, 29.7, 15.6.

HRMS (ESI): *m/z* calcd for $C_{13}H_{15}O_3$ $[M+H]^+$ 219.1016, found 219.1015.

(1*S*,8a*R*)-7-Hydroxy-1,8a-dimethyl-1,8a-dihydronaphthalene-2,6-dione (1):



(±)-botryosphaeridione **1**

To a solution of **16** (20 mg, 0.092 mmol) in dry CH_2Cl_2 (5 mL) was added a solution of BBr_3 (1M in CH_2Cl_2, 0.18 mL, 0.183 mmol) dropwise at −78 °C. The reddish reaction mixture was stirred for 2 h at same temperature and was then quenched by adding saturated aqueous $NaHCO_3$ solution (3 mL). The cooling bath was removed and the mixture was stirred at room temperature for another 30 min. The mixture was diluted with CH_2Cl_2 (10 mL) and the organic phase was washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 3.0:7.0 ethyl acetate: petroleum ether as eluent) afforded (±)-botryosphaeridione **1** (16

mg, 85%) as pale yellow solid.

M.p.: 118–120 °C.

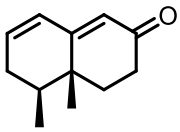
IRυ_{max} (film): 2973, 1665, 1644 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 10.2 Hz, 1H), 6.63 (s, 1H), 6.42 (s, 1H), 6.20 (d, *J* = 10.2 Hz, 1H), 6.05 (s, 1H), 2.64 (q, *J* = 7.2 Hz, 1H), 1.38 (d, *J* = 7.2 Hz, 3H), 0.91 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 201.5, 181.4, 158.2, 148.2, 141.5, 129.7, 128.1, 123.8, 53.4, 45.7, 29.4, 15.6.

HRMS (ESI): *m/z* calcd for $C_{12}H_{13}O_3$ $[M+H]^+$ 205.0859, found 205.0858.

(4a*R*, 5*S*)-4a,5-Dimethyl-4,4a,5,6-tetrahydronaphthalen-2(3H)-one (18):

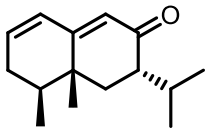


A solution of *α, β*–unsaturated ketone **11** (4.4 g, 0.025 mol) in THF (60 mL) was added to liquid ammonia (120 mL) at −78 °C. Lithium (2.1 g, 0.300 mol) was added in small pieces and reaction mixture was stirred at −78 °C for 1 h. After consumption of starting material (by TLC), solid NH_4Cl (3.0 g) was added and ammonia was allowed to evaporate at room temperature. Water (30 mL) was added and reaction mixture was extracted with EtOAc (2 x 60 mL). Combined organic layer was washed with water (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to afford ketone **17** (3.6 g, 81%) which was treated with IBX (17 g, 0.060 mmol) in DMSO (80 mL) at room temperature and stirred for 24 h. After the completion (by TLC), the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (25 mL). The aqueous layer was extracted with EtOAc (3 x 60 mL) and combined organic layer was washed with water (30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 1.0:9.0 ethyl acetate: petroleum ether as eluent) afforded **18** (2.3 g, 65%) as colorless oil.

IRυ_{max} (film): 2978, 1671 cm^{-1}.

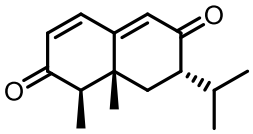
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23–6.11 (m, 2H), 5.68 (s, 1H), 2.61–2.40 (m, 2H), 2.25–2.18 (m, 1H), 2.12–2.01 (m, 2H), 1.76–1.68 (m, 2H), 1.03 (s, 3H), 0.95 (d, *J* = 6.8 Hz, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 199.8, 163.7, 138.3, 128.3, 123.8, 38.2, 36.2, 34.2, 34.1, 32.7, 15.1, 14.5.
HRMS (ESI): *m/z* calcd for $C_{12}H_{17}O$ $[M+H]^+$ 177.1274, found 177.1274.

(3*S*,4a*R*,5*S*)-3-Isopropyl-4a,5-dimethyl-4,4a,5,6-tetrahydronaphthalen-2(3H)-one (19):



To a solution of diisopropylamine (0.72 mL, 5.11 mmol) in dry THF (10 mL) was added a solution of *n*-butyllithium in hexane (1.6 M, 3.2 mL, 5.11 mmol) at –78 °C. The reaction was stirred for 30 min at –78 °C and **18** (300 mg, 1.70 mmol) in THF (5 mL) was added dropwise via syringe. After stirring for 30 min, HMPA (0.9 mL, 5.11 mmol) was added. After stirring for a further 20 min, isopropyl iodide (1.7 mL, 17.0 mmol) was added slowly. After stirring for 2 h at –78 ºC the mixture was warmed to room temperature and then stirred for a further 16 h. The reaction was quenched with 1N aqueous HCl (5 mL) extracted with EtOAc (3 x 10 mL) and combined organic layer was washed with water (5 mL), brine (5 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether as eluent) afforded **19** (115 mg, 40% brsm, 31%) as colorless oil.
IRυ_{max} (film): 2973, 1671, 1216 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.21–6.17 (m, 1H), 6.12–6.09 (m, 1H), 5.66 (s, 1H), 2.65–2.57 (m, 1H), 2.44–2.38 (m, 1H), 2.25–2.17 (m, 1H), 2.11–2.04 (m, 1H), 1.94–1.89 (m, 1H), 1.74–1.68 (m, 1H), 1.52–1.45 (m, 1H), 1.02 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H).
^{13}C NMR (100 MHz, $CDCl_3$): δ 201.2, 162.4, 137.9, 128.0, 124.4, 47.8, 38.4, 36.4, 33.6, 32.6, 26.2, 20.4, 17.7, 15.6, 14.6.
HRMS (ESI): *m/z* calcd for $C_{15}H_{23}O$ $[M+H]^+$ 219.1743, found 219.1743.

(1*R*,7*S*,8a*R*)-7-Isopropyl-1,8a-dimethyl-1,7,8,8a-tetrahydronaphthalene-2,6-dione (5):



(±)-pleodendione **5**

To a stirred solution of **19** (20 mg, 0.092 mmol) in benzene (6 mL) were added *t*-BuOOH (5 M in decane), (0.09 mL, 0.458 mmol), 4 Å MS (20 mg) at room temperature. After 5 min, PDC (172 mg, 0.458 mmol) and reaction mixture was stirred for 16 h. The reaction mixture was diluted ethyl acetate (5 mL), filtered through a celite bed, and washed with ethyl acetate (2 x 5 mL). The filtrate was concentrated in *vacuo*. Purification by column chromatography (silica gel 100–200, 2.0:8.0 ethyl acetate: petroleum ether as eluent) afforded (±)-pleodendione **5** (6 mg, 46% brsm, 28%) as yellow solid.

M.p.: 103–105 °C.

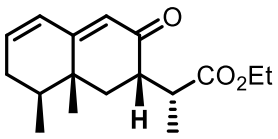
IRυ_{max} (film): 2963, 1671, 1595, 772 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.96 (d, *J* = 9.8 Hz, 1H), 6.19 (d, *J* = 9.8 Hz, 1H), 6.00 (s, 1H), 2.63–2.54 (m, 1H), 2.56 (q, *J* = 6.8 Hz, 1H), 2.36–2.31 (m, 1H), 1.97 (dd, *J* = 12.9, 4.7 Hz, 1H), 1.74 (t, *J* = 13.8 Hz, 1H), 1.14 (d, *J* = 6.8 Hz, 3H), 1.12 (s, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.81 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.2, 200.1, 158.3, 142.3, 132.1, 129.7, 52.5, 47.4, 40.3, 34.4, 26.0, 20.3, 18.6, 17.7, 7.2.

HRMS (ESI): *m/z* calcd for $C_{15}H_{21}O_2$ $[M+H]^+$ 233.1536, found 233.1534.

Ethyl(S)-2-((2*S*,8*S*,8a*R*)-8,8a-dimethyl-3-oxo-1,2,3,7,8,8a-hexahydronaphthalen-2-yl) propanoate (20):



To a solution of diisopropylamine (2.0 mL, 14.2 mmol) in dry THF (15 mL) was added a solution of *n*-butyllithium in hexane (1.6 M, 9.0 mL, 14.2 mmol) at –78 °C. The reaction was stirred for 30 min at –78 °C and **18** (500 mg, 2.84 mmol) in THF (10 mL) was added

dropwise via syringe. After stirring for 30 min, HMPA (2.5 mL, 14.2 mmol) was added. After stirring for a further 20 min, ethyl-2-iodopropanoate (3.2 g, 14.2 mmol) was added slowly. The resulting mixture was maintained at –20 °C for 2 h. After the completion (by TLC), reaction was quenched with saturated NH_4Cl (5 mL) and extracted with EtOAc (3 x 15 mL) and combined organic layer was washed with water (10 mL), brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by column chromatography (silica gel 100–200, 0.5:9.5 ethyl acetate: petroleum ether as eluent) afforded **20** (533 mg, 68%) as colorless oil.

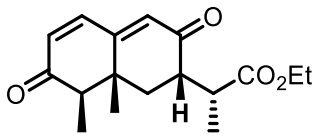
IRυ_{max} (film): 1725, 1661 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.22–6.17 (m, 1H), 6.12–6.09 (m, 1H), 5.67 (s, 1H), 4.22–4.11 (m, 2H), 3.19–3.12 (m, 1H), 3.08 (ddd, *J* = 14.1, 4.7, 4.7 Hz, 1H), 2.24–2.17 (m, 1H), 2.11–2.03 (m, 1H), 1.94–1.90 (m, 1H), 1.73–1.67 (m, 1H), 1.58–1.52 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.08 (d, *J* = 8.0 Hz, 3H), 1.07 (s, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.1, 176.2, 162.9, 138.3, 127.9, 123.5, 60.5, 44.9, 38.5, 38.4, 36.8, 36.0, 32.6, 15.4, 14.5, 14.4, 12.7.

HRMS (ESI): *m/z* calcd for $C_{17}H_{25}O_3$ $[M+H]^+$ 277.1798, found 277.1796.

Ethyl (S)-2-((2*S*,8*R*,8a*R*)-8,8a-dimethyl-3,7-dioxo-1,2,3,7,8,8a-hexahydronaphthalen-2-yl)propanoate (21):



To a solution of **20** (300 mg, 1.08 mmol) in benzene (30 mL) were added *t*-BuOOH (5M in decane), (1.1 mL, 5.43 mmol), 4 Å MS (200 mg) at room temperature. After 5 min, PDC (2.0 g, 5.43 mmol) was added and reaction mixture was stirred for 16 h. The reaction mixture was diluted with EtOAc (20 mL), filtered through a celite bed, and washed with ethyl acetate (2 x 10 mL). The filtrate was concentrated in *vacuo*. Purification by column chromatography (silica gel 100–200, 2.0:8.0 ethyl acetate: petroleum ether as eluent) afforded **21** (87 mg, 43% brsm, 28%) as yellow solid.

M.p.: 121–123 °C.

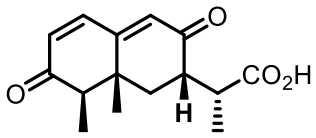
IRυ_{max} (film): 1730, 1668 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (d, *J* = 10.0 Hz, 1H), 6.22 (d, *J* = 10.0 Hz, 1H), 6.03 (s, 1H), 4.23–4.13 (m, 2H), 3.15–3.11 (m, 1H), 3.03 (ddd, *J* = 14.1, 4.7, 4.7 Hz, 1H), 2.57 (q, *J* = 6.8 Hz, 1H), 2.04–2.00 (m, 1H), 1.86–1.79 (m, 1H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.19 (s, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.11 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.7, 198.5, 175.6, 158.8, 142.1, 132.3, 128.7, 60.8, 52.4, 44.4, 40.5, 38.1, 36.6, 18.5, 14.4, 13.0, 7.12.

HRMS (ESI): *m/z* calcd for $C_{17}H_{23}O_4$ $[M+H]^+$ 291.1591, found 291.1588.

(S)-2-((2*S*,8*R*,8a*R*)-8,8a-Dimethyl-3,7-dioxo-1,2,3,7,8,8a-hexahydronaphthalen-2-yl) propanoic acid (22):



To a solution of **21** (50 mg, 0.172 mmol) in THF (3 mL): MeOH (3 mL) was added $LiOH.H_2O$ (36 mg, 0.862 mmol) in H_2O (3 mL) at 0 °C. The mixture was warmed up to room temperature and stirred for 12 h. The solvent was evaporated *in vacuo* and mixture was acidified to pH 2 with 1N HCl and extracted with EtOAc (3 x 10 mL). The combined organic layer was washed by brine (5 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*. Purification by flash chromatography over silica gel (0.5:9.5; MeOH–DCM) afforded **22** (32 mg, 72%) as white solid.

M.p.: 135–138 °C.

IRυ_{max} (film): 3023, 1717, 1655, 1620 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (d, *J* = 9.8 Hz, 1H), 6.24 (d, *J* = 9.8 Hz, 1H), 6.06 (s, 1H), 3.23–3.19 (m, 1H), 3.04 (ddd, *J* = 14.4, 4.8, 4.8 Hz, 1H), 2.58 (q, *J* = 6.8 Hz, 1H), 2.08–2.05 (m, 1H), 1.89–1.84 (m, 1H), 1.22 (s, 3H), 1.18 (d, *J* = 7.2 Hz, 3H), 1.15 (d, *J* = 6.8 Hz, 3H).

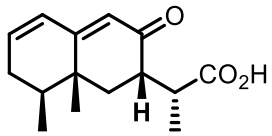
^{13}C NMR (100 MHz, $CDCl_3$): δ 199.7, 198.3, 181.2, 159.1, 142.1, 132.4, 128.6, 52.4, 44.3, 40.6, 38.1, 36.7, 18.6, 13.0, 7.2.

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.2 (s, 1H), 7.27 (d, *J* = 9.8 Hz, 1H), 6.23 (d, *J* = 9.8 Hz, 1H), 6.16 (s, 1H), 2.97–2.91 (m, 2H), 2.73 (q, *J* = 6.7 Hz, 1H), 1.91-1.89 (m, 2H), 1.10 (s, 3H), 1.02 (d, *J* = 7.0 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6): δ 199.7, 198.5, 176.4, 158.8, 142.3, 131.7, 128.3, 51.3, 43.8, 37.6, 35.7, 18.0, 13.0, 6.9.

HRMS (ESI): *m/z* calcd for $C_{15}H_{18}O_4$ $[M+Na]^+$ 285.1097, found 285.1096.

(S)-2-((2*S*,8*S*,8a*R*)-8,8a-Dimethyl-3-oxo-1,2,3,7,8,8a-hexahydronaphthalen-2-yl) propan- oic acid (23):



Compound **23** was prepared from **20** using the similar experimental procedure as described above for preparation of **22**.

Yield: 82%.

M.p.: 122–124 °C.

IRυ_{max} (film): 1717, 1664 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.24–6.11 (m, 2H), 5.70 (s, 1H), 3.26–3.20 (m, 1H), 3.10 (ddd, *J* = 14.4, 4.8, 4.8 Hz, 1H), 2.26–2.19 (m, 1H), 2.13–2.05 (m, 1H), 2.00–1.96 (m, 1H), 1.76–1.69 (m, 1H), 1.62–1.55 (m, 1H), 1.13 (d, *J* = 7.1 Hz, 3H), 1.09 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.1, 181.6, 163.4, 138.6, 127.9, 123.3, 44.7, 38.4, 36.8 (2C), 36.0, 32.6, 15.4, 14.5, 12.7.

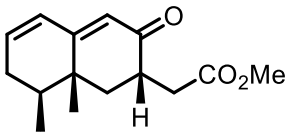
HRMS (ESI): *m/z* calcd for $C_{15}H_{21}O_3$ $[M+H]^+$ 249.1485, found 249.1483.

Single X-ray Crystal Structure of 23: X-ray intensity data measurements of compound **23** were carried out on a Bruker SMART APEX II CCD diffractometer with graphite-monochromatized (MoK_α= 0.71073Å) radiation. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from three sets of 36 frames. Data were collected with ω scan width of 0.5° at different settings of φ and 2θ with a frame time of 15 secs keeping the sample-to-detector

distance fixed at 5.00 cm. The X-ray data collection was monitored by APEX2 program (Bruker, 2006). All the data were corrected for Lorentzian, polarization and absorption effects using SAINT and SADABS programs (Bruker, 2006). SHELX-97 was used for structure solution and full matrix least-squares refinement on *F*. All the hydrogen atoms were placed in geometrically idealized position and constrained to ride on their parent atoms. ORTEP views of both compounds were drawn with 30% probability displacement ellipsoids and H atoms are shown as small spheres of arbitrary radii.

Crystal data of **23:** $C_{15}H_{20}O_3$, M = 248.31, colorless plate, 0.36 x 0.26 x 0.20 mm^3, triclinic, space group *P*-1, *a* = 7.2066(3) Å, *b* = 9.6275(3)Å, *c* = 9.8499(3)Å, α=84.696(2)° β = 74.028(2)°, γ=81.042(2)°, *V* = 648.12(4) $Å^3$, Z = 2, *T* = 150(2)K, $2\theta_{max}$=50.0°, D_{calc} (g cm^{-3}) = 1.272, *F*(*000*) = 268, μ (mm^{-1}) = 0.087, 8037 reflections collected, 2238 unique reflections (R_{int}= 0.0316), 1859 observed ($I > 2\sigma(I)$) reflections, multi-scan absorption correction, T_{min} = 0.9693, T_{max} = 0.9828, 170 refined parameters, *S* = 1.071, *R*1 = 0.0513, *wR*2 = 0.1021 (all data *R* = 0.0659, *wR*2 = 0.1081), maximum and minimum residual electron densities; $\Delta\rho_{max}$ = 0.255, $\Delta\rho_{min}$= -0.176 ($eÅ^{-3}$).

Methyl2-((2S,8S,8aR)-8,8a-dimethyl-3-oxo-1,2,3,7,8,8a-hexahydronaphthalen-2-yl) acetate (24):



Compound **24** was prepared from **18** using the similar experimental procedure as described above for preparation of **20**.

Yield: 78%.

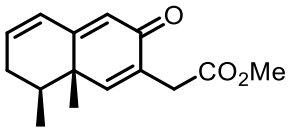
IRυ_{max} (film): 1737, 1661, 1168 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.22–6.10 (m, 2H), 5.68 (s, 1H), 3.70 (s, 3H), 3.09–3.01 (m, 1H), 2.96–2.91 (m, 1H), 2.34–2.28 (m, 1H), 2.24–2.17 (m, 1H), 2.11–2.03 (m, 2H), 1.74–1.68 (m, 1H), 1.59–1.53 (m, 1H), 1.10 (s, 3H), 0.93 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 199.3, 173.3, 163.2, 138.3, 128.0, 123.0, 51.8, 40.7, 39.8, 38.2, 37.0, 34.9, 32.5, 15.5, 14.5.

HRMS (ESI): *m/z* calcd for $C_{15}H_{21}O_3$ $[M+H]^+$ 249.1485, found 249.1485.

Methyl-2-((8*S*,8a*S*)-8,8a-dimethyl-3-oxo-3,7,8,8a-tetrahydronaphthalen-2-yl)-acetate (25):



To a solution of **24** (150 mg, 0.604 mmol) in dry 1, 4 dioxane (10 mL) was added DDQ (412 mg, 1.81 mmol) at room temperature. The resulting mixture was refluxed for 24 h. After completion (by TLC), reaction mixture was allowed to cool at room temperature and diluted with CH_2Cl_2. The resulting mixture was filtered and filtrate was concentrated in *vacuo*. Purification by column chromatography (silica gel 100–200, 2.0:8.0 ethyl acetate: petroleum ether as eluent) afforded **25** (126 mg, 85%) as yellow solid.

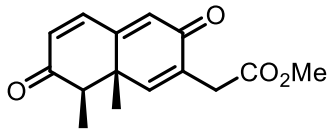
IRυ_{max} (film): 1739, 1619 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.95 (s, 1H), 6.26–6.23 (m, 1H), 6.13–6.08 (m, 1H), 6.01 (s, 1H), 3.67 (s, 3H), 3.35 (d, *J* = 16.5 Hz, 1H), 3.30 (d, *J* = 16.5 Hz, 1H), 2.30–2.23 (m, 1H), 2.12–2.04 (m, 1H), 1.96–1.89 (m, 1H), 1.11 (s, 3H), 1.10 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 185.3, 171.9, 162.1, 150.9, 135.7, 132.0, 127.7, 123.2, 52.1, 41.2, 35.2, 35.1, 32.7, 19.4, 15.6.

HRMS (ESI): *m/z* calcd for $C_{15}H_{19}O_3$ $[M+H]^+$ 247.1329, found 247.1327.

Methyl-2-((8*R*,8a*R*)-8,8a-dimethyl-3,7-dioxo-3,7,8,8a-tetrahydronaphthalen-2-yl) acetate (6):



(±)-Hoaensieremodione **6**

Compound **6** was prepared from **25** using the similar experimental procedure as described above for preparation of **21**.

Yield: 58% brsm (40%).

M.p.: 123–125 °C.

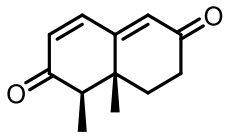
IRυ_{max} (film): 1739, 1661, 1619 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, *J* = 9.8 Hz, 1H), 7.02 (s, 1H), 6.44 (s, 1H), 6.27 (d, *J* = 9.8 Hz, 1H), 3.71 (s, 3H), 3.41 (d, *J* = 16.5 Hz, 1H), 3.35 (d, *J* = 16.5 Hz, 1H), 2.63 (q, *J* = 6.8 Hz, 1H), 1.33 (d, *J* = 6.8 Hz, 3H), 1.24 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.2, 184.1, 171.1, 157.4, 151.3, 142.1, 132.6, 131.2, 128.3, 52.0, 49.5, 44.3, 34.7, 22.3, 7.8.

HRMS (ESI): *m/z* calcd for $C_{15}H_{17}O_4$ $[M+H]^+$ 261.1121, found 261.1120.

(1*R*,8a*R*)-1,8a-Dimethyl-1,7,8,8a-tetrahydronaphthalene-2,6-dione (26):



Compound **26** was prepared from **18** using the similar experimental procedure as described above for preparation of **15**.

Yield: 44% brsm.

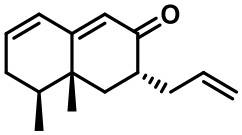
IRυ_{max} (film): 3045, 1664 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (d, *J* = 9.8 Hz, 1H), 6.23 (d, *J* = 9.8 Hz, 1H), 6.05 (s, 1H), 2.61–2.52 (m, 3H), 2.16–2.11 (m, 1H), 2.04–1.96 (m, 1H), 1.16 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.0, 198.8, 159.5, 142.4, 132.3, 129.2, 52.2, 40.0, 34.5, 33.5, 18.3, 7.1.

HRMS (ESI): *m/z* calcd for $C_{12}H_{15}O_2$ $[M+H]^+$ 191.1067, found 191.1066.

(3R,4aR,5S)-3-Allyl-4a,5-dimethyl-4,4a,5,6-tetrahydronaphthalen-2(3H)-one (27):



Compound **27** was prepared from **18** using the similar experimental procedure as described above for preparation of **19**.

Yield: 78%.

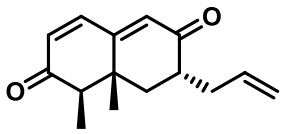
IRυ_{max} (film): 3019, 2864, 1673 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.21–6.10 (m, 2H), 5.83–5.73 (m, 1H), 5.67 (s, 1H), 5.08–5.02 (m, 2H), 2.77–2.71 (m, 1H), 2.57–2.49 (m, 1H), 2.23–2.03 (m, 4H), 1.70–1.65 (m, 1H), 1.45–1.38 (m, 1H), 1.04 (s, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.6, 163.0, 138.1, 136.6, 128.0, 123.6, 116.8, 42.0, 39.7, 38.3, 36.8, 34.3, 32.5, 15.6, 14.5.

HRMS (ESI): *m/z* calcd for $C_{15}H_{21}O$ $[M+H]^+$217.1587, found 217.1586.

(1*R*,7*R*,8a*R*)-7-Allyl-1,8a-dimethyl-1,7,8,8a-tetrahydronaphthalene-2,6-dione (28):



Compound **28** was prepared from **27** using the similar experimental procedure as described above for preparation of **5**.

Yield: 46% brsm.

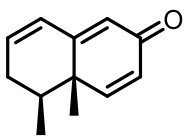
IRυ_{max} (film): 3020, 2929, 1673 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00 (d, *J* = 9.6 Hz, 1H), 6.22 (d, *J* = 9.6 Hz, 1H), 6.05 (s, 1H), 5.83–5.72 (m, 1H), 5.12–5.07 (m, 2H), 2.79–2.72 (m, 1H), 2.59–2.49 (m, 2H), 2.24–2.11 (m, 2H), 1.73–1.67 (m, 1H), 1.17 (s, 3H), 1.14 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.0, 199.8, 158.8, 142.2, 135.7, 132.2, 129.0, 117.5, 52.3, 41.5, 40.6, 40.0, 33.7, 18.7, 7.10.

HRMS (ESI): *m/z* calcd for $C_{15}H_{19}O_2$ $[M+H]^+$ 231.1380, found 231.1379.

(4a*R*,5*R*)-4a,5-Dimethyl-5,6-dihydronaphthalen-2(4aH)-one (Trienone-I):



Compound **Trienone-I** was prepared from **11** using the similar experimental procedure as described above for preparation of **14**.

Yield: 73%.

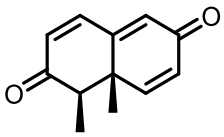
IRυ_{max} (film): 2975, 1660 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, *J* = 10.0 Hz, 1H), 6.26–6.23 (m, 2H), 6.13–6.10 (m, 1H), 6.00 (s, 1H), 2.31–2.23 (m, 1H), 2.13–2.04 (m, 1H), 1.92–1.86 (m, 1H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.10 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 186.7, 162.3, 153.2, 135.7, 128.3, 128.0, 123.8, 41.1, 35.2, 32.7, 19.3, 15.5.

HRMS (ESI): *m/z* calcd for $C_{12}H_{15}O$ $[M+H]^+$ 175.1117, found 175.1116.

(1*S*,8a*S*)-1,8a-Dimethyl-1,8a-dihydronaphthalene-2,6-dione (29):



Compound **29** was prepared from **Trienone-I** using the similar experimental procedure as described above for preparation of **15**.

Yield: 63% brsm.

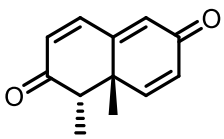
IRυ_{max} (film): 2975, 1661, 1626 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.19 (d, *J* = 10.0 Hz, 1H), 7.11 (d, *J* = 10.0 Hz, 1H), 6.41 (s, 1H), 6.37 (dd, *J* = 10.1, 1.8 Hz, 1H), 6.27 (d, *J* = 10.0 Hz, 1H), 2.60 (q, *J* = 6.8 Hz, 1H), 1.32 (d, *J* = 6.8 Hz, 3H), 1.23 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.5, 185.3, 157.7, 153.5, 142.6, 131.4, 129.1, 128.7, 49.7, 44.4, 22.4, 7.9.

HRMS (ESI): *m/z* calcd for $C_{12}H_{13}O_2$ $[M+H]^+$ 189.0910, found 189.0910.

(1*R*,8a*S*)-1,8a-Dimethyl-1,8a-dihydronaphthalene-2,6-dione (30):



Compound **30** was prepared from **29** using the similar experimental procedure as described above for preparation of **16**.

