PHYTOCHEMICAL INVESTIGATION OF *ALSTONIA BOONEI* AND *SCHIZOZYGIA COFFAEOIDES* FOR CYTOTOXIC PRINCIPLES

BY

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RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN CHEMISTRY OF THE UNIVERSITY OF NAIROBI

2018

DECLARATION

I declare that this thesis is my original work and has never been presented for a degree award in any higher University. Where other people's work has been used, this has properly been acknowledged and referenced in accordance with University of Nairobi Requirements.

17-10-2018

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DEDICATION

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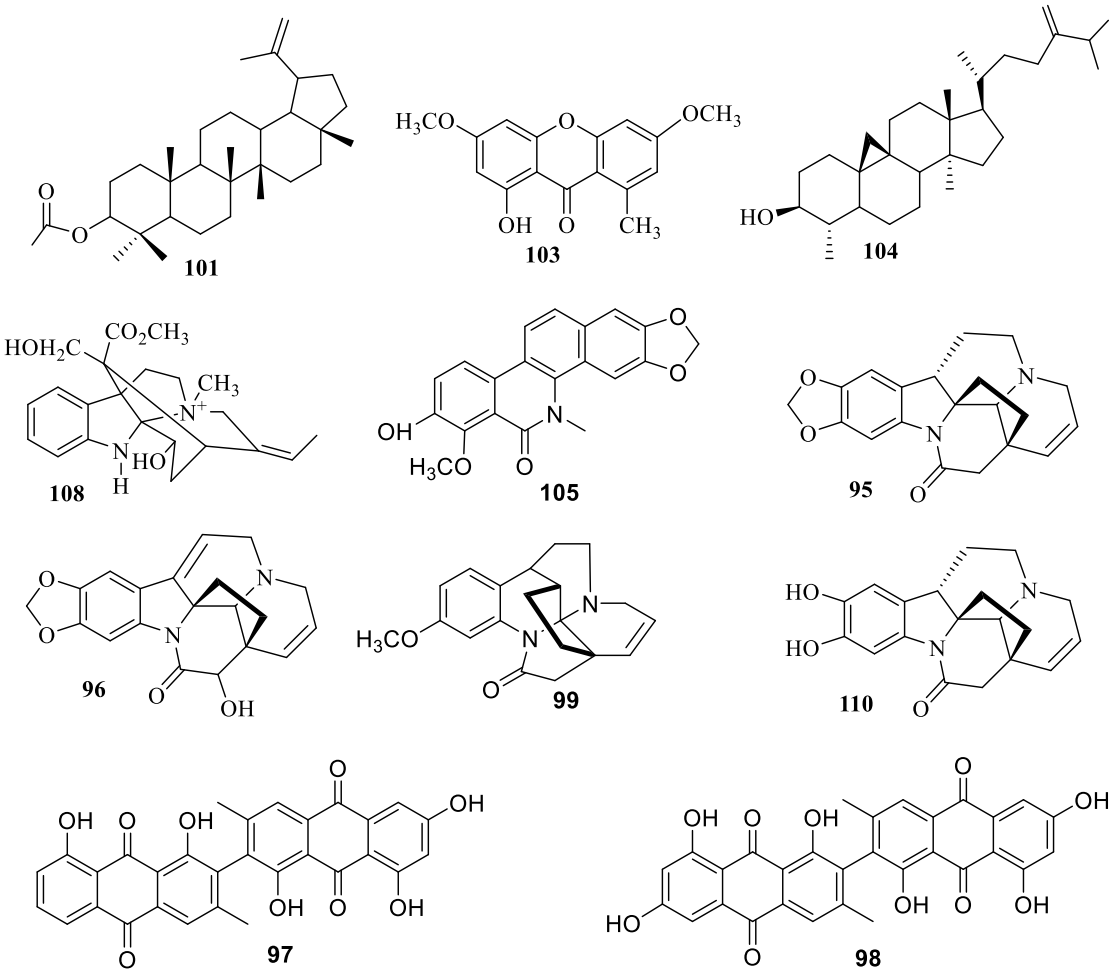
ABSTRACT

The air dried and ground stem bark and root bark of *Alstonia boonei* were extracted, separately, with CH_2Cl_2/MeOH (1:1) by cold percolation. After which, the crude extract of the stem bark was subjected to a combination of chromatographic separation which resulted in the isolation of two terpenes: lupeol acetate (**101**) and cycloeucalenol (**104**), a steroid: stigmasterol, a xanthone: lichexanthone (**103**) and an alkaloid: phenanthridine-**6**(**5***H*)-one (**105**). Similarly, the root bark extract resulted into isolation of three triterpenes: **101**, **104** and lupeol (**106**), a steroid: *β*-sitosterol and an alkaloid: Echitamine (**108**). Chromatographic separation of the leaf extract of *Schizozygia coffaeoides* resulted into isolation of stigmasterol, three alkaloids: schizozygine (**95**), isoschizozygaline (**99**) and 6,7-dehydro-19*β*-hydroxyschizozygine (**96**) and two anthraquinones: cassamin A (**97**) and cassamin B (**98**). Compounds **104** and **105** are hereby reported for the first time from Apocynaceae family. All of the compounds structures where elucidated using various spectroscopic/spectrometric techniques; proton and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, NOESY, HMBC, ORD and MS.

The crude extract of the root bark from *Alstonia boonei* exhibited good cytotoxic activity against HepG2 liver cancer cells (IC_{50} = 12.8 µg/ml) but was toxic to the LO2 normal liver cells (IC_{50} = 1.19 µg/ml). The crude extract was also toxic to the BEAS-2B normal lung cells but inactive against the A549 human lung cancer cells. Similarly, the crude extract of the stem bark of *Alstonia boonei* was toxic to the normal cells from both the lung and the liver but not active against the respective cancerous cells. Lupeol was inactive against the A549 cancer cells but toxic to BEAS-2B normal lung cells (IC_{50} = 0.71 µM). Furthermore, lupeol was very active HepG2 liver cancerous cells (IC_{50} = 1.65 µM) cells but highly toxic against LO2 normal liver cells (IC_{50} = <0.1 µM).

Treatment of schizozygine (**95**) with boron tribromide in an inert atmosphere at -18 ^{o}C led to cleavage of the methylenedioxy group leading to the formation the Schizozygine derivative (**110**) with 29% yield.



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ABBREVIATIONS

CD	:	Circular Dichroism
DCM	:	Dichloromethane
DMSO		Dimethylsulphoxide
DNA	:	Deoxyribonucleic acid
EIMS	:	Electron Ionization Mass Spectrometry
HCOSY:		Homonuclear correlation spectroscopy
HMBC:		Heteronuclear multiple bond correlation
HRMS	:	High Resolution Mass Spectrometry
HSQC:		Heteronuclear Single quantum coherence
IC_{50}	:	50% Inhibition Concentration
IR	:	Infrared
MeOH	:	Methanol
NMR	:	Nuclear Magnetic Resonance
PTLC	:	Preparative Thin Layer Chromatography
UV	:	Ultra Violet
WHO	:	World Health Organization

CHAPTER ONE

INTRODUCTION

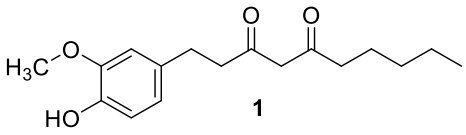
1.1 Background

Cancer for long has been taken to be ‘a disease of the rich’, considering their lifestyle. However, the situation seems to have changed, given the increasing cancer cases in developing countries (WHO, 2008). Cancer may result from genetic instability and epigenetic alterations caused by different types of DNA damage induced by exposure to radiation and other carcinogenic agents (Villanueva *et al*., 2011). Apparently, everyone is potentially at risk of developing some form of cancer because of exposure to various external factors such as tobacco smoking, infectious diseases and unhealthy diet. Internal factors, including heredity, genetic mutations, hormones and susceptible conditions are also causes of cancer. The long period it takes for detection of cancer (more than nine years of exposure to external factors to detect cancer) complicates the situation (American Cancer Society, 2016).

It was reported that out of 14.1 million new cancer cases, 58% cancer deaths were registered worldwide in 2012 (Globocan, 2012). Cancer is becoming a major health problem in developing countries where it is projected to shoot up by 55% by the year 2020 (Szic *et al*., 2011). The death rate among cancer patients has not declined significantly even with advances in the existing cancer treatment methods (American Cancer Society, 2016). Hence, prevention remains an essential strategy in the fight against cancer worldwide. The increasing incidences of cancer in the world today call for interventions including phytochemical chemoprevention. This involves the use of natural dietary phytochemicals that restrain, obstruct or reverse tumour growth at different stages - initiation, promotion or progression of carcinogenesis (Ugbogu *et al.*, 2013).

Phytochemicals lower the risk of cancer advancement in humans by radical scavenging, anti-inflammatory, cytotoxicity, anti-proliferative and anti-oxidation mechanisms (Ugbogu *et al*., 2013). Therefore, phytochemicals are a potential stand-in source for safer agents having anti-carcinogenic effects. The mode of action of phytochemicals in chemoprevention is either as blocking or suppressing agents (Ugbogu *et al*., 2013).

Surgery, radiotherapy and chemotherapy are the most commonly used cancer treatment methods (WHO, 2014; WHO, 2008). Even so, these can evoke various side effects and complete cure is not always realized for most patients suffering from different types of cancers (American Cancer Society, 2016). It has recently been reported that phyto-compounds from different foods can activate or deactivate molecular signaling cascades by targeting molecular cancer cells (Chung *et al*., 2013). A number phytochemicals, including gingerol (**1)**, indole-3-carbinol (**2**), genistein (**3**), resveratrol (**4**) and curcumin (**5**) have been considered anticancer chemo-preventive agents (Ugbogu *et al*., 2013).



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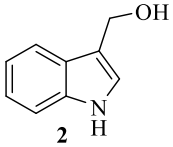

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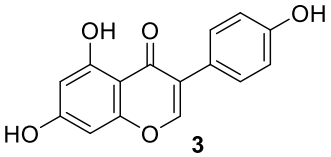

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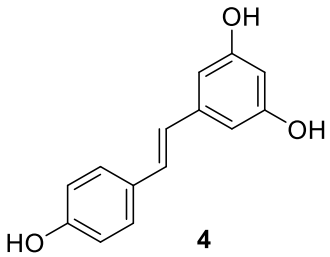

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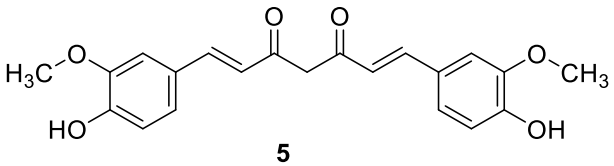

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The metastatic stage of cancer complicates its management by either surgery or radiotherapy. This requires metastasis gene suppression through epigenetic mechanisms that can alter DNA methylation, histone acetylation and methylation, a condition only brought about by dietary natural substances (Szic *et al*., 2011). There seem to be no clear therapeutic approaches for

managing metastasis (Ugbogu *et al*., 2013). However, phytochemicals in edible plants are known to restrain metastatic of cancer progression (Meadows, 2012). In 2012, the American Cancer Society recommended two guidelines that could reduce cancer mortality by 22%: eating foods such as non-starchy vegetables, fruits and legumes containing various anticancer phytochemicals and low consumption of red meat as a source of protein (Meadows, 2012).

Members of the family Apocynaceae, including some *Alstonia* species produce compounds with cytotoxic activities (Zhang *et al.,* 2014). Hence, in this project the stem bark and roots of *A. boonei* and the leaves of *Schizozygia coffaeoides* were investigated for cytotoxicity with the aim of identifying lead compounds that might have a different mode of action from the current anticancer drugs.

1.2 Statement of the Problem

Despite advances in surgery, radiotherapy and chemotherapy, which are the most commonly used cancer treatment methods, the cancer death rate has not declined significantly (American Cancer Society, 2016). At the same time, these common methods can cause a number of side effects, and yet complete cure is not realized for most cancer patients. In addition, cancer drugs have encountered resistance especially after the initial chemotherapy and this has become a problem for the new targeted agents (Cree and Charlton, 2017). For this reason, cancer is becoming a major health problem, not only in developed countries, but in developing countries as well. It is estimated that the incidence of cancer will have shot-up by 55% in the year 2020 (Szic *et al*., 2011). Therefore, this research was aimed at searching for new cytotoxic agents of plant origin, which can be used as templates in management of cancer related illnesses.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to identify cytotoxic metabolites from *Alstonia boonei* and *Schizozygia coffaeoides*.

1.3.2 Specific Objectives

The specific objectives of this study were:

i. To isolate and characterize metabolites from the stem and root bark of *Alstonia boonei* and leaves of *Schizozygia coffaeoides;*
ii. To establish the cytotoxity of the crude extract and isolated metabolites;
iii. To improve the cytotoxity of the promising compounds through derivatization.

1.4 Justification

Due to the estimated high cancer cases by the year 2020 (Szic *et al*., 2011), it is necessary to search for alternative anticancer agents. In this regard, plants remain an excellent source of anticancer agents (Ugbogu *et al*., 2013). Some cytotoxic compounds have been reported from the family Apocynaceae (Khyade *et al*., 2014), including *Alstonia* species (Khyade *et al*., 2014). However, there is no report on the phytochemistry and cytotoxicity of *Alstonia boonei* and *Schizozygia coffaeoides.* Therefore in this study, the stem bark and root bark of *Alstonia boonei*; and the leaves of *Schizozygia coffaeoides* were investigated for cytotoxic phytochemicals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cancer

Cancer is a collection of diseases resulting from the uncontrolled growth of abnormal and irreparable cells (American Cancer Society, 2016). The root cause of cancer can be due to internal or external factors (lifestyle and environmental). Internal factors may include alteration of DNA structure (Tringali, 2001), high concentration of free radical agents (Boik, 2001) and epigenetic changes (Miller *et al*., 2016). Lifestyle includes tobacco smoking – mortality from various types of cancer have been attributed to smoking (Kuper *et al*., 2002; Stewart & Kleihues, 2003). Diet also plays a very big role in contributing to cancer risks and apparently accounting for about 30% of the cancer cases in western countries and 20% of cases in developing countries (Key *et al*., 2004; Stewart & Kleihues, 2003). Heavy alcohol consumption contributes to the cancer through formation of carcinogenic metabolites to humans, according to the International Agency for Research on Cancer (IARC) (Testino *et al*., 2012; WHO, 2008; Danaei *et al*., 2005). Environmental factors such as exposure to radiation, pathogens (for example; *Helicobacter pylori*, papilloma viruses and Epstein-Barr virus) also contribute to cancer (Tringali, 2001). Non-modifiable factors such as old age and family history also contribute to cancer development (WHO, 2014; WHO, 2008).

2.1.2 Types of Cancer

Cancer manifests in various forms which can closely be related to the cause and the organ/tissue affected. Globocan (2012) reported an estimate of over 28 cancer types spread over 184 countries across the world. Of these, the most common and the most devastating cancer forms

are lung cancer (responsible for 13.0% of the global total), breast cancer (responsible for 11.9%), and colorectal cancer (accounting for 9.7%). Liver cancer is yet another type causing 9.1% cancer deaths, and stomach cancer which accounted for 8.8% global mortality. In women, about 528,000 cervical cancer cases are registered each year making it fourth, with mortality of about 266,000 worldwide in 2012 (Globocan, 2012). Leukaemia, lymphoma and choriocarcinoma cancers are commonly reported in children (WHO, 2014; WHO, 2008). Other common types of cancer include prostate, Kaposi sarcoma, ovary and gastric (WHO, 2014; WHO, 2008).

2.1.3 Cancer Prevalence

Globally, 14.1 million new cancer cases were recorded in 2012, and of these 58% were fatal. By 2020 cancer cases are projected to rise to about 22 million globally. A great majority of cancer cases were recorded in under-developed countries (Globocan, 2012). As a result, cancer is becoming a major health problem in developing countries where it is projected to rise by 55% by the year 2020 (Szic *et al*., 2011). Annually 37,000 new cancer cases are registered in Kenya with 28,000 deaths which are 7% of the total annual mortality (Kenya cancer control, 2017). Thus cancer is rated the second highest cause of mortality (below cardiovascular disease) in Kenya as for other developing countries (Kenya Nation Cancer Control, 2017).

2.2 Cancer Therapy

Cancer therapy involves both the preventive and treatment measures. Treatment of cancer depends on the cancer type, the stage of cancer, health status, age and additional personal traits of the patient. Preventive measures such as vaccination (American cancer society, 2016), minimizing exposure to intense radiation (American Cancer Society, 2016), systematic screening (Maseri *et al*., 1978) and chemoprevention (Ugbogu *et al*., 2013) reduces cancer incidences. Such preventive measures have worked against leukaemia, non-Hodgkin's lymphoma and Wilms tumour (WHO, 2014).

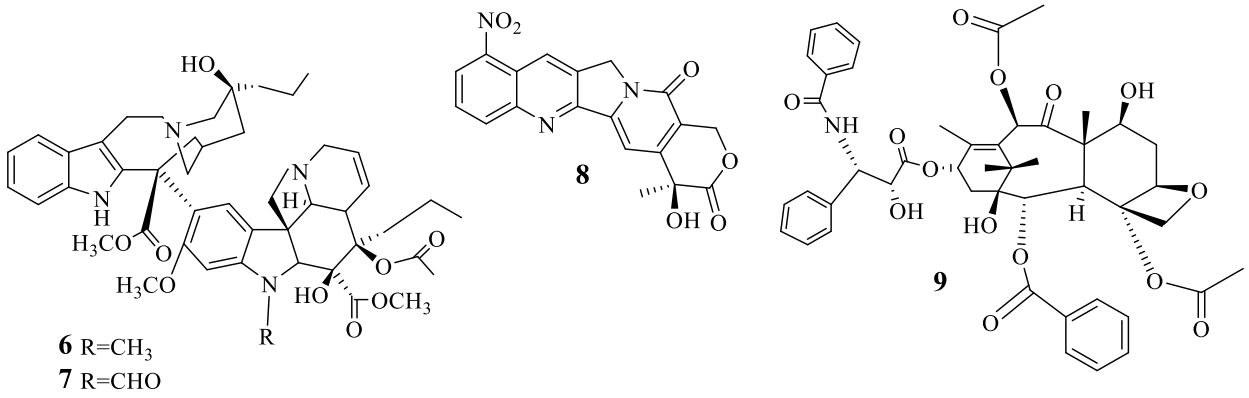
The existing methods for cancer treatment include radiotherapy, immunotherapy, surgery, targeted therapy (Ugbogu *et al*., 2013), chemotherapy, gene therapy (Bagchi & Preuss, 2005), and hormone therapy (American Cancer Society, 2016). These treatments are very expensive to the common population; as such treatment involves both direct and indirect costs. For example, in the UK, an average of £570 is spent monthly per individual upon cancer diagnosis (Macmillan Cancer Support, 2016). Moreover, up to £860 is spent per person as indirect cost associated with cancer treatment. The situation is even worse in under-developed countries, because treatment is often sought overseas in developed countries (Macmillan cancer support, 2016). The average treatment cost for cancer in Kenya is estimated at KShs 300,000 (approx. 3000 USD) (Atieno *et al*., 2018). Besides the cost, the present treatment methods are not well tolerated by the patients. Even after such treatment, cancer often re-occur in a short period of time because of aggressive metastasis (Macmillan cancer support, 2016). In addition to cost, the side effects associated to these treatments are painful and are also sometimes the cause of death to patients after being cured of cancer (American cancer society, 2016).

2.4 Cancer Treatment using Natural Products

Natural products have played an important role in the treatment of different diseases including cancer (Ugbogu *et al*., 2013). The management of cancer cases is still costly-unaffordable to many patients; and the drugs have severe side effects. Therefore the use of phyto-chemotherapy in cancer management is a reasonable option. Most of the natural products used in cancer treatment, work as blocking or suppressing agents in chemoprevention mechanisms. The systematic use of natural products in cancer treatment can be traced to the isolation of the vinca alkaloids vinblastine (**6**) and vincristine (**7**). To date, several natural product-derived drugs are used in the treatment of various cancers. Table 2.1 shows some of the most important natural product derived anti-cancer drugs.

Table 2. 1: Natural products derived anti-cancer drugs

Compound	**Source**	**Form of cancer treated**	**Reference**
Vinblastine (**6**)	*Catharanthus roseus*	Breast, lung, leukaemia	Prakash *et al*., 2013
Vincristine (**7**)	*Catharanthus roseus*	Leukaemia in children	Prakash *et al*.,2013
Camptothecin (**8**)	*Camptotheca acumminata*	Ovarian, lung, breast	Itokawa *et al*., 2008
Taxol (**9**)	*Taxus brevifolia nut*	Colorectal	Itokawa *et al*., 2008



2.5 The Apocynaceae Family

Plants of the family Apocynaceae can be tropical trees, shrubs and vines among dogbane family of flowering plants of the gentian order (Wong *et al*., 2013). The subfamilies of Apocynaceae include; Secamonoideae, Asclepiadoideae, Apocynoideae, Rauvolfioideae and Periplocoideae (Kashef *et al*., 2015). The family Apocynaceae comprises of more than 500 genera and 15,000 species with 22 genera and 40 species reported in Kenya (Kashef *et al*., 2015, Omino and Kokwaru, 1993). Some plants from this family have been reported to possess cytotoxic properties. The genera with cytotoxic activity include; *Allamanda*, *Alstonia*, *Calotropis*, *Catharanthus*, *Cerbera, Nerium, Plumeria, Tabernaemontana* and *Vallaris* (Wong *et al*., 2013). For example, *Catharanthus roseus* has been reported as a source of the natural product-derived

anticancer drugs including vinblastine (**6**) and vincristine (**7**) which are used in the treatment of leukaemia in children (Aruna *et al*., 2015).

2.5.1 The Genus *Alstonia*

The genus *Alstonia* has diverse pharmacological properties including cytotoxicity, antiplasmodial and antifungal (Pratyush *et al*., 2011). The genus consists of about 40 species widely distributed in the tropics especially in Africa, South Asia (Adotey *et al*., 2012), Australia and the Central America (Pan *et al*., 2014). *Alstonia* species are rich in alkaloids, steroids and triterpenoids, and phenolic compounds (Pratyush *et al.*, 2011).

2.5.1.1 Alstonia Boonei

Alstonia boonei is widely distributed throughout the tropics, the rain forest of west and Central Africa (Opoku and Akoto, 2014). The species is widely available in Ethiopia, Uganda, Senegal and Sudan (Orwa *et al.*, 2009). It has also been considered as a medicinal plant in the areas where it grows (Opoku and Akoto, 2015). The plant is commonly known as stool wood, cheese wood and De wild. In Central Uganda, it is known as "*Mubajangalabi*" (Luganda) (Orwa *et al*., 2009). It grows best in the damp areas with moist to well-drained soils and also favoured by the altitude between 500-1000 m above sea level, with mean annual rainfall between 1500-2000 mm (Orwa *et al*., 2009).

2.5.2 Ethnobotanical Uses of the Genus *Alstonia*

Plants of the genus *Alstonia* are widely used as medicinal plants for the treatment of various diseases. Table 2.2 summarizes the various ethnomedical uses of the different *Alstonia* species

Table 2. 2: Ethnobotanical uses of *Alstonia* species

Species	**Plant part**	**Use**	**Reference**
A. boonei	Leaves	Anti-diabetic	Adotey *et al*., 2012
	Stem bark	Anti-inflammatory	
	Root bark	Antipsychotic, insecticidal, anti-snake venom, anthelminthic, antidiarrheal properties	Miracle *et al*., 2014
A. scholaris	Leaves	Used for the treatment of Ulcer, rheumatic pain, asthma, diabetes and dropsy	Pratyush *et al*., 2011
	Roots	Used for treatment of enlarged liver with pain and leprosy	
	Stem bark	Tonic, aphrodisiac, febrifuge, stimulant, expectorant, alterative, carminative, antiperiodic, astringent and stomach ache. For treatment of leprosy, dyspepsia, malarial fever, leishmania infection. Ulcers, tuberculosis, toothache, post-pregnancy fever, menstrual disorder, skin disease, dog bite, cough, asthma, hepatitis, snake bites, tumors, itching, rheumatism	Khyade *et al*., 2014
	Flowers	Management of asthma and other respiratory problems	

Table 2.2: Continued ….

A. macrophylla	Leaf	Used for treatment of stomach ache, bruise, dislocated joints, skin diseases, urinary infections, sprain, ulcers, vulnerary properties	Khyade *et al.*, 2014
	Stem bark	Used for treatment of swelling, malaria fever, bone fracture, vulnerary properties, diabetes, fatigue, liver disease, general tonic, fatigue.	
	Roots	Fractured bones, fever, ulcers	
A. angustifolia	Leaves	Used as remedy for fever	Pan *et al*., 2014
	Stem bark	Anti-malarial remedy	
A. rupestris	Leaves	Known for its cytotoxicity, anti-bacterial and antifungal activities	Zhang *et al*., 2014

2.6 Phytochemistry of the Genus *Alstonia*

A variety of phytochemicals have been reported from the genus *Alstonia* including alkaloids, steroids, terpenoids and phenolic compounds (Pratyush *et al*., 2011; Zhang *et al*.,2014). These classes of compounds are presented in sub-sections 2.6.1 - 2.6.3.

2.6.1 Alkaloids of the genus *Alstonia*

More than 76 alkaloids have been isolated from the genus *Alstonia*. Some of these have exhibited antimicrobial (Khyade *et al*., 2014), anticancer (cytotoxic activities), antitussive (Zhang *et al*., 2014) and anthelminthic activities (Miracle *et al*., 2014**)**. Some of the alkaloids reported from this genus are listed in Table 2.3.

Table 2.3: Alkaloids reported from *Alstonia* species

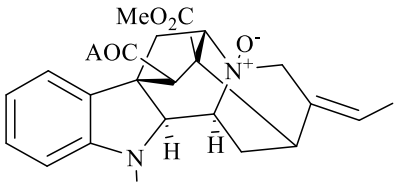
Alkaloids	Source (plant part)	References
Alstiphyllanine A (**10**)	*A. Macrophylla* (L)	Hirasawa *et al*., 2009
Alstiphyllanine B (**11**)		
Alstiphyllanine C (**12**)		
Alstiphyllanine D (**13**)		
Alstiphyllanine G (**14**)	*A. Macrophylla* (L)	Khyade *et al*.,2014
Alstonoxine A (**15)**	*A. Macrophylla* (L)	Lim *et al*., 2014
Alstonoxine B (**16)**		
Alstonoxine C (**17)**		
Alstonoxine D (**18)**		
Alstofolinine A (**19**)		
Alstonerine (**20**)		
Quaternine (**21**)		
11-demethoxy quaternine (**22**)		
Vincamajine (**23**)		
Vincamajine *N*(4)-oxide (**24**)		
Vincamajine17-*O*-verate *N*(4)-Oxide(**25**)		
Vincamajine17-*O*-verate (**26**)		
Cathafoline (**27**)	*A. macrophylla* (Sb)	Khyade *et al*.,2014
2(*S*)- Cathafoline (**28**)		
2(*R*)-3-Hydroxy-Cathafoline (**29**)		
2(*S*)-10-Methoxy Cathafoline (**30**)		
Lumutinine A (**31**)		
Macralstonine (**32**)		
Macrocarpine A(**33**)		
Macrocarpine B (**34**)		
Macrocarpine D (**35**)		
Macrodasine A (**36**)		
Macrodasine F (**37**)		
Macrodasine H(**38**)	*A. macrophylla* (Sb)	Kam *et al.*,1998
Alstonal (**39**)		
Alstophylline (**40**)		
Alstonisine (**41**)		
Alstonerinal(**42**)		
Lochnerine (**43**)		
10-methoxyaffinisine (**44**)		
Alstomacrophylline (**45**)	*A. macrophylla* (Rb)	Niwat *et al*., 1998
Alstomacroline (**46**)		

Table 2.3 continued…

Alstoniascholarine A (**47**)	*A. scholaris* (L)	Qin *et al*., 2015
Alstoniascholarine B (**48**)		
Alstoniascholarine C (**49**)		
Alstoniascholarine D (**50**)		
Alstoniascholarine E (**51**)		
Alstoniascholarine F (**52**)		
Alstoniascholarine G (**53**)		
Alstoniascholarine H (**54**)		
Alstoniascholarine I (**55**)		
Alstoniascholarine J (**56**)		
Alstoniascholarine K (**57**)		
Akuammidine (**58**)	*A. scholaris* (L)	Feng *et al*., 2009
19-*Epi*-scholaricine (**59**)		
Vallesamine (**60**)	*A. scholaris* (L)	Qin *et al*.,2015
Echitamine (**61**)	*A. scholaris* (Sb)	Khyade *et al*.,2014
Scholaristine B (**62**)	*A. scholaris* (Sb)	Feng *et al*.,2009
Scholaristine C (**63**)		
Scholaristine D (**64**)		
Scholaristine E (**65**)		
Scholaristine F (**66**)		
Scholaristine G (**67**)		
Picralinal (**68**)	*A. scholaris (L)*	Liang *et al*., 2013
Picraline (**69**)		
Scholarsine A (**70**)	*A. scholaris (Rb)*	Arora & Rai, 2015
Alistonitrine A (**71**)		
6,7-Epoxy-8-oxo-vincadiformine (**72**)	*A. rupestris*(aerialparts)	Zhang *et al*.,2014
11-Acetyl-6,7-epoxy-8-0xo-vincadifformine (**73**)		
11,15-Dihydroxy-14-chloro-vincadifformine (**74**)		
Perakine $N_{1,}N_4$-dioxide (**75**)		
11-Hydroxy-6,7-epoxy-8-oxo-vincadifformine (**76**)		
Vinorine N_1,N_4-doixide (**77**)		
N_4-Methyltalpinine (**78**)	*A. angustifolia* (Sb)	Pan *et al*., 2014
Villastonine (**79**)		
Villastonine *N* (4)-oxide (**80**)		

Table 2.3 continued…

Villastonine D (**81**)	*A. angustifolia* (Sb)	Pan *et al*.,2014
Villastonine E (**82**)		
Alstogustine (**83**)	*A. angustifolia* (Sb)	Hu *et al*., 1989
19-Epicalstogustine (**84**)		
Echitamidine (**85**)		
Akuamicine (**86**)		



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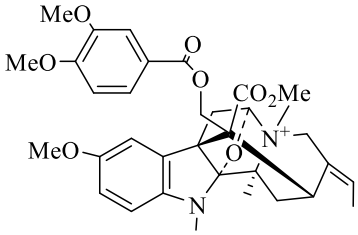

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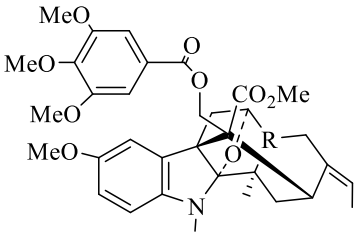
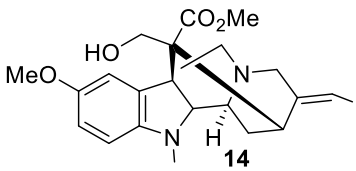


12 R=+N
13 R=N



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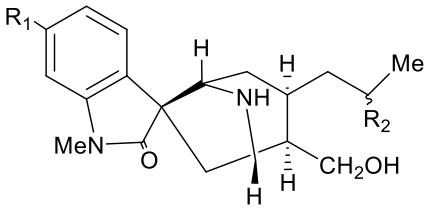
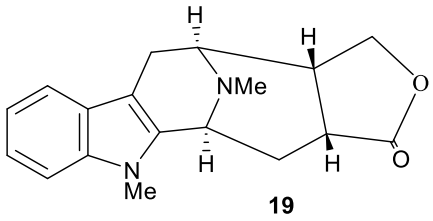


15 R_1=H, R_2= C=O
16 R_1=H, R_2= a-OH, 19S
17 R_1=OMe, R_2= C=O
18 R_1=OMe, R_2= a-OH, 19S



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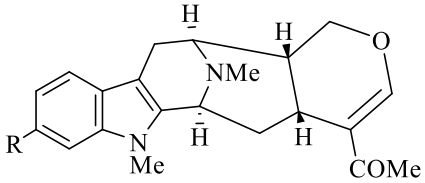
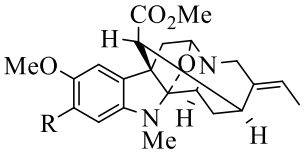
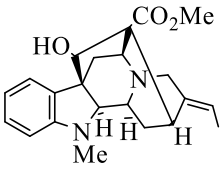


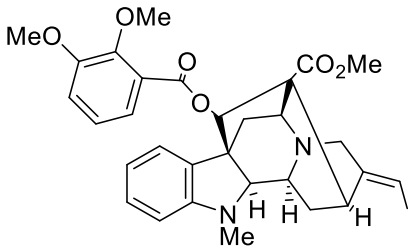
20 R=H
39 R=OMe



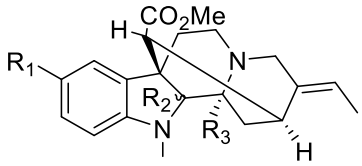
21 R=H
22 R=OMe



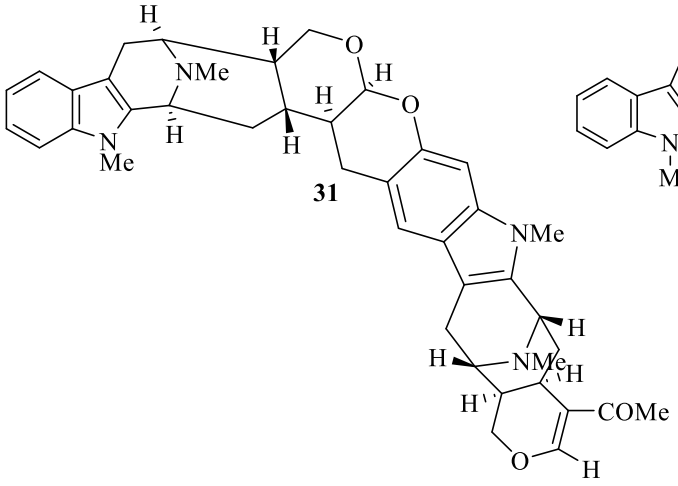
23
24 N(4) → O



25 N(4)→O
26



27 R_1=H, R_2= b-H, R_3= H, 2R
28 R_1=H, R_2= a-H, R_3= H, 2S
29 R_1=H, R_2= b-H, R_3= OH, 2R
30 R_1=OMe, R_2= a-H, R_3= H, 2S



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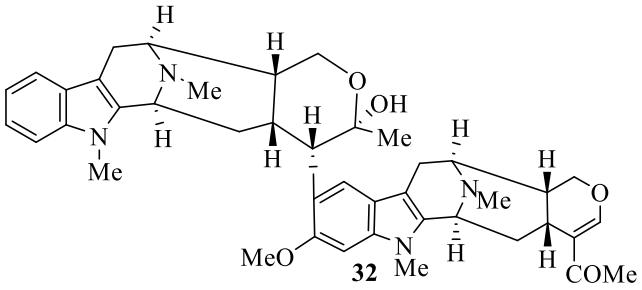

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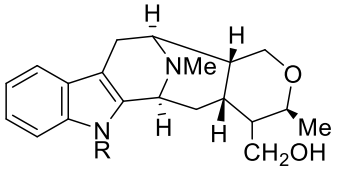
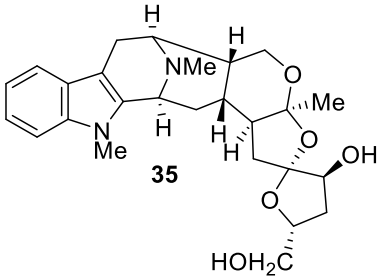


33 R=H
34 R=Me



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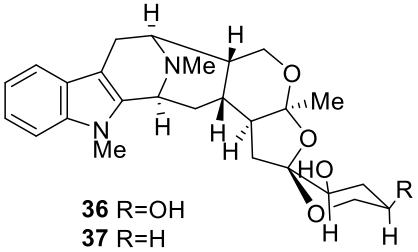
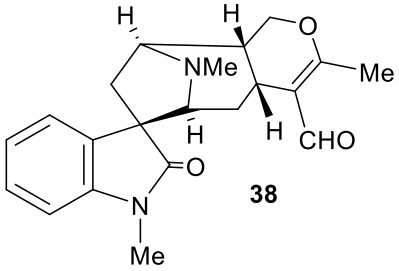


36 R=OH
37 R=H



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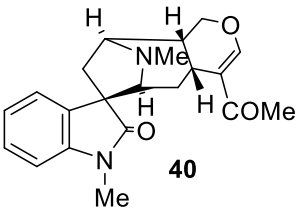

