# Studies in Antibacterial Natural Products and their Analogs

**Thesis Submitted to AcSIR**

*For the Award of the Degree of*

**DOCTOR OF PHILOSOPHY**

*In*

**CHEMICAL SCIENCES**


By

**Gajanan N. Raut**
(AcSIR Roll No: 10CC11J26094)

Under the guidance of
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August 2016

*Dedicated*

*To My Family*

सीएसआईआर - राष्ट्रीय रासायनिक प्रयोगशाला
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# Thesis Certificate

This is to certify that the work incorporated in this Ph.D. thesis entitled **"Studies in Antibacterial Natural Products and their Analogs"** submitted by **Mr. Gajanan N. Raut** (AcSIR Roll No. **10CC11J26094**) 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, "**Studies in Antibacterial Natural Products and their Analogs**" 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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# *Acknowledgment*

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*The financial assistance in the form of fellowship by **CSIR**, New Delhi is gratefully acknowledged.*

*Gajanan*

# List of Abbreviations

| | |
|---|---|
| Amu | atomic mass unit |
| AcOH | acetic acid |
| AcCl | acetyl chloride |
| $Ac_2O$ | acetic anhydride |
| Å | angstrom |
| Ar | aryl |
| ACN | acetonitrile |
| Bn | benzyl |
| Boc | *tertiary*-butyloxycarbonyl |
| Br | bromo |
| brs | broad singlet |
| Bu | butyl |
| *t*-Bu | *tertiary*-butyl |
| calcd. | Calculated |
| $cm^{-1}$ | 1/centimetre |
| C–C | carbon-carbon |
| C–H | carbon-hydrogen |
| C–N | carbon-nitrogen |
| C–O | carbon-oxygen |
| DCM | Dichloromethane |
| $CHCl_3$ | Chloroform |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| DMAP | 4-dimethyl aminopyridine |
| DABCO | diazabicyclo[2.2.2]octane |
| DMF | *N*,*N*-dimethylformamide |
| DMSO | dimethylsulphoxide |

| | |
|---|---|
| DMSO-$d_6$ | deutriated dimethylsulphoxide |
| dd | doublet of doublet |
| d | doublet (in NMR) |
| Et | ethyl |
| EtOAc | ethyl acetate |
| EtOH | ethanol |
| equiv | equivalent |
| EWG | electron withdrawing group |
| g | gram(s) |
| h | hour(s) |
| HRMS | high resolution mass spectrometry |
| HSQC | homonuclear single bond correlation |
| COSY | Correlation spectroscopy |
| HMBC | Heteronuclear Multiple Bond Correlation |
| TOCSY | Total Correlated SpectroscopY |
| Hz | hertz |
| IR | infrared |
| *J* | coupling constant (in NMR) |
| mass (ESI+) | electron spray ionization mass spectroscopy |
| min | minute(s) |
| m | multiplet |
| mL | milliliter(s) |
| mmol | millimole(s) |
| mp | melting point |
| m/z | mass to charge ratio |
| Me | methyl |
| MHz | megahertz |
| m/z | mass to charge ratio |
| N | normality |
| nM | nanomolar(s) |

| | |
|---|---|
| NMR | nuclear magnetic resonance |
| NMP | *N*-methyl pyrrolidin-2-one |
| NIS | ***N***-Iodosuccinimide |
| Ph | phenyl |
| ppm | parts per million |
| Pr | propyl |
| q | quartet |
| $R_f$ | retention factor |
| rt | room temperature |
| s | singlet |
| $S_N$ | nucleophilic substitution |
| *sec* | secondary |
| t | triplet |
| *tert* | tertiary |
| TBHP | *tert*-Butyl hydroperoxide |
| TMEDA | tetramethylethylene diamine |
| TEA | triethyl amine |
| THF | tetrahydrofuran |
| TFA | trifluroacetic acid |
| TFAA | trifluroacetic anhydride |
| TLC | thin layer chromatography |
| TEA | triethyl amine |
| Ts | *para*-toluenesulphonyl |
| UV | ultraviolet |
| v/v | volume by volume |
| w/v | weight by volume |
| $^{\circ}C$ | degree celsius |
| µM | micromolar(s) |
| MIC | Minimum inhibitory concentration |

# 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** | **Gajanan N. Raut** |
| **Degree Enrolment No. & Date** | **Ph. D in Chemical Sciences (10CC11J26094); January 2011** |
| **Title of the Thesis** | **Studies in Antibacterial Natural Products and their Analogs** |
| **Research Supervisor** | **Dr. D. Srinivasa Reddy** |

The thesis is divided into four chapters. Chapter 1 gives a brief introduction to the importance of natural products and natural product derivatives in antibacterial drug discovery. Chapter 2 describes studies toward the latocillin and its structurally simplified analogs. Chapter 3: Section I describes the total synthesis of antituberculosis natural product diaportheone B, its absolute configuration and SAR study against the *mycobacterium tuberculosis*[2] and various gram positive bacterial strains. Section II includes the total synthesis and SAR studies of dehydroxydiversonol. The total synthesis of diarylheptanoid natural product was described in chapter 4.

**Chapter 1: Natural Products and Natural Product derived Antibacterial Agents in Modern Drug Discovery**

From several decades, natural products proved to be a rich source of biologically active compounds. In recent times, in particular, from the year 2000 onwards there are 22 new drugs that were approved belong to five new classes of antibiotics.[1] Out of 22 newly approved antibacterials, nine drugs have been originated from either natural products or natural product derivatives which show their importance in the modern drug discovery. Therefore, we became interested in synthesis and evaluation of antibacterial natural products and their analogs.

**Chapter 2: Studies toward Total Synthesis of Lactocillin and its Structurally Simplified Analogs**

Bacteria continuously develop resistance to the existing drugs and treatments. So, there is always a need to develop new antibacterial drugs with a novel mode of actions. The natural product isolated from the vaginal pathogens *lactobacillus gassari* showed potent antibacterial activity against the series of gram-positive bacteria including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *enterococcus* (VRE).

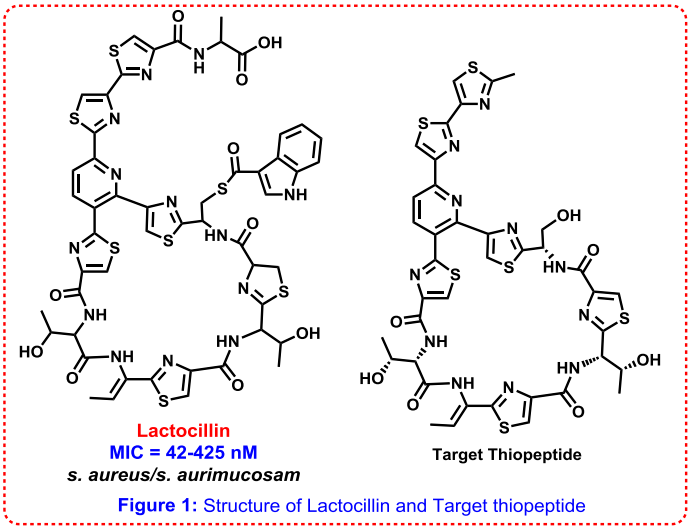
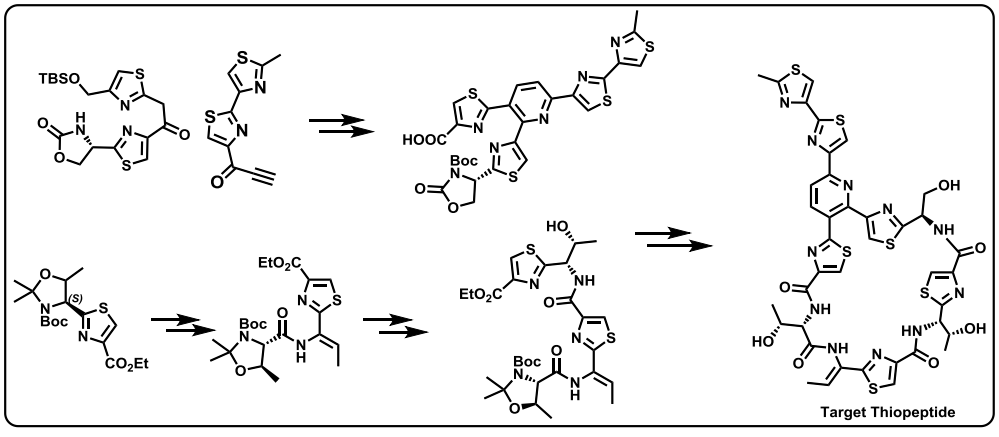



**Figure 1:** Structure of Lactocillin and Target thiopeptide

**Methodology used:**

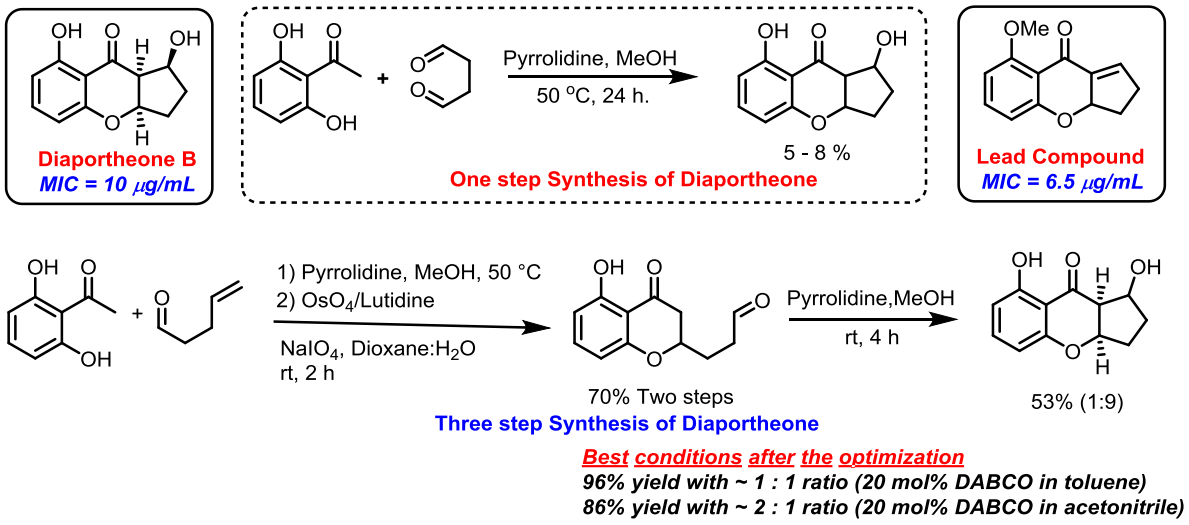
We have planned to synthesize lactocillin and its structurally simplified analogs. The lactocillin and other related thiopeptides contain similar structural features such as canonical 26-membered thiopeptide, trithiazolylpyridine core, macrocycle with four cysteine derived heterocycles and single dehydrobutyrine residue. Based on this we have planned for the synthesis of simplified target molecule having all key features of thiopeptide natural products. The planned target molecule was synthesized from two key fragments A and B. Fragment A is synthesized using Eiden-Herdies pyridine synthesis as a key step and fragment B is a peptide fragment, synthesized from L-threonine using the protection-deprotection chemistry and acid amine coupling reactions (Scheme 1). Both fragments were coupled using peptide coupling agents (HATU, DIPEA) and then subjected to macrocyclization under standard lactamization conditions using DPPA to get the desired molecule.




**Scheme 1**: Synthesis of lactocillin analogs

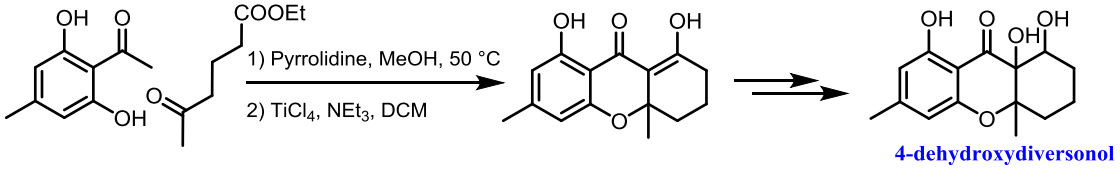
**Chapter 3: Section I: Antituberculosis agent diaportheone B: Synthesis, Absolute Configuration and SAR Study.**[2]

First and short synthesis of antituberculosis agent diaportheone B following two complementary methods were achieved starting from bench-top chemicals (Scheme 2). We then determined the absolute configuration of the natural isomer through X-ray crystal structure analysis of its dibromo derivative. We also synthesized > 20 analogs of diaportheone B and screened for antitubercular and antibacterial activity. The lead compound obtained was shown in scheme 2.




**Scheme 2**: Synthesis of diaportheoneone B by one step and three step sequence

**Chapter 3: Section II: Total Synthesis and SAR Studies of 4-Dehydroxydiversonol and its Analogs.** The racemic total synthesis of dehydroxydiversonol was achieved using a short sequence of reactions involving domino-reaction sequence with the help of an organocatalyst and Claisen-type condensation with good overall yields (Scheme 3). We also synthesized a library of compounds around this scaffold and currently profiling them for their antibacterial activity. The biological screening results are awaited.

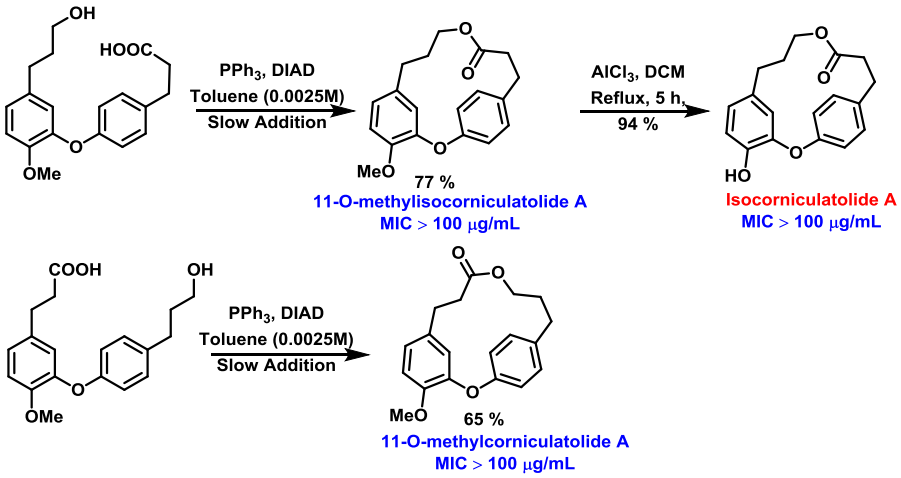



**Scheme 3**: Synthesis of dehydroxydiversonol

**Chapter 4: Synthesis and SAR Studies of Diarylheptanoid Natural Products**[3]

Synthesis of three natural diarylheptanoids 11-O-methylcorniculatolide A, 11-O-methylisocorniculatolide A and isocorniculatolide A was achieved using simple, straight forward and high yielding route which involves macrolactonization as key step (Scheme 4). All the synthesized compounds were tested against the *Mtb* and found to be inactive with MIC > 100

μg/mL. The probable reasons have been analyzed and compared to active natural product pterocarine.




**Scheme 4**: Synthesis of methylisocorniculatolide A, isocorniculatolide A and methylcorniculatolide A

**Noteworthy Findings:**

a) Macrocyclic thiopeptide core of lactocillin was synthesized towards identifying the novel antibacterial agent and is ready to be screened against several bacterial strains.

b) Accomplished the first total synthesis of diaportheone B, determined its absolute configuration and developed SAR around this scaffold.

c) Total synthesis of dehydroxydiversonol was achieved in short reaction sequence and synthesized several new analogs for SAR Study.

d) Total synthesis of macrocyclic diarylheptanoid natural product methylcorniculatolide A, isocorniculatolide A and methylisocorniculatolide A was accomplished and synthesized compounds was tested against the *Mtb* strains.

**References:**

1. M. S. Butler, M. A Blaskovich and M. A. Cooper, *J. Antibiot.*, 2013, **66**, 571.

2. P. S. Swaroop, **G. N. Raut**, R. G. Gonnade, P. Verma, R. S. Gokhale and D. S. Reddy; *Org. Biomol. Chem.*, 2012, **10**, 5385.

3. **G. N. Raut**, K. Chakraborty, P. Verma, R. S. Gokhale and D. S. Reddy, *Tett. Lett.*, 2012, **53**, 6343.

4. **G. N. Raut**, S. B. Wagh and D. S. Reddy, *ARKIVOC*, 2016, **2**, 1.

# Table of Contents

*Chapter 1*

# Natural Products and Natural Product derived Antibacterial Agents in Modern Drug Discovery

## 1. Introduction

Natural products have attracted the attention of different branches of science, particularly biologists and chemists over the last several decades. It is one of the most important and reliable source of pharmacologically active compounds. Research in natural products continues to explore a variety of pharmaceutically active lead structures which can be used for the development of new drugs by the drug hunters, especially against life-threatening diseases such as bacterial infections of various pathogens, HIV, cardiac diseases, cancer, diseases of the circulatory system and central nervous system. Although it is very time-consuming and laborious process, the chances of success of becoming lead molecules to drugs are high. There is no doubt that natural products will continue to be very important source of new pharmaceutical lead compounds in the years to come.

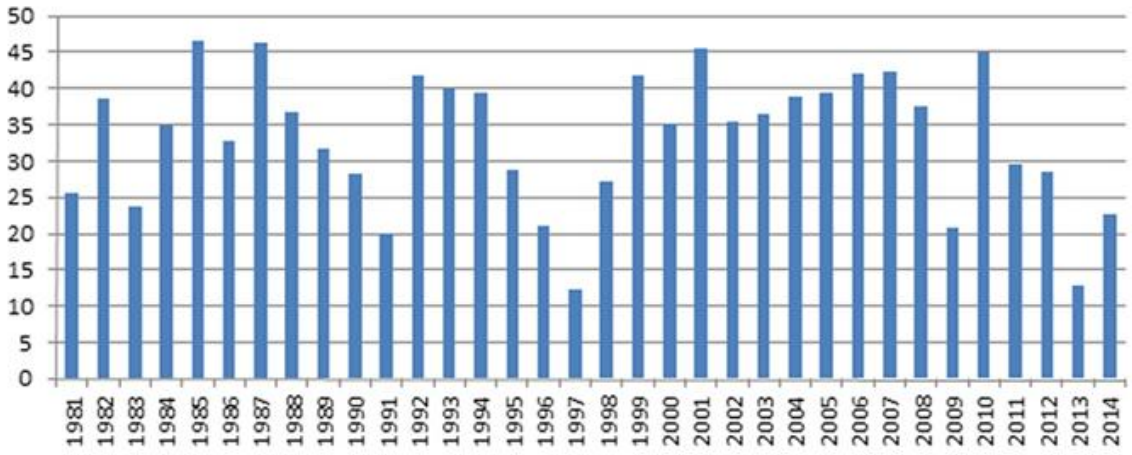



**Figure 1:** Percentage of natural products/ natural product-derived drugs from 1981-2014.[4a]

According to a recent review published in Journal of Natural Products, analysis of all the drugs approved by US FDA in last three decades, there are several natural products/natural product derivatives which have been approved as drugs against different diseases (Figure 1). The contribution of these natural products remains high which is almost 35-40% of the total approved drugs.[4-6] If we consider the natural product mimics

and botanical drugs, their contribution increases to around 60%, thus highlighting their importance in drug discovery (Figure 2).

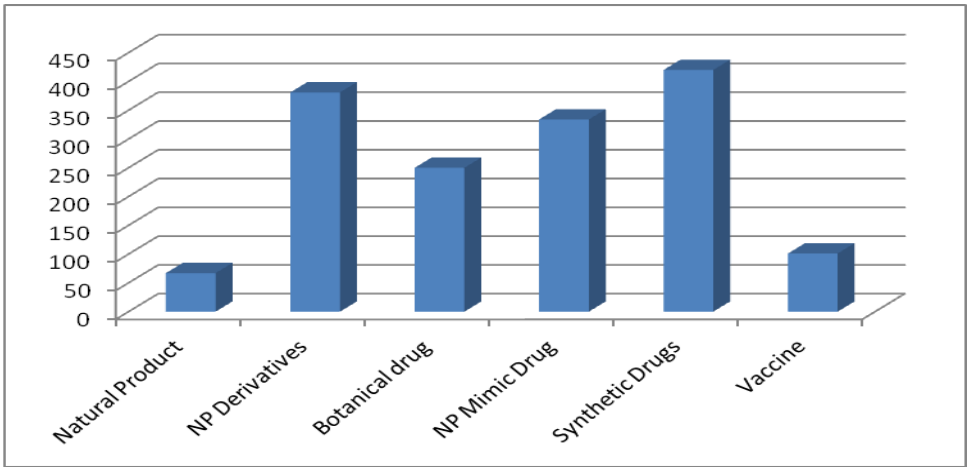



**Figure 2**: Number of newly approved drug from 1981-2015[4a]

The most famous and well-known example of natural product-derived drugs would be the anti-inflammatory agent, acetylsalicylic acid (aspirin) (**1**)[7] derived from the natural product, salicin (**2**) isolated from the bark of the willow tree *Salix alba L*. In 1803, the commercially important drug morphine along with several alkaloids has been isolated from the *Papaver somniferum L.* (opium poppy). It was in 1870 that the accidental investigation of heroin (**4**), as crude morphine (**3**) was boiled with acetic anhydride to yield diacetylmorphine (heroin, **4**) and readily converted to the codeine (**5**) the well-known painkiller.[8]

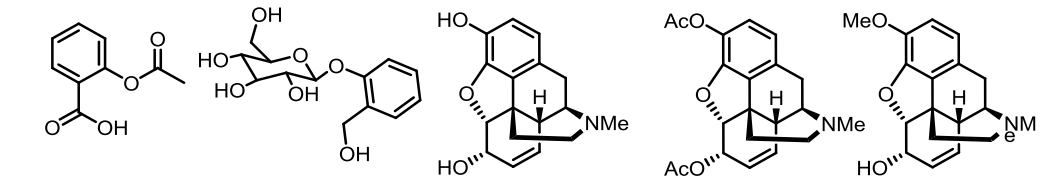



**Figure 3**: Structures of natural product derived drugs-classic examples

The well-known natural product derived from the fungus (microorganism) is penicillin (**6**) from the fungus, *Penicillium notatum* discovered by Fleming in 1929.[9] This discovery led to the re-isolation and clinical studies by Chain, Florey and co-workers in the 1940s and commercialization of synthetic penicillins,[10] which ultimately revolutionized the drug discovery research and won Nobel Prize in the year 1945 in physiology and medicine by Chain and Florey together with Fleming. After publication of the first clinical data on penicillin,[9] there was a worldwide endeavor to discover new antibacterials from microorganisms and bioactive natural products and till 1968 several *β*-lactam antibacterials such as norcardicin (**7**), imipenem (**8**) were discovered and developed. Although initial antibacterials were originated from microorganisms, continuous efforts to meet the society needs led to the discovery of novel and interesting antibacterial natural products from different sources. Before discussing further details on antibacterial natural products, a brief discussion on historical background and overview of the antibacterial drugs will be useful.

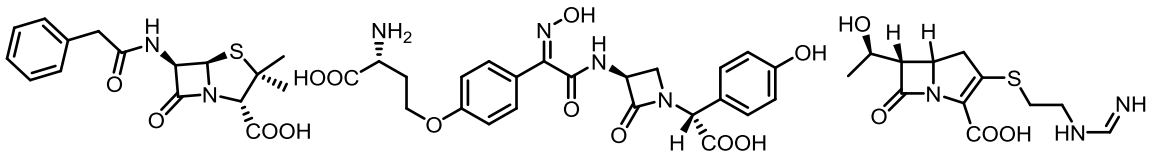



**Figure 4**: *β*-Lactam antibacterials

## 1.1. Historical overview of antibacterial agents

The most acceptable and appropriate definition for an antibiotic drug is "An antibacterial drug represents a chemical substance derived from a biological source or produced by chemical synthesis that is able to destroy or to inhibit the development/growth of bacteria".[11] A recent review from Högberg in Trends in Pharmacological Sciences[12] and on antibiotic development timeline[13] beautifully captured the historical overview of antibacterial drug discovery and development of bacterial resistance to marketed drugs in schematic diagram as shown in Figure 5. The antibiotic drug discovery started with the introduction of sulfa drug prontosil (**10**) into the market in early 1935 and widely used as

an antibacterial agent and as a results the bacteria developed the resistance to prontosil (**10**) in 1942. Similarly, several other classes of drugs entered the market and the bacterial resistance occurred within few years (Figure 5), as a result of the natural phenomenon of bacteria through different mechanisms, like alteration of bacterial proteins, changes in membrane permeability towards antibiotics and by acquiring the resistance from other bacteria.[14-21] The bacterial-resistance observed to almost all the antibacterial agents currently available in the market including the recently developed antibiotics from various classes.

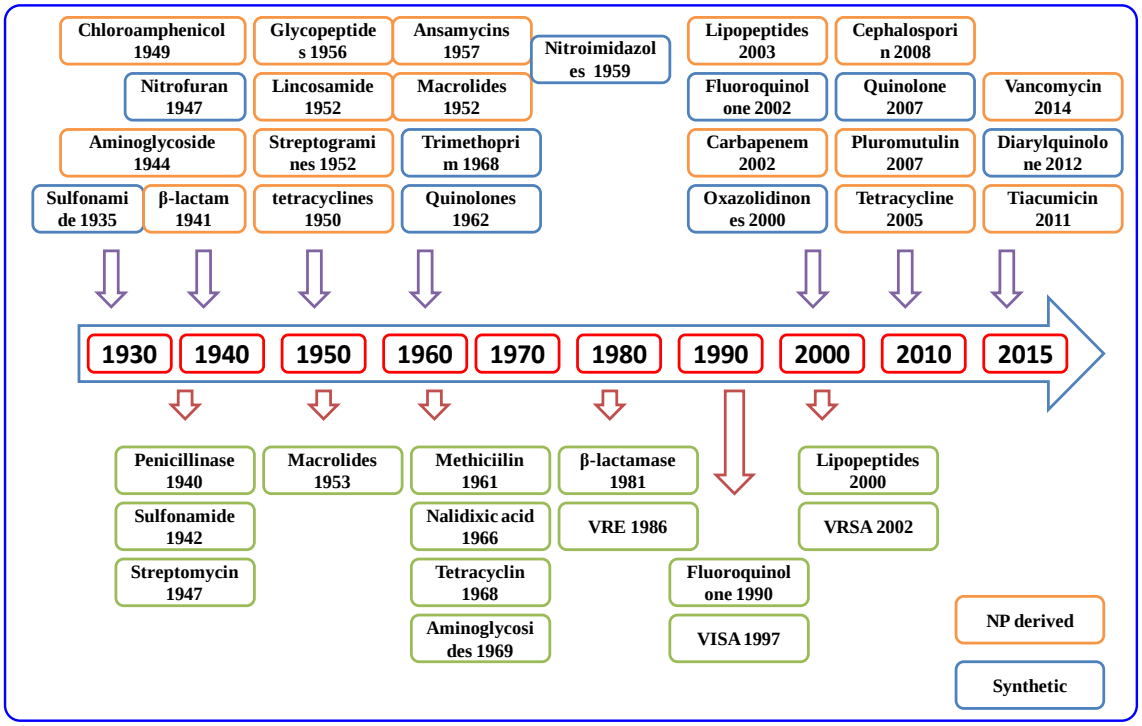
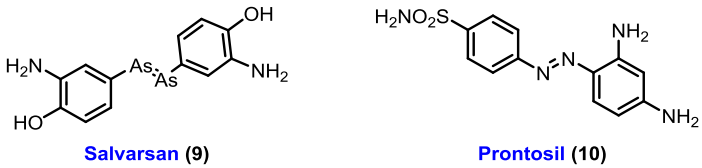



**Figure 5:** Historical overview of antibacterial drug discovery and development of bacterial resistance to marketed drugs.[13]

The era of antibacterial chemotherapy began with the discovery of salvarsan (**9**), first synthesized by Bertheim and Ehrlich in 1907, used to treat *syphilis*, *trypanosomiasis* in early 1910 and this organic compound is considered as a first chemotherapeutic agent.[22] A sulphonamide drug prontosil (**10**) was the first systemically active antibacterial drug, discovered in 1933 by Domagk,[23] for which he was awarded the Nobel Prize in 1939.

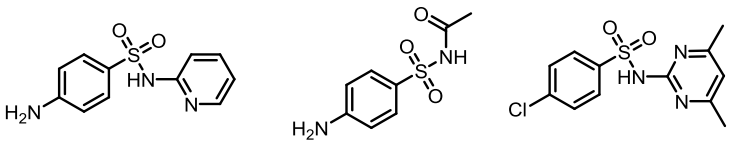
The discovery of first three antimicrobials salvarsan (**9**), prontosil (**10**) and penicillin (**6**) were exemplary for the future drug discovery research, which ultimately led to the discovery of several other antibacterial drugs which fall under following categories based on their chemical structures.[13, 24-35]




**Salvarsan (9)** **Prontosil (10)**

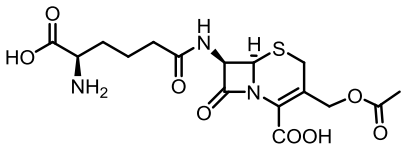
**Figure 6**: Structures of salvarson (**9**) and prontosil (**10**)

**1.1.1. Sulfonamide antibacterials:** This is the synthetic class of drugs, where first sulfa drug prontosil (**10**) was discovered in the year 1933 by Domagk. Prontosil (**10**) is a synthetic dye which showed activity against the gram-positive bacteria of genus *streptococcus.* Later, structural derivatization of sulphanilamide series resulted in the development of a family of highly successful antibacterials that have saved millions of lives around the world. Sulfonamides interfere with the folic acid synthesis which is essential for nucleic acid synthesis in bacteria. e.g. sulfapyridine (**11**) was shown to be effective against pneumonia, sulfacetamide (**12**) was found effective against the urinary tract infections. Another example of sulfa drugs includes sulfadimidine (**13**).

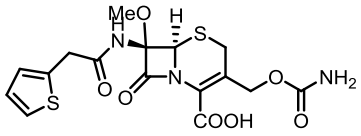



**Sulfapyridine (11)** **Sulfacetamide (12)** **Sulfadimidine (13)**

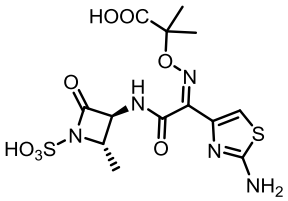
**1.1.2. *β*-Lactam antibacterials:** It is a broad class of antibacterials, consists of a *β*-lactam ring in their molecular structures. This class includes well-known penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. *β*-lactam antibacterials work by inhibiting cell wall biosynthesis in the bacteria and are the most widely used group of antibacterials. e.g. cephalosporin C (**14**), cefoxitin (**15**), aztreonam (**16**) etc.



**Cephalosporin C (14)**

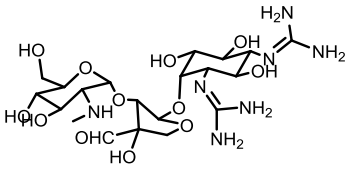


**Cefoxitin (15)**

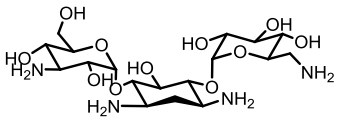


**Aztreonam (16)**

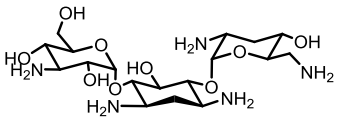
**1.1.3. Aminoglycosides:** It contain an amino-modified glycoside as a structural unit, this is the traditional gram-negative antibacterial agents which inhibit the protein synthesis of bacteria. Streptomycin (**17**) was the first aminoglycoside antibiotic derived from the *Streptomyces griseus.* Aminoglycosides bind to the bacterial ribosome inhibiting the protein synthesis. e.g. kanamycin (**18**), tobramycin (**19**), gentamycin (**20**), amikacin (**21**).



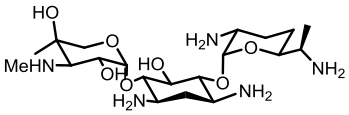
**Streptomycin (17)**



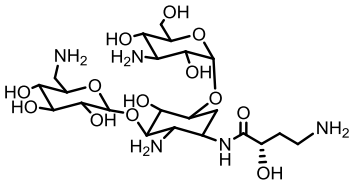
**Kanamycin (18)**



**Tobramycin (19)**

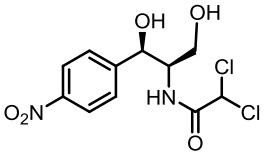


**Gentamycin (20)**



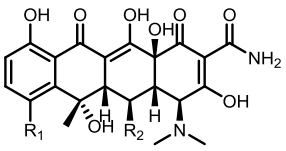
**Amikacin (21)**

**1.1.4. Chloramphenicol:** The antibacterial agent chloramphenicol is a broad spectrum antibiotic introduced to the market in 1949 against the *Staphylococcus aureus*, *Streptococcus pneumonia*, and *Escherichia coli*. It is also effective against the vancomycin-resistant *enterococcus* (VRE). The chloramphenicol works by inhibiting the protein synthesis during chain elongation stage.

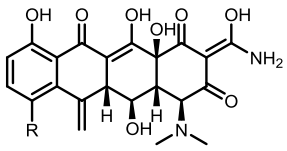


**Chloroamphenicol (22)**

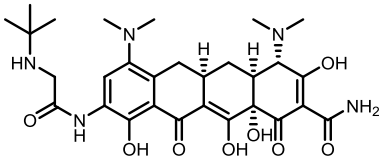
**1.1.5. Tetracycline antibacterial**: It is a group of broad spectrum antibacterials, its use is limited but remains the choice of the drug for patients with allergic to *β*-lactam and macrolide antibacterials. Tetracycline antibacterials bind with the 30s ribosomal subunit of the mRNA translation complex resulting in the inhibition of bacterial protein synthesis. e.g. tetracycline (**23**), terramycin (**24**), demeclocycline (**25**) are natural product drugs along with the semisynthetic tetracyclines like methacycline (**26**), and meclocycline (**27**). A few years ago, tigecycline (**28**) was approved, is the first member of a new subgroup of tetracyclines named glycylcyclines. Also, it was the latest in the class of tetracyclines marketed for human use.



$R_1$, $R_2$ = H,Tetracycline (**23**)
$R_1$ = H, $R_2$ = OH Terramycin (**24**)
$R_1$ = Cl, $R_2$ = H Demeclocycline (**25**)

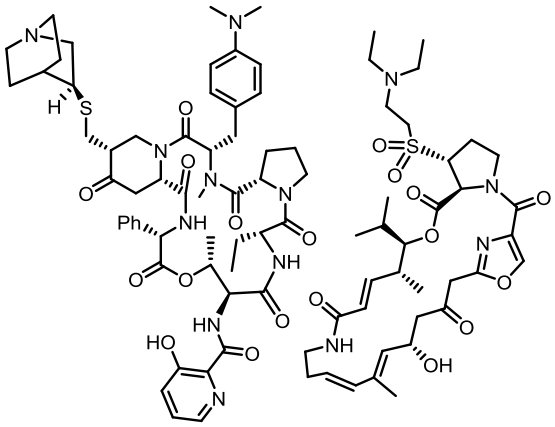


R = H, Methacycline (**26**)
R = Cl, Meclocycline (**27**)

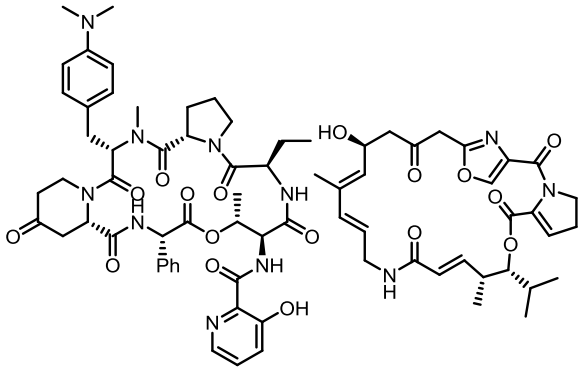


Tigecycline (**28**)

**1.1.6. Streptogramins:** It consists of mixtures of two structurally distinct compounds, streptogramin A and streptogramin B. Streptogramin A contains a 23-membered unsaturated ring with lactone and peptide bonds. It is a polyketide structural framework with some amino acids while streptogramin B are cyclic hexa or hepta depsipeptides. Streptogramins A and B are bacteriostatic when used in conjunction with one another, the streptogramins can inhibit bacterial growth and are bactericidal by inhibiting the protein synthesis (synergistic mechanism). Streptogramins are effective against two of the most growing multi-drug resistant bacterial strains vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant enterococcus (VRE). e.g. quinupristin/dalfopristin (**29**) and pristinamycin (**30**).

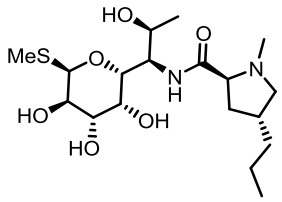


**Quinupristin/Dalfopristin (29)**

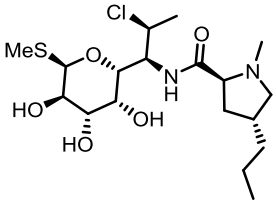


**Pristinamycin (30)**

**1.1.7. Lincosamide:** These are narrow spectrum antibacterial agents and active only against the gram-positive bacteria like *staphylococci* and *streptococci*. Lincosamides prevent the replication of bacteria by inhibiting the protein synthesis on the ribosomal level. There are two lincosamide drugs available lincomycin (**31**) and clindamycin (**32**). Clindamycin exhibits the improved antibacterial activity compared to lincomycin (**31**) and also shows the activity against some protozoa, toxoplasmosis, and malaria.

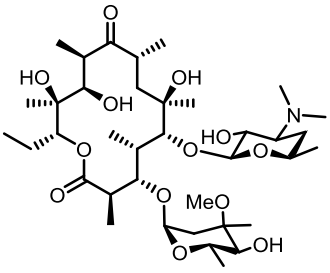


**Lincomycin (31)**

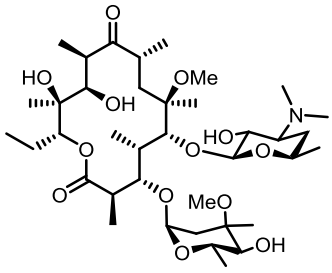


**Clindamycin (32)**

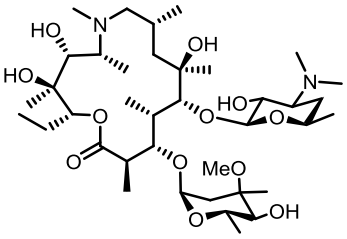
**1.1.8. Macrolides:** Macrolides are a group of antibacterial agents produced by various strains of *Streptomyces* and have a complex macrocyclic structure. These are bacteriostatic antibacterials with a broad spectrum of activity against many gram-positive bacteria. Macrolides inhibit protein synthesis, specifically by blocking the 50s ribosomal subunit. e.g. erythromycin (**33**), clarithromycin (**34**), azithromycin (**35**).



**Erythromycin (33)**

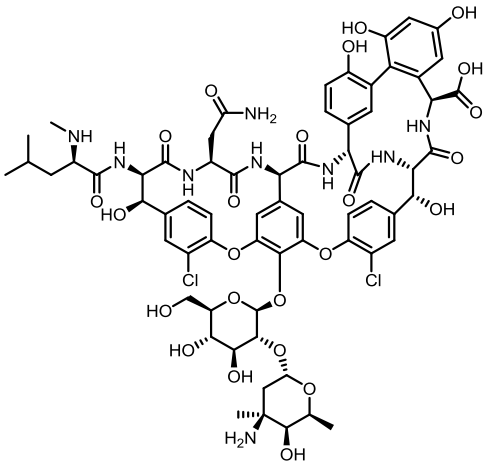


**Clarithromycin (34)**

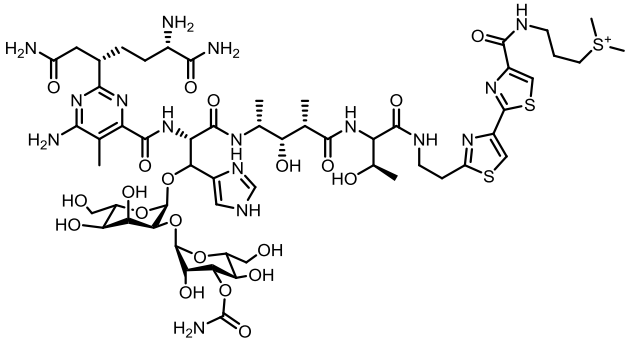


**Azithromycin (35)**

**1.1.9. Glycopeptide:** These are drugs of microbial origin that are composed of glycosylated cyclic or polycyclic nonribosomal peptides. The first glycopeptide vancomycin was isolated in 1953 and approved by FDA in 1958 to treat penicillin-resistant *staphylococci*. Glycopeptides inhibit the synthesis of the cell wall by inhibiting peptidoglycan synthesis.[33, 34] e.g. vancomycin (**36**), bleomycin (**37**).

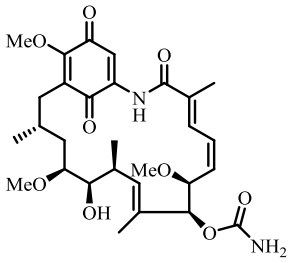


**Vancomycin (36)**

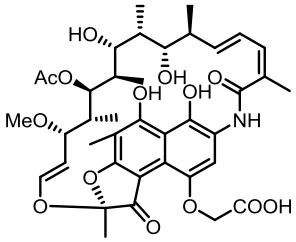


**Bleomycin (37)**

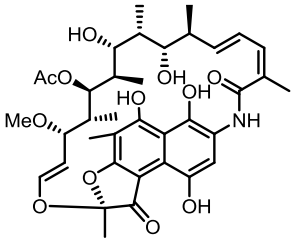
**1.1.10. Ansamycin:** It constitutes a class of drugs having an aliphatic bridge linking two non-adjacent positions of an aromatic moiety. They show antimicrobial activity against the several gram-positive and some gram-negative bacteria. The two major groups of this are streptovaricins and rifamycins. Rifamycin is active against the mycobacteria and used to treat the *mycobacterium tuberculosis*, *mycobacterium avium*. e.g. Geldanamycin (**38**), Rifamycin B (**39**), Rifamycin SV (**40**).



**Geldanamycin (38)**

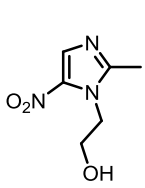


**Rifamycin B (39)**

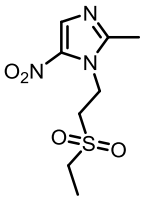


**Rifamycin SV (40)**

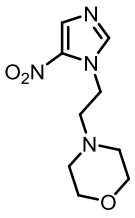
**1.1.11. Nitroimidazole:** Nitroimidazole antibacterials are used to treat anaerobic bacteria and parasitic infections. It can be classified according to the location of the nitro functional group. 5-nitro imidazole derivatives include metronidazole (**41**), tinidazole (**42**), nimorazole (**43**) while 2-nitromidazoles include benznidazole (**44**).



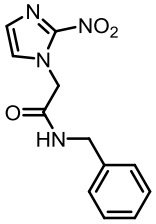
**Metronidazole (41)**



**Tinidazole (42)**



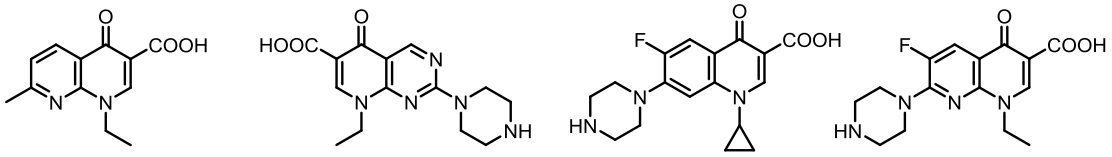
**Nimorazole (43)**



**Benznidazole (44)**

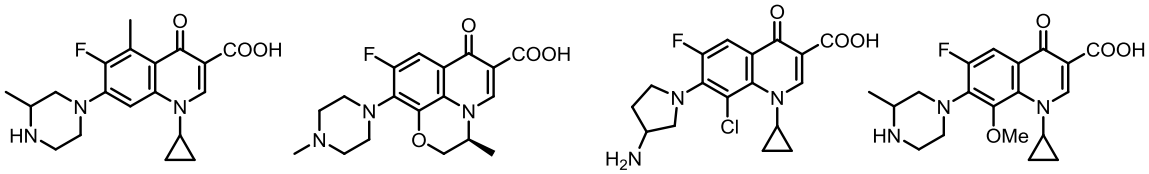
**1.1.12. Quinolones:** Quinolones or fluoroquinolones are synthetic broad-spectrum antibiotic drugs. The first quinolone antibiotic nalidixic acid (**45**) was discovered in 1962 by Lesher and used for the treatment of urinary tract infections. Later during 1970 pipemidic acid (**46**), ciprofloxacin (**47**), enoxacin (**48**), grepafloxacin (**49**), levofloxacin (**50**), clinafloxacin (**51**) and gatifloxacin (**52**), were introduced in the market. The addition of the fluorine atom at C6 position distinguishes the successive-generations of fluoroquinolones from the first-generation of quinolones. The quinolones show activity against both gram-positive and gram-negative bacteria. They act on the topoisomerases in gram-positive bacteria while in the case of gram-negative bacteria they inhibit the DNA gyrase enzymes, which ultimately result in the inhibition of DNA replication.

**First Generation** **Second Generation**



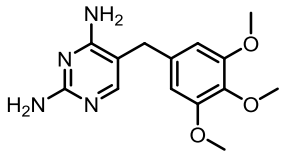
**Nalidixic acid (45)** **Pipemidic acid (46)** **Ciprofloxacin (47)** **Enoxacin (48)**

**Third Generation** **Fourth Generation**



**Grepafloxacin (49)** **Levofloxacin (50)** **Clinafloxacin (51)** **Gatifloxacin (52)**

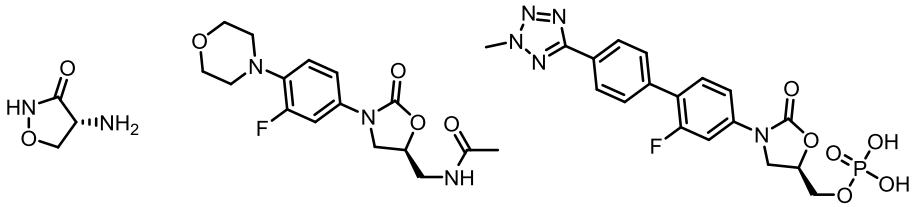
**1.1.13. Trimethoprim:** It was discovered in 1962 and used to treat the bladder (lower urinary tract) infection. It binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid which is an essential precursor in the thymidine synthesis, hence resulting in the inhibition of DNA synthesis.



**Trimethoprim (53)**

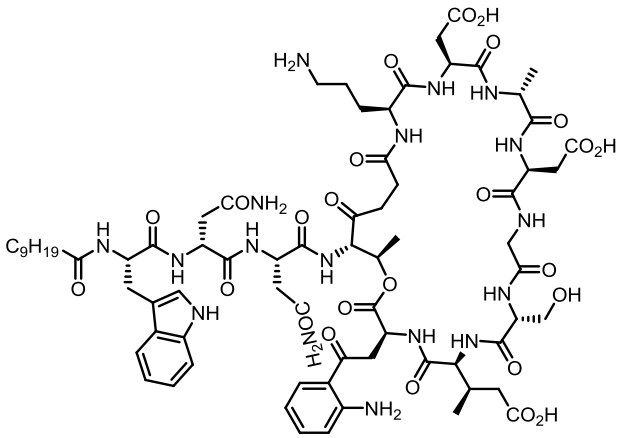
**1.1.14. Oxazolidinones:** Cycloserine (**54**) is a first member of oxazolidinone class antibacterials under the brand name of seromycin in 1956 which is used as a second-line drug to treat the infections of *mycobacterium tuberculosis*. The first synthetic drug from oxazolidinone family linezolid (**55**), discovered by Pfizer, was approved in 2000 for various gram-positive bacteria, including *streptococc*i, vancomycin-resistant *enterococci* (VRE), and methicillin-resistant *Staphylococcus aureus* (MRSA). While tedizolid phosphate (**56**) was approved in 2014 as prodrug against the acute skin infections. The few molecules from the oxazolidinone family are currently in development stage. The

oxazolidinones inhibit the protein synthesis by binding with the *N*-formyl methionyl-tRNA to the ribosome at an early stage of protein synthesis in bacteria.



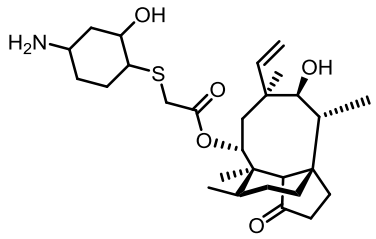
Cycloserine (54) Linezolid (55) Tedizolid Phosphate (56)

**1.1.15. Lipopeptides:** These are molecules consisting of a lipid connected to a peptide and used in the treatment of systemic and life-threatening infections caused by gram-positive bacteria. Daptomycin (**57**) is the first member of lipopeptide family approved in 2003 for the treatment of acute skin infections. A few other members of lipopeptide family such as amphomycin, friulimicin, laspartomycin are in developmental stages. The lipopeptide mainly works on the bacterial cell wall synthesis.



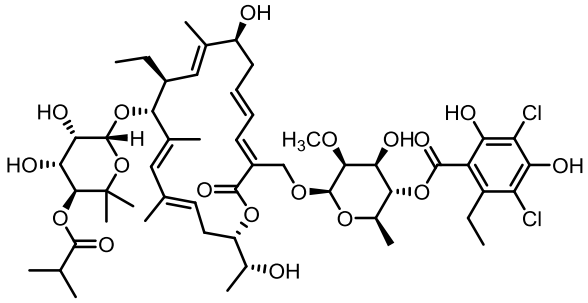
Daptomycin (57)

**1.1.16. Pleuromutilin:** This class was discovered as antibacterial in 1950 where tiamulin was the first pleuromutilin compound to be approved for veterinary use in 1979, followed by valnemulin in 1999. While in 2007 the first pleuromutilin antibiotic retapamulin (**58**) was approved for use in humans for bacterial skin infections as an ointment. It selectively inhibits the bacterial protein synthesis by interacting with a site on the 50s subunit of the bacterial ribosome through an interaction that differs from other antibacterials.



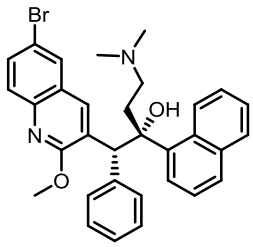
**Retapamulin (58)**

**1.1.17. Tiacumicin:** This is a group of antibacterial agents with 18-membered macrocyclic structures. Tiacumicins B and C are the initial members of this family, where fidaxomicin (**59**) is the first member approved in 2011 as an antibacterial agent against the non-systemic gram positive bacterial pathogens. Fidaxomicin (**59**) binds to the "switch regions" of bacterial RNA polymerase and prevents movement of DNA: RNA clamp which is required during RNA transcription.



**Fidaxomicin (59)**

**1.1.18. Diarylquinolone:** This is a synthetic class of antibacterial agents; the only member of this family bedaquiline (**60**) was approved very recently, in the year 2012 against tuberculosis. Bedaquiline (**60**) shows the potent activity towards multi-drug-resistant tuberculosis (MDR-TB), and extensively drug-resistant tuberculosis (XDR-TB). It inhibits the DNA gyrase enzymes which result in the inhibition of DNA replication and also affects the proton pump for ATP synthase.




**Bedaquiline (60)**

## 1.2. Classification of antibacterial agents

In addition to above classification based on structures, antibacterial agents are also classified in several ways, like based on their spectrum of activity, the effect on bacteria and their mode of action.[36-43] Based on the spectrum of activity antibacterial agents are classified as broad spectrum and narrow spectrum antibacterial agents. The drugs which show the activity against various bacterial strain of gram-positive and gram-negative bacteria are considered as **broad spectrum antibacterial** e.g. tetracyclines, phenicols, fluoroquinolones, "third-generation" and "fourth-generation" cephalosporins. **Narrow spectrum antibacterial** agents have limited activity and are useful against particular species of microorganisms e.g. glycopeptides, aminoglycosides, nitroimidazoles and sulfonamides.

Effect of drugs on bacteria divides the antibacterials to (1) bacteriostatic and (2) bactericidal agents. Bacteriostatic agents inhibit the bacterial growth e.g. tetracyclines, sulfonamides, and macrolides and the bactericidal agents kill the bacteria e.g. aminoglycosides, cephalosporins, penicillins, and quinolones. While some antibacterial agents show the bacteriostatic and bactericidal effect depending on the dose and duration of exposure e.g. aminoglycosides, fluoroquinolones, and metronidazole.

The antibacterials are also classified on the basis of their mode of action into five different groups as captured below.[36-43]

1) Cell wall synthesis inhibitors
2) Inhibitors of cell membrane functions
3) Protein synthesis inhibitors
4) Nucleic acid synthesis inhibitors
5) Inhibitors of other metabolic processes

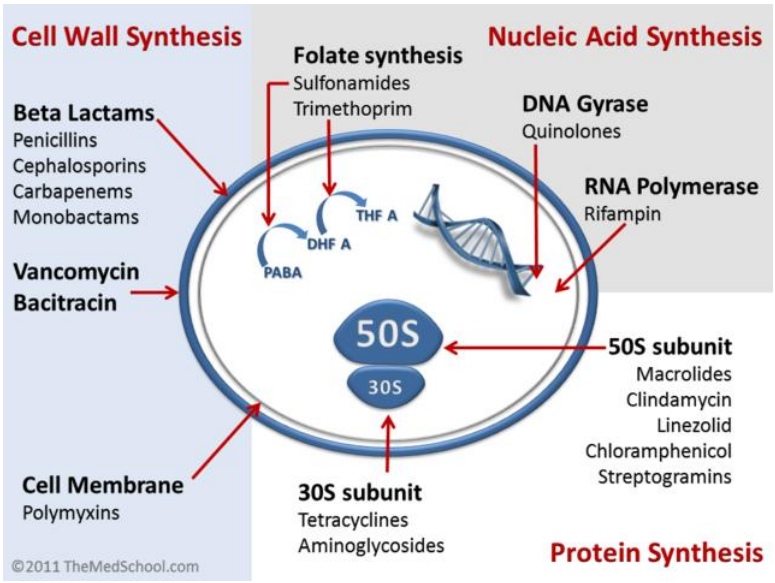



**Figure 7:** Antibacterial agents showing different mode of action[43]

## 1.3. Antibacterial natural products in modern drug discovery (2000-2015)

Antibacterial agents have saved millions of lives since the discovery of penicillin and sulphonamides during the 1930s. The discovery of these two drugs initiated the golden era of antibiotics which lasted for 40 years, during this period most of the current classes of antibiotics were discovered.[25] However, the innovative antibiotic pipeline dried up from early 1970 to 1999, while some derivatives of existing classes of drugs have been launched except mupirocin, a gram-positive topical antibiotic launched in 1985.[35, 42] Since 2000, the condition has improved with the insertion of five new classes of antibacterials. Linezolid (**55**) is a systemic drug approved in 2000 from oxazolidinone class, daptomycin (**57**) is a lipopeptide which is also a systemic drug approved in 2003, retapamulin (**58**) is a topical drug approved in 2007 for gram positive bacterial infection from the pleuromutilin class of antibacterials. A tiacumicin class drug fidaxomicin (**59**) was approved in 2010 against the *Clostridium difficile* infections and bedaquiline (**60**) is a purely synthetic drug approved in 2012 from diarylquinoline class, which was fast-tracked through clinical trials and used in combination with other drugs to treat

tuberculosis. All the newly launched class of antibacterials are limited mostly to treat gram-positive bacteria and are ineffective against the gram-negative bacteria.[35, 42] The approved drugs for treating various bacterial infections are compiled in Table 1 along with their source (synthetic/natural product /NP derivative) during the period of last fifteen years (2000-2015). Analysis of Table 1 clearly demonstrates that natural products are the best for discovering new antibacterial agents even today.

**Table 1:** Drugs approved from 2000-2015 as antibacterial agents.[44, 45]

| Year approved | Drug Name | Class | Lead source | NP lead organism | Bacteria Type |
|---|---|---|---|---|---|
| 2000 | Linezolid | Oxazolidinone | Synthetic | - | G +ve |
| 2001 | Telithromycin | Macrolide | NP derivative | Actinomycete | G +ve /G -ve |
| 2002 | Biapenem | Carbapenem | NP derivative | Actinomycete | G +ve /G -ve |
| 2002 | Ertapenem | Carbapenem | NP derivative | Actinomycete | G +ve /G -ve |
| 2002 | Prulifloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2002 | Pazufloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2002 | Balofloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2003 | Daptomycin | Lipopeptide | NP | Actinomycete | G +ve |
| 2004 | Gemifloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2005 | Doripenem | Carbapenem | NP derivative | Actinomycete | G +ve /G -ve |
| 2005 | Tigecycline | Tetracycline | NP derivative | Actinomycete | G +ve /G -ve |
| 2007 | Retapamulin | Pleuromutilin | NP derivative | Fungus | G +ve |
| 2007 | Garenoxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2008 | Ceftobiprole | Cephalosporin | NP derivative | Fungus | G +ve /G -ve |
| 2008 | Sitafloxacin | Fluoroquinolone | Synthetic | - | G +ve |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | /G -ve |
| 2009 | Tebipenem pivoxil | Carbapenem | NP derivative | Actinomycete | G +ve /G -ve |
| 2009 | Telavancin | Glycopeptide | NP derivative | Actinomycete | G +ve |
| 2009 | Antofloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2009 | Besifloxacin | Fluoroquinolone | Synthetic | - | G +ve /G -ve |
| 2010 | Ceftaroline fosamil | Cephalosporin | NP derivative | Fungus | G +ve /G -ve |
| 2011 | Fidaxomicin | Tiacumicin | NP | Actinomycete | G +ve |
| 2012 | Bedaquiline | Diarylquinoline | Synthetic | - | G +ve (TB) |
| 2014 | Dalbavancin | Glycopeptide | NP | | G +ve |
| 2014 | Oritavancin | Glycopeptide | NP | | G +ve |
| 2014 | Tedizolid phosphate | Oxazolidinone | Synthetic | - | G +ve /G -ve |
| 2014 | Ceftolozane-tazobactam | Cephalosporin | NP derivative | - | G +ve /G -ve |
| 2015 | Ceftazidime-avibactam | Cephalosporin / diazabicycloctane | NP-Synthetic | | G +ve /G -ve |

In the past one and half decades, total 27 new chemical compounds were approved for the treatment of bacterial infections. Out of 27 drugs recently approved as antibacterial drugs a significant percentage (16 nos., 60%) of those is either of natural products themselves or were derived from natural product scaffolds (Table 1). Telithromycin (Ketek®) (Figure 8) is a semi-synthetic derivative of erythromycin, approved by European Commission (EC) in 2001 to treat community-acquired pneumonia.[26] It is macrolide class antibiotic which prevents the growth of bacteria by inhibiting protein synthesis, it binds to the 50s subunit of the bacterial ribosome, and blocks the elongation of growing polypeptide chain. There are total four $\beta$-lactam (carbapenem) class of antibacterial drugs which originated from the natural products, mainly biapenem (Omegacin®, 2002),

ertapenem(Invanz®, 2002), doripenem (Finibax®, 2005) and Tebipenem (Orapenem®, 2009).

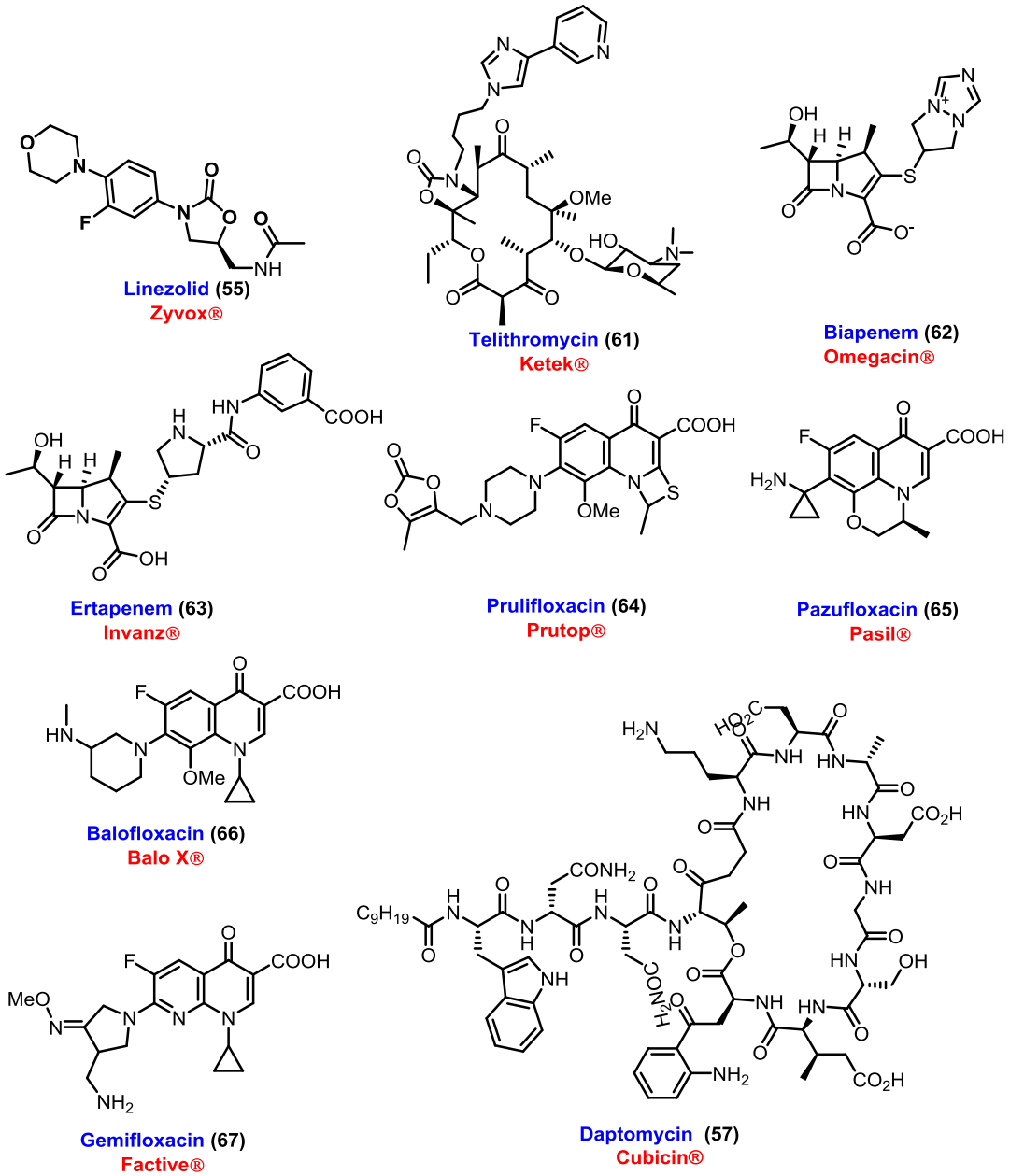



**Figure 8:** Structures of selected antibacterial drugs approved during the period of 2000-2015

Ceftobiprole (Zeptera®) and Ceftaroline fosamil (Teflaro®) are β-lactam antibacterials of the fifth generation cephalosporin subclass approved in 2008 and 2010, respectively for the treatment of the methicillin-resistant *Staphylococcus aureus* and other gram-positive

bacterial strains. Ceftolozane-tazobactam (Zerbaxa®), Ceftazidime-avibactam (Avycaz®) are both fixed-dose combination drugs containing the cephalosporin class of

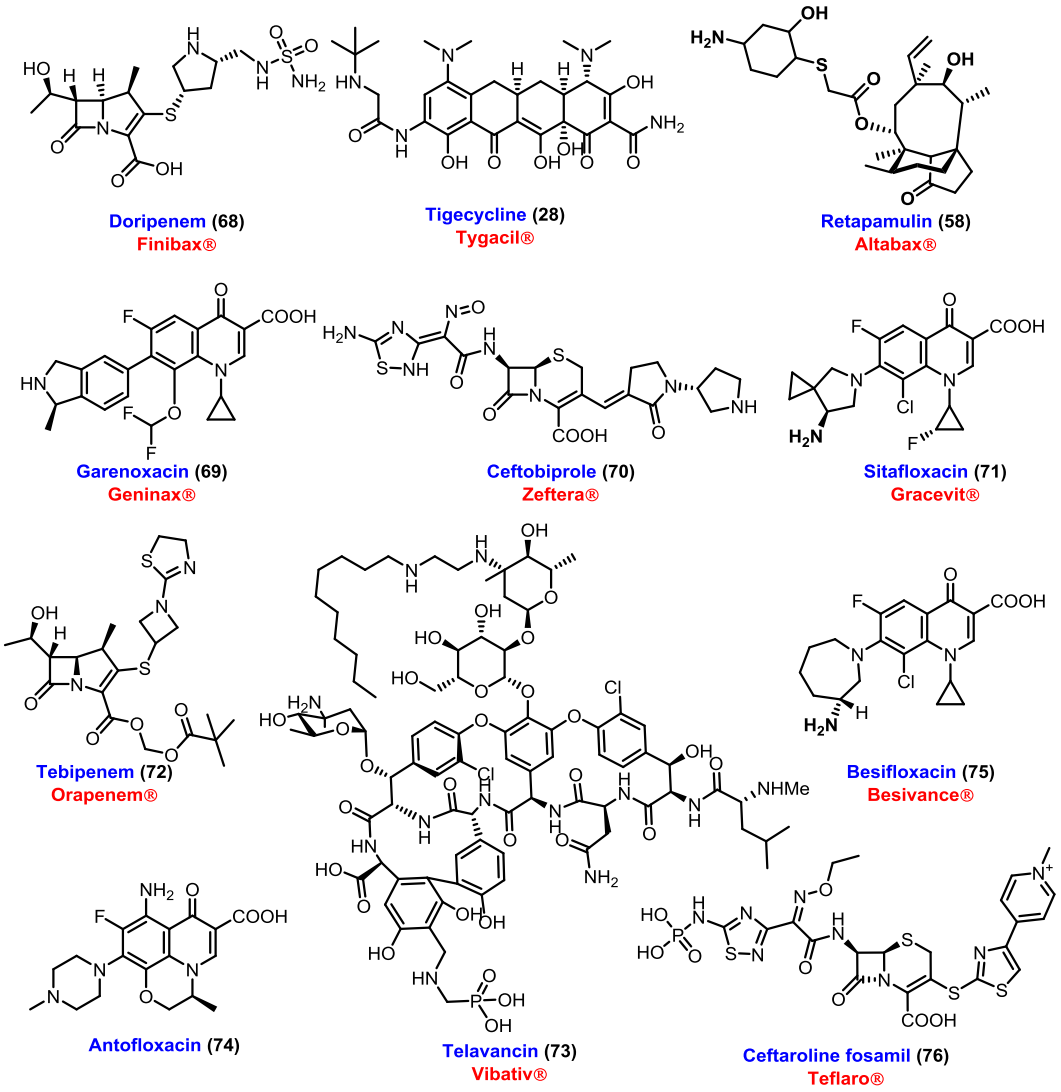



**Figure 9:** Structures of selected antibacterial drugs approved during the period of 2000-2015

drugs mixed with tazobactam and avibactam respectively which are non-$\beta$-lactam $\beta$-lactamase inhibitors. All these drugs have a broad spectrum of activity inhibiting the bacterial cell wall synthesis. A natural product daptomycin (Cubicin®, 2003) belonging to lipopeptide class of antibiotics, was approved as a drug against the systemic and life-threatening infections caused by gram-positive organisms. Tigecycline (Tygacil®) is a

tetracycline antibacterial drug of new subclass glycylcycline approved in 2005 that is administered intravenously.

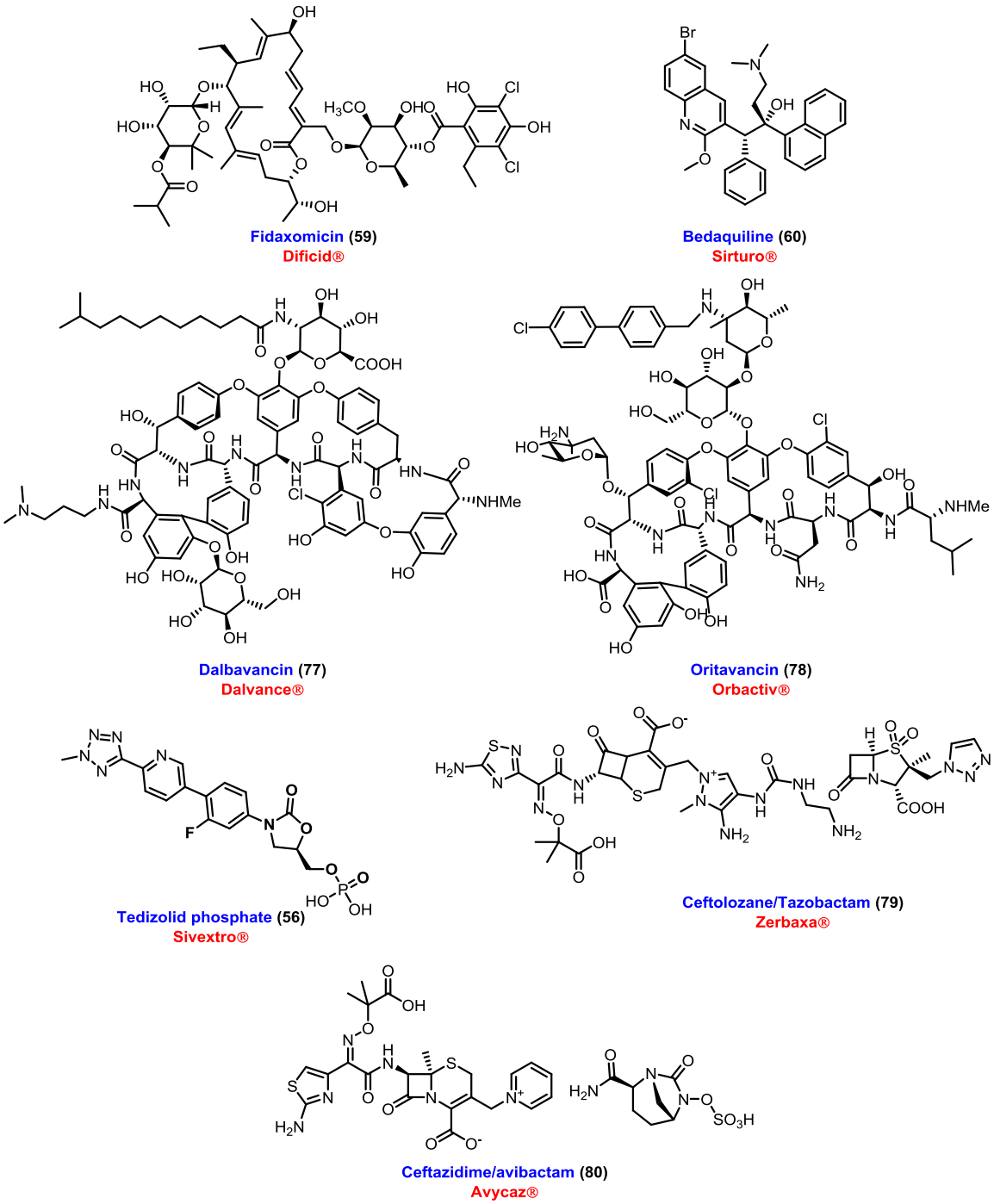



**Figure 10:** Structures of selected antibacterial drugs approved during the period of 2000-2015

Retapamulin (Altabax®) is another drug of a new class of antibacterial agent pleuromutilin which is approved in 2007 for the treatment of bacterial skin infections and it selectively inhibits the bacterial protein synthesis. The glycopeptide antibacterial, telavancin was approved in 2009, while dalbavancin and oritavancin were approved in 2014. Telavancin is a drug derived from natural product vancomycin while dalbavancin and oritavancin are natural products of the same family. All the three drugs work through the cell wall synthesis inhibition. Fidaxomicin (Dificid®) is a tiacumicin class of natural product approved in 2011, for the treatment of *Clostridium difficile* infection. It shows the bactericidal activity by binding and preventing the motion of switch region of bacterial RNA polymerase.

The natural products which are currently in clinical development (Phase-III) are listed in table 2. The omadacycline and ervacycline are a tetracycline based natural product in clinical studies, where omadacycline is being developed for the treatment of serious community-acquired infections while ervacycline is developing against the complicated intra-abdominal infections and complicated urinary tract infections.[23] Lifamulin is a pleuromutilin class of drug in phase-III clinical studies. It is a semi-synthetic compound that inhibits the synthesis of bacterial protein, which is essential for the bacterial growth.

**Table 2:** Antibacterial natural products in clinical development (Phase-III)[44, 45]

| Development Phase | Drug Name | Class | Lead source | Bacteria Type |
|---|---|---|---|---|
| Phase -III | Omadacycline | Tetracycline | NP derivative | G +ve |
| Phase -III | Eravacycline | Tetracycline | NP derivative | G +ve |
| Phase -III | Lefamulin | Pleuromutilin | NP derivative | G +ve |
| Phase -III | Plazomicin | Aminoglycoside | NP derivative | G +ve |
| Phase -III | Solithromycin | Macrolides | NP derivative | G +ve |
| Phase -III | Surotomycin | Lipopeptide | NP derivative | G +ve /G -ve |
| Phase -III | Imipenem/ cilastatin+relebactam | Carbapenem | NP derivative | G +ve /G -ve |

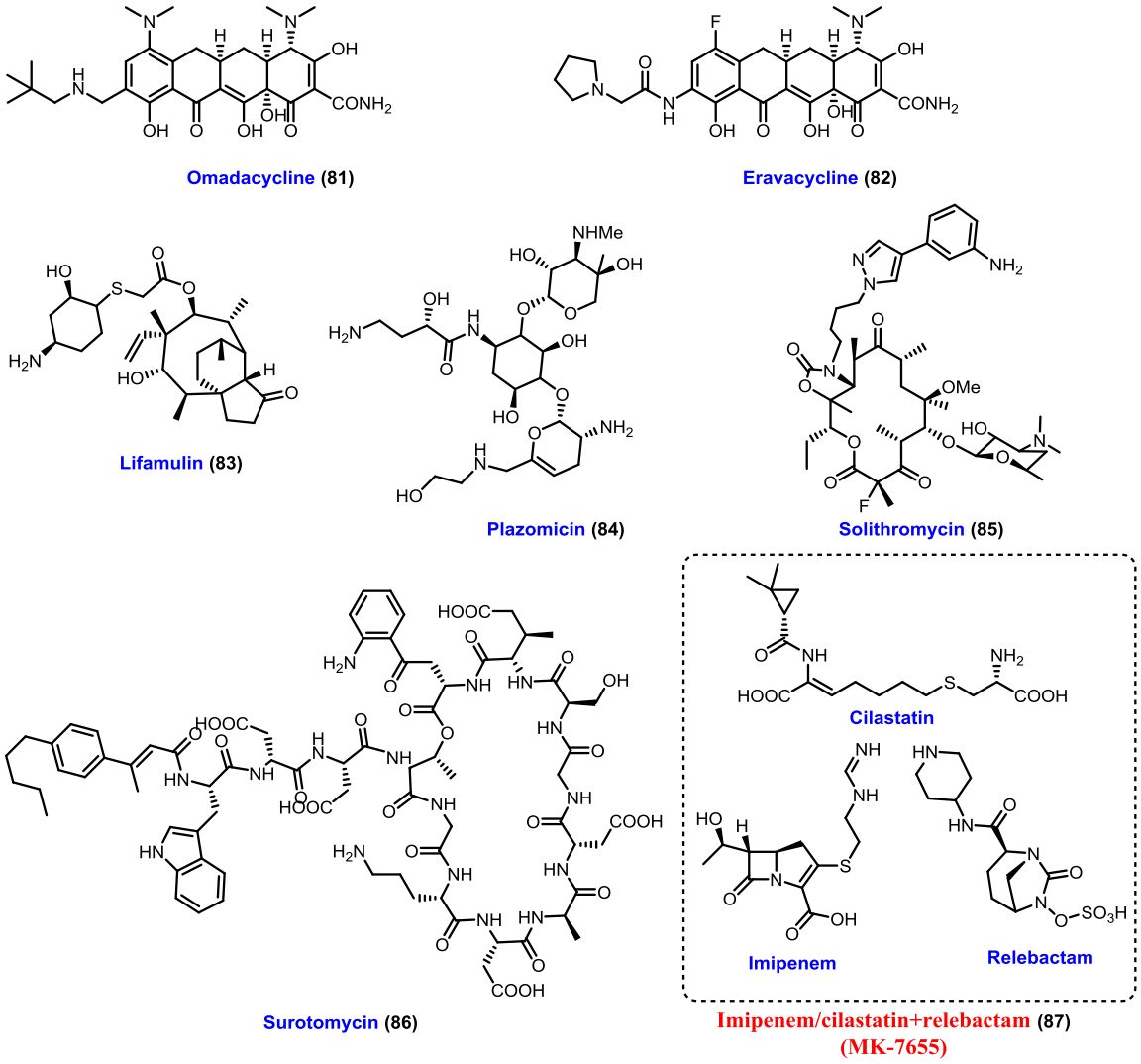



**Figure 11:** Antibacterial natural product/ NP derivatives currently in phase-III[44, 45]

The macrolide class antibacterial agent solithromycin is also undergoing the phase-III clinical trials which showed excellent in vitro activity against a broad spectrum of gram-positive respiratory tract pathogens including the macrolide-resistant strain. Plazomycin is next generation aminoglycoside antibacterial undergoing the clinical studies for the treatment of the urinary tract infections. The lipopeptide antibacterial agent surotomycin is developing against the gram positive bacterial strain of *C. difficile* associated diarrhea

while a fixed dose combination drug imipenem/cilastatin+relebactam (MK-7655) is undergoing the clinical studies for the treatment of complicated intra-abdominal infections and urinary tract infections.

There are several molecules from natural sources or based on natural product scaffolds which are under active development at various stages of development (Phase-II, Phase-I and preclinical). Due to high numbers and space limitation in present thesis, our discussion is limited to only approved/marketed drugs and molecules in Phase-III clinical trials. In general, most of the antibacterial natural products are complex in nature and pose a challenge to synthetic chemists. However, a significant number of natural product scaffolds of small in size and less complex structures have been discovered over decades of natural product research. Often they were isolated in small quantities which are not sufficient for biological evaluations. Hence there is a need for making the natural products through total synthesis, particularly which is scarcely available from nature. In addition, these already discovered scaffolds as antibacterial agents provide potential starting points for making new compounds/derivatives that could provide leads in the short period, which ultimately can provide much desired antibacterial drugs. Along these lines, present thesis work is focused and details are provided in next three chapters. Chapter 2 describes efforts toward latocillin related compounds; Chapter 3 describes on diaportheone B, dehydroxydiversonol, and their analogs. The final chapter includes the synthesis of diarylheptanoid class natural products.

## 1.4. Conclusions

As discussed above, the natural products remain to play an important role in the discovery of new chemical entities as most of the antibacterial agents were derived from the natural product leads such as $\beta$-lactams, aminoglycosides, cephalosporins, chloramphenicol, tetracyclines, macrolides, lincosamides, streptogramins, glycopeptides, rifamycins and lipopeptides. Despite a substantial decline in natural product research at many pharmaceutical companies over the last few decades, natural products have unquestionably been a fertile and unmatched source for new leads as antibacterial agents. The emergence of antimicrobial resistance has lead to the ineffectiveness of a large number of the current antimicrobials in use. There is an urgent need for complete understanding of the various aspects of drug resistance in microbes which can help in the choice of good targets and vital for the discovery of new antibacterial drugs. Continuous research in the area of natural products has been an important area for the antibacterial drug discovery. With recent developments in methods for uncovering new natural products, improved organic synthesis tools, and chemical biology tools for target elucidation, this area of antibacterial research can continue to provide new medicines for the human use. The present thesis is aligned towards the identification of novel antibacterial leads based on natural products through chemical synthesis.

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# *Chapter 2*

# Studies toward Total Synthesis of Lactocillin and its Structurally Simplified Analogs

## 2.1. Introduction to thiopeptides

Thiopeptide antibiotics are interesting and are a growing class of sulfur-rich polythiazolyl peptides. These are a group of macrocyclic peptides produced by various bacteria, mainly soil derived bacteria but also from the marine sources. Many members of thiopeptide family exhibit potent biological activities against various drug-resistant bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococci* (VRE). In addition to the antibacterial activity, thiopeptides also possess antimalarial and anticancer activity.[1]

The well known thiopeptide antibiotic thiostrepton was isolated from *Streptomyces azureus* by Donovick et al. in 1954 and its structure was solved by Hodgkin in 1970.[2,3] Till date almost 100 thiopeptide based natural products are known in the literature and possess very complex structures and contain a characteristic macrocyclic core that consists of a six-membered nitrogen-containing heterocycle, central to multiple thiazoles, oxazoles or thiazolines, and dehydroamino acids but vary in side chains or rings. Most of the thiopeptides are differentiated based on their central heterocyclic ring system which is also responsible for the biological activity of thiopeptides.

## 2.2. Classification of thiopeptides

Thiopeptide antibiotics are classified according to structure, in particular, using the nature of the central heterocyclic domain into five different sub classes.[4]

**a-series thiopeptides:** This series contains thiopeptide compounds with totally reduced central six-membered heterocyclic ring as piperidine. The first thiopeptide antibiotics thiostrepton (thiactin/bryamycin) along with Sch18640 and some thiopeptins like thiopeptin A1a, thiopeptin A3a, thiopeptin A4a and thiopeptin Ba contain the completely saturated central piperidine ring fall into this category.

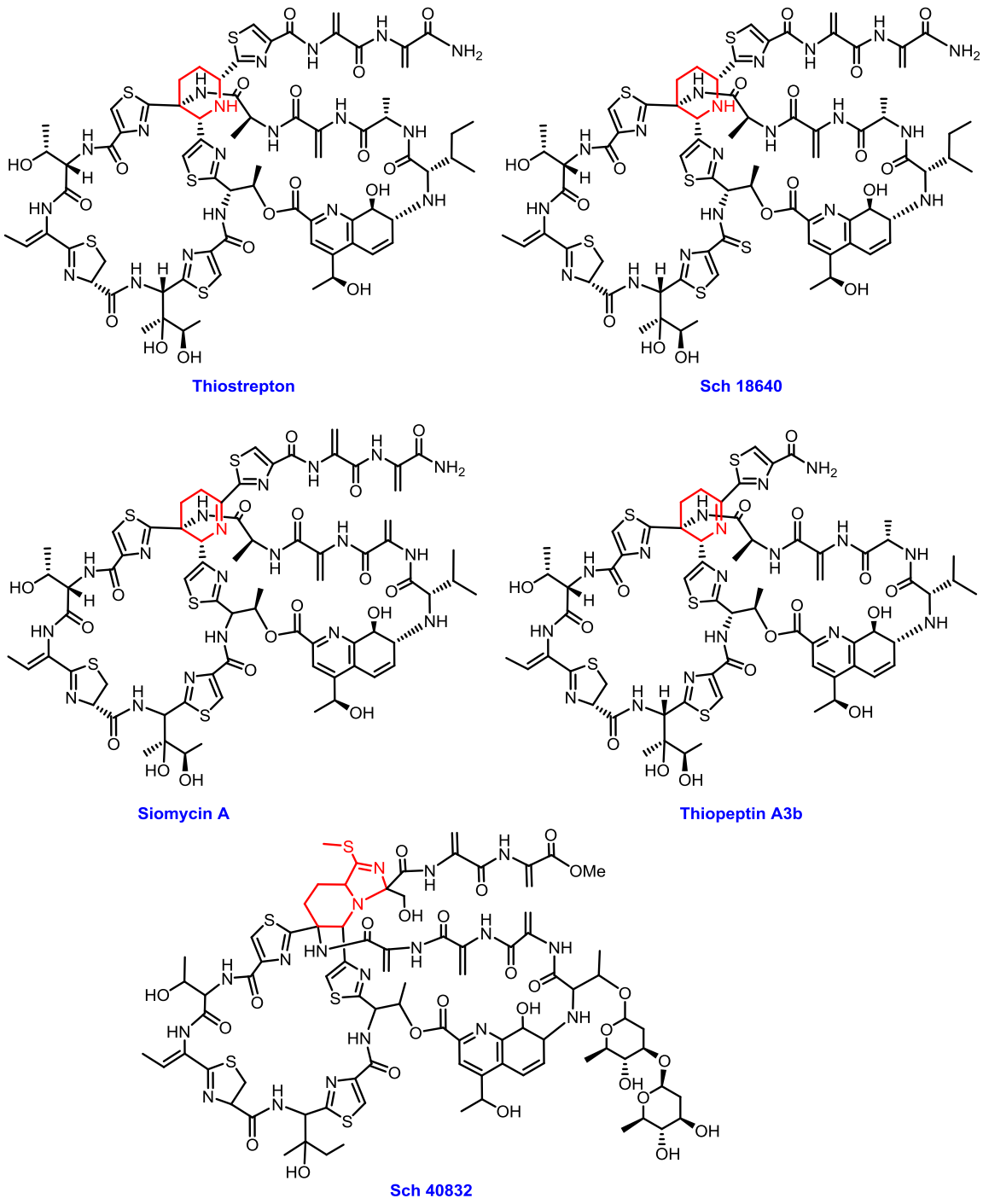



**Figure 1:** Structures of 'a', 'b' and 'c-series' thiopeptides

**b-series thiopeptides:** This series is oxidized form of six-membered heterocycle and contains a 1,2-dehydropiperidine ring. Some thiopeptins like thiopeptin A1b, thiopeptin

A3b, thiopeptin A4b and thiopeptin Bb and siomycin A contain the oxidized form of a central heterocyclic ring as 1,2-dehydropiperidine belong to this class.

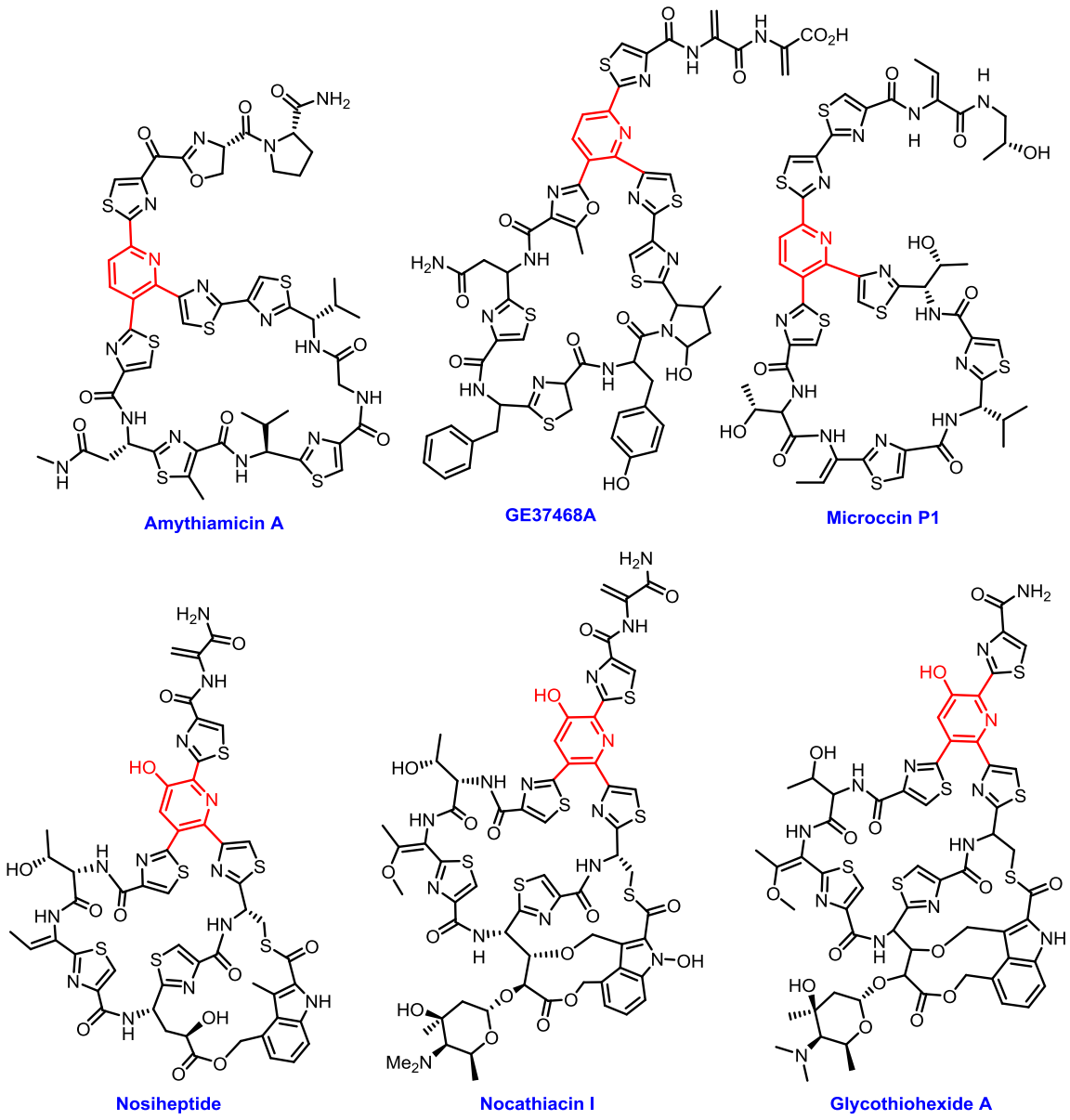



**Figure 2:** Structures of selected 'd' and 'e-series' thiopeptides

**c-series thiopeptides:** Only one member of c-series thiopeptide, Sch 40832 has been reported and it contains a piperidine ring fused with imidazoline ring.

**d-series thiopeptides:** The 'd-series' contains trisubstituted pyridine ring, predominantly only one macrocycle loop and centered around 2,3,6-trisubstituted pyridine clustered with thiazole or oxazole heterocycles. There are several thiopeptide antibiotics (~50 nos.) belong to this class and show the diversity in their structures as well as biological activities. e.g. amythiamicin, micrococcin P1, promothiocin, radamycin, thiocillin and recently isolated thiopeptide lactocillin.

**e-series thiopeptides:** The 2,3,5,6-tetrasubstituted pyridine core is a central heterocyclic ring in this class of thiopeptides. In most cases, the 5-position is substituted with hydroxy or alkoxy functionality. In this series, the peptide backbone is divided into two loops and one of them is made up with indole or hydroxy indole connected by ester or thioester. A dozen compounds which are structurally distinct belong to this class. e.g. nosiheptide, nocathiacin, glycothiohexide.

## 2.3. Mechanism of action of thiopeptide antibacterials

Thiopeptides are well known to be antibacterial agents, but their medicinal potential is broad and known to possesses a wide variety of biological functions. It is well known that thiopeptides exert their antibacterial functions by inhibiting the ribosomal protein synthesis. The mechanism of thiopeptide antibiotics depends on the size of the macrocycle and it may be 26-membered, 29-membered or 35 membered ring, depending on the number of residual aminoacids present in the macrocyclic loop. The thiopeptides containing 26-membered macrocycle,[5] such as thiostrepton, micrococcin P1,[6] siomycin A are known to bind the guanosine triphosphatase (GTPase) associated region of ribosomes or 60s ribosomal L11 protein complex. By binding with GTPase or forming a complex with the L11 protein, the thiopeptides block binding region of the elongation factor G (EF-G) and inhibits the translocation of growing peptide chain or *t*-RNA complex in the ribosome.[5] The thiopeptides with 29-membered macrocycles like GE37468A, binds to thermo unstable elongation factor (EF-Tu), blocking its *t*-RNA or aminoacyl complex binding site, as a result, the complex can not be delivered in the ribosome and peptide elongation does not take place.[7] The thiopeptides with the 35-membered macrocycles

show potent antibacterial activity; however, their mode of action or molecular target is yet to be discovered.[8]

## 2.4. Lactocillin: isolation, characterization and biological activity

Lacticillin (**1**) is a thiopeptide antibiotic isolated from the *lactobacillus gassari* as a part of human microbiome project (HMP) by Fischbach and co-workers in the year 2014.[9] HMP is a United States National Institutes of Health's initiation towards the identifying and characterizing the genomes of human-associated bacteria.[10a] Thiopeptide antibiotics are widely distributed in the genomes of human microbiota. Fischbach and co-workers purified and determined the structure of new thiopeptide antibiotic called lactocillin (**1**) from the vaginal commensal *lactobacillus gasseri*, a gram-positive, rod-shaped, non-spore-forming bacteria. This is anaerobic bacteria and found in the gastrointestinal tracts of humans and animals due to its fermentative function, although it is common in the gastrointestinal tract, its very important location is a vaginal tract of pregnant or normal women and it is one of the prominent members of women vaginal microbiota.

**Scientific Classification**

Kingdom: Bacteria
Phylum : Firmicutes
Class : Bacilli
Order : Lactobacillales
Family : Lactobacillaceae
Genus : *Lactobacillus*
Species : *gassari*

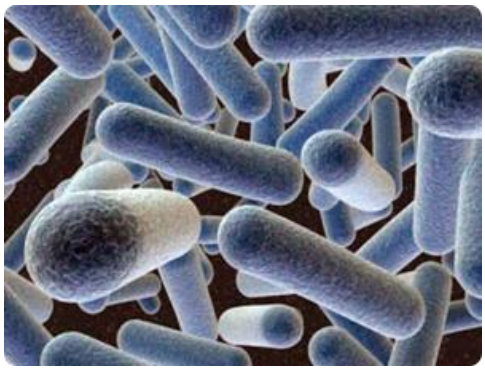


**Figure 3:** *Lactobacillus gassari* a rod-shaped bacteria[10b]

In order to obtain milligram quantities of the lactocillin (**1**) for the structural characterization and biological profiling, Fischbach and co-workers cultivated in laboratory conditions (50 L of *L. gassari* JV-V03) but initial experiments were failed because of its low solubility in different NMR solvents and apparent instability which

made them to hypothesize that the lactocillin (**1**) contains free carboxylic acid, probably responsible. By considering this, crude material obtained from the solid-phase extraction was subjected TMS-diazomethane reaction to convert any free carboxylic acid to corresponding methyl esters. The methyl ester remarkably improved stability and solubility of the compound in the NMR solvents. The crude reaction mixture of methyl ester was purified by the reverse phase HPLC to yield small pure samples of lactocillin methyl ester (~ 0.5 mg).

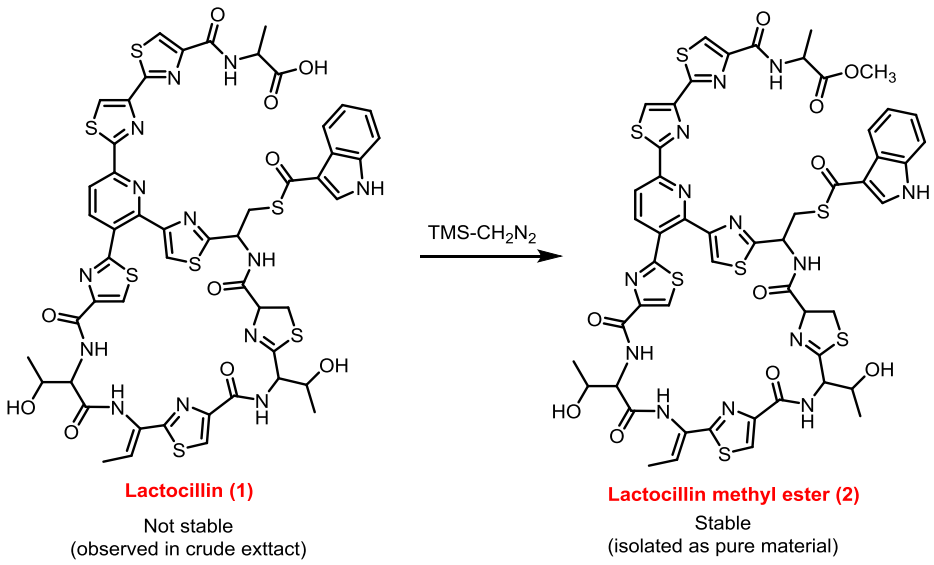



**Figure 4:** Structures of lactocillin (**1**) and lactocillin methyl ester (**2**)

Structural elucidation of the lactocillin methyl ester was done with the help of various analytical tools. Initially, with the help of high resolution-ESI orbitrap data where $[M+H]^+$ was observed at m/z 1238.16916, (calculated for $[M+H]^+$ at m/z 1238.16916, Δ ppm = - 0.02) which was consistent with the empirical formula $C_{52}H_{47}N_{13}O_{10}S_7$. Based on the computational study and $\lambda_{max}$ at 350 nm, it was predicted that lactocillin would have a similar core structure to 'd-series' thiopeptides with trisubstituted pyridine core along with a 26-membered macrocycle in which peptide chain undergoes a series of cyclization, dehydration, and pyridine ring-forming post-translational modifications. With the help of different 1D and 2D NMR experiments, high-resolution orbitrap experiments, L-tryptophan-$d_5$ (indole-$d_5$) feeding experiments and isotope labeling

methods, the structure of lactocillin was established as drawn in Figure 4. The stereochemistry of lactocillin (**1**) at various locations was not determined by the authors because of the scarcity of material and low solubility of the compound in NMR solvents. However, the stereochemistry of residues present in the lactocillin is expected to be same as that of closely related and structurally established molecules which have been documented in the literature because of biogenetic considerations. Similar to other thiopeptides of d-series, lactocillin (**1**) also showed potent antibacterial activity against the variety of gram-positive bacterial pathogens ranging from the 42-425 nM.[9] Antibacterial activity of lactocillin determined by Fischbach and co-workers is captured here for the ready reference.

**Table 1**: Antibacterial activity of lactocillin (**1**) against different bacterial pathogens.

| **Bacteria** | **Description** | **Lactocillin MIC (nM)** |
|---|---|---|
| *Staphylococcus aureus* | Pathogen | 42 |
| *Enterococcus faecalis* | Pathogen | 425 |
| *Corynebacterium aurimucosum* | vaginal pathogen | 42 |
| *Gardnerella vaginalis* | vaginal pathogen | 212 |
| *Streptococcus sanguinis* | oral commensal | 212 |
| *Streptococcus sobrinus* | oral commensal/pathogen | 85 |
| *Streptococcus mutans* | oral commensal/pathogen | 425 |

## 2.5. Present work

The other two antibiotic thiopeptides which are closely related to the structure of lactocillin are (i) micrococcin P1 (MP1) and (ii) thiocillin I. The MP1 thiopeptide was discovered by T. L. Su from the sewage collected from the city of oxford, UK.[6] It has unique structure of ‘d-series’ thiopeptide with 2,3,6-trisubstituted pyridine core with only one loop of 26-membered macrocycle containing the dehydroamino acids and cyclic thiazole residues. MP1 showed a wide range of activities against a variety of gram-positive bacterial strains, but none of the gram-negative bacteria were affected.[11] The

second thiopeptide called thiocillin I, a product of posttranslational modification of ribosomally generated peptides, having 50-60 residues also has similar structural units[12] as that of MP1 except the *L*-valine in MP1 versus the hydroxybutyric acid derivative in thiocillin I in the 26-membered macrocyclic loop. Thiocillin I also showed potent antibacterial activity with minimum inhibitory concentrations (MICs) of 0.2-0.9 μg/mL and < 0.03-0.1 μg/mL, respectively against the *Bacillus subtilis* and two MRSA strains. The binding study of thiostrepton also showed that the 26-membered loop binds to the apicoplast 23s rRNA which inhibits the bacterial protein synthesis.[13]

As discussed above, lactocillin (**1**) share a common structural core as 2,3,6-trisubstituted pyridine core and 26-membered macrocyclic loop with a dehydrobutyrine unit similar to the MP1 and thiocillin I. It differs only amino acid residues of macrocycle as *L*-valine in MP1 replaced with the *L*-threonine, thiazole ring with thiazoline ring and cysteine unit instead of threonine thiazole ring attached at 2-position of pyridine core. The open peptide chain of MP1 and thiocillin I was replaced with the simple alanine unit in lactocillin (**1**).

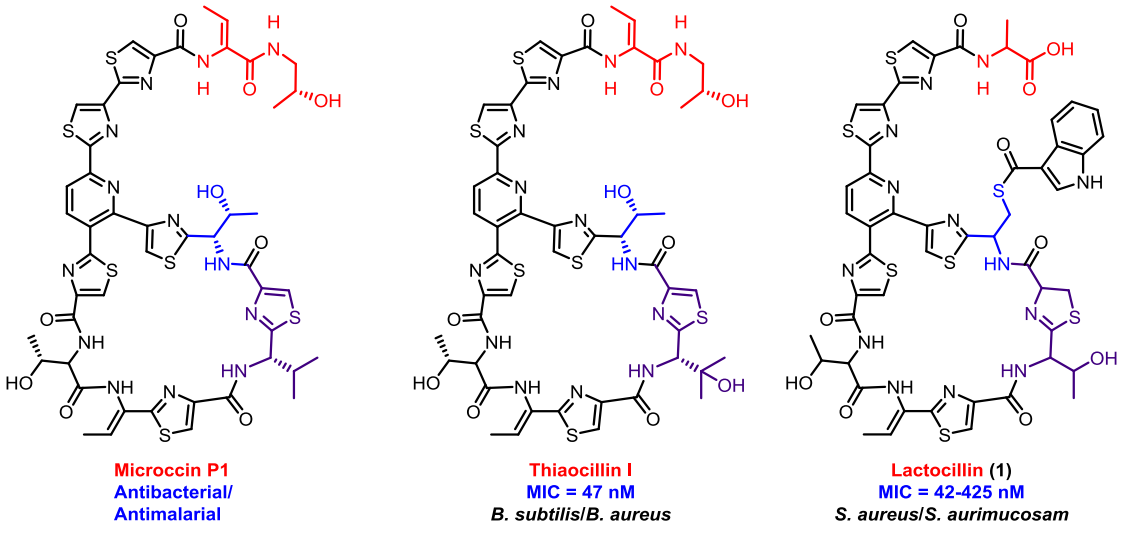



**Figure 5:** Comparative structural analysis of MP1, thiocillin I and lactocillin (**1**)

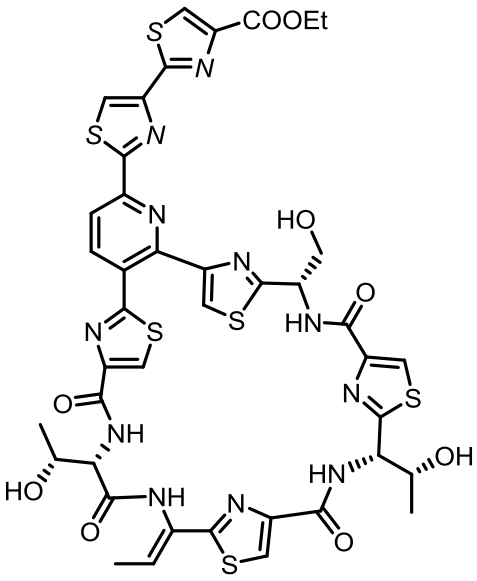
The key structural features of lactocillin (**1**) and other related d-series thiopeptides are

- 2,3,6-Trithiazolyl substituted pyridine core.
- Canonical 26-membered thiopeptide loop.

- Single dehydrobutyrine residue in the macrocyclic loop.
- Macrocycle with four cysteine derived heterocycles.

Considering the close structural resemblance of thiopeptides, its wide range of biological activities and the importance of thiopeptides as emerging novel class of antibiotics, we became interested in the total synthesis of newly isolated thiopeptide lactocillin (**1**) and it's structurally simplified analogs with all key features of d-series thiopeptide. initially, we were interested in the synthesis of model thiopeptide (**3**) and similar strategy later can be used for the total synthesis of lactocillin (**1**).

Based on the above information such as structural information and impressive biological activities, we have designed a model thiopeptide (**3**), having all the key features of thiopeptide antibiotics, in particular, those of MP1, thiocillin I and lactocillin. As the stereochemistry of lactocillin (**1**) was not determined, based on the MP1 and thiocillin I which are again considering by biogenetic considerations, we have decided to start with all natural amino acids. While designing model peptide **3** and synthetic route to access the target, we also considered the synthetic feasibility of the target, the possibility of generating a small focused library of compounds around the skeleton with an eventual plan of making original lactocillin. According to our plan, the designed target thiopeptide **3** is expected to show similar biological properties as that of MP1, thiocillin, lactocillin (**1**) and other d-series thiopeptide antibiotics.[14] It is also expected to take care of problems such as poor stability, scarcity of the material for biological profiling, systematic structure-activity relationship studies and ultimately pave the way towards discovering antibiotics based on thiopeptides.




