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Synthesis of Small Molecule Inhibitors of Janus Kinase 2, Phosphodiesterase IV, GABAA and NMDA receptors: Investigation of Mcmurry, Mannich and Chemoenzymatic Strategies

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Synthesis of Small Molecule Inhibitors of Janus Kinase 2, Phosphodiesterase IV, $GABA_A$ and NMDA receptors: Investigation of Mcmurry, Mannich and Chemoenzymatic Strategies

by

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A dissertation is submitted in partial fulfillment
of the requirements for the degree of
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Dedication

I dedicate this dissertation to my parents, who supported me to achieve greater goals in life and always there with me to uplift my strength when I am at troughs of life. I could not have done this without them. I would also like to thank my brother for his support. My friends are my strength and thank you all for valuable help provided to me throughout the Ph.D.

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List of Symbols and Abbreviations

δ	Chemical shift in parts per million units
^{1}H	Isotope of hydrogen with mass of 1 amu
^{13}C	Isotope of carbon with mass of 13 amu
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
Bn	benzyl
BuLi	butyl lithium
$CDCl_3$	Deuterated chloroform
CH_3OD	Deuterated methanol
CNS	central nervous system
COSY	COrrelation SpectroscopY
DCM	dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DEPT-135	Distortionless Enhancement by Polarization Transfer at a flip angle of 135 degrees
DMAP	N, N-dimethyl aminopyridine
DME	1,2-dimethoxyethane (glyme)
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DMSO-d_6	Deuterated dimethylsulfoxide

e. e	enantiomeric excess
Et	ethyl
EtOAc	ethyl acetate
EA	ethyl acetate
EtOH	ethanol
FDA	Food and Drug Administration
GABA	γ-Amino butyric acid
Hz	a unit of frequency defined as the number of cycles per second
MHz	Megahertz
hrs	hour(s)
HPLC	high pressure (performance) liquid chromatography
HTS	high throughput screening
^{i}Pr	isopropyl
^{i}PrOH	isopropanol
LAH	lithium aluminum hydride
LC-MS	liquid chromatography-mass spectrometry
Me	methyl
MeOH	methanol
NaH	Sodium hydride
NMDA	N-Methyl-D-Aspartate
NMR	nuclear magnetic resonance
Novozyme 435	Lipase from Candida Antarctica
Pd	Palladium

Ph	phenyl
PPh_3	triphenylphosphine
Ppm	parts per million
PPTS	Pyridinium p-toluene sulfonate
Pr	propyl
Ps-30	*Pseudomonas Sepacia* Lipase
PTSA	*p*-Toluene sulfonic acid
Pyr	pyridine
R	R enantiomer
rt	room temperature
S	S enantiomer
SAR	structure activity relationship
SPR	structure property relationship
^{t}Bu	*tert*-butyl
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	Tetramethylsilane
USF	University of South Florida
WHO	World Health Organization

Abstract

Stilbenoids possess a wide range of biological properties such as, anticancer, antiplatelet aggregation, antiestrogenic, antibacterial, antifungal and antiatherogenic, etc. Owing to these therapeutic values, a great deal of attention attracted in the synthesis of derivatives of stilbenes. During the course of the study, **G6** a novel stilbenoid was discovered, through high throughput screening, to be a potent inhibitor of mutated JAK2-V617F. The mutated JAK2 variant has been implicated in various myeloproliferative disorders (MPDs) including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) has been targeted by therapeutics. Chapter **2** describes the synthesis of analogs of the stilbenoid G6 and N-substituted stilbenes bisoxazines by utilizing Mcmurry reaction and Mannich condensation methods. The main emphasis of this work is to develop novel stilbenoids as inhibitors of JAK2-V617F mutated Jak2 enzyme in Human erythroleukemia cells (HEL) since this mutation is discovered in the majority of patients with myeloproliferative disorders (MPDs). Using Mcmurry reaction, five novel *trans*-hydroxystilbenes have been synthesized from carbonyl compounds. Subsequently using Mannich coupling with five secondary amines and five primary amines, 25 novel stilbenoids and 9 novel N-substituted stilbene bisoxazines have been synthesized. In HEL cell assay, 8 stilbenoid analogues have been identified as potent inhibitors of Jak2 enzyme.

Chapter **3** describes the modification of ketamine structurally for the synthesis of novel analogues to study for their agonist activity at $GABA_A$ receptors and antagonist activity at NMDA receptors. Ligand gated ion channels like $GABA_A$ and NMDA receptors are membrane-embedded proteins at synaptic cleft which controls intercommunication among neurons and plays an important role in motor control activity, learning. $GABA_A$ receptors are responsible for inhibitory action potentials while NMDA receptors are responsible for excitory action potentials. Ketamine, known as dissociative anesthetic, produces profound analgesia at low doses to a unique cardiovascular stimulation and a cataleptic state at higher doses with dose dependent side effects like vivid dreams, disruptions of cognitive functions. The main emphasis of this work is the synthesis of novel analogues of ketamine by transforming carbonyl group in ketamine to imine functionality with small to bulkier groups and to identify an analogue of ketamine which is highly potent in its activity at the both $GABA_A$ and NMDA receptors and improved clinical actions. Studies of analogues activity against $GABA_A$ subtypes $\alpha6\beta2\delta$, $\alpha1\beta2\gamma2$ receptors and NMDA subtypes NR1/2A, NR1/2B, NR1/2D receptors have been described.

Chapter **4** describes the formal synthesis of (±)-Rolipram and the chemoenzymatic synthesis of β-aryl-γ-lactone, a Rolipram analogue. The key steps, Pd catalyzed arylation of diethylmalonate and the efficient use of selective acylation of 1, 3-diol entails the formal synthesis of (±)-Rolipram. The regioselective deacylation of 2-aryl-1, 4-diacetate by lipase *Pseudomonas Sepacia* entails the formation of β-aryl-γ-lactone. The efficient use of various methods including halogen exchange, Heck arylation

of diethylmaleate and lactonization for the synthesis of β-aryl-γ-lactone have been discussed. The present work provides an efficient and general route to γ-lactones.

Chapter 1

A short review of small molecule inhibitors of Janus Kinase 2, Phosphodiesterase IV, and $GABA_A$ and NMDA receptors

1.1 Significance of small molecule inhibitors.

A number of small molecule inhibitors[1] have been discovered and are currently used in therapy of many diseases including cancer[2] and inflammation.[3] The successful treatment of chronic myeloid leukemia by imitinib[4] (**1**, **Fig. 1**-1) among other different types of cancers and treatment of non small lung cancer (NSCLC) by gefitinib (**2**)[5] and erlotinib (**3**)[6] serves as examples that small molecule inhibitors can be effective drugs. Currently, Most of the drug discovery efforts are focused on developing small molecule inhibitors with exquisite selectivity, specificity, affinity for binding with proteins and to identify off-target interactions. Small molecule inhibitors have a greater advantage for the elucidation of the mechanism of cellular processes and so are very useful tools. Since they are more stable than peptide inhibitors and are often easily cell permeable, use of them as inhibitors of protein-protein interactions[7] is particularly helpful.

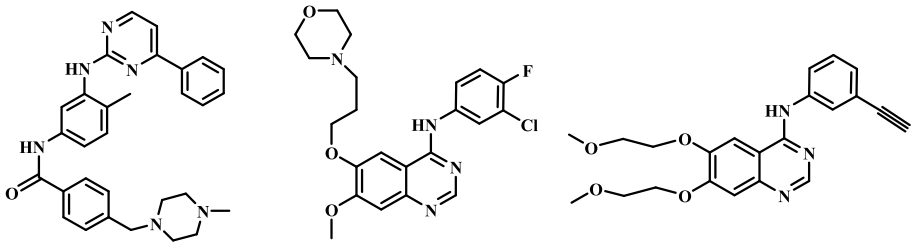
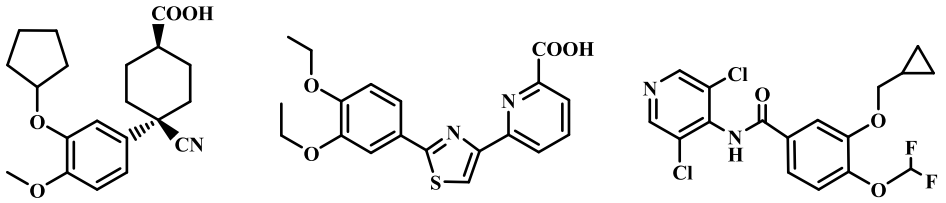


Fig. 1-1. Structures of kinase inhibitors

Imitinib **1** (Fig**. 1-1**) is a potent inhibitor of three tyrosine kinases: ABL (Ableson), platelet-derived growth factor receptor (PDGFR) and KIT. The main feature that prompted the use of imitinib in clinical trials[8] is its inhibitory action against ABL tyrosine kinase. Constitutively activated Janus Kinase 2 (Jak2) has been implicated in myeloproliferative neoplasms[10] (MPNs), i.e. polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). A large number of patients with myeloproliferative neoplasms (MPNs) have mutated JAK2-V617F,[11] which prompted the development of Jak2 inhibitors[12] for the treatment of MPNs. Several groups are working on Jak2 inhibitors with favourable pharmacokinetics and toxicity profiles which are good for clinical evaluation.



Cilomilast **Tetomilast** **Roflumilast**

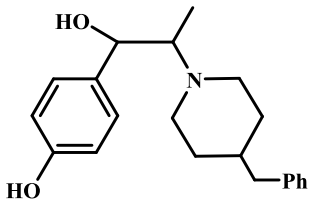
4 **5** **6**

Fig. 1-2. Structures of Phosphodiesterase-4 inhibitors.

Phosphodiesterase-4 (PDE4), an enzyme belonging to the family of eleven phosphodiesterase isoenzymes identified so far, has absolute specificity for cyclic adenosine-3',5'-monophosphate (cAMP) and is considered potential therapeutic targets[13] for the treatment of chronic inflammatory disorders,[14] such as chronic obstructive pulmonary disease (COPD).[15] PDE4 is abundant and is the major regulator of cAMP metabolism[16] in almost every proinflammatory and immune cell. PDE4 inhibitors, of varied structural classes, suppress a myriad of in vitro responses, such as proliferation and the generation and/or release of histamine.

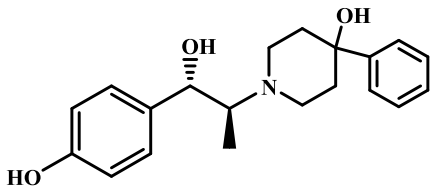
For example small molecule Cilomilast[17] (**4**) (**Fig. 1-2**), a selective phosphodiesterase-4 inhibitor, has demonstrated encouraging therapeutic efficacy in clinical trials of COPD. Now, several other selective PDE4 inhibitors are in clinical trials such as tetomilast[18] (**5**)**,** roflumilast[19] (**6**) (**Fig. 1-2**).

Antagonists of NMDA receptors[20] are currently under constant development to treat neurogenerative disorders such as Parkinson's, Alzheimer's, Huntington's diseases, cerebral ischemia, epilepsy, neuropathic pain, brain stroke and multiple sclerosis[21]. The rational for design of inhibitors is strongest for the acute treatment of ischemia[22]. A majority of NMDA antagonists developed so far have adverse effects such as hallucinations, increase in blood pressure, catatonia and anesthesia. Fortunately, all of the side effects so far identified are dose-limiting. A number of potent small molecule NMDA antagonists have been developed. For example, Ifenprodil[23] (**7**) proved to be neuroprotective in animal models of stroke without the severe side effects of earlier drugs.



Ifenprodil

7



Traxoprodil

8

Fig. 1-3. Structures of Antagonists of NMDA receptors.

Various animal models also revealed that Ifenprodil (**7**) is beneficial and significantly superior in neuroprotection of Anoxia (Hypoxia), Seizures, Ischemia and brain injury. Traxoprodil[24] (**8**) (**Fig. 1-3**) is another small molecule NMDA antagonist which also has neuroprotective, analgesic and anti-Parkinson's effects in animal studies although human studies proved less beneficial for the treatment of brain injury after stroke. The clinical trial, however, revealed another important therapeutic benefit of Traxoprodil (**8**), as a rapid acting antidepressant. NMDA subtype selectivity has been the driving force for development of small molecule antagonist; similar to that of benzodiazepines (such as Valium **9**) which enhance activation of $GABA_A$ receptors. Several small molecules have also been developed which are agonists for $GABA_A$ receptors with subtype selectivity such as sedatives, hypnotics, anxiolytics, anxiogenic, insomnia and memory enhancers. For example, Zolpidem[25] (**10,** Ambien), Zaleplon[26] (**11**), Eszopiclon[27] (**12,**(Lunesta) and Indiplone[28] (**13**) (**Fig. 1-4**) have shown to be therapeutically beneficial in insomnia treatment. These findings reveals that small molecule inhibitors have significant importance in design and development of drug targets of protein-protein interactions.

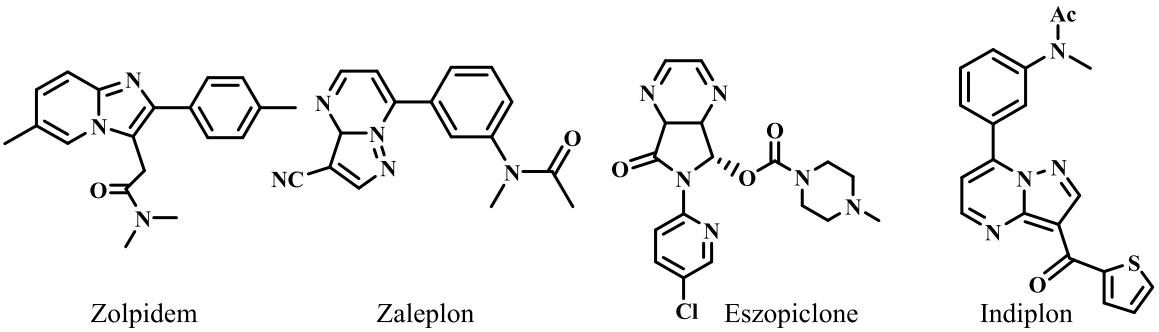


Fig. 1-4. Structures of Agonists of $GABA_A$ receptors.

1.2. Small molecules as Jak2 inhibitors.

Small-molecule kinase inhibitors most commonly target the highly conserved adenosine triphosphate (ATP)-binding domain in enzyme. Since the discovery of the mutation JAK2-V617F[29] in Jak2 and its implication in myeloproliferative neoplasms[30] (MPNs), many number of Jak2 inhibitors have been developed and some are under clinical trials. Majority of them are small molecules.

Pyrrolopyrimidine

INCB018424 (**14** in **Fig. 1-5**) has shown potent inhibition activity against Jak2 with IC_{50} of 2.8 nM. However, the selectivity has been an issue and it has also shown potent to moderate activity against Jak1, Tyk2 kinase proteins.

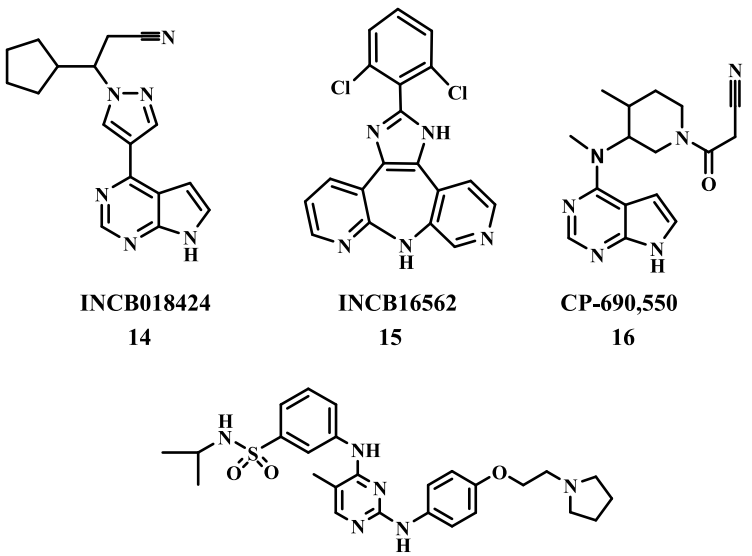


Fig. 1-5. Structures of Jak2 inhibitors in clinical studies.

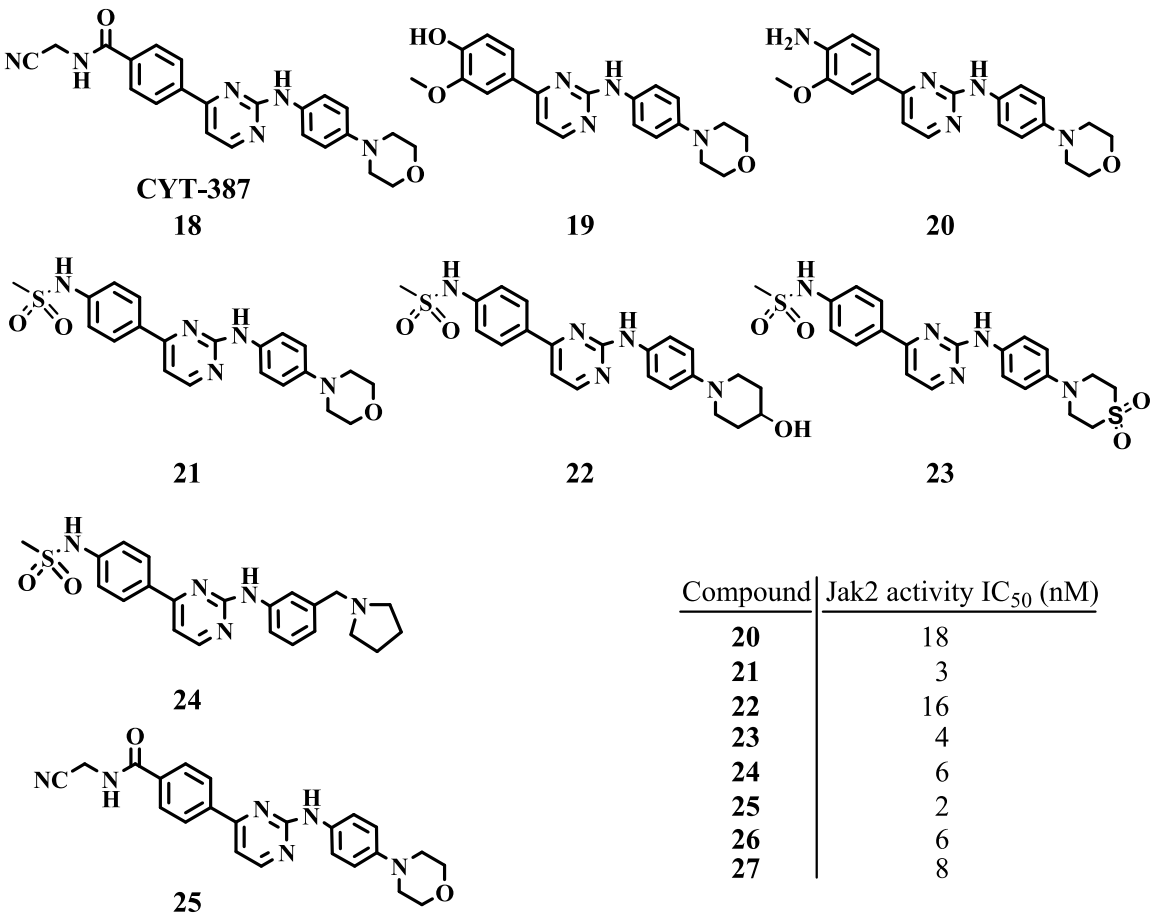
INCB018424[31] (**14**) treatment of murine model of JAK2-V617F driven malignancy resulted in significant attenuation of spleen growth and significantly increased mice survival compared with mice treated with vehicle alone. These inhibitors belongs to pyrrolopyrimidine class compounds.

Another clinical candidate CP-690,550[33] (**16** in **Fig. 1-5**) for rheumatoid arithritis, also been shown to have potent inhibition activity against Jak2 enzyme with IC_{50} of 20 nM. It has also shown higher degree of selectivity for Jak2 over Jak1/3 enzymes. This inhibitor has been identified as a lead candidate at Pfizer for rheumatoid arthritis treatment from a screening of over 400,000 small molecules library. Interestingly this compound, like INCB018424 (**14** in **Fig. 1-5**) also belongs to the pyrrolopyrimidines class of molecules. Based on this pyrrolopyrimidine ring system, several structure-activity studies have revealed other potent Jak family inhibitors.

Aminopyrimidines

Extensive *in vitro* and *in vivo* studies have revealed an interesting aminopyrimidine class compound TG101348[34] (**17** in **Fig. 1-5**) as a potent Jak2 inhibitor with IC_{50} of 3 nM. This inhibitor also turned out to be highly selective for Jak2 when profiled against over 230 kinases.

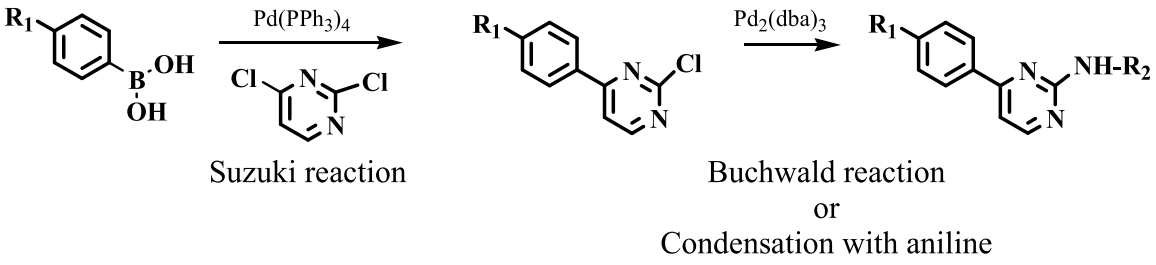
The potency and promising clinical efficacy reported for TG101348 (**17**) has led to structure-activity relationship investigations and new compounds have been developed. For example CYT-387[35] **18** (**Fig. 1-6**) has been recently found to be another potent Jak2 inhibitor which belongs to the aminopyrimidine class of compounds. Series of other aminopyrimidines (**19-25**) developed in structure-active relationship studies[35] with CYT-387 (**19**) have shown high potency than **19** (**Fig. 1-6**).



Compound	Jak2 activity IC_{50} (nM)
20	18
21	3
22	16
23	4
24	6
25	2
26	6
27	8

Fig. 1-6. Aminopyrimidine compounds as potent Jak2 inhibitors.

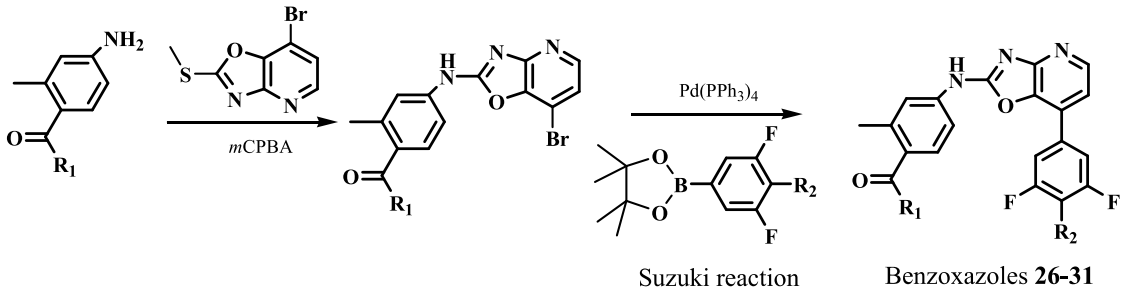
Synthesis of these aminopyrimidines (**19-25**) involves two steps, starting from various phenyl boronicacids. Regioselective cross-coupling of phenylboronic acids with pyrimidine dichloride and condensation with anilines or palladium catalyzed Buchwald reaction yields various aminopyrimidines (**19-25**) as shown in **Scheme 1-1**.



Scheme 1-1. Synthesis of various pyrimidine analogues by Burns *et. al.*

Benzoxazoles

Benzoxazole compounds also have shown attractive Jak2 inhibitory profiles in cellular assays. For example novel benzoxazole **26-31** (**Fig. 1-7**) synthesized by Gerspacher *et. al.*[36] showed high inhibitory potency against Jak2 with IC_{50} values in 3.0 to 8.0 nM range. Synthesis of the benzoxazoles **26-31** was reported to be relatively straightforward and convergent (Scheme **1-2**).



Scheme 1-2. Synthesis of various benzoxazole analogues by Gespacher *et. al.*

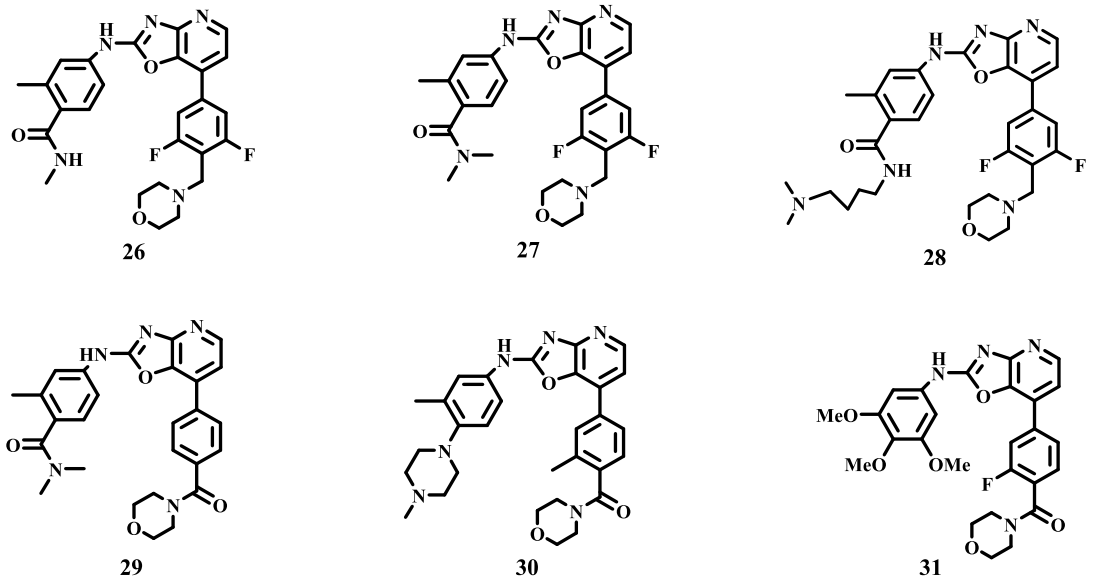
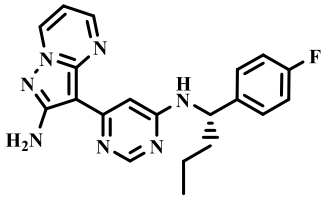


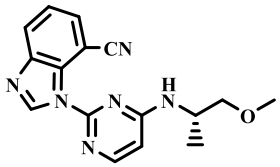
Fig. 1-7. Benzoxazole compounds as Jak2 inhibitors.

Miscellaneous

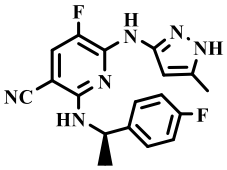
Apart from aminopyrimidines and benzoxazoles, small molecules belonging to various other scaffolds such as Tricyclic pyridones, pyrrolotriazines, purines, aminopyrazolopyrimidines, monocyclic pyridines and pyrimidines (**Fig. 1-8**) have also shown promising therapeutic efficiency in anti Jak2 activity. For example, INCB16562[32] **15** (**Fig. 1-5**) is a potent inhibitor of Jak2 enzyme with IC_{50} of 0.25 nM. In a combination studies, the growth of myeloma xenografts in mice was suppressed and antitumor activity was enhanced by oral administration of INCB16562 (**15**) which belongs to the pyridine class of compounds.



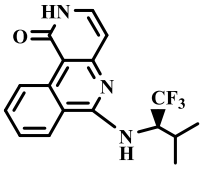
Aminopyrazolo pyrimidines



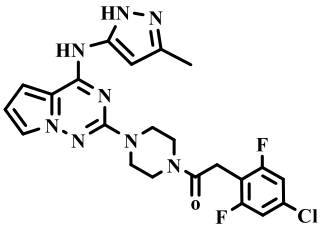
Pyrimidines



Pyridines



Tricyclic pyridones



Pyrrolotriazines

Fig. 1-8. Recently reported potent Jak2 inhibitors.

1.3 Phosphodiesterase 4 inhibitors as anti-inflammatory drugs.

Phosphodiesterase-4[14] (PDE4) enzyme is ubiquitous among inflammatory and immune cells. Inhibition of PDE4 effectively increases the intracellular cyclic adenosine-3',5'-monophosphate (cAMP) level, which in turn provides critical negative regulation of various cellular functions in these immune cells. Regulation of cAMP level by PDE4 enzyme is also critical in airway smooth muscle, and pulmonary nerves. PDE4 inhibition suppresses the recruitment and activation of several inflammatory cells such as neutrophils, CD8 T cells, macrophages, and eosinophils, that are thought to be important in the pathobiological processes that take place in the airway diseases such as chronic obstructive pulmonary disease (COPD) and asthma. PDE4 inhibitors also show suppressive activity on various in-vitro responses, including production of cytokines, cell proliferation and chemotaxis, release of inflammatory mediators, and NADPH oxidase activity. Owing to this therapeutic benefits, development of PDE4 inhibitors as anti-inflammatory drugs has attracted extensive research efforts.

Small molecules like Cilomilast[17a] **4** and Roflumilast[19] **6** (**Fig. 1-2**) have promising efficacy in PDE4 inhibition and are in phase-III clinical trials for the treatment of COPD. Despite significant progress in this area, designing new pharmacophores for the development of PDE4inhibitors such that they cover most of pulmonary diseases and increasing therapeutic index is a major effort in medicinal chemistry[17b]. Various small molecules such as oxazoles, triazolodiazines, quinazolines and pyridinemethanlos have been developed as a selective and potent inhibitors of PDE4 enzyme. For example, kuang *et. al.*[37] identified several oxazole compounds (**32-37**) for this purpose (**Fig. 1-9**).

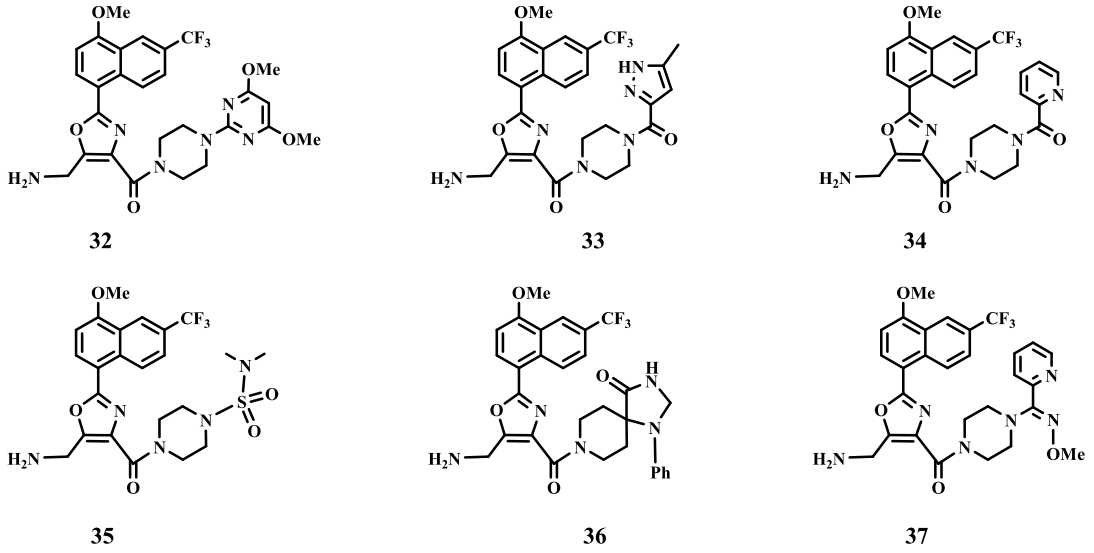
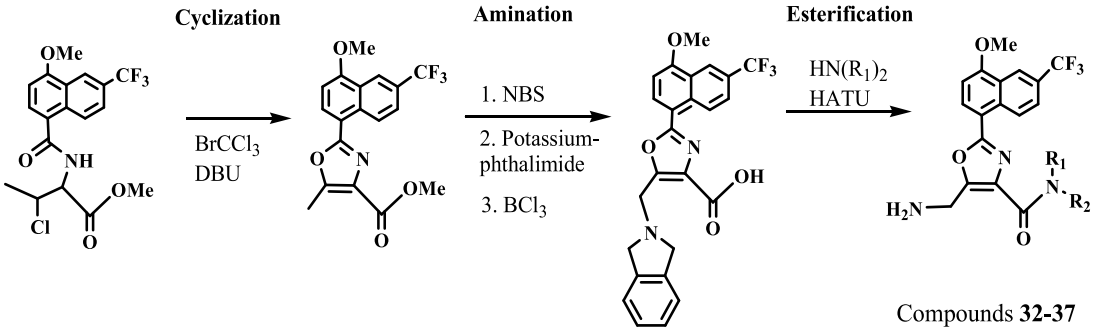


Fig. 1-9. Oxazoles as Phosphodiesterase-4 inhibitors.

The oxazole-4-carboxyamide analogs **32-37** were synthesized as shown in **scheme 1-3** and found to have potent inhibition activity against PDE4 enzyme with IC_{50} values in 0.5 to 2.2 nM range.



Scheme 1-3. Synthesis of Oxazole compounds as potent Phosphodiesterase-4 inhibitors.

Triazolodiazines have also shown potent inhibition of PDE4 enzyme. For example, skoumbourdis *et. al.*[38] synthesized several triazoloindoles (**38-40**) (**Fig. 1-10**) with high potency and selectivity for PDE4 enzyme. The triazoloindazoles **40-42** were highly selective even for PDE4 enzyme isoforms with IC_{50} values in the 1.5 to 3.0 nM range. Synthesis of these novel triazolodiazines was accomplished in two steps. Cyclization of pyridazine benzamide to intermediate triazolopyridazine and cross coupling of this intermediate with various phenyl boricacids via Suzuki reaction yields triazolodiazines efficiently (**Scheme 1-4**).

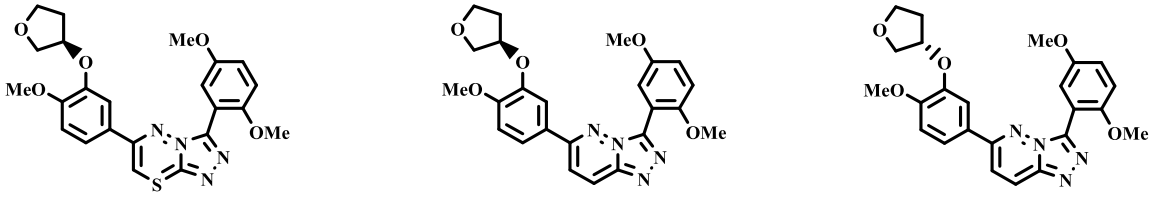
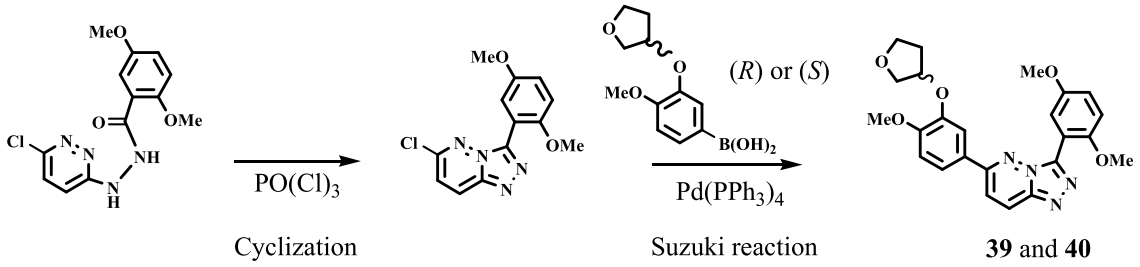


Fig. 1-10. Triazolodiazines as inhibitors of phosphodiesterase 4 inhibitors.



Scheme 1-4. Synthesis of Triazolodiazines in two steps by skoumbourdis *et. al.*

1.4 Small molecules as agonists of $GABA_A$ receptors

$GABA_A$ receptors have been therapeutic target[39] of many drugs. Majority of them are small molecules which interact with $GABA_A$ receptors as allosteric modulators. For example, benzodiazepines, barbiturates and neurosteroids interact at these receptors. $GABA_A$ receptors are functionally characterized as ligand gated Cl^- ion channels with a GABA (γ-amino butyric acid) recognition site. Normally, GABA binds to $GABA_A$ receptor fecilitating the Cl^- ion flux into the cells leading to inhibitory neurotransmission. These receptors also have binding sites for external modulators called allosteric modulators.

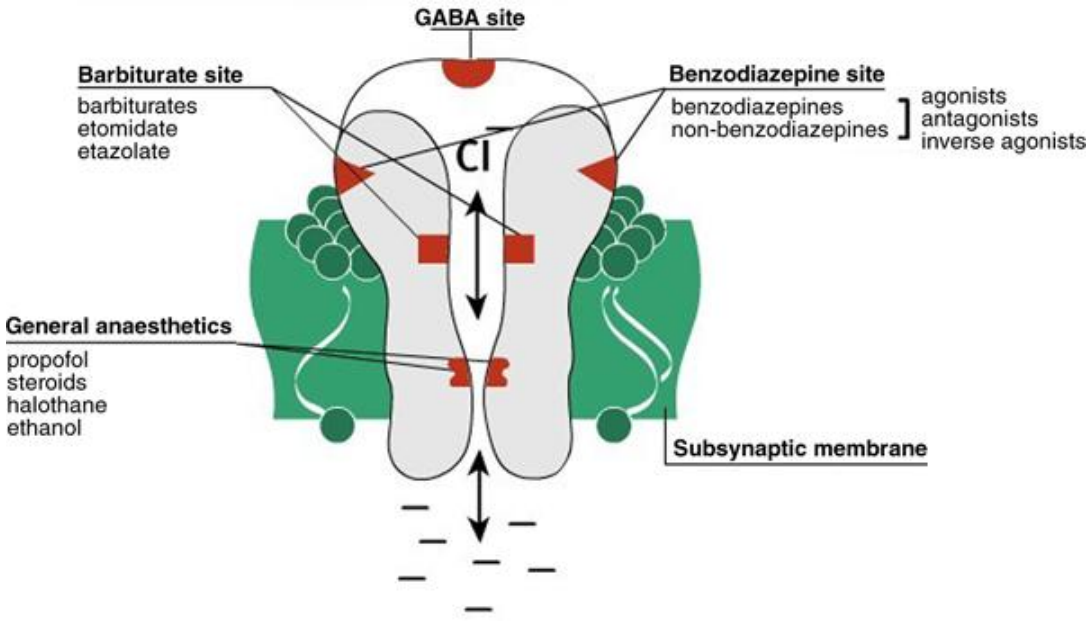


Fig. 1-11. Diagramme representing $GABA_A$ receptor with binding sites for allosteric modulators (Reprinted from Richards. G, Schoch. P, Haefely. W; *Seminars in Neurosciences.* **1991**, *3*, 191. with permission from Elsevier).

Allosteric modulators typically modulates Cl^- ion flux along with GABA at these receptors resulting many beneficial effects for the treatment of anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and premedication for medical, dental procedures. Thus barbiturates, benzodiazepines, neurosteroids and their analogues have been developed as small molecule agonists and inverse agonists for therapeutic benefits. Benzodiazepines[40] such as diazepam **41**, midazolam **42**, lorazepam **43**, oxazepam **44** and quazepam **45** (**Fig. 1-12**) are extensively used as sedatives, hypnotics and anticonvulsants which enhance the effect of GABA potentiation at $GABA_A$ receptors and thus characterized as agonists or positive modulators. Flumazenil **46** and Imidazenil **47** (**Fig. 1-12**) are other benzodiazepines which acts as antagonists mainly and reverse the effects of benzodiazepines by competitive inhibition at their binding site on $GABA_A$ receptors and thus used as antidote for benzodiazepine overdose. Benzodiazepines are heterocycles derived by fusion of benzene and 1, 4-diazepines.

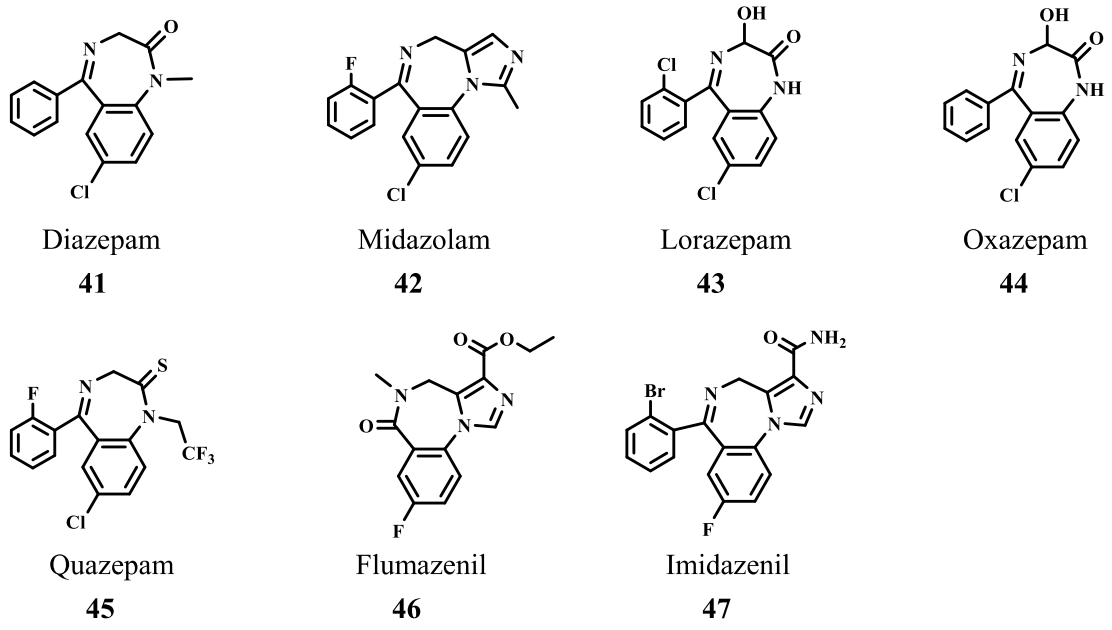
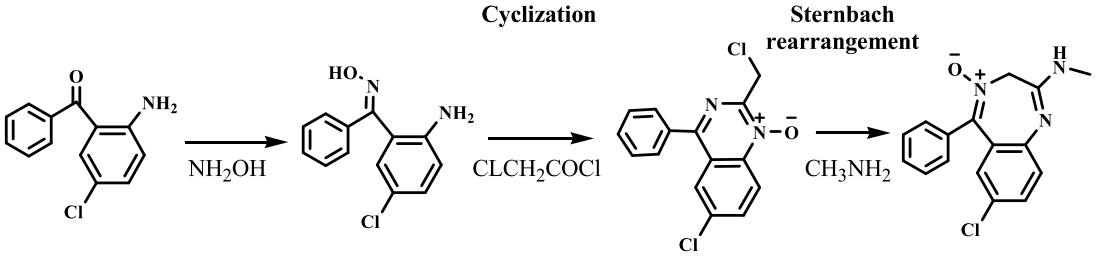


Fig. 1-12. Benzodiazepine class of compounds as agonist and antagonists of $GABA_A$ Receptors.

The synthesis of benzodiazepines, a short three steps process, is the major attraction for the development of benzodiazepines as allosteric modulators of $GABA_A$ receptors apart from their unique anticonvulsant effects. For example, the synthesis of first benzodiazepine derivative, chlordiazepine[41] **50** was achieved as depicted in Scheme **1-5** via Sternbach rearrangement.



Scheme 1-5. Chlordiazepoxide **48** synthesis by Sternbach rearrangement.

Neurosteroids are another group of small molecules which acts on $GABA_A$ receptors as allosteric modulators[42] apart from NMDA and Sigma receptors. Because of this pharmacological property, they have been used as sedatives for the purpose of general anesthesia for surgical procedures [43]. Neurosteroids such as minaxolone **49**, alphaxolone **50** and alphadolone **51** (**Fig. 1-13**) are mainly composed of four cycloalkane rings and have wide range of pharmacological properties from sedatives to treatment of epilepsy.

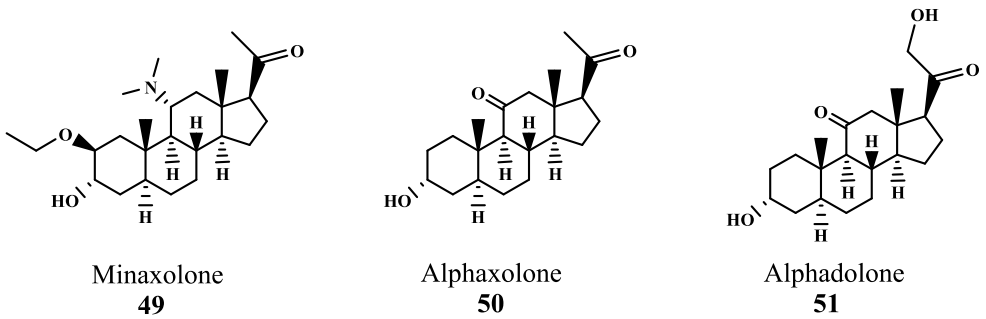


Fig. 1-13. Neurosteroids as agonists of $GABA_A$ receptors for anesthesia.

Phenobarbitol **52** and Pentobarbitol **53 (Fig. 1-14**) are also small molecules which at submicromolar concentration act on $GABA_A$ receptors as agonists producing anesthesia. Etomidate **54** and propofol **55 (Fig. 1-14**) are also well known small molecules which primarily acts as agonists at $GABA_A$ receptors and thus used extensively as intravenous anesthetic agents.

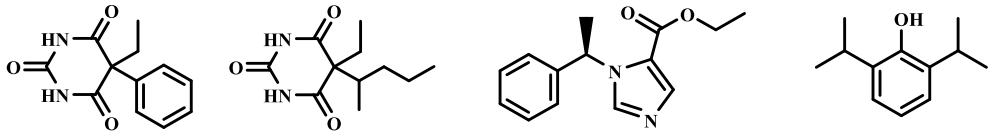


Fig. 1-14. Barbiturates and other small molecules as agonists for $GABA_A$ receptors.

Many number of small molecules are being constantly developed for the allosteric modulation of $GABA_A$ receptors since beneficial effects of targeting $GABA_A$ receptors therapeutically encompasses over a wide range from hypnotics to neuropathic pain management.

1.5 NMDA antagonists

NMDA receptors as therapeutic target by antagonists plays critical role in pain management and anesthesia. Vast number of small molecule antagonists exerts this pharmacological purpose. This is feasible because protein-protein interactions have been better understood mechanistically over the last several years. Due to the enhanced mechanistic understanding, significant progress in the development of small, drug like molecules that are capable of blocking NMDA receptor has been made. Functionally, NMDA receptors also have several binding sites that can bind to other antagonists.

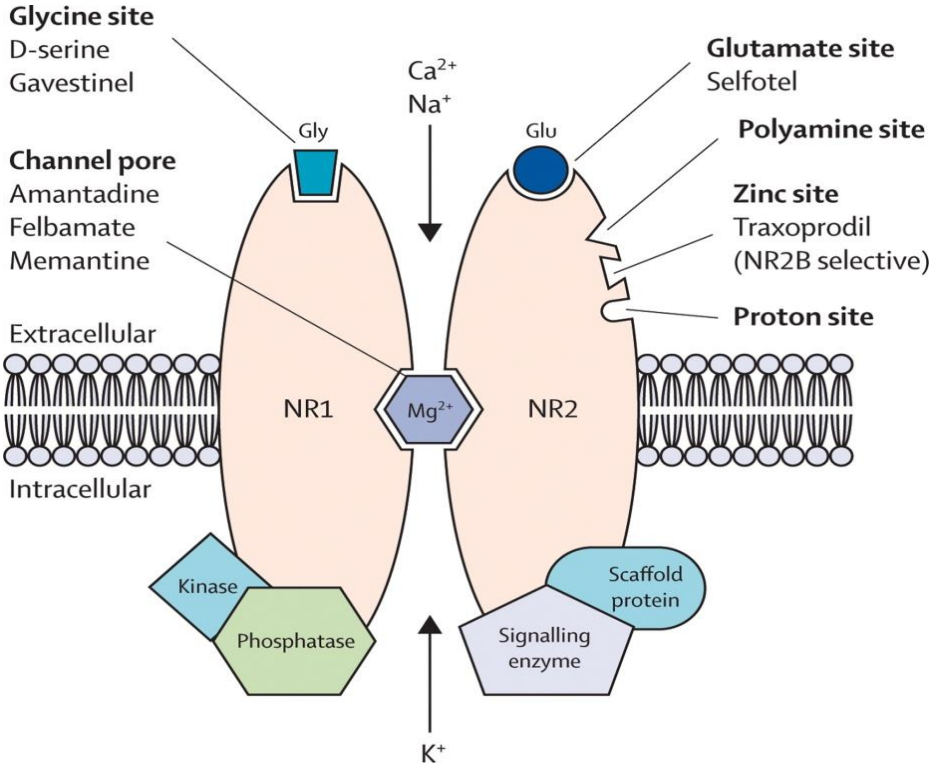


Fig. 1-15. Schematic representation of potential sites for drug action within the NMDAR protein complex. (Reprinted from Kalia, L. V.; Kalia, S. K.; Salter, M. W. *The Lancet Neur.* **2008**, *7*, 742. with permission from Elsevier)

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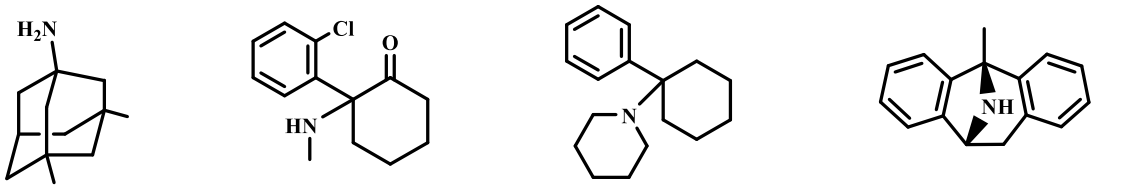
So modulation of NMDA receptors can be achieved through actions at different recognition sites such as the primary transmitter site (competitive), the phencyclidine site located inside the cation channel(uncompetitive), the polyamine modulatory site and the strychnine-insensitive, co agonistic glycine site (glycine) (**Fig. 1-15**).

Following few small molecules exemplify the purpose of negative modulation of NMDA receptors. Memantine **56** (**Fig. 1-16**) is fused cycloalkane ring system which function at NMDA receptors as uncompetitive antagonist. Memantine has been widely accepted as a very well tolerated medicine towards the treatment of Parkinson's disease and Alzheimer's dementia. Memantine functions as antagonist at NMDA receptor with low affinity. This unique feature results in its fast binding and dissociating from the receptor and thus less psychotomimetic effects. Memantine, an aminoadamantane, has been used in more than 200000 patients for the treatment of dementia.



Memantine **56** | Ketamine **57** | Phencyclidine **58** | Dizocilpine (MK-801) **59**

Fig. 1-16. Small cycloalkane rings with phenyl substituents as NMDA antagonists.

Ketamine **57** is an another NMDA receptor blocker which has been used in clinical practice for many years. Being used as dissociative anesthetic, ketamine has also been in clinical trials for neuropathic pain treatment. Phencyclidine **58** is another dissociative anesthetic which was used as surgical anesthetic but discontinued due to adverse side effects. Dizocilpine (MK-801) **59** is also another antagonist which is in clinical trials as an antidepressant.

Various other small molecule drugs have been in clinical practice and many more are undergoing clinical trials as antagonists of NMDA receptors. The significance of using small molecules as drugs comes with many advantages from wide range of libraries being screened for the lead compound to ready access to preparation.

In summary, the development of various small molecules as drugs has advantage over large molecules such as peptides. Small molecules can cross cell membranes easily than large molecules, provided enough lipophilicity. Large molecules such as peptides can be degraded easily if orally administered and cannot cross cell membranes easily. Since fewer interactions like hydrogen bonds are enough to inhibit or activate enzymes and receptors activity of the molecules can be modulated by small changes in its structures. This advantage combined with the ease of preparation of the library of small molecules for screening makes small molecules as attractive drugs.

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Chaper-2

Synthesis of Novel Stilbenoids as Inhibitors of Jak2 enzyme: Application of Mcmurry Reaction and Mannich Condensation

2.1 Janus Kinase and its role in cell signaling

Janus kinases (Jaks) are cytoplasmic protein kinases bound to the cytoplasmic tails of cytokine receptors. Upon binding of the ligand in the extracellular portion of the cell, JAK undergo conformational changes causing autophopshorylation of tyrosine residues on the C-terminal end of the cytokine receptors and further activation of Signal Transducers and Activators of Transcription (STAT) proteins (**Fig. 2-1**), which function as a gene transcription factors.[1] The mammalian JAK kinase family comprises four members: JAK1, JAK2, JAK3 and TYK2.[2] JAKs are expressed ubiquitously, except JAK3 which is primarily expressed in hematopoietic cells.[3] JAKs comprises seven regions (JH1-JH7) with significant sequence homology which are referred to as the Jak homology domains (**Fig. 2-1**).[4] JH1 domain, also known as tyrosine kinase domain, binds ATP and harbors phosphor-transferase activity of the protein. JH2 domain, which lacks tyrosine kinase activity and also known as pseudo kinase domain, negatively regulates the kinase activity of JH1 domain.[4b,5] JH4-JH7 domains, collectively referred to FERM domain, directly mediate the interaction of the Janus kinases with other cellular proteins such as cytokine receptors.[6]

Genetic knockout studies of Jaks to gain insight into the function of each Jaks have shown importance of Jak2 implied in the death of Jak2 deficient mice

embryonically due to impaired erythropoiesis and profound anemia.[7] Apart from playing a critical role in embryonic development,[7] the Jaks have been implicated in several cancers.[8] The discovery of a somatic mutation in Jak2 resulting in valine to phenylalanine substitution at position 617 (Jak2-V617F)[9] in majority of patients with myeloproliferative disorders (MPDs) including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) has been targeted by therapeutics.[10]

Its enhanced activity is usually associated with abnormal cell proliferation in a series of hematologic malignancies including lymphoid and myeloid leukemias, Hodgkin's lymphoma and various B-cell non-Hodgkin's lymphomas.[11] Several molecular inhibitors of constitutively activated Jak2 have been under study in high throughput screening and significant structural improvement in these inhibitors could be the clinical benefit in treating this MPDs at molecular pathogenesis.

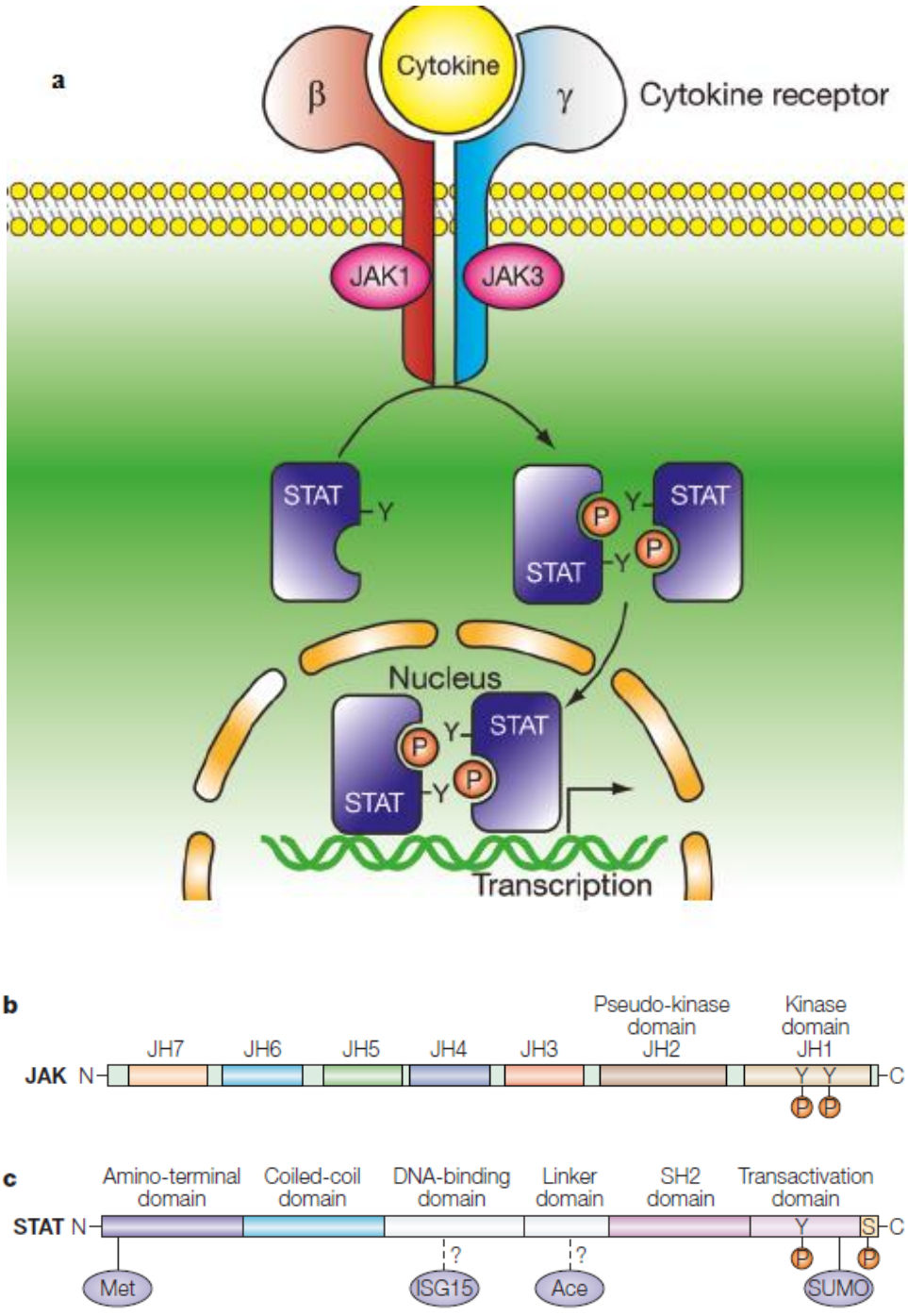


Fig. 2-1. The JAK–STAT pathway.[12] a). A schematic representation of the Janus kinase (JAK)– (STAT) pathway. b). The domain structure of JAKs. c). The domain structure of STATs. (Reprinted by permission from Macmillan Publishers Ltd.: Nat. Rev. Immunol.**2003**, 3, 900−911, copyright 2003).

2.2 Jak2 inhibition by small molecular inhibitors

Given the critical role that Jak2 plays in the pathophysiology, identification of specific Jak2 inhibitors has become an important step towards the development of an effective targeted therapy for myeloproliferative disorders. Reports on JAK2 inhibition have revealed structural platforms (**Fig. 2-3**) like AG490,[8a] LFM-A13,[13] INCB20,[14] WP1066,[15] SD-1008,[16] aminoindazoles,[17] phenylaminopyrimidines,[18] and lestaurtinib (CEP701).[19] However, only a small number of JAK2 inhibitors have been reported and face issues with target specificity. For example, AG490, lacks sufficient target specificity and may not be limited to JAK2 inhibition.[20] Specific JAK2 inhibition is highly desirable since off-target effects may cause serious immuno-modulative or proliferative side effects.

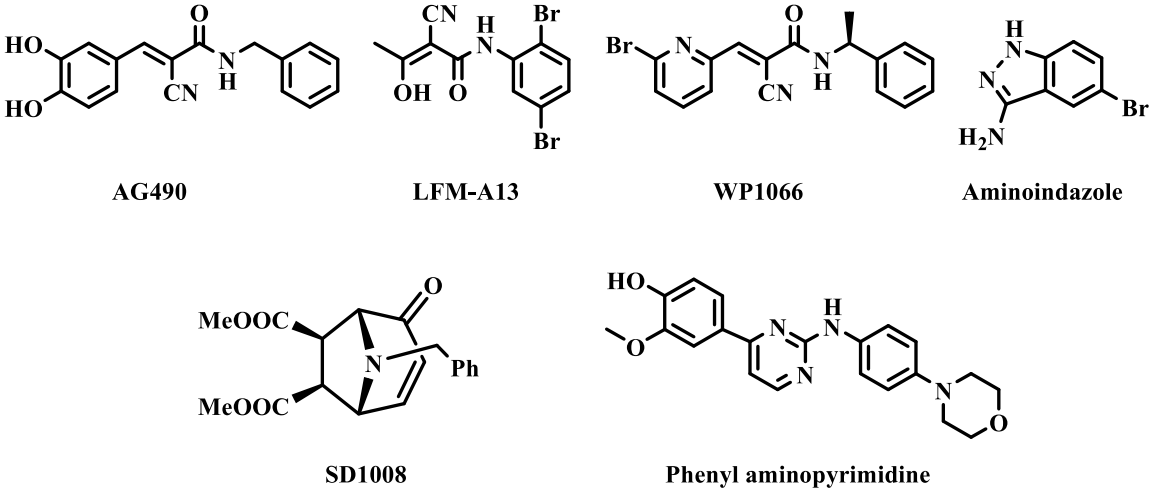


Fig. 2-2. Structures of some Jak2 inhibitors.

2.3 Significance of stilbenes in biological applications

The activity of stilbenes has been widely studied for their biological role against pathogens and for pharmacological properties.[21] The biological activity encompasses a wide range that includes anticancer therapy. Resveratrol (**Fig. 1-4**), present in grapes and other medicinal plants[22], is a well known stilbene as chemopreventive agent against major stages of carcinogenesis[23]. It's other biological properties[24] include antifungal, antibacterial, anticancer,[25] antiviral,[26] estrogenic,[27] platelet antiaggregating[28](**Fig. 2-4**) and heart protecting activities.[29] Resveratrol was found to be highly beneficial to the cardiovascular system and has shown potent anti tumorigenic effect.[30] Piceatannol, a naturally occurring phenolic stilbenoid (**Fig. 2-6**), is the only stilbene that is a known protein tyrosine kinase inhibitor. It inhibits LMP2A, a viral tyrosine kinase implicated in diseases associated with the Epstein- Barr virus.[31]

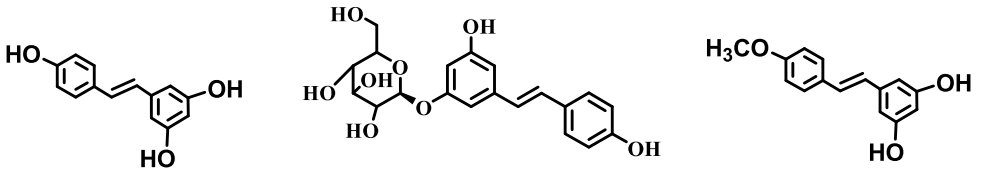
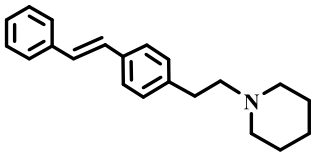


Fig. 2-3. Resveratrol and its analogues as inhibitors of platelet aggregation induced by collagen.

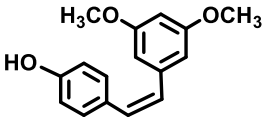
Antimicrobial compounds[32] in grape wines belong to family of stilbenes. For example, 3,5-dimethoxy-4′-hydroxy stilbene (Dimethoxy Resveratrol in **Fig. 2-5**), present in grapevine leaves has relatively high activity as antifungal compound.[32a]

It has been shown that the total phenolic compounds extracted from red wine inhibit the oxidation of human low-density lipoproteins (LDL) which otherwise may promote atherogenesis.[33] Analogues of resveratrol,[34] like piceatannol,[35] are also known to

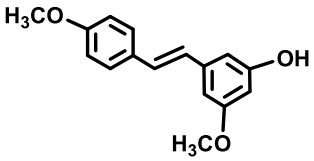
possess antimicrobial activities. The presence of phenolic hydroxyl/methoxyl groups in this stilbenes, which have high affinity for proteins, is thought to cause scavenging of the the reactive oxygen species (ROS) and partly explains their associated biological roles.[36] Previous studies also showed that hydroxystilbenes are capable of DNA binding and cleavage can also occurs in combination with metals.[37] Owing to these therapeutic values, the synthesis of derivatives of resveratrol has attracted a great deal of attention recently since they cannot be obtained in large quantities by extractive procedures. Thus several stilbene derivatives have been synthesized and found to be showing good antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis* and *Bacillus anthracis.*[32d,38] For example, Dimethoxy Resveratrol derivatives were shown to be active against several gram-positive and negative bacteria. Piperidino stilbenes was also found to be active against *Stephylococccus Aureas* with MIC of 5 mG (**Fig. 1-5**).



Piperidino-(*E*)-Stilbene
Stephylococcus. Aureas



DiMethoxy-(*Z*)-Resveratrol
Micrococcus Luteus
Neisseria gonorrhoeae



DiMethoxy-(*E*)-Resveratrol
Neisseria gonorrhoeae

Fig. 2-4. Structures and Antimicrobial activities of Piperidino stilbenes and Resveratrol analogues against Gram-positive and Gram-negative bacteria.

Since Resveratrol has been studied extensively for its antiproliferative activity,[39] there have been many number of other stilbene analogues studied. Combretastatin A4 is a naturally found stilbene (**Fig. 2-6**) in the bush willow tree *Comretum caffrum* and shown to be a potent inhibitor of tubilin assembly (IC_{50}=1.2 μM)[40] and strongly cytotoxic against selected human cancer cell lines. It's evaluation against DU-145 prostate cancer

cell lines showed activity with GI_{50} = 2 nM. Phosphate prodrug of Combretastatin A4(CA4P),[41] with water soluble property, is currently being evaluated as a vascular disrupting agent, which targets and damages tumor vasculature, in human clinical trials.

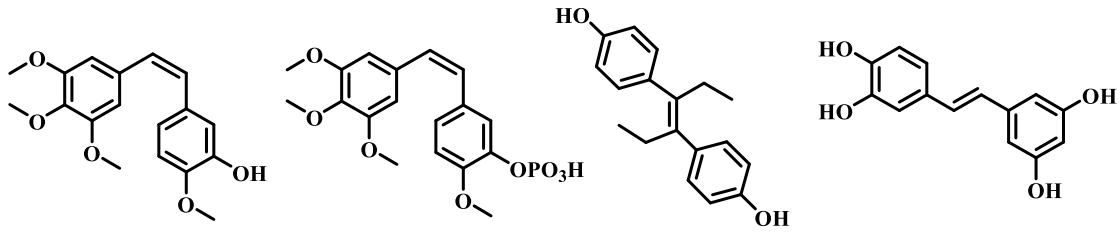


Fig. 2-5. Stilbenes as inhibitors of tubulin assembly and with activity in prostate cancer cell lines.

Diethylstilbestrol was found to be antiandrogen in MOP cell line evaluation[42] with EC_{50}=1 μM though it does not have a significant effect on tubulin polymerization.[43] Piceatannol has been reported to possess substantial antileukemic[44] activity and also shown to be a potent inducer of apoptotic cell death in BJAB (Burkitt-like lymphoma cells) cell line with an ED_{50}=25 μM.[45] It is also found to be a potent inhibitor of protein tyrosine kinases (p56^{lck} and Syk) with an inhibitory constant (K_i) of 15 μM.[31a,46] Further it was shown to suppress TNF (tumor necrosis factor-α) induced activation of NF-κB (nuclear transcription factor) at 50 μM concentration.[47]

Tamoxifen,[48] belongs to stilbene class of compounds, was discovered to be a selective estrogen receptor modulator. It is widely used currently for the treatment of all stages of hormone-responsive breast cancer[49] and currently approved by the FDA for the chemoprevention of this disease.[50]

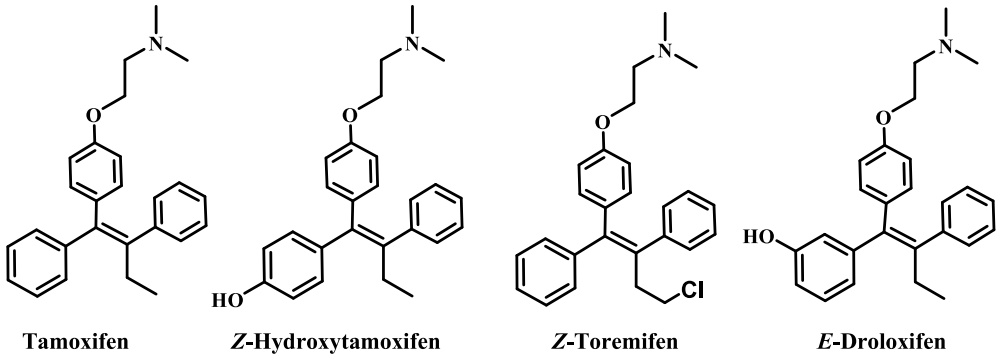


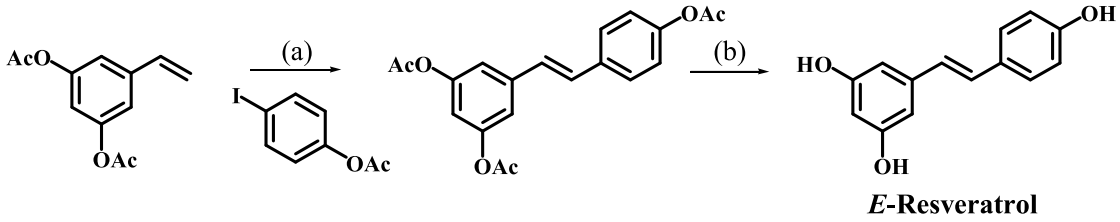
Fig. 2-6. Tamoxifen and its analogues as antiestrogen compounds for the treatment of advanced breast cancer.

Z-Hydroxytamoxifen,[51] an active metabolite of Tamoxifen,[52] was found to be an active antiestrogenic stilbene.[53] It also possess a high *in-vitro* potency for the estrogenic receptor[54] and is a weaker agent than the tamoxifen *in-vivo* due to rapid clearance[55] from cell line. *Z*-Toremifen was also found to be effective for the treatment of estrogen receptor positive advanced breast cancer[56] and has been marketed for the treatment of advanced breast cancer.[57] E-Droloxifen is another stilbene found to be a new antiestrogen[58] and is under clinical trials for the treatment of breast cancer in postmenopausal women. Potential uses of *E*-Droloxifen[59] could be in endometriosis, Alzheimer's disease, Uterine fibroid disease among others. The pronounced biological activities of tamoxifen analogues have encouraged the synthesis of many novel structural congeners.[60]

2.4 Synthetic approaches towards stilbenes

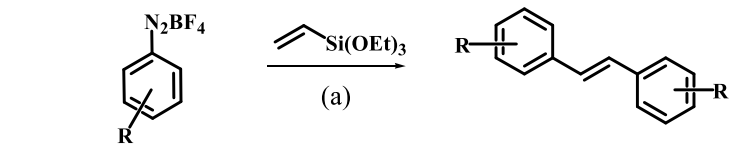
Stilbenes have been synthesized via various methods.[61] These methods include reactions catalyzed by Pd, Ru, Ti, Zn, Rh, Ni, Mo and ylide formation in wittig reaction. Following sections will describe a few methods that are widely used.

Guiso *et. al.* [62] have synthesized *E*-Resveratrol (**Scheme 2-1**) via a Heck reaction between 3,5-diacetoxy styrene and 4-acetoxy phenyl iodide catalyzed by $Pd(OAc)_2$ and PPh_3 in 70% yield. Since aryl iodide is more reactive than ArBr and ArCl in Heck reaction, it was chosen in this reaction. This reaction also yielded small amounts of partially deacylated by products. The resulted triacetoxy stilbene was hydrolyzed to *E*-Resveratrol with NaOMe.



Scheme 2-1. Synthesis of E-Resveratrol by Guiso *et. al.*via heck reaction. (a). $Pd(OAc)_2$, PPh_3, Et_3N, CH_3CN, 85^0C. (b). NaOMe, MeOH, THF.

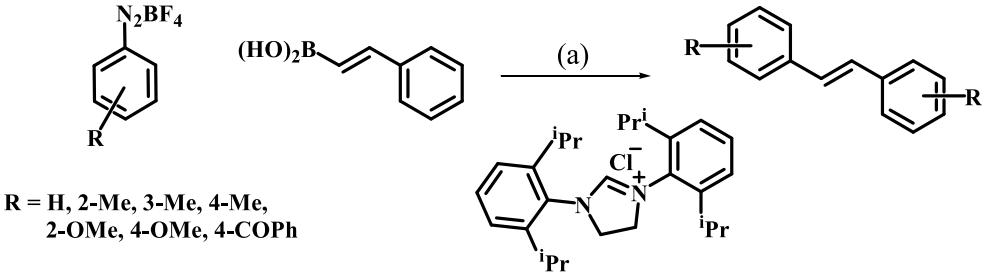
Sengupta *et. al.* [63] used aryldiazonium salts in double heck reaction (**Scheme 2-2**) with vinyltriethoxysliane to synthesize symmetrical *trans*-stilbenes with 40-70% yields for various substituents.



R = 2-Me, 2-CO_2Me, 4-Cl, 4-Br, 4-I, 4-Me, 4-OMe, 4-NO_2.

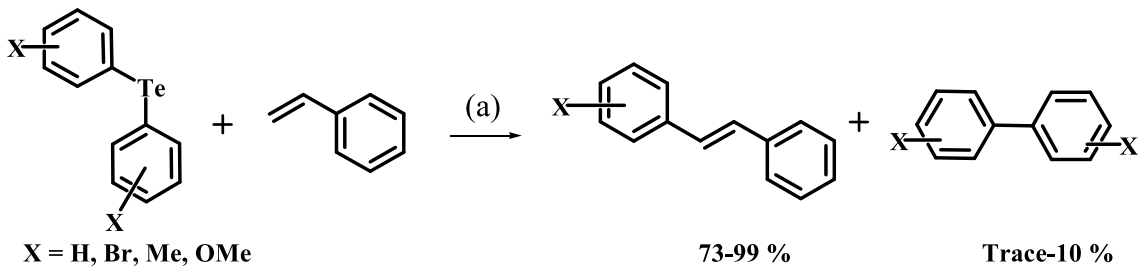
Scheme 2-2. Synthesis of Stilbenes by sengupta *et. al.*via heck reaction. (a). $Pd(OAc)_2$, MeOH, 60^0C, 1hr.

Suzuki-Miyaura reaction has also been used for the synthesis of stilbenes since the method is highly selective, tolerant of functional groups on either coupling partners and reagents is fairly insensitive towards water. Andrus *et. al.* [64] (**Scheme 2-3**) used imodozolium chloride catalyst in combination with $Pd(OAc)_2$ to couple aryldiazonium salts and aryl vinylboronic acids in suzuki reaction for the synthesis of *trans*-stilbenes.



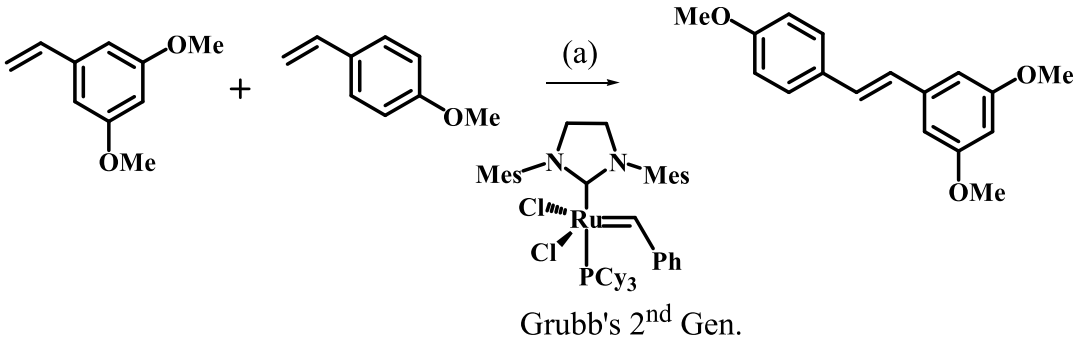
Scheme 2-3. Synthesis of Stilbenes by Andrus *et. al.*via suzuki reaction. (a). $Pd(OAc)_2$, THF, 0^0C to room temperature, imidazolium chloride, 3hrs, 68-87% yields.

Stille coupling[65] with modified reagents is also an useful method for the synthesis of stilbenes. For example, Nishibayashi *et. al.* [66] (**Scheme 2-4**) used organotellurides instead of organostannes in Stille type coupling for the synthesis of *trans*-stilbenes. Various organotellurides reacted with alkenes in the presence of palladium salts and oxidant like AgOAc for the efficient synthesis of stilbenes.



