Synthesis and Antibacterial Activity of Stilbene Derivatives

By

Yilkal Matebie

A Thesis Submitted to Department of Applied Chemistry,

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of

Master‘s in Applied Chemistry (Synthetic Organic Chemistry)

Office of Graduate Studies

Adama Science and Technology University

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Approval of Board of Examiners

We, the undersigned, members of the Board of Examiners of the final open defense by Yilkal Matebie have read and evaluated his thesis entitled "synthesis and antibacterial activity of stilbene derivatives" and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master's in Applied Chemistry (Synthetic Organic Chemistry).

Advisor	Signature	Date
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.		
External Examiner	Signature	Date

DECLARATION

I hereby declare that this MSc Thesis is my original work and has not been presented for any degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Yilkal Matebie

Signature:_____

This MSc has been submitted for examination with my approval as a thesis advisor.

Name: _____

Signature:_____

Date of submission:……..

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LIST OF ABBREVIATIONS

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
AD	Alzheimer's disease
Dba	Dibenzylideneacetone
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
DME	Dimethoxyethane
DMSO	Dimethyl sulfoxide
FTIR	Fourier Transformation Infrared Spectroscopy
H	Hour
M.W	micro wave
n-BuLi	n-Butyllithium
NMP	N-Methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance Spectroscopy
PEG	Polyethylene glycol
Ppm	part per million
Rut-cat	Rutinium catalyst
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane

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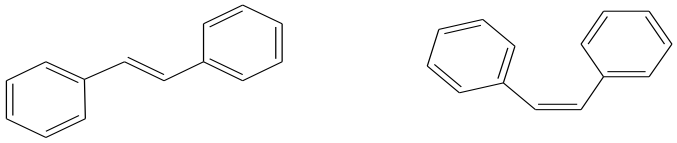
ABSTRACT

The stilbene scaffold is a basic element for a number of biologically active natural and synthetic compounds, and it is considered as a privileged structure. Stilbene derivatives have reported to have enormous biological activities including antimicrobial, antifungal, antioxidant, hepatoprotective, anti-viral, cytotoxic, anti-inflammatory and antiplatelet. The current research project is therefore designed to synthesize methoxy, chlorine and nitro containing stilbene derivatives and evaluated their antibacterial activities. In view of this substituted stilbene derivatives were prepared from benzyl triphenyl phosphonium chloride and substituted benzaldehyde using Wittig reaction. The compounds were obtained in yields ranging from 64-92 % .The structures of the synthesized compounds viz stilbene, 4'-nitrostilbene, 4'-methoxystilbene, 4'-chlorostilbene, 3',4'-dichlorostilbene, 4''-methoxy-4'-nitrostilbene and 4'-chloro-4''-methoxystilbene were done employing spectroscopic methods including NMR (^{1}H-NMR and ^{13}C-NMR) and FT-IR. Furthermore, the synthesized compounds were evaluated for their in vitro antibacterial activity against one Gram-positive strains (Staphylococcus aureus) and three Gram-negative strains (Escherichia coli, P. aeroginosa and K. puemonia) using disc diffusion method. Compoud 3',4'-dichlorostilbene displayed pronounceable activity against S. aureus and E.coli with inhibition zones of 24 mm and 22 mm, respectively. The result is significant compared with ceftriaxone used as positive control.

Key words: Antibacterial Activity, Stilbene Derivatives, Wittig Reaction,

1. INTRODUCTION

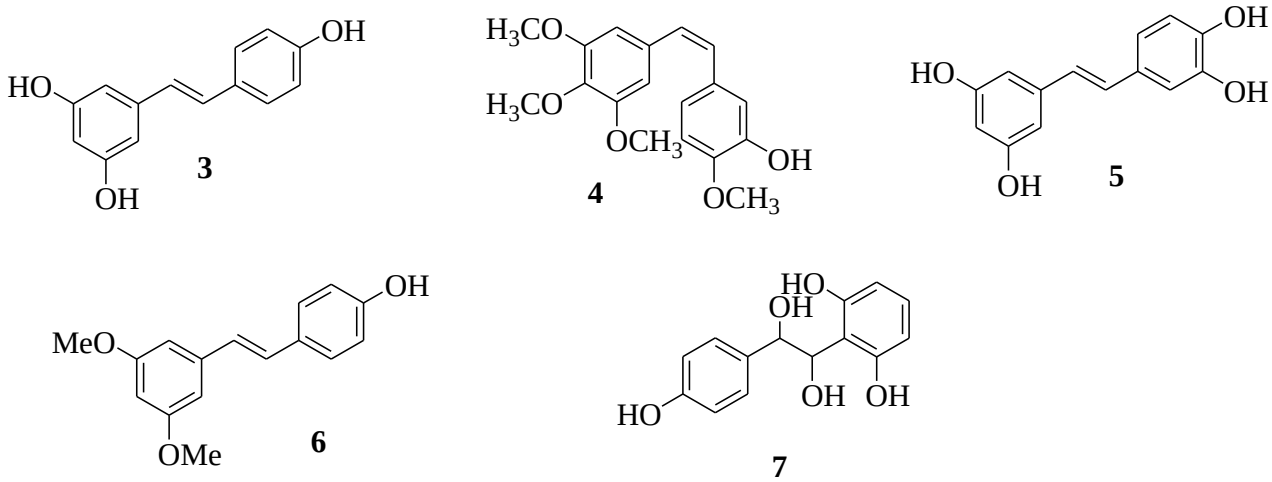
The name stilbene was derived from the Greek word stilbos, which means shining. Stilbenes are chemical derivatives of *trans*-1, 2-diphenylethylene. There are two isomeric forms of 1, 2-diphenylethylene: (*E*)-stilbene (*trans*-stilbene) (**1)** which is not sterically hindered, and (*Z*)-stilbene (*cis*-stilbene) (**2)**, which is sterically hindered and therefore less stable (**Figure 1**).



1 **2**

Figure 1: Two isomeric forms of stilbene

Stilbene based compounds are widely represented in nature and have become of particular interest because of their wide range of biological activities [1]. Stilbene derivatives, such as resveratrol (**3**), combrestatin A-4 (**4)**, Piceatannol **(5**), pterostilbene (**6)** and (1-(2',6'-dihyroxphenyl-2-(4"-hdroxyphenyl))-1,2-ethane-diol) (**7**) are compounds found in numerous medicinal plants and food products (**Figure 2**) [2-5].



3 **4** **5** **6** **7**

Figure 2: Stilbenes derivatives found in medicinal plants

The natural stilbene is the most relevant and more described in the literature as resveratrol (**3**), which was first isolated from the roots of *Polygonum cuspidatum* Chinese and Japanese medicinal plants in 1963 [6]. In 1992, this compound was postulated to explain some of the cardioprotective effects of red wine (the so-called-French paradox) [7-9]. Since then, dozens of studies have indicated that resveratrol plays an important role in preventing or slowing the progression of many diseases and illnesses, such as inflammation [10-13], cancer [12, 14 and 15] and heart diseases [8, 16]. Additional properties of resveratrol have been documented, such as radical scavenging antioxidant activity [15,17], neuroprotection [15,18], antiviral activity [15, 19], antibacterial activity [20-22] and antitubercular activity [23-25]. The immense biological activities reported in the literature by stilbene derivatives make them a good prospect to further synthesis and explore their antibacterial activities.

1.1. Statement of Problem

Infectious diseases are one of the major health problems of the world population. Certainly, the rapid development of resistance to the existing antimicrobial drugs is the root cause of this global issue. This opens the gateway for the medicinal chemists for the development of novel antimicrobial drugs having a different mechanism of action to combat the problem of multidrug resistance. In view of this some stilbene derivatives were known to have some established biological activities including antimicrobial, antifungal, anti-oxidant, hepatoprotective, anti-viral, cytotoxic, anti-inflammatory and antiplatelet. Therefore it is necessary to synthesis and explores the antibacterial activities of stilbene derivatives. The current research project is therefore designed to synthesize some methoxy, chlorine and nitro containing stilbene derivatives by using Wittig reaction. The antibacterial activities of the synthesized stilbene derivatives were also evaluated.

1.2. Objectives of the Study

1.2.1. General objective

- ✓ To synthesize and evaluate the antibacterial activities of stilbene derivatives.

1.2.2. Specific objectives

- ✓ To synthesize stilbene derivatives using Wittig reaction.
- ✓ To characterize the synthesized compounds using melting point, FTIR and NMR (^{1}H-NMR and ^{13}C-NMR).
- ✓ To evaluate the antibacterial activities of synthesized stilbene derivatives using disc diffusion method against one Gram positive strain *(Staphylococcus aureus)* and *three* Gram-negative strains *(Escherichia coli, P.aeroginosa and K. puemonia).*

1.3. Significance of the Study

Our interest in stilbene derivatives stems from the fact that they have found a range of applications including antimicrobial, antifungal, antioxidant, hepatoprotective, anti-HIV, cytotoxic, anti-inflammatory and antiplatelet and used in the manufacture of industrial dyes, dye lasers and optical brighteners. Stilbene derivatives are playing an increasingly prominent role in the area of photophysical, photochemical, biophysical, and biomedical investigations. Substituted stilbene derivatives that were synthesized in this work may give compounds with pronounced antibacterial activity.

2. LITERATURE REVIEW

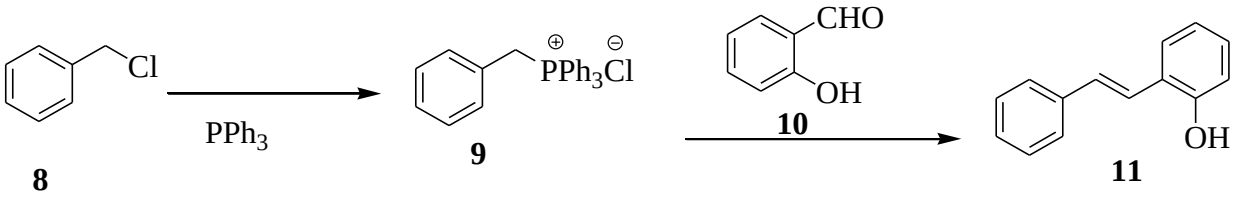
2.1. Synthesis of stilbene derivatives

A variety of synthetic methods have been employed to prepare stilbene derivatives with substituted aromatic rings.

2.1.1. **Wittig reaction**

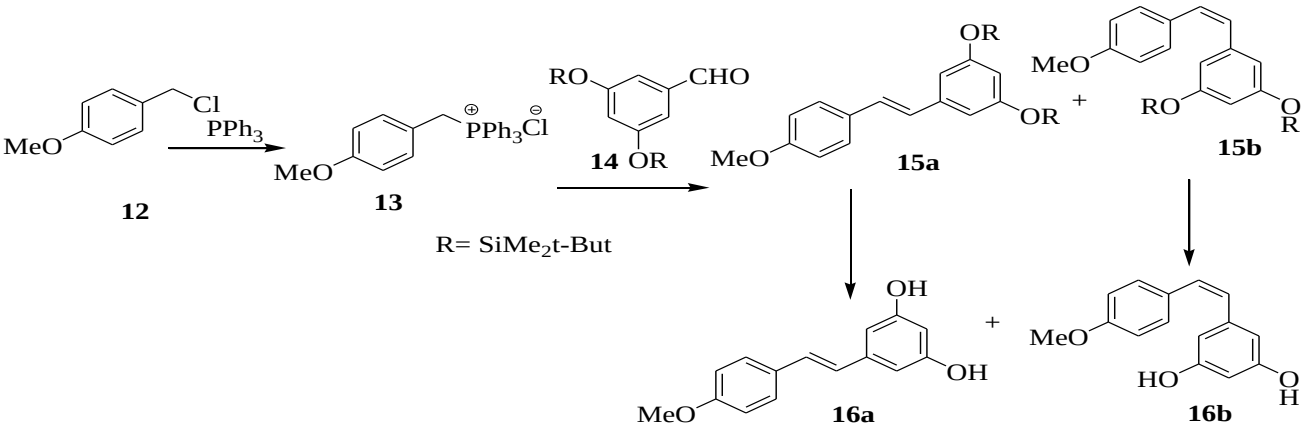
The Wittig reaction is the reaction of an aldehyde or ketone with a triphenyl phosphonium ylide to give an alkene and triphenylphosphine oxide. The Wittig reaction was discovered in 1954 by Georg Wittig and described in his pioneering publication titled "Über Triphenyl-phosphin-methyleneals olefin bildende Reagenzien I" [26]. The Wittig reaction has proved to be quite versatile in the preparation of different substituted stilbene derivatives [27]. This reaction is not sensitive to atmospheric oxygen, thus allowing simpler experimental procedures. It furnishes the trans- isomer in the steriospecific reaction. Moreover, the trans-isomer can be separated from the cis-isomer in the course of reaction because it is less soluble in the reaction solvent (usually, methanol, if sodium/lithium methoxide is used as a base) and precipitates on standing.

Moreno *et al.* synthesized stilbene derivatives by using Wittig reaction, but the yield was very low (10%) [28]. Kikumoto *et al*. (1990) have synthesized stilbenes derivatives (**11**) as an intermediate of the final compound [2 (ω-Aminoalkoxy) phenyl] ethyl] benzene [29]. The starting material was benzyl triphenyl phosphonium salt (**9**) obtained from benzyl chloride (**8**) and triphenyl phosphine (PPh_3) then reacst with aldehyde (**10**) by Wittig reaction (**Scheme 1**).



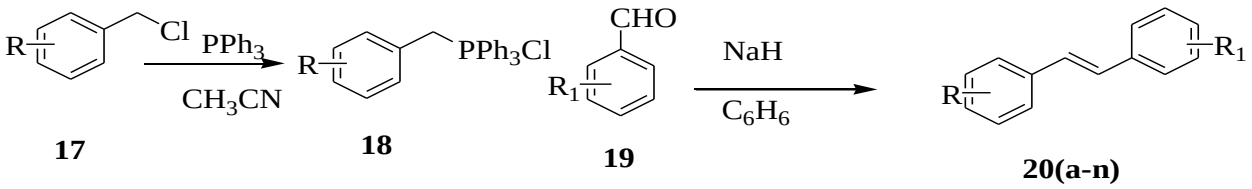
Scheme 1: Synthesis of stilbene derivative via Wittig reaction

The relative reactivity and stereoselectivity of substituted benzaldehydes in the Wittig reaction with benzylidene triphenyl phospharane were reported [30]. A synthesis of stilbene derivatives by a Wittig reaction between the 3,5-bis–(tert-butyldimethylsilyloxy) benzaldehyde (**14**) and (4-methoxybenzyl)- triphenyl-phosphonium chloride (**13**) obtained from 4-methoxybenzyl chloride (**12**) and PPh_3 is depicted in **Scheme 2**. The Wittig product obtained in 98% yields as a 2.3:1 mixture of the (Z/E)-isomers (**15b**) and (**15a**) was directly desilylated with tetrabutylammonium fluoride to afford 3,5–di-hydroxy-4'-methoxystilbene (**16a**) and (**16b**) [31]. They have synthesized a new derivative of resveratrol and shown the antiplatelet aggregation activity.



Scheme 2: Synthesis of stilbene derivative using Wittig reaction

Subhas *et al.* synthesized halogenated stilbene derivatives (**20** (**a-n**)) by using Wittig reaction of substituted benzyl chloride (**17**) with PPh_3 to obtained substituted benzyltriphenylphosphonium chloride (**18**) and react with substituted aryl aldehyde (**19**) (**Scheme 3**) [32].



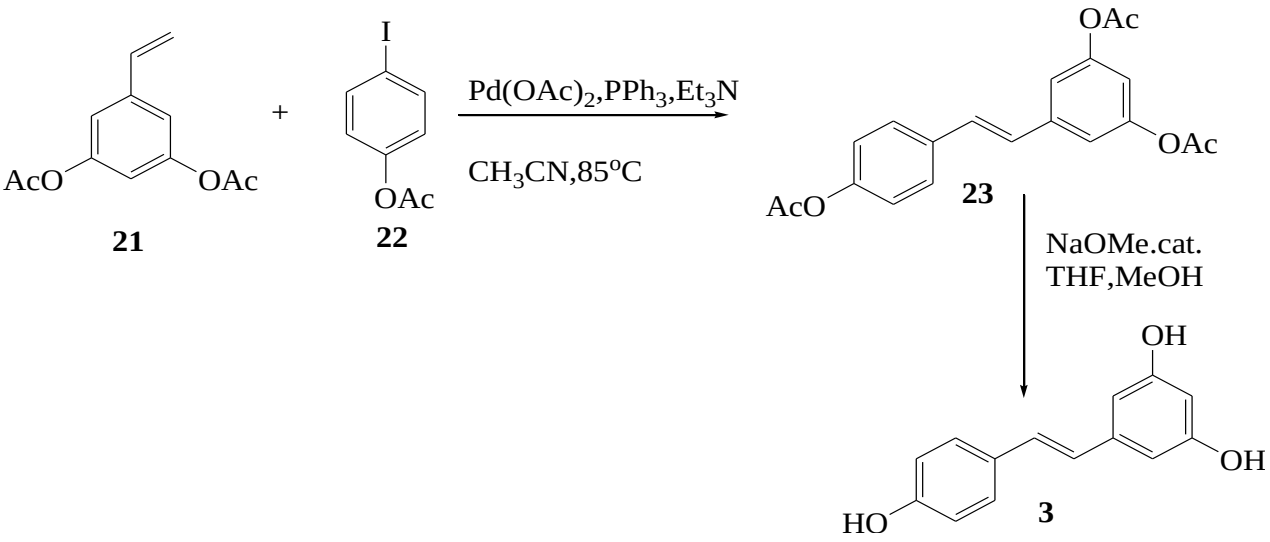
17 **18** **19** **20(a-n)**

20a, R=4-F, R_1= 4'-F
20b, R=4-F, R_1= 3'-F
20c, R=4-F, R_1= 3', 4'-F
20d, R=4-F, R_1= 2', 4'-F
20e, R=2,4,Cl, R_1= 2', 4'-Cl
20f, R= 2, 4-Cl, R_1= 3', 4'-Cl
20g, R= 2, 4-Cl, R_1= 3'-Cl
20h, R= 2,4,Cl, R_1= 4'-Cl
20i, R= 4-Me, R_1= 4'-Me
20j, R= 2,4-Cl, R_1 = 4'-F
20k, R= 2,4-Cl, R_1= 4'-Me
20l, R= 4-F, R_1 = 3'-Cl
20m, R= R=4-F, R_1 = 4'-Me
20n, R= 4-F, R_1 = 4'-Cl

Scheme 3: Synthesis of halogenated stilbene derivatives by using Wittig

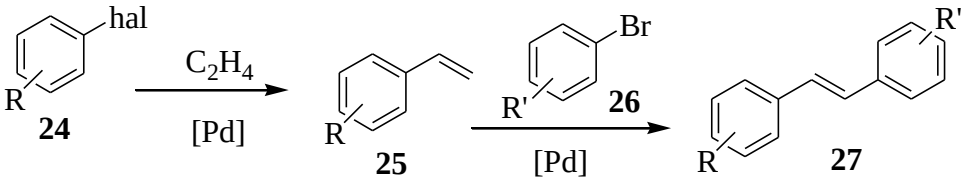
2.1.2. **Heck reaction**

The Heck reaction (Mizoroki–Heck reaction) is the reaction of an unsaturated halide (or triflate) with an alkene and a strong base and palladium catalyst to form a substituted alkene [33, 34]. Guiso *et al*. reported the synthesis of fully protected resveratrol (**23**) (70%) by coupling reaction of 3,5-diacetoxystyrene (**21**) with *p*-iodophenyl acetate (**22**) in acetonitrile containing $Pd(OAc)_2$ as catalyst, PPh_3 as ligand and Et_3N as base **(Scheme 4**) [35]. A standard deacetylation provides the targeted resveratrol (**3**).