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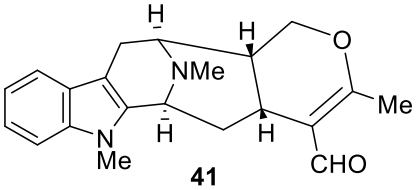

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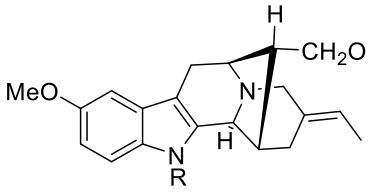
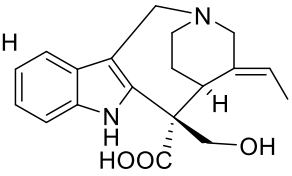
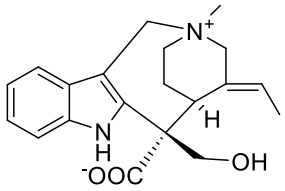


42 R=H
43 R=Me



46
47 Nb-Oxide



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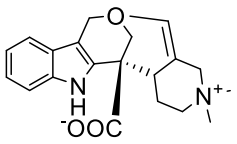

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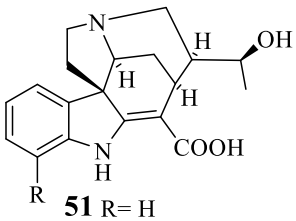
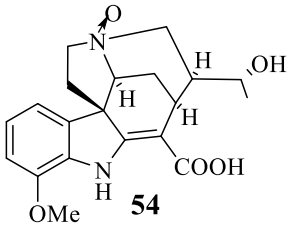


51 R= H
52 R= OH
53 R= OMe



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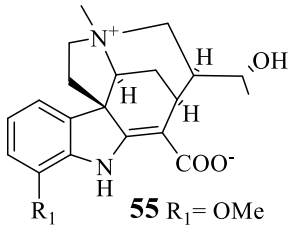
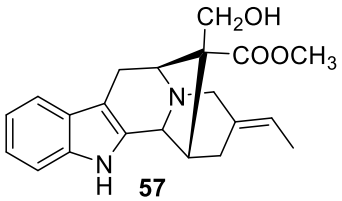


55 R_1= OMe
56 R_1= H



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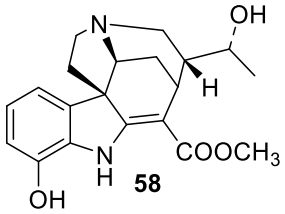

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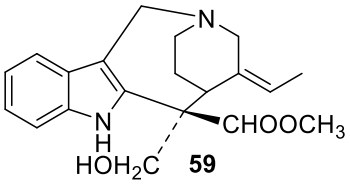

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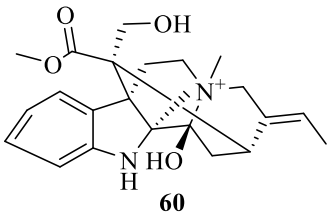

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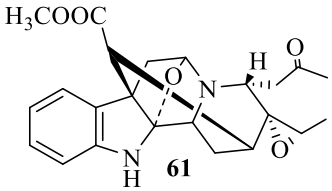

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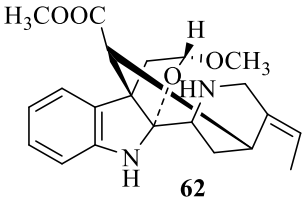

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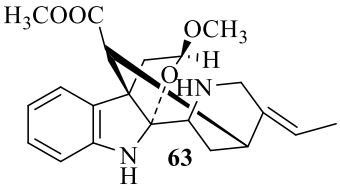

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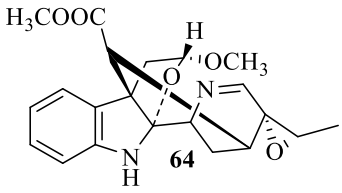

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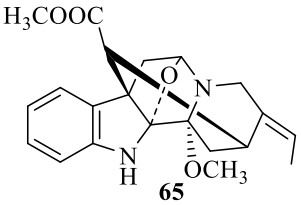

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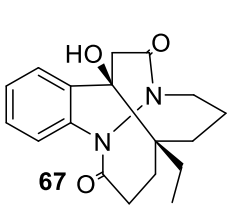

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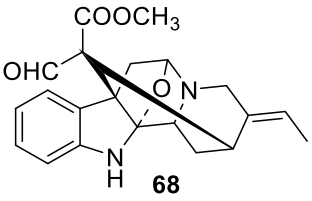

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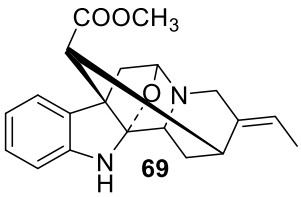

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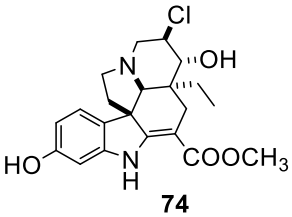

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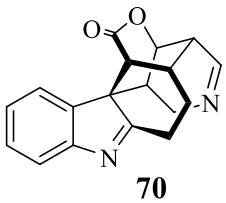

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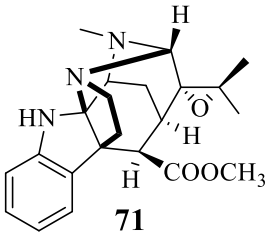

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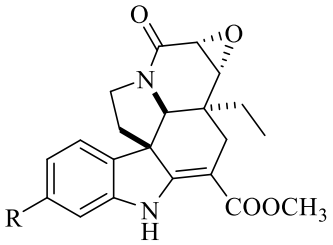
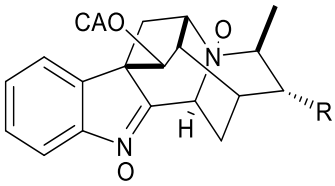
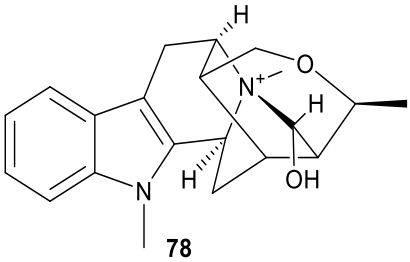


72 R=H
73 R=OAC
76 R=OH



75 R= CHO
77 R= COOH



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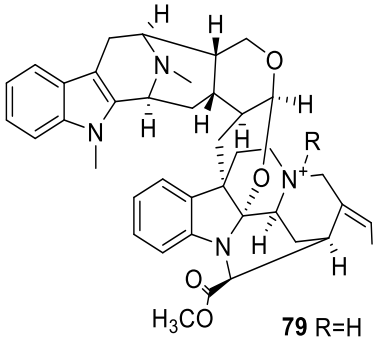
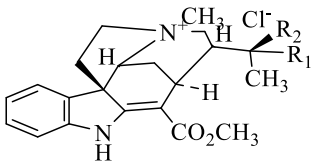
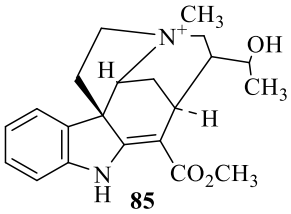


79 R=H
80 R=O
81 R=CH_3
82 R=CH_2Cl



83 R_1=OH, R_2=H
84 R_1=H, R_2=OH



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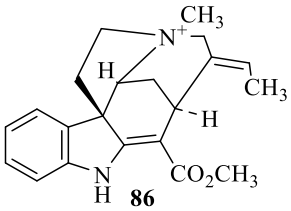


86

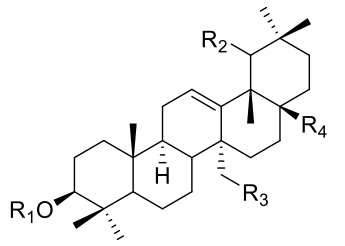
2.6.2 Triterpenes of the genus *Alstonia*

More than 13 triterpenes have been reported from the genus *Alstonia*. Some of the triterpenoids, such as lupeol acetate and *β*-amyrin acetate, have exhibited anti-inflammatory activity (Adotey *et al*., 2012). Other terpenoids isolated include *α*-amyrin, *α*-amyrin acetate, alstoprenylene, 20(30)-

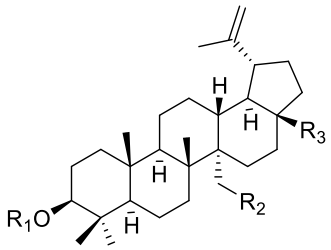
ursa-ene-3-ol, lupeol, betulin, betulinic acid, ursolic acid (Kashef *et al*., 2015), alstonic acid A, alstonic acid B, (-)-lyoniresinol-3*α*-*O*-*β*-D-glucopyranoside (Khyade *et al*., 2014) and *α*-amyrin palmitate (Adotey *et al*., 2012). Some of the terpenoids from this genus are represented in the Table 2.4

Table 2. 4: Triterpenoids reported from *Alstonia* species

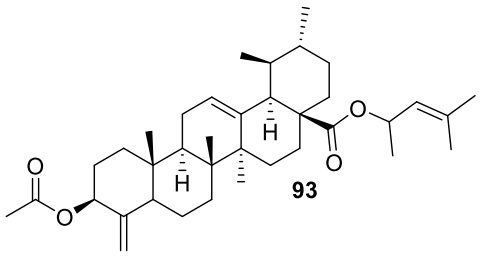
Triterpenes	**Source**	**Reference**
Betulin (**87**)	*A. scholaris* (L)	(Kashef *et al.*, 2015)
Betulinic acid (**88**)	*A. scholaris* (L)	(Kashef *et al*., 2015)
α-Amyrin (**89**)	*A. scholaris* (Rb)	(Kashef *et al*., 2015)
β-Amyrin acetate(**90)**	*A. scholaris* (L)	(Kashef *et al*., 2015)
Alstonic acid A (**91)**	*A. scholaris*	(Khyade *et al*., 2014)
Alstonic acid B (**92)**	*A. scholaris*	(Khyade *et al*., 2014)
Alstoprenylene (**93)**	*A. scholaris* (Fl)	(Kashef *et al*., 2015)
Ursolic acid (**94**)	*A. macrophylla*	(Adotey *et al*., 2012)

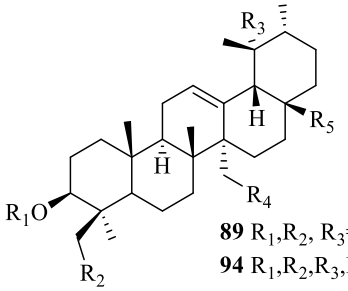


90 R_1=Acetyl,R_2, R_3=H, R4=CH_3



87 R_1,R_2,=H, R_3=CH_2OH
88 R_1,R_2,=H, R_3=COOH





89 R_1,R_2, R_3=H, R_4=H,R_5=CH_3
94 R_1,R_2,R_3,R_4=H,R_5=COOH

2.7 Biological Activities of Compounds Isolated from *Alstonia* Species

Previous studies on *Alstonia* species have resulted in the identification of bioactive compounds from this genus. The activities include antidiabetic, antipyretic, anti-fertility, antioxidant, anti-inflammatory, antimicrobial, antiprotozoal and antidiarrheal (Khyade *et al*., 2014), anticancer (cytotoxic activities) and antitussive activities (Zhang *et al.*, 2014).

2.7.1 Cytotoxic Compounds from *Alstonia* Species

Six cytotoxic monoterpenoid indole alkaloid derivatives have been reported from the aerial parts of *Alstonia rupestris* (Zhang *et al*., 2014). These monoterpenoid indole alkaloids include 6,7-epoxy-8-oxo-vincadifformine (**72**), 11-acetyl-6,7-epoxy-8-oxo-vincadifformine (**73**), 11-hydroxy-14-chloro-15-hydroxy-vincadifformine (**74**), perakine-N_1,N_4-dioxide**(75)**, 11-hydroxy-6, 7-epoxy-8-oxo- vincadifformine (**76**) and vincorine-N_1,N_4-dioxide (**77**). *In vitro* cytotoxicity tests on these compounds were carried out against seven tumor cell lines using MTT assay (Zhang *et al*., 2014). The cytotoxic activities are presented in Table 2.5.

Table 2. 5: *In vitro* Cytotoxicity of compounds from *Alstonia* species against seven human tumor cell lines ($IC_{50,}$ μM) [a].

Compounds	**Cell lines (IC_{50}, μM)**						
	Hep-2	SCL-1	CAL-27	UMSCC-27	Detroit-562	SCC-PKU	TCA-83
6,7-Epoxy-8-oxo-vincadifformine (**72**)	10.3	11.3	9.2	12.0	10.7	13.7	13.0
11-Ecetyl-6,7-epoxy-8-oxo- vincadifformine (**73**)	12.9	12.3	10.8	12.7	11.3	12.9	14.9
11-Hydroxy-14-chloro-15-hydroxy-vincadifformine (**74**)	52.7	51.8	49.0	59.4	54.3	59.7	59.5
Perakine N_1,N_4-dioxide **(75)**	44.1	40.8	44.8	50.7	48.9	47.0	40.1
11-Hydroxy-6,7-epoxy-8-oxo-vincadifformine (**76**)	16.3	15.7	14.8	17.2	14.7	11.2	15.5
Vincorine N_1,N_4-dioxide (**77**)	47.8	51.5	44.8	49.1	53.2	43.6	48.2
Doxorubicin	**18.3**	**14.7**	**22.0**	**31.7**	**24.9**	**35.4**	**15.9**

[a] The activities of doxorubicin are expressed as IC_{50} in nM and those of compounds **72-77** are expressed as IC_{50} in μM.

2.8 Schizozygia coffaeoides

Schizozygia coffaeoides a monotypic shrub, family Apocynaceae, is indigenous to Comoro islands, central and East Africa, including Kenya, Tanzania Somalia and Angola (Barink, 1984). The plant has wide ethnomedicinal uses.

2.8.1 Ethnobotanical Uses of *Schizozygia coffaeoides*

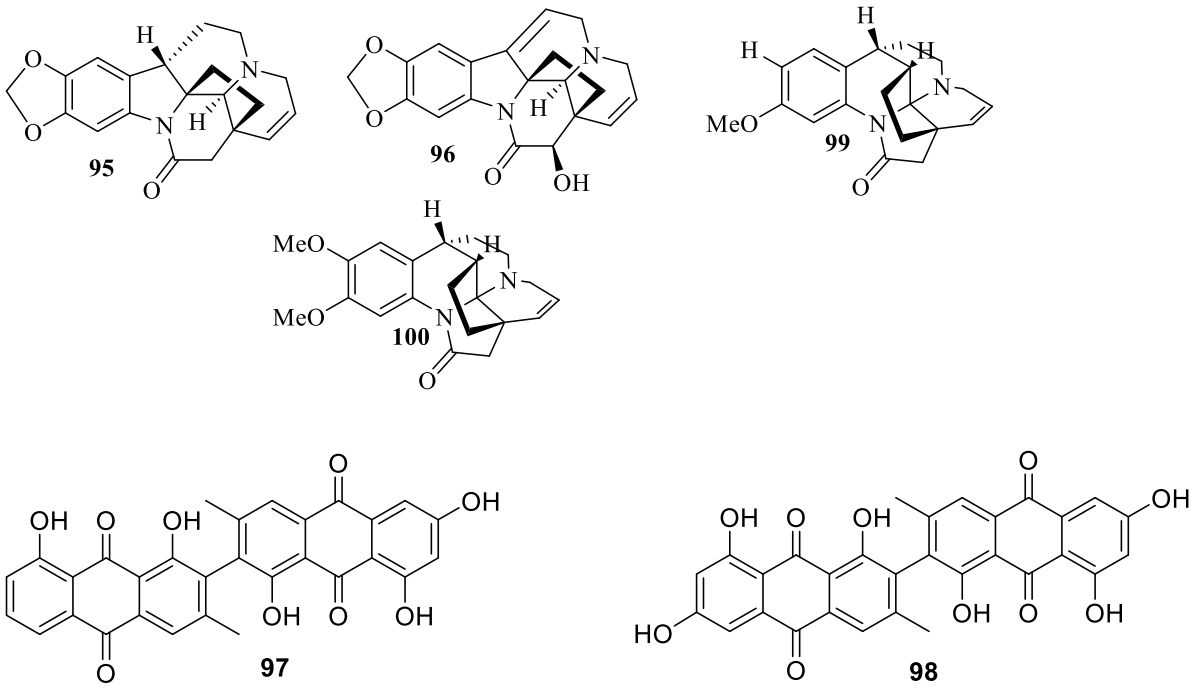
The root infusion is used in the treatment of dizziness, Steam from boiled leaves treats inflamed eyes, a mixture of *S. coffaeoides* and coconut oil is applied in the sore treatment and lastly in Kenya, the aqueous extract of leaves treats infected skins (Barink, 1984; Omino and Kokwaro, 1993).

2.8.2 Phytochemistry of *Schizozygia coffaeoides*

Among the compounds reported from this shrub, includes alkaloids which are considered to be the major class compared to the triterpenes, quinones and steroids. The alkaloids isolated from this monotypic shrub are known as hexacyclic *N*-acyl indole alkaloids (Atilaw *et al*., 2014). Table 2.6 show some of the alkaloids isolated from different parts of *Schizozygia coffaeoides*;

Table 2. 6**:** Reported Alkaloids from *Schizozygia coffaeoides*

Compound	**Plant part**	**Reference**
Schizozygine (**95**)	Leaves, roots and stem bark	Atilaw *et al*., 2014; Kariba *et al*., 2002
6,7-Dehydro-19*β* hydroxyschizozygine (**96)**	Leaves and roots	Atilaw *et al*., 2014; Kariba *et al*., 2002
Cassamin A (**97**)	Roots	Atilaw *et al*., 2014
Cassamin B (**98**)	Roots	Atilaw *et al*., 2014
Isoschizogaline (**99**)	Stem, roots and leaves	Atilaw *et al*., 2014; Kariba *et al*., 2002
Isoschizogamine (**100**)	Roots and stem bark	Atilaw *et al*., 2014; Kariba *et al*., 2002



2.8.3 Biological Activities of Metabolites of *Schizozygia coffaeoides*

Different activities of the metabolites from *Schizozygia coffaeoides* have been reported by various researchers mainly focusing on antifungal, antimicrobial and antiplasmodial activities (Atilaw *et al*., 2014; Kariba *et al*., 2002). In one of these reports (Kariba *et al*., 2002), 6,7-dehydro-19*β*-hydroxyschizozygine (**96**) was the most active compound as an antifungal agent, while isoschizogaline (**99**) was active against bacteria. The results obtained by Kariba *et al*. (2002) showed that 6,7-dehydro-19*β*-hydroxyschizozygine (**96**) was also more active than Ketoconazole (standard drug). Of all the test fungi, *T. mentagrophyles* (MIC= <1.95 µg/ml), *M. gyseum* (MIC= 1.96 µg/ml) and *E. floccosum* (MIC= >1.96 µg/ml) were the most inhibited. Isoschizogaline (**99**) showed weak activity against *Bacillus subtilis* (MIC= 62.5 µg/ml) and *Staphylococcus aureas* (MIC= 125 µg/ml) as compared to ampicillin (standard drug) (Kariba *et al*., 2002). The dichloromethane extract of *Schizozygia coffaeoides* leaves was active against

fungi such as *Tichophyton mentagrophytes*, *Micosporium gypseum*, *Cladosporim cucumerium* and *Candida albicans* using disc diffusion method (Kariba *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 General

Solvents used in extraction and chromatographic separation were glass distilled. The proton and carbon spectra were obtained on a Bruker Avance 500 MHz Spectrometer using TMS (Teteramethyl silane) or residual solvent signals as reference. UV/VIS spectra were recorded using UV-1601 (UV-Visible spectrophotometer). HCOSY, HSQC and HMBC spectra were processed using the standard software, Mestronova or Topspin, EI-MS was done as direct inlet at 70 eV on micro mass GC-TOF Wythenshawe, waters ink, UK.

3.2 Chromatographic Conditions

Column chromatography was performed on silica gel 60G (70-230 mesh, Merck) and Sephadex LH-20. Analytical TLC was done on silica gel 60 (F_{254} Merck) pre-coated aluminium plates. The visualization of the spots on the TLC was carried out using the UV light (254 or 366 nm), iodine vapour and/or by spraying with Dragendroff's reagent.

3.4 Plant materials

The root bark and stem bark of *Alstonia boonei* were collected from Nsanule village, Kaliro district in Eastern Uganda in August 2016. The plant material was identified by Dr. Paul Ssegawa of the Herbarium, Department of Botany, Makerere University, where a voucher specimen (IG001) was deposited.

The leaves of *Schizozygia coffaeoides* were collected from Kenya around Shimba hills in 2009. The plant material was verified by Mr. Simon Mathenge, of the Herbarium, School of Biological Sciences, University of Nairobi, where a voucher specimen (AYT-SM-036-2009) was deposited.

3.5 Extraction and Isolation of Compounds

3.5.1 Extraction and Isolation of Compounds from the Stem Bark of *Alstonia boonei*

The air dried and ground stem bark (1 kg) of *Alstonia boonei* was extracted using CH_2Cl_2/MeOH (1:1) by cold percolation (3 x 3L) to give 73 g of crude extract after the removal of the solvent. The crude extract was partitioned between EtOAc and water giving 38 g of the EtOAc extract which was separated by column chromatography over silica gel (400 g) eluting with *n*-hexane containing increasing amounts of EtOAc. A total of 230 fractions were collected and combined into 40 fractions based on their TLC profile. Lupeol acetate (**101,** 70 mg) precipitated as white amorphous solids from the fraction eluted with 1% EtOAc in *n*-hexane. From the combined fractions eluted with 2% EtOAc in *n*-hexane stigmasterol (**102**, 30 mg) precipitated as white amorphous powder. 6% EtOAc eluent in *n*-hexane were combined and purified by Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) and gave a light yellow powder of lichexanthone (**103** 10.1 mg). From fractions eluted with 7% EtOAc in *n*-hexane cycloeucalenol (**104**, 20 mg) precipitated as white amorphous solids, while 60% EtOAc in *n*-hexane eluent yielded a brown solid of phenanthridine-6(5*H*)-one (**105**, 10 mg).

3.5.2 Extraction and Isolation of compounds from the root bark of *Alstonia boonei*

The air dried and ground root bark (900 g) of *Alstonia boonei* was extracted using CH_2Cl_2/MeOH (1:1) by cold percolation to give 74 g of extract. A portion of the extract (68 g) was subjected to fractionation using column chromatography using silica gel (600 g) and eluting with *n*-hexane containing increasing percentages of EtOAc. A total of 300 fractions were collected and combined to 30 fractions based on their TLC profile. The fraction eluted with 3% EtOAc gave white amorphous solids of stigmasterol (**102**, 10 mg). Lupeol (**106,** 30 mg) was obtained as a white amorphous solid from the fraction eluted with 3-5% EtOAc in *n*-hexane. The

combined fractions eluted with 6-9% EtOAc in *n*-hexane yielded white amorphous solids of β–stitosterol (**107,** 6 mg). Sucrose (**109**, 20 mg) was eluted at 50% EtOAc in *n*-hexane as white crystals. Echitamine (**108**, 500 mg) was obtained from the column when eluted with 100% MeOH as white solids.

3.5.3 Extraction and Isolation of Compounds from the Leaves of *Schizozygia coffaeoides*

The air dried and ground leaves (800 g) of *Schizozygia coffaeoides* were extracted with CH_2Cl_2/MeOH (1:1) by cold percolation. The extract (53.4 g) was subjected to column chromatography over silica gel (600 g) and eluted with a mixture of CH_2Cl_2 and EtOAc. Three major fractions collected; F1 (100% CH_2Cl_2), F2 (50% EtOAc) and F3 (100% EtOAc).

F1 (4.9 g) was applied on silica gel (400 g) column and eluted with petroleum ether (boiling point: 50-70 ^oC) containing increasing percentages of EtOAc. The fraction eluted with 4-7% EtOAc in petroleum ether were combined, from which a white solid of stigmasterol (**102**, 25 mg) was obtained; also fractions eluted with 10-16% EtOAc in petroleum ether were purified, combined and further separated on Sephadex LH-20 (eluent CH_2Cl_2/MeOH; 1:1) to give 6,7-dehydro-19β-hydroxyschizozygine (**96**, 15 mg); the fraction eluted with 17-25% EtOAc in petroleum ether was combined and crystallized from CH_2Cl_2/petroleum ether to give schizozygine (**95**, 500 mg). The fractions eluted with 20-24% EtOAc in petroleum ether were combined and purified on Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) to give cassamin B (**98**, 18 mg).

The second major fraction (F2, 4 g) eluted at 50% EtOAc in CH_2Cl_2 was also separated over silica gel (300 g) eluting with petroleum ether containing increasing percentages of EtOAc. The fractions eluted with 22-24% EtOAc in petroleum ether were combined and purified over Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) to give (**99,** 9 mg); and the fractions eluted with 26-32%

EtOAc in petroleum ether were combined and purified by column chromatography over Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) to give cassamin A (**97**, 16 mg).

3.5.4 Physical and Spectroscopic Data of Compounds Isolated from the Stem and Root Bark of *Alstonia boonei*

Lupeol acetate (101)

White amorphous solids. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 4.47 (1H, *dd* , *J* = 5.3, 11.3 Hz, H-3), 4.70 (*s*, 29a), 4.59 (*s*, 29b), 0.88 (*s*, 23-CH_3), 0.86 (*s*, 24-CH_3), 1.05 (*s*, 25-CH_3), 0.85 (*s*, 25-CH_3), 0.95 (*s*, 27-CH_3), 0.80 (*s*, 28-CH_3); ^{13}C NMR (125 MHz): δ_C 38.4 (C-1), 25.1 (C-2), 81.0 (C-3), 38.0 (C-4), 55.4 (C-5), 18.2 (C-6), 34.2(C-7), 40.8 (C-8), 50.3 (C-9), 37.1 (C-10), 20.9 (C-11), 23.7 (C-12), 37.8 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 150.9 (C-20), 29.8(C-21), 40.0 (C-22), 28.0 (C-23), 16.5 (C-24), 16.2 (C-25),16.0 (C-26), 14.5 (C-27), 18.0 (C-28), 19.3 (C-29), 109.4 (C-30), 171.0 (C=O in OAc), 21.3 (CH_3 in OAc).

Stigmasterol (102)

White solid. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 5.37 (1H, *d*, *J* = 6.0 Hz, H-6), 5.17 (1H, *dd*, *J* = 15.0, *J* = 9.0 Hz, H-22), 5.04(1H, *dd*, *J* = 15.0, 9.0 Hz, H-21) and 3.55 (1H, *m*, H-3). ^{13}C NMR (CD_2Cl_2, 125 MHz)**:** δ_C 37.2 (C-1), 29.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.7 (C-5), 121.7 (C-6), 31.7 (C-7), 29.4 (C-8), 50.1 (C-9), 36.2 (C-10), 24.7 (C-11), 39.8 (C-12), 42.2 (C-13), 56.9 (C-14), 24.3 (C-15), 28.9 (C-16), 56.0 (C-17), 12.1 (C-18), 19.4 (C-19), 40.5 (C-20), 23.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51,2 (C-24), 33,7 (C-25), 21.1 (C-26), 22.8 (C-27), 25.4 (C-28) and 12.2 (C-29).

Lichexanthone (103)

Light yellow amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 6.71 (1H, *d*, *J* = 2.5Hz, H-5), 6.68 (1H, *dd*, *J* = 2.5 Hz, 10.7Hz, H-7), 6.35 (1H, *d*, *J* = 2.5 Hz, H-4), 6.32 (1H, *d*, *J* = 2.5 Hz, H-2), 3.92 (3H, *s*, 6-OCH_3), 3.89 (3H, *s*, 3-OCH_3) and 2.83 (3H, *s*, 8-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz)**:** δ_C 163.7 (C-1), 182.4 (C-9), 104.1(C-9a), 156.9 (C-4a), 98.5 (C-5), 96.9(C-2), 165.8 (C-3), 92.1 (C-4), 112.9 (C-8a), 143.5 (C-8), 115.4 (C-7), 163.8 (C-6), 98.5 (C-5'), 159.4 (C-10a), 55.7(6-OCH_3), 55.6(3-OCH_3), 23.5(8- CH_3).

Cycloeucalenol (104)

White amorphous solid. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 4.72 (1H, *brs*, H-30a), 4.67 (1H, *brs*, H-30b), 3.20 (1H, *m*, H-3), 1.03 (6H, *d*, *J* = 6.8 Hz, 26, 27-CH_3), 0.38 (1H, *d*, *J* = 3.6 Hz, H-19a), 0.14 (1H, *d*, *J* = 3.6 Hz, H- 19b), 1.03, 1.02, 0.97, 0.97, 0.89 (5 x CH_3). ^{13}C NMR (CD_2 Cl_2, 125MHz): δ_C 30.9 (C-1), 34.9 (C-2), 76.7 (C-3), 44.7 (C-4), 43.5 (C-5), 24.8 (C-6), 28.3 (C-7), 46.0 (C-8), 23.7 (C-9), 29.6 (C-10), 25.3 (C-11), 35.5 (C-12), 45.5 (C-13), 48.3 (C-14), 33.0 (C-15), 27.4 (C-16), 52.3 (C-17), 17.9 (C-18), 27.1 (C-19), 36.3 (C-20),18.5 (C-21), 35.1 (C-22), 31.4 (C-23), 157.1 (C-24), 33.9 (C-25), 21.9 (C-26, C-27), 19.3 (C-28), 14.5 (C-29), 106.0(C-30).

Phenanthridin-6(5*H*)-one (105)

Brown solids**.** ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 7.35 (1H, *d*, *J* = 8.0 Hz, H-3), 8.09 (1H, *d*, *J* = 8.0 Hz, H-9), 8.14 (1H, *d*, *J* = 8.0 Hz, H-11), 7.61 (1H, *d*, *J* = 8.0 Hz, H-12), 7.38 (1H, *s*, H-1), 7.69 (1H, *s*, H-4), 6.17 (2H, *s*, OCH_2O), 3.76 (3H, *s*, 5-CH_3), 3.86 (3H, *s*, MeO-7), 9.68 (1H, *brs*, HO-2). ^{13}C NMR (DMSO-d_6 , 125 MHz): δ_C 147.2 (C-7), 150.8 (C-8), 122.5 (C-10), 119.1 (C-12), 127.6 (C-10a), 117.2 (C-11a), 135.2 (C-4a), 161.7 (C-6), 119.3 (C-6a), 119.0 (C-9), 123.2 (C-10), 131.2 (C-10a), 104.4 (C-1), 147.56(C-3), 141.21 (C-2), 102.76 (C-4), 120.3 (C-5a), 102.06 (OCH2O), 40.5 (7-CH_3), 61.7 (7-OCH_3).

Lupeol (**106**)

White amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 4.69 (1H, *s*, H-30a), 4.56 (1H, *s*, H-30b), 3.17 (1H, *m*, H-3), 2.37 (1H, *m*, H-19), 1.38 (2H, *m*, H-21), 1.55 (2H, *m*, H-2) and the methyl protons δ_H 0.74, 0.80, 0.83, 0.95, 0.95, 1.04 and 1.68. ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 151.1 (C-20), 109.1 (C-30), 78.8 (C-3), 55.2 (C-5), 50.4 (C-9), 48.3 (C-18), 48.0 (C-19), 43.0 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.8 (C-4), 38.7 (C-1), 38.1 (C-13), 37.1 (C-10) 35.6 (C-16), 34.3 (C-7), 29.8 (C-21), 27.8 (C-23), 27.5 (C-2), 27.4 (C-15), 25.2 (C-12), 20.9 (C-11), 19.1 (C-29), 18.30 (C-6), 17.8 (C-28), 15.9 (C-25), 15.8 (C-26), 15.2 (C-24), 14.30 (C-27)

β-Sitosterol (107)

White amorphous solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 5.35 (1H, *t*, *J* = 6.4 Hz, H-5), 3.52 (1H, *tdd*, *J* = 4.5 Hz, 4.2Hz, 3.8 Hz, H-6), 1.0 (3H, *s*, CH_3), 0.92 (3H, *d*, *J* = 6.5 Hz, CH_3), 0.85 (3H, *t*, *J* = 7.2 Hz, CH_3), 0.83 (3H, *d*, *J* = 6.4 Hz, CH_3), 0.83 (3H, *d*, *J* = 6.4 Hz) and 0.68 (3H, *s*, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 140.9 (C-5), 121.9 (C-6), 72 (C-3), 57.0 (C-14), 56.2 (C-17), 50.3 (C-9), 46.0 (C-22), 42.5 (C-13), 42.4 (C-4), 40.0 (C-12), 37.4 (C-1), 36.6 (C-10), 36.3 (C-18), 34.1 (C-20), 32.1 (C-7), 32.0 (C-8), 31.8 (C-13), 29.3 (C-25), 28.4 (C-16), 26.2 (C-21), 24.4 (C-15) , 23.2 9C-23), 21.2 (C-11), 20.0 (C-26), 19.5 (C-27), 19.2 (C-19), 18.9 (C-28), 12.1 (C-24), 12.0 (C-29).

Echitamine (108)

Pale yellow crystals. $[\alpha]_D$ = -49.1 (*c* 0.02, MeOH). UV (MeOH) λ_{max} nm: 246.5, 293.5 and 332. IR ν_{max} cm^{-1}: 3427 (OH), 3215 (OH), 1734 (C=O), 1470, 1032 and 75. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 4.41 (1H, *dd*, *J* = 10.7, 6.3 Hz, H-3), 3.39 (1H, *m*, H-5), 2.37 (IH, *dd*, *J* = 13.62, 8.72 Hz, H-6β), 2.07 (1H, *dd*, *J* = 13.62, 8.72 Hz, H-6α), 7.63 (1H, *d*, *J* = 8.0 Hz, H-9), 6.76 (1H, *t*, *J* = 7.6 Hz, H-10), 7.09 (1H, *t*, *J* = 7.6 Hz, H-11), 6.70 (1H, *d*, *J* = 8.0 Hz, H-12), 2.48 (1H, *m*, H-14β), 1.60 (1H, *m*, H-14α), 3.87 (1H, *d*, *J* = 6.85 Hz, H-15), 3.80 (1H, *d*, *J* = 12.3 Hz, H-17β),

3.27(1H, *d*, *J* = 12.3 Hz, H-17α), 1.66 (1H, *d*, *J* = *6*.90 Hz, H-18), 5.73 (1H, *dd*, *J* = 13.83, 6.78 Hz, H-19), δ_H 4.39 (1H, *br d*, 1H-21β), 3.90 (1H, *br d*, H-21α), 3.72 (1H, *s*, OCH_3) and 3.22 (1H, *s*, N-CH_3). ^{13}C NMR (CD_2Cl_2, 125 MHz)**:** δ_C 100.7 (C-2), 70.1 (C-3), 63.9 (C-5), 42.3 (C-6), 62.3 (C-7), 130.5 (C-8), 128.2 (C-9), 121.5 (C-10), 130.6 (C-11), 111.7 (C-12), 148.1 (C-13), 31.6 (C-14), 35.9 (C-16), 65.7 (C-17), 15.5 (C-18), 132.2 (C-19), 131.6(C-20), 66.9 (C-21), 175.6 (C=O), 53.5 (OCH_3) and 50.5 (N-CH_3). HRESIMS *m/z*: 385.2119 [theoretical mass for $C_{22}H_{29}N_2O_4$, 385.2119].