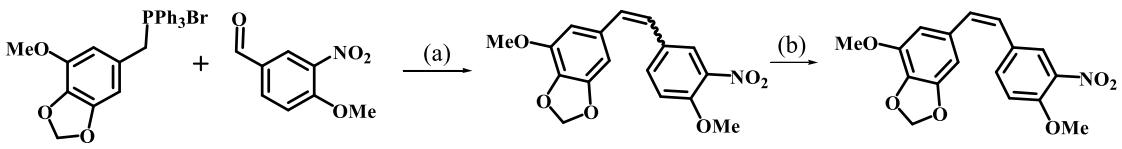
Scheme 2-4. Synthesis of Stilbenes by Nishibayashi *et. al.*via Stille coupling. (a). $Pd(Cl)_2$, AgOAc, Et_3N, MeOH, 25^0C, 20 hrs.

Cross metathesis reaction presents another possible method for the synthesis of both symmetrical and unsymmetrical stilbenes.[67] Since schrock *et. al.* [68]demonstrated that trans-stilbenes can be synthesized by using Mo-based catalyst, this method has garnered attention recently. Chang *et. al.* [69] synthesized symmetrical stilbenes from the corresponding para-substituted styrene by using Ru-based Grubbs 2nd generation catalyst. Velder *et. al.* [70] synthesized a broad spectrum of unsymmetrically substituted stilbenes (**Scheme 2-5**) using Grubbs 2nd gen. catalyst.



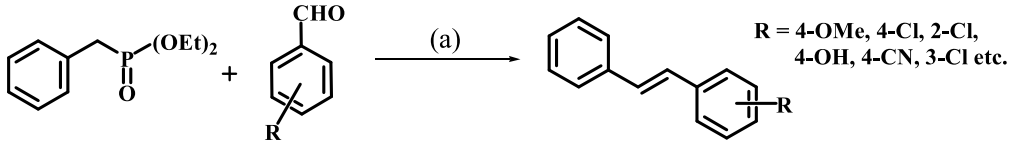
Scheme 2-5. Synthesis of Stilbenes by Velder *et. al.* [70] via cross-metathesis. (a). Grubb's 2nd generation cat.(2 mol%), CH_2Cl_2, 40^0C, 1.5 hrs.

Wittig or Horner-Wadsworth-Emmons reactions have attracted greater attention for the synthesis of stilbenes from two aromatic building blocks although it affords both (E)- and (Z)-alkenes. This reaction of benzyl ylides with substituted benzyl aldehydes proved to be unsatisfactory since it afforded poor control over the configuration of the newly formed C=C double bond yielding mixtures. This is mainly attributed to semi stability or instability of the ylides. Pettit *et. al.* [71] synthesized *cis*-stilbenes (**Scheme 2-6**) using wittig reaction with a *Z* to *E* ratio of 1.4:1. Both isomers were isolated by column chromatography. Photochemical isomerization of trans isomer proceeded to yield cis-stilbenes with 62% overall yield.



Scheme 2-6. Synthesis of (*Z*)-Stilbenes by Pettit *et. al.*[71] via wittig reaction. (a). *n*-BuLi, THF, 0^0C then aldehyde in THF, Argon. Z to E ratio of products 1.4:1. (b). Benzil, Benzene, 254 nm lamp, Argon. (c). Zn/AcOH. (d).

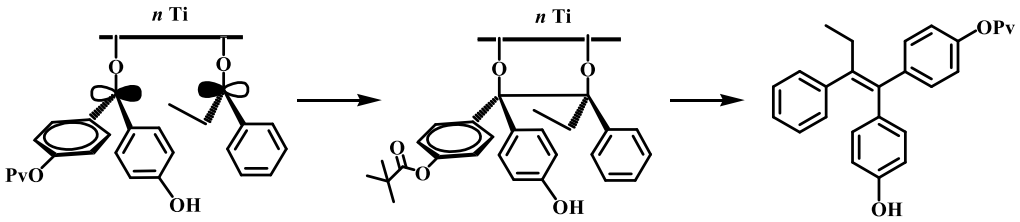
Horner−Emmons−Wadsworth olefination is another reaction which provides greater selectivity for the synthesis of trans-stilbenes and avoids the formation of byproduct triphenylphosphine oxide, a byproduct of wittig reaction. For example, Heynekamp *et. al.* [72] synthesized substituted (E)-stilbenes (**Scheme 2-7**) by reacting benzylphosphonic acid diethylester with various substituted benzaldehydes using sodium methoxide as a base.



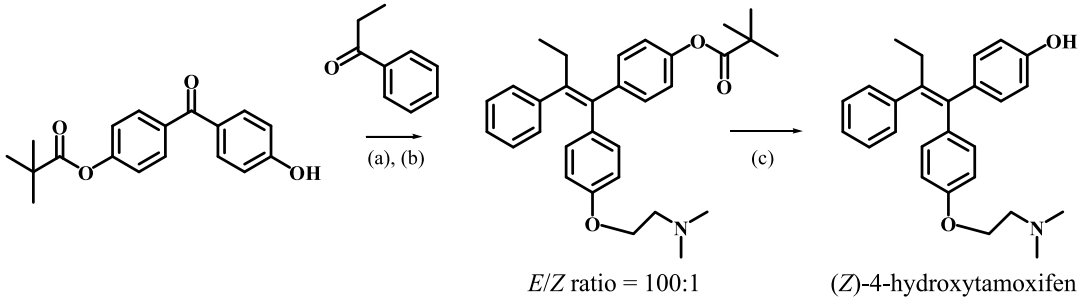
Scheme 2-7. Synthesis of (*Z*)-Stilbenes by Heynekamp[72] *et. al.*via Horner-Emmons-Wadsworth reaction. (a). NaOMe, 18-crown-6, DMF.

There are also several methods for the synthesis of stilbenes that are catalyzed by transition metals. Among them, Mcmurry reaction[60b,73] is versatile in the formation of stilbenes via coupling of carbonyl compounds, such as diarylketones and aldehydes. It provides ready access to symmetrical stilbenes by avoiding byproducts as is the case in other methods. It is also efficient in terms of functional group tolerance, solid-liquid biphasic work up and does not require expensive metal catalysts, or ligand. Mcmurry reaction,[74] a reductive coupling of carbonyl groups, is catalyzed by low valent titanium.

The formation of the stilbene is the result of a induced reductive dimerization to form carbon-carbon bond which is followed by the deoxygenation of the pinacolate intermediate. The Mcmurry reaction has been exploited in organic synthesis and usually provides (*E*)-stilbenes. (*Z*)-stilbenes[75] can be preferentially obtained if geometric constraints control the orientation of the two carbonyl moieties. Thus this reaction is versatile in terms of controlling stereochemistry of the double bond and functional group tolerance. Some of the stilbenes synthesized by this method have shown potent nematocidal activity. [76] Mcmurry reaction has been used in the synthesis of highly functionalized stilbenes such as Tamoxifen, [73a] Droloxifen[59] and related compounds. [52]



Scheme 2-8. Observed stereoselectivity in the synthesis of (Z)-4-Hydroxytamoxifen.

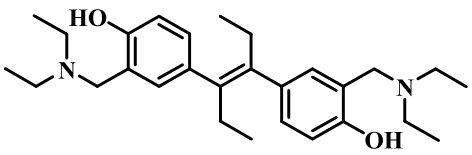


Scheme 2-9. Synthesis of (*Z*)-4-Hydroxytamoxifen by Gautheir *et. al.* [52] via Mcmurry reaction with modified reaction conditions. (a). $TiCl_4$, Zn, THF, 0^0C to reflux. (b). $Cl(CH_2)_2N(CH_3)_2$, K_2CO_3, acetone, H_2O. (c). MeLi, THF, -78^0C.

Mcmurry reaction yields mixed results for synthesis of unsymmetrical stilbenes especially when the carbonyl compound used is diaryl ketone and is reacted with equimolar amounts of other ketone or aldehyde. However, by using diaryl ketone in less than equimolar amount, the desired unsymmetrical stilbene can be obtained in good stereoselectivity and yield. For example, Gauthier *et. al.* [52]demonstrated that using diaryl ketone and propiophenone in 1:3 ratio, the cross coupled product was obtained with *E*/*Z* ratio of 100:1 in 82% yield (**Scheme 2-9**). The mechanistic investigation[52,59] of the reaction revealed a steric preference in the transition state in which the sterically smaller alkyl chain aligned with the bulkier substituted phenol group (**Scheme 2-8**) thus minimizing the steric interaction. This steric preference was elegantly exploited by Gauthier *et. al.* in the design of the synthetic route to (Z)-4-Hydroxytamoxifen and other analogues like (*E*)-Droloxifen.

2.5 Synthesis of novel stilbenes via Mcmurry reaction

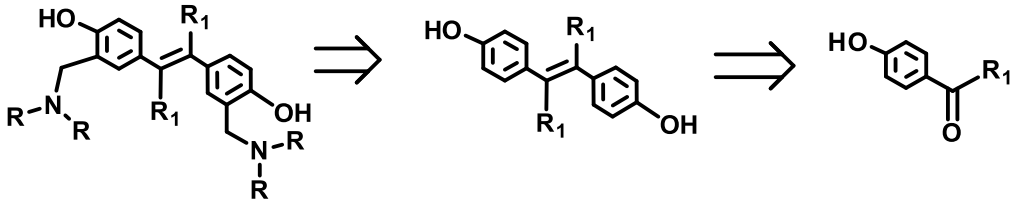
Recently, we have identified a novel small-molecule inhibitor of Jak2, named G6, (**Fig. 2-8**) using structure-based virtual screening. [77] We showed that G6 has a specific inhibitory effect on Jak2 kinase activity as measured by *in vitro* enzyme assays. In an *in vivo* and *ex vivo* study, G6 greatly reduced growth of Jak2-Val617phe mutated human pathological cells isolated from the bone marrow of a polycythemia vera patient who was Jak2-V617F positive in a dose dependent manner with IC_{50} value of 50 nM. Our laboratories have also reported that the core stilbenoid ring system[78] present in G6 is essential for maintaining its ability to inhibit Jak2 kinase activity. In the course of our studies towards the synthesis of G6 and its analogs, our efforts were led on to the investigation of the Mcmurry reaction.



G 6 (11)

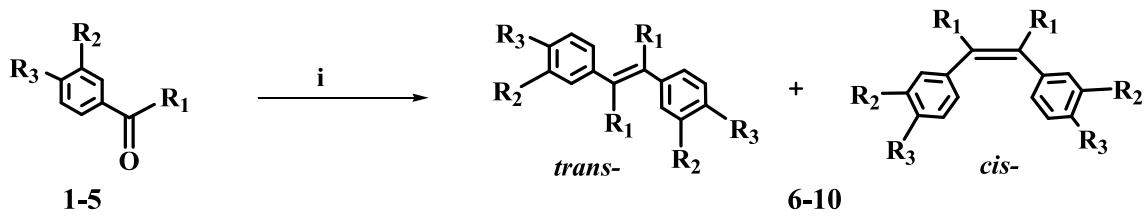
Fig. 2-7 Structure of G6 (compound 11).

As illustrated in **Scheme 2-10**, synthesis of the various G6 analogs was envisioned using Mcmurry coupling of the hydroxyl benzaldehyde or ketones to synthesize the corresponding hydroxystilbene followed by the Mannich coupling with the appropriate amine. Five stilbenes, **6-10** (see **Table. 2-1**, **Scheme 2-11**) were synthesized. Mcmurry coupling of the ketone and aldehyde in the presence of Zinc and titanium tetrachloride in dry THF led to a mixture of *E*- and *Z*- stilbenoids to a varying degree.



Scheme 2-10. Synthesis of stilbenoids from ketones and aldehydes.

Separation of these isomers involving column chromatography proved to be a difficult task due to the dynamic isomerization on the column. Next, we tried various solvents for recrystallization to isolate the isomers selectively. However, several attempts to separate and isolate the *Z*- isomer were unsuccessful. The *E*-stilbenoid, being more stable, was the only isomer isolated in these recrystallization. When 4'-hydroxy propiophenone **1** was treated with the low valent titanium reagent, prepared from $TiCl_4$/Zn, at reflux, diethylstilbestrol was obtained as an inseparable *E/Z* mixture. This *E/Z* mixture upon recrystallization from 15% ethylacetate/hexane resulted **6** (>100:1 *E*/*Z* ratio) with pure *E* isomer in 73% yield. [79]



1-5 6-10

Scheme 2-11. Reagents and Conditions. i. TiCl4, Zn, Dry. THF, reflux.

Table 2-1. Stilbenes **6-10** from various carbonyl compounds.

Aldehyde or ketone	R_1	R_2	R_3	Stilbene	*E/Z* ratio	% Yield
4'-Hydroxy propiophenone (**1**)	$-C_2H_5$	-H	-OH	**6**	100 : 1	73
4'-Hydroxy acetophenone (**2**)	$-CH_3$	-H	-OH	**7**	100 : 1	65
4'-Hydroxy benzaldehyde (**3**)	-H	-H	-OH	**8**	100 : 1	71
3'-Hydroxy acetophenone (**4**)	$-CH_3$	-OH	-H	**9**	85 : 15	76
3'-Hydroxy benzaldehyde (**5**)	-H	-OH	-H	**10**	100 : 1	84

<u>*trans*- 4,4'-Diethyl stilbestrol(**6**)</u>: The ^{1}H NMR of *trans*- diethylstilbestrol **6** was in complete agreement with the one in literature (**Fig. 2-9b**).[79a] A NMR spectral comparison of the *cis*- and *trans*- mixture before and after crystallization is shown in figure 1-8. The methylene (H**a'**) and methyl (**Hb'**) protons in the *trans* isomer were upfield than of those (**Ha** and **Hb**) in the *cis*-stilbestrol (**Fig. 1-8a**). Methyl (**Hb'**) protons in *trans*- **6** were at 0.6 ppm while those (**Hb**) were at 0.7 ppm in the *cis*-isomer. This upfield shift in the resonance of the methyl and methylene in the trans isomer can be attributed to the diamagnetic shielding due to their proximity to the aromatic ring. The diamagnetic shielding was also observed in the upfield shift in the resonance position of the aromatic protons in the cis- isomer (**Fig. 2-9a**). [79a]

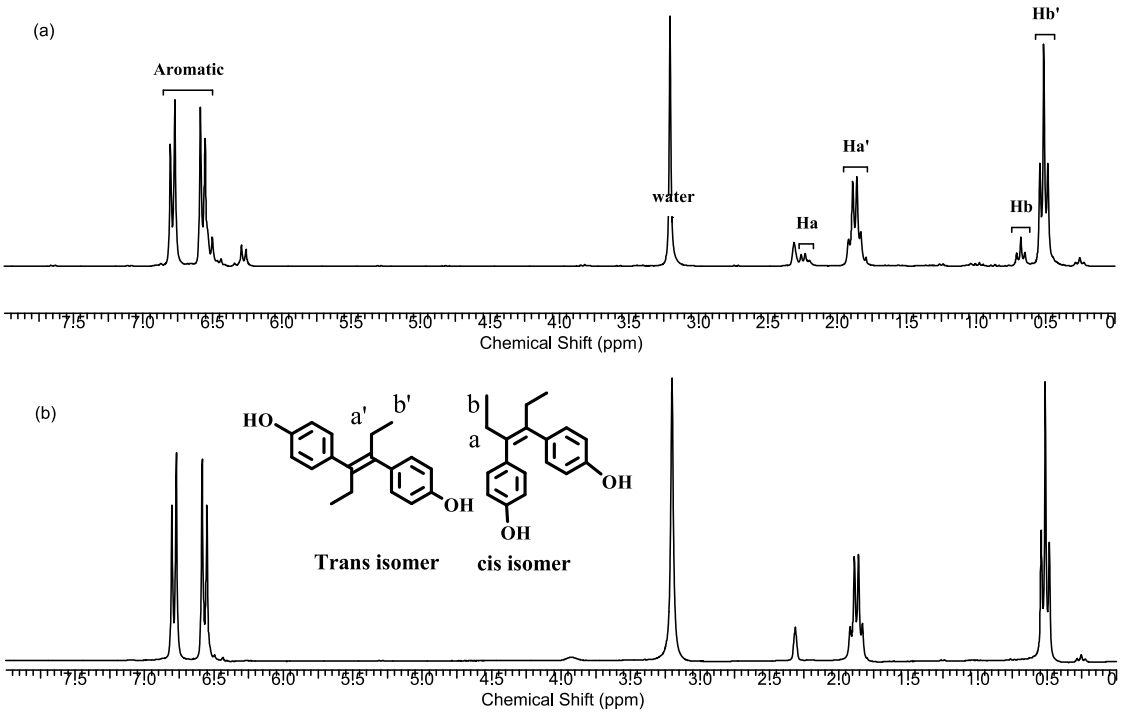


Fig. 2-8. ^{1}H NMR spectra of Diethyl stilbestrol **6** (a). trans and cis mixture . (b). isolated trans isomer after recrystallization.

<u>*trans*- 4,4'-Dihydroxy dimethylstilbene (**7**):</u> Using reaction conditions similar to as previously described the 4'-Hydroxy acetophenone (**2**) in Mcmurry coupling also gave E/Z mixture which upon recrystallization from (9:1) methanol and water furnished **7** (>99 : 1 ratio) with 65% yield. In the ^{1}H-NMR spectrum, the methyl groups in the *trans*-isomer of **7** also showed signal upfield than that of in the *cis*-isomer (**Fig. 2-10**); as seen in the case of Diethylstilbestrol **6**. Similar trend was also observed for aromatic protons of **7** in which were upfield in the cis-isomer that in the *trans*- isomer. This could also be attributed to diamagnetic shielding effect as explained in the case of Diethylstilbestrol **6**.

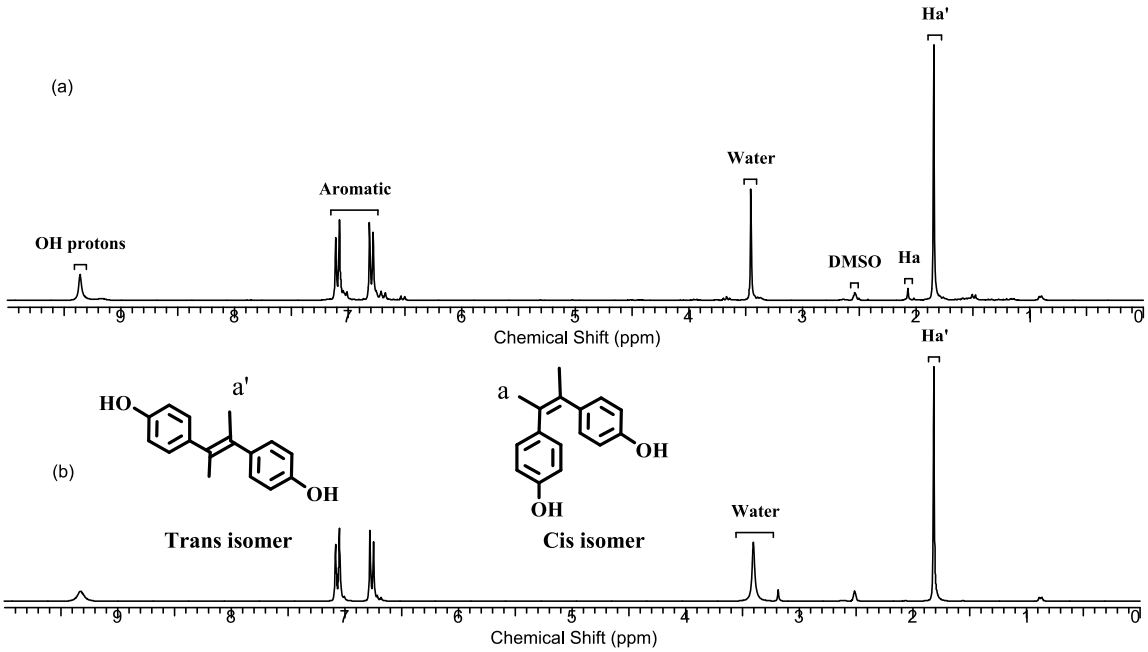


Fig. 2-9. Comparisons of ^{1}H NMR spectrums of 4,4'-dihydroxy dimethylstilbene **7** as mixture and trans isolated. (a). Mixture of isomers trans and cis. (b). Pure trans isomer after recrystallization.

Next, 4' and 3'-Hydroxy benzaldehydes (**3** and **5**) also gave E/Z mixtures which upon recrystallization from methanol furnished pure isomers with 71% and 84% yields. Crude product from 3'-Hydroxy acetophenone in recrystallization from methanol resulted in 85:15 (*E*/*Z* ratio) which used in next step directly. Unambiguous structural characterization of the stilbene was carried out using the ^{1}H and ^{13}C NMR spectroscopic analysis . [76,79]

In all of the stilbenes synthesized, trans-isomer was obtained predominantly. This predominant formation of trans-product suggests that the orientation of phenyl rings plays an important role in the transition state as shown in **Fig. 2-11**. The generally accepted mechanism, proposed by Mcmurry[73b,80] and several other, [59] is that the carbonyl compounds are reduced by metallic titanium to give a radical anion species leading to

their homolytic coupling followed by the deoxygenation of the pinocolic intermediate to result in the desired stilbene. It is noteworthy to mention that unless the orientation of carbonyl compounds are constrained by geometrical restraints[81] like it was observed in the cases such as cyclophanes, [82] calix[4]arenes[75] and 2,3-dicarbonyl substituted benzene derivatives[83] with rigid scaffold structures, the carbonyl compounds prefer to orient themselves such that with phenyl groups are always syn to the smaller alkyl groups. So this could explain the predominant formation of trans-isomers as this orientation could minimize the interaction between bulkier phenyl rings.

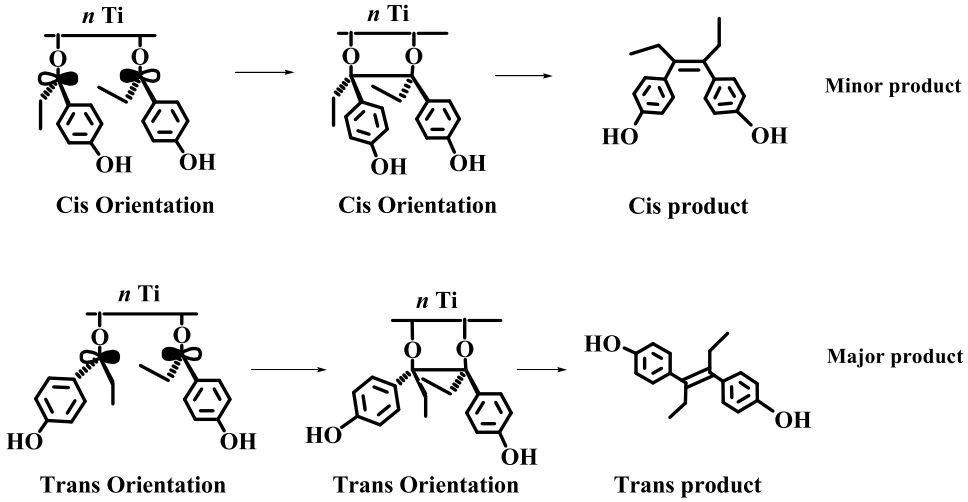


Fig. 2-10. Observed stereoselectivity in the synthesized stilbene in Mcmurry reaction.

2.6 Synthesis of novel stilbenoids via Mannich condensation

Stilbenes **6-10** synthesized have been coupled with various amines in Mannich condensation as shown in **scheme 2-12** and the list of compounds is included in Table **2-2**. As an example of the process, Diethylstilbestrol **6** was treated with 2.1 molar equivalent of dimethylamine and 2.2 molar equivalent of paraformaldehyde at reflux to synthesize stilbenoid **11**. The stilbenoid **11** showed proton signals in ^{1}H NMR spectrum (**Fig. 2-12a**) at 3.6 ppm for benzylic protons (He), 2.8 and 1.3 ppm for ethylamine

protons (Hc and Hd), aromatic protons at 6.8-7.0 ppm, the Ha at 2.1 ppm and the Hb protons at 0.7ppm. ^{13}C NMR spectrum also confirmed the structure of the symmetrical stilbenoid **11**. Importantly, the NMR data confirmed that the reflux condition used in this reaction did not alter the trans-configuration of this product.

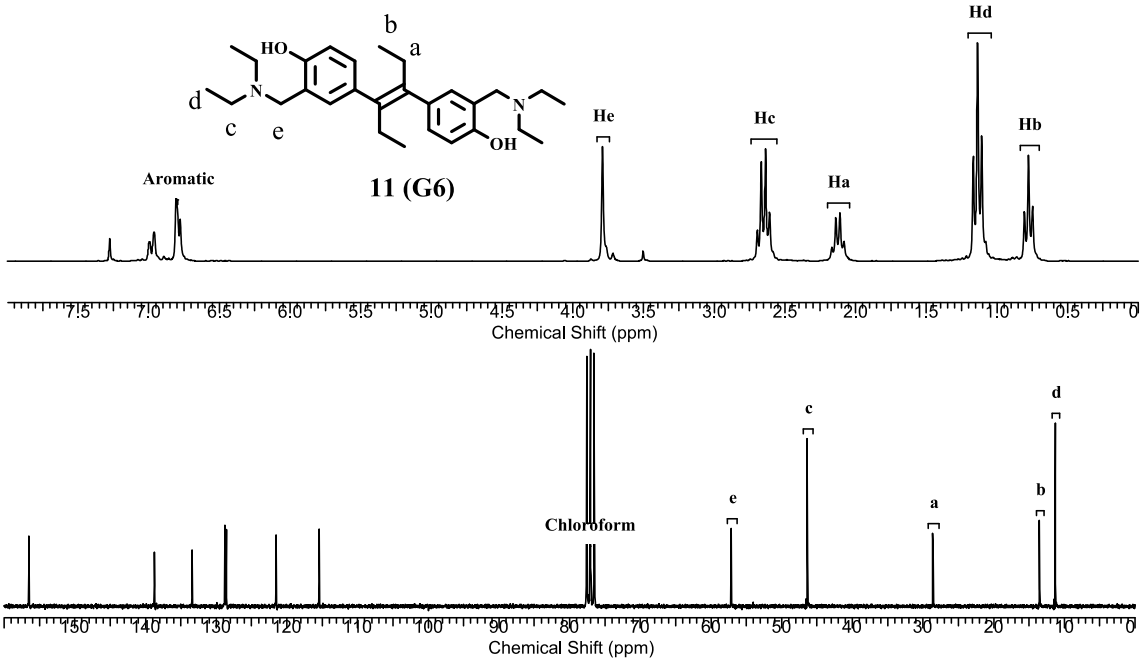
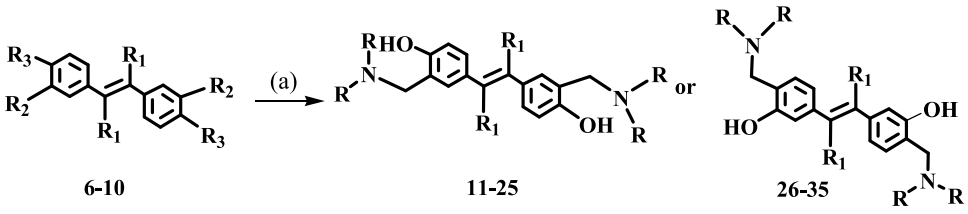


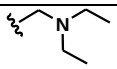
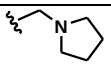
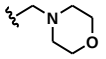
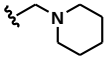
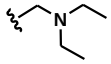
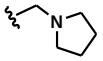
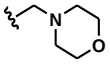
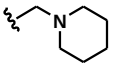
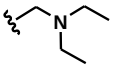
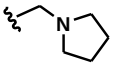
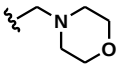
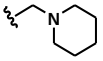
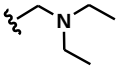
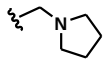
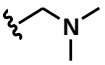
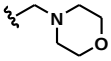
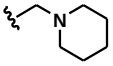
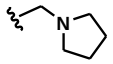
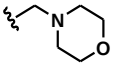
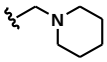
Fig. 2-11. Compound **11** spectrums. (a). ^{1}H NMR spectrum. (b). ^{13}C NMR spectrum.

Following the general synthetic protocol established for the synthesis of **11** above, Diethyl stilbestrol **6** was treated with four other amines dimethyl amine, piperidine, pyrrolidine and morpholine to synthesize four other stilbenoids **12-15** (**Table 2-2**). All of them obtained in excellent yield with preserved *trans*- configuration.



Scheme 2-12. Reagents and Conditions. (a). amine, paraformaldehyde, MeOH, reflux.

Table 2-2. List of synthesized stilbenoids (**11-35**) and stereo selectivity.

Compound	R_1	R_2	*E*/*Z* ratio	Compound	R_1	R_2	*E*/*Z* ratio
11 (G6)	C_2H_5		*E*	**24**	H		*E*
12	C_2H_5		*E*	**25**	H		*E*
13	C_2H_5		*E*	**26**	CH_3		4 : 1
14	C_2H_5		*E*	**27**	CH_3		3.5 : 1.5
15	C_2H_5		*E*	**28**	CH_3		4 : 1
16	CH_3		*E*	**29**	CH_3		4 : 1
17	CH_3		*E*	**30**	CH_3		4.5 : 1.5
18	CH_3		*E*	**31**	H		*E*
19	CH_3		*E*	**32**	H		*E*
20	CH_3		*E*	**33**	H		*E*
21	H		*E*	**34**	H		*E*
22	H		*E*	**35**	H		*E*
23	H		*E*				

In a similar manner, 4,4'-dihydroxy-dimethyl stilbene **9 (***E*/*Z* ratio as 85:15) was treated with five different amines diethyl and dimethyl amine, piperidine, pyrrolidine and morpholine to synthesize stilbenoids **26**-**30**. In this mannich condensation, stilbenoid **26** was obtained with 4:1 *E*/*Z* ratio. Stilbenoid **27**, **28** and **30** were obtained with 7:3, 4:1 and 3:1 *E*/*Z* ratios, respectively. In the case of stilbenoid **29**, *E*/*Z* ratio was 4:1 (**Fig. 2-13)**.

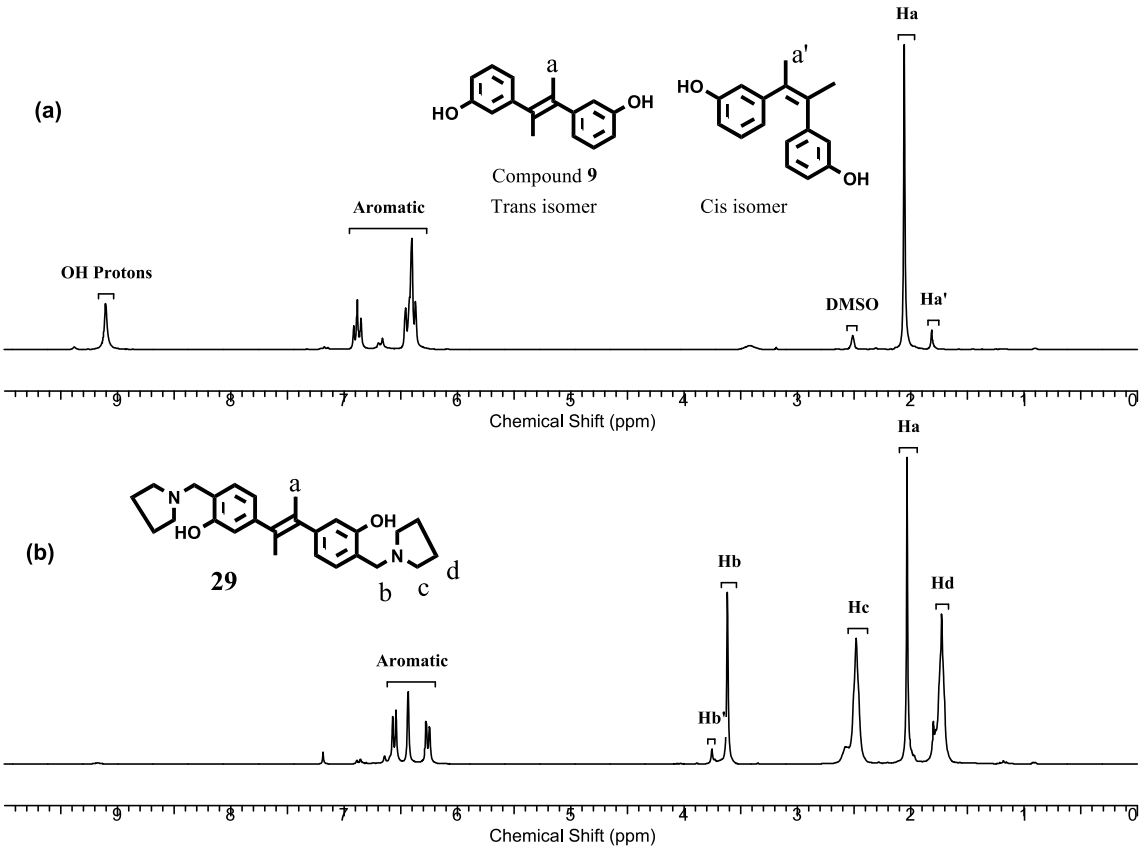


Fig. 2-12. 1H NMR spectrums. a. Compound **9**. b. Signals for stilbenoid **29**.

Stilbenes **7**, **8**, and **10** were also treated with 2.2 mmols of appropriate amines (dimethyl and diethylamine, pyrrolidine, piperidine and morpholine) and paraformaldehyde in methanol at 80^0 C to synthesize the 11 analogues in good to excellent yield (**Scheme 2-12**, **Table 2-2**).

The chemical structures of the stilbenoids obtained were confirmed by ^{1}H- and ^{13}C- NMR analysis. For example, ^{1}H NMR spectra of stilbenoids **17**, **22** and **34** shown below (**Fig. 2-14**) indicates excellent purity and the absence of any side products.

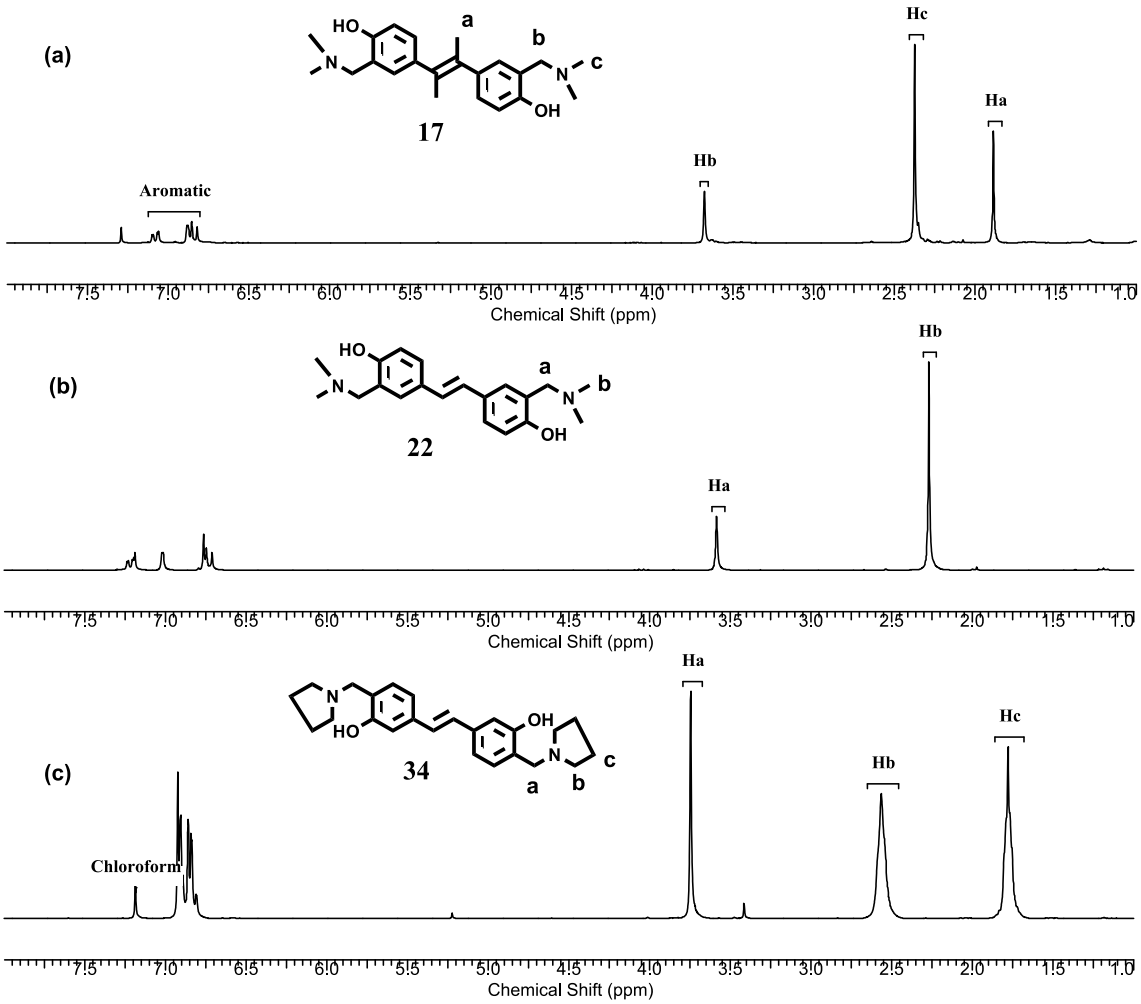


Fig. 2-13. 1H NMR spectrums. (a). Compound **17**. (b). Compound **22**. (c). Compound **34**.

2.7 Synthesis of novel N-substituted stilbenebisoxazine analogues via double condensation

Stilbenes (**8** and **10**) were also converted to N-substituted bisoxazines by Mannich condensation with various primary amines. As an illustrative procedure, stilbene **8** was treated with 2 equivalents of *p*-methoxy benzyl amine and 4.2 equivalents of paraformaldehyde to form bisoxazine ring as shown in scheme **2-13**. This reaction gave 1,3-bisoxazone **39** as the major product. In ^{1}H NMR spectrum (**Fig. 2-15**), the

corresponding signals for methylene protons (Hb, Hc and Hd) were observed at 4.15, 4.0 and 4.9 ppm respectively. Methoxy group protons(Ha in **Fig. 2-15**) showed signal at 3.75 ppm and aromatic proton between 6.75-7.35 ppm range. This product **39** was also obtained as the only isomer as there is no presence of *E*/*Z* mixture or side products from the ^{1}H NMR spectrum (**Fig. 2-15**).

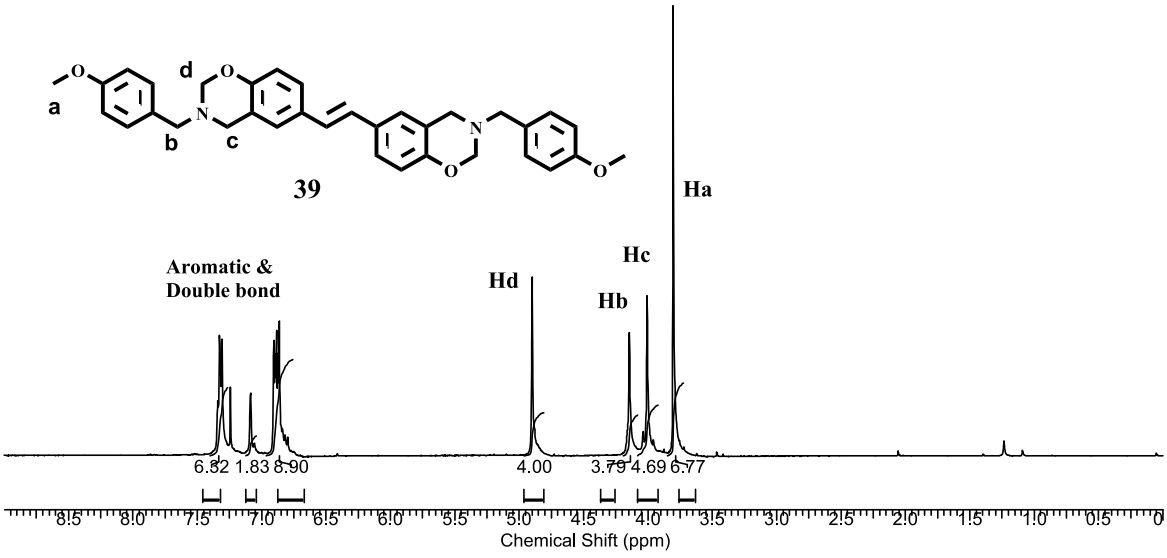
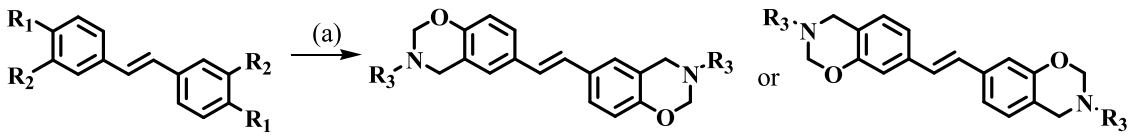


Fig. 2-14. ^{1}H NMR spectrum of Compound **39**.

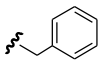
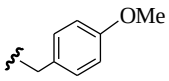
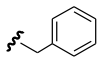
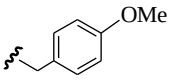
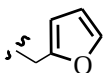
Encouraged by this result, Stilbenes (**8** and **10**) were treated with 2.0 equivalents of various primary amines and 4.2 equivalents of paraformaldehyde in Mannich condensation in MeOH at reflux. Five different amines- cyclohexyl and cyclopentyl amines, benzyl and *p*-methoxy benzyl amines, Furfuryl amine- were used in this mannich condensation to synthesize various N-substituted stilbenebisoxazines (**36-44**) as shown in scheme **2-13** and Table **2-3** in good to excellent yields.



8 or **10** **36-39** **40-44**

Scheme 2-13. Reagents and Conditions. (a). amine, paraformaldehyde, MeOH, reflux.

Table 2-3. List of synthesized stilbenoid rings (**36-44**) and stereo selectivity.

Stilbene	R_1	R_2	Product	R_3
8	OH	H	**36**	
8	OH	H	**37**	
8	OH	H	**38**	
8	OH	H	**39**	
9	H	OH	**40**	
9	H	OH	**41**	
9	H	OH	**42**	
9	H	OH	**43**	
9	H	OH	**44**	

The chemical structures of all the N-substituted stilbenebisoxazines were confirmed by ^{1}H- and ^{13}C- NMR analysis. ^{1}H NMR spectra of N-substituted stilbenebisoxazines **38**, **41** and **43** shown below (**Fig. 2-16**) indicates excellent purity and the absence of any side products.

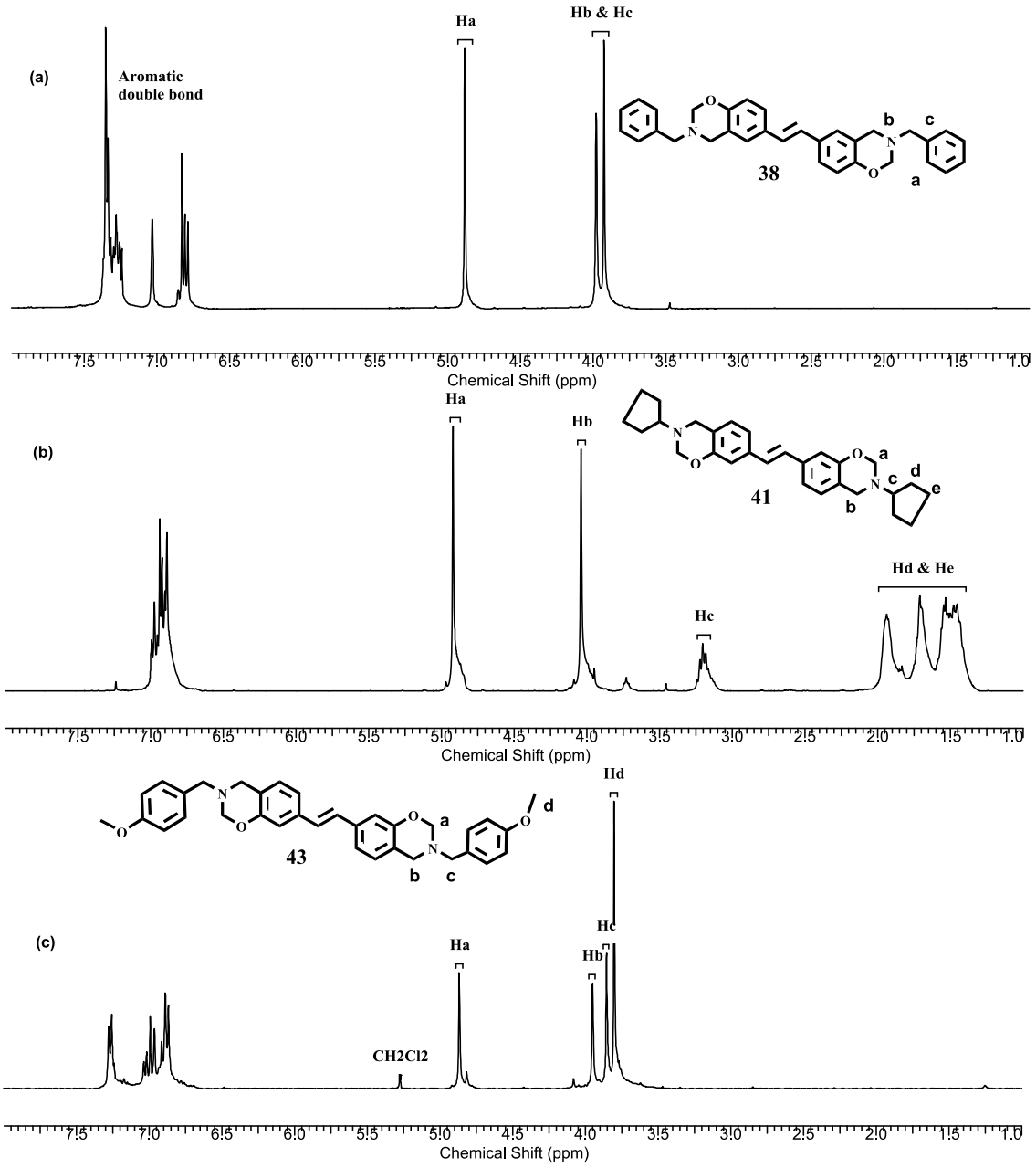


Fig. 2-15. ^{1}H NMR spectrums. (a). N-Benzyl stilbenebisoxazine **38**. (b). N-Cyclopentyl stilbenebisoxazine **41**. (c). N-(*p*-methoxybenzyl) stilbenebisoxazine **43**.

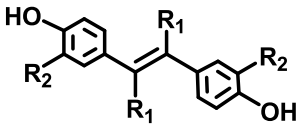
2.8 Evaluation of the novel stilbenes and stilbenoids against JAK2-V617F Mutated JAK2 Enzyme

Stilbenes (**6** to **10**) and stilbenoids (**11-35**) synthesized were evaluated for JAK2 enzyme activity. Proliferation of HEL cells is mediated by the constitutively active Jak2-V617F signaling which promotes a G1/S phase transition, thereby leading to increased cellular proliferation.[10a-e] G6 (Stilbenoid **11**) has been identified as a potent inhibitor of Jak2 enzyme in structure based virtual screening. In an *in vivo* and *ex vivo* study, G6 greatly reduced growth of JAK2-Val617phe mutated human pathological cells. Elaborated details of this work have been discussed at the end of this chapter. We observed that the phenol OH in G6 forms strong hydrogen bonds with the backbone NH group of Leu932 and a salt bridge is formed between N of 3^0 amine in G6 and Asp994 (see **Fig. 2-20** and **Fig. 2-21**). These interactions might be the important to consider during further modification of the G6. To study these interactions and tolerance of various groups on the G6, we here describe structural modification of G6 in an effort to derive a structure activity relationship (**Fig. 2-8**). G6 and its structurally related derivatives were therefore first analyzed for their ability to inhibit the Jak2-V617F dependent proliferation of HEL cells. Viable cell numbers were determined by trypan blue exclusion and hemocytometer after 72 hrs. Each sample was measured in triplicate. Inhibition by G6 (Compound **11**) was arbitrarily set at 100% and the percent inhibition for all the other compounds relative to G6 was defined as 1.00 - (Δ drug / Δ vehicle control). To determine the ability of each of these compounds to inhibit Jak2-V617F mediated HEL cell proliferation, cells were treated with G6 or its derivatives. Viable cell numbers for each treatment were determined.

Anti Jak2 activity of 4,4'-dihydroxy stilbenoids in terms of HEL (Human Erythro Leukemia) cell assay were shown in Table **2-4**. The results of anti Jak2 activity of 3,3'-dihydroxy stilbenoids were shown in Table **2-5** in terms of HEL cell assay. This cell line is homozygous for the V617F mutation which induces constitutive Jak2 phosphorylation and drives HEL cell proliferation. We investigated five different amines, i.e. dimethylamine, diethylamine, piperidine, pyrrolidine and morpholine to probe the importance of the interactions observed in **G6** (**11**) between N atom of amine and **Asp994** in Jak2 catalytic activation loop[77].

In an HEL cell inhibition assay of 4,4'-dihydroxy stilbenoids (Table **2-4**), The favourable configuration around alkene found to be disubstitution than tetrasubstitution. This can be observed in compounds **22** and **24** in comparison with compounds **12-20**. In other words, When R_1 is ethyl and methyl (**12-20**), the enzyme inhibitory potency against JAK2 is low. If alkene has only phenyl groups (**22** and **24**), the potency is similar to **G6 (11)**. Small alkyl groups like methyl and pyrrolidine (**22** and **24**) in amine proved to be better fit in enzyme binding site than larger rings like pyrrolidine and morpholine. It was also found that 4,4'-dihydroxy stilbene (**8**) itself is highly potent inhibiting Jak2 enzyme than **G6 (11).** This is also true with 3,3'-dihydroxy stilbene (**10**). This suggests that hydroxyl groups are efficient enough to make enhanced interactions with JAK2 enzyme binding site to improve potency. Structures of potent active compounds **8**, **10**, **22** and **24** are shown in **Fig. 2-18.**

Table 2-4. Jak2 activity of G6 and its 4,4'-Dihydroxy stilbenoid analogues.



11-25

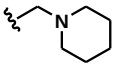
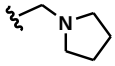
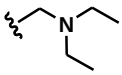
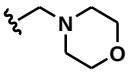
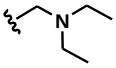
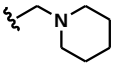
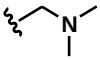
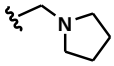
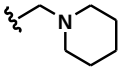
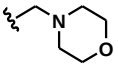
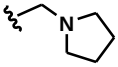
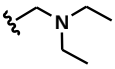
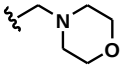
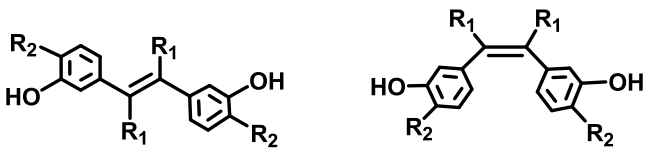
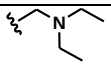
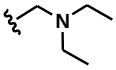
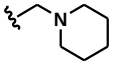
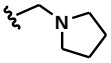
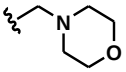
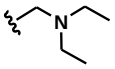
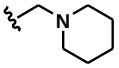
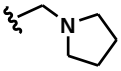
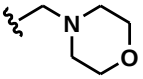
Comp.	R_1	R_2	% HEL Inhibition (10 µM) at 72 hrs	Comp.	R_1	R_2	% HEL Inhibition (10 µM) at 72 hrs
6	C_2H_5	H	65	**17**	CH_3		60
7	CH_3	H	63	**18**	CH_3		72
8	H	H	106	**19**	CH_3		74
11 (G6)	C_2H_5		100	**20**	CH_3		nd
12	C_2H_5		74	**21**	H		85
13	C_2H_5		80	**22**	H		99
14	C_2H_5		89	**23**	H		nd
15	C_2H_5		48	**24**	H		96
16	CH_3		26	**25**	H		nd

Table 2-5. Jak2 activity of G6 and its 3,3'-Dihydroxy stilbenoid analogues .



26-35

Compound	R_1	R_2	*E*/*Z* ratio	% HEL Inhibition (10 µM) at 72 hrs
11 (G6)	C_2H_5		100 : 1	100
9	CH_3	H	85 : 15	35
10	H	H	100 : 1	106
26	CH_3		4 : 1	61
27	CH_3		3.5 : 1.5	55
28	CH_3		4 : 1	84
29	CH_3		4 : 1	68
30	CH_3		4.5 : 1.5	39
31	H		*E*	74
32	H		*E*	36
33	H		*E*	50
34	H		*E*	35
35	H		*E*	25

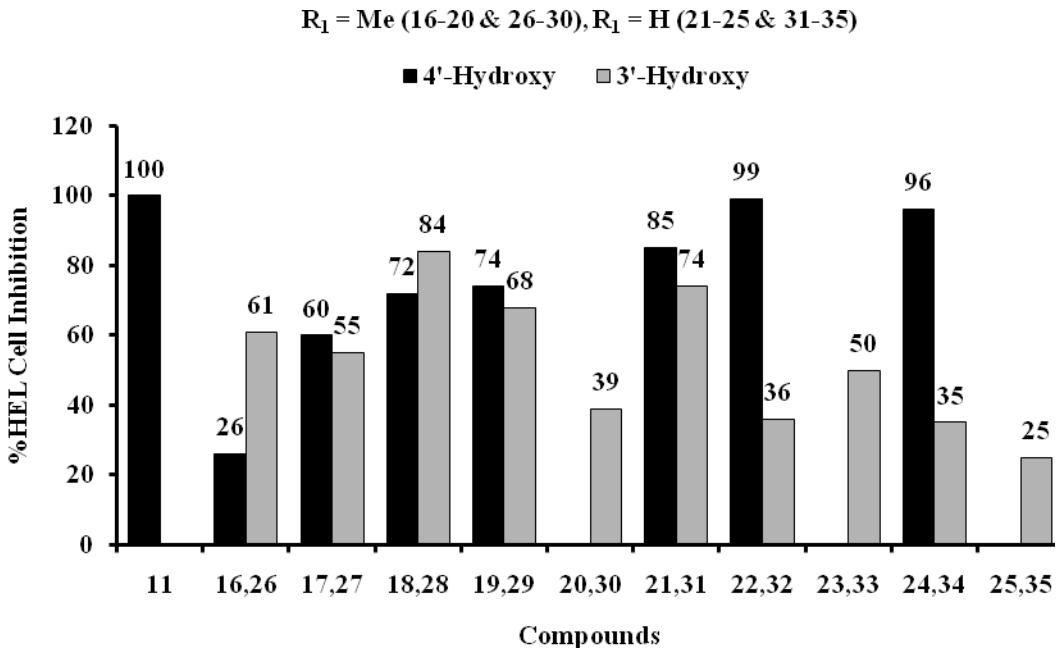


Fig. 2-16. Activity Comparison of 3,3' and 4,4'-Dihydroxy Stilbenoids.

Inhibitory activity comparison of 4,4' and 3,3'-dihydroxy stilbenoids (**Fig. 2-17**) with same alkyl groups on alkene and amines reveals several trends. In 4,4'-dihydroxy stilbenoids, The potency increases sequentially over diethylamine, dimethyl, piperidine, pyrrolidine (**16-19**) with methyl on alkene and with disubstitution on alkenes (**21-24**) over diethylamine, piperidine, pyrrolidine. As previously said, small alkyl groups methyl and pyrrolidine (**22** and **24**) showed increased potency. 3,3'-dihydoxy stilbenoids assay (**Table. 2-5** and **Fig. 2-17**) revealed that while free amine bases are not able to increase the potency but few salts (Table 3) showed very potent activity than **G6.**

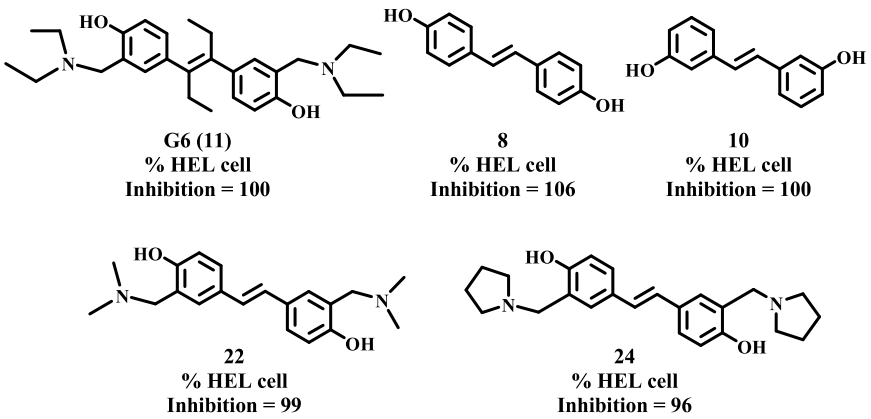


Fig. 2-17. Stilbenoids which shows potent activity against JAK2 enzyme.

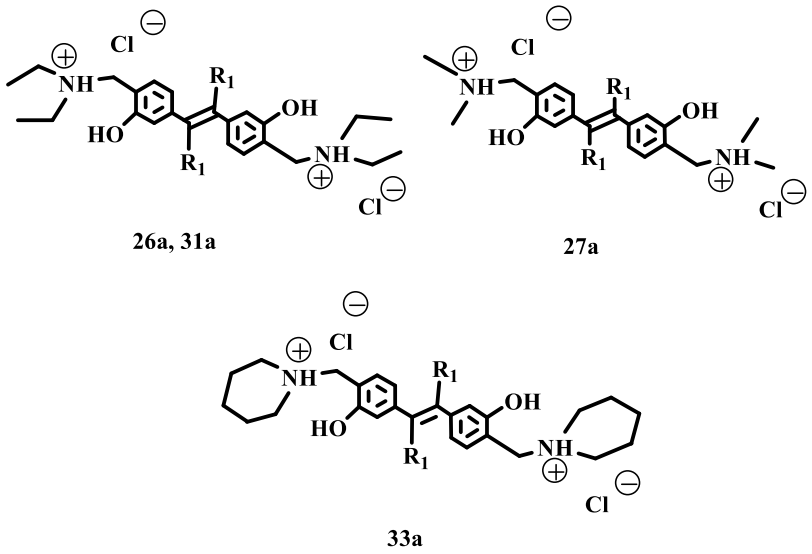


Fig. 2-18. Structures of salts of stilbenoids.

The assay of few salts (**Fig. 2-19**) was done to follow the inhibition activity (**Table 2-6**) dependence on solubility. Even in salts, large alkyl ring on N did not improve the inhibitory activity as evident in **33a** than **G6 (Compound 11)**. But dimethyl and diethyl amine, showed good inhibitory activity and greatly increased the potency than **G6**. This may be due to fact that salts have the advantage of having charges on N already making them more soluble in carrier polar media and making better interactions in the binding pocket.

Table 2-6. Anti Jak2 activity of salts of 3,3'-Dihydroxy stilbenoid analogues .

Comp.	-R_1	% HEL Inhibition(10 µM) at 72 hrs	Comp.	-R_1	% HEL Inhibition(10 µM) at 72 hrs
26a	-Methyl	112	**26**	-Methyl	61
31a	-H	112	**31**	-H	74
27a	-Methyl	114	**27**	-Methyl	55
33a	-H	62	**33**	-H	50

In this Anti Jak2 activity, Salts of stilbenoids **26a**, **31a** and **27a** have shown two times increased potency than their corresponding free amines **26**, **31**, **27 (**see **Fig. 2-19** and **Table 2-6**). This strongly indicates that there is enhanced interaction of small alkyl groups and free hydroxyl groups on stilbenoids with Jak2 enzyme and so better solubility.

2.9 Conclusions

In summary we have developed the methodology for efficient synthesis of stilbenoids based on Mcmurry and Mannich condensation protocol. The assay studies of these compounds has provided that substitution around alkene in the stilbenoid molecule has a critical role in deciding inhibitory activity and revealed several potent inhibitors of Jak2 enzyme. Also by varying the alkyl groups in amine substituent we were able to identify the potent inhibitors (compounds **8, 10**, **22** and **24).** These assay studies also able to demonstrate that not only amine and their salts also active inhibiting Jak2 as observed enhanced potency which is evident in **26a, 31a** and **27a.**

2.9 Inhibition of JAK2-V617F kinase activity by G6 (Stilbenoid 11)

Recently, In collaboration with Dr. Peter, P. Sayeski, we identified G6 (Stilbenoid **11**) as a inhibitor of JAK2 enzyme using structure-based virtual screening.[29] It was shown that G6 has a specific inhibitory effect on Jak2 kinase activity as measured by *in vitro* enzyme assays.[29] In an *in vivo* and *ex vivo* study, G6 greatly reduced growth of JAK2-Val617phe mutated human pathological cells isolated from the bone marrow of a polycythemia vera patient who was Jak2-V617F positive in a dose dependent manner with IC_{50} value of 50 nM (**Fig. 1-20**). We have also reported that the core stilbenoid ring system present in G6 is essential for maintaining its ability to inhibit Jak2 kinase activity. From analysis of the G6 binding mode in Jak2 active site[77], The human erythroleukemia (HEL 92.1.7) cell line is homozygous for the Jak2-V617F mutation and this gain-of-function mutation is responsible for its transformed phenotype.[10d]

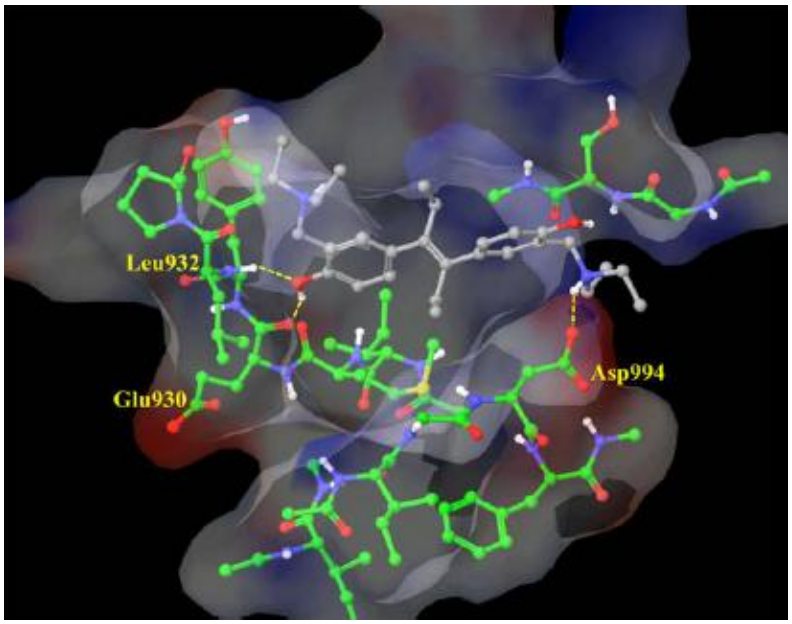


Fig. 2-19. Binding mode of **G6**(grey) at the Jak2(green) vanderwall surface.[77]

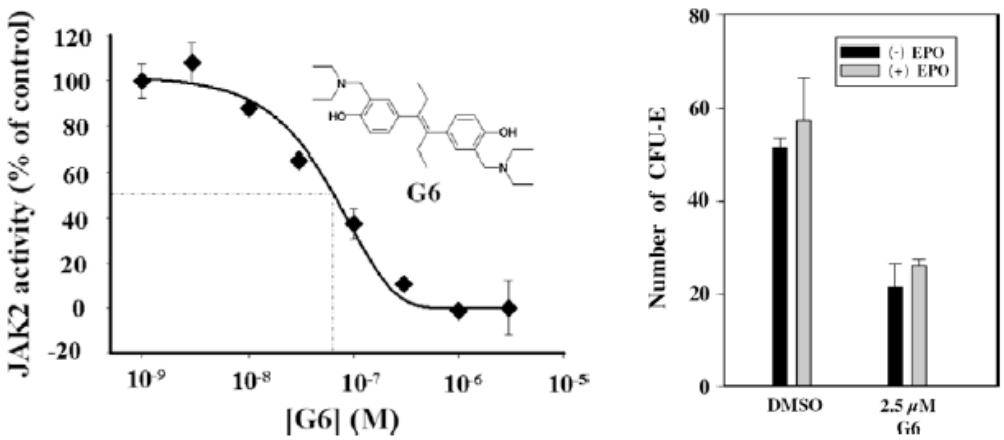


Fig. 2-20. Anti Jak2 enzyme activity of G6 (stilbenoid 11)[77]. **Left.** Inhibition of JAK2 Val617Phe kinase activity by G6 (IC50 = 60 ± 4.5 nM). **Right.** Growth inhibition of G6 on a human pathological cells isolated from polycythemia vera patient .

2.10 Antimicrobial activity of stilbenoids

Previous studies revealed that hydroxy stilbenes and analogues of Resveratrol possess antimicrobial activities. The presence of phenolic hydroxyl/methoxyl groups in these stilbenes, which have high affinity for proteins, generally attributed to scavenge the reactive oxygen species(ROS) and partly explain their associated biological roles.[36] Studies also shown that hydroxystilbenes are capable of DNA binding and cleavage can also occurs in combination with metals.[37] Thus pronounced biological role of stilbenes encouraged us to screen several stilbenoids synthesized in our lab. Each of the stilbenoid synthesized, as illustrated in Scheme **2-12** and Table **2-2**, and N-substituted stilbenebisoxazine synthesized, as illustrated in Scheme **2-13** and Table **2-3**, was assayed for the ability to inhibit the growth of select strain of clinically significant bacteria.

The invitro MIC determination for each compound were performed against a multi drug resistant hospital acquired methicillin resistant S. aureus (MRSA) and methicillin resistant S. epidermidis (MRSE). From the results of these MIC (minimum inhibitory concentration) assays, most active compounds are presented in Table **2-7**. To explore the broad spectrum activity of these compounds (**Fig. 2-22**), we also employed the modified Kirby-Bauer assay using two gram-positive pathogens, *E. Faecalis* and *B. Anthracis,* Gram-negative pathogen, *E. Coli*, and the eukaryote *Saccharomyces cerevisiae* (yeast) (see Table **2-7**).

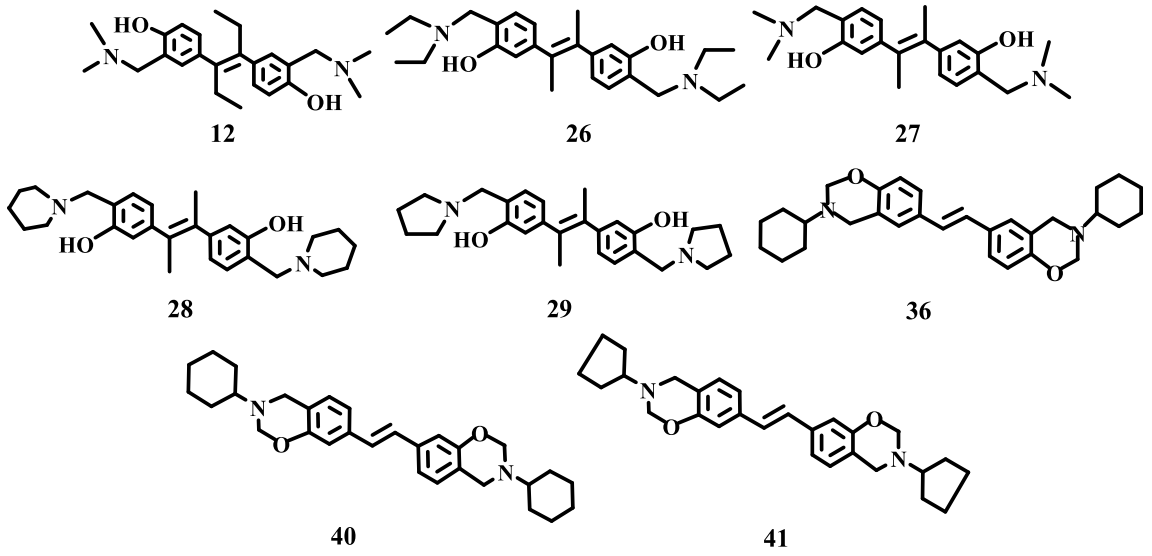


Fig. 2-21. Structures of active stilbenoids and N-Substituted stilbenebisoxazines.

From the analysis of data, Most observable feature is that not only stilbenes with free hydroxyl groups are able to show potent antibacterial activity but also stilbenes with bisoxazine rings on phenyl groups were able to show moderate antimicrobial activity. This fact is quite interesting than previously predicted observation that free hydroxyl moiety in stilbenes is vital to show enhanced activity. Alkene with methyl group and dimethyl groups on tertiary amine in stilbenoid (Compound **27**) was able to show

enhanced activity in MRSA (MIC 21.25 μg/mL) and MRSE (MIC 15 μg/mL) studies than other stilbenoids with larger rings like piperidine, pyrrolidine, cyclohexyl and cyclopentyl groups. This compound also showed potent activity against both Gram-positive bacteria and Gram-negative bacteria than other compounds. Another exciting feature in this data is that all of the N-substituted stilbenebisoxazine analogues(**36**, **40**-**41**) were equally potent in MRSE assays although stilbenebisoxazine (**40**) with cyclohexyl ring shown greater potency (MIC 25 μg/mL) than other stilbenebisoxazine with rigid cyclopentyl group(Compound **41**) in MRSA studies. In conclusion from these data, these novel scaffolds certainly serve as a starting points for the development of new drugs that are certainly needed to combat MRSA and anthrax infections and may promote additional SAR studies of stilbenoid compounds for therapeutic use.

Table 2-7. Antimicrobial assay of stilbenoids. Minimum inhibitory concentration values (μg/mL) against MRSA and MRSE bacteria and Zone of inhibition values (millimeters) against various bugs. [c]

Compound No.	MIC (μG/mL)		Zone of Inhibition (mm)			
	MRSA[a]	MRSE[b]	B. Anthracis	E. Faecalis	E.Coli	Yeast
12	62.5	33.33333	ND[d]	ND[d]	ND[d]	ND[d]
26	25	25	23.33	27.33	0.00	24.00
27	21.25	15	30.67	36.67	33.33	27.33
28	33.75	25	16.00	27.33	0.00	23.33
29	25	25	24.00	32.00	27.33	31.33
40	25	25	14.67	27.33	30.67	24.00
41	50	25	23.33	26.00	25.33	0.00
36	50	25	0.00	26.67	0.00	0.00

[a] Methicillin-resistant Staphylococcus aureus. [b] Methicillin-resistant Staphylococcus epidermidis.
[c] Positive control is DMSO (dimethyl sulfoxide). [d] ND = not determined.

2.11 Experimental Procedures

All solvents were dried and distilled prior to use and organic solvent extracts dried over Na_2SO_4. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker 250 MHz spectrometer in CDCl3 and DMSO-d6 with TMS as the standard. Chemical shifts are reported in parts per million, multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and bs (broad singlet). Thin-layer chromatography (TLC) was performed on glass plates coated with 0.25 mm thickness of silica gel.

2.12.1 General Procedure for the synthesis of Stilbenes:

In a flame dried 2 neck round bottom flask fitted with magnetic stirrer bar and reflux condensor, dry THF (180 mL or 1.5 Molar) and Zinc (8 equivalents) was taken under N_2 atmosphere. Then reaction mixture was cooled to 0^Oc in an ice bath. To this mixture $TiCl_4$ (4 equivalents) was added dropwise while maintaining the reaction temperature at 0^OC. After addition was complete, reaction mixture was refluxed for 2 hours. Then this brown color reaction mixture was cooled again to 0^Oc. Aldehyde or Ketone(1 equivalent) was taken in 20 mL of dry THF was added to this ice cooled reaction mixture slowly portion wise. Then this reaction mixture was allowed to reflux until TLC (2:3 mixture of ethylacete/hexane) shows that all the aldehyde or ketone is consumed in the reaction. After reaction is complete, reaction mixture was concentrated and ethylacetate was added. To this mixture saturated K_2CO_3 solution was added and allowed to stir for another 7 hours. Reaction mixture was filtered and residue was washed with ethylacetate. Filtrate was extracted with ethylacetate. Organic layer was washed with saturated NaCl solution, water, dried over anhydrous Na_2SO_4 and concentrated. This

crude mixture was chromatographed on silica column eluting with 1:9 mixture of ethylacetate:hexane mixture to receive *E* and *Z* stilbenes as a mixture.

Representative procedure for *E*-4,4'-[1,2-diethyl-1,2-ethenediyl]bis Phenol (6):[79a]

To a solution of Zinc (20.9 g, 0.319 mol) in dry THF (150 mL) was added $TiCl_4$ (22.73 g, 0.119 mol) dropwise at 0^0C under N_2 atmosphere. After addition was complete, reaction mixture was refluxed for 2 hours. Then this brown color reaction mixture was cooled again to 0^Oc. A solution of 4'-hydroxy propiophenone (6 g, 0.039 mol) in dry THF (50 mL) was added to this ice cooled reaction mixture slowly portion wise. Then this reaction mixture was allowed to reflux for 6 hrs. Then the reaction mixture was concentrated and ethylacetate was added. To this mixture sat. K_2CO_3 solution was added and allowed to stir for another 7 hours. Reaction mixture was filtered and residue was washed with ethylacetate. Filtrate was extracted with ethylacetate. Organic layer was washed with saturated NaCl solution, water, dried over anhydrous Na_2SO_4 and concentrated. This crude mixture was chromatographed on silica column eluting with 1:9 mixture of ethylacetate:hexane mixture to receive cis/trans mixture. This cis/trans mixture received in column has been recrystallized from 15% ethylacetate/hexane mixture to receive pure ***E*** isomer with >99% purity, which is also confirmed from literature values, with 73% yield. ^{1}H NMR (d_6-DMSO, MHz): δ 9.1 (bs, 2H), 6.7-6.6 (d, 4H, *j*=8.5 Hz), 6.5-6.4 (d, 4H, *j*=8.5 Hz), 1.8 (q, 4H, *j*=7.5 Hz), 0.5 (t, 6H, *j*=7.5 Hz). ; ^{13}C NMR (62.5 MHz, d_6-DMSO): δ 155.7, 137.9, 132.5, 129.3, 114.8, 28.0, 13.3.

***E/Z*-4,4'-[1,2-dimethyl-1,2-ethenediyl]bis Phenol (7):**

Cis/trans mixture received in column has been recrystallized from (9:1) methanol: water to receive *E*/*Z* mixture as 99 : 1 ratio with 65% yield. ^{1}H NMR (d_6-DMSO): 7.0 (dd, 4H, *J* = 2, 1.75 Hz), 6.7 (dd, 4H, *J* = 2, 1.75 Hz), 1.8 (s, 6H); ^{13}C NMR (62.5 MHz, d_6-DMSO): 155.6, 134.4, 131.3, 129.1, 128.1, 114.8, 22.4.

***E*- 4,4'-(1,2-ethenediyl)bis Phenol (8):[76]**

Crude product has been recrystallized from methanol to receive pure isomer with 71 % yield. ^{1}H NMR (250 MHz, MeOD): 7.3 (d, 4H, *J* = 8 Hz), 6.9 (s, 2H), 6.7 (d, 4H, *J* = 8 Hz); ^{13}C NMR (62.5 MHz, d_6-DMSO): 156.7, 128.6, 127.2, 125.2, 115.4.

***E/Z*-3,3'-[1,2-dimethyl-1,2-ethenediyl]bis Phenol (9):**

Crude product has been recrystallized from methanol to receive *E/Z* mixture as 85 : 15 ratio with 76 % yield . ^{1}H NMR (250 MHz, d_6-DMSO): 9.1 (bs, 2H), 7.1 (m, *J* = 7.75 Hz), 6.88 (t, 2H, *J* = 7.75 Hz), 6.6 (m), 6.4 (m, 6H), 2.0 (s, 6H), 1.8 (s); ^{13}C NMR (62.5MHz, d_6-DMSO): 157.1, 156.5, 145.6, 131.9, 129.1, 128.4, 119.6, 115.5, 112.6, 22.0, 21.4.

***E*- 3,3'-[1,2-ethenediyl]bis Phenol (10):[76]**

Crude product recrystallized from methanol to receive *E* isomer with 84% yield. ^{1}H NMR (250 MHz, MeOD): 7.1 (t, 2H *J* = 8 Hz), 7.0 (s, 2H), 6.9 (ddd, 2H, *J* = 8.5, 2.5, 1.5 Hz), 6.9 (dd, 2H, *J* = 2.75, 1.75 Hz), 6.6 (ddd, 2H, *J* = 8, 2, 1 Hz).

2.12.2 General Procedure for the synthesis of Stilbenoids:

Stilbene(1 equivalent) was taken in one neck round bottom flask and 15 mL of methanol was added. Paraformaldehyde(2.1 equivalents) and appropriate amine(2.2 equivalents) was added. Then this reaction mixture was allowed to reflux until TLC (2:3 mixture of ethylacetate:hexane) shows that reaction is complete. Then mixture is cooled to room temperature and concentrated. To this mixture ethylacetate and 1M HCl solution is added. Aqueous phase is separated and treated with 1M NaOH solution until pH is 7. Then this aqueous solution is extracted with ethylacetate. Organic layer is washed with saturated NaCl solution, water and dried over anhydrous Na_2SO_4. This organic layer is concentrated and dried in vacuuo. This mixture containing mannich products has been purified by flash chromatography over silicagel characterized by eluting with dichloromethane : methanol : triethylamine mixture(99:0.5:0.5) to receive pure product yields ranging from 80 to 95 %.