Scheme 4: Palladium acetate catalyzed Heck coupling

Non-symmetrically substituted stilbene derivatives (**27**) have synthesized using one-pot two-step double Heck reaction of substituted Styrene (**25)** and aryl bromide (**26**). Substituted Styrene (**25**) also obtained via Heck reaction from reaction of aryl halide (**24**) and ethene (**Scheme 5**) [36].



hal= I, Br,

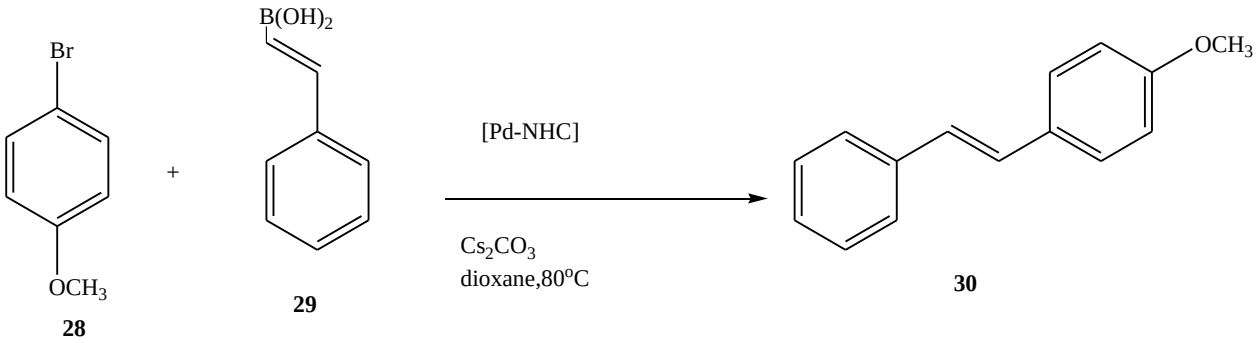
R= OMe, Me,COMe and NH_2

R'= COMe, OMe,Me

Scheme 5: Synthesis of Stilbene derivative via one-pot two-step double Heck reaction

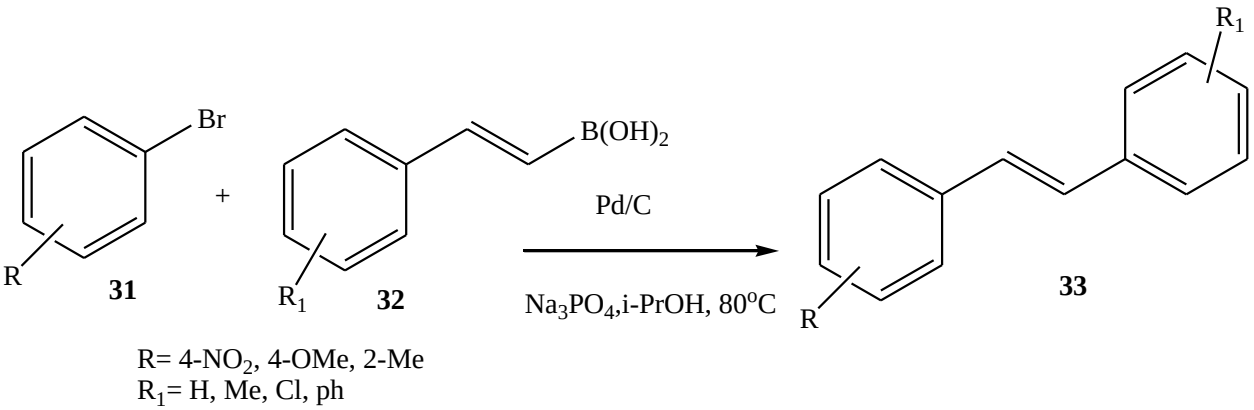
2.1.3. **Suzuki-Miyaura reaction**

Tudose *et al*. performed the Suzuki-Miyaura reaction of aryl halides (**28**) with *trans*-2-phenylvinylboronic acid (**29**) to generate stilbene derivative (**30**) (**Scheme 6**) [37]. They carried out this coupling in 1,4-dioxane using Cs_2CO_3 as a base together with in situ generated N-heterocyclic carbene palladium (II) complexes (Pd-NHC). However, the nature of the catalyst system significantly affects the reaction efficiency.



Scheme 6: Stilbene synthesis promoted by Suzuki-Miyaura coupling

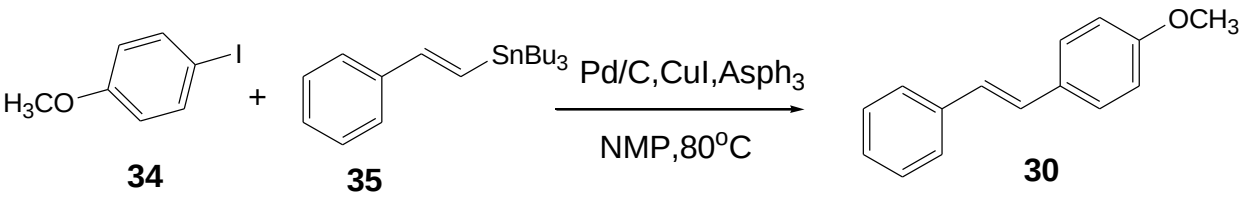
Buchwald *et al*. have synthesis stilbene derivatives (**33**) from substituted aryl bromide [**31**] and various aryl vinyl boronic acid (**32**) via Pd/C-catalyzed Suzuki-Miyaura coupling in reaction conditions (Na_3PO_4 in 50% iPrOH at 80^{o}C) and they obtained 80%-91% yield (**Scheme 7**) [38].



Scheme 7: Stilbene derivatives synthesized via Pd/C catalysted Suzuki-Miyaura reaction

2.1.4. **Stille reaction**

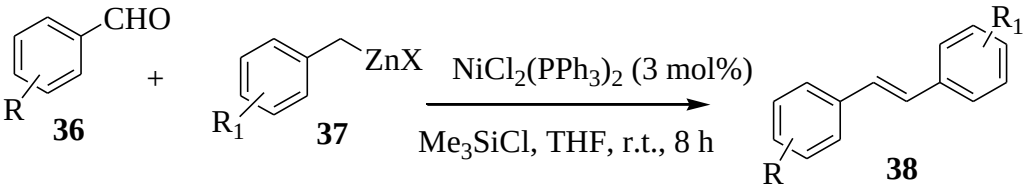
The Stille coupling is the palladium-catalyzed cross-coupling between organotin and alkenyl or aryl halide or triflate [39]. Roth and Farina have optimized the Stille reaction of 4-methoxy-phenyl iodide (**34**) with aryl vinyl tertbutylsinnel (**35**) by addition of co-catalytic copper iodide and ligand triphenylarsine to produce stilbene derivative **30** in 82% yield (**Scheme 8)** [40].



Scheme 8: Stilbene synthesis by Stille coupling

2.1.5. **Negishi coupling**

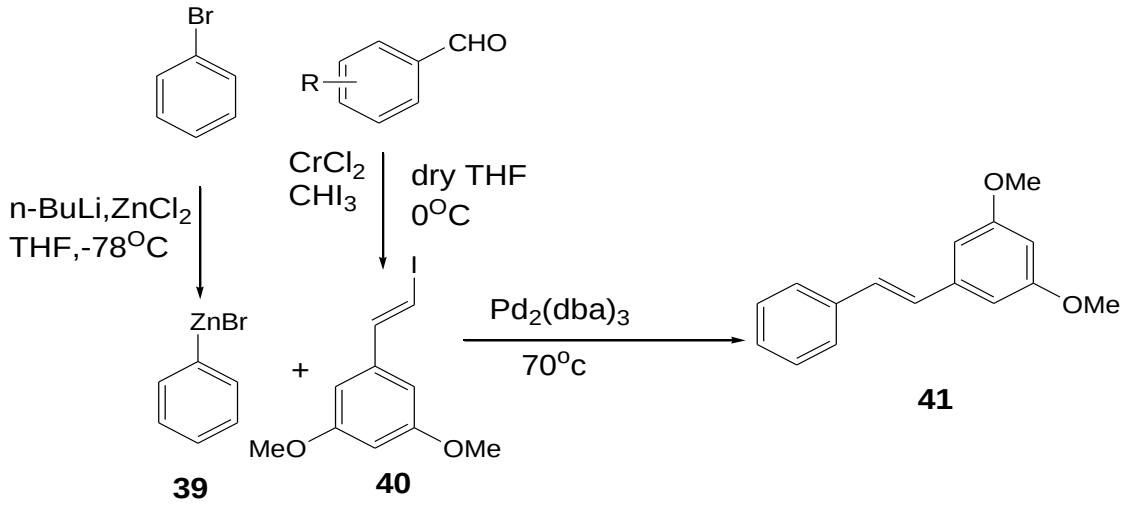
A convenient method for the efficient and highly stereoselective synthesis of E-stilbene derivatives [**38**] from the corresponding aldehydes (**36**) and functionalized organozinc reagents [**37**] in the presence of a silylating agent and a catalytic amount of $NiCl_2(PPh_3)_2$ was devised (**Scheme 9)** [41]. For the benzylic zinc halides, reactions could be carried out at room temperature and afforded the corresponding E-stilbene derivatives in good yields after 8 h at room temperature (63–92%,) .



R= H, 2-OMe, 4-OH, 4-Cl,4-OMe
X= Cl, Br
R_1=H, 3-Cl, Br, 4-Br

Scheme 9: Stereoselective synthesis of E-stilbene derivatives via Negishi reaction

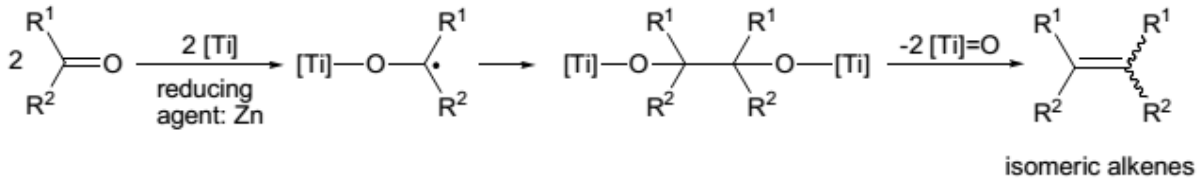
Kabir *et al*. prepared a series of substituted stilbenes with electron donor and withdrawing groups via Negishi cross-coupling without any additional ligand [42]. The active species, aryl zinc reagent (**39**) is reacted with arylvinyl iodide (**40**) in the presence of Pd $(PPh_3)_4$ to give stilbene derivatives (**41**). The aryl zinc reagent is prepared by treating aryl bromide with $ZnCl_2$ in basic medium (**Scheme 10**).



Scheme 10: Stilbene synthesis by Negishi cross-coupling

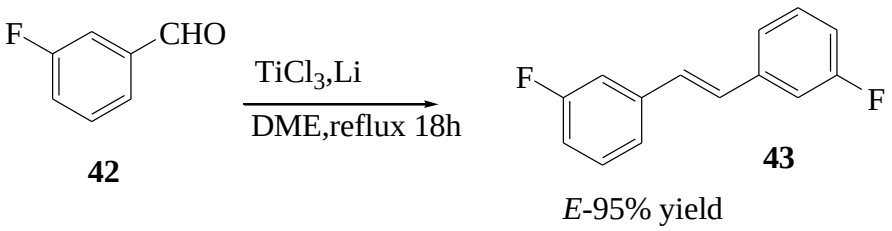
2.1.6. **McMurry coupling reaction**

The McMurry reductive coupling reaction is an organic reaction in which two ketone or aldehyde groups are coupled to produce alkenes in the presence of titanium (III) chloride and a reducing agent. Most commonly, low-valent titanium [mixture of Ti (II) and Ti (0)] is prepared by reducing $TiCl_3$ with a zinc-copper couple (Zn-Cu) in DME [43]. . The mechanism of the McMurry coupling consists of metallopinacol formation and deoxygenation to the alkene (**Scheme 11**). This reaction is typically a homocoupling leading to symmetrical alkenes. Heterocoupling is only possible when one of the substrates is introduced in excess.



Scheme 11: Mechanism of the McMurry coupling

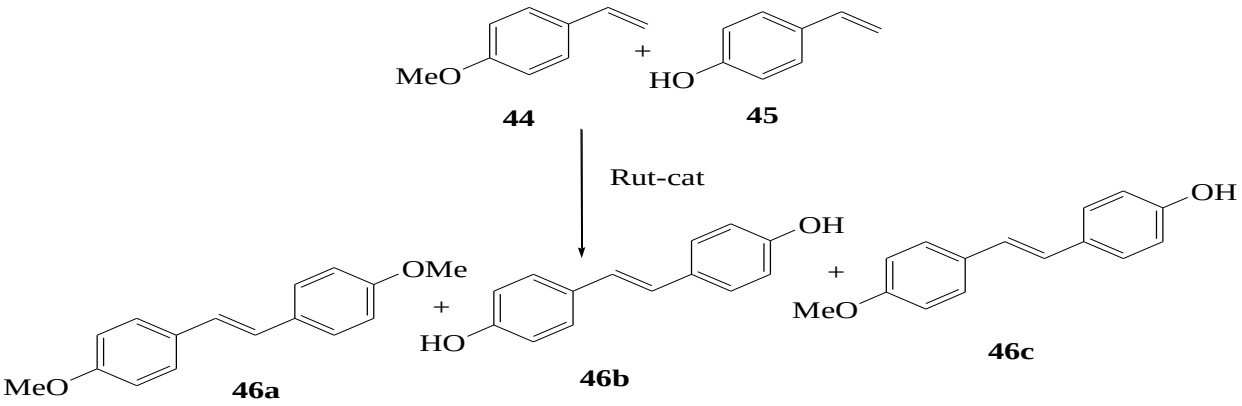
The symmetrical E-stilbene [**43**] can be made in 95% yield and in almost perfect (99.92:0.04 E: Z) E-selectivity from 3-fluorobenzaldehyde [**42**]. The low-valent titanium is produced by reduction of Ti (III) with lithium metal (**Scheme 12**) [44].



Scheme 12: E-selective synthesis of stilbene derivative in McMurry coupling reaction

2.1.7. **Ruthenium-catalyzed cross-metathesis**

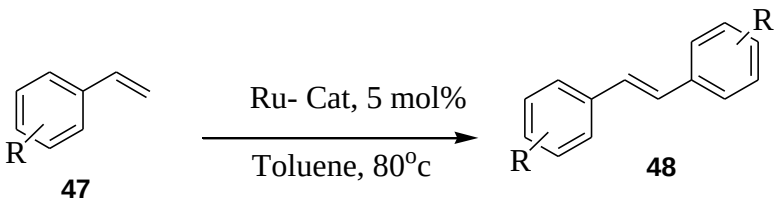
Ferré-Filmon *et al*. reported the construction of symmetrical and unsymmetrical (E)-methoxystilbene and (E)-hydroxystilbene derivatives (**46(a-c)**) from the reaction of 4-Methoxy-styrene (**44**) and 4-Hydroxy- styrene (**45**) by ruthenium-catalyzed cross-metathesis with help of second generation Grubbs catalysts (**Scheme 13**) [45]. They were of the opinion that the selectivity of the unsymmetrical cross-product can be enhanced almost up to 95% yield by introducing one of the reacting substrates in excess.



Ratio: 13/22/65 with 100% conversion of starting material

Scheme 13: Synthesis of (E)-hydroxystilbenoids by metathesis.

Cheikh *et al*. synthesis of symmetrical stilbene derivaties (**48**) by using catalytic self-metathesis reaction was screened with a series of styrene derivatives (**47**) bearing electrondonating and electron-withdrawing substituents (**Scheme 14**) [46].

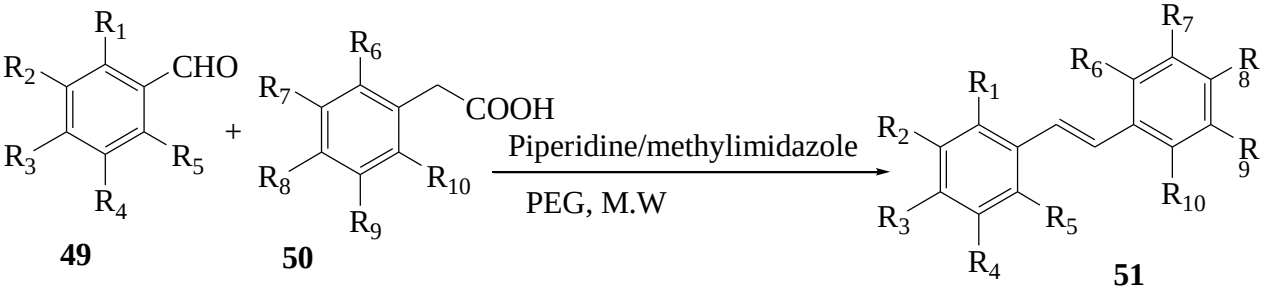


R= H, 2-Br, 3-Br, 2-OMe, 4-NO2, 4-CN, 2,4-diMe

Scheme 14: synthesis of symmetrical stilbene derivaties by using self-metathesis

2.1.8. **Modified Perkin reaction**

The Perkin reaction is an organic reaction developed by William Henry Perkin that can be used to make cinnamic acids by the aldol condensation of aromatic aldehydes and acid anhydrides in the presence of an alkali salt of the acid [47, 48]. Sinha *et al*. synthesized a series of hydroxylated stilbenes in moderate yields through a one-pot modified Perkin reaction [49]. The standard Perkin reaction involves the condensation of aromatic aldehydes with the anhydrides of aliphatic carboxylic acids in the presence of a weak base to yield α,β-unsaturated carboxylic acids. aryl aldehydes (**49**) treated with phenylacetic acids (**50**) in polyethylene glycol (PEG) containing piperidine and methylimidazole under microwave irradiation to produce hydroxylated stilbene derivatives (**51**) via simultaneous condensation-decarboxylation pathway (**Scheme 15**).



at least one of R_1or R_3 or R_5 or R_6 or R_8 or R_{10}= OH and R_1-R_{10}= H, OMe, H, Cl etc.

Scheme 15: Hydroxylated stilbene synthesis via a modified Perkin reaction

2.1.9. **Knoevenagel condensation**

Knoevenagel condensation is a classic C-C bond formation reaction in organic chemistry [50]. These condensations occur between aldehydes or ketones and active methylene compounds. Al-Shihry, reported the synthesis of stilbene derivatives (**54**) (80%-95%) with electron withdrawing groups via Knoevenagel condensation (**Scheme 16**) [51]. This reaction is carried out between aldehyde (**52**) and active methylene compounds (**53**) in polar solvent, ethanol added with K_3PO_4.

52 X= F, Cl, NO_2 **53**
Y= H, Cl
R_1= H,OEt
R= H, Cl, Br, NO_2, $N(CH_3)_2$

54

Scheme 16: Stilbenes derivatives synthesized via Knoevenagel condensation

2.2. Biological Activities Stilbene Derivatives

Stilbene-based compounds are largely present in nature and have attracted chemist and biologist, because of their wide range of biological activities [52]. Stilbene derivatives such as the 3, 5, 4′-trihydroxy-trans stilbene and combretastatin A-4 are the plant microcomponents, and they exhibit a variety of biological activities including antineoplastic, chemopreventive, antioxidant and antiestrogenic [53].

