# SYNTHESIS, CHARACTERIZATION AND STUDY OF BIOLOGICAL ACTIVITY OF SOME LACTOL, BENZIL AND OXINDOLE DERIVATIVES

**A Thesis Submitted to**

**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA**

**For the Award of the Degree of**

**DOCTOR OF PHILOSOPHY**
**IN**
**APPLIED CHEMISTRY**


**By**

**GAUTAMKUMAR MAHESHBHAI PATEL**

**Research Supervisor**

**Prof. PRADEEP T. DEOTA**




**APPLIED CHEMISTRY DEPARTMENT**
**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA, VADODARA (GUJARAT) - 390001, INDIA**


**December – 2013**

Tel: 2434188 extn.415, 212

APPLIED CHEMISTRY DEPARTMENT
UGC-SAP DEPARTMENT
Faculty of Technology & Engineering
The Maharaja Sayajirao University of Baroda
POST BOX NO. 51, KALABHAVAN
VADODARA-390001 (India)
Date: 17/12/2013

## DST-FIST SPONSORED DEPARTMENT

# CERTIFICATE

This is to certify that the work presented in the thesis entitled **"Synthesis, characterization and study of biological activity of some lactol, benzil and oxindole derivatives"** submitted by **Mr. Gautamkumar Maheshbhai Patel** for the award of the degree of **Doctor of Philosophy in Applied Chemistry** is the authentic and original research work carried out by him under my guidance and supervision in the Applied Chemistry Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara - 390001 India.

PDeota

**Prof. P. T. Deota**
**Research Supervisor**

APPLIED CHEMISTRY DEPARTMENT THE M. S. UNIVERSITY VADODARA FACULTY OF TECH. & ENGG.

PDeota

**Head**
**Applied Chemistry Department**

HEAD
APPLIED CHEMISTRY DEPARTMENT
FACULTY OF TECHNOLOGY & ENGINEERING
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
POST BOX NO. 51, KALABHAVAN,
VADODARA - 390 001. (INDIA)

17/12

**I/C Dean**
**Faculty of Technology and Engineering**

FACULTY OF TECHNOLOGY AND ENGINEERING VADODARA

Dean
Faculty of Tech. & Engg.
M. S. University of Baroda,
.Baroda.

# DECLARATION

I hereby declare that all the information in the thesis entitled **"Synthesis, characterization and study of biological activity of some lactol, benzil and oxindole derivatives"** is my own research work conducted under the supervision of **Prof. Pradeep T. Deota** in **Applied Chemistry Department, Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara (Gujarat)-390001 India**.

I further declare that to the best of my knowledge no part of this thesis has been submitted elsewhere for the award of any equivalent degree or Diploma.

**Gautamkumar M. Patel**

**Research Scholar**

***DEDICATED***
***TO***
***MY FATHER***

***LATE MAHESHBHAI V. PATEL***

## ACKNOWLEDGEMENTS

It is a pleasant aspect that I have now the opportunity to express my gratitude for all of them who accompanied and supported me in this journey of completing my PhD.

First of all, I would like to express my deepest sense of gratitude and everlasting respect to my research advisor **Prof. Pradeep T. Deota,** Head, Applied Chemistry Department, Faculty of Technology and Engineering, under whose pre-eminent guidance, I have carried out my research work. It was his keen interest, constant encouragement and valuable suggestions and guidelines, through supervision in every matter, which enabled me to complete this work. His scholarly suggestions, prudent admonitions, immense interest, constant help and affectionate behavior have been a beacon of light for me. His suggestions will remain with me as an inexhaustible source of scientific learning throughout my life.

It gives me immense pleasure to acknowledge the help, co-operation, continuous support, suggestions, encouragement and involvement rendered by my group colleagues **Dr. Piyush Upadhyay**, **Dr. Hemant Parmar, Mr. Deepak Singh and Mr. Umesh V. Chaudhari** during my Ph.D. work.

I would like to thank Dean, Faculty of Technology and Engineering, The M. S. University of Baroda, for providing the facilities for research.

It is my pleasure to acknowledge **Prof. B. V. Kamath, Prof. N. D. Kulkarni, Prof. S. R. Shah** and **Prof. A. V. Bedekar**, Department of Chemistry, Faculty of Science, The M. S. University of Baroda, for allowing me to use their instrumental facilities during my research work.

I owe a debt of gratitude to **Dr. H. C. Srivastav**, Head and **Dr. Chandrashekhar S. Pant**, Technical officer, National Institute of Malaria Research (NIMR), Nadiad, Gujarat, India for his assistance in carrying out mosquito pathogenicity activity.

Special thanks are extended to **Mr. Raju Patil**, Sr. Manager, QA Department, and **Mr. Ajay Kolte**, R&D officer, Transpek Industries Ltd., from their help in HPLC analysis.

I am also grateful to Microcare Laboratory, Surat for providing antimicrobial activity.

Sincere thanks to **Dr. Devkar,** Zoology Department, The M. S. University of Baroda for their help in cytoxicity study.

I would like to thank my colleagues **Mr. Srinivas Ghodke**, **Mr. Brijesh Shah**, **Mr. Tarun Parangi**, **Mr. Gangadhar Tammana**, **Mrs. Vaishali Suthar**, **Ms. Renu Singh**, **Mr. Himanshu Bhatt, Mr. Purvang Patel, Dr. Parimal Patel, Dr. Shrusti Deshmukh**, **Dr. Mayur Patel**, **Dr. Murli, Dr. Harish Talele, Ms. Ankita Solanki, Dr. Zala Mahendra, Mr. Kushan Parikh, Mr. Nishant Anasne, Mr. Umesh, Ms Ruchita Thakor, Mr. Shardul Bhatt, Dr. Mayur Valodkar, Mr. Akeel Saiyed, Dr. Ketan Patel, Mr. Soyeb Pathan, Ms Arti Singh** and all other research scholars for their help and support.

Thanks are also due to **Prof. C. N. Murthy, Prof. J. S. Dave, Prof. A. K. Prajapati, Dr. D. P. Bharambe, Dr. R. B Yadav, Dr. R. K. Sharma, Dr.(Mrs.) S. Dixit, Dr. Pankaj Sharma** and all other teaching staff and **Mr. Parag Vaishnav, Mrs. Kamini Patel, Mrs. Bhavna Desai** non-teaching staff of Applied Chemistry Department for their support and co-operation.

I am also grateful to **Mr. Sandip Jaiswal** and **Mrs. Bhoomika Jaiswal** for their help.

Finally, I would like to express my deepest gratitude to my grandfather **Mr. Vitthalbhai Patel**, my grandmother **Shardaben Patel**, my mother **Mrs. Taralikaben Patel** and my wife **Mrs. Rinku Patel** for their endless love, support, and countless sacrifices.

I would like to thank my family members **Mr. Sanjay Patel**, **Mrs. Nisha Patel**, **Mr. Dushyant Patel**, **Mrs. Damini Patel**, **Mr. Manish Patel**, **Mrs. Jagruti Patel**, **Avisha**, **Shivam**, **Prachi**, **Pavan**, **Shivangi** and **Shivansh** without whose affection, the present endeavor would have remained an unrealized dream.

**Gautam Patel**

# Contents

# List of Abbreviations and Symbols

| | |
|---|---|
| cm | Centimeter |
| FTIR | Fourier transform infrared spectroscopy |
| Mp. | Melting point |
| min | Minute |
| NMR | Nuclear magnetic resonance |
| RT | Room temperature |
| TLC | Thin layer chromatography |
| CHN | Elemental analyzer |
| m/z | Mass-to-charge ratio |
| mmol | Millimoles |
| mL | Milliliters |
| α | Alpha |
| β | Beta |
| δ | Delta |
| ° | Degree |
| ppm | parts per million |
| MHz | Mega hertz |
| $\theta$ | Theta |
| Å | Angstrom |
| °C | Degree Celsius |
| K | Kelvin |
| mg | Milligrams |
| g | Grams |

# Chapter 1

## Serendipitous one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one from indene and synthesis of its novel acetal derivatives

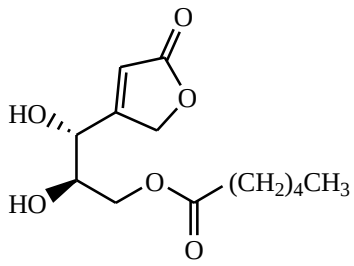
## 1.1 Abstract

One-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one (lactol) **20** from indene using tungstic acid-hydrogen peroxide is reported and a plausible mechanism of its formation is also proposed by different experimental studies. The lactol was further converted into its novel acetal derivatives **46 (a-h)**.

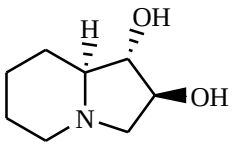
## 1.2 Introduction

The dihydroxylation of alkenes is one of the most important transformations in organic synthesis. The product obtained from the dihydroxylation of alkene i.e. 1,2-diol plays an important role in the production of numerous commodity materials which find wide application in all areas of life for example polymers, fine chemicals, pharmaceuticals, cosmetics, fragrant, photographic plates, lubricants, cleaners, etc. The diol compounds are also employed as solvents and additives in various synthetic reactions.[1]

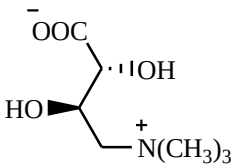
The 1,2-diol is found as a basic structural unit in many natural products, that exerts a wide spectrum of biological and pharmaceutical activities such as syributin[2] **1** acts as nonproteinaceous C-glycosidic elicitors; lentiginosine[3] **2** inhibits the amyloglucosidase enzyme and shows good glycosidase inhibitory activity; anthopleurine[4] **3** is the alarm pheromone of the sea anemone; circumcin A[5] **4** posseses neuroactivity; dopamine[6] **5** is a neurotransmitter involved in the regulation of a range of physiological functions, including motor control, cognition and the ability to experience pleasure. (Figure 1.1)



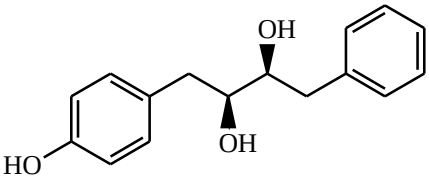

Syributin **1**



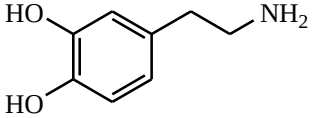

Lentiginosine **2**




Anthopleurine **3**



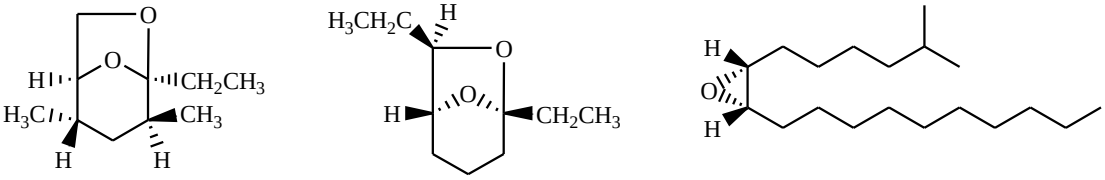

Circumcin A **4**




Dopamine **5**

**Figure 1.1:** Structures of some bioactive natural products having 1,2-diol moiety

The compounds containing 1,2-diol moiety have enormous synthetic potential to act as a precursor in the synthesis of various natural products like multistriatin[7] **6**, brevicomin[8] **7**, disparlure[9] **8**, etc. (Figure 1.2)




Multistriatin **6** Brevicomin **7** Disparlure **8**

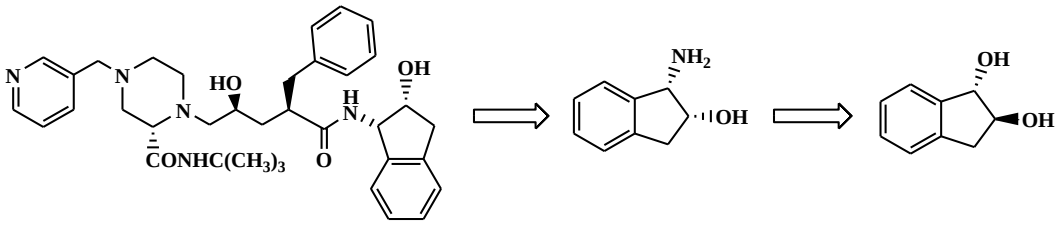
**Figure 1.2:** 1,2-diol mediated synthesis of natural products

Owing to its importance, a number of methods have been developed for the synthesis of 1,2-diols from various alkenes such as Prevost reaction, Woodward reaction, Upjohn dihydroxylation, Sharpless dihydroxylation and many more.[10] Depending on the reagent used, *cis*-diol or *trans*-diol is formed.

Generally, potassium permanganate, osmium tetraoxide and iodine-silver benzoate are used for the synthesis of *cis*-diols from alkenes.[11] Among the reagents available, none have achieved more success than osmium tetraoxide for the synthesis of *cis*-diols. A variety of co-oxidants have been used in the conjugation with osmium tetroxide in order to improve this transformation such as hydrogen peroxide,[12] metal chlorates,[13] *t*-butyl hydroperoxide,[14] *N*-methylmorpholine *N*-oxide[15] and molecular oxygen.[16] Despite of the widespread popularity of osmium tetroxide, the toxicity, high cost, volatile nature and high levels of inorganic waste are limitations of its extensive uses.[17] Therefore, the metal catalysts like palladium,[18] iron,[19] ruthenium,[20] manganese,[21] copper,[22] cobalt[23] and molybdenum[24] have been used to convert alkenes into *cis*-1,2-diols as an alternate pathways. A variety of peroxy acids are also employed for the synthesis of *trans*-diols from alkenes such as performic acid, perbenzoic acid, potassium peroxymonosulfate and others.[25]

The significance of HIV protease inhibitors in the treatment of acquired immune deficiency syndrome (AIDS) is now well known in the literature.[26] The crixivan **9**, a leading drug was developed for the treatment of AIDS. The *cis*-1-amino-

2-indanol **10** is an important synthon for the synthesis of crixivan which can be synthesized from *trans*-1,2-indan diol **11**.[26] (Scheme 1.1)



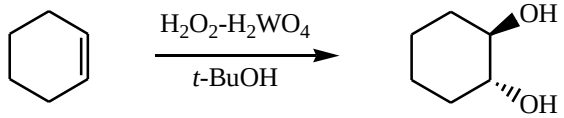

Crixivan **9** *cis*-1-amino-2-indanol **10** *trans*-1,2-indan diol **11**

**Scheme 1.1:** *trans*-1,2-indandiol as precursor

Numerous methods have been developed for the synthesis of *trans*-1,2-indandiol **11** either chemically or biologically.[27-29] Whalen *et al*[28] have used Prevost dihydroxylation for the synthesis of *trans*-1,2-indandiol **11**. Asano *et al*[29] have prepared *trans*-1,2-indandiol **11** from indene in two steps. The bio-transformation of indene to *trans*-1,2-indandiol **11** using *Rhodococcus strain I24* is reported by Sinskey *et al*.[27] Though these methods work well, many of them involve use of expensive reagents, tedious workup procedure, toxic effluent.

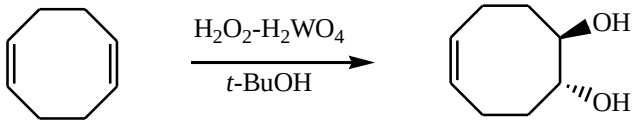
Tungstic acid and hydrogen peroxide are commercially available reagents. Water is the only byproduct and the tungstic acid is easily recovered after completion of the reaction. The tungstic acid catalyzed *trans* addition of hydrogen peroxide to alkenes is well known for a long time.[30]

The first synthesis of *trans*-1,2-cyclohexane diol **13** using tungstic acid-hydrogen peroxide was reported by Payne *et al* in 1957.[31] (Scheme 1.2)




**12** **13**

**Scheme 1.2:** Synthesis of *trans*-1,2-cyclohexane diol

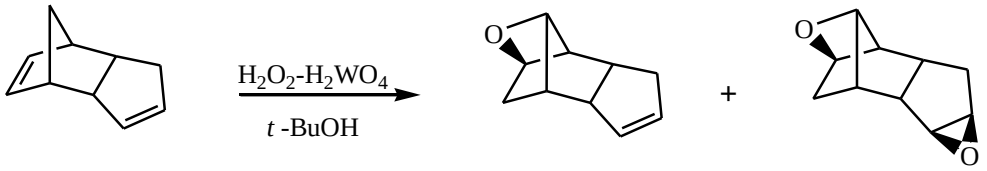



**14** **15**

**Scheme 1.3:** Synthesis of *trans*-diol of (*Z*,*Z*)-cycloocta-1,5-diene

Earlier our group has demonstrated the synthetic utility of tungstic acid–hydrogen peroxide to obtain the corresponding *trans*-diol **15** of (*Z*,*Z*)-cycloocta-1,5-diene **14**.[32] (Scheme 1.3) (*Z*,*Z*)-cycloocta-1,5-diene is known to pose considerable problems in hydroxylation with other hydroxylating agents owing to its propensity towards *trans*-annular cyclization.[32]

The treatment of this reagent combination with *endo*-dicyclopentadiene **16** in *t*-butanol unexpectedly resulted in the formation of novel polycyclic oxetanes (**17, 18**).[33](Scheme 1.4)




**16** **17** **18**

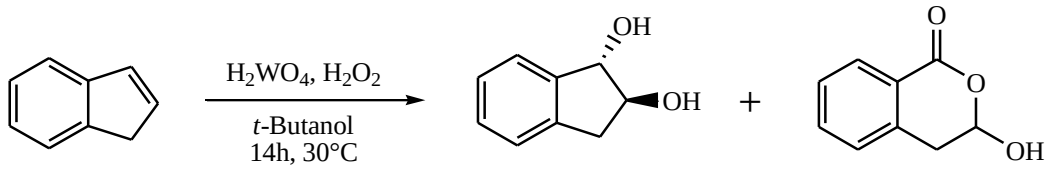
**Scheme 1.4:** Reaction of *endo*-dicyclopentadiene with $H_2O_2$-$H_2WO_4$

Our continued interest in the dihydroxylation of olefins prompted us to explore the reaction of indene **19** with tungstic acid-hydrogen peroxide in order to prepare its *trans*-diol **11** which is an important intermediate for crixivan **9**, an HIV protease inhibitor.

### 1.3 Results and discussion

In pursuing above objective, indene **19** was treated with tungstic acid–hydrogen peroxide in *t*-butanol for 14h which resulted in the formation of not only

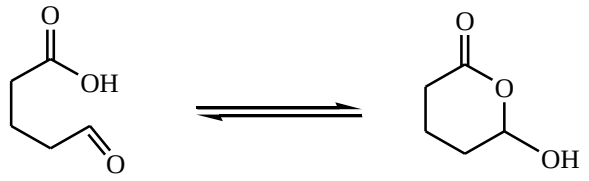
*racemic* indan-*trans*-1,2-diol **11** but also racemic 3,4-dihydro-3-hydroxyisochroman-1-one (lactols or pseudoacid) **20**. (Scheme 1.5)




**19** **11** **20**

**Scheme 1.5:** Reaction of indene with hydrogen peroxide-tungstic acid

The structures of compounds **11** and **20** were confirmed by mp, FTIR, $^{1}$H NMR, $^{13}$C NMR, Mass and elemental analysis.



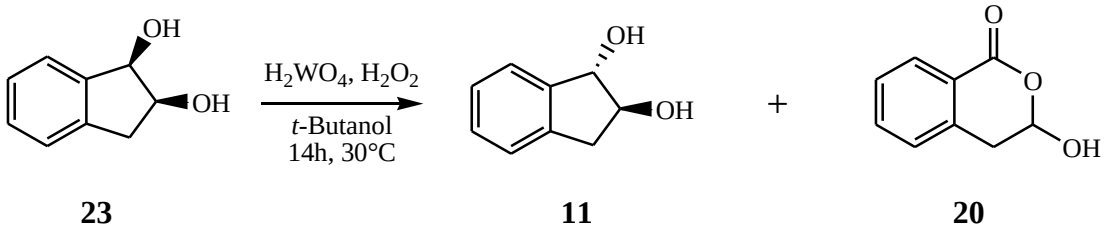

5-oxo acid **21** Cyclic lactol **22**

**Scheme 1.6:** 5-oxoacids exist as cyclic lactols

During the reaction, the serendipitous formation of lactol encouraged us to investigate its route of formation. Since it is already known that certain 5-oxoacids such as **21** exist as pseudoacids or cyclic lactols **22**,[34] (Scheme 1.6) it was clear that **20** was formed as a result of cleavage of indene followed by *in situ* cyclization of the intermediate 2-carboxyphenylethanal **24** similar to **21**.

To ascertain the source of the lactol **20**, a few experiments were carried out. Initially, it was thought that indan-*trans*-1,2-diol **11** could be giving rise to **20** *via* 2-carboxyphenylethanal but the treatment of **11** with tungstic acid-hydrogen peroxide at 30°C in *t*-butanol left it unchanged even after 14 h. It was then contemplated that *cis*-diol of indene could be the source of **20.** Thus, indan-*cis*-1,2-diol **23** was prepared by a reported method[35] and was treated with tungstic acid–hydrogen peroxide under the

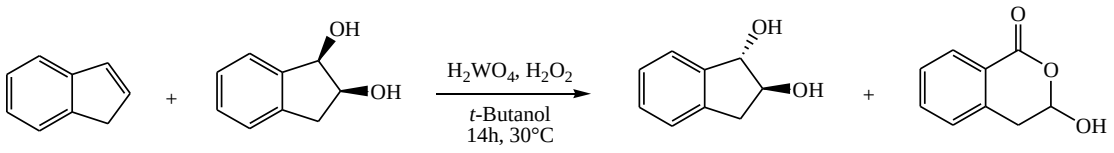
reaction condition which indeed gave **20** along with **11** in 28% and 63% yields respectively (Scheme 1.7). A similar conversion of **23** to **11** in the presence of nickel at 60°C was reported.[36] It should be noted that the present method (Scheme 1.7) furnishes **11** from **23** in better yield (63%) under milder conditions.




**23** **11** **20**

**Scheme 1.7:** Reaction of indan-*cis*-1,2-diol with hydrogen peroxide - tungstic acid.

That the lactol **20** originates from **23** was further confirmed by spiking studies as described below. We added varying amounts of **23**, a proposed precursor of **20**, to the reaction mixture in order to prove its intermediacy or involvement in the formation of **20**. Our contention was supported when enhancement in the yields of **20** and **11** was obtained on treatment of the mixtures of varying amounts of **23** and **19** under reaction condition (Scheme 1.8, Table 1.1).

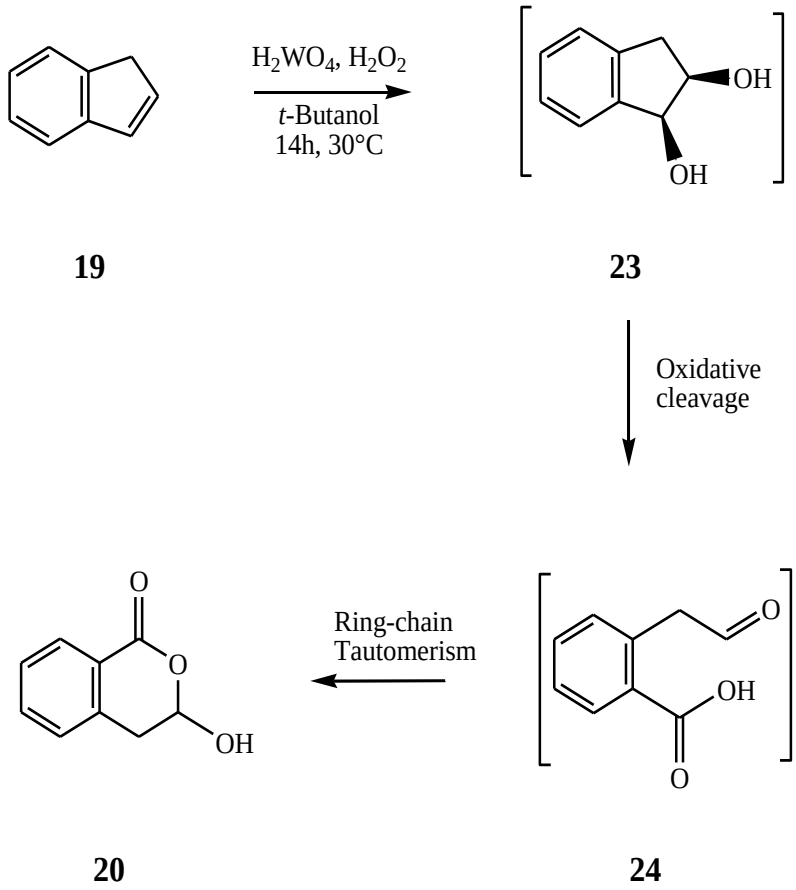



**19** **23** **11** **20**

**Scheme 1.8:** Treatment of mixtures of **23** and **19** with hydrogen peroxide-tungstic acid

**Table 1.1:** Results of spiking study

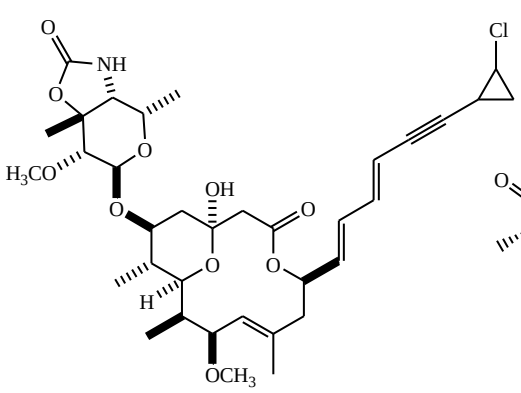
| Indan-*cis*-1,2-diol (**23**) | Indan-*trans*-1,2-diol (**11**) | Lactol (**20**) |
|---|---|---|
| No. of moles added | Rise found in no. of moles | Rise found in no. of moles |
| 0.007 | 0.0036 (61.85%) | 0.0020 (26.66%) |
| 0.014 | 0.0081 (61.81%) | 0.0037 (26.95%) |
| 0.021 | 0.0155 (61.92%) | 0.0056 (26.98%) |



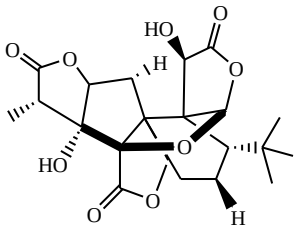

**Scheme 1.9**: Possible steps in formation of lactol from indene

These observations also implied that **23** gives rise to lactol **20** via oxidative cleavage followed by cyclization under reaction condition. (Scheme 1.9) Similar cleavage of **11** does not take place during the reaction, perhaps due to the *trans* geometry of the hydroxyl groups in it.

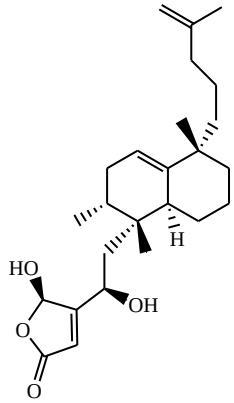
Lactol scaffold is widely distributed in nature and is found as a core structural unit in various biologically active compounds (Figure 1.3) such as callipeltoside A **25**,[37] ginkgolides **26**,[38] dysidiolide **27**,[39] cladocorans B **28**,[40] acuminolide **29**,[41] spongianolide A **30**,[42] manoalide **31**,[43] cacospongionolide $B_2$ **32**,[44] peniolactol **33**.[45] Lactols are also employed as important precursors of several compounds such as illudalic acid,[46] benzopyran-1-ones,[47] caronaldehyde,[48] mevalonate and mevaldate.[49]



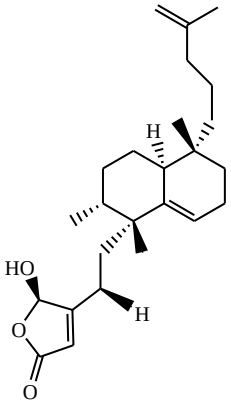
Callipeltoside A **25**



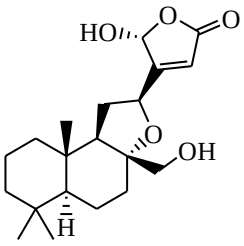
Ginkgolide **26**



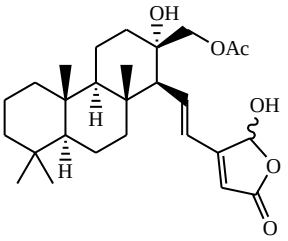
Dysidiolide**27**



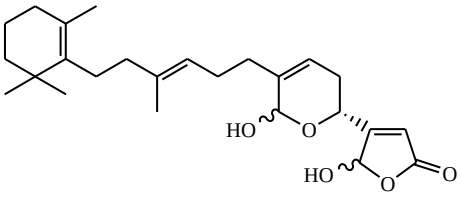
Cladocorans B **28**



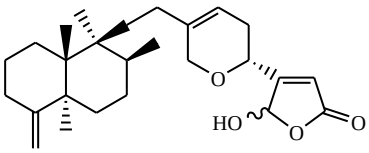
Acuminolide **29**



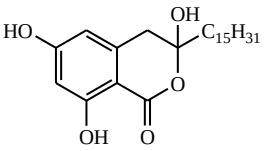
Spongianolide A **30**



Manolide **31**



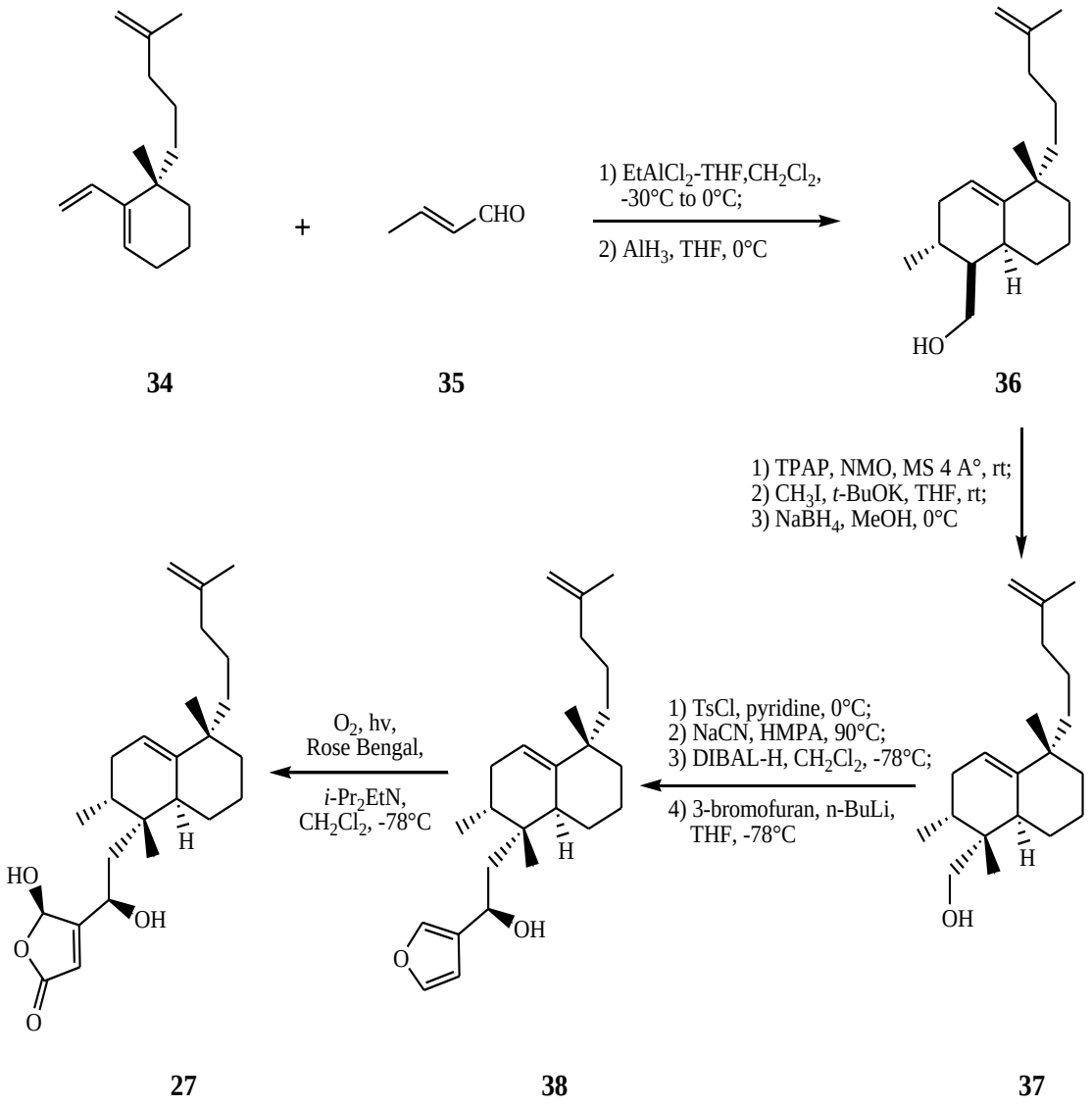
Cacospongionolide B **32**



Peniolactol **33**

**Figure 1.3**: Various biologically active compounds having lactol scaffold

In past, lactol unit has been assembled via different routes by various research groups.[46-50] Boukouvalas *et al*[39] has reported the total synthesis of dysidiolide **27** in which lactol unit has been generated by photosensitized oxygenation of furan (Scheme 1.10) and in the same way the lactol units of cladocorans B **28**, acuminolide **29**, spongianolide A **30**, manoalide **31** and cacospongionolide $B_2$ **32** have also been synthesized.

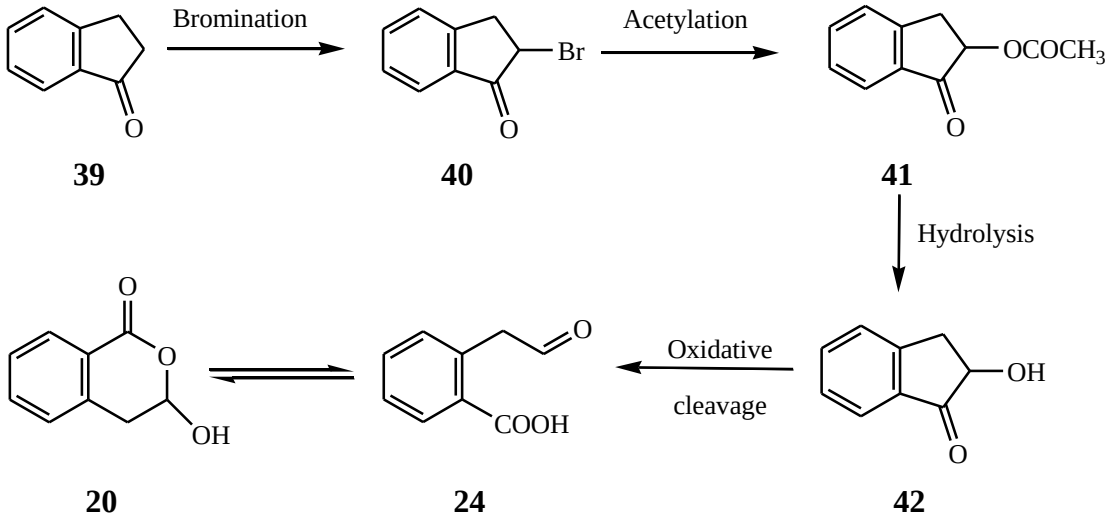



**34** **35** **36** **37** **38** **27**

**Scheme 1.10:** Total synthesis of dysidiolide

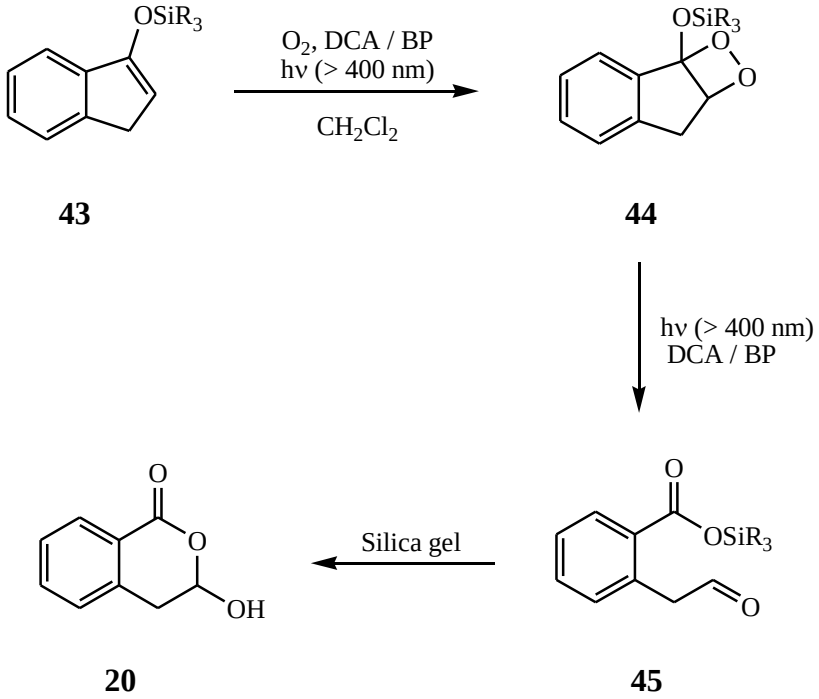
Many other route are also available in the literature like oxidative cleavage of olefins or diols followed by cyclization using various reagents like sodium metaperiodate,[46] aqueous potassium hydroxide,[48] triflouroacetic acid,[49] and ozonolysis followed by addition of dimethyl sulphate.[50]

Schöpf and Kühne[51] were the first to report lactol **20** in four steps (Scheme 1.11) and its crystal structure was subsequently reported much later by Valente *et al*.[34]




**Scheme 1.11:** Synthesis of lactol **20** reported by Schöpf and Kühne

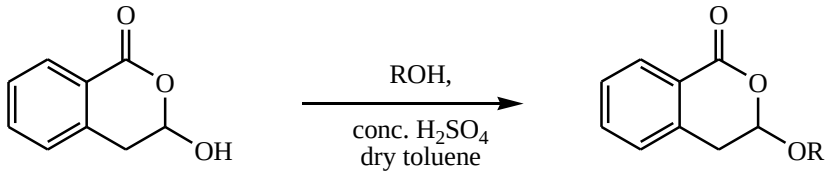
Abe *et al*[52] have also synthesized the lactol **20** from silyl enol ethers **43** by photooxygenation sensitized by 9,10-dicyanoanthracene (DCA) or bipyridyl (BP) in three steps (Scheme 1.12).




**Scheme 1.12:** Synthesis of lactol **20** reported by Abe *et al*

To the best of our knowledge, one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one **20** from indene **19** under such mild conditions (Scheme 1.5) is hitherto unknown in the literature.

Literature survey revealed that various lactol derivatives show wide variety of biological activities, for example dysidiolide **27** acts as the inhibitor of phosphatase CDC25A and inhibits the growth of A-549 human lung carcinoma,[39] cladocorans B **28** are used as inhibitor of protein phosphatase CDC25A,[40] acuminolide **29** displays cytotoxic activity in human cancer cell lines and cultured P388 cells,[41] spongianolide A **30** inhibits proliferation of the mammary tumor cell line MCF-7,[42] manoalide **31** is an irreversible inhibitor of phospholipase A2 (PLA2),[43] cacospongionolide B **32** shows a significant activity on recombinant human synovial PLA2,[44] illudalic acid is a potential human leukocyte common antigen-related (LAR) phosphatase inhibitor.[46]




**20** **46(a-h)**

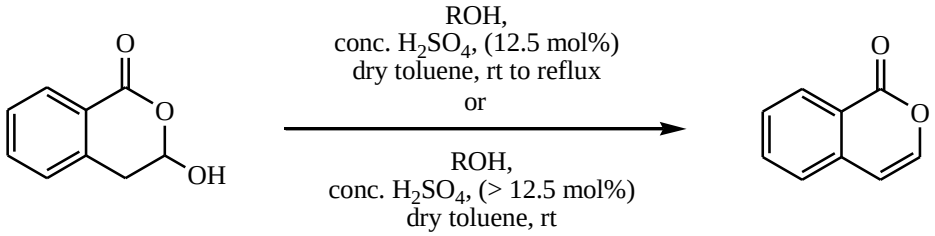
**a)** R = n-$C_2H_5$; **b)** R = n-$C_3H_7$; **c)** R = n-$C_4H_9$; **d)** R = n-$C_5H_{11}$; **e)** R = n-$C_8H_{17}$; **f)** R = n-$C_{12}H_{25}$; **g)** R = n-$C_{14}H_{29}$; **h)** R = n-$C_{16}H_{33}$

**Scheme 1.13:** Synthesis of acetal derivatives of lactol

We also found that the acetals of nepetalic acid exhibit prominent mosquito repellency.[53] These interesting biological properties of lactol derivatives impelled us to prepare the acetal derivatives **46(a-h)** (Scheme 1.13) and examine their antimicrobial activity as well as mosquito pathogenicity. As mentioned earlier, the lactols are known to coexist as open chain structure having free carboxylic acid functionality that can react with alcohols to form acetals.

The structures of acetal derivatives **46(a-h)** were also confirmed by mp, FTIR, $^1$H NMR, $^{13}$C NMR, mass and elemental analysis. The FTIR spectrum of **46a** showed bands at 1628, 1464 cm$^{-1}$ for aromatic ring and a strong band at 1729 cm$^{-1}$ for the carbonyl group. The $^1$H NMR spectrum of **46a** displayed triplet of three methyl protons at δ 1.20, two doublet of doublet at δ 3.15 and 3.35 for two methylene protons and triplet at δ 5.55 for one methine proton. The multiplets between δ 7.2-8.2 showed the presence of four aromatic protons. The $^{13}$C NMR spectrum of **46a** exhibited

signals at δ 15.30 for methyl carbon, at δ 33.71, 65.35 for methylene carbon, at δ 101.14 for methine carbon and at δ 125.04, 127.70, 128.34, 129.97, 134.12 and 136.68 for aromatic carbons along with signal at δ 164.22 for carbonyl carbon. The structure of **46a** was further confirmed by its mass spectrum which gave a molecular ion peak at 192. The elemental analysis was in good agreement with the required molecular formula for $C_{11}H_{12}O_3$ and it was found as C, 68.76; H, 6.23 and calculated; C, 68.74; H, 6.29.




**20** **47**

**Scheme 1.14:** Dehydration of 3,4-dihydro-3-hydroxyisochroman-1-one

The catalyst quantity and the reaction temperature play an important role in the formation of acetal derivatives. It was observed that lactol **20** underwent dehydration by increasing either the temperature or the catalyst quantity beyond 12.5 mol%. (Scheme 1.14) Various acetal derivatives of lactol (**46a-h**) were prepared to examine the effect of spacer groups on their biological potency.

## 1.4 Experimental

### *General*

FTIR spectra were recorded on a Shimadzu 8400S FTIR spectrometer using KBr. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker-400MHz NMR spectrometer (100 MHz for $^{13}$C NMR) using $CDCl_3$ or DMSO-$d_6$ (TMS as an internal standard). Mass spectra were obtained on a Shimadzu QP-5050 mass spectrometer. Column chromatography was carried out on Acme's silica gel (60-120 mesh size) and eluted using mixtures of light petroleum and ethyl acetate. Thin layer chromatography was performed using Acme's silica gel for TLC and spots were visualized in the iodine vapor. Percentage yields were reported based upon the recovery of starting materials.

The structures of all the compounds were confirmed by their mp, elemental analysis, FTIR, $^{1}$H NMR, $^{13}$C NMR and mass spectrometric data.

***Synthesis of 2,3-dihydro-1H-indan-trans-1,2-diol and 3,4-dihydro-3-hydroxyisochroman-1-one:***

To a stirred solution of indene (**19)** (43 mmol) in *t*-butanol (25 ml) was added a suspension of tungstic acid (0.5 g) and hydrogen peroxide (30%, 140 mmol). The reaction mixture was stirred for 14 hrs at room temperature (~30°C). After completion of the reaction (TLC), reaction mixture was filtered through a celite pad to remove the suspended catalyst. The filtrate was diluted with water (20 ml) and extracted with ethyl acetate (4 × 25 ml). The combined organic extracts were washed with water (15 ml), brine (10 ml) and dried over anhydrous sodium sulphate. Removal of solvent and column chromatography of the residue furnished the indan-*trans*-1,2-diol (**11**, 39%) and 3,4-dihydro-3-hydroxyisochroman-1-one (**20**, 16%). Prolonged reaction periods did not appreciably alter the product ratio.

***2,3-Dihydro-1H-indan-trans-1,2-diol (11)***

White crystalline solid (39%), mp. 156-160 °C; IR ($\nu_{max}$, $cm^{-1}$): 3226, 2911, 2849, 1559, 1477, 1458, 1354, 1057, 746, 645; $^{1}$H NMR: $\delta_H$ 2.74 (dd, 1H, $CH_2$ geminal, $J_1$ = 15.5 Hz, $J_2$ = 7.5 Hz), 3.18 (dd, 1H, $CH_2$ geminal, $J_1$ = 15.3 Hz, $J_2$ = 7.4 Hz), 4.25 (m, 1H, CH–OH), 5.04 (d, 1H, Ar-CH-OH, $J$ = 5.6 Hz), 4.88 and 4.86 (s, 2H, OH, both exchange with $D_2O$), 7.10-7.40 (m, 4H, aromatic H); $^{13}$C NMR: $\delta_C$ 37.69 ($CH_2$), 80.58, 81.10 (CH-OH), 123.89, 124.29, 126.31, 127.40 (aromatic), 138.99, 143.12 (quaternary aromatic). MS (EI): m/z 150($M^+$). Anal. Calcd for $C_9H_{10}O_2$: C, 71.98; H, 6.71. Found: C, 71.80; H, 6.74.

***3,4-Dihydro-3-hydroxyisochroman-1-one (20)***

White crystalline solid (16%), mp. 95 °C; IR ($\nu_{max}$, $cm^{-1}$): 3276, 2923, 1702, 1603, 1439, 1393, 1133, 1067, 735. $^{1}$H NMR: $\delta_H$ 1.70 (broad s, 1H, OH, exchanges with $D_2O$), 3.15 (dd, 1H, $CH_2$, $J_1$= 16.6 Hz, $J_2$= 4.6 Hz), 3.35 (dd, 1H, $CH_2$, $J_1$= 16.8 Hz, $J_2$= 4.2 Hz), 5.95 (t, 1H, CH–OH, $J$ = 4.8 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 34.20 ($CH_2$), 96.17 (CH-OH), 124.76, 127.94, 128.66, 130.21(aromatic),

134.51, 136.67 (quaternary aromatic), 165.57(C=O). MS (EI): m/z 164($M^+$). Anal. Calcd for $C_9H_8O_3$: C, 65.85; H, 4.91. Found: C, 65.78; H, 4.84.

***Synthesis of Acetal derivatives of 3,4-dihydro-3-hydroxyisochroman-1-one 46(a-h):***

To a mixture of the lactol (**20)** (3.05 mmol) and the alcohol (3.65 mmol) in dry toluene (20 ml) was added conc.$H_2SO_4$ (0.4 ml) at room temperature under stirring. After completion of the reaction (TLC), solvent was removed and the reaction mixture was neutralized with saturated solution of sodium bicarbonate and extracted using ethyl acetate. The combined organic extracts were washed with water, brine, dried over anhydrous sodium sulphate. Removal of solvent and chromatography of the residue furnished **5** as light yellow liquid.

***3-Ethoxy-3,4-dihydroisochroman-1-one (46a)***

Light yellow liquid (72%), IR ($\nu_{max}$, $cm^{-1}$): 2946, 2912, 2834, 1729, 1628, 1464, 1310, 1274, 1104, 1092, 738; $^1$H NMR: $\delta_H$ 1.20 (t, 3H, $OCH_2\underline{CH_3}$, $J$= 6.9 Hz), 3.15 (dd, 1H, $CH_2$, $J_1$= 16.2 Hz, $J_2$= 4.8 Hz), 3.35 (dd, 1H, $CH_2$, $J_1$= 16.8 Hz, $J_2$= 4.2 Hz), 3.70 (m, 1H, $O\underline{CH_2}CH_3$), 3.98 (m, 1H, $O\underline{CH_2}CH_3$), 5.55 (t, 1H, $\underline{CH}$–OR, $J$= 4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 15.30 ($OCH_2\underline{CH_3}$) 33.71 ($CH_2$), 65.35 (CH-$O\underline{CH_2}CH_3$), 101.14 ($\underline{CH}$-$OCH_2CH_3$), 125.04, 127.70, 128.34, 129.97 (aromatic), 134.12, 136.68 (quaternary aromatic), 164.22 (C=O). MS (EI): m/z 192($M^+$). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.76; H, 6.23.

***3,4-Dihydro-3-propoxyisochroman-1-one (46b)***

Light yellow liquid, IR ($\nu_{max}$, $cm^{-1}$): 2964, 2933, 2879, 1728, 1610, 1462, 1379, 1271, 1016, 910, 731. $^1$H NMR: $\delta_H$ 0.88 (t, 3H, $OCH_2CH_2\underline{CH_3}$, $J$ = 7.2 Hz), 1.60 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 3.12 (dd, 1H, $CH_2$, $J_1$ = 16.3 Hz, $J_2$ = 4.4 Hz), 3.31 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.6 Hz), 3.59 (m, 1H, $O\underline{CH_2}CH_2CH_3$), 3.91 (m, 1H, $O\underline{CH_2}CH_2CH_3$), 5.57 (t, 1H, $\underline{CH}$–$OCH_2CH_2CH_3$, $J$ = 4.4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 15.30 ($OCH_2CH_2\underline{CH_3}$), 22.62, 33.41 ($CH_2$), 71.17 (CH-$O\underline{CH_2}CH_2CH_3$), 101.06 ($\underline{CH}$-$OCH_2CH_2CH_3$), 124.93, 127.57, 128.15, 129.90 (aromatic), 133.97, 136.51 (quaternary aromatic), 164.23 (C=O). MS (EI): m/z 206($M^+$). Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.79; H, 6.88.

***3-Butoxy-3,4-dihydroisochroman-1-one (46c)***

Light yellow liquid, IR ($\nu_{max}$, $cm^{-1}$): 2958, 2935, 2874, 1728, 1610, 1460, 1379, 1271, 1128, 1053, 732, 692. $^1$H NMR: $\delta_H$ 0.89 (t, 3H, $OCH_2CH_2CH_2\underline{CH_3}$, $J$ = 7.2 Hz), 1.30 (m, 2H, $OCH_2CH_2\underline{CH_2}CH_3$), 1.60 (m, 2H, $OCH_2\underline{CH_2}CH_2CH_3$), 3.11 (dd, 1H, $CH_2$, $J_1$ = 18.2 Hz, $J_2$ = 4.4 Hz), 3.30 (dd, 1H, $CH_2$, $J_1$ = 18.6 Hz, $J_2$ = 4.6 Hz), 3.62 (m, 1H, $O\underline{CH_2}CH_2CH_2CH_3$), 3.97 (m, 1H, $O\underline{CH_2}CH_2CH_2CH_3$), 5.56 (t, 1H, $\underline{CH}$–$OCH_2CH_2CH_2CH_3$, $J$ = 4.1 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 13.78 ($OCH_2CH_2CH_2\underline{CH_3}$), 19.09, 31.36, 33.44 ($CH_2$), 69.39 (CH-$O\underline{CH_2}CH_2CH_2CH_3$), 101.06 ($\underline{CH}$-$OCH_2CH_2CH_2CH_3$), 124.96, 127.58, 128.13, 129.95 (aromatic), 133.95, 136.49 (quaternary aromatic), 164.21 (C=O). MS (EI): m/z 220($M^+$). Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.81; H, 7.26.

***3,4-Dihydro-3-(pentyloxy)isochroman-1-one (46d)***

Light yellow liquid, IR ($\nu_{max}$, $cm^{-1}$): 2956, 2933, 2872, 1732, 1620, 1460, 1383, 1271, 1136, 1072, 732. $^1$H NMR: $\delta_H$ 0.86 (t, 3H, $OCH_2(CH_2)_3\underline{CH_3}$, $J$ = 6.4 Hz), 1.28 (m, 4H, $OCH_2CH_2(\underline{CH_2})_2CH_3$), 1.56 (m, 2H, $OCH_2\underline{CH_2}(CH_2)_2CH_3$), 3.10 (dd, 1H, $CH_2$, $J_1$ = 17.4 Hz, $J_2$ = 4.4 Hz), 3.29 (dd, 1H, $CH_2$, $J_1$ = 17.1 Hz, $J_2$ = 4.2 Hz), 3.60 (m, 1H, $O\underline{CH_2}(CH_2)_3CH_3$), 3.94 (m, 1H, $O\underline{CH_2}(CH_2)_3CH_3$), 5.55 (t, 1H, $\underline{CH}$–$OCH_2(CH_2)_3CH_3$, $J$ = 4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 12.37 ($OCH_2(CH_2)_3\underline{CH_3}$), 22.50, 27.91, 29.00, 34.21 ($CH_2$), 69.65 (CH-$O\underline{CH_2}(CH_2)_3CH_3$), 101.05 ($\underline{CH}$-$OCH_2(CH_2)_3CH_3$), 123.43, 127.57, 128.14, 129.91 (aromatic), 133.95, 136.51 (quaternary aromatic), 164.21 (C=O). MS (EI): m/z 234($M^+$). Anal. Calcd for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.68; H, 7.71.

***3,4-Dihydro-3-(octyloxy)isochroman-1-one (46e)***

Light yellow liquid, IR ($\nu_{max}$, $cm^{-1}$): 3018, 2928, 1723, 1612, 1464, 1320, 1244, 1045, 794, 689. $^1$H NMR: $\delta_H$ 0.86 (t, 3H, $OCH_2(CH_2)_6\underline{CH_3}$, $J$ = 6.4 Hz), 1.25 (m, 10H, $OCH_2CH_2(\underline{CH_2})_5CH_3$), 1.55 (m, 2H, $OCH_2\underline{CH_2}(CH_2)_5CH_3$), 3.09 (dd, 1H, $CH_2$, $J_1$ = 17.1 Hz, $J_2$ = 4.4 Hz), 3.28 (dd, 1H, $CH_2$, $J_1$= 17.4 Hz, $J_2$= 4.6 Hz), 3.59 (m, 1H, $O\underline{CH_2}(CH_2)_6CH_3$), 3.93 (m, 1H, $O\underline{CH_2}(CH_2)_6CH_3$), 5.54 (t, 1H, $\underline{CH}$–$OCH_2(CH_2)_6CH_3$, $J$ = 3.6 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 14.08 ($OCH_2(CH_2)_6\underline{CH_3}$), 22.60, 25.85, 29.15, 29.29, 31.73, 33.41 ($CH_2$), 69.61 (CH-$O\underline{CH_2}(CH_2)_6CH_3$), 101.02 ($\underline{CH}$-$OCH_2(CH_2)_6CH_3$), 124.98, 127.50, 128.12, 129.85

(aromatic), 133.88, 136.52 (quaternary aromatic), 164.07 (C=O). MS (EI): m/z 276($M^+$). Anal. Calcd for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 73.82; H, 8.78.

***3-(Dodecyloxy)-3,4-dihydroisochroman-1-one (46f)***

Light yellow liquid, IR ($\nu_{max}$, $cm^{-1}$): 2968, 2931, 2868, 1728, 1637, 1487, 1251, 1045, 792, 688. $^1$H NMR: $\delta_H$ 0.89 (t, 3H, $OCH_2(CH_2)_{10}\underline{CH_3}$, *J* = 6.8 Hz), 1.26 (m, 18H, $OCH_2CH_2(\underline{CH_2})_9CH_3$), 1.60 (m, 2H, $OCH_2\underline{CH_2}(CH_2)_9CH_3$), 3.12 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.8 Hz), 3.31 (dd, 1H, $CH_2$, $J_1$= 16.6 Hz, $J_2$= 4.6 Hz), 3.62 (m, 1H, $O\underline{CH_2}(CH_2)_{10}CH_3$), 3.96 (m, 1H, $O\underline{CH_2}(CH_2)_{10}CH_3$), 5.56 (t, 1H, $\underline{CH}$–$OCH_2(CH_2)_{10}CH_3$, *J* = 4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 14.13 ($OCH_2(CH_2)_{10}\underline{CH_3}$), 22.67, 25.76, 29.33, 29.44, 29.59, 29.61, 29.65, 31.89, 32.77 ($CH_2$), 62.91 (CH- $O\underline{CH_2}(CH_2)_{10}CH_3$), 107.04 ($\underline{CH}$-$OCH_2(CH_2)_{10}CH_3$), 125.58, 128.56, 129.52, 134.81 (aromatic), 136.43, 144.66 (quaternary aromatic), 162.25 (C=O). MS (EI): m/z 332($M^+$). Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.78; H, 9.66.

***3,4-Dihydro-3-(tetradecyloxy)isochroman-1-one (46g)***

White crystalline solid, IR ($\nu_{max}$, $cm^{-1}$): 3020, 2938, 1720, 1637, 1487, 1330, 1251, 1045, 794, 688. $^1$H NMR: $\delta_H$ 0.89 (t, 3H, $OCH_2(CH_2)_{12}\underline{CH_3}$, *J* = 6.8 Hz), 1.28 (m, 22H, $OCH_2CH_2(\underline{CH_2})_{11}CH_3$), 1.58 (m, 2H, $OCH_2\underline{CH_2}(CH_2)_{11}CH_3$), 3.12 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.4 Hz), 3.31 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.5 Hz), 3.62 (m, 1H, $O\underline{CH_2}(CH_2)_{12}CH_3$), 3.95 (m, 1H, $O\underline{CH_2}(CH_2)_{12}CH_3$), 5.56 (t, 1H, $\underline{CH}$–$OCH_2(CH_2)_{12}CH_3$, *J* = 4.4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 14.17 ($OCH_2(CH_2)_{12}\underline{CH_3}$), 22.72, 25.89, 29.31, 29.39, 29.55, 29.58, 29.68, 29.69, 29.71, 31.95, 33.46 ($CH_2$), 69.70 (CH-$O\underline{CH_2}(CH_2)_{12}CH_3$), 101.02 ($\underline{CH}$-O $CH_2(CH_2)_{12}CH_3$), 124.99, 127.58, 128.12, 129.97 (aromatic), 133.93, 136.49 (quaternary aromatic), 164.19 (C=O). MS (EI): m/z 360($M^+$). Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.58; H, 10.10.

***3-(Hexadecyloxy)-3,4-dihydroisochroman-1-one (46h)***

White crystalline solid, IR ($\nu_{max}$, $cm^{-1}$): 3090, 2941, 2837, 1720, 1637, 1487, 1251, 1045, 794, 688. $^1$H NMR: $\delta_H$ 0.89 (t, 3H, $OCH_2(CH_2)_{14}\underline{CH_3}$, *J* = 6.9 Hz), 1.26 (m, 26H, $OCH_2CH_2(\underline{CH_2})_{13}CH_3$), 1.57 (m, 2H, $OCH_2\underline{CH_2}(CH_2)_{13}CH_3$), 3.11 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.4 Hz), 3.30 (dd, 1H, $CH_2$, $J_1$ = 16.6 Hz, $J_2$ = 4.2 Hz), 3.61 (m, 1H,

O$\underline{CH_2}(CH_2)_{14}CH_3$), 3.96 (m, 1H, O$\underline{CH_2}(CH_2)_{14}CH_3$), 5.56 (t, 1H, $\underline{CH}$–$OCH_2(CH_2)_{14}CH_3$, $J$ = 4 Hz), 7.20-8.20 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 14.16 ($OCH_2(CH_2)_{14}\underline{CH_3}$), 22.72, 25.75, 25.89, 29.31, 29.39, 29.46, 29.55, 29.58, 29.64, 29.69, 29.72, 31.95, 32.81, 33.46 ($CH_2$), 69.70 (CH- O$\underline{CH_2}(CH_2)_{14}CH_3$), 101.03 ($\underline{CH}$-$OCH_2(CH_2)_{14}CH_3$), 124.99, 127.58, 128.13, 129.96 (aromatic), 133.94, 136.50 (quaternary aromatic), 164.19 (C=O). MS (EI): m/z 388($M^+$). Anal. Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.22; H, 10.30.

***1H-isochromen-1-one (47)***

White crystalline solid, mp. 46 °C; IR ($\nu_{max}$, $cm^{-1}$): 2962, 1732, 1610, 1462, 1246, 1008, 731. $^1$H NMR: $\delta_H$ 6.52 (d, 1H, $\underline{CH}$=CH-O, $J$ = 5.6 Hz), 7.28 (d, 1H, CH=$\underline{CH}$-O, $J$ = 5.2 Hz), 7.20-8.30 (m, 4H, aromatic H). $^{13}$C NMR: $\delta_C$ 107.04, 121.89 (olefinic), 125.56, 128.63, 129.72, 134.83 (aromatic), 136.48, 144.71 (quaternary aromatic), 162.29 (C=O).

## 1.5 Conclusion

First one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one (lactol) **20** from indene **19** is reported. A probable steps of its formation is also proposed by various experimental studies. Various acetal derivatives of lactol (**57a-h**) were prepared by acid catalyzed reaction with different aliphatic alcohols. It was further found that similar reaction with phenols resulted in the dehydration of **20** to **47**.