Representative procedure:

***E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (Pure isomer) (11) :**

To a solution of **6**(0.388g, 1.447 mmol) in MeOH was added paraformaldehyde(0.095 g, 3.183 mmol) and diethyl amine(0.232g, 3.183 mmol). The reaction mixture was refluxed for 8 hrs. Then reaction mixture was cooled to room temperature and concentrated under reduced pressure. To this mixture ethylacetate and 1M HCl solution is added. Aqueous phase is separated and treated with 1M NaOH solution until pH is 7. Then this aqueous solution is extracted with ethylacetate. Organic layer is washed with saturated NaCl solution, water and dried over anhydrous Na_2SO_4.

This organic layer is concentrated and dried in vacuuo. Crude reaction mixture chromatographed over silica gel by eluting with dichloromethane : methanol : triehtylamine mixture(99:0.5:0.5) to receive pure product **11** as white color solid with 92 % yield. ^{1}H NMR ($CDCl_3$): δ 6.9 (d, 2H, J = 1.5 Hz), 6.8 (d, 4H, J = 8 Hz), 3.79 (S, 4H), 2.6-2.6 (q, 8H, J = 7.25 Hz), 2.1-2.08 (q, 4H, J = 7.5 Hz), 1.1-1.0 (t, 12H, J = 7.25 Hz), 0.8-0.7 (t, 6H, J = 7.5 Hz) ; ^{13}C NMR (CDCl3): δ 156.4, 138.7, 133.3, 129.8, 128.7, 128.5, 121.5, 115.4, 57.1, 54.0, 46.5, 46.3, 28.6, 13.5, 11.4, 11.3.

***E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (Pure isomer) (12) :**

^{1}H NMR ($CDCl_3$): δ 6.7 (d, 2H, J = 8.25 Hz), 6.7 (d, 4H, J = 3Hz), 3.5 (s, 4H), 2.2 (s, 12H), 2.0 (q, 4H, J = 7.25 Hz), 0.6 (t, 6H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 156.2, 138.7, 133.4, 129.0, 128.4, 121.4, 115.4, 63.0, 44.5, 28.5, 13.4;

***E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol (Pure isomer) (13) :**

^{1}H NMR ($CDCl_3$): δ 6.9 (d, 1H, J = 2 Hz), 6.8 (d, 1H, J = 2 Hz), 6.7 (d, 2H, J = 2.5 Hz), 6.7 (d, 2H, J = 3.5 Hz), 3.7 (s, 4H), 2.5 (bs, 8H), 2.0 (q, 4H, J = 7.5 Hz), 1.7 (m, 8H, J = 3.25 Hz), 0.6 (t, 6H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 156.2, 138.7, 133.3, 128.8, 128.0, 121.9, 115.2, 59.0, 53.5, 28.5, 23.6, 13.5.

***E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol (Pure isomer) (14) :**

^{1}H NMR ($CDCl_3$): δ 6.90 (d, 1H, J = 2 Hz), 6.8 (d, 1H, J = 2 Hz), 6.7 (d, 2H, J = 2 Hz), 6.7 (d, 2H, J = 2 Hz), 3.5 (s, 4H), 2.4 (bs, 8H), 2.03 (q, 4H, J = 7.5 Hz), 1.5 (m,

8H, *J* = 5 Hz), 1.4 (bs, 4H), 0.6 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 156.3, 138.7, 133.4, 128.8, 128.6, 121.0, 115.3, 62.3, 54.1, 53.9, 28.5, 25.9, 24.0, 13.5.

***E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol (Pure isomer) (15) :**

^{1}H NMR ($CDCl_3$): δ 6.9 (d, 1H, *J* = 2 Hz), 6.9 (d, 1H, *J* = 2 Hz), 6.7 (m, 4H), 3.7 (t, 8H, *J* = 4.5 Hz), 3.6 (s, 4H), 2.5 (bs, 8H), 2.0 (q, 4H, *J* = 7.5 Hz), 0.6 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 155.7, 138.7, 133.7, 129.3, 128.9, 120.1, 115.5, 66.8, 62.0, 52.9, 28.5, 13.4.

4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (16) :

(*E*/*Z*: 5:1) ^{1}H NMR ($CDCl_3$): δ 6.9-6.9 (m, 3H), 6.7-6.6 (m, 6H), 6.5-6.3(m, 1H), 3.7 (s, 4H), 3.4 (s), 2.6-2.5 (q, 8H, *J* = 7.5 Hz), 2.3 (q, 2H, *J* = 7.5 Hz), 2.0 (s), 1.7 (s, 6H) 1.0 (t, 6H, *J* = 7.5 Hz), 1.0-0.9 (m) ; ^{13}C NMR ($CDCl_3$): δ 156.4, 156.1, 155.8, 154.8, 135.6,135.2, 132.2, 131.6, 131.5, 129.9, 129.6, 121.5, 121.1, 115.0, 114.4, 57.1, 56.7, 46.3, 46.1, 22.7, 21.4, 11.3, 11.0 .

4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (17) :

(*E*/*Z* 100 : 1) ^{1}H NMR ($CDCl_3$): δ 7.1 (dd, 2H, *J* = 2 Hz), 6.8 (d, 2H, *J* = 2 Hz), 6.8 (s, 1H), 6.8 (s, 1H), 3.6 (s, 4H), 2.3 (s, 12H), 1.8 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 156.2, 135.3, 132.1, 128.6, 128.1, 121.4, 115.5, 63.0, 44.5, 22.6.

4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol (18) :

(*E*/*Z* 100 : 1) ^{1}H NMR ($CDCl_3$): δ 6.9 (dd, 2H, *J* = 2 Hz), 6.7 (d, 2H, *J* = 2 Hz), 6.7 (s, 1H), 6.7 (s, 1H), 3.7 (s, 4H), 2.5 (bs, 8H), 1.8-1.7 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 156.2, 135.2, 132.1, 128.4, 127.7, 121.9, 115.3, 58.9, 53.5, 23.6, 22.6.

4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(Piperidine)methyl]-Phenol (19) :

(*E*/*Z* 100 : 1) ^{1}H NMR ($CDCl_3$): δ 6.9 (d, 1H, *J* = 2 Hz), 6.9 (d, 1H, *J* = 2 Hz), 6.8 (d, 2H, *J* = 2 Hz), 6.7 (s, 1H), 6.6 (s, 1H), 3.6 (s, 4H), 2.4 (bs, 8H), 1.7 (s, 6H), 1.5 (m, 8H, *J* = 5.25 Hz), 1.4 (bs, 4H); ^{13}C NMR ($CDCl_3$): δ 156.3, 135.3, 132.1, 128.4, 128.3, 121.1, 115.4, 62.3, 53.9, 25.9, 24.0, 22.7.

***E*-4,4'-(1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (21) :**

^{1}H NMR ($CDCl_3$): δ 7.3 (d, 1H, *J* = 2 Hz), 7.2 (d, 1H, *J* = 2 Hz), 7.1 (s, 2H), 6.8 (s, 2H), 6.8 (d, 2H, *J* = 8.25 Hz), 3.8 (s, 4H), 2.6 (q, 8H, *J* = 7 Hz), 1.1 (t, 12H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 157.8, 128.9, 126.5, 126.1, 125.6, 122.2, 116.3, 57.0, 46.3, 11.2.

***E*-4,4'-(1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (22) :**

^{1}H NMR ($CDCl_3$): δ 7.2 (d, 1H, *J* = 2 Hz), 7.1 (d, 1H, *J* = 2 Hz), 7.0 (s, 2H), 6.7 (s, 2H), 6.7 (d, 2H, *J* = 8.25 Hz), 3.5 (s, 4H), 2.2 (s, 12H); ^{13}C NMR ($CDCl_3$): δ 157.6, 128.9, 126.7, 126.1, 125.6, 122.0, 116.3, 62.9, 44.5.

***E*-4,4'-(1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol (23) :**

^{1}H NMR ($CDCl_3$): δ 7.2 (d, 1H, *J* = 2 Hz), 7.1 (d, 1H, *J* = 2 Hz), 7.0 (s, 2H), 6.7 (s, 2H), 6.7 (d, 2H, *J* = 8.25 Hz), 3.7 (s, 4H), 2.5 (bs, 8H), 1.7 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 157.5, 128.9, 126.5, 125.6, 122.5, 116.1, 58.9, 53.5, 23.6.

***E*-4,4'-(1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol (24) :**

^{1}H NMR ($CDCl_3$): δ 7.2 (d, 1H, *J* = 2 Hz), 7.0 (s, 2H), 6.7 (s, 2H), 6.7 (d, 2H, *J* = 8.25 Hz), 3.6 (s, 4H), 2.4 (bs, 8H), 1.5 (m, 8H, *J* = 5 Hz), 1.4 (bs, 4H); ^{13}C NMR ($CDCl_3$): δ 157.6, 128.9, 126.5, 126.2, 125.6, 121.7, 116.2, 62.2, 53.9, 25.8, 24.0.

***E*-4,4'-(1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol (25) :**

^{1}H NMR ($CDCl_3$): δ 7.2 (d, 1H, J = 2 Hz), 7.2 (d, 1H, J = 2 Hz), 7.0 (s, 2H), 6.7 (s, 2H), 6.7 (d, 2H, J = 8.25 Hz), 3.6 (t, 8H, J = 4.5 Hz), 3.6 (s, 4H), 2.5 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 157.0, 129.2, 126.9, 126.6, 125.7, 120.8, 116.4, 66.8, 61.9, 52.9.

3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (26) :

(*E/Z* : 4:1) ^{1}H NMR ($CDCl_3$): δ 6.6-6.5 (m, 2H), 6.4 (s, 2H), 6.2 (dd, 2H, J = 2 Hz), 3.5 & 3.3 (2s, 4H), 2.4 (q, 8H, J = 7.5 Hz), 2.0 & 1.8 (2s, 6H), 1.0 (t, 12H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$): δ 157.3, 145.3, 132.3, 127.2, 124.2, 120.2, 119.2, 116.5, 56.7, 48.3, 46.2, 23.2, 21.4, 11.2 .

3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (27) :

(*E/Z* : 7:3) ^{1}H NMR ($CDCl_3$): δ 6.8 (s, 1H,), 6.7 (s, 1H), 6.6 (s, 2H), 6.6-6.5 (2s, 2H), 3.5 (s, 4H), 3.4 (s, 2H), 2.2 & 2.1 (2s, 12H), 2.0 & 1.7 (2s, 6H) ; ^{13}C NMR ($CDCl_3$): δ 157.7, 145.3, 132.5, 127.9, 119.8, 119.7, 118.9, 116.2, 115.8, 62.7, 44.5, 44.4, 22.4, 21.3.

3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol (28) :

(*E/Z :* 4:1) ^{1}H NMR ($CDCl_3$): δ 6.6 (S, 1H), 6.5 (s, 1H), 6.4 (s, 1H), 6.3 (s, 1H), 6.2 (d, 1H, J = 2 Hz), 6.2 (d, 1H, J = 2 Hz), 3.8 & 3.6 (2s, 4H), 2.4 (bs, 8H), 2.0 & 1.8 (2s, 6H), 1.7 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 157.7, 157.1, 145.3, 132.3, 127.4, 126.8, 120.1, 119.6, 118.8, 116.4, 115.8, 58.5, 53.5, 53.4, 23.6, 22.4, 21.4.

3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol (29) :

(*E/Z :* 4:1) ^{1}H NMR ($CDCl_3$): δ 6.5 (s, 1H), 6.4 (s, 1H), 6.3 (s, 1H), 6.3 (s, 1H), 6.2 (d, 1H, J = 2 Hz), 6.2 (d, 1H, J = 2 Hz), 3.6 & 3.4 (2s, 4H), 2.4 (bs, 8H), 2.0 & 1.7 (2s, 6H), 1.5 (m, 8H, J = 5 Hz), 1.4 (bs, 4H); ^{13}C NMR ($CDCl_3$): δ 157.7, 157.1, 145.3,

145.1, 132.5, 132.3, 128.0, 127.3, 120.2, 119.4, 118.9, 118.7, 116.4, 115.8, 61.9, 61.7, 53.9, 53.8, 25.9, 24.0, 22.4, 21.4.

3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol (30) :

(*E/Z :* 4.5:1.5) ^{1}H NMR ($CDCl_3$): δ 6.6 (s, 1H), 6.5 (s, 1H), 6.4 (s, 1H), 6.3 (s, 1H), 6.2 (d, 1H, J = 2 Hz), 6.1 (d, 1H, J = 2 Hz), 3.6 (t, 8H, J = 4.75 Hz), 3.5 (s, 4H), 2.4 & 2.3 (bs, 8H), 2.0 & 1.7 (2s, 6H); ^{13}C NMR ($CDCl_3$): δ 157.2, 156.6, 145.7, 145.3, 132.3, 128.5, 127.8, 120.5, 119.3, 118.5, 117.8, 116.6, 115.9, 66.8, 61.7, 61.6, 53.2, 52.8, 22.4, 21.4.

***E*-3,3'-(1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (31) :**

^{1}H NMR ($CDCl_3$): δ 6.9 (m, 6H), 6.8 (d, 2H, J = 2.75 Hz), 3.7 (s, 4H), 2.5 (q, 8H, J = 7.25 Hz), 1.0 (t, 12H, J = 7.25 Hz) ; ^{13}C NMR ($CDCl_3$): δ 158.4, 138.0, 128.6, 128.3, 121.6, 117.6, 113.7, 56.6, 46.3, 11.1.

***E*-3,3'-(1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (32) :**

^{1}H NMR ($CDCl_3$): δ 7.0-6.9 (m, 8H), 3.6 (s, 4H), 2.2 (s, 12H); ^{13}C NMR ($CDCl_3$): δ 158.2, 138.1, 128.5, 128.3, 121.5, 117.7, 113.5, 62.6, 44.5.

***E*-3,3'-(1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol (33) :**

^{1}H NMR ($CDCl_3$): δ 6.9-6.9 (m, 4H), 6.8-6.8 (m, 4H), 3.7 (s, 4H), 2.5 (bs, 8H), 1.7 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 158.2, 138.0, 128.3, 128.0, 122.1, 117.5, 113.4, 58.6, 53.5, 23.6.

***E*-3,3'-(1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol (34) :**

^{1}H NMR ($CDCl_3$): δ 7.0-6.8 (m, 8H), 3.7 (s, 4H), 2.5 (bs, 8H), 1.3-1.2 (m, 12H); ^{13}C NMR ($CDCl_3$): δ 158.2, 157.9, 157.8, 138.3, 138.0, 129.8, 128.6, 128.3, 128.2, 128.1, 121.2, 119.6, 117.6, 117.3, 166.3, 114.3, 113.5, 61.9, 54.4, 53.9, 25.8, 24.0.

***E*-3,3'-(1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol (35) :**

^{1}H NMR ($CDCl_3$): δ 7.2-6.9 (m, 8H), 3.7 (s, 8H), 3.6 (s, 4H), 2.6 (bs, 8H); ^{13}C NMR ($CDCl_3$): δ 157.6, 138.3, 129.0, 128.4, 120.2, 118.0, 113.6, 66.8, 61.6, 52.9.

2.12.3 General procedure for the synthesis of stilbenebisoxazine analogues:

Stilbene (1 equivalent) was taken in one neck round bottom flask and 15 mL of methanol was added. Paraformaldehyde(4.2 equivalents) and appropriate amine(2 equivalents) was added. Then this reaction mixture was allowed to reflux until TLC (2:3 mixture of ethylacetate:hexane) shows that reaction is complete. Then mixture is cooled to room temperature. Then the precipitation observed was collected by filtration and the residue was washed with cold. Methanol several times. This solid received, pure product in all cases, was dried in vacuuo and characterized by ^{1}H and ^{13}C NMR.

Representative procedure :

***E*-6,6'-(vinylene)bis[3-cyclohexyl-3,4-dihydro- 2H-1,3-Benzoxazine] (36) :**

To a solution of 4,4'-dihydroxystilbene **8** (0.2 g, 0.952 mmol) in MeOH (15 mL) was added paraformaldehyde (0.12 g, 4.0 mmol) and cyclohexylamine (0.22 mL, 1.904 mmol). The reaction mixture was refluxed for 8 hrs. Then reaction mixture was cooled to room temperature. The resulting white color precipitate was collected by filtration and washed with cold MeOH (5 mL). This precipitate was pure enough in NMR spectra and obtained yield was 93% as white color precipitate. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.1 - 7.2 (m, 2H), 7.0 - 7.1 (m, 2H), 6.7 - 6.8 (m, 3H), 6.6 - 6.7 (m, 2H), 4.9 - 5.0 (m, 4H), 4.0 - 4.1 (m, 4H), 2.6 - 2.7 (m, 2H), 1.8 - 2.0 (m, 5H), 1.6 - 1.8 (m, 7H), 1.5 - 1.6 (m, 3H), 0.9 -

1.3 (m, 12H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 154.8, 130.3, 126.4, 125.7, 124.9, 122.0, 116.9, 80.7, 59.0, 47.6, 31.8, 26.3, 25.7.

***E*-6,6'-(vinylene)bis[3-cyclopentyl-3,4-dihydro- 2H-1,3-Benzoxazine] (37):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.1 - 7.3 (m, 2H), 6.9 - 7.1 (m, 2H), 6.6 - 6.9 (m, 4H), 4.9 (br. s., 4H), 4.0 (br. s., 4H), 3.1 - 3.2 (m, 2H), 1.8 - 2.0 (m, 4H), 1.6 - 1.8 (m, 5H), 1.3 - 1.6 (m, 10H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 154.2, 130.2, 126.1, 125.6, 125.1, 120.5, 116.5, 81.7, 59.5, 49.5, 31.4, 23.8.

***E*-6,6'-(vinylene)bis[3-benzyl-3,4-dihydro- 2H-1,3-Benzoxazine] (38):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2 - 7.4 (m, 13H), 7.0 (m, 2H), 6.7 - 6.8 (m, 4H), 4.8 - 4.9 (m, 4H), 3.8 - 4.0 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.5, 130.5, 129.0, 128.5, 127.5, 126.2, 125.8, 125.4, 116.7, 82.2, 55.5, 49.6.

***E*-6,6'-(vinylene)bis[3-(4-methoxy benzyl)-3,4-dihydro- 2H-1,3-Benzoxazine] (39):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2 - 7.3 (m, 6H), 7.0 - 7.1 (m, 2H), 6.8 - 6.9 (m, 12H), 4.8 - 4.9 (m, 4H), 4.1 - 4.1 (m, 4H), 3.9 - 4.0 (m, 4H), 3.7 - 3.8 (m, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.0, 153.7, 130.2, 127.6, 126.2, 125.7, 125.4, 120.0, 116.1, 113.8, 82.2, 55.3, 54.9, 49.5.

***E*-7,7'-(vinylene)bis[3-cyclohexyl-3,4-dihydro- 2H-1,3-Benzoxazine] (40):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.8 - 7.0 (m, 10H), 4.9 - 5.0 (m, 4H), 4.0 (s, 4H), 2.6 - 2.7 (m, 2H), 1.8 - 2.0 (m, 7H), 1.6 - 1.8 (m, 8H), 1.5 (dd, J = 3.71, 15.81 Hz, 4H), 0.9 - 1.3 (m, 17H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 136.8, 127.9, 121.3, 118.7, 114.0, 80.3, 58.7, 47.3, 31.6, 25.9, 25.5.

***E*-7,7'-(vinylene)bis[3-cyclopentyl-3,4-dihydro- 2H-1,3-Benzoxazine] (41):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.7 - 7.0 (m, 8H), 4.8 - 4.9 (m, 4H), 3.9 - 4.0 (m, 4H), 3.0 - 3.2 (m, 2H), 1.8 - 2.0 (m, 5H), 1.3 - 1.7 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.1, 137.2, 128.7, 127.8, 120.3, 119.1, 114.1, 81.8, 59.8, 49.7, 31.6, 24.0.

***E*-7,7'-(vinylene)bis[3-benzyl-3,4-dihydro- 2H-1,3-Benzoxazine] (42):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.1 - 7.4 (m, 21H), 6.8 - 7.0 (m, 11H), 4.8 - 4.9 (m, 4H), 3.8 - 4.0 (m, 9H), 3.6 - 3.7 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.3, 138.2, 137.2, 129.0, 128.5, 128.3, 128.2, 127.9, 127.4, 119.1, 114.0, 82.3, 73.7, 57.0, 55.7.

***E*-7,7'-(vinylene)bis[3-(4-methoxy benzyl)-3,4-dihydro- 2H-1,3-Benzoxazine] (43):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2 - 7.3 (m, 6H), 6.8 - 7.0 (m, 10H), 4.8 - 4.9 (m, 4H), 3.9 (s, 4H), 3.8 (m, 5H), 3.7 - 3.8 (m, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 154.6, 137.4, 130.4, 119.7, 119.3, 114.2, 114.1, 113.8, 82.3, 55.5, 55.1, 49.1.

***E*-7,7'-(vinylene)bis[3-furfuryl-3,4-dihydro- 2H-1,3-Benzoxazine] (44):**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 - 7.4 (m, 2H), 6.8 - 7.0 (m, 10H), 6.2 - 6.3 (m, 2H), 4.8 - 4.9 (m, 4H), 3.9 - 4.0 (m, 4H), 3.8 - 3.9 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.1, 151.5, 142.6, 137.2, 128.1, 119.2, 114.1, 110.2, 109.0, 81.9, 49.5, 48.2.

2.12 References

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Chapter 3

Synthesis of Novel Ketamine Analogues and Their Activity at $GABA_A$ and NMDA Receptors.

3.1 Introduction

Ligand gated ion channels are membrane-embedded proteins at synaptic cleft which controls intercommunication among neurons. Each receptor has a function of allowing the flow of ions like Cl^-, Na^+, K^+ resulting in excitation or inhibition. When a neurotransmitter like γ-aminobutyric acid (GABA) binds to its respective receptor-channel (GABA receptors), chloride ions flow through the receptor [1] channel reducing the likelihood of initiating action potentials. $GABA_a$ receptors[2] are most ubiquitously expressed ligand gated Cl^- ion channels in the mammalian central nervous system.[3] These receptors are assembled from a diverse family of 19 homologous subunits[4] ($\alpha_{(1-6)}$, $\beta_{(1-3)}$, $\gamma_{(1-3)}$, δ, π, θ, ε and $\rho_{(1-3)}$), which are differentially expressed throughout the brain.[5] The $GABA_a$ receptor is a pentameric complex composed of closely related subunits in 2:2:1 stoichiometry in which α, β and γ subunits expressed as most abundant subunits.[6] From previous studies with the transgenic mice and with subtype selective subunits, it has been established that while α1-containing receptors are responsible for mediating sedative and muscle relaxant properties, α3 and/or α2-containing receptors are responsible for anxiety.

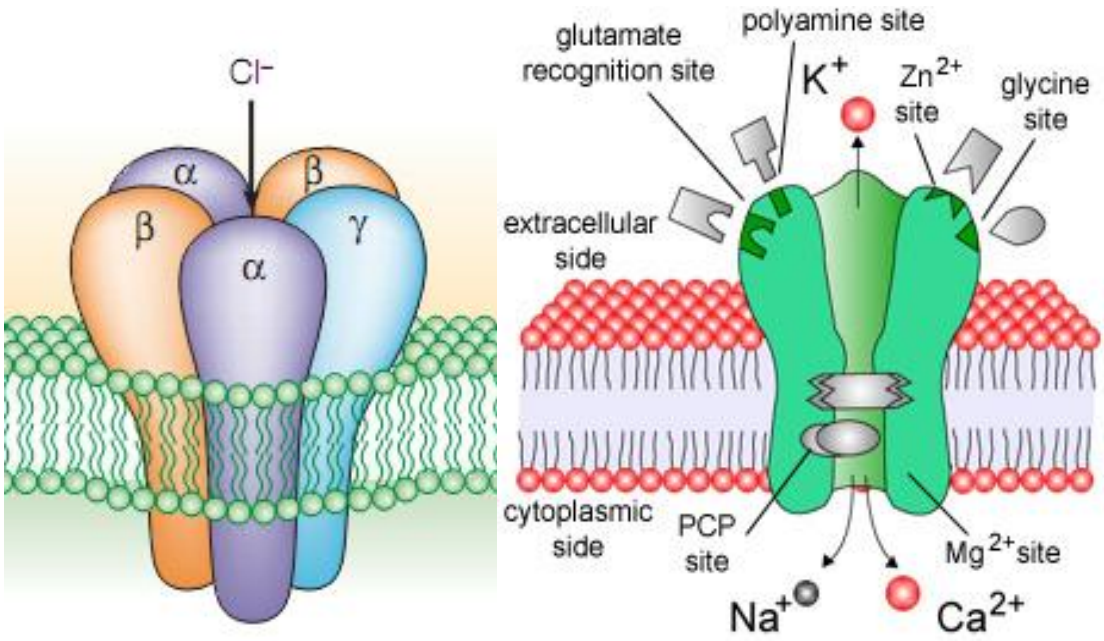

Fig.3-1. General representation of $GABA_a$ (Left) and NMDA (Right) receptors.

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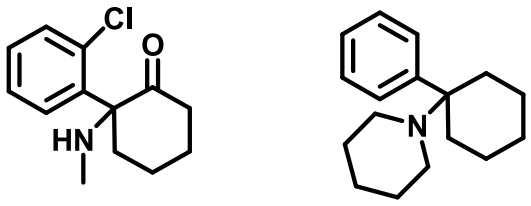
Subtypes $\alpha_6\beta_{2/3}\delta$ in the $GABA_a$ receptor are expressed at high levels within mature cerebellar granule neurons.[7] Cerebellar granule cells are the most abundant neurons in the CNS. They play pivotal role in the cerebellar motor control[8] and learning activity. Upon binding agonist γ-Aminobutyric acid (GABA) to receptors, a major inhibitory neurotransmitter, the pore opens to allow Cl^- ion influx[9] which leads to inhibitory neurotransmission. $GABA_a$ receptors also contains a site of action for a number of allosteric modulators like barbiturates, neurosteriods, ethanol, benzodiazepines,[5,10] barbiturates,[11] picrotoxin,[12] zinc cations,[13] loreclazole[14] and anesthetics including thiopental,[15] propofol[16] and etomidate.[17]

In contrast N-methyl-D-aspartate (NMDA) receptors[18] are glycine and glutamate gated receptors. When a neurotransmitter binds to NMDA receptors, the pore opens to allow influx of cations[19] like a Ca^+ ions which are implicated in synaptic plasticity and learning.[20] The influx of Ca^+ ions into neuronal cell results membrane depolarization causing the propagation of excitory signal and also triggering intracellular signaling pathways.[21] Overactivation of these receptors has been implicated in conditions such as ischemic stroke and traumatic brain injury,[22] where the glutamate levels are elevated. In a central nervous system (CNS), subunits like NR1, NR2 and NR3 forms the heterotetrameric NMDA receptor complex. [23] Two NR1 and two NR2A or NR2B subunits assembles in a hetereotetrameric complex to form most common NMDA receptors in adult CNS. In general, NMDA receptors contributes to differing biophysical and pharmacological properties.[23] Ketamine and Phencyclidine (PCP) (**Fig. 3-2**), known as dissociative anesthetics, produce profound analgesia at low doses to a unique cardiovascular stimulation and a cataleptic state at higher doses.[24] Both ketamine and PCP, primarily acting at NMDA receptors as uncompetitive antagonists[25] via reducing excitory input, show differing CNS depression levels. PCP shows markedly higher affinity[26] for this target than ketamine while the ketamine possesses a higher CNS depressant activity and produce a better quality of anesthesia than PCP.[27] Neurotransmitter systems like dopamine D2 receptors,[28] 5-HT2 receptors,[29] mu and kappa opioid receptors,[30] sigma receptors and muscarinic cholinergic receptors,[31] voltage gated K^+, Na^+ and Ca^{2+} channels[32] are also shown to get affected by ketamine and PCP besides NMDA receptors though at significantly higher concentrations.



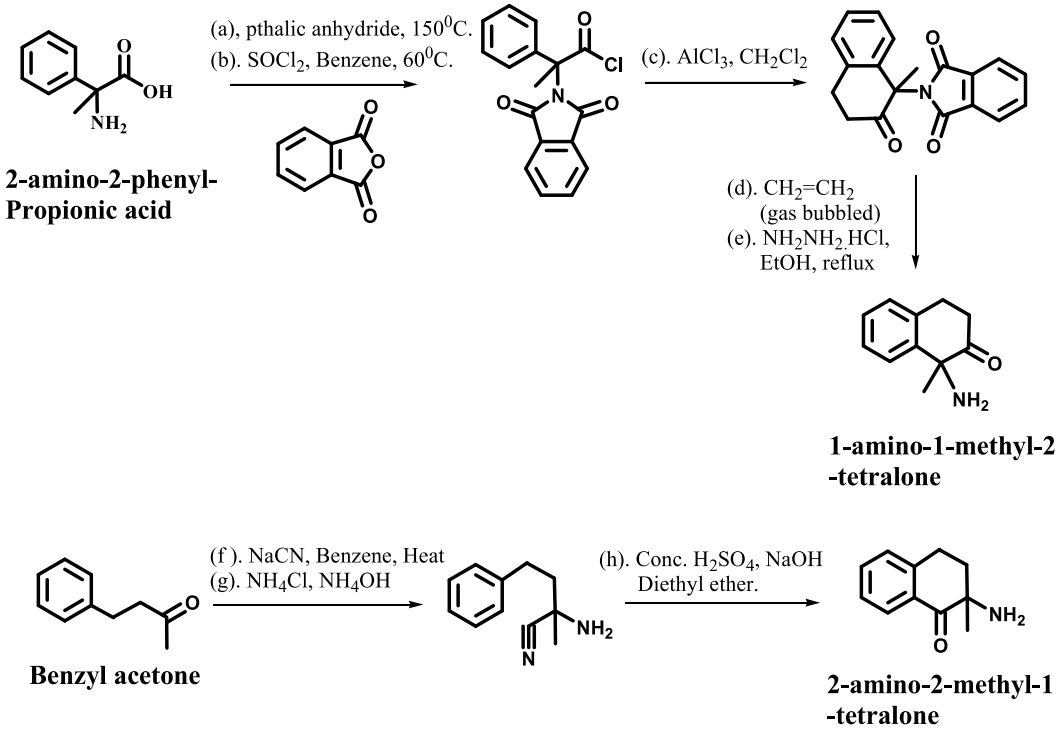
Ketamine **Phencyclidine (PCP)**

Fig.3-2. Structures of Ketamine and Phencyclidine (PCP).

Studies have yet to find about the mechanism by which ketamine produces a higher CNS depression than PCP while at anesthetic doses.[33] Dose-dependent side effects like vivid dreams, illusions, disruptions of cognitive function, mood changes have been reported with ketamine administration which are also observed in schizophrenia.[34] The psychotomimetic effects of the ketamine have been shown or proposed to mediated by its inhibitory effect on GABAergic interneurons leading to increase in the glutamate release.[35] Administration of subanesthetic doses of the ketamine shown to have therapeutic efficacy for the treatment resistant major depression.[36] Other symptoms associated with ketamine like saccade, nystagmus, hyperlocomotion and prepulse inhibition deficit found to be a model system[37] to study schizophrenia and to identify even hit-to-lead compounds for the inhibition of a ketamine induced hyperlocomotion for the schizophrenic studies in animals. However, the spectrum of ketamine analogues[38] is narrow so far in terms of broadening ketamine's desired effects to improve clinical actions, decreasing its incidence of side effects and therapeutic efficacy on the selective affinity towards receptors.

3.2 Synthetic approaches towards ketamine analogues

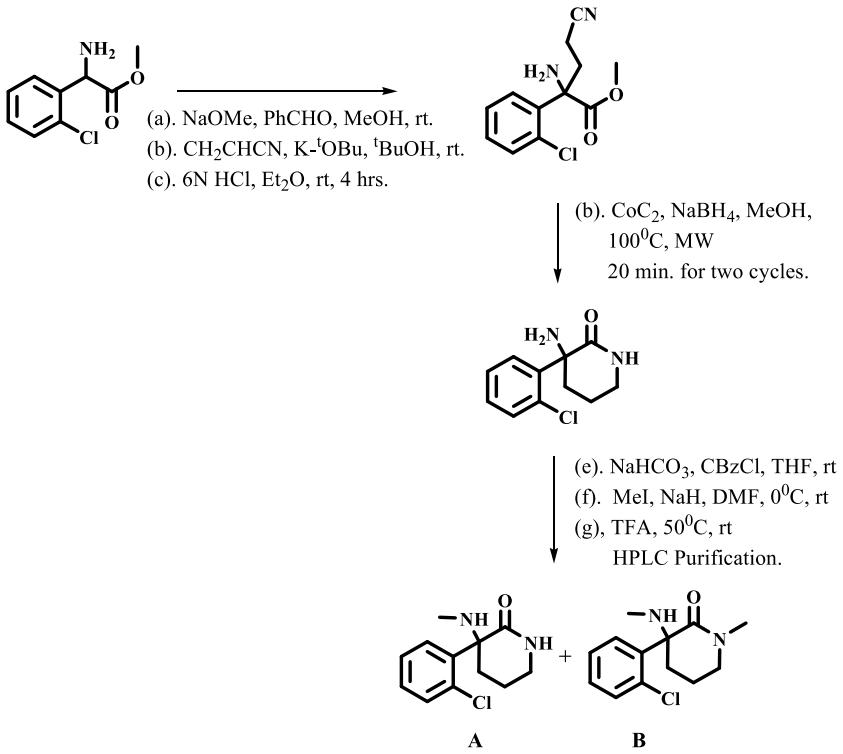
Only few reports exist toward the efforts to synthesize ketamine analogues. Yang *et. al.* synthesized amino tetralone analogues of ketamine (**Scheme. 3-1**) for locomotor activity tests in mice.[38b] In these compounds, benzene ring was fused with cyclohexanone ring restricting conformational flexibility. 1-amino-1-methyl-2-tetralone was synthesized starting from 2-amino-2-phenyl-propionic acid via intermediate pthalimidophenyl acetylchloride. 2-amino-2-methyl-1-tetralone was synthesized from benzyl acetone via amino cyanide intermediate (see **Scheme 3-1**).



Scheme 3-1. Synthesis of tetralone analogues of ketamine by Yang *et. al.*

Though none of the tetralones produced hypnosia or profound ataxia, 2-amino-2-methyl-1-tetralone caused an increased locomotor activity while 1-amino-1-methyl-2-tetralone decreased but not significant when compared with ketamine.

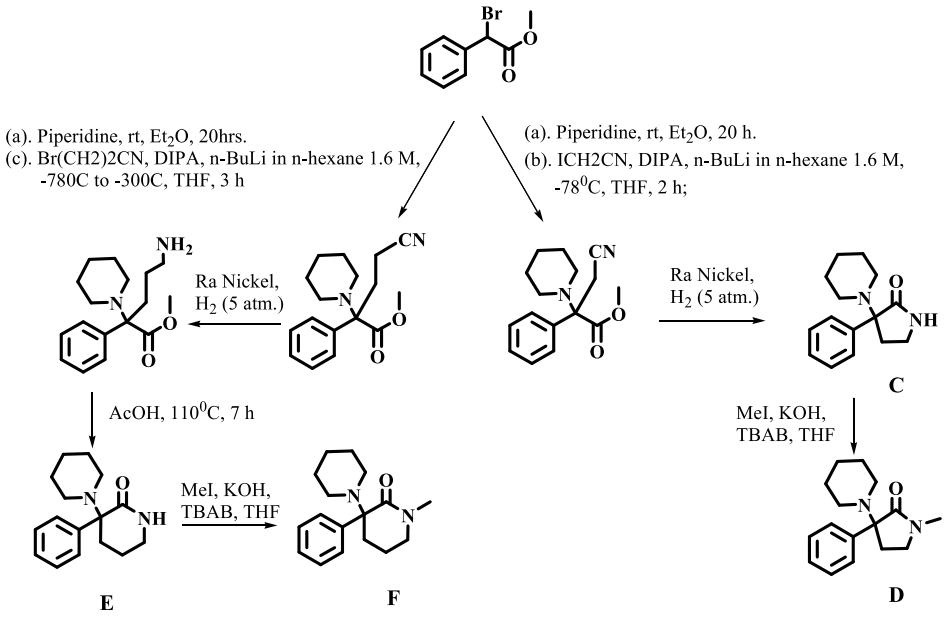
Recently, Zarantonello *et.al*. reported novel ketamine analogues. In their strategy, ketamine was modified by introducing amide in cyclohexanone ring and with various tertiary and secondary amines attached in the place of methylamine for evaluation as NMDA receptor antagonists.[39] Ketamine analogues **A** and **B,** which have amide group in cyclohexanone ring (**Scheme. 3-2)** were synthesized from chlorophenyl amine acetic acid. Intermediate amino nitrile was synthesized from chlorophenyl amineacetic acid by alkylation. Upon reductive cyclization of this intermediate in the presence of $CoCl_2$ and $NaBH_4$, the resulting cyclic amide was further methylated with methyl iodide to isolate novel analogues **A** and **B** as a mixture which were separated by HPLC purification.



Scheme 3-2. Synthesis of lactam with methylamine analogues of ketamine by Zarantonello *et. al.*

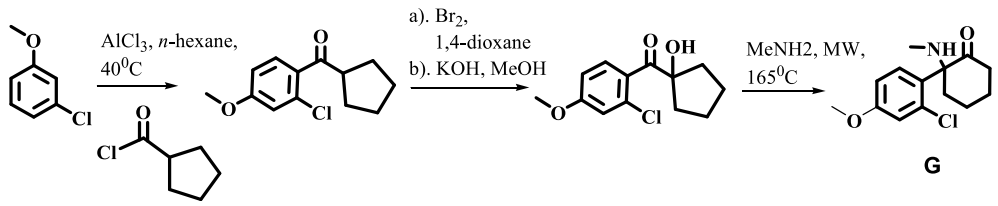
In a similar strategy, analogues of phencyclidine (**C** and **D**) were synthesized from bromophenyl methylacetate via piperidine nitrile intermediate (see **Scheme 3-3**). Nitrile group in this intermediate was then reduced and cyclized in the case of synthesis of six membered ring as in **C** and cyclized directly by reductive hydrogenation in the case of synthesis of 5 membered ring as in **D**. Methylated amides (**E** and **F**) were synthesized from the amides (**C** and **D**) by methylation.

The evaluation of **A** and **B** against NMDA receptor with NR2-A and NR2-B subunits showed that amide functionality in cyclohexanone ring was not tolerated. The same loss of functional activity was observed even in the case of **C-F** in comparison to ketamine.



Scheme 3-3. Synthesis of lactam with piperidine analogues of ketamine by Zarantonello *et. al.*

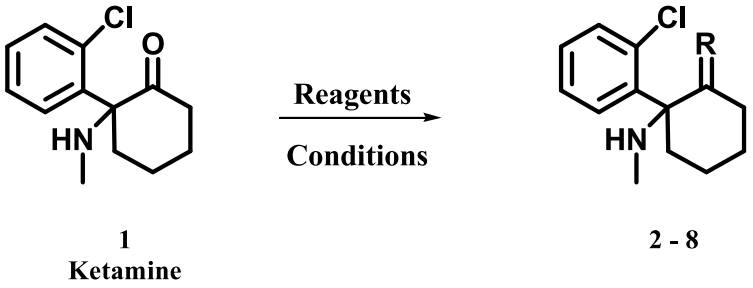
From this structure and activity relationship studies, any appreciable activity was observed only in the analogue (**G**) in which cyclohexyl ring was preserved and phenyl group has methoxy substituent (**Scheme 3-4)**.



Scheme 3-4. Synthesis of ketamine analogue with phenyl group substitution.

3.3 Synthesis of Novel ketamine analogues:

Ketamine analogues with modification of cyclohexyl ring to amide[39] (**Scheme 3-2** and **3-3)** or fused cyclohexane with benzene[38b] (**Scheme 3-1**) resulted in a loss of functional activity. The functional activity was sustained only when cyclohexyl ring is preserved (**Scheme. 3-4**) in ketamine or phencyclidine. When the phenyl group was introduced with various substitutions, the activity was modestly retained in comparison with ketamine. So to synthesize novel analogues of ketamine with cyclohexyl ring intact, we transformed the carbonyl group in cyclohexane to few other analogues with imine substitutions as depicted in Scheme **3-5** and Table **3-1**.

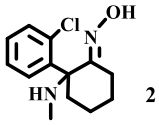
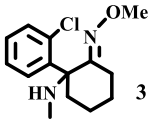
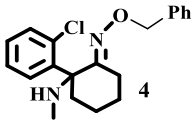
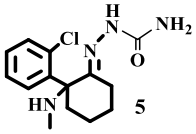
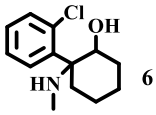


1
Ketamine

2 - 8

Scheme 3-5. Synthesis of ketamine analogues. See **Table 3-1** for Appropriate reagents and conditions.

Table 3-1: Reagents and Conditions to synthesize ketamine analogues.

Entry	Reagents	Solvent	Conditions	Yield	Product
1	$NH_2OH.HCl$, $CH_3COONa.H_2O$,	MeOH: H_2O (7:3)	reflux	70 %	 2 Oxime
2	$NH_2OMe.HCl$, $CH_3COONa.H_2O$.	MeOH: H_2O (7:3)	reflux	78 %	 3 Methoxime
3	$NH_2OPh.HCl$, $CH_3COONa.H_2O$	MeOH: H_2O (7:3)	reflux	90 %	 4 Benzoxime
4	$NH_2CONHNH_2.HCl$, $CH_3COONa.H_2O$	MeOH: H_2O (7:3)	reflux	60 %	 5 Semicarbazone
5	$NaBH_4$	MeOH	r. temp.	80 %	 6 Hydroxy
6	$(Ph_3P)_3PCH_3Br$, *n*-BuLi,	dry. THF	-78^0C to r. temp.		No Product
7	$MeNH_2$. HCl, $CH_3COONa.H_2O$,	MeOH or CH_2Cl_2 or Toluene	reflux		Product is unstable
8	NH_2-NH_2. HCl, Pyridine	MeOH	reflux		Product is unstable
9	$NH_2CSNHNH_2.HCl$, $CH_3COONa.H_2O$,	MeOH: H_2O (7:3)	reflux		No Product

Ketamine carbonyl group was converted to the oxime by treating with hydroxylamine in the presence of sodium acetate at reflux to synthesize oxime **2** in 70% yield as a white color solid. The structure of the oxime **2** was confirmed by its NMR spectra (**Fig. 3-3**). In a ^{1}H NMR spectrum, methylene hydrogens (Ha and Ha') next to C=N group showed resonance at 2.5 and 2.8 ppm. Carbon in C=N-OH groups showed resonance at 161 ppm in ^{13}C NMR. The absence of any signal between 200-220 confirms that ketamine has been transformed to oxime **2**. Absence of any isomers of hydroxyl group on C=N could be observed in both ^{1}H and ^{13}C NMR spectra (**Fig. 3-3**).

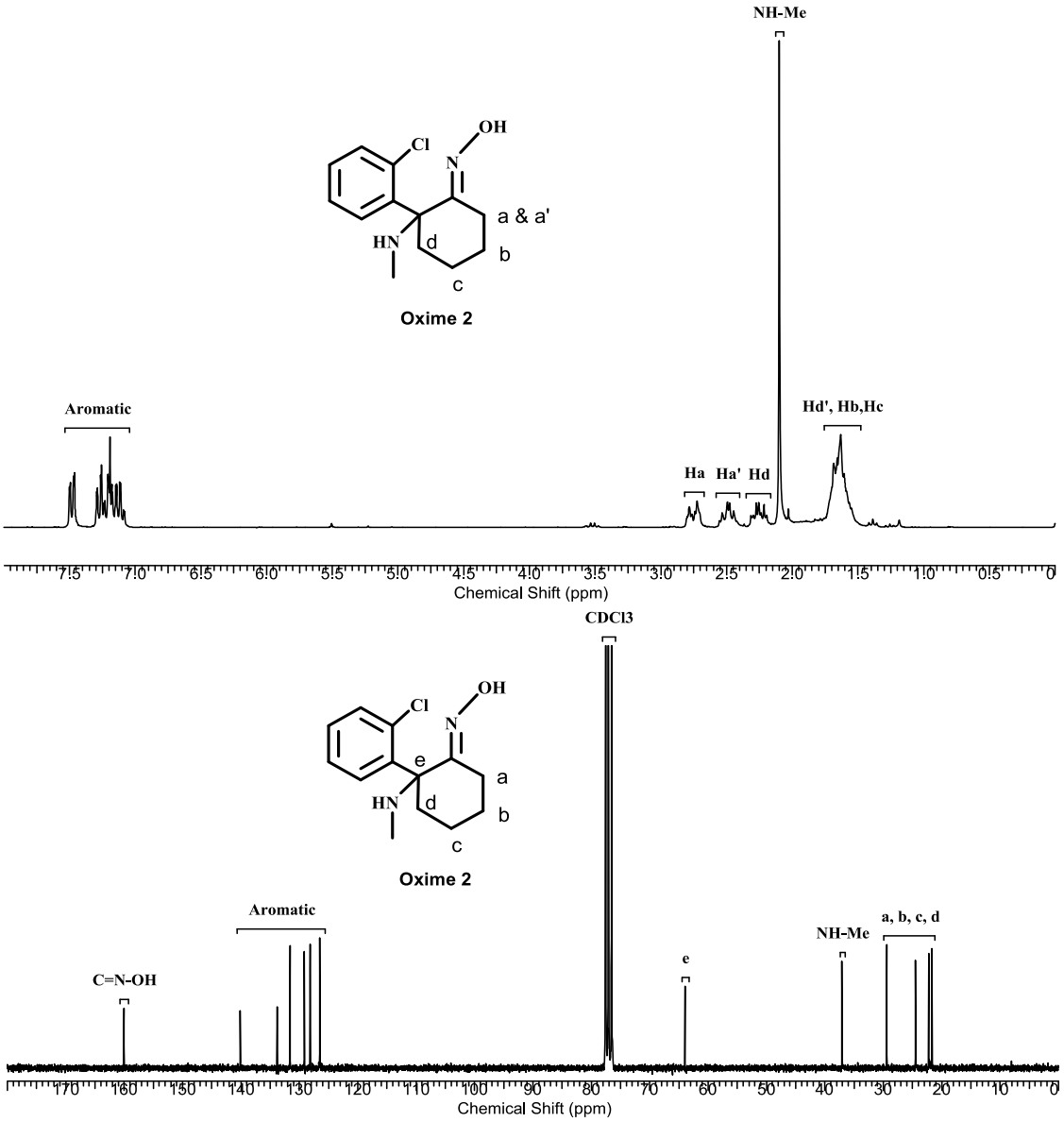


Fig.3-3. NMR Spectra of Oxime **2**. TOP. ^{1}H NMR. BOTTOM. ^{13}C NMR.

In a similar fashion, ketamine **1** was treated with methoxylamine to synthesize methoxime **3.** In this reaction, methoxime **3** has obtained in 78% yield as a light yellow color solid. In the structural determination of methoxime **3** by NMR spectra, methylene hydrogens (H**a** and H**a'**) have showed resonance at 3.3 ppm and methoxy hydrogens showed resonance at 3.9 ppm (**Fig. 3-4**). In both ^{1}H and ^{13}C NMR spectra, isomers of methoxime 3 were also not present (**Fig. 3-4**).

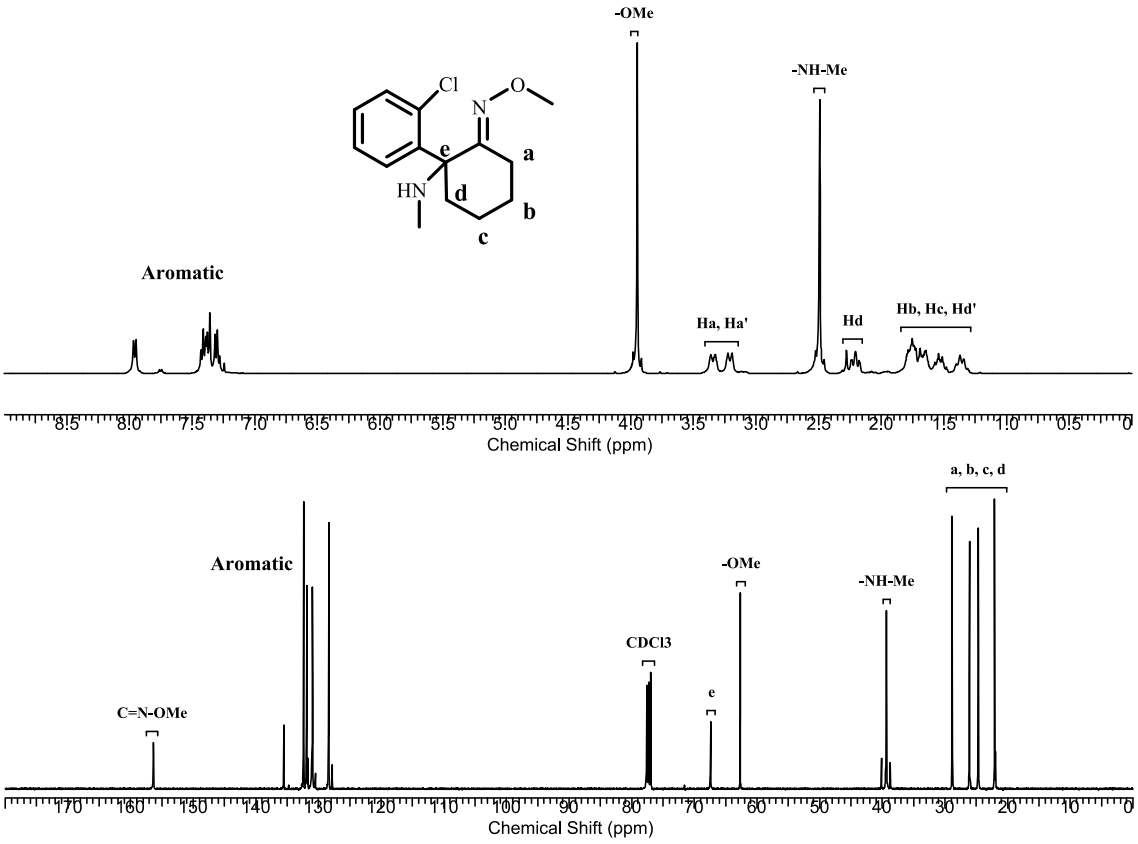


Fig.3-4. NMR Spectra of Methoxime **2**. TOP. ^{1}H NMR. BOTTOM. ^{13}C NMR.

When the ketamine **1** was treated with benzoxylamine in the presence of sodium acetate, benzoxime **4** was isolated as white color solid. Although benzoxylamine group is bulky, the reaction proceeded smoothly yielding the product in 90% yield. Presence of resonance for benzyl methylene hydrogens (Hf) at 5.4 ppm in ^{1}H NMR spectrum and C=N carbon at 158 ppm in ^{13}C NMR spectrum confirmed the product. It could be worthy to assume that bulkier benzoxyl group allows the formation of only one isomer which could be observed in ^{1}H and ^{13}C NMR spectra (**Fig. 3-5**).

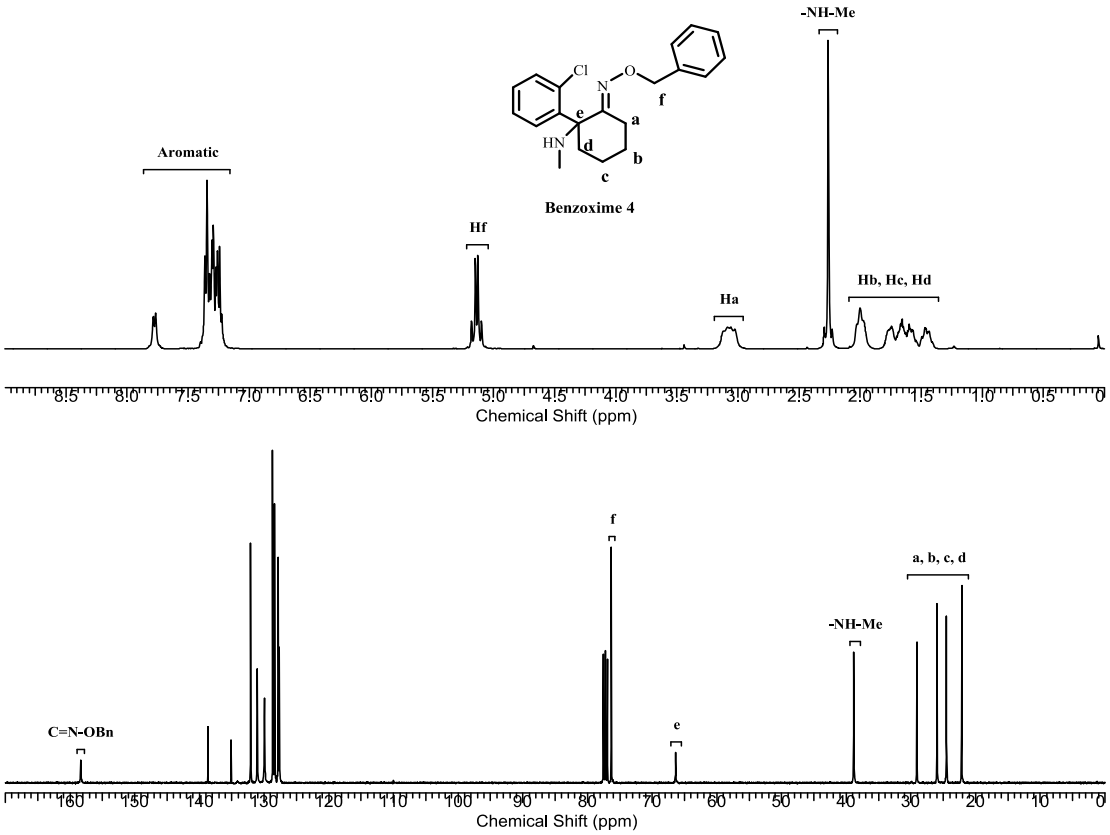


Fig.3-5. NMR Spectra of Benzoxime **4**. TOP. ^{1}H NMR. BOTTOM. ^{13}C NMR.

Semicarbazone **5** was also synthesized by treating the ketamine **1** with semicarbazide as light yellow color solid. In a wittig reaction with $(PPh_3)_3CH_2PBr$ reagent, the ketamine carbonyl was attempted to convert into a terminal alkene by treating wittig reagent and *n*-BuLi at low temperature (-78^0C). But the product could not be observed as only the reactant ketamine **1** was recovered. This reaction was repeated with varying equivalent ratios of reagents and temperatures. But in all cases, only ketamine **1** was recovered. So to test the reagent purity, wittig reagent and *n*-BuLi was treated with benzophenone. In this case, the reaction mixture with 1 hr stirring at room temperature, after addition of benzophenone to wittig reagent and *n*-BuLi, showed the product on TLC (1:4 EtOAc:Hexanes) which was confirmed in both ^{1}H NMR and ^{13}C NMR spectra.

Even in the reactions of ketamine **1** with $HOCH_2CH_2NH_2$. HCl and $NH_2CSNHNH_2$. HCl (Thiosemicarbazide), the product was not observed. In all these reactions, only the reactant ketamine **1** was observed on TLC and NMR spectra. From these reactions, one can assume that ketamine carbonyl group is sterically crowded to be reached by bulky reagent. Methyl amine was also treated with the ketamine **1** to convert into N-methylimine. But the product was not stable even under dry conditions. So the ketamine was recovered as the end product.

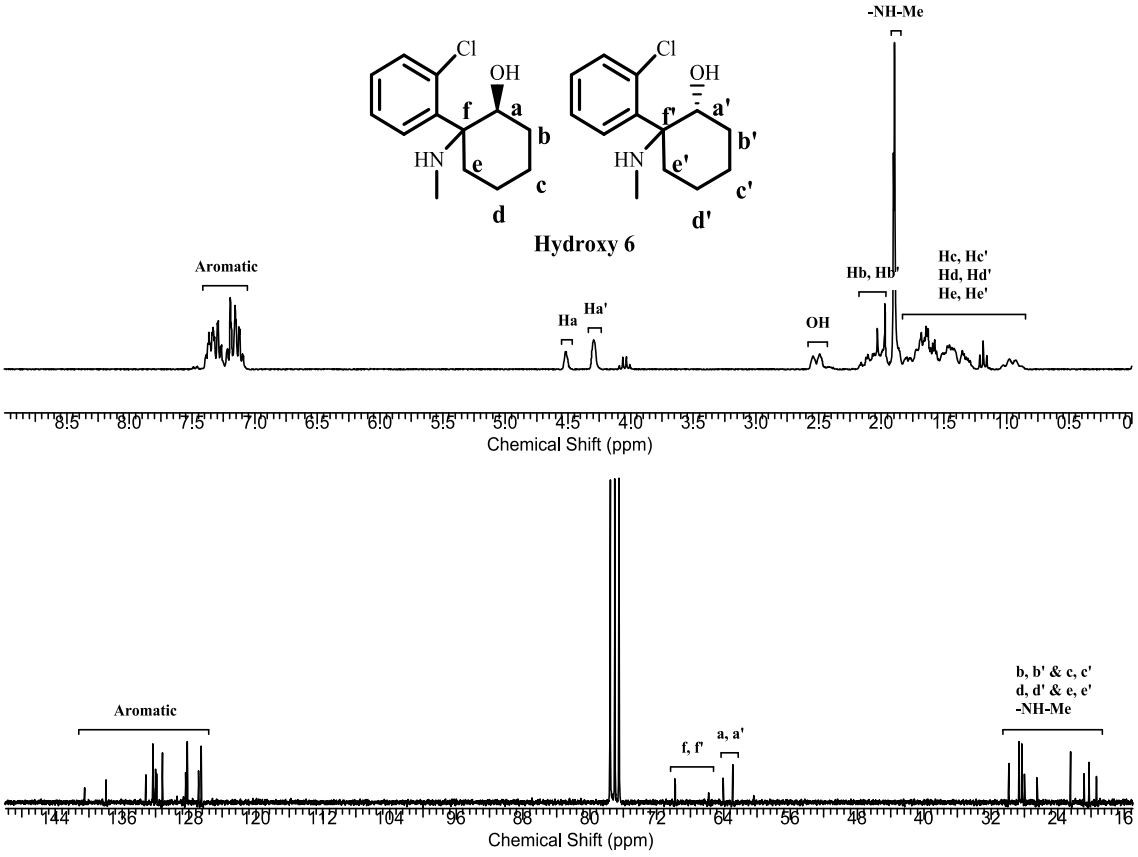


Fig.3-6. NMR Spectra of Hydroxy **6 (**Diastereomer ratio 1:0.5). TOP. ^{1}H NMR. BOTTOM. ^{13}C NMR.

The hydroxy analogue **6** was synthesized by treating ketamine **1** with sodium borohydride. This reaction yielded both diastereomers. The presence of the diastereomers was observed on both ^{1}H and ^{13}C NMR spectra (**Fig. 3-6**). The CH-OH hydrogen (H**a** and H**a'** in **Fig. 3-6**) for both diastereomers showed resonances at 4.5 and 4.25 ppm in the ^{1}H NMR spectrum and the respective CH-OH carbon at 64 and 63 ppm in the ^{13}C NMR spectrum. The ratio of diastereomers has been calculated as 7:4 from ^{1}H NMR spectrum. All of the ketamine analogues synthesized was used for the $GABA_A$ and NMDA receptors assay to evaluate their agonist activity at $GABA_A$ receptors and antagonist activity at NMDA receptors. Hydroxy **6** analogue, which received as diastereomeric mixture was used for receptor assay as such without separation.

3.4 Agonist activities of ketamine analogues on α6β2δ and α1β2γ2 receptors:

The $GABA_a$ subtypes α6β2δ and α1β2γ2 receptors are expressed at high levels exclusively within mature cerebellar granule neurons. In the previous study,[40] ketamine has potentiated the GABA current arising from α6- and δ-containing $GABA_a$ receptors in oocytes, dissociated granule neurons and cerebellar slices isolated from rodents within anesthecally relevant concentration. This study also shown that PCP (Phencyclidine) and Mk-801 (a potent non-competitive inhibitor of NMDA receptor) was less active compared to ketamine in potentiating tonic chloride current arising from $GABA_A$ receptors containing α6- and δ-subtypes. These subtypes were chosen to test against new analogues within the oocyte expression system since their association with cerebellum has a pivotal role in motor control activity.

Table 3-2: Agonist activity of ketamine analogues on $GABA_a$ receptors.

Conc.	α6β2δ[a]				α1β2γ2[a]	
	Ketamine	Oxime (Monosalt)	Oxime (Disalt)	Hydroxy	Ketamine	Oxime (Disalt)
10	116.2	128.2	165.4	100	-	-
20	157.1	193.746	239	100	0	122.678
50	218.3	312.04	418	123.98	105	153.41
100	-	-	-	135.41	119	214.5

a = values depicted are $I_{Average}$ (n. amp). $I_{Average} = (I_{GABA + Drug} / I_{GABA})$ 100.

Agonist activities of ketamine analogues are shown in Table **3-2**. At various concentrations, Oxime **2** has shown high sensitivity as shown in Figure **3-7**. Oxime **2** in both forms has greatly potentiated the $GABA_A$ current relatively than ketamine at various concentrations. On α6β2δ subtype receptors, Oxime **2** disalt has shown greater potentiation at 20 μM concentration. Even on α1β2γ2 receptors, Oxime **2** has greatly potentiated $GABA_a$ current at 20 μM when ketamine is not able to potentiate any current. It has shown nearly two fold increase at 50 μM concentration on α6β2δ subtype receptor and similarly on α1β2γ2 receptors at 100 μM concentration.

Methoxime **3** and benzoxime **4** analogues have not evoked considerable response from receptors. Even Semicarbazone **5** has also been less sensitive than ketamine towards receptors potentiating $GABA_A$ current. Hydroxy analogue **6** has shown less sensitivity than ketamine towards both $GABA_a$ receptors. This could be seen even at higher concentration like 100 μM (**Table 3-2**).

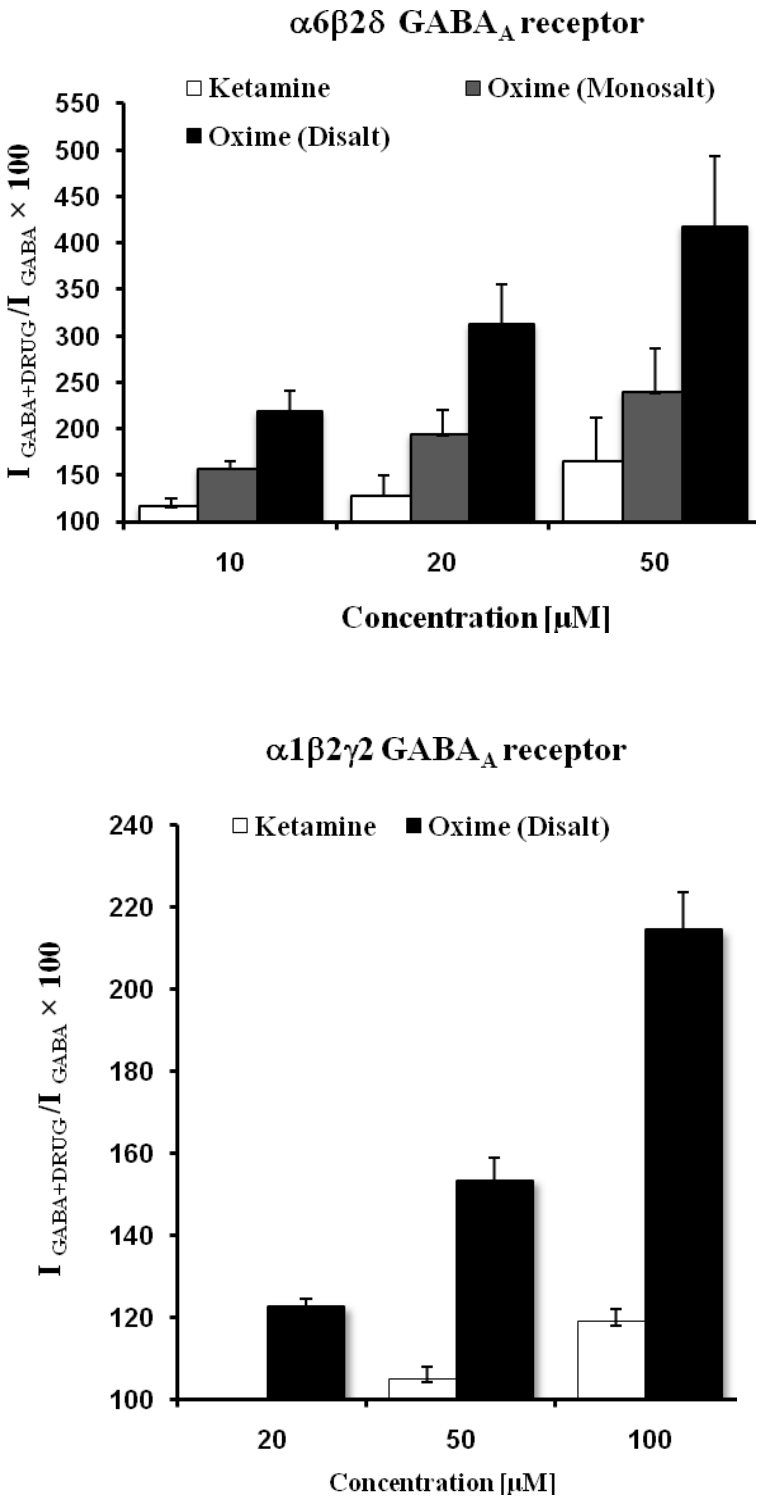


Fig 3-7. Agonist activities of ketamine **1** and oxime **2** on $GABA_a$ receptors. TOP. α6β2δ receptors. BOTTOM. α1β2γ2 receptors.

From this analysis, Bulkier groups like methoxime and benzoxime are less effective in potentiation. Among polar groups, Bulkier group like one in semicarbazone **5** and smaller group like hydroxyl were also insensitive towards $GABA_a$ receptors. It indicates that double bond and the presence of small polar groups as in oxime **2** are crucial to potentiate. The increased potentiation can be attributed to the reduced lipophilicity in oxime **2** than ketamine.

3.5 Antagonist activities of ketamine analogues on NMDA Receptors:

Initially, new analogues synthesized were tested at 50 μM concentration against three different receptors (**Table 3-3 and Fig. 3-8**) for their antagonist activity. Modification of the carbonyl group in ketamine to oxime has increased inhibition at NMDA-A and –B receptors while the comparable activity can be observed at NMDA-D receptors (**Table 3-3 and Fig. 3-8**). Particularly it had a greater inhibition at NMDA-A receptors than ketamine and similar to PCP. It even had a similar inhibition at NMDA-B and –D receptors. This could establish the fact that oxime **2** is target specific towards NMDA-A receptors at 50 μM concentration. Replacement of the carbonyl group in ketamine with other analogues like methoxime, benzoxime and semicarbazone has resulted decreased effect on all receptors (Table **3-3**, Fig. **3-8**). % Recovery of current measured post drug treatment to reflect anesthetically effectiveness of drug has also shown that oxime **2** retains similar effect on A and B receptors (**Fig. 3-9**) while showing ineffectiveness at D receptors compared to ketamine. Oxime **2** also shown comparable activity with PCP (Phencyclidine) at NMDA-A and –D receptors in inhibition. At NMDA-B and –D receptors, it shown greater affinity than PCP as obvious from % recovery of current measured at these receptors (**Table 3-3 and Fig. 3-9**).

Table 3-3: Antagonist activities of ketamine analogues on NMDA receptors.[a, b, c]

Compound	NMDA-A (NR1/2A)		NMDA-B (NR1/2B)		NMDA-D (NR1/2D)	
	% Inhibition	% Recovery	% Inhibition	% Recovery	% Inhibition	% Recovery
Ketamine	57.627	84.745	80.512	34.564	88.09	26.19
Oxime	89.23	86.1538	83.0188	33.9622	86.666	52.272
Methoxime	25.257	98.453	75.597	71.6417	57.5	82.5
Benzoxime	-	-	40.993	77.639	49.315	79.452
Semicarbazone	33.557	-	50.757	98.484	33.3333	-
PCP	88.372	93.953	96.774	59.677	87.5	66.666

a = % Inhibition = $(I_{Glu/Gly} - I_{Drug}/I_{Glu/Gly})$ 100. b = % Recovery = $(I_{Post\ treatment} - I_{Glu/Gly})$ 100.
c = Drug concentration is 50 μM at receptor.

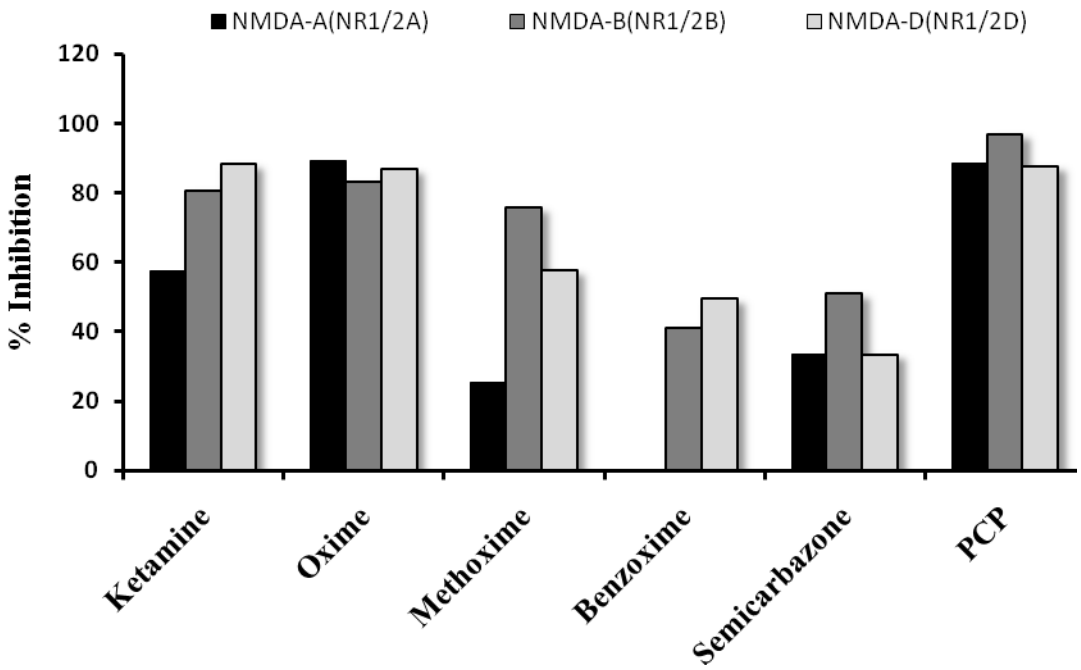


Fig 3-8. Activity of ketamine analogues against NMDA receptors at 50 μM concentration inhibiting NMDA response.

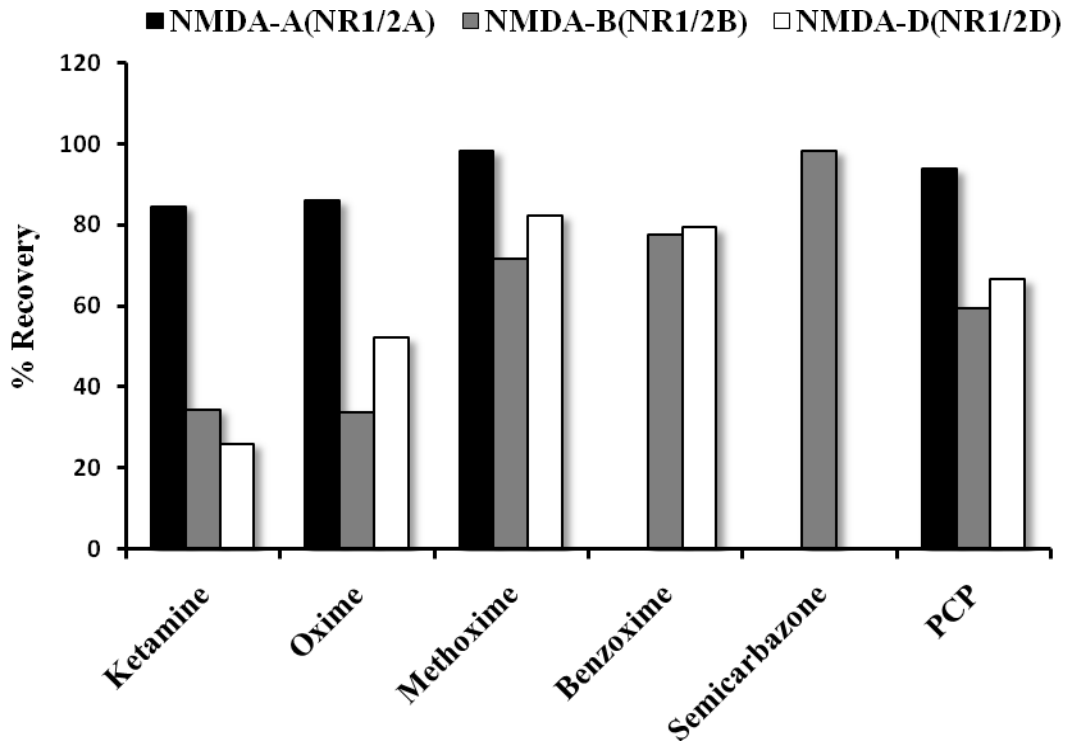


Fig. 3-9. % Recovery of response from NMDA receptors post drug(50 mM conc.) treatment .

Thus encouraged by this result, oxime **2** affinity studies have been conducted at various concentrations to determine IC_{50} values (**Table 3-4 and Fig. 3-10**). The % recovery reflects long lasting effect of drug as anesthetic to determine anesthetically relevant concentration. Though oxime **2** showed greater or similar inhibition at 50 μM concentration with ketamine at all NMDA receptors, it's affinity was attenuated over a range of concentrations from 0.1 to 200 μM in terms of % recovery of response (**Table 3-4** and **Fig. 3-10**) by observing IC_{50} values.

Table 3-4 : Affinity studies of ketamine and oxime against NMDA receptors with IC_{50} values.

NMDA Receptor	% Recovery (Ketamine)	% Recovery (Oxime)	IC_{50} (Ketamine)	IC_{50} (Oxime)
NMDA-NR1/2A	28	74	2.32	5.911
NMDA-NR1/2B	19	31	0.82	1.068
NMDA-NR1/2C	-	44	-	0.747
NMDA-NR1/2D	24	68	0.76	1.068

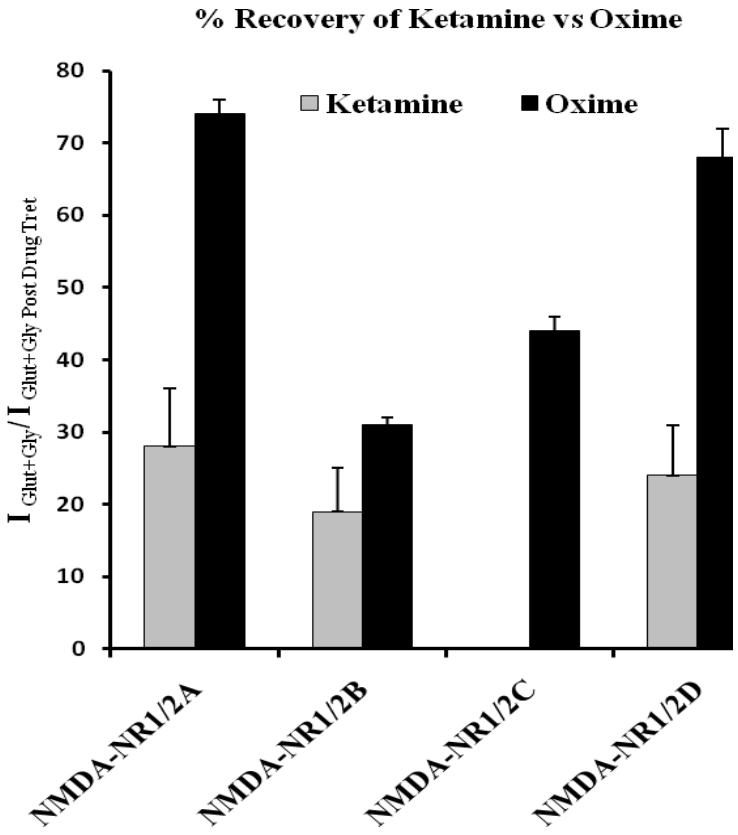


Fig 3-10. % Recovery of response from NMDA receptors after post drug treatment (over various conc.).