Sucrose (109)

White solids. IR ν_{max} cm^{-1}: 3390 (OH), 3325 (OH), 2941, 1065, 1050 and 988, ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 5.25 (1H, *d*, *J* = 3.8 Hz, H-2), 3.73 (1H, *m*, H-3), 4.05 (1H, *d*, *J* =8.8 Hz, H-4), 3.89 (1H, *t*, *J* = 8.58Hz, H-5), 3.59 (1H, *t*, *J* = 9.61Hz, H-6), 3.65 (1H, *m*, H-7), 3.39 (1H, *dd*, *J* = *9*.98, 3.8 Hz, H-8), 3.31 (1H, *t*, *J* = 9.49Hz, H-9), 3.66, 3.52 and 3.65. ^{13}C NMR (CD_2Cl_2, 125 MHz): δ_C 104.6 (C-1), 93.1 (C-2), 82.3 (C-3), 77.3 (C-4), 74.9 (C-5), 73.5 (C-6), 73.3 (C-7), 73.3 (C-7), 72.0 (C-8), 70.1 (C-9), 63.3 (C-10), 62.2 (C-11) and 61.0 (C-12). ESIMS *m/z*: 265.1049 [M^++Na] [theoretical mass for $C_{12}H_{22}NaO_{11}$, 365.1054]

3.5.5 Physical and Spectroscopic Data of Compounds isolated from the Leaves of *Schizozygia coffaeoides*

Schizozygine (95)

White crystals. $[\alpha]_D$ = 17.5 (*c* 0.02, CH_2Cl_2). UV (CH_2Cl_2) λ_{max} nm: 229, 2670, 316. IR ν_{max} cm^{-1}: 2935, 1639 (amidic C=O), 1466, 1392, 1256 and 1158 ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 7.61 (1H, *s*, H-12), 6.67 (1H, *d*, *J* = 0.7 Hz, H-9), 5.92 (1H, *d*, *J* = 1.3 Hz, OCH_2O), 5.91 (1H, *d*, *J* = 1.3 Hz, OCH_2O), 5.73 (1H, *ddd*, *J* = 10.0, 4.4, 2.0 Hz, H-15), 5.57 (1H, *dt*, *J* = 10.0, 2.2 Hz, H-14), 3.37 (1H, *d*, *J* = 16.83 Hz, H-3β), 2.80 (1H, *d*, *J* = 16.80 Hz, H-3α), 3.02 (1H, *bs*, H-5β), 2.27 (1H, *m*, H-5α), 3.20 (1H, *t*, *J* = 6.70 Hz, H-7β), 2.03 (1H, *m*, H-7α), 2.28 (1H, *m*, H-16),

1.87 (*ddd*, *J* = 12.8, 8.7, 5.4 Hz, H-17α), 2.04 (1H, *m*, H-17β), 2.45 (1H, *dd*, *J* = 18.0, 2,7 Hz, H-19α), 2.61 (1H, *d*, *J* = 18.0 Hz, H-19β) and 2.25 (1H, *s*, H-21). ^{13}C NMR (CD_2Cl_2, 125 MHz); δ_C 73.0 (C-2), 53.9 (C-3), 50.4 (C-5), 26.2 (C-6), 42.5 (C-7), 125.8 (C-8), 104.2 (C-9), 144.6 (C-10), 147.2 (C-11), 98.4 (C-12), 137.4 (C-13), 124.2 (C-14), 130.6 (C-15), 39.0 (C-16), 38.0 (C-17), 169.2 (C-18), 47.3 (C-19), 45.1 (C-20), 68.4 (C-21) and 102.0 (OCH_2O). EIMS *m/z* (rel. int.): 336 $[M]^+$ (100), 307 (30), 337 (20). HRMS *m/z* 336.1481 (theoretical mass for $C_{20}H_{20}O_3N_2$, 336.1474)

6,7-Dehydro-19β-hydroxyschizozygine (96)

Purple amorphous solid**.** $[\alpha]_D$ = -20.1 (*c* 0.02, CH_2Cl_2). UV ($CHCl_3$) λ_{max} nm: 277, 316, 545. IR ν_{max} cm^{-1}: 3431 (OH), 2936, 1653 (amidic C=O), 1436 1223, 1035 and 1021. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 2.85 (1H, *d*, *J* = 16.9 Hz, H-3α), 3.45 (1H, *dd*, *J* = 16.9, 4.6 Hz, H-3β), 2.94 (1H, *d*, *J* = 16.3 Hz, H-5α), 3.59 (1H, *dd*, *J* = 16.3, 4.8 Hz, H-5β), 5.24 (1H, *dd*, *J* = 4.75, 2.2 Hz, H-6), 6.5 (1H, *s*, H-9), 8.35 (1H, *s*, H-12), 5.73 (1H, *ddd*, *J* = 10.0, 4.6, 1.9 Hz, H-14), 6.03 (1H, *d*, *J* = 10.25 Hz, H-15), 2.06 (1H, *m*, H-16α), 2.35 (1H, *m*, H-16β), 1.84 (1H, *m*, H-17α), 2.44 (1H, *m*, h-17β), 4.02 (1H, *d*, *J* = 1.6 Hz, H-19), 2.50 (1H, *s* H-21), 5.91 (1H, *d*, *J* = 1.3 Hz, $OCH_2O\alpha$) and 5.92 (1H, *d*, *J* = 1.3 Hz, $OCH_2O\beta$). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ_C 64.4 (C-2), 52.4 (3), 52.3 (C-5), 100.6 (C-6), 145.1 (C-7), 116.8 (C-8), 98.3 (C-9), 144.9 (10), 142.5 (C-11), 103.0 (C-12), 138.5 (C-13), 123.6 (C-14), 127.5 (15), 38.2 (16), 30.0 (C-17), 171.1 (C-18), 76.2 (C-19), 46.9 (20), 68.1 (C-21) and 102.2 (OCH_2O).

Cassamin A (97)

Yellow amorphous solid: $[\alpha]_D$ = -49.8 (*c* 0.02, CH_2Cl_2). UV (CH_2Cl_2) λ_{max} nm: 271, 308 and 445. IR ν_{max} cm^{-1}: 3352 (OH), 2498, 1672 (C=O), 1604, 1446, 1264, 1128 and 1041. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 7.83 (1H, *s*, H-4), 7.82 (1H, *dd*, *J* = 7.7, 1.2 Hz, H-5), 7.72 (1H, *dd*, *J* =

8.47, 7.9 Hz, H-6), 7.30 (1H, *dd*, *J* = 8.47, 1.21 Hz, H-7), 7.78 (1H, *s*, H-4'), 7.24 (1H, *d*, *J* = 2.5 Hz, H-5'), 6.61 (1H, *d*, *J* = 2.5Hz, H-7'), 2.21 (3H, *s*, 3-CH_3) and 2.20 (3H, *s*, 3'-CH_3). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ_C 160.3 (C-1), 114.6 (C-1a), 131.2 (C-2), 149.0 (C-3), 122.2 (C-4), 133.6 (C-4a), 120.5 (C-5), 134.2 (C-5a), 137.8 (C-6), 125.1 (C-7), 162.8 C-(8), 116.5 (C-8a), 193.3 (C-9), 182.9 (C-10), 160.0 (C-1'), 114.6 (C-1'a), 130.9 (C-2') 147.7 (C-3'), 121.9 (C-4'), 133.6 (C-4'a), 110.1 (C-5'), 136.1 (C-5'a), 166.4 (C-6'a), 108.9 (C-7'), 165.8 (C-8'), 110.1 (C-8'a), 191.2 (C-9'), 182.9 (C-10'), 20.9 (3-CH_3) and 20.7 3'-CH_3). EIMS *m/z* (rel. int.): 522 $[M]^+$(100), 520 (20), 523 (10). HRMS *m/z* 522.0968 (theoretical mass for $C_{30}H_{18}O_9$, 522.0950).

Cassamin B (98)

Yellow amorphous solid. $[\alpha]_D$ = 27.0 (*c* 0.02, CH_2Cl_2). (CH_2Cl_2). UV (CH_2Cl_2) λ_{max} nm: 264, 291, 450. IR ν_{max} cm^{-1}: 3328 (OH), 2975, 1720, 1621, 1600, 1517, 1478, 1456 and 1031. ^{1}H NMR (CD_2Cl_2/CD_3OD, 500 MHz): δ_H 7.76 (1H, *s*, H-4), 7.22 (1H, *s*, H-5), 6.59 (1H, *s*, H-7) and 2.22 (3H, *s*, 3-CH_3). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ_C 160 (C-1), 131.1 (C-2), 147.7 (C-3), 121.9 (C-4), 133.4 (C-4a), 110.1 (C-5), 136.0 (C-5a), 166.4 (C-6), 108.9 (C-7), 165.8 (C-8), 109.9 (C-8a), 191.2 (C-9), 182.9 (C-10) and 20.7 (3-CH_3). EIMS *m/z* (rel. int.): 538 $[M]^+$(100), 539 (30). HRMS *m/z* 538.0907 (theoretical mass for $C_{30}H_{18}O_{10}$, 538.0900).

Isoschizozygaline (99)

White amorphous solid. $[\alpha]_D$ = -222.7 (*c* 0.02, CH_2Cl_2). UV ($CHCl_3$) λ_{max} nm: 252.5, 295.2. IR ν_{max} $cm^{-1:}$ 2965, 1567, 1426, 1327 and 1153. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ_H 8.43 (1H, *d*, *J* = 2.6 Hz, H-12), 7.06 (1H, *d*, *J* = 8.35 Hz, H-9), 6.67 (1H, *dd*, *J* = 8.33, 2.63 Hz, H-10), 5.75 (1H, *ddd*, *J* = 10.13, 2.63, 1.8 Hz, H-15), 5.62 (*ddd*, *J* = 10.14, 4.64, 1.84 Hz, H-14), 3.83 (3H, *s*,OCH_3), 3.44 (1H, *ddd*, *J* = 17.4, 3.0, 1.64 Hz, H-3β), 3.29 (1H, *ddd*, *J* = 17.3, 2.8, 1.43 Hz, H-3α), 3.25 (1H, *t*, *J* = 5.91 Hz, H-7), 2.84 (1H, *ddd*, *J* = 14.6, 12.9, 3.31 Hz, H-5β), 2.78 (1H, *d*, *J* = 12.35 Hz, H-19β), 2.52 (1H, *ddd*, *J* = 14.3, 2.81, 1.82 Hz, H-5α), 2.50 (*dd*, *J* = 19.0, 2.9 Hz, H-

19α), 2.34 (1H, *m*, H-2), 2.24 (1H, *m*, H-6β), 1.86 (1H, *m*, H-17 β), 1.76 (1H, *m*, H-17α), 1.69 (1H, *m*, H-16β), 1.19 (1H, *m*, H-6α) and 1.13 (1H, *m*, H-16α). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ_C 174.8 (C-18), 159.2 (C-11), 139.3 (C-13), 131.3 (C-15), 129.6 (C-9), 120.7 (C-14), 118.9 (C-8), 110.1 (C-10), 103.5 (C-12), 85.1 (C-21), 55.8 (O-CH_3), 48.2 (C-3), 46.5 (C-19), 44.7 (C-20), 37.8 (C-7), 36.8 (C-17), 35.1 (C-2), 27.3 (C-6) and 25.3 (C-16). EIMS *m/z* (rel. int.): 322 $[M]^+$(100), 323 (20). HRMS *m/z* 322.1685 (theoretical mass for $C_{20}H_{22}N_2O_2$, 322.1681).

3.6 Modification of compound 95

3.6.1 Preparation of 10, 11-Dihydroxyschizozygine Derivative

Schizozygine (50 mg, 0.15 mmol) in dry dichloromethane (30 ml) was stirred at -18 ^oC under nitrogen atmosphere for 30 minutes. Boron tribromide (1 g, 4.5 mmol) in dry dichloromethane (10 ml) was added to the mixture then stirred for 24 hours. MeOH (20 ml) was then added to quench the reaction. The mixture was then concentrated *in vacuo* and crystallized from CH_2Cl_2/MeOH to yield a brown solid of nor-schizozygine derivative (15 mg) in 29% yield.

3.7 Cytotoxic activity

The cell lines; A549, HepG2 and two non-tumoral cells purchased from ATCC were cultured in RPMI 1640 medium. The medium was supplemented with 10% fetal bovine serum, antibiotics penicillin (50 U/mL) and streptomycin (50 µg/mL; Invitrogen, Paisley, Scotland, UK). All the cell cultures were incubated at 37 ^oC in a 5% humidified CO_2 incubator. Cytotoxicity on the A549, HepG2 and two non-tumoral cells was carried out according to Atilaw *et al.*, (2017) using 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide (MTT) (5.0 mg/mL). The test compounds were dissolved in DMSO (50 mmol/L) and stored at -20 ^oC until when needed for use.

An optimal $4\text{^}10^3$ cells per well were seeded in 96-well plates to ensure exponential growth for the duration of the assay. The cells were exposed, after overnight culture, to different concentrations of the compounds (0.039-100 µmol/L) for 72 hours. The untreated cells were used as control. MTT solution (10 µL) was added to each cell and incubated at 37 ^{o}C for 4 hours with the addition of 100 µL solubilisation buffer (10% SDS in 0.01 mol/L HCl) then incubated overnight. After 24 hours, the concentration of A_{570} nm was measured on each well.

The percentage cell viability was obtained from a formula: cell viability = $(A_{treated} / A_{control}) * 100$. The results obtained were expressed as mean ± standard error for the eight independent experiments.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Introduction

The crude extracts of the root bark of *Alstonia boonei* and the leaves of *Schizozygia coffaeoides* were analysed by TLC which showed the presence of several spots. These spots were visualized by exposure to iodine vapour or under UV light (254 and 366 nm) and by spraying with dragendorff reagent (for alkaloids) and ammonium solution (for quinones). The constituents of these extracts were isolated and characterized and tested for cytotoxicity. The structural elucidation and cytotoxicity results of the isolated metabolite are discussed below.

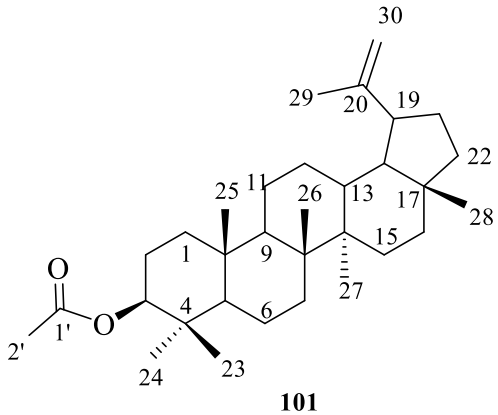
4.1 Characterization of Compounds Isolated from the Stem Bark of *Alstonia boonei*

The air dried and ground stem bark of *Alstonia boonei* was extracted with CH_2Cl_2/MeOH (1:1) by cold percolation at room temperature. The crude extract was subjected to a combination of chromatographic techniques which resulted in isolation of two triterpenes, a steroid, a xanthone and an alkaloid.

4.1.1 Lupeol acetate (101)

Compound **101** was isolated as a white solid, which was not visible at 254 nm on TLC. The ^{1}H NMR spectral data (Table 4.1) shows the presence of six methyl singlets at δ_H 0.84, 0.83, 0.85, 1.05, 0.94, 0.78, and a vinylic methyl at δ_H 1.70 ppm. The ^{13}C NMR spectrum displayed 32 carbon signals (Table 4.1), consistent with a triterpene acetate skeleton. The presence of an acetate group was supported by methyl signal at δ_H 2.05 (3H, s). The oxymethine proton signal at δ_H 4.47 (*dd, J* = 10.7 Hz, 5.5 Hz) was assigned to H-3 as in several triterpenes (Ahmad *et al*., 2013).

The presence of two exo-methylene protons (δ_H 4.69, 4.57, H_2-29) and one methyl signal at δ_H 1.70 (H-30) represents a lupane skeleton. Two of the ^{13}C NMR signals at δ_C 150.9 and 109.4 ppm were attributed to olefinic carbon atoms, a feature consistent with the presence of a terminal double bond. All the other signals were for sp^3 hybridized carbon atoms, one of which appeared at δ_C 81.0 ppm was for an acetoxymethine (C-3) of a triterpene. On the basis of NMR spectral data (Table 4:1) and comparison with the literature values, the compound **101** was identified as lupeol acetate (Ahmad *et al*., 2013; Ragasa *et al*., 2012).



101

Table 4.1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **101** ($CDCl_3$)

Position	$\delta_{H\ in}$ ppm (*mult*, *J* in Hz)	δ_C Experimental	δ_C literature (Ragasa *et al*., 2012)
1	0.99 (*m*)	38.4	38.7
2	1.62 (*m*)	25.1	21.3
3	4.47 (*dd*, *J* = 5.3, 11.3 H_Z)	81.0	81.0
4		38.0	38.0
5	0.80 (*m*)	55.4	55.4
6	1.52 (*m*), 1.42 (*m*)	18.2	18.2
7	1.49 (*m*), 1.38 (*m*)	34.2	34.2
8		40.8	40.8
9	1.30 (*m*)	50.3	50.3
10		37.1	37.1
11	1.42 (*m*), 1.23 (*m*)	20.9	20.9
12	1.91 (*m*), 1.63 (*m*)	23.7	23.7
13	1.66 (*m*)	37.8	37.1
14		42.8	42.8
15	1.69 (*m*), 1.01 (*m*)	27.4	27.4
16	1.49 (*m*), 1.36 (*m*)	35.6	35.6
17		43.0	43.0
18	1.37 (*m*)	48.3	48.3
19	2.39 (*m*)	48.0	48.0
20		150.9	151.0
21	1.93 (*m*), 1.34 (*m*)	29.8	29.8
22	1.39 (*m*), 1.20 (*m*)	40.0	40.0
23	0.88 (*s*)	28.0	28.0
24	0.86 (*s*)	16.5	16.5
25	1.05 (*s*)	16.2	16.2
26	0.85 (*s*)	16.0	16.0
27	0,95 (*s*)	14.5	14.5
28	0.80 (*s*)	18.0	18.0
29	1.70 (*s*)	19.3	19.3
30	4.70 (*s*, 29a), 4.59 (*s*, 29b)	109.4	109.3
2'	2.06 (*s*)	21.3	21.3
1'		171.0	171.3

4.1.2 Stigmasterol (**102**)

Compound **102** was isolated as a white amorphous solid, which was iodine active on TLC. Its ^{13}C NMR spectral data (Table 1.2) showed twenty nine signals, and this is consistent with a steroid skeleton (Pierre and Moses, 2015). Four of these signals resonating at δ_C 141.5, 122.0, 139.0 and 129.8 ppm were for olefinic carbon atoms, which are consistent with the presence of two carbon-carbon double bonds. The other signals included six methyl carbons resonating at δ_C 12.2, 12.3, 19.1, 19.7, 20.6 and 21.6, an oxymethine carbon at δ_C 71.8 ppm was typical of C-3 of steroids (Chaturvedula and Prakash, 2012). By direct comparison of its NMR spectral data (Table 4.2) with literature, compound **102** was identified as stigmasterol (Chaturvedula and Prakash, 2012; Pierre & Moses, 2015).

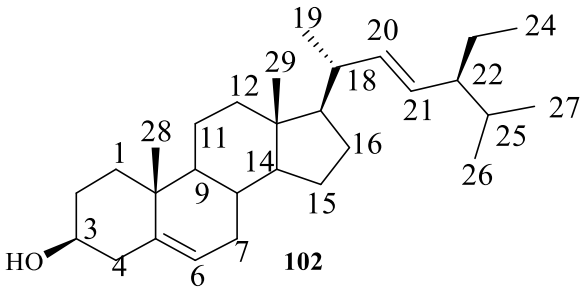


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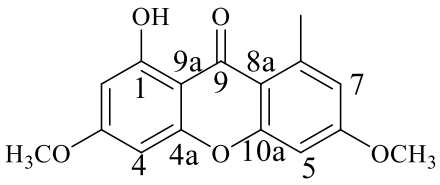
Table 4.2: ^{13}C NMR ($CDCl_3$: 125 MHz) data for compound **102**

Position	**δ_H in ppm (*mult, J* in Hz)**	**δ_C Experimental**	**δ_C Literature (Chaturvedula & Prakash, 2012)**
1		37.8	37.6
2		32.2	32.1
3		72.2	72.1
4		42.9	42.4
5		141.5	141.1
6	5.37 (1H, *d*, *J* = 6.0 Hz)	122.0	121.8
7		32.5	31.8
8		32.5	31.8
9		50.7	50.2
10		36.7	36.6
11		21.6	21.5
12		40.3	39.9
13		41.1	42.4
14		57.4	56.8
15		24.8	24.4
16		29.5	29.3
17		56.6	56.2
18		40.4	40.6
19		21.6	21.7
20	5.17 (1H, *dd*, *J* = 15.0, *J* = 9.0 Hz)	139.0	138.7
21		129.0	129.6
22		46.4	46.1
23		26.0	25.4
24		12.2	12.1
25		29.7	29.6
26		20.1	20.2
27		19.7	19.8
28		19.1	18.9
29		12.3	12.2

4.1.3 Lichexanthone (**103)**

Compound **103** was isolated as a light yellow solid which was visualized under UV light (on TLC at 254 nm). Furthermore, from the ^{1}H and ^{13}C NMR spectral data (Table 4:3), it is evident that this compound is a xanthone derivative (Le Pogam and Boustie, 2016). Its ^{1}H NMR spectral data (Table 4.3) displayed a signal for a chelated hydroxy group (δ_H 13.4, 1-OH).



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In addition, there was a pair of *meta*-coupled aromatic protons of ring A at δ_H 6.32 (*d*, 1H, *J* = 2.3 Hz, H-2) and δ_H 6.35 (*d*, 1H, *J* = 2.5 Hz, H-4), in agreement with the biogenetic expected oxygenation at C-1 (δ_C 163.7) and C-3 (δ_C 165.8) of ring A. Two *meta*-coupled protons at δ_H 6.68 (*d*, 1H, *J* = 2.5, Hz, H-7) and δ_H 6.71 (*d*, *J* = 2.5 Hz, H-5) indicated a C-6 and C-10a substituted ring B of a xanthone. The presence of two methoxy (δ_H 3.89, δ_C 55.7 and δ_H 3.92, δ_C 55.6), a hydroxyl (δ_C 163.7) and methyl (δ_C 23.5) substituents were observed in the NMR spectra (Table 4.3). The placement of the methoxy groups at C-3 and C-6 was established from HMBC spectrum, where 3-OCH_3 (δ_H 3.89) correlated with C-3 (δ_C 165.8) and 6-OCH_3 (δ_H 3.92) with C-6 (δ_C 163.8). The long range coupling of the methyl protons (δ_H 0.79) with H-7 and its HMBC correlation with C-7 and C-8a confirmed the placement of the methyl group at C-8. Upon irradiation of the methoxy groups, in an NOE difference experiment, enhancements were observed in the signals for H-2 and H-4 and H-5 and H-7 confirming the placement of methoxyl groups at C-3 and C-6, respectively. Based on the spectroscopic data and literature, compound **103** was identified as lichexanthone (Le Pogam and Boustie, 2016). This compound has been reported for the first time from the family Apocynaceae.

Table 4.3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data with HMBC and NOE correlation for **103** ($CDCl_3$)

Position	**δ_H (*m*, *J* in Hz)**	**δ_C**	**HMBC**	**NOE**
1		163.7		
2	6.32 (*d*, 2.3)	96.8	C-1, C-3, C-4, C-9a	3-OCH_3
3		165.8		
4	6.35 (*d*, 2.5)	92.1	C-2, C-3, C-4a,C-9a	3- OCH_3
4a		156.9		
5	6.71 (*d*, 2.5)	98.5	C-6, C-7, C-8a, C-10a	6-OCH_3
6		163.8		
7	6.68 (*d*, 2.5)	115.4	C-5, C-6, C-8, C-8a	8-CH_3, 6-OCH_3
8		143.5		
8a		112.9		
9		182.4		
9a		104.1		
10a		159.4		
3-OCH_3	3.89, *s*	55.6	C-3	
6-OCH_3	3.92, *s*	55.7	C-6	
8-CH_3	2.87, *s*	23.5	C-7, C-8a	

4.1.4 Cycloeucalenol (104)

The ^{13}C NMR spectrum of this compound exhibited 30 carbon signals, of which two of these are olefinic carbon atoms resonating at δ_C 105.6 (C-30) and 156.2 (C-24) ppm consistent with a triterpene having one carbon-carbon double bond, five methyl protons at δ_H 1.03, 1.02, 0.97, 0.97, 0.89, 0.89 and a hydroxy proton resonating at δ_H 7.21 ppm. In agreement with this, the ^{1}H NMR exhibited the presence of two olefinic protons resonating at δ_H 4.72 and 4.67 ppm which is typical of terminal methylene protons (CH_2-30), The presence of cyclopropyl group was deduced from the ^{1}H NMR spectrum δ_H 0.39 and δ_H 0.14 ppm. The methylene carbon of the cyclopropyl moiety appeared at δ_C 27.1 ppm (C-19) and is a characteristic of cycloartane triterpenoids (and the oxymethine carbon at δ_C 76.7 ppm (C-3). Comparing the NMR spectral data (Table 4.4) with the literature confirms that compound **104** is cycloeucalenol (Ragasa *et al*., 2012).

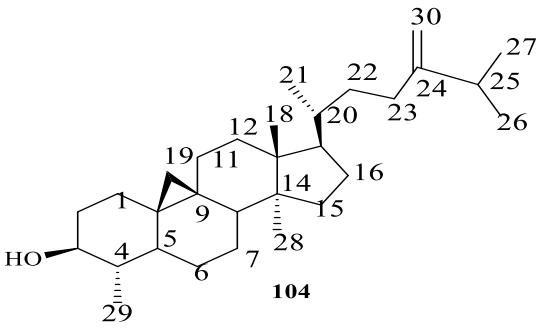

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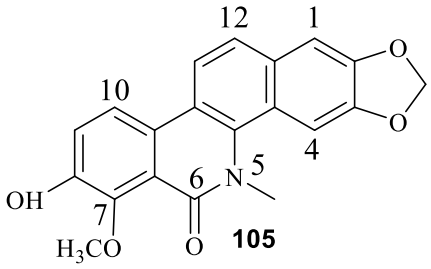
Table 4.4: ^{13}C NMR (125 MHz) data of compound **104** ($CDCl_3$)

Position	**δ_C-literature (Ragasa *et al.*, 2012)**	**δ_C-experimental**
1	30.8	30.9
2	34.8	34.9
3	76.6	76.7
4	44.6	44.7
5	43.3	43.5
6	24.7	24.8
7	28.0	28.3
8	46.9	47.0
9	23.5	23.7
10	29.5	29.6
11	25.1	25.3
12	35.3	35.5
13	45.3	45.5
14	48.6	49.3
15	32.9	33.0
16	27.0	27.4
17	52.2	52.3
18	17.8	17.9
19	27.0	27.1
20	36.1	36.3
21	18.3	18.5
22	35.0	35.1
23	31.3	31.4
24	156.9	157.1
25	33.8	33.9
26	21.9	22.1
27	21.9	22.0
28	19.3	19.3
29	14.4	14.5
30	105.9	106.0

4.1.5 Phenanthridine-6(5*H*)-one (**105**)

Compound **105** was isolated as a brown solid and identified as an alkaloid using dragendorff reagent. The ^{1}H NMR spectrum in the aromatic region showed two sets of *ortho*-coupled protons including one set resonating at δ_H 7.35 and δ_H 8.09 (J = 8.0 Hz) while the other set at δ_H 8.14 and 7.61 (1H, *d*, J = 8.8 Hz). In addition, two *para*-oriented proton singlets at δ_H 7.38 (1H, s) and δ_H 7.69 (1H, s) were also observed. The ^{13}C NMR spectral data (Table 4.5) showed a methylenedioxy, a methoxy, *N*-methyl, amidic carbonyl and 16 sp^2-hybridized carbon atoms suggesting a typical skeleton of benzophenanthridine alkaloids (Vavreková *et al*., 1986). In agreement with this, the *N*-CH_3 protons correlated with C-6 and C-8 in the HMBC spectrum. The placement of the methylenedioxy group at C-2/C-3 is consistent with the HMBC and HSQC spectra (Appendix 92). Compound **105** was identified as phenanthridine-6(5*H*)-one based on the above evidence and comparison of the spectroscopic data with literature data (Krane *et al*., 1984).



105

Table 4.5: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **105** (DMSO-d_6,)

Position	**δ_C**	**δ_H**	**HMBC**
1	104.8	7.38 (*s*)	C-3, C-4, C-4a, C-12
2	147.6		
3	147.2		
4	102.8	7.69 (*s*)	C-1, C-2, C-12a
6	161.7		
7	147.6		
8	150.8		
9	119.1	8.09 (*d*, *J* = 8.0 Hz)	C-7, C-10a
10	122.5	7.35 (d, *J* = 8.0 Hz)	C-6a, C-8, C-11a
11	119.1	8.14 (*d*, *J* = 8.0 Hz)	C-5a, C-10a, C-12a
12	123.6	7.61 (*d*, *J* = 8.0 Hz)	C-1, C-4a , C-11a
4a	135.2		
5a	120.8		
6a	119.3		
10a	127.6		
11a	117.2		
12a	131.6		
OCH_2O	102.1	6.18 (*s*)	C-2, C-3
OCH_3	61.4	3.86(*s*)	C-7
N-CH_3	40.8	3.76 (*s*)	C-6, C-8

4.2 Characterization of Compounds Isolated from the Stem Bark of *Alstonia boonei*

4.2.1 Lupeol (106)

Compound **106** was isolated as a white amorphous solid; the ^{1}H NMR spectrum showed the presence of seven methyl singlets at δ_H 0.74, 0.80, 0.83, 0.95, 0.95, 1.04 and 1.68 ppm, and an oxymethine proton signal at δ_H 3.17 (1H, *m*, H-3) ppm. A vinylic proton at δ_H 1.68 (H-30) and the presence of two exo-methylene protons (δ_H 4.69, 4.55, H-29) showed that compound has the lupine triterpene skeleton. In agreement with this, the ^{13}C NMR spectrum showed the presence of thirty carbon atoms with the olefinic carbons appearing at δ_C 151.1 (C-20) and δ_C 109.1 (C-29) consistent with a terminal double bond. The signal at δ_C 78.7 confirms a highly deshieled carbon due to the presence of oxygenation at C-3 in this triterpene. The NMR data (Table 4.6) is in complete agreement with the literature report for lupeol (Abdullahi *et al*., 2013).

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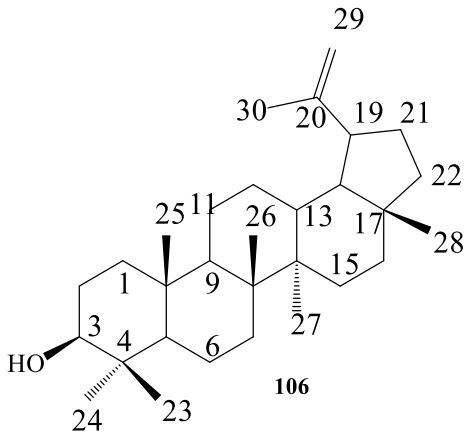


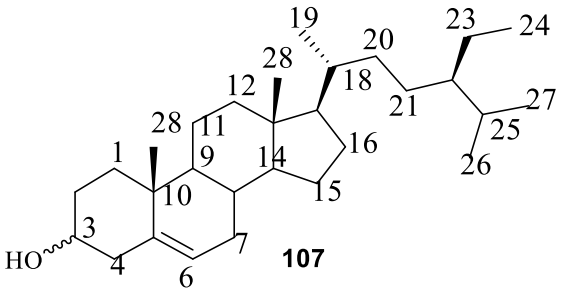
106

Table 4.6: ^{13}Carbon (125 MHz) NMR data of compound **106** ($CDCl_3$)

Position	**δ_C -literature (Abdullahi *et al*., 2013).**	**δ_C-experimental**
1	38.7	38.7
2	27.4	27.5
3	79.0	78.8
4	38.9	38.8
5	55.5	55.2
6	18.5	18.3
7	34.2	34.3
8	40.9	40.8
9	50.5	50.4
10	37.2	37.1
11	21.0	20.9
12	25.2	25.2
13	38.1	38.1
14	42.9	42.8
15	27.1	27.4
16	35.5	35.6
17	43.0	43.0
18	48.3	48.3
19	48.0	48.0
20	150.0	151.1
21	29.9	29.8
22	40.0	39.9
23	28.0	27.8
24	15.5	15.2
25	16.1	15.9
26	16.0	15.8
27	14.8	14.3
28	18.0	17.8
29	109.0	109.1
30	19.5	19.1

4.2.2 β-Sitosterol (107)

Compound **107** was isolated as a white amorphous solid. It's ^{1}H NMR spectrum exhibited one deshieled olefinic proton δ_H 5.38 (*t, J* = 6.7 Hz) and also a proton attached to the oxygenated carbon δ_H 3.53 (*m*) together with six methyl protons at δ_H 1.0, 0.92, 0.85, 0.83, 0.80 and 0.68. Among the twenty nine carbons observed in the ^{13}C NMR; one carbon (δ_C 72.0) was oxygenated, two were olefinic (δ_C 140.9 and 121.9) and the remaining where sp^3 carbons. Based on the spectroscopic data (Table 4.7) and the comparisons with the literature (Chaturvedula & Prakash, 2012) the compound was identified as β-sitosterol (**107**).



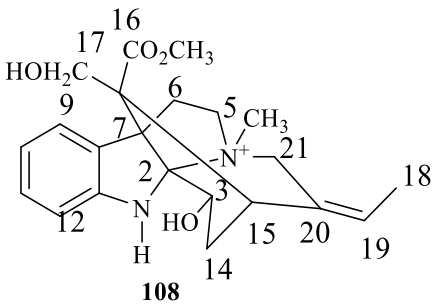
107

Table 4.7: ^{13}C NMR (125 MHz) data of compound **107** ($CDCl_3$)

Position	**δ_C-literature** (Chaturvedula & Prakash, 2012)	**δ_C-experimental**
1	37.5	37.4
2	31.9	31.8
3	72.0	72.0
4	42.5	42.4
5	140.9	140.9
6	121.9	121.9
7	32.1	32.1
8	32.1	32.0
9	50.3	50.3
10	36.7	36.6
11	21.3	21.3
12	39.9	40.0
13	42.6	42.5
14	56.9	57.0
15	26.3	24.4
16	28.5	28.4
17	56.3	56.2
18	36.3	36.3
19	19.2	19.2
20	36.2	34.1
21	26.3	26.2
22	46.1	46.0
23	23.3	23.2
24	12.2	12.1
25	29.4	29.3
26	20.1	20.0
27	19.6	19.5
28	18.9	18.9
29	12.0	12.0

4.2.3 Echitamine (108)

Compound **108** was isolated as a brown crystalline powder. The TLC spot changed to orange on spraying with dragendorff's reagent suggesting an alkaloid.



108

The ^{1}H NMR spectral data (Table 4.8) of **108** was fully assigned by means of a H,H-COSY experiment, it showed signals for one olefinic proton δ_H 5.73 ((*dd*, *J*= 13.83, 6.780 Hz, H-19) together with four mutually coupled aromatic protons resonating at δ_H 7.63 (H-9), 7.09 (H-11), 6.76 (H-10) and 6.70 (H-12) expressing the lack of substituents on the aromatic ring. The presence of three methyl groups were also evident from the ^{1}H NMR signals at δ_H 3.72 (3H, s), 3.22 (3H, s) and 1.66 (3H, s) with the corresponding ^{13}C NMR signals appearing at δ_C 55.6, 51.5 and 49.9 ppm. These were assigned to a methyl ester, a quaternary *N*-methyl group and allylic methyl groups, respectively.

The ^{13}C NMR spectrum also displayed seven quaternary carbon signals at δ_C 100.7, 62.3, 130.5, 148, 57 and 131.6, including an ester C=O resonating at 175.6. In the HMBC spectrum, H-9 correlated with C-7, C-11 and C-13; H-10 correlated with C-8 and C-12; H-11 correlated with C-9 and C-13; and H-12 correlated with C-8 and C-10. Also in the HMBC spectrum, H-14 correlates with C-2, C-3, C-6, C-15, C-16 and C-20 while H-5 correlates with C-3, C-7, C-14, C-19 and C-21. The HREIMS of compound **108** showed a molecular ion peak at *m/z* 385.2119 in line with the molecular formula of $C_{20}H_{29}N_2O_4^+$. Thus this compound was identified as echitamine based on comparison of NMR data (table 4.8) with literature (Ashok *et al*., 2015, Niwat *et al*., 1994).

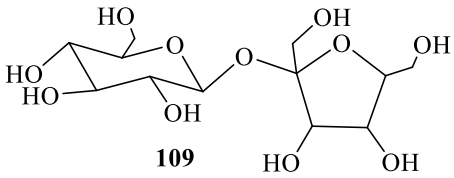
Table 4.8: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **108** (D_2O)

Position	δ_C	δ_H (*m*, *J* in Hz)	HMBC
2	100.7		
3	70.1	4.41 (*dd*, 10.7, 6.3)	
5	63.9	3.39 (*m*)	
6	42.3	2.37 (*dd*, 13.6, 8.72)	C-5, C-16
		2.07 (*dd*, 13.6, 8.72)	C-2, C-5, C-8
7	62.3		
8	130.5		
9	128.2	7.63 (*d*, 8.0)	C-7, C-11, C-13
10	121.5	6.76 (*t*, 7.6)	C-8, C-12
11	130.6	7.09 (*t*, 7.6)	C-9, C-13
12	111.7	6.70 (*d*, 8.0)	C-8, C-10
13	148.1		
14	31.6	2.48 (*m*)	C-2, C-6, C-20
		1.60 (*m*)	C-3, C-15, C-16, C-20
15	35.9	3.87 (*d*, 6.85)	C-3, C-7, C-14, C-19,C-21
16	57		
17	65.7	3.80 (*d*, 12.3)	
		3.27 (*d*, 12.3)	
18	15.5	1.66 (*d*, 6.90)	C-19, C-30
19	132.2	5.73 (*dd*, 13.83, 6.780)	C-15, C-18, C-21
20	131.6		
21	66.9	4.39 (*br d*)	
		3.90 (*br d*)	
C=O	175.6		
OCH_3	53.5	3.72 (*s*)	C=O
N-CH_3	50.5	3.22 (*s*)	C-2, C5, C-21

4.2.4 Sucrose (109)

Compound **109** was isolated as a white solid. The ESI of compound **109** showed a molecular ion peak *m/z* 365.1049 $[M+Na]^+$ corresponding to the molecular formula **$C_{12}H_{22}O_{11}$.** The ^{1}H NMR and HMBC spectrums showed the linkage of the hexose and pentose sugar units through H-2 (δ_H 5.25) (table 4.9). The HMBC spectrum showed correlation of H-2 with C-1 and C-6 and this is characteristic of sucrose (Bruyn, 1991). The ^{13}C exhibited twelve oxygenated carbon atoms which belong to sugar nucleus with three methylene oxygenated carbons C-10 (63.3), C-11

(62.2) and C-12 (61.0) biogenetically belonging to sucrose moiety. Based on the spectroscopic data (Table 4.9) and the literature (Bruyn, 1991), compound **109** was confirmed as sucrose.