Yield: 86%.

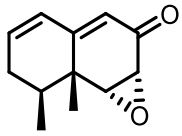
IR υ_{max} (film): 2975, 1661, 1626 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.21 (d, *J* = 9.8 Hz, 1H), 6.80 (d, *J* = 9.8 Hz, 1H), 6.52 (s, 1H), 6.42 (dd, *J* = 10.0, 1.7 Hz, 1H), 6.16 (d, *J* = 10.0 Hz, 1H), 2.63 (q, *J* = 7.2 Hz, 1H), 1.36 (s, 3H), 0.94 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 201.3, 186.0, 155.0, 154.6, 142.7, 131.2, 129.8, 129.3, 52.4, 45.2, 28.2, 15.7.

HRMS (ESI): *m/z* calcd for $C_{12}H_{13}O_2$ $[M+H]^+$ 189.0910, found 189.0910.

(1a*R*,7*R*,7a*S*,7b*R*)-7,7a-Dimethyl-6,7,7a,7b-tetrahydronaphtho[1,2-b]oxiren-2(1aH)-one (31):



Compound **31** was prepared from **12** using the similar experimental procedure as described above for preparation of **14**.

Yield: 62%.

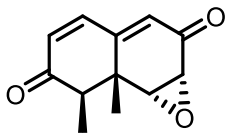
IR υ_{max} (film): 2968, 1665, 1210 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.16–6.02 (m, 2H), 5.66 (s, 1H), 3.62 (d, *J* = 3.9 Hz, 1H), 3.37 (d, *J* = 3.9 Hz, 1H), 2.32–2.31 (m, 1H), 2.11–2.07 (m, 2H), 1.24 (s, 3H), 1.11 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 192.3, 162.0, 136.0, 128.0, 120.3, 60.6, 54.5, 38.3, 34.4, 33.4, 16.1, 14.8.

HRMS (ESI): *m/z* calcd for $C_{12}H_{15}O_2$ $[M+H]^+$ 191.1067, found 191.1066.

(1a*R*,7*S*,7a*S*,7b*R*)-7,7a-Dimethyl-1a,7,7a,7b-tetrahydronaphtho[1,2-b]oxirene-2,6-dione (32):



Compound **32** was prepared from **31** using the similar experimental procedure as described above for preparation of **15**.

Yield: 50% brsm.

IR υ_{max} (film): 2975, 1660, 1622 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (d, *J* = 10.0 Hz, 1H), 6.20 (d, *J* = 10.0 Hz, 1H), 6.01 (s, 1H), 3.67 (d, *J* = 3.4 Hz, 1H), 3.48 (d, *J* = 3.4 Hz, 1H), 2.73 (q, *J* = 6.6 Hz, 1H), 1.35 (s, 3H), 1.34 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 198.0, 191.6, 157.3, 142.8, 131.9, 124.6, 60.9, 54.4, 49.5, 41.0, 19.2, 7.8.

HRMS (ESI): *m/z* calcd for $C_{12}H_{13}O_3$ $[M+H]^+$ 205.0859, found 205.0858.

Methodology

1) Cell culture

Mouse microglial cell line N9 was kindly gifted by Prof. Maria Pedroso de Lima, Center for Neuroscience and Cell Biology, University of Coimbra, Portugal and maintained in laboratory at 37 °C in RPMI-1640 media supplemented with 10% heat-in activated fetal bovine serum (FBS) and penicillin/streptomycin.

2) Cytotoxicity assay

Viability of cultured cells was determined by-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) as described earlier (1). N9 was seeded in triplicate at a density of 2×10^4 cells per well on a 96-well plate. After 12 h, cells were treated with varying concentrations (0-100 µM) of all the compounds in a serum free condition for another 24 h. The MTT solution (0.5 mg/mL) was then added to each well and incubates for 4 h at 37 °C. After incubation period, the medium was separate and the resulting purple formazan was solubilised using 0.1 N HCl in absolute isopropanol, and the

absorbance was read at 570 nm using Biorad Microplate reader (Biorad, USA).

3) Nitric oxide (NO) measurement

Nitrite, a stable oxidized product of NO, was measured in culture supernatant using Griess reagent (Sigma Aldrich) according to a previously reported method (2). After overnight seeding in 96-well plate (2× 10^4 cells/well), N9 cell was treated with lipopolysaccharide (LPS; Sigma) at a concentration of 1µg/ml along with different doses of compounds (as determined from cytotoxicity assay) and standard curcumin serum-free culture for 24 h. Following treatment, media was collected and to remove cellular debris, it was centrifuged at 2,000 rpm for 5 min. From this media, 50 µL was then reacted with equal volume of Griess reagent for 15 min at room temperature in dark and absorbance was taken at 540 nm using Microplate reader (Biorad, USA). The standard solutions of sodium nitrite prepared in cell culture medium were used for nitrite concentrations determination.

4) Assessment of reactive oxygen species (ROS)

Intracellular ROS generation is a significant marker of microglial inflammation. Here, the effects of compounds on LPS (1 µg/mL) induced ROS level was evaluated using the cell permeable, non-polar hydrogen peroxide-sensitive dye 5-(and-6)-chlromethyl-2', 7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA;Sigma Aldrich) as described previously (3). N9 cell was seeded in 6-well plate (2× 10^5 cells/well) for overnight and then replaced with serum free media containing LPS and varying doses of compounds. After 24 h of treatment, the cells were washed with PBS and incubated with 5 µM CM-H_2DCFDA for 30 min at 37 °C in the dark. Following washing with PBS, cells were harvested and the MFIs (mean fluorescent intensities) were recorded on the FL-1 channel on a FACS (fluorescence-activated cell sorting) Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

5) Cytokine bead array

The cytokine bead array (CBA) kit (BD Biosciences, NJ, USA) was selected to quantitatively determine cytokine levels in N9 cell lysates. After overnight seeding of cells in 6-well plate, they were treated with LPS along with compounds for 24 h and then cells were harvested for protein isolation. 30 µL of bead mix, containing a population of beads

that have been coated with capture antibodies for cytokines, along with equal volume of PE-conjugated detection antibodies were incubated with 30 µg protein samples for 2 h at room temperature in dark and then the beads were collected using Cell Quest Pro Software in FACS Calibur and analysis done using BD CBA software (Becton Dickinson, San Diego, CA) as indicated earlier (4).

4.6 References

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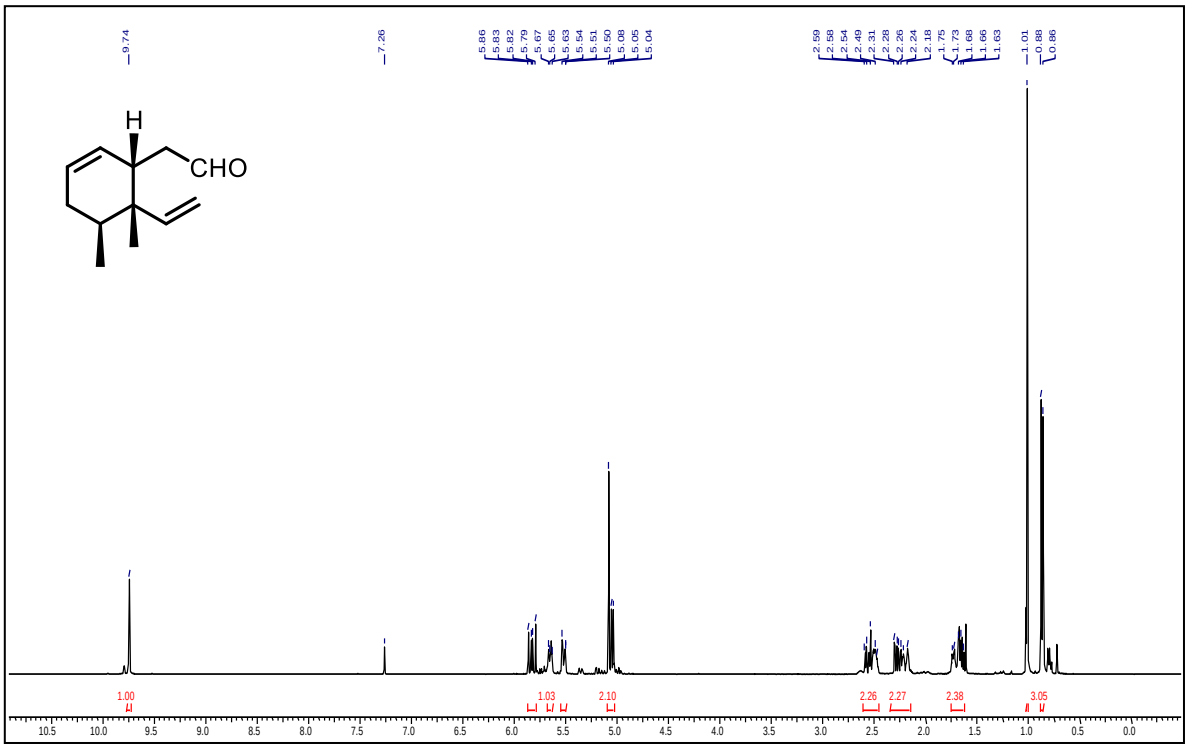
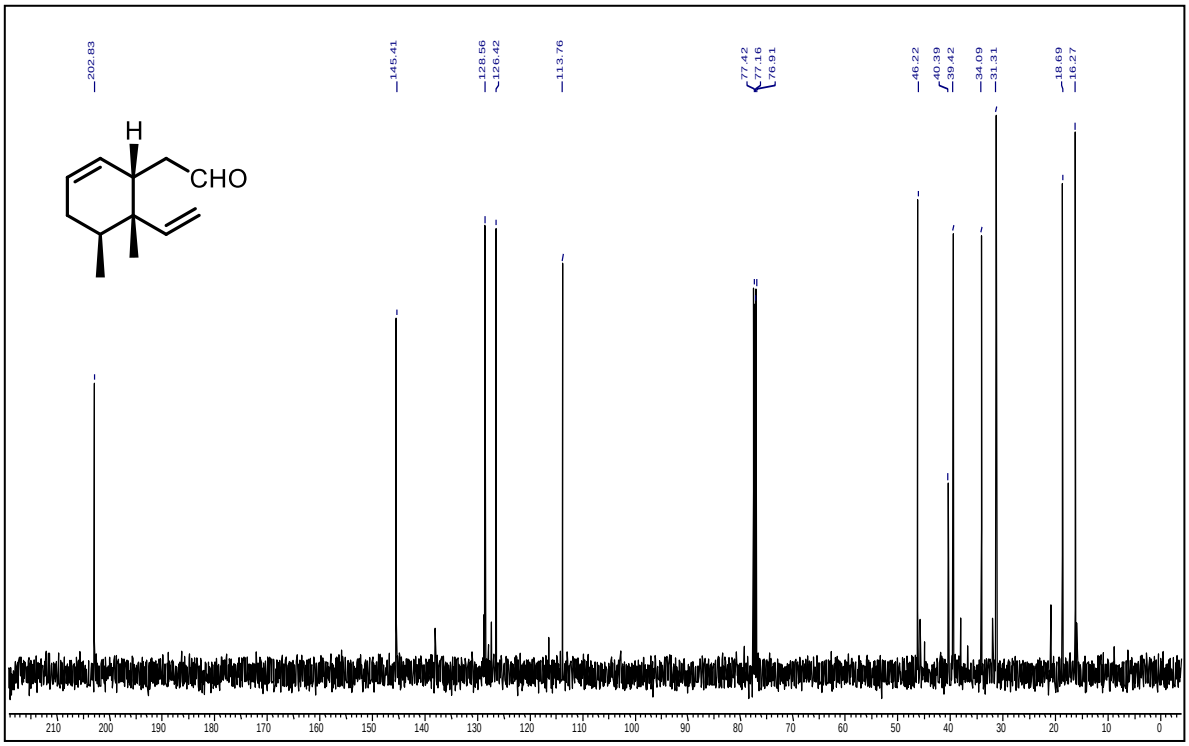
4.7 Selected Copies NMR spectra

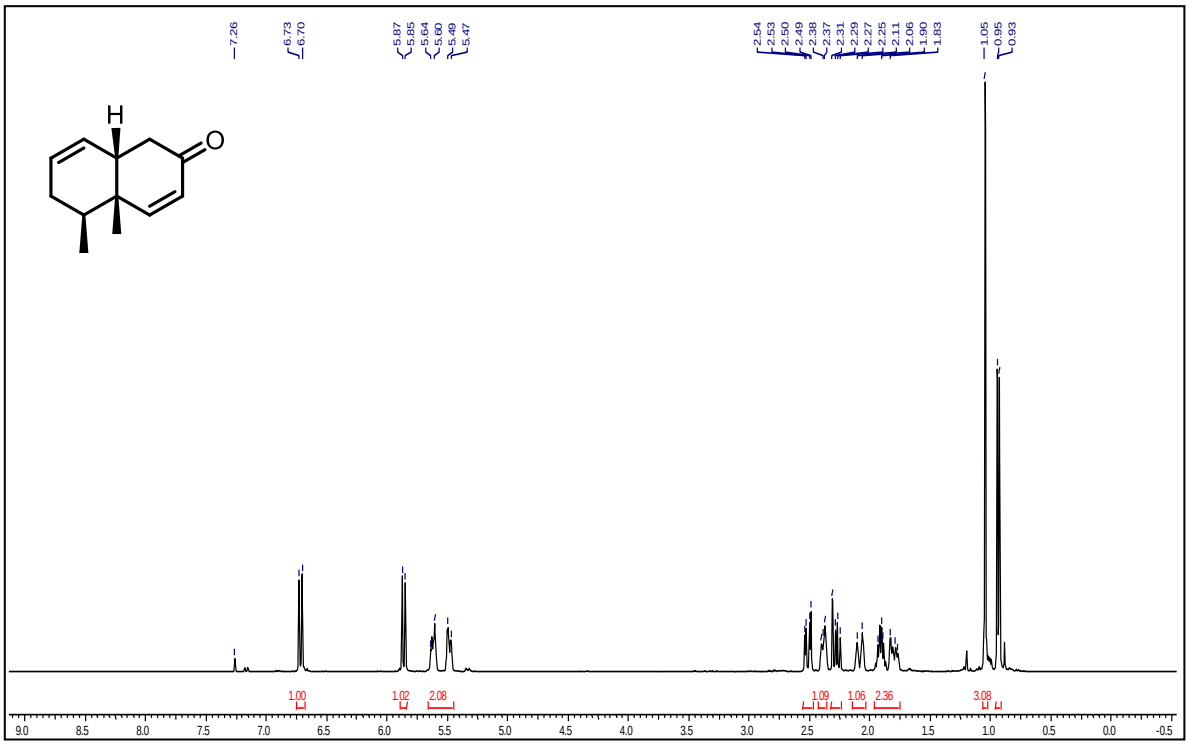
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 10



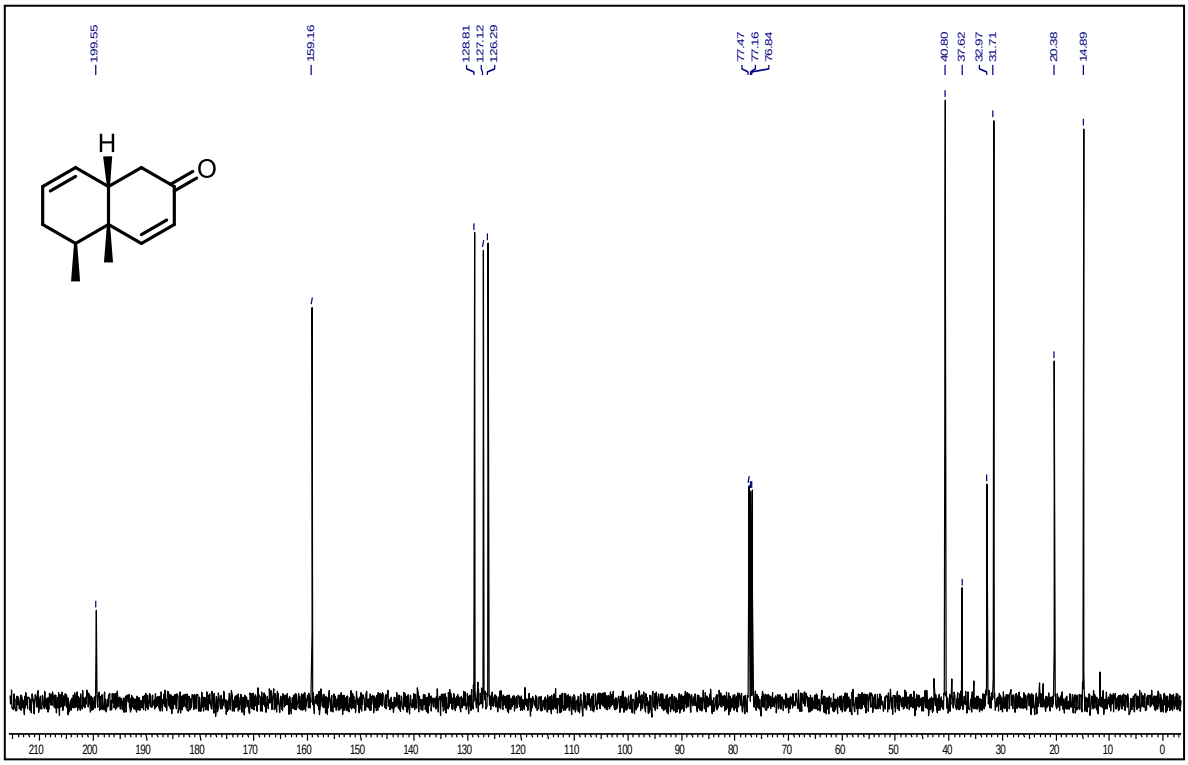
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 10



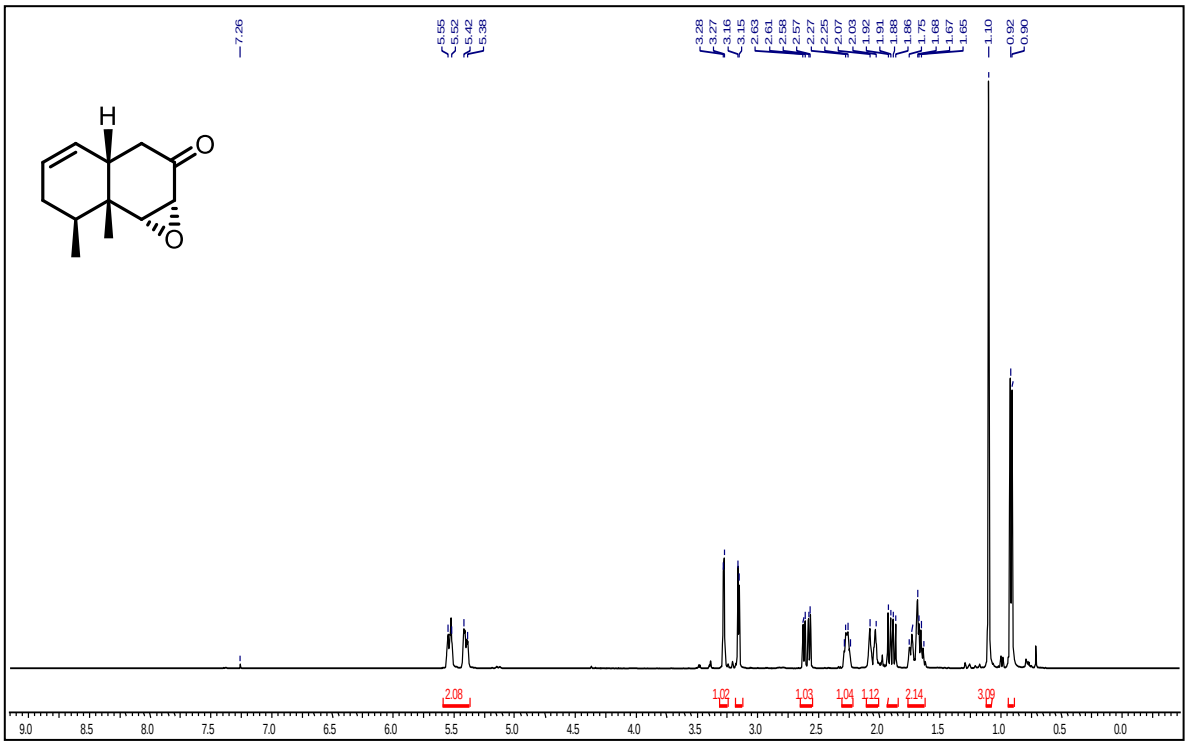
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 11



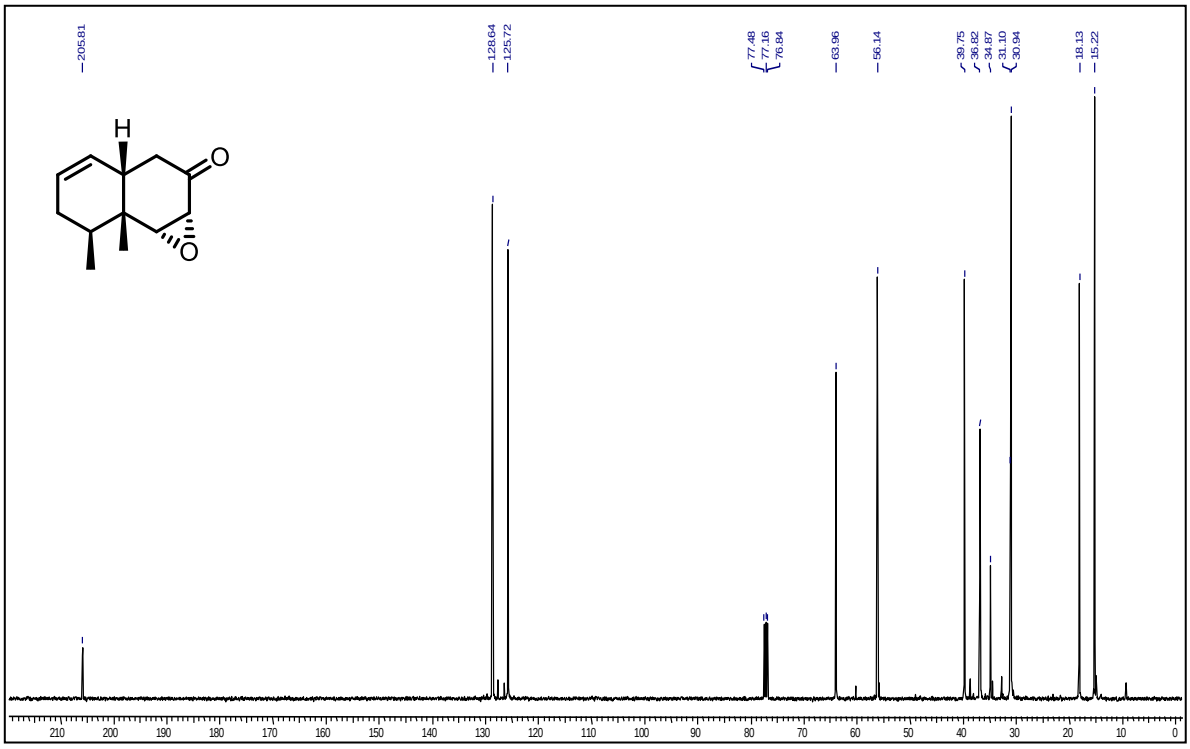
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 11



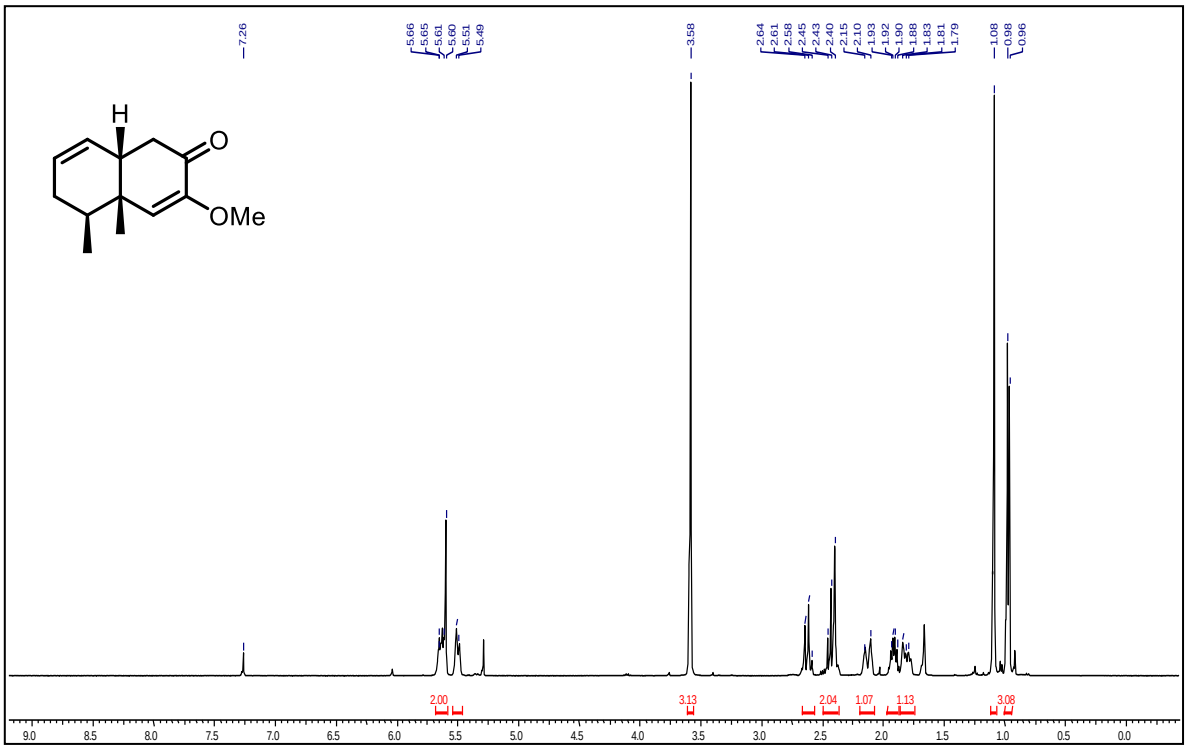
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 12