# 1.6 Spectra

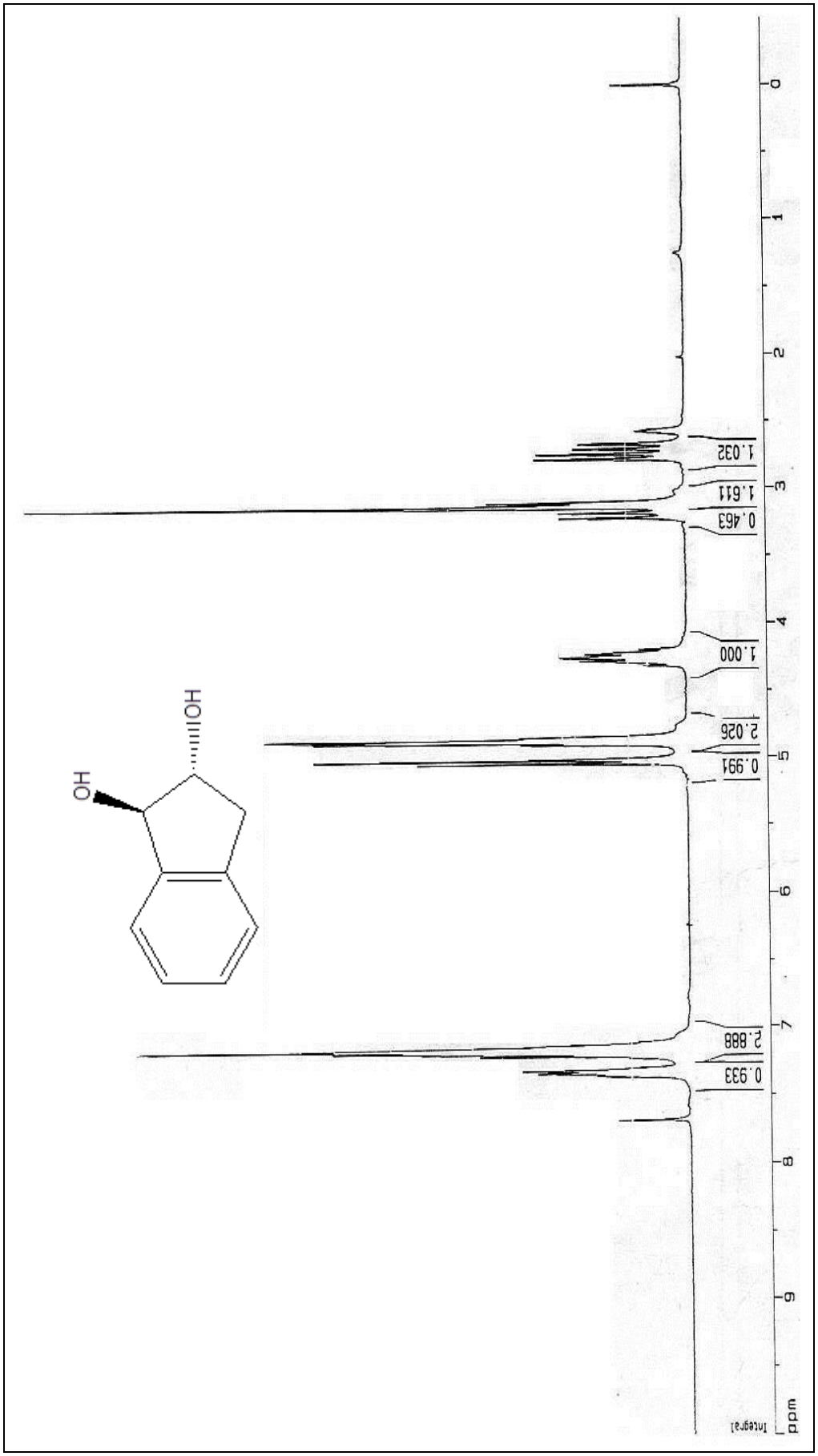



**Figure 1.4**:- $^{1}$H NMR of 2,3-dihydro-1H-indan-trans-1,2-diol **(11)**

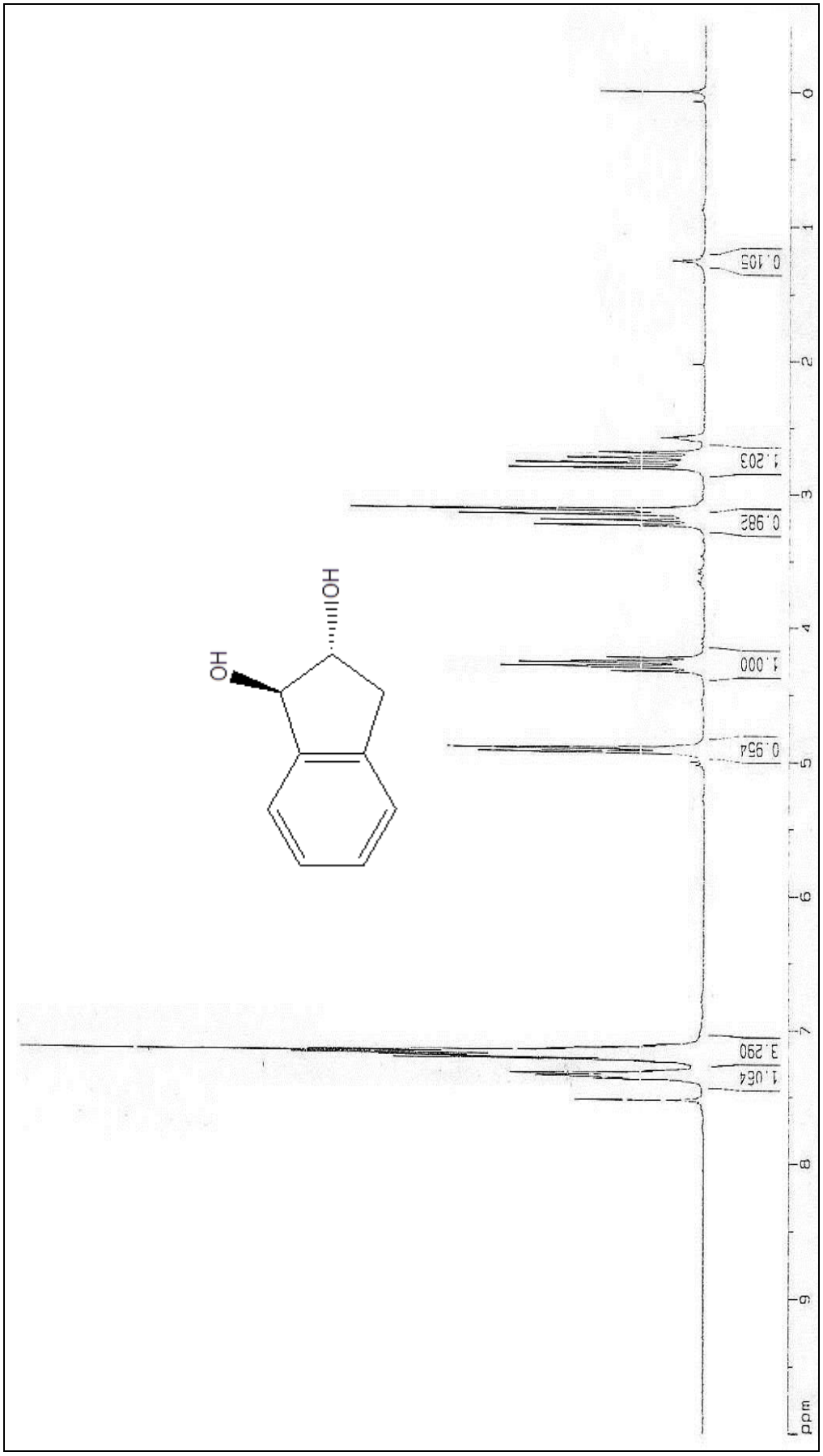



**Figure 1.5**: $^{1}$H NMR with $D_2O$ exchange of 2,3-dihydro-1H-indan-trans-1,2-diol **(11)**

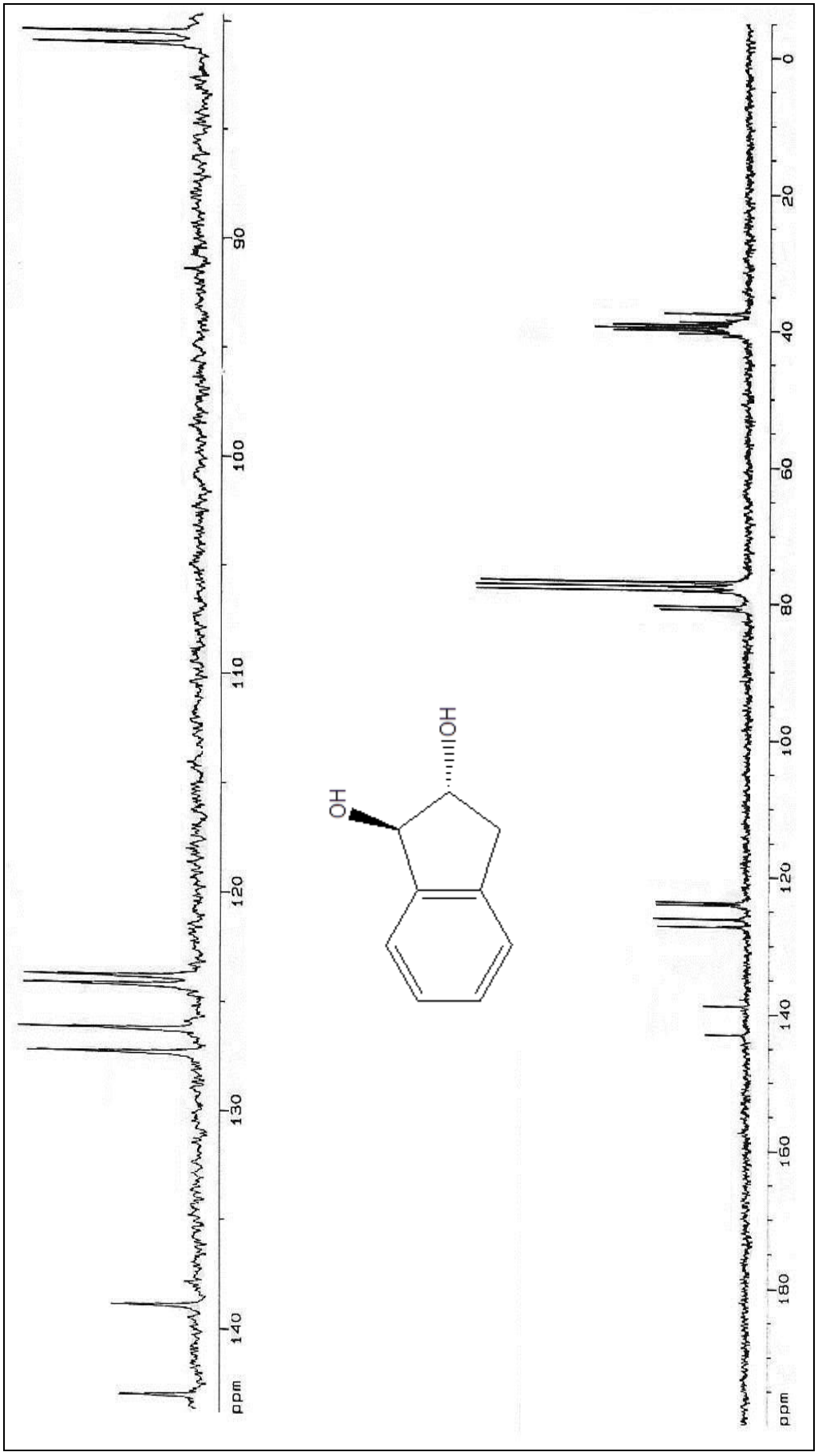



**Figure 1.6**: $^{13}C$ NMR of 2,3-dihydro-1H-indan-trans-1,2-diol **(11)**

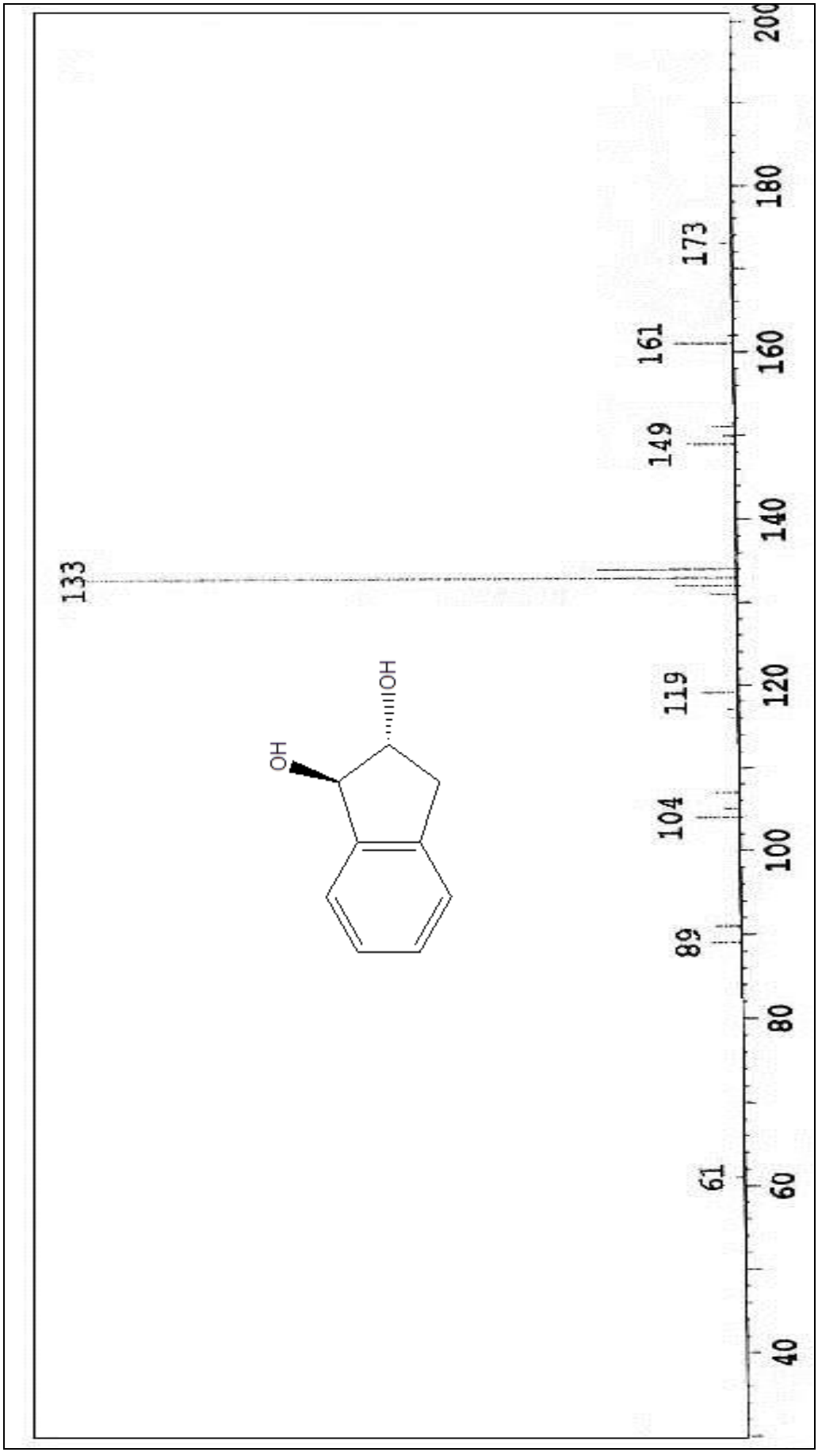



**Figure 1.7**: Mass Spectrum of 2,3-dihydro-1H-indan-trans-1,2-diol **(11)**

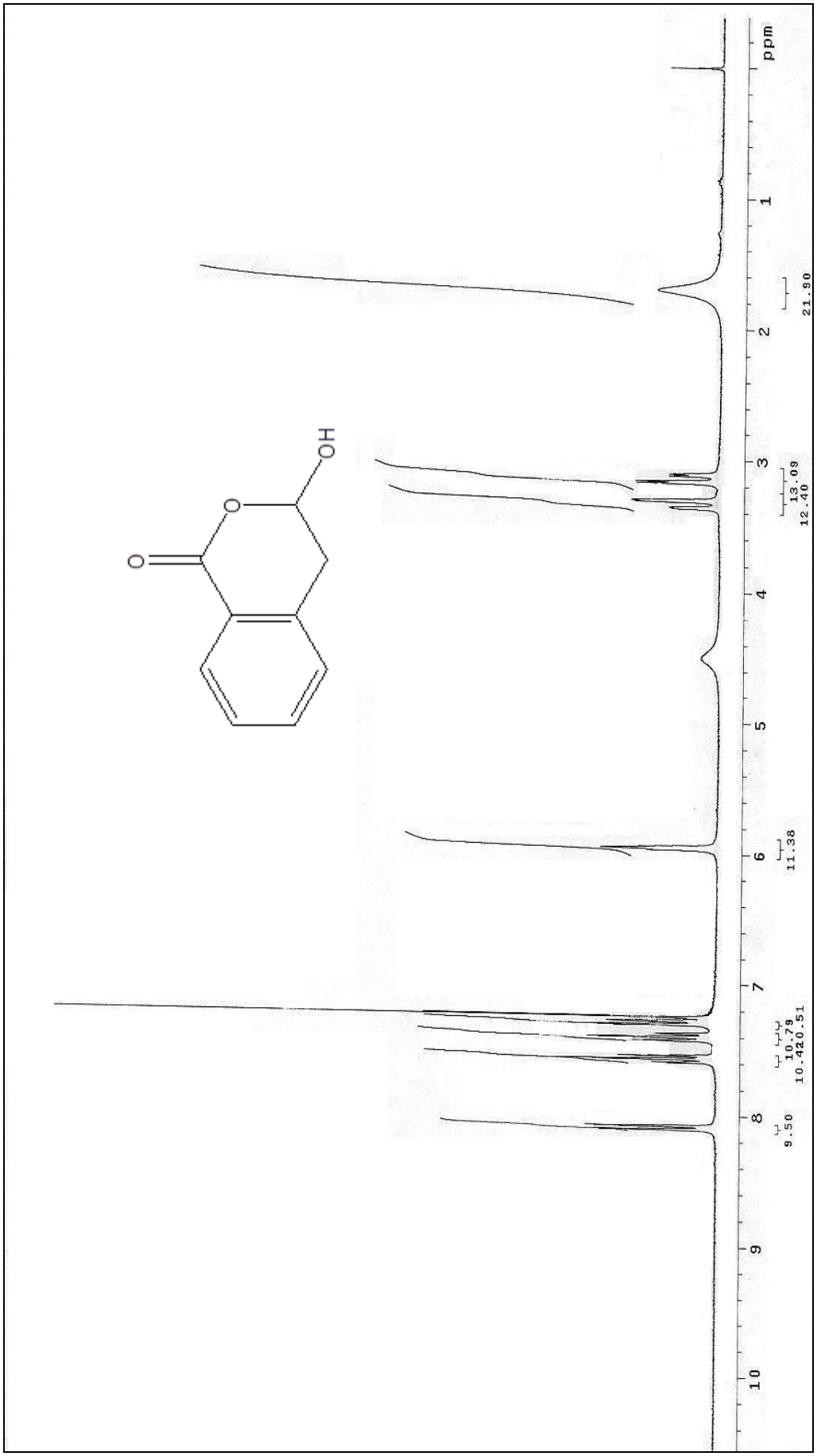



**Figure 1.8**: $^{1}$H NMR of 3,4-Dihydro-3-hydroxyisochroman-1-one **(20)**

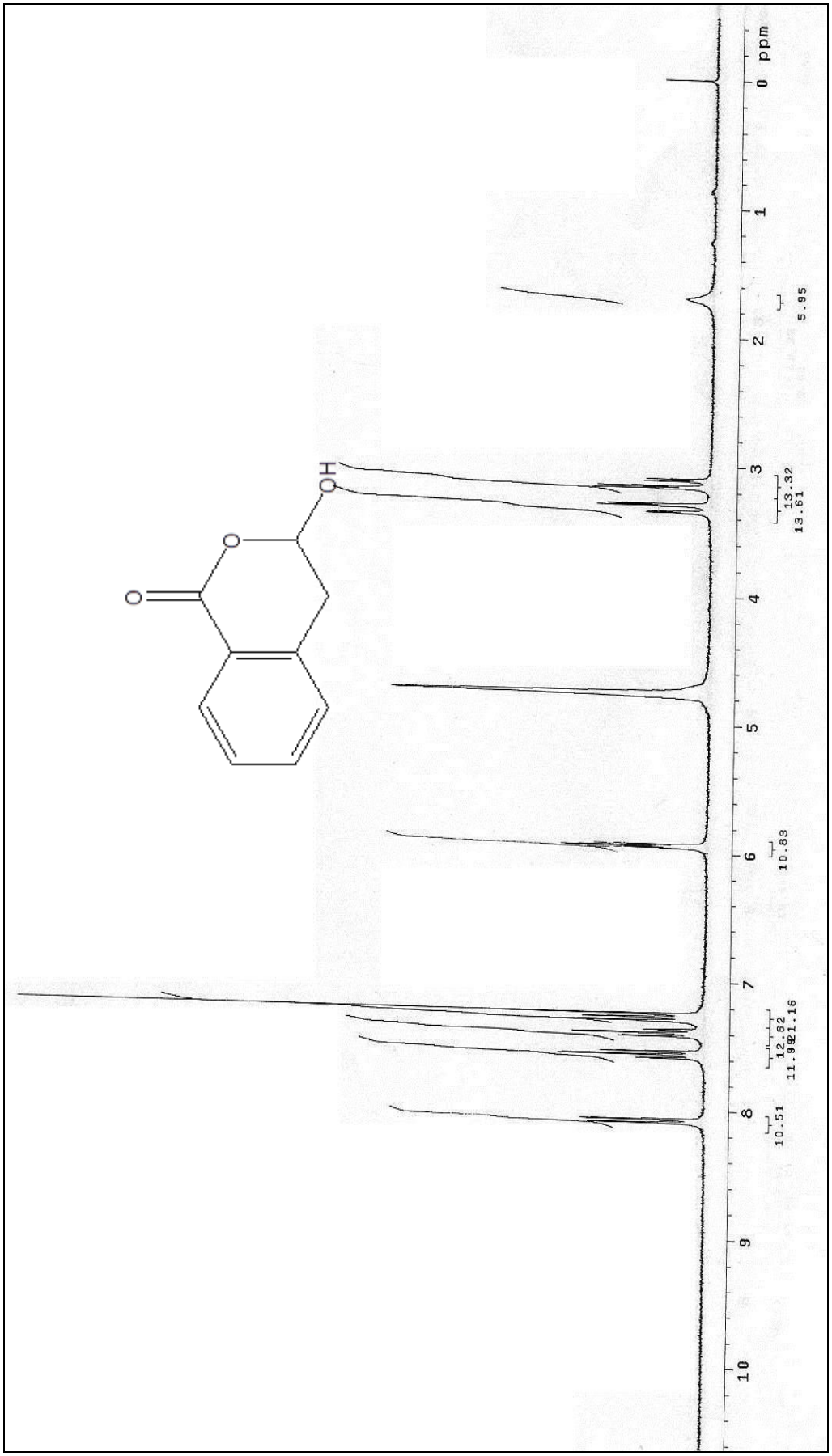



**Figure 1.9**: $^{1}H$ NMR with $D_2O$ exchange of 3,4-Dihydro-3-hydroxyisochroman-1-one **(20)**

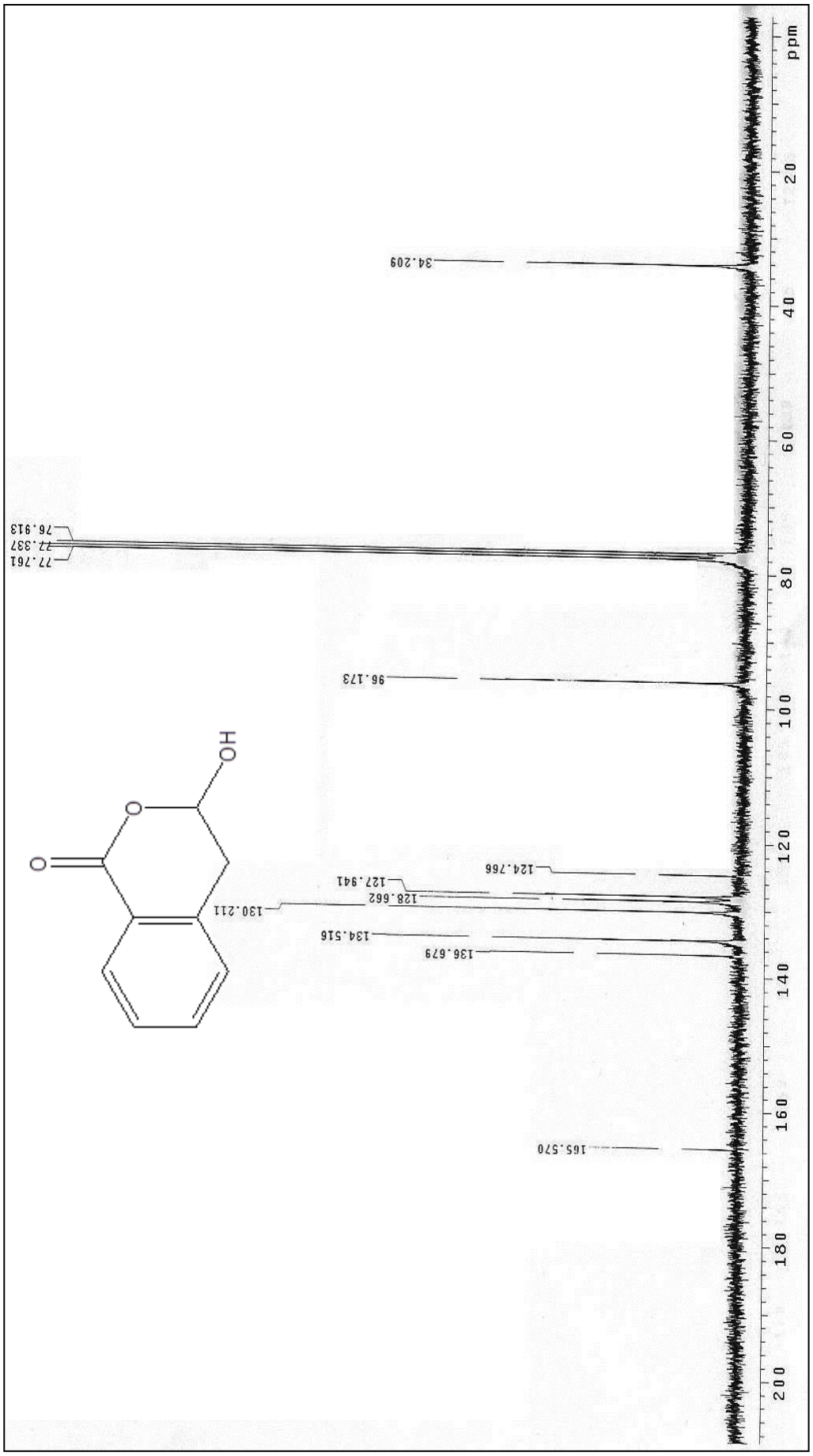



**Figure 1.10**: $^{13}$C NMR of 3,4-Dihydro-3-hydroxyisochroman-1-one **(20)**

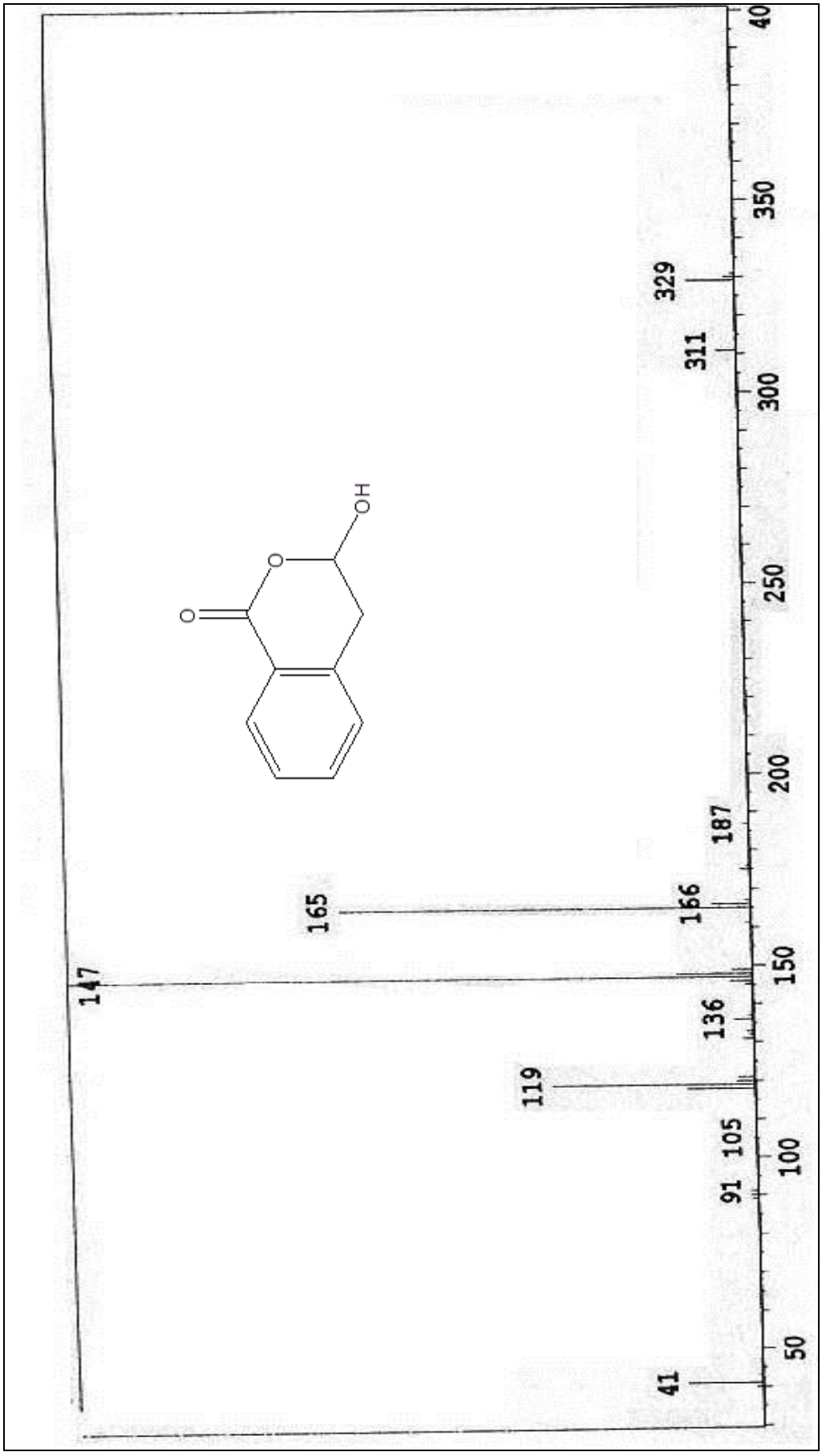



**Figure 1.11**: Mass Spectrum of 3,4-Dihydro-3-hydroxyisochroman-1-one **(20)**

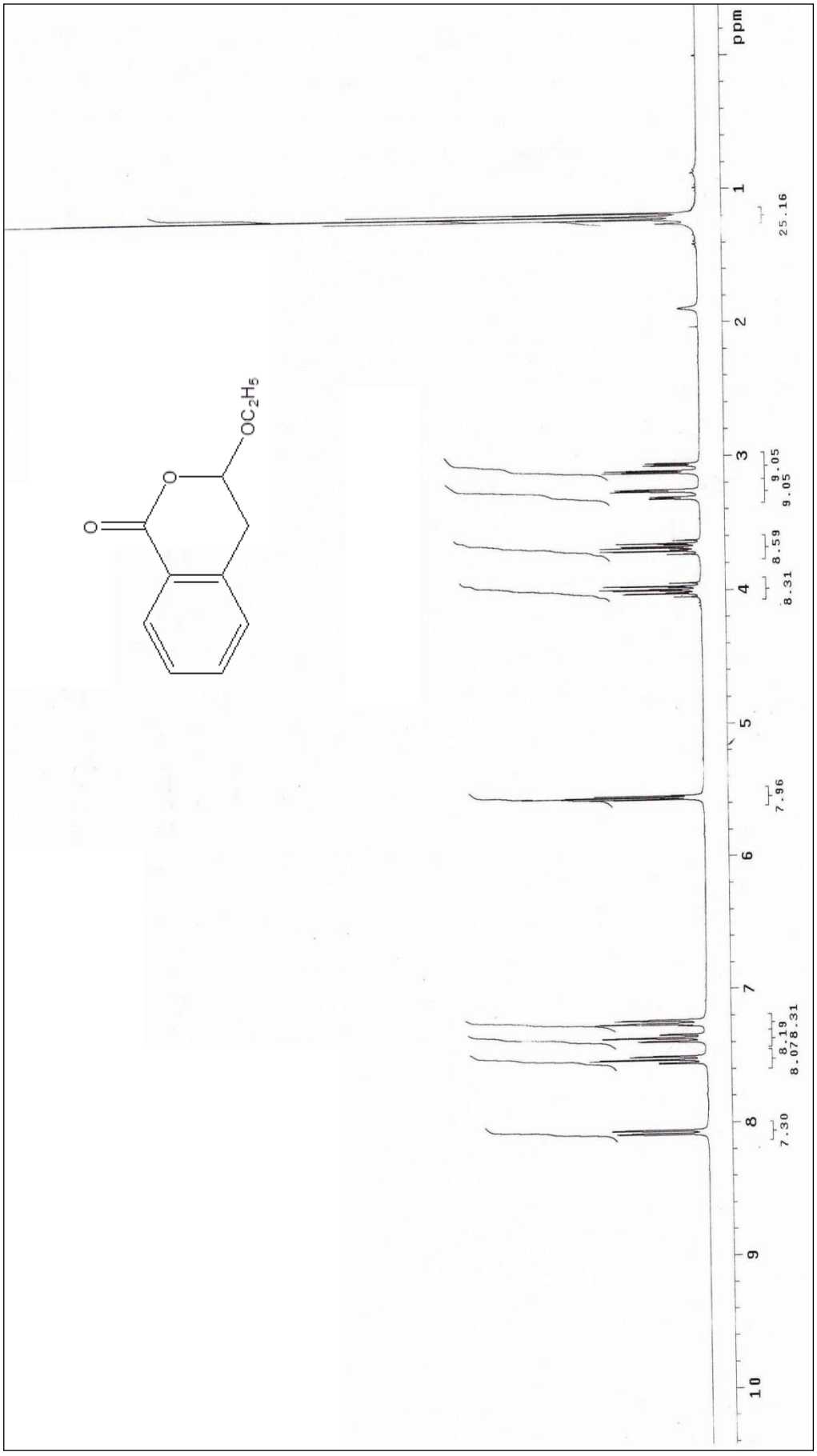



**Figure 1.12**:- $^{1}$H NMR of 3-ethoxy-3,4-dihydroisochroman-1-one **(46a)**

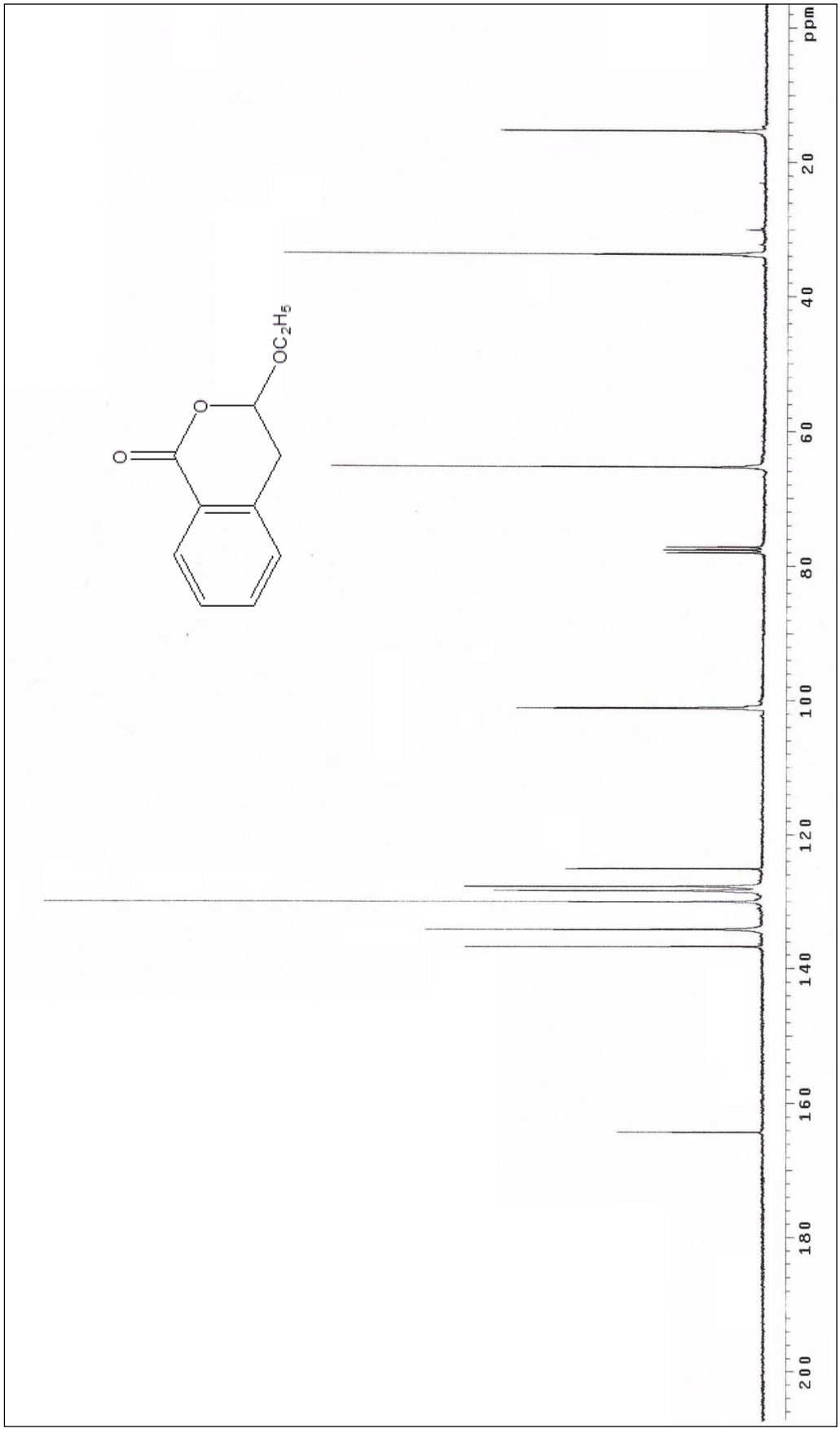



**Figure 1.13**:- $^{13}$C NMR of 3-ethoxy-3,4-dihydroisochroman-1-one **(46a)**

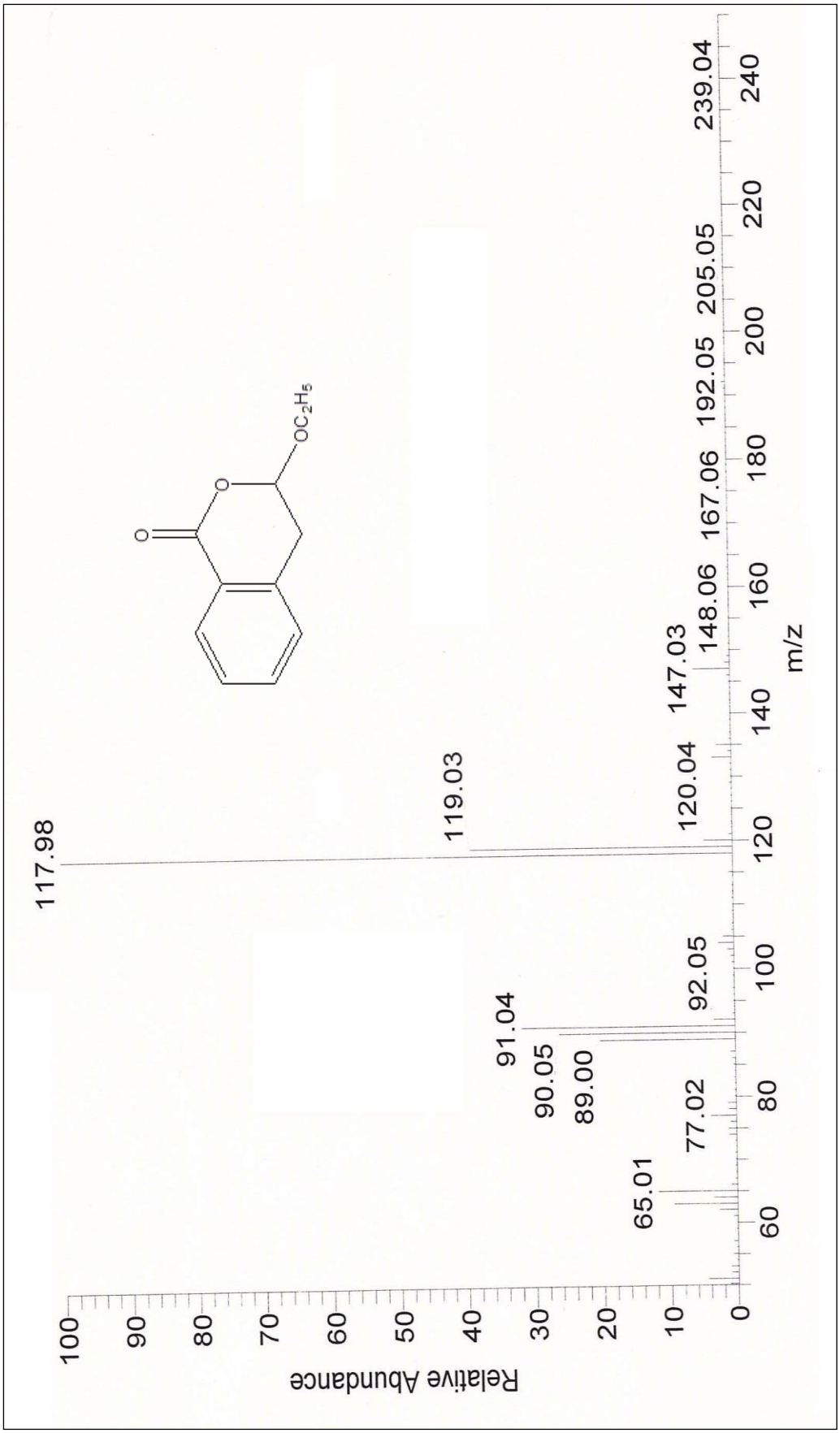



**Figure 1.14**:- Mass Spectrum of 3-ethoxy-3,4-dihydroisochroman-1-one **(46a)**

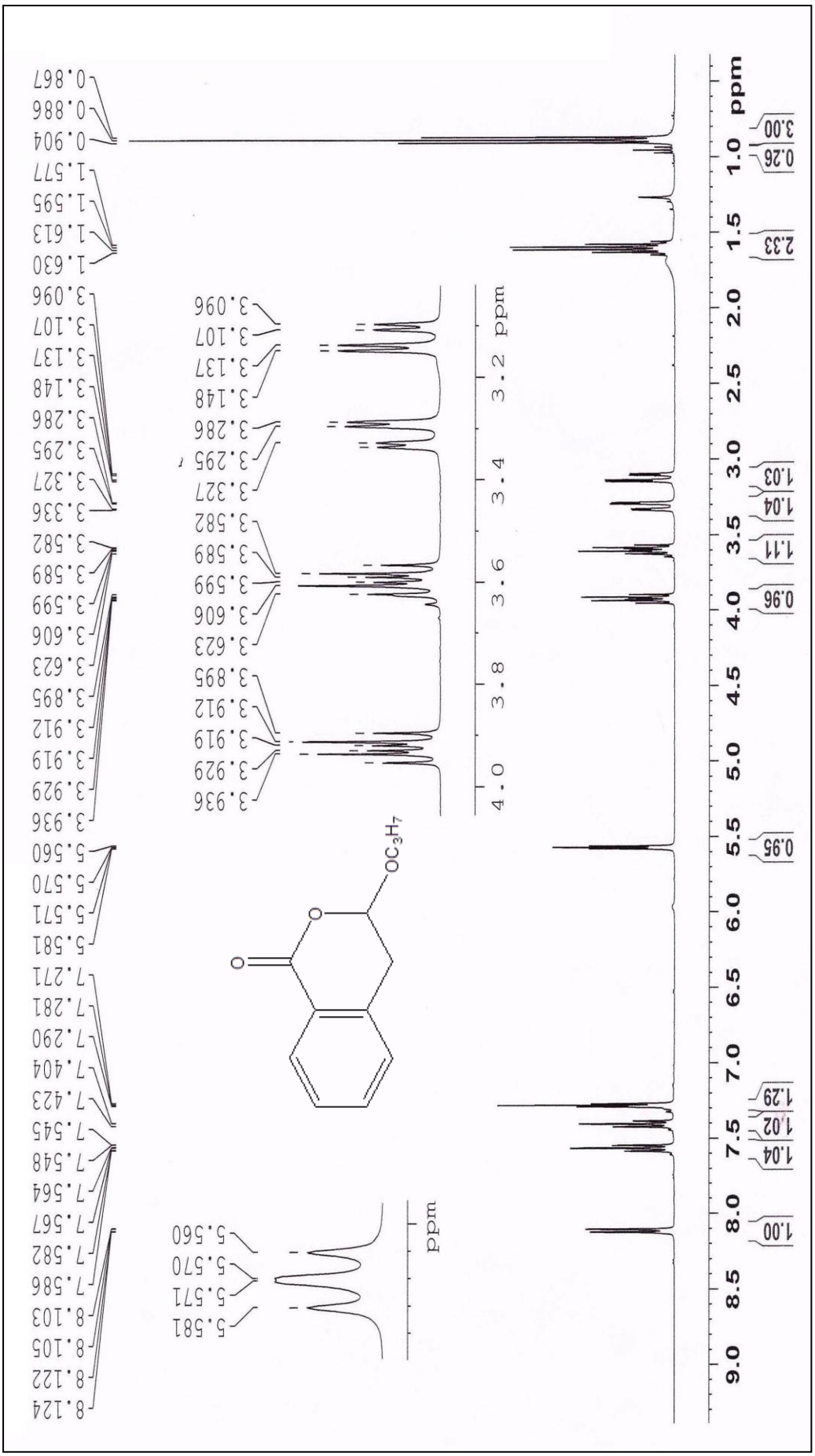



**Figure 1.15**:- $^{1}$H NMR of 3,4-dihydro-3-propoxyisochroman-1-one **(46b)**

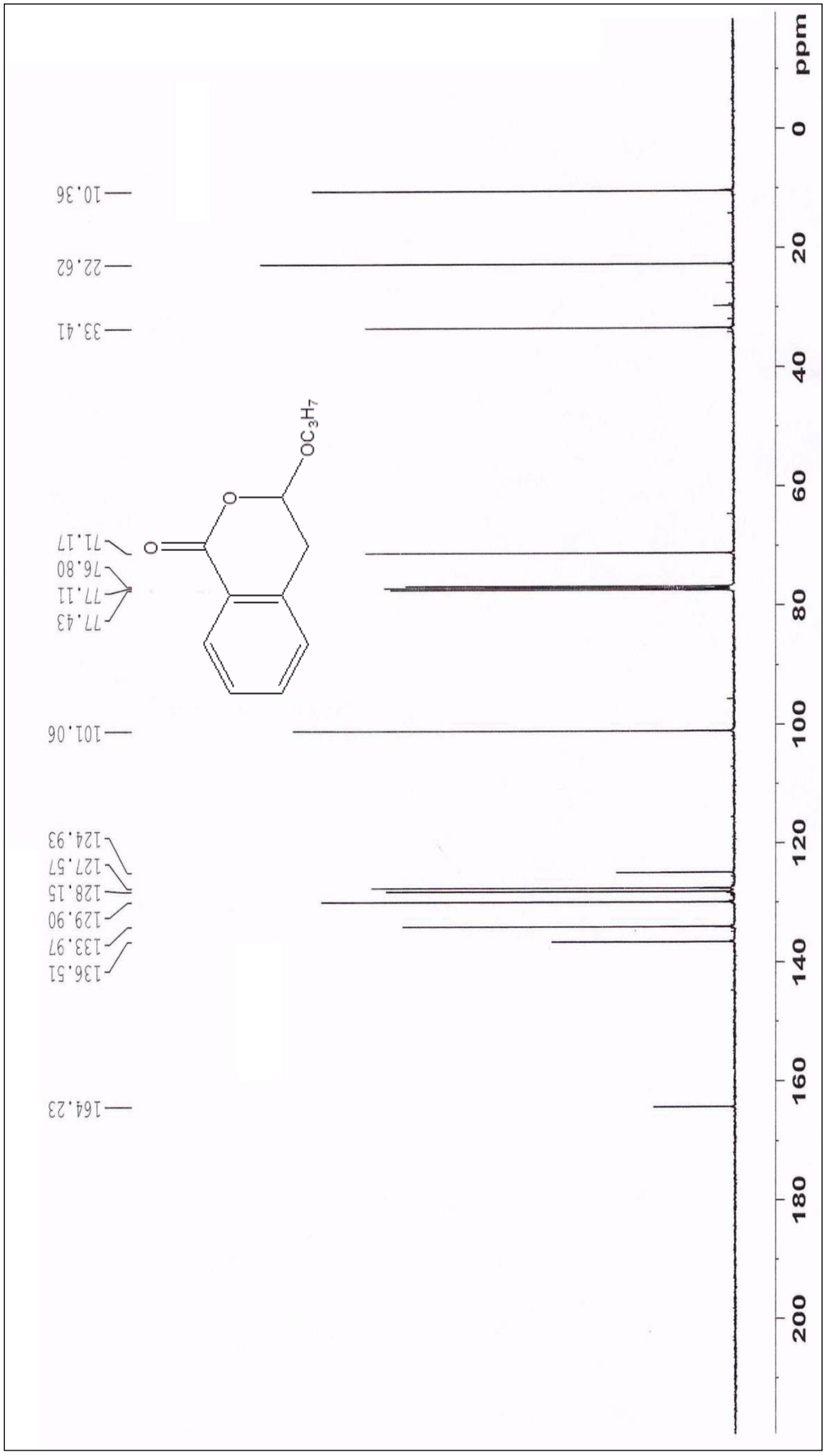



**Figure 1.16**:- $^{13}C$ NMR of 3,4-dihydro-3-propoxyisochroman-1-one **(46b)**

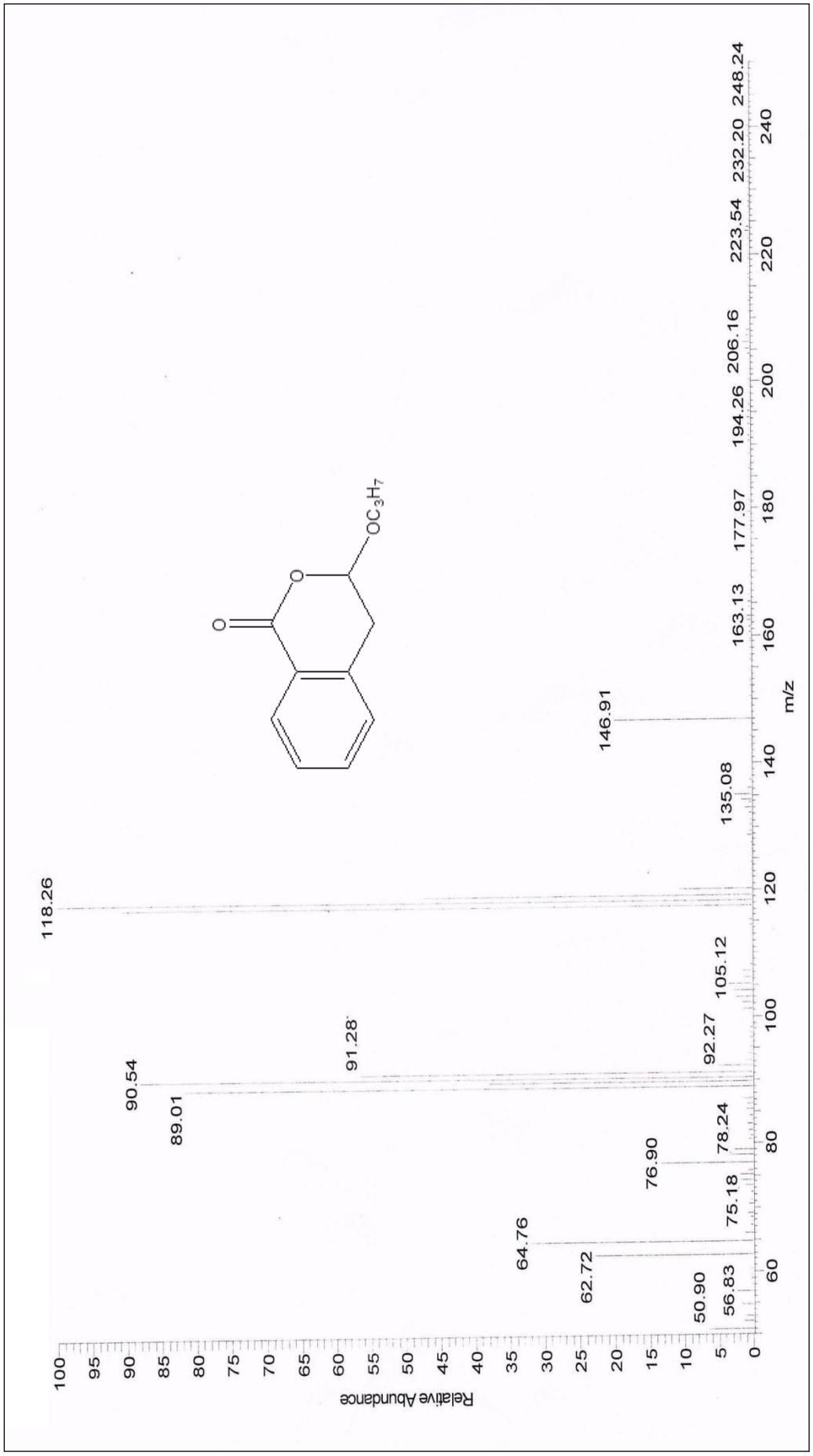



**Figure 1.17**:- Mass Spectrum of 3,4-dihydro-3-propoxyisochroman-1-one **(46b)**

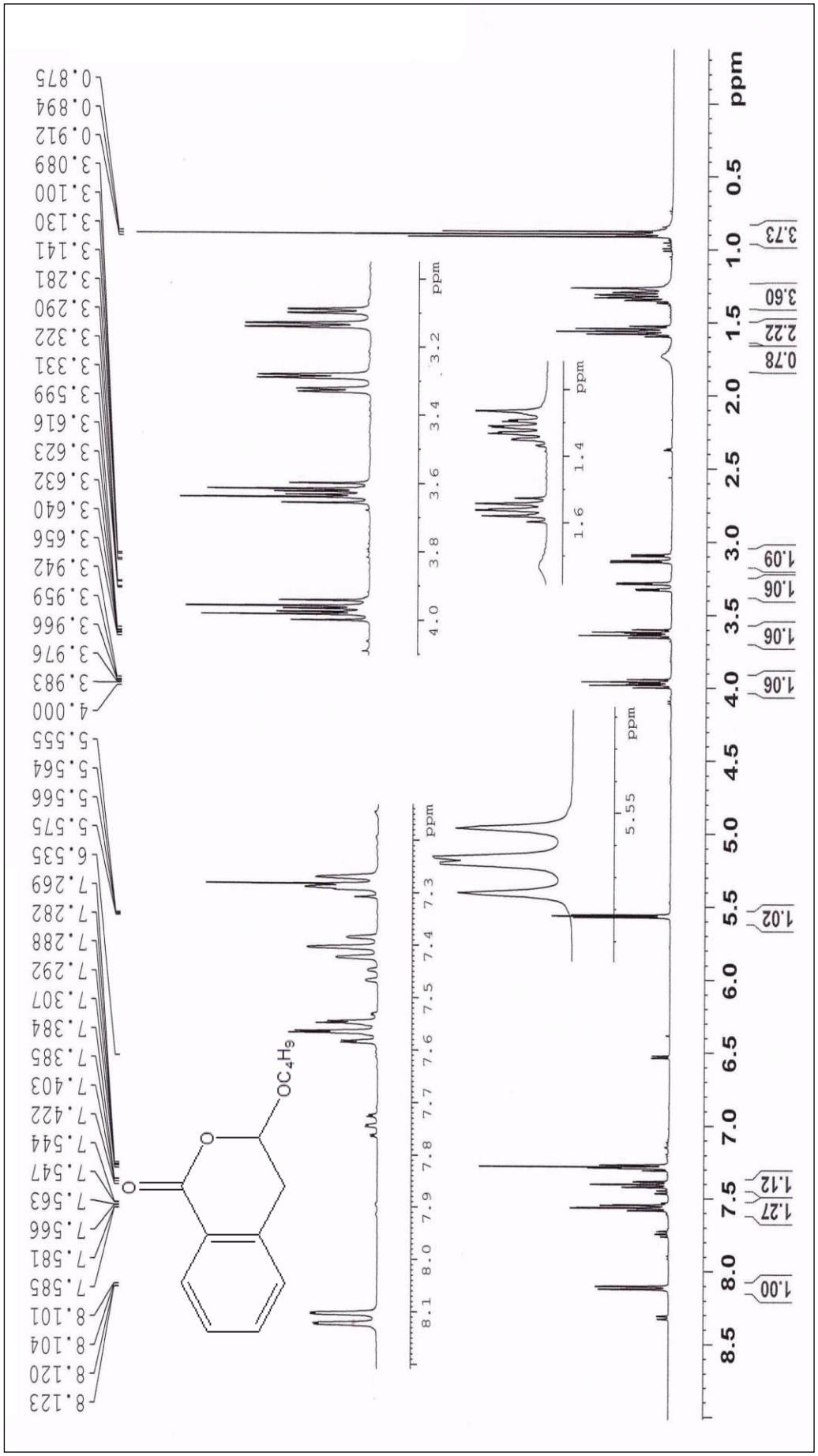



**Figure 1.18:-** $^{1}$H NMR of 3-butoxy-3,4-dihydroisochroman-1-one **(46c)**

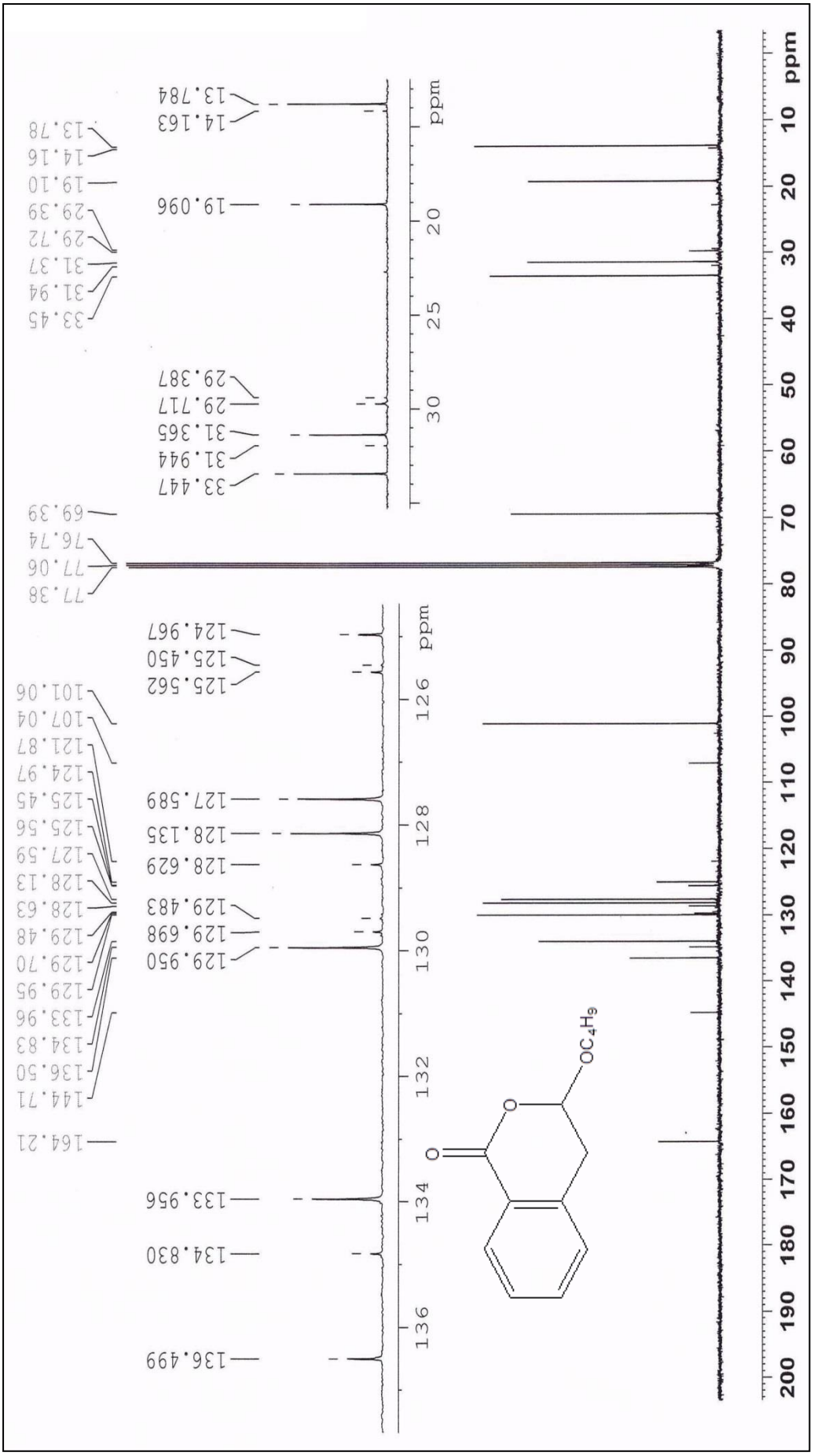



**Figure 1.19**:- $^{13}C$ NMR of 3-butoxy-3,4-dihydroisochroman-1-one **(46c)**

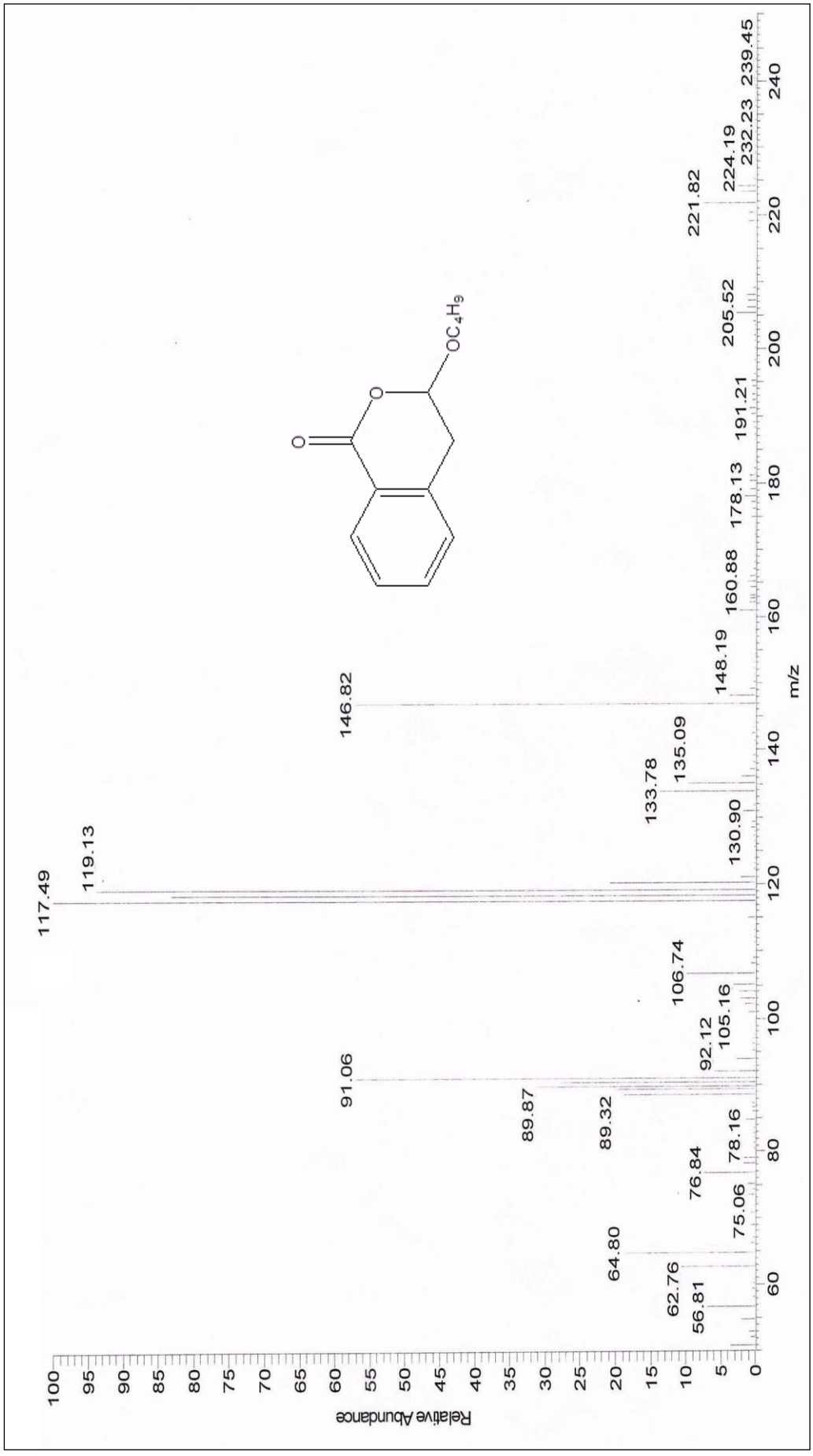



**Figure 1.20**:- Mass Spectrum of 3-butoxy-3,4-dihydroisochroman-1-one**(46c)**

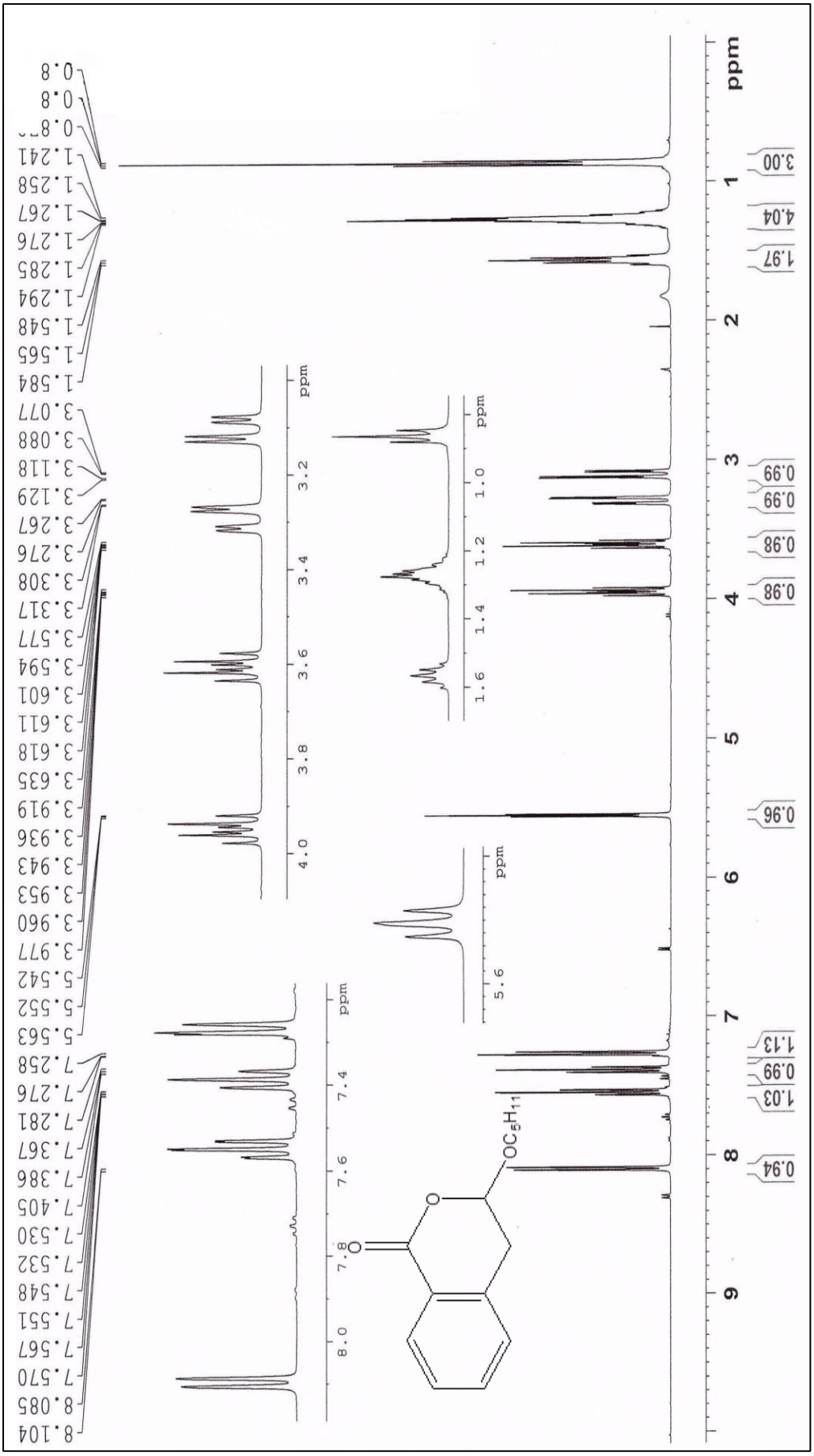



**Figure 1.21:-** $^{1}$H NMR of 3,4-dihydro-3-(pentyloxy)isochroman-1-one **(46d)**

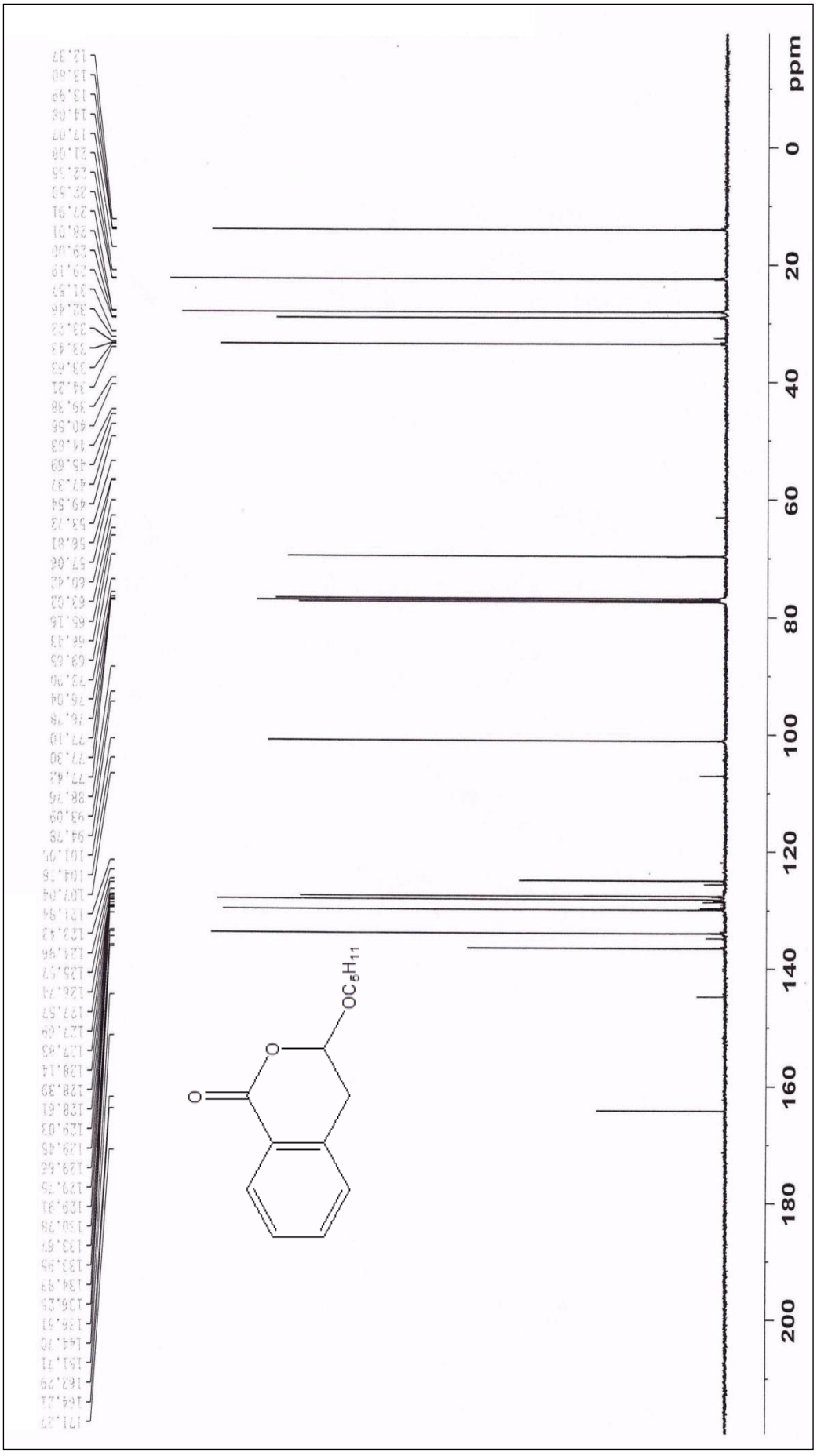



**Figure 1.22**:- $^{13}C$ NMR of 3,4-dihydro-3-(pentyloxy)isochroman-1-one (**46d**)

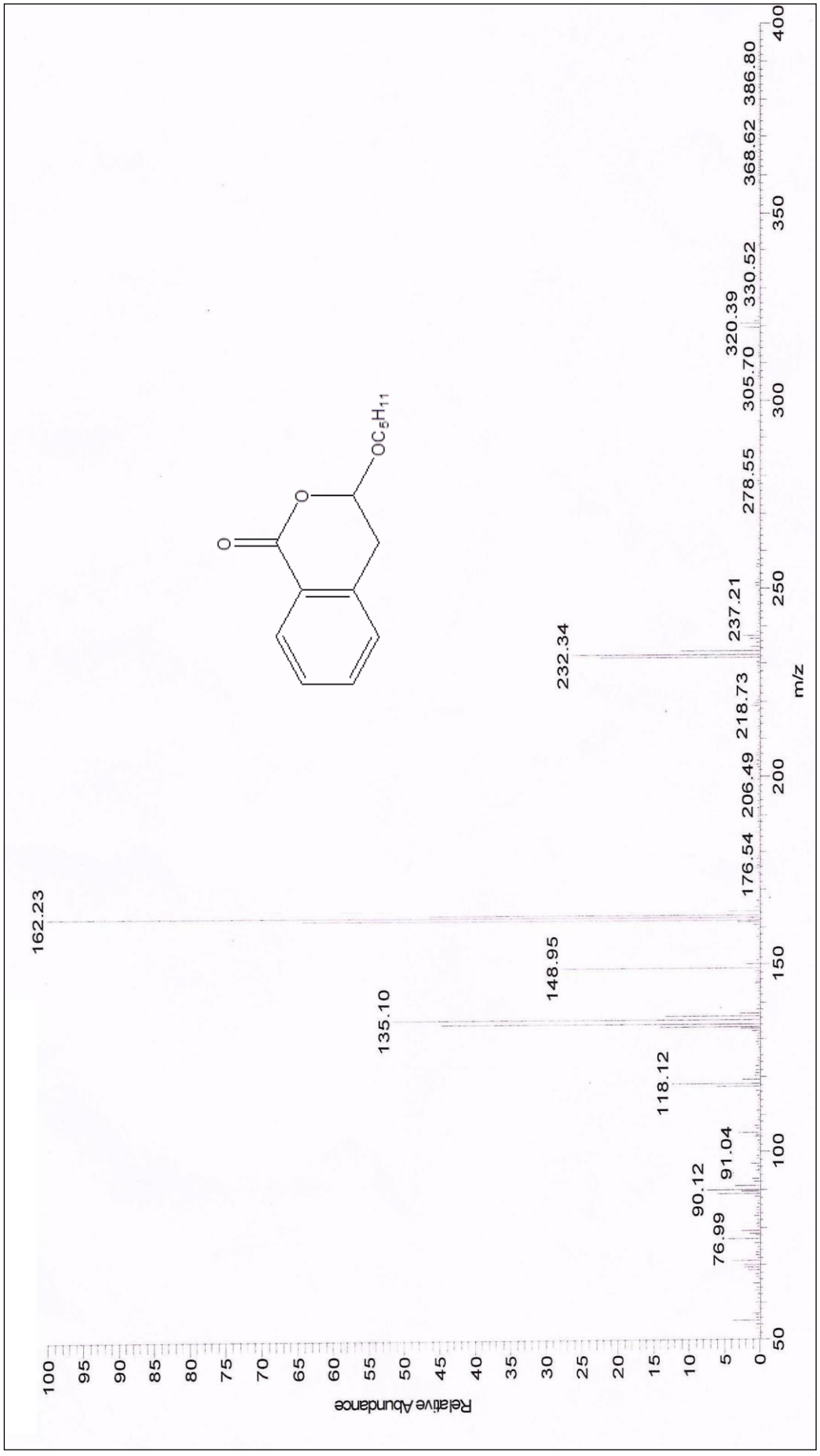



**Figure 1.23**:- Mass Spectrum of 3,4-dihydro-3-(pentyloxy)isochroman-1-one (**46d**)

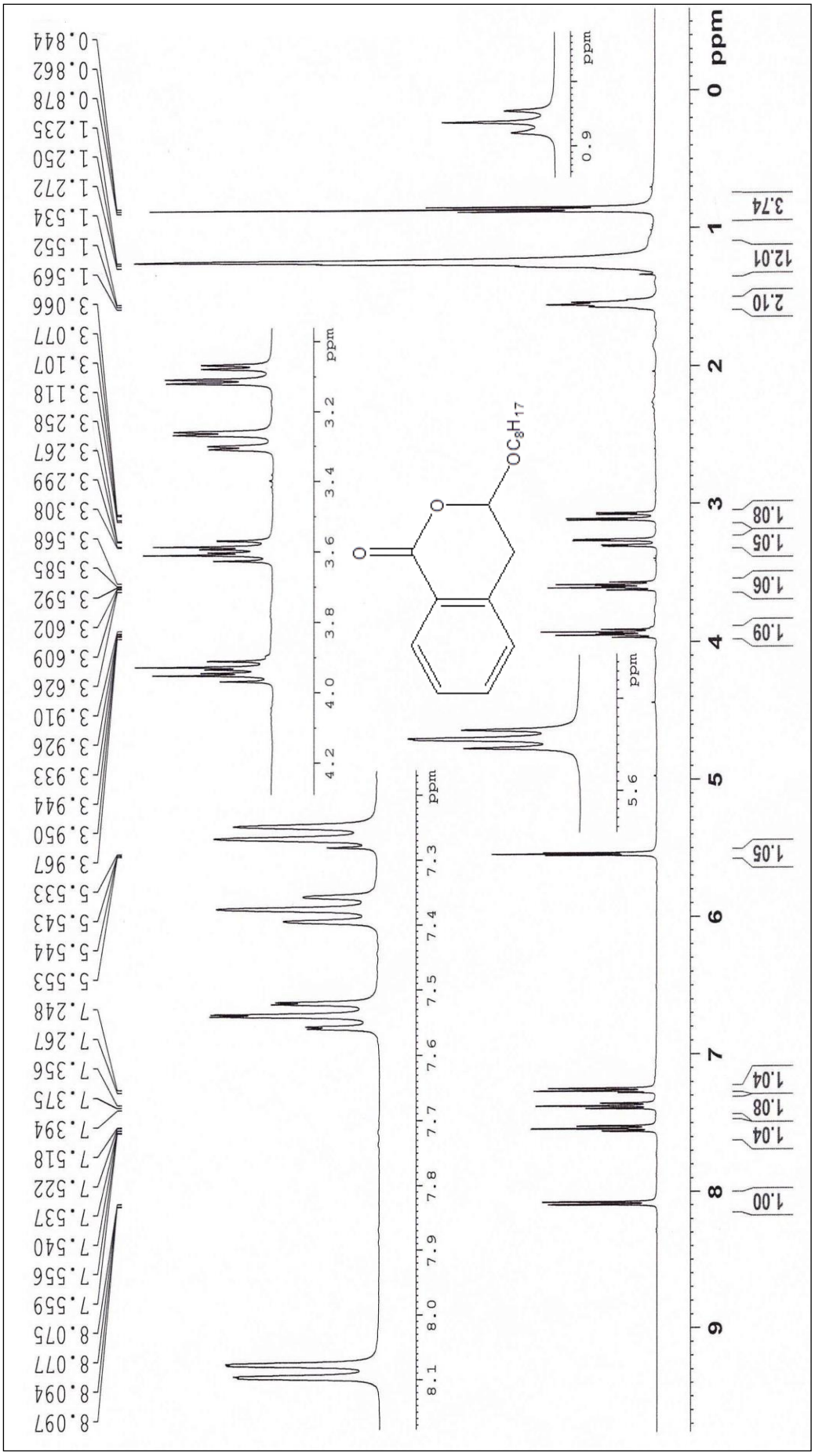



**Figure 1.24:-** $^{1}$H NMR of 3,4-dihydro-3-(octyloxy)isochroman-1-one **(46e)**

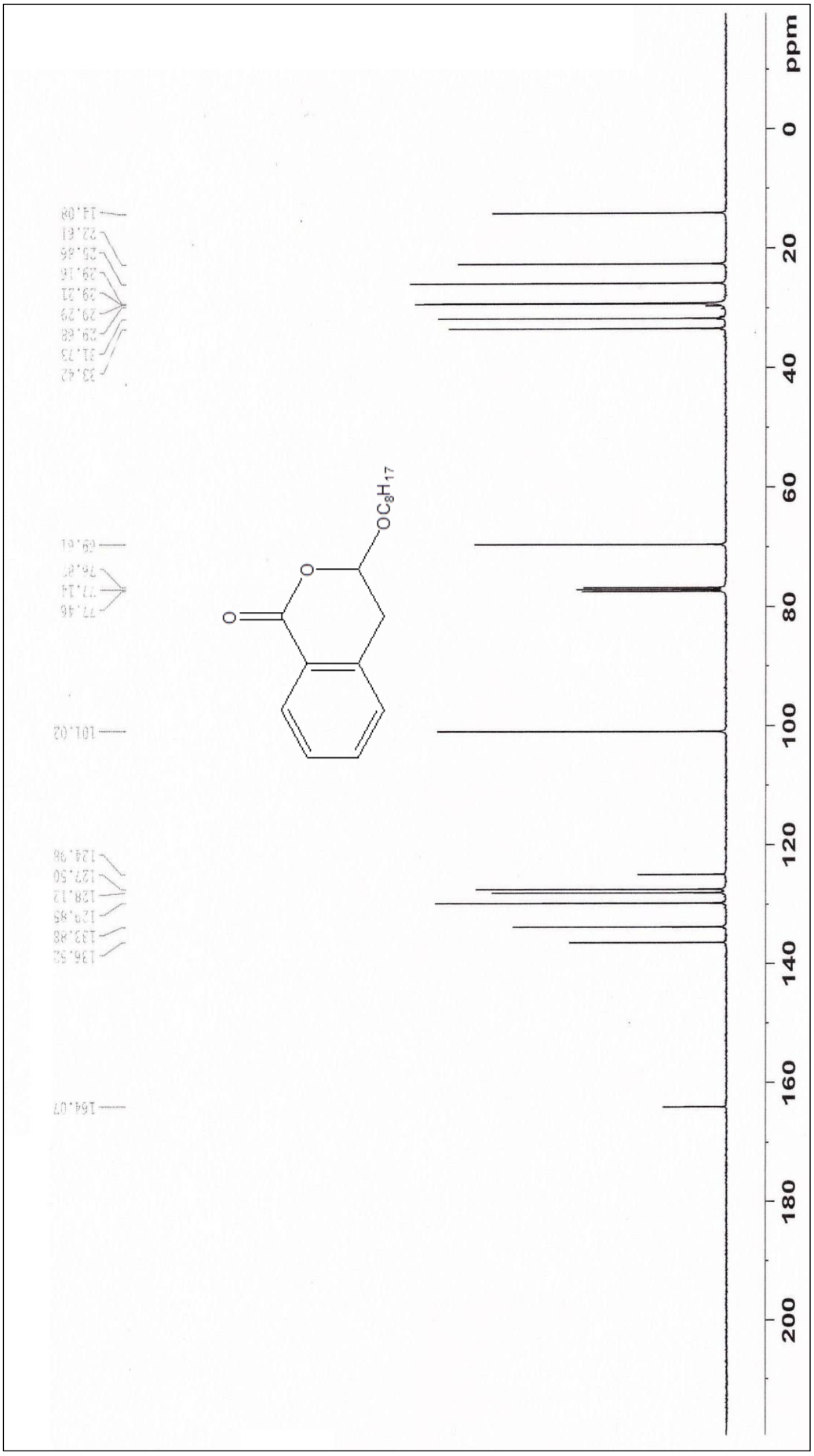



**Figure 1.25**:- $^{13}C$ NMR of 3,4-dihydro-3-(octyloxy)isochroman-1-one **(46e)**

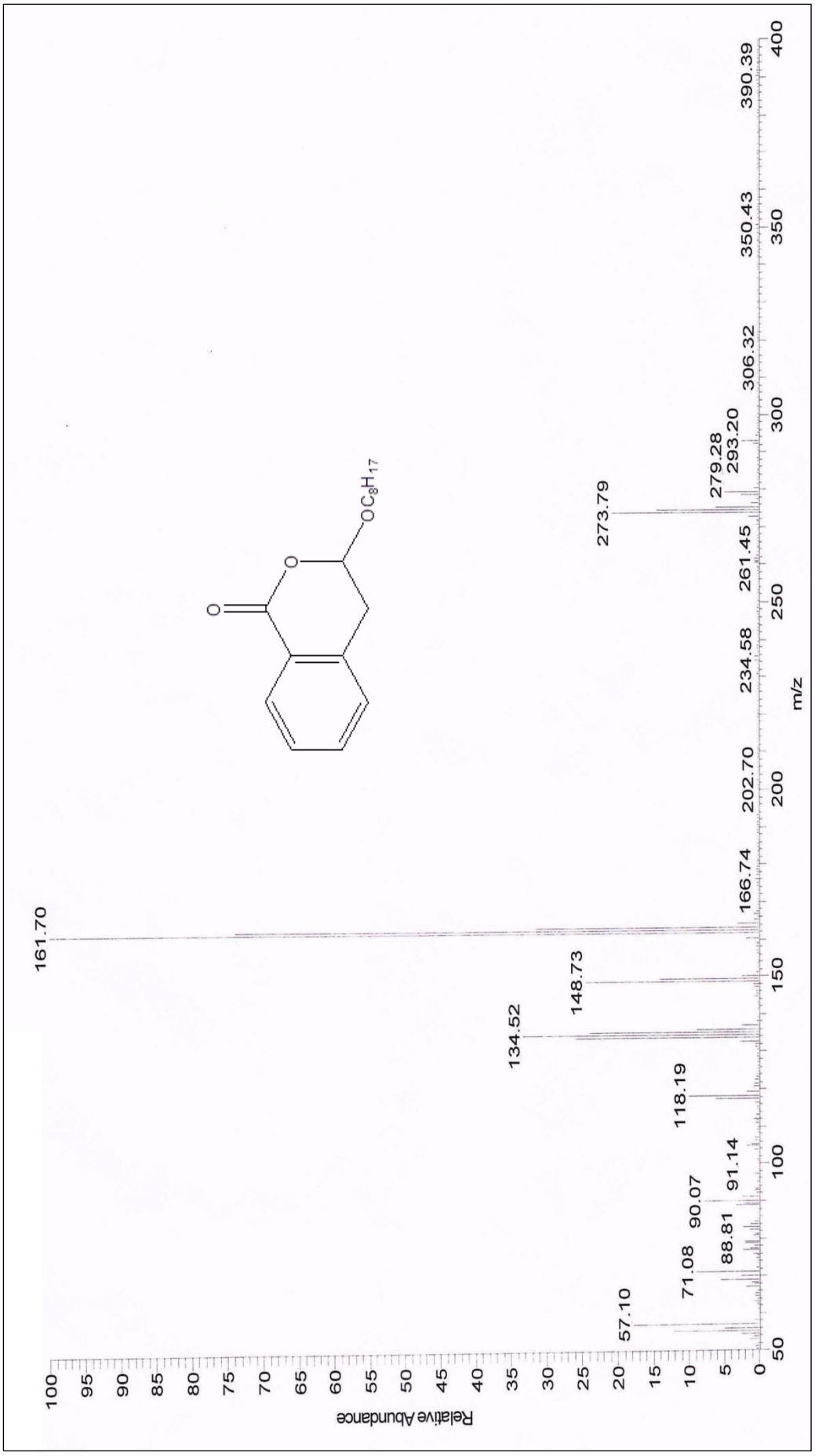



**Figure 1.26**:- Mass Spectrum of 3,4-dihydro-3-(octyloxy)isochroman-1-one **(46e)**

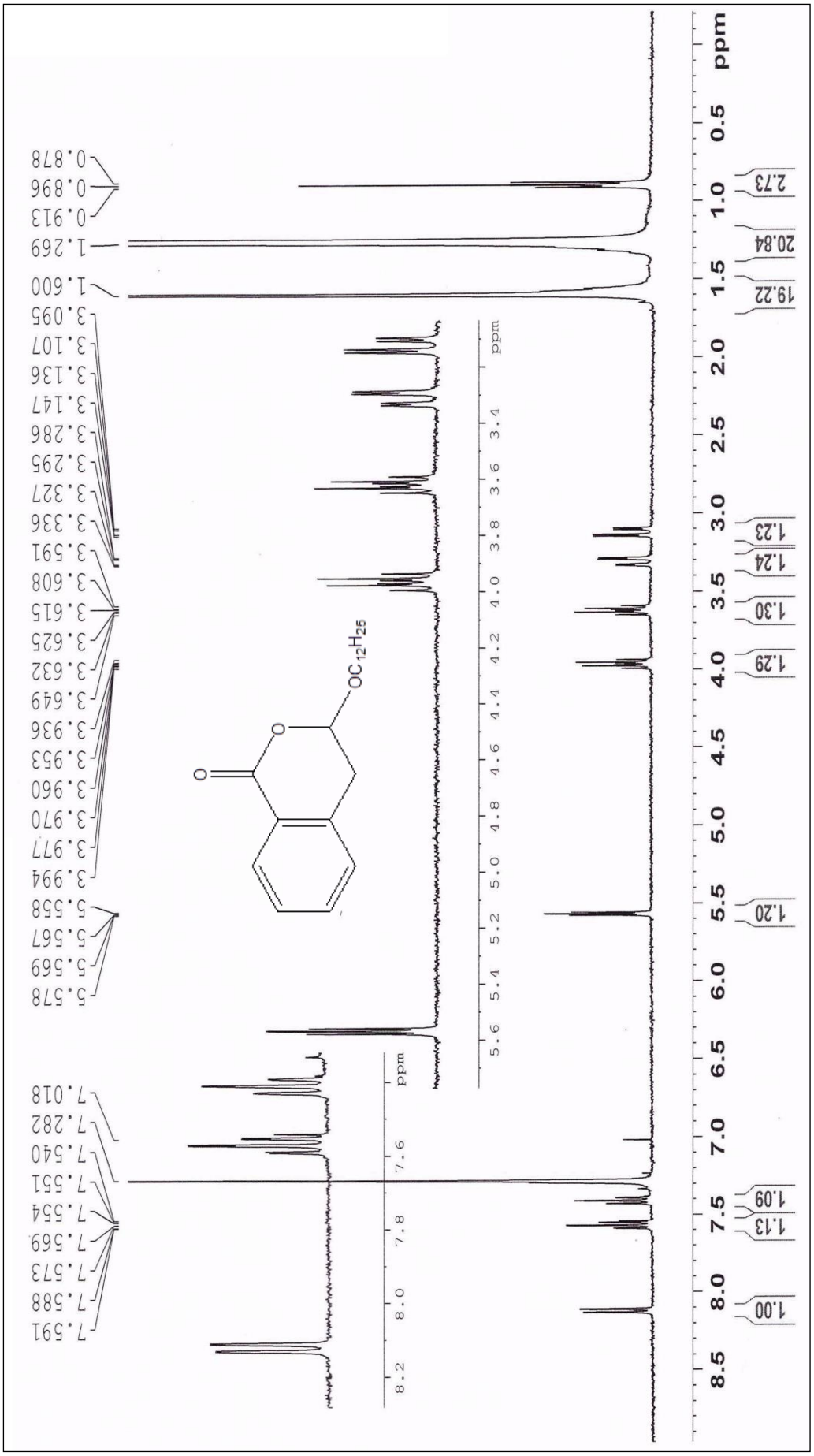



**Figure 1.27:-** $^{1}$H NMR of 3-(dodecyloxy)-3,4-dihydroisochroman-1-one **(46f)**

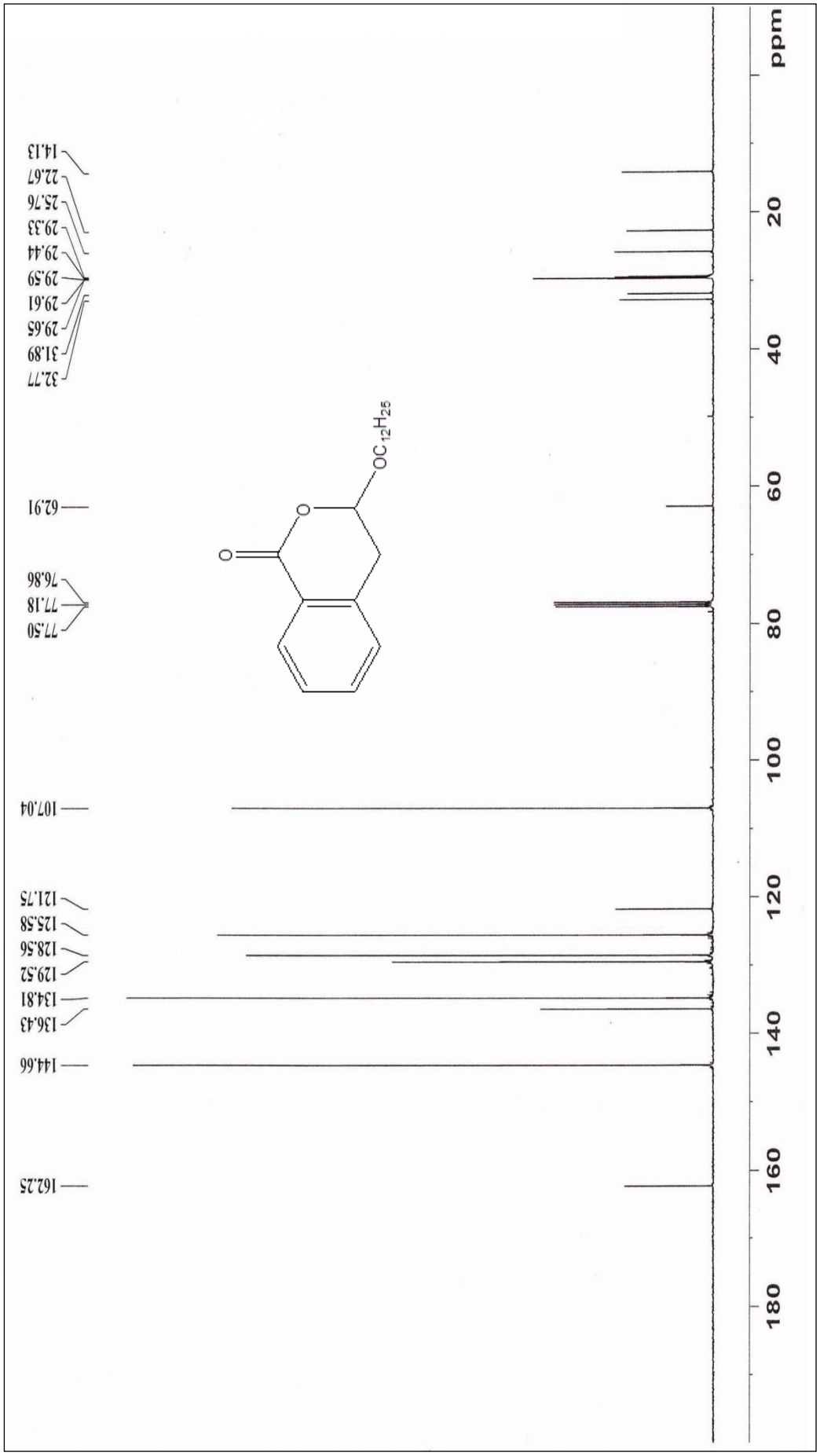



**Figure 1.28**:- $^{13}C$ NMR of 3-(dodecyloxy)-3,4-dihydroisochroman-1-one **(46f)**

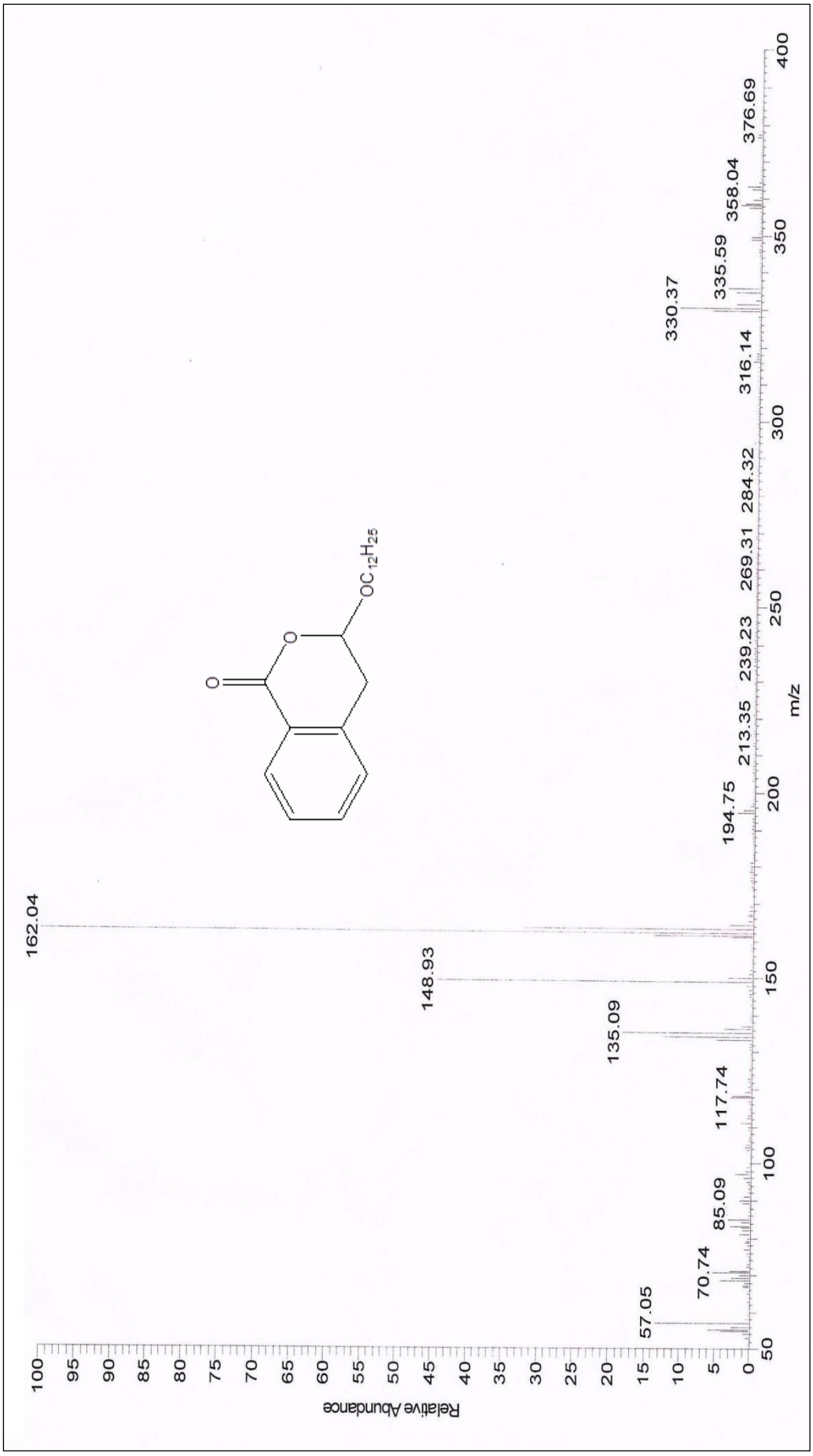



**Figure 1.29**:- Mass Spectrum of 3-(dodecyloxy)-3,4-dihydroisochroman-1-one **(46f)**

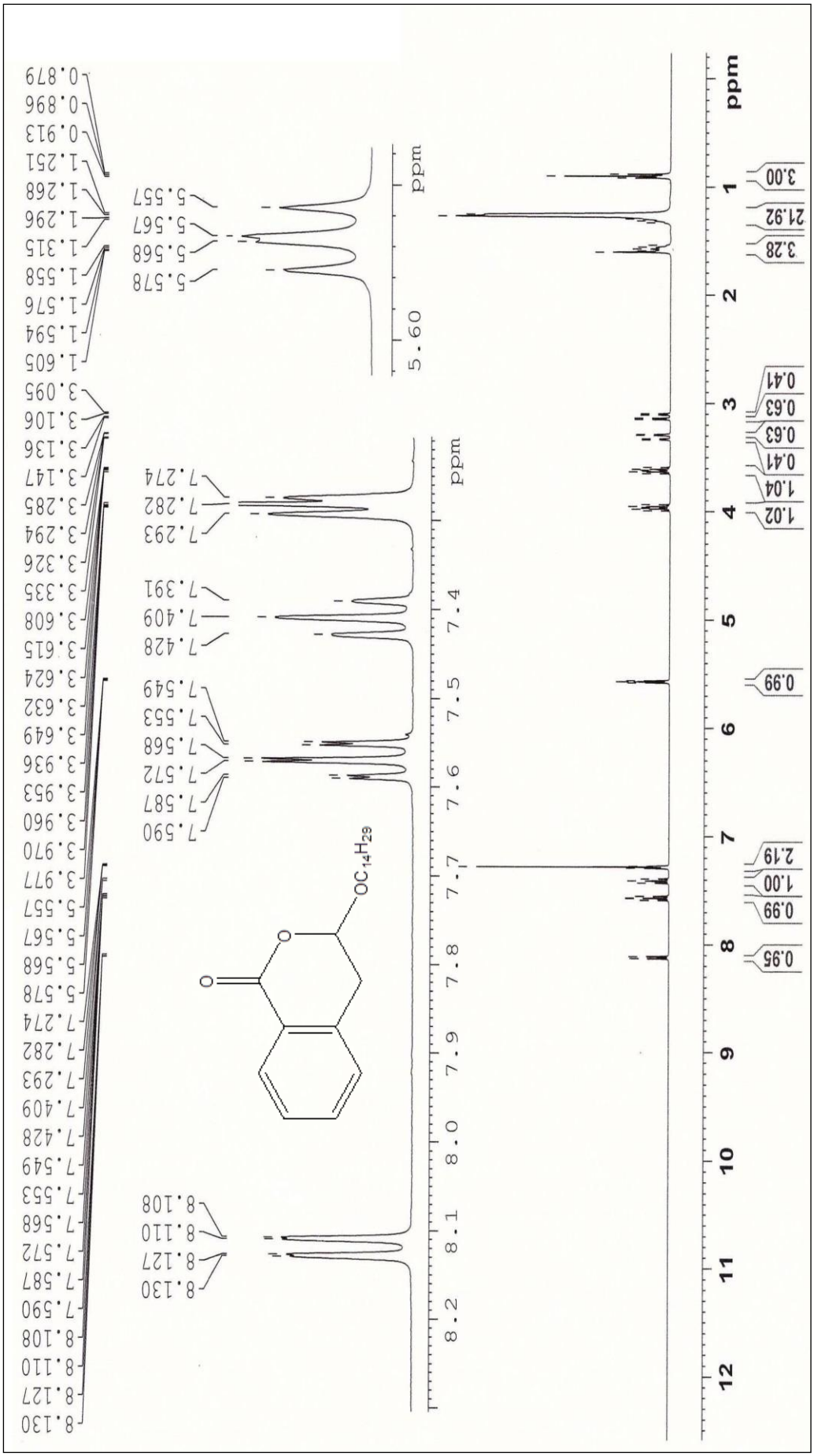



**Figure 1.30:-** $^{1}$H NMR of 3,4-dihydro-3-(tetradecyloxy)isochroman-1-one **(46g)**

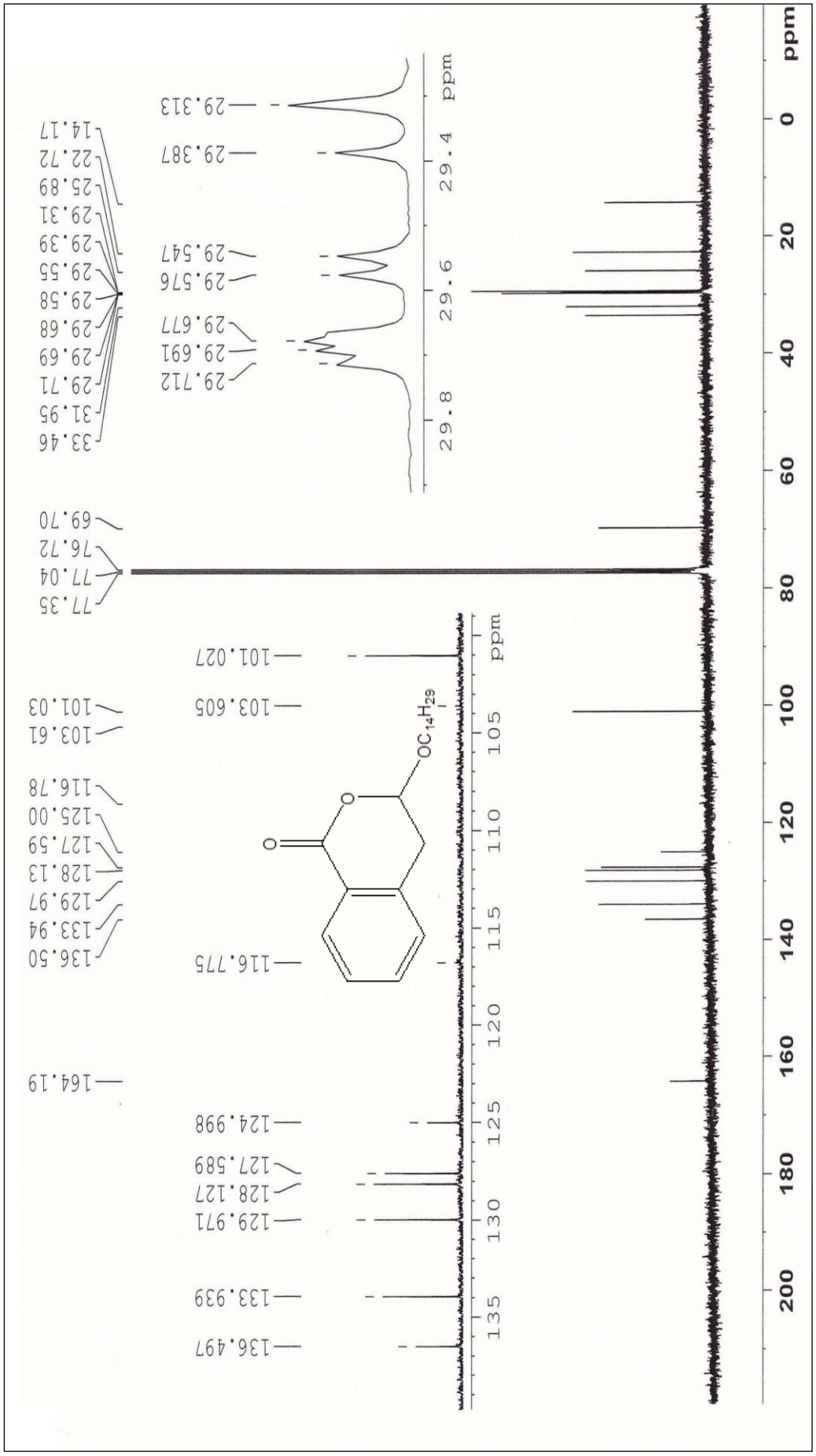



**Figure 1.31**:-$^{13}$C NMR of 3,4-dihydro-3-(tetradecyloxy)isochroman-1-one **(46g)**

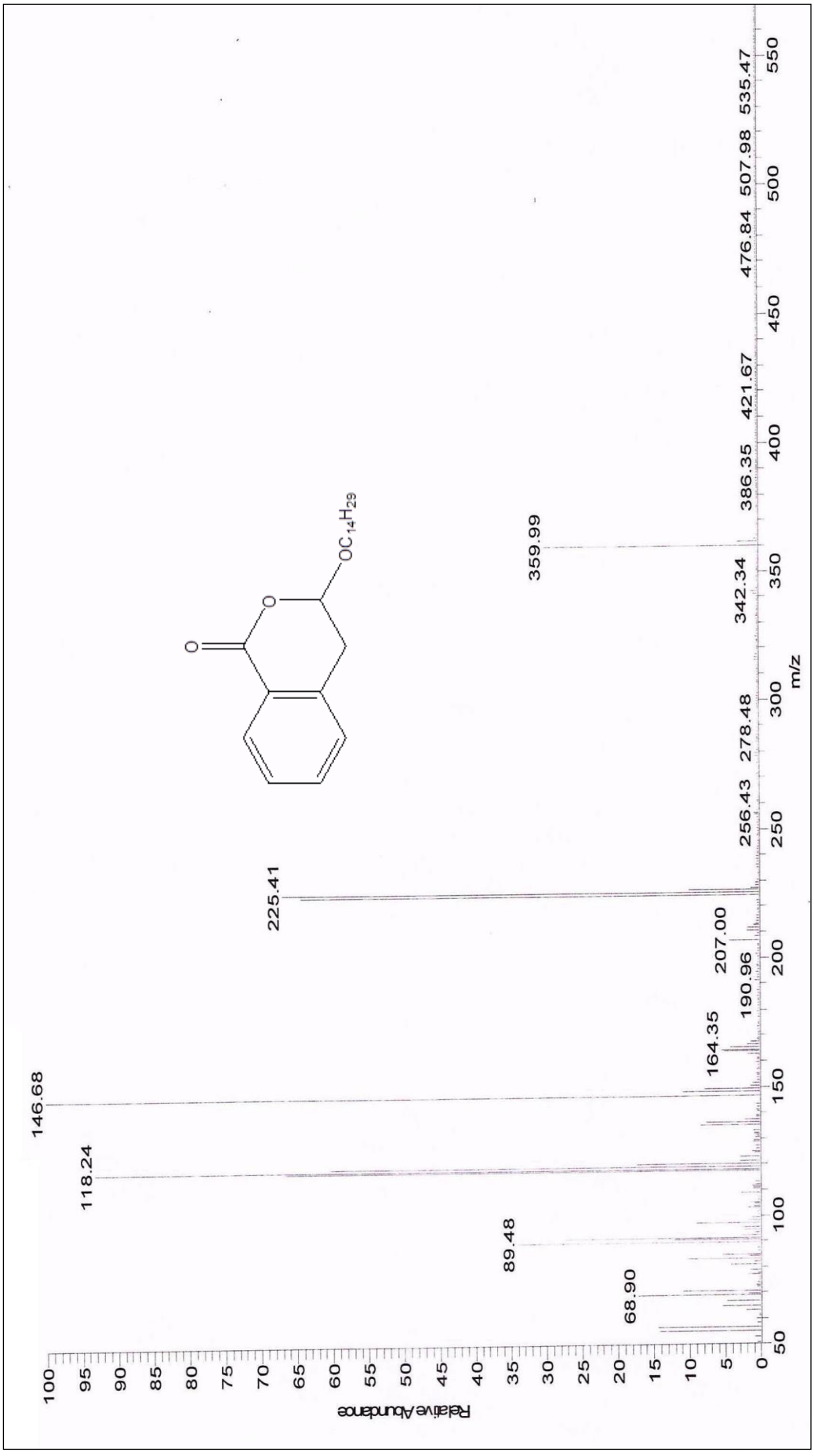



**Figure 1.32**:- Mass Spectrum of 3,4-dihydro-3-(tetradecyloxy)isochroman-1-one **(46g)**

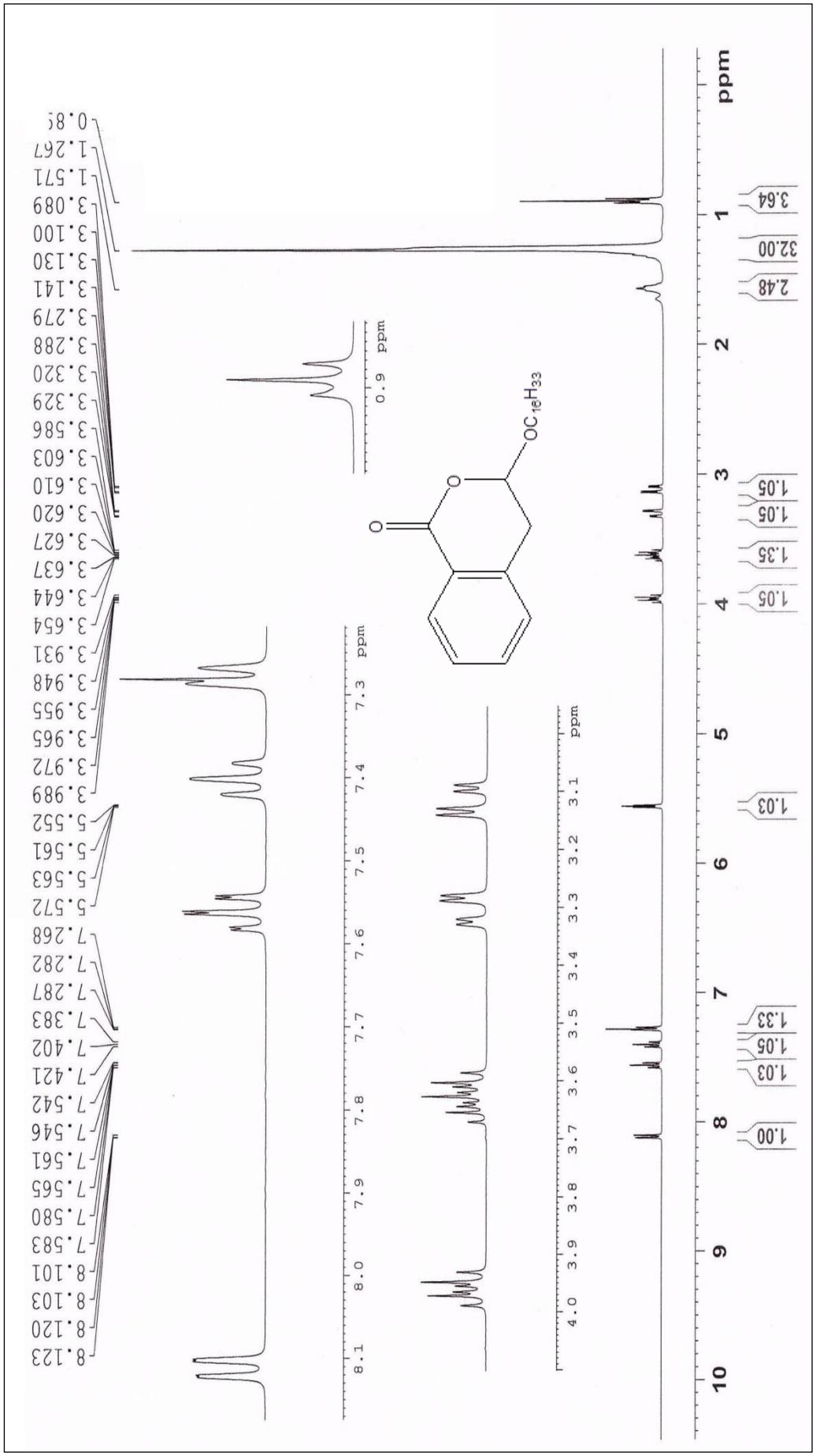



**Figure 1.33:-** $^{1}$H NMR of 3-(hexadecyloxy)-3,4-dihydroisochroman-1-one **(46h)**

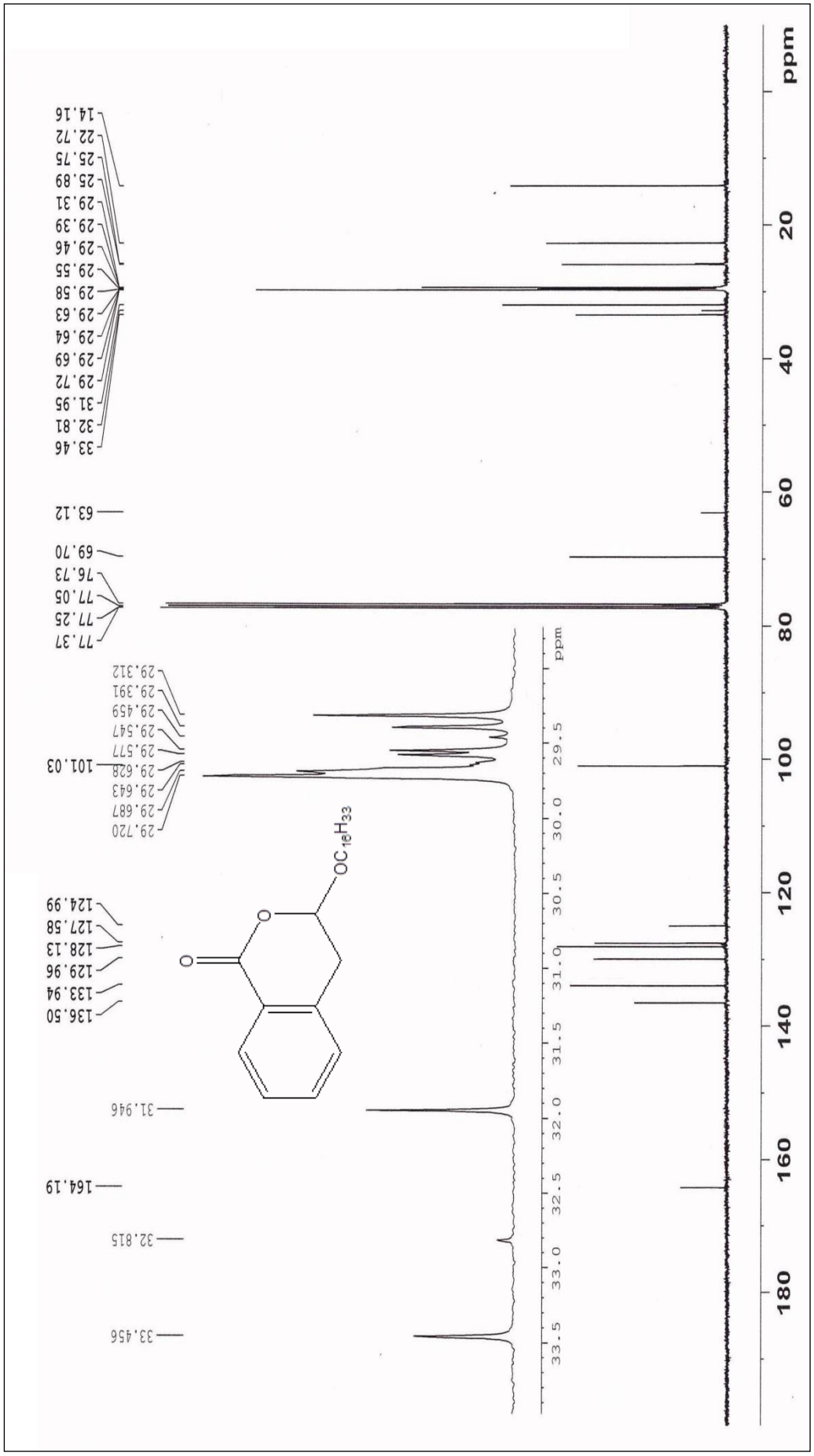



**Figure 1.34**:- $^{13}C$ NMR of 3-(hexadecyloxy)-3,4-dihydroisochroman-1-one **(46h)**

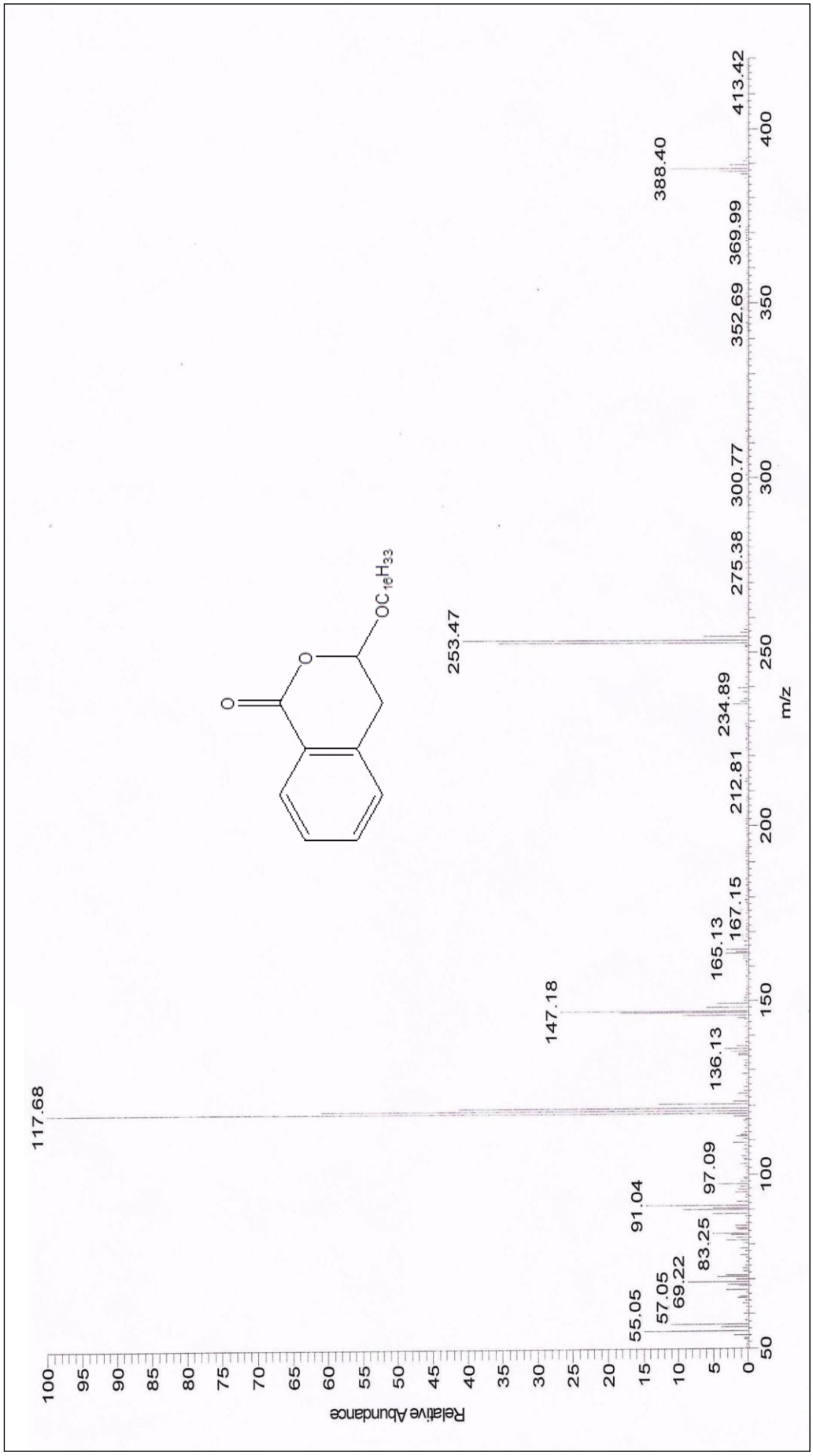



**Figure 1.35**:- Mass Spectrum of 3-(hexadecyloxy)-3,4-dihydroisochroman-1-one **(46h)**

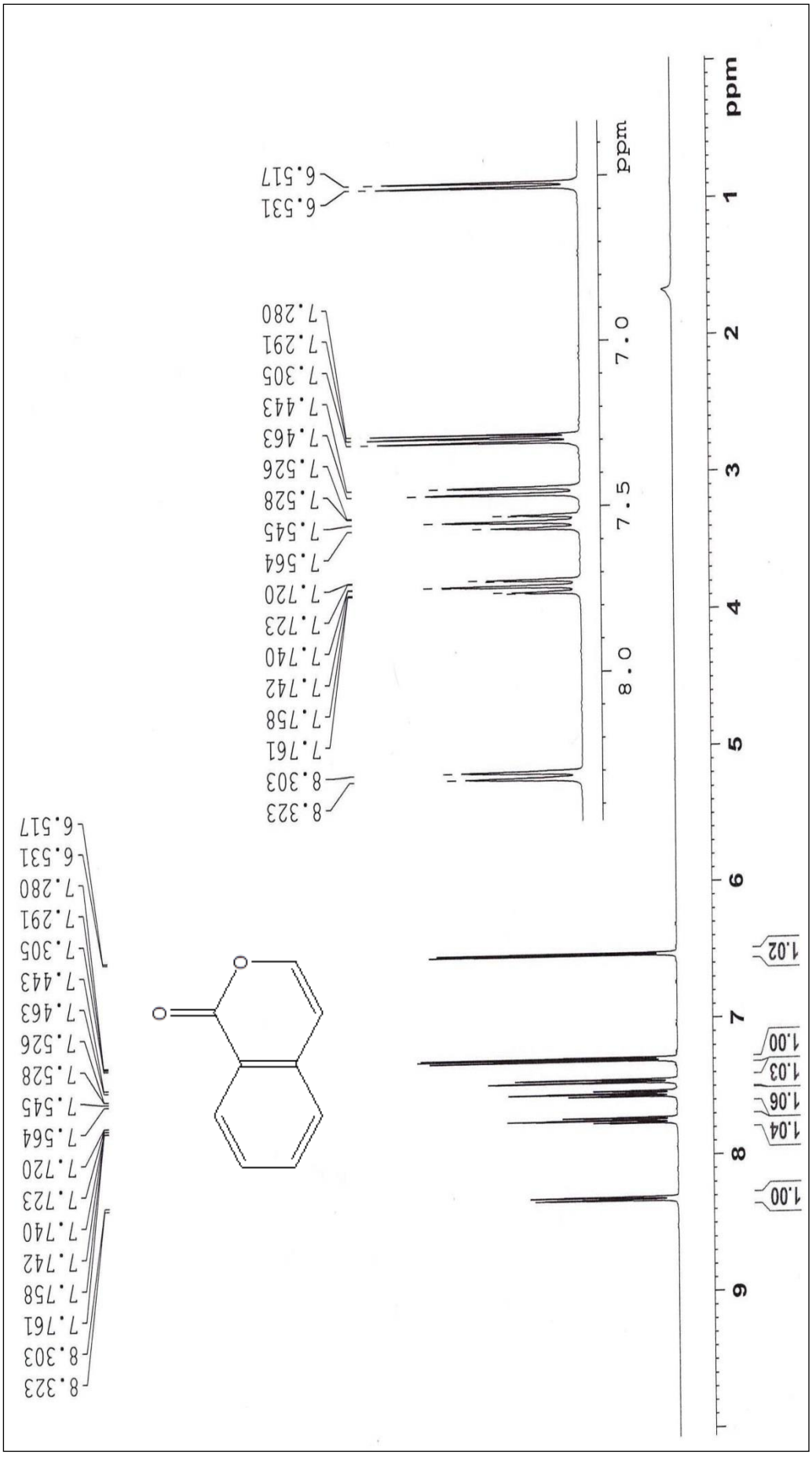



**Figure 1.36:-** $^{1}$H NMR of 1H-isochromen-1-one **(47)**

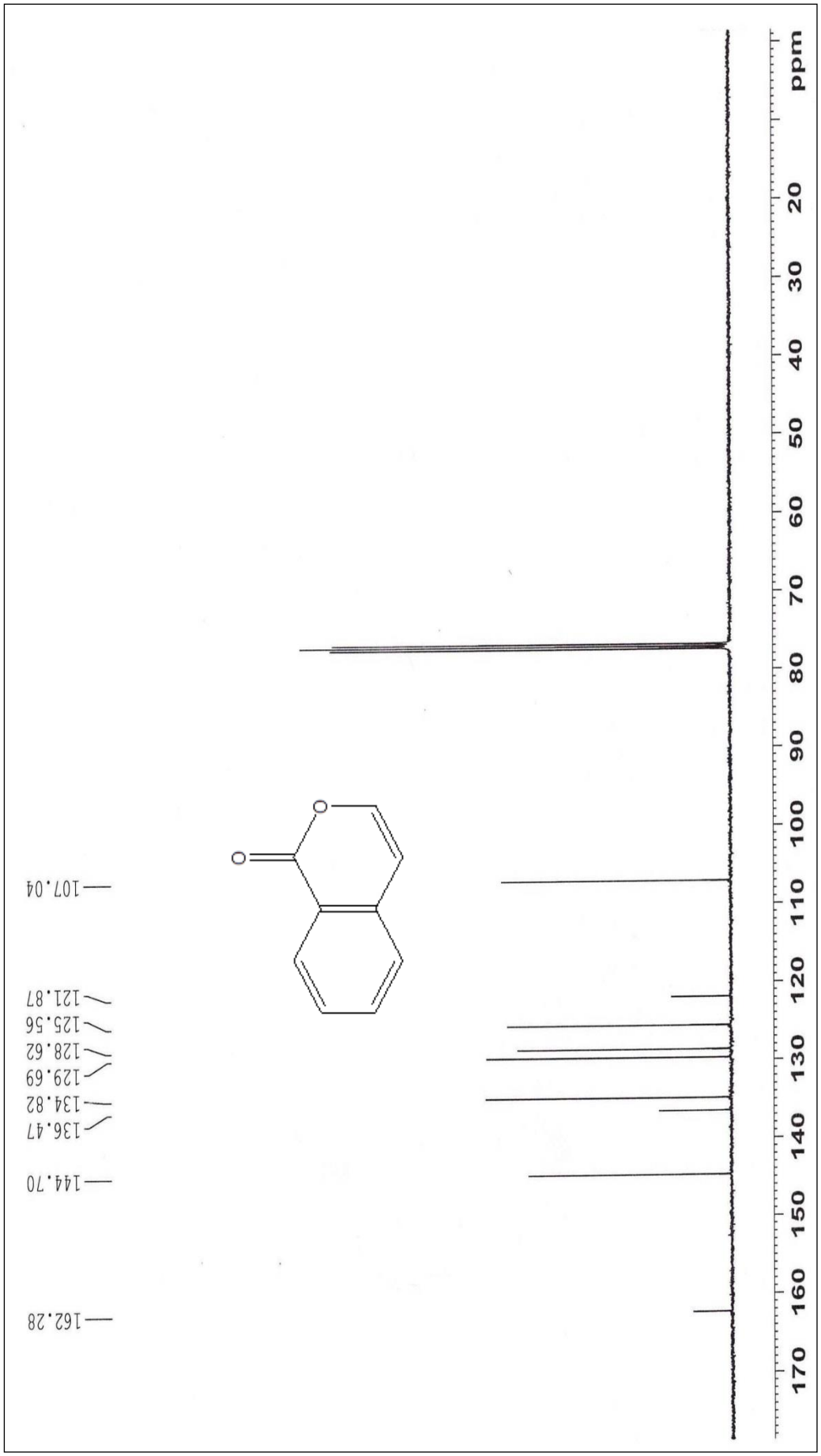



**Figure 1.37**:- $^{13}C$ NMR of 1H-isochromen-1-one **(47)**

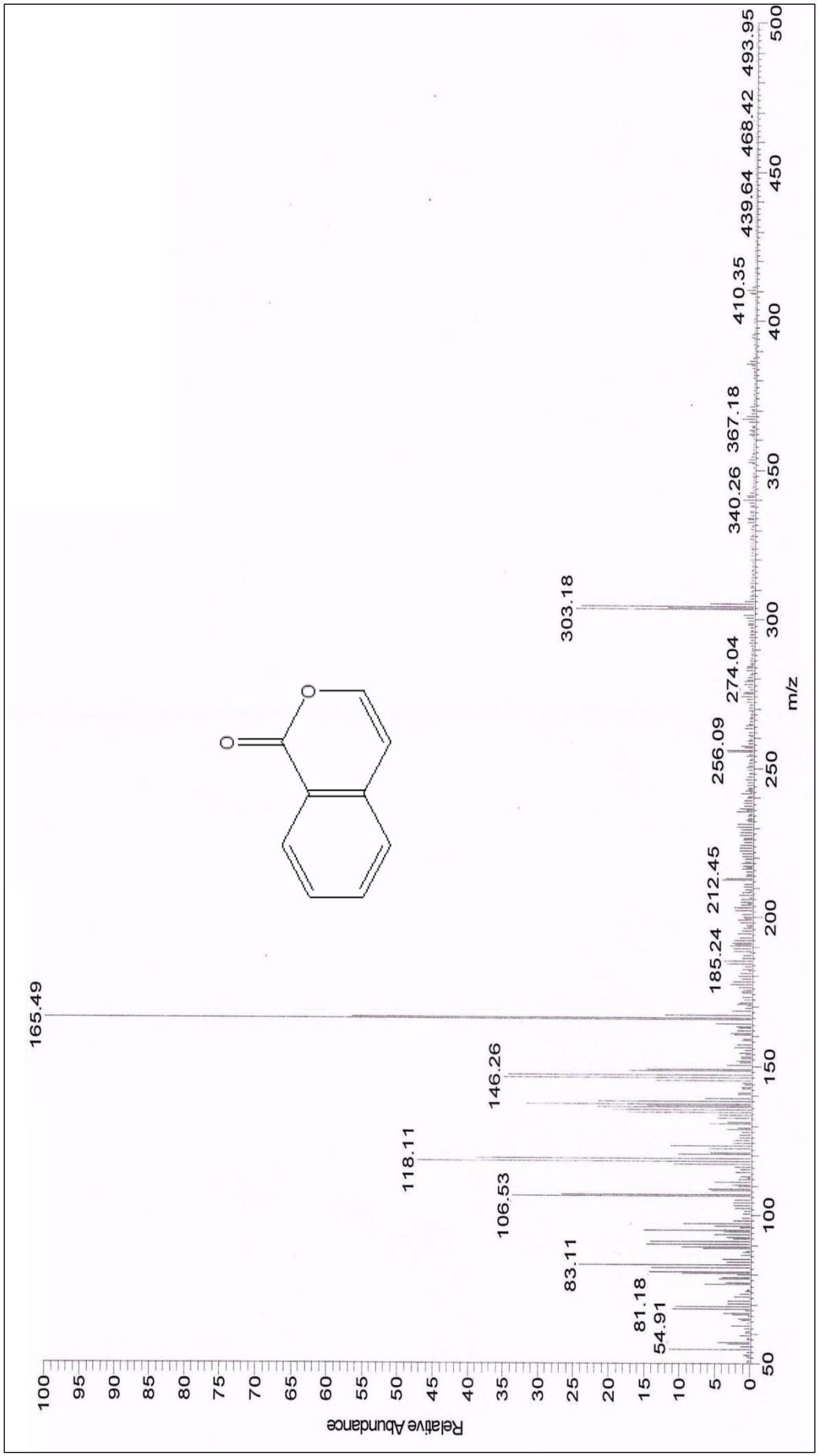



**Figure 1.38**:- Mass spectrum of 1H-isochromen-1-one **(47)**

## 1.7 References


1. Weissermel, K.; Arpe, H. J. Industrial Organic Chemistry, 4th ed.; Wiley-VCH: Federal Republic of Germany, **2003**.; Yuan, C.; Axelrod, A.; Varela, M.; Danysh, L.; Siegel D. *Tetrahedron Letters*, **2011**, *52*, 2540.; Dobler, C.; Mehltretter, G. M.; Sundermeier, U.; Beller, M. *J. Am. Chem. Soc.* **2000**, *122*, 10289; Kulkand, K.; Dittrick, J. W. *Cosmet. Perfum.* **1975**, *90*, 90; Kirk Othmer *Encyclopedia of Chemical Technology*, 4th ed. Kroscwitz, J. I., Howe Grant, M., Eds.; Wiley: New York, **1991**, Vol 12.; Hirshman, J. L.; Garcia, D. S.; Kazemizadeh, M. R. *US Patent*, 5,324,846, **1994**; *Chem. Abstr.* **1994**, *120*, 136143c; Zhou, Y.; Shan, Z. *J. Org. Chem.* **2006**, *71*, 9510.
2. Honda, T.; Mizutani, H.; Kanai, K. *J. Org. Chem.*, **1996**, *61* (26), 9374.
3. Chandra, K. L.; Chandrasekhar, M.; Singh, V. K. *J. Org. Chem.*, **2002**, *67* (13), 4630.
4. Musich, J. A.; Rapoport, H. *J. Am. Chem. Soc.*, **1978**, *100* (15), 4865.
5. Lin, Z.; Marett, L.; Hughen, R. W.; Flores, M.; Forteza, I.; Ammonc, M.; Concepcion, G. P.; Espino, S.; Olivera, B. M.; Rosenberg, G.; Haygood, M. G.; Light, A. R.; Schmidt, E. W. *Bioorganic & Medicinal Chemistry Letters*, **2013**, *23*, 4867.
6. Clark, A. H.; McCorvy, J. D.; Watts, V. J.; Nichols, D. E. *Bioorganic & Medicinal Chemistry*, **2011**, *19*, 5420.
7. Bartlett, P. A.; Myerson, J. *J. Org. Chem.*, **1979**, *44 (10)*, 1625.
8. Raghavan, S.; Joseph, S. C. *Tetrahedron Letters*, **2003**, *44(45)*, 8237.
9. Keinan, E.; Sinha, S. C.; Bagchia, A. S.; Wang, Z. M.; Zhang, X. L.; Sharpless, K. B. *Tetrahedron Letters*, **1992**, *33(43)*, 6411.
10. Prevost, C. *Compt. Rend.* **1933**, *196*, 1129.; Woodward, R. B.; Brutcher, F. V. *J. Am. Chem. Soc.* **1958**, *80*, 209.; Jacobsen, E. N.; Markó, I.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968.; Wai, J. S. M.; Markó, I.; Svenden, J. S.; Finn, M. G.; Jacobsen, E. N.; Sharpless, K. B. *J. Am. Chem. Soc.* **1989**, *111*, 1123.; Kim, N.-S.; Choi, J.-R.; Cha, J. K. *J. Org. Chem.* **1993**, *58*, 7096.; Kolb, H. C.; VanNiewenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.; Corey, E. J.; Noe, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 319.; DelMonte, A. J.; Haller, J.; Houk, K. N.; Sharpless, K. B.; Singleton, D. A.; Strassner, T.; Thomas, A. A. *J. Am. Chem. Soc.* **1997**, *119*, 9907.

11. Brewster, D.; Myers, M.; Ormerod, J.; Otter, P.; Smith, A. C. B.; Spinner, M. E.; Turner, S. J. Chem. Soc. Perkin Trans 1. 1973, 2796.; Woodward, R. B.; Brutcher, F. V. *J. Am. Chem. Soc.* **1958**, *80*, 209.; VanNiewenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
12. Milas, N. A.; Sussman, S. *J. Am. Chem. Soc.*, **1936**, *58*, 1302.; Milas, N. A.; Sussman, S. *J. Am. Chem. Soc.*, **1937**, *59*, 2345.; Milas, N. A.; Sussman, S.; Mason, H. S. *J. Am. Chem. Soc.*, **1939**, *81*, 1844.
13. Hofmann, K. A. *Chem. Ber.*, **1912**, *45*, 3329.; Hofmann, K. A.; Ehrhart, O.; Schneider, O. *Chem. Ber.*, **1913**, *46*, 1657.; Milas, N. A.; Terry, E. M. *J. Am. Chem. Soc.*, **1925**, *47*, 1412.; Buchi, G.; Demole, E.; Thomas, A. F. *J. Org. Chem.*, **1973**, *38*, 123.
14. Byers, A.; Hickinbottom, W. J. *J. Chem. Soc.*, **1948**, 1328.; McCasland, G. E.; Turuta, S.; Durham, L. J. *J. Org. Chem.*, **1968**, *33*, 2835.
15. Van Rheenan, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.*, **1976**, 1973.; Corey, E. J.; Danheiser, R. L.; Chandrasekaran, S.; Siret, P.; Keck, G. E.; Gras, J. L. *J. Am. Chem. Soc.*, **1978**, *100*, 8031.
16. Willstatter, R.; Sonnenfeld, E. *Chem. Ber.*, **1913**, *48*, 2952.
17. Dobler, C.; Mehltretter, G. M.; Sundermeier, U.; Beller, M. J. *Organomet. Chem.*, **2001**, *70*, 621.
18. Li, Y.; Song, D.; Dong, V. M. *J. Am. Chem. Soc.* **2008**, *130*, 2962.; Wang, A.; Jiang, H.; Chen, H. *J. Am. Chem. Soc.* **2009**, *131*, 3846.; Wang, W.; Wang, F.; Shi, M. *Organometallics* **2010**, *29*, 928.
19. Chen, K.; Costas, M.; Kim, J.; Tipton, A. K.; Que, L. Jr. *J. Am. Chem. Soc.* **2002**, *124*, 3026.; Oldenburg, P. D.; Que, L. Jr. *Catal. Today*, **2006**, *117*, 15.
20. Plietker, B.; Niggemann, A.; Pollrich, A. *Org. Biomol. Chem.* **2004**, *2*, 1116.; Plietker, B.; Niggemann, A. *J. Org. Chem.* **2005**, *70*, 2402.; Neisius, N. M.; Plietker, B. *J. Org. Chem.* **2008**, *73*, 3218.
21. Boer, J. W.; Brinksma, J.; Browne, W. R.; Meetsma, A.; Alsters, P. L.; Hage, R.; Feringa, B. L. *J. Am. Chem. Soc.* **2005**, *127*, 7990.; Boer, J. W.; Brinksma, J.; Browne, W. R.; Harutyunyan, S. R.; Bini, L.; Tiemersma Weyman, T. D.; Alsters, P. L.; Hage, R.; Feringa, B. L. *Chem. Commun.* **2008**, 3747.
22. Seayad, J.; Seayad, A. M.; Chai, C. L. L. *Org. Lett.* **2010**, *12*, 1412.
23. Samantaa, S.; Lahab, S. C.; Malc, N. K.; Bhaumika, A. *J. Mol. Catal. A: Chem.* **2004**, *222*, 235.

24. Biradar, A. V.; Sathe, B. R.; Umbarkar, S. B.; Dongare, M. K. *J. Mol. Catal. A: Chem.* **2008**, *285*, 111.
25. Usui, Y.; Sato, K.; Tanaka, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 5623.; Hrdina, R.; Muller, C. E.; Wende, R. C.; Wanka, L.; Schreiner, P. R. *Chem. Commun.*, **2012**, *48*, 2498.; Morgan, J. B.; Miller, S. P.; Morken, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 8702.; Sampson, K.; Paik, A.; Duvall, B.; Whalen, D. L. *J. Org. Chem.* **2004**, *69*, 5204.
26. Senanayake, C. H. Aldrichimica Acta. **1998**, *31*, 1.
27. Patel, R. N. *Food Technol. Biotechnol.* **2004**, *42*, 305.; Treadway, S. L.; Yanagimachi, K. S.; Lankenau, E.; Lessard, P. A.; Stephanopoulos, G.; Sinskey, A. J. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 786.;
28. Whalen, D. L.; Sampson, K.; Paik, A.; Duvall, B. *J. Org. Chem.* **2004**, *69*, 5204.
29. Asano, Y.; Kato, Y. *J. Mol. Catalysis B: Enzymatic.*, **2001**, *13*, 27.
30. Mugdan, M.; Young, D. P. *J. Chem. Soc.*, **1949**, 2988.; Church, J. M.; Blumberg, R. *Ind. Eng. Chem.*, **1951**, *43*, 1780.; Young, D. P. *Brit. Patent* 654,764; *Chem. Abstr.*, **1952**, *46*, 7115.; Owen, L. N.; Smith, P. N. *J . Chem. Soc.*, **1952**, 4041.; Smith, C. W. *US. Patent* 2,838,575, **1958**.
31. Payne, G. B.; Smith, C. W. *J. Org. Chem.*, **1957**, *22 (12)*, 1682.
32. Deota, P. T.; Singh, V. K. *Synth. Commun.* **1988**, *18*, 617.; Paquette, L. A. *Top. Curr. Chem.*, **1979**, *59*, 43.; Trayham, J. G.; Greene, P. M. *J. Am. Chem. Soc.*, **1964**, *86*, 2657.
33. Deota, P. T.; Desai, R.; Valodkar, V. B. *J. Chem. Res. (S)*. **1998**, 562.
34. Valente, E. J.; Fuller, J. E.; Ball, J. D. *Acta Cryst.* **1998**, *B54*, 162.
35. Brewster, D.; Myers, M.; Ormerod, J.; Otter, P.; Smith, A. C. B.; Spinner, M. E.; Turner, S. *J. Chem. Soc. Perkin Trans 1*. **1973**, 2796.
36. Taylor, J. E. *Synthesis*. **1985**, 1142.
37. Evans, D. A.; Hu, E.; Tedrow, J. S. *Org. Lett.* **2001**, *3(20)*, 3133, and references cited therein.
38. Stromgaard, K.; Nakanishi, K. *Angew. Chem. Int. Ed.* **2004**, *43*, 1640; Nakanishi, K. *Bioorg. Med. Chem.* **2005**, *13*, 4987.
39. Boukouvalas, J.; Cheng, Y. X.; Robichaud, J. *J. Org. Chem.* **1998**, *63*, 228.
40. Yamada, Y.; Miyaoka, H.; Yamanishi, M.; Kajiwara, Y. *J. Org.Chem.* **2003**, *68*, 3476.

41. Lee, I. S.; Ma, X.; Chai, H; Madulid, D. A.; Lamont, R. B.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. *Tetrahedron.* **1995**, *51*, 21.
42. He, H.; Kulanthaivel, P.; Baker, B. J. *Tetrahedron lett.* **1994**, *35*, 7189.
43. De Silva, E. D.; Scheuer, P. J. *Tetrahedron Lett.* **1980**, *21*, 1611.
44. De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Pronzato, R.; Zavodnik, N. J. Nat. Prod. 1995, *58*, 1776.
45. Saeed, A. *Monatshefte fur Chemie.* **2003**, *134*, 457.
46. Shen, J.; Ling, Q.; Huang, Y.; Zhou, Y.; Cai, Z.; Xiong, B.; Zhang, Y.; Maa, L.; Wang, X.; Li, X.; Li, *J. Bioorg. Med. Chem.* **2008**, *16*, 7399.
47. Hauser, F. M.; Baghdanov, V. M. *J. Org. Chem.* **1988**, *53*, 4676.
48. Tkachev, A. V.; Kolesnik, V. D.; Rukavishnikov, A. V. *Mendeleev Commun.* **1995**, *5(5)*, 179.
49. Kondratov, I. S.; Gerus, I. I.; Kukhar, V. P.; Manoilenko, O. V. *Tetrahedron: Asymmetry*. **2007**, *18*, 1918.
50. Rousha, W. R.; Roth, J.; Madoux, F.; Hodderb, P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2628.
51. Schöpf, C.; Kühne, R. *Chem. Ber.* **1950**, *83*, 390.
52. Einaga, H.; Nojima, M.; Abe, M. *Main Group Metal Chem.* **1999**, *22(9)*, 539.; Einaga, H.; Nojima, M.; Abe, M. *J. Chem. Soc., Perkin Trans.1: Org. and Bioorg. Chem.* **1999**, *17*, 2507.; Abe, M.; Inakazu, T.; Munakata , J.; Nojima, M. *J. Amer. Chem. Soc.* **1999**, *121(28)*, 6556.; Akbutina, F. A.; Davydova, V. A.; Zardii, F. A.; Sagitdinov, I. A.; Miftakhov, M. S. *Khimiko Farmatsevticheskii Zhurnal.* **1998**, *32(5)*, 26.; Miftakhov, M. S.; Akbutina, F. A.; Tolstikov, A. G.; Anpilogov, A. P.; Tolstikov, G. A. *Zhurnal Organicheskoi Khimii.* **1987**, *23(12)*, 2559.
53. Scialdone, M.; Liauw, A. *US. Patent* 7,776,912 B2, **2010**.

# Chapter 2

## Design, synthesis and application of novel benzil derivatives as photostabilizers for Chlorpyrifos

## 2.1 Abstract

Design and synthesis of novel benzil derivatives is reported and furthermore, they are employed as photostabilizers to study the photostabilization of chlorpyrifos under UV light. The percentage recovery of chlorpyrifos after UV irradiation is obtained by HPLC. Results indicate significant enhancement in the photostabilization of chlorpyrifos using these benzil derivatives in comparison with 2,4-dihydroxy benzophenone, a reference photostabilizer.

## 2.2 Introduction

The sunlight emits broad range of electromagnetic radiations such as gamma rays to radio waves. The sunlight plays an important role in the routine life processes of living organisms on the earth by providing energy in the form of light such as plant photosynthesis and production of Vitamin D in the human body. In spite of its importance, it is also causing serious adverse effects in the organisms and molecules on exposure to UV radiations. The presence of effective ozone gas layer in the stratosphere allows light only with the wavelength greater than 290 nm to reaches the earth surface.[1] At the earth's surface, sunlight is composed of about 7% UV, 44% visible and 49% other radiations (Figure 2.1). Although only a relatively small amount of the sunlight energy is in the UV region of 290-400, it is ample to induce photochemical reaction/degradation of many organic molecules.[1]

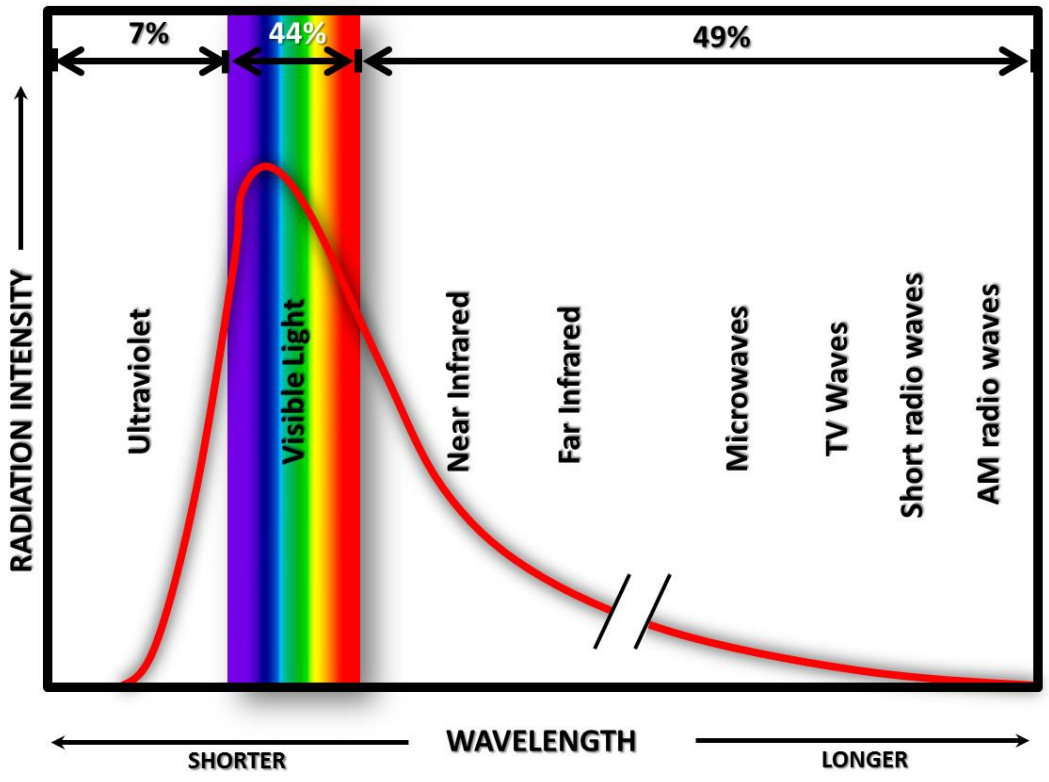



**Figure 2.1.** Solar radiation spectrum[1]

The damaging UV radiation in the sunlight is responsible for the discoloration of dyes and pigments, weathering and yellowing of plastics, loss of gloss and

mechanical properties (cracking), sunburnt skin, degradation of pesticides/bio-pesticides and other problems associated to UV light.

A long standing problem in agricultural field is menace of various pests damaging the useful crops. The use of insecticides in agriculture is continuously increasing with simultaneous addition of new types of insecticides. The development of effective insecticides has obviously been a major activity in the past decades for controlling the ruinous attack by the pests. A wide range of insecticides including organophosphorus, carbamates, pyrethroids and other class of pesticides have been used on crops to control insects.

There are many factors which affect the insecticidal activity when exposed to external environment like microbial decomposition, hydrolysis, volatilization and photolysis. Photodegradation due to sunlight is one of the major pathways which lessen insecticidal activity after their application in the field. On exposure to sunlight, the insecticide molecules undergo a variety of primary processes often leading to their degradation (Figure 2.2).[1,2]

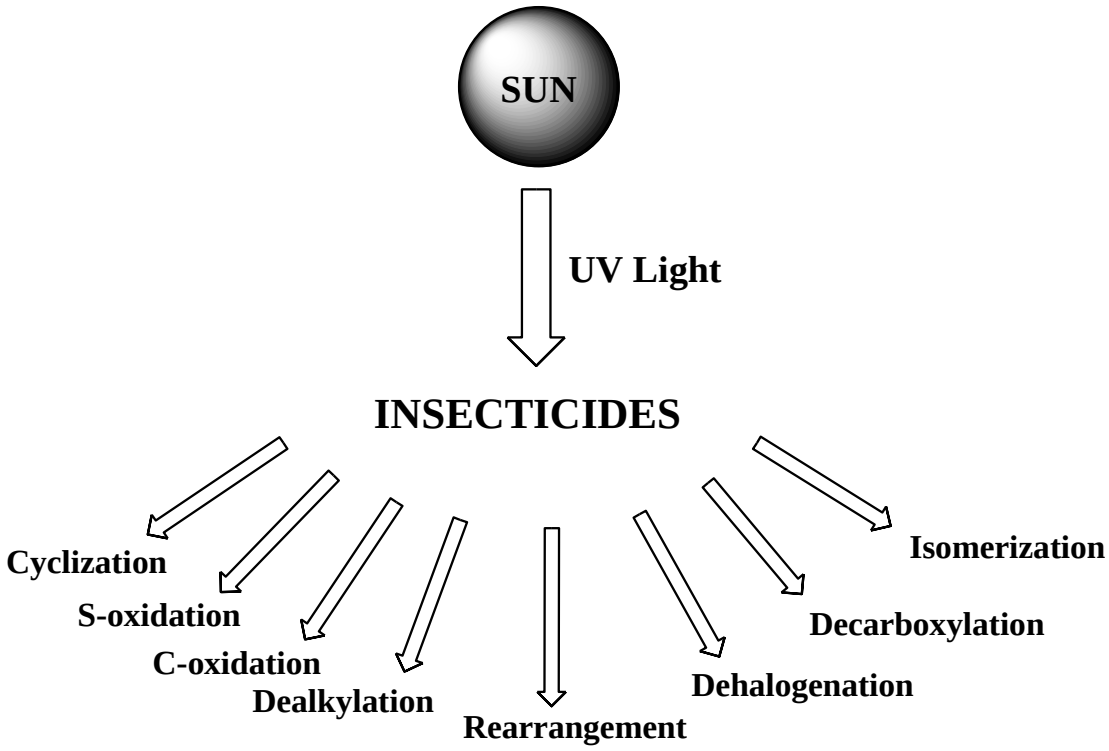
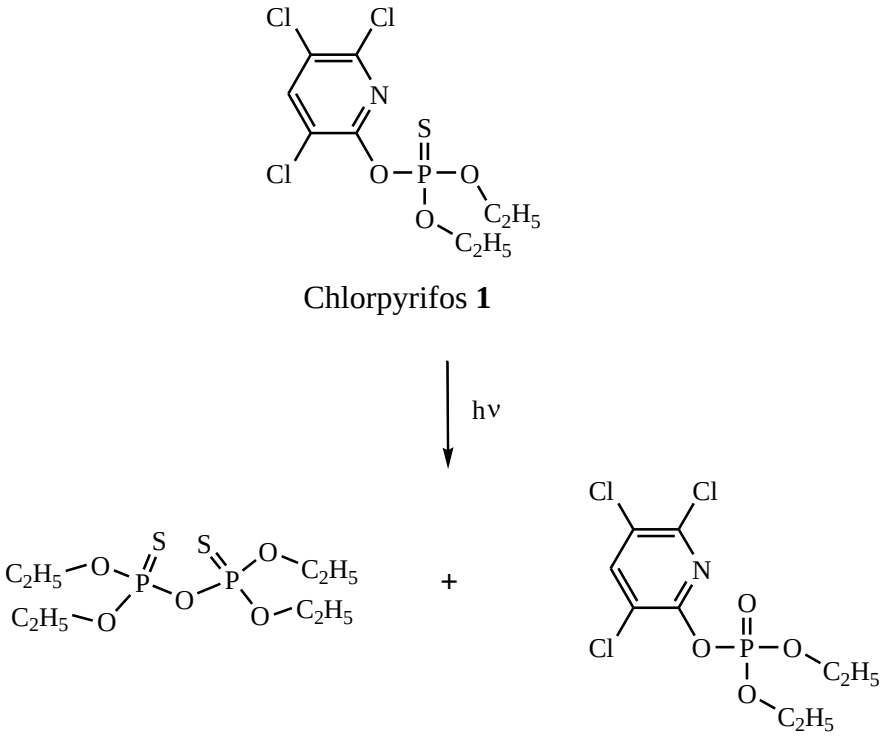



**Figure 2.2.** Various primary processes of insecticides on exposure to UV light

To overcome these problem chemical modifications were attempted[3] which seriously affected the insecticidal activity and also caused ecological problems.[4] Alternatively, the UV absorbing molecules, also known as photostabilzers were used in the formulations to extend the environmental life of the insecticides.[5-8]

Chlorpyrifos **1**, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate ($C_9H_{11}Cl_3NO_3PS$), is most widely used insecticide all over the world for the protection against variety of pests. It is used both for agriculture and household purposes. US alone uses almost 30 million pounds per year[9] while in Europe, more than 50,000 kg/year are used.[10] It is used for various crops such as corn, alfalfa, cotton, soyabeans, cereals, tobacco, peaches, vegetables and citrus fruits to control a wide spectrum of chewing, sucking and boring insects like aphids, caterpillars, Helicoverpa spp, mites, moths, jassids, budworm, stem borer and locusts.



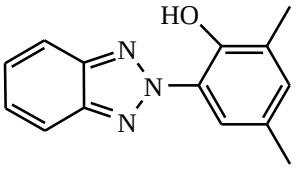

Chlorpyrifos **1**

Sulfotep **2** Chlorpyrifos-oxon **3**

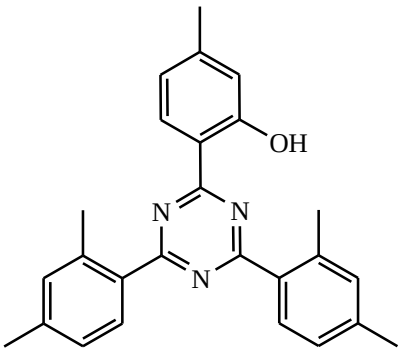
**Scheme 2.1.** Photodegradation of chlorpyrifos to chlorpyrifos-oxon and sulfotep

Chlorpyrifos is also subject to degradation on exposure to sunlight resulting in formation of various photoproducts which are more stable to UV radiation than chlorpyrifos itself. Chlorpyrifos-oxon **3** is one such photoproduct which is more persistent and about 3000 times more toxic to humans than chlorpyrifos (Scheme 2.1).[11-13] On exposure to UV light, sulfotep **2** is also formed from chlorpyrifos (Scheme 2.1) which is highly toxic and often exists as an impurity in chlorpyrifos.[14]

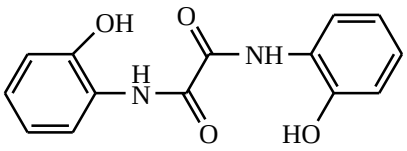
In order to extend environmental life of chlorpyrifos and consequently to minimize the formation of toxic, UV stable photoproducts, use of effective and efficient photostabilizers is indispensable. The photostabilizers absorb UV radiation and dissipate the absorbed energy harmlessly and also persist in the matrix for the expected lifetime. Photostabilization of the insecticide can take place either by preferential absorption of light by photostabilizer, thereby preventing photo-excitation of the insecticide molecules or transfer of excess energy from the excited insecticide molecules to the photostabilizers through various energy transfer mechanisms such as excited-state intramolecular proton transfer (ESIPT) or keto-enol tautomerism.[15, 16]



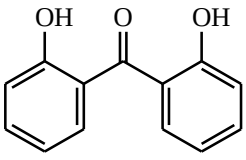

Hydroxyphenyl-benzotriazole **4**




Hydroxyphenyl-s-triazine **5**



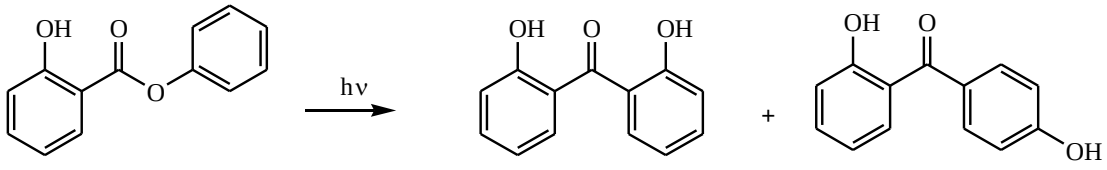

Oxanilide **6**




2,2'-Dihydroxy benzophenone **7**

**Figure 2.3.** Intramolecularly H-bonded photostabilizers

Intramolecularly H-bonded photostabilizers such as hydroxyphenyl-benzotriazoles **4**, hydroxyphenyl-s-triazines **5**, oxanilides **6** and 2-hydroxybenzophenones **7** are widely used for the protection of various products against photodegradation (Figure 2.3).[17-19]

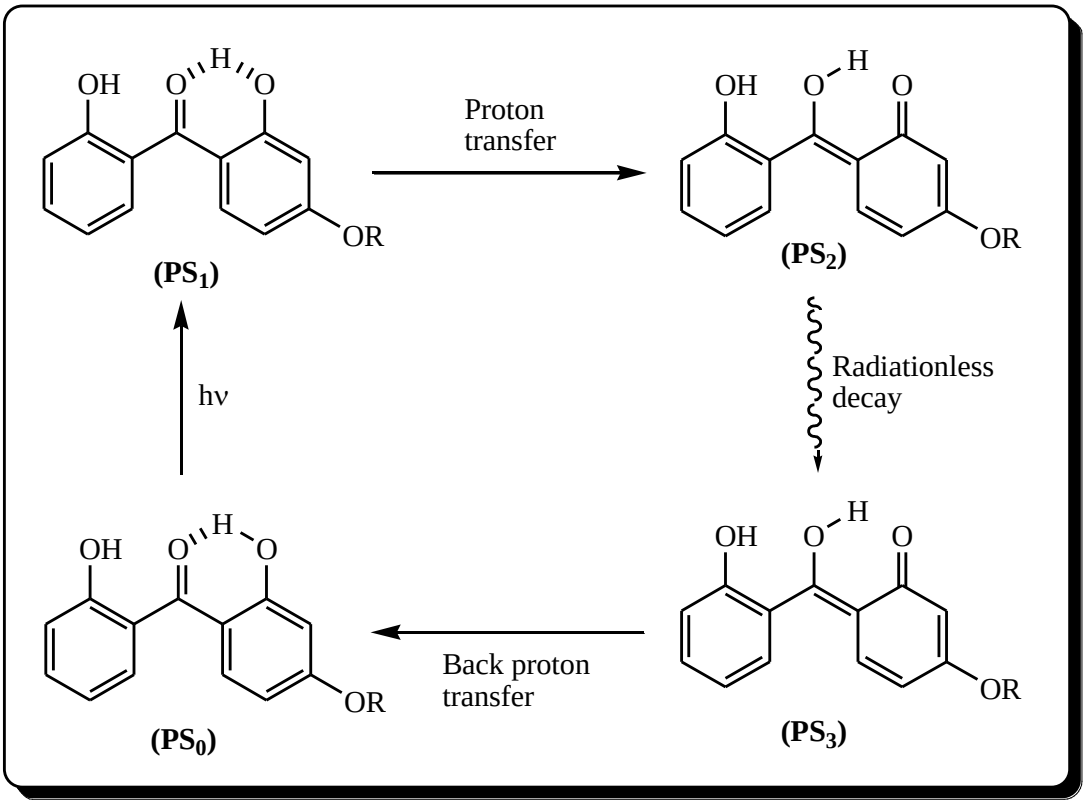



Phenyl Salicylate **8** | 2,2'-Dihydroxy benzophenone **9** | 2,4'-Dihydroxy benzophenone **10**

**Scheme 2.2.** Photo-Fries type rearrangement of phenyl salicylate under UV light

Earlier our group has studied the effect of photostabilization of azadirachtin-A on exposure to UV light in the presence of four structurally different photostabilizers, namely 4-aminobenzoic acid, 2,4-dihydroxybenzophenone, 4,4'-dihydroxybenzophenone and phenyl salicylate.[20, 21] It was found that among all the photostabilizers studied, only phenyl salicylate provided the best photostabilization. It was proposed that on exposure to UV light, phenyl salicylate **8** does not dissipate absorbed energy by direct absorption of UV light but instead undergoes Photo-Fries type rearrangement to form strongly absorbing 2,2'- and 2,4'-dihydroxy benzophenones (Scheme 2.2). These two molecules then dissipate absorbed energy through ESIPT.