2.2.1. **Antibacterial and antifungal activities of stilbene derivatives**

Infectious diseases are one of the major health problems of the world population. Certainly, the rapid development of resistance to the existing antimicrobial drugs is the root cause of this global issue. This opens the gate for the medicinal chemists for the development of novel antimicrobial drugs having a different mechanism of action to combat the problem of multidrug resistance [54]. In a wide variety of stilbene nucleus constitutes an important class displaying a broad spectrum of biological activities. Subhas *et a*l. synthesized halogenated stilbene derivatives (**20** (**a**-**n**)) by using Wittig reaction and evaluate the antibacterial and antifungal activities and they was reported that Compound (**20c**) showed equipotent antibacterial activity against *S. aureus as* compared to penicillin, while (**20b**), (**20d**), and (**20f)** compounds showed moderate antibacterial activity against *S. aureus*. And (**20a**), (**20b**), (**20f**), (**20g**), (**20j**), (**20l**) and (**20n**) inhibited the growth of *P. chrysogenum* which proved their antifungal potential [32].

2.2.2. **Antioxidant activity**

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. Fauconneau et al.(1997) assessed the antioxidant activity of the flavonoids (anthocyanins, catechins) and non-flavonoids (stilbenes) by their capacity to prevent Fe^{2+}-induced lipid per-oxidation in microsomes and their action on Cu^{2+}-induced lipid per-oxidation in low-density lipoproteins [55]. Cai *et al*. (2003) synthesized 4-hydroxy-trans-stilbene (4-HS) (**55**), 3, 4-dihydroxy-trans-stilbene (3, 4-DHS) (**56**), 4, 4'-dihydroxy-*Trans*-stilbene (4, 4'-DHS) (**57**) and 3, 5-dihydroxy-trans-stilbene (3, 5-DHS) (**58**) and studied their antioxidant activity through the free radical-induced peroxidation of rat liver microsomes in vitro [56]. They found that these derivatives are effective antioxidants against both AAPH- and iron-induced per-oxidation in rat liver microsomes [56, 57]. Besides, they also found that the activity sequence follows the order: 3, 4-DHS > 4, 4'-DHS > resveratrol > 4-HS > 3, 5-DHS (**Figure 3**).

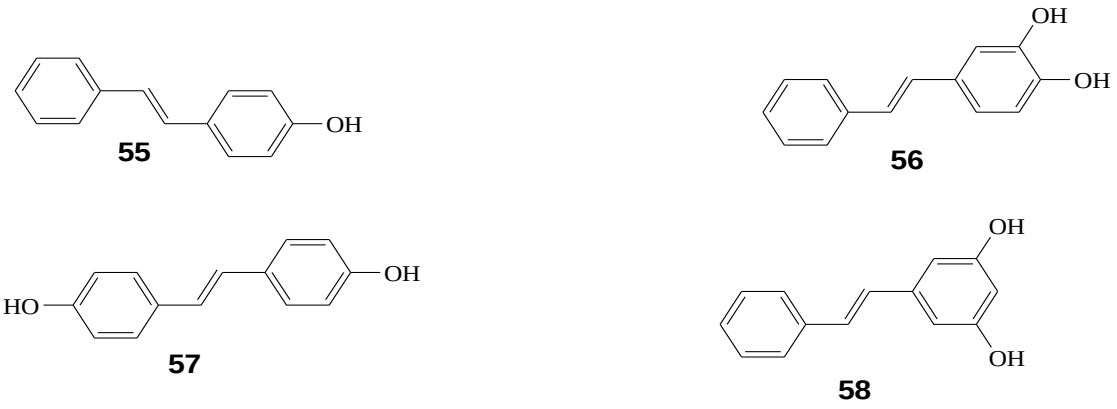
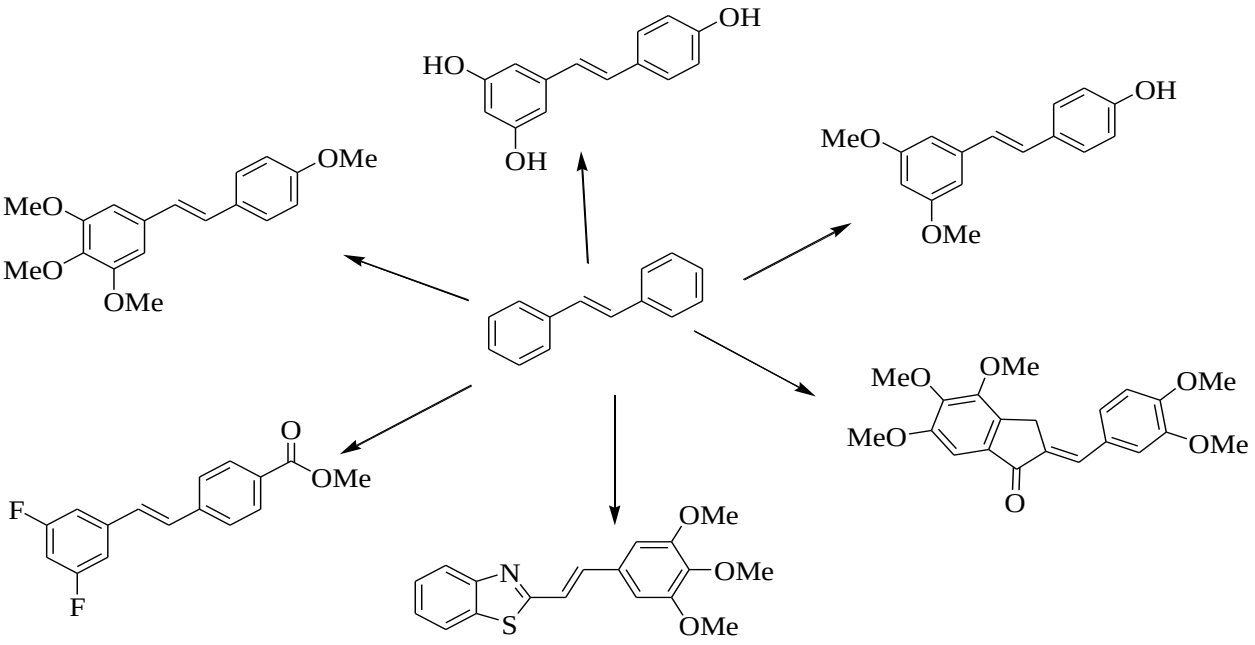


Figure 3: Antioxidant Activity hydroxyl stilbene derivatives

Subhas *et al.* (2011) synthesis halogenated stilbene derivatives (**20(a-n)**) via Wittig reaction and evaluated antioxidant activity by DPPH-free radical scavenging assay and the synthesis compounds showed moderate (%RSA>40) to significant (%RSA>50) radical scavenging activity [32]. Compounds (**20a**),(**20d**), (**20e**),(**20f**) and(**20g**) showed %RSA>50% radical scavenging activity, while compounds (**20b)**, (**20c**), (**20h**), (**20m**) and (**20n**) showed %RSA>40% activity.

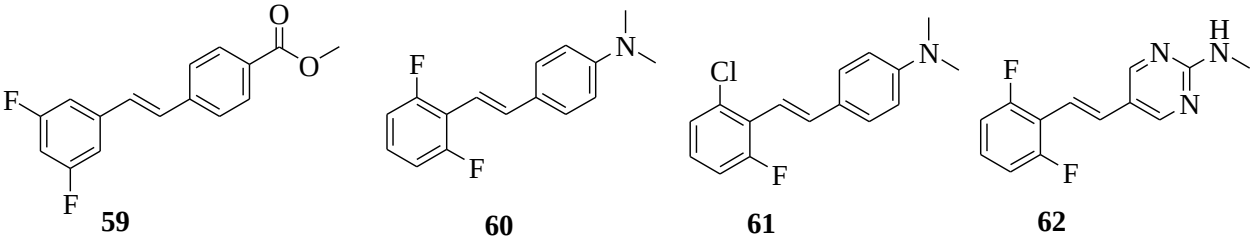
2.2.3. **Anticancer Activity of Stilbene Derivatives**

Cancer is the term used for diseases in which abnormal cells divide without control and are able to invade other tissues. All cancers begin in cells, when the DNA of a cell becomes damaged or changed, it will produce mutations that will affect normal cell growth and division. In recent years, cancer has become one of the main causes of death to the human being. New stilbene derivatives have been designed and synthesized in order to find stilbene analogues with cancer chemopreventive and/or therapeutic activities superior to that of the parent compound (**Scheme17**) [58].



Scheme 17: Stilbene containing anti cancer compounds

Moran *et al*. prepared aseries of trans-fluorinated analogues of stilbene derivatives (**Figure 4**), some with hydroxy groups and others with protected hydroxy, amino, ether and/or nitro groups [59]. These compounds were assayed in lung cancer and melanoma cell lines. Among them, compound (**59**) displayed greater potency than that of the parent compound resveratrol.

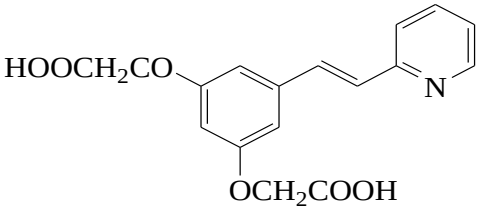


59 **60** **61** **62**

Figure 4: Fluorinated analogues of stilbene derivatives with anti-cancer activities

2.2.4. **Anti-Inflammatory Activity of stilbene Derivatives**

In order to find new potent anti-inflammatory agents, Chen *et al*. synthesized stilbene derivatives and tested them on xylene-induced mouse ear edema, the results indicated that compound (**63**) (**figure 5**) showed almost the same inhibition rate as resveratrol by 37.0% [60]. The study showed that resveratrol pyridyl-substituted analogs and the Mannich base displayed potent anti-inflammatory activity.

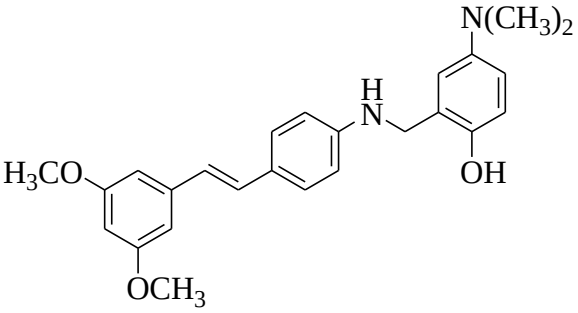


63

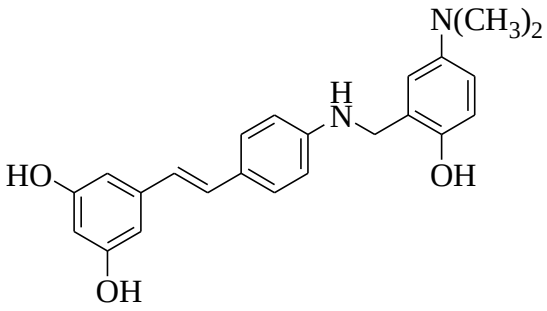
Figure 5: Anti-Inflammatory Activity of stilbene derivatives

2.2.5. **Neuroprotective Effect of stilbene Derivatives**

There is no effective therapy for Alzheimer's disease (AD), the most common fatal neurodegenerative disorder. In order to reduce the risk of this neurodegenerative disorder, Lu *et al*. synthesized and evaluated a novel series of resveratrol derivatives [61]. They found that most of the synthetic compounds exhibited significant inhibition on self-induced β-amyloid (Aβ) aggregation and Cu (II)-induced Aβ1-42 aggregation. Especially, compounds (**64**) and (**65**) (**Figure 6**) are potential lead compounds for AD therapy.



64



65

Figure 6: Neuroprotective effect of stilbene derivatives

Besides, compound (**64**) could cross the blood-brain barrier in vitro and did not exhibit any acute toxicity in mice at doses up to 2000 mg/kg. The results gave us an insight into the neuroprotective effect of resveratrol derivatives.

3. MATERIALS AND METHODS

3.1. General

All the chemicals used were procured from Alcheme import plc, Ranchem industry and trading and scientific chemical supplier Addis Ababa, Ethiopia and all chemicals were used without further purification. Melting points were recorded in Melting Point Apparatus. IR spectra were recorded on KBr disk on the 'PerkinElmer IR' instrument and are reported in cm^{-1}, NMR spectra were recorded on a 'Bruker-400' instrument with tetramethylsilane (TMS) as the internal standard and DMSO-*d*6 and $CDCl_3$ as a solvents.

3.2. Reagents and chemicals

The chemicals and reagents used in this study were aryl aldehyde (benzaldehyde, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde and 3,4-dichlorobenzaldehyde), substituted benzyl-triphenylphosphonium chloride, sodium hydroxide, dichloromethane, anhydrous sodium sulphate, dimethyl sulfoxide (DMSO), hexane and EtOH.

3.3. Apparatus and instruments

The apparatus and instruments used in this study were rotary evaporator, gravity filtration apparatus, Whatmann filter paper, digital beam balance, TLC plate, Beaker, Separating funnel, stirrer bar, melting point apparatus and ice bath.

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3.4. General procedure for synthesis of stilbene derivatives

To a well-stirred suspension of substituted benzyl triphenylphosphonium chloride (1.5g, 3.866 mmol) in dichloromethane (20 mL) was added substituted aryl aldehyde (3.866 mmol). To the mixture, sodium hydroxide (308.0 mg, 7.7 mmol) was added in an ice bath and allowed to warm to room temperature for 10 min. After the additional stirring for 1 hr, excess sodium hydroxide was neutralized by the addition of 1M hydrochloric acid. The progress of reaction was controlled by TLC. Then DCM (20 mL) and water (10 mL) was added. The organic layer was separated using separatory funnel, dried over anhydrous Na_2SO_4 (30 min), filtered and concentrated using rotary evaporator. The residue was purified by using silica gel column chromatography using n-hexane:ethyl acetate (4:1 v/v) as eluent. Then dried and the percent yield was calculated. Stilbene derivatives that were synthesized in this work were characterized by using melting point, FT-IR and NMR (^{1}H-MNR and ^{13}C-NMR) spectroscopy.

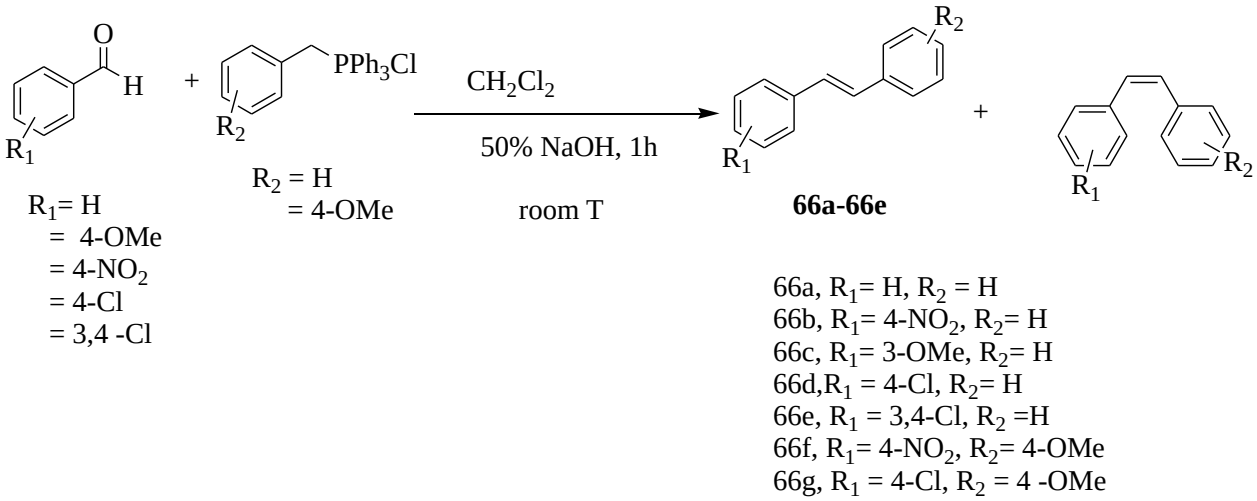
3.5. Antibacterial activities of stilbene derivatives

The *in vitro* anti-bacterial activity of the synthesized stilbene derivatives were evaluated using the American Test Culture Collection (ATCC) bacterial strains following standard procedure described hereunder [62]. Four bacterial strains were obtained from Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory Center, Adama, Ethiopia. Three Gram-negative bacterial strains (*E. coli, P.aeroginosa and K. puemonia)* and one Gram-positive strain (*S. aureus*) human bacterial pathogens were selected for the study. Mueller hinton agar was prepared by using pour plate technique and wells were prepared using a sterile cork borer. A 2% concentration of the synthesized compounds in dimethyl sulfoxide (DMSO) was used. A 2% solution of Ceftriaxone and DMSO was used as positive and negative control, respectively. The values of zone of inhibition (mm) were determined after 24 h of static incubation at 37^{o}C.

4. RESULTS AND DISCUSSIONS

4.1. Synthesis of stilbene derivatives

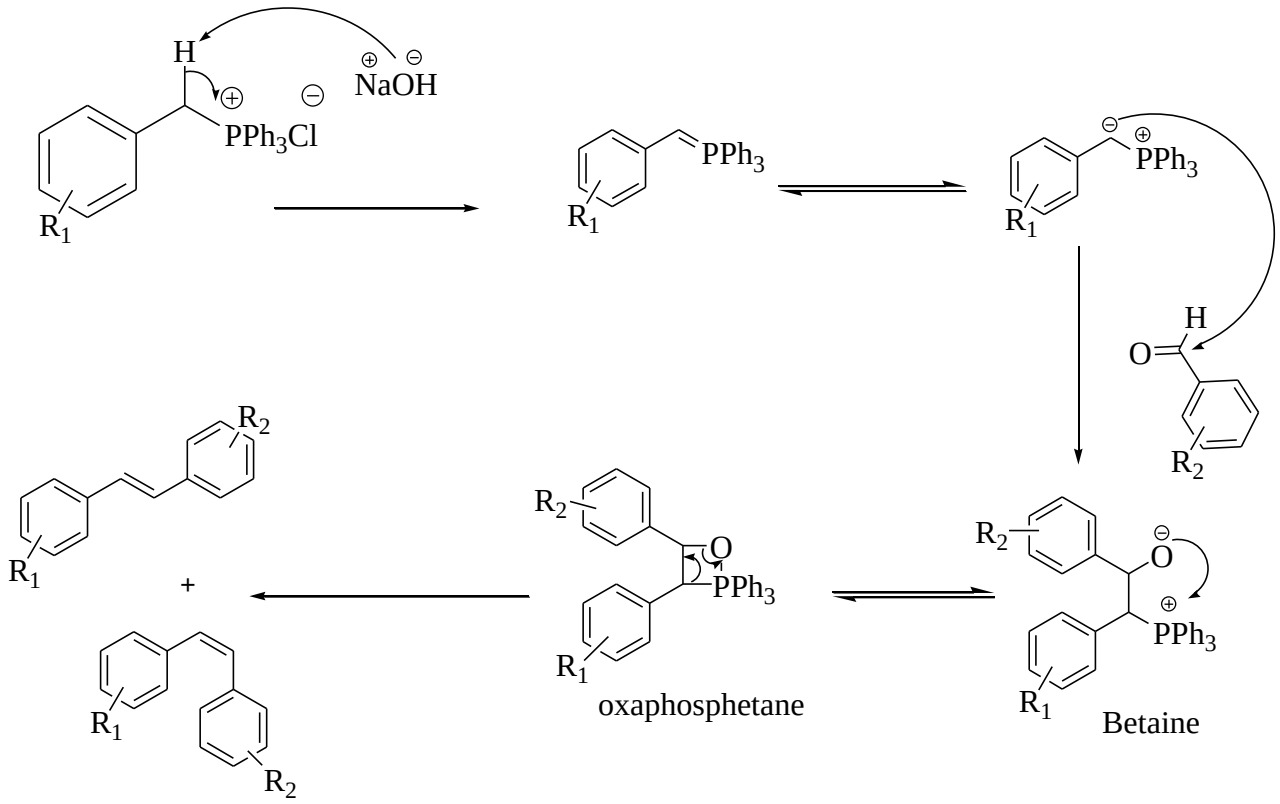
The synthesis of stilbene derivatives namely stilbene (**66a**), *p*-nitrostilbene (**66b**), *p*-methoxystilbene (**66c**), *p*-chlorostilbene (**66d**), 3',4'-dichlorostilbene (**66e**)**,** 4''-methoxy-4'-nitrostilbene **(66f**) and 4'-chloro-4''-methoxystilbene **(66g**) were carried out by reactions between benzyl triphenylphosphonium chloride and substituted benzaldehydes using NaOH as base by Wittig reaction (**Scheme 18)**. The products thus formed were purified over silica gel column chromatography with n-hexane and ethyl acetate (4:1) as eluent.