Recovery is very less for ketamine than oxime means ketamine has long lasting effect and so more affinity. Oxime shown similar inhibition at NMDA-B, C, D receptors while it has two fold increase in terms of IC_{50} values than ketamine at NMDA-A receptors.

3.6 Conclusions

Modification of carbonyl group in ketamine to oxime, methoxime, benzoxime, semicarbazone and hydroxyl groups has provided an insight into the effect of size of the groups towards $GABA_a$ receptors. Oxime has shown to reduce lipophilicity while methoxime, benzoxime shown increased lipophilicity. This study has also shown that polar groups like semicarbazone and hydroxyl were not able to increase the potency towards $GABA_a$ receptors. Thus oxime **2** has been better agonist at $GABA_a$ receptors exhibiting two times more excitory post synaptic potential than ketamine. From this study , though anesthetic efficacy of ketamine and PCP (Phencyclidine) as non-competitive inhibitors of NMDA (N-Methyl-D-Aspartate) led to classifying them as dissociate anesthetics, Oxime **2** has shown improved or similar potency towards various NMDA receptors. This could be beneficial to study further anesthetic efficacy of oxime **2** in epileptic seizures, other convulsant side effects which are associated with ketamine. Ketamine is known to be more effective anesthetic across different animal species but in humans it is associated with various adverse effects like inducing catalepsy at higher doses and hallucinations at lower doses. Since oxime has shown greater or comparable potency at lower doses as antagonist exciting postsynaptic potential at various NMDA receptors and less duration of affinity, it could be interesting to study its efficacy across various animals.

3.7 Experimental Section

General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 250 MHz and Varian 400 MHz spectrometer in $CDCl_3$ and DMSO-d6 with TMS as the standard. Chemical shifts are reported in ppm, multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (quintet), h (sextet), m (multiplet) and bs (broad singlet) coupling constants reported in hertz (Hz). All 13C NMR spectra were proton decoupled. Thin-Layer chromatography (TLC) was performed on glass plates coated with 0.25 mm thickness of silica-gel. All solvents were dried and distilled prior to use and organic solvent extracts were dried over Anhydrous. Na_2SO_4. Toluene and tetrahydrofuran were distilled from sodium and benzophenone and were stored in a dry box. Chromatographic purifications were performed by flash chromatography using silica gel (63-200μ) from Dynamic Adsorbents Inc. $Pd(dba)_2$ and $P(t\text{-}Bu)_3$ were purchased from Aldrich Inc. All reagents and bases were purchased from Aldrich, Fisher Science, VWR and used without further purification. Aryl bromide **5** was prepared using literature procedure. Ketamine was purchased from Aldrich chemical company and used as received.

2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone oxime (2): To as solution of NH_2OH. HCl and CH_3COONa. H_2O in MeOH:H_2O (7:3) stirred at room temperature for 15 minutes, was added ketamine and the resulting solution was stirred at reflux for 16 hrs before cooling to room temperature. The reaction mixture was filtered and the filtrate was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The filtrate was extracted with CH_2Cl_2 (2×30 mL) and the combined organic layer was washed with brine solution. The

organic layer was dried over anhydrous. Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture received was purified over silica gel column chromatography by eluting with initially CH_2Cl_2 and later with (1:9) MeOH:CH_2Cl_2 to receive oxime **2** as pale yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4 - 7.5 (m, 1H), 7.0 - 7.3 (m, 3H), 2.6 - 2.8 (m, 1H), 2.4 - 2.5 (q, *J* = 5.8 Hz, 1H), 2.1 - 2.3 (m, 1H), 2.0 - 2.1 (m, 3H), 1.5 - 1.7 (m, 5H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.1, 140.1, 133.8, 131.6, 129.1, 128.1, 126.5, 64.0, 37.1, 29.4, 24.5, 22.2, 21.7.

2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-methyl-oxime (3): To as solution of NH_2OMe. HCl and CH_3COONa. H_2O in MeOH:H_2O (7:3) stirred at room temperature for 15 minutes, was added ketamine and the resulting solution was stirred at reflux for 16 hrs before cooling to room temperature. The reaction mixture was filtered and the filtrate was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The filtrate was extracted with CH_2Cl_2 (2×30 mL) and the combined organic layer was washed with brine solution. The organic layer was dried over anhydrous. Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture received was purified over silica gel column chromatography by eluting with initially CH_2Cl_2 and later with (1:9) MeOH:CH_2Cl_2 to receive methoxime **3** as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.9 - 8.0 (m, 1H), 7.2 - 7.4 (m, 3H), 3.91 - 4.0 (m, 3H), 3.3 (td, *J* = 4.10, 13.96 Hz, 1H), 3.2 (td, *J* = 4.49, 13.73 Hz, 1H), 2.4 - 2.5 (m, 3H), 2.1 - 2.3 (m, 1H), 1.6 - 1.8 (m, 3H), 1.4 - 1.5 (m, 1H), 1.2 - 1.4 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.3, 135.5, 132.4, 132.2, 131.9, 131.0, 128.3, 67.4, 62.7, 39.4, 28.8, 26.1, 24.7, 22.0.

2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-benzyl-oxime (4): To as solution of NH_2OCH_2Ph. HCl and CH_3COONa. H_2O in MeOH:H_2O (7:3) stirred at room temperature for 15 minutes, was added ketamine and the resulting solution was stirred at reflux for 16 hrs before cooling to room temperature. The reaction mixture was filtered and the filtrate was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The filtrate was extracted with CH_2Cl_2 (2×30 mL) and the combined organic layer was washed with brine solution. The organic layer was dried over anhydrous. Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture received was purified over silica gel column chromatography by eluting with initially CH_2Cl_2 and later with (1:9) MeOH:CH_2Cl_2 to receive benzoxime **4** as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.7 (d, *J* = 8.06 Hz, 1H), 7.1 - 7.4 (m, 8H), 5.0 - 5.2 (q, *J* = 8.2 Hz, 2H), 2.9 - 3.1 (m, 2H), 2.2 - 2.3 (m, 3H), 1.9 - 2.0 (m, 2H), 1.3 - 1.8 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.3, 138.7, 135.1, 132.1, 131.1, 129.9, 128.7, 128.4, 127.8, 127.6, 66.4, 38.8, 29.1, 26.0, 24.5, 22.1.

2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-semicarbazone (5): To as solution of NH2CONHNH$_2$. HCl and CH_3COONa. H_2O in MeOH:H_2O (7:3) stirred at room temperature for 15 minutes, was added ketamine and the resulting solution was stirred at reflux for 16 hrs before cooling to room temperature. The reaction mixture was filtered and the filtrate was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution. The filtrate was extracted with CH_2Cl_2 (2×30 mL) and the combined organic layer was washed with brine solution. The organic layer was dried over anhydrous. Na_2SO_4 and concentrated under reduced pressure. The crude reaction mixture received was purified

over silica gel column chromatography by eluting with initially CH_2Cl_2 and later with (1:9) MeOH:CH_2Cl_2 to receive semicarbazone **5** as pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.6 (br. s., 1H), 7.7 - 7.8 (m, 1H), 7.4 - 7.5 (m, 3H), 4.0 - 4.1 (m, 2H), 3.0 - 3.1 (d, *J* = 4.9 Hz, 2H), 2.1 (s, 3H), 1.5 - 1.8 (m, 3H), 1.2 - 1.4 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.3, 149.1, 134.8, 133.2, 133.0, 132.3, 128.7, 67.8, 36.8, 28.2, 26.7, 25.9, 22.3.

2-(2-Chloro-phenyl)-2-methylamino-cyclohexanol (6): To a solution of ketamine in MeOH at 0^OC was added $NaBH_4$ portion wise and allowed to stir at room temperature for 12 hrs. The reaction mixture was cooled to 0^OC and quenched with sat. NH_4Cl solution by adding dropwise. The resulting reaction mixture was partitioned between CH_2Cl_2 and sat. NH_4Cl solution and extracted with CH_2Cl_2 (2×30 mL), The organic layer was dried over anhydrous. Na_2SO_4 and concentrated at reduced pressure. The crude reaction mixture received was purified over silica gel column chromatography by eluting with (2:8) EtOAc:Hexanes to receive hydroxyl analogue **6** as amorphous white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.2 - 7.4 (m, 4H), 7.0 - 7.2 (m, 4H), 4.4 - 4.5 (m, 1H), 4.2 - 4.3 (m, 1H), 2.4 - 2.5 (m, 1H), 1.9 - 2.1 (m, 2H), 1.8 - 1.9 (m, 3H), 1.2 - 1.7 (m, 5H), 0.8 - 1.0 (m, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 140.5, 137.9, 133.2, 132.3, 132.0, 131.9, 131.2, 128.3, 126.7, 69.8, 64.0, 62.9, 29.9, 28.7, 28.3, 28.0, 26.5, 22.5, 20.8, 20.3, 19.3.

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Chapter - 4

Selective Acylation of Diols: Formal Synthesis of (±)-Rolipram and its (±)-γ-Lactone Analogue

4.1 General Introduction

Rolipram is a potent and selective inhibitor of phosphodiesterase IV (PDE 4). It was initially developed and marketed as antidepressant by Schering AG.[1] PDE4 is the main enzyme which regulates the concentration of secondary messenger cAMP (cyclic adenosine-3',5'-monophosphate). Rolipram (**Fig. 4-1)** was known to exert its effect by increasing the levels of cAMP within mature brain cells. In its course of action, it inhibits PDE4 enzyme which hydrolyzes the phosphodiester bridge in cAMP which is the cause of inflammatory processes.[2] PDE4 enzyme is also known to be involved in pathologies related to multiple sclerosis, coronary failure, asthma, pulmonary diseases and diabetes.[3] So, Rolipram as a pharmacological probe is an important tool to further study the structural requirements for selective inhibition of PDE 4.[2b,3a] Though both enantiomers of Rolipram are active against PDE4, the (*R*)-isomer is the more active one.[4] Numerous approaches [4-5] have been reported for the synthesis of Rolipram.

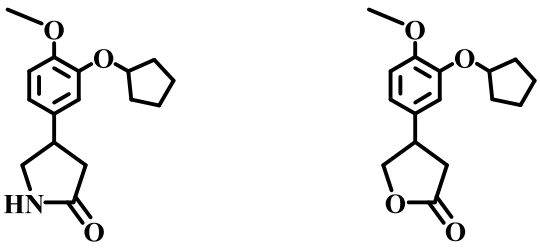
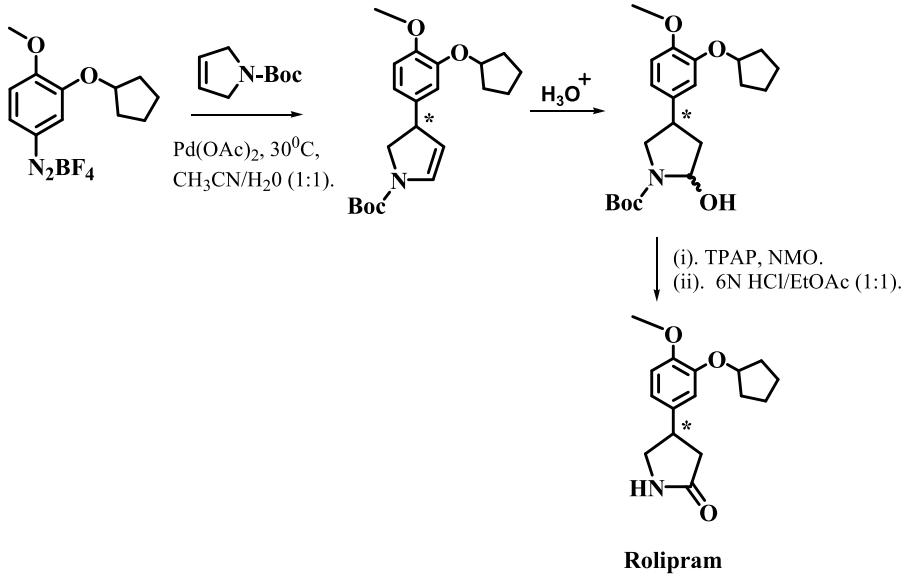


Fig. 4-1. Structure of Rolipram and its analogue γ-Lactone.

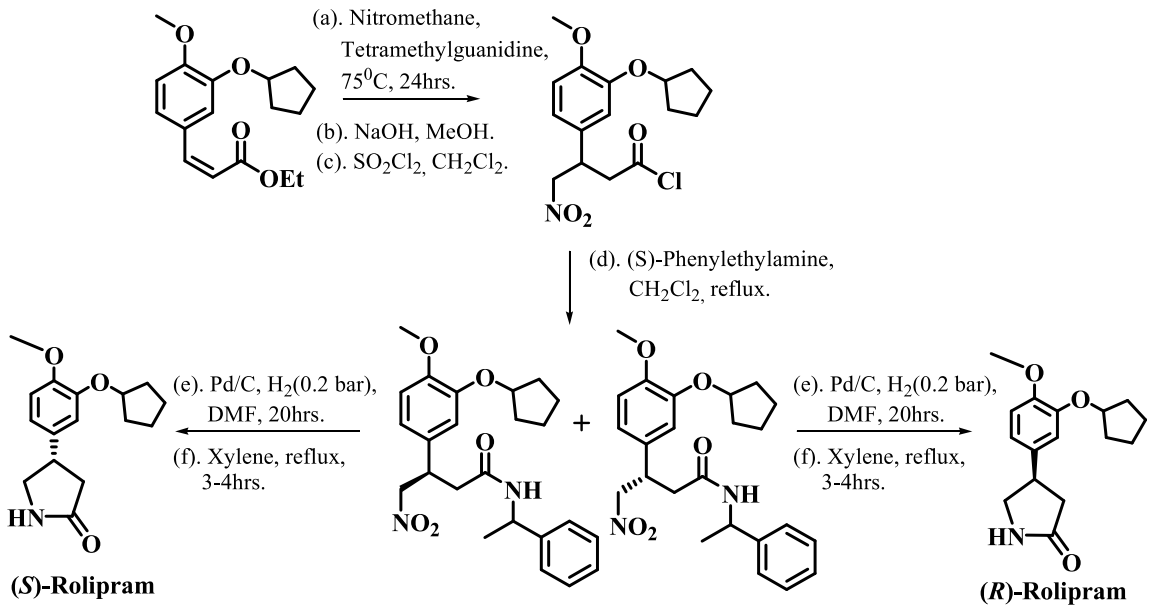
4.2 Synthetic approaches towards Rolipram and β-substituted lactone

Several methods exist to synthesize β- Substituted γ-lactams and γ-lactones in both racemic and optically pure forms. Metal catalysts like Rh, Zn, $CoCl_2$, $NiCl_2$ or organo catalysts like epicinchonine have been widely used. Various methods including cross-coupling between 4-aryl pyrrolidones and aryl halides, Michael addition to α,β-unsaturated esters or aryl nitroolefines and metal catalyzed 1,4-addition of arylboronic acids to α,β-unsaturated γ-lactams have been applied to synthesis of β- substituted γ-lactams and specifically Rolipram. In following sections, a short review of a few important methods for the synthesis of rolipram and β- substituted γ-lactone is included.

Garcia *et. al* [1] reported a racemic synthesis of rolipram by employing Heck-Matsuda arylation of 3-pyrroline with aryldiazonium tetrafluoroborates (**Scheme 4-1**). The racemic rolipram was then resolved into its enantiomers by chromatographic resolution.



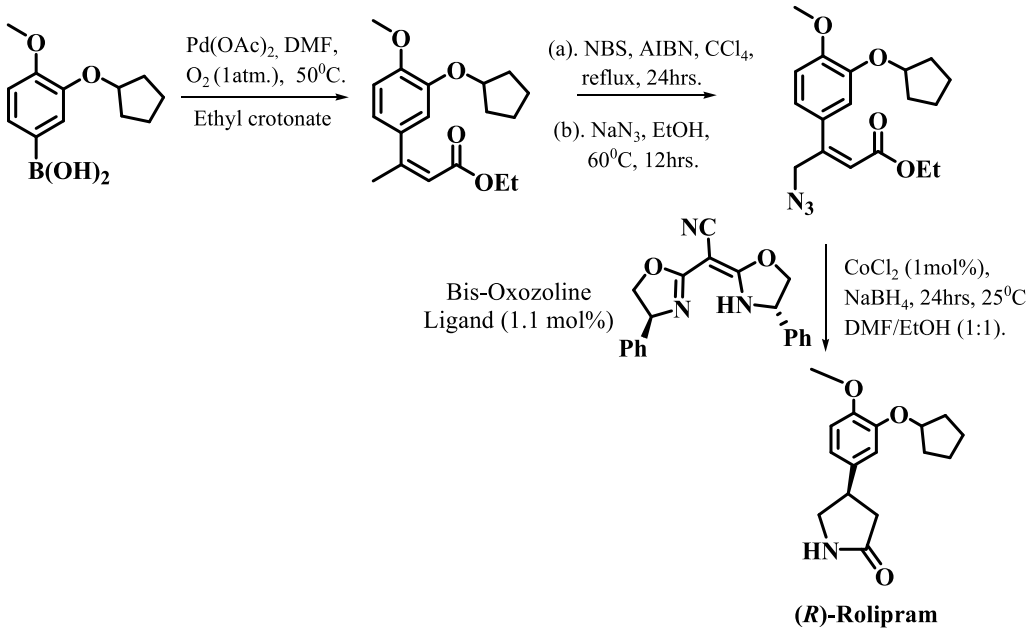
Scheme 4-1. Garcia *et. al* synthesis of (±)-Rolipram via Heck-Matsuda arylation of 3-pyrroline.



Scheme 4-2. Demnitz *et. al* synthesis of (*R*) & (*S*)-Rolipram via chromatographic separation of two diastereomers.

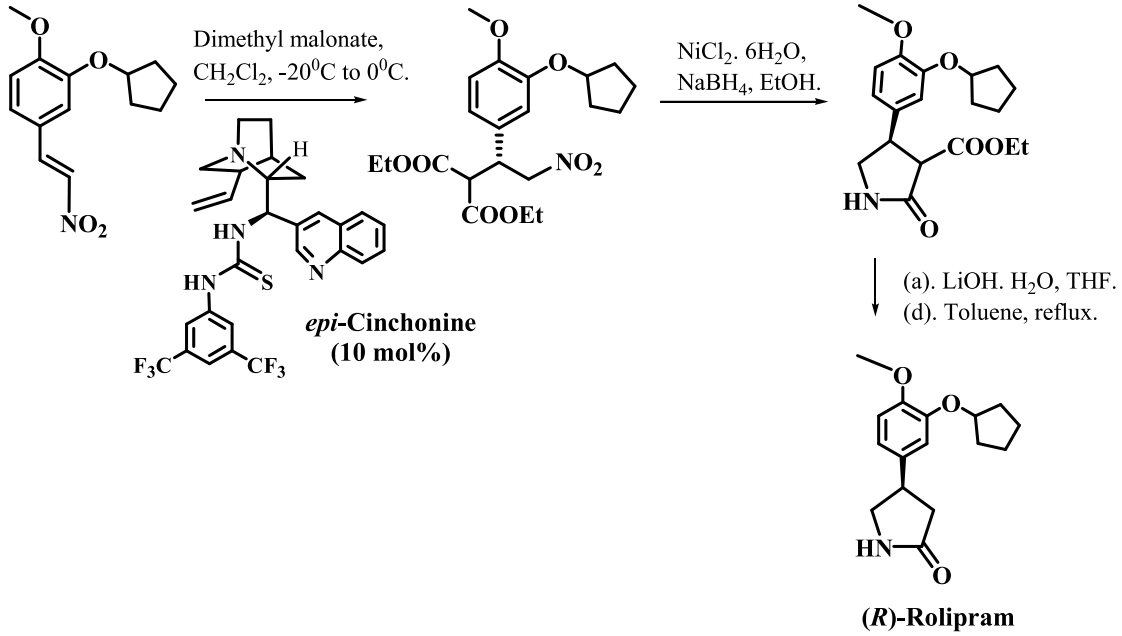
Demnitz *et. al.* [5g] (**Scheme 4-2**) added nitromethane to aryl α,β-unsaturated esters via Michael addition. The resulting racemic nitrobutyric acid was resolved via chromatographic separation of two readily separable diastereomeric amides obtained with (S)-(-)-phenyl ethylamine. Reduction followed by a reflux resulted in formation of Rolipram.

Paraskar *et. al.* [5l] (**Scheme 4-3**) used $NaBH_4$ in combination with $CoCl_2$ catalyst and chiral ligand for reductive cyclizations of a suitably substituted azido α,β-unsaturated ester in the key step to synthesize (*R*)-Rolipram. The intermediate, azido α,β-unsaturated ester, was synthesized via suzuki reaction from Guaiacol.



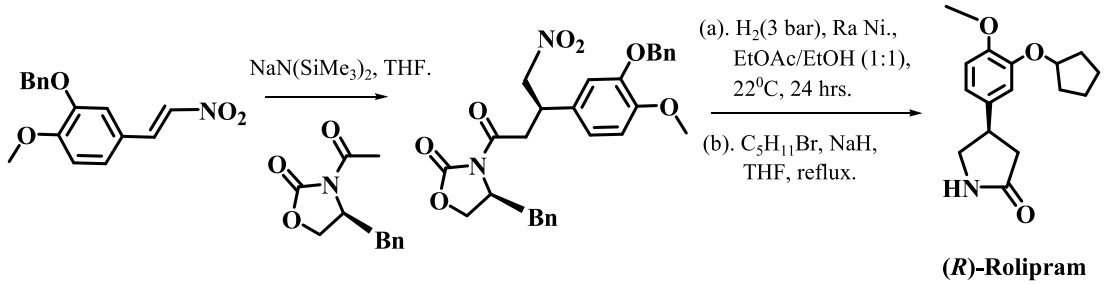
Scheme 4-3. Paraskar *et. al* synthesis of (R)-Rolipram via chiral bis-oxozoline ligand, $NaBH_4$ and $CoCl_2$.

Michael addition is another alternative method used in the synthesis of rolipram. Hynes *et. al* [5m] (**Scheme 4-4**) utilized organocatalyst epicinchonine derivative for the Michael addition of malonate nucleophile to aryl nitro olefin enantioselectively in the key step. The resulting nitro ester was further reduced and cyclized using $NiCl_2$ and $NaBH_4$ followed by decarboxylation to (*R*)-rolipram.



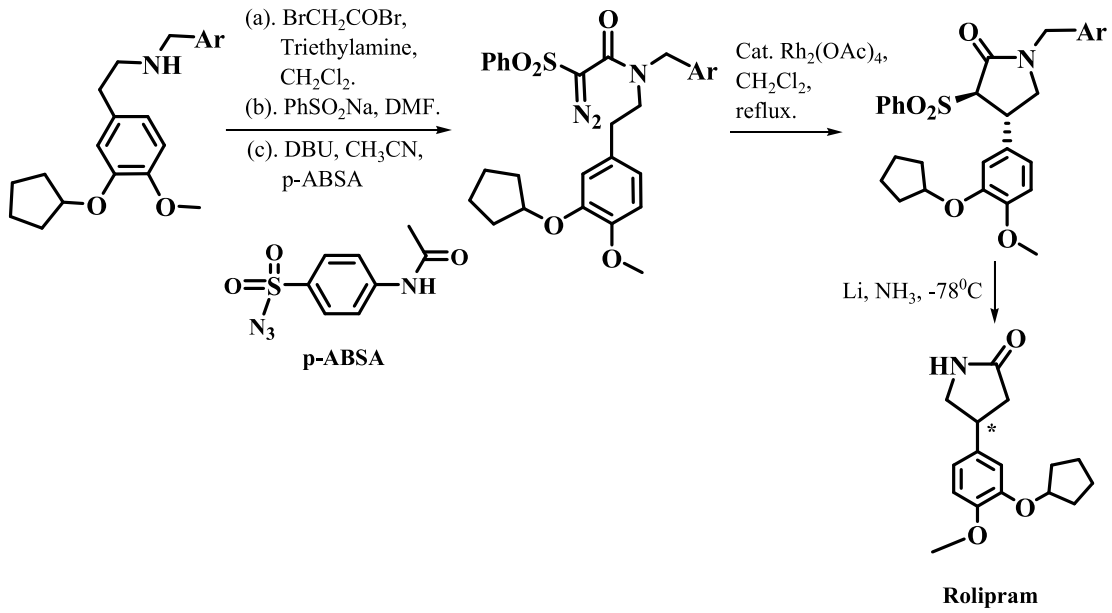
Scheme 4-4. Hynes *et. al* synthesis of (*R*)-Rolipram via Michael addition.

Mulzer *et. a*l [5a] chose enolate of *N*-acetyloxozolidone to add to the nitroolefine in a Michael addition (**Scheme 4-5**). Resulting nitro oxozolidone was recrystallized from methanol to receive single diastereomer which upon catalytic hydrogenation yielded (*R*)-rolipram.



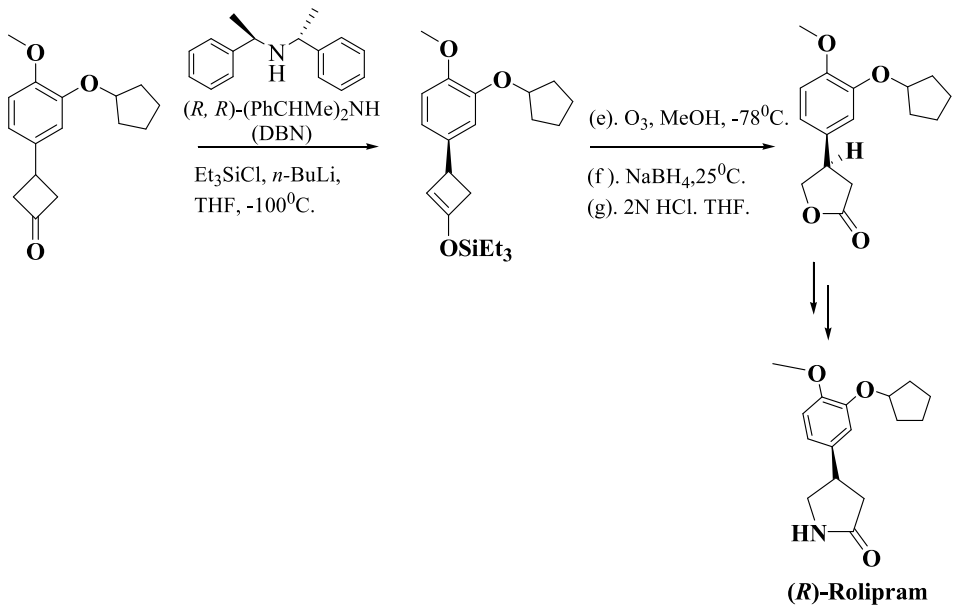
Scheme 4-5. Mulzer *et. al* synthesis of (*R*)-Rolipram via Michael addition.

Yoon *et. al* [5j] utilized Rhodium catalyzed regioselective intramolecular C-H insertion of a α-diazo-α-(phenylsulfonyl)-acetamide in the key step for the synthesis of rolipram (**Scheme 4-6**).



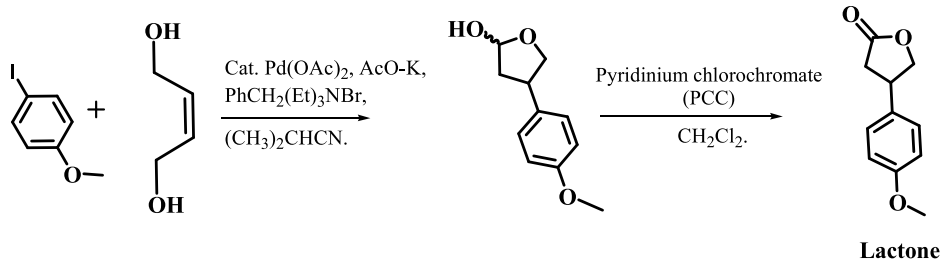
Scheme 4-6. Yoon *et. al* synthesis of (±)-Rolipram via Rhodium catalyzed C-H insertion.

Honda *et. al.* [5d] used the enantioselective silyl ether formation from phenyl cyclobutanone in a key step to synthesize β-substituted γ-butyrolactones. This lactone is further used to synthesize (*R*)-Rolipram (**Scheme 4-7**).



Scheme 4-7. Honda *et. al* [5d] synthesis of (*R*)-Rolipram via β-Aryl-γ-butyrolactone.

Mandai *et. al.* [5p] used $Pd(OAc)_2$ catalyzed coupling of (*Z*)-2-butene-1,4-diols with aryl iodide to synthesize tetrahydro-2-furanols (**Scheme 4-8**) which upon oxidation with PCC led to β-substituted γ-butyrolactones.

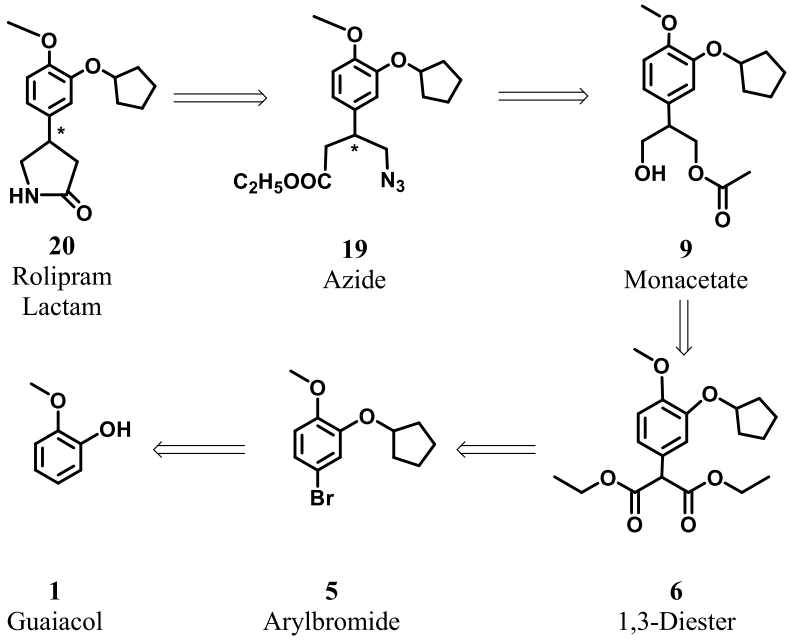


Scheme 4-8. Mandai *et. al* synthesis of β-Aryl-γ-butyrolactone.

4.3 Formal synthesis of (±)-Rolipram

Selective protection of diols[6] is an important concept in which one hydroxyl group is protected while the other hydroxyl could be converted to various functional groups. This selective protection of hydroxyl groups has been reported in various symmetric and unsymmetric diols. Selective protection of alcohols can be achieved with groups like acetates, benzoates and carbonates, e.g., the boc group. Adsorbents like silica gel (SiO_2),[7] Alumina (Al_2O_3),[8] Phase transfer catalysts, [8a] metal catalysts[6c] and lipases[9] have been widely used as catalysts to achieve monoprotection in diols. Infact, lipases have been used in our lab previously for selective protection of diols. Lipase catalyzed disymmetrization of *meso* 1,3-diols is an efficient strategy which has been used in our lab to synthesize (*S*)-Imperanene[10] and Carbafuranomycin[11] analogues. In our effort to synthesize rolipram, we applied the monoacylation of 1,3-diols by silicagel (SiO_2) catalyst to achieve maximum yield. The strategy for the formal synthesis of (±)-Rolipram is shown in Scheme (4-**9**). The synthesis of the azido ester (**19**) from the monoacetate (**9**) represents the formal synthesis of Rolipram. The synthesis of the diester (**6**) was

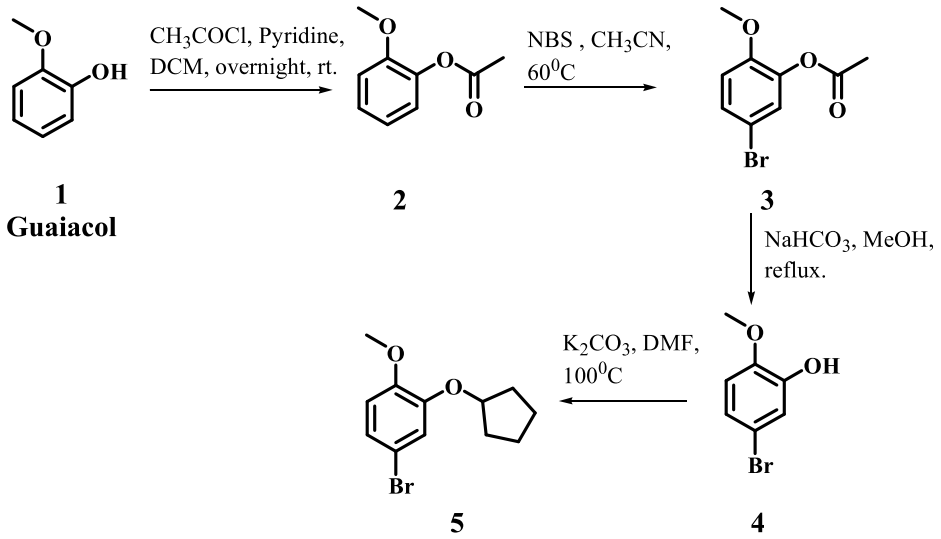
envisioned through cross coupling of the aryl bromide (**5**) to diethylmalonate via Hartwig protocol. The precursor aryl bromide (**5**) was synthesized from guaiacol. The diester (**6**) would then be reduced to the prochiral 1,3-diol (**7**) which would be monoacylated with SiO_2 catalyst. The monoacetate(**9**) would then be converted to the azidoester (**19**) through homologation of free hydroxyl group and azide substitution. The synthesis of the Rolipram from azidoester (**19**) can then be accomplished through the reported protocol described by Honda *et. al.*[5d] The following sections document our effort to accomplish the synthesis of Rolipram by selective protection of diols.



Scheme 4-9. Retrosynthetic analysis for the synthesis of γ-Lactam or (±)-Rolipram.

4.3.1 Synthesis of arylbromide (5)

Aryl bromide (**5**) was synthesized from guaiacol (**1**) in 4 steps by following the reported literature procedure as shown in scheme (4-**10**). [51]

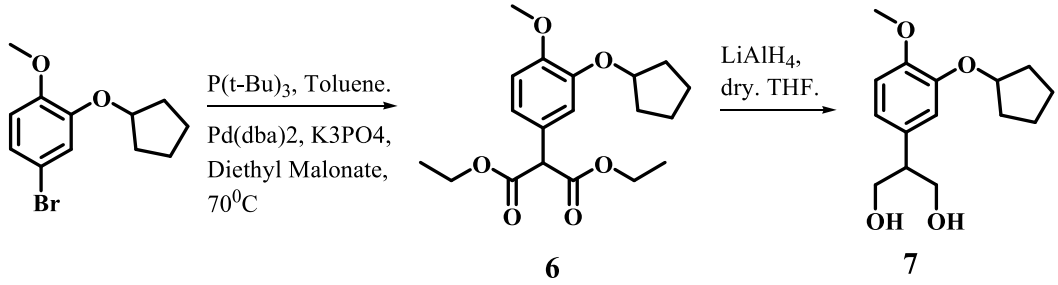


Scheme 4-10. Synthesis of Aryl Bromide from Guaiacol

Phenolic group in Guaiacol **1** was acylated to fecilitate bromine substitution at para to the methoxy group. The acylated guaiacol (**2**) was then treated with NBS (N-Bromosuccinimide) in acetonitrile at 60^0C to receive compound **3** in 85% yield as a light yellow color solid. The deacylation in compound **3** was performed by treating with $NaHCO_3$ in methanol at reflux to receive bromophenol **4** as a light yellow color solid in 95% yield. The cyclopentyl group was introduced in bromophenol **4** by treating **it** with cyclopentyl bromide and K_2CO_3 in DMF at 100^0C to receive aryl bromide **5** as light brown color liquid in 96% yield (**Scheme 4-10**).

4.3.2 Synthesis of the prochiral 1,3-diol (7)

The 1,3-Diester (**6**) was synthesized by palladium catalyzed reaction of aryl bromide (**5**) with diethyl malonate. The coupling reaction was successfully accomplished using $Pd(dba)_2$ catalyst and ligand $P(t\text{-}Bu)_3$ with K_3PO_4 as a base in toluene solvent by following Hartwig protocol[12] (**Scheme 4-11**). Importantly, no product was formed in the coupling of diethyl malonate with aryl bromide (**5**) with various other palladium catalysts e.g., $Pd_2(dba)_3$, $Pd(PPh_3)_4$ and $Pd(OAc)_2$ in combination with PPh_3 and $P(t\text{-}Bu)_3$ ligands. The structure of the 1,3-dister(**6**) was established from NMR spectral data (**Fig. 4-2**). The resonance for the active methylene hydrogen (He) was at 4.4 ppm in the ^1H NMR and at 57.1 ppm in the ^{13}C NMR spectrum. The reduction of 1,3-diester was accomplished by $LiAlH_4$ in dry THF to yield the prochiral 1,3-Diol (**7**) (**Scheme 4-11**).



Scheme 4-11. Synthesis of 1,3-diol **7** via 1,3-diester **6**.

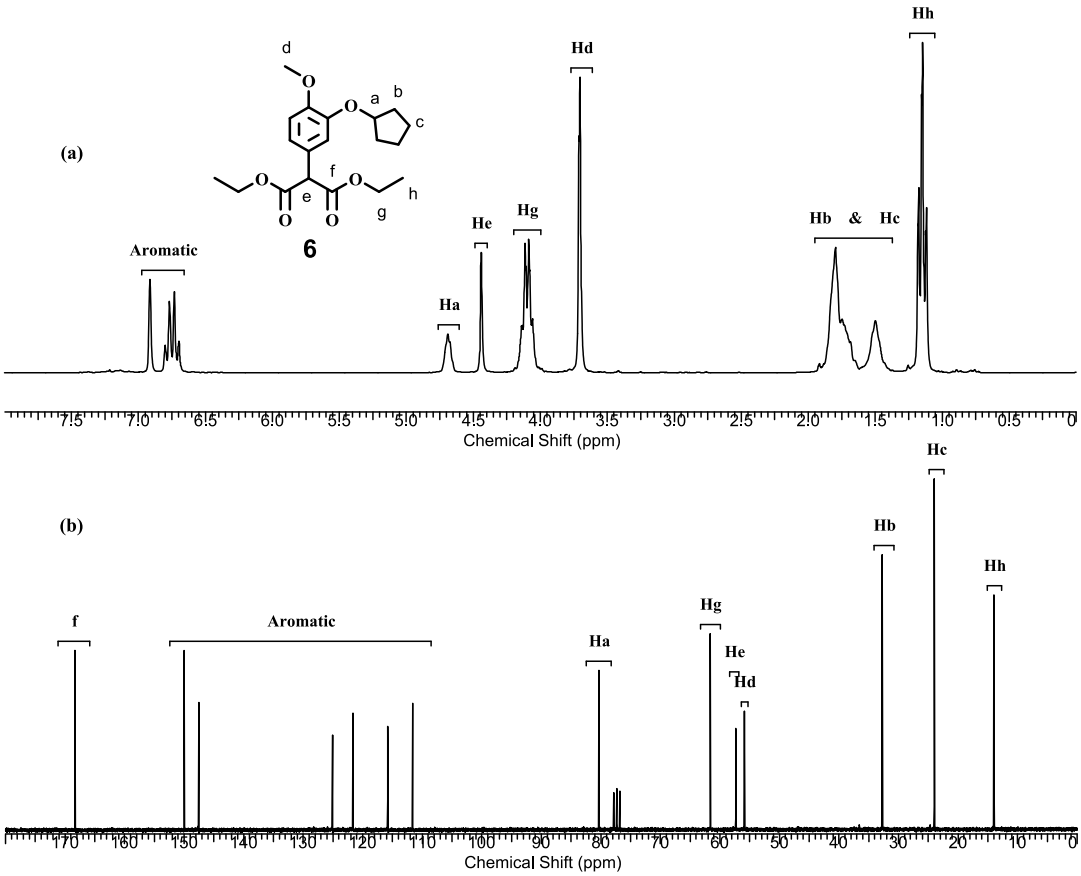


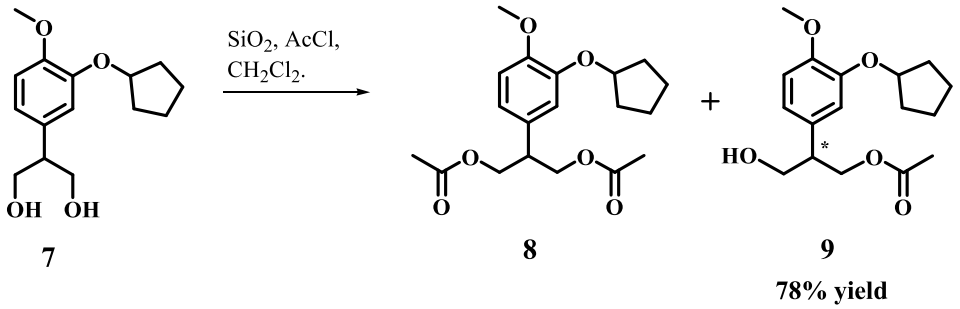
Fig. 4-2. NMR Spectra of 1,3-diester **6.** (a). ^{1}H NMR. (b). ^{13}C NMR

This 1,3-diol is prochiral[9b,c,10] and can be used to introduce enantiomeric center by enantioselective acylation or it can also be diacylated and the resulting 1,3-diacetate (**8**) can be disymmetrized to yield enantiomeric center. The importance of this disymmetrization is that the quantitative conversion (~ 100%) can be achieved as against resolution where the highest yield of enantiopure compound is limited to 50%.

4.3.3 Synthesis of the monoacetate (9) via selective protection

Absorbents such as silicagel (SiO_2) [7] and alumina (Al_2O_3) [8] are reported to be efficient catalysts for the selective monoacetylation of various symmetric diols, hindered alcohols in unsymmetric diols and even per-*O*-acylation of hydroxyl groups in carbohydrates.[6d] Following this approach, 1,3-Diol (**7**) was preferentially acylated to

monoacetate (**9**) using SiO_2 (0.6 equival.) and acetyl chloride (0.94 equival.) in CH_2Cl_2 solvent. The monoacetate (**9**) was isolated in 78% yield along with 1,3-diacetate (**8**) and 1,3-Diol (**7**). The reaction procedure was optimized after screening with various SiO_2 and acetyl chloride ratios to synthesize monoacetate (**9**) in high yield.



7 **8** **9**

78% yield

Scheme 4-12. Synthesis of monoacetate via selective acylation of 1,3-Diol.

The structure of the monoacetate (**9**) was established from its NMR spectrum and its comparison with 1,3-diol (**7**) and 1,3-diacetate (**8**) (**Fig. 4-3**). The methylene hydrogen (He) in 1,3-diol (**7**) and in monoacetate (**9**) was observed at 2.9 and 3.0 ppm respectively, while it was at 3.3 ppm in the 1,3-diacetate (**8**) (**Fig. 4-3**). The resonance for CH_2-OH protons (Hi) in monoacetate (**9**) appears at 3.6 ppm while it is at 3.8 ppm (Hf) in 1,3-diol (**7**). Having the acetoxy methylene (Hf) hydrogens at 4.5 ppm and the CH_2-OH hydrogens at 3.6 ppm (Hi) in monoacetate **9** confirms its structure (**Fig. 4-3**).

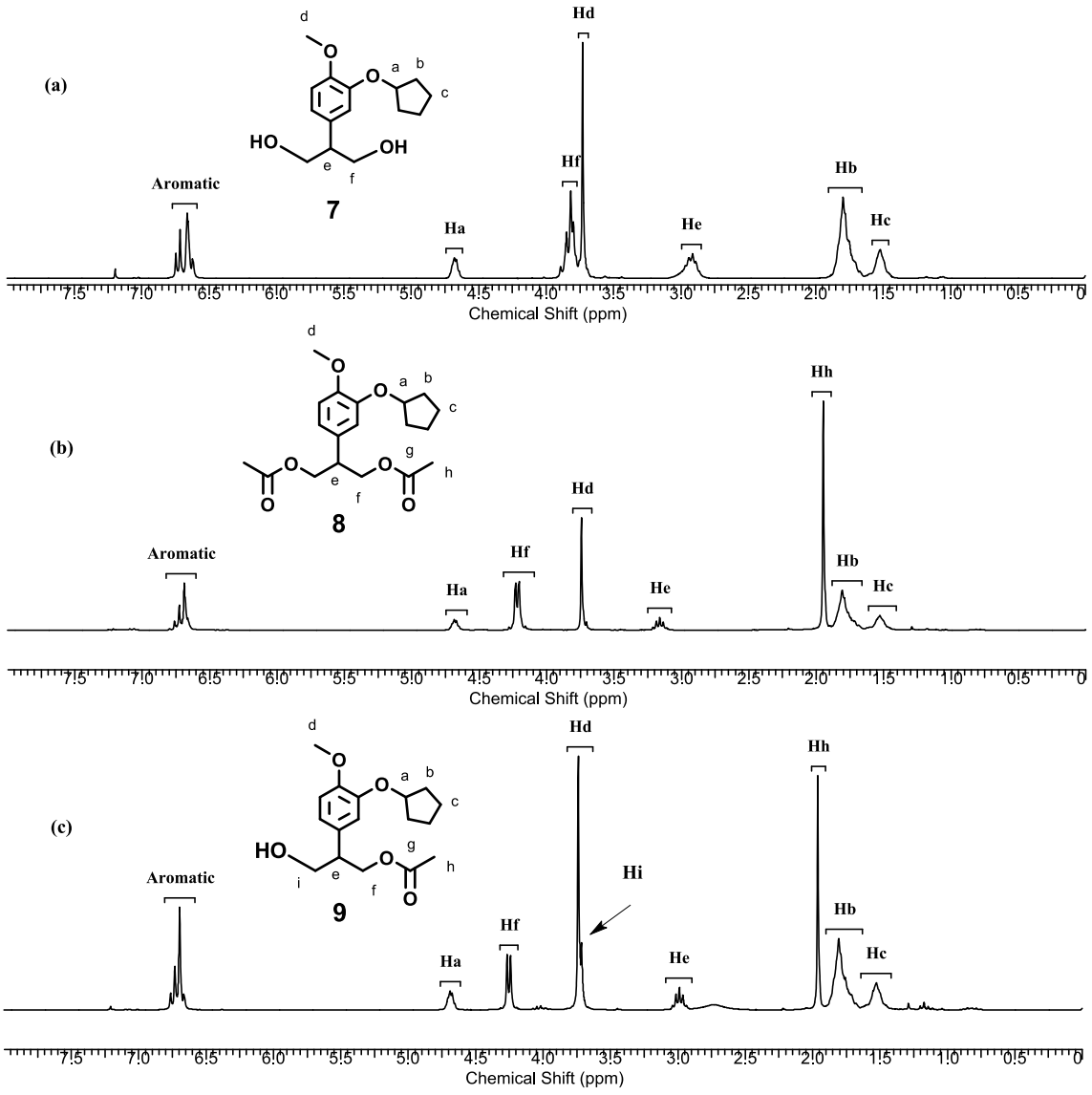
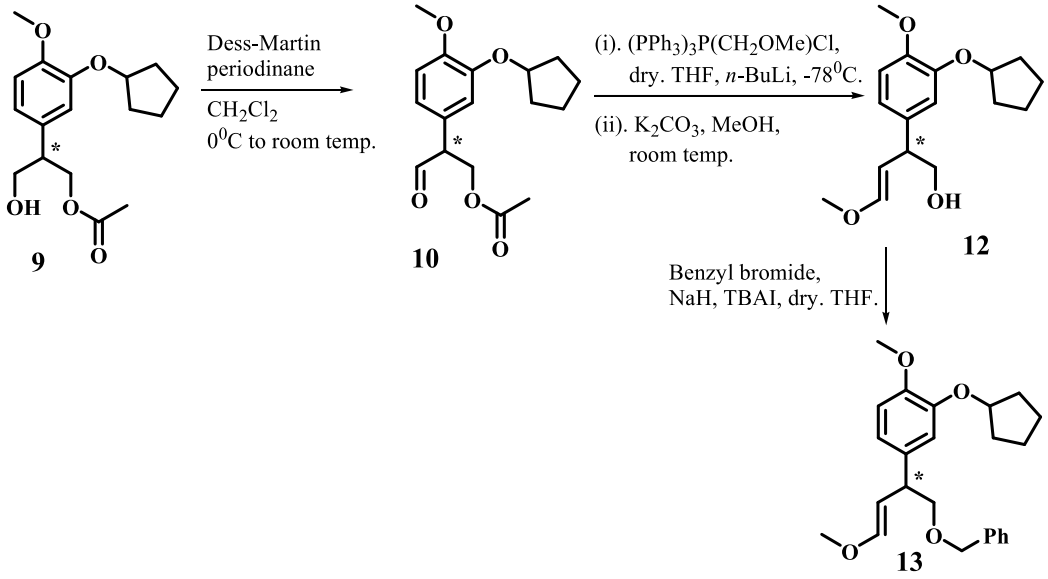


Fig. 4-3. NMR Spectra. (a). 1,3-diol **7** .(b). 1,3-diacetate **8** .(c). monoacetate **9.**

4.3.4 Synthesis of methoxyalkene (13) via homologation

The monoacetate (**9**) was oxidized to the aldehyde (**10**) with dess-martin periodinane[13] in dry dichloromethane in 96% yield (**Scheme 4-13**). The structure of the aldehyde was confirmed by its ^{1}H NMR spectrum in which aldehyde hydrogen (Hj) resonance was present at 9.6 ppm and acetoxy methylene hydrogens (Hf) resonance was present at 4.2 ppm (**Fig. 4-4).**



Scheme 4-13 Synthesis of Alkene **13** from monoacetate **9** via homologation.

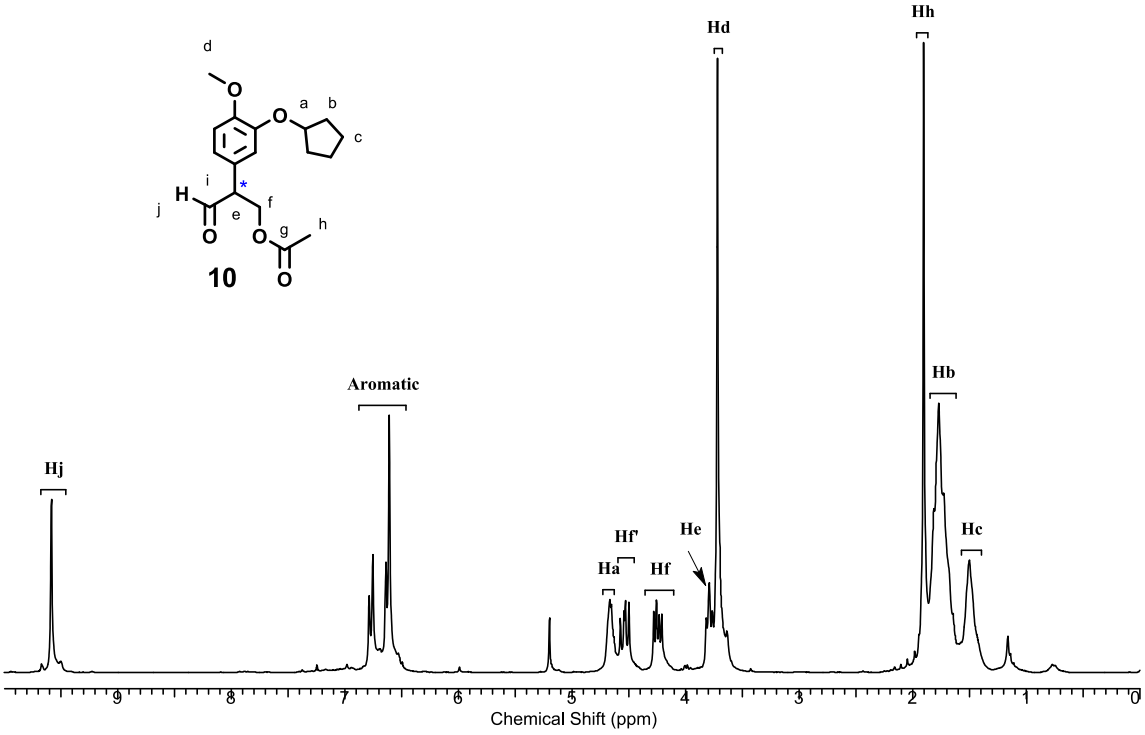


Fig. 4-4. 1H NMR spectra of Aldehyde **10**.

In a homologation reaction, the aldehyde **10** was treated with wittig reagent (methoxymethyl)triphenylphosphonium chloride,[14] at -78^{0}C with *n*-BuLi to yield the vinyl ether ***12*** as a *E/Z* mixture.[15] Presence of both isomers was confirmed by its ^{1}H NMR spectrum (**Fig. 4-5**). For example, the alkene hydrogens showed resonance at 6.4 (Hj) and 4.8 (Hi) in the E isomer and at 6.0 (Hj) and 4.5 (Hi) ppm in the Z- isomer (**Fig. 4-5**). Since this methoxy alkene moiety will be hydrolyzed further to aldehyde, (*E/Z*)-homologated alkene (**11**) was used in next step without separation of the isomers.

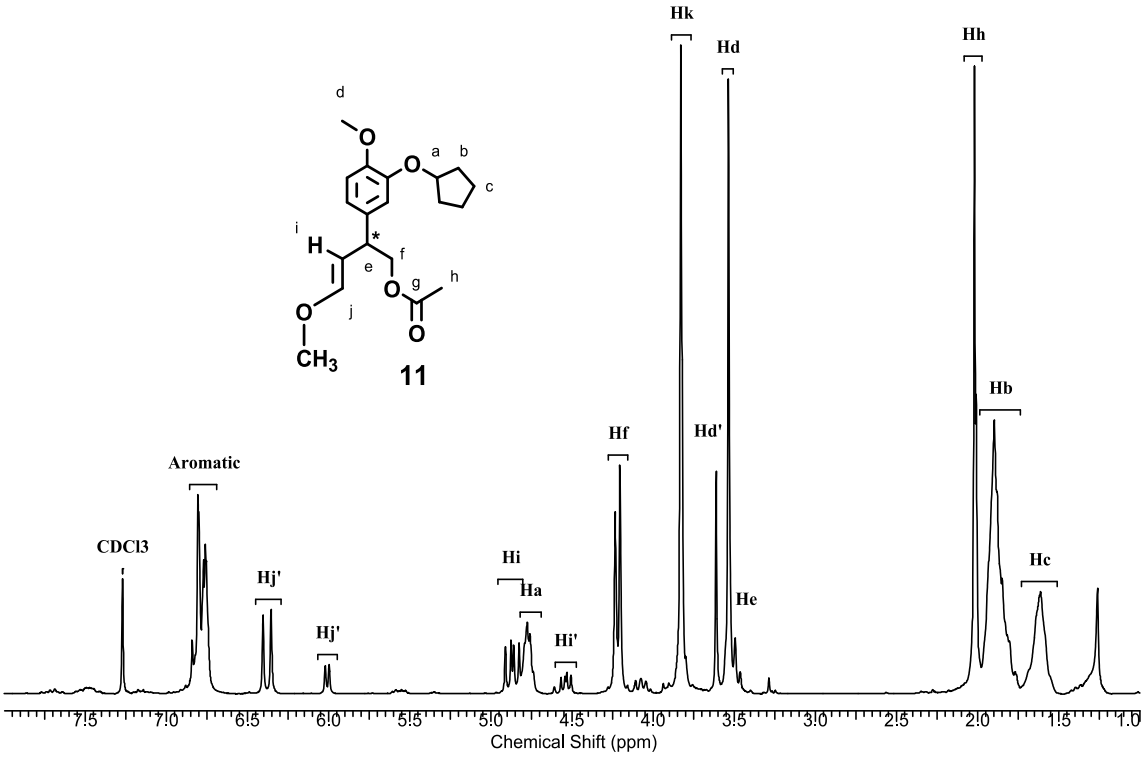
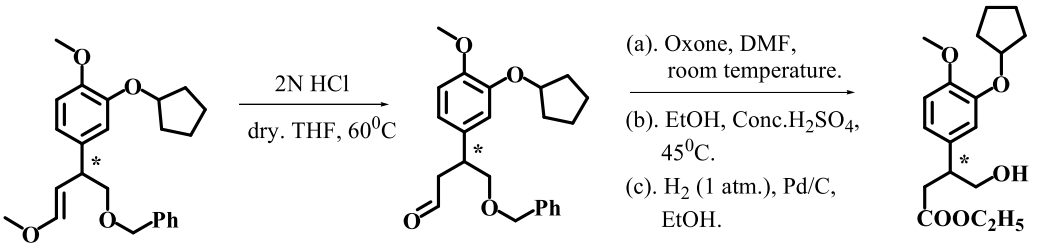


Fig. 4-5. 1H NMR spectra of (*E/Z*)-homologated alkene (**11**).

Since acetate group is labile in acidic condition needed in subsequent steps, (*E/Z*)-homologated alkene (**11**) was hydrolyzed to the (*E/Z*) -homologated alcohol (**12**) by K_2CO_3 and methanol. The hydroxyl group in **12** was then protected with benzyl group[16] by treating with benzyl bromide to yield the benzylated vinylether (**13**) (**Scheme 4-13**).

4.3.5 Synthesis of esteralcohol (17)

Hydrolysis[17] of vinyl ether in **13** with 1N HCl in dry THF at 60^0c led to the aldehyde (**14**) in 97% yield (**Scheme 4-14**). Temperature higher than 60^0c led to cleavage of benzyl protection in the hydrolysis reaction. The presence of resonance for aldehyde proton (Hi) at 9.6 ppm and benzylic protons (Hg) at 4.4 ppm in ^{1}H NMR (**Fig. 4-6**) confirmed the structure of aldehyde (**14**).



13 **14** **17**

Scheme 4-14 Synthesis of Ester alcohol **17**.

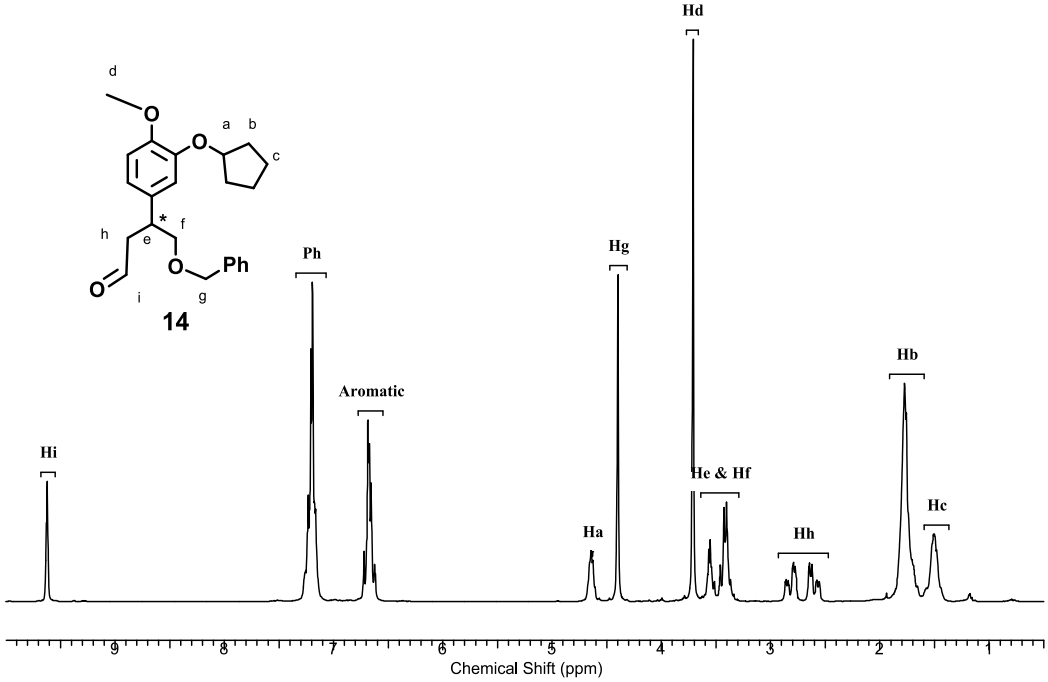


Fig. 4-6. 1H NMR spectra of Aldehyde **14.**

Aldehyde (**14**) was then oxidized[18] with Oxone® (Potassium peroxymonosulfate)[19] to receive carboxylic acid (**15**) in 96% yield. The carboxylic acid was further esterified with ethanol in the presence of conc. H_2SO_4 at 45^0c (**Scheme 4-14**). In this reaction maintaining temperature below 50^0c and excess solvent was crucial to retain benzyl group protection on the alcohol since reflux led to removal of benzyl group on the alcohol.

Once the desired ester (**16**) was received, benzyl protection on the alcohol was removed by hydrogenolysis over palladium and charcoal (Pd/C 10 wt%) (**Scheme 4-14**). The debenzylation was monitored using TLC, after shaking reaction mixture for 12 hrs, all of the starting material was debenzylated. The structure of ester alcohol (**17**) was confirmed by its ^{1}H NMR spectrum (**Fig. 4-7**). The CH_2-OH hydrogens (Hf) showed resonance at 3.6 ppm and ethyl hydrogens (Hi and Hj) showed resonances at 4.0 ppm and 1.2 ppm respectively.

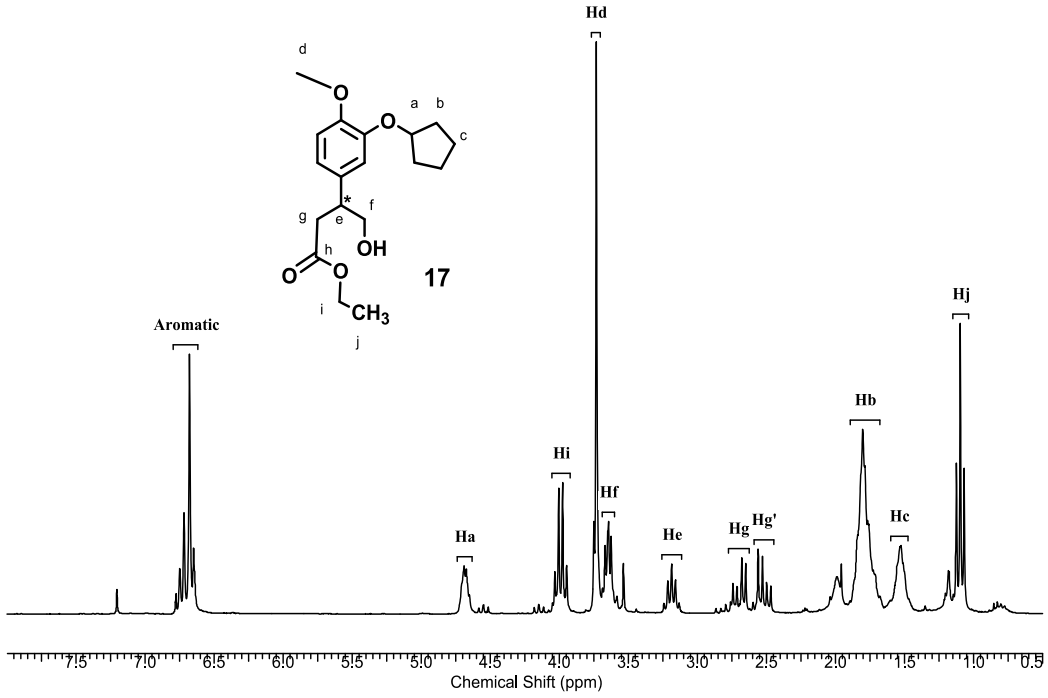
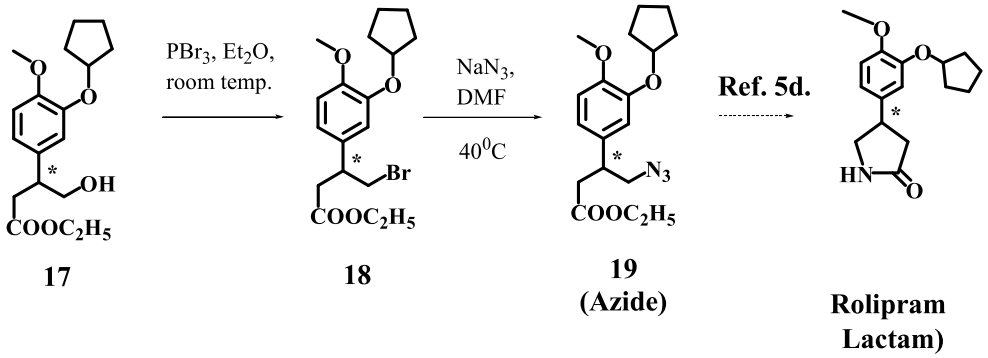


Fig. 4-7. 1H NMR spectra of Ester **17.**

4.3.6 Synthesis of azide (19) for rolipram



Scheme 4-15 Synthesis of azide for the Rolipram from Esteralcohol **17**.

Having established a synthetic route to the ester alcohol (**17**), the next task was to introduce the azide group **(Scheme 4-15**). The bromide serves as facile leaving group to substitute with azide nucleophile,[20] so the alcohol (**17**) was treated with PBr_3 to synthesize bromide (**18**). The bromide was then treated with NaN_3 in dimethylformamide to synthesize the azide (**19**). The structure of the azide(**19**) was established from its ^1H NMR spectrum (**Fig. 4-8**). The resonance for CH_2-N_3 hydrogens (Hf) was present at 3.4 ppm while the methylene (Hg) hydrogens showed resonance at 2.6 ppm. Since azide (**19**) was already transformed into Rolipram by Honda *et. al.*,[5d] the synthesis of azide (**19**) constitutes the formal synthesis to Rolipram.

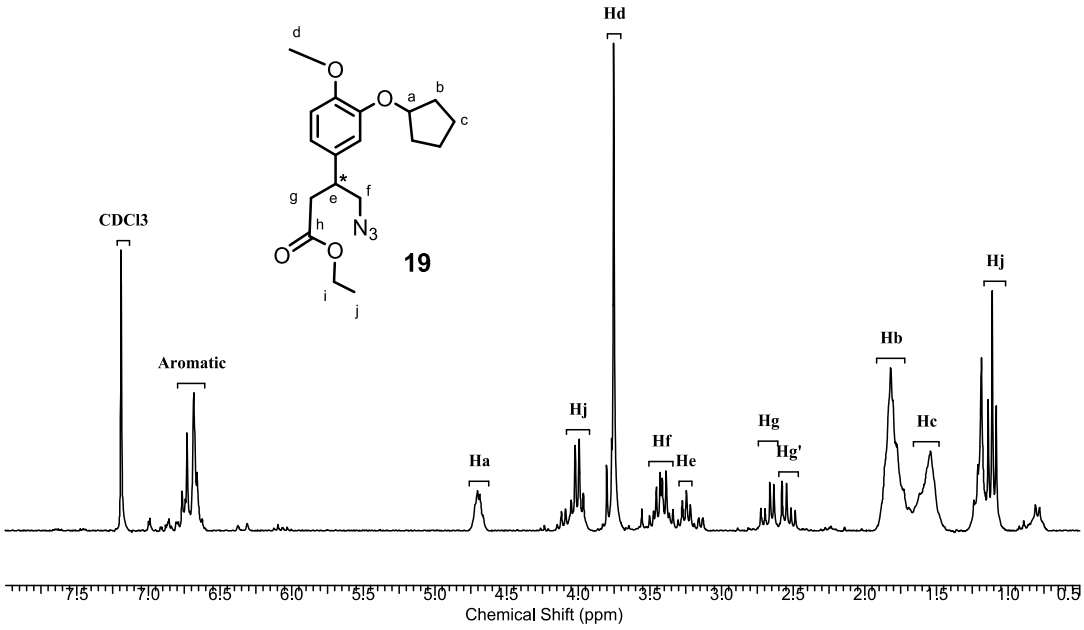
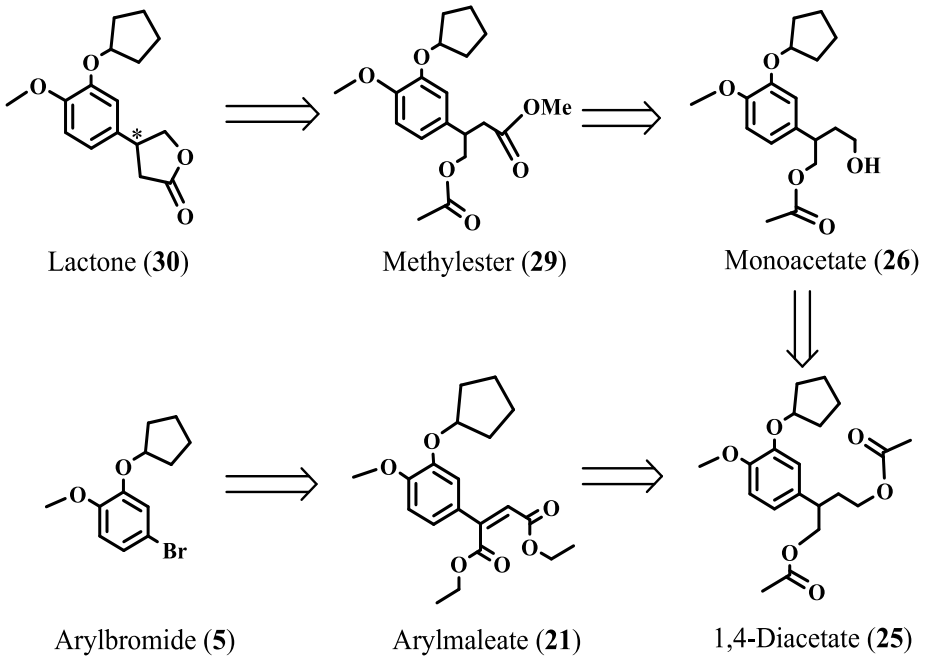


Fig. 4-8. ^{1}H NMR spectra of Azide **19.**

4.4 Synthesis of β-substituted-γ-(±)-Lactone

Previous sections described the utilization of selective protection of hydroxyl group in 1,3-diol for the formal synthesis of Rolipram. In a chemoenzymatic study for the synthesis of γ-lactone, unsymmetric 1,4-diol was also regioselectively deacylated with lipases. The following sections demonstrate the regioselective deacylation of 1,4-diols by *Ps*-30 lipase for the synthesis of γ-lactone. The strategy for the synthesis of β-aryl-γ-lactone was shown in scheme (**4-16).** The synthesis of arylmaleate (**21**) from aryl bromide (**5**) was envisioned via Heck cross coupling protocol. The synthesis of the precursor aryl bromide (**5**) from guaiacol (**1**) was shown in scheme **4-10.** The arylmaleate (**21**) would be converted to the monoacetate (**26**) via lipase catalyzed regioselective deacylation. The monoacetate (**26**) would then be converted to the methyl ester (**29**) via oxidation and esterification. The methyl ester (**29**) would finally be lactonized to the lactone (**30**).



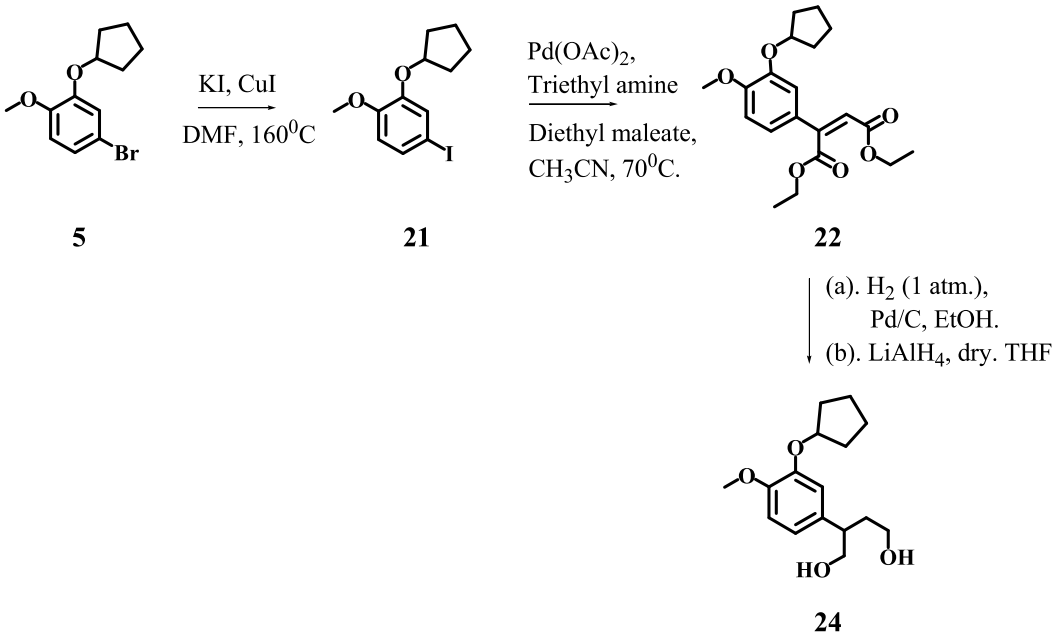
Scheme 4-16 Retrosynthetic analysis for the synthesis of Lactone (**30**) from Aryl bromide (**5**) via monoacetate (**26**).

4.4.1 Synthesis of 1,4-diol (24) via heck reaction

The aryl bromide (**5**) was used in heck cross coupling reaction with diethyl maleate with $Pd(OAc)_2$ catalyst and triethylamine base. No product was observed even at prolonged reaction times and elevated temperatures in the heck reaction. It is not surprising that the ability of aryl halides to undergo oxidative addition with palladium(0) catalysts in heck reaction depends on the bond strengths of Ar-X. The established trend for the ease of cross coupling of aryl halides with activated alkenes in heck reaction is Ar-Cl<Ar-Br<Ar-I.[21]

Aryl bromide (**5**) also has electron donating groups which also led them to participate poorly in heck reaction. So, the aryl bromide was converted to aryl iodide (**21**) since iodide is better leaving group in palladium(0) catalyzed heck reaction. Bromine in aryl bromide was converted to aryl iodide (**21**) in halogen exchange

reaction[22] with KI in the presence of CuI catalyst. The aryl iodide (**21**) was received as pale yellow oil. The structure of the aryl iodide (**21**) was confirmed by its ^{13}C NMR spectrum (**Fig. 4-9**). The presence of resonance for the carbon in aromatic ring with iodide at 82.5 ppm confirmed the structure of aryl iodide (**21**). Same carbon in aromatic ring attached to bromide was showed resonance at 112 ppm in ^{13}C NMR spectrum (**Fig. 4-9**).



5 **21** **22** **24**

Scheme 4-17 Synthesis of 1,4-Diol **24** via Heck arylation of diethylmaleate.

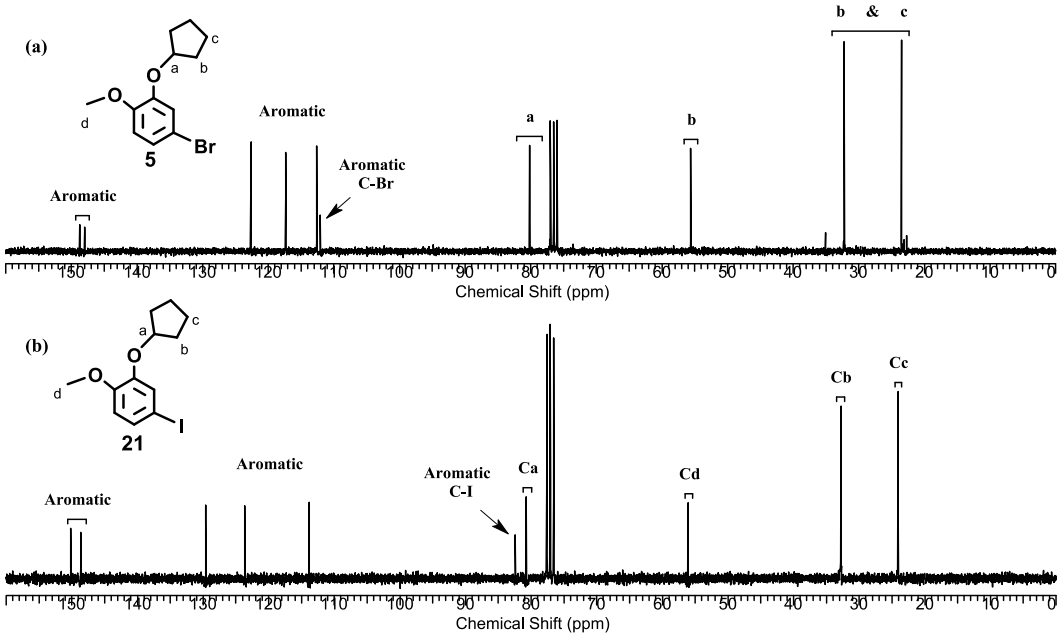
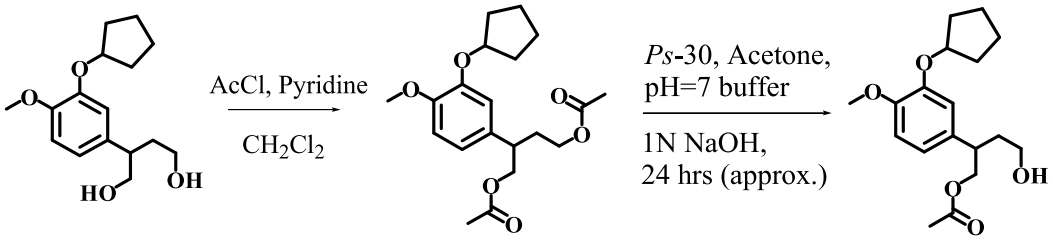


Fig. 4-9. ^{13}C NMR spectra. (a). Aryl bromide **5.** (b). Aryl iodide **21.**

The heck reaction[23] between aryl iodide (**21**) and diethyl maleate was successfully accomplished with $Pd(OAc)_2$ and triethylamine base in CH_3CN yielding aryl maleate (**22**) with 90% yield[24](**Scheme 4-17**). In this reaction, the aryl maleate (**22**) was received as *E/Z* mixture which was used directly in next step without separation of the isomers since alkene will be hydrogenated in the following reaction. The double bond in the aryl maleate (**22**) was hydrogenated to 1,4-diester (**23**) in the presence of Pd/C and H_2 gas at 1 atm pressure.[25] Further reduction of ester groups in 1,4-diester (**23**) was accomplished by using lithium aluminiumhydride (LAH)[26] to 1,4-diol (**24**) (**Scheme 4-17**).