**Model thiopeptide (3)**

**Figure 6:** Structure of model thiopeptide (**3**).

## 2.5.1. Retrosynthetic approach

Retrosynthetically the designed model thiopeptide (**3**) was planned by stitching two different fragments together, fragment A (**4**) and fragment B (**5**). The fragment A (**4**) is a trithiazolylpyridine core which can be synthesized using the Eiden-Herdies pyridine ring synthesis method[15] from the ynone partner (**6**) and ketone partner (**7**). The synthesis of ynone partner (**6**) could be achieved from the dithiazolyl ester (**8**) which can ultimately synthesized from the monothiazole ester (**9**). The ketone partner (**7**) could be synthesized from the thiazole **10** and TBS-protected thiazole alcohol (**11**) using the literature methods.[15, 17]

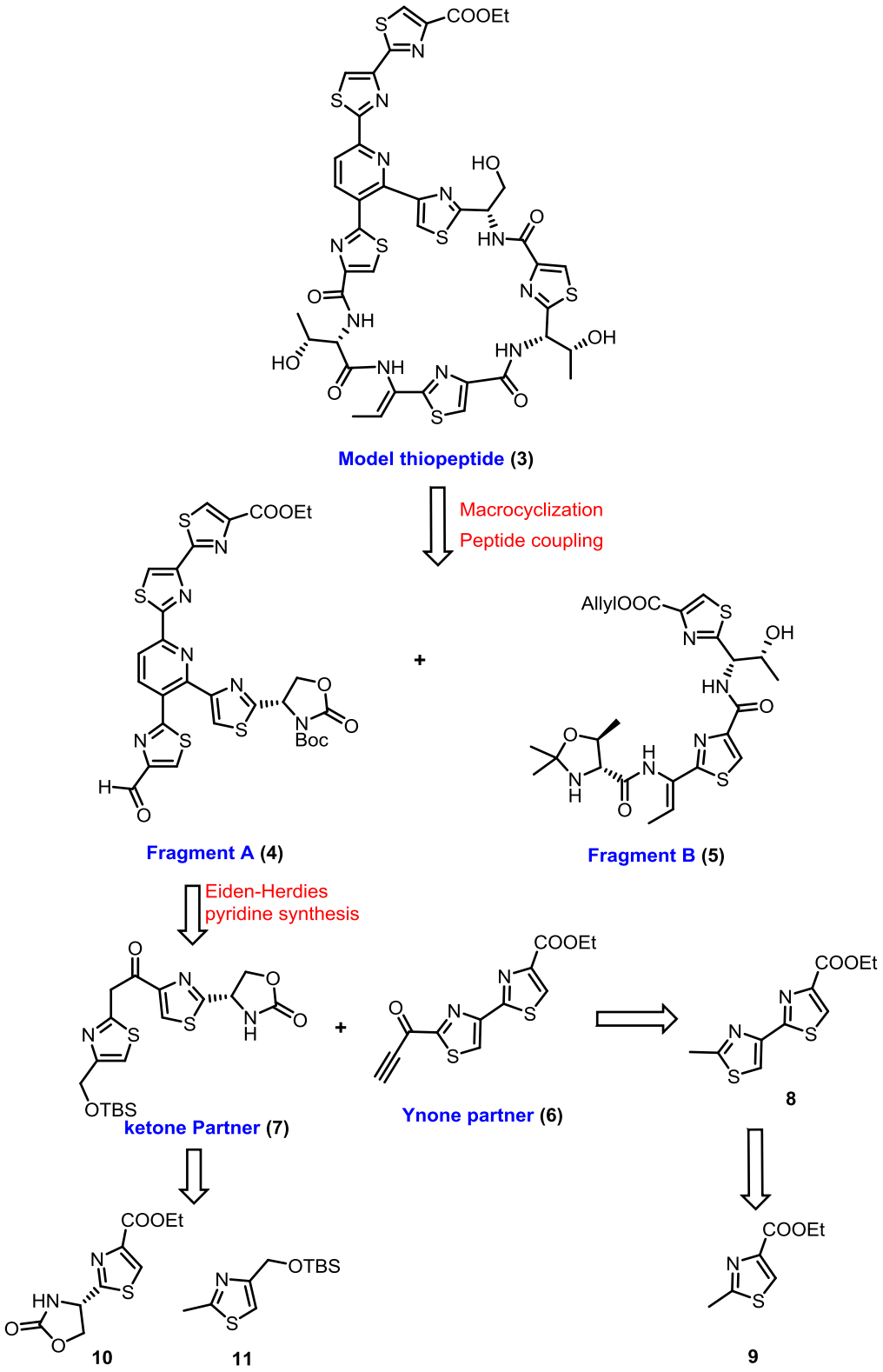



**Figure 7:** Retrosynthetic approach for the designed thiopeptide (**3**).

The fragment B (**5**) is a peptide fragment containing the two thiazole rings. The synthesis of peptide fragment was planned through the coupling reactions between the allyl ester of threonine thiazole (**12**) and compound **13**. The compound **13** could be synthesized from the Boc-acetonide protected threonine (**14**) with the free amine of the ethyl ester of thiazole **15** which in turn could be synthesized using the known reaction sequence starting from the *L*-threonine.[19] Compound **12** is an analog of thiazole ester **15** where ethyl ester exchanged with allyl ester.

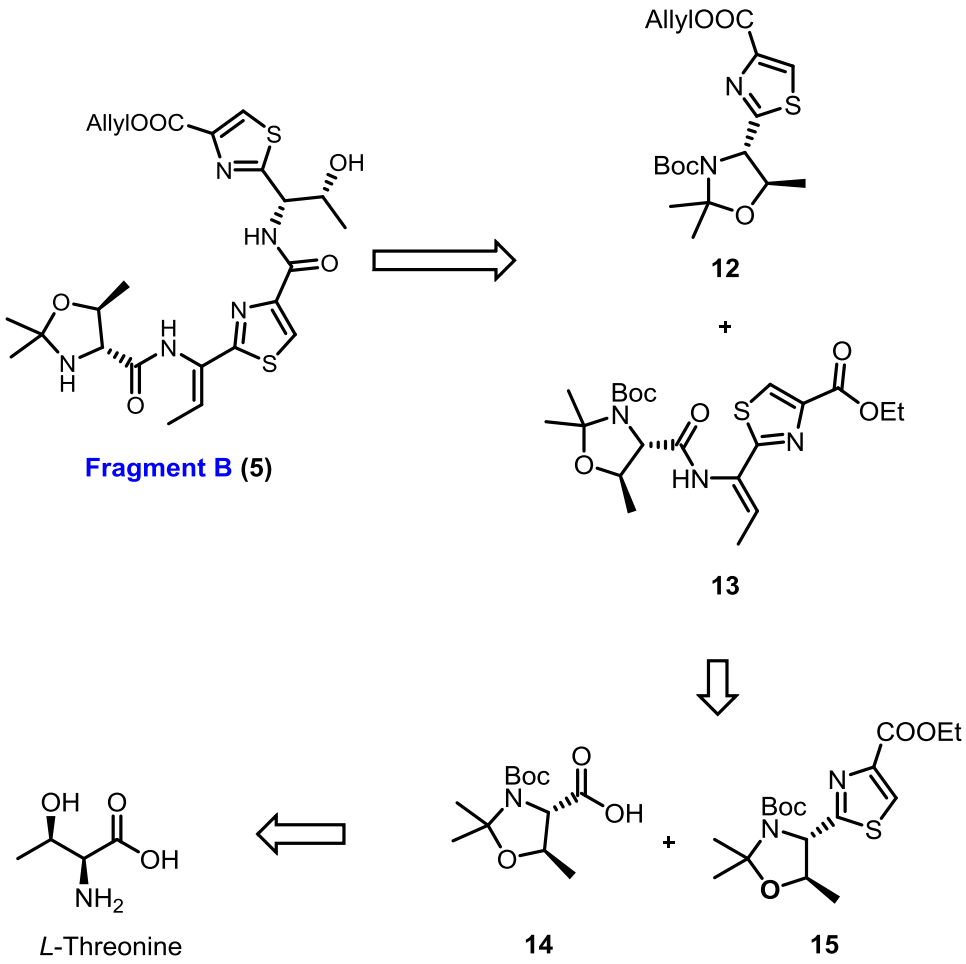
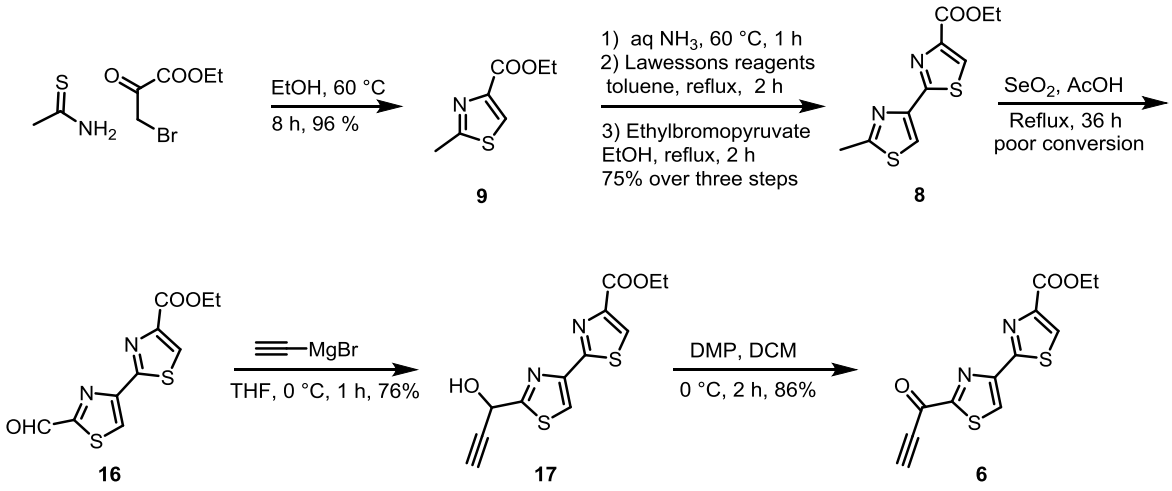



**Figure 8:** Retrosynthetic analysis of fragment B (**5**)

## 2.5.2. Synthetic attempts toward model thiopeptide

### 2.5.2.1. Synthesis of fragment A

Our planned synthesis started with the synthesis of thiazole ester (**9**) which was synthesized by treatment of ethyl-3-bromopyruvate with thioacetamide in ethanol at 60 ºC following the known procedure.[16] The mono thiazole ethyl ester (**9**) is then treated with the aqueous ammonia to get the corresponding amide as pure product, which was confirmed by MS analysis which showed a peak at m/z of $[M+H]^+$ at 143 corresponding to the molecular formula $C_5H_7N_2OS$. The amide obtained was converted to thioamide using the Lawesson's reagent in refluxing benzene for 2 h, The crude thioamide was then treated with ethyl-3-bromopyruvate which gave us the dithiazole ethyl ester (**8**)[17] and it

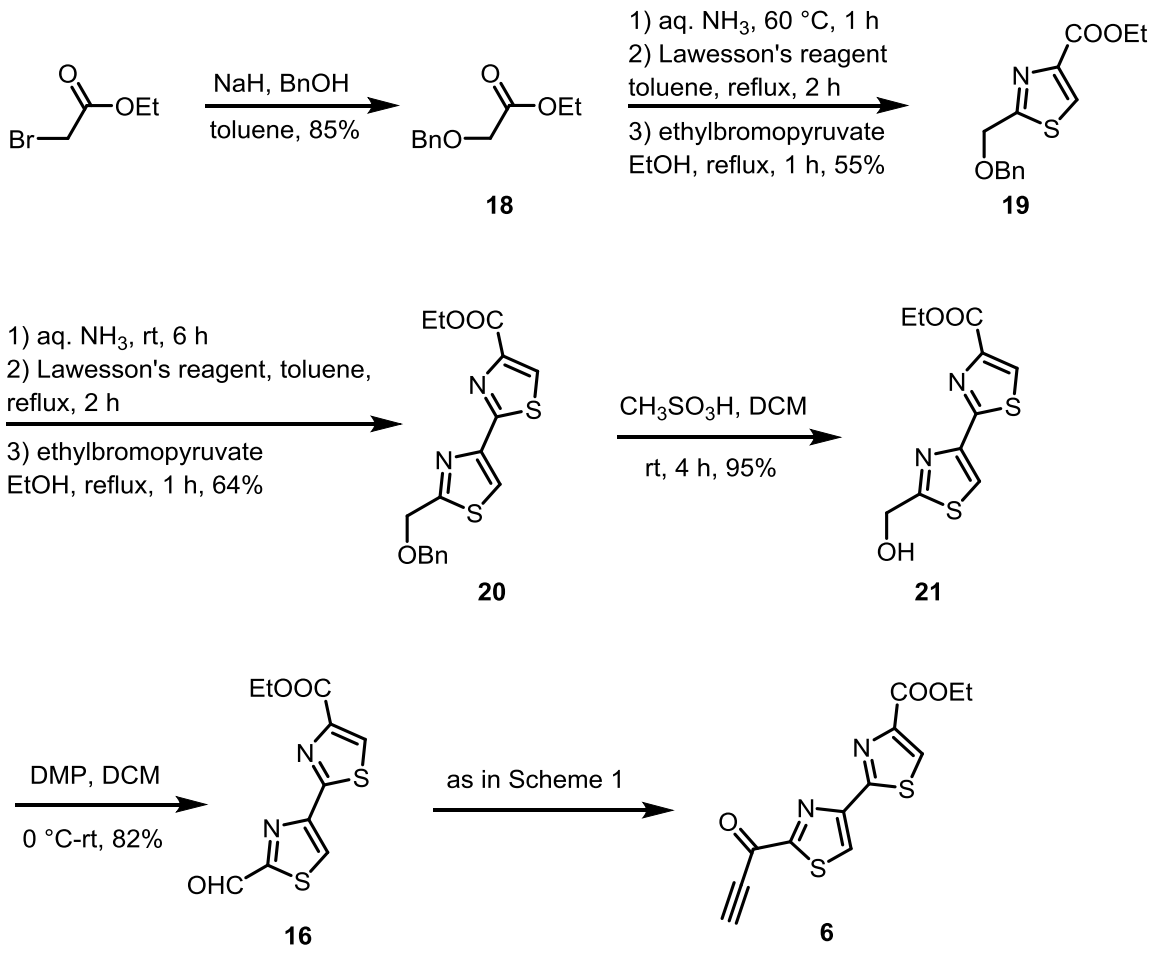



**Scheme 1:** Synthesis of ynone partner (**6**)

was supported by corresponding mass which showed a base peak at m/z 277 of $[M+Na]^+$ corresponding to molecular formula $C_{10}H_{10}N_2O_2S_2Na$. The compound **8** was further confirmed by comparing the $^1H$ and $^{13}C$ NMR with the literature reports. The compound **8** was subjected to the benzylic oxidation using selenium dioxide in acetic acid at 120 ºC using literature procedures[15a] to get the aldehyde **16**. All the spectral data of compound **16** was in agreement with the structure and compared with that of reported

data and found identical. Although it was reported in moderate yield, we have observed the formation of the desired aldehyde in poor yields in our hands. We observed very poor conversion even after refluxing for 36 h. We also attempted this reaction by adding the selenium dioxide after regular interval of time but we could not improve the conversion/yield of this reaction. The aldehyde **16** was treated with the ethynylmagnesium bromide in dry THF at 0 ºC for 30 minutes to furnish the propargylic alcohol **17**, which on oxidation with Dess-Martin periodinane resulted into the required ynone **6** in excellent yield (Scheme 1). In the $^{1}$H NMR spectrum presence of characteristic proton attached to sp-carbon at δ 3.68 ppm and $^{13}$C NMR spectra, where the acetylene carbon was observed at δ 84.0 and 79.1 ppm confirmed the formation of compound **6**, the observed data was also compared with literature reports and found to be identical.[17] It was further confirmed by using HRMS which showed a peak at m/z 314.9862 corresponding to the molecular formula $C_{12}H_8O_3N_2S_2Na$, $[M+Na]^+$.

To address the problem of poor yields and conversion and also to get the aldehyde **16** in good quantities, we decided to develop the new route for the gram-scale synthesis of aldehyde **16** and the developed alternate synthetic route is described in Scheme 2. We started with the commercially available starting material benzyl alcohol, which on alkylation using the NaH and ethyl bromoacetate furnished the compound **18** in excellent yield. The ester **18** which on similar series of reactions used for the synthesis of compound **8**, as amide formation followed by conversion to thioamide and treatment with ethyl-3-bromopyruvate resulted in the mono thiazole ester **19**. All the spectral data was in agreement with the assigned structure. The mono thiazole ester **19** was then converted to the dithiazole ester **20** by repeating the similar sequence of reaction with overall good yield. The compound **20** was subjected for benzyl deprotection with methanesulfonic acid in dry DCM at room temperature gave us the alcohol **21** with excellent yield. Although this condition was reported in the literature, it was used on very few occasions.[22] The resulting alcohol was oxidized to the corresponding aldehyde **16** under Dess-Martin periodinane conditions with excellent yield. All the spectral data of the compound **16** was

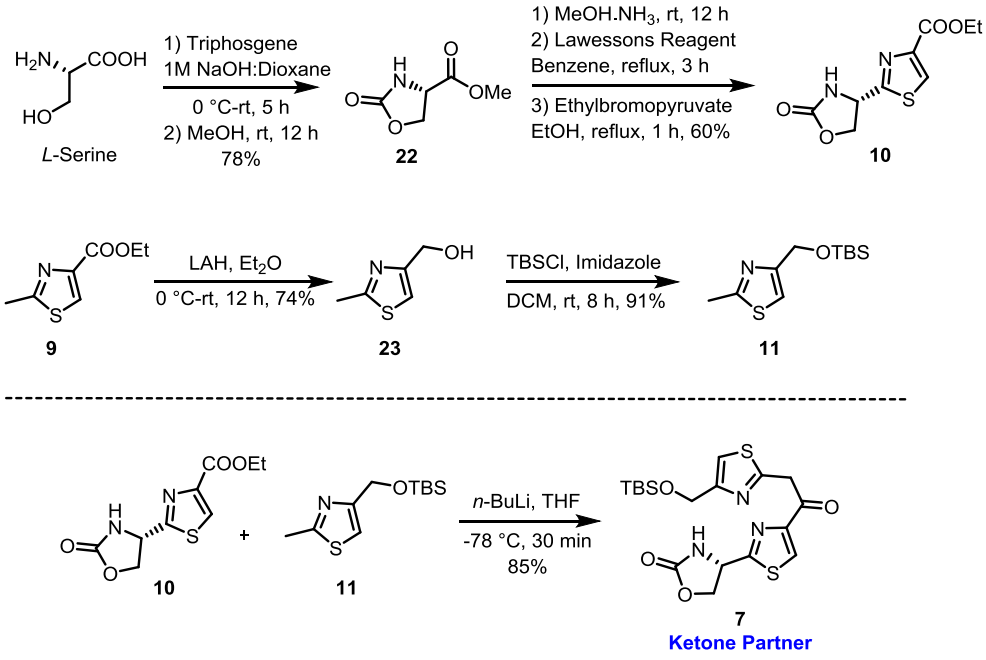



**Scheme 2:** Gram-scale synthesis of ynone partner (**6**)

identical in all respects with the compound prepared using the route described in Scheme 1. Having developed the route for the gram-scale synthesis of aldehyde **16** with an overall yield of 25% (Scheme 2), it was later converted to the ynone compound **6** as described in Scheme 1.

The synthesis of ketone partner was started from the *L*-serine, which on carbamate protection using triphosgene and 1N NaOH in dioxane at 0 ºC, the crude residue after evaporation of the solvent was stirred with methanol to furnish methyl ester (**22**).[18] The formation of ester **22** was confirmed by comparing its $^{1}H$ and $^{13}C$ NMR spectral data with literature reported values. The compound **22** was then treated with a catalytic amount of

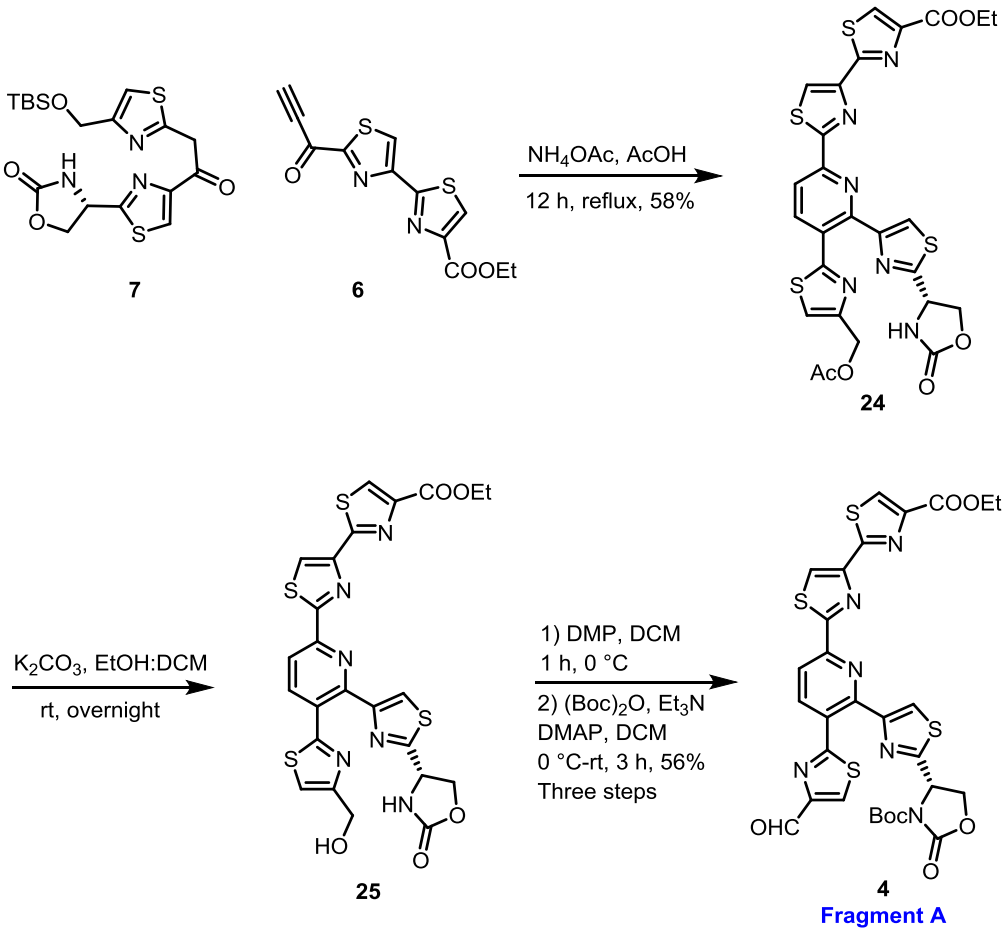
DMAP and saturated solution of ammonia in methanol to furnish the corresponding amide. The formation of amide was confirmed by mass which shows a peak at m/z 131 corresponding to molecular formula $C_4H_6N_2O_3$, $[M+H]^+$. The amide on treatment with Lawesson's reagent in benzene under reflux conditions was converted to the corresponding thioamide and the resulting crude thioamide was again treated with ethyl-3-bromopyruvate in refluxing ethanol to furnish the desired thiazole ethyl ester (**10**) with 60% yield over three steps. The spectral data of **10** was in agreement the product formation. The compound **10** was then treated with the compound **23** (prepared from the compound **9** by LAH reduction followed by TBS protection of alcohol) in presence of *n*-butyl lithium in dry THF at -78 ºC resulted into the keto-enol mixture of compound **7** (Scheme 2), the compound **7** formation was indicated by the mass spectrum which shows a peak at m/z 440 $[M+H]^+$ corresponding to molecular formula $C_{18}H_{26}N_3O_4S_2Si$, The structure was further confirmed by the $^1H$ NMR and $^{13}C$ NMR data in which the keto-enol mixture was observed with ~3:1 ratio. The observation of keto-enol equilibrium was also documented the literature reports on similar compounds.[17]




**Scheme 3:** Synthesis of ketone partner **7**.

**Synthesis of Fragment A Using Eiden-Herdies Pyridine Synthesis**

Having both ynone partner (compound **6**) and ketone partner (compound **7**) in hand, we subjected both the fragments (**6** and **7**) for Eiden-Herdies pyridine synthesis by following the modified procedures.[15] In this reaction, the ynone compound **6** and ketone compound **7** were treated with ammonium acetate in acetic acid under refluxing conditions for 12 h to obtain pyridine derivative (**24**) in moderate yield. The $^{1}$H NMR spectrum showed the presence of characteristic four singlets at δ 8.29, 8.28, 7.97, 7.40 ppm which indicates the presence of four disubstituted thiazole rings in the molecule. It was further confirmed by HRMS which showed the base peak at m/z 583.0344 corresponding to molecular formula $C_{24}H_{19}O_4N_6S_4$ $[M+H]^+$. The obtained compound **24** is then subjected to the acetate hydrolysis with $K_2CO_3$ in ethanol:DCM (1:1) to furnish the alcohol **25**. The obtained crude alcohol **25** was then converted to corresponding aldehyde using Dess-Martin periodinane in DCM followed by treatment of crude aldehyde with $(Boc)_2O$ and $Et_3N$ in a catalytic amount of DMAP in dry DCM to get the fragment A (**4)** (Scheme 4). The $^{1}$H NMR spectrum in which the characteristic proton of aldehyde was observed at δ 10.06 ppm and ethyl ester shows the characteristic signals at δ 4.46 and 1.44 ppm as a quartet and triplet respectively indicates the formation of compound **4**, $^{13}$C NMR spectrum shows the carbonyl carbon of aldehyde at δ 184.2 ppm which supports the formation of aldehyde, it was further confirmed by HRMS showed a base peak at m/z 719.0471 corresponding to the molecular formula $C_{29}H_{24}O_7N_6S_4$, $[M+Na]^+$. The fragment A (**4**) is a structurally interesting compound with six heterocyclic rings along with central pyridine moiety which is common and makes a major component of different members of thiopeptide class of antibiotics.

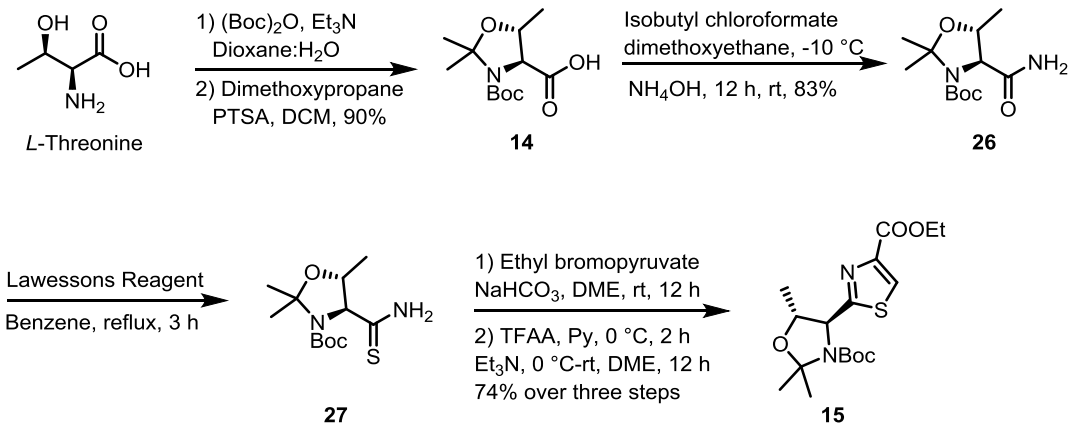



**Scheme 4:** Synthesis of fragment A (**4**) using Eiden-Herdies pyridine synthesis.

### 2.5.2.2. Synthesis of fragment B

The fragment B (**5**) of targeted thiopeptide is a peptide fragment containing the two thiazole rings. Our synthesis of fragment B (**5**) started from the commercially available *L*-threonine which was converted to the threonine thiazole ethyl ester **15** following reported procedure.[19] First, the *L*-threonine was protected using $(Boc)_2O$, $Et_3N$ in 1,4-dioxane-water, the obtained *N*-Boc-threonine was protected as acetonide using 2,2-dimethyoxypropane in DCM and a catalytic amount of PTSA. The product **14** was confirmed by comparing the $^1H$ and $^{13}C$ NMR spectra with the literature reports and found to be identical. The acid **14** was converted to amide **26** using the isobutyl chloroformate in dimethoxyethane followed by treatment with aqueous ammonia with

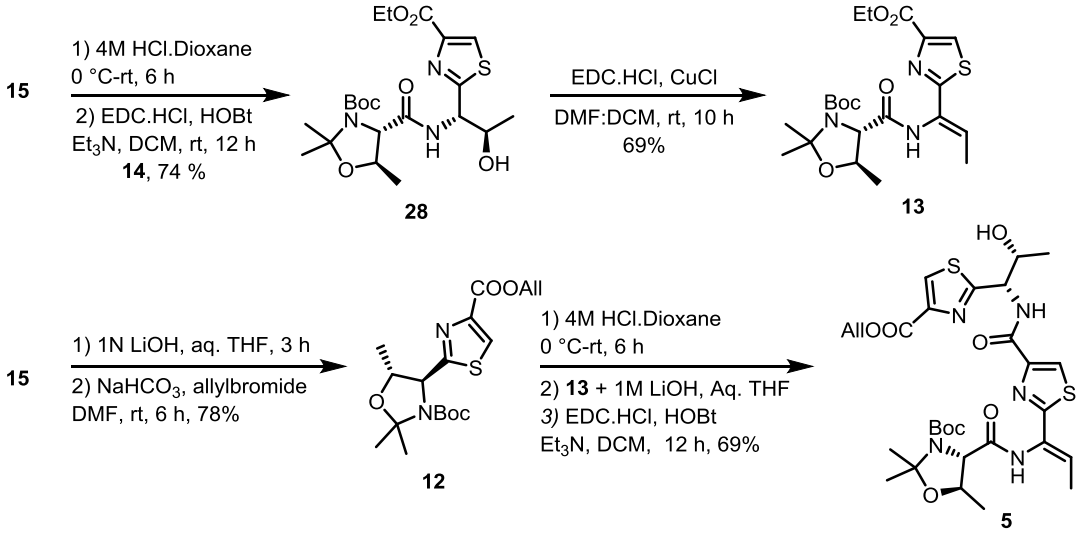
excellent yield. The amide **26** was converted to thiazole **15** via thioamide followed by treatment with ethyl-3-bromopyruvate which was later treated with TFAA and pyridine (Scheme 5). The spectral data of compound **15** was compared with the reported data and found to be identical.




**Scheme 5:** Synthesis of thiazole (**15**)

To accomplish the synthesis of fragment B (**5**), the *N*-Boc and acetonide of thiazole **15** was deprotected using the 4M HCl in dioxane and the resulting free amine was coupled with the protected acid **14** to furnish the compound **28**, the formation of compound **28** was confirmed by mass which showed a peak at m/z 494 corresponding to the formula $C_{21}H_{33}O_7N_3SNa$ $[M+Na]^+$. The structure of compound **28** was further confirmed using the $^1H$ NMR and $^{13}C$ NMR spectroscopic methods. The elimination of secondary alcohol from the compound **28** was achieved using the EDC.HCl and CuCl in 1:1 mixture of DMF:DCM resulted in the formation of *Z*-olefin compound **13**. The formation of **13** was confirmed by comparing the $^1H$ and $^{13}C$ NMR data with the literature reports[20] which was found to be identical. The compound **13** was further confirmed by HRMS which showed a base peak at m/z 454.2006 corresponding to the molecular formula $C_{21}H_{32}O_6N_3S$ $[M+H]^+$. The compound **13** was then subjected to hydrolysis using LiOH in aqueous THF at 0 $^oC$ to result in carboxylic acid, which was coupled with an amine (prepared by deprotection of compound **12** by treatment with the 4M HCl in dioxane) using the standard coupling reaction (EDC.HCl, HOBt) gave us the peptide fragment B (**5**) in 69%

yield (Scheme 6). The formation of **5** was established by using $^1$H NMR characteristic signal at δ 1.87 ppm corresponding to protons of the methyl group of dehydrobutyrine moiety along with signals of olefin at δ 5.37 (dd) ppm correspond to allyl ester group. It was further confirmed by HRMS showed base peak at m/z 650.2308 and m/z 672.2124 corresponding to the molecular formula $C_{28}H_{40}N_5O_8S_2[M+H]^+$ and $C_{28}H_{39}N_5O_8S_2Na$ $[M+Na]^+$ respectively.

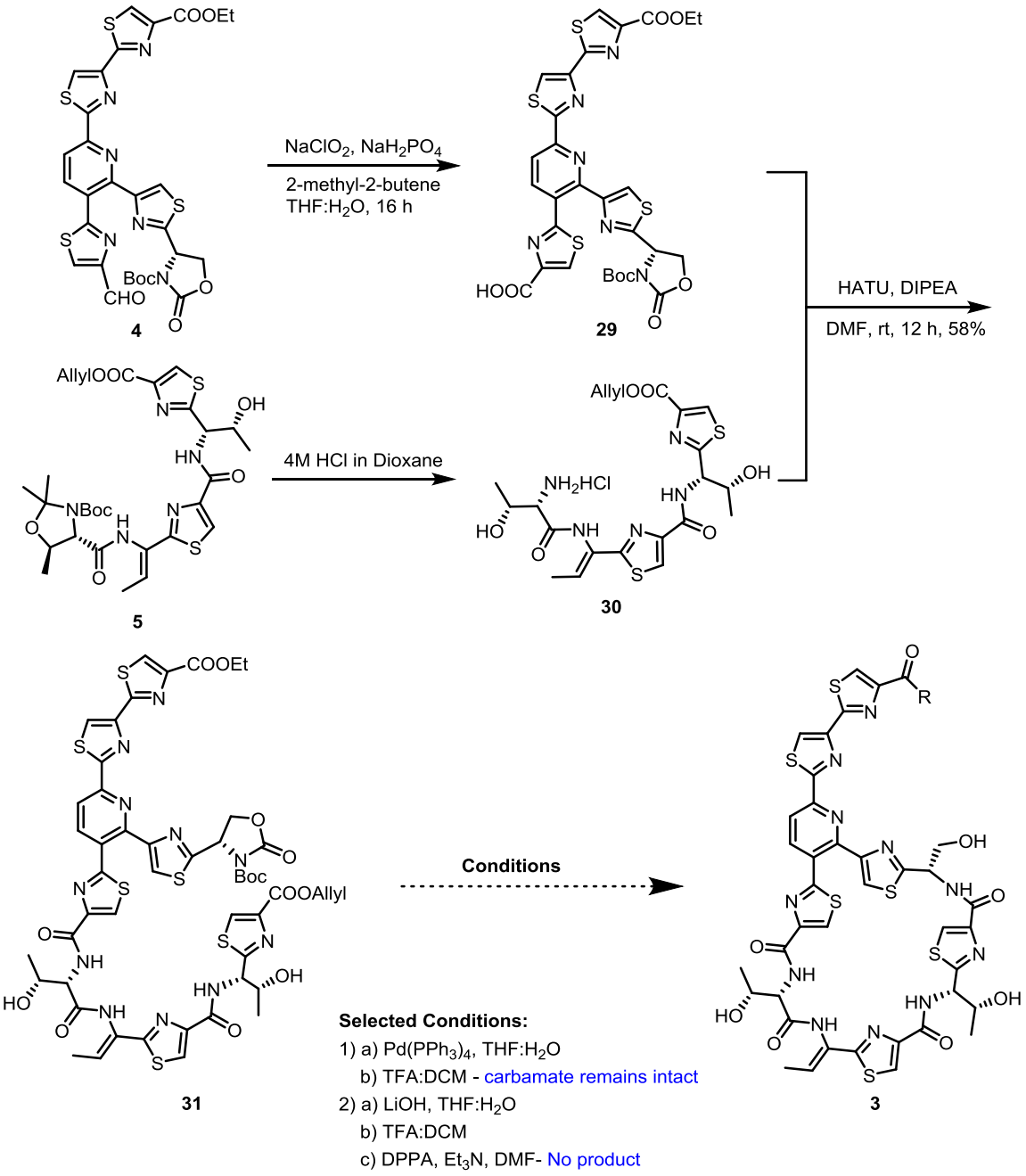



**Scheme 6:** Synthesis of fragment B (**5**)

### 2.5.2.3. Attempts towards synthesis of model thiopeptide (3)

Having both the fragments A (**4**) and B (**5**) in hand, the next task was to accomplish the synthesis of target model thiopeptide **3**. The aldehyde **4** was oxidized under standard Pinnick oxidation conditions[21] to give the acid **29**, which was coupled with free amine **30** (prepared from the treatment of fragment B (**5**) with 4M HCl in dioxane) under HATU/DIPEA in dry DMF to furnish the key precursor **31** for the desired cyclization. The formation of **31** was confirmed using $^1$H NMR analysis which showed characteristic protons of methyl group of dehydrobutyrine unit at δ 1.86 ppm and protons of terminal olefin of allyl ester at δ 5.96 and 5.44 ppm, the six protons of disubstituted thiazoles from pyridine core and peptide core was observed in the range of δ 8.83 to 8.09 ppm, which

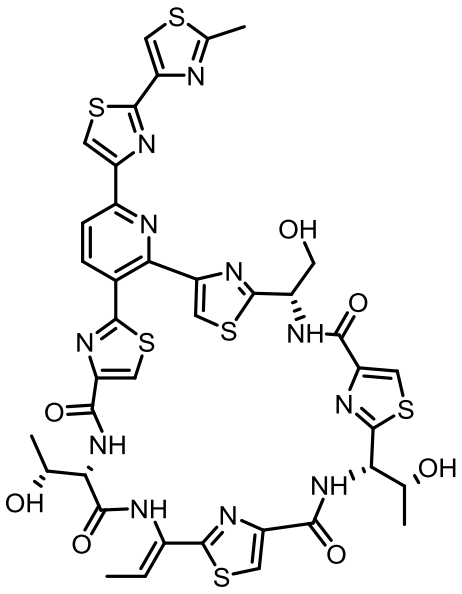
was merged with the pyridine and amide protons. The $^{13}C$ NMR analysis showed the characteristic ester carbonyl carbons at δ 171.4 and 168.8 ppm. The compound **31** was further confirmed by HRMS which showed a base peak at m/z 1204.1934 corresponding to the molecular formula $C_{50}H_{50}O_{13}N_{11}S_6$ $[M+H]^+$. Towards completion of the task of macrocyclization, the allyl ester was deprotected using catalytic amount of $Pd(PPh_3)_4$ in THF:$H_2O$ and it was confirmed using mass spectrum where it showed a peak at m/z 1163 corresponding to the molecular formula $C_{47}H_{45}N_{11}O_{13}S_6$, followed by HRMS. The crude acid was then subjected to one pot *N*-Boc and carbamate deprotections using TFA:DCM following the literature reports.[17b] However, the expected deprotection of both *N*-Boc and carbamate under the same condition were not observed but only *N*-Boc was deprotected even after exposer to TFA: DCM for a long time. This was monitored and confirmed by HRMS and showed a peak at m/z 1064.1049 corresponding to the molecular formula $C_{42}H_{38}N_{11}O_{11}S_6$ $[M+H]^+$. The failure of carbamate deprotection in acidic conditions forced us to use a strong base such as lithium hydroxide to deprotect the carbamate moiety. Here we hydrolysed the carbamate along with allyl and ethyl esters in compound **31** using the LiOH in aqueous THF to give crude di carboxylic acid, which was confirmed using mass spectrum. Then *N*-Boc was deprotected using TFA: DCM to yield amine salt which was again confirmed using the HRMS and showed a base peak at m/z 1010.0971 corresponding to molecular formula $C_{39}H_{36}N_{11}O_{10}S_6$ $[M+H]^+$. The resulting crude material was subjected to macrolactamization reaction using the DPPA/$Et_3N$ in dilute conditions to furnish the required thiopeptide, but we could not observe the expected product. We also tried a few more conditions but they were not successful.




**Scheme 7:** Attempts towards synthesis of model thiopeptide (**3**)

Having encountered the problem during final deprotections and macrolactamization, we have modified target thiopeptide macrocycle and came with a compound (**32**), where the ester functionality on pyridine core was replaced with the simple methyl group with a

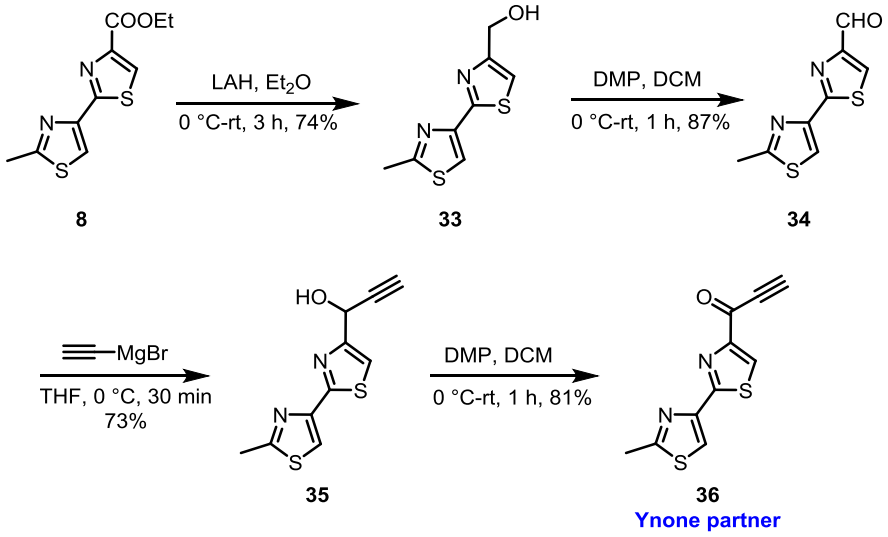
minor change in the positions of heteroatom of thiazole rings. As the revised model thiopeptide also shows all characteristic features of thiopeptide similar to that of previous macroccyle **3** and hence it is expected to show a similar biological profile as that of lactocillin (**1**), thiocillin I and micrcoccin P1.



**Revised model thiopeptide (32)**

The synthesis of planned thiopeptide (**32**) could be accomplished using similar strategy used for the synthesis of compound **3**, with minor modifications in the functional group of ynone partner and peptide fragment B. A previously synthesized thiazole ester **8** was subjected to LAH reduction in diethyl ether at 0 ºC to provide corresponding alcohol **33**. The $^{1}$H NMR spectrum of **33** showed the characteristic protons of hydroxy attached 2H at δ 4.81 ppm and absence of carbonyl in IR and $^{13}$C NMR confirmed the formation of compound **33**. The alcohol **33** was converted to aldehyde **34** using Dess-Martin periodinane oxidation in excellent yield. The aldehyde **34** was treated with commercially available ethynylmagnesium bromide in dry THF at 0 ºC for 30 minutes to get the propargylic alcohol **35**, which on oxidation with Dess-Martin periodinane yielded the required ynone **36** in very good yield (Scheme 8). The $^{1}$H NMR spectrum of ynone **36** showed the characteristic proton attached to sp-carbon at δ 3.48 ppm and $^{13}$C NMR

spectrum where the acetylene carbon observed at δ 80.7 and 80.5 ppm. It was further confirmed by HRMS which showed a base peak at m/z 234.9993 corresponding to the molecular formula $C_{10}H_7ON_2S_2$, $[M+H]^+$.

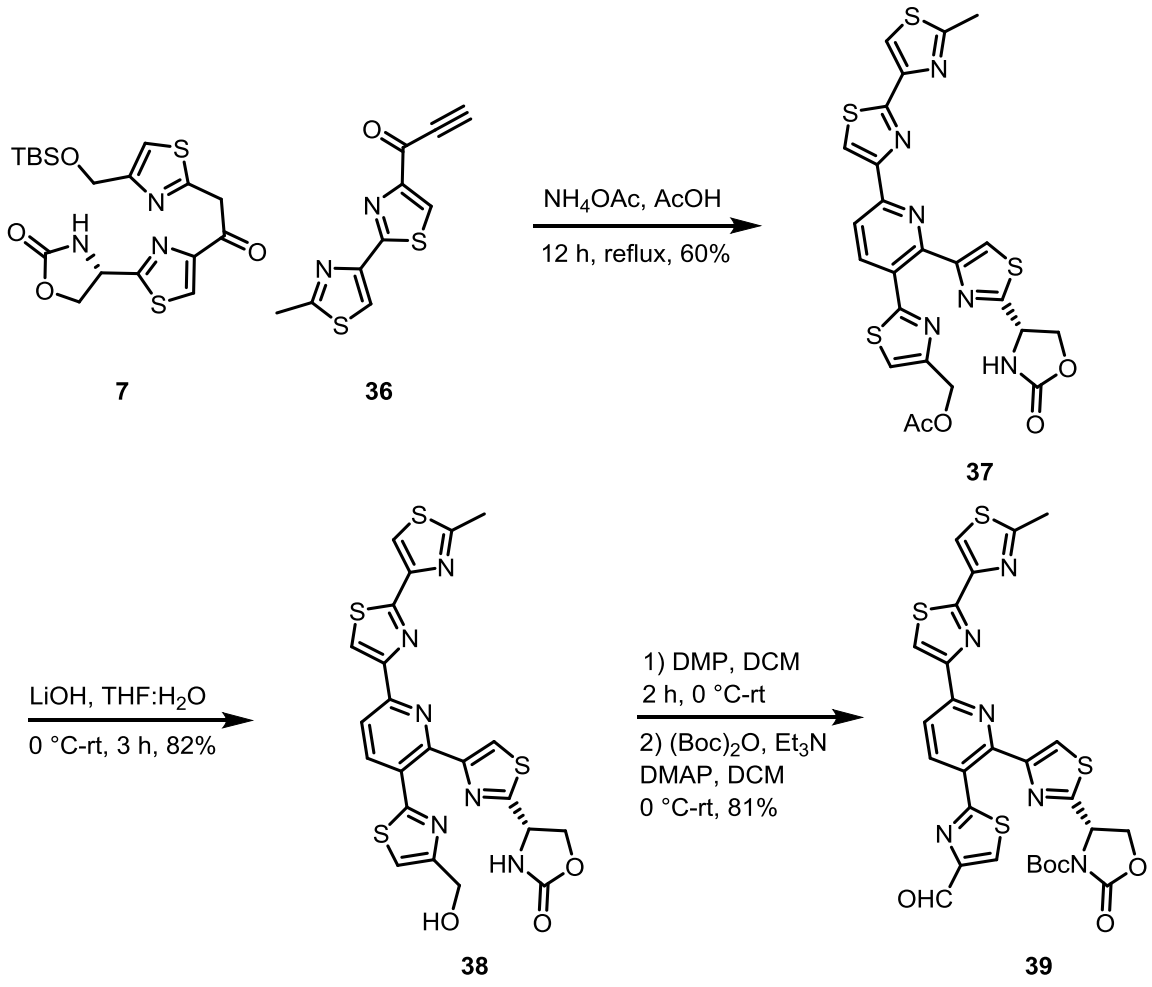



**Scheme 8:** Synthesis of ynone partner (**36**)

### 2.5.2.4. Synthesis of trisubstituted pyridine 39

Both compounds ynone partner (**36**) and ketone partner (**7**) were subjected to Eiden-Herdies pyridine synthesis under similar conditions as described in Scheme 4. The $^1$H NMR spectrum of resulting product **37** showed the characteristic four singlets in aromatic region at δ 8.07 (s), 7.88 (s), 7.36 (s) and 7.28 (s) which indicate the presence of four disubstituted thiazole rings in the compound and compound **37** was further confirmed by HRMS which showed the base peak at m/z 583.0344 corresponding to molecular formula $C_{24}H_{19}O_4N_6S_4$ $[M+H]^+$. The compound **37** was then transformed to **39** under similar conditions through the intermediary of compound **38** (Scheme 9). The $^1$H NMR spectrum of compound **39** in which the characteristic proton of aldehyde was observed at δ 10.03 ppm and singlet for methyl protons present on thiazole ring is observed at δ 2.80 ppm along with the six characteristic protons in aromatic region of δ 8.32-7.94 ppm

corresponding to four disubstituted thiazole rings and two protons of pyridine ring. $^{13}C$ NMR spectrum also shows the carbonyl carbon of aldehyde at δ 184.3 ppm, which supports the formation of aldehyde. It was further confirmed by HRMS and showed a base peak at m/z 639.0607 corresponding to the molecular formula $C_{27}H_{23}O_5N_6S_4$ $[M+H]^+$.

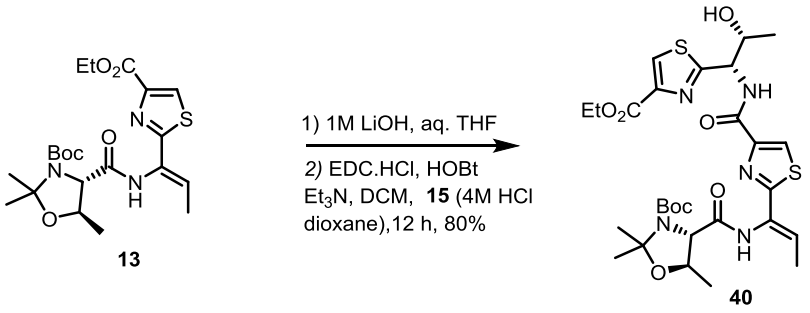



**Scheme 9:** Synthesis of pyridine compound (**39**) using Eiden-Herdies pyridine synthesis.

### 2.5.2.5. Synthesis of peptide fragment 40

The peptide fragment (**40**) of targeted thiopeptide was synthesized from the previous intermediates compound **13** and compound **15**. The crude acid obtained after the

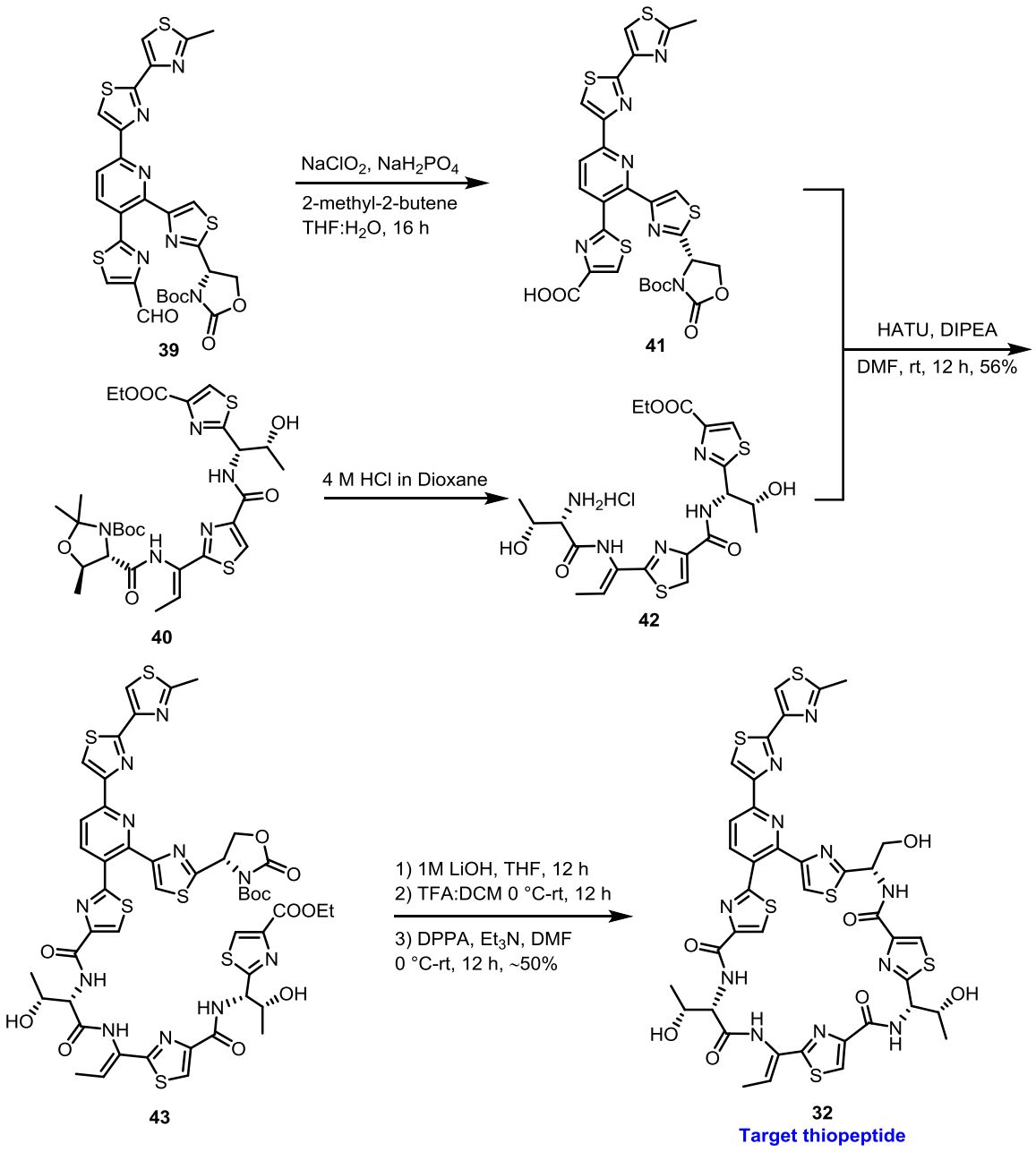
hydrolysis of ester present in **13** was coupled with amine (prepared from deprotection of compound **15** by treatment with the 4M HCl in dioxane) using the EDC.HCl and HOBt as a coupling reagents to furnish the peptide fragment (**40**) with excellent yield (Scheme 10). The $^1$H NMR spectrum of compound **40** showed the characteristic proton of the methyl group of the dehydrobutyrine unit at δ 1.87 ppm along with the ethyl ester protons at δ 4.39 (2H) and 1.31 (3H) ppm and doublet for 6H at δ 1.61 ppm corresponding to acetonide protection confirms the formatiom of petide fragment **40**, which is further confirmed by HRMS showed a base peak at m/z 660.2119 corresponding to the molecular formula $C_{28}H_{39}N_5O_8S_2Na$ $[M+Na]^+$.




**Scheme 10:** Synthesis of peptide fragment (**40**)

### 2.5.2.6. Synthesis of thiopeptide (32)

The carboxylic acid prepared from aldehyde **39** using Pinnick oxidation conditions was coupled with free amine **42** (prepared from **40**) under HATU/DIPEA in dry DMF conditions to obtain the precursor **43**. The compound **43** was confirmed by HRMS which showed a base peak at m/z 1134.1870 corresponding to the molecular formula $C_{47}H_{48}O_{11}N_{11}S_6$ $[M+H]^+$. However, $^1$H NMR analysis showed broad peaks probably due to its low solubility in NMR solvents ($CDCl_3$, DMSO-$d_6$ and $CD_3OD$). Having acyclic thiopeptide **43** in hand the ester hydrolysis and carbamate deprotection was achieved using LiOH in aqueous THF to have free acid arm and the *N*-Boc protecting group was deprotected using TFA:DCM at room temperature resulted to amine salt in the same molecule. Deprotections were monitored using HMRS before the material taking




**Scheme 11:** Synthesis of target thiopeptide (**32**)

forward for next step. At this stage, the crude material was dried under reduced pressure using toluene as an azeotrope and the obtained residue was dissolved in dry DMF

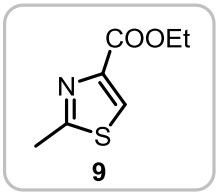
(0.025M) and subjected to intramolecular macrolactamization conditions. The macrolactamization was accomplished using the DPPA and triethylamine at room temperature to furnish designed target thiopeptide **32** in ~ 50% yield (Scheme 11). The formation of thiopeptide **32** was monitored and confirmed by HRMS which showed a peak at m/z 962.1111 corresponding to the molecular formula $C_{39}H_{36}O_7N_{11}S_6$ $[M+H]^+$. The formation of **32** was further confirmed by using the $^1H$ NMR in DMSO-d6 (overnight scanning) which showed all broad peaks. Here also we faced solubility problem with thiopeptide **32** similar to the acyclic precursor **43**. Although it was not completely characterized, based on HRMS data, we conclude that product isolated from this reaction correspond to the structure of model thiopeptide **32**.

## 2.6. Summary

- The synthetic route for macrocyclic thiopeptides has been developed. Problems encountered for the synthesis of initial macrocycle were addressed by new design and synthesis target thiopeptide.
- Further fine tuning of the synthetic route can provide access to lactocillin and related thiopeptides which are otherwise difficult to access for biological profiling.
- Several intermediates prepared during the present exercise may have potential biological activities which are not limited to bacteria. Further work along these lines is underway in our research group.

## 2.7. Experimental

**Ethyl 2-methylthiazole-4-carboxylate (9)**[17]




**9**

To a solution of thioacetamide (1.96 g, 26.15 mmol,) in absolute ethanol (20 mL), ethyl 3-bromopyruvate (90%, 5.0 g, 25.15 mmol) was added dropwise over 30 minutes. After being stirred for 12 h at 60 ºC, the reaction mixture was poured onto 2.5 N HCl (20 mL), stirred for 30 minutes and extracted with diethyl ether (10 mL x 3). The aqueous solution was cautiously neutralized with excess solid $NaHCO_3$ and re-extracted with diethyl ether (10 mL x 3). The combined extracts were washed with brine, dried over $Na_2SO_4$ and concentrated under reduced pressure to give a yellow solid, which was purified by flash column chromatography eluting with ethyl acetate: pet ether (1:9) to afford thiazole **9** (4.21 g, 96%) as a white solid.

**Mp:** 52-54 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3089, 2979, 1716, 1698, 1480, 1223, 1107

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.02 (s, 1H), 4.41 (q, *J* = 6.95 Hz, 2H), 2.76 (s, 3H), 1.39 (t, *J* = 6.95 Hz, 3H)

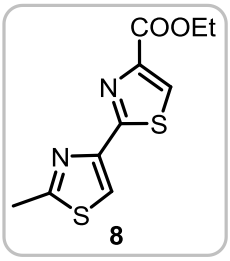
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 166.8, 161.4, 146.9, 127.2, 61.4, 19.3, 14.3

**MS:** 194 $[M+Na]^+$

**HRMS:** Calculated for $C_7H_{10}O_2NS$, $[M+H]^+$: 172.0427 found 172.0425 and $C_7H_9O_2NSNa$, $[M+Na]^+$: 194.0246 found 194.0245.

The $^1$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**Ethyl 2'-methyl-[2,4'-bithiazole]-4-carboxylate (8)**[17]




**8**

Ethyl 2-methylthiazole-4-carboxylate (**9**) (9.7 g, 56.7 mmol) was dissolved in aqueous $NH_4OH$ solution (40 mL) and the mixture was heated to 60 °C for 1 h. The solution was cooled to room temperature and extracted with ethyl acetate (20 mL x 3). The combined extracts were evaporated and the residue of 2-methylthiazole-4-carboxamide was directly treated with the Lawesson's reagent (11.45 g, 28.36 mmol) in refluxing benzene (50 mL) for 2 h. Reaction mixture cooled to room temperature, the solvent was removed under reduced pressure to get the thioamide as a brown solid. The crude thioamide was treated with ethyl-3-bromopyruvate (11.06 g, 7.1 mL, 56.7 mmol) in refluxing ethanol (50 mL) for 2 h. The cooled reaction mixture was evaporated and the residue was suspended in ethyl acetate (100 mL), neutralized with an aqueous saturated $NaHCO_3$ solution (50 mL) and the organic layer was separated, aqueous layer re-extracted with ethyl acetate (20 mL x 3). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered, evaporated and purified by crystallization with ethyl acetate: pet ether (1:3) to afford the compound **8** (11.6 g, 81% over three steps) as a white solid.

**Mp:** 94-98 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3124, 3019, 1724, 1218, 760

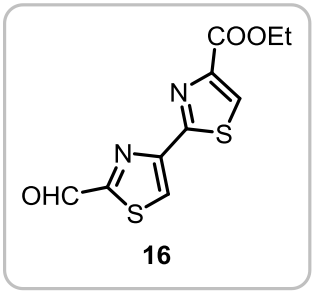
**$^1$H NMR (400 MHz, $CDCl_3$):** 8.14 (s, 1H), 7.99 (s, 1H), 4.42 (q, *J* = 7.04 Hz, 2H), 2.75 (s, 3H), 1.41 (t, *J* = 7.04 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** 166.7, 163.3, 161.4, 148.0, 147.9, 127.5, 117.0, 61.4, 19.1, 14.3

**MS:** 277 $[M + Na]^+$

The $^1$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**Ethyl 2'-formyl-[2,4'-bithiazole]-4-carboxylate (16)**[15]




**16**

To a solution of compound **8** (200 mg, 0.79 mmol) in glacial acetic acid (2 mL) was added selenium dioxide (262 mg, 3.36 mmol). The reaction mixture was heated to reflux temperature for 36 h, the solvent was removed under reduced pressure, the residue was suspended in ethyl acetate and neutralised with saturated $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (5 mL x 3). The combined organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by flash column chromatography (silica gel 230-400 mesh, 1:9, ethyl acetate: pet ether) to afford **16** as a yellow solid (32 mg, 50% brsm) along with the starting material (138 mg).

**Mp**: 154-154 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3037, 2995, 1719, 1685

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.04 (s, 1H), 8.55 (s, 1H), 8.25 (s, 1H), 4.45 (q, *J* = 7.05 Hz, 2H), 1.43 (t, *J* = 7.05 Hz, 3H)

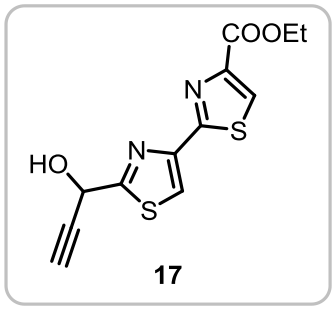
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 183.2, 165.9, 161.9, 161.2, 151.1, 148.2, 128.4, 123.9, 61.7, 14.3

**MS:** 291 $[M+Na]^+$

**HRMS:** Calculated for $C_{10}H_9O_3N_2S_2$, $[M+H]^+$: 269.0049 found 269.0042

The $^1H$ and $^{13}C$ NMR values are compared with reported values and found to be identical.

**Ethyl 2'-(1-hydroxyprop-2-yn-1-yl)-[2,4'-bithiazole]-4-carboxylate (17)**[17]




**17**

A solution aldehyde **16** (270 mg, 1.37 mmol) in dry THF (15 mL) were cooled to 0 ºC and a 0.5M solution of ethynylmagnesium bromide (2.75 mL, 1.37 mmol) were added dropwise over the period of 10 minutes under argon. The reaction mixture was stirred for 30 minutes at the same temperature and saturated solution of $NH_4Cl$ was added (2 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (10 mL x 3), the combined organic layers were washed with saturated $NH_4Cl$, water, and brine. The organic layer was dried over $Na_2SO_4$, evaporated and purified by column chromatography eluting with ethyl acetate: pet ether, (1:2) to afford the alcohol **35** (310 mg, 76% yield) as fibrous solid.

**Mp:** 142-146 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3431, 2930, 2210, 1732, 1630, 1618

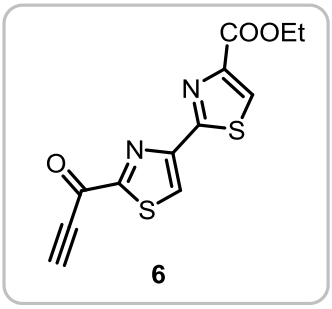
**$^1$H NMR (500 MHz, $CDCl_3$):** δ 8.19 (s, 1H), 8.18 (s, 1H), 5.79 (d, *J* = 2.2 Hz, 1H), 4.45 (q, *J* = 7.28 Hz, 2H), 2.75 (d, *J* = 2.2 Hz, 1H), 1.43 (t, *J* = 7.28 Hz, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 170.3, 162.9, 161.3, 148.3, 147.9, 127.8, 118.6, 80.9, 75.73 61.7, 61.6, 14.3

**HRMS:** Calculated for $C_{12}H_{11}O_3N_2S_2$, $[M+H]^+$: 295.0206 found 295.0200 and $C_{12}H_{10}O_3N_2S_2Na$, $[M+Na]^+$: 317.0025 found 317.0018

The $^1H$ and $^{13}C$ NMR values are compared with reported values and found to be identical.

**Ethyl 2'-propioloyl-[2,4'-bithiazole]-4-carboxylate (6)**[17]




**6**

To a 100 mL flask equipped with a septum, a magnetic stirring bar, was charged with alcohol **17** (1.9 g, 6.46 mmol) and dry DCM (50 mL). It was cooled to 0 ºC and Dess-Martin periodinane (3.0 g, 7.1 mmol) was added portion wise. The reaction mixture was stirred at room temperature for 1 h, diluted with water and the organic layer was washed well with water (10 mL x 2), a saturated solution of $NaHCO_3$ (10 mL x 2) and brine (10 mL). The combined organic layer was dried over $Na_2SO_4$, evaporated and purified by column chromatography eluting with the ethyl acetate: pet ether (1:3) to get the ynone **6** (1.6 g, 86% yield) as colourless solid.

**Mp:** 105-106 °C

**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3231, 1788, 1632, 1519, 1015

**$^1H$ NMR (400 MHz, $CDCl_3$):** δ 8.53 (s, 1H), 8.24 (s, 1H), 4.45 (q, *J* = 7.21 Hz, 2H), 3.68 (s, 1H), 1.42 (t, *J* = 7.21 Hz, 3H)

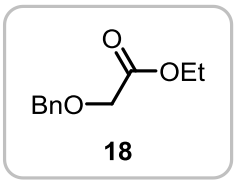
**$^{13}C$ NMR (100 MHz, $CDCl_3$):** δ 168.5, 165.7, 161.9, 161.1, 151.0, 148.1, 128.5, 124.7, 84.0, 79.1, 61.7, 14.3

**MS:** 314 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_9O_3N_2S_2$, $[M+H]^+$: 293.0049 found 292.0044 and $C_{12}H_8O_3N_2S_2Na$, $[M+Na]^+$: 314.9869 found 314.9862

The $^1H$ and $^{13}C$ NMR values are compared with reported values and found to be identical.

**Ethyl 2-(benzyloxy) acetate (18)**




**18**

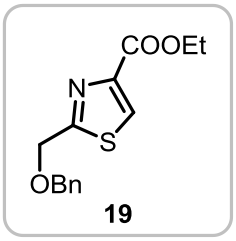
To a suspension of sodium hydride (0.92 g, 38.5 mmol) in dry toluene (20 mL) was added dropwise a solution of benzyl alcohol (2.32 mL, 19.23 mmol, in 10 mL of toluene) at 0 ºC under nitrogen, over 30 minutes and the resulting mixture was stirred for 4 h at room temperature. The reaction mixture was cooled with ice cold water, and a solution of ethyl bromoacetate (1.81 mL, 19.23 mmol, in 10 mL toluene) was added over 30 minutes and the resulting mixture was stirred for additional 1 h at 0 ºC. The reaction mixture was then poured into a solution of 20 mL of cold water and 0.5 mL of con. HCl, followed by extraction with toluene. The organic layer was washed with brine (10 mL) and dried over anhydrous $Na_2SO_4$. Filtration, evaporation, and purification by flash chromatography gave **18** as yellow oil (3.2 g, 85% yield).

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 7.37-7.31 (m, 5H), 4.64 (s, 2H), 4.23 (q, *J* = 6.96 Hz, 2H), 4.09 (S, 2H), 1.29 (t, *J* = 6.96 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$)**: δ 170.3, 137.1, 128.5 (2C), 128.0 (2C), 128.0, 73.3, 67.2, 60.8, 14.2

**HRMS:** Calculated for $C_{11}H_{15}O_3$, $[M+H]^+$: 195.1013 found 195.1016.

**Ethyl 2-((benzyloxy)methyl)thiazole-4-carboxylate (19)**




**19**

Compound **19** was prepared by following the similar procedure used for the synthesis of compound **8** with 55% yield over three steps.

**Mp:** 112-115 °C

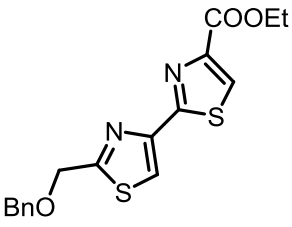
**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3038, 2925, 1738, 1614, 1519, 1015

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 8.18 (s, 1H), 7.38-7.26 (m, 5H), 4.87 (s, 2H), 4.68 (s, 2H), 4.42 (q, *J* = 7.25, 2H), 1.40 (t, *J* = 7.25, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.2, 161.3, 146.9, 136.9, 128.5 (2C), 128.1, 127.8, 127.8 (2C), 73.3, 69.0, 61.4, 14.3

**HRMS:** Calculated for $C_{14}H_{16}O_3NS$ $[M+H]^+$: 278.0844 found 278.0845 and $C_{14}H_{15}O_3NSNa$, $[M+Na]^+$: 300.0661 found 300.0665

**Ethyl 2'-((benzyloxy)methyl)-[2,4'-bithiazole]-4-carboxylate (20)**




**20**

Compound **20** was prepared by following the similar procedure used for the synthesis of compound **8** from compound **19** with 64% yield over three steps.

**Mp:** 136-140 °C

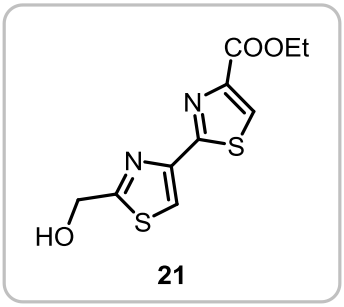
**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3036, 2920, 1738, 1614, 1007

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.16 (s, 2H), 7.40-7.33 (m, 5H), 4.85 (s, 2H), 4.70 (s, 2H), 4.44 (q, *J* = 7.17 Hz, 2H), 1.42 (t, *J* = 7.17 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 169.9, 163.2, 161.4, 148.2, 147.9, 137.0, 128.5 (2C), 128.1, 127.9 (2C), 127.64, 73.26, 68.70, 61.50, 14.31

**HRMS:** Calculated for $C_{17}H_{17}O_3N_2S_2$, $[M+H]^+$: 361.0675 found 361.0669 and $C_{17}H_{16}O_3N_2S_2Na$, $[M+Na]^+$: 383.0495 found 383.0486

**Ethyl 2'-(hydroxymethyl)-[2,4'-bithiazole]-4-carboxylate (21)**




**21**

To a solution of compound **20** (2.0 g, 5.5 mmol) in dry DCM (50 mL) was added a methanesulfonic acid (7.2 mL, 111 mmol) dropwise at room temperature. The reaction mixture was stirred for 4 h and slowly diluted with water. The organic layer was separated and washed with $H_2O$ (50 mL), saturated $NaHCO_3$ (10 mL x 3) and brine (20 mL), dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Purification of the residue by column chromatography (silica gel, 230-400 mesh, ethyl acetate: pet ether, 1:2) afforded the compound **21** (1.43 g, 95% yield) as white solid.

**Mp:** 120-121 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3233, 2975, 1730, 1599

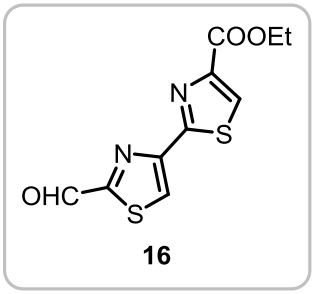
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.17 (s, 1H), 8.15 (s, 1H), 5.02 (s, 2H), 4.45 (q, *J* = 7.14 Hz, 2H), 1.43 (t, *J* = 7.14 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 172.3, 163.2, 161.4, 148.1, 147.8, 127.7, 117.6, 62.1, 61.6, 14.3

**MS:** 293 $[M+Na]^+$

**HRMS:** Calculated for $C_{10}H_{11}O_3N_2S_2$,$[M+H]^+$: 271.0206 found 271.0201 and $C_{10}H_{10}O_3N_2S_2Na$, $[M+Na]^+$: 293.0025 found 293.0019

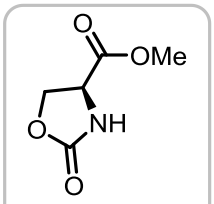
**Ethyl 2'-formyl-[2,4'-bithiazole]-4-carboxylate (16)**[15]




**16**

Compound **16** was prepared by following the similar procedure used for synthesis of compound **6** from compound **21** with 82% yield.

**Methyl (*S*)-2-oxooxazolidine-4-carboxylate (22)**[17c]




**22**

*L*-Serine (4.0 g, 38.0 mmol) was dissolved in a 1 M aqueous solution of NaOH (105 mL, 105 mmol) and a solution of triphosgene (11.3 g, 38 mmol) in dioxane (100 mL) was added dropwise at 0 ºC over a period of 30 minutes. The reaction was stirred at room temperature for 5 h before being concentrated. The residue was dissolved in methanol (200 mL) and stirred for overnight. The reaction mixture was concentrated to dryness and dissolved in ethyl acetate, washed with saturated solution of $NaHCO_3$, water and brine.

The organic layer was dried over $Na_2SO_4$ and solvent was evaporated under reduced pressure to afford the methyl ester **22** (4.1 g, 78% yield) as a colourless oil.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3019, 2937, 1735, 1690, 1557

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 6.44 (brs, 1H), 4.60 (t, *J* = 9.29 Hz, 1H), 4.51 (dd, *J* = 8.80, 4.40 Hz, 1H), 4.42 (m, 1H), 3.80 (s, 3H)

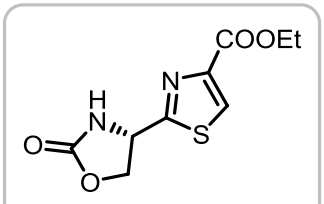
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.5, 159.0, 66.7, 53.7, 53.0

**MS:** 168 $[M+Na]^+$

**HRMS:** Calculated for $C_5H_8O_4N$ $[M+H]^+$: 146.0448, found 146.0447

The $^1$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**Ethyl (S)-2-(2-oxooxazolidin-4-yl)thiazole-4-carboxylate (10)**




**10**

To a solution of compound **22** (3.8 g, 26.2 mmol) in methanol (50 mL) was saturated with ammonia after DMAP (20 mg) was added. The mixture was stirred at room temperature for 12 h before being concentrated. The resulting amide and Lawesson's reagent (5.3 g, 13.1 mmol) suspended in benzene (40 mL) were heated to reflux for 3 h. The concentration of the crude yielded the thioamide, which was directly treated with ethyl-3-bromopyruvate (3.29 mL, 26.2 mmol) in refluxing ethanol (40 mL). After 1 h, the solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$ (100 mL), washed with saturated aqueous $NaHCO_3$ and brine (80 mL each), dried over $Na_2SO_4$ and concentrated. The crude mixture was purified by flash column

chromatography eluting with ethyl acetate: pet ether (1:1) to afford **10** (3.75 g, 60% yield over three steps) as sticky mass.

**$[\alpha]_D^{27}$:** - 53.9 (*c* 1.14, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3244, 1755, 1694, 1525, 1464

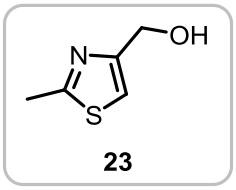
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.20 (s, 1H), 6.48 (brs, 1H), 5.44 (m, 1H), 4.86 (t, *J* = 9.03 Hz, 1H), 4.39 (m, 3H), 1.40 (t, *J* = 7.14 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 171.9, 160.9, 159.2, 147.9, 127.9, 70.7, 61.8, 54.1, 14.3

**MS:** 265 $[M+Na]^+$

**HRMS**: Calculated for $C_9H_{11}O_4N_2S$ $[M+H]^+$: 243.0434 found 243.0439.

**(2-methylthiazol-4-yl)methanol (23)**[17]




**23**

To a suspension of lithium aluminum hydride (244 mg, 6.43 mmol) in dry diethyl ether (10 mL) at 0 °C was added slowly a solution of compound **9** (1.0 g, 5.84 mmol) in diethyl ether (10 mL). The reaction mixture was allowed to stir at 0 ºC for 10 minutes and then at room temperature for 12 h. Cooled to 0 ºC and diluted with ethyl acetate (5 mL), the resulting suspension were added slowly to an aqueous solution of potassium sodium tartrate (30% w/v, 10 mL) and stirred for 1 h. The mixture was diluted with 50 mL of ethyl acetate, the layers were separated and the aqueous layer was re-extracted with ethyl acetate (10 mL x 2). The combined organic layers were dried over $Na_2SO_4$, filtered, evaporated and purified by column chromatography (silica gel, 100-200 mesh, ethyl acetate: pet ether, 1:2) to give alcohol **23** (560 mg, 74% yield) as colourless oil.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3413, 1682, 1575

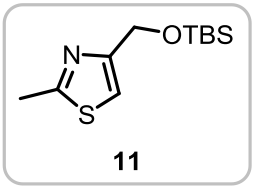
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.00 (s, 1H), 4.69 (s, 2H), 3.92 (brs, 1H), 2.68 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 166.9, 155.9, 114.4, 60.3, 18.9

**MS:** 152 $[M+Na]^+$

The $^{1}$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**4-(((tert-butyldimethylsilyl)oxy)methyl)-2-methylthiazole (11)**[17]




**11**

To a solution of **23** (300 mg, 2.32 mmol) in DCM (5 mL), imidazole (240 mg, 3.5 mmol), TBSCl (385 mg, 2.55 mmol) and a catalytic amount of DMAP (2.8 mg, 0.025 mmol) was added and reaction mixture was stirred at room temperature for 8 h. The reaction mixture was diluted with DCM (5 mL), washed with water (5 mL) and brine (5 mL), dried over anhydrous $Na_2SO_4$. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100-200, using pet ether) to afford **11** (510 mg, 91%) as a colourless liquid.

**IR $\upsilon_{max}$(film): $cm^{-1}$** 3245, 2950, 1679, 1608, 1043

**$^{1}$H NMR (400 MHz, $CDCl_3$): δ** 6.99 (s, 1H), 4.82 (s, 2H), 2.68 (s, 3H), 0.94 (s, 9H), 0.11 (s, 6H).

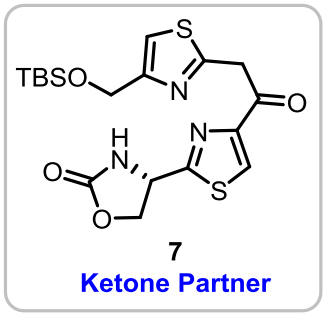
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 166.0, 156.8, 112.9, 62.3, 25.9 (3C) 19.1, -5.4 (2C)

**MS:** 266 $[M+Na]^+$

**HRMS:** Calculated for $C_{11}H_{22}ONSSi$, $[M+H]^+$: 244.1186 found 244.1184 and $C_{11}H_{21}ONSSi$ Na, $[M+Na]^+$: 266.1005 found 266.0999.

The $^{1}$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**(S)-4-(4-(2-(4-(((tert-butyldimethylsilyl)oxy)methyl)thiazol-2-yl)acetyl)thiazol-2-yl)oxazolidin-2-one (7)**




**7**
**Ketone Partner**

A solution of **11** (3.0 g, 13.4 mmol) in dry THF (10 mL) were cooled to -78 ºC and *n*-BuLi (1.6 M, 7.74 mL, 13.4 mmol) were added dropwise. The dark red solution was stirred for 15 minutes at the same temperature and a solution of compound **10** (1.0 g, 4.13 mmol) in dry THF (10 mL) were added dropwise, over the period of 10 minutes. The reaction mixture was slowly warmed to -30 ºC over a period of 3 h and quenched with the water (5.0 mL), stirred for 5 minutes and warmed to room temperature. The organic layer was separated and aqueous layer re-extracted with the ethyl acetate (10 mL x 2). The combined organic layer was dried over anhydrous $Na_2SO_4$. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100-200, 1:3 ethyl acetate: pet ether) to afford **7** (1.53 g, 85%) as a keto-enol mixture with 3:1 ratio. The $^1$H NMR and $^{13}$C NMR data was given only for major (ketone) compound.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3294, 2937, 1718, 1676, 1609, 1169, 1045

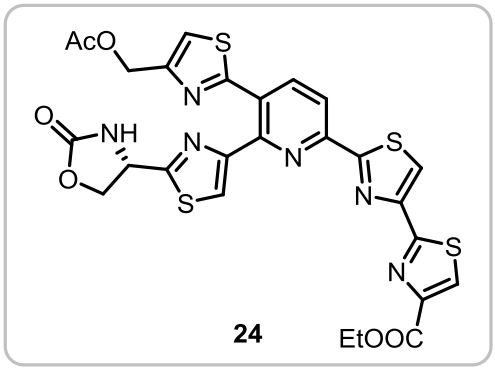
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 7.73 (s, 1H), 6.97 (s, 1H), 6.90 (s, 1H), 6.66 (s, 1H), 5.30 (m, 1H), 4.85-4.74 (m, 4H), 4.49 (m, 1H), 0.95 (s, 9H), 0.13 (s, 6H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.3, 167.7, 159.2, 155.2, 154.4 (2C), 151.9, 118.1, 110.5, 93.5, 70.6, 61.7, 54.1, 25.8 (3C), -5.36 (2C)

**MS:** 440 $[M+H]^+$

**HRMS:** Calculated for $C_{18}H_{25}N_3O_4S_2SiNa$, [M+Na]+: 462.0954 found 462.0946

**Ethyl (S)-2'-(5-(4-(acetoxymethyl)thiazol-2-yl)-6-(2-(2-oxooxazolidin-4-yl)thiazol-4-yl)pyridin-2-yl)-[2,4'-bithiazole]-4-carboxylate (24)**




**24**

To a solution of compound **7** (200 mg, 0.46 mmol) in glacial acetic acid (3 mL), $NH_4OAc$ (53 mg, 0.68 mmol) and compound **6** (133 mg, 0.46 mmol) was added and reaction mixture was stirred for 12 h at 120 ºC. Cooled to room temperature, solvent was removed under reduced pressure and the residue was neutralized with saturated $NaHCO_3$ solution (pH ~10) and extracted with ethyl acetate (10 mL x 3). The combined organics were dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure and purified by column chromatography with ethyl acetate: pet ether (1:1) to afford **24** (170 mg, 58% yield).

**Mp:** 195-199 °C

**$[\alpha]_D^{27}$:** + 26.1 (*c* 1.0, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 1780, 1733, 1716

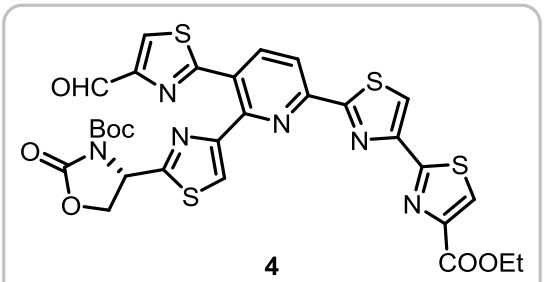
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.28 (m, 2H), 8.20 (s, 1H), 8.18 (d, *J* = 7.71 Hz, 1H), 7.97 (s, 1H), 7.40 (s, 1H), 6.50 (s, 1H), 5.22 (s, 2H), 5.11 (brs, 1H), 4.66 (s, 1H), 4.45 (q, *J* = 7.05 Hz, 2H), 4.30 (brs, 1H), 2.12 (s, 3H), 1.43 (t, *J* = 7.05 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.8, 169.0, 168.3, 165.2, 163.1, 161.3, 158.7, 154.0, 151.5, 150.6, 150.5, 149.8, 147.9, 139.9, 129.4, 127.9, 121.6, 120.6, 119.3, 118.71, 70.3, 61.7, 61.6, 53.6, 20.9, 14.3

**MS:** 663 $[M+Na]^+$

**HRMS:** Calculated for $C_{26}H_{21}O_6N_6S_4$, $[M+H]^+$: 641.0400 found 641.0388 and $C_{26}H_{20}O_6N_6S_4$ Na, $[M+Na]^+$: 663.0219 found 663.0204

***tert*-butyl 4-(4-(6-(4-(ethoxycarbonyl)-[2,4'-bithiazol]-2'-yl)-3-(4-formylthiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2-oxooxazolidine-3-carboxylate (4)**




**4**

To a solution of compound **24** (200 mg, 0.32 mmol) in ethanol: DCM (1:1, 10 mL) was added a $K_2CO_3$ (0.42 g, 3.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h and solvent removed under reduced pressure. The residue was suspended in ethyl acetate (5 mL) and solid was filtered out, filtrate was washed with water (5 mL), brine (3 mL), dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. A solution of obtained alcohol **25** in dry DCM (10 mL) was cooled to 0 ºC and Dess-Martin periodinane (155 mg, 0.38 mmol) was added portionwise. The reaction mixture was stirred at room temperature for 1 h and diluted with water; the organic layer was washed well with water (5 mL), saturated solution of $NaHCO_3$ (5 mL x 3) and brine (10 mL). The combined organic layer was dried over $Na_2SO_4$, evaporated in vacuo to get the aldehyde.

The aldehyde were dissolved in DCM (10 mL) and cooled to 0 °C, $(Boc)_2O$ (104 mg, 0.48 mmol), $Et_3N$ (0.07 mL, 0.48 mmol) and catalytic amount of DMAP (2 mg) was added. The reaction was allowed to stir at 0 ºC for 30 minutes and 2 h at room temperature. 1N HCl was added and organic layer was separated, the aqueous layer was re-extracted with DCM (10 mL x 3). The combined organic layer was dried over $Na_2SO_4$, filtered, and concentrated. The crude material obtained after removal of solvent was

purified by flash column chromatography (silica gel, 230-400, 1:2 ethyl acetate: pet ether) to afford **4** (128 mg, 56% yield).

**$[\alpha]_D^{26}$:** + 29.10 (*c* 1.0, $CHCl_3$)

**Mp:** 187-190 ºC

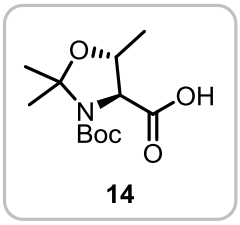
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3023, 1774, 1714, 1597

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.06 (s, 1H), 8.35 (m, 3H), 8.22 (m, 2H), 8.17 (m, 1H), 5.42 (m, 1H), 4.46 (m, 3H), 4.27 (m, 1H), 1.47 (s, 9H), 1.44 (t, *J* = 7.74 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 184.2, 168.2, 166.4, 166.0, 163.1, 161.4, 154.7, 153.4, 151.1, 150.9, 150.1, 150.0, 148.5, 148.0, 140.5, 131.2, 128.4, 127.9, 122.6, 120.6, 118.6, 85.0, 66.3, 61.6, 55.1, 27.9 (3C), 14.3

**HRMS**: Calculated for $C_{29}H_{24}O_7N_6S_4$, $[M+Na]^+$: 719.0482 found 719.0471

***(*4S,5R)-3-(tert-butoxycarbonyl)-2,2,5-trimethyloxazolidine-4-carboxylic acid (14)**[19]




**14**

To a solution of *L*-threonine (10 g, 84 mmol) in dioxane: water (1:1, 200 mL) was added triethylamine (15.25 mL, 110 mmol) and $(Boc)_2O$ (18.31 mL, 92.4 mmol) at 0 °C and reaction mixture was stirred at room temperature for 12 h. Solvent was evaporated and aqueous layer was acidified with 1N HCl ($P^H$ 2-3) and extracted with ethyl acetate (100 mL x 3). The combined organic layers were dried over $Na_2SO_4$, filtered, and concentrated to afford (tert-butoxycarbonyl)-*L*-threonine as colourless foam.

The obtained (tert-butoxy carbonyl)-*L*-threonine was dissolved in DCM (100 mL), a catalytic amount of PTSA (0.5 g) and 2,2-dimethoxy propane was added. The reaction

mixture was stirred at room temperature for 1 h and slowly evaporated on rota vapour to remove solvents. The procedure was repeated once again to convert the remaining starting material to product. The residue was dissolved in DCM and washed with water (30 mL), brine (30 mL), the combined organic layer was dried over anhydrous $Na_2SO_4$. The crude material obtained after removal of solvent was crystallised with ethyl acetate to afford **14** as a white crystalline solid (19.8 g, 90% for two steps).

**Mp**: 90-94 °C

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 4.23 (brs, 1H), 3.90 (brs, 1H), 1.58 (m, 6H), 1.44 (m, 12H)

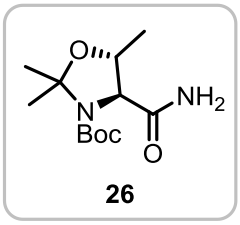
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 177.0, 150.9, 95.3, 80.8, 73.9, 66.0, 28.2 (3C), 26.4, 23.9, 18.9

**MS:** 282 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{21}O_5N$ $[M+Na]^+$: 282.1312, found 282.1307

The $^1$H NMR and $^{13}$C NMR and rotation values are identical with the reported values.

***tert*-butyl (4S,5R)-4-carbamoyl-2,2,5-trimethyloxazolidine-3-carboxylate (26)**[19]




**26**

To a stirred solution of compound **14** (1.0 g 3.86 mmol) in THF (15 mL) at -10 °C, $Et_3N$ (0.6 mL, 4.25 mmol) and isobutyl chloroformate (0.56 mL, 4.25 mmol) were sequentially added and the reaction mixture was stirred for 1.5 h. The aqueous $NH_3$ (4 mL, 35 mmol) was added and the solution was allowed to warm to room temperature over 12 h. The volatile organics were removed in vacuo and resulting aqueous solution was extracted with ethyl acetate (10 mL x 3). The combined organic layers were washed with water (10

mL), brine (10 mL), dried over anhydrous $Na_2SO_4$, concentrated and purified by column chromatography (ethyl acetate: pet ether, 1:1), to afford amide **26** (0.83 g, 83%) as a colourless solid.

**Mp**: 144-148 °C

**$[\alpha]_D^{27}$:** -7.6 (*c* 0.27, $CHCl_3$)

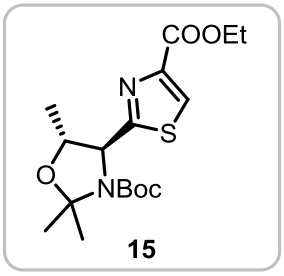
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 5.95 (brs, 2H), 4.21 (brs, 1H), 3.79 (brs, 1H), 1.61 (s, 3H), 1.57 (s, 3H), 1.45 (s, 9H), 1.39 (d, *J* = 6.11 Hz, 3H)

**MS:** 281 $[M+Na]^+$

**HRMS**: Calculated for $C_{12}H_{23}O_4N_2$ $[M+H]^+$: 259.1652 found 259.1647 and $C_{12}H_{22}O_4N_2Na$ $[M+Na]^+$: 281.1472 found 281.1465

The $^1$H NMR and melting point values are compared with reported values and found to be identical.

***tert*-butyl (4S,5R)-4-(4-(ethoxycarbonyl)thiazol-2-yl)-2,2,5-trimethyloxazolidine-3-carboxylate (15)**[19]




**15**

To a solution of amide **26** (1.0 g, 3.87 mmol) in dry benzene (10 mL) was added a Lawesson's reagent (0.78 g, 1.93 mmol) and reaction mixture were refluxed for 3 h. Cooled to room temperature, the solvent was removed under reduced pressure and crude thioamide **27** was forwarded to next step without further purification.

The solid $NaHCO_3$ (2.6 g, 30.9 mmol) was added to a solution of thioamide **27** in anhydrous DME (10 mL). Ethyl-3-bromopyruvate (1.45 mL, 2.26 g, 11.6 mmol) was

added and the solution was stirred at room temperature for 12 h. The reaction mixture was evaporated in vacuo and the residue partitioned between $H_2O$ (20 mL) and ethyl acetate (10 mL). The aqueous layer was further extracted with ethyl acetate (10 mL x 3) and the combined organic extracts were washed with brine (10 mL), dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. The residue was dissolved in anhydrous DME (10 mL) and the solution was cooled to 0 ºC. Pyridine (2.8 mL, 34.8 mmol) was added slowly, followed by dropwise addition of TFAA (2.2 mL, 15.48 mmol) and the solution was stirred at 0 ºC for 2 h before addition of $Et_3N$ (1.0 mL, 7.74 mmol), the reaction mixture was warmed to room temperature and evaporated in vacuo. The residue was dissolved in $CHCl_3$ (10 mL), washed with $H_2O$ (10 mL) and brine (5 mL), dried over anhydrous $Na_2SO_4$ and evaporated in vacuo. Purification of the residue by column chromatography eluting with ethyl acetate: pet ether (1:3) to afford the compound **15** (1.07 g, 74% yield) as white solid.

**Mp**: 92-98 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3443, 2957, 1756, 1612, 1473

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.13 (s, 1H), 4.77 (m, 1H), 4.39 (m, 2H), 4.13 (m, 1H), 1.67 (s, 6H), 1.38 (m, 9H), 1.16 (m, 6H)

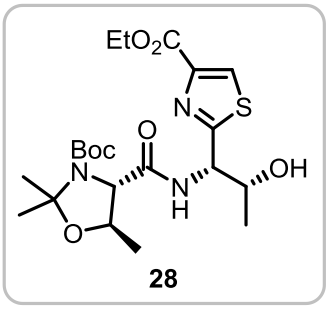
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 173.4, 161.1, 151.3, 146.7, 127.2, 95.2, 80.7, 77.8, 65.9, 61.4, 28.0 (3C), 26.5, 25.8, 17.8, 14.3

**MS:** 393 $[M+Na]^+$

**HRMS**: Calculated for $C_{17}H_{27}O_5N_2S$ $[M+H]^+$: 371.1635 found 371.1626 and $C_{17}H_{26}O_5N_2SNa$ $[M+Na]^+$: 393.1455 found 393.1444

The $^1$H and $^{13}$C NMR values are compared with reported values and found to be identical.

***tert*-butyl (4S,5R)-4-(((1S,2R)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-2-hydroxypropyl) carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (28)**




**28**

To a stirred solution of compound **14** (260 mg, 1.0 mmol) in dry DCM (5 mL) was added EDC.HCl (211 mg, 1.1 mmol), HOBt (149 mg, 1.1 mmol) at 0 ºC. The amine salt (prepared from the treatment of compound **15 (**230 mg, 1.0 mmol) with 4M HCl in dioxane) was added followed by $Et_3N$ (0.56 mL, 4.0 mmol). The reaction mixture was stirred for 16 h at room temperature and diluted with DCM (5 mL). The organic layer was seperated and aqueous layer extracted with the DCM (5 mL x 3), combined organic layers were washed with 1N HCl (5 mL), saturated $NaHCO_3$ (5 mL), water (5 mL) and brine (5 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by column chromatography (silica gel 230-400, 1:2, ethyl acetate: pet ether) to afford **28** (350 mg, 74%) as a viscous liquid.

**$[\alpha]_D^{26}$:** -72.3 (*c* 0.5, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3342, 2920, 1756, 1256, 1100

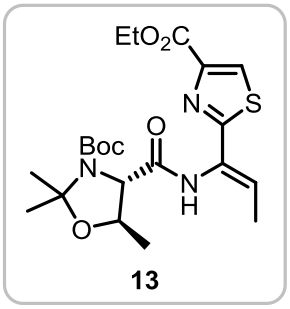
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.06 (s, 1H), 5.23 (brs, 1H), 4.54 (brs, 1H), 4.34 (q, *J* = 7.09 Hz, 2H), 3.90 (d, *J* = 7.34 Hz, 1H), 1.57 (d, *J* = 6.11 Hz, 6H), 1.35 (m, 15H), 1.25 (m, 6H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.2 (2C), 161.0, 152.1, 146.5, 127.6, 94.7, 81.0, 73.9, 69.0, 67.3, 61.3, 55.9, 27.5 (3C), 25.2, 19.3, 18.7, 14.2

**MS:** 494 $[M+Na]^+$

**HRMS:** Calculated for $C_{21}H_{34}O_7N_3S$ $[M+H]^+$: 472.2112, found 472.2103.

***tert*-butyl (4S,5R)-4-(((Z)-1-(4-(ethoxycarbonyl)thiazol-2-yl)prop-1-en-1-yl)carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (13)**[20]




**13**

To a solution of compound **28** (500 mg, 1.06 mmol) in 1:1 DMF: DCM (10 mL) was added the EDC.HCl (2.03 g, 10.6 mmol) and CuCl (314 mg, 3.18 mmol) at room temperature. The reaction mixture was stirred for 10 h, solvent was removed under reduced pressure and diluted with water. The resulting solution was filtered through celite bed and filtrate was extracted with ethyl acetate (10 mL x 3), washed with water (10 mL x 3), brine (10 mL x 2), dried over anhydrous $Na_2SO_4$. The crude material obtained after removal of solvent was purified by flash column chromatography (silica gel 230-400 mesh, 1:3, ethyl acetate: pet ether) to afford **13** (330 mg, 69% yield) as a viscous liquid.

**$[\alpha]_D^{26}$ :** -62.0 (*c* 1.1, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3223, 2910, 1690, 1230, 1103

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.01 (s, 1H) 7.82 (s, 1H) 6.51 (brs, 1H) 4.35 (q, *J* = 7.09 Hz, 3H) 3.99 (d, *J* = 7.82 Hz, 1H) 1.86 (d, *J* = 6.87 Hz, 3H) 1.65 (s, 6H) 1.48 (d, *J* = 6.11 Hz, 3H) 1.42 (s, 9H) 1.36 (t, *J* = 7.09 Hz, 3H)

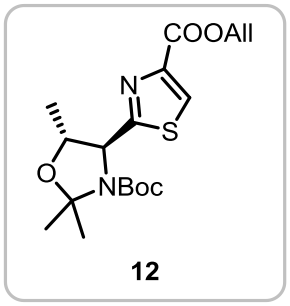
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 168.2, 166.9, 161.1, 152.1, 147.0, 127.9, 126.8 (2C), 95.0, 81.0, 74.2, 67.8, 61.3, 28.2 (3C), 27.1, 25.5, 18.9, 14.2 (2C)

**MS:** 376 $[M+Na]^+$

**HRMS:** Calculated for $C_{21}H_{32}O_6N_3S$ $[M+H]^+$: 454.2006, found 454.1995.

The $^{1}H$ and $^{13}C$ NMR values are compared with reported values and found to be identical.

***tert*-butyl (4S,5R)-2,2,5-trimethyl-4-(4-((((E)-prop-1-en-1-yl)oxy)carbonyl)thiazol-2-yl)oxazolidine-3-carboxylate (12)**




**12**

To a solution of compound **15** (500 mg, 1.35 mmol) in THF (5.0 mL) was added 1N LiOH (4.0 mL, 4.0 mmol) at 0 ºC. The reaction mixture was stirred for 3 h at room temperature and solvent was removed under reduced pressure. The aqueous layer was acidified to $P^{H}$ 2 and extracted with ethyl acetate (10 mL x 3), combined organics were dried over anhydrous $Na_2SO_4$. The crude acid (390 mg) obtained after removal of solvent was forwarded to next step without further purification.

To a solution of acid (390 mg, 1.14 mmol) in dry DMF (5.0 mL) was added a solid $NaHCO_3$ (670 mg, 8.0 mmol) and allyl bromide (2.1 mL, 23.9 mmol) at room temperature. The reaction mixture were stirred for 6 h and diluted with water, extracted with ethyl acetate (5 mL x 3). Combined organic layers were washed with saturated $NaHCO_3$ (10 mL x 3), water (10 mL x 3), brine (10 mL) and dried over anhydrous $Na_2SO_4$, the crude material obtained after removal of solvent was purified by flash column chromatography (silica gel 230-400 mesh, 1:3, ethyl acetate: pet ether) to afford **12** (400 mg, 78% two steps).

**$[\alpha]_D^{26}$:** -29.10 (*c* 1.0, $CHCl_3$)

**Mp:** 155-158 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3123, 1765, 1620, 1597

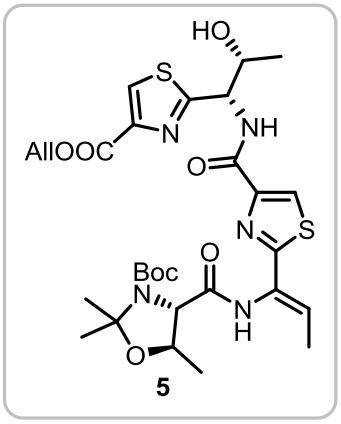
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 8.17 (brs, 1H), 6.02 (tdd, *J* = 5.6, 11.0, 16.9 Hz, 1H), 5.39 (dd, *J* = 1.3, 17.2 Hz, 1H), 5.28 (d, *J* = 10.5 Hz, 1H), 4.85 (d, *J* = 4.9 Hz, 2H), 4.79 (brs, 1H), 4.16 (brs, 1H), 1.69 (brs, 6H), 1.42 (d, *J* = 5.9 Hz, 6H), 1.18 (brs, 6H)

**$^{13}$C NMR (100MHz, $CDCl_3$):** δ 173.5, 173.4, 160.8, 146.4, 131.8, 127.5, 118.8, 95.3, 80.8, 77.8, 65.9 (2C), 28.1 (3C), 26.5, 25.9, 17.8

**MS**: 405 $[M+Na]^+$

**HRMS**: Calculated for $C_{18}H_{27}O_5N_2S$ $[M+H]^+$: 383.1635 found 383.1628 and $C_{18}H_{26}O_5N_2SNa$ $[M+Na]^+$: 405.1455 found 405.1447

***tert*-butyl (4S,5R)-4-(((Z)-1-(4-(((1S,2R)-2-hydroxy-1-(4-((((E)-prop-1-en-1-yl)oxy)carbonyl)thiazol-2-yl)propyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)carbamoyl)-2,2,5-trimethyloxazolidine-3-carboxylate (5)**




**5**

To a solution of compound **13** (200 mg, 0.44 mmol) in 1:1 THF: $H_2O$ (6 mL) was added 1M $LiOH.H_2O$ (2.2 mL, 2.2 mmol) at 0 ºC. The reaction mixture was stirred for 2 h at room temperature and solvent was removed under reduced pressure. The aqueous layer was acidified to $P^H$ 2 and extracted with ethyl acetate (5 mL x 3), combined organics were dried over anhydrous $Na_2SO_4$. The crude acid obtained after removal of solvent was forwarded to next step without further purification.

To a stirred solution of acid in dry DCM (10 mL) was added EDC.HCl (102 mg, 0.54 mmol), HOBt (72 mg, 0.54 mmol) and stirred for 10 minutes at 0 ºC. The amine salt (125 mg, 0.44 mmol) (prepared from the treatment of compound **12** with 4M HCl in dioxane) was added, followed by $Et_3N$ (0.25 mL, 1.8 mmol) and reaction mixture was stirred for 16 h at room temperature. Reaction mixture was diluted with DCM (5 mL) and water (5 mL), organic layer was seperated and aqueous layer re-extracted with the DCM (5 mL x 3), combined organic layers were washed with 1N HCl (5 mL), saturated $NaHCO_3$ (5 mL), water (5 mL) and brine (5 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by column chromatography (silica gel, 230-400 mesh, 1:2 ethyl acetate: pet ether) to afford **5** (196 mg, 69%) as a viscous liquid.

**$[\alpha]_D^{27}$:** - 22.73 (*c* 1.0, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3323, 1756, 1650, 1217

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.21 (brs, 1H), 8.10 (s, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.81 (brs, 1H), 6.57 (brs, 1H), 5.37 (dd, *J* = 1.8, 8.7 Hz, 1H), 4.77-4.67 (m, 1H), 4.39 (q, *J* = 7.3 Hz, 4H), 4.02 (d, *J* = 7.3 Hz, 1H), 3.83 (brs, 1H), 1.86 (s, 3H), 1.61 (d, *J* = 6.4 Hz, 6H), 1.46-1.39 (m, 12H), 1.31 (d, *J* = 6.4 Hz, 3H)

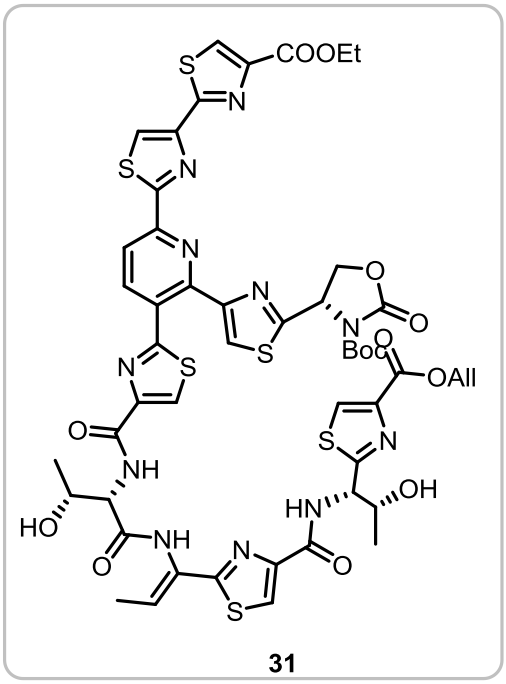
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 171.1, 168.3 (2C), 167.0, 166.4, 161.1, 149.0, 146.8, 128.0, 127.9, 127.8 (2C), 127.7, 123.8, 95.1, 81.3, 74.4, 68.2, 67.73, 61.4, 55.3, 31.5, 28.3 (3C), 25.8, 22.6, 19.5, 14.3

**MS:** 672 $[M+Na]^+$

**HRMS:** Calculated for $C_{29}H_{39}O_8N_5S_2Na$ $[M+H]^+$: 650.2313, found 650.2308 and $C_{29}H_{40}O_8N_5S_2Na$ $[M+Na]^+$: 672.2132, found 672.2124

**tert-butyl (S)-4-(4-(6-(4-(ethoxycarbonyl)-[2,4'-bithiazol]-2'-yl)-3-(4-(((2S,3R)-3-hydroxy-1-(((Z)-1-(4-(((1S,2R)-2-hydroxy-1-(4-((((E)-prop-1-en-1-yl)oxy)carbonyl) thiazol-2-yl)propyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)amino)-1-oxobutan-2-**

**yl)carbamoyl)thiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-2-oxooxazolidine-3-carboxylate (31)**




**31**

To a solution of compound **4** (50 mg, 0.071 mmol) in 1:1 THF: $H_2O$ (5 mL) was added a 2-methyl-2-butene (0.076 mL, 0.71 mmol), $NaH_2PO_4$ (32 mg, 0.035 mmol) and $NaClO_2$ (43 mg, 0.35 mmol) at 0 ºC. The reaction mixture was stirred for 16 h at room temperature and solvent was removed under reduced pressure. The solid product was filtered out and aqueous layer was acidified, the obtained solid product was filtered and the combined acid **29** was forwarded to the next step without further purification.

To a stirred solution of acid **29** in dry DMF (3 mL) was added HATU (32 mg, 0.08 mmol) and stirred for 10 minutes at 0 ºC. The amine salt **30** (39 mg, 0.62 mmol) (prepared by the treatment of compound **5** with 4M HCl in dioxane) followed by DIPEA (0.049 mL, 0.28 mmol) was added and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with DCM (3 mL) and water (3 mL). The organic layer was separated and aqueous layer re-extracted with the DCM (3 mL x 3), combined organic layers were washed with 1N HCl (3 mL), saturated $NaHCO_3$ (3 mL), water (2 mL) and brine (2 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and

the crude material obtained after removal of solvent was purified by flash column chromatography (silica gel, 230-400 mesh, 1:20, MeOH: DCM) to afford **31** (49 mg, 58%).

