UNIVERSITY OF NAIROBI
COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES
DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL EVALUATION OF *TEPHROSIA HILDEBRANDTII* FOR ANTIMICROBIAL PRINCIPLES

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY

2018

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination. Where other people's work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

Signature: ..... Date: 17/10/2018

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DEDICATION

To my wonderful and beautiful baby girl Nahsael NGOY LISENGE, this is the fruit of both our sacrifices.

To my father IKALA Medard and my late mother KABANGA MUIBU Lea, my brothers and sisters DJEMA IKALA Murielle, MUIBU Guelord, MESU IKALA Romelie, TEWO IKALA Melissa and IKALA Prunel; family MUNYWOKI, my family in-law NEMO, who showed me total support and reassurance, may Yahweh redeem them.

To my beloved husband B.P. NGOY, who never stopped to give me inspiration for this scientific life, may you stay blessed.

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Finally special thanks to my husband and my family who lent a hand in their own ways to facilitate me to accomplish this work.

ABSTRACT

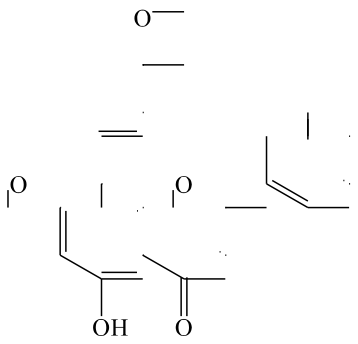
Pathogenic diseases caused by microbes are a main public health problematic around the world causing death. Medicinal plants contain bioactive compounds (metabolites) that serve an important therapeutic role in curing and healing human maladies caused by these microbes.

Plant metabolites such as flavonoids have been reported to display antimicrobial properties against some strains of microbes. The genus *Tephrosia* (Fabaceae) is known to be rich in flavonoids. In this study, flavonoids and other secondary metabolites from the aerial parts of *Tephrosia hildebrandtii* were isolated, characterized and assayed for their antimicrobial activities.

The investigation of phytochemicals the aerial parts of this plant led to the isolation of four flavone derivatives, a sterol, a triterpene and a carotenoid. One of the isolated compounds is new and the structures were established using a combination of techniques including NMR, UV and MS.

Both the crude extract ($MeOH/CH_2Cl_2$ 1:1 v/v) and the isolated compounds were tested for antimicrobial activity against *Escherichia coli*, *Bacillus cereus* and *Candida albicans*. The crude extract, compounds **1** and **2** showed significant activity against *Escherichia coli*. The crude extract and compound **1** also exhibited good activity against *Candida albicans*, while compounds **4**, **5** and **6** showed moderate activity. Compound **1** and a mixture of compounds **2** and **3** showed moderately good activities against *Bacillus cereus*, while the crude extract and compound **2** were moderately active.

Compound **4** was treated with hydrazine hydrate and guanidine hydrochloride in presence of alkali to yield a new pyrazoline derivative **8** and a new guanidine derivative **9**, respectively. Both derivatives showed activities against the tested bacterial and fungal strains. Compound **8** showed not only the highest antifungal activity against *C. albicans* compared to compound **9**, it also was the most active on the gram negative and gram positive bacteria tested.



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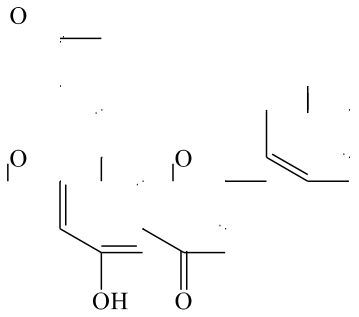

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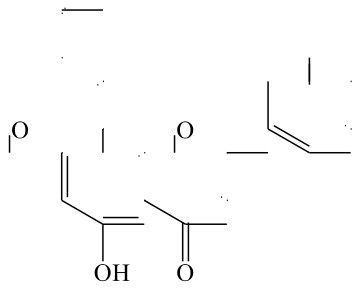

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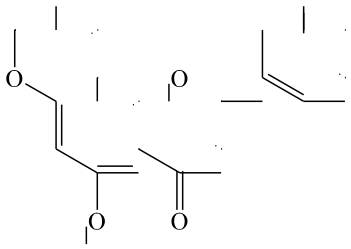

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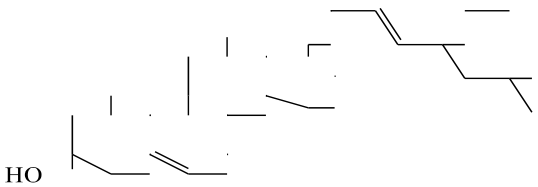

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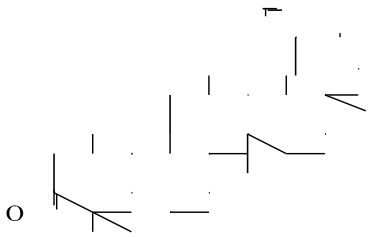

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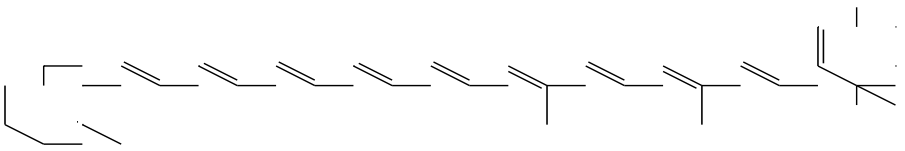

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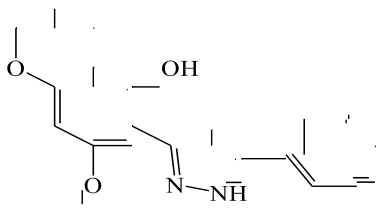

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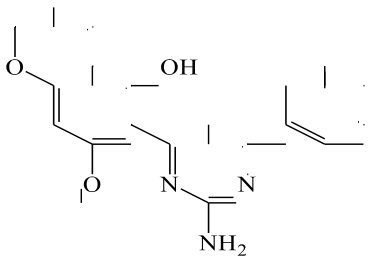

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LIST OF ABBREVIATIONS AND SYMBOLS

B. cereus: *Bacillus cereus*

°C: Degree Celsius

CC: Column Chromatography

COSY: Correlation Spectroscopy

d: doublet

dd: double doublet

δ: Chemical shift

DEPT: Distortionless Enhancement by Polarization Transfer

EtOAc: Ethyl Acetate

HMBC: Heteronuclear Multiple Bond Correlation

HPLC: High Pressure Liquid Chromatography

HRESI-MS: High Resolution Electron-Spray Ionization -Mass Spectrometry

HSQC: Heteronuclear Single Quantum Coherence

Hz: Hertz

J: coupling constant

λ_{max}: Wavelength at maximum absorption

LC-MS: Liquid Chromatography-Mass Spectroscopy

m: Multiplet

m/z: Mass to charge ratio

$[M]^+$: Molecular ion peak

$[M+1]^+$: *Pseudo*-Molecular ion peak

MeOH: Methanol

MeO: Methoxyl group

MHz: Mega Hertz

MIC: Minimum Inhibitory Concentration

MS: Mass Spectrometry

NMR: Nuclear Magnetic Resonance

s: Singlet

TLC: Thin Layer Chromatography

t: Triplet

UV-Vis : Ultraviolet-visible

v/v: Volume to volume ratio

WHO: World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The vintage society used plant materials for curative purposes for centuries (Farnsworth and Soejarto, 1991;Olowokudejo *et al.*, 2008; Schmelzer *et al.*, 2008). In essence, the use of medicinal plants for healing some diseases is as old as mankind. Several studies have estimated that more than 25% of medications prescribed today are based on plant-derived constituents yet there is still a major lack of research information about it (WHO, 1999; Miller, 2001).

Some plants play a valuable role as in primary healthcare in the treatment and prevention of various diseases. These medicinal plants have a potential influence on economic development, affordable healthcare and conservation of vital biodiversity, a vital cause of all varieties of medicines as well as traditional medicines, modern medicines, nutraceuticals and also provides new chemical substances (Lawal *et al.*, 2010; Ahmad and Wajid, 2013; Mezui *et al.*, 2015).

The useful medicinal effects of plant materials naturally result from the groupings of secondary metabolites present inside the plant. These secondary metabolites include alkaloids, tannins and other phenols, flavonoids, steroids, resins, fatty acids and gums which are biologically active (Kumar and Santhi, 2012; Kumar *et al.*, 2016). These natural compounds are of utmost significance presently because of their abundant functionalities (Bariş *et al.*, 2006; Jegede and Kunle, 2011). It has been demonstrated that among the 252 drugs considered as indispensable by the World Health Organization, 11% have been derived from plants (Rates, 2001; Burris, 2011). Therefore, there is need to investigate the potential therapeutic properties of the phytochemicals contained in medicinal plants (Kadiri, 2009;Merculieff *et al.*, 2014; Kowa *et al.*, 2015).

Medicinal plants are used to treat many illnesses such as fever, ulcers, infertility, indigestion and microbial infections. Microbial diseases are a universal health problem that cause death of over 17 million people in the world (Omobuwajo *et al.*, 2008; Ngbolua *et al.*, 2014). They include ebola hemorrhagic fever, polio, cholera, tuberculosis, meningitis, malaria, athlete's foot and parasitic worms (National Institutes of Health, 2007). Microbes such as bacteria, viruses, fungi and protozoa are the causative agents for these diseases (National Institutes of Health, 2007). These diseases can be spread through food and water, by animals and insects, by person-to-person contact, by sexual contact, blood and body fluids (WHO, 2001).

In spite of the rise of diagnostics that have improved the understanding of disease origin, pathogenesis, and molecular epidemiology, which offer suitable recognition, prevention, and control measures as well as balanced design of vaccine, there is still the development of drug resistance by the infective agents such as *Escherichia coli*, *Neisseria gonorrhoeae*, and *Pneumococcus* (Nii-Trebi, 2017). Since drug resistance has limited the use of many well-known antimicrobials drugs, there is need for continual development of new antiviral and antimicrobial products (Stewart and William, 2001; Narayan *et al.*, 2010; Nii-Trebi, 2017). Moreover, there are reports about widespread drug counterfeits which cause therapeutic failure and compound the drug resistance problem (Chika *et al.*, 2011).

In most developing countries, conventional drugs are either not available or unaffordable to the rural communities (Cameron *et al.*, 2009). Consequently 60-90% of the population use medicinal plants to cure many ailments (Koochak *et al.*, 2010; Wendakoon *et al.*, 2012). In an effort to address the problem of microbial resistance to antimicrobials, many studies have been done on crude plant products (Nii-Trebi, 2017). The potential of indigenous plants to heal diverse diseases is widely recognized by traditional herbal medicine practitioners (Anitha *et al.*, 2012; Anyanwu *et al.*, 2015). Indeed, many bioactive compounds have been identified from these plants (Jayaprakasha *et al.*, 2006; Murugan *et al.*, 2012), of which the genus *Tephrosia* has shown significant activities.

Tephrosia species have been reported of having antibacterial and anti-fungal activity (Touqeer *et al.*, 2013). The roots and leaves of *Tephrosia vogelii* show antimicrobial activity with MIC between 0.25 and 6.4 μg/mL against *S. aureus, E. coli and F. phaseolida* (Wanga *et al.*, 2006) and the ethanolic and aqueous extracts of its seeds have antibacterial efficacy at high doses when tested on *E. coli, S. aureus and S. paratyphi B* (Touqeer *et al.*, 2013). Ganapaty *et al*. (2008) demonstrated that the root extract from *Tephrosia villosa* had antibacterial and antifungal potency. An alternative study on *Tephrosia villosa* showed an activity of the fruit, leaf and root extract against *C.neoformans*, *E. coli* and *B. anthracis* respectively and the ethanolic extract of the twig was extremely active against *C.neoformans* and *S. typhi*.(Nondo *et al.*, 2011). A study on *Tephrosia purpurea* proved that the roots had antimicrobial activity against *P. aeruginosa*, antifungal against *A. niger* and *C. albicans* but inactive against *S. aureus* and *E.coli* (Touqeer *et al.*, 2013). *Tephrosia tinctoria* exhibited activity against *C. albicans, B. subtilis, A. niger* and *S. marceseans*, (Lakshmi *et al.*, 2010; Touqeer *et al.*, 2013). *Tephrosia*

deflexa and its isolated compounds were studied for antibacterial activity (Kare *et al.*, 2006). The antibacterial activity of *Tephrosia linearis* has also been reported (Touqeer *et al.*, 2013).

The antifungal activity of *Tephrosia hildebrandtii* against *C. cucumerinum* has also been studied and this activity was associated to the isolated compound from its roots (Lwande *et al.*, 1986). However, the phytochemicals responsible for the antimicrobial activity of the plant were not established, therefore, in this study, the aerial part of *Tephrosia hildebrandtii* was investigated for antimicrobial principles. Some of the active compounds were structurally modified with the aim of enhancing their antimicrobial potency.

1.2 Statement of the Problem

Microbial diseases are still the major killer diseases globally and yet the causative agents have developed resistance to the available drugs used for their management (Nascimento *et al.*, 2000; Burris, 2011). In 2013, 480 000 cases of multi-drug resistant tuberculosis were recorded (WHO, 2015). In 2017, WHO raised concern about a new critical priority group comprising of multi-resistant *Pseudomonas*, *Enterobacteriaceae* and *Acinetobacter* which are responsible for severe infections (WHO, 2017). Also bacterial resistance has continued to increase at a high rate especially for microbes that are origin of communal infections such as pneumonia and urinary tract infections (WHO, 2014).

In order to contain this problem that threatens human health, there is an urgent necessity to identify other alternative antimicrobial agents that can be developed into new and more effective drugs.

1.3 Objectives of the Study

1.3.1 General Objective

The main objective of this study was to identify the antimicrobial principles of the aerial part of *Tephrosia hildebrandtii*.

1.3.2 Specific Objectives

The specific objectives of this study were to:

i. Establish the antimicrobial efficacy of the crude extract of *Tephrosia hildebrandtii.*

ii. Isolate and characterize the phytochemical principles of *Tephrosia hildebrandtii* and assess their antimicrobial potency.

iii. Enhance the efficacy of the most potent isolated compounds through structural modification.

1.4 Justification and Significance

The rapidly increasing incidences of microbial resistance to antimicrobial agents has become a serious problem worldwide (Narayan *et al.*, 2010). Thus there is need to develop alternative antimicrobial agents especially from plant extracts which have not only different structures but also a different mechanism of action since some phytochemicals are known to be potent against some of microbial pathogens (Stefanović *et al.*, 2012; Wendakoon *et al.*, 2012; Zige *et al.*, 2013).

Preliminary investigations show that the genus *Tephrosia* is credited for a long list of ethnomedicinal properties due to the presence of flavonoids, flavanones, isoflavanoids, chalcones, rotenoids and essential oils giving the species diverse biological activities such as antifungal, antibacterial, antiplasmodial, antiviral, anthelminthic, anticancer, antioxidant, larvicidal, anti-inflammatory, antinociceptive, cytotoxic and antiproliferative. Thus the use of *Teprosia* species for the control of microbial infections may make available a convenient deterrent against proliferations of microbial diseases (Kare *et al.*, 2006; Wanyama, 2010; Manikandan *et al.*, 2014).

The antimicrobial and phytochemical investigation of the aerial part of *T. hildebrandtii* is scanty, therefore this study addressed this deficiency. The aerial part of the plant was investigated to establish whether *T. hildebrandtii* crude extract and isolated compounds had the potential to inhibit the growth of the tested clinical pathogenic fungi/bacteria (*Candida albicans*, *Escherichia coli* and *Bacillus cereus*).

CHAPTER TWO: LITERATURE REVIEW

2.1 Microbial Diseases

Microbes are microscopic organisms some of which cause infectious diseases in humans, plants and animals (NIAID, 2009). Infectious diseases are currently the principal cause to human morbidity and mortality (Kaur *et al.*, 2011). They account for 17 million deaths and disability worldwide and in some regions they continue to be the greatest source of ill health (Saker *et al.*, 2004; Dye, 2014). Infective diseases affect persons of all ages. They inflict a specific burden on the young children below five, since they are vulnerable than adults by the lack of defensive mechanisms to lessen the impact of these infections. Subsequently, in areas where the majority of the population are youths like in Africa, infective diseases have a high toll on the population (Saker *et al.*, 2004).

The four major groups of microbes that cause diseases are: bacteria, viruses, fungi and protozoa. The most common infectious diseases caused by microbes are summarized in Table 2.1.

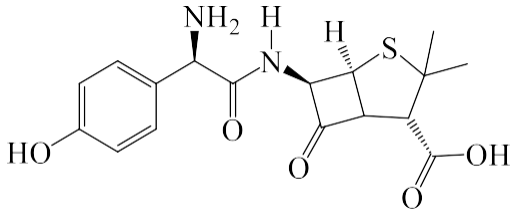
Table 2.1: Communicable diseases and their causative infectious agents (NIAID, 2009)

Disease/Infection	**Fungus**	**Virus**	**Protozoa**	**Bacteria**
Diarrhea		+	+	+
Lower respiratory tract infections	+	+		+
Malaria			+	
Vaginal infections	+		+	+
Urinary tract infections	+			+
Meningococcal meningitis				+
Skin diseases	+		+	+
Typhoid fever				+
Yellow fever		+		
Hepatitis A & E		+		

These infectious diseases have become one of the greatest burden to the healthcare systems in the African region. There are a number of emerging and reemerging infectious diseases such as monkey pox, Rift valley fever virus, malaria, tuberculosis, yellow fever and trypanosomiasis in Africa (Institute of Medicine, 2001).The treatment of these infectious diseases is commonly through the use of antimicrobial drugs (NIAID, 2009; Gupta *et al.*, 2010; Ahmad and Wajid, 2013).

2.2 Antimicrobial Drugs

Antimicrobial drugs used to cure diseases caused by microbes are either synthetic drugs, antibiotics (natural drugs synthesized by bacteria or fungi) or semi synthetic (Kunin, 1993; Chopra and Roberts, 2001; Gupta *et al.*, 2010; Stefanović *et al.*, 2012). There are several categories of antimicrobial drugs; these include antibiotics such as amoxicillin (**10**); antivirals, e.g. acyclovir (**11**); antifungals, e.g. griseofulvin (**12**) and antiprotozoals, e.g. artemisinin (**13**) (Huber and Gottlieb, 1968; Gnann *et al.*, 1983; NIAID, 2009; eMedExpert.com, 2016)



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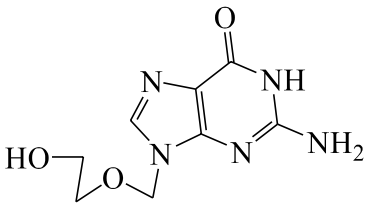

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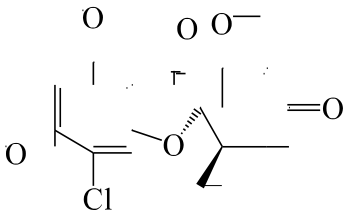

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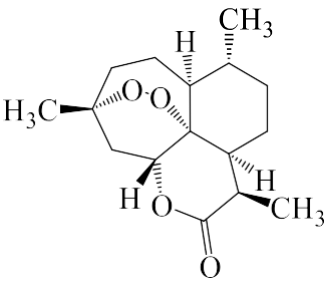

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Figure 2.1: Antimicrobial drugs

Among the several groups of antimicrobial drugs, antibiotics are commonly used. These drugs can either be broad spectrum, which destroy a extensive range of microbes e.g. penicillin, or narrow spectrum that exterminate a specific type of microbes e.g. Isoniazid (Kunin, 1993). Antibiotics have two mechanisms of action on the pathogens: bacteriostatic (those acting by arresting the bacteria multiplication e.g. tetracyclines) and bactericidal (those which act by killing the bacteria e.g. penicillins) (Chopra and Roberts, 2001; Kaufman, 2011).

Although these antimicrobial drugs have been effective, their efficacy has been compromised by the arrival of drug-resistant microorganisms (Ncube *et al.*, 2008).

2.3 Antimicrobial Drug Resistance

This is the resistance of a disease-causing microbe to the antimicrobial agents that used to cure the infectious disease it causes (Leung *et al.*, 2011; Dubey *et al.*, 2012). Currently most of the antimicrobials that were first used in the 1940s and 1950s are no longer used clinically (Fluit *et al.*, 2000; Silvério and Lopes, 2012). Above and beyond the misuse of antimicrobials and the problem of counterfeit drugs, a number of drug-resistant microbes have appeared (Brent, 2005; Khan *et al.*, 2009; Bapela *et al.*, 2014). For example, the use of artemisinin monotherapy as an antimalarial drug has led to the emergence of artemisinin resistance. Instances of resistance of *Escherichia coli* to the commercial drugs is also on the rise, while methicillin-resistant *Staphylococcus aureus* is becoming gradually recurrent (WHO, 2014). Generally, about 90% of the bacteria strains have become more and more resistant to drugs of first choice that are currently in the market (Bodenstein and Du Toit, 2012; Glienke *et al.*, 2012; Panda *et al.*, 2016). This therefore calls for the identification of new drugs against these drug-resistant microbes.

Plants have been reliable sources of antimicrobial agents, such as *Artemisia annua* for antimalarial activity and *Acacia nilotica* for antimicrobial principles (Krishna *et al.*, 2004; Khan *et al.*, 2009; Anyanwu *et al.*, 2013), therefore there is need to explore more plant derived antimicrobial agents and in this study we have explored *T. hildebrandtii*, a plant from the genus *Tephrosia* for antimicrobial principles.

2.4 Natural Products in Antimicrobial Drug Discovery

More than 80% of the entire world depends on phytotherapy (herbal medicine) by utilizing plants and herbs that possess beneficial pharmacological properties for human welfare. The activities of these therapeutic plants are valued by their chemical components (Daniel, 2006).

Plants produce two specifically different types of chemical compounds; the first one, called primary metabolites, comprises of compounds like sugar and proteins that are commonly found in most organisms and are indispensable for functional metabolism. The second type, secondary metabolites, are unique to a particular species or related group of organisms. They serve in a wide number of purposes such as nourishment, conservation, reproduction, healing, defense and offense.

Secondary metabolites are phytochemicals that plants naturally synthesize and stock, these comprise of flavonoids, alkaloids, sterols, terpenes, volatile oils, saponins, tannins, etc. Most of these secondary metabolites are derived through polyketide/fatty acid, isoprenoid and phenylpropanoid pathways (Motaleb, 2011). Medicinal plants are a potential source of new drugs and templates for development of synthetic analogues, however many plant species have not yet been phytochemically screened for their biological potencies.

Previous studies have also shown that some secondary metabolites have varied levels of antimicrobial activities. Thus, there is need to investigate medicinal plants that have been and still being used in the healing practices and treatment of illness since the beginning of human civilization to identify the bioactive compounds in those medicinal plants (Abdel-Mageed *et al.*, 2014; Bamidele *et al.*, 2014).

2.5 The genus *Tephrosia*

The genus *Tephrosia* belongs to the family Leguminosae (*Fabaceae*) in the *Papilionoideae* (*Faboideae*) sub-family (Wanyama, 2010; Chen *et al.*, 2014). It is big a tropical and subtropical genus of woody shrubs that contains up to 400 species distributed as follows; 30 occurring in South America, 35 in India, 70 in South Africa, and among the 50 species found in equatorial Africa, 30 are found in Kenya these include: *T. elata, T. hildebrandtii, T. pumila, T. aequilata, T. villosa, T. purpurea,* among others (Tarus *et al.*, 2002).

Plants of this genus are known as sources of unique and biologically active natural products, which possess various biological activities (Peter and Sami, 1980; Peng *et al.*, 2014). The genus *Tephrosia* is known to harbor plenty of flavonoid derivatives, isoflavones particularly pterocarpans and isoflavans, isoflavonoids and rotenoids (Arriaga *et al.*, 2008), which possess a expansive spectrum of biological potencies including antibacterial, insecticidal, antifungal, antifeedant, antiviral and oestrogenic properties (Jianping Wu; Ammar and El-Diwany, 1988; Abreu and Luis, 1996; Nenaah, 2014).

2.6 Ethnomedicinal Uses of *Tephrosia*

The genus *Tephrosia* has diverse uses in traditional medicine. Some members of the genus are commonly used as antimalarials, bronchial asthma, inflammation, ulcers, eczema, stomach pains, antidiabetes, fish poisons, rat poisons, arrow poisons, antibacterial and antifungal (Bentley *et al.*, 1987; Emmanuel Sami Ragbib, 2011). The ethnomedicinal uses of this genus are briefed in Table 2.2.

Table 2.2: Ethnomedical uses of *Tephrosia* species

Species	Plant part used	Disease condition	Reference
T. nubica	Roots, leaves, bark stem bark	Antifungal	Ammar and El-Diwany, 1988
T. cinerea	leaves	larvicidal	Arriaga *et al.*, 2008
T. purpurea	Roots, leaves	Dental pain, fish poison, anthelmintic, antipyretic, wounds, skin diseases, cough, fever ulcers, tumors, diarrhea, inflammation of liver, asthma, urinary disorders	Chang *et al.*, 1997; Omobuwajo *et al.*, 2008; B.N.L.D. Rangama *et al.*, 2009; Khalafalah *et al.*, 2010; Dalwadi *et al.*, 2014; Anyanwu *et al.*,

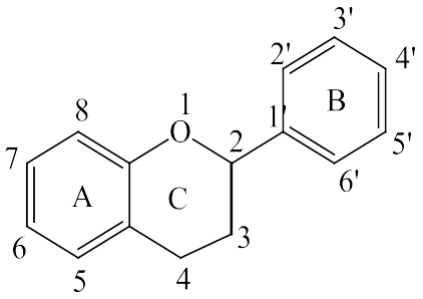
			2015; Bhardwaj and Shrivastava, 2017
T.elata	bark, leaves	Insecticidal, fish poison, fever, rat poison, stomach pains, general weakness	Bentley *et al.*, 1987
T. villosa	leaves	Fish poison, dropsy and diabetes, insecticidal	Kamal and Jain, 1980; Ganapaty *et al.*, 2008; Madhusudhana *et al.*, 2010
T. appolinea		Fish poison, antibacterial, insecticidal	Hisham *et al.*, 2006
T. tinctoria		Fish poison, anticancer, insecticidal, repellent	Khalivulla *et al.*, 2008
T. aequilata		Liver pain, venereal diseases Medicine for babies	Tarus *et al.*, 2002
T. linearis		For strength to women after childbirth	Touqeer *et al.*, 2013
T. interrupta		Cold in chest, venereal diseases	Touqeer *et al.*, 2013
T. pumila			Chen *et al.*, 2014

2.7 Phytochemistry of the genus *Tephrosia*

Previous studies on the phytochemistry of the genus *Tephrosia* has shown the presence of flavones, isoflavones, rotenoids, pterocarpans, isoflavanones, flavanes and chalcones as the most common flavonoids (Lwande *et al.*, 1985; Kare *et al.*, 2006; Chen *et al.*, 2014).

2.7.1 Flavonoids

Flavonoids stand for a class of secondary metabolites with various phenolic structures. They have a fifteen-carbon skeleton comprising of two benzene rings connected *via* a heterocyclic pyrane ring as shown in figure 2.2 below.



14

Figure 2.2: Basic structure of flavonoids

Flavonoids are classified into several groups such as flavones, flavonols, flavanones, flavans, isoflavones, etc which depend on the level of oxidation and pattern of substitution in ring C, whereas individual compounds within the same class deviate in the pattern of substitution of ring A and ring B (Agrawal, 1989; Kumar and Panday, 2013).

Flavonoids are well known to hold multiple biological potencies such as Antioxidant activity; Anti-inflammatory activity; Antiviral activity; Hepatoprotective activity; Anticancer activity and Antibacterial activity.

Flavonoids are synthesized by plants in retort to microbial infection, therefore this proves their *in vitro* effective antimicrobial activity against a wide range of germs. The antibacterial flavonoids can have several cellular targets, and one of their mode of actions is to create complex with proteins by hydrogen bonding, hydrophobic effects or covalent bonds. The antimicrobial mode of action might be linked to the aptitude of flavonoids to disable microbial adhesins, enzymes, cell envelope transport proteins, and so on (Kumar and Panday, 2013).

2.7.1.1 Flavones from genus *Tephrosia*

Flavones, from the word *flavus* meaning yellow, are a class of flavonoids composed of 2-phenylchromen-4-one.They are responsible for the color of many flowers ranging from yellow to white and mainly found in cereals and herbs and are biologically active compounds. To date, they constitute the most abundant class of flavonoids (Shaikh and Nazeruddin, 2014). Flavones from *Tephrosia* are given in Table 2.3 and figure 2.3 below.

Table 2.3: Flavones from genus *Tephrosia*

Name	Source	Reference
Tephrostachin (15**)**	*T. polystachoides*	Chen *et al.*, 2014
Emoroidone (16**)**	*T. emoroides*	Chen *et al.*, 2014
Tephroapollin C (17**)**	*T. apollinea*	Abou-Douh *et al.*, 2005
Tephroapollin D (18**)**	*T. apollinea*	Abou-Douh *et al.*, 2005
5-Methoxy-6,6-dimethylpyrano[2,3:7,6]flavone (19**)**	*T. praecans*	Camele *et al.*, 1980; Chen *et al.*, 2014
Purleptone (20**)**	*T. purpurea*	Atilaw . *et al.*, 2017
Tephroglabin (21**)**	*T. purpurea*	Chen *et al.*, 2014
Hookerianin (22**)**	*T. hookeriaa*	Touqeer *et al.*, 2013; Chen *et al.*, 2014
Apollinine (23**)**	*T. apollinea*	Khalafalah *et al.*, 2010
Tachrosin (24**)**	*T. polystachoides*	Smalberg *et al.*, 1974
Tephroapollin E (25**)**	*T. apollinea*	Abou-Douh *et al.*, 2005
Tephroapollin F (26**)**	*T. apollinea*	Abou-Douh *et al.*, 2005
Tephroapollin G (27**)**	*T. apollinea*	Abou-Douh *et al.*, 2005
Tephropurpulin A (28**)**	*T. apollinea*, *T. purpurea*	Khalafalah *et al.*, 2010; Chen *et al.*, 2014
Semiglabrinol (29**)**	*T. semiglabra*	Chen *et al.*, 2014

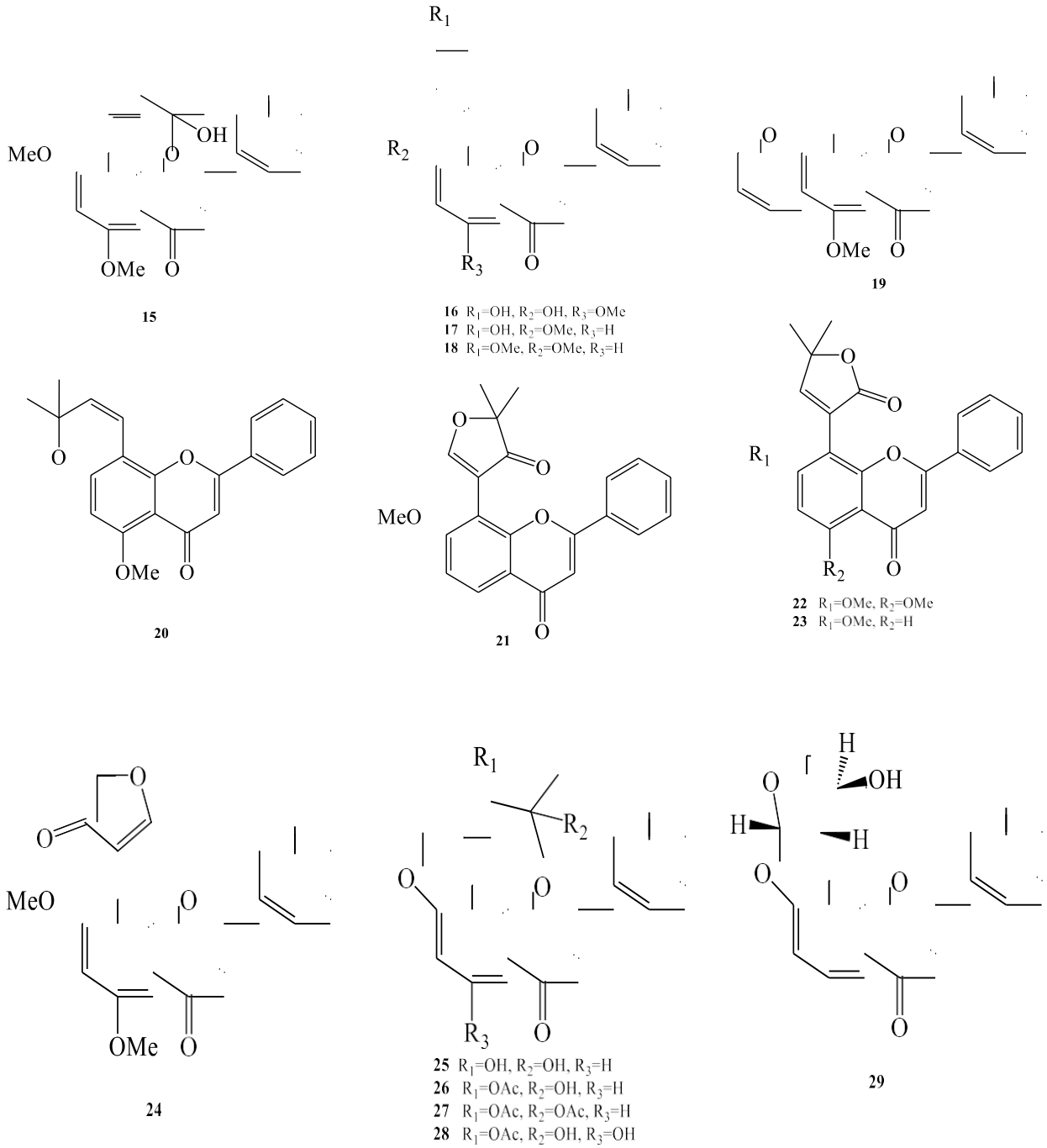


Figure 2.3: Structures of flavones from genus *Tephrosia*

2.7.1.2 Isoflavones from genus *Tephrosia*

Isoflavones are diphenolic compounds of plant origin, isoflavonoids are basically dissimilar from other flavonoid groups because their C_{15} skeleton derived from 3-phenylchromen-4-one structure. The isoflavonoid structure is proven by an aryl migrating enzyme named as isoflavone synthase. Isoflavones from *Tephrosia* are illustrated in Table 2.4 and figure 2.4 below.

Table 2.4: Isoflavones from genus *Tephrosia*

Name	Source	Reference
3,4:8,9-dimethylenedioxypterocarpan (30**)**	*T. aequilata*	Chen *et al.*, 2014
Hildecarpin (31**)**	*T. hildebrandtii*	Lwande *et al.*, 1987
7,4'-Dihydroxy-3',5'-dimethylisoflavone (32**)**	*T. purpurea*	Chang *et al.*, 1997
Elongatin (33**)**	*T. elongata*	Chen *et al.*, 2014
Pumilaisoflavone D (34**)**	*T. pumila*	Yenesew *et al.*, 1989
Barbigerone (35**)**	*T. barbigera*	Vilain, 1980
4'-Demethyltoxicarol isoflavone (36**)**	*T. polyphylla*	Dagne *et al.*, 1992
Maxima isoflavone J (37**)**	*T. maxima*	Yejella and Velagapudi, 2004
Villosol (38**)**	*T. villosa*	Madhusudhana *et al.*, 2010
Villosin (39**)**	*T. villosa*	Krupadanam *et al.*, 1977
Tephrosol (40**)**	*T. villosa*	Rao and Srimannarayana, 1980
2-Methoxy-3,9-dihydroxy coumestone (41**)**	*T. hamiltonii*	Rajani and Sarma, 1988

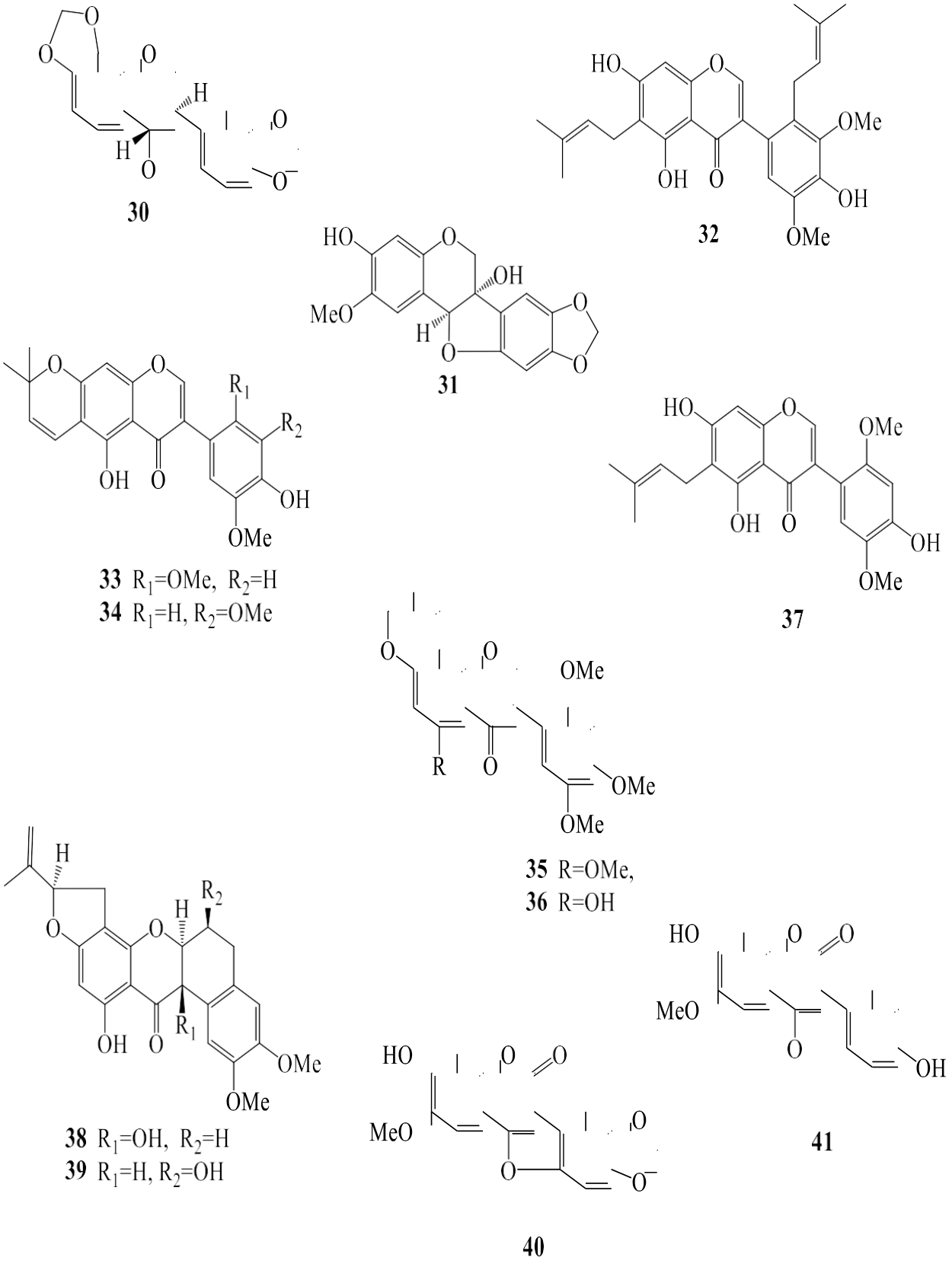


Figure 2.4: Isoflavones from genus *Tephrosia*

2.7.1.3 **Flavanones from genus *Tephrosia***

Flavonoids class possessing 2-phenylchromanone as basic skeleton are called flavanones. Actually, a large number of flavanones have been elucidated whereby half of them are prenylated at C-8. Flavans, Flavenes and Flavanones from *Tephrosia* are proven in Table 2.5 and in figure 2.5 below.