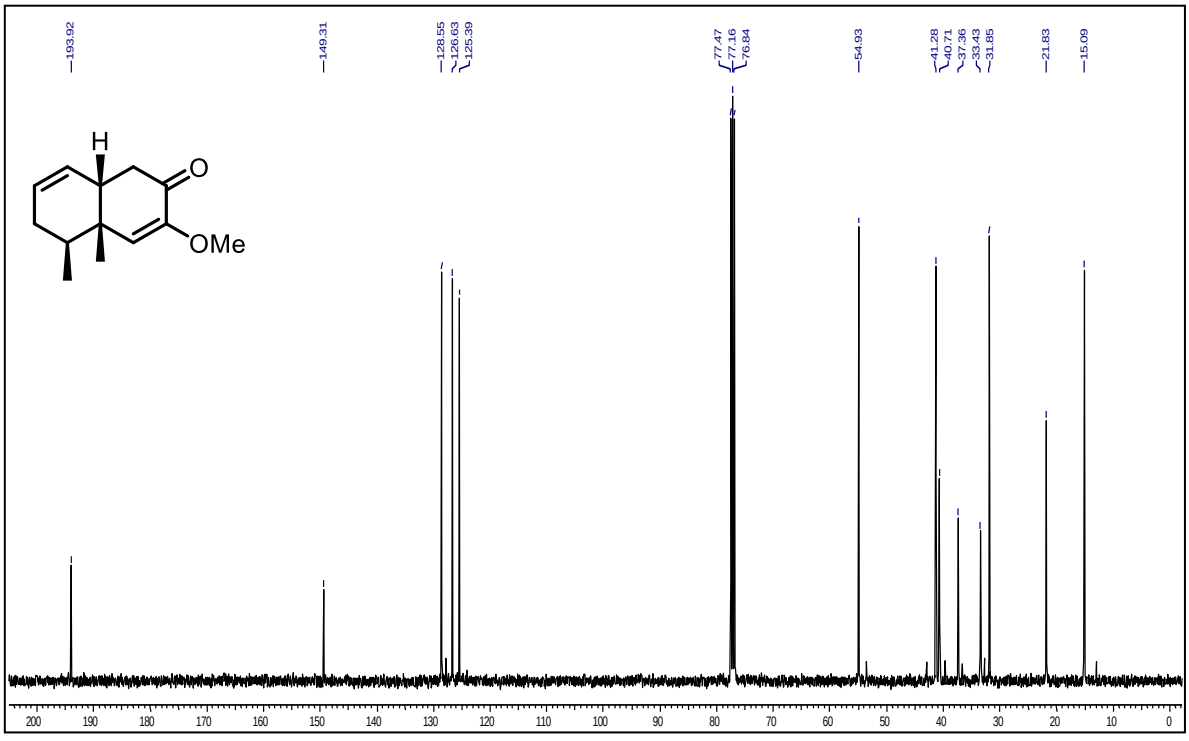
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 12



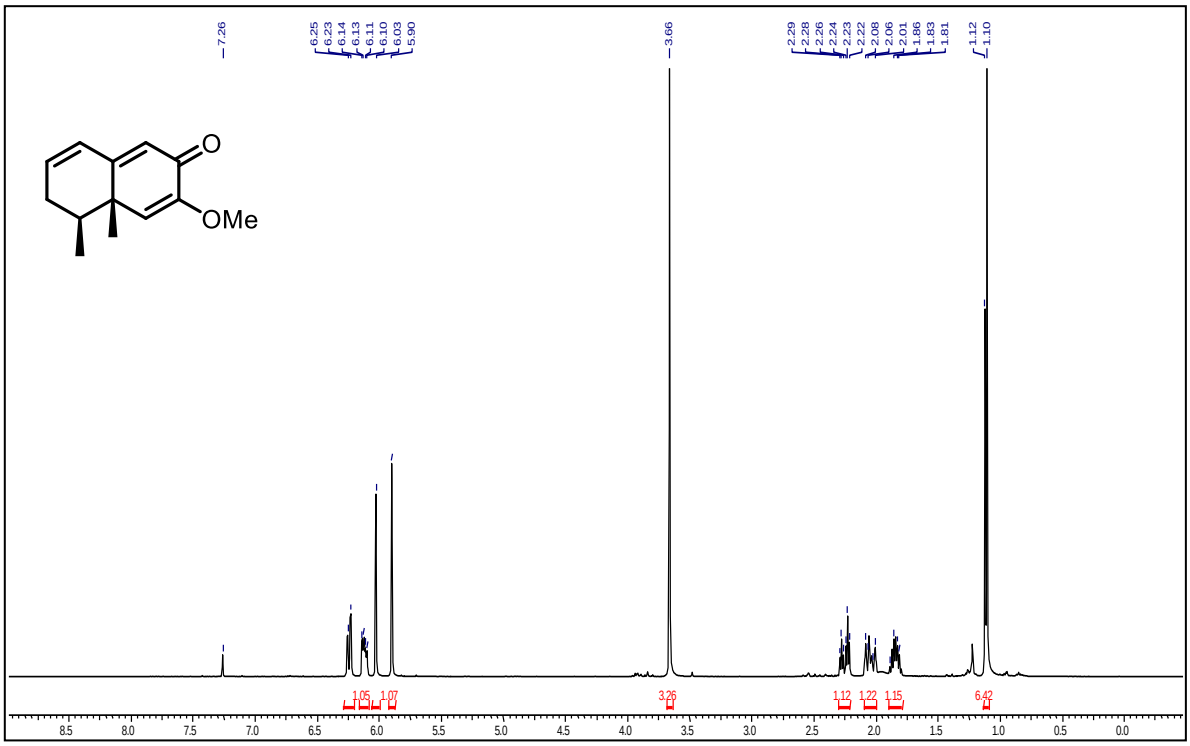
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 13



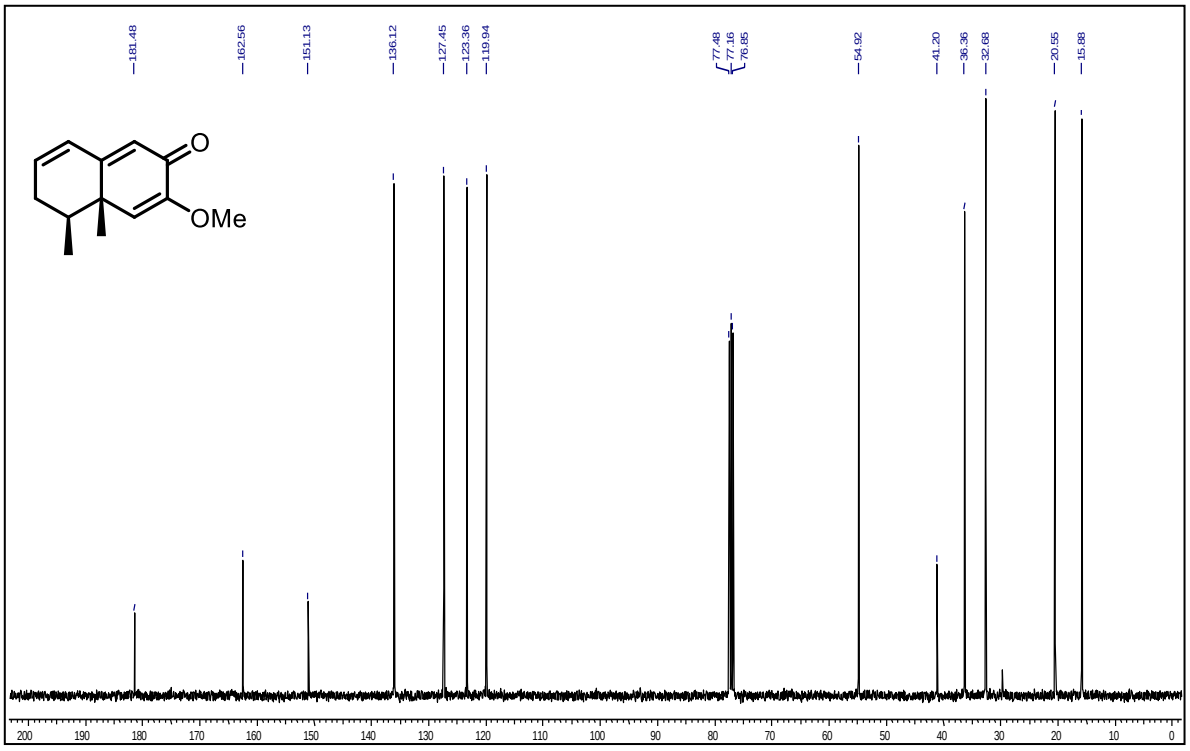
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 13



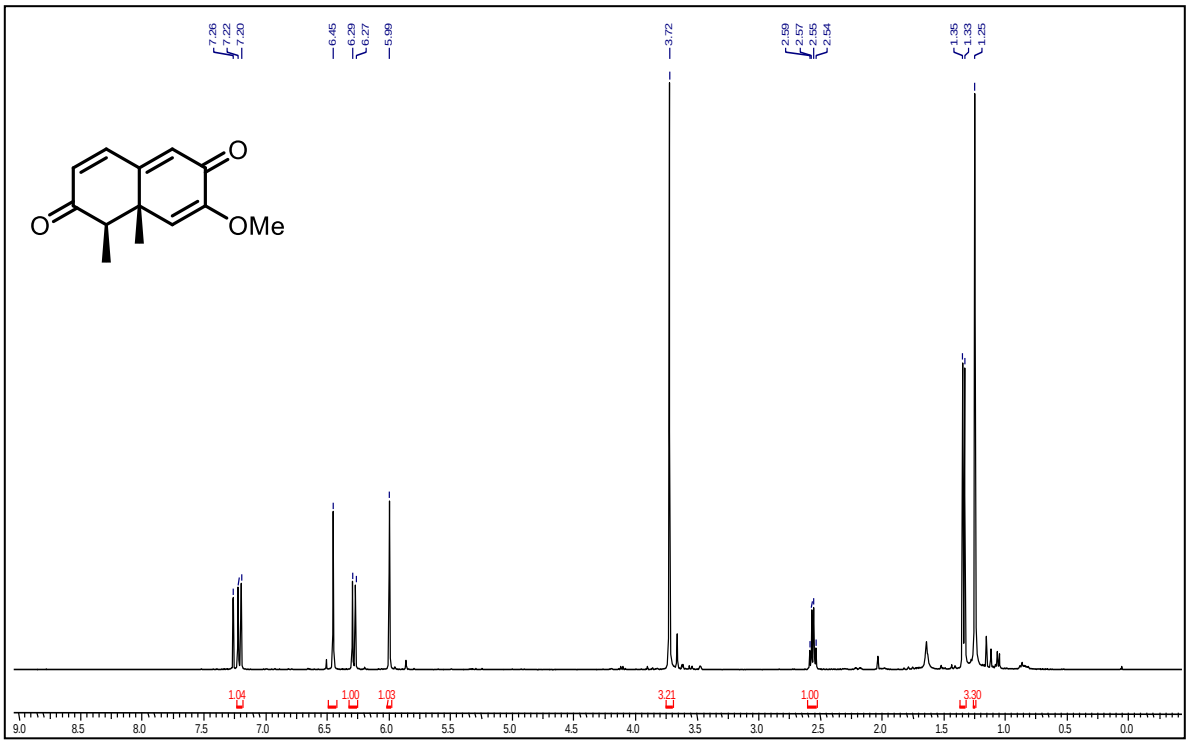
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 14



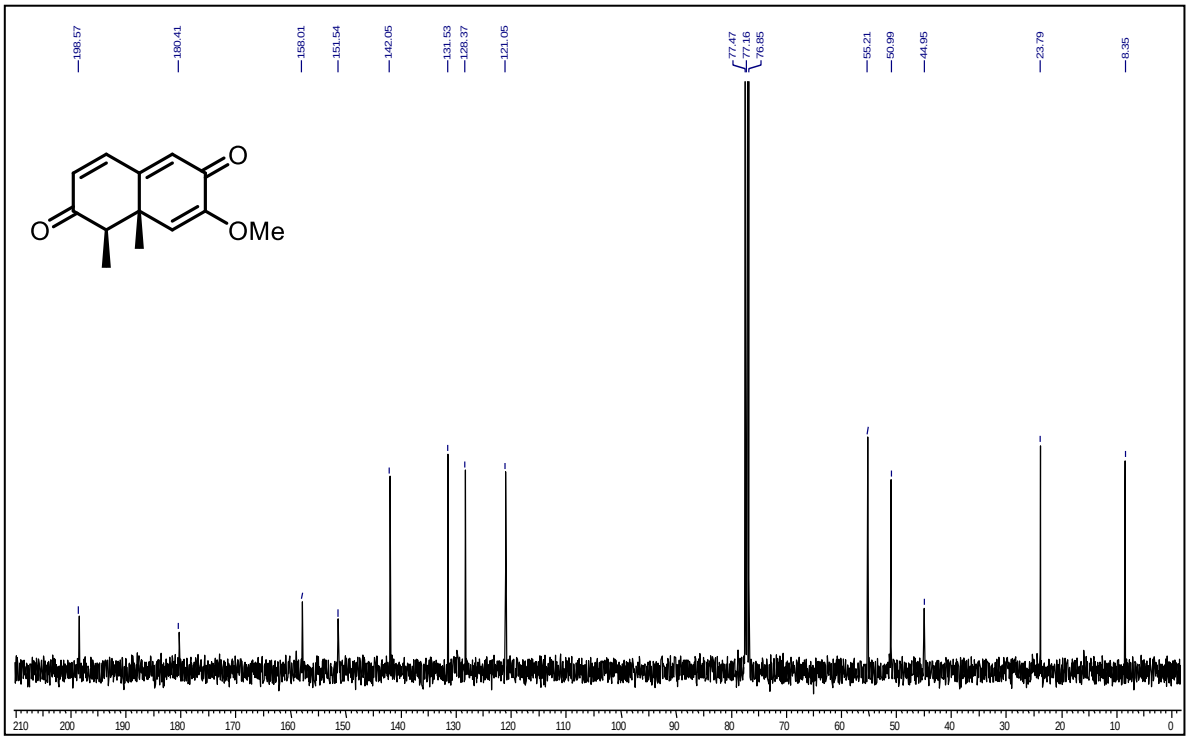
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 14



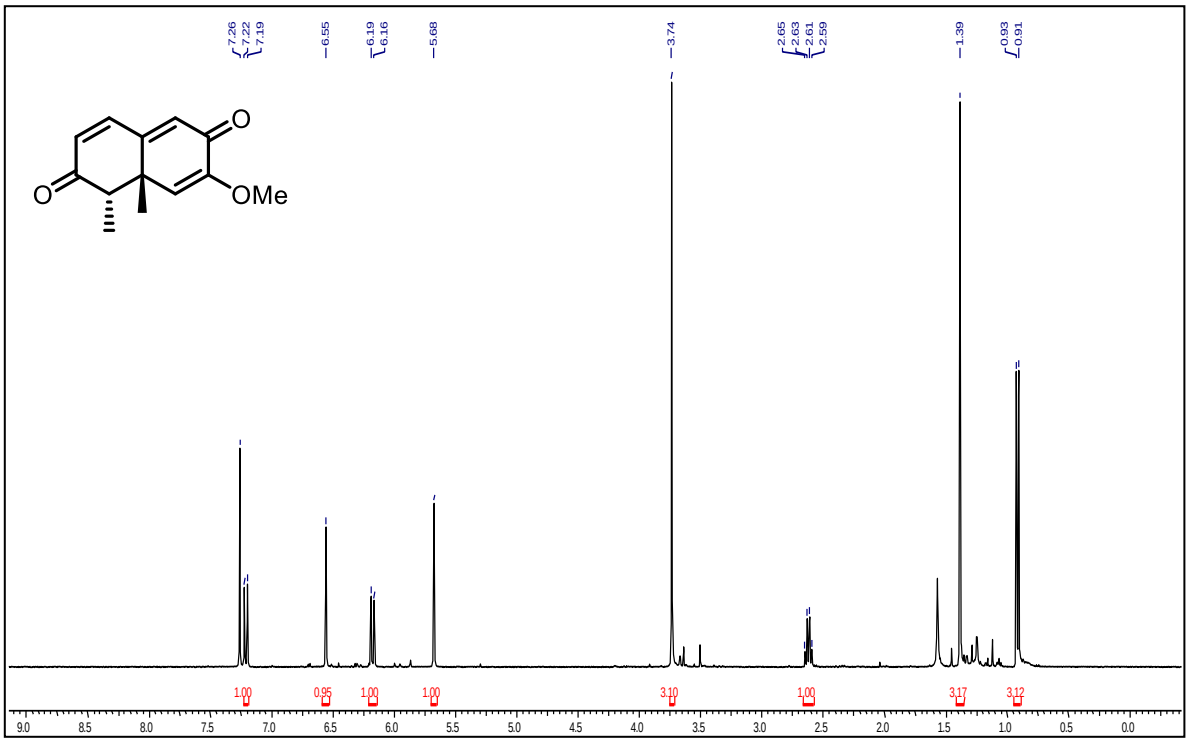
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 15



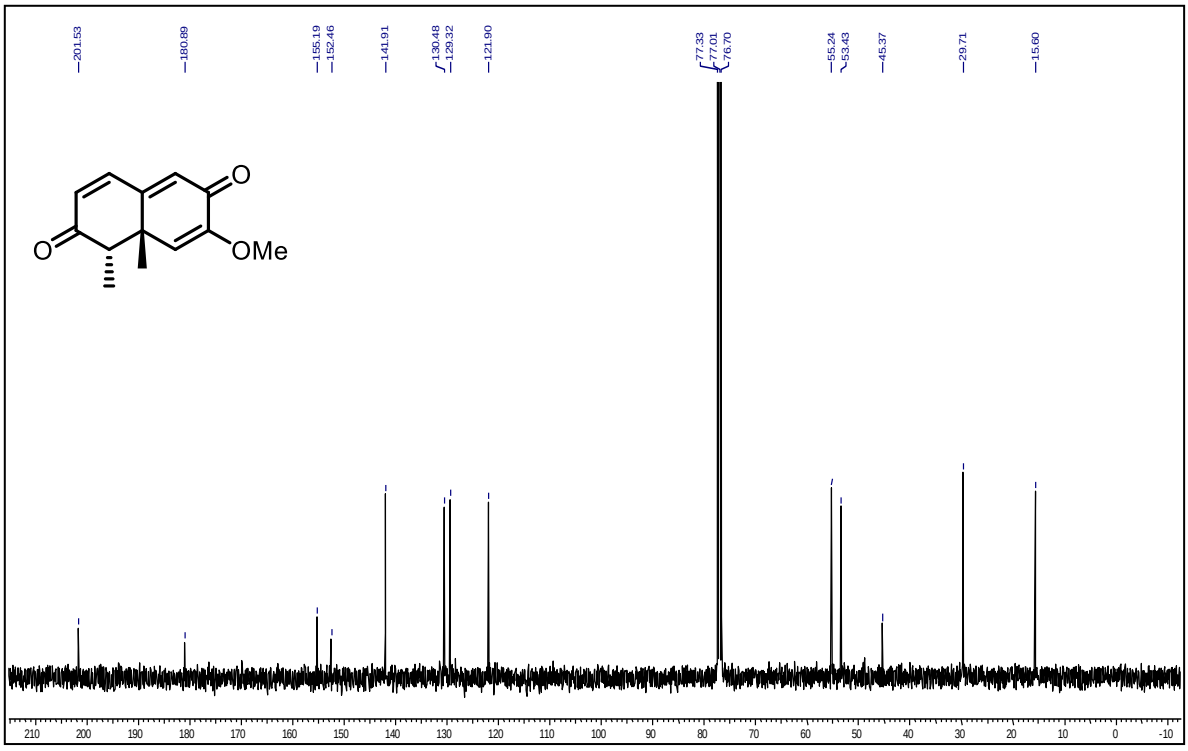
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 15



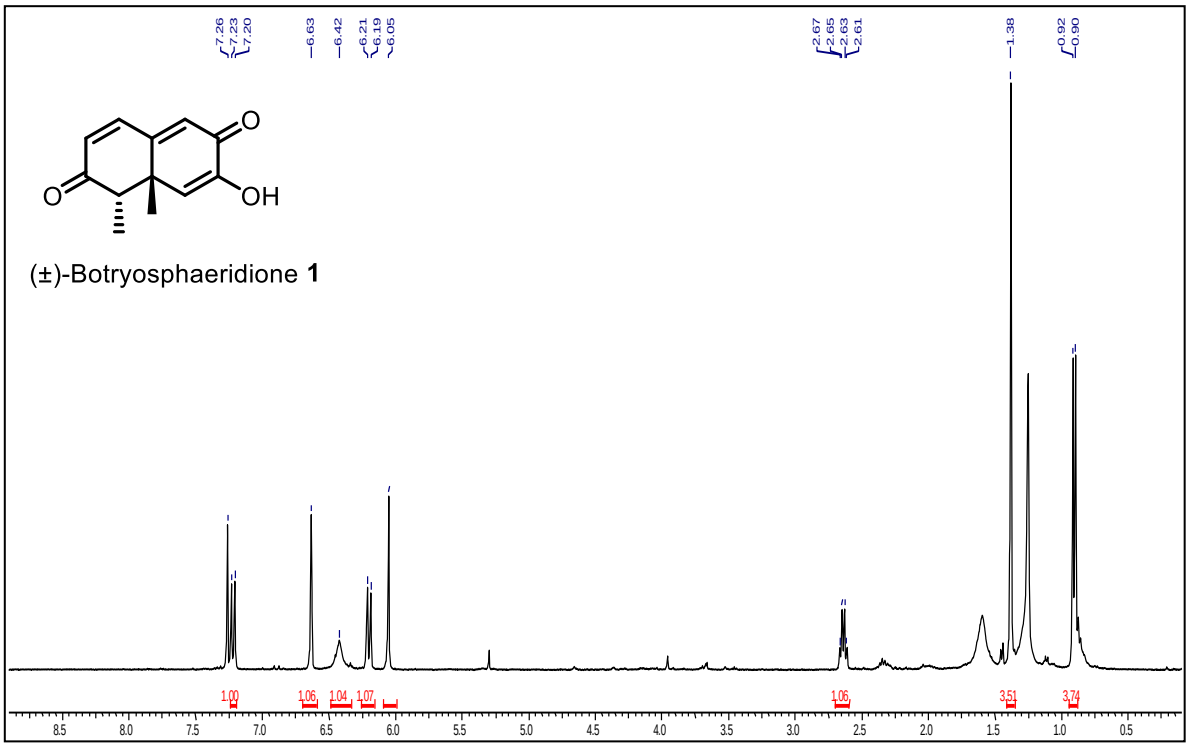
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 16



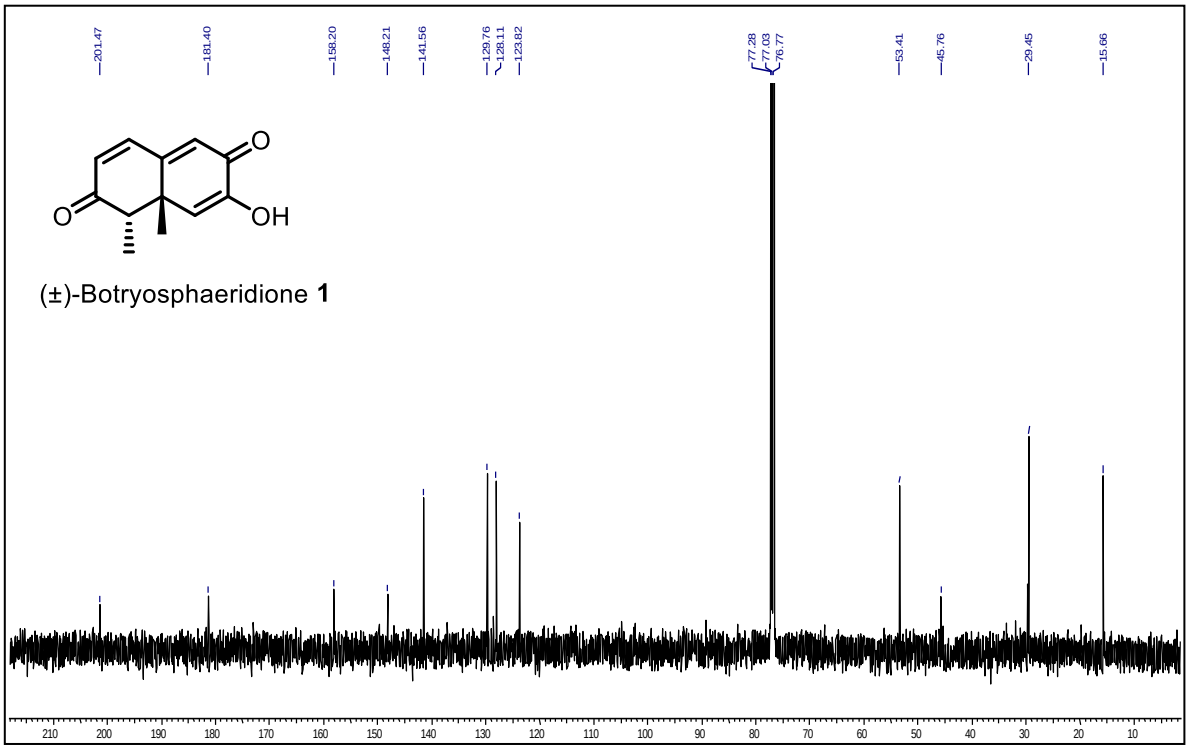
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 16



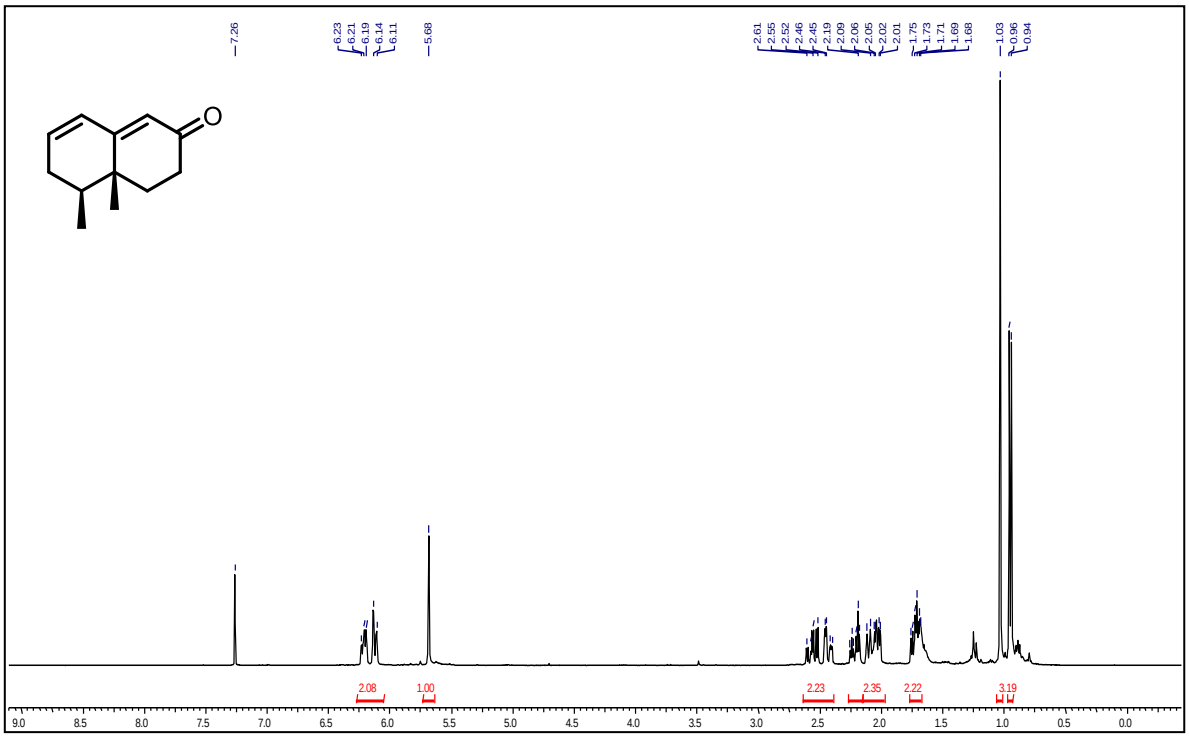
^{1}H NMR ($CDCl_3$, 400 MHz) of (±)-Botryosphaeridione 1