109

Table 4.3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **109** (D_2O)

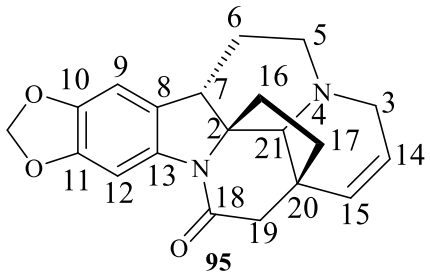
Position	**δ_C**	**δ_H (*m*, *J* in Hz)**	**HMBC (H →C)**
1	104.6		
2	93.1	5.25 (*d*, 3.8)	C-1, C-6
3	82.3	3.73	
4	77.3	4.05 (*d*, 8.8)	C-3, C-5, C-11
5	74.9	3.89 (*t*, 8.6)	C-3, C-4, C-10
6	73.5	3.59 (*t*, 9.6)	C-2, C-8, C-9
7	73.3	3.65	
8	72.0	3.39 (*dd*, 9.9, 3.8)	C-6
9	70.1	3.31 (*t*, 9.5)	C-7, C-10
10	63.3	3.66	
11	62.2	3.52	C-1, C-4
12	61.0	3.65	C-3, C-11

4.3 Characterization of compounds isolated from the leaves of *Schizozygia coffaeoides*

The air dried ground leaves of *Schizozygia coffaeoides* were extracted with CH_2Cl_2/MeOH (1:1) by cold percolation at room temperature. The crude extract was subjected to a combination of chromatographic techniques which resulted into isolation of a steroid, three alkaloids and two anthraquinones. Then using the spectroscopic techniques, the structures were determined.

4.3.1 Schizozygine (**95**)

Compound **95** was obtained as white crystals from CH_2Cl_2/petroleum ether. When sprayed with Dragendorff's reagent, the TLC spot of this compound turned orange suggesting that it is an alkaloid.



95

The ^1H and ^{13}C NMR spectral data (Table 4.10) of this compound suggested that it is a schizozygine alkaloid (Kariba *et al*., 2002). The ^1H NMR spectrum exhibited two aromatic protons *para* to each other resonating at δ_H 7.61 (*s*) and 6.66 (*bs*) and were assigned to H-9 and H-12, with C-10 and C-11 having a methylenedioxy group, δ_H 5.92 and 5.92 (2 x *d, J* = 1.3 Hz, OCH_2O). The long range coupling observed between the signal at δ_H 6.66 (H-9) and H-7 allowed the assignment of the aromatic signal to H-9 and hence the singlet at δ_H 7.61 to H-12. The two olefinic proton signals observed at δ_H 5.57 (1H, *dt, J* = 10.0, 2.2 Hz,) and δ_H 5.73 (1H, *ddd, J* = 10.0, 4.4, 2.0 Hz) were assigned to H-14 and H-15. In agreement with this assignment, the corresponding ^{13}C NMR signals appeared at δ_C 124.2 (C-14) and 130.6 (C-15). The ^{13}C NMR

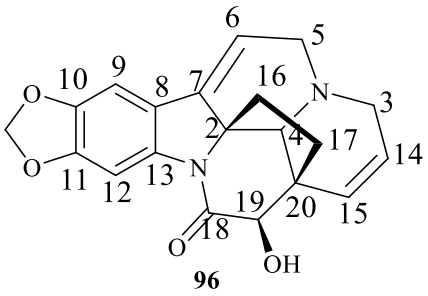
spectrum further displayed seven quaternary carbons δ_C 45.1, 73.0, 125.8, 137.4, 144.6, 147.2 and 169.2, of which a carbonyl resonance appeared at δ_C 169.2 (C-18). The HMBC spectrum shows correlation of H-7 with C-2, C-5, C-6, C-8, C-9, C-13, C-16 and C-21 and H-9 with C-7, C-10, C-11 and C-13 confirming that H-7 and H-9 are in close proximity as shown by the long range coupling between the two protons (Table 4.10). Also in the HMBC spectrum, correlations of H-7 (δ_H 3.2, *t*, *J* = 6.7 Hz) with C-2 (δ_C 73.0), C-5 (δ_C 50.4), C-6 (δ_C 26.2), C-8 (δ_C 125.8), C-9 (δ_C 104.2), C-13 (δ_C 137.4), C-16 (δ_C 39.0) and C-21 (δ_C 68.4) is in agreement with a piperidine ring fused to the indole moiety at the C-2/C-7 junction. Furthermore, the annulation with tetrahydropyridine ring C-21/N-4 was evident from the HMBC data (Table 4.10). Using the above spectroscopic data and the literature (Kariba *et al*., 2002), the compound was identified as schizozygine and is the major compound from the roots and leaves of *Schizozygia coffaeoides* (Kariba *et al*., 2002).

Table 4.4:^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **95** (CD_2Cl_2)

Carbon No	**^{1}H NMR δ_H (*m, J* in Hz)**	**^{13}C NMR**	**HMBC**
2		73.0	
3	2.80 (*d*, 16.8)	53.9	
	3.37 (*d*, 16.8)		
5	2.27 (*m*)	50.4	C-2, C-3, C-19
	3.02 (*bs*)		
6	2.03 (*m*)	26.2	
7	3.20 (*t*, 6.7)	42.5	C-2, C-5, C-6, C-8, C-9,C-13, C-16, C-21
8		125.8	
9	6.67 (*bs*)	104.2	C-7, C-10, C-11,C-13
10		144.6	
11		147.2	
12	7.61 (*s*)	98.4	C-8, C-10, C-11, C-13
13		137.4	
14	5.57 (*dt*, 10.0, 2.2)	124.2	C-3, C-15
15	5.73 (*ddd*, 10.0, 4.4, 2.0)	130.6	C-3, C-14, C-21
16	2.28 (*m*)	39.0	
17	1.87 (*ddd*, 12.8, 8.7, 5.4)	38.0	
	2.04 (*m*)		
18		169.2	
19	2.45 (*dd*, 18.0, 2,7)	47.3	C-15, C-17, C-18, C-20
	2.61 (*d*, 18.0)		C-17, C-18, C-20, C-21
20		45.1	
21	2.25 (*s*)	68.4	C-3, C-5, C-7, C19
OCH_2O	5.91 (*d*, 1.3)	102.0	C-10, C-11
	5.92 (*d*, 1.3)		C-10, C-11

4.3.2 6,7-Dehydro-19β-hydroxyschizozygine (96)

Compound **96** was isolated as a pink amorphous solid whose spot on TLC turned red when sprayed with Dragendorff's reagent, indicating that it is an alkaloid. The only difference between compounds **95** and **96** is the presence in the latter of a hydroxy group C-19 and a double bond between C-6 and C-7.



96

As in compound **95**, the ^{1}H NMR spectrum exhibited two aromatic proton singlets resonating at δ_H 6.5 (H-9) and 8.35 (H-12) with C-10 (144.9) and C-11 (142.5) being substituted with methylenedioxy group. Three olefinic protons were evident from the signals at δ_H 5.73 (*ddd, J* = 10.0, 4.6, 1.9 Hz, H-14), δ_H 6.03 (*d, J* = 10.3 Hz, H-15) and δ_H 5.24 (*dd, J* = 4.8, 2.2 Hz, H-6). The proton resonating at δ_H 4.02 (1H, *d, J* = 1.6 Hz, H-19) was assigned to hydroxymethine proton (H-19). The ^{13}C NMR spectrum showed one amidic carbonyl resonating at δ_C 171.1 (C-18), four olefinic carbons resonating at δ_C 100.6 (C-6), 145.1 (C-7), 123.6 (C-14) and 127.5 (15) together with three oxygenated carbons resonating at δ_C 144.9 (C-10), 142.5 (C-11) and 76.2 (C-19).

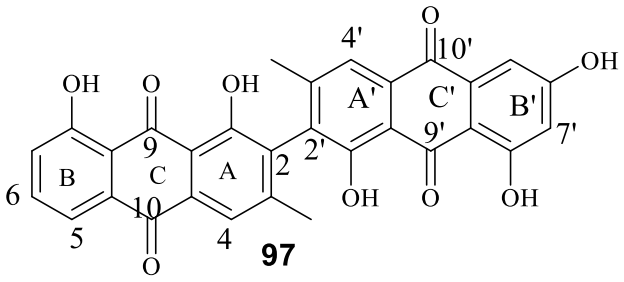
In the HMBC spectrum, H-19 correlates with C-15 (δ_C 127.5), C-17 (δ_C 30.0), amidic carbonyl C-18 (δ_C 171.1), C-20 (δ_C 46.9) and C-21 (δ_C 68.1) supporting the fact that the hydroxy group is attached to C-19 adjacent to C-18 (Table 4.11). The β-orientation of OH-19 was established from long range (*W*) coupling (*J*=1.8 Hz) between H-19 (δ_H 4.02) and H-17 (δ_H 1.84) that requires H-19 be in α-equatorial configuration and hence the 19-OH has to be β-oriented. The compound was therefore identified as 6,7-dehydro-19β-hydroxyschizozygine based on the spectroscopic data and comparison with literature (Kariba *et al*., 2002).

Table 4.5: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR for compound **96** (CD_2Cl_2)

Position	**δ_C**	**δ_H (*m*, *J* in Hz)**	**HMBC**
2	64.4		
3	52.4	3.45 (*dd*, 16.9, 4.6)	
		2.85 (*d*, 16.9)	
5	52.3	3.59 (*dd*, 16.3, 4.8)	
		2.94 (*d*, 16.3)	
6	100.6	5.24 (*dd*, 4.8, 2.2)	C-2, C-5, C-7
7	145.1		
8	116.8		
9	98.3	6.5 (*s*)	C-7, C-8, C-10, C-13
10	144.9		
11	142.5		
12	103.0	8.35 (*s*)	C-8, C-9, C-11, C-13
13	138.5		
14	123.6	5.73 (*ddd*, 10.0, 4.6, 1.9)	C-3, C-20
15	127.5	6.03 (*d*, 10.25)	
16	38.2	2.35 (*m*)	
		2.06 (*m*)	
17	30.0	2.44 (*m*)	C-16, C-19, C-20, C-21
		1.84 (*m*)	C-2, C-16, C-19, C-20
18	171.1		
19	76.2	4.02 (*d*, 1.6)	C-15, C-17, C-18, C-20, C-21
20	46.9		
21	68.1	2.50 (*s*)	C-5, C-16, C-17, C-19
OCH_2O	102.2	5.92 (*d*, 1.3)	C-10, C-11
		5.91 (*d*, 1.3)	C-10, C-11

4.3.3 Cassamin A (97)

Compound **97** was isolated as a yellow amorphous solid; and a yellow TLC spot turned red on exposure to ammonia vapour indicating that it could be quinone derivative.



97

The HRESIMS molecular ion peak at *m/z* 522.0954 and the NMR data (Table 4.12) was consistent with the molecular formula $C_{30}H_{18}O_9$. The MS, ^{1}H NMR (which showed two methyl groups at δ_H 2.20 and 2.21) and ^{13}C NMR, which showed two methyl carbons at δ_C 20.7 and 20.9 and four carbonyl resonances (δ_C 182.9, 182.9, 191.2 and 193.3) suggested that this compound is a dimeric anthraquinone.

In one half of the molecule, the ^{1}H NMR spectrum showed three mutually coupled aromatic protons (Table 4.12) for ring B (structure **97**) which is substituted with hydroxy group at C-8 (δ_C 162.8) as in chrysophanol. Ring A has only one aromatic proton (δ_H 7.83) where C-1 (hydroxy group, δ_C 160.3) and C-3 (methyl group (δ_H 2.21; δ_C 20.9) are substituted as expected biogenetically. This leaves C-2 (δ_C 131.2) as the point of attachment to the other half of the molecule.

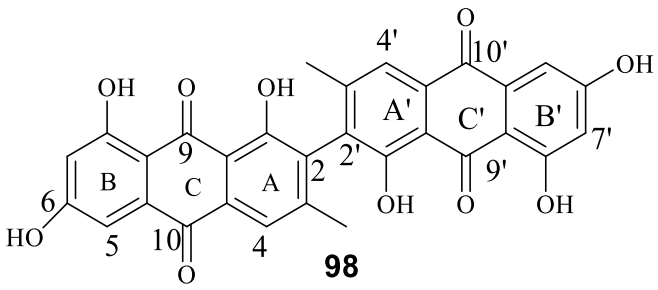
The other half of the molecule has three hydroxy substituents (δ_C 160, 166.4 and 165.8) in addition to the methyl (δ_H 2.20; δ_C 20.7) at C-3' as expected from biogenesis. In ring A', the presence of only one aromatic proton (δ_H 7.78 for H-4'), having hydroxy group at C-1' (from biogenetic point of view) suggested that C-2' is the point of attachment with the other half. In ring B, two *meta*-coupled aromatic protons resonating at δ_H 6.61 and 7.24 would place the remaining hydroxy group at C-7 (δ_C 125.1) as in emodin. Thus based on the above evidence in addition to detailed ^{1}H,^{1}HCOSY, HSQC and HMBC analyses, along with literature comparison, compound **97** was identified as 2,2'-crysophanol-emodin dimer (trivial name cassamin A) (Atilaw *et al*., 2014). This compound is optically active, $[\alpha]_D$ = -49.8, due to restricted rotation at the bi-aryl linkage. However, the absolute configuration remains undetermined.

Table 4.6: ^{1}H (500 MHz) and ^{13}C (125MHz) NMR data for compound **97** (CD_2Cl_2/ CD_3OD; 1:1)

Position	**δ_H (*m*, *J* in Hz)**	**δ_C**	**HMBC**
1		160.3	
1a		114.6	
2		131.2	
3		149.0	
4	7.83 (*s*)	122.2	C-1a, C-2, C-3
4a		133.6	
5	7.82 (*dd*, 7.7, 1.2)	120.5	C-7, C-8a, C-10
5a		134.2	
6	7.72 (*dd*, 8.5, 7.9)	137.8	C-5a, C-8
7	7.30 (*dd*, 8.5, 1.2)	125.1	C-5, C-8a
8		162.8	
8a		116.5	
9		193.3	
10		182.9	
1'		160.0	
1'a		114.6	
2'		130.9	
3'		147.7	
4'	7.78 (*s*)	121.9	C-1'a, C-2', C-10', C-3'
4'a		133.6	
5'	7.24 (*d*, 2.5)	110.1	C-7', C-10'
5'a		136.1	
6'		166.4	
7'	6.61 (*d*, 2.5)	108.9	C-5'
8'		165.8	
8'a		110.1	
9'		191.2	
10'		182.9	
1-OH			
1'-OH			
3-CH_3	2.21	20.9	C-2, C-3 C-4
3'-CH_3	2.20	20.7	C-2', C-3', C-4'
6'-OH			
8-OH			
8'-OH			

4.3.4 Cassamin B (98)

Compound **98** was isolated as a yellow amorphous solid, as in compound **97**, a yellow TLC spot turned red when exposed to ammonia vapour, indicating a quinone moiety. The HRESIMS of this compound showed a molecular ion peak of 538.0917 consistent with the molecular formula $C_{30}H_{18}O_{10}$, also indicating a dimeric anthraquinone.



98

The ^{1}H NMR spectrum showed three aromatic protons resonating at δ_H 7.76 (*s*, H-4), δ_H 7.22 (*s*, H-5) and δ_H 6.59 (*s*, H-7). ^{13}C NMR only showed fifteen carbon signals of which; two were carbonyl (δ_C 191.2 and 182.9), three oxygenated carbons (δ_C 166.4, 165.8 and 160.0), a methyl group (δ_C 20.7) and three methine aromatic carbon signals (δ_C 121.9, 110.1, 108.9) and biogenetically expected methyl group (δ_H 2.22) at C-3. In the HMBC spectrum, the methyl protons showed correlation with C-2, C-3 and C-4 (table 4.13).

The NMR spectra are clearly in agreement with the compound being a symmetrical dimer of emodin; the only difference is the absence of a signal for H-2 in compound **98** indicating C-2 is the point of attachment to the other half of the molecule. In support of this, in the HMBC spectrum, H-4 correlated with C-1a, C-2, C-10 and 3-CH_3 and H-2 was a point of attachment to the half of molecule since it was not observed in the ^{1}H NMR spectrum consistent with the compound **98** being a symmetric dimer of emodin. In ring C, H-5 correlated with C-8a, C-10 and H-7 correlated with C-8a confirming the identity of this ring. Compound **98** was therefore

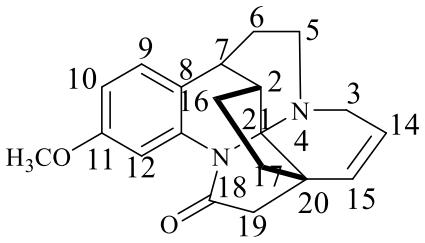
characterized as 2,2'-biemodin (Atilaw *et al*., 2014). This compound is also optically active, $[\alpha]_D$ = 27.0, and the absolute configuration is yet to be determined.

Table 4.7: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR for compound **98** (CD_2Cl_2/ CD_3OD; 1:1)

Position	**δ_C**	**δ_H (*m*, *J* in Hz)**	**HMBC**
1	160.0		
1a	114.5		
2	131.1		
3	147.7		
4	121.9	7.76 (*s*)	C-1a, C-2, C-10, 3- CH_3
4a	133.4		
5	110.1	7.22	C-8a, C-10
5a	136.0		
6	166.4		C-8a
7	108.9	6.59	
8	165.8		
8a	109.9		
9	191.2		
10	182.9		
3-CH_3	20.7	2.22 (*s*)	C-2, C-3, C-4
1-OH			
6-OH			
8-OH			

4.3.5 Isoschizozygaline (99)

Compound **99** was isolated as a white amorphous solid and is also an isoschizozygane alkaloid as shown from NMR spectral data (Table 4.14). The HREIMS of compound **99** exhibited the molecular ion peak at *m/z* 322.1685 corresponding to the molecular formula **$C_{20}H_{22}O_2N_2$.**



99

The ^{1}H NMR spectrum of compound **99** is similar to that of schizozygine except that the two *para*-oriented aromatic protons in the latter are replaced by an AMX spin system resonating at δ_H 8.43 (1H, *d*, *J* = 2.6 Hz, H-12), δ_H 7.06 (1H, *d*, *J* = 8.4 Hz, H-9) and δ_H 6.67 (1H, *dd*, *J* = 8.3, 2.6 Hz, H-10). Also the methylenedioxy signals in compound **99** are now replaced by a methoxy group (δ_H 3.8; δ_C 55.8).

The ^{1}H NMR spectrum of compound **99** also showed two olefinic protons resonating at δ_H 5.75 (1H, *ddd*, *J* = 10.1, 2.6, 1.8 Hz, H-15) and δ_H 5.62 (*ddd*, *J* = 10.1, 4.6, 1.8 Hz, H-14). The ^{13}C NMR spectrum exhibited two olefinic carbons C-15 (δ_C 131.3) and C-14 (δ_C 120.7), one methoxy group (δ_C 55.8), and one carbonyl group (δ_C 174.8). In the HMBC spectrum, (H-9 correlated with C-7, C-11 and C-13 confirming the long range coupling of the protons (H-7 and H-9) and also guided in the confirmation of the substitution pattern of the aromatic ring. That the compound has a five-membered lactam ring, rather than six-member as in compound **95** was established based on the chemical shift value of the carbonyl (δ_C 178.4) as discussed in Kariba *et*

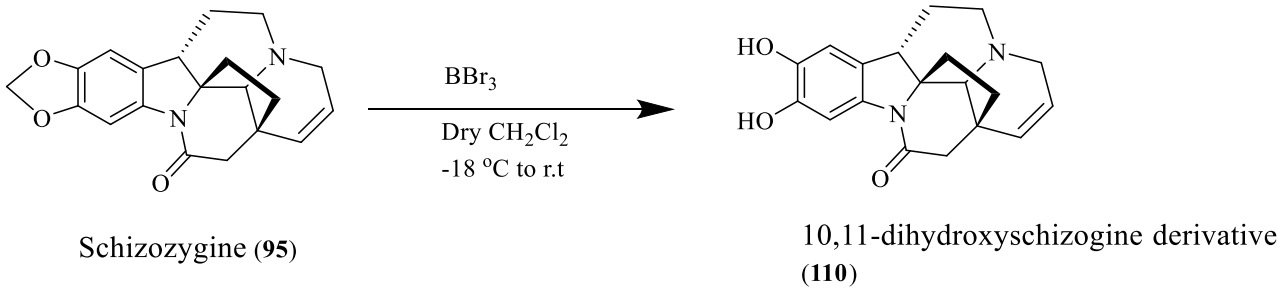
al., (2002). The compound was therefore characterized as isoschizogaline (Atilaw *et al*., 2014; Kariba *et al*., 2002).

Table 4.14: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR for compound **99** (CD_2Cl_2)

Position	δ_C	δ_H (***m, J*** **in Hz)**	**HMBC**
2	35.1	2.34 (*m*)	
3	48.2	3.44 (*ddd*, 17.4, 3.0, 1.6*)*	C-5, C-14, C-15
		3.29 (*ddd*, 17.3, 2.8, 1.4))	C-14, C-15
5	44.6	2.84 (*ddd*, 14.6, 12.9, 3.3)	C-5,
		2.52 (*ddd*, 14.3, 2.81, 1.8)	
6	27.3	2.24 (*m*)	
		1.19 (*m*)	
7	37.8	3.25 (*t*, 5.9)	
8	118.9		
9	129.6	7.06 (*d*, 8.4)	C-7, C-11, C-13
10	110.1	6.67 (*dd*, 8.3, 2.6)	C-8, C-11, C-12
11	159.2		
12	103.5	8.43 (*d*, 2.6)	C-8, C-10, C-10, C-11
13	139.3		
14	120.7	5.62 (*ddd*, 10.14, 4.6, 1.8)	
15	131.3	5.75 (*ddd*, 10.13, 2.6, 1.8)	C-3, C-7, C-20, C-21
16	25.3	1.69 (*m*)	
		1.13 (*m*)	
17	36.8	1.86 (*m*)	C-15
		1.76 (*m*)	C-5, C-17
18	174.8		
19	46.5	2.78 (*d*, 12.4)	C-5, C-13, C-17, C-18
		2.50 (*dd*, 19.0, 2.9)	C-5, C-17,C-18, C-18
20	44.7		
21	85.1		
OCH_3	55.8	3.83 (*s*)	C-11

4.4 10,11-Dihdroxyschizozygine Derivative (110)

Compound **110**, a white amorphous solid, was prepared from schizozygine using boron tribromide as Lewis acid in dry dichloromethane as illustrated in Scheme 2.1.

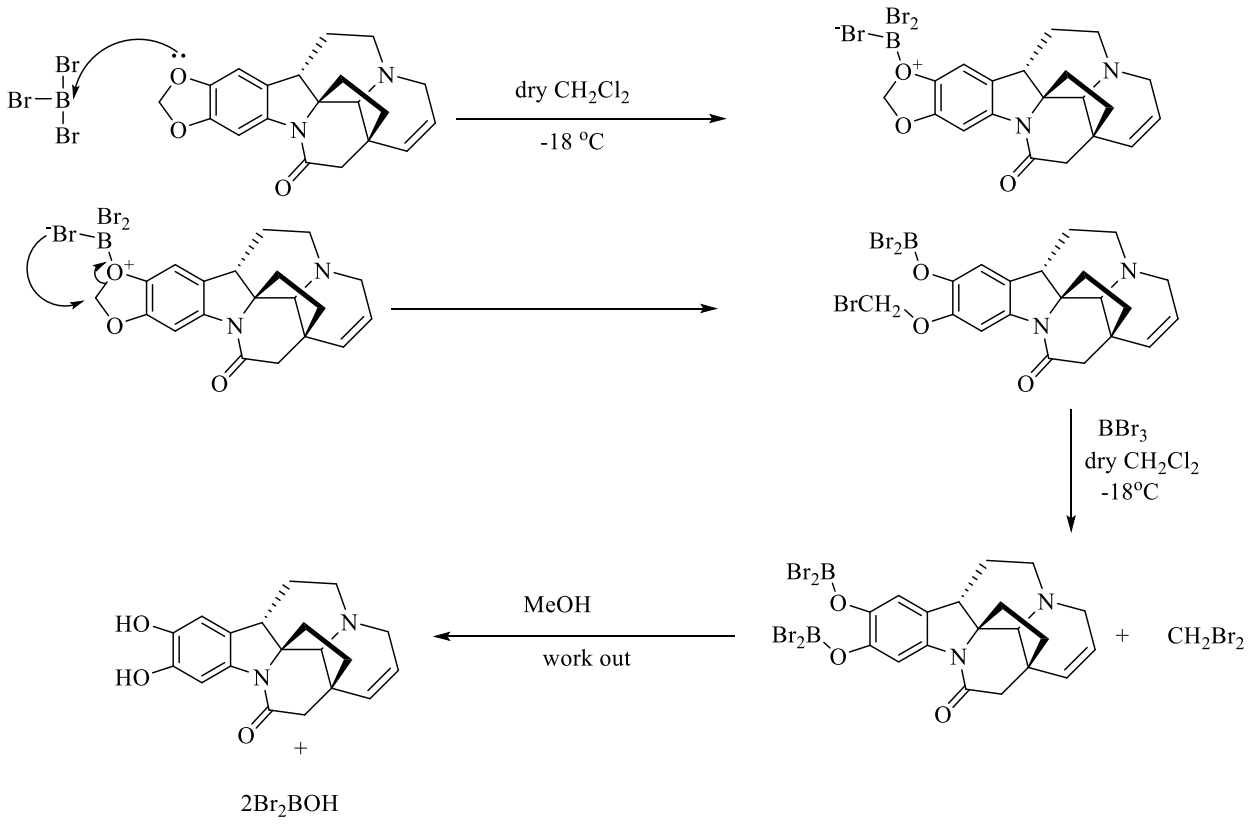


Schizozygine (**95**)

10,11-dihydroxyschizogine derivative (**110**)

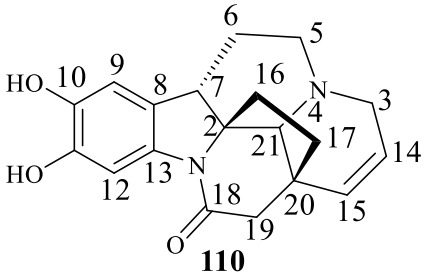
Scheme 2.1: Reaction equation of Schizozygine with boron tribromide

Preliminary attempts that used lower concentrations of boron tribromide (0.2 g, 1 mmol and 0.4 g, 2 mmol) in 10mL of dry dichloromethane were not successful in cleaving the methylenedioxy linkage. The reaction was successful when 1 g (4.5 mmol) of boron tribromide was used in the same volume of dry dichloromethane. This was a sign that the methylenedioxy is a very stable group hence required excess boron tribromide for its cleavage. The proposed reaction mechanism involves boron tribromide acting as a Lewis acid to activate the methylenedioxy linkage for nucleophilic cleavage as shown in Scheme 2.2.



Scheme 2.2: proposed mechanism of synthesis of 10,11-dihydroxyschizozygine derivative

Based on the spectroscopic data, mass and IR, the compound was characterized as 10,11-dihydroxyschizozygine derivative.



110

The ^{1}H NMR of 10, 11-dihydroxyschizozygine derivative is similar to that of schizozygine (**95**), except for the absence of the methylenedioxy protons resonating at δ_H 5.92. The absence of the methylenedioxy carbon resonating at δ_C 102.0 in the ^{13}C NMR spectrum (Appendix 146) of the product was evident, reducing the number of carbons to nineteen, further confirming the

cleavage of the methylenedioxy. The IR ν_{max} cm^{-1} were as follows; 3290 (OH), 3119 (OH), 2906, 1636 (amidic C=O), 1501 and 1413 confirming the existence of the hydroxyl groups.

The HREIMS of compound **110** showed a molecular ion peak at *m/z* 324.1484 consistent with the molecular formula $C_{19}H_{20}O_3N_2$, thereby confirming that the cleavage was successful.

4.5 Cytotoxicity

The crude extract of the stem bark and root bark and some of the compounds isolated from *A. boonei* were tested for cytotoxicity against four cell lines (Table 4.15).

Table 4.15: *In vitro* cytotoxicity of compounds from *Alstonia boonei* against 2 normal and two cancer cell lines (IC_{50} in µM)

Samples	Cell lines			
	BEAS-2B	LO2	A549	HepG2
Stem bark extract	19.70±0.59 µg/ml	6.29 µg/ml	>100 µg/ml	84.1 µg/ml
Root bark extract	7.48±2.83 µg/ml	1.19±0.25 µg/ml	>100 µg/ml	12.8 µg/ml
Lupeol acetate (**101**)	>100	8.07±0.72	>100	>100
Lupeol (**106**)	0.71±0.19	<0.10	>100	1.65
Cycloeucalenol (**104**)	>100	91.60±9.8	>100	>100
Phenanthridine-6(5H)-one (**105**)	39±1.10	2.58±0.30	>100	34.90
Lichexanthone (**103**)	>100	84.50±8.40	>100	85.40
Stigmasterol (**103**)	94.60±7.90	11.30±`1.84	>100	>100

LO2: non-tumor (normal) liver cell; BEAS-2B: immortal cell line (normal lung cell). Cancer cells; A549 (adenocarcinomic human epithelial cells) and HepG2 (human liver cancer cell line).

The crude extract of the root bark exhibited good activity against HepG2 liver cancer cells (IC_{50} = 12.8 µg/ml) but was also toxic to both LO2 normal liver cells (IC_{50} = 1.19 µg/ml) and BEAS-2B lung cells. However, the crude extract of the stem was toxic to the normal cells from both the lung and the liver but inactive against the respective cancerous cells. Lupeol was active against

the HepG2 human liver cancer cells (IC_{50} = 1.65 µM). Furthermore, lupeol was toxic to both LO2 normal (IC_{50} = <0.1 µM) and BEAS-2B normal (IC_{50} = 0.71 µM) cells. Lupeol has been reported to inhibit growth and proliferation of highly aggressive pancreatic cancer and melanoma cells through modulation of several signaling pathways in cap cells (Saleem *et a*l., 2009). Lupeol acetate was inactive against any cancerous cells but toxic to the LO2 cells. Cycloeucalenol and lichexanthone were inactive against any cell line but phenanthridine-6(5H)-one was moderately active on HepG2 and toxic to both BEAS-2B and LO2 cells. Stigmasterol was toxic to LO2 cells but inactive against other cell lines. Both the crude extracts and the isolated compounds were inactive against the A549 cancerous cells. Lupeol being one of the active phytochemical shows the reason why *Alstonia boonei* is used for the treatment of cancer traditionally but also has side effects on the normal cells.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

1. Five compounds were isolated and characterized from the stem bark of *Alstonia boonei* including; lupeol acetate (**101**), stigmasterol (**102**), lichexanthone (**103**), cycloeucalenol (**104**) and phenanthridine-6(5H)-one (**105**). Of these, compound (**103)** and **105** are reported for the first time from the family Apocynaceae.
2. From the root bark of *Alstonia boonei*, a total of four compounds were isolated and characterized as lupeol (**106**), β-sitosterol (**107**), echitamine (**108**), sucrose (**109**) and stigmasterol (**102**) was re-isolated.
3. Five compounds were isolated and characterized from *Schizozygia coffaeoides* including schizozygine (**95**), 6,7-dehydro-19β-hydroxyschizozygine (**96**), cassamin A (**97**), cassamin B (**98**) and isoschizozygaline (**99**).
4. The dimeric anthraquinones; Cassamin A and Cassamin B are reported for the first time from the leaves of *S. coffaeoides*
5. The crude extract of the root bark and lupeol from *Alstonia boonei* showed moderate cytotoxity against HepG2 cancer cell line but toxic to the normal cells. However, both the crude extract and isolated compounds were inactive against A549 cancerous cell line.
6. 10,11-Dihydroxyschizozygine (**110**) was prepared from schizozygine (**95**) through cleavage of the methylene dioxide using boron tribromide. Compound **110** is a new compound.

5.2 RECOMMENDATIONS

- The crude extracts and isolated compounds from *Alstonia boonei* and *Schizozygia coffaeoides* should be tested against other cell lines.
- The absolute configuration of the Cassamin A (**97**) and Cassamin B (**98**) should be determined.