**$[\alpha]_D^{27}$:** - 10.19 (*c* 0.5, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3394, 2977, 1794, 1720, 1700, 1501

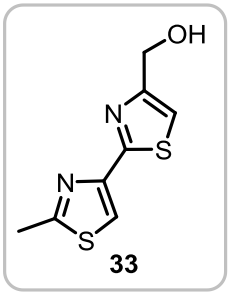
**$^1$H NMR (500 MHz, $CDCl_3$):** δ 8.83 (s, 1H), 8.52 (d, *J* = 8.9 Hz, 1H), 8.33 (m, 3H), 8.25 (m, 3H), 8.13 (d, *J* = 5.8 Hz, 1H), 8.09 (d, *J* = 7.3 Hz, 2H), 7.98 (m, 1H), 6.44 (m, 1H), 5.96 (m, 1H), 5.44 (dd, *J* = 2.7, 8.2 Hz, 1H), 5.38-5.31 (m, 2H), 5.24 (d, *J* = 10.4 Hz, 1H), 4.84 (m, 2H), 4.75 (m, 3H), 4.53 (dd, *J* = 2.9, 6.3 Hz, 1H), 4.46 (q, *J* = 7.2 Hz, 3H), 4.28 (dd, *J* = 2.6, 8.7 Hz, 1H), 1.86 (d, *J* = 7.0 Hz, 3H), 1.46 (s, 9H), 1.44 (s, 3H), 1.34 (d, *J* = 6.4 Hz, 3H), 1.24 (brs, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 171.4, 168.8, 168.2, 166.6, 166.4, 165.0, 163.1, 161.4, 161.2, 160.7, 153.3, 151.1, 150.8, 150.3, 150.0, 149.4, 148.5, 148.4, 148.0, 146.2, 140.3, 131.7, 128.7, 128.1, 128.0, 127.1, 126.1, 123.4, 122.3, 120.6, 118.9, 118.8, 85.0, 68.8, 67.2, 66.6, 66.0, 66.0, 61.6, 57.8, 55.4, 27.9, 27.8 (3C), 19.5, 18.5, 14.8, 14.3

**MS:** 1226 $[M+Na]^+$

**HRMS:** Calculated for $C_{50}H_{50}O_{13}N_{11}S_6$ $[M+H]^+$: 1204.1908, found 1204.1934

**(2'-methyl-[2,4'-bithiazol]-4-yl)methanol (33)**




**33**

To a suspension of lithium aluminum hydride (750 mg, 19.82 mmol) in dry diethyl ether (20 mL) at 0 °C was added slowly a solution of compound **8** (3.6 g, 14.16 mmol) in

diethyl ether (20 mL). The reaction mixture was allowed to stir at 0 ºC for 10 minutes and then at room temperature for 3 h. Cooled to 0 ºC and diluted with ethyl acetate (15 mL), the resulting suspension were added slowly to an aqueous solution of potassium sodium tartrate (30% w/v, 50 mL) and stirred for 1 h. The mixture was diluted with 50 mL of ethyl acetate, the layers were separated and the aqueous layer was re-extracted with ethyl acetate (10 mL x 2). The combined organic layers were dried over $Na_2SO_4$, filtered, evaporated and purified by column chromatography (silica gel, 100-200 mesh, ethyl acetate: pet ether, 1:2) to give alcohol **33** (2.2 g, 74% yield) as white solid.

**Mp:** 114-116 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3349, 3014, 1598, 1495, 1611

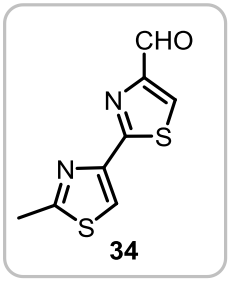
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 7.81 (s, 1H), 7.19 (s, 1H), 4.81 (s, 2H), 2.76 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$): δ** 166.8, 163.3, 157.1, 148.7, 115.8, 115.2, 60.9, 19.2

**MS:** 235 $[M+Na]^+$

**HRMS:** Calculated for $C_8H_9ON_2S_2$ $[M+H]^+$: 213.0151, found 213.0150

**2'-methyl-[2,4'-bithiazole]-4-carbaldehyde (34)**




**34**

To a 100 mL flask equipped with a septum, a magnetic stirring bar, was charged with alcohol **33** (2.0 g, 9.42 mmol) and dry DCM (20 mL). It was cooled to 0 ºC and Dess-Martin periodinane (4.49 g, 10.36 mmol) was added portion wise. The reaction mixture was stirred at room temperature for 1 h and diluted with water. The organic layer was washed well with water (10 mL x 2), a saturated solution of $NaHCO_3$ (10 mL x 2) and

brine (10 mL x 1). The combined organic layer was dried over $Na_2SO_4$, evaporated and purified by column chromatography eluting with ethyl acetate: pet ether (1:4) to get the aldehyde **34** (1.7 g, 87% yield) as colourless solid.

**Mp:** 136-140 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3259, 1720, 1615, 1587

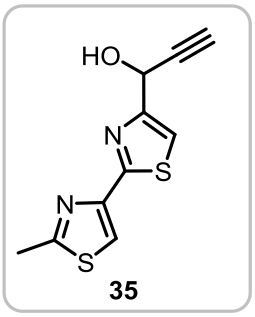
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.05 (s, 1H), 8.17 (s, 1H), 7.96 (s, 1H), 2.78 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 184.7, 167.2, 164.0, 155.5, 147.8, 128.2, 117.2, 19.2

**MS:** 232 $[M+Na]^+$

**HRMS:** Calculated for $C_8H_7ON_2S_2$ $[M+H]^+$: 210.9994, found 210.9994.

**1-(2'-methyl-[2,4'-bithiazol]-4-yl)prop-2-yn-1-ol (35)**




**35**

A solution aldehyde **34** (1.5 g, 7.13 mmol) in dry THF (15 mL) were cooled to 0 ºC and a 0.5M solution of ethynylmagnesium bromide (14.26 mL, 7.13 mmol) were added dropwise over the period of 10 minutes under argon. The reaction mixture was stirred for 30 minutes at the same temperature and saturated solution of $NH_4Cl$ was added (2 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (10 mL x 3), the combined organic layers were washed with saturated $NH_4Cl$, water, and brine. The organic layer was dried over $Na_2SO_4$, evaporated and purified by column chromatography eluting with ethyl acetate: pet ether, (1:2) to afford the alcohol **35** (1.24 g, 73% yield) as fibrous solid.

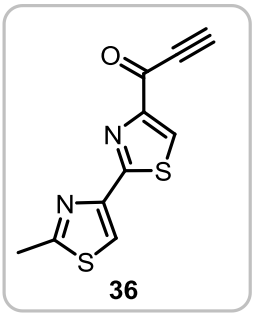
**$^{1}$H NMR (400 MHz, $CD_3OD$):** δ 8.02 (s, 1H), 7.58 (s, 1H), 5.55 (brs, 1H), 3.05 (s, 1H), 2.74 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 166.8, 163.9, 155.8, 148.3, 116.4, 82.2, 74.3, 60.5, 29.7, 19.2

**MS:** 259 $[M+Na]^+$

**HRMS:** Calculated for $C_{10}H_9ON_2S_2$ $[M+H]^+$: 237.0151, found 237.0148.

**1-(2'-methyl-[2,4'-bithiazol]-4-yl)prop-2-yn-1-one (36)**




**36**

To a 100 mL flask equipped with a septum, a magnetic stirring bar, was charged with alcohol **35** (1.1 g, 4.65 mmol) and dry DCM (10 mL). It was cooled to 0 ºC and Dess-Martin periodinane (2.17 g, 5.12 mmol) was added portion wise. The reaction mixture was stirred at room temperature for 1 h, diluted with water and the organic layer was washed well with water (10 mL x 2), a saturated solution of $NaHCO_3$ (10 mL x 2) and brine (10 mL). The combined organic layer was dried over $Na_2SO_4$, evaporated and purified by column chromatography eluting with the ethyl acetate: pet ether (1:3) to get the ynone **36** (0.87 g, 81% yield) as colourless solid.

**Mp:** 166-170 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3135, 2926, 2100, 1730, 1645

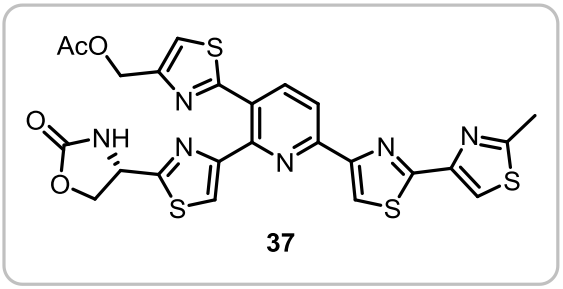
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 8.36 (s, 1H), 7.99 (s, 1H), 3.48 (s, 1H), 2.73 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 169.3, 166.9, 163.9, 154.9, 147.5, 131.1, 117.6, 80.7, 80.5, 19.0

**MS:** 257 $[M+Na]^+$

**HRMS:** Calculated for $C_{10}H_7ON_2S_2$ $[M+H]^+$: 234.9994, found 234.9993

**(S)-(2-(6-(2'-methyl-[2,4'-bithiazol]-4-yl)-2-(2-(2-oxooxazolidin-4-yl)thiazol-4-yl)pyridin-3-yl)thiazol-4-yl)methyl acetate (37)**




**37**

To a solution of compound **7** (500 mg, 1.14 mmol) in glacial acetic acid (5 mL), $NH_4OAc$ (132 mg, 1.71 mmol) and compound **36** (267 mg, 1.14 mmol) was added and reaction mixture was stirred for 12 h at 120 ºC. Cooled to room temperature, solvent was removed under reduced pressure and the residue was neutralized with saturated $NaHCO_3$ solution (pH ~10) and extracted with ethyl acetate (10 mL x 3). The combined organics were dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure and purified by column chromatography with ethyl acetate: pet ether (1:1) to afford **37** (395 mg, 60% yield).

**$[\alpha]_D^{27}$:** + 41.63 (*c* 1.0 $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3307, 2933, 1745, 1714, 1669, 1610

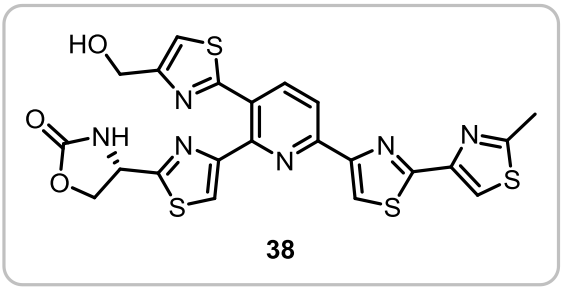
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.17 (m, 2H), 8.07 (s, 1H), 7.88 (s, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 6.47 (brs, 1H), 5.21 (m, 2H), 5.13 (m, 1H), 4.68 (t, *J* = 8.80 Hz, 1H), 4.32 (dd, *J* = 8.93, 5.26 Hz, 1H), 2.81 (s, 3H), 2.13 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 170.8, 169.0 (2C) 166.4, 165.9, 158.7 (2C), 154.8, 153.9, 152.8, 151.3, 150.4 (2C) 139.5, 127.4, 120.9, 120.1, 119.0, 118.4, 70.4, 61.8, 53.7, 20.9, 19.4

**MS:** 605 $[M+Na]^+$

**HRMS:** Calculated for $C_{24}H_{19}O_4N_6S_4$ $[M+H]^+$:583.0345, found 583.0344.

**(S)-4-(4-(3-(4-(hydroxymethyl)thiazol-2-yl)-6-(2'-methyl-[2,4'-bithiazol]-4-yl)pyridin-2-yl)thiazol-2-yl)oxazolidin-2-one (38)**




**38**

To a solution compound **37** (100 mg, 0.17 mmol) in THF: $H_2O$ (5 mL, 1:1) was added a $LiOH.H_2O$ (15 mg, 0.34 mmol) at 0 ºC. The reaction mixture was stirred for 3 h at room temperature and solvent was removed under reduced pressure. The aqueous layer was extracted with ethyl acetate (5 mL x 3). The combined organic layer was washed with saturated $NaHCO_3$ (3 mL), brine (3 mL) and dried over anhydrous $Na_2SO_4$. The crude material obtained after removal of solvent was purified by column chromatography (silica gel 100-200, 2:1 ethyl acetate: pet ether) to afford **38** (75 mg, 82% yield) as yellow solid.

**Mp**: 185-188 ºC

**$[\alpha]_D^{26}$:** -7.5 (*c* 0.053, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3310, 2924, 1710, 1632, 1040

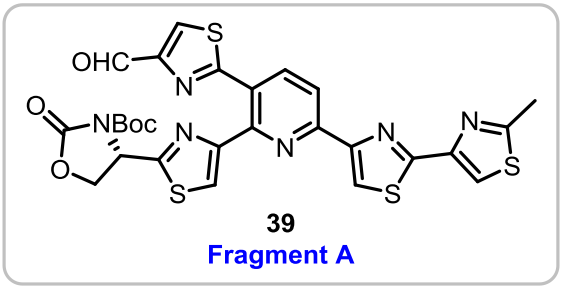
**$^{1}$H NMR (400 MHz, DMSO-d6):** δ 8.69 (s, 1H), 8.45 (m, 2H), 8.25 (m, 3H), 7.49 (s, 1H), 5.37 (t, *J* = 5.75 Hz, 1H), 5.23 (dd, *J* = 8.56, 4.89 Hz, 1H), 4.63 (m, 3H), 4.16 (dd, *J* = 8.56, 4.89 Hz, 1H), 2.76 (s, 3H)

**$^{13}$C NMR (100 MHz, DMSO-d6):** δ 171.3, 167.3, 163.4, 162.6, 158.4, 157.8, 154.4, 153.5, 151.5, 150.6, 147.5, 138.9, 128.0, 121.8, 120.3, 120.0, 117.3, 117.1, 69.6, 59.7, 52.9, 18.8

**MS:** 563 $[M+Na]^+$

**HRMS:** Calculated for $C_{22}H_{17}O_3N_6S_4$ $[M+H]^+$: 541.0239, found 541.0237

***tert*-butyl (S)-4-(4-(3-(4-formylthiazol-2-yl)-6-(2'-methyl-[2,4'-bithiazol]-4-yl) pyridin-2-yl)thiazol-2-yl)-2-oxooxazolidine-3-carboxylate (39)**




**39**

**Fragment A**

A solution of compound **38** (50 mg, 0.09 mmol) in dry DCM (3 mL) was cooled to 0 ºC and Dess-Martin periodinane (44 mg, 0.1 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and diluted with water, the organic layer was washed well with water (3 mL x 3), saturated solution of $NaHCO_3$ (3 mL) and brine (3 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to afford the aldehyde which was forwarded to next step without further purification.

The obtained aldehyde were dissolved in DCM (3 mL) and cooled to 0 °C, $(Boc)_2O$, $Et_3N$ and catalytic amount of DMAP (1 mg) was added. The reaction was allowed to stir at 0 ºC for 30 minutes and 2 h at room temperature. 1N HCl (1 mL) was added and organic layer was separated, the aqueous layer was extracted with DCM (3 mL x 3). The combined organic layers were dried over anhydrous $Na_2SO_4$, filtered and concentrated.

The crude material obtained after removal of solvent was purified by column chromatography (silica gel, 230-400, 1:2 ethyl acetate: pet ether) to afford **39** (40 mg, 81% yield).

**$[\alpha]_D^{27}$:** -7.6 (*c* 0.27, $CHCl_3$)

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3230, 2981, 2931, 1710, 1690

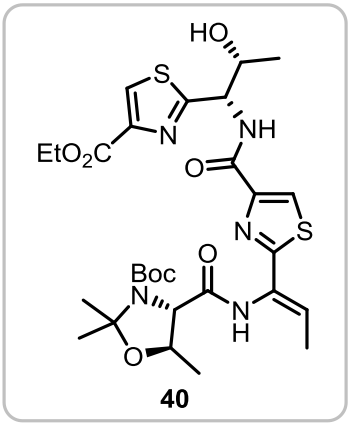
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.03 (s, 1H), 8.32 (m, 2H), 8.27 (s, 1H), 8.15 (m, 2H), 7.94 (s, 1H), 5.43 (dd, *J* = 8.44, 3.06 Hz, 1H), 4.44 (t, *J* = 8.56 Hz, 1H), 4.29 (dd, *J* = 8.80, 3.18 Hz, 1H), 2.80 (s, 3H) 1.47 (s, 9H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 184.3, 167.1, 166.9, 166.0, 163.0, 155.0, 154.6, 154.2, 153.1, 151.0, 150.0, 148.7, 148.5, 140.0, 130.7, 126.5, 121.8, 120.1, 119.3, 116.0, 84.9, 66.4, 55.3, 27.9 (3C) 19.2

**MS:** 661 $[M+Na]^+$

**HRMS:** Calculated for $C_{27}H_{23}O_5N_6S_4$ $[M+H]^+$: 639.0607, found 639.0602

***tert*-butyl (4S,5R)-4-(((Z)-1-(4-(((1S,2R)-1-(4-(ethoxycarbonyl)thiazol-2-yl)-2-hydroxypropyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)carbamoyl)-2,2,5-trimethyl oxazolidine-3-carboxylate (40)**




**40**

To a solution of compound **13** (470 mg, 1.1 mmol) in 1:1 THF: $H_2O$ (6 mL) was added LiOH.$H_2O$ (92 mg, 2.2 mmol) at 0 ºC. The reaction mixture was stirred for 2 h at room temperature and solvent was removed under reduced pressure. The aqueous layer was acidified to $P^H$ 2 and extracted with ethyl acetate (10 mL x 3), combined organics were dried over anhydrous $Na_2SO_4$. The crude acid obtained after removal of solvent was forwarded to next step without further purification.

To a stirred solution of  acid in dry DCM (10 mL) was added EDC.HCl (254 mg, 1.33 mmol), HOBt (179 mg, 1.33 mmol) and stirred for 10 minutes at 0 ºC. The amine salt (294 mg, 1.1 mmol) (prepared from the treatment of compound **15** with 4M HCl in dioxane) was added, followed by $Et_3N$ (0.6 mL, 4.42 mmol) and reaction mixture was stirred for 16 h at room temperature. Reaction mixture was diluted with DCM (10 mL) and water (10 mL), organic layer was seperated and aqueous layer re-extracted with the DCM (10 mL x 3), combined organic layers were washed with 1N HCl (10 mL), saturated $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by column chromatography (silica gel, 230-400 mesh, 1:2 ethyl acetate: pet ether) to afford **40** (560 mg, 80%) as a viscous liquid.

**$[\alpha]_D^{27}$:** -50.2 (*c* 1.0, $CHCl_3$)

**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3310, 2936, 1785, 1420, 1013

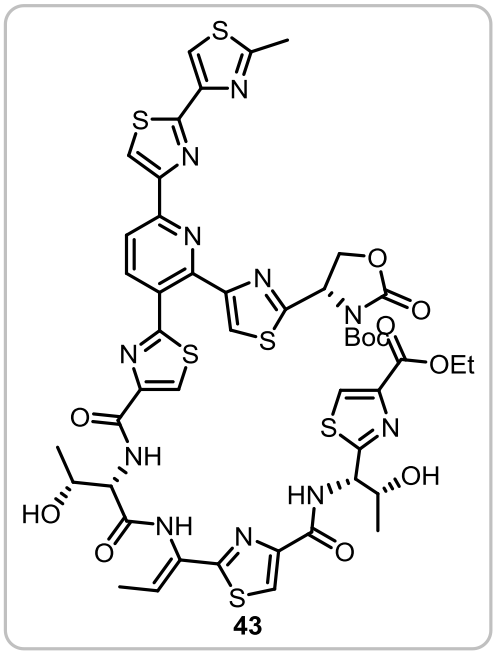
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 8.20 (brs, 1H), 8.10 (s, 1H), 8.03 (s, 1H), 7.81 (brs, 1H), 6.57 (brs, 1H), 5.37 (dd, *J* = 8.70, 1.83 Hz, 1H), 4.73 (m, 1H), 4.39 (m, 4H), 4.02 (d, *J* = 7.33 Hz, 1H), 1.87 (d, *J* = 7.33 Hz, 3H), 1.61 (d, *J* = 6.41 Hz, 6H), 1.42 (m, 15H), 1.31 (d, *J* = 6.41 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 171.1, 168.3 (2C), 167.0, 166.4, 161.1 (2C), 149.0, 146.8, 128.0, 127.8, 127.7, 123.8, 95.1, 81.3, 68.2, 67.7, 61.4, 55.3, 31.5, 28.3 (3C), 25.8, 22.6, 19.5, 19.3, 14.3

**MS:** 660 $[M+Na]^+$

**HRMS:** Calculated for $C_{28}H_{39}O_8N_5S_2$ $[M+Na]^+$: 660.2132, found 660.2119

***tert*-butyl (S)-4-(4-(3-(4-(((2S,3R)-1-(((Z)-1-(4-(((1S,2R)-1-(4-(ethoxycarbonyl) thiazol-2-yl)-2-hydroxypropyl)carbamoyl)thiazol-2-yl)prop-1-en-1-yl)amino)-3-hydroxy-1-oxobutan-2-yl)carbamoyl)thiazol-2-yl)-6-(2'-methyl-[2,4'-bithiazol]-4-yl)pyridin-2-yl)thiazol-2-yl)-2-oxooxazolidine-3-carboxylate (43)**




**43**

To a solution of compound **39** (40 mg, 0.062 mmol) in 1:1 THF: $H_2O$ (5 mL) was added a 2-methyl-2-butene (0.07 mL, 0.62 mmol), $NaH_2PO_4$ (38 mg, 0.31 mmol) and $NaClO_2$ (29 mg, 0.31 mmol) at 0 ºC. The reaction mixture was stirred for 16 h at room temperature and solvent was removed under reduced pressure. The solid product was filtered out and aqueous layer was acidified, the obtained solid product was filtered and the combined acid **41** was forwarded to the next step without further purification.

To a stirred solution of acid **41** (40 mg, 0.062 mmol) in dry DMF (2 mL) was added HATU (28 mg, 0.07 mmol) and stirred for 10 minutes at 0 ºC. The amine salt **42** (32 mg, 0.62 mmol) (prepared by the treatment of compound **40** with 4M HCl in dioxane) followed by DIPEA (0.032 mL, 0.18 mmol) was added and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with DCM (3 mL)

and water (3 mL). The organic layer was separated and aqueous layer re-extracted with the DCM (3 mL x 3), combined organic layers were washed with 1N HCl (3 mL), saturated $NaHCO_3$ (3 mL), water (2 mL) and brine (2 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by flash column chromatography (silica gel, 230-400 mesh, 1:20, MeOH: DCM) to afford **43** (39 mg, 56%).

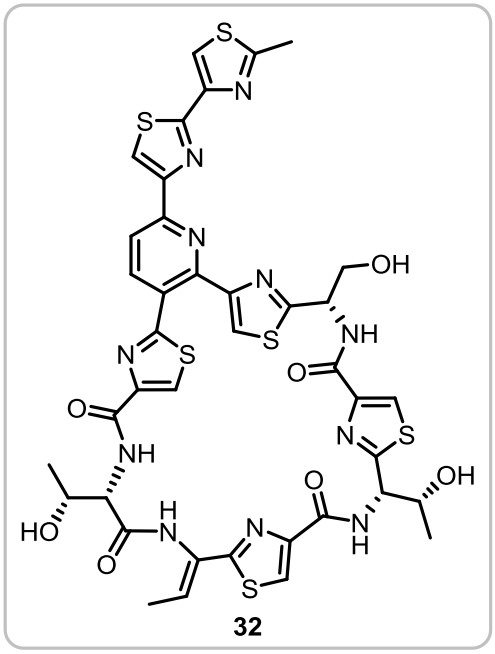
**$[\alpha]_D^{27}$:** +7.2 (*c* 0.5, $CHCl_3$)

**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3310, 2936, 1735, 1710, 1690

**$^1$H NMR (400 MHz, $CD_3OD$):** δ 8.72 (brs, 1H), 8.32-8.08 (m, 9H), 6.01 (brs, 1H), 5.60 (brs, 1H), 5.49 (brs, 1H), 5.34 (brs, 2H), 5.27 (brs, 1H), 4.51 (brs, 2H), 4.35 (brs, 1H), 4.19 (brs, 1H), 2.74 (brs, 3H), 2.31 (brs, 1H), 2.03 (brs, 1H), 1.87 (brs, 1H), 1.61 (brs, 3H), 1.28 (brs, 18H)

**HRMS:** Calculated for $C_{47}H_{48}O_{11}N_{11}S_6$ $[M+H]^+$:1134.1854, found 1134.1870 and $C_{47}H_{47}O_{11}N_{11}S_6Na$ $[M+Na]^+$:1156.1673, found 1156.1689.

**Target Thiopeptide (32)**




**32**

To a solution of compound **43** (30 mg, 0.026 mmol) in 1:1 THF: $H_2O$ (5 mL) was added $LiOH.H_2O$ (11 mg, 0.26 mmol) at 0 ºC. The reaction mixture was stirred for 12 h at room temperature and solvent was removed under reduced pressure. The aqueous layer was acidified to $P^H$ 2 and extracted with 1:20 MeOH: DCM (3 mL x 3), combined organics were dried over anhydrous $Na_2SO_4$. The crude acid obtained after removal of solvent was forwarded to next step without further purification.

The product of the first step was dissolved in dry DCM (3 mL) and trifluoroacetic acid (0.6 mL) was added dropwise at 0 ºC. The reaction mixture stirred for 30 minutes at 0 ºC and 12 h at room temperature. The solvent was evaporated under reduced pressure followed by co-evaporation with the azeotrope of toluene to get the amine salt. The obtained salt was then dissolved in dry DMF and cooled to 0 ºC, the DPPA (10 mg, 0.039 mmol) and $Et_3N$ (0.007 mL, 0.10 mmol) were added and the reaction mixture stirred for 12 h at room temperature. The reaction mixture was diluted with DCM (3 mL) and water (3 mL). The organic layer was separated and aqueous layer re-extracted with the DCM (3 mL x 3), combined organic layers were washed with 1N HCl (3 mL), saturated $NaHCO_3$ (3 mL), water (2 mL) and brine (2 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and the crude material obtained after removal of solvent was purified by flash column chromatography (silica gel 230-400 mesh, 1:20, MeOH: DCM) to afford **32** (13 mg, 50% over three steps).

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3310, 2936, 1720, 1707, 1693

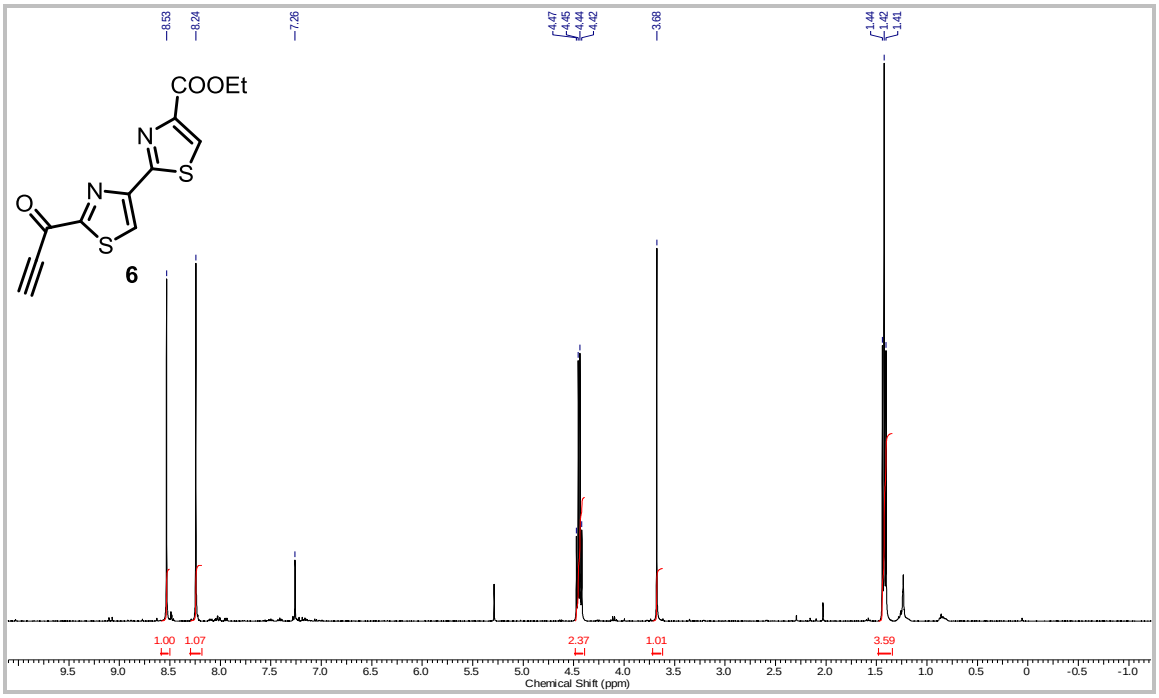
**$[\alpha]_D^{27}$:** +7.2 (*c* 0.05, $CHCl_3$)

**$^1$H NMR (400 MHz, DMSO-d6):** δ 8.50-8.25 (m, 8H), 7.49 (m, 1H), 7.18 (m, 3H), 6.83 (m, 1H), 5.47-5.21 (m, 3H), 4.57 (brs, 1H), 4.30 (m, 2H), 3.37 (m, 3H), 2.76 (s, 3H), 1.78 (m, 3H), 1.23 (brs, 3H), 1.16 (d, *J* = 5.87 Hz, 3H)

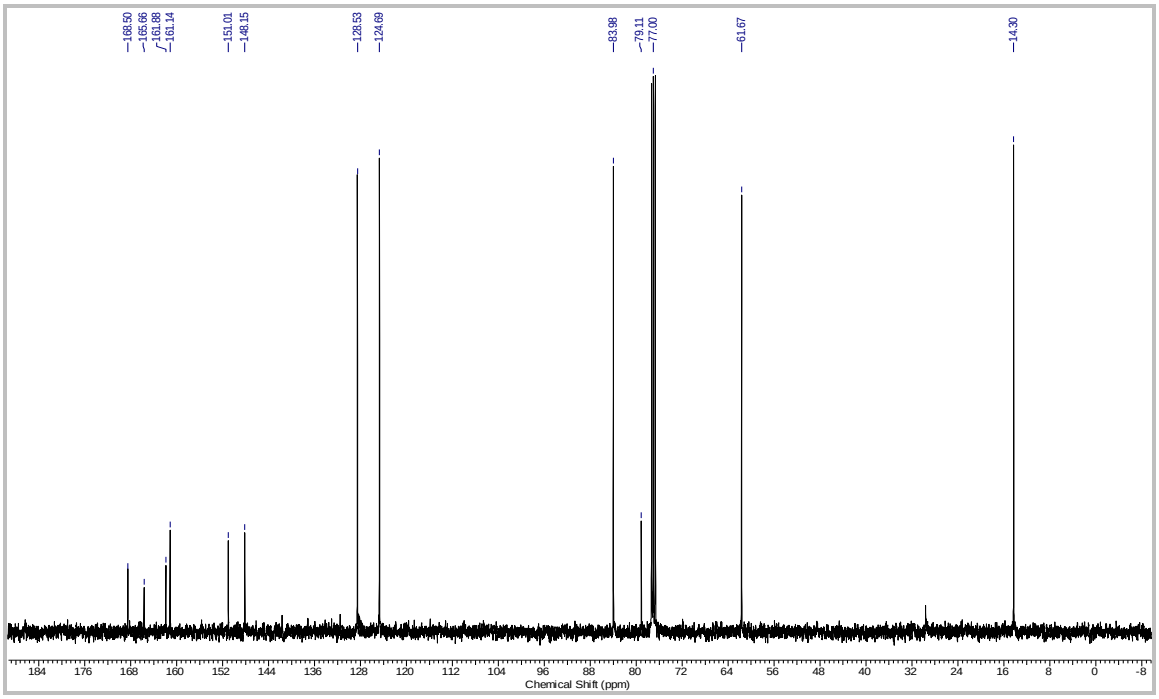
**HRMS:** Calculated for $C_{39}H_{36}O_7N_{11}S_6$ $[M+H]^+$: 962.1118, found:962.1111.

## 2.8. Spectra

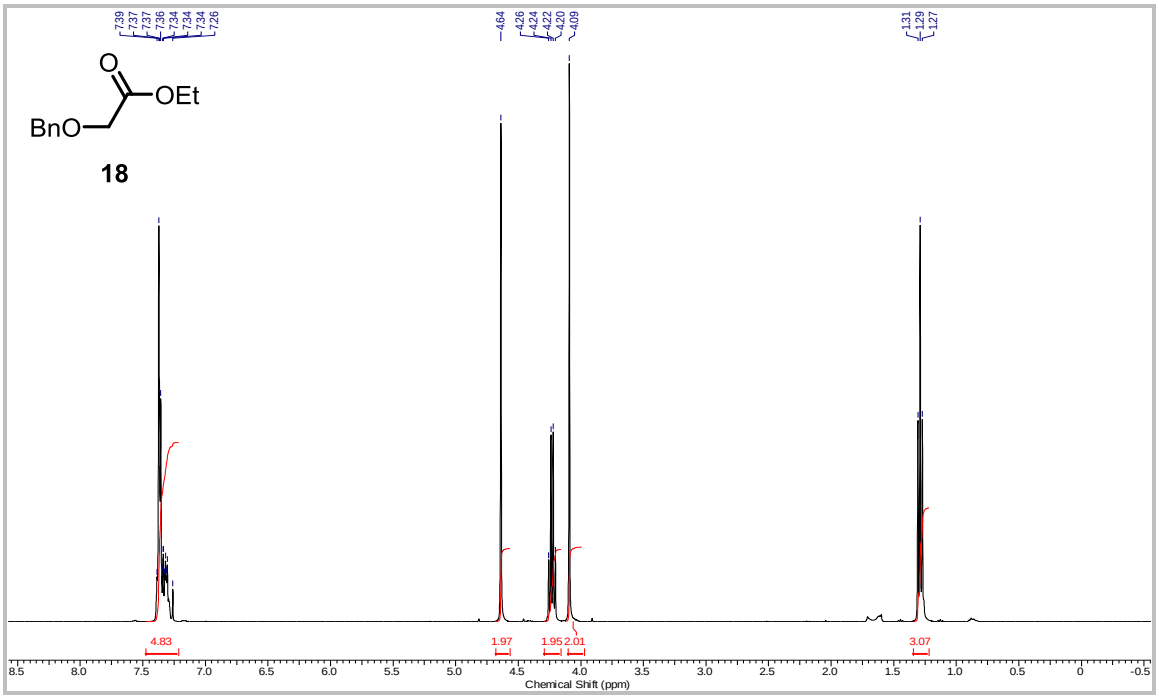
**$^{1}$H NMR (400 MHz, $CDCl_3$) of compound 6**



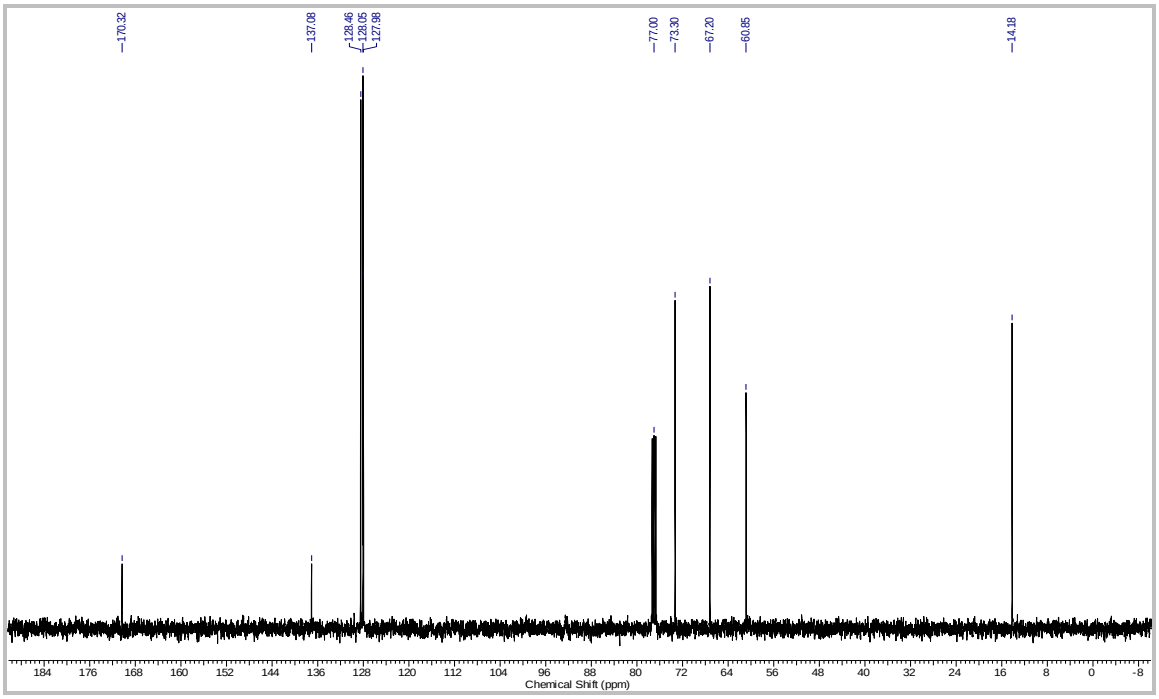

**$^{13}$C NMR (100 MHz, $CDCl_3$) of compound 6**



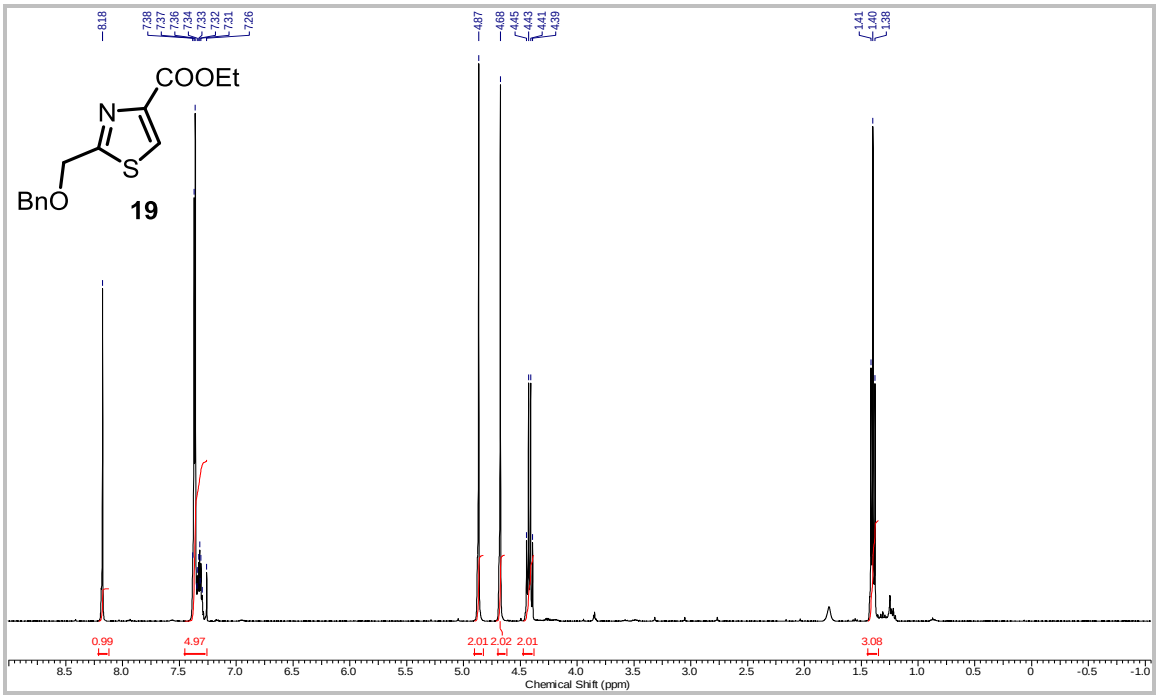
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 18



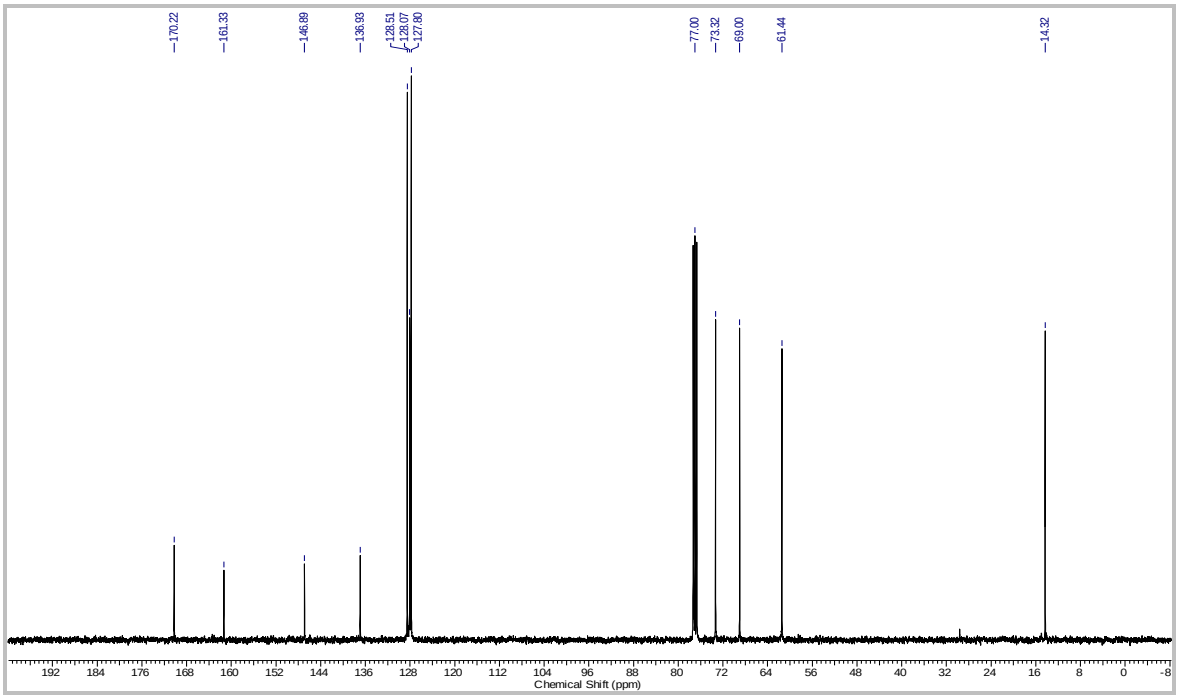

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 18



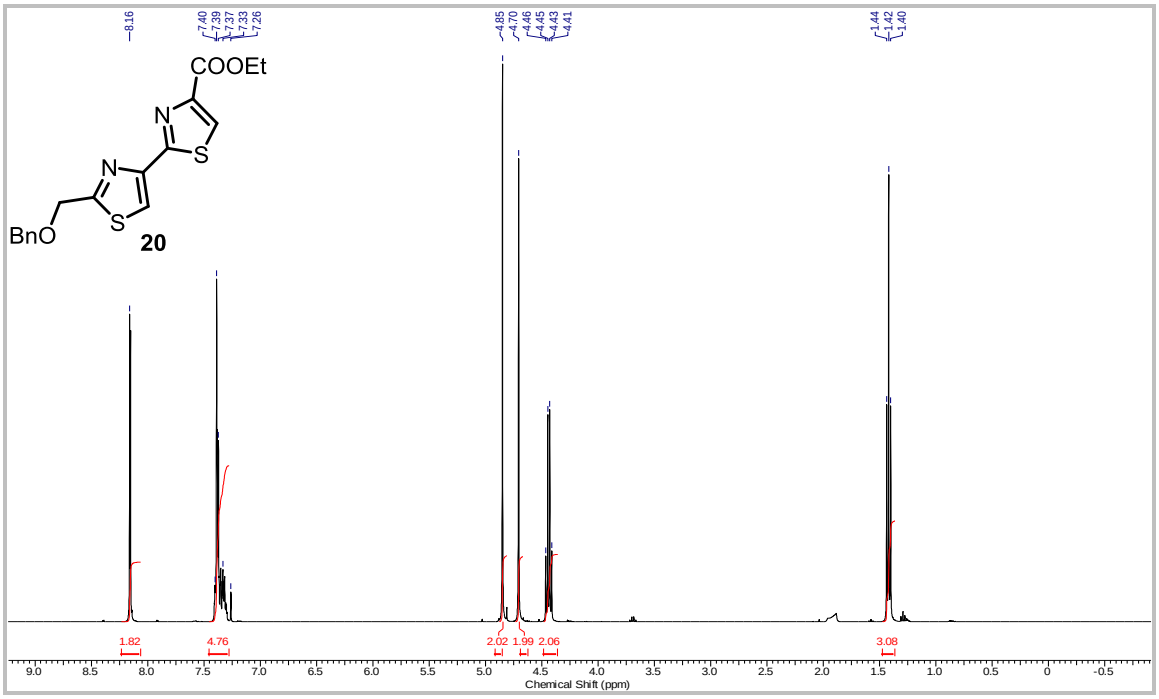
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 19



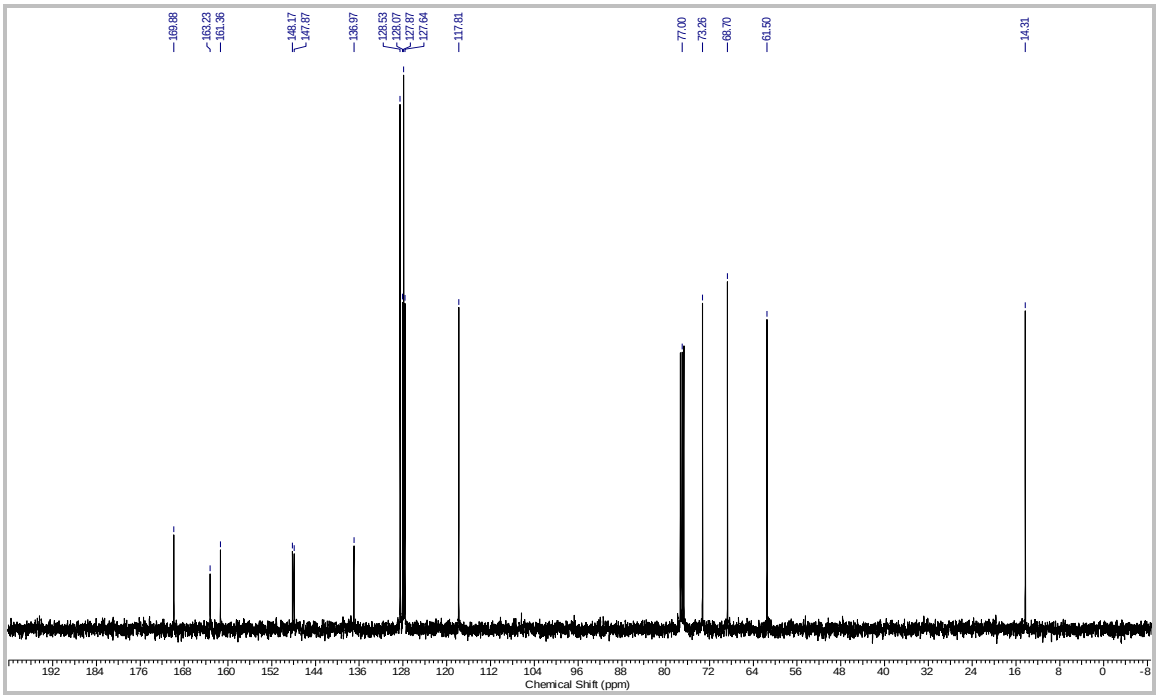

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 19



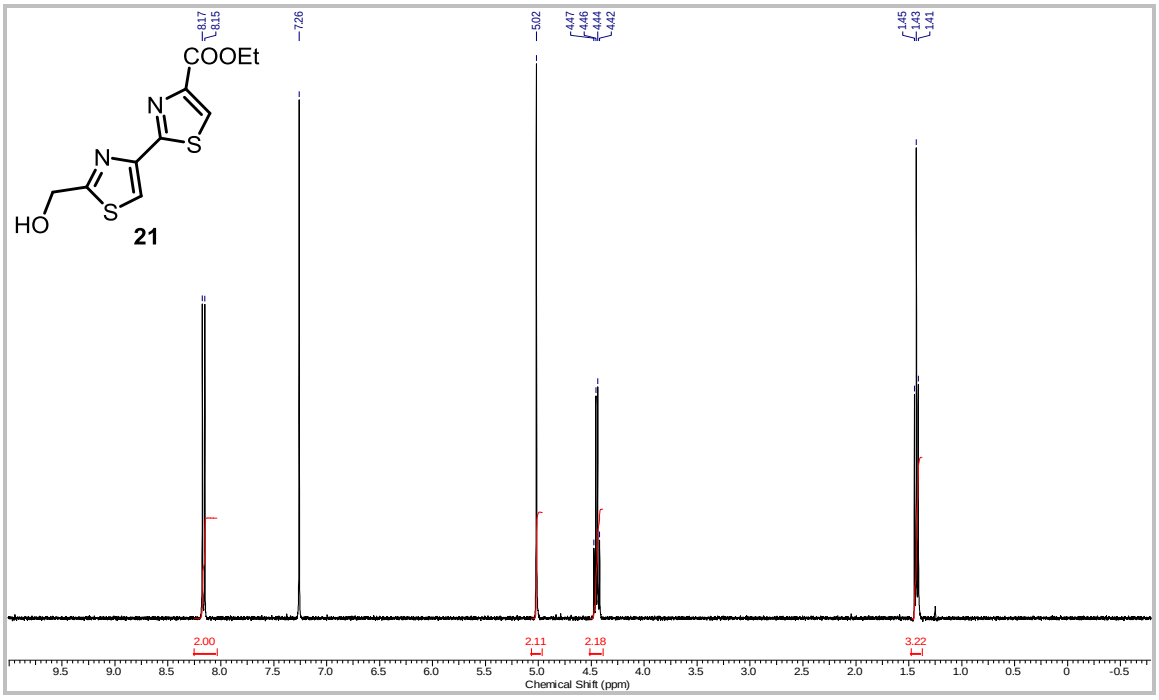
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 20



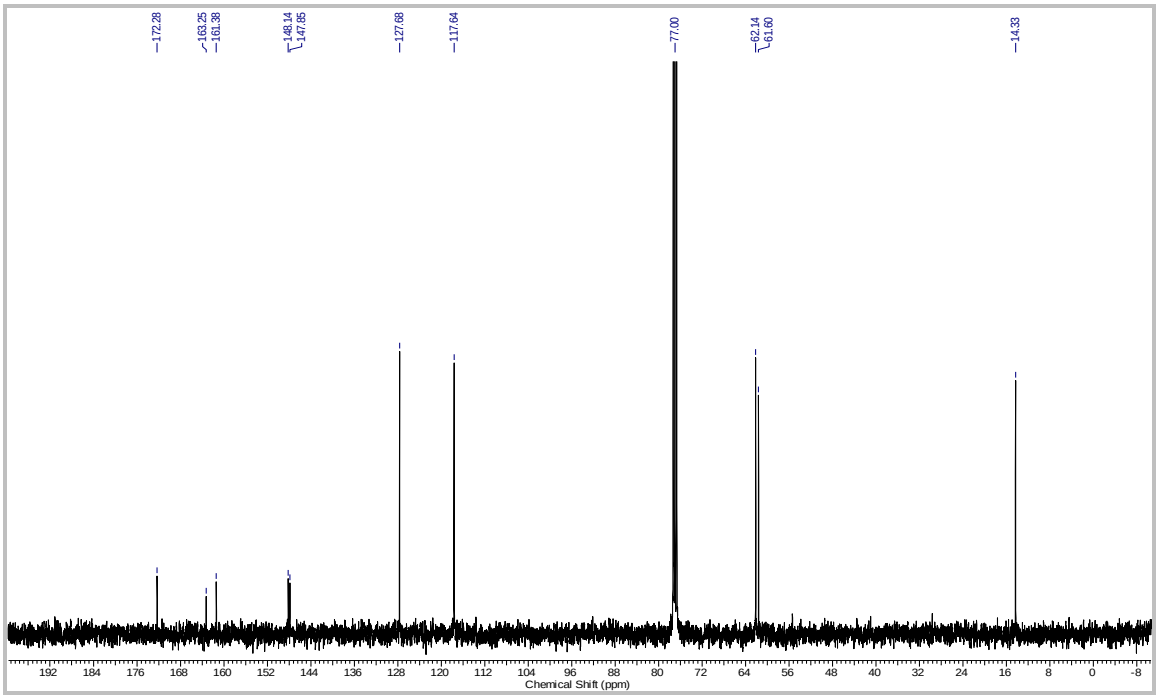

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 20



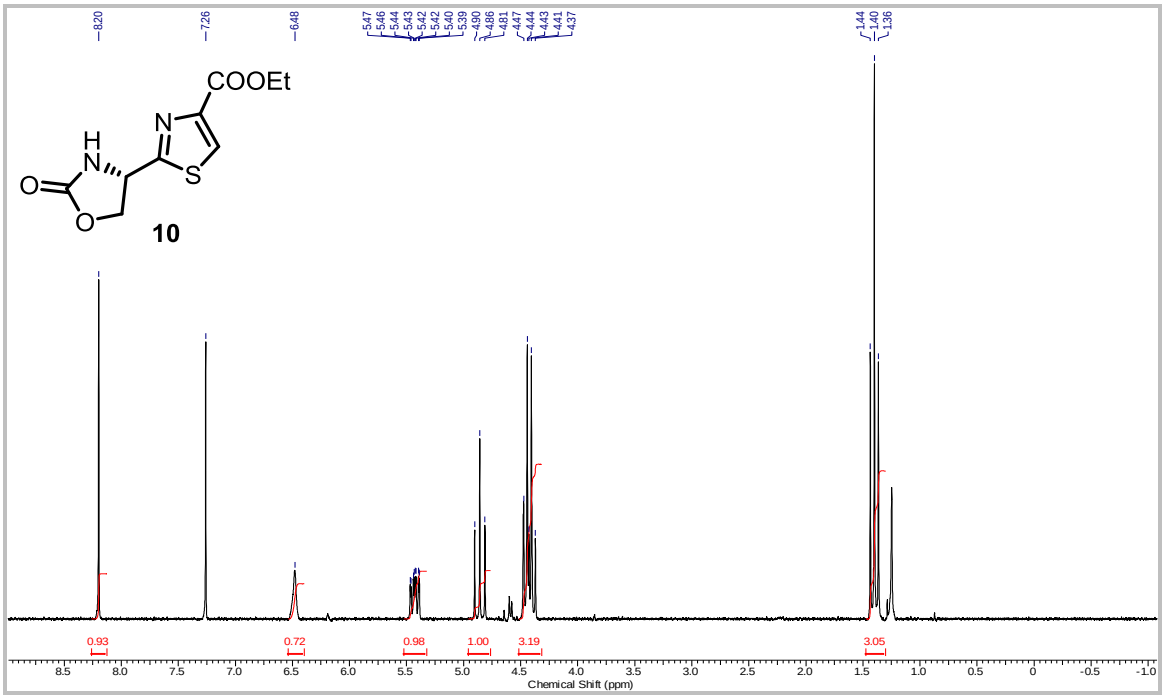
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 21



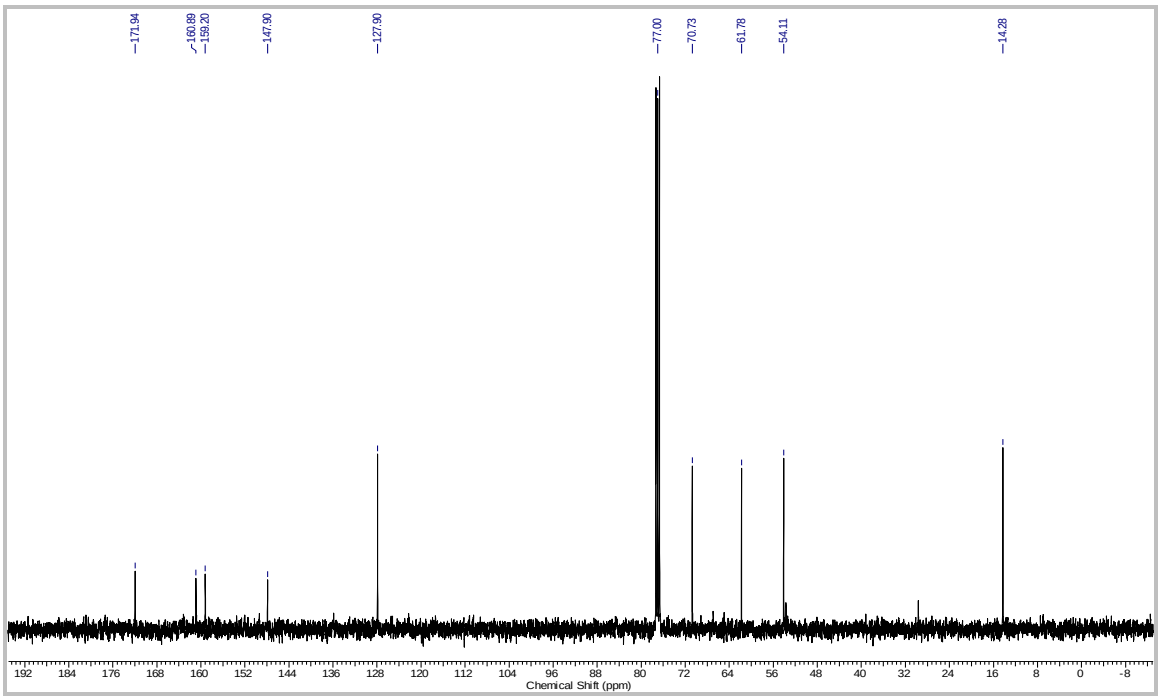

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 21



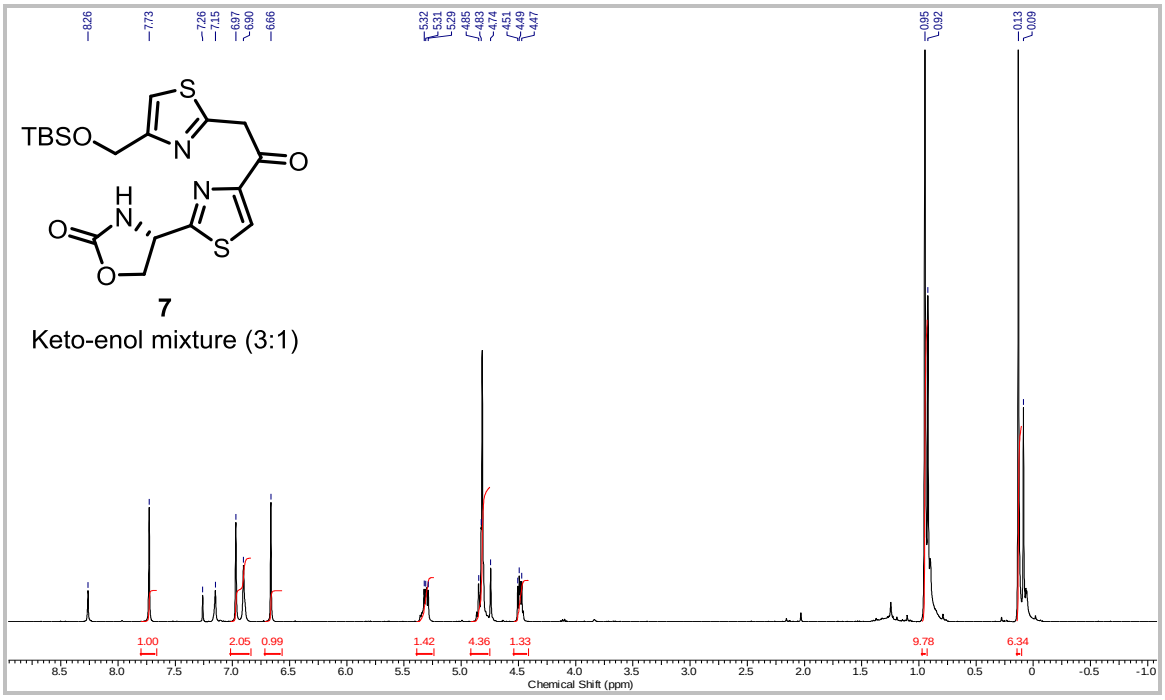
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 10



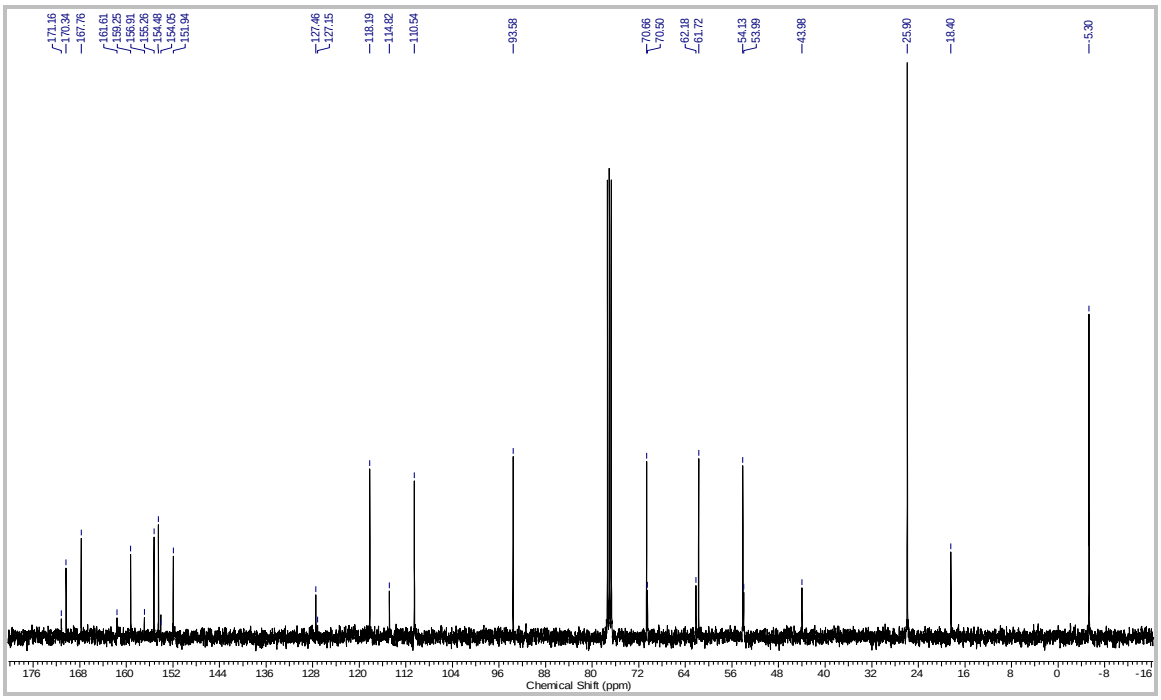

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 10



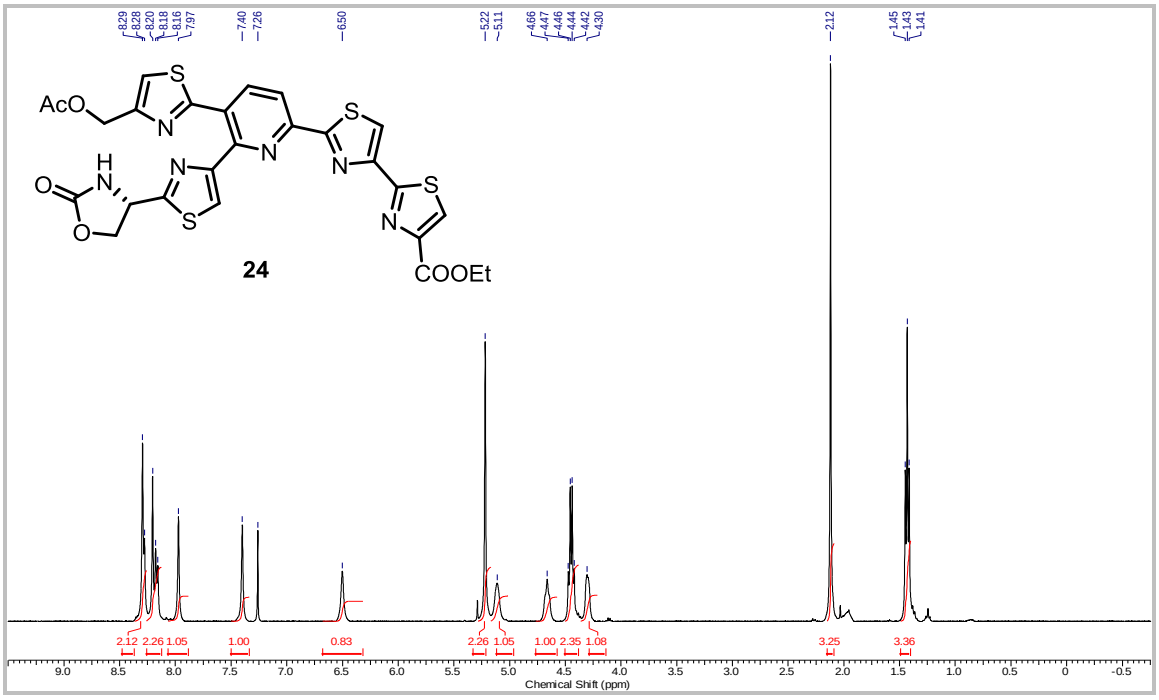
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 7



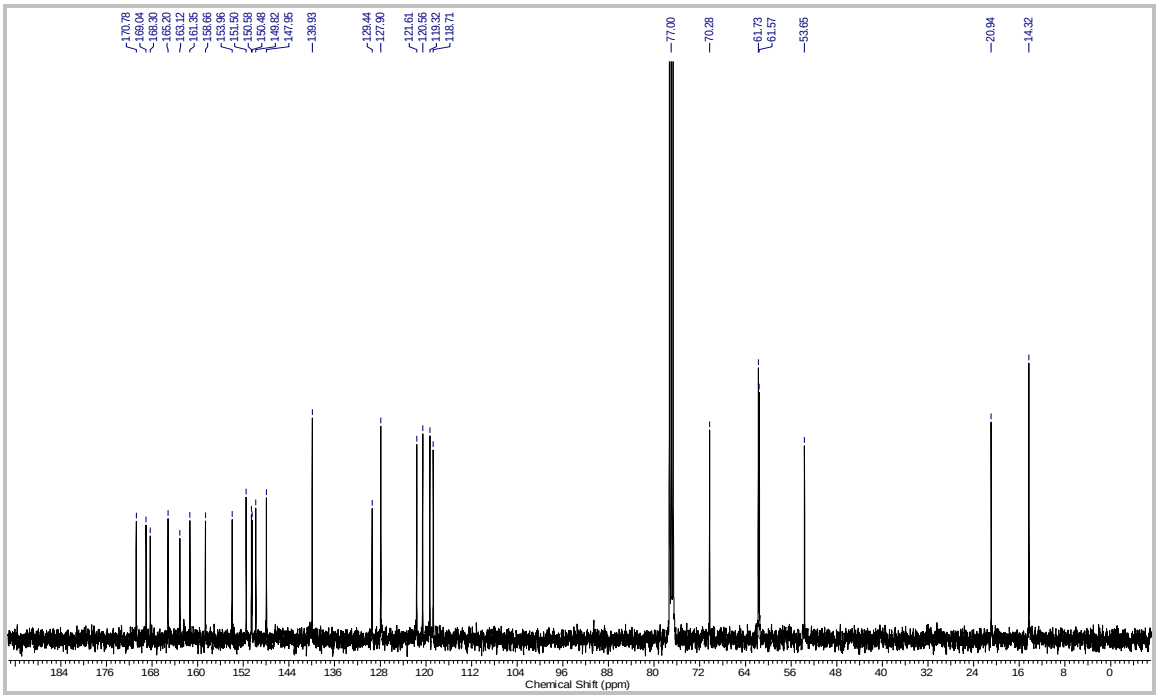

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 7



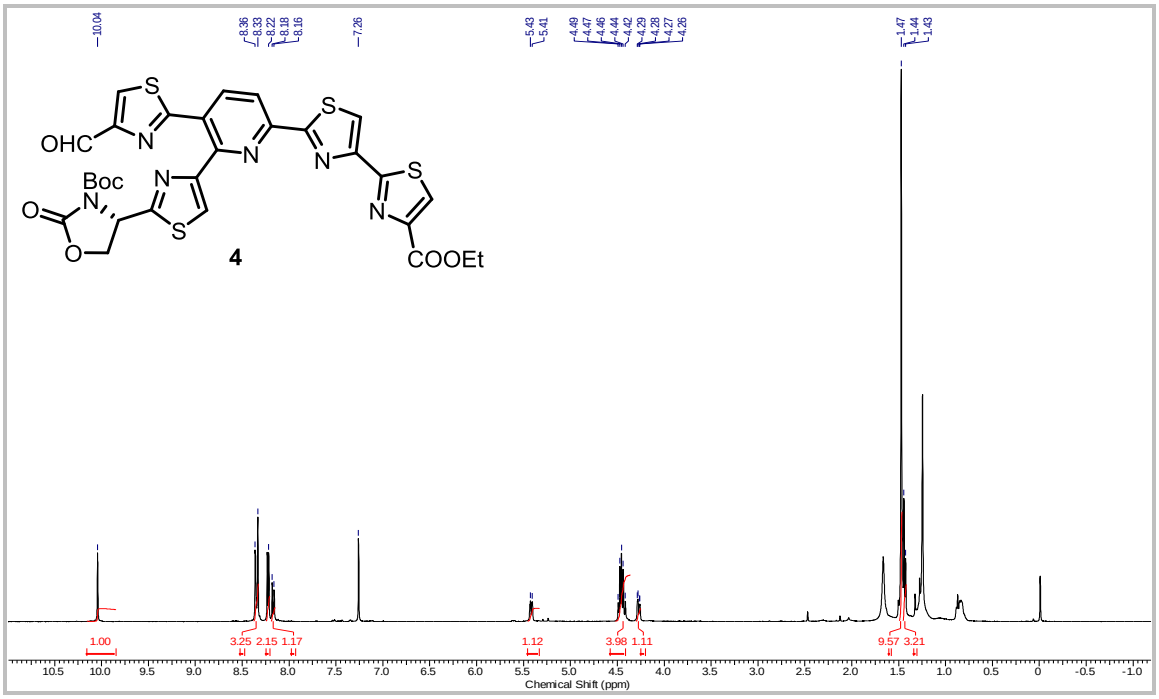
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 24



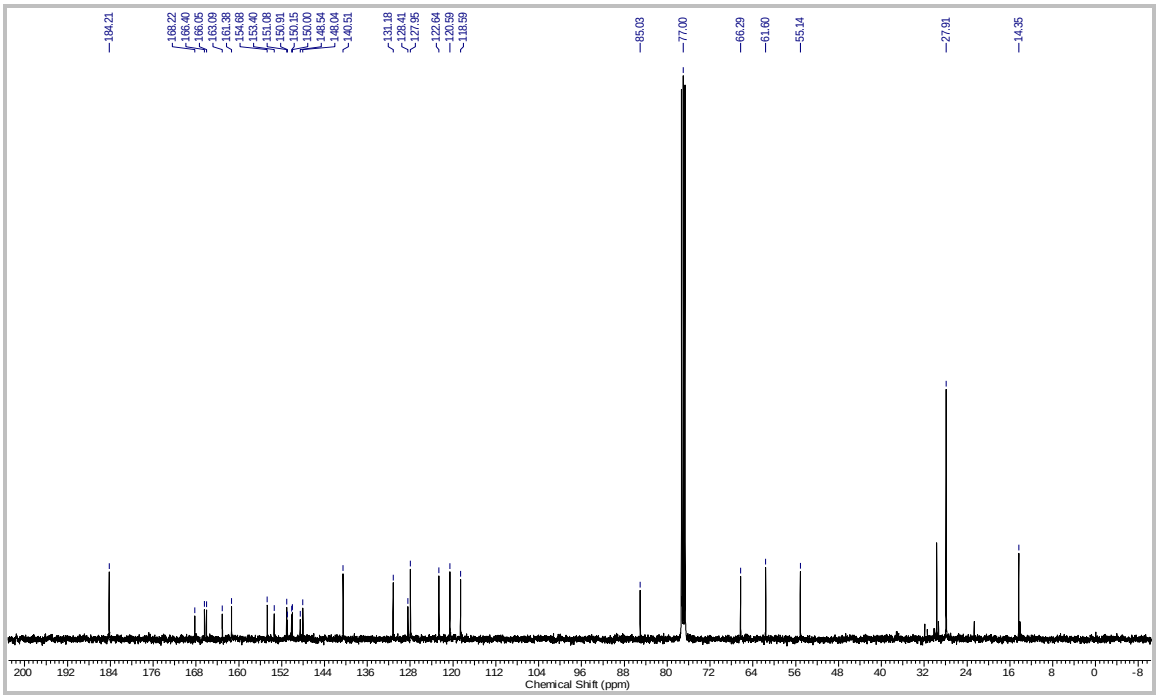

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 24



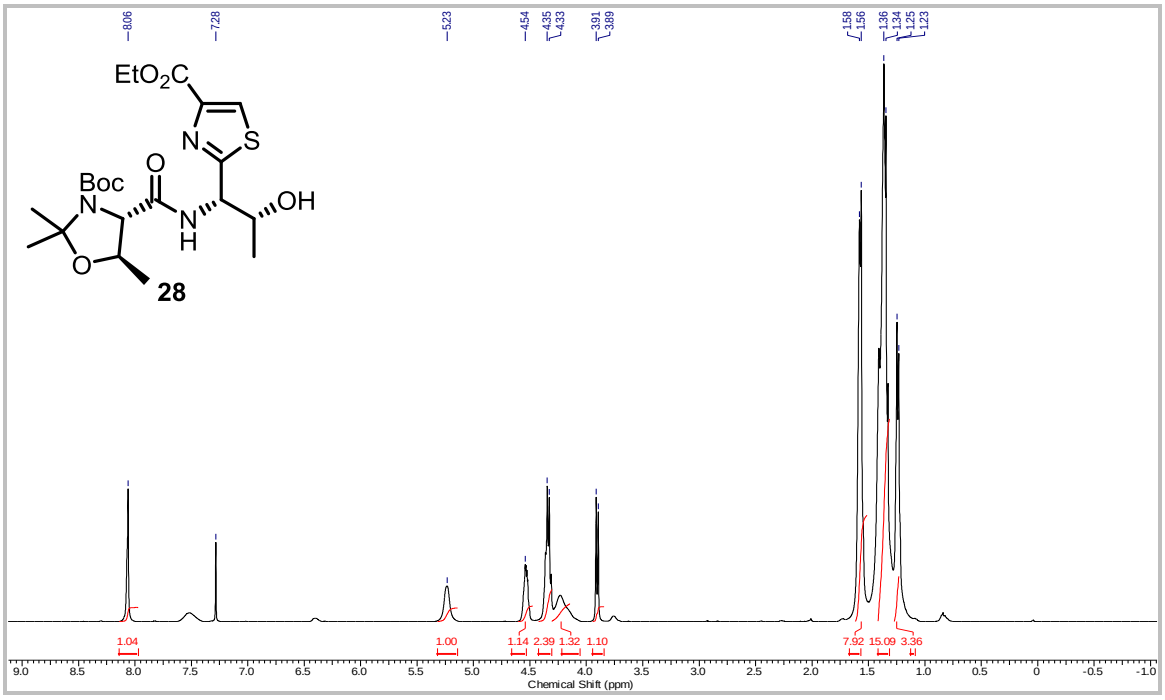
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 4



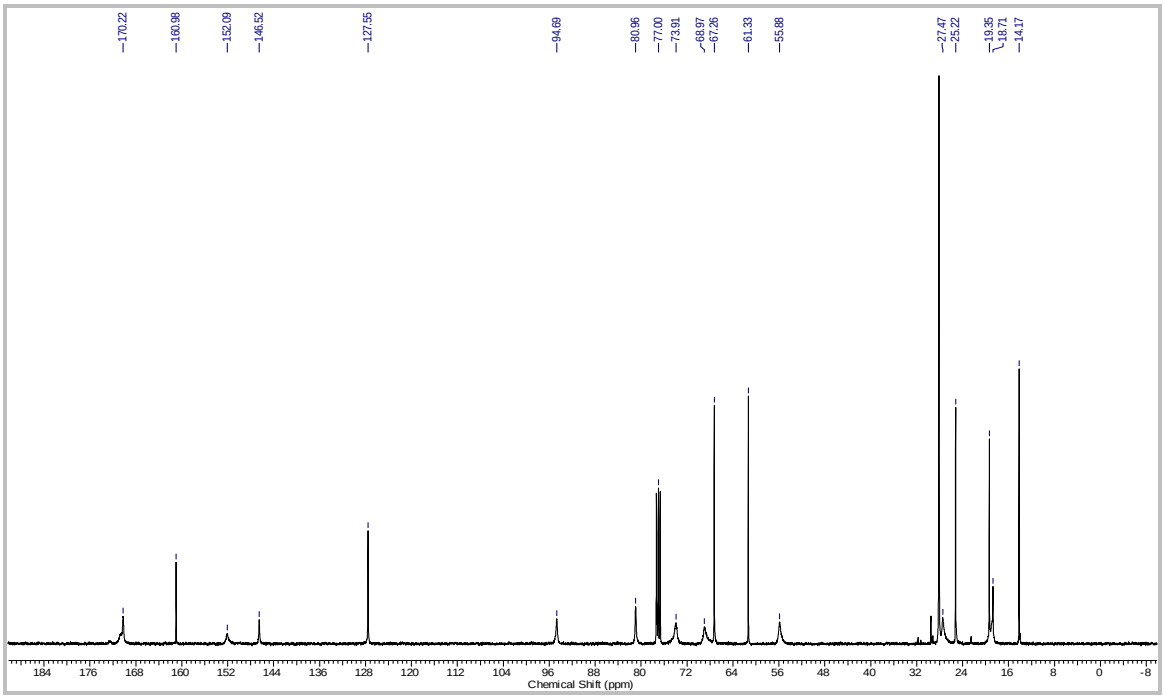

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 4



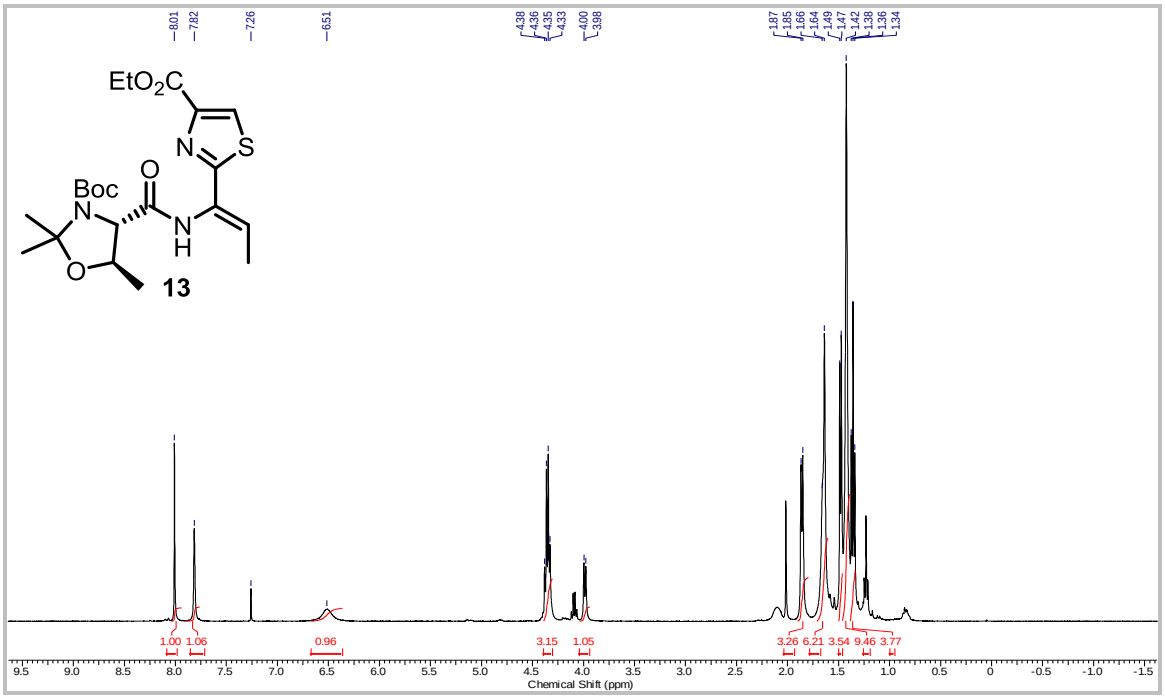
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 28




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



## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 13

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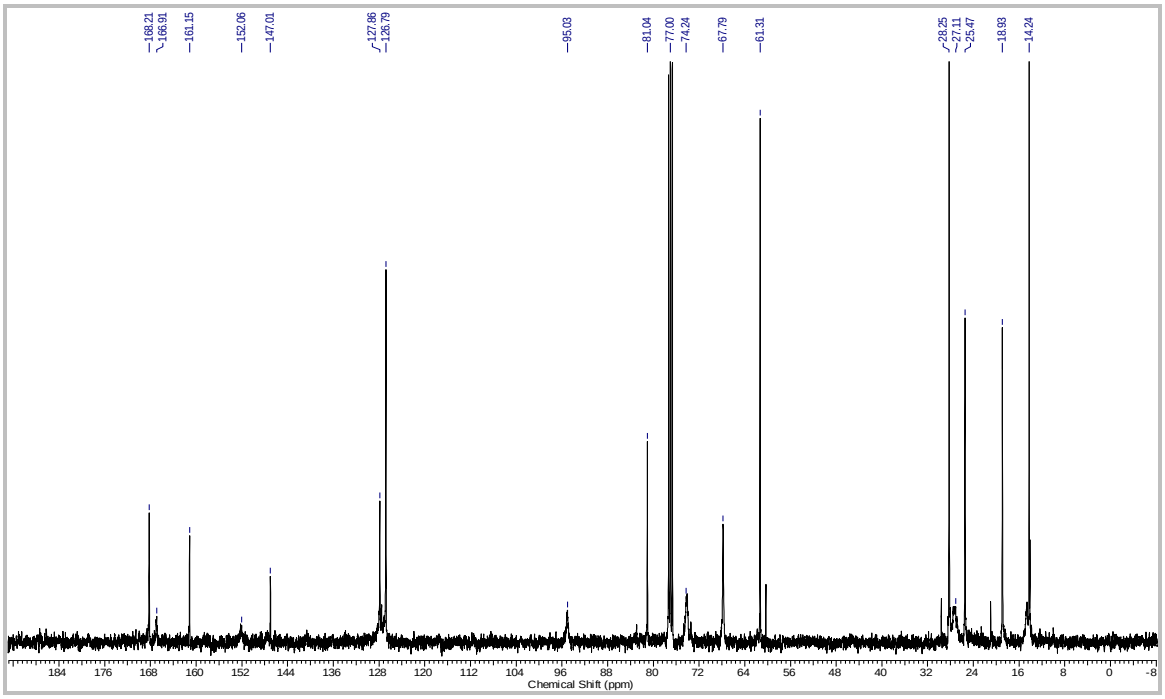

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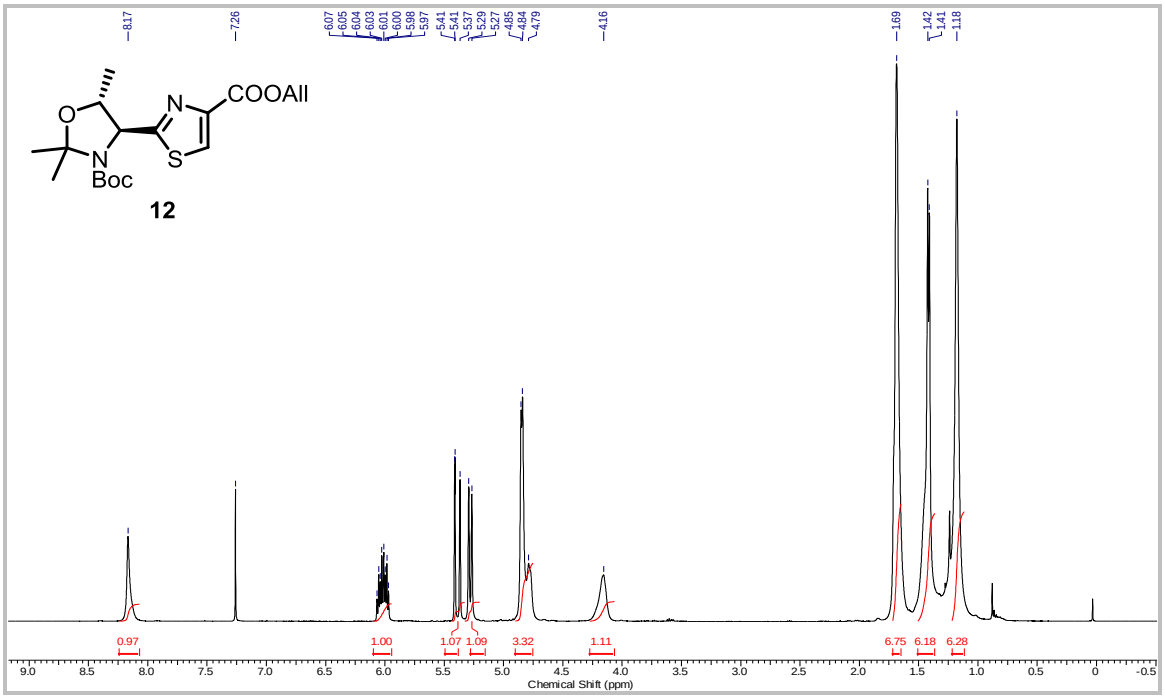


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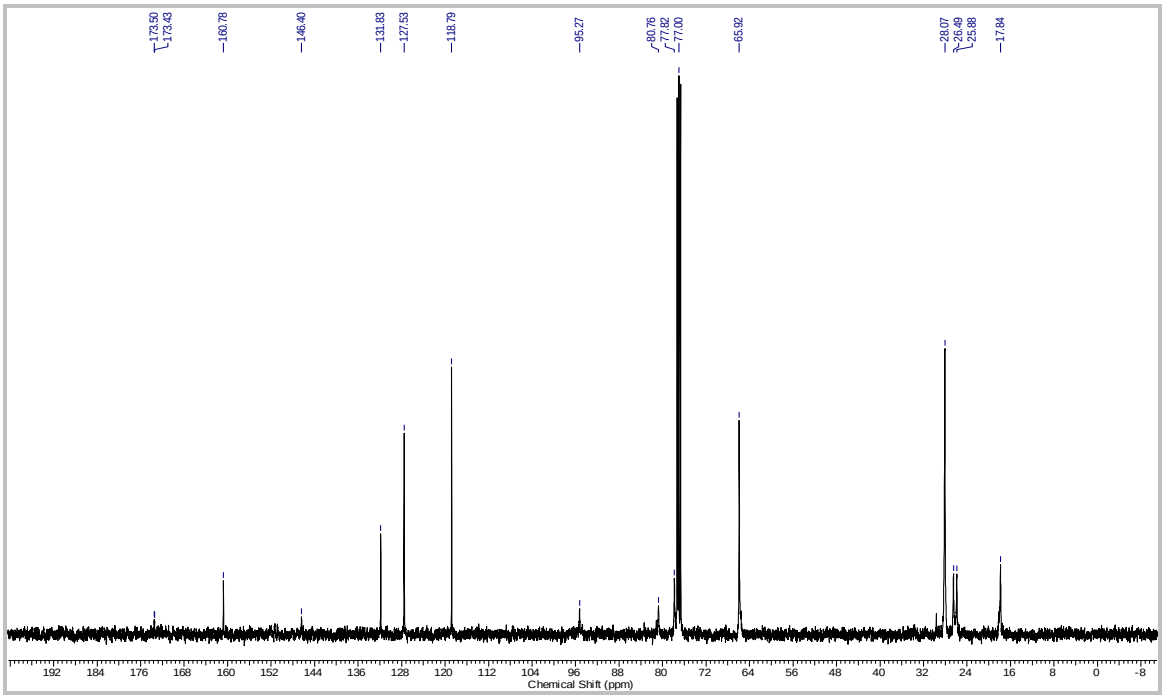

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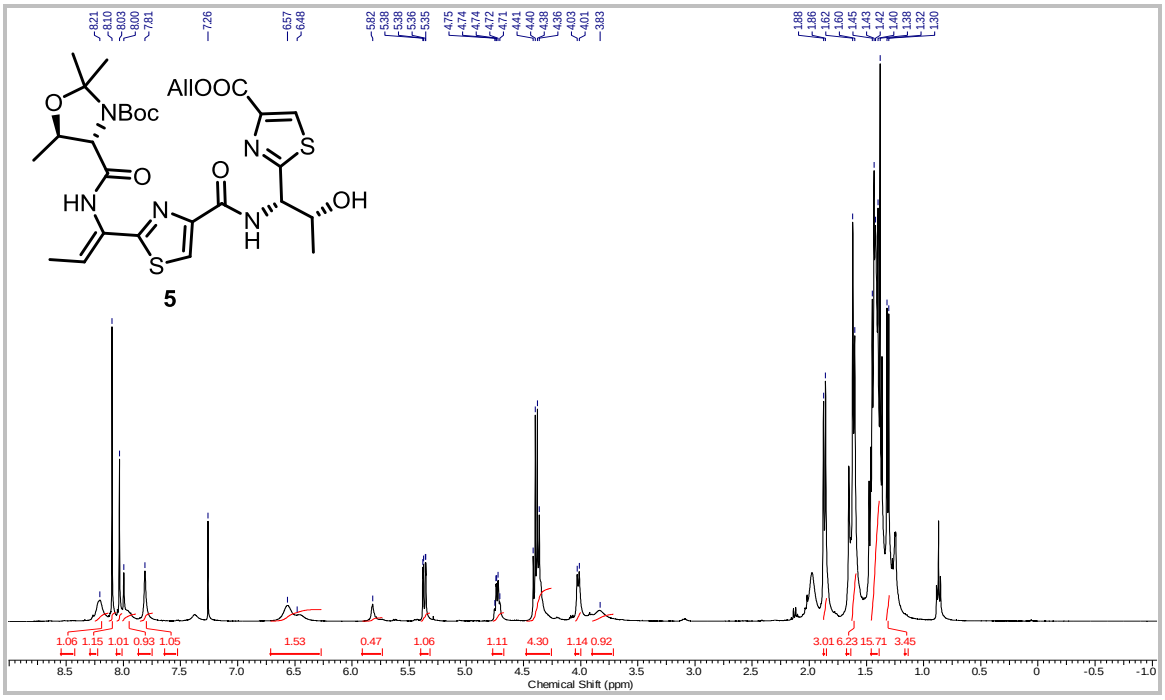


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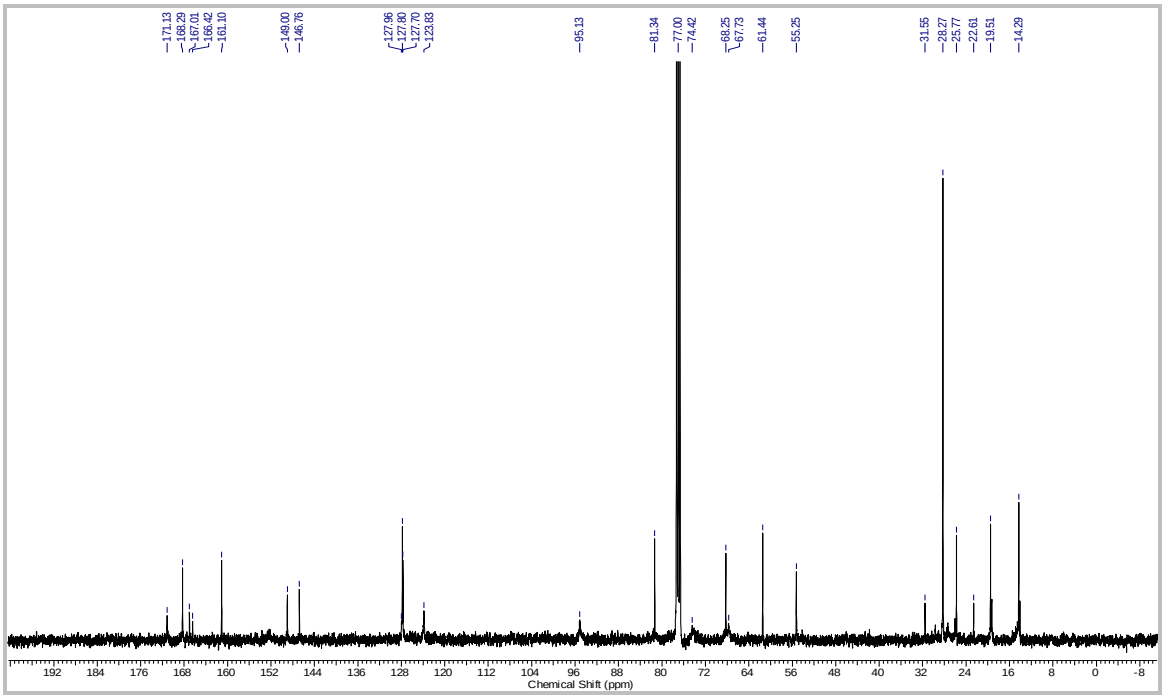

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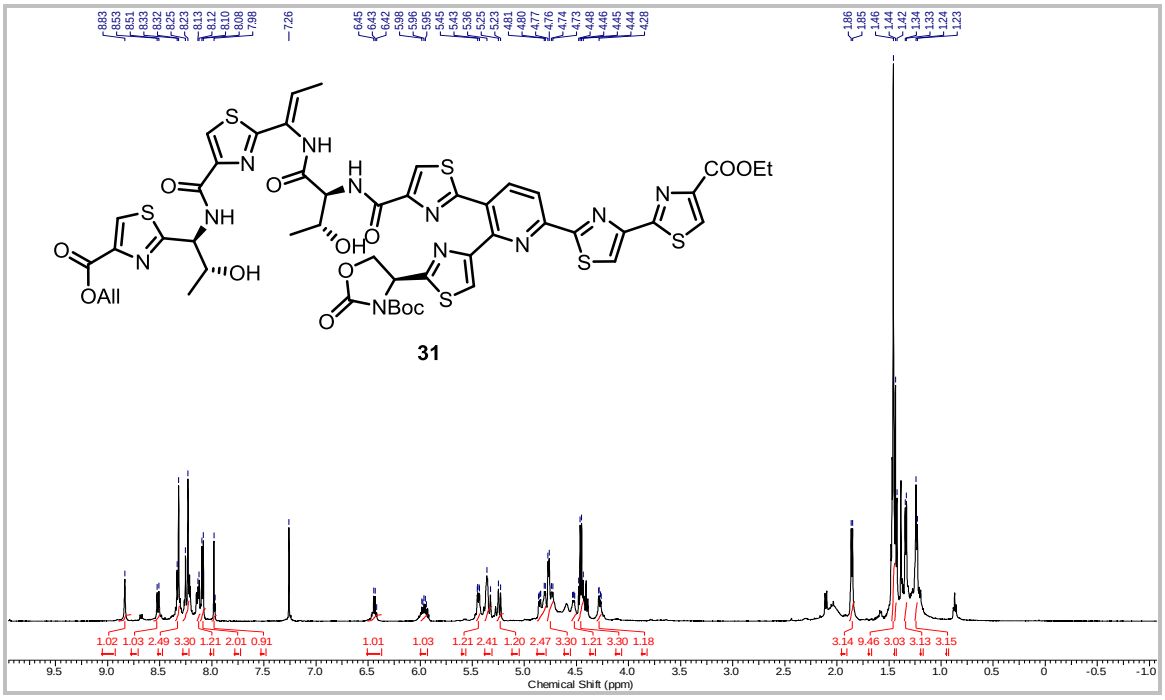


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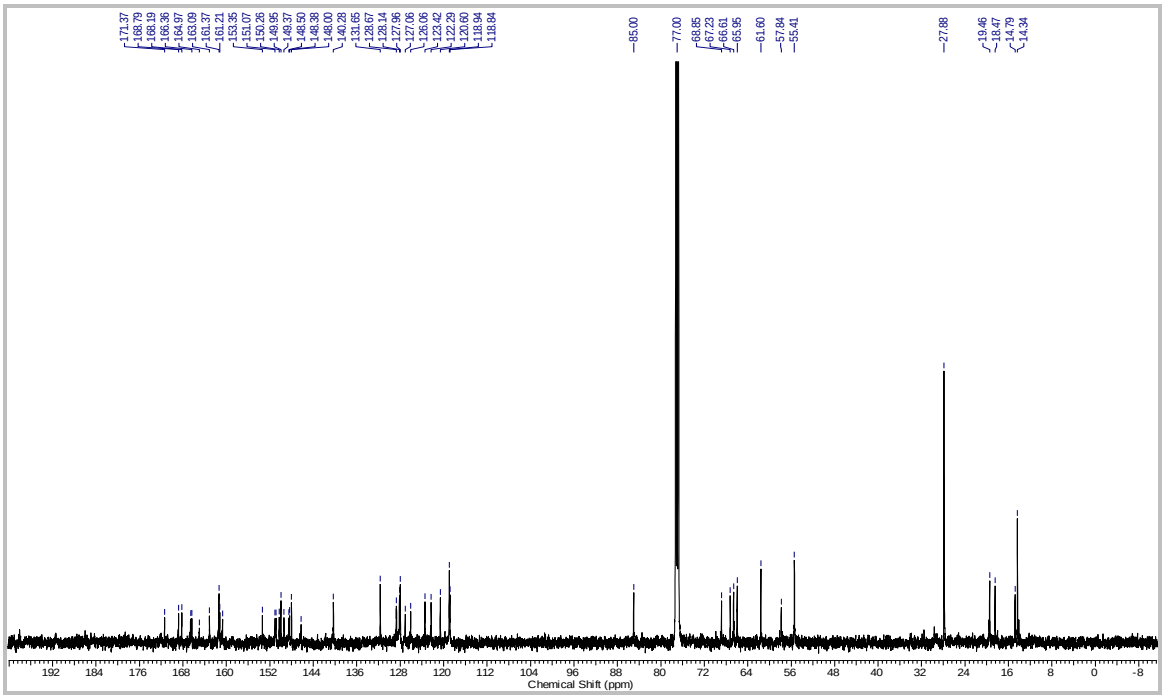

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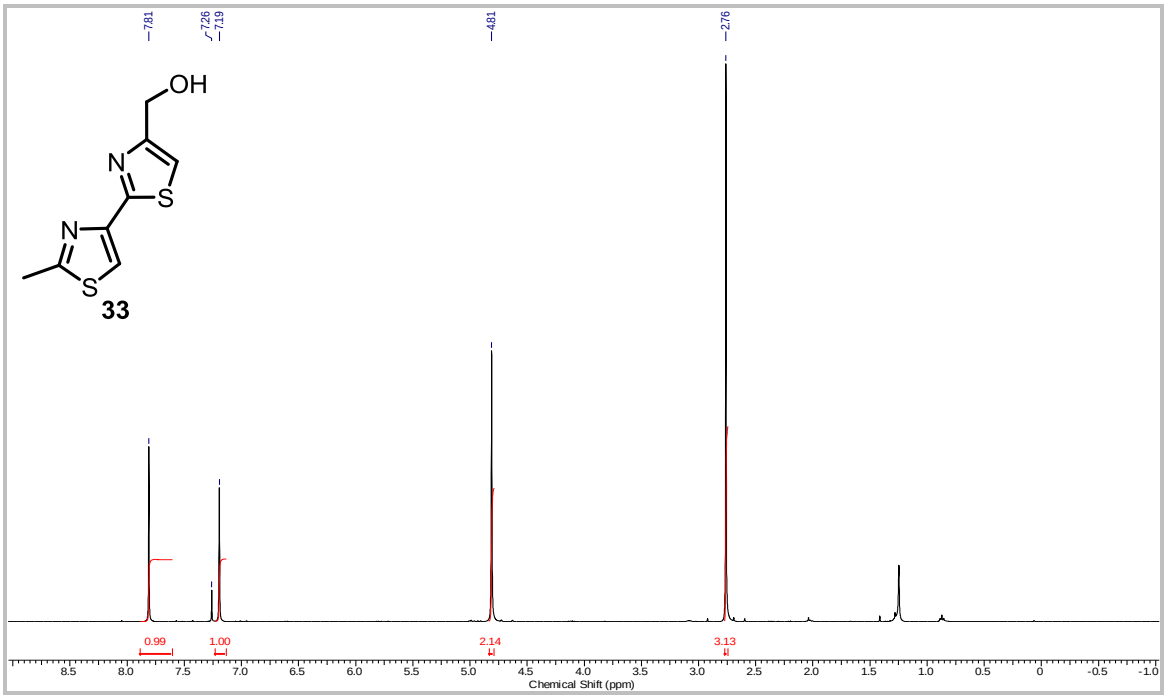


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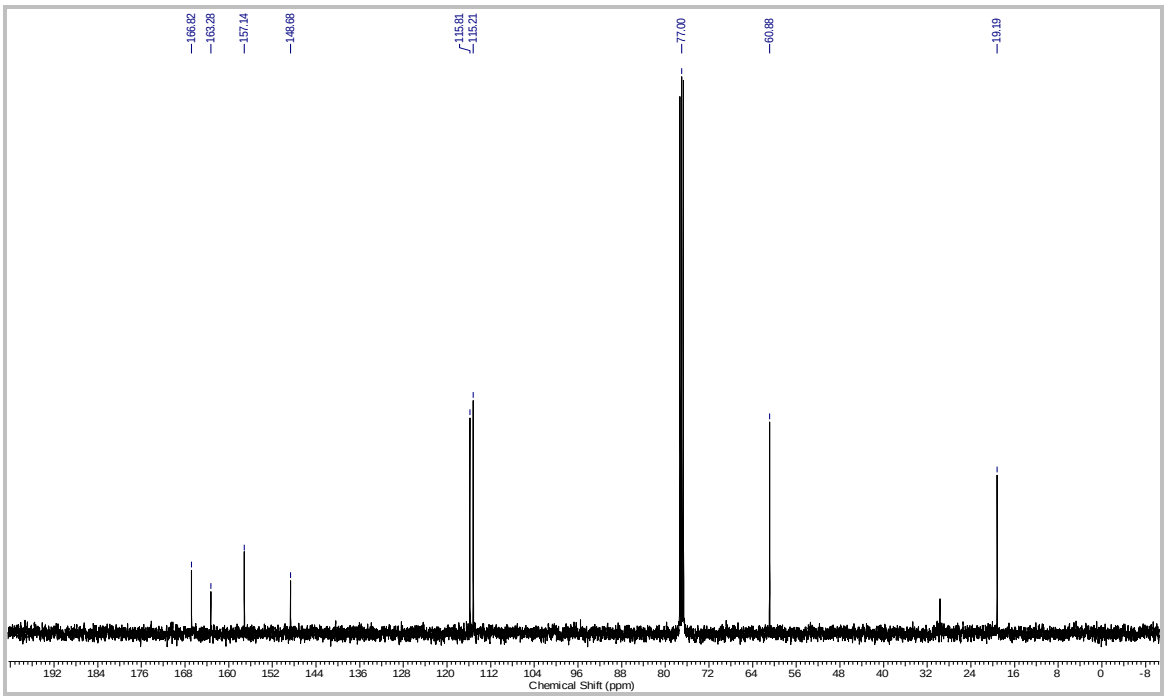

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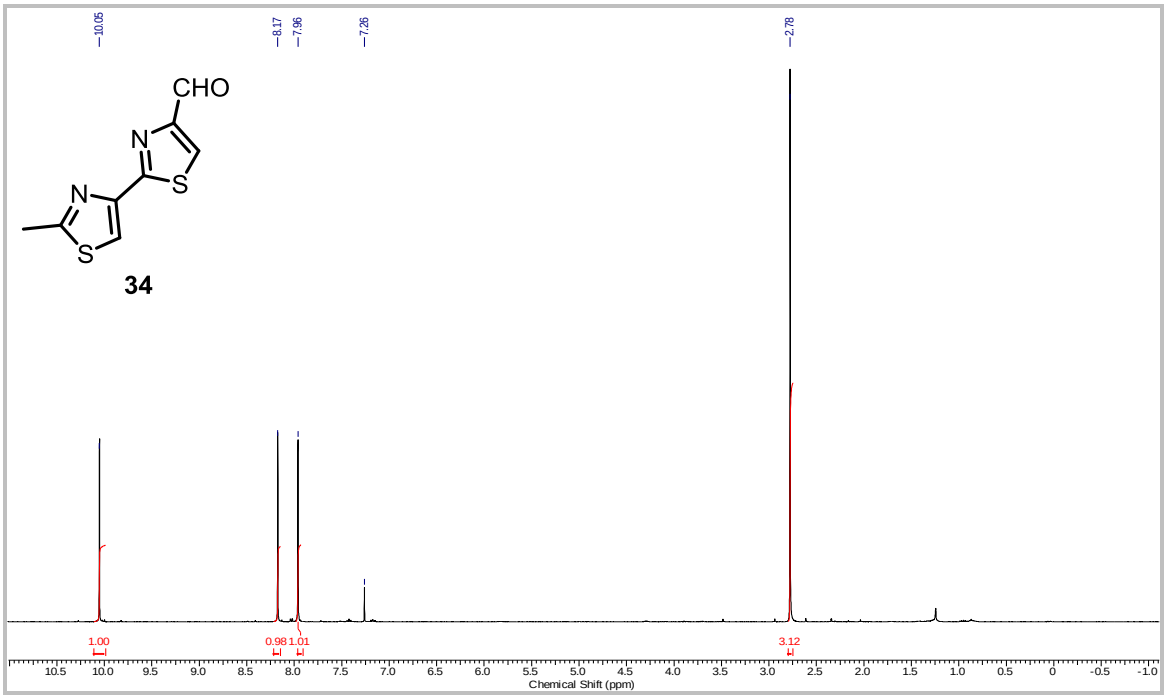