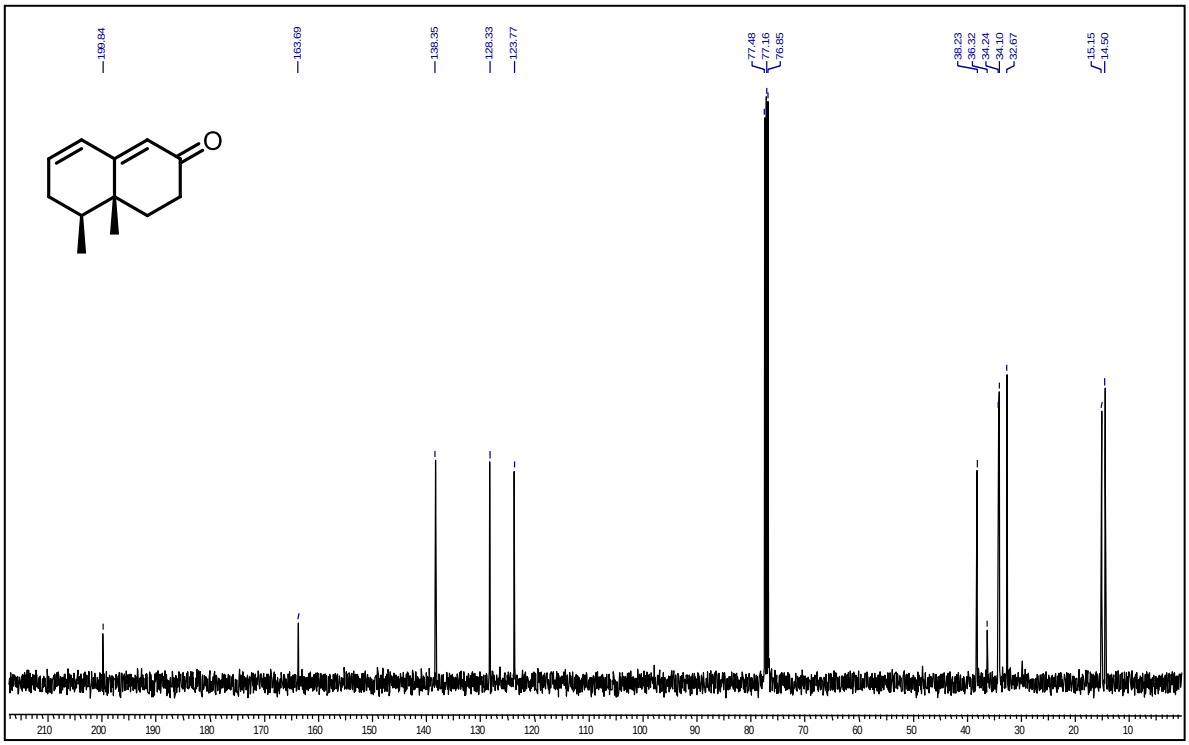
^{13}C NMR ($CDCl_3$, 100 MHz) of (±)-Botryosphaeridione 1



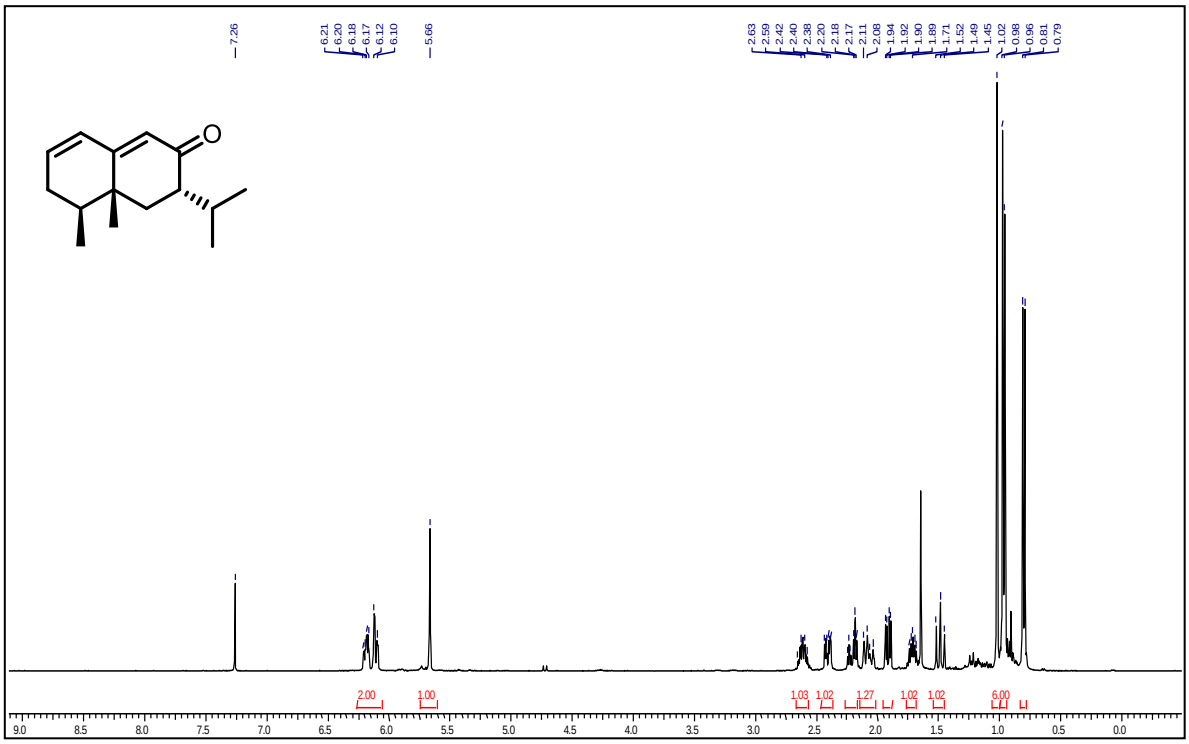
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 18



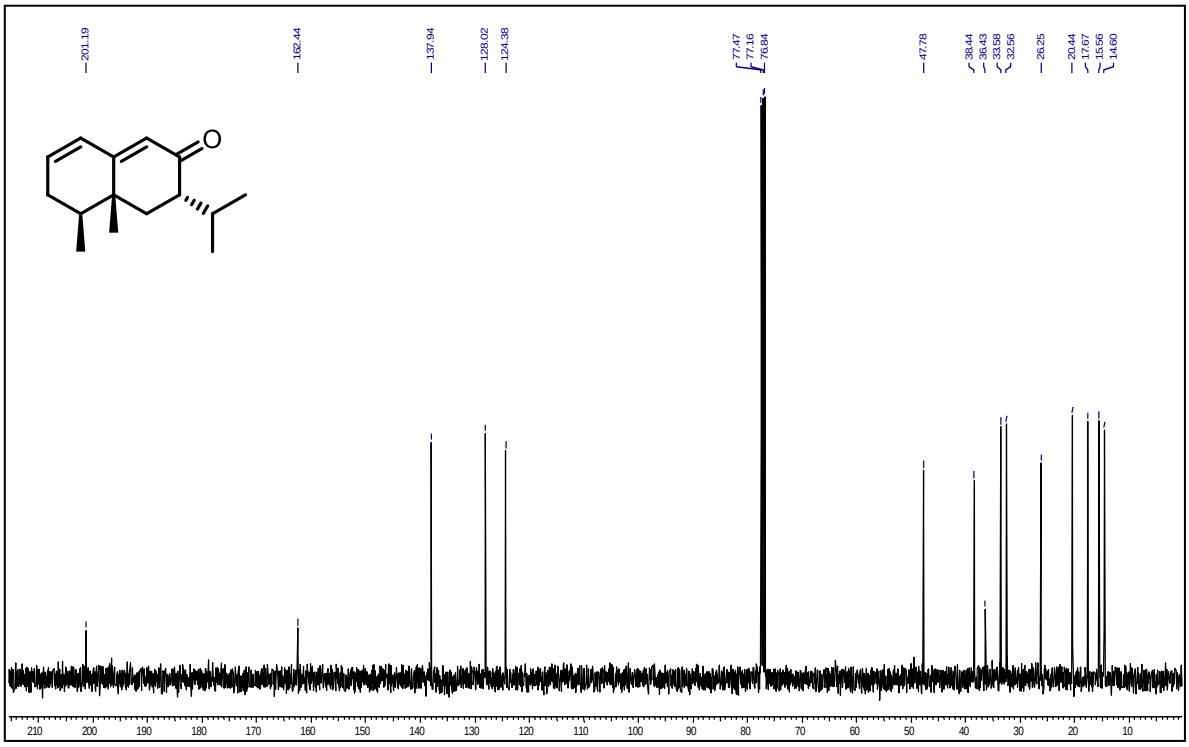
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 18



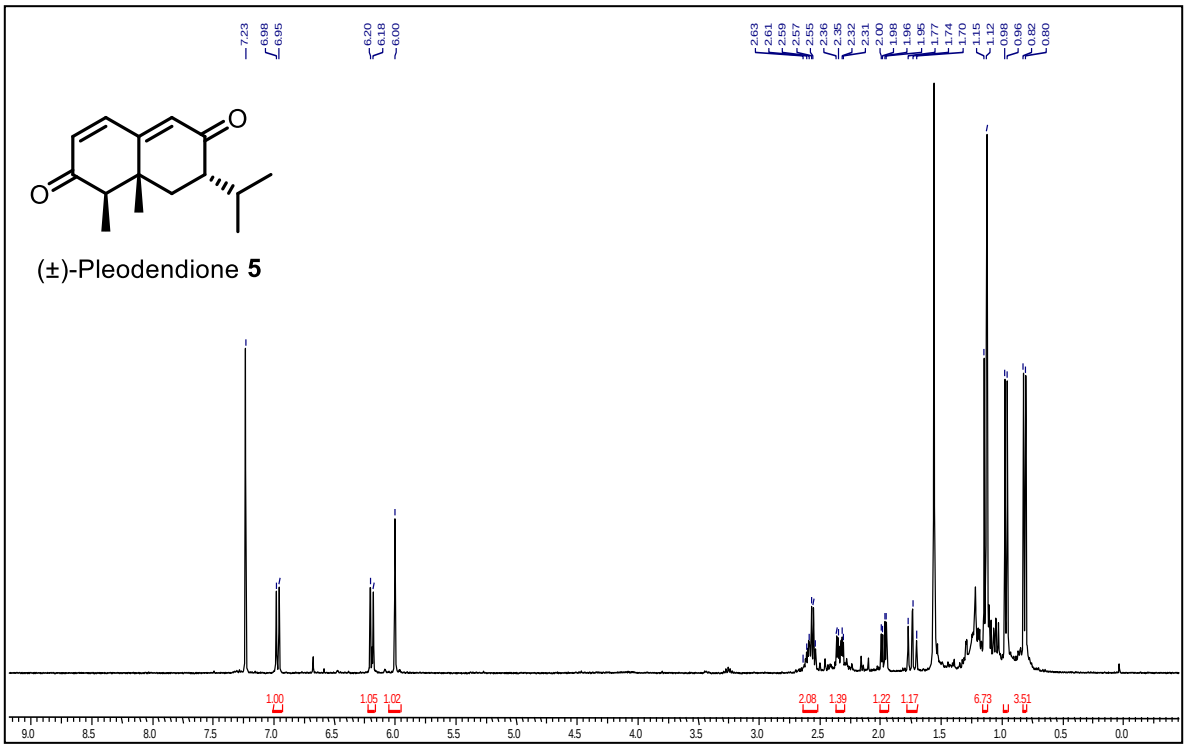
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 19



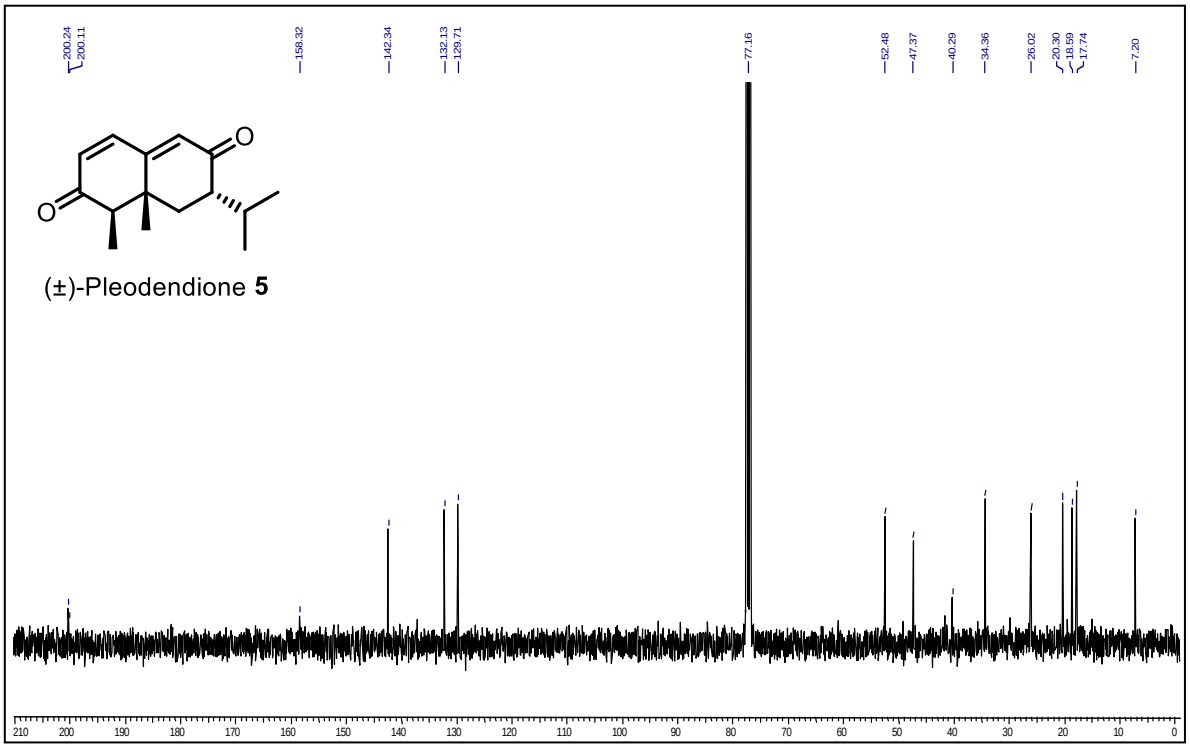
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 19



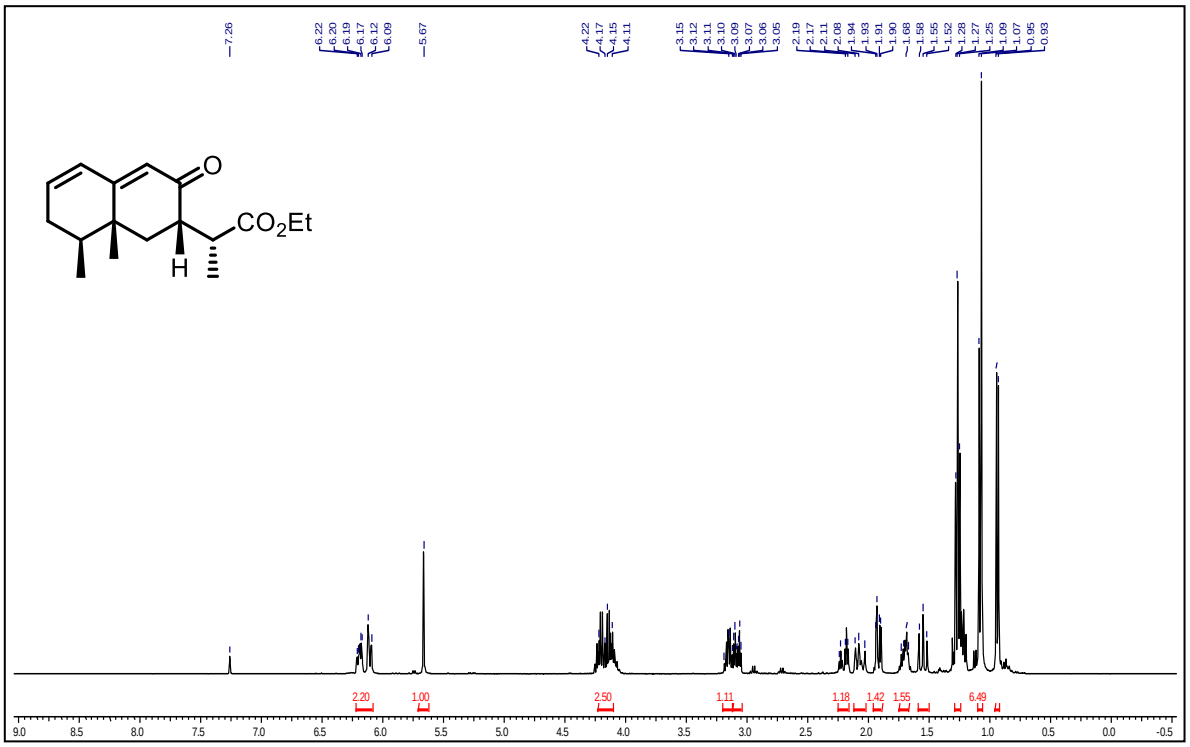
^{1}H NMR ($CDCl_3$, 400 MHz) of (±)-Pleodendione 5



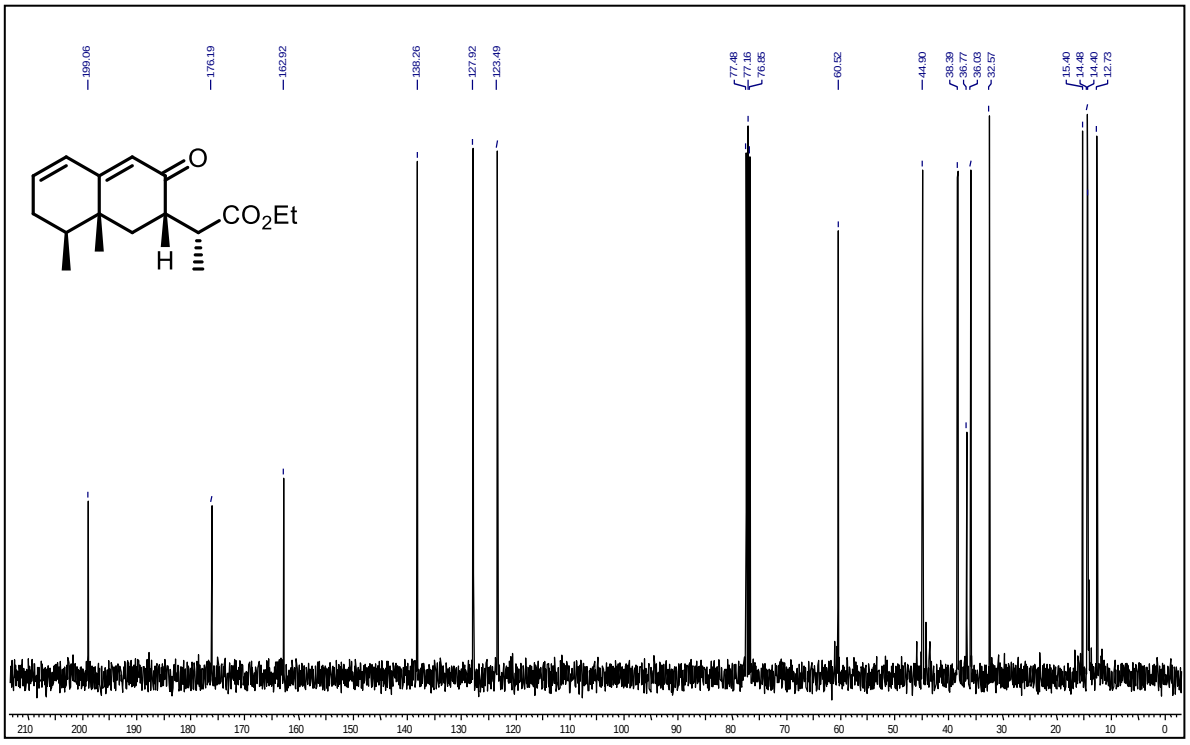
^{13}C NMR ($CDCl_3$, 100 MHz) of (±)-Pleodendione 5



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 20



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



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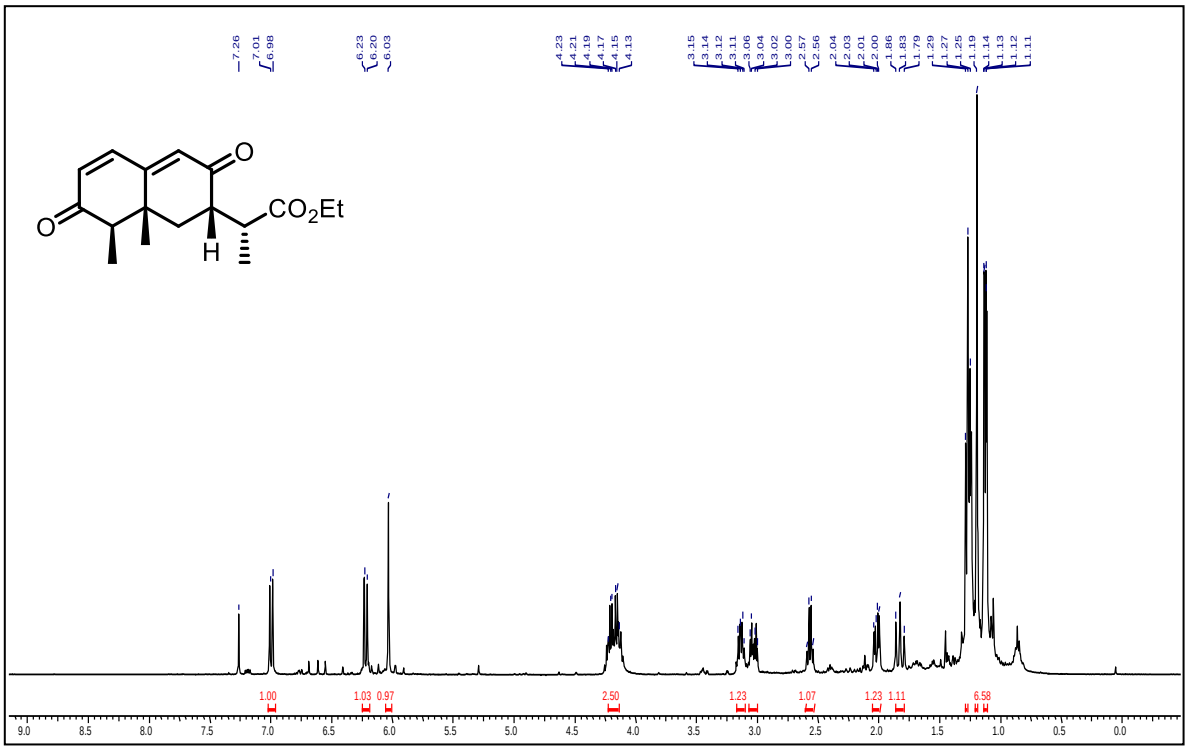
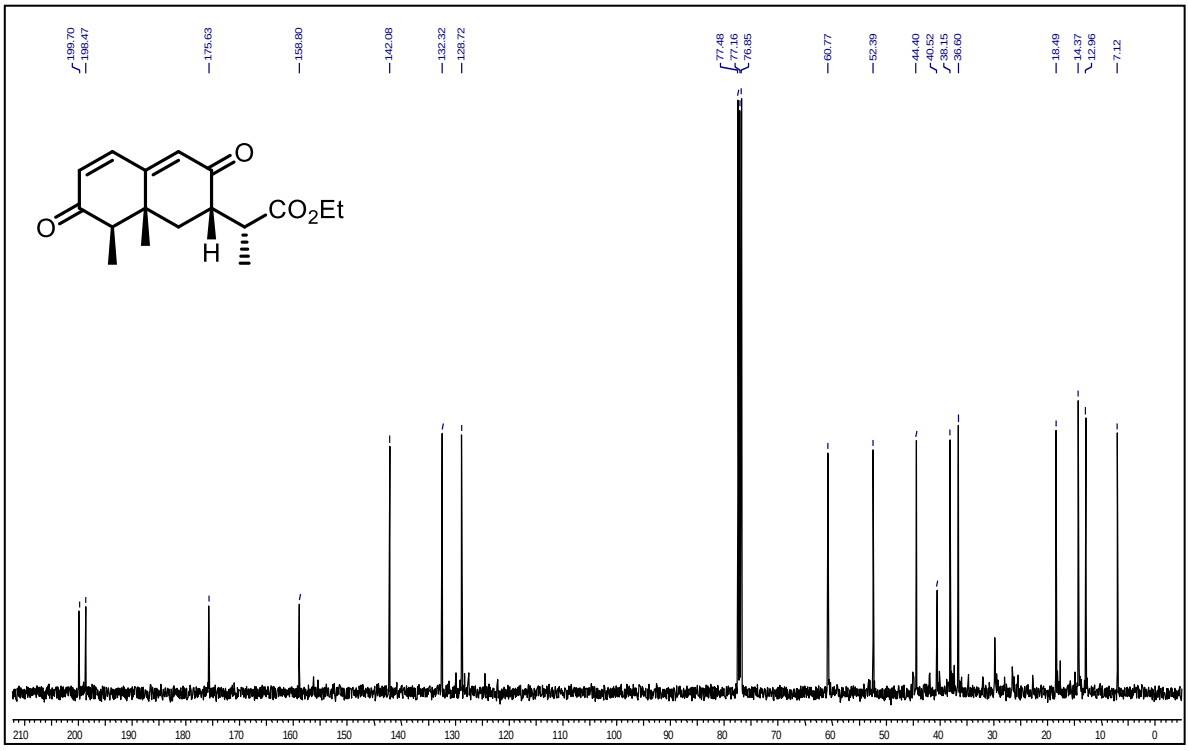
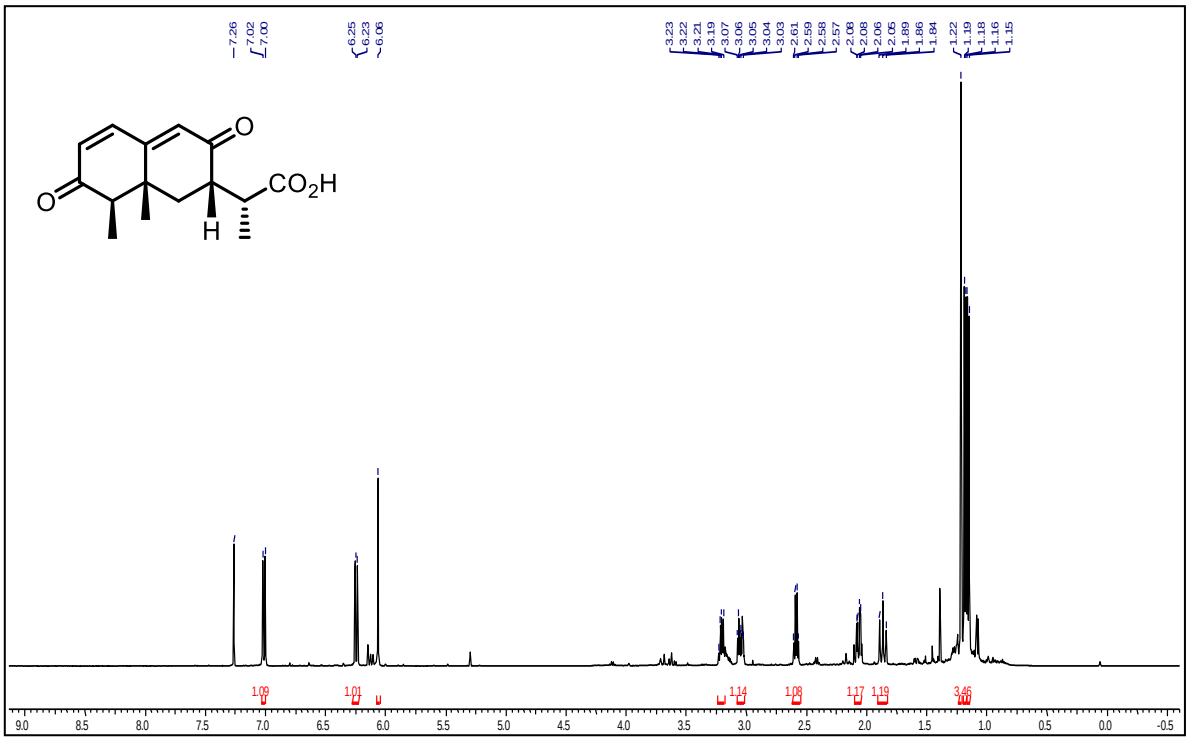