Table 2.5: Flavans, Flavenes and Flavanones from genus *Tephrosia*

Compound name	Source	Reference
Methylhilgardtol B (42**)**	*T. hildebrandtii*	Chen *et al.*, 2014
Hildgardtol B (43**)**	*T. hildebrandtii*	Chen *et al.*, 2014
Hildgardtene (44**)**	*T. hildebrandtii*	Monache *et al.*, 1986
Methylhildgardtol A (45**)**	*T. hildebrandtii*	Lwande *et al.*, 1985
Hildgardtol A (46**)**	*T. hildebrandtii*	Lwande *et al.*, 1986
Tephrocandidin A (47**)**	*T. candida*	Mohamed-Elamir *et al.*, 2011
Falciformin (48**)**	*T. falciformis*	Hamid *et al.*, 1986
Quercetol C (49**)**	*T. quercetorum*	Gomes-Garibay *et al.*, 1988
Purpurin (50**)**	*T. purpurea*	Gupta *et al.*, 1980
Nitenin (51**)**	*T. nitens*	Gomes *et al.*, 1984

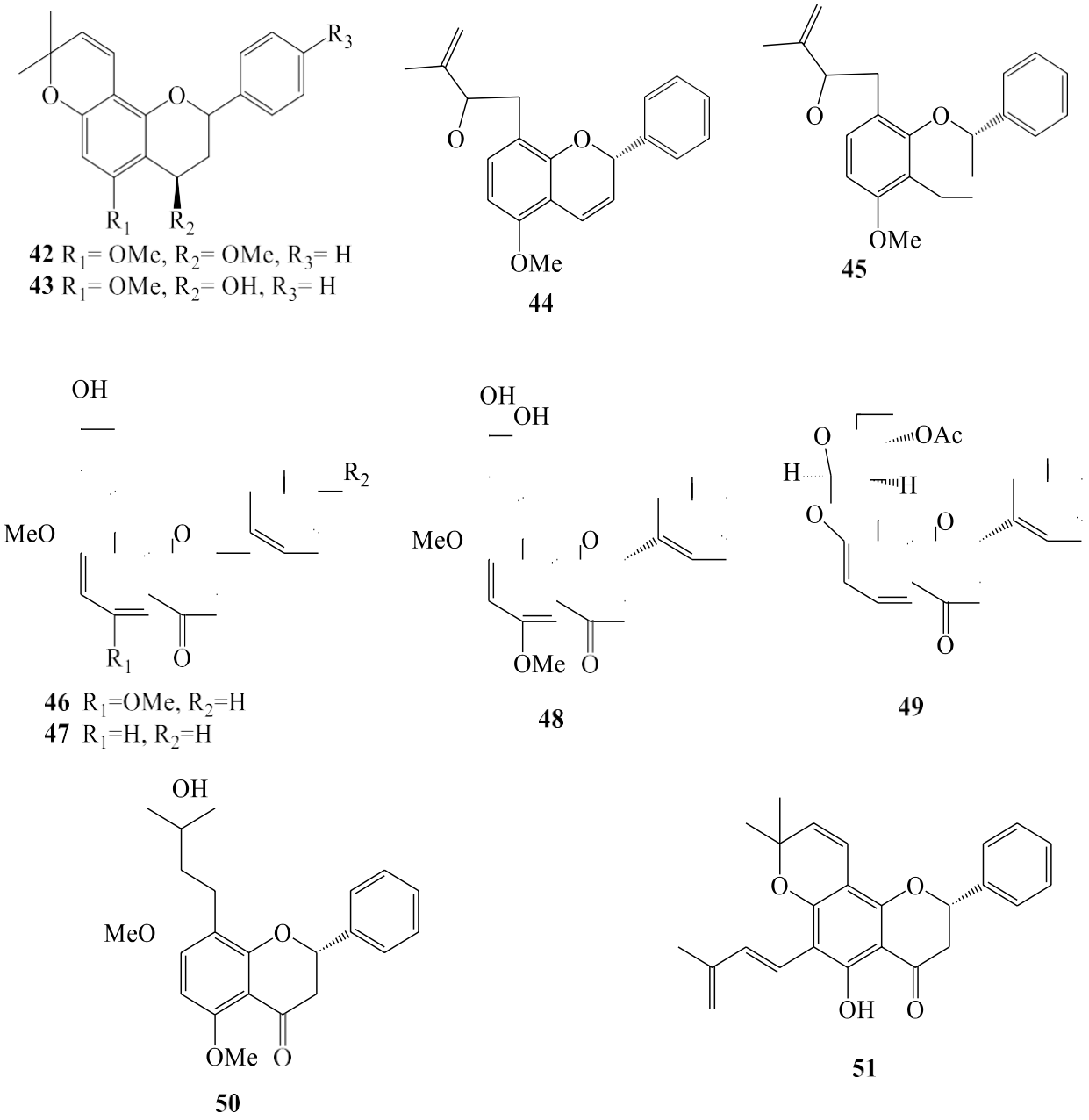


Figure 2.5: Flavans, Flavenes and Flavanones from genus *Tephrosia*

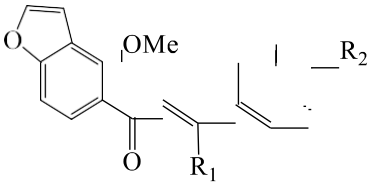
2.7.1.4 Chalcones, Flavonols and Triterpenes and other compounds from genus *Tephrosia*

Thus far, others compounds such flavonols, steroids, chalcones, flavonols have been also reported from genus *Tephrosia*. Examples include the structures below. Chalcones, Flavonols, Flavanonols and Triterpenes and other compounds from genus *Tephrosia* are shown in Table 2.6 and in figure 2.6 below.

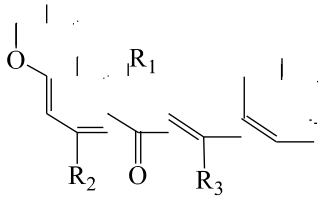
Table 2.6: Chalcones, Flavonols, Flavanonols and Triterpenes and other compounds from genus *Tephrosia*

Name	Source	Reference
***O*-Methylpongamol (**52**)**	*T. purpurea*	(Pelter *et al.*, 1981)
Purpuritenin (53**)**	*T. purpurea*	(Sinah *et al.*, 1982)
2',6'-Dimethoxy-4',5'-(2''2''dimethyl)-pyranochalcone (54**)**	*T. pulcherrima*	(Chen *et al.*, 2014)
Praecansone A (55**)**	*T. praecans*	(Camele *et al.*, 1980)
Praecansone B (56**)**	*T. praecans*	(Camele *et al.*, 1980)
Obovatachalcone (57**)**	*T. obovata*	(Chen *et al.*, 2014)
Oaxacacin (58**)**	*T. woodii*	(Chen *et al.*, 2014)
6'-Dimethoxypraecansone B (59**)**	*T. purpurea*	(Rao and Raju, 1984)
Tephrone (60**)**	*T. candida*	(Touqeer *et al.*, 2013)
Spinochalcone A (61**)**	*T. spinosa*	(Rao and Prasad, 1992)
3',5'-Diisopentenyl-2',4'-dihydroxychalcone (62**)**	*T. spinosa*	(Sharma and Rao, 1992)
Lupinifolinol (63**)**	*T. lupinifolia*	(Smalberg *et al.*, 1974)
Lupinifolinol triacetate (64**)**	*T. lupinifolia*	(Smalberg *et al.*, 1974)
Candidol (65**)**	*T. candida*	(Dutt and Chibber, 1983)
Candirone (66**)**	*T. candida*	(Chen *et al.*, 2014)
7-Ethoxy-3,3',4'-trihydroxyflavone (67**)**	*T. procumbens*	(Chen *et al.*, 2014)
Oleanolic acid (68**)**	*T. strigosa*	(Chen *et al.*, 2014)
1*β*-hydroxy-6,7*α*-dihydroxyeudesm-4(15)-ene (69**)**	*T. candida*	(Mohamed-Elamir *et al.*, 2011)
Linkitriol (70**)**	*T. purpurea*	(Chen *et al.*, 2014)
1β,6α,10α-guai-4(15)-ene-6,7,10-triol (71**)**	*T. vogelii*	(Chen *et al.*, 2014)
Purpureamethied (72**)**	*T. purpurea*	(Sinah *et al.*, 1982)
2-Propenoic acid, 3-(4-(acetyloxy) -3-methoxypheny)-3(4-actyloxy)-3-methoxyphenyl)-2-propenyl ester (73**)**	*T. purpurea*	(Chen *et al.*, 2014)

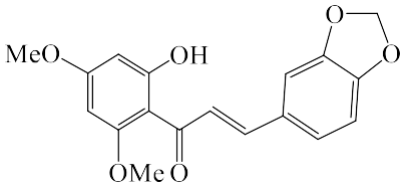
Tephrospiroketone II (74**)**	*T. candida*	(Chen *et al.*, 2014)
Astraciceran (75**)**	*T. strigosa*	(Chen *et al.*, 2014)



52 R_1= OMe, R_2= H
53 R_1= H, R_2= OMe



54 R_1= OMe, R_2= OMe, R_3= H
55 R_1= OMe, R_2= OMe, R_3= OMe
56 R_1= OMe, R_2= OMe, R_3= OH
57 R_1= OH, R_2= OMe, R_3= H
58 R_1= OMe, R_2= OH, R_3= H
59 R_1= OMe, R_2= H, R_3= OH



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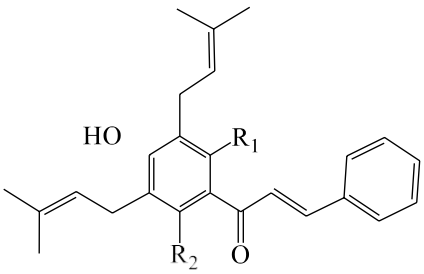
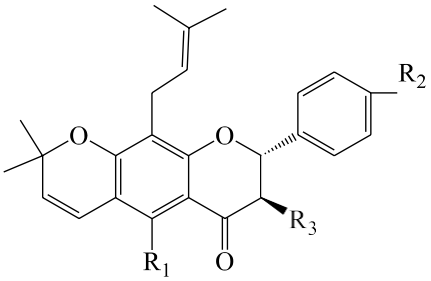
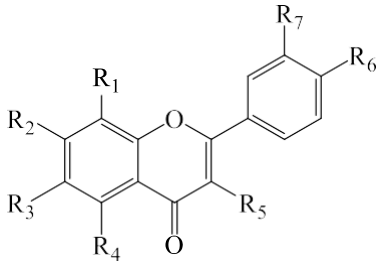


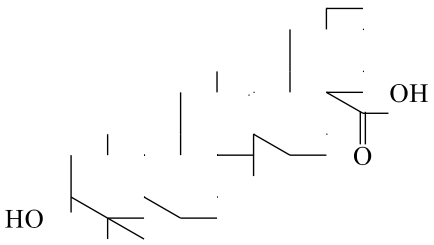
61 R_1= H, R_2= OH
62 R_1= OH, R_2= H



63 R_1= OH, R_2=OH, R_3= OH
64 R_1= OAc, R_2= OAc, OAc



65 R_1= H, R_2= OMe, R_3= OMe, R_4= OMe, R_5= OH, R_6= OH, R_7= H
66 R_1= OMe, R_2= H, R_3=OMe, R_4= OH, R_5= OMe, R_6= OH, R_7= H
67 R_1= H, R_2= OEt, R_3= H, R_4=H, R_5= OH, R_6= OH, R_7= OH



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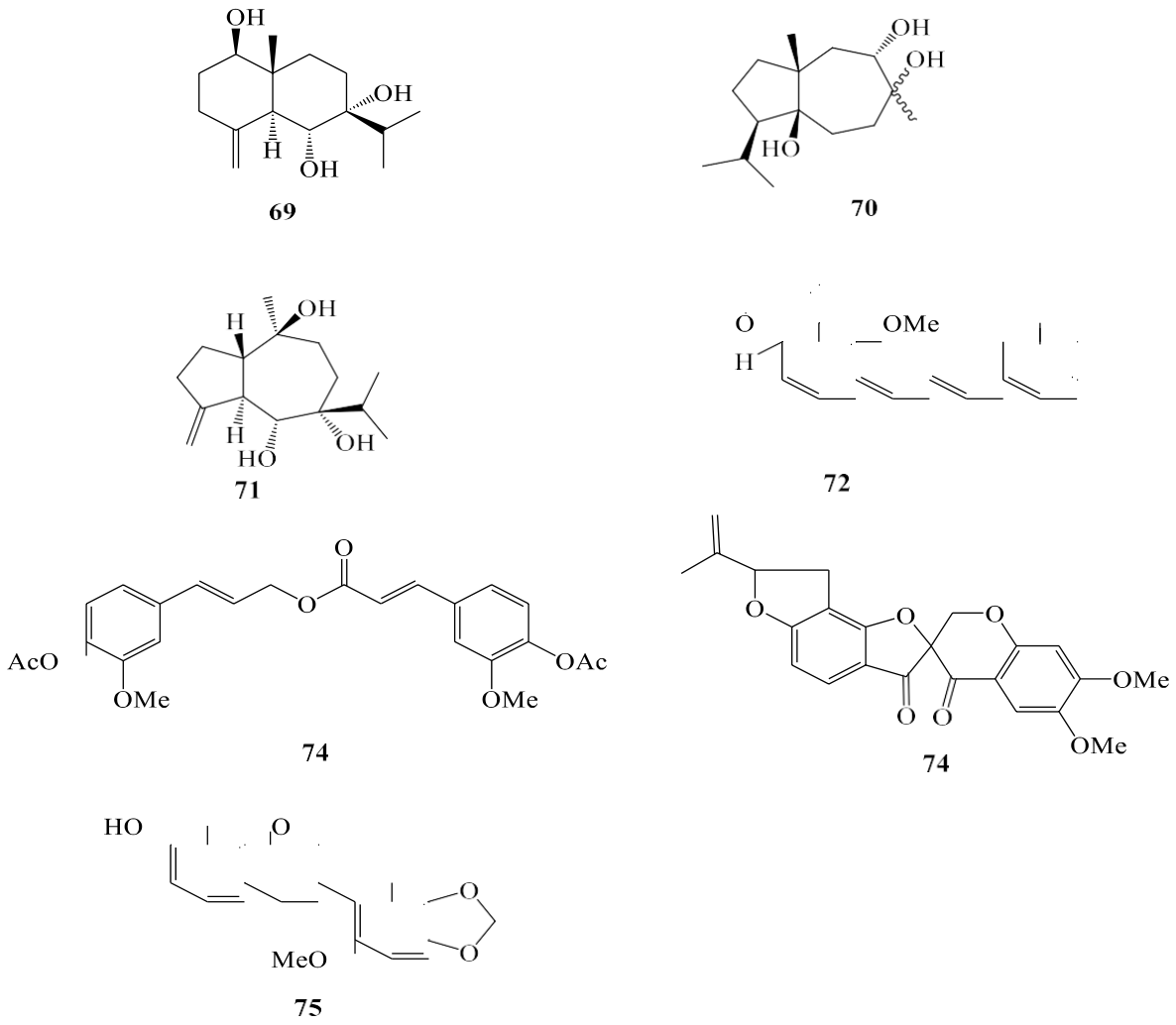


Figure 2.6: Chalcones, Flavonols, Flavanonols and Triterpenes and other compounds from genus *Tephrosia*

2.8 Antimicrobial activity of phytochemicals isolated from genus *Tephrosia*

The phytochemicals from the genus *Tephrosia* have been shown to exhibit various bioactivities, such as antimicrobial activities. Tephroapollin C (**17)** and Apollinine (**23)** both isolated from *Tephrosia apollinea* have showed positive effect when tested for antifungal activity against *Aspergillus niger, Penicilleuim funiculosum* et *Fusarium moniliform* (Ammar *et al.*, 1988). 2',6'-Dimethoxy-4',5'-(2",2"-dimethyl)-pyranochalcone (**54**) from *T. pulcherrima* showed significant antimicrobial activity when tested against a series of micro-organisms. 3,4:8,9-Dimethylenedioxypterocarpan (**30**) from *T. aequilata* exhibited little activity against gram-positive bacteria, *B. subtilis* and *Micrococcus lutea*. Hildecarpin (**31**) from *T. hildebrandtii* exhibited antifungal activity against *Cladosporium cucumerinum* (Chen *et al.*, 2014).

2.9 Tephrosia hildebrandtii

Tephrosia hildebrandtii is a species belonging to Fabaceae family, Faboideae subfamily, Millettieae tribe and in the *Tephrosia* genus is an annual regular bushy herbaceous plant growing in East Africa (Lwande *et al.*, 1986) and distributed in the tropical and subtropical regions of the world (Lwande *et al.*, 1987). It is a shrub of 1-3 m height. It is a short-lived, bushy perennial that occurs mostly in Acacia bushland, on grassland and formerly cultivated land of Kenya. *Tephrosia hildebrandtii* image is shown in fig 2.7 below.

Figure 2.7: Image of Tephrosia hildebrandtii

CHAPTER THREE: MATERIALS AND METHODS

3.1 General Experimental

Column chromatography (CC) was carried out using silica gel as stationary phase and analytical thin layer chromatography (TLC) was done on silica gel 60 F254 (Merck) coated on aluminium plates which, after development with an appropriate solvent system, spots were detected under UV light (254 nm and 366 nm) or by using iodine. Further purifications were achieved using a Chromatotron. Gel filtration was carried out over Sephadex® LH-20 (Pharmacia) suspended in CH_2Cl_2 /MeOH (1:1).

The isolates were dissolved in deuterated chloroform and run on a Bruker Avance 600 MHz NMR spectrometer to obtain both one-dimensional (^1H, ^{13}C NMR) and two-dimensional (^1H-^1H COSY, HSQC, HMBC) spectra which were processed by using MestReNova-8.1.1 or Topspin softwares. High-resolution mass spectral data was obtained on a Bruker LC-MS/MS TOF using acetonitrile as the eluent and processed using compass software version 4.4. The UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer.

3.2 Plant Materials

3.2.1 Collection

The aerial part of *Tephrosia hildebrandtii* was collected from Machakos in Delmonte pineapple plantation, in Thika, Nairobi, Kenya on February 2017 and identified at the Herbarium of the University of Nairobi where a voucher specimen was deposited. The plant material was shade-dried at room temperature and pulverized before extraction. The GPS data are 1°00'22.3"S and 37°04'46.6"E.

3.2.2 Extraction and Isolation

The air-dried and pulverized plant material (4.6 Kg) was extracted using MeOH/CH_2Cl_2 (1.5L x 4, 1:1v/v) at intervals of 12 hours for a period of 48 hrs. The combined extracts were concentrated on a rotary evaporator to provide 373.58g (6.0%) of a brown coloured crude extract. 286.44g of the crude extract was adsorbed on 209 g of silica gel 60 (230-400 mesh, Merck) then subjected to CC eluting with 100% *n*-hexane followed by increasing concentration of ethyl acetate. The first CC fraction eluted at 100% *n*-hexane was purified on Sephadex

(MeOH: CH_2Cl_2 1:1 v/v) to yield compound **7** (10 mg). The second CC fraction eluted at 1% EtOAc in *n*-hexane was loaded on Sephadex (MeOH: CH_2Cl_2 1:1 v/v) then subjected to small CC on silica gel (solvent: 10-40% EtOAc in *n*-hexane) to yield compounds **2** (6.27 mg) and compound **5** (48.30 mg). Fraction 3 which eluted with 2% EtOAc in *n*-hexane was subjected to Chromatotron (eluent 10-30% EtOAc in *n*-hexane) followed by size exclusion chromatography over Sephadex LH-20 (eluent MeOH: CH_2Cl_2 1:1 v/v) giving compound **90** (12.41 mg), compound **6** (23.87 mg) and compound **3** (19.42 mg). The fraction eluted with 15-40% EtOAc in *n*-hexane was subjected over Sephadex LH-20 (eluent MeOH: CH_2Cl_2 1:1 v/v) then subjected to Chromatotron [eluent (2:6:8 *n*-hexane: EtOAc: CH_2Cl_2)] to yield compound **4** (37.41 mg).

3.3 Biological Assays

3.3.1 Test Organisms

The standard test organisms (*Escherichia coli*, *Bacillus cereus* and *Candida albicans*) were obtained from the School of Biological Sciences, University of Nairobi.

3.3.2 Antimicrobial Assays

The antimicrobial screening was done for the crude extracts, pure isolated compounds and the structurally modified compounds. These were tested against three microbial pathogens, namely: *Escherichia coli*, *Bacillus cereus* and *Candida albicans*, using the Agar Well Diffusion technique. Organic solvents used to extract these samples were used for the preparation of the concentrations.

The Agar Well Diffusion medium was prepared by suspending 28.00 g of nutrient agar in 1000 mL of distilled water then the mixture was boiled to dissolve completely the medium which was sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Afterwards, the medium was well mixed before pouring in petri dishes to let it solidify for 24 hours.

100µL of inoculums (microbial suspensions) were inoculated on the surface of the solidified agar and holes of 6 mm in diameter were made on it using sterile Pasteur pipettes and from the stock solution of 10 mg/mL, 100 µL (1000 µg of crude extract) was transferred into the holes. Similarly, stock solutions of 1 mg/ml of each pure compound and modified compounds were prepared and 100 µL (100 µg compound) were transferred into the holes and allowed to dry.

Serial dilutions [10.0, 5.0, 2.5, 1.25, 0.625 mg/mL for the crude extract and 1.0, 0.5, 0.25, 0. 125, 0.0625 mg/mL for pure compounds and modified compounds] were prepared in order to determine the minimum inhibition concentrations (MIC). This was done in duplicate under sterile conditions, and the plates were incubated at 37°C for the three microbe strains. 100 µL of 0.5 mg/ml (50 µg) of the antibiotics (Chloramphenicol for bacteria and Fluconazole for fungi) were used as positive controls for comparison of the antimicrobial activities with those of the test samples and solvents used to prepare the samples were used as negative controls (Buyinza, 2012; Komolafe, 2014).

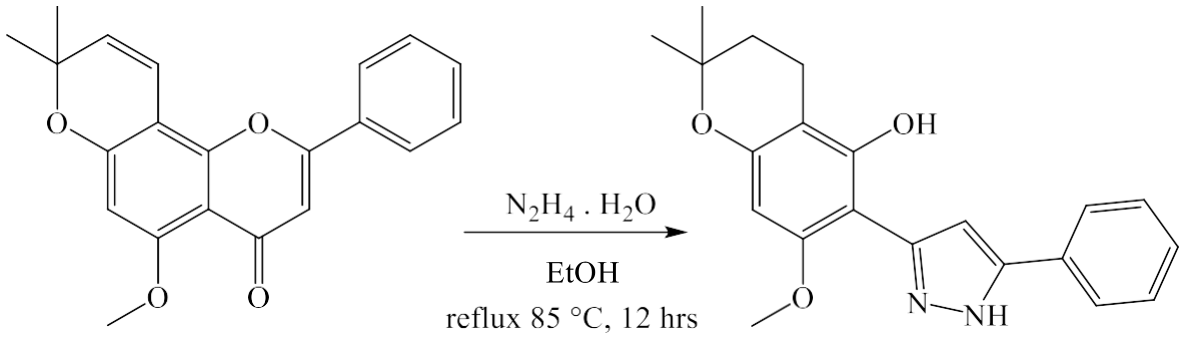
The antimicrobial activity was determined by measuring the diameter (mm) of the clear inhibition zone formed around holes using a transparent 30 cm ruler. This was done after 24 and 48 hours for bacteria and fungi, respectively (Babu *et al.*, 2011; Buyinza, 2012).

3.4 Structural Modification

The compounds earmarked for structural modification were selected based on the initial biological activity of the isolated pure compounds and the modification determined by the functional groups that were present in the active compounds.

3.4.1 Synthesis of the Pyrazoline Derivative of Compound 4

(10 mg) of compound **4** (0.03 mmol) was dissolved in 5 mL of ethanol and 0.5 mL hydrazine monohydrate (1.04 mmol) was added dropwise. The mixture was then heated to reflux at 85°C for 12 hours. The reaction mixture was concentrated *in vacuo*, diluted with H_2O (100 mL) and extracted into CH_2Cl_2 (3 x 15 mL). The CH_2Cl_2 layer was subjected to Chromatotron eluting with (0-20% ethyl acetate in *n*-hexane) to give compound **8** (7.03 mg). Scheme 3.1 below illustrate the preparation of the derivative **8.**

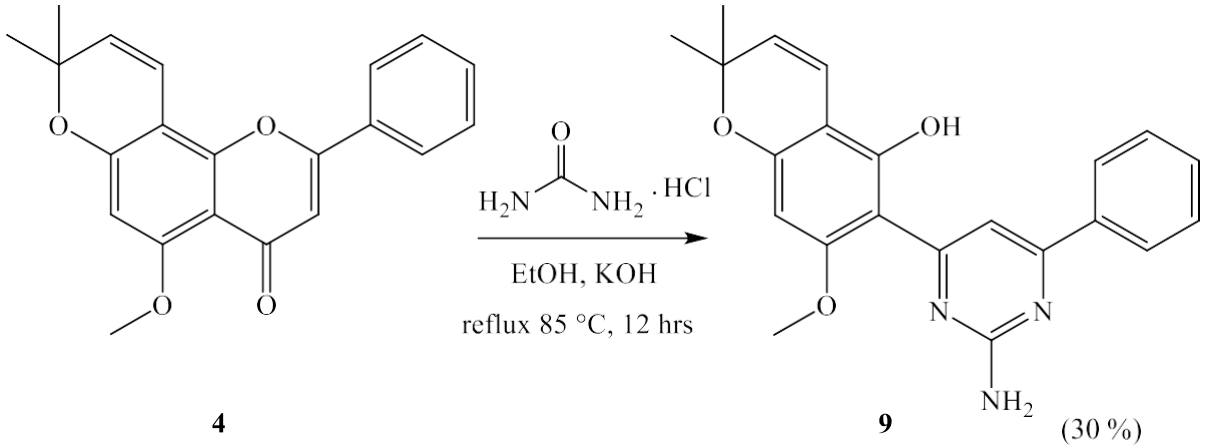


4 **8** (75%)

Scheme 3.1 Preparation of the pyrazoline derivative of compound 4

3.4.2 Synthesis of Guanidine Derivative of Compound 4

(10 mg) of compound **4** (0.03 mmol) was mixed with guanidinium hydrochloride (1g, 1.04 mmol) and dissolved in 10 mL of ethanol. Catalytic amount of KOH was added and the mixture heated to reflux for 12 hours. The reaction mixture was transferred to a beaker (250 mL) containing crushed ice and few drops of HCl were then added. The reaction mixture was then subjected to Chromatotron eluting with (0-20% ethyl acetate in *n*-hexane) to give compound **9** (3.23 mg). A scale-up reaction was done to increase the amount of the product. Scheme 3.1 below illustrate the preparation of the derivative **9.**



4 **9** (30 %)

. **Scheme 3.2** Preparation of pyrimidine derivative

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Compounds isolated from the Aerial Part of *T. hildebrandtii*

The aerial parts of *Tephrosia hildebrandtii* yielded seven compounds that included four flavones, one sterol, one triterpene and one retinoid. One of the flavones was new and the other known compounds are reported here for the first time from the plant.

4.1.1 Flavones

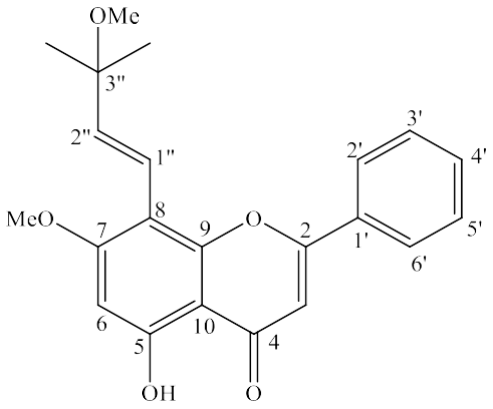
In this study a total of four flavones were isolated. These are compounds **1** – **4**, whose structure elucidations will be discussed in the following sections.

4.1.1.1 Hildflavone A (1)

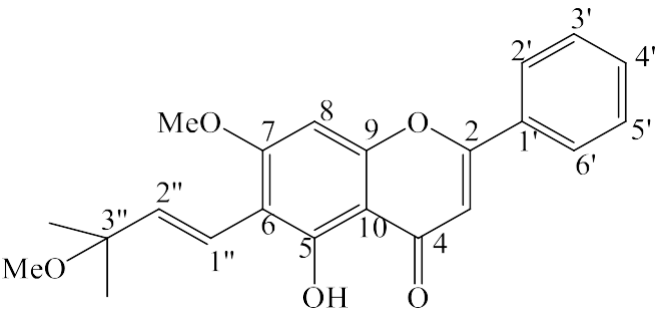
Compound **1** was obtained as yellow needles, with a protonated molecular ion peak $[M+1]^+$ at *m/z* = 367.1631 in the HREI-MS corresponding to a molecular formula $C_{22}H_{22}O_5$ (Appendix A). The skeleton of this compound was determined as a 5-hydroxyflavone from the UV (λ_{max} 356 nm), the ^{1}H NMR δ_H 6.69 (1H, *s*, for H-3) and 13.11 (1H, *s*, 5-OH) and the ^{13}C NMR [δ_C 164.3 (C-2), 105.6 (C-3) and 183.0 (C-4)] along with its HSQC correlations.

The NMR spectra further exhibited signals for a methoxyl group at δ_H 3.95 (3H, *s*) and δ_C 56.2. The occurrence of a methoxyisopentyl $(CH_3)_2$-C(OCH_3)-CH=CH- moiety was suggested by the presence of *trans* olefinic protons at δ_H 6.75 and 6.54 (1H, *d*, *J* = 16.7 Hz), *gem*-methyl at δ_H 1.44 (6H, *s*) and a methoxyl group at δ_H 3.27 (3H, *s*). The corresponding carbons for this moiety resonated at δ_C 117.6, 138.7, 75.8, 26.0 and 50.6. In the aromatic region, the ^{1}H NMR exhibited two multiplets at δ_H 7.54 (3H) and 7.92 (2H) representing an unsubstituted ring B of the flavone. The singlet aromatic proton peak at δ_H 6.44 (1H) in the ^{1}H NMR was assigned to a trisubstituted ring A. This is consistent with a flavone with an unsubstituted ring B and a trisubstituted ring A.

The methoxyisopentyl moiety could either be at position C-6 or C-8 as in **1** and **1a**. HMBC correlation of the singlet proton at δ_H 6.44 with C-5 and C-10 placed the methoxyisopentyl moiety at C-8. This led to the identification of this compound as (*E*)-5-hydroxy-6-(3-methoxy-3-methylbut-1-en-1-yl)-7-methoxy-2-phenyl-4H-chromen-4-one (**1**). This is the first report of this compound in literature and is given the trivial name hildflavone A (**1**). The assignment of the structure of hildflavone A (**1**) was based on the ^{1}H, ^{13}C, COSY, HSQC and HMBC as given in Table 4.1 and Appendices A1, A2, A3, A4 and A5.



1



1a

Table 4.1: ^{1}H and ^{13}C NMR (600 MHz) data along with HMBC correlations for compound **1** in $CDCl_3$

Atom	**δ_H, m, (^nJ), HSQC**	**^1H- ^1H COSY**	**δ_C**	**HMBC Correlations**
2			164.3	
3	6.69, *s*		105.6	C-10, C-1', C-2, C-4
4			183.0	
10			105.4	
5			154.2	
6			105.3	
7			163.3	
8	6.44, *s*		95.4	C-5, C-10
9			161.4	
1'			131.6	
2'/6'	7.92, *m*	7.54	126.5	C-2'/6', C-4', C-2
3'/5'	7.54, *m*	7.92	129.2	C-3'/5'/4', C-2'/6'
4'	7.54, *m*		132.0	
1"	6.75, *d* (16.7)	6.54	117.6	C-3", C-5, C-7
2"	6.54, *d* (16.7)	6.75	138.7	C-6, Me-3"
3"			75.8	
MeO-7	3.94, *s*		56.2	C-7
MeO-3"	3.27, *s*		50.6	C-3"
Me-3"	1.44, *s*		26.0	C-3", C-2", Me-3"
Me-3"	1.44, *s*		26.0	
OH-5	13.11			

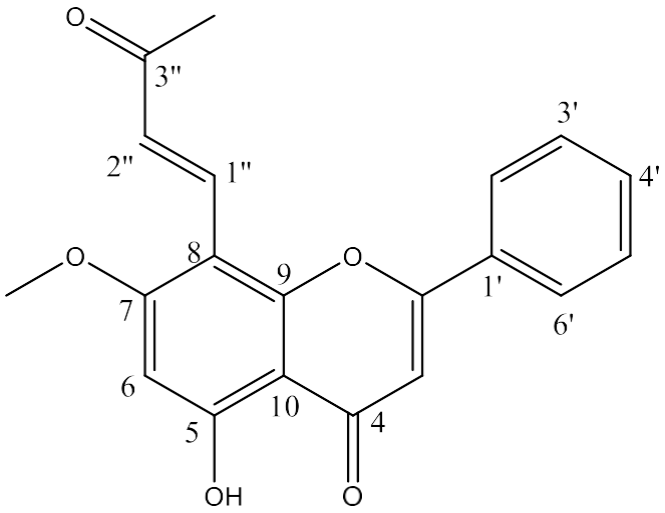
4.1.1.2 Purleptone (2)

Compound **2** was isolated as a yellow solid. EI-MS/MS analysis showed a $[M+1]^+$ peak at *m/z* = 337.1113 corresponding to the molecular formula of $C_{20}H_{16}O_5$ (Appendix B). Its UV spectrum (λ_{max} 361 nm), NMR spectra of δ_H 6.76 (1H, *s*, for H-3), δ_C 164.7 (C-2), δ_C 106.2 (C-3) and δ_C 182.6 (C-4)] and δ_H 13.44 (1H, *s*, 5-OH) (Table 4.2) along with its HSQC correlation showed that compound **2** is a 5-hydroxyflavone.

The ^{1}H NMR spectrum indicated the presence of two substituents; a methoxyl (δ_H 4.03 (*s*) and δ_C 56.5)) the rare *trans*-3-oxo-1-butenyl group. In the later substituent, the *trans* olefinic protons appeared deshielded at δ_H 8.01 and 7.19 (1H, *d*, *J* = 16.3 Hz) and the methyl protons appear at δ_H 2.43 (3H, s). The corresponding carbons of this substituent appeared at δ_C 27.9 (methyl) with the olefinic protons resonating at δ_C 132.0 and 128.9 while the carbonyl resonating at δ_C 199.3. The exhibition of two multiplets in the aromatic region at δ_H 7.61 (3H) and δ_H 7.94 (2H) supported that ring B of the flavone was unsubstituted while ring A was trisubstituted resulting from the presence of a singlet aromatic peak at δ_H 6.48 (1H).

The HMBC correlation of the olefinic proton, δ_H 8.01 (*d*, *J* = 16.3 Hz) of the *trans*-3-oxo-1-butenyl with C-9 (δ_C 156.0) and C-7 (δ_C 165.0) led to the placement of this substituent at C-8 similar to what is reported for purleptone isolated from *Tephrosia purpurea* (Atilaw *et al*., 2017). Consistent with this placement the singlet proton at δ_H 6.44 showed HMBC correlations with C-6 (δ_C 103.5), C-5 (δ_C 164.2) and C-10 (δ_C 105.4) allowing its assignment to H-8.

In agreement with this, compound **2** was characterized as purleptone whose structure was assigned on the basis of its ^{1}H, ^{13}C, COSY, HSQC and HMBC spectrum given in Table 4.2 and Appendices B1, B2, B3, B4 and B5. This is the first report of this compound **2** in this plant.



2

Table 4.2: ^{1}H and ^{13}C NMR (600 MHz) data for compound **2** in $CDCl_3$ along with HMBC Correlations

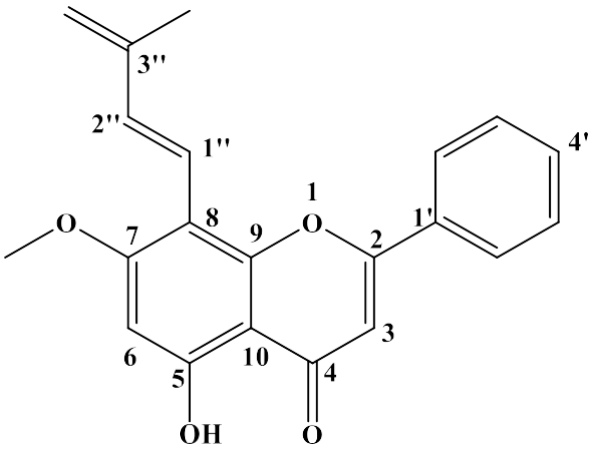
Atom	**δ_H, m, (^nJ), HSQC**	**^{1}H-^{1}H COSY**	**δ_C**	**HMBC Correlations**
2			164.7	
3	6.76, *s*		106.2	C-10, C-1', C-2
4			182.6	
10			105.4	
5			156.0	
6			103.5	
7			165.0	
8	6.48, *s*		95.7	C-9, C-10, C-6
9			164.2	
1'			131.2	
2'/6'	7.94, *m*	7.61	126.5	C-2'/6', C-4', C-2
3'/5'	7.61, *m*	7.94	129.4	C-3'/5'/4', C-2'/6', C-1
4'	7.61, *m*		132.3	
1"	8.01, *d* (16.3)	7.19	132.0	C-3",C-2", C-5, C-7
2"	7.19, *d* (16.3)	8,01	128.9	C-6, C-3"
3"			199.3	
MeO-7	4.03, *s*		56.5	C-7
Me-3"	2.43, *s*		27.9	C-3"
OH-5	13.44			

4.1.1.3 (*E*)-5-hydroxyanhydrotephrostachin (3)

Compound **3** was isolated as a yellow solid with its NMR spectra closely similar to those of compounds **3** and **2** (Table 4.3) indicating that it is a 5-hydroxyflavone derivative. Just as in compounds **1** and **2**, it has a methoxyl substituents at C-7. In addition, it also has similar aromatic substitutions pattern to those of compounds **1** and **2.**

What makes this compound different from the previous two is the presence of an isoprenyl substituent at C-6. This is evidenced from the ^{1}H NMR which showed *trans* olefinic protons resonating at δ_H 7.16 and δ_H 6.45 (1H, *d, J* = 16.2 Hz), a methylene at 5.10 (2H, *dd, J* = 1.2, 3.6 Hz) and a single methyl peak at δ_H 2.41 (3H, *d, J* = 1.2). In agreement with the ^{13}C NMR showed signals for four olefinic carbons at δ_C 143.1, 135.6, 117.6 and 117.1 and a signal for a methyl group at 18.4. These spectroscopic data are similar to that reported for tephrostachin **15** isolated from *Tephrosia polystachoides* (Chen *et al.*, 2014).

Compound **3** was characterized as (*E*)-5-hydroxyanhydrotephrostachin. This is the first report of this compound **3** in this plant. The assignment of the structure of compound **3** was based on the ^{1}H, ^{13}C, COSY, HSQC and HMBC as given in Table 4.3 and Appendices C1, C2, C3, C4 and C5.



3

Table 4.3: ^{1}H and ^{13}C NMR (600 MHz) data for compounds **3** in $CDCl_3$

Atom	δ_H, m, (^nJ), HSQC	^1H- ^1H COSY	δ_C	HMBC Correlations
2			164.8	
3	6.83, *s*		105.7	C-4, C-1', C-2
4			182.8	
10			103.6	
5			154.4	
6			106.4	
7			163.3	
8	6.71, *s*		95.6	C-9, C-10, C-6
9			161.5	
1'			131.7	
2'/6'	8.04, *m*	7.57	126.9	C-2'/6', C-4', C-2
3'/5'	7.57, *m*	8.04	129.3	C-3'/5'/4', C-2'/6', C-1
4'	7.92, *m*		132.4	
1"	7.16, *d* (16.2)	6.45	117.6	C-3",C-2", C-5, C-7, C-
2"	6.45, *d* (16.2)	7.16	135.6	6
3"			143.1	C-6, C-3", C-4", C-5"
4"	5.10, *dd* (1.2, 3.6)		117.1	
5-OH	13.11, *s*			C-5", C-2"
MeO-7	3.95, *s*		56.4	
Me-3"	2.41, *d* (1.2)		18.4	C-7 C-3", C-4", C-2"

4.1.1.4 Isopongaflavone (4)

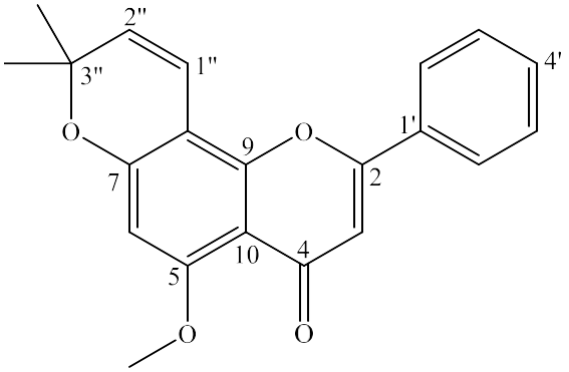
Compound **4** was isolated as yellow solid. The NMR of this compound (δ_H 7.03 (1H, *s*) and δ_C 177.8 (C-4), 161.2 (C-2), 108.2 (C-3)) indicated that this compound is a flavone, Table 4.4.

The ^{1}H NMR showed the presence of a methoxyl (δ_H 3.96) and a 3",3"-dimethylpyrano ring (δ_H 5.64 and 6.85 (*d*, *J*= 10 Hz, H-1") and 1.53 (6H, s). The methoxyl carbons resonated at 56.6 while the carbons of the 3",3"-dimethylpyrano ring resonated at 115.1 (C-1"), 127.8 (C-2"), 78.7 (C-3") and 28.3 ppm (Me-3").