Scheme 18: Synthesis of stilbene derivatives (**66a–66g**) using Wittig reaction

4.2. Reaction mechanism

Phosphonium ylides are broadly categorized according to the nature of the substituent (s) attached to the α-carbon. Wittig reactions of the most commonly encountered semi-stabilized ylides, those derived from triphenylphosphine, are generally not very selective for one isomer or another. The general mechanism for the synthesis of these stilbene derivatives were depicted in **Scheme 19.**



R_1= H, 4-OMe
R_2= H,4-NO_2, 4-OMe, 4-Cl and 3,4-Cl

Scheme 19: Reaction Mechanism of Wittig Reaction

4.3. Characterization of stilbene derivatives

4.3.1. Physical properties of the synthesized compounds

The synthesized stilbene derivatives were obtained as solid crystal with their melting points, percent of yields and color presented in **Table 1**.

Table 1: Melting points and percent yields of the synthesized compounds

Compounds	Melting point in ^{o}C		% of yield	Color
	Experimental	Literature		
66a	120-122	122-124	64.7	White
66b	63-66	61-65	92.0	Yellowish
66c	64-67	-	69.6	White
66d	80-82	-	71.4	White
66e	88-91	-	73.2	White
66f	129-132	131-134	82.0	Yellowish
66g	92-94	-	66.0	White

As seen from Table 1, the percent yield of the Wittig product is affected by the nature of the substituent's on the aldehydes. Electron withdrawing groups on the aldehydes such as nitro (NO_2) increases the yield of the stilbene derivatives compared with electron donating substituents on the aldehydes. The electron withdrawing group increase the electrophilicity of the C=O towards nucleophilic attack by the ylides.

The result of melting point indicates *cis* isomers melt at relatively lower temperature that *trans* isomer. This is likely due to the nature of intermolecular forces aren't as effective as they should be and so less energy is needed to melt.

4.3.2. **Characterization of Stilbene Derivatives**

In the course of this project work seven stilbene derivatives including stilbene (**66a**), *p*-nitrostilbene (**66b**), *p*-methoxystilbene (**66c**), *p*-chlorostilbene (**66d**), 3',4'-dichlorostilbene (**66e**)**,** 4''-methoxy-4'-nitrostilbene (**66f**) and 4'-chloro-4''-methoxystilbene (**66g)** were synthesized. Herein is the characterization of each of these compounds one by one.

4.3.2.1. **Stilbene (66a)**

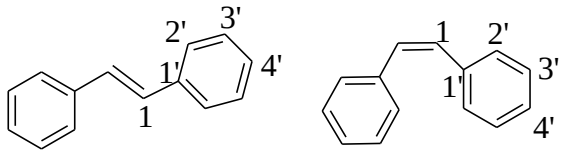
The FT-IR spectrum (**Appendix 1**) of compound **66a** showed two bands at 1595 cm^{-1} for C=C and 3019 cm^{-1} for sp^2C-H stretching. The ^{1}H-NMR (400MHz, $CDCl_3$) spectrum (**Table 1, Appendix 2**) of compound **66a** showed peaks in the aromatic and olefinic region. The proton signal at δ_H 6.75 and 7.26 (2H, *s*) is due to the proton on olefinic carbon for cis and trans, respectively. The remaining peaks observed in aromatic region at δ_H 7.67 (4H, *t*, *J* = 7.2 Hz, H-2'), 7.50 (4H, *t*, *J* = 7.2 Hz, H-3') and 7.4 (2H, *m*, H-4').

The proton decoupled ^{13}C-NMR spectrum (**Appendix 3**) of compound **66a** with aid of DEPT-135 (**Appendix 4**) showed the presence ten well resolved carbon resonances of which eight are due to methine carbons. The signals at δc 130.4, 128.88, 128.3, and 127.2 were ascribed to C-3', C-4', C-2' and C-1, respectively for *Trans* isomer and 129.0, 128.84, 127.7 and 126.7 were ascribed to C-3', C-4', C-2' and C-1, respectively for *cis* isomer. The remaining quaternary carbon observed at δc 137.41 and 137.49 are accounted to C-1' for cis and trans isomer respectively. From the result of NMR data the synthesized compound is a mixture of *cis* and *trans* stilbene.

Table 2: ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) spectral data of stilbene (**66a**)

Carbon position	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR data δ in ppm		Multiplic
		Cis	*trans*	
1	6.75 (2H, *s*)	126.7	127.2	CH
1'	-	137.41	137.49	Q
2'	7.67 (4H, *t*, *J* = 7.2 Hz)	127.7	128.3	CH
3'	7.52 (4H, *t*, J = 7.2 Hz)	129.0	130.4	CH
4'	7.41 (2H, *m*,)	128.84	128.88	CH

The above spectral data is consistent with a mixture of *cis* and *trans* stilbene whose structures are shown in F**igure 7**.



66a

Figure 7: The structure of compound 66a

4.3.2.2. 4'-Nitrostilbene (66b)

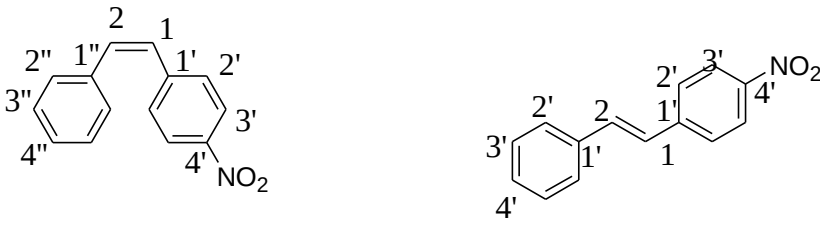
The FT-IR spectrum (**Appendix 5**) of compound **66b** showed two bands at 1340 cm^{-1} and 1506 cm^{-1} due to symmetric and asymmetric stretching of the nitro group. The weak bands at 1590 and 3100 cm^{-1} are accounted to the stretching of C=C and sp^2-C-H, respectively. The ^{1}H-NMR (400 MHz, DMSO-*d*6) spectrum (**Table 3, Appendix 6)** of compound **66b** showed the presence of peaks in aromatic and olefinic region. The proton signals at δ_H 6.71 (1H, *d*, *J* = 12.4 Hz, H-1) and 6.82 (1H, *d*, *J* =12.4 Hz, H-2) are due to the proton on olefinic carbon. The coupling constant obtained herein established the stereochemistry of the product as *cis*. The remaining peaks observed in aromatic region at δ_H 7.19-7.40 (2H, *m*, H-3''), 8.19 (2H, *d*, *J* = 8.8 Hz, H-3'), 8.06 (2H, *d*, *J* = 8.8 Hz, H-2'), 7.82 (2H, *d*, *J* = 8.8 Hz, H-2''), and 7.65 (1H, *d*, *J* = 7.2 Hz, H-4''). The proton NMR spectrum also showed that the product obtained is a mixture of *cis* and *trans* isomers with diastereoselectivity of 2:1.

The proton decoupled ^{13}C-NMR spectrum (**Appendix 7**) of compound **66b** with the aid of DEPT-135 spectrum (**Appendix 8**) showed the presence of twenty well resolved carbon resonances of which fourteen are due to methine carbons while the remaining six carbons are quaternary. The signal at δc 123.9, 126.7, 133.6, 129.2, 129.0, 128.3 and 127.5 were ascribed to C-1, C-2, C-3'', C-4'', C-2', C-2'' and C-3' respectively. The remaining quaternary carbon observed at δc 146.5, 144.4 and 136.3 are accounted to C-1', C-4' and C-1'' respectively.

Table 3:[1]H-NMR (400MHz, DMSO-*d*6) and [13]C-NMR (100MHz, DMSO-*d*6) spectral data of stilbene derivative (66b)

Carbon position	1H-NMR δ in ppm and J in Hz	13C-NMR data δ in ppm	Multiplicity
1	6.71 (1H,*d*, *J* = 12.4 Hz)	123.9	CH
2	6.82 (1H,*d*, *J* = 12.4 Hz)	126.7	CH
1'	-	146.5	Q
2'	8.05(2H,*d*, *J* = 8.8 Hz)	129.0	CH
3'	8.19(2H, *d*, *J* = 8.8 Hz)	127.5	CH
4'	-	144.4	Q
1"	-	136.3	Q
2"	7.82(2H, *d*, J = 8.8 Hz)	128.3	CH
3"	7.19-7.40(2H, *m*,)	133.6	CH
4"	7.65(1H, *d*, *J* = 8.8 Hz)	129.2	CH

The NMR spectral data is in agreement with the stilbene derivatives (**66b1 and 66b2**) whose structures are shown in **Figure 8**.



66b

Figure 8: The structure of compound **66b**

4.3.2.3. **4'-Methoxystilbene (66c)**

The FT-IR spectrum (**Appendix 9**) of compound **66c** showed band 1511 cm^{-1} C=C for aromatic ring and 1605 cm^{-1} for olefinic carbon. The weak bands at 3020 cm^{-1} and 1250 cm^{-1} are accounted for stretching of sp^2-C-H and C-O, respectively. The [1]H-NMR (400MHz, $CDCl_3$) spectrum (**Table 4, Appendix 10**) of compound **66c** showed proton signals in aromatic, olefinic and methyl region.

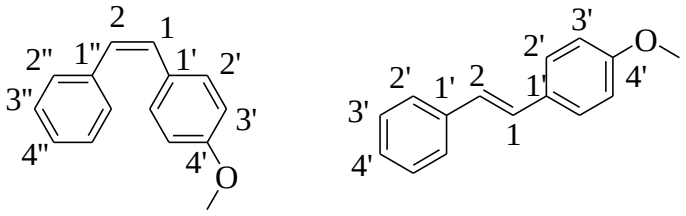
The proton signals observed at δ_H 6.91 (1H, *d*, *J* = 8.8Hz, H-1) and 7.02 (1H, *d*, *J* =8.8 Hz, H-2) are due to the proton on olefinic carbon. The coupling constant established the configuration of the double bond as *cis*. The signals observed at δ_H 3.87(*s*, 3H) accounted for oxygenated methyl carbon. The remaining peaks observed on aromatic region at δ_H 6.68 (2H, *d*, *J* =1.6Hz, H-3'), 7.2 (1H, *m*, H-4''), 7.36 (2H, *m*, H-3''), 7.44 (2H, *m*, H-2'), and 7.64 (2H, *m*, H-2''). Close inspection of the proton NMR spectrum also revealed the presence of trans product as a minor constituents.

The proton decoupled ^{13}C-NMR spectrum **(Appendix 11**) of compound **66c** with the aid of DEPT-135 spectrum **(Appendix 12**) showed the presence of twenty two well resolved carbon resonances of which fourteen are due to methine carbons. The signals at δc 113.7, 126.4, 127.9, 128.4, 128.9, 129.9 and 130.3 were ascribed to C-3', C-1, C-2, C-2', C-2'', C-4'' and C-3'' respectively. The signals observed at δc 55 are accounted for methoxy group. The remaining quaternary carbon observed at δc 158.8, 137.8 and 130.2 are accounted to C-4', C-1'' and C-1' respectively.

Table 4: ^{1}H-NMR (400MHz, $CDCl_3$) and ^{13}C-NMR (100MHz, $CDCl_3$) spectral data of stilbene derivative (66c)

Carbon position	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR data δ in ppm	Multiplicity
1	7.02 (*d*, 1H,J = 8.8)	126.4	CH
2	6.91 (*d*, 1H, *J* = 8.8)	127.9	CH
1'	-	130.2	Q
2'	7.44 (2H, *m*)	128.4	CH
3'	6.68(*d*, 2H, *J* = 1.6)	113.7	CH
4'	-	158.8	Q
1"	-	137.8	Q
2"	7.64 (2H, *m*)	128.9	CH
3"	7.36 (2H, *m*)	130.3	CH
4"	7.20 (1H, *m*)	129.9	CH
3	3.87 (3H, *s*)	55.3	CH_3

The NMR spectral data is in agreement with the stilbene derivatives (**66c**) whose structure is shown in **Figure 9**.



66c

Figure 9: The structure of compound 66c

4.3.2.4. 4'-Chlorostilbene (66d)

The FT-IR spectrum **(Appendix 13)** of compound **66d** showed bands at 1550 cm^{-1} and 1598 cm^{-1} ascribed to stretching frequency for C=C of aromatic and olefinic carbon, respectively. The weak band at 3025 cm^{-1} is accounted to sp^2C-H stretching. The C-Cl stretching frequency is evident at 820 cm^{-1}. The ^{1}H-NMR (400MHz, DMSO-*d*6) spectrum (**Table 5, Appendix 14)** of compound **66d** showed proton peaks in Aromatic and olefinic region. The proton signals observed at δ_H 6.60 (1H, *d*, *J* =12.4 Hz, H-1) and 6.65 (1H, *d*, *J* =12.4 Hz, H-2) are due to the proton on olefinic carbon oriented *cis* to one another. The remaining peaks observed in aromatic region at δ_H 7.60 (2H, *t*, *J* =8 Hz, H-2''), 7.40 (2H, *d*, *J* =8 Hz, H-2'), 7.37 (2H, *d*, *J* = 7.2 Hz, H-3'), 7.27(2H, *m*, H-3'') and 7.20 (1H, *m*, H-4''). Also observed in the ^{1}H-NMR spectrum is an additional 9 proton signals in the olefinic and aromatic region evident for the presence of *trans* isomer.

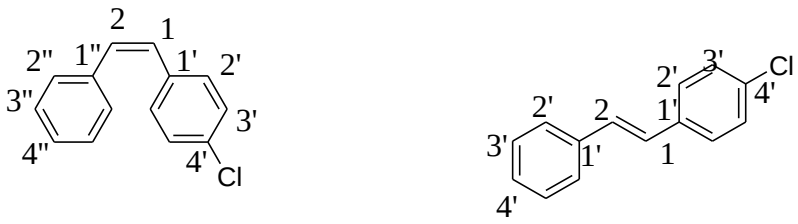
The proton decoupled ^{13}C-NMR spectrum(**Appendix 15**) of compound **66d** with the aid of DEPT-135 (**Appendix 16**) showed the presence of carbons signals for both *cis* and *trans* 4-chlorostilbene. The signals due to quaternary carbons are evident at δc136.9 (C-1''), 136.1 (C-1') and 132.1 (C-4'). Also observed in the ^{13}C-NMR spectrum is at δc 127.0 and 127.5 which are accounted to the olefinic carbon C-1 and C-2, respectively. The reaming signal observed in aromatic region at δc 130.7 (C-3'), 129.1 (C-3''), 128.8 (C-4''), 128.5 (C-2') and 128.3, C-2'').

The NMR spectral data reported herein is in good agreement with the NMR spectral data perversely reported for the same compound [**64**].

Table 5: ^{1}H-NMR (400MHz, DMSO d-6) and ^{13}C-NMR (100MHz, DMSO-*d*6) spectral data of stilbene derivative (66d)

Carbon position	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR data δ in ppm	Multiplicity
1	6.60 (1H, *d*, *J*= 12.4 Hz)	127.0	CH
2	6.65 (1H, *d*, *J* = 12.4 Hz)	127.5	CH
1'	-	136.1	Q
2'	7.40 (2H, *d*, *J* = 8 Hz)	128.5	CH
3'	7.37 (2H, *d*, *J* =7.2 Hz)	130.7	CH
4'	-	132.1	Q
1"	-	136.9	Q
2"	7.64 (2H, *t*, *J* = 8 Hz)	128.3	CH
3"	7.27 (2H, *m*,)	130.7	CH
4"	7.20 (1H, *m*,)	128.8	CH

The NMR spectral data is in agreement with the stilbene derivatives (**66d**) whose structure is shown in **Figure 10**.



66d1 **66d2**

Figure 10: The structure of compound 66d

4.3.2.5. 3',4'-Dichlorostilbene (66e)

The FTIR spectrum **(Appendix 17)** of compound **66e** showed band at 1500 cm^{-1} C=C for aromatic ring and 1548 cm^{-1} for olefinic carbon. The weak band at wave number 3086 cm^{-1} is showed for sp^2C-H stretching and at 820 cm^{-1} showed for C-Cl stretching. The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum (**Table 6, Appendix 18)** of compound **66e** showed the presence of eight proton peaks in the aromatic and olefinic region. The proton signals observed at δ_H 6.51 (1H, *d*, *J*

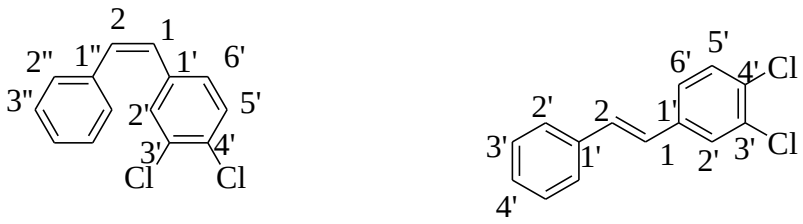
= 12.4 Hz, H-1) and 6.72 (1H, *d*, *J* =12.4 Hz, H-2) are due to the proton on olefinic carbon establishing the configuration of the double bond as *cis*. The remaining peaks observed in aromatic region at δ_H 7.61(2H, *d*, *J* = 2Hz, H-2''), 7.54(1H, *d*, *J* =7.6 Hz, H-2'), 7.43(2H, *m*, H-3''), 7.36(2H, *m*, H-6'), 7.30 (1H, *m*, H-5') and 7.09(1H, *m*, H-4'').

The proton decoupled ^{13}C-NMR spectrum (**Appendix 19**) of compound **66e** with the aid of DEPT-135 (**Appendix 20**) showed the presence of twenty two well resolved carbon resonances in the olefinic and aromatic region. The carbon signals due to quaternary carbons were observed at at δc =137.5 (C-1''), 136.5 (C-1'), 132.8 (C-3'), and 132.3(C-4'). The signal observed at δc 125.6(C-1) and 126.1(C-2) are due to olefinic carbon. The remaining aromatic carbon signals were evident at δc126.7 (C-6'), 127.6(C-2''), 128.2(C-4''), 128.4(C-2'), 128.8(C-3'') and 130(C-5').

Table 6: ^{1}H-NMR (400MHz, $CDCl_3$) and ^{13}C-NMR (100MHz, $CDCl_3$) spectral data of stilbene derivative (66e)

Carbon position	^{1}H-NMR δ in ppm and J in Hz	^{13}C-NMR data δ in ppm	Multiplicity
1	6.51(1H, d, J = 12.4 Hz)	125.6	CH
2	6.72(d,1H, J = 12.4 Hz)	126.1	CH
1'	-	136.5	Q
2'	7.54(1H, d, J = 7.6 Hz)	128.4	CH
3'	-	132.8	Q
4'	-	132.3	Q
5'	7.30(1H, *m*)	130	CH
6'	7.36(1H, *m*)	126.7	CH
1"		137.5	Q
2"	7.61(2H, d, *J*= 2 Hz)	127.6	CH
3"	7.43(m,2H)	128.8	CH
4"	7.09(m, 1H)	128.2	CH

The NMR spectral data is in agreement with the stilbene derivatives [66e] whose structure shown in **Figure 11**.