4.4.2 Synthesis of the monoacetate (26) via regioselective deacylation

After synthesizing 1,4-diol successfully via heck reaction, the next step was to introduce selective protection of one hydroxyl group. Monoacylation of the unsymmetric 1,4-diol in a regioselective fashion was proved to be difficult with SiO_2 (silicagel) and acetyl chloride (AcCl). The reaction was yielded the mixture of monoacetates. Next, we studied the effect of lipases on regioselective deacylation of 1,4-diacetate (**25**) since lipases[27] were also proved to be suitable catalysts.



24 **25** **26**

Scheme 4-18 Synthesis of Monoacetate **26** via Regioselective deacylation of 1,4-Diacetate by Lipase *Ps*-30 (Lipase *Pseudomonas Sepacia*).

Acylation of both hydroxyl groups in 1,4-Diol (**24**) was done with AcCl and DMAP to synthesize key intermediate 1,4-Diacetate (**25**). The presence of both acetoxy methylene hydrogens (Hf and Hh) resonance at 4.2 ppm and 3.8-4.0 ppm respectively in the ^{1}H NMR spectrum (**Fig. 4-10)** confirmed the structure of 1,4-diacetate (**25**). The 1,4-Diacetate (**25**) was then regioselectively deacylated with *Pseudomonas Sepacia* (PS-30) lipase in acetone solvent while maintaining the pH of reaction mixture at 7 with 1N NaOH.

The selective deacylation in the diacetate (**25**) was occurred at 4-acetyl group regioselectively in 24 hrs yielding monoacetate (**26**) in 49% yield (**Scheme 4-18**). The structure of the monoacetate (**26**) was confirmed by its ^{1}H NMR (**Fig 4-10**). Previous reports in the literature claimed either slow reaction rates (>40 hrs) or preferential hydrolysis of 1-acetyl group regioselectively when aryl group is present at C2 carbon in 1,4-Diacetate with lipases.[28]

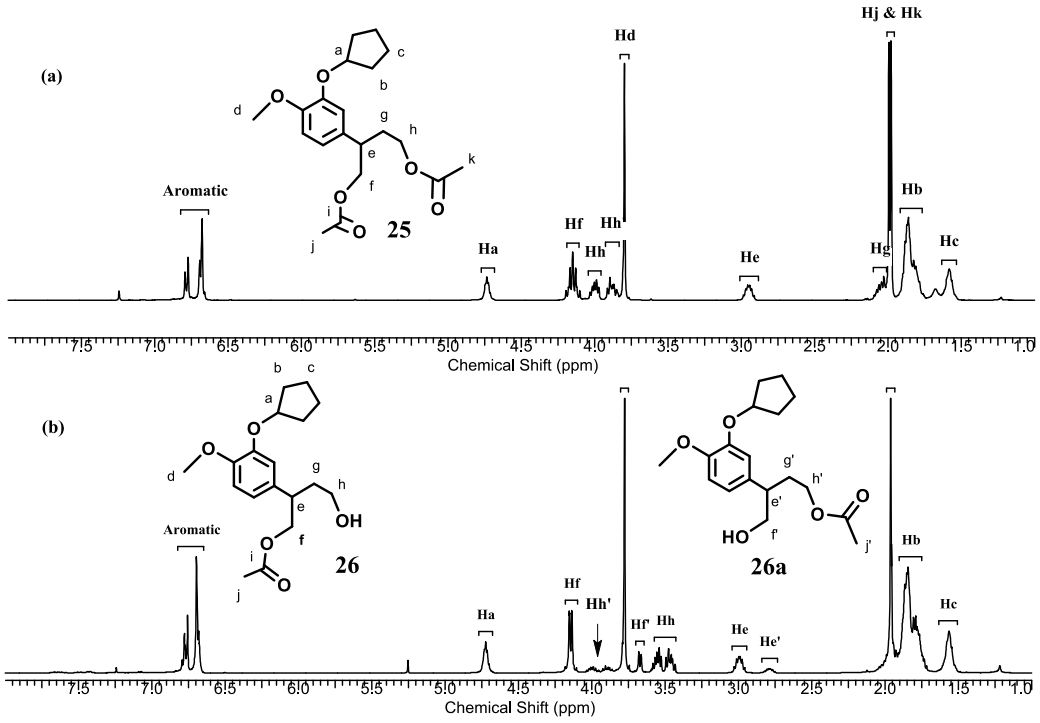
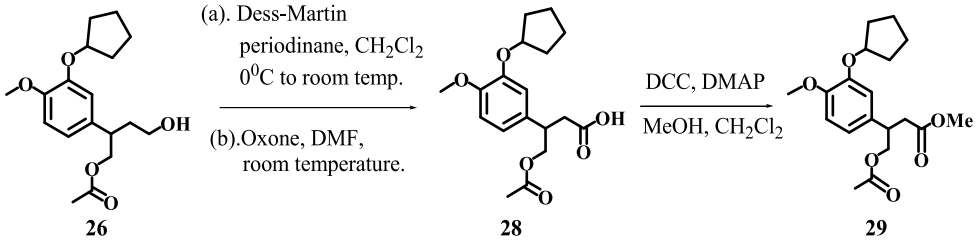


Fig. 4-10. ^{1}H NMR spectra. (a). 1,4-Diacetate **25.** (b). Mixtures of monoacetates **26** and **26a**.

The monoacetate **26** was then taken immediately in the next step since storing of this compound longer than 48 hrs led to transesterification of hydroxyl group yielding both isomers. The presence of both isomers of monoacetate was confirmed by the ^{1}H NMR spectrum [(b) in **Fig. 4-10**] after monoacetate **26** was stored for 50 hrs.

4.4.3 Synthesis of ester (29)



Scheme 4-19. Synthesis of Ester acetate **29** from monoacetate **26**.

The free hydroxyl group in the monoacetate (**26**) was oxidized by treating with dess-martin periodinane[29] to the aldehyde (**27**) which was then further oxidized with Oxone to the carboxylic acid (**28**) (**Scheme 4-19**). Direct esterification[19,30] of the aldehyde (**27**) to the methyl ester (**29**) was not proceded with Oxone rather it yielded the acid (**28**). So the esterification of the acid (**28**) to the methyl ester (**29**) was accomplished with DCC (dicyclohexylcarbodiimide), DMAP (dimethylamino pyridine) and MeOH in CH_2Cl_2 solvent (**Scheme 4-19**). The presence of the resonance for methoxy group (Hk) in ^{1}H NMR spectrum at 3.6 ppm was confirmed the structure of ester (**29**) (**Fig. 4-11**).

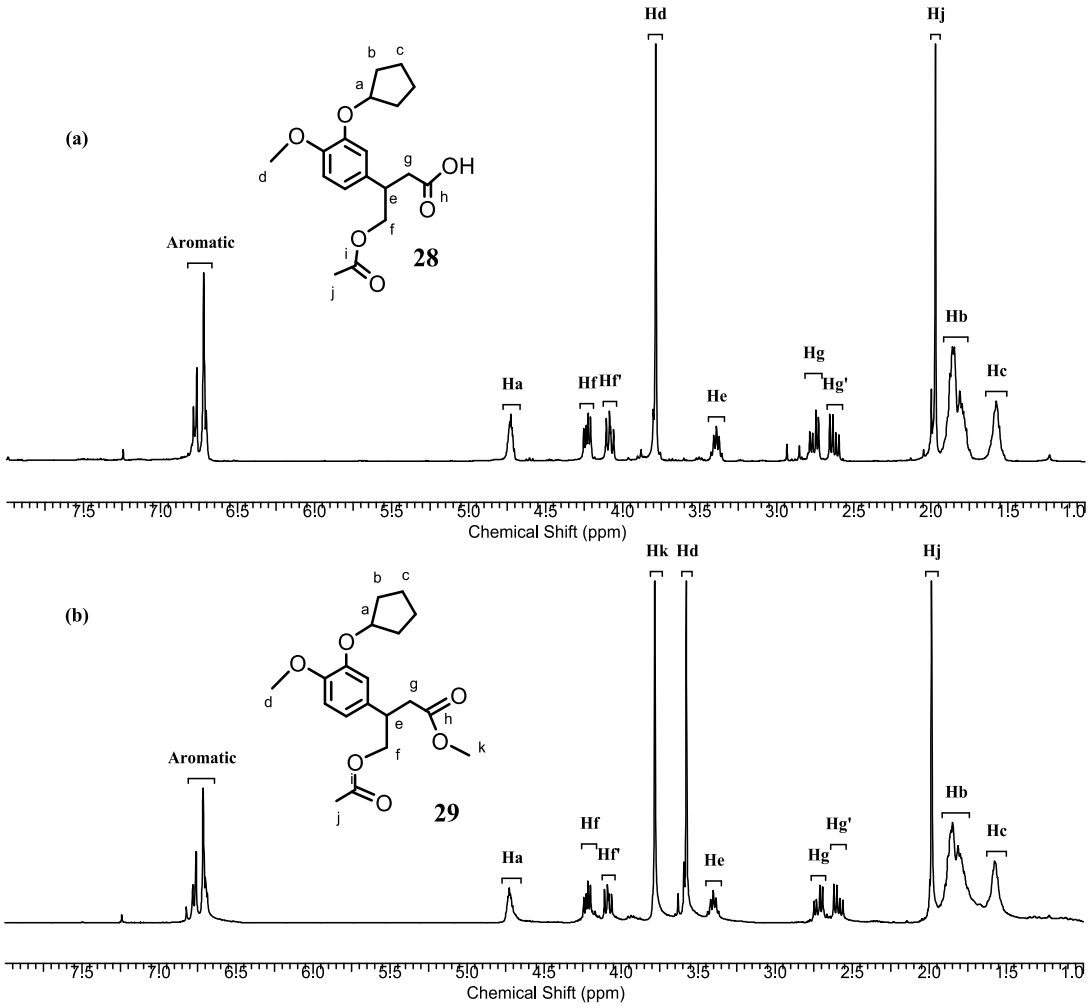
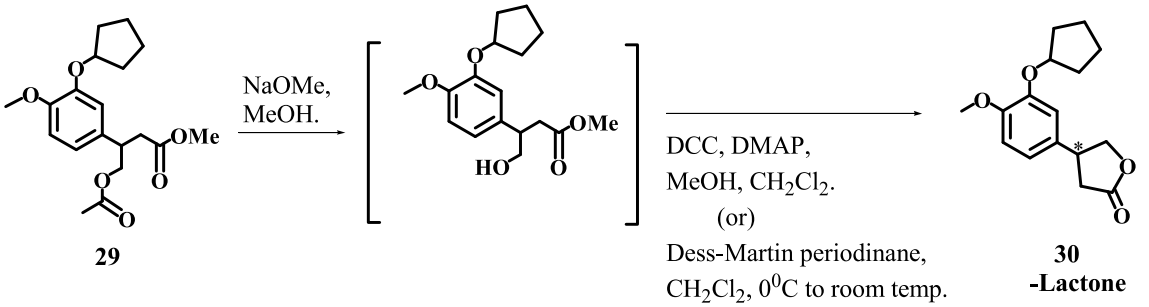


Fig. 4-11. ^{1}H NMR spectra. (a). Carboxylic acid **28**. (b). Ester **29**.

4.4.4 Synthesis of lactone (30) via lactonization

The deacylation of 1-acetoxy group in the ester (**29**) was performed to fecilitate the final assembly of the lactone (**30**). No reaction was observed when the lipase PPL (*Pigliver pancreatic lipase*)[31] was used at pH=7 to hydrolyse 1-acetoxy group.[32] The deacylation of 1-acetoxy group was also unsuccessful even with the Novozyme 435 lipase[33] in the presence of n-butanol and dry. tetrahydrofuran at prolonged reaction times (>60 hrs). Mild hydrolysis of this acetate group with sodium acetate (CH_3COONa)[34] or LiCl[35] was also not proceeded to the product.



Scheme 4-20. Synthesis of γ-Lactone **30** via Ester acetate **29**.

Finally the acetate group in the ester (**29**) was hydrolyzed to alcohol by sodium methoxide (NaOMe)[36] in methanol. After quenching the reaction with dil. acetic acid, the reaction mixture was extracted with dichloromethane. The organic layer was then added by dicyclohexylcarbodiimide (DCC), *N*,*N*-dimethylamino pyridine (DMAP), MeOH and stirred at room temperature for 16 hrs. The lactone (**30**) was then isolated as a major product. Alternatively treating the reaction mixture isolated above with the dess-martin periodinane also yielded the lactone (**30**) as major product with 62% yield instead of aldehyde (**Fig. 4-20**).

4.5 Conclusions

In Summary, We were able to establish the formal synthesis of the Rolipram by employing the selective acylation of the 1,3-diol (**7**) by SiO_2 catalyst. Optical purity in the 1,3-diol (**7**) can also be established via disymmetrization of the corresponding 1,3-diacetate via enzyme catalysis. Key steps for the efficient synthesis of the azide (**19**) starting from the aryl bromide (**5**) includes Palladium catalyzed cross-coupling of the aryl bromide (**5**) with diethylmalonate and selective acylation of the 1,3-diol (**7**). β-substituted-γ-Lactone (**7**), an analogue of the Rolipram, was also synthesized efficiently by the regioselective deacylation of the 1,4-diacetate (**25**). Key steps for the synthesis of

β-substituted-γ-Lactone (**30**) includes the heck arylation of diethyl maleate, regioselective deacylation of β-substituted-1,4-diacetate (**25**) via lipase catalysis and lactonization.

4.6 Experimental Section

4.6.1 General

Lipase PS-30 was generous gifts from Amano Enzymes. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 250 MHz and Varian 400 MHz spectrometer in $CDCl_3$ and DMSO-d6 with TMS as the standard. Chemical shifts are reported in ppm, multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), h (sextet), m (multiplet), dd (doublet of doublet), td (triplet of doublet), qd (quartet of doublet) and bs (broad singlet). Coupling constants are reported in hertz (Hz). All ^{13}C NMR spectra were proton decoupled. Thin-Layer chromatography (TLC) was performed on glass plates coated with 0.25 mm thickness of silica-gel. All solvents were dried and distilled prior to use and organic solvent extracts were dried over anhydrous Na_2SO_4. Toluene and tetrahydrofuran were distilled from sodium and benzophenone and were stored in a dry box. Chromatographic purifications were performed by flash chromatography using silica gel (63-200μ) from Dynamic Adsorbents Inc. $Pd(dba)_2$ and $P(t\text{-}Bu)_3$ were purchased from Aldrich Inc. All reagents and bases were purchased from Aldrich, Fisher Scientific, VWR and used without further purification.

Acetic acid 2-methoxy-phenyl ester (2): To a solution of a guaiacol **1** (15 g, 0.12 mol) and pyridine (22.75 mL, 0.279 mol) in CH_2Cl_2 (300 mL) at 0^0C was added AcCl (13 mL, 0.181 mol) portion wise and the resulting mixture was allowed to stir for overnight at room temperature. The reaction mixture was poured into 1M H_3PO_4 solution (100 mL)

and the aqueous layer was extracted with CH_2Cl_2 (4×50 mL). The Combined organic phases were then washed with brine solution (80 mL), water (2×30 mL), dried over anhydrous Na_2SO_4 and filtered. The resulting light filtrate was concentrated under reduced pressure and chromatographed over silica gel column by eluting with EtOAc:Hexanes (1:9) to receive compound **2** as light brown color oil in 95% yield (18.9 g, 0.114 mol). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.0 - 7.1 (m, 1H), 6.7 - 6.9 (m, 3H), 3.6 - 3.7 (bs, 3H), 2.1 - 2.2 (bs, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 169.1, 151.1, 139.8, 126.9, 122.8, 120.8, 112.4, 55.8, 20.7.

Acetic acid 5-bromo-2-methoxy-phenyl ester (3): To a solution of **2** (13.639 g, 0.082 mol) in CH_3CN (180 mL) was added N-Bromosuccinimide (17.533 g, 0.098 mol) under N_2 atmosphere and the reaction mixture was allowed to stir overnight at 60^0C and filtered. The resulting filtrate was added by EtOAc in a separatory funnel and (200 mL), washed with brine solution (2×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated under reduced pressure and chromatographed over silicagel column to receive **3** as a light yellow color solid (16.17 g, 85% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.3 (dd, *J* = 2.4, 8.7 Hz, 1H), 7.1 (d, *J* = 2.3 Hz, 1H), 6.8 (d, *J* = 8.6 Hz, 1H), 3.8 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 168.6, 150.6, 140.3, 129.6, 126.1, 113.7, 112.0, 56.1, 20.6.

5-Bromo-2-methoxy-phenol (4): To a solution of **3** (1.05 g, 4.3 mmol) in MeOH (6 mL) was added $NaHCO_3$ (0.6 g, 8.0 mmol) and the reaction mixture was allowed to stir overnight at reflux and filtered. The filtrate was then poured into 1N HCl solution (10

mL) and the aqueous layer was extracted with diethyl ether (3×10 mL). The combined organic phases were then washed with brine solution (2×10 mL), dried over anhydrous Na_2SO_4 and filtered. The resulting filtrate was then concentrated under reduced pressure and chromatographed over silicagel column by eluting with EtOAc:Hexanes (1:9) to receive compound **4** as light yellow color solid (0.829 g, 95% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.0 (m, 1H), 6.9 (m, 1H), 6.8 (m, 1H), 6.7 (d, *J* = 8.5 Hz, 1H), 3.8 (s, 3H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 146.5, 145.9, 122.8, 117.8, 113.2, 111.9, 56.1.

4-bromo-2-(cyclopentyloxy)-1-methoxybenzene (5): To a mixture of bromo phenol **4** (23.5 g, 0.115 mol) and K_2CO_3 (31.79 g, 0.225 mol) in DMF (100 ml) was added cyclopentyl bromide (16.5 mL, 0.149 mol) using a syringe at 50^0C with vigorous stirring. After completion of the reaction (monitored by TLC (1:4 EtOAc:Hexanes), the reaction mixture was allowed to cool to room temperature. The reaction mixture was then poured into 1M NaOH solution (100 mL) and EtOAc (200 mL) added to it. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×50 ml). The combined organic extracts were washed with water (30 ml) followed by the brine solution (50mL), dried over anhydrous $MgSO_4$ and filtered. The organic layer was then concentrated under reduced pressure to give crude product (30 g), which was further purified by column chromatography on silica gel using hexanes/EtOAc (9:1) as eluent to afford the aryl bromide **5** (26.5 g, 96% yield, yellowish liquid). ^{1}H NMR (250MHz, $CDCl_3$) δ = 6.9 - 6.8 (m, 2 H), 6.6 (d, *J* = 8.5 Hz, 1 H), 4.6 (bs, 1 H), 3.7 (s, 3 H), 1.9 - 1.6 (m, 6 H), 1.5 (bs., 2 H). ^{13}C NMR (62.5 MHz , $CDCl_3$) δ = 148.7, 148.0, 122.6, 117.3, 112.6, 112.1, 80.1, 55.6, 32.2, 23.5.

2-(3-Cyclopentyloxy-4-methoxy-phenyl)-malonic acid diethyl ester (6): To a schlenk tube containing diethyl malonate (0.176 g, 1.1 mmol) and aryl bromide **5** (0.271 g, 1.0 mmol) were added $P(t\text{-}Bu)_3$ (0.009 g, 0.040 mmol), $Pd(dba)_2$ (0.0115 g, 0.020 mmol), and K_3PO_4 (0.636 g, 3.0mmol) followed by toluene (3.0 mL) under N_2 atmosphere. The tube was sealed with the stopper and the heterogeneous reaction mixture was stirred at 70 °C for 16 hrs and monitored by TLC (1:4 EtOAc:Hexanes). The crude reaction mixture was filtered through a plug of celite and concentrated in vacuuo. The residue was purified by column chromatography on silica gel (10:90 EtOAc/hexanes) to give the desired product **6 (**yellowish liquid, 60% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.8 - 6.95(m, 1H), 6.7 (d, *J* = 9.6 Hz, 2H), 4.6 - 4.7 (m, 1H), 4.4 - 4.4 (m, 1H), 4.1 (d, *J* = 7.1 Hz, 4H), 3.7 (m, 3H), 1.6 - 1.9 (m, 6H), 1.4 - 1.5 (m, 2H), 1.1 (d, *J* = 2.2 Hz, 3H) ^{13}C NMR (62.5 MHz , $CDCl_3$) δ = 168.3, 150.0, 147.5, 125.1, 121.7, 115.8, 111.6, 80.3, 61.6, 57.3, 55.9, 32.7, 24.0, 14.0.

2-(3-Cyclopentyloxy-4-methoxy-phenyl)-propane-1,3-diol (7): To a solution of 6 (0.573 g, 1.63 mmol) in diethyl ether, cooled at 0^0C was added lithium aluminiumhydride (0.745 g, 19.64 mmol). The reaction warmed to room temperature and allowed to stir for a further 8 hours. The reaction was quenched at 0^0C with water (3 mL) and 2N NaOH solution (6 mL) until white color suspension received and filtered. The filtrate was extracted with ethyl acetate (3×25 ml). The organic layer was dried over sodium sulfate and filtered. The organic layer was concentrated under reduced pressure and purified over column chromatography on silica gel (3:7 EtOAc/ Hexanes) to receive 1,3-Diol as a

colorless viscous liquid (68% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.5 - 6.7 (m, 3H), 4.6 - 4.7 (m, 1H), 3.6 - 3.9 (m, 7H), 2.8 - 3.0 (m, 1H), 1.6 - 1.8 (m, 6H), 1.4 - 1.5 (m, 2H).

Acetic acid 3-acetoxy-2-(3-cyclopentyloxy-4-methoxy-phenyl)propyl ester (8): To a solution of 1,3-Diol **(7)** (0.827 g, 3.109 mmol) in CH_2Cl_2 was added acetylchloride (0.8 mL, 12.43 mmol) and pyridine (1.01 mL, 12.43 mmol). The reaction was stirred for 6 hrs at room temperature and quenched with $NaHCO_3$ (20 mL). The reaction mixture was extracted with CH_2Cl_2 (2×25 mL). Combined organic layer was washed with 1N HCl, brine solution and dried over Anhydrous Na_2SO_4. The resulting crude reaction mixture from evaporation under rotavap was purified by column chromatography on silica gel by eluting with EtOAc/Hexanes (1:9) to receive **8** as yellowish liquid (85% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.62 - 4.7 (m, 1H), 4.2 (d, *J* = 6.79 Hz, 4H), 3.7 - 3.8 (m, 3H), 3.1 (t, *J* = 6.56 Hz, 1H), 1.97 (s, 6H), 1.70 - 1.89 (m, 6H), 1.46 - 1.60 (m, 2H)

Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-hydroxy-propyl ester (9): Acetyl chloride (0.183 ml, 2.57 mmol) was added to a stirred solution of diol (0.720 g, 2.7 mmol), SiO_2 (0.100 g, 1.664 mmol) in CH_2Cl_2 (5 ml) under a nitrogen atmosphere and the resulting reaction mixture was stirred at room temperature. When judged complete by TLC (approx. 3 hrs 1:1 EtOAc:Hexanes), the reaction was diluted with Et_2O and washed twice with a saturated $NaHCO_3$ solution and once with brine. The organic layer was dried over anhydrous. $MgSO_4$ and filtered. The organic layer was concentrated under reduced pressure and purified via flash column chromatography (1:4 EtOAc–

hexane mixtures) to receive monoacetate **9** (64% yield, 78% yield based on recovered 1,3-Diol **7**). ^{1}H NMR (250MHz , $CDCl_3$) δ = 6.8 - 6.6 (m, 3 H), 4.7 - 4.6 (m, 1 H), 4.2 (d, *J* = 6.8 Hz, 2 H), 3.8 - 3.6 (m, 5 H), 2.9 (t, *J* = 6.5 Hz, 1 H), 2.0 - 1.9 (m, 3 H), 1.9 - 1.6 (m, 6 H), 1.6 - 1.4 (m, 2 H).

Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-oxo-propyl ester (10): To a solution of **9** (0.156 g, 1.75 mmol) in dry dichloromethane (5 ml) at 0^0C was added Dess-Martin periodinane (5ml, 15%w/v). The reaction was allowed to stir at 0^0C for a further 90 minutes then allowed to warm to room temperature and stirred for an additional 2 hours. A solution of sodium thiosulfate (15ml) was added followed by a solution of sat. $NaHCO_3$ and the solution stirred for an additional 15 minutes, then vacuum-filtered over celite. An additional 30 ml of dichloromethane was added and extracted with aqueous sodium chloride (20 ml). The organic layer was dried over sodium sulfate, concentrated, and the product (amber liquid, 0.508 g, 1.73 mmol, 96% yield) used immediately in the next reaction without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.5 - 9.6 (m, 1H), 6.7 - 6.8 (m, 1H), 6.5 - 6.6 (m, 2H), 4.6 - 4.7 (m, 1H), 4.4 - 4.6 (m, 1H), 4.2 (dd, *J* = 4.4, 5.8 Hz, 1H), 3.6 - 3.8 (m, 5H), 1.8 - 1.9 (m, 3H), 1.6 - 1.8 (m, 6H), 1.4 - 1.5 (m, 2H). ^{13}C NMR (62.5 MHz , $CDCl_3$) δ = 190.8, 170.6, 154.9, 147.8, 128.0, 121.9, 113.1, 110.5, 80.6, 65.8, 56.1, 56.0, 32.7, 24.1, 20.6.

(*E*/Z)-Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-methoxy-but-3-enyl ester (11): To a solution of the Wittig reagent [(Methoxymethyl) triphenylphosphoniumchloride] (2.97 g, 8.68 mmol) in dry THF under a nitrogen atmosphere at -78^{0}C was added n-butyl lithium (5.64 mL, 9.03 mmol). The reaction was allowed to stir for an additional 30 minutes and a solution of the aldehyde **10** (0.508 g, 1.736 mmol) in dry THF (10 ml) was added to the sanguineous solution at -78^{0}C. The reaction was allowed to warm to room temperature and stirred for an additional 19 hours. The solution was then concentrated and added by ethyl acetate (50 ml),brine solution (20 ml). The organic layer was concentrated under reduced pressure and purified over wet silica chromatography using ethyl acetate: hexane (2:8) to receive compound **11** as a viscous, amber liquid (88% yield) as *E/Z* mixture. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.7 - 6.8 (m, 3H), 6.3 (d, *J* = 12.6 Hz, 1H), 6.0 (d, *J* = 8.3 Hz, 1H), 4.8 (d, *J* = 8.3 Hz), 4.7 - 4.8 (bs, 1H), 4.2 (d, *J* = 7.4 Hz, 1H), 3.7 - 3.8 (m, 3H), 3.6 (s, 2H), 3.5 - 3.6 (m, 3H), 1.9 - 2.0 (m, 3H), 1.7 - 1.9 (m, 6H), 1.5 - 1.7 (m, 2H)

(*E*/Z)-2-(3-Cyclopentyloxy-4-methoxy-phenyl)-4-methoxy-but-3-en-1-ol (12): To a solution of the alkene **11** (0.35 g, 1.04 mmol) in dry methanol (20 ml) was added anhydrous potassium carbonate (0.3 g). The reaction was allowed to stir at room temperature for 3 hours upon which it was quenched with sat. $NaHCO_3$ solution (10 mL) and extracted with dichloromethane. The organic layer was dried over anhydrous. Na_2SO_4, concentrated and purified by flash column chromatography using ethyl acetate: hexane (4:6) to receive viscous colorless liquid **12** (0.22 g, 0.75 mmol, 72% yield) ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 6.3 (d, *J* = 12.00 Hz, 1H), 5.9 (d, *J* = 7.27

Hz,), 4.7 (dd, *J* = 8.37, 10.43 Hz, 1H), 4.6 - 4.7 (bs, 1H), 3.7 - 3.7 (m, 3H), 3.5 - 3.7 (m, 2H), 3.4 - 3.5 (bs, 3H), 3.2 (d, *J* = 8.53 Hz, 1H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (bs, 2H)

4-(1-Benzyloxymethyl-3-methoxy-allyl)-2-cyclopentyloxy-1-methoxy-benzene (13): To a solution of alcohol(0.22 g, 0.75 mmol) in dry THF (150 ml) at 0^0C was added sodium hydride (0.04 g, 1.575 mmol) portion wise under a nitrogen atmosphere. The reaction was allowed to stir for 20 minutes and a solution of TBAI and Benzyl bromide (0.154 g, 0.9 mmol) in dry THF (100 ml) was added dropwise over a 10 minute period. The solution was warmed to room temperature and allowed to stir at room temperature for a further 16 hrs. The solution was concentrated under reduced pressure and the viscous liquid taken up in Et_2O (20 ml) and extracted with aqueous ammonium chloride (30 ml). The organic layer was dried over sodium sulfate and concentrated. The product was purified by silica gel chromatography using a gradient eluent of ethyl acetate: hexane (10:90) followed by ethyl acetate: hexane (20:80) to afford **13** (0.2 g, 0.54 mmol, 72% yield) as light yellow viscous oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.1 - 7.3 (m, 5H), 6.6 - 6.8 (m, 3H), 6.3 (d, *J* = 12.8 Hz, 1H), 5.9 (d, *J* = 6.2 Hz), 4.8 (dd, *J* = 7.9, 12.64 Hz, 1H), 4.6 (quin, *J* = 4.58 Hz, 1H), 4.4 (s, 2H), 3.7 - 3.8 (bs, 3H), 3.5 - 3.6 (m, 2H), 3.3 - 3.4 (bs, 3H), 1.7 - 1.8 (m, 6H), 1.4 - 1.6 (m, 2H).

4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyraldehyde (14): To the solution of **13** (0.3 g, 0.78 mmol) in 10 mL dry. THF was added 1 mL 2N HCl. The reaction mixture was then stirred at 60^0C for 16 hrs. After the reaction was completed, and then concentrated, 25 mL of EtOAc and 15 mL of water was added. The phases were

separated and the aqueous phase was extracted with ethyl acetate (3×10 mL). The combined organic phases were washed with 10 mL brine, dried over Na_2SO_4 and filtered. The organic layer was concentrated under reduced pressure and purification by silica gel column chromatography (hexanes/ethyl acetate 1:9) provided 0.28 g (0.759 mmol) (97% yield) of **14** as a colorless oil. ^{1}H NMR (250 MHz, CHLOROFORM-d) δ 9.5 - 9.6 (m, 1H), 7.1 - 7.3 (m, 5H), 6.6 - 6.7 (m, 3H), 4.6 (quin, *J* = 4.50 Hz, 1H), 4.3 - 4.4 (m, 2H), 3.6 - 3.7 (m, 3H), 3.5 - 3.6 (m, 1H), 3.3 - 3.4 (m, 2H), 3.3 - 3.3 (m, 0H), 2.8 (dd, *J* = 10.27, 11.06 Hz, 0H), 2.6 (dd, *J* = 7.42, 8.85 Hz, 1H), 1.6 - 1.8 (m, 6H), 1.4 - 1.5 (m, 2H).

4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid (15): The aldehyde (0.2 g, 0.542 mmol) was dissolved in DMF (6 mL). Oxone (0.217 g, 0.705 mmol) was added in one portion and stirred at room temperature for 3 days. The reaction was monitored by TLC (1:4 EtOAc:Hexanes) and 1N HCl (3 mL) was added to dissolve the salts and EtOAc was added to extract the products. The organic layer was washed with 1N HCl (3x10 mL), brine, dried over Na_2SO_4 and filtered. The organic layer was concentrated under reduced pressure and purified by silica gel column chromatography to receive **15** as a colorless liquid (0.2 g, 0.52 mmol, 96% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.1 - 7.3 (m, 5H), 6.5 - 6.7 (m, 3H), 4.5 - 4.7 (m, 1H), 4.4 (s, 2H), 3.6 - 3.7 (m, 3H), 3.3 - 3.61 (m, 2H), 3.2 - 3.3 (m, 1H), 2.7 - 2.9 (m, 2H), 2.4 - 2.6 (dd, *J* = 11.8, 7.9 Hz, 1H), 1.6 - 1.8 (m, 6H), 1.3 - 1.6 (m, 2H).

4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester (16): The acid **15** (0.115 g, 0.3 mmol) was dissolved in ethanol and stirred at 50^0C for 10 min. 5 drops of Conc. H_2SO_4 was added to above solution at 45^0C and allowed to stir for 16 hrs. The reaction was monitored by TLC (1:4 EtOAc:Hexanes). After starting material was consumed completely, reaction was concentrated and partitioned between diethyl ether and sat. $NaHCO_3$. The organic layer was dried over anhydrous. Na_2SO_4 and concentrated. Product was purified over column chromatography on silicagel by eluting with EtOAc/hexanes (15:85) to receive **16** as colorless liquid (0.108 g, 0.261 mmol, 87% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.1 - 7.3 (m, 5H), 6.5 - 6.8 (m, 3H), 4.5 - 4.7 (m, 1H), 4.3 - 4.5 (m, 2H), 3.8 - 4.0 (m, 2H), 3.6 - 3.8 (m, 3H), 3.2 - 3.6 (m, 3H), 2.7 - 2.8 (dd, *J* = 8.1, 8.1 Hz,, 1H), 2.4 - 2.6 (dd, *J* = 8.1, 8.1 Hz,,,1H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H), 0.9 - 1.1 (m, 3H).

3-(3-Cyclopentyloxy-4-methoxy-phenyl)-4-hydroxy-butyric acid ethyl ester (17): To a solution of **16** (0.108 g, 0.262 mmol) in absolute ethanol (20 mL) was added palladium/charcoal(0.05 g, 10wt%), and the suspension was shaken for 12 hrs under H_2 atmosphere (45 psi) in a Parr apparatus. The suspension was filtered over a short pad of silica gel and the filtrate was evaporated to an oily residue. Product was purified over column chromatography on silica gel to receive alcohol **17** (0.6 g, 0.186 mmol, 72% yield) as yellowish color liquid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.5 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 3.9 - 4.0 (q, *J* = 6.8 Hz, 2H), 3.5 - 3.7 (m, 5H), 3.1 (t, *J* = 7.11 Hz, 1H), 2.4 - 2.7 (dd, *J* = 10.4, 8.2 Hz,2H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H), 1.0 - 1.1 (m, 3H).

4-Bromo-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester (18): To a solution of **17** (0.045 g, 0.1395 mmol) in anhydrous Et_2O (30 mL) was added slowly PBr_3 (0.04 mL, 0.3 mmol) at 0^0C. After stirring for 6 hrs at room temperature, MeOH (5 mL) was added and the resulting mixture was allowed to stir for 1 hr. The organic layer was concentrated under reduced pressure and purified by flash column chromatography on silica gel by eluting with hexanes/EtOAc (9:1) to afford **18** (0.04 g, 96%) as a pale yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.5 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 3.9 - 4.1 (m, 2H), 3.6 - 3.7 (m, 3H), 3.2 - 3.6 (m, 3H), 2.8 - 2.9 (m, 1H), 2.4 - 2.6 (dd, *J* = 8.2, 6.0 Hz 1H), 1.6 - 1.9 (m, 6H), 1.5 - 1.6 (m, 2H), 1.0 (t, *J* = 7.19 Hz, 3H)**.** ^{13}C (DEPT) NMR (62.5 MHz, $CDCl_3$) δ 118.3, 113.6, 111.1, 79.6, 59.5, 55.0, 42.2, 37.9, 37.0, 31.9, 23.0, 13.0.

4-Azido-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester (19): To a solution of Bromide **18** (0.04 g, 0.103 mmol) in DMF (3 mL) was added sodium azide (0.010 g, 0.1557 mmol) and the reaction mixture was heated to 40^0C over night. The mixture was diluted with water (5 mL) and extracted with ethyl acetate (3×5 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The organic layer was concentrated under reduced pressure and purified over column chromatography on silica gel to receive azide **19** (0.02 g, 0.057 mmol, 55% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.6 (d, *J* = 1.11 Hz, 3H), 4.6 - 4.7 (m, 1H), 3.9 - 4.0 (m, 2H), 3.7 (s, 3H), 3.1 - 3.5 (m, 3H), 2.4 - 2.7 (dd, *J* = 8.8, 7.3 Hz 2H), 1.7 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H), 1.1 (t, *J* = 7.11 Hz, 3H). ^{13}C NMR (DEPT) (62.5 MHz, $CDCl_3$) δ 118.5, 113.7, 111.1, 79.5, 59.5, 55.4, 55.0, 40.4, 37.0, 31.8, 23.0.

2-Cyclopentyloxy-4-iodo-1-methoxy-benzene (21): To a solution of aryl bromide **5** (1 g, 3.7 mmol) in DMF (10 mL) was added potassium iodide (9.213 g, 55.5 mmol) and copper(I) iodide (3.52 g, 18.5 mmol). The heterogeneous reaction mixture was stirred at 160^0C for 24 hrs. After the reaction was quenched with 1N HCl, EtOAc (25 mL) was added and insoluble copper(I) was filtered over celite. The filtrate was extracted with EtOAc (50 mL). The combined organic phase was washed with 1N HCl, brine, dried over anhydrous. Na_2SO_4 and filtered. The organic layer was concentrated under reduced pressure and purified over column chromatography on silica gel to receive aryl iodide **21** as a pale yellow oil (0.9 g, 2.83 mmol, 76% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.9 - 7.2 (m, 2H), 6.5 (d, *J* = 8.37 Hz, 1H), 4.6 (dt, *J* = 3.00, 5.92 Hz, 1H), 3.6 - 3.7 (m, 3H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 150.1, 148.7, 129.6, 123.6, 113.7, 82.4, 80.7, 56.1, 32.7, 24.1.

2-(3-Cyclopentyloxy-4-methoxy-phenyl)-but-2-enedioic acid diethyl ester (22): To a solution of aryl iodide **21** (0.9 g, 2.83 mmol) in CH_3CN (5 mL) was added diethyl maleate (1.3 mL, 7.86 mmol), Triethylamine (1.11 ml, 7.86 mmol), $Pd(OAc)_2$ (0.007 g, 0.03 mmol) and stirred at 70^0C for 16 hrs. The reaction mixture was cooled and filtered over celite plug. The filtrate was concentrated in vacuuo and purified over column chromatography on silica gel by eluting with EtOAc/hexanes (1:4) to receive aryl maleate **22** as yellowish liquid (0.92 g, 2.547 mol, 90% yield). ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.8 - 7.0 (m, 1H), 6.8 (s, 1H), 6.6 - 6.8 (m, 2H), 6.1 (s, 1H), 4.5 - 4.7 (m, 1H), 4.3 (q, *J* = 7.11 Hz, 1H), 4.1 (qd, *J* = 7.16, 10.58 Hz, 2H), 3.9 - 4.0(q, *J* = 9.2 Hz, 1H) , 3.6 - 3.7 (m, 3H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H), 0.9 - 1.3 (t, *J* = 8.5 Hz, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 166.5, 165.9, 165.1, 152.3, 150.4, 148.7, 147.8, 146.8, 143.3, 128.1, 126.2, 125.8, 121.1, 116.1, 114.8, 112.7, 111.6, 111.0, 80.5, 61.4, 56.0, 32.7, 24.0, 14.0.

2-(3-Cyclopentyloxy-4-methoxy-phenyl)-succinic acid diethyl ester (23): To a solution of **22** (6.8 g, 18.75 mmol) in absolute ethanol (50 mL) was added palladium/charcoal (0.8 g, 10wt%), and the suspension was shaken for 12 hrs under H_2 atmosphere (45 psi) in a Parr apparatus. The suspension was filtered over a short pad of silica gel and the filtrate was evaporated to an oily residue. The product was purified over column chromatography on silica gel to receive 1,4-diester **23** (6.45 g, 17.7 mmol, 95% yield) as yellowish color liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.7 - 6.8 (m, 3H), 4.7 (td, *J* = 3.1, 6.2 Hz, 1H), 4.0 - 4.1 (m, 4H), 3.9 (dd, *J* = 5.0, 10.1 Hz, 1H), 3.7 - 3.8 (m, 3H), 3.1 (dd, *J* = 10.1, 16.7 Hz, 1H), 2.6 (dd, *J* = 5.4, 16.78 Hz, 1H), 1.7 - 1.9 (m, 6H), 1.5 - 1.6 (m, 2H), 1.1 - 1.2 (q, *J* = 11.3 Hz, 6H).

2-(3-Cyclopentyloxy-4-methoxy-phenyl)-butane-1,4-diol (24): To a solution of **23** (6.45 g, 18.75 mmol) in dry. THF (40 mL), cooled at 0^0C was added lithium aluminiumhydride (2.82 g, 74.32 mmol). The reaction was warmed to room temperature and allowed to stir for a further 8 hours. The reaction was quenched at 0^0C with water and 2N NaOH solution dropwise until white color suspension received and filtered. The filtrate was extracted with ethyl acetate (3×25 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified over column chromatography on silica gel (3:7 EtOAc/ Hexanes) to receive 1,4-Diol as a colorless

viscous liquid (4 g, 14.466 mmol, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 3.7 - 3.8 (d, *J* = 5.9 Hz, 3H), 3.4 - 3.7 (m, 4H), 2.8 (quin, *J* = 6.9 Hz, 1H), 2.5 - 2.7 (m, 1H), 1.7 - 1.9 (m, 8H), 1.4 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.9, 147.7, 134.7, 119.8, 114.9, 112.1, 80.5, 67.5, 61.0, 56.1, 45.4, 35.9, 32.7, 24.0.

Acetic acid 4-acetoxy-2-(3-cyclopentyloxy-4-methoxy-phenyl)-butyl ester (25): To a solution of 1,4-Diol **(24)** (4 g, 14.466 mmol) in CH_2Cl_2 was added acetylchloride (2.05 mL, 28.93 mmol) and pyridine (2.35 mL, 28.93 mmol). The reaction was stirred for 6 hrs at room temperature and quenched with $NaHCO_3$ (20 mL). The reaction mixture was extracted with CH_2Cl_2 (2×25 mL). Combined organic layer was washed with 1N HCl, brine solution and dried over Anhydrous. Na_2SO_4. Resulting crude reaction mixture from evaporation under rotavap was purified by column chromatography on silica gel by eluting with EtOAc/Hexanes (10:90) to receive **25** as yellowish liquid (4.4 g, 12.06 mmol, 84% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 6.7 - 6.8 (m, 1H), 6.6 - 6.7 (m, 2H), 4.6 - 4.7 (m, 1H), 4.1 - 4.2 (m, *J* = 7.0 Hz, 2H), 3.9 - 4.0 (m, 1H), 3.8 - 3.9 (m, 1H), 3.7 - 3.8 (m, 3H), 2.8 - 3.0 (m, 1H), 1.95 - 2.11 (m, 7H), 1.7 - 1.9 (m, 7H), 1.5 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 149.1, 147.6, 132.8, 119.8, 114.8, 112.1, 80.5, 68.3, 62.4, 56.0, 41.3, 32.7, 31.4, 24.0, 20.9.

Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-hydroxy-butyl ester (26): 10.23g (0.0284 mol) of 1,4-diacetate **25**, was stirred in a mixture of phosphate buffer (pH 7.0; 60ml) and acetone (3ml) in a round bottom flask at room temperature. Lipase PS-30 (1.0 g) was added while maintaining the pH of the reaction mixture at 7.0 using 1N NaOH solution. The reaction was stopped when no change in the pH of the reaction medium occurred (approx. 24 hrs). The reaction was monitored by TLC (1:4 EtOAc:Hexanes). The reaction mixture was extracted with ethyl acetate (3 x 200 mL). The organic layer was dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and purified by column chromatography over silica gel using ethylacetate: hexane (1:3) to isolate the monoacetate **26** (49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 4.1 - 4.2 (m, 2H), 3.7 - 3.8 (m, 3H), 3.4 - 3.6 (m, 2H), 2.9 - 3.0 (m, 1H), 1.9 - 2.0 (m, 3H), 1.7 - 1.9 (m, 6H), 1.5 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.1, 149.0, 147.7, 133.5, 119.9, 115.0, 112.2, 80.4, 68.4, 60.5, 56.0, 41.0, 35.3, 32.8, 24.0, 20.9.

Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-oxo-butyl ester (27): To a solution of monoacetate **25** (4.3 g, 13.33 mmol) in dry dichloromethane (20 ml) at 0^0C was added Dess-Martin periodinane (40ml, 15%w/v). The reaction was allowed to stir at 0^0C for a further 90 minutes then allowed to warm to room temperature and stirred for an additional 3 hours. A solution of sodium thiosulfate (30ml) was added followed by a solution of sat. $NaHCO_3$ (30 mL) and the solution stirred for an additional 60 minutes, then vacuum-filtered over celite. An additional 30 ml of dichloromethane was added and extracted with aqueous sodium chloride (20 ml). The organic layer was dried over

sodium sulfate, concentrated, and the product (yellow liquid, 4.2 g, 13.02 mmol, 98% yield) is pure enough by ^{1}H NMR and used immediately in the next reaction without further purification.^{1}H NMR (400 MHz, $CDCl_3$) δ 9.6 - 9.7 (m, 1H), 6.6 - 6.8 (m, 3H), 4.6 - 4.8 (m, 1H), 4.0 - 4.3 (m, 2H), 3.7 - 3.8 (m, 3H), 3.4 - 3.5 (m, 1H), 2.7 - 2.8 (m, 2H), 1.7 - 2.0 (m, 9H), 1.5 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.7, 170.8, 149.3, 147.7, 132.2, 119.6, 114.8, 112.1, 80.4, 67.8, 56.0, 46.8, 38.5, 32.8, 24.02, 20.9.

4-Acetoxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid (28): The aldehyde **27** (5.62 g, 17.541 mmol) was dissolved in DMF (20 mL). The Oxone (5.059 g, 26.312 mmol) was added to above solution in one portion and stirred at room temperature for 24 hrs. The reaction was monitored by TLC (1:4 EtOAc:Hexanes) and 1N HCl (3 mL) was added to dissolve the salts and EtOAc was added to extract the products. The organic layer was washed with 1N HCl (3x10 mL), brine and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and purified by silica gel column chromatography to receive **28** as a colorless liquid (5.510 g, 16.38 mmol, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.7 (tt, *J* = 3.17, 6.00 Hz, 1H), 4.1 - 4.2 (dd, *J* = 7.8, 10.9 Hz, 1H), 4.0 (dd, *J* = 7.8, 10.9 Hz, 1H), 3.7 - 3.8 (m, 3H), 3.3 - 3.4 (m, 1H), 2.7 - 2.8 (m, 1H), 2.5 - 2.6 (m, 1H), 1.7 - 2.0 (m, 9H), 1.5 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.3, 170.8, 149.2, 147.6, 132.3, 119.5, 114.8, 112.1, 80.5, 67.7, 56.1, 40.5, 37.5, 32.7, 24.0, 20.7.

4-Acetoxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid methyl ester (29): To a solution of the acid **28** (5.51 g, 16.38 mmol) in dichloromethane (30 mL) was added dicyclohexyldicarbodiimide (DCC) (4.4 g, 21.3 mmol), dimethylaminopyrimidine (DMAP) (0.2 g, 1.638 mmol) MeOH (13.2 mL) and stirred at room temperature for 24 hrs. The resulting reaction mixture was quenched by adding 1N HCl (20 mL) and extracted with CH_2Cl_2 (2×50 mL). Combined organic phase was washed with brine, dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure and purified by silica gel column chromatography to receive **29** as a colorless liquid (4.87 g, 13.923 mmol, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 4.1 - 4.3 (m, 1H), 4.0 (dd, *J* = 7.61, 11.13 Hz, 1H), 3.7 - 3.8 (m, 3H), 3.5 - 3.6 (m, 3H), 3.3 - 3.4 (m, 1H), 2.6 - 2.7 (dd, *J* = 7.8, 7.8 Hz, 1H), 2.5 - 2.6 (m, 1H), 1.9 - 2.0 (m, 3H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2, 170.7, 149.2, 147.5, 132.6, 119.5, 115.0, 112.1, 80.6, 67.5, 56.0, 51.5, 40.6, 37.6, 32.6, 24.0, 20.8.

4-(3-Cyclopentyloxy-4-methoxy-phenyl)-dihydro-furan-2-one (30): To a solution of ester acetate **29** (0.160 g, 0.456 mmol) in dry. MeOH (4 mL) was added a solution prepared from sodium (0.1 g) and dry. MeOH (4 mL) and the resulting mixture was stirred for 3 hrs at room temperature. The reaction was quenched with glacial acetic acid until pH=7 and water (5 mL) was added. The resulting reaction mixture was extracted with CH_2Cl_2. To this reaction mixture was added DCC (0.147 g), DMAP (0.025 g) and stirred at 40^0C for 6 hrs. The reaction mixture was then concentrated without heat under reduced pressure and the crude product was purified by column chromatography on silica

gel by eluting with EtOAc/Hexanes (10:90) to receive **30** as yellowish liquid (0.103 g, 3.74 mmol, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.6 - 6.8 (m, 3H), 4.6 - 4.7 (m, 1H), 4.6- 4.5 (m, 1H), 4.0-4.2 (m,1H), 3.7 - 3.8 (m, 3H), 3.5 - 3.6 (m, 1H), 2.7 - 2.9 (m, 1H), 2.5 - 2.6 (m, 1H), 1.6 - 1.9 (m, 6H), 1.4 - 1.6 (m, 2H).

4.7. References

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Appendices

Appendix-A

Spectroscopic data for compounds of chapter 2

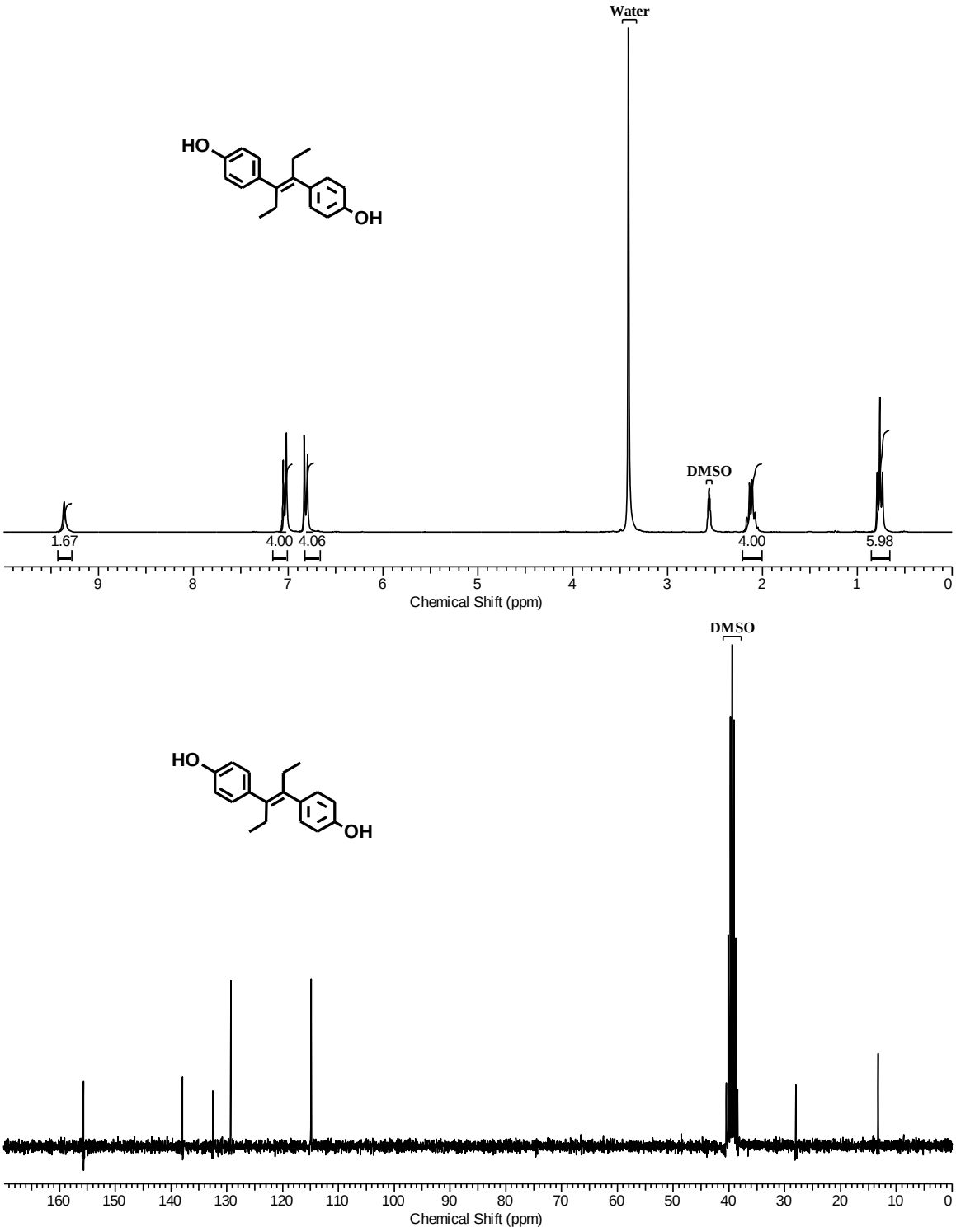


Fig. A-1 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-[1,2-diethyl-1,2-ethenediyl]bis Phenol (**6**).

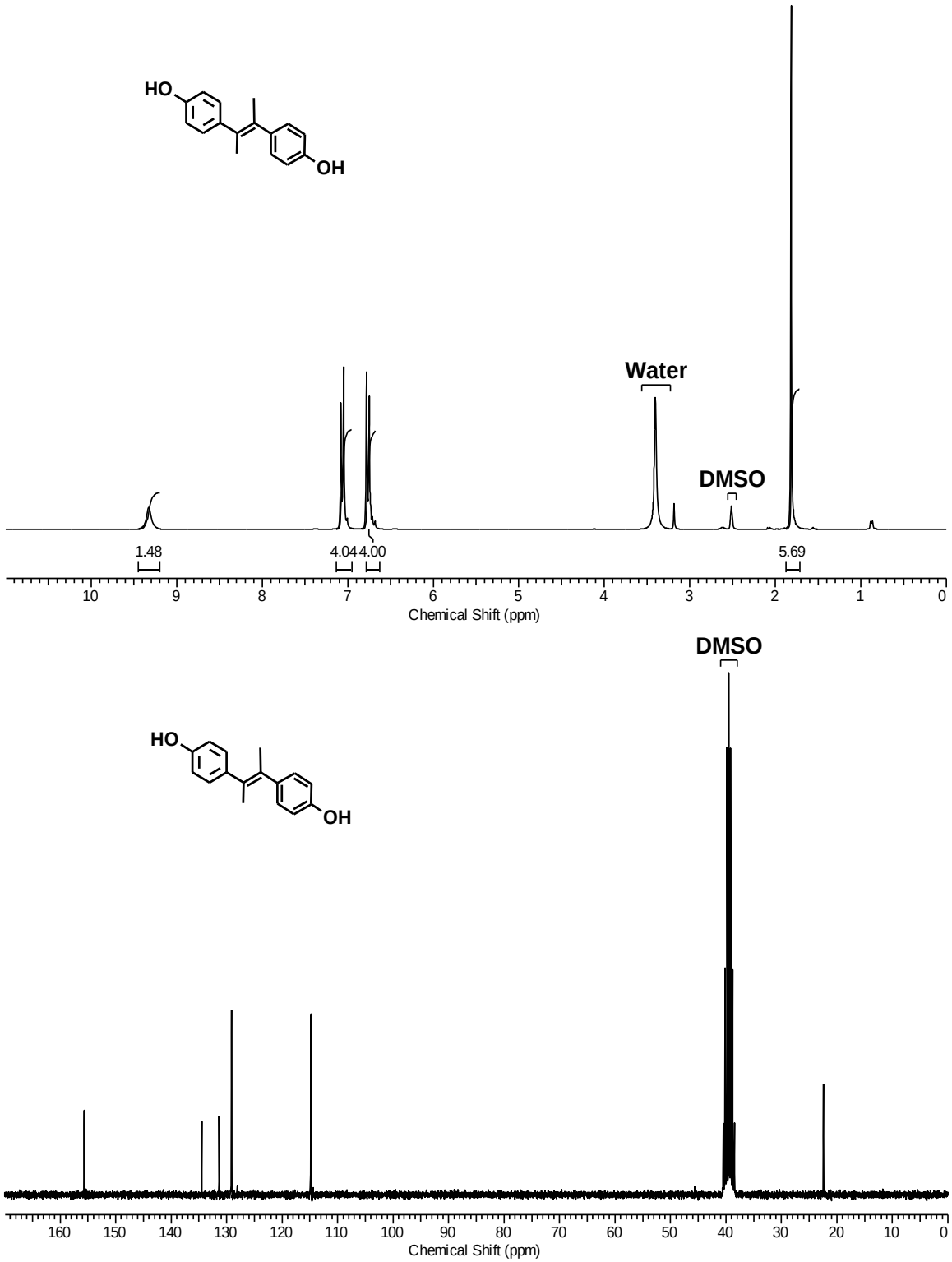


Fig. A-2 ^{1}H and ^{13}C NMR spectra of *E/*Z-4,4'-[1,2-dimethyl-1,2-ethenediyl]bis Phenol **(7)**.

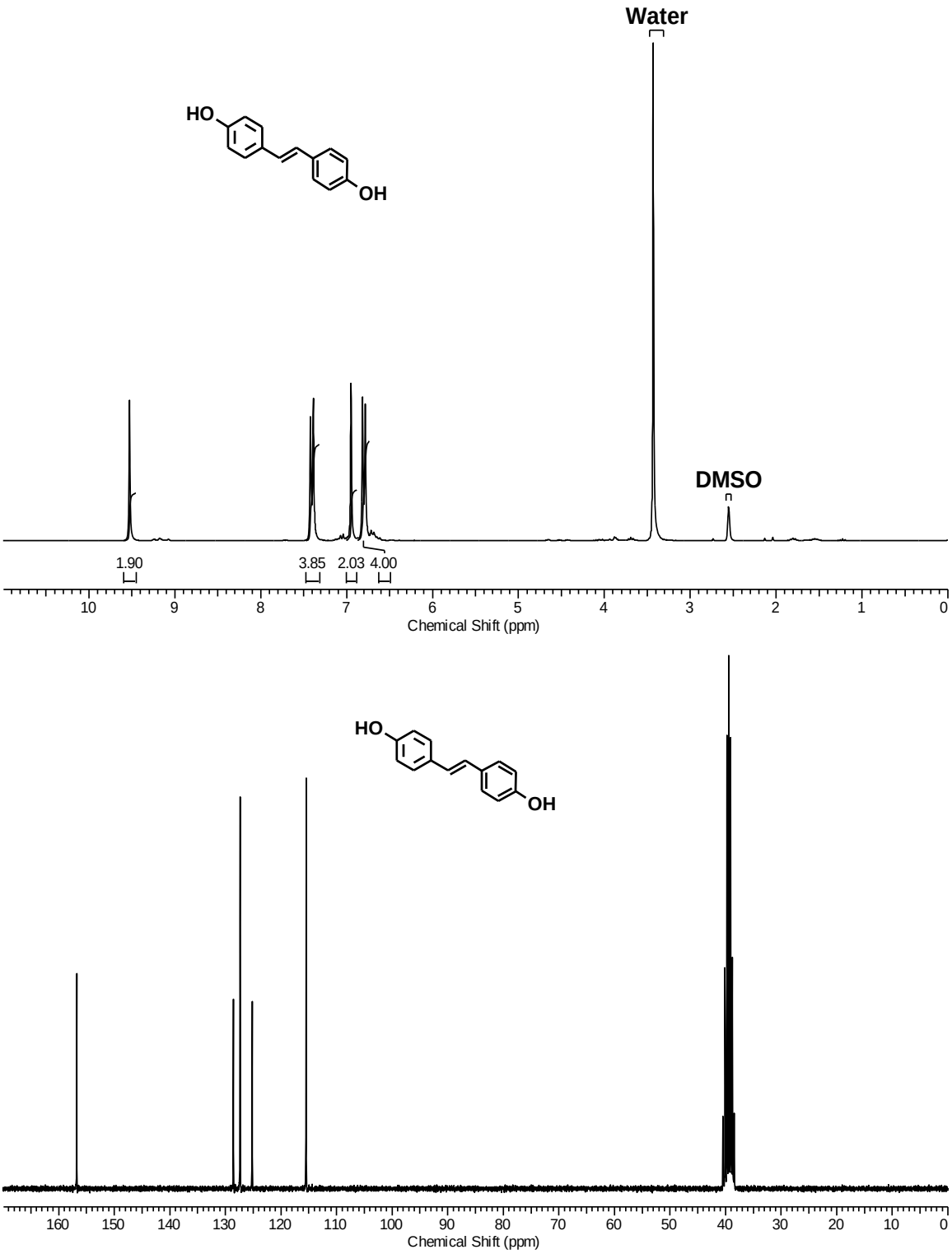


Fig. A-3 ^{1}H and ^{13}C NMR spectra of *E*- 4,4'-(1,2-ethenediyl)bis Phenol **(8)**.

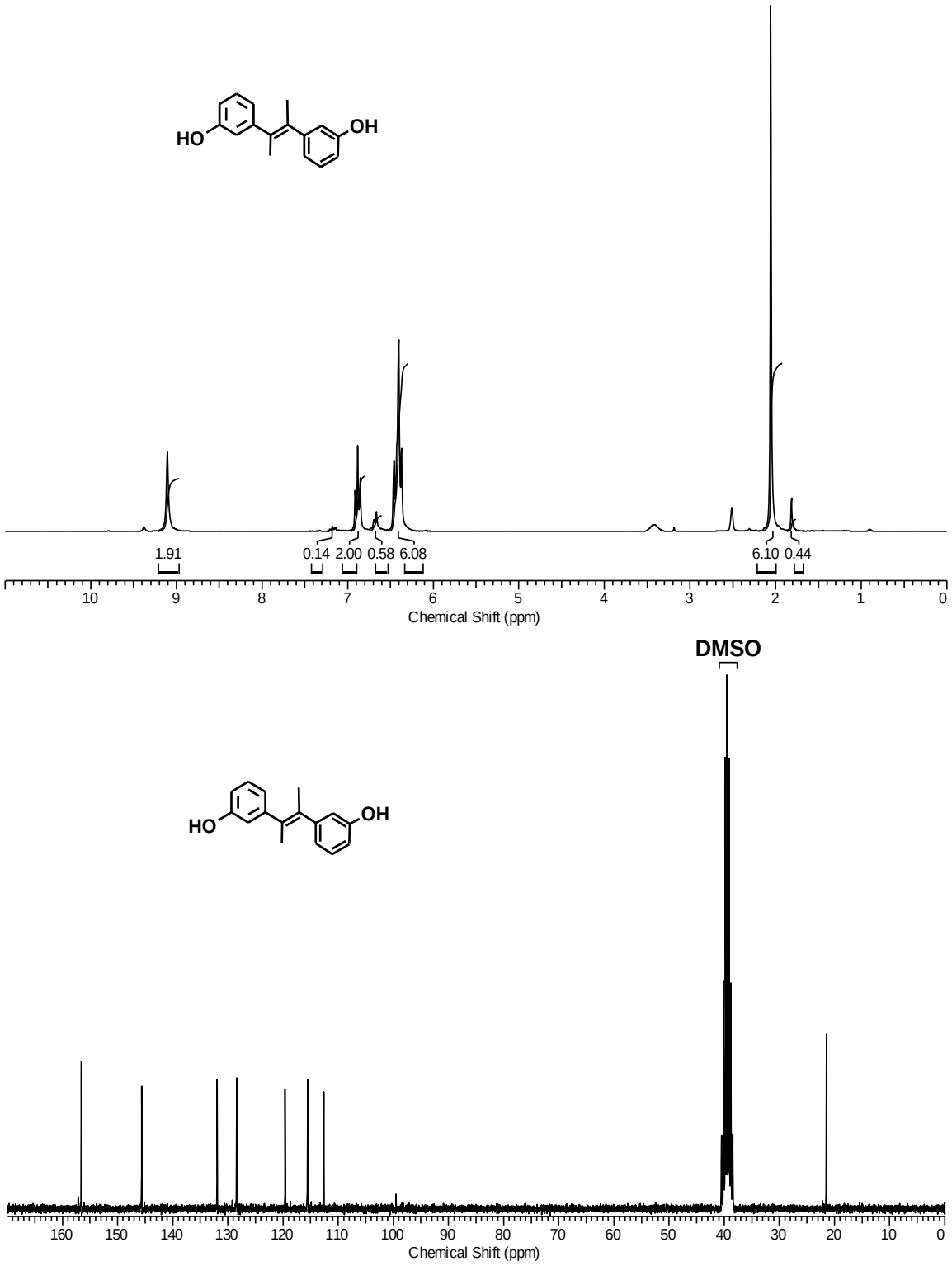


Fig. A-4 ^{1}H and ^{13}C NMR spectra of *E*/*Z*-3,3'-[1,2-dimethyl-1,2-ethenediyl]bis Phenol **(9)**.

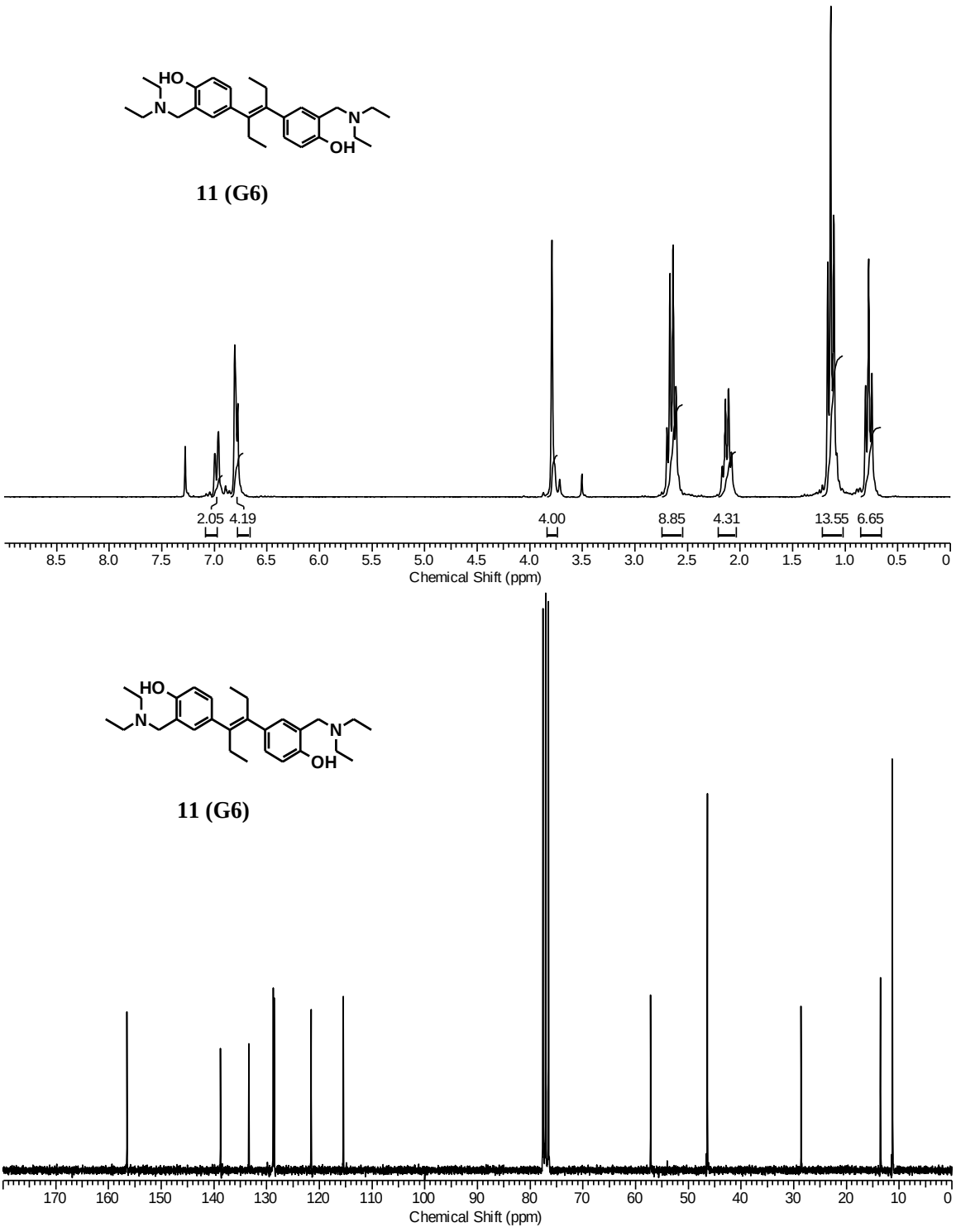


Fig. A-5 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol (11).

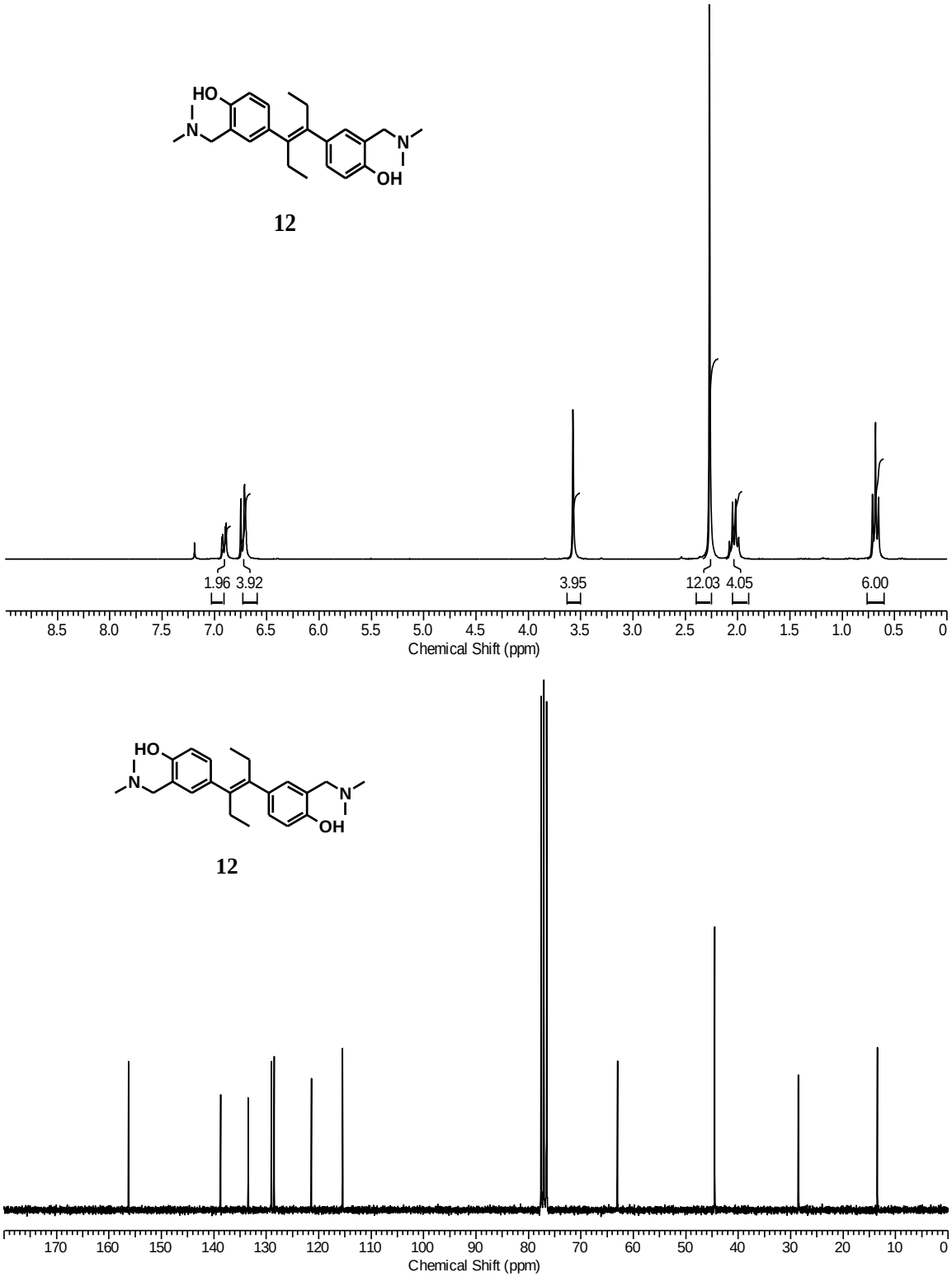


Fig. A-6 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol (**12)**.