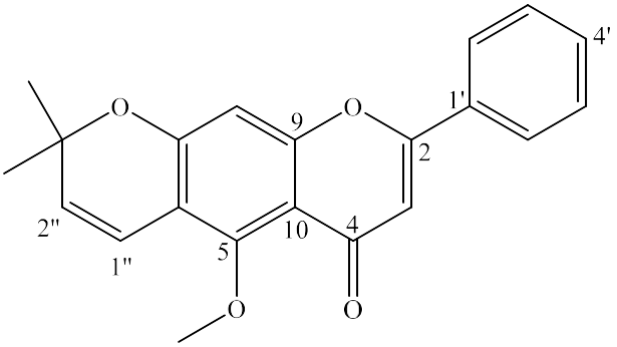
The ^{1}H NMR indicated that ring A is unsubstituted (δ_H 7.89 (*m*, 2H) and δ_H 7.53 (*m*, 3H)) while ring B is trisubstituted (δ_H 6.36 (*s*)).

Consistent with this, the 3",3"-dimethylpyrano substituent could either be at C-7/C-8 (**4)** or C-6/C-7 (**4a**). The position of the 3",3"-dimethylpyrano substituent is fixed at C-7/C-8 since had it been at C-6/C-7 this will make the methoxyl substituent to be disubstituted and therefore resonate at around δ_C 60. The methoxyl substituent in this compound resonated at δ_C 56.6 consistent with compound **4**.

Both carbon and proton spectra along with HMBC (Table 4.4 and Appendices D1, D2, D3, D4 and D5) data were in agreement with those reported for isopongaflavone previously isolated from *T. elata* (Bentley *et al.*, 1987).



4



4a

Table 4.4: ^{1}H and ^{13}C NMR (600 MHz) data along with HMBC correlations for compound 4 in $CDCl_3$

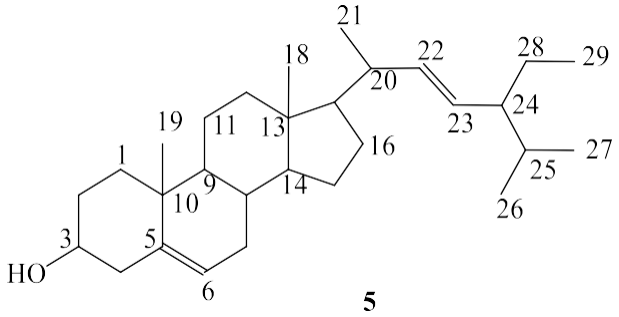
Atom	**δ_H, m, (^nJ), HSQC**	**^1H- ^1H COSY**	**δ_C**	**HMBC Correlations**
2			161.2	
3	7.03, *s*		108.2	C-10, C-1', C-2, C-4
4			177.8	
10			108.0	
5			160.8	
6	6.36, *s*		99.0	C-8, C-10, C-7, C-4, C-5
7			158.8	
8			102.7	
9			161.4	
1'			131.4	
2'/6'	7.89, *m*	7.52	126.3	C-2'/6', C-4', C-2, C-1'
3'/5'	7.53, *m*	7.89	129.1	C-3'/5'/4', C-2'/6', C-1'
4'	7.53, *m*		131.8	
1"	6.85, *d* (10.0)	5.65	115.1	C-3", C-9, C-7, C-8
2"	5.64, *d* (10.0)	6.85	127.8	C-8, Me-3", C-3"
3"			75.7	
MeO-5	3.96, *s*		56.6	C-5
Me-3"	1.53, *s*		28.3	C-3", C-2", Me-3"
Me-3"	1.53, *s*		28.3	

4.1.2 Sterol

One sterol was isolated and identified from the aerial part of *T. hildebrandtii*, and this was identified as stigmasterol (**5**).

4.1.2.1 Stigmasterol (5)

Compound **5** was obtained as white needles and identified as a triterpene based on its ^{13}C NMR spectrum that had twenty nine carbon peaks along with ^{1}H NMR spectrum that had peaks in region of 0.69 to 5.35 ppm. The ^{1}H NMR signals δ_H 0.69 (3H, d); 0.80 (3H, d); 0.84 (3H, d); 0.92 (3H, d); 1.01 (6H, d); 3.52 (1H, m); 5.01 (1H, m); 5.15 (1H, m) and 5.35 (1H, m) together with the ^{13}C NMR/DEPT experiment (CH_3 groups at δ_C 12.04, 12.30, 19.03, 19.86 x 2, 21.24); an oxygenated methine at δ_C 71.54 and four sp^2 carbons at δ_C 121.73 (CH), 129.24 (CH), 138.35 (CH) and 140.74 (quaternary) revealed the presence of six methyl groups, a hydroxymethine group and olefinic functionalities. Comparison of ^{1}H and ^{13}C NMR spectral data (Table 4.5 and Appendices E1, E2 and E3) of this compound with published literature led to the identification of the compound as stigmasterol (Luhata and Munkombwe, 2015).



5

Table 4.5: ^{13}C NMR (600 MHz) and DEPT 135 spectral data for compound **5** in $CDCl_3$

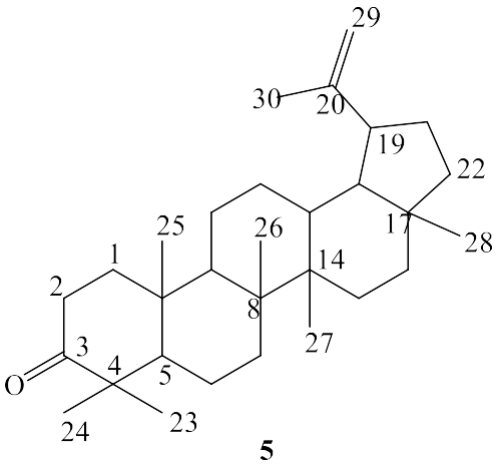
Atom	**δ_C**	**DEPT**
1	37.2	CH_2
2	31.6	CH_2
3	71.8	CH
4	42.3	CH_2
5	140.7	C
6	121.7	CH
7	31.9	CH_2
8	29.1	CH
9	50.1	CH
10	36.1	C
11	21.1	CH_2
12	38.8	CH_2
13	40.6	C
14	56.9	CH
15	24.3	CH_2
16	29.0	CH_2
17	56.0	CH
18	12.1	CH_3
19	19.9	CH_3
20	39.7	CH
21	23.0	CH_3
22	138.4	CH
23	129.2	CH
24	51.2	CH
25	31.9	CH
26	21.2	CH_3
27	19.0	CH_3
28	25.4	CH_2
29	12.3	CH_3

4.1.3 Triterpene

One triterpene was isolated and identified from the aerial part of *T. hildebrandtii*, and this was identified as lupenone (**6**).

4.1.3.1 Lupenone (6)

Compound **6** was also obtained as colorless needles. The ^{1}H NMR (Table 4.6) spectrum exhibited methyl protons resonating at δ 0.73, 0.86, 0.88, 0.96 (each 3H, *s*, for C-27, C-25, C-26, C-28, respectively), 1.00 (6H, *s*, for C-23 and C-24), 1.61 (3H, *s*, for C-30), a peak at δ 2.36 (2H, *m*, for H-2) and two olefinic protons due to geminal methylene protons of a terminal double bond appearing at δ 4.50 and 4.62 (each 1H, *s*, for H-29a and H-29b, respectively). The ^{13}C NMR spectrum showed typical signals at δ 218.2 corresponding to a saturated carbonyl group and at δ 150.9 and 109.4 corresponding to alkene carbons, these suggesting a lupine triterpene skeleton which has a carbonyl group. Considering the ^{1}H NMR and ^{13}C NMR spectra alongside the values of DEPT (Appendices F1, F2 and F3), the structure of compound **6** was assigned as the known lupenone in accordance with the data in the literature.



5

Table 4.6: ^{13}C NMR (600 MHz) and DEPT 135 spectral data for compound **6** in $CDCl_3$

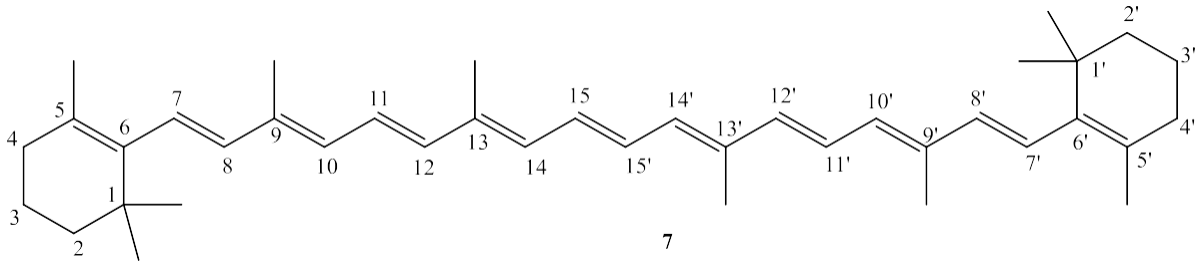
Atom	δ_C	DEPT
1	39.6	CH_2
2	34.1	CH_2
3	218.6	C=O
4	47.3	C
5	54.7	CH
6	19.7	CH_2
7	33.7	CH_2
8	40.8	C
9	49.8	CH
10	36.9	C
11	21.5	CH_2
12	25.2	CH_2
13	38.1	CH
14	42.9	C
15	27.5	CH_2
16	35.5	CH_2
17	43.0	C
18	48.3	CH
19	47.9	CH
20	151.2	C
21	29.8	CH_2
22	40.0	CH_2
23	26.7	CH_3
24	21.1	CH_3
25	15.8	CH_3
26	15.9	CH_3
27	14.5	CH_3
28	18.0	CH_3
29	109.3	CH_2
30	19.3	CH_3

4.1.4 Tetraterpenoid derivative

4.1.4.1 β-Carotene (7)

Compound **7** was isolated as a needle-like orange solid with NMR spectral features characteristic of a highly unsaturated compound comparable to that of a carotenoid. The ^1H NMR spectrum showed fourteen olefinic protons signals at δ_H 6.11-6.18 (6H, *d*, *J*= 16.0, 10.0, 16.0 Hz, for H-7/7'; H-8/8' and H-10/10' respectively); δ_H 6.25 (2H, *d*, *j* = 8 Hz, for H-14/14');

δ_H 6.37 (2H, *d*, *j* = 15.0 Hz , for H-12/12') and at δ_H 6.64-6.67 (4H, *m*, for H-11/11' and H-15/15'). The ^{1}H NMR spectrum further exhibited three singlets at δ_H 1.03, 1.72 and 1.97 (due to methyl groups), a peak at (δ_H 1.46, 4H, *d*, *J*= 6.0 Hz for methylene protons H-2/2') and two triplet resonances at δ_H 2.02 and 1.61 (4H, *t*, *J*= 6.0 Hz for methylene protons H-4/4' and H-3/3' respectively).The symmetric ^{13}C NMR spectrum revealed a total of twenty carbons which were suggestive of a tetraterpenoid derivative. The presence, in the ^{13}C NMR spectrum, of eleven sp^2 hybridized carbons at δ_C 129.9 (C-5), δ_C 138.1 (C-6), δ_C 127.0 (C-7), δ_C 138.0 (C-8), δ_C 136.3 (C-9), δ_C 131.2 (C-10), δ_C 125.3 (C-11), δ_C 137.5 (C-12), δ_C 136.7 (C-13), δ_C 132.7 (C-14) and δ_C 130.2 (C-15) along with 2D data were consistent with the structure of all *trans* β-carotene. The data are shown in Table 4.7 and Appendices G1 and G2.



7

Table 4.7: ^{1}H and ^{13}C NMR spectra (600 MHz) spectral data for compound **7** in $CDCl_3$

Atom	**δ_H (multiplicity and ^nJ)**	**δ_C**
1/1'		34.5
2/2'	1.46, *d* (6.0)	39.8
3/3'	1.60, *t* (6.0)	19.5
4/4'	2.02, *t* (6.0)	33.3
5/5'		129.6
6/6'		138.1
7/7'	6.11, *d* (16.0)	129.6
8/8'	6.16, *d* (16.0)	138.0
9/9'		136.3
10/10'	6.18, *d* (10.0)	131.1
11/11'	6.67, *dd* (8.0, 12.0)	125.3
12/12'	6.35, *d* (15)	137.5
13/13'		136.7
14/14'	6.25, *d* (8)	132.7
15/15'	6.64, *dd* (8.0, 12.0)	130.2
1/1'-Me	1.03, *s*	29.2
5/5'-Me	1.72, *s*	22.0
9/9'-Me	1.97, *s*	13.0
13//13'-Me	1.97, *s*	13.1

4.2 Structure Modification of Selected Natural Products

Attempts to enhance the bioactivity of compound **4** by preparing its pyrazoline and guanidine derivatives were made using guanidine hydrochloride and hydrazine hydrate, respectively, in presence of alkali.

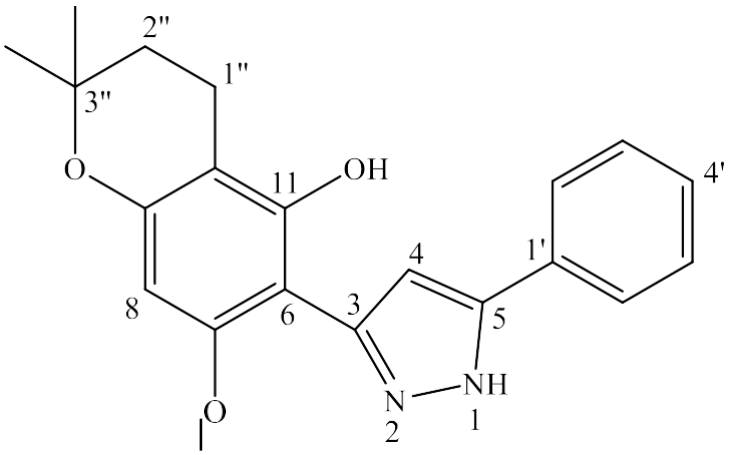
4.2.1 Synthesis of Pyrazoline Derivative 8

Compound **8** was obtained as needle-like solid in 65% yield. The NMR spectral features confirmed that compound **8** was a pyrazoline derivative of compound **4**. The ^{1}H NMR data (Table 4.8) showed a singlet peak resonating at δ_H 7.34 (1H, *s*, for N-H), a singlet peak at δ_H 7.29 (1H, *s*, for H-4) and ^{13}C NMR 142.8 (C-5), 103.8 (C-4) and 150.8 (C-3) along with its HSQC correlations.

The ^{1}H NMR spectrum indicated the presence of a methoxyl (δ_H 3.80) and a 3",3"-dimethylpyrano ring δ_H 2.64 and 1.74 (2H, *dd*, *J*= 6.0, 6.0 Hz, H-1"and H-2") and 1.28 (6H, s). The methoxyl carbon resonated at 55.6 while the carbons of the 3",3"-dimethylpyrano ring resonated at 17.0 (C-1"), 32.7 (C-2"), 74.9 (C-3") and 26.8 ppm (Me-3").

The ^{1}H NMR also indicated that ring B is unsubstituted [δ_H 7.53 (*m*, 2H) and δ_H 7.37 (*m*, 3H)] while ring A is trisubstituted (δ_H 5.97 (*s*)). The 3",3"-dimethylpyrano ring was found to be reduced in compound **8**.

In accordance with this, compound **8** was identified as 7-methoxy-2,2-dimethyl-6-(5-phenyl-1*H*-pyrazol-3-yl)-chroman-5-ol.



8

Table 4.8: ^{1}H and ^{13}C NMR (600 MHz) data along with HMBC correlations for compound **8** in $CDCl_3$

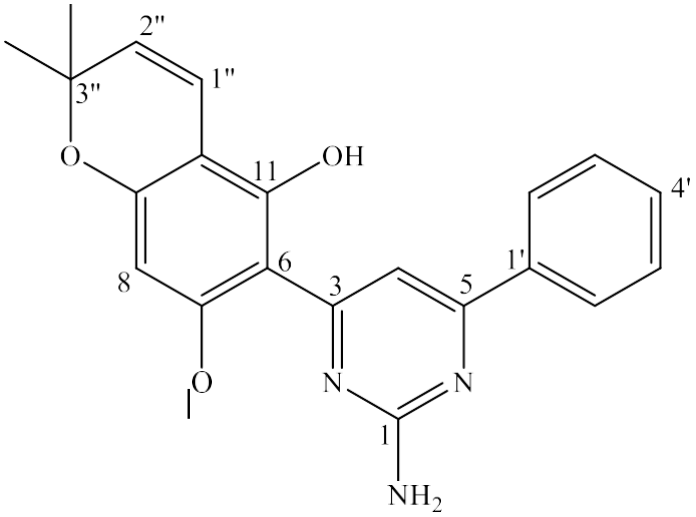
Atom	**δ_H (multiplicity and ^nJ) HSQC**	**^1H- ^1H COSY**	**δ_C**	**HMBC Correlations**
3			150.8	
4	7.29, *s*		103.9	C-3, C-5
5			142.8	
6			98.9	
7			156.2	
8	5.98, *s*		91.9	C-6, C-7, C-9, C-10
9			154.8	
10			102.1	
11			157.2	
1'			129,4	
2'/6'	7.52, *m*		125.8	C-2'/6', C-4', C-2, C-1'
3'/5'	7.37, *m*		129.4	C-3'/5'/4', C-2'/6', C-1'
4'	7.37, *m*		128.9	
1"	2.64, *dd* (6.0, 6.0)	1.74	17.02	C-2", C-3", C-9, C-10
2"	1.74, *dd* (6.0, 6.0)	2.64	32,7	C-1", Me-3", C-3", C-10
3"			74.8	
MeO-7	3.80, *s*		55.6	
Me-3"	1.28, *s*		26.8	C-7
Me-3"	1.28, *s*		26.8	C-3", C-2", Me-3"
1-NH	7.34, *s*			

4.2.2 Synthesis of Pyrimidine Derivative 9

Compound **9** was obtained as a yellow solid in 30% yield. The establishment of compound **9** as a pyrimidine derivative of compound **4** was from the ^{1}H NMR data (Table 4.9) showing a characteristic singlet peak at δ_H 8.02 (1H, *s*, for H-4) and another singlet peak resonating at δ_H 5.06 (2H, *s*, for NH_2), and ^{13}C NMR 159.6 (C-1), 166.0 (C-3) and 164.1 (C-5) along with its HSQC correlations.

The ^{1}H NMR spectrum indicated the presence of a methoxyl (δ_H 3.84) and a 3",3"-dimethylpyrano ring δ_H 6.67 and 5.38 (1H, *d*, *J*= 9.0 Hz, H-1"and H-2") and δ_H 1.39 (6H, s). The methoxyl carbon resonated at 55.8 while the carbons of the 3",3"-dimethylpyrano ring resonated at 116.8 (C-1"), 125.5 (C-2"), 77.3 (C-3") and 28.2 ppm (Me-3").

The ^{1}H NMR also indicated that ring B is unsubstituted δ_H 7.92 (*m*, 2H) and δ_H 7.42 (*m*, 3H) while ring A is trisubstituted [δ_H 5.96 (*s*)]. In accordance with this, compound **9** was identified as 6-(2-amino-6-phenylpyrimidin-4-yl)-7-methoxy-2,2-dimethyl-2*H*-chromen-5-ol.



9

Table 4.9: ^{1}H and ^{13}C NMR (600 MHz) data along with HMBC correlations for compound **9** in $CDCl_3$

Atom	**δ_H (multiplicity and ^nJ) HSQC**	**^1H- ^1H COSY**	**δ_C**	**HMBC Correlations**
1			159.6	
3			166.3	
4	8.02, *s*		107.5	C-1', C-3, C-5, C-6
5			164.1	
6			101.5	
7			161.4	
8	5.92, *s*		91.7	C-6, C-7, C-9, C-10
9			15697	
10			103.8	
11			158.9	
1'			138.1	
2'/6'	7.90, *m*	7.39	127.3	C-2'/6', C-4', C-2, C-1'
3'/5'	7.39, *m*	7.90	128.8	C-3'/5'/4', C-2'/6', C-1'
4'	7.39, *m*		130.4	
1"	6.63, *d* (9.0)	5.38	116.8	C-3", C-9, C-11
2"	5.38, *d* (9.0)	6.63	125.5	Me-3", C-3", C-10
3"			77.3	
MeO-7	3.84, *s*		55.8	C-7
Me-3"	1.39, *s*		28.2	C-3", C-2", Me-3"
Me-3"	1.39, *s*		28.2	
1-NH_2	5.06, *s*			

4.3. Antimicrobial Activity

The MeOH/DCM (1:1 v/v) crude extract, the isolated and the modified compounds from the aerial part of *T. hildebrandtii* were all assayed for their antifungal and antibacterial activities against *Candida albicans*, *Bacillus cereus* and *Escherichia coli* and the zone of inhibition and the MICs results are summarized in (Table 4.10).

Table 4.10: Antibacterial and Antifungal efficacy tests results

Compound	**Concentration (µg/well)**	**Zone of inhibition (mm)**		
		EC	**BC**	**CA**
Crude extract	1000 500 250	15 13.5 11.6	11 10.5 9.5	18 14 -
90	100 50 25	18 14.3 7	14 12 -	21 17.5 11
91	100 50 25	15 10 4.7	11 9 -	12 7.8 -
92	100 50 25	- - -	17 14 10	- - -
93	100 50	- -	- -	13 10.6
94	100 50	- -	- -	14 11
95	100 50	- -	- -	14 8.3
96	100	-	-	-
97	100 50 25	13 - -	17 7.5 -	20 11 8.1
98	100 50 25	11 6.2 -	14 7 -	18 11 -
Chloramphenicol	50	26	26	
Fluconazole	50			25.5

The sensitivity criteria of each strain of microbes to the plant crude extract, the isolated compounds and the modified compounds was based on the zone of inhibition (ZI) data expressed in millimeters as follows: no activity for ZI diameter < 7 mm; low activity for 7 mm ≤ ZI diameter ≤ 9 mm; intermediate activity for 10 mm ≤ ZI diameter ≤ 13 mm and high activity for ZI diameter ≥ 14 mm (Babu *et al.*, 2011).

The MeOH/DCM (1:1 v/v) crude extract of *T. hildebrandtii* showed better antimicrobial activity against *E coli* and *C. albicans* compared to *B cereus* at 500 μg/well. However, the inhibition of *E. coli* was higher than the inhibition of *B cereus* comparing the inhibition zone of both strains. This behavior assured that the crude contained some bioactive compounds which are responsible for this antimicrobial potency.

On one hand, compound **1** showed the highest activity against *E coli* and *C. albicans* of all the isolated compounds when compared to the standard, an outcome that supports the above hypothesis about the crude extract. This antimicrobial potency of compound **1** might be due to the presence of the two methoxyl substituents on its structure. This compound appears to be one of the flavonoids responsible for the antimicrobial activity of the crude extract.

On the other hand, compound **2** also showed to be effective against *E. coli* compared to the other microorganisms, while the mixture of compounds **2** & **3** exhibited the most effective activity against *B cereus* but remained completely ineffective against *E. coli* and *C. albicans*. These positive and negative effects of the compounds **2** & **3** could be due to the combination of the two compounds put together.

Compound **93** was only bioactive against *C. albicans* and this is also supported by the methoxyl group at position C-5 of this flavone and the susceptibility of the gram negative and gram positive bacteria.

E coli, a gram negative bacteria, has generally showed susceptibility to most of the compounds and this might be due to the possession of outer membrane by gram negative bacteria that serves as an effective barrier whereas *B cereus*, gram positive bacteria susceptibility is due to their naked cell wall. This is also called resistance of the microbes.

For the same reason, the behavior of compounds **5**, **6** and **7** can be explained but adding to it the lack of possible active sites on their respective structures.

Both the modified compounds **8** and **9** have enhanced activities compared to their starting material compound **4** which was only active on the tested fungus.

Therefore, in agreement with Kumar *et al.,* 2013, we can assume that the mode of action for antimicrobial activity of some of the isolated flavonoids in this study is by inactivation of proteins.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study investigated the extract of the aerial part of *T. hildebrandtii* for antimicrobials principles. The key findings of this study are summarized below:

A total of seven compounds were isolated and characterized. These include; four flavones compounds **1**, **2**, **3** and **4**; one steroid, compound **5**; one triterpene, compound **6** and one carotenoid, compound **7**. Of these, compound **1**: flavone was new and this is the first report on the occurrence of compounds **2, 3, 6** and **7** in *Tephrosia hildebrandtii*.

The crude extract exhibited a good antimicrobial activity that indicated that there were phytochemical contained in it that were responsible for antibacterial and antifungal potencies, That the new compounds **1** and **2** exhibited promising antibacterial and antifungal activities supports the potent antimicrobial activity exhibited by the crude extract.

Structural modification of compound **4** provided compound **8**, a pyrazole derivative and compound **9**, pyrimidine derivative, which have enhanced activities compared to compound **4** which was only active on the tested fungus.

5.2 Recommendations

Having closely examined the results of this study, I recommend:

1. The testing of the isolated and the modified compounds and the crude extract against different pathogens and cancer cells along with their toxicity to establish the scope of their efficacy and their safety.

2. *In vivo* studies of the crude extract and the isolated compounds should be carried out in order to determine their efficacy.

3. A comprehensive structure-activity relationship by structural modification for compounds **1**, **2**, **8** and **9** to determine the functional groups responsible of their antimicrobial activities.

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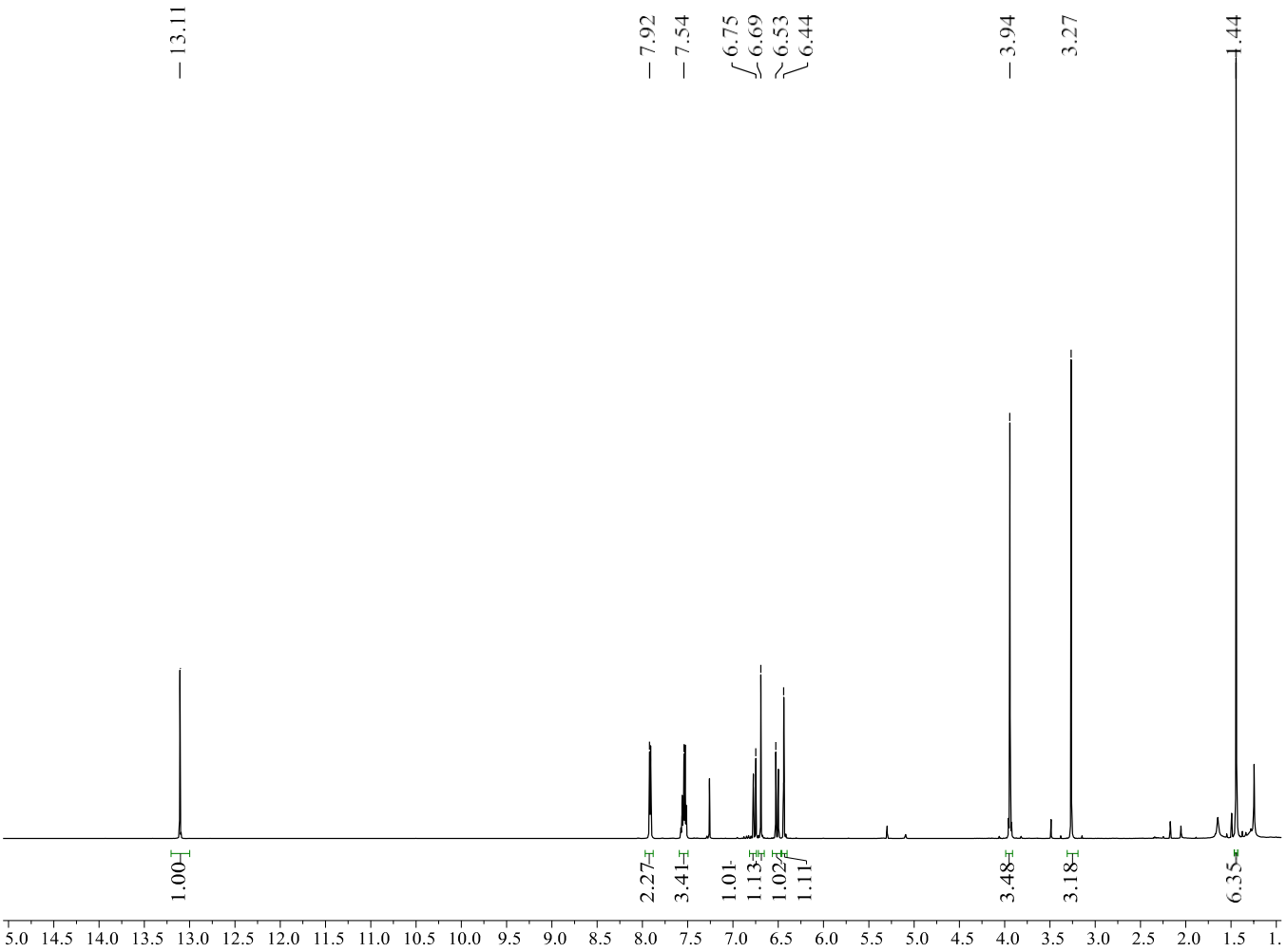
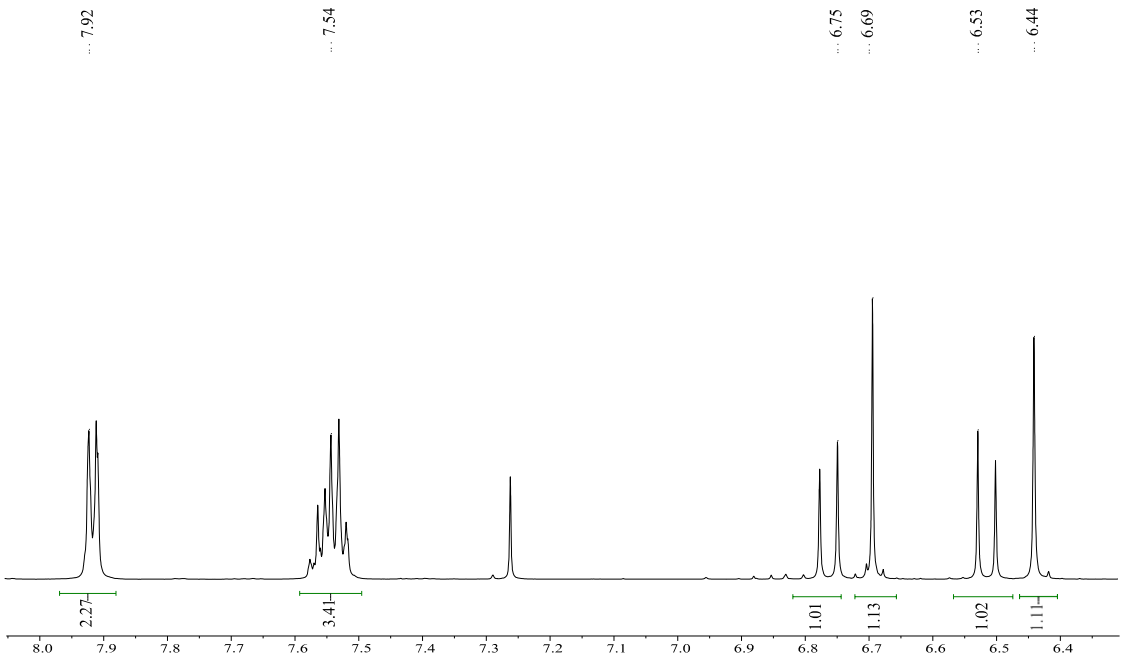
APPENDICES

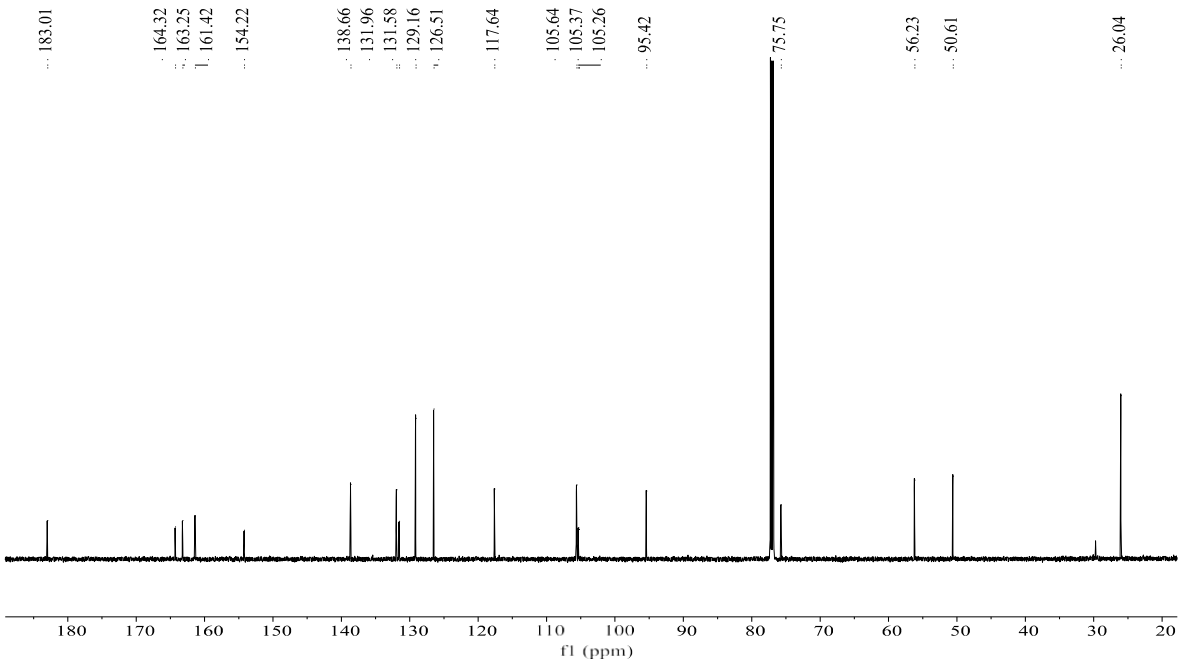
APPENDIX A: SPECTRA FOR COMPOUND 1

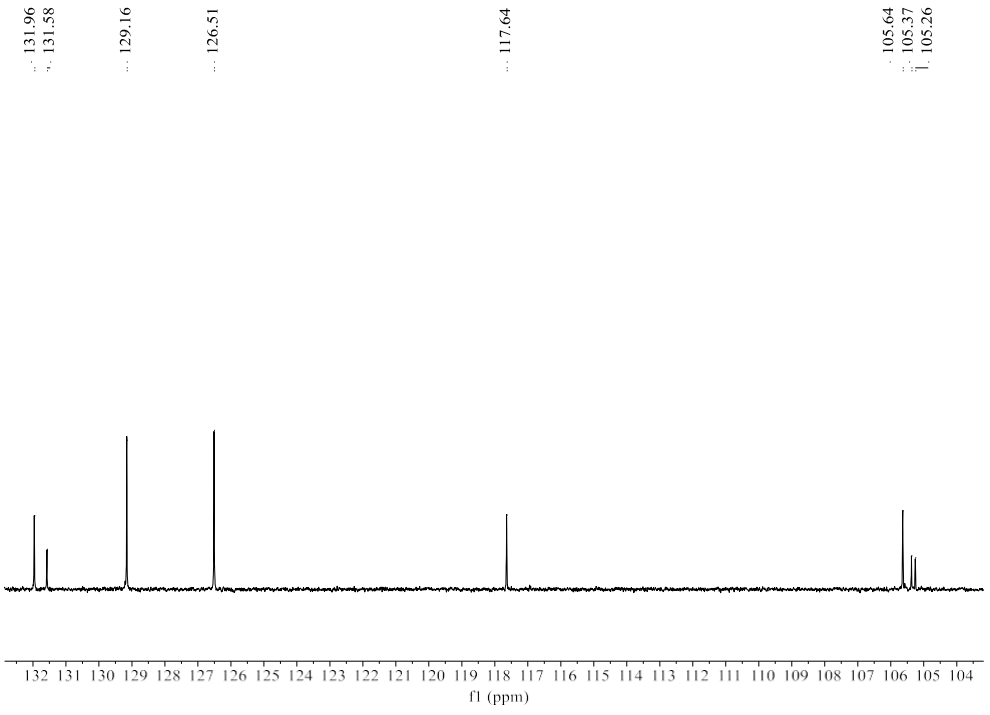
Appendix A1: ^{1}H NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 1



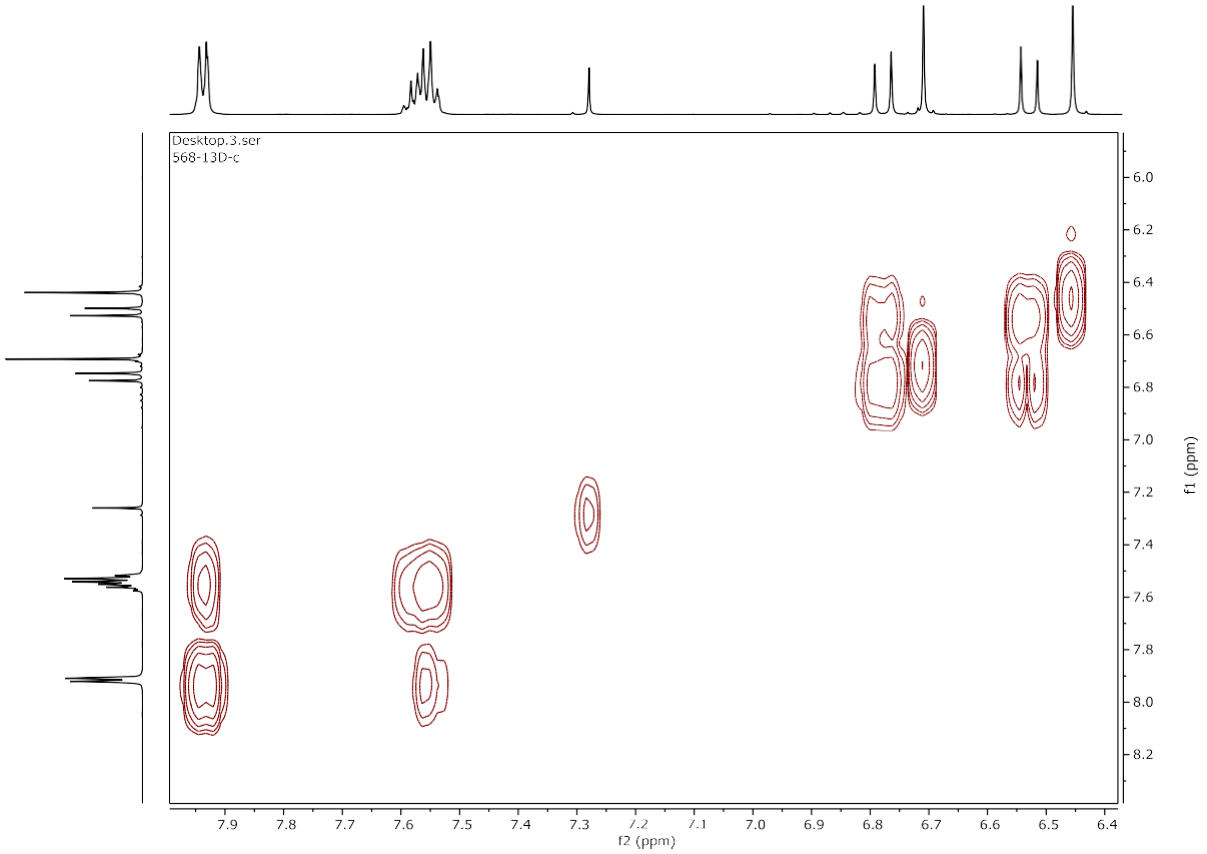

7.92
7.54
6.75
6.69
6.53
6.44
2.27
3.41
1.01
1.13
1.02
1.11
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4