2-Hydroxybenzophenones are known to act as photostabilizer via ESIPT involving dissipation of absorbed light energy through nonphotochemical pathways.[22-26] These compounds possess an efficient radiationless mechanism (keto-enol tautomerism) of energy dissipation. The molecule in the first excited state $\mathbf{PS_1}$ undergoes ESIPT to generate another species in its first excited singlet state $\mathbf{PS_2}$. This excited proton-transferred species loses its energy by a nonradiative decay process to form $\mathbf{PS_3}$. It should be noted that the contribution of this energy towards thermal degradation of the compound is negligible compared to the much stronger thermal energy reaching the compound from the solar radiation.[22] The original form of the



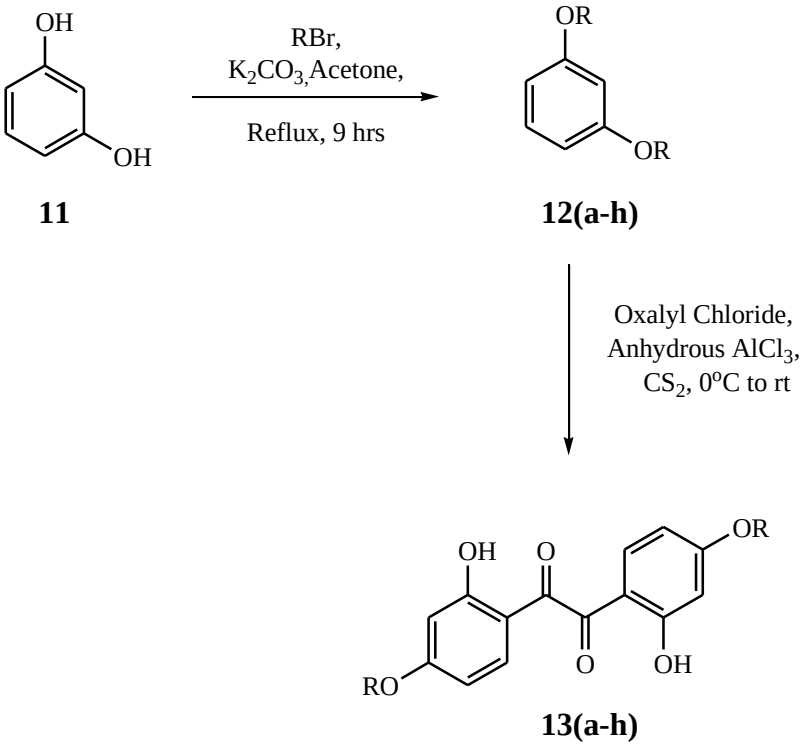

**Scheme 2.3.** Excited State Intramolecular Proton Transfer (ESIPT) of o-hydroxy benzophenones

photostabilizer $\mathbf{PS_0}$ is regenerated by a reverse proton transfer mechanism.[23-26] (Scheme 2.3) In 2-hydroxybenzophenone, it is the proximity of hydroxyl and keto groups in the molecule which is thought to be responsible for such photostabilization.

In continuation with our research directed towards design and synthesis of novel photostabilizers, we conceived benzils having structures of the type 3 (Scheme 2.4) with two such hydroxy and keto pairs assembled into a single structure. It was envisioned that these benzils would possess enhanced efficiency and usefulness as photostabilizers due to the inherent structural features.

## 2.3 Results and discussion

To ascertain our contemplation, novel 2,2'-dihydroxy-4,4'-dialkoxy benzils **13(a-h)** were synthesized from various 1,4-dialkoxy benzenes **12(a-h)** (Scheme 2.4) by an intermolecular Friedel-Crafts acylation with oxalyl chloride, using $CS_2$ as solvent.[27]




**a**: R= $CH_3$; **b**: R= $C_2H_5$; **c**: R= n-$C_3H_7$; **d**: n-$C_4H_9$; **e**: R= n-$C_6H_{13}$; **f**: R= n-$C_8H_{17}$;

**g**: R= n-$C_{10}H_{21}$; **h**: R= n-$C_{12}H_{25}$

**Scheme 2.4.** Synthesis of novel benzil derivatives

The structures of compounds **13(a-h)** were confirmed by FTIR, $^1$H NMR, $^{13}$C NMR, mass and elemental analysis. The FTIR spectrum of **13g** showed bands at 1471, 1600 $cm^{-1}$ for aromatic ring and a strong band at 1633 $cm^{-1}$ for the carbonyl group. The $^1$H NMR spectrum of **13g** displayed a singlet at δ 0.89 for two methyl protons and multiplets at δ 1.34, 1.45, 1.80 for 32 methylene protons of alkyl chain. The triplet at δ 4.0 showed the presence of 4 methylene protons attached to oxygen and a singlet at δ 11.86 for two protons of hydroxyl group. The $^{13}$C NMR spectrum of **13g** exhibited signal at δ 14.14 for carbon of methyl group, signals at δ 22.69, 25.89,

28.85, 29.30, 29.31, 29.54, 31.89, 68.77 for methylene carbon of alkyl chain, signals at δ 101.58, 109.35, 110.79, 134.00, 166.76, 167.38 for aromatic carbons along with signal at δ 194.45 for carbonyl carbon. The structure of **13g** was further confirmed by its mass spectrum which gave a molecular ion peak at 554. The elemental analysis was in good agreement with the required for $C_{34}H_{50}O_6$ and it was found: C, 73.4; H, 9.2 and calculated: C, 73.6; H, 9.1.

The FTIR spectrum of **13h** showed bands at 1471, 1581 $cm^{-1}$ for aromatic ring and a strong band at 1635 $cm^{-1}$ for the carbonyl group. The $^{1}H$ NMR spectrum of **13h** displayed a singlet at δ 0.80 for two methyl protons and multiplets at δ 1.21, 1.35, 1.71 for 40 methylene protons of alkyl chain. The triplet at δ 3.9 showed the presence of 4 methylene protons attached to oxygen and a singlet at δ 11.77 for two protons of hydroxyl group. The $^{13}C$ NMR spectrum of **13h** exhibited signal at δ 14.13 for carbon of methyl group, signals at δ 22.69, 25.89, 28.85, 29.29, 29.35, 29.52, 29.57, 29.63, 29.64, 31.92, 68.77 for methylene carbon of alkyl chain, signals at δ 101.58, 109.34, 110.80, 134.00, 166.76, 167.38 for aromatic carbons along with signal at δ 194.45 for carbonyl carbon. The structure of **13h** was further confirmed by its mass spectrum which gave a molecular ion peak at 610. The elemental analysis was in good agreement with the required for $C_{38}H_{58}O_6$ and it was found: C, 74.6; H, 9.7 and calculated: C, 74.7; H, 9.6.

The novel benzils **13(a-h)** are then subjected to photostabilization study. The photostability of chlorpyrifos in the presence of benzils **13(a-h)** was compared with that using the reference photostabilizer *i.e.* 2,4-dihydroxybenzophenone. The standard solutions of pure chlorpyrifos with (1:1) and without (1:0) benzil photostabilizers were irradiated in a Pyrex immersion–well type photochemical reactor (Figure 2.4) using a high-pressure mercury vapor lamp (MPMV, 250W, Bajaj, India). After 10h, the irradiated solutions were analyzed for their chlorpyrifos content by analytical HPLC. The percentage remaining of chlorpyrifos recovered from the solutions after 10 hours of exposure to UV radiation are shown in Table 1.

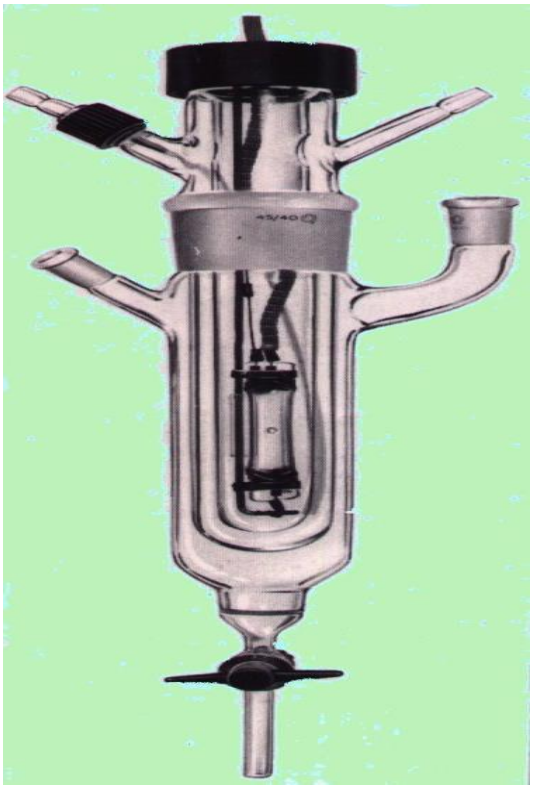


**Figure 2.4.** Immersion-well type photo reactor

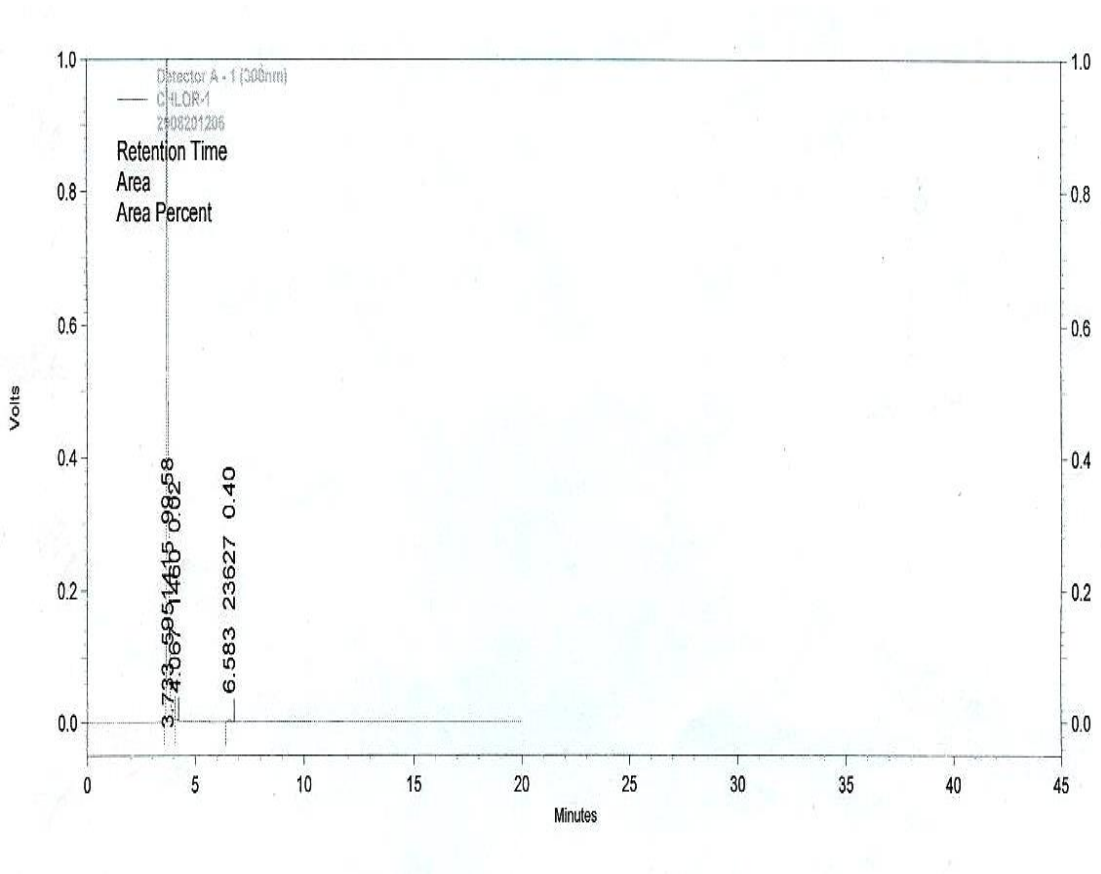



**Figure 2.5.** HPLC chromatogram of pure chlorpyrifos

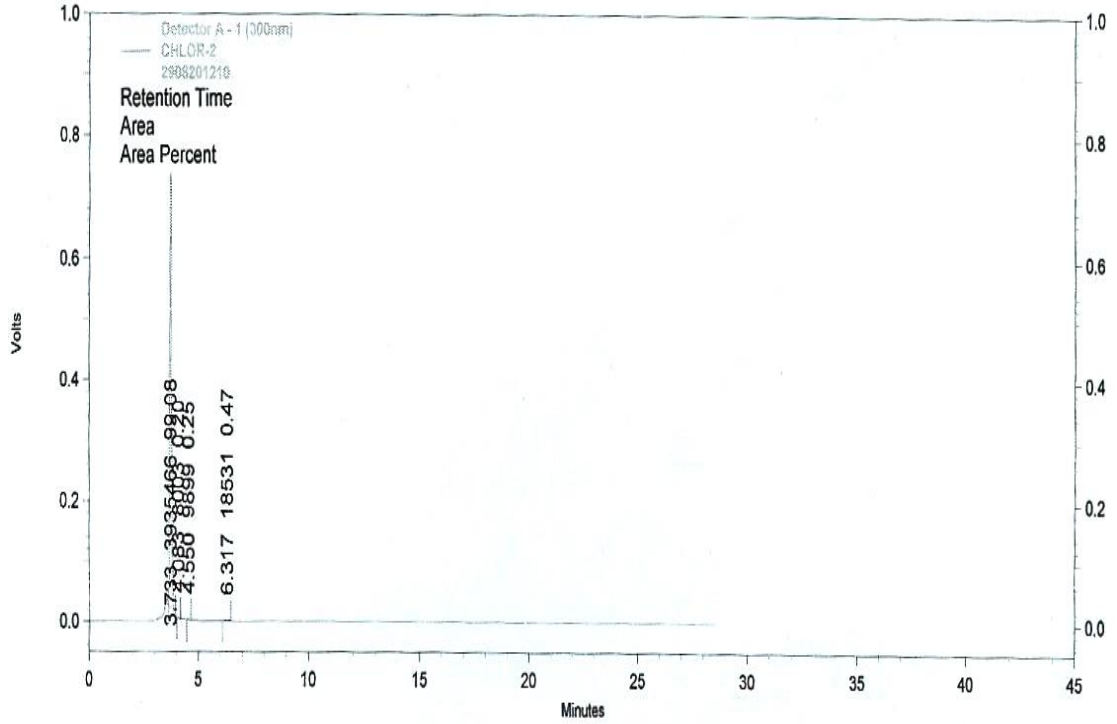



**Figure 2.6.** HPLC chromatogram of pure chlorpyrifos (no photostabilizer) after 10h irradiation under UV light

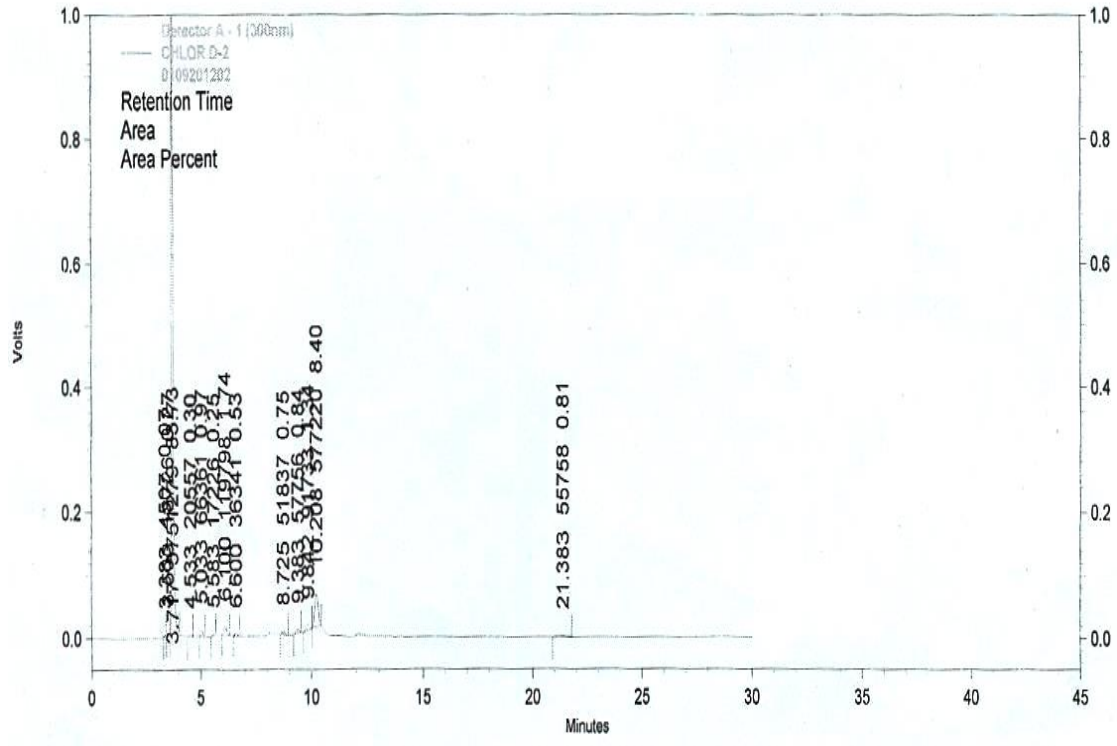



**Figure 2.7.** HPLC chromatogram of chlorpyrifos (1 chlorpyrifos : 1 photostabilizer **13h**) after 10h irradiation under UV light

Figure 2.5, 2.6 and 2.7 shows HPLC chromatogram of pure chlorpyrifos, pure chlorpyrifos (no UV absorber) and pure chlorpyrifos (1 chlorpyrifos **:** 1 UV absorber **13h**) following ten hours of exposure of UV light, respectively.

In case of pure chlorpyrifos (no photostabilizer) following ten hours of exposure to UV radiation, HPLC chromatogram showed reduction of the peak height at 3.733 ($t_R$) corresponding to pure chlorpyrifos and appearance of a number of unidentified peaks (Figure 2.6).

The recovery of chlorpyrifos was found to be only 66.12 % in the absence of photostabilizers when exposed to UV light while that in the presence of known photostabilizer, 2,4-dihydroxybenzophenone was 78.80 %. Thus the photostabilization of chlorpyrifos induced by 2,4-dihydroxybenzophenone was found to be upto 12.68 %. We further found that benzil derivatives (**13a-h**), provided photostabilizing effect up to 30.51 % *i.e.* up to 96.63 % of chlorpyrifos was recovered after irradiation experiments as compared to UV exposure of bare chlorpyrifos. (Table 2.1)

**Table 2.1.** Percentage recovery of Chlorpyrifos in presence and absence of photostabilizer on exposure to UV radiation

(Chlorpyrifos: UV absorber, 1:1 mole ratio)

| **Sr. No.** | **Samples** | **% recovery** |
|---|---|---|
| 1 | Chlorpyrifos (no stabilizer ) | 66.12 |
| 2 | 2,4-dihydroxy benzophenone $C_{13}H_{10}O_3$ | 78.80 |
| 3 | $C_{16}H_{14}O_6$ **(13a)** | 89.83 |
| 4 | $C_{18}H_{18}O_6$ **(13b)** | 89.00 |
| 5 | $C_{20}H_{22}O_6$ **(13c)** | 95.53 |
| 6 | $C_{22}H_{26}O_6$ **(13d)** | 94.42 |
| 7 | $C_{26}H_{34}O_6$ **(13e)** | 96.44 |
| 8 | $C_{30}H_{42}O_6$ **(13f)** | 96.62 |
| 9 | $C_{34}H_{50}O_6$ **(13g)** | 89.90 |
| 10 | $C_{38}H_{58}O_6$ **(13h)** | 96.63 |

**Absorption spectra of chlorpyrifos and photostabilizers**

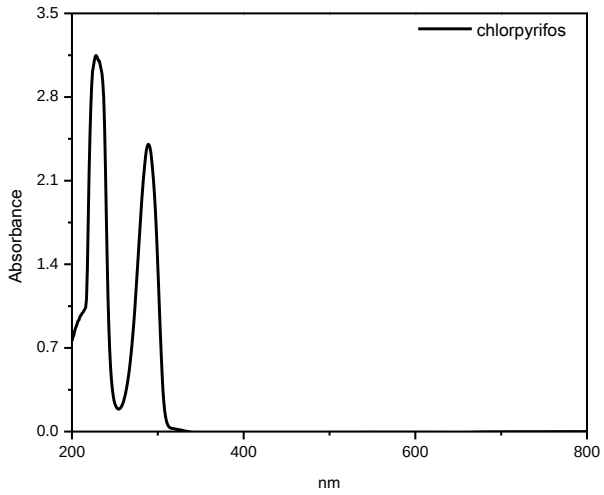



**Figure 2.8.** UV spectrum of chlorpyrifos

Figure 2.8 and 2.9 show the absorption spectra of pure chlorpyrifos, 2,4-dihydroxybenzophenone and benzil (**13a**) photostabilizers in methanol. The UV spectra of pure chlorpyrifos and benzils show that both of them absorb strongly near 289 nm and 287 nm respectively. Effective photostabilization of chlorpyrifos by benzil photostabilizers appears to be due to competitive energy absorption of UV photons which cause degradation of chlorpyrifos.

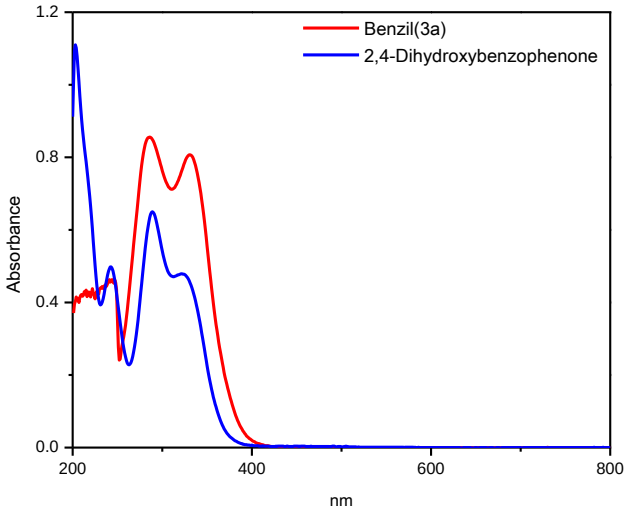



**Figure 2.9.** UV spectra of benzil and 2,4-dihydroxybenzophenone

The UV spectra of 2,4-dihydroxybenzophenone and benzil photostabilizers are almost identical and absorb strongly near 287 nm (Figure 2.9). Less recovery of chlorpyrifos was observed in case of 2,4-dihydroxybenzophenone which has one hydroxy-keto pair that can photostabilize chlorpyrifos through ESIPT. However in case of benzil derivatives there are two such hydroxy-keto pairs which are perhaps responsible for more efficient photostabilization of chlorpyrifos. The use of such photostabilizers in the formulations of expensive pesticides might reduce use of their excessive quantities during actual field application on larger scale thereby adding attractive economical and environmental benefits.

## 2.4 Experimental

### *Chemicals*

Chlorpyrifos (99 %) was gifted by the United Phosphorus Limited (India) while the rest of the chemicals and HPLC solvents were purchased from Glaxo (Qualigens) India Ltd.

### *HPLC Instrumentation*

The HPLC used in the study was Shimadzu LC 20AT equipped with a variable wavelength detector (SPD 20A), flow controller and Class-VP software. The instrument employed dual solvent system and dual pump heads with common drive which gave sable and reproducible flows. The Class-VP provided the chromatogram, percent area and retention time ($t_R$) for each peak.

### *Standard Solutions*

Standard solutions of pure chlorpyrifos (0.5 mg/mL) were prepared along with photostabilizers in the mole ratio of 1:1 (Chlorpyrifos : Photostabilizer) and 1:0 (no

photostabilizer) in dry methanol. The solutions were stored in amber colored bottles between 0-4$^{o}$C and the chlorpyrifos content was determined by analytical HPLC.

***Irradiation Experiments***

The standard solutions of pure chlorpyrifos prepared as above with and without photostabilizers in methanol (20 ml) were placed in a Pyrex immersion–well type photochemical reactor and irradiated separately using a high-pressure mercury vapor lamp (HPMV, 250 W, Bajaj India) at a distance of 3.8 cm from the source. The solutions were withdrawn after irradiation for 10h and analyzed for the chlorpyrifos content by analytical HPLC. Control samples were irradiated and analyzed similarly.

***General***

UV spectra were recorded on a Shimadzu UV-2450 UV/Visible Spectrophotometer. FTIR spectra were recorded on a Shimadzu 8400S FTIR spectrometer using KBr. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker-400MHz NMR spectrometer (100 MHz for $^{13}$C NMR) using $CDCl_3$ or DMSO-$d_6$ (TMS as an internal standard). Mass spectra were obtained on a Shimadzu QP-5050 mass spectrometer. Column chromatography was carried out on Acme's silica gel (60-120 mesh size) and eluted using mixtures of light petroleum and ethyl acetate. Thin layer chromatography was performed using Acme's silica gel for TLC and spots were visualized in the iodine vapor. Percentage yields were reported based upon the recovery of starting materials. The structures of all the compounds were confirmed by their mp, elemental analysis, FTIR, $^{1}$H NMR, $^{13}$C NMR and mass spectrometric data.

Identification and quantitative analysis of chlorpyrifos in the sample solution was done using methanol as mobile phase by high performance liquid chromatography (HPLC) equipped with a SPD 20A variable wavelength UV-Vis detector. The HPLC column was fitted with a 4.6 mm ID, 250 mm length, Hypersil ODS and 5 micron particle size.

***General procedure for the synthesis of Dialkoxy benzenes:***

A mixture of 45 mmol of 1,3-dihydroxy benzene **1**, 108 mmol of appropriate alkyl bromide and 135 mmol of powdered potassium carbonate was stirred in dry acetone (50ml) for 9h under reflux. After completion of the reaction (TLC), the reaction mixture was allowed to cool to room temperature, filtered through a celite pad and acetone was distilled off. Water was added to the residue and the aqueous layer was extracted with ethyl acetate (4x25 ml) and the combined organic layers were washed with water (2x20 ml), brine (2x20 ml), dried over anhydrous sodium sulphate. The solvent was removed on rotavapor and the resulting residue was chromatographed over silica gel using a mixture of light petroleum and ethyl acetate as eluents.

***1,3-Diethoxybenzene (12b)***

Colourless liquid, $\lambda_{max}$ (EtOAc)/nm: 270; IR ($\nu_{max}$, $cm^{-1}$): 2980 (CH), 1047 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 1.44 (t, 6H, *J* = 7.2 Hz, $CH_3$), 4.04 (q, 4H, $CH_2$), 6.51(m, 3H, Ar), 7.19 (t, 1H, *J* = 8.4 Hz, Ar).

***1,3-Di-n-propoxybenzene (12c)***

Colourless liquid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2964 (CH), 1012 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 1.07 (t, 6H, *J* = 7.2 Hz, $CH_3$), 1.84 (m, 4H, $CH_2$), 3.93 (t, 4H, *J* = 6.8 Hz, O-$CH_2$), 6.52 (m, 3H, Ar), 7.19 (t, 1H, *J* = 8.4 Hz, Ar).

***1,3-Di-n-butoxybenzene (12d)***

Colourless liquid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2968 (CH), 1031 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 1.04 (t, 6H, *J* = 7.6 Hz, $CH_3$) , 1.55 (m, 4H, $CH_2$), 1.82 (m, 4H, $CH_2$), 4.0 (t, 4H, *J* = 6.8 Hz, O-$CH_2$), 6.55 (m, 3H, Ar), 7.20 (t, 1H, *J* = 8.4 Hz, Ar).

***1,3-Bis-n-(hexyloxy)benzene (12e)***

Colourless liquid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2965 (CH), 1045 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 0.96 (t, 6H, *J* = 7.2 Hz, $CH_3$) , 1.36 (m, 8H, $CH_2$), 1.50 (m, 4H, $CH_2$), 1.81 (m, 4H, $CH_2$), 3.96 (t, 4H, *J* = 6.8 Hz, O-$CH_2$), 6.52 (m, 3H, Ar), 7.20 (t, 1H, *J* = 8.0 Hz, Ar).

***1,3-Bis-n-(octyloxy)benzene (12f)***

White crystalline solid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2953 (CH), 1043 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 0.99 (t, 6H, J = 7.2 Hz, $CH_3$), 1.35 (m, 16H, $CH_2$), 1.45 (m, 4H, $CH_2$), 1.78 (m, 4H, $CH_2$), 3.94 (t, 4H, J = 6.4 Hz, O-$CH_2$), 6.49 (m, 3H, Ar), 7.20 (t, 1H, J = 8.0 Hz, Ar); $^{13}C$ NMR (100MHz, $CDCl_3$): $\delta_C$ 14.14 ($CH_3$), 22.69, 26.08, 29.27, 29.28, 29.39, 31.84 ($CH_2$), 67.97 (O-$CH_2$), 101.36, 106.59, 129.75 (Ar), 160.37 (C-OR).

***1,3-Bis-n-(decyloxy)benzene (12g)***

White crystalline solid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2961 (CH), 1022 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 0.90 (t, 6H, *J* = 7.2 Hz, $CH_3$), 1.35 (m, 24H, $CH_2$), 1.45 (m, 4H, $CH_2$), 1.75 (m, 4H, $CH_2$), 3.94 (t, 4H, *J* = 6.8 Hz, O-$CH_2$), 6.49 (m, 3H, Ar), 7.17 (t, 1H, *J* = 8.4 Hz, Ar).

***1,3-Bis-n-(dodecyloxy)benzene (12h)***

White crystalline solid, $\lambda_{max}$ (EtOAc)/nm: 274; IR ($\nu_{max}$, $cm^{-1}$): 2955 (CH), 1045 (C-O); $^1H$ NMR (400MHz, $CDCl_3$): $\delta_H$ 0.90 (t, 6H, *J* = 7.2 Hz, $CH_3$), 1.29 (m, 32H, $CH_2$), 1.46 (m, 4H, $CH_2$), 1.78 (m, 4H, $CH_2$), 3.94 (t, 4H, *J* = 6.8 Hz, O-$CH_2$), 6.49 (m, 3H, Ar), 7.17 (t, 1H, *J* = 8.4 Hz, Ar); $^{13}C$ NMR (100MHz, $CDCl_3$): $\delta_C$ 14.17 ($CH_3$), 22.74, 26.09, 29.31, 29.40, 29.45, 29.63, 29.65, 29.69, 29.71, 31.97 ($CH_2$), 67.95 (O-$CH_2$), 101.35, 106.58, 129.75 (Ar), 160.37 (C-OR).

***General procedure for the synthesis of Dialkoxy Benzils:***

To a mechanically stirred suspension of 7.2 mmol of dialkoxy benzene and 7.9 mmol of anhydrous aluminium chloride in carbon disulfide (100 ml) at $0^oC$ was added a solution of 4.7 mmol of oxalyl chloride in carbon disulfide (50 ml) over a period of 4 h under a constant stream of nitrogen. Stirring was continued for 18 h after which the resulting mixture was poured onto ice and carbon disulfide was distilled off. The aqueous mixture was extracted with ethyl acetate (4x25 ml) and the combined organic layers were washed with water (2x20 ml), brine (2x20 ml), dried over anhydrous sodium sulphate. The solvent was removed on rotavapor and the resulting residue was chromatographed over silica gel using a mixture of light petroleum and ethyl acetate as eluents.

***1,2-Bis(2-hydroxy-4-methoxyphenyl)ethane-1,2-dione (13a)***

White crystalline solid; mp 150°C; $\lambda_{max}$ (EtOAc)/nm: 284; IR ($\nu_{max}$, $cm^{-1}$): 1024 (C-O), 1629 (C=O), 3076 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 3.89 (s, 6H, O-$CH_3$), 6.45 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.52 (d, 2H, $J$ = 2.4 Hz, Ar), 7.42 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100MHz, $CDCl_3$): $\delta_C$ 55.86 (O-$CH_3$), 101.17, 109.03, 110.95, 134.04, 166.80 (C-OH), 167.76 (C-OR), 194.47 (Keto carbon); MS: m/z 301.13($M^+$). Anal. Calc. for $C_{16}H_{14}O_6$: C, 63.6; H, 4.7. Found: C, 63.4; H, 4.8.

***1,2-Bis(4-ethoxy-2-hydroxyphenyl)ethane-1,2-dione (13b)***

White crystalline solid; mp 130°C; $\lambda_{max}$ (EtOAc)/nm: 287; IR ($\nu_{max}$, $cm^{-1}$): 1043 (C-O), 1633 (C=O), 2983 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 1.43 (t, 6H, $J$ = 7.2 Hz, $CH_3$), 4.14 (q, 4H, O-$CH_2$), 6.42 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.4 Hz, Ar), 7.42 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, -OH); $^{13}$C NMR (100MHz, $CDCl_3$): $\delta_C$ 14.49 ($CH_3$), 64.33 (O-$CH_2$), 101.55, 109.33, 110.81, 134.04, 166.76 (C-OH), 167.18 (C-OR), 194.45 (Keto carbon); MS: m/z 330.22 ($M^+$). Anal. Calc. for $C_{18}H_{18}O_6$: C, 65.4; H, 5.5. Found: C, 65.3; H, 5.5.

***1,2-Bis(2-hydroxy-4-n-propoxyphenyl)ethane-1,2-dione (13c)***

White crystalline solid, mp 117°C; $\lambda_{max}$ (EtOAc)/nm: 285; IR ($\nu_{max}$, $cm^{-1}$): 1018 (C-O), 1622 (C=O), 2966 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 0.89 (t, 6H, $J$ = 6.8 Hz, $CH_3$), 1.80 (m, 4H, $CH_2$), 4.04 (t, 4H, $J$ = 6.8 Hz, O-$CH_2$), 6.44 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.4 Hz, Ar), 7.40 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100MHz, $CDCl_3$): $\delta_C$ 14.14 ($CH_3$), 22.69 ($CH_2$), 68.77 (O-$CH_2$), 101.57, 109.35, 110.79, 134.00, 166.76 (C-OH), 167.38 (C-OR), 194.45 (Keto carbon); MS: m/z 358.11 ($M^+$). Anal. Calc. for $C_{20}H_{22}O_6$: C, 67.0; H, 6.2. Found: C, 67.1; H, 6.3.

***1,2-Bis(4-n-butoxy-2-hydroxyphenyl)ethane-1,2-dione (13d)***

White crystalline solid, mp 107°C; $\lambda_{max}$ (EtOAc)/nm: 287; IR ($\nu_{max}$, $cm^{-1}$): 1058 (C-O), 1630 (C=O), 2962 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 1.49 (t, 6H, $J$ = 7.2 Hz, $CH_3$), 1.52 (m, 4H, $CH_2$), 1.80 (m, 4H, $CH_2$), 4.04 (t, 4H, $J$ = 6.8 Hz, O-$CH_2$), 6.44

(dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.0 Hz, Ar), 7.40 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100MHz, $CDCl_3$): $\delta_C$ 13.77 ($CH_3$), 19.12, 30.86 ($CH_2$), 68.45 (O-$CH_2$), 101.55, 109.37, 110.78, 134.01, 166.76(C-OH), 167.39 (C-OR), 194.46 (Keto carbon); MS: m/z 385.88 ($M^+$). Anal. Calc. for $C_{22}H_{26}O_6$: C, 68.4; H, 6.8. Found: C, 68.6; H, 6.7.

***1,2-Bis(4-n-hexyloxy-2-hydroxyphenyl)ethane-1,2-dione (13e)***

White crystalline solid, mp 94$^o$C; $\lambda_{max}$ (EtOAc)/nm: 287; IR ($\nu_{max}$, cm$^{-1}$): 1022 (C-O), 1633 (C=O), 2964 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 0.93 (t, 6H, $J$ = 6.8 Hz, $CH_3$), 1.34 (m, 8H, $CH_2$), 1.46 (m, 4H, $CH_2$), 1.80 (m, 4H, $CH_2$) 4.03 (t, 4H, $J$ = 6.8 Hz, O-$CH_2$), 6.44 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.4 Hz, Ar), 7.40 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta_C$ 14.04 ($CH_3$), 22.57, 25.57, 28.82, 31.47 ($CH_2$), 68.76 (O-$CH_2$), 101.56, 109.35, 110.78, 134.01, 166.76 (C-OH), 167.38 (C-OR), 194.45 (Keto carbon); MS: m/z 441.93 ($M^+$). Anal. Calc. for $C_{26}H_{34}O_6$: C, 70.6; H, 7.7. Found: C, 70.7; H, 7.6.

***1,2-Bis(2-hydroxy-4-n-octyloxyphenyl)ethane-1,2-dione (13f)***

White crystalline solid, mp 98$^o$C; $\lambda_{max}$ (EtOAc)/nm: 287; IR ($\nu_{max}$, cm$^{-1}$): 1030 (C-O), 1620 (C=O), 2945 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 0.91 (t, 6H, $J$ = 6.8 Hz, $CH_3$), 1.34 (m, 16H, $CH_2$), 1.46 (m, 4H, $CH_2$), 1.80 (m, 4H, $CH_2$) 4.03 (t, 4H, $J$ = 6.8 Hz, O-$CH_2$), 6.44 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.4 Hz, Ar), 7.40 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta_C$ 14.12 ($CH_3$), 22.66, 25.90, 28.85, 29.20, 29.26, 31.79 ($CH_2$), 68.77 (O-$CH_2$), 101.56, 109.36, 110.78, 134.04, 166.76 (C-OH), 167.38 (C-OR), 194.45 (Keto carbon); MS: m/z 497.84 ($M^+$). Anal. Calc. for $C_{30}H_{42}O_6$: C, 72.2; H, 8.5. Found: C, 72.3; H, 8.3.