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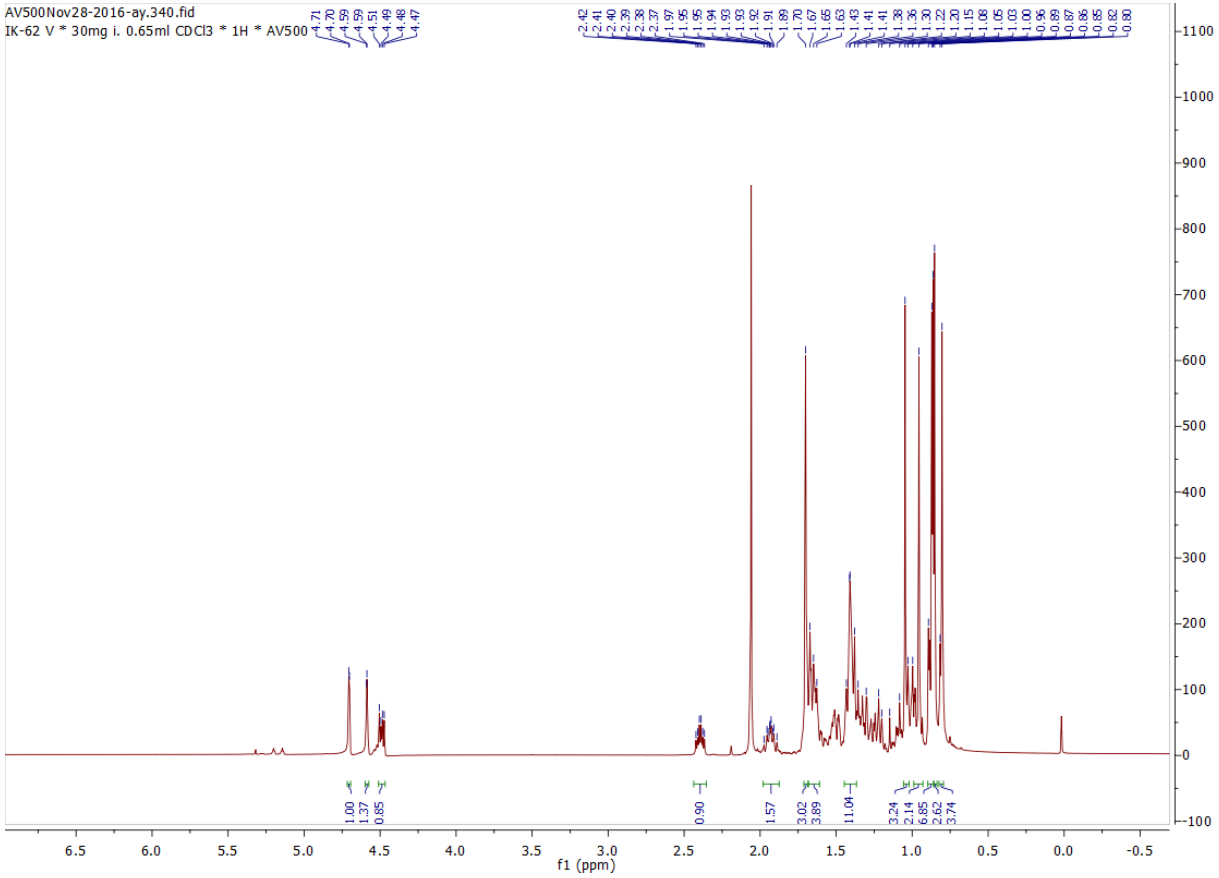
SPECTRA APPENDICES

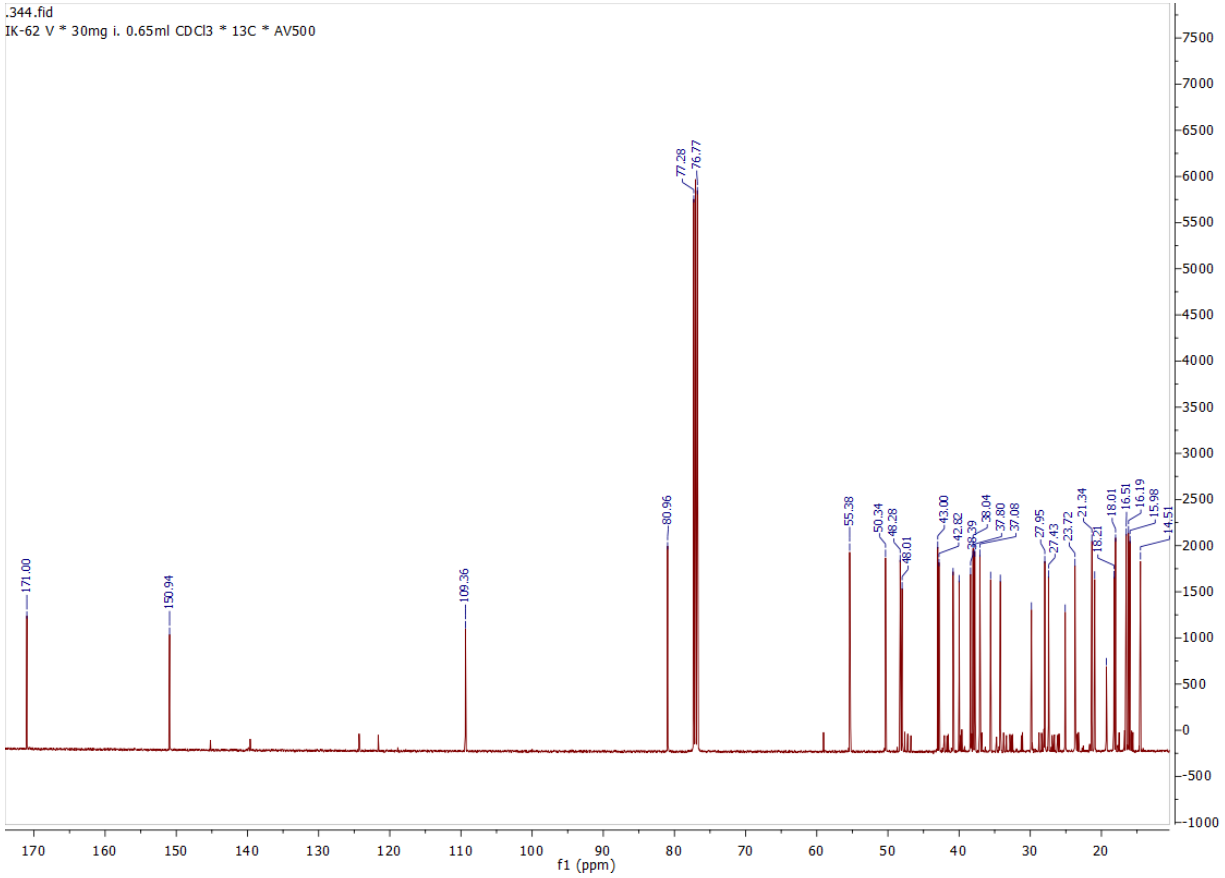
APENDIX A

SPECTRA FOR COMPOUND 101

^{1}H NMR SPECTRA FOR COMPOUND 101 ($CDCl_3$, 500 MHz)



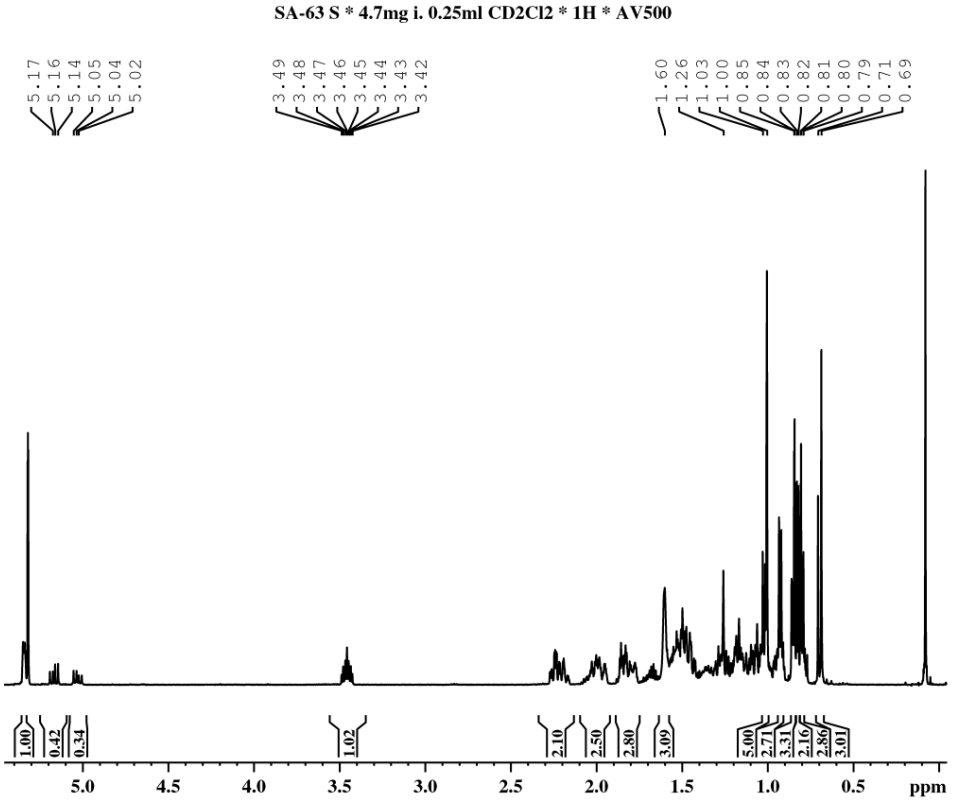
^{13}C NMR SPECTRUM FOR COMPOUND 101 ($CDCl_3$, 125 MHz)



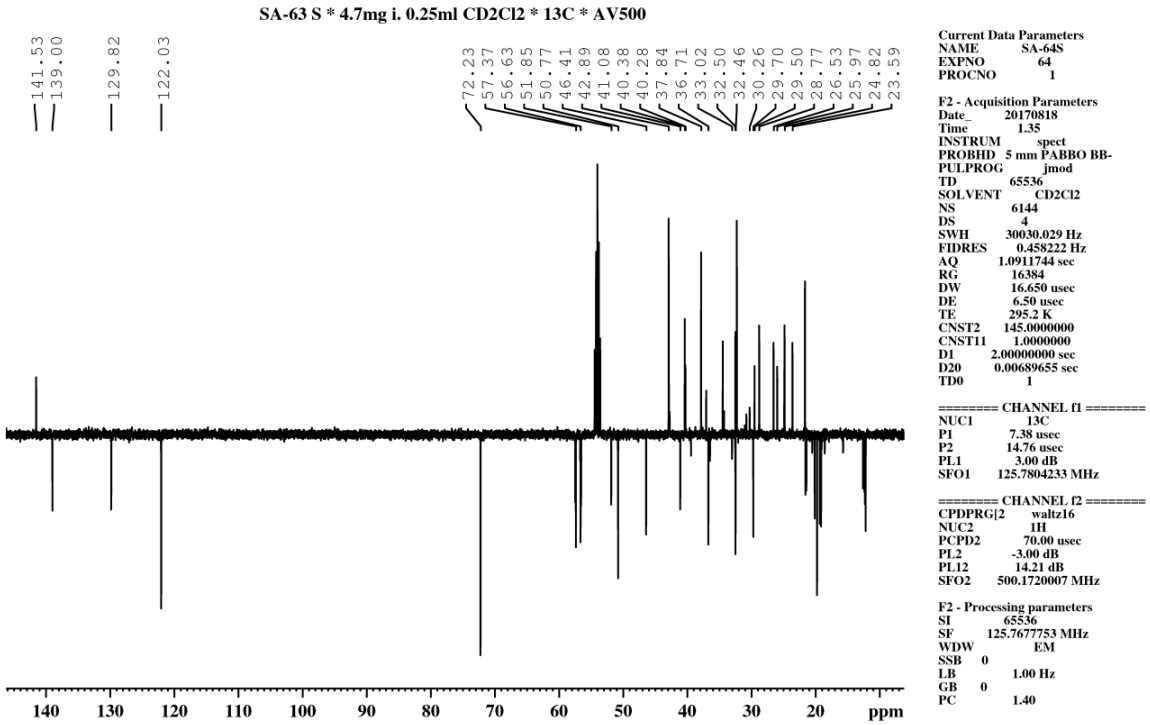
APPENDIX B

SPECTRA FOR COMPOUND 102

^{1}H NMR SPECTRA FOR COMPOUND 102 (CD_2Cl_2, 500 MHz)



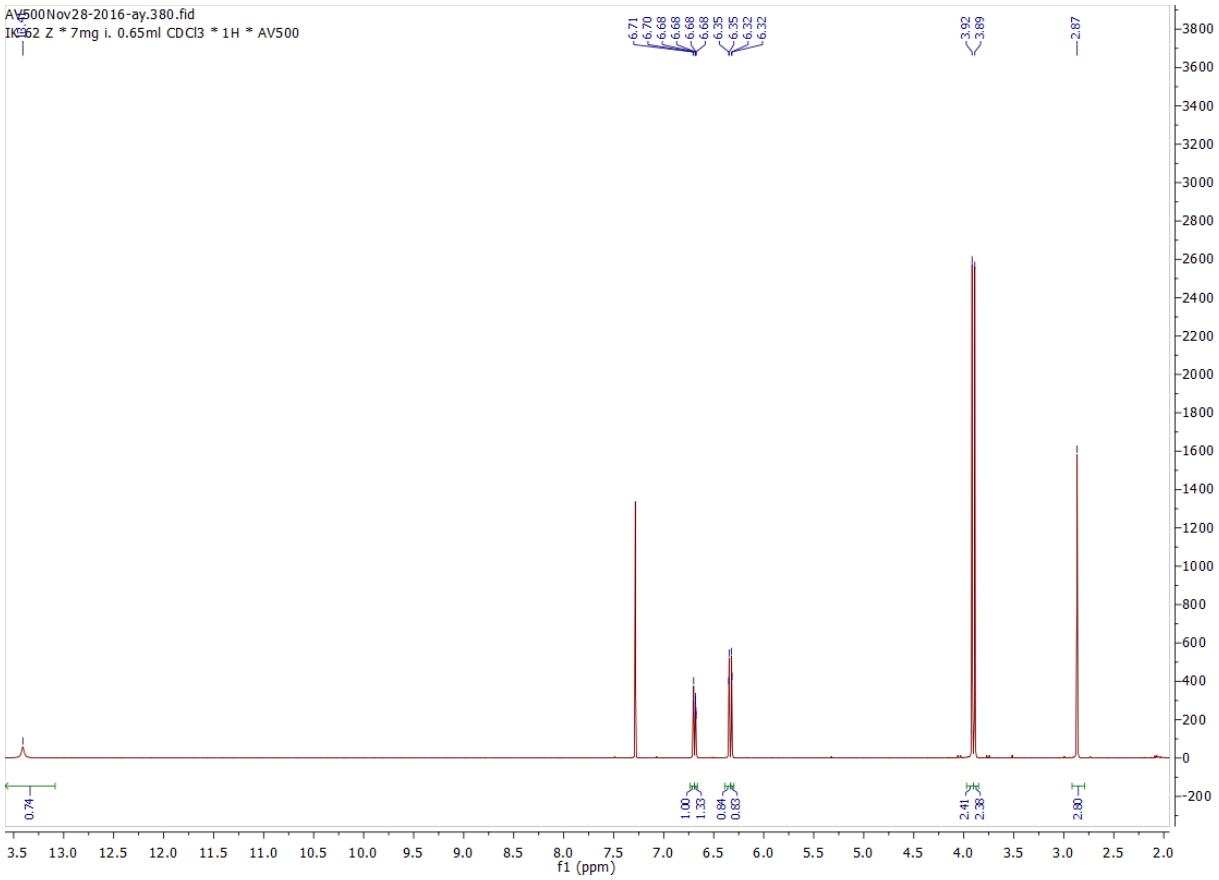
^{13}C NMR SPECTRUM FOR COMPOUND 102 (CD_2Cl_2, 125 MHz)



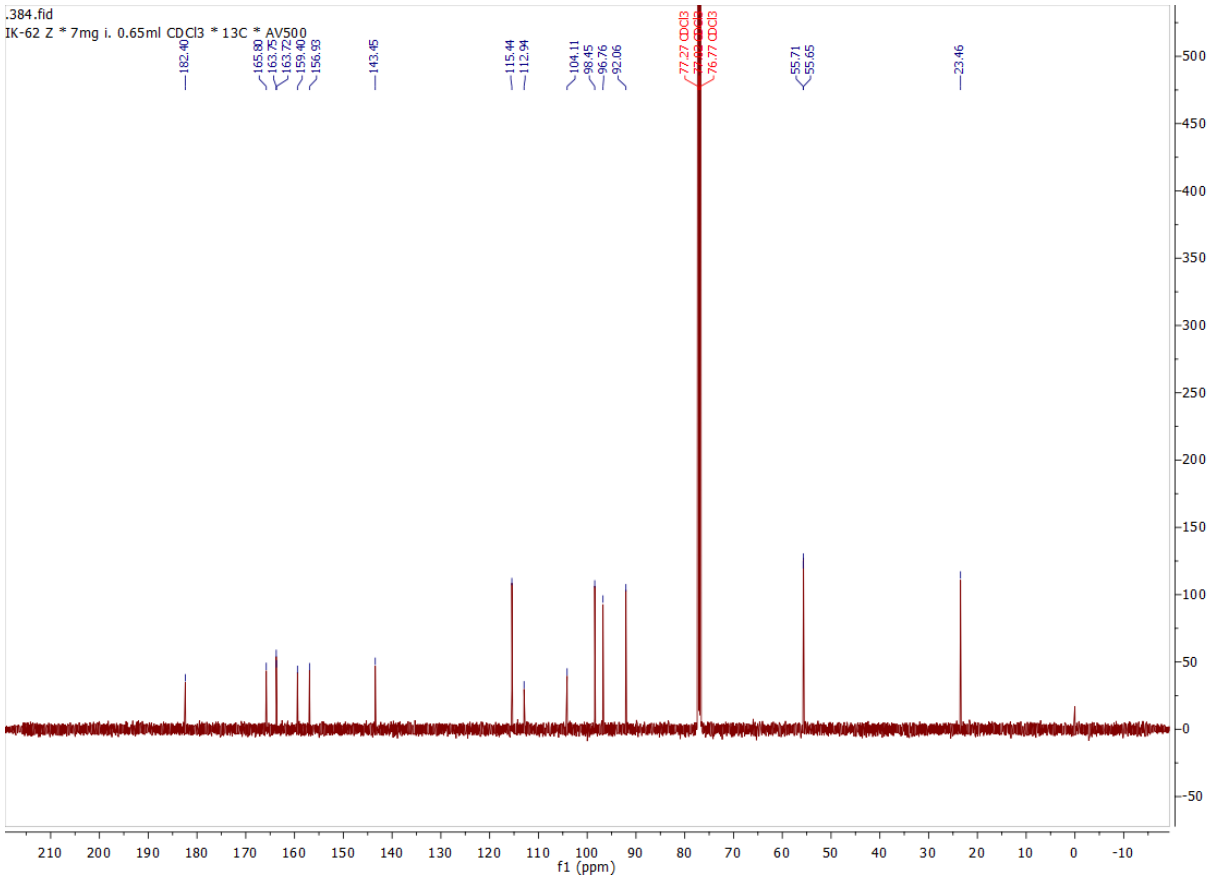
APPENDIX C

SPECTRA FOR COMPOUND 103

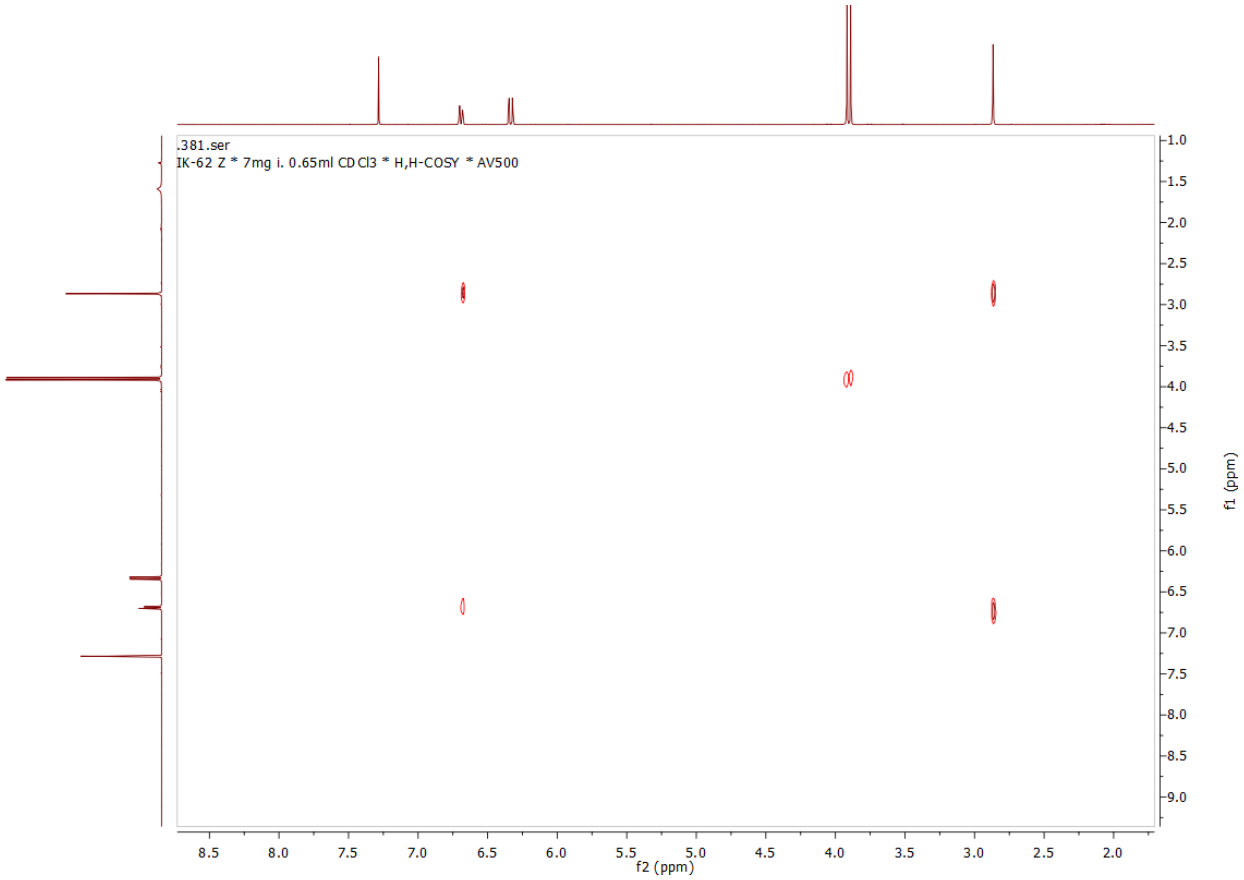
^{1}H NMR SPECTRUM FOR COMOPUND 103 ($CDCl_3$, 500 MHz)



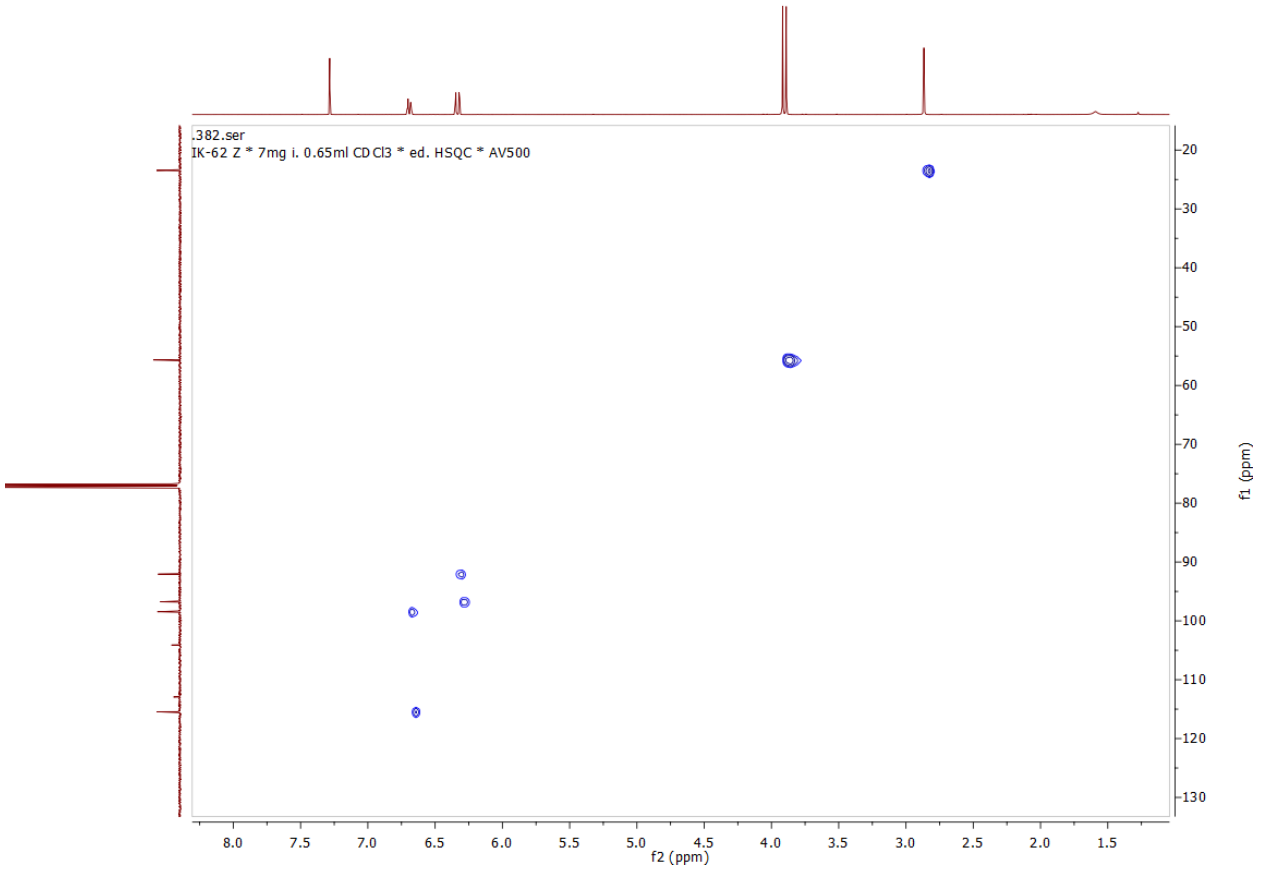
^{13}C NMR SPECTRUM FOR COMPOUND 103 ($CDCl_3$, 125 MHz)



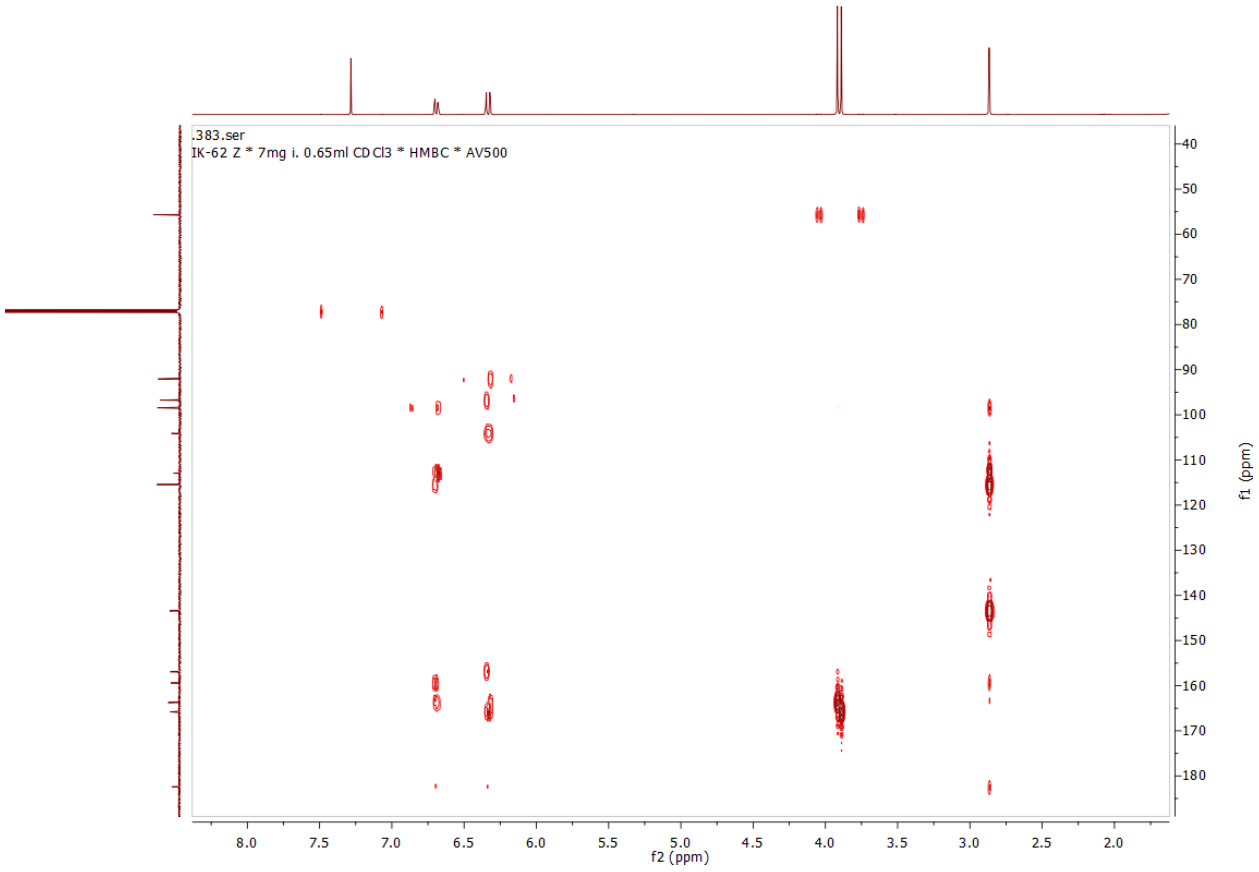
HH-COSSY SPECTRUM FOR COMPOUND 103 ($CDCl_3$, 500 MHz)



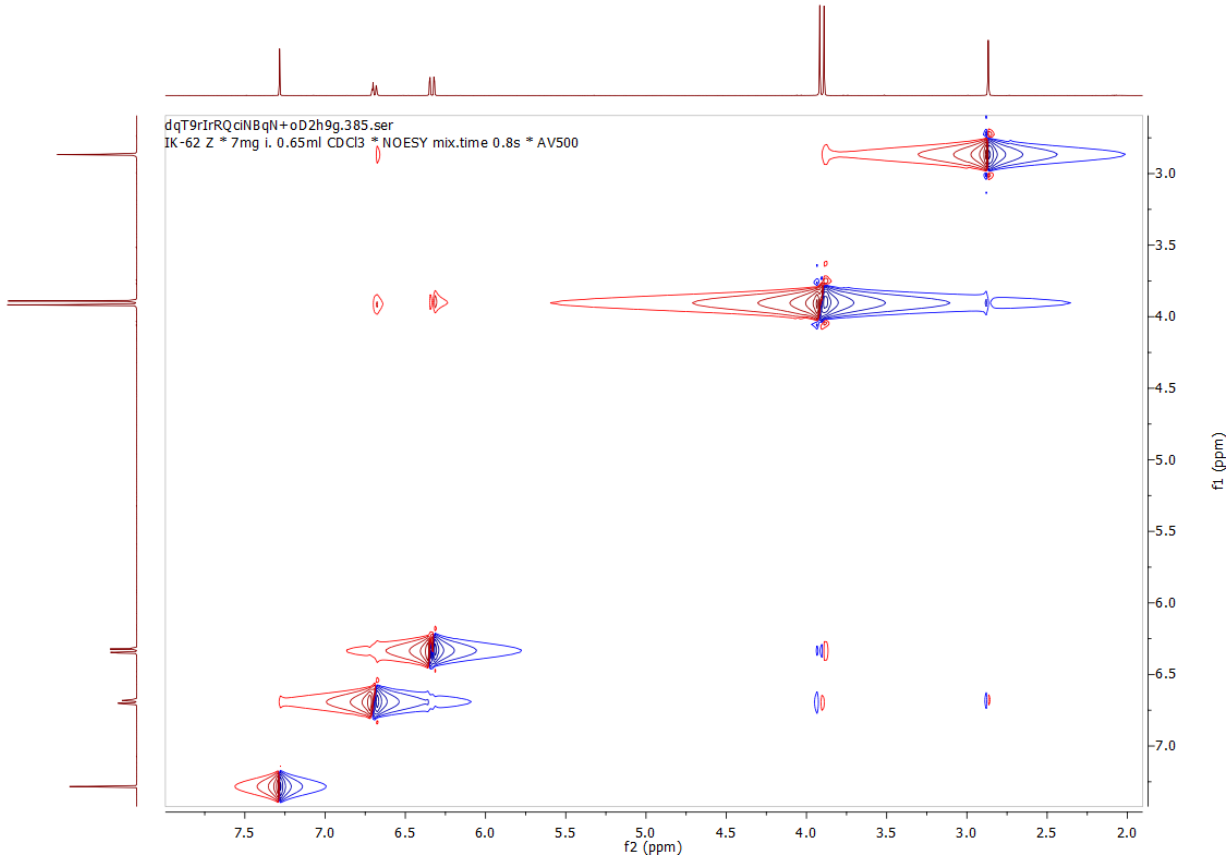
HSQC SPECTRUM FOR COMPOUND 103 ($CDCl_3$, 500 MHz)



HMBC SPECTRUM FOR COMPOUND 103 ($CDCl_3$, 500 MHz)



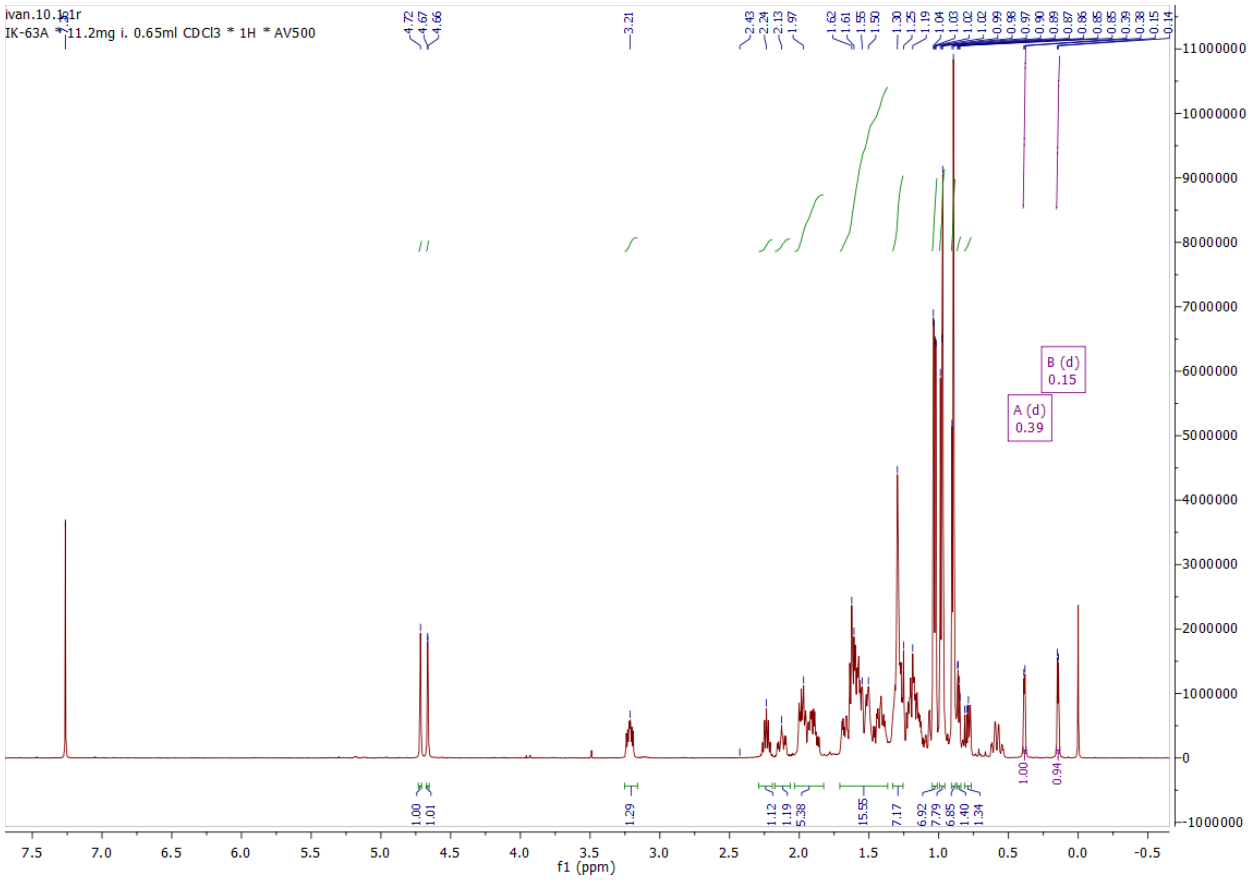
NOESY SPECRUM FOR COMPOUND 103 ($CDCl_3$, 500 MHz)



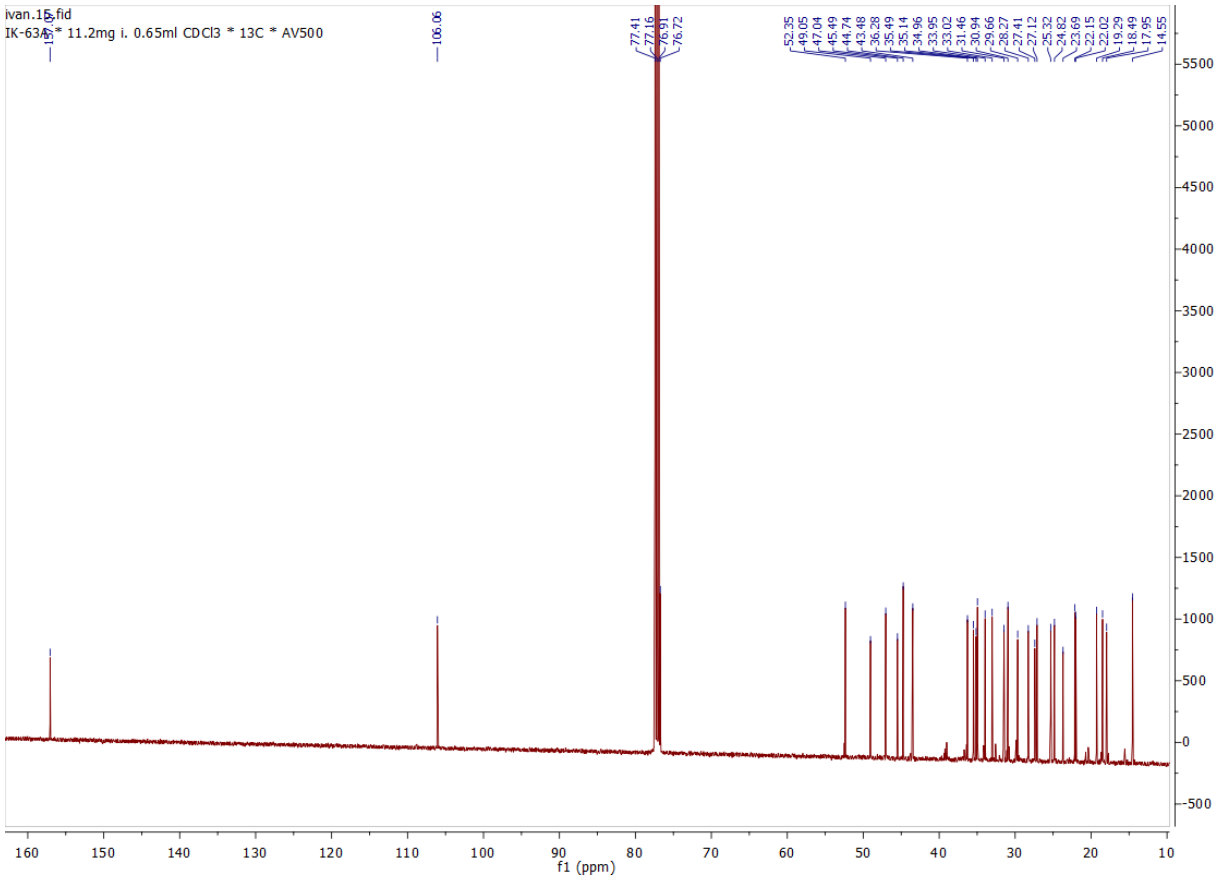
APPENDIX D

SPECTRA FOR COMPOUND 104

^{1}H NMR SPECTRUM FOR COMPOUND 104 ($CDCl_3$, 500 MHz)