Appendix A2: ^{13}C NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 1

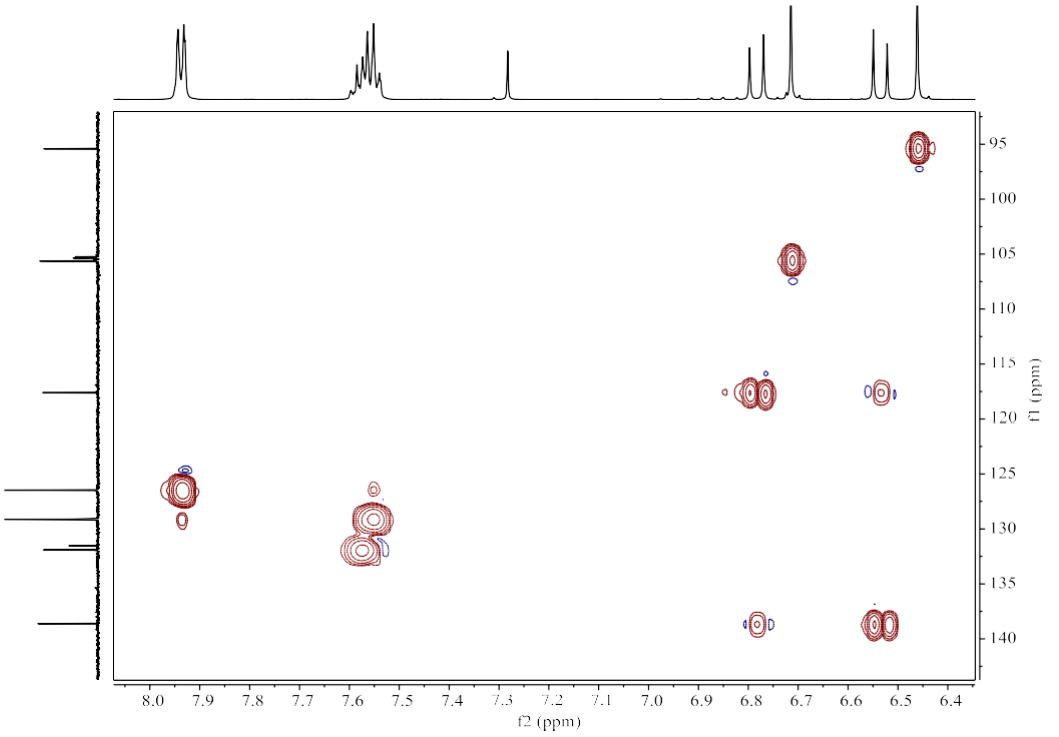


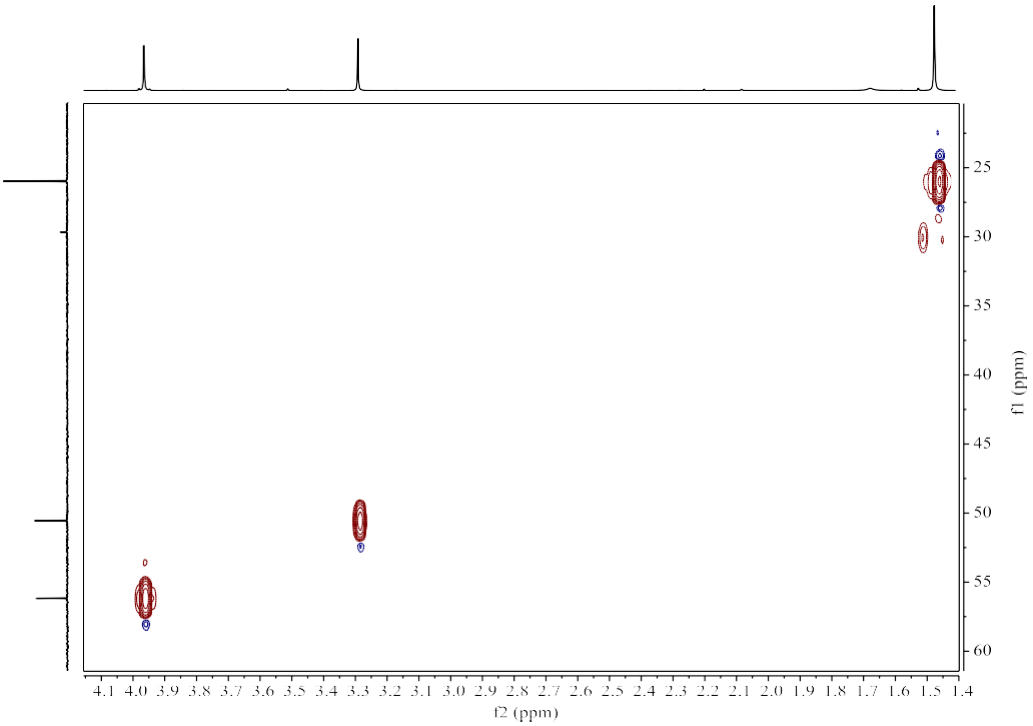


Appendix A3: ^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 1

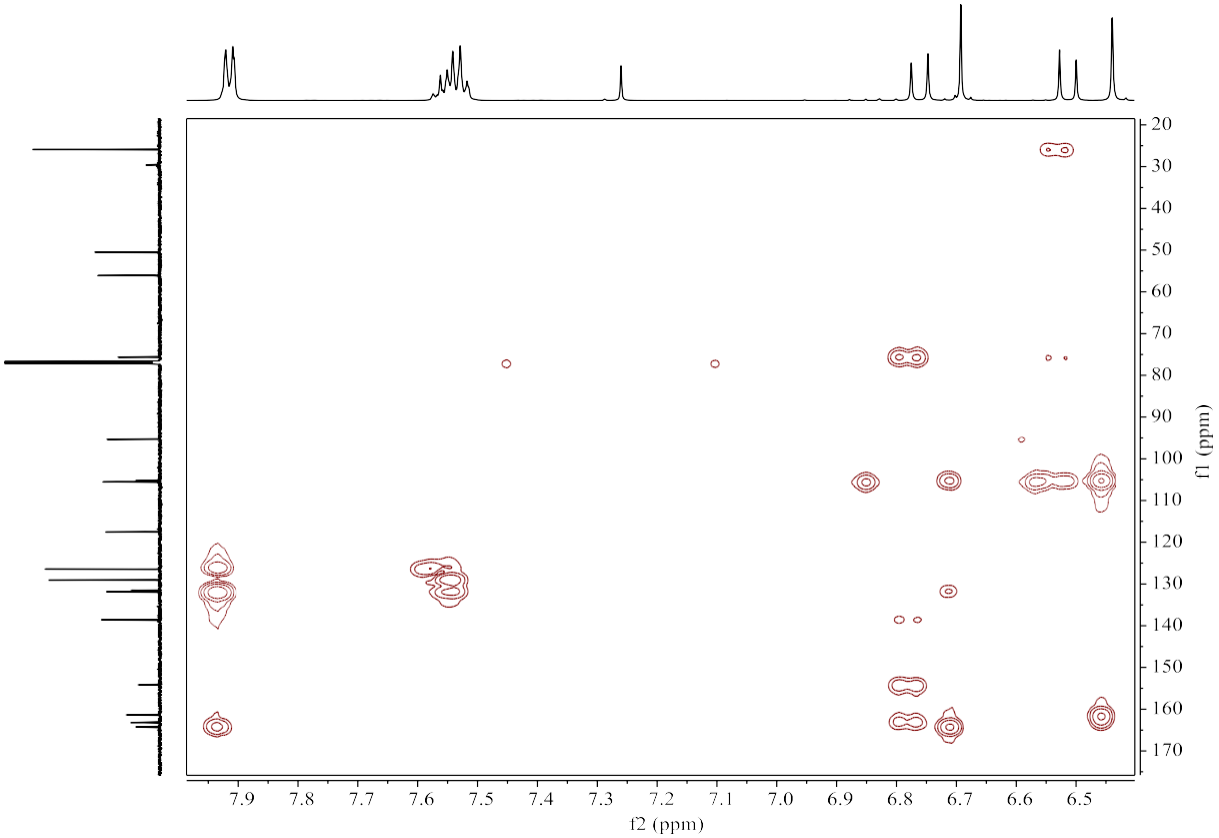


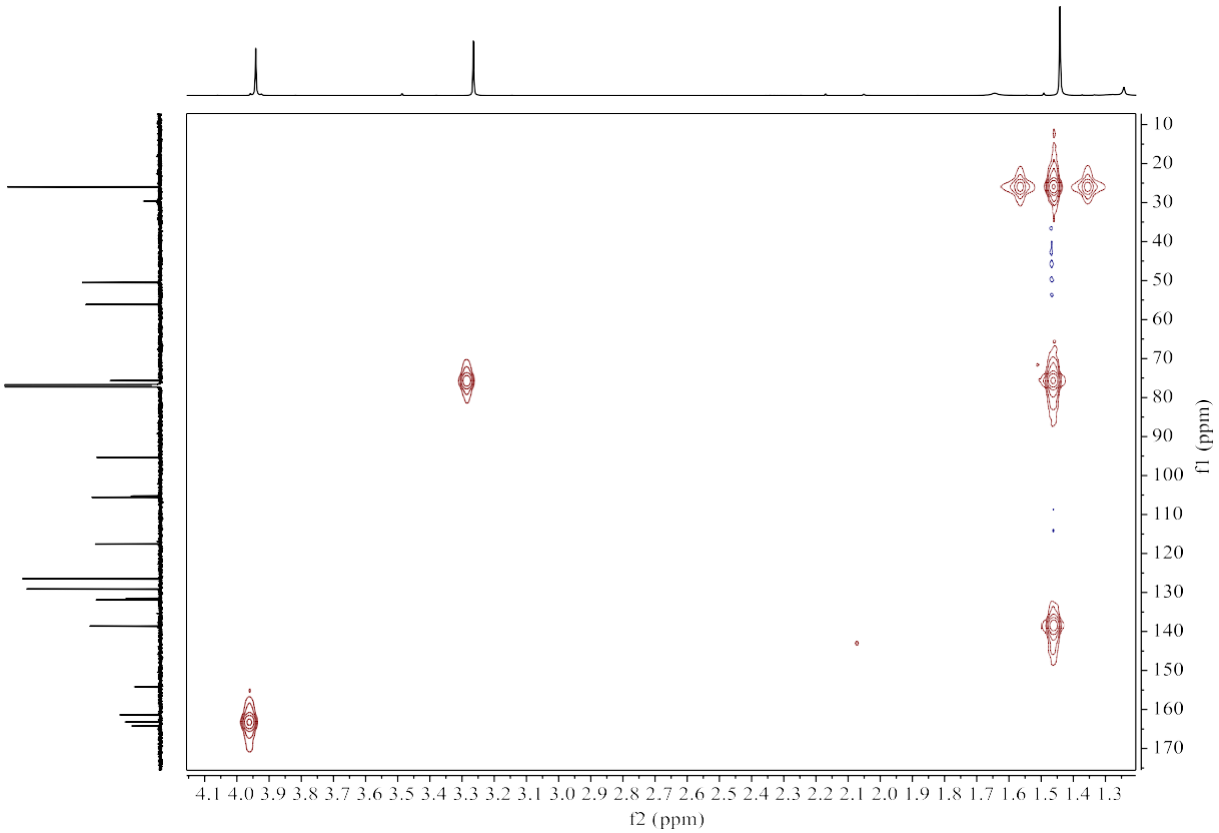
Appendix A4: HSQC SPECTRUM (EXPANSION) FOR COMPOUND 1



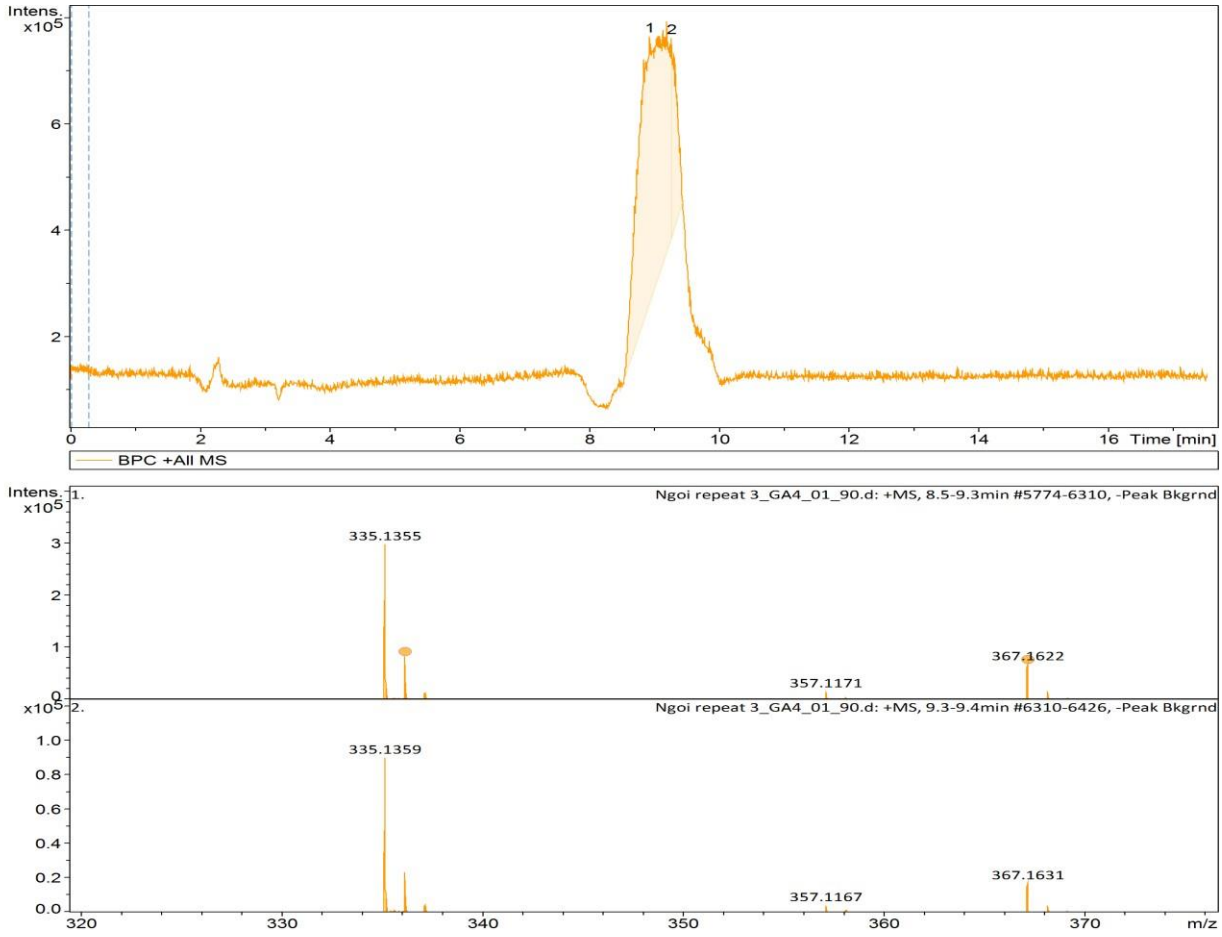


Appendix A5: HMBC SPECTRUM (EXPANSION) FOR COMPOUND 1

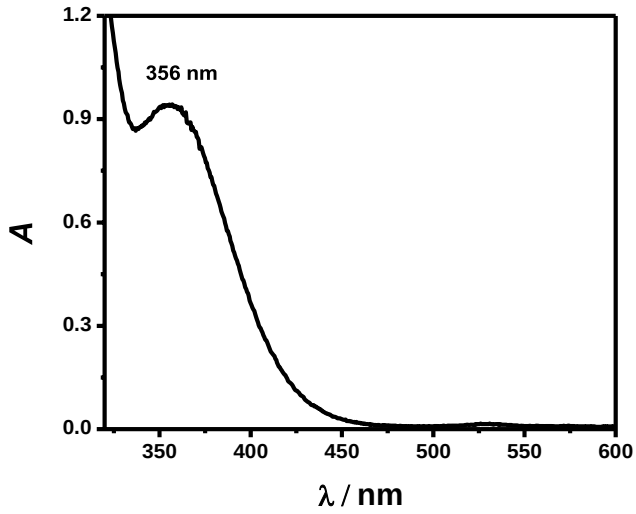




Appendix A6: MS SPECTRUM FOR COMPOUND 1

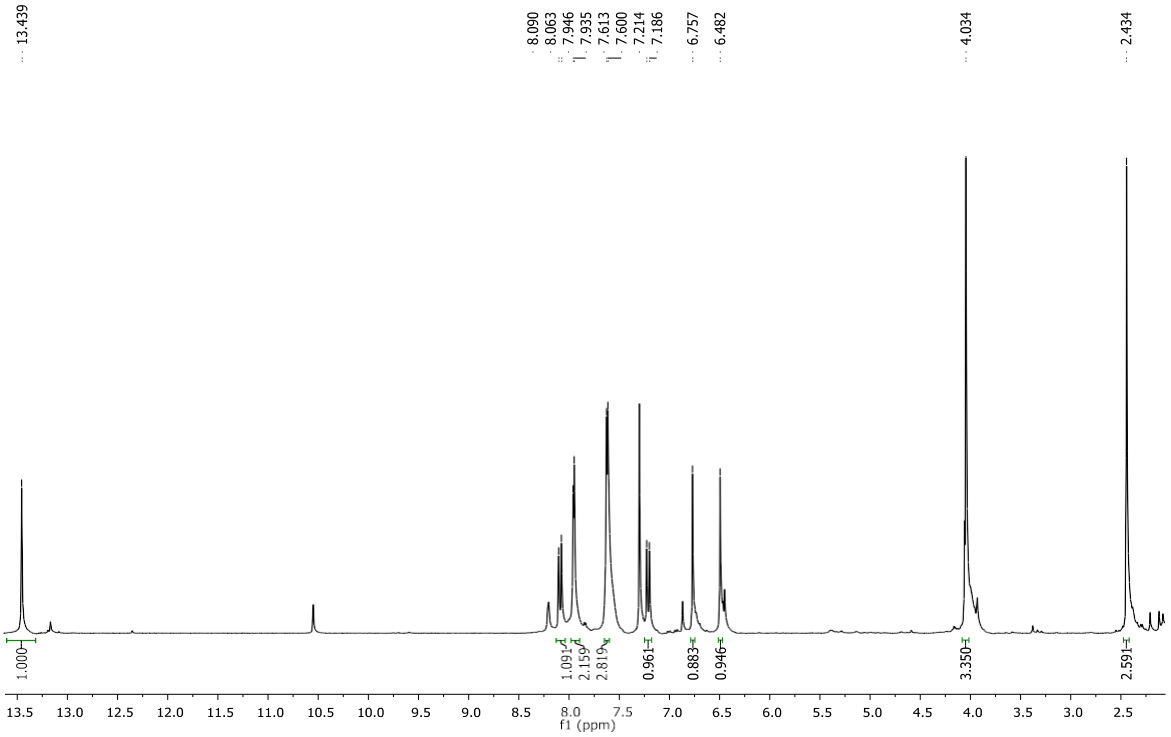


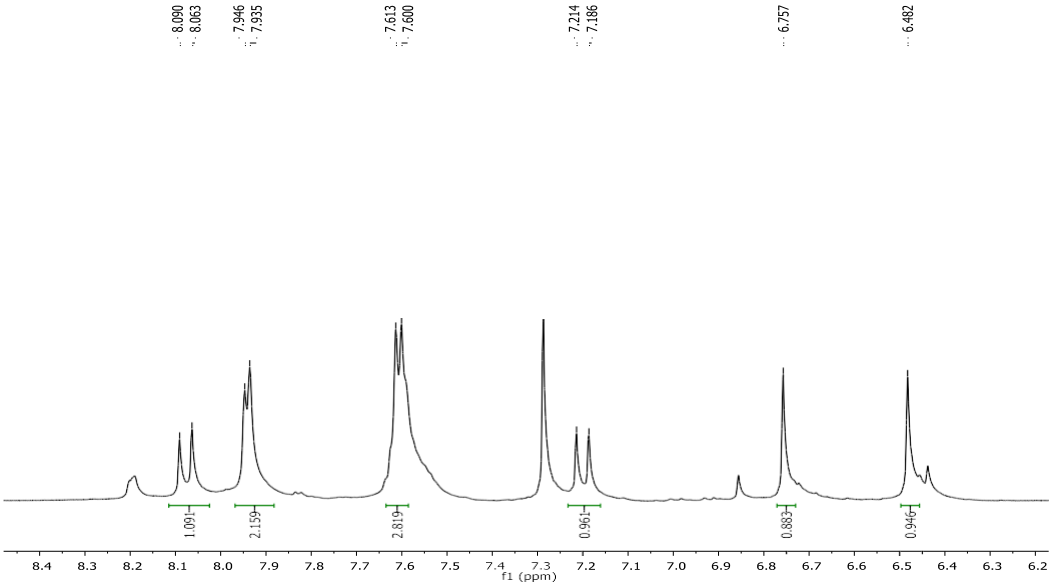
Appendix A7: UV SPECTRUM FOR COMPOUND 1



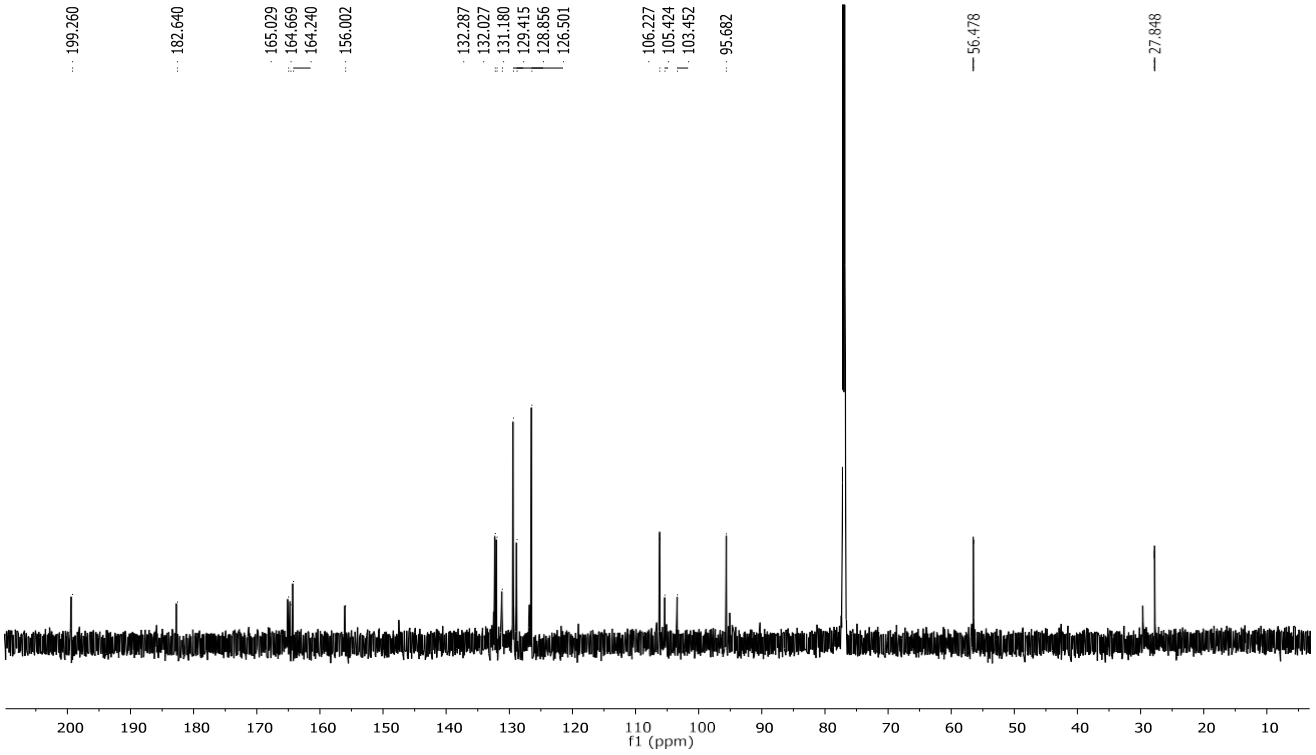
APPENDIX B: SPECTRA FOR COMPOUND 2

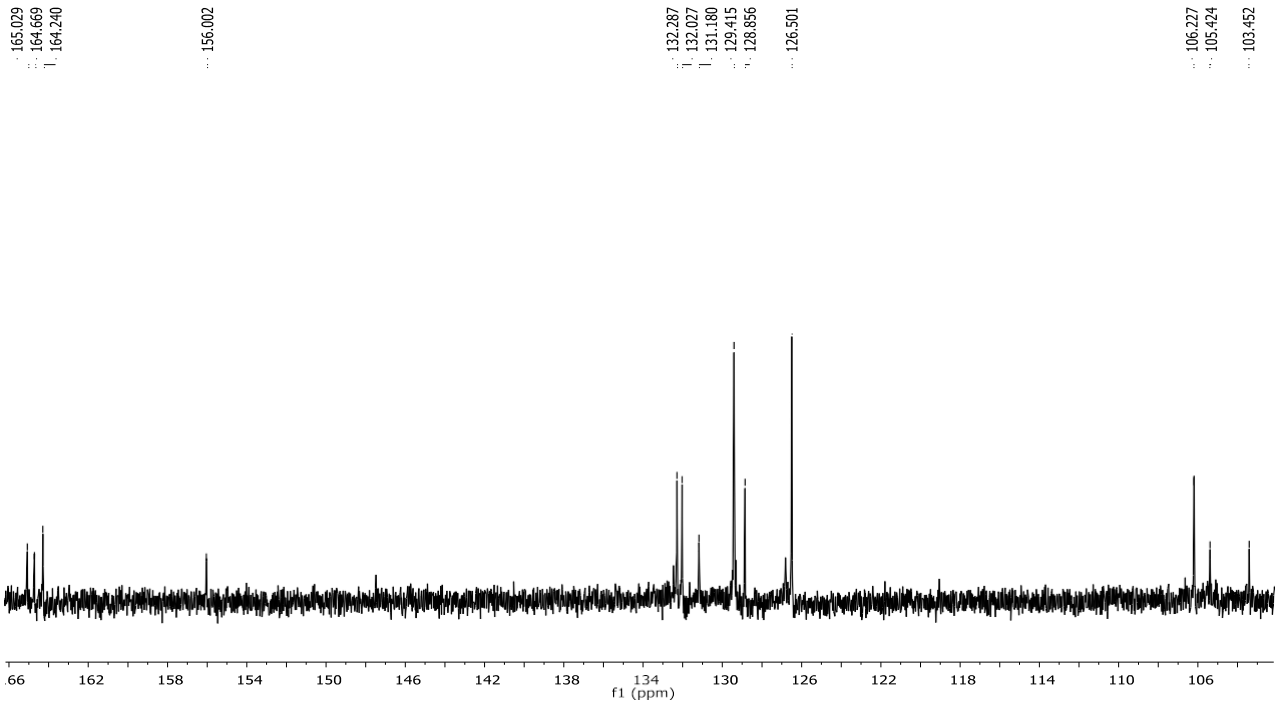
Appendix B1: ^{1}H NMR SPECTRUM (600 MHz, $CDCl_3$) EXPANSION FOR COMPOUND 2



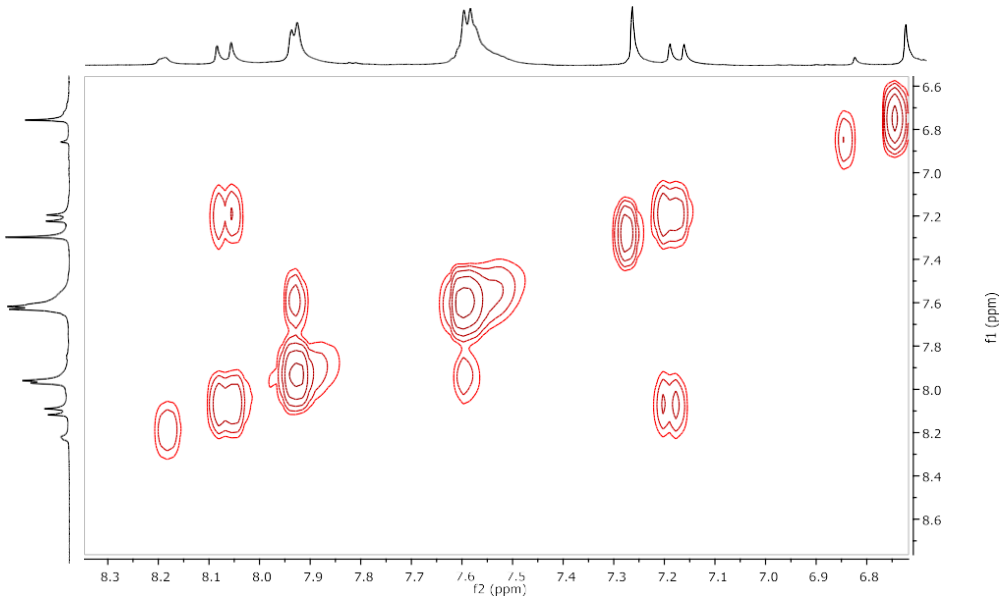


Appendix B2: ^{13}C NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 2

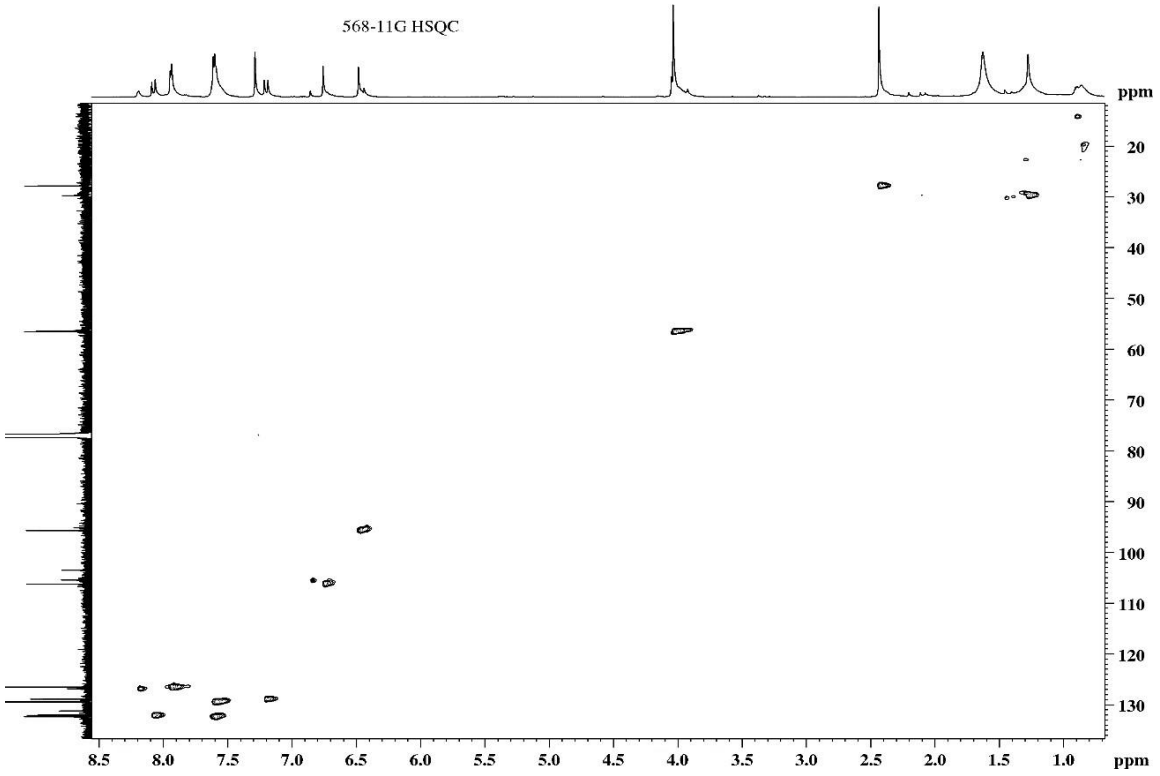


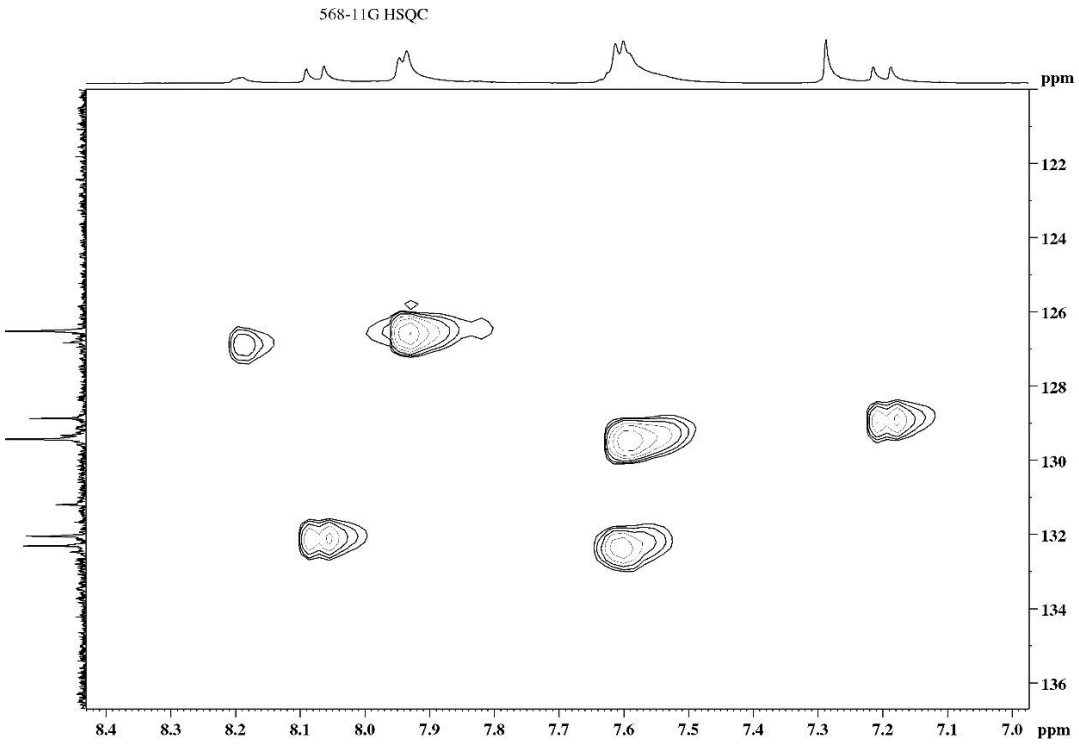


Appendix B3: ^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 2

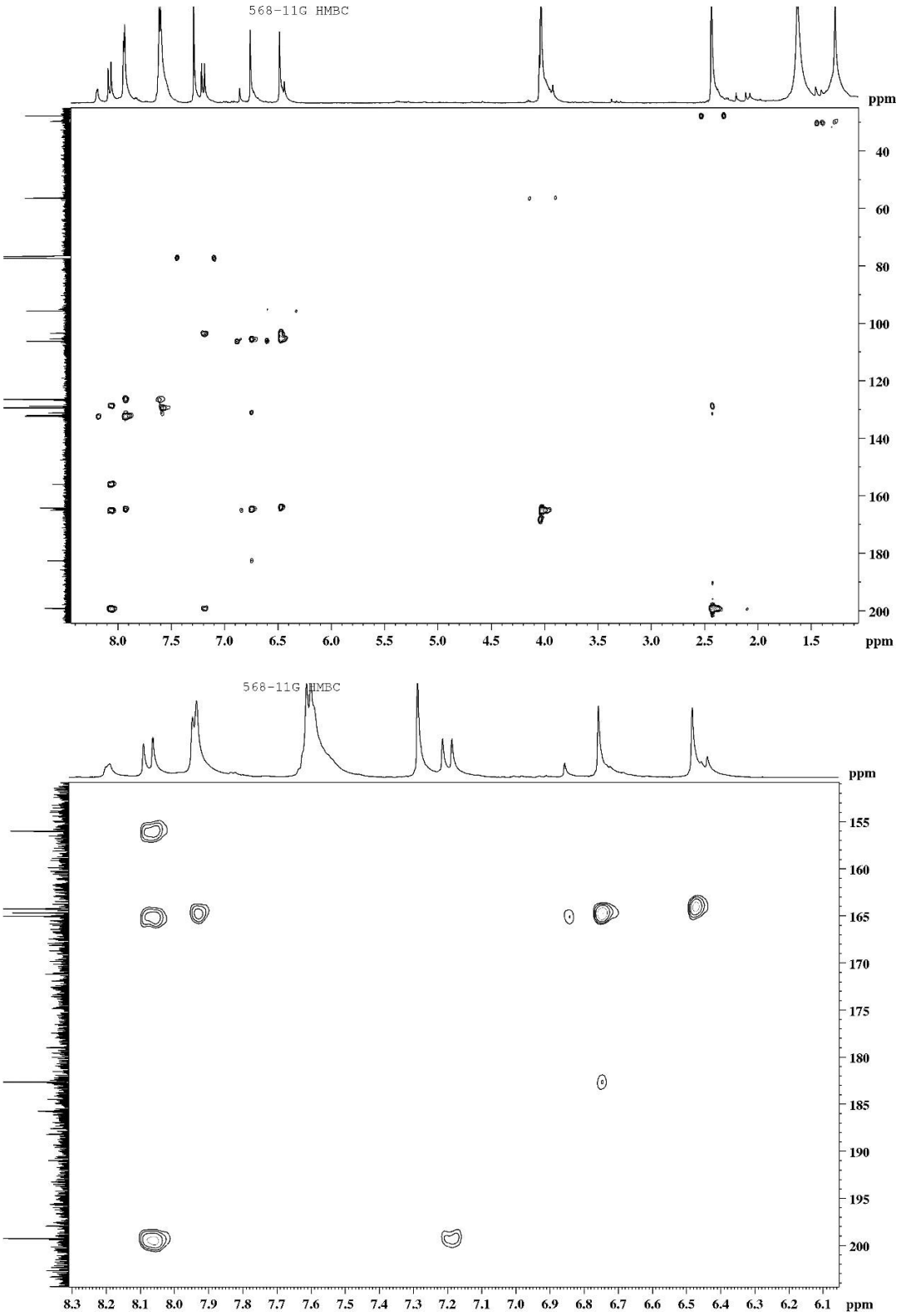


Appendix B4: HSQC SPECTRUM (EXPANSION) FOR COMPOUND 2





Appendix B5: HMBC SPECTRUM (EXPANSION) FOR COMPOUND 2



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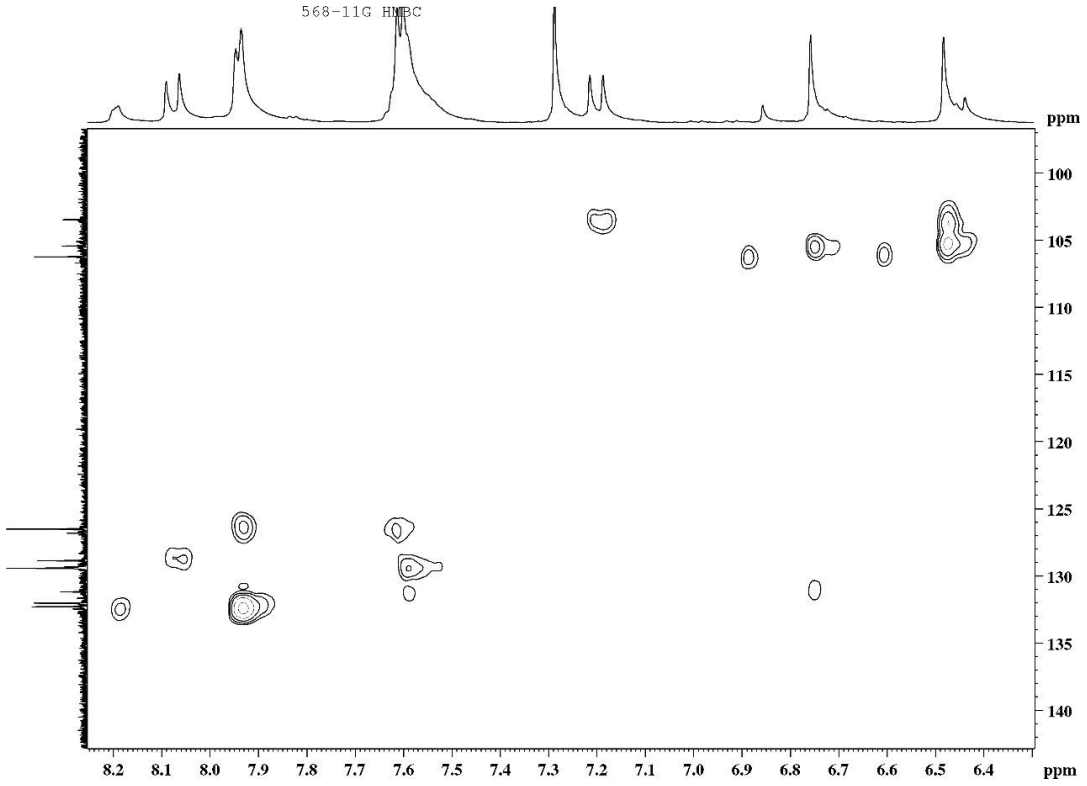
568-11G HMBC
ppm
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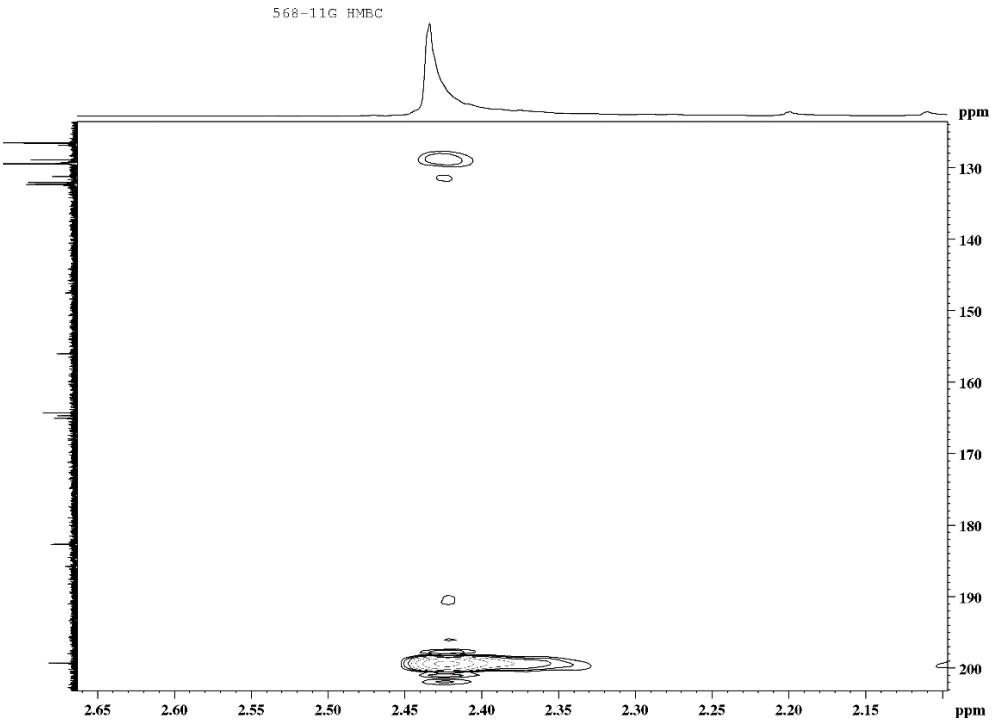
105
110
115
120
125
130
135
140
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
ppm