66e

Figure 11: The structure of compound 66e

4.3.2.6. 4’’-Methoxy-4’-nitrostilbene(66f)

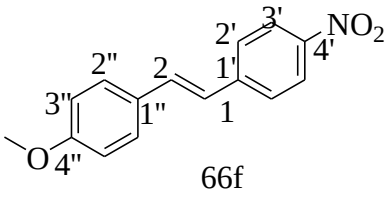
FTIR spectrum **(Appendix 21)** of Compound **66f** were showed the absorption band at 1339 and 1513cm^{-1} indicates nitro-containing functional group and the absorption band observed at 1589 cm^{-1} for C=C stretching. The band was observed at wave number 1250 cm^{-1} for C-O stretching. The absorption band was observed at 2933 cm-1 due to sp^3C-H stretching. The ^{1}H-NMR (400MHz, $CDCl_3$) spectrum (**Table 7, Appendix 22)** of compound **66f** showed the presence of seven proton signals in Aromatic, olefinic and methyl region. The proton signals observed at δH 7.04 (1H, d, *J* = 16.4 Hz, H-1) and 7.28 (1H, *d, J* =16.4 Hz, H-2) are due to the proton on olefinic carbon. The signal observed at δH 3.86(s, 3H) accounted to oxygenated methyl and the remaining signal were observed in aromatic region at δ_H 8.22 (2H, *d, J* =8.8 Hz, H-3’), 7.62(2H, *d, J* =8.8 Hz, H-2’), 7.52(2H, *d, J* =8.8 Hz, H-2’’), and 6.96 (2H, *d, J* = 8.8, H-3’’).

The proton decoupled ^{13}C-NMR spectrum (**Appendix 23**) of compound **66f** with the aid of DEPT- (**Appendix 24**) showed the presence of eleven well resolved carbon resonances of which six are due to methine carbons. The signals at δc 114.3, 124.1, 124.16, 126.5, 128.4 and 133.9 were ascribed to C-3’’, C-1, C-2, C-3’, C-2’, and C-2’’ respectively. The signal observed at δc 55.3 due to oxygenated methyl carbon(C-3). The remaining quaternary carbon observed at δc = 160.2, 144.3, 146.4 and 128.9 are accounted to C-4’’, C-1’, C-4’ and C-1’’ respectively. The NMR spectral data reported herein is in good agreement with the NMR spectral data perversely reported for the same compound [**64**].

Table 7: ^{1}H-NMR (400MHz, $CDCl_3$) and ^{13}C-NMR spectral data of stilbene derivative (66f)

Carbon position	1H-NMR δ in ppm and J in Hz	13C-NMR data δ in ppm	Multiplicity
1	7.04(1H, *d*, *J*= 16.4 Hz)	124,1	CH
2	7.28(1H, *d* , *J* = 16.4 Hz)	124.16	CH
1'	-	144.3	Q
2'	7.62 (2H, *d* , *J* = 8.8 Hz)	128.4	CH
3'	8.22 (*d* , 2H, *J j* = 8.8 Hz)	126.5	CH
4'	-	146.4	Q
1"	-	128.9	Q
2"	7.52 (2H, *d*, *J* =8.8 Hz)	132.9	CH
3"	6.96(2H, *d*, *J* = 8.4 Hz)	114.3	CH
4"	-	160.2	Q
3	3.86(3H, *s*)	55.3	CH_3

Based on the above spectral data the compound fits with the stilbene derivative (**66f)** whose structure is shown in **Figure 12.**



66f

Figure 12: The structure of compound 66f

4.3.2.7. 4'-Chloro,4''-methoxystilbene(66g)

FTIR spectrum **(Appendix 25)** of Compound **66g** were showed the absorption band at 1513 cm-1 and 1605 cm^{-1} for C=C are due to Aromatic ring and olefinic carbon. The band was observed at wave number 1255 cm^{-1} for C-O stretching. The absorption band was observed at 2935 cm-1 due to sp^3 C-H stretching for Aliphatic. Furthermore the absorption band observed at 834 cm^{-1} for C-Cl stretching. The ^{1}H-NMR (400MHz, $CDCl_3$) spectrum (**Table 8, Appendix 26)** of compound **66g** showed the proton signals in Aromatic, olefinic and methyl region. The proton signals observed at δH 6.47 (1H, d, *J* = 12.4 Hz, H-1) and 6.59 (1H, *d*, *J* =12.4 Hz, H-2)

are due to the proton on olefinic carbon. The signal observed at δH 3.81 (s, 3H) and 3.86 (s, 3H) accounted to oxygenated methyl carbon for *cis* and *trans* isomer respectively. The remaining signal were observed in aromatic region at δ_H 7.48 (2H, *d*, *J* = 8.4, H-2'), 7.44 (2H, *d*, *J*= 8.4, H-2''), 6.80 (2H, *d*, *J* =8.8, H-3''), 6.96 (*d*, *J* = 16.8 Hz, 1H, H-1), 7.08 (*d*, *J* =16.4, 1H, H-2), and 7.22 (2H, *d*, *J* = 8.4, H-3').

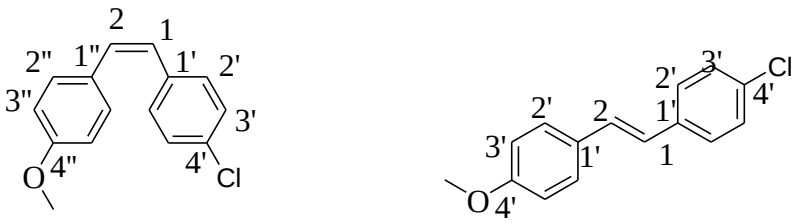
The proton decoupled ^{13}C-NMR spectrum (**Appendix 27**) of compound **66g** with the aid of DEPT- 135 (**Appendix 28**) showed the presence of twenty two well resolved carbon resonances of which twelve are due to methine carbons. The signals at δc 114.2, 127.4, 127.8, 128.8, 130.13 and 130.5 were ascribed to C-3'', C-1, C-2, C-2'', C-2', and C-3' respectively for *trans* isomer and the signal at δc 113.7, 125.2, 127.45, 128.4, 128.89 and 130.18 were ascribed to C-3'', C-1, C-2, C-2'', C-2', and C-3' respectively for *cis* isomer . The signal observed at δc 55.3 and 55.2 due to oxygenated methyl carbon (3) for *Trans* and *cis* isomer respectively. The remaining quaternary carbon observed at δc 159.5, 136.2, 132.7 and 129.8 are accounted to C-4'', C-4', C-1' and C-1'' respectively for *trans* isomer and the signal at δc 158.8, 136.0, 132.5 and 129.3 are accounted to C-4'', C-4', C-1' and C-1'' respectively for *cis* isomer. From the result of NMR data the synthesized compound is a mixture of *cis* and *trans* stilbene derivative [66g].

Table 8: ^{1}H-NMR (400MHz, $CDCl_3$) and ^{13}C-NMR spectral data of stilbene derivative (66g)

Position	1H-NMR δ in ppm and J in Hz		13C-NMR data δ in ppm		Multiplicity
	Cis	*Trans*	*Cis*	*Trans*	
1	6.47 (1H, *d*, *j*= 12.4 Hz)	6.96(1H, *J*= 16.8Hz	125.2	127.4	CH
2	6.59 (*d*, 1H, *j*= 12.4 Hz)	7.08(1H, *d*, *J=16.4*Hz	127.45	127.8	CH
1'	-		132.5	132.7	Q
2'	7.48 (2H, *d*, *J* = 8.4 Hz)		128.89	130.13	CH
3'	7.22 (2H,*d*, *J* =2.4 Hz)		130.18	130.5	CH
4'	-		136.0	136.2	Q
1"	-		129.2	129.8	Q
2"	7.44 (2H, *d*, *J* = 8.4 Hz)		128.4	128.8	CH
3"	6.80 (*d*, 2H, *J* = 8.8 Hz)		113.7	114.2	CH
4"	-		158.8	159.5	Q

3	3.81(s, 3H)	3.86(s, 3H)	55.2	55.3	CH_3

The NMR spectral data is in agreement with the stilbene derivatives (**66g)** whose structure is shown in **Figure 13.**



66g

Figure 13: The structure of compound 66g

4.4. Antibacterial Activity

The antibacterial activity of the synthesized stilbene derivatives were determined by screening against three Gram-negative strains (*E. coli, P.aeruginosa and K. puemonia*) and one Gram-positive strains (*S. aureus)* using the disc diffusion method. The antibacterial activities of the stilbene derivatives are shown in **Table 9**.

Table 9: Inhibition zone of diameter in mm of the synthesis stilbene derivatives,

compounds	Zone of inhibition (mm)			
	S.aureus	*E. coli*	*P.aeroginosa*	*K. puemonia*
66a	6	6	6	6
66b	6	6	6	6
66c	6	6	6	6
66d	6	6	6	6
66e	24	22	6	6
66f	6	6	6	6
66g	6	6	6	6
Ceftriaxone	21	32	20	15
DMSO	0	0	0	0

Ceftriaxone are used as positive control

The antibacterial activities of the synthesized compounds differ considerably. Among the compounds evaluated, compound **66e** exhibited pronounceable activity against two strains of bacterial pathogens such as *S. aureus* and *E. coli* with zone of inhibition of 24 and 22 mm, respectively (**Figure 15**). The result is significant compared with ceftriaxone with inhibition zone of 21mm and 32 mm against *S. aureus and E. coli*, respectively. In contrast all the other compounds were not active against all tested pathogens. But inhibit the growth of all bacteria species.

5. CONCLUSIONS AND RECOMMENDATIONS

In the course of this work, seven stilbene derivatives viz stilbene, 4'-nitrostilbebe, 4'-methoxystilbene,4'-chlorostibene,3',4'-dichlorostilbene, 4''methoxy,4'-nitrostilbene and 4'-chloro,4''-methoxystilbene were synthesized using substituted benzaldehydes and various ylides using Wittig reactions. The synthesized stilbenes were evaluated for their antibacterial activities against *S. aureus, E. coli*, *P.aeruginosa and K. puemonia*. Comparable antibacterial activity was displayed by 3,4-dichlorostilbene (**66e**) against *S. aureus* and *E.coli* with ceftraxone used as positive control.

As stilbene derivatives may have immense biological activities, it is necessary to evaluate the antioxidant, anticancer, antifungal and anti-inflammatory activities of these compounds.

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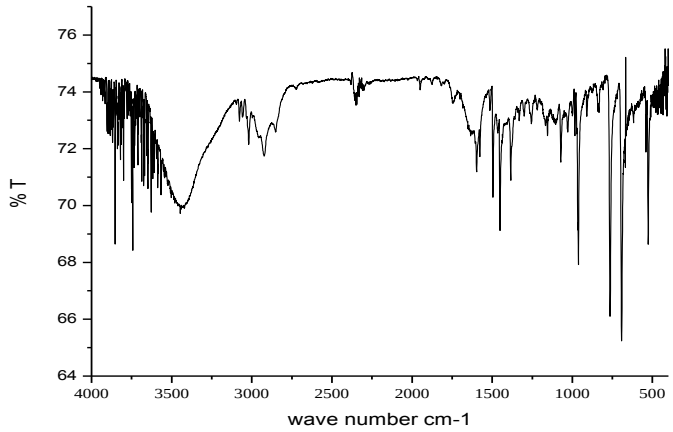
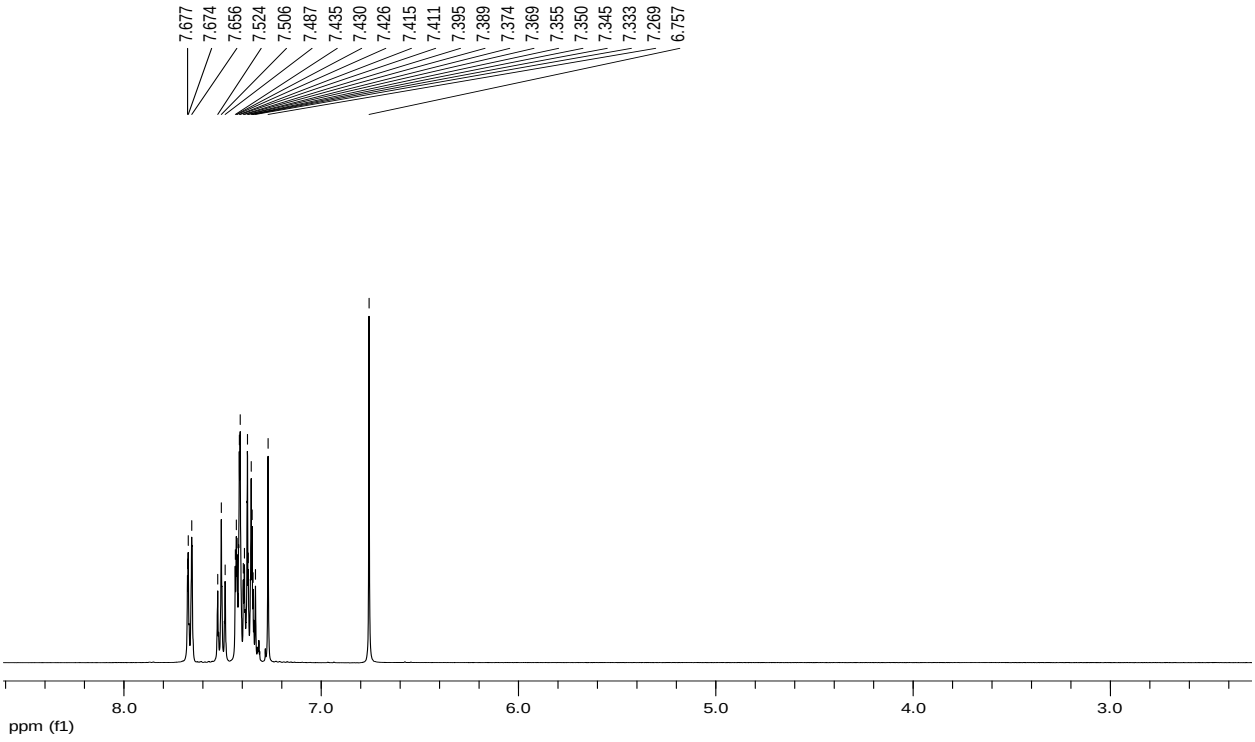
7. APPENDIXES

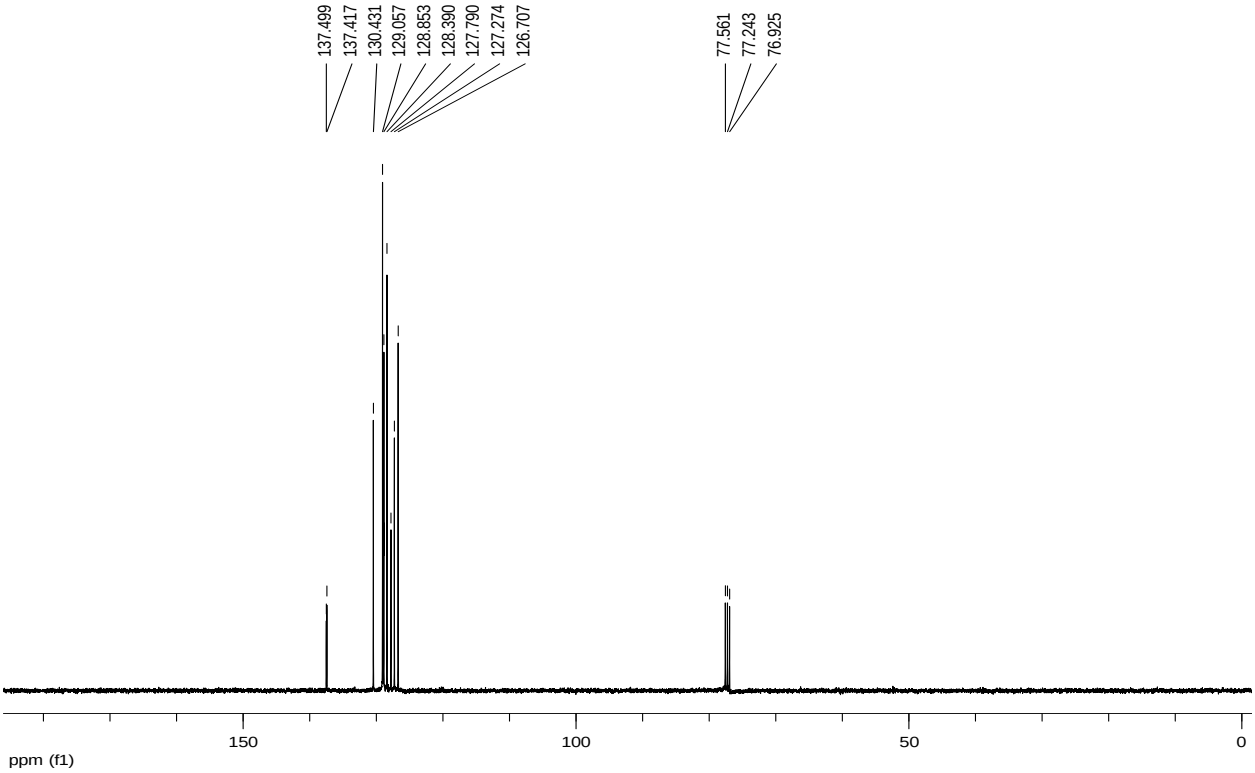
Appendix 1: FTIR spectrum of compound 66a



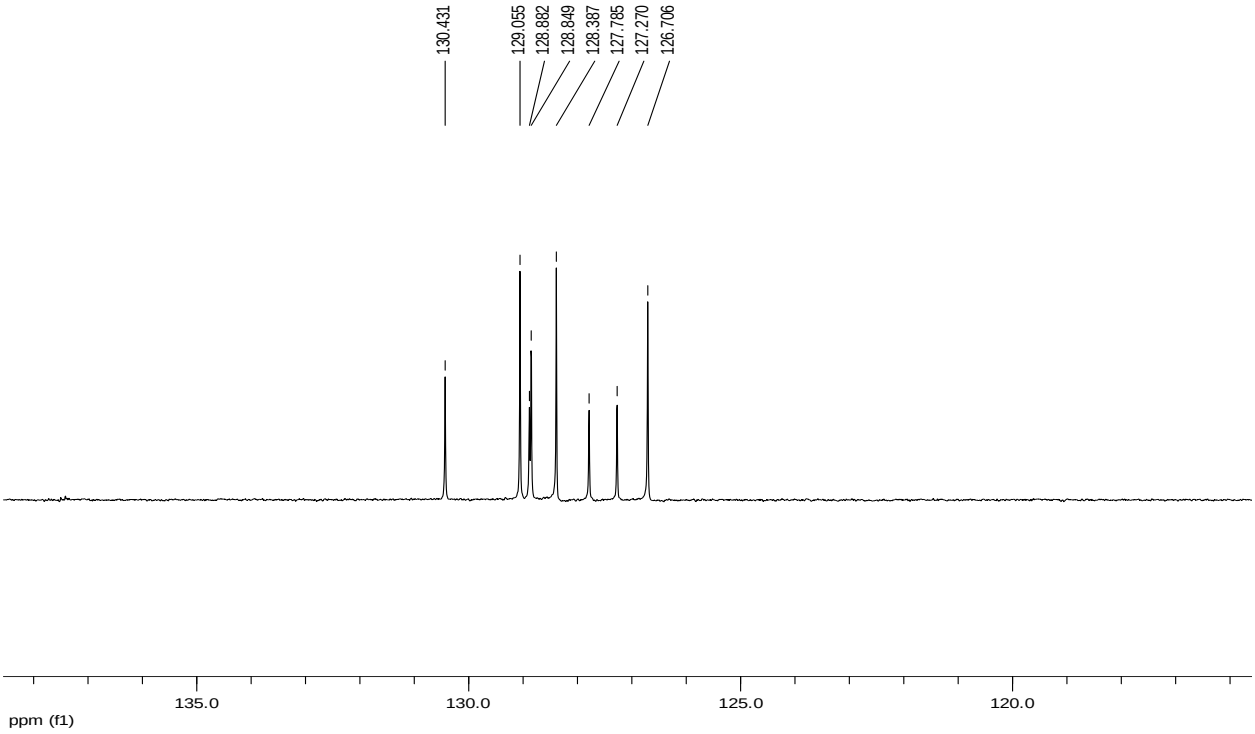
Appendix 2: ^{1}H-NMR spectrum of compound 66a