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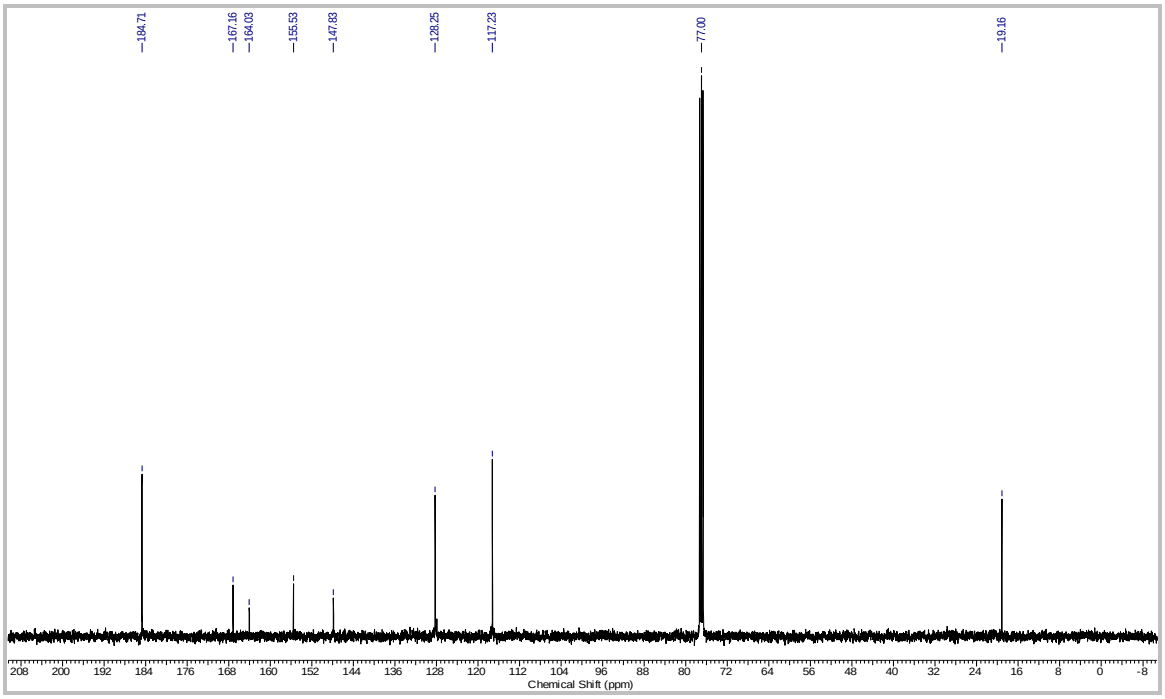

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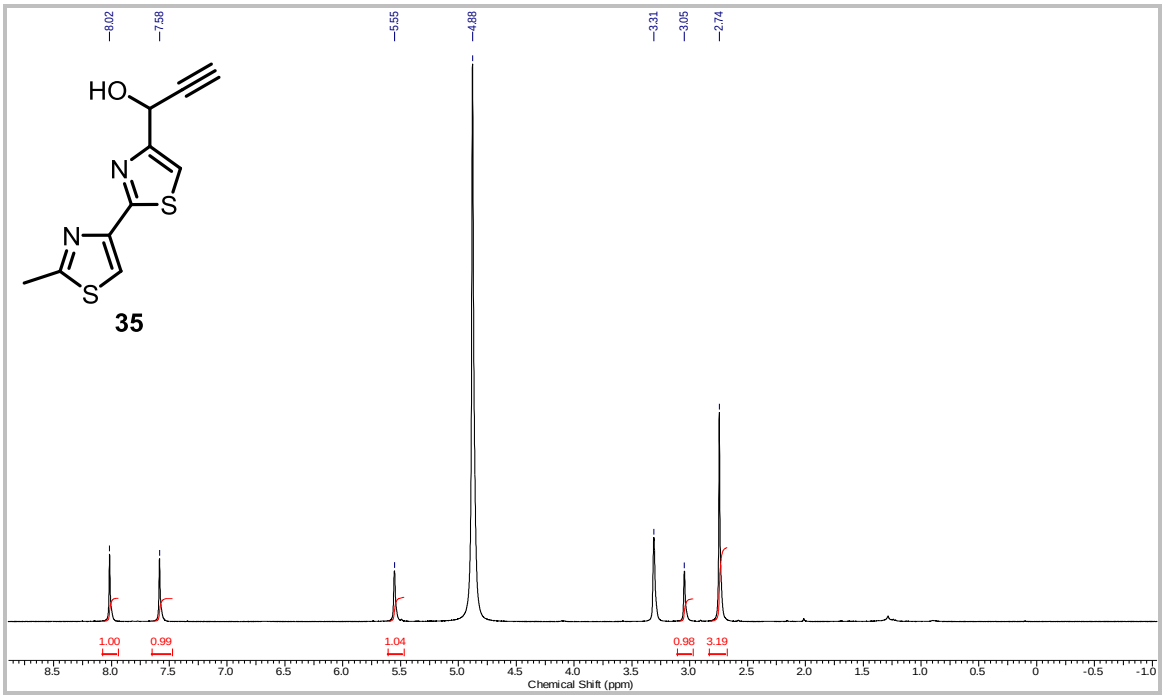


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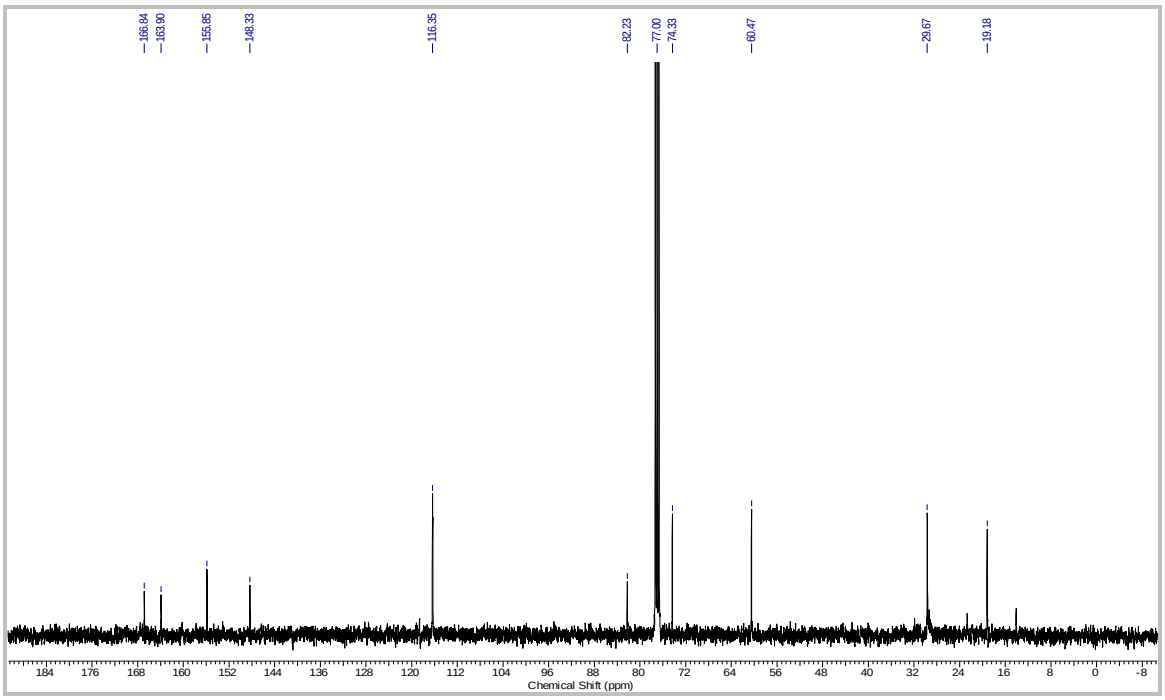

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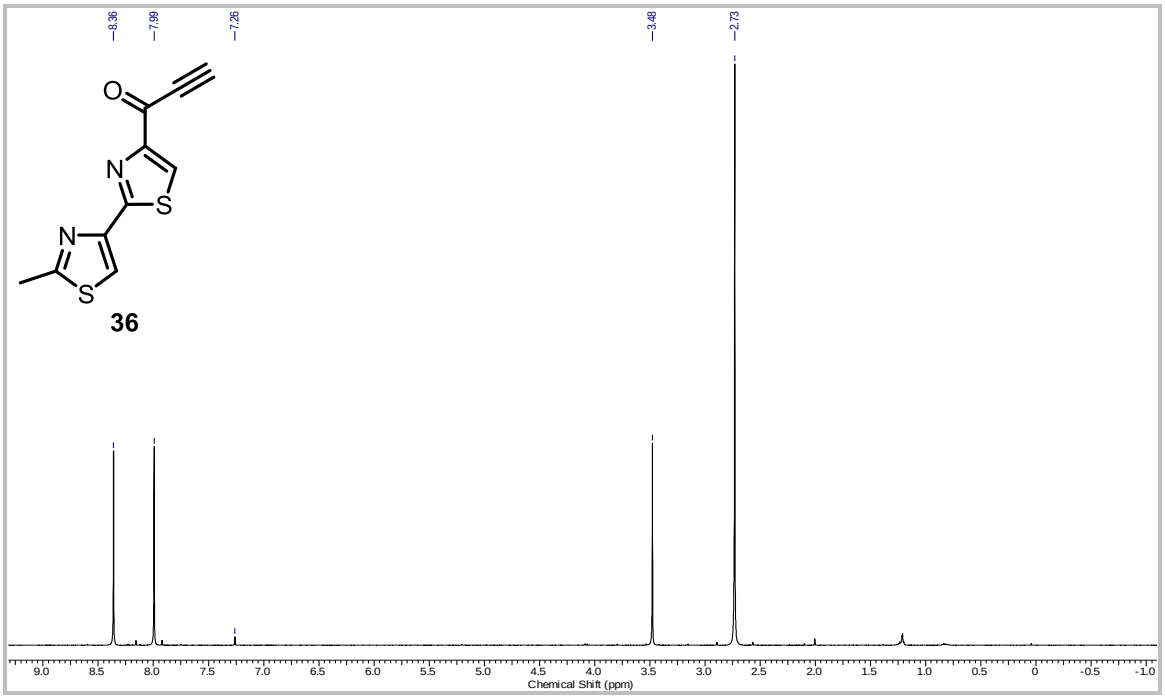


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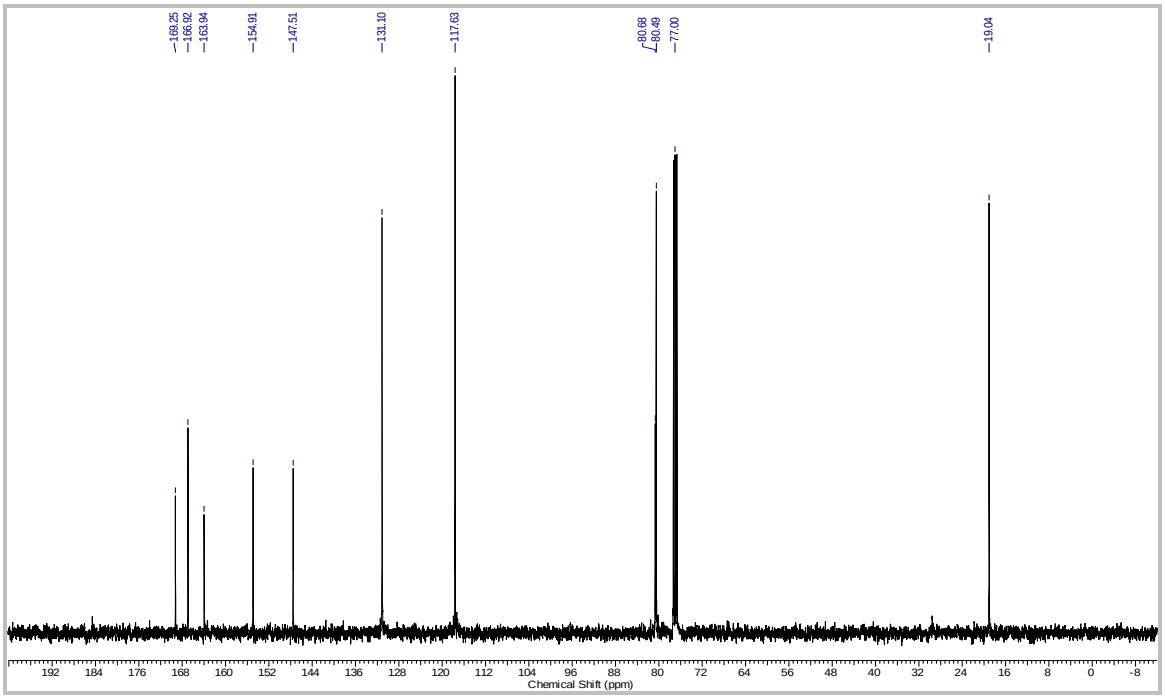

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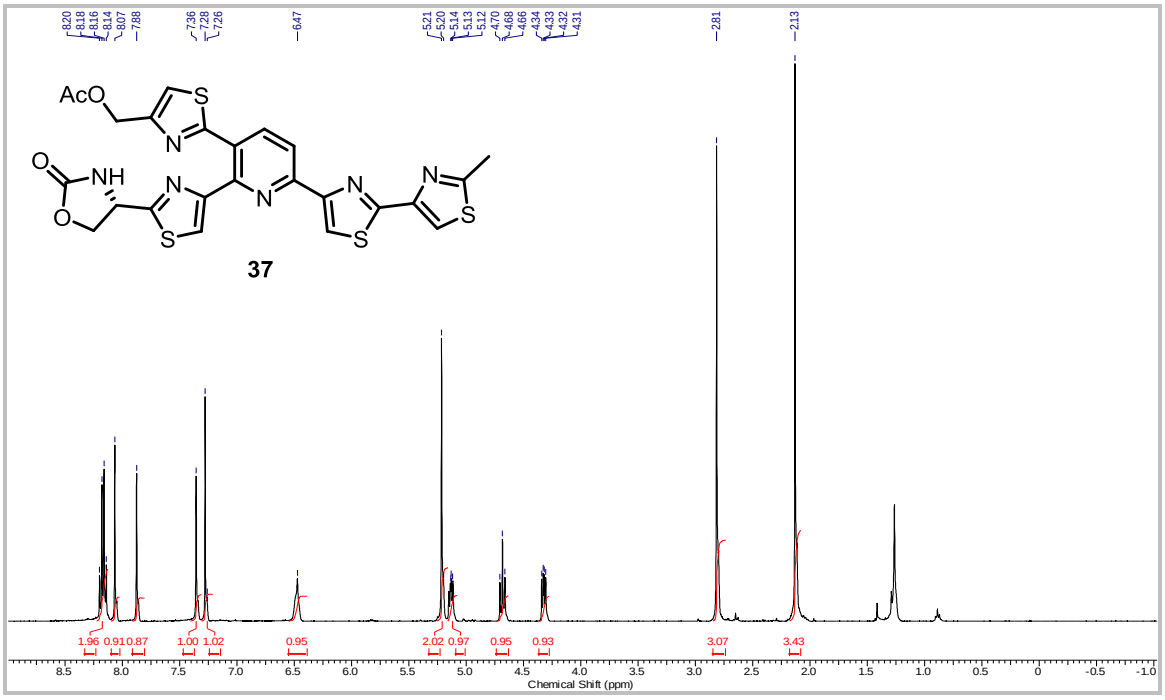


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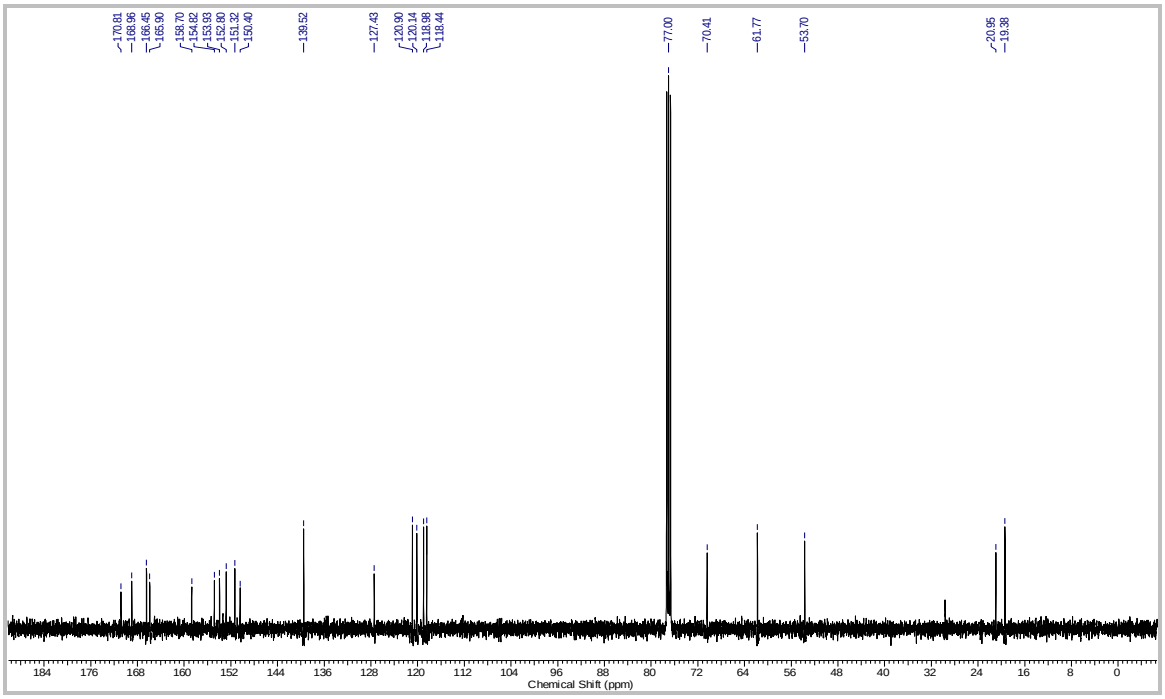

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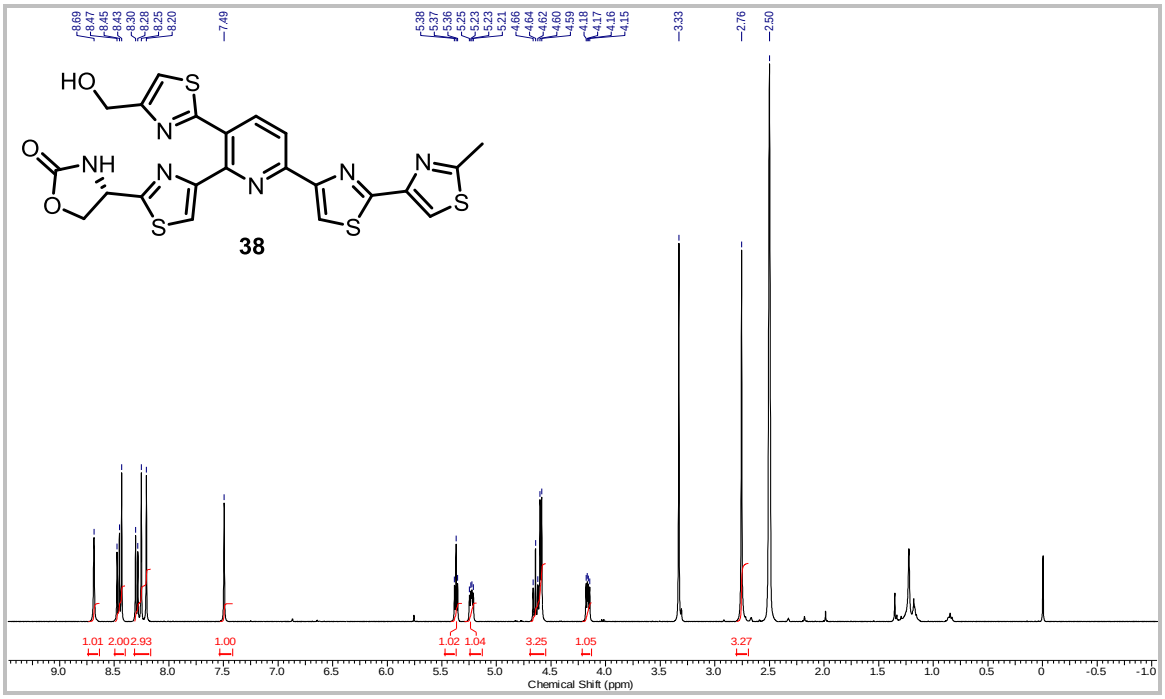


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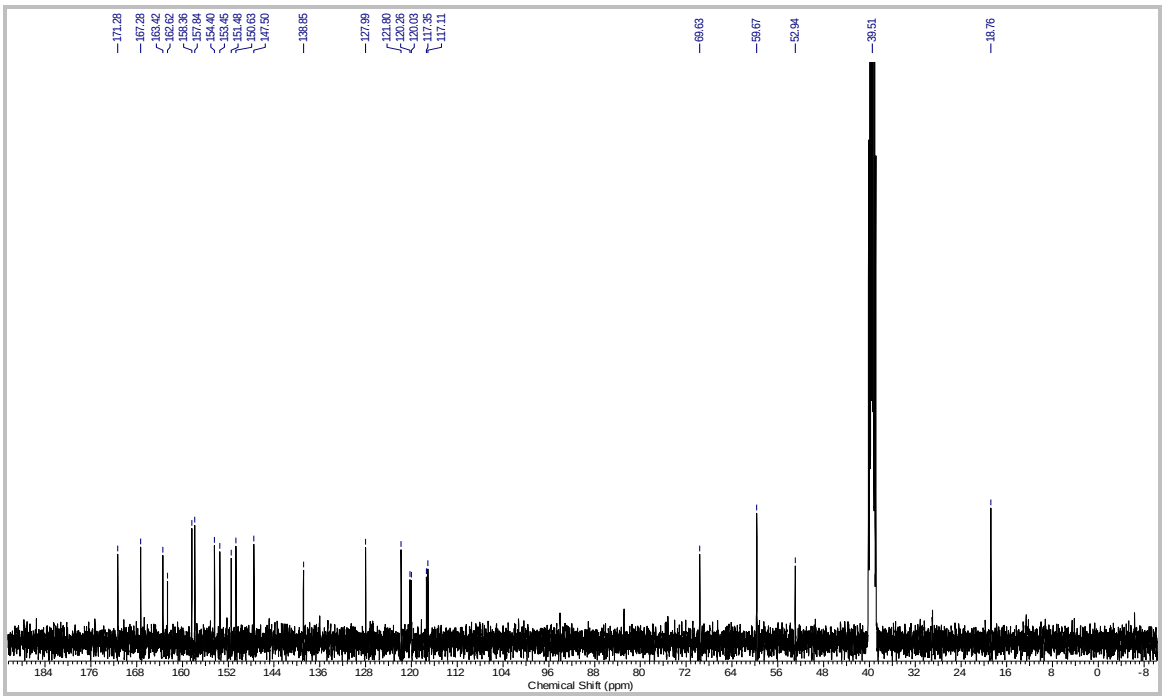

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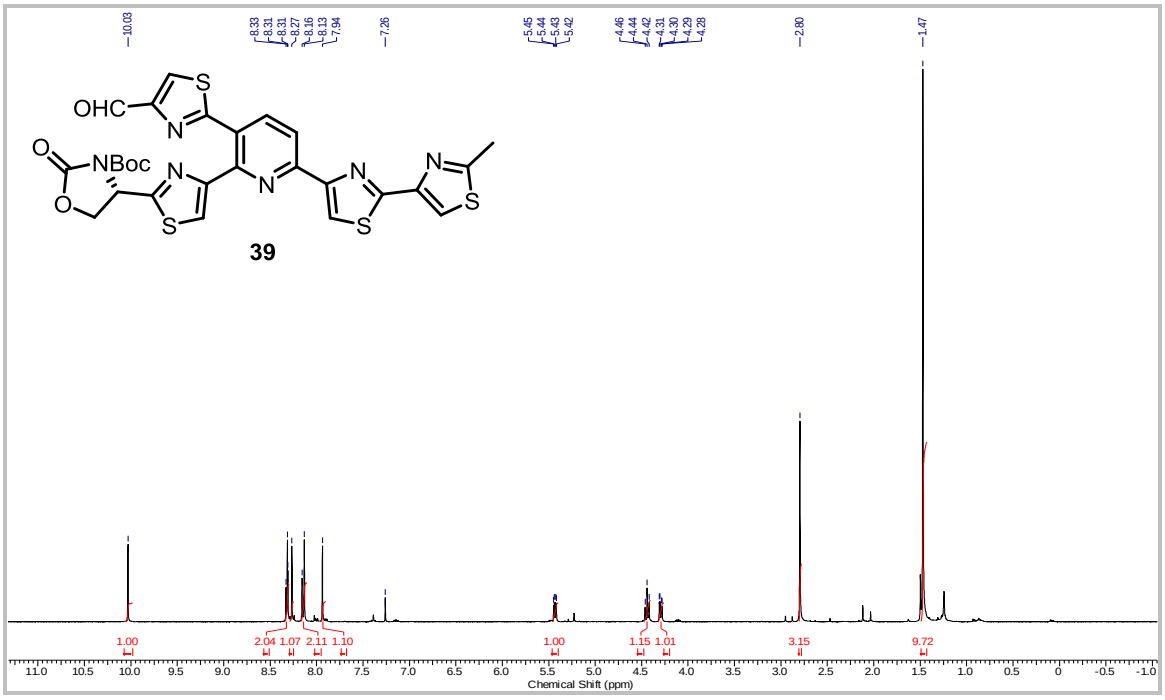


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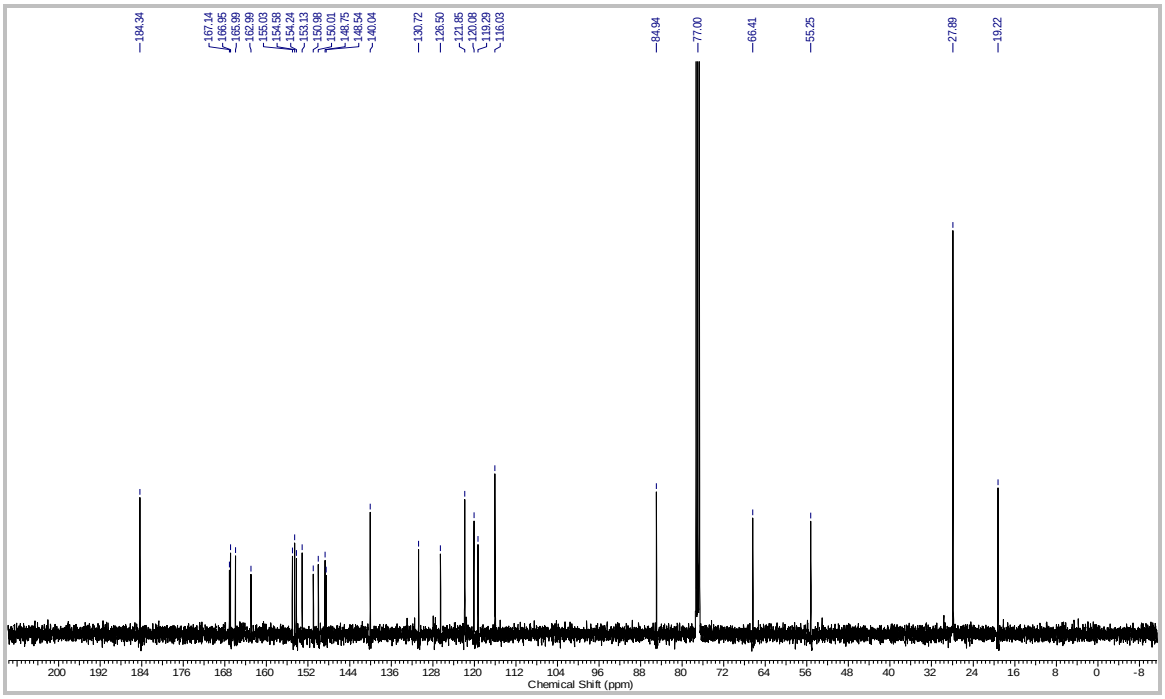

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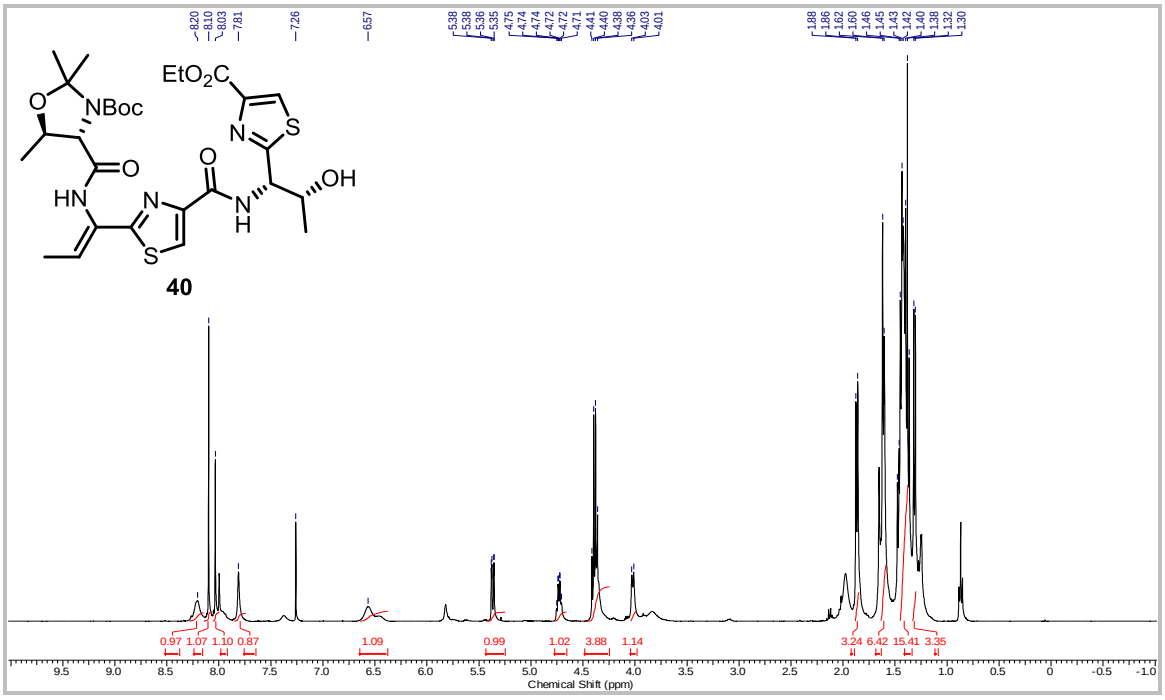


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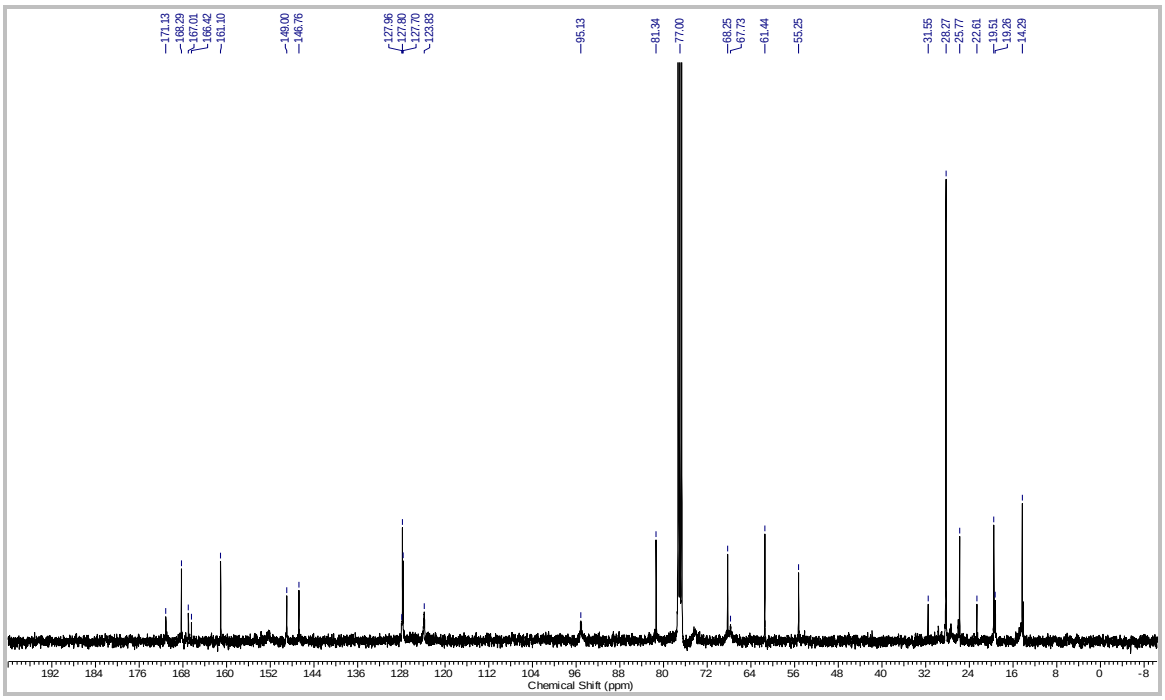

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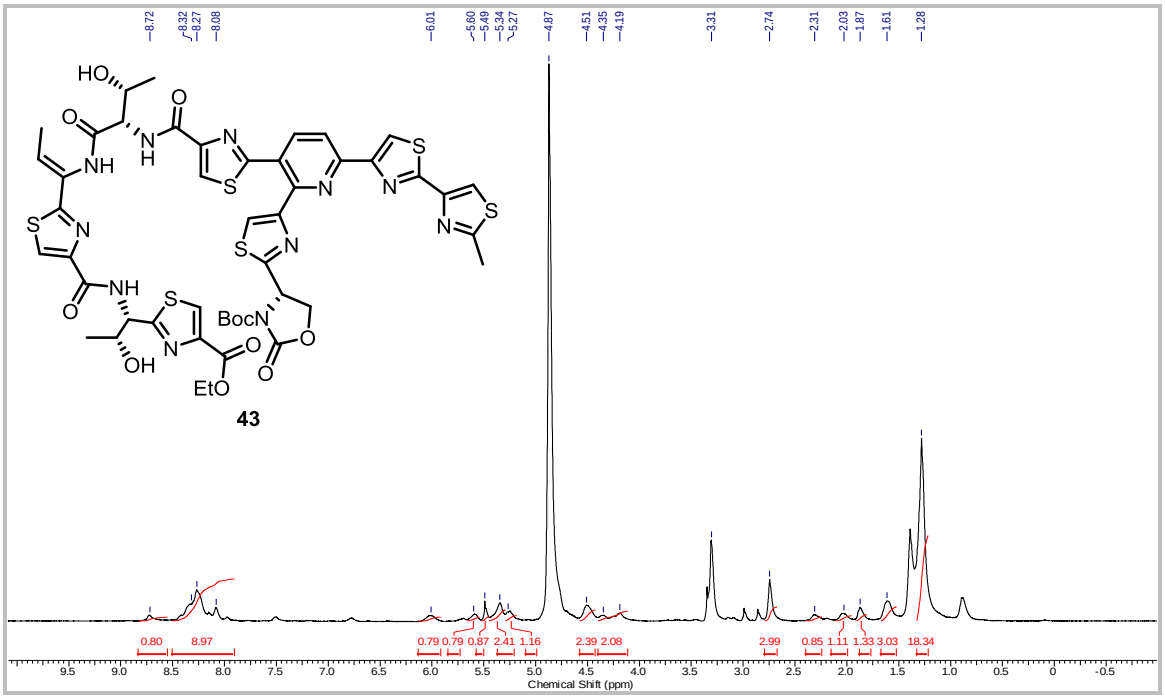
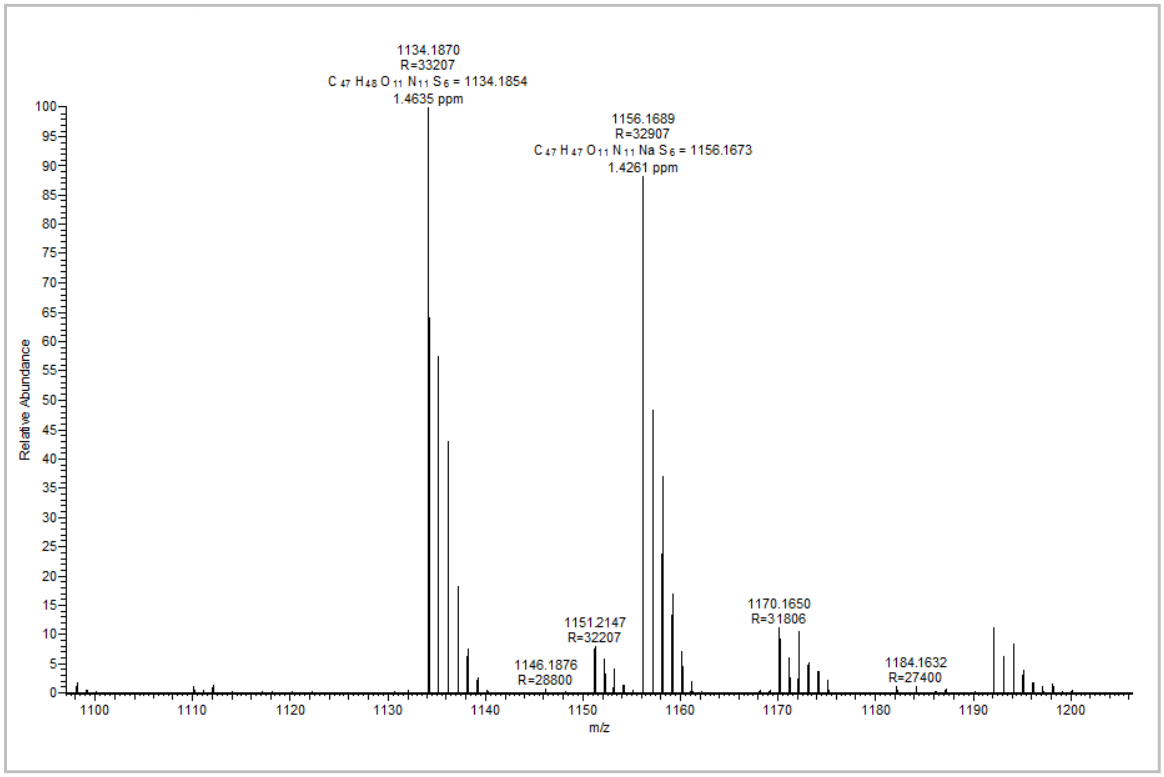



## HRMS of compound 43



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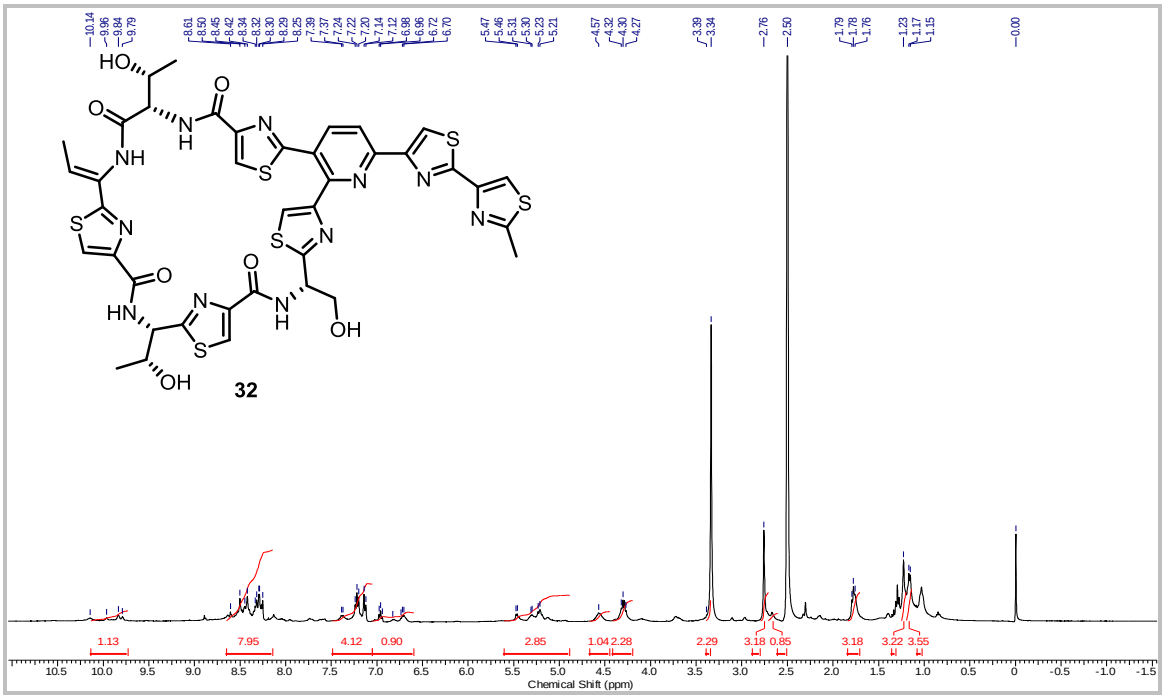


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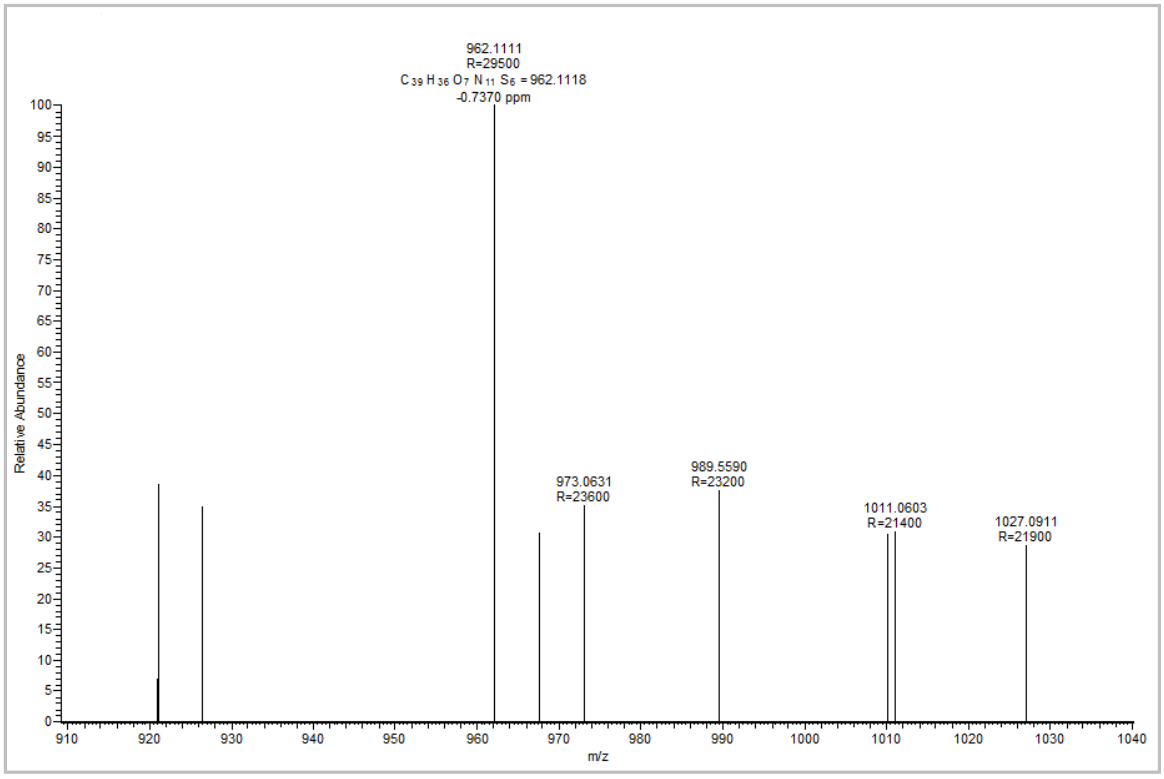

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# *Chapter 3 Section I*

# Antituberculosis Agent Diaportheone B: Synthesis, Absolute Configuration and SAR Study

## 3.1. Chromanones

Benzopyranone or chromanone are the benzene ring fused with pyranone, these compounds are widely distributed in nature. Most of the benzopyranone compounds have interesting biological properties, particularly those isolated from the plant pigments. These compounds are well documented in the literature that they exhibit various biological properties such as antioxidant,[1] antibacterial,[2] anti-HIV,[3] anti-inflammatory,[4] amebicidal[5] and $I_{Kr}$ (rectified potassium current) inhibitory properties.[6]

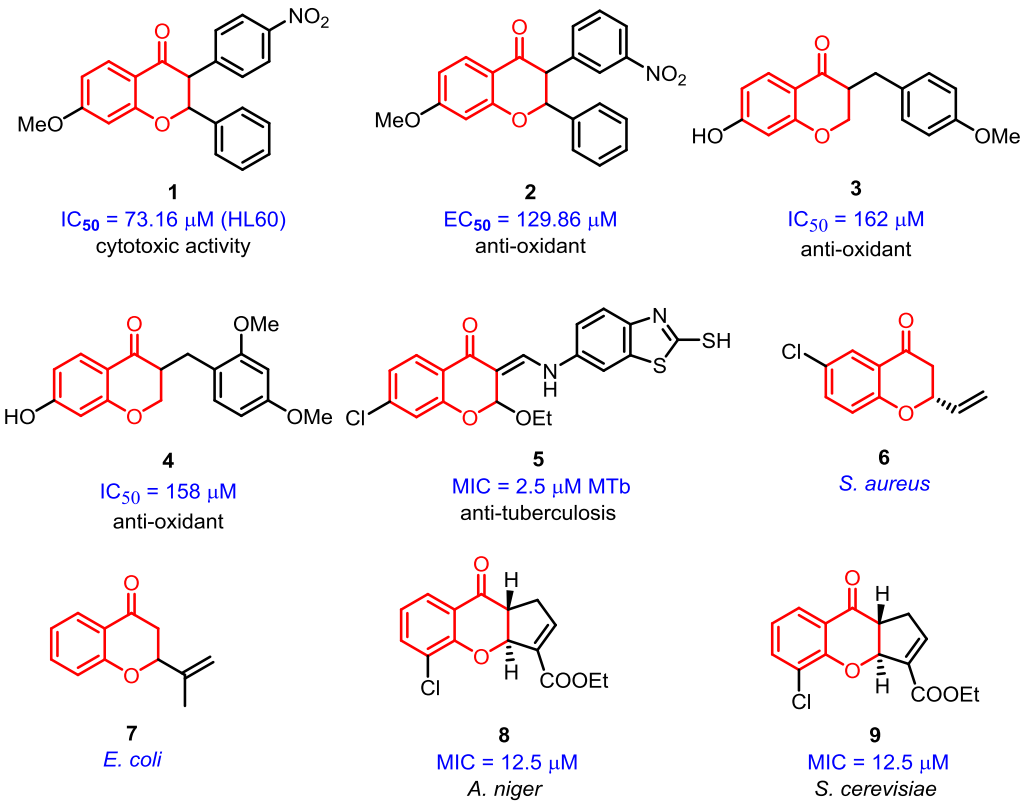



**Figure 1**: Bioactive compounds containing chromanone moiety

## 3.2. Tricyclic natural products containing chromanone moiety

The mother nature produces several tricyclic compounds with chromanone core and several of them with this core moiety have been isolated and well characterized. The majority of them were isolated from the fungal strains collected from various plants.

Analysis of various medicinally important molecules suggests that the chromanone is the key scaffold in the medicinal chemistry, as several fused bicyclic and tricyclic chromanone compounds are known to show potent activity against the various diseases.[1-6] A bicyclic diaryl chromanone compound **1** exhibited the potent cytotoxic activity with $IC_{50}$ = 73.16 μM and its regioisomer compound **2** was found to be a potent anti-oxidant with $EC_{50}$ = 129.8 μM, it also showed the potent radical modulation activity (Figure 1). The compounds **3** and **4** possess a 3-benzylchromanone with phenolic hydroxyl group showed the potent anti-oxidant activity. A 3-formylchromanone **5** showed the significant antimycobacterial activity (Mtb) comparable to the marketed drug isoniazid (2.5 μM), while the bicyclic and tricyclic chromanones (**6**-**9**) are known to be potent antibacterial agents.

Compound **10**-**32** are the tricyclic chromanone core natural products isolated from the various sources, mostly from various fungal strains. Compounds **10**, **11** and **12**, the 6-6-5 fused tricyclic natural products, in which diaportheone A (**10**) and B (**11**) are known to be antituberculosis agents with MIC of 100 μM and 3.5 μM activity while the preussochromones D (**12**) showed potent cytotoxicity against A549 cells. The compound **13**-**27** which contains the fused 6-6-6 tricyclic skeleton and this is also called as xanthones. Most of the xanthone derivatives are known to be good antifungal agents, it also showed the potent antibacterial, cytotoxicity, anticancer activities.[7] The lachnone (**28**) and gonytolide (**29**) are also biologically important natural products with similar scaffold (Figure 2), secalonic acids are the dimeric xanthones which showed the broad range of activity like antitumor (compound **30**), cytotoxicity (compound **30**, **31**), inhibition of DNA topoisomerase I (compound **32**) etc.[8] The structural simplicity and biologically diverse properties of tricyclic chromanone compounds or xanthones increase the importance of this class in medicinal chemistry point of view.

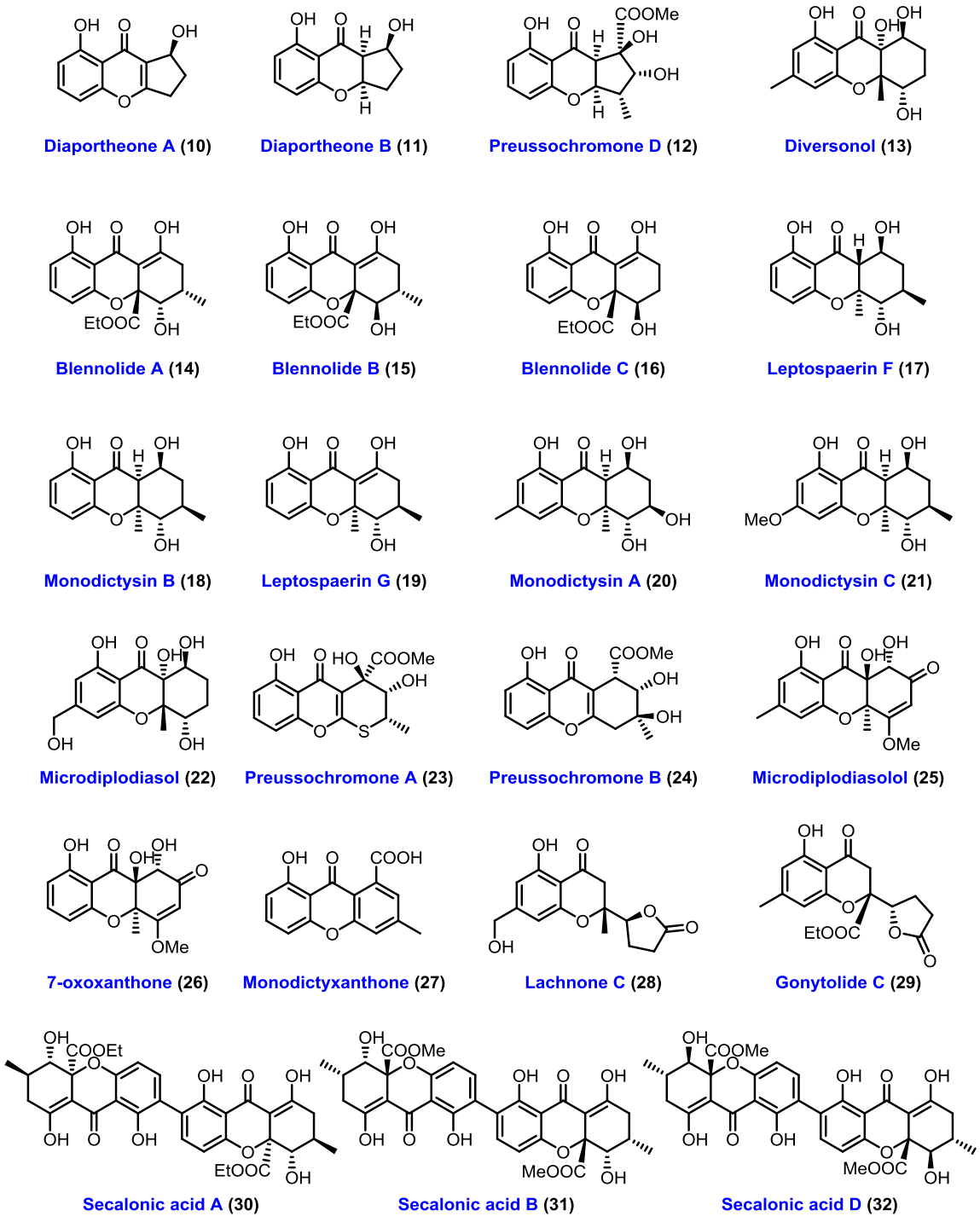



**Figure 2:** Structures of tricyclic natural products.

## 3.3. Antituberculosis agents diaportheones

Diaportheone A (**10**) and B (**11**) are the two recently isolated benzopyranone based natural products which showed the potent antituberculosis activity against the virulent strain of *mycobacterium tuberculosis* H37Rv with MIC of 101 μM and 3.5 μM activity respectively.[9] Although structurally simple molecule, because of interesting biological activity, we became interested in the total synthesis of this natural product and development of a library of related analogs towards the lead optimization. Details are discussed in the following sections.

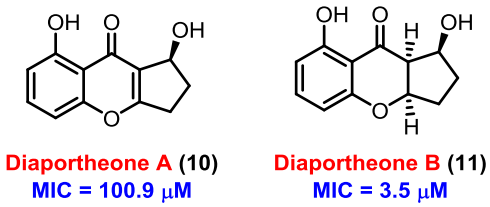



**Figure 3:** Structures of diaportheone A (**10**) and B (**11**).

### 3.3.1. Isolation, characterization and biological activity of diaportheones

Endophytic fungi are found in the calm state within the plant tissues, dominantly in the rainforest plants. This shows the symbiotic relationship with the host by different ways, like protecting the host from the various soil-borne pathogens and toxic compounds, attack of herbivores or by enhancing plant responses.[10] For this reason, most of the endophytes produce secondary metabolites and that were harnessed for their biological activity to treat the different human or animal diseases.

Diaportheone A (**10**) and B (**11**) are the two benzopyranones isolated from the endophytic fungus *diaporthe sp P133* by Bungihan et al in 2011. The fungus *diaporthe sp P133* was isolated from the *Pandanus amaryllifolius* leaves by surface sterilization method.[9] In order to obtain the milligram quantities of the diaportheones for the structural elucidation and biological evaluation, The freshly cut mature and healthy leaves of *Pandanus amaryllifolius* was washed to remove the debris and fungus growing out of the

plant tissue was cultured using potato dextrose agar. The growing fungal strains were identified by using DNA extraction protocols using the 18s rRNA genes with the primers LRI1 and SRLR and generating the homology online by BLAST at NCBI.

The identified fungal strain *diaporthe sp P133* was cultured in sterile potato dextrose broth (1.5 L) for three weeks. the culture broth was repeatedly extracted with ethyl acetate. The extracts were filtered and the solvent was removed in vacuo to obtain the crude residue. The residue was further purified by column chromatography using chloroform: methanol gradient affording the fraction A-F. The active fraction C was further purified by column chromatography and then by medium pressure liquid chromatography method to obtain the diaportheone A (**10**) (5.9 mg) and B (**11**) (5.4 mg).

Structural elucidation of the diaportheone A and B started with the analysis of the high resolution-ESI Orbitrap data where $[M+Na]^+$ was observed at m/z 241.0466, in agreement with the corresponding molecular formula $C_{12}H_{10}O_4$ for diaportheone A and m/z 220.0750 $[M]^+$ (calculated for $C_{12}H_{12}O_4$, 220.0736) in agreement with the molecular formula of $C_{12}H_{12}O_4$ of diaportheone B. The IR spectrum showed absorptions for hydroxyl at 3318, for carbonyl at 1745 and a double bond at 1644 $cm^{-1}$ functional groups. The further confirmation of function group attachment was elucidated with the help of $^1H$ NMR, $^{13}C$ NMR and DEPT. The further confirmation of C-C and C-H was accomplished by using the $^1H$-$^1H$ COSY, HMQC and HMBC spectroscopic analyses. An NOE differential experiment for diaportheone B showed significant correlations among the methine protons at H-1, H-4, and H-13

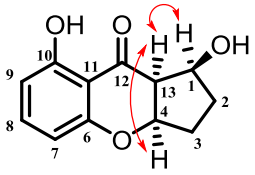



**Figure 4:** Key NOEs of diaportheone B (**11**).

The crude ethyl acetate extract obtained from bioassay-guided isolation was preliminary screened against *Mycobacterium tuberculosis*, *Gordonia terrae*, *Staphylococcus aureus*

and *Escherichia coli* and showed good activity. The purified natural products diaportheone A (**10**) and B (**11**) was tested for antitubercular assay against *Mycobacterium tuberculosis* H37Rv using the Microplate Alamar blue assay method and showed the potent activity with MIC 100.9 μM and 3.5 μM, respectively.[9]

## 3.3.2. Present work

Although both natural products diaportheone A (**10**) and B (**11**) showed potent activity, the diaportheone B (**11**) is a good starting point for a medicinal chemistry program which contains additional two chiral centers. In addition, when we compared the biological activity with marketed TB-drug rifampin (**33**) (MIC 0.25 μM), the diaportheone B (**11**) showed only 14-fold less potent and it is a structurally simple molecule. Moreover, diaportheone B (**11**) has a low molecular weight (220) and clogP value (1.38) which are perfect starting points for medicinal chemists for the lead optimization to develop a druggable candidate as it follow Lipinski rule of five which is meant to evaluate the drug-

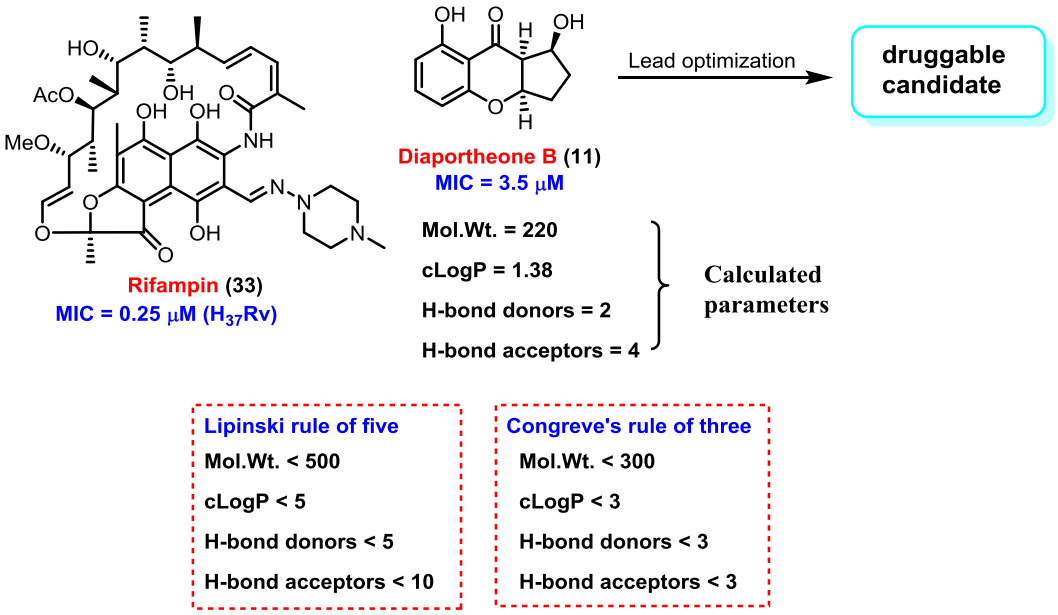



**Figure 5:** Structures and activity of diaportheone B (**11**) and rifampin (**33**)

likeness of biologically active compound that would make it a likely orally active drug in humans[11] and Congreve's rule of three described by M. Congreve for fragment-based

drug discovery.[12] Hence, we were interested in the total synthesis of diaportheone B (**11**), determination of its absolute configuration and development of the lead compound by synthesizing the library of analogs related to this scaffold

## 3.3.2.1. Retrosynthetic approach

Retrosynthetically, we planned the target molecule **11** using a simple strategy, single domino-reaction sequence with the help of an organocatalyst starting from commercially available 2,6-dihydroxyacetophenone (**34**) and succinaldehyde (**35**). A plausible rationale for the desired transformation is shown in figure 6. Ultimately, our goal was to use chiral organocatalyst[13] to access enantiopure material of natural product **11** and its analogs.

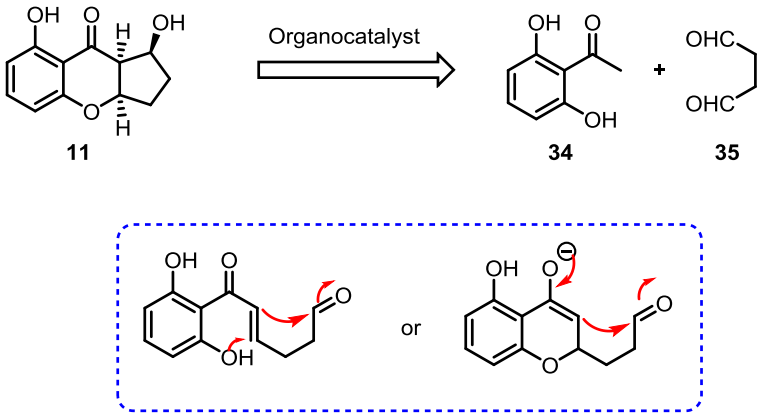


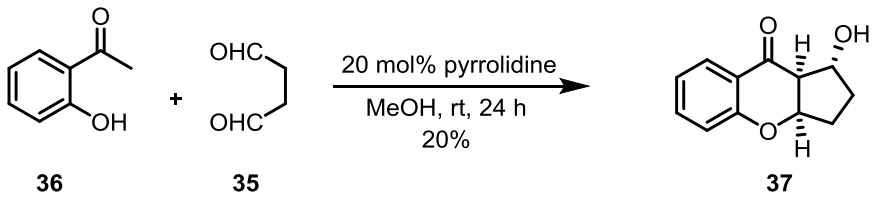

**Figure 6:** Retrosynthetic analysis of diaportheone B (**11**)

## 3.3.2.2. Synthesis of diaportheone B

### 3.3.2.2.1. Synthesis of desired tricyclic core of diaportheone

Initially, we have attempted the reaction on *o*-hydroxy acetophenone (**36**) with scuccinaldehyde (**35**) in the presence of 20 mol% of pyrrolidine to obtain the desired tricyclic core structure through a cascade reaction.[14] In this reaction, compound **37** was obtained in 20% yield as a major one along with trace amounts of its epimer. The formation of **37** was indicated by $^{1}$H NMR which showed the three characteristic protons at δ 5.02 (dt, 1H), 4.59 (m, 1H) and 2.73 (dd, 1H) and in $^{13}$C NMR the characteristic

signals, oxygen attached carbons were observed at 81.8 and 75.1 δ. Which was further confirmed by mass and HRMS which showed the peak at m/z 227.0694 corresponding to the molecular formula $C_{12}H_{12}O_3Na$ $[M+Na]^+$ with the calculated value of 227.0683. We have tried to standardize the yield of this reaction by varying reaction conditions, we observed the formation of required product in case of pyrrolidine in methanol, acetonitrile, DMSO, ethanol and DBU in methanol but with very poor yield. The best conditions for this reaction are pyrrolidine in methanol and results in 20% yield. However, all our efforts towards the improvement of yield of cascade reaction by varying the bases, temperature and solvents were not successful and the details are captured below (table 1).




**Scheme 1:** Synthesis of desired tricyclic core of diaportheone

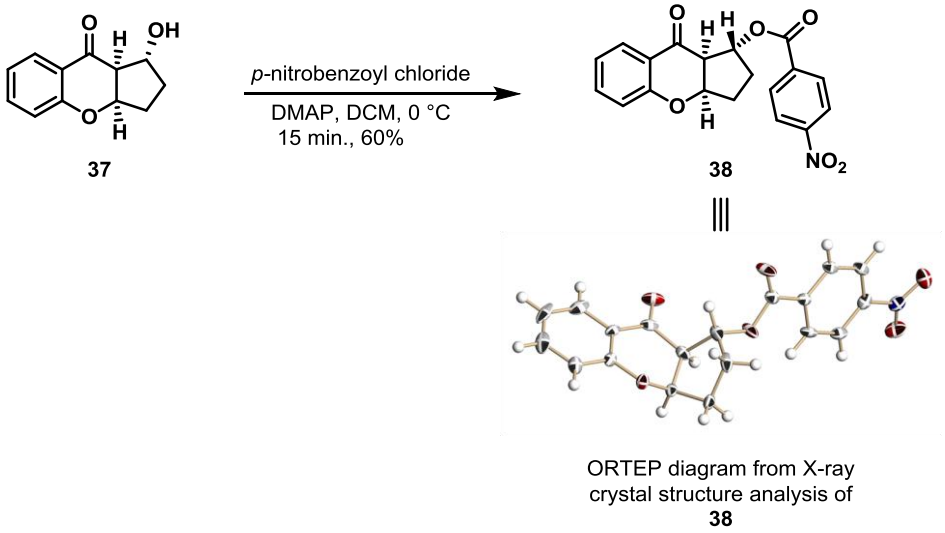
**Table 1:** Conditions followed for standardization of the cascade reaction

| S.No | Base | Solvent | Time (h) | Temp ($^oC$) | Result |
|---|---|---|---|---|---|
| 1 | Pyrrolidine | MeOH | 24 | 50 | 20% |
| 2 | DBU | MeOH | 24 | 50 | <10% |
| 3 | DABCO | MeOH | 24 | 50 | No desired Product |
| 4 | Morpholine | MeOH | 24 | 50 | No desired Product |
| 5 | Proline | MeOH | 24 | 50 | No desired Product |
| 6 | Pyrrolidine | Toluene | 24 | 50 | No desired Product |
| 7 | Pyrrolidine | THF | 24 | 50 | No desired Product |
| 8 | Pyrrolidine | Acetonitrile | 24 | 50 | 12% |
| 9 | Pyrrolidine | DMF | 24 | 50 | No desired Product |
| 10 | Pyrrolidine | DMSO | 24 | 50 | 8% |

| | | | | | |
|---|---|---|---|---|---|
| 11 | Pyrrolidine | EtOH | 24 | 50 | 15% |
| 12 | Pyrrolidine | IPA | 24 | 50 | No desired Product |
| 13 | Pyrrolidine | BuOH | 24 | 50 | No desired Product |

### 3.3.4.2.2. Determination of relative stereochemistry

To determine the relative stereochemistry of the compound **37**, first, it was converted to its *p*-nitrobenzoyl ester **38** by using the *p*-nitrobenzoyl chloride and DMAP in DCM at 0 ºC for 15 minutes. The obtained compound **38** was then crystallized by using DCM: pentane (3:1) to have suitable crystals for diffraction. The relative stereochemistry in compound **38** was unambiguously proved with the help of X-ray crystal structure analysis (Scheme 2).

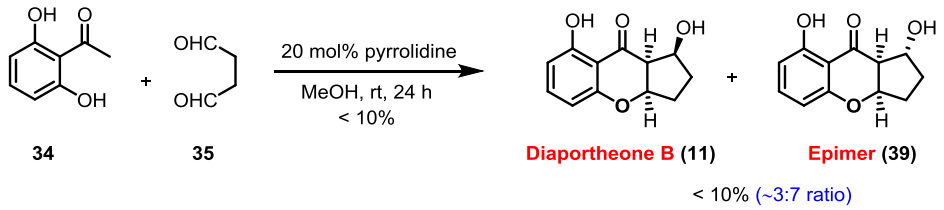



**Scheme 2:** Synthesis of *p*-nitrobenzoyl ester and determination of relative stereochemistry through X-ray analysis

### 3.3.2.2.3. Synthesis of diaportheone B by tandem reaction

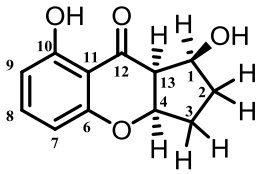
Having seen a positive result from the tandem reaction on a model substrate, we have reacted the 2,6-dihydroxyacetophenone **34** with succinaldehyede **35** under similar conditions to furnish the natural product diaportheone B (**11**) along with its epimer **39** but with poor yield (<10%) (Scheme 3). The formation of diaportheone was confirmed by

mass which showed the peak at m/z 243 corresponding to the molecular formula $C_{12}H_{12}O_4Na$ $[M+Na]^+$. Both diaportheone B and its diastereomer were separated by silica gel (230-400 mesh) column chromatography eluting with the pure DCM, in 3:7 diastereomeric ratio with natural products as minor one.




**Scheme 3:** Synthesis of diaportheone B (**11**)

**Table 2:** $^1$H NMR Spectral data comparison between reported and synthesized Diaportheone B



Diaportheone B (11)

| S. N | H | $^1$H NMR of Diaportheone B (Reported) | | $^1$H NMR of Diaportheone B (400 MHz) (Synthesized) | |
|---|---|---|---|---|---|
| 1 | 1-OH | 1.95 | 1H, d, *J* = 7.0 Hz | 1.98-2.09 | 3H, multiplet |
| 2 | H-2b | 2.02-2.05 | 1H, overlapped | | |
| 3 | H-3b | 2.05-2.10 | 1H, overlapped | | |
| 4 | H-2a | 2.23-2.28 | 1H, m | 2.21-2.29 | 1H, m |
| 5 | H-3a | 2.37-2.42 | 1H, m | 2.32-2.41 | 1H, m |
| 6 | H-13 | 2.78 | 1H, dd, *J* = 5.0, 7.0 Hz | 2.78 | 1H, dd, *J* = 5.2, 6.2 Hz |
| 7 | H-1 | 4.76 | 1H, ddd, *J* = 2.5, 6.5, 13.0 Hz | 4.74 | 1H, m |
| 8 | H-4 | 4.97 | 1H, td, *J* = 3.2, 4.0 Hz | 4.95 | 1H, m |
| 9 | H-7 | 6.40 | 1H, dd, *J* = 1.5, 8.5 Hz | 6.39 | 1H, d, *J* = 8.2 Hz |

| | | | | | |
|---|---|---|---|---|---|
| 10 | H-9 | 6.50 | 1H, dd, *J* = 1.0, 8.0 Hz | 6.48 | 1H, d, *J* = 8.2 Hz |
| 11 | H-8 | 7.34 | 1H, t, *J* = 8.0, Hz | 7.33 | 1H, t, *J* = 8.2 Hz |
| 12 | 10-OH | 11.80 | 1H, s | 11.86 | 1H, s |

**Table 3:** $^{13}$C NMR Spectral data comparison between reported and synthesized Diaportheone B (**11**)

| Sr. No. | Carbon No. | $^{13}$C NMR of Diaportheone B (ppm) (Reported) | $^{13}$C NMR of Diaportheone B (400 MHz) (ppm) (Synthesized) |
|---|---|---|---|
| 1 | C-12 | 198.3 | 198.3 |
| 2 | C-10 | 161.9 | 161.9 |
| 3 | C-6 | 161.3 | 161.2 |
| 4 | C-8 | 138.6 | 138.5 |
| 5 | C-9 | 109.6 | 109.5 |
| 6 | C-11 | 109.3 | 109.2 |
| 7 | C-7 | 107.6 | 107.6 |
| 8 | C-4 | 81.8 | 81.7 |
| 9 | C-1 | 75.5 | 75.4 |
| 10 | C-13 | 55.4 | 55.4 |
| 11 | C-2 | 33.8 | 33.7 |
| 12 | C-3 | 31.8 | 31.7 |

Although we have synthesized the target compound in one step, synthesis suffered from poor yields and selectivity. We have tried several conditions to improve the yield of diaportheones via tandem reaction by varying the bases, temperature, and solvents, but all our efforts to improve the yield and selectivity of the reaction to favor the natural product were in vain. All the conditions are compiled in table 4. The chiral organocatalysts (entries 6-11) also did not produce expected results in terms of yield or selectivity. We suspected instability or polymerization associated with the succinaldehyde (**35**) under the

reaction conditions and hence attempted a few efforts to address this concern, but they were also not successful.

**Table 4:** Standardization of the cascade reaction to improve the yield.

| Sr. No | Base/Acid | Solvent | Time (h) | Temp. | Observation |
|---|---|---|---|---|---|
| 1 | Pyrrolidine | MeOH | 24 | 50 ºC | <10% yield |
| 2 | KOH | DMSO | 24 | 50 ºC | Complex mixture |
| 3 | NaOEt | EtOH | 24 | 50 ºC | No desired Product |
| 4 | CSA | MeOH | 24 | 50 ºC | No specific change |
| 5 | Amberlyst15 | MeOH | 24 | 50 ºC | No specific change |
| 6 | L-Proline | MeOH | 24 | rt | Complex mixture |
| 7 | (*S*)-diphenylprolinol | MeOH | 24 | rt | Complex mixture |
| 8 | Quinine amine | ACN | 48 | rt | No desired Product |
| 9 | Quinine amine | DMSO | 48 | rt | No desired Product |
| 10 | 1-(pyrrolidin-2-ylmethyl) pyrrolidine | ACN | 24 | rt | No desired Product |
| 11 | 1-(pyrrolidin-2-ylmethyl) pyrrolidine | DMSO | 48 | rt | No desired Product |

CSA- Camphor sulfonic acid

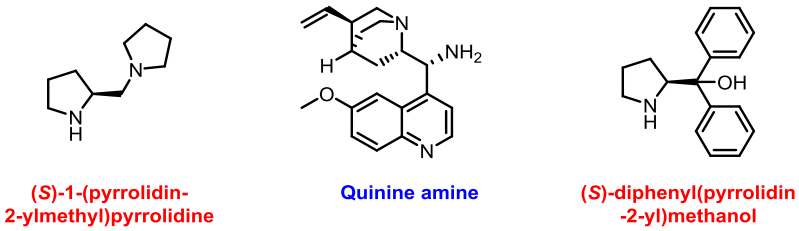
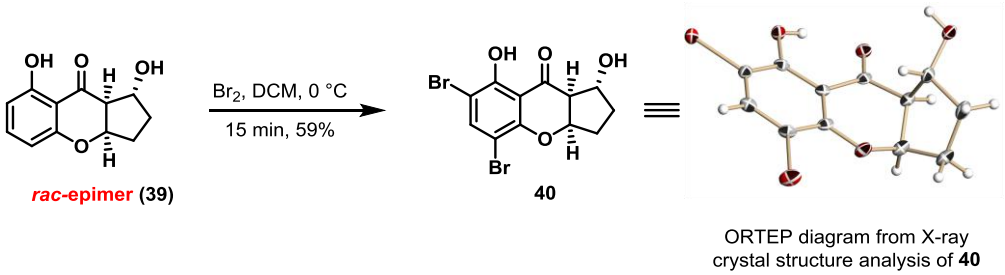



**Figure 7:** Structures of chiral amine bases

### 3.3.2.2.4. Determination of relative stereochemistry of epimer (39)

The racemic epimer **39** obtained through the column chromatography purification was converted to its dibromo derivatives **40** by using bromine in DCM.[15] The reaction gives a dibromo product which was directed by phenolic -OH group on the aromatic ring at C-7 and C-9 positions, which was confirmed by presence only one aromatic proton at δ 7.83 (m, 1H) followed by HRMS calculated for $C_{12}H_{10}O_4Br_2Na$ $[M+Na]^+$: 398.8843, and observed (398.8831) in agreement. This reaction was very fast which completed in 15 minutes because of the presence of phenolic hydroxy group on the aromatic ring. The relative stereochemistry of compound **39** was proved with the help of X-ray crystal structure analysis of **40** as shown in scheme 4.

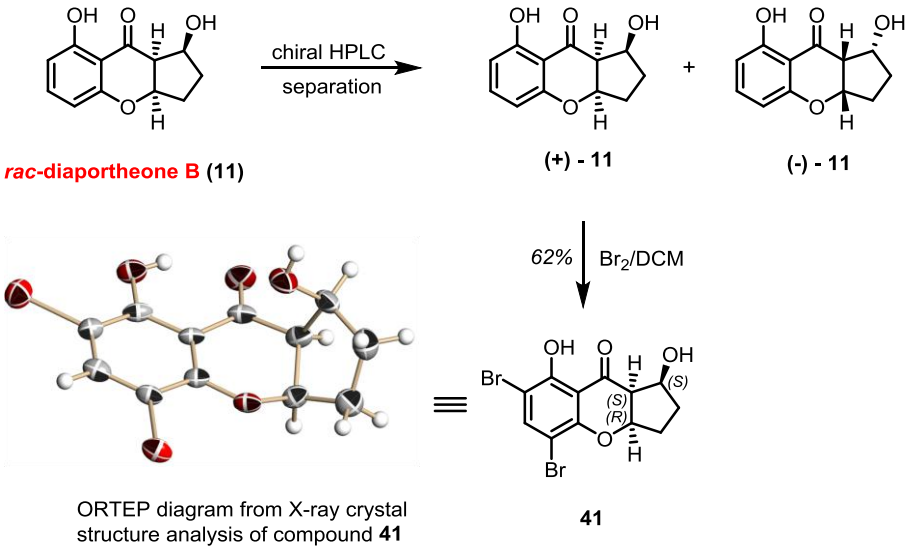



**Scheme 4:** Determination of relative stereochemistry of compound **39**

### 3.3.2.2.5. Determination of absolute stereochemistry of diaportheone B

To determine the absolute configuration of the natural diaportheone (+)-isomer, we decided to separate both the enantiomers of racemic diaportheone **11** using chiral HPLC column. After few attempts, we were successful in separating all the four possible stereoisomers from **11** (+**11** and ent -**11**) and its epimer **39** (+**39** and ent -**39**) using chiral HPLC [Kromasil 5-AmyCoat (250 x 4.6 mm), using IPA: n-Hexane (15:85) as eluting solvent at 254 nm wavelength with 0.7 mL/min of flow rate and 1.0 mg/mL concentration. $R_t$ = 13.033 for (+)-diaportheone B (**11**) and $R_t$ = 20.083 for (-)-enantiomer of **11**). The natural enantiomer (+)-**11** was converted to its dibromo derivative **41** by the same procedure which followed for the racemic compound.[15] The absolute configuration of (+)-**11** was determined by single-crystal X-ray diffraction analysis of **41** as

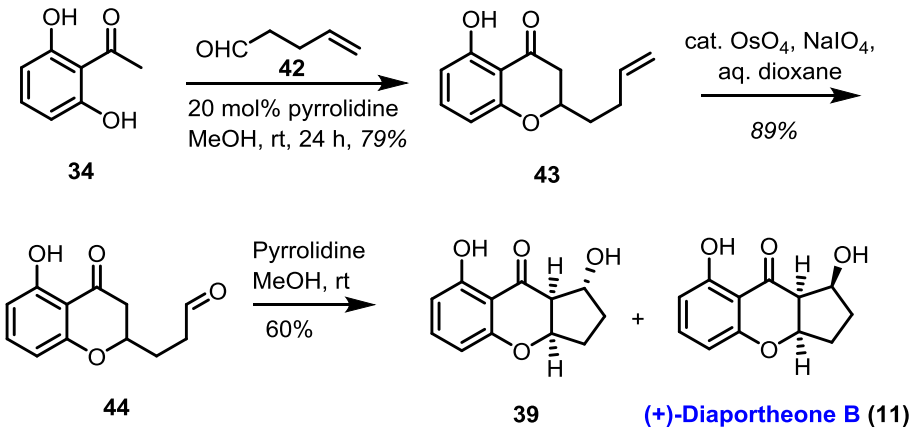
*(1S,3aR,9aS)-1,8-dihydroxy-1,2,3,3a tetrahydro- cyclopenta[b]chromen-9(9aH)-one*, by anomalous dispersion effect (Flack parameter of -0.0032) in X-ray diffraction measurements which is caused by the presence of bromine atom in the molecule.




**Scheme 5:** Determination of absolute stereochemistry of (+)-diaportheone B (**11**)

### 3.3.2.2.6. Synthesis of diaportheone B by three-step sequence

Although we have achieved the first total synthesis of diaportheone B (**11**) using the one-step procedure with bench-top chemicals, we were interested to improve the yield of natural product **11** by following an alternate route. Considering the polymerization observed with succinaldehyde (**35**) we planned for the synthesis of diaportheones using masked aldehyde. We have treated the 2,6-dihydroxyacetophenone (**34**) with 4-pentenal (**42**) in the presence of pyrrolidine (20 mol%) resulted in benzopyranone **43** in 79% yield. The formation of olefin was confirmed by using $^{1}$H NMR and $^{13}$C NMR spectroscopic data, which was further confirmed using the HRMS which showed a peak at m/z 219.1029 corresponding to the molecular formula $C_{13}H_{14}O_3$ $[M+H]^+$. Olefin cleavage of compound **43** using Lemieux-Johnson oxidation[16] to produce aldehyde **44**, the aldehyde **44** was confirmed by $^{1}$H NMR in which the characteristic aldehyde proton was observed at δ 9.81 ppm followed by $^{13}$C NMR at δ 200.7 ppm. The aldehyde **44** was then exposed to 20 mol% of pyrrolidine in methanol to furnish the compound **39** and **11** in ~9:1 ratio.



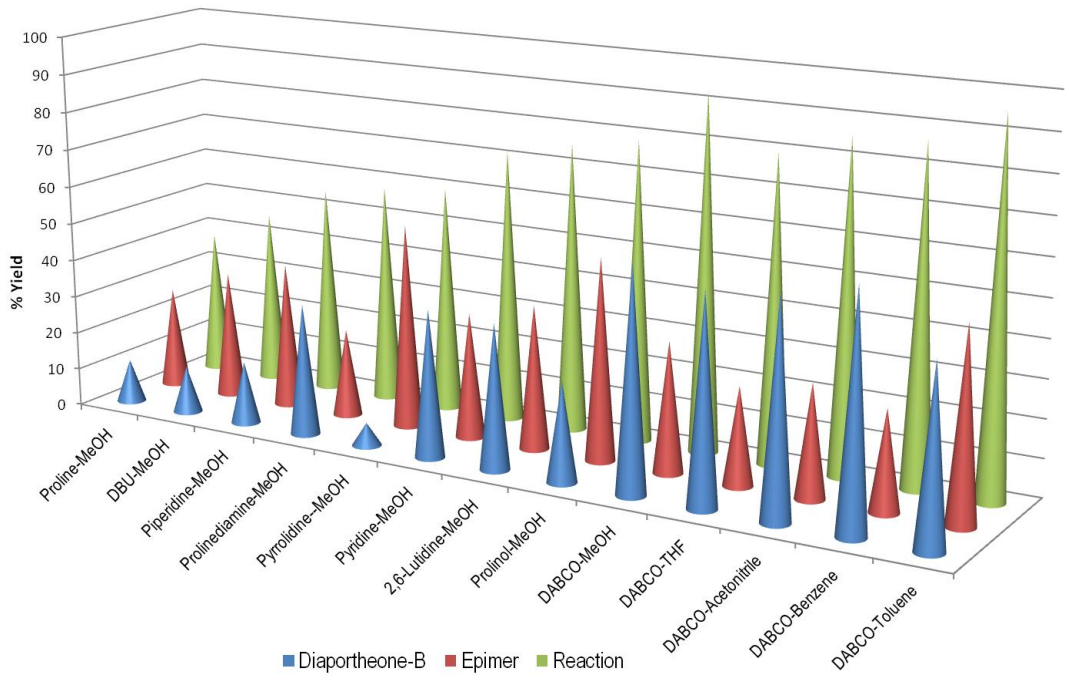

**Scheme 6:** Synthesis of diaportheone B (**11**) by three-step sequence

To improve the yield and ratio to favor the natural product (+)-diaportheone B (**11**), we have explored various conditions by changing the base, solvent, etc (see table 5). Which are summarized as a graphical representation (Figure 8). Our initial attempts towards standardization started with the treatment of aldehyde **44** with various bases for aldol reaction keeping the methanol as common solvent. Here, we screened the various secondary and tertiary amine bases such as pyrrolidine, proline, piperidine, proline diamine, prolinol, pyridine, DBU and DABCO at room temperature. The best result observed is DABCO in methanol treated for 15 minutes gave highest yield of 93% based on recovery of starting material and with 37:63 as the diastereomeric ratio of **39**:**11** favoring diaportheone B (**11**). Having the best condition in hand we decided to keep the DABCO as a base and varying the solvents from polar protic (methanol) to polar aprotic solvent (tetrahydrofuran, acetonitrile) followed by using non-polar solvents (benzene, toluene). While monitoring the reaction by TLC we observed that the continuing reaction for a longer time resulted in the β-hydroxy elimination from the diaportheone to give α, β-unsaturated compounds. The best conditions found after the optimization was that 20 mol% of DABCO in acetonitrile resulted in the higher ratio (~1:2) favoring diaportheone **11** and the 20 mol% of DABCO in toluene furnished the highest yield (96%) of the reaction. Also, we have made few attempts to convert the epimer **39** to the natural product diaportheone **11** with no success.

**Table 5**: Conditions followed for standardization and improvement of yield

| Sr.No | Base | Solvent | Reaction Time | Ratio **39:11**[b] | Yield % (brsm) | Recovered SM % |
|---|---|---|---|---|---|---|
| 1 | Proline[a] | MeOH | 12 h | 70:30 | 38 | 38 |
| 2 | DBU | MeOH | 30 min | 73:27 | 46 | 18 |
| 3 | Piperidine | MeOH | 18 h | 70:30 | 55 | 42 |
| 4 | Proline Diamine[a] | MeOH | 10 h | 40:60 | 58 | 17 |
| 5 | Pyrrolidine | MeOH | 2.0 h | 90:10 | 60 | 25 |
| 6 | Pyridine | MeOH | 36 h | 46:54 | 72 | 64 |
| 7 | 2,6-Lutidine | MeOH | 24 h | 50:50 | 76 | 42 |
| 8 | Prolinol | MeOH | 15 h | 67:33 | 79 | 13 |
| 9 | DABCO | MeOH | 15 min | 37:63 | 93 | 50 |
| 10 | DABCO | THF | 4 h | 32:68 | 80 | 14 |
| 11 | DABCO | ACN | 3 h | 34:66 | 86 | 12 |
| 12 | DABCO | Benzene | 5 h | 30:70 | 87 | 14 |
| 13 | DABCO | Toluene | 6 h | 48:52 | 96 | 00 |

Note: (a) 50 mol% of base used. (b) diastereomeric ratio was determined using HPLC




***best conditions after the optimization***

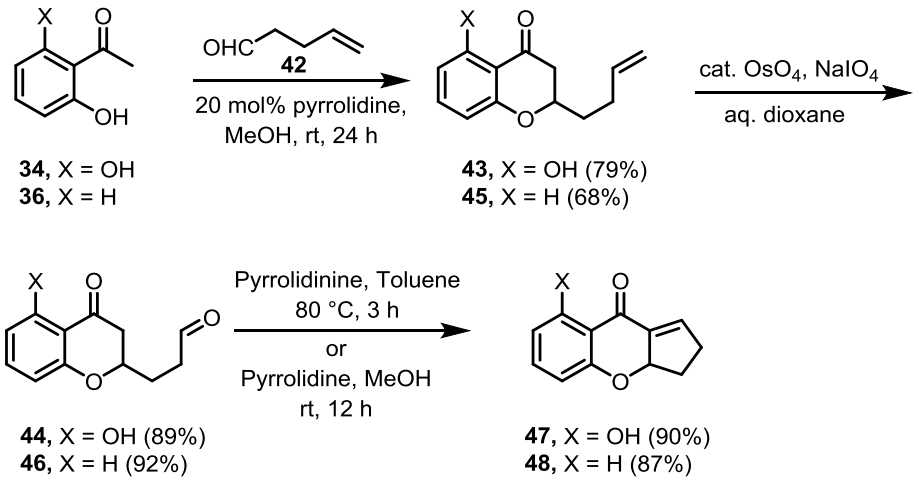
*96% yield with ~ 1 : 1 ratio (20 mol% DABCO in toluene)*

*86% yield with ~ 1 : 2 ratio (20 mol% DABCO in acetonitrile)*

**Figure 8:** Optimization of diastereomeric ratio during final aldol reaction

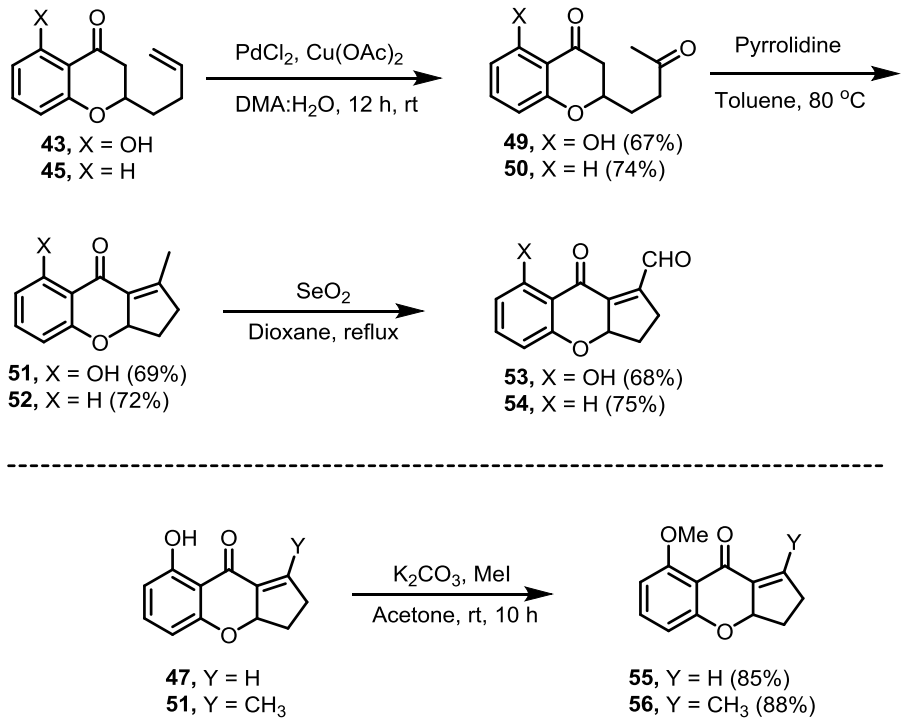
## 3.3.2.3. Synthesis of analogs

Having developed methods for the easy access to natural diaportheone B (**11**) and its epimer **39**, we were interested in the synthesis of their close analogs to evaluate their antituberculosis potential. The synthesis of the dehydro analogs **47** and **48** was accomplished by treatment of aldehydes **44** and **46** with 20 mol% pyrrolidine in toluene at 80 °C for 3 h. The formation of the product was confirmed by $^{1}$H NMR and $^{13}$C NMR data, which was further confirmed by HRMS analysis which showed a peak at m/z 203.0720 corresponding to $[M+H]^+$ with molecular formula $C_{12}H_{11}O_3$ for **47** and at m/z 187.075 found corresponding to $[M+H]^+$ with molecular formula $C_{12}H_{11}O_2$ for **48**.[17] This same transformation can also be achieved by conducting the reaction in methanol for prolonged time (Scheme 7).



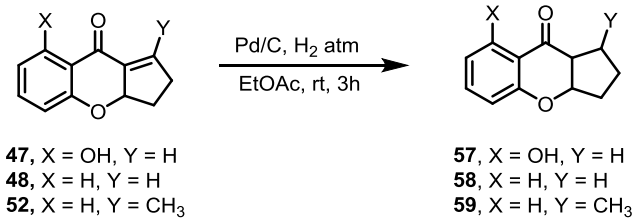

**Scheme 7:** Synthesis of diaportheone analogs

To incorporate a substitution on the 5-membered ring of tricyclic compounds, olefins **43** and **45** were subjected to Wacker oxidation in DMA:water to produce the corresponding ketones **49** and **50**, respectively. Cyclization of **49** and **50** using pyrrolidine in toluene resulted in the desired analogs **51** and **52** in good yields. The formation of **51** and **52** was confirmed by corresponding mass of $[M+Na]^+$ followed by the $^1H$ NMR and $^{13}C$ NMR spectroscopic methods. Allylic oxidation ($SeO_2$) of the methyl groups on **51** and **52** resulted in the corresponding aldehydes **53** and **54**. The compounds **53** and **54** were confirmed by HRMS which showed the corresponding $[M+H]^+$ peak at m/z 231.0651 and 215.0697 respectively. Methylation of a phenolic hydroxy group of compounds **47** and **51** using potassium carbonate and methyl iodide in acetone results in the methoxy analogs **55** and **56** (Scheme 8) which was confirmed by mass corresponding to $[M+H]^+$ followed by $^1H$ NMR and $^{13}C$ NMR.




**Scheme 8:** Synthesis of diaportheone analogs

Hydrogenation of the double bond present in compounds **47**, **48**, and **52** using 10% Pd/C under a hydrogen atmosphere (using balloon pressure) produced the corresponding saturated analogs **57**, **58**[18] and **59**[19] in good yields, respectively. The $^{1}H$ and $^{13}C$ NMR data of compound **58** and **59** was compared with the literature reports and found to be in complete agreement.




**Scheme 9:** Synthesis of diaportheone analogs

## 3.3.3. Biological activity and SAR of diaportheone analogs

The natural product diaportheone B (**11**), its isomers, and all other synthesized analogs were tested for antitubercular activity in collaboration with Dr. Rajesh Gokhale's group at CSIR-IGIB, New Delhi and antibacterial activity with the help of Dr. Sidharth Chopra's group at CSIR-CDRI Lucknow (the results are compiled in table 6). The anti-TB activity for inhibition of growth of a virulent strain of *Mycobacterium tuberculosis* H37Rv using the Alamar-blue assay method was determined. The natural isomer (+)-**11**, its enantiomer (-)-**11**, and rac-**11** showed very similar activity (10-12.5 μg $ml^{-1}$). While the (+)-**39**, its enantiomer (-)-**39** showed the two-fold less potent compared to the racemic **39**. The aldol condensation product **47** was also found to be equipotent compared to natural diaportheone, on the other hand, the methyl substituted product on the five-membered ring (compound **51**) was found to be almost inactive towards the *mycobacterium tuberculosis*. Out of all the tested analogs, methoxy analog **55** showed superior activity compared to natural (+)-diaportheone B (**11**). The synthesized compounds **52**, **53**, **54**, **56**, **57, 58** and **59** were found to be completely inactive against the *Mycobacterium tuberculosis*. Based on this limited SAR on its anti-TB activity, this novel tricyclic scaffold seems promising; with potential that needs further systematic exploration with a larger number of compounds.

The Synthesized analogs were also screened for antibacterial assay against the *Staphylococcus aureus* ATCC29213. The dibromo derivatives of diaporteone B and its epimer (compound **40** and **41**) showed the considerable activity with MIC = 4.0 μg/mL. While compound **47** and **48** also found to be active with MIC 16 μg/mL.

**Table 6:** Antituberculosis and antibacterial activity of synthesized compounds

| Sr. No. | Compound | Activity (µg/mL) Mtb H37Rv | Activity(µg/mL) *S. aureus* ATCC29213 |
|---|---|---|---|
| 1 | *rac*-11 | 12.5 | >32 |
| 2 | (+)-11 | 10.0 | >32 |
| 3 | (-)-11 | 12.5 | >32 |
| 4 | *rac*-39 | 12.5 | >32 |
| 5 | (+)-39 | 25 | >32 |
| 6 | (-)-39 | 25 | >32 |
| 7 | 37 | 50 | >64 |
| 8 | 40 | 25 | 4.0 |
| 8 | 41 | 25 | 4.0 |
| 9 | 47 | 12.5 | 16 |
| 10 | 48 | 25 | 16 |
| 11 | 51 | 50 | >64 |
| 12 | 52 | 100 | >64 |
| 13 | 53 | >200 | >64 |
| 14 | 54 | 100 | >64 |
| 15 | 55 | 6.25 | >64 |
| 16 | 56 | >200 | >64 |
| 17 | 57 | >200 | >64 |
| 18 | 58 | >200 | >64 |
| 19 | 59 | >200 | >64 |

## 3.3.4. Summary

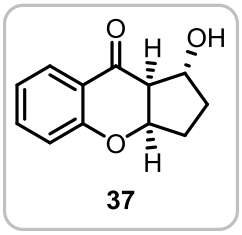
- First total synthesis of diaportheone B was achieved in one-step and three-step sequence using bench-top chemicals. The second route is suitable for the scaleup of the natural product.
- The absolute configuration of natural diaportheone B was determined as *(1S,3aR,9aS)-1,8-dihydroxy-1,2,3,3a tetrahydro- cyclopenta[b]chromen-9(9aH)-one* through separation of enantiomers, conversion to bromo derivative of natural enantiomer followed by its X-ray structure analysis with the help of Flack factor.
- Several analogs related to the tricyclic core of diaportheones have been synthesized and screened as anti-tuberculosis and anti-bacterial agents against *Mycobacterium tuberculosis* and *Staphylococcus aureus*, respectively.

## 3.3.5. Experimental

**General procedure for tandem aldol reaction**

To a mixture of succinaldehyde (2 mmol) and hydroxy acetophenone (1 mmol) in dry methanol (4 mL), was added a catalytic amount of pyrrolidine (0.2 mmol) dropwise at room temperature. The reaction mixture was warmed to 50 $^{o}$C and stirring continued for 24h. Methanol from the reaction mixture was removed under reduced pressure and the crude mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL) and the combined organic extracts were washed with 1N HCl (2 mL), water (5 mL), brine (2 mL), dried over anhydrous $Na_2SO_4$, concentrated to a crude mixture which was further purified by flash column chromatography (230-400 mesh) by using ethyl acetate: pet ether to afford pure compound along with the recovery of unreacted acetophenone (see table 1 and table 4 for conditions used for standardization of yield).

**(1R*,3aR*,9aS*)-1-hydroxy-1,2,3,3a-tetrahydrocyclopenta[b]chromen-9(9aH)-one (37)**




**37**

Prepared by following the general procedure described above in 20% yield as colourless gummy mass.

**Mp:** 58-60 $^{o}$C

**IR $\upsilon_{max}$(film):** cm$^{-1}$ 3684, 3611, 3020, 1677, 1607, 1464

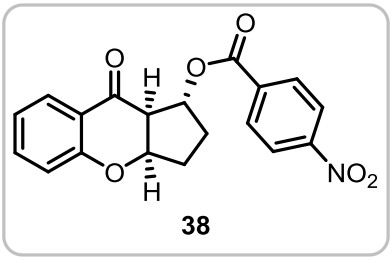
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.89 (dd, *J* = 1.6, 7.9 Hz, 1H), 7.51-7.39 (m, 1H), 7.07-6.98 (m, 1H), 6.92 (d, *J* = 8.3 Hz, 1H), 5.03 (dt, *J* = 2.1, 5.1 Hz, 1H), 4.64-4.51 (m, 1H), 2.75 (dd, *J* = 5.0, 7.7 Hz, 1H), 2.40 (d, *J* = 8.6 Hz, 1H), 2.39-2.22 (m, 2H), 2.17-2.06 (m, 1H), 1.86-1.77 (m, 1H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 193.3, 160.7, 136.5, 126.9, 121.5, 119.5, 118.2, 81.8, 75.1, 59.0, 31.8, 30.4

**MS:** 227 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{12}O_3$, $[M+Na]^+$: 227.0683 found 227.0694

**(1*R**,3a*R**,9a*R**)-9-oxo-1,2,3,3a,9,9a-hexahydrocyclopenta[b]chromen-1-yl 4-nitrobenzoate (38)**




**38**

To a mixture of **37** (1 mmol) and DMAP (3 mmol) in DCM (10 mL) at 0 °C was added *p*-nitrobenzoyl chloride (3 mmol) and allowed to stir for 15 minutes. After the completion of the reaction, water was added to the reaction mixture and the aqueous layer was separated and extracted with DCM (5 mL x 2). The combined organic extracts were washed with 1N HCl (5 mL), water (10 mL), brine (5 mL ), dried over anhydrous $Na_2SO_4$, concentrated to a crude mixture and the compound purified using silica gel column chromatography (100-200 mesh) to afford **38** as white crystals (60%).

**Mp:** 136-138 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3155, 2929, 2254, 1727, 1686, 1608, 1529, 1464, 1350

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 8.28 (d, *J* = 8.7 Hz, 2H), 8.21 (d, *J* = 8.7 Hz, 2H), 7.88 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.50 (m, 1H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.95 (d, *J* = 8.2 Hz, 1H), 5.71 (m, 1H), 5.12 (m, 1H), 3.14 (dd, *J* = 7.2, 4.7 Hz, 1H), 2.70-2.60 (m, 1H), 2.30-2.22 (m, 2H), 1.97-1.89 (ddd, *J* = 14.3, 10.0, 5.2 Hz, 1H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 191.0, 164.1, 160.4, 150.6, 136.6, 135.2, 130.9, 127.2, 127.1, 123.5, 121.8, 119.2, 118.2, 81.3, 77.9, 55.4, 30.4, 30.0

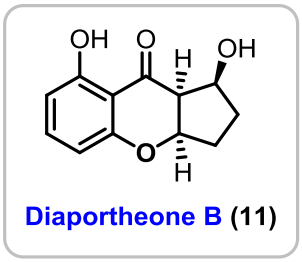
**MS:** 376 $[M+Na]^+$

## Details of X-Ray Crystal Structure analysis

Single crystals of compounds **38** were obtained from DCM/Pentane by diffusion crystallization method. 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.

**Crystallographic data for 38:** ($C_{19}H_{15}N_1O_6$): *M* = 353.32, Crystal dimensions 0.49 x 0.16 x 0.10 $mm^3$, monoclinic, space group $P\ 2_1/c$, *a* = 8.0391(17), *b* = 18.123(3), *c* = 11.275(2) Å, $\beta$ = 105.499(9), *V* = 1582.9(5) $Å^3$, *Z* = 4, $\rho_{calcd}$ = 1.483 $gcm^{-3}$, μ (Mo-$K_\alpha$) = 0.112 $mm^{-1}$, *F(000)* = 736, $2\theta_{max}$ = 50.00°, *T* = 100(2) K, 9119 reflections collected, 2705 unique, 2128 observed ($I > 2\sigma(I)$) reflections, 236 refined parameters, *R* value 0.0718, *wR*2 = 0.1112, (all data *R* = 0.0967, *wR*2 = 0.1192), S = 1.171, minimum and maximum transmission 0.9472 and 0.9889; maximum and minimum residual electron densities +0.225 and -0.236 e $Å^{-3}$.

**(1*S*,3a*R*,9a*S*)-1,8-dihydroxy-1,2,3,3a-tetrahydrocyclopenta[b]chromen-9(9aH)-one (11)**




Diaportheone B (11)

Prepared from 2,6-dihydroxy acetophenone (**34**) (200 mg, 1.31 mmol), succinaldehyde (**35**) (226 mg, 2.63 mmol), pyrrolidine (0.02 mL, 0.26 mmol) by following the general procedure described above, yielding a mixture of **11** and **39** (3:7) (23 mg, 8%). The diastereomeric mixture of **11** and **39** was separated by silica gel column chromatography using DCM (100%) gave us **11** and **39** as colourless solids.