***1,2-Bis(4-n-decyloxy-2-hydroxyphenyl)ethane-1,2-dione (13g)***

White crystalline solid, mp 105$^o$C; $\lambda_{max}$ (EtOAc)/nm: 287; IR ($\nu_{max}$, cm$^{-1}$): 1018 (C-O), 1633 (C=O), 3082 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 0.89 (t, 6H, $J$ = 6.8 Hz, $CH_3$), 1.34 (m, 24H, $CH_2$), 1.45 (m, 4H, $CH_2$), 1.80 (m, 4H, $CH_2$), 4.0 (t, 4H, $J$ = 6.8 Hz, O-$CH_2$), 6.44 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.49 (d, 2H, $J$ = 2.4 Hz, Ar), 7.40 (d, 2H, $J$ = 8.8 Hz, Ar), 11.86 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta_C$ 14.14 ($CH_3$), 22.69, 25.89, 28.85, 29.30, 29.31, 29.54, 31.89, 68.77 (O-$CH_2$), 101.58,

109.35, 110.79, 134.00, 166.76 (C-OH), 167.38 (C-OR), 194.45 (Keto carbon); MS: m/z 553.99 ($M^+$). Anal. Calc. for $C_{34}H_{50}O_6$: C, 73.6; H, 9.1. Found: C, 73.4; H, 9.2.

***1,2-Bis(4-n-dodecyloxy-2-hydroxyphenyl)ethane-1,2-dione (13h)***

White crystalline solid, mp 90°C; $\lambda_{max}$ (EtOAc)/nm: 285; IR ($\nu_{max}$, $cm^{-1}$): 1026 (C-O), 1635 (C=O), 2955 (CH); $^1$H NMR (400MHz, $CDCl_3$): $\delta_H$ 0.80 (t, *J* = 6.8 Hz, 6H, $CH_3$), 1.21 (m, 32H, $CH_2$), 1.35 (m, 4H, $CH_2$), 1.71 (m, 4H,$CH_2$), 3.9 (t, *J* = 6.8 Hz, 4H, O-$CH_2$), 6.35 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar), 6.40 (d, 2H, *J* = 2.4 Hz, Ar), 7.31 (d, 2H, *J* = 8.8 Hz, Ar), 11.77 (s, 2H, OH); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta_C$ 14.13 ($CH_3$), 22.69, 25.89, 28.85, 29.29, 29.35, 29.52, 29.57, 29.63, 29.64, 31.92, 68.77 (O-$CH_2$), 101.58, 109.34, 110.80, 134.00, 166.76 (C-OH), 167.38 (C-OR), 194.45 (Keto carbon); MS: m/z 610.63 ($M^+$). Anal. Calc. for $C_{38}H_{58}O_6$: C, 74.7; H, 9.6. Found: C, 74.6; H, 9.7.

## 2.5 Conclusion

Synthesis and application of novel benzil derivatives as photostabilizers is reported. A systematic photostabilization study of known and widely used insecticide, chlorpyrifos was carried out using novel benzils under UV light. The percentage recovery of chlorpyrifos showed a significant enhancement in photostabilization of chlorpyrifos by benzil derivatives as compared to 2,4-dihydroxybenzophenone as a reference. Thus it is possible to minimize generation of toxic impurities resulting from photochemical decomposition of chlorpyrifos in field by employing suitable photostabilizers such as benzil derivatives presented in this chapter.