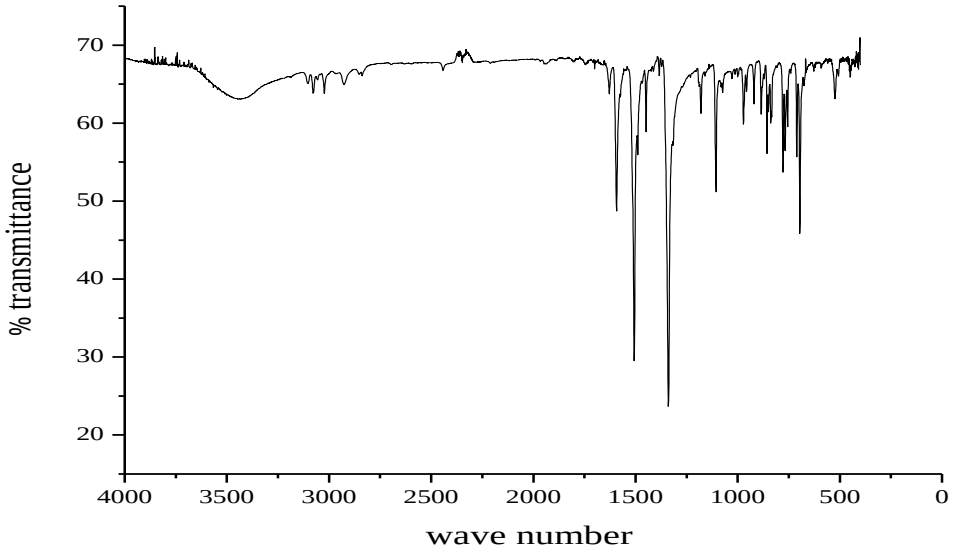
Appendix 3: ^{13}C-NMR spectrum of compound 66a



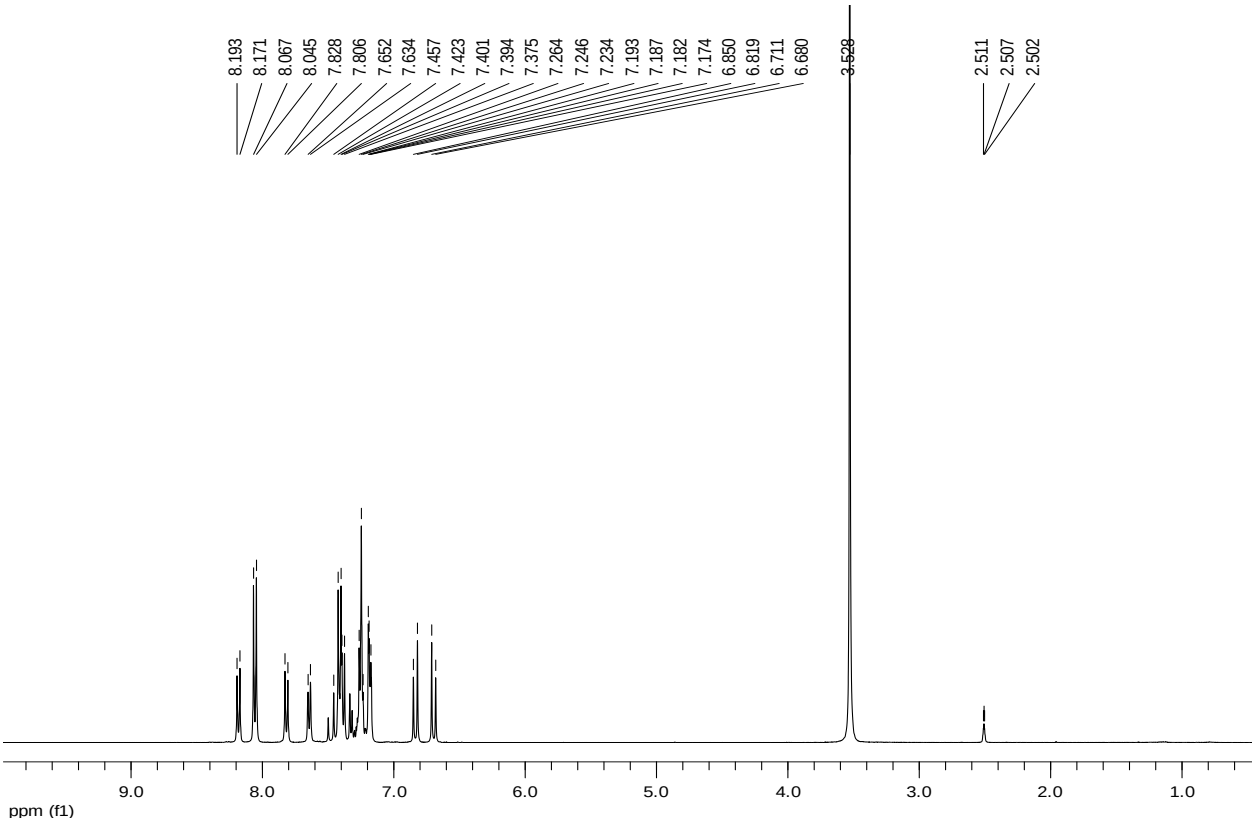
Appendix 4: DEPT-135 NMR spectrum of compound 66a



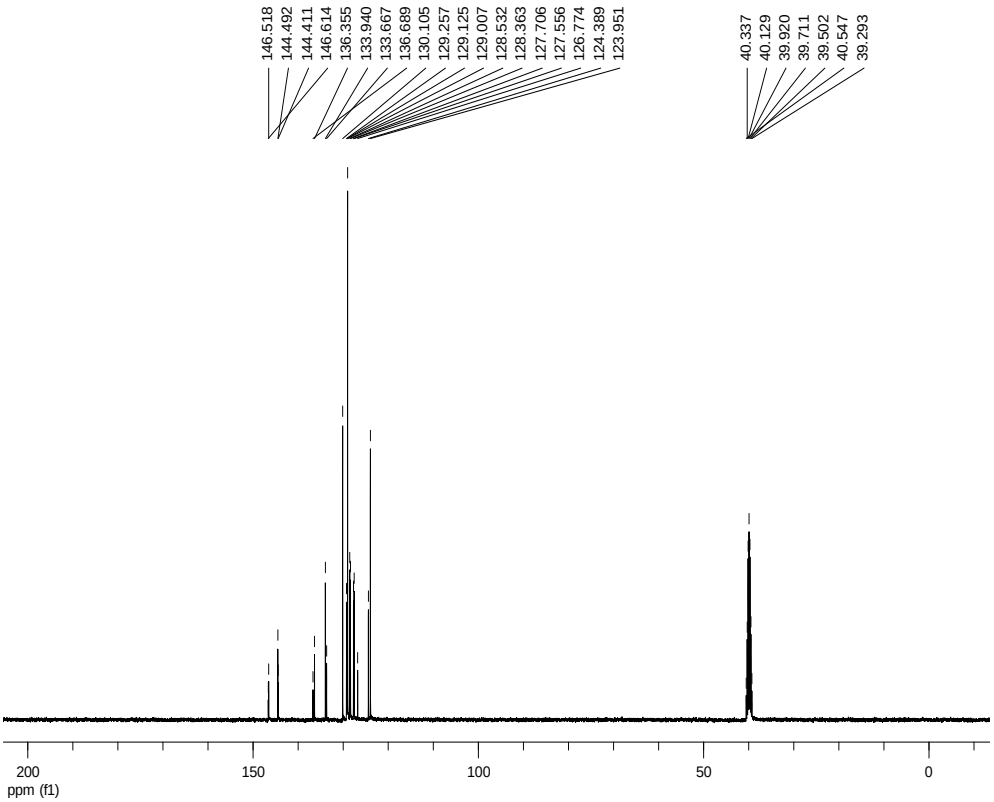
Appendix 5: FTIR spectrum of compound 66b



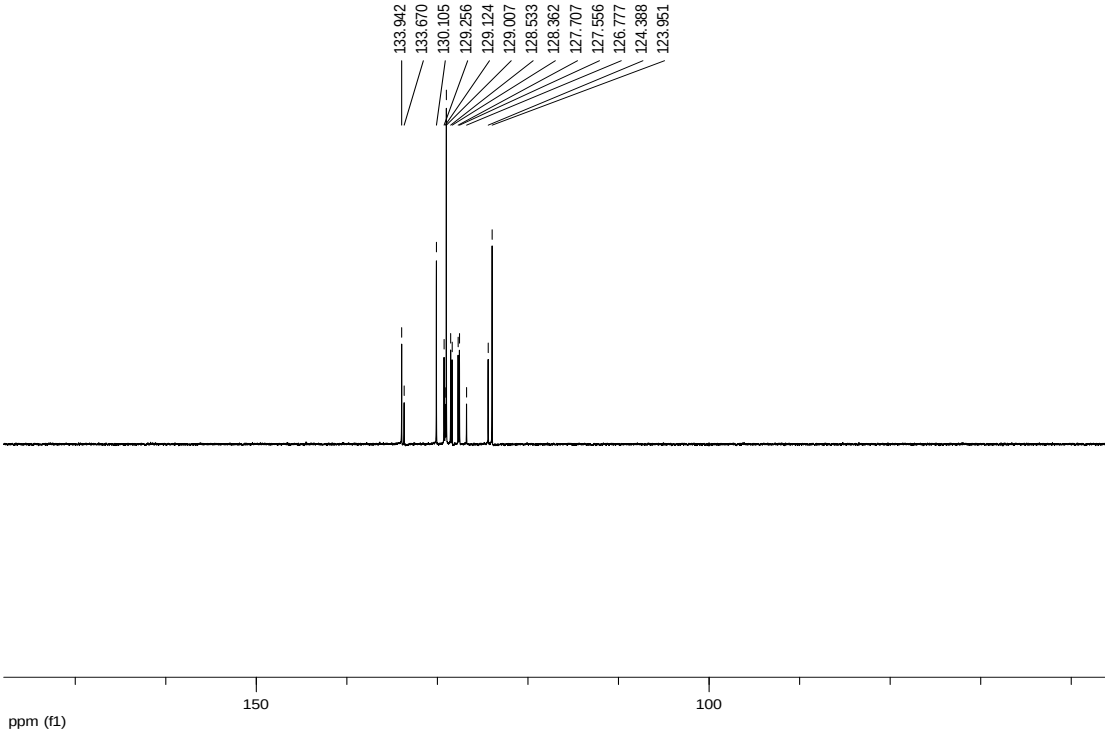
Appendix 6: ^{1}H-NMR spectrum of compound 66b



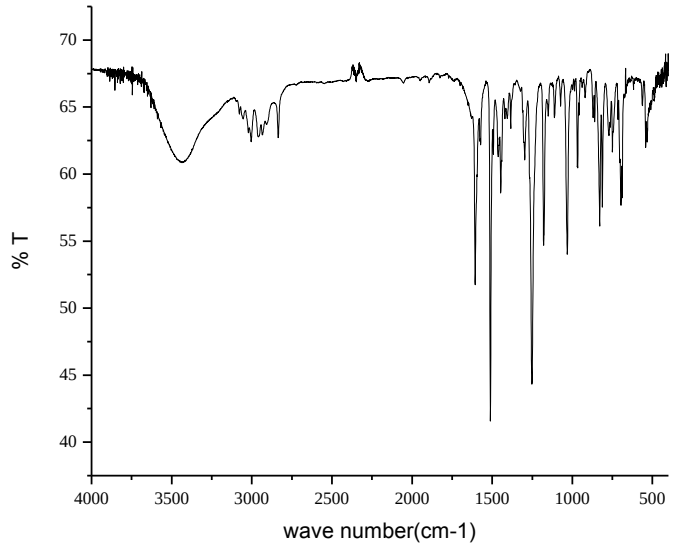
Appendix 7: ^{13}C-NMR spectrum of compound 66b



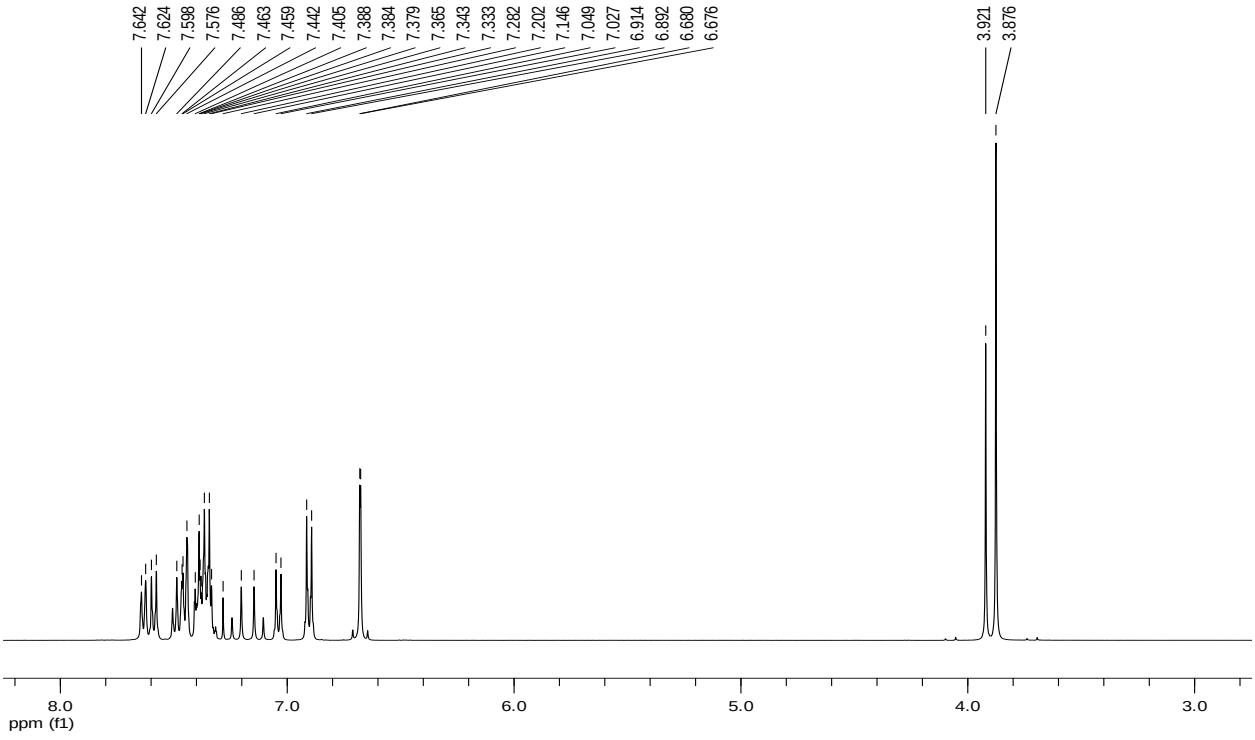
Appendix 8: DEPT-135 spectrum of compound 66b



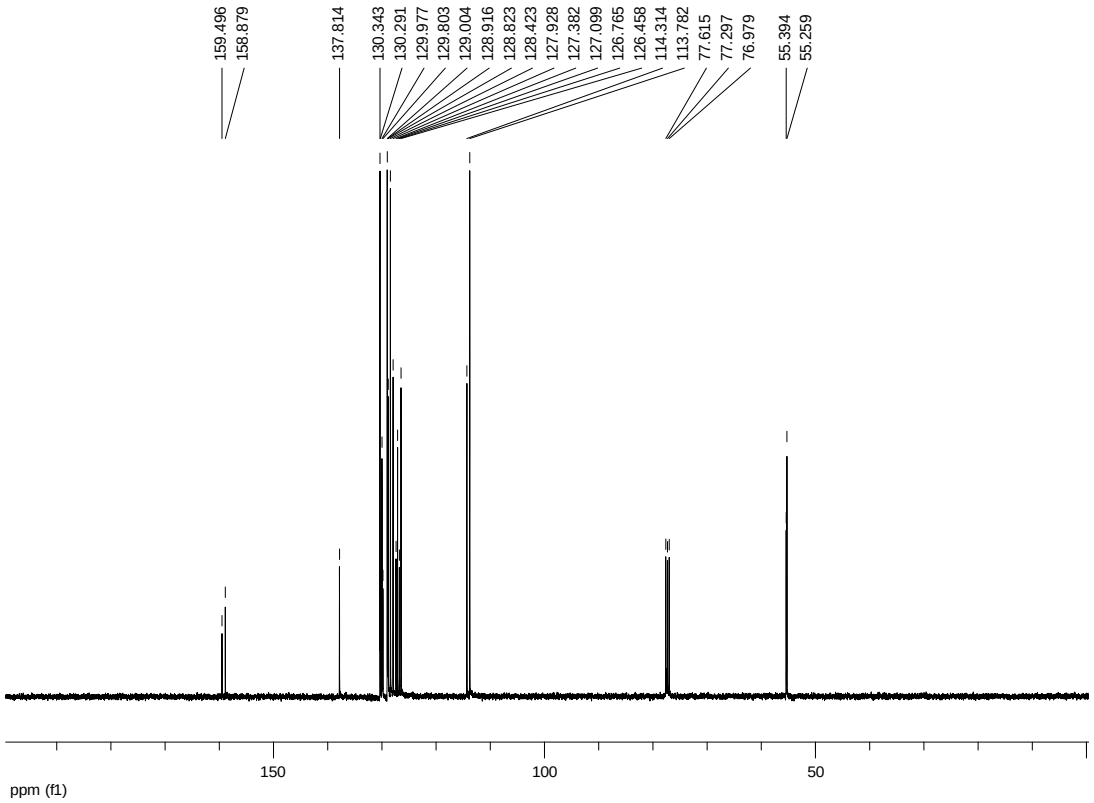
Appendix 9: FT-IR spectrum of compound 66c