568-11G HMBC
ppm
130
140
150
160
170
180
190
200
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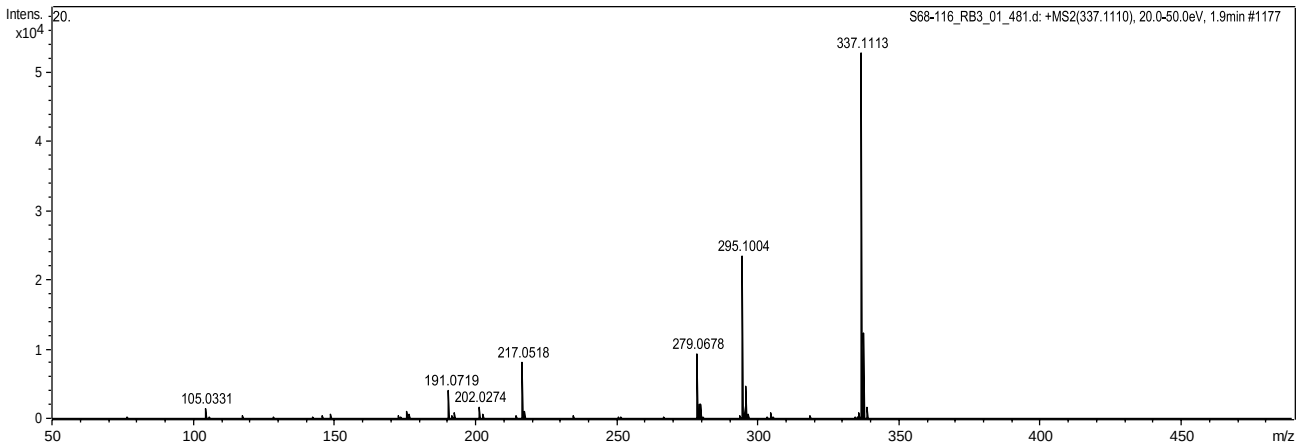
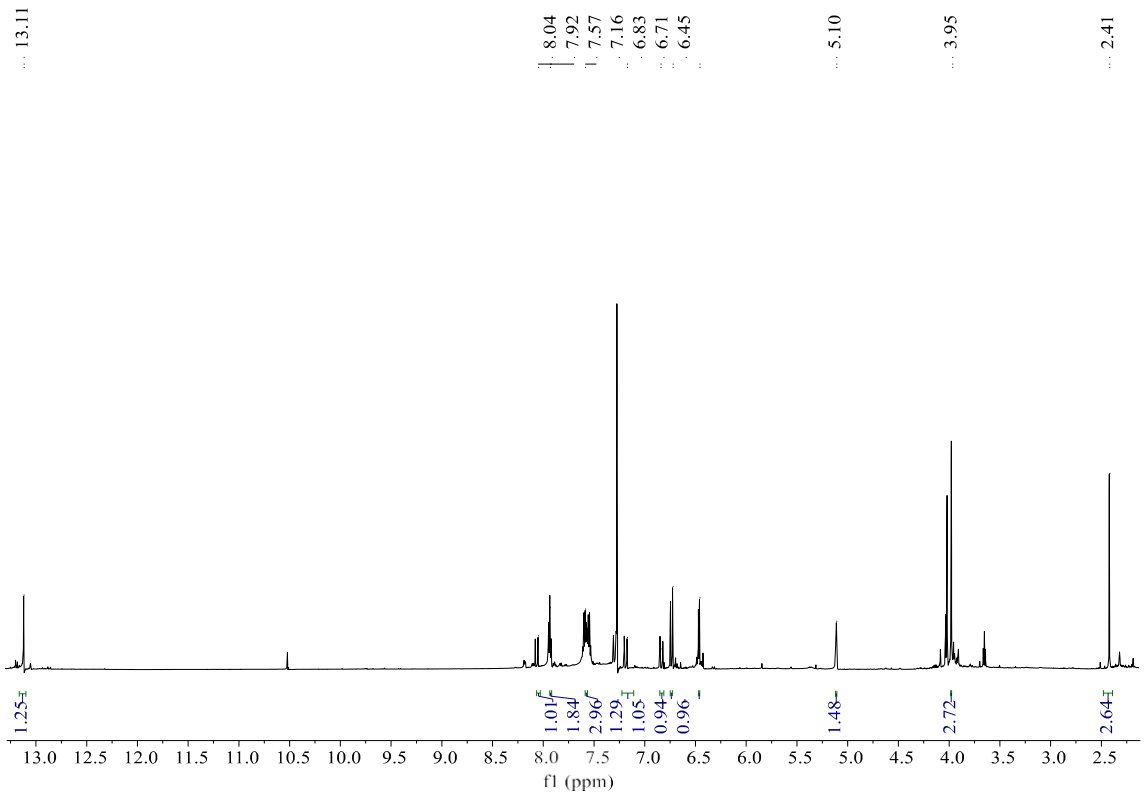
2.60
2.55
2.50
2.45
2.40
2.35
2.30
2.25
2.20
2.15
ppm

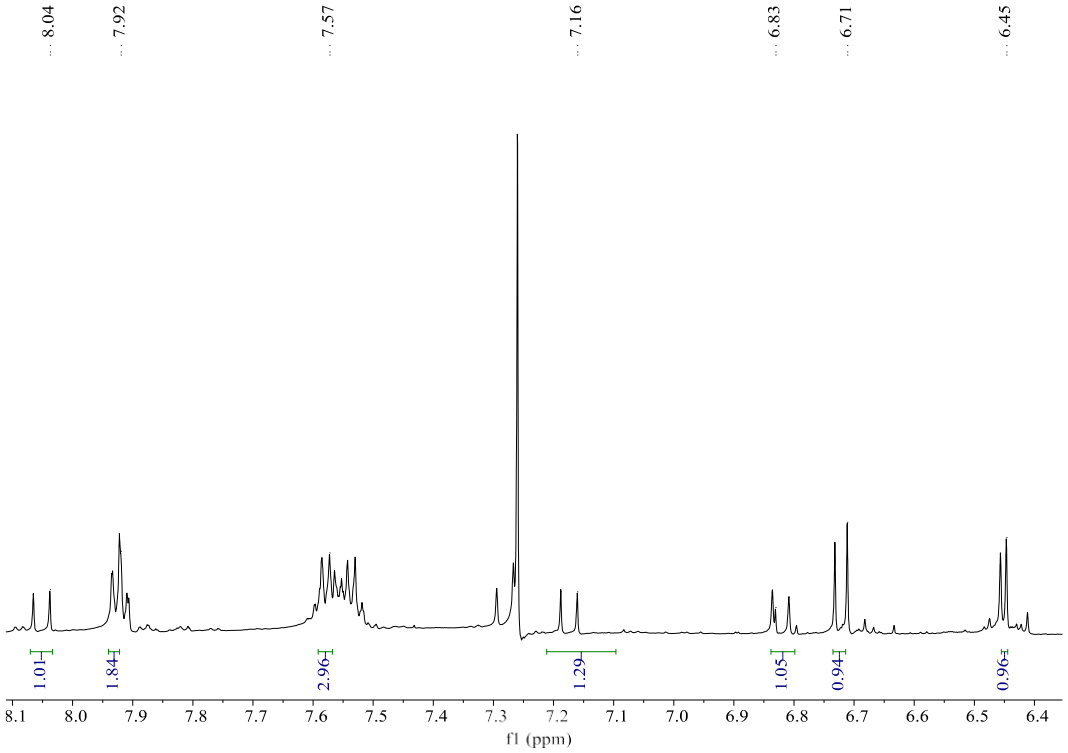
Appendix B6: MASS SPECTRUM FOR COMPOUND 2



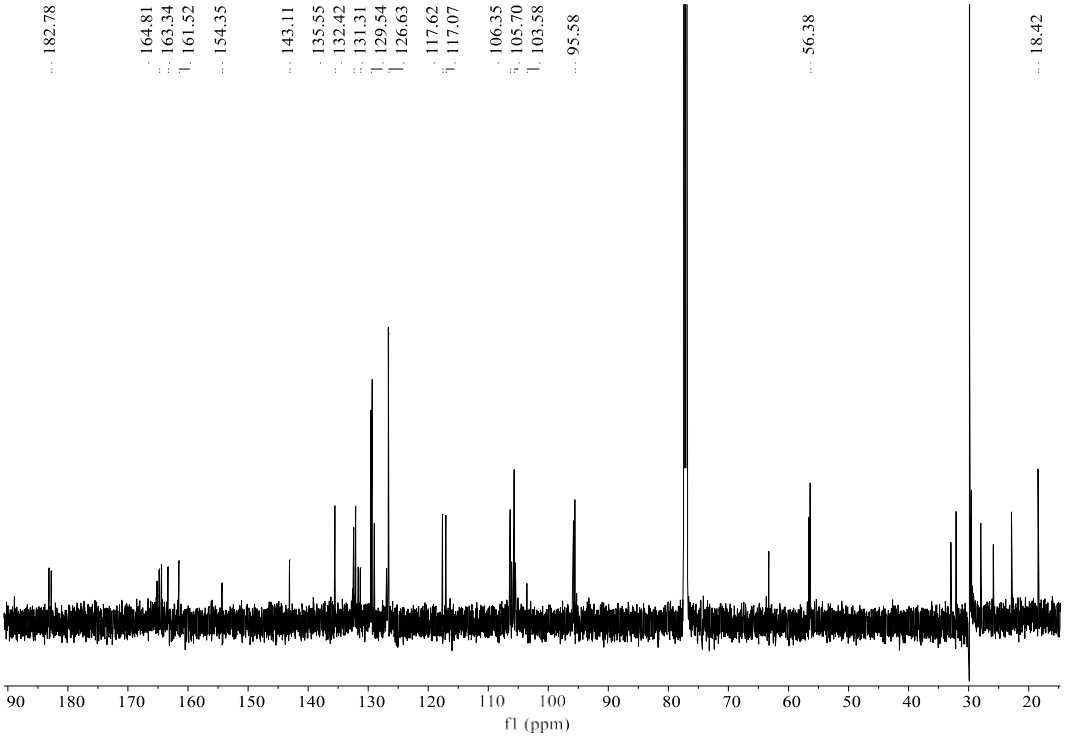
APPENDIX C: SPECTRA FOR COMPOUND 3

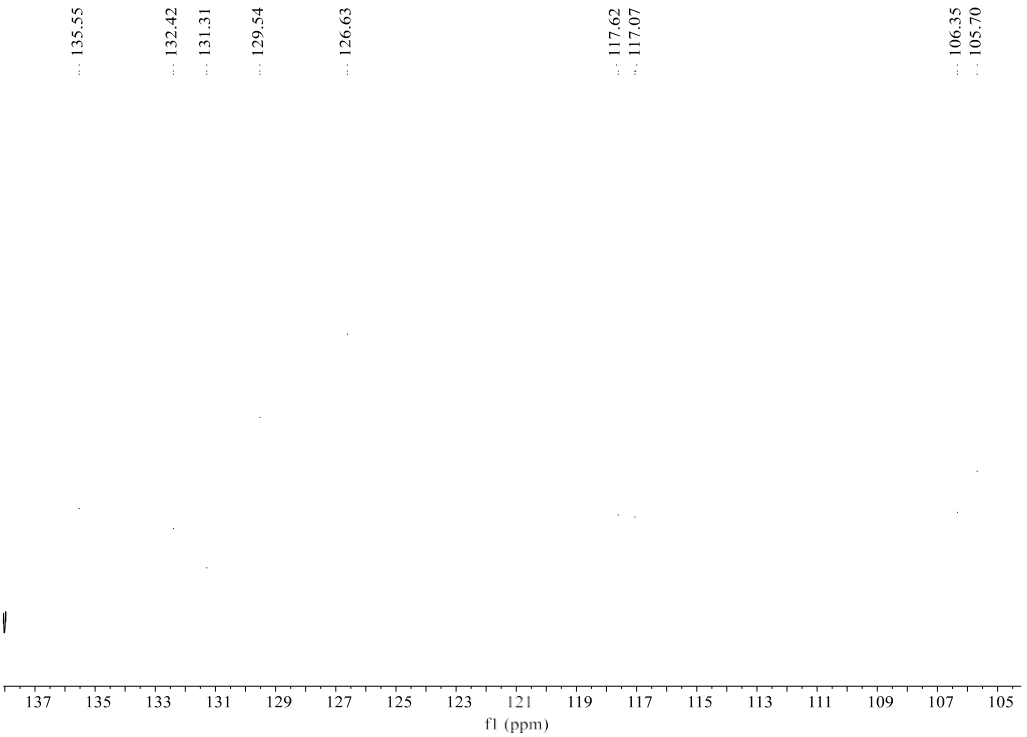
Appendix C1: ^{1}H NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 3



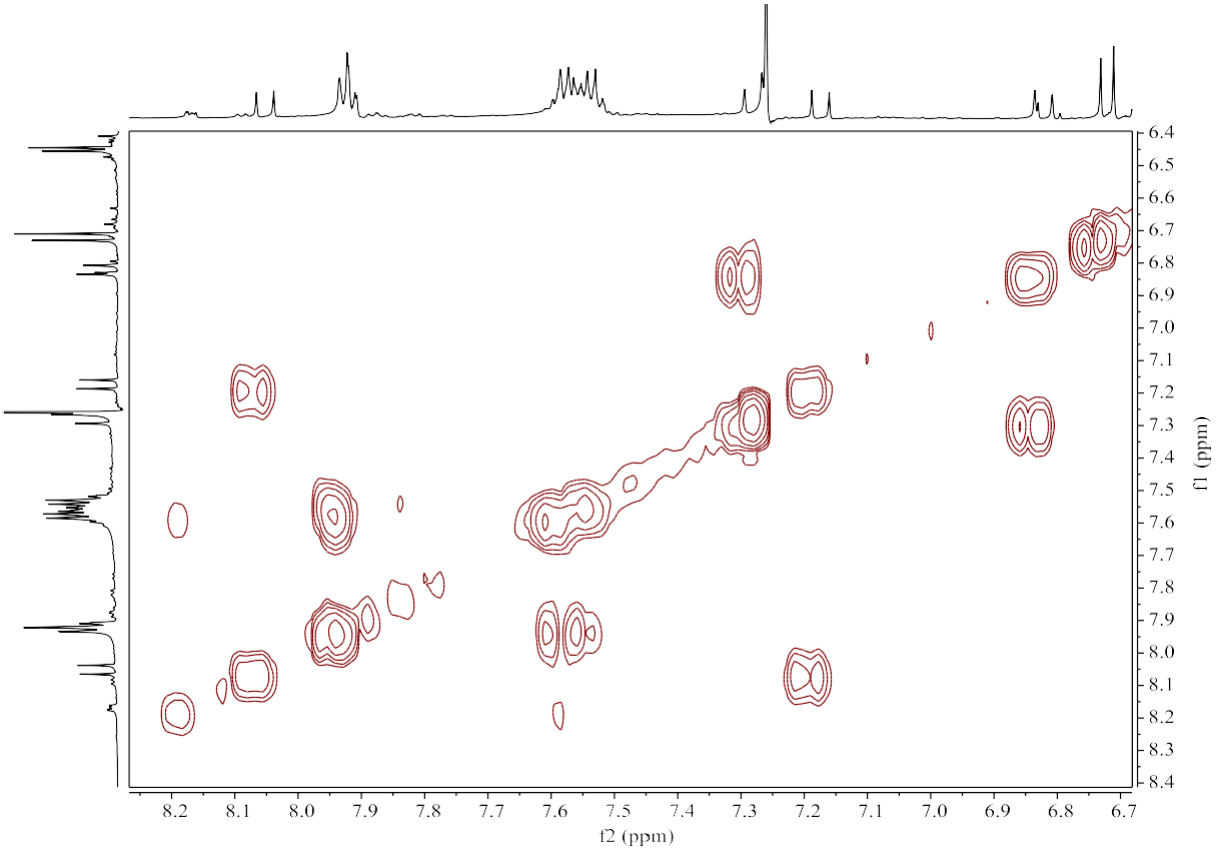


Appendix C2: ^{13}C NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 3

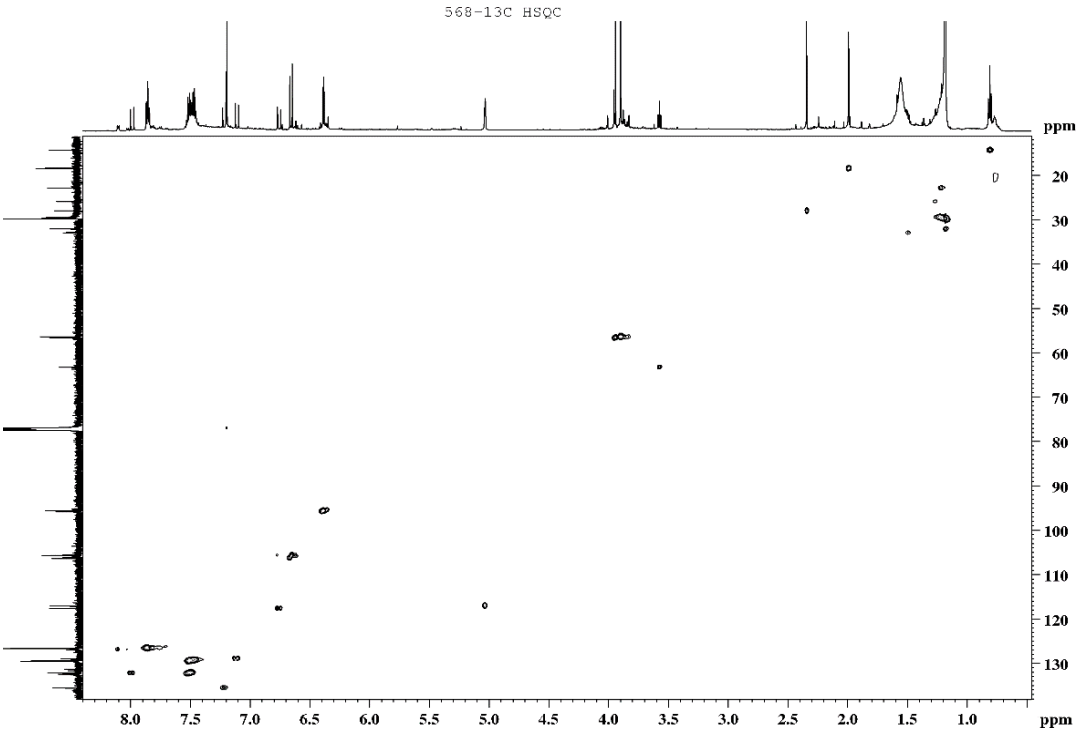


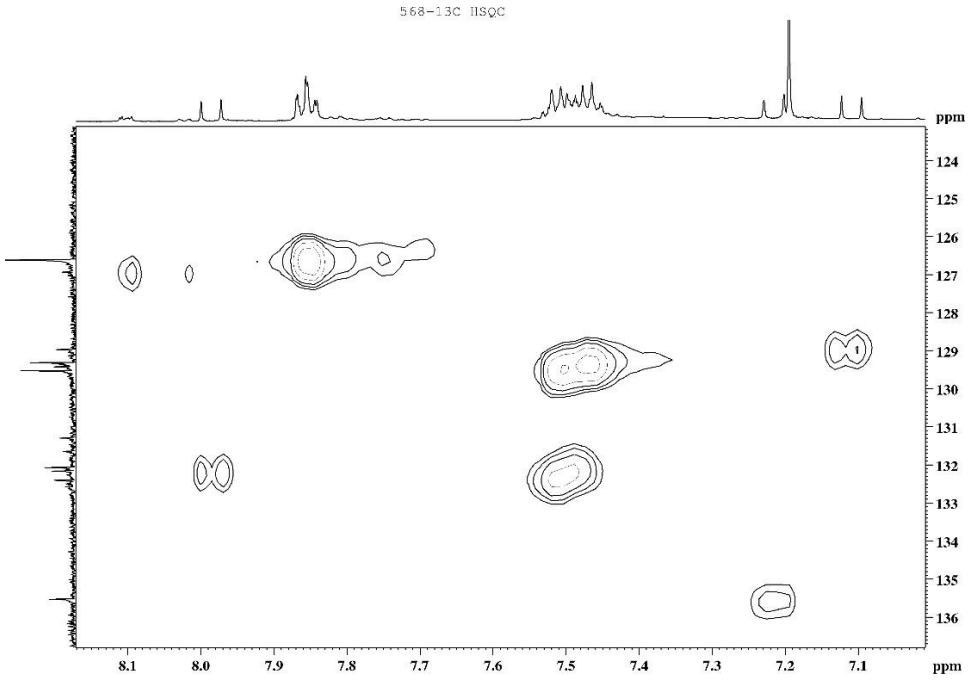


Appendix C3: ^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 3

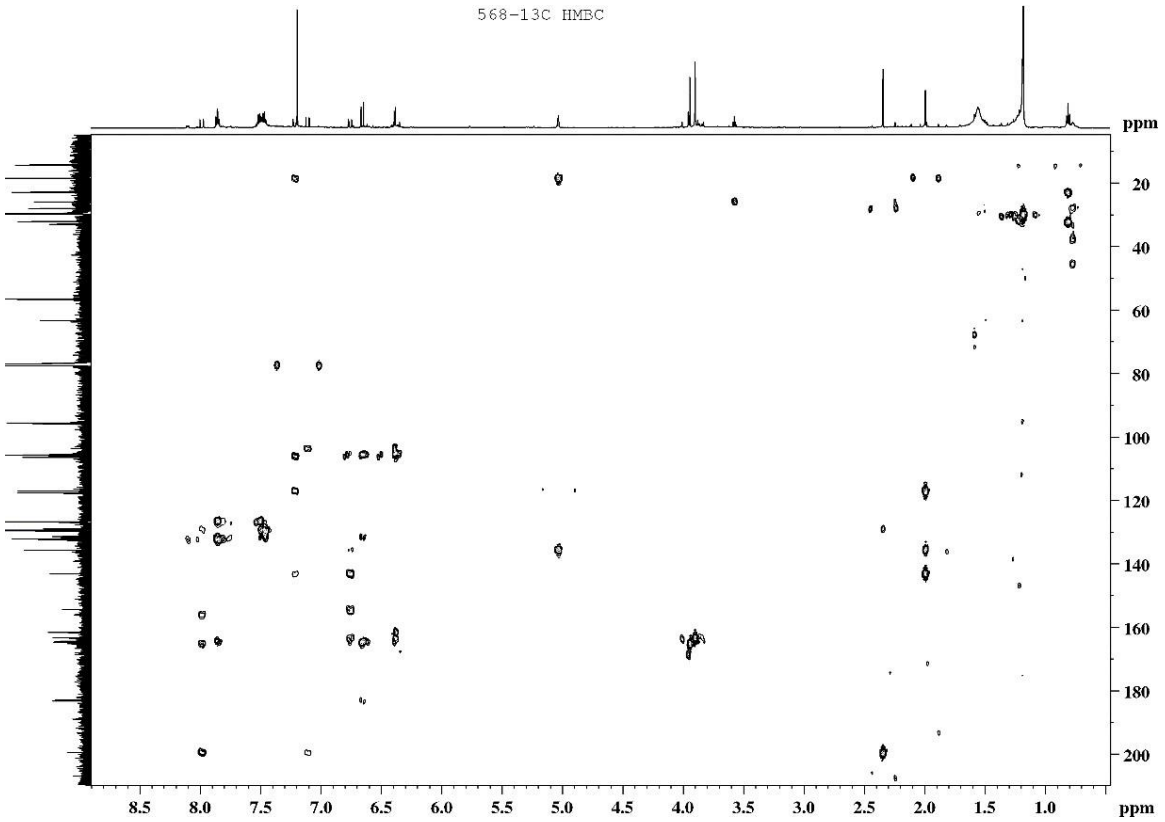


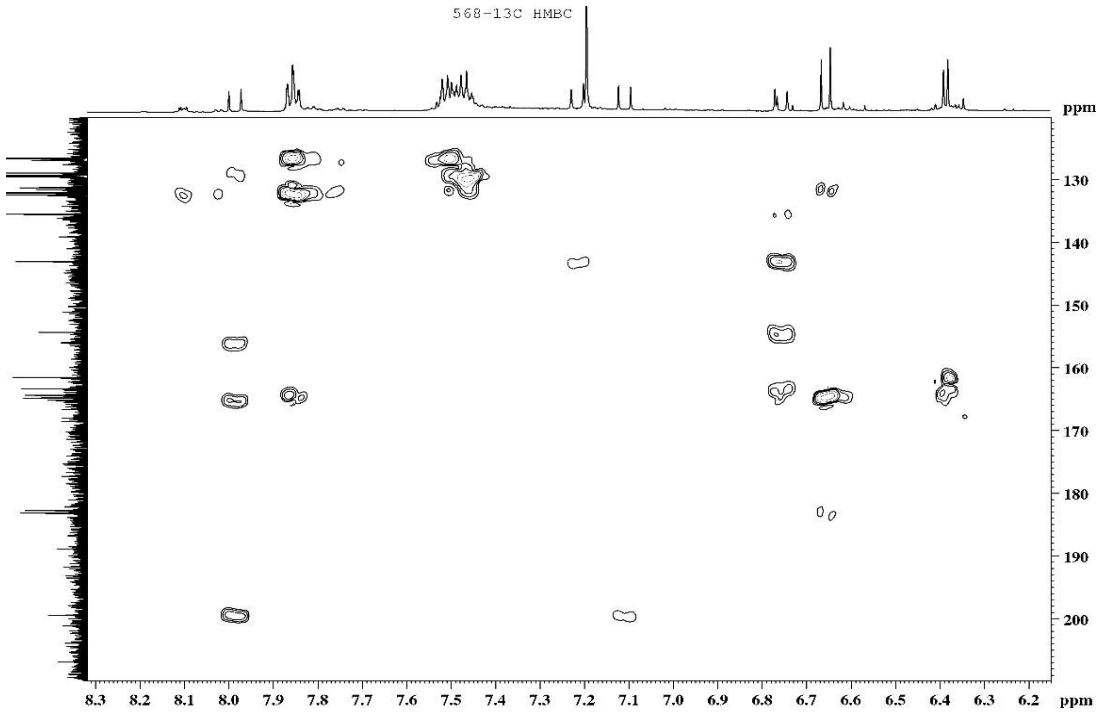
Appendix C4: HSQC SPECTRUM FOR COMPOUND 3





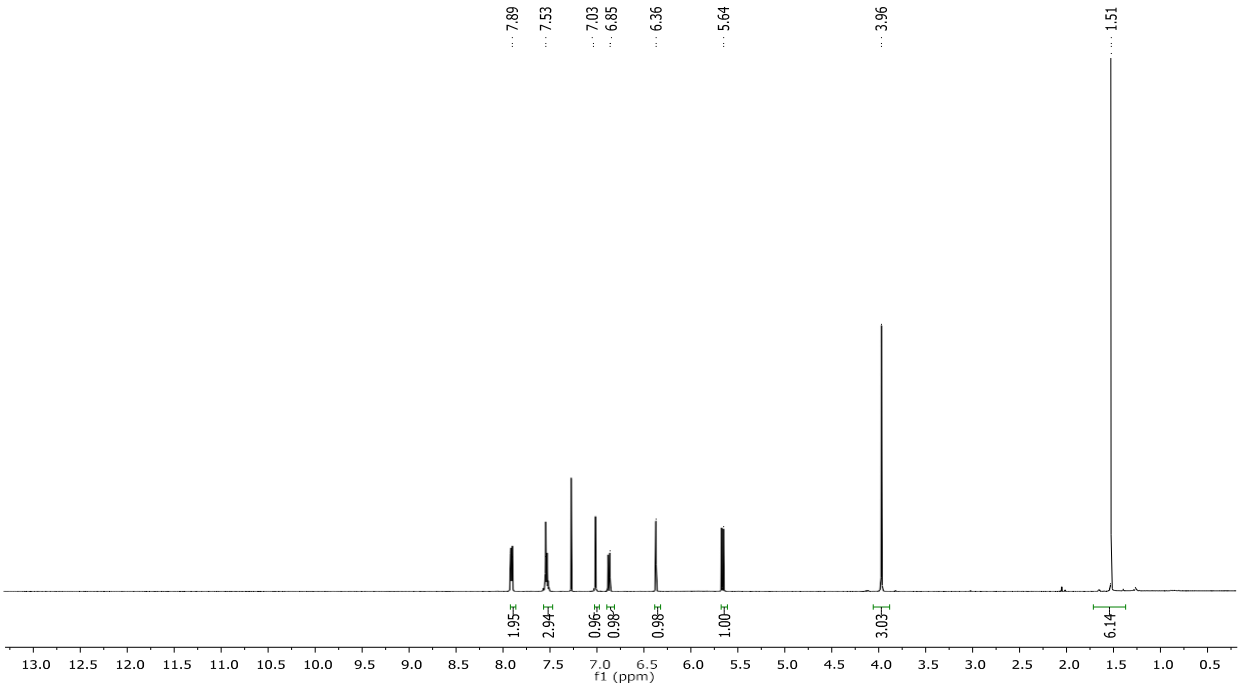
Appendix C5: HMBC SPECTRUM FOR COMPOUND 3

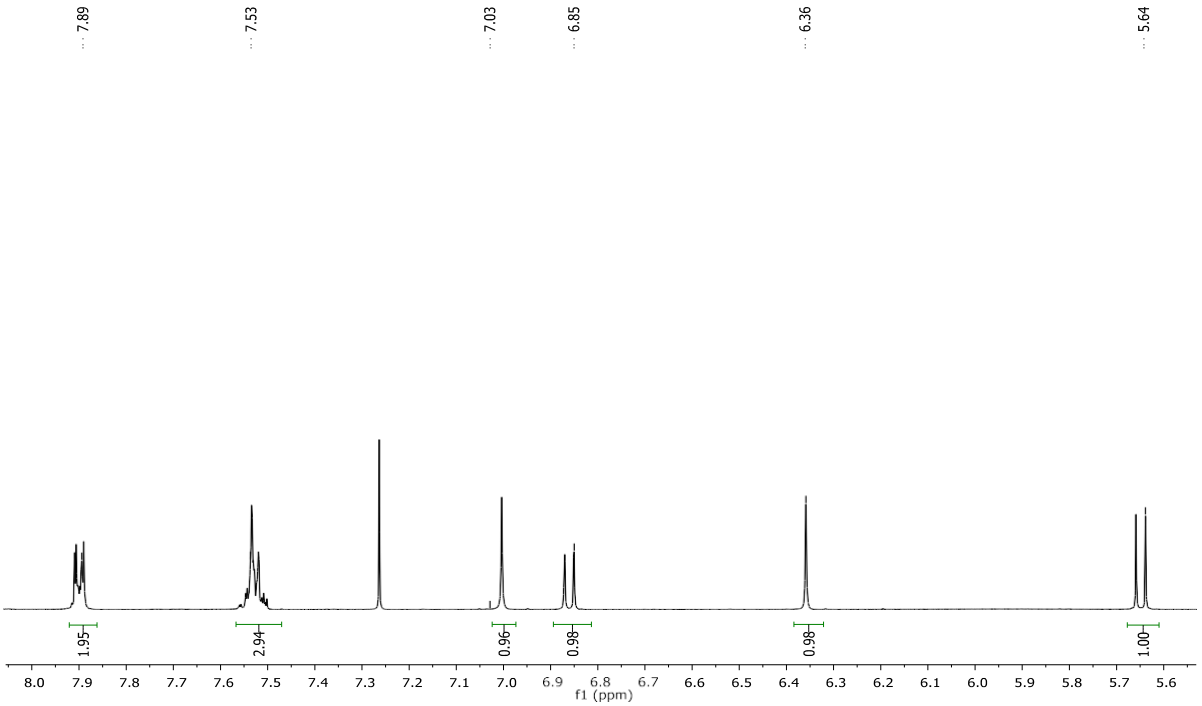




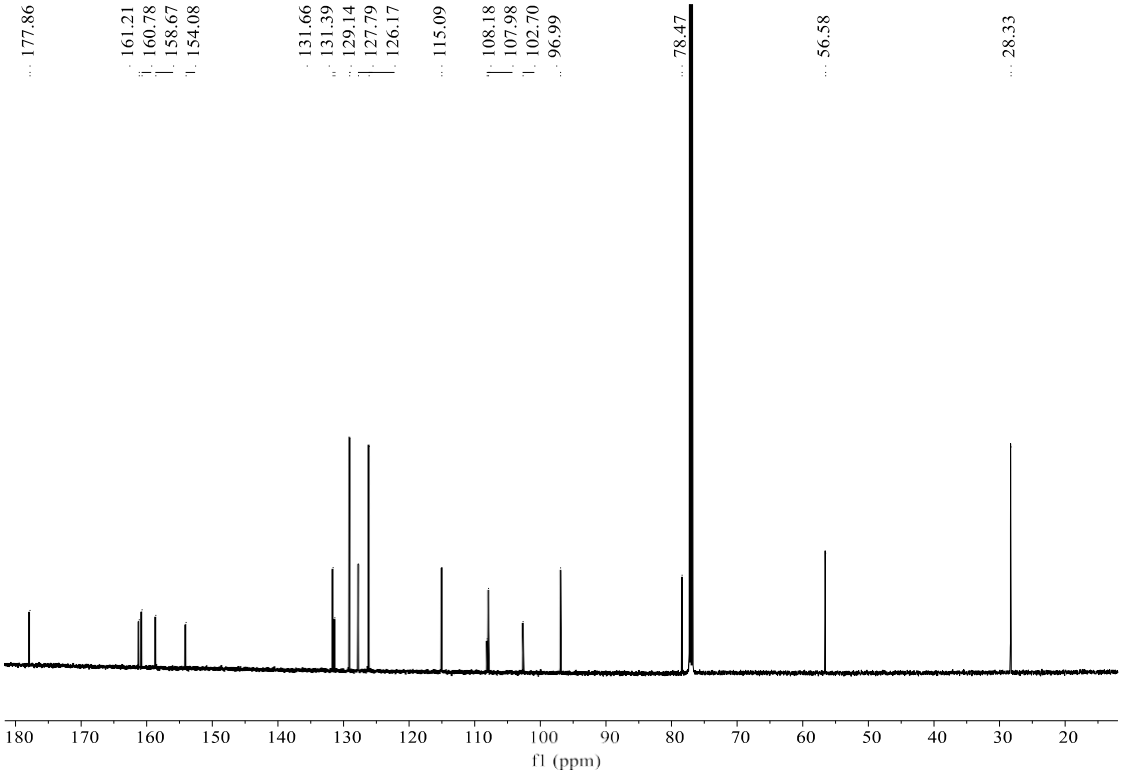
APPENDIX D: SPECTRA FOR COMPOUND 4

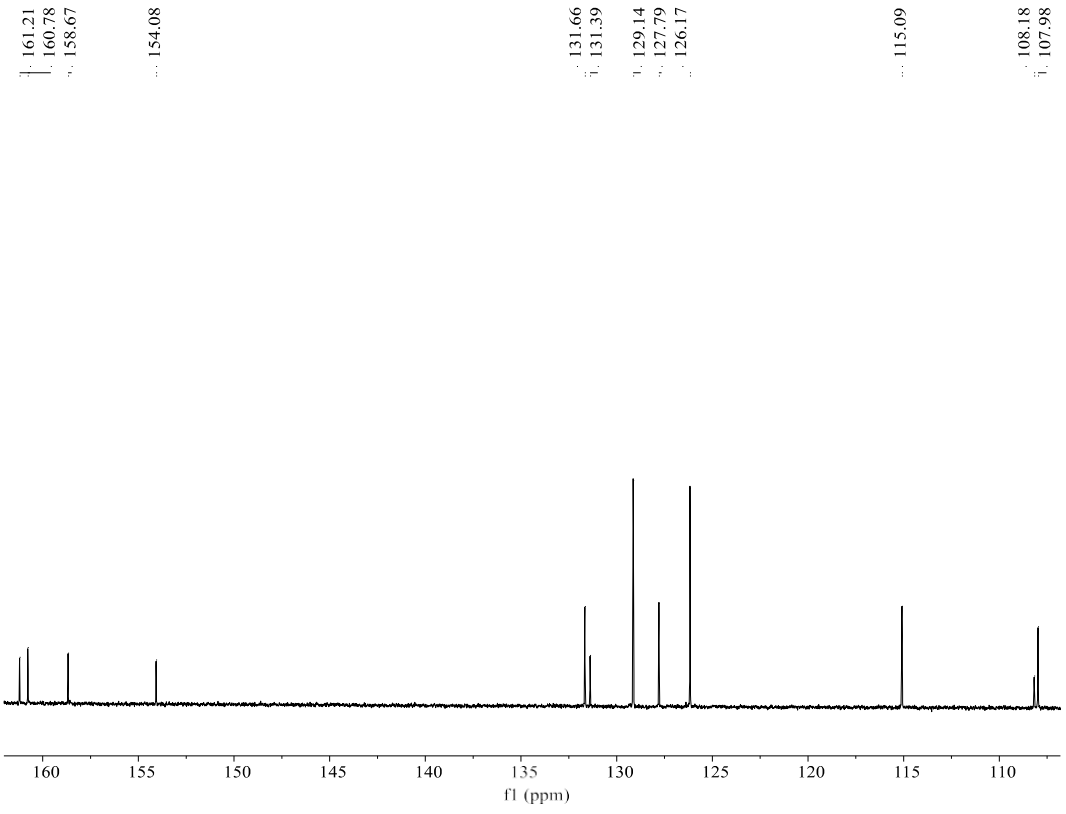
Appendix D1: ^{1}H NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 4