# 2.6 Spectra

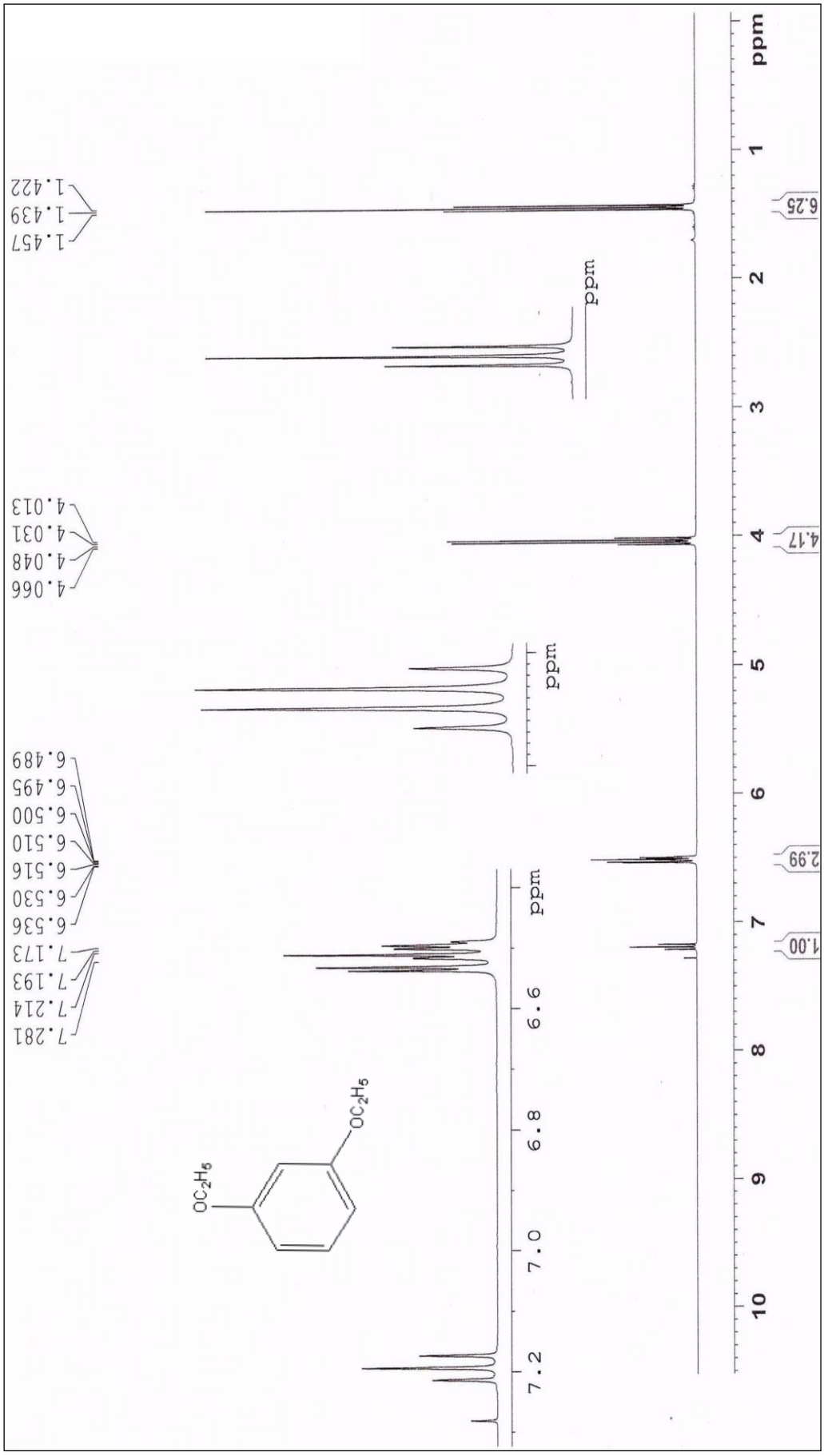



**Figure 2.10:-** $^1$H NMR of 1,3-diethoxybenzene **(12b)**

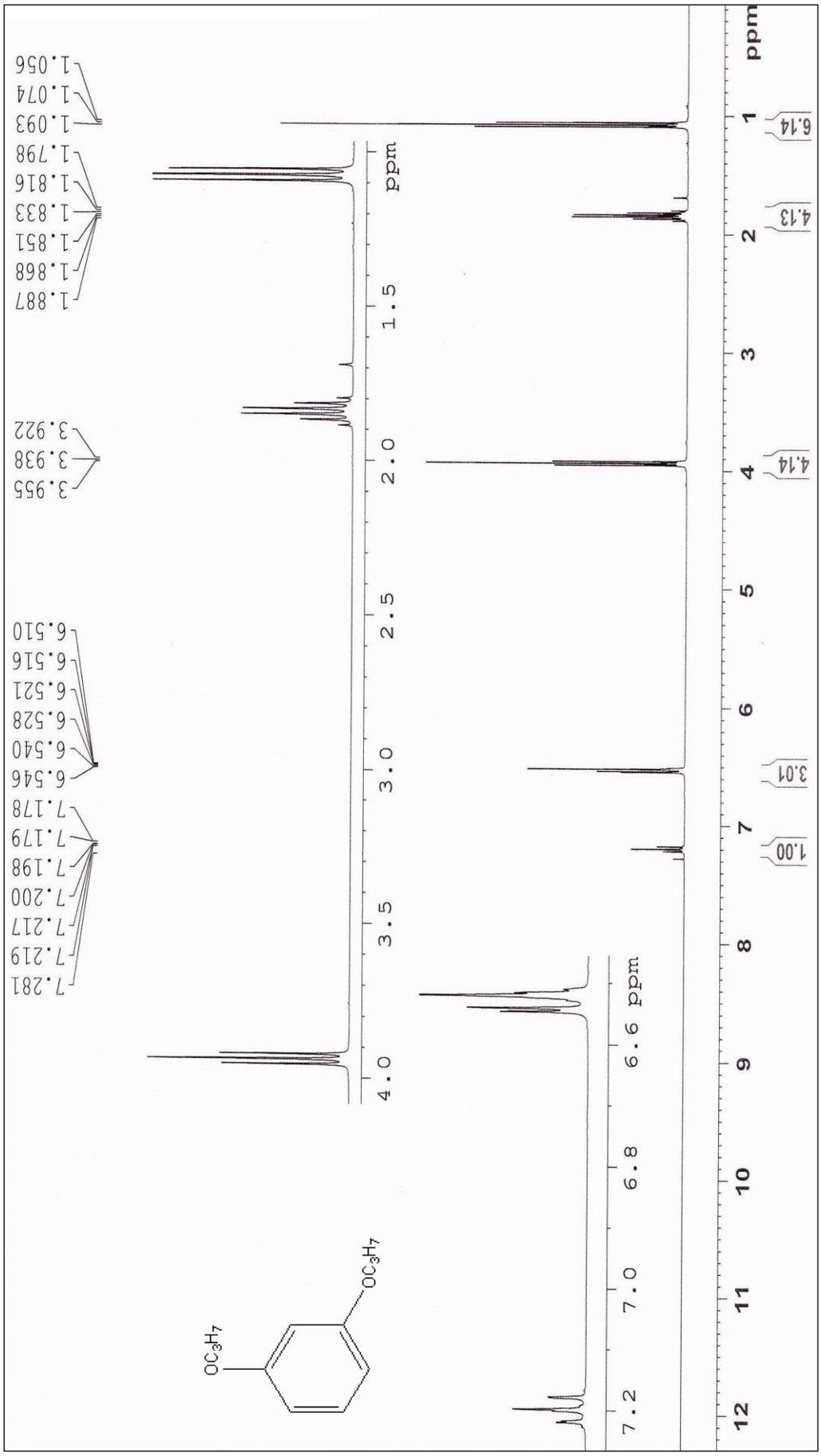



**Figure 2.11:-** $^{1}$H NMR of 1,3-di-n-propoxybenzene **(12c)**

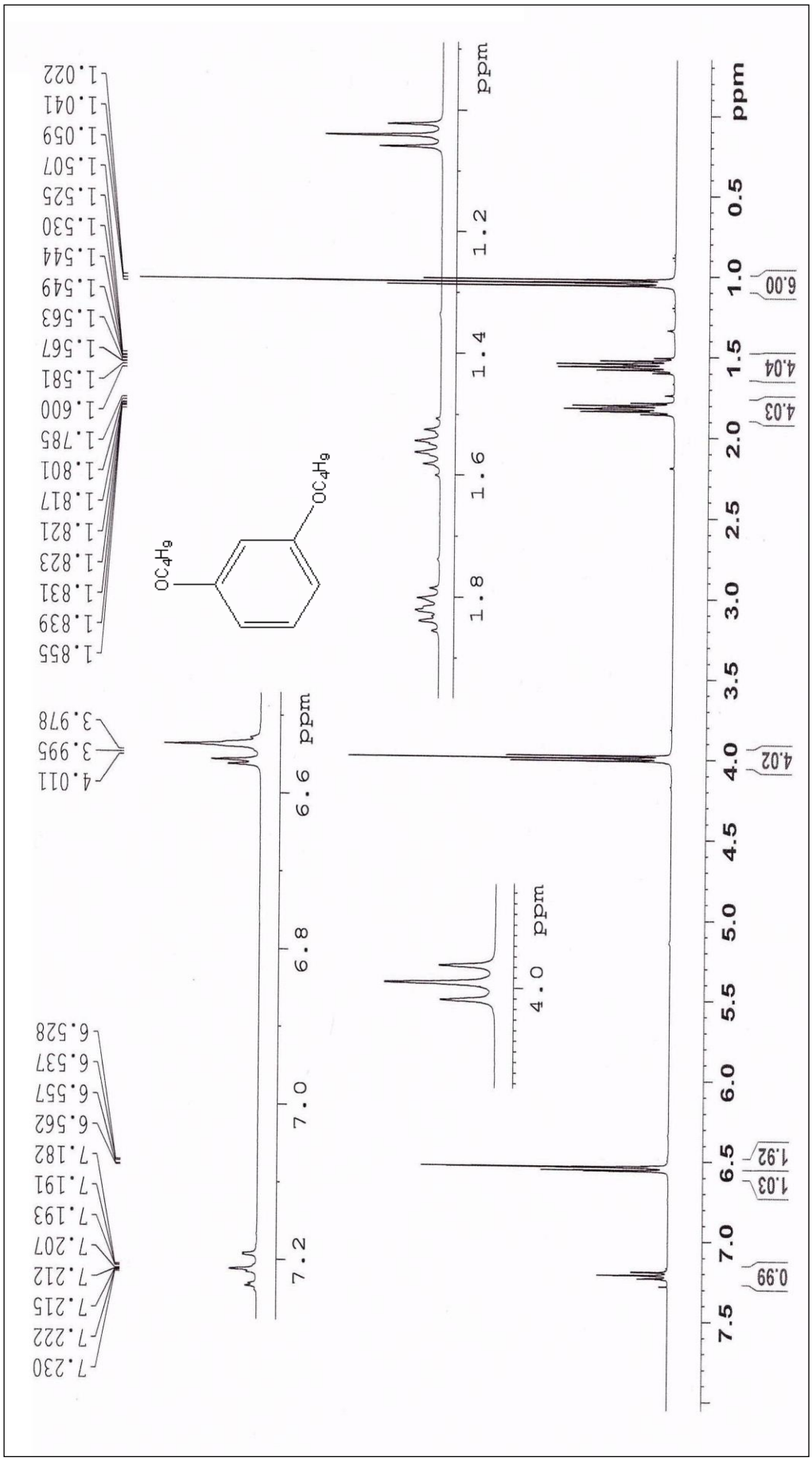



**Figure 2.12:-** $^{1}$H NMR of 1,3-di-n-butoxybenzene **(12d)**

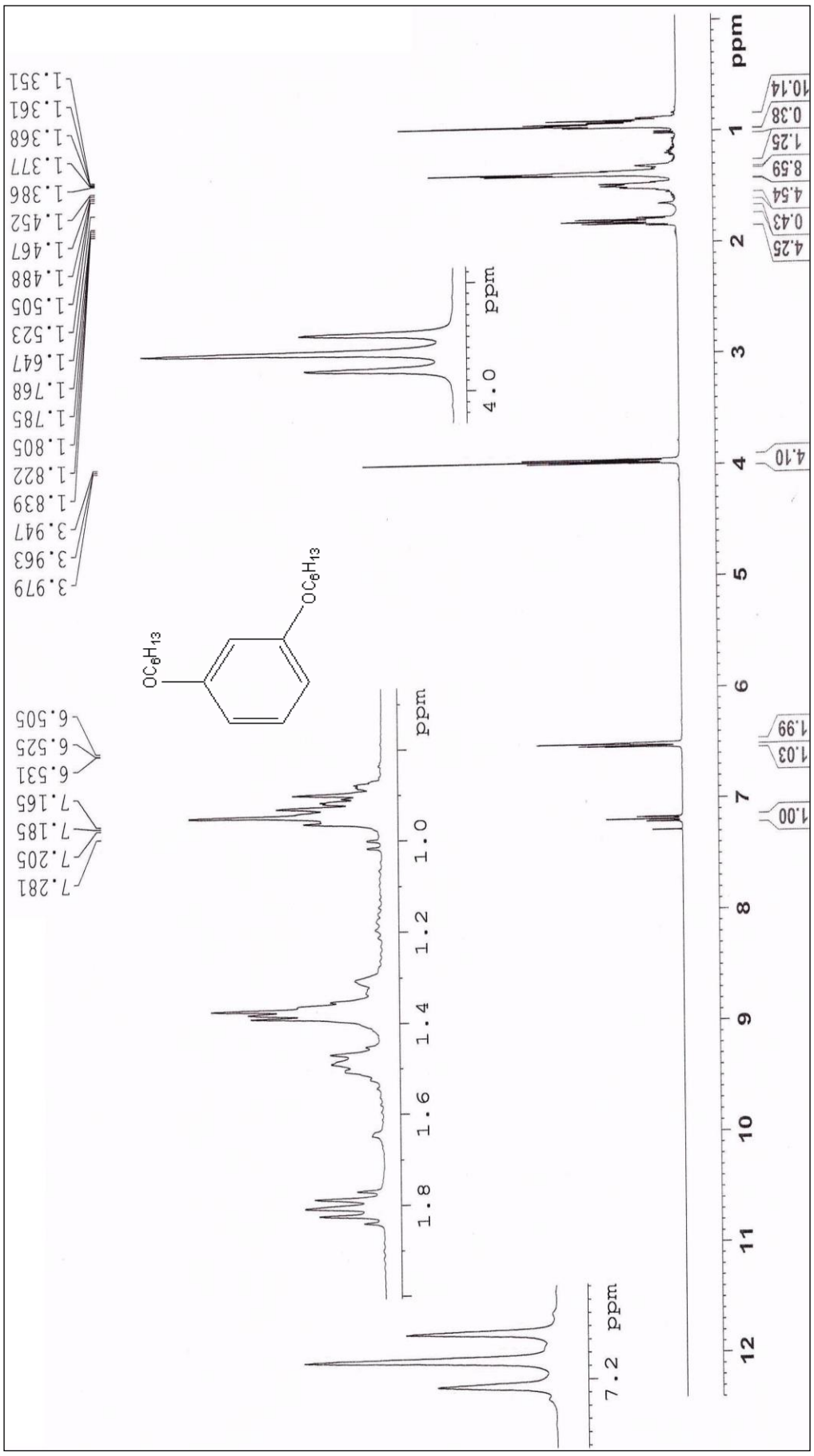



**Figure 2.13:-** $^{1}$H NMR of 1,3-bis-n-(hexyloxy)benzene **(12e)**

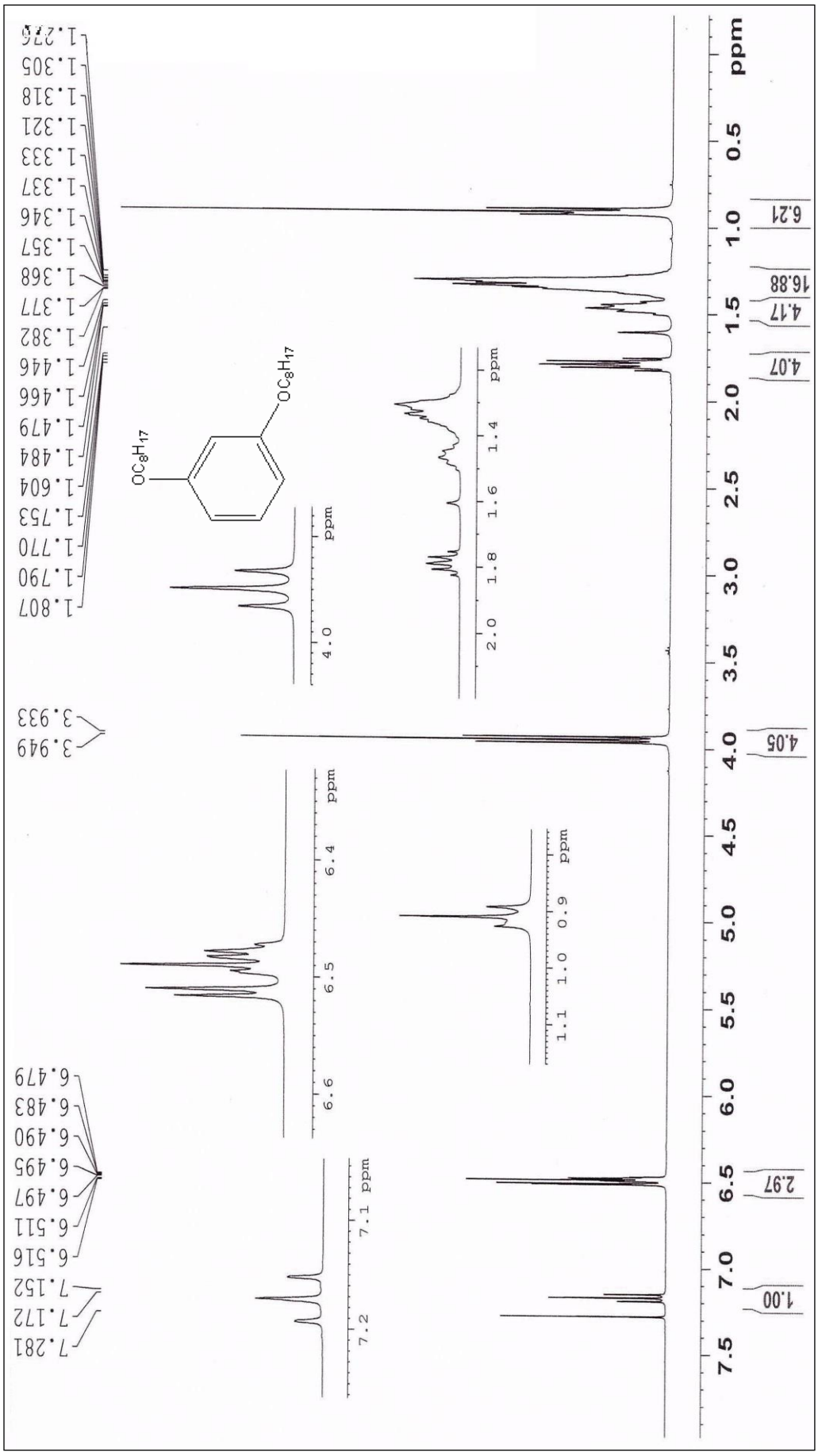



**Figure 2.14:-** $^{1}$H NMR of 1,3-bis-n-(octyloxy)benzene **(12f)**

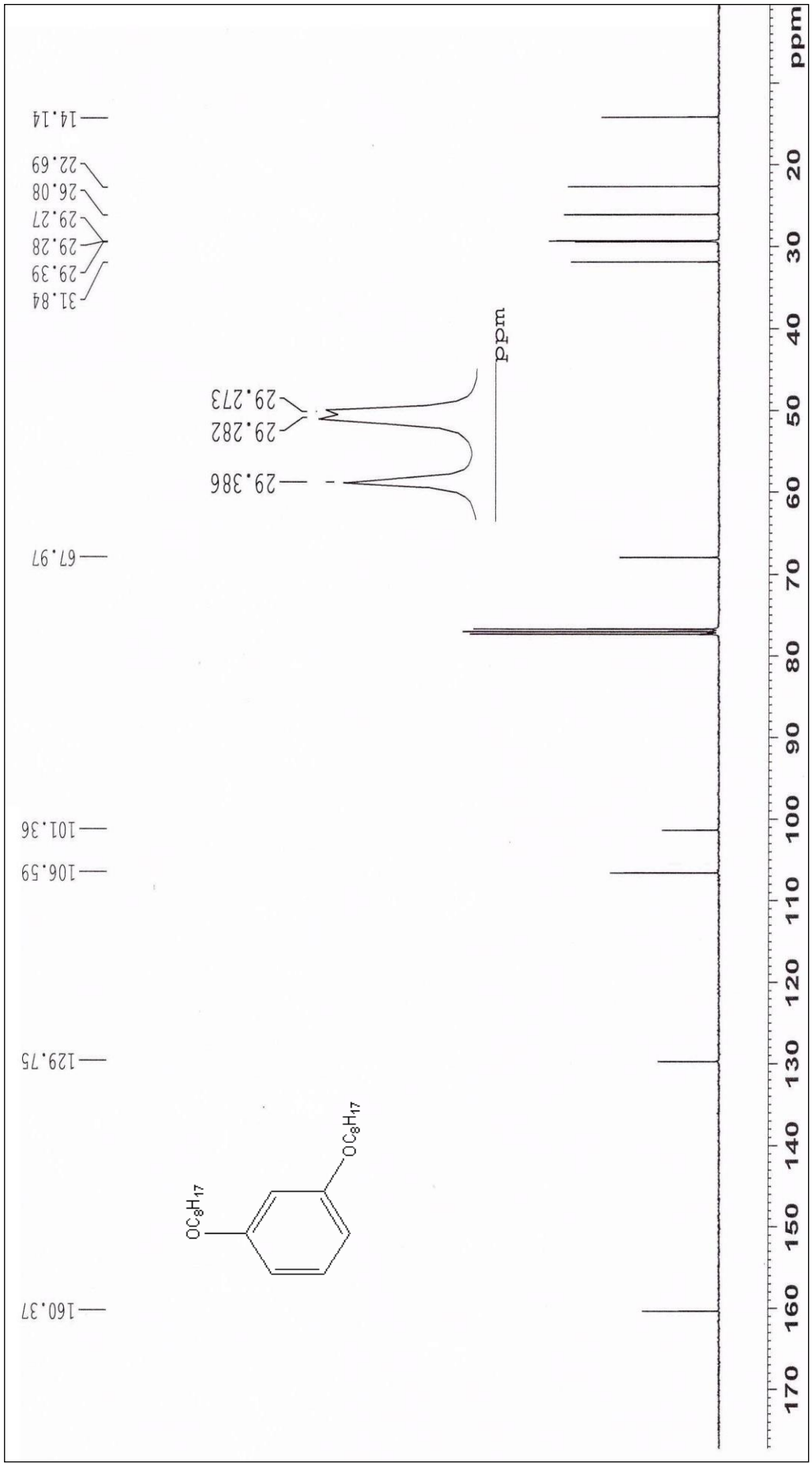



**Figure 2.15:-** $^{13}C$ NMR 1,3-bis-n-(octyloxy)benzene **(12f)**

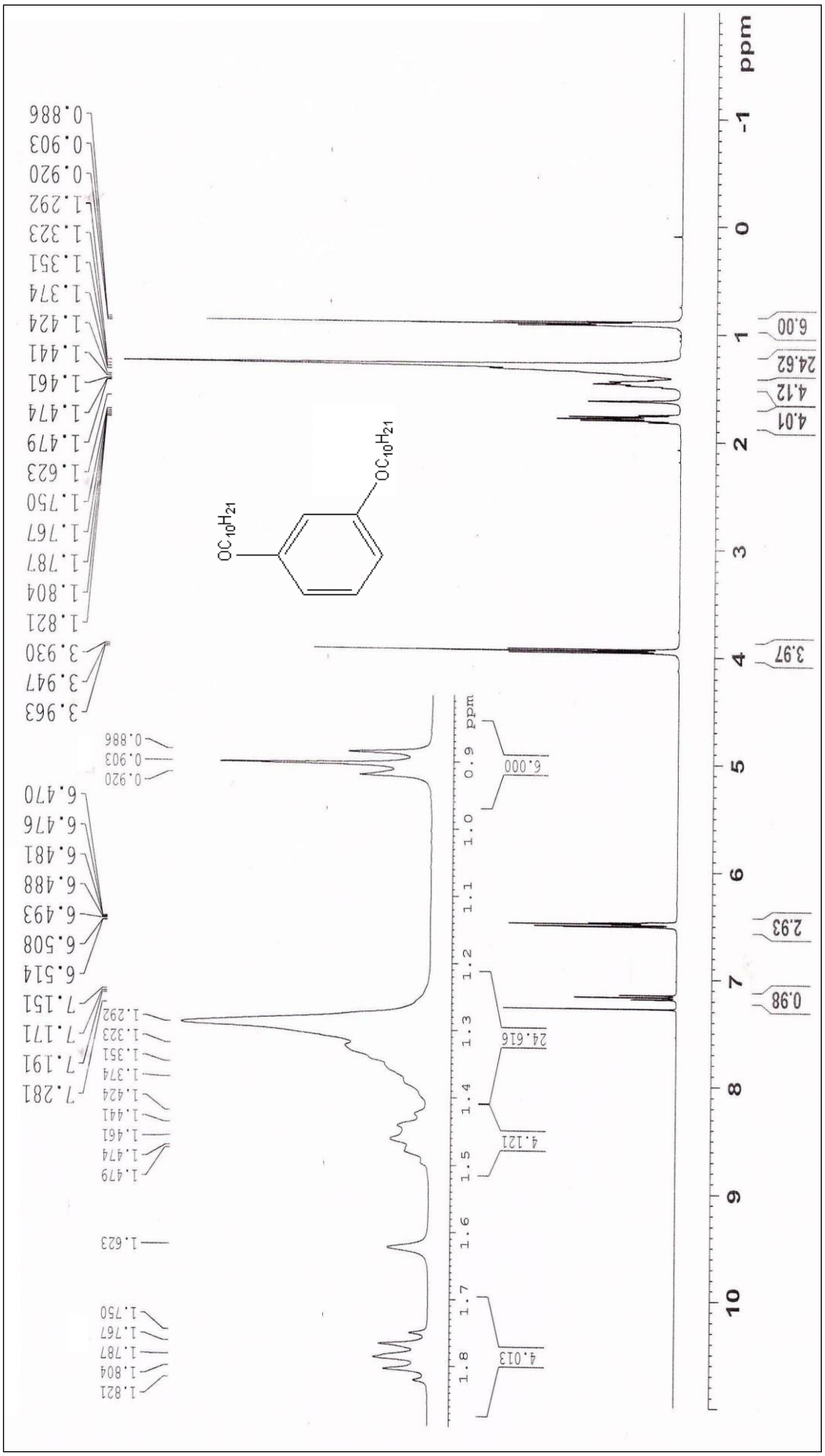



**Figure 2.16:-** $^{1}$H NMR of 1,3-bis-n-(decyloxy)benzene **(12g)**

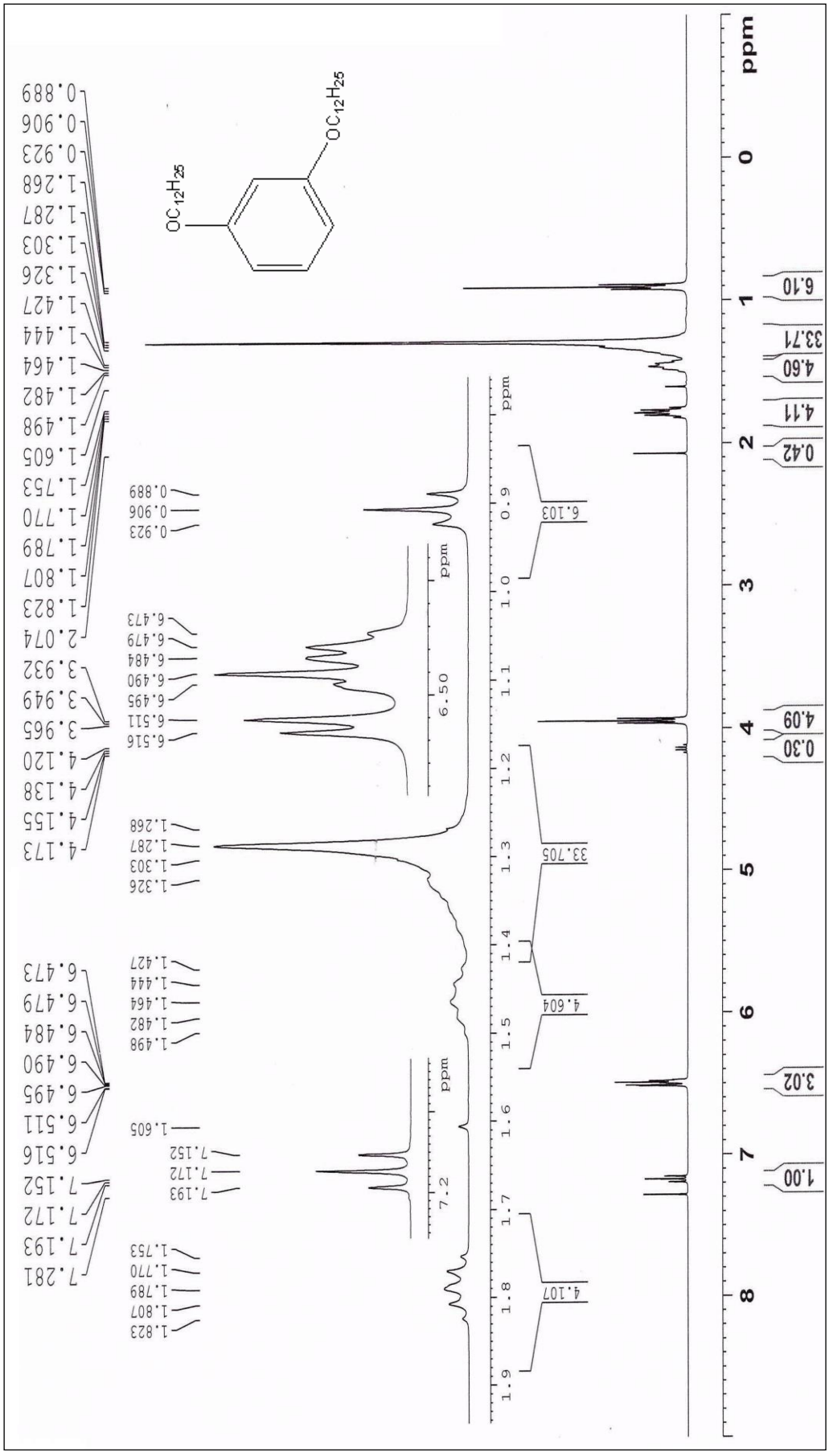



**Figure 2.17:-** $^{1}$H NMR of 1,3-bis-n-(dodecyloxy)benzene **(12h)**

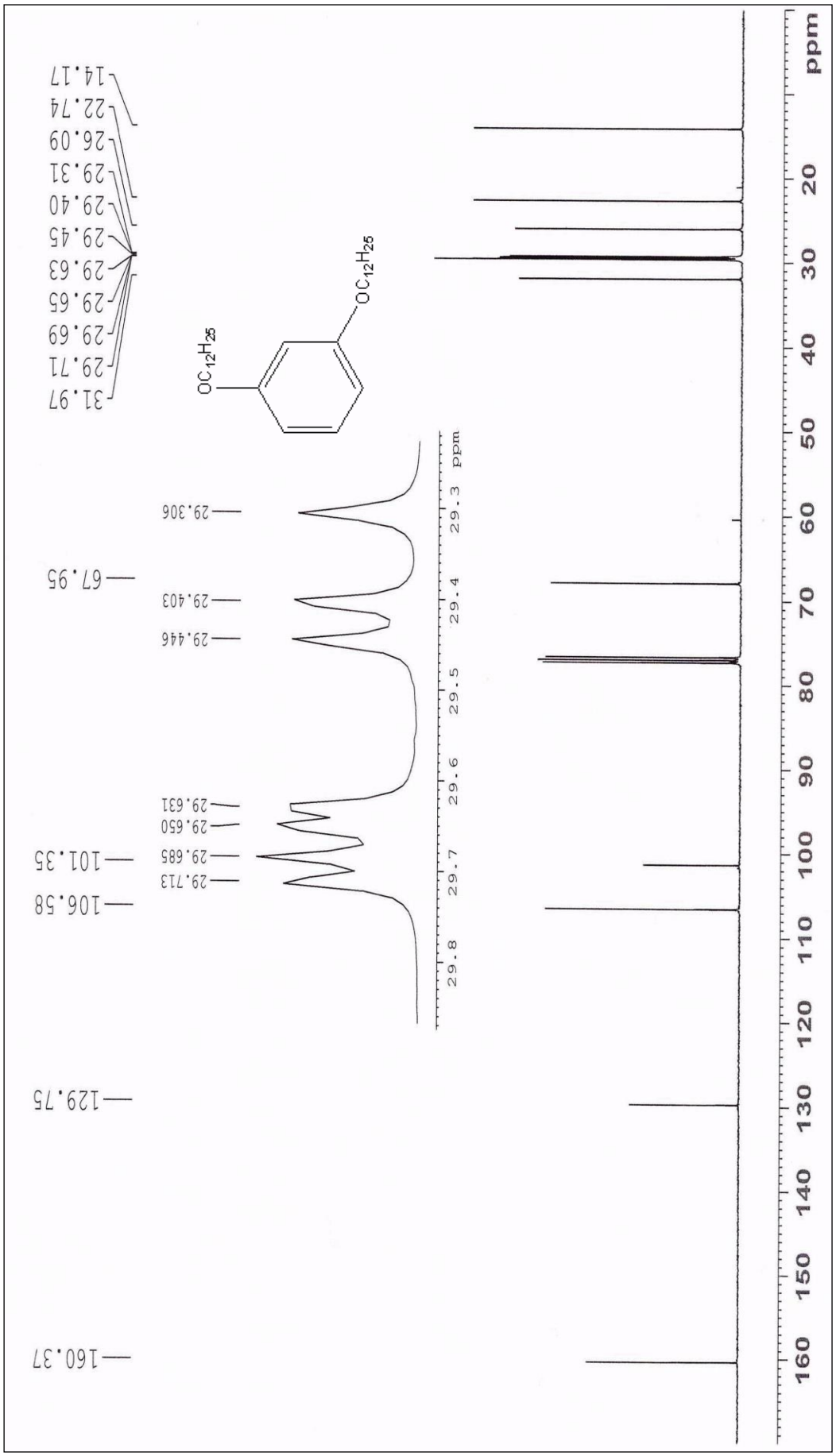



**Figure 2.18:-** $^{13}C$ NMR of 1,3-bis-n-(dodecyloxy)benzene **(12h)**

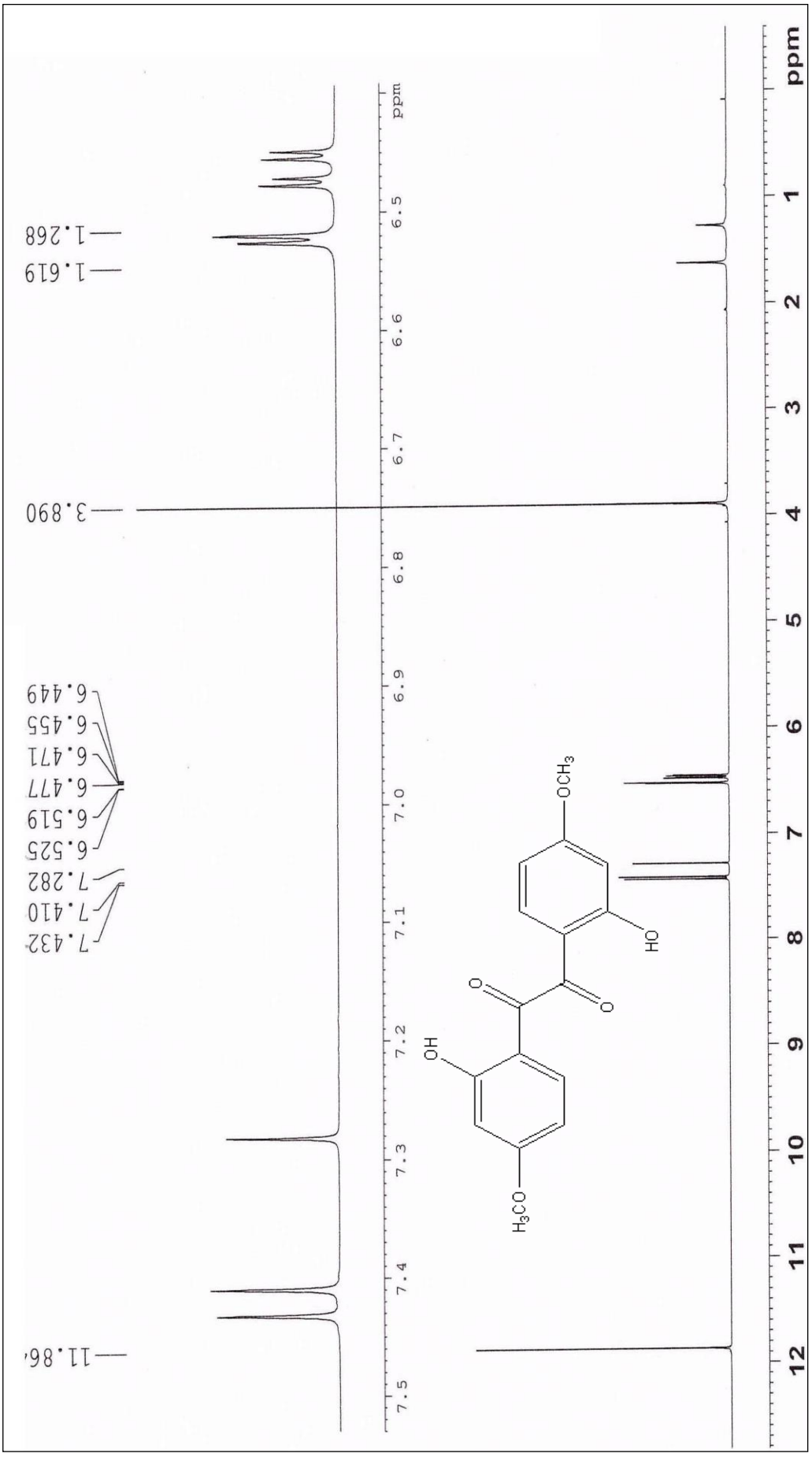



**Figure 2.19:-** $^{1}$H NMR of 1,2-bis(2-hydroxy-4-methoxyphenyl)ethane-1,2-dione **(13a)**

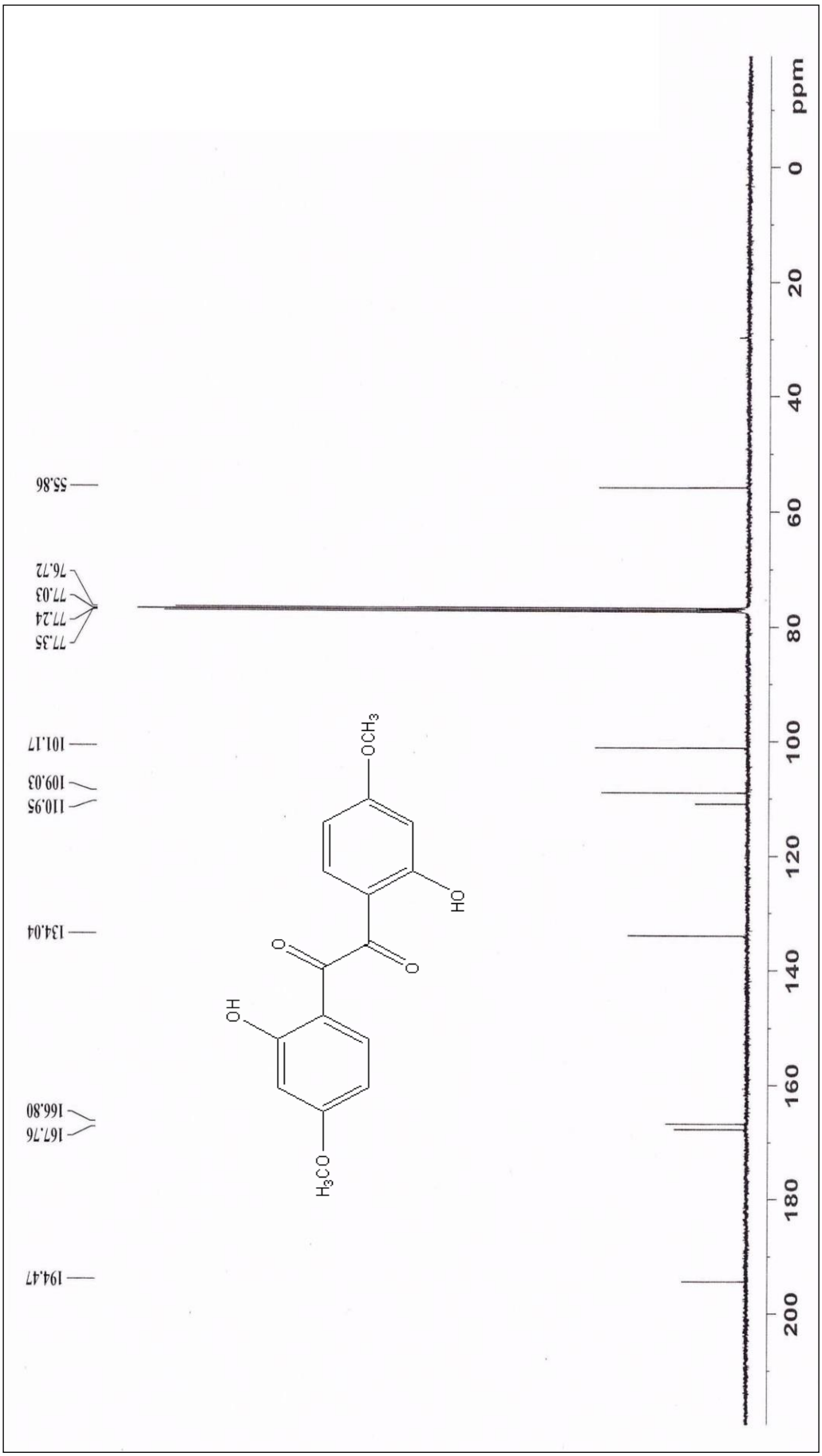



**Figure 2.20:-** $^{13}$C NMR of 1,2-bis(2-hydroxy-4-methoxyphenyl)ethane-1,2-dione **(13a)**

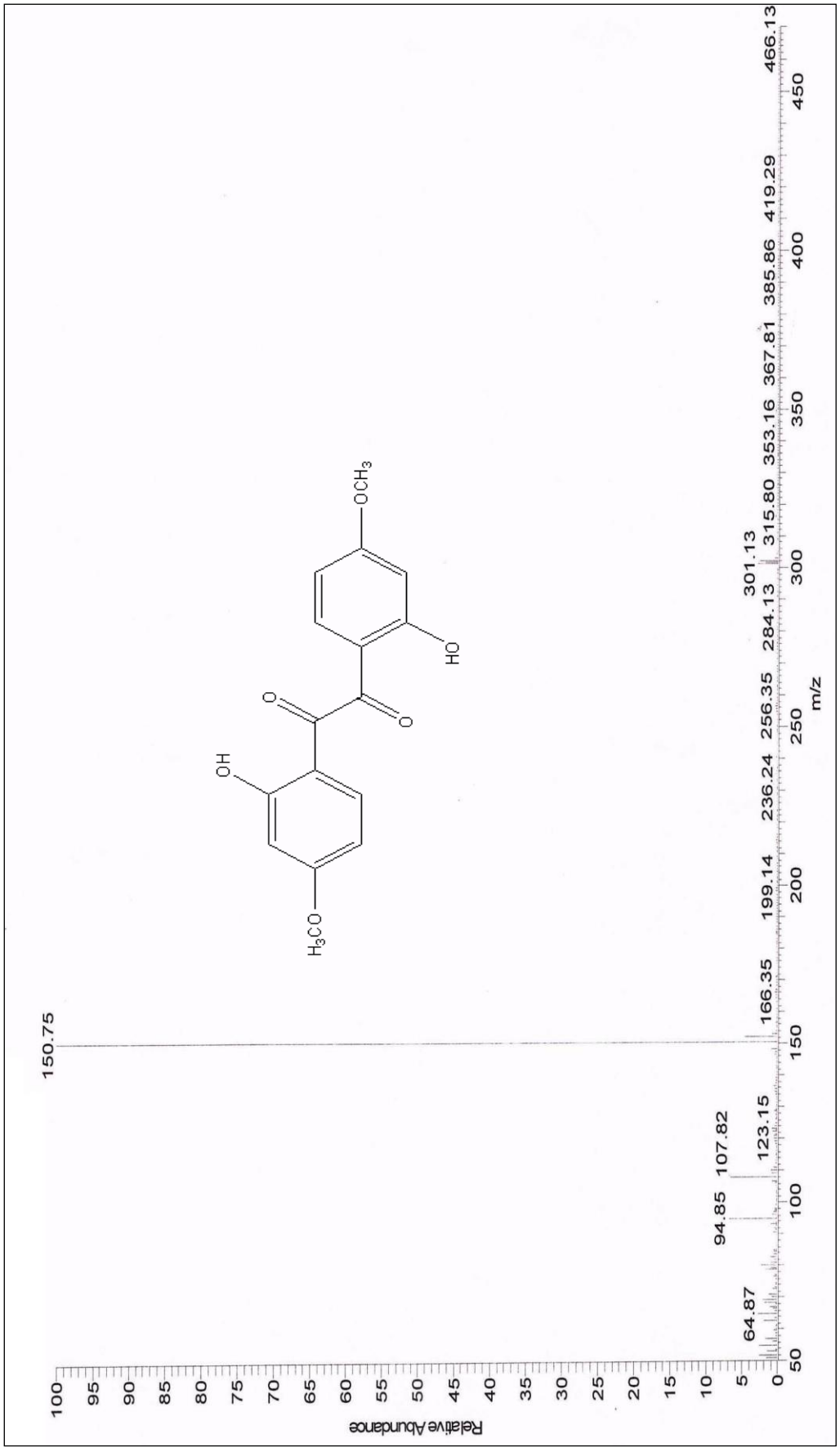



**Figure 2.21:-** Mass Spectrum 1,2-bis(2-hydroxy-4-methoxyphenyl)ethane-1,2-dione **(13a)**

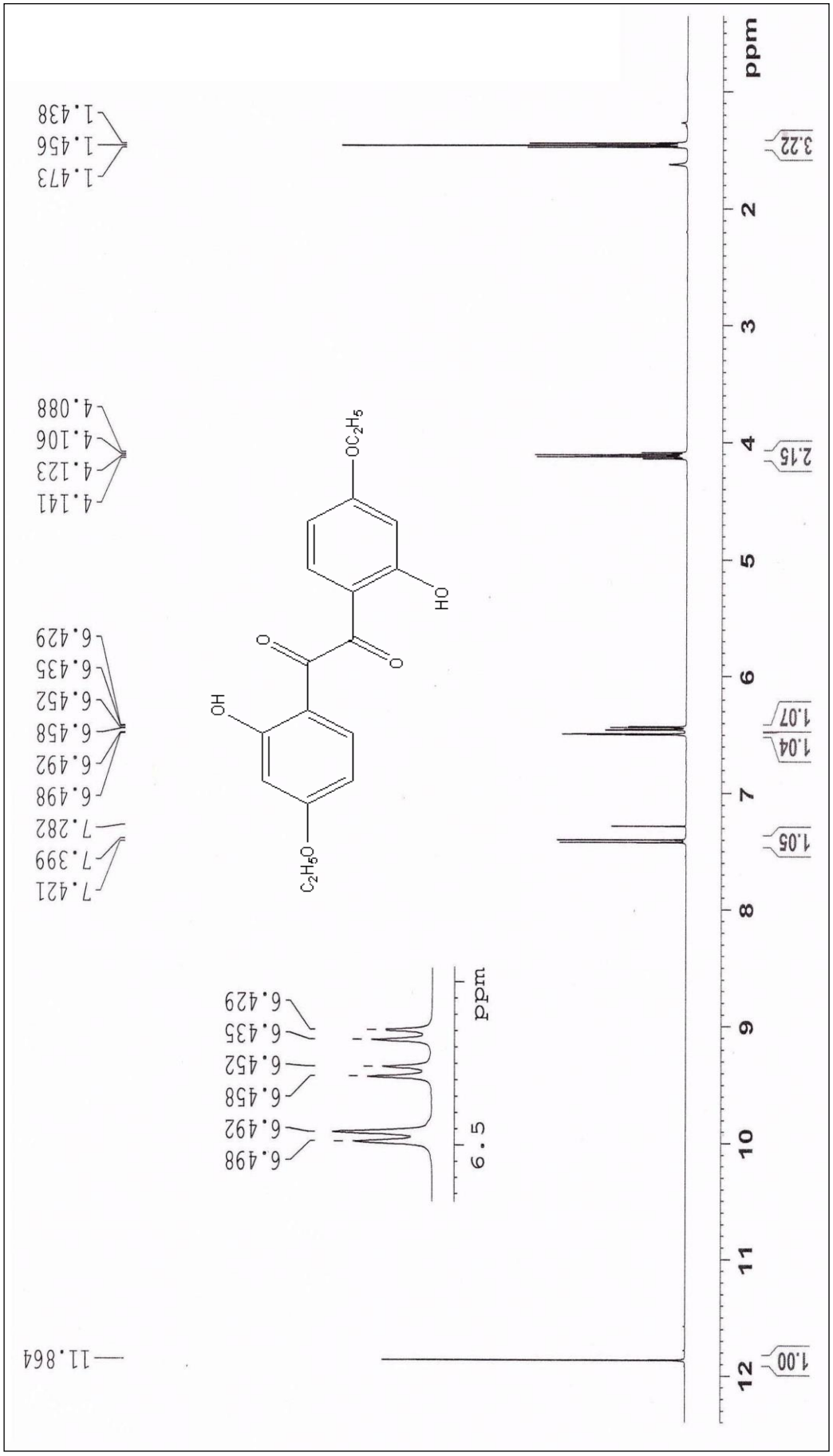



**Figure 2.22:-** $^{1}$H NMR of 1,2-bis(4-ethoxy-2-hydroxyphenyl)ethane-1,2-dione **(13b)**

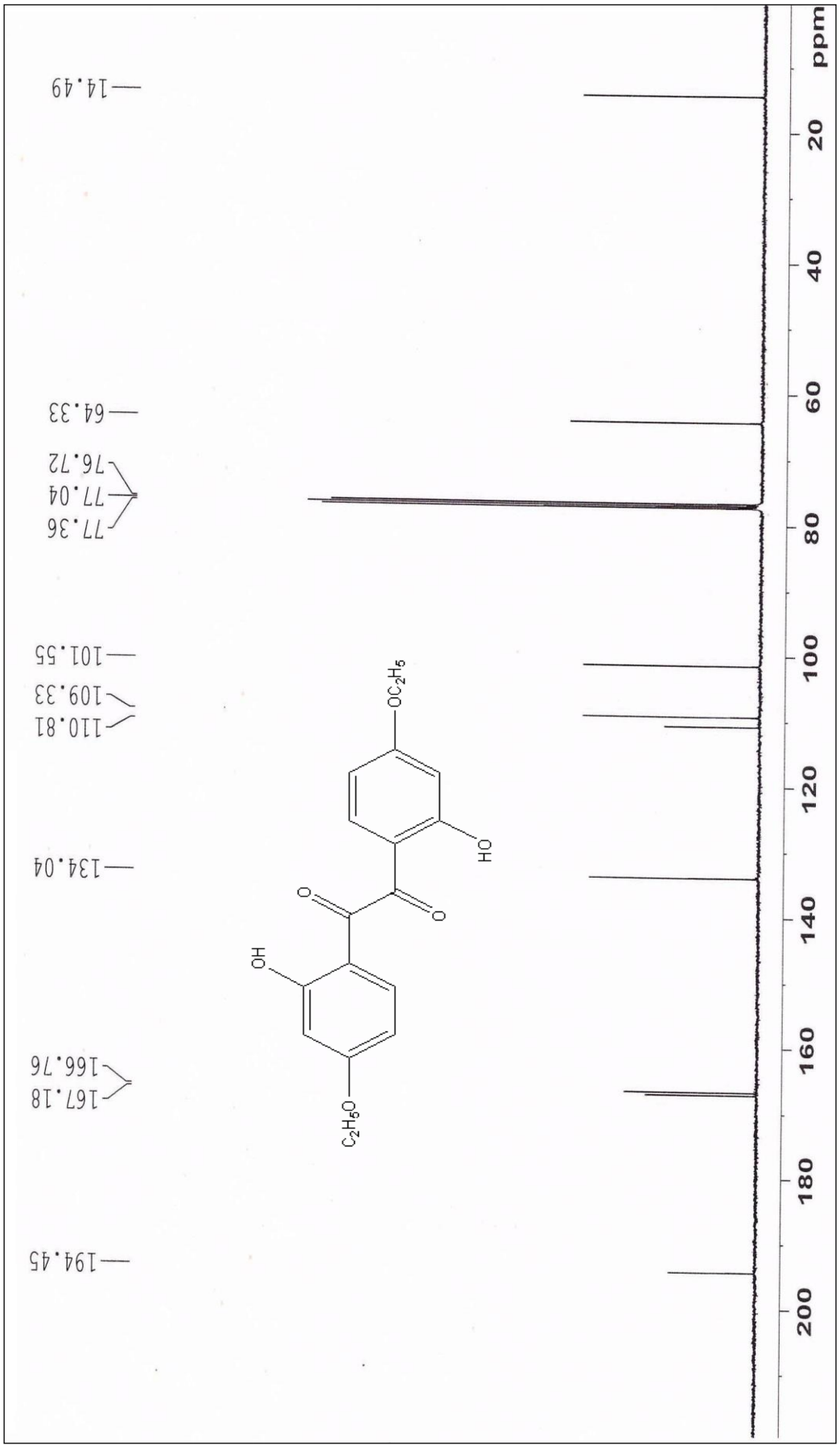



**Figure 2.23:-** $^{13}$C NMR of 1,2-bis(4-ethoxy-2-hydroxyphenyl)ethane-1,2-dione **(13b)**

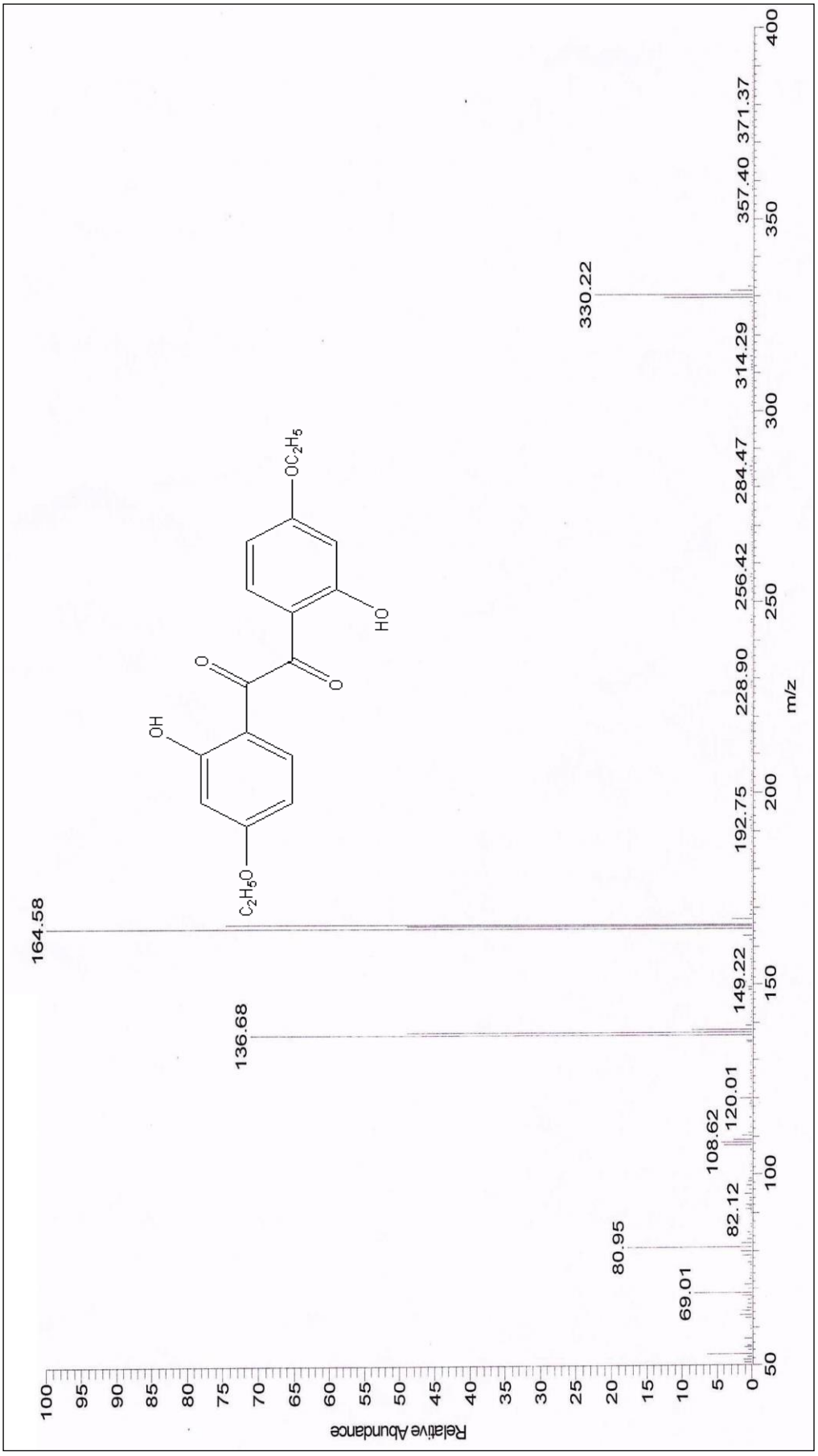



**Figure 2.24:-** Mass Spectrum of 1,2-bis(4-ethoxy-2-hydroxyphenyl)ethane-1,2-dione **(13b)**

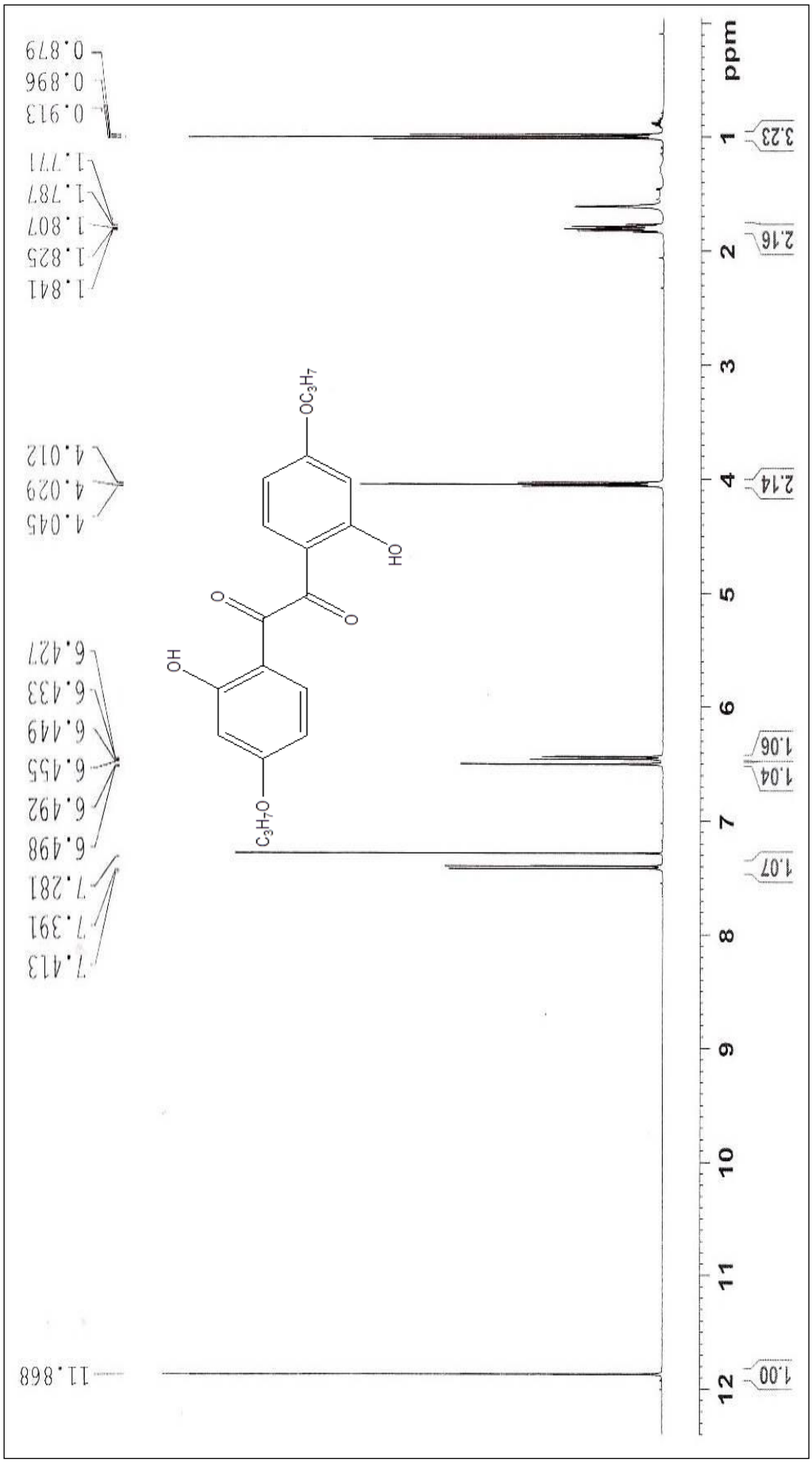



**Figure 2.25:-** $^{1}$H NMR of 1,2-bis(2-hydroxy-4-n-propoxyphenyl)ethane-1,2-dione **(13c)**

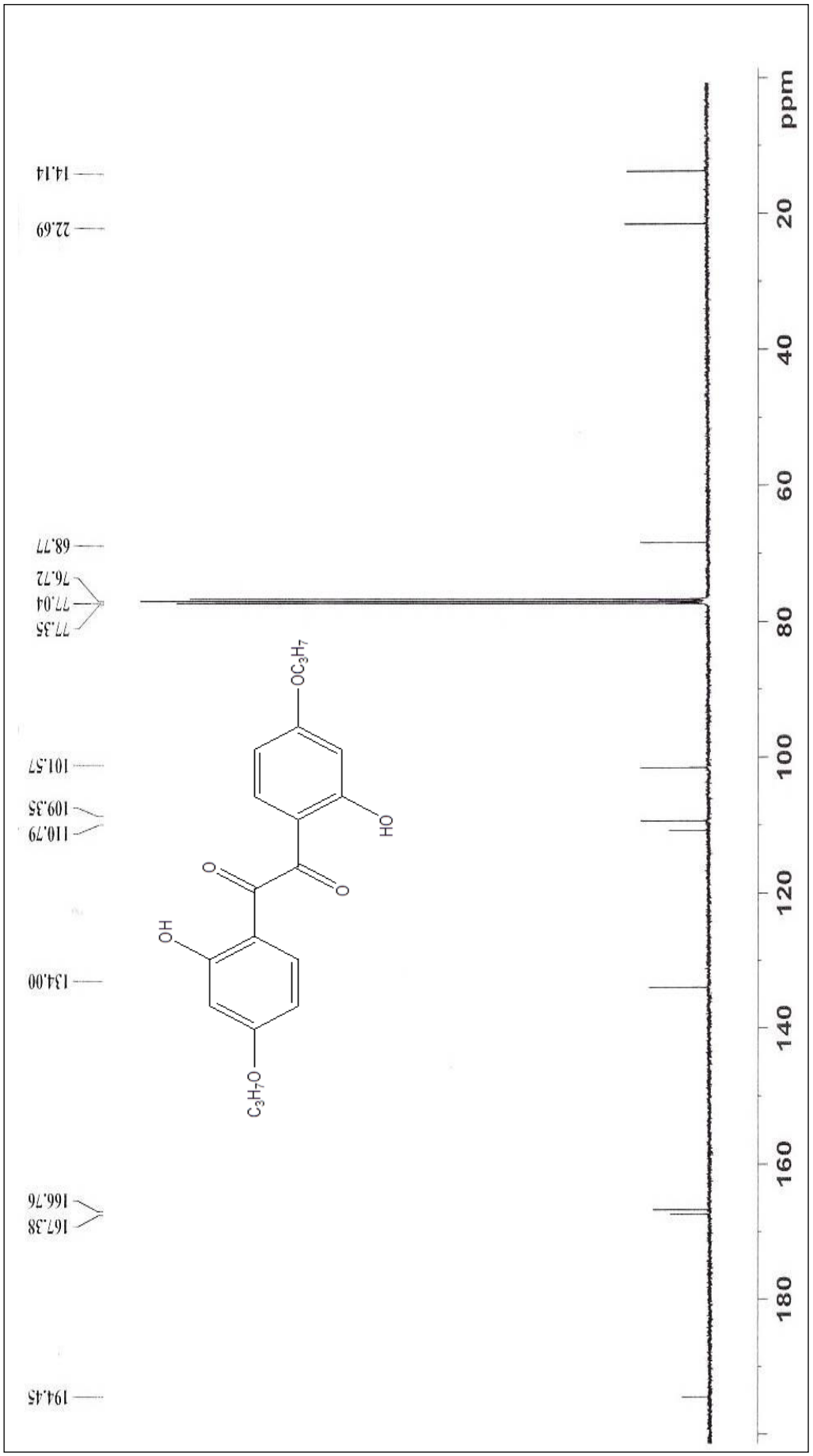



**Figure 2.26:-** $^{13}C$ NMR of 1,2-bis(2-hydroxy-4-n-propoxyphenyl)ethane-1,2-dione **(13c)**

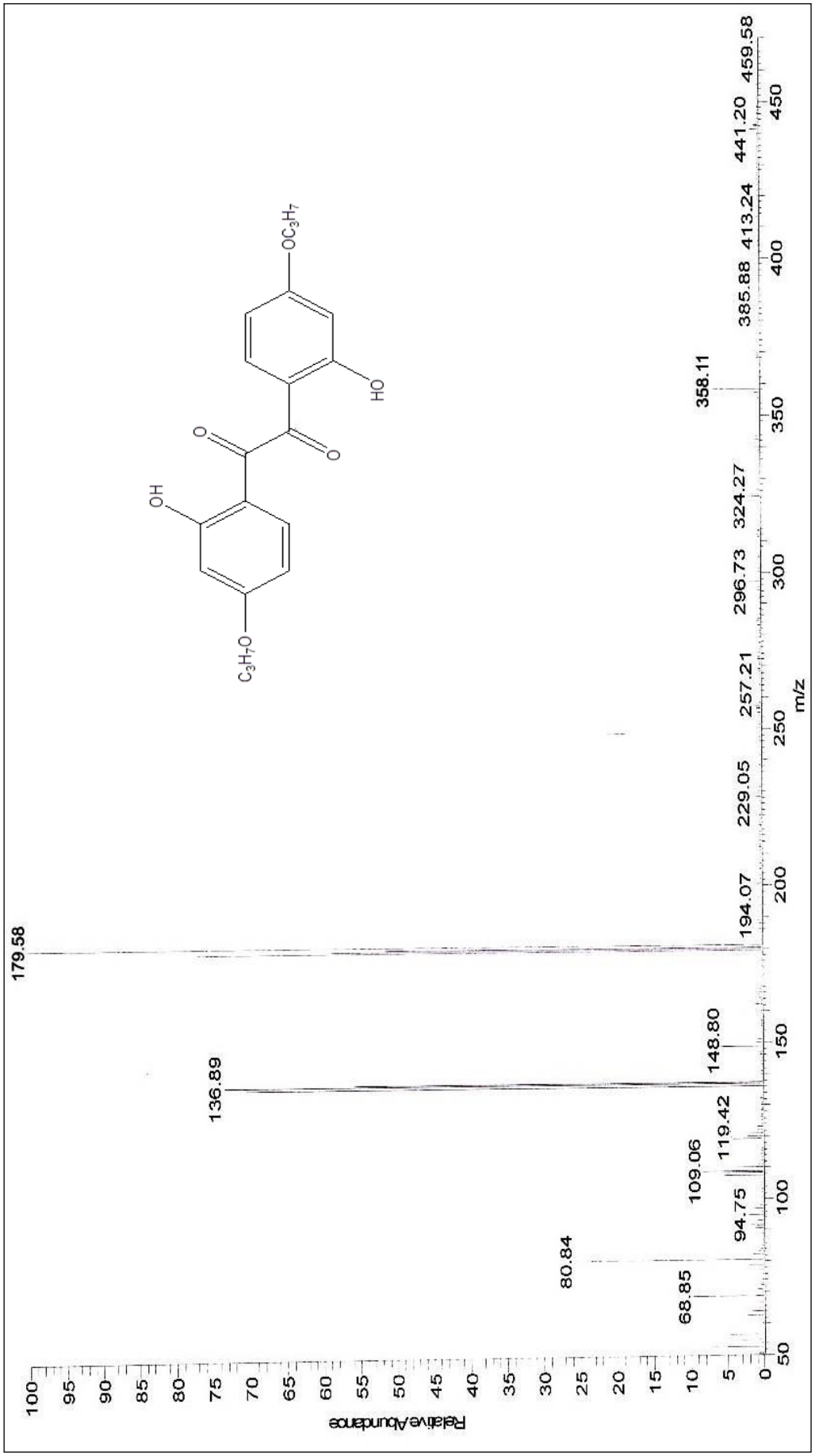



**Figure 2.27:-** Mass Spectrum of 1,2-bis(2-hydroxy-4-n-propoxyphenyl)ethane-1,2-dione **(13c)**

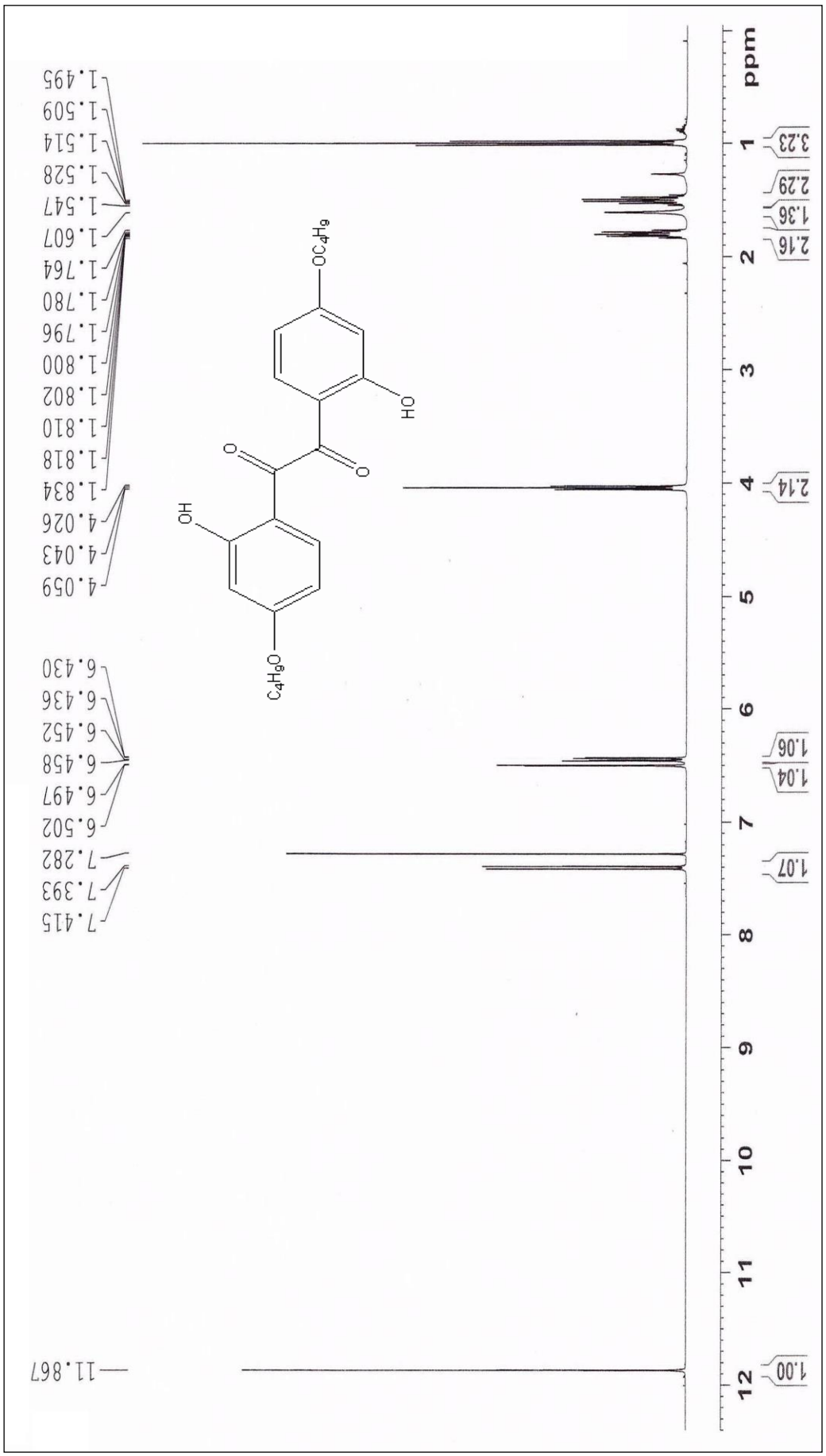



**Figure 2.28:-** $^{1}$H NMR of 1,2-bis(4-n-butoxy-2-hydroxyphenyl)ethane-1,2-dione **(13d)**

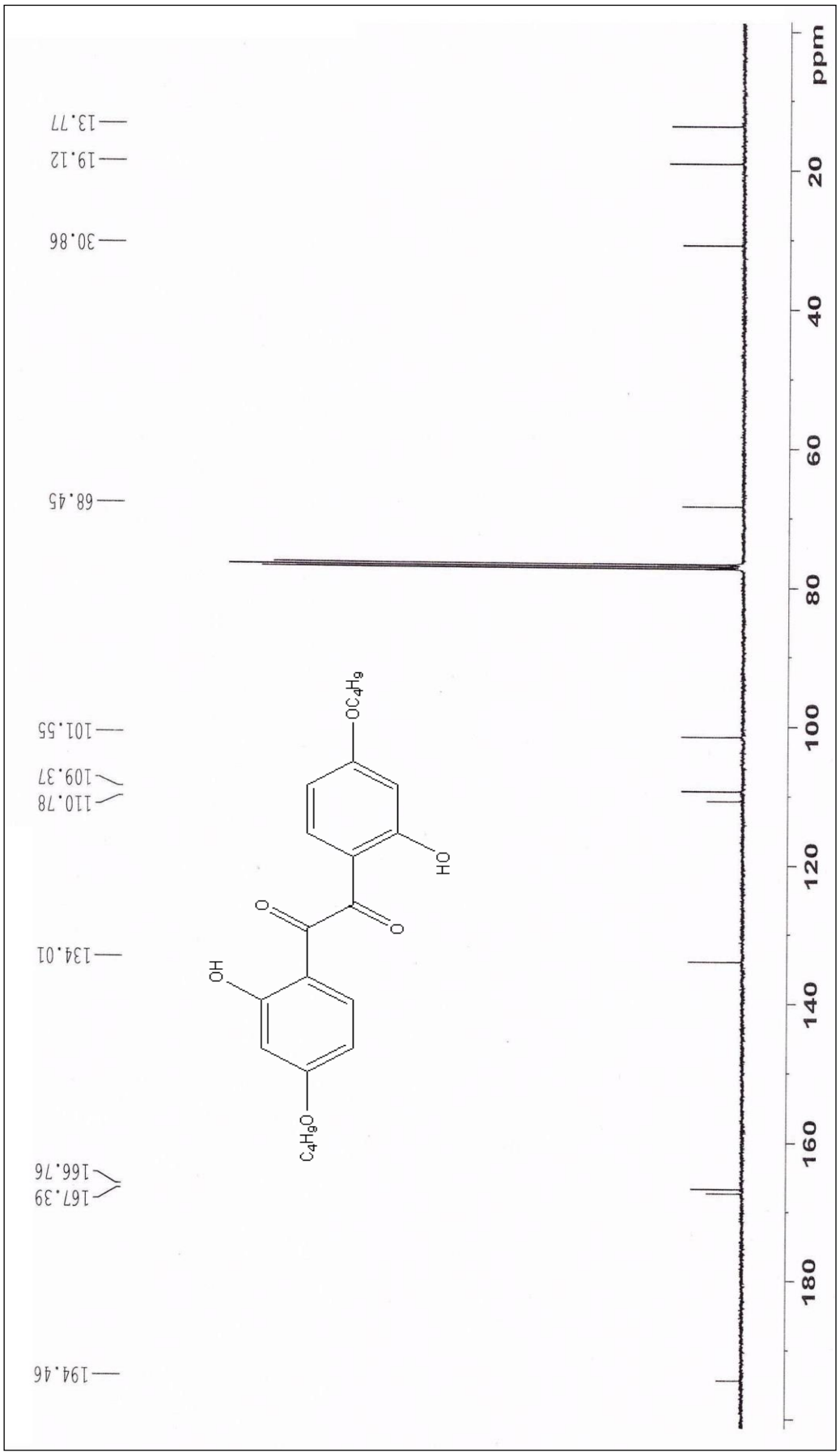



**Figure 2.29:-** $^{13}C$ NMR of 1,2-bis(4-n-butoxy-2-hydroxyphenyl)ethane-1,2-dione **(13d)**

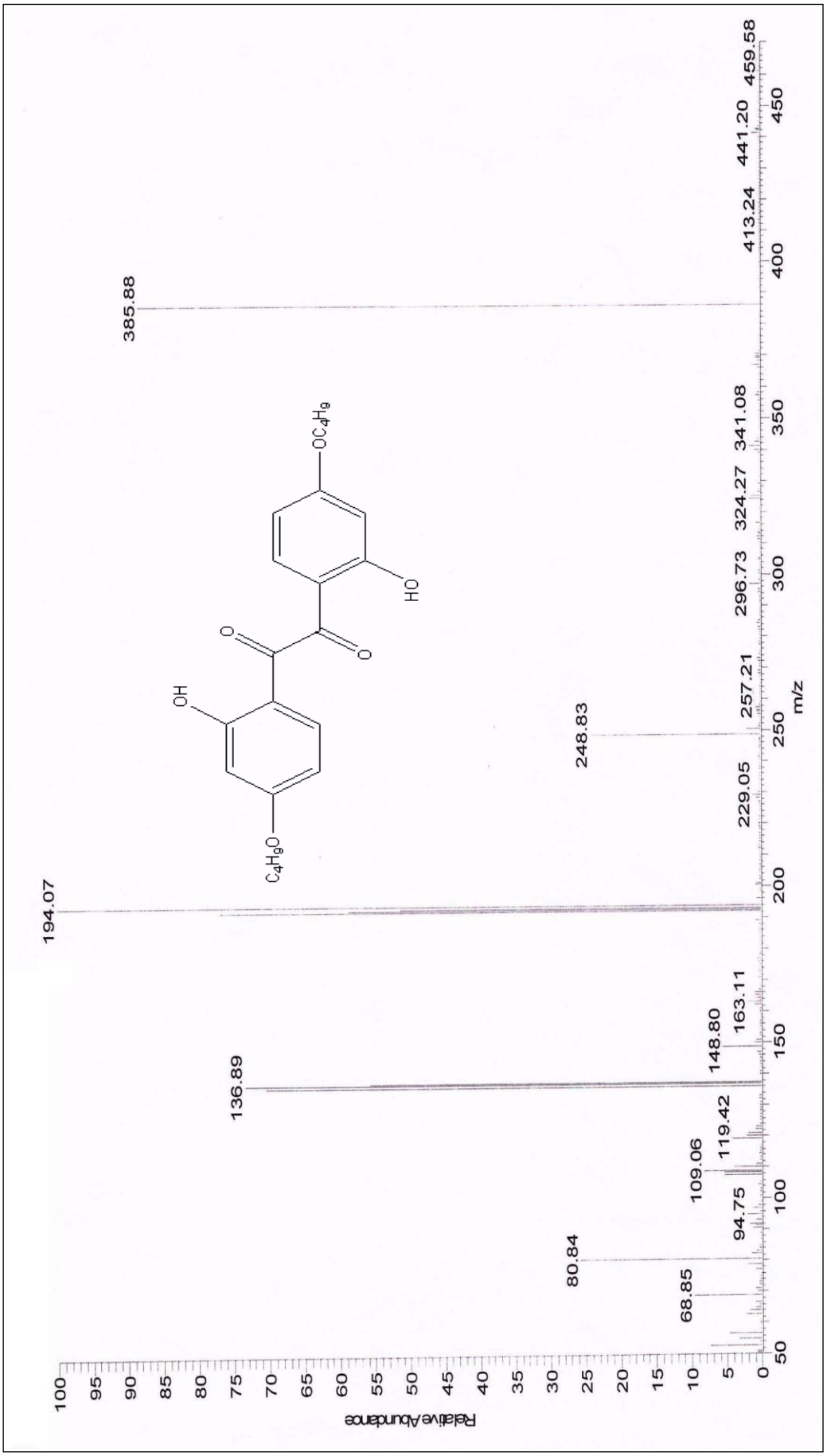



**Figure 2.30:-** Mass Spectrum of 1,2-bis(4-n-butoxy-2-hydroxyphenyl)ethane-1,2-dione **(13d)**

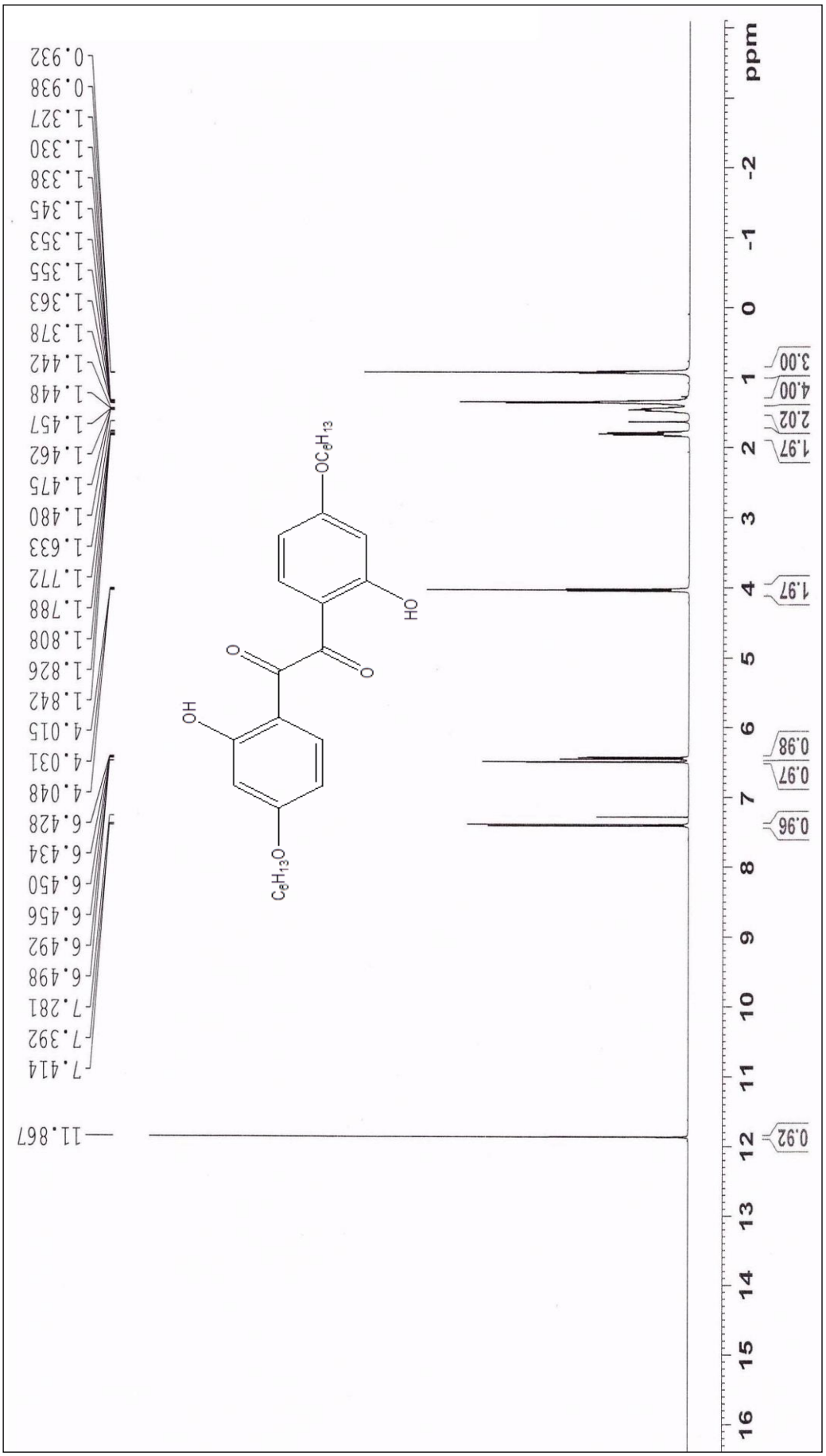



**Figure 2.31:-** $^{1}$H NMR of 1,2-bis(4-n-hexyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13e)**

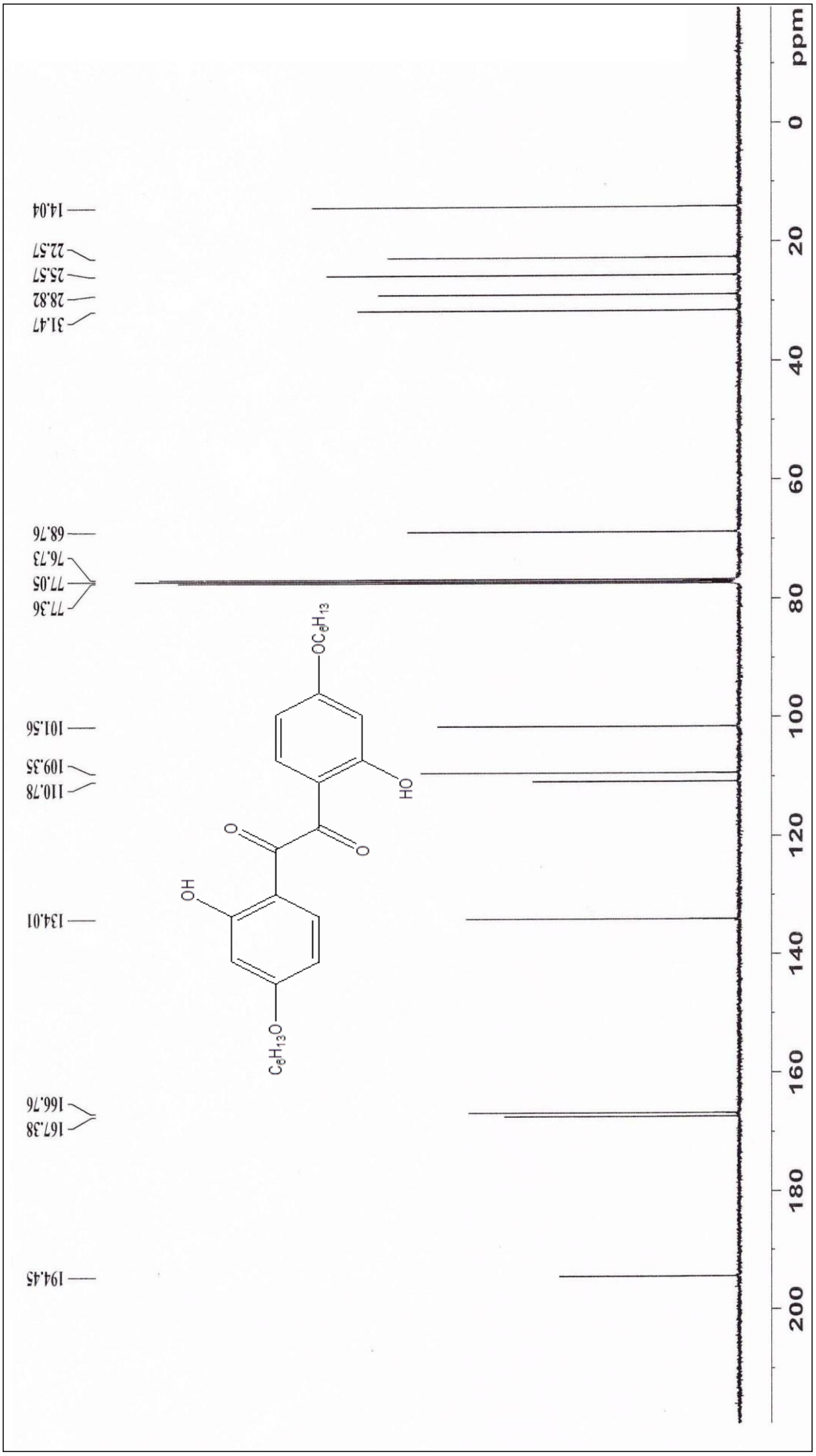



**Figure 2.32:-** $^{13}$C NMR of 1,2-bis(4-n-hexyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13e)**

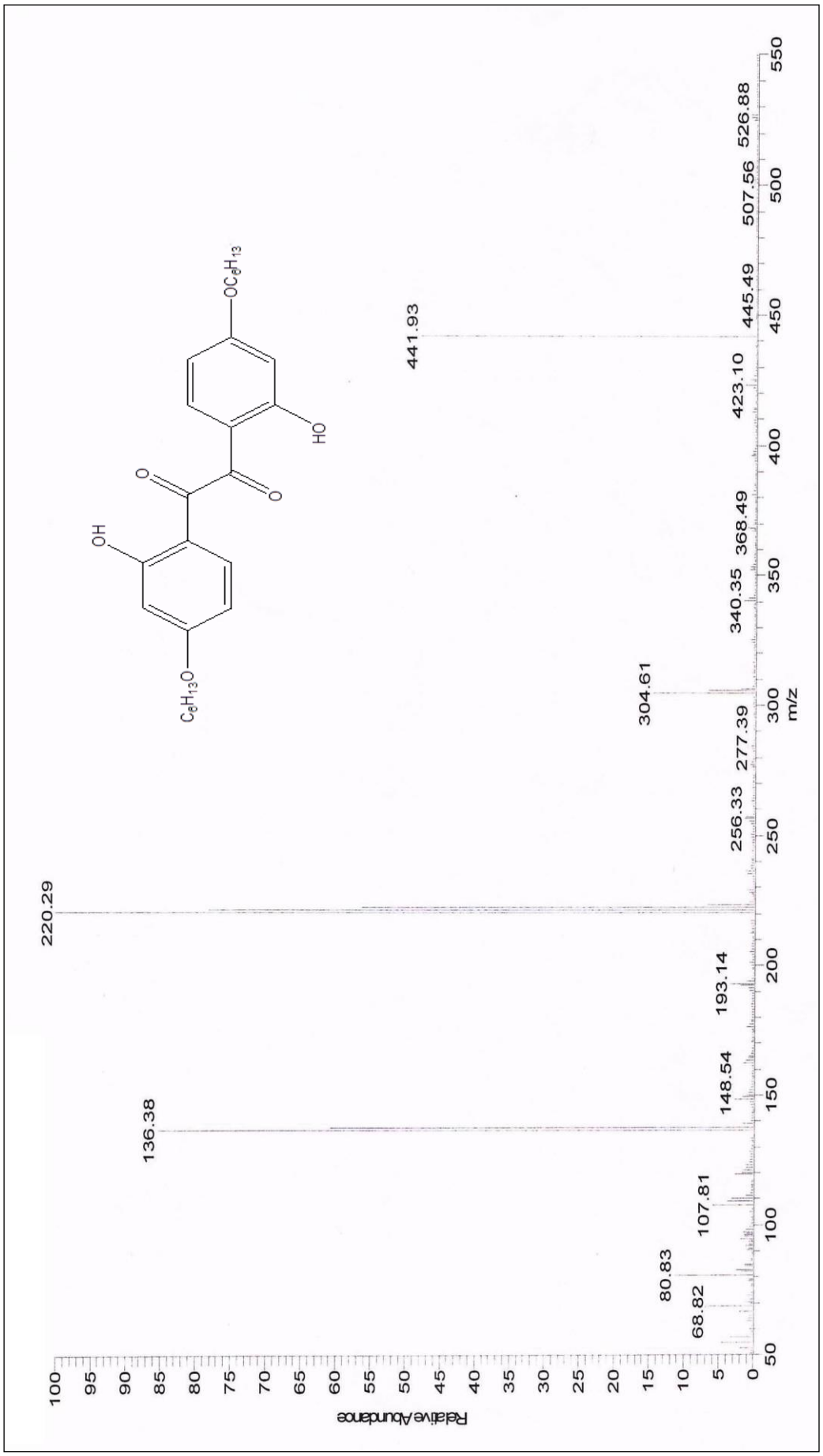



**Figure 2.33:-** Mass Spectrum of 1,2-bis(4-n-hexyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13e)**

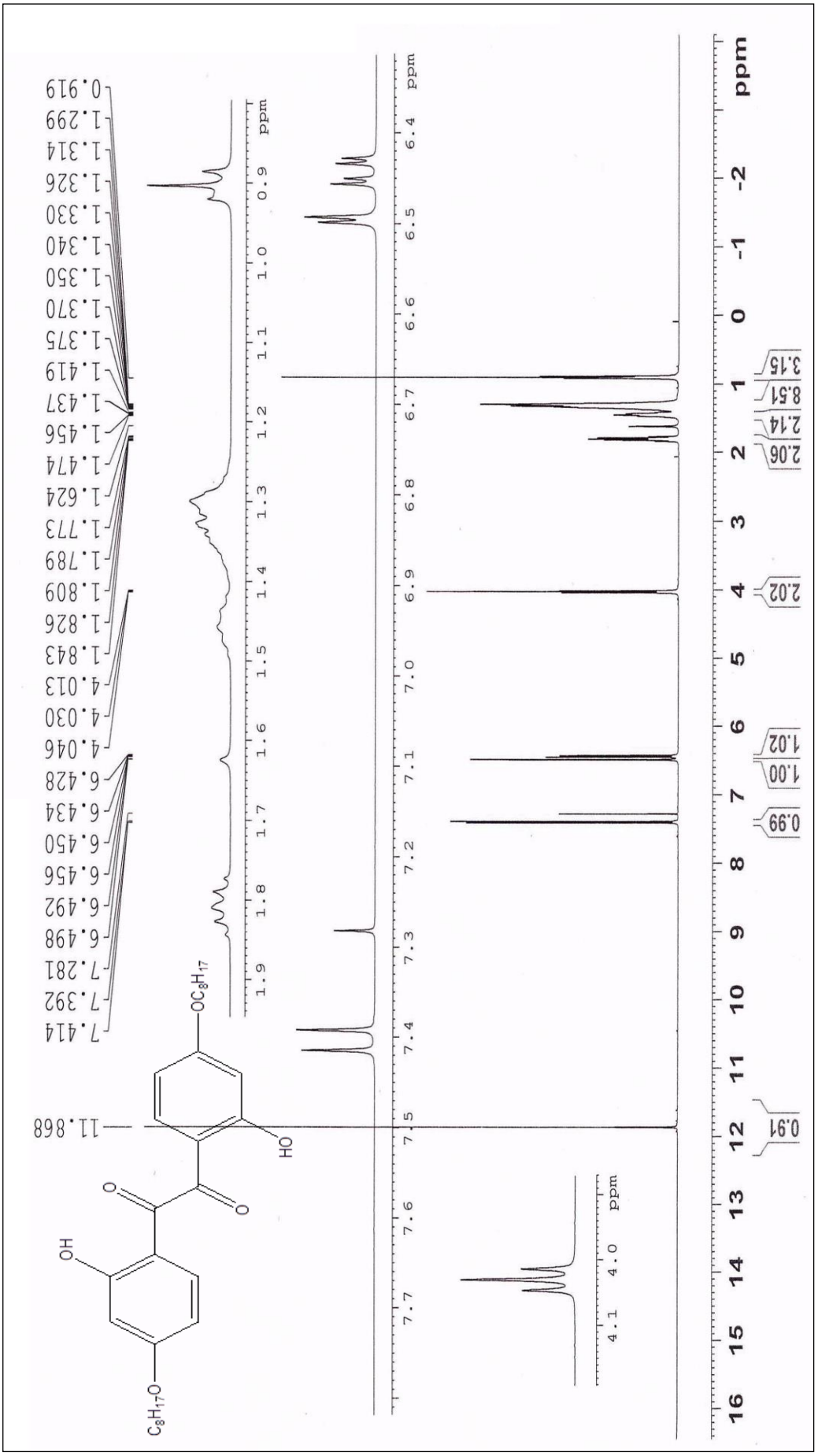



**Figure 2.34:-** $^{1}$H NMR of 1,2-bis(2-hydroxy-4-n-octyloxyphenyl)ethane-1,2-dione **(13f)**

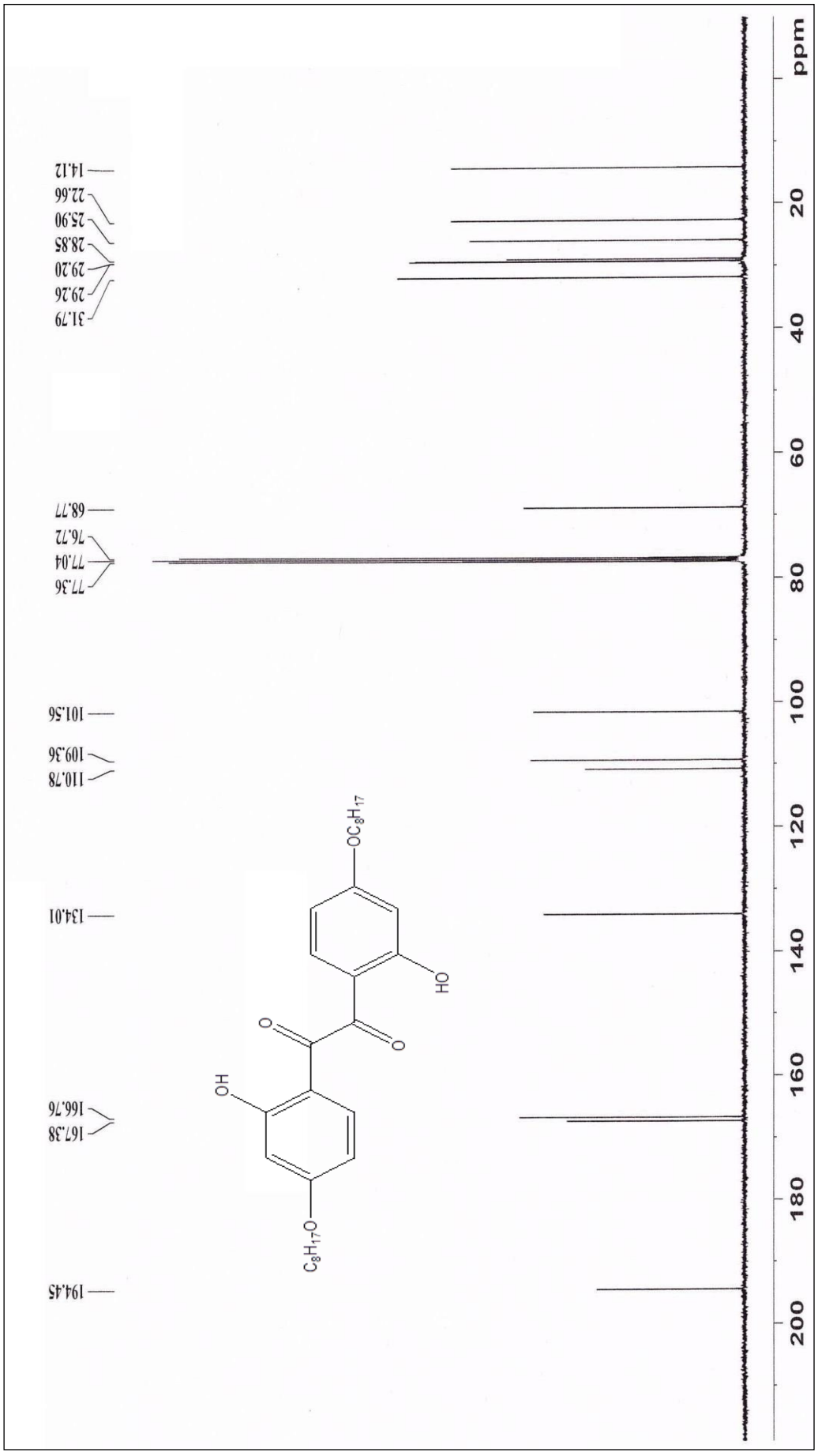



**Figure 2.35:-** $^{13}$C NMR of 1,2-bis(2-hydroxy-4-n-octyloxyphenyl)ethane-1,2-dione **(13f)**

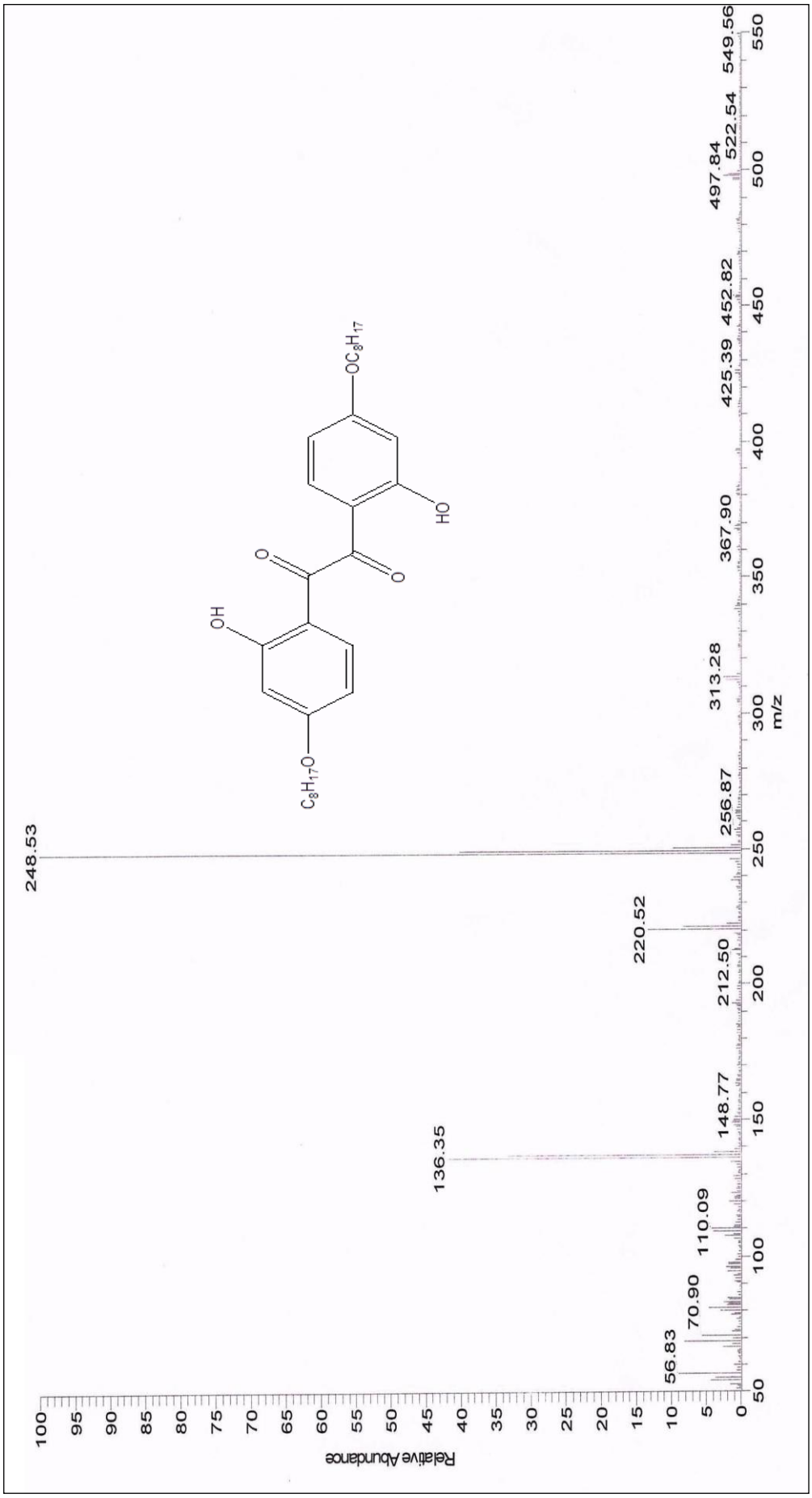



**Figure 2.36:-** Mass Spectrum of 1,2-bis(2-hydroxy-4-n-octyloxyphenyl)ethane-1,2-dione **(13f)**

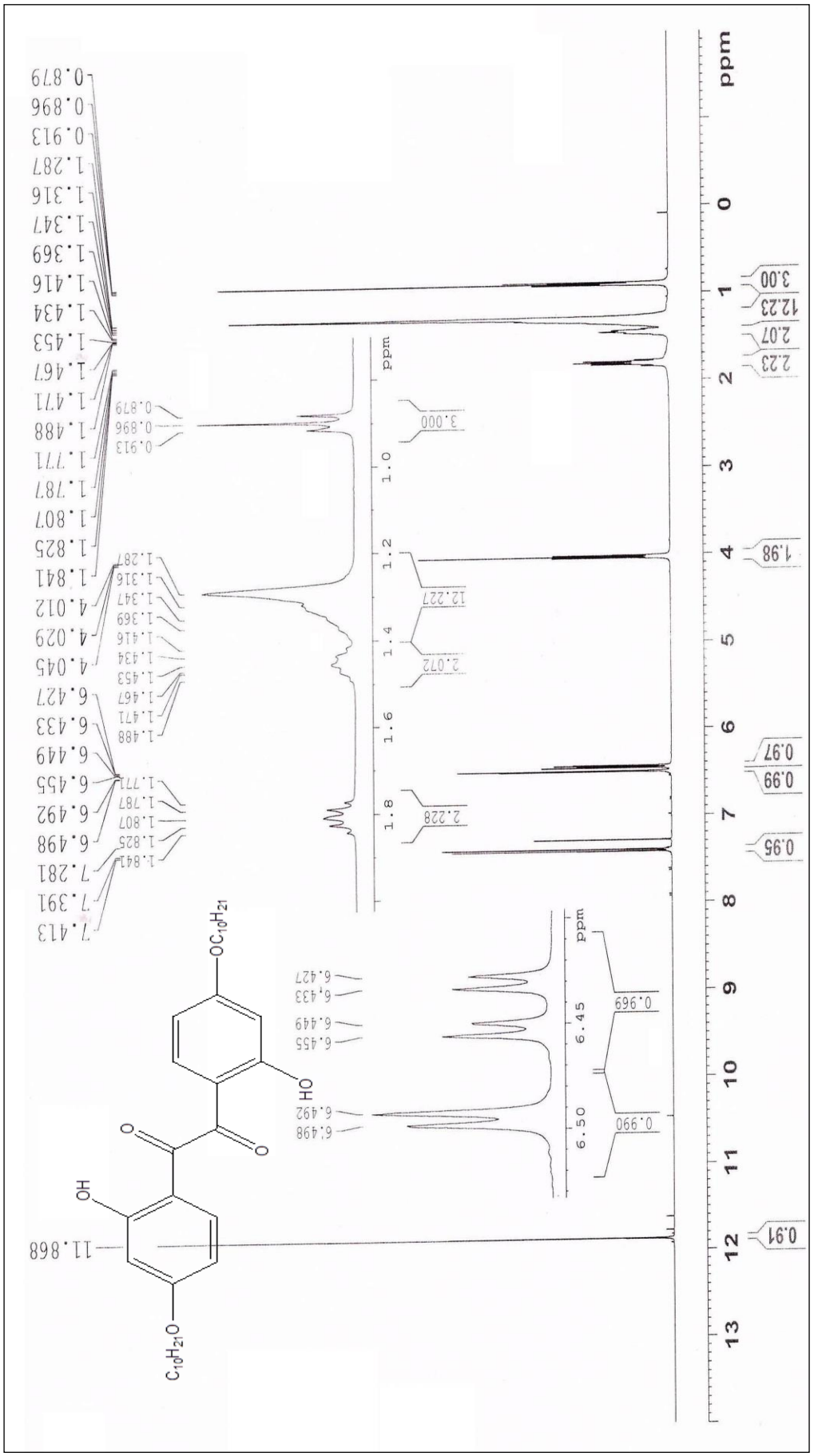


**Figure 2.37:-** $^{1}$H NMR of 1,2-bis(4-n-decyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13g)**

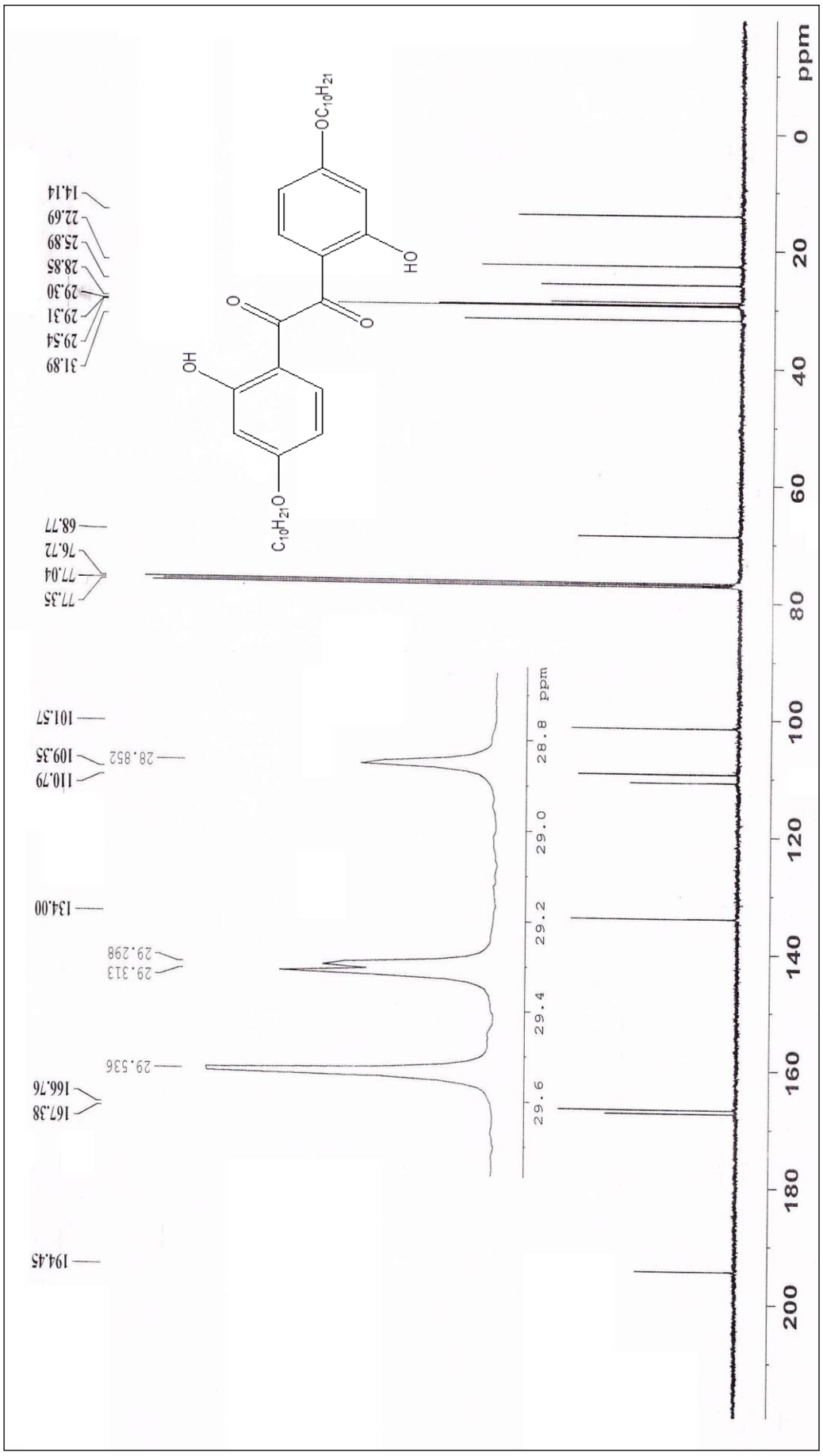



**Figure 2.38:-** $^{13}C$ NMR of 1,2-bis(4-n-decyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13g)**

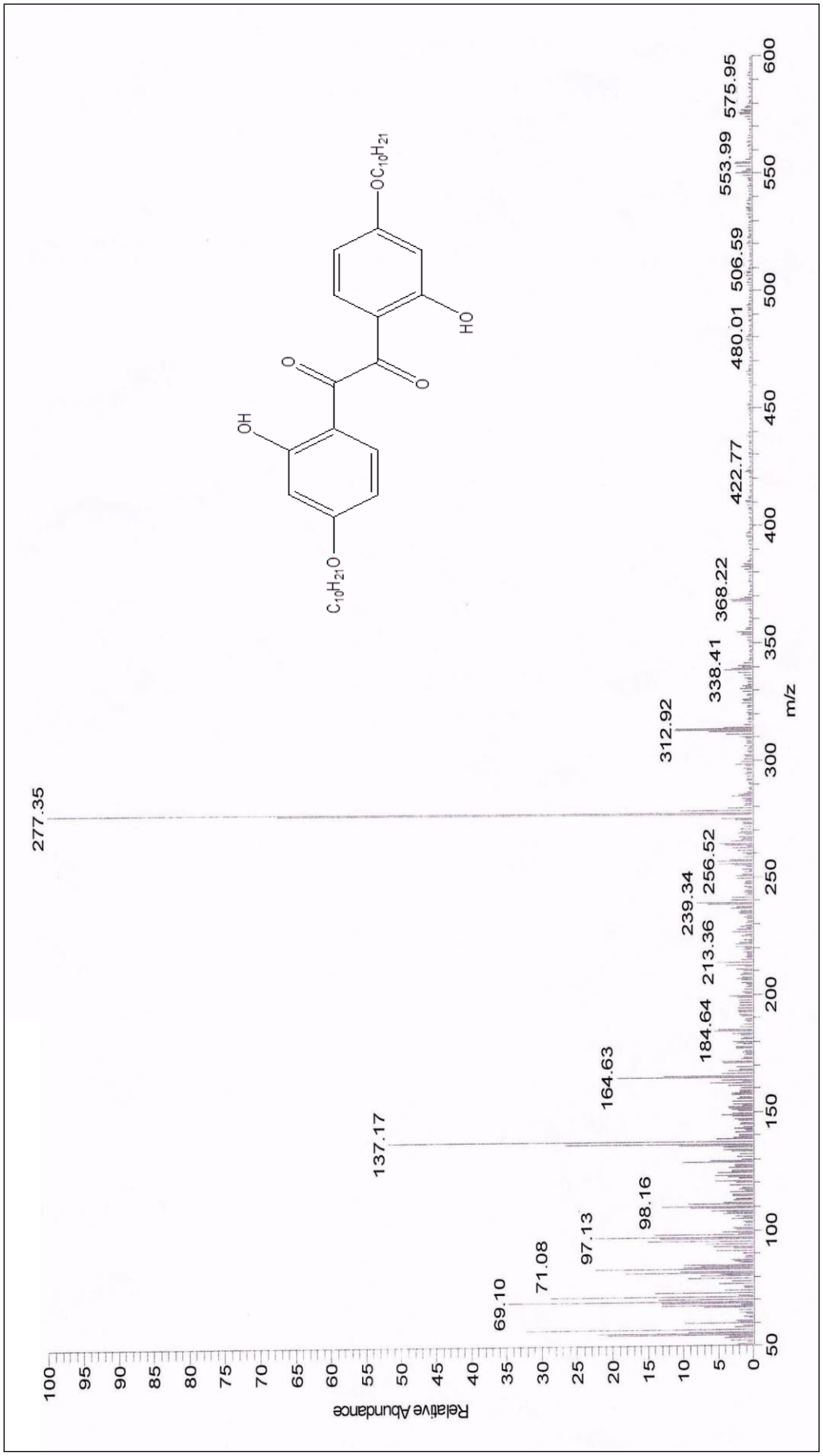



**Figure 2.39:-** Mass Spectrum of 1,2-bis(4-n-decyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13g)**

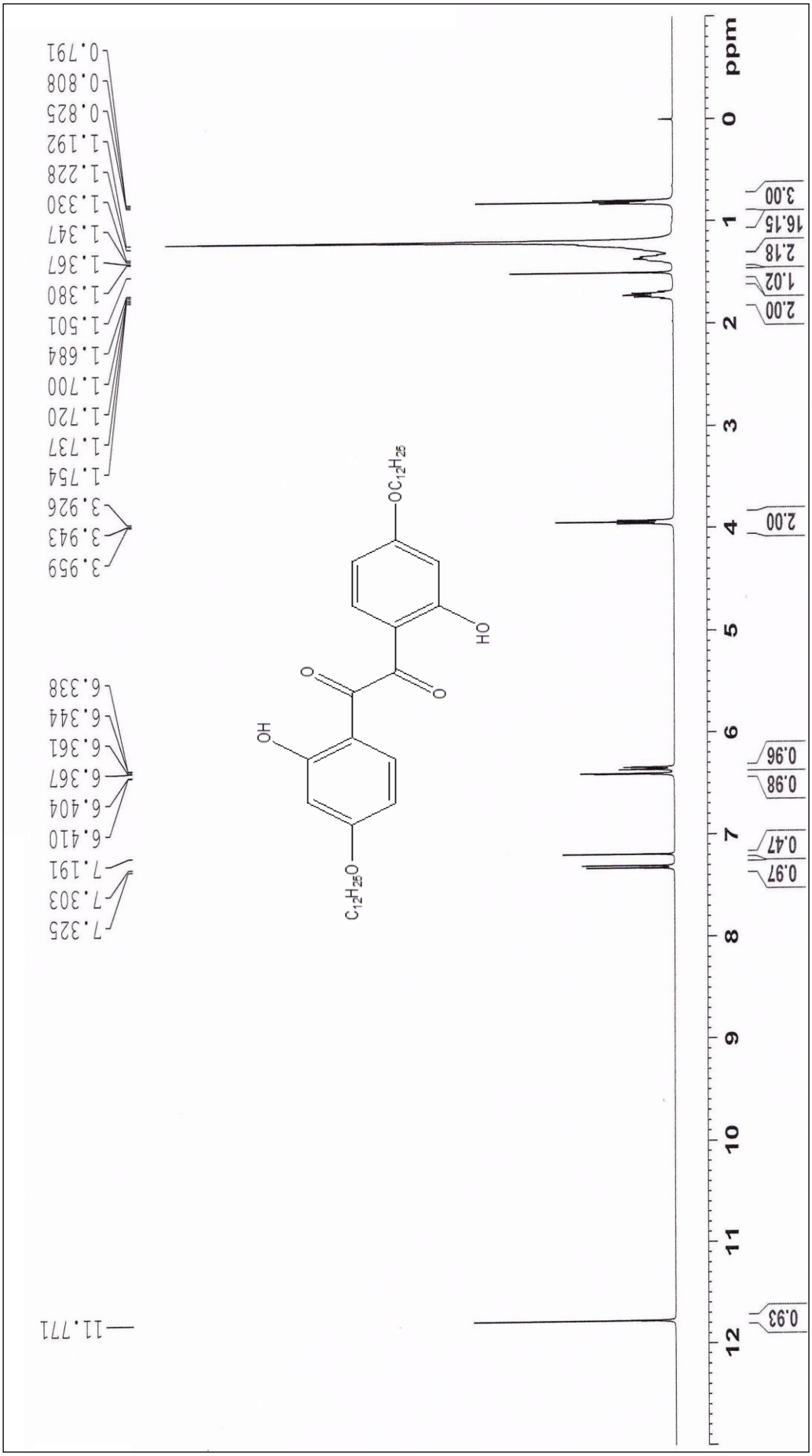



**Figure 2.40:-** $^{1}$H NMR of 1,2-bis(4-n-dodecyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13h)**

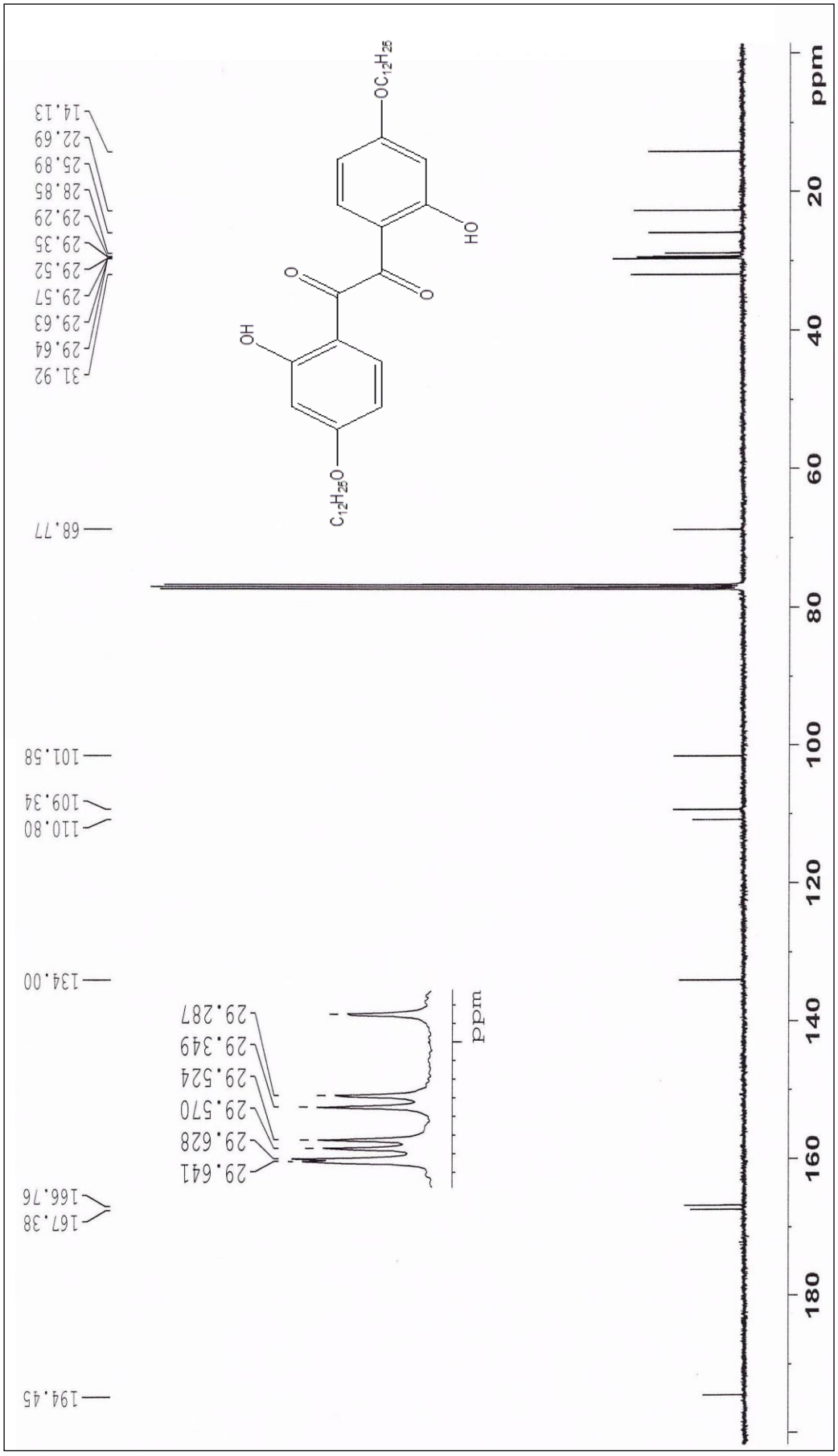



**Figure 2.41:-** $^{13}$C NMR of 1,2-bis(4-n-dodecyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13h)**

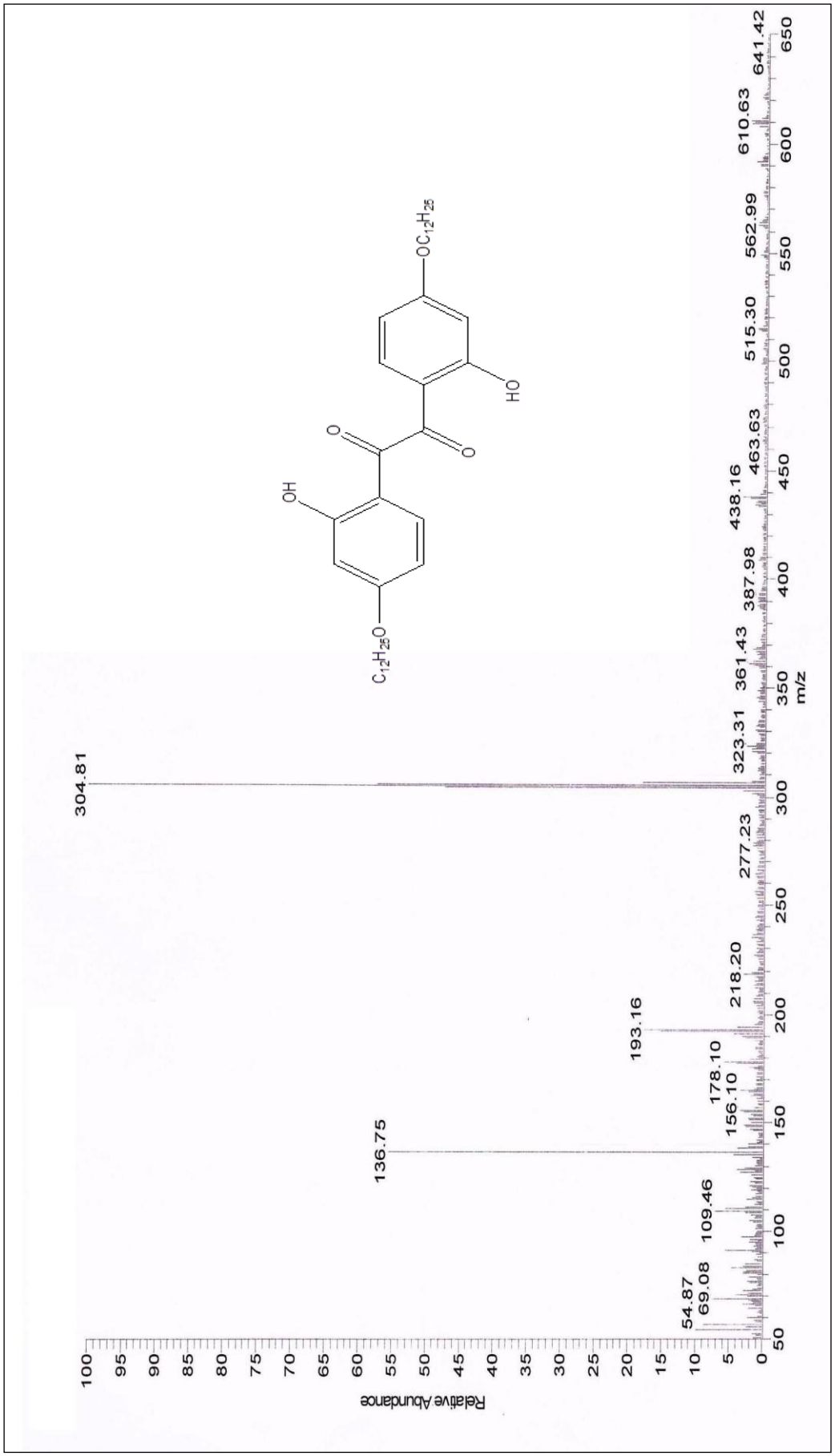



**Figure 2.42:-** Mass Spectrum of 1,2-bis(4-n-dodecyloxy-2-hydroxyphenyl)ethane-1,2-dione **(13h)**

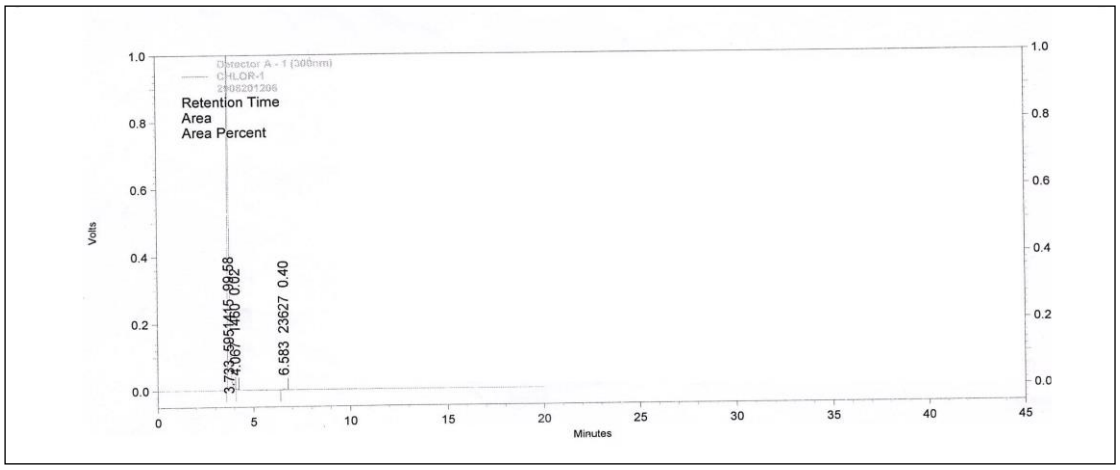



**Figure 2.43**:- HPLC Chromotagram of pure chlorpyrifos without irradiation

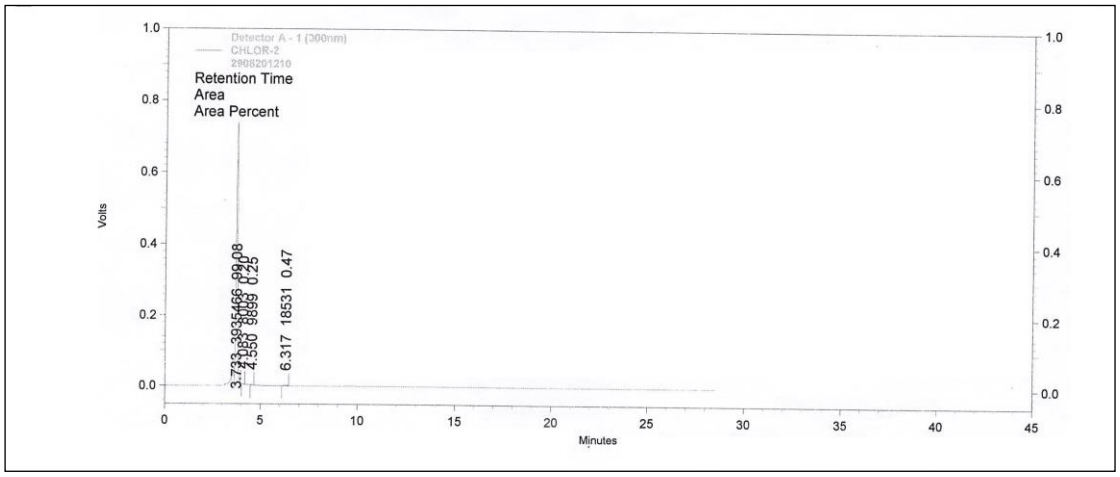



**Figure 2.44**:- HPLC Chromotagram of chlorpyrifos (without photostabilizer) after irradiation

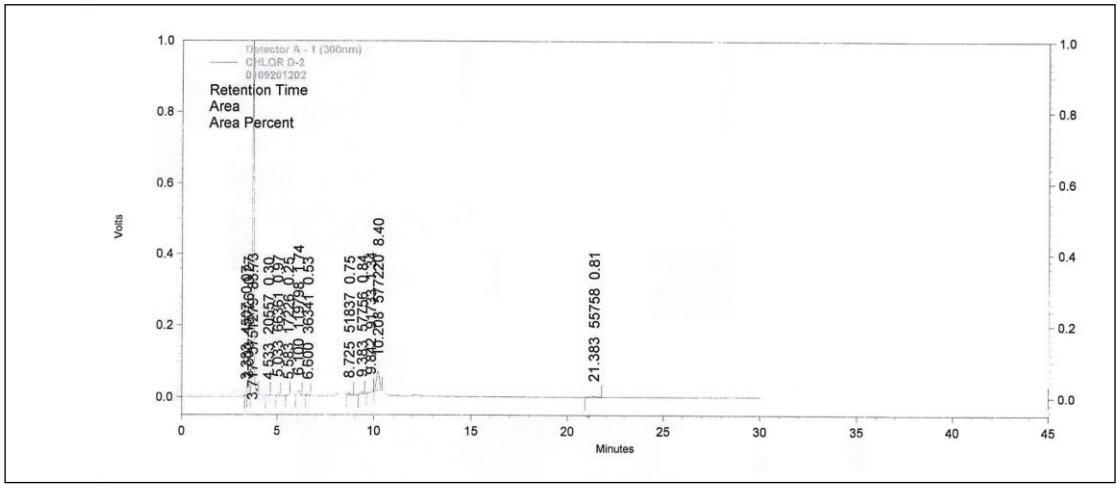



**Figure 2.45**:- HPLC Chromotagram of chlorpyrifos with **13a** (1:1) after irradiation

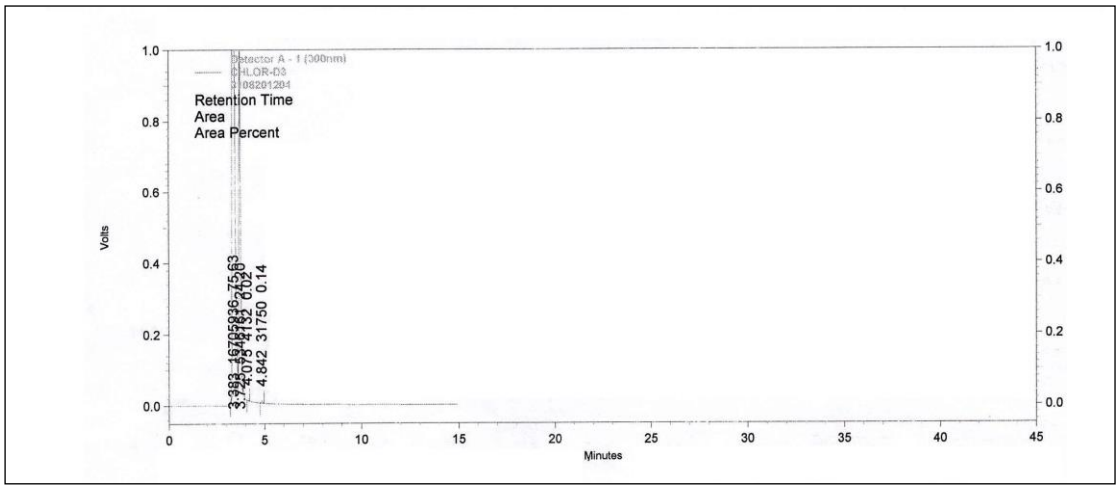



**Figure 2.46**:- HPLC Chromotagram of chlorpyrifos with **13b** (1:1) after irradiation

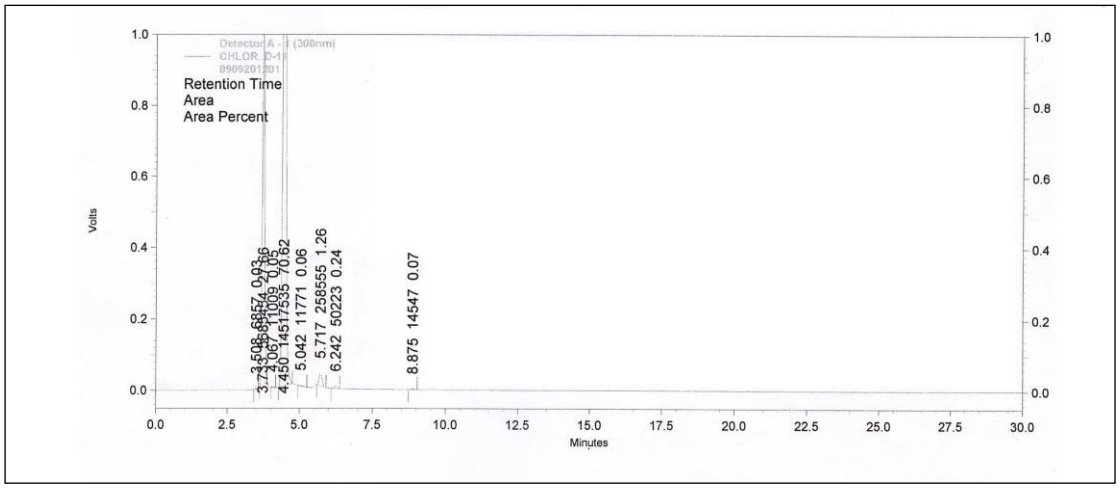



**Figure 2.47**:- HPLC Chromotagram of chlorpyrifos with **13c** (1:1) after irradiation

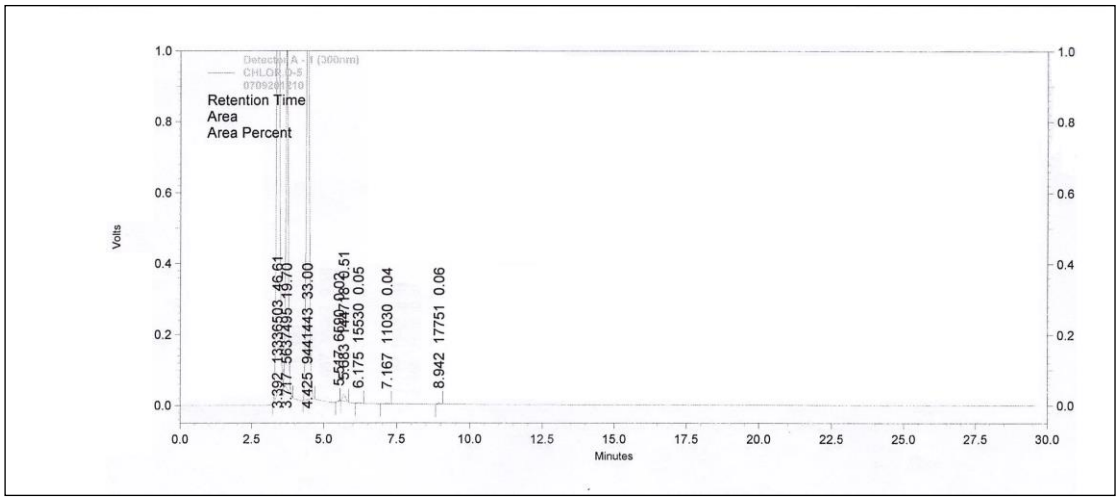



**Figure 2.48**:- HPLC Chromotagram of chlorpyrifos with **13d** (1:1) after irradiation

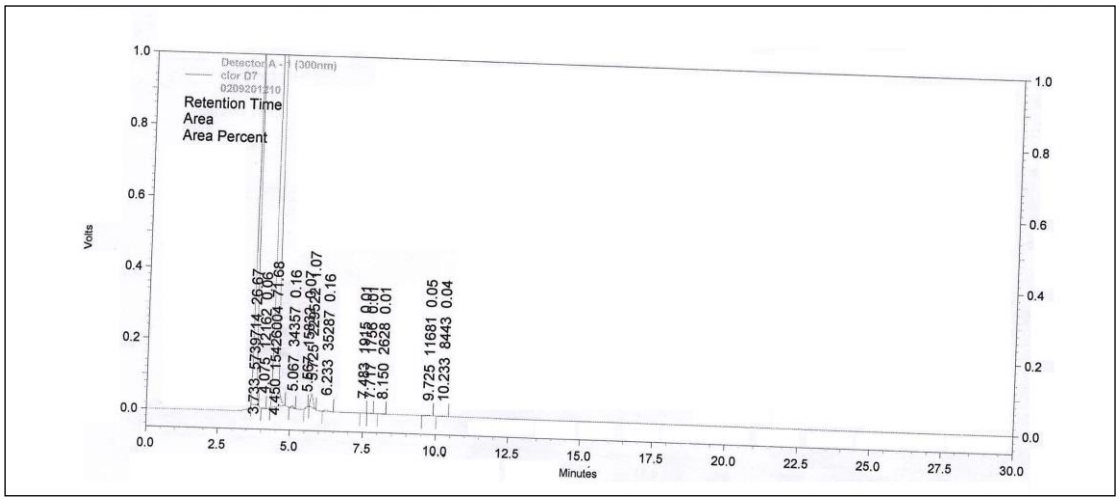



**Figure 2.49**:- HPLC Chromotagram of chlorpyrifos with **13e** (1:1) after irradiation

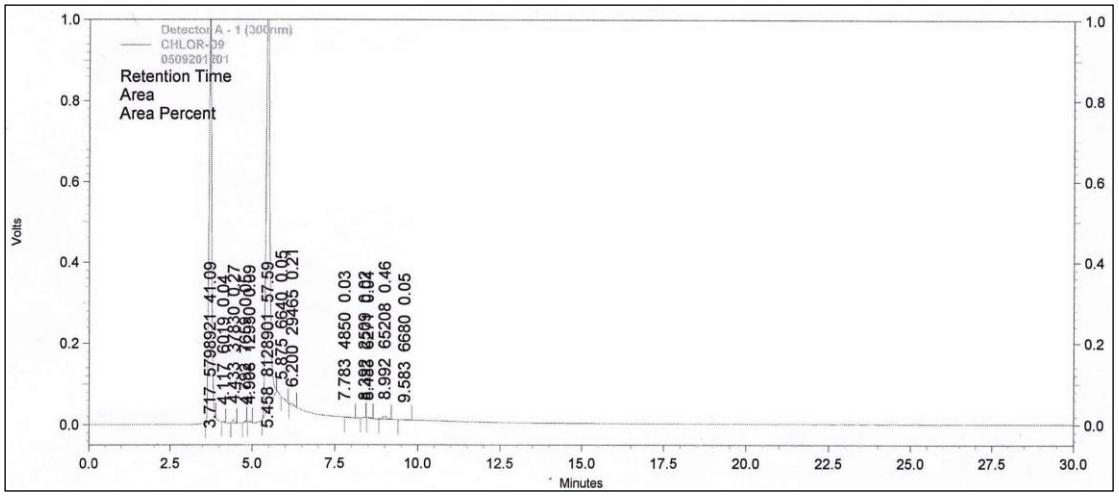



**Figure 2.50**:- HPLC Chromotagram of chlorpyrifos with **13f** (1:1) after irradiation

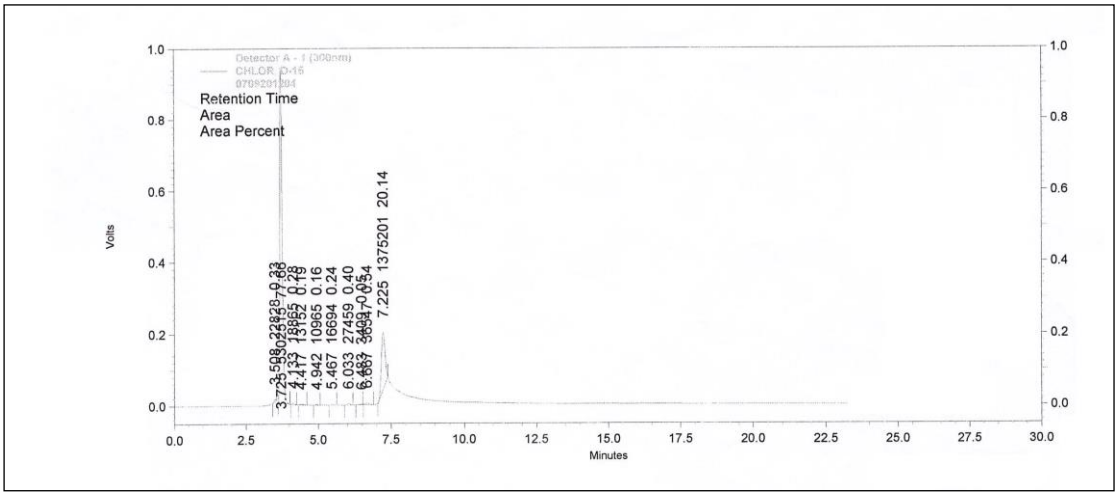



**Figure 2.51**:- HPLC Chromotagram of chlorpyrifos with **13g** (1:1) after irradiation

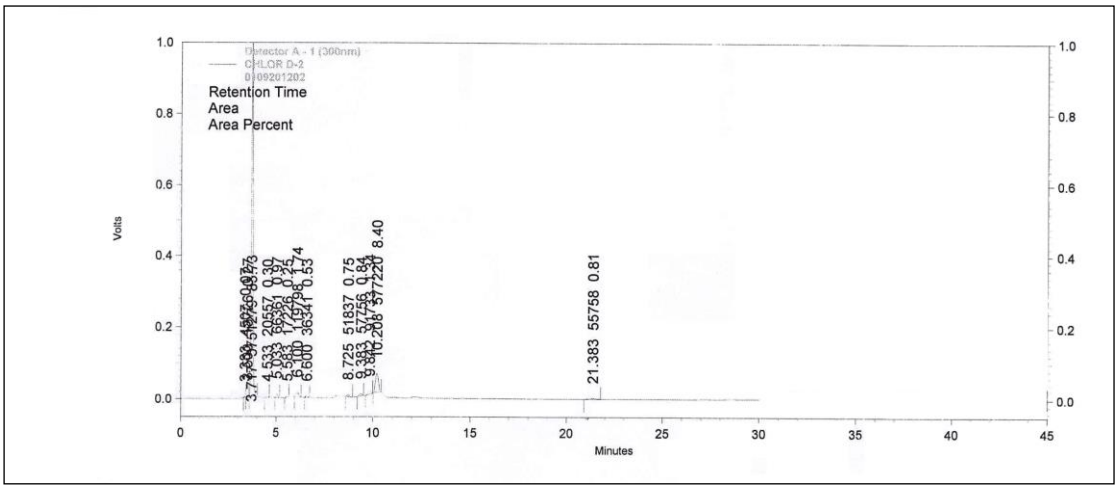



**Figure 2.52**:- HPLC Chromotagram of chlorpyrifos with **13h** (1:1)after irradiation

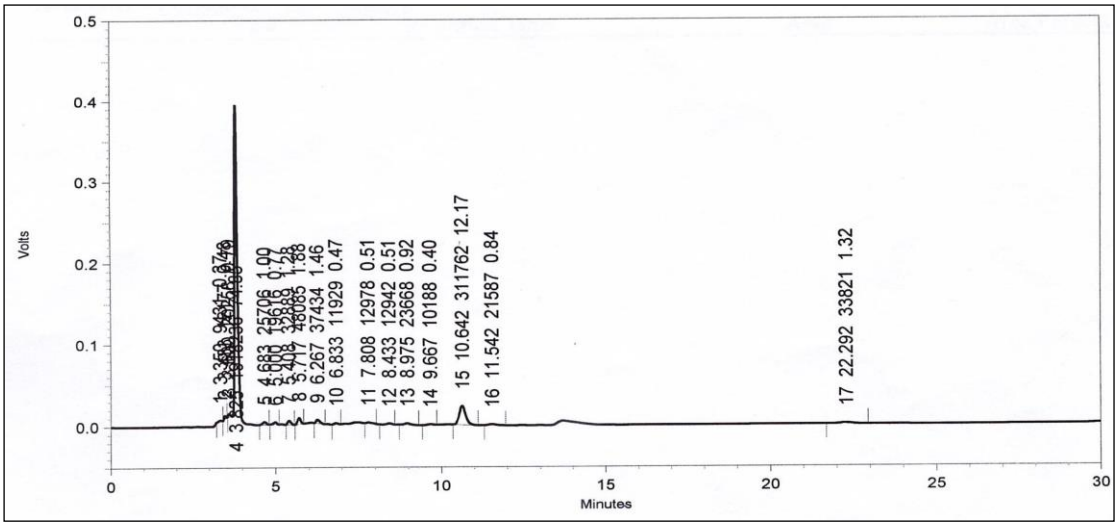



**Figure 2.53**:- HPLC Chromotagram of chlorpyrifos with **13h** (1:0.1) after irradiation

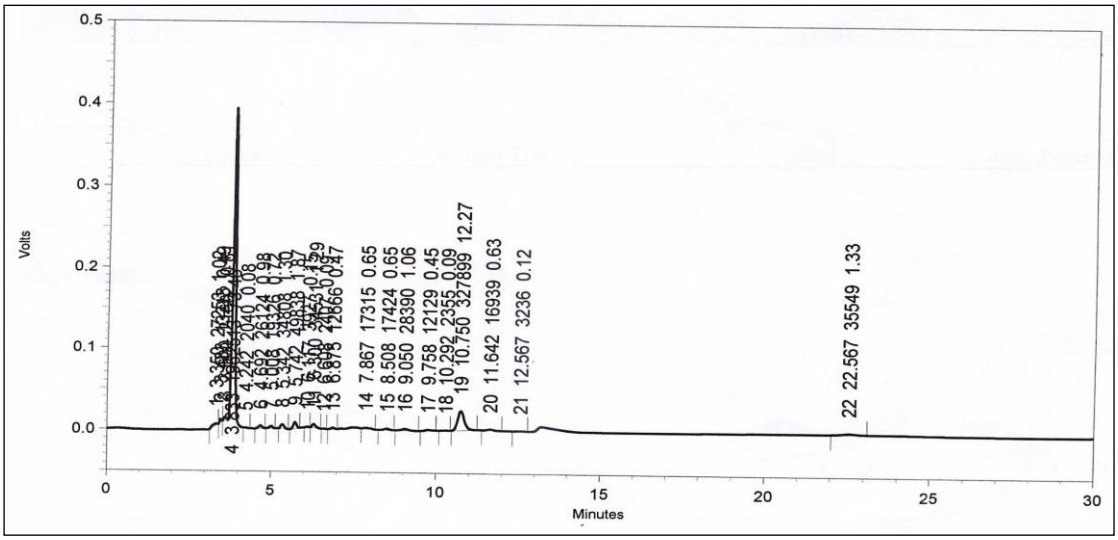



**Figure 2.54**:- HPLC Chromotagram of chlorpyrifos with **13h** (1:0.3) after irradiation

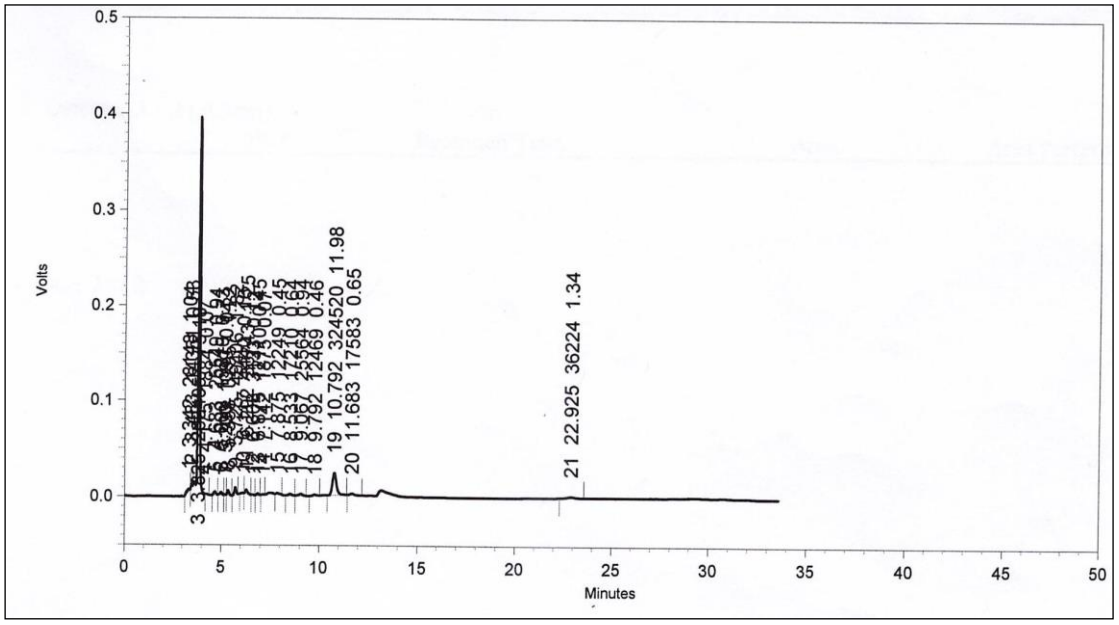
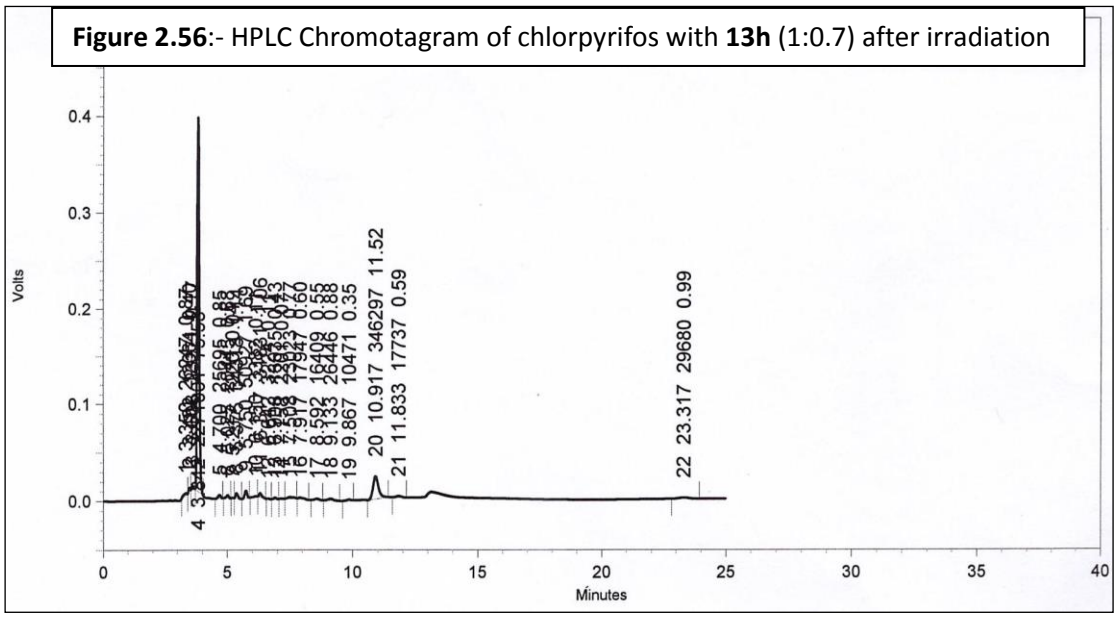



**Figure 2.55**:- HPLC Chromotagram of chlorpyrifos with **13h** (1:0.5) after irradiation

**Figure 2.56**:- HPLC Chromotagram of chlorpyrifos with **13h** (1:0.7) after irradiation



## 2.7 References


1. Kirk-Othmer. *Encyclopedia of Chem. Tech., 3rd Ed.*, 23, Wiley (Inter Science), New York, **1984**, 615; Katagi T. *Rev Environ Contam Toxicol.* **2004**, *182*, 1.
2. Zepp R. G.; Cline D. M. *J. Agric. Food. Chem.* **1977**, *11*, 359.
3. Elliot, M.; Farnham, A. W.; Janes, N. F.; Needham, P. H.; Pullman, D. A. *Nature*. **1974**, *248*, 710.
4. Banerjee, K.; Dureja, P. *Pestic. Sci.* **1995**, *43*, 333.
5. Hussain, M.; Persche, H.; Kutscher, R. *Pestic. Sci.* **1990**, *28*, 345.
6. Hussain, M.; Gan, J.; Perschke, H.; Rather, M. N. *Chemosphere.* **1990**, *21*, 589.
7. Sundaram, K. M. S.; Curry, J. *Chemosphere.* **1996**, *32(4)*, 649.
8. Sundaram, K. M. S.; Curry, J. *J. Environ. Sci. Health B.* **1996**, *31 (5)*, 1041.
9. Caroline, C. *J.Pesticide Reform/ winter.* **1994**, *14(4)*, 15.
10. Barcelo´, D. *J. Chromatography A.*, **1993**, *643*, 117.
11. Chambers, J. E.; Carr. R. L. *Fund.and Appl. Toxicol.* **1993**, *21(1)*, 111.
12. Sultatos, L. G. *Toxicology*. **1991**, *68(1)*, 1.
13. Hirayama, Y.; Sayato, Y.; Nakamuro, K. *Jap. J. Toxicol. Environ. Health*. **1998**, *44(6)*, 451.
14. Allender, W. J.; Keegan, J. *Bull. Environ. Contam. Toxicol*. **1991**, *46*, 313.
15. Dexter, D. L. *J. Chem. Phys.* **1953**, *21*, 836.
16. Kavarnos, G. J.; Turro, N. J. *Chem. Rev*. **1986**, *86*, 401.
17. Leppard, D.; Hayoz, P.; Vogel, T.; Wendeborn, F. *Chimia* **2002**, *56*, 216.
18. Leppard, D.; Waiblinger, F.; Keck, J.; Stein, M.; Fluegge, A. P.; Kramer, H. E. A. *J. Phys. Chem. A* **2000**, *104*, 1100.
19. Paterson, M. J.; Robb, M. A.; Blancafort, L.; DeBellis, A. D. *J. Phys. Chem. A*, **2005**, *109 (33)*, 7527.

20. Deota, P. T.; Upadhyay, P. R.; Patel, K. B.; Mehta, K. J.; Varshney, A. K.; Mehta, M. H. *Natural Product Letters*. **2002**, *16(5)*, 329.

21. Deota, P. T.; Upadhyay, P. R.; Valodkar, V. B. *Natural Product Research*. **2002**, *17(1)*, 21.

22. Pickett, J. E.; Hamid H. S. H*andbook of polymer degradation*, 2nd ed., New York, ed. Marcel Dekker, **2000**, 163.

*23.* Mosquera, M.; Penedo, J. C.; Rodriguez, M. C. R.; Rodriguez-Prieto V. *J. Phys. Chem.* **1996**, *100*, 5398.

24. Woessner, G.; Goelle, G.; Kollat, P.; Stezowski, J. J.; Hauser, M.; Klein, U. K. A.; Kramer, H. E. A. *J. Phys. Chem.* **1984**, *88*, 5544.

25. Ghiggino, K. P.; Scull, A. D.; Leaver, I. H. *J. Phys Chem.* **1986**, *90*, 5089.

26. Otterstedt, J. E. A. *J. Chem. Phys.* **1973**, *58*, 5716.

27. Wegner, G.; Enkelmann, V.; Mohr, B. *J. Org. Chem.* **1994**, *59*, 635.

# Chapter 3

## Biological evaluation of lactol and benzil derivatives

# Chapter 3

## Biological evaluation of lactol and benzil derivatives

# Part A

# Study of Mosquitocidal Activity

### 3A.1 Abstract

The mosquito species, *Anopheles Culicifacies*, a major vector for malaria was reared in the insectary laboratory. All the compounds reported in chapter 1 (**11**, **20**, **46a-h**) were evaluated for their mosquitocidal activity on *Anopheles Culicifacies* using standard WHO specified adult susceptibility test and tunnel test. The results revealed that all the compounds encompassed excellent mosquitocidal activity. Further all the compounds are tested for cytotoxicity on human lung cell line L132. The results of the studies exhibited compounds **20** and **46(a-h)** are less toxic to human body than existing mosquitocidal compounds.

## 3A.2 Introduction

Insect transmitted diseases remain a major source of illness and death worldwide. Mosquito transmit serious human diseases, causing millions of deaths every year.[1] Many mosquito-borne diseases, such as malaria, dengue fever (DF), dengue hemorrhagic fever (DHF) and filariasis, are serious public health problems in tropical regions, especially in Africa and Asia.[2]

Mosquito alone transmit diseases to more than 700 million persons annually.[3] Research shows that malaria kills about 3 million persons each year, including one child every 30 seconds.[4] Although insect borne diseases currently represent a greater health problem in tropical and subtropical climate, no part of the world is immune to their risks.[5]

These diseases are transmitted to human beings through mosquito bite only. Since there is no effective vaccine available for the control of these diseases, prevention of mosquito bites is one of the main strategies to control or minimize incidence of these diseases. The use of insecticide can provide a practical and economical means of preventing mosquito-borne diseases.[2]

### Diseases spread by mosquitoes

### Malaria[6]

Malaria is the most well known mosquito-transmitted illness. Malaria is spread by a particular type of mosquito (the infected *Anopheles* mosquito) found in different parts of the world. People living in or visiting those places can reduce the risk of malaria by taking anti-malarial tablets and taking measures to avoid being bitten.

### Dengue fever[7]

The mosquitoes that transmit dengue fever breed in containers that hold water, and bite during the day, not mainly at dusk or evening like other types of mosquito. People infected with the virus may have no symptoms, but others may experience

high fever, severe headache, muscle and joint pain, rash and extreme fatigue. In rare cases, dengue fever can be severe and even fatal (dengue hemorrhagic fever).

There is no vaccine against dengue fever, so mosquito prevention measures are essential. The mosquito responsible for dengue fever (the infected *Aedes* mosquito) is found in many tropical and subtropical areas. The World Health Organization reports that dengue is found in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific.

**Japanese encephalitis**[8]

Japanese encephalitis is an infection of the brain caused by a virus that is spread by infected mosquitoes. The mosquitoes become infected after biting infected pigs with the virus. Japanese encephalitis occurs in parts of Asia and Papua New Guinea. There have also been cases in north Queensland in Australia. Most infected people have symptoms like headaches, high fever, convulsions and coma. There is no treatment, but a vaccine is available to protect against the infection in people travelling to or resident in, areas where the virus is found.

**Rose River virus infection**[9]

Occurring widely in Australia, the Ross River virus is spread from animals to humans by several different types of mosquitoes. Although many people infected with this virus have symptoms such as fever, joint pain and swelling and a rash. There is no specific treatment but medicines may be taken to help relieve the symptoms.

**Barmah Forest virus infection**[10]

The Barmah Forest Virus is also widespread in Australia and causes a similar illness to Ross River virus infection but the symptoms usually last for a shorter length of time. The virus is spread from animals to humans by mosquitoes. Again there is no specific treatment for this infection, but medicines may be taken to help manage the symptoms.

**Murray Valley encephalitis**[11]

Murray Valley encephalitis is a very rare diseases involving swelling of the brain tissue. The disease is caused by infection with a virus that is spread by the bite of infected mosquitoes. The mosquito responsible is found throughout Australia and breeds in surface pools of water. Water birds, such as herons, are a natural reservoir of the virus. Most people infected with the Murray Valley encephalitis virus do not develop symptoms, but others may have high fever, severe headache, neck stiffness, irritability, seizures (or fits), and drowsiness. Immediate medical advice should be sought for these symptoms.

**Yellow fever**[12]

Yellow fever occurs in area such as Africa and South America, and is spread by infected *Aedes* and *Haemogogus* mosquitoes. It can be spread by mosquitoes in jungles/rural areas as well as urban areas. Yellow fever causes a flu-like illness, but some people develop a more severe form which can be life threatening. The 'yellow' relates to jaundice which occurs as part of the severe illness. No specific treatment is available other than supportive measures.

Among all, malaria is infecting approximately 110 million people annually and causes 2-3 million deaths.[13] The worldwide threat of arthropod-transmitted diseases, with their associated morbidity and mortality, underscores the need for effective mosquito control.

**Mosquito control**

Many techniques have been developed for the mosquito control like mechanical methods, non-chemical methods and chemical methods.

**(A) Mechanical Methods**

Recently two mechanical methods (Electric mosquito zapper and Mosquito magnet) are developed and they are widely used in the daily life.

**1. Electric mosquito zapper**

An electric zapper works by using ultraviolet light to lure in bugs and then kills them upon contact with its lethal dose of electrical charge.[14]

**2. Mosquito Magnet**[15]

The mosquito magnet mimics mammals by giving off carbon dioxide, heat and moisture. Once the mosquito gets close to the magnet, it is sucked in and eventually dies of dehydration. These are combined with an attractant, octenol which is a natural plant pheromone. As an advantage, the mosquito magnet not only captures mosquitoes, but also kills biting midges, black flies, and sand flies. It vacuums the insects into a net where they dehydrate and die.

**(B) <u>Non-chemical methods</u>**

**1. Physical method**

Emptying the stagnant water in rain gutters, old tires, buckets, plastic covers, in bird baths, fountains, pools, rain barrels etc are the common physical methods for controlling the mosquito breeding. During the dawn and dusk times, full-sleeved clothing is also highly essential as the chance of mosquito bite is highest during this period.

**2. Mosquito Net**

Mosquito nets are considered as a better protection from mosquitoes than other mosquitocidal and mosquito repellents that cause health hazards. There are two type of mosquito nets available, non medicated and medicated nets.

- **Non Medicated Net**[16]**:** Mosquito netting is a protective covering that prevents mosquitoes and other insects from biting. Different shapes and sizes of mosquito netting are available and they also come in different materials such as cotton, polyester, and polyamide. It is crucial to find a net that has a mesh size larger enough to allow air to circulate, but small enough to keep the mosquitoes out. Mosquito nets can be used to cover small and large areas such

as bed, porch, etc. Mosquito nets are generally considered as an effective way to naturally combat mosquitoes.

- **Medicated Net**[17]**:** Existing mosquito nets could be medicated by using mosquitocidal compounds such as deltmethrin, alpha-cypermethrin, cyfluthrin, etc. The nets are medicated by soaking them in the solution of mosquitocidal compound for 10 minutes followed by drying. The effect of the medicine lasts for about six months. Window meshes could also be medicated in a similar fashion.

- **Mosquito traps**[18]**:-**

  Mosquito traps lure and capture female mosquitoes. The traps mimic the different mosquito-attractants such as exhaled carbon dioxide, human scents and body heat. Attracted by these chemicals, the insect approaches and an impeller fan draws it in. It then adheres to a sticky surface on the device and is eventually electrocuted. Mosquito traps are powered by electricity and are a safe, chemical free method of mosquito control.

**(C) Chemical methods**

A number of natural and chemical compounds are used for the control of mosquitoes. Some compounds repel the mosquitoes whereas some of them impart mosquitocidal activity for the mosquitoes.

**1. Use of repellents**[19]

Generally, the repellents are applied to skin, clothing or other surfaces which discourages insects (and arthropods in general) from landing or climbing on that surface. Synthetic as well as natural chemical compounds are used as repellents. Synthetic repellents are more effective or long lasting than natural repellents as natural repellents evaporates in short period of time. DEET (N,N-diethyl-m-toluamide), Icaridin, Nepetalactone (catnip oil), IR3535 (3-(N-Butyl-N-acetyl)-

aminopropionic acid, ethyl ester), dimethyl *cis*-5-norbornene-2,3-dicarboxylate, 2-hydroxy-α,α,4-trimethylcyclohexanemethanol, Tricyclodecenyl allyl ether are widely used as repellents. Along with their effectiveness, they also impart several human health problems such as rashes, swelling, eye irritation, brain swelling in children, anaphylactic shock and low blood pressure. DEET exposure was more likely to have insomnia, mood disturbances, impaired cognitive function, cause dizziness and can severely irritate the skin. DEET may even cause cancer and defect in child birth.

**2. Use of insecticides**[20]

Insecticides act upon the nervous system of the mosquito or act as growth regulators or endotoxins. Different synthetic insecticides are used to kill the mosquitoes such as DDT (dichlorodiphenyltrichloroethane), fenitrothion, malathion, pirimiphos-methyl, propoxur, bendiocarb, alpha-cypermethrin, cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and bifenthrin. Like repellents, insecticides also effect the human health severely. They cause abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems.

Among all the mosquito control methods, chemical method gives the best effect in the short period of time and also lasting for a long time. The natural insecticides are quite impressive in terms of human health hazards but they are not as much effective as synthetic insecticides for killing the mosquitoes. The synthetic insecticides kill the mosquitoes effectively but also adversely impart toxicity to human body. It is necessary to develop some novel class of synthetic mosquitocidal compounds which have less human toxicity.

### 3A.3 Results and discussion

Genus Anopheles consists of 420 species of which 58 species are found in India. Out of these 58 species, 6 species viz. *An.Culicifacies* (rural), *An. stephensi* (urban), *An. fluviatilis* (foothill), *An. dirus* (deep forest), *An minimus* (foothill and fringe) *and An. Sundaicus* (coastal, only in Anfaman and Nicobar Island) transmit one of major disease of the country i.e. malaria to the human body.

**Figure 3A.1.** Anopheles mosquito

Malaria control strategies are specific therefore identification of mosquitoes has great significance. Head wings, legs and abdomen are the main part of the body of mosquitoes. Genus *Anopheles* is identified[21] by palpi characters of them. *Anopheles* mosquitoes (Figure 3A.1) are morphologically identified elongated palpi, which are equal to proboscis, slender in females and club shaped in males. Subgenus and species identified by wing observation. In *Anopheles culcifacies* specking in fore leg and hind leg, band on fore leg tarsomeres absent and wing vein is mostly dark and inner quarter of costa is interrupted. *Anopheles* mosquitoes are active between sunset and sunrise. Each species has specific peak biting hours, and there are also variations in their preference for biting indoors or outdoors.[22]

The anopheles mosquitoes that enter houses to feed are often take rest indoors for a few hours after feeding. They may then leave for outdoor sheltered resting sites such as vegetation, rodent burrows, cracks and crevices in trees or in the ground, caves and the undersides of bridges. Alternatively, they may stay indoors for the whole period needed to digest the blood-meal and produce eggs. Indoor resting is most common in dry or windy areas where safe outdoor resting sites are scarce. Once the eggs are fully developed the gravid mosquitoes leave their resting sites and try to find a suitable breeding habitat.

## Life cycle of the Anopheles mosquito

The immature and adult stages of mosquitoes are passed in two completely different environments. The immature stages (i.e., eggs, larvae, and pupae) require an aquatic environment whereas adult stage require non aquatic environment. The four successive stages of development in the life cycle of *anopheles mosquitoes* are briefly described as under.

### Eggs[23]:

The female anopheles mosquito will lay 60-100 eggs after a blood meal. *Anophelines* lay their eggs separately over the surface of water (Figure 3A.2), each eggs having lateral air floats to keep it afloat. *Culicines* of genus *Culex* and *Mansonia* lay their eggs on the water, in a boat-shaped mass referred to as an egg raft whereas those of genus *Aedes* are laid separately, often in dry hollows or containers or discarded containers which become flooded after rain. These "dry-laid" eggs are able to retain their viability without water for very long periods.

**Figure 3A.2.** Eggs of anopheles

**Larva**[23]**:**

Eggs of mosquitoes generally hatch after two or three days in contact with water. The larva is about 1.5 mm long when newly hatched and about 10mm long when fully grown (Figure 3A.3). During growth, the larva cast its skin four times, the stages between successive moult being known as instars. The larva of mosquito is made up of head, thorax and abdomen- the last being composed of nine distinct segments. A mosquito larva breathe through two orifice, called spiracles; those of the anopheline being situated on the eight abdominal segments so that in order to breathe, the larva rests in horizontal position at the surface of water.

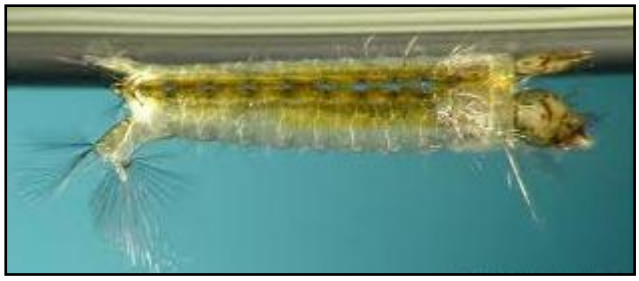


**Figure 3A.3.** Larva of anopheles mosquito

**Pupa**[23]**:**

The pupa is a non-feeding stage, required for the morphological and physiological changes to occur for the transformation of the larvae to the adult. The general appearance of the pupa is of a comma with an exaggerated "dot" and small "tail". The "dot" is occupied by the head and thorax while the "tail" encases the abdomen which terminates in a pair of paddles (Figure 3A.4). The pupa is mobile and able to dive rapidly when disturbed. When quiescent, the pupa rests at the surface of water, suspended by a large air cavity within its body. Breathing is carried out, at the surface of water, by a pair of respiratory

**Figure 3A.4.** Pupa of anopheles mosquito

trumpelts extending from the thoracic area. In general, *culicines* pupae can be distinguished from *anopheline* pupae by their considerably longer respiratory trumplets.