*Racemic*-**11**

**Mp:** 93-95 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3445, 3020, 2980, 2940, 1731, 1635, 1578, 1462

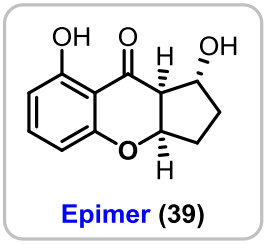
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 11.86 (s, 1H), 7.33 (t, *J* = 8.2 Hz, 1H), 6.48 (d, *J* = 8.2 Hz, 1H), 6.39 (d, *J* = 8.2 Hz, 1H), 4.95 (m, 1H), 4.74 (m, 1H), 2.78 (dd, *J* = 6.2, 5.2 Hz, 1H), 2.32-2.41 (m, 1H), 2.21-2.29 (m, 1H), 1.98-2.09 (m, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 198.3, 161.9, 161.3, 138.5, 109.6, 109.3, 107.6, 81.7, 75.5, 55.4, 33.8, 31.8

**MS:** 243 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{12}O_4Na$ $[M+Na]^+$: 243.0628 found 243.0640

**(1R,3aR,9aS)-1,8-dihydroxy-1,2,3,3a-tetrahydrocyclopenta[b]chromen-9(9aH)-one (39)**




Epimer **(39)**

**Mp:** 92-96 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3445, 3020, 2980, 2940, 1731, 1635, 1578, 1462

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 11.77 (s, 1H), 7.34 (t, *J* = 8.2 Hz, 1H), 6.48 (d, *J* = 8.2 Hz, 1H), 6.36 (d, *J* = 8.2 Hz, 1H), 4.98 (td, *J* = 4.7, 1.7 Hz, 1H), 4.64 (dd, *J* = 13.5, 7.5 Hz, 1H), 2.76 (dd, *J* = 7.5, 5.0 Hz, 1H), 2.63 (brs, 1H), 2.40-2.33 (m, 1H), 2.29-2.21 (m, 1H), 2.15-2.07 (m, 1H), 1.82-1.75 (m, 1H)

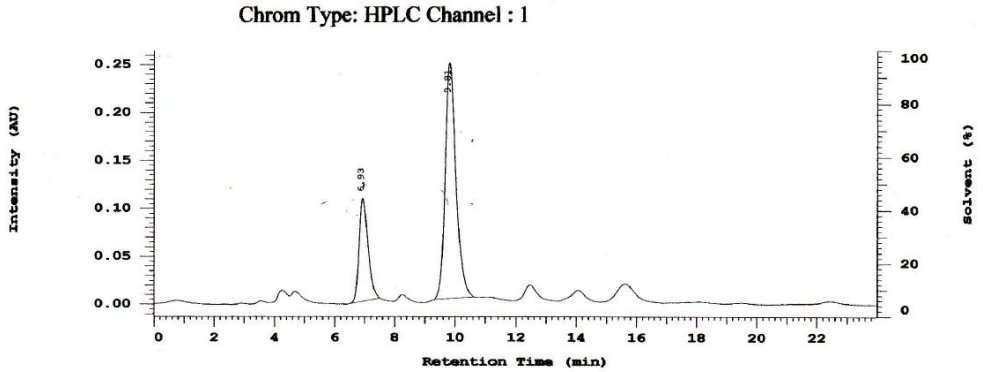
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 199.0, 162.4, 160.6, 138.8, 109.4, 107.5, 106.6, 81.7, 75.6, 58.4, 32.2, 30.4

**MS:** 243 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{12}O_4Na$ [M+Na]+: 243.0628 found 243.0635

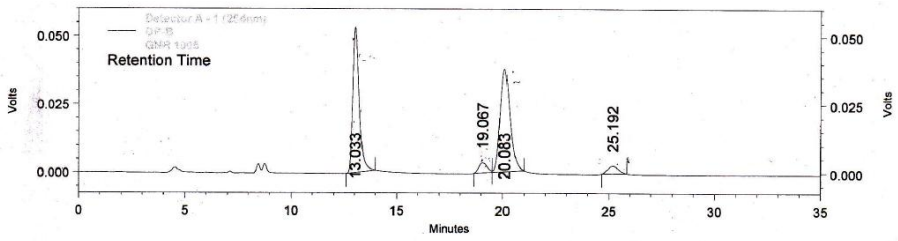
**Determinaton of diastereomeric ratio of diapotheones by reverse phase HPLC (Tandem Reaction)**

Column: Atlantis RP-18 (250 x 4.6 mm)
Mobile phase: MeOH: $H_2O$ (50:50)
Wavelength: 254 nm
Flow rate: 1.0 mL/min (2900 psi)
Sample conc: 1.0 mg/mL
Injection volume: 10 µL




| No. | RT | Height | Area | Area % |
|---|---|---|---|---|
| 1 | 6.93 | 53704 | 1100132 | 26.386 |
| 2 | 9.81 | 122636 | 3069198 | 73.614 |
| | | 176340 | 4169330 | 100.000 |

**Separation of enantiomers for absolute configuration determination of diaportheone B (11)**




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 13.033 | 1149396 | 45.451 |
| 19.067 | 101063 | 3.996 |
| 20.083 | 1177268 | 46.553 |
| 25.192 | 101158 | 4.000 |
| Totals | 2528885 | 100.000 |

Column: Kromasil 5-AmyCoat (250 x 4.6 mm)

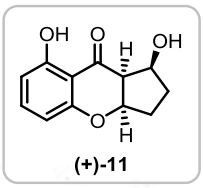
Mobile phase: IPA: n-Hexane (15:85)

Wavelength: 254 nm

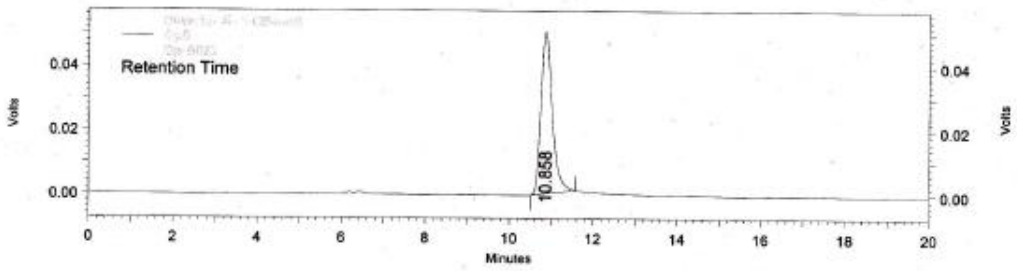
Flowrate: 0.7 mL/min 470 psi

Concentration: 1.0 mg/mL

Inj volume: 10µL

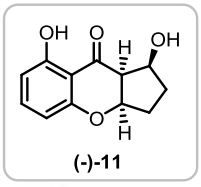



(+)-11

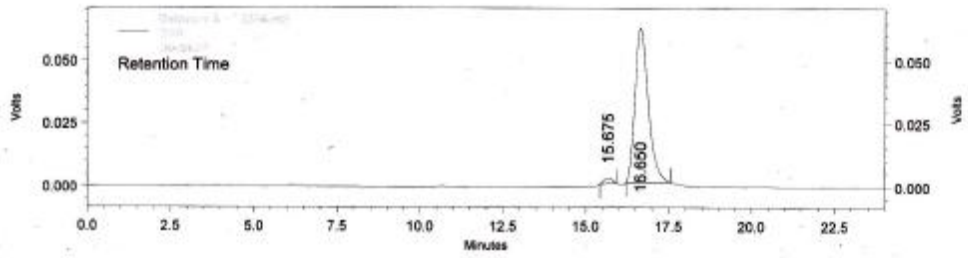



**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 10.858 | 944985 | 100.000 |
| Totals | 944985 | 100.000 |




(-)-11




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 15.675 | 35815 | 2.046 |
| 16.650 | 1714609 | 97.954 |
| Totals | 1750424 | 100.000 |

Column: Kromasil 5-AmyCoat (250 x 4.6 mm)

Mobile phase: IPA: n-Hexane (12:88)

Wavelength: 254 nm

Flow Rate: 0.7 mL/min, 470 psi

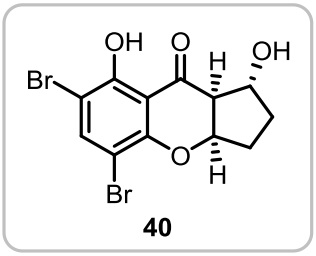
Concentration: 1.0 mg/mL

Injection volume: 10µL

**General procedure for bromination reaction**

A solution of bromine (1 mmol) in DCM (5 mL) was slowly added to the compound (1 mmol), in dry DCM (5 mL) and allowed to stir for 15 min at 0 ºC. The reaction mixture was treated with saturated aqueous solution of $Na_2S_2O_5$ (5 mL) followed by addition of water (5 mL). The organic phase was separated and aq. phase was extracted with DCM (5 mL) and combined organic extracts were washed with water (5 mL), brine (2 mL), dried over $Na_2SO_4$ and concentrated to a crude mixture. The crude mixture was purified by flash column chromatography (230-400 mesh) to obtain yellow coloured solid.

***rac*-5,7-dibromo-1,8-dihydroxy-1,2,3,3a-tetrahydrocyclopenta[b]chromen-9(9aH)-one (40)**




**40**

Prepared from **39** (5 mg, 0.022 mmol) and bromine (2.30 µl, 0.045 mmol) following the general procedure for the bromination reaction described above yielding **40** (5 mg, 58%) as a yellow solid.

**Mp:** 136-140 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3585, 3019, 2931, 1638, 1606, 1448

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 12.52 (s, 1H), 7.83 (m, 1H), 5.09 (td, *J* = 4.7, 2.0 Hz, 1H), 4.63 (m, 1H), 2.84 (dd, *J* = 7.5, 4.7 Hz, 1H), 2.45-2.36 (m, 2H), 2.34-2.20 (m, 2H), 1.86-1.76 (m, 1H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 198.5, 157.9, 155.9, 143.0, 107.3, 101.7, 99.8, 82.9, 75.5, 57.8, 32.1, 30.3

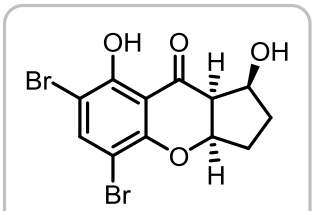
**MS:** 398 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{10}O_4Br_2Na$ $[M+Na]^+$: 398.8843, found 398.8831

**Crystallographic data for 40:**

Single crystals of compounds **40** were obtained from DCM/Pentane by diffusion crystallization method. 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. ($C_{12}H_{10}Br_2O_4$): $M$ = 378.02, Crystal dimensions 0.56 x 0.07 x 0.07 $mm^3$, triclinic, space group $P$ -1, $a$ = 7.6833(5), $b$ = 12.1129(8), $c$ = 13.7371(9) Å, $\alpha$ = 97.440(3), $\beta$ = 97.440(3), $\gamma$ = 102.311(3), $V$ = 1222.16(14) $Å^3$, $Z'$ = 2, $Z$ = 4, $\rho_{calcd}$ = 2.054 $gcm^{-3}$, μ (Mo-$K_\alpha$) = 6.636 $mm^{-1}$, $F(000)$ = 736, $2\theta_{max}$ = 54.00°, $T$ = 100(2) K, 20413 reflections collected, 5313 unique, 4563 observed ($I > 2\sigma(I)$) reflections, 329 refined parameters, $R$ value 0.0225, $wR2$ = 0.0492, (all data $R$ = 0.0306, $wR2$ = 0.0521), S = 1.045, minimum and maximum transmission 0.1186 and 0.6538; maximum and minimum residual electron densities +0.682 and -0.638 e $Å^{-3}$.

**(1S,3aR,9aS)-5,7-dibromo-1,8-dihydroxy-1,2,3,3a-tetrahydrocyclopenta[b]chromen-9(9aH)-one (41)**




**41**

Prepared from (+)-**11** (10 mg, 0.04 mmol) and bromine (4.60 µl, 0.09 mmol) following the general procedure for the bromination reaction described above yielding **41** (10.6 mg, 62%) as a yellow solid.

**$[\alpha]_D^{25}$:** +107.3° (*c* 1, $CHCl_3$).

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3584, 3019, 2930, 1641, 1607, 1448

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 12.61 (s, 1H), 7.82 (m, 1H), 5.05 (m, 1H), 4.79 (m, 1H), 2.85 (t, *J* = 5.7 Hz, 1H), 2.56-2.49 (m, 1H), 2.33-2.24 (m, 1H), 2.16-2.03 (m, 2H), 1.81 (d, *J* = 6.0 Hz, 1H)

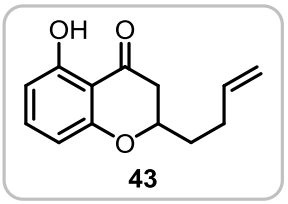
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 198.0, 157.5, 155.5, 143.0, 109.9, 101.6, 99.8, 82.8, 75.6, 54.9, 33.8, 32.0.

**MS:** 398 $[M+Na]^+$

**Crystallographic data for 41:**

Single crystals of compounds **41** were obtained from DCM/Pentane by diffusion crystallization method. 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. ($C_{12}H_{10}Br_2O_4$): *M* = 378.02, Crystal dimensions 0.35 x 0.10 x 0.03 $mm^3$, orthorhombic, space group *P* $2_12_12_1$, *a* = 13.574(6), *b* = 17.699(8), *c* = 21.533(9) Å, *V* = 5173(4) $Å^3$, *Z'* = 4, *Z* = 16, $\rho_{calcd}$ = 1.942 $gcm^{-3}$, µ (Mo-$K_\alpha$) = 6.271 $mm^{-1}$, *F(000)* = 2944, $2\theta_{max}$ = 50.00°, *T* = 100(2) K, 79943 reflections collected, 9365 unique, 6701 observed ($I > 2\sigma(I)$) reflections, 657 refined parameters, *R* value 0.0352, *wR*2 = 0.0513, (all data *R* = 0.0702, *wR*2 = 0.0589), *S* = 1.020, minimum and maximum transmission 0.2176 and 0.8342; maximum and minimum residual electron densities +0.413 and -0.382 e $Å^{-3}$. The absolute configuration was established as *(1S,3aR,9aS)-1,8-dihydroxy-1,2,3,3a tetrahydro-cyclopenta[b]chromen-9(9aH)-one* by anomalous dispersion effect (Flack parameter of -0.0032 (65)) in X-ray diffraction measurements which is caused by the presence of bromine atom in the molecule.

**2-(But-3-enyl)-5-hydroxychroman-4-one (43)**




**43**

To a mixture of **34** (5 g, 33 mmol) and 4-penten-1-al (**42**) (6.5 mL, 66 mmol) in dry methanol (200 mL) was added pyrrolidine (6.6 mmol, 0.5 mL) dropwise at room temperature. The reaction mixture was warmed to 50 °C and was further allowed to stir for 24 h at the same temperature. Methanol was removed under reduced pressure and the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (50 mL x 2). The organic layer was washed with 1 N HCl (20 mL), water (20 mL), brine (10 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure to give a crude residue which was further purified by silica gel column chromatography (100-200 mesh) using ethyl acetate: pet ether (2:98) affording 4.3 g of compound **43** as yellow oil (75% yield based on the recovered starting material of 1 g).

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3079, 3019, 2933, 1647, 1628, 1463

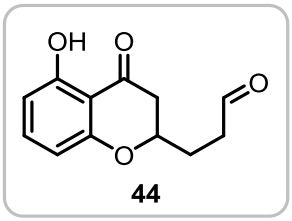
**$^1$H NMR (200 MHz, $CDCl_3$):** δ 11.69 (s, 1H), 7.35 (t, *J* = 8.3 Hz, 1H), 6.44 (d, *J* = 7.3 Hz, 1H), 6.49 (d, *J* = 8.3 Hz, 1H), 5.98-5.71 (m, 1H), 5.18-4.98 (m, 2H), 4.55-4.35 (m, 1H), 2.79-2.66 (m, 2H), 2.38-2.21 (m, 2H), 2.09-1.92 (m, 1H), 1.91-1.70 (m, 1H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 198.5, 162.0, 161.5, 138.2, 137.0, 115.7, 109.1, 108.2, 107.3, 76.6, 42.2, 33.8, 28.9

**MS:** 241 $[M+Na]^+$

**HRMS:** Calculated for $C_{13}H_{14}O_3$ $[M+H]^+$: 219.1021, found 219.1029

**3-(5-Hydroxy-4-oxochroman-2-yl)propanal (44)**




**44**

To a mixture of **43** (0.2 g 0.9 mmol) in dioxane: water (4:1, 10 mL) was added 2,6-lutidine (0.2 mL, 1.8 mmol), $OsO_4$ (cat) (0.01 mmol) and $NaIO_4$ (0.78 g, 3.7 mmol). The reaction was stirred at 25 °C for 1 h. After completion of the reaction (monitored by TLC), water (10 mL) and DCM (4 mL) were added. The organic layer was separated and the water layer was extracted with DCM (4 mL x 3). The combined organic extracts were washed with 1 N HCl (4 mL), brine (2 mL) and dried over $Na_2SO_4$. The solvent was removed under reduced pressure, and the crude mixture was purified on silica gel column (100-200 mesh) using ethyl acetate: pet ether (1:4) affording 0.18 g of pure compound **44** (89%) as an opaque low melting solid.

**Mp:** 68-69 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 2932, 2832, 2731, 1726, 1647, 1629, 1463, 1357

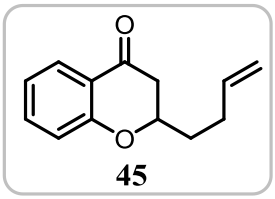
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 11.62 (s, 1H), 9.82 (s, 1H), 7.31 (t, *J* = 8.2 Hz, 1H), 6.46 (d, *J* = 8.2 Hz, 1H), 6.38 (d, *J* = 8.2 Hz, 1H), 4.43 (m, 1H), 2.73 (m, 3H), 2.65 (dd, *J* = 17.0, 3.6 Hz, 1H), 2.08 (m, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 200.7, 197.8, 161.9, 161.0, 138.1, 109.3, 107.9, 107.1, 76.1, 42.1, 39.1, 27.0

**MS:** 243 $[M+Na]^+$

**HRMS**: Calculated for $C_{12}H_{12}O_4$ $[M+H]^+$: 221.0814 found 221.0823.

**2-(but-3-en-1-yl)chroman-4-one (45)**[14g]



**45**

Prepared from **36** and Penten-4-al (**42**) by following the procedure described for the preparation of **43** with 92% yield (brsm) as yellow oil.

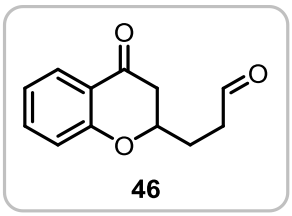
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3413, 1682, 1575

**$^1$H NMR (200 MHz, $CDCl_3$):** δ 7.87 (dd, *J* = 1.7, 7.9 Hz, 1H), 7.47 (dt, *J* = 1.7, 7.7 Hz, 1H), 6.98 (m, 2H), 5.85 (m, 1H), 5.05 (m, 2H), 4.47 (m, 1H), 2.70 (m, 2H), 2.33 (m, 2H), 1.99 (m, 1H), 1.84 (m, 1H)

**MS:** 225 $[M+Na]^+$

The $^1$H NMR compared with the literature values and found to be identical.

**3-(4-oxochroman-2-yl)propanal (46)**



**46**

Prepared from **45** by following the procedure described for the preparation of **44** as yellow oil with 92% yield.

**IR $\upsilon_{max}$(film): $cm^{-1}$** 3020, 2831, 2730, 1724, 1691, 1609, 1465

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 9.84 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.45 (m, 1H), 6.99 (m, 1H), 6.94 (d, *J* = 8.2 Hz, 1H), 4.47 (m, 1H), 2.71 (m, 4H), 2.11 (m, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 200.9, 191.8, 161.1, 136.0, 126.9, 121.4, 120.8, 117.7, 76.6, 42.9, 39.3, 27.2

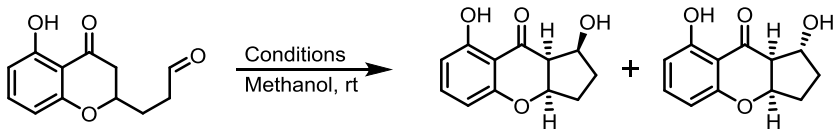
**MS:** 227 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{12}O_3Na$ $[M+Na]^+$: 227.0683 found 227.0669

**General procedure for the intramolecular aldol reaction**

To a solution of **44** (1 mmol) in a specified solvent (Table 4) (5 mL/mmol) at room temperature, was added amine (0.2 mmol) dropwise and the reaction mixture was allowed to stir for required time (Table 4), after considerable amount of starting material is consumed with no further improvement in the product formation (TLC monitoring), the solvent was removed under reduced pressure from the reaction mixture and diluted with ethyl acetate (10 mL). The organic layer was washed with 1 N HCl (5 mL), water (5 mL), brine (2 mL), dried over anhydrous $Na_2SO_4$, concentrated to a crude mixture. The crude diastereomeric mixture was purified by silica gel column chromatography (100-200 mesh) by using 100% DCM to afford pure **11** and **39**.

**Note:** for determining the diastereomeric ratios of **11** and **39**, the crude mixture was initially passed through silica gel column (100-200 mesh) using 100% DCM until **44** (starting material) was removed, then the mixture of **11** and **39** were eluted together by increasing the polarity of the solvent to 10% ethyl acetate: DCM.




**44** **11** **39**

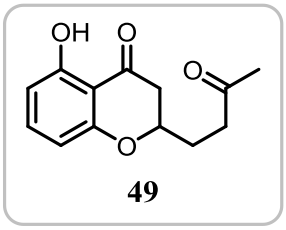
**Graphical representation of optimization of diastereomeric ratio** (Scheme 6 and Figure 8)

| Base/Solvent | Diaportheone-B (**11**) % | Epimer (**39**) % | Yield % |
|---|---|---|---|
| Proline- MeOH | 11.44 | 26.60 | 38 |
| DBU- MeOH | 12.42 | 33.58 | 46 |
| Piperidine- MeOH | 16.50 | 38.50 | 55 |
| Proline Diamine[a]- MeOH | 34.80 | 23.20 | 58 |
| Pyrrolidine- MeOH | 06.00 | 54.00 | 60 |
| Pyridine- MeOH | 38.88 | 33.12 | 72 |
| 2,6-Lutidine- MeOH | 38.00 | 38.00 | 76 |
| Prolinol- MeOH | 26.07 | 52.93 | 79 |
| DABCO-MeOH | 58.60 | 34.10 | 93 |
| DABCO-THF | 54.40 | 25.60 | 80 |
| DABCO- Acetonitrile | 56.80 | 29.40 | 86 |
| DABCO- Benzene | 60.90 | 26.10 | 87 |
| DABCO- Toluene | 46.10 | 49.90 | 96 |

## General procedure for the Wacker oxidation of olefin

A suspension of alkene (5 mmol), $PdCl_2$ (0.5 mmol) and $Cu(OAc)_2.H_2O$ (7.5 mmol) in dimethylacetamide: water (4:1) (75 mL), was placed under oxygen (1atm) and the green colored mixture was allowed to stir for 12h at room temperature. The crude mixture was diluted with ether (10 mL), filtered through Celite bed and washed with an additional amount of ether (30 mL) and the filtrate was poured into water, extracted with ether (3 X 10 mL). The combined organic extracts were washed with brine (1 X 10 mL), dried over $Na_2SO_4$, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (100-200 mesh) using ethyl acetate: pet ether as eluent to afford the pure compound.

**5-hydroxy-2-(3-oxobutyl)chroman-4-one (49)**




**49**

Prepared by following the general procedure for Wacker oxidation described above from compound **43** with 67% yield as white solid.

**Mp:** 82-83 $^{o}$C

**IR $\nu_{max}$(film):** cm$^{-1}$ 3017, 2929, 1716, 1648, 1629, 1578, 1463, 1225, 1054, 771

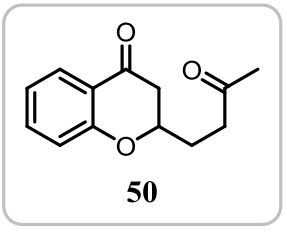
**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 11.67 (s, 1H), 7.34 (t, *J* = 8.3 Hz, 1H), 6.51-6.39 (m, 2H), 4.50-4.36 (m, 1H), 2.77-2.69 (m, 4H), 2.20 (s, 4H), 2.12-1.99 (m, 2H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 207.4, 198.1, 162.0, 161.2, 138.1, 109.3, 108.0, 107.1, 76.2, 42.2, 38.5, 30.0, 28.4

**MS:** 235 $[M+H]^+$

**HRMS:** calculated for $C_{13}H_{14}O_4$ $[M+Na]^+$: 257.0789 found 257.0768.

**2-(3-oxobutyl)chroman-4-one (50)**




**50**

Prepared by following the general procedure for Wacker oxidation described above from compound **45** to furnish **50** with 74% yield.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 1715, 1689, 1608, 1465, 1215

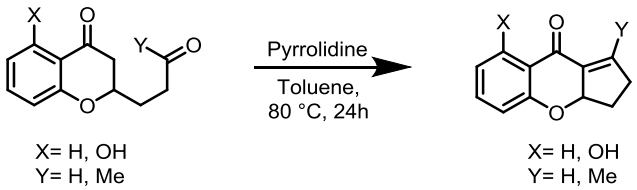
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.86 (m, 1H), 7.46 (m, 1H), 7.0 (m, 1H), 6.94 (m, 1H), 4.46 (m, 1H), 2.75-2.67 (m, 4H), 2.20 (s, 3H), 2.05 (m, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 207.4, 192.0, 161.3, 136.0, 127.0, 121.4, 120.9, 117.8, 76.8, 43.0, 38.7, 30.0, 28.6

**MS:** 219 $[M+H]^+$

**HRMS**: Calculated for $C_{13}H_{14}O_3Na$ $[M+Na]^+$: 241.0840 found 241.0848

**General procedure for the synthesis of substituted 3,3a-dihydrocyclopenta[b]chromen-9(2H)-ones**



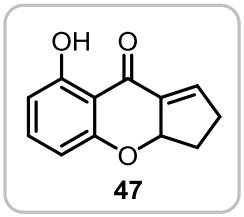

**Procedure-I**

To a solution of aldehyde (1.0 mmol) in dry toluene (20 mL) at room temperature, was added pyrrolidine (0.2 mmol) dropwise. The reaction mixture was heated to 80 $^{o}$C and stirred for 24h. After completion of the reaction, the solvent was removed under reduced pressure from the reaction mixture and diluted with ethyl acetate. The organic layer was washed with 1N HCl (5 mL), water (10 mL), brine (2 mL), dried over $Na_2SO_4$, concentrated under reduced pressure and the crude residue was purified by silica gel column chromatography (100-200 mesh) eluting with ethyl acetate: pet ether as eluent to afford a pure compound.

**Procedure-II**

To a solution of aldehyde (1.0 mmol) in dry methanol (20 mL) at room temperature, was added pyrrolidine (0.5 mmol) dropwise. The reaction mixture was stirred at room temperature for 8h. After completion of the reaction, methanol was removed under reduced pressure and diluted with ethyl acetate. The organic layer was washed with 1N HCl (5 mL), water (10 mL), brine (2 mL), dried over $Na_2SO_4$, concentrated under reduced pressure and the crude residue was purified using silica gel column chromatography (100-200 mesh; ethyl acetate: pet ether) to afford the pure compound.

**8-hydroxy-3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (47)**




**47**

Prepared by following the general procedure described above (Procedure I/II) from compound **44** in 90% yield as yellow viscous mass.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 2982, 2932, 2867, 1635, 1619, 1459

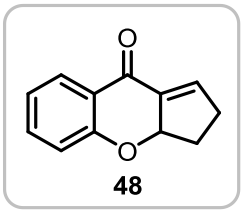
**$^1$H NMR (200 MHz, $CDCl_3$):** δ 12.14 (s, 1H), 7.34 (t, *J* = 8.3 Hz, 1H), 7.0 (m, 1H), 6.52 (dd, *J* = 8.3, 0.8 Hz, 1H), 6.42 (dd, *J* = 8.3, 0.8 Hz, 1H), 5.41 (m, 1H), 2.84-2.43 (m, 3H), 2.27-2.09 (m, 1H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 186.0, 163.3, 161.3, 142.4, 138.2, 137.8, 109.7, 109.4, 107.7, 84.0, 31.6, 31.2

**MS:** 225 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{11}O_3$ $[M+H]^+$: 203.0708 found 203.0720

**3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (48)**[14f]




**48**

Prepared from **46** (50 mg, 0.24 mmol) and pyrrolidine (0.04 mL, 0.05 mmol) in toluene (2 mL) by following the general procedure described above yielding **48** (40 mg, 87%) as a yellow viscous mass.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 2979, 3019, 1667, 1638, 1607, 1462

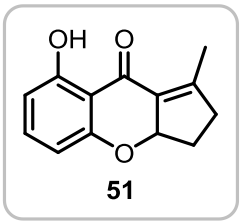
**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 7.96 (dd, *J* = 7.83, 1.77 Hz, 1H), 7.51-7.42 (ddd, *J* = 8.3, 7.2, 1.7 Hz, 1H), 7.05 (m, 1H), 6.97 (m, 2H), 5.46 (m, 1H), 2.82-2.65 (m, 2H), 2.62-2.43 (m, 1H), 2.30-2.11 (m, 1H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 181.0, 161.2, 141.3, 139.0, 135.9, 127.6, 122.8, 121.6, 118.3, 84.5, 31.7, 30.9

**MS:** 209 $[M+Na]^+$

**HRMS:** Calculated for $C_{12}H_{11}O_2$ $[M+H]^+$: 187.0759 found 187.0754

**8-hydroxy-1-methyl-3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (51)**




**51**

Prepared by following the general procedure described above (procedure I) from compound **49** in 69% yield as yellow solid.

**Mp**: 100-102 ºC

**IR $\nu_{max}$ (film):** $cm^{-1}$ 3020, 2981, 2946, 2867, 2840, 1655, 1633, 1610

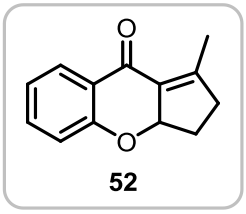
**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 12.40 (s, 1H), 7.30 (t, *J* = 8.2 Hz, 1H), 6.49 (d, *J* = 8.2 Hz, 1H), 6.38 (d, *J* = 8.2 Hz, 1H), 5.38 (br. s., 1H), 2.69-2.48 (m, 3H), 2.27 (s, 3H), 2.16-2.05 (m, 1H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 187.8, 163.3, 161.1, 158.8, 137.6, 129.1, 109.9, 109.6, 107.4, 85.1, 37.1, 29.9, 16.6

**MS:** 239 $[M+Na]^+$

**HRMS:** Calculated for $C_{13}H_{13}O_3$ $[M+H]^+$: 217.0864 found 217.0859

**1-Methyl-3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (52)**



**52**

Prepared from **50** (500 mg, 0.23 mmol) and pyrrolidine (0.37 mL, 0.46 mmol) in toluene (10 mL) by following the general procedure described above yielding **52** (330 mg, 72%) as a yellow crystalline solid.

**Mp**: 98-100 °C

**IR $\nu_{max}$ (film):** $cm^{-1}$ 3019, 2979, 2945, 2867, 2842, 1669, 1638, 1605, 1462

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 7.97 (m, 1H), 7.44 (m, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 5.43 (m, 1H), 2.60-2.52 (m, 3H), 2.26 (s, 3H), 2.15 (m, 1H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 182.3, 160.9, 157.2, 135.3, 130.4, 127.4, 123.6, 121.4, 118.0, 85.6, 36.9, 29.9, 16.4

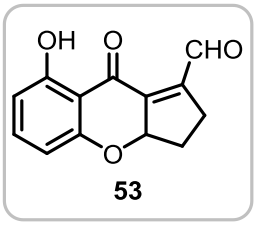
**MS:** 223 $[M+Na]^+$

**HRMS**: Calculated for $C_{13}H_{13}O_2$ $[M+H]^+$: 201.0915 found 201.0930

## General procedure for the $SeO_2$ mediated allylic oxidation

To a stirred solution of compound (0.25 mmol) in 1, 4-dioxane (2 mL) was added Selenium dioxide (0.4 mmol) and the reaction mixture was refluxed for 3h. After completion of reaction, the reaction mixture was allowed to cool to room temperature. The deposited selenium metal was filtered off and residue was washed with dioxane (10 mL). The filtrate was concentrated and diluted with ethyl acetate (5 mL) and water (5 mL). The organic layer was separated and aqueous layer was extracted with ethyl acetate (2 x 2 mL) and the combined organic extracts were washed with saturated solution of $NaHCO_3$ (2 mL), water (2 mL), brine (2 mL), dried over $Na_2SO_4$ and concentrated to a crude mixture which was further purified by silica gel column chromatography by using ethyl acetate: pet ether to afford pure compound.

**8-hydroxy-9-oxo-2,3,3a,9-tetrahydrocyclopenta[b]chromene-1-carbaldehyde (53)**




**53**

Compound **53** prepared by following the $SeO_2$ mediated allylic oxidation general procedure described above from compound **51** in 68% yield as yellow solid.

**Mp**: 180-182 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 2954, 2874, 1715, 1681, 1634, 1615, 1459

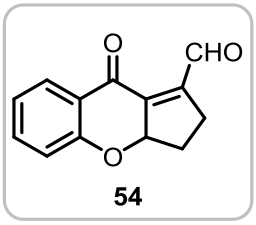
**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 11.96 (s, 1H), 10.57 (s, 1H), 7.42 (t, *J* = 8.2 Hz, 1H), 6.57 (dd, *J* = 8.2, 0.9 Hz, 1H), 6.47 (dd, *J* = 8.2, 0.6 Hz, 1H), 5.55 (m, 1H), 2.96 (m, 1H), 2.68 (m, 2H), 2.20 (m, 1H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 189.8, 185.4, 163.6, 161.0, 149.8, 143.7, 139.4, 110.3, 109.9, 107.8, 84.8, 30.1, 28.1

**MS:** 253 $[M+Na]^+$

**HRMS**: Calculated for calculated for $C_{13}H_{11}O_4$ $[M+H]^+$: 231.0657 found 231.0651

**9-oxo-2,3,3a,9-tetrahydrocyclopenta[b]chromene-1-carbaldehyde (54)**




**54**

Prepared from **52** (50 mg, 0.25 mmol) and $SeO_2$ (44 mg, 0.40 mmol) by following the general procedure for $SeO_2$ mediated allylic oxidation described above yielding **54** (40 mg, 75%) as a yellow solid.

**Mp:** 118-120 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 1680, 1633, 1608

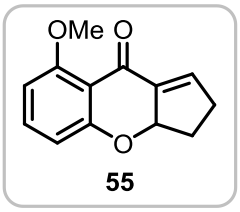
**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 10.57 (s, 1H), 8.02 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.55 (m, 1H), 7.09 (m, 1H), 7.02 (m, 1H), 5.58 (m, 1H), 3.03-2.88 (m, 1H), 2.75-2.66 (m, 2H), 2.27-2.16 (m, 1H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 190.3, 180.6, 161.1, 149.0, 144.9, 136.9, 127.7, 122.6, 122.3, 118.4, 85.2, 30.0, 27.8

**MS:** 237 $[M+Na]^+$

**HRMS:** Calculated for $C_{13}H_{11}O_3$ [M+H]+: 215.0708 found 215.0697

**8-methoxy-3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (55)**




**55**

To a solution of compound **47** (100 mg, 0.46 mmol) in acetone (2 mL) was added an oven dried potassium carbonate (254 mg, 1.85 mmol) and methyl iodide (0.12 mL, 1.85 mmol) and the reaction mixture was stirred at room temperature for 10 h. After completion of reaction, acetone was evaporated and residue was diluted with ethyl acetate (2 mL). The organic layer washed with water, brine, dried over $Na_2SO_4$, concentrated and purified by column chromatography by eluting the crude mixture with ethyl acetate: pet ether (8:92) afforded 90 mg of pure compound **55** (85%) as a white powdery solid.

**Mp:** 176-178 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3019, 3931, 1664, 1636, 1602, 1471

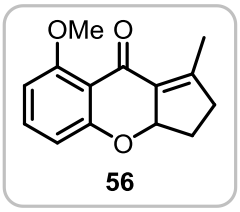
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 7.37 (t, *J* = 8.2 Hz, 1H), 6.89 (m, 1H), 6.57 (d, *J* = 8.2 Hz, 1H), 6.53 (d, *J* = 8.2 Hz, 1H), 5.35 (m, 1H), 3.91 (s, 3H), 2.73-2.59 (m, 2H), 2.54-2.45 (m, 1H), 2.20-2.11 (m, 1H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 180.0, 163.1, 161.6, 140.5, 140.1, 135.7, 113.5, 110.5, 104.4, 83.9, 56.2, 31.5, 30.8

**MS:** 217 $[M+H]^+$

**HRMS:** Calculated for $C_{13}H_{12}O_3Na$ $[M+Na]^+$: 239.0683 found 239.0658.

**8-methoxy-1-methyl-3,3a-dihydrocyclopenta[b]chromen-9(2H)-one (56)**




**56**

The compound was prepared from **51** by following the procedure used for the synthesis of **56** in yield 88% as yellow solid.

**Mp:** 145-147 $^{o}$C

**IR $\upsilon_{max}$ (film):** cm$^{-1}$ 3019, 2944, 2842, 1667, 1634, 1601, 1470

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.31 (t, *J* = 8.2 Hz, 1H), 6.53 (m, 2H), 5.31 (m, 1H), 3.90 (s, 3H), 2.57-2.45 (m, 3H), 2.24 (s, 3H), 2.11-2.01 (m, 1H)

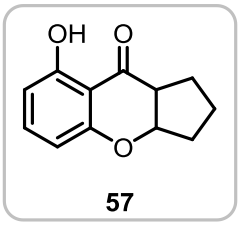
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 181.7, 162.8, 161.5, 156.6, 135.2, 131.0, 114.2, 110.3, 104.3, 85.2, 56.1, 37.1, 29.5, 16.3

**MS:** 231 $[M+H]^+$

**HRMS:** Calculated for $C_{14}H_{14}O_3Na$ $[M+Na]^+$: 253.0840 found 253.0830

**General procedure for the reduction of 3,3a-dihydrocyclopenta- [b]chromen-9(2H)-ones:** To a solution of chromenone (1 mmol) in ethyl acetate (3 mL) was added a catalytic amount of 10% Pd on activated charcoal (10 mg) and the reaction mixture was stirred under $H_2$ (1 atm) for 0.5-3 h. After completion of reaction, the reaction mixture was filtered through a short celite bed. The filtrate was concentrated under reduced pressure and the crude mixture was purified by silica gel column chromatography (100-200 mesh) eluting with ethyl acetate: pet ether to give pure compound.

**8-hydroxy-2,3,3a,9a-tetrahydrocyclopenta[b]chromen-9(1H)-one (57)**




**57**

Prepared from **47** (100 mg, 0.49 mmol) and 5% Pd/C (cat) under hydrogen atmosphere (1 atm) by following the general procedure described above yielding **57** (87 mg, 86%) as a colourless mass.

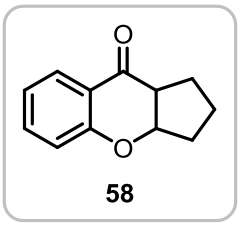
**IR $\nu_{max}$ (film):** $cm^{-1}$ 3020, 2929, 2877, 1641, 1578, 1555, 1462

**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 11.90 (s, 1H), 7.33 (t, *J* = 8.3 Hz, 1H), 6.47 (dd, *J* = 8.3, 0.8 Hz, 1H), 6.37 (dd, *J* = 8.3, 0.8 Hz, 1H), 4.91 (m, 1H), 2.73 (m, 1H), 2.22-2.12 (m, 2H), 2.04-1.89 (m, 4H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 200.7, 162.4, 160.6, 138.3, 109.1, 107.3, 106.2, 82.9, 50.4, 32.9, 28.0, 22.5

**HRMS:** Calculated for $C_{12}H_{12}O_3$ [M+Na]+: 227.0683 found 227.0668

**2,3,3a,9a-tetrahydrocyclopenta[b]chromen-9(1H)-one (58)**[18]




**58**

Prepared from **48** (80 mg, 0.43 mmol) and 10% Pd/C (cat) under hydrogen atmosphere (1 atm) by following the general procedure described above yielding **58** (79 mg, 98%) as a colourless viscous mass. The spectral data matched according to the literature reported data.

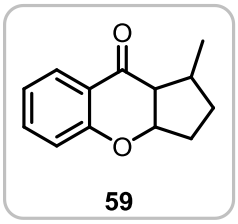
**IR $\upsilon_{max}$ (film):** $cm^{-1}$ 3310, 2936, 1735, 1710, 1690

**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 7.88 (m, 1H), 7.46 (m, 1H), 7.0 (m, 1H), 6.91 (m, 1H), 4.94 (m, 1H), 2.74 (m, 1H), 2.16 (m, 2H), 2.03-1.91 (m, 3H), 1.82 (m, 1H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 194.6, 160.6, 136.0, 127.1, 121.2, 119.1, 118.0, 83.2, 51.1, 33.0, 27.5, 22.4

**MS:** 189 $[M+H]^+$

**1-methyl-2,3,3a,9a-tetrahydrocyclopenta[b]chromen-9(1H)-one (59)**[19]



**59**

Prepared from **52** (100 mg, 0.50 mmol) and 5% Pd/C (cat) under hydrogen atmosphere (1 atm) by following the general procedure described above yielding **59** (96 mg, 95%) as a colourless liquid. The spectral data is in complete agreement with the literature reported data.

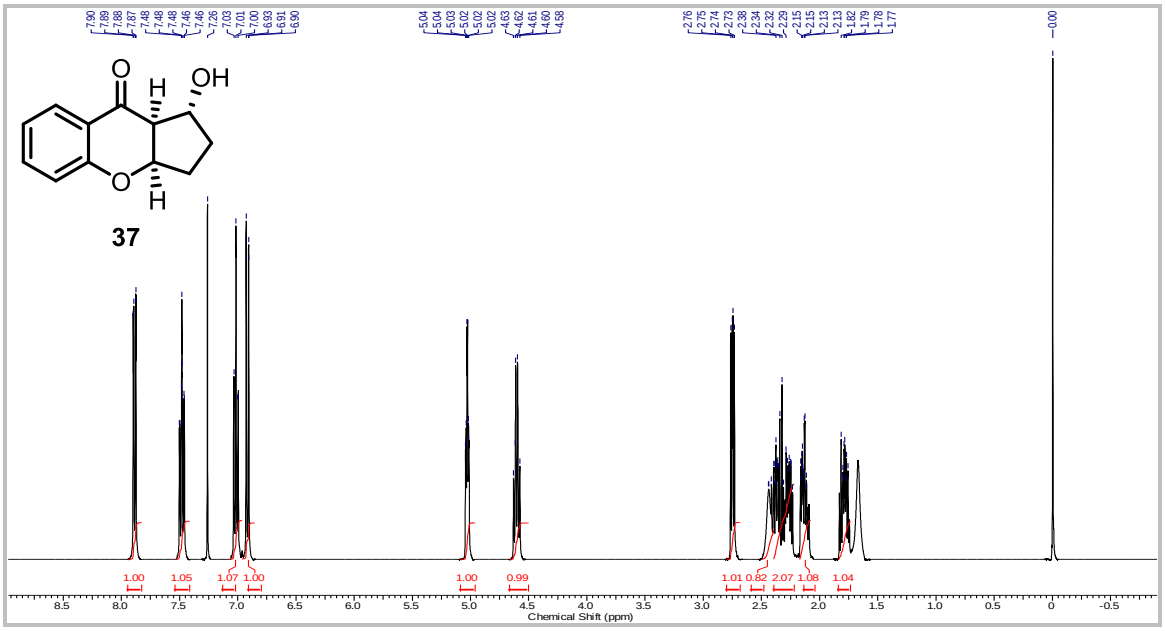
**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 7.89 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.46 (dt, *J* = 1.8, 7.8 Hz, 1H), 7.05-6.86 (m, 2H), 4.94 (t, *J* = 4.4 Hz, 1H), 2.91-2.58 (m, 2H), 2.37-1.89 (m, 3H), 1.82-1.65 (m, 1H), 0.88 (d, *J* = 7.1 Hz, 3H)

**$^{13}$C NMR (50 MHz, $CDCl_3$):** δ 194.5, 161.4, 136.0, 126.5, 121.5, 121.2, 118.1, 83.4, 54.0, 36.0, 33.3, 32.4, 18.3

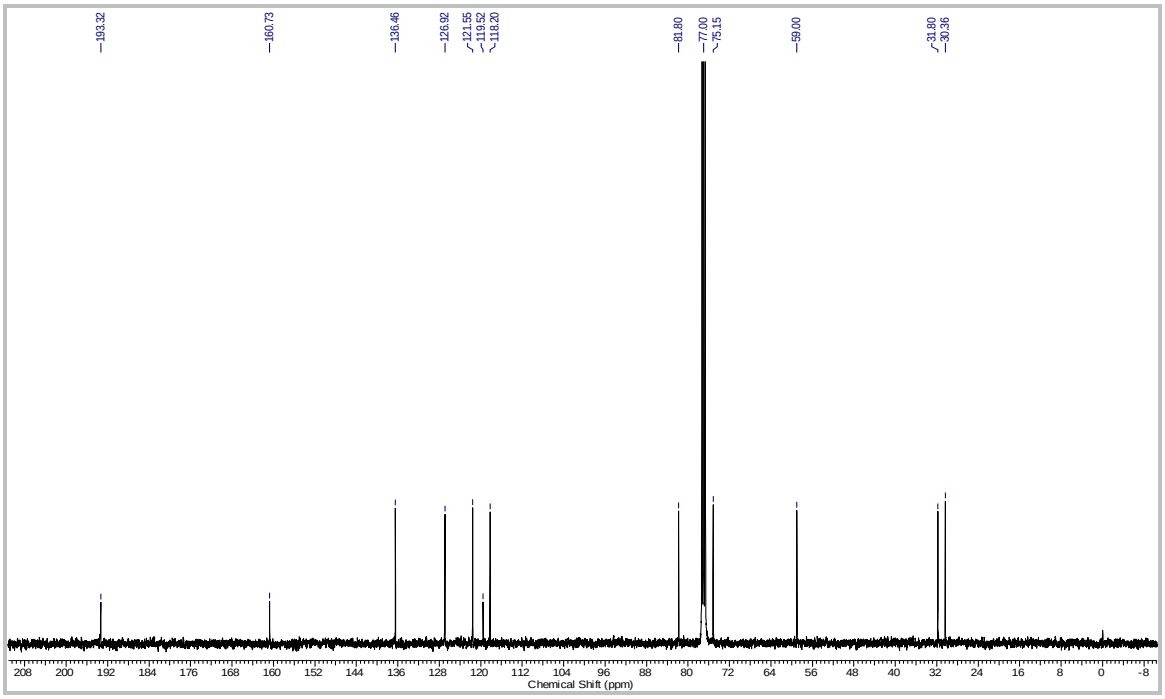
**MS:** 203 $[M+H]^+$

## 3.3.6. Spectra

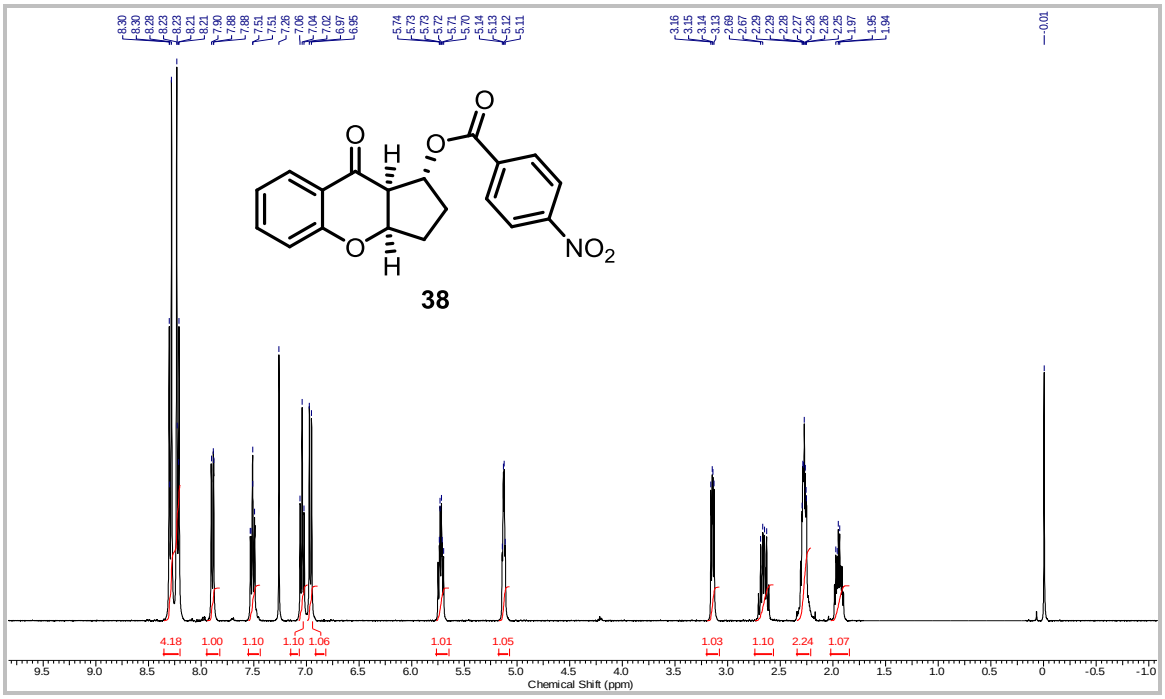
**$^{1}$H NMR (400 MHz, $CDCl_3$) of compound 37**



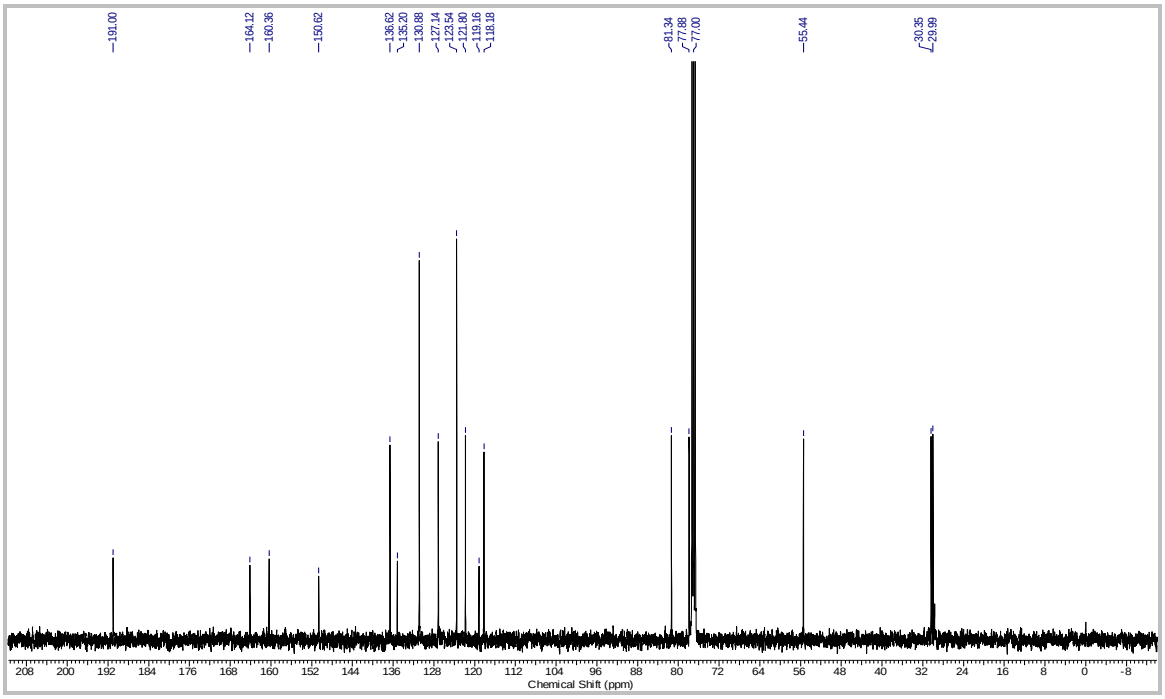

**$^{13}$C NMR (100 MHz, $CDCl_3$) of compound 37**



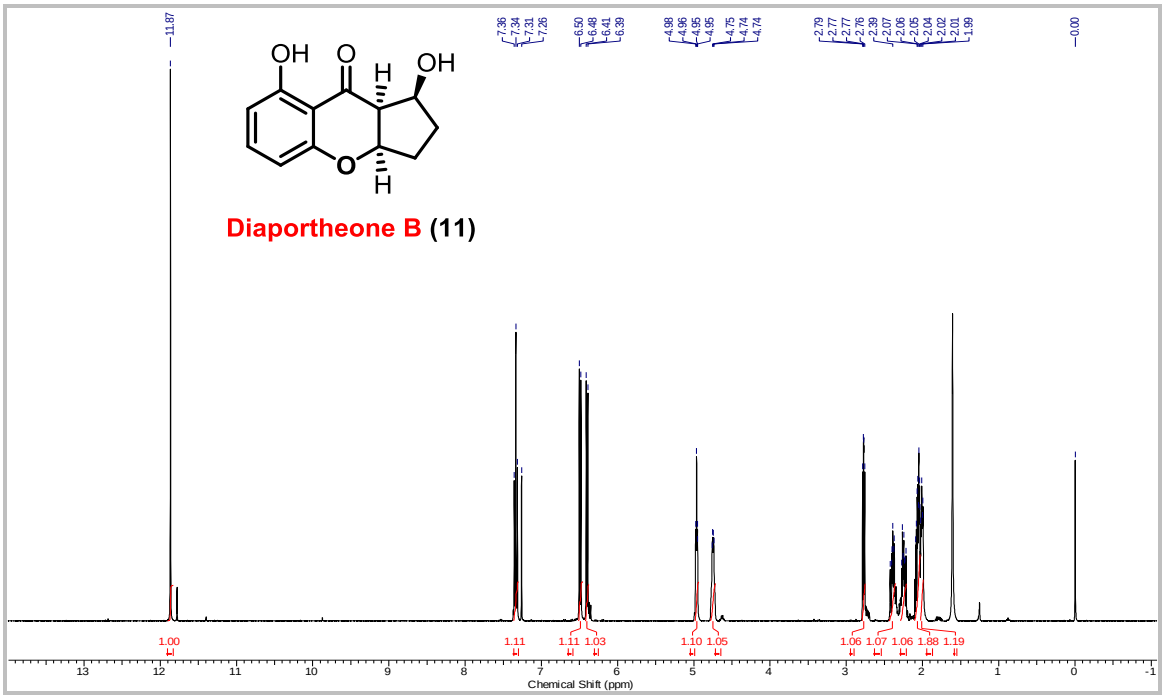
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 38



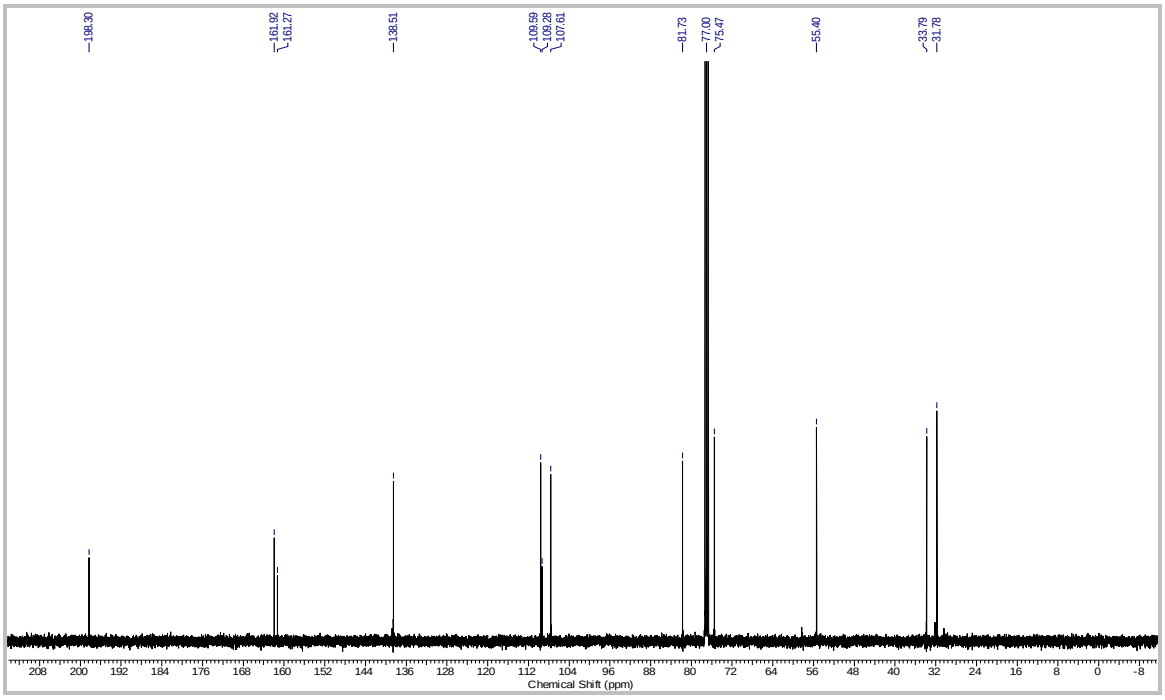

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 38



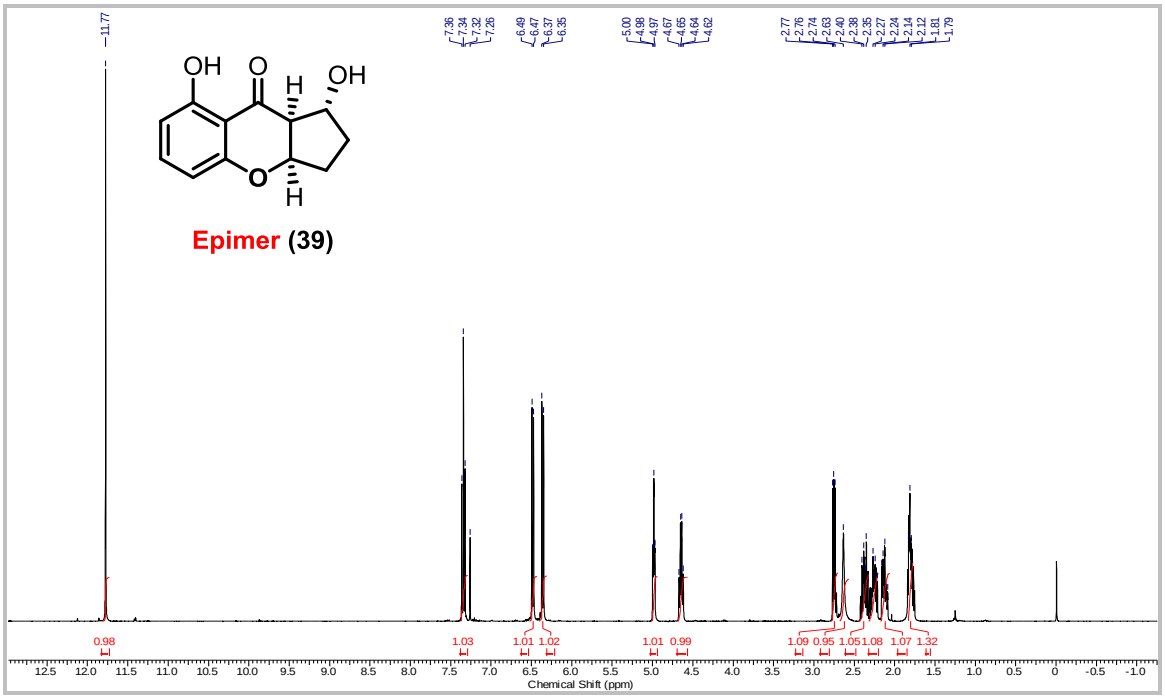
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 11



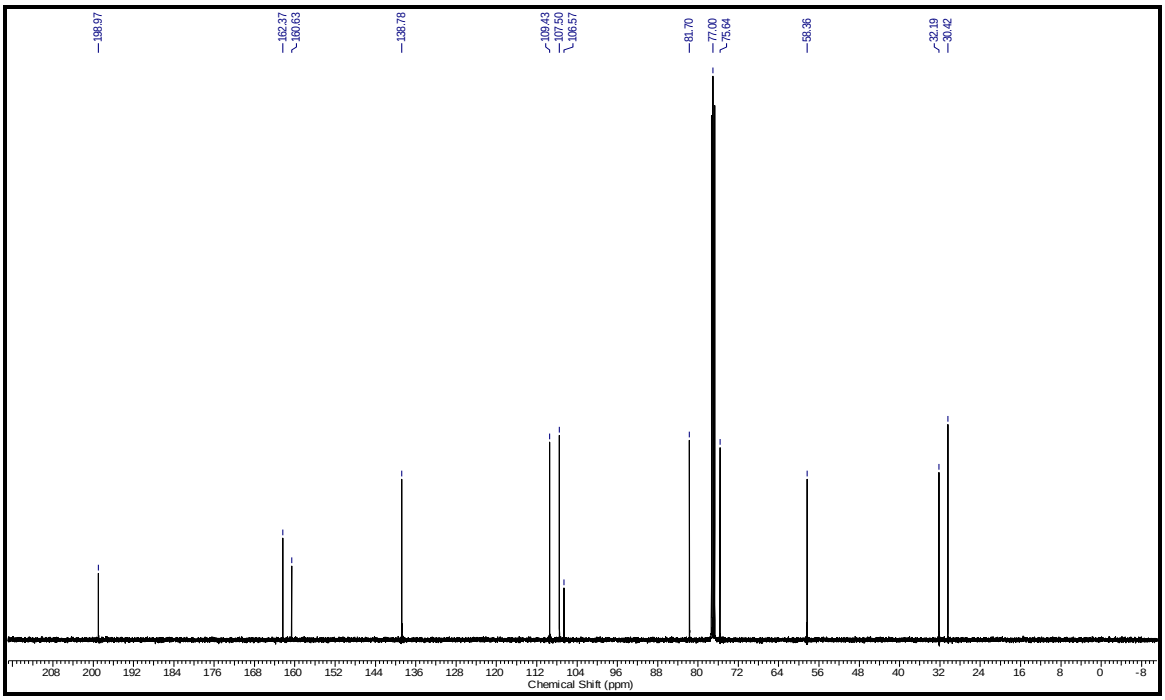

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 11



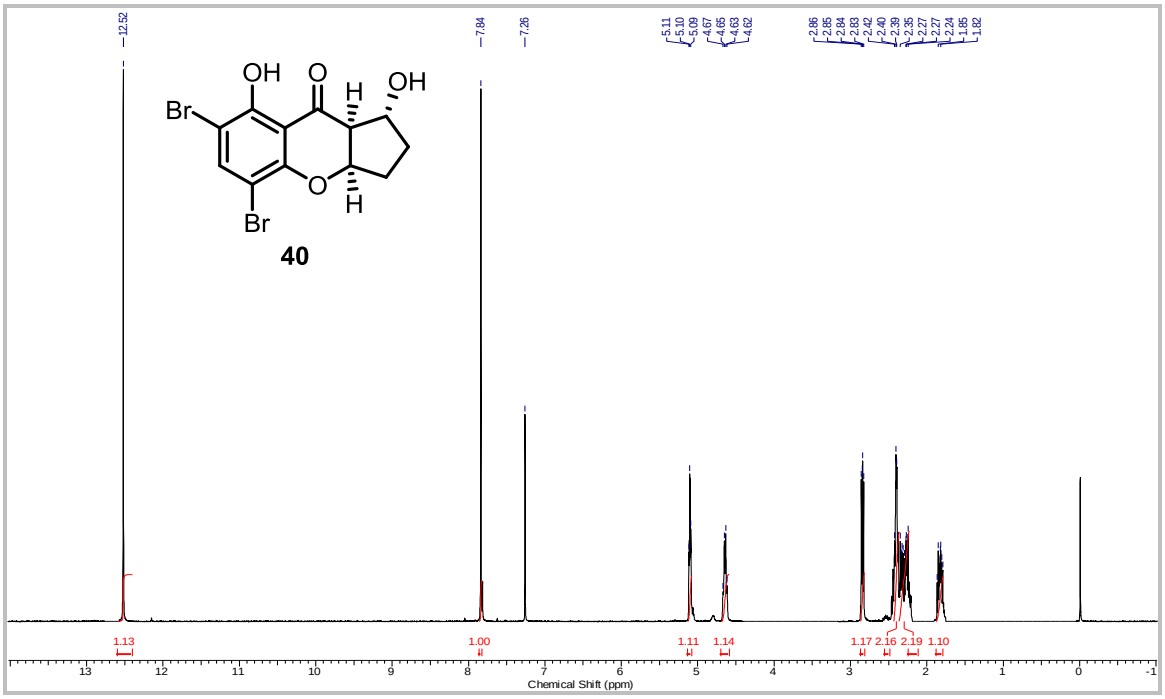
## $^{1}H$ NMR (400 MHz, $CDCl_3$) of compound 39



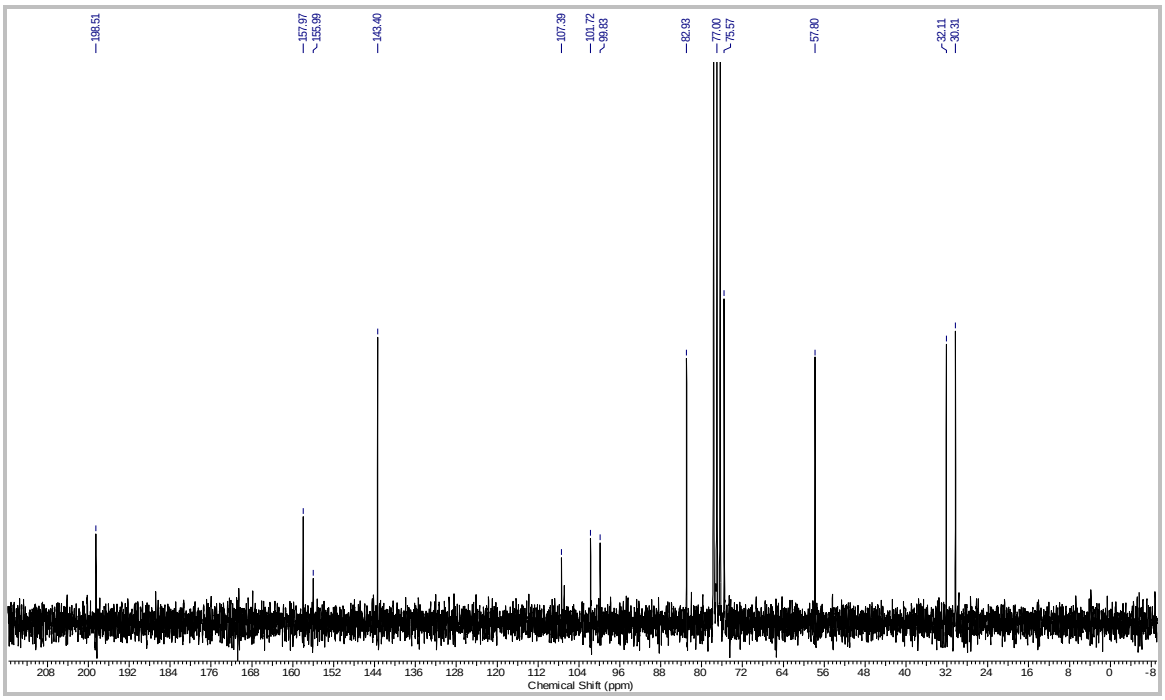

## $^{13}C$ NMR (100 MHz, $CDCl_3$) of compound 39



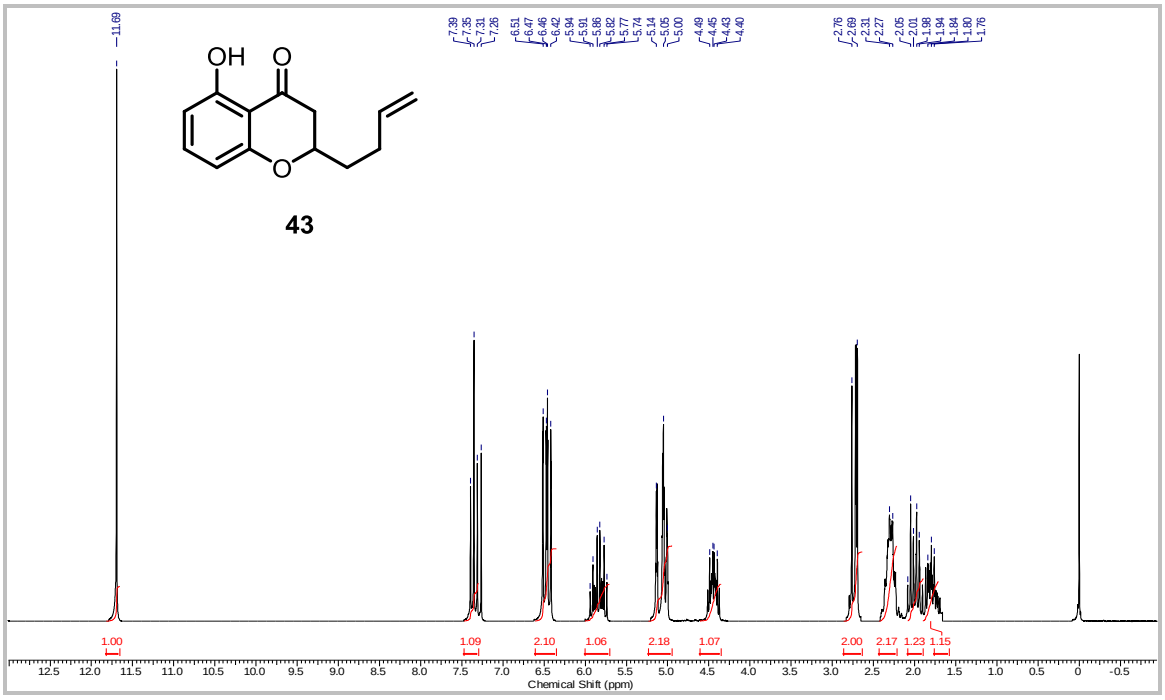
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 40



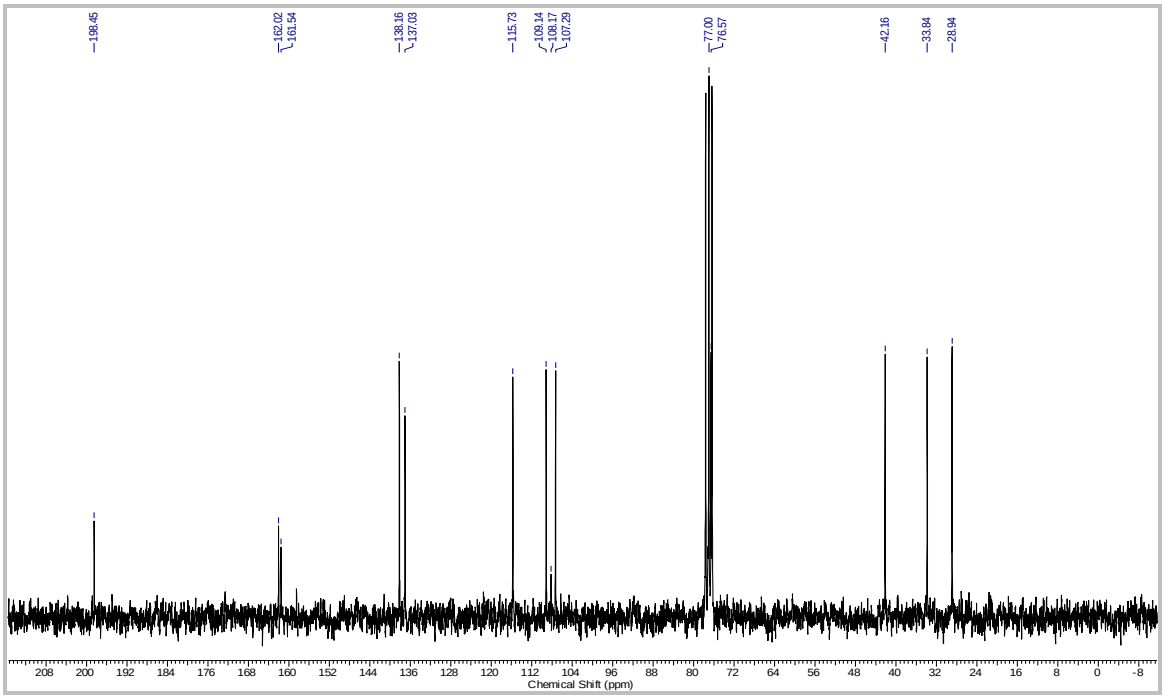

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 40



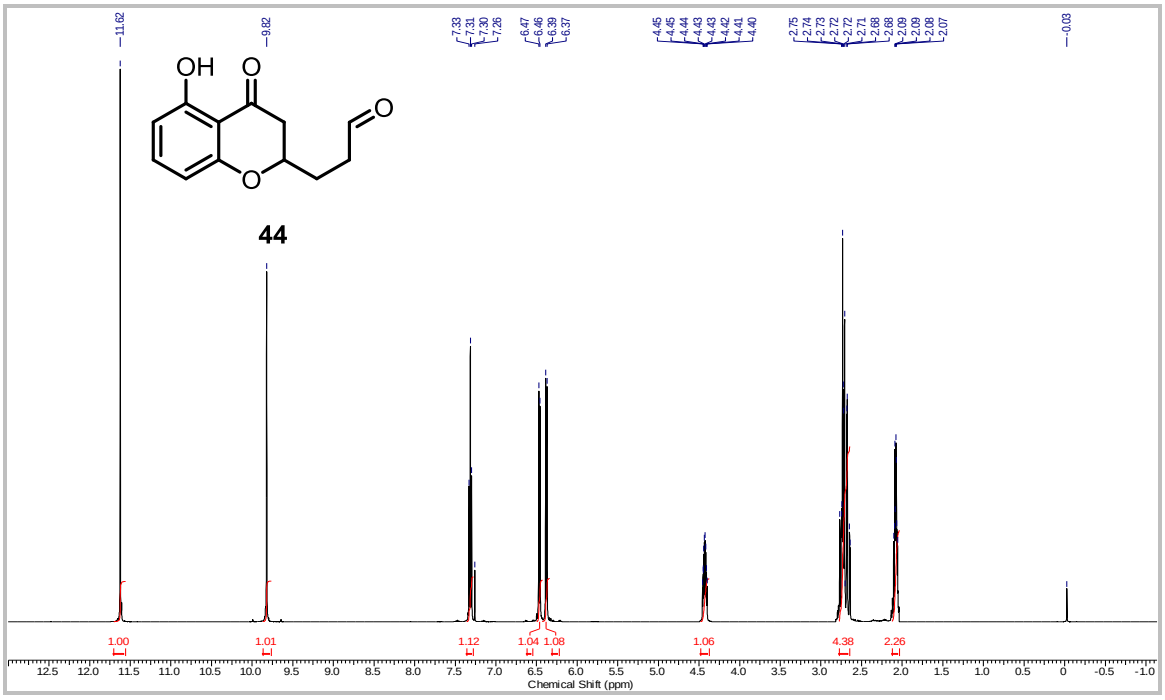
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 43



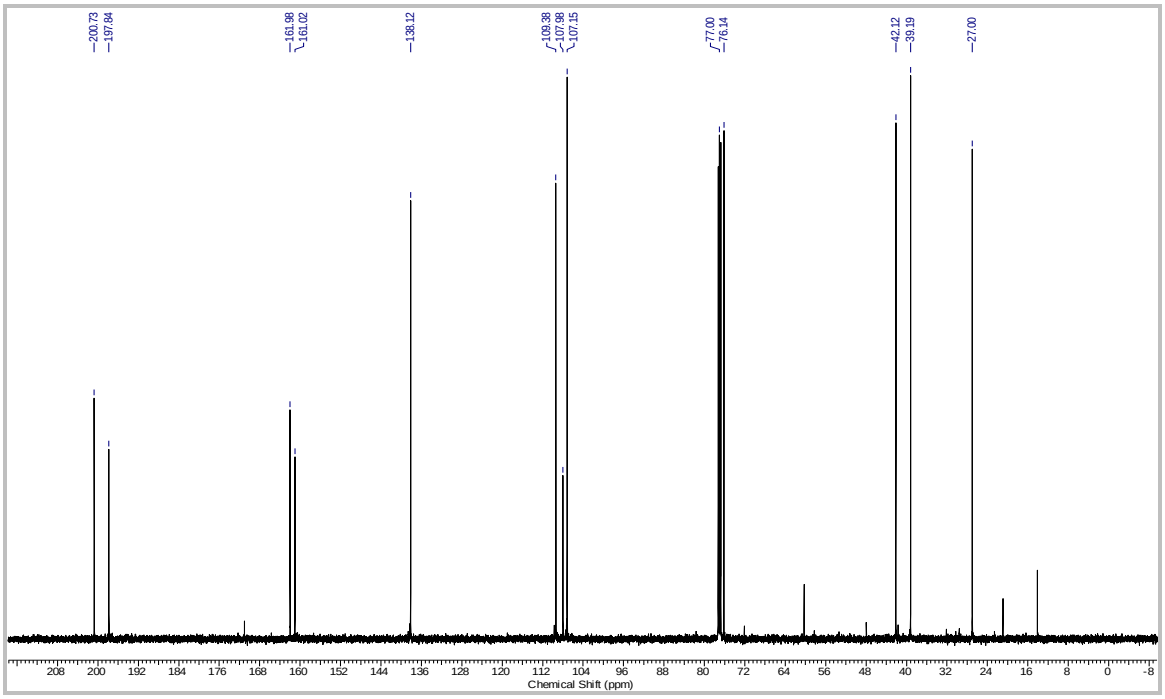

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 43



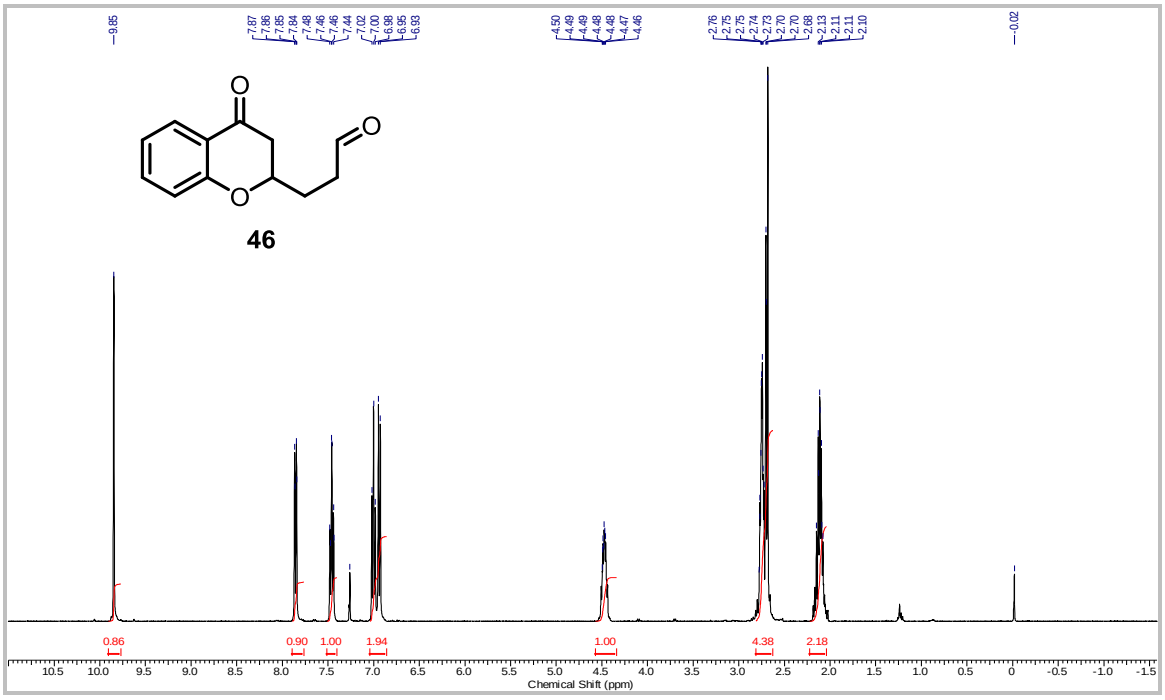
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 44



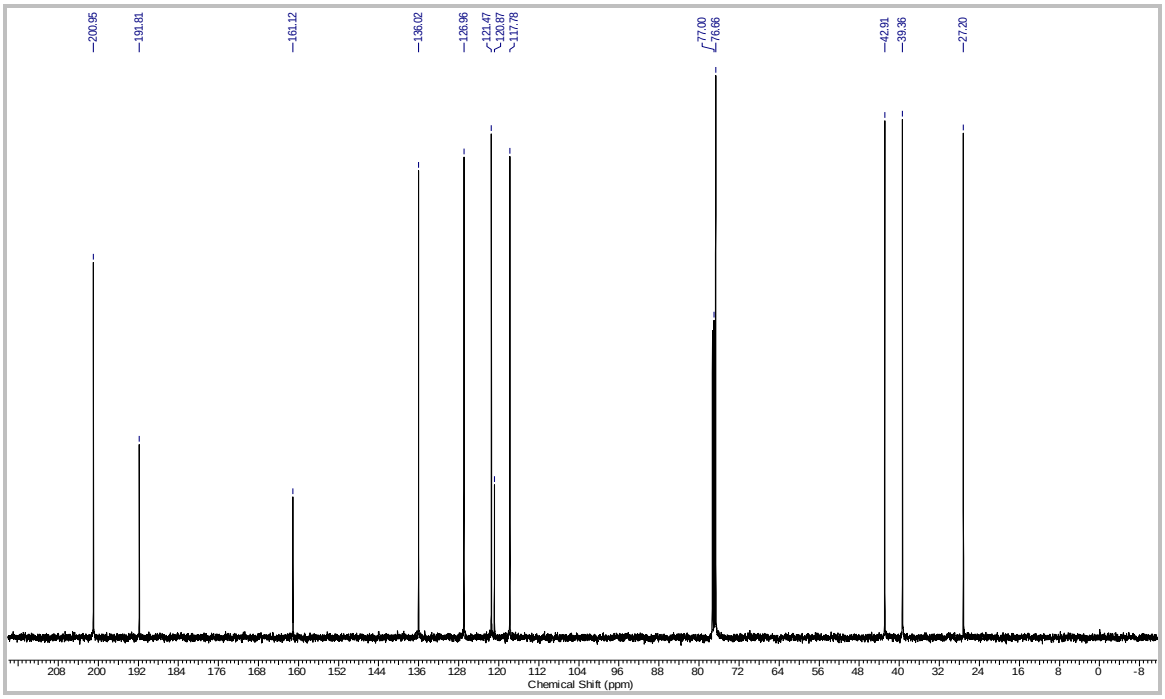

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 44



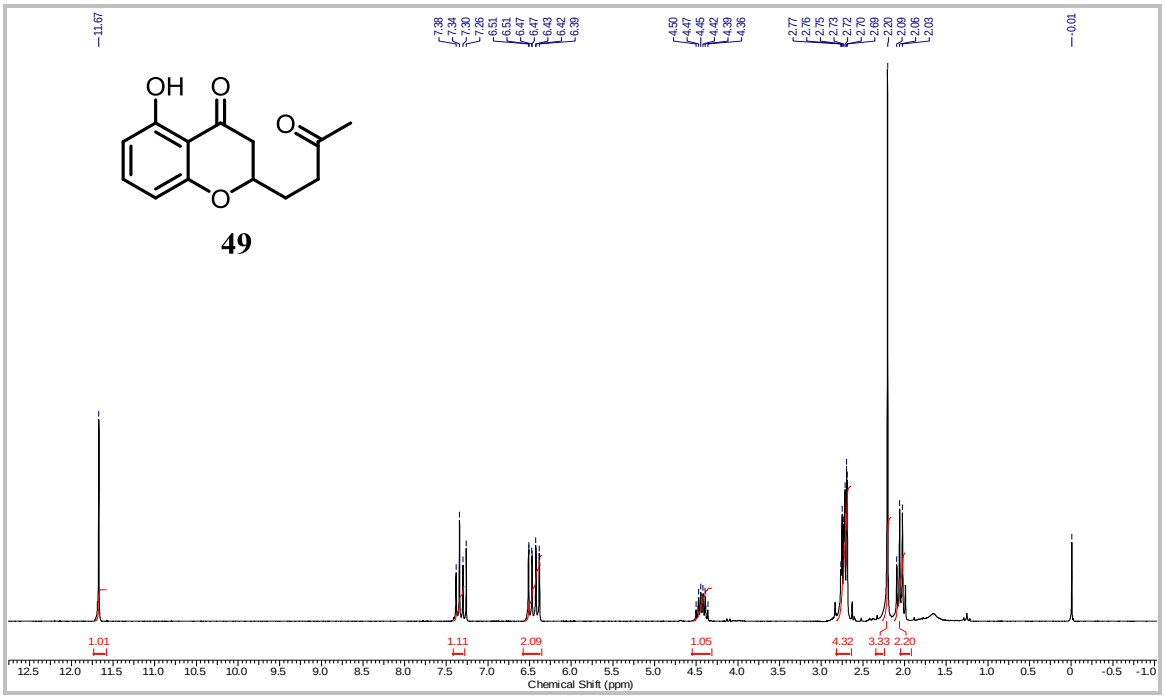
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 46



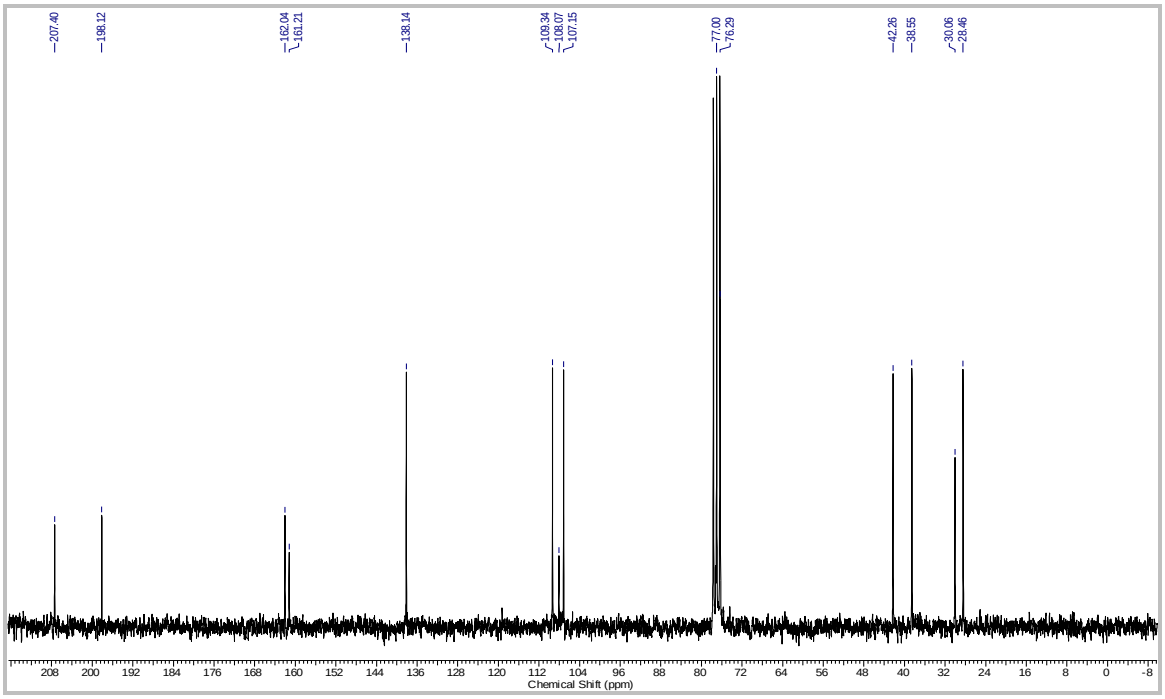

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 46



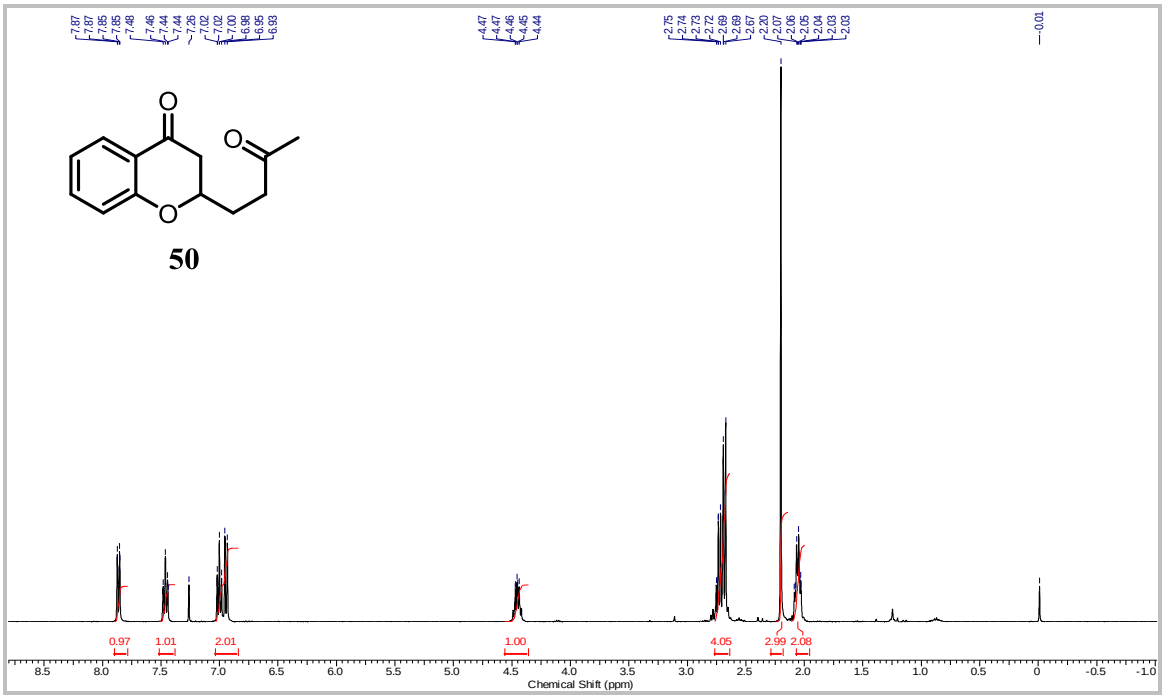
## $^{1}$H NMR (200 MHz $CDCl_3$) of compound 49



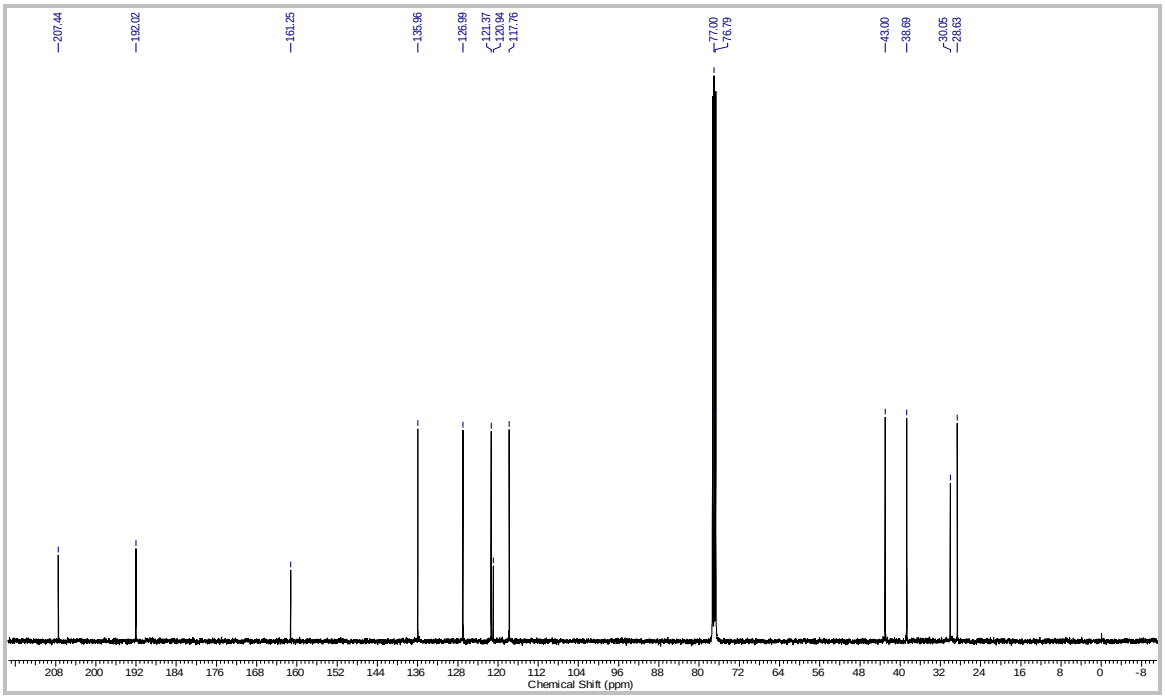

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 49



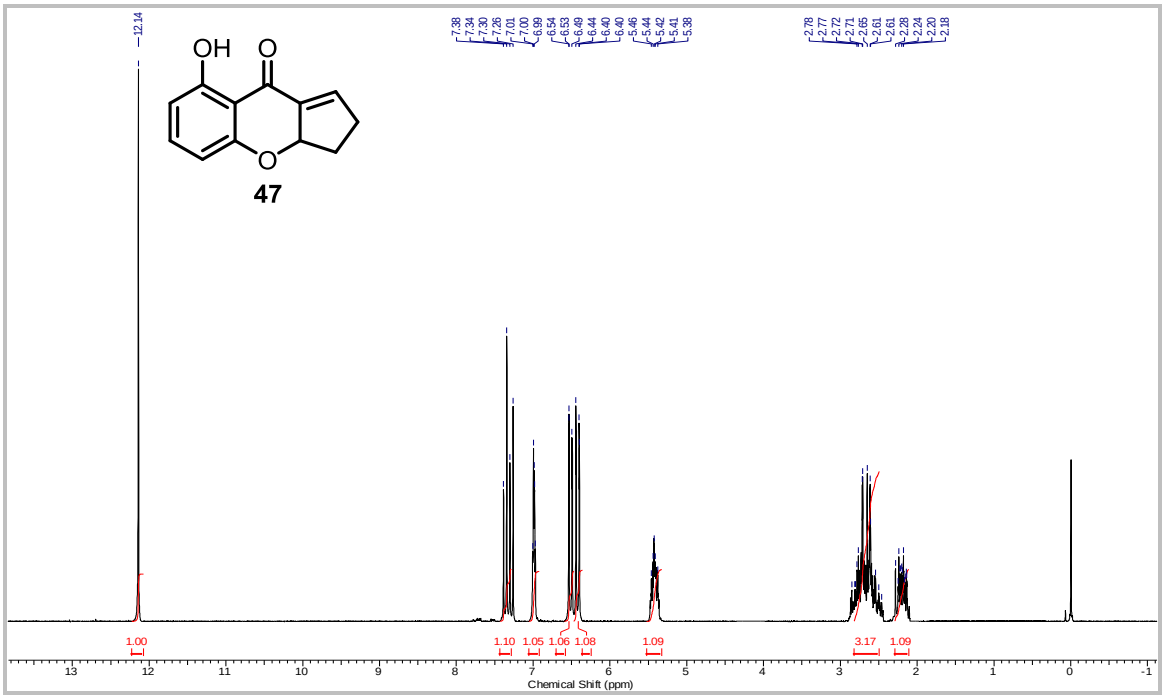
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 50



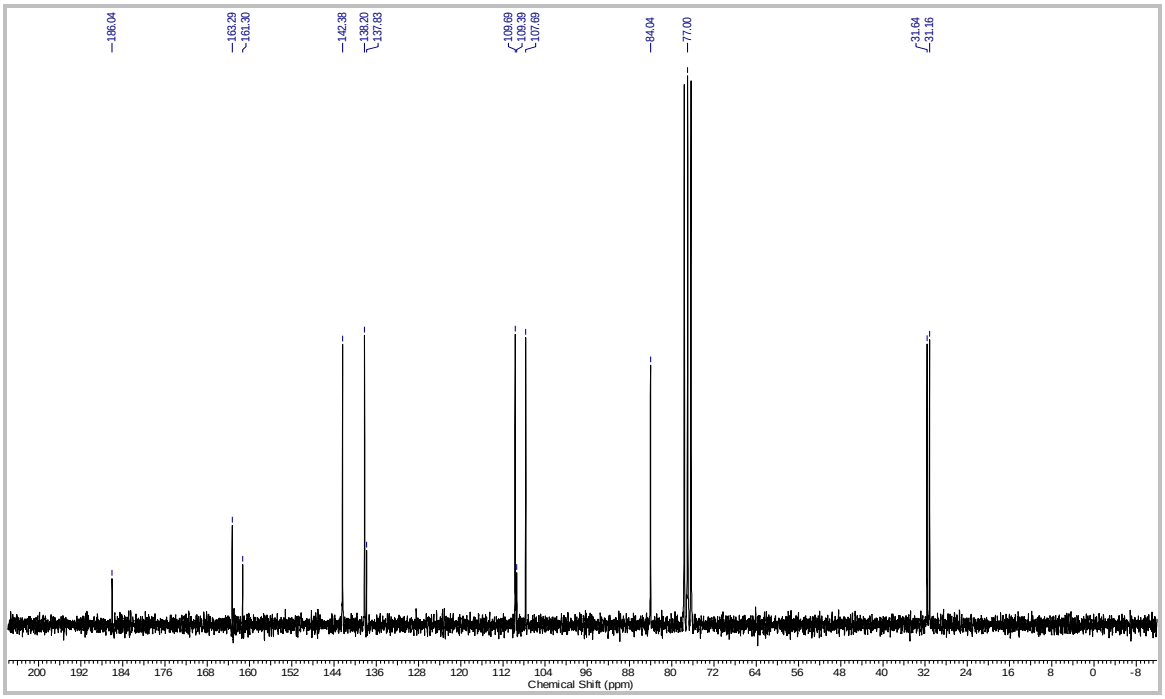

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 50



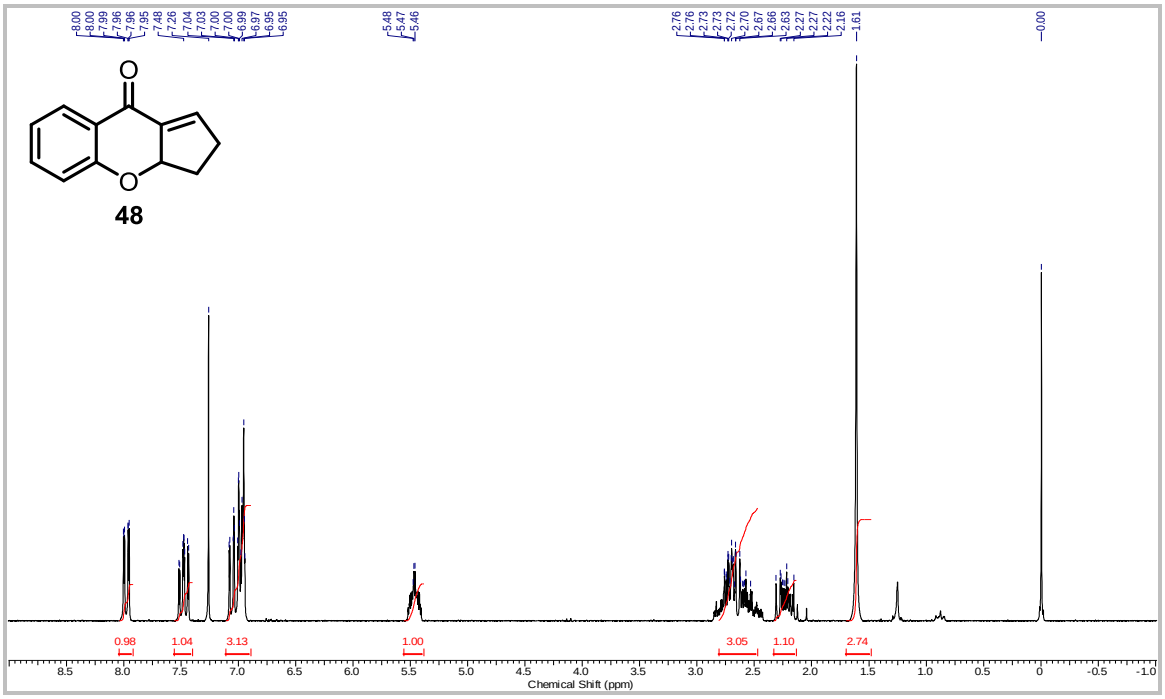
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 47



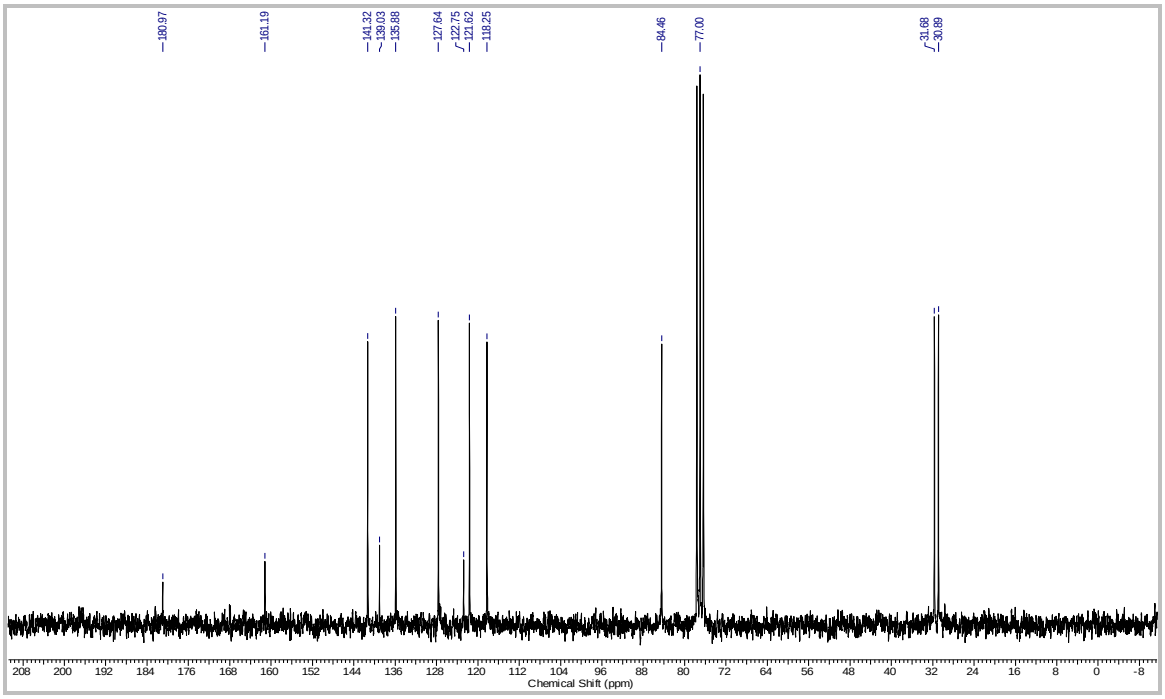

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 47



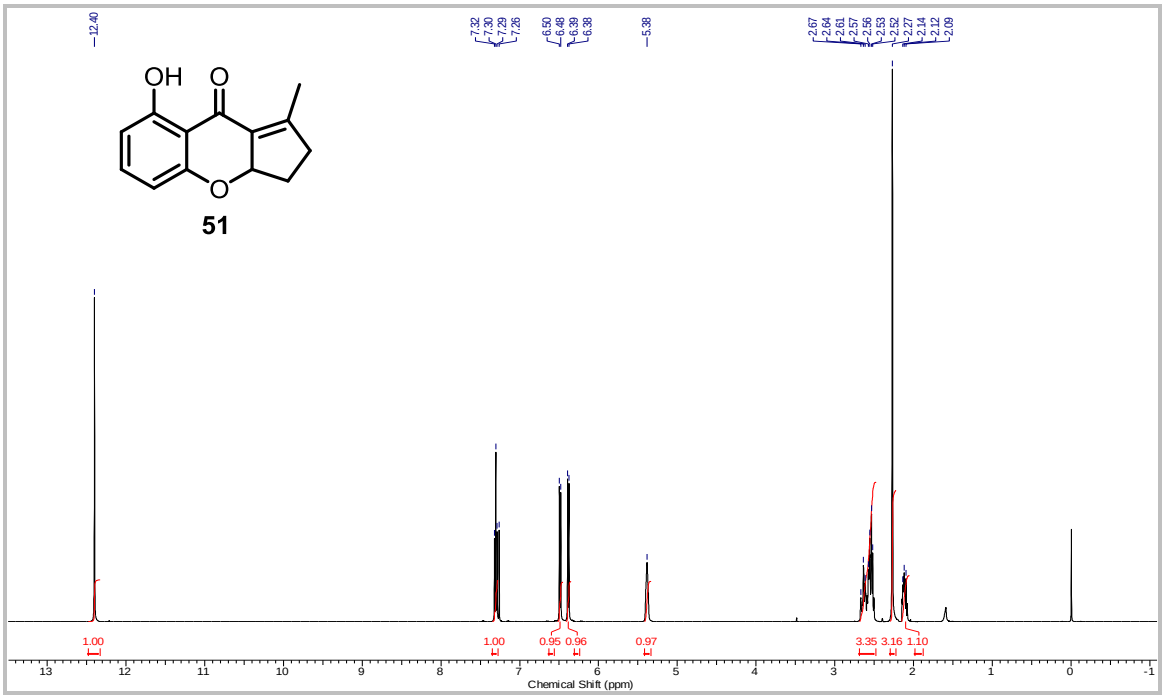
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 48



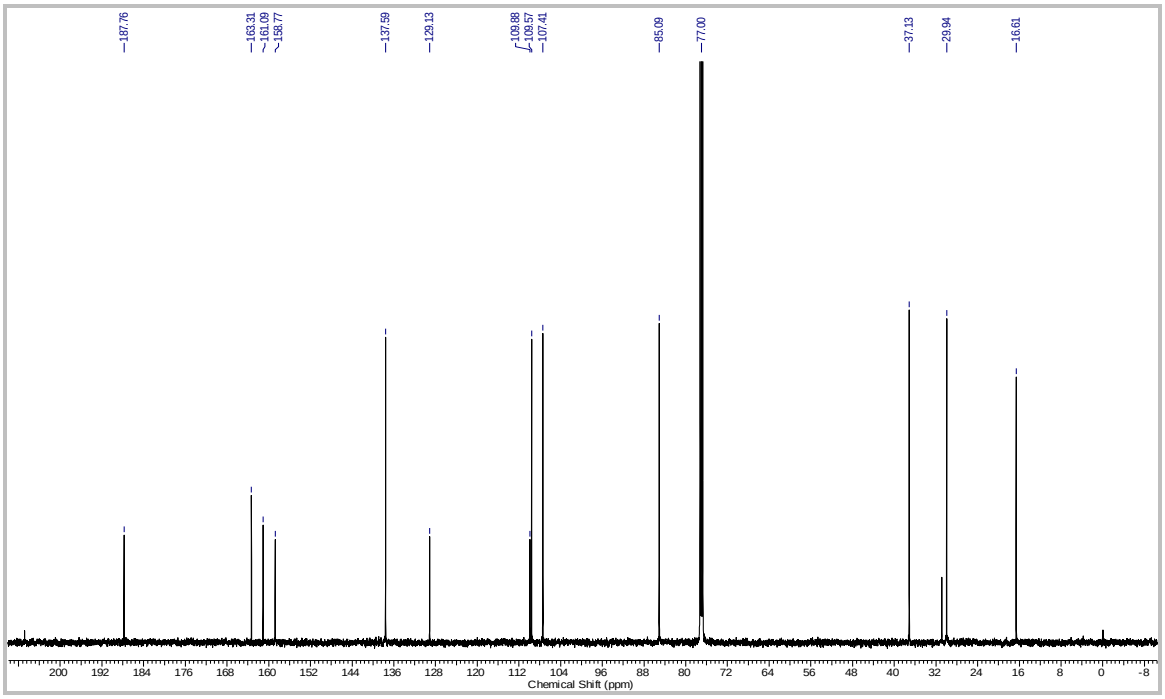

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 48



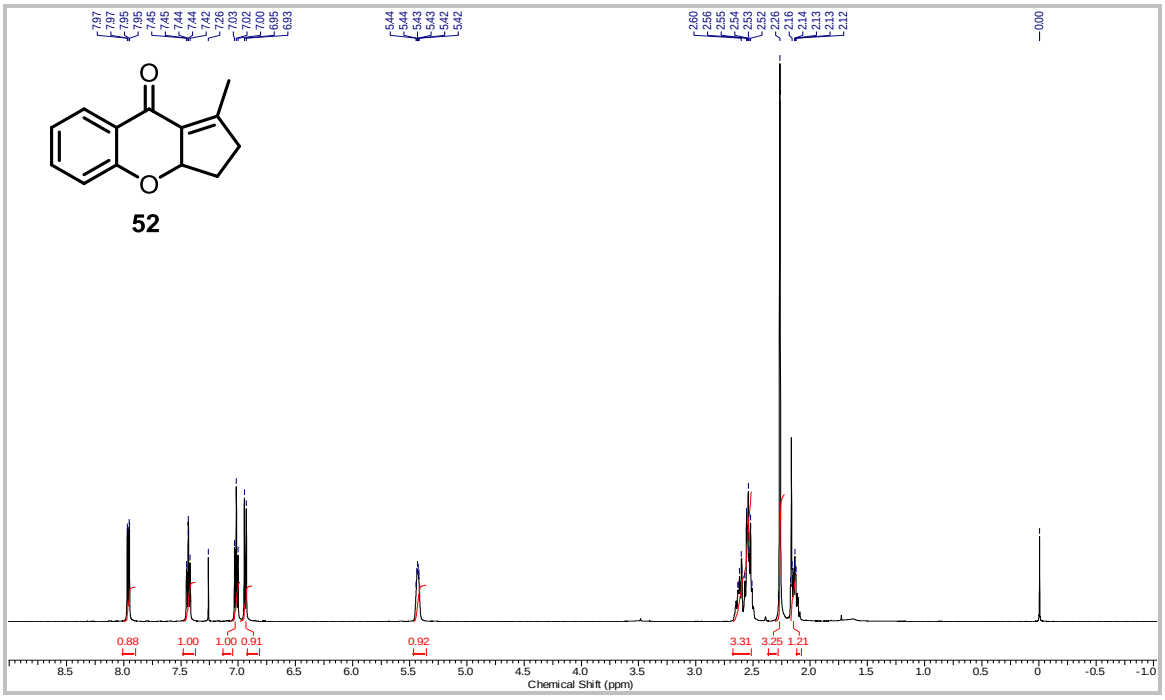
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 51