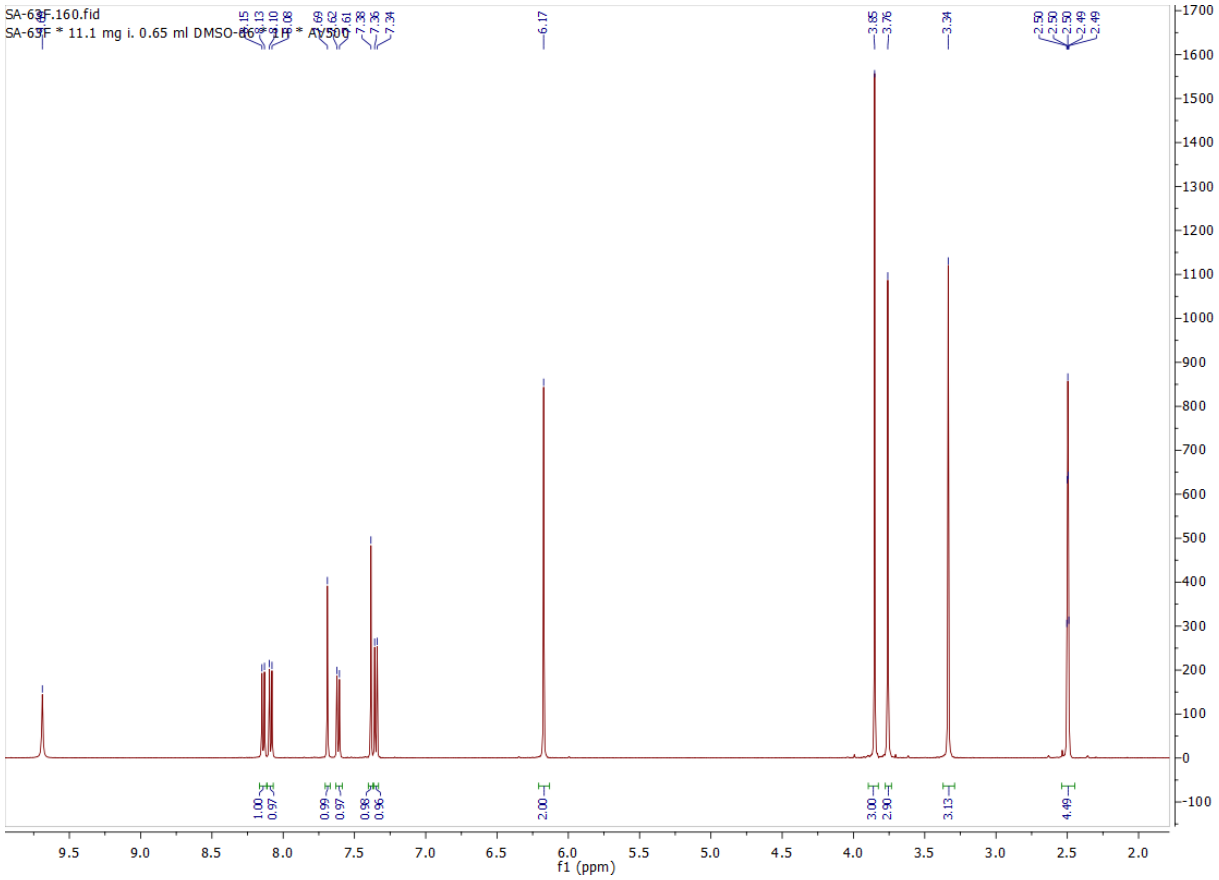
^{13}C NMR SPECTRUM FOR COMPOUND 104 ($CDCl_3$, 125 MHz)



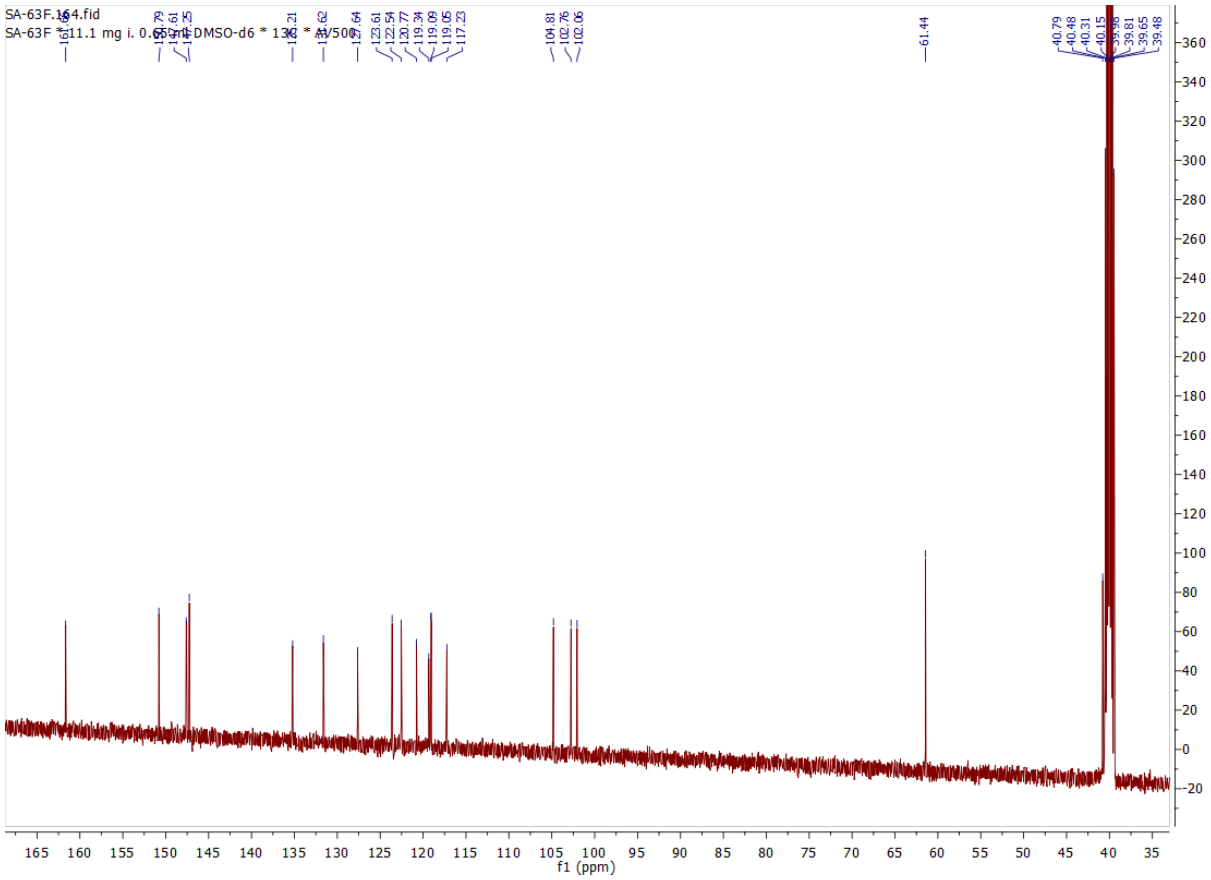
APPENDIX E

SPECTRA FOR COMPOUND 105

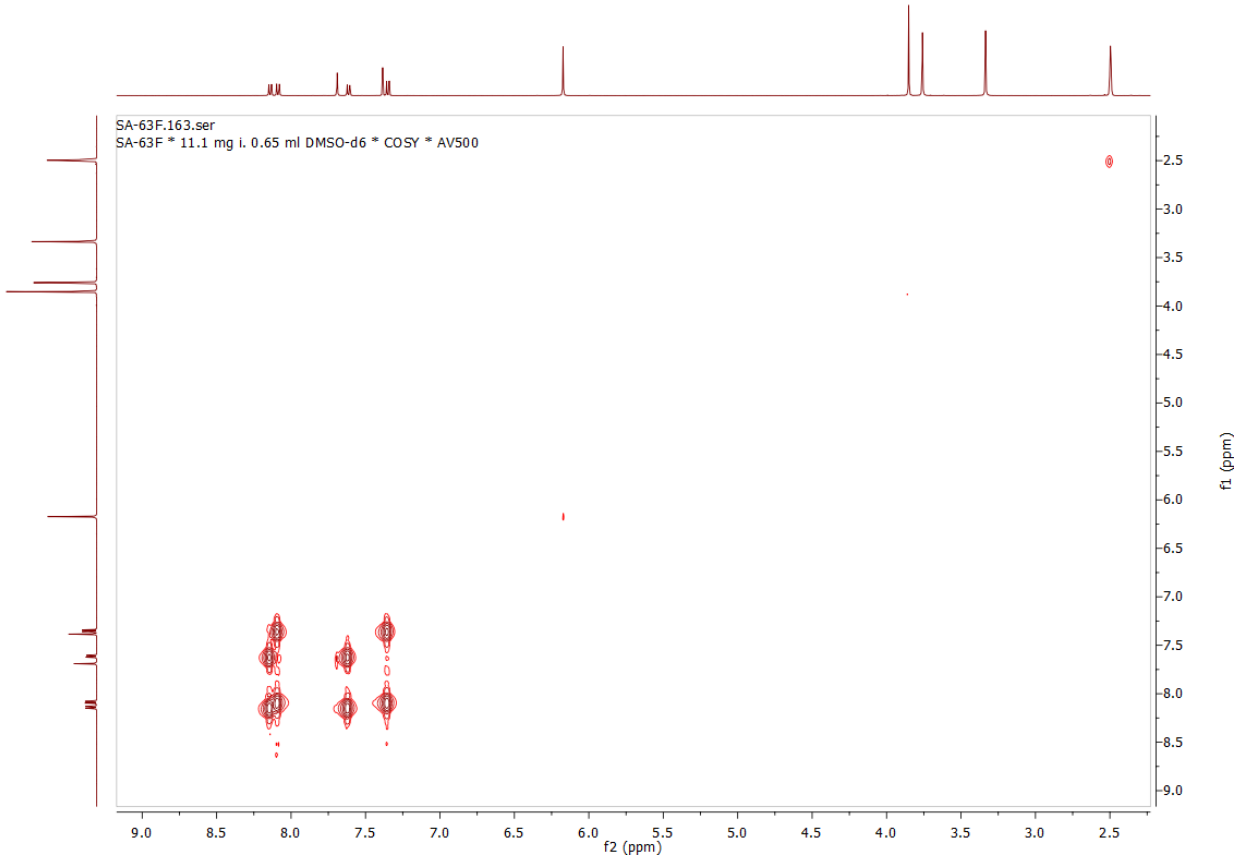
^{1}H NMR SPECTRA FOR COMPOUND 105 (DMSO-d6, 500 MHz)



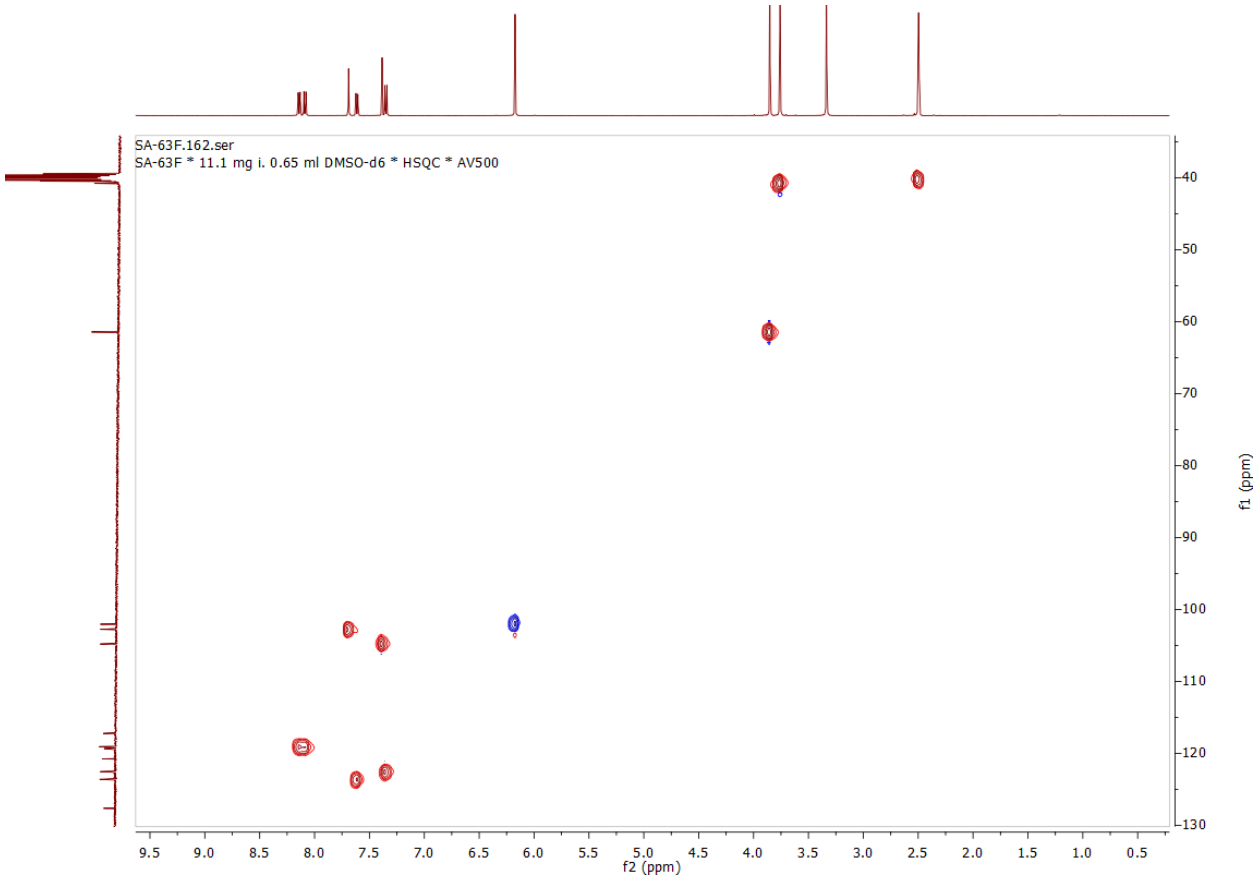
^{13}C NMR SPECTRUM FOR COMPOUND 105 (DMSO-d6, 125 MHz)



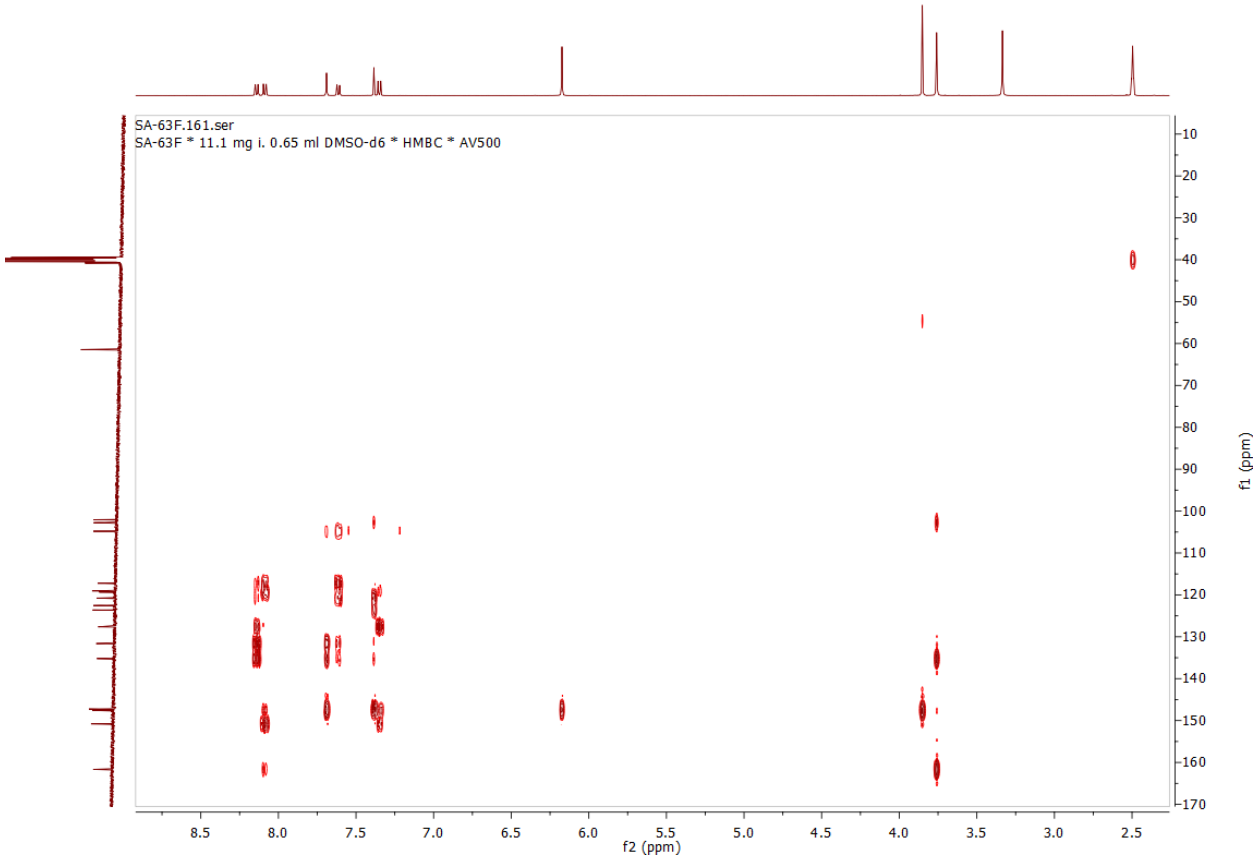
HH-COSSY SPECTRUM FOR COMPOUND 105 (DMSO-d6, 500 MHz)



HSQC SPECTRUM FOR COMPOUND 105 (DMSO-d6, 500 MHz)



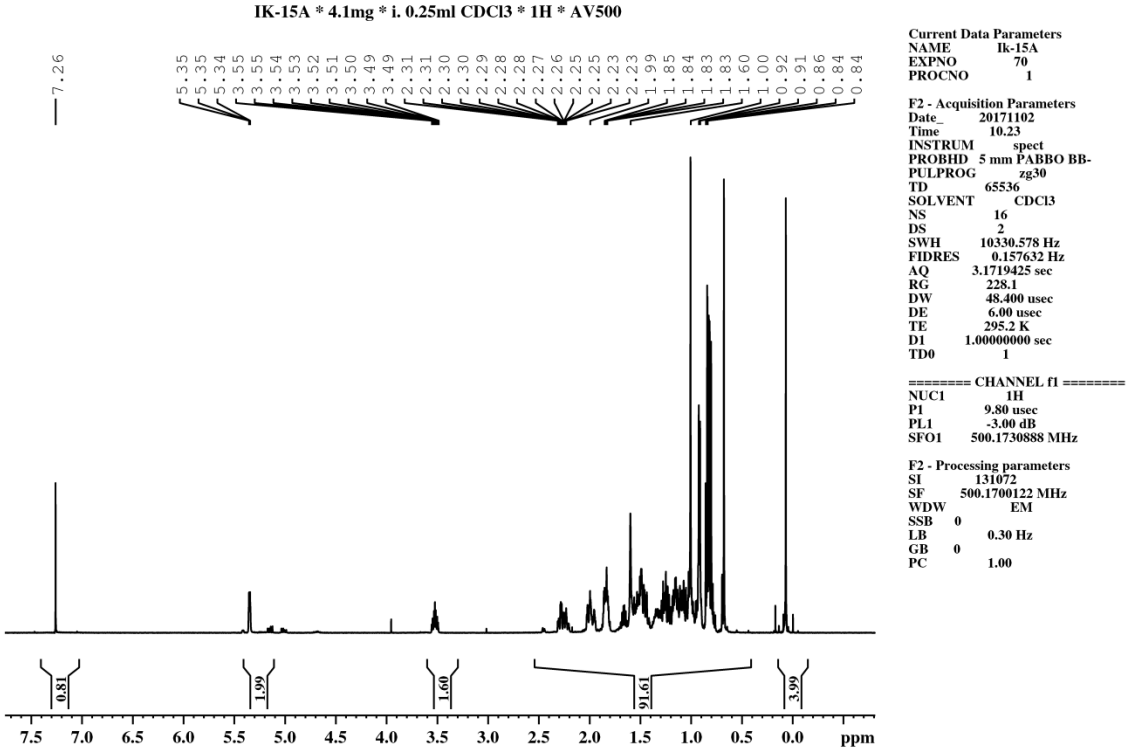
HMBC FOR COMPOUND 105 (DMSO-d6, 500 MHz)



APPENDIX F

SPECTRUM FOR COMPOUND 106

^{1}H NMR SPECTRUM FOR COMPOUND 106 ($CDCl_3$, 500 MHz)



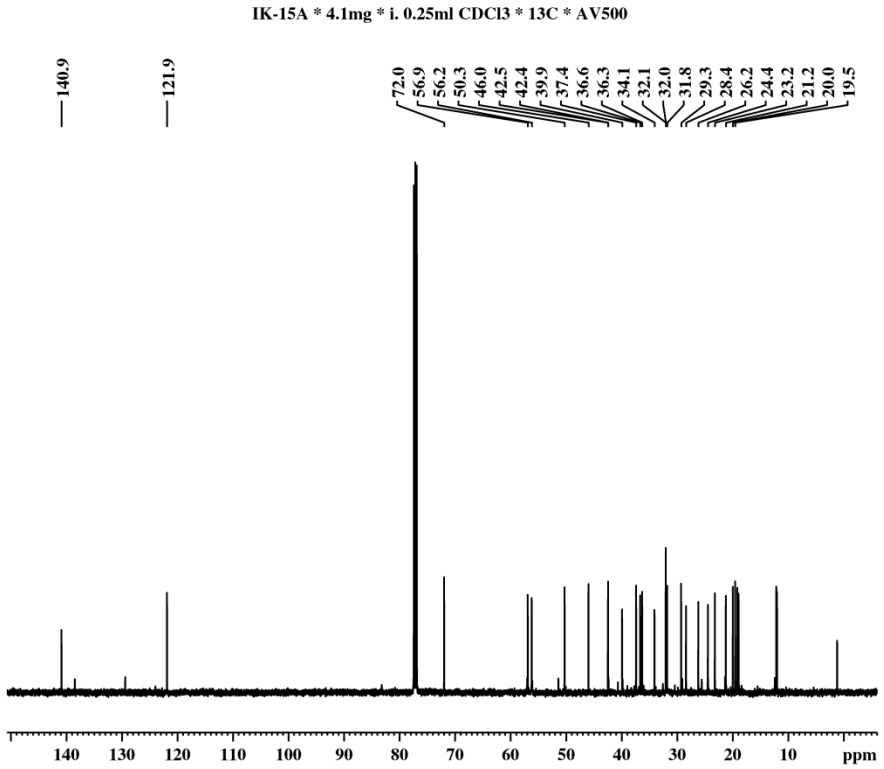
Current Data Parameters
NAME Ik-15A
EXPNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171102
Time 10.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 228.1
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700122 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR SPECTRUM FOR COMPOUND 106 ($CDCl_3$, 125 MHz)



Current Data Parameters
NAME Ik-15A
EXPNO 74
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171103
Time 3.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 10240
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0911744 sec
RG 322.5
DW 16.650 usec
DE 6.00 usec
TE 295.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 7.38 usec
PL1 3.00 dB
SFO1 125.7804233 MHz

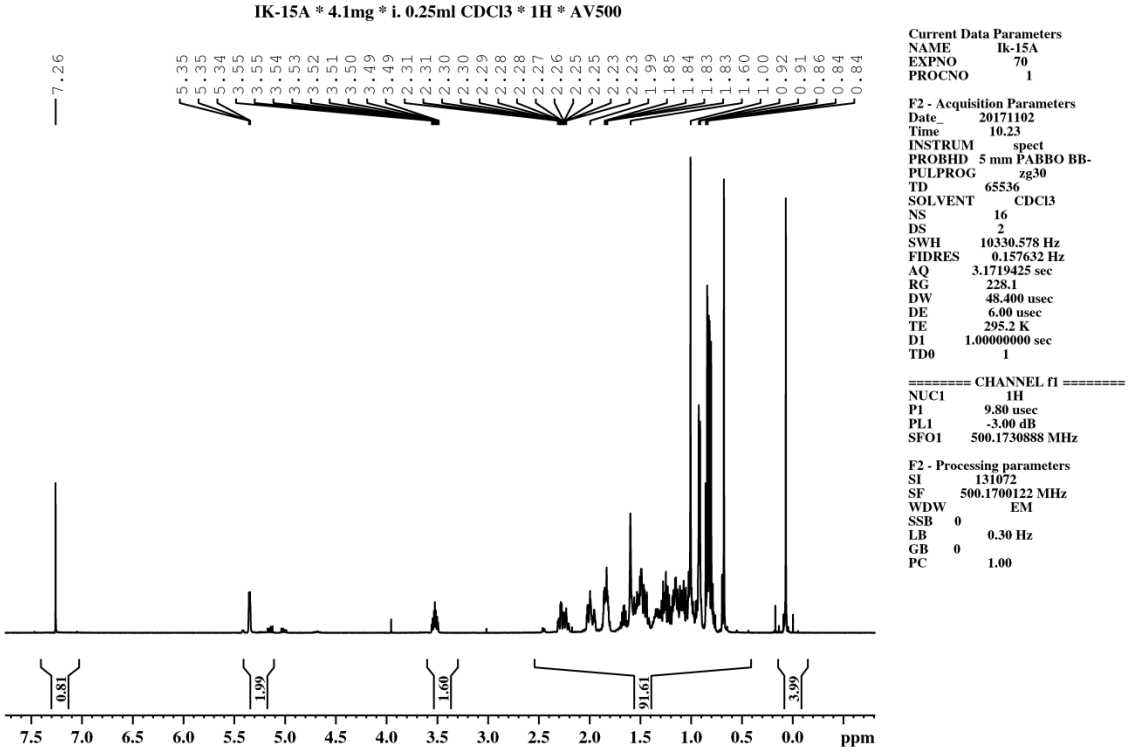
======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -3.00 dB
PL12 14.21 dB
PL13 17.30 dB
SFO2 500.1720007 MHz

F2 - Processing parameters
SI 65536
SF 125.7678300 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

APPENDIX G

SPECTRUM FOR COMPOUND 107

^{1}H NMR SPECTRUM FOR COUMPOUND 107 (CD_2Cl_2, 500 MHz)



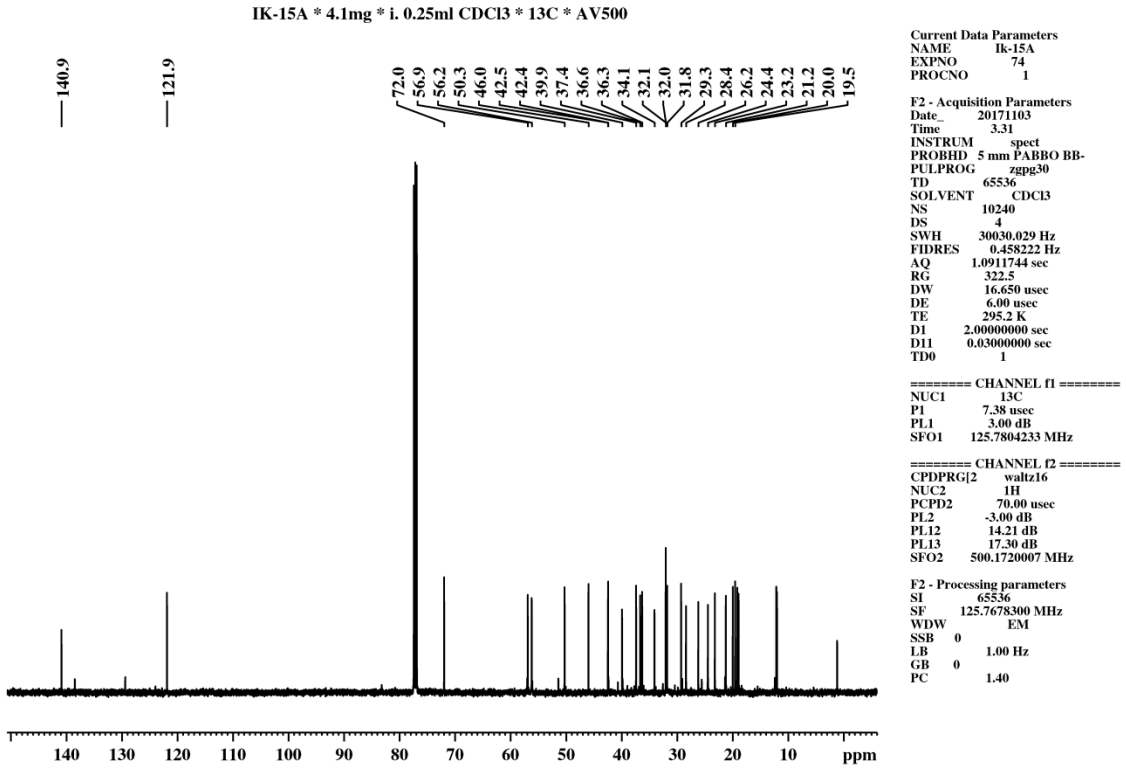
Current Data Parameters
NAME Ik-15A
EXPNO 70
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171102
Time 10.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 228.1
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700122 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

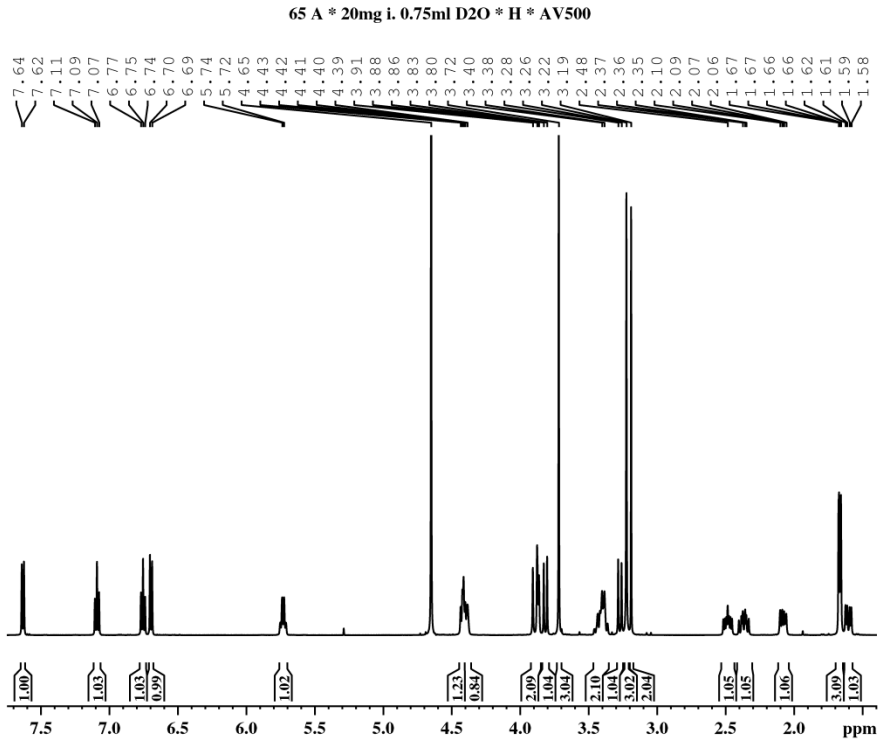
^{13}C NMR SPECTRUM FOR COMPOUND 107 (CD_2Cl_2, 125 MHz)



APPENDIX H

SPECTRUM OF COMPOUND 108

^{1}H NMR SPECTRUM OF COMPOUND 108 (CD_2Cl_2, 500 MHz)



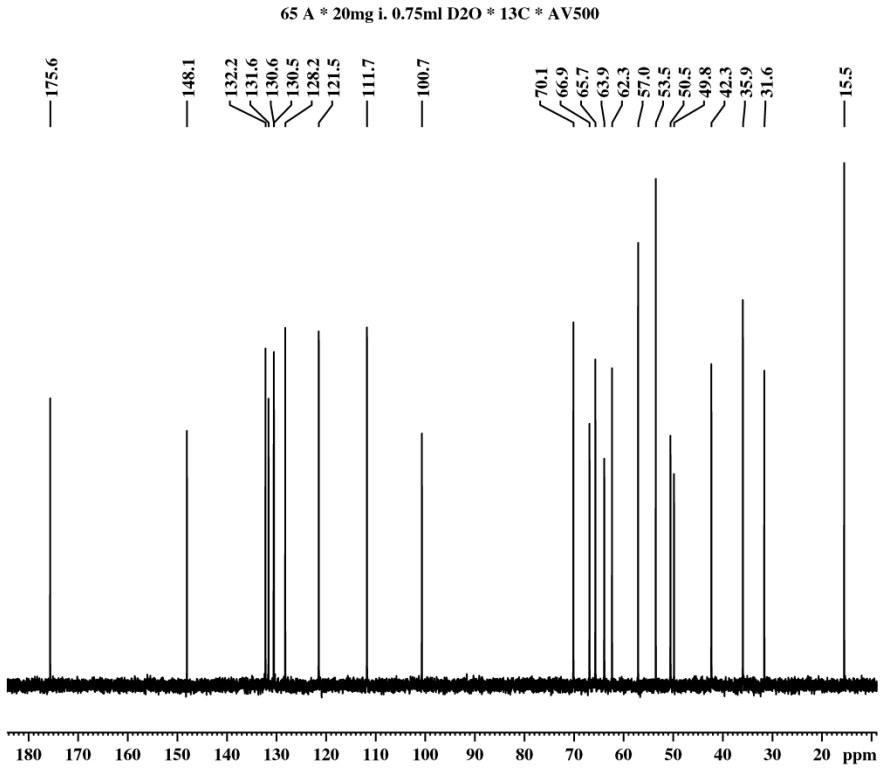
Current Data Parameters
NAME Ik-65A
EXPNO 10
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171206
Time 15.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 114
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

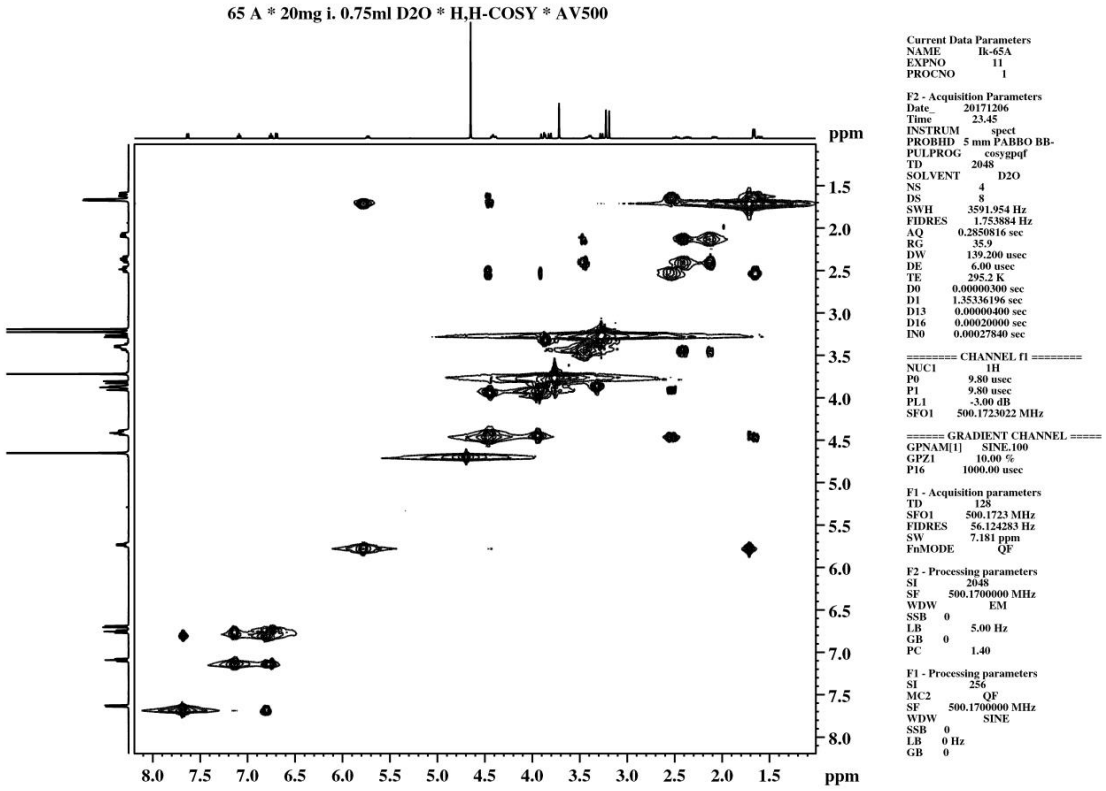
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700250 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