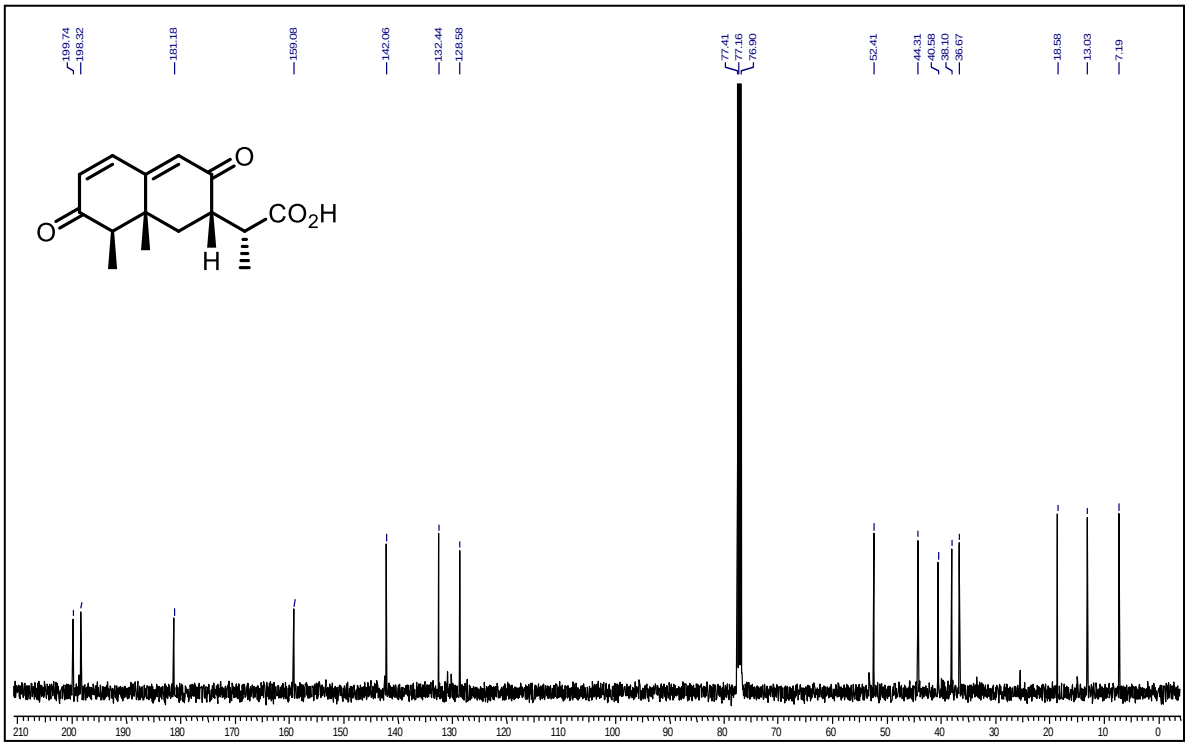
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 21



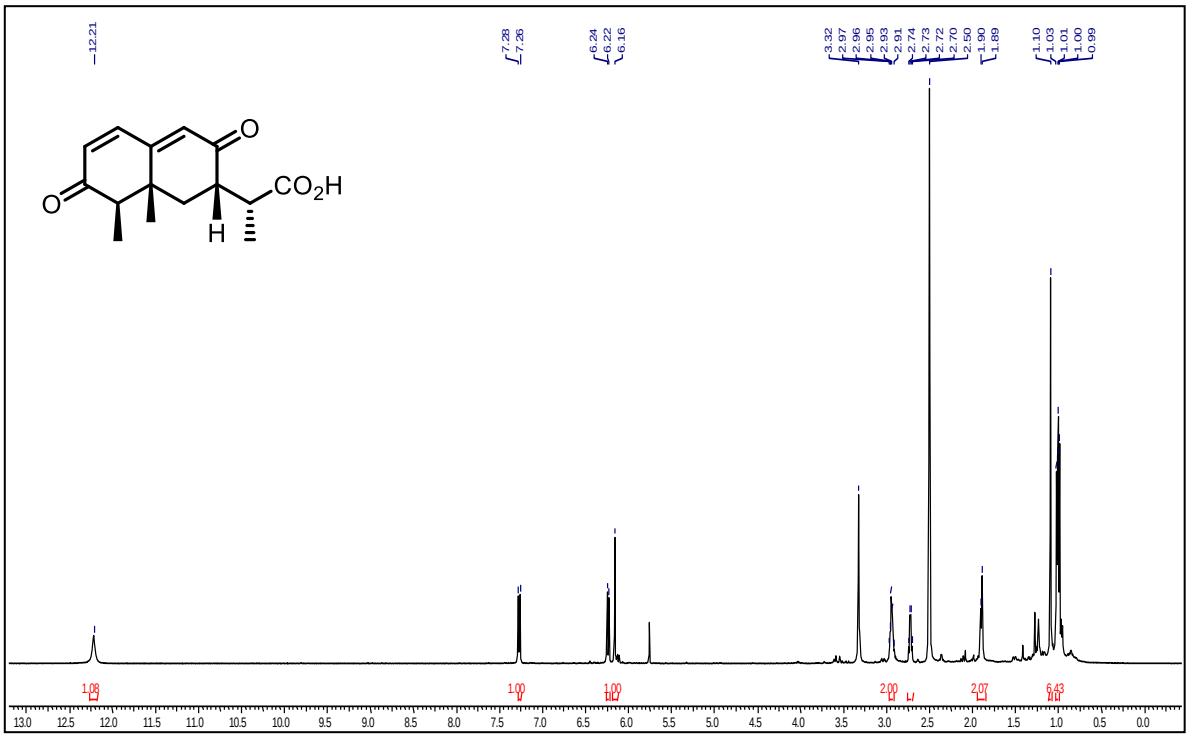
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 22



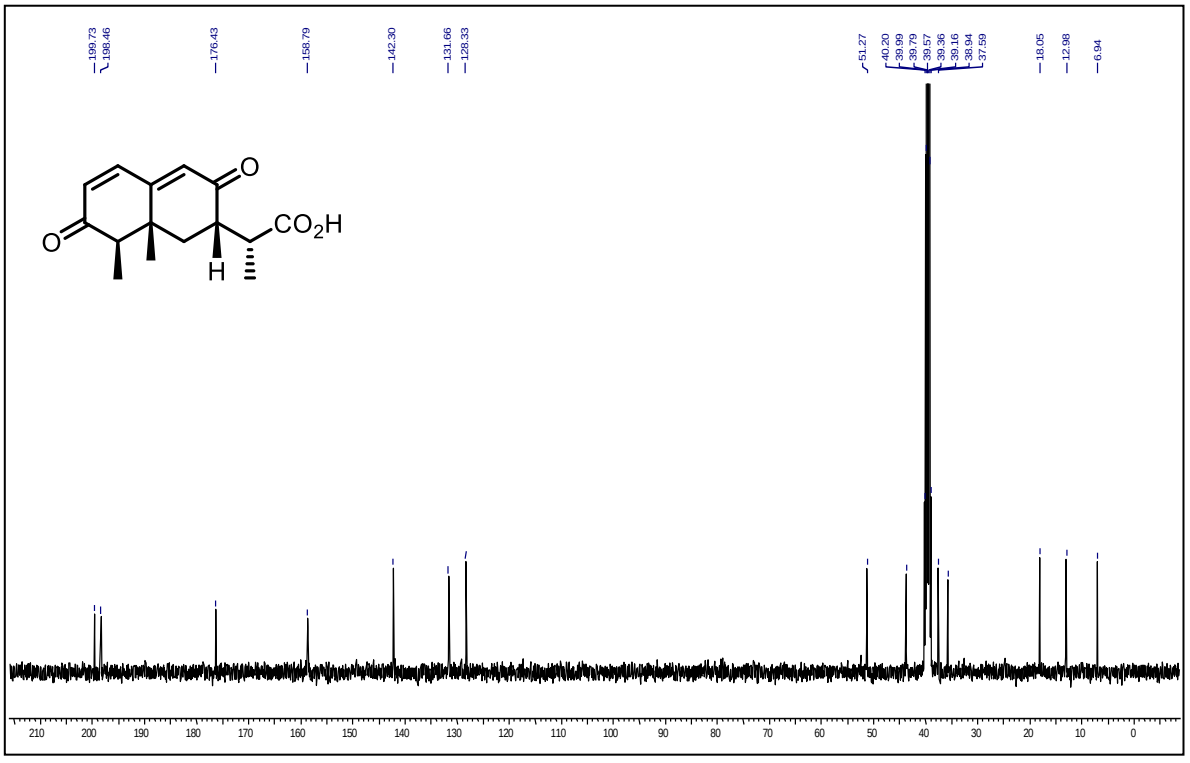
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 22



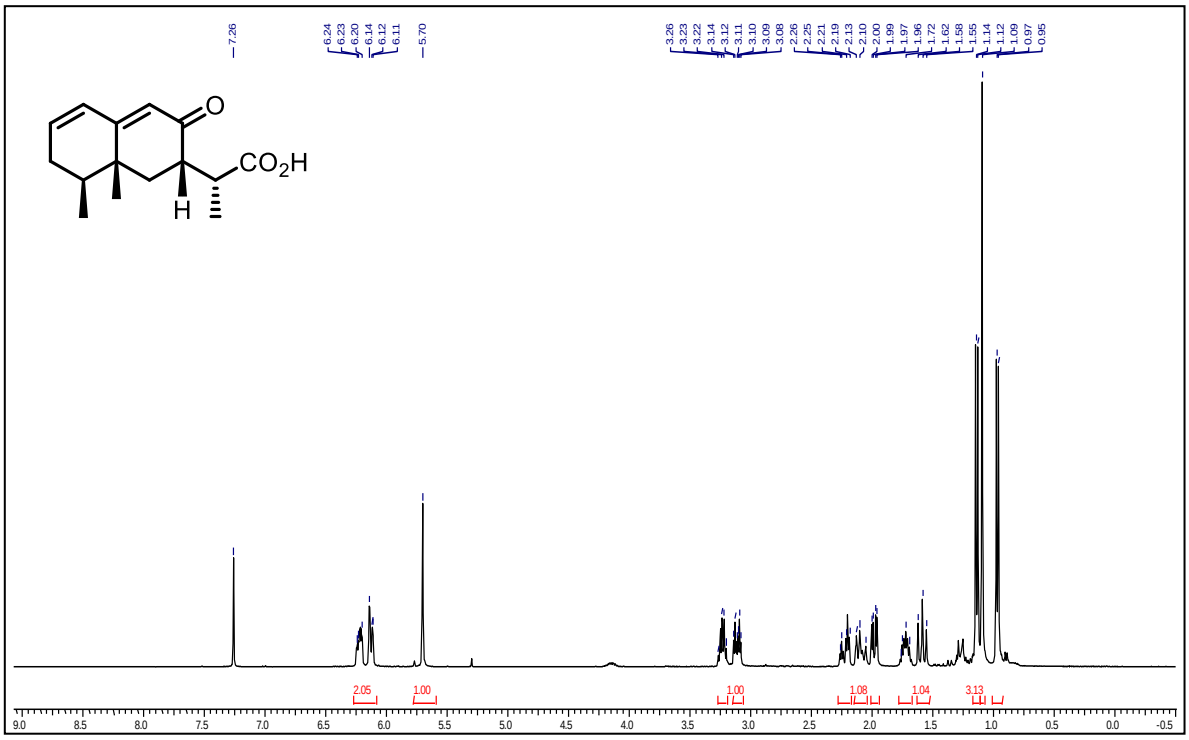
^{1}H NMR (DMSO-d_6, 400 MHz) of compound 22



^{13}C NMR (DMSO-d_6, 100 MHz) of compound 22



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 23



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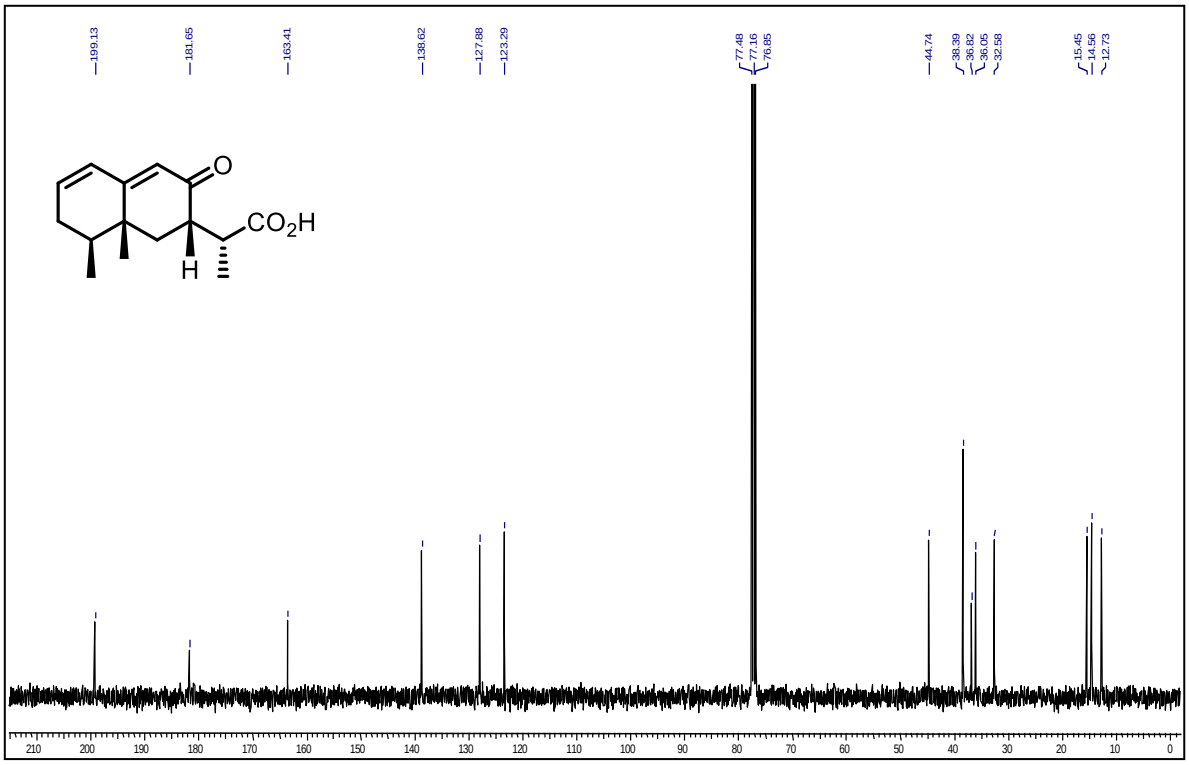
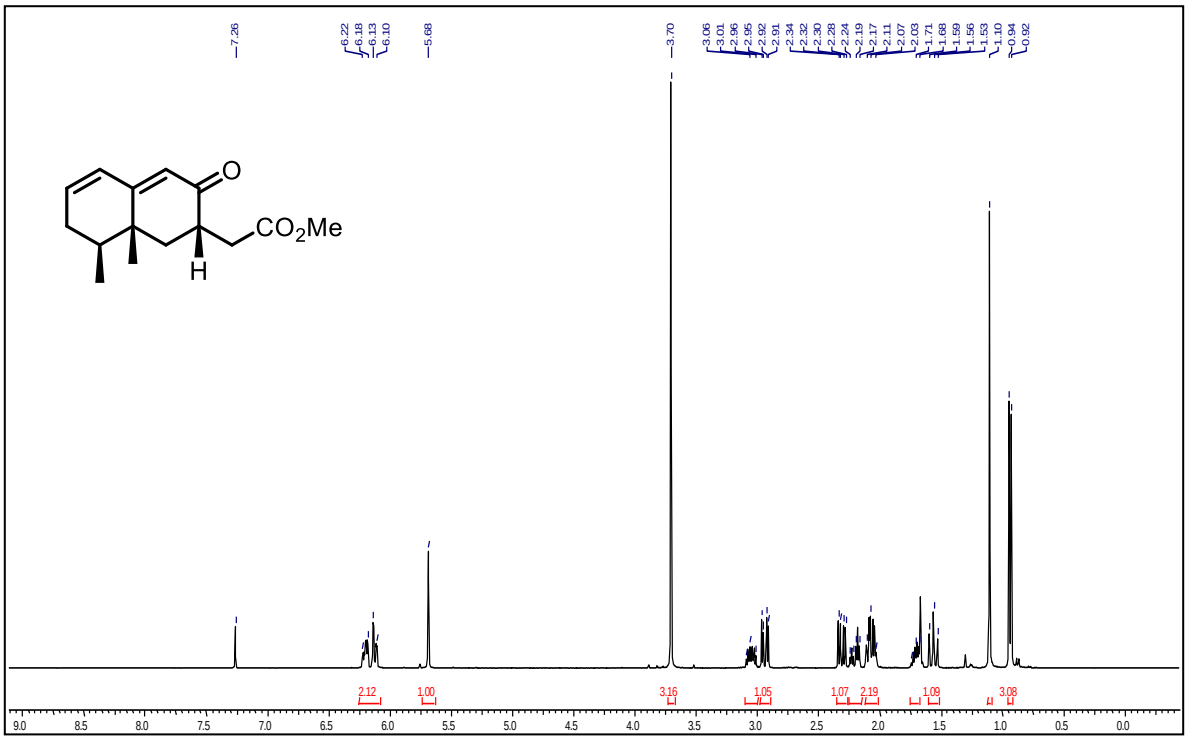
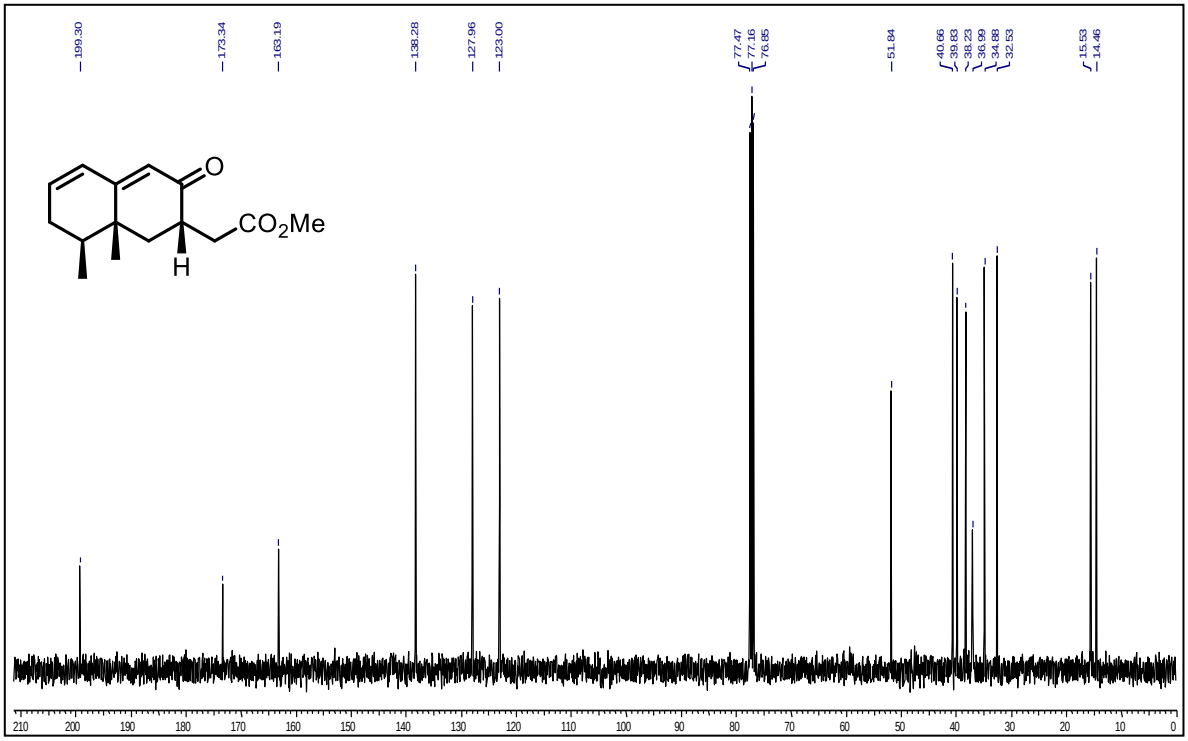


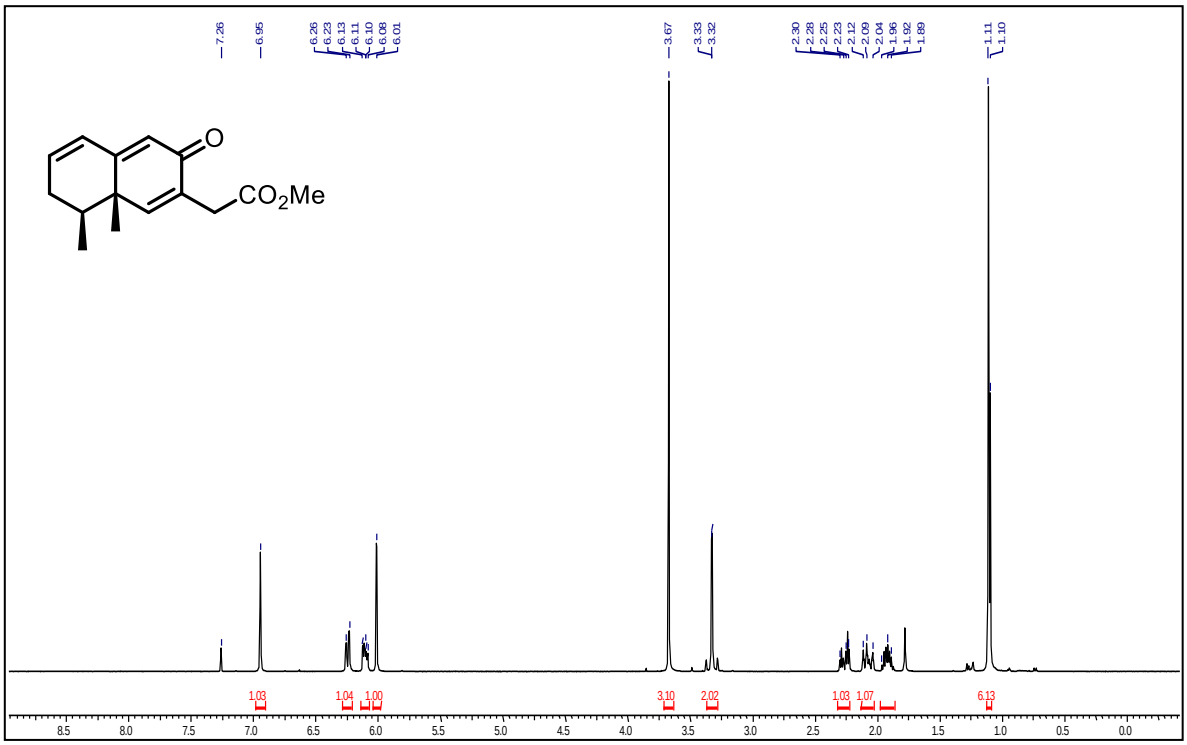
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 24



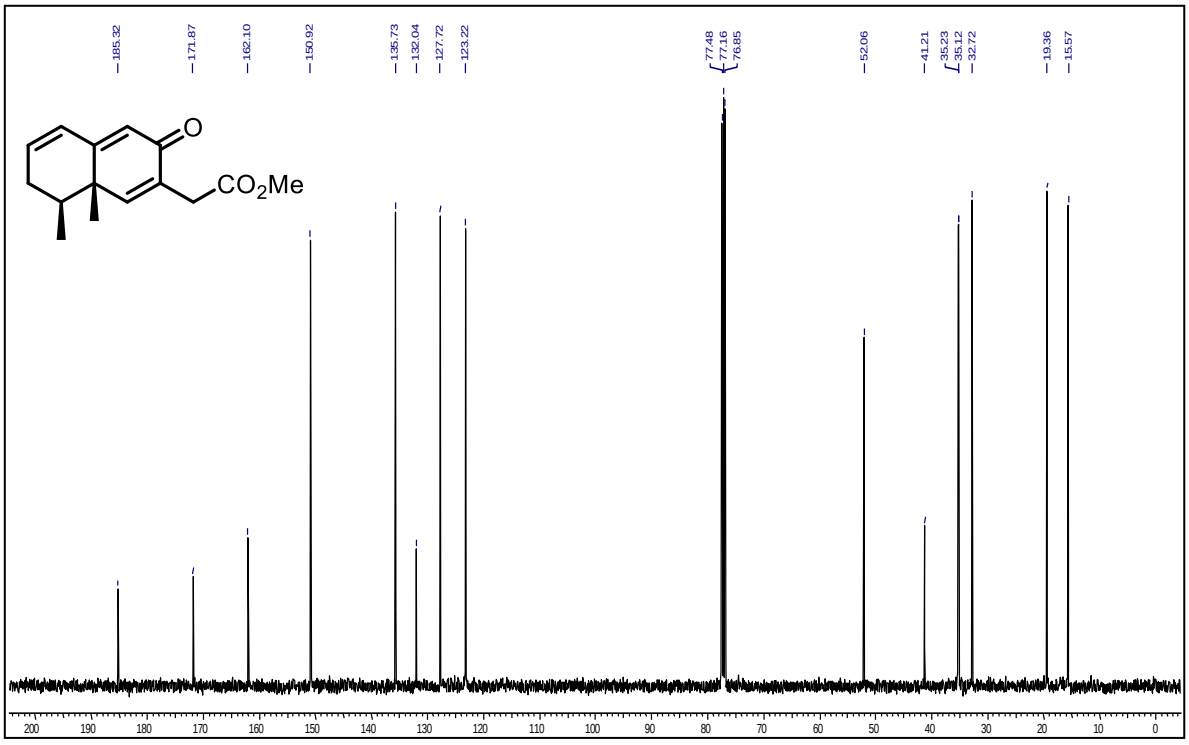
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 24