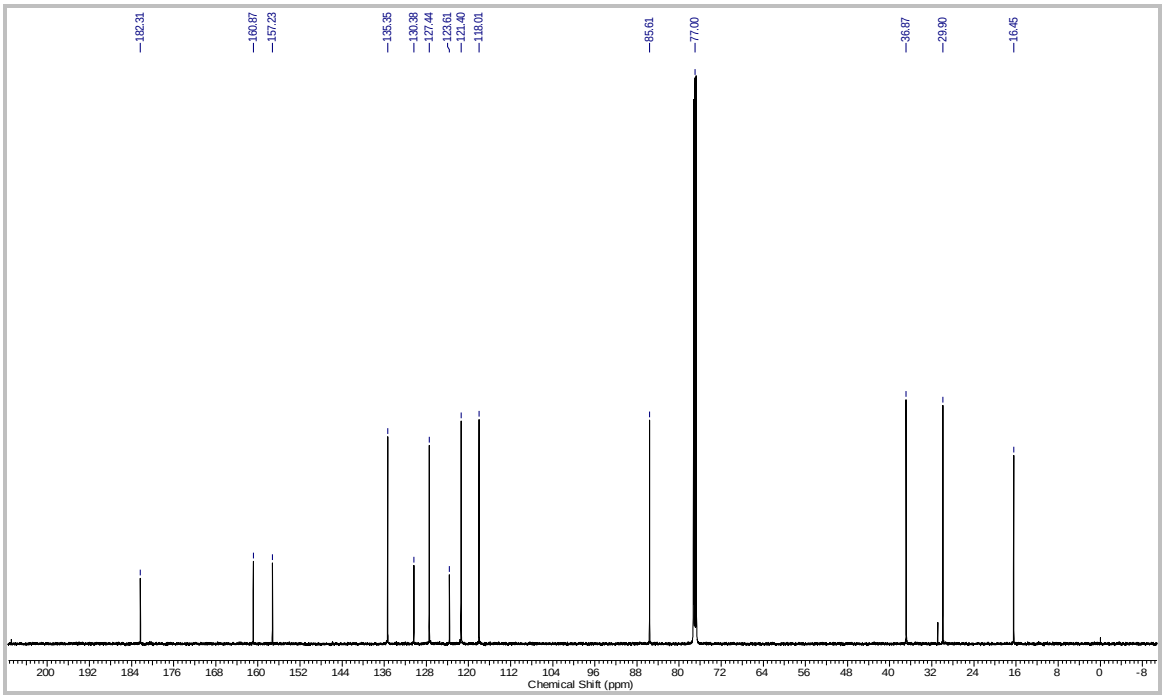

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 51



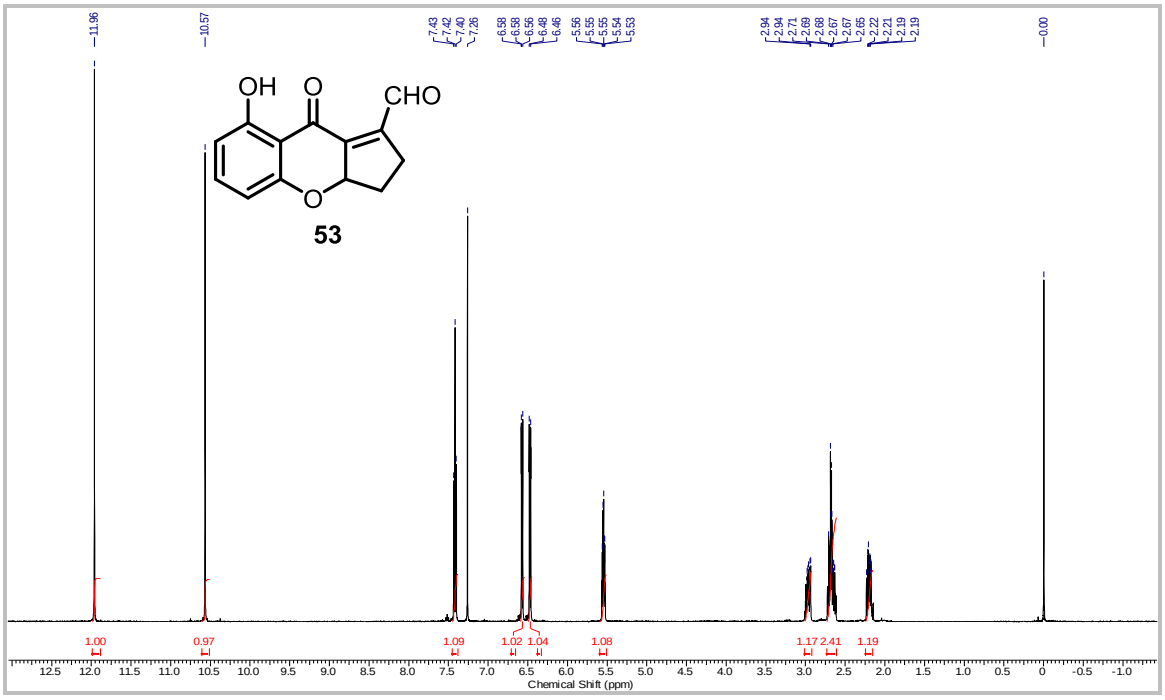
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 52



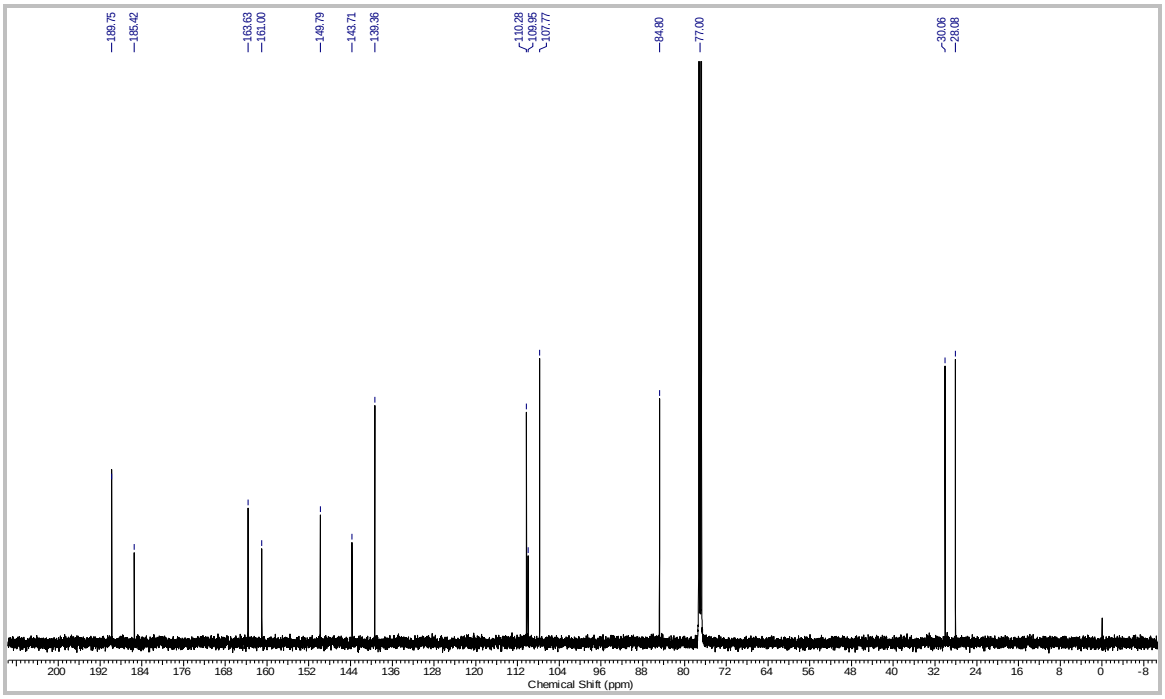

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 52



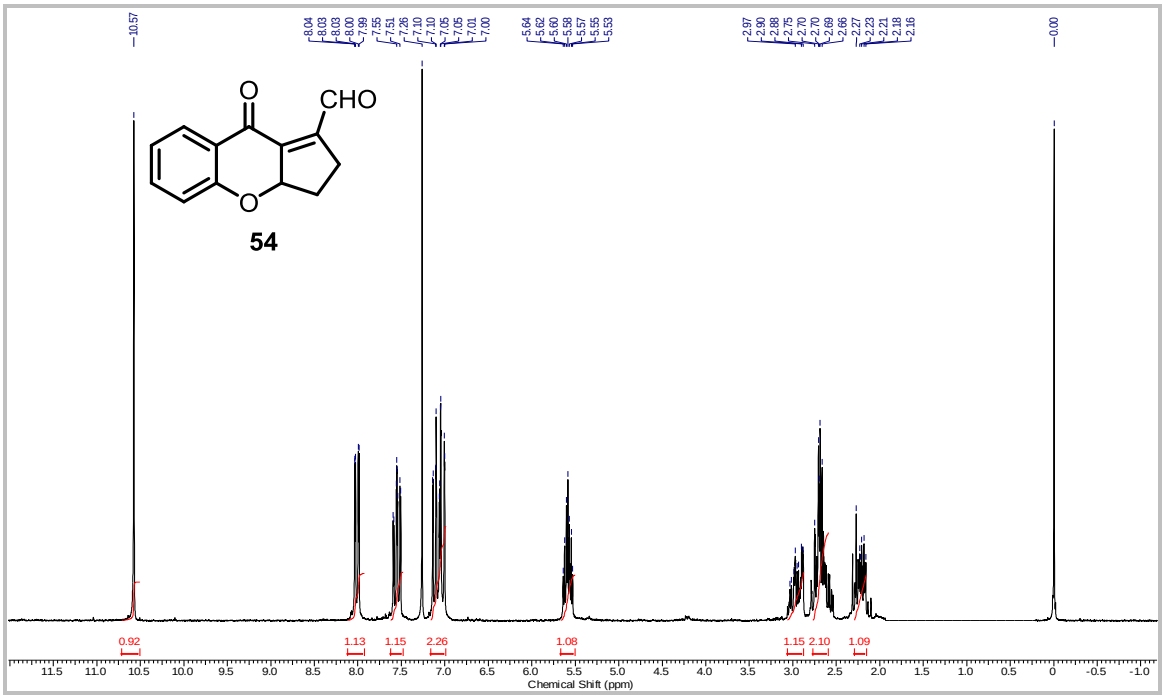
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 53



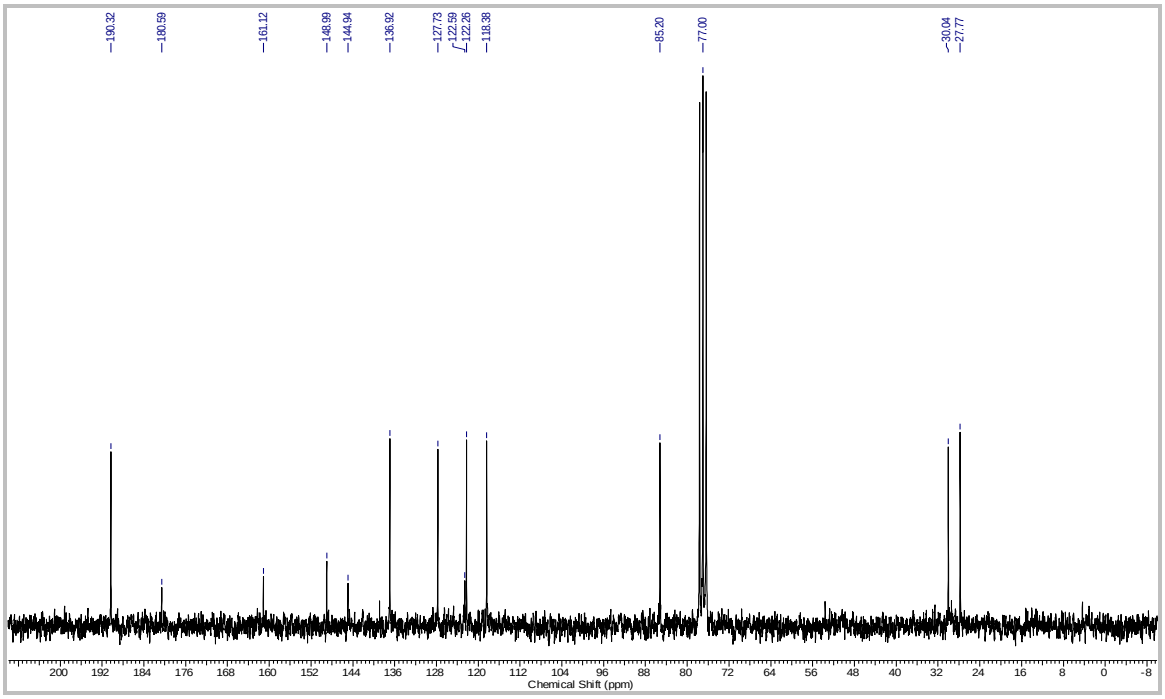

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 53



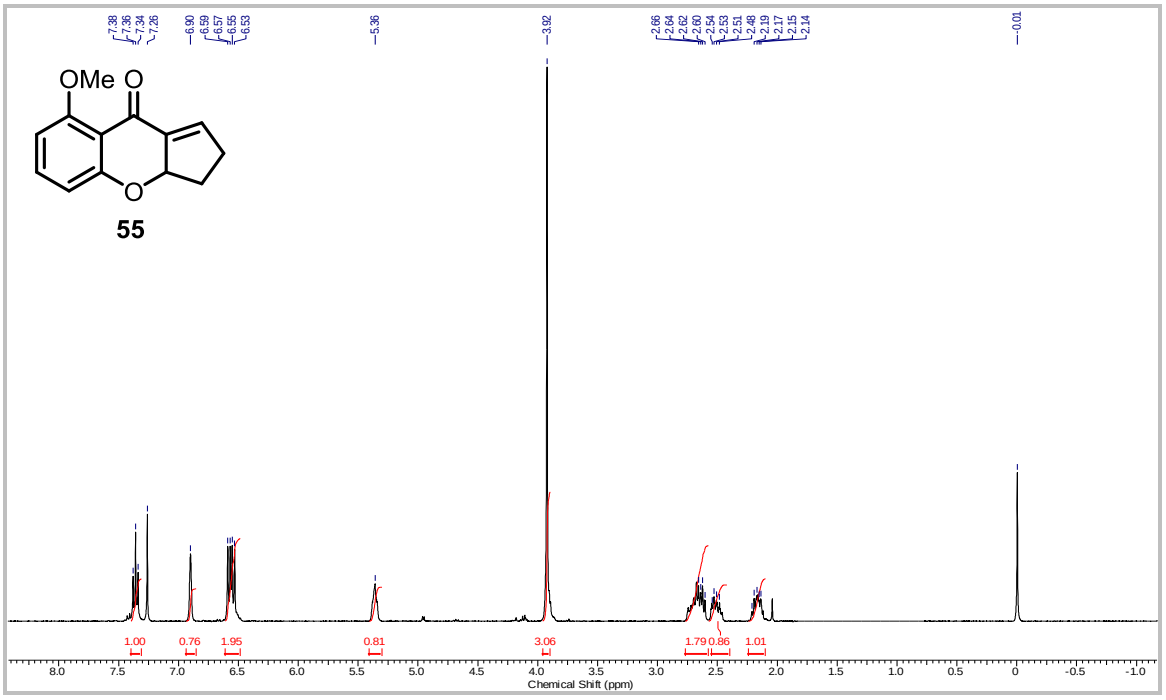
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 54



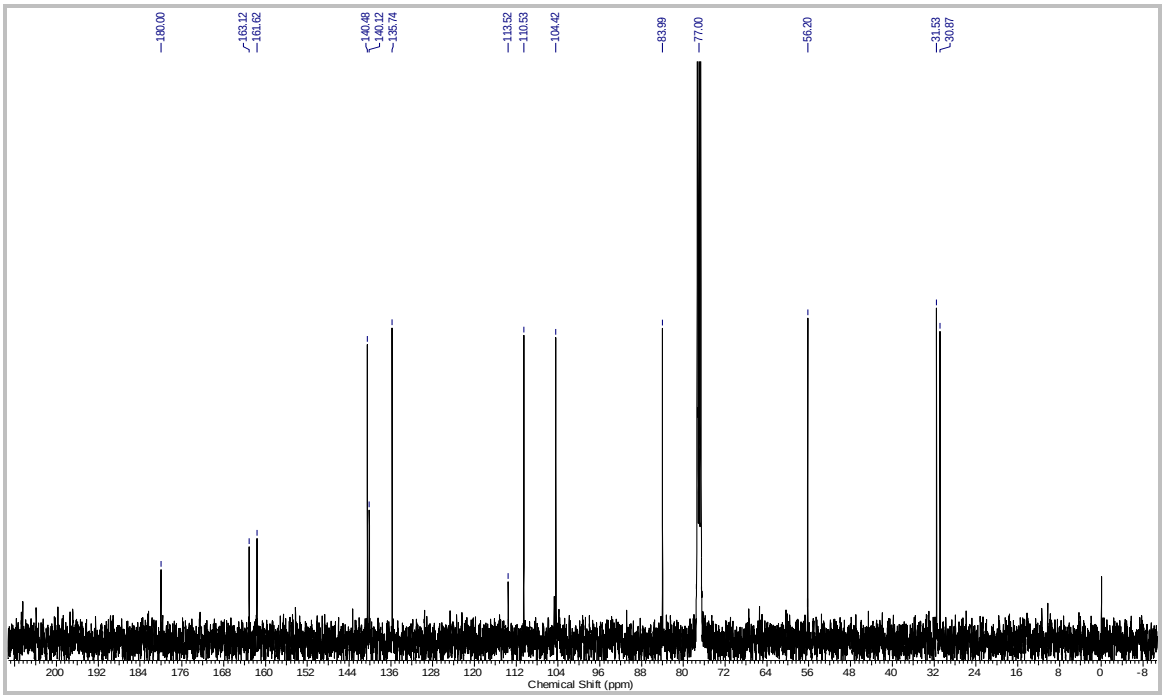

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 54



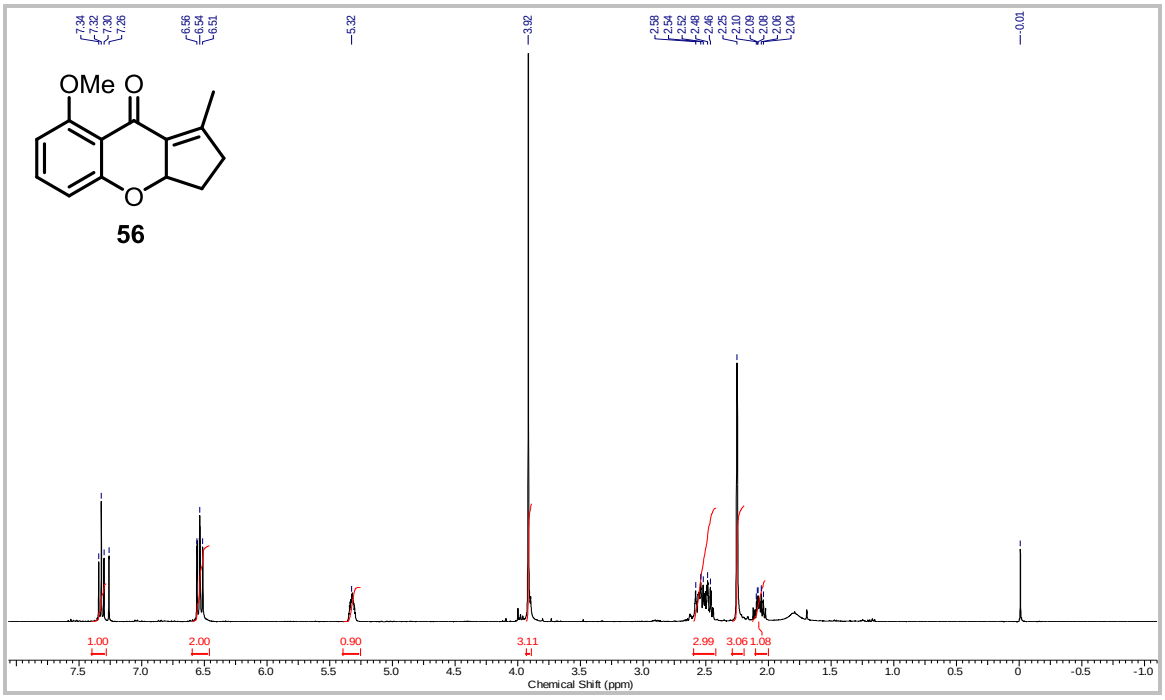
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 55



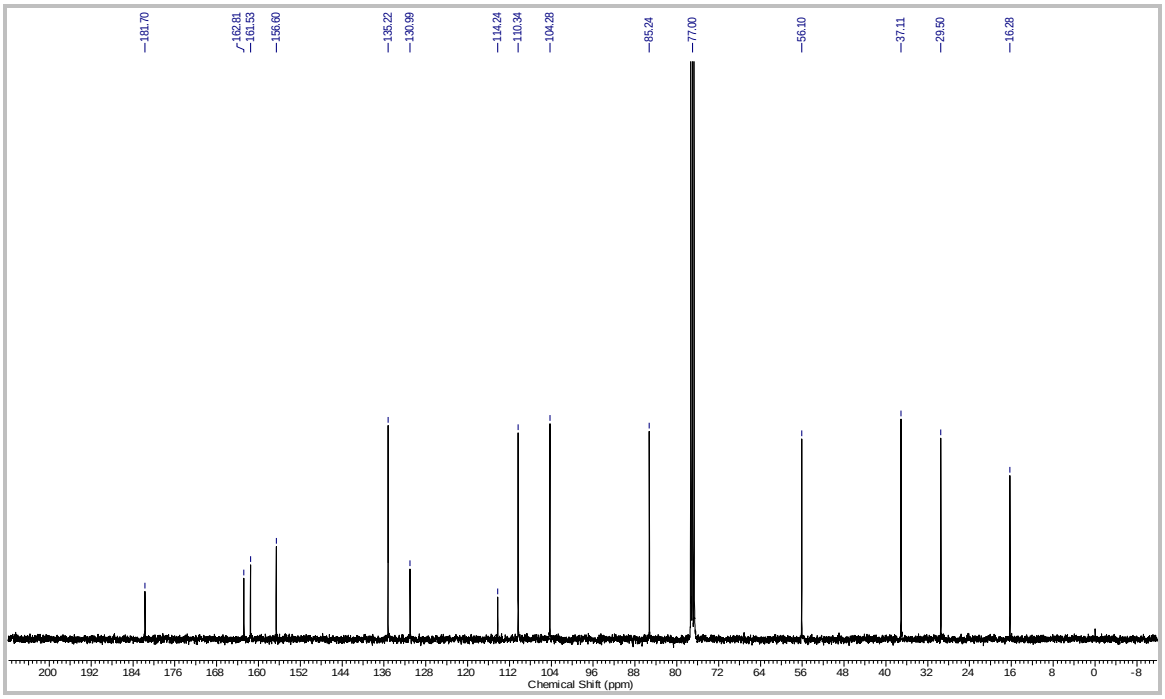

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 55



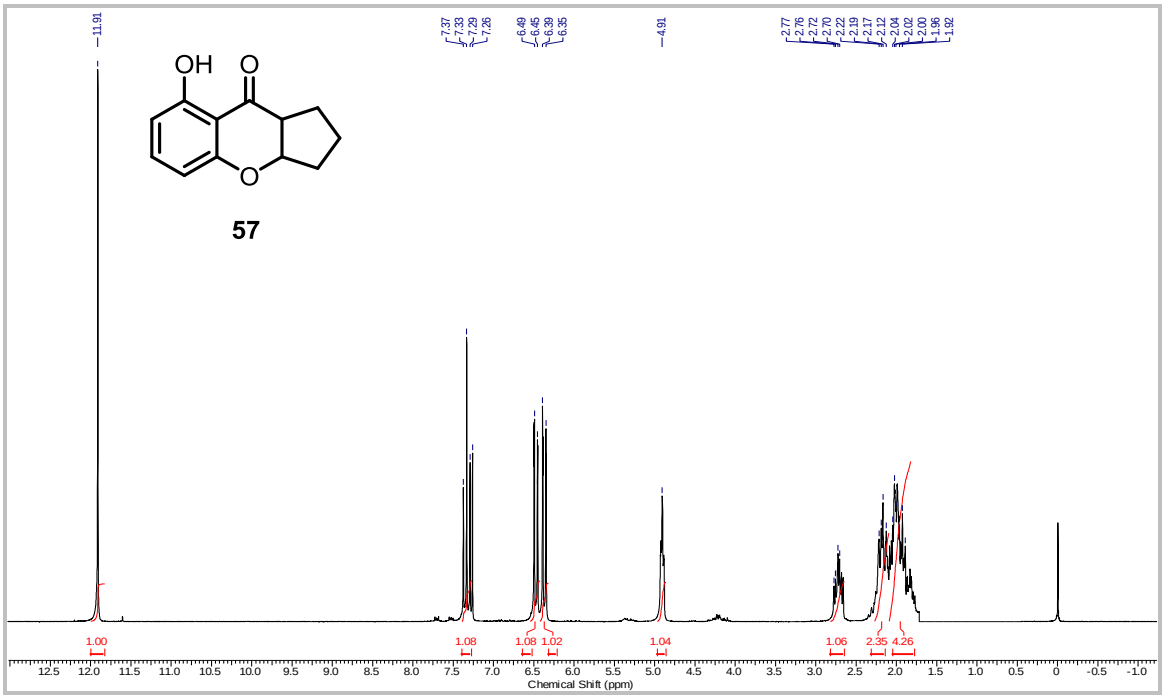
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 56



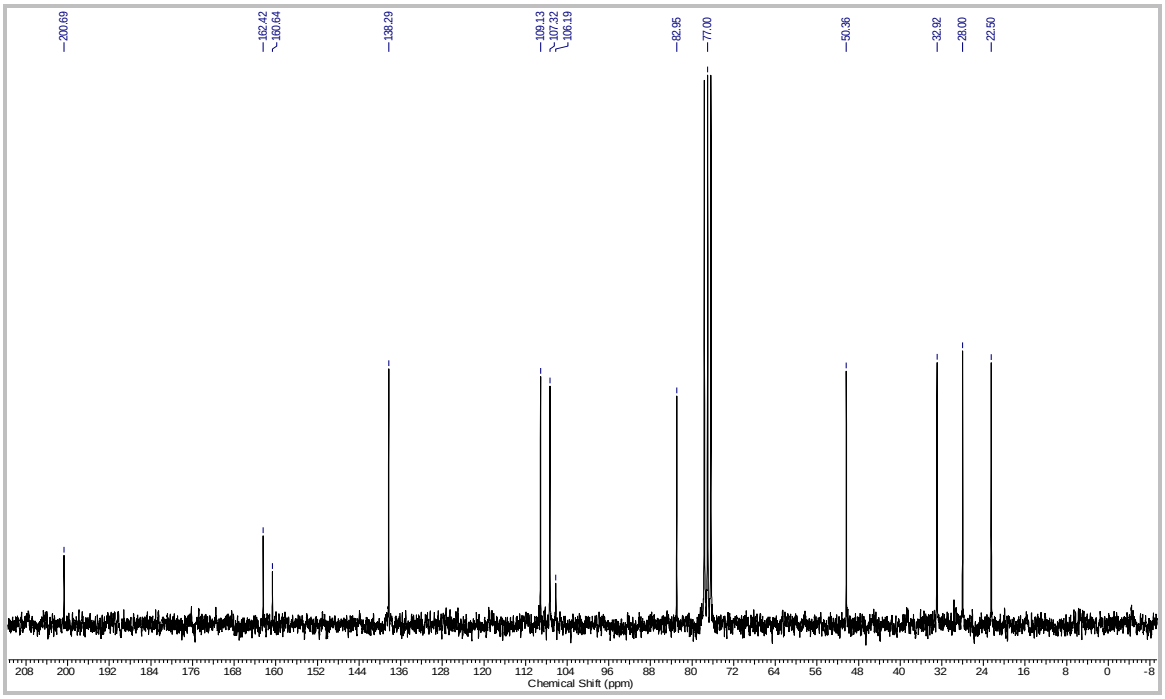

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 56



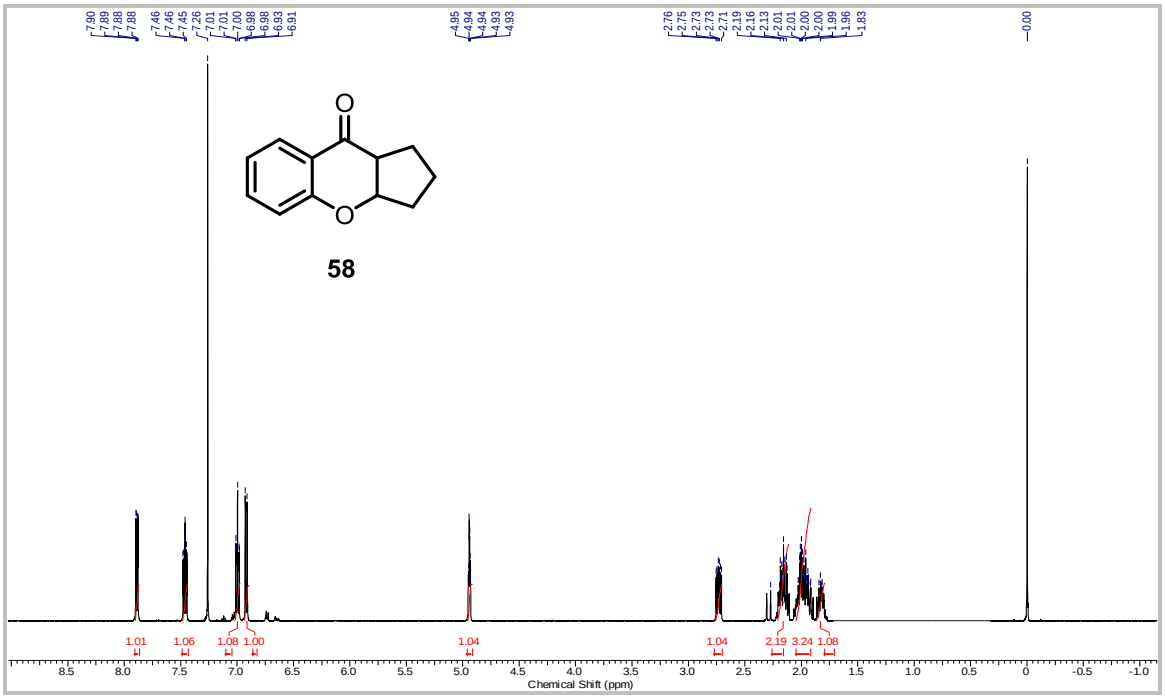
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 57



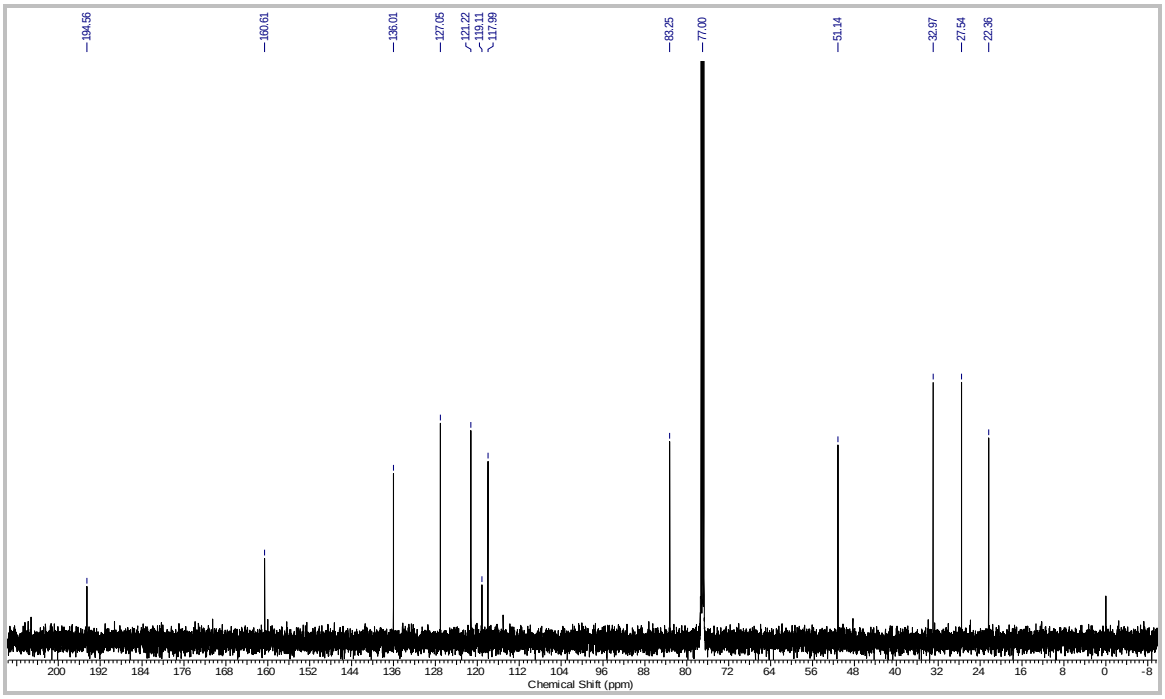

## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 57



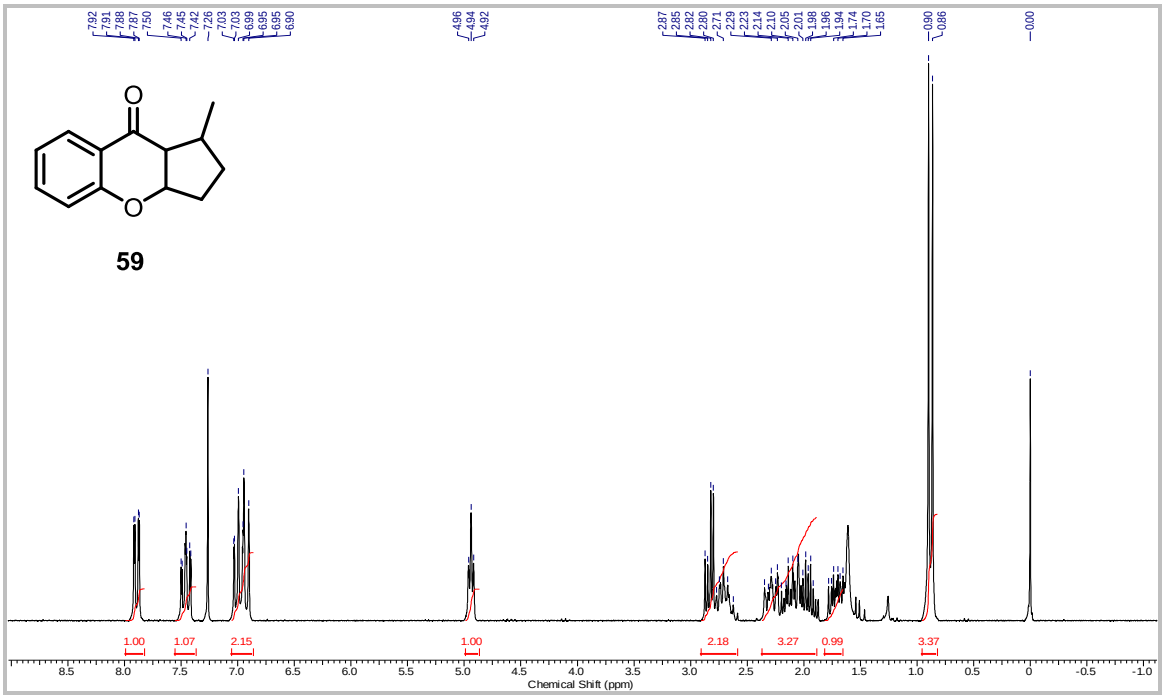
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 58



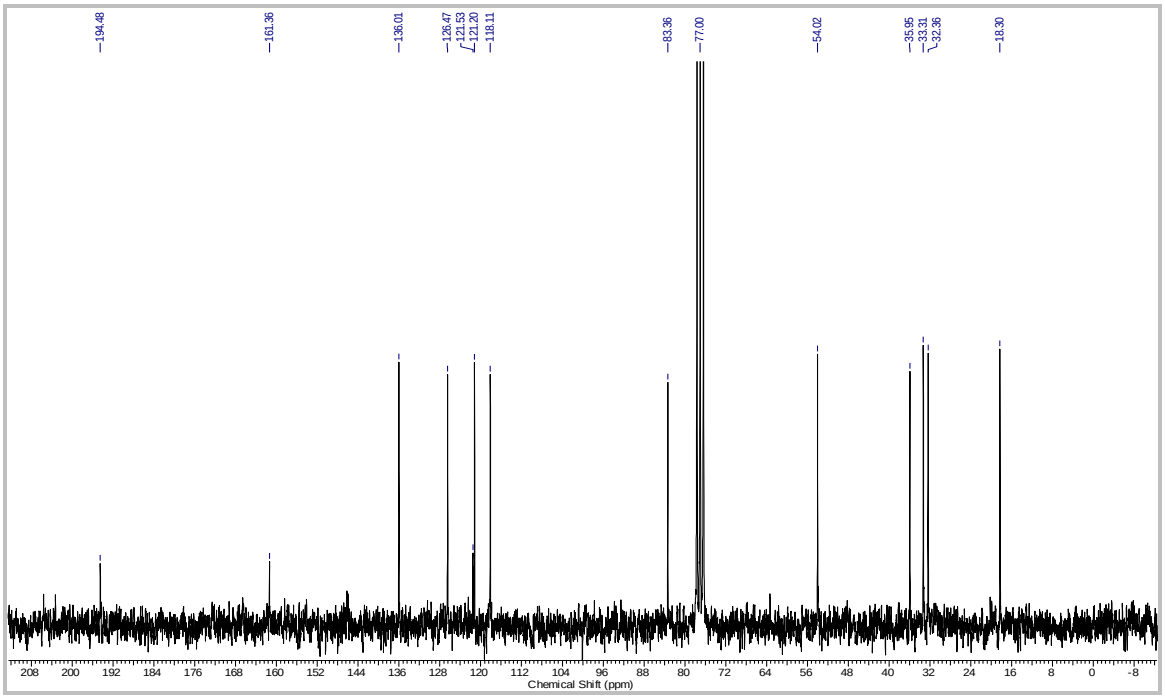

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 58



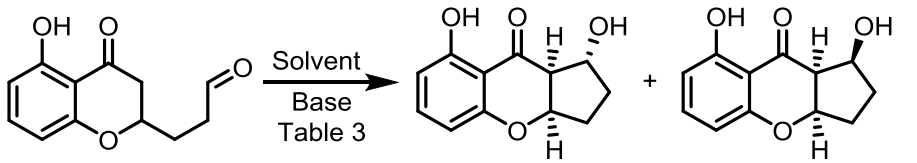
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 59




## $^{13}$C NMR (50 MHz, $CDCl_3$) of compound 59



**Selective HPLC chromatograms for determination of diastereomeric ratio**




**44** **39** **Diaportheone B (11)**

Column: Chiralcel OJ-H (250 x 4.6 mm)

Mobile phase: IPA:n-Hexane (10:90)

Wavelength: 254 nm

Flow Rate: 0.7 mL/min, 475 psi

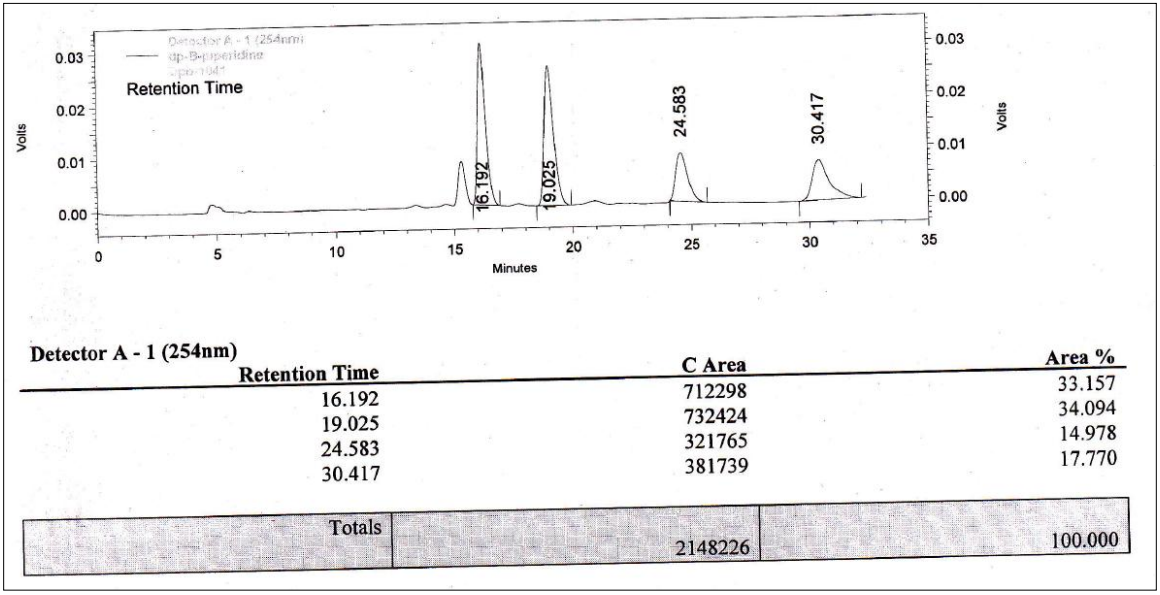
Concentration: 1.0 mg/mL

Injection volume: 5.0 µL

$R_t$ = 16.192 min & $R_t$ = 19.025 min peak shows the enantiomers of epimer (**47**)

$R_t$ = 24.583 min & $R_t$ = 30.417 min peak shows the enantiomers of diapothrone B (**12**)

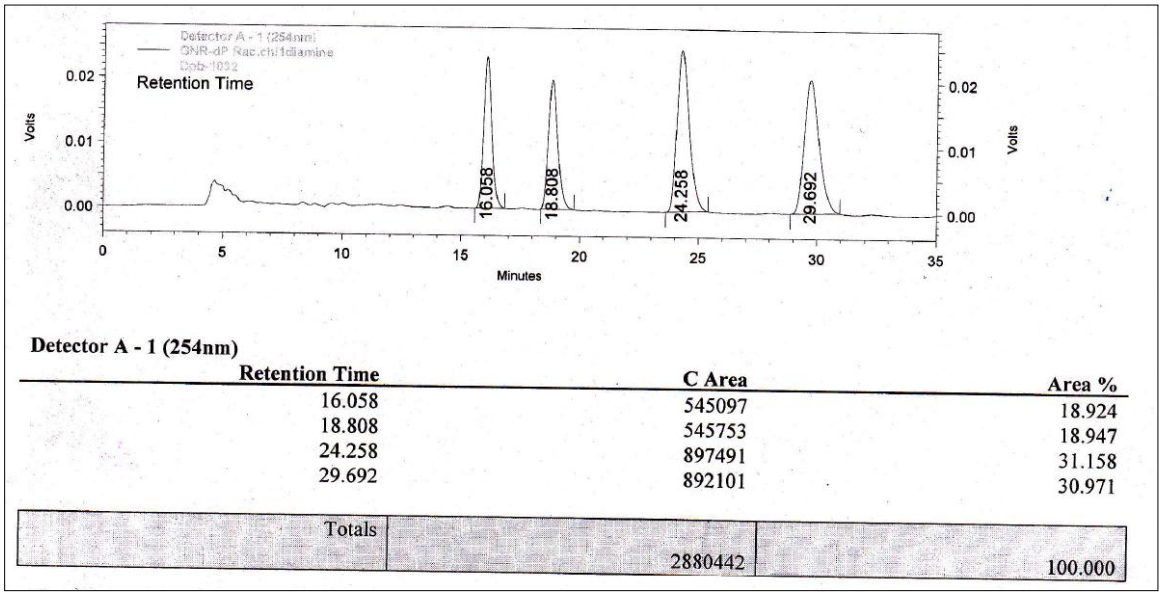
**Piperidine in methanol**




Detector A - 1 (254nm)

| Retention Time | C Area | Area % |
|---|---|---|
| 16.192 | 712298 | 33.157 |
| 19.025 | 732424 | 34.094 |
| 24.583 | 321765 | 14.978 |
| 30.417 | 381739 | 17.770 |
| Totals | 2148226 | 100.000 |

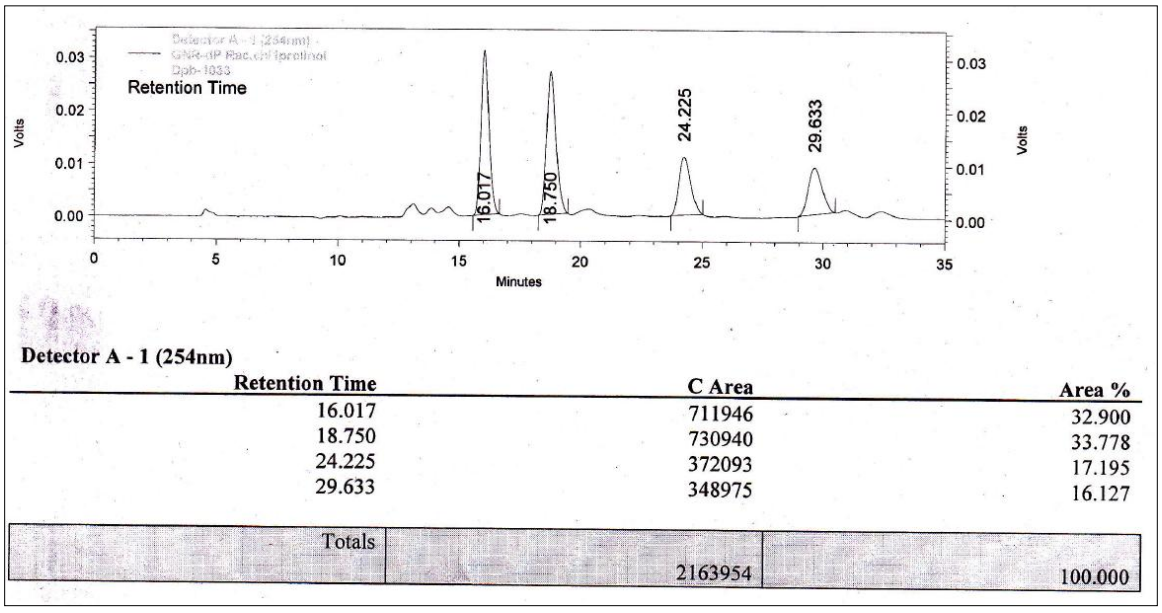
## Proline diamine in methanol




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 16.058 | 545097 | 18.924 |
| 18.808 | 545753 | 18.947 |
| 24.258 | 897491 | 31.158 |
| 29.692 | 892101 | 30.971 |
| Totals | 2880442 | 100.000 |

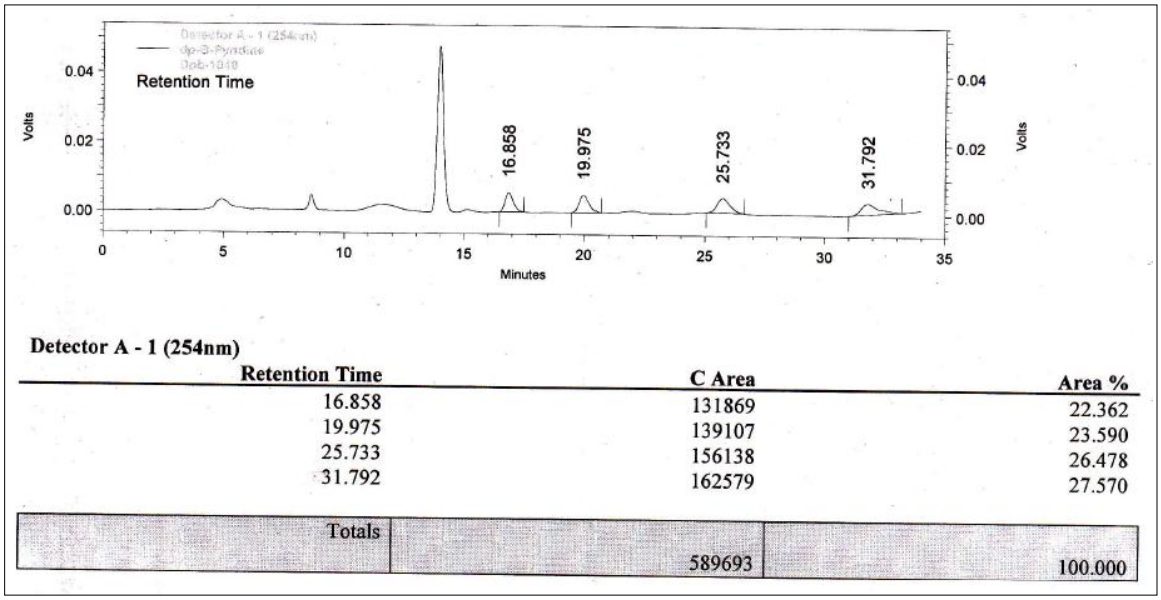
## Prolinol in methanol




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 16.017 | 711946 | 32.900 |
| 18.750 | 730940 | 33.778 |
| 24.225 | 372093 | 17.195 |
| 29.633 | 348975 | 16.127 |
| Totals | 2163954 | 100.000 |

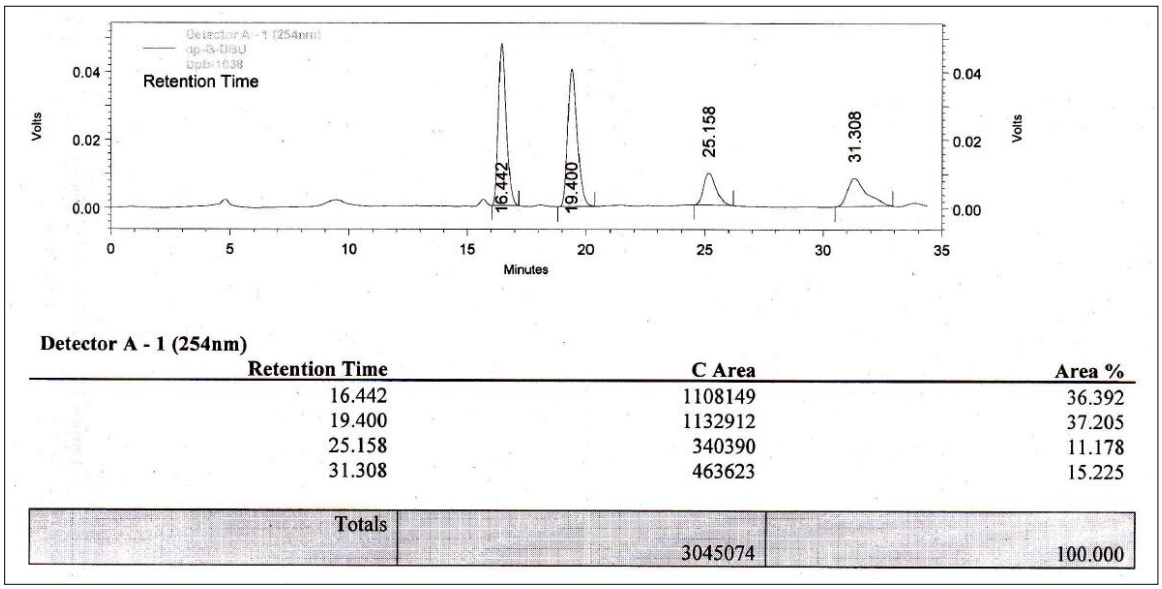
## Pyridine in methanol




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 16.858 | 131869 | 22.362 |
| 19.975 | 139107 | 23.590 |
| 25.733 | 156138 | 26.478 |
| 31.792 | 162579 | 27.570 |
| Totals | 589693 | 100.000 |

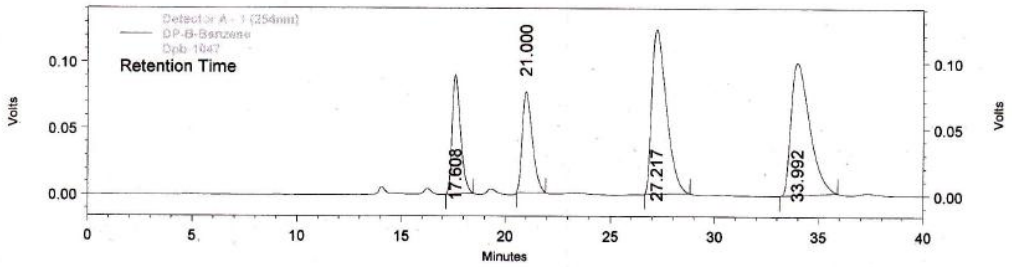
## DBU in methanol




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 16.442 | 1108149 | 36.392 |
| 19.400 | 1132912 | 37.205 |
| 25.158 | 340390 | 11.178 |
| 31.308 | 463623 | 15.225 |
| Totals | 3045074 | 100.000 |

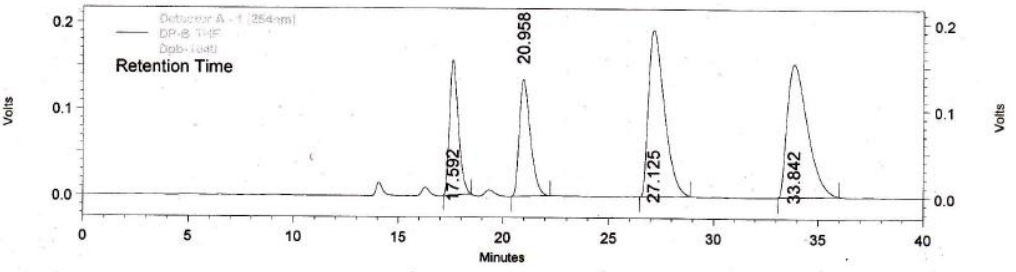
## DABACO in benzene




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 17.608 | 2532993 | 14.634 |
| 21.000 | 2554018 | 14.756 |
| 27.217 | 6051673 | 34.963 |
| 33.992 | 6170062 | 35.647 |
| Totals | 17308746 | 100.000 |

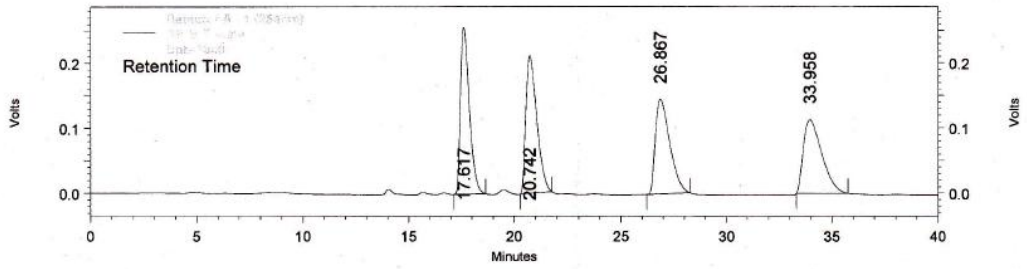
## DABACO in THF




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 17.592 | 4591210 | 15.370 |
| 20.958 | 4893236 | 16.381 |
| 27.125 | 10105692 | 33.831 |
| 33.842 | 10281203 | 34.418 |
| Totals | 29871341 | 100.000 |

**DABACO in toluene**




**Detector A - 1 (254nm)**

| Retention Time | C Area | Area % |
|---|---|---|
| 17.617 | 7542048 | 26.039 |
| 20.742 | 7623293 | 26.320 |
| 26.867 | 6931602 | 23.932 |
| 33.958 | 6867357 | 23.710 |
| Totals | 28964300 | 100.000 |

## 3.3.7. References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*Chapter 3 Section II*

# Total Synthesis and SAR Studies of 4-Dehydroxydiversonol and its Analogs

## 3.4. Synthesis of 4-dehydroxydiversonol and its analogs

### 3.4.1. Isolation and characterization of diversonol

Endophytic fungi are known to be a rich source of biologically active secondary metabolites. Xanthones and partially hydrogenated as di- or tetra-hydroxanthones are the major class of secondary metabolites which occur in endophytic fungi. Several tetrahydroxanthones isolated from the fungi showed prominent activity as anticancer, antibacterial agents and several other pharmaceutically important properties.

**Scientific Classification**

Kingdom: Fungi

Order : Eurotiales

Family : Trichocomaceae

Genus : *Penicillium*

Species : *P. diversum*

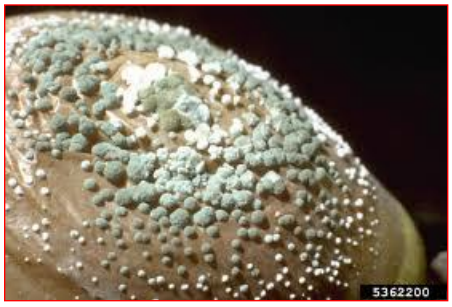



**Figure 9:** *Penicillium diversum* fungi

Diversonol (**13**) is a polyketide metabolite first isolated from *Penicillium diversum* in 1978 by Turner[1] and later on by Krohn in 2011 reisolated the diversonol (**13**) from the *Microdiplodia sp.* obtained from the shrub *Lycium intricatum*.[2] Krohn and his group isolated the diversonol (**13**) in enantiopure form and determined its absolute configuration. Since the diversonol was obtained as a crystalline solid, the stereostructure of natural product was determined using the ECD spectra in solution and in the microcrystalline solid state as a KCl pellet, which was later confirmed with the help of X-ray crystal structure analysis. Diversonol (**13**) is a monomeric tetrahydroxanthone derivative and showed antibacterial activity against *Legionella pneumophila*, it is also found to be antifungal agent against *Microbotryum violaceum*.[3-5] Even though the target was attracted by many groups which resulted in the synthesis of diversonol (**13**),[7] dehydroxydiversonol (**60**)[8] and other several related natural products (see figure 1) of tetrahydroxanthone class,[2,7,8] the detailed biological evaluation yet to be explored further.

## 3.4.2. Reported approaches for synthesis of diversonol and 4-dehydroxydiversonol

Diversonol (**13**) and 4-dehydroxydiversonol (**60**) are tetrahydroxanthone derivatives, the biological activity of diversonol (**13**) was not found to be very interesting but related dimeric natural products secalonic acids (**30**, **31**, **32**) which have interesting pharmacological potential as antibacterial, anti-HIV and cytostatic activity. As of now, there are four total syntheses and one formal synthesis of diversonol (**13**)[7] along with two syntheses of 4-dehydroxydiversonol (**60**)[8] are reported in the literature (Figure 10).

Bräse and co-workers reported the first racemic total synthesis in 2006 followed by enantioselective total synthesis of diversonol (**13**) in 2011. The first racemic synthesis reported by using the domino oxa-Michael-aldol reaction as a key step starting from the 2-hydroxy-6-methoxy-4-methylbenzaldehyde and 4-hydroxycyclohex-2-en-1-one in 14-steps of linear sequence with poor over all yield (0.36%).[7a] The first asymmetric synthesis of diversonol (**13**), lachnone C (**28**) and *epi*-lachnone C have been reported by his group in 2011.[7c] The enantioselective aldol-oxa-Michael domino reaction using Jørgensen's catalysts was used as a key step and synthesis was accomplished in 12-linear steps. Bräse group also reported the enantioselective total synthesis of 4-dehydroxydiversonol (**60**) in 2009[8b] using asymmetric aldol-oxa-Michael reaction using Jørgensen's catalysts, followed by two carbon Wittig reaction starting from the commercially available aldehyde and 3-methylbut-2-enal. The total synthesis of 4-dehydroxydiversonol (**60**) was achieved in eight steps with an overall yield of 16%. Nicolaou and Li reported the synthesis of diversonol (**13**) along with the structural revision of α- and β-diversonolic ester in 2008 starting from the chiral starting material (*R*)-4-((tert-butyldimethylsilyl)oxy)-3-methylcyclohex-2-en-1-one and substituted aldehyde. They obtained the diastereomeric mixture during the Pd-catalyzed intramolecular 1,4-addition reaction of phenolic oxygen to α, β-unsturated olefin, the required diastereomer was separated and forwarded to complete the synthesis with good overall yield (38%).[7b]

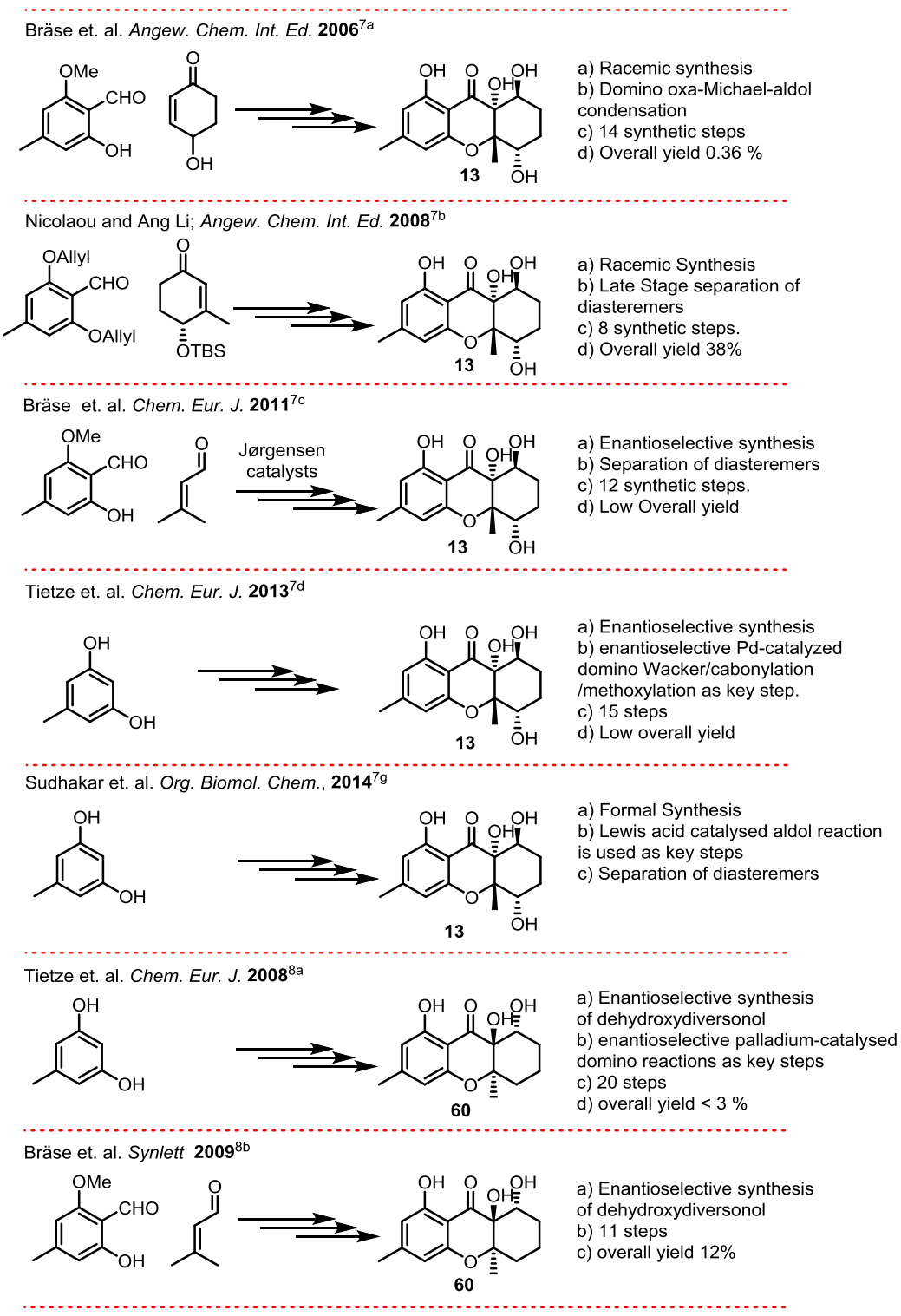



**Figure 10:** Previously reported approaches of diversonol (**13**) and 4-dehydroxydiversonol (**60**)

In the year 2008, Tietze and co-workers reported the enantioselective total synthesis of 4-dehydroxydiversonol (**60**) starting from the orcinol. The Pd-catalyzed cyclization-carbonylation reaction is used as key step using the (S,S)-Bn-BOXAX as a chiral catalyst.[8a] Tietze group also reported the enantioselective synthesis of diversonol (**13**) in 2013 by using the similar strategy.[7d] In this synthesis, the hydroxy group at 4-position was installed by using the treatment of corresponding terminal olefin with AD-mix α followed by using a similar sequence of reactions to get the diversonol (**13**) in overall 13 steps. Recently, Sudhakar's group also reported the total synthesis of lachnone C (**28**), gonytolide C (**29**) and gonytolide G along with the formal synthesis of blennolide C (**16**) and diversonol (**13**) using Lewis acid catalyzed aldol reaction as a key step.[7g]

## 3.4.3. Present work

Considering the close structural resemblance of diversonol (**13**) with diaportheone B (**11**), its wide range of biological activities and the importance of xanthones in the pharmaceutical industry, we became interested in diversonol (**13**) and related natural products along with the synthesis of a library of analogs. The syntheses of analogs were based on the simplicity and close structural resemblance with natural products. Our initial interest was to synthesize the 4-dehydroxydiversonol (**60**) in the racemic form using a simple and scalable route which will give us all possible stereoisomers for biological screening.

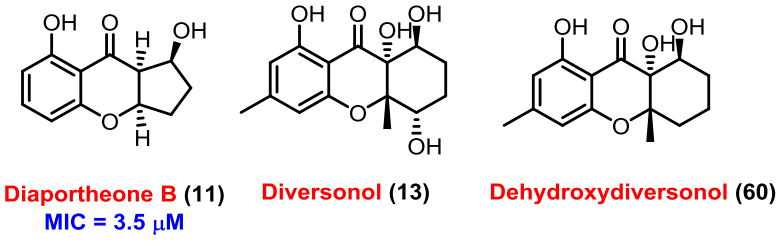



**Figure 11:** Structures of diaportheone B (**11**), diversonol (**13**) and 4-dehydroxydiversonol (**60**)

### 3.4.3.1. Retrosynthetic approach

Retrosynthetically, we planned the target tetrahydroxanthone compound **60** by using our previously reported approach as an aldol-oxa-Michael reaction as a key step.[6] The compound **60** could be synthesized from the compound **61** by using the oxidation of enol to α-hydroxy ketone followed by the selective reduction of the carbonyl group to alcohol. The compound **61** could be obtained through the intramolecular Dieckmann condensation[8] reaction of ester **62,** which can ultimately synthesized using aldol-oxa-Michael reaction[8] of commercially available hydroxy acetophenone **63** and ketoester **64** in the presence of an organocatalyst. The similar strategy can also be used for the synthesis of analogs.

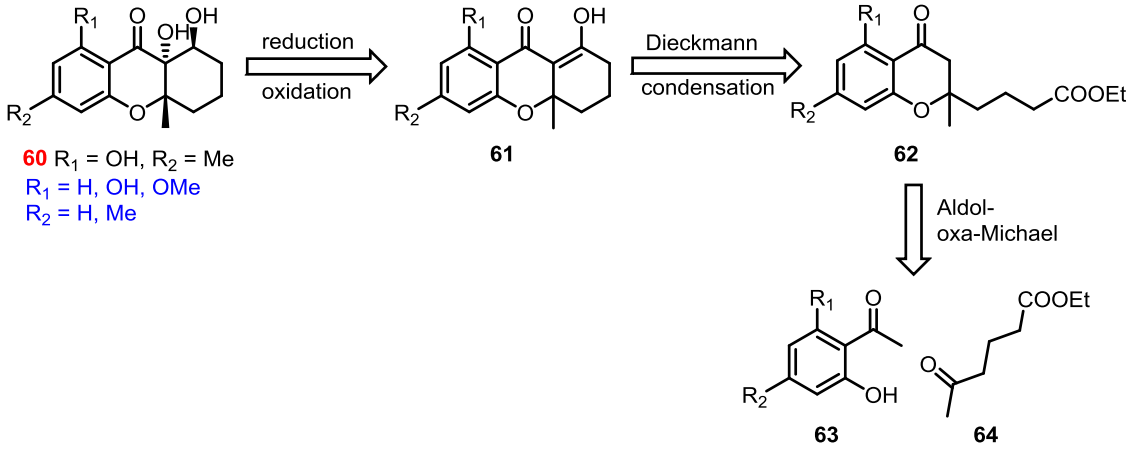
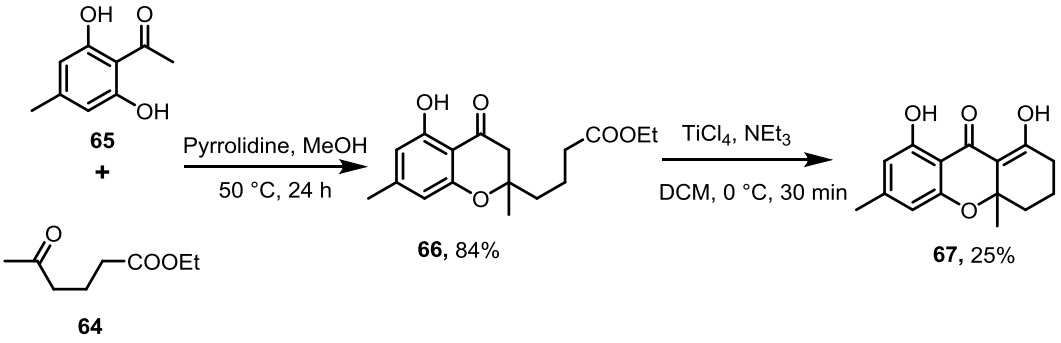



**Figure 12:** Retrosynthetic approach for the 4-dehydroxydiversonol (**60**).

### 3.4.3.2. Synthesis of 4-dehydroxydiversonol

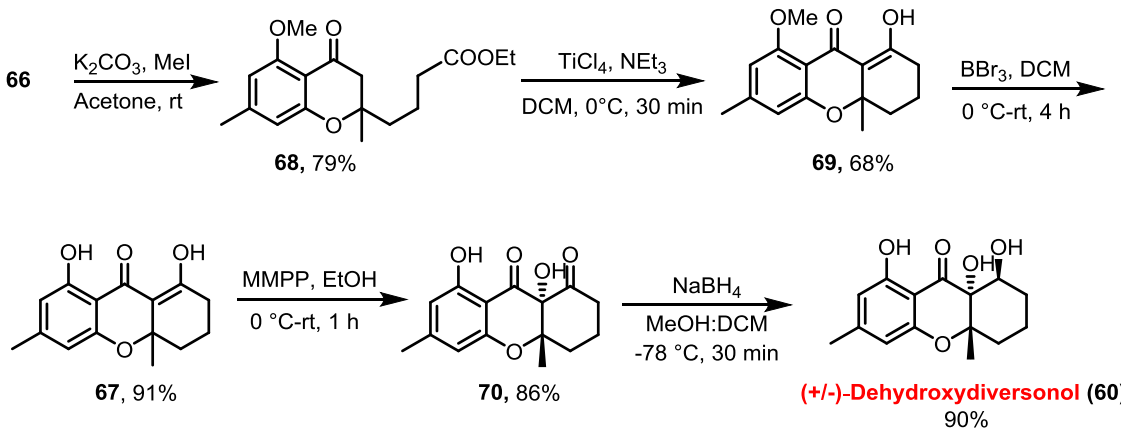
Our planned synthesis started with the key step of our synthesis as an aldol-oxa-Michael reaction to construct the substituted chromanone ester **66** by treatment of ethyl 5-oxohexanoate (**64**) with 2,6-dihydroxy-4-methyl acetophenone (**65**) in the presence of 20 mol% of pyrrolidine in methanol at 50 ºC. The formation of compound **66** was confirmed using the mass which showed the peak at m/z 329 $[M+Na]^+$ corresponding to molecular formula $C_{17}H_{22}O_5Na$. The compound was further confirmed using the $^1H$ NMR which

showed the characteristic enolic proton in the highly downfield region and $^{13}C$ NMR indicate the characteristic ester carbonyl at δ 173.1 ppm. The ester **66** was kept for intramolecular Dieckmann condensation using titanium tetrachloride ($TiCl_4$) and triethylamine ($Et_3N$) in DCM at 0 ºC for 30 minutes to get enol **67**. The Dieckmann condensation reaction on free phenolic hydroxy group resulted into poor yield (25%) (Scheme 10). Compound **67** was confirmed using the MS, the corresponding peak of $[M+H]^+$ was observed at 261, which was further confirmed by $^1H$ NMR in which the both hydroxy groups showed signals in the down field region at δ 13.75 and 11.45 ppm.




**Scheme 10:** Synthesis of trihydroxanthone **67**

To increase the yield of this reaction we have protected the phenolic OH group of compound **66** as methyl ether using methyl iodide and potassium carbonate in dry acetone to obtain the methoxy compound **68** with 79% yield. The compound **68** was confirmed using the $^1H$ NMR which showed the disappearance of the enolic proton observed in compound **66** and three methyl protons attached to phenolic oxygen were found at δ 3.86 ppm. The obtained compound **68** was then subjected to intramolecular Dieckmann condensation to get the compound **69** with 68% yield, which was confirmed by $^1H$ and $^{13}C$ NMR spectroscopic methods and it was further confirmed by HRMS which showed the peak at m/z 275.1276 corresponding to molecular formula $C_{16}H_{19}O_4$ $[M+H]^+$(275.1278).



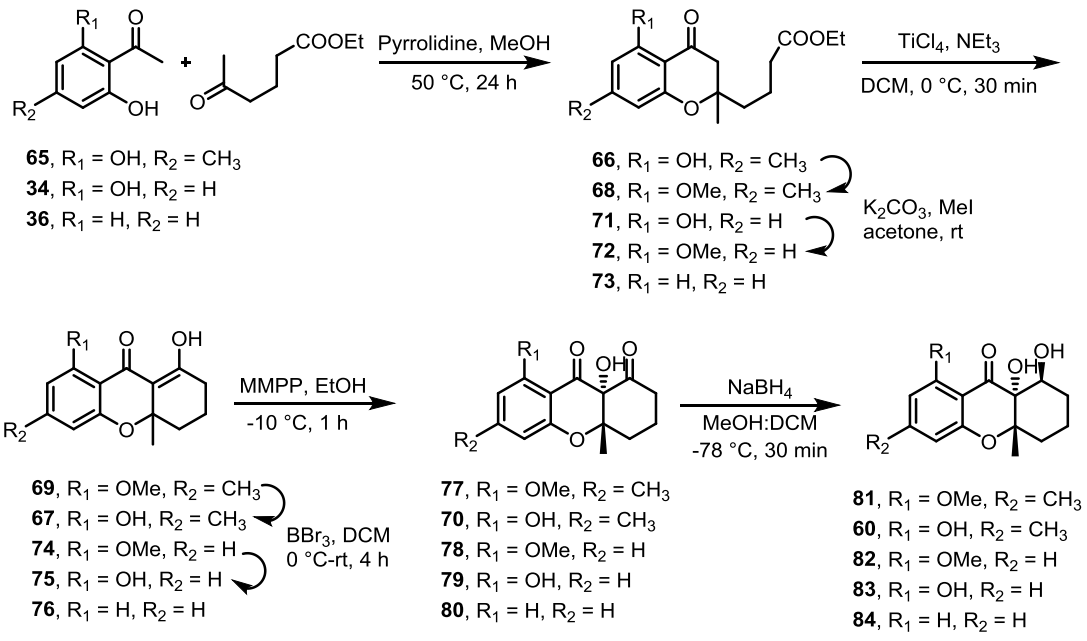

**Scheme 11:** Synthesis of 4-dehydroxydiversonol (**60**)

The methyl ether was deprotected with boron tribromide in DCM to get the compound **67** with good yield. The compound **67** was oxidized under the reported conditions[7,8] with magnesium monoperoxyphthalate (MMPP) in ethanol at -10 ºC resulting into the hydroxy diketone **70**. Here relative stereochemistry of methyl and hydroxyl group should be *trans* based on the literature reports[7c] and we also observed the single diastereomer during reaction which was confirmed based on the $^{1}H$ and $^{13}C$ NMR analysis followed by the HRMS which showed the base peak at m/z 277.1080 which is in agreement with the corresponding molecular formula $C_{15}H_{17}O_5$ $[M+H]^+$ (277.1071). The compound **70** was reduced under controlled conditions to get the 4-dehydroxydiversonol (**60**) with excellent yield. The formation of 4-dehydroxydiversonol (**60**) was confirmed by $^{1}H$ NMR which showed the characteristic protons attached to hydroxy attached carbon is at δ 4.45 ppm and $^{13}C$ NMR which is in complete agreement with the literature reports, it was further confirmed by HRMS which showed the base peak at m/z 279.1238 which is in agreement with the molecular formula $C_{15}H_{19}O_5$ $[M+H]^+$ ( 279.1227).

## 3.4.3.3. Synthesis of 4-dehydroxydiversonol analogs

Having developed methods for the easy access of 4-dehydroxydiversonol (**60**), we were interested in the synthesis of a library of its close analogs to evaluate their antibacterial potential. The synthesis of analogs was achieved by following the similar sequence of reactions used for the synthesis of 4-dehydroxydivesonol (**60**) by changing the hydroxy

acetophenone partners as **65**, **34** and **36** as shown in scheme 12. The synthesis of tricyclic enolic compounds **66**, **68**, **71**, **72** and **73** was achieved by using Dieckmann condensations of its corresponding esters using the $TiCl_4$ and $Et_3N$ in DCM. The obtained enols were then kept for MMPP oxidation in ethanol at -10 ºC resulted into the only one diastereomers with relative stereochemistry of junction methyl and the hydroxy group as trans to each other, which is confirmed by comparing the $^1H$ and $^{13}C$ NMR with the literature reports.[7,8] The $NaBH_4$ reduction of diketones was accomplished at -78 ºC to fix the stereochemistry of vicinal diol as anti and we did not observe the other diastereomer. These observations are very similar to that of literature reports.[7] All the synthesized analogs were confirmed by MS, $^1H$ NMR, $^{13}C$ NMR followed by HRMS.




**Scheme 12:** Synthesis of analogs

## 3.4.4. Biological activity SAR of 4-dehydroxydiversonol analogs

The 4-dehydroxydiversonol (**60**) and all the synthesized tetrahydroxanthone analogs were tested for their antibacterial activity with the help of Dr. Sidharth Chopra's group at CSIR-CDRI, Lucknow and the results are compiled in table 7. The compounds were tested against the five different gram-positive / gram-negative bacterial pathogens like

*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*. The only compound **76** showed a better activity against *Staphylococcus aureus* with MIC = 8 μg/mL. The all other synthetic compounds including the 4-dehydroxydiversonol (**60**) were not active at a tested concentration for the *Staphylococcus aureus.* None of the synthetic tetrahydroxanthone compounds were active against the other four gram-positive / gram-negative bacterial pathogens of *Escherichia coli, Klebsiella Pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa.*

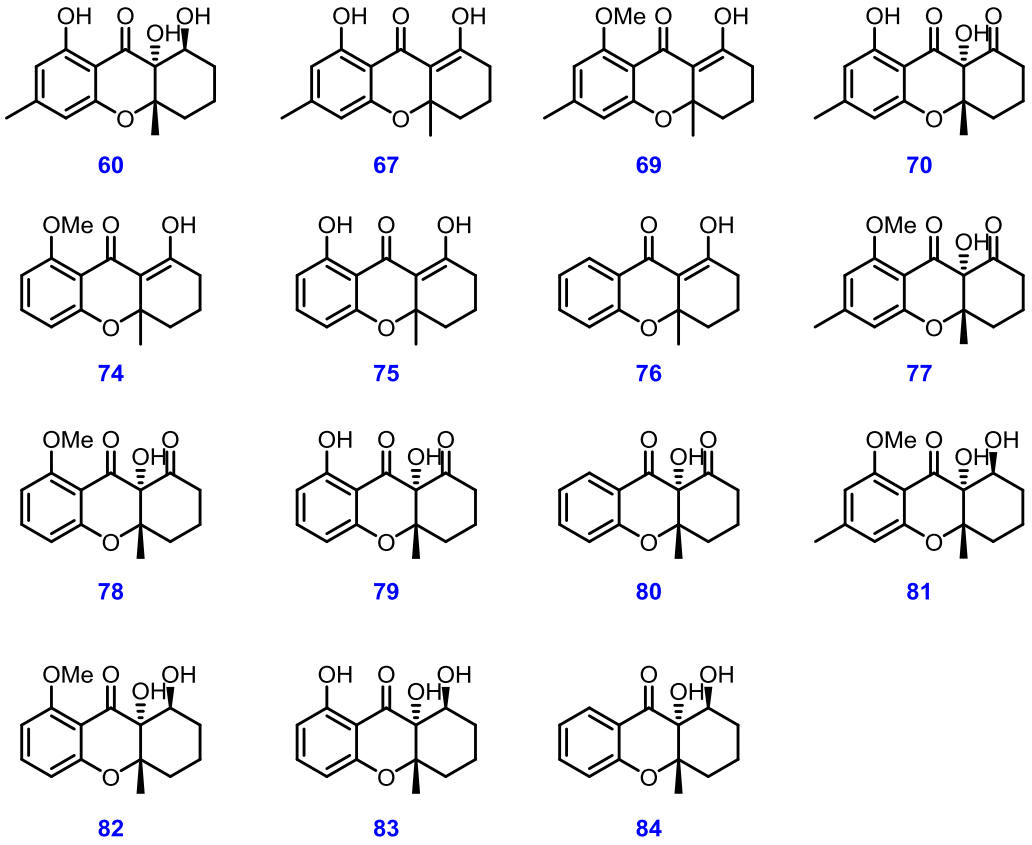



**Figure 13:** Synthesized analogs for biological screening.

**Table 7:** Antibacterial activity of synthesized compounds

| Sr. No. | Comp. No. | Activity (µg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | *E. coli* | *S. aureus* | *K. pneumoniae* | *A. baumannii* | *P. aeruginosa* |
| 1 | **60** | >64 | >64 | >64 | >64 | >64 |
| 2 | **67** | >64 | >64 | >64 | >64 | >64 |
| 3 | **69** | >64 | >64 | >64 | >64 | >64 |
| 4 | **70** | >64 | >64 | >64 | >64 | >64 |
| 5 | **74** | >64 | >64 | >64 | >64 | >64 |
| 6 | **75** | >64 | >64 | >64 | >64 | >64 |
| 7 | **76** | >64 | **8** | >64 | >64 | >64 |
| 8 | **77** | >64 | >64 | >64 | >64 | >64 |
| 9 | **78** | >64 | >64 | >64 | >64 | >64 |
| 10 | **79** | >64 | >64 | >64 | >64 | >64 |
| 11 | **80** | >64 | >64 | >64 | >64 | >64 |
| 12 | **81** | >64 | >64 | >64 | >64 | >64 |
| 13 | **82** | >64 | >64 | >64 | >64 | >64 |
| 14 | **83** | >64 | >64 | >64 | >64 | >64 |
| 15 | **84** | >64 | >64 | >64 | >64 | >64 |
| 12 | Levo | >0.5 | >0.5 | 64 | 8 | >0.5 |

Levo- Levofloxacin used as standard

## 3.4.5. Summary

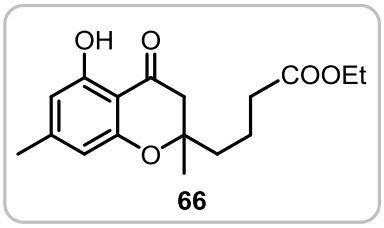
- A short and efficient route for the synthesis of 4-dehydroxydiversonol has been developed using aldol-oxa-Michael reaction as a key step.
- A focused library of fifteen closely related analogs of 4-dehydroxydiversonol was synthesized and characterized, among them, seven are new compounds.
- All the synthesized compounds were tested against four bacterial pathogens and found fourteen compounds were not active at a tested concentration (64 μg/mL). However, only compound **76** showed moderate activity against gram-positive bacteria *Staphylococcus aureus*.

## 3.4.6. Experimental

**General Procedure for Aldol-oxa-Michael Reaction** (Procedure A)

To a mixture of ketone (1 mmol) and hydroxy acetophenone (1 mmol) in dry methanol (4 mL), was added a catalytic amount of pyrrolidine (0.2 mmol) dropwise at room temperature. The reaction mixture was warmed to 50 $^{o}$C and stirring continued for 24h. Methanol from the reaction mixture was removed under reduced pressure and the crude mixture was diluted with ethyl acetate (10 mL) and water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL) and the combined organic extracts were washed with 1N HCl (2 mL), water (5 mL), brine (2 mL), dried over anhydrous $Na_2SO_4$, solvent was removed under reduced pressure and crude residue were purified by flash column chromatography (230-400 mesh) by using ethyl acetate: pet ether to afford pure compound.

**Ethyl 4-(5-hydroxy-2,7-dimethyl-4-oxochroman-2-yl)butanoate (66)**




**66**

Compound **66** was prepared by following the general procedure from the 2,6-dihydroxy-4-methyl acetophenone (**65**) and ethyl 5-oxohexanoate (**64**) in 84% yield as a yellow oil.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3289, 2979, 1745, 1107

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 11.58 (s, 1H), 6.27 (s, 1H), 6.20 (s, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.77 (d, *J* = 16.9 Hz, 1H), 2.61 (d, *J* = 16.9 Hz, 1H), 2.31 (t, *J* = 6.4 Hz, 2H), 2.25 (s, 3H), 1.81 - 1.67 (m, 4H), 1.40 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 197.3, 173.1, 161.6, 159.5, 150.4, 109.3, 108.5, 105.4, 80.3, 60.4, 46.5, 38.7, 34.0, 23.8, 22.5, 19.0, 14.2

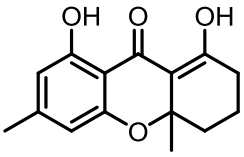
**MS:** 329 $[M+Na]^+$

**HRMS:** Calculated for $C_{17}H_{23}O_5$ $[M+H]^+$: 307.1540 found 307.15378

**General procedure for Dieckmann condensation reaction** (Procedure B)

To a solution of ester (1 mmol) in DCM (5 mL) was cooled to 0 ºC and $TiCl_4$ (1M solution in DCM, 2.2 mmol) was added dropwise, followed by $Et_3N$ (2.5 mmol) and reaction mixture was stirred for 30 minutes at same temperature. Quenched with saturated solution of $NH_4Cl$ (2 mL) and extracted with the DCM (5 mL x 3). The combined organic layer was washed with water (3 mL), brine (3 mL) and dried over anhydrous $Na_2SO_4$. Solvents were removed under reduced pressure and residue was purified by silica gel (100 - 200) column chromatography eluting with the ethyl acetate: pet ether (1:9) to afford the compound as gummy mass.

**1,8-dihydroxy-4a,6-dimethyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (67)**




**67**

Compound **67** was prepared by following the general procedure B from the compound **66** in 25% yields as yellow viscous oil.

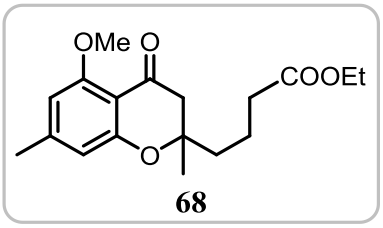
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3324, 3019, 1724, 1612, 1218, 760

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 13.75 (s, 1H), 11.45 (s, 1H), 6.31 (s, 1H), 6.20 (s, 1H), 2.56 - 2.47 (m, 1H), 2.39 (m, 1H), 2.26 (s, 3H), 2.03 (m, 2H), 2.01 (brs, 1H), 1.80 (m, 1H), 1.49 (s, 3H)

**MS:** 283 $[M+Na]^+$

**HRMS:** Calculated for $C_{15}H_{16}O_4$ $[M+H]^+$: 261.1140 found 261.1127

**Ethyl 4-(5-methoxy-2,7-dimethyl-4-oxochroman-2-yl)butanoate (68)**[7]




**68**

To a solution of compound **66** (100 mg, 0.32 mmol) in acetone (2 mL) was added an oven dried potassium carbonate (172 mg, 1.25 mmol) and methyl iodide (0.10 mL, 1.25 mmol) and the reaction mixture was stirred at room temperature for 10 h. After completion of the reaction, acetone was evaporated and the residue was diluted with ethyl acetate (3 mL). The organic layer was washed with water (2 mL), brine (2 mL), dried over anhydrous $Na_2SO_4$, concentrated and purified by column chromatography by eluting with ethyl acetate: pet ether (8:92) afforded 83 mg of pure compound **68** (79% yield) as a colorless oil.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3350, 3014, 1685, 1612

**$^1$H NMR (500 MHz, $CDCl_3$):** δ 6.32 (s, 1H), 6.26 (s, 1H), 4.09 (q, *J* = 7.0 Hz, 2H), 3.86 (s, 3H), 2.69 (d, *J* = 15.6 Hz, 1H), 2.56 (d, *J* = 15.6 Hz, 1H), 2.30 - 2.25 (m, 5H), 1.76 (d, *J* = 6.5 Hz, 2H), 1.70 (brs, 1H), 1.65 (d, *J* = 11.4 Hz, 1H), 1.36 (s, 3H), 1.23 - 1.20 (m, 3H)

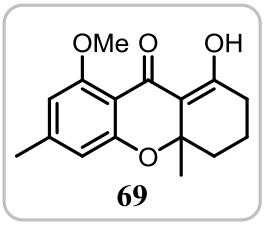
**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 190.7, 173.1, 161.2, 160.1, 147.4, 110.7, 108.4, 104.3, 80.0, 60.3, 55.9, 48.7, 38.6, 34.1, 23.5, 22.3, 19.0, 14.1

**MS:** 343 $[M+Na]^+$

**HRMS:** Calculated for $C_{18}H_{24}O_5$ $[M+H]^+$: 321.1713, found 321.1702

$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**1-hydroxy-8-methoxy-4a-methyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (69)**[7]




**69**

Compound **69** was prepared by following the general procedure B from the compound **68** in 68% yields as opaque solid.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 2953, 1609, 1469, 1108, 878

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 15.99 (s, 1H), 6.34 (s, 2H), 3.93 (s, 3H), 2.55 - 2.43 (m, 1H), 2.42 - 2.34 (m, 1H), 2.31 (s, 3H), 2.09-2.01 (m, 2H), 1.97 (dd, *J* = 6.5, 16.4 Hz, 1H), 1.83 - 1.74 (m, 1H), 1.45 (s, 3H)

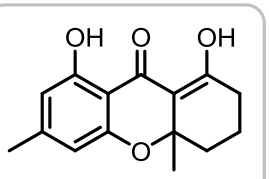
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 182.1, 180.3, 160.6, 160.2, 147.2, 111.2, 108.7, 108.2, 105.4, 78.2, 56.1, 35.8, 30.2, 25.5, 22.4, 18.3

**MS:** 297 $[M+Na]^+$

**HRMS:** Calculated for $C_{16}H_{19}O_4$ $[M+H]^+$: 275.1278, found 275.1276

$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**1,8-dihydroxy-4a,6-dimethyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (67)**




**67**

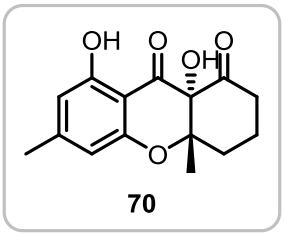
A solution of methyl ether **69** (100 mg, 0.37 mmol) in DCM (5 mL) was cooled at 0 ºC and $BBr_3$ (1.1 mL, 1.1 mmol) was added slowly. The resulting dark-red solution was stirred for 15 min at 0 ºC and further 30 min at room temperature before being quenched

with water (10 mL). The organic layer was separated and the aqueous layer was extracted with DCM (5 mL x 2). The combined extracts were dried over anhydrous $Na_2SO_4$ and the solvent was evaporated in vacuo. After column chromatography on silica gel (ethyl acetate: pet ether 1:9) afforded compound **67** (65 mg, 68% yield) as a yellow viscous oil.

**General Procedure for MMPP Oxidation (**Procedure C**)**

A solution of enol (1 mmol) in ethanol (6.5 mL) was treated with MMPP (0.55 mmol) at -10 ºC and stirred for 2h. The reaction was quenched by addition of silica gel (0.5 g) and the solvent was evaporated under reduced pressure. The purification by column chromatography (230-400 mesh) eluting with ethyl acetate: pet ether (1:4) to yield the diketones.

***Racemic*-8,9a-dihydroxy-4a,6-dimethyl-3,4,4a,9a-tetrahydro-1H-xanthene-1,9(2H)-dione (70)**




**70**

Compound **70** was prepared by following the general procedure C from the compound **69** in 86% yields as white solid.

**Mp:** 164-168 ºC

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3340, 3020, 1720, 1702, 1495, 1611

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 11.29 (s, 1H), 6.36 (s, 1H), 6.25 (s, 1H), 4.02 (s, 1H), 3.31 (dt, *J* = 7.6, 12.9 Hz, 1H), 2.67 (dt, *J* = 5.0, 13.3 Hz, 1H), 2.28 (s, 3H), 2.24 (d, *J* = 4.1 Hz, 1H), 2.06 (dd, *J* = 6.6, 13.1 Hz, 1H), 1.85 (dd, *J* = 3.4, 13.1 Hz, 1H), 1.70-1.60 (m, 1H), 1.30 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 205.8, 192.2, 163.0, 157.1, 151.3, 110.8, 108.9, 104.6, 84.3, 77.6, 37.3, 31.2, 22.5, 20.4, 17.9

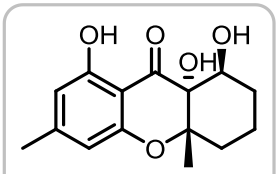
**MS:** 299 $[M+Na]^+$

**HRMS:** Calculated for $C_{15}H_{17}O_5$ $[M+H]^+$: 277.1071, found 277.1080

**General Procedure for $NaBH_4$ Reduction** (Procedure D)

A solution of diketone (1 mmol) in MeOH: DCM (2:1, 7.5 mL) was treated slowly with powdered $NaBH_4$ (1.1 mmol) at -78 ºC. The resulting mixture was stirred at -78 ºC for further 15 minutes before being quenched with water (5 mL). Water (10 mL) was added and the aqueous layer was extracted with DCM (10 mL x 3). The combined extracts were dried over anhydrous $Na_2SO_4$ and concentrated in vacuo. After column chromatography on silica gel eluting with ethyl acetate: pet ether (1:3) gave us diol as a colourless solid.

***Racemic*-1,8,9a-trihydroxy-4a,6-dimethyl-1,2,3,4,4a,9a-hexahydro-9H-xanthen-9-one (60)[8]**




**60**

Compound **60** was prepared by following the general procedure D from the compound **70** in 90% yields as white solid.

**Mp:** 194-196 °C

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3567, 3342, 2935, 1634, 1567, 1395, 1202, 1099, 962

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.95 (s, 1H), 6.37 (s, 1H), 6.26 (s, 1H), 4.45 (brs, 1H), 3.01 (s, 1H), 2.56 (brs, 1H), 2.28 (s, 3H), 2.15-2.09 (m, 1H), 2.01-1.93 (m, 1H), 1.81-1.67 (m, 4H), 1.57 (s, 3H)

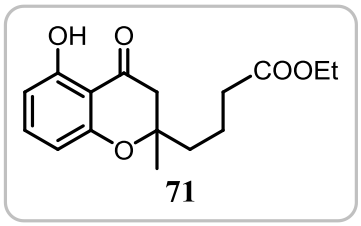
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 197.8, 162.3, 157.6, 150.8, 110.5, 109.2, 104.7, 83.6, 74.1, 67.3, 32.2, 26.7, 22.6, 19.8, 17.9

**MS:** 301 $[M+Na]^+$

**HRMS:** Calculated for $C_{15}H_{19}O_5$ $[M+H]^+$: 279.1227, found 279.1238

$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**Ethyl 4-(5-hydroxy-2-methyl-4-oxochroman-2-yl)butanoate (71)**




**71**

Compound **71** was prepared by following the general procedure A from the compound **34** and **64** in 78% yield as a colorless oil.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3290, 2979, 1745, 1716, 1107

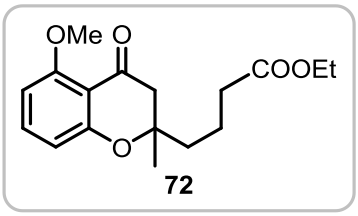
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 11.61 (s, 1H), 7.31 (t, *J* = 8.2 Hz, 1H), 6.43 (d, *J* = 8.3 Hz, 1H), 6.35 (d, *J* = 8.1 Hz, 1H), 4.09 (d, *J* = 7.3 Hz, 2H), 2.80 (d, *J* = 16.9 Hz, 1H), 2.65 (d, *J* = 16.9 Hz, 1H), 2.33-2.27 (m, 2H), 1.83-1.64 (m, 4H), 1.41 (s, 3H), 1.25-1.21 (m, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 198.1, 173.0, 161.7, 159.7, 138.3, 108.6, 107.8, 107.4, 80.4, 60.3, 46.7, 38.6, 34.0, 23.7, 19.0, 14.1

**MS:** 315 $[M+Na]^+$

**HRMS:** Calculated for $C_{16}H_{21}O_5$ $[M+H]^+$: 293.1389, found 293.1379

**Ethyl 4-(5-methoxy-2-methyl-4-oxochroman-2-yl)butanoate (72)**




**72**

Compound **72** was prepared by following the same procedure used for synthesis of **68** from compound **71** in 82% yield as viscous oil.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3019, 2937, 1735, 1716, 1557

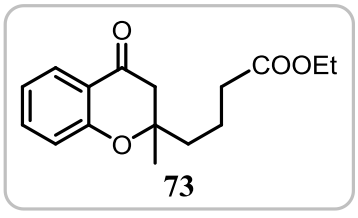
**$^{1}$H NMR (500 MHz, $CDCl_3$):** δ 7.34 (t, *J* = 8.4 Hz, 1H), 6.50 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.88 (s, 3H), 2.74 (d, *J* = 15.6 Hz, 1H), 2.60 (d, *J* = 15.6 Hz, 1H), 2.29 (t, *J* = 6.9 Hz, 2H), 1.79-1.68 (m, 4H), 1.38 (s, 3H), 1.24 - 1.21 (m, 3H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 191.2, 173.1, 161.3, 160.2, 135.9, 110.6, 110.4, 103.1, 80.2, 60.3, 56.1, 48.7, 38.6, 34.1, 23.5, 19.0, 14.2

**MS:** 329 $[M+Na]^+$

**HRMS:** Calculated for $C_{17}H_{23}O_5$ $[M+H]^+$: 307.1545, found 307.1541

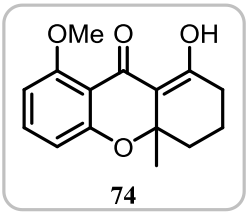
**Ethyl 4-(2-methyl-4-oxochroman-2-yl)butanoate (73)**[7]




**73**

Compound **73** was prepared by following the general procedure A from compound **36** and **64** in 76% yield as viscous oil. The $^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**1-hydroxy-8-methoxy-4a-methyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (74)**




**74**

Compound **74** was prepared by following the general procedure B from the compound **72** in 65% yields as yellow viscous oil.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3510, 2937, 1701, 1650, 1557

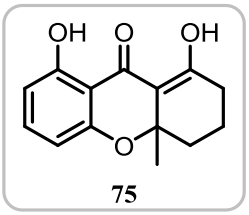
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 15.98 (s, 1H), 7.32 (t, *J* = 8.2 Hz, 1H), 6.51 (dd, *J* = 4.6, 8.2 Hz, 2H), 3.92 (s, 3H), 2.55-2.34 (m, 2H), 2.07 - 1.93 (m, 3H), 1.82-1.70 (m, 1H), 1.44 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 181.8, 181.2, 160.7, 160.3, 135.4, 110.7, 110.5, 108.9, 104.2, 78.1, 56.1, 35.8, 30.3, 25.4, 25.4, 18.2

**MS:** 283 $[M+Na]^+$

**HRMS:** Calculated for $C_{15}H_{17}O_4$ $[M+H]^+$: 261.1127, found 261.1122

**1,8-dihydroxy-4a-methyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (75)**




**75**

Compound **75** was prepared by following the similar procedure used for the synthesis of compound **67** from the compound **74** in 86% yield.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3516, 3435, 1695, 1557

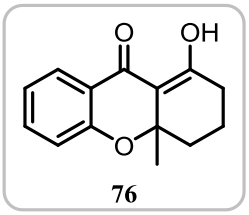
**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 13.78 (s, 1H), 11.50 (s, 1H), 7.29 (t, *J* = 8.2 Hz, 1H), 6.47 (d, *J* = 8.5 Hz, 1H), 6.36 (d, *J* = 8.5 Hz, 1H), 2.56 - 2.47 (m, 1H), 2.43 - 2.35 (m, 1H), 2.06 (d, *J* = 7.3 Hz, 2H), 2.00 (d, *J* = 11.0 Hz, 1H), 1.81 (dd, *J* = 6.7, 17.7 Hz, 1H), 1.50 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 188.1, 175.8, 162.1, 159.0, 137.6, 109.5, 108.2, 108.1, 107.3, 78.5, 35.4, 28.8, 26.4, 18.2

**MS:** 269 $[M+Na]^+$

**HRMS:** Calculated for $C_{14}H_{15}O_4$ $[M+H]^+$: 247.0970, found 247.0966

**1-hydroxy-4a-methyl-2,3,4,4a-tetrahydro-9H-xanthen-9-one (76)[7]**




**76**

Compound **76** was prepared by following the general procedure B from the compound **73** in 71% yield as gummy mass.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3413, 1739, 1716, 1575

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 15.26 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.42 (t, *J* = 7.7 Hz, 1H), 7.01 (m, 1H), 6.89 (d, *J* = 8.3 Hz, 1H), 2.56-2.40 (m, 2H), 2.11 (m, 2H), 2.01 (d, *J* = 13.9 Hz, 1H), 1.83 (brs, 1H), 1.49 (s, 3H)

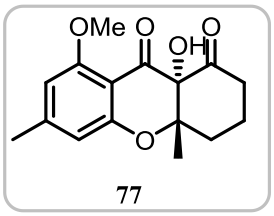
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 182.6, 180.2, 158.5, 135.2, 126.5, 121.3, 120.4, 118.0, 108.8, 78.5, 35.7, 30.5, 26.3, 18.4

**MS:** 253 $[M+Na]^+$

**HRMS:** Calculated for $C_{14}H_{15}O_3$ $[M+H]^+$: 231.1021 found 221.1029

$^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**9a-hydroxy-8-methoxy-4a,6-dimethyl-3,4,4a,9a-tetrahydro-1H-xanthene-1,9(2H)-dione (77)**[7]




**77**

Compound **77** was prepared by following the general procedure C from the compound **69** with 78% yield.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3114, 2931, 1726, 1709, 1530

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 6.34 (s, 1H), 6.23 (s, 1H), 4.92 (s, 1H), 3.70 (s, 3H), 3.41 (m, 1H), 2.72 (m, 1H), 2.28 (s, 3H), 2.18 (m, 1H), 2.03 (m, 1H), 1.79 (m, 1H), 1.66-1.59 (m, 1H), 1.25 (s, 3H)

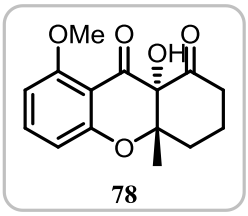
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 207.2, 186.5, 161.6, 159.2, 147.9, 110.6, 107.6, 105.0, 83.6, 77.9, 55.6, 37.4, 31.3, 22.4, 20.6, 17.7

**MS:** 313 $[M+Na]^+$

**HRMS:** Calculated for $C_{16}H_{19}O_5$ $[M+H]^+$: 291.1233, found 291.1242

The $^{1}$H NMR and $^{13}$C NMR compared with the literature values and found to be identical.