**Adult:**

After one to three days, the adult mosquito is ready to emerge. The pupal skin splits along the top of the case. The adult mosquito slowly and carefully works its way out of the pupal case. After emerging it will float on the surface of the water and rest there until its body and wings harden. Once the body has hardened the mosquito will fly off to begin its new life.

**Collection of mosquito from field**

It is necessary to collect the mosquitoes from the field for rearing in the entomology laboratory. There are two different way of rearing the mosquito into the laboratory. One way is to collect the larvae from the field, separate them according their species and emerge them. Another way is to collect the adult mosquito from the field and allow them to mate in the laboratory to obtain egg.

**Larvae collection:**

The dipping method is used for collection of mosquito larvae. A dipper of 9.5 cm diameter and 300 ml made up of stainless steel with long handle or white enamel bowl is used to collect larvae from the edge of mosquito breeding habitats. A rectangular or round frying pan about 25 cm in diameter with a long handle, is useful for collecting larvae from more inaccessible parts of the breeding places. Ladles with diameter 5 to 10 cm were used only for collecting larvae from very small breeding places. The collecting instruments (enamel bowl, Frying pan and ladle) were immersed in the breeding places at an angle of 45%. The surface water is flown in to the cavity but care should be taken not to fill this completely as otherwise some larvae would have been washed out. If the dipper is immersed too slowly, the larvae would be distributed and would go to the bottom with the result that may escape the larvae. Therefore, the site was approached carefully. There was an interval of 2 to 3 minutes between each dip to allow larvae and pupa to return to the surface. When water is

cool, larvae may remain deep within the breeding place for several minutes and was not collected through repeated dipping at short intervals.

Small pipettes (glass tube with a rubber tube) may be used for collecting larvae from the surface of the breeding place. This method mostly used with combination if dipping.

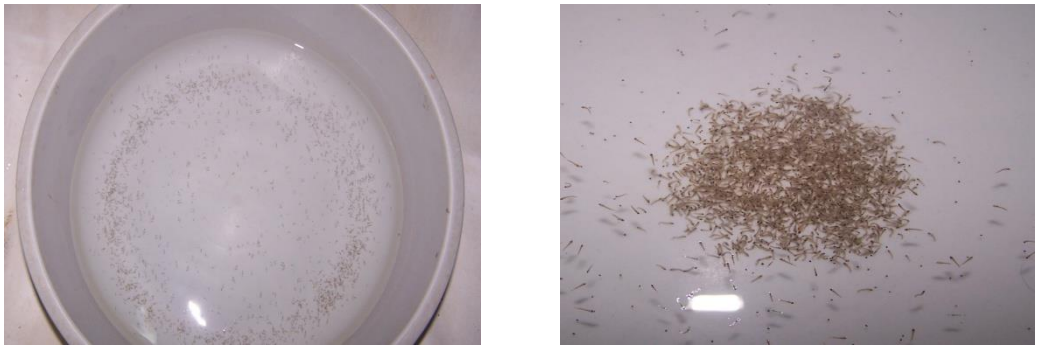


**Figure 3A.5.** Larvae collected from the field

**Figure 3A.6.** Transfer of larvae from field water to fresh water

Larva and pupa is collected in plastic container (Figure 3A.5) and then transferred into bowl containing fresh water (Figure 3A.6) and reared in laboratory with optimum temperature condition of 28±1 °C and 75±3% relative humidity. Larvae fed on mixture of powder form of dog biscuits and yeast tablets at the rate of 60:40 respectively.

**Adult mosquito collection:**

In the present study hand collection and Pyrethrum spray collection methods were being used to collect the adult mosquitoes from the field.

**1) Hand collection:**

Mosquito resting on different surfaces (indoor or outdoor) are collected by using a sucking tube (suction tube or mouth aspirator). In this method most commonly used type, the suction tube or aspirator is composed of glass or plastic tube of 15 mm diameter, a rubber tube and a mouth piece. The mosquito is located with the help of a torch light and the end of glass tube is brought near the mosquito and the air is sucked in with the help of mouthpiece. The mosquito is blown in the glass tube with the air current and a fingertip is placed over the opening of the glass tube to prevent the escape of the mosquito. The specimen thus collected can then be transferred to the test tube or a plastic cup, and then immediately plug the test tube with cotton plug or plastic cup covered with net, so that the mosquitoes in live form can be brought to the laboratory.

**2) Pyrethrum spray collection:**

This method consists of the collection of indoor resting mosquitoes on white cotton sheet (bad sheet) after knock-down by space spraying a pyrethrum solution. The mosquitoes are collected during the day time, usually early in the morning between 06.30 hours, and 10.00, depending on the situation and the objective. The floor surfaces of the room selected for the spray are completely covered from wall to wall with white sheets. All other opening in the door, window, and eaves are closed with available material so as to minimize escape of mosquitoes through them. The collector starts to spray pyrethrum solution (0.2% in kerosene) with the help of hand atomizer (hand pump) in space. After filling the room with the insecticidal mist, the collector leaves the room/hut and closes the door. Ten or fifteen minutes after the door is opened and the mosquitoes are collected with the help of torch and entomological forceps, starting at the door and moving to the interior of the room. Mosquitoes are picked up from the sheet and transferred either to paper cup or Petri dish which are prepared to ensure enough humidity for the mosquitoes.

**Rearing of *Anopheles culicifacies***

The *Anopheles culicifacies*, a major vector for malaria was reared in the entomology laboratory at the National Institute of Malaria Research, Nadiad field

**Figure 3A.7.** Mosquito cage

**Figure 3A.8.** Larvae of *Anopheles Culicifacies* collected from the field in fresh water

**Figure 3A.9.** Pupa of *Anopheles Culicifacies* collected from the field in fresh water

station, Gujarat, India. The adults of *Anopheles culicifacies* collected from human dwellings in Nadiad town in the morning hours were transferred into a mosquito cage (60 cm x 60 cm x 60 cm) (Figure 3A.7). The cyclic colony was maintained at optimum condition of 28±1°C temperature and 75±3% relative humidity. The mosquitoes were fed on water soaked raisins and 10% glucose solution soaked in cotton pads. The females were offered blood meal during night on alternate days on back and belly shaved rabbits. Eggs were collected in plastic bowls containing water and held in the same water for emergence of first instars larvae. The newly emerged larvae were transferred to rearing trays (30 cm x 25 cm x 5 cm) made up of plastic (Figure 3A.8 and 3A.9). The larvae were fed on a mixture of powdered dog biscuits and powdered yeast. The water of the rearing trays was changed on alternate days to avoid decay and larval mortality. The pupae were collected from the rearing trays and were transferred to the adult cages for emergence. Light was provided to the colony by two 40-watt fluorescent tubes and a 60-watt bulb with a photoperiodicity of 12 hours of darkness and 12 hours of light.

Once mosquitoes are reared in the entomology laboratory, all female mosquitoes are kept without blood meal in the cage. They were fed on water soaked raisins and 10% glucose solution soaked in cotton pads. These non blood fed 5-8 days old mosquitoes are used for the mosquito pathogenicity studies.

**Tunnel tests**

The tunnel has three components, two cages at both ends and central glass case as shown in Figure 3A.10. For the tunnel test, at one third of the length, a disposable cardboard frame is placed with the treated net (5% **46a**). Then 100 non-blood fed female anopheles mosquitoes, aged 5–8 days are released in a tunnel at 18:00 (Figure 10). Females are free to fly in the tunnel but have to make contact with the piece of netting and locate the holes in it before passing through to reach the rabbit. The following morning, at 09:00, the mosquitoes are removed and counted separately from each section of the tunnel and the immediate mortality is recorded. Live females are placed in plastic cups with honey solution; delayed mortality is recorded after 24 hours. During tests, cages are maintained at 28±1°C and 75±3%

relative humidity under subdued light. Several tunnels will be used simultaneously, one tunnel with untreated netting always being used as a negative control. Blood feeding inhibition is assessed by comparing the proportion of blood fed females (alive or dead) in treated and control tunnels. Overall mortality is measured by pooling the immediate and delayed (24-hour)

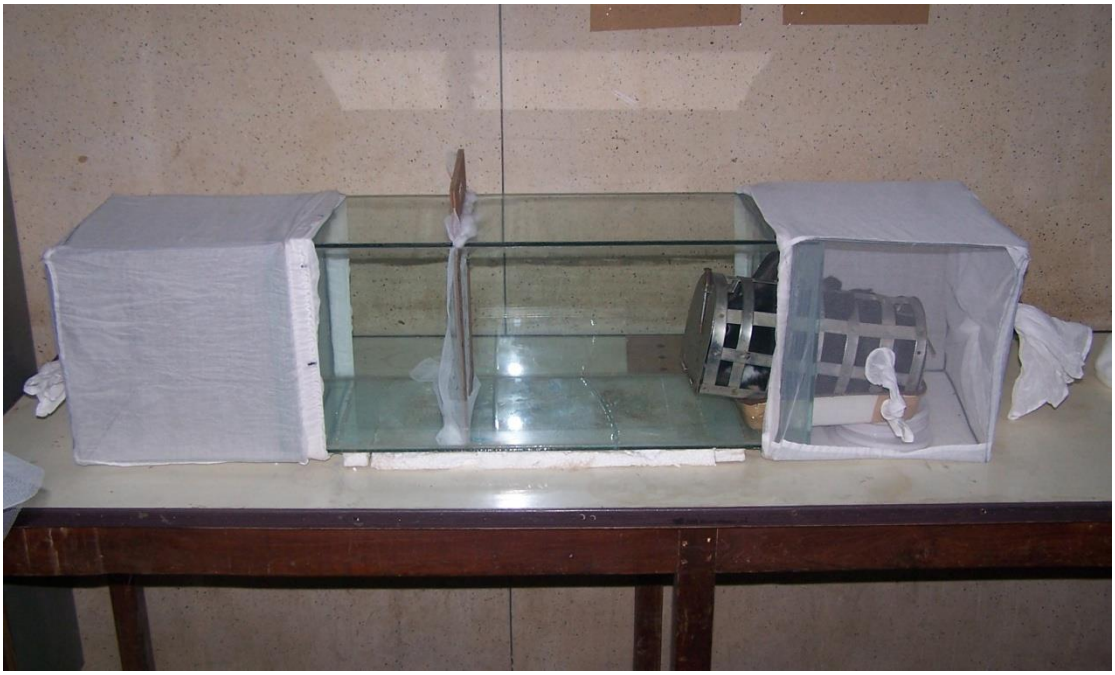


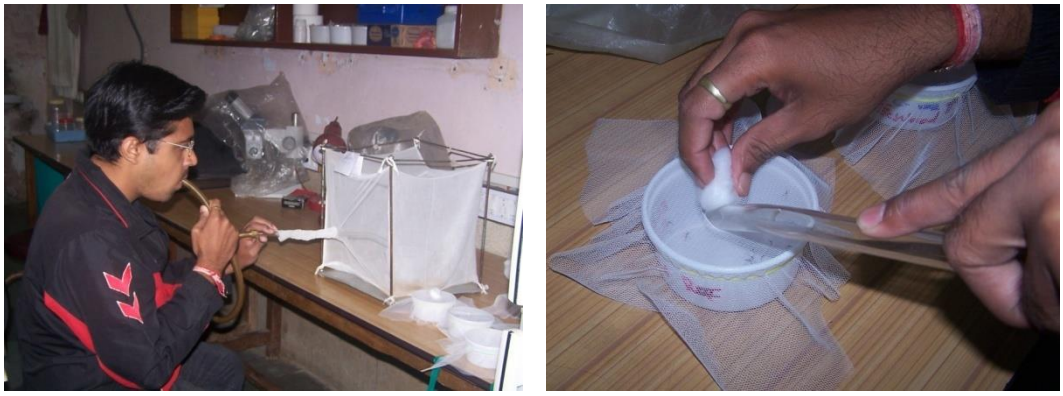
**Figure 3A.10.** Tunnel test assembly with rabbit at one end

mortalities of mosquitoes from the two sections of the tunnel. All the mosquitoes are found dead (i.e. 100% mortality) after 24h of exposure. Only 10 % of the mosquitoes are able reached to the rabbit side and they all are died at that end. This test was performed to check the compound is mosquito repellent or mosquito pathogen. Once it was known (from the tunnel test) that this compound (**46a**) show mosquito pathogennicity, the Adult susceptibility test was done next in order to check the exact concentration required for mortality and exposure time.

**Adult susceptibility test**

Further studies involved testing of mosquitocidal activity of all the synthesized compounds (**11, 20, 46a-h**) using standard WHO specified adult susceptibility test method and kit.[24] The 5-10 days adult glucose-fed healthy female mosquitoes were used to check the adult susceptibility test (Figure 3A.11, 3A.12). Mosquitoes are

introduced into the holding tubes through the small orifice in the slide and closed (Figure 3A.12).



**(a)** **(b)**

**Figure 3A.11.** (a) Collecting mosquito from cage using suction tube; (b) transferring mosquito from suction tube to bowl

**(a)** **(b)**

**Figure 3A.12.** (a) Mosquito counting in bowl; (b) Transferring mosquito from bowl to adult susceptibility kit

**(a)** **(b)**

**Figure 3A.13.** (a) Exposure of mosquitoes on impregnated paper; (b) Exposure of mosquito on non-impegnated paper

The holding tubes are appropriately labeled with locality, species, etc. and provided with glucose source. After 30 min (to 2.5 hrs) holding period to observe for injured and dead mosquitoes, green dot tubes with insecticide-control papers and red dot tubes with insecticide impregnated papers are screwed to the respective holding tubes (Figure 3A.13). During exposure glucose source should be removed. Exposures are made for the specified period and held for 24 h of holding period and mosquitoes should be provided with glucose source. Susceptibility levels are determined by scoring the dead and alive mosquitoes and expressed as percent mortality.

The adult susceptibility test parameters like concentration of impregnated paper and exposure time were optimized. The **46a** was used as model insecticide for the optimization of concentration of impregnated paper and the exposure time.

**Table 3A.1.** Optimization of concentration of impregnated paper and exposure time

| **Concentration** | **No. of mosquitoes exposed** | **No. of mosquitoes knocked down in 0.5 h** | **No. of mosquitoes dead after 1 h exposure** | **No. of mosquitoes dead after 1.5 h exposure** | **No. of mosquitoes dead after 2 h exposure** | **No. of mosquitoes dead after 2.5 h exposure** |
|---|---|---|---|---|---|---|
| Control[a] | 20 | 0 | 0 | 0 | 0 | 0 |
| 1%[b] | 20 | 20 | 8 | 9 | 11 | 12 |
| 2.5%[b] | 20 | 20 | 10 | 12 | 15 | 16 |
| 5%[b] | 20 | 20 | 14 | 15 | 18 | 20 |

[a] two replicates were used; [b] five replicates were used

**Village:**Kanjari; **District:**Anand; **Temperature:** 28±1°C; **Humidity:** 75±3%;
**Test species:** *Anopheles culicifacies*.

First the concentration of impregnated paper was optimized. The results of the optimization study reveal that **46a** exhibits 100 % mortality at 5% concentration of the impregnated paper. Then optimization of exposure time was done. On 30 min exposure of **46a**, the entire group of mosquitoes were found to become unconscious. After the completion of the holding period, it was observed that many of them become alive. In order to get the exact exposure time for 100% mortality, 1h, 1.5h, 2h and 2.5h exposure of impregnated **46a** were given to mosquitoes. Among all, 2.5h exposure time gave 100% mortality. Thus, 5% concentration of impregnated paper and 2.5h exposure time were used as an optimized condition for the adult susceptibility test (Table 3A.1).

By following the optimized condition, all other compounds were studied for mosquito pathogenicity. The five test replicate of each compounds and respective two controls were run simultaneously. Mortality was determined by scoring the dead and alive mosquitoes after 24 hours and results were expressed as percent mortality. At 5% concentration on 2.5 hours of exposure time, the *trans*-diol **11** exhibited only 10% and lactol **20** showed 55% mortality whereas all the acetal derivatives **46(a-h)** imparted 100% mortality (Table 3A.2).

**Table 3A.2:** Adult susceptibility test for **11, 20, 46(a-h)**

| Sample Code | No. of mosquitoes exposed | % Knocked down in 0.5 h | % Knocked down in 2.5 h | % Mortality after 24 h |
|---|---|---|---|---|
| Control[a] | 40 | 5 | 10 | 0 |
| 11[b] | 100 | 30 | 30 | 10 |
| 20[b] | 100 | 60 | 60 | 55 |
| 46a[b] | 100 | 100 | 100 | 100 |
| 46b[b] | 100 | 95 | 100 | 100 |
| 46c[b] | 100 | 95 | 100 | 100 |
| 46d[b] | 100 | 100 | 100 | 100 |
| 46e[b] | 100 | 95 | 100 | 100 |
| 46f[b] | 100 | 85 | 100 | 100 |
| 46g[b] | 100 | 95 | 100 | 100 |
| 46h[b] | 100 | 100 | 100 | 100 |

[a] two replicates were used; [b] five replicates were used; each replicate having 20 mosquitoes.

**Village:** Kanjari; **District:** Anand; **Insecticide dose (%):** 5 %; **Temperature:** 28±1°C; **Humidity:** 75±3%; **Test species:** *Anopheles culicifacies*.

**Table 3A.3:** Mosquitocidal activity of existing insecticides

| Insecticide | Concentration | Exposure time |
|---|---|---|
| DDT | 4% | 1h |
| Malathion | 5% | 2h |
| Propoxur | 0.1% | 1h |
| Deltamethrin | 0.05% | 1h |
| Permethrin | 0.75% | 1h |

**Test species:** *Anopheles culicifacies*

The comparison of existing mosquitocidal compounds and the synthesized compounds shows that all the acetal derivatives **46(a-h)** of lactol showing equipotent mosquitocidal activity as compared to DDT and malathion but exhibited modest mosquitocidal activity against pyrethroids and carbamates insecticides (Table 3A.3)

**Study of cytotoxicity**

The excellent mosquito pathogenicity of all acetal derivatives of lactol prompted us to investigate its human toxicity. All the existing mosquitocidal compounds impart less to acute toxicity to human body either by contact or inhalation or ingestion.

The cytotoxicity of lactol **20** and its all acetal derivatives **46(a-h)** were studied on human lung cell line L132. The cytotoxicity study revealed that as the concentration increases, the

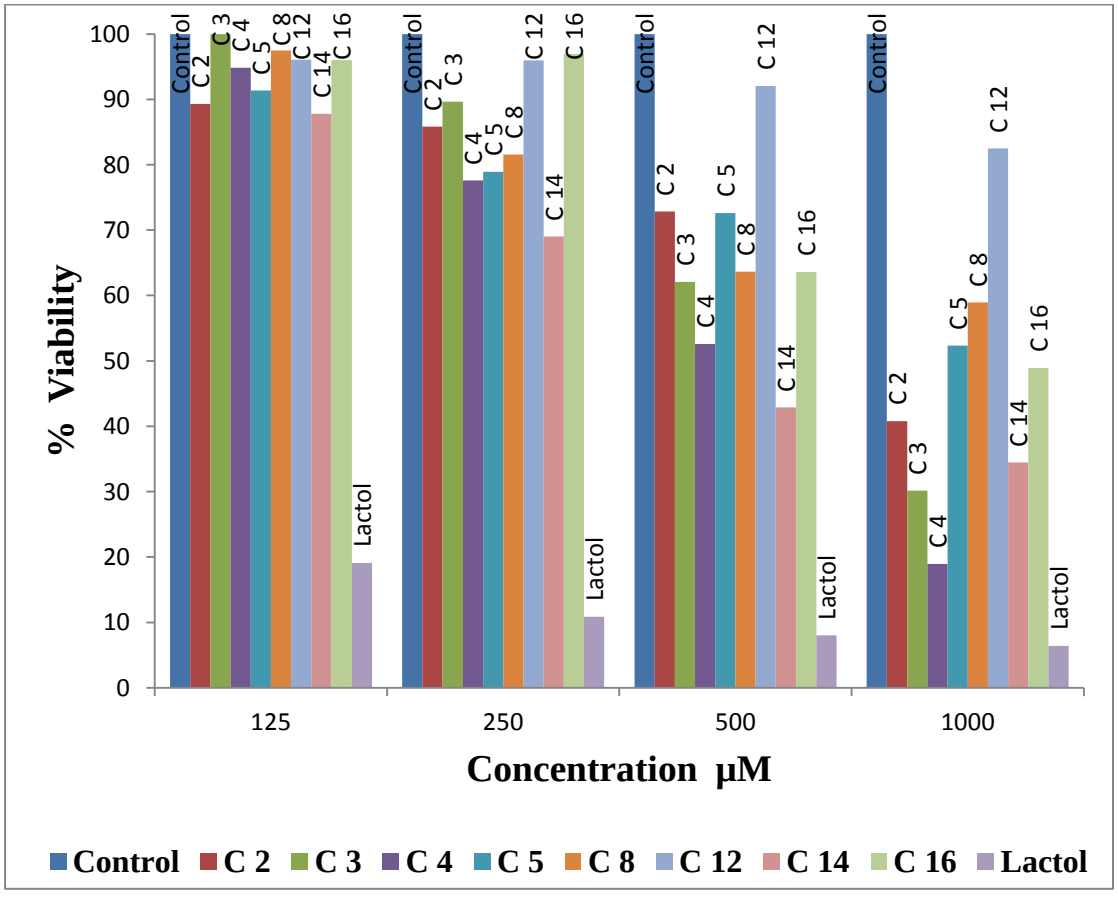



**Figure 3A.14.** Cytotoxicity study of lactol **20** and its acetal derivatives **46(a-h)**

% viability of the cell decreases. At 125 µM concentration, lactol showed 19% viability i.e. more than 80% cells were dead whereas all acetal derivatives of lactol showed more than 90% viability i.e. only 10 % cells were dead. All the acetal derivatives of lactol **46(a-h)** are less toxic to human lung cell line than lactol (Figure 3A.14).

Literature survey revealed that mosquitocidal compounds like pyrethriod, organochlorine, organophosphorous insecticides exhibit cytotoxicity. They display 100% mortality i.e 0% viability in the range of 0.5 µM to 100 µM concentration on human cells.[25] It should be noted that all the compound (**20**, **46a-h**) exhibit only 10% mortality even at 125 µM concentration which is much lesser than the existing mosquitocidal compounds.

### 3A.4 Experimental

**Tunnel test**

The tunnel has three components, two cages at both ends and central glass case as shown in Figure 3A.10. At each end of the tunnel, a 25-cm square cage is fitted (extension) and covered with polyester netting. At one third of the length, a disposable cardboard frame is placed with the treated net (5% of **46a**). The surface of netting "available" to mosquitoes is 400 $cm^2$ (20 cm x 20 cm), with nine holes each 1 cm in diameter: one hole is located at the centre of the square and the other eight are equidistant and located at 5 cm from the border. In the shorter section of the tunnel, a rabbit is placed, unable to move. In the cage at the end of the longer section of the tunnel, 100 females are introduced. During tests, cages are maintained at 28±1°C and 75±3% relative humidity under subdued light. Several tunnels were used simultaneously, one tunnel with untreated netting always being used as a negative control. Blood feeding inhibition was assessed by comparing the proportion of blood fed females (alive or dead) in treated and control tunnels. Overall mortality was measured by pooling the immediate and delayed (24-hour) mortalities of mosquitoes from the two sections of the tunnel.

**Adult Susceptibility Test**

The kit for conducting the test is supplied by Vector Control Research Unit, School of Biological Sciences, 11800 University Sains Malaysia (USM), Penang, Malaysia.

**Kit:** The kit supplied by WHO include green dot and red dot plastic tubes of 125 mm (length) and 44 mm (breadth) with one end with 16 mm mesh screen; slide-units with screw cap on either side with a large orifice to transfer the mosquitoes and a small orifice for introduction of mosquitoes by aspirator; copper and steel clips; instruction sheet; report forms; glass aspirators with 60 cm rubber tubing and mouth piece; roll of adhesive tape and white paper sheets (12 x 15 cm).

**Impregnated papers:**

The determination of diagnostic concentrations was done with a graded series of dosages of compounds **11**, **20, 46a-h** applied to sheets of filter-paper. Rectangular pieces of filter paper measuring 12 x 15 cm (Whatman No. 1 or equivalent) were impregnated with 2 ml of solvent (ethanol) and mixed with a non-volatile carrier (silicon oil). Oil allows the production of a stable, thin and homogeneous layer of the active ingredient on the paper and prevents crystallization of those active ingredients that are solid at room temperature. The concentrations are generally expressed as the percentage of active ingredient per unit volume of carrier on the filter-paper (the acetone being volatile). Filter papers impregnated with different diagnostic dose (1 %, 2.5 %, and 5 %) of all synthesized compounds **(11, 20, 46a-h)** are used.

**Method:** Tubes with green dot were used for holding of mosquitoes and for control exposures. Tubes with red dot were used for synthesized compounds or insecticide exposures. The green dot tube was lined from inside with a plain paper fastened with a steel clip and later fixed to the slide by threading into screw cap. As needed, the required number of green dot tubes were lined from inside with insecticide-control papers duly fastened with steel clips and red dot tubes lined with insecticide impregnated papers of the designated dose and fastened with copper clip. Mosquitoes were introduced into the holding tubes through the small orifice in the slide and closed. The holding tubes were appropriately labeled with locality, species, etc. and provided with glucose source. After 30 min (to 2.5 hrs) holding period to observe for

injured and dead mosquitoes, green dot tubes with insecticide-control papers and red dot tubes with insecticide impregnated papers were screwed to the respective holding tubes. During exposure glucose source was removed. Exposures were made for the specified period and held for 24 h of holding period and mosquitoes were provided with glucose source. Susceptibility levels were determined by scoring the dead and alive mosquitoes and expressed as percent mortality. After each exposure the kit was washed with soap and clean water and dried.

**Method for cytotoxicity study**

Thiazolyl Blue Tetrazolium Bromide (MTT) is soluble in water (10 mg/ml), ethanol (20 mg/ml) and is also soluble in buffered salt solutions and culture media (5 mg/ml). Reconstituted MTT solution is stable for at least 6 months when stored at -0°C. Storage at 4°C for more than four days will result in decomposition and will yield erroneous results.

MTT Solution: 5 mg/ml MTT was added in PBS (Phosphate Buffered Saline) and then the solution were filter and sterilized after adding MTT.

**Procedure:**

Short 96 well assay: Each condition was done in triplicate.

Day one

Trypsinize one T-25 flask was taken and added 5 ml of complete media to trypsinized cells followed by centrifugation in a sterile 15 ml falcon tube at 500 rpm in the swinging bucked rotor for 5 min. Then the media was removed and re-suspended cells to 1.0 ml with complete media. The cells per ml were counted and recorded. The cells (cv = cv) were then diluted to 75,000 cells per ml using complete media. Then 100 µl of cells (7500 total cells) were added into each well and allowed to incubate overnight.

Day two

The cells were treated on day two with the synthesized compounds (**11, 20, 46a-h)**.

Day three

The 20 µl of 5 mg/ml MTT was added to each well including one set of wells with MTT but no cells (control). Then they were incubated for 3.5 hours at 37°C in culture hood. After completion of incubation period, media was carefully removed without

disturbing cells. Then 150 µl MTT solvent were added, covered it with tinfoil and agitate cells on orbital shaker for 15 min. The absorbance at 590 nm was recorded with a reference filter of 620 nm.

### 3A.5 Conclusion

All the synthesized compounds reported in chapter 1 (**11, 20, 46a-h)** were evaluated for their mosquitocidal activity on *Anopheles Culicifacies* using standard WHO specified adult susceptibility test and tunnel test. The results revealed that all the compounds displayed excellent mosquito pathogenicity except **11** and **20**. Further studies of cytotoxicity of lactol and its acetal derivatives on human lung cell line L132 exhibited compound **20** and **46(a-h)** are less toxic to human body than the existing mosquitocidal compounds.

## 3A.6 References


1. Pozharskii, A. F.; Soldatenkov, A. T.; Katritzky, A. R. "An Introduction to Heterocyclic Chemistry and Biochemistry and the Role of Heterocycles in Science, Technology, Medicine and Agriculture". John Wiley & Sons Ltd, **1997**.
2. Richard, J. P.; Anthony, E. K.; Andrew, S. *N. Eng. J. Med.* **2002**, *347*, 2.
3. Tawatsin, A.; Asavadachanukorn, P.; Thavara, U. *Southest Asian Trop Med Public Health* **2006**, *37(5)*, 915.
4. Taubes, G., "A mosquito bites back". New York Times Magazine. August 24, **1997**, 40.
5. Shell, E. R., "Resurgence of a deadly disease", Atlantic Monthly, August **1997**, 45.
6. Nayyar, G. M. L.; Breman, J. G.; Newton, P. N.; Herrington, J. *Lancet Infectious Diseases* **2012**, *12(6)*, 488; Nadjm, B.; Behrens, R. H. *Infectious Disease Clinics of North America* **2012**, *26 (2)*, 243; Beare, N. A.; Taylor, T. E.; Harding, S. P.; Lewallen, S.; Molyneux, M. E. *American Journal of Tropical Medicine and Hygiene* **2006**, *75 (5)*, 790.
7. Whitehorn, J.; Farrar, J. *Br. Med. Bull.* **2010**, *95*, 161; Ranjit, S.; Kissoon, N. *Pediatr. Crit. Care Med.* **2011**, *12 (1)*, 90; Simmons, C. P.; Farrar, J. J.; Nguyen, V.; Wills, B. *N Engl J Med* **2012**, *366 (15)*, 1423; Chen, L. H.; Wilson, M. E. *Current Opinion in Infectious Diseases* **2010**, *23 (5)*, 438.
8. Ghoshal, A.; Das, S.; Ghosh, S.; Mishra, M. K.; Sharma, V.; Koli, P.; Sen, E.; Basu, A. *Glia* **2007**, *55 (5)*, 483; Jelinek, T. *Expert Rev Vaccines* **2008**, *7 (5)*, 689; Gambel, J. M.; DeFraites, R.; Hoke, C. *J Infect Dis* **1995**, *171(4)*, 1074.
9. Morrison, T. E.; Fraser, R. J.; Smith, P. N.; Mahalingam, S.; Heise, M. T. *J. Virol.* **2007**, *81 (10)*, 5132.
10. Kostyuchenko, V. A.; Jakana, J.; Liu, X.; Haddow, A. D.; Aung, M.; Weaver, S. C.; Chiu, W.; Lok, S. M. *Journal of Virology* **2011**, *85 (18)*, 9327.
11. Marshall, I.D.; Brown, B. K.; Keith, K.; Gard, G. P.; Thibos, E. *Aust J Exp Biol Med Sci* **1982**, *60 (5)*, 471; Hurrelbrink, R. J.; Nestorowicz, A.; McMinn, P. C. *J. Gen. Virol.* **1999**, *80(12)*, 3115.

12. Oldstone, M. B. A. *Viruses, Plagues, and History* (1st ed.). Oxford University Press. **2000**, 45; Barrett, A. D.; Higgs, S. *Annu. Rev. Entomol.* **2007**, *52*, 209; Monath, T. P. *Antiviral Res.* **2008**, *78 (1)*, 116.
13. World Health Organization. Malaria Fact sheet **1999**, *94*. Geneva http://www.who.int/inf-fs/en/fact094.html.
14. Urban, J. E.; Alberto, B. *Current Microbiology* **2000**, *41*, 4.
15. For mosquito magnet link accessed: http://www.mosquitomagnet.com
16. Tawrell, P. *Camping & wilderness survival : the ultimate outdoors book* (2nd ed.) **2006**, 92.; Also referred: http://www.mosquito-netting.com
17. World Health Organization: Annex VII : Procedure for Treating Mosquito Nets and curtains"
18. Qiu, Y. T.; Smallegange, R. C.; Braak, C. J. F.; Spitzen, J.; Van Loon, J. J. A. *J Med Entomol* **2007**, 44; Krockel, U.; Rose, A.; Eiras, A. E.; Geier, M. *J Am Mosq Cont Assoc* **2006**, *22*, 229.
19. Fradin, M. S.; Day, J. F. *N Engl J Med* **2002**, *347 (1)*, 13; Collins, D. A.; Brady, J. N.; Curtis, C. F. *Phytotherapy Research* **1993**, *7 (1)*, 17; Cilek, J. E.; Petersen, J. L.; Hallmon, C. E. *J Am Mosq Control Assoc* **2004**, *20 (3)*, 299.
20. Vijverberg, H.; Van Der Zalm, J. *Nature* **1982**, *295*, 601; Class, T. J.; Kintrup, J. *Fresenius' Journal of Analytical Chemistry* **1991**, 340; Rosemary, A. C. *Phytochemutry*, **1976**, *15*, 759.
21. Harzsch, S.; Hafner, G. *Arthropod Structure and Development* **2006**, *35 (4)*, 319.
22. Kaufmann, C.; Briegel, H. *Journal of Vector Ecology* **2004**, *29 (1)*, 140–153.
23. Huang, J.; Walker, E. D.; Vulule, J. M.; James, R. *Malaria Journal* **2006**, *5*, 87.
24. Manual on practical entomology in malaria. Pt. II. Methods and techniques. Geneva: World Health Organization **1975**, 191.; Test procedures for insecticide resistance monitoring in malaria vectors. Bio-efficacy and persistence of insecticides on treated surfaces. Report of the WHO informal consultation. Geneva: World Health Organization **1998**. WHO/CDC/MAL/98.12.
25. Marcos, R.; Surralles, J.; Xamena, N.; Creus, A.; Catalan, J.; Norppa, H. *Mutation Research* **1995**, *341*, 169.; Gabliks, J.; Friedman, L. *Exp Biol Med*

*(Maywood)* **1965,** *120*, 163; Gabliks, J.; BantugJurilla, M.; Friedman, L. *Exp Biol Med (Maywood)* **1967**, *125*, 1002; Gabliks, J. *Exp Biol Med (Maywood)* **1965**, *120,* 168.

# Part B

# Antimicrobial activity of lactol and benzil derivatives

## 3B.1 Abstract

All the synthesized compounds of chapter 1 (**11**, **20**, **46a-h**, **47**) and chapter 2 (**13a-h**) were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes* (Gram-positive), *Escherichia coli, Pseudomonas aeruginosa* (Gram-negetive) strains and antifungal activity against two fungal strains (*Candida albicans, Aspergillus niger)* by broth dilution method. The compounds showed potent antibacterial activity against *Staphylococcus aureus* bacterial strain in comparison with ampicillin and exhibited modest antifungal activity.

## 3B.2 Introduction

Microorganisms are found everywhere in the taxonomic organization of life on the earth. Microorganisms are important because they produce antibiotics, recycle elements, remove organic substances from sewage and break down waste, produce vitamins and many other important products. The bacteria in our bodies help to digest the food. Microorganisms are indispensable components of earth's ecosystem. They make possible the cycles of carbon, oxygen, nitrogen, and sulfur that take place in terrestrial and aquatic systems. They also are a source of nutrients at the base of all ecological food chains.[1]

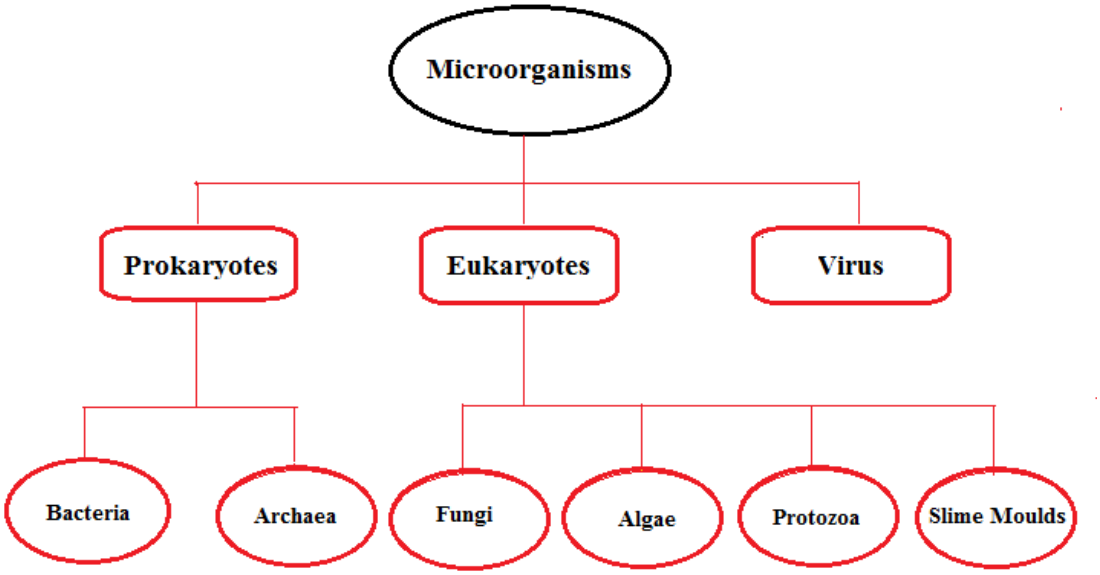



**Figure 3B.1.** General classification of microorganisms

The microorganisms are generally classified as prokaryotes, eukaryotes and virus depending on their cell structure (Figure 3B.1).

### Prokaryotes

A group of organisms whose cells does not contain membrane-bound nucleus are called prokaryotes. Most prokaryotes are unicellular organisms. There are two subtypes of prokaryotes i.e.(a) bacteria and (b) archaea.[2,3]

**(a) Bacteria**

All bacteria are unicellular prokaryotes. They do not have a defined cellular nucleus. Their genetic information is in their nucleoid, a single, circular tightly packed DNA molecule. According to their shape, bacteria are divided into three groups: (1) spirilla (with a spiral body shape); (2) bacillus (with a rod or stick shaped body); (3) cocci (with a spherical body shape) (Figure 3B.2).[4,5]

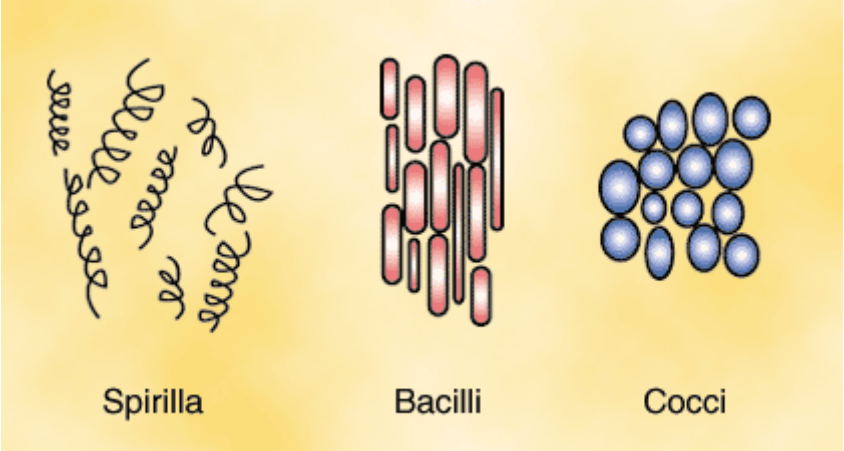



**Figure 3B.2.** Classification of bacteria according to their shape

Some types of bacteria live on their own and others form colonies. Some bacteria are quite mobile and others stay put for their whole life. Bacteria move using their cytoplasmic tail - flagella, or by secreting slimy substances that allow them to slide along surfaces.[6,7,8]

The cell walls of most bacteria contain a polysaccharide called peptidoglycan. A difference in their cell wall structure is a major feature used in classifying these organisms.[9] The staining abilities of bacteria are also based on their cell wall structure. According to the way they stain, bacteria can be classified as:

- Gram - positive
- Gram – negative

Based on their response to gaseous oxygen, all bacteria can be divided into the following groups:

- Aerobic - living in the presence of oxygen;
- Anaerobic - living without oxygen;
- Facultative anaerobes - can live in both environments.

According to the way they obtain energy, bacteria are classified as heterotrophs or autotrophs. Autotrophs make their own food by using the energy from sunlight or chemical reactions, in which case they are called chemoautotrophs. Heterotrophs obtain their energy by consuming other organisms.[10]

**(b) Archaea**

Archaea are prokaryotes having no cell nucleus or any other membrane-bound organelles within their cells. Archaea and bacteria are quite similar in size and shape. Despite this visual similarity to bacteria, archaea possess genes and several metabolic pathways like the enzymes involved in transcription and translation. Archaea use a much greater variety of sources of energy than eukaryotes ranging from familiar organic compounds such as sugars, to ammonia, metal ions or even hydrogen gas. Salt-tolerant archaea (the Haloarchaea) use sunlight as an energy source. Archaea reproduce asexually by binary fission, fragmentation, or budding. Archaea are now recognized as a major part of Earth's life and play roles in both the carbon cycle and the nitrogen cycle. No clear examples of archaeal pathogens or parasites are known, but they are often mutualists or commensals.[11,12,13]

**Eukaryotes**

A group of organisms having membrane-bound nucleus in their cells are called Eukaryotes. Eukaryotes are unicellular or multicellular organisms contain a nucleus and membrane-bound organelles. A nucleus is a membrane sac within the cell which holds cell's DNA. Membrane-bound organelles within the cell contain chloroplasts,

mitochondria, and several other types of organelle. Eukaryotes are subdivided into four different groups: (a) Fungi (b) Algae (c) Protozoa (d) slime moulds.[14]

**(a) Fungi**

Fungi are found in a variety of shapes and sizes and different types. They can range from single cells to enormous chains of cells that can stretch for miles. Fungi include single-celled living things that exist individually such as yeast and multicellular clusters such as molds or mushrooms. Fungi usually grow best in slightly acidic environments. They can grow on substances with very low moisture. Fungi live in the soil and on human body and on plants and animals, in freshwater and seawater. [15]

Fungi are basically stationary but they can spread either by forming reproductive spores that are carried on wind and rain or by growing and extending their hyphae. Hyphae grow as new cells form at the tips, creating even longer chains of cells.[16]

Fungi absorb nutrients from living or dead organic matter that they grow on. They absorb simple, easily dissolved nutrients, such as sugars, through their cell walls. They give off special digestive enzymes to break down complex nutrients into simpler forms that they can absorb.[17]

Several kinds of fungi are used to make antibiotics to fight bacterial infections. These antibiotics are based on natural compounds that the fungi produce to compete against bacteria for nutrients and space. Fungi break down dead plants and animals and keep the world tidier.[18]

**(b) Algae**

Algae are distinguished from animals, fungi, and protozoans by their ability to make their own food through photosynthesis and are distinguished from plants by their relative simplicity of structure. All algae contain the green pigment chlorophyll and the organelles chloroplasts, both of which are essential for photosynthesis.[19]

Algae may be either unicellular or multicellular. Generally unicellular algae are important in water treatment. All algae contain a rigid cell wall and some also

have sheaths (or thin gelatinous coatings) outside the cell wall. Algae may be non-motile, but many are able to move using flagella.[20]

Most algae are aquatic, living in salt or fresh water, though a few live in soil or on the bark of trees. In natural waters, algae are an important source of food for other organisms. They also produce oxygen during photosynthesis, adding to the dissolved oxygen content of the water during the day.[21]

**(c) Protozoa**

Protozoa mainly feed on bacteria, but they also eat other protozoa and sometimes fungi. Some protozoa absorb food through their cell tissues. Others have openings called mouth pores into which they sweep food. All protozoa digest their food in stomach-like compartments. As they digest, they make and give off nitrogen which is an element that plants and other higher creatures can use.[22]

Protozoa vary in size from 1/5,000 to 1/50 of an inch in diameter. They can be classified into three general groups based on their shape. One group is the Ciliates, which are generally the largest protozoa. They have hair-like projections called cilia and they eat the other protozoa as well as bacteria. The second group is the Amoebae. The third group is the Flagellates, which are usually the smallest of the protozoa and have one or several long, whip-like projections called flagella extending from their cells.[22]

To hunt, protozoa have to be able to move about. Amoebas ooze about by extending parts of their cells. Amoebae have fluid cell membranes or coverings that they can stretch out, bend and curve. As the membrane moves outward, the fluid and other parts inside the cell follow, flowing into the new bulge created by the moving membrane. Many Ciliates swim along by beating their cilia in a rhythmic pattern, like so many tiny oars. Flagellates swim by waving their flagella, using them much like a fish uses its tail to push itself through water.[22]

Most protozoa are not harmful but there are a few that cause diseases. One type of amoeba can live in human intestines. It feeds on red blood cells and causes a disease known as dysentery.[22]

**(d) Slime moulds**

Slime mould is a broad term describing some organisms that use spores to reproduce. They found in a wide variety of colors. More than 900 species of slime mould occur all over the world. They live in any type of dead plant material. They contribute to the decomposition of dead vegetation and feed on bacteria, yeasts, and fungi. For this reason, these organisms are usually found in soil, lawns and on the forest floor, commonly on deciduous logs.[23]

Most slime molds are smaller than a few centimeters, but some species may reach sizes of up to several square meters and masses of up to 30 grams. When food is abundant a slime mold exists as a single-celled organism, but when food is in short supply, slime molds congregate and start moving as a single body. In this state they are sensitive to airborne chemicals and can detect food sources. They can readily change the shape and function of parts and may form stalks that produce fruiting bodies, releasing countless spores, light enough to be carried on the wind or hitch a ride on passing animals.[24]

**Virus**

Viruses are non-living organisms which can only reproduce in a living host cell. As a result, all viruses are obligate parasites and all cause some sort of disease. Infectious hepatitis, polio, influenza, smallpox, AIDS, and a variety of intestinal disturbances are all caused by viruses.[25]

Viruses can attack many different kinds of organisms ranging from bacteria through plants and animals, though each type of virus is specific in its type of host. For example, a plant virus will not attack an animal and a dog virus is unlikely to attack a human.[26]

Viruses are too small to be to be seen with a light microscope so their presence is usually recognized only by the harm they cause. They often found in animal faeces and are thus expected to be present in domestic wastes. In addition, viruses can often survive for long periods of time in natural waters. Viruses are a public health concern in water and wastewater treatment since many are not removed by conventional treatment methods such as disinfection.[27]

**Pathogens**

The microorganisms which are capable of producing diseases in host are known as pathogen. There are several subtrates and pathways whereby pathogens can invade a host. Pathogens have certain characteristics that they need and use, to cause disease. They may be bacteria, fungi, protozoa, virus, etc.

**Bacterial pathogens**

Bacteria that cause disease are called pathogenic bacteria. Bacteria can cause diseases in humans, in other animals and also in plants. Some bacteria can only make one particular host ill others cause trouble in a number of hosts, depending on the host specificity of the bacteria. The disease caused by bacteria are almost as diverse as the bugs themselves and include infectious diseases such as pneumonia, food borne illnesses, tetanus, typhoid fever, diphtheria, syphilis and leprosy and even certain forms of cancer. Bacterial cells grow and divide, replicating repeatedly to form large numbers, present during an infection or on the surfaces of the body. To grow and divide, organisms must synthesize or take up many types of biomolecules.

**Gram positive bacterial pathogens**

***Streptococcus pyogenes*[28]**

*Streptococcus pyogenes* is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells. Individual cells are round-to-ovoid cocci, 0.6-1.0 micrometer in diameter (Figure 3B.3). Streptococci divide in one plane and thus occur

in pairs or in chains of varying lengths. The metabolism of *S. pyogenes* is fermentative and requires enriched medium containing blood in order to grow.

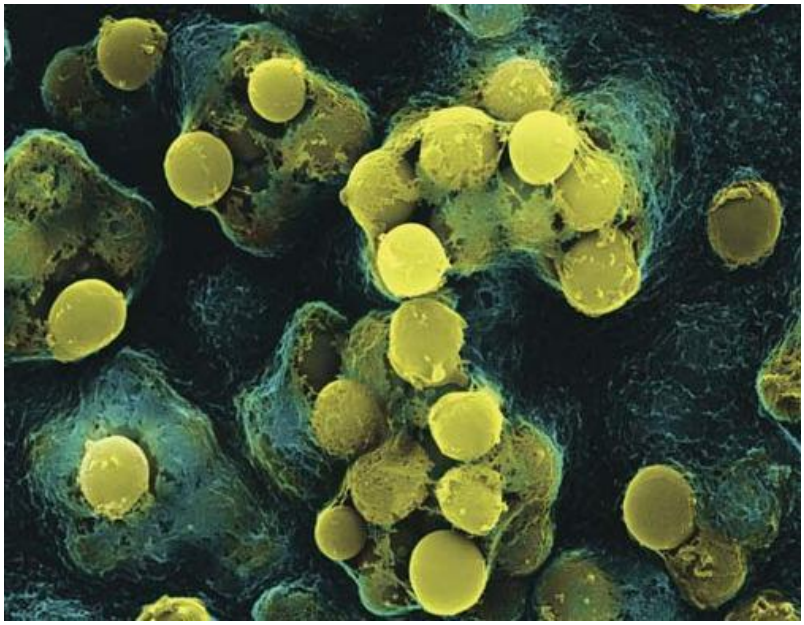


**Figure 3B.3.** Streptococcus pyogenes bacteria

*Streptococcus pyogenes* cause several infections in the human body like pharyngitis (strep throat), scarlet fever (rash), impetigo (infection of the superficial layers of the skin) or cellulitis (infection of the deep layers of the skin).

### *Staphylococcus aureus*[29]

Staphylococcus aureus are bacteria that produce toxin (a poisonous chemical substance produced and released by the bacteria during its normal life and growth) , which causes illness by causing inflammation of the intestine wall. The individual cells of S. aureus are 0.8 to 0.9 micro in diameter. They are ovoid or spherical (Figure 3B.4), non motile, non capsulated, typically arranged in groups of irregular clusters like branches of groups found in puts, singly or in pairs , grows best in the presence of oxygen but can grow anaerobically (absence of oxygen). The optimum temperature for the growth is 37°C; optimum pH is 7.4 to 7.6.

It is a food poisoning organism that causes nausea, vomiting and abdominal cramps which may be followed by diarrhea. In severe cases, headaches, sweating and fever may occur. Changes in blood pressure and pulse rate may also occur.

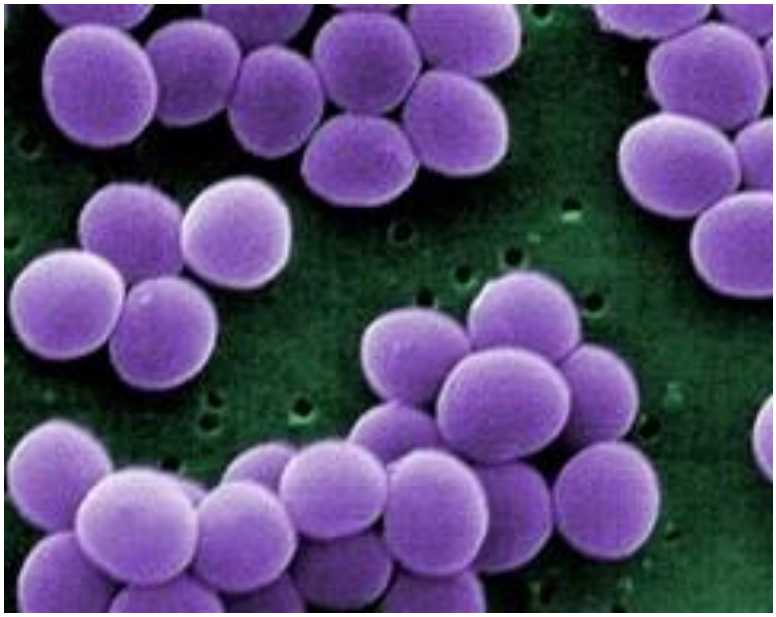


**Figure 3B.4.** Staphylococcus aureus bacteria

It also causes toxic shock syndrome, pyoregenic of pus forming conditions, mastitis of women and cows, boils, carbuncles infantile impetigo and internal abscess. Some strains are capable of producing a highly heat stable protein toxin in food that can cause the illness (staphyloenterotoxaemia) when ingested.

**Gram negative bacterial pathogens**

***Pseudomonas aeruginosa***[30]

**Figure 3B.5.** Pseudomonas aeruginosa bacteria

*Pseudomonas aeruginosa* is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm (Figure 3B.5). Almost all strains are motile by means of a single polar flagellum.

The bacterium is ubiquitous in soil and water, and on surfaces in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of oxygen also.

It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. Pseudomonas aeruginosa infection is a serious problem in patients hospitalized with cancer, cystic fibrosis, and burns.

***Escherichia coli*[31]**

They are rod shape, 2 to 4 µm by 0.4 µm in size, commonly seen in coccobacillary form and rarely in filamentous form (Figure 3B.6). Colonies are circular, raised and smooth. It grows best at 37°C, through a pH range of 4.4 to 9.0, in the presence or absence of oxygen.

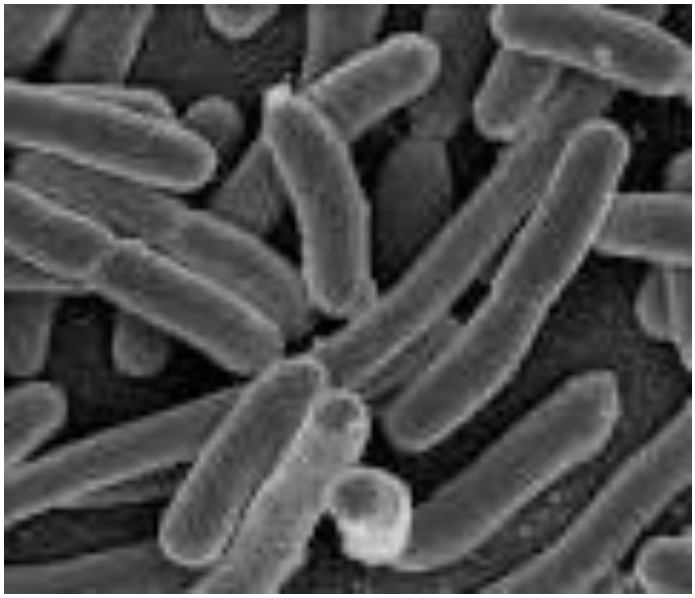


**Figure 3B.6.** Escherichia coli bacteria

They are normally present in the intestine without causing problems, but a few types cause illness after consuming contaminated food or water, when the bacteria produces toxin in the intestine causing diarrhea. It causes infantile diarrhea, gastroenteritis, traveller's diarrhea, bacillary dysentery, hemorrhagic colitis, hemolytic uremic syndrome (HUS) or thrombocytopenic purpura. It does not form toxin in food but in the intestine of infected people. Illness is caused after ingestion of a sufficient number of E. coli when bacteria travels through the stomach and small intestine, attaches itself to the inside surface of the large intestine and causes inflammation of the intestinal wall.

### *Fungal Pathogens*

Most fungal infections are due to opportunistic pathogens. These affect people who are already ill or have a suppressed immune system (e.g. in patients who have been given an organ transplant or AIDS patients). Fungi are common problems in the immune competent population as the causative agents of skin, nail or yeast infections. Most commonly, fungi grow as pathogen on the skin of animals or humans. This is sometimes called Ringworm symptom. Fungi also cause a number of plant and animal diseases e.g. in human's, ringworm, athlete's foot and several more serious diseases are caused by fungi. As fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Plant diseases caused by fungi include rusts, smuts and rottening in leaf, root and stem and may also cause severe damage to crops. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections due to the fact that fungi and their hosts both have eukaryotic cells. The typical fungal spore size is 1-40 µm in length.

### *Candida albicans*[32]

Candida albicans is found among the normal flora of the mouth, digestive tract and vagina of perfectly healthy people (Figure 3B.7). It may cause severe and even fatal infections, with lesions and eruptions of the skin, nails, mouth, bronchial tubes and lungs under some circumstances. The reason for this outbreak is difficult to pinpoint since the fungus is generally present on and within the body of healthy individuals.

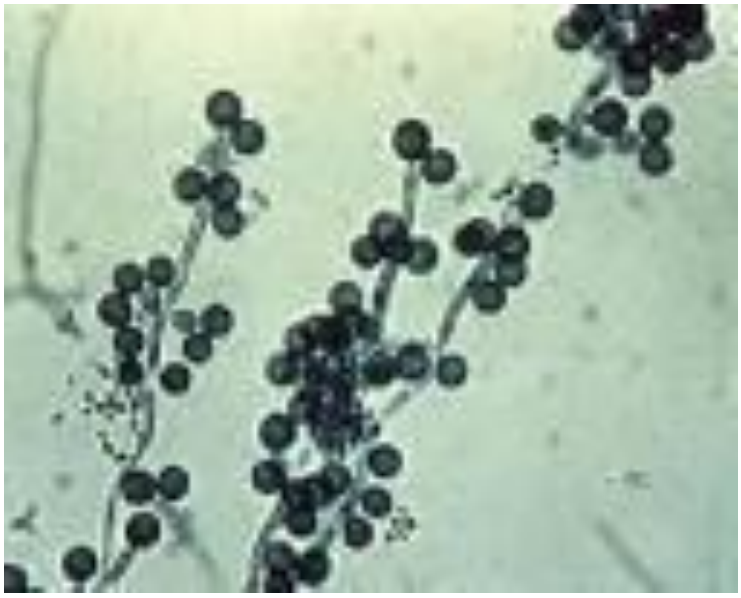


**Figure 3B.7.** Candida albicans fungi

***Aspergillus niger***[33]

Aspergillus niger is fungus and one of the most common species of the genus Aspergillus (Figure 3B.8). It causes black mould on certain types of fruits and vegetables and is a common contaminant of food. Aspergillus includes a set of fungi that are generally considered asexual although the perfect forms have been found. Aspergillus is ubiquitous in nature. They are geographically widely distributed and have been observed in a broad range of habitats because they can colonize a wide variety of substrates.

**Figure 3B.8.** Aspergillus niger fungi

A. niger is commonly found as a saprophyte growing dead leaves, stored grain, compost piles and other decaying vegetation. The spores are widespread and are often associated with organic materials and soil. The primary uses of A. niger are for the production of enzymes and organic acids by fermentation. A. niger is also used to produce organic acids such as citric acid and gluconic acid and the enzymes glucoamylase and agalactosidase.

All of our internal fluid, organs and body structures are sterile under normal circumstances and the presence of bacteria, fungi, virus, etc. in these parts is diagnostic evidence of infection. Micro-organisms are harmful to mankind in many ways either when they come in contact and invade the tissues and cause diseases or if they find suitable conditions for their growth. Therefore, one must constructively do for prevention and cure of such infectious diseases. Protection against such infection can be achieved by inhibition of microbial growth or by killing them. This can be done by using various physical agents, physical processes or chemical agents. The major physical agents or processes used for the control of microorganisms are temperature, desiccation, osmotic pressure, radiation and filtration. A large number of chemical compounds have the ability to inhibit the growth of metabolism of microorganisms or to kill them. Research and development in different areas of chemistry have shown that several classes of chemical substrates are used to reduce the microbial flora.

The microorganisms are controlled by various physical agents, physical processes or chemical agents. A variety of techniques and agents are available which act in many different ways and each has its own limits of application. The chemical agents are also known as antimicrobial agents. Antimicrobial agents may be either bactericidal, killing the target bacterium or fungus or bacteriostatic, inhibiting its growth. Bactericidal agents are more effective, but bacteriostatic agents can be extremely beneficial since they permit the normal defenses of the host to destroy microorganisms. Antimicrobial agents may be classified according to the type of organism against which they are active i.e. antibacterial, antiviral, antifungal, antiprotozoal, and anthelmintic drugs. It can also be useful to combine various antimicrobial agents for broadening activity spectrums and to minimize the possibility of the development of bacterial resistance.

Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease: antibiotics, which are natural substances produced by certain groups of microorganisms and chemotherapeutic agents, which are chemically synthesized. A hybrid substance is a semi synthetic antibiotic, wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. In the medical and pharmaceutical worlds, all these antimicrobial agents used in the treatment of disease are referred to as antibiotics, chemicals that are produced by living organisms which, even in minute amounts, inhibit the growth of or kill another organism.

***Antibacterial and Antifungal activity***

During the last few years a very large number of organic compounds have been tested for their possible fungicidal and bactericidal activity (the ability to kill or inhibit their growth). In earlier days due to the lack of reliable testing methods for the fungicidal activity, the progress in this field was slow. The bioassay technique should be such that (i) the laboratory trials must be reproducible (ii) the laboratory bioassay and the field performance of the test chemical must produce uniform and congruent results.

The modern methods give reliable and reproducible results regarding protective values of a test fungicide or bactericide under field condition. The present method of the laboratory bioassay requires only a few milligrams of the test chemicals and screen out unsuccessful candidates by trials against specific phytopathogens.

Various methods have been used from time to time by several workers to evaluate the antimicrobial activity either in the form of a zone size or minimum inhibitory concentration (MIC). They are as follows:

**Turbidimetric Method**

In this method, a uniform solution of an antibiotic is made. The microbial culture is added to the fluid. The biggest advantage of this method is that it requires a relatively shorter incubation period. However, there is also a big disadvantage. The presence of foreign material that may be inhibitory to the growth of microbes may influence the results of this assay. This method is therefore appropriate when the samples are clear.

**Agar Streak dilution method**

The aim of agar dilution methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents. Agar dilution involves the incorporation of different concentrations of the antimicrobial substance into a nutrient agar medium followed by the application of a standardized number of cells to the surface of the agar plate.

**Serial dilution method**

A serial dilution is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. Serial dilutions are used to accurately create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale.

**Agar diffusion method**

Also known as Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing) is a test which uses antibiotic-impregnated wafers to test whether particular bacteria are susceptible to specific antibiotics. A known quantity of bacteria is grown on agar plates in the presence of thin wafers containing relevant antibiotics. If the bacteria are susceptible to a particular antibiotic, an area of clearing surrounds the wafer where bacteria are not capable of growing (called a zone of inhibition)

**E-Test dilution and diffusion method**

The Epsilometer test (usually abbreviated E-test) is a laboratory test used by microbiologists to determine whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic. This is most commonly used in the setting of medicine, where a particular organism has been found to infect a patient, and the doctor treating the patient is seeking guidance on what concentration of antibiotic is suitable.

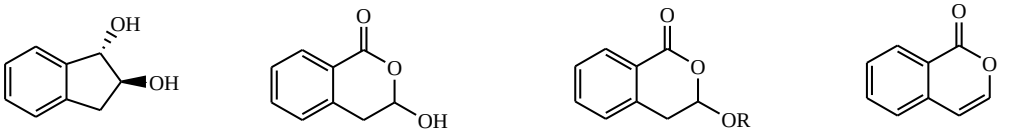
**Broth Dilution Method**

Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for test bacteria. 2% DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. In the present study of antimicrobial activity this method was used.

**3B.3 Results And Discussion**

All the synthesized compounds of chapter 1 (**11, 20, 46a-h, 46**) and chapter 2 (**13a-h**) were tested for their *in vitro* antimicrobial activities. The minimum inhibitory concentration (MIC) of various synthesized compounds were tested against two Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), two Gram-negetive bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Aspergillus niger*) by broth dilution method.[34] Standard antibacterial drug (Amplicillin) and antifungal drug (Griseofulvin) were also screened under identical conditions for comparison. The evaluation of antimicrobial activity has been carried out using Broth Dilution method for antimicrobial study recommended by the National Committee for Clinical Laboratory Standards (NCCLS).

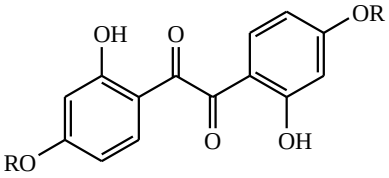
Individual MIC values of all the compounds along with the standards are listed in Table 3B.1. The results reveal that all the compounds show significant antibacterial activity with high degree of variations. All the compounds show good activity against *Staphylococcus aureus,* gram positive bacteria whereas in case of *Streptococcus*



**11** **20** **46 (a-h)** **47**

a) R = n-$C_2H_5$; b) R = n-$C_3H_7$; c) R = n-$C_4H_9$; d) R = n-$C_5H_{11}$; e) R = n-$C_8H_{17}$; f) R = n-$C_{12}H_{25}$; g) R = n-$C_{14}H_{29}$; h) R = n-$C_{16}H_{33}$

**Figure 3B.9.** All the synthesized compounds of chapter 1 (**11, 20, 46a-h, 47**)



**13 (a-h)**

a) R= $CH_3$; b) R= $C_2H_5$; c) R= n-$C_3H_7$; d) n-$C_4H_9$; e) R= n-$C_6H_{13}$; f) R= n-$C_8H_{17}$; g) R= n-$C_{10}H_{21}$; h) R= n-$C_{12}H_{25}$

**Figure 3B.10.** All the synthesized compounds of chapter 2 (**13a-h**)

*pyogenes* only compounds **20**, **46b**, **46g**, **47**, **13a** and **13b** are showing potent antibacterial activity (MIC = 100, 100, 100, 62.5, 100 and 100 respectively).

Among the tested compounds, **46d**, **47**, **13d**, **13c** and **13g** are potent against *Escherichia coli* (MIC = 62.5, 100, 62.5, 100, 62.5 µg/mL respectively) in comparision with ampicillin and compounds **46d** and **46e** are potent against *Pseudomonas aeruginosa* (MIC = 100 and 62.5 µg/mL respectively).

The antifungal screening data shows that a few compounds are potent against fungal strains. The compounds **11**, **46d**, **46e**, **13f**, **13g** and **13h** are equipotent against *Candida albicans* in comparison with Griseofulvin, a standard drug.

**Table 3B.1.** Results of antibacterial and antifungal screening of compounds **11**, **20**, **46(a-h)**, **47** and **13(a-h)**

| Compounds | Minimum Inhibitory Concentration (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | **E. Coli** | **P. Aeruginosa** | **S. Aureus** | **S. Pyogenus** | **C. Albicans** | **A. Niger** |
| | **MTCC 443** | **MTCC 1688** | **MTCC 96** | **MTCC 442** | **MTCC 227** | **MTCC 282** |
| 11 | 200 | 500 | **200** | 250 | **500** | > 1000 |
| 20 | 125 | 250 | **100** | **100** | > 1000 | 500 |
| 46a | 250 | 200 | **200** | 200 | 1000 | > 1000 |
| 46b | 200 | 250 | **100** | **100** | 1000 | 1000 |
| 46c | 200 | 250 | **200** | 200 | 1000 | > 1000 |
| 46d | **62.5** | **100** | **200** | 200 | **500** | > 1000 |
| 46e | 200 | **62.5** | **250** | 250 | **500** | 500 |
| 46f | 200 | 200 | **250** | 250 | > 1000 | 200 |
| 46g | 250 | 250 | **100** | **100** | > 1000 | 250 |
| 46h | 250 | 200 | **250** | 250 | > 1000 | 1000 |
| 47 | **100** | 200 | **100** | **62.5** | 1000 | 1000 |
| 13a | 200 | 250 | **125** | **100** | 1000 | >1000 |
| 13b | 200 | 500 | **200** | **100** | >1000 | 500 |
| 13c | **100** | 250 | **250** | 250 | >1000 | 250 |
| 13d | **62.5** | 250 | **200** | 200 | 1000 | >1000 |
| 13e | 200 | 250 | **250** | 250 | >1000 | >1000 |
| 13f | 125 | 200 | **200** | 500 | **500** | 1000 |
| 13g | **62.5** | 125 | **250** | 200 | **250** | >1000 |
| 13h | 125 | 250 | **200** | 250 | **250** | >1000 |
| Ampicillin | **100** | **100** | **250** | **100** | - | - |
| Griseofulvin | - | - | - | - | **500** | **100** |

## 3B.4 Experimental

The in *vitro* antimicrobial activity of the synthesized compounds and standard drugs were assessed against two representative of Gram-positive bacteria viz. *Bacillus subtillis* (MTCC 441) and *staphylococcus aureus* (MTCC 96), two Gram-negative bacteria viz. *Escherichia coli* (MTCC 443) and *Salmonella typhi* (MTCC 98) and two fungi viz. *Aspergillus niger* (MTCC 282) and *candida albicans* (MTCC 227) and the strains employed for the activity were procured from (MTCC – Micro Type Culture Collection) Institute of Microbial Technology, Chandigarh.

### Antibacterial assay

All the compounds **(11, 20, 46a-h, 47 and 13a-h)** were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)) and Gram-negative bacteria (*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688). All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh. The activity of compounds was determined as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller Hinton Broth (Becton Dickinson, USA). Compounds were screened for their antibacterial activity as primary screening in five sets against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* at different concentrations of 1000, 500, 250 μg/mL. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to $10^6$ CFU/mL (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for test bacteria. 2% DMSO was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Synthesized compounds were diluted to 2000 μg/mL concentration, as a stock solution. Control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of test organisms. The tubes were then put for incubation at 37 °C for 24 h for bacteria. 10 μg/mL suspensions were further inoculated on an appropriate media and growth was noted after 24 h and 48 h. The highest dilution (lowest concentration) preventing appearance of turbidity was considered as minimum inhibitory concentration (MIC, μg/mL) i.e. the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. A set of tubes containing only seeded broth and solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this was greatly affected by size of inoculum. Test mixture should contain $10^6$ CFU/mL organisms. Standard drug used in the present study was 'Ampicillin' for evaluating antibacterial activity which showed 100, 100, 250 and 100 μg/mL MIC against *E. coli, P. aeruginosa, S. aureus* and *S. pyogenes* respectively.

**Antifungal assay**

Same compounds **(11, 20, 46a-h, 47 and 13a-h)** were tested for antifungal activity as primary screening in five sets against *Candida albicans* and *Aspergillus niger* at various concentrations of 1000, 500, 250 µg/mL. Compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 µg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. For fungal growth, in the present protocol, Sabourauds dextrose broth was used at 28 °C in aerobic condition for 48 h. Griseofulvin was used as a standard drug for antifungal activity which showed 500 and 100 µg/mL MIC against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282) respectively.

**3B.5 Conclusion**

All the synthesized compounds were tested for antibacterial and antifungal activity. They exhibited good antibacterial activity and did not show splendid antifungal activity. They exhibited good antibacterial activity against Gram positive bacteria, *Staphylococcus aureus* while showing modarate activity against other bacterial strains in comparision with commercial antimicrobial agents. As for the fungi, the majority of compounds showed the worst activity against *A. Niger* while modest antifungal activity against *C. Albican*. The antimicrobial activity study showed that novel class of compounds **46(a-h)** and **13(a-h)** might be useful as antimicrobial agents.

**3B.6 References**


1. Prescott. L. M. Microbiology, 5th edition, **2002**.
2. Kaiser, D. *Nat. Rev. Microbiol.* **2003**, *1 (1)*, 45.
3. Sapp, J. *Microbiol. Mol. Biol. Rev.* **2005**, *69 (2)*, 292.
4. Schulz, H.; Jorgensen, B. *Annu Rev Microbiol* **2001**, *55*, 105.
5. Robertson, J., Gomersall, M., Gill, P. *J Bacteriol.* **1975**, *124 (2)*, 1007.
6. Bardy, S.; Jarrell, K. *Microbiology* **2003**, *149*, 295.
7. Merz, A.; Sheetz, M. *Nature* **2000**, *407*, 98.
8. Macnab, R. M. *J. Bacteriol.* **1999**, *181 (23)*, 7149.
9. Van, H. J. *Glycobiology* **2001**, *11(3)*, 25; Koch, A. *Clin Microbiol Rev* **2003**, *16 (4)*, 673.
10. Zumft, W. *Microbiol Mol Biol Rev* **1997**, *61 (4)*, 533.
11. Theron, J.; Cloete, T. E. *Crit. Rev. Microbiol.* **2000**, *26 (1)*, 37.
12. Robertson, C. E.; Harris, J. K.; Spear, J. R.; Pace, N. R. *Current Opinion in Microbiology* **2005**, *8 (6)*, 638.
13. Koga, Y.; Morii, H. *Microbiol. Mol. Biol. Rev.* **2007**, *71 (1)*, 97.
14. Thomas, C. S. *European Journal of Protistology* **2006**, *39 (4)*, 338.
15. Bruns, T. *Nature* **2006**, *443*, 758.
16. Taylor, T. N.; Taylor, E. L. *Review of Palaeobotany and Palynology* **1996**, *95*, 83.
17. Zabriskie, T. M.; Jackson, M. D. *Natural Product Reports* **2000**, *17 (1)*, 85.
18. Celio, G. J.; Padamsee, M.; Dentinger, B. T.; Bauer, R.; Mc Laughlin, D. J. *Mycologia* **2006**, *98 (6)*, 850.
19. Patrick, J. K. *American Journal of Botany* **2004**, *91 (10)*, 1481.
20. Wellman, C. H.; Osterloff, P. L.; Mohiuddin, U. *Nature* **2003**, *425*, 282.
21. Bigogno, C.; Khozin-Goldberg, I.; Boussiba, S.; Vonshak, A.; Cohen, Z. *Phytochemistry* **2002**, *60 (5)*, 497.
22. Honigberg, B. M.; Balamuth, W.; Bovee, E. C.; Corliss, J. O.; Gojdics, M.; Hall, R. P.; Kudo, R. R.; Levine, N. D.; Lobblich, A. R.; Weiser, J. *Journal of Eukaryotic Microbiology* **1964**, *11 (1)*, 7; Dogiel, V. A. *General Protozoology*, 2nd ed., Oxford University Press, **1965**.
23. Deasey, M. C.; Olive, L. S. *Science* **1981**, *213*, 561.
24. Worley, A. C.; Raper, K. B.; Hohl, M. *Mycologia* **1979**, *71 (4)*, 746.
25. Edwards, R. A.; Rohwer, F. *Nature Reviews Microbiology*. **2005**, *3(6)*, 504.

26. Barman, S.; Ali, A.; Hui, E. K.; Adhikary, L.; Nayak, D. P. *Virus Res.* **2001**, *77(1)*, 61.
27. Matter, L.; Kogelschatz, K.; Germann, D. *J. Infect. Dis.* **1997**, *175(4)*, 749.
28. Ryan, K. J.; Ray, C. G. *Sherris Medical Microbiology* 4th edition. **2004**; Kellogg, J. A.; Bankert, D. A.; Elder, C. J.; Gibbs, J. L.; Smith, M. C. *J. Clin. Microbiol.* **2001**, *39 (9)*, 3373.; Falagas, M. E.; Vouloumanou, E. K.; Matthaiou, D. K.; Kapaskelis, A. M.; Karageorgopoulos, D. E. *Mayo Clin Proc* **2008**, *83 (8)*, 880.
29. Ogston, A. *Rev Infect Dis* **1984**, *6 (1)*, 122.; Kluytmans, J.; Van Belkum, A.; Verbrugh, H. *Clin. Microbiol. Rev.* **1997**, *10 (3)*, 505.; Vigliani, G. A.; Campion, M. *Clin Infect Dis* **2009**, *48 (6)*, 713.; Carter, A. P.; Clemons, W. M.; Brodersen, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 340.
30. Itah, A. Y.; Essien, J. P. *World Journal of Microbiology and Biotechnology* **2005**, *21 (6)*, 1317.; Vander, W. C.; Piérard, A.; Kley-Raymann, M.; Haas, D. *J. Bacteriol.* **1984**, *160 (3)*, 928.; Miyata, S.; Casey, M.; Frank, D. W.; Ausubel, F. M.; Drenkard, E. *Infect. Immun.* **2003**, *71 (5)*, 2404.; Rahme, L. G.; Stevens, E. J.; Wolfort, S. F.; Shao, J.; Tompkins, R. G.; Ausubel, F. M. *Science* **1995**, *268*, 1899.; Poole, K. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **2004**, *10 (1)*, 12.
31. Bentley, R.; Meganathan, R. *Microbiol. Rev.* **1982**, *46 (3)*, 241.; Eckburg, P. B.; Bik, E. M.; Bernstein, C. N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S. R.; Nelson, K. E.; Relman, D. A. *Science* **2005**, *308*, 1635.; Fotadar, U.; Zaveloff, P.; Terracio, L. *J. Basic Microbiol.* **2005**, *45 (5)*, 403.; Darnton, N. C.; Turner, L.; Rojevsky, S.; Berg, H. C. *J. Bacteriol.* **2007**, *189 (5)*, 1756.; Nataro, J. P.; Kaper, J. B. *Clin. Microbiol. Rev.* **1998**, *11 (1)*, 142.
32. Zadik, Y.; Burnstein, S.; Derazne, E.; Sandler, V.; Ianculovici, C.; Halperin, T. *Oral Dis* **2010**, *16 (2)*, 172.; Sudbery, P. E. *Nature Reviews Microbiology* **2011**, *9 (10)*, 737.; Berman, J.; Sudbery, P. E. *Nature Reviews Genetics* **2002**, *3 (12)*, 918.; Lingappa, B. T.; Prasad, M.; Lingappa, Y.; Hunt, D. F.; Biemann, K. *Science* **1969**, *163*, 192.
33. Abarca, M.; Bragulat, M.; Castellá, G.; Cabañes, F. *Appl Environ Microbiol* **1994**, *60 (7)*, 2650.; Schuster, E.; Dunn-Coleman, N.; Frisvad, J. C.;

Van Dijck, P. W. *Applied microbiology and biotechnology* **2002**, *59 (4)*, 426.; Varga, J.; Kocsube, S.; Toth, B.; Frisvad, J. C.; Perrone, G.; Susca, A.; Meijer, M.; Samson, R. A. *International Journal of Systematic and Evolutionary Microbiology* **2007**, *57 (8)*, 1925.; Pel, H.; de Winde, J.; Archer, D. *Nat Biotechnol* **2007**, *25 (2)*, 221.

34. National committee for clinical laboratory standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, third ed. NCCLS Publication M7-A3, Villanova, PA. **1993**.; National committee for clinical laboratory standards, Reference Method for Broth Dilution Antifungal Testing of Yeasts, Proposed Standard. NCCLS Document M27-P, Villanova, PA. **1992**.

# Chapter 4

## Tungstic acid catalyzed route for the synthesis of

## 3, 3-di(1*H*-indol-3-yl)indolin-2-one derivatives

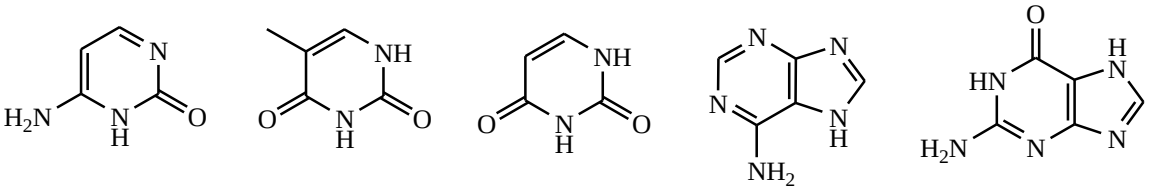
## 4.1 Abstract

Tungstic acid, a low-cost and readily available heterogeneous catalyst is thoroughly characterized by various analytical and spectral techniques and employed for the synthesis of 3,3-di(1*H*-indol-3-yl)indolin-2-one derivatives from indoles. The reaction parameters like catalyst quantity, solvents, reaction temperature and time have been optimized. The present method has several advantages such as mild conditions, simple work-up, elimination of anhydrous condition, easy recovery of catalyst and its recyclability.

## 4.2 Introduction

Heterocycles are prevalent division of the organic chemistry. As heterocycles are of enormous importance biologically and industrially, they largely contribute to the development of society from biological and industrial point of view. Heterocycles are present in all kind of materials of general interest which are used for various routine applications in different fields such as biology, pharmacology, electronics, material science and so on. The occurrence of heterocycles in natural substances, find extensive applications in fine chemicals, medicines, analysis, agriculture and in many fields. [1,2]

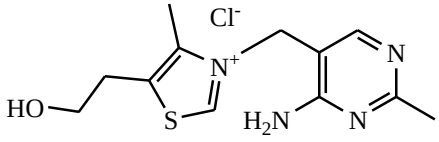
The knowledge of heterocycles helps mankind to understand the life processes and to improve the quality of life. Many natural and synthetic heterocyclic compounds participate in chemical reactions of the human body. The fundamental processes of life such as provision of energy, transmission of nerve impulses, sight, metabolism and the transfer of hereditary information are based on chemical reactions involving the participation of many heterocyclic compounds like vitamins, enzymes, coenzymes, nucleic acids, ATP and serotonin. [3]



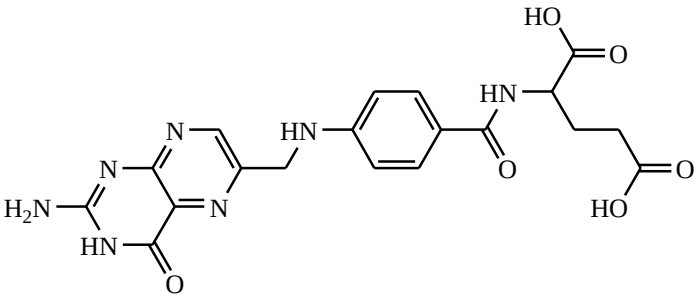
Cytosine **1** Thymine **2** Uracil **3** Adenine **4** Guanine **5**

**Figure 4.1:** Structures of pyrimidine and purine derivatives

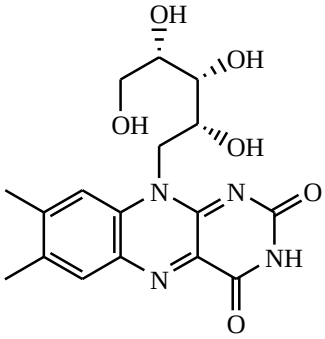
Pyrimidine derivatives (cytosine **1**, thymine **2** and uracil **3**) and purine derivatives (adenine **4** and guanine **5**) derivatives are heterocycles with two and four nitrogen atoms, respectively (Figure 4.1). They are key components of the deoxyribonucleic acid (DNA) molecules and participate directly in the encoding of genetic information. They also pass information to the related ribonucleic acid (RNA) molecules that control the protein synthesis and the sequence of amino acids. [4]



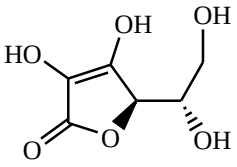
Thiamine **6**



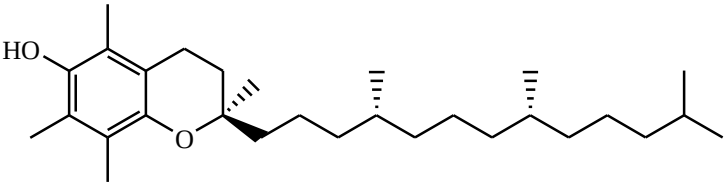
Folic acid **7**



Riboflavin **8**



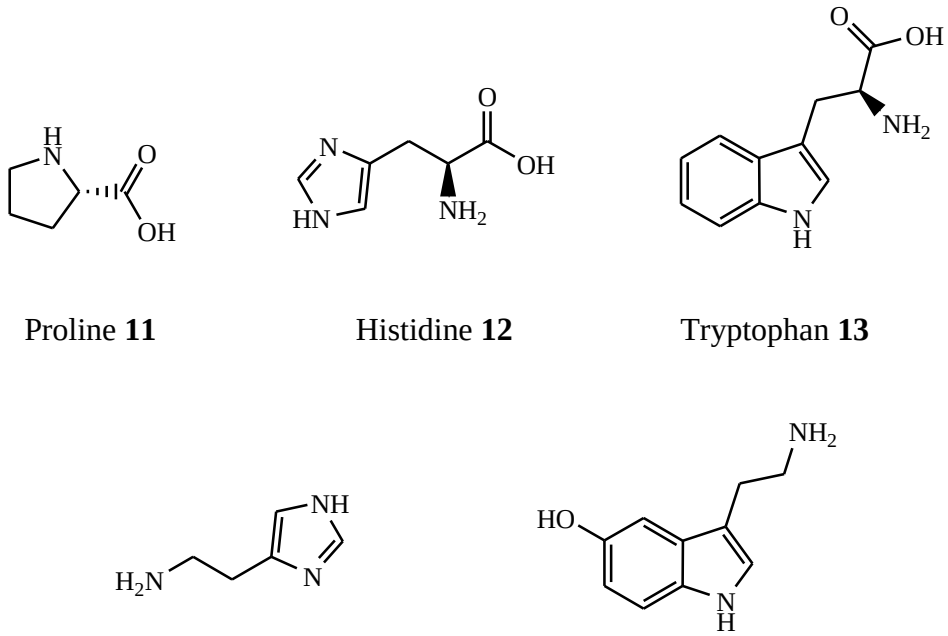
Ascorbic acid **9**



Tocopherol **10**

**Figure 4.2:** Structures of different N- and O- heterocyclic vitamins

The need for minute quantities of vitamins in diet is well-known for normal growth and nutrition in the human body. Vitamins in the B group thiamine **6**, folic acid **7** and riboflavin **8** are nitrogen heterocycles (Figure 4.2) and function either as coenzymes or their precursors. Other vitamins such as ascorbic acid **9** (vitamin C) and tocopherol **10** (vitamin E) are oxygen heterocycles (Figure 4.2) which are used in maintaining healthy connective tissue and in the stabilization of cell membranes, respectively. [5]



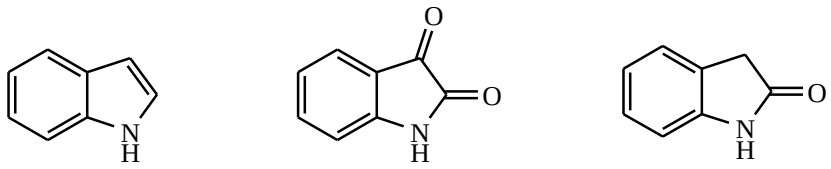

Proline **11** Histidine **12** Tryptophan **13**

Histamine **14** Serotonin **15**

**Figure 4.3:** Structures of essential amino acids and neurotransmitters

The essential amino acids like proline **11**, histidine **12**, tryptophan **13**, neurotransmitter histamine **14** and serotonin **15** are successful applications of heterocyclic compounds (Figure 4.3). [6] The photosynthesizing pigment chlorophyll, the oxygen transporting pigment haemoglobin, the hormones kinetin, heteroauxin, cytokinins are also heterocycles.