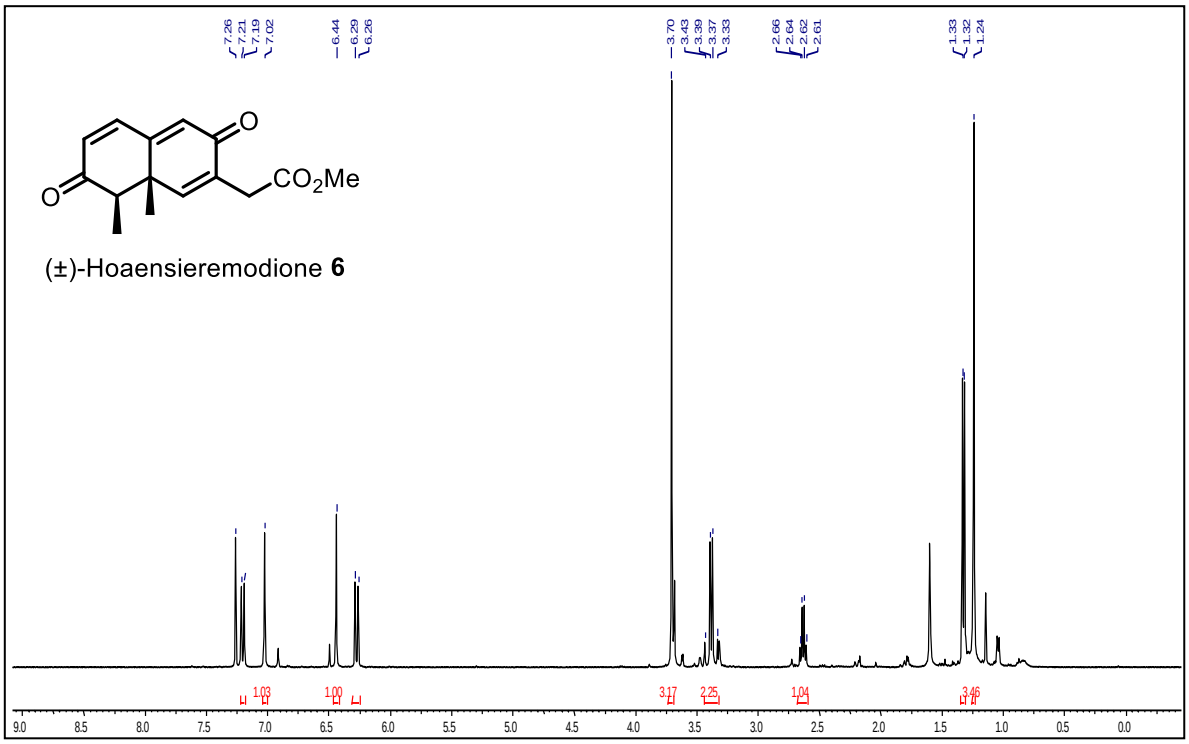
^{1}H NMR ($CDCl_3$, 400 MHz) of compound 25



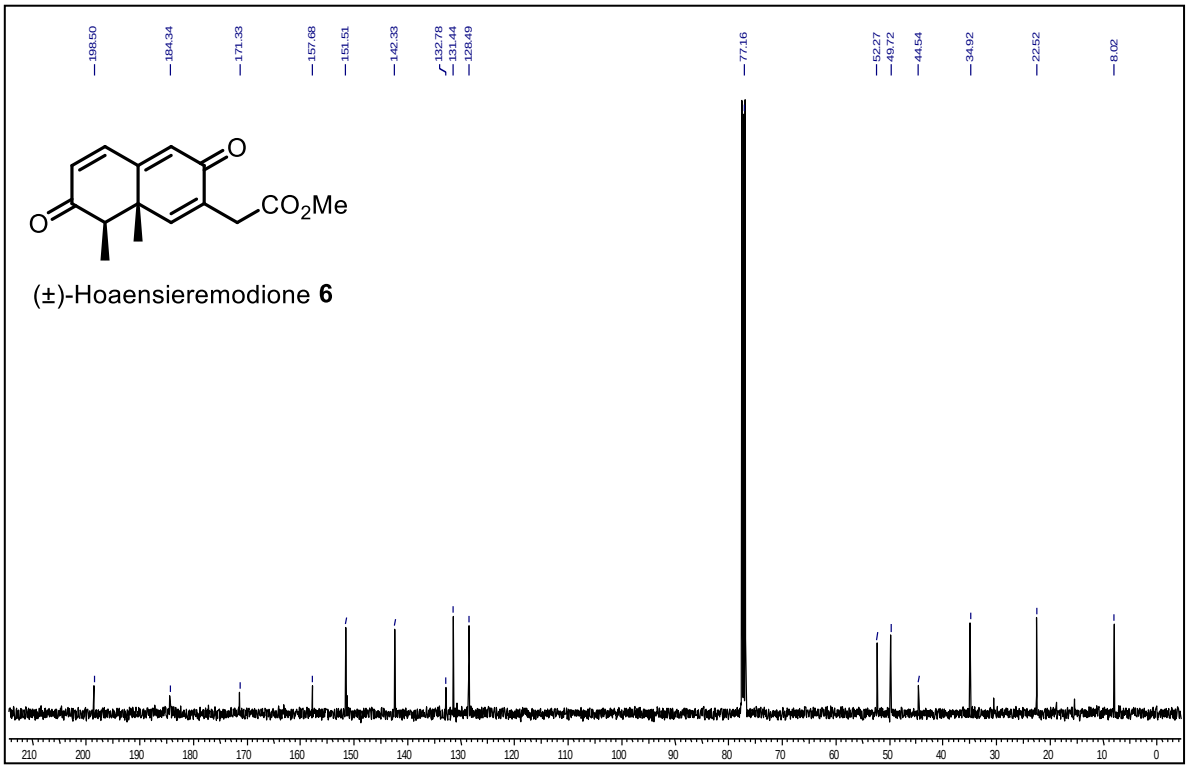
^{13}C NMR ($CDCl_3$, 100 MHz) of compound 25



^{1}H NMR ($CDCl_3$, 400 MHz) of (±)-Hoaensieremodione 6



^{13}C NMR ($CDCl_3$, 100 MHz) of (±)-Hoaensieremodione 6



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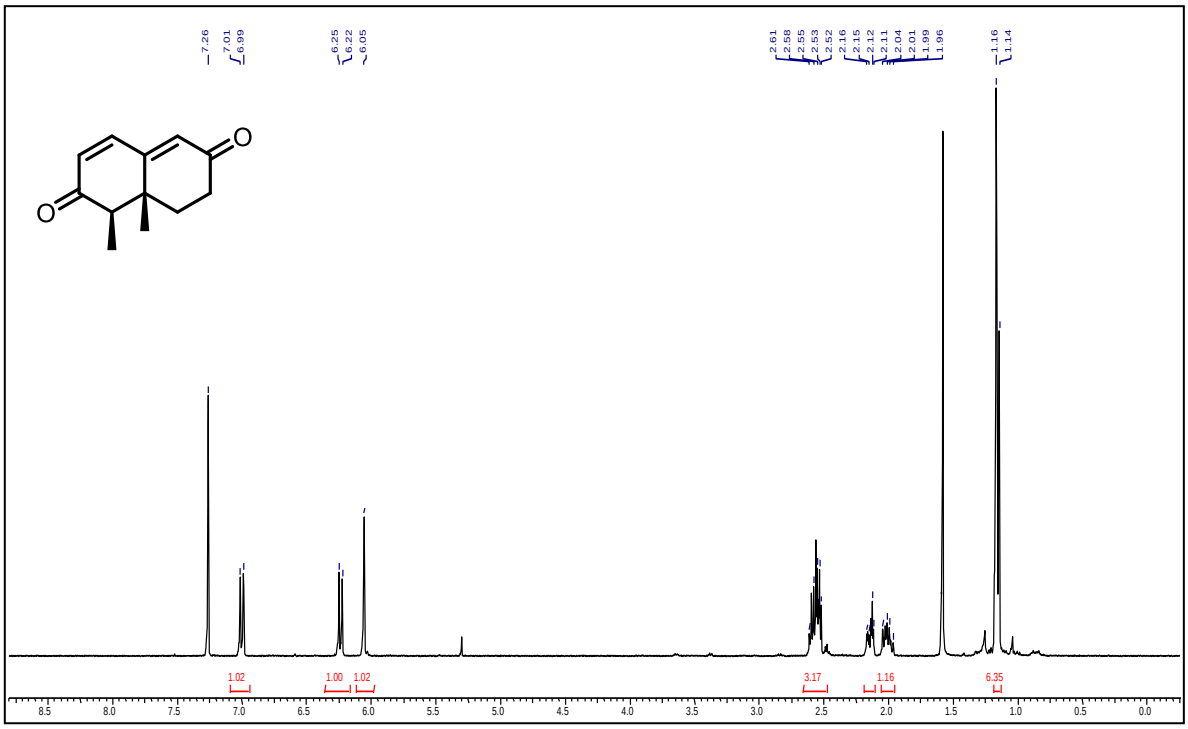
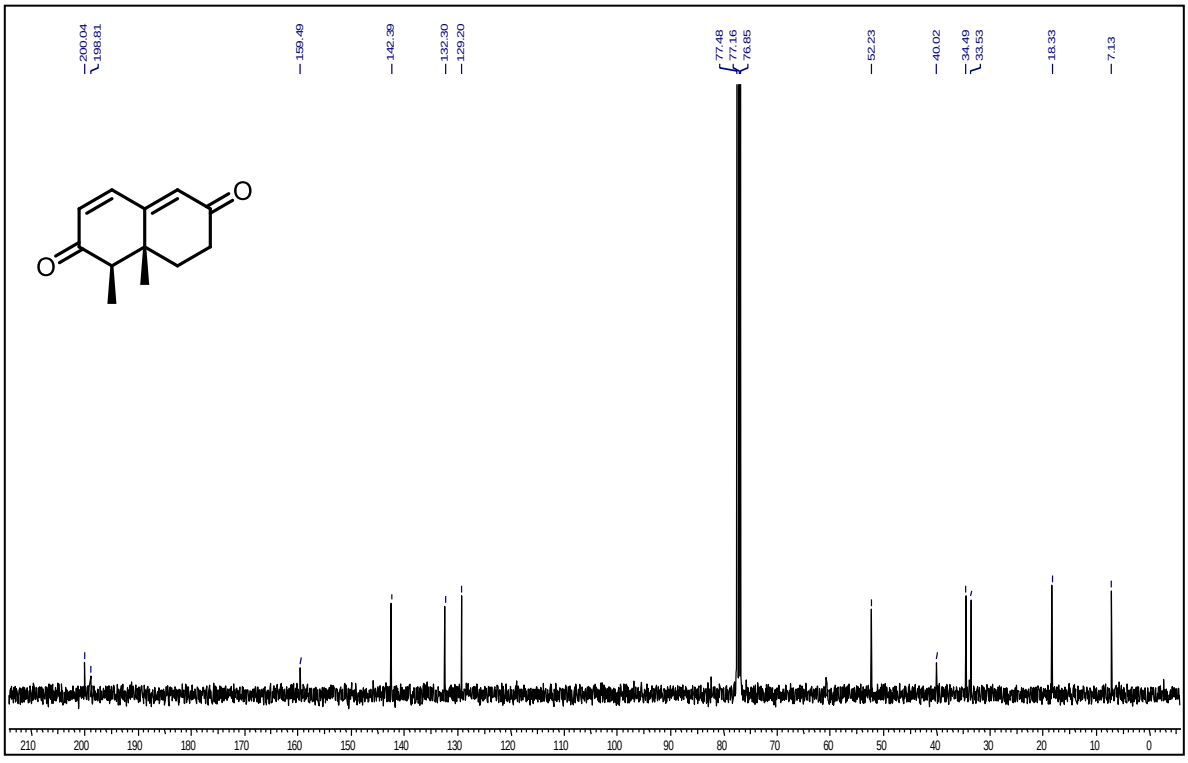
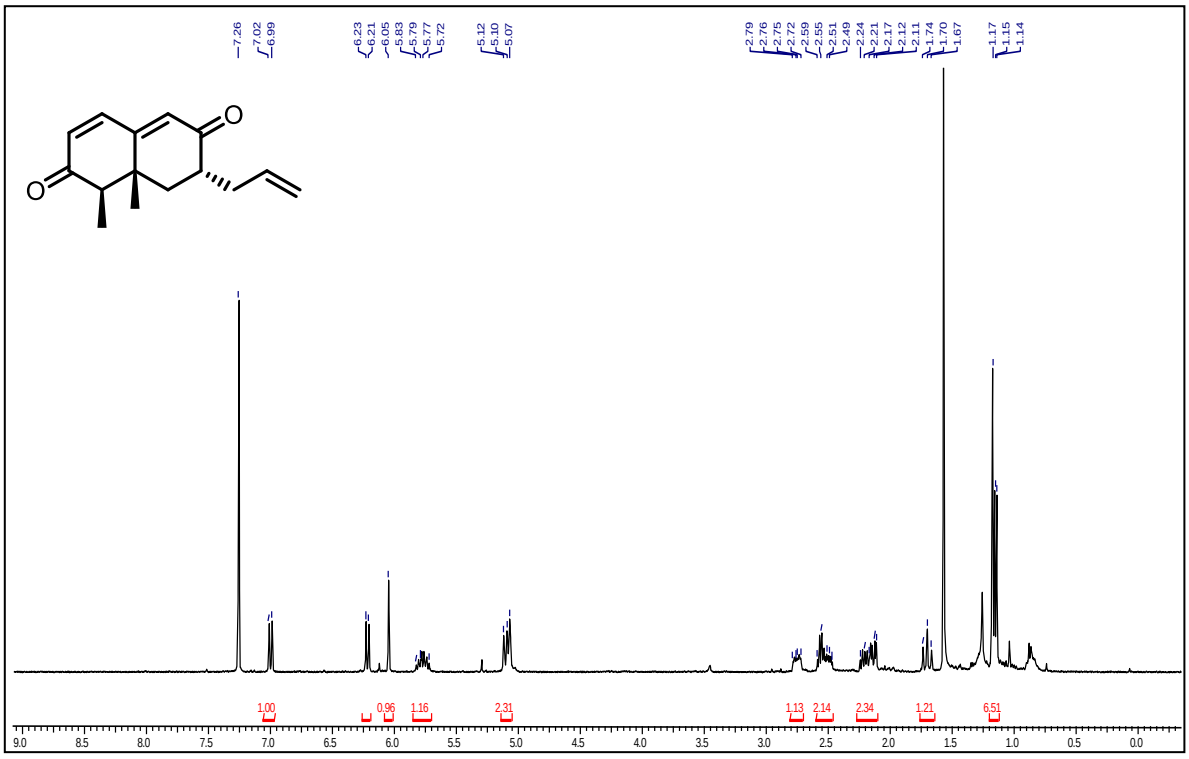


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 26



^{1}H NMR ($CDCl_3$, 400 MHz) of compound 28



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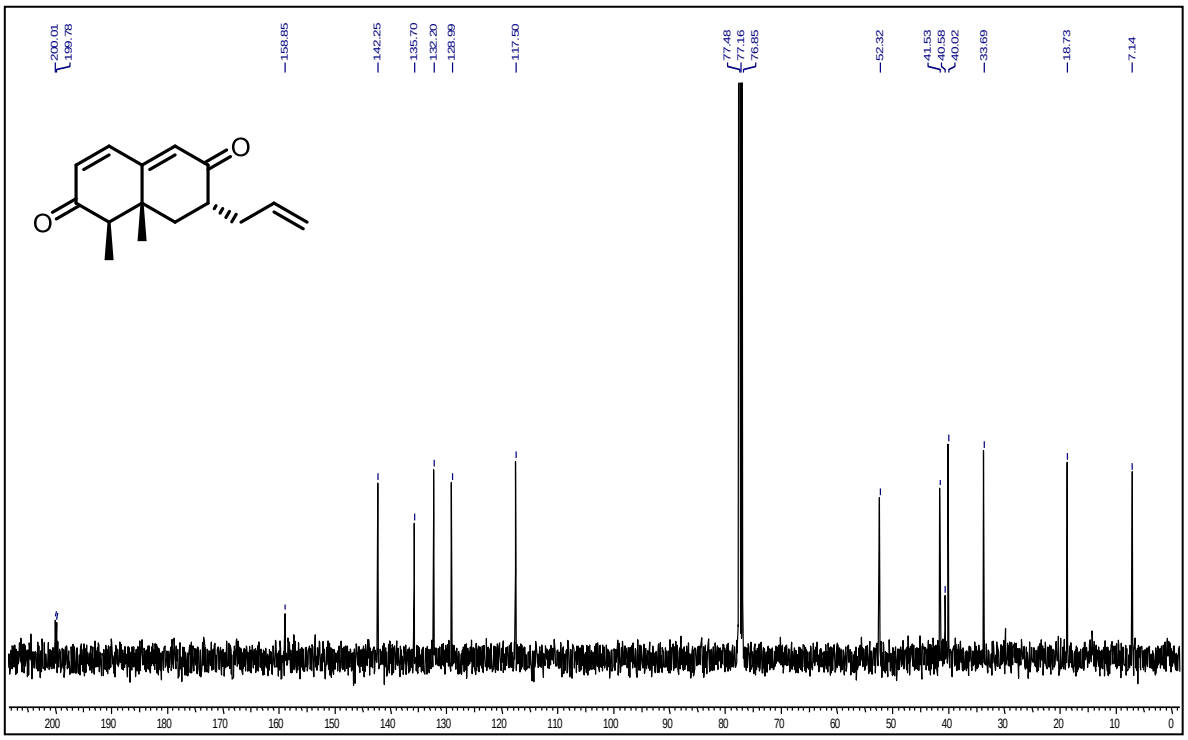

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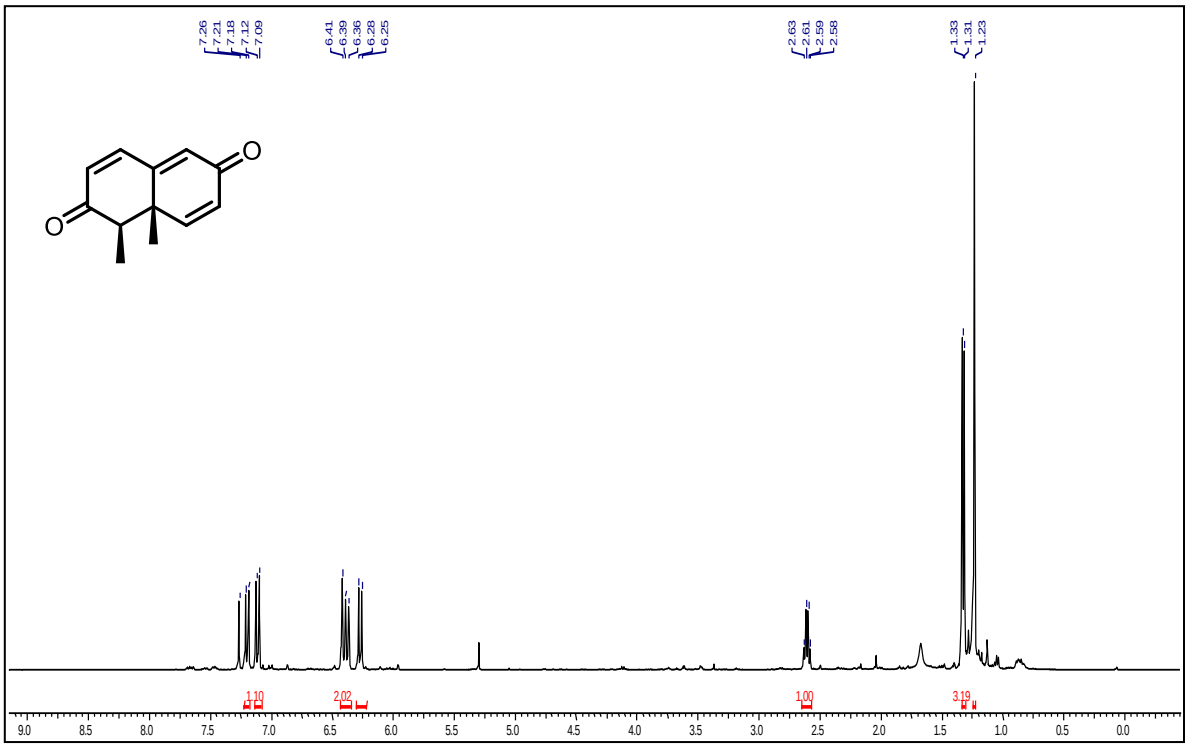

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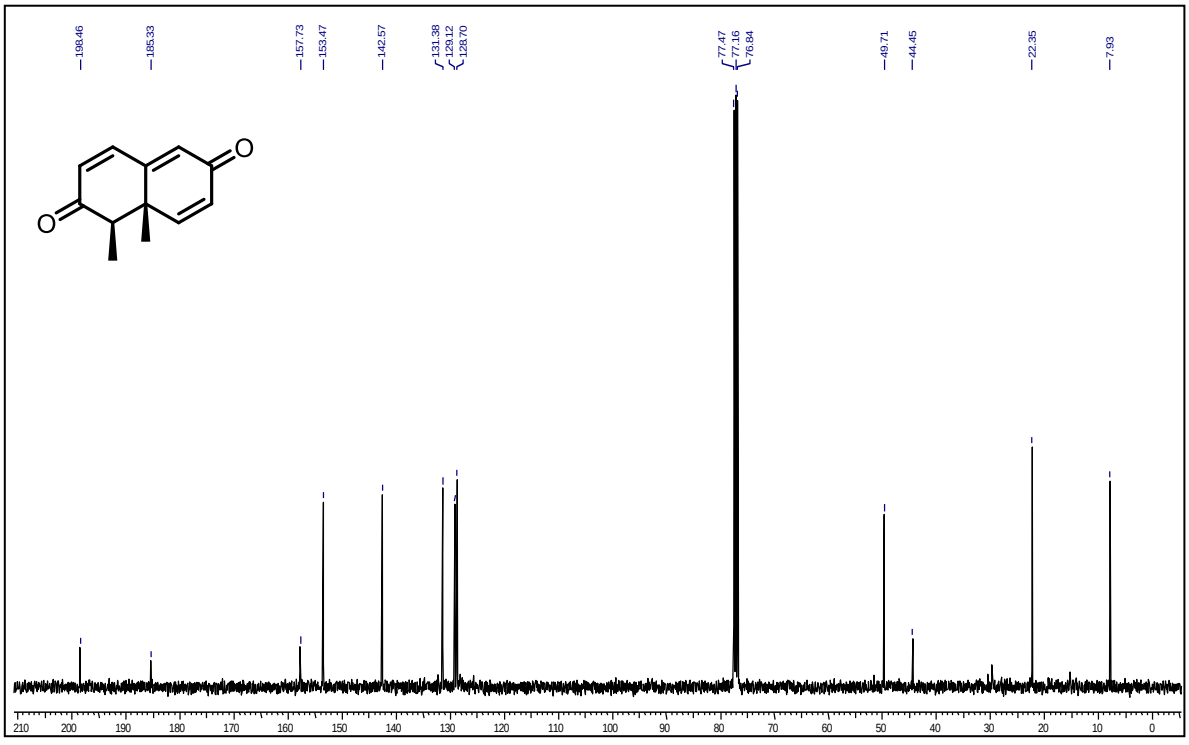

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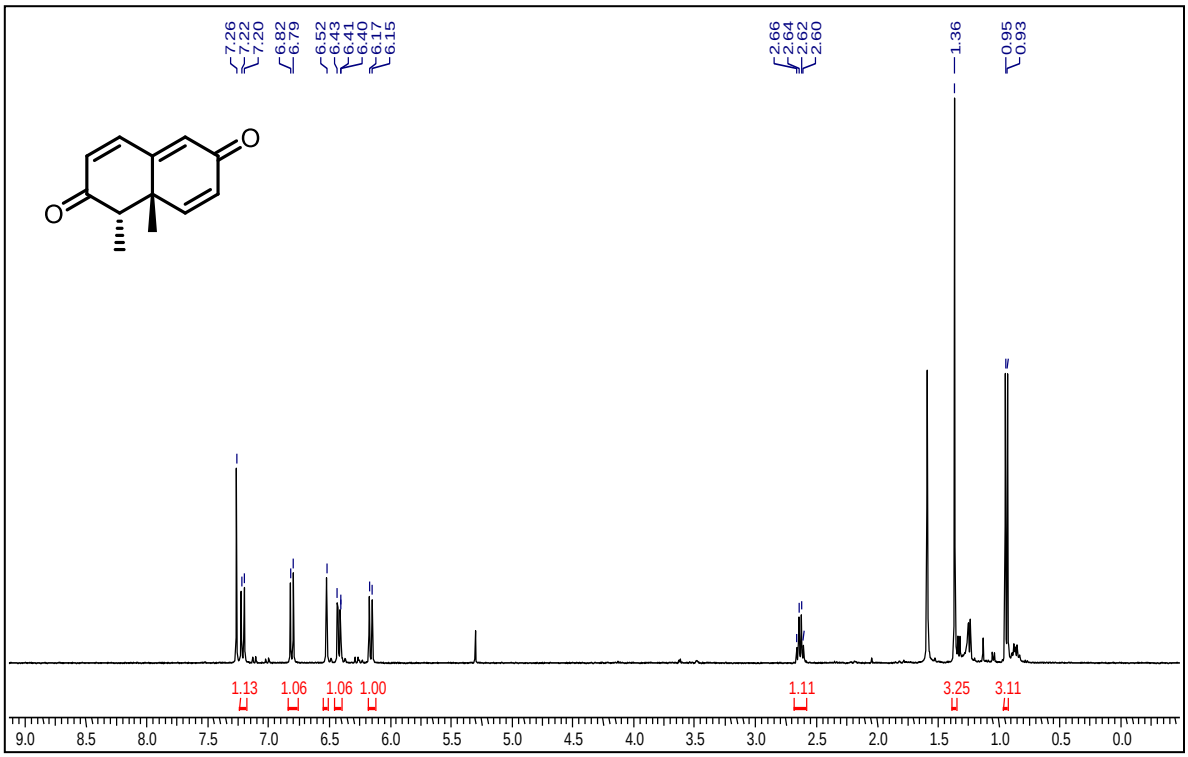
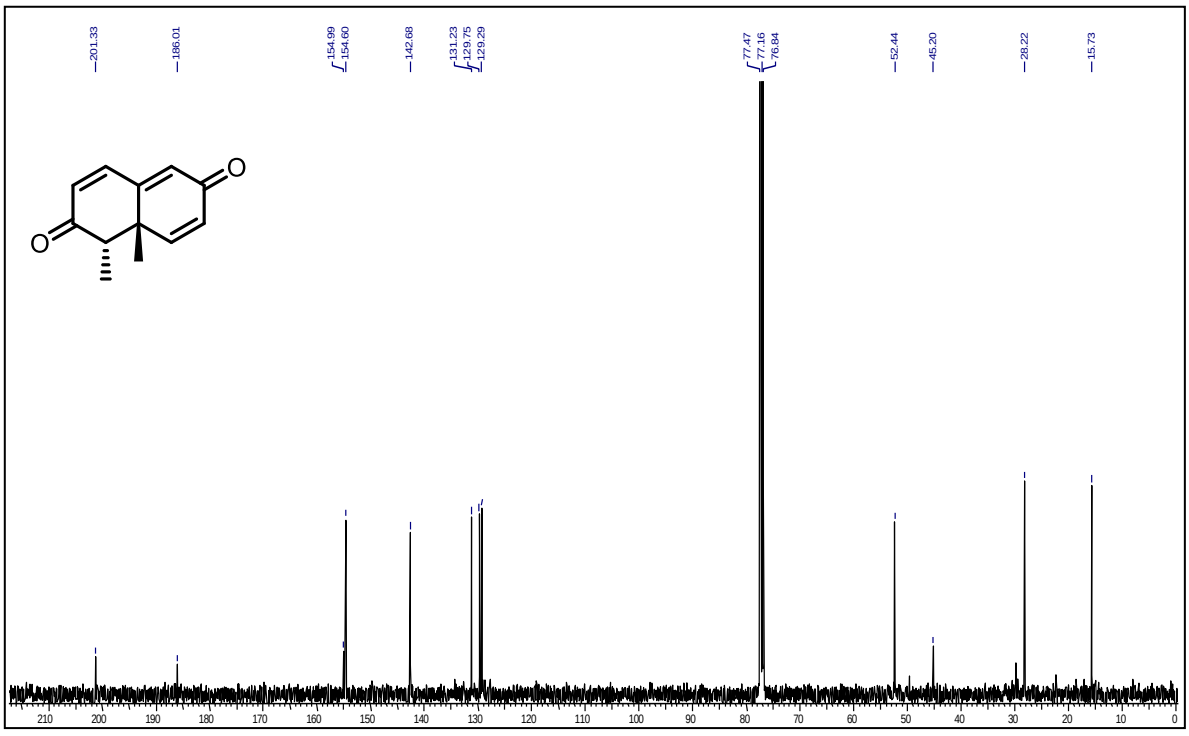