**9a-hydroxy-8-methoxy-4a-methyl-3,4,4a,9a-tetrahydro-1H-xanthene-1,9(2H)-dione (78)**[7]




**78**

Compound **78** was prepared by following the general procedure C from the compound **74** in 76% yield as sticky material.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3120, 2935, 1725, 1710, 1535

**$^{1}$H NMR (400 MHz, DMSO-d$_6$):** δ 7.44 (t, *J* = 8.1 Hz, 1H), 6.65 (d, *J* = 7.8 Hz, 1H), 6.51 (d, *J* = 7.8 Hz, 1H), 3.79 (s, 3H), 3.12 (d, *J* = 7.3 Hz, 1H), 2.52 (m, 1H), 1.96 (m, 2H), 1.73 (d, *J* = 11.2 Hz, 1H), 1.55 (d, *J* = 13.2 Hz, 1H), 1.14 (s, 3H)

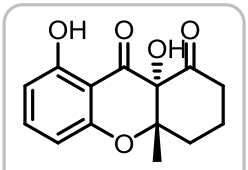
**$^{13}$C NMR (100 MHz, DMSO-d$_6$):** δ 206.5, 185.0, 161.0, 158.7, 135.8, 109.7, 109.5, 104.3, 83.4, 77.1, 55.8, 37.3, 30.9, 19.9, 17.3

**MS:** 299 $[M+Na]^+$

**HRMS:** Calculated for $C_{15}H_{17}O_5$ $[M+H]^+$: 277.1076, found 277.1079

$^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**8,9a-dihydroxy-4a-methyl-3,4,4a,9a-tetrahydro-1H-xanthene-1,9(2H)-dione (79)**




**79**

Compound **79** was prepared by following the general procedure C from the compound **75** with 68% yield.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3219, 2950, 1719, 1693, 1560

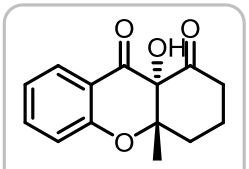
**$^{1}$H NMR (400 MHz, CDCl$_3$):** δ 11.32 (s, 1H), 7.38 (t, *J* = 8.2 Hz, 1H), 6.53 (d, *J* = 7.9 Hz, 1H), 6.42 (d, *J* = 7.9 Hz, 1H), 4.10 (s, 1H), 3.30 (dt, *J* = 7.6, 13.0 Hz, 1H), 2.68 (dt, *J* = 5.5, 13.4 Hz, 1H), 2.26 (dd, *J* = 4.6, 12.5 Hz, 1H), 2.06 (m, 1H), 1.87 (d, *J* = 9.8 Hz, 1H), 1.70 - 1.63 (m, 1H), 1.31 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 205.7, 192.9, 163.1, 157.3, 138.9, 110.2, 108.1, 106.6, 84.4, 77.6, 37.3, 31.2, 20.4, 17.8

**MS:** 285 $[M+Na]^+$

**HRMS:** Calculated for $C_{14}H_{15}O_5$ $[M+H]^+$: 263.0919, found 263.0920

**9a-hydroxy-4a-methyl-3,4,4a,9a-tetrahydro-1H-xanthene-1,9(2H)-dione (80)** [7, 8]




**80**

Compound **80** was prepared by following the general procedure C from the compound **76** with 81% yield as sticky material.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3120, 2922, 1716, 1698, 1562

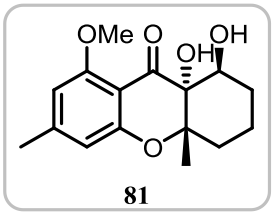
**$^1$H NMR (400 MHz, $CD_3OD$):** δ 7.86 (d, *J* = 7.8 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 3.28 (brs, 1H), 2.75 (dt, *J* = 4.5, 13.3 Hz, 1H), 2.14 (m, 1H), 2.06 (m, 1H), 1.86 (d, *J* = 12.5 Hz, 1H), 1.68 (d, *J* = 13.7 Hz, 1H), 1.24 (s, 3H)

**$^{13}$C NMR (100 MHz, $CD_3OD$):** δ 209.2, 189.3, 159.3, 137.5, 128.4, 128.0, 122.7, 121.1, 119.5, 85.8, 38.7, 32.6, 21.7, 17.9

**MS:** 269 $[M+Na]^+$

$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**1,9a-dihydroxy-8-methoxy-4a,6-dimethyl-1,2,3,4,4a,9a-hexahydro-9H-xanthen-9-one (81)**[7]




**81**

Compound **81** was prepared by following the general procedure D from the compound **77** with 82% yield.

**IR $\upsilon_{max}$(film): cm$^{-1}$** 3350, 2937, 1703, 1551

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 6.36 (d, *J* = 9.3 Hz, 2H), 4.80 (s, 1H), 4.71 (brs, 1H), 4.34 (brs, 1H), 3.87 (s, 3H), 3.33 (brs, 1H), 2.35 (s, 1H), 2.30 (s, 3H), 2.06-1.91 (m, 3H), 1.69 (brs, 1H), 1.65 (s, 3H)

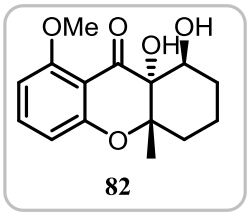
**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 158.7, 151.6, 140.4, 111.2, 109.6, 104.2, 78.6, 73.4, 70.9, 70.1, 55.6, 34.6, 28.0, 21.8, 19.7, 17.9

**MS:** 315 $[M+Na]^+$

**HRMS:** Calculated for $C_{16}H_{21}O_5$ $[M+H]^+$: 293.1389, found 293.1398

$^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**(1S,4aS,9aR)-1,9a-dihydroxy-8-methoxy-4a-methyl-1,2,3,4,4a,9a-hexahydro-9H-xanthen-9-one (82)**




**82**

Compound **82** was prepared by following the general procedure D from the compound **78** with 86% yield as solid.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3244, 1699, 1525, 1464

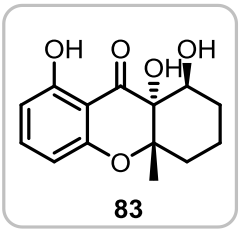
**$^1$H NMR (400 MHz, $CD_3OD$):** δ 7.15 (t, *J* = 8.3 Hz, 1H), 6.54 (d, *J* = 7.8 Hz, 1H), 6.42 (d, *J* = 8.3 Hz, 1H), 4.75 (s, 1H), 4.60 (brs, 1H), 4.25 (brs, 1H), 3.85 (s, 3H), 2.16-1.98 (m, 3H), 1.98-1.87 (m, 1H), 1.64 (s, 3H)

**$^{13}$C NMR (100 MHz, $CD_3OD$):** δ 161.0, 154.2, 130.6, 113.9, 111.4, 103.5, 78.6, 76.2, 71.1, 70.6, 56.1, 35.8, 29.4, 21.2, 19.1

**MS:** 301 $[M+Na]^+$

**HRMS**: Calculated for $C_{15}H_{18}O_5Na$ $[M+Na]^+$: 301.1051 found 301.1045

**1,8,9a-trihydroxy-4a-methyl-1,2,3,4,4a,9a-hexahydro-9H-xanthen-9-one (83)**




**83**

Compound **83** was prepared by following the general procedure D from the compound **79** in 76% yields as sticky material.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3344, 1705, 1620, 1525, 1464

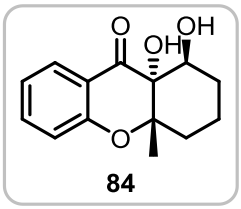
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 10.99 (s, 1H), 7.39 (t, *J* = 8.4 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 6.43 (d, *J* = 8.4 Hz, 1H), 4.47 (brs, 1H), 3.01 (brs, 1H), 2.53 (brs, 1H), 2.19-2.11 (m, 1H), 2.02-1.93 (m, 1H), 1.82-1.69 (m, 4H), 1.59 (s, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 198.5, 162.5, 157.8, 138.5, 110.0, 108.4, 106.8, 83.7, 74.1, 67.3, 32.2, 26.8, 19.7, 17.9

**MS:** 287 $[M+Na]^+$

**HRMS**: Calculated for $C_{14}H_{16}O_5$ $[M+H]^+$: 264.0998 found 264.1004

**1,9a-dihydroxy-4a-methyl-1,2,3,4,4a,9a-hexahydro-9H-xanthen-9-one (84)**[7]




**84**

Compound **84** was prepared by following the general procedure D from the compound **80** in 88% yield as sticky material.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3317, 2933, 1710, 1669

**$^1$H NMR (400 MHz, $CD_3OD$):** δ 7.83 (dd, *J* = 1.7, 7.8 Hz, 1H), 7.51 (m, 1H), 7.01 (t, *J* = 7.9 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 4.37 (brs, 1H), 2.31 (dt, *J* = 4.3, 13.0 Hz, 1H), 2.04 (m, 1H), 1.82 (m, 1H), 1.67 (m, 3H), 1.51 (s, 3H)

**$^{13}$C NMR (100 MHz, $CD_3OD$):** δ 194.1, 160.2, 137.1, 128.2, 121.9, 121.7, 119.5, 84.1, 75.2, 69.1, 33.6, 28.7, 20.4, 19.1

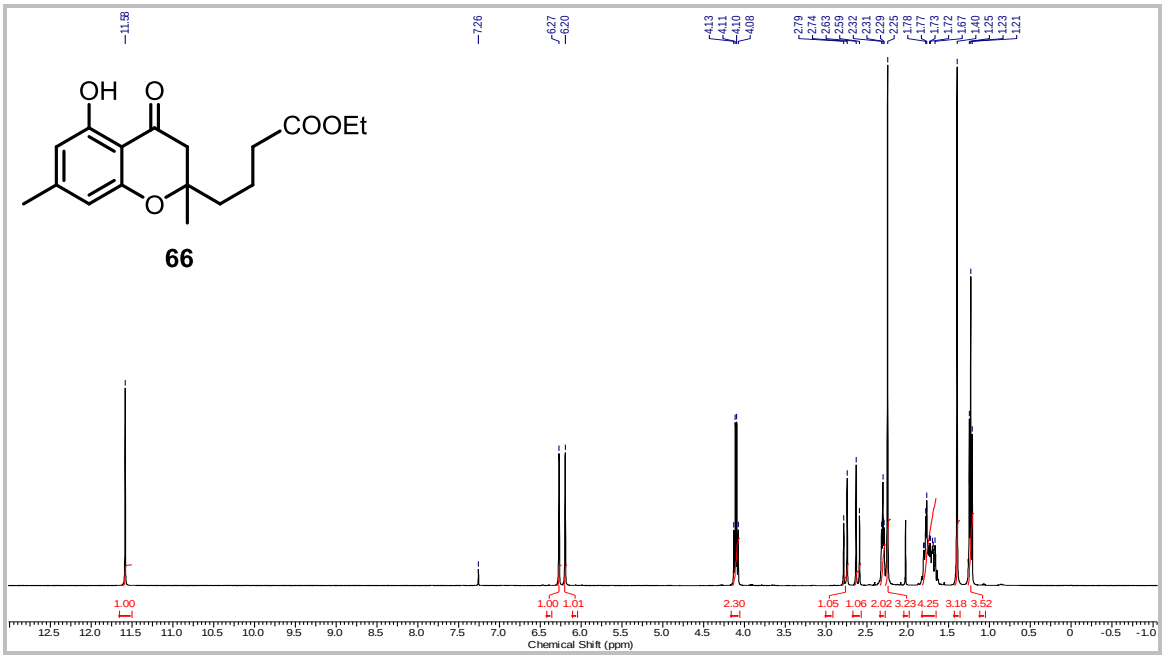
**MS:** 271 $[M+Na]^+$

**HRMS:** Calculated for $C_{14}H_{17}O_4$ $[M+H]^+$: 249.1127, found 249.1131

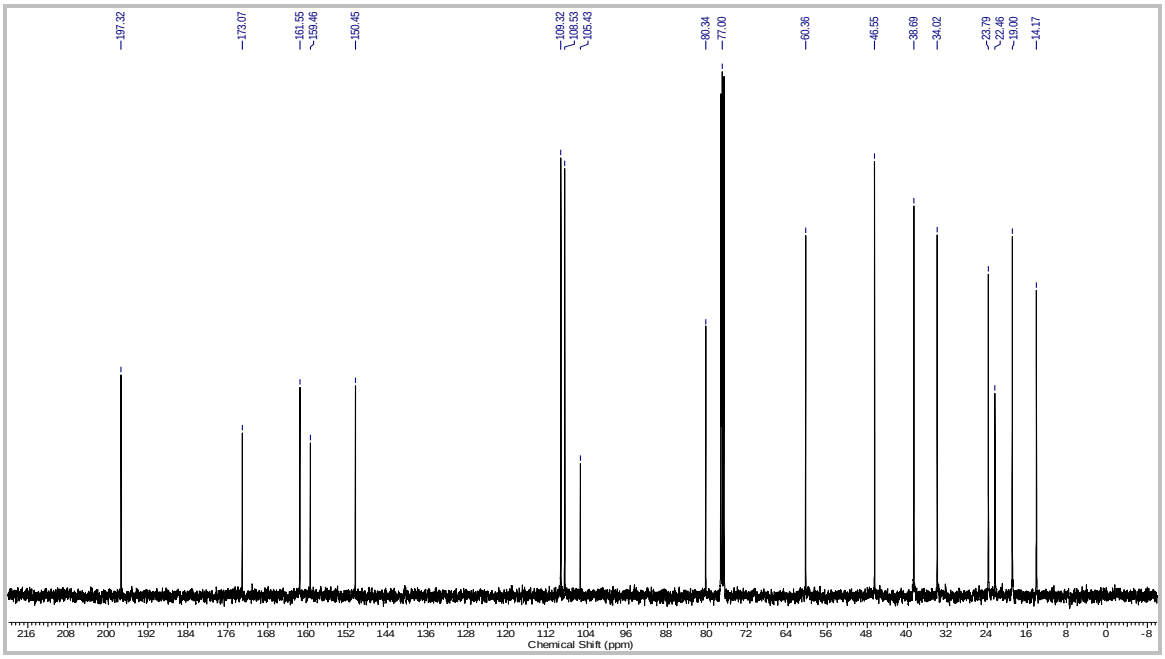
$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

## 3.4.7. Spectra

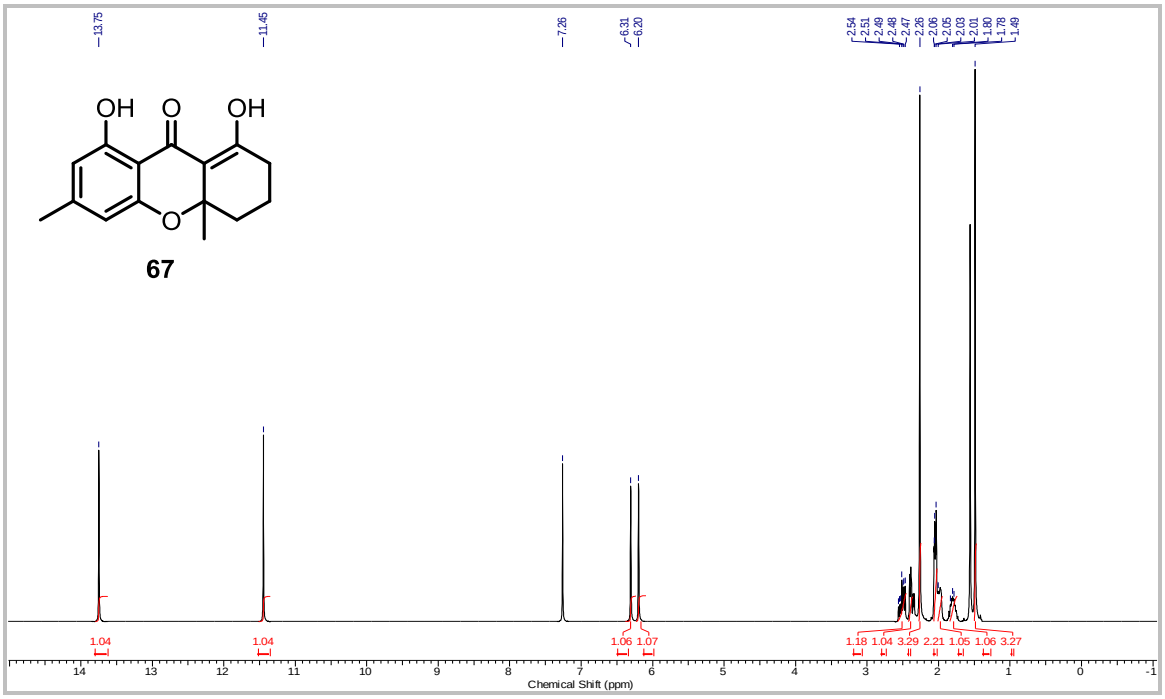
**$^{1}$H NMR (400 MHz, $CDCl_3$) of compound 66**



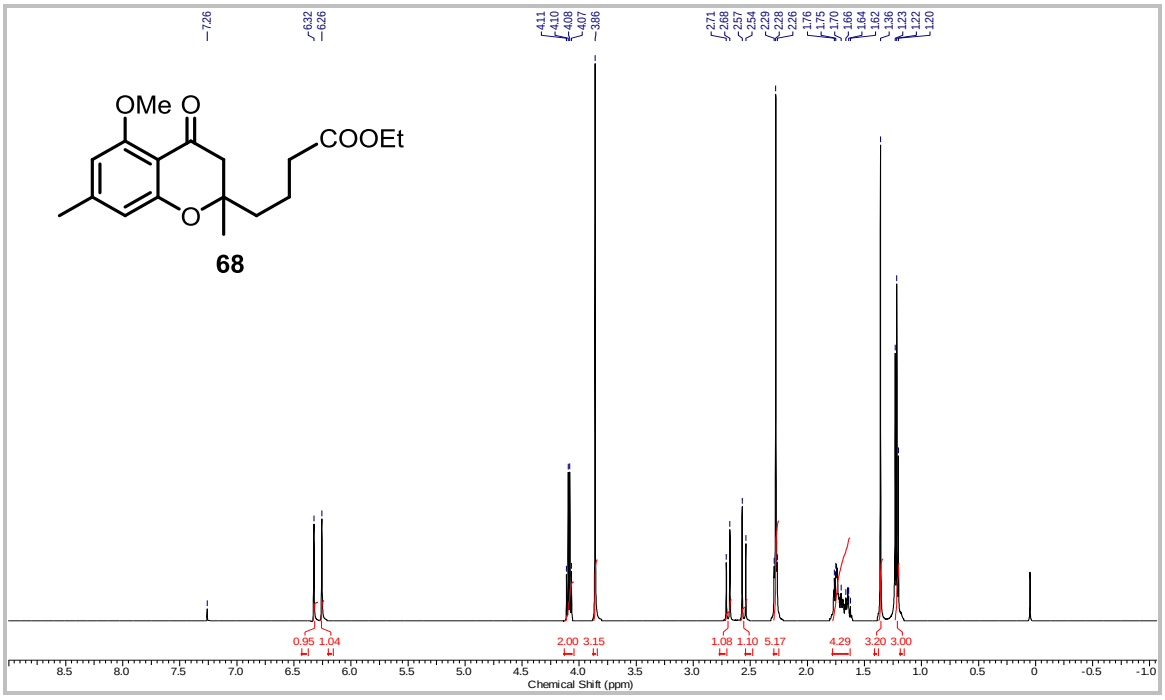

**$^{13}$C NMR (100 MHz, $CDCl_3$) of compound 66**



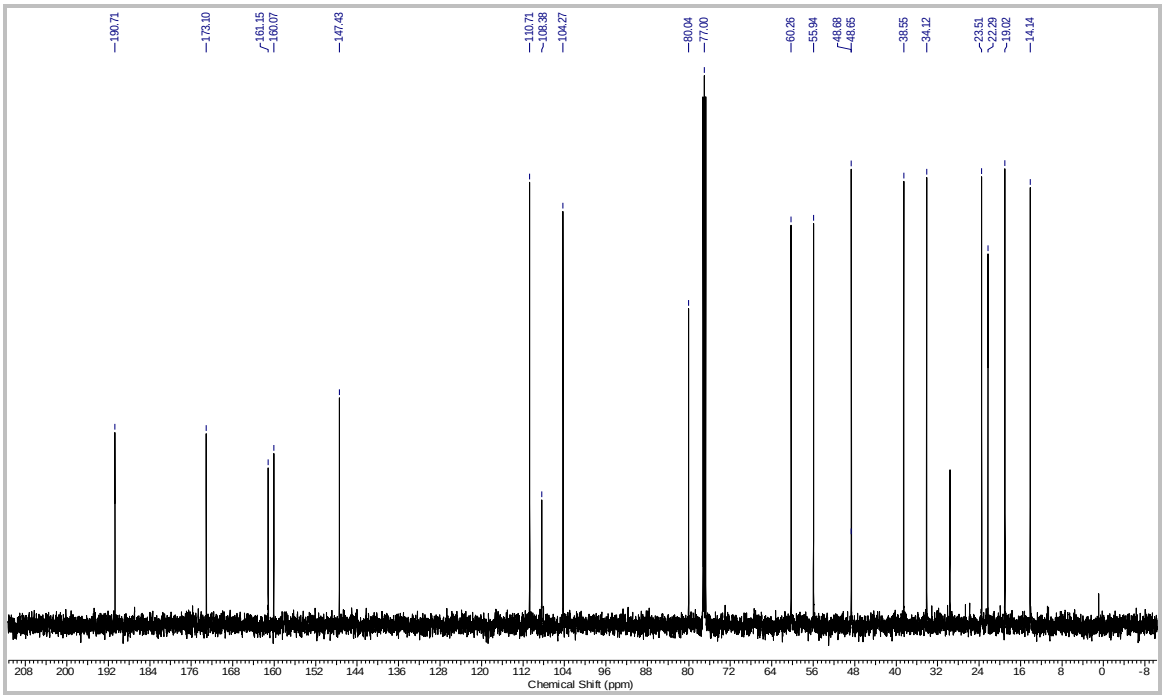
**$^1$H NMR (400 MHz, $CDCl_3$) of compound 67**



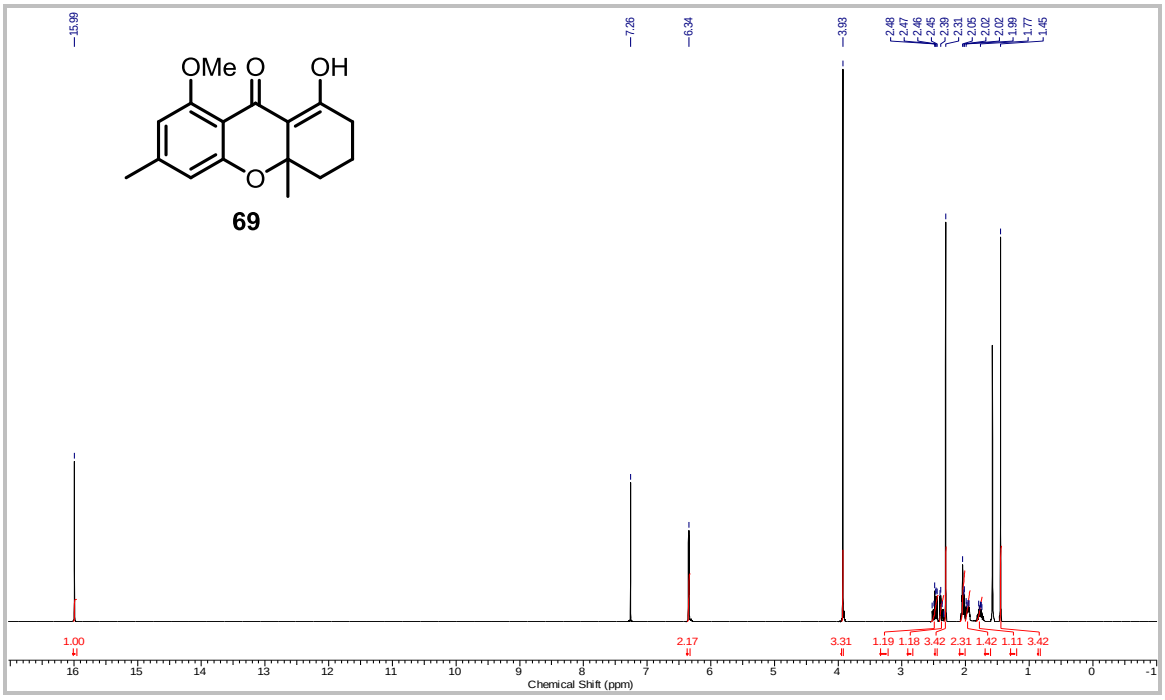
## $^{1}$H NMR (500 MHz $CDCl_3$) of compound 68



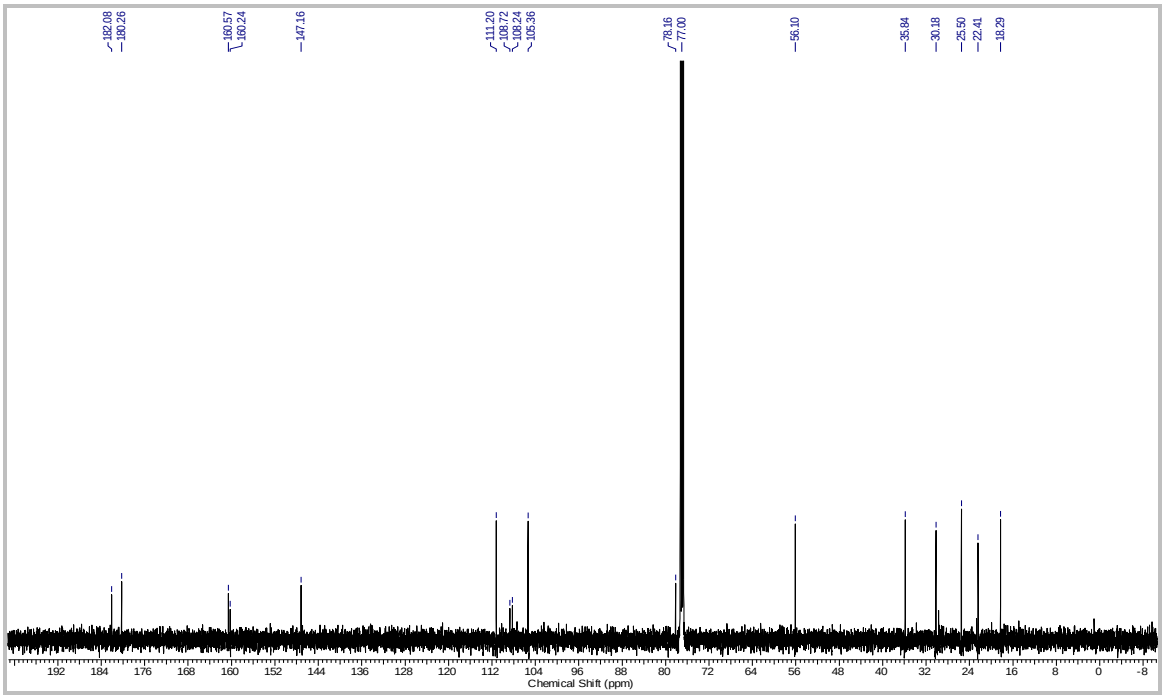

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 68



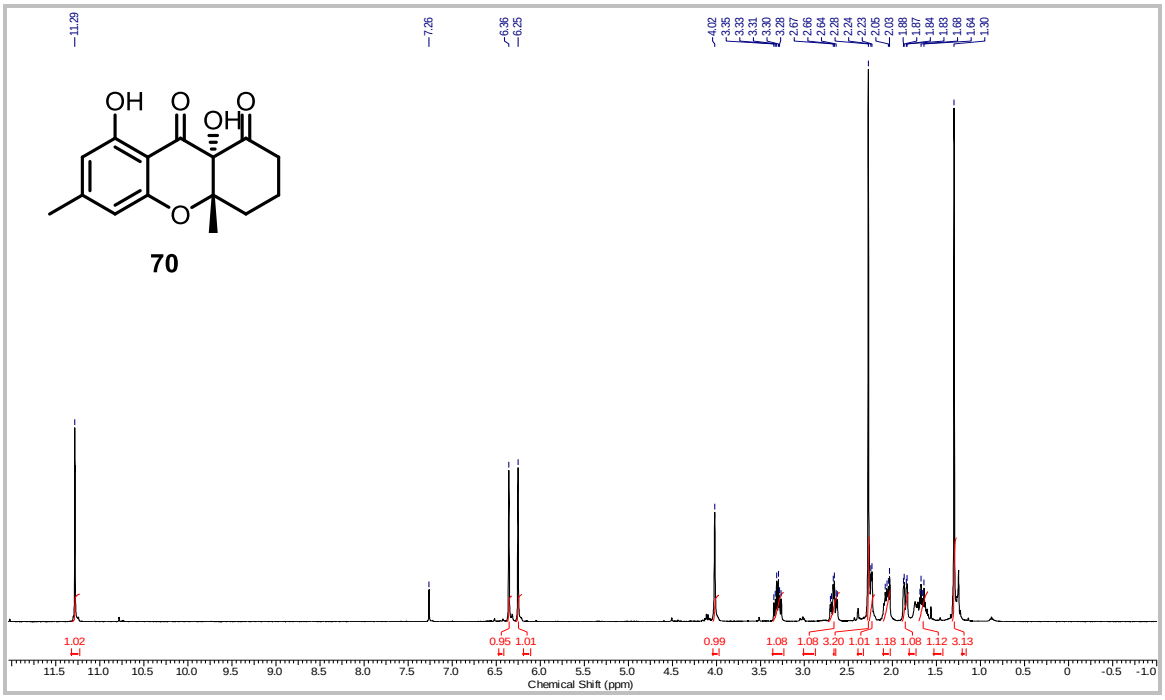
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 69



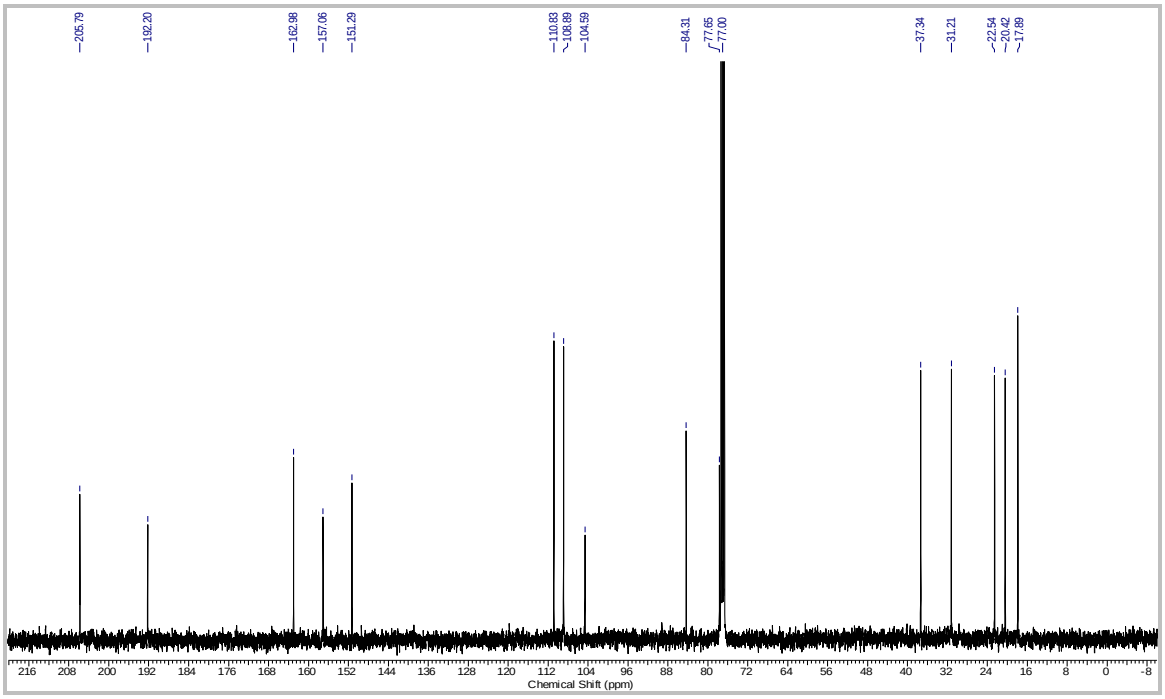

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 69



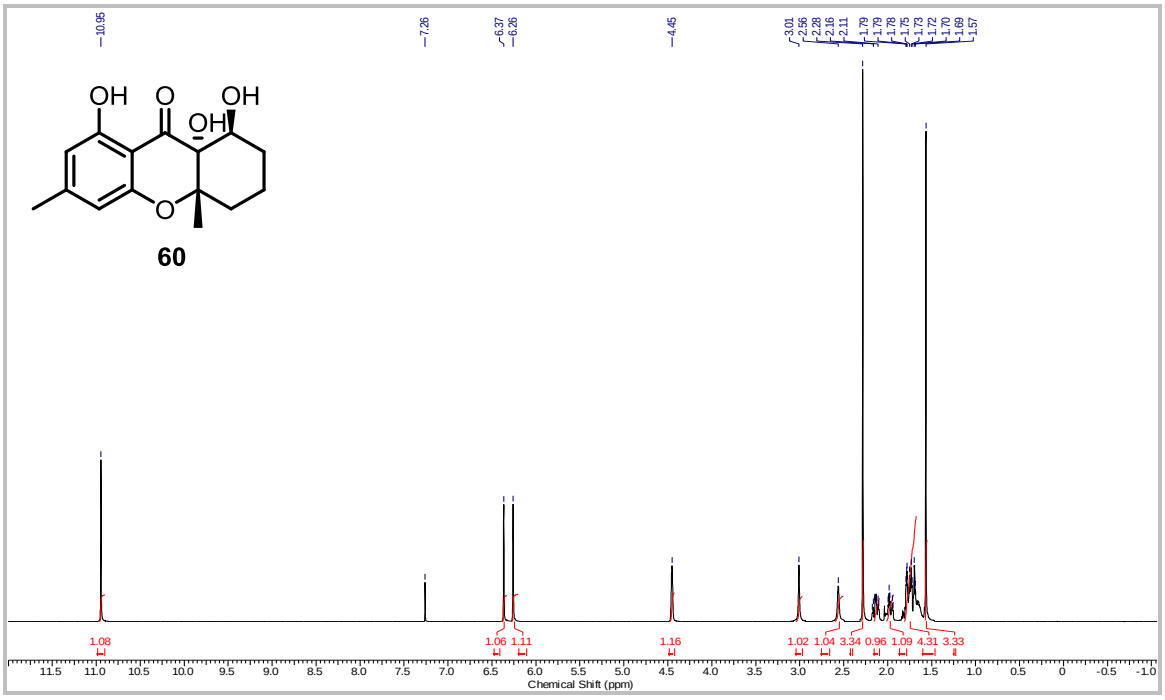
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 70



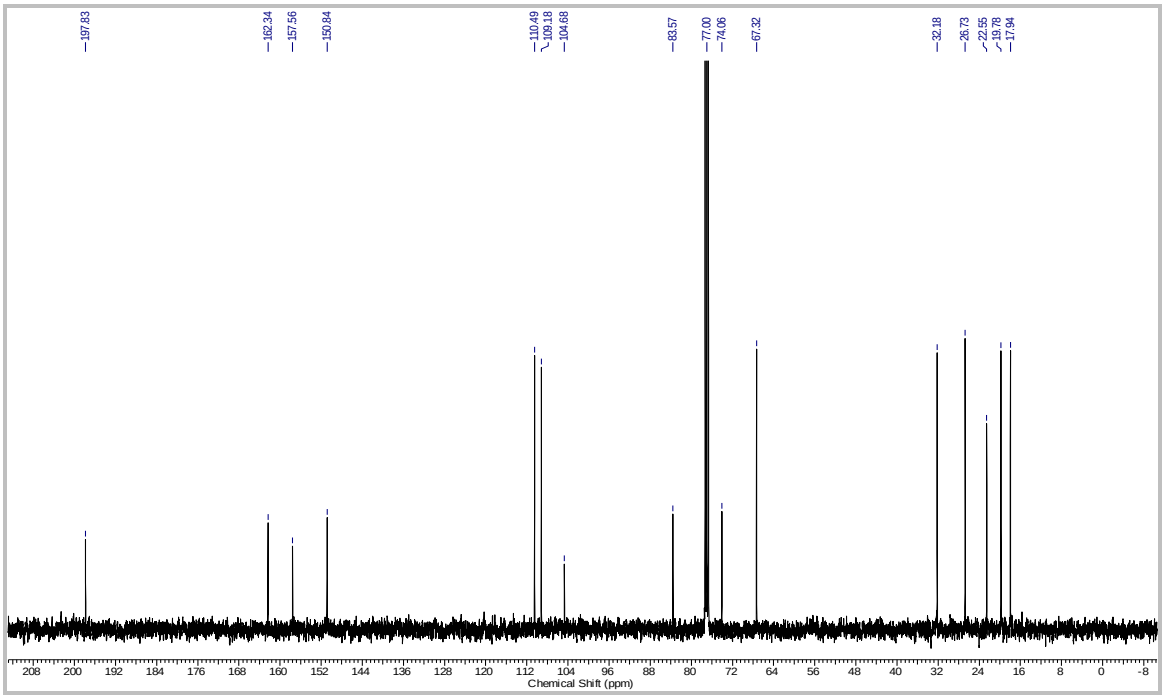

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 70



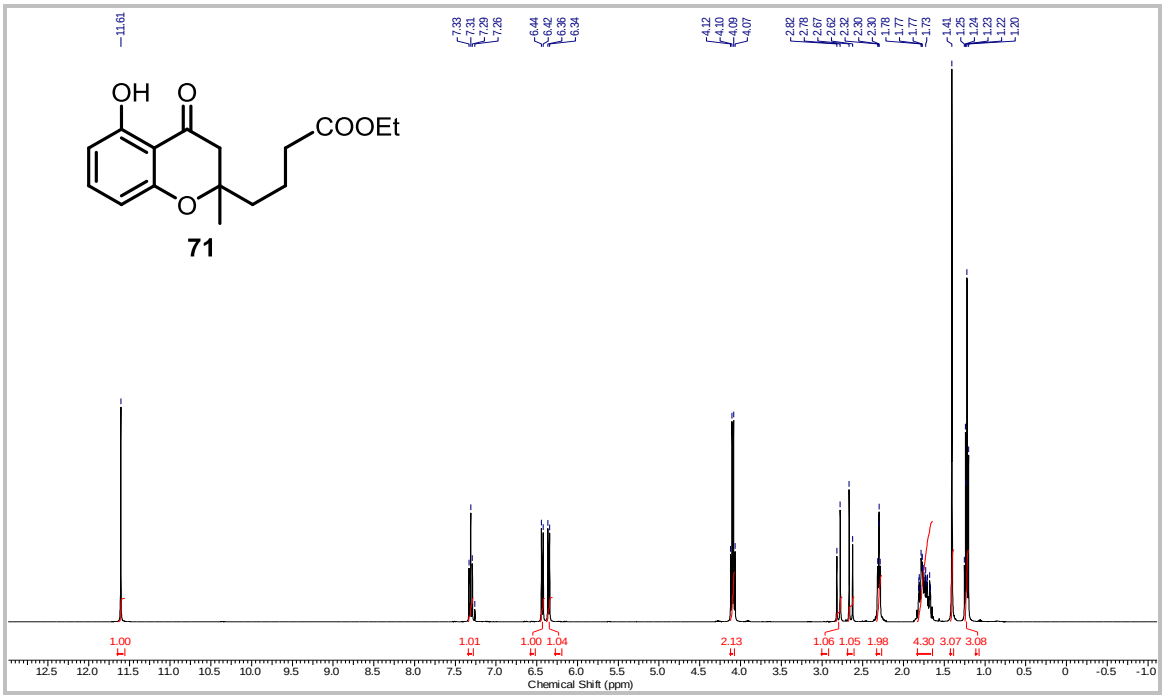
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 60



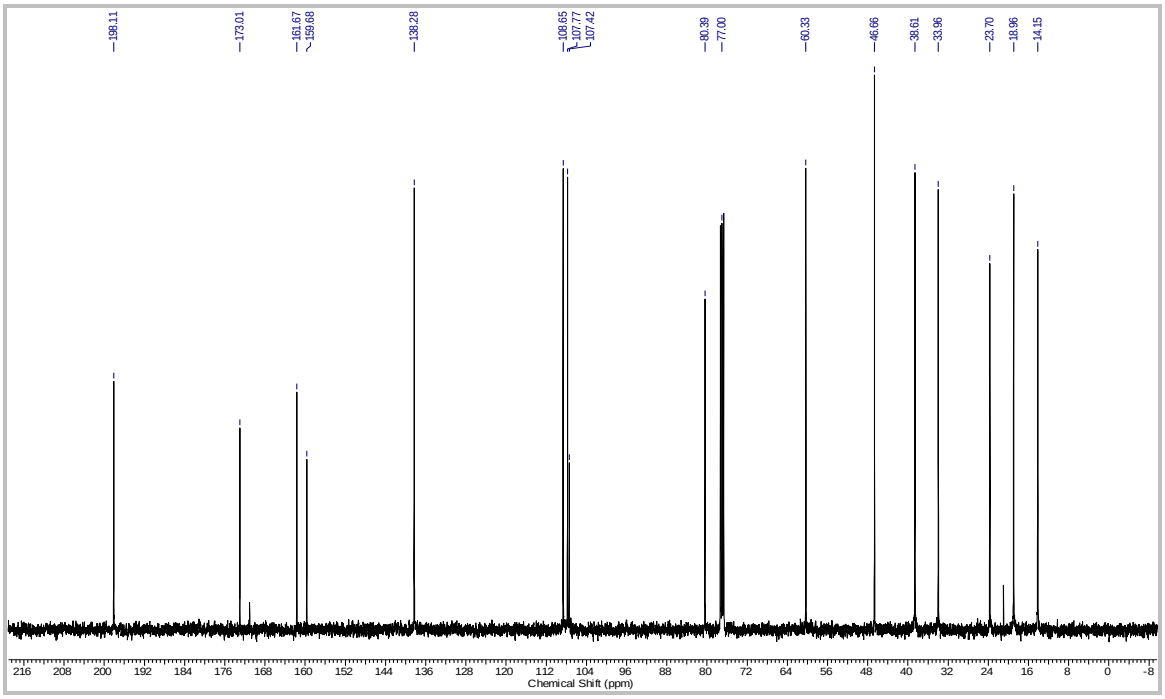

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 60



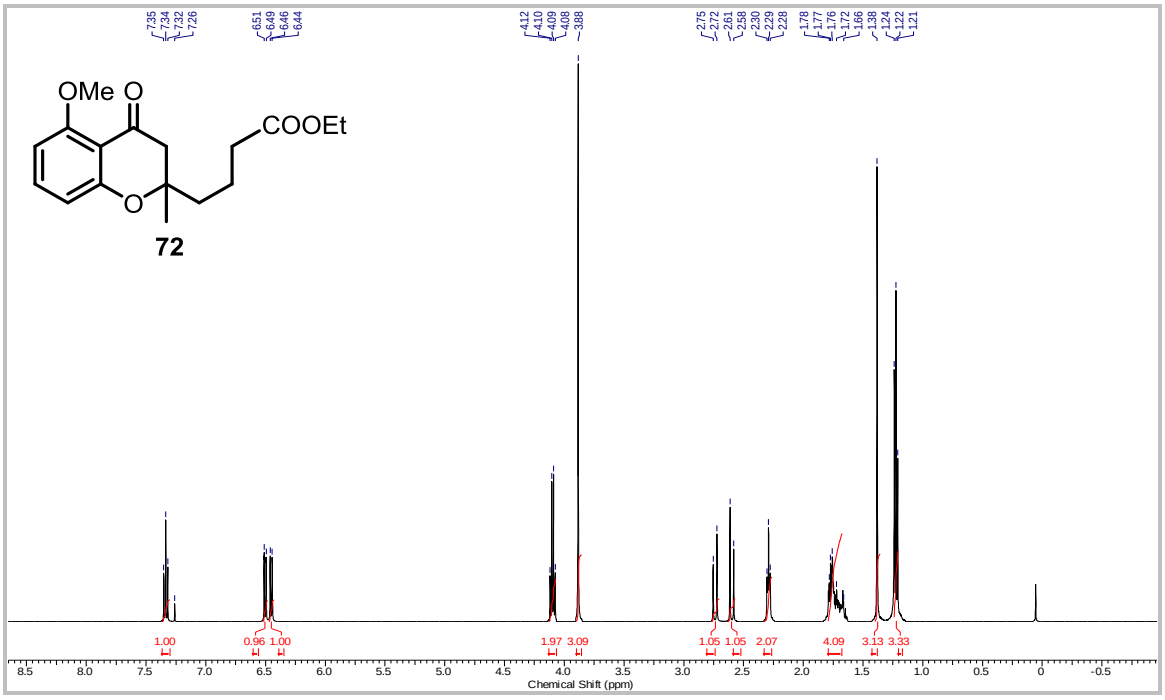
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 71



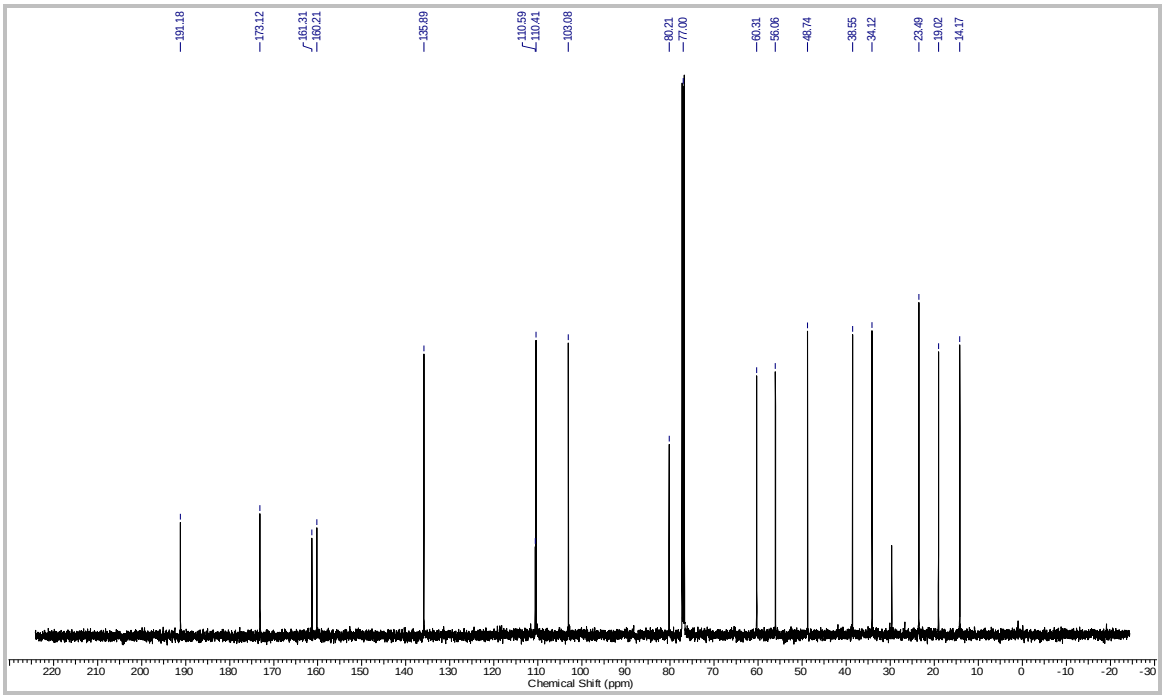

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 71



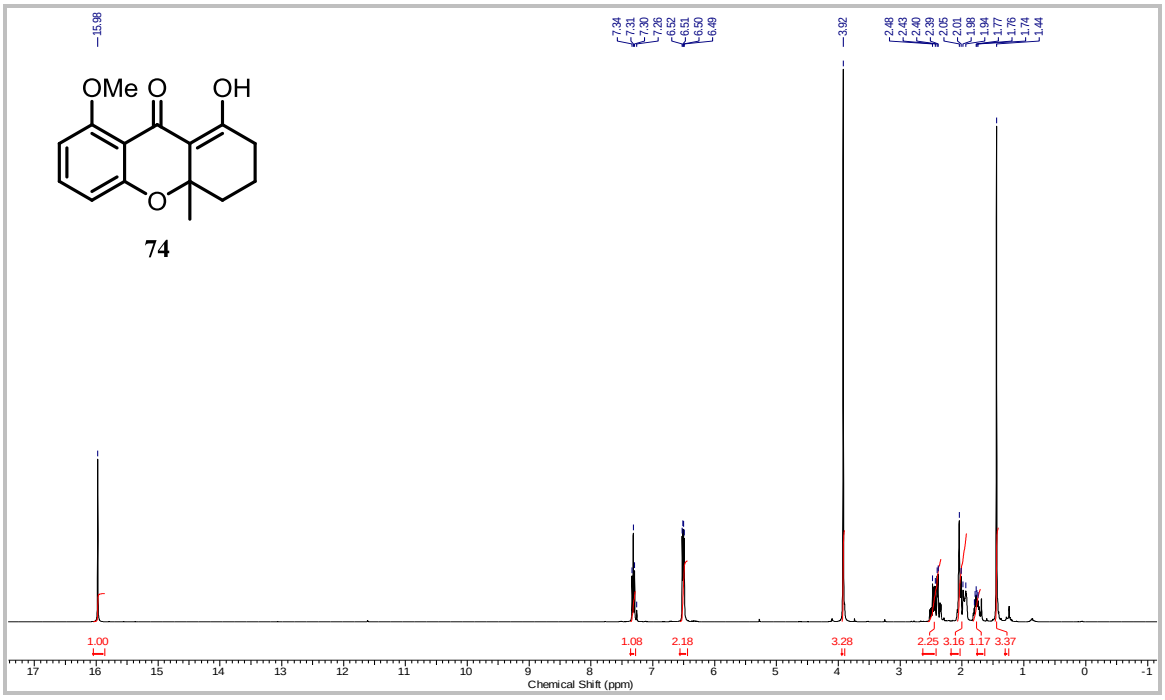
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 72



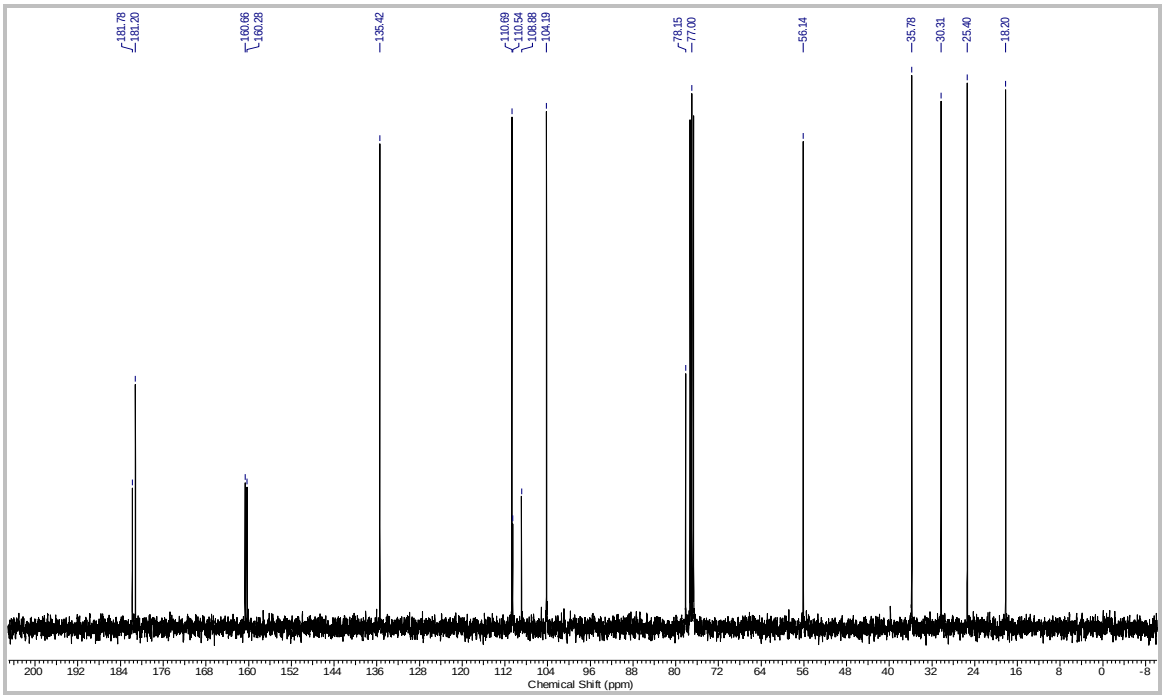

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 72



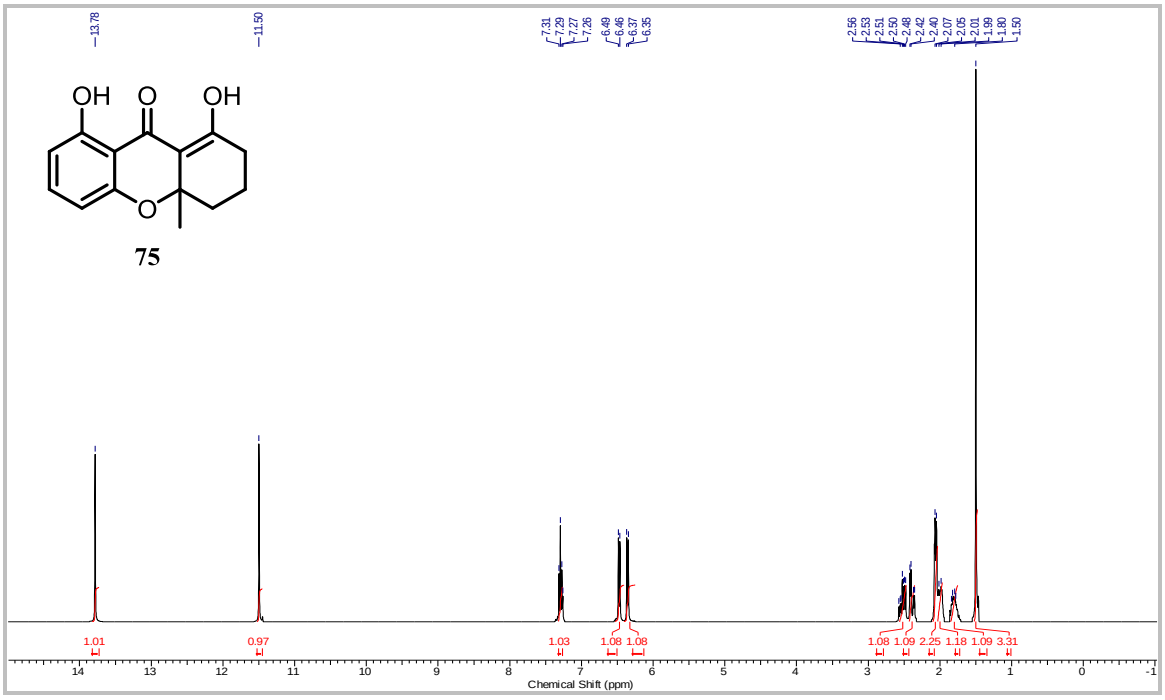
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 74



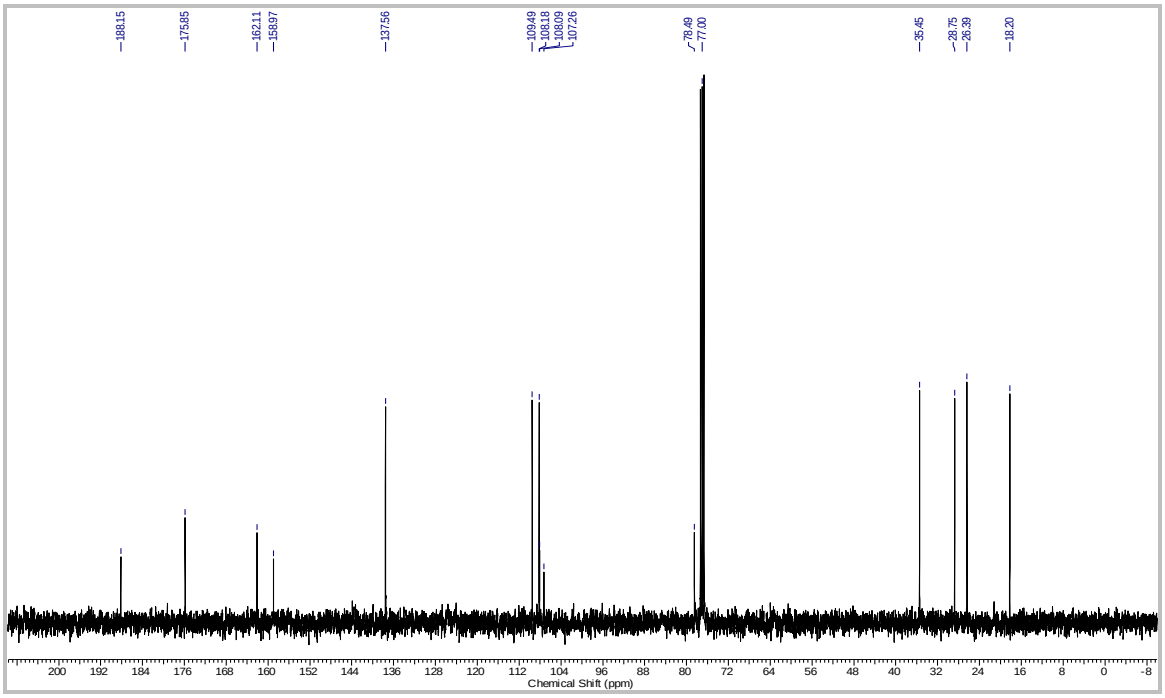

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 74



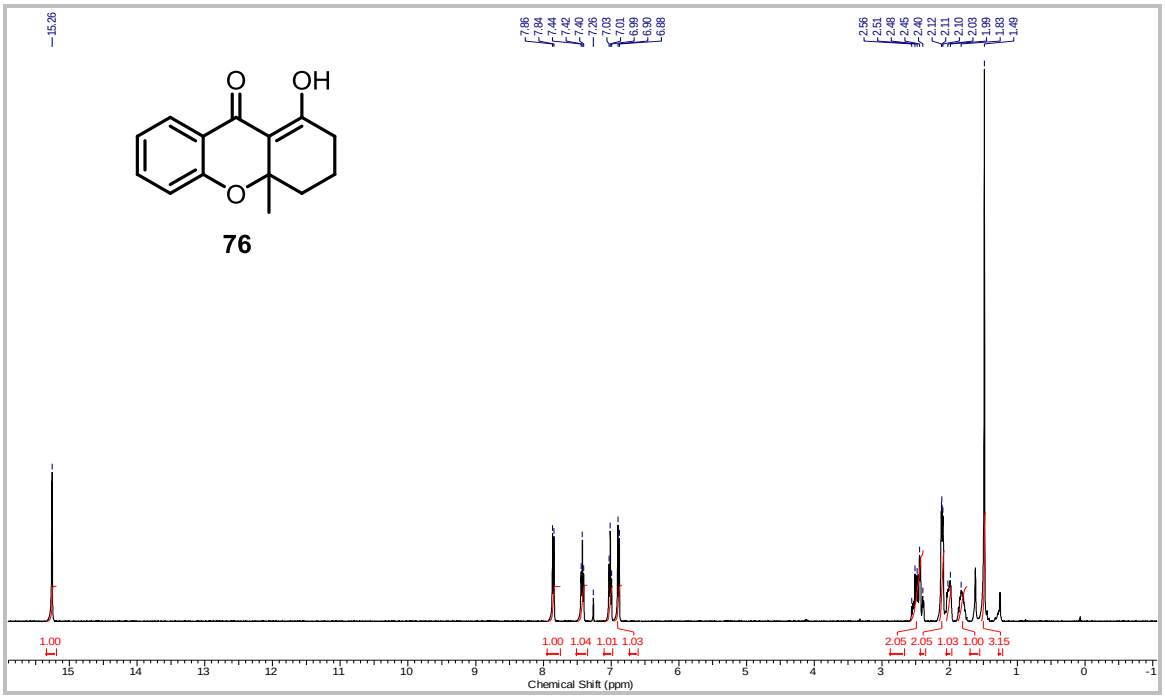
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 75



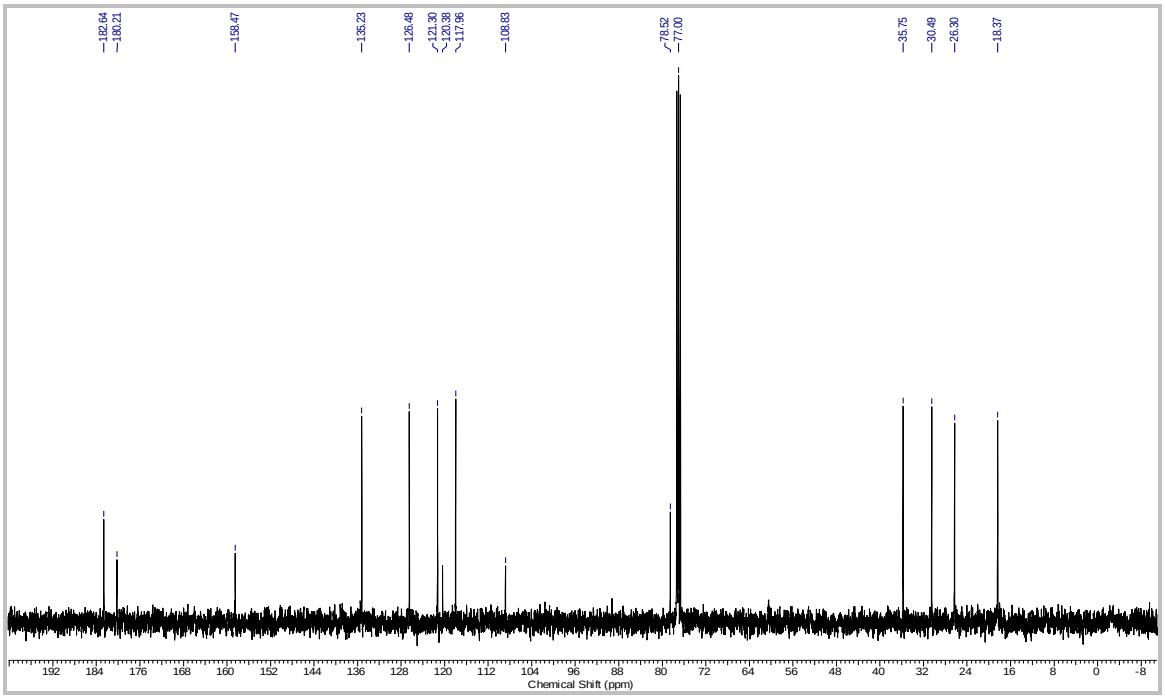

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 75



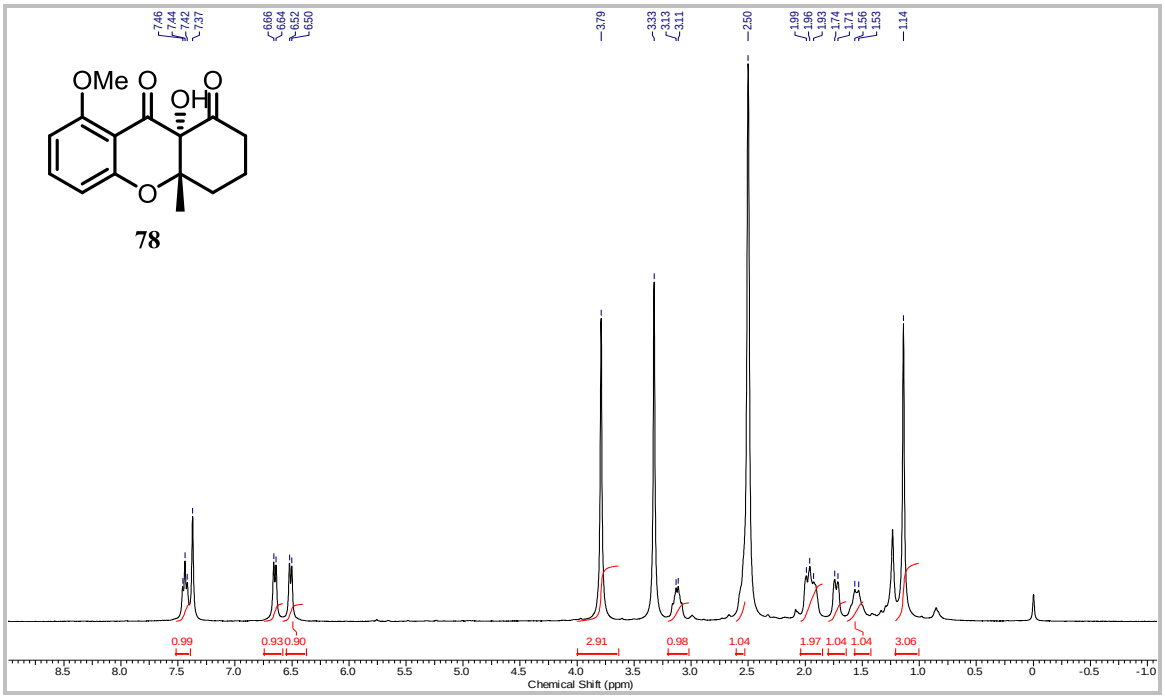
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 76



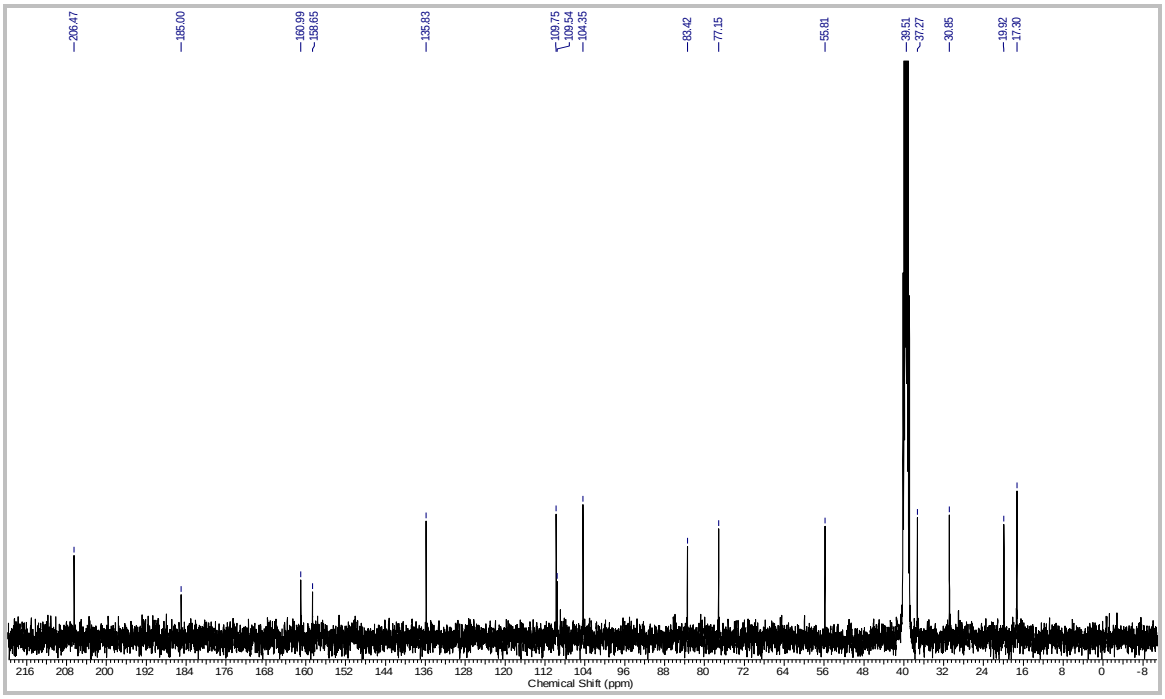

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 76



## $^{1}$H NMR (400 MHz, DMSO-$d_6$) of compound 78

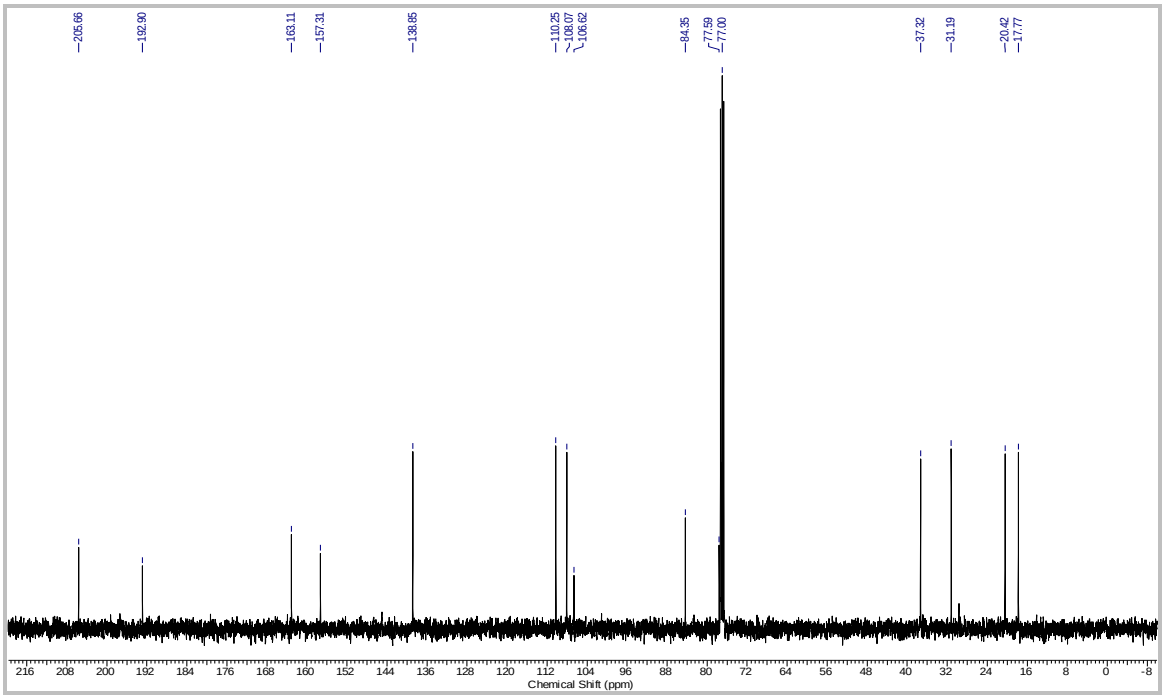



## $^{13}$C NMR (100 MHz, DMSO-$d_6$) of compound 78

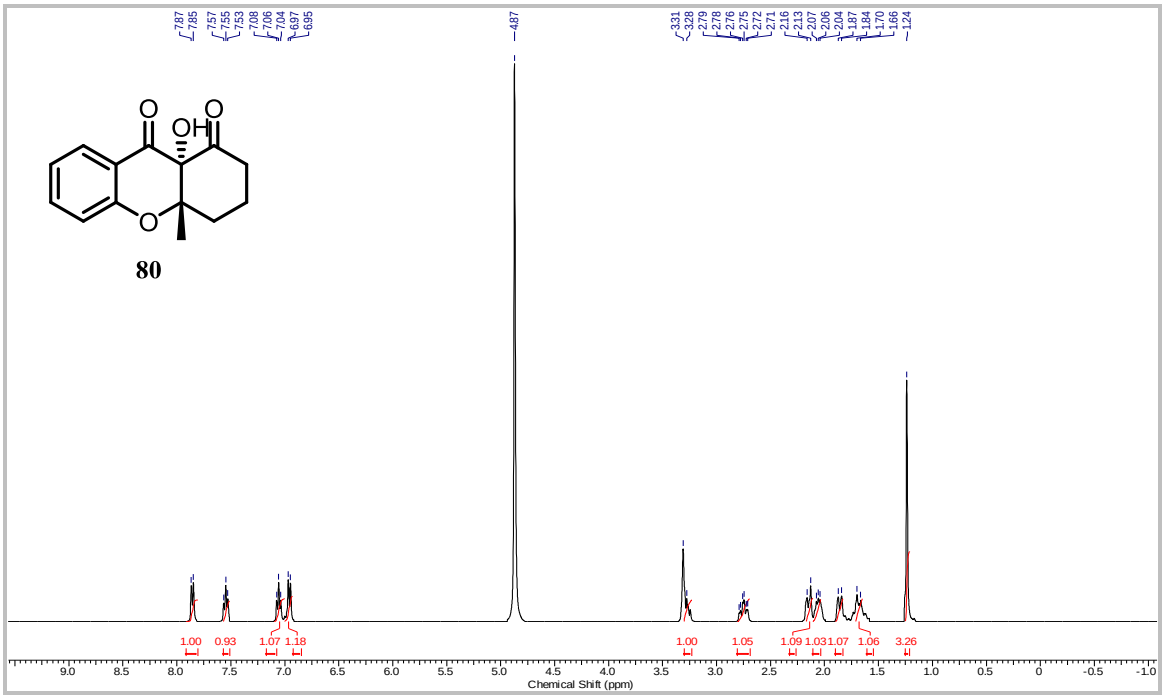


## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 79

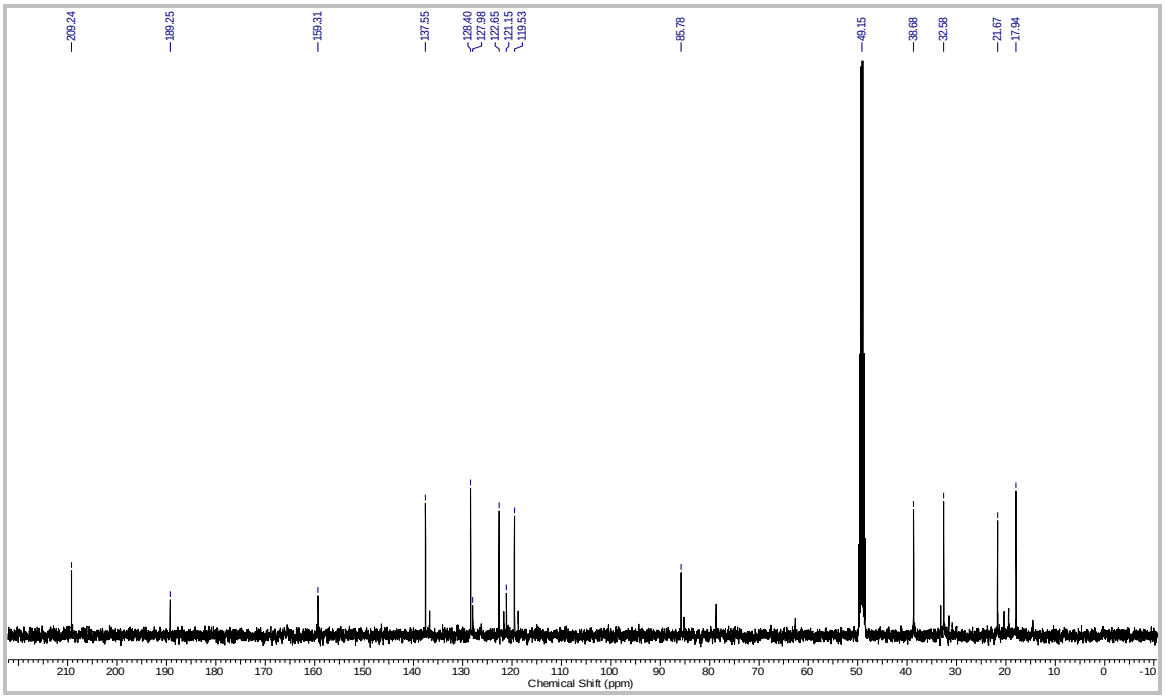

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 79



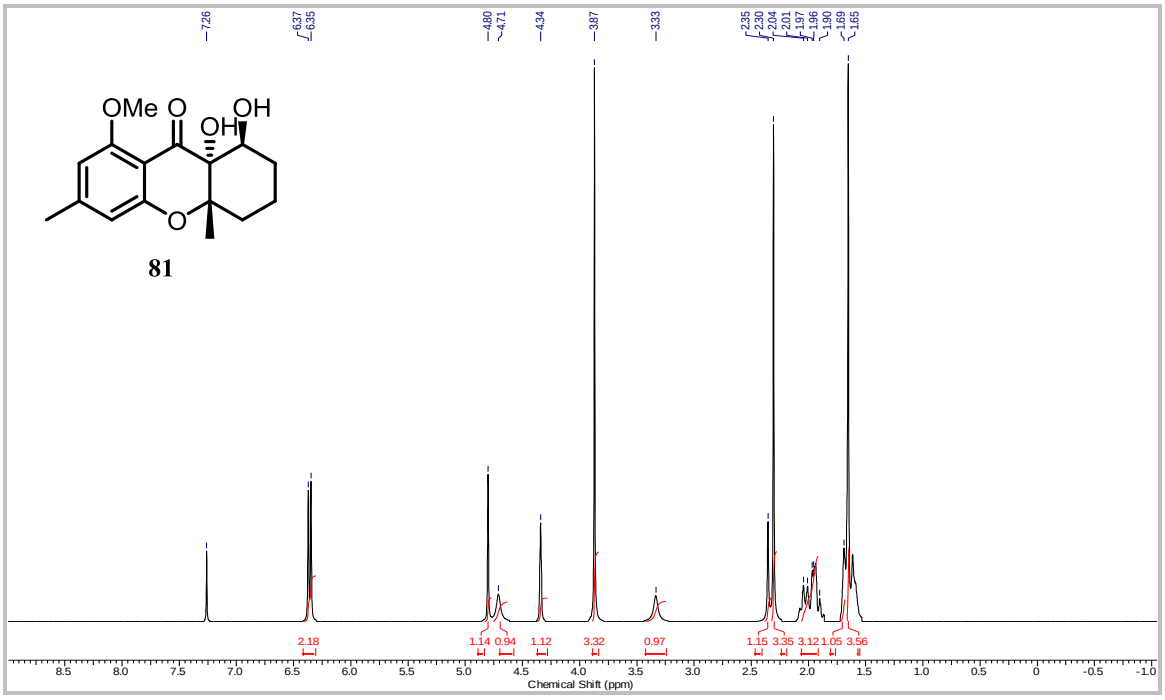
## $^{1}$H NMR (400 MHz, $CD_3OD$) of compound 80



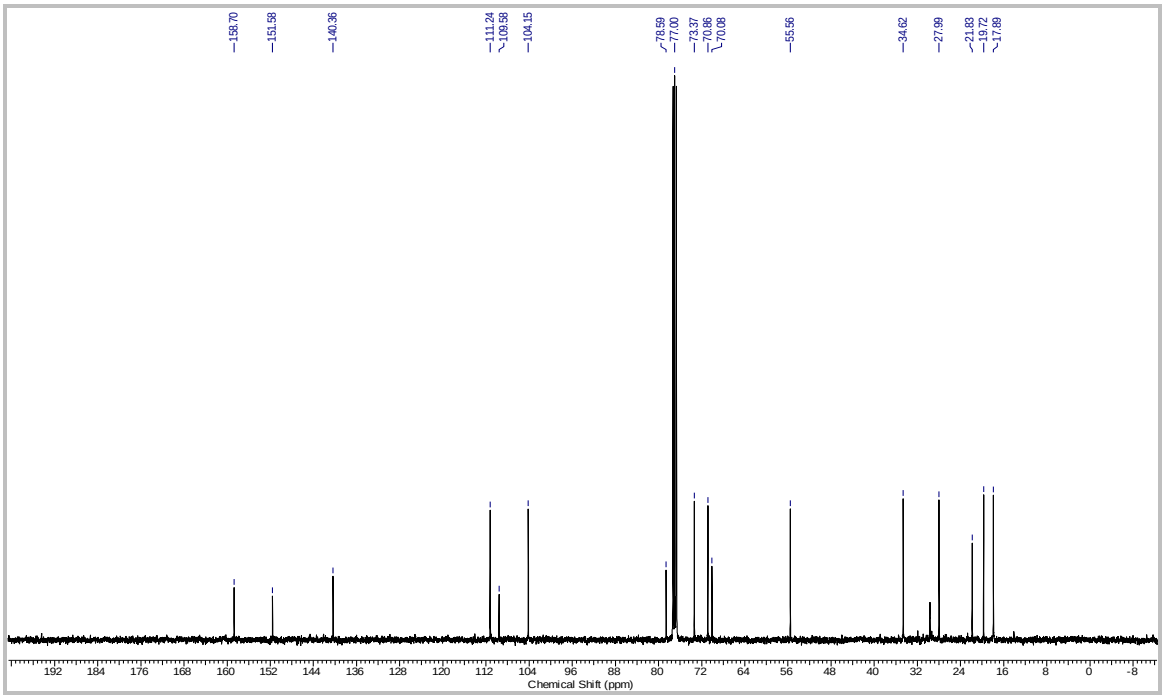

## $^{13}$C NMR (100 MHz, $CD_3OD$) of compound 80



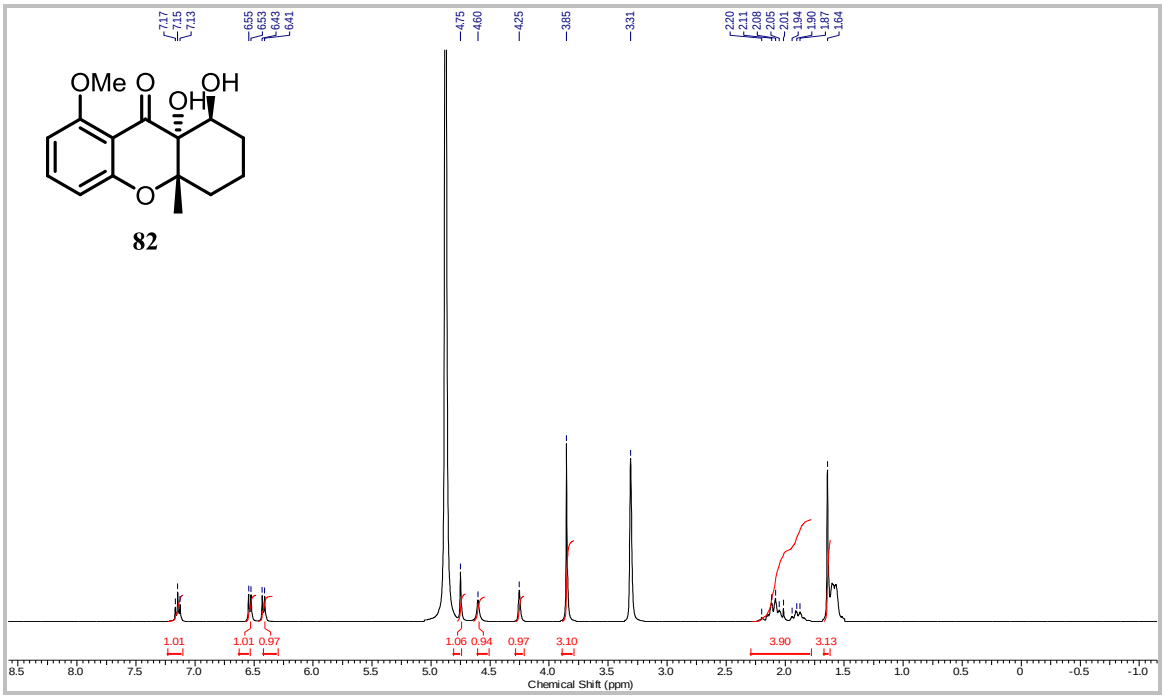
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 81



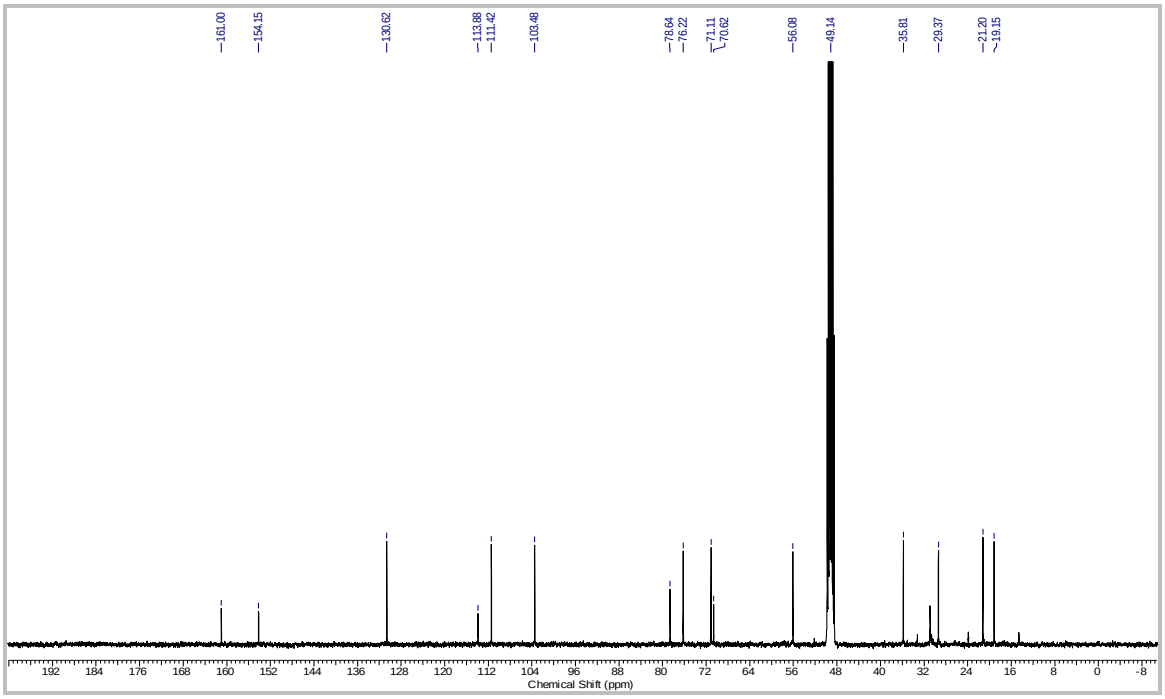

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 81



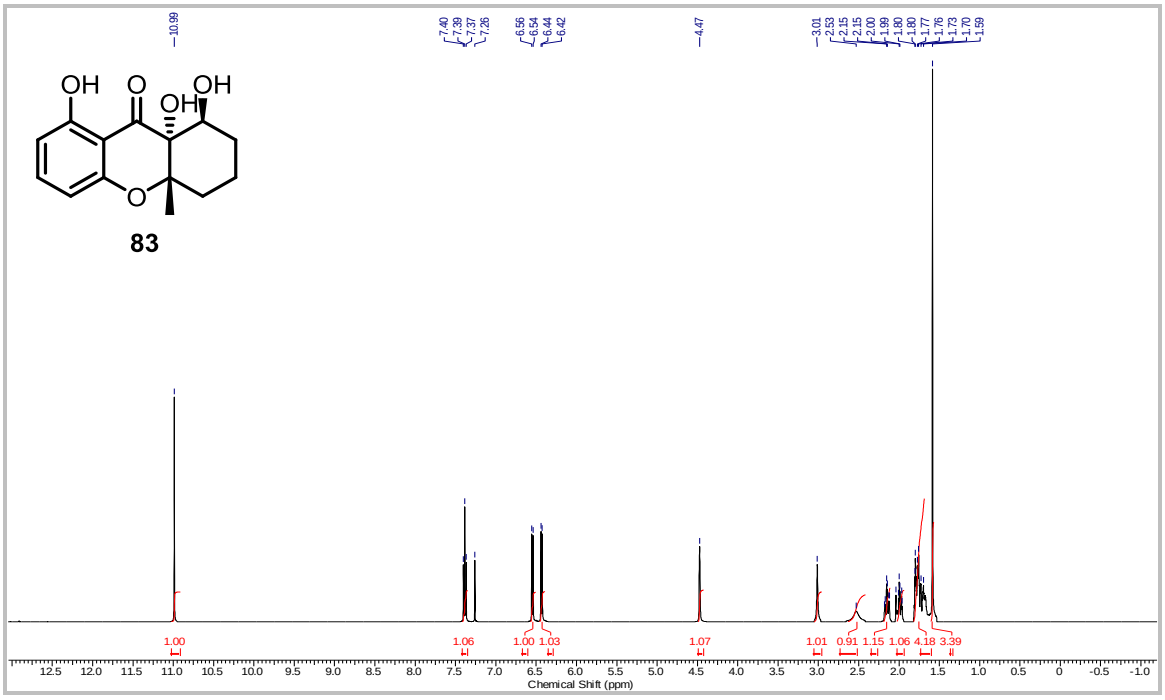
## $^{1}$H NMR (400 MHz, $CD_3OD$) of compound 82



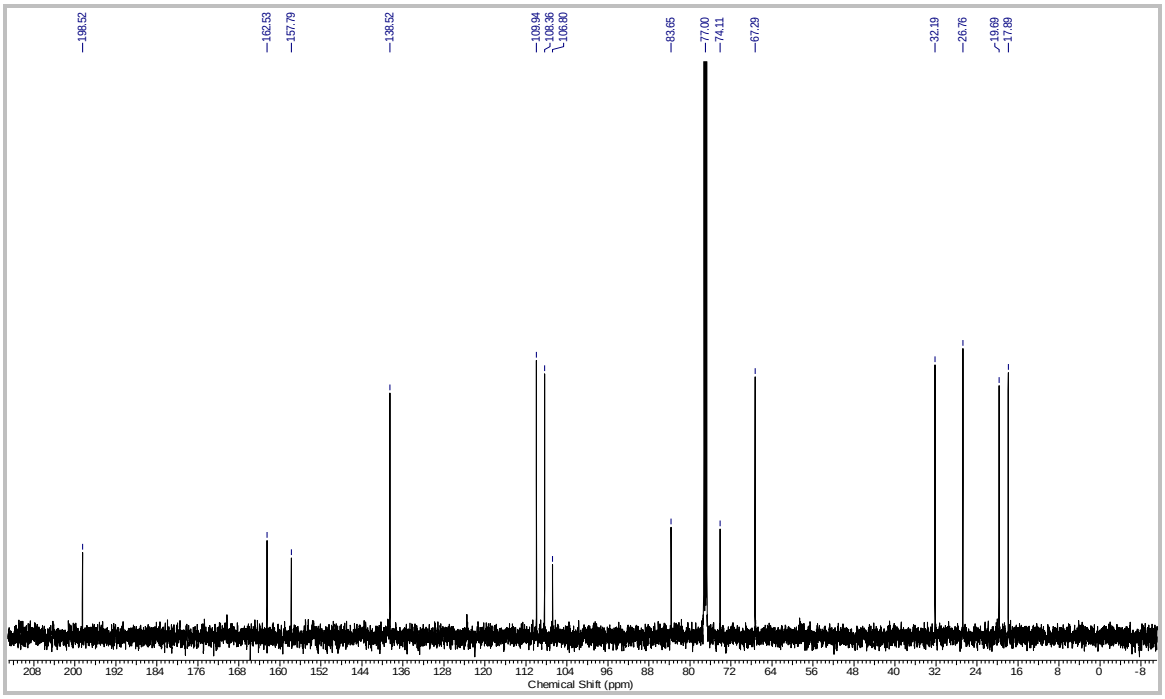

## $^{13}$C NMR (100 MHz, $CD_3OD$) of compound 82



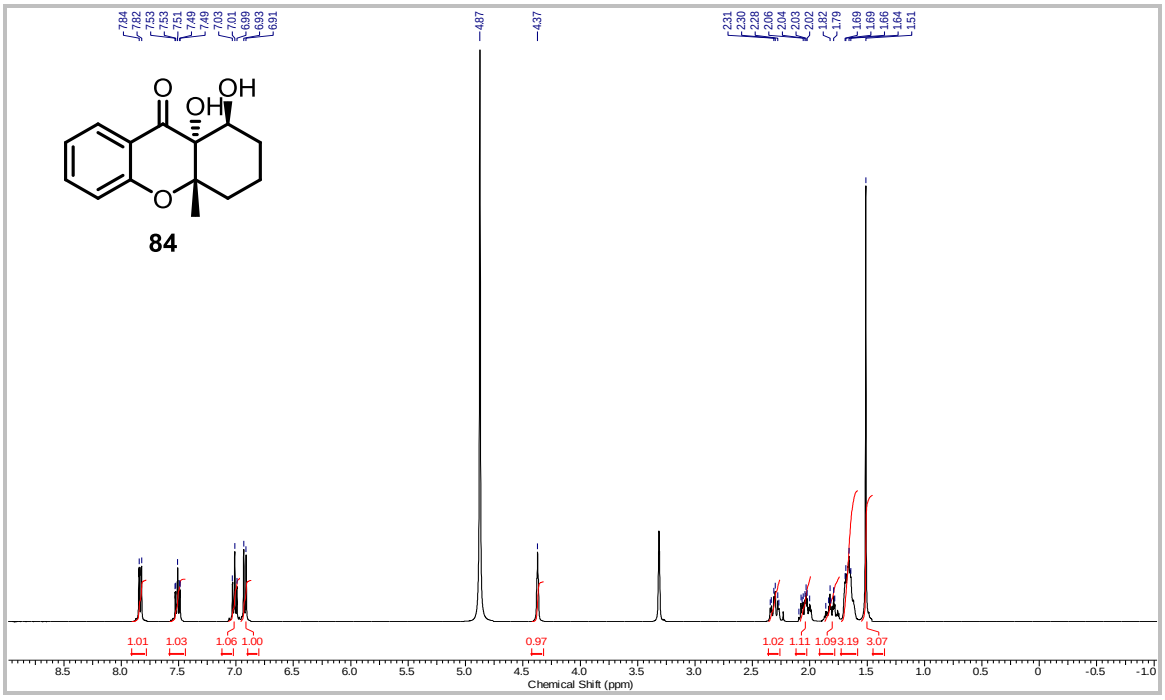
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 83



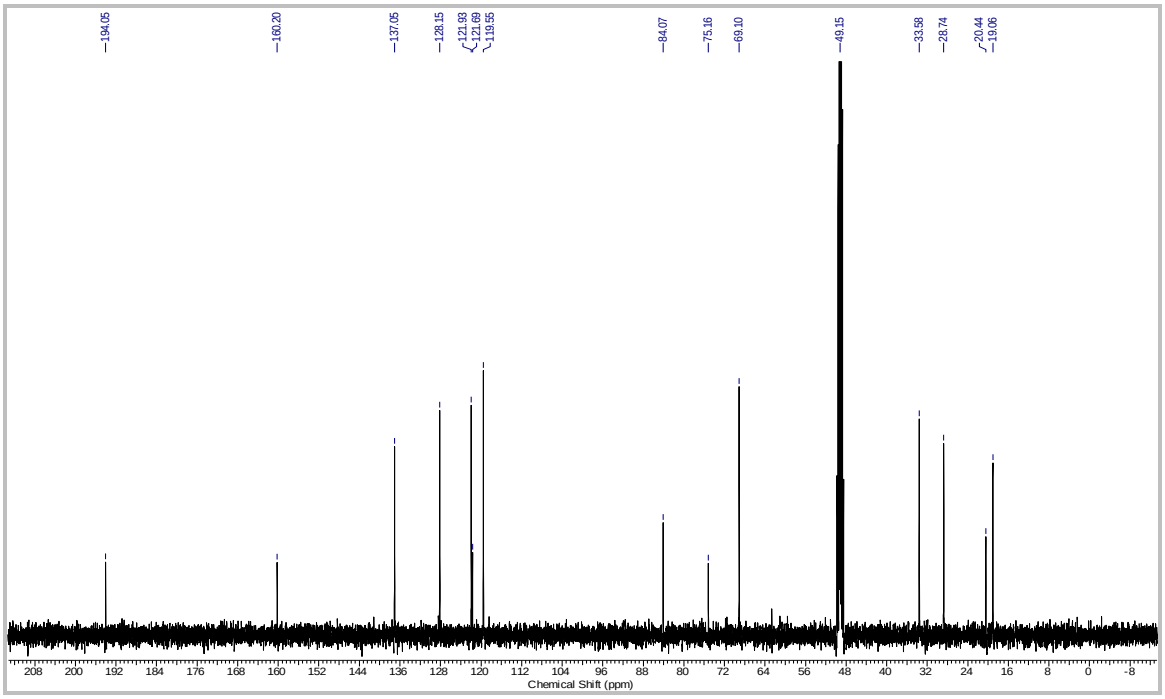

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 83



## $^{1}$H NMR (400 MHz, $CD_3OD$) of compound 84




## $^{13}$C NMR (100 MHz, $CD_3OD$) of compound 84



## 3.4.8. References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# *Chapter 4*

# Synthesis and SAR Studies of Diarylheptanoid Natural Products

## 4.1. Macrocyclic diarylheptanoids

Diarylheptanoid is a group of natural products which contain 1,7-diphenylheptane core as special characteristics. This class of the natural products is increasingly recognized as potential therapeutic agents such as antiinflammatory,[1] antioxidant, antitumor, estrogenic, leishmanicidal, melanogenesis, hepatoprotective and neuroprotective activities.[2] Diarylheptanoids are divided into two groups as (1) open chain and (2) macrocyclic diarylheptanoids. In the case of cyclic diarylheptanoids, the aromatic rings are connected to form a biaryl or a diaryl ether moiety.

Particularly, the cyclic diarylheptanoids are an important class of macrocycles which exhibit a broad range of potent biological activities.[2,3] The most of the cyclic diarylheptanoids have been isolated from the plant species such as *Aceraceae* (aceroside **1** and acerogenins **2**-**6**), *Betulaceae*, *Zingiberaceae* (*Curcuma*), *Leguminosae*, *Juglandaceae* and *Myricaceae* etc.[4] The extracts of these plants have been widely used in folk medicine in Asia for the treatment of cancer, stomachic, nitric oxide production and hepatic disorders. The extracts from the *Curcuma* were used against the biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorders, rheumatism, sinusitis, abdominal pains, icterus etc.[5a] The isolated and purified natural compounds like galeon (**7**) and pterocarine (engelhardione) (**8**) are potent inhibitors of *Mycobacterium tuberculosis* with MIC 0.2 μg/mL against the virulent strain H37Rv. The further studies of pterocarine/engelhardione (**8**) and its derivatization by Sun et al showed that even though the compound **8** did not show the considerable activity, its derivatives made by this group were found to be moderately active against the *Mycobacterium tuberculosis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli*.[5b] In addition galleon (**7**) and engelhardione (**8**) are also known to show the potent cytotoxic activity,[4b] while the biological properties of garuganin I (**9**), garugamblin I (**10**) and garugamblin II (**11**) are not well explored.[6] Myricanone (**12**) is a biphenyl class of diarylheptanoid and has anticancer effects on two different cancer cell lines HeLa and PC3 and it is considered to be a suitable candidate for possible use in the formulation of a therapeutic agent for

treating cancer.[7] The combretastatin $D_1$ (**13**), $D_2$ (**14**) and $D_3$ (**15**) are cyclic analogs of the combretastatin family isolated from the *Combretaceae* and the cytotoxicity study of these compounds in mouse leukemia P388 showed modest activity ($ED_{50}$ 3-5 $\mu gml^{-1}$).[8] Several other cyclic diarylheptanoids are also known in the literature and known to show a wide range of biological properties.[8,9] Selected compounds from the class of cyclic diarylheptanoids are listed below in figure 1.

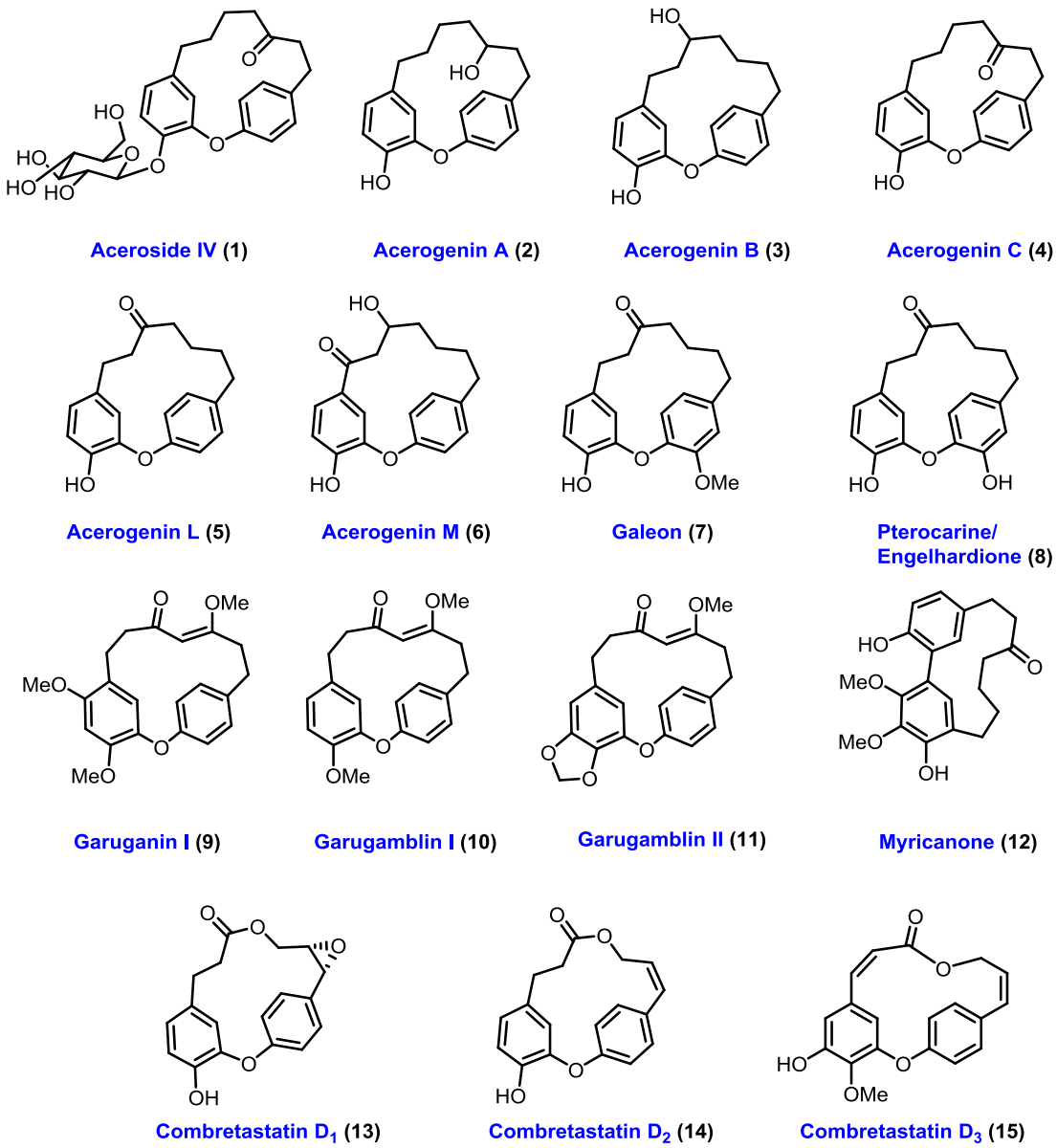



**Figure 1:** Structures of cyclicdiarylheptanoid natural products

## 4.2. Isolation and characterization of isomeric corniculatolides

*Aegiceras corniculatum* also called as black mangrove is a rich source of biologically active secondary metabolites. Traditionally this mangrove has been used in several folk medicines in Australia and south Asia to treat asthma, diabetes, inflammation, and rheumatism.[10] The crude extract of this mangrove also showed active against cytotoxicity, itchytoxicity, as antiinflammatory and antioxidant.

**Scientific Classification**

Kingdom: Plantae
Order : Ericales
Family : Primulaceae
Genus : *Aegiceras*
Species : *A. corniculatum*

**Figure 2:** *Aegiceras corniculatum* shrub

Recently, Gowri's research group from India isolated four new isomeric macrolides of combretastatin $D_2$ (**14**) congeners called 11-*O*-methylisocorniculatolide A (**16**), 11-*O*-methylcorniculatolide A (**17**), isocorniculatolide A (**18**), 12-hydroxy-11-*O*-methylcorniculatolide A (**20**) along with previously known compound corniculatolide A (**19**) from the bark of a mangrove *Aegiceras corniculatum.*[11]

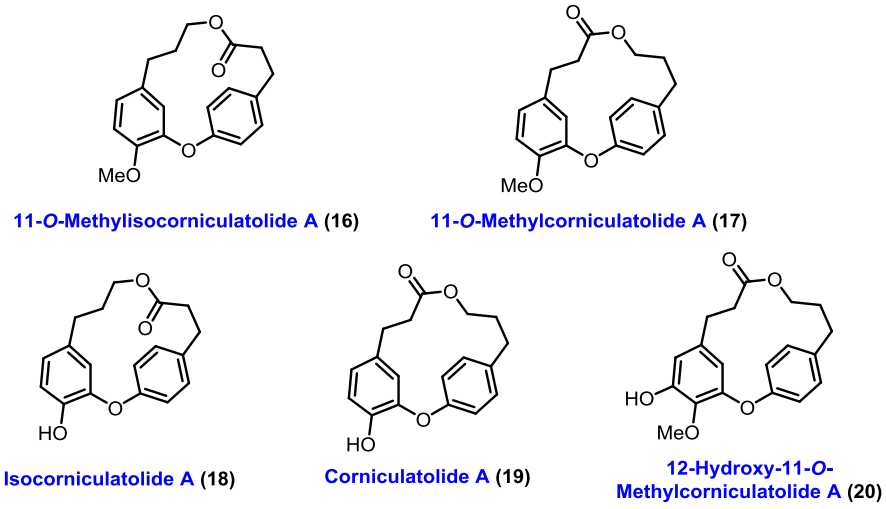
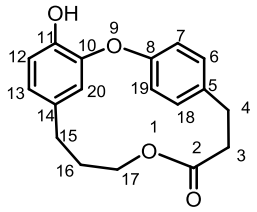



**Figure 3:** Structures of isomeric corniculatolides (**16-20**)

The structural assignments were done with the help of detailed spectroscopic methods. The obtained molecular formula and similarities in NMR spectra suggested that the compounds are isomeric to corniculatolides. The structure of all these compounds (**16**-**20**) were further confirmed by using 2D NMR interpretations such as COSY, HMBC. Since the compound **16**-**17** and **18**-**19** are regioisomers, to differentiate between them the $^{1}$H-$^{1}$H NOESY spectrum of compound **18** showed the spatial correlation between the H-20 and H-16/H-17/H-19 which indicates the position of lactone carbonyl is at C-2. While the position of lactone moiety between C-2 and C-17 was further confirmed by single crystal X-ray crystal structure determination of isocorniculatolide (**18**)[11]




Isocorniculatolide **(18)**

## 4.3. Reported approaches for the synthesis of macrocyclic diarylheptanoids

As the cyclic diarylheptanoids are an important class of natural products, they attracted the attention of many synthetic organic chemists. Although the several groups[8] reported the total syntheses of macrocyclic diarylheptanoid natural products like acerogenins A (**2**), B (**3**), C (**4**), L (**5**), M (**6**), galeon C (**7**), pterocarine/engelhardione (**8**) along with the macrolactones combretastatin $D_1$ (**13**), $D_2$ (**14**) and $D_3$ (**15**), the key steps for all of them fall in either a) final cyclization using diaryl ether formation or b) macrolactonization of corresponding acid. The selective synthetic approaches are highlighted in figure 4.