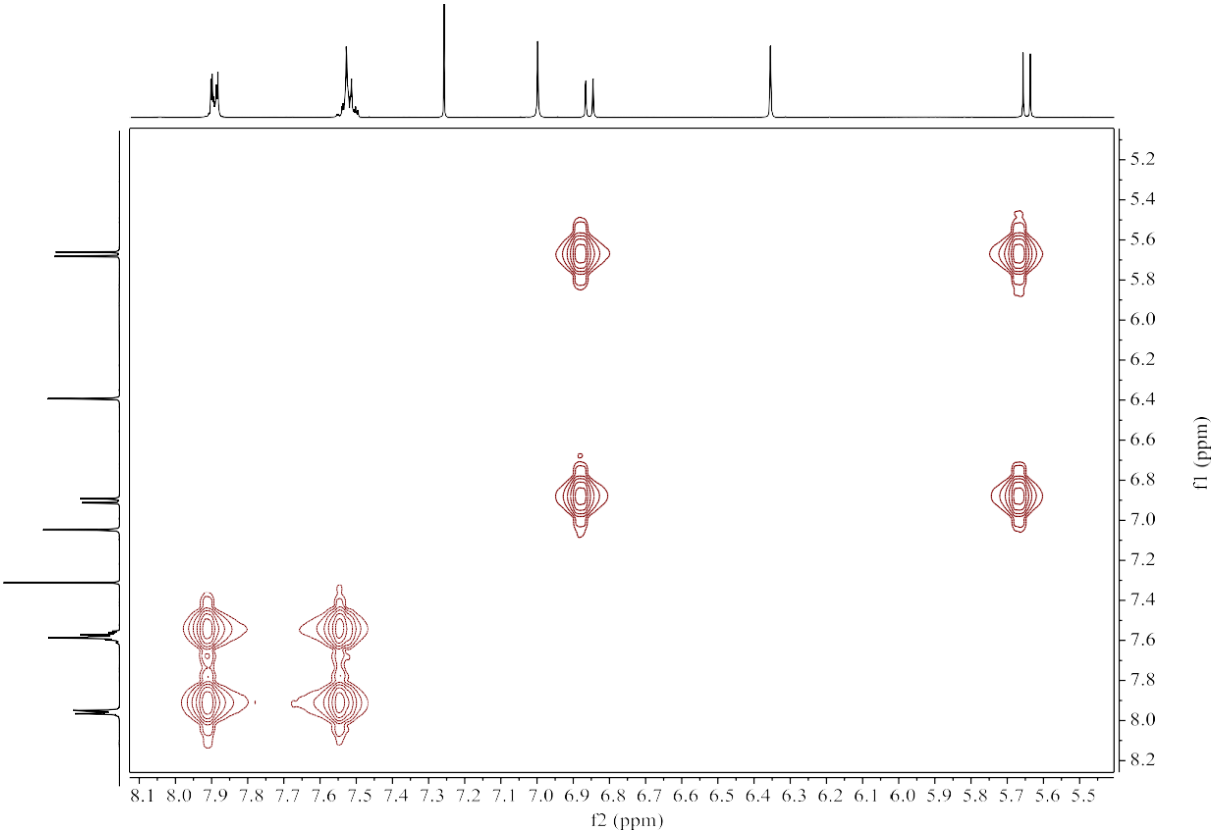


Appendix D2: ^{13}C NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 4

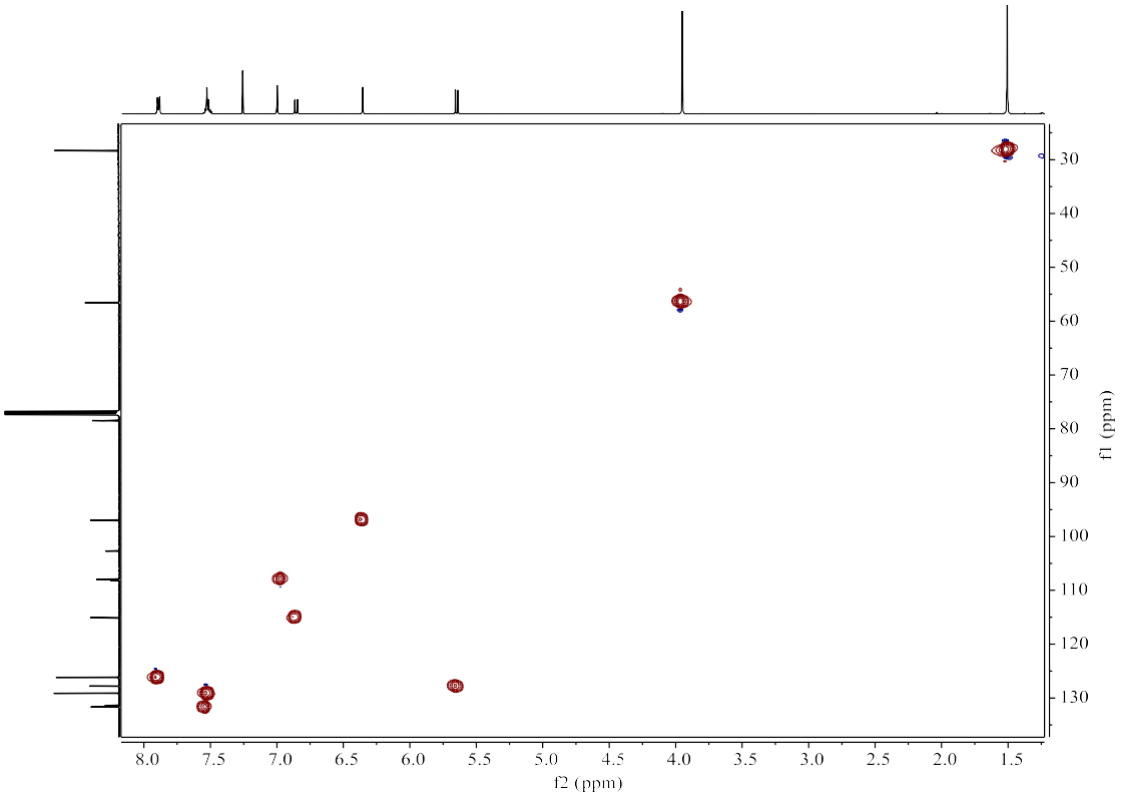


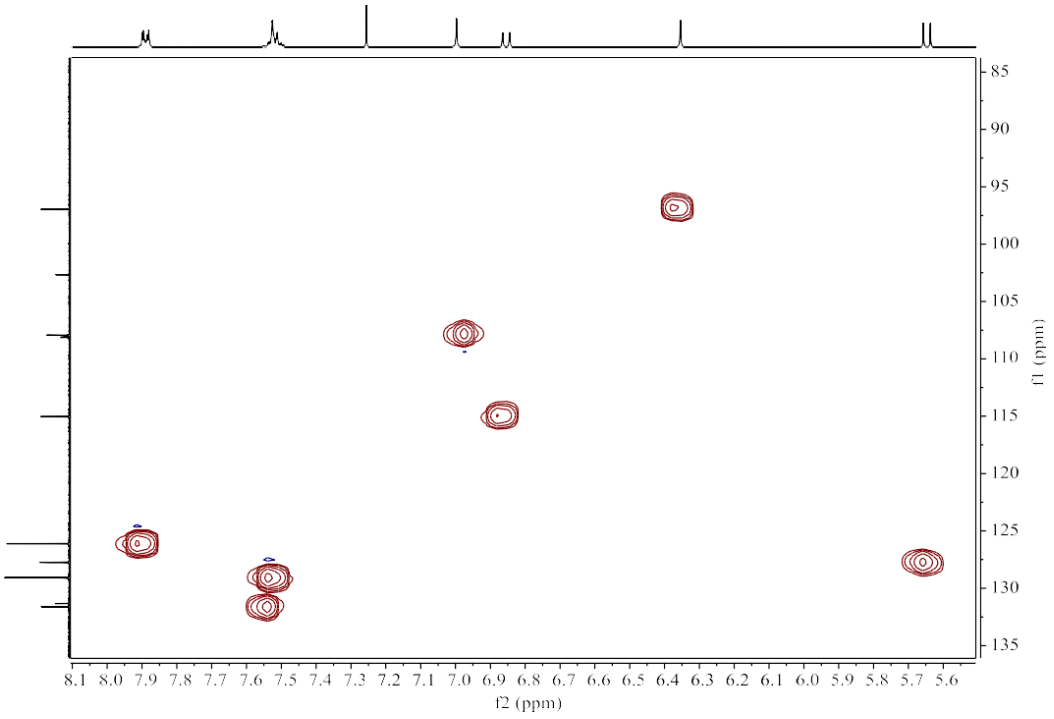


Appendix D3: ^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 4

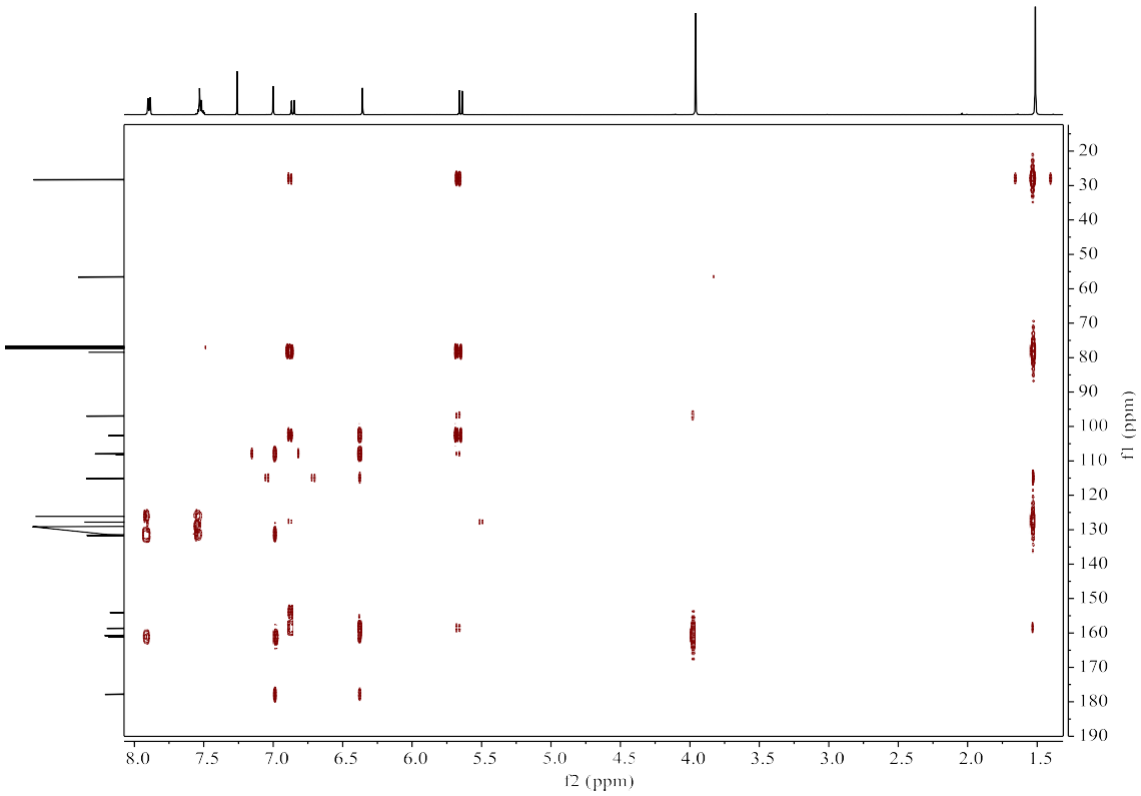


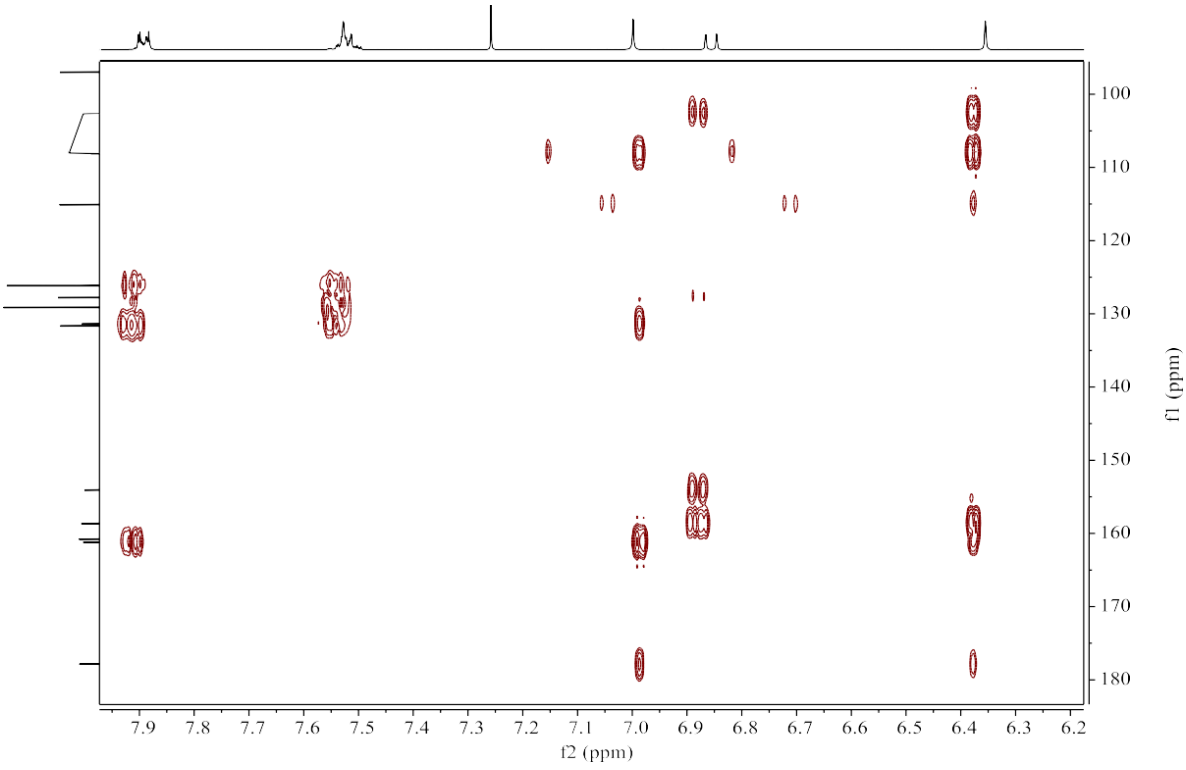
Appendix D4: HSQC COSY SPECTRUM FOR COMPOUND 4





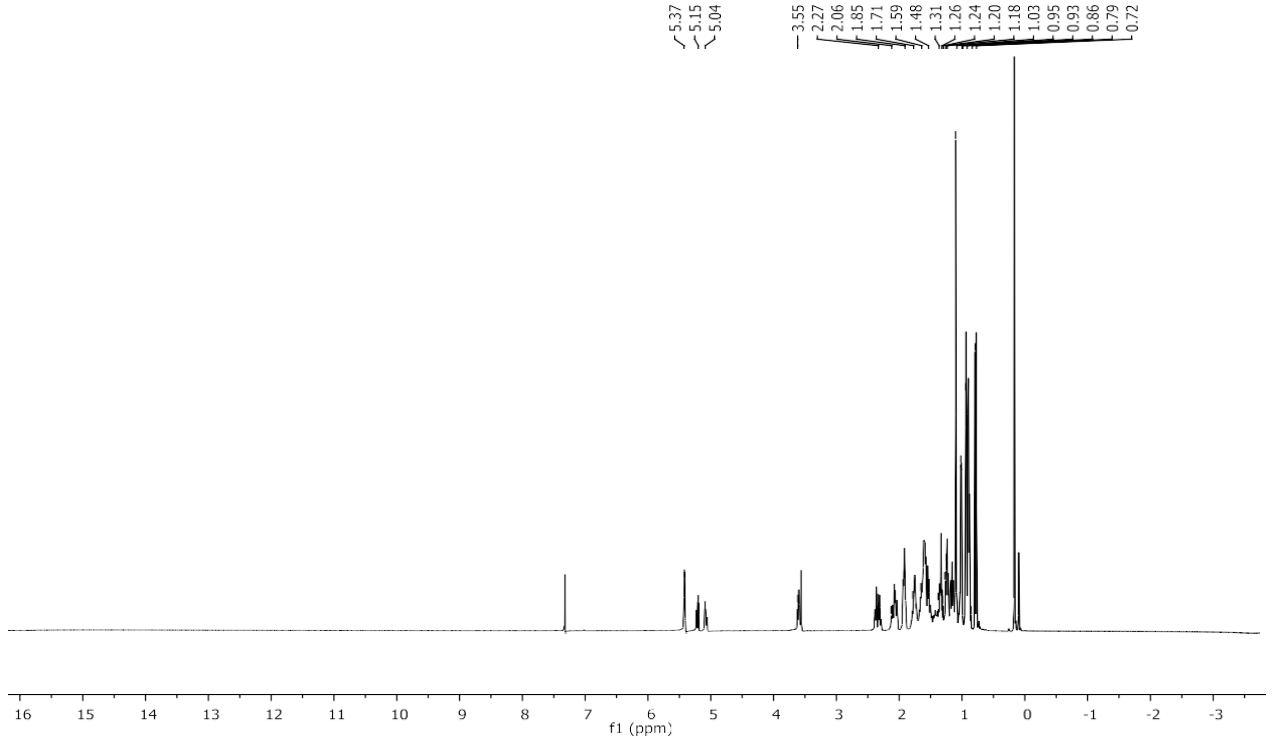
Appendix D5: HMBC COSY SPECTRUM FOR COMPOUND 4



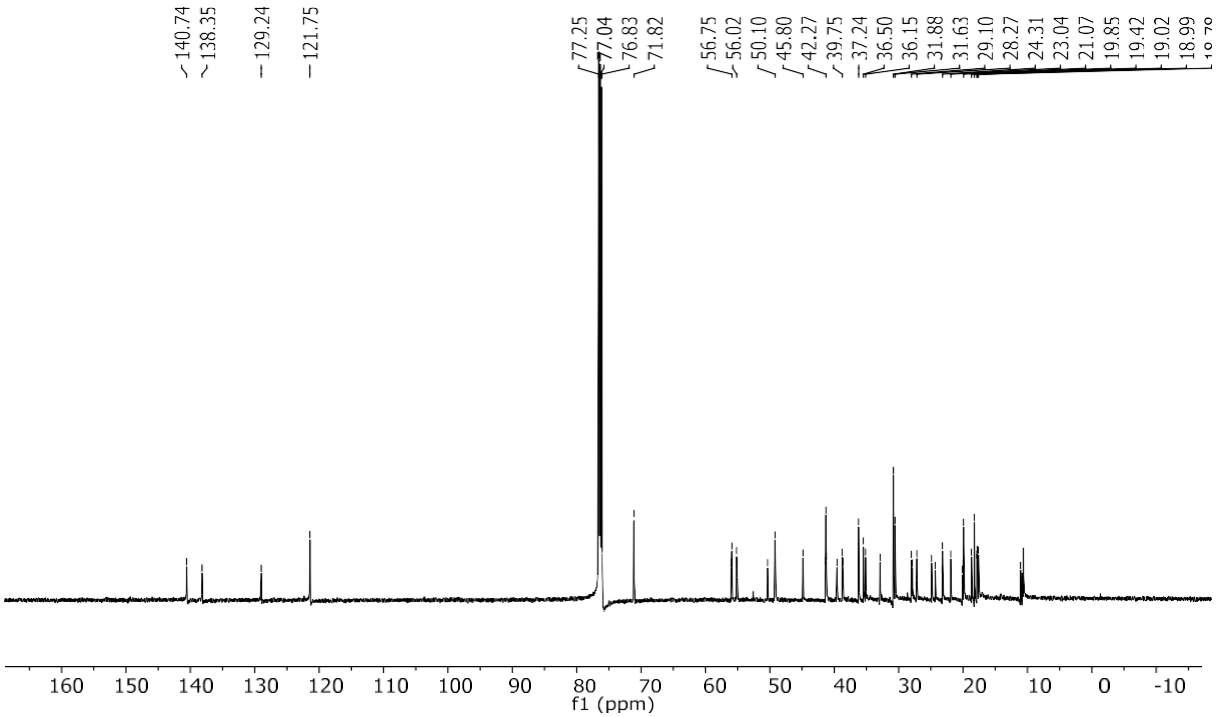


APPENDIX E: SPECTRA FOR COMPOUND 5

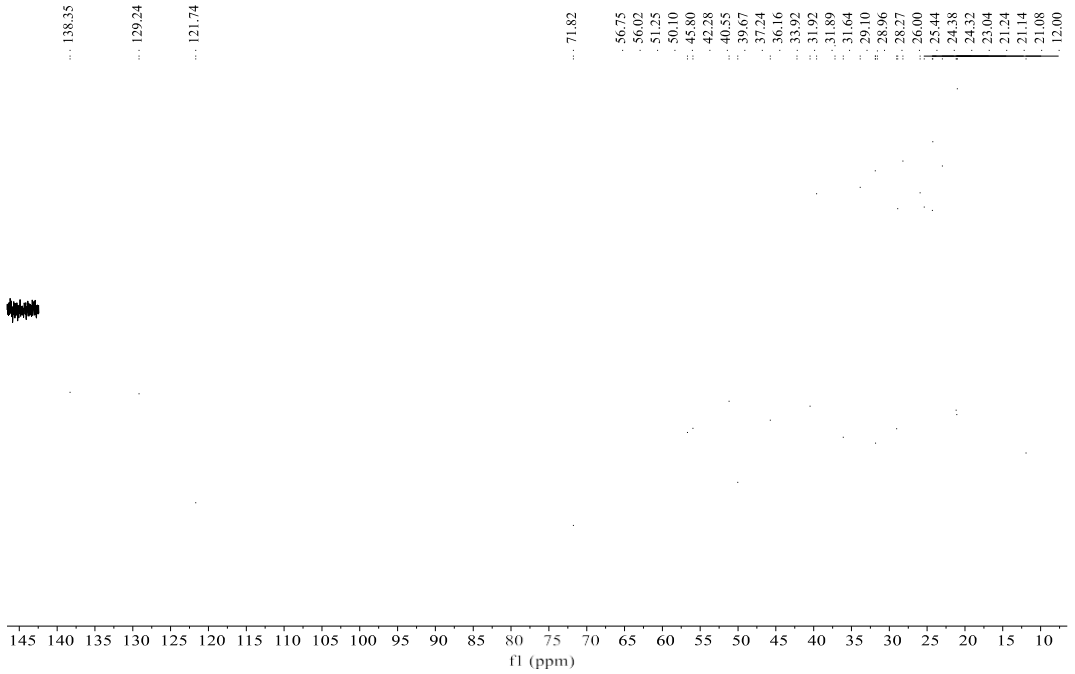
Appendix E1: ^{1}H NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 5



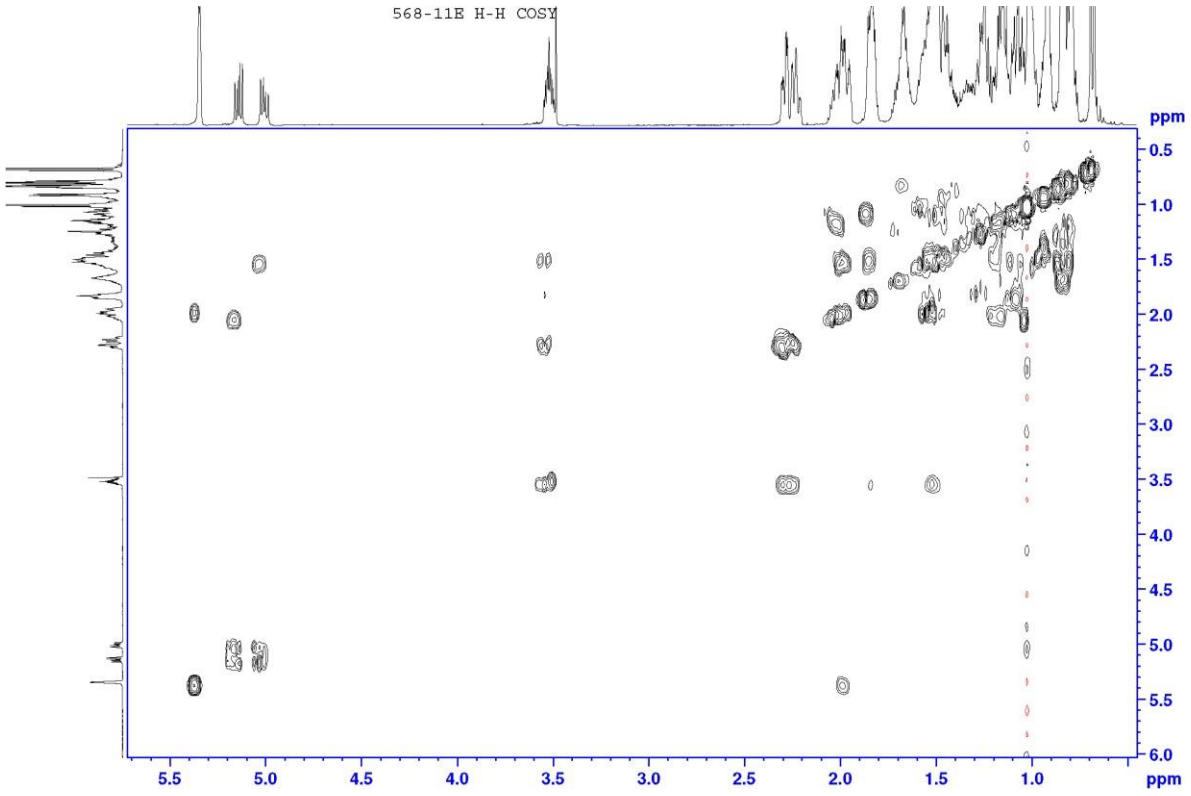
Appendix E2: ^{13}C NMR SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 5



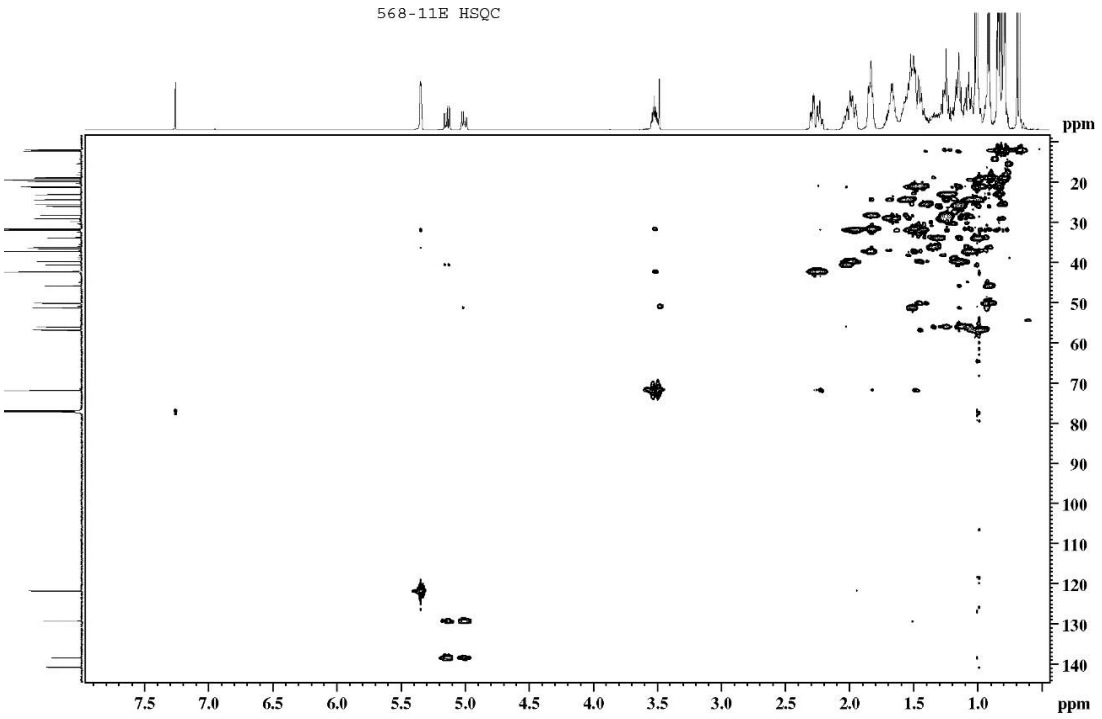
Appendix E3: DEPT 135 SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 5

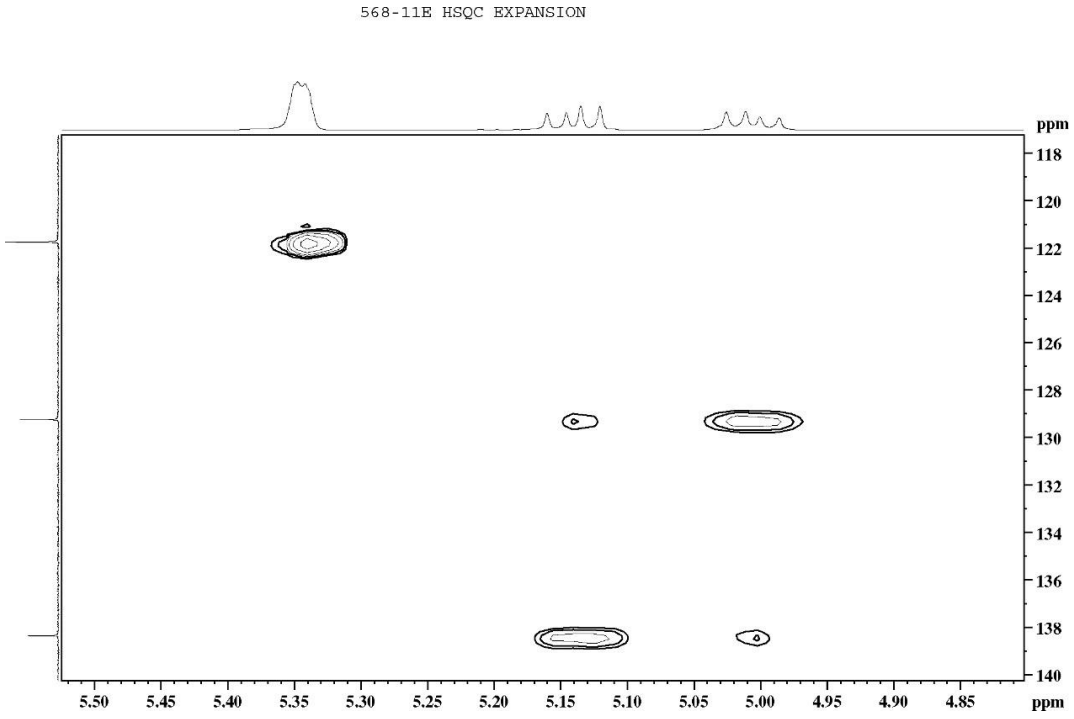


Appendix E4: ^{1}H-^{1}H COSY SPECTRUM FOR COMPOUND 5

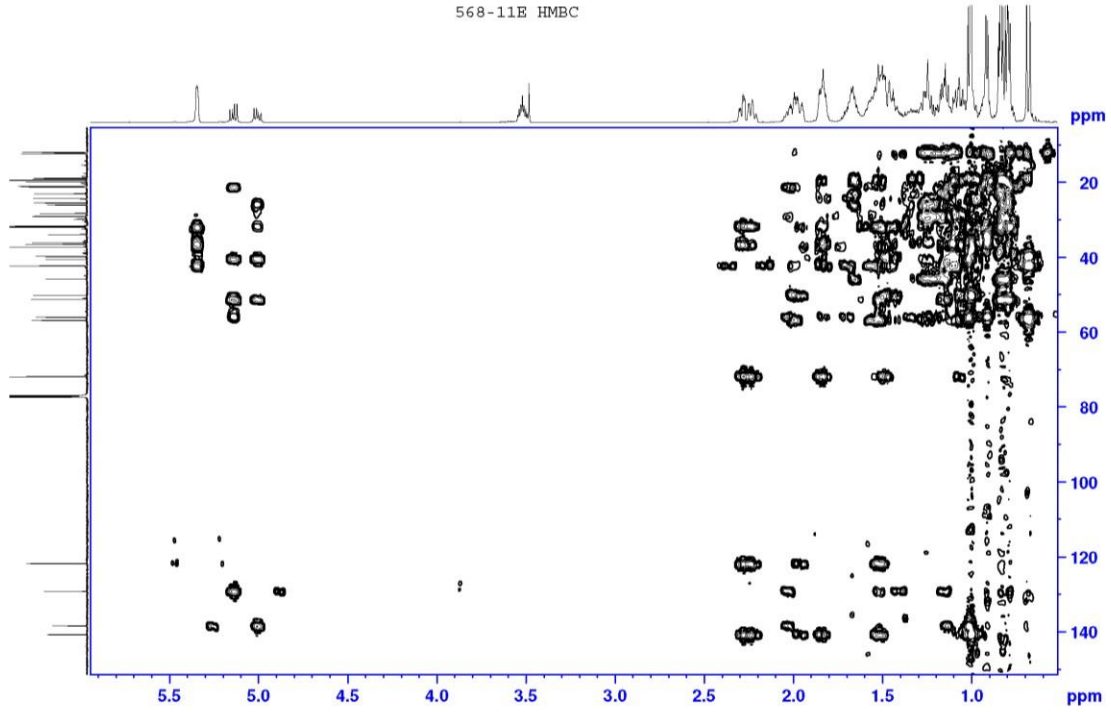


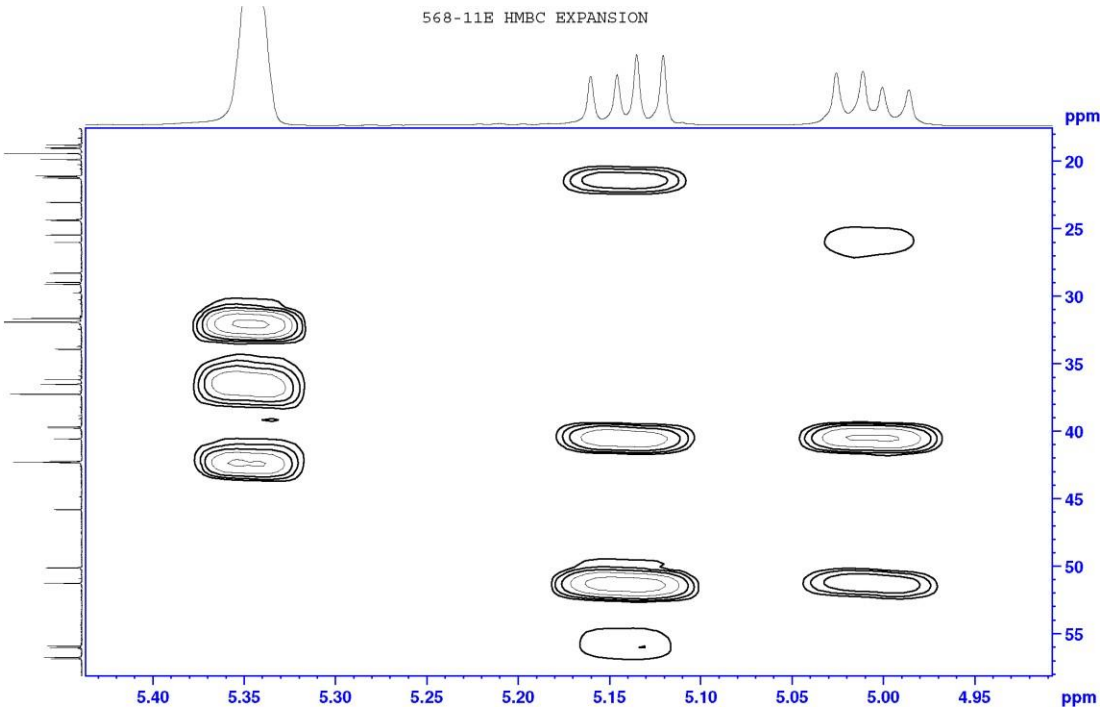
Appendix E5: HSQC SPECTRUM FOR COMPOUND 5





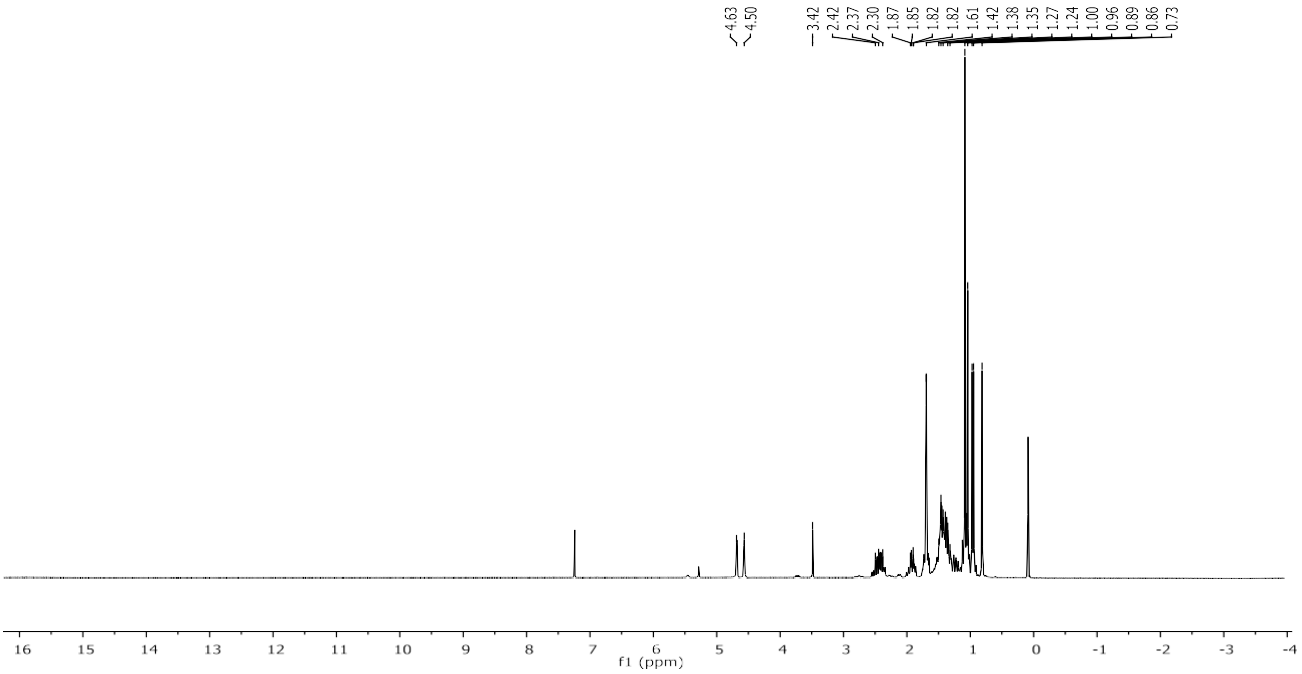
Appendix E6: HMBC SPECTRUM FOR COMPOUND 5



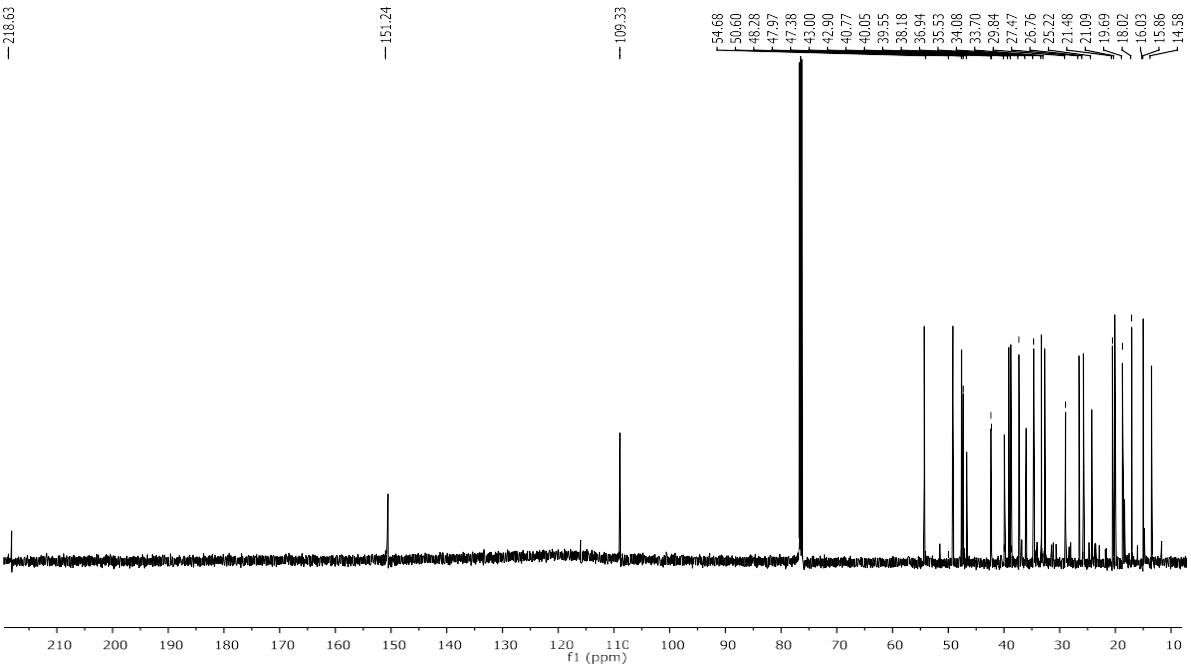


APPENDIX F: SPECTRA FOR COMPOUND 6

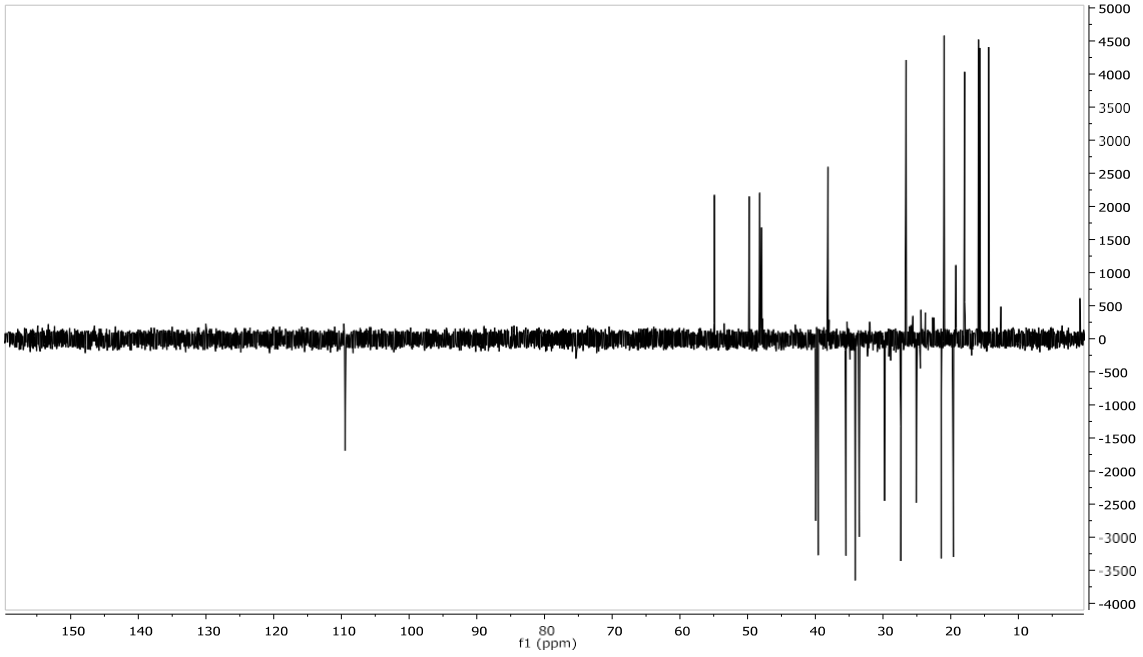
Appendix F1: ^{1}H SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 6