Heterocycles have widespread therapeutic uses such as antibacterial, antifungal, antimycobacterial, trypanocidal, antiHIV, antileishmanial, genotoxic, antitubercular, antimalarial, analgesic, antiinflammatory, musclerelaxants, anticonvulsant, anticancer, lipid peroxidation inhibitor, hypnotics, antidepressant, anthelmintic, herbicidal, and insecticidal agents. [7] There are many heterocyclic compounds with other important applications such as fungicides, herbicides, anticorrosive agents, photostabilizers, agrochemicals, dyestuff, copolymer, photographic developers, fluorescent whiteners, sensitizers, booster agent, antioxidant and flavouring agent. [8]

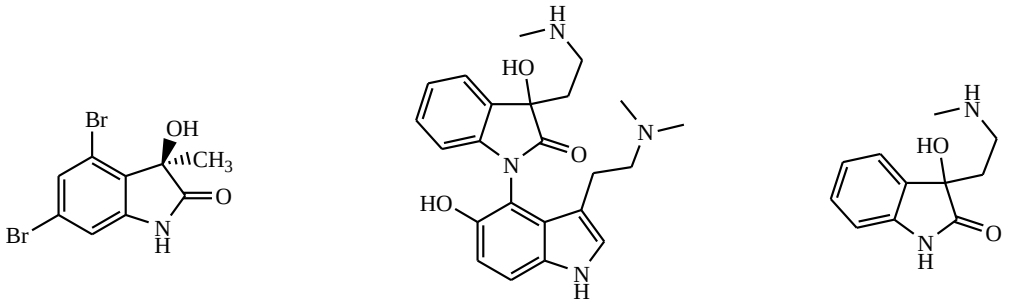


Indole **16** Isatin **17** Oxindole **18**

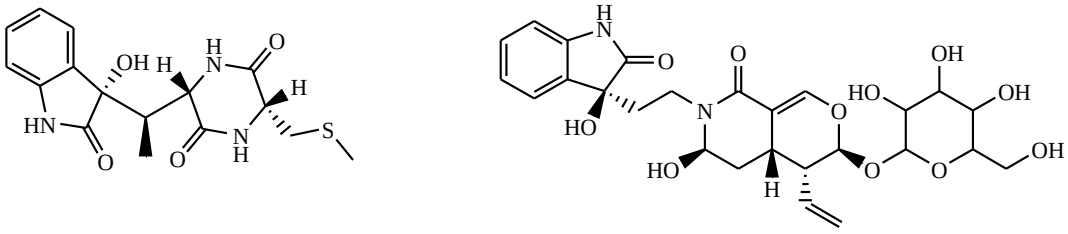
Benzimidazole **19** Pyrimidone **20**

**Figure 4.4:** Structures of various N-heterocyclic moieties

Various N-heterocyclic moieties (Figure 4.4) such as indole **16**, isatin **17**, oxindole (indolin-2-one) **18**, benzimidazole **19**, pyrimidone **20** and many more are known to be responsible for a variety of biological activities. [9] Among all, oxindole **18** attracts much attention as it exhibits wide range of diverse biological activities like



Convolutamydine C **21** Arundaphine **22** Donaxaridine **23**

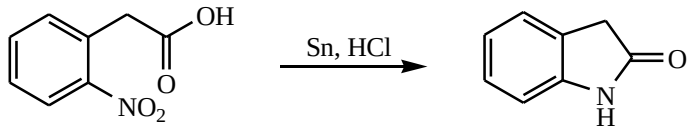


Maremycin B **24** Paratunamide D **25**

**Figure 4.5:** Presence of oxindole moiety in various biologically active compounds

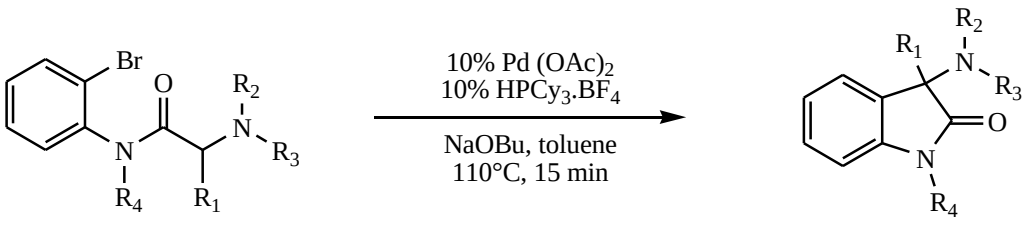
antiproliferative, [10] antibacterial, [11] antiprotozoal, [12] antiinflammatory, [13] antiHIV, [14] antiviral, [15] antitumor, [16] antifungal, [17] antiandrogenics, [18] anticonvulsants, [19] and laxatives. [20] Oxindole **18** moiety is also present as a core structural unit in many natural products and biologically active compounds (Figure 4.5) such as convolutamydines **21**, [21] arundaphine**22**, [22] donaxaridine **23**, [23] maremycins **24**, [24] paratunamide **25**, [25] 3-hydroxy welwitindolinones, [26] celogentin K [27] and neuroprotectin B. [28]

The biological potency of oxindole derivatives prompted many efforts towards their syntheses. Baeyer has developed different routes for the synthesis of oxindole derivatives.[29] One of the syntheses of oxindole reported by him was the reduction of 2-nitrophenylacetic acid **26** with tin and hydrochloric acid. [30] (Scheme 4.1)




2-nitrophenylacetic acid **26** oxindole **18**

**Scheme 4.1:** Synthesis of oxindole **18** by Baeyer

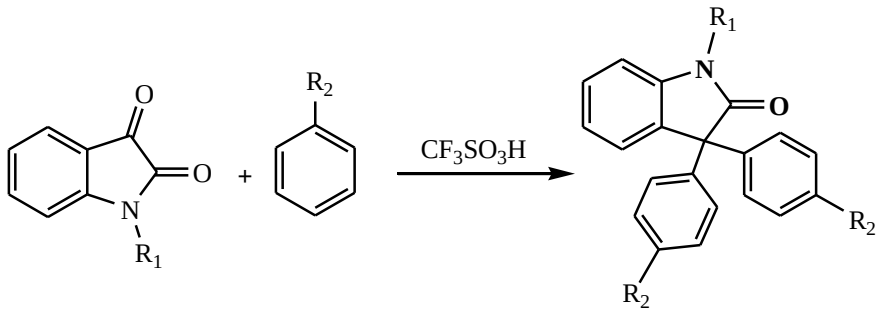



**27** **28**

$R_1 = C_2H_5, C_3H_7, C_4H_9, C_6H_5$; $R_2 = CH_3, C_2H_5$; $R_3 = C_2H_5, C_6H_5$; $R_4 = CH_3$

**Scheme 4.2:** Synthesis of 3-aminooxindole **28**

Since than, a variety of methods have been developed for the preparation of oxindole [31-35] for example, Marsden *et al* [32] have synthesized the quaternary 3-aminooxindole **28** skeleton on the basis of intramolecular arylation of enolates of substituted amino acids **27** (Scheme 4.2). Klumpp *et al* [33] have prepared 3,3-diaryloxidoles **31** by the reaction of substituted isatins **29** with aromatics **30** in triflic acid (Scheme 4.3).




**29** **30** **31**

$R_1 = CH_3$, Ph; $R_2 = CH_3$, $C_2H_5$, $C_3H_7$, $C_4H_9$

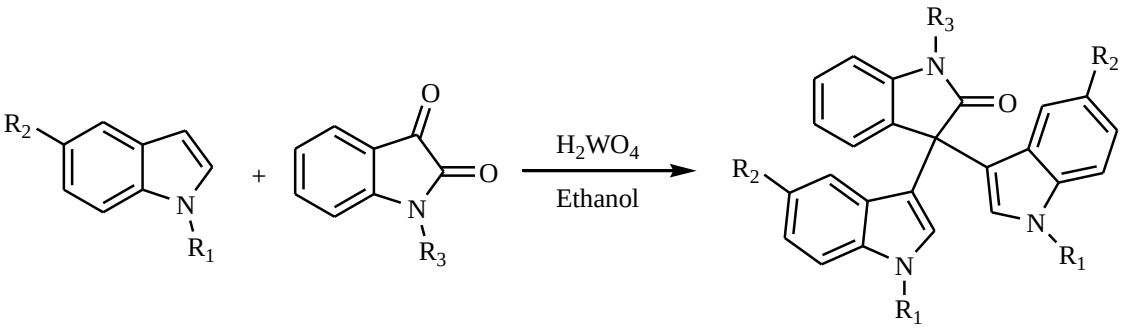
**Scheme 4.3:** Synthesis of 3,3-diaryloxidoles **31**

Among them particularly, the 3,3-di(indol-3-yl)indolin-2-ones **34a** (Scheme 4.4), display wide range of biological activities including spermicidal activity[36] and anticancer.[37] The 3,3-di(indol-3-yl)indolin-2-ones **34a** have been synthesized by condensation of indoles **32a** and isatins **33a** in the presence of several Brønsted [38] and Lewis acids [$AlCl_3$, $BF_3$, $LnCl_3$, $Ln(OTf)_3$, $Dy(OTf)_3$, $In(OTf)_3$, etc.], [39] amino catalysts, [40] ionic liquids, [41] aluminiumdodecatungstophosphate ($AlPW_{12}O_{40}$), [42] ceric ammonium nitrate (CAN), [43] $LiClO_4$ [44] and iodine. [45] Though these methods work well, they involve stronger reaction conditions, longer reaction time, possessing no catalyst recyclability, use of Lewis acids and strong Brønsted acids. Most of the Lewis acid catalysts being moisture sensitive require usually more than stoichiometric amounts, inert atmosphere and get easily decomposed.

We have been involved in the study of the catalytic activity of tungstic acid in organic reactions. [46, 47] Many tungstic acid catalyzed organic transformations are

known and well-documented.[48] Tungstic acid, a low cost and readily available heterogeneous catalyst exhibits high catalytic efficiency.

In this chapter, the tungstic acid was thoroughly characterized by various analytical and spectral techniques and was employed for the synthesis of oxindole derivatives **34(a-h)** by condensation of indoles **32(a-h)** with isatins **33(a-h)** (Scheme 4.4).




**32** a-h **33** a-h **34** a-h

**Scheme 4.4:** Synthesis of 3,3-di(indol-3-yl)indolin-2-one derivatives **34 (a-h)**

Where **a)** $R_1$= H, $R_2$= H, $R_3$= H; **b)** $R_1$= H, $R_2$= Br, $R_3$= H;

**c)** $R_1$= H, $R_2$= $OCH_3$, $R_3$= H; **d)** $R_1$= $CH_3$, $R_2$= H, $R_3$= H;

**e)** $R_1$= H, $R_2$= Br, $R_3$= $CH_3$; **f)** $R_1$= H, $R_2$= $OCH_3$, $R_3$= $CH_3$;

**g)** $R_1$= H, $R_2$= H, $R_3$= $CH_3$; **h)** $R_1$= $CH_3$, $R_2$= H, $R_3$= $CH_3$

## 4.3 Results and Dıscussıon

### *Catalyst characterization*

Tungstic acid, a heterogeneous catalyst is thoroughly characterized by FTIR, TGA, SEM, EDX, surface area (BET) and surface acidity ($NH_3$-TPD).

### *FTIR:*

The FTIR spectrum of tungstic acid exhibited a broad band at 3400 $cm^{-1}$ for -OH stretching. The bands at 1083 $cm^{-1}$ and 727 $cm^{-1}$ showed the presence of W=O and W-O stretching, respectively. (Figure 4.6)

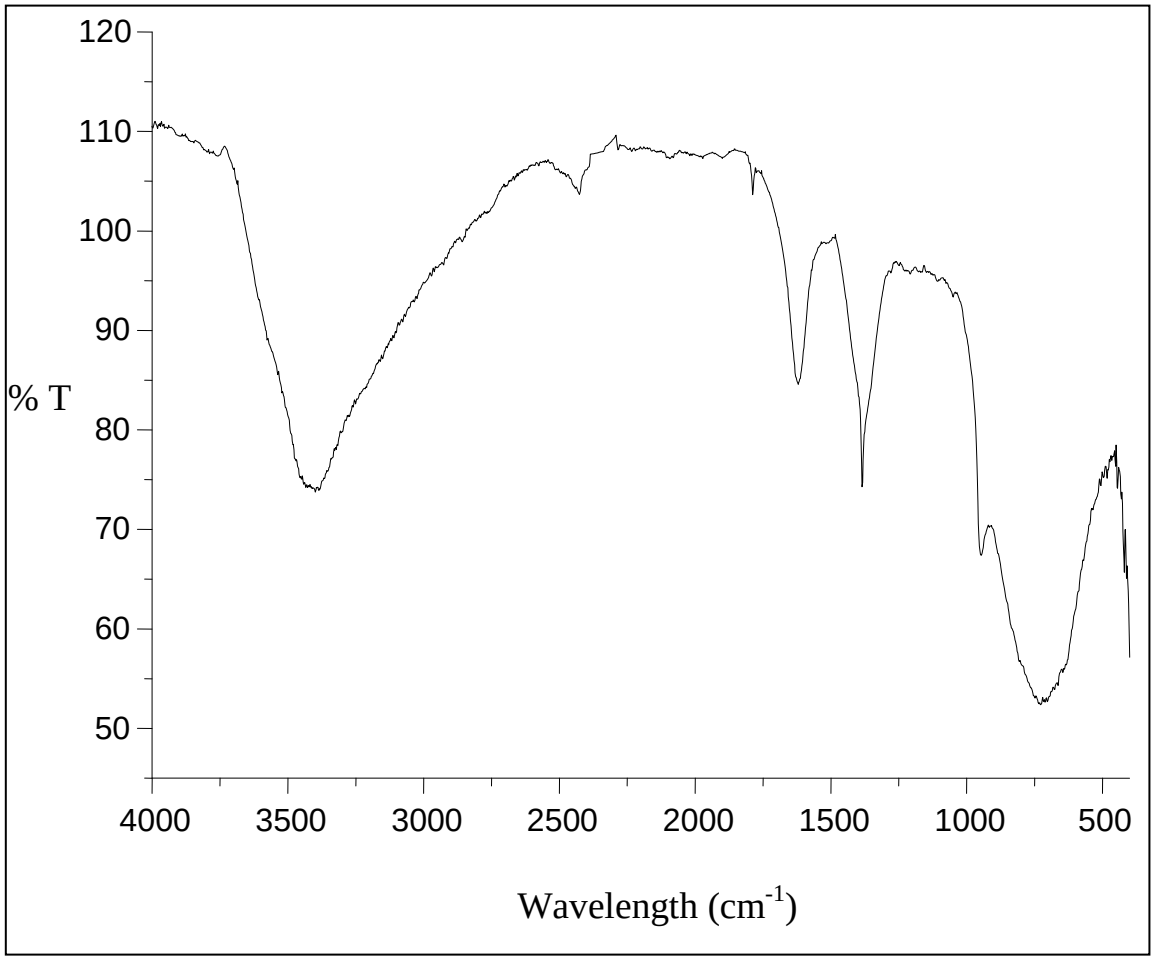



**Figure 4.6:** FTIR Spectrum of Tungstic acid

***Thermal analysis (TGA):***

TGA thermogram of tungstic acid showed 14.07 % weight loss, which was due to the loss of surface moisture and hydrated water. (Figure 4.7)

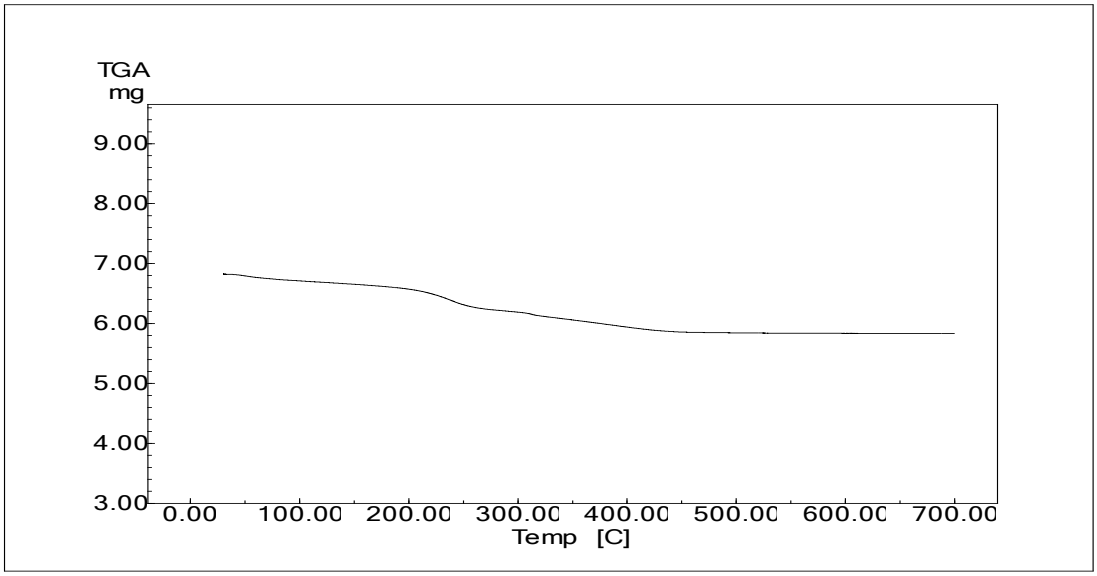



**Figure 4.7:** TGA Thermogram of tungstic acid

***Scanning Electron Microscopy (SEM):***

The SEM image of tungstic acid exhibited irregular morphology of the catalyst. (Figure 4.8)

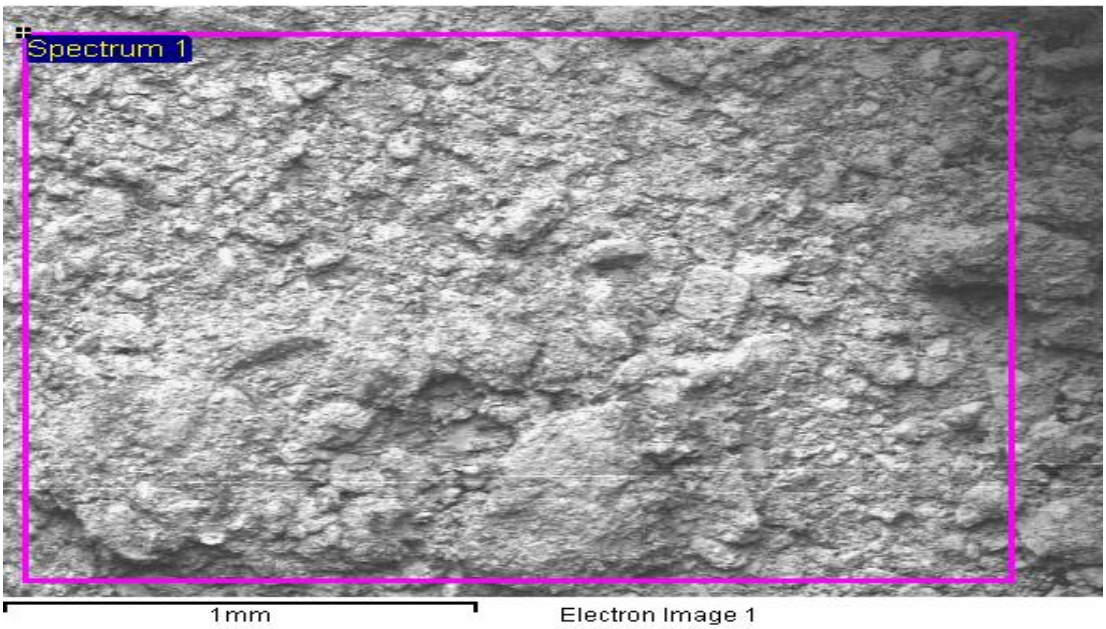



**Figure 4.8:** SEM image of tungstic acid

***Energy Dispersive X-ray spectroscopy (EDX)*:**

EDX graph of tungstic acid showed that the atomic % of W and O was found to be 18.93 and 81.07 % respectively. (Figure 4.9)

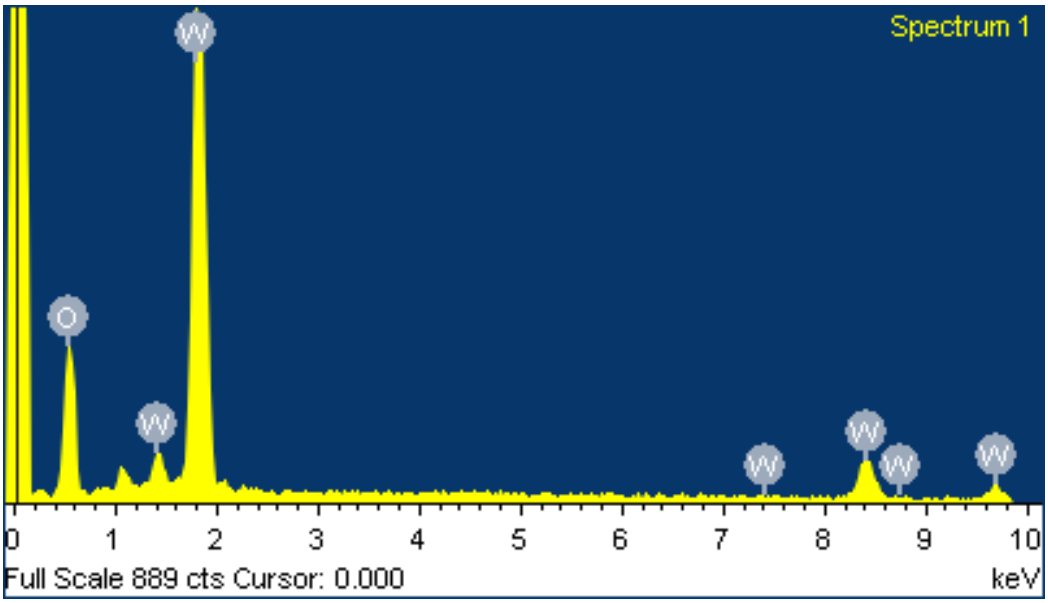



**Figure 4.9:** EDX graph for tungstic acid

***Surface acidity by Ammonia Temperature Programmed Desorption (TPD) method:***

Tungstic acid exhibited broad desorption peak (Figure 4.10) at higher temperature (at ~ 433 $^{o}$C) indicating the presence of strong acid sites in it. Surface acidity for tungstic

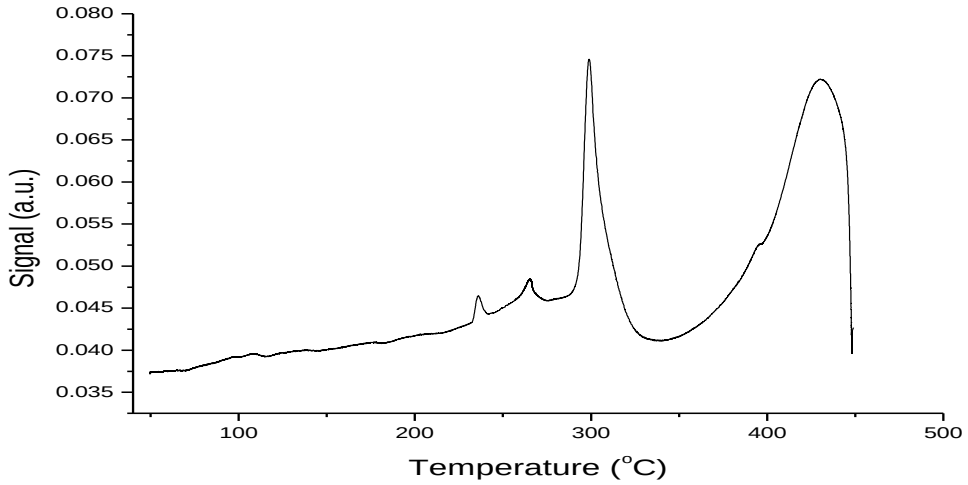



**Figure 4.10:** Surface acidity of tungstic acid by ammonia TPD method

acid at ~ 305 $^{o}$C and ~ 433 $^{o}$C were found to be 4.13 ml/g and 6.55 ml/g respectively (Figure 4.10). Surface area (BET method) for tungstic acid was found to be 7.54m$^2$/g.

***Optimization of reaction conditions***

The reaction of indole **32a** with isatin **33a** in the presence of tungstic acid to form of 3,3-di(indol-3-yl)indolin-2-one **34a** was taken as a model reaction for the optimization study.

**Table 4.1:** Optimization of reaction parameters for **32a** and **33a**

| Entry | Solvents | Amount of catalyst (mol %) | Reaction time (h) | Reaction temp (°C) | % Yield |
|---|---|---|---|---|---|
| 1 | Ethanol | 2.5 | 6 | 30 | 70.82 |
| 2 | Ethanol | 5 | 6 | 30 | 82.00 |
| 3 | Ethanol | 7.5 | 6 | 30 | 90.16 |
| **4** | **Ethanol** | **10** | **6** | **30** | **91.83** |
| 5 | Ethanol | 12.5 | 6 | 30 | 92.08 |
| 6 | Ethanol | 15 | 6 | 30 | 92.31 |
| 7 | Ethanol | 10 | 2 | 30 | 58.34 |
| 8 | Ethanol | 10 | 4 | 30 | 78.42 |
| 9 | Ethanol | 10 | 8 | 30 | 91.96 |
| 10 | Ethanol | 10 | 10 | 30 | 92.10 |
| 11 | Toluene | 10 | 6 | 30 | 58.82 |
| 12 | DMF | 10 | 6 | 30 | 69.38 |
| 13 | Chloroform | 10 | 6 | 30 | 79.20 |
| 14 | Dichloromethane | 10 | 6 | 30 | 83.54 |
| 15 | t- butyl alcohol | 10 | 6 | 30 | 88.46 |
| 16 | Methanol | 10 | 6 | 30 | 90.12 |
| 17 | Ethanol | 10 | 6 | 45 | 92.10 |
| 18 | Ethanol | 10 | 6 | 60 | 92.22 |
| 19 | Ethanol | 10 | 6 | Reflux | 92.38 |

In search of the best catalytic condition for this conversion, optimization of various parameters like catalyst quantity, solvent, temperature and time was carried out (Table 4.1).

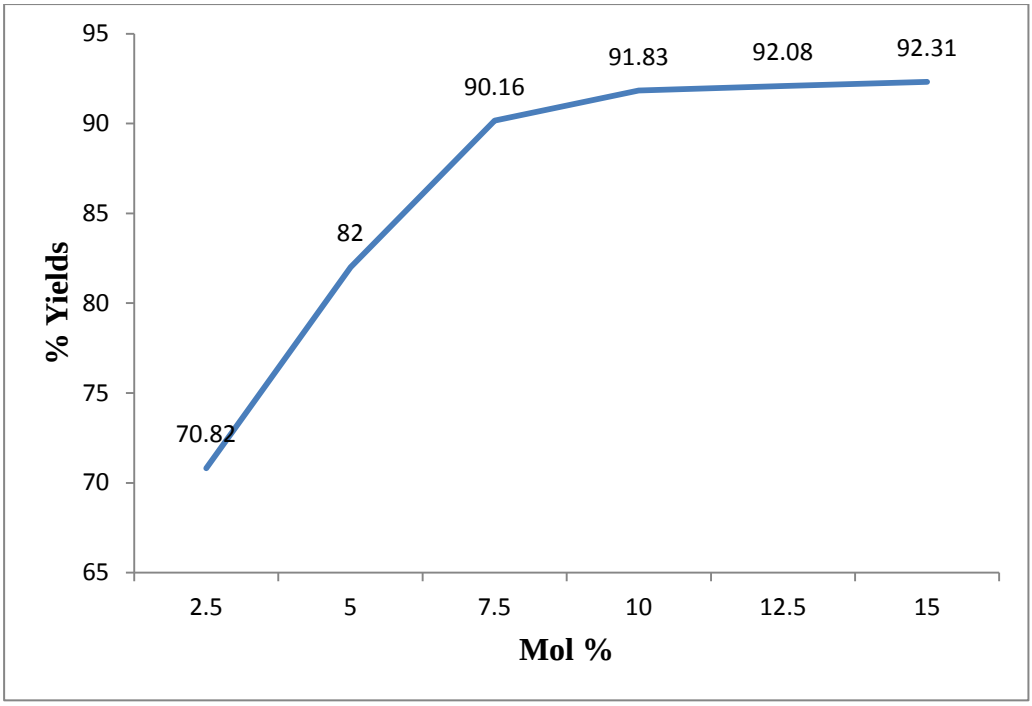



**Figure 4.11:** Optimization of mol % of catalyst

In order to evaluate the appropriate catalyst quantity, a reaction of **32a** and **33a** was carried out using various amounts of catalyst as shown in Table 4.1. It was found that 10 mol % of catalyst showed the maximum yield in minimum time. The use of larger amounts of the catalyst neither increased the yield nor shortened the conversion time (Figure 4.11). Thus, the 10 mol % of catalyst was found to be the optimal quantity.

Having found 10 mol % as the best catalyst quantity for this reaction, solvent optimization was done next. Various solvents such as toluene, chloroform, DMF, dichloromethane, methanol, *t*-butyl alcohol and ethanol were used. Among all the solvents studied, ethanol gave the best yield (91.83%) at 30°C. The use of methanol was avoided due to its toxicity. Increase in the yield of **34a** was observed up to 6 h. However, no substantial increase in the yield of **34a** was noted when the reaction was continued beyond 6 h (Figure 4.12). Thus, 6 h was taken as optimum reaction time. A marginal rise in the yield was also recorded as temperature was increased from 30°C to refluxing in ethanol.

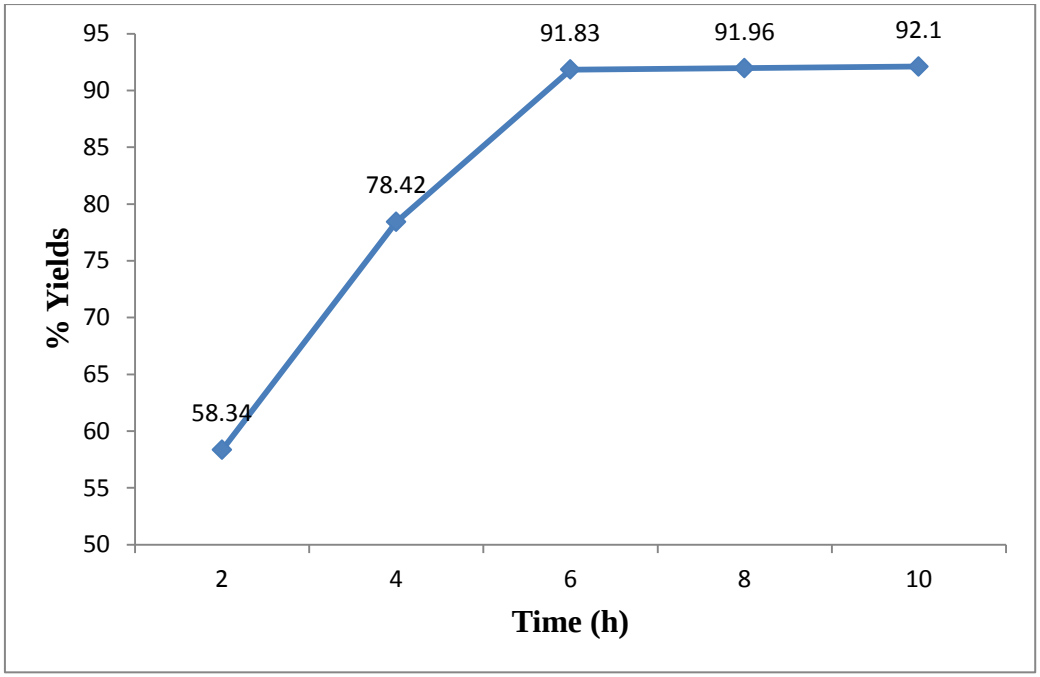



**Figure 4.12:** Optimization of reaction time

By following the optimized reaction parameters, we synthesized various oxindole derivatives **34**(**a-h**). The structures of the compounds **34(a-h)** were confirmed by FTIR, $^{1}$H NMR, $^{13}$C NMR, mass and elemental analysis. The FTIR spectrum of **34e** showed bands at 1608, 1456 $cm^{-1}$ for aromatic ring and a strong band at 1686 $cm^{-1}$ for the carbonyl of amide group along with a broad band at 3354 $cm^{-1}$ due to the presence of N-H groups. The $^{1}$H NMR spectrum of **34e** displayed a singlet at δ 3.25 for the three methyl protons and a singlet at δ 6.91 for two olefinic protons. The multiplets between δ 7.06-7.40 showed the presence of ten aromatic protons and a singlet at δ 11.25 for two amine protons. The $^{13}$C NMR spectrum of **34e** exhibited signals at δ 26.76 for carbon of methyl group, at δ 52.16 for quaternary carbon, at δ 109.47, 111.55, 113.97, 114.34, 123.01, 124.15, 125.00, 126.37, 127.65, 128.92, 133.14, 136.11 and 143.04 for aromatic carbons along with a signal at δ 177.18 for carbonyl carbon of amide group. The structure of **34e** was further confirmed by its mass spectrum which gave a molecular ion peak at 534. The elemental analysis was in good agreement with the required molecular formula for $C_{25}H_{17}Br_2N_3O$ and it was found as C, 56.12; H, 3.24; N, 7.82 and calculated; C, 56.10; H, 3.20; N, 7.85.

The FTIR spectrum of **34f** showed bands at 1606, 1481 $cm^{-1}$ for aromatic ring and strong band at 1693 $cm^{-1}$ for carbonyl of amide group along with broad band at

3398 $cm^{-1}$ due to the presence of N-H group. The $^{1}H$ NMR spectrum of **34f** displayed singlets at δ 3.27, 3.51 for methyl protons and singlet at δ 6.58 for two olefinic protons. The multiplets in the range of δ 6.59-7.37 showed the presence of aromatic protons and singlet at δ 10.84 for two amine protons. The $^{13}C$ NMR spectrum of **34f** exhibited signals at δ 26.63 and 52.51 for carbon of methyl groups, signal at δ 55.46 for quaternary carbon, signals at δ 103.31, 109.00, 111.05, 112.62, 113.76, 122.74, 125.12, 125.62, 126.40, 128.49, 132.56, 134.04, 143.27 and 152.88 for aromatic carbon along with signal at δ 177.41 for carbonyl carbon of amide group. The structure of **34f** was further confirmed by its mass spectrum which gave a molecular ion peak at 437. The elemental analysis was in good agreement with the required molecular formula for $C_{27}H_{23}N_3O_3$ and it was found as C, 74.12; H, 5.30; N, 9.60 and calculated; C, 74.08; H, 5.32; N, 9.58.

**Recyclability and reusability of the catalyst:**

For regeneration and reusability, the catalyst was heated in ethanol at reflux temperature for half an hour, washed with ethanol and dried at room temperature (~ 30°C). The catalyst reusability study showed that tungstic acid can be reused as such up to three cycles without significant decrease in the yield. (Table 4.2)

**Table 4.2:** Reusability of tungstic acid

| Entry | Amount of catalyst (mol %) | Cycles | % Yield |
|---|---|---|---|
| 1 | 10 | 1 | 91.83 |
| 2 | 10 | 2 | 82.64 |
| 3 | 10 | 3 | 70.81 |
| 4 | 10 | 4 | 24.36 |

Some of the reactant molecules on the surface of the catalyst enter into reaction to give a product while a few of them get adsorbed on the surface. This is perhaps responsible for a change in the color of the catalyst which was observed to be green after use. It was also noted that after every regeneration cycle, there was a decrease in yields of the product which may be due to the deactivation of the catalyst by adsorption of the substrate molecules on the surface. The FTIR of the spent catalyst revealed that there were some additional bands throughout the spectrum which

indicated the presence of organic molecules adsorbed on the surface of the catalyst (Figure 4.13).

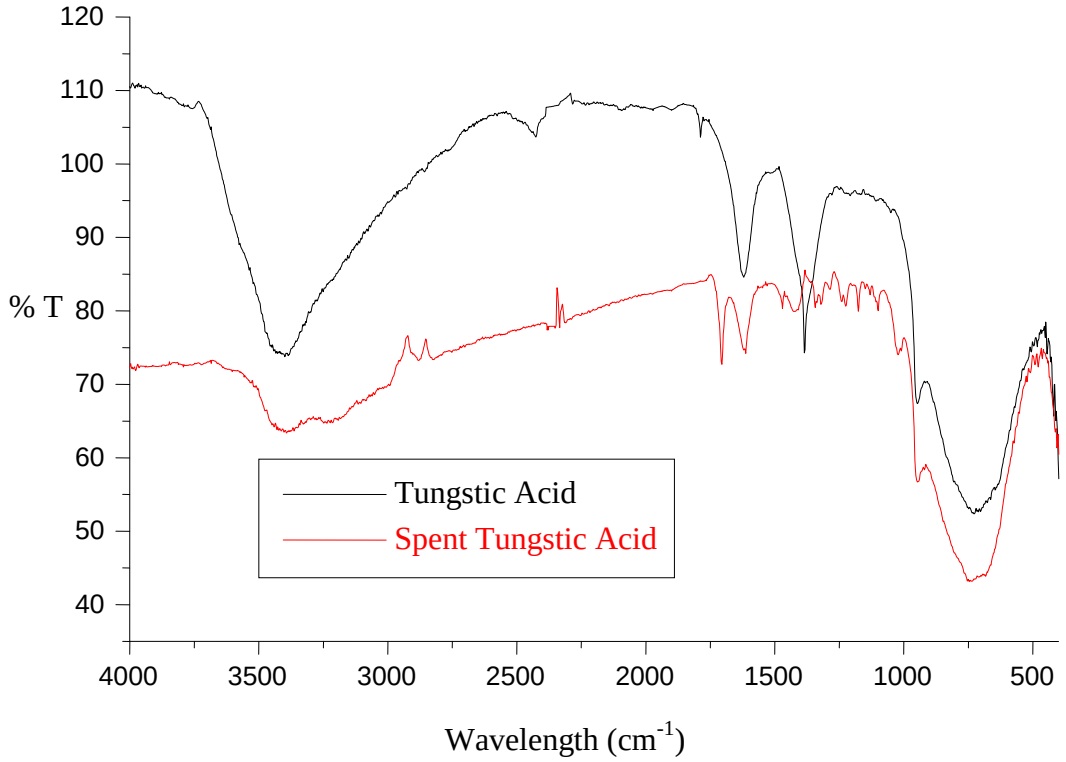



**Figure 4.13:** Overlap FTIR Spectra of tungstic acid and spent tungstic acid

Further, EDX of the spent tungstic acid (Figure 4.14) showed a decrease in atomic % of W from 18.93 to 18.10 %.

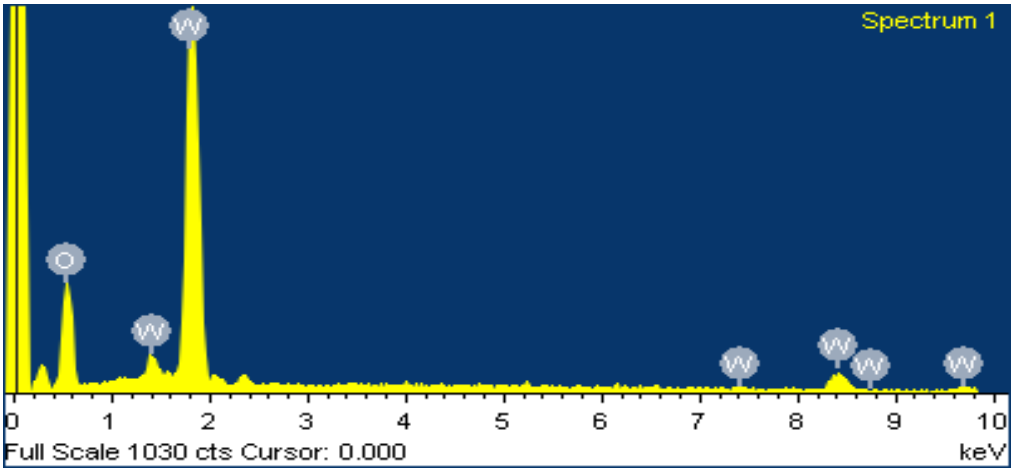
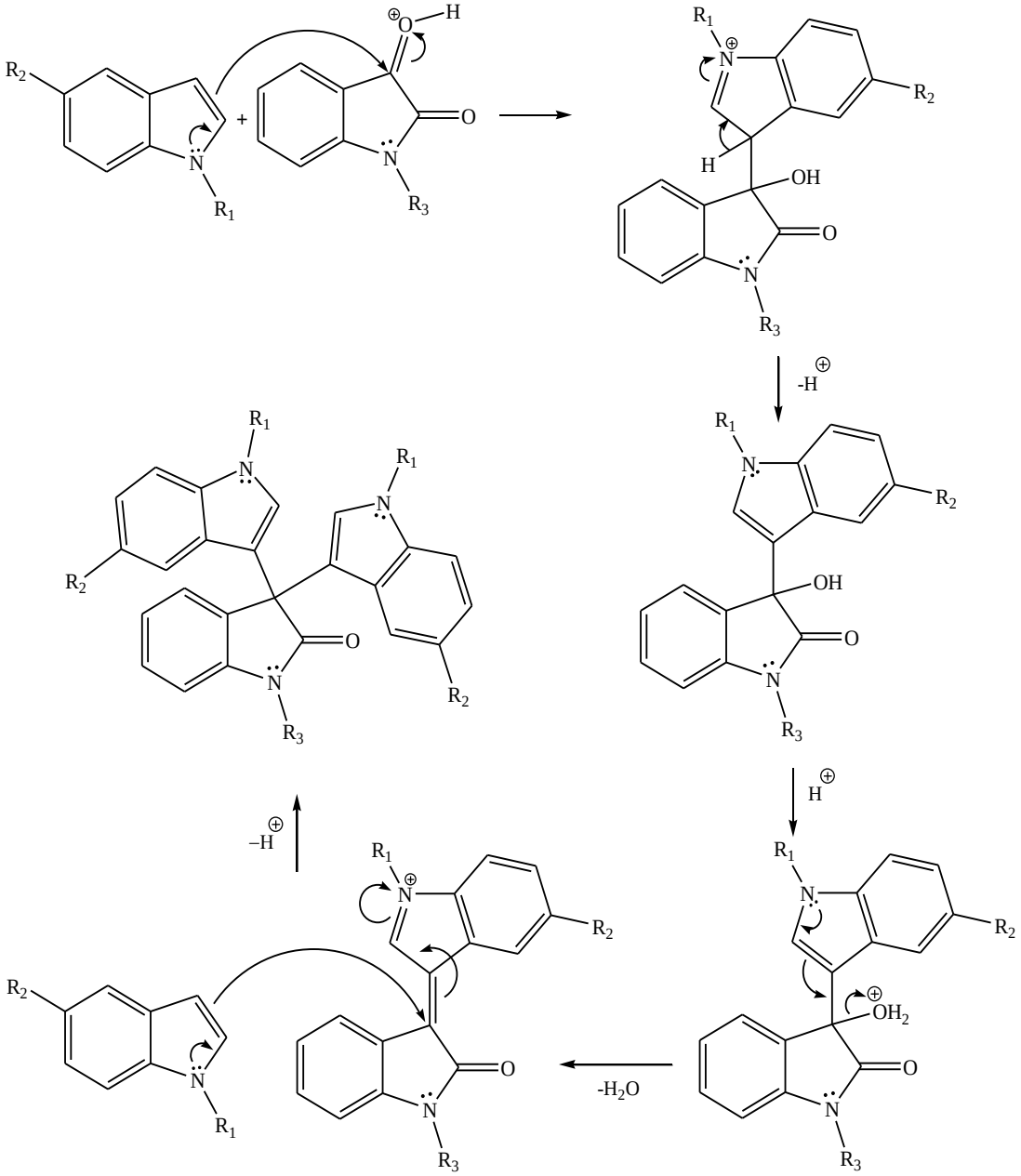



**Figure 4.14:** EDX graph for spent tungstic acid



**Scheme 4.5:** Mechanism of condensation of indoles with isatins

The Scheme 4.5 shows a plausible mechanism of formation of 3,3-di(indol-3-yl)indolin-2-one derivatives (**34a-h**) by the condensation of indoles (**32a-h**) with isatins (**33a-h**) in the presence of tungstic acid.

## 4.4 Experimental

### *General*

FTIR spectra were recorded on a Shimadzu 8400S FTIR spectrometer using KBr. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker-400MHz NMR spectrometer (100 MHz for $^{13}$C NMR) using DMSO-$d_6$ (TMS as an internal standard). Mass spectra were obtained on a Shimadzu QP-5050 mass spectrometer. Column chromatography was carried out on Acme's silica gel (60-120 mesh size) and eluted using mixtures of light petroleum and ethyl acetate. Thin layer chromatography was performed using Acme's silica gel for TLC and spots were visualized in the iodine vapor. Percentage yields were reported based upon the recovery of starting materials. The structures of all the compounds were confirmed by their mp, FTIR, $^{1}$H NMR, $^{13}$C NMR and mass spectrometric data.

### *Catalyst Characterization*

Thermal analysis (TGA) was carried out on a Shimadzu (Model TGA 50) thermal analyzer at a heating rate of 10°C min$^{-1}$. SEM and EDX were obtained on a Jeol JSM-5610-SLV scanning electron microscope. Surface area measurement (by BET method) was measured on Micromeritics Gemini at -196°C using nitrogen adsorption isotherms. Surface acidity was determined on Micromeritics Chemisorb 2720 by a temperature programmed desorption of ammonia.

### *Typical experimental procedure for the synthesis of oxindole derivatives*

To a stirred solution of isatin (**33a**, 1.0 g, 6.79 mmol) and indole (**32a**, 1.59 g, 13.58 mmol) in ethyl alcohol (10 ml), tungstic acid (10 mol %) was added. The reaction mixture was stirred for 6 h at room temperature (~ 30 °C). After completion of reaction (TLC), it was filtered through a celite pad to remove the suspended catalyst. Removal of the solvent and chromatography of the residue furnished the 3,3-di(1*H*-indol-3-yl)indolin-2-one **34a**.

***3,3-Di(1H-indol-3-yl)indolin-2-one (34a)***

White solid, 92%, mp > 300 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3429, 3327, 3281, 1708, 1614, 1469, 1437, 1330, 1219, 1107, 1012, 759, 613; $^{1}$H NMR: $\delta_H$ 6.78 (t, 2H, *J* = 7.2Hz), 6.81 (s, 2H), 6.95 (m, 4H), 7.22 (t, 4H, *J* = 7.6Hz), 7.34 (d, 2H, *J* = 8.0Hz), 10.61 (s, 1H), 10.97 (s, 2H); MS: m/z 363 ($M^+$), 334 ($M^+$ - [HC=O]), 247 ($M^+$ - [indole]), 218 (334 – [indole]), 116.

***3,3-Bis(5-bromo-1H-indol-3-yl)indolin-2-one (34b)***

White solid, 92%, mp 264-266 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3435, 3323, 3284, 1716, 1616, 1467, 1334, 1242, 1099, 887, 752, 650, 582, 486; $^{1}$H NMR: $\delta_H$ 6.92 (s, 2H), 7.00 (m, 2H), 7.16 (m, 3H), 7.27 (t, 1H, *J* = 7.6 Hz), 7.36 (m, 4H), 10.77 (s, 1H) 11.25 (s, 2H); $^{13}$C NMR: $\delta_C$ 52.61, 110.32, 111.52, 114.25, 114.30, 122.31, 123.09, 124.11, 125.31, 126.43, 127.70, 128.75, 134.03, 136.13, 141.63, 178.91 (Keto carbon); MS: m/z 519 ($M^+$), 490 ($M^+$ - [HC=O]), 325 ($M^+$ - [bromoindole]), 298 (490 – [bromoindole]), 193.

***3,3-Bis(5-methoxy-1H-indol-3-yl)indolin-2-one (34c)***

White solid, 92%, mp 240- 244 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3377, 3327, 2939, 1685, 1618, 1483, 1301, 1253, 1215, 1035, 802, 756, 632; $^{1}$H NMR: $\delta_H$ 3.52 (s, 6H, O$\underline{C}H_3$), 6.69 (m, 4H), 6.85 (s, 2H), 6.97 (m, 2H), 7.23 (m, 4H), 10.62 (s, 1H), 10.82 (s, 2H); $^{13}$C NMR: $\delta_C$ 52.94, 55.54 (O$\underline{C}H_3$), 103.77, 109.92, 110.86, 112.54, 114.02, 121.98, 125.41, 125.60, 126.53, 128.32, 132.60, 134.96, 141.83, 152.84, 179.24 (Keto carbon); MS: m/z 423 ($M^+$), 394 ($M^+$ - [HC=O]), 277 ($M^+$ - [5-methoxyindole]), 250 (394 – [5-methoxyindole]), 147.

***3,3-Bis(1-methyl-1H-indol-3-yl)indolin-2-one (34d)***

White solid, 89%, mp > 300 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3184, 2933, 2881, 1707, 1614, 1537, 1471, 1330, 1207, 1132, 1016, 758, 738; $^{1}$H NMR: $\delta_H$ 3.70 (s, 6H, $CH_3$), 6.83 (m, 2H), 6.88 (s, 2H), 6.95 (m, 2H), 7.08 (m, 2H), 7.22 (m, 4H), 7.37 (d, 2H, *J* = 8.4 Hz), 10.63 (s, 1H); $^{13}$C NMR: $\delta_C$ 32.80 ($\underline{C}H_3$), 52.82, 110.09, 110.24, 113.82, 118.87, 121.35, 121.55, 122.07, 125.32, 126.43, 128.39, 128.89, 134.92, 137.74, 141.65, 179.03 (Keto carbon); MS: m/z 391 ($M^+$), 362 ($M^+$ - [HC=O]), 261 ($M^+$ - [1-methylindole]), 232 (362 – [1-methylindole]), 130.

***3,3-Bis(5-bromo-1H-indol-3-yl)-1-methylindolin-2-one (34e)***

White solid, 91%, mp 280-284 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3354, 3117, 1686, 1608, 1456, 1371, 1286, 1089, 794, 746, 690, 601, 501, 418; $^{1}$H NMR: $\delta_H$ 3.25 (s, 3H, $CH_3$), 6.91 (s, 2H), 7.07 (m, 3H), 7.14 (m, 2H), 7.26 (m, 2H), 7.38 (m, 3H), 11.25 (s, 2H). $^{13}$C NMR: $\delta_C$ 26.76 ($CH_3$), 52.16, 109.47, 111.55, 113.97, 114.34, 123.01, 124.15, 125.00, 126.37, 127.65, 128.92, 133.14, 136.11, 143.04, 177.18 (Keto carbon); MS: m/z 534 ($M^+$), 505 ($M^+$ - [HC=O]), 342 ($M^+$ - [bromoindole]), 312 (505 – [bromoindole]), 193; Anal. Calcd. for $C_{25}H_{17}Br_2N_3O$: C, 56.10; H, 3.20; N, 7.85. Found: C, 56.12; H, 3.24; N, 7.82.

***3,3-Bis(5-methoxy-1H-indol-3-yl)-1-methylindolin-2-one (34f)***

White solid, 88%, mp 236-240$^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3398, 2999, 2821, 1693, 1606, 1481, 1354, 1220, 1124, 1020, 817, 752; $^{1}$H NMR: $\delta_H$ 3.27 (s, 3H, $CH_3$), 3.51 (s, 6H, $OCH_3$), 6.58 (s, 2H), 6.76 (m, 2H), 6.84 (d, 2H, *J* = 2.4Hz), 7.03 (m, 2H), 7.27 (m, 4H), 10.84 (s, 2H); $^{13}$C NMR: $\delta_C$ 26.63 ($CH_3$), 52.51, 55.46 ($OCH_3$), 103.31, 109.00, 111.05, 112.62, 113.76, 122.74, 125.12, 125.62, 126.40, 128.49, 132.56, 134.04, 143.27, 152.88, 177.41 (Keto carbon); MS: m/z 437 ($M^+$), 408 ($M^+$ - [HC=O]), 291 ($M^+$ - [5-methoxyindole]), 262 (408 – [5-methoxyindole]), 146; Anal. Calcd. for $C_{27}H_{23}N_3O_3$: C, 74.12; H, 5.30; N, 9.60. Found: C, 74.08; H, 5.32; N, 9.58.

***3,3-Di(1H-indol-3-yl)-1-methylindolin-2-one (34g)***

White solid, 90%, mp > 300 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3358, 3051, 1670, 1610, 1469, 1354, 1240, 1091, 916, 732; $^{1}$H NMR: $\delta_H$ 3.26 (s, 3H, $CH_3$), 6.78 (m, 2H), 6.80 (s, 2H), 6.99 (m, 3H), 7.09 (m, 3H), 7.36 (m, 4H), 10.99 (s, 2H); $^{13}$C NMR: $\delta_C$ 26.70 ($CH_3$), 52.65, 109.21, 112.08, 114.48, 118.77, 121.11, 121.45, 122.81, 124.76, 126.03, 128.58, 134.25, 137.35, 143.32, 177.42 (Keto carbon); MS: m/z 377 ($M^+$), 348 ($M^+$ - [HC=O]), 261 ($M^+$ - [indole]), 232 (348 – [indole]), 116.

***1-Methyl-3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one (34h)***

White solid, 88%, mp 230-232 $^{o}$C; IR ($\nu_{max}$, cm$^{-1}$): 3050, 2931, 1722, 1604, 1469, 1332, 1251, 1128, 1008, 908, 746; $^{1}$H NMR: $\delta_H$ 3.25 (s, 3H, $CH_3$), 3.70 (s, 6H, $CH_3$), 6.81 (m, 2H), 6.87 (s, 2H), 7.00 (m, 1H), 7.04 (m, 2H), 7.08 (m, 3H), 7.17 (m, 1H),

7.30 (m, 3H); $^{13}$C NMR: $\delta_C$ 26.74 ($CH_3$), 32.81 ($CH_3$), 52.41, 109.19, 110.26, 113.59, 118.95, 121.28, 121.59, 122.77, 125.00, 126.36, 128.53, 128.92, 133.98, 137.76, 143.08, 177.17 (Keto carbon); MS: m/z 405 ($M^+$), 376 ($M^+$ - [HC=O]), 275 ($M^+$ - [1-methylindole]), 247 (376 – [1-methylindole]), 130.

## 4.5 Conclusion

An efficient and expeditious method is reported for the synthesis of 3,3-di(indol-3-yl)indolin-2-one derivatives (**34a-h**) using tungstic acid as a heterogeneous catalyst. The present method has several advantages like high yields, relatively mild conditions and easy work-up procedure. The catalyst can be easily regenerated and reused up to three cycles without significant loss in the yield.