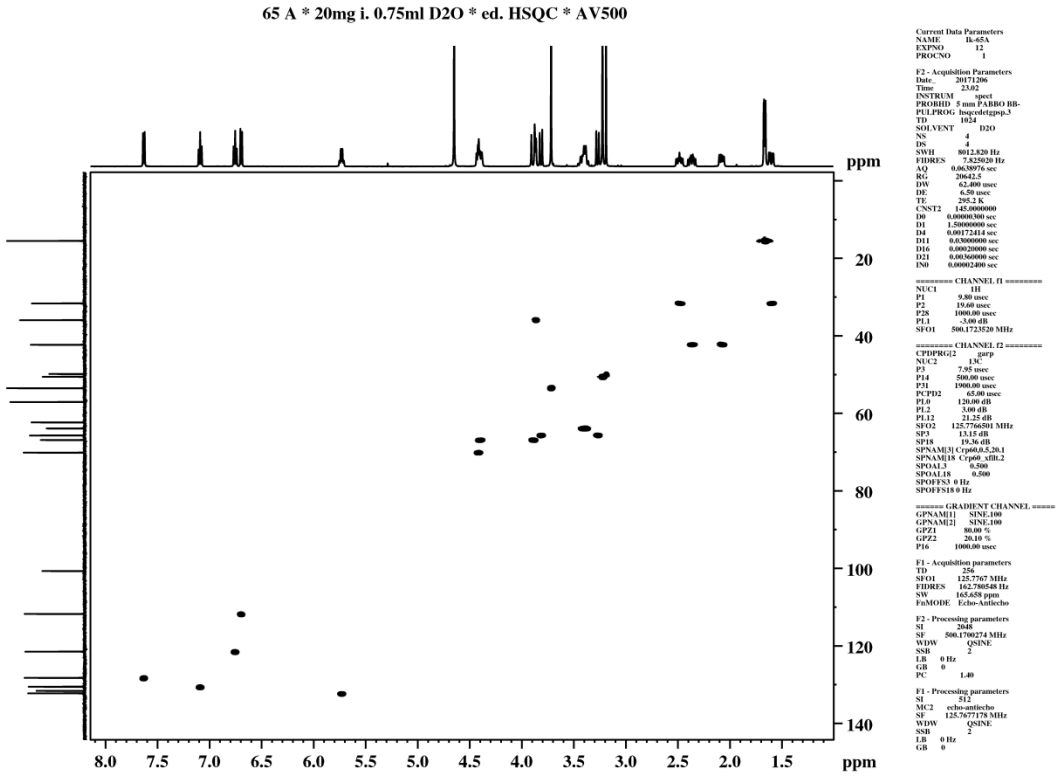
^{13}C NMR SPECTRUM FOR COMPOUND 108 (CD_2Cl_2, 125 MHz)



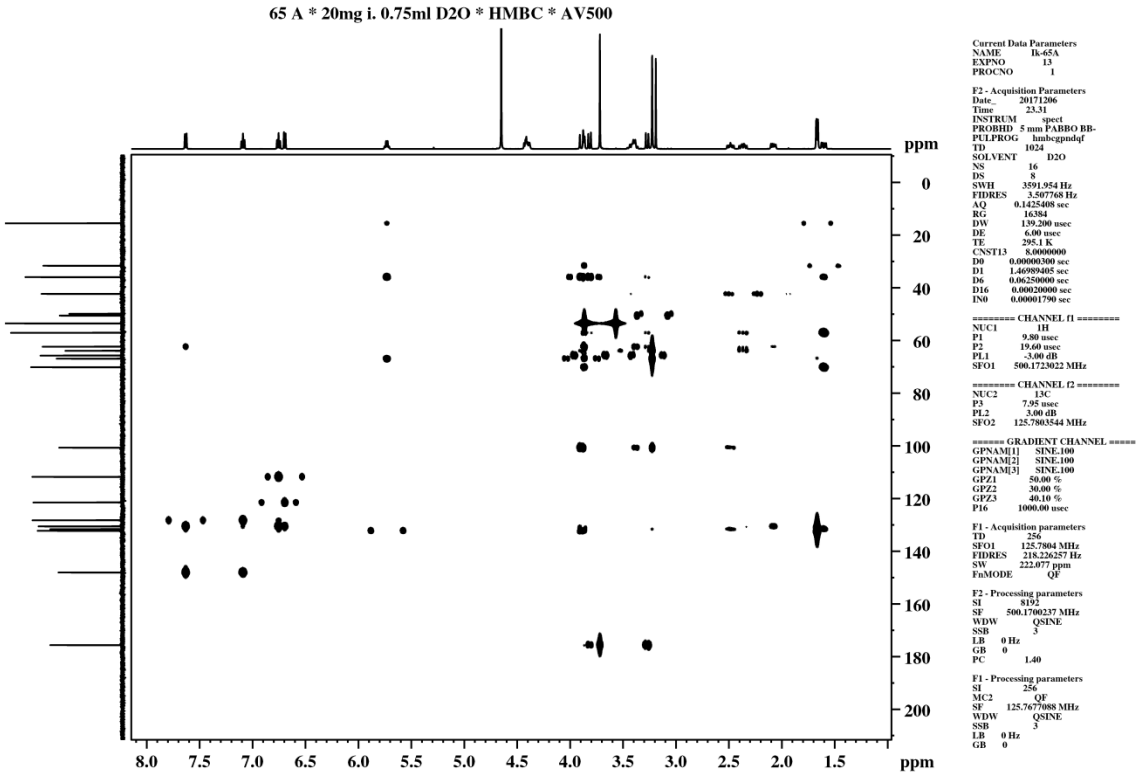
HH-COSSY SPECTRUM FOR COMPOUND 108(CD_2Cl_2, 500 MHz)



HSQC SPECTRUM FOR COMPOUND 108 (CD_2Cl_2, 500 MHz)



HMBC SPECTRUM FOR COMPOUND 108 (D_2O, 500 MHz)



Current Data Parameters
NAME Ik-65A
EXPNO 13
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171206
Time 23.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hmbcgpndqf
TD 1024
SOLVENT D2O
NS 16
DS 8
SWH 3591.954 Hz
FIDRES 3.507768 Hz
AQ 0.1425408 sec
RG 16384
DW 139.200 usec
DE 6.00 usec
TE 295.1 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.46989405 sec
D6 0.06250000 sec
D16 0.00020000 sec
IN0 0.00001790 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
PL1 -3.00 dB
SFO1 500.1723022 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 7.95 usec
PL2 3.00 dB
SFO2 125.7803544 MHz

====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7804 MHz
FIDRES 218.226257 Hz
SW 222.077 ppm
FnMODE QF

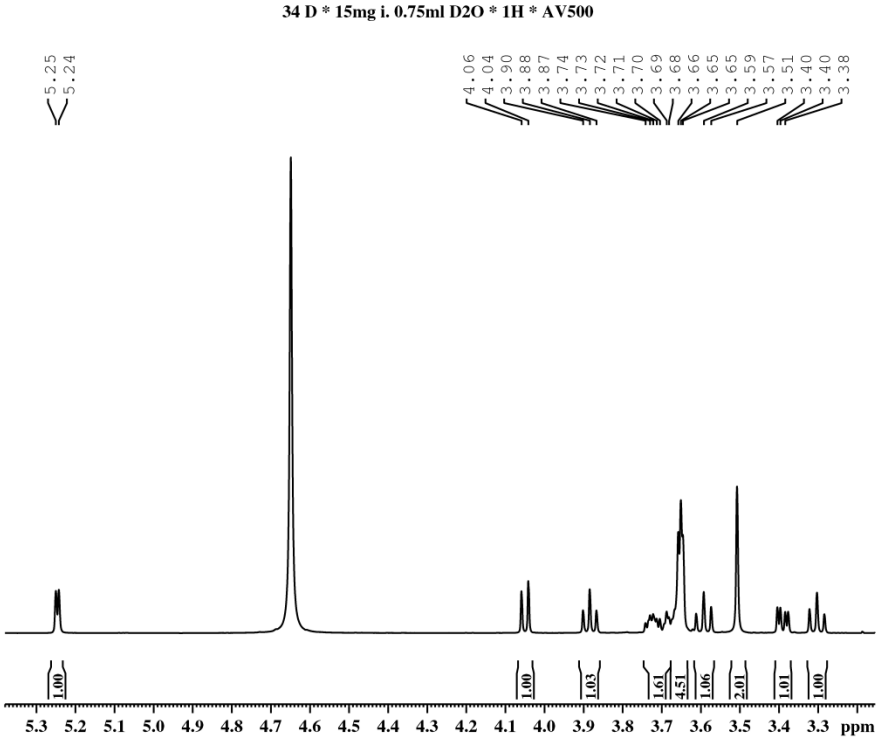
F2 - Processing parameters
SI 8192
SF 500.1700237 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 125.7677088 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0

APPENDIX I

SPECTRUM FOR COMPOUND 109

^{1}H NMR SPECTRUM FOR COMPOUND 109 (D_2O, 500 MHz)



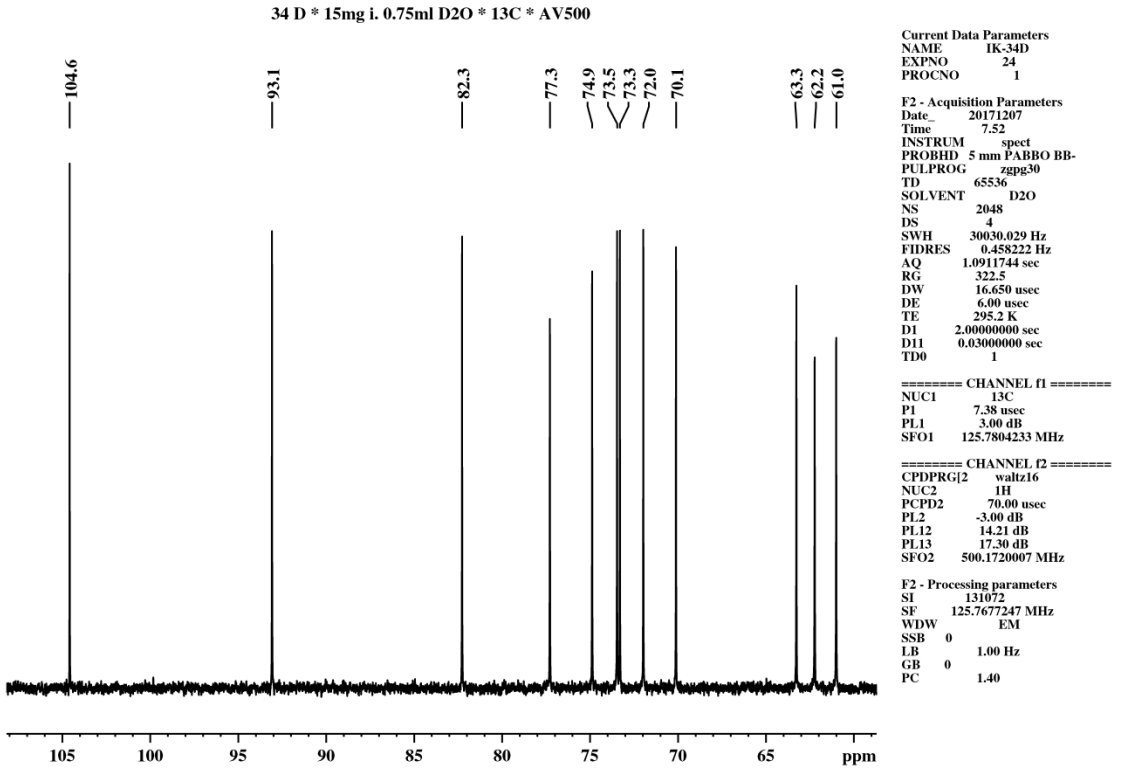
Current Data Parameters
NAME IK-34D
EXPNO 20
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171206
Time 15.55
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 128
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

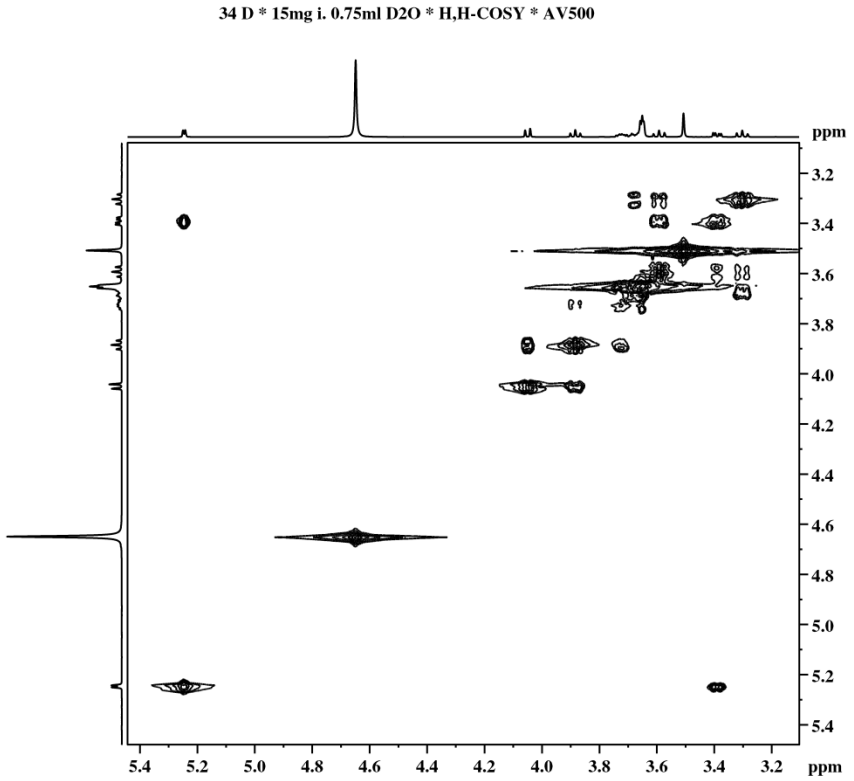
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700239 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR SPECTRUM FOR COMPOUND 109 (D_2O, 125 MHz)



HH-COSSY SPECTRUM FOR COMPOUND 109 (D_2O, 500 MHz)



Current Data Parameters
NAME IK-34D
EXPNO 21
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171207
Time 3.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygpqf
TD 2048
SOLVENT D2O
NS 4
DS 8
SWH 1260.081 Hz
FIDRES 0.615274 Hz
AQ 0.8126464 sec
RG 114
DW 396.800 usec
DE 6.00 usec
TE 295.1 K
D0 0.00000300 sec
D1 0.82293040 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00079360 sec

======== CHANNEL f1 ========
NUC1 1H
P0 9.80 usec
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1721521 MHz

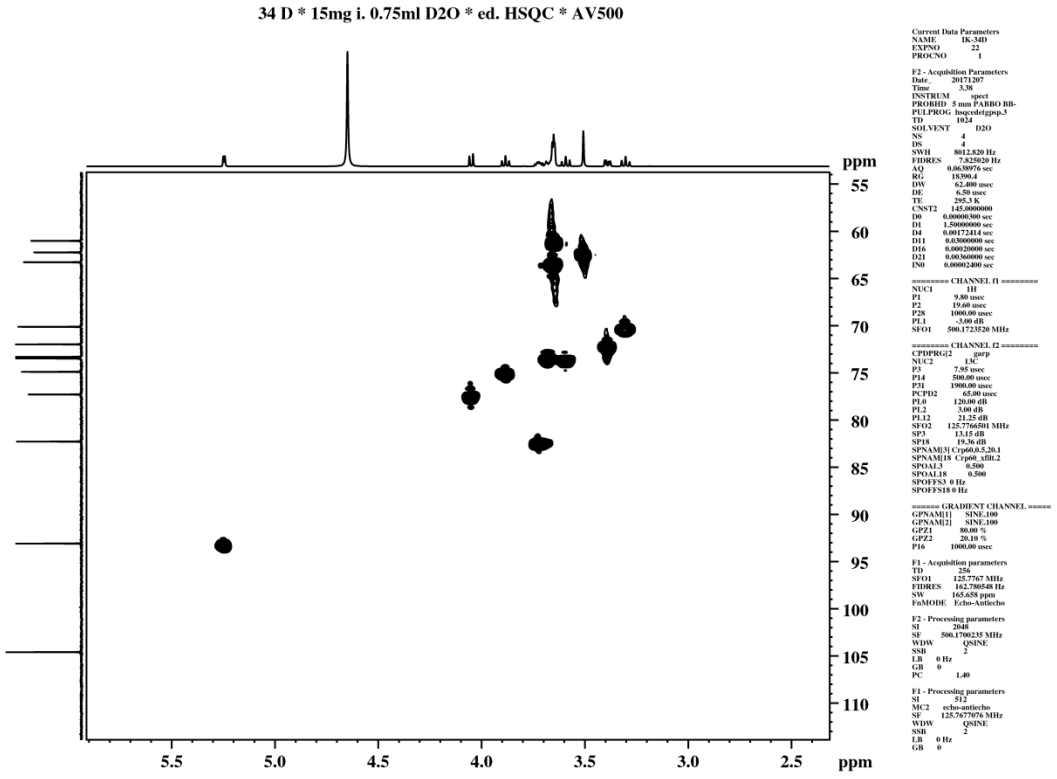
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 500.1722 MHz
FIDRES 19.688761 Hz
SW 2.519 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 500.1700222 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 500.1700233 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

HSQC SPECTRUM FOR COMPOUND 109 (D_2O, 500 MHz)



Current Data Parameters
NAME IK-34D
EXPNO 22
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171207
Time 3.38
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT D2O
NS 4
DS 4
SWH 8012.820 Hz
FIDRES 7.825020 Hz
AQ 0.063897 6 sec
RG 18390.4
DW 62.400 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
PJ1 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfilt.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

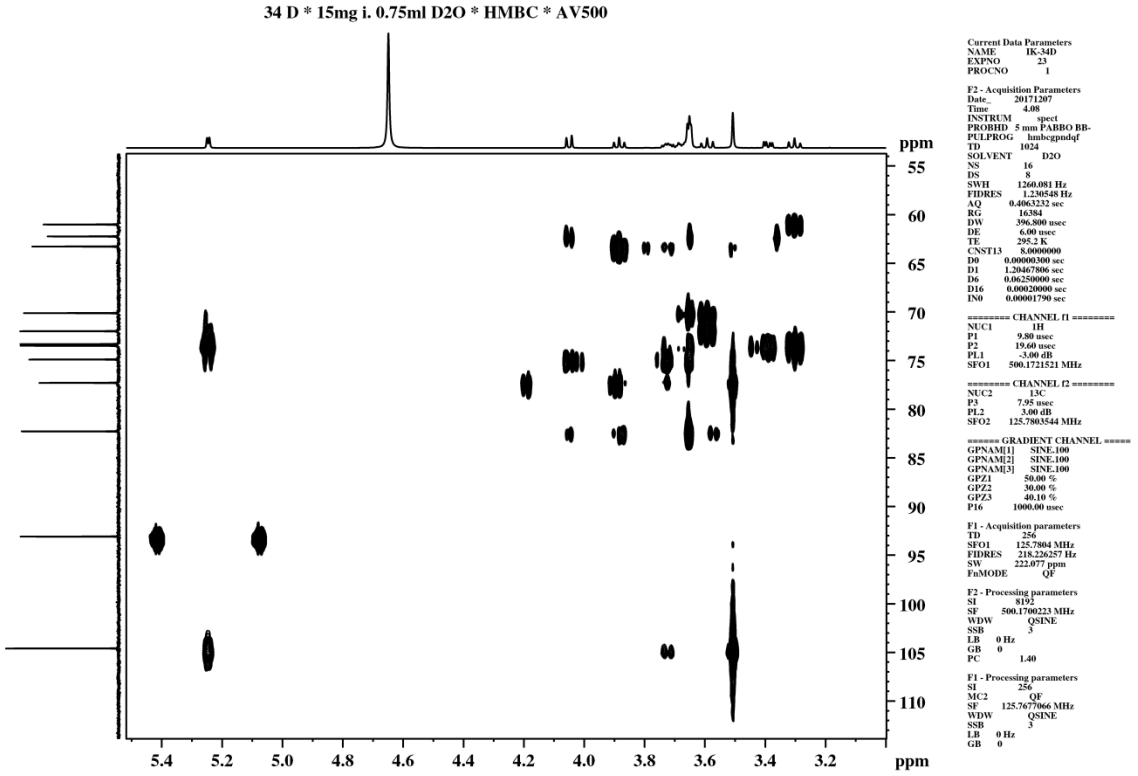
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1700235 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677076 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

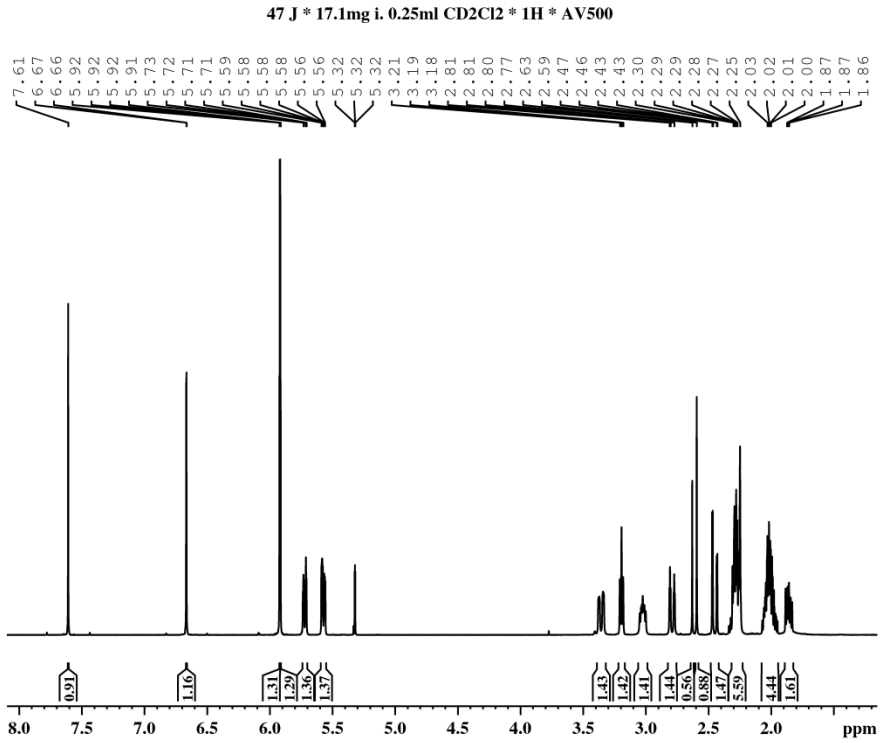
HMBC SPECTRUM FOR COMPOUND 109 (D_2O, 500 MHz)



APPENDIX J

SPECTRUM FOR COMPOUND 95

^{1}H NMR SPECTRUM FOR COMPOUND 95 (CD_2Cl_2, 500 MHz)



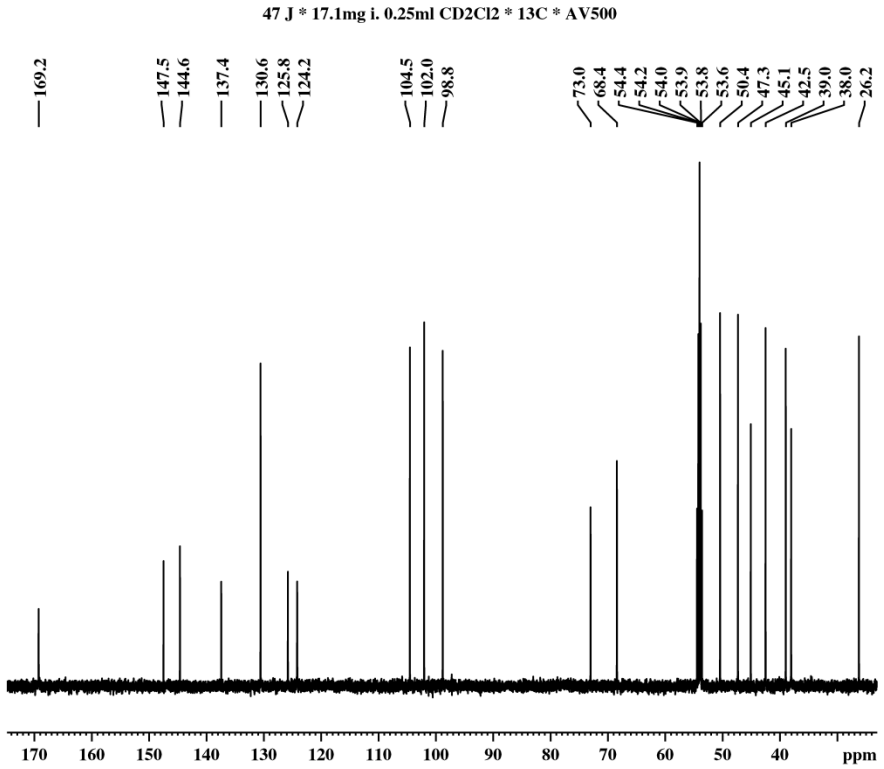
Current Data Parameters
NAME IK-47J
EXPNO 30
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171116
Time 14.29
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CD2Cl2
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 181
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

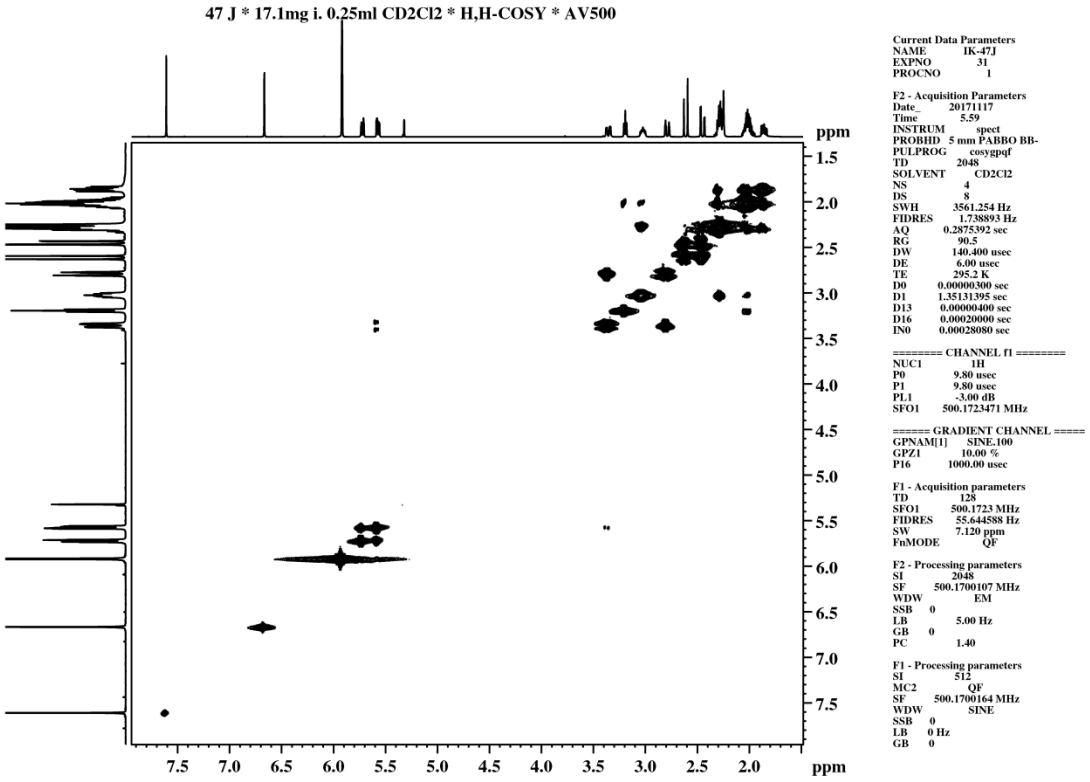
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700191 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

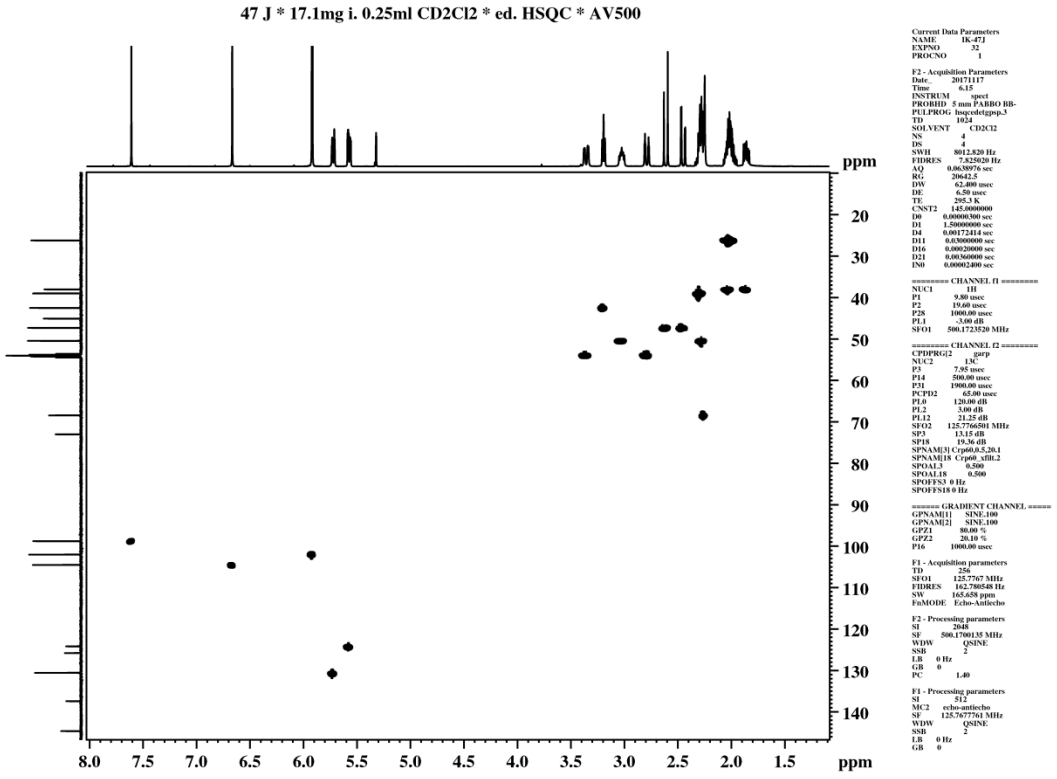
^{13}C NMR SPECTRUM FOR COMPOUND 95 (CD_2Cl_2, 125 MHz)



HH-COSSY SPECTRUM FOR COMPOUND 95 (CD_2Cl_2, 500 MHz)



HSQC SPECTRUM FOR COMPOUND 95 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-471
EXPNO 32
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171117
Time 6.15
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT CD2Cl2
NS 4
DS 4
SWH 8012.820 Hz
FIDRES 7.825020 Hz
AQ 0.063897 6 sec
RG 20642.5
DW 62.400 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
P31 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfilt.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

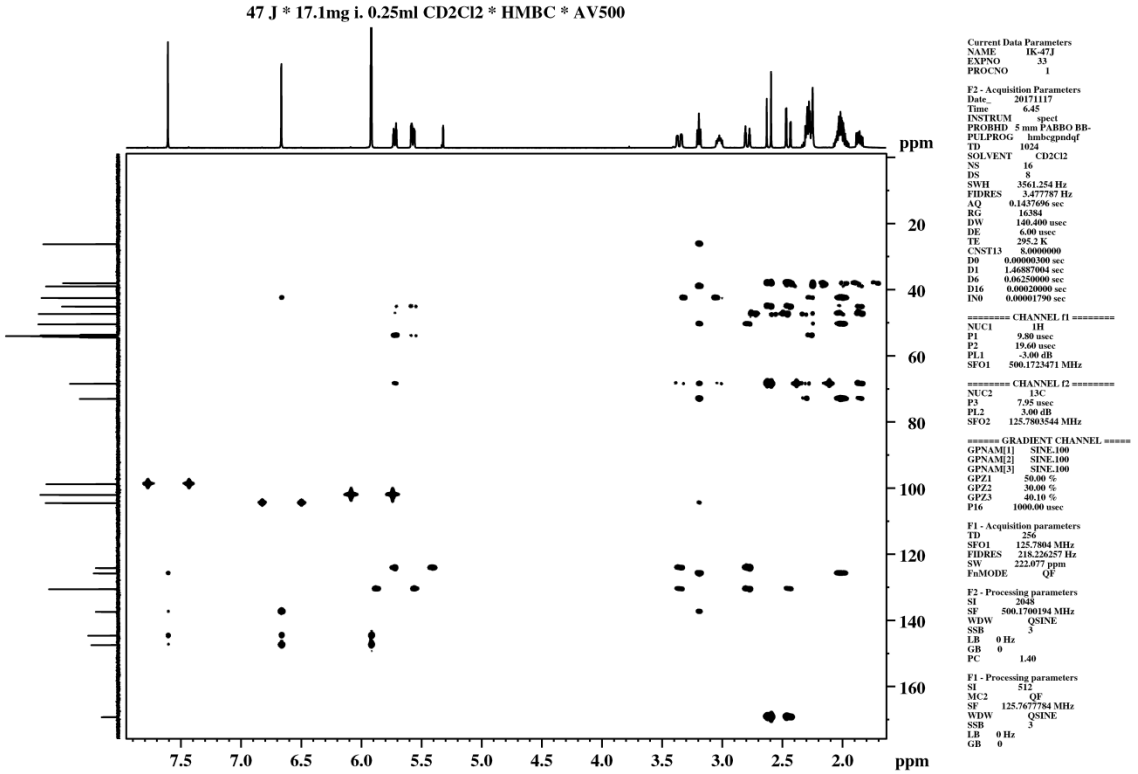
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1700135 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677761 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

HMBC SPECTRUM FOR COMPOUND 95 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-47J
EXPNO 33
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171117
Time 6.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hmbcgpndqf
TD 1024
SOLVENT CD2Cl2
NS 16
DS 8
SWH 3561.254 Hz
FIDRES 3.477787 Hz
AQ 0.1437696 sec
RG 16384
DW 140.400 usec
DE 6.00 usec
TE 295.2 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.46887004 sec
D6 0.06250000 sec
D16 0.00020000 sec
IN0 0.00001790 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
PL1 -3.00 dB
SFO1 500.1723471 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 7.95 usec
PL2 3.00 dB
SFO2 125.7803544 MHz

====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7804 MHz
FIDRES 218.226257 Hz
SW 222.077 ppm
FnMODE QF

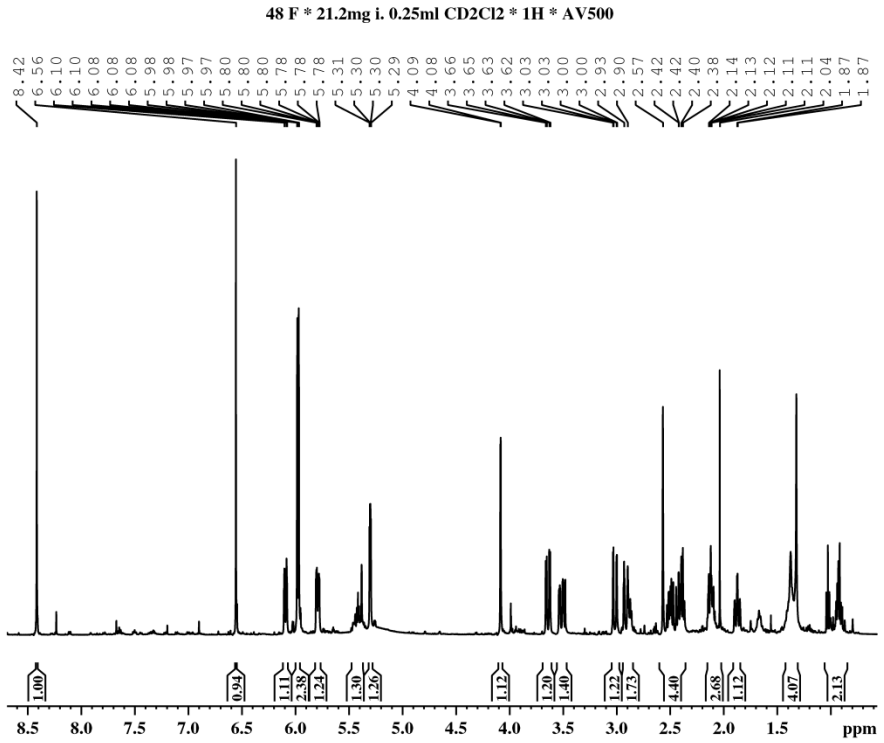
F2 - Processing parameters
SI 2048
SF 500.1700194 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 QF
SF 125.7677784 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0