Appendix F2: ^{13}C SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 6

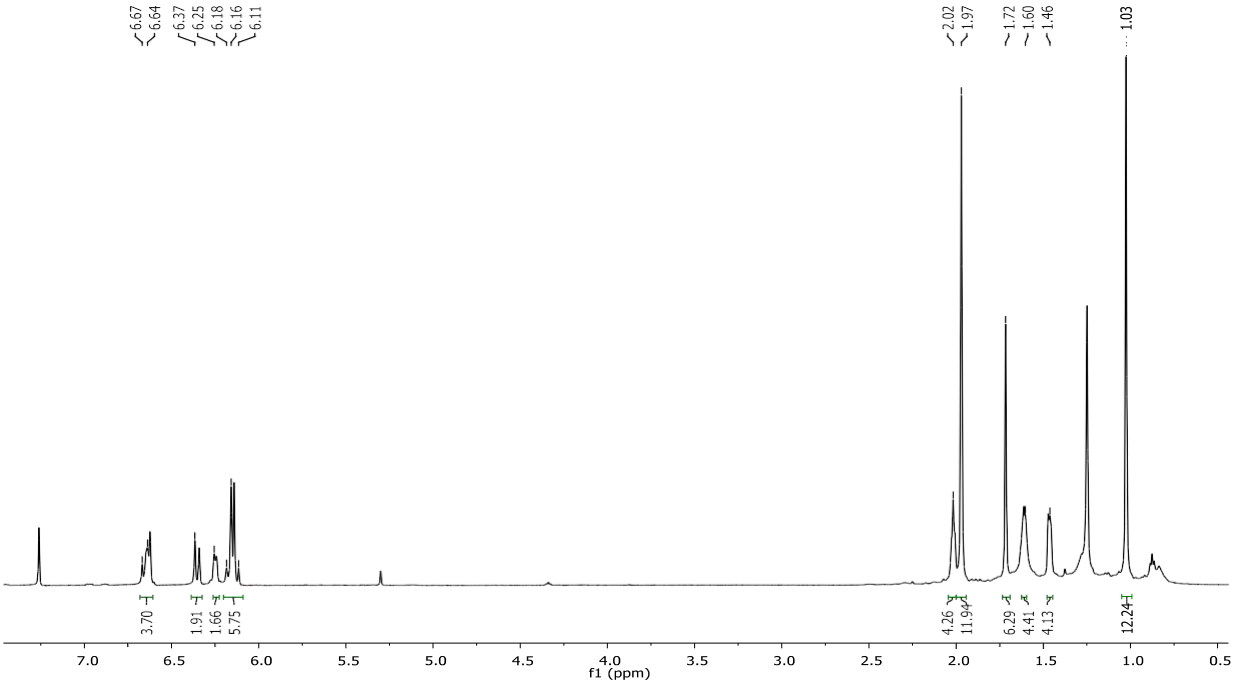


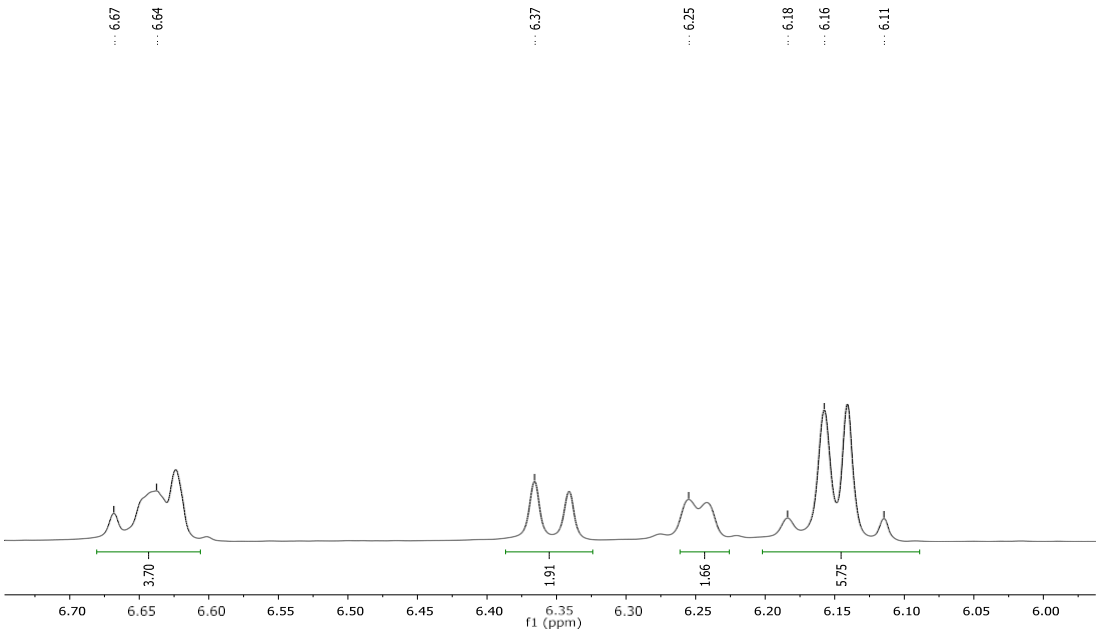
Appendix F3: DEPT 135 SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 6



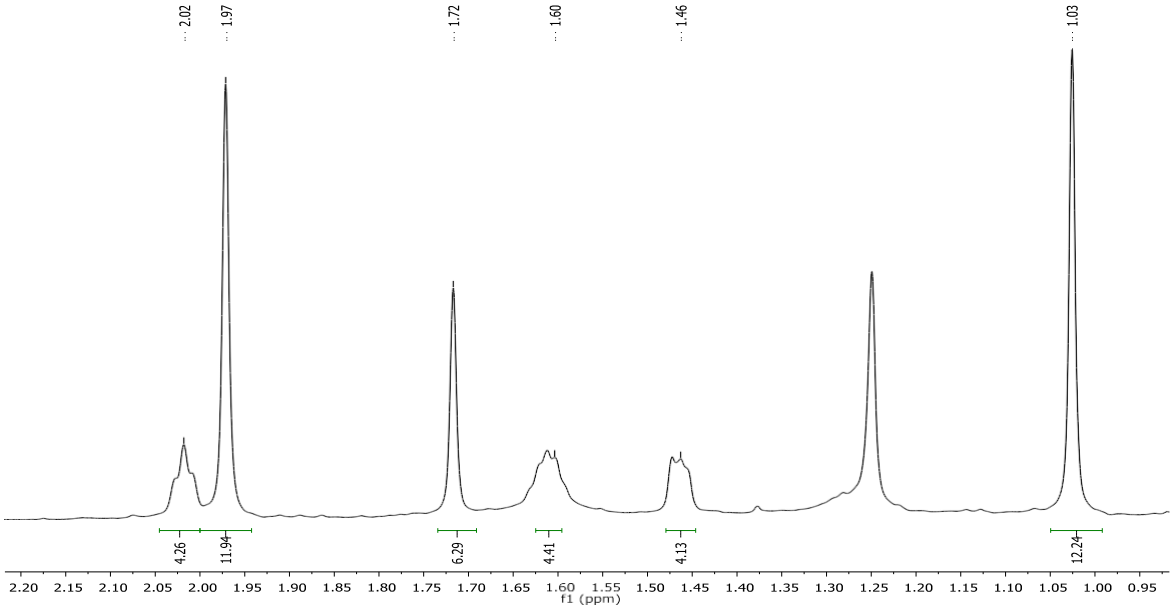
APPENDIX G: SPECTRA FOR COMPOUND 7

Appendix G1: ^{1}H SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 7

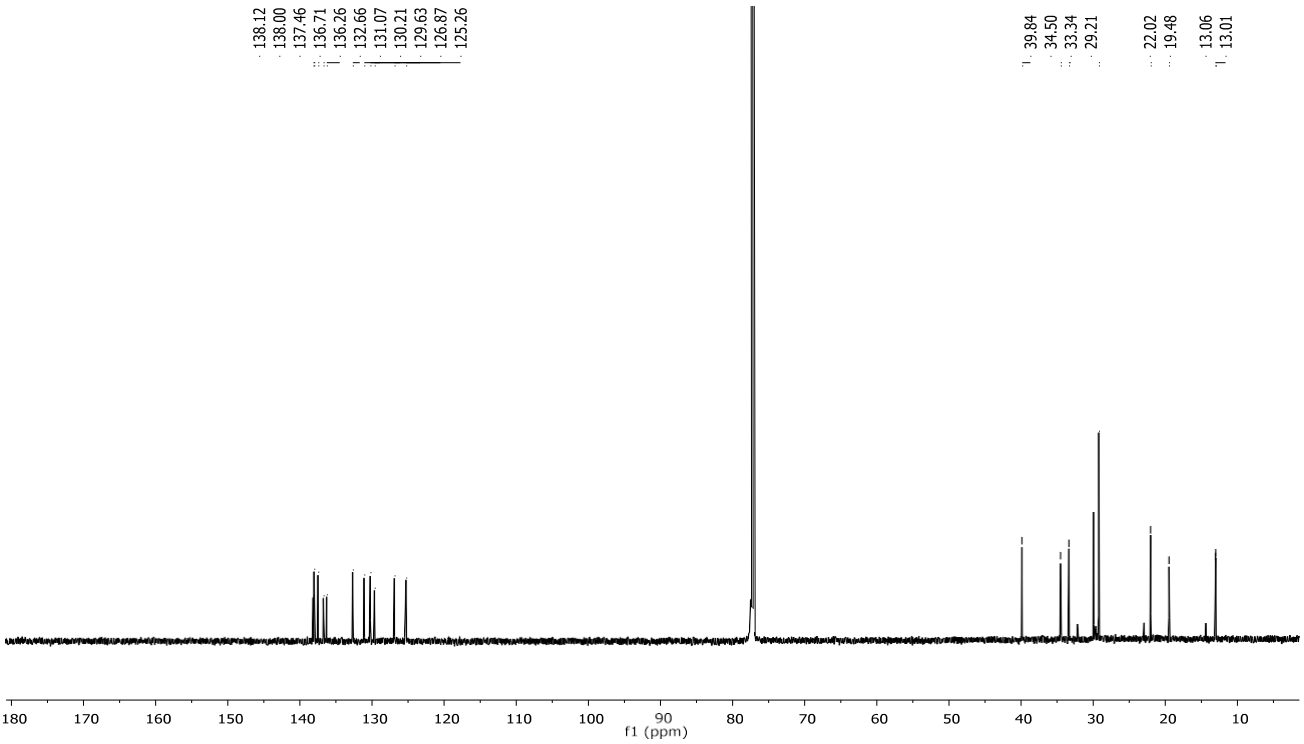




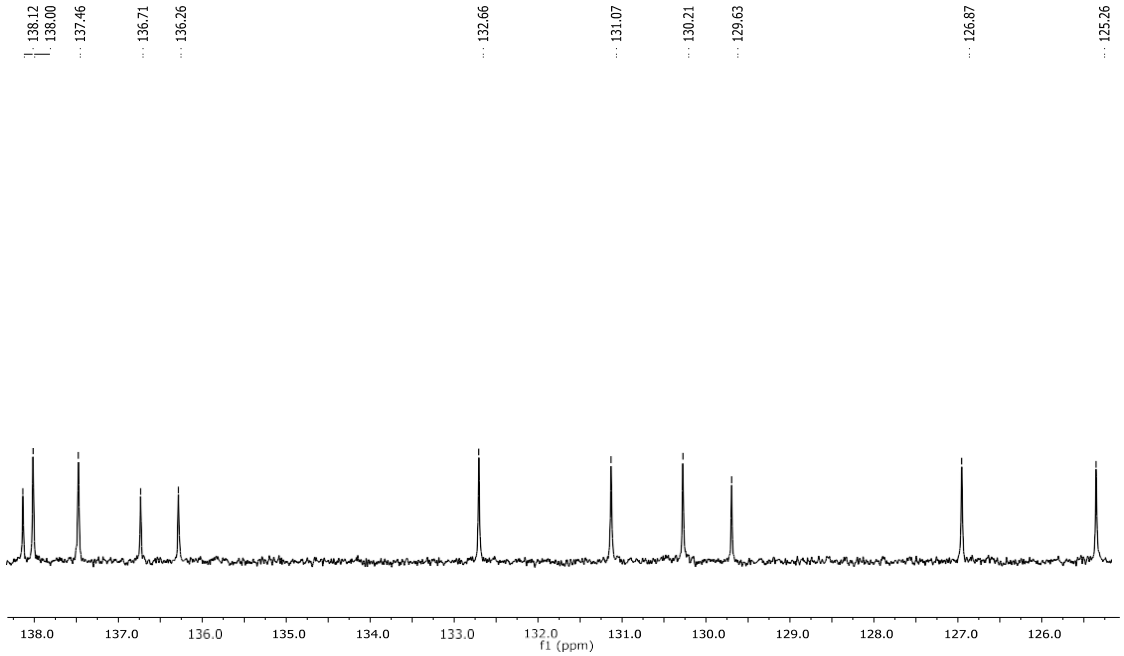
^{1}H SPECTRUM ($CDCl_3$, 600MHz) EXPANSION FOR COMPOUND 7



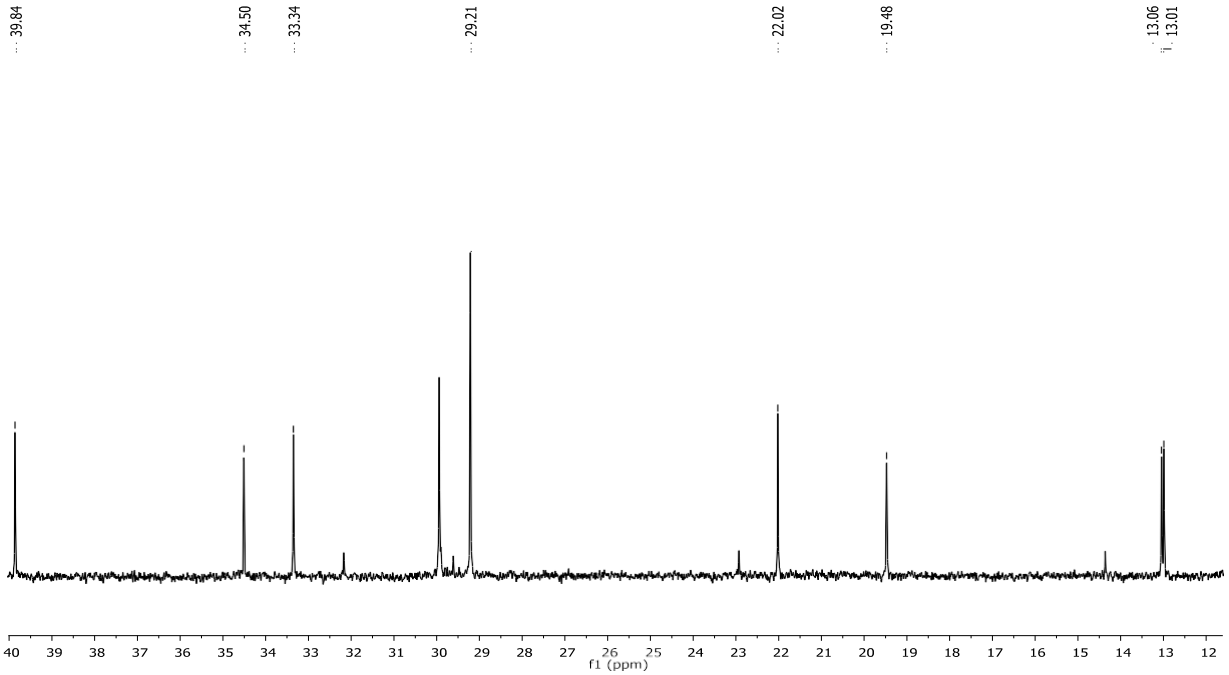
Appendix G2: ^{13}C SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 7



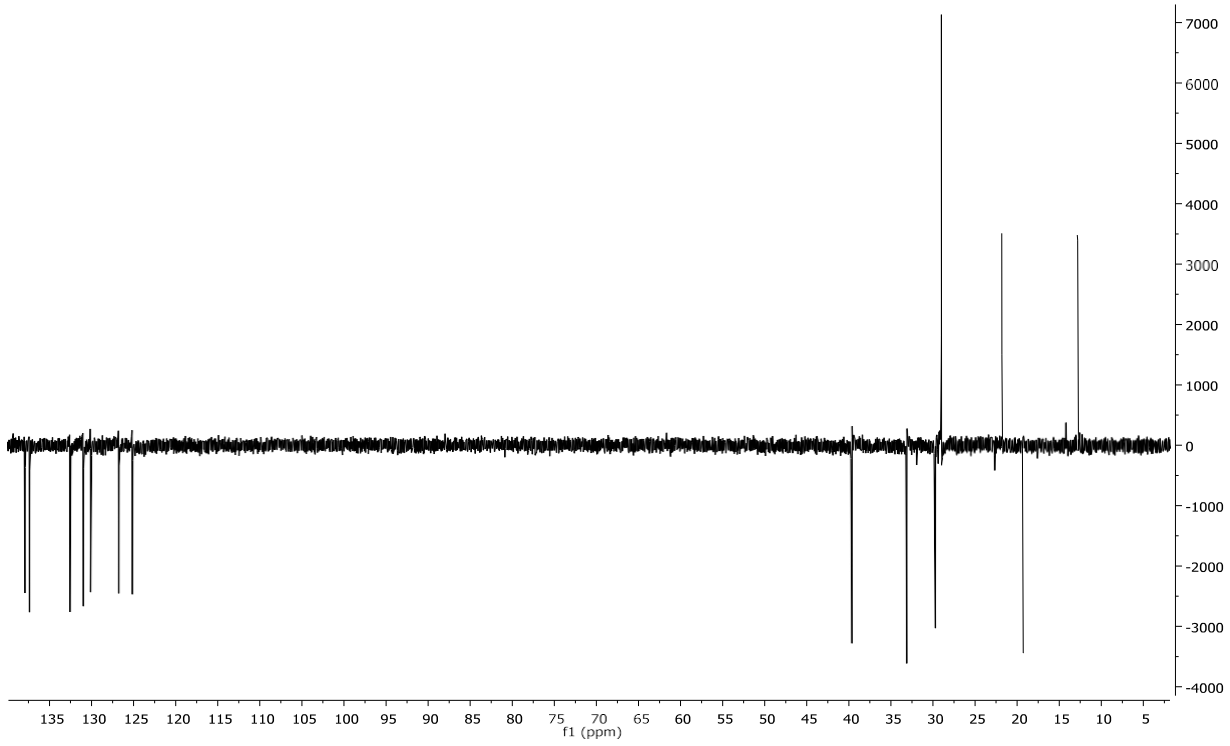
^{13}C SPECTRUM ($CDCl_3$, 600MHz) EXPANSION FOR COMPOUND 7



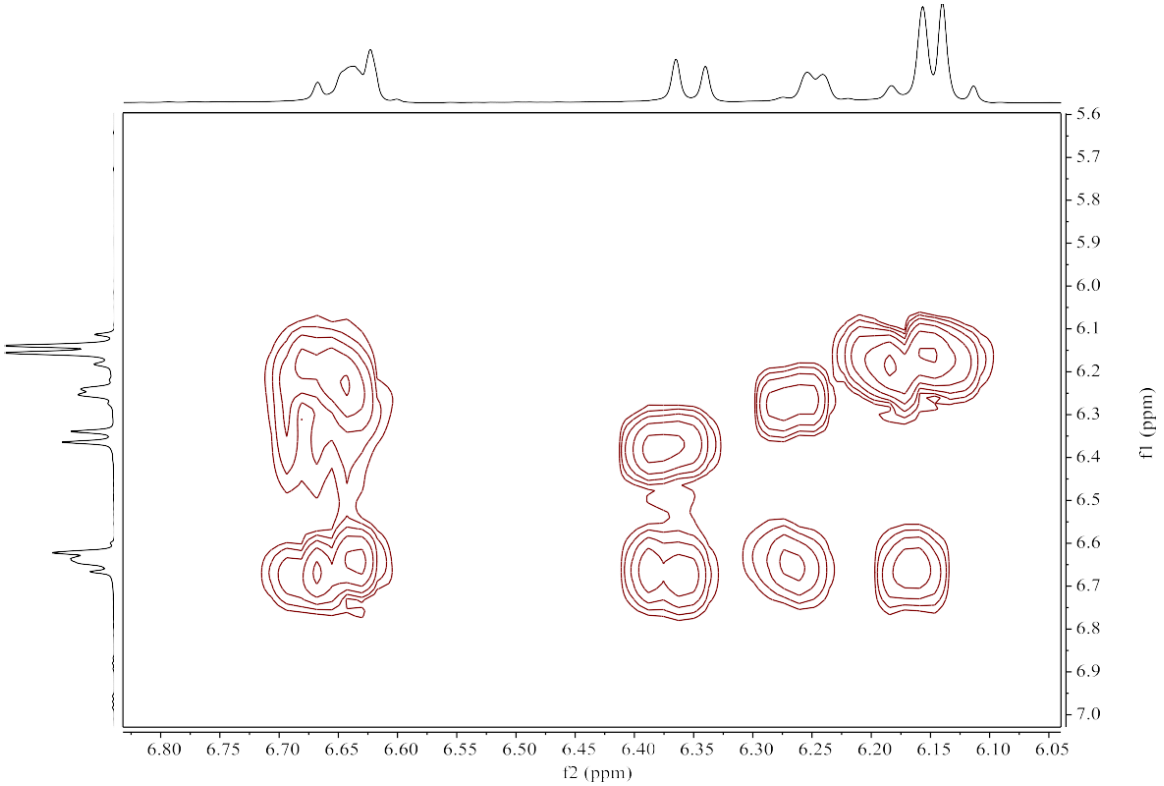
^{13}C SPECTRUM ($CDCl_3$, 600MHz) EXPANSION FOR COMPOUND 7

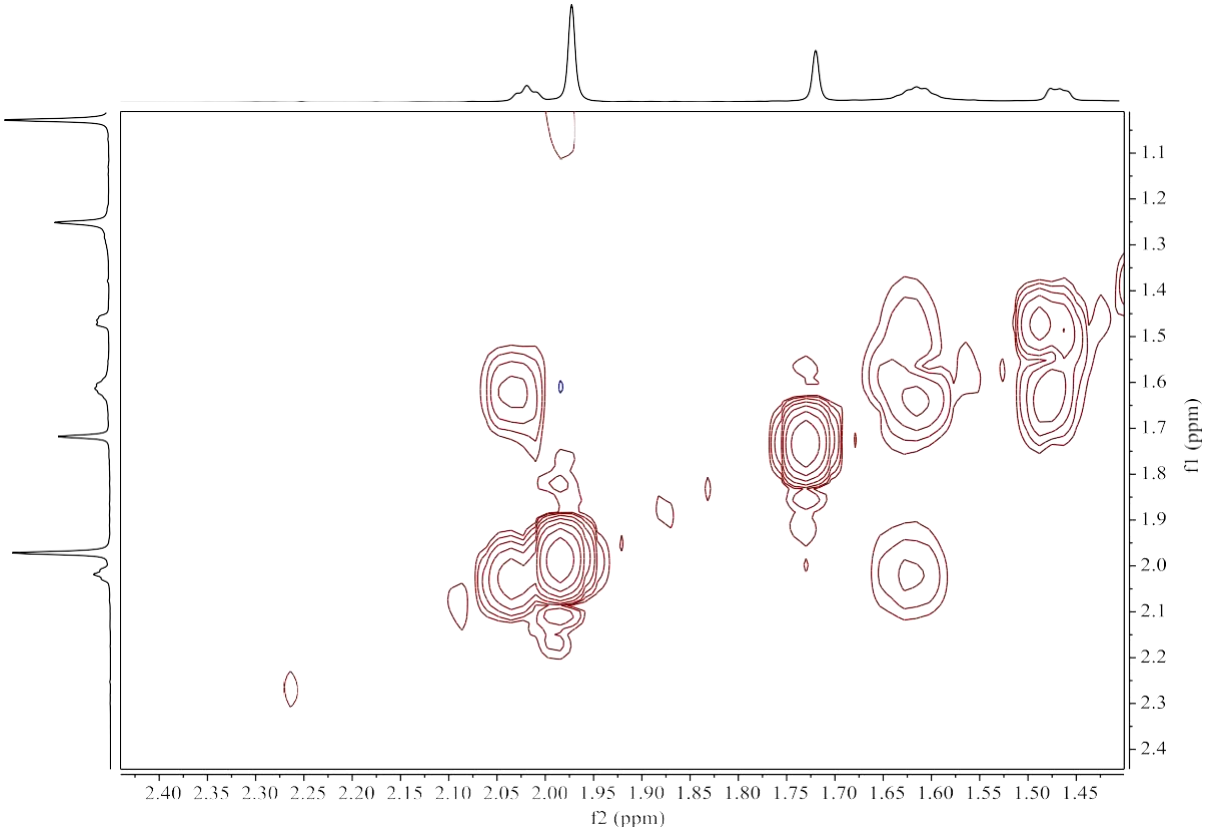


Appendix G3: DEPT 135 SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 7

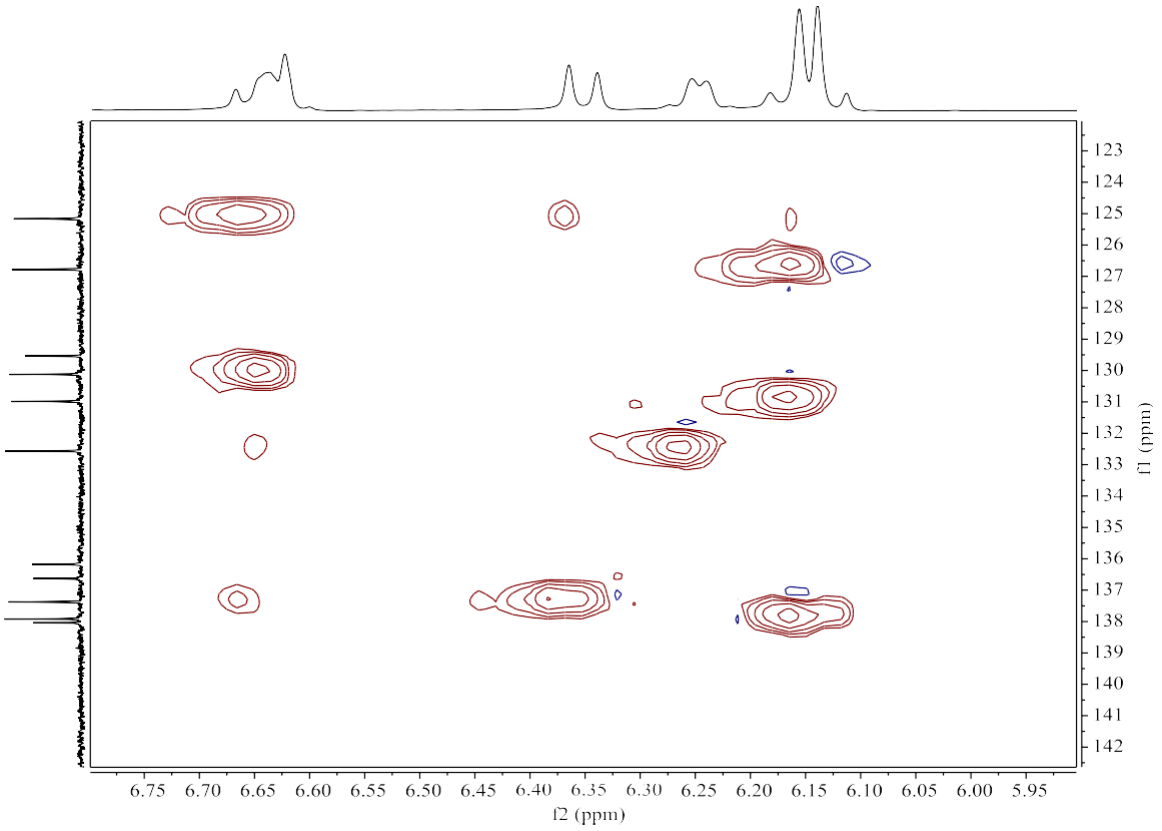


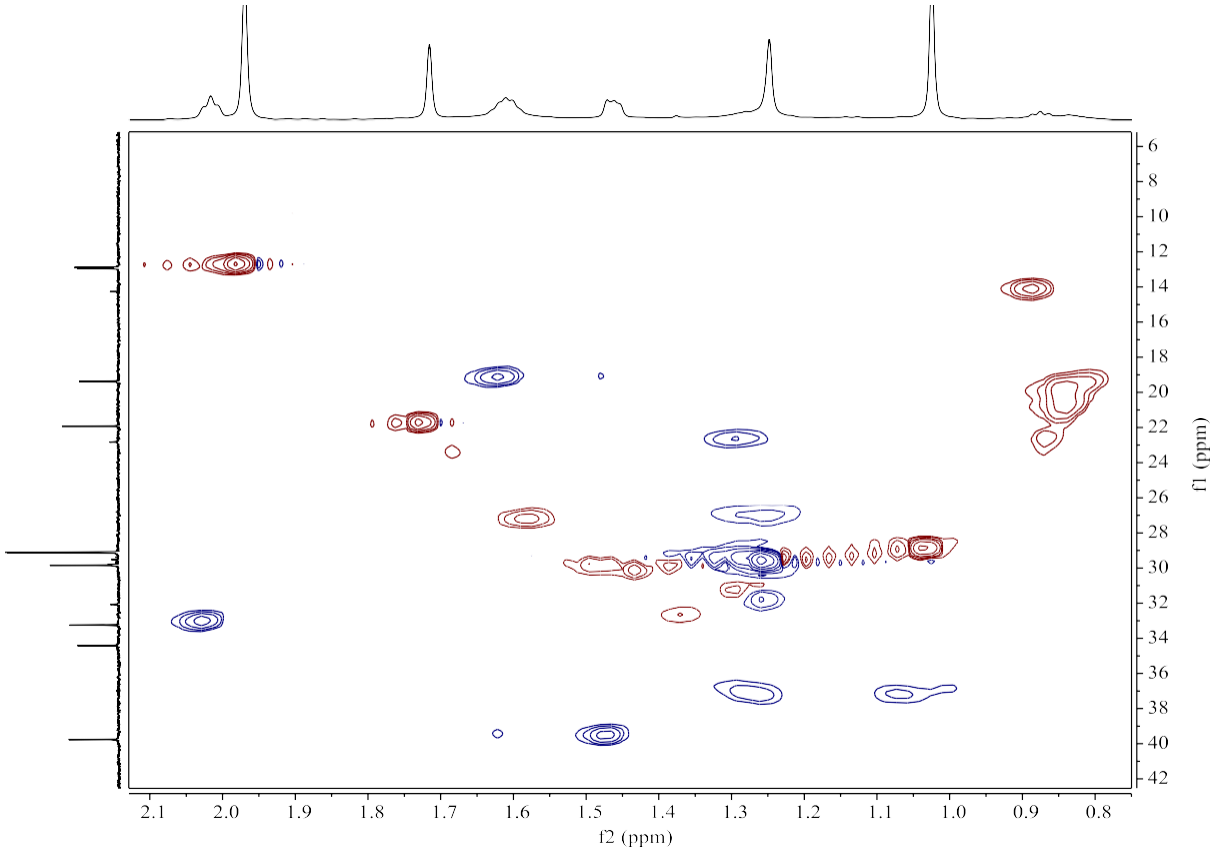
Appendix G4: ^{1}H-^{1}H COSY SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 7



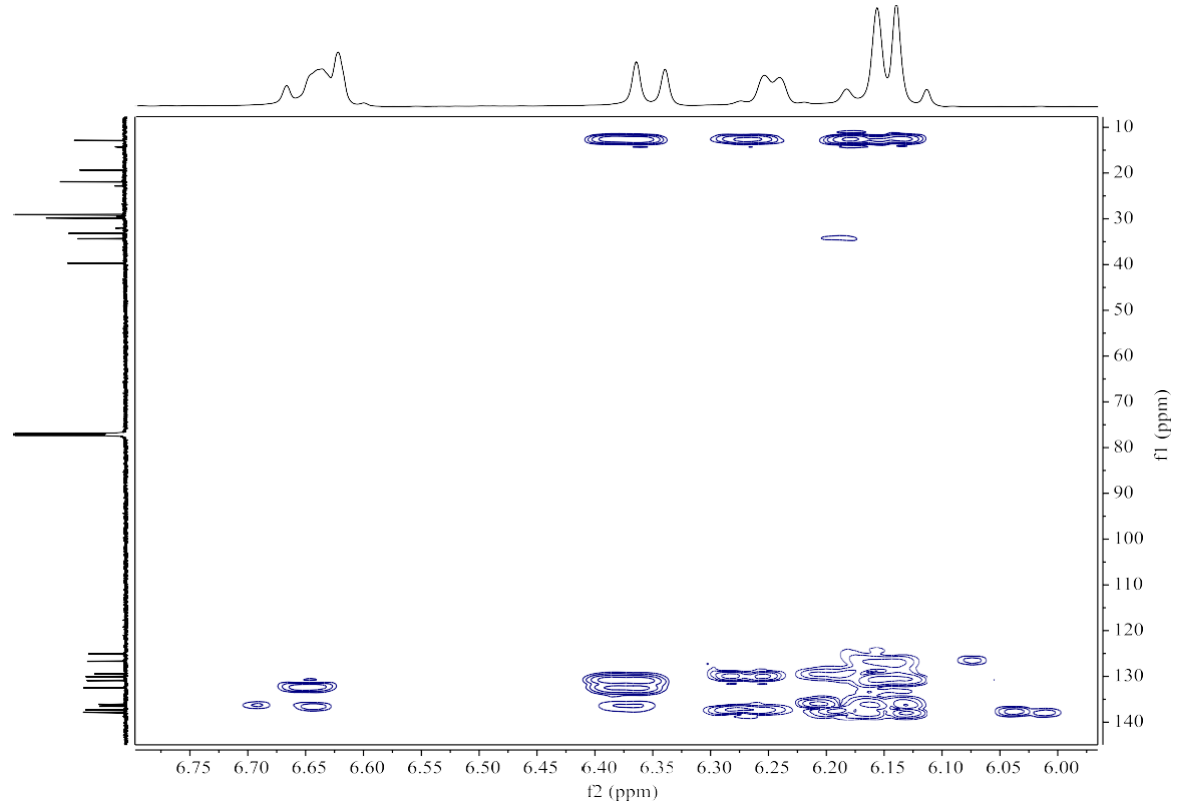


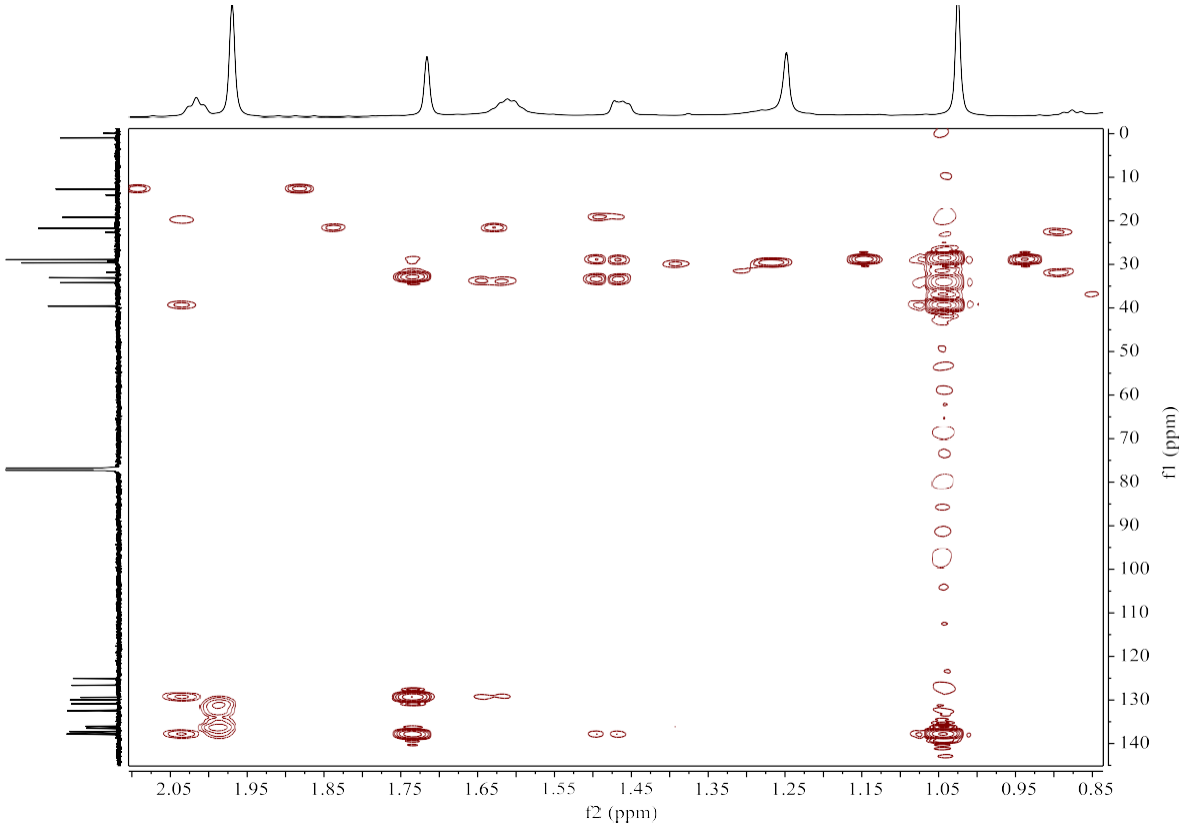
Appendix G5: HSQC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 7





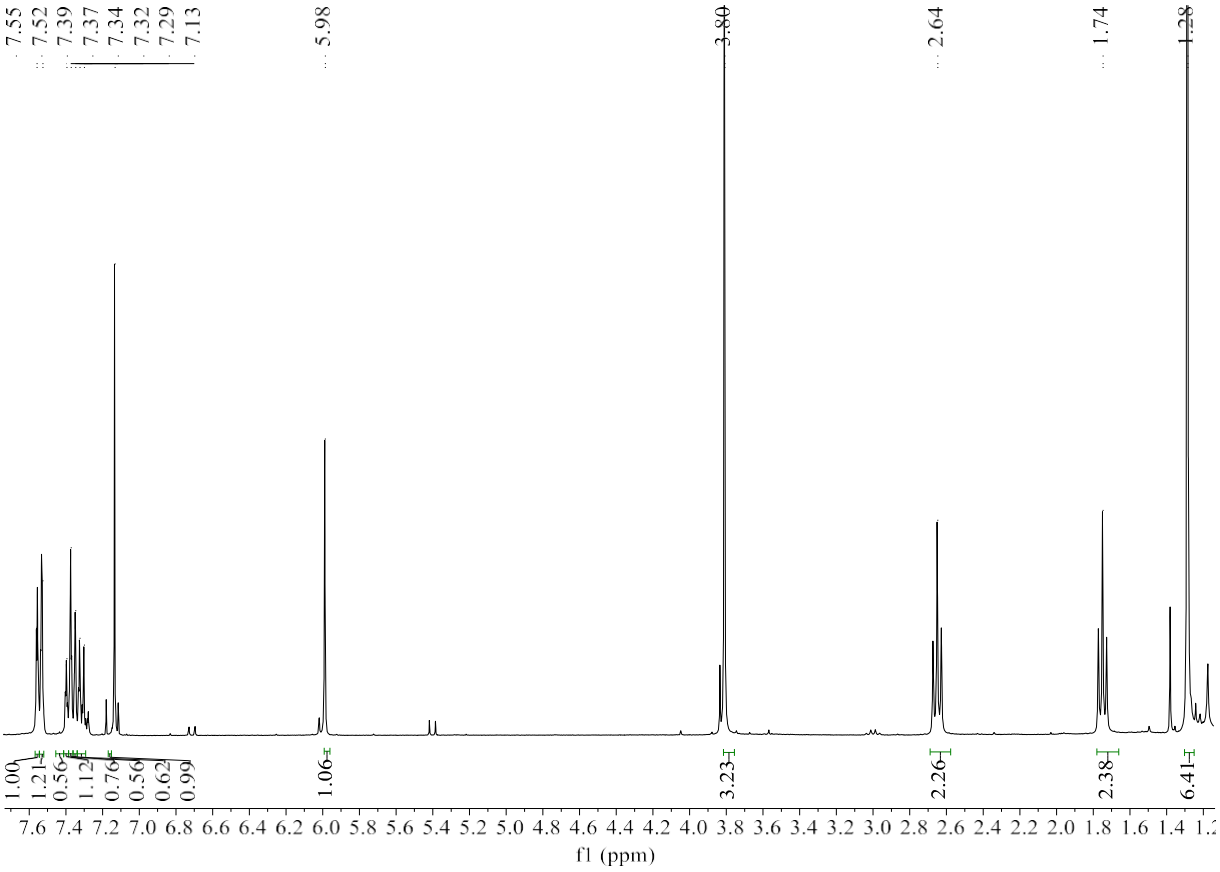
Appendix G6: HMBC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 7