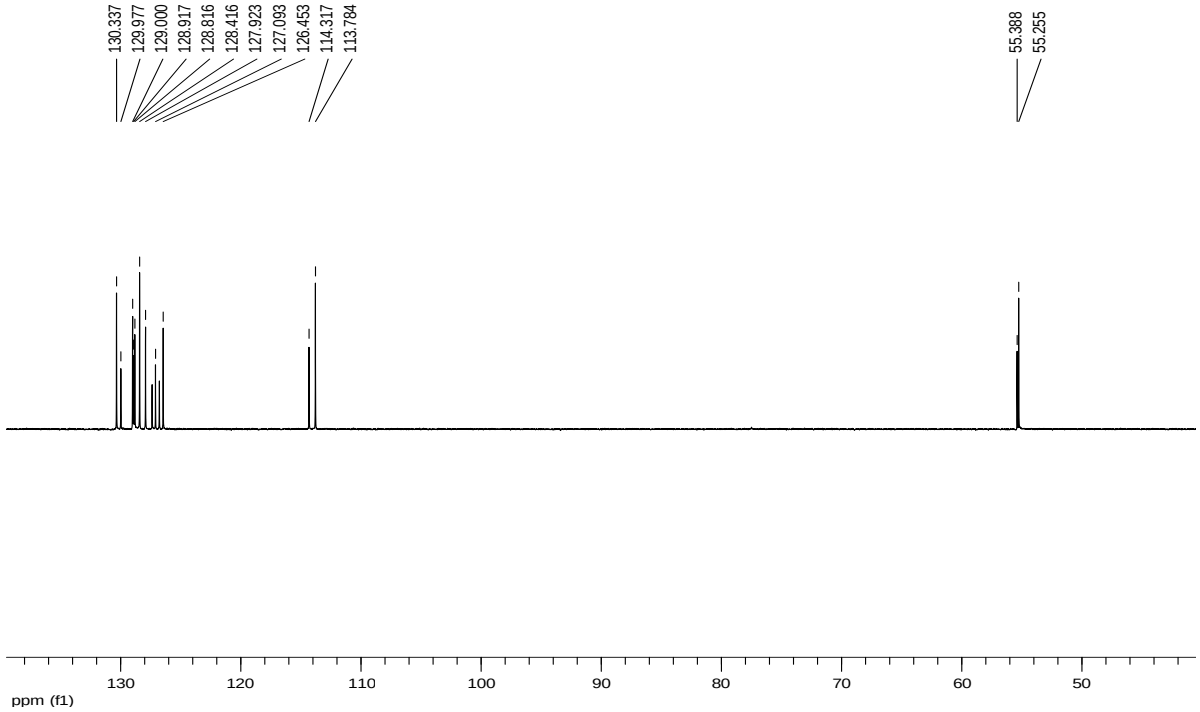
Appendix 10: ^{1}H-NMR spectrum of compound 66c



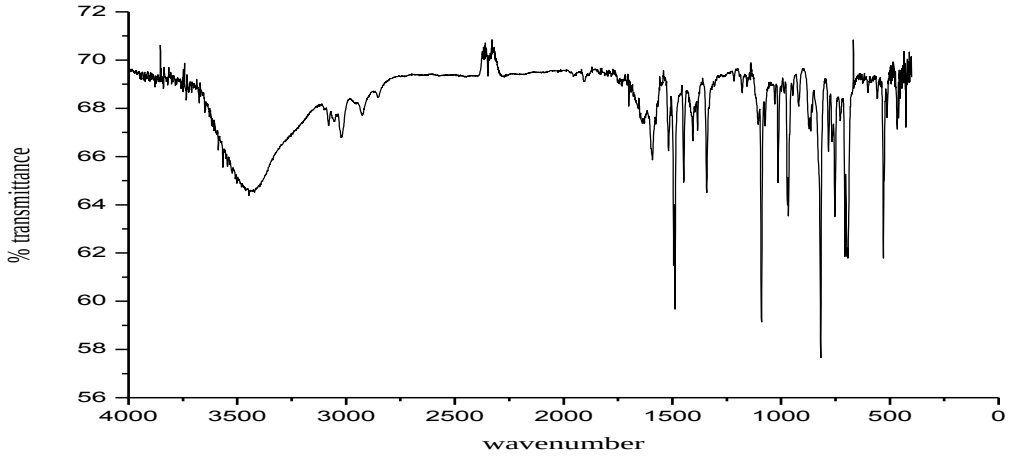
Appendix 11: ^{13}C-NMR spectrum of compound 66c



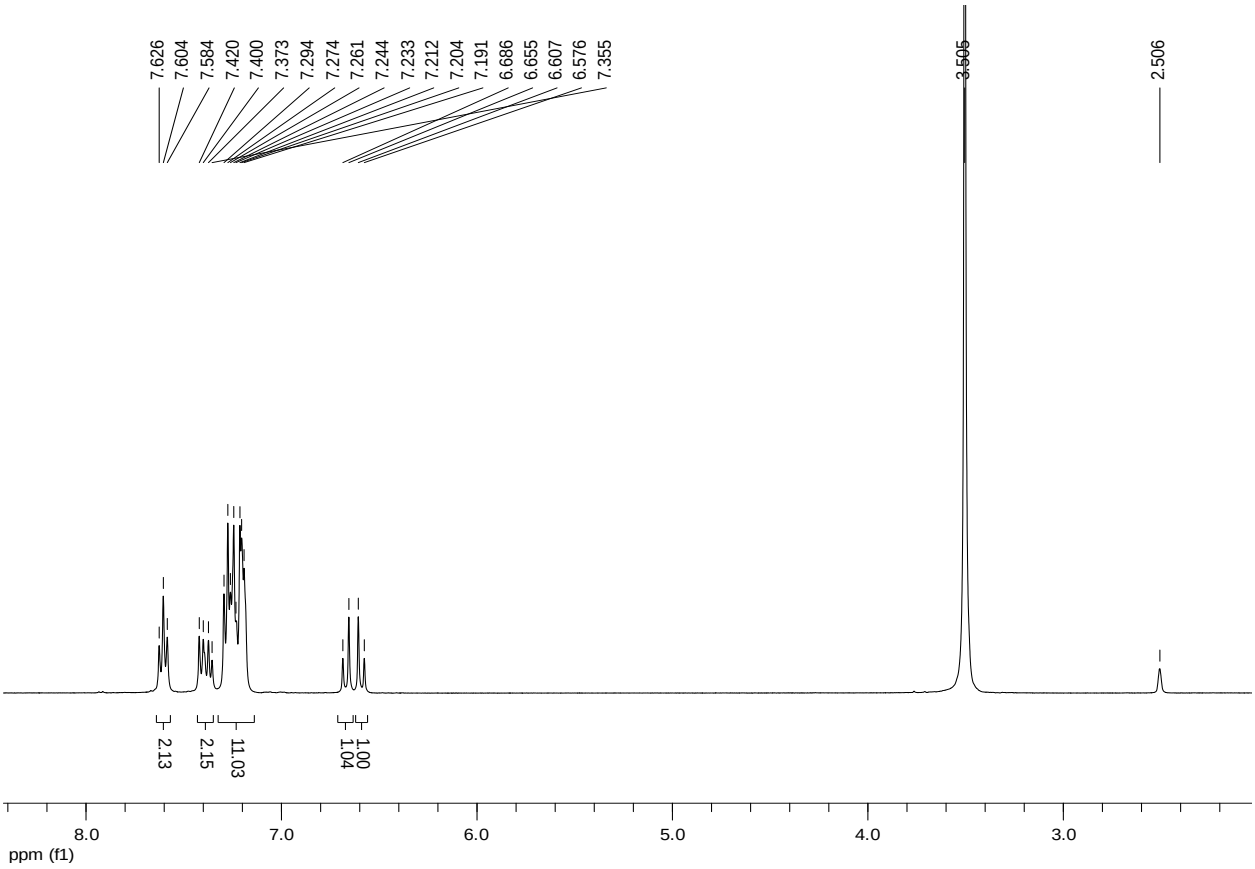
Appendix 12: DEPT-135 NMR spectrum of compound 66c



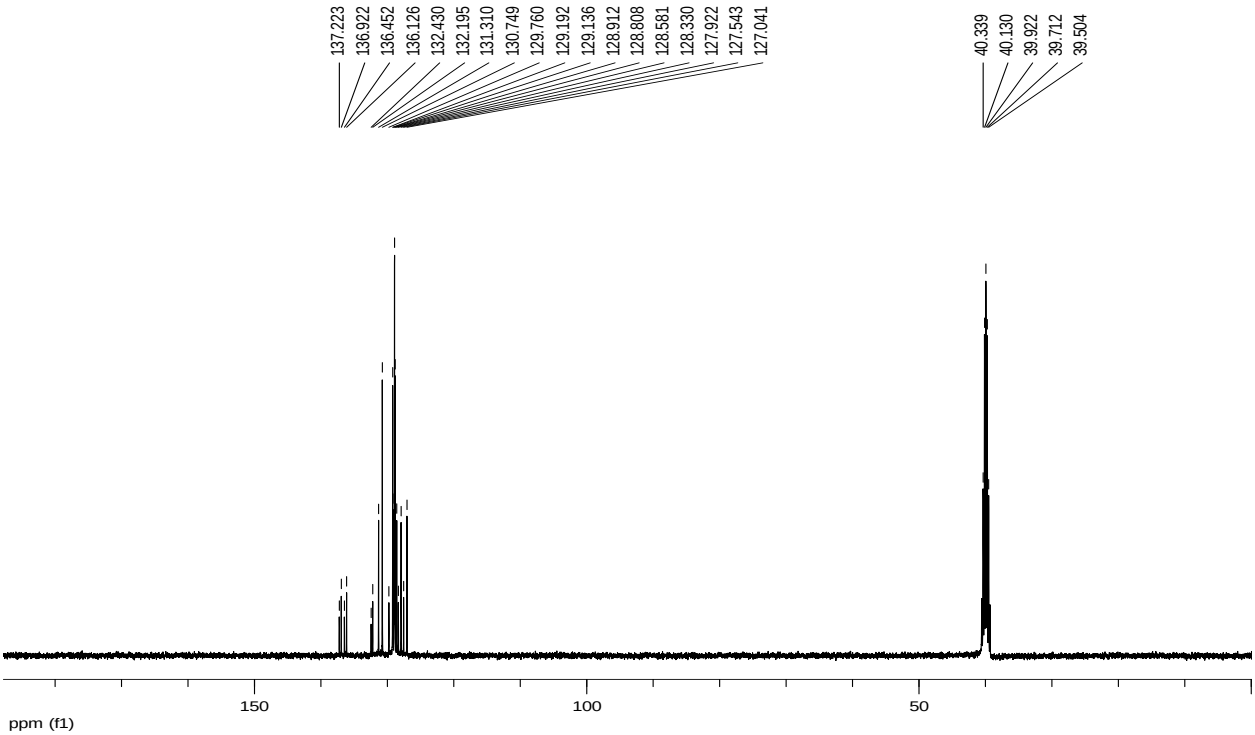
Appendix 13: FTIR spectrum of compound 66d



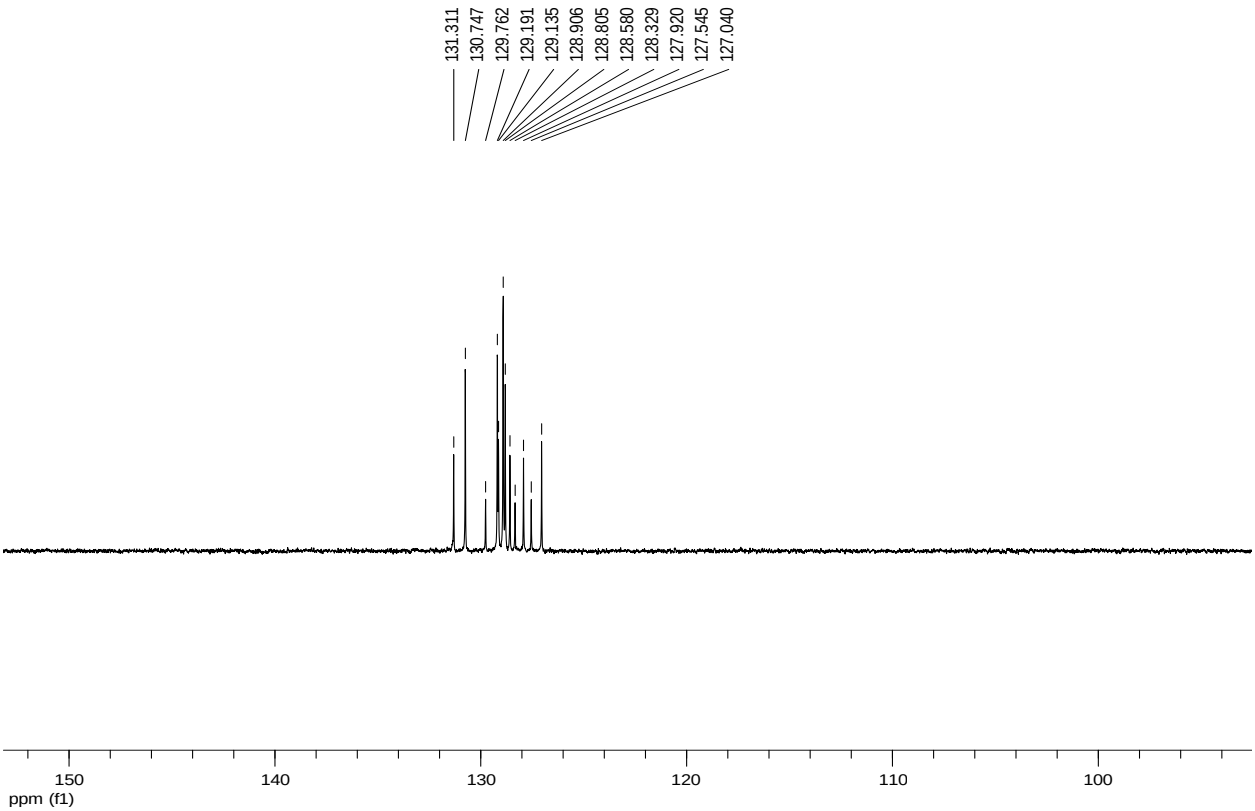
Appendix 14: ^{1}H-NMR spectrum of compound 66d



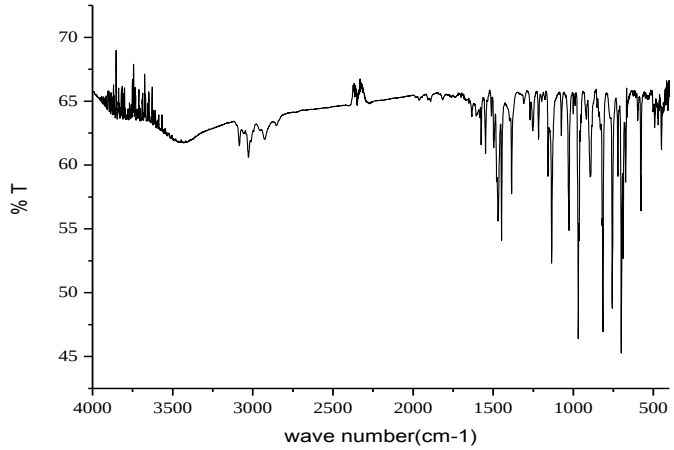
Appendix 15: ^{13}C-NMR spectrum of compound 66d



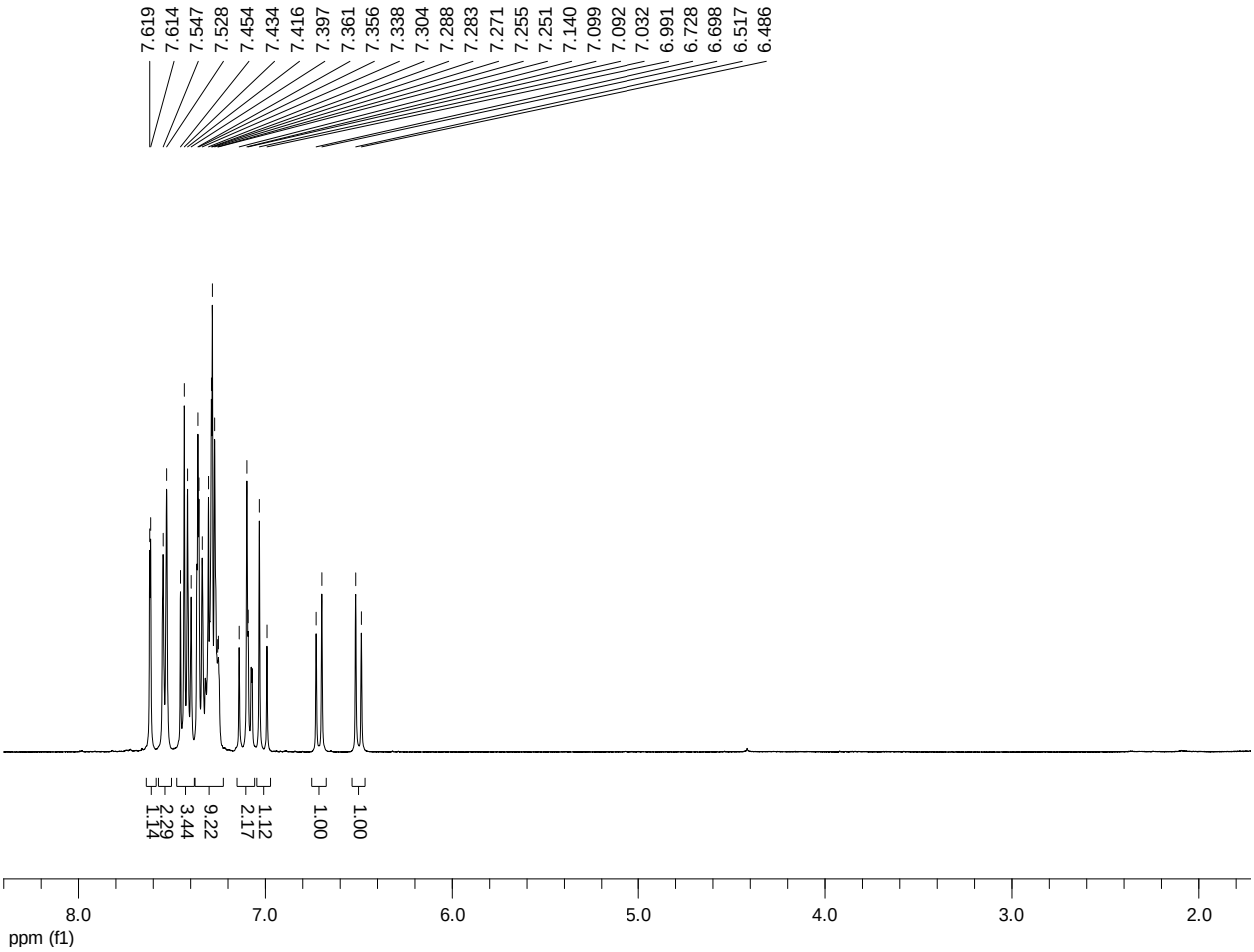
Appendix 16: DEPT-135 NMR spectrum of compound 66d



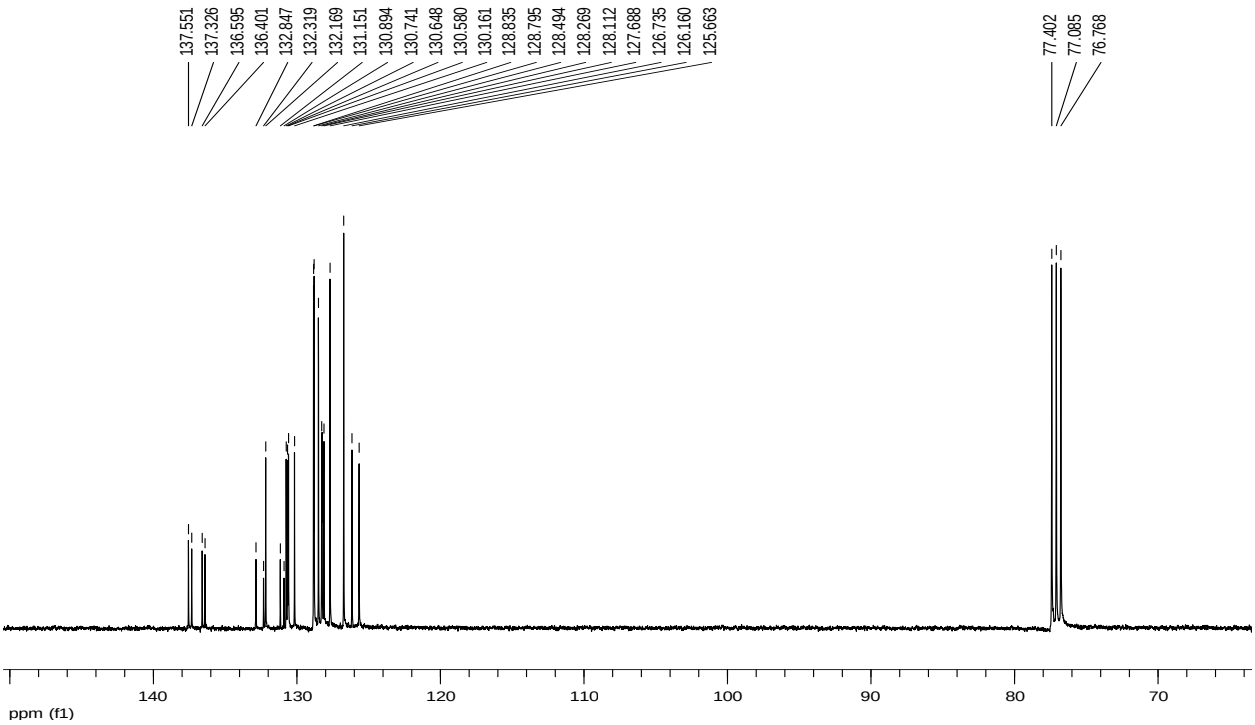
Appendix 17: The FTIR spectrum of compound 66e