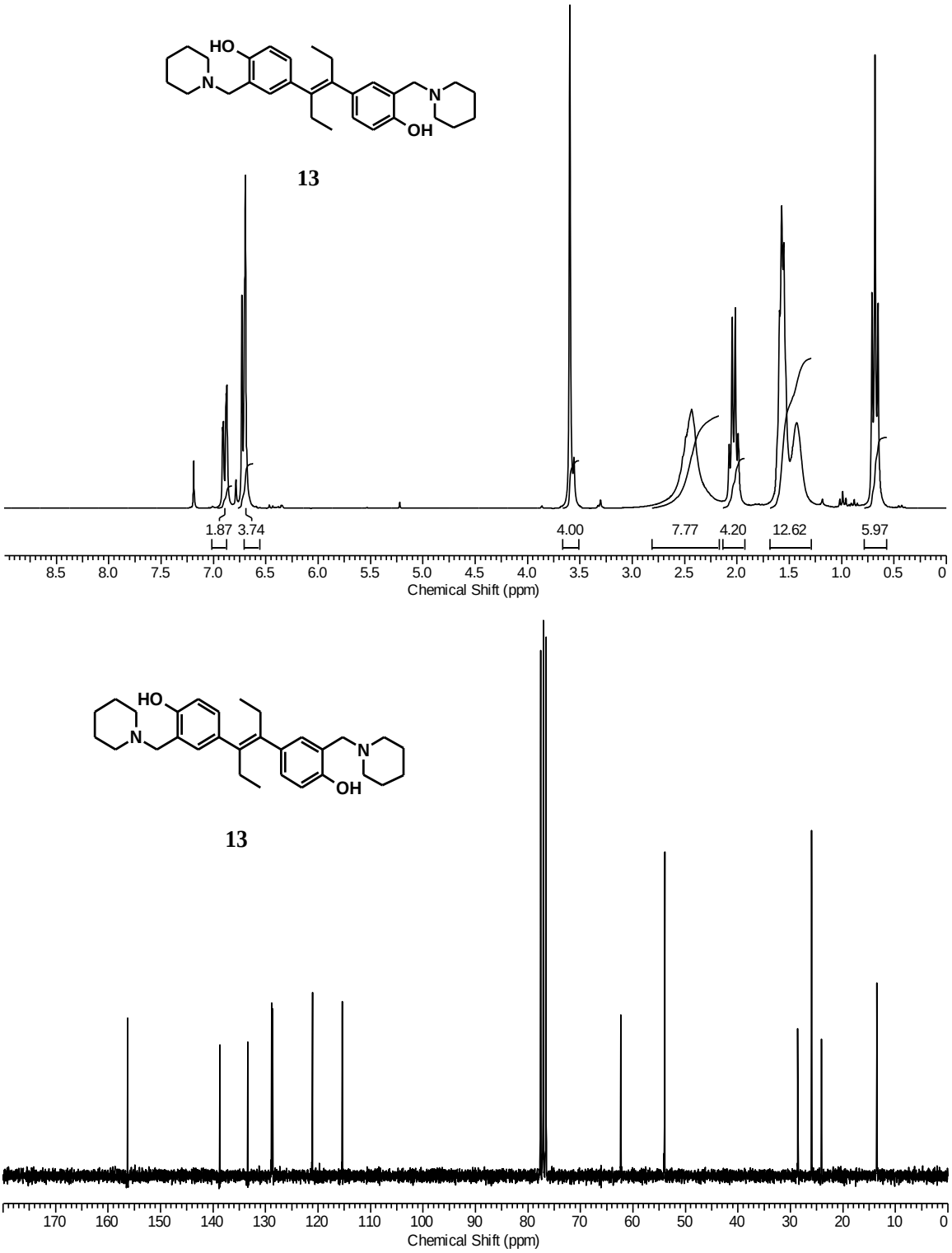


Fig. A-7 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol **(13).**

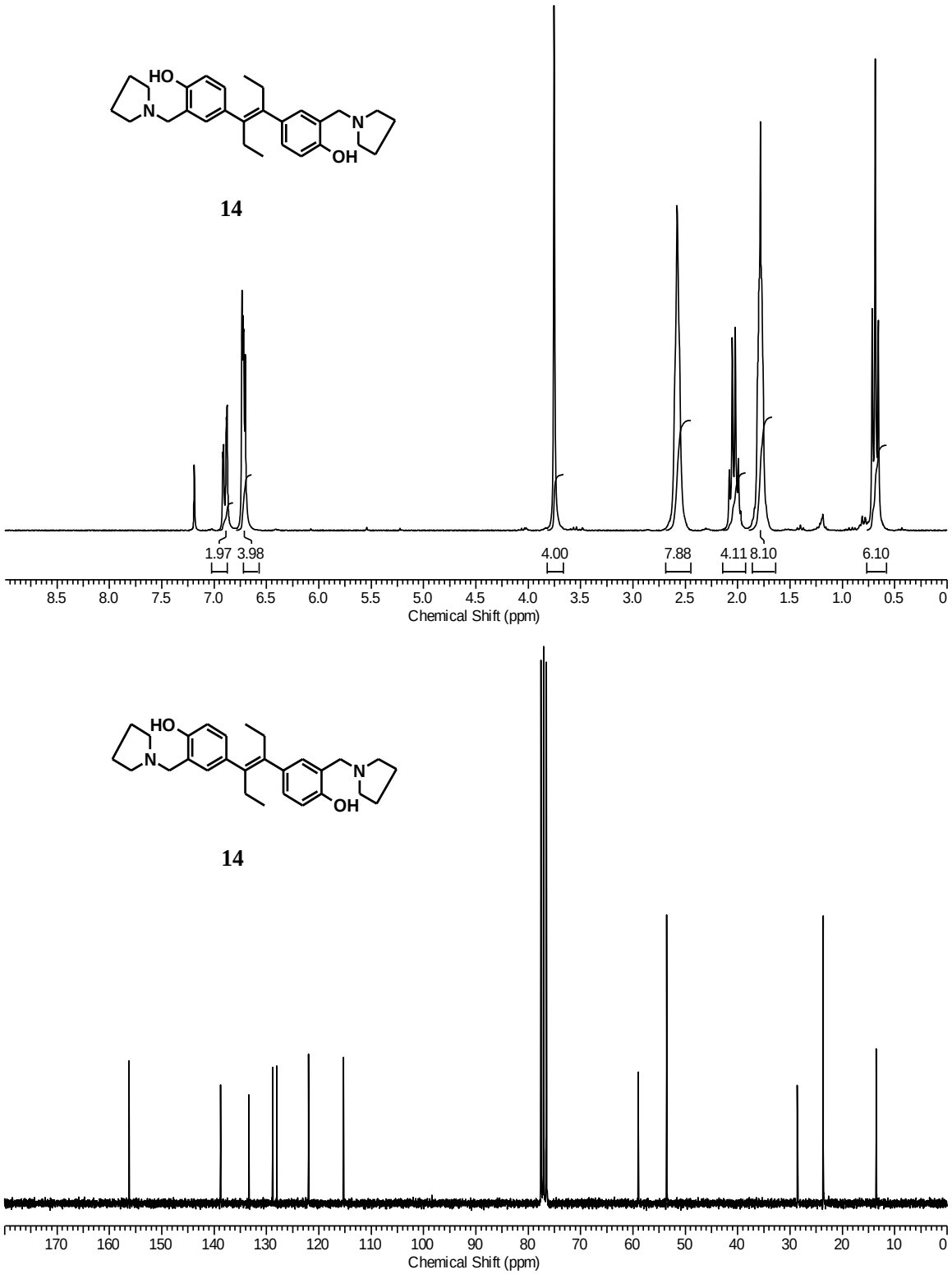


Fig. A-8 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol **(14)**.

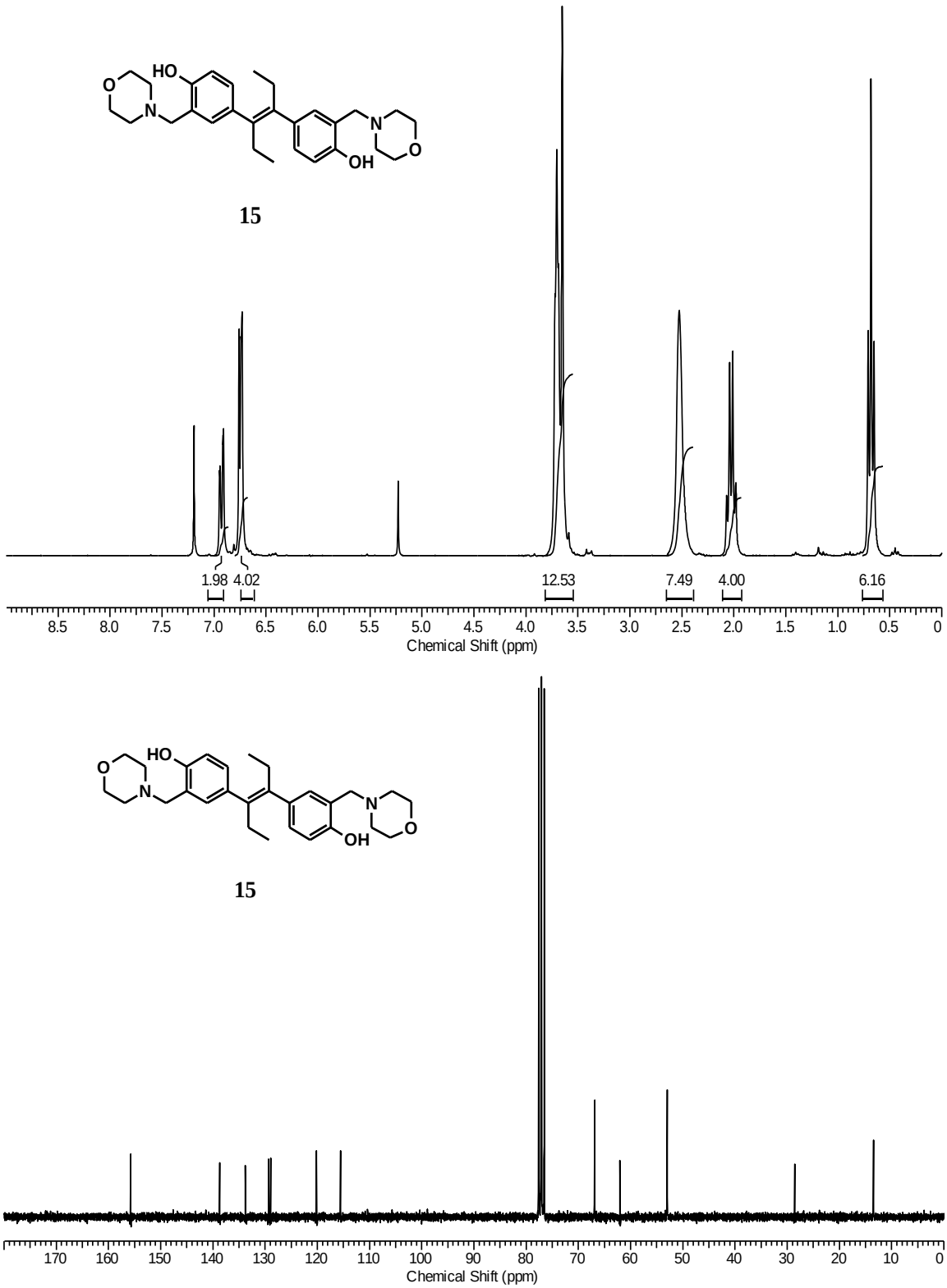


Fig. A-9 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-diethyl-1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol **(15)**.

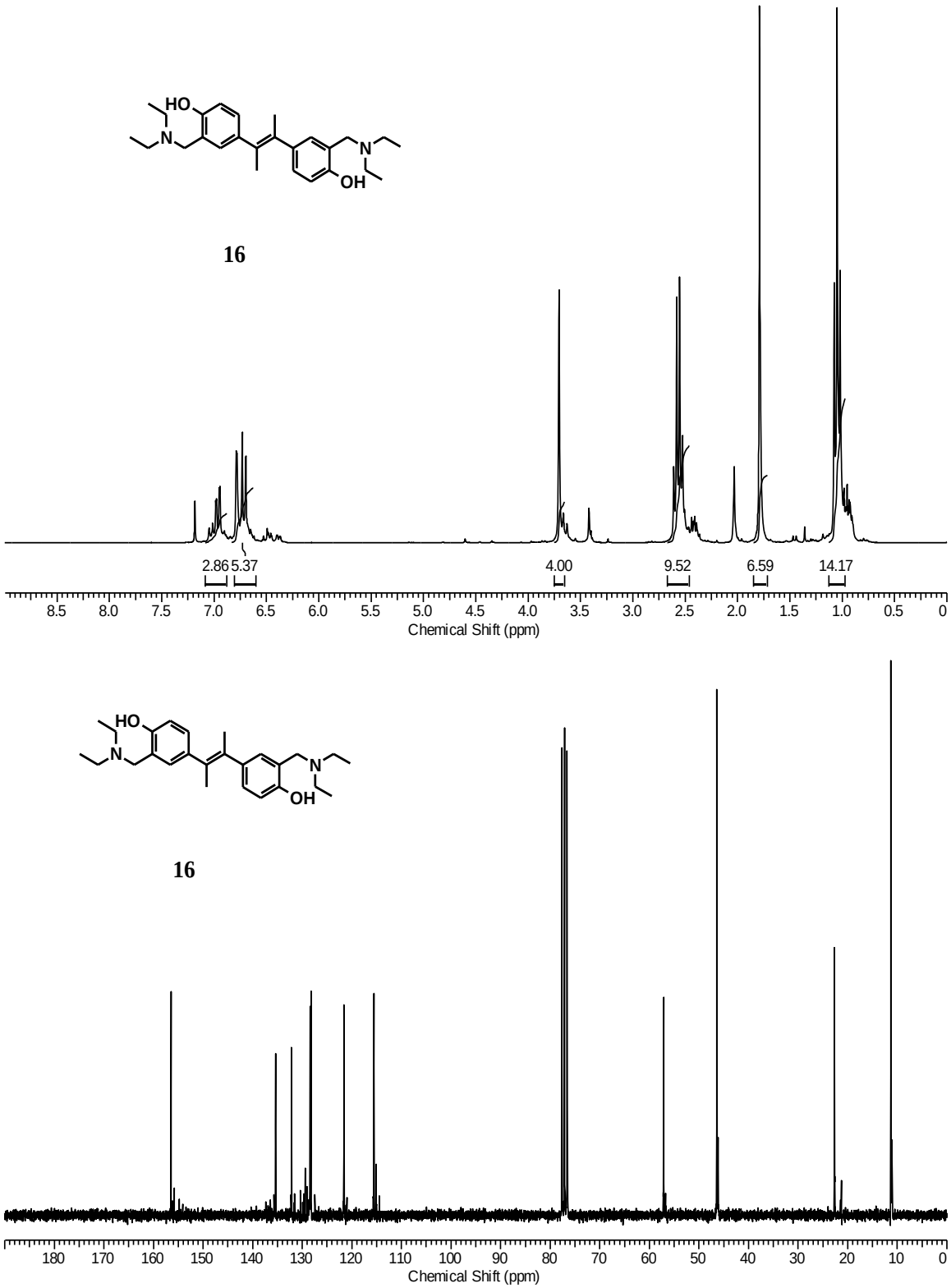


Fig. A-10 ^{1}H and ^{13}C NMR spectra of 4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol **(16)**.

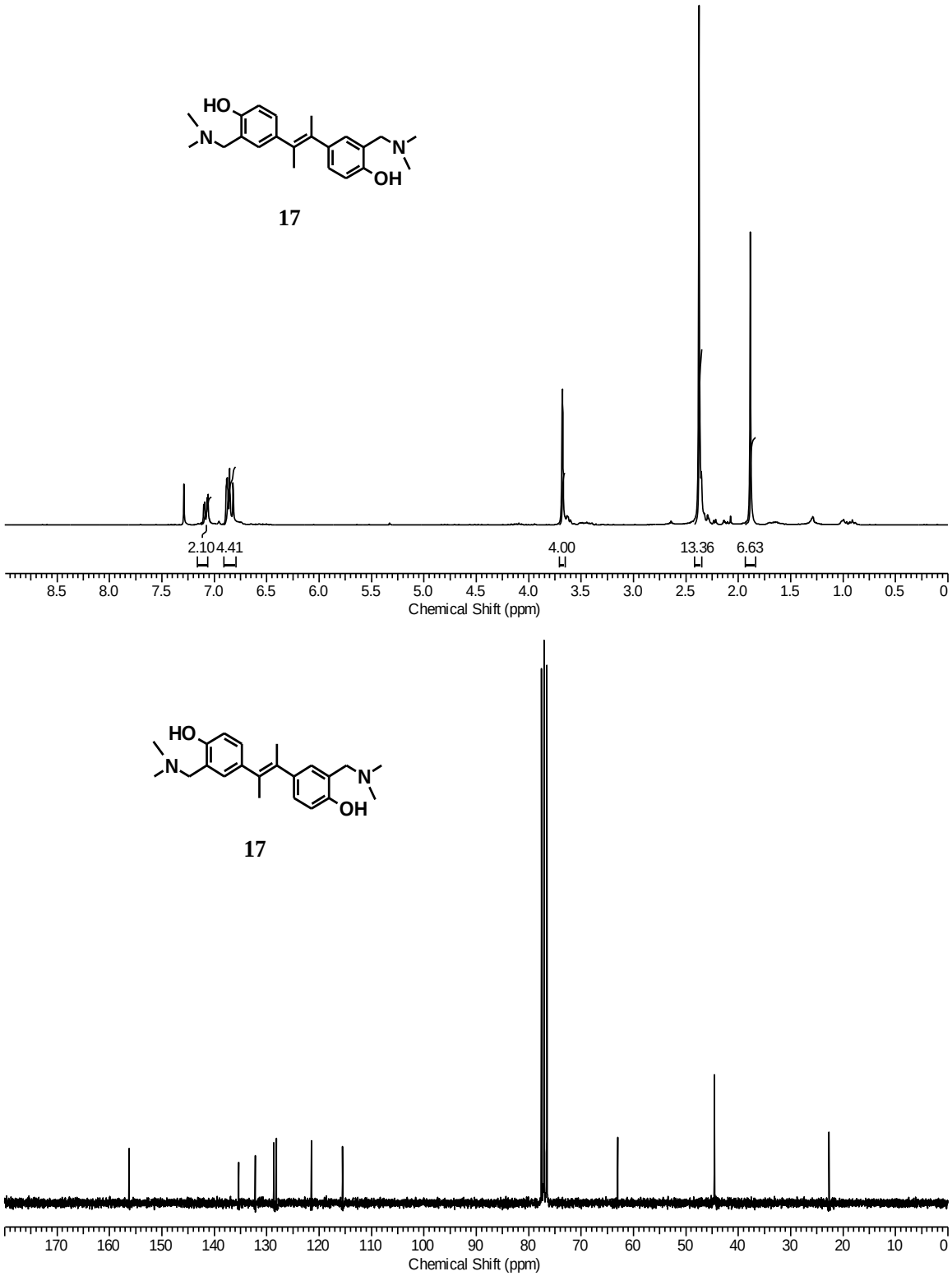


Fig. A-11 ^{1}H and ^{13}C NMR spectra of 4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol **(17)**.

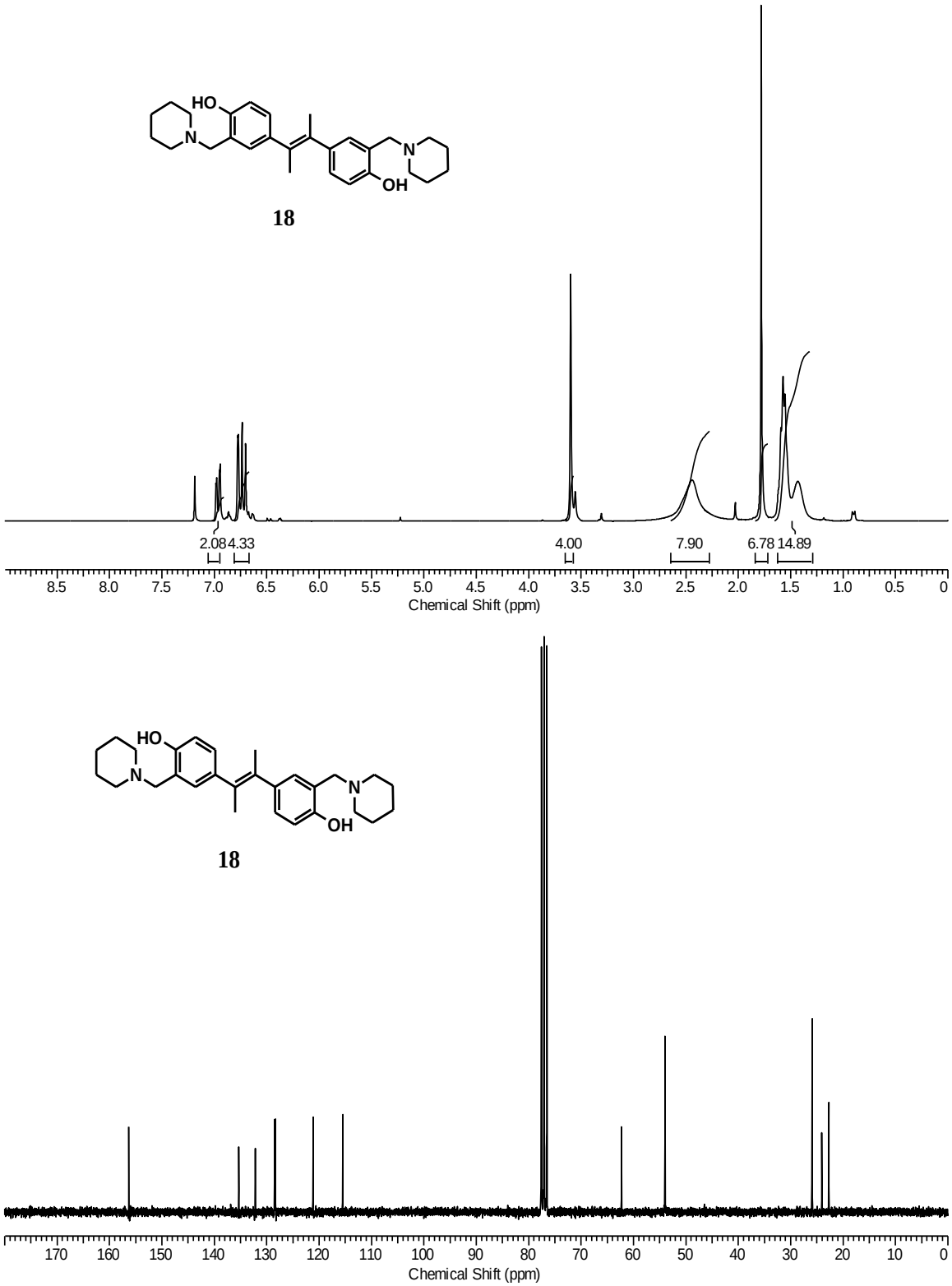


Fig. A-12 ^{1}H and ^{13}C NMR spectra of 4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol **(18)**.

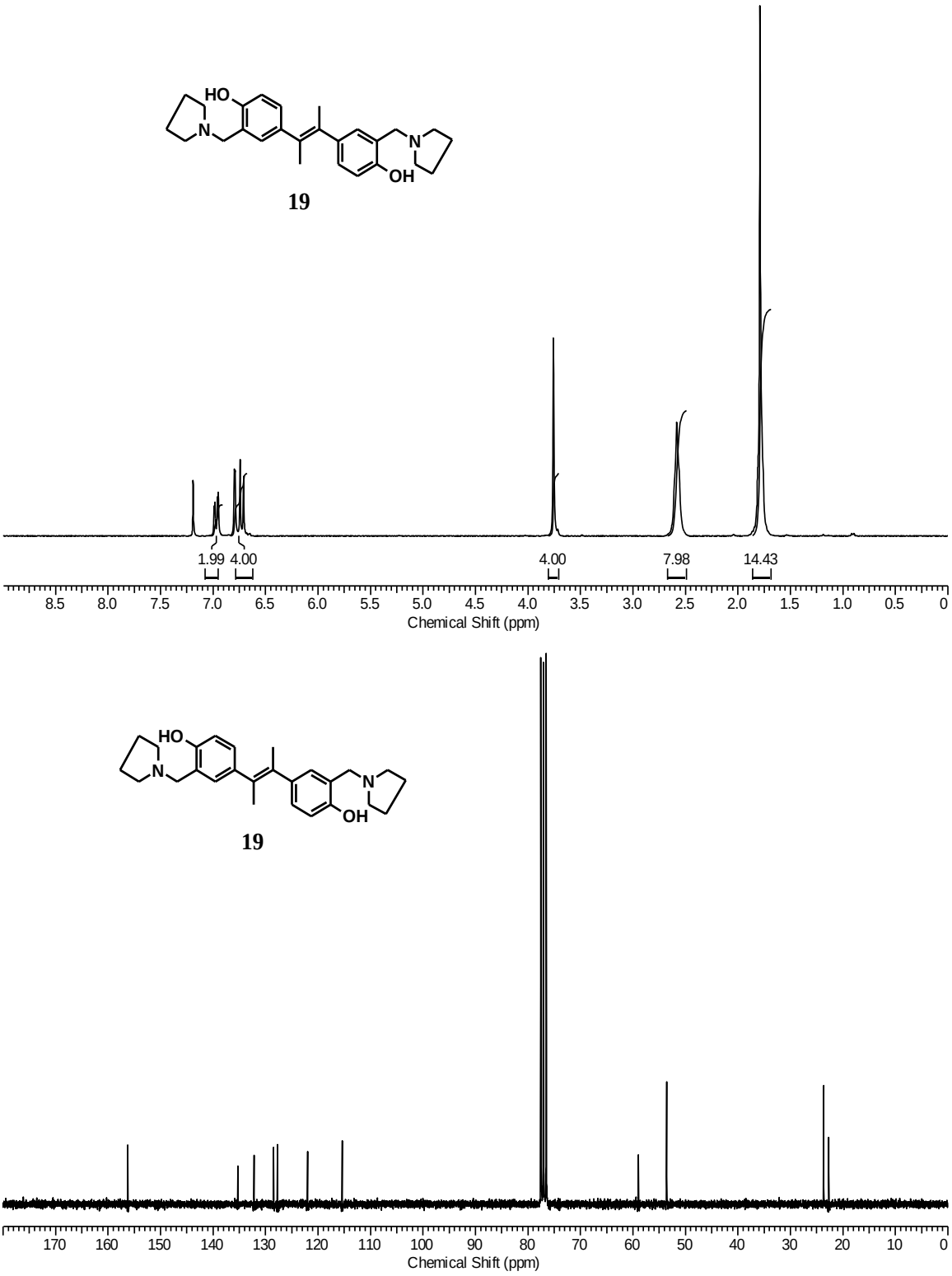


Fig. A-13 ^{1}H and ^{13}C NMR spectra of 4,4'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(Piperidine)methyl]-Phenol **(19)**.

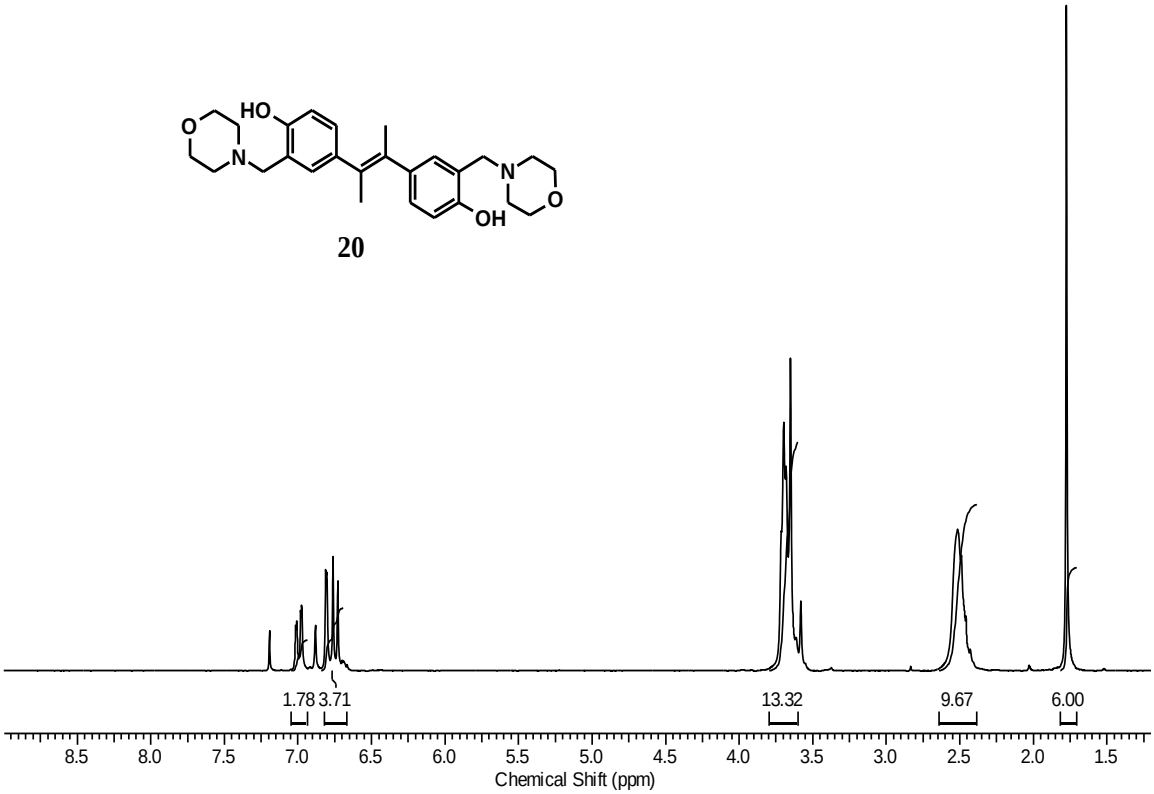


Fig. A-14 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol **(20)**.

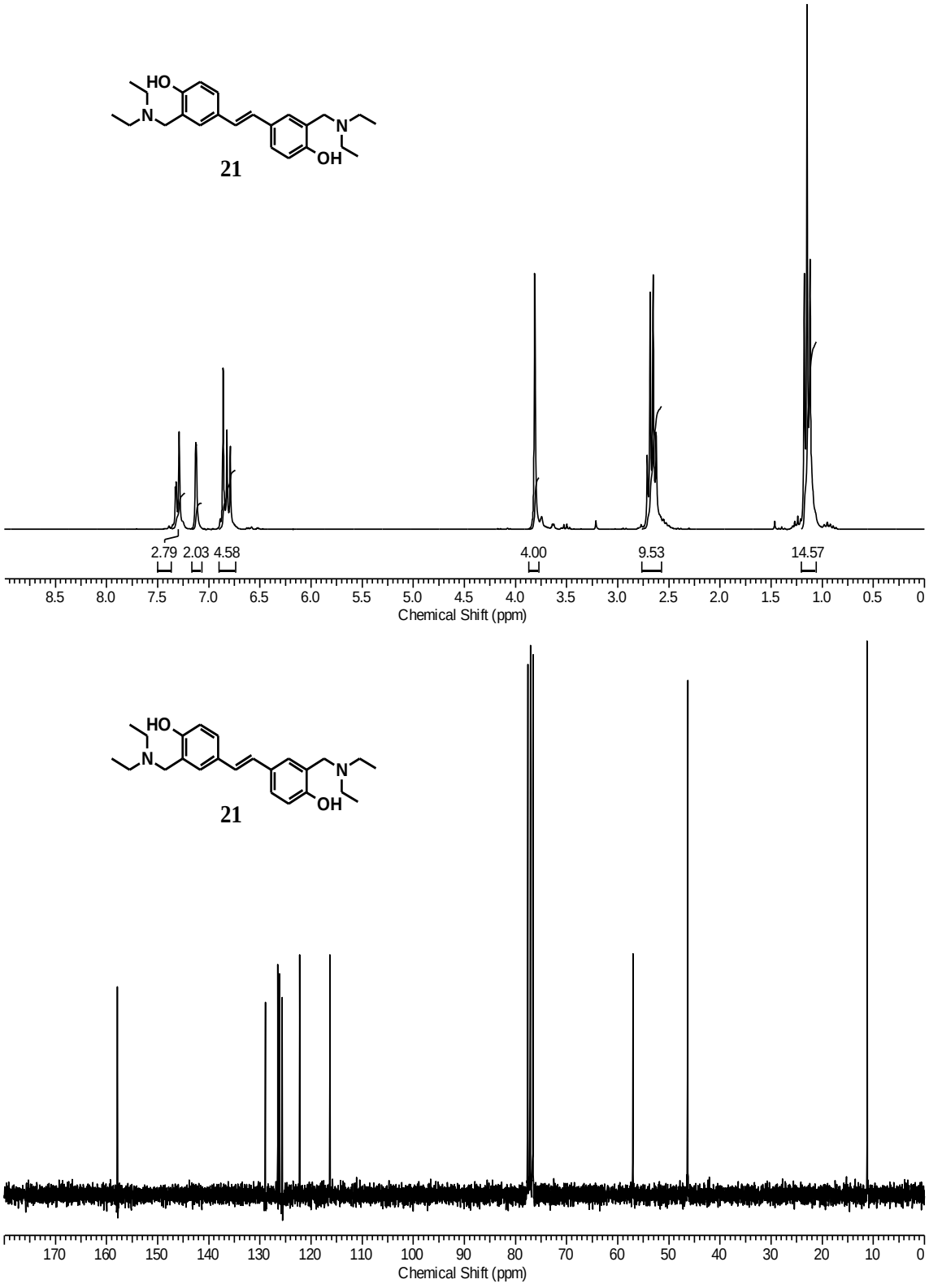


Fig. A-15 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol **(21)**.

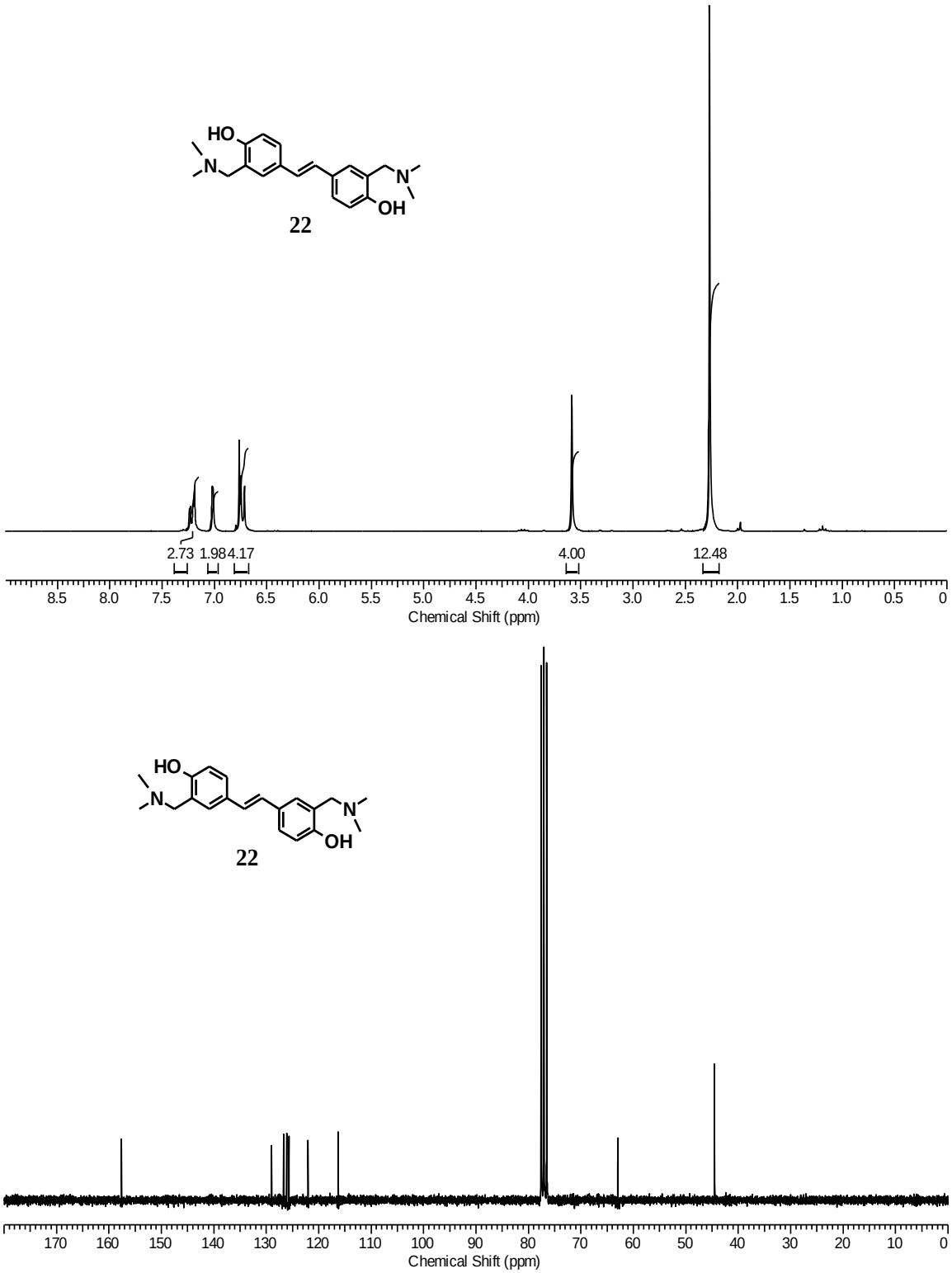


Fig. A-16 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol **(22)**.

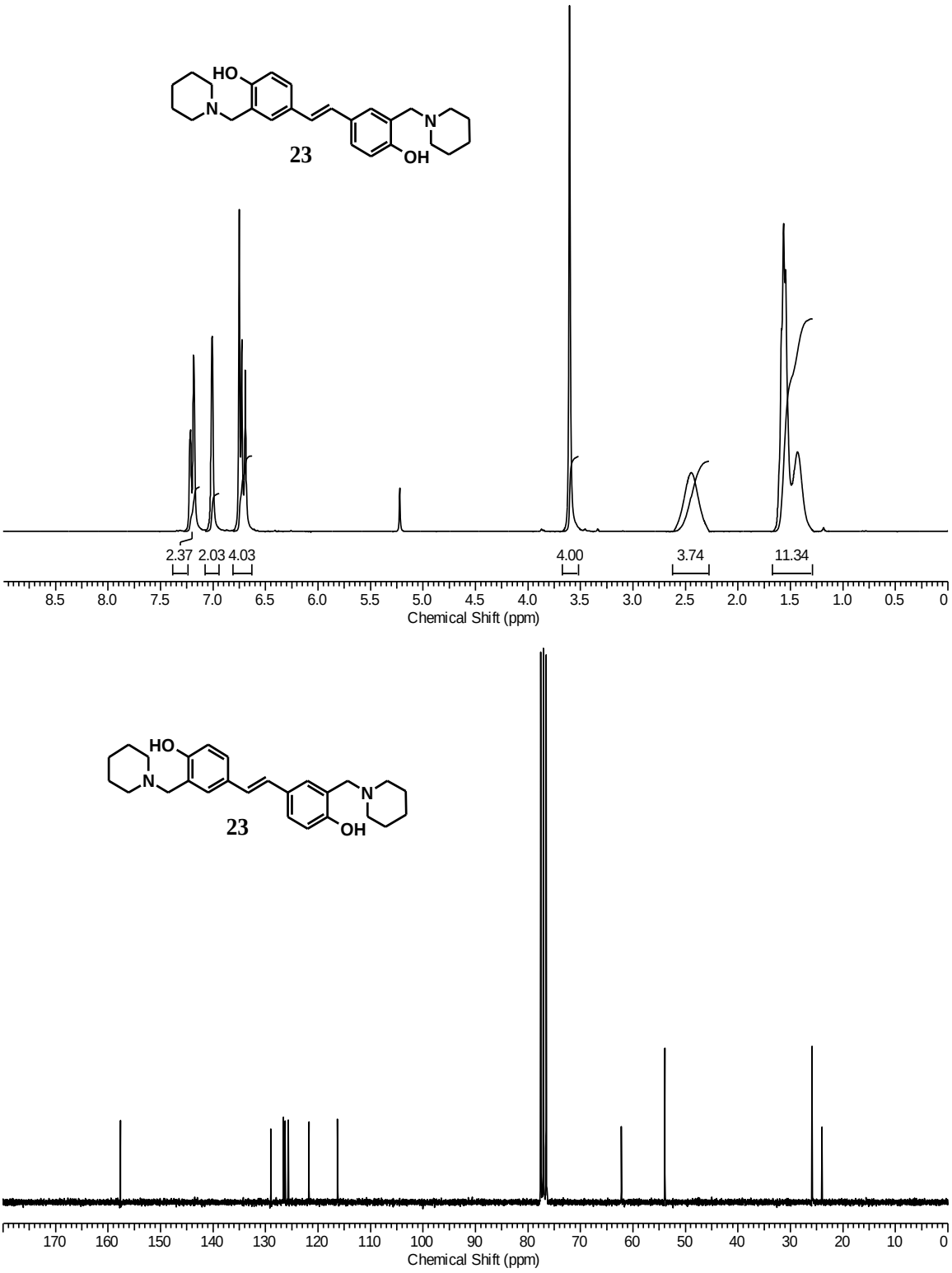


Fig. A-17 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol **(23)**.

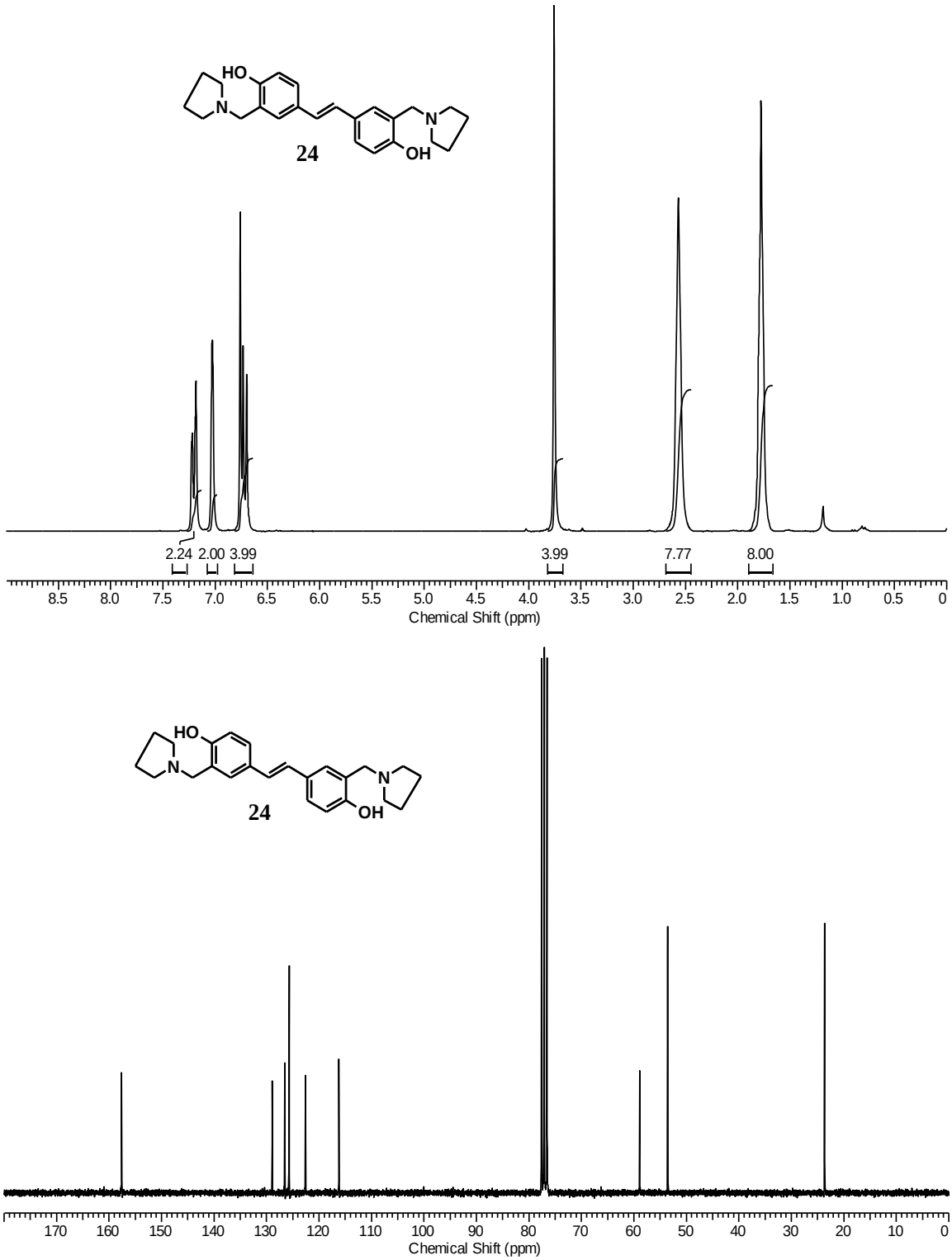


Fig. A-18 ^{1}H and ^{13}C NMR spectra of *E-4*,4'-(1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol **(24)**.

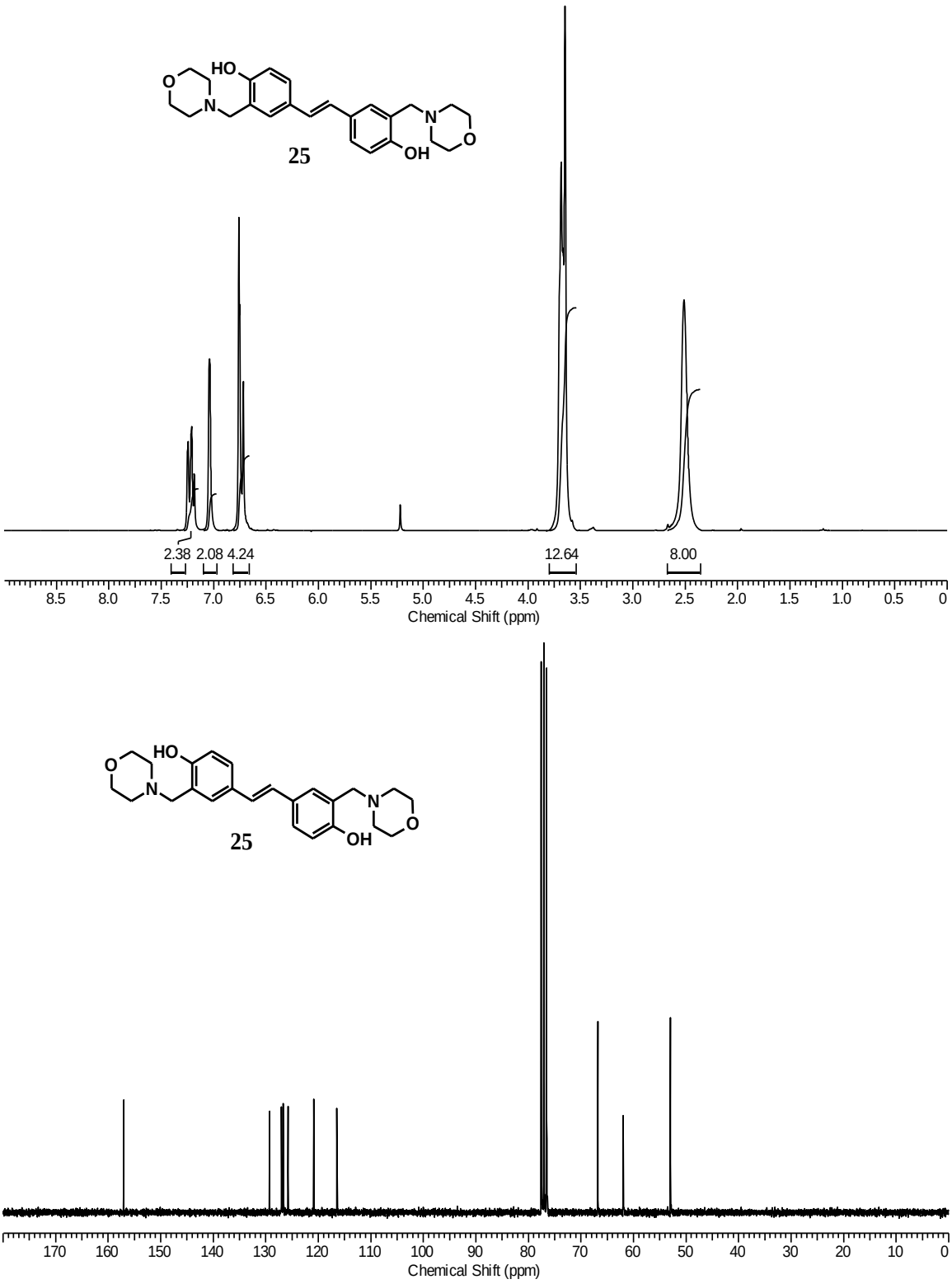


Fig. A-19 ^{1}H and ^{13}C NMR spectra of *E*-4,4'-(1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol **(25)**.

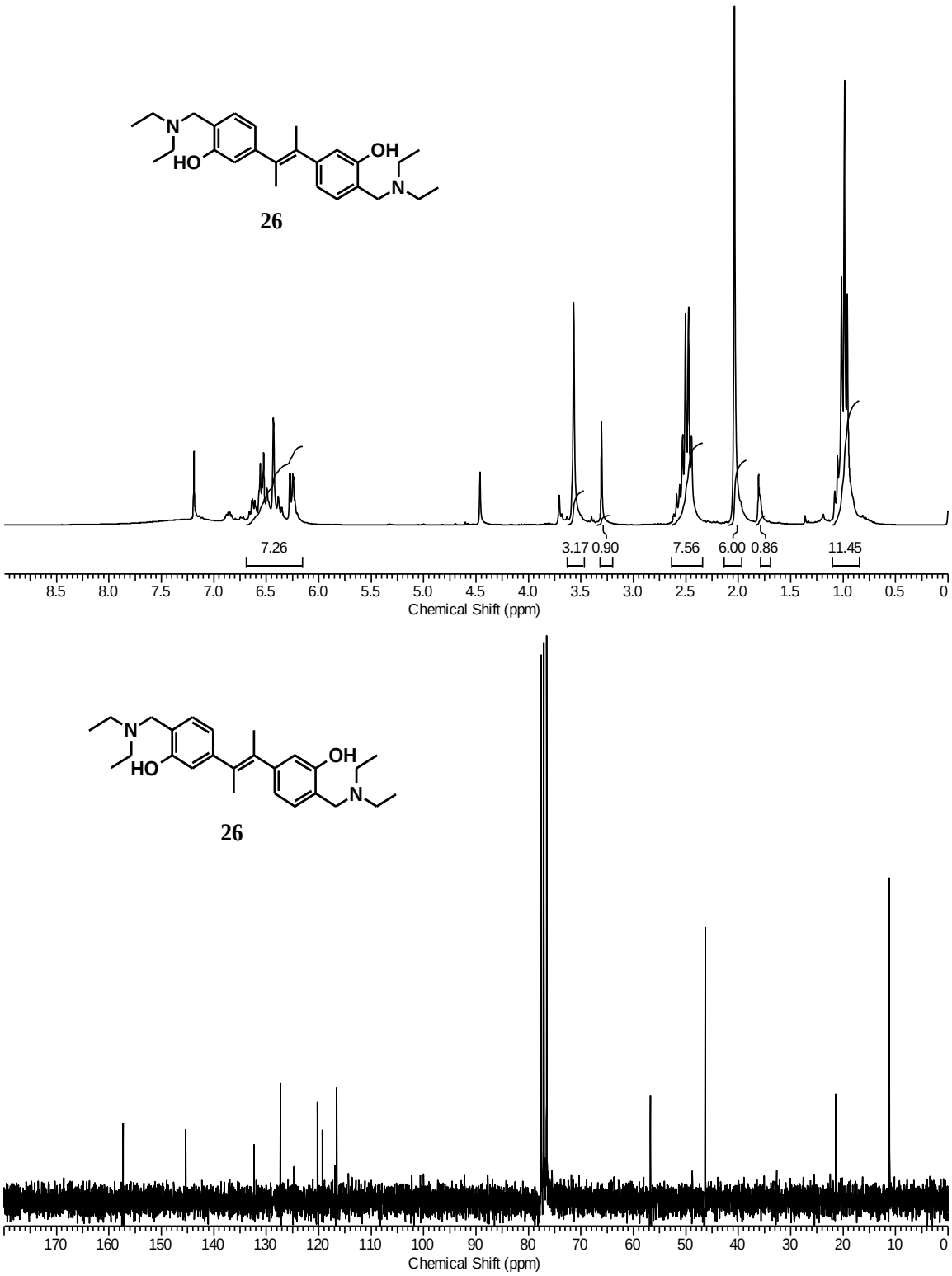


Fig. A-20 ^{1}H and ^{13}C NMR spectra of 3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol **(26)**.

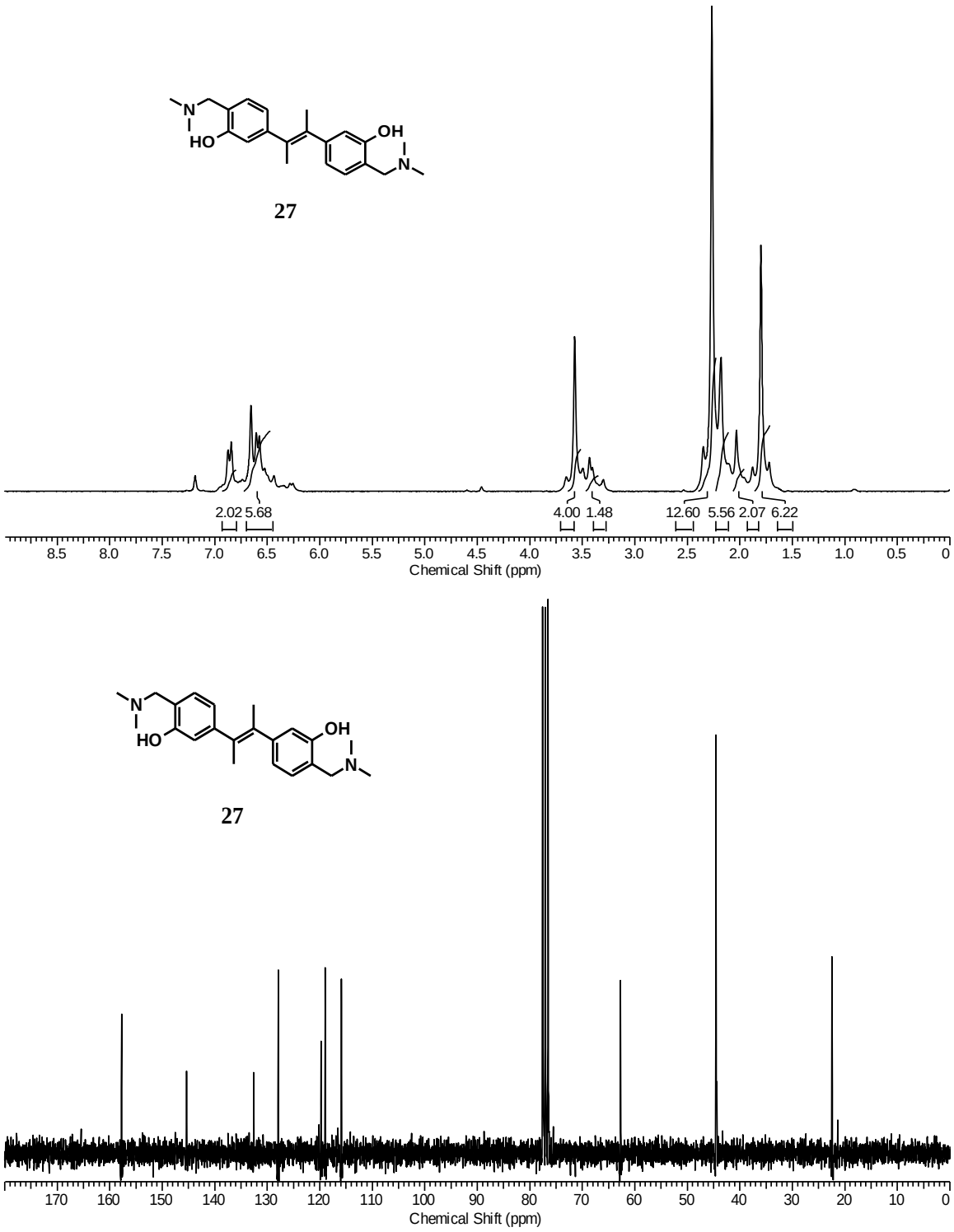


Fig. A-21 ^{1}H and ^{13}C NMR spectra of 3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol **(27)**.

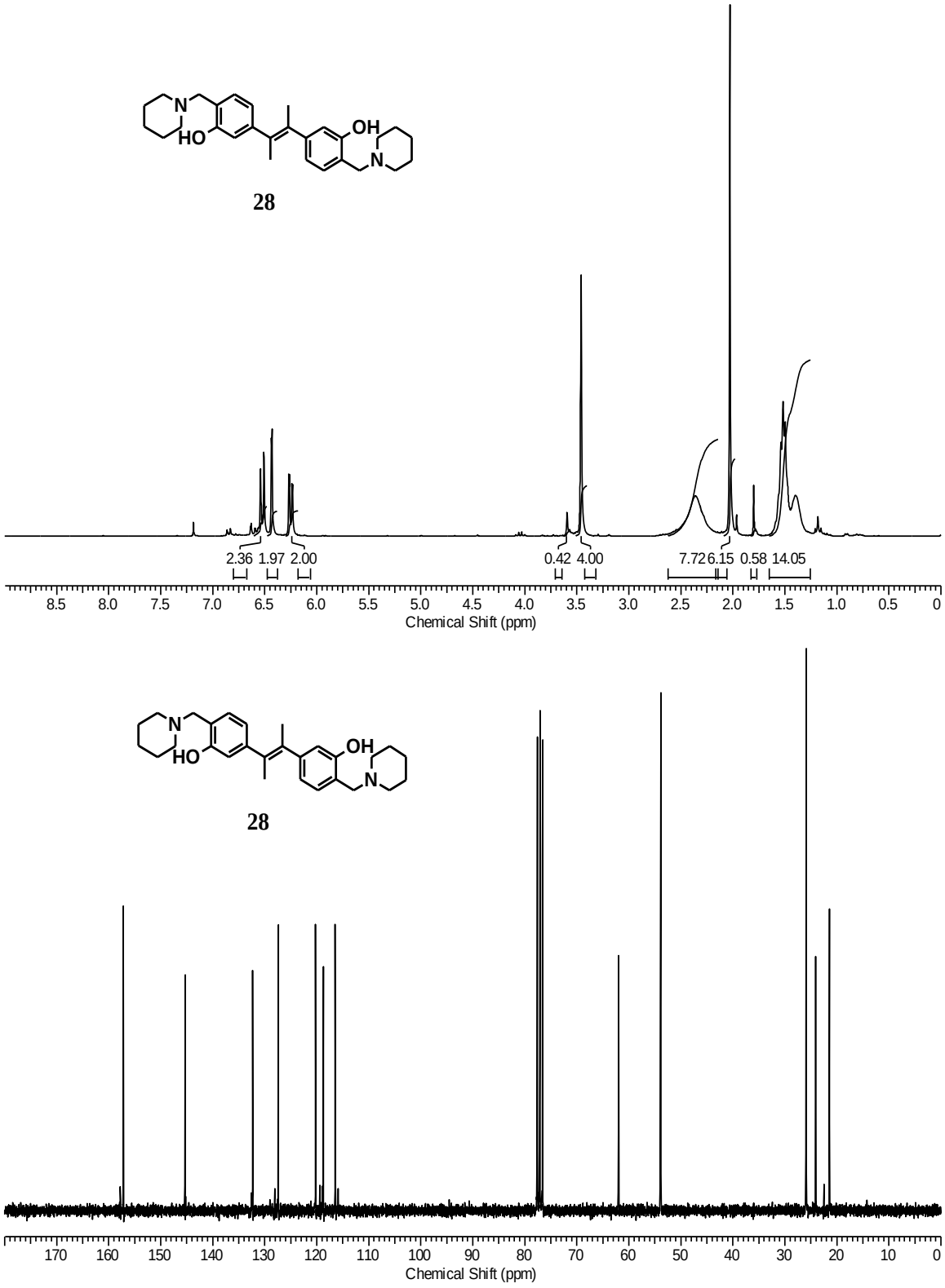


Fig. A-22 ^{1}H and ^{13}C NMR spectra of 3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol **(28)**.

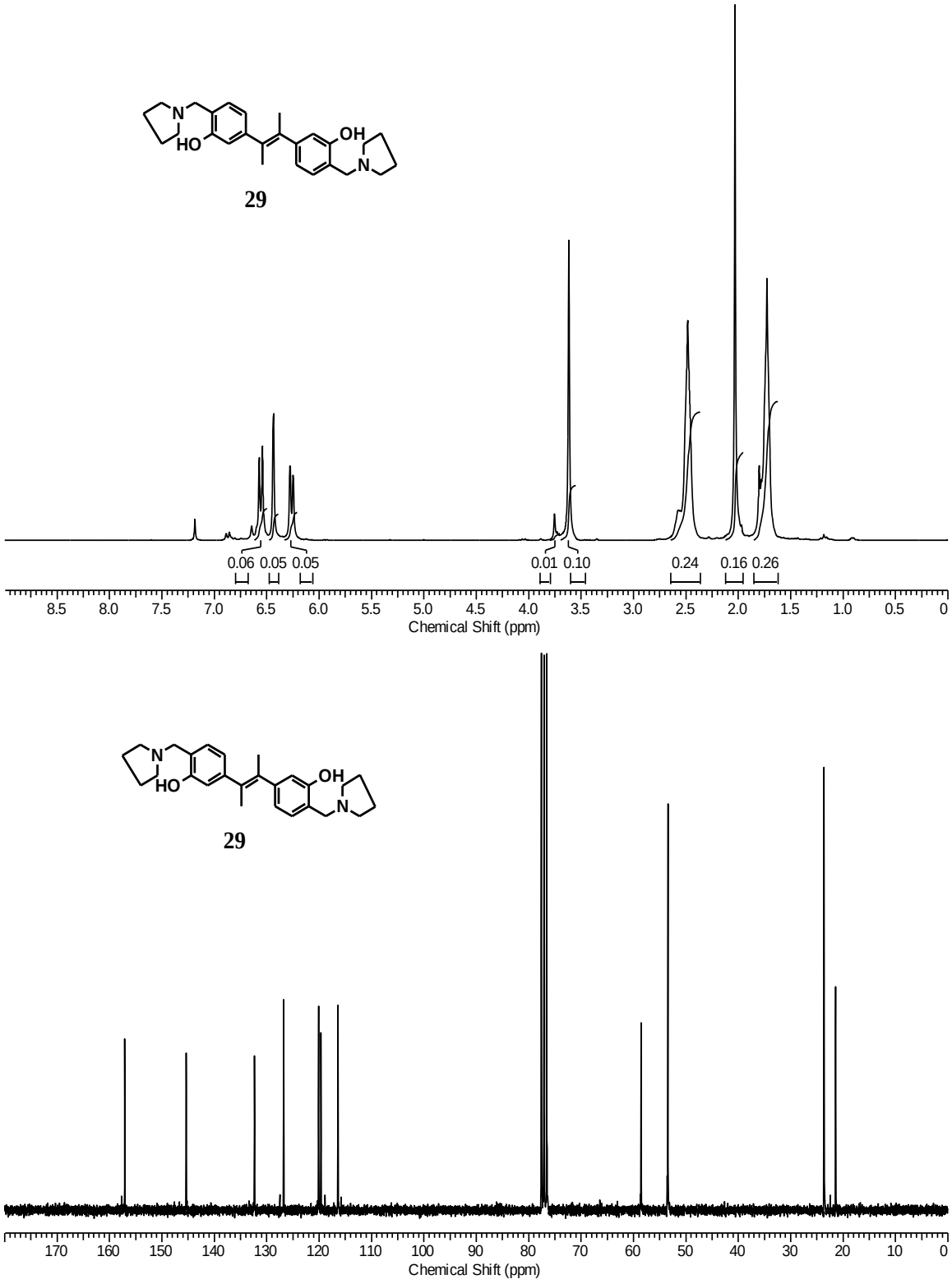


Fig. A-23 ^{1}H and ^{13}C NMR spectra of 3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol **(29)**.

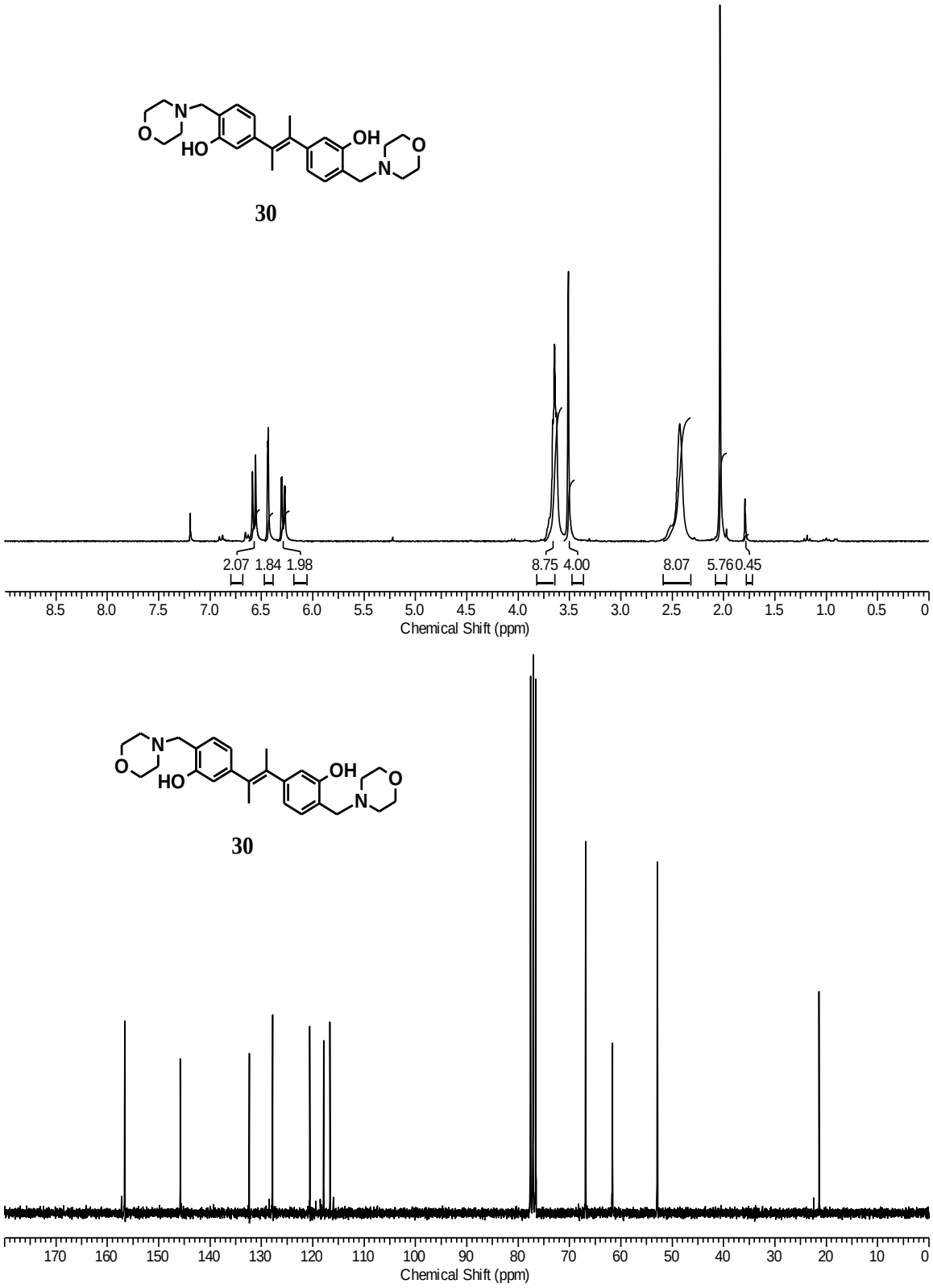


Fig. A-24 ^{1}H and ^{13}C NMR spectra of 3,3'-(1,2-dimethyl-1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol **(30)**.

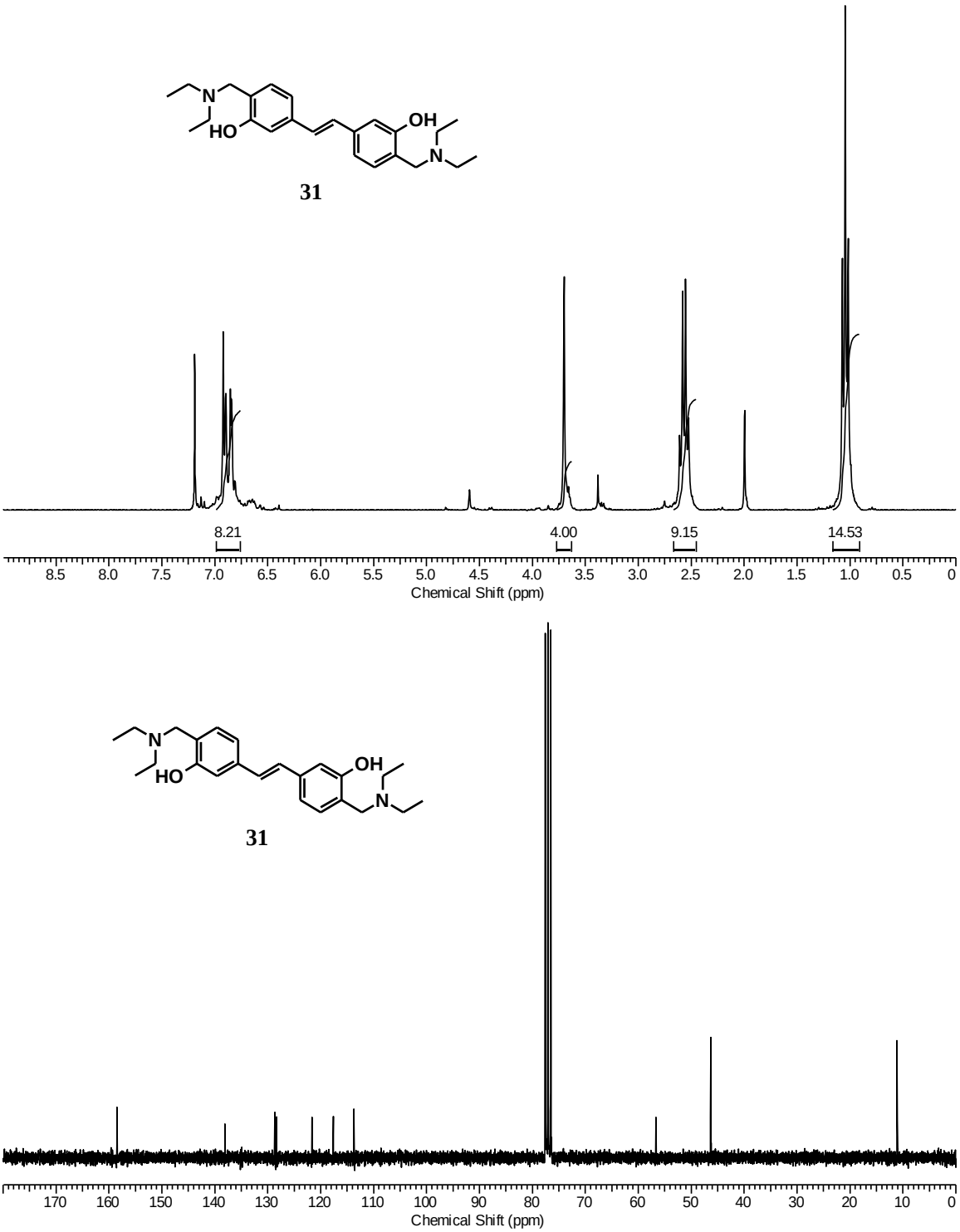


Fig. A-25 ^{1}H and ^{13}C NMR spectra of *E*-3,3'-(1,2-ethenediyl)bis[2-[(diethylamino)methyl]-Phenol **(31)**.

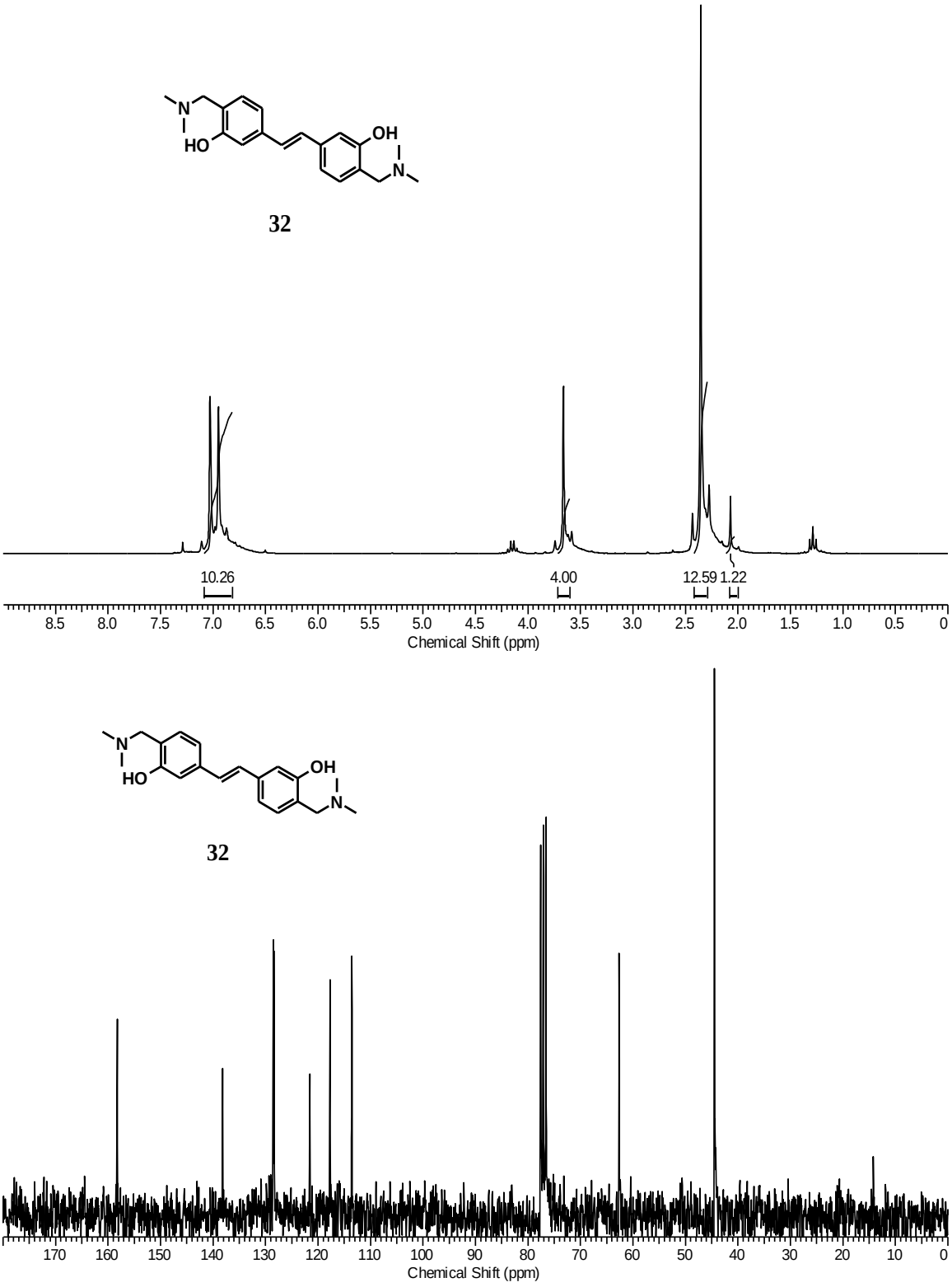


Fig. A-26 ^{1}H and ^{13}C NMR spectra of *E*-3,3'-(1,2-ethenediyl)bis[2-[(dimethylamino)methyl]-Phenol **(32)**.

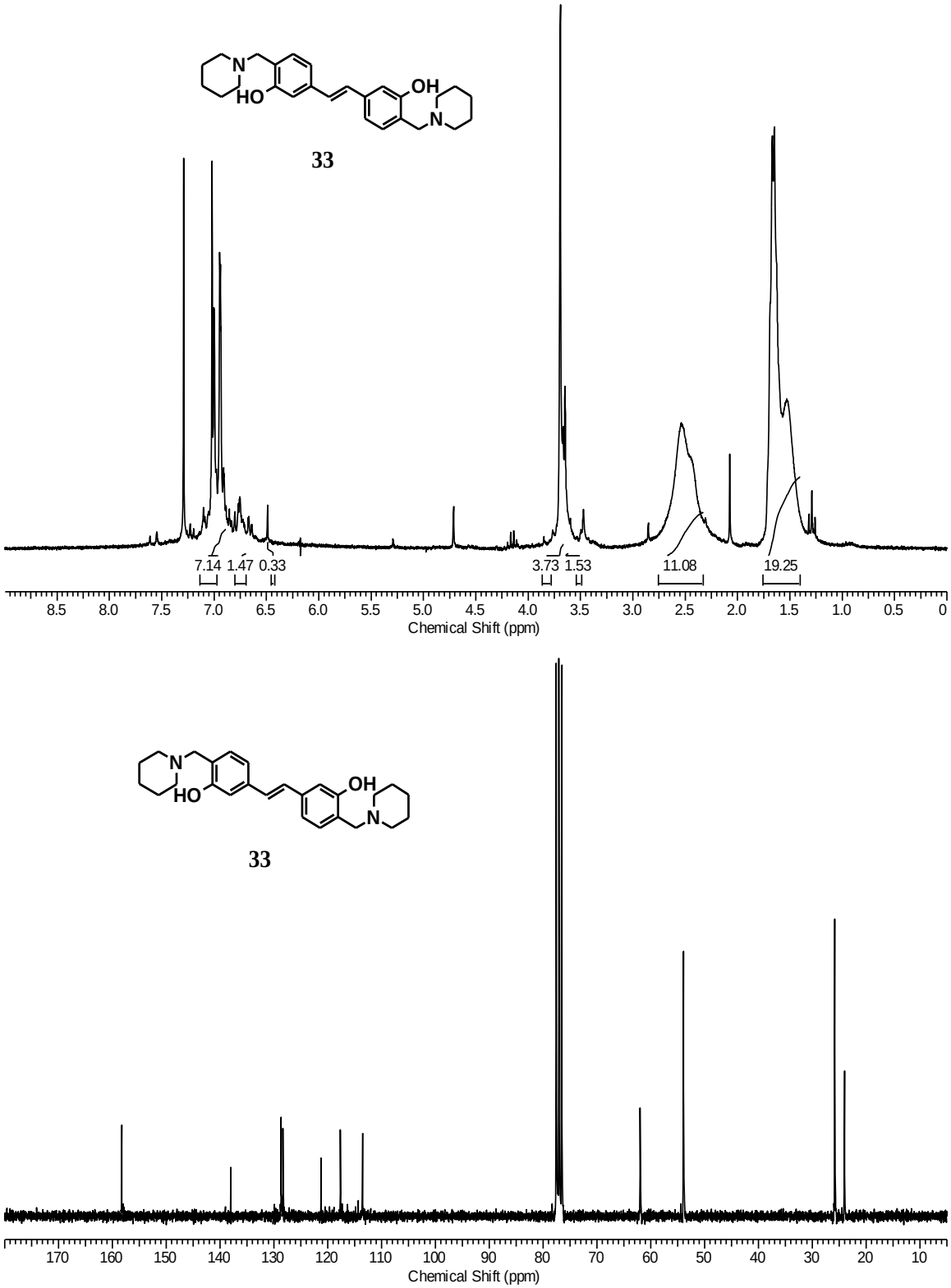


Fig. A-27 ^{1}H and ^{13}C NMR spectra of *E*-3,3'-(1,2-ethenediyl)bis[2-[(pyrrolidine)methyl]-Phenol **(33)**.