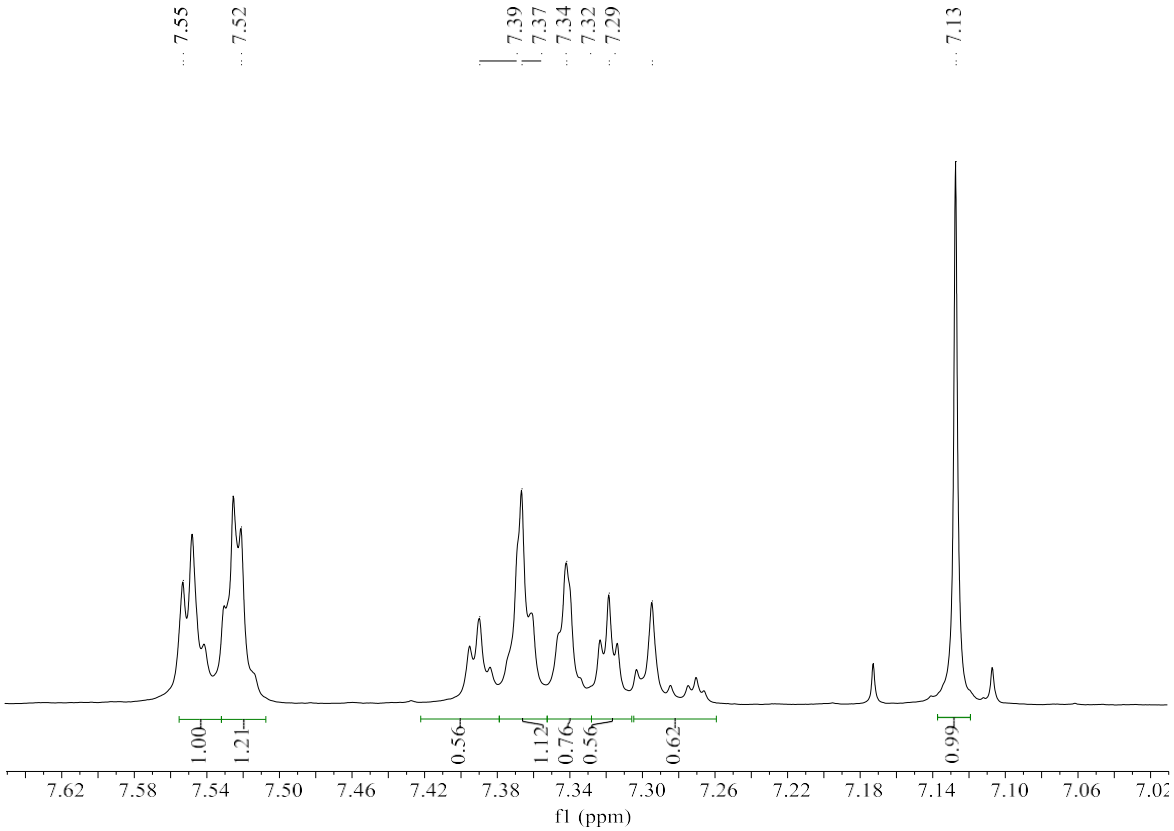

10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)
6.75 6.70 6.65 6.60 6.55 6.50 6.45 6.40 6.35 6.30 6.25 6.20 6.15 6.10 6.05 6.00
f2 (ppm)


0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)
2.05 1.95 1.85 1.75 1.65 1.55 1.45 1.35 1.25 1.15 1.05 0.95 0.85
f2 (ppm)

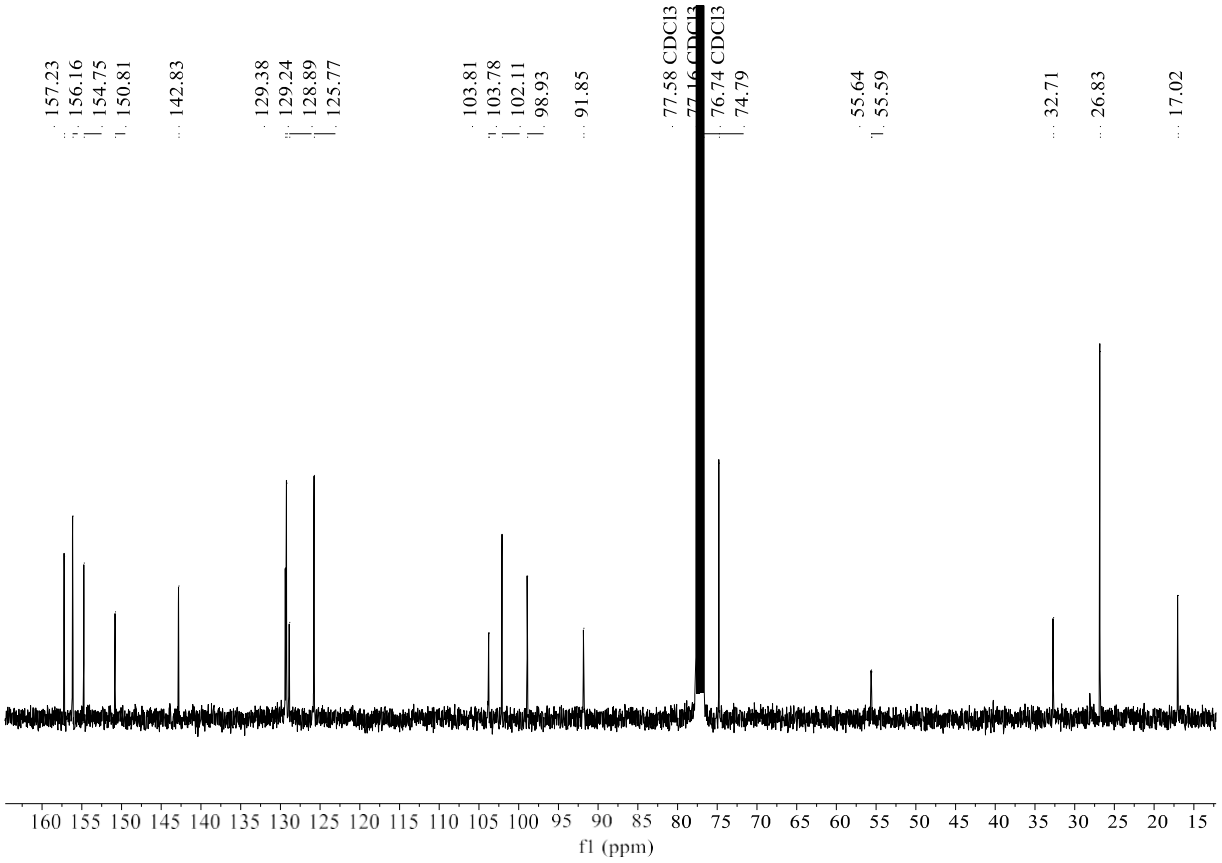
APPENDIX H: SPECTRA FOR COMPOUND 8

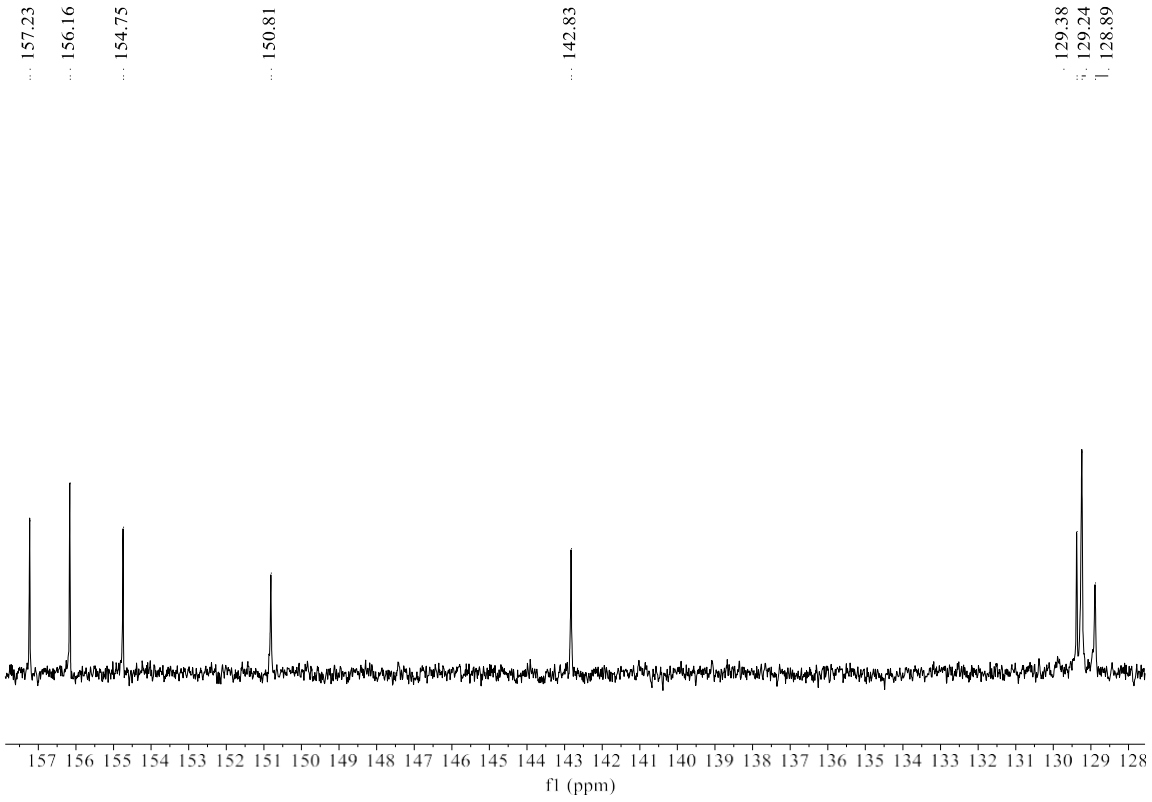
Appendix H1: ^{1}H SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 8



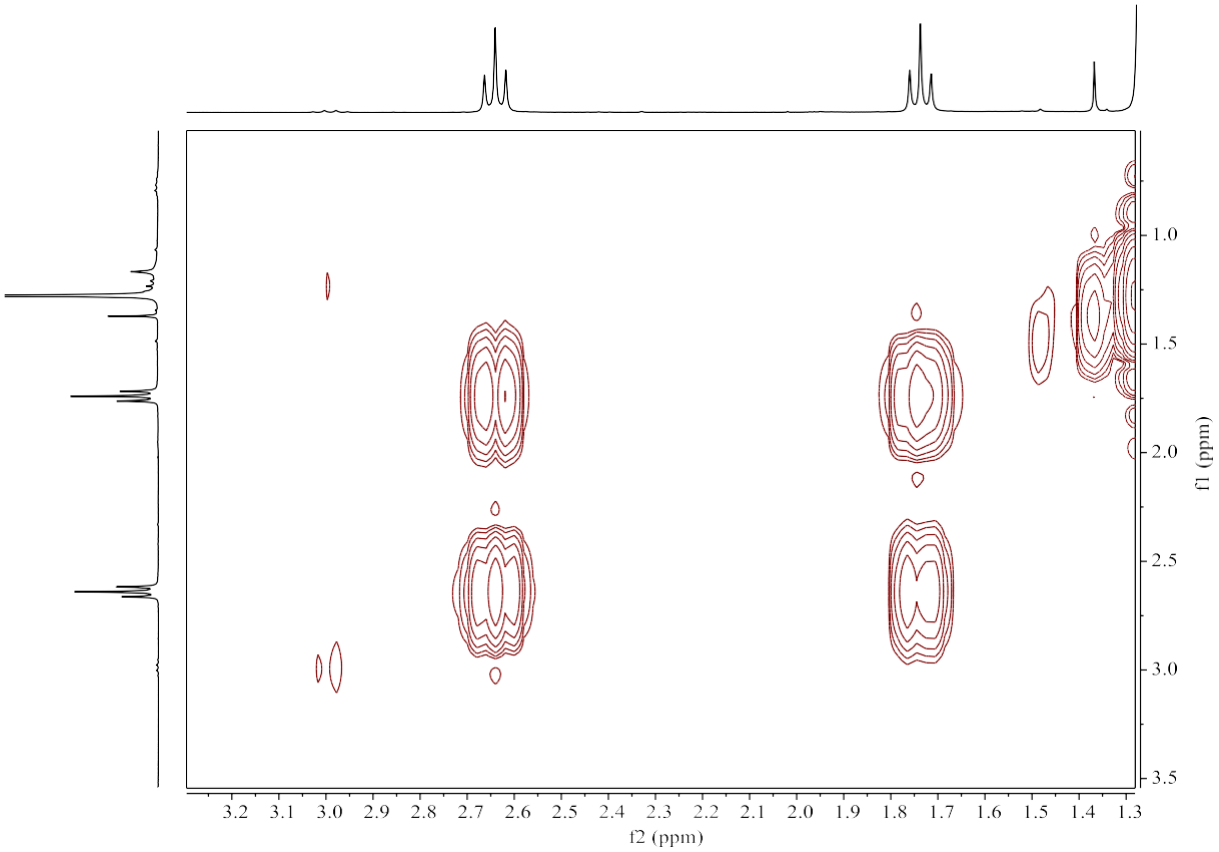


Appendix H2: ^{13}C SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 8

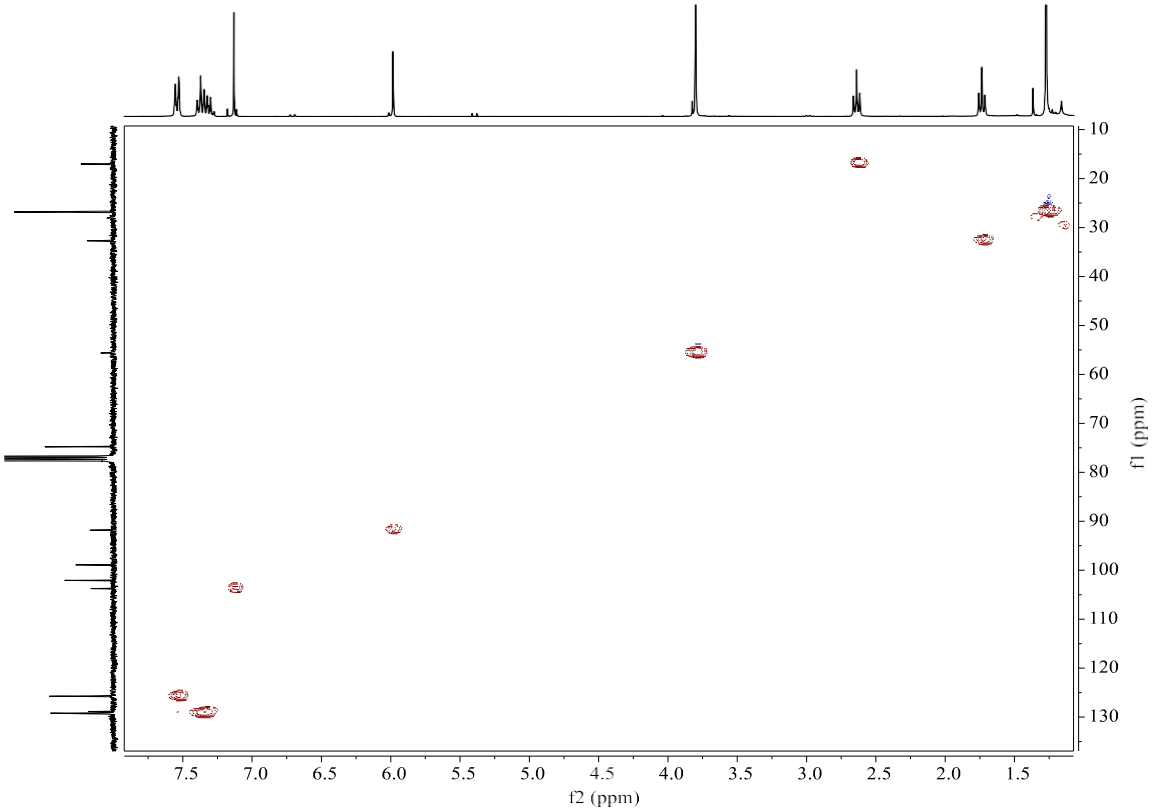




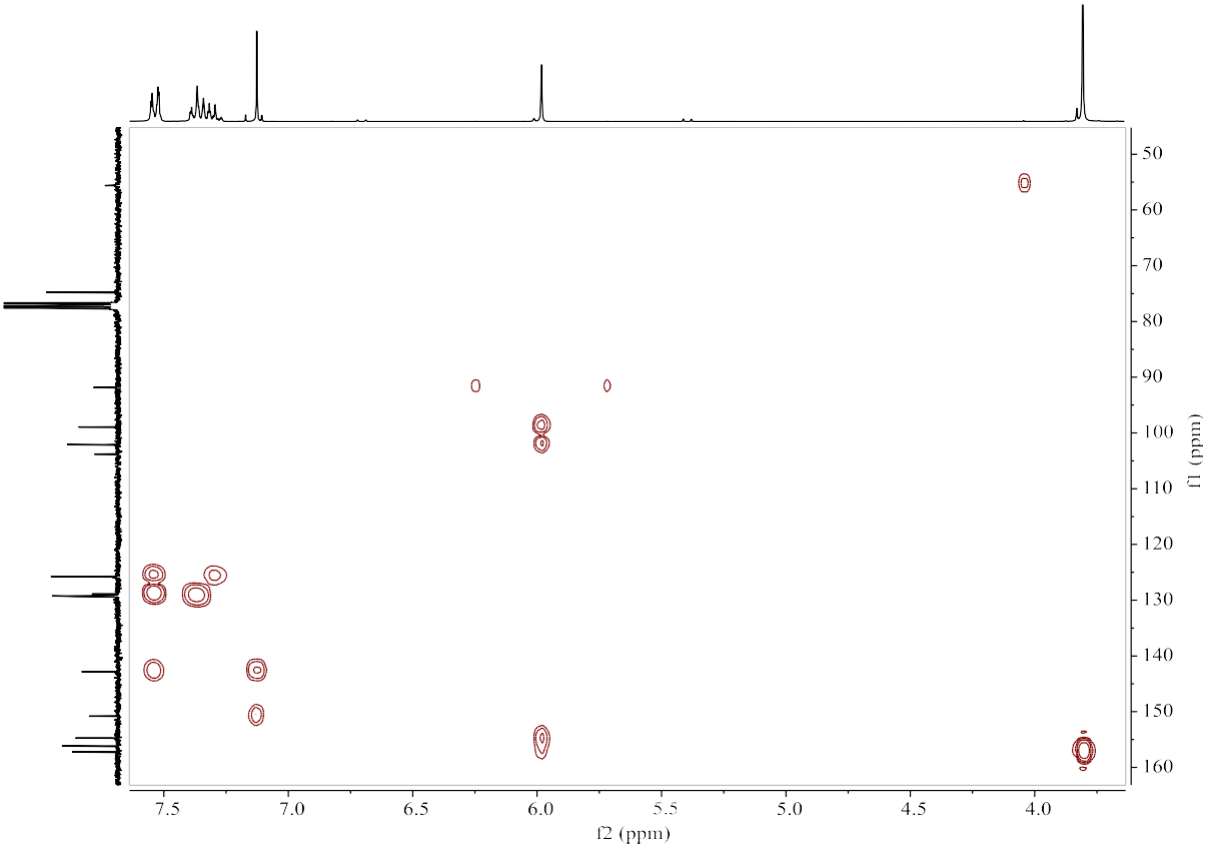
Appendix H3: ^{1}H-^{1}H COSY SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 8

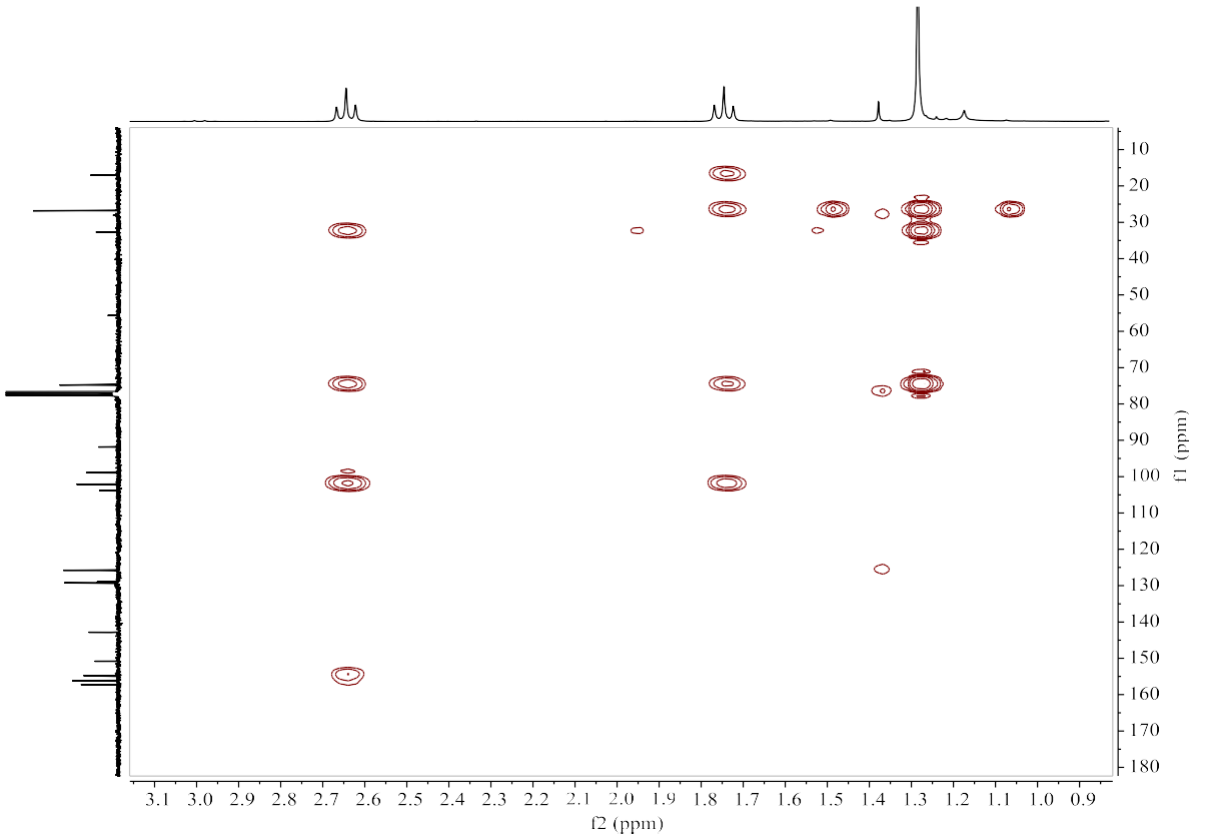


Appendix H4: HSQC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 8



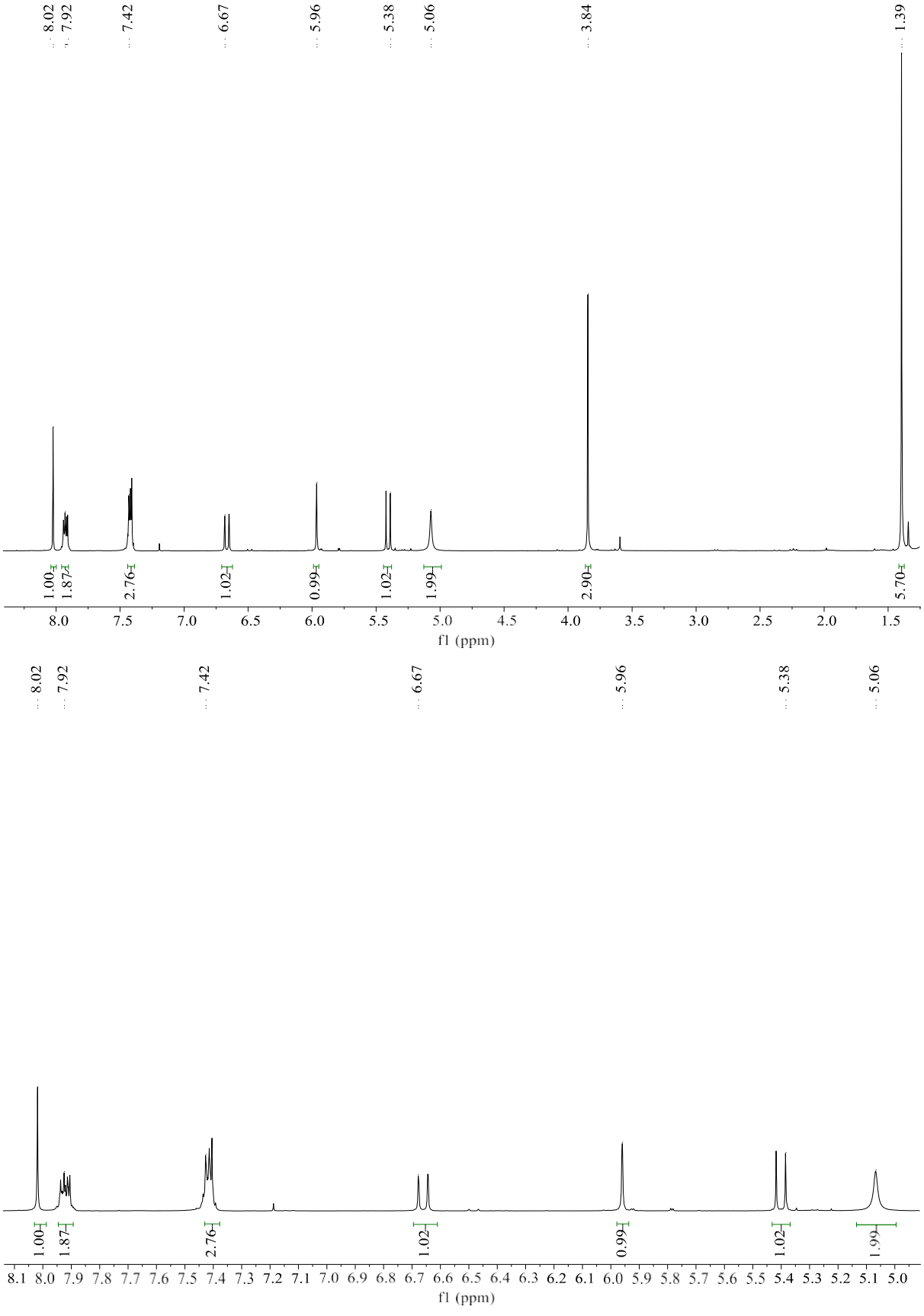
Appendix H5: HMBC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 8



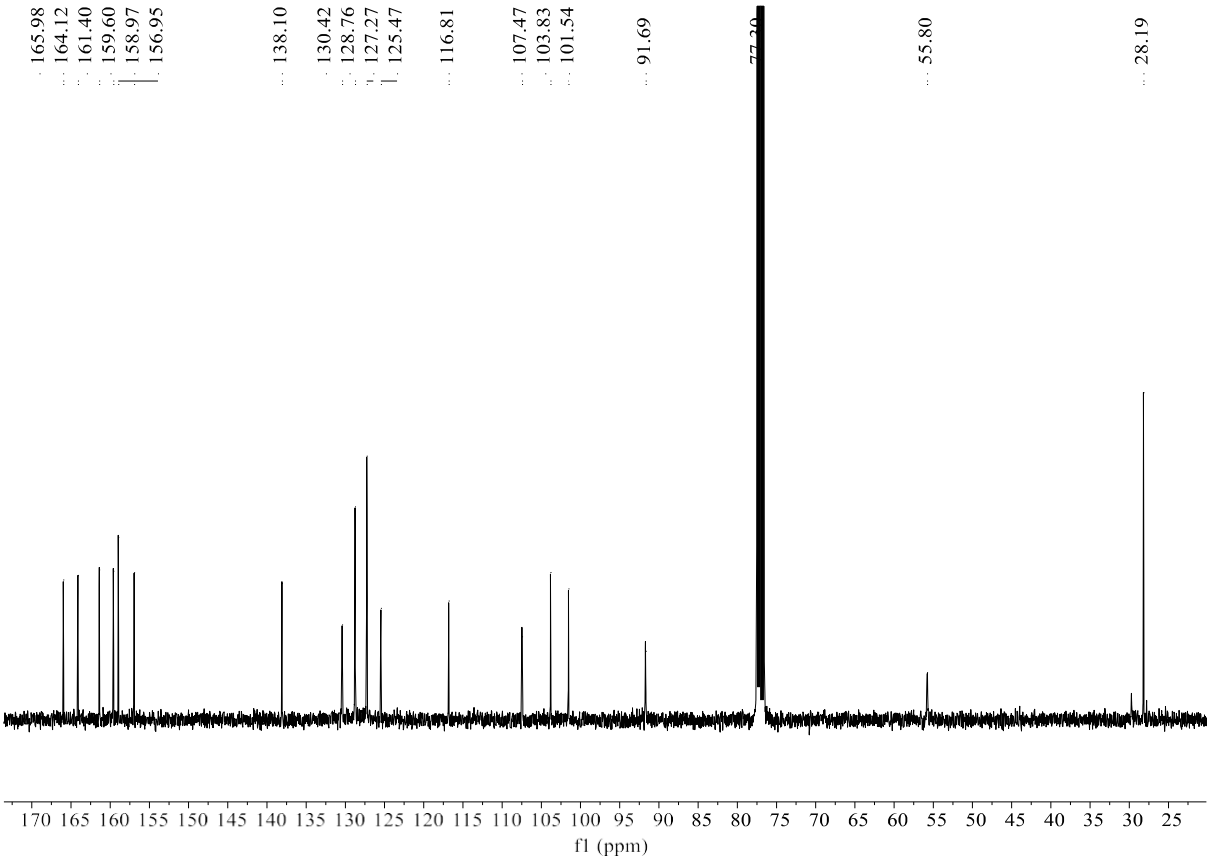


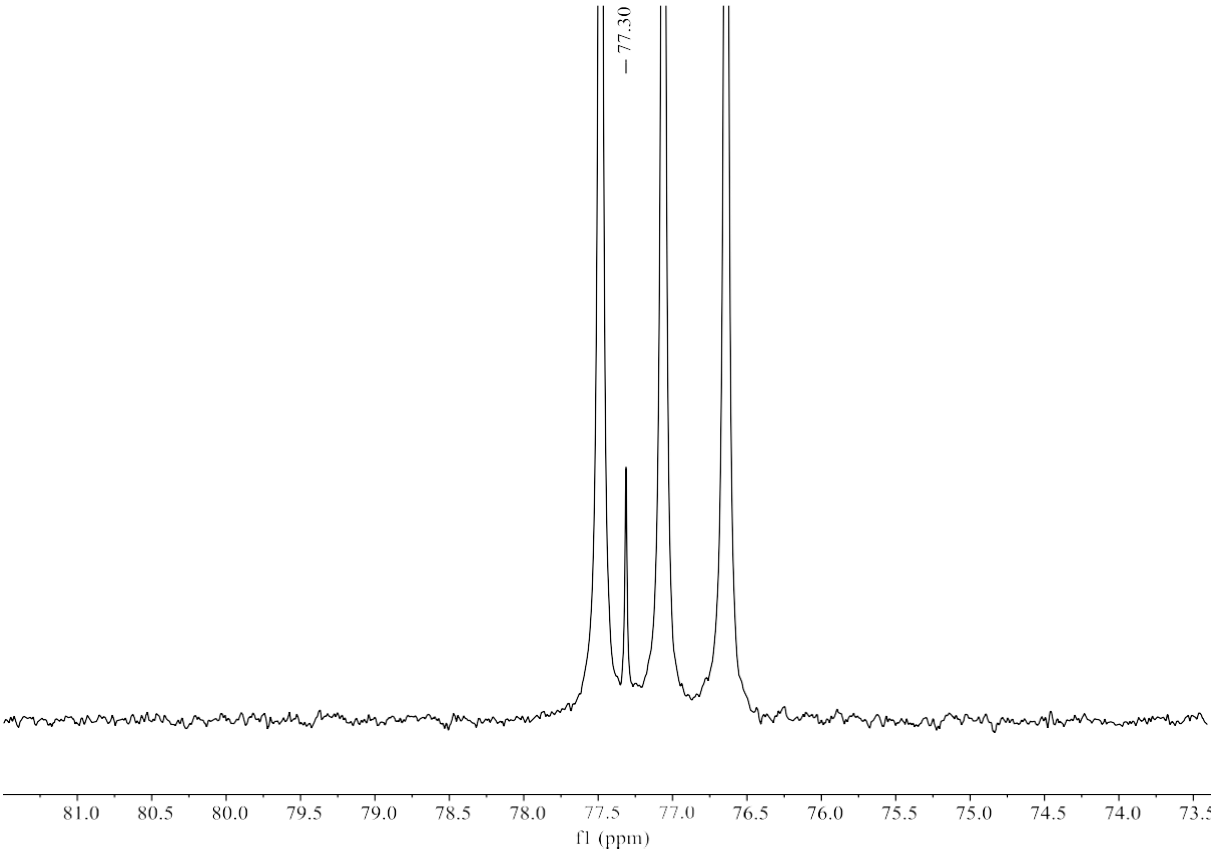
APPENDIX I: SPECTRA FOR COMPOUND 9

Appendix I1: ^{1}H SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 9

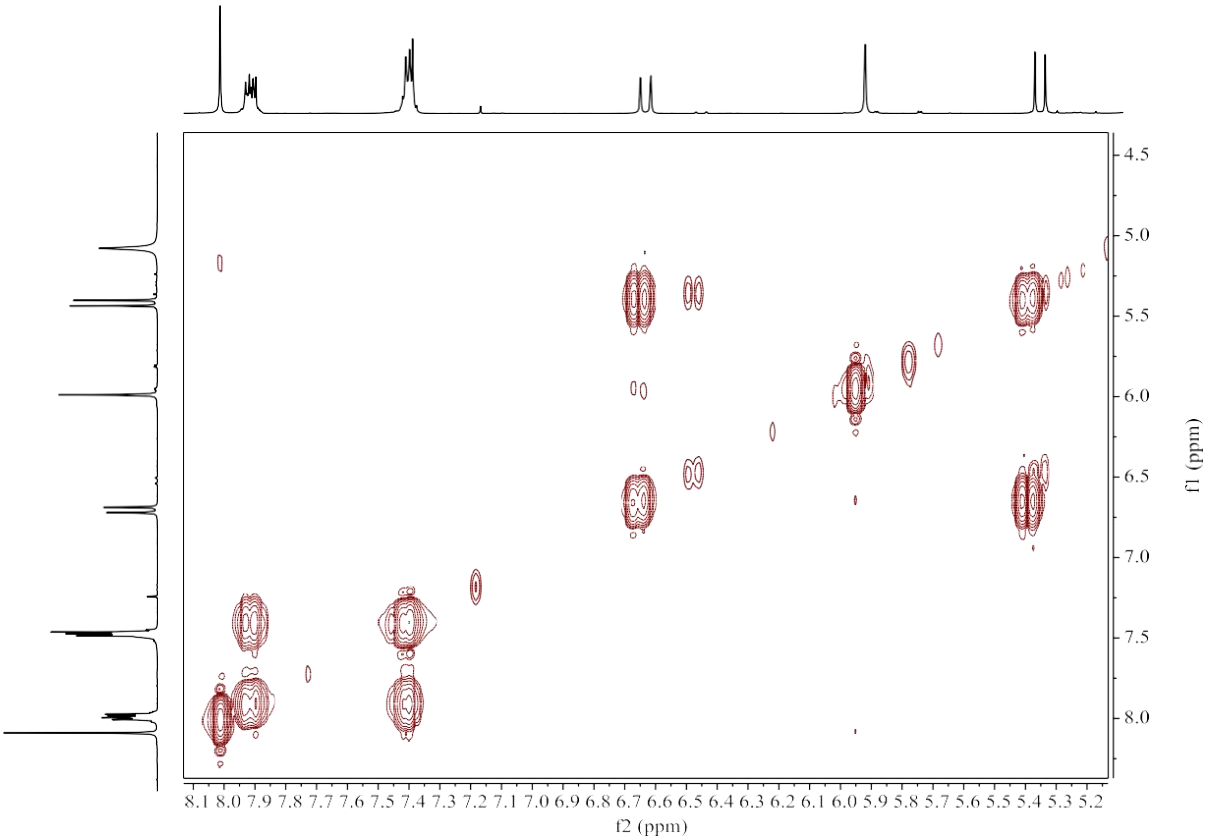


Appendix I2: ^{13}C SPECTRUM ($CDCl_3$, 600MHz) FOR COMPOUND 9

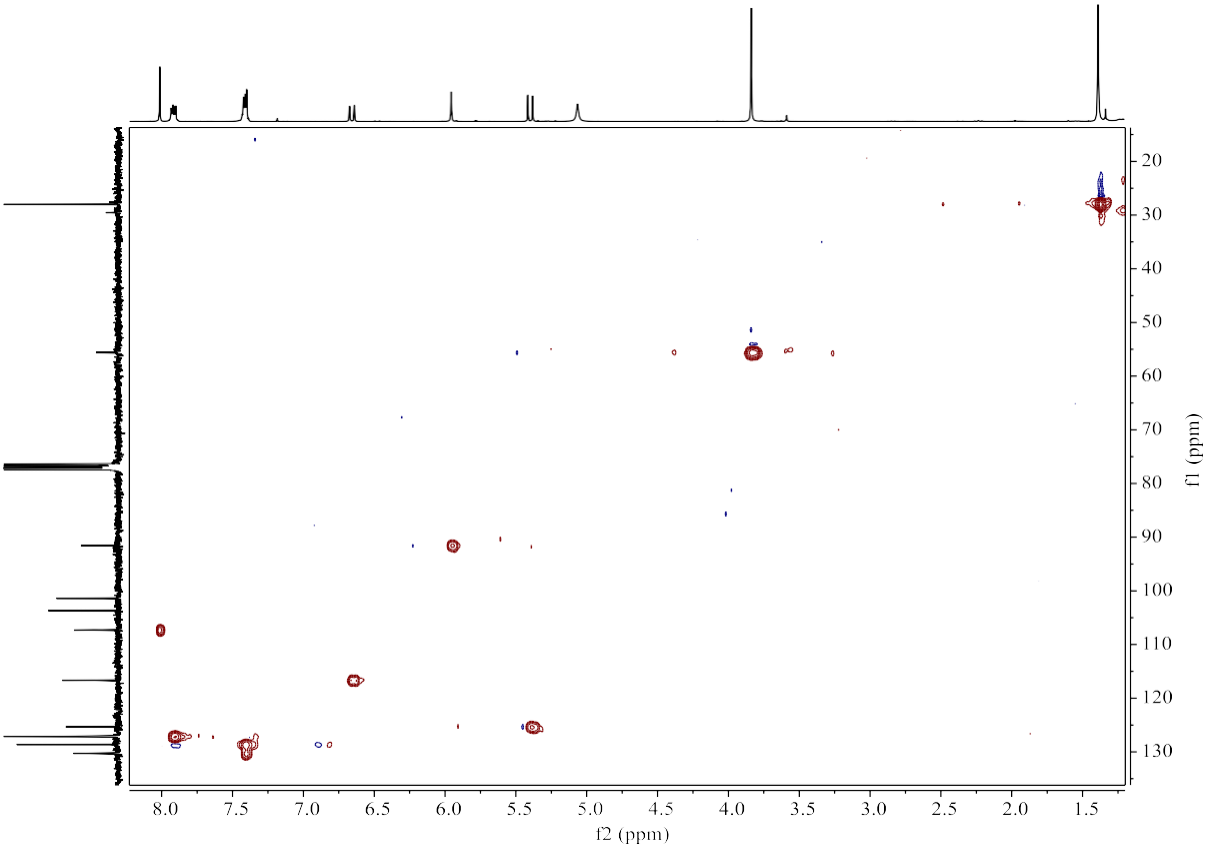




Appendix I3: ^{1}H-^{1}H COSY SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 9



Appendix I4: HSQC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 9



Appendix I5: HMBC SPECTRUM (600 MHz, $CDCl_3$) FOR COMPOUND 9