^{13}C NMR ($CDCl_3$, 100 MHz) of compound 30



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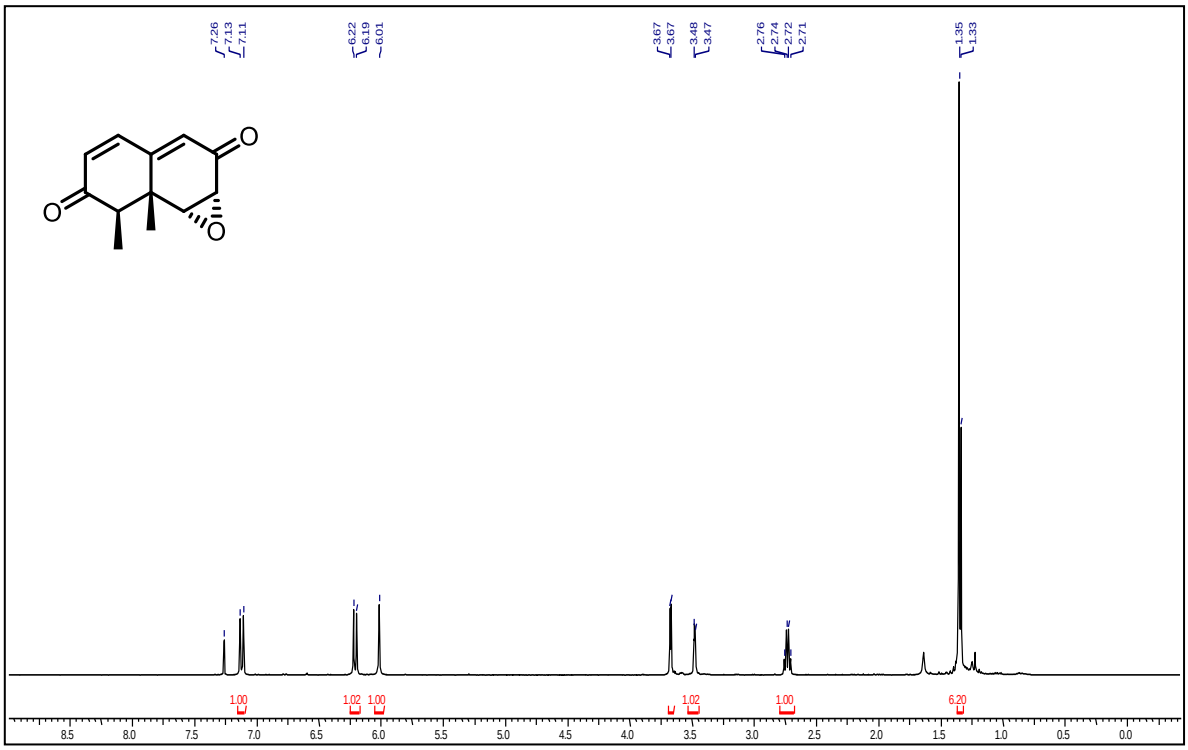

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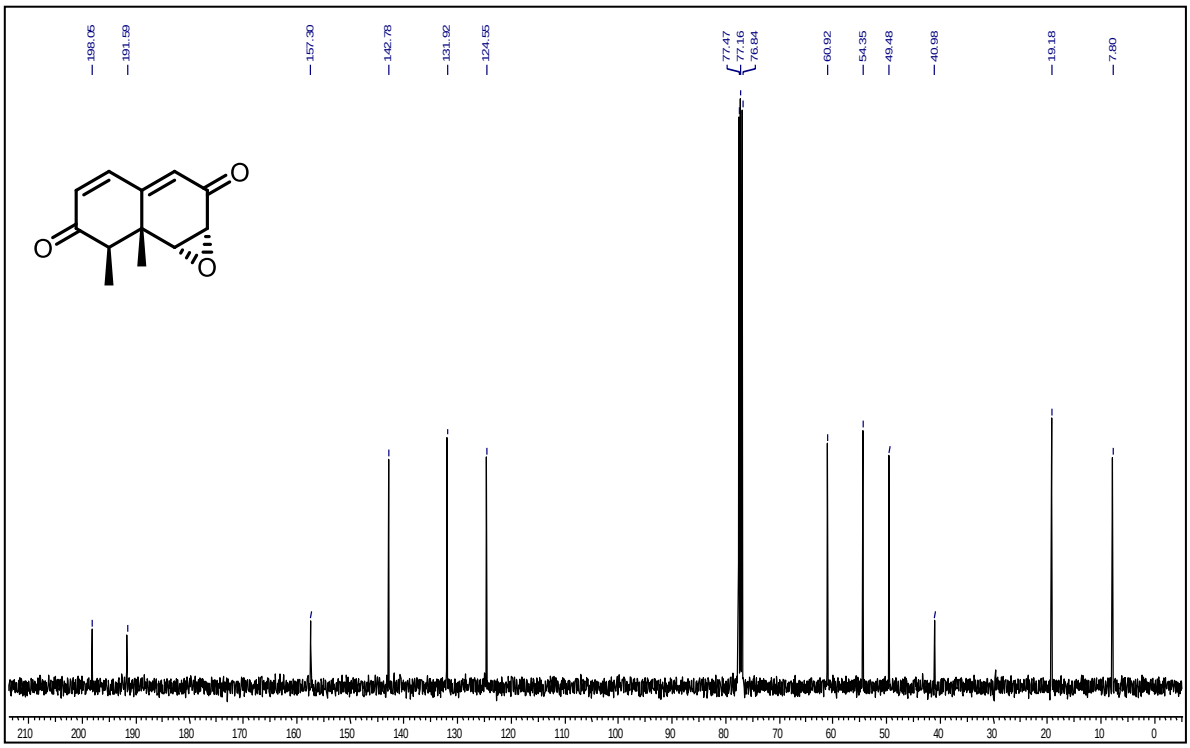
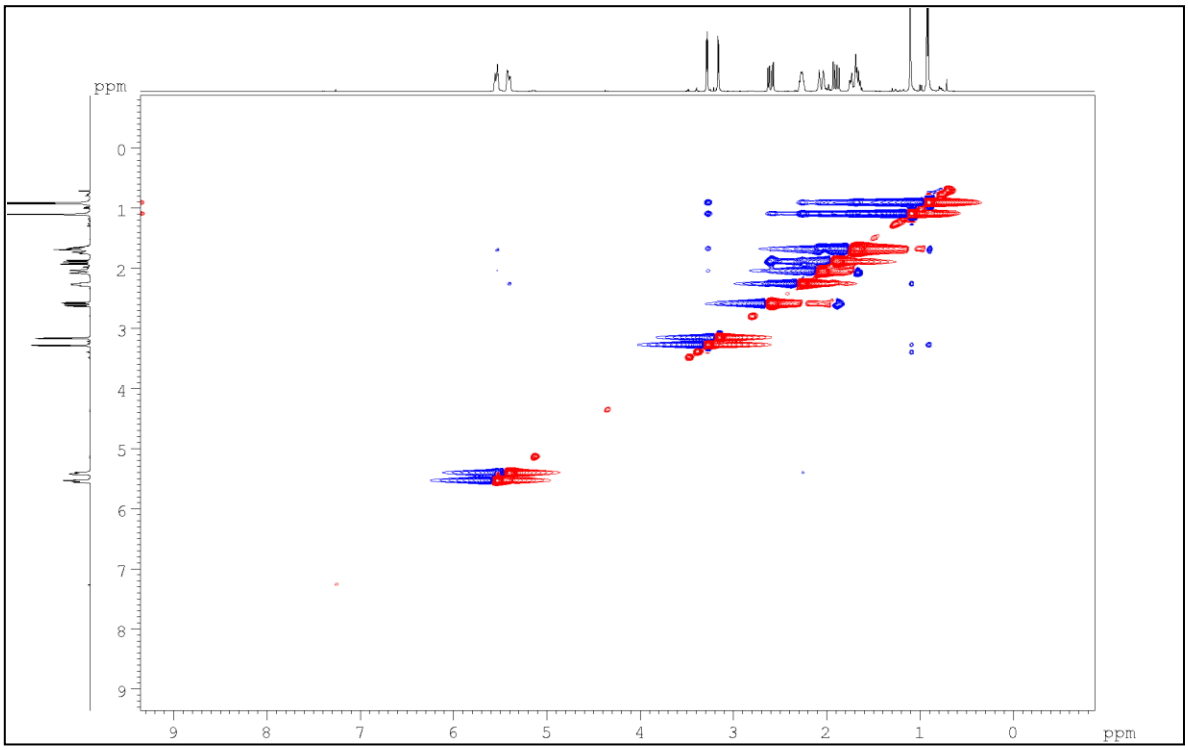
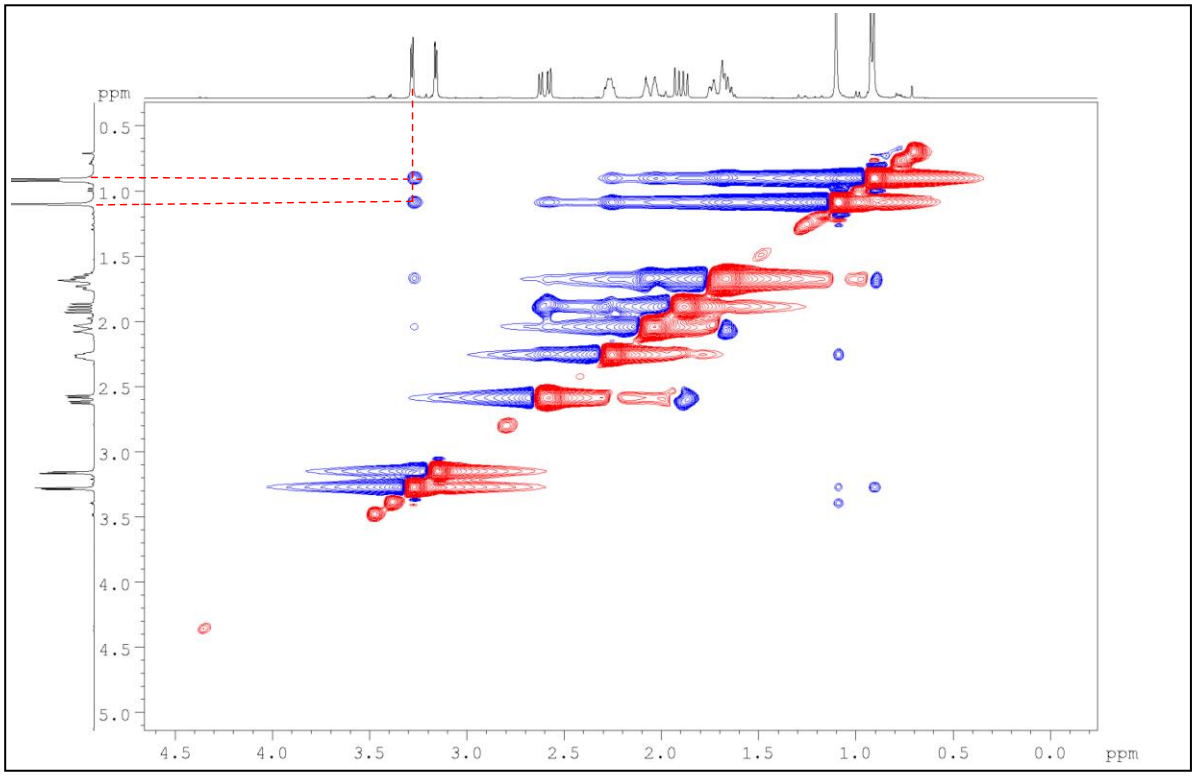


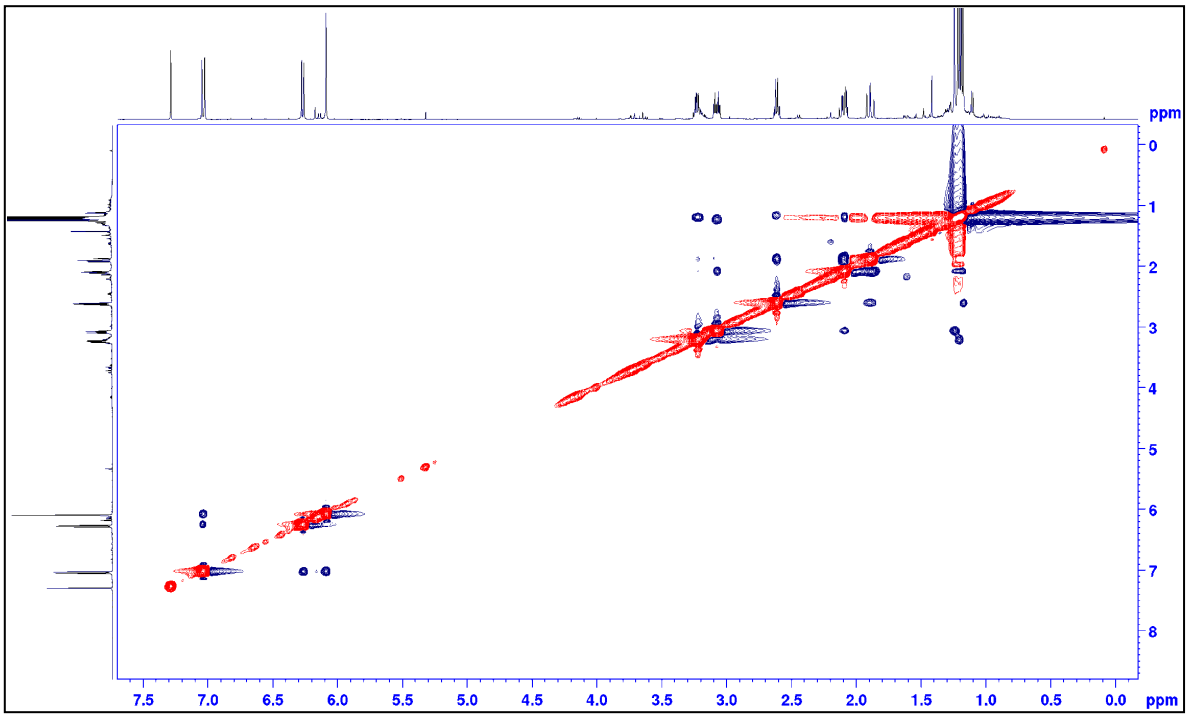
NOESY Spectrum (400 MHz) of 12 in $CDCl_3$



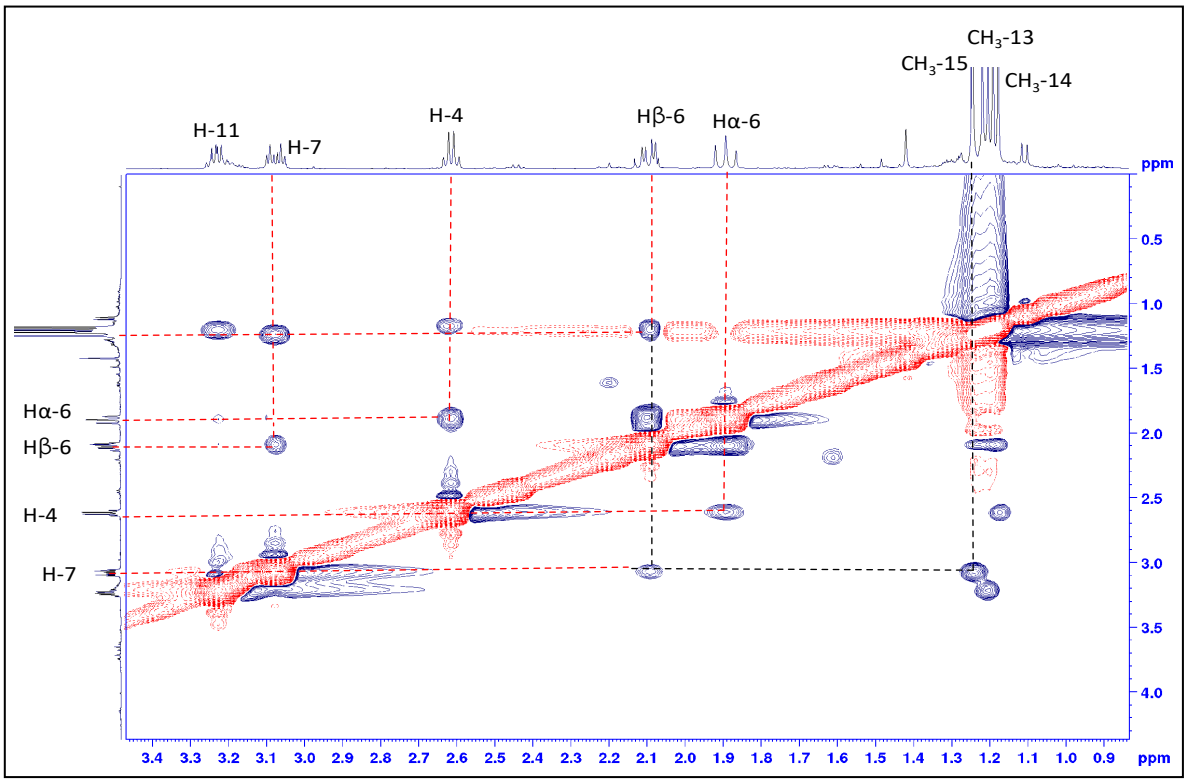
Enlarged NOESY Spectrum (400 MHz) of 12 in $CDCl_3$



NOESY Spectrum (400 MHz) of 22 in $CDCl_3$



Enlarged NOESY Spectrum (400 MHz) of 22 in $CDCl_3$



List of Publications and Patents

1. A Diverted Total Syntheses of Potent Cell Adhesion Inhibitor Peribysin E Analogues. **Kishor L. Handore** and D. Srinivasa Reddy, *Org. Lett.*, **2013**, *15*, 1894–1897.
2. Ready Access to Functionally Embellished *cis*-Hydrindanes and *cis*-Decalins: Protecting group-Free Total Syntheses of (±)-Nootkatone and (±)-Noreremophilane. **Kishor L. Handore**, B. Seetharamsingh and D. Srinivasa Reddy, *J. Org. Chem.*, **2013**, *78*, 8149–8154.
3. Total Synthesis of (±)-Nardoaristolone B and Its Analogues. **Kishor L. Handore** and D. Srinivasa Reddy, *Org. Lett*., **2014**, *16*, 4252–4255.
4. Total Syntheses and Biological Evaluation of (±)-Botryosphaeridione, (±)-Pleodendione, 4-*epi*-Periconianone B and Analogs. **Kishor L. Handore**, Prakash D. Jadhav, Bibhabasu Hazra, Anirban Basu and D. Srinivasa Reddy. *ACS Med. Chem. Lett.*, **2015**, *6*, 1117–1121.
5. Identification of Noreremophilane-Based Inhibitors of Angiogenesis using Zebrafish Assays. Kalai Mangai Muthukumarasamy,* **Kishor L. Handore**,* Dipti N. Kakade, Madhuri Shinde, Shashi Ranjan, Naveen Kumar, Seema Sehrawat, Chetana Sachidanandan and D. Srinivasa Reddy. *Org. Biomol. Chem.*, **2016**, *14*, 1569–1578. (*equal contribution, *cover page article*).
6. A Total Synthesis of (–)-Nardoaristolone B. Rohini Ople, **Kishor L. Handore**, Nidhi Kamat and D. Srinivasa Reddy. *Eur. J. Org. Chem.*, **2016**, 3804–3808.
7. A Solution-Phase Synthesis of Macrocyclic Core of Teixobactin. Santu Dhara,* Vidya B. Gunjal,* **Kishor L. Handore*** and D. Srinivasa Reddy. (*equal contribution). (*Eur. J. Org. Chem.*, **2016**, 4289–4293.
8. Anticancer Compounds and Process for the Preparation thereof. D. Srinivasa Reddy, **Kishor Handore,** *WO2014128723 A2.*
9. Insect Repellents. D. Srinivasa Reddy, **Kishor Handore**, B. Seetharamsingh, Avalokiteswar Sen, P.V. Pawar, M. Joseph, *WO 2014170915 A1*.
10. Tricyclic Compounds and Process for Preparation thereof. D. Srinivasa Reddy, **Kishor Handore**, *WO2016013032A1*.

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A Diverted Total Syntheses of Potent Cell Adhesion Inhibitor Peribysin E Analogues

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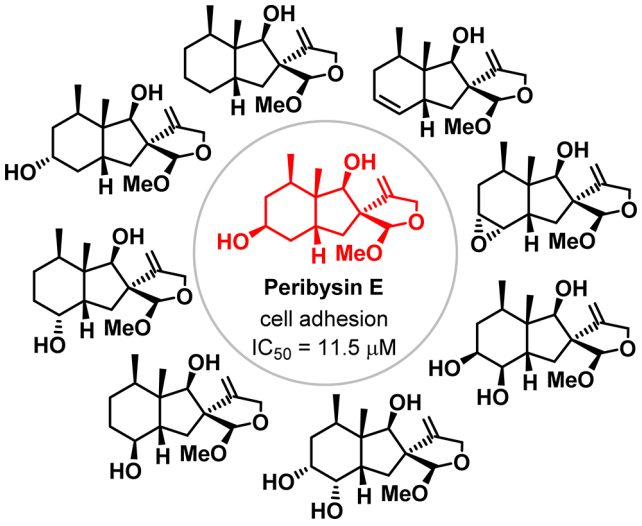
ABSTRACT



Preliminary results from a program aimed at the creation of a focused library of analogues around the natural product peribysin E, a potent biologically active and structurally fascinating molecule, are reported. The total synthesis of (±)-peribysin E was accomplished using a short route. Eight new analogues of the natural compound have been accomplished by means of "diverted total synthesis" in less than 10 steps. The present effort highlights protecting-group-free total syntheses and the shortest route to access these functionally embellished hydrindanes.

A group of natural products called peribysins (A−G) were isolated by Yamada's group from a strain of *Periconia byssoides* OUPS-N133 originally separated from the sea hare, *Aplysia kurodai*.[1] The structurally interesting natural products, especially peribysin E (**1**), attracted our attention toward developing anticancer and anti-inflammatory agents owing to its potent cell adhesion inhibitory activity (Figure 1).[2] It was claimed that the natural product (−)-peribysin E has shown more potent activity than the gold standard herbimycin **2** in the cell adhesion inhibition assay (Figure 1). Due to its attractive biological activity, coupled with intriguing structural features and scarcity of the material, compound **1** has already attracted the attention of groups such as Danishefsky and Sha. Danishefsky and co-workers achieved the first total synthesis of peribysin E which helped in reassigning the absolute configuration of the natural product. In their elegant synthesis, the Diels−Alder reaction followed by semipinacol-type ring contraction served to secure the stereochemistry of peribysin E (**1**).[3] In another interesting effort, Sha's group executed the synthesis of racemic peribysin E by using α-carbonyl radical cyclization as a key step.[4] Here, we disclose the synthesis of several novel analogues of peribysin E through "diverted total synthesis"[5] in less than 10 steps starting from readily accessible materials (Figure 1).

(1) Yamada, T.; Doi, M.; Miura, A.; Harada, W.; Hiramura, M.; Minoura, K.; Tanaka, R.; Numata, A. *J. Antibiot.* **2005**, *58*, 185.

(2) The cell adhesion inhibition was measured in leukemia HL-60 cells to human-umbilical-vein endothelial cells (HUVEC).

(3) Angeles, A. R.; Dorn, D. C.; Kou, C. A.; Morre, M. A. S.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 1451. (b) Angeles, A. R.; Waters, S. P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 13765.

(4) Lee, H.-Y.; Sha, C.-K. *J. Org. Chem.* **2012**, *77*, 598.

10.1021/ol400555m
Published on Web 04/04/2013

JOC The Journal of Organic Chemistry

Note

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Ready Access to Functionally Embellished *cis*-Hydrindanes and *cis*-Decalins: Protecting Group-Free Total Syntheses of (±)-Nootkatone and (±)-Noreremophilane†

Kishor L. Handore, B. Seetharamsingh, and D. Srinivasa Reddy*

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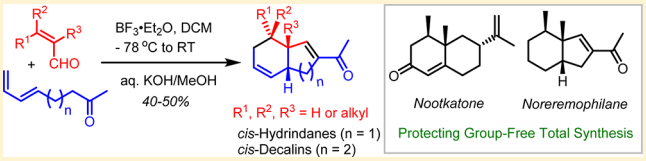
Supporting Information

ABSTRACT: A simple and efficient synthesis of functionalized *cis*-hydrindanes and *cis*-decalins was achieved using a sequential Diels–Alder/aldol approach in a highly diastereoselective manner. The scope of this method was tested with a variety of substrates and was successfully applied to the synthesis of two natural products in racemic form. The highlights of the present work provide ready access to 13 new *cis*-hydrindanes/*cis*-decalins, a protecting group-free total synthesis of an insect repellent Nootkatone, and the first synthesis of a Noreremophilane using the shortest sequence.



Natural products based entirely on the *cis*-hydrindane/*cis*-decalin skeleton or embodying this system as the core unit in their gross structures are frequently encountered in the literature. Many of these compounds exhibit interesting biological activities and are endowed with various functionalities and stereochemical patterns. All of these features aroused considerable interest in the synthetic community (Figure 1).[1] Along these lines, one of us (D.S.R.) recorded a simple method to access the *cis*-hydrindane skeleton using a Diels–Alder/aldol sequence in a highly diastereoselective manner and applied it to the synthesis of bakkenolide A in the year 2004.[2] Later, it was used for the synthesis of other natural products.[3] Here, we would like to report a fresh extension of this work to access several new *cis*-hydrindanes/*cis*-decalins and a short synthesis of two natural products starting from readily accessible materials.

Retrosynthetically, natural products based on the *cis*-hydrindane/*cis*-decalin skeleton could be constructed from the key intermediates, such as *cis*-hydrindane (**A**) or *cis*-decalin (**B**), which possess chemically differentiated double bonds. The intermediates **A** and **B** could be prepared by reacting appropriate dienophiles **C** with dienes **1** and **2**, respectively, using a previously developed Diels–Alder/aldol sequence, (Scheme 1). The starting components **1**, **2**, and **C** are commercially available or can be prepared using known literature procedures.

Our fresh exploration began with a $BF_3{\cdot}Et_2O$ mediated intermolecular Diels–Alder reaction[4] between diene **1**[2] and 2.5 equiv of dienophile methacrolein. Although the reaction works well with $MeAlCl_2$, we preferred using $BF_3{\cdot}Et_2O$ because of availability and safety. The crude Diels–Alder adduct was subjected to an intramolecular aldol condensation reaction with 15% aq KOH in MeOH to furnish *cis*-hydrindane **3** in a highly diastereoselective fashion (dr ~ 98:2) with a moderate yield of 53%. Similarly, *cis*-decalin **4** was prepared by the reaction between **2**[5] and methacrolein in 50% yield with a dr ~ 98:2 ratio.[6] The *cis*-hydrindane **5** and *cis*-decalin **6** were prepared from tiglic aldehyde by reacting with diene **1** and **2**, respectively. The diastereomeric ratio was found to be ~95:5 in both cases. This methodology was successfully applied for the construction of various *cis*-hydrindanes/*cis*-decalins, and the details are compiled in Scheme 2. The observed diastereo- and regioselectivity can be explained on the basis of secondary orbital interactions and atomic coefficient preferences, respectively.[7] Accordingly, the stereochemistry was assigned to major isomers of all the hydrindanes (**3**, **5**, **7**, **9**, **11**, **13**, **15**) and decalins (**4**, **6**, **8**, **10**, **12**, **14**, **16**), as shown in drawings (Scheme 2).[8] The assigned *cis*-stereochemistry of the ring junction in compounds **7**, **8**, **9**, and **10** was further confirmed by 2D-NMR analysis to exclude any possibility of epimerization before the aldol condensation.[8] In the majority of hydrindane cases, the observed diastereoselectivity was high compared with that of decalin cases. In the case of hydrindanes **13** and **15**, low diastereoselectivity was observed, which may be explained by the presence of bulky substitution at α- and β-positions of the dienophile. It is worth mentioning that all of these compounds can be subjected to selective functional group transformations and stereoselective manipulations as they possess chemically differentiated double bonds present in two different rings and a rigid framework.