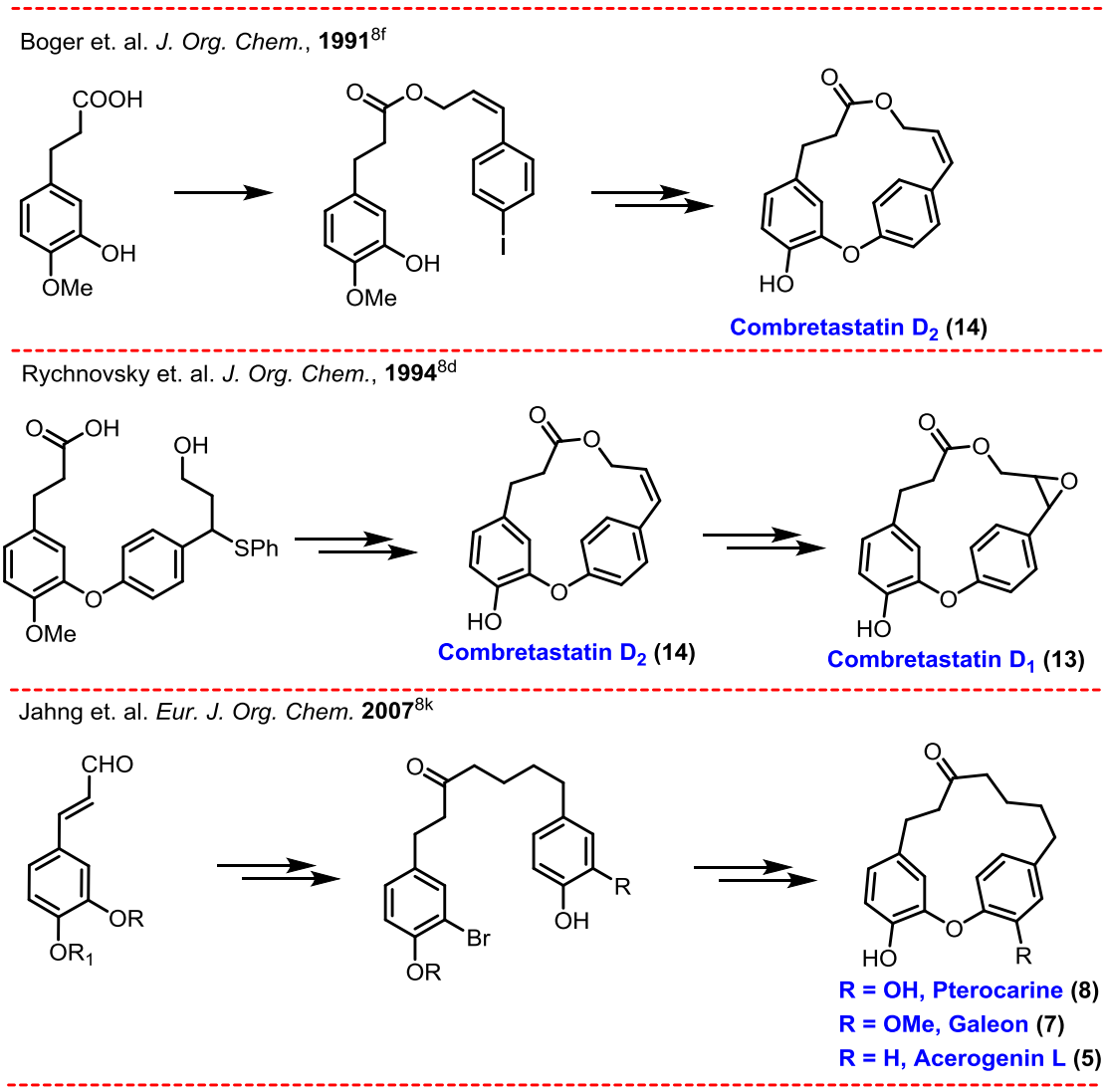



**Figure 4:** Reported approaches for macrocyclic diarylheptanoids

The Boger group attempted a synthesis of combretastatin $D_2$ (**14**)[8f] by using the conventional macrolactonization reaction to form the cyclic 15-membered diarylheptanoid but they did not succeed, but they achieved the synthesis using the intramolecular Ullmann coupling to form a diaryl ether using high dilution and pyridine as a solvent.

Rychnovsky's group in 1994 used the modified Mitsunobu cyclization (also called a Steghlich-Mitsunobu cyclization) of the hydroxy acid to produce macrolactone.[8d] In this synthesis the Ullmann-type coupling was used for the initial diaryl ether formations.

Jahng and co-workers reported the total synthesis of acerogenins C (**4**), L (**5**), galeon (**7**), and pterocarine (**8**) in 2007 by using the Ullmann diaryl ether formation reaction as a key step of the synthesis from linear diarylheptanoids.[8k] The required linear diarylheptanoids were synthesized using the series of cross-aldol condensation reactions.

## 4.4. Present work

All the five newly isolated cyclic diarylheptanoids are congeners of combretastatin $D_2$ (**14**) which have been known to be a potent anticancer agent. Pterocarine/engelhardione (**8**) a biaryl ether containing natural product is a well-known anti-tuberculosis agent, which showed the potent activity against the *Mycobacterium tuberculosis* H37Rv. The compounds **16**-**20** are closely related with the combretastatin $D_2$ (**14**) and engelhardione (**8**) and are expected to show the similar activities like anticancer and antituberculosis properties. Therefore, we became interested in the total synthesis of newly isolated macrocyclic compounds (listed in figure 3). Our ultimate goal is to develope the short, efficient and scalable route for the synthesis of this class of natural products and development of SAR around this scaffold as an antituberculosis agent.

### 4.4.1. Retrosynthetic approach

Retrosynthetically we have planned the synthesis of the target macrocycles **16**-**20** based on two key steps (i) diaryl ether formation[12] and (ii) macrolactonization[13] starting from readily available building blocks. The targeted macrocycles could be achieved by using the intramolecular macrolactonization of acid and alcohol using Mitsunobu reaction.[8l] The required fragments for the synthesis of the acid precursor was synthesized starting from appropriate phenols and aryl halides, following simple functional group conversions. The other regioisomer also could be achieved using a similar sequence.

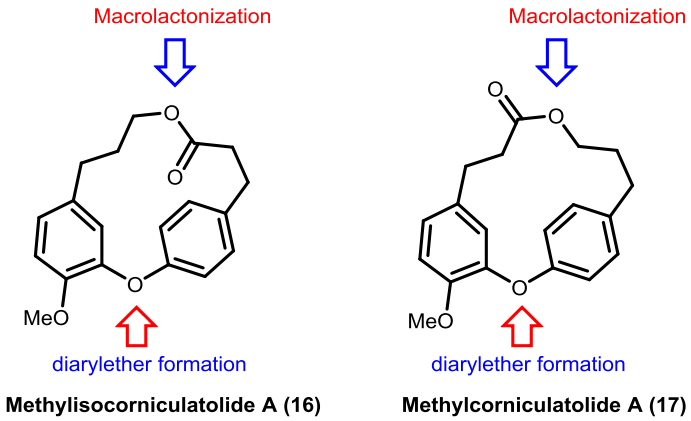
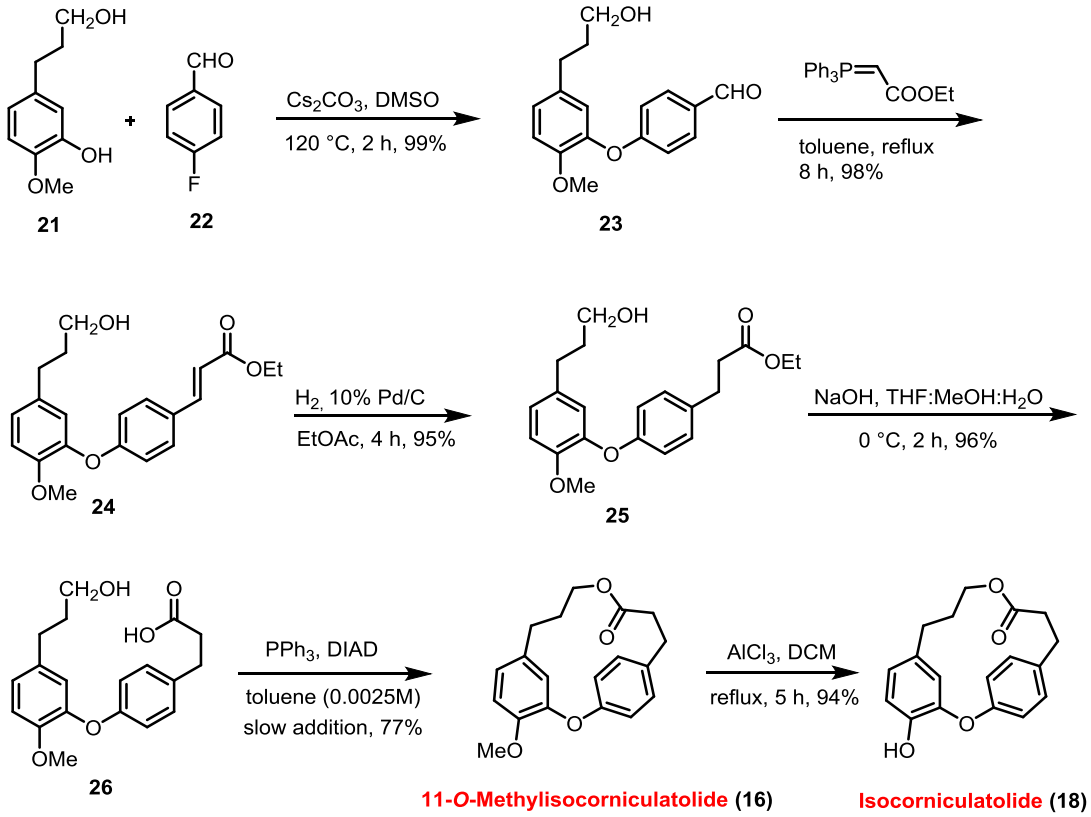



**Figure 5:** Retrosynthetic approach for the 11-*O*-methylisocorniculatolide (**16**) and 11-*O*-methylcorniculatolide (**17**).

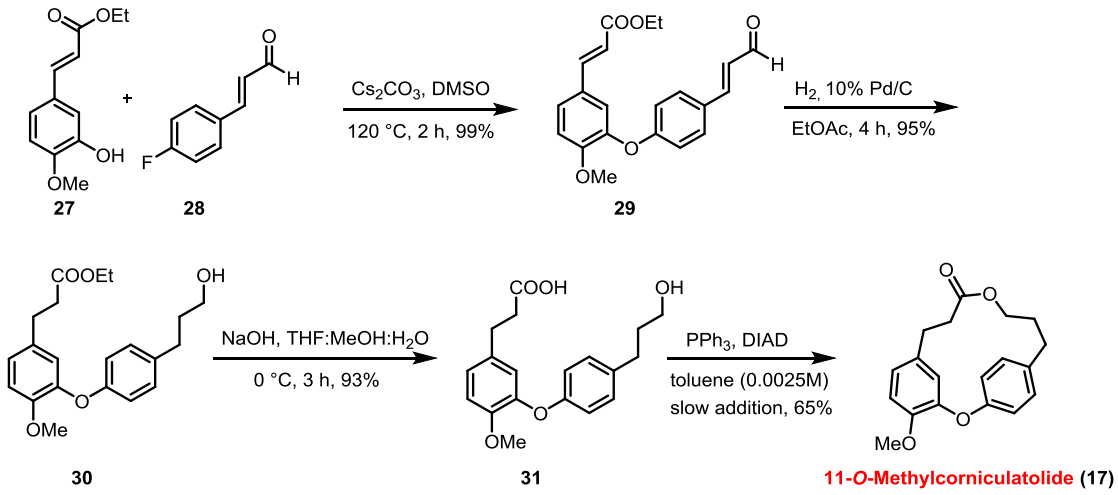
## 4.4.2. Synthesis of methylisocorniculatolide A, methylcorniculatolide A and isocorniculatolide A,

The planned synthesis began with the first key step diaryl ether formation between the readily accessible partners, compound **21** and *para*-fluoro benzaldehyde **22**. The reaction between **21** and **22** using $Cs_2CO_3$ in DMSO at 120 °C for 8 h was very clean and resulted in a high yield of desired compound **23**. The formation of compound **23** was confirmed by $^{1}H$ NMR which showed the characteristic aldehyde proton at δ 9.86 ppm and $^{13}C$ NMR shows the carbonyl carbon of aldehyde at δ 190.8 ppm. The formation of compound **23** was further confirmed by using HRMS which showed a peak at m/z 287.1286 corresponding to the molecular formula $C_{17}H_{18}O_4$ $[M+H]^+$ which is in agreement with the calculated (287.1278) molecular weight. Horner-Wittig reaction on aldehyde **23** produced the α,β-unsaturated ester **24** which was further transformed to saturated ester **25** using 10% Pd/C under the hydrogen atmosphere. Horner-Wittig reaction on aldehyde **23** also produced small amounts of the corresponding Z-isomer of **24**. As it is not relevant for the present purpose, we reduced the mixture to furnish **25** as a single compound.




**Scheme 1:** Synthesis of 11-*o*-methylisocorniculatolide (**17**) and isocorniculatolide (**16**)

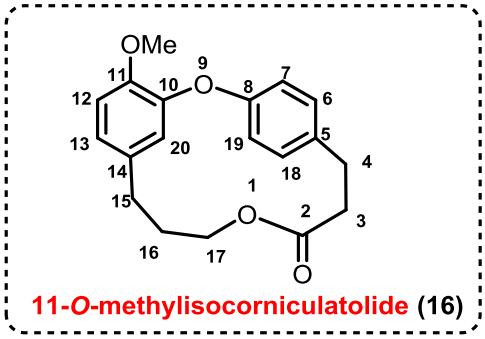
The compound **25** was confirmed by $^{1}$H NMR where the characteristic ethyl ester shows the quartet for 2H at δ 4.12 ppm and $^{13}$C NMR the carbonyl of ester was observed at δ 172.9 ppm. It is further confirmed by HRMS which gave the corresponding $[M+Na]^{+}$ at m/z 381.1688. Ester hydrolysis of compound **25** using aqueous NaOH in THF-MeOH mixture produced the acyclic acid precursor **26** in 93% yield. The macrocyclization under Mitsunobu conditions[14,8l] ($PPh_3$, DIAD) with high dilution (0.0025 M final concentration of a solution) in toluene at room temperature resulted in the formation of 11-*O*-methylisocorniculatolide A (**16**) with high yield. The $^{1}$H NMR and $^{13}$C NMR data were compared with the reported data and found to be identical (see table 1 and 2). The compound **16** was converted to the second natural product isocorniculatolide A (**18**) through demethylation using $AlCl_3$ in DCM under reflux conditions. The spectral data of isocorniculatolide (**18**) were compared with that of reported data and they were found to be identical (see table 3 and 4).




**Scheme 2:** Synthesis of 11-*O*-methylcorniculatolide (**17**)

Having the successful results in both the planned key reactions, we attempted the synthesis of isomeric corniculatolides **17**. The reaction between **27** and **28** using $Cs_2CO_3$ at standardized conditions resulted in a high yield of desired compound **29**. The formation of compound **29** was confirmed by $^{1}H$ NMR and $^{13}C$ NMR characterization followed by HRMS which showed peak at m/z 353.1382 $[M+H]^+$ which is in agreement with the calculated for $C_{21}H_{20}O_5$ (353.1384). The compound **29** was subjected to hydrogenation using 10% Pd/C under hydrogen balloon pressure to remove both the unwanted double bonds in **29**. We were pleased to find saturated alcohol **30** in very high yield during the hydrogenation step which is the result of the further reduction of intermediate saturated aldehyde.[15] The saturated alcohol **30** was confirmed using $^{1}H$ NMR and $^{13}C$ NMR data followed by HRMS which showed a base peak corresponding to $[M+H]^+$ at m/z 353.1382. The acyclic compound **31** obtained from **30** through hydrolysis of ester was subjected to the second key step macrolactonization under the standardized Mitsunobu conditions which resulted in 11-*O*-methylcorniculatolide (**17**) in good yield. The spectral data of synthetic compound **17** were compared with the reported data and found to be identical (see table 5 and 6). Thus, we have synthesized three new natural products.

### 4.4.2.1. $^{1}$H and $^{13}$C NMR comparison Tables of 11-*O*-methylisocorniculatolide (16) isocorniculatolide (18) and 11-*O*-methylcorniculatolide (17)



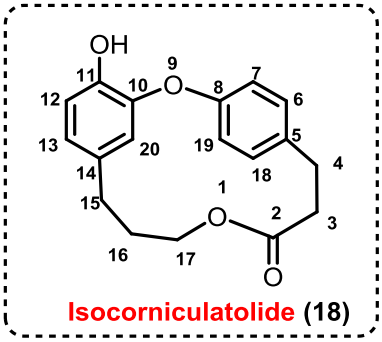

11-*O*-methylisocorniculatolide (16)

**Table 1:** $^{1}$H NMR data comparison between reported and synthesized 11-*O*-methylisocorniculatolide (**16**)

| Position | $^{1}$H NMR of 11-*O*-methylisocorniculatolide (ppm) (Reported) | | $^{1}$H NMR of 11-*O*-methylisocorniculatolide (400 MHz) (ppm) (Synthesized) | |
|---|---|---|---|---|
| 6,18 | 7.23 | d, 2H, *J* = 8.5 Hz | 7.24 | d, 2H, *J* = 8.5 Hz |
| 7,19 | 7.05 | d, 2H, *J* = 8.5 Hz | 7.05 | d, 2H, *J* = 8.5 Hz |
| 12 | 6.83 | d, 1H, *J* = 8.1 Hz | 6.83 | d, 1H, *J* = 8.3 Hz |
| 13 | 6.65 | dd, 1H, *J* = 8.1, 1.7 Hz | 6.66 | dd, 1H, *J* = 8.0, 2.0 Hz |
| 20 | 5.38 | d, 1H, *J* = 1.7 Hz | 5.35 | d, 1H, *J* = 2.0 Hz |
| -OMe | 3.95 | s, 3H | 3.95 | s, 3H |
| 17 | 3.63 | t, 2H, *J* = 6.8 Hz | 3.63 | t, 2H, *J* = 6.8 Hz |
| 4 | 3.03 | t, 2H, *J* = 6.7 Hz | 3.02 | t, 2H, *J* = 6.7 Hz |
| 3 | 2.53 | t, 2H, *J* = 6.7 Hz | 2.47- | m, 4H |
| 15 | 2.49 | t, 2H, *J* = 6.8 Hz | 2.53 | |
| 16 | 1.73 | m, 2H | 1.73 | m, 2H |

**Table 2:** $^{13}$C NMR data comparison between reported and synthesized 11-*O*-methylisocorniculatolide (**16**)

| Carbon No. | $^{13}$C NMR of 11-*O*-methylisocorniculatolide (ppm) (Reported) | (400 MHz) $^{13}$C NMR of 11-*O*-methylisocorniculatolide (ppm) (Synthesized) |
|---|---|---|
| C-2 | 173.1 | 173.1 |
| C-3 | 38.6 | 38.6 |
| C-4 | 32.4 | 32.4 |
| C-5 | 137.5 | 137.5 |
| C-6 | 130.5 | 130.5 |
| C-7 | 123.9 | 123.9 |
| C-8 | 156.2 | 156.2 |
| C-10 | 151.7 | 151.4 |
| C-11 | 146.4 | 146.4 |
| C-12 | 112.1 | 112.0 |
| C-13 | 121.4 | 121.4 |
| C-14 | 132.2 | 132.2 |
| C-15 | 28.2 | 28.2 |
| C-16 | 26.7 | 26.7 |
| C-17 | 62.1 | 62.1 |
| C-18 | 130.5 | 130.5 |
| C-19 | 123.9 | 123.9 |
| C-20 | 116.2 | 116.2 |
| -OMe | 56.2 | 56.2 |



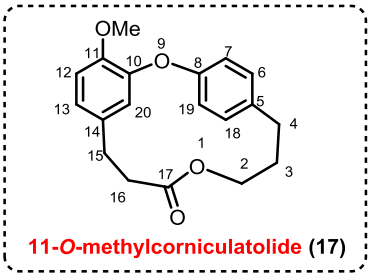

**Isocorniculatolide (18)**

**Table 3:** $^{1}$H NMR data comparison between reported and synthesized isocorniculatolide (**18**)

| Position | $^{1}$H NMR of isocorniculatolide (ppm) (Reported) | | $^{1}$H NMR of isocorniculatolide (200 MHz) (ppm) (Synthesized) | |
|---|---|---|---|---|
| 6,18 | 7.25 | d, 2H, *J* =8.3 Hz | 7.24 | d, 2H, *J* = 8.3 Hz |
| 7,19 | 7.02 | d, 2H, *J* =8.3 Hz | 7.02 | d, 2H, *J* = 8.5 Hz |
| 12 | 6.85 | d, 1H, *J* =8.3 Hz | 6.85 | d, 1H, *J* = 8.0 Hz |
| 13 | 6.60 | dd,1H, *J* =8.3, 2.3 Hz | 6.60 | dd, 1H, *J* = 8.3, 2.1 Hz |
| -OH | 5.57 | brs, 1H | 5.56 | brs, 1H |
| 20 | 5.38 | d, 1H, 2.3 Hz | 5.38 | d, 1H, *J* = 2.1 Hz |
| 17 | 3.58 | t, 2H, *J* =6.7 Hz | 3.59 | t, 2H, *J* =6.8 Hz |
| 4 | 3.03 | t, 2H, *J* = 6.8 Hz | 3.03 | d, 2H, *J* = 6.8 Hz |
| 3 | 2.52 | t, 2H, *J* = 6.8 Hz | 2.47-2.52 | m, 4H |
| 15 | 2.48 | t, 2H, *J* = 6.7 Hz | 1.73 | |
| 16 | 1.73 | m, 2H | | m, 2H |

**Table 4:** $^{13}$C NMR data comparison between reported and synthesized isocorniculatolide (**18**)

| Carbon No. | $^{13}$C NMR of isocorniculatolide (ppm) (Reported) | $^{13}$C NMR of isocorniculatolide (125 MHz) (ppm) (Synthesized) |
|---|---|---|
| C-2 | 173.1 | 173.1 |
| C-3 | 38.6 | 38.6 |
| C-4 | 32.3 | 32.3 |
| C-5 | 137.9 | 137.9 |
| C-6 | 130.6 | 130.6 |
| C-7 | 123.7 | 123.7 |
| C-8 | 155.9 | 155.9 |
| C-10 | 149.2 | 149.2 |
| C-11 | 142.7 | 142.8 |
| C-12 | 115.3 | 115.3 |
| C-13 | 122.1 | 122.2 |
| C-14 | 131.5 | 131.5 |
| C-15 | 28.2 | 28.2 |
| C-16 | 26.3 | 26.4 |
| C-17 | 62.2 | 62.2 |
| C-18 | 130.6 | 130.6 |
| C-19 | 123.7 | 123.7 |
| C-20 | 115.4 | 115.5 |




**11-*O*-methylcorniculatolide (17)**

**Table 5:** $^{1}$H NMR data comparison between reported and synthesized 11-*O*-methylcorniculatolide (**17**)

| Position | $^{1}$H NMR of 11-*O*-methylcorniculatolide (ppm) (Reported) | | $^{1}$H NMR of 11-*O*-methylcorniculatolide (400 MHz) (ppm) (Synthesized) | |
|---|---|---|---|---|
| 6,18 | 7.29 | d, 2H, *J* = 8.3 Hz | 7.29, | d, 2H, *J* = 8.3 Hz |
| 19 | 7.05 | d, 1H, *J* = 8.3 Hz | 7.04 | d, 2H, *J* = 8.3 Hz |
| 7 | 7.01 | d, 1H, *J* = 8.3 Hz | | |
| 12 | 6.81 | d, 1H, *J* = 8.1 Hz | 6.81 | d, 1H, *J* = 8.2 Hz |
| 13 | 6.66 | dd, 1H, *J* = 8.1, 1.8 Hz | 6.66 | d, 1H, J = 8.3 Hz |
| 20 | 5.30 | d, 1H, *J* =1.8 Hz | 5.35 | s, 1H |
| 2 | 4.06 | m, 2H | 4.08 | m, 2H |
| -OMe | 3.95 | s, 3H | 3.97 | s, 3H |
| 15 | 2.85 | m, 2H | 2.79-2.86 | m, 4H |
| 4 | 2.81 | t, 2H, *J* = 6.5 Hz | | |
| 16 | 2.26 | m, 2H | 2.26 | m, 2H |
| 3 | 2.10 | m, 2H | 2.10 | m, 2H |

**Table 6:** $^{13}$C NMR data comparison between reported and synthesized 11-*O*-methylcorniculatolide (**17**)

| Carbon No. | $^{13}$C NMR of 11-*O*-methylcorniculatolide (ppm) (Reported) | $^{13}$C NMR of 11-*O*-methylcorniculatolide (400 MHz) (ppm) (Synthesized) |
|---|---|---|
| C-2 | 63.9 | 63.9 |
| C-3 | 28.6 | 28.6 |
| C-4 | 33.9 | 33.9 |
| C-5 | 137.4 | 137.4 |
| C-6 | 131.0 | 131.0 |
| C-7 | 123.6 | 123.6 |
| C-8 | 154.5 | 154.5 |
| C-10 | 151.1 | 151.2 |
| C-11 | 146.1 | 146.1 |
| C-12 | 113.2 | 113.3 |
| C-13 | 120.8 | 120.8 |
| C-14 | 133.1 | 133.2 |
| C-15 | 26.9 | 26.9 |
| C-16 | 32.7 | 32.7 |
| C-17 | 173.9 | 173.8 |
| C-18 | 131.0 | 131.0 |
| C-19 | 123.6 | 123.6 |
| C-20 | 111.6 | 111.8 |
| -OMe | 56.1 | 56.2 |

## 4.5. Biological activity and SAR of corniculatolides

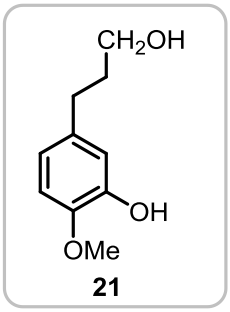
All the three natural products were tested for antitubercular assay with the help of Dr. Rajesh Gokhale's group at CSIR-IGIB Delhi. MIC values of all the three synthesized compounds 11-*O*-methylisocorniculatolide A (**16**), 11-*O*-methylcorniculatolide A (**17**) and isocorniculatolide A (**16**), against H37Rv was determined in 7H9-OADC media supplemented with 0.5% glycerol and 1 mg/ml tryptone at 37 °C in 96-well microtiter plates using the colorimetric resazurin microtiter assay, and growth was measured by visual readout. Rifampicin was used as a positive drug control. The compounds are found to be inactive towards the *Mycobacterium tuberculosis* H37Rv with a tested concentration of >100 μg/mL. The reports from the Sun group[5b] in 2013 also show that the original compound pterocarine/engelhardione (**8**) did not reproduce results reported for the same and found to be inactive against the *Mycobacterium tuberculosis* with MIC = 200 μg/mL.

## 4.6. Summary

- We have achieved the synthesis of three out of four recently isolated isomeric corniculatolide natural products using a simple, efficient and scalable synthetic route.
- The present route is useful for the synthesis of most of the related compounds of this family and their analogs towards developing interesting macrocyclic chemotypes for drug discovery programs.
- All the three synthesized natural products were tested against the *Mycobacterium tuberculosis* H37Rv and found to be inactive.

## 4.7. Experimental

**5-(3-hydroxypropyl)-2-methoxyphenol (21)**




**21**

To a solution of ethyl 3-(3-hydroxy-4-methoxyphenyl) propanoate (2.5 g, 11.16 mmol) in dry THF (50 mL) was added LAH (636 mg, 16.74 mmol) in THF (10 mL) at 0 ºC. The reaction mixture warmed to room temperature and stirred for 1.5 h before being diluted with ethyl acetate (10 mL) and quenched with saturated solution of $Na_2SO_4$ at 0 ºC. The suspension was filtered through the celite bed and washed with ethyl acetate (50 mL). The filtrate was dried over anhydrous $Na_2SO_4$, concentrated and purified by silica gel column chromatography (100-200 mesh) eluting with ethyl acetate: pet ether (1:3) to afford the alcohol **21** (1.96 g, 91% yield) as oil.

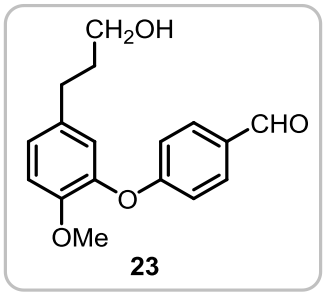
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3450, 2930, 1599, 1028

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 6.76 (m, 2H), 6.66 (d, *J* = 9.3 Hz, 1H), 5.79 (s, 1H), 3.85 (s, 3H), 3.65 (t, *J* = 6.4 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 1.87-1.79 (m, 2H), 1.71 (brs, 1H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 145.5, 144.8, 135.1, 119.6, 114.7, 110.7, 62.2, 56.0, 34.2, 31.3

**HRMS:** Calculated for, $C_{10}H_{14}O_3Na$ $[M+Na]^+$: 205.0840 found 205.0837

**4-(5-(3-hydroxypropyl)-2-methoxyphenoxy)benzaldehyde (23)**




**23**

To a mixture of **21** (500 mg, 2.58 mmol) and cesium carbonate (840 mg, 2.58 mmol) in DMSO (10 mL), *p*-fluorobenzaldehyde (**22**) (0.3 mL, 2.83 mmol) were added and the resulting mixture was heated to 120 ºC for 2 h and cooled to room temperature. It was then quenched with ice-water and extracted with ethyl acetate (10 mL x 3) and the combined organic layer was washed with brine (5 mL), dried over $Na_2SO_4$, concentrated and purified by flash column chromatography using 3:7 ethyl acetate: pet ether to afford **23** (730 mg, 99%) as colorless oil.

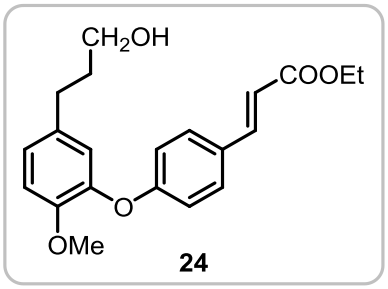
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3445, 2937, 2839, 2741, 1695, 1599, 1028

**$^1$H NMR (500 MHz, $CDCl_3$):** δ 9.86 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 2H), 7.05 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.94-6.98 (m, 4H), 3.75 (s, 3H), 3.65 (t, *J* = 6.3 Hz, 2H), 2.66 (t, *J* = 7.3 Hz, 2H), 1.85 (m, 2H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 190.8, 163.5, 149.5, 142.4, 135.2, 131.7 (2C), 130.6, 126.0, 122.3, 116.0 (2C), 112.9, 61.7, 55.8, 34.0, 30.9

**HRMS:** calculated for, $C_{17}H_{18}O_4$ $[M+H]^+$: 287.1278 found 287.1286

**(*E*)-ethyl-3-(4-(5-(3-hydroxypropyl)-2-methoxyphenoxy) phenyl)acrylate (24)**




**24**

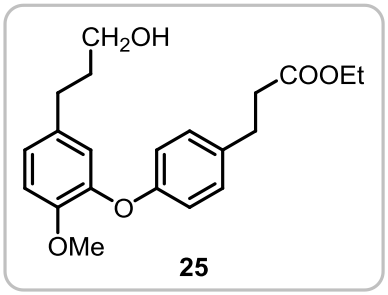
The solution of **23** (700 mg, 2.45 mmol ) and ethyl 2-(triphenylphosphoranylidene) acetate (1.28 g, 3.67 mmol) in toluene (12 mL) was refluxed for 8 h under argon atmosphere. The solvent was removed under reduced pressure and crude product was purified by flash column chromatography eluting with 1:3 ethyl acetate: pet ether to afford **24** (855 mg, 98%) as colorless oil.

**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3500, 3005, 2939, 1703, 1640, 1599, 1223, 1034

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.64 (d, *J* = 15.8 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.01 (dd, *J* = 8.53, 2.3 Hz, 1H), 6.94-6.83 (m, 4H), 6.32 (d, *J* = 15.8 Hz, 1H), 4.25 (q, *J* = 7.0 Hz, 2H), 3.78 (s, 3H), 3.65 (t, *J* = 6.3 Hz, 2H), 2.64 (t, *J* = 7.3 Hz, 2H), 1.84 (m, 2H), 1.33 (t, *J* = 7.0 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 167.2, 160.1, 149.7, 144.0, 143.4, 135.1, 129.5 (2C), 128.4, 125.3, 121.9, 116.6

**Ethyl-3-(4-(5-(3-hydroxypropyl)-2-methoxyphenoxy)phenyl)propanoate (25)**




**25**

The solution of **24** (400 mg, 1.12 mmol) and catalytic amount of 10% Pd/C in ethyl acetate (10 mL) was stirred for 1h at room temperature under hydrogen atmosphere. The

reaction mixture was then filtered through celite bed, washed with ethyl acetate (10 mL x 2) and concentrated under reduced pressure to afford **25** (380 mg, 95%) as colorless oil.

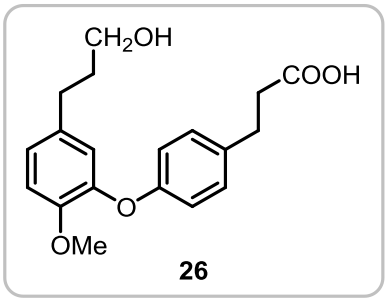
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3568, 2952, 2932, 2856, 1734, 1508, 1226

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.12 (d, *J* = 8.5 Hz, 2H), 6.80-6.93 (m, 5H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 3H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.91 (t, *J* = 7.8 Hz, 2H), 2.60 (m, 4H), 1.82 (m, 2H), 1.63 (brs, 1H), 1.23 (t, *J* = 7.1 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 172.9, 156.3, 149.5, 145.0, 134.8, 134.5, 129.3 (2C), 124.2, 120.8, 117.2 (2C), 112.8, 62.1, 60.3, 56.1, 36.1, 34.1, 31.1, 30.2, 14.1

**HRMS:** Calculated for $C_{21}H_{26}O_5$, $[M+Na]^+$: 381.1672 found 381.1688.

**3-(4-(5-(3-hydroxypropyl)-2-methoxyphenoxy)phenyl)propanoic acid (26)**




**26**

NaOH (84 mg, 2.1 mmol) was added to a solution of **25** (500 mg, 1.40 mmol) in 3:2:1 mixture of THF: MeOH: $H_2O$ at 0 ºC and stirred for 3 h. Solvent was removed under reduced pressure, the residue was dissolved in water (2 mL) and acidified by 1N HCl to pH ~2, followed by extraction with ethyl acetate (5 mL x 3). The combined organic layer was washed with brine (3 mL), dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure, and purified by column chromatography (1:1 mixture of ethyl acetate: pet ether) to afford **26** (430 mg, 93%) as sticky material.

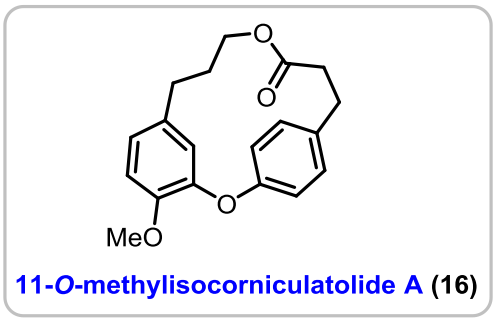
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 2963, 1714, 1608, 1507, 1270, 1216, 1029

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.11 (d, *J* = 8.3 Hz, 2H), 6.77-6.93 (m, 5H), 3.80 (s, 3H), 3.62 (t, *J* = 6.5 Hz, 2H), 2.91 (t, *J* = 7.5 Hz, 2H) 2.64 (t, *J* = 7.5 Hz, 2H), 2.58 (t, *J* = 7.3 Hz, 2H), 1.80 (quintet, *J* = 6.5 Hz, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 177.9, 156.3, 149.4, 145.1, 134.8, 134.2, 129.3 (2C), 124.2, 120.7, 117.4 (2C), 112.8, 61.9, 56.1, 35.7, 33.9, 31.00, 29.9

**HRMS:** Calculated for $C_{19}H_{22}O_5$, $[M+Na]^+$: 353.1359 found 353.1374

**11-*O*-methylisocorniculatolide A (16)**




**11-*O*-methylisocorniculatolide A (16)**

The solution of $Ph_3P$ (4.13 g, 15.15 mmol) and DIAD (3.1 mL, 15.76 mmol) in toluene (1.2 L, 0.0025 M final concentration) was stirred for 20 minutes at room temperature and solution of **26** (1.0 g, 3.03 mmol) in toluene-THF (20 mL, 3:1) was added over the period of 8 h (half of the portion) and reaction mixture was stirred vigorously for 8 h. Second portion of $Ph_3P$ (1.98 g, 7.57 mmol), DIAD (1.47 mL, 7.45 mmol) followed by remaining part of acid was added over 8 h. After stirring for additional 10 h, the solvent was removed under reduced pressure and product was directly purified by flash column chromatography eluting with 1:20 mixture of ethyl acetate: pet ether to afford the 11-*O*-methylisocorniculatolide A (**16**) (724 mg, 77%) as white solid.

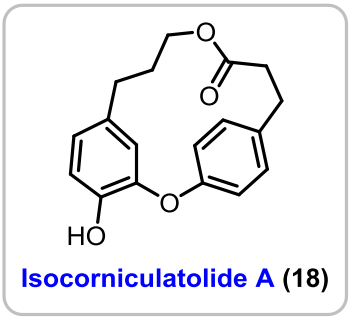
**Mp:** 138-140 ºC

**$^{1}$H NMR (400 MHz, $CDCl_3$):** δ 7.24 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.3 Hz, 1H), 6.65 (dd, *J* = 8.0, 2.0 Hz, 1H), 5.37 (d, *J* = 2.0 Hz, 1H), 3.95 (s, 3H), 3.62 (t, *J* = 6.8 Hz, 2H), 3.02 (t, *J* = 6.8 Hz, 2H), 2.53-2.47 (m, 4H), 1.72 (m, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 173.1, 156.2, 151.4, 146.3, 137.5, 132.2, 130.5 (2C), 123.9 (2C), 121.4, 116.2, 112.0, 62.1, 56.1, 38.6, 32.4, 28.2, 26.7

$^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**Isocorniculatolide A (18)**




**Isocorniculatolide A (18)**

To a solution of 11-*O*-methylisocorniculatolide A **(16)** (60 mg, 0.192 mmol) in dry DCM (2 mL), $AlCl_3$ (128 mg, 0.96 mmol) was added and reaction mixture was refluxed for 5 h, cooled to room temperature and quenched with ice cooled water. The organic layer was separated and aqueous layer was extracted with DCM (2 mL x 3). The combined organic layer was dried over anhydrous $Na_2SO_4$, concentrated and purified by column chromatography (230 - 400 mesh) eluting with 1:20 ethyl acetate: pet ether to afford **18** (54 mg, 94% yield) as white crystalline solid.

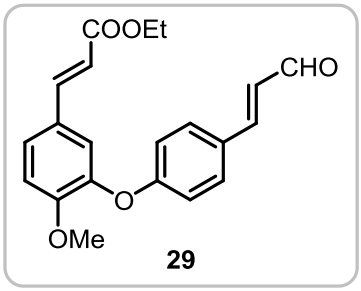
**Mp:** 196-199 ºC

**$^{1}$H NMR (200 MHz, $CDCl_3$):** δ 7.24 (d, *J* = 8.3 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.60 (dd, *J* = 8.3, 2.1 Hz, 1H), 5.56 (brs, 1H), 5.38 (d, *J* = 2.1 Hz, 1H), 3.59 (t, *J* = 6.8 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H), 2.47-2.52 (m, 4H), 1.73 (m, 2H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 173.1, 155.9, 149.2, 142.8, 137.9, 131.5, 130.6 (2C), 123.7 (2C), 122.2, 115.5, 115.3, 62.2, 38.6, 32.3, 28.2, 26.4

$^{1}$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

**(*E*)-Ethyl-3-(4-methoxy-3-(4-((*E*)-3-oxoprop-1-en-1-yl) phenoxy) phenyl)acrylate (29)**




**29**

The mixture of ethyl 3-(3-hydroxy-4-methoxyphenyl)acrylate (**27**) (740 mg, 3.33 mmol), *p*-fluorocinnamaldehyde (**28**) (500 mg, 3.33 mmol) and cesium carbonate (868 mg, 2.66 mmol) in dry DMSO (10 mL) was heated to 120 ºC for 8 h till the starting materials were consumed. The reaction mixture was then cooled to room temperature, quenched with ice-water and extracted with ethyl acetate (10 mL x 3). The combined organic layer was washed with brine, dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure, and purified by flash column chromatography using 2:8 mixture of ethyl acetate: pet ether to afford **29** (1.05 g, 90%) as pale brown solid.

**Mp:** 116-118 ºC

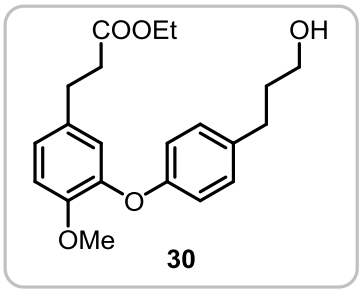
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3020, 2964, 1728, 1675, 1600, 1216, 1011

**$^1$H NMR (400 MHz, $CDCl_3$):** δ 9.67 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 16.1 Hz, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 15.8 Hz, 1H), 7.37 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.25 (m, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 2H), 6.64 (dd, *J* = 15.8, 7.5 Hz, 1H), 6.27 (d, *J* = 15.8 Hz, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 193.6, 166.9, 160.4, 153.2, 152.2, 143.9, 143.2, 130.3 (2C), 128.5, 128.1, 127.3, 126.6, 120.8, 117.0 (3C), 112.8, 60.4, 56.0, 14.3

**HRMS:** Calculated for, $C_{21}H_{20}O_5$ $[M+H]^+$: 353.1384 found 353.1382

**Ethyl-3-(3-(4-(3-hydroxypropyl)phenoxy)-4-methoxyphenyl) propanoate (30)**




**30**

The solution of **29** (500 mg, 1.42 mmol) and catalytic amount (~20 mg) of 10% Pd/C in ethyl acetate (10 mL) was stirred for 4 hours at room temperature under hydrogen atmosphere. The reaction mixture was filtered through celite bed and washed with ethyl acetate (10 mL x 2) and concentrated under reduced pressure to afford **30** (480 mg, 95%) as colorless oil.

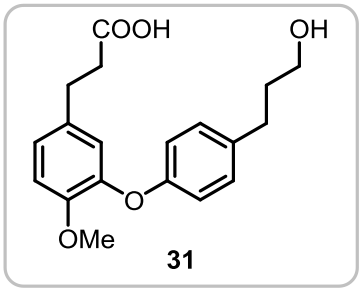
**IR $\upsilon_{max}$(film):** $cm^{-1}$ 3568, 2952, 2932, 2856, 1734, 1508, 1226, 650

**$^1$H NMR (200 MHz, $CDCl_3$):** δ 7.11 (d, *J* = 8.5 Hz, 2H), 6.78-6.93 (m, 5H), 4.09 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 3H), 3.67 (t, *J* = 6.5 Hz, 2H), 2.84 (dt, *J* = 8.1, 7.3 Hz, 2H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.54 (t, *J* = 8.1 Hz, 2H), 1.85 (m, 2H), 1.21 (t, *J* = 7.2 Hz, 3H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 172.8, 155.9, 149.7, 145.3, 135.9, 133.5, 129.4 (2C), 124.0, 120.5, 117.4 (2C), 112.8, 62.2, 60.4, 56.1, 36.0, 34.3, 31.2, 30.1, 14.2

**HRMS:** Calculated for, $C_{21}H_{26}O_5$ $[M+H]^+$: 359.1853 found 359.1847

**3-(3-(4-(3-hydroxypropyl)phenoxy)-4-methoxyphenyl) propanoic acid (31)**




**31**

To a solution of **30** (500 mg, 1.40 mmol) in 3:2:1 mixture of THF: MeOH: $H_2O$, NaOH (84 mg, 2.1 mmol) was added at 0 ºC and stirring was continued for 3h, solvent was removed under reduced pressure, residue was dissolved in water (10 mL) and acidified by 1N HCl to pH ~2, and extracted with ethyl acetate (10 mL x 3). The combined organic layer was washed with brine (5 mL), dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure, and purified by column chromatography (1:1 mixture of ethyl acetate: pet ether) to afford **31** (420 mg, 90%) as sticky material.

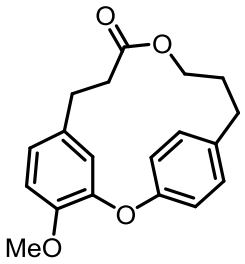
**IR υmax(film):** $cm^{-1}$ 2936, 1714, 1608, 1507, 1270, 1216, 1029

**$^1$H NMR (500 MHz, $CDCl_3$):** δ 7.10 (d, *J* = 8.5 Hz, 2H), 6.91 (m, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 1.5 Hz, 1H), 3.80 (s, 3H), 3.65 (t, *J* = 6.4 Hz, 2H), 2.83 (t, *J* = 7.6 Hz, 2H), 2.65 (dt, *J* = 7.9, 7.3 Hz, 2H), 2.58 (dt, *J* = 7.9, 7.6 Hz, 2H), 1.86 (m, 2H), 1.60 (t, 1H)

**$^{13}$C NMR (125 MHz, $CDCl_3$):** δ 177.5, 155.7, 149.6, 145.5, 136.0, 133.2, 129.4 (2C), 123.9, 120.3, 117.6 (2C), 112.8, 62.2, 56.1, 35.5, 34.1, 31.7, 29.7

**HRMS:** Calculated for $C_{19}H_{22}O_5$ $[M+Na]^+$: 353.1359 found 353.1355.

**11-*O*-Methylcorniculatolide (17)**




**11-*O*-methylcorniculatolide A (17)**

To a solution of $Ph_3P$ (790 mg, 3.03 mmol) and DIAD (0.6 mL, 3.15 mol) in toluene (242 mL, 0.0025M final concentration) was stirred for 20 minutes at room temperature and solution of acid **31** (200 mg, 0.606 mmol) in toluene-THF (8 mL, 3:1 ratio) was added slowly (half of the portion) over the period of 8 h. The reaction mixture was stirred vigorously for 8 h and second portion of $Ph_3P$ (400 mg, 1.52 mmol) and DIAD (0.3 mL, 1.5 mmol) followed by remaining part of acid were added over period of 7-8 h. After stirring additional 10 h, the solvent was removed under reduced pressure and product was directly purified by flash column chromatography eluting with 1:20 mixture of ethyl acetate: pet ether to afford the macrocycle 11-*O*-Methylcorniculatolide (**17**) (123 mg, 65%) as white solid.

**Mp:** 142-145 ºC

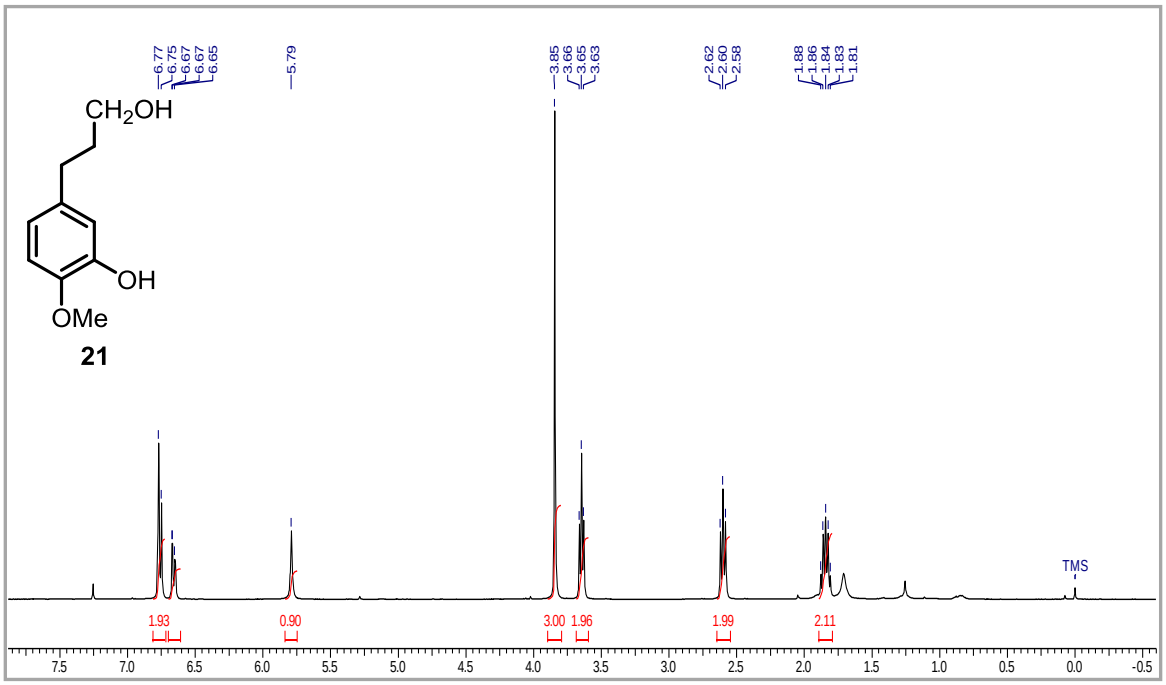
**$^1$H NMR (400 MHz, $CDCl_3$):** δ 7.29 (d, *J* = 8.3 Hz, 2H), 7.04 (d, *J* = 8.3 Hz, 2H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.66 (d, *J* = 8.3 Hz, 1H), 5.35 (s, 1H), 4.08 (m, 2H), 3.97 (s, 3H), 2.79-2.86 (m, 4H), 2.26 (m, 2H), 2.10 (m, 2H)

**$^{13}$C NMR (100 MHz, $CDCl_3$):** δ 173.8, 154.5, 151.2, 146.1, 137.4, 133.2, 131.0 (2C), 123.6 (2C), 120.8, 113.3, 111.8, 63.9, 56.2, 33.9, 32.7, 28.6, 26.9

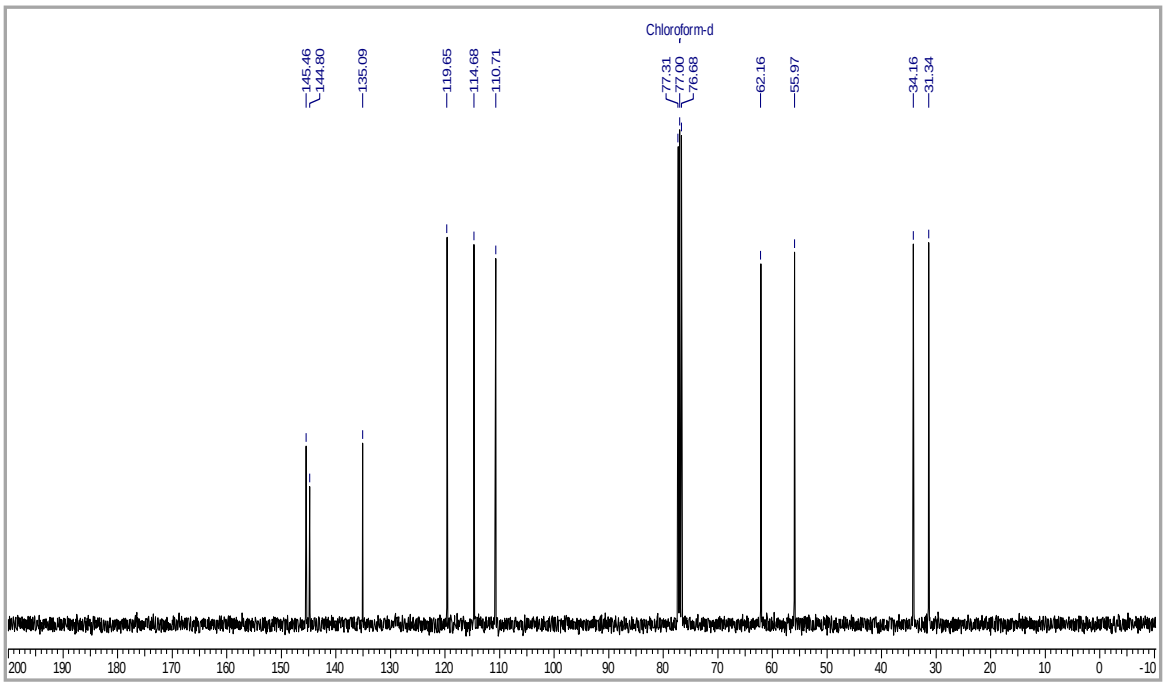
$^1$H and $^{13}$C NMR data was compared with the literature reports and found to be identical.

## 4.8. Spectra

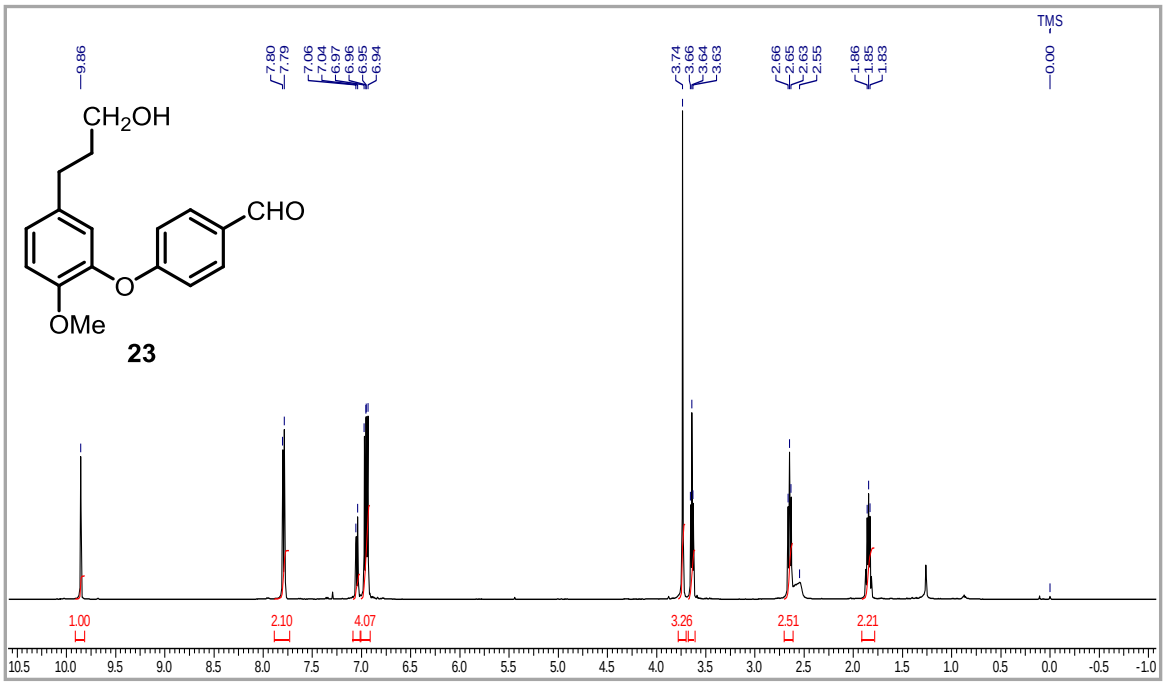
**$^{1}$H NMR (400 MHz, $CDCl_3$) of compound 21**



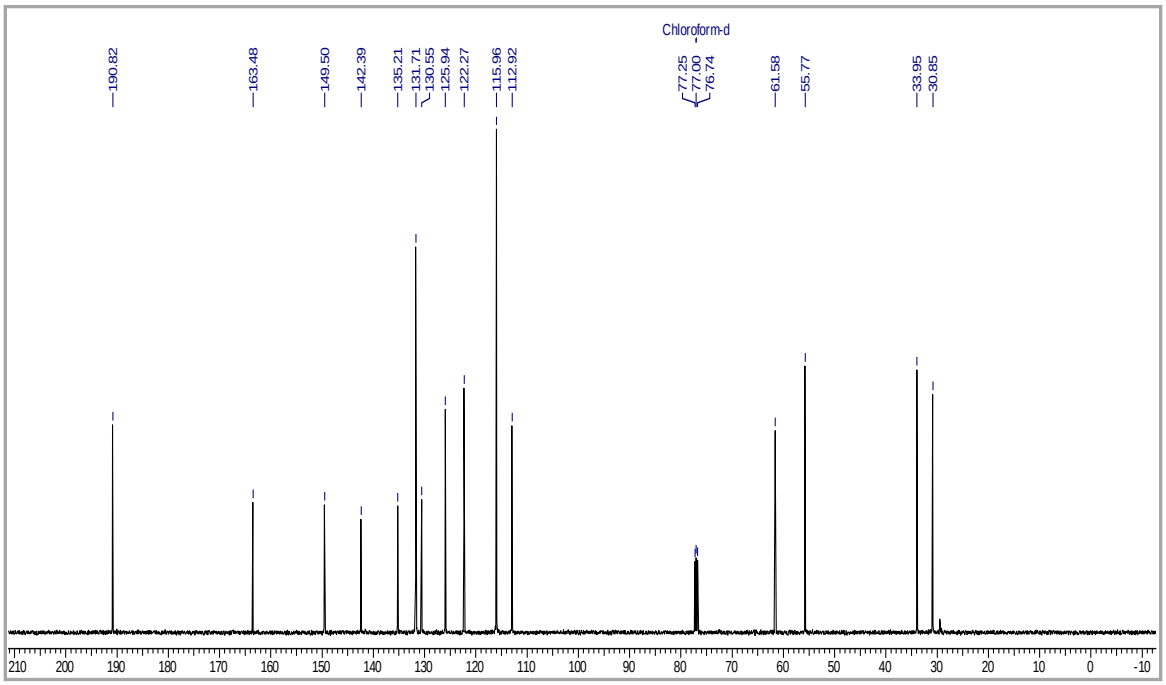

**$^{13}$C NMR (100 MHz, $CDCl_3$) of compound 21**



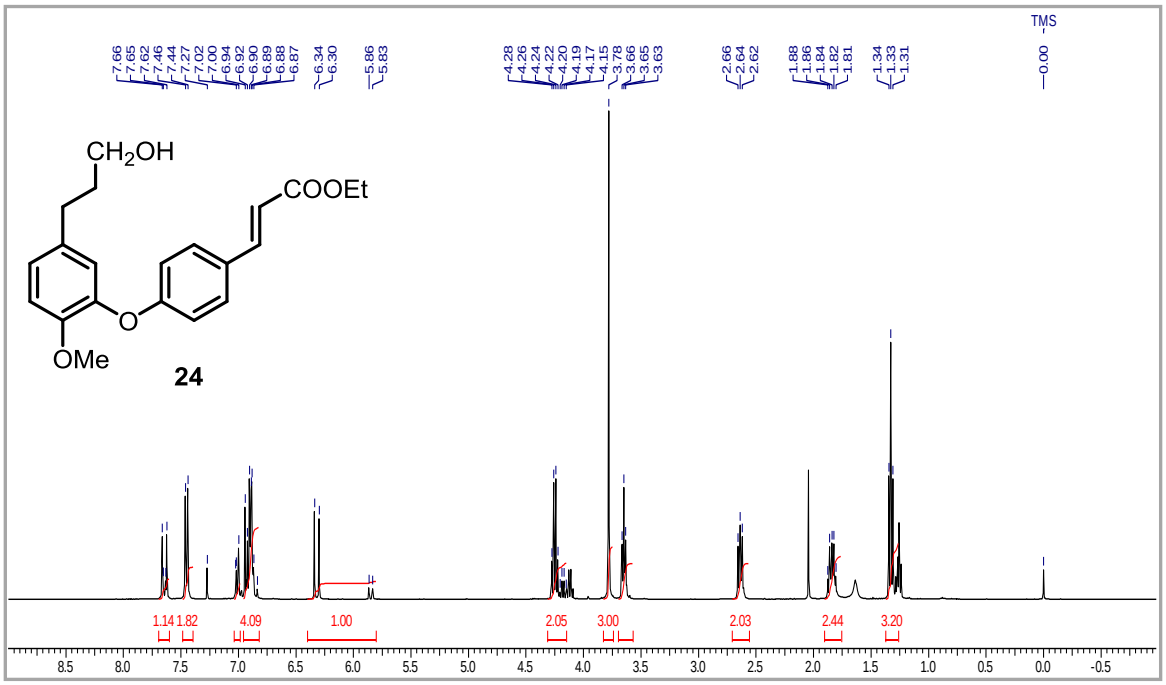
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 23



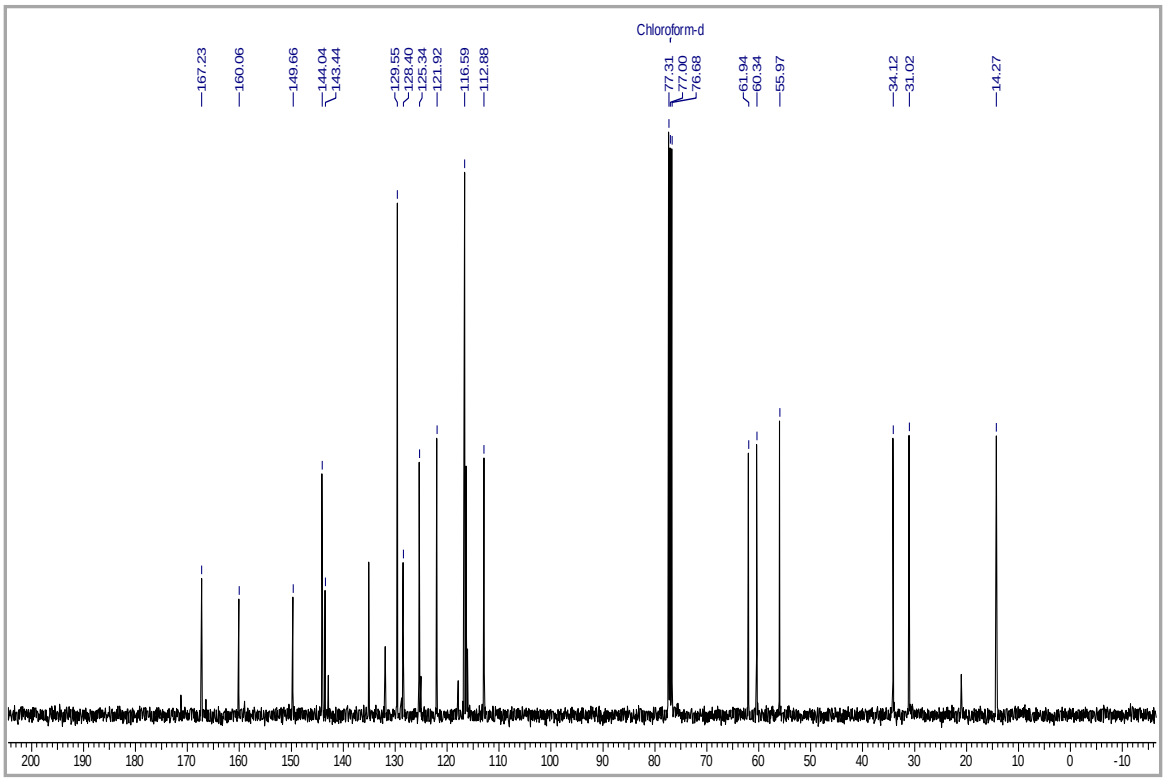

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 23



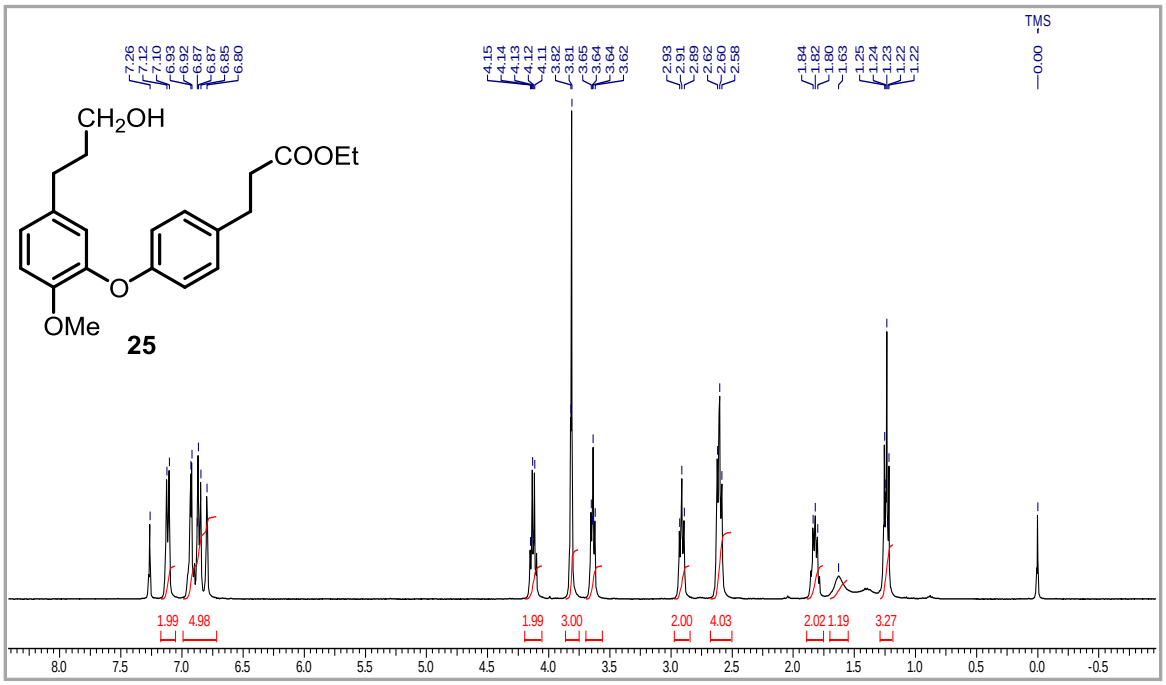
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 24



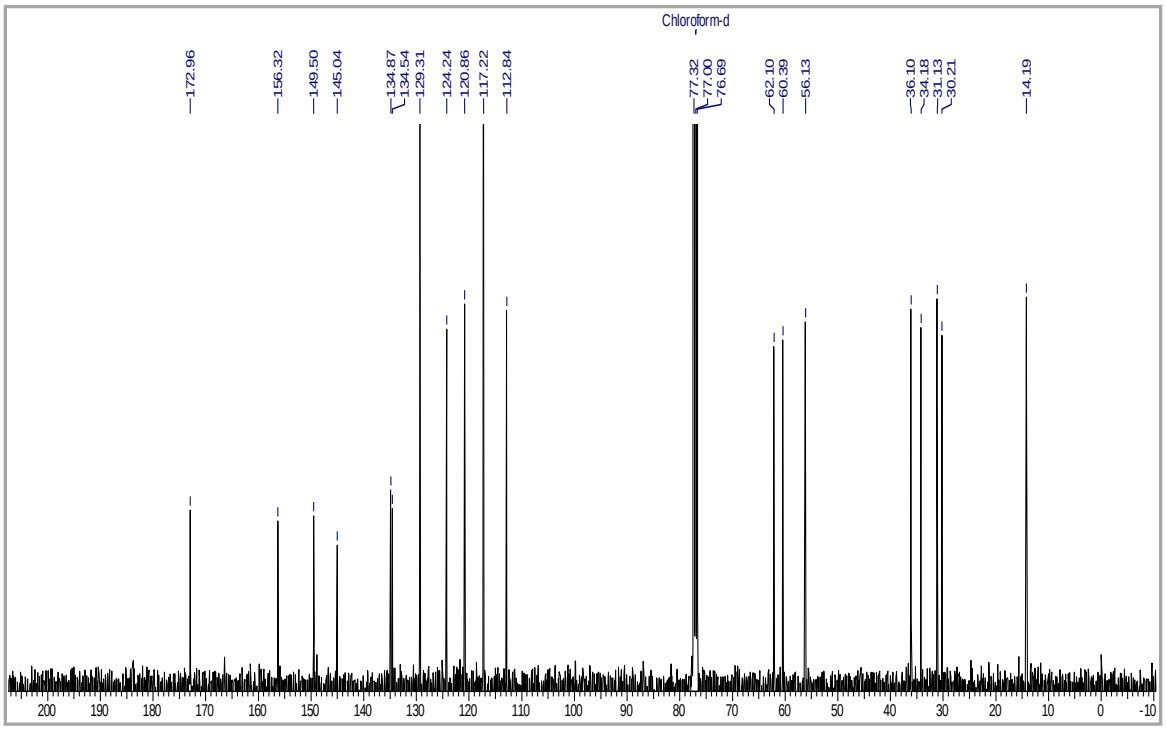

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 24



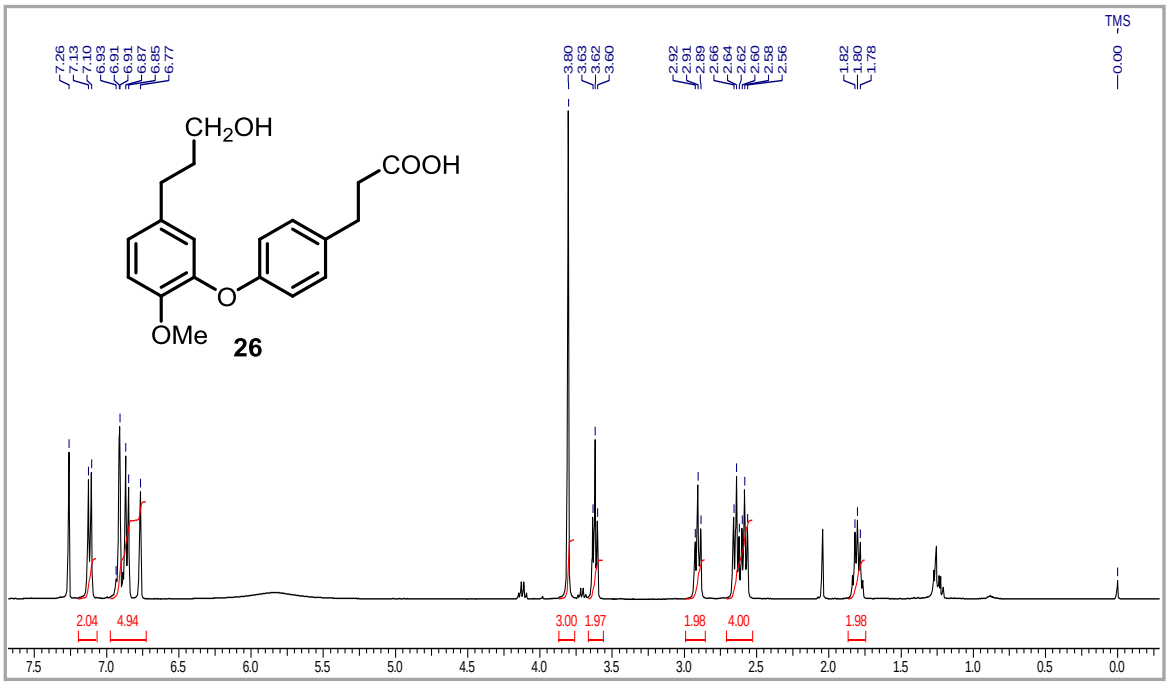
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 25



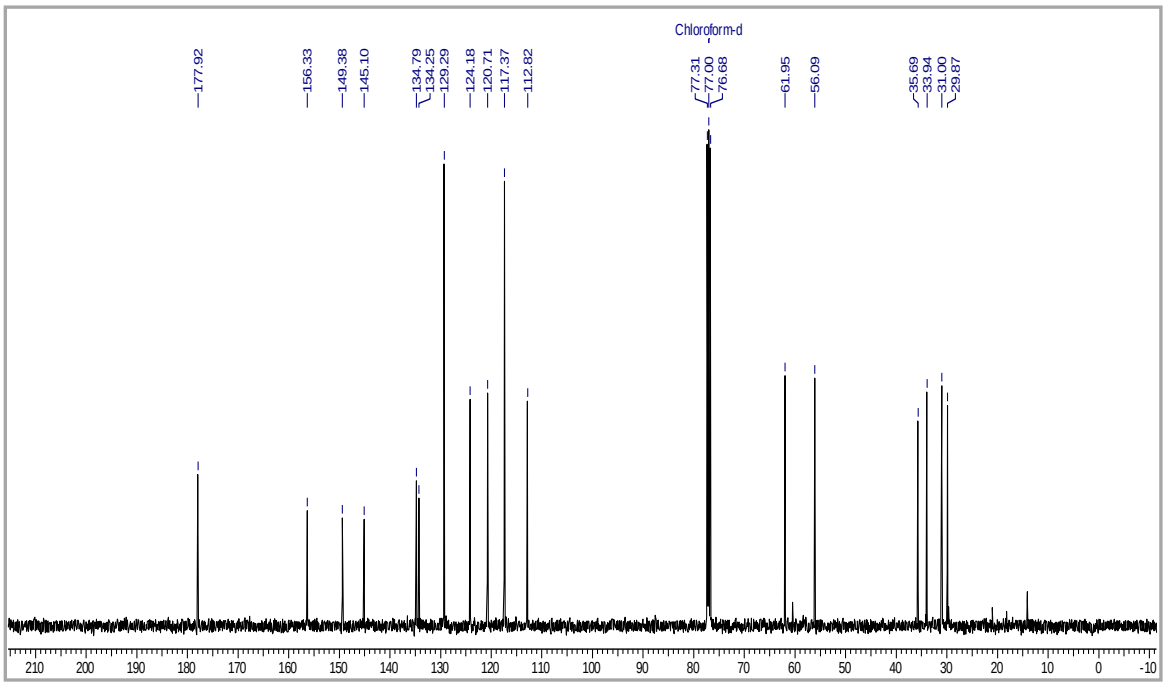

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 25



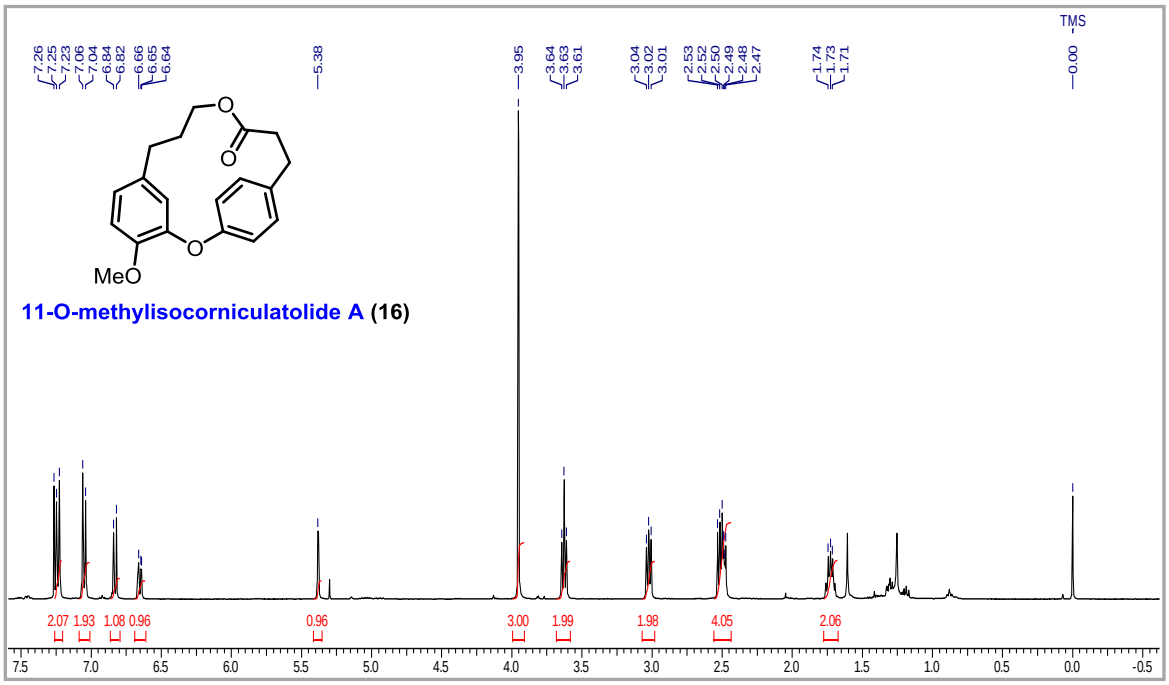
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 26



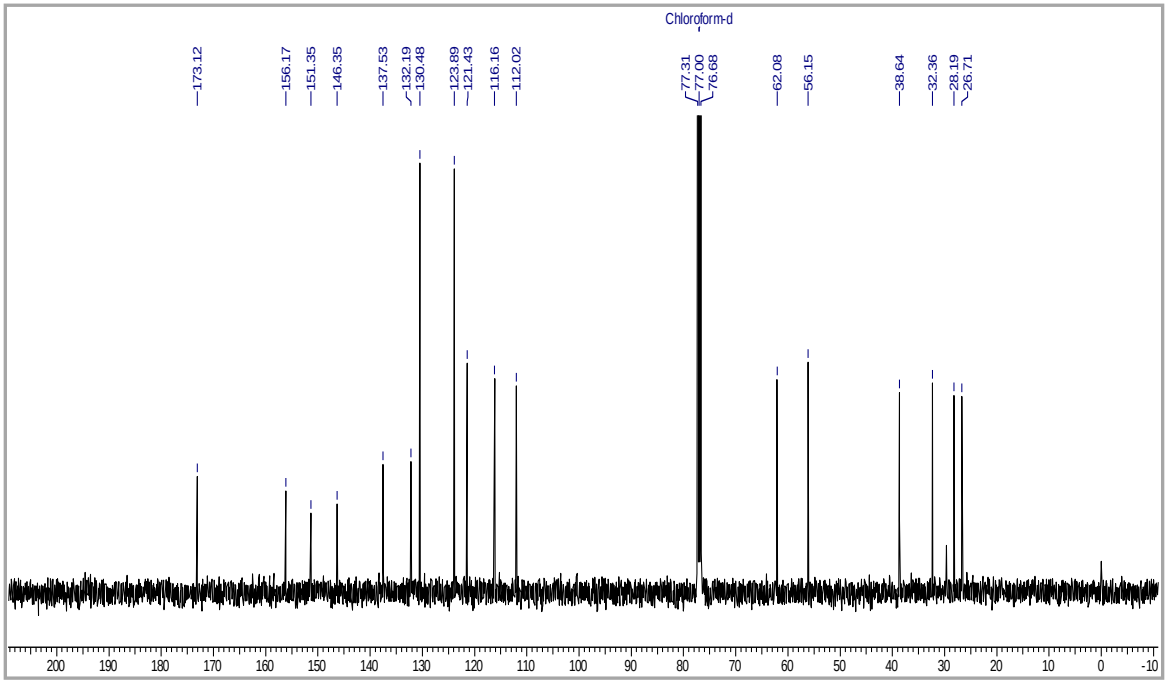

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 26



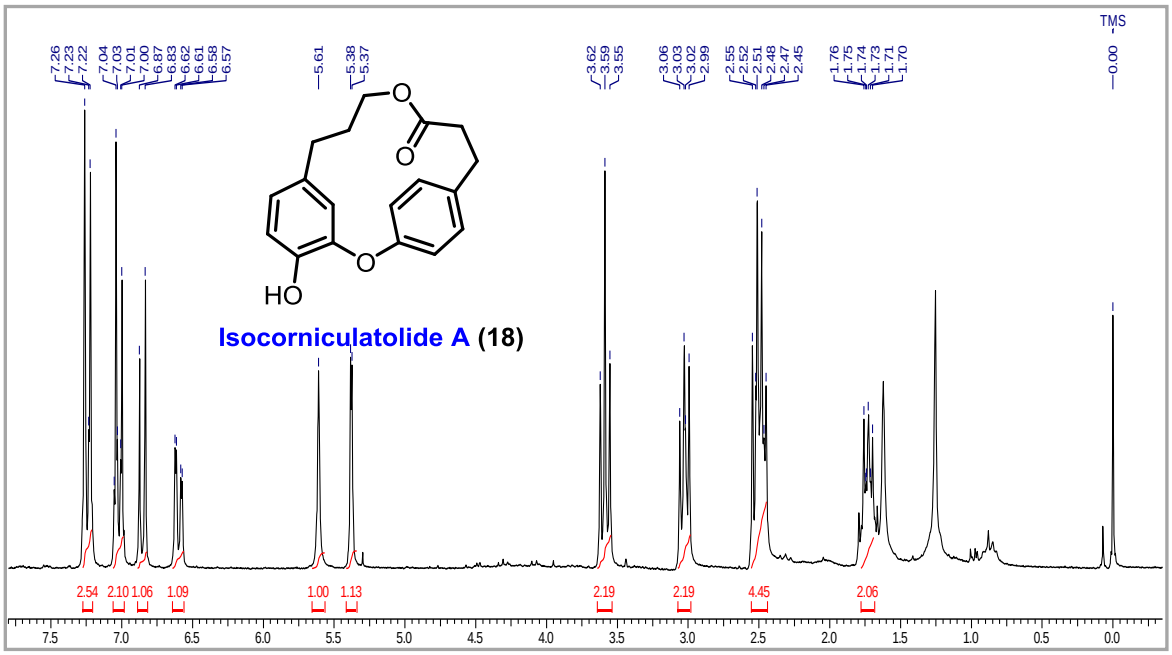
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 16



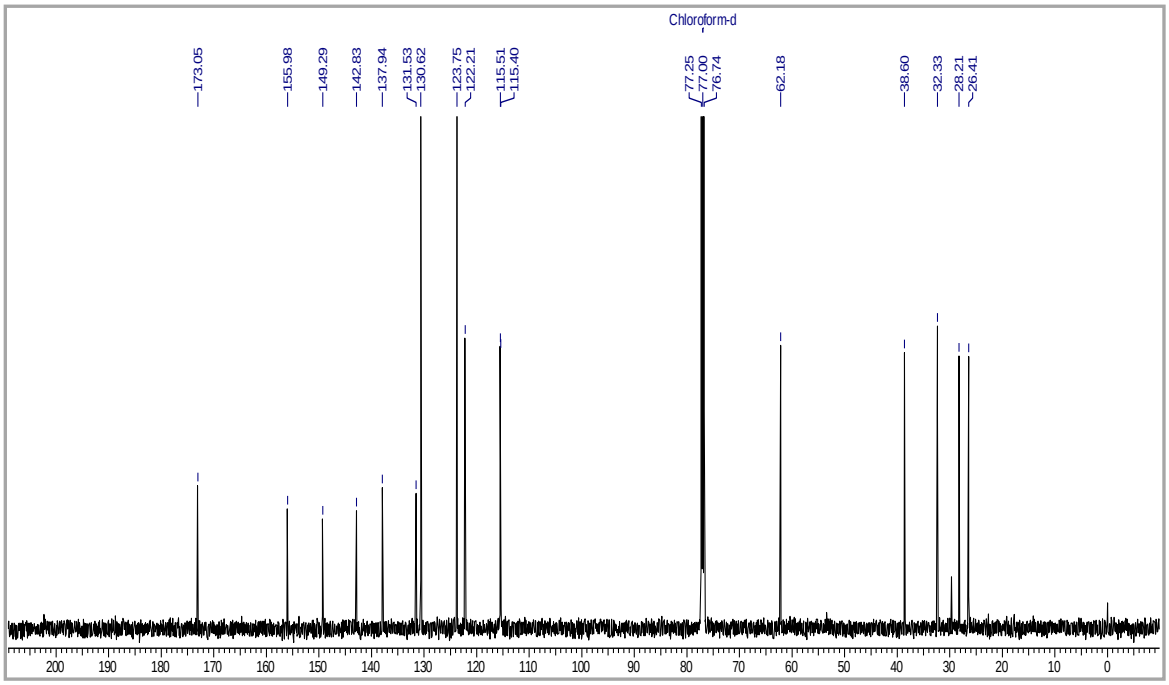

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 16



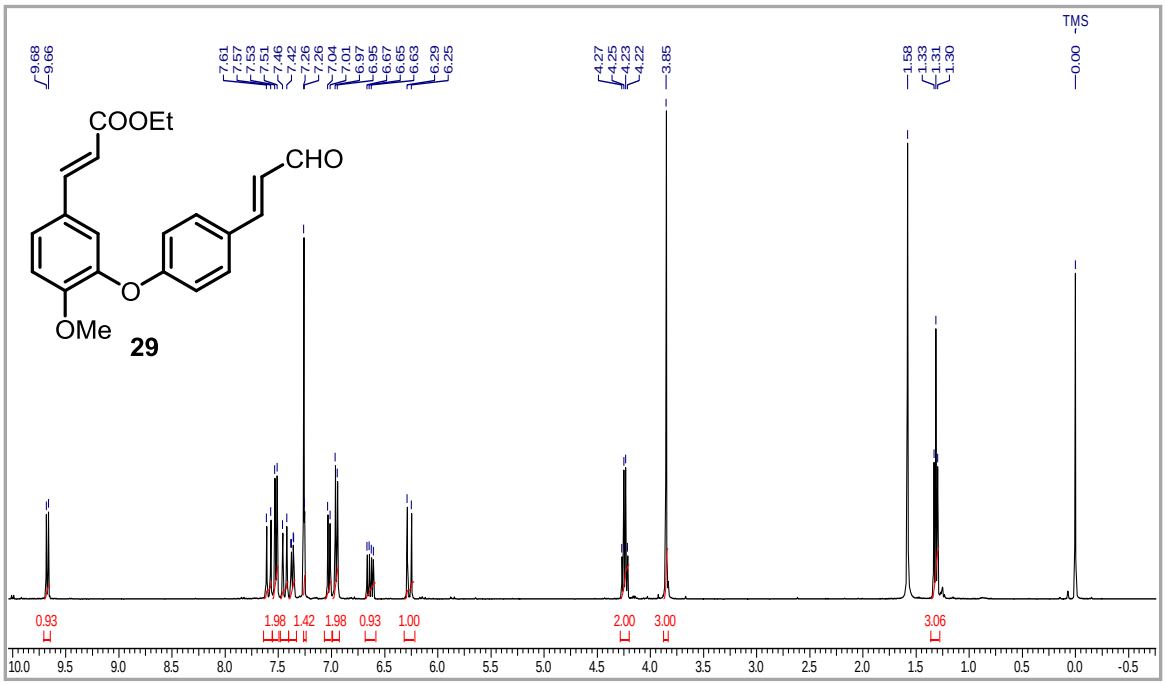
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 18



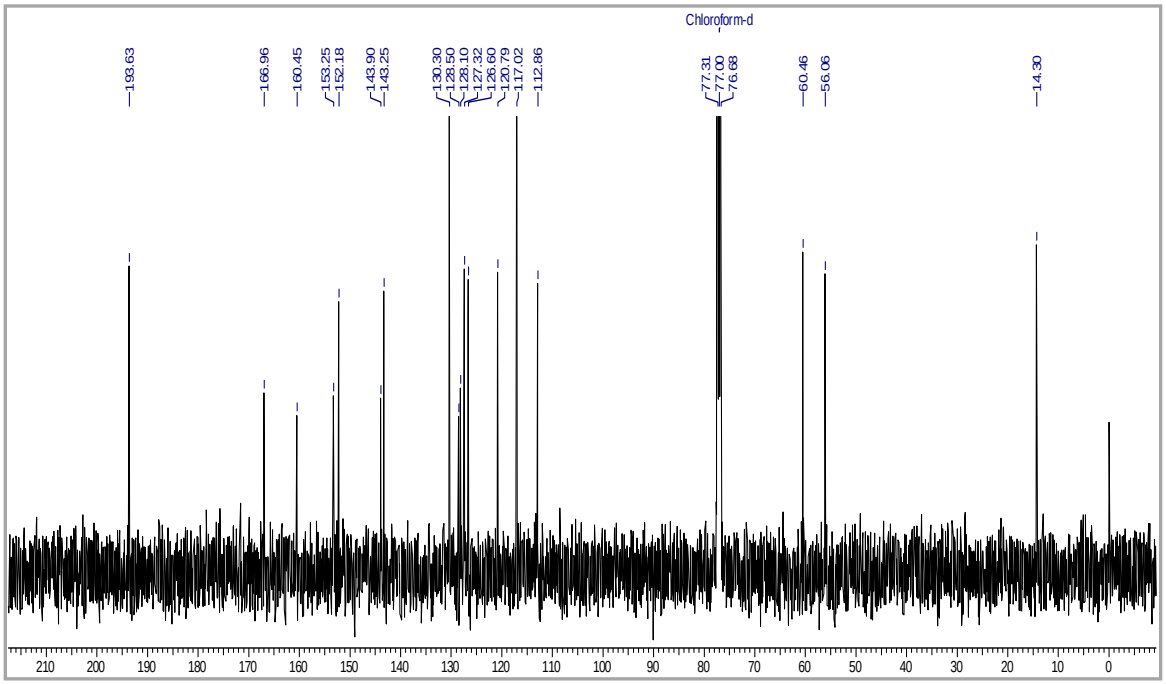

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 18



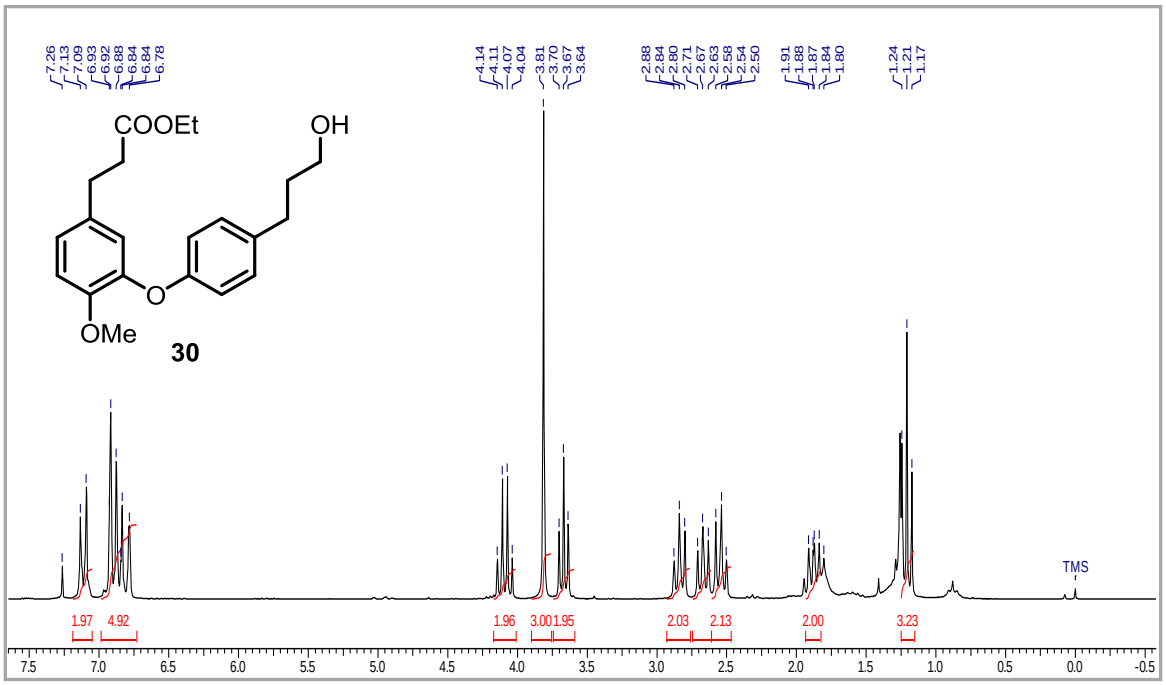
## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 29



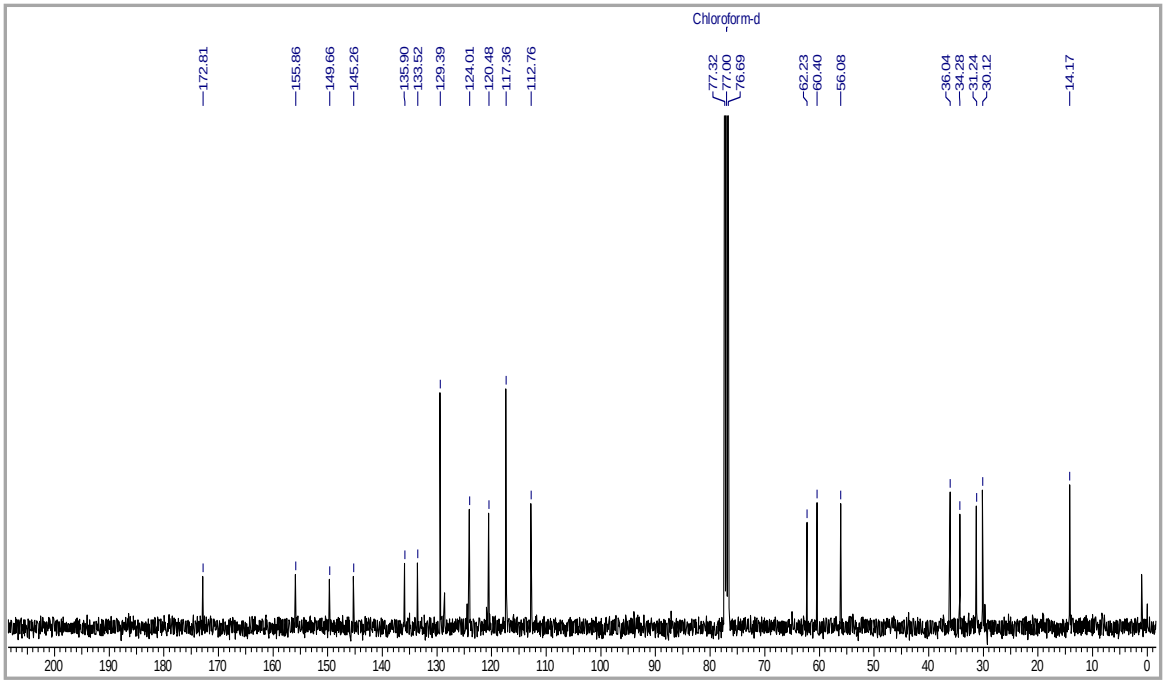

## $^{13}$C NMR (100 MHz, $CDCl_3$) of compound 29



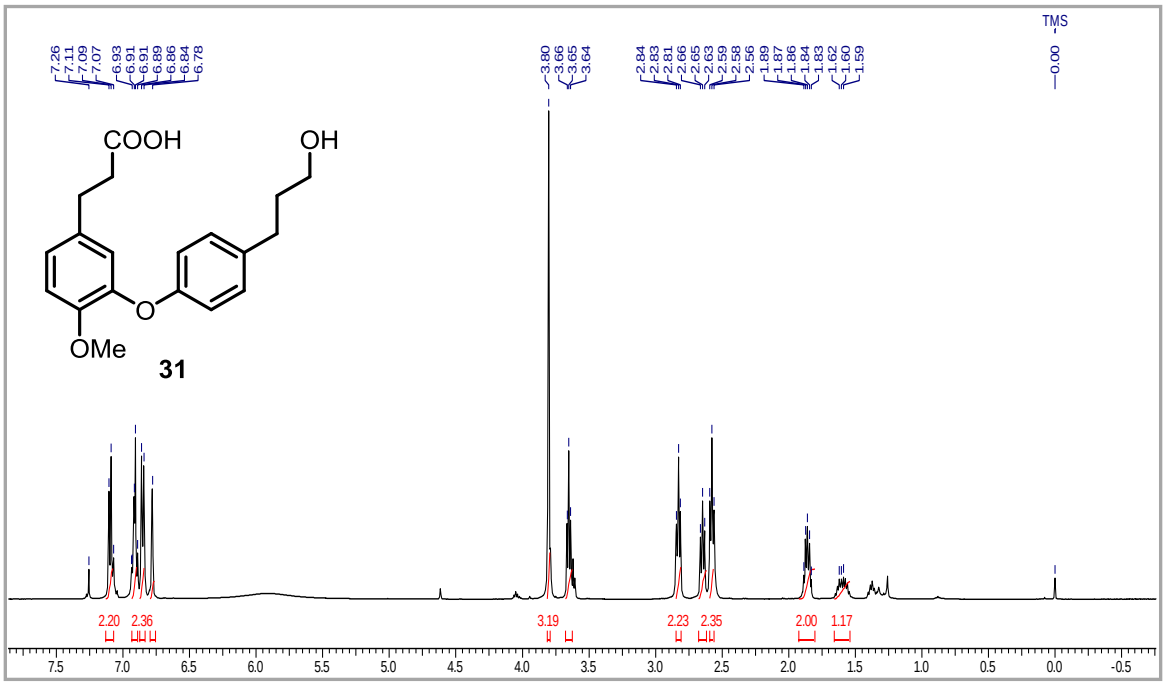
## $^{1}$H NMR (200 MHz, $CDCl_3$) of compound 30



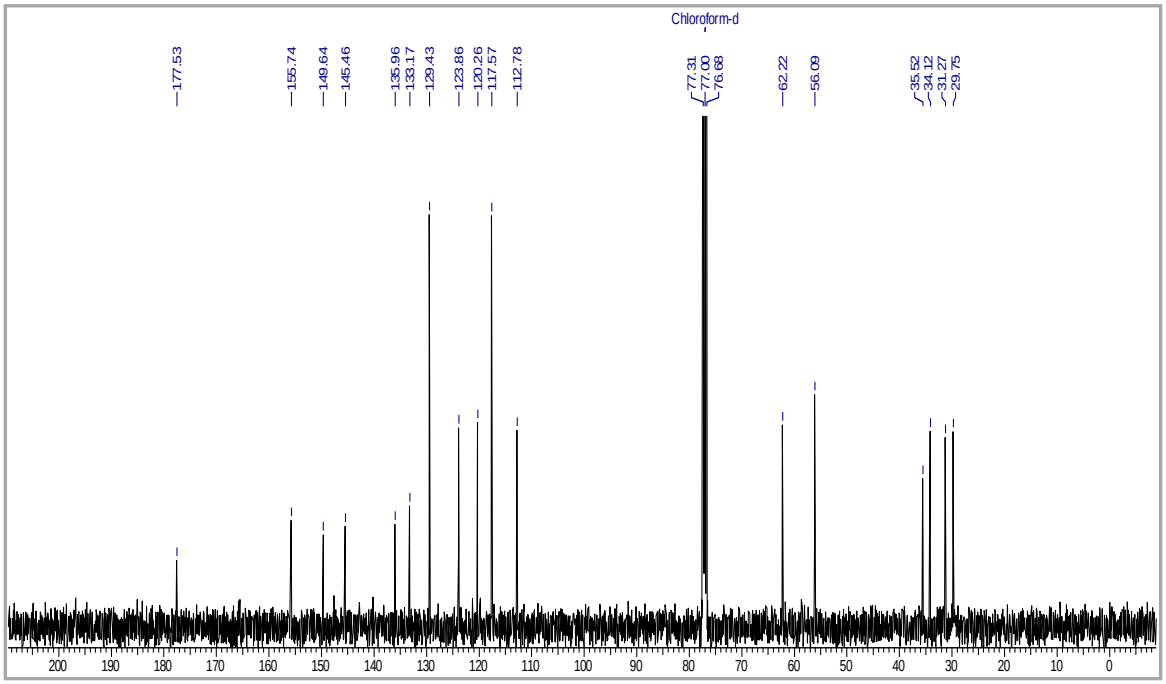

## $^{13}$C NMR (100 MHz $CDCl_3$) of compound 30



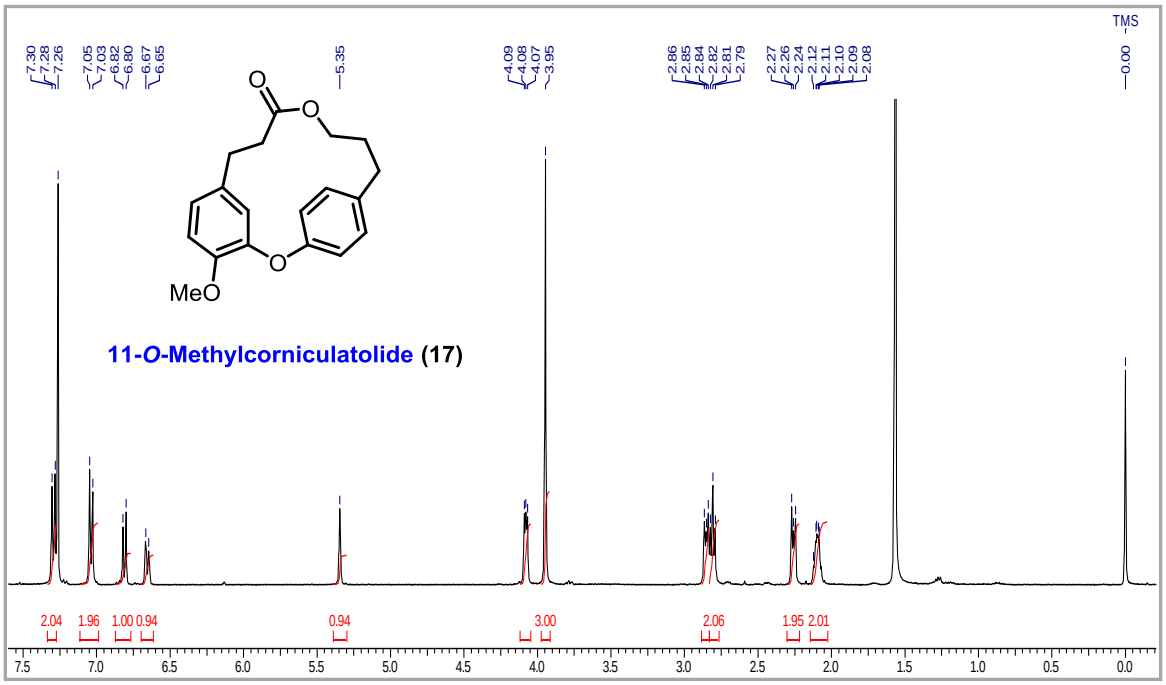
## $^{1}$H NMR (500 MHz, $CDCl_3$) of compound 31



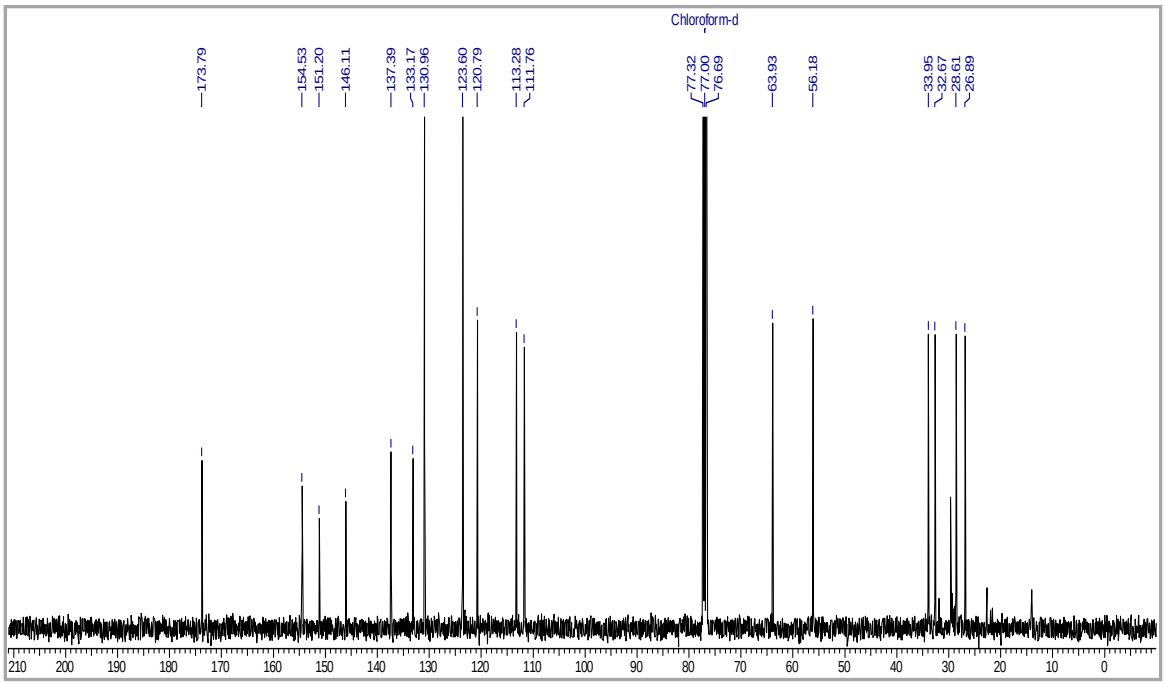

## $^{13}$C NMR (125 MHz, $CDCl_3$) of compound 31



## $^{1}$H NMR (400 MHz, $CDCl_3$) of compound 17




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



## 4.9. References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## List of Publications:

1. Antituberculosis agent diaportheone B: synthesis, absolute configuration assignment, and anti-TB activity of its analogues, Pandrangi Siva Swaroop, **Gajanan N. Raut**, Rajesh G. Gonnade, Priyanka Verma, Rajesh S. Gokhale, and D. Srinivasa Reddy. ***Org. Biomol. Chem.***, 2012, 10, 5385.
2. Synthesis of isomeric corniculatolides, **Gajanan N. Raut,** Kasturi Chakraborty, Priyanka Verma, Rajesh S. Gokhale, D. Srinivasa Reddy, *Tetrahedron Lett.*, 2012, **53**, 6343.
3. Synthesis of revised structure of klaivanolide (acetylmelodorinol), **Gajanan N. Raut**, Sachin B. Wagh, D. Srinivasa Reddy, *ARKIVOC*, 2016, **2**, 1.
4. Synthesis and Biological Evaluation of Structurally Simplified Analogs of Thiopeptide Antibiotic Lactocillin, **Gajanan N. Raut**, D. Srinivasa Reddy (**to be communicated**)
5. 4-Dehydroxydiversonol: Total Synthesis and SAR Studies of Related Tetrahydroxanthone Analogs, **Gajanan N. Raut**, Pankaj V. Khairnar, D. Srinivasa Reddy (**manuscript under preparation**)