# 4.6 Spectra

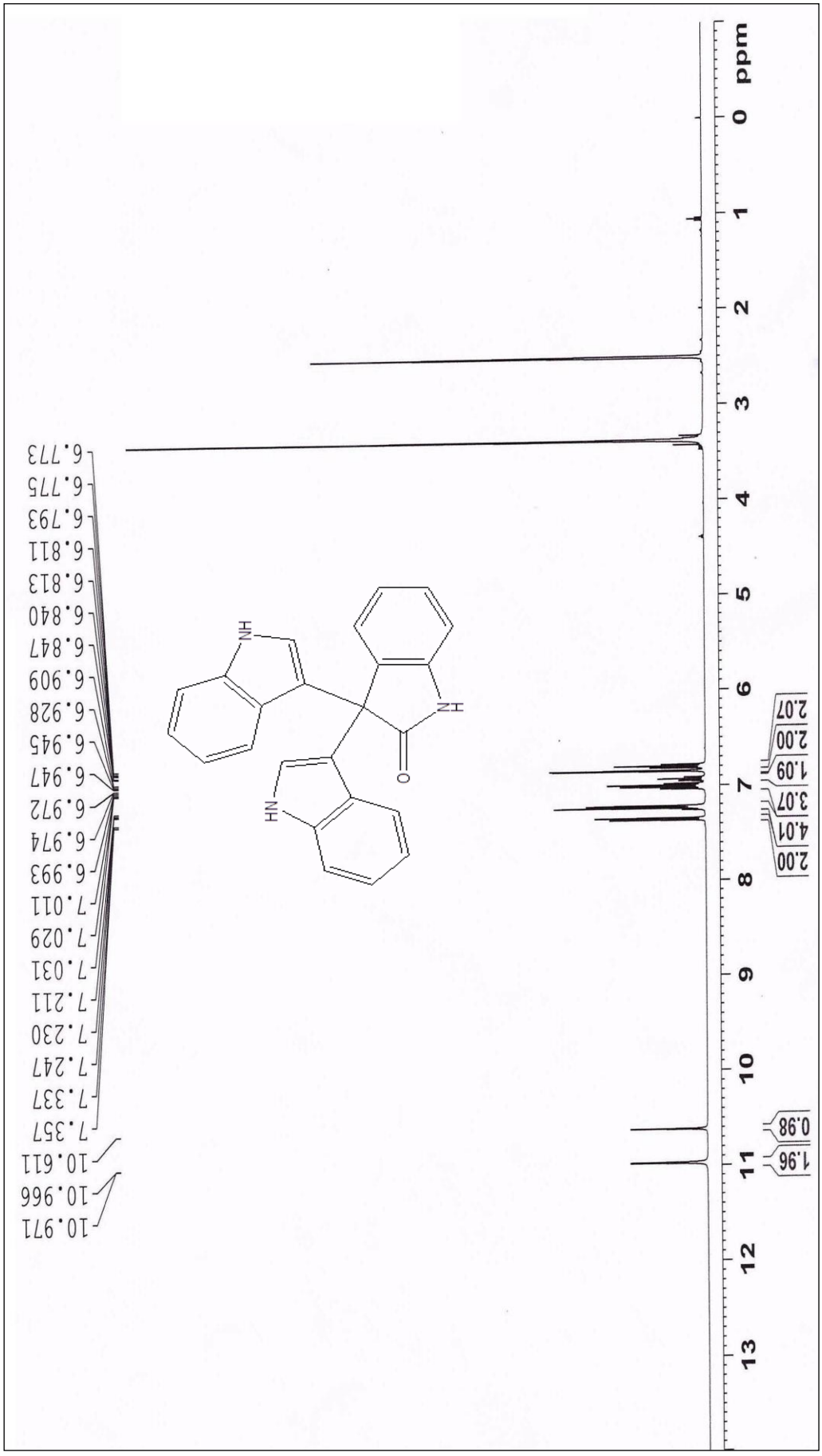



**Figure 4.15**:- $^{1}$H NMR of 3,3-di(1H-indol-3-yl)indolin-2-one **(34a)**

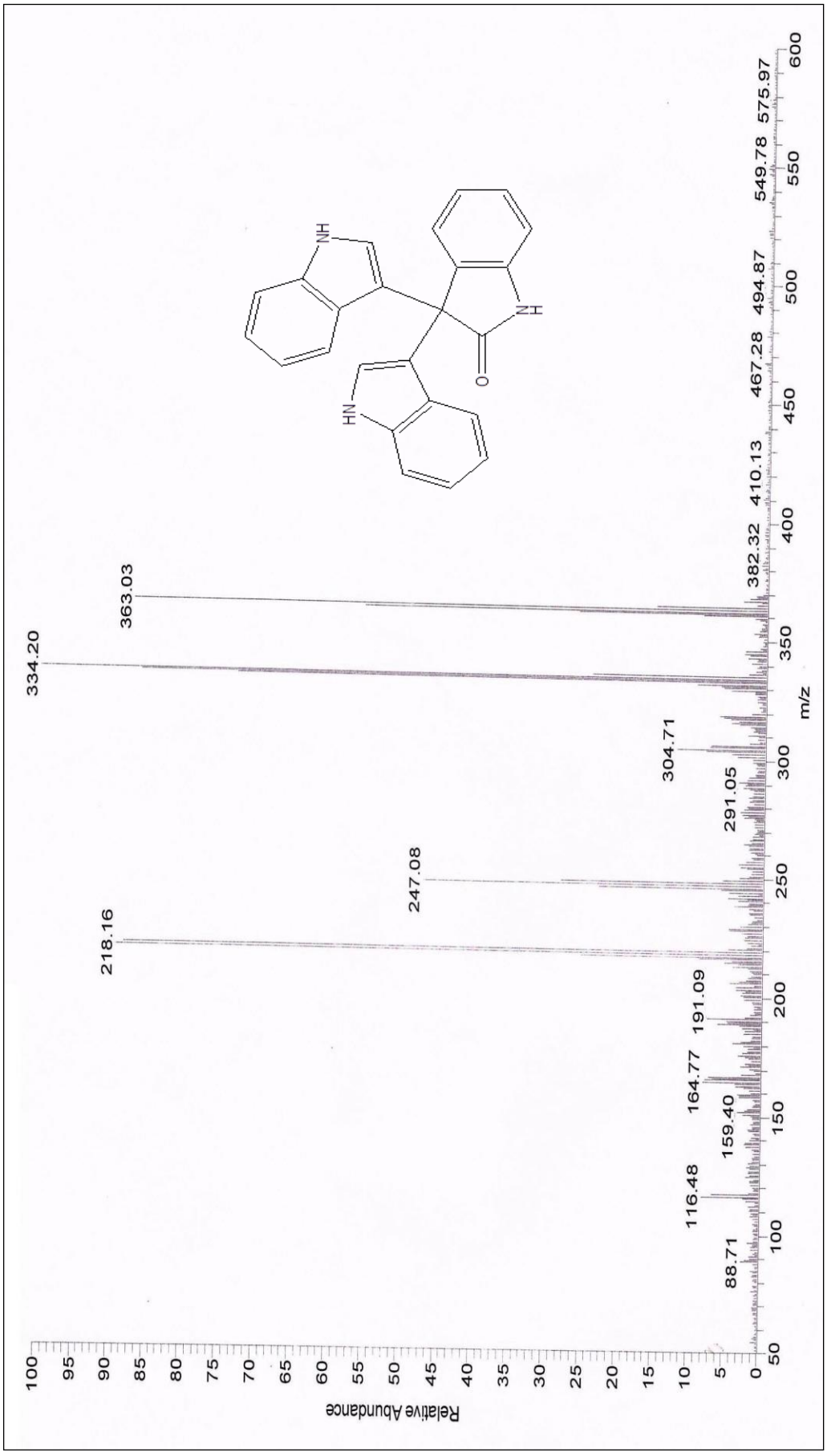



**Figure 4.16**:- Mass Spectrum of 3,3-di(1H-indol-3-yl)indolin-2-one **(34a)**

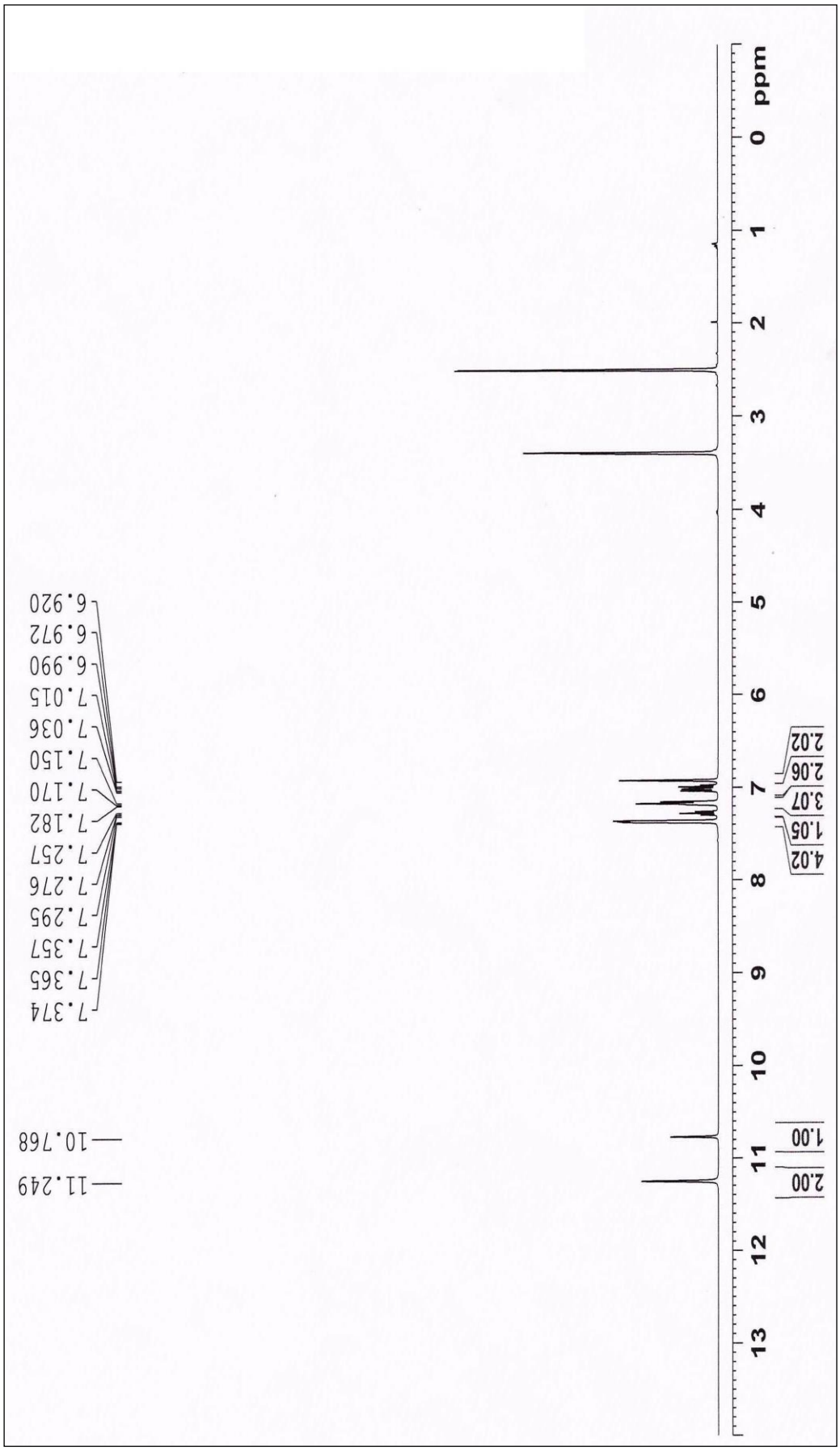



**Figure 4.17**:- $^{1}$H NMR of 3,3-bis(5-bromo-1H-indol-3-yl)indolin-2-one **(34b)**

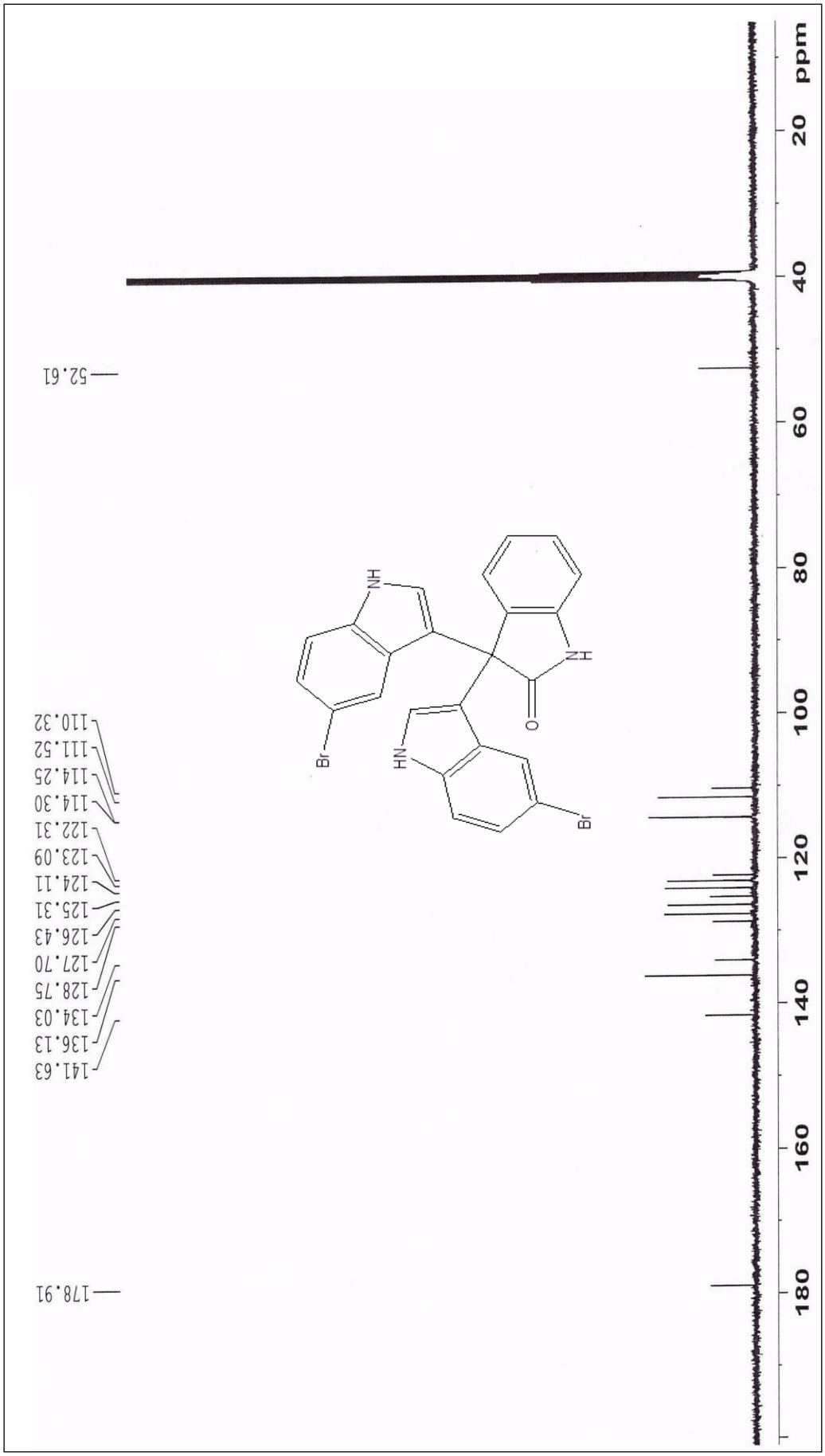



**Figure 4.18**:- $^{13}C$ NMR of ,3-bis(5-bromo-1H-indol-3-yl)indolin-2-one **(34b)**

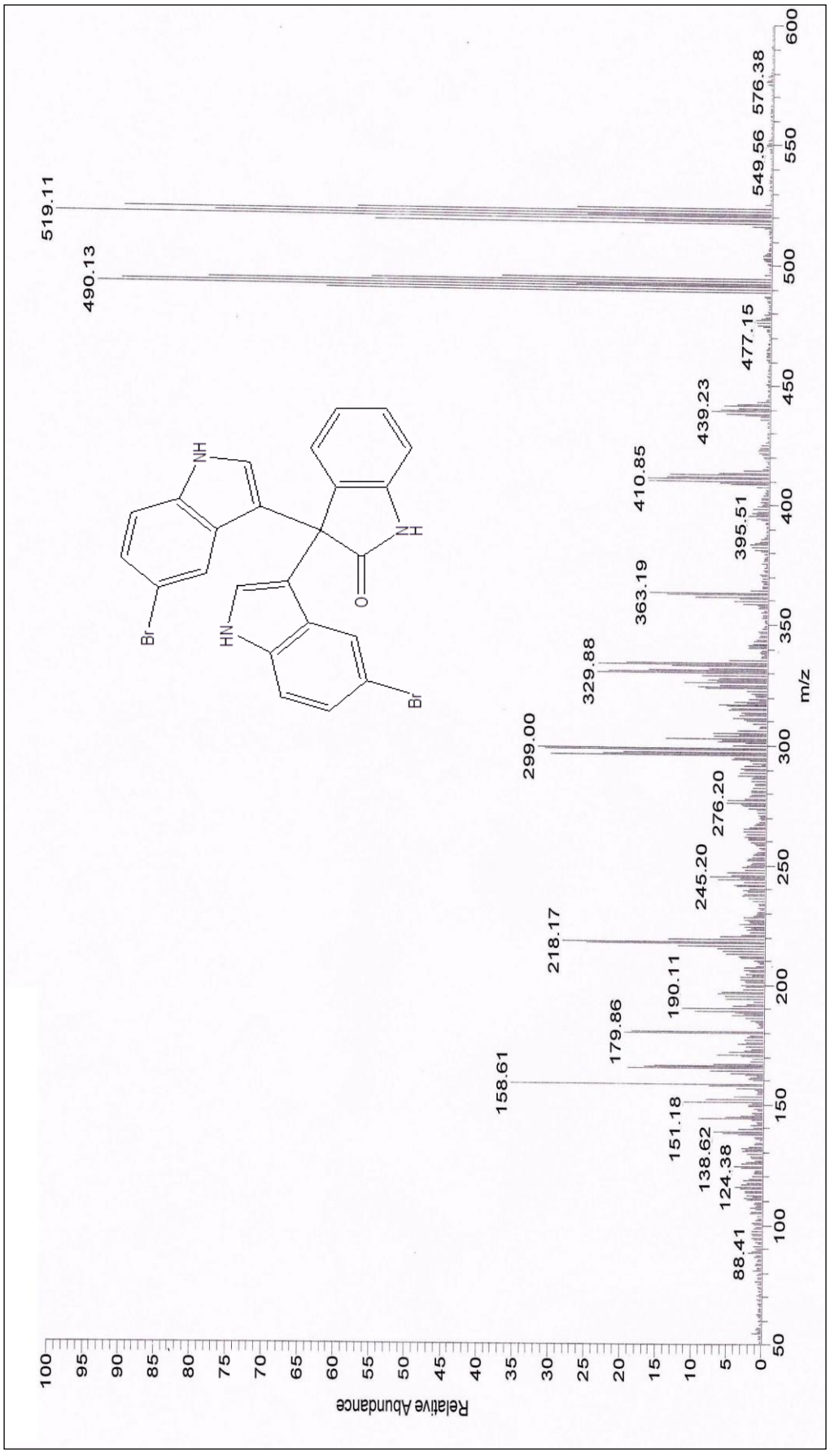



**Figure 4.19**:- Mass Spectrum of 3,3-bis(5-bromo-1H-indol-3-yl)indolin-2-one **(34b)**

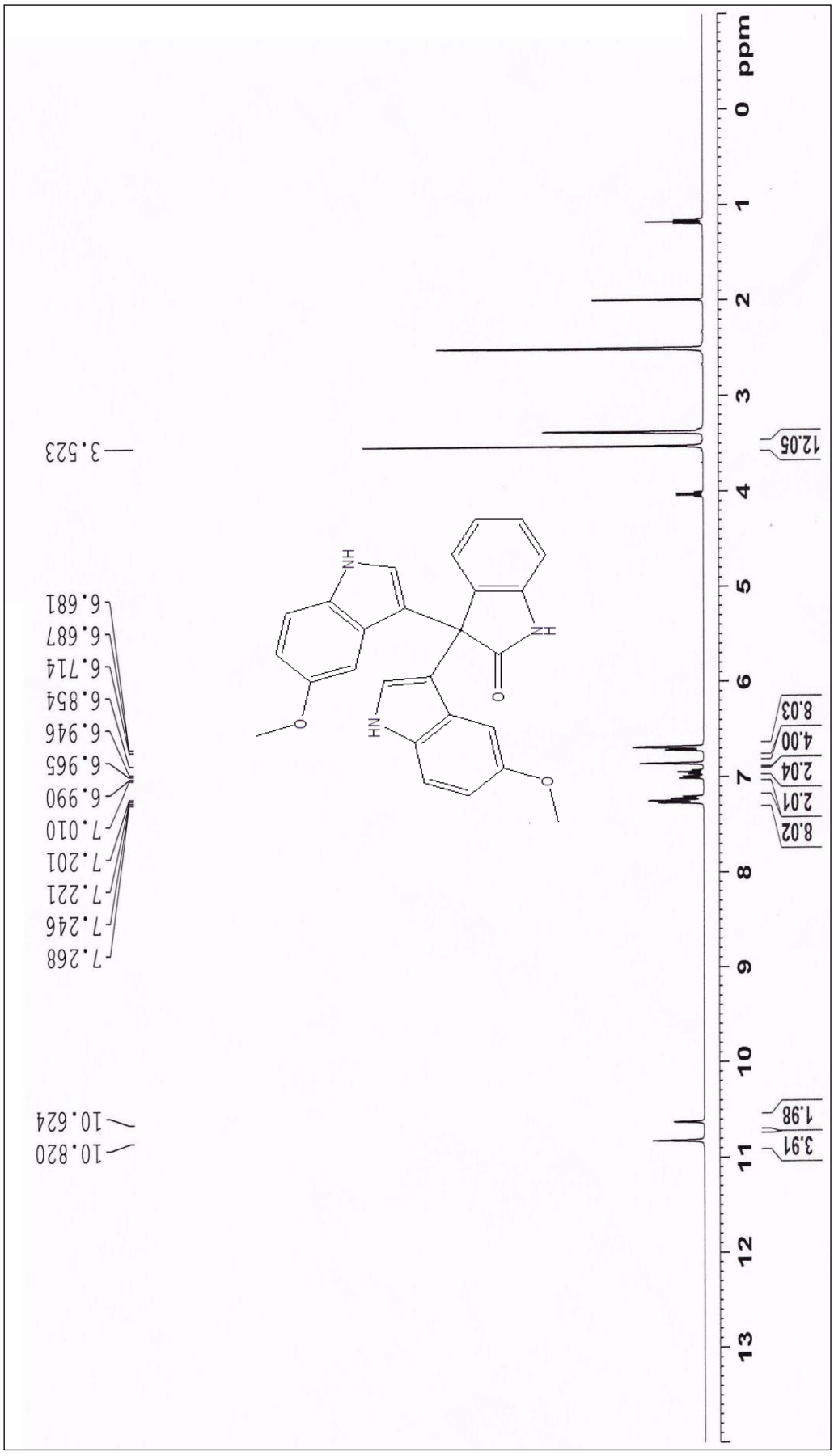



**Figure 4.20**:- $^{1}$H NMR of 3,3-bis(5-methoxy-1H-indol-3-yl)indolin-2-one **(34c)**

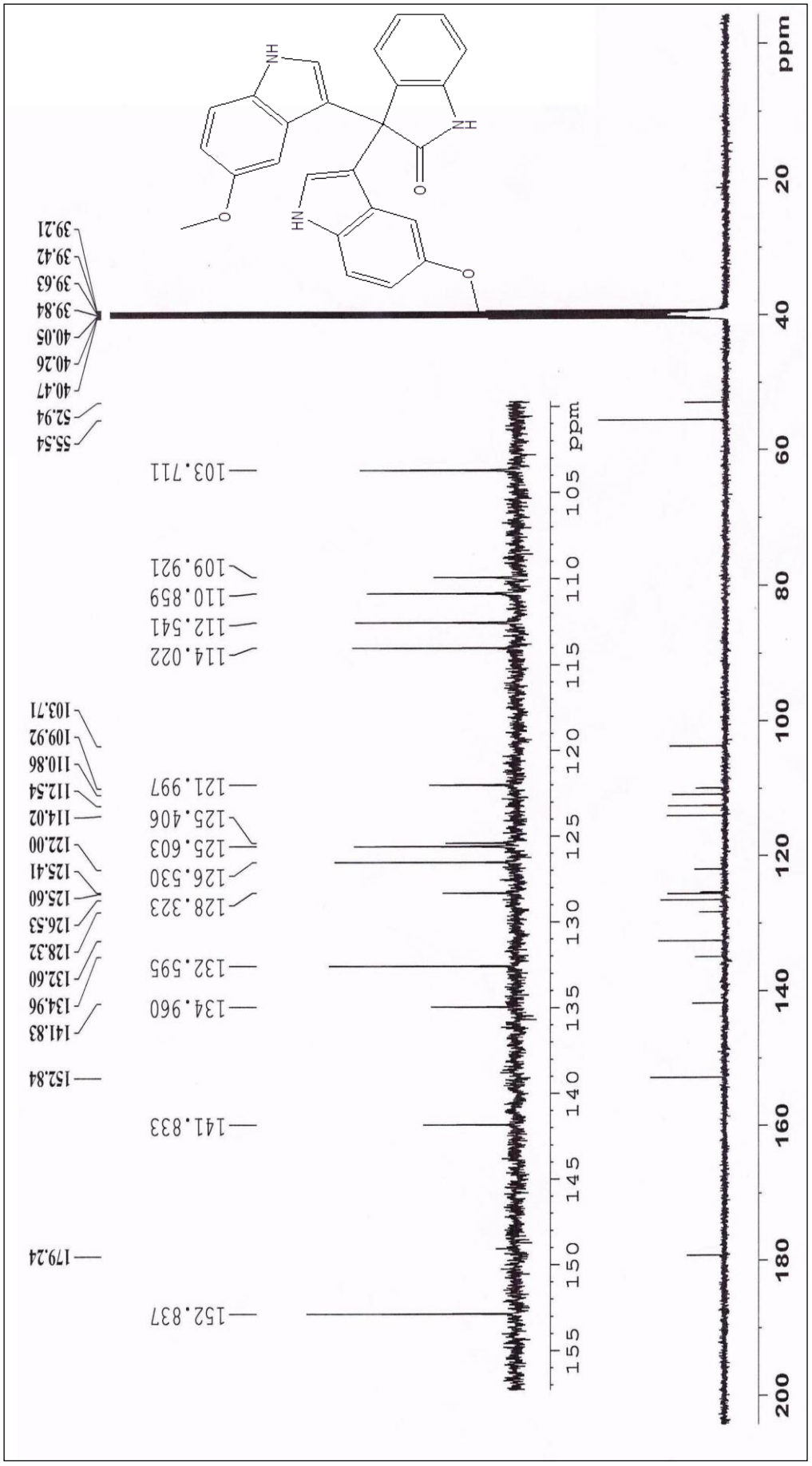



**Figure 4.21**:- $^{13}C$ NMR of 3,3-bis(5-methoxy-1H-indol-3-yl)indolin-2-one (**34c**)

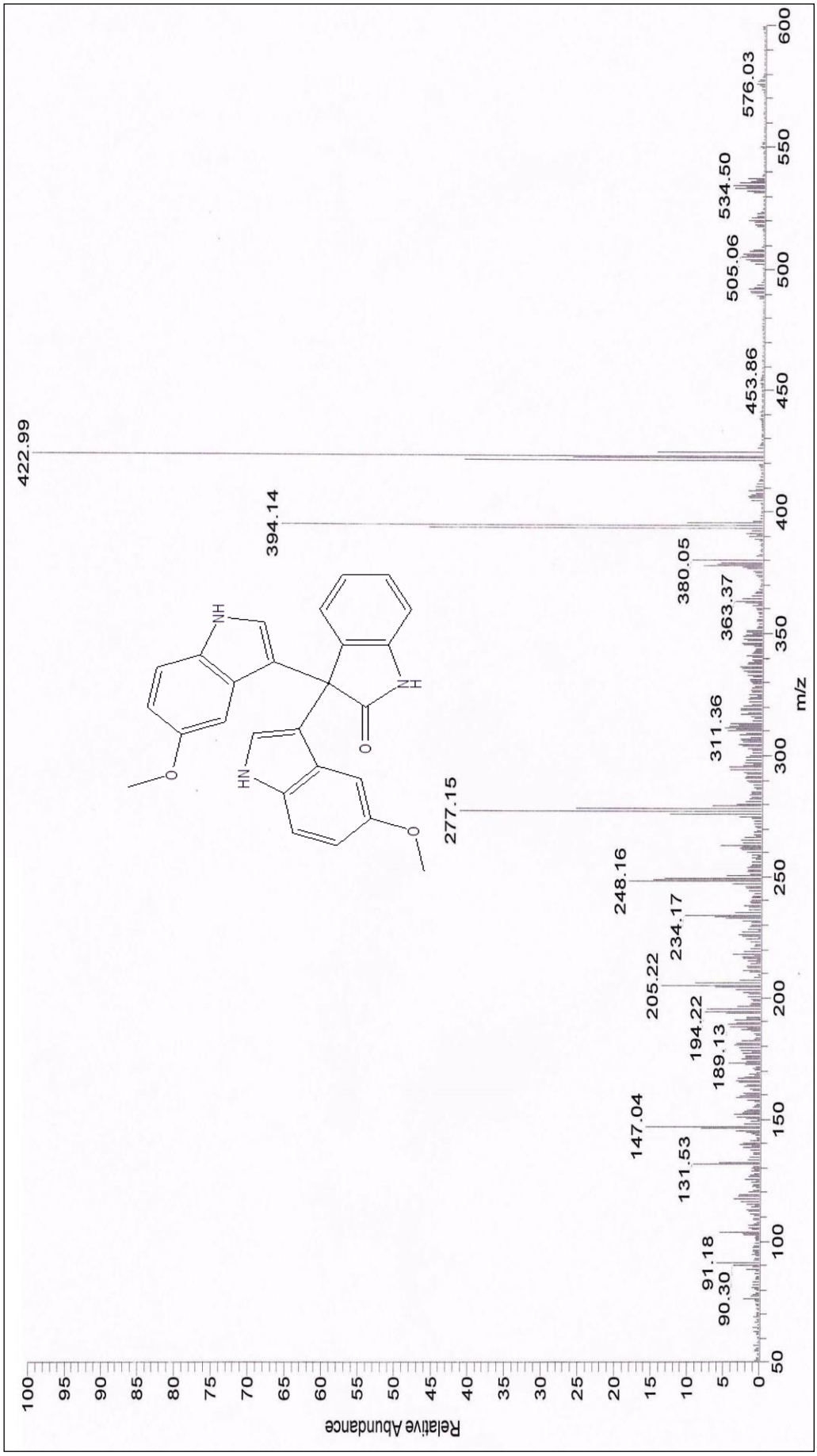



**Figure 4.22**:- Mass Spectrum of 3,3-bis(5-methoxy-1H-indol-3-yl)indolin-2-one **(34c)**

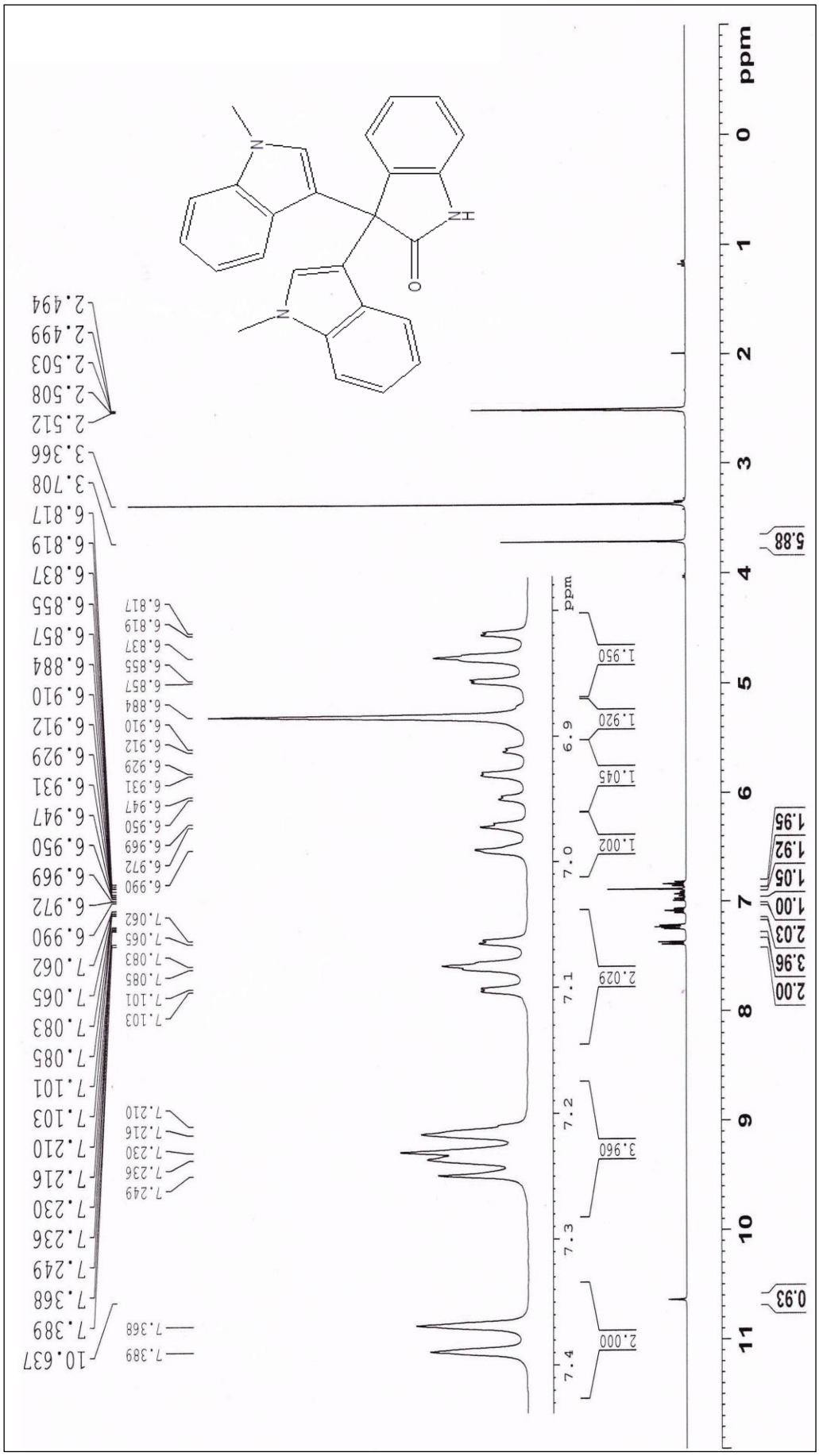


**Figure 4.23**:- $^{1}$H NMR of 3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34d)**

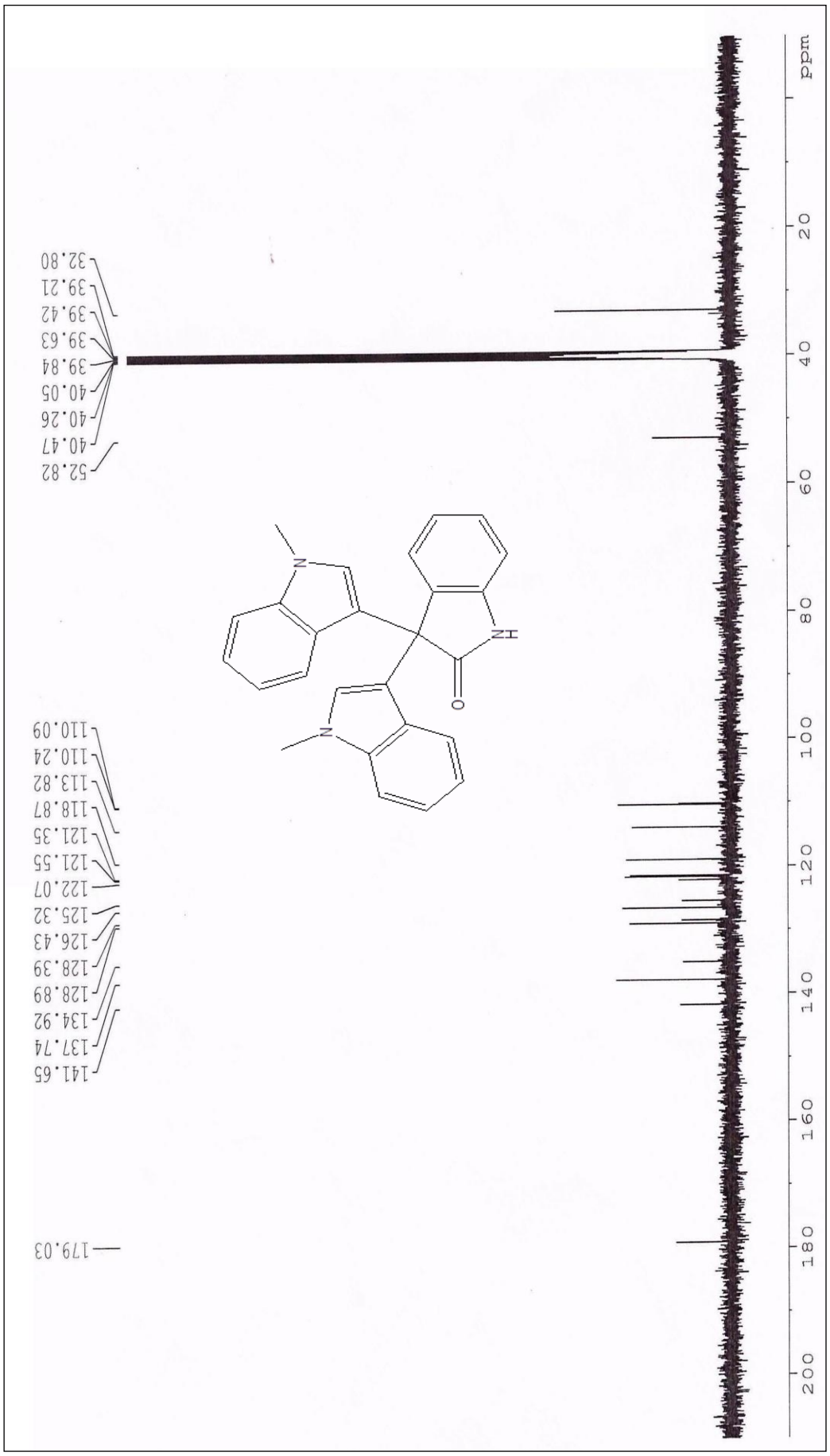



**Figure 4.24**:- $^{13}$C NMR of 3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34d)**

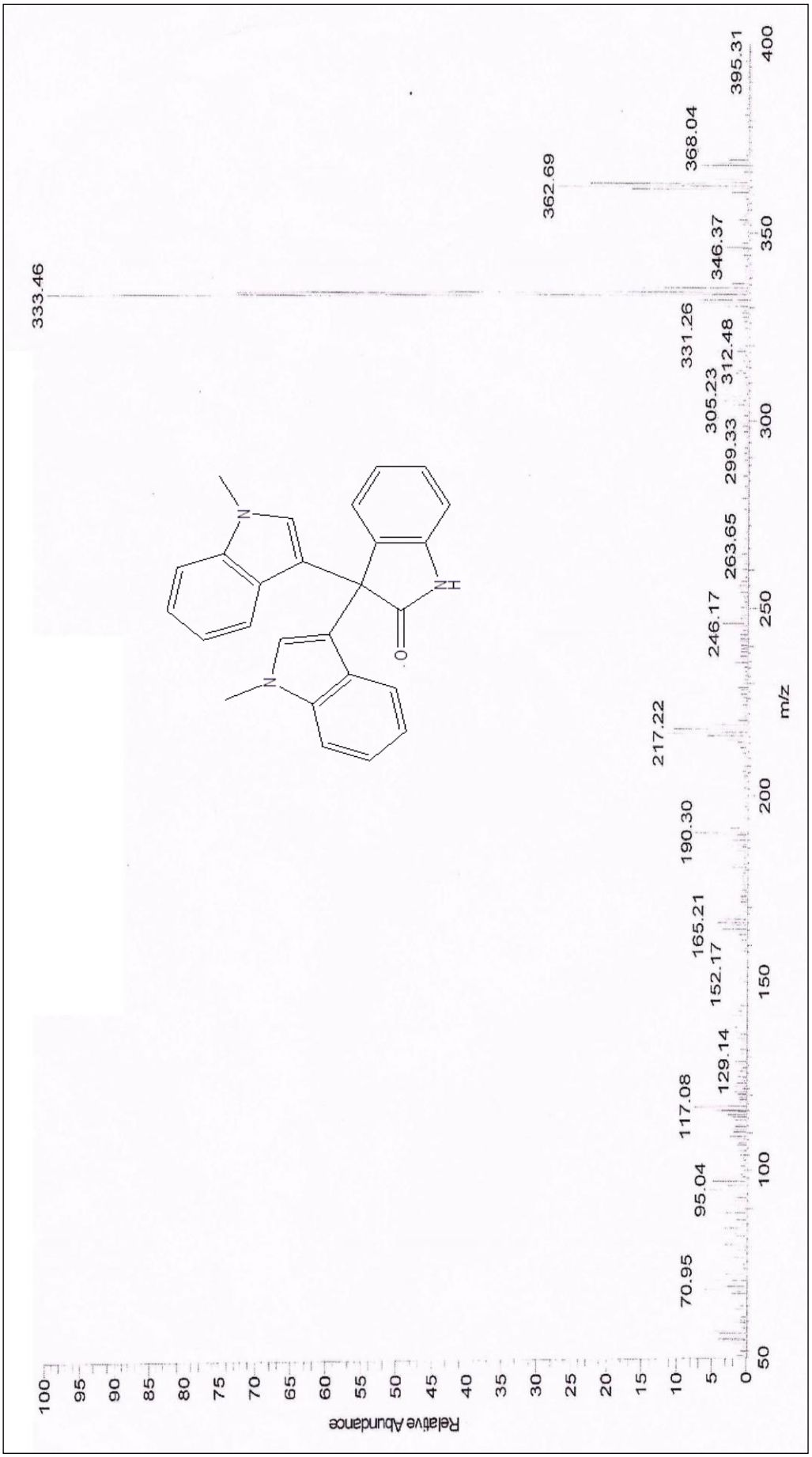



**Figure 4.25**:- Mass Spectrum of 3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34d)**

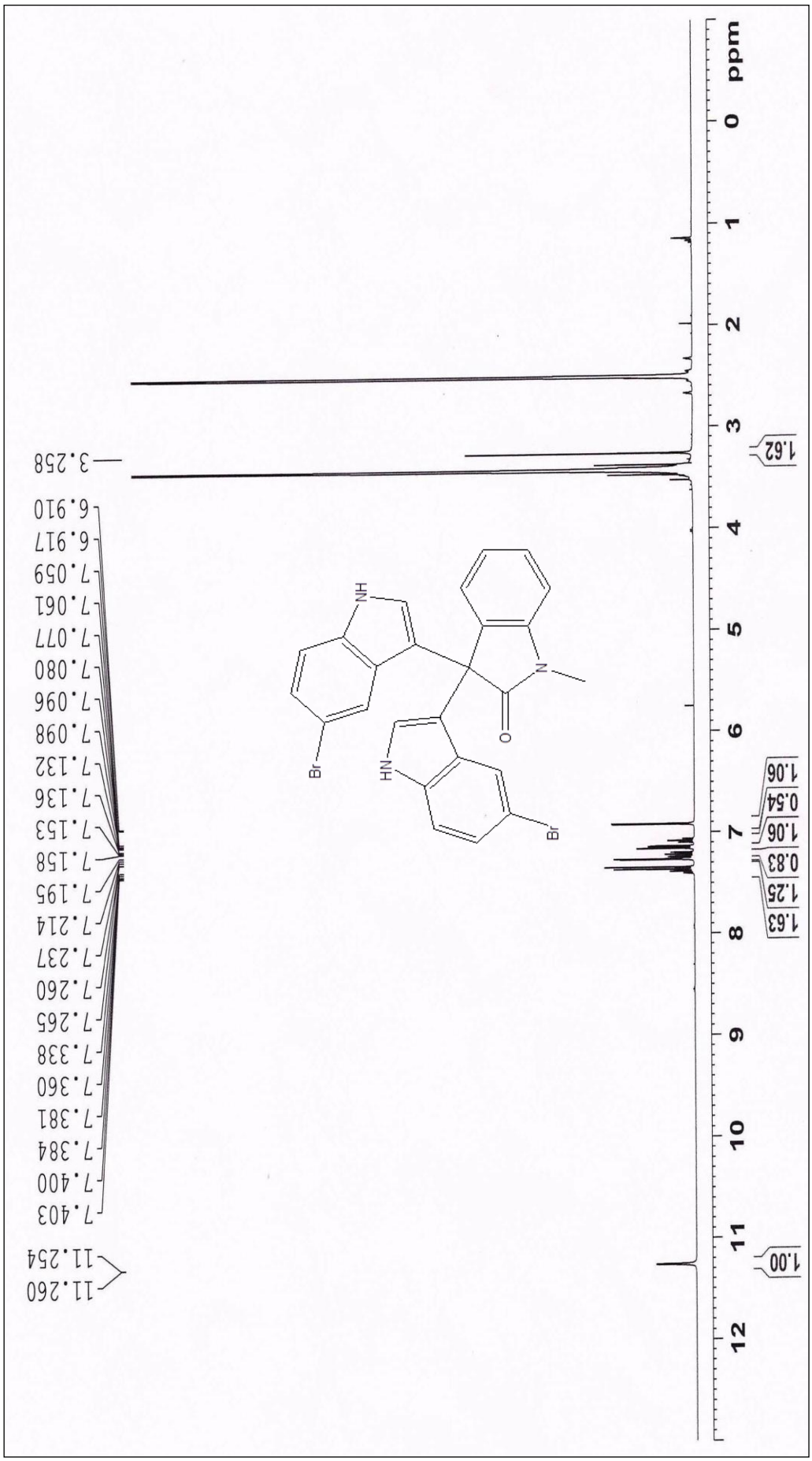



**Figure 4.26**:- $^{1}$H NMR of 3,3-bis(5-bromo-1H-indol-3-yl)-1-methylindolin-2-one **(34e)**

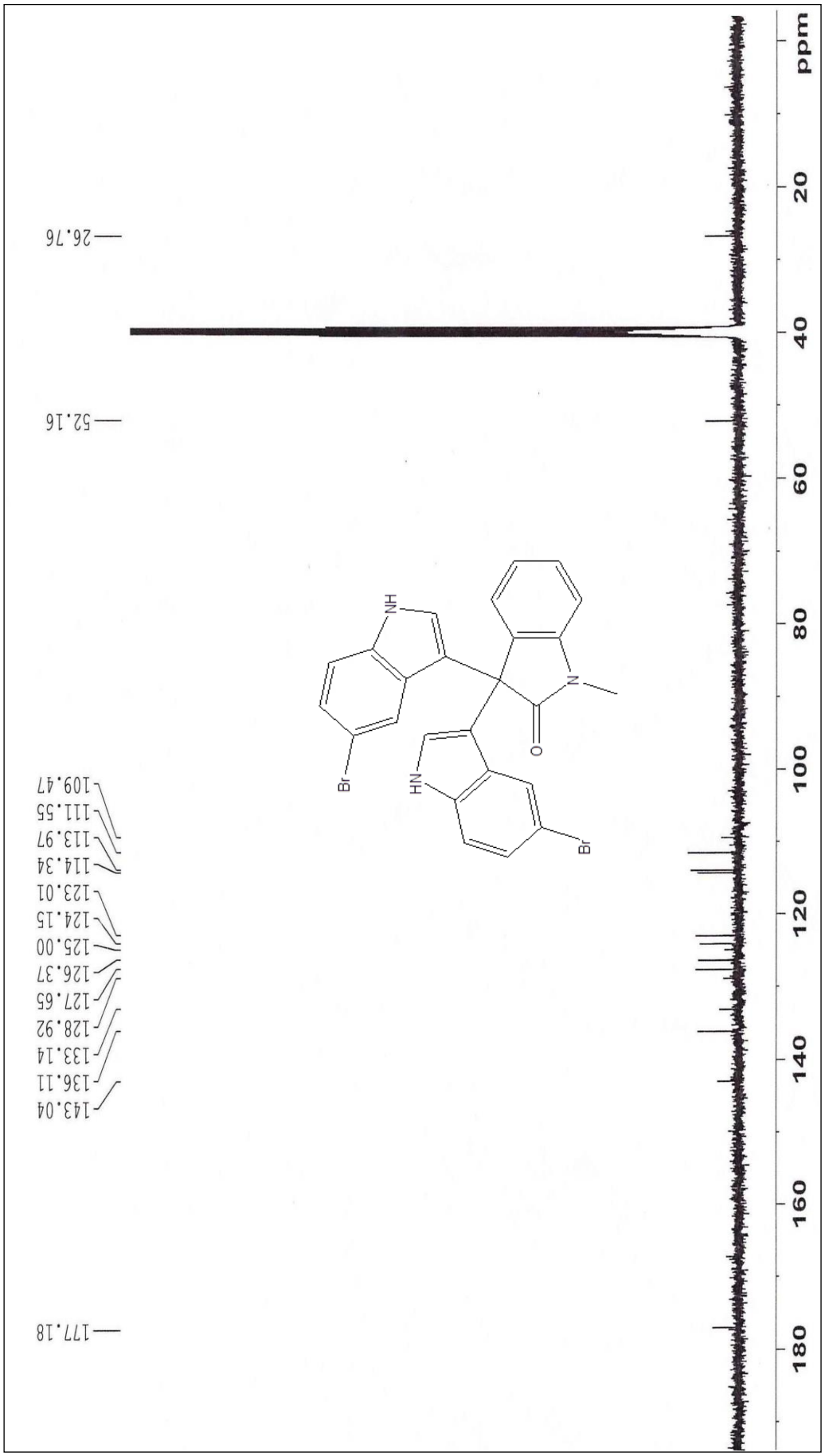



**Figure 4.27**:- $^{13}C$ NMR of 3,3-bis(5-bromo-1H-indol-3-yl)-1-methylindolin-2-one **(34e)**

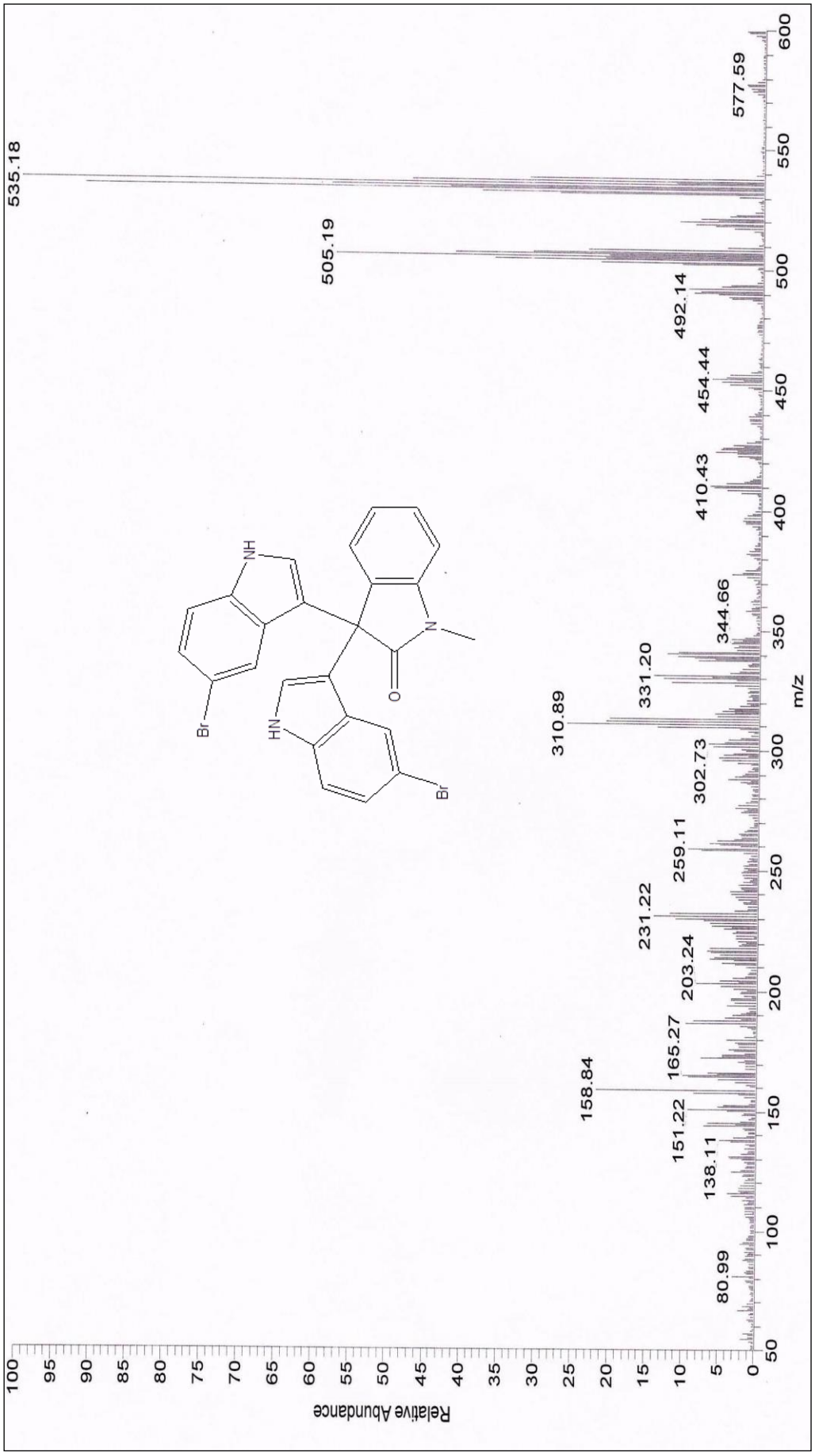



**Figure 4.28**:- Mass Spectrum of 3,3-bis(5-bromo-1H-indol-3-yl)-1-methylindolin-2-one **(34e)**

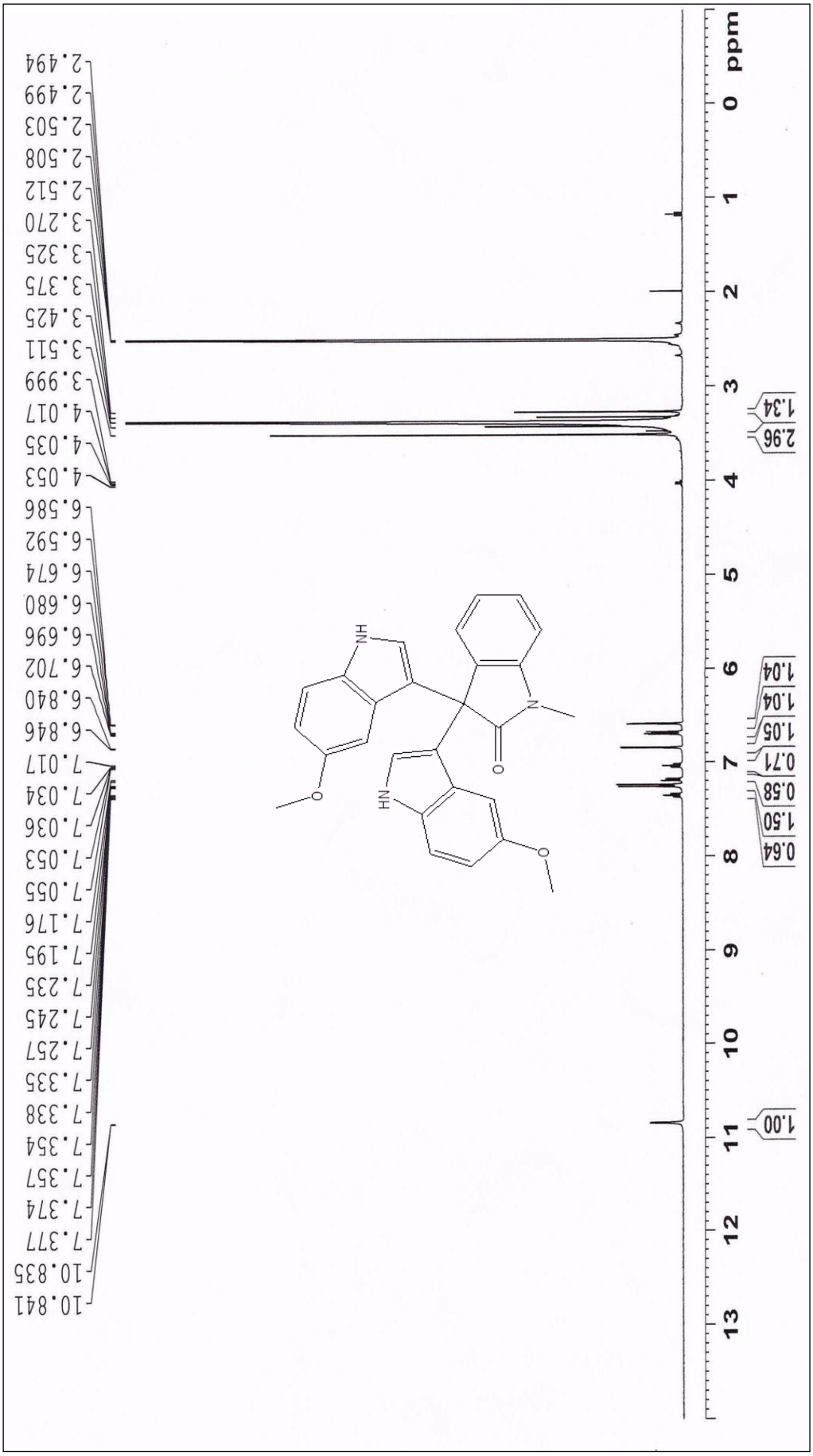



**Figure 4.29**:- $^{1}$H NMR of 3,3-bis(5-methoxy-1H-indol-3-yl)-1-methylindolin-2-one **(34f)**

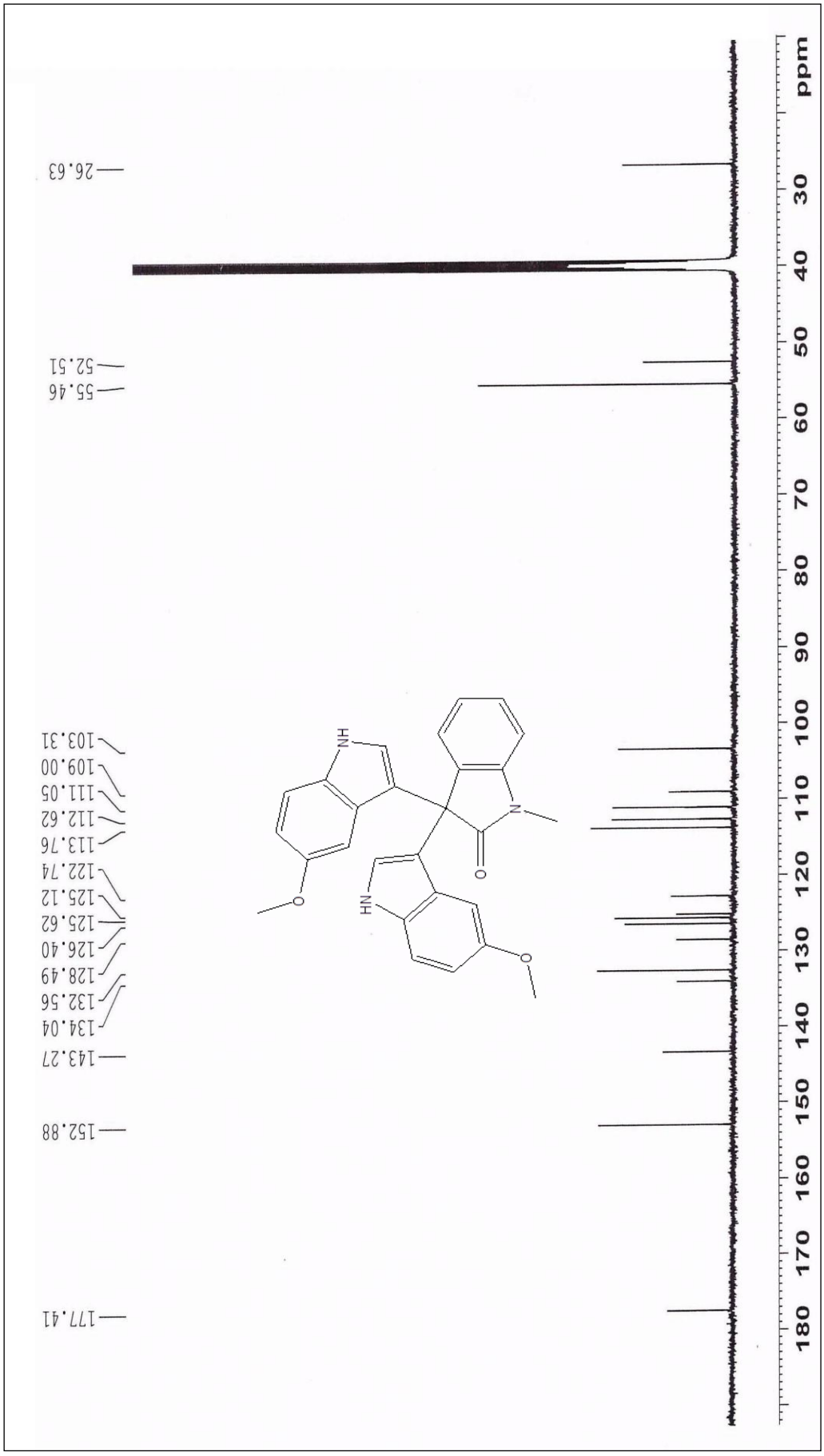



**Figure 4.30**:- $^{13}$C NMR of 3,3-bis(5-methoxy-1H-indol-3-yl)-1-methylindolin-2-one **(34f)**

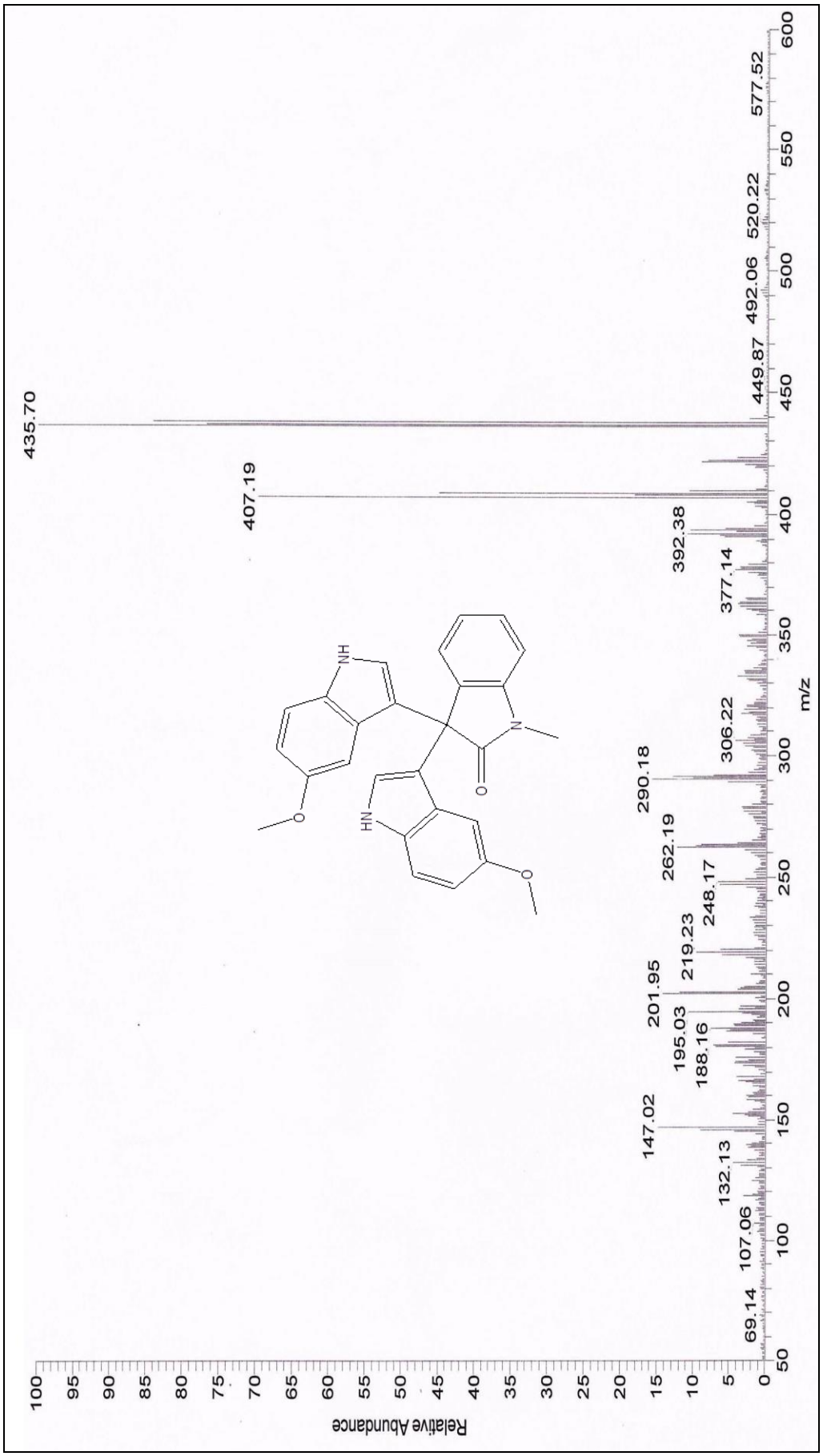



**Figure 4.31**:- Mass Spectrum of 3,3-bis(5-methoxy-1H-indol-3-yl)-1-methylindolin-2-one **(34f)**

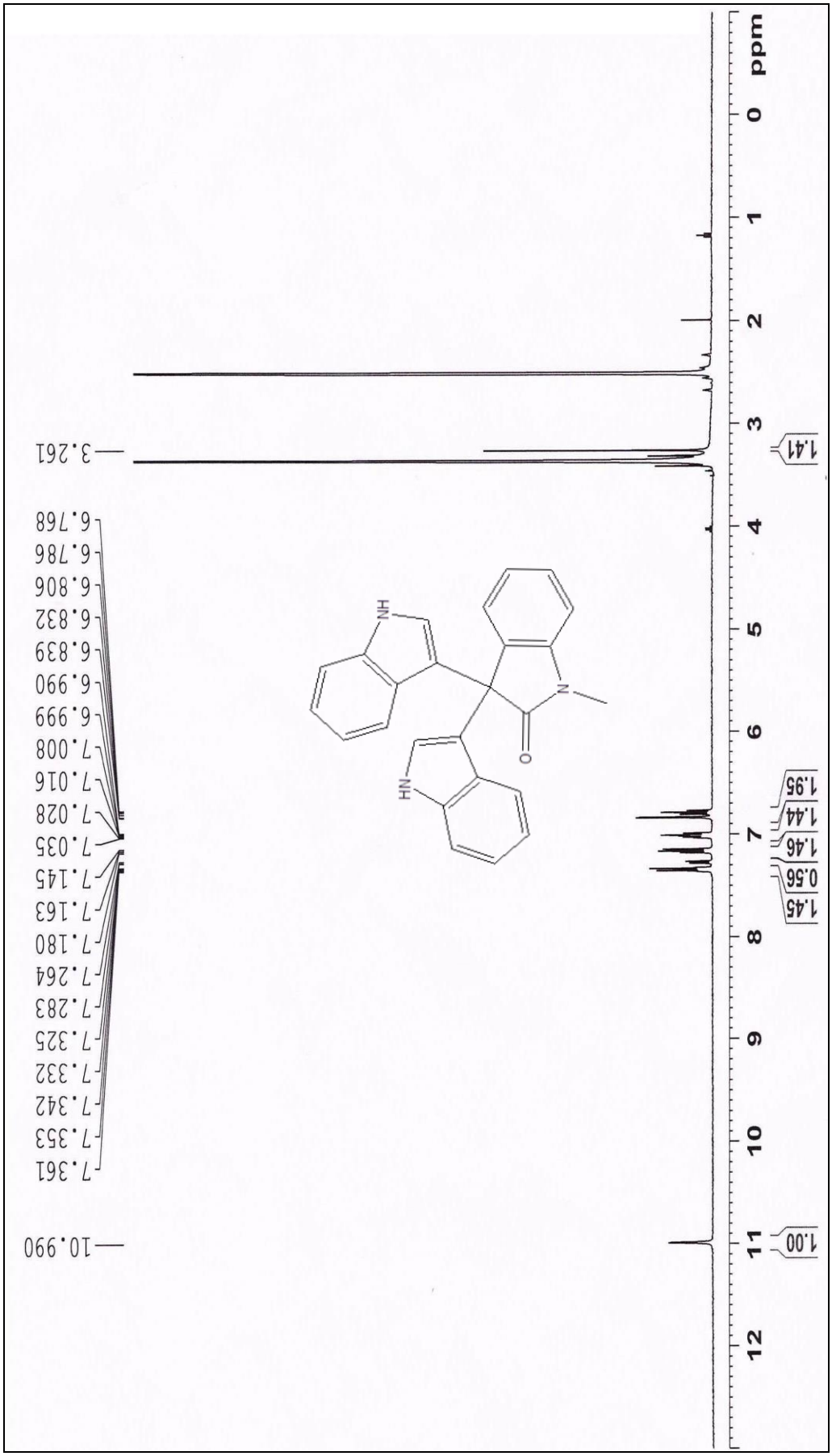



**Figure 4.32**:- $^{1}$H NMR of 3,3-di(1H-indol-3-yl)-1-methylindolin-2-one (**34g**)

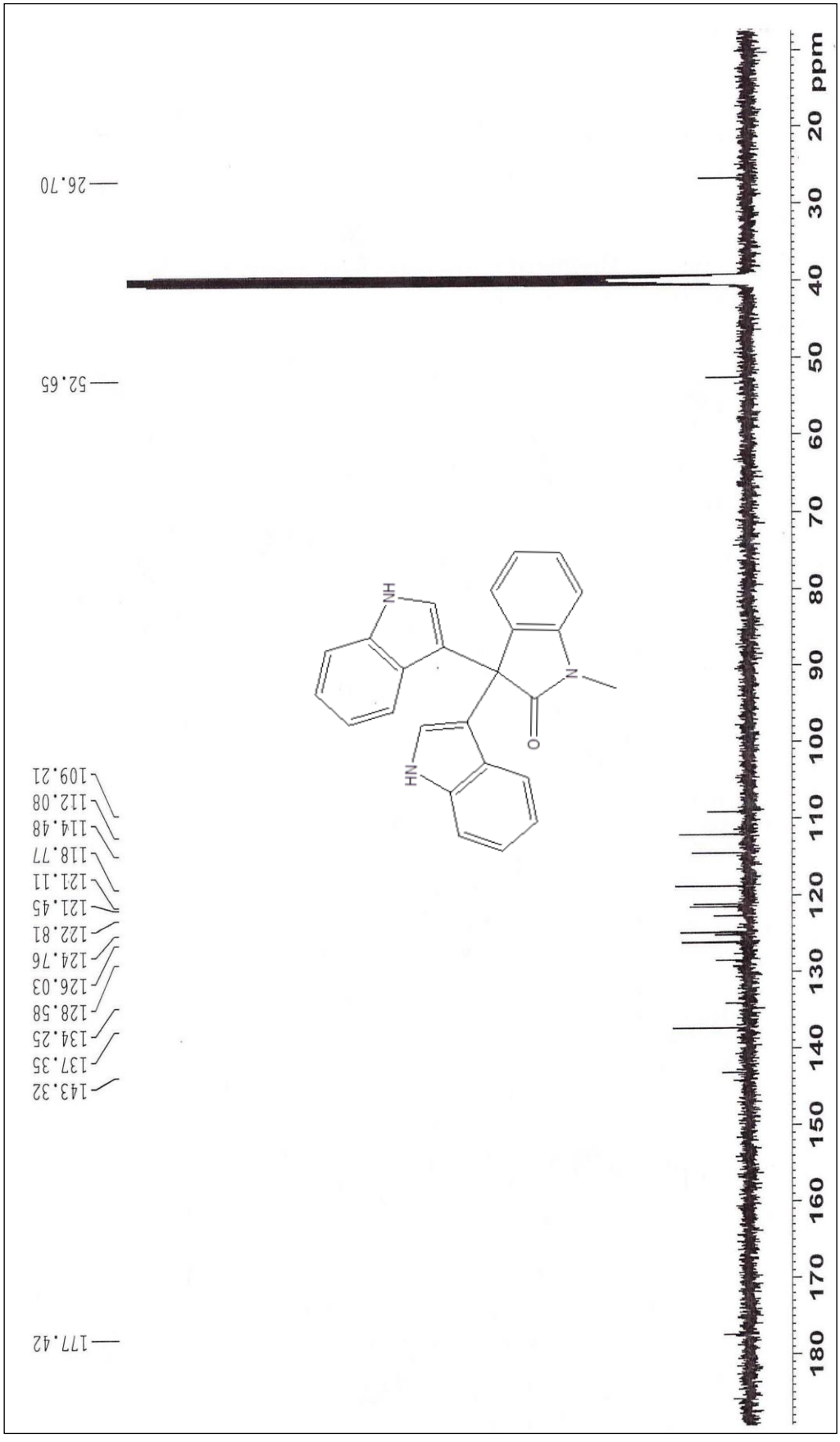



**Figure 4.33:-** $^{13}$C NMR of 3,3-di(1H-indol-3-yl)-1-methylindolin-2-one **(34g)**

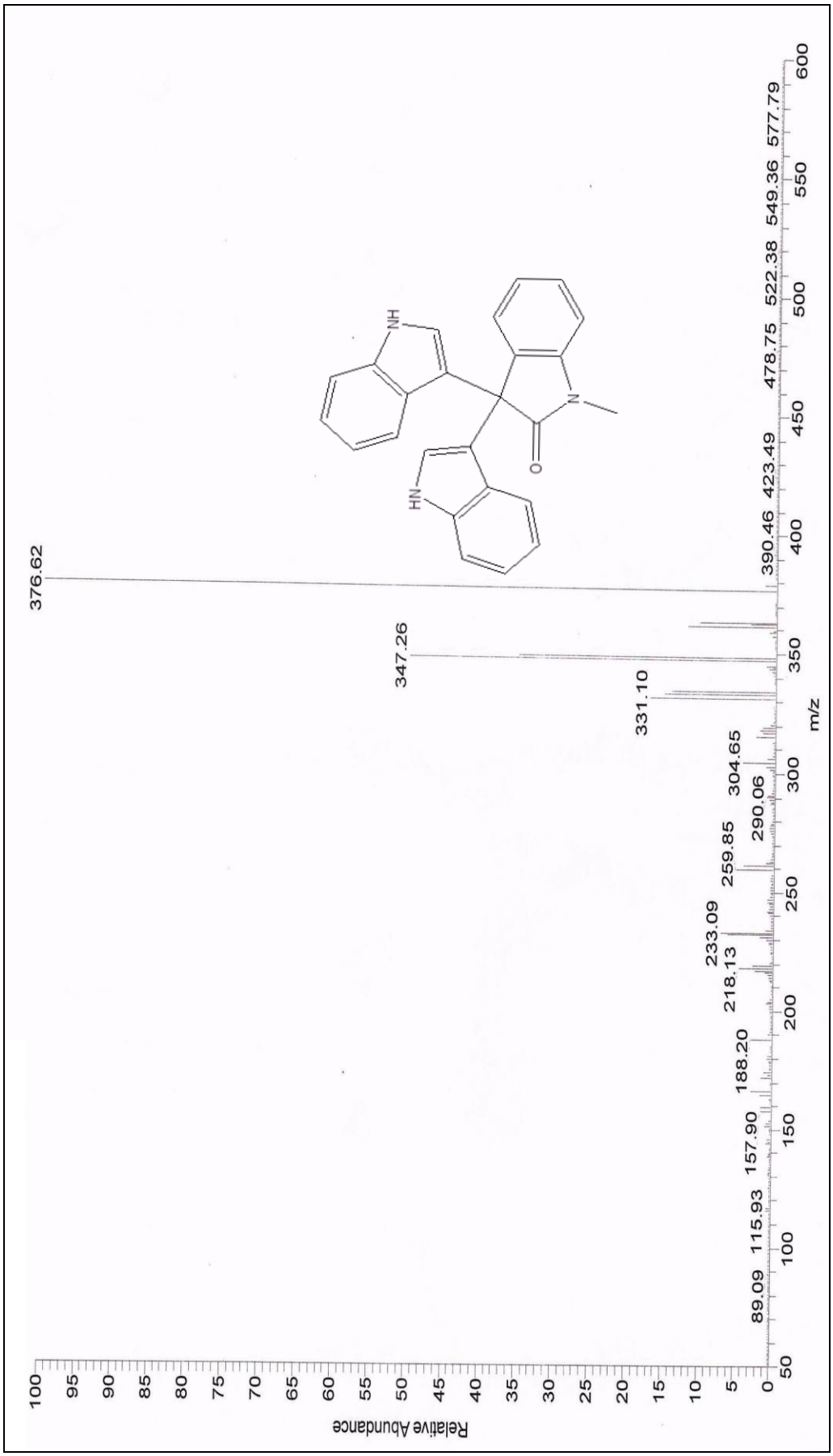



**Figure 4.34**:- Mass Spectrum of 3,3-di(1H-indol-3-yl)-1-methylindolin-2-one **(34g)**

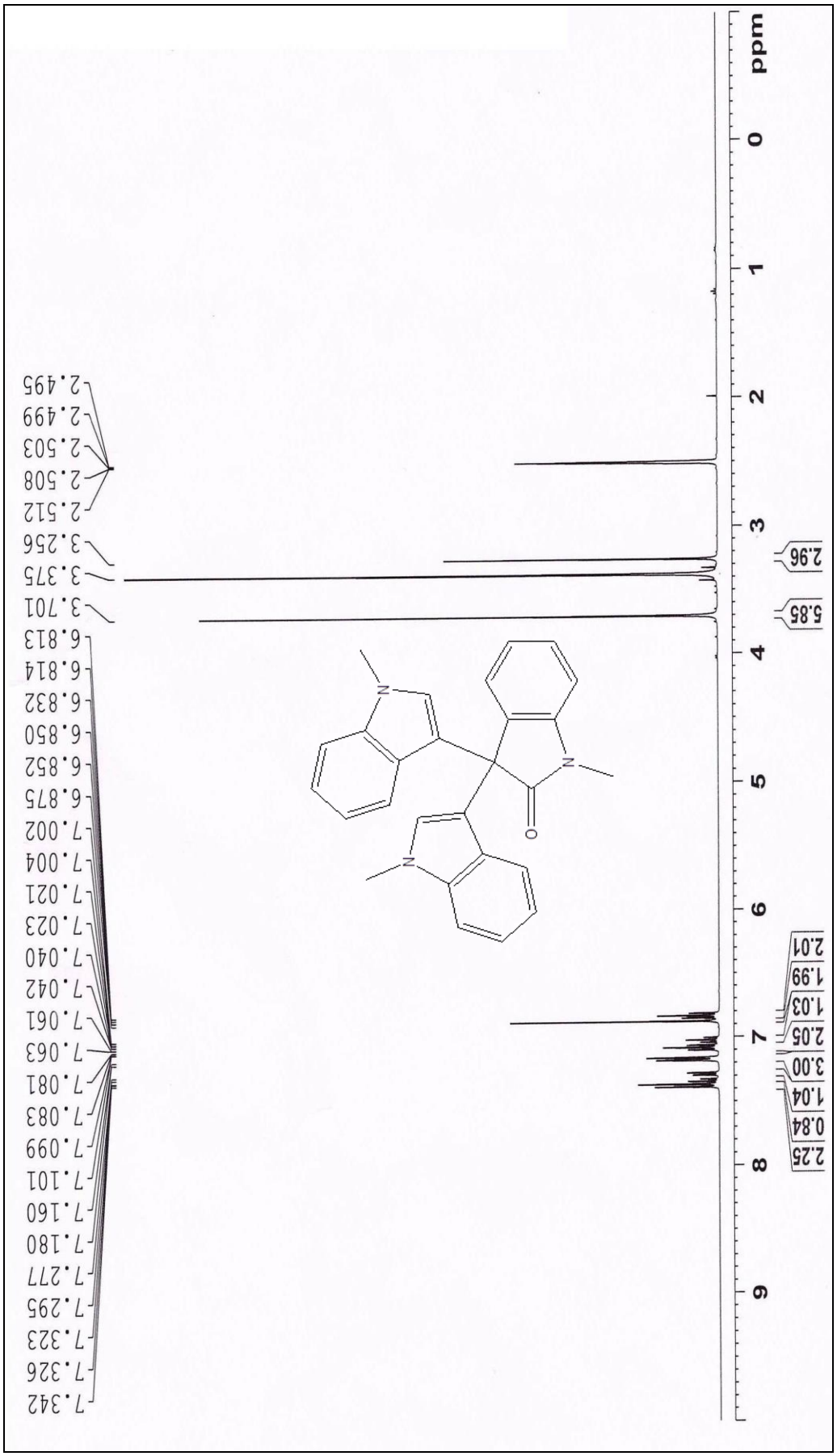



**Figure 4.35**:- $^{1}$H NMR of 1-methyl-3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34h)**

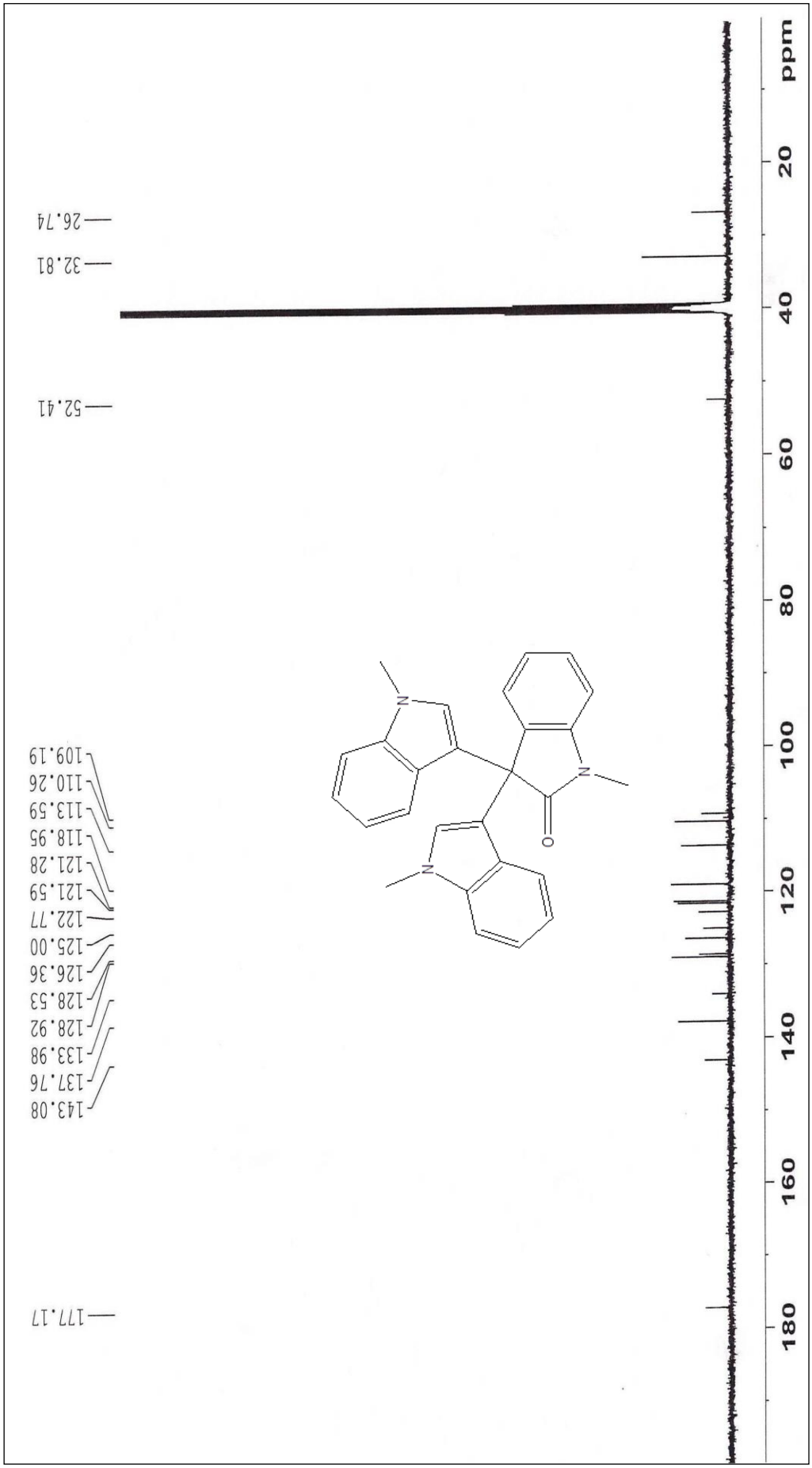



**Figure 4.36**:- $^{13}C$ NMR of 1-methyl-3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34h)**

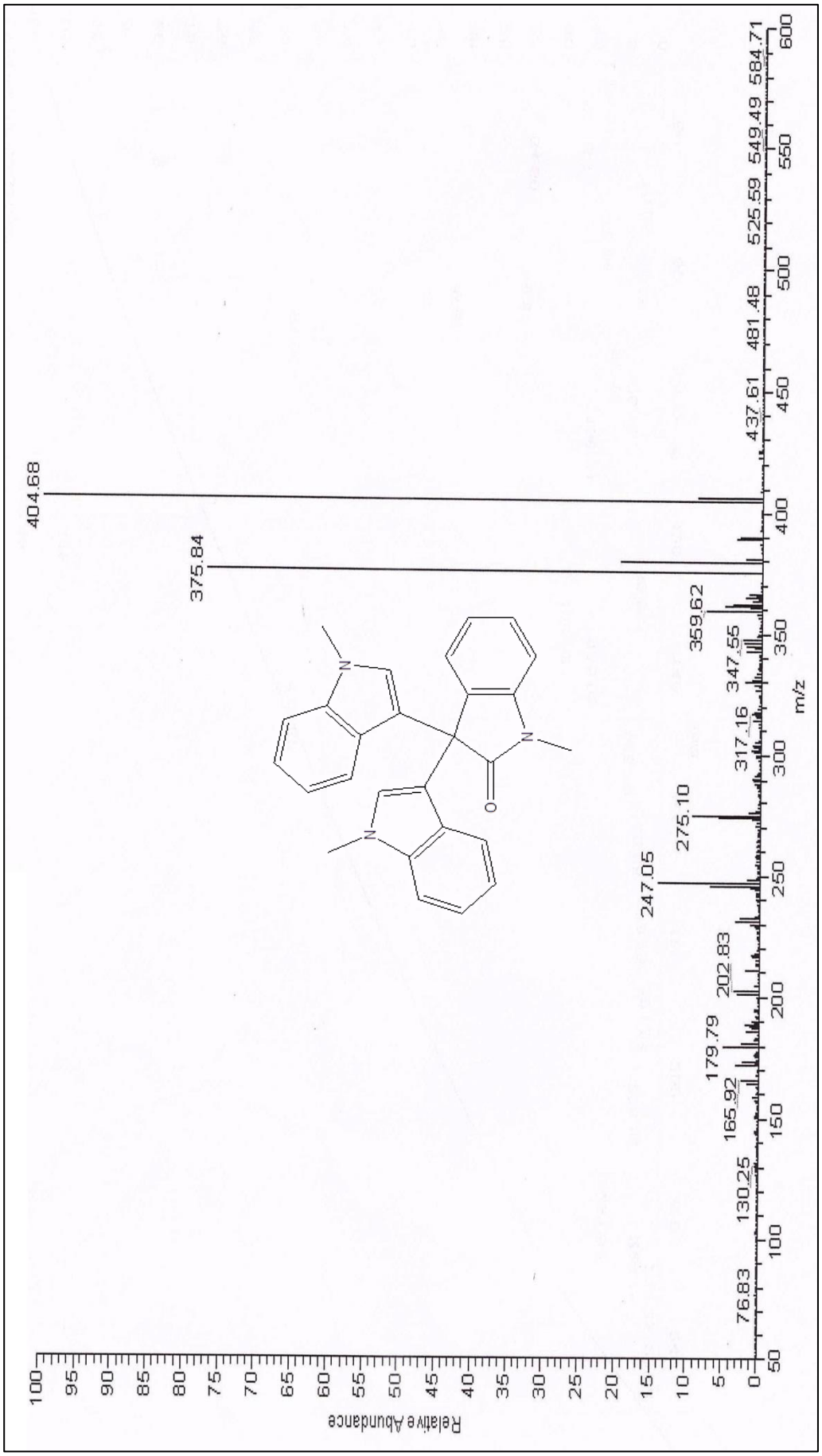



**Figure 4.37**:- Mass Spectrum of 1-methyl-3,3-bis(1-methyl-1H-indol-3-yl)indolin-2-one **(34h)**

## 4.7 References


1. Pozharskii, A. F.; Soldatenkov, A. T.; Katritzky, A. R. An Introduction to Heterocyclic Chemistry and Biochemistry and the Role of Heterocycles in Science, Technology, Medicine and Agriculture. John Wiley & Sons Ltd, **1997**.
2. Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discov.*, **2005**, *4(3)*, 206.; Cordell, G. A., Quinn-Beattie, M. L.; Farnsworth, N. R. *Phytother Res.*, **2001**, *15(3)*, 183.; Hughes, E. H.; Shanks, J. V. *Metab. Eng.*, **2002**, *4(1)*, 41.
3. Norman, S. R., *Drug Development Res.*, **2008**, *69*, 15.
4. Dahm, R., *Human Genetics*, **2008**, *122(6)*, 565.; Watson, J. D.; Crick, F. H. *Nature*, **1953**, *171*, 737.
5. Evans, H. M., Emerson, O. H.; Emerson, G. A. *J. Biological Chemistry*, **1936**, *113(1)*, 319.; Davies, M. B., Austin, J.; Partridge, D. A. The Royal Society of Chemistry, **1991**, 48.
6. Furst, P.; Stehle, P. *J. Nutrition*, **2004**, *134*, 1558S.; Brian, P. W., Review Lecture: Hormones in Healthy and Diseased Plants Proceedings of the Royal Society of London. Series B, *Biological Sci.*, **1978**, *200*, 231.
7. Mittal, A. *Sci. Pharm.*, **2009**, *77*, 497.; Nagalakshmi, G. *Indian J. Pharm. Sci.*, **2008**, *70(1)*, 49.; Joule, J. A.; Mills, K. *Heterocyclic Chemistry*, 4thEd., Blackwell Publishing, **2000**, 369.; Nekrasov, D. D. *Chemistry of Heterocyclic Compounds*, **2001**, *37(3)*, 263.; Sperry, J. B.; Wright, D. L. *Current Opinion in Drug Discovery and Development*, **2005**, *8*, 723.; Polshettiwar, V.; Varma, R. S. *Pure Appl. Chem.*, **2008**, *80(4)*, 777.; Katritzky, A. R. *Chemistry of Heterocylic Compounds*, **1992**, *28(3)*, 241.
8. Fan, W. Q.; Katritzky, A. R. *Comprehensive Heterocyclic Chemistry II*, Oxford, Elsevier, **1996**, *4*, 1.; Dehne, H. *In Methoden der Organischen Chemie (Houben-Weyl)*, Schaumann, Stuttgart, Thieme, **1994**, *8*, 305.; Eicher, T.; Hauptmann, S. *The Chemistry of Heterocycles: Structure, Reactions, Syntheses and Applications*. Wiley-VCH, 2 ed. **2003**, 371.; Finley, K. T., *The Chemistry of Heterocyclic Compounds*, John Wiley and Sons, New York, **1980**, 1.; Wamhoff, H., *Comprehensive Heterocyclic Chemistry*, Pergamon Press: Oxford, **1984**, *5*, 669.

9. Zhang, H. C.; Ye, H.; Moretto, A. F.; Brumfield, K. K.; Maryanoff, B. E. *Org. Lett*, **2000**, *2*, 89; Gilchrist, T. L. *Heterocyclic Chemistry*; Academic Press: London, **1997**, 231; Humphrey, G. R.; Kuethe, J. T., *Chem. Rev.*, **2006**, *106 (7)*, 2875.; Shimazawa, R.; Kuriyama, M.; Shirai, R. *Bioorg. Med. Chem. Lett.*, **2008**, 18, 3350; Abadi, A. H.; AbouSeri, S. M.; AbdelRahman, D. E.; Klein, Ch.; Lozach, O.; Meijer, L. *Eur. J. Med. Chem.* **2006**, *41*, 296.; Demirayak, S.; Kayagil, I.; Yurttas, L. *European Journal of Medicinal Chemistry*, **2011**, *46*, 411; Ito, H.; Sakakibara, J.; Ueda T. *Cancer Letters*, **1985**, *28*, 61.
10. Joshi, K. C.; Pathak, V. N.; Jain, S. K. *Pharmazie*. **1980**, *35*, 677; Bolotov, V. V.; Rugovina, V. V.; Yakovleva, L. V.; Bereznyakova, A. I. *Khim. Farm. Zh.* **1982**, *16*, 58.
11. William, C. E. *US Patent* 3, 558, 653. **1971**; Pajouhesh, H.; Parsons, R.; Popp, F. D. *J. Pharm. Sci.* **1983**, *72*, 318.
12. Garrido, F.; Ibanez, J.; Gonalons, E.; Giraldez, A. *Eur. J. Med. Chem.* **1975**, *10*, 143.
13. Natarajan, A.; Fan, Y. H.; Chen, H.; Guo, Y.; Iyasere, J.; Harbinski, F.; Christ, W. J.; Aktas, H.; Halperin, J. A. *J. Med. Chem.* **2004**, *47*, 1882.
14. RatanBal, T.; Anand, B.; Yogeeswari, P.; Sriram, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4451.
15. Jiang, T.; Kuhen, K. L.; Wolff, K.; Yin, H.; Bieza, K.; Caldwell, J.; Bursulaya, B.; Tuntland, T.; Zhang, K.; Karanewsky, D.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2109.
16. Tripathy, R.; Reiboldt, A.; Messina, P. A.; Iqbal, M.; Singh, J.; Bacon, E. R.; Angeles, Th. S.; Yang, Sh. X.; Albom, M. S.; Robinson, C.; Chang, H.; Ruggeri, B.A.; Mallamo, J. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2158.; Cane, A.; Tournaire, M. C.; Barritault, D.; Crumeyrolle Arias, M. *Biochem. Biophys. Res. Commun.* **2000**, *276*, 379.; Silveira, V. C.; Luz, J. S.; Oliveira, C. C.; Graziani, I.; Ciriolo, M. R.; Costa Ferreira, A. M. *J. Inorg. Biochem.* **2008**, *102*, 1090.
17. Amal Raj, A.; Raghunathan, R.; Sridevikumaria, M. R.; Raman, N. *Bioorg. Med. Chem.* **2003**, *11*, 407.; Rodriguez Arguelles, M. C.; Mosquera Vazaquez, S.; Touron Touceda, P.; Sanmartin Matalobos, J.; Garcia Deibe, A. M.;

Belicchi Ferraris, M.; Pelosi, G.; Pelizzi, C.; Zani, F. *J. Inorg. Biochem.* **2007**, *101*, 138.

18. Maskell, L.; Blanche, E. A.; Colucci, M. A.; Whatmore, J. L.; Moody, C. *J. Bioorg. Med. Chem. Lett.* **2007**, *17*, 1575.
19. Verma, M.; Nath Pandeya, S.; Nand Singh, K.; Stables, J. P. *Acta Pharm.* **2004**, *54*, 49.
20. Popp, F. D. *J. Heterocycl. Chem.* **1984**, *21 (5)*, 1367.; Garrido, F.; Ibanez, J.; Gonalons, E.; Giraldez, A. *Eur. J. Med. Chem.* **1975**, *10*, 143.
21. Kamano, Y., Zhang, H. P., Ichihara, Y., Kizu, H., Komiyama, K., Pettit, G. R. *Tetrahedron Lett.*, **1995**, *36*, 2783.; Zhang, H. P., Kamano, Y., Ichihara, Y., Kizu, H., Komiyama, K., ltokawa, H., Pettit, G. R. *Tetrahedron*, **1995**, *51*, 5523.
22. Khuzhaev, V. U., Zhalolov, I., Turguniv, K. K., Tashkhodzhaev, B.; Levkovich, M. G., Arpova, S. F., Shashkov, A. S. *Chem. Nat. Compd.* **2004**, *40*, 269.
23. Rasmussen, H. B., MacLeod, J. K. *J. Nat. Prod.*, **1997**, *60*, 1152.
24. Tang, Y. Q., Sattler, I., Thiericke, R., Grabley, S., Feng, X. Z. *Eur. J. Org. Chem.*, **2001**, *66*, 261.
25. Kagata, T., Saito, S., Shigemori, H., Ohsaki, A., Ishiyama, H., Kubota, T., Kobayashi, J. *J. Nat. Prod.*, **2006**, *69*, 1517.
26. Jimenez, J. I., Huber, U., Moore, R. E., Patterson, G. M. L. *J. Nat. Prod.*, **1999**, *62*, 569.
27. Suzuki, H., Morita, H. Shiro, M., Kobayashi, J., *Tetrahedron*, **2004**, *60*, 5307.
28. Kobayashi, H., Kazuo, S. Y., Furihata, K., Nagai, K., Suzuki, K. I., Hayakawa, Y., Seto, H. Yun, B. S., Ryoo, I. J., Kim, J. S., Kim, C. J., Yoo, I. D. *J. Antibiot.*, **2001**, *54*, 1019.; Kobayashi, H., Kazuo, S. Y., Furihata, K., Nagai, K., Suzuki, K. I., Hayakawa, Y., Seto, H., Yun, B. S., Ryoo, I. J., Kim, J. S., Kim, C. J., Yoo, I. D. *J. Antibiot.* **2001**, *54*, 1013.
29. Baeyer, A. *Ber*. **1868**, *1*, 17.; Baeyer, A. *Ber*. **1879**, *12*, 456.; Baeyer, A. *Ann.* **1866**, *140*, 296.; Baeyer, A. *Ber*. **1879**, *12*, 1312.
30. Bayer, A. *Ber*. **1878**, *11*, 532.
31. Jursic, B. S.; Stevens, E. D. *Tetrahedron Lett.* **2002**, *43*, 5681.
32. Marsden, S. P.; Watson, E. L.; Raw, S. A. *Org. Lett.* **2008**, *10*, 2905.

33. Klumpp, D. A.; Yeung, K. Y.; Prakash, G. K. S.; Olah, G. A. *J. Org. Chem.* **1998**, *63*, 4481.
34. Sharma, I.; Saxena, A.; Ojha, C. K.; Paradasani, C. K. P.; Paradasani, R. T.; Mukherjee, T. *Proc. Indian Acad. Sci. (Chem. Sci.)* **2002**, *114*, 523.
35. Felpin, F. X.; Ibarguren, O.; Nassar Hardy, L.; Fouquet, E. *J. Org. Chem.*, **2009**, *74*, 1349.; Luppi, G.; Cozzi, P. G.; Monari, M.; Kaptein, B.; Broxterman, Q. B.; Tomasini, C. *J. Org. Chem.* **2005**, *70*, 7418.; Malkov, A. V.; Kabeshov, M. A.; Bella, M.; Kysilka, O.; Malyshev, D. A.; Pluhackova, K.; Kocovsky, P. *Org. Lett.* **2007**, *9*, 5473.; Bergman, J.; Eklund, N. *Tetrahadron*. **1980**, *36*, 1445; Azizian, J.; Mohammadi, A. A.; Karimi, A. R.; Mohammadizadeh, M. R. *J. Chem. Res. Synop.* **2004**, *6*, 424.
36. Mondal, N. B.; Paira, P.; Saha, P. *Bio. Med. Chem. Lett.* **2009,** *19*, 4786.
37. Kamal, A.; Shaik, T. B. *Bio. Med. Chem. Lett.* **2010,** *20*, 5229.
38. Ke, B.; Qin, Y.; Wang, Y.; Wang, F. *Synth. Commun.* **2005**, *35*, 1209; Nagarajan, R.; Perumal, P. T. *Chem.Lett.* **2004**, *33*, 288; Bandgar, B. P.; Shaikh, K. A. *Tetrahedron Lett.* **2003**, *44*, 1959; Chakrabarty, M.; Ghosh, N.; Basak, R.; Harigaya, Y. *Tetrahedron Lett.* **2002**, *43*, 4075.
39. Wang, L.; Han, J.; Tian, H.; Sheng, J.; Fan, Z.; Tang, X. *Synlett*. **2005**, *2*, 337; Shi, M.; Cui, S. C. Li, J. Q. *Tetrahedron*. **2004**, *60*, 6679; Bartoli, G.; Bosco, M.; Foglia, G.; Giuliani, A.; Marcantoni, E.; Sambri, L. *Synthesis.* **2004**, *6*, 895; Chatterjee, A.; Manna, S.; Benerji, J.; Prange, T.; Shoolery, J. *J. Chem. Soc. Perkin Trans. I*, **1980**, 553; Babu, G.; Sridhar, N.; Perumal, P. T. *Synth. Commun.* **2000**, *30*, 1609; Nagarajan, R.; Perumal, P. *Tetrahedron.* **2002**, *58*, 1229; Chen, D. P.; Yu, L. B.; Wang, P.G. *Tetrahedron Lett.* **1996**, *37*, 4467; Mi, X.; Lu, S.; He, J.; Cheng, J. P. *Tetrahedron Lett.* **2004**, *45*, 4567.
40. Gibbs, T. J. K.; Tomkinson, N. C. O. *Org. Biomol. Chem.* **2005**, *3*, 4043.
41. Yadav, J. S.; Reddy, B. V. S.; Sunitha, S. *Adv. Synth. Catal.* **2003**, 345, 349.
42. Firouzabadi, H.; Iranpoor, N.; Jafari, A. A. *J. Mol. Catal.* **2006**, *244*, 168.
43. Ko, S.; Lim, C.; Tu, Z.; Wang, Y. F.; Wang,C. C.; Yao, C. F. *Tetrahedron Lett.* **2006**, *47*, 487.
44. Yadav, J. S.; Reddy, B. V. S.; Murthy, C. V. S.; Kumar, G. M.; Madan, C. *Synthesis*. **2001**, *5*, 783.
45. Bandgar, B. P.; Shaikh, K. A. *Tetrahedron Lett.* **2003**, *44*, 1959.
46. Singh, V. K.; Deota, P. T., *Synth. Commun.* **1988**, *18*, 617.

47. Deota, P. T.; Desai, R.; Valodkar, V. B., *J. Chem. Res. (S)* **1998**, 562.
48. Hui C, WeiLin D, Ruihua G, Yong C, Hexing L, Kangnian F, *Applied Catalysis A: General* **2007**, *328*, 226; Crosman, A.; Gelbard, G.; Poncelet, G.; Parvulescu, V. I. *Applied Catalysis A: General* **2004**, *264*, 23; Gelbard, G.; Raison, F.; RoditiLachter, E.; Thouvenot, R.; Ouahab, L.; Grandjean, D. *Journal of Molecular Catalysis A: Chemical* **1996**, *114*, 77; Yahdih, M.; Laurent, S.; JeanYves, P.; Emmanuel, B.; Ahmed, A.; JeanMarie, B. *Journal of Catalysis*. **2007**, *249*, 338.

Compounds containing lactol functionality are widely distributed in many natural product and biologically active compounds[1] such as callipeltoside A isolated from the lithistid sponge *Callipelta sp.*; ginkgolides present in Ginkgobiloba; dysidiolide, a novel sesterterpenoid isolated from the caribbean sponge *Dysidea etheria de Laubenfels*, an antitumor agent active at the micro molar level; cladocorans A and B, isolated from the mediterranean coral *Cladocora cespitosa.*; acuminolide, a cytotoxic labdane diterpene, isolated from the stem bark of *Neouvaria acuminatissima*; spongianolide A, a cytotoxic sesterterpene isolated from marine sponge; manoalide and cacospongionolide $B_2$, sesterterpenes isolated from soft sponges; peniolactol isolated from the wood attacked by the fungus *Peniophora sanguinea Bres*.

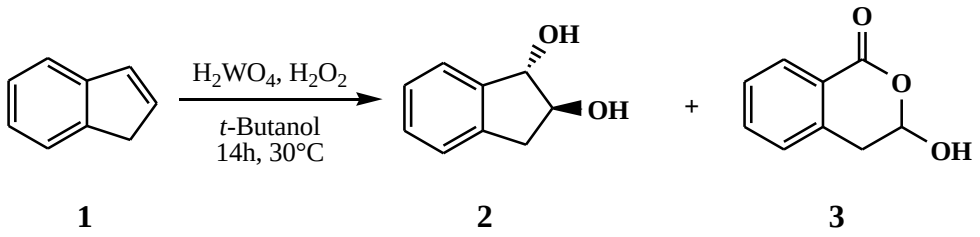
Lactol is also important precursor for many biologically active compounds such as in the synthesis of illudalic acid, a potential LAR inhibitor, synthesis of small molecule inhibitors of the orphan nuclear receptor steroidogenic factor-1 (NR5A1) based on isoquinolinone scaffolds, synthesis of benzopyran-1-ones, caronaldehyde, synthesis of mevalonate and mevaldate.

Construction of lactol unit has been reported earlier in several steps by different research groups, the earliest being from Schöpf and Kühne in four steps; its crystal structure was subsequently reported by Valente *et al.*[2]

The synthetic utility of tungstic acid–hydrogen peroxide in 2-methyl-2-propanol to furnish the corresponding *trans*-diol of (*Z,Z*)-cycloocta-1,5-diene in 65 % yield has been demonstrated by our group earlier[3]. Further investigation of this reagent combination revealed that its treatment with *endo*-dicyclopentadiene unexpectedly resulted in the formation of polycyclic oxetanes.[4] Our continued interest in the dihydroxylation using tungstic acid-hydrogen peroxide prompted us to explore the hydroxylation of indene in order to prepare indan-*trans*-1,2-diol which is an important intermediate for Indinavir, an HIV protease inhibitor.

It was interesting to observe that the treatment of tungstic acid–hydrogen peroxide with indene resulted in the formation of not only *racemic* indan-*trans*-1,2-

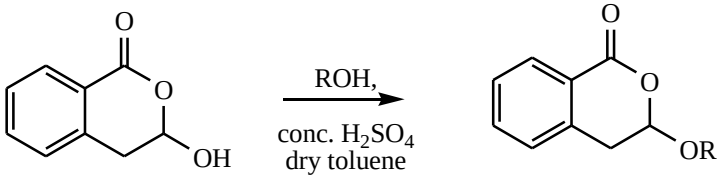
diol but also *racemic* 3,4-dihydro-3-hydroxyisochromen-1-one (also known as lactol or pseudoacid).




**1** **2** **3**

**Scheme 1.** Reaction of indene with hydrogen peroxide-tungstic acid

Various lactol derivatives show wide variety of biological activities such as dysidiolide acts as the inhibitor of phosphatase cdc25A and inhibits the growth of A-549 human lung carcinoma; Cladocorans **A** and **B**, are also used as a inhibitor of protein phosphatase cdc25A; illudalic acid is a potential LAR inhibitor; manoalide is a potent, irreversible inhibitor of phospholipase A2 (PLA2); cacospongionolide **B** has shown a comparable activity on recombinant human synovial PLA2 *in vitro*; (+)- acuminolide display cytotoxic activity in human cancer cell lines and cultured P388 cells; (-)- spongianolide **A** inhibits proliferation of the mammary tumor cell line MCF-7 and protein kinase; The acetals of nepetalic acid exhibits prominent mosquito repellency. These interesting biological properties prompted us to prepare the acetal derivatives of lactol.




**3** **4 (a-h)**

Where a) R = n-$C_2H_5$; b) R = n-$C_3H_7$; c) R = n-$C_4H_9$; d) R = n-$C_5H_{11}$; e) R = n-$C_8H_{17}$; f) R = n-$C_{12}H_{25}$; g) R = n-$C_{14}H_{29}$; h) R = n-$C_{16}H_{33}$

**Scheme 2.** Synthesis of acetal derivatives of 3,4-dihydro-3-hydroxyisochroman-1-one

The **first chapter** of the thesis provides synthesis of *racemic* indan-*trans*-1,2-diol **2** and *racemic* lactol **3** from indene **1** (Scheme 1). Several experiments were carried out to discuss probable mechanism of formation of the products from indene.

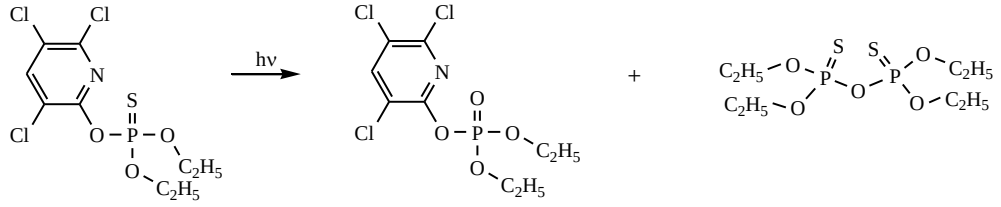
One-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one **3** from indene **1** under such mild conditions (Scheme 1) is hitherto unknown in the literature. Further work involves reaction of lactol **3** with various alcohols in the presence of acid furnished different acetal derivatives of lactol **4(a-h)** (Scheme 2).

A long standing problem in agricultural field is menace of various pests damaging the useful crops. The use of insecticides in agriculture is continuously increasing with simultaneous addition of new types of insecticides. The development of effective insecticides has obviously been a major activity in the past decades for controlling the ruinous attack by the pests. A wide range of insecticides including organophosphorus, carbamates, pyrethroids and other class of pesticides have been used on crops to control insects.

There are many factors which affect the insecticidal activity when exposed to external environment like microbial decomposition, hydrolysis, volatilization and photolysis.[5] Photodegradation due to sunlight is one of the major pathways which lessen insecticidal activity after their application in the field. On exposure to sunlight, the insecticide molecules undergo a variety of primary processes often leading to their degradation. To overcome these problem chemical modifications[6] were attempted which seriously affected the insecticidal activity and also caused ecological problems.[7] Alternatively, the UV absorbing molecules, also known as photostabilzers were used in the formulations to extend the environmental life of the insecticides.

Chlorpyrifos **5**, *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate ($C_9H_{11}Cl_3NO_3PS$), is most widely used insecticide all over the world for the protection against variety of pests. It is used both for agriculture and household purposes. US alone uses almost 30 million pounds per year while in Europe, more than 50,000 kg/year are used. It is used for various crops such as corn, alfalfa, cotton, soyabeans, cereals, tobacco, peaches, vegetables and citrus fruits to control a wide spectrum of chewing, sucking and boring insects like aphids, caterpillars, Helicoverpa spp, mites, moths, jassids, budworm, stem borer and locusts.

Chlorpyrifos is also subject to degradation on exposure to sunlight resulting in formation of various photoproducts which are more stable to UV radiation than chlorpyrifos itself. Chlorpyrifos-oxon **6** is one such photoproduct which is more persistent and about 3000 times more toxic to humans than chlorpyrifos (Scheme 3). On exposure to UV light, sulfotep **7** is also formed from chlorpyrifos (Scheme 3) which is highly toxic and often exists as an impurity in chlorpyrifos.



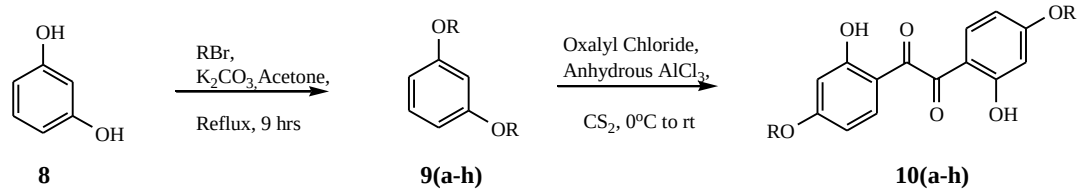

Chlorpyrifos **5** Chlorpyrifos-oxon **6** Sulfotep **7**

**Scheme 3.** Photodegradation of chlorpyrifos to chlorpyrifos-oxon and sulfotep

In order to extend environmental life of chlorpyrifos and consequently to minimize the formation of toxic, UV stable photoproducts, use of effective and efficient photostabilizers is indispensable. The photostabilizers absorb UV radiation and dissipate the absorbed energy harmlessly and also persist in the matrix for the expected lifetime. Photostabilization of the insecticide can take place either by preferential absorption of light by photostabilizer, thereby preventing photo-excitation of the insecticide molecules or transfer of excess energy from the excited insecticide molecules to the photostabilizers through various energy transfer mechanisms such as excited-state intramolecular proton transfer (ESIPT) or keto-enol tautomerism. Intramolecularly H-bonded photostabilizers such as hydroxyphenyl-benzotriazoles, hydroxyphenyl-s-triazines, oxanilides and 2-hydroxybenzophenones are widely used for the protection of various products against photodegradation.

Earlier our group has studied the effect of photostabilization of azadirachtin-A on exposure to UV light in the presence of four structurally different photostabilizers, namely 4-aminobenzoic acid, 2,4-dihydroxybenzophenone, 4,4'-dihydroxybenzophenone and phenyl salicylate.[8] In continuation with our research directed towards design and synthesis of novel photostabilizers, we conceived benzils

having structures of the type 10 (Scheme 4) with two such *hydroxy* and *keto* pairs assembled into a single structure. It was envisioned that these benzils would possess enhanced efficiency and usefulness as photostabilizers due to the inherent structural features.




**8** **9(a-h)** **10(a-h)**

**a**: R= $CH_3$; **b**: R= $C_2H_5$; **c**: R= n-$C_3H_7$; **d**: n-$C_4H_9$; **e**: R= n-$C_6H_{13}$; **f**: R= n-$C_8H_{17}$; **g**: R= n-$C_{10}H_{21}$; **h**: R= n-$C_{12}H_{25}$

**Scheme 4.** Synthesis of novel benzil derivatives

The **second chapter** includes design and synthesis of novel benzil compounds from resorcinol and their study of photostabilization property. Photostabilization study of chlorpyrifos insecticide was carried out using these novel benzils under irradiation with HPMV-lamp for 10 h and percentage recovery of these insecticides in methanol was analyzed by HPLC.[9]

It is estimated that every year at least 500 million people in the world suffer from one or the other tropical diseases that include malaria, chikungunya, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis. One to two million deaths is reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia, and Pacific Islands. These diseases not only cause high levels of mortality, but also inflict great economic loss and social disruption on developing countries such as India, China, etc. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about Rs. 720 crore.

Mosquitoes are the most important single group of insects well-known for their public health importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis and encephalitis of different types including, Japanese encephalitis. *Anopheles stephensi*, *Aedes aegypti and Culex quinquefasciatus* are the major urban vectors of malaria,

dengue and lymphatic filariasis, respectively. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides. Generally, two types of vector control methods, namely indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LNs) are used for controlling malaria transmission.

The **part A** of the **third chapter** is focused on exploring mosquitocidal activity of lactol and its acetal derivatives.[10] *Anopheles* species of mosquito, responsible for malaria was reared into the entomology laboratory at National Institute of Malaria Research (NIMR), Nadiad, Gujarat, India and used for studying mosquitocidal activity. The study includes various testing methods such as tunnel test, and adult susceptibility test for the above activities.

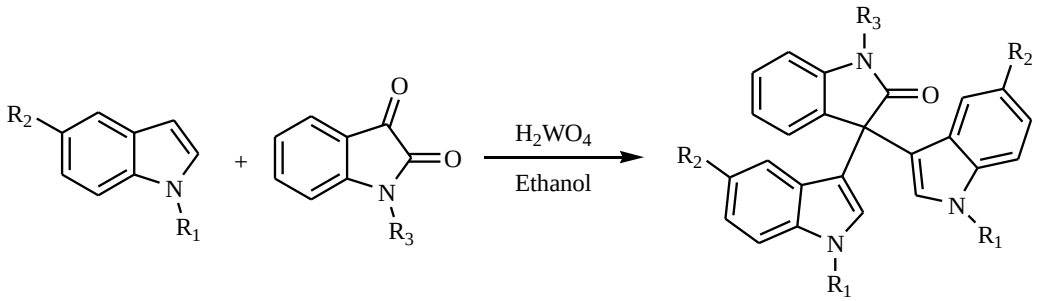
Human body is closely influenced by the activities of microorganisms. Microorganisms are a part of our lives in many ways. They are employed in the manufacture of dairy products, certain foods, processing of certain medicines and therapeutic agents, in manufacture of certain chemicals and in numerous other ways. Despite of the established useful functions and potentially valuable activities of microorganism, they are also harmful to mankind in many ways. Different microorganisms are responsible for many diseases such as Acquired Immune Deficiency Syndrome [AIDS], herpes, legionnaire's disease, influenza, jaundice, tuberculosis, typhoid, dermatomycoses, dysentery, malaria etc. Microorganisms can cause diseases either when they come in contact and invade the tissues or if they find suitable condition for their growth. Therefore control of microbial population is necessary to prevent transmission of disease and infection. Protection against such diseases can be achieved by inhibition of microbial growth or by killing them.

The work incorporated in **part B** of the **third chapter** is the study of antimicrobial activity of benzils, lactol and acetals of lactol prepared in first and third chapters of the thesis. The compounds were tested on a variety of microorganisms such as gram positive and gram negative bacteria and on fungi. For the testing of antibacterial activity two species of gram positive bacteria i.e. *Staphylococcus*

*aureus* and *Streptococcus pyogenes* and two species of gram negative bacteria i.e. *Escherichia coli* and *Pseudomonas aeruginosa* have been used. For antifungal activity two fungi i.e. *Candida albicans* and *Aspergillus niger* have been employed.

Among biologically active heterocyclic compounds, derivatives of oxindole [3,3-bis(1*H*-indol-3-yl) indolin- 2-one, **3a** in Scheme 1) attract much attention as anti-inflammatory, anti-HIV and antitumor agents, among other things. Oxindole is also an integral component of many natural products including convolutamydines, arundaphine, donaxaridine, paratunamide and maremycins.

There are many reactions known for the synthesis of oxindole derivatives by condensation of isatins and indoles in the presence of various catalysts. Although these methods work well, many of them involve harsh reaction conditions, long reaction time, or the use of corrosive acids. Most of the Lewis acid catalysts, being moisture sensitive, require usually more than stoichiometric amounts, the use of inert atmosphere, and easily undergo decomposition.




**11 a-h** **12 a-h** **13 a-h**

**Scheme 5:** Synthesis of 3,3-di(indol-3-yl)indolin-2-one derivatives **13 (a-h)**

Where **a)** $R_1$= H, $R_2$= H, $R_3$= H; **b)** $R_1$= H, $R_2$= Br, $R_3$= H;

**c)** $R_1$= H, $R_2$= $OCH_3$, $R_3$= H; **d)** $R_1$= $CH_3$, $R_2$= H, $R_3$= H;

**e)** $R_1$= H, $R_2$= Br, $R_3$= $CH_3$; **f)** $R_1$= H, $R_2$= $OCH_3$, $R_3$= $CH_3$;

**g)** $R_1$= H, $R_2$= H, $R_3$= $CH_3$; **h)** $R_1$= $CH_3$, $R_2$= H, $R_3$= $CH_3$

We have been involved in the study of the catalytic activity of tungstic acid in organic reactions. Many tungstic acid-catalyzed organic transformations are known. Tungstic acid, a low-cost and readily available heterogeneous catalyst, exhibits high catalytic efficiency. We found that tungstic acid efficiently catalyzes condensation of indoles with isatins to form corresponding oxindole derivatives in high yields. The

present method involves relatively mild conditions and easy work-up procedure (Scheme 5).

The **fourth chapter** describes thorough characterization of tungstic acid, a heterogeneous catalyst and its application for the synthesis of various oxindole derivatives.[11]

**References**


1. Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis,C. *J. Am. Chem. Soc.* **1996**, *118*, 11085-11088; Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C. *Tetrahedron* **1997**, *53*, 3243-3248.; Stromgaard, K.; Nakanishi, K. *Angew. Chem., Int. Ed.*, 2004, **43**, 1640; Nakanishi, K. *Bioorg. Med. Chem.*, 2005, **13**, 4987.
2. Schöpf, C.; Kühne, R. *Chem. Ber.*, **1950**, *83*, 390.; Valente, E. J.; Fuller, J. E.; Ball, J. D. *Acta Cryst.*, **1998**, *B54*, 162.
3. Singh, V. K.; Deota, P. T., **1988**. Synth. Commun., 18, 617.;
4. Deota, P. T.; Desai, R.; Valodkar, V. B., **1998**. J. Chem. Res. (S), 562.
5. Katagi T. *Rev Environ Contam Toxicol.* **2004**, *182*, 1.; Zepp R. G.; Cline D. M. *J. Agric. Food. Chem.* **1977**, *11*, 359.
6. Elliot, M.; Farnham, A. W.; Janes, N. F.; Needham, P. H.; Pullman, D. A. *Nature*. **1974**, *248*, 710.
7. Banerjee, K.; Dureja, P. *Pestic. Sci.* **1995**, *43*, 333.
8. Deota, P. T.; Upadhyay, P. R.; Patel, K. B.; Mehta, K. J.; Varshney, A. K.; Mehta, M. H. *Natural Product Letters*. **2002**, *16(5)*, 329.; Deota, P. T.; Upadhyay, P. R.; Valodkar, V. B. *Natural Product Research*. **2002**, *17(1)*, 21.
9. Patel, G. M.; Parmar, H. S.; Deota, P. T. *Benzil derivatives as Photostabilizers*, **Indian Patent**, Application no. 1256/MUM/2013.
10. Patel, G. M.; Parmar, H. S.; Deota, P. T. *Lactol derivatives as mosquitocidal compounds*, **Indian Patent**, Application no. 3019/MUM/2013.
11. Patel, G. M.; Deota, P. T. *Heterocyclic Communications*, **2013**, *19(6)*, 421.

# List of Publication

## Patents

1. Water soluble photostabilizers, Gautam M. Patel, Hemant S. Parmar and Pradeep T. Deota, **Indian Patent**, Application no. 3718/MUM/2012.

2. Benzil derivatives as Photostabilizers, Gautam M. Patel, Hemant S. Parmar and Pradeep T. Deota, **Indian Patent**, Application no. 1256/MUM/2013.

3. Lactol derivatives as mosquitocidal compounds, Gautam M. Patel and Pradeep T. Deota, **Indian Patent**, Application no. 3019/MUM/2013.

## Research Papers

1. Gautam M. Patel, Pradeep T. Deota "**Tungstic acid-catalyzed synthesis of 3,3-bis(1*H*-indol-3-yl)indolin-2-one derivatives**" *Heterocyclic Communications*, **2013**, *19(6)*, 421–424.

2. Gautam M. Patel, Pradeep T. Deota "**Serendipitous one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one from indene**" (*Manuscript under preparation*)

3. Gautam M. Patel, Chandrasekhar Pant, Pradeep T. Deota "**Synthesis, characterization and application of novel class of mosquitocidal compounds**" (*Manuscript under preparation*)

4. Gautam M. Patel, Hemant Parmar, Pradeep T. Deota "**Design, synthesis and application of novel benzil derivatives as photostabilizers for chlorpyrifos**" (*Manuscript under preparation*)

5. Gautam M. Patel, Hemant Parmar, Pradeep T. Deota "**Novel water soluble photostabilizers for disulfoton**" (*Manuscript under preparation*)

## Paper presented in conferences

1. **Oral** presentation in **National Conference** on **Green Chemistry** 6-8 February 2009 at Veer Narmad South Gujarat University, Surat, Gujarat, India.

2. **Poster** presentation in **Regional Science Congress** on "**Science for Shaping the Future of India"** 15-16 September 2012 at The M. S. University of Baroda, Vadodara, Gujarat, India.

3. **Poster** presentation in **44$^{th}$ world chemistry congress (IUPAC)** 11-16 August 2013 at Istanbul, Turkey.