To enhance the utility of this simple method, we have taken up the protecting group-free total synthesis of Nootkatone, a popular natural product for several years. (+)-Nootkatone is a sesquiterpene first isolated from the heartwood of Alaskan yellow cedar (*Chamaecyparis nootkatensis*) and was later found in trace amounts in grapefruit (*Citrus paradise*), pummelo (*Citrus grandis*), and vetiver oil (*Vetiveria zizanioides*).[9] In addition to its applications in the flavor and fragrance fields, (+)-Nootkatone possesses very impressive insect repellent and/or insecticidal activity against various ticks, mosquitos, termites,

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Total Synthesis of (±)-Nardoaristolone B and Its Analogues

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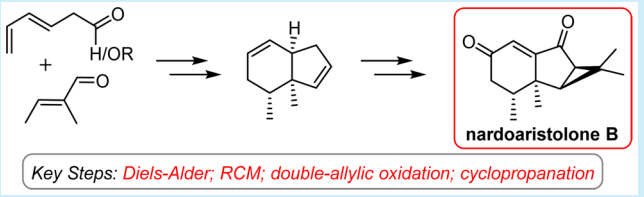
Supporting Information

ABSTRACT: The first total synthesis of nardoaristolone B, a nor-sesquiterpenoid with an unusual fused ring system and having protective effects on the injury of neonatal rat cardiomyocytes, has been accomplished. Stereoselective synthesis of its novel analogues inlcuding *exo*-cyclopropyl ring fusion is also part of this disclosure. In addition, an alternate and more efficient one-step method to make a 3/5/6 tricyclic ring system using the Robinson annulation method has been developed toward the generation of a library of compounds around this skeleton.



Very recently, the nardoaristolone B (**1**) natural product was isolated from the underground parts of *Nardostachy chinensis* plants[1] which have been used as sedative and analgesic agents in traditional Chinese medicine for centuries (Figure 1).[2] The structure elucidation of **1** was carried out using various

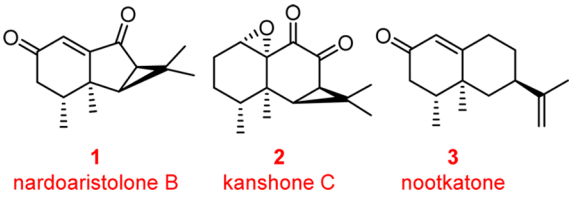
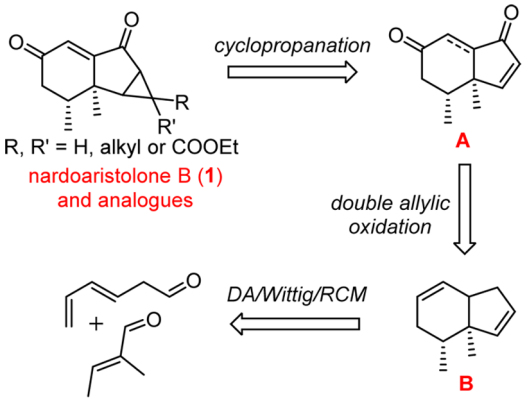


Figure 1. Structures of natural products.

spectral and X-ray diffraction methods, and it is believed to be biogenetically derived from kanshone C (**2**).[1] The nardoaristolone B has shown protective effects on the injury of neonatal rat cardiomyocytes in a dose-dependent manner.[1] In view of its potential biological activity and a rare skeleton with a 3/5/6 tricyclic fused ring system, nardoaristolone B (**1**) attracted our attention and this program was initiated. It is also worth highlighting that the structure of **1** is close to that of nootkatone (**3**), an interesting natural product with effective insect repellent/insecticidal activity[3] and AMPK (Adenosine Monophosphate Kinase) activity.[4] As the structural features are similar to that of nootkatone **3**, compound **1** and its analogues are expected to show a range of biological activities.[5] The synthesis of the nardoaristolone B and its close analogues are described here.

The retrosynthetic analysis is shown in Scheme 1. Nardoaristolone B (**1**) and its analogues could be prepared using stereoselective cyclopropanation of dienone **A**. The dienone **A** could be prepared from diene **B** through double allylic oxidation. The requisite hydrindane scaffold **B** could be constructed from tiglic aldehyde, and the appropriate diene, through a sequence of Diels–Alder (DA), Wittig, and ring-closing metathesis (RCM) reactions.

Scheme 1. Retrosynthetic Analysis



The synthesis began with a borontrifluoride-mediated Diels–Alder reaction between the diene **4**[6] and tiglic aldehyde to provide the Diels–Alder adduct,[7,8] which was immediately subjected to one carbon Wittig reaction to give the desired diene **6** in poor yield (8%–10%) but with very high diastereoselectivity (>9:1). The observed low yield could probably be due to inter/intramolecular condensation of both starting aldehydes. After a few trials, the same transformation was achieved in ∼41% overall yield by replacing the diene **4** with more stable diene **5**[9] and by the addition of two more steps (DIBAL-H reduction and Wittig reaction). The DA reaction of **5** also produced high diastereoselectivity (>9:1) as in the case of **4**. The Lewis acid mediated intermolecular Diels–Alder reaction produces the *endo*-adduct having both arms on the same side. The diastereo- and regioselectivity can be explained on the basis of secondary orbital interactions and atomic coefficient preferences, respectively.[10] The diene **6** was subjected to ring-closing metathesis (RCM) using the Grubbs'

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Total Syntheses and Biological Evaluation of (±)-Botryosphaeridione, (±)-Pleodendione, 4-*epi*-Periconianone B, and Analogues

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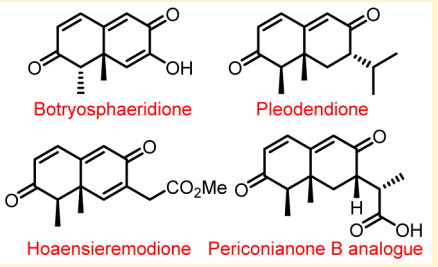
Supporting Information

ABSTRACT: The total syntheses of (±)-botryosphaeridione, (±)-pleodendione, (±)-hoaensieremodione, 4-*epi*-periconianone B, and their analogues have been accomplished for the first time. All the synthesized target compounds were screened in neural anti-inflammatory assays using LPS induced microglia cells (N9). Among them, compounds **1** and **21** were identified as potential lead compounds for further profiling.



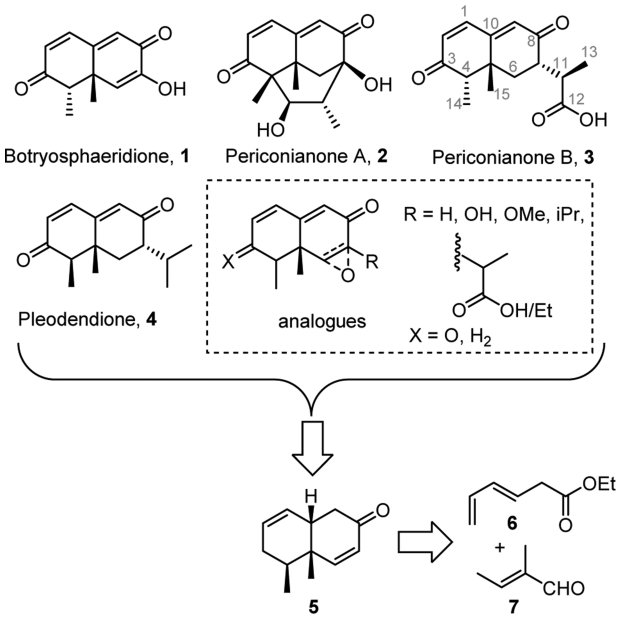
KEYWORDS: *Natural product, total synthesis, antineuroinflammatory agents, botryosphaeridione, pleodendione*

The bioassay-guided fractionation of the ethyl acetate extract from the fermentation broth of the endophytic fungus *Periconia sp.* F-31, which was derived from the medicinal plant *Annonsa muricata*, resulted in the isolation of the natural products called botryosphaeridione **1**, periconianone A **2**, and periconianone B **3** by the research group led by Jungui Dai.[1] Compound **1** and another closely related eremophilane-type sesquiterpene pleodendione **4** were isolated previously.[2,3] The structures and absolute configurations were established by extensive spectroscopic analyses, ECD calculations, and single-crystal X-ray diffraction as shown in Scheme 1. Compounds **1**–**3** were reported to have shown neural anti-inflammatory activity (mouse microglia BV2 cells) with impressive IC_{50} values of 0.23, 0.15, and 0.38 μM, respectively.[1] This interesting biological activity suggests that dihydro-, tetrahydro-naphthalene-2,6-dione scaffolds can be promising lead structures for the treatment of CNS disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD).[4,5] Therefore, we became interested in this novel chemotype and synthesized several compounds and tested their neural anti-inflammatory potential.

The retrosynthetic analysis for the target compounds is shown in Scheme 1. The target compounds and their closely related analogues could be prepared from the key dienone intermediate **5** through a series of steps viz. alkylation of the dienolate, epoxidation, allylic oxidation, and base-mediated ring opening of epoxide. The key intermediate enone **5** could be prepared from diene **6** and tiglic aldehyde **7** through a Diels–Alder chemistry,[6] which is extensively practiced in our group, followed by a few functional group transformations including Wittig, Grignard, and ring-closing metathesis (RCM) reactions.

The present synthesis began with the aldehyde **8**, which was previously reported from our group[7] through a sequence of $BF_3{\cdot}Et_2O$-mediated Diels–Alder reaction,[8,9] Wittig reaction, and DIBAL-H reduction. The aldehyde **8** on a Grignard

Scheme 1. Structures of Targets and Retrosynthetic Plan



reaction, using vinylmagnesium bromide, gave an alcohol that was subjected to RCM[10] to obtain the *cis*-fused decalin, which was further oxidized to the desired enone **5** in overall 44% yield. The conjugated double bond in enone **5** was subjected to selective epoxidation (H_2O_2–NaOH) to produce **9** in 71% yield. Assigned stereochemistry of the epoxide in compound **9**

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Identification of noreremophilane-based inhibitors of angiogenesis using zebrafish assays†

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Noreremophilanes are a rare class of *cis*-hydrindanes produced by genus *Ligularia* herbaceous plants which are known to exhibit interesting biological activities. We synthesized *cis*-hydrindanes based on a naturally occurring noreremophilane scaffold using a Diels–Alder/aldol sequence and screened them for multiple biological activities using high-content zebrafish embryonic development assays. We discovered a noreremophilane that has strong anti-angiogenic effects on the developing zebrafish embryos as well as on tumor-induced angiogenesis in a zebrafish xenograft model. We synthesized several derivatives of this class of noreremophilanes and performed structure–activity relationship studies in zebrafish to identify more potent and less toxic analogs of the original structure.

Introduction

Nature is an abundant and underexplored source of small molecules with a variety of bioactivities. Many of these small molecules when taken out of their context and applied on live cells yield unexpected and potentially useful activities.[1] Among natural products certain scaffolds are found to reoccur in several species, but studying their activities remains a challenge due to the miniscule quantities that can be extracted from their natural source. Moreover, the discovery of new bioactivities necessitates multiple assays to hunt for therapeutically important activities. Here we circumvent these challenges by synthesizing derivatives of natural-product-based scaffolds and testing them using whole-organism assays in zebrafish embryos. Zebrafish has emerged as an ideal model organism that is accessible for high throughput assays while being complex enough to model the vertebrate biology.[2,3] A number of recent studies have illustrated the power of whole-organism screening in zebrafish to identify unexpected bioactivities of small molecules.[4,5]

The *cis*-hydrindane backbone has been extensively used in nature, in particular in sesquiterpenoids.[6] The *cis*-hydrindane motifs are found in many natural products with known biological activities.[6,7] For example, peribysin-E **1** (Fig. 1), isolated from marine organisms was shown to be a potent cell adhesion inhibitor with potential for use as anti-inflammatory and anti-cancer agents.[8] Bakkenolides **2** and **3** are another interesting family of natural products (Fig. 1) with multiple bioactivities.[9,10] Similarly noreremophilane-type sesquiterpenes **4–6** also contain the *cis*-hydrindane framework which were isolated from the roots of *Ligularia* herbaceous plants. Some of these have attracted our attention previously due to

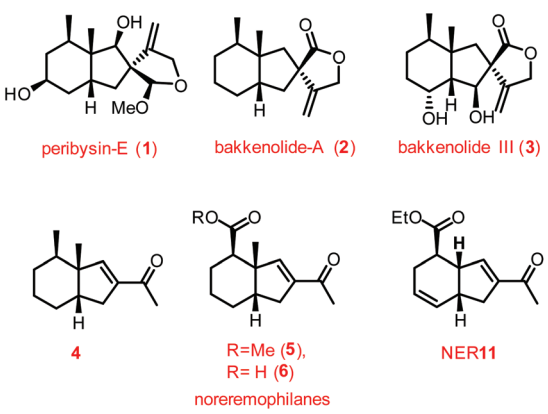

Fig. 1 Selected natural products with the *cis*-hydrindane skeleton.

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†Electronic supplementary information (ESI) available: Characterization data, NMR spectra, detailed experimental procedures, and CIF file of X-ray crystal structures. CCDC 1009597. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c5ob01594d

‡Both authors contributed equally.

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Full Paper

Natural Product Synthesis

A Total Synthesis of (–)-Nardoaristolone B

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Abstract: A stereoselective total synthesis of (–)-Nardoaristolone B, a nor-aristolane sesquiterpenoid natural product with an unusual 3/5/6 tricyclic ring system is described. The highlights of the present work includes use of (+)-(*R*)-Pulegone as a chiral-pool starting material, ring-closing metathesis, allylic oxidation and stereoselective cyclopropanation. In addition, a new analogue of Nardoaristolone B (minor product from the final step) was isolated in pure form and fully characterized with the help of single-crystal X-ray analysis.

Introduction

Sesquiterpenoids are a class of terpenoids that consist of three isoprene units followed by biochemical modifications such as oxidation or rearrangement. Isolation of such a sesquiterpenoid natural product, Nardoaristolone B (**1**), was reported in 2013 by Yao and co-workers from the underground parts of the *Nardostachys chinensis* plant.[1a] The investigation of this particular plant suggests that it is a source of a series of natural products such as aristolane,[1b] nardosinane,[1c–1e] guaiane types,[1f,1g] lignans,[1h] sesquiterpenoids, debilon,[1i] and kanshone A.[1d] The roots and rhizomes of *Nardostachys chinensis* Batalin (*Valerianaceae* family) have been used as stomachic and sedative agents[2a] in Chinese traditional medicine for centuries.[2b] In addition, it exhibits antimalarial, antinociceptive,[1g] and cytotoxic activities[1i] and also helps to enhance the nerve growth factor.[2c] The natural product Nardoaristolone B isolated from the same species exhibits a protective activity on the injury of neonatal rat cardiomyocytes in a dose-dependent manner and is believed to be biogenetically derived from Kanshone C (**4**).[1a] This compound is also expected to have many interesting biological activities such as 5′-adenosine monophosphate activated protein kinase (AMPK) activation, insect repellent, and insecticidal activities due to its structural resemblance to that of Nootkatone (**5**).[2d–2h] In fact, in our hands, compound **1** in recemic form showed excellent mosquito-repellent activity against the *Aedes* mosquito, which is the vector for Dengue fever and the Zika virus.[3] Isolation of the closely related sesquiterpene (–)-Aristolone (**6**)[4] was reported in 1955 from the roots of *Aristochia debilis*, and its several syntheses were reported in the literature.[5] There are a few more natural products reported in the literature like Lindenene (**2**)[6] and Chloranthalactone A (**3**),[7] having a similar 3/5/6 structural core as Nardoar-

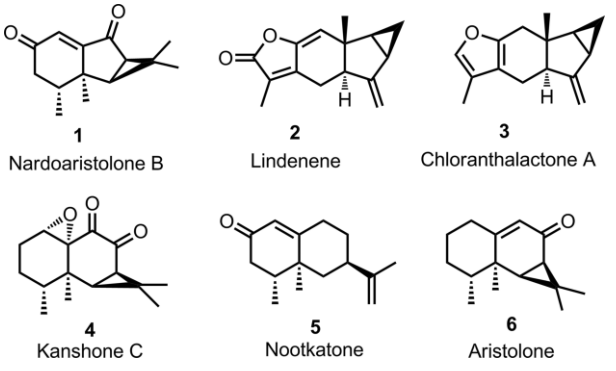

Figure 1. Structures of selected natural products.

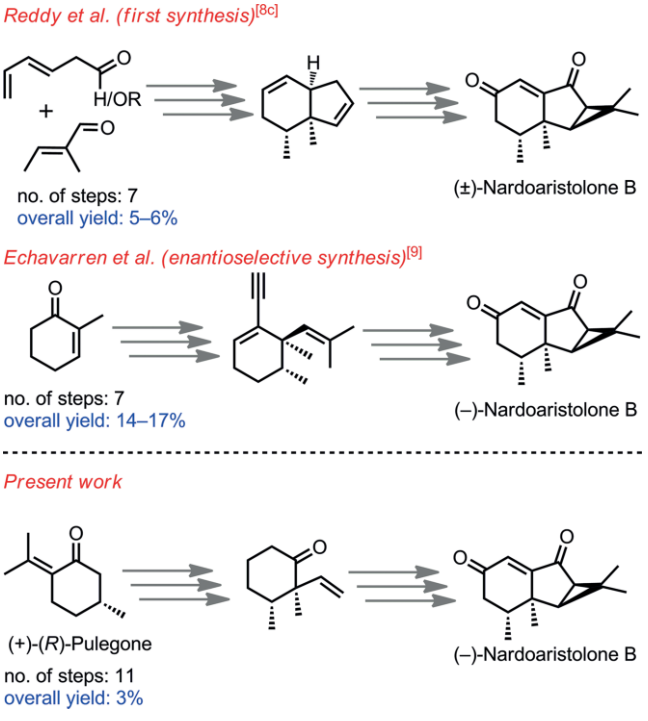

Figure 2. Previous and present synthetic approaches.

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Communication

Macrocycles

Solution-Phase Synthesis of the Macrocyclic Core of Teixobactin

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Abstract: Towards the total chemical synthesis of the exceptionally potent antibiotic teixobactin, we synthesized the macrocyclic core of a natural product by using a solution-phase approach. The gramscale synthesis of L-*allo*-enduracididine and Shiina macrolactonization are highlights of the present disclosure.

Introduction

Humanity continues to suffer as bacteria continue to develop resistance against known antibiotic drugs.[1,2] The increasing costs of drug development and bacterial resistance are limiting antibiotic research (in particular, in pharma companies).[2] As a consequence, there is an urgent need for more efforts to develop antibacterial drugs, in particular, to identify novel antimicrobial compounds with distinct modes of action. Since the discovery of penicillin by Alexander Fleming in 1929,[3] mankind has benefited from a variety of natural-product-based antibacterials.[4] Very recently, a Nature publication reported the discovery and pharmacological characterization of teixobactin (**1**) as a potential antibiotic of the future (Figure 1).[5] This compound has received the attention of scientific and social media across the world with the buzzword "super-antibiotic".[6] Teixobactin is an 11-amino-acid macrocyclic depsipeptide that was isolated from uncultured bacteria by using sophisticated iChip technology.[5a] Compound **1** displays an exceptional activity profile against various bacterial pathogens including *Mycobacterium tuberculosis* and *Staphylococcus aureus*. Lewis and coworkers established that teixobactin interacts with the precursors of peptidoglycan and teichoic acid of the cell wall by binding to the pyrophosphate sugar moiety of these molecules.[6a] As the molecular targets of teixobactin are not endogenous proteins but are instead present across the outer membranes, it is suggested that any emergence of resistance to teixobactin will be very little or none. This is evident in view of the nonobservance of any drug resistance in mice that were infected with *S. aureus* or *M. tuberculosis* even after treatment with teixobactin for four weeks.[5a] The distinct pharmacological profile of teixobactin and interesting structural features with several unnatural amino acid residues attracted us towards this target compound. While we were working on this project and preparing the manuscript, four publications (three analogue syntheses

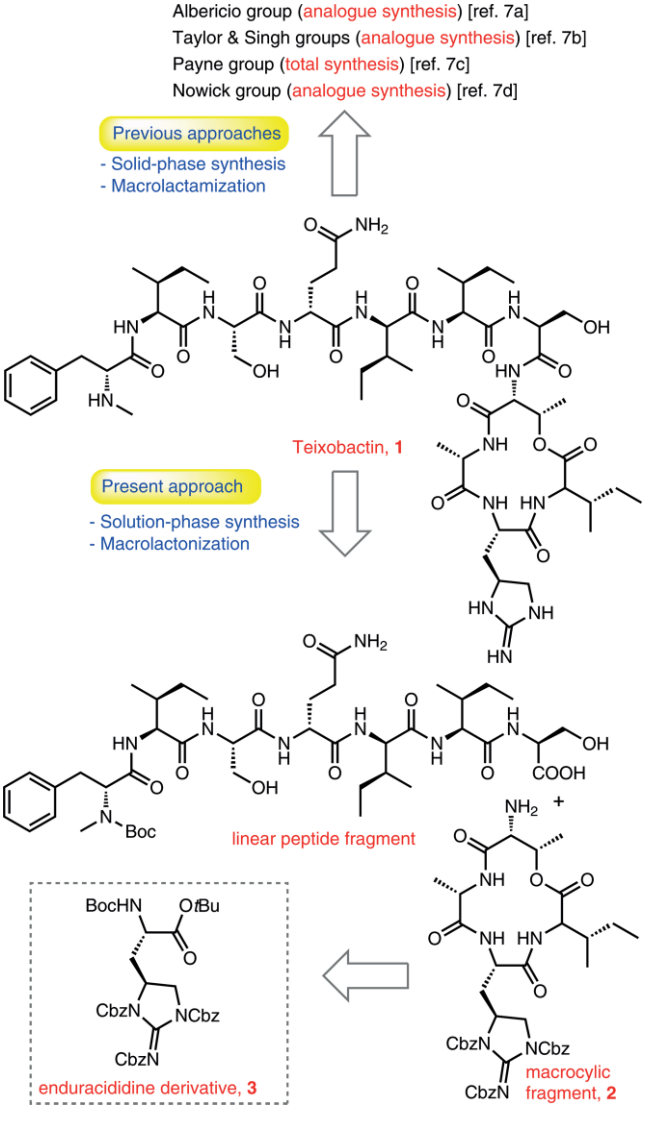

Figure 1. Structure of teixobactin and retrosynthetic plan.

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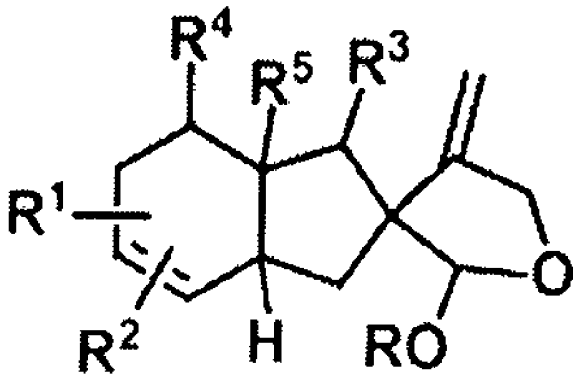
(54) **Title**: ANTICANCER COMPOUNDS AND PROCESS FOR THE PREPARATION THEREOF

(57) **Abstract**: The invention disclosed herein relates to novel Peribysin E analogues of general formula-I. Further the invention provides simple, economical and short synthesis of Peribysin E and its analogues of Formula I, in good yield and purity leading to the identification of more potent cell adhesion inhibitors.



General formula-I

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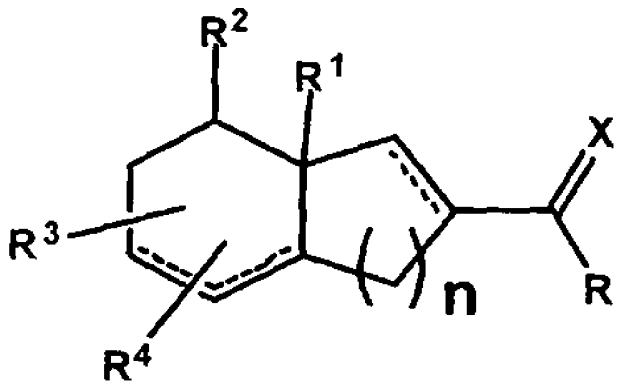
— *of inventorship (Rule 4.17(iv))*

[Continued on next page]

(54) Title: INSECT REPELLENTS



[I]

(57) Abstract: Disclosed herein are the novel insect repellents of formula (I) to control the spread of various tropical diseases and to the process of preparation thereof wherein R, Rl, R3, R4 represents hydrogen or alkyl; R2 is selected from hydrogen, alkyl, C02R, C02H; 'n' is 1, 2, or 3; wherein any two of Rl, R2, R3 or R4 may form a 3-8 membered carbocyclic ring which may optionally be substituted or may contain a heteroatom; X is selected from O, S or CH2; ' ' represents a single or double bond; wherein, either of the ring in formula (I) may additionally contain at least one carbonyl group.

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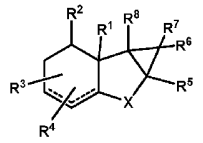
[Continued on next page]

(54) Title: TRICYCLIC COMPOUNDS AND PROCESS FOR PREPARATION THEREOF



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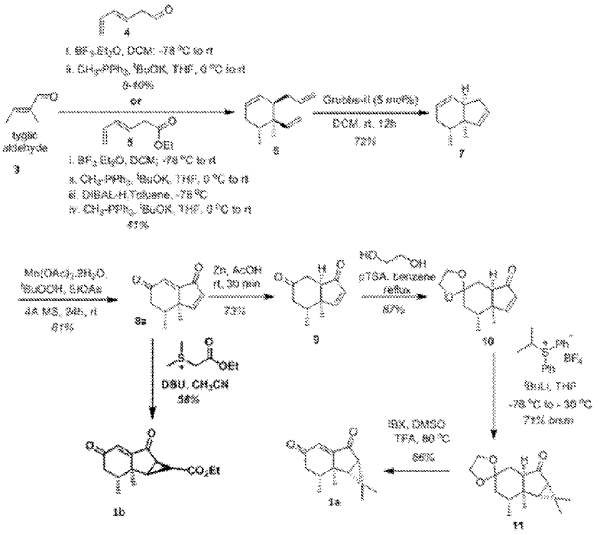

Scheme: 1

(57) Abstract: The present invention discloses tricyclic compounds of formula (I) or salt thereof and their process for synthesis. Further, the present invention relates to the use of these novel tricyclic compounds of formula (I) or salt thereof as insect repellents.

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