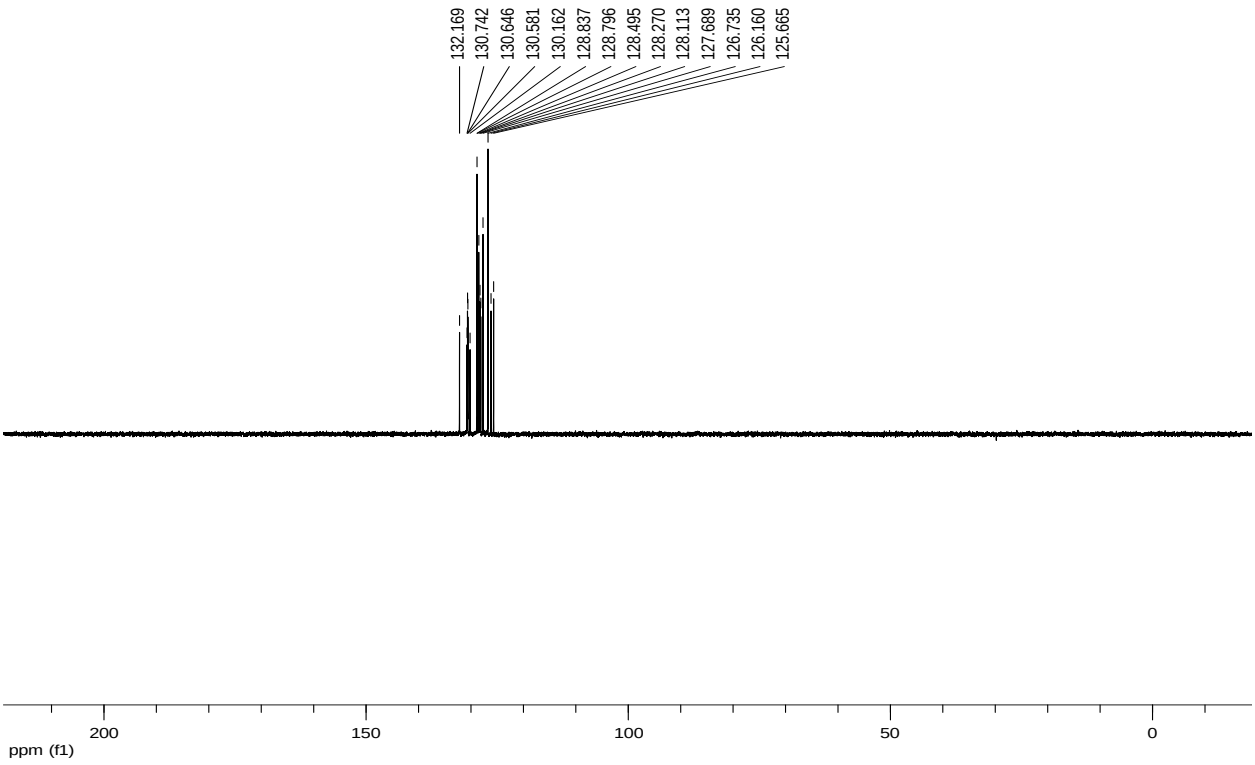
Appendix 18: ^{1}H-NMR spectrum of compound 66e



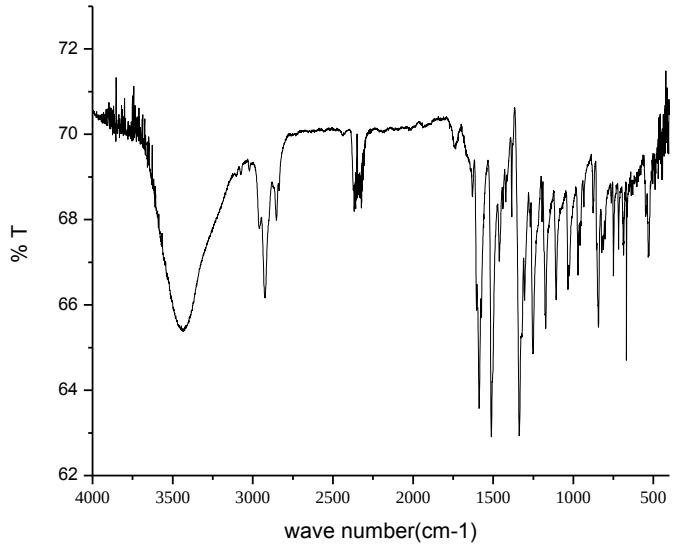
Appendix 19: ^{13}C-NMR spectrum of compound 66e



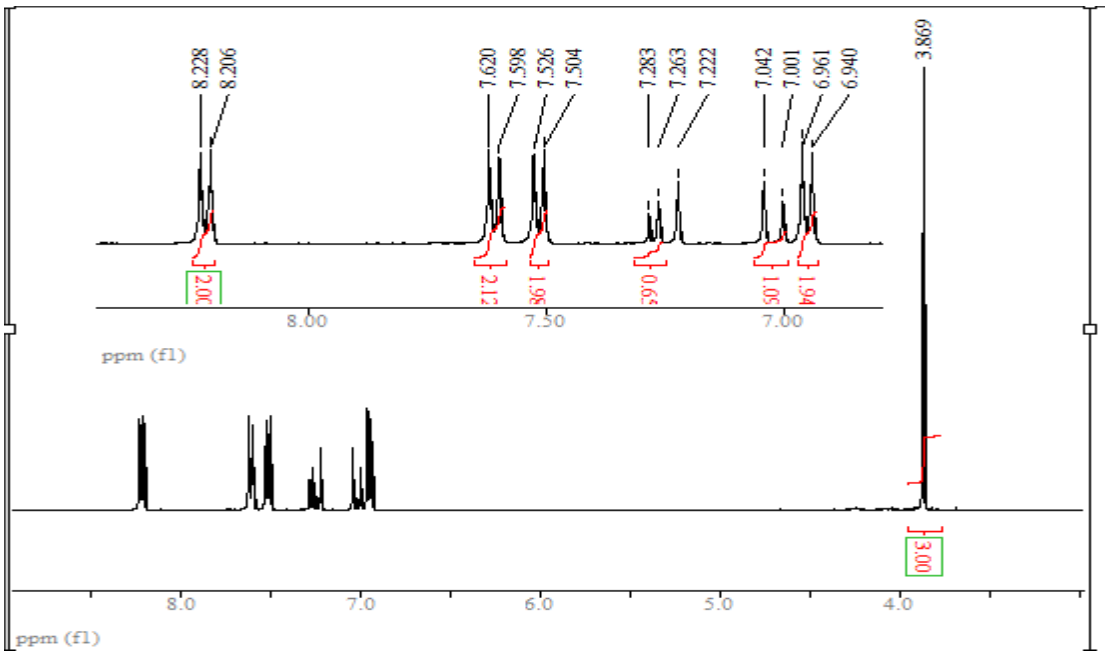
Appendix 20: DEPT-135 NMR spectrum of compound 66e



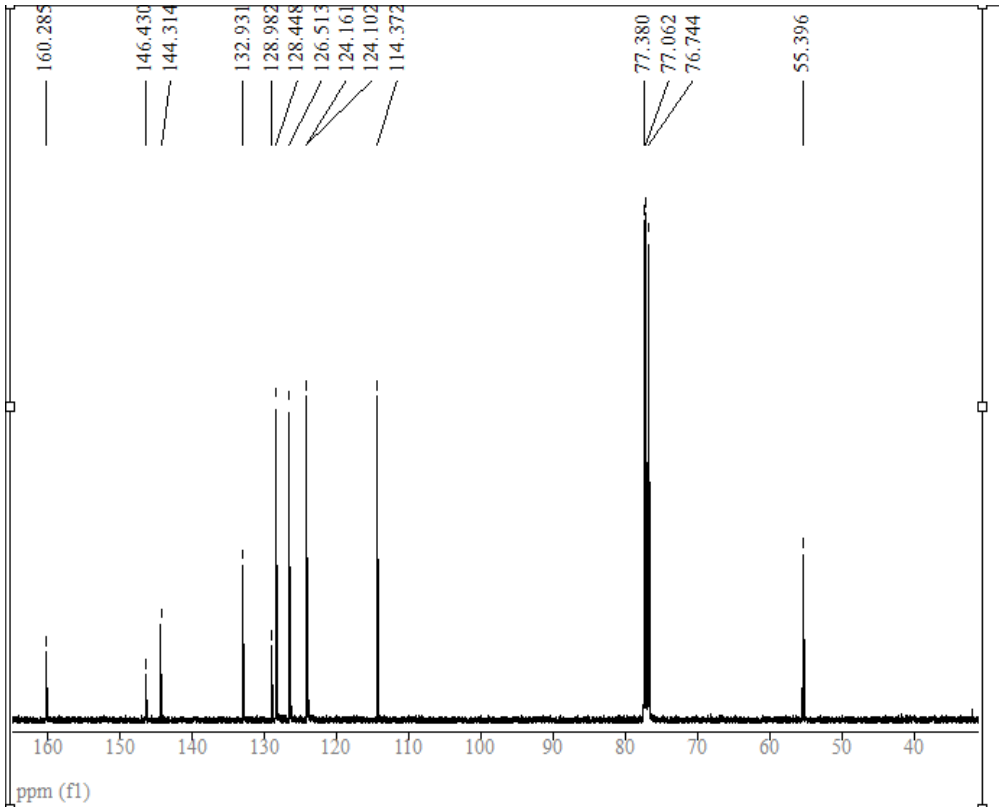
Appendix 21: The FTIR spectrum of compound 66f



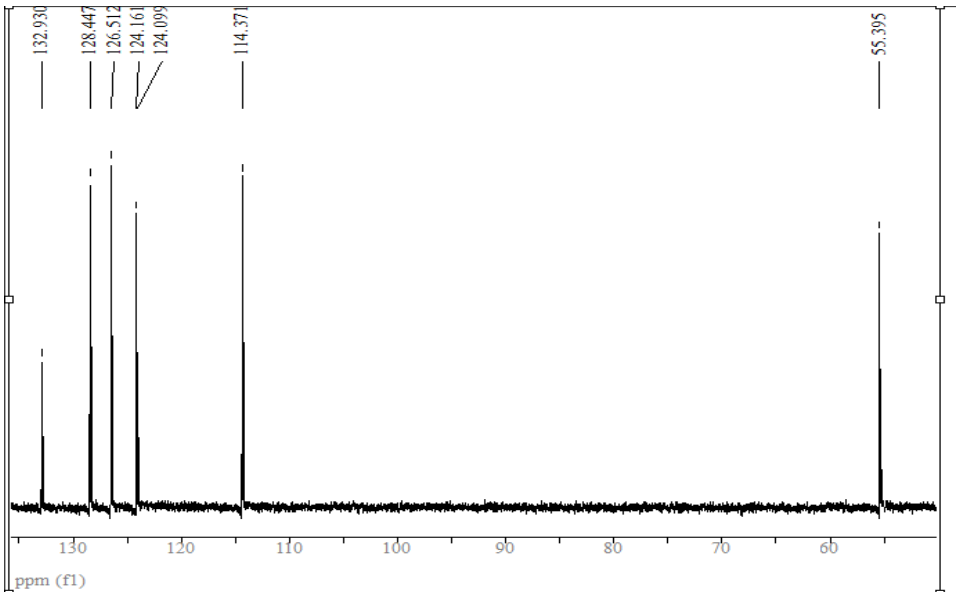
Appendix 22: 1H-NMR spectrum of compound 66f



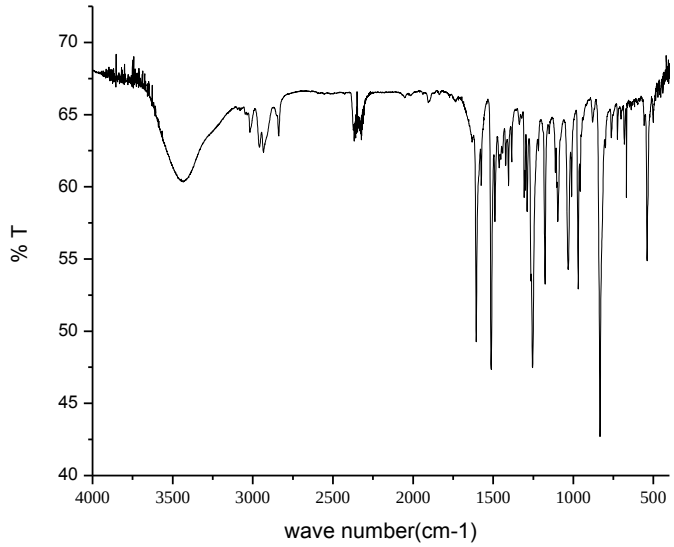
Appendix 23: ^{13}C-NMR spectrum of compound 66f



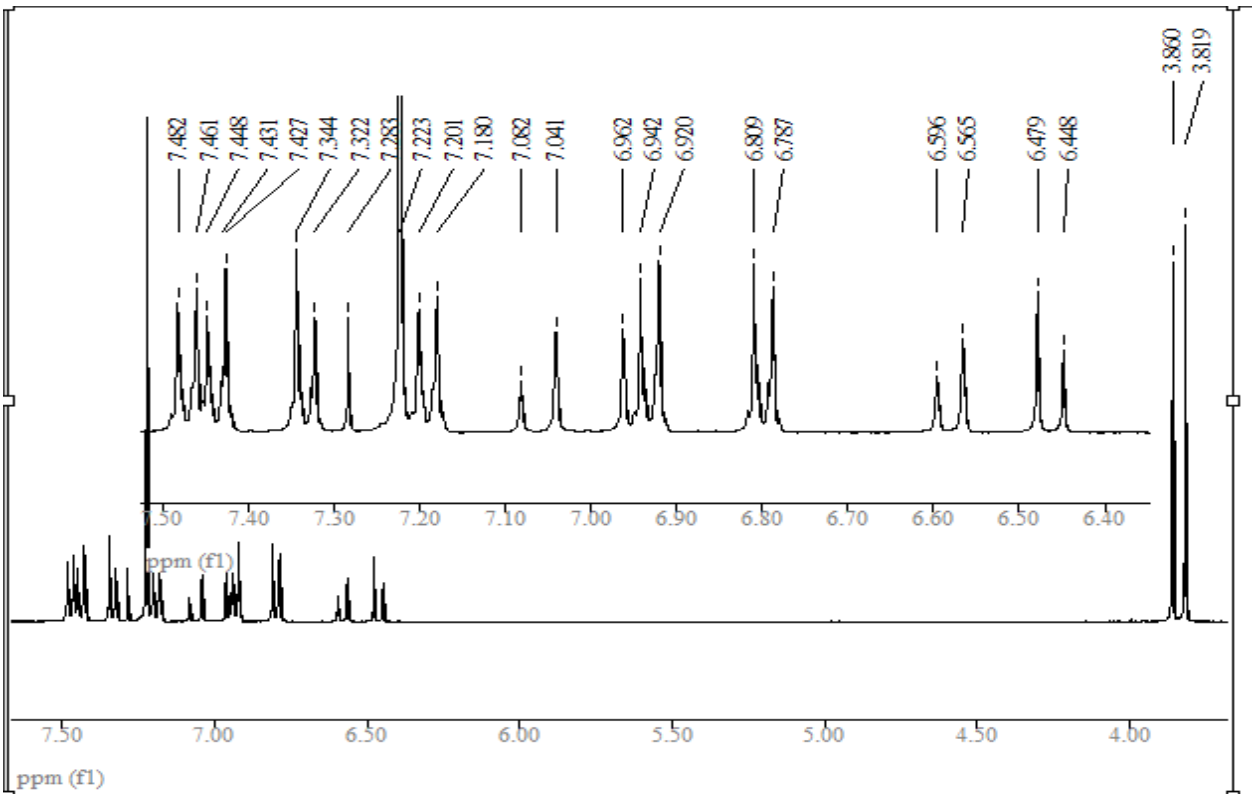
Appendix 24: DEPT-135 spectrum of compound 66f



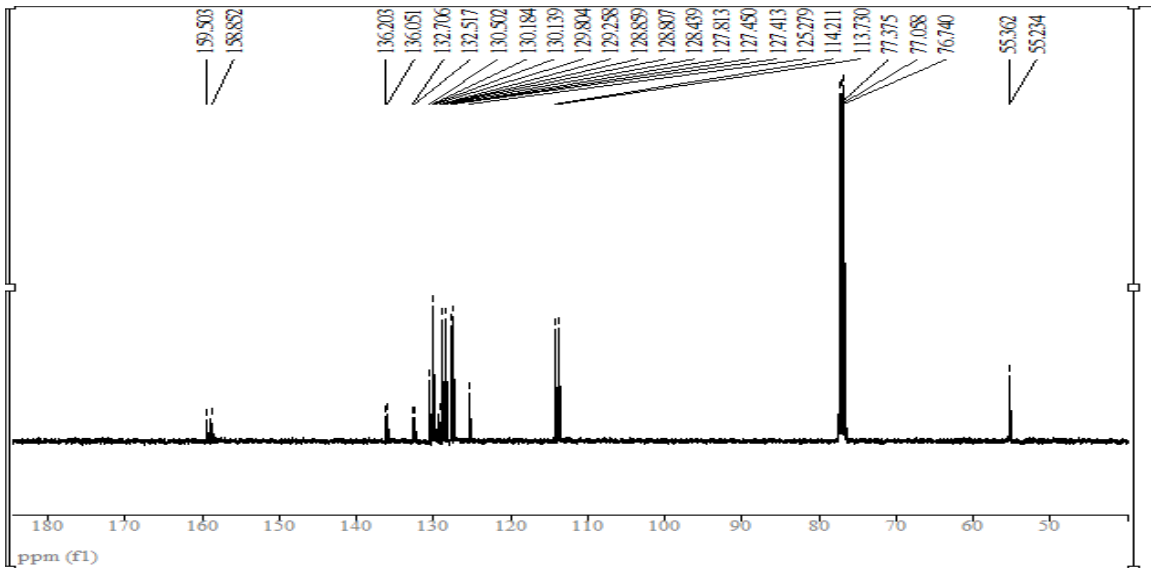
Appendix 25: The FTIR spectrum of compound 66g



Appendix 26: ^{1}H-NMR spectrum of compound 66g



Appendix 27: ^{13}C-NMR spectrum of compound 66g



Appendix 28: DEPT-135 spectrum of compound 66g