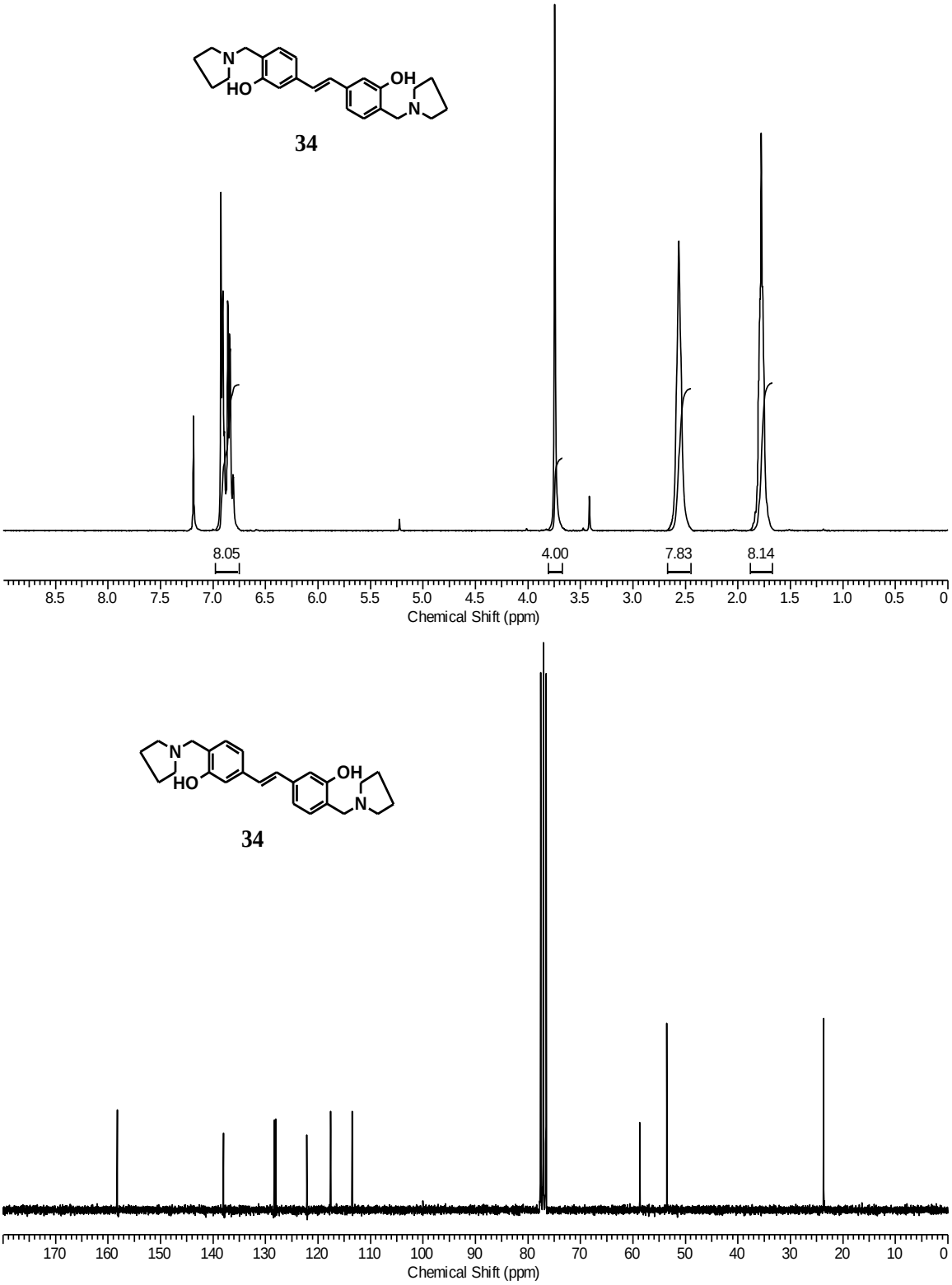


Fig. A-28 ^{1}H and ^{13}C NMR spectra of *E*-3,3'-(1,2-ethenediyl)bis[2-[(piperidine)methyl]-Phenol **(34)**.

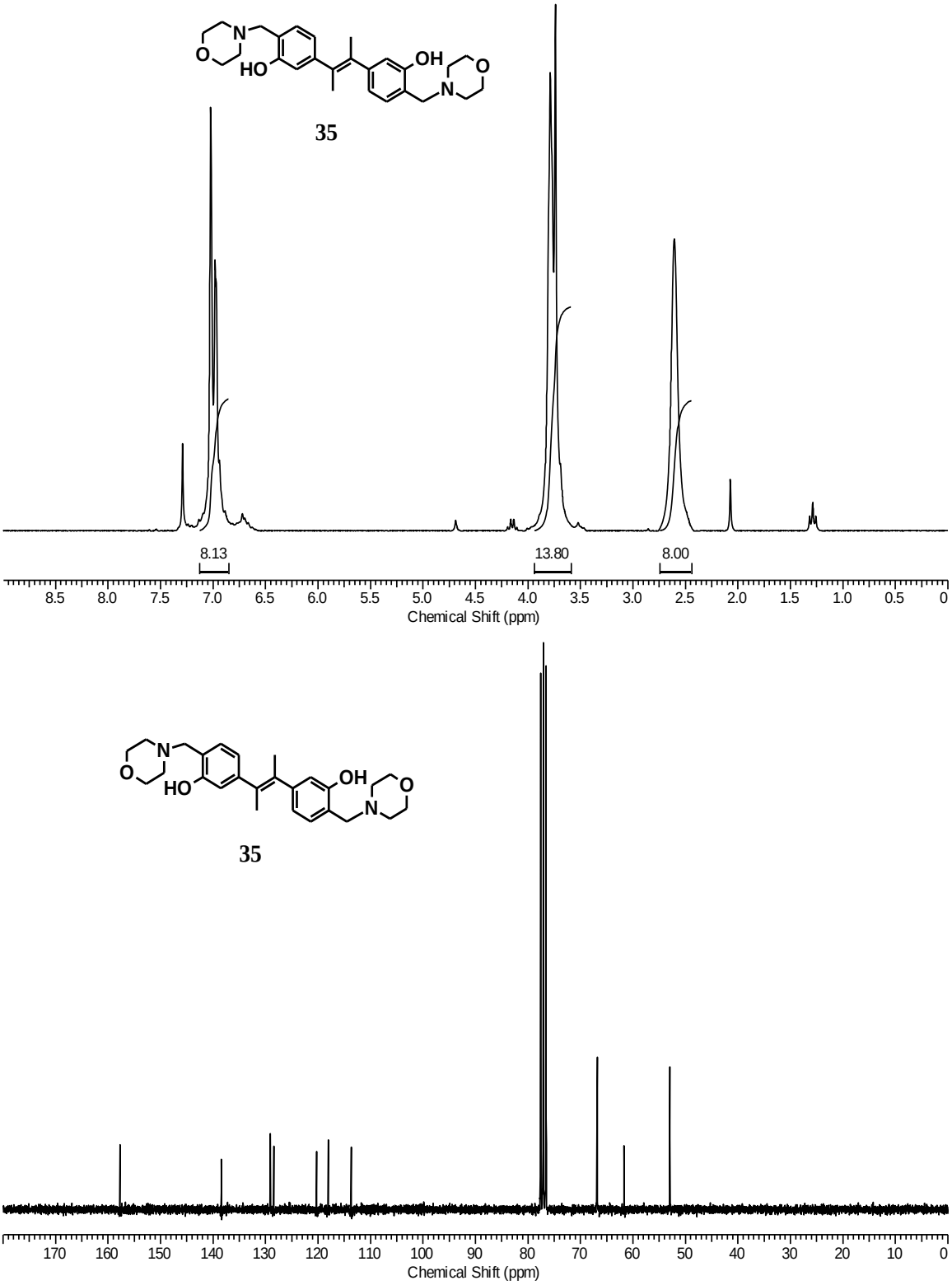


Fig. A-29 ^{1}H and ^{13}C NMR spectra of *E*-3,3'-(1,2-ethenediyl)bis[2-[(morpholine)methyl]-Phenol **(35)**.

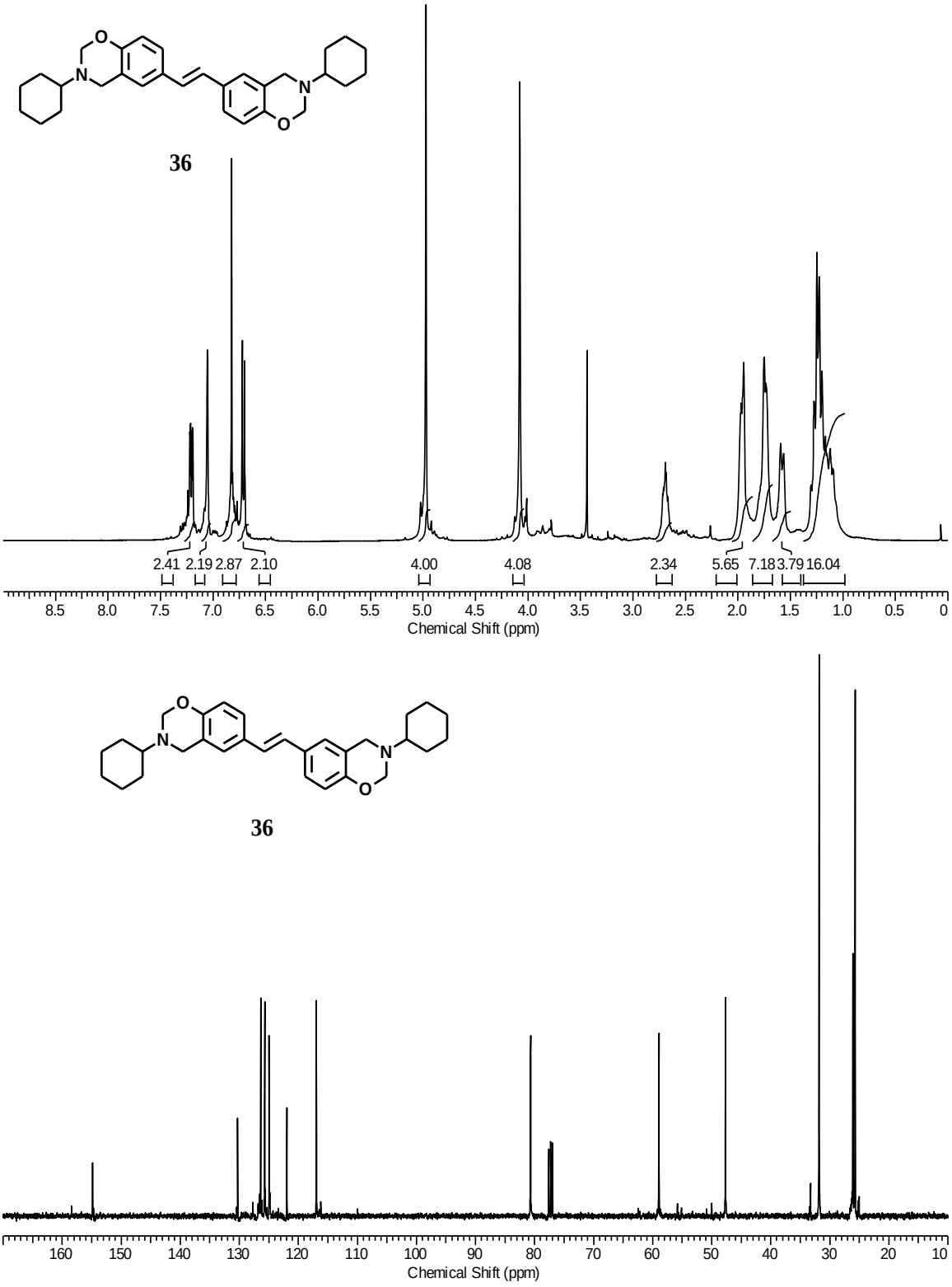


Fig. A-30 ^{1}H and ^{13}C NMR spectra of *E*-6,6'-(vinylene)bis[3-cyclohexyl-3,4-dihydro-2H-1,3-Benzoxazine] **(36).**

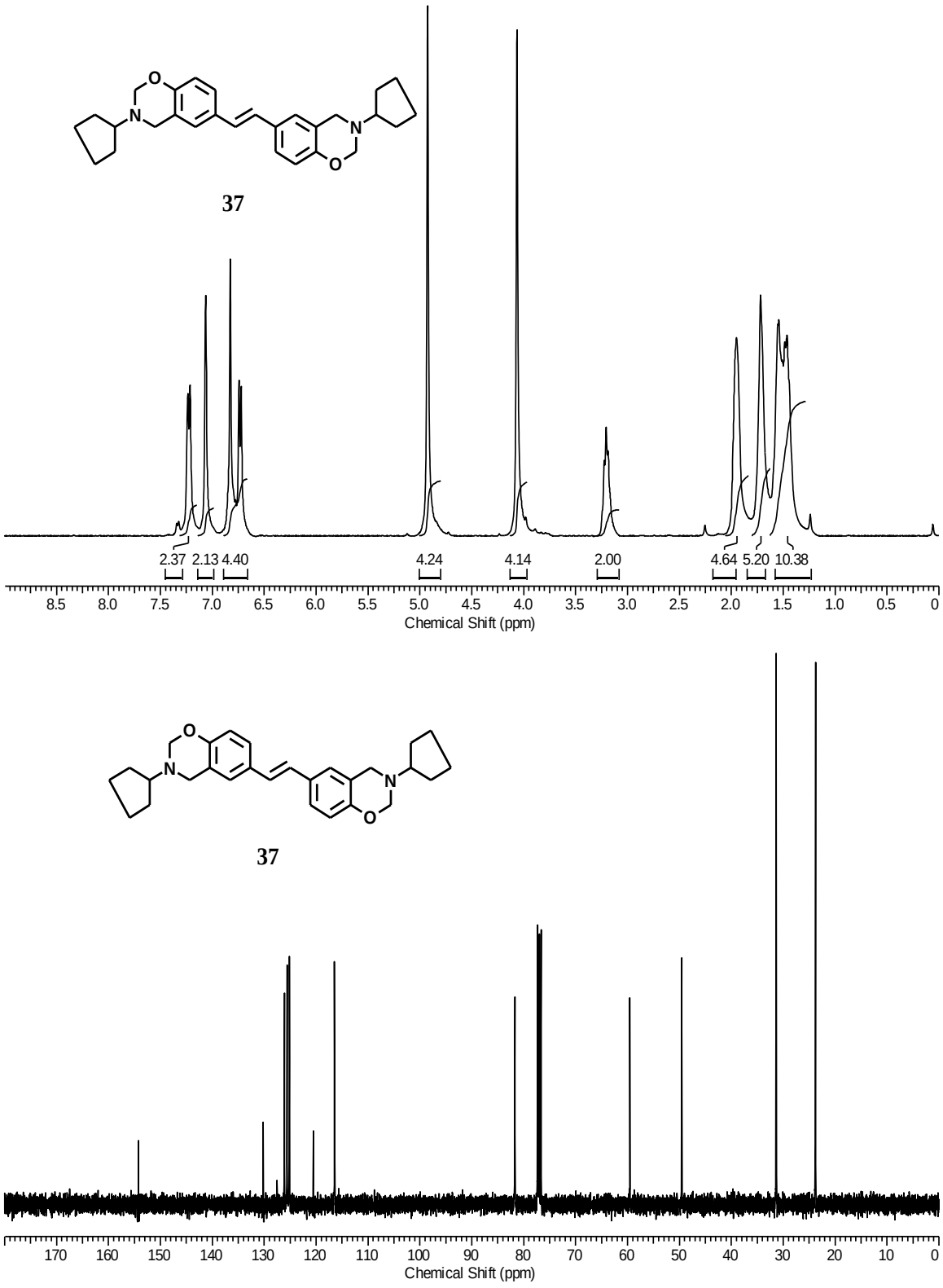


Fig. A-31 ^{1}H and ^{13}C NMR spectra of *E*-6,6'-(vinylene)bis[3-cyclopentyl-3,4-dihydro-2H-1,3-Benzoxazine] **(37)**.

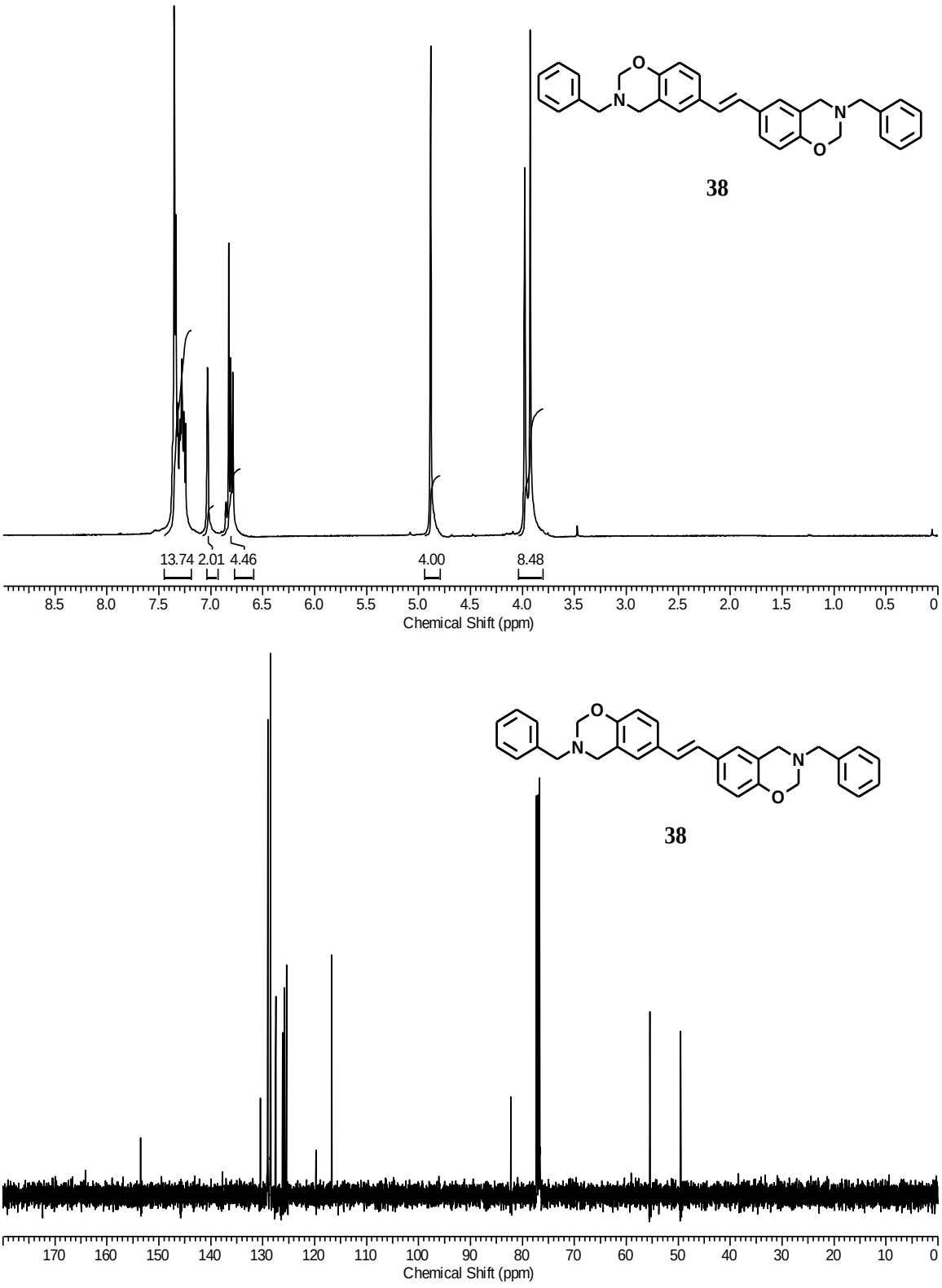


Fig. A-32 ^{1}H and ^{13}C NMR spectra of *E*-6,6'-(vinylene)bis[3-benzyl-3,4-dihydro- 2H-1,3-Benzoxazine] **(38)**.

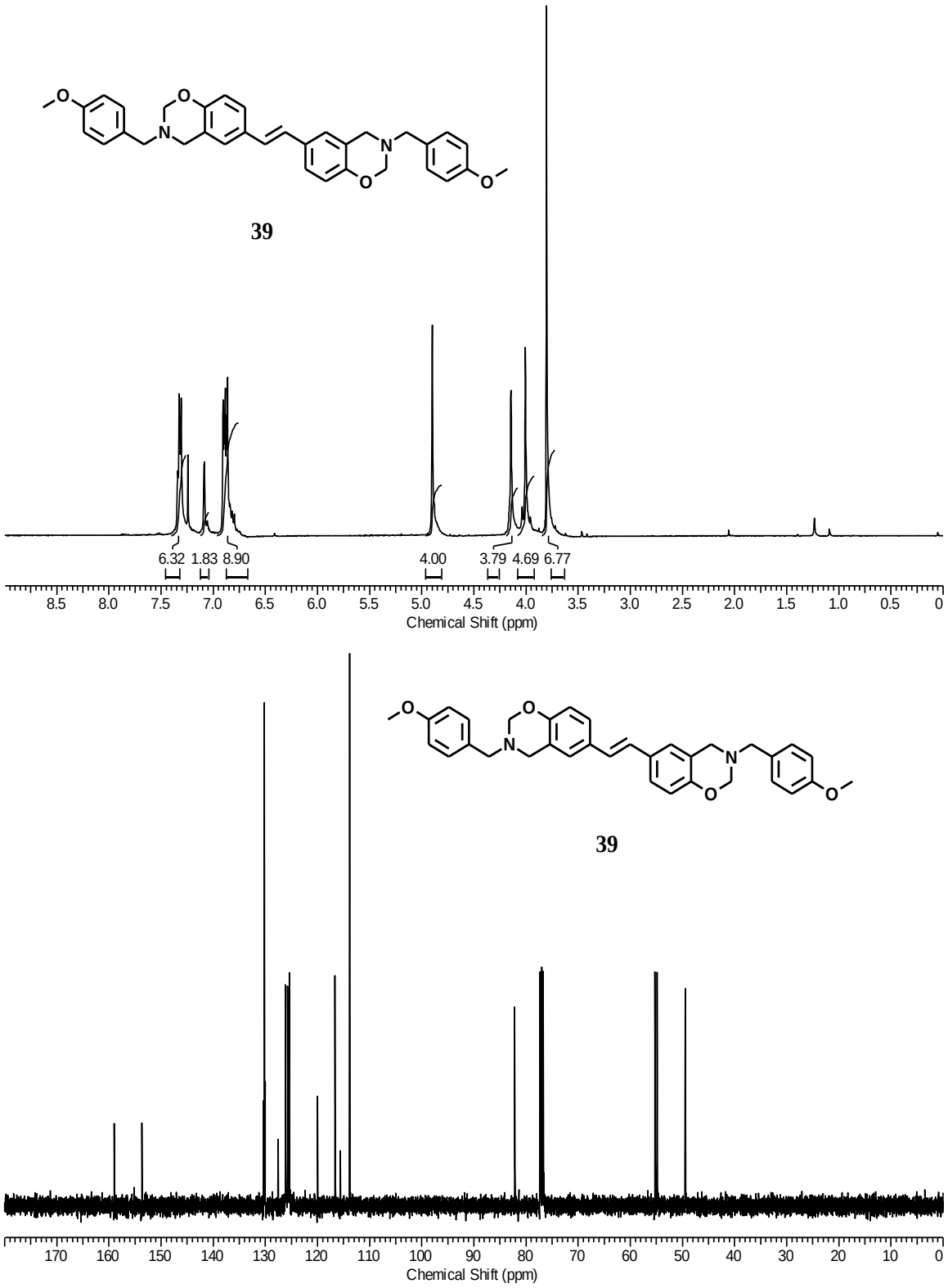


Fig. A-33 ^{1}H and ^{13}C NMR spectra of *E*-6,6'-(vinylene)bis[3-(4-methoxy benzyl)-3,4-dihydro- 2H-1,3-Benzoxazine] **(39)**.

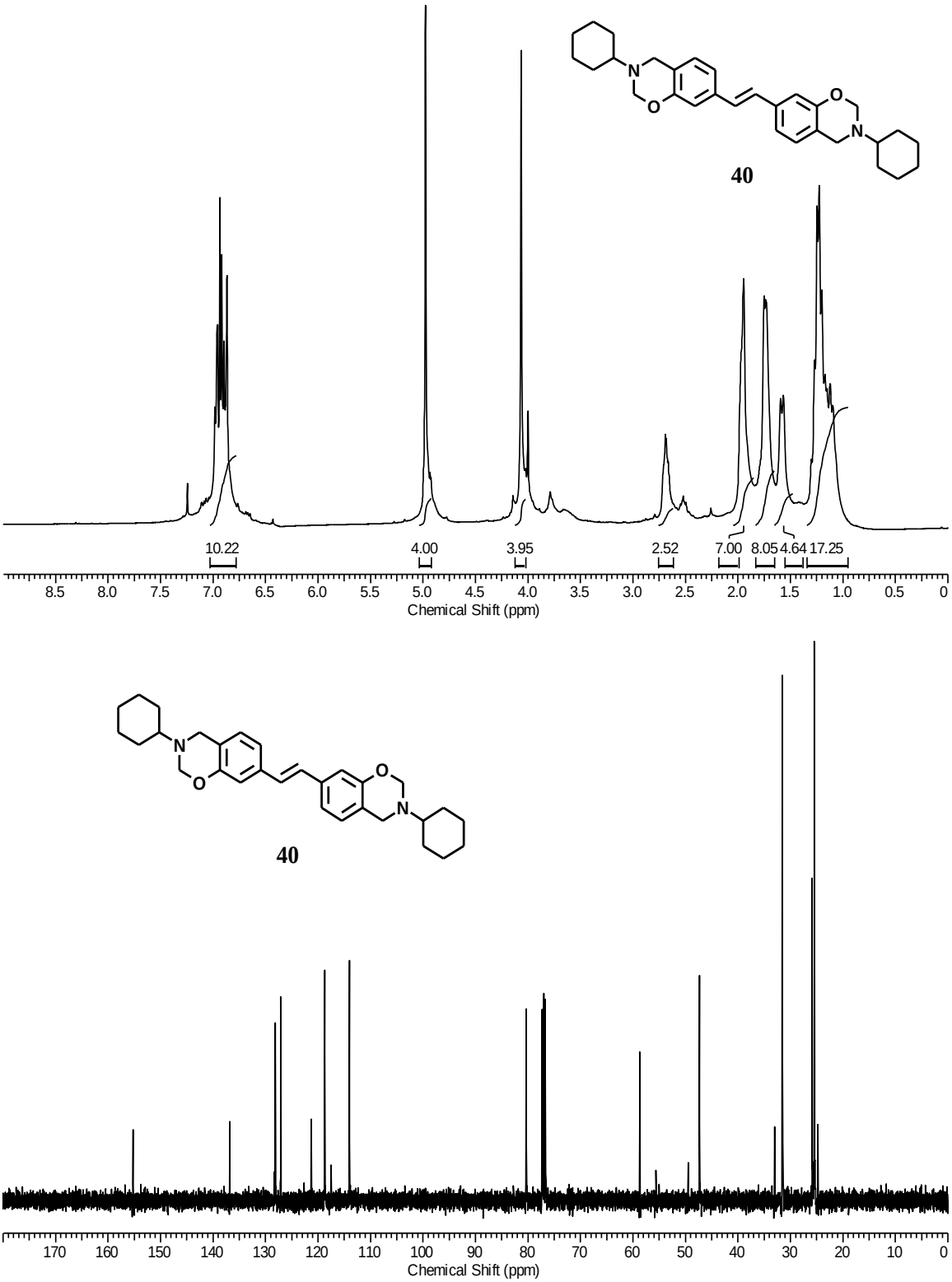


Fig. A-34 ^{1}H and ^{13}C NMR spectra of *E*-7,7'-(vinylene)bis[3-cyclohexyl-3,4-dihydro-2H-1,3-Benzoxazine] **(40)**.

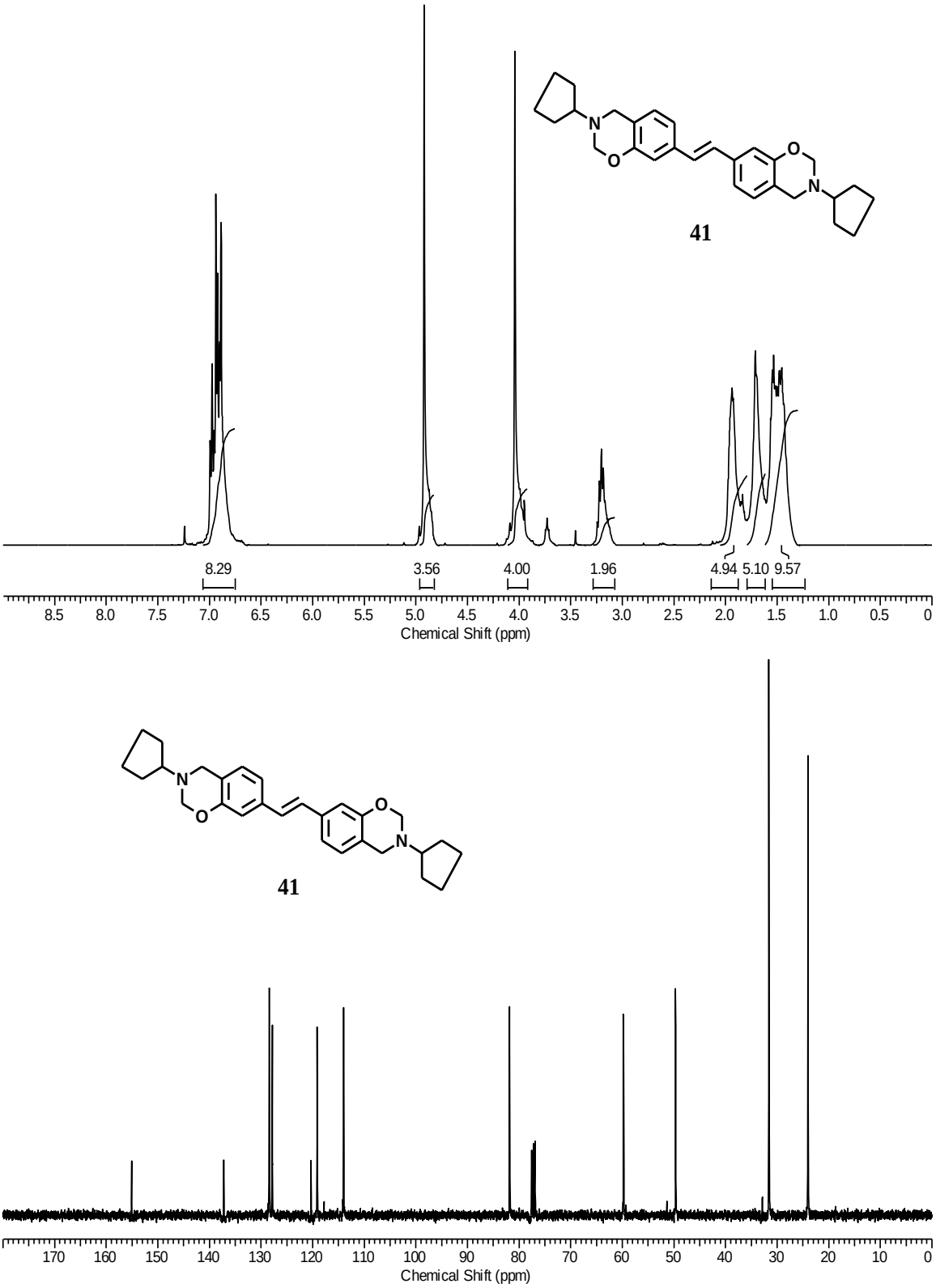


Fig. A-35 ^{1}H and ^{13}C NMR spectra of *E*-7,7'-(vinylene)bis[3-cyclopentyl-3,4-dihydro-2H-1,3-Benzoxazine] **(41)**.

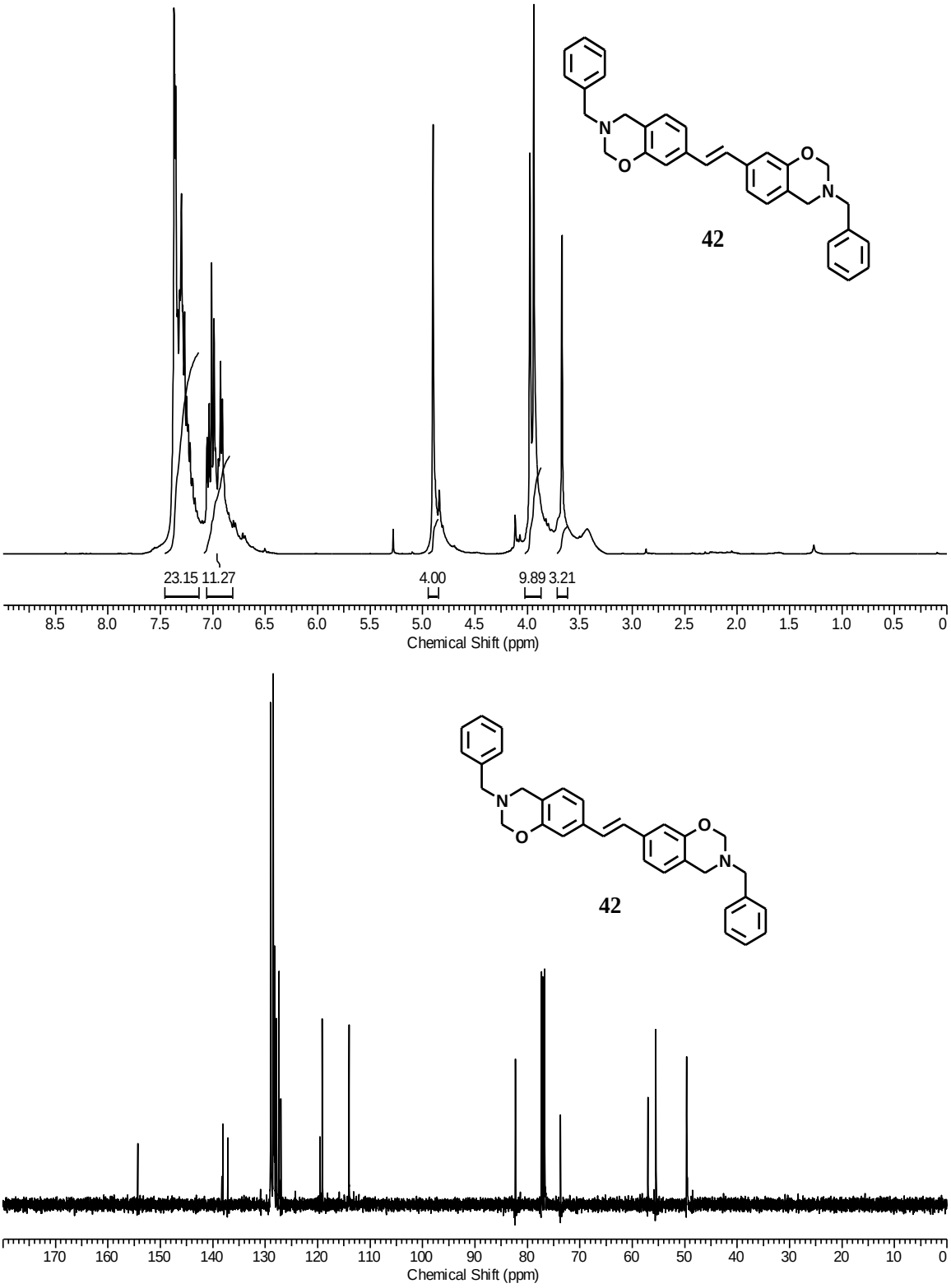


Fig. A-36 ^{1}H and ^{13}C NMR spectra of *E*-7,7'-(vinylene)bis[3-benzyl-3,4-dihydro- 2H-1,3-Benzoxazine] **(42)**.

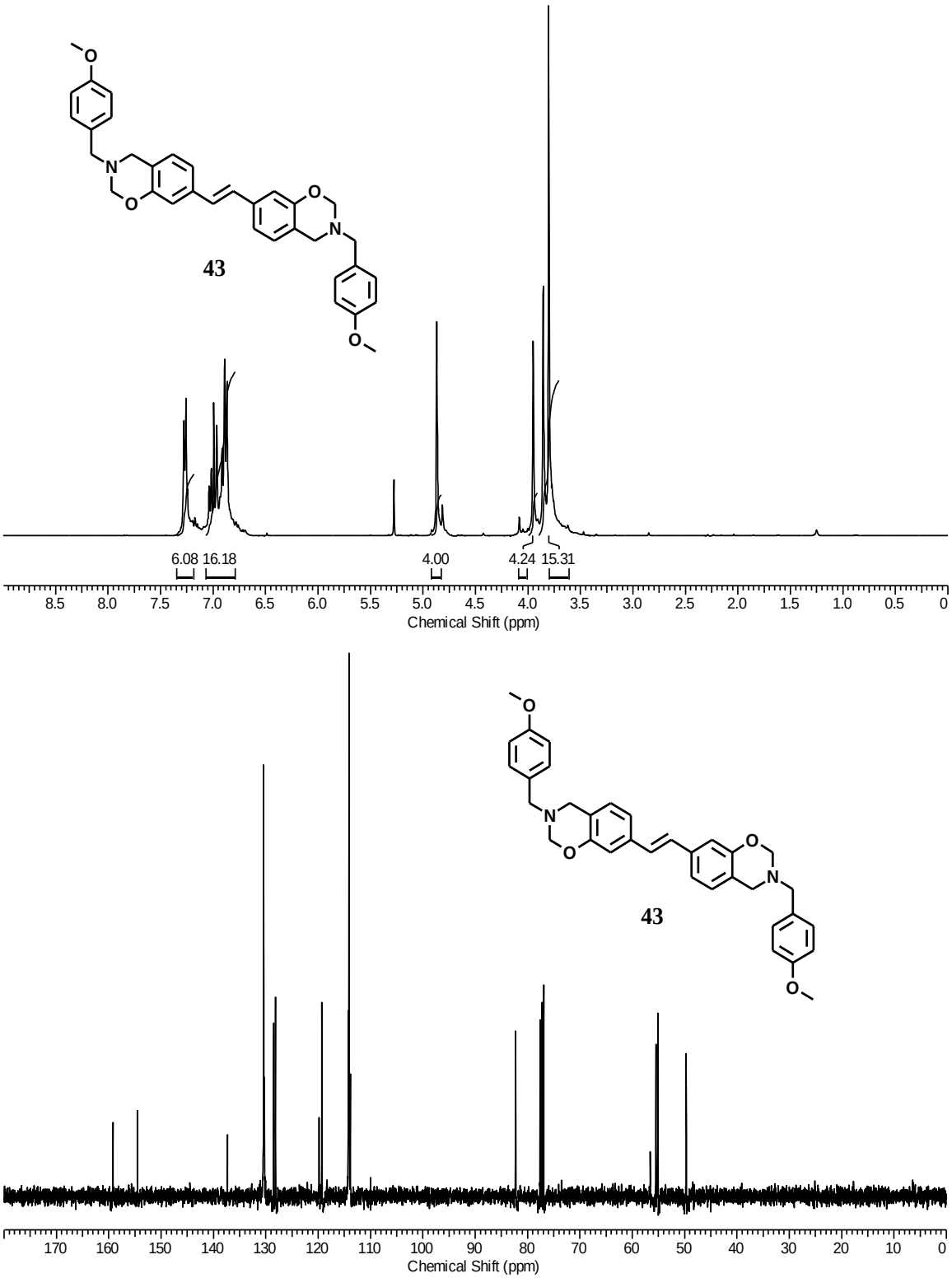


Fig. A-37 ^{1}H and ^{13}C NMR spectra of *E*-7,7'-(vinylene)bis[3-(4-methoxy benzyl)-3,4-dihydro- 2H-1,3-Benzoxazine] **(43)**.

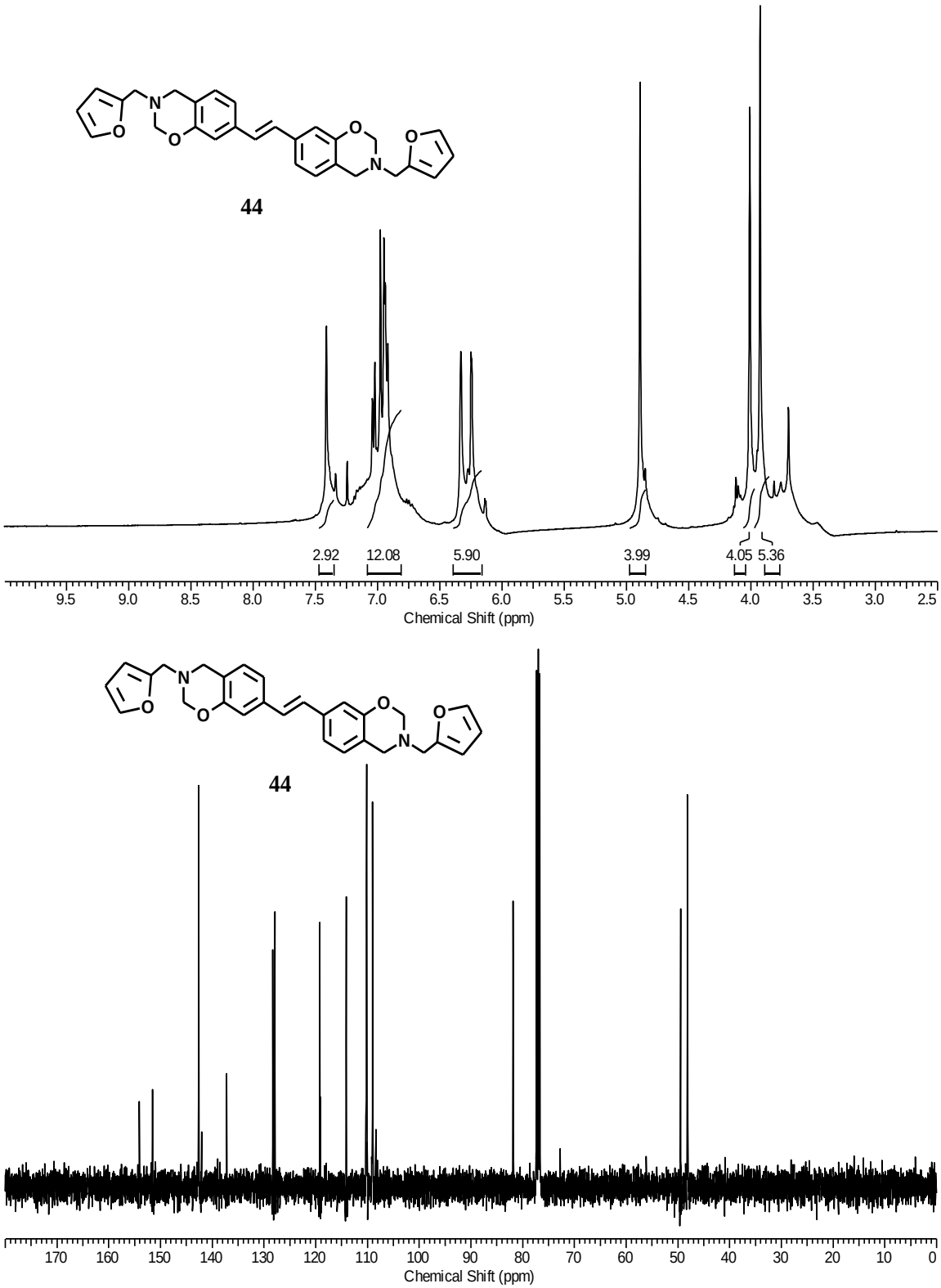


Fig. A-38 ^{1}H and ^{13}C NMR spectra of *E*-7,7'-(vinylene)bis[3-furfuryl-3,4-dihydro- 2H-1,3-Benzoxazine] **(44)**.

APPENDIX B

Spectroscopic Data for Compounds of Chapter 3

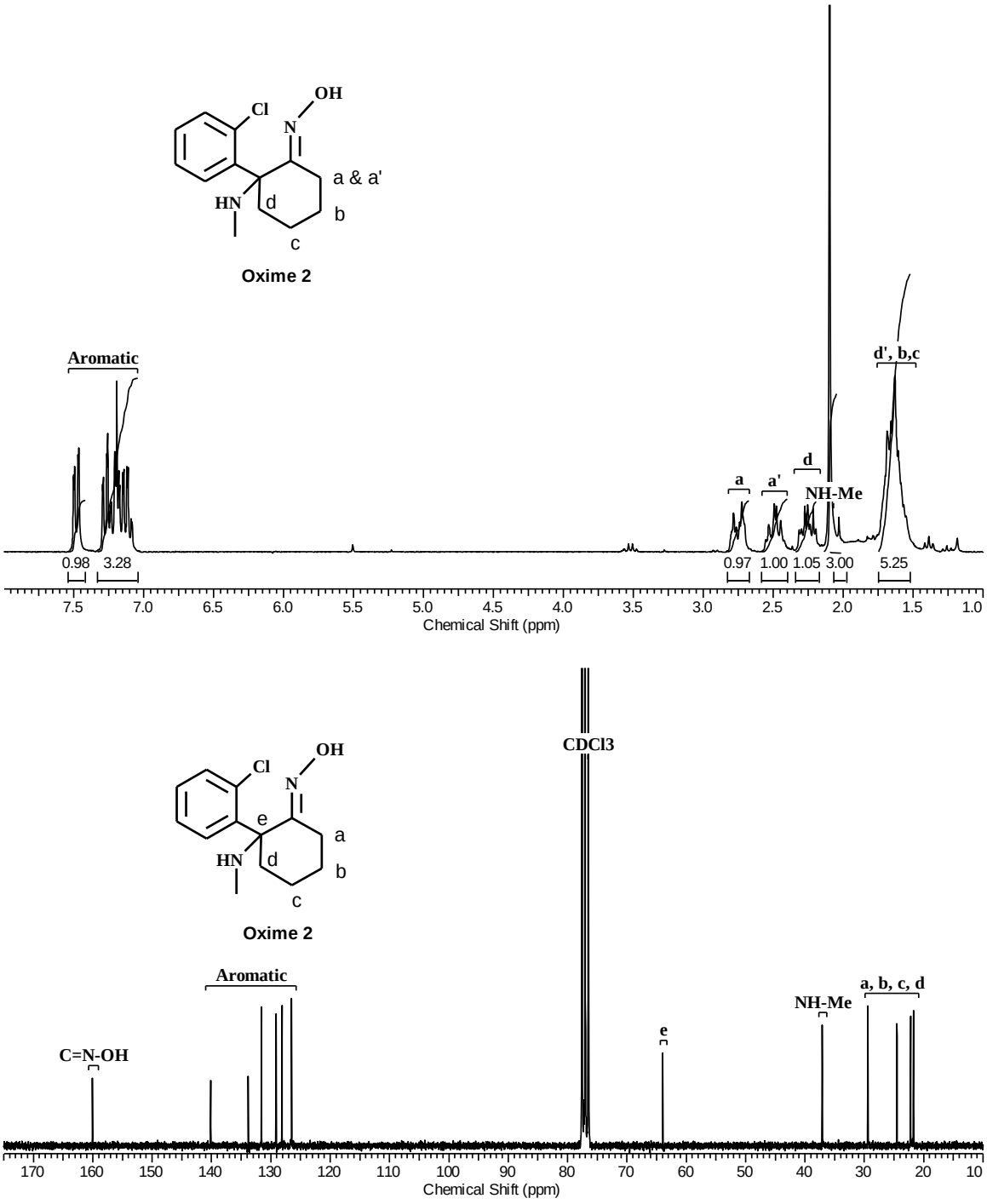


Fig. B-1 ^{1}H and ^{13}C-NMR spectra of 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone oxime **(2).**

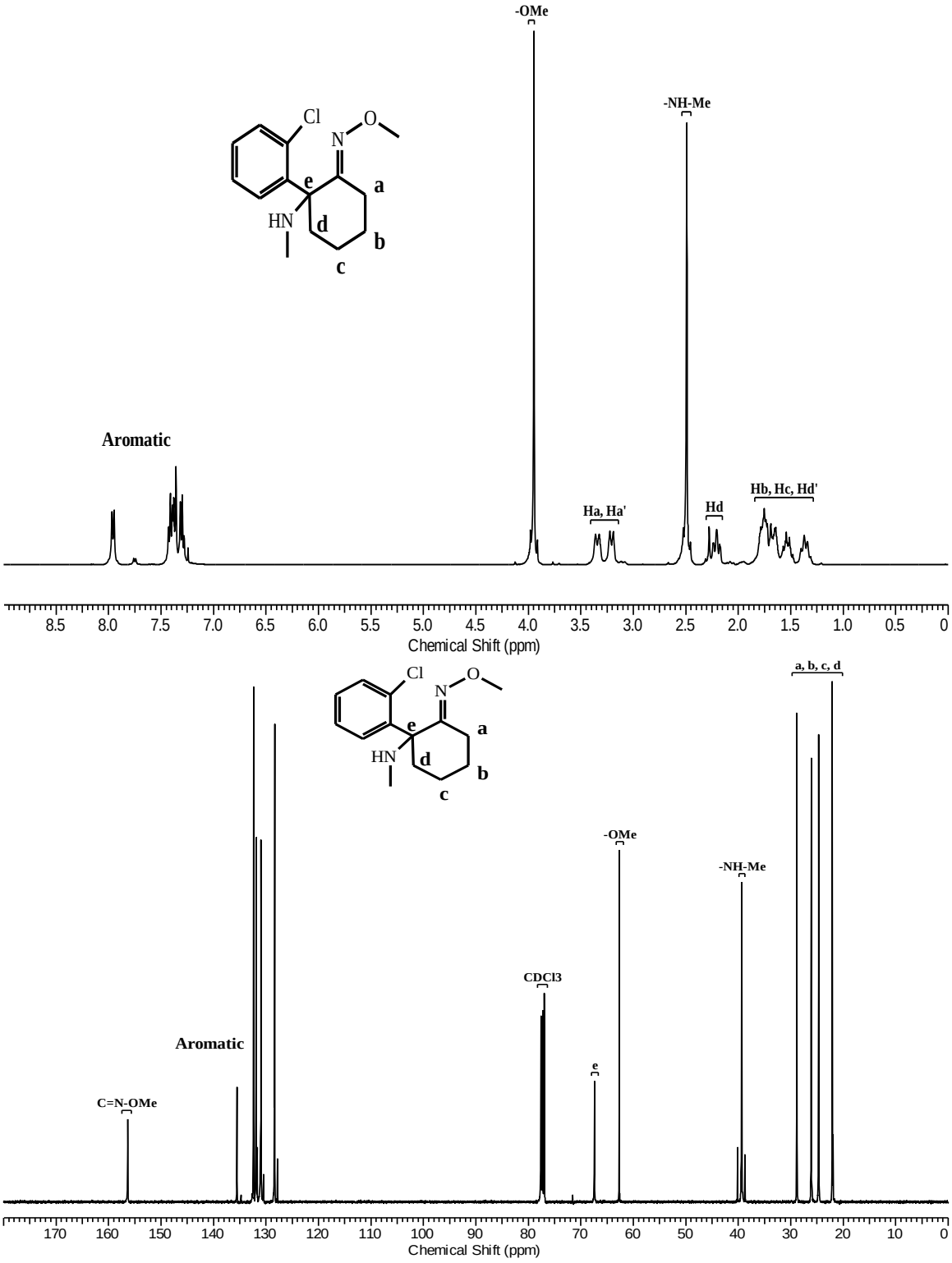


Fig. B-2 ^{1}H and ^{13}C-NMR spectra of 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-methyl-oxime **(3).**

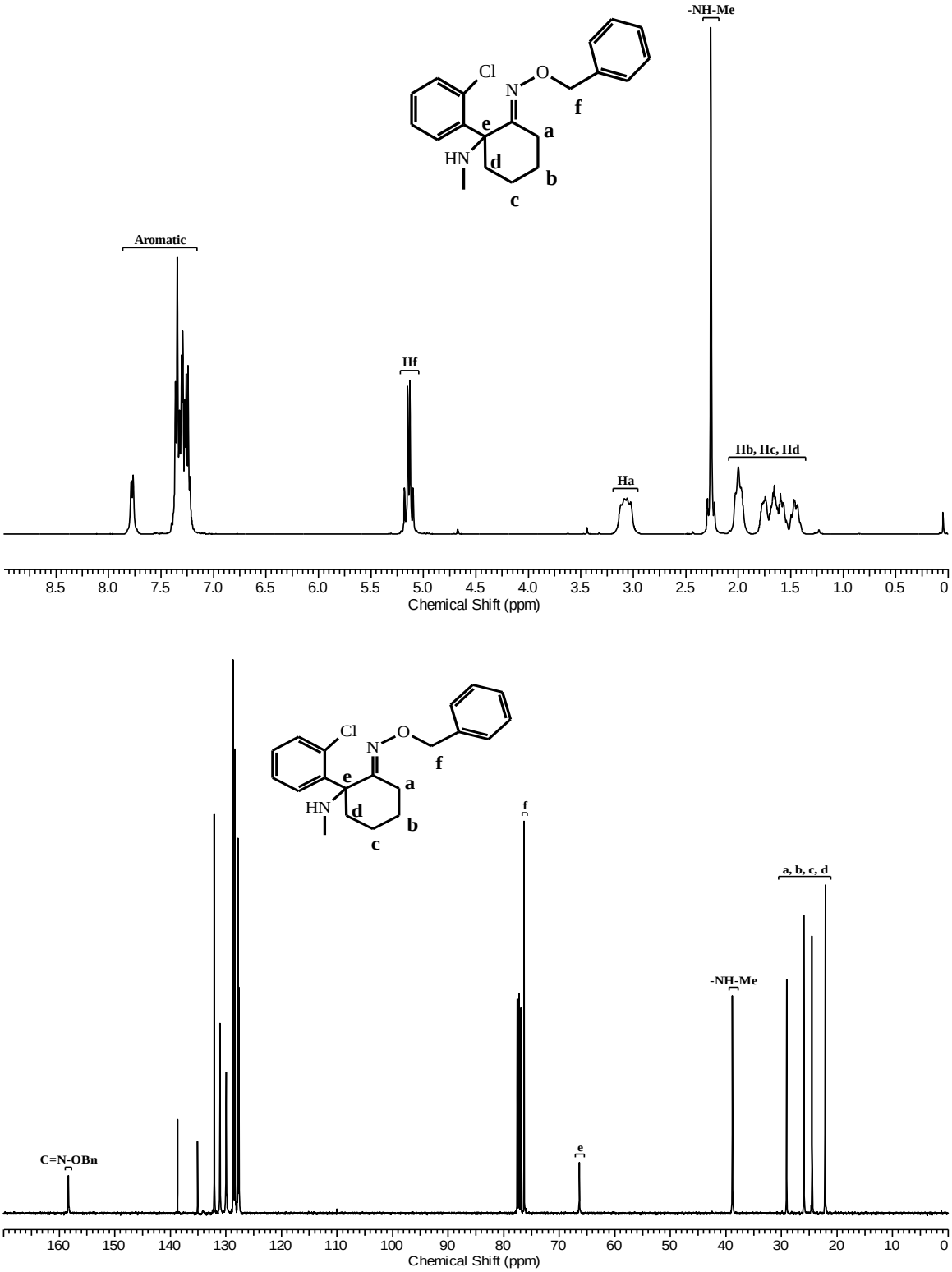


Fig. B-3 ^{1}H and ^{13}C-NMR spectra of 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-benzyl-oxime **(4).**

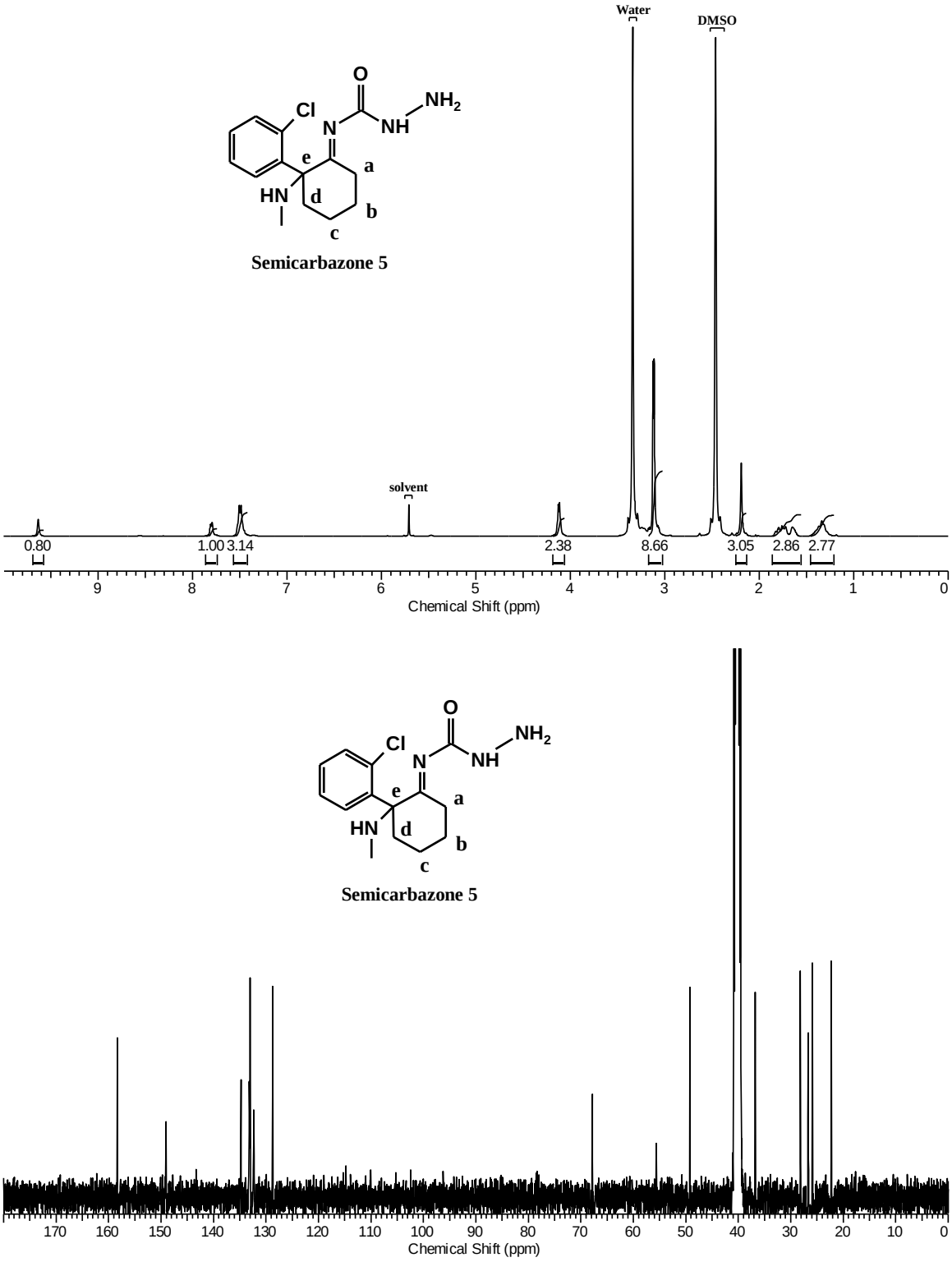


Fig. B-4 ^{1}H and ^{13}C-NMR spectra of 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanone O-semicarbazone **(5)**

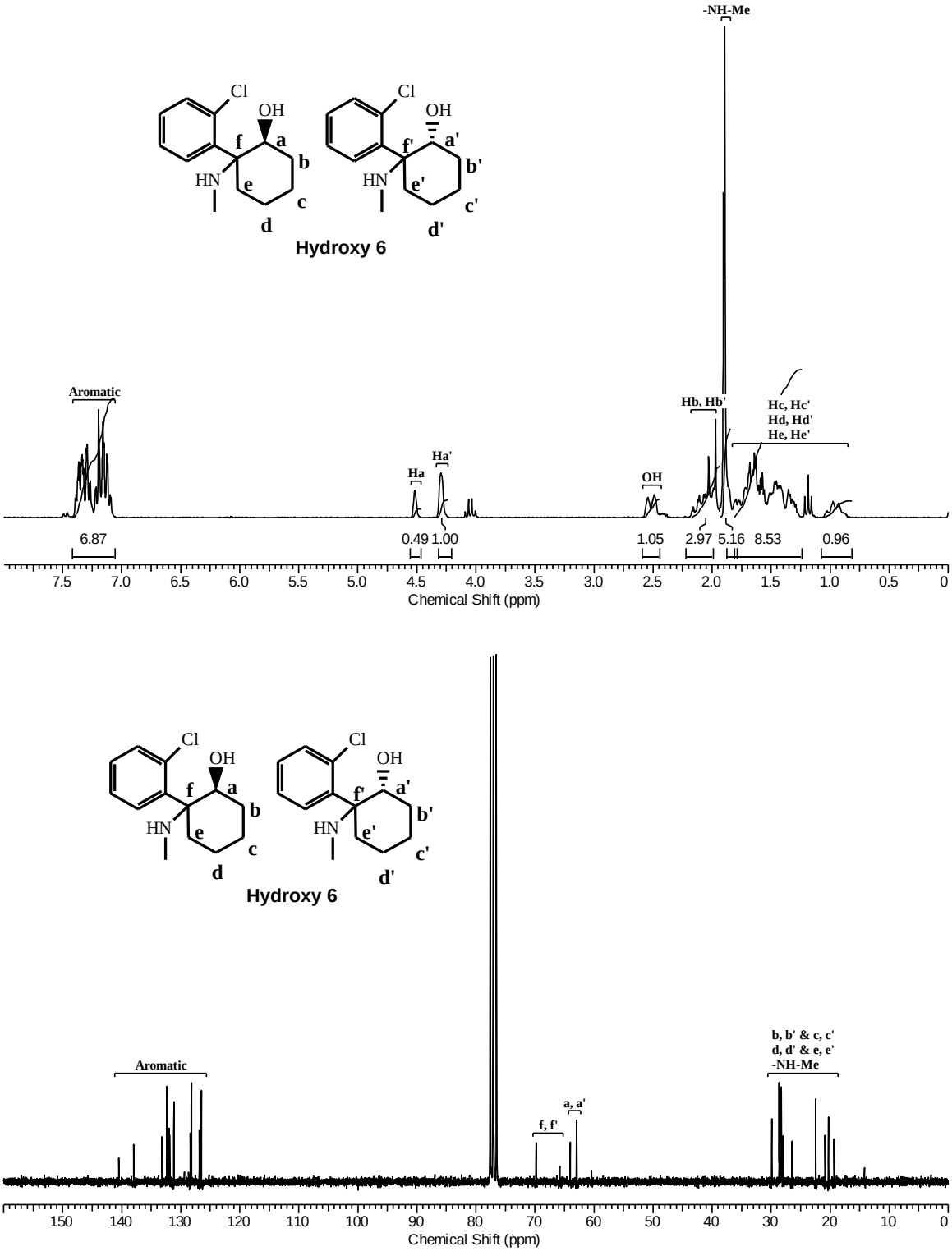


Fig. B-5 ^{1}H and ^{13}C-NMR spectra of 2-(2-Chloro-phenyl)-2-methylamino-cyclohexanol **(6).**

APPENDIX C

Spectroscopic Data for Compounds of Chapter 4

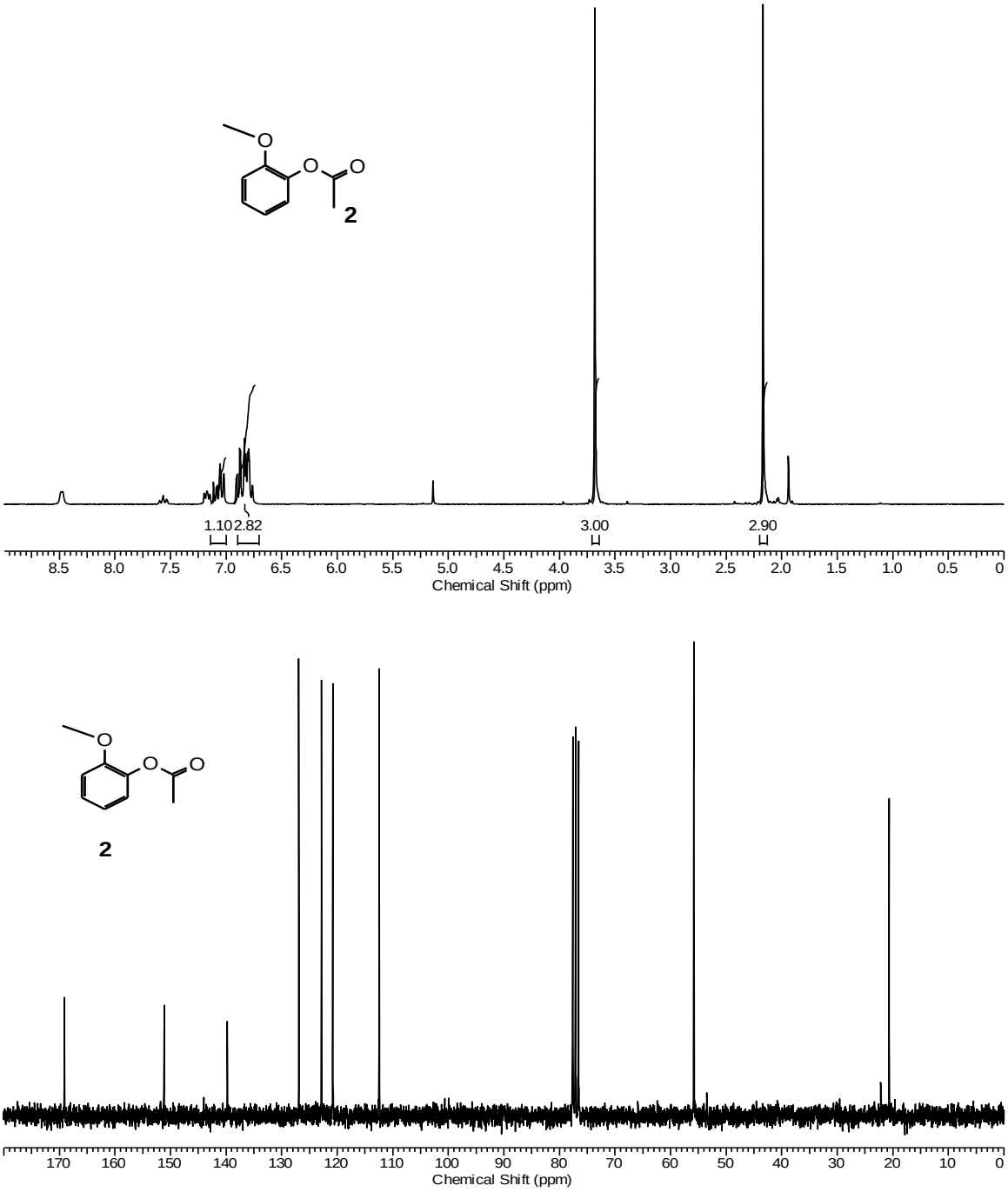


Fig. C-1 ^{1}H and ^{13}C-NMR spectra of Acetic acid 2-methoxy-phenyl ester **(2).**

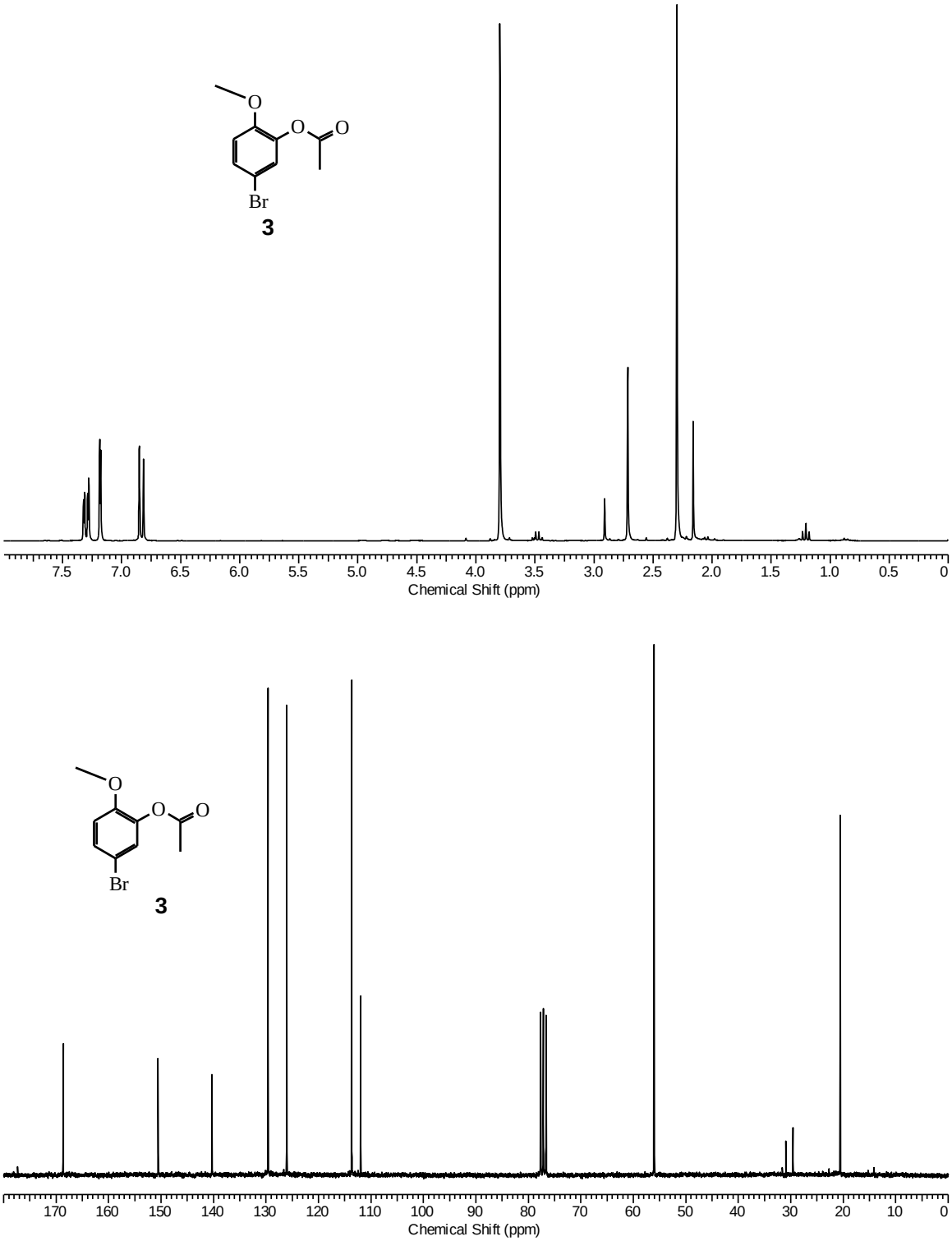


Fig. C-2 ^{1}H and ^{13}C-NMR spectra of Acetic acid 5-bromo-2-methoxy-phenyl ester **(3).**

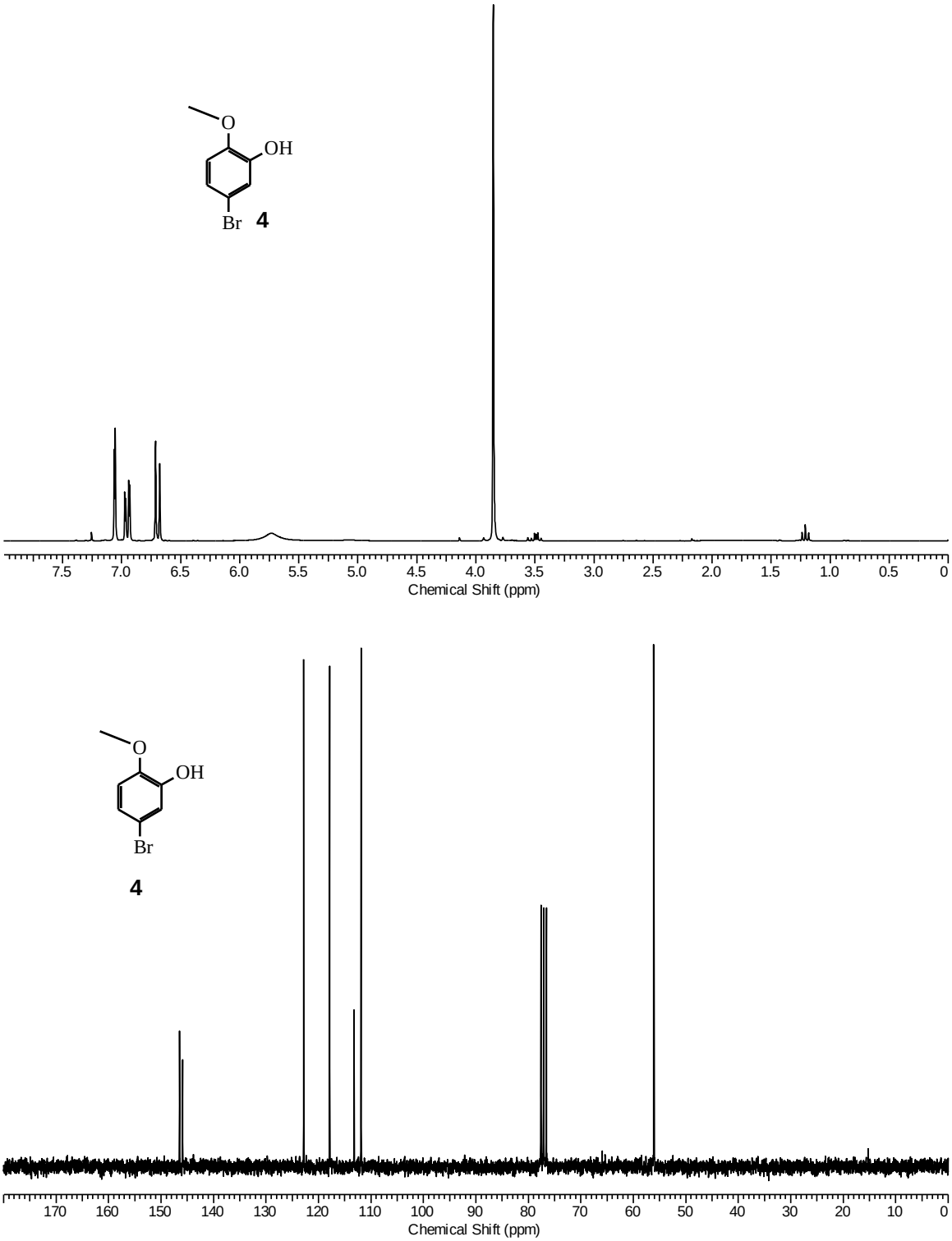


Fig. C-3 ^{1}H and ^{13}C-NMR spectra of 5-Bromo-2-methoxy-phenol **(4).**

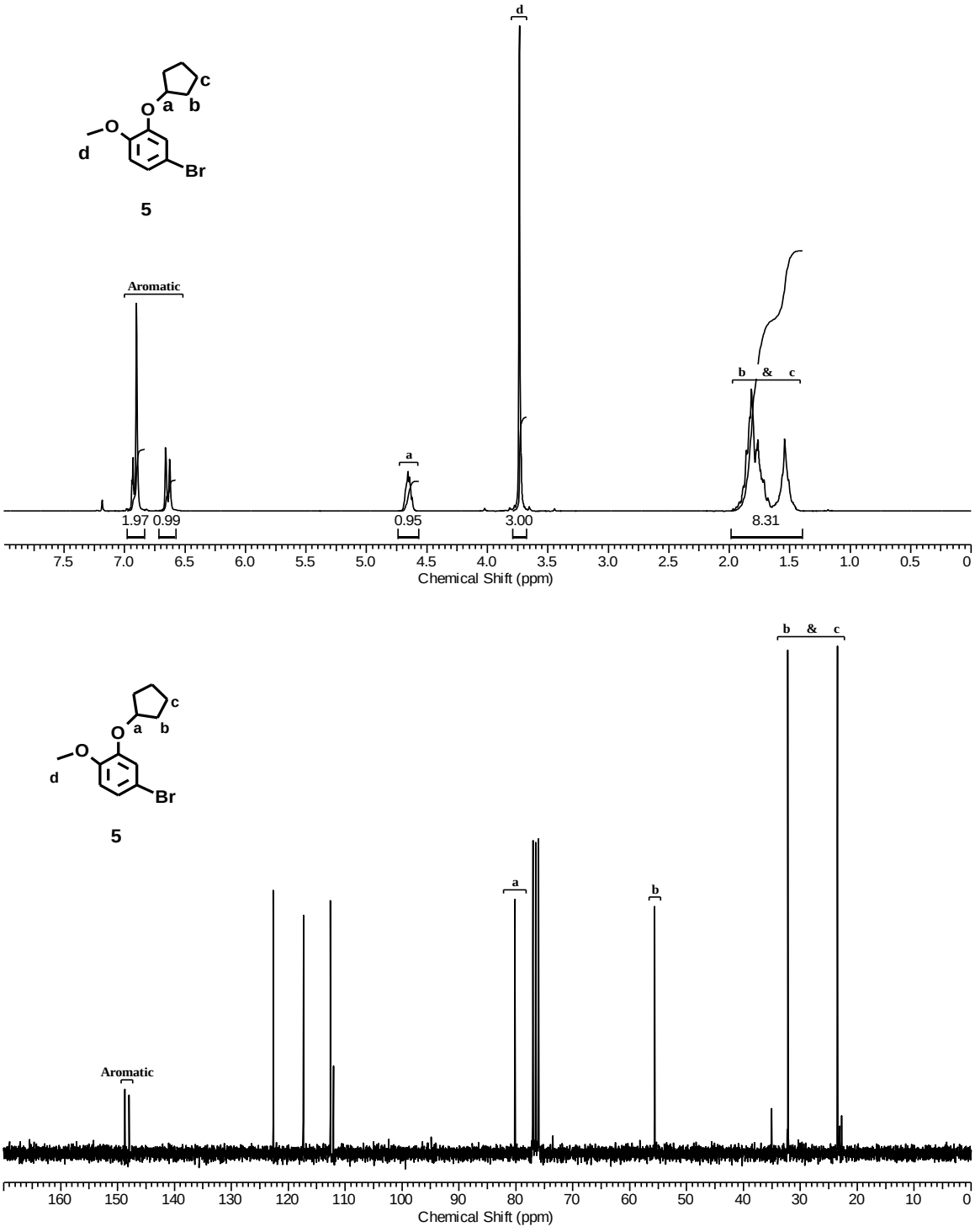


Fig. C-4 ^{1}H and ^{13}C-NMR spectra of 4-bromo-2-(cyclopentyloxy)-1-methoxybenzene **(5).**

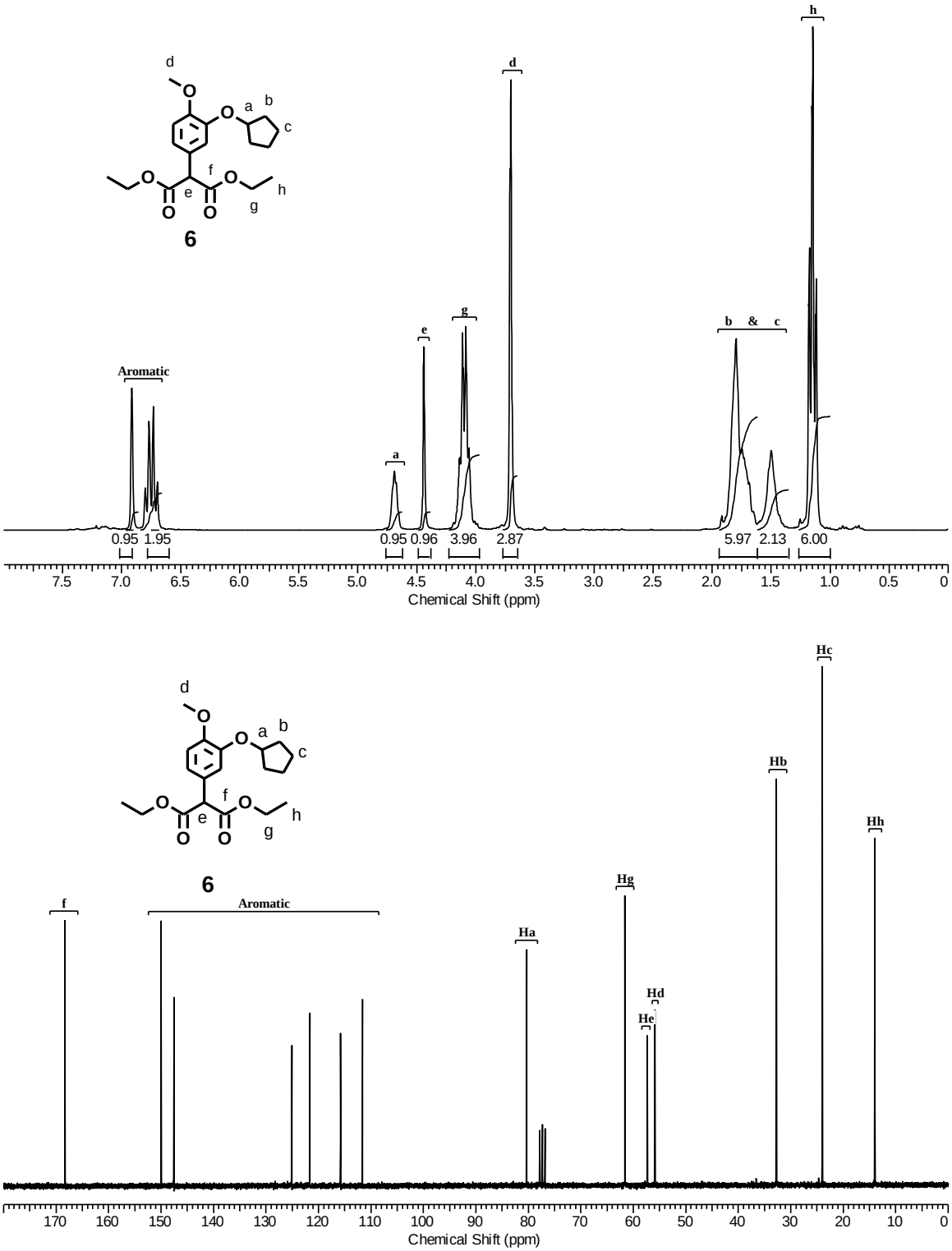


Fig. C-5 ^{1}H and ^{13}C-NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-malonic acid diethyl ester **(6).**

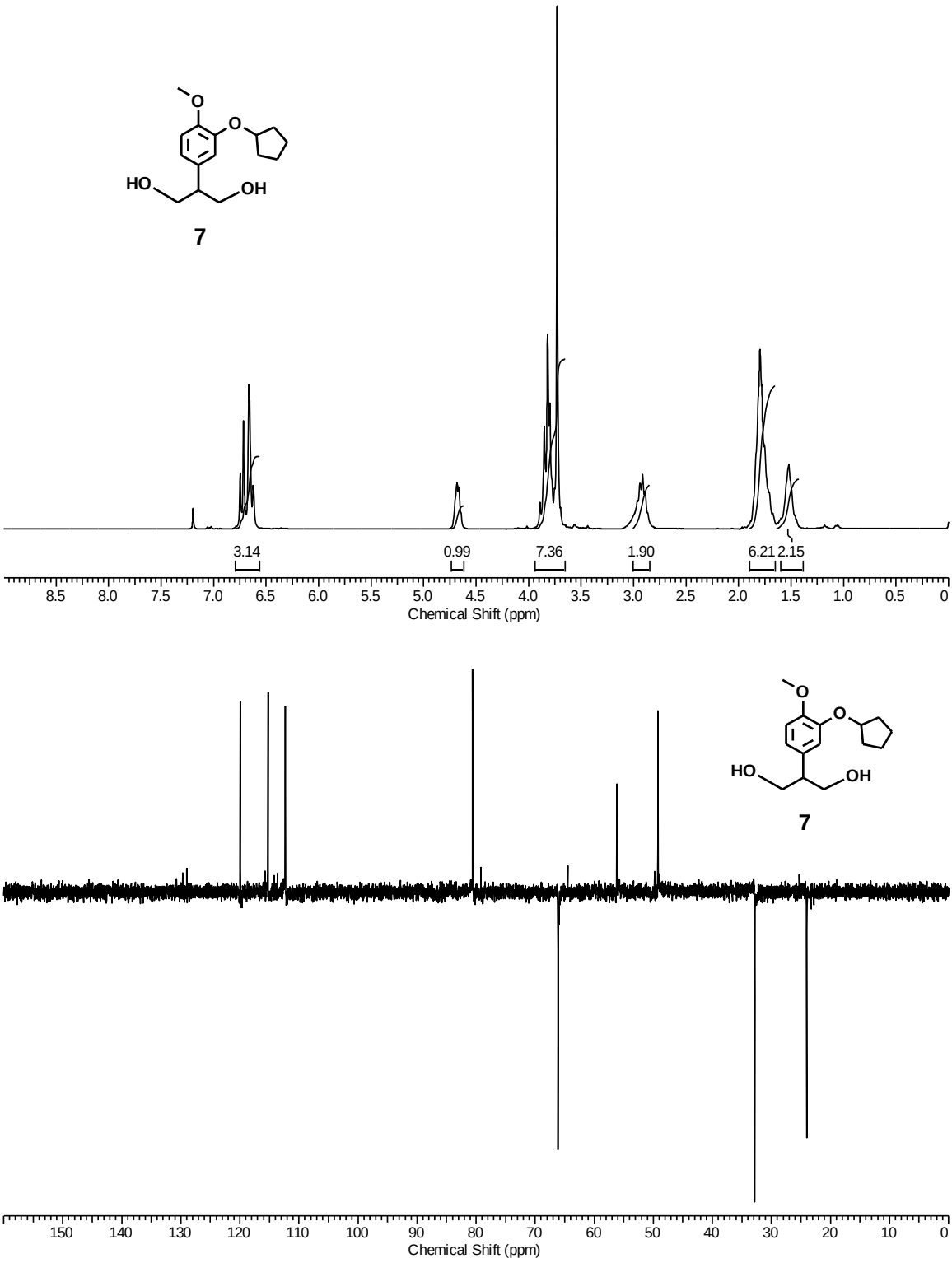


Fig. C-6 ^{1}H and DEPT-NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-propane-1,3-diol **(7).**

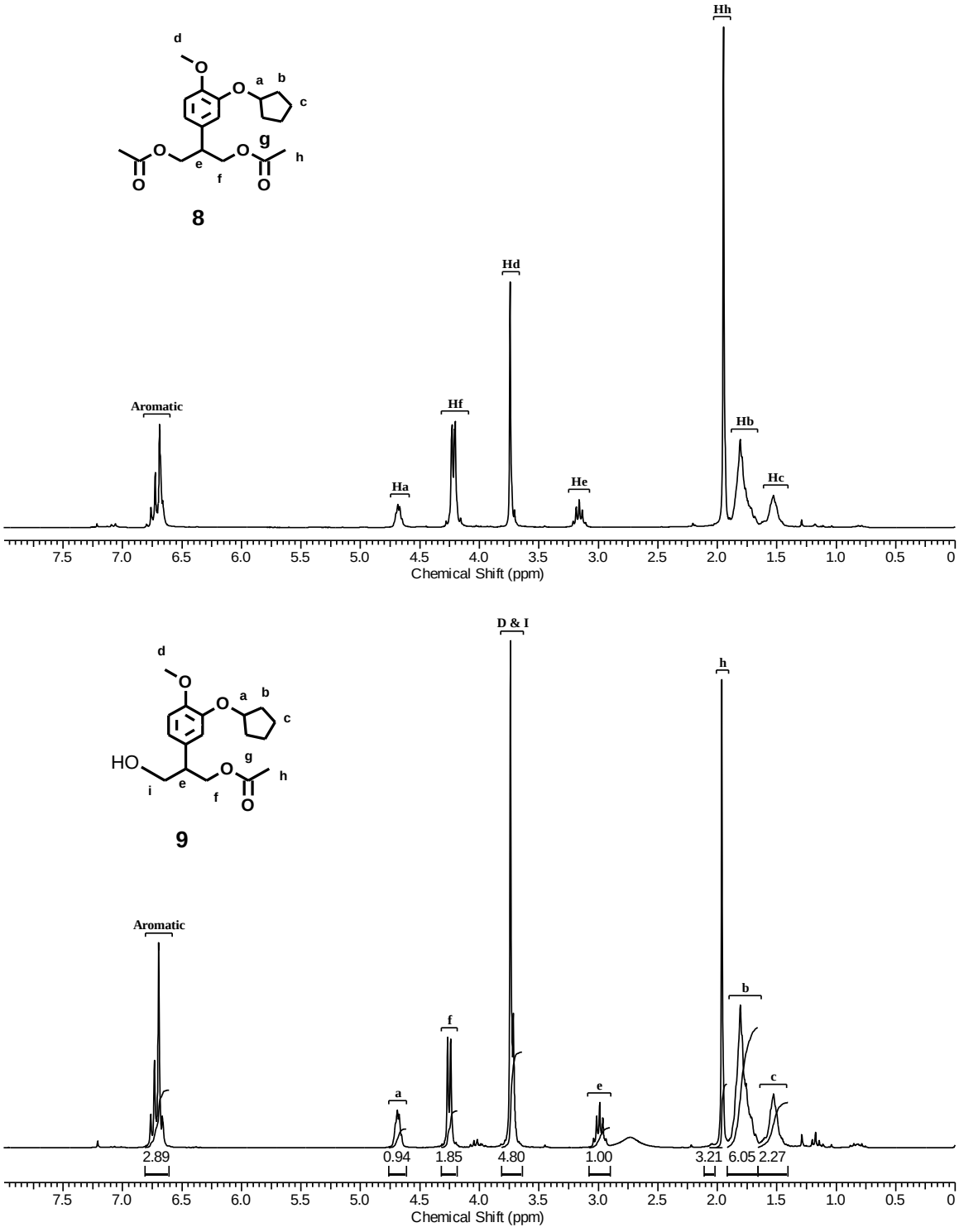


Fig. C-7 ^{1}H NMR spectra of Acetic acid 3-acetoxy-2-(3-cyclopentyloxy-4-methoxy-phenyl)propyl ester **(8)** and ^{1}H -NMR spectra of Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-hydroxy-propyl ester **(9).**

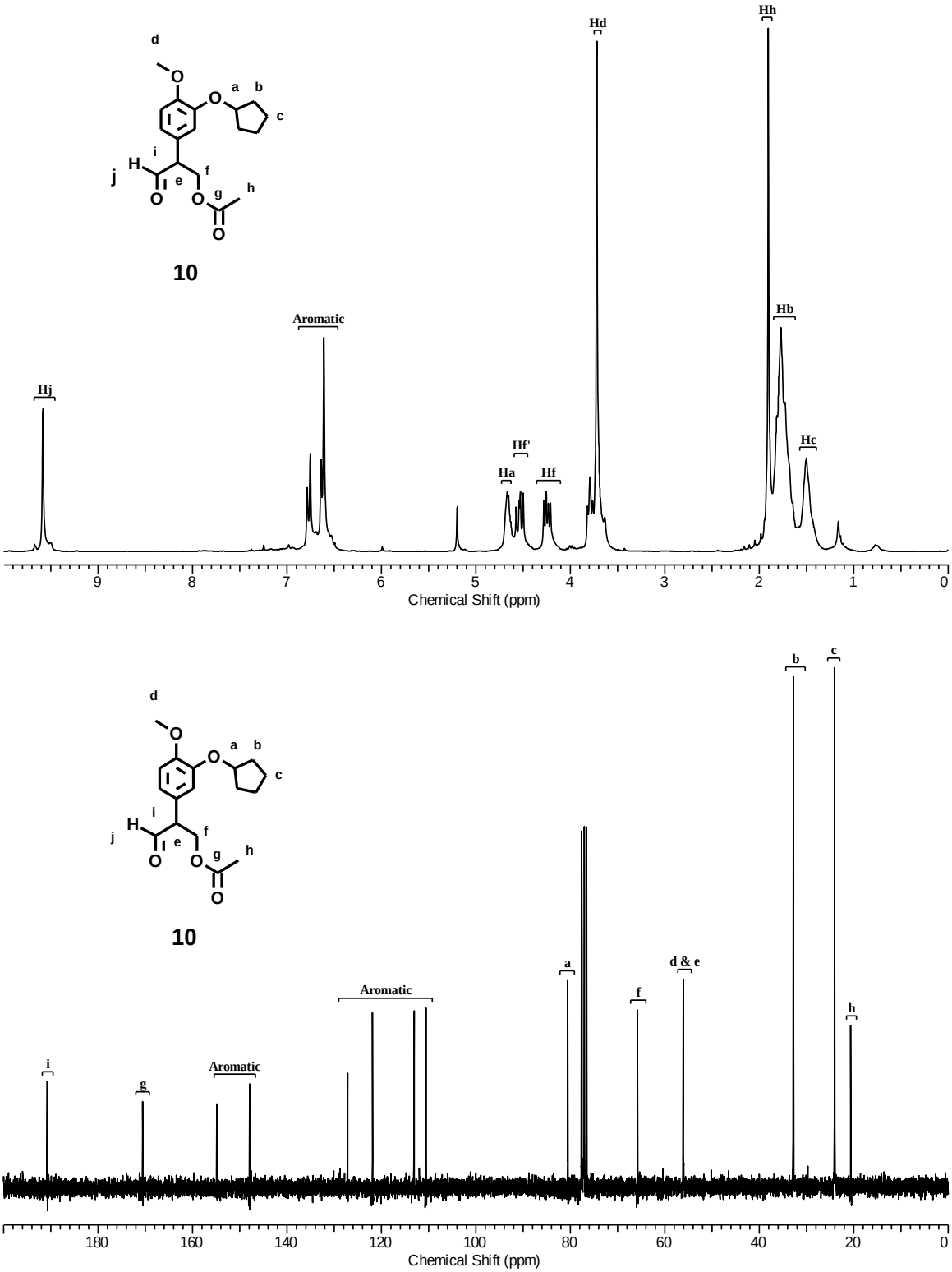


Fig. C-8: 1H and 13C-NMR spectra of Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-3-oxo-propyl ester **(10).**

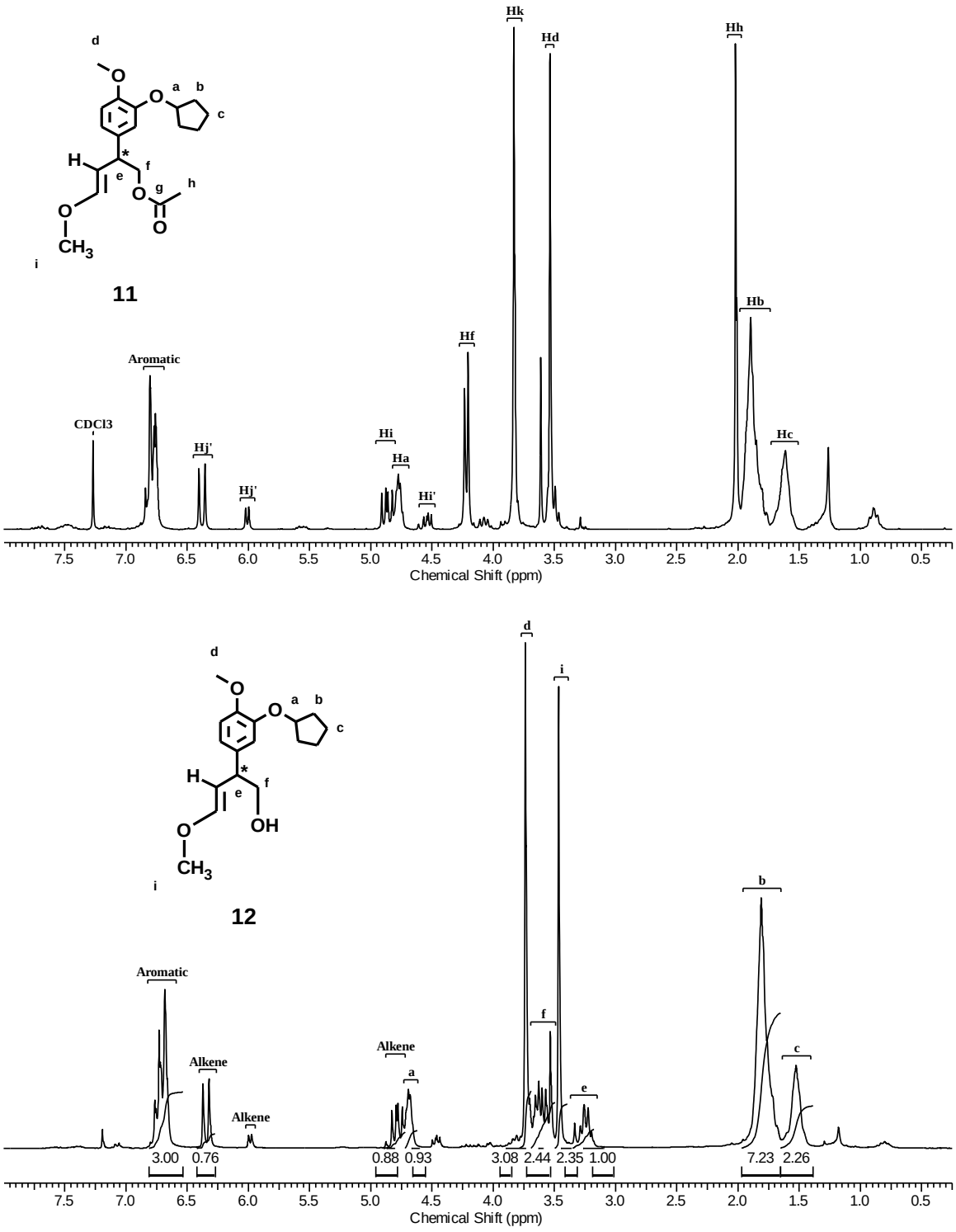


Fig. C-9 ^{1}H NMR spectra of (*E*/Z)-Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-methoxy-but-3-enyl ester **(11)** and ^{1}H -NMR spectra of (*E*/Z)-2-(3-Cyclopentyloxy-4-methoxy-phenyl)-4-methoxy-but-3-en-1-ol **(12).**

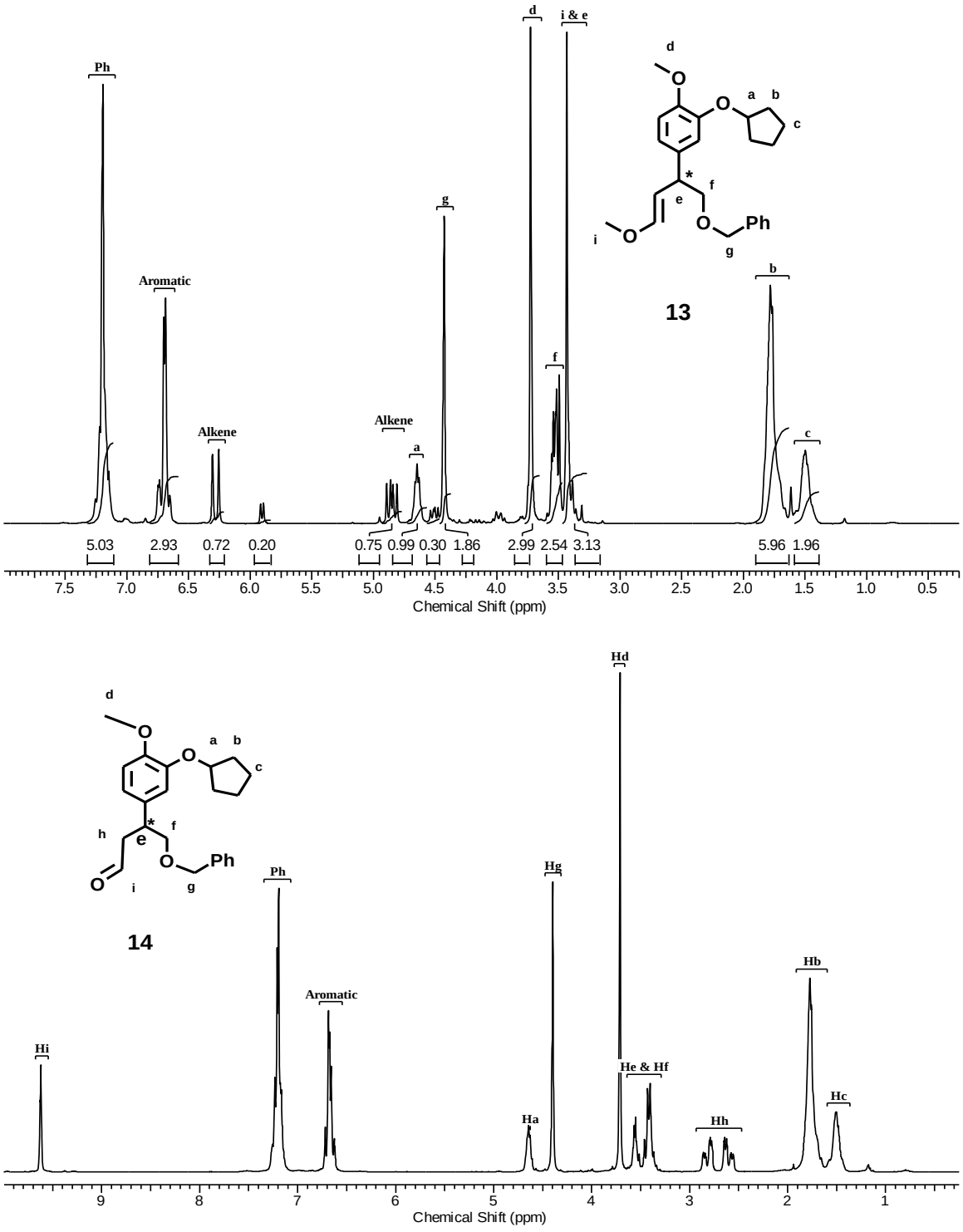


Fig. C-10 ^{1}H NMR spectra of 4-(1-Benzyloxymethyl-3-methoxy-allyl)-2-cyclopentyloxy-1-methoxy-benzene **(13)** and ^{1}H -NMR spectra of 4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyraldehyde **(14).**

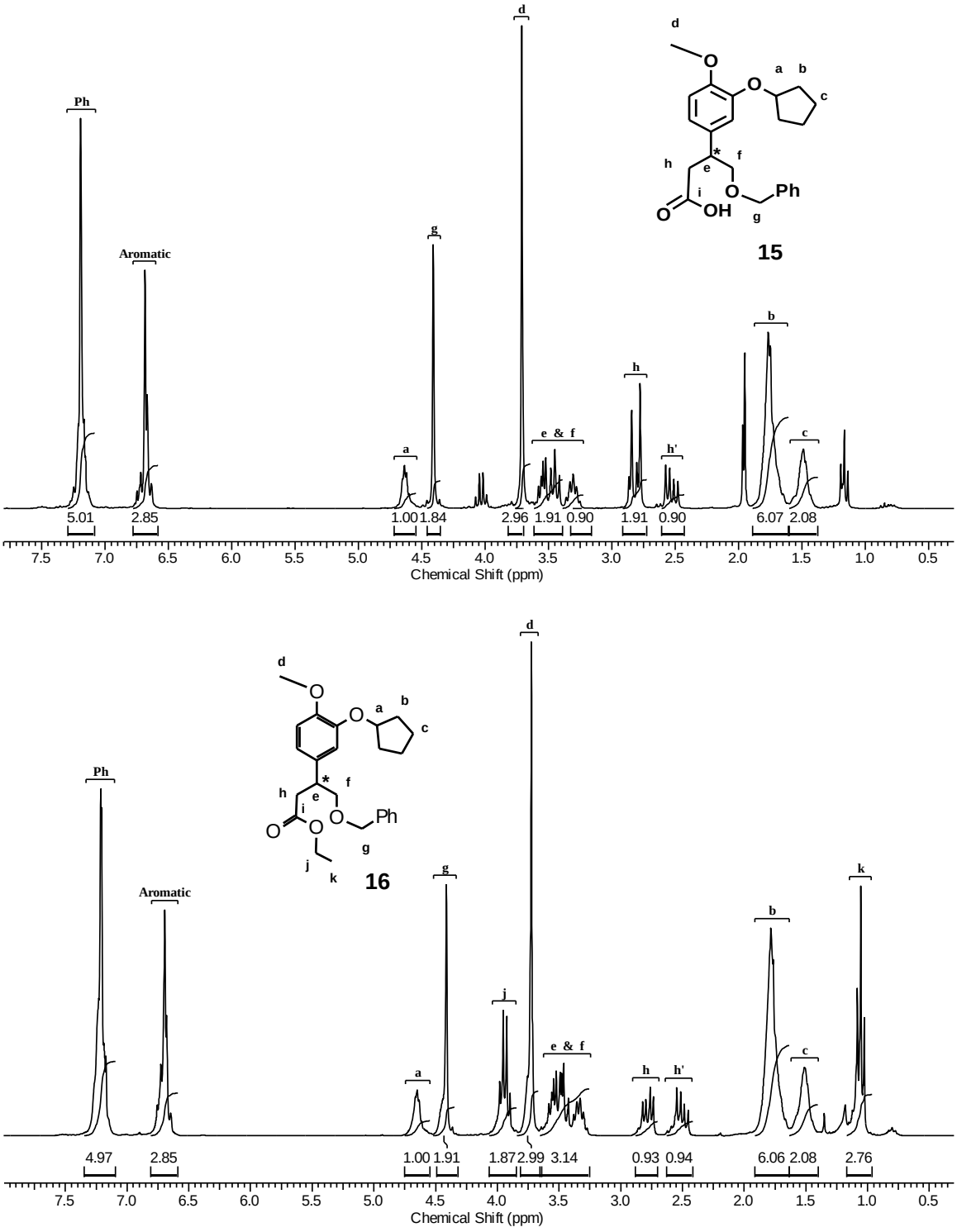


Fig. C-11 ^{1}H NMR spectra of 4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid **(15)** and ^{1}H -NMR spectra of 4-Benzyloxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester **(16).**

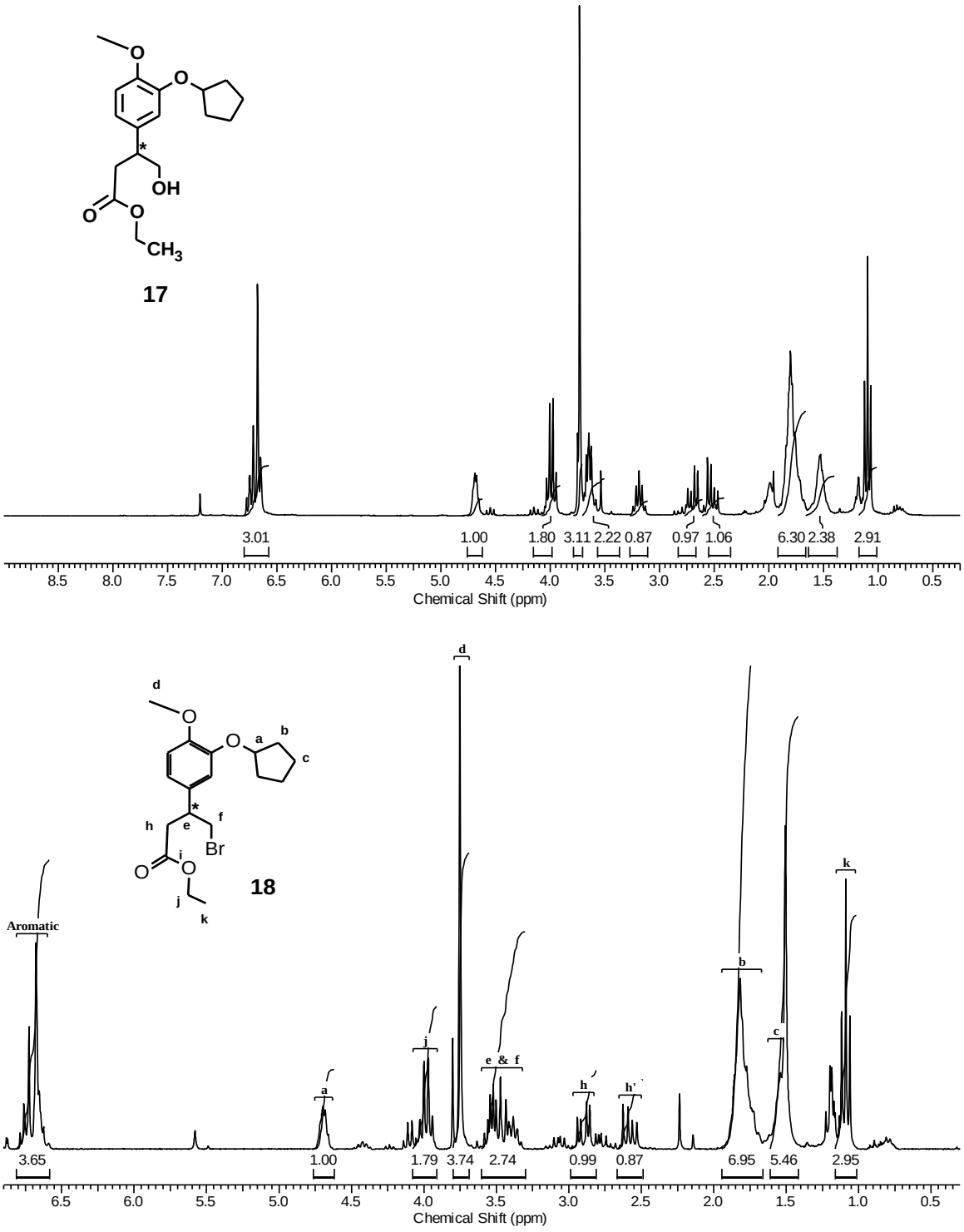


Fig. C-12 ^{1}H NMR spectra of 3-(3-Cyclopentyloxy-4-methoxy-phenyl)-4-hydroxy-butyric acid ethyl ester **(17)** and ^{1}H -NMR spectra of 4-Bromo-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester **(18).**

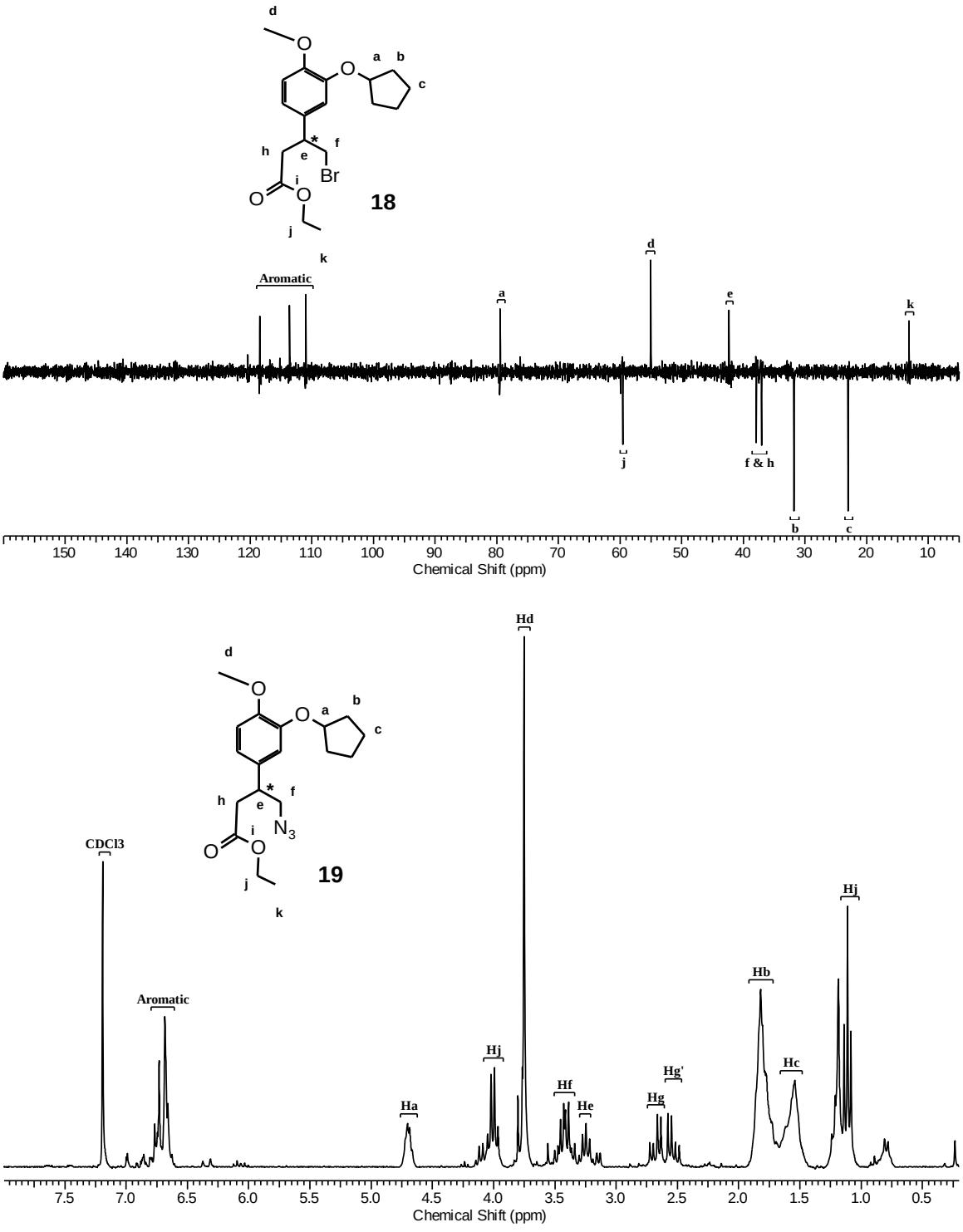


Fig. C-13 DEPT- NMR spectra of 4-Bromo-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester **(18)** and ^{1}H -NMR spectra of 4-Azido-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester **(19).**

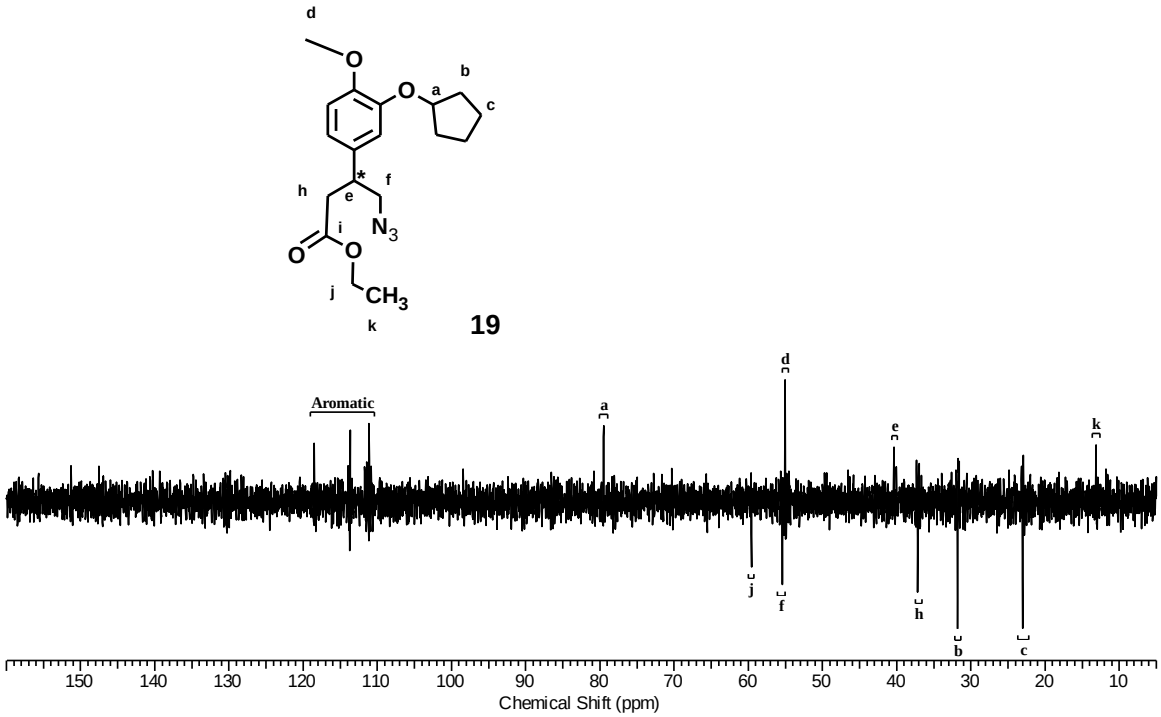


Fig. C-14 DEPT- NMR spectra of 4-Azido-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid ethyl ester **(19).**

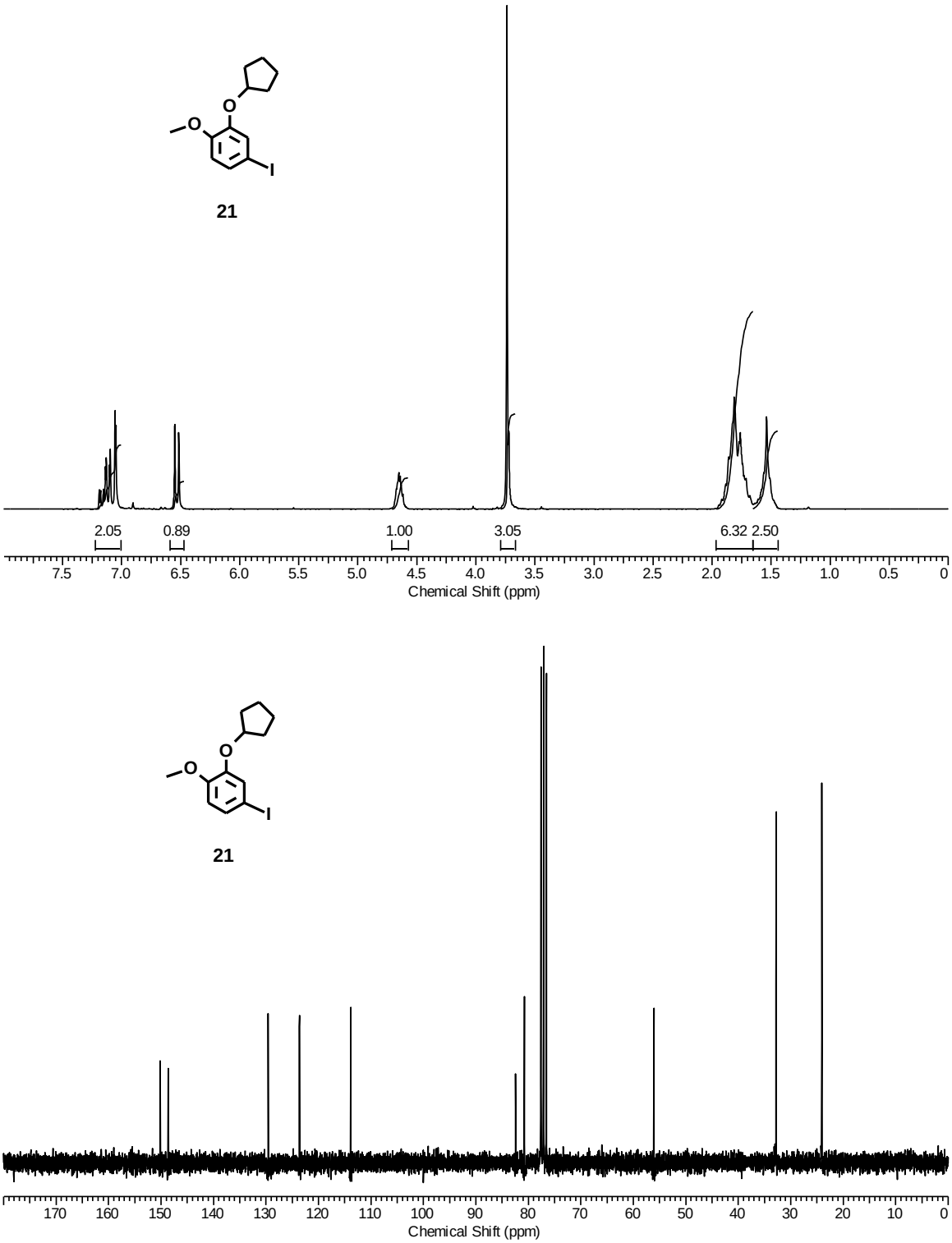


Fig. C-15 ^{1}H and ^{13}C-NMR spectra of 2-Cyclopentyloxy-4-iodo-1-methoxy-benzene **(21).**

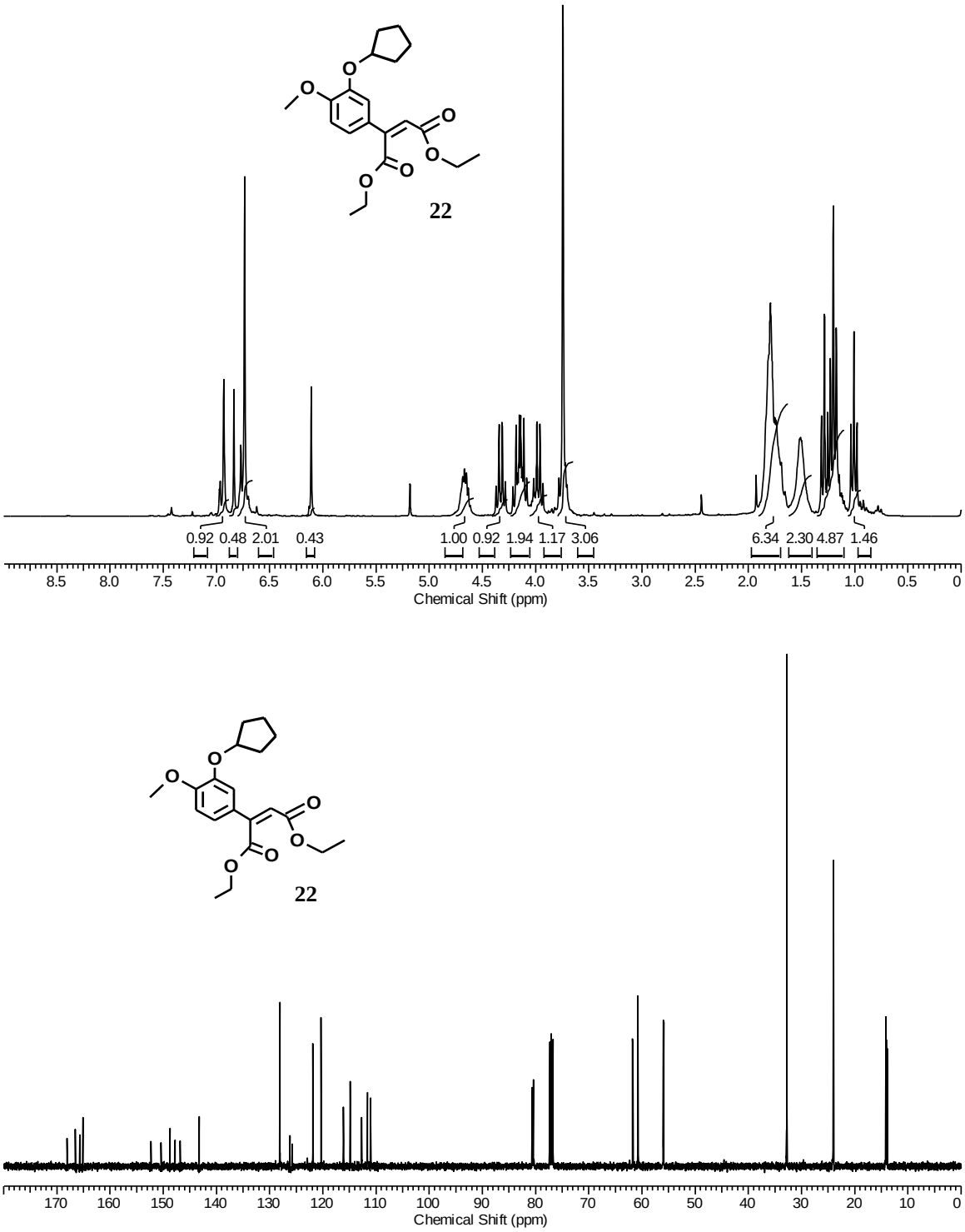


Fig. C-16 ^{1}H and ^{13}C-NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-but-2-enedioic acid diethyl ester **(22).**

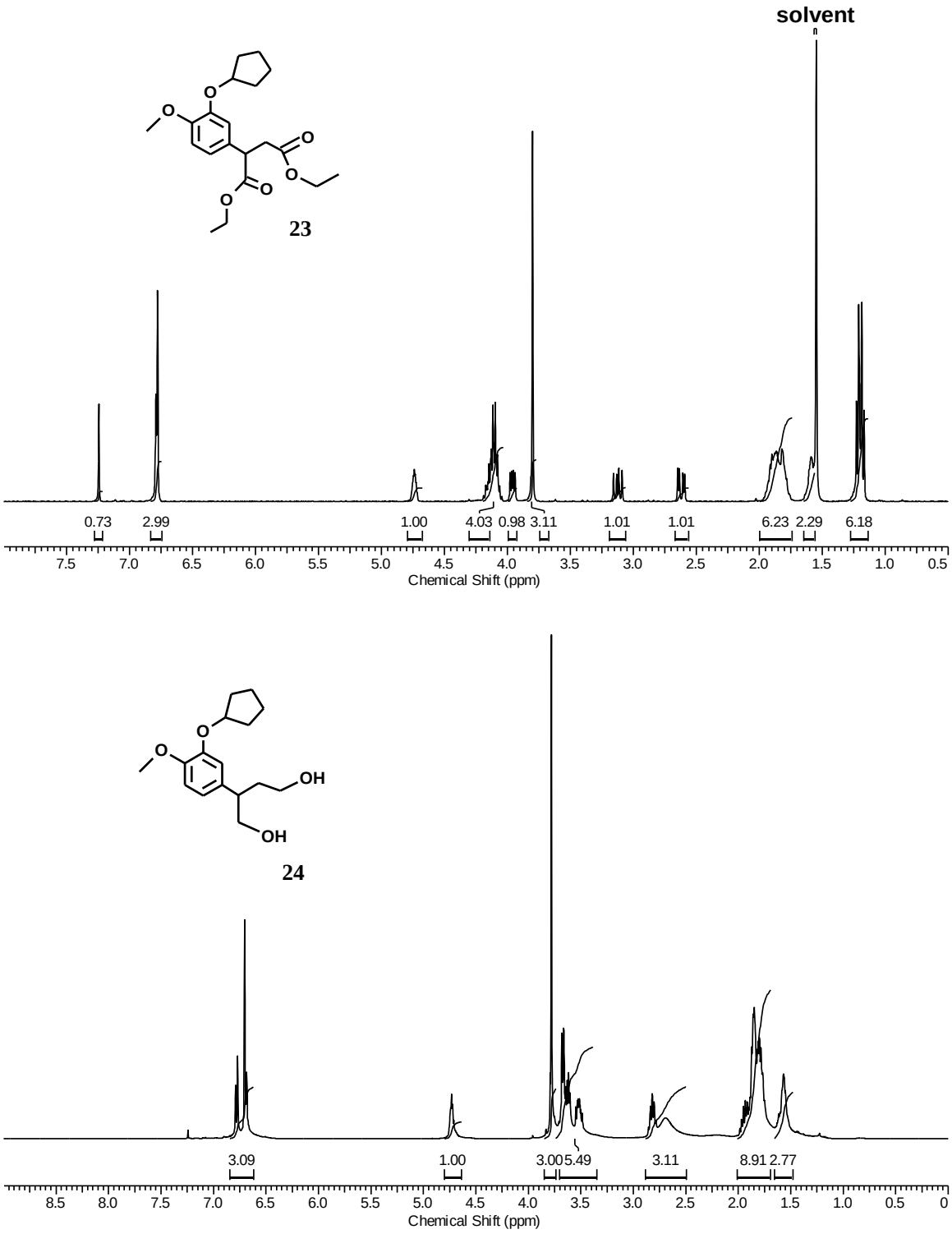


Fig. C-17 ^{1}H NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-succinic acid diethyl ester **(23)** and ^{1}H -NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-butane-1,4-diol **(24).**

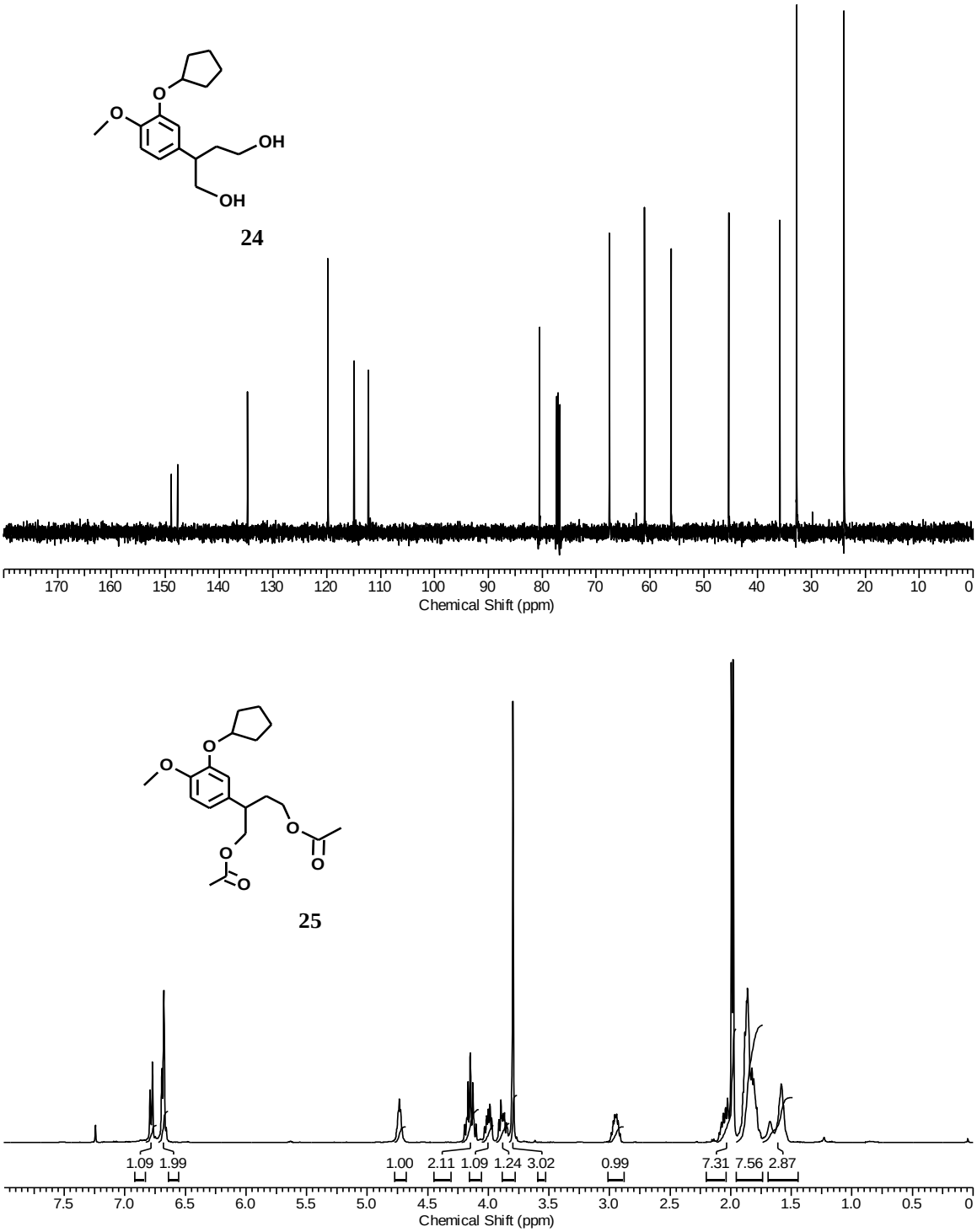
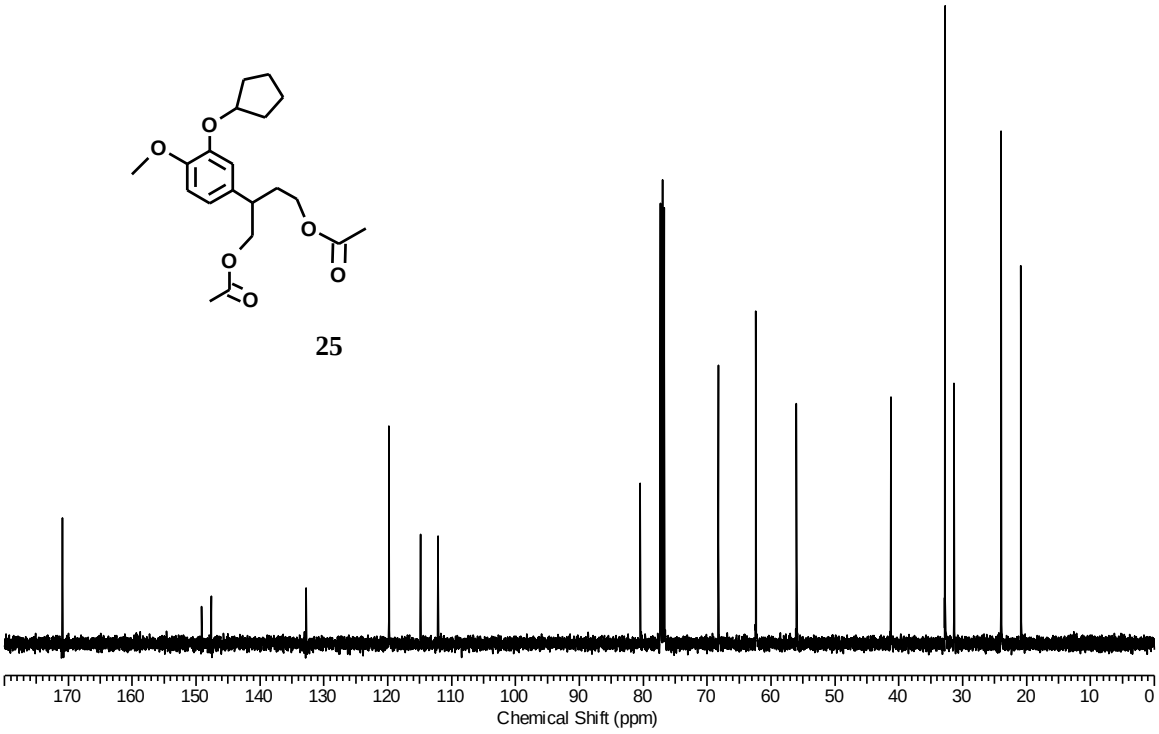


Fig. C-18 ^{13}C -NMR spectra of 2-(3-Cyclopentyloxy-4-methoxy-phenyl)-butane-1,4-diol **(24)** and ^{1}H -NMR spectra of Acetic acid 4-acetoxy-2-(3-cyclopentyloxy-4-methoxy-phenyl)-butyl ester (**25).**



25

Fig. C-19 ^{13}C -NMR spectra of Acetic acid 4-acetoxy-2-(3-cyclopentyloxy-4-methoxy-phenyl)-butyl ester (**25).**

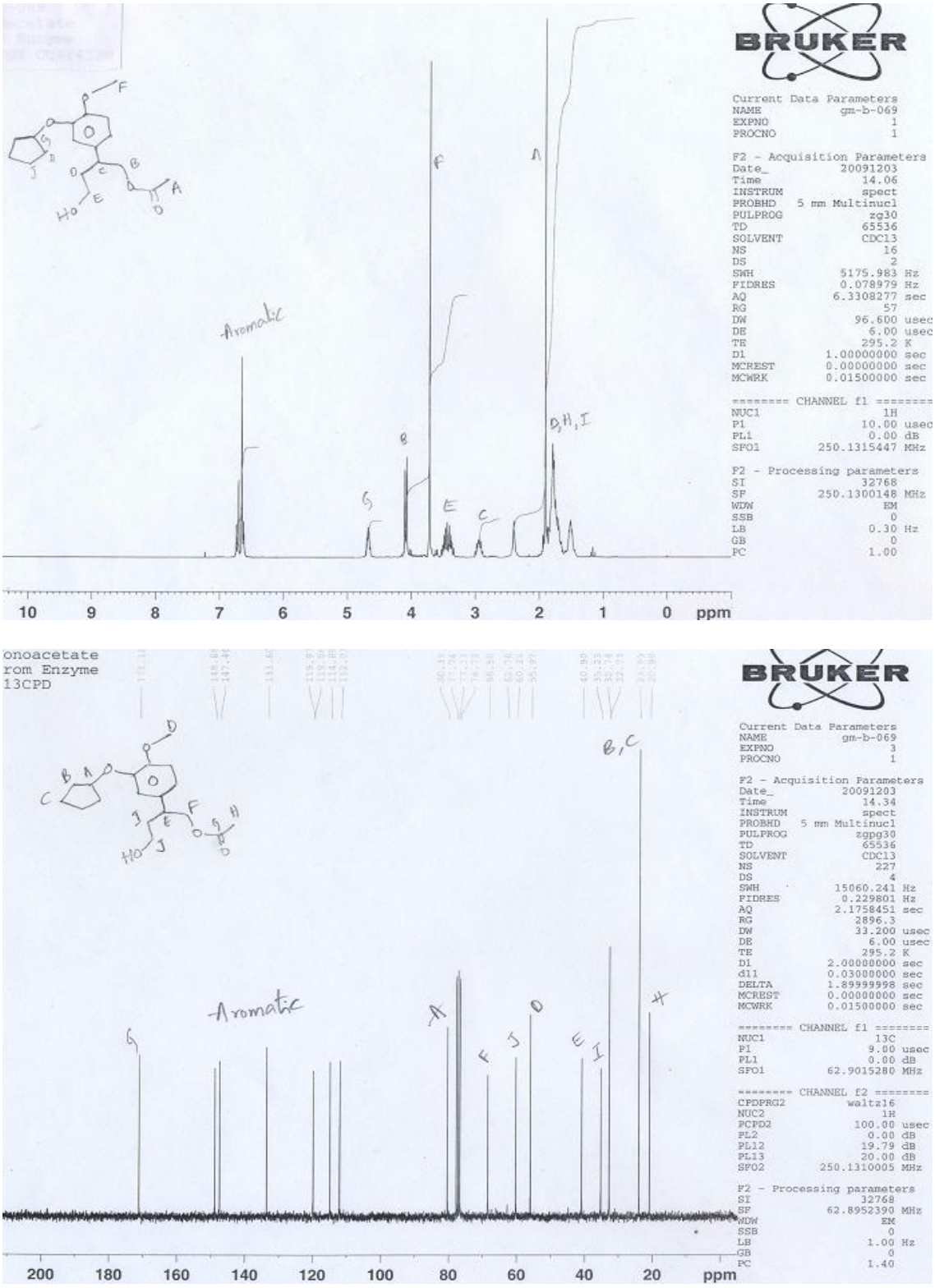


Fig. C-20 ^{1}H and ^{13}C-NMR spectra of Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-hydroxy-butyl ester **(26).**

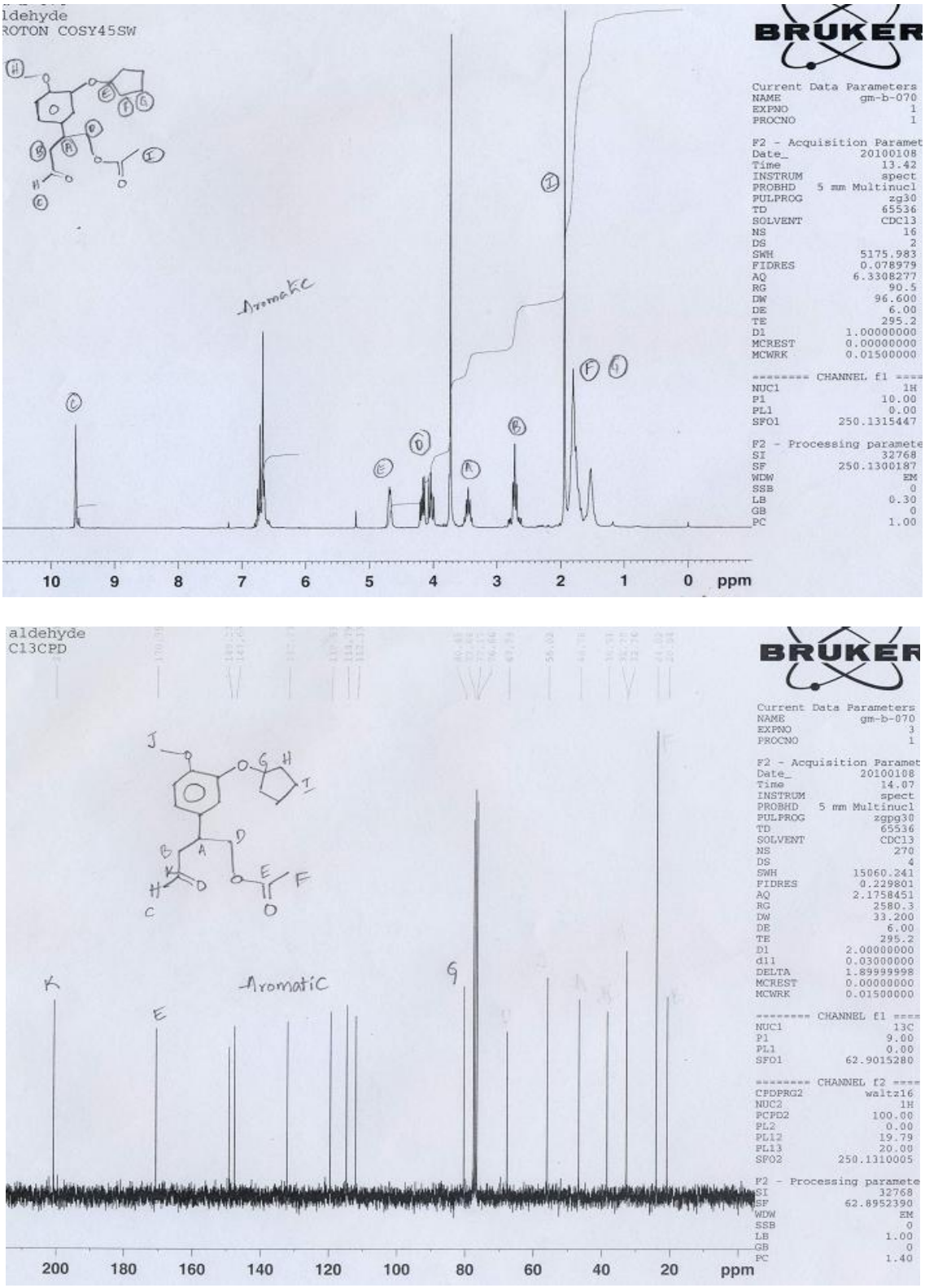


Fig. C-21 ^{1}H and ^{13}C-NMR spectra of Acetic acid 2-(3-cyclopentyloxy-4-methoxy-phenyl)-4-oxo-butyl ester **(27).**

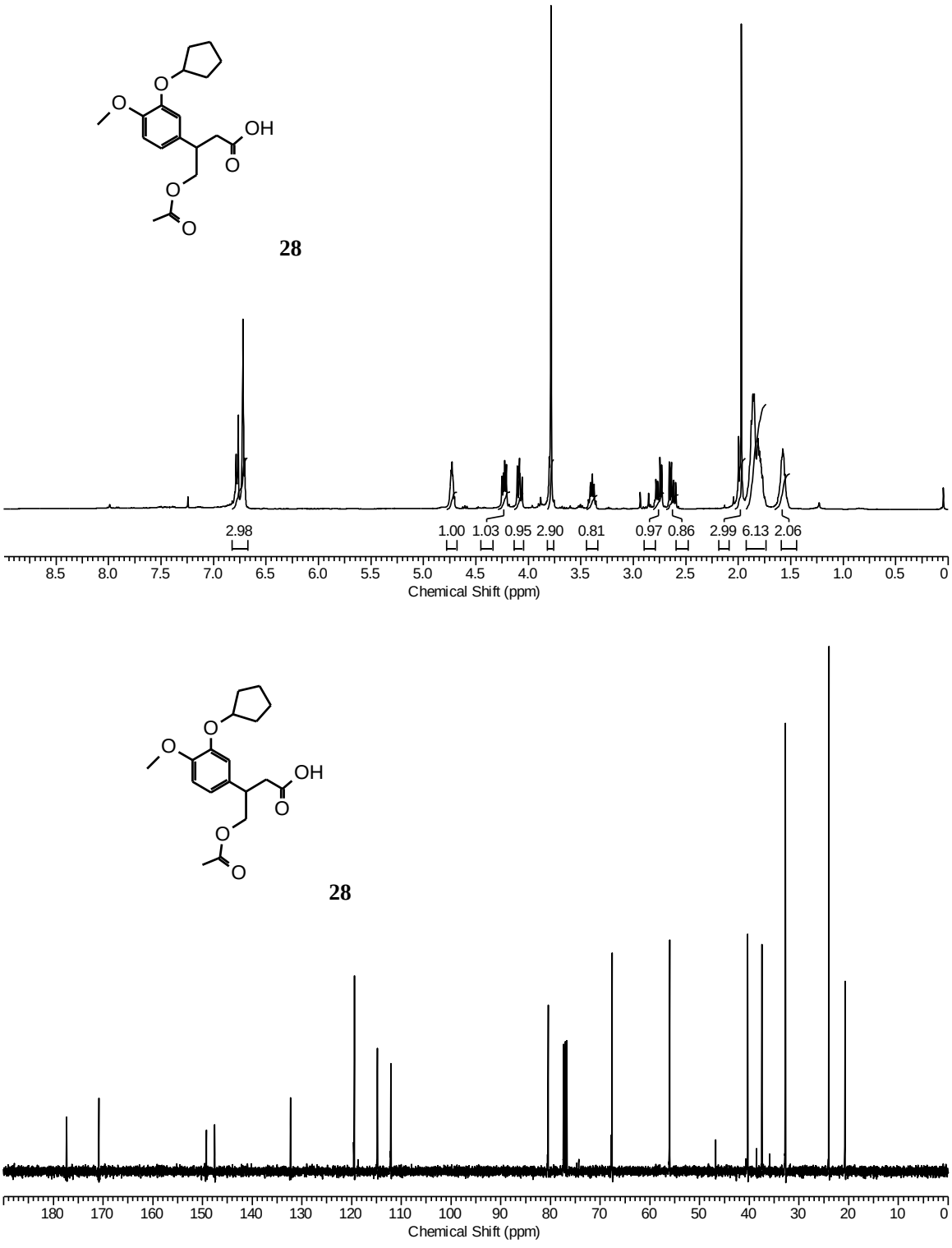


Fig. C-22 ^{1}H and ^{13}C-NMR spectra of 4-Acetoxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid **(28).**

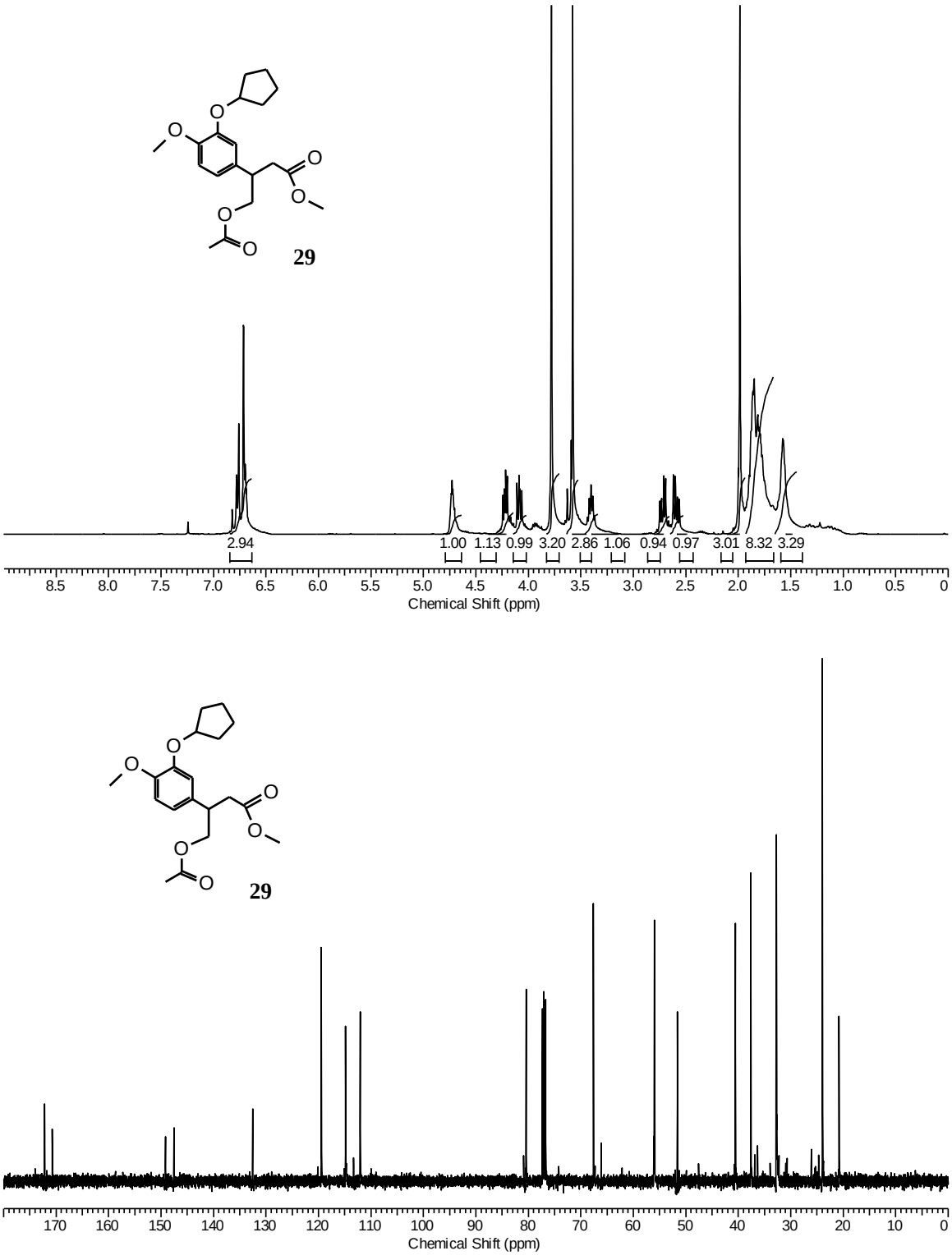


Fig. C-23 ^{1}H and ^{13}C-NMR spectra of 4-Acetoxy-3-(3-cyclopentyloxy-4-methoxy-phenyl)-butyric acid methyl ester **(29).**

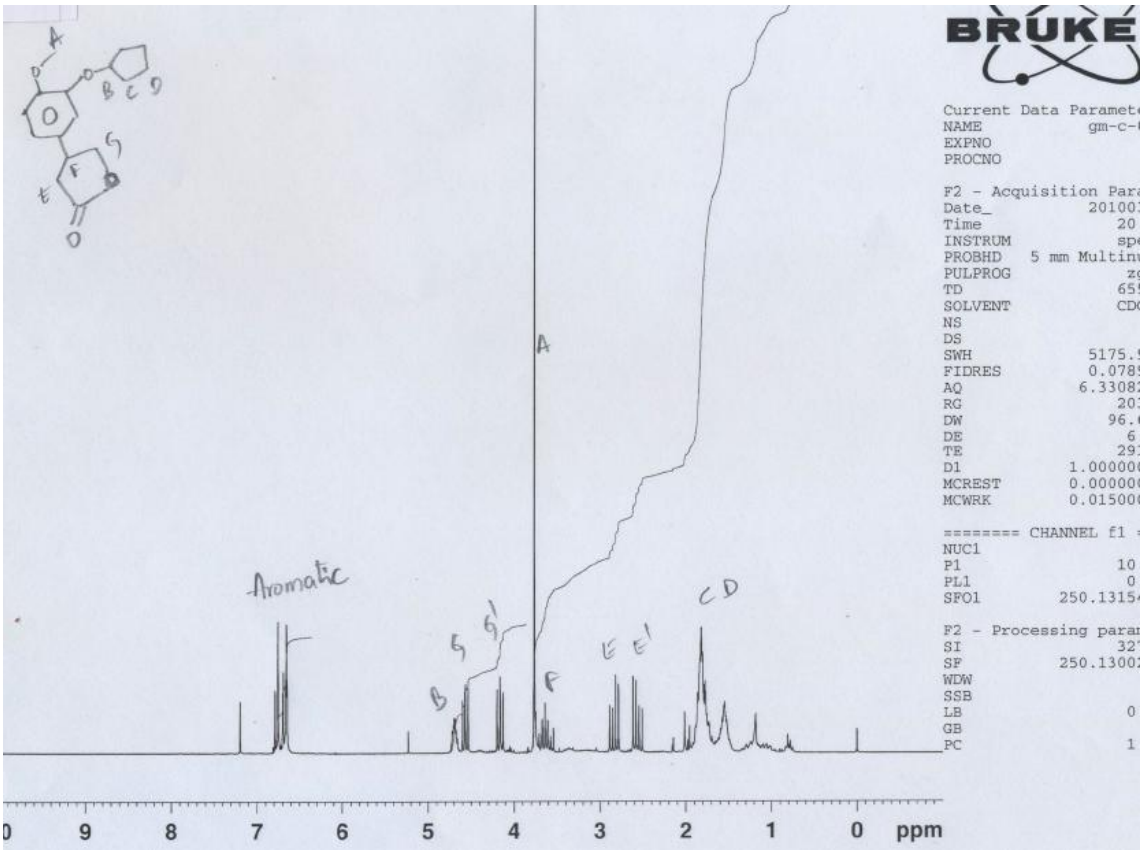


Fig. C-24 ^{1}H NMR spectra of 4-(3-Cyclopentyloxy-4-methoxy-phenyl)-dihydro-furan-2-one **(30).**