APPENDIX L

SPECTRUM FOR COMPOUND 96

^{1}H NMR SPECTRUM FOR COMPOUND 96 (CD_2Cl_2, 500 MHz)



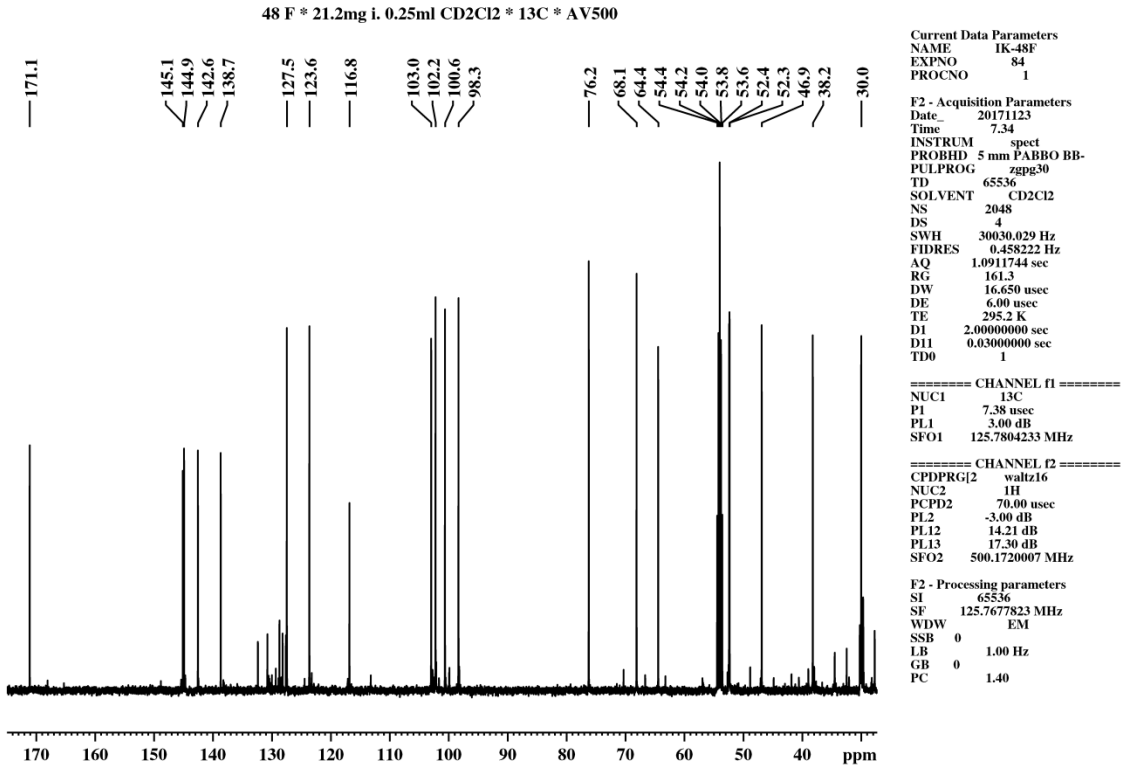
Current Data Parameters
NAME IK-48F
EXPNO 80
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171122
Time 16.54
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CD2Cl2
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 71.8
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

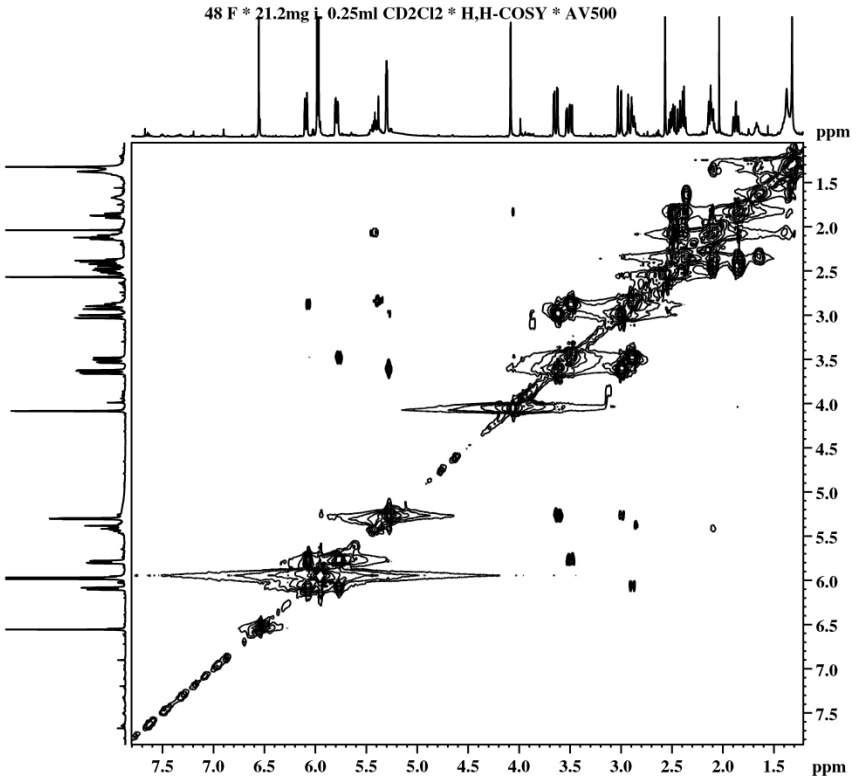
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1699886 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR SPECTRUM FOR COMPOUND 96 (CD_2Cl_2, 125 MHz)



HH-COSSY SPECTRUM FOR COMPOUND 96 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-48F
EXPNO 81
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171123
Time 3.03
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygpqf
TD 2048
SOLVENT CD2Cl2
NS 4
DS 8
SWH 5081.301 Hz
FIDRES 2.481104 Hz
AQ 0.2015232 sec
RG 35.9
DW 98.400 usec
DE 6.00 usec
TE 295.2 K
D0 0.00000300 sec
D1 1.43733001 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00019680 sec

======== CHANNEL f1 ========
NUC1 1H
P0 9.80 usec
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1719629 MHz

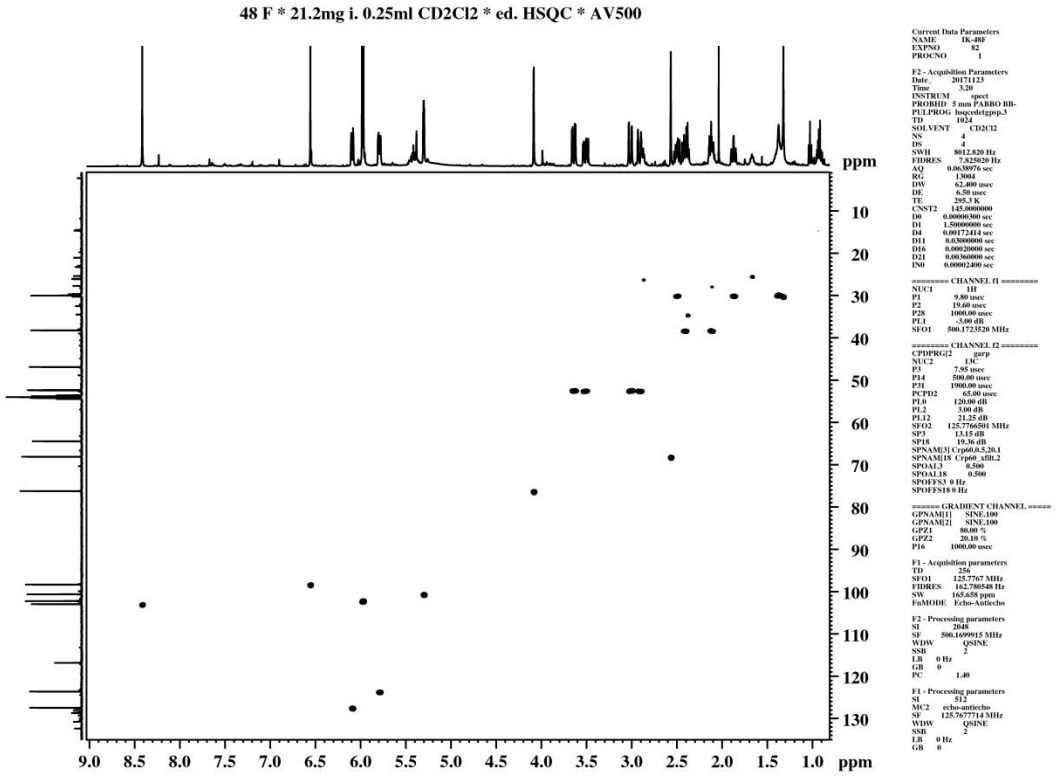
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 500.172 MHz
FIDRES 79.395325 Hz
SW 10.159 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 500.1700000 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 500.1700000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

HSQC SPECTRUM FOR COMPOUND 96 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-d8F
EXPNO 82
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171123
Time 3.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT CD2Cl2
NS 4
DS 4
SWH 8012.820 Hz
FIDRES 7.825020 Hz
AQ 0.063897 6 sec
RG 13004
DW 62.400 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
P31 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfilt.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

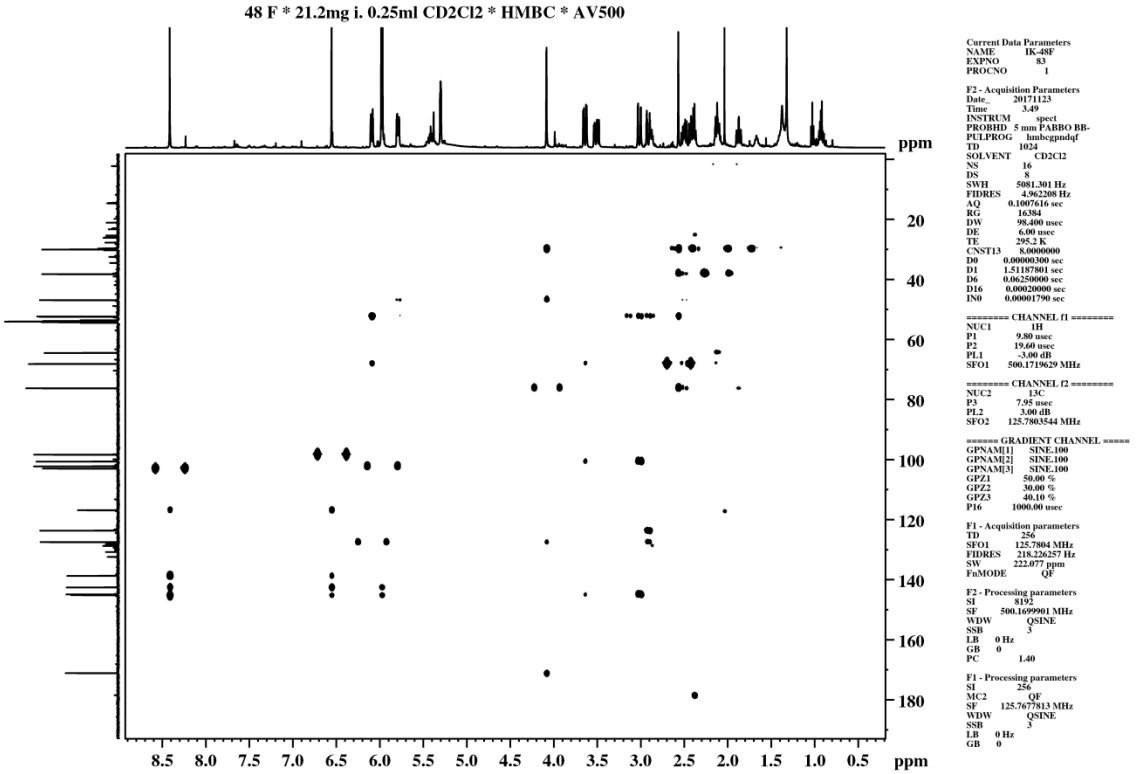
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1699915 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677714 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

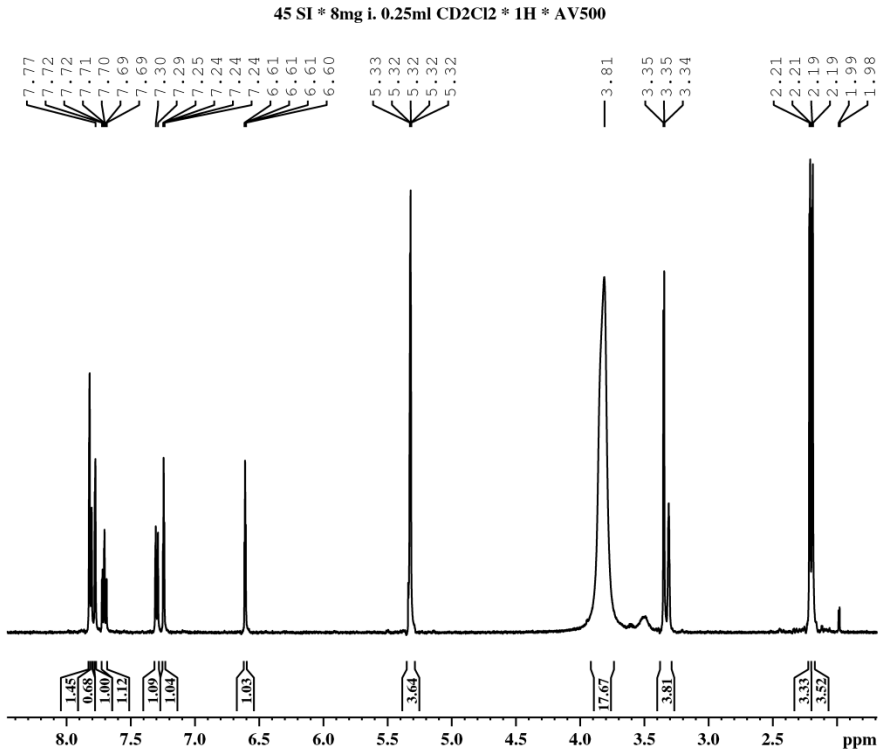
HMBC SPECTRUM FOR COMPOUND 96 (CD_2Cl_2, 500 MHz)



APPENDIX M

SPECTRUM FOR COMPOUND 97

^{1}H NMR SPECTRUM FOR COMPOUND 97 (CD_2Cl_2/CD_3OD, 500 MHz)



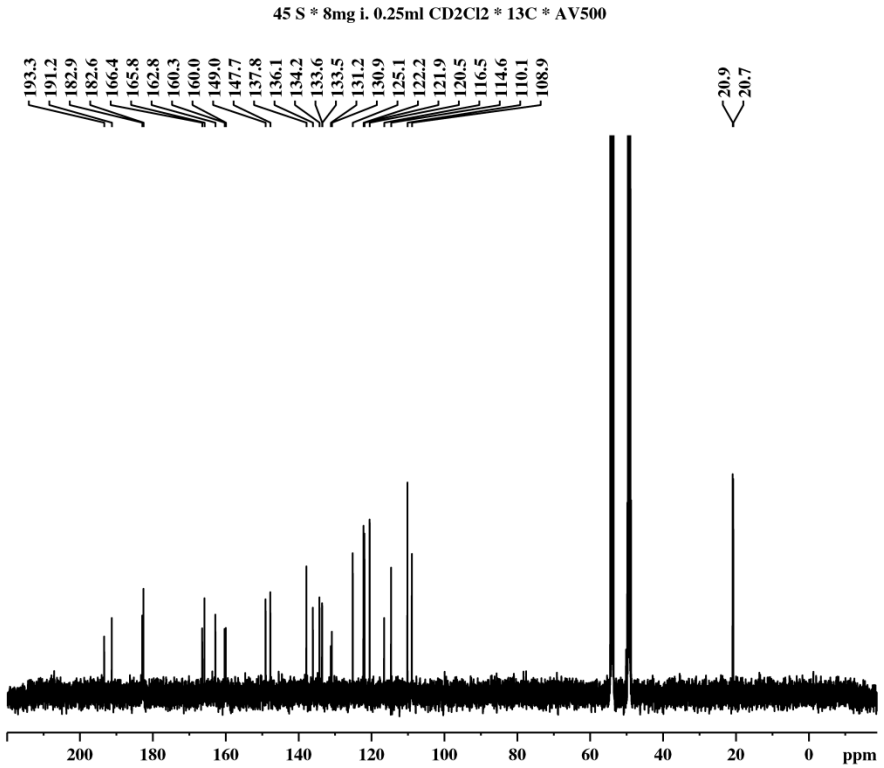
Current Data Parameters
NAME Ik45S
EXPNO 50
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171117
Time 9.47
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CD2Cl2
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 322.5
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

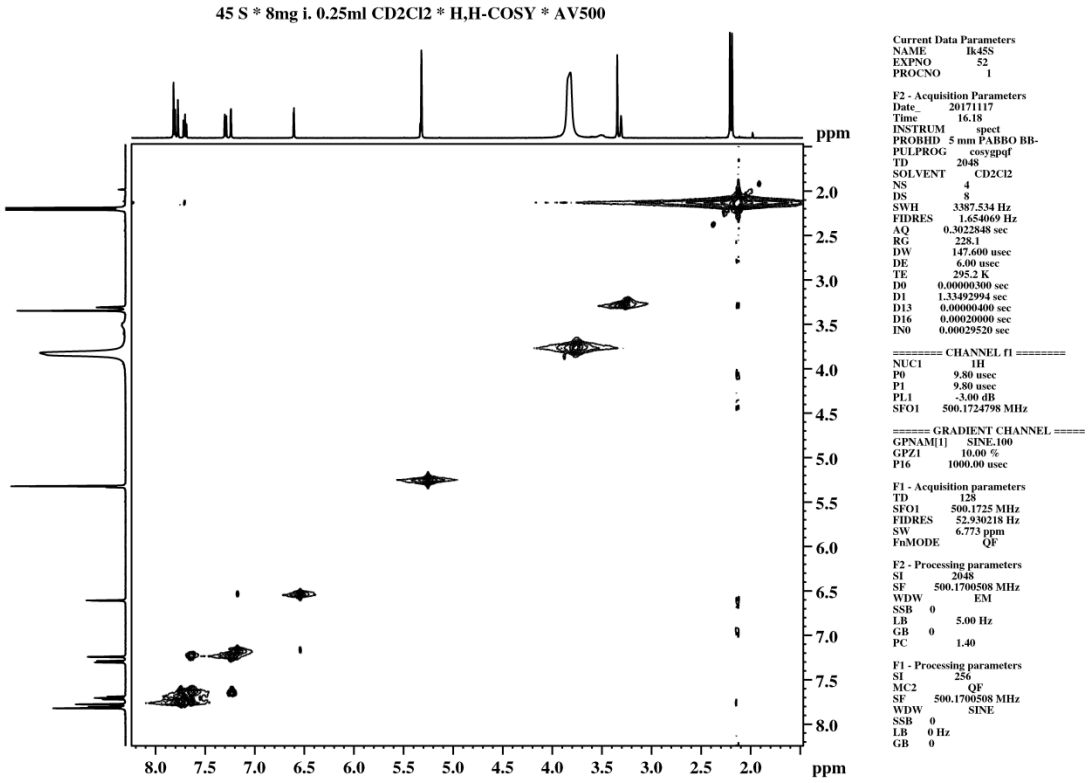
F2 - Processing parameters
SI 131072
SF 500.1700184 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR SPECTRUM FOR COMPOUND 97 (CD_2Cl_2/CD_3OD, 125 MHz)

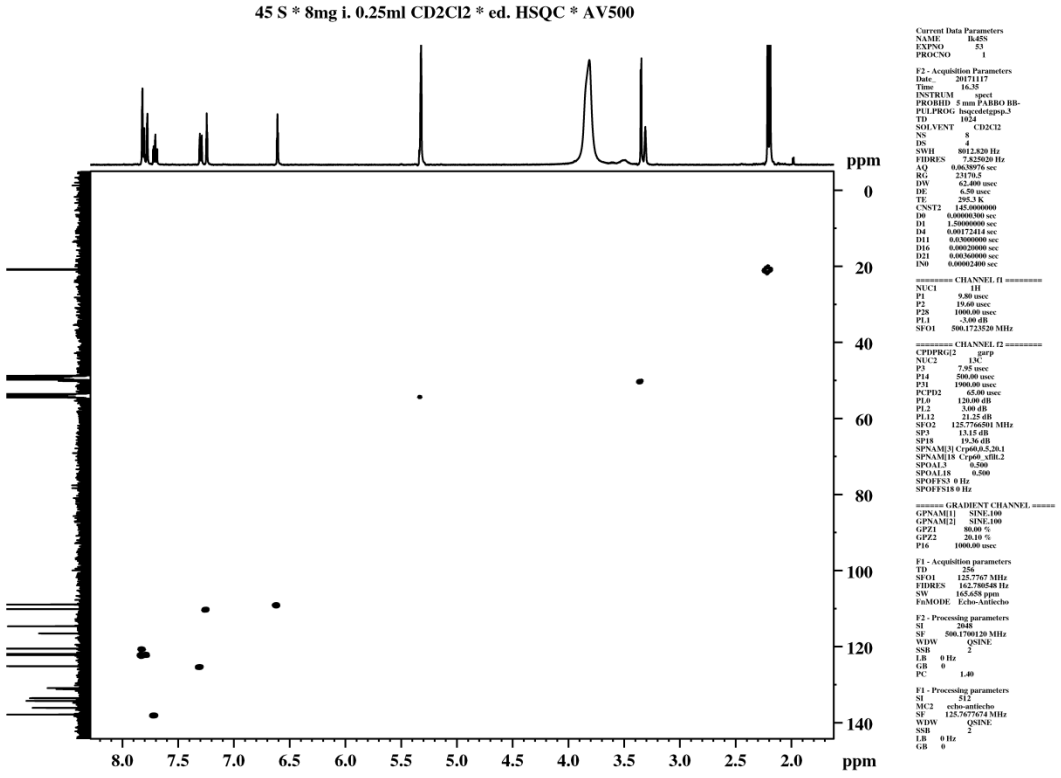



```
Current Data Parameters
NAME            Ik45S
EXPNO             55
PROCNO             1

F2 - Acquisition Parameters
Date_         20171118
Time              4.27
INSTRUM          spect
PROBHD   5 mm PABBO BB-
PULPROG       zgpg30
TD             65536
SOLVENT         CD2Cl2
NS             10240
DS                 4
SWH          30030.029 Hz
FIDRES         0.458222 Hz
AQ           1.0911744 sec
RG               181
DW             16.650 usec
DE              6.00 usec
TE             295.2 K
D1          2.00000000 sec
D11         0.03000000 sec
TD0                1

======== CHANNEL f1 ========
NUC1              13C
P1              7.38 usec
PL1             3.00 dB
SFO1        125.7804233 MHz

======== CHANNEL f2 ========
CPDPRG[2        waltz16
NUC2              1H
PCPD2           70.00 usec
PL2             -3.00 dB
PL12            14.21 dB
PL13            17.30 dB
SFO2        500.1720007 MHz

F2 - Processing parameters
SI              65536
SF          125.7677668 MHz
WDW                EM
SSB      0
LB              1.00 Hz
GB       0
PC              1.40
```

HH-COSSY SPECTRUM FOR COMPOUND 97 (CD_2Cl_2/CD_3OD, 500 MHz)



HSQC SPECTRUM FOR COMPOUND 97 (CD_2Cl_2/CD_3OD, 500 MHz)



Current Data Parameters
NAME Ik45S
EXPNO 53
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171117
Time 16.35
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT CD2Cl2
NS 8
DS 4
SWH 8012.820 Hz
FIDRES 7.825020 Hz
AQ 0.063897 6 sec
RG 23170.5
DW 62.400 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
PJ1 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfilt.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

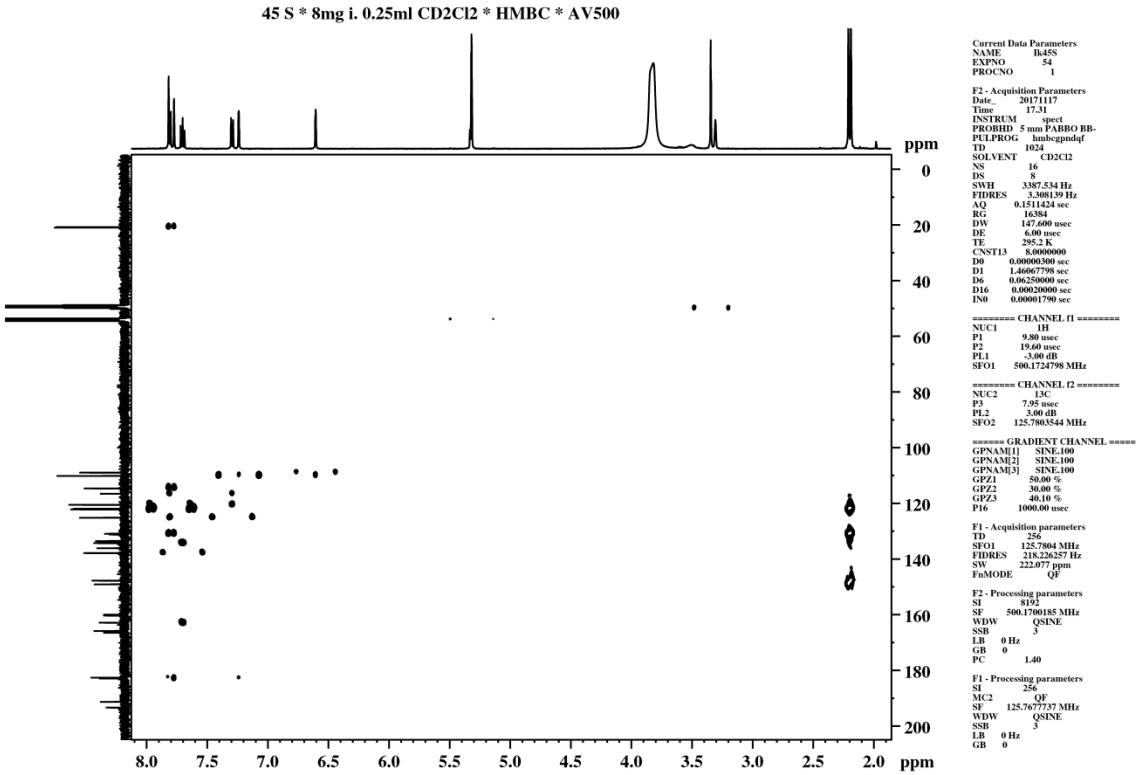
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1700120 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677674 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

HMBC SPECTRUM FOR COMPOUND 97 (CD_2Cl_2/CD_3OD, 500 MHz)



Current Data Parameters
NAME lk45S
EXPNO 54
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171117
Time 17.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hmbcgpndqf
TD 1024
SOLVENT CD2Cl2
NS 16
DS 8
SWH 3387.534 Hz
FIDRES 3.308139 Hz
AQ 0.1511424 sec
RG 16384
DW 147.600 usec
DE 6.00 usec
TE 295.2 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.46067798 sec
D6 0.06250000 sec
D16 0.00020000 sec
IN0 0.00001790 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
PL1 -3.00 dB
SFO1 500.1724798 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 7.95 usec
PL2 3.00 dB
SFO2 125.7803544 MHz

====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7804 MHz
FIDRES 218.226257 Hz
SW 222.077 ppm
FnMODE QF

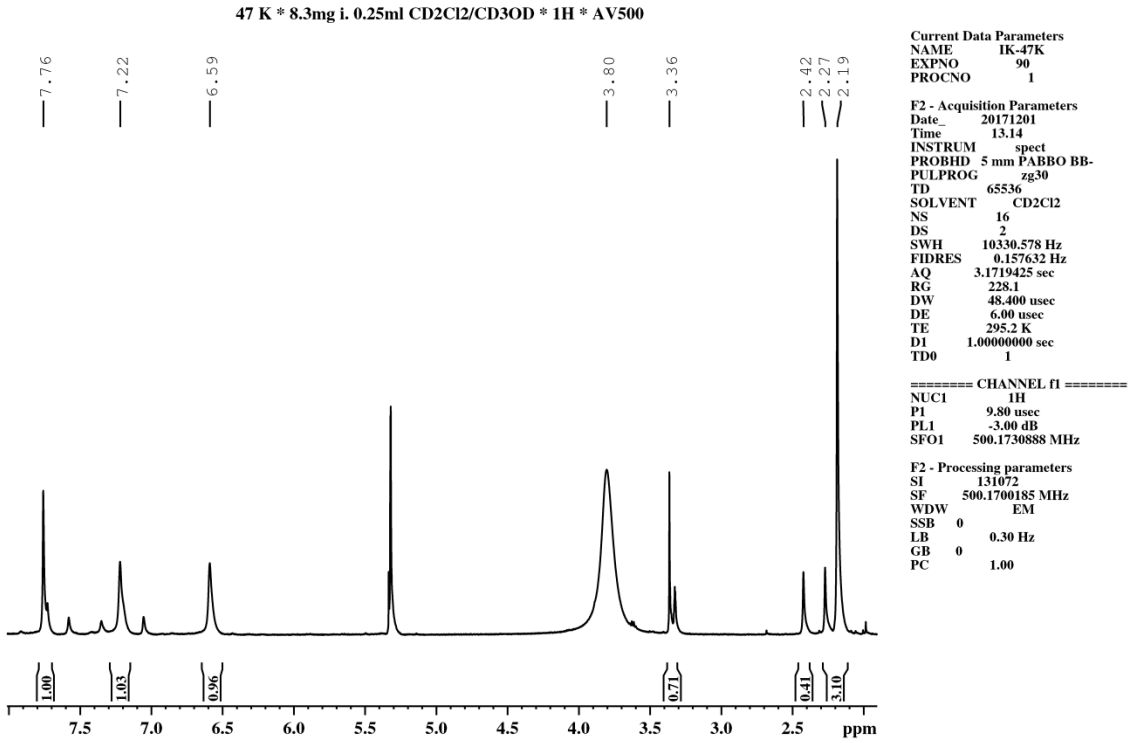
F2 - Processing parameters
SI 8192
SF 500.1700185 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 125.7677737 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0

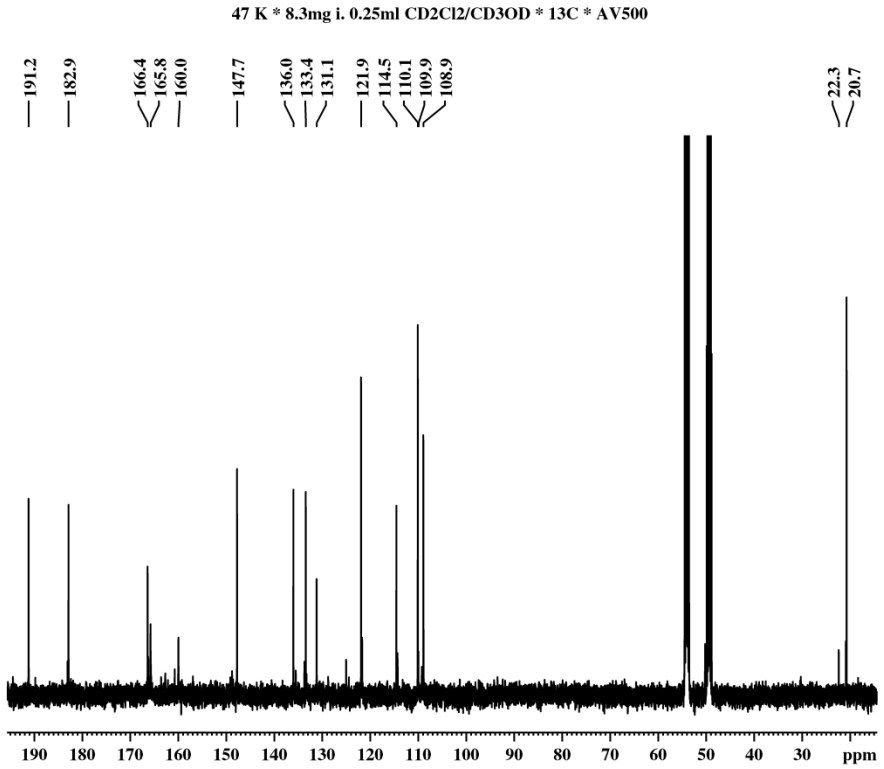
APPENDIX N

SPECTRUM FOR COMPOUND 98

^{1}H NMR SPECTRUM FOR COMPOUND 98 (CD_2Cl_2/CD_3OD, 500 MHz)



^{13}C NMR SPECTRUM FOR COMPOUND 98 (CD_2Cl_2/CD_3OD, 125 MHz)



Current Data Parameters
NAME IK-47K
EXPNO 94
PROCNO 1

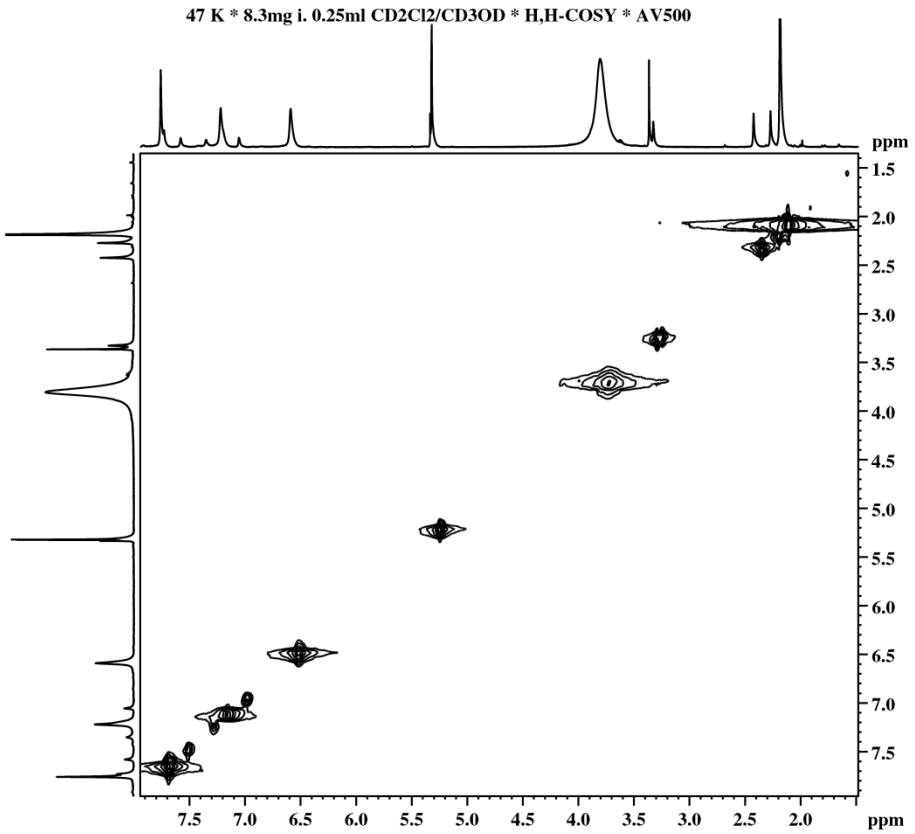
F2 - Acquisition Parameters
Date_ 20171203
Time 2.54
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT CD2Cl2
NS 10240
DS 4
SWH 30030.029 Hz
FIDRES 0.458222 Hz
AQ 1.0911744 sec
RG 181
DW 16.650 usec
DE 6.00 usec
TE 295.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 7.38 usec
PL1 3.00 dB
SFO1 125.7804233 MHz

======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 70.00 usec
PL2 -3.00 dB
PL12 14.21 dB
PL13 17.30 dB
SFO2 500.1720007 MHz

F2 - Processing parameters
SI 65536
SF 125.7677709 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

HH-COSSY SPECTRUM FOR COMPOUND 98 (CD_2Cl_2/CD_3OD, 500 MHz)



Current Data Parameters
NAME IK-47K
EXPNO 91
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171202
Time 15.13
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygpqf
TD 2048
SOLVENT CD2Cl2
NS 4
DS 8
SWH 6510.417 Hz
FIDRES 3.178914 Hz
AQ 0.1572864 sec
RG 114
DW 76.800 usec
DE 6.00 usec
TE 295.2 K
D0 0.00000300 sec
D1 1.48033798 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00015360 sec

======== CHANNEL f1 ========
NUC1 1H
P0 9.80 usec
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1734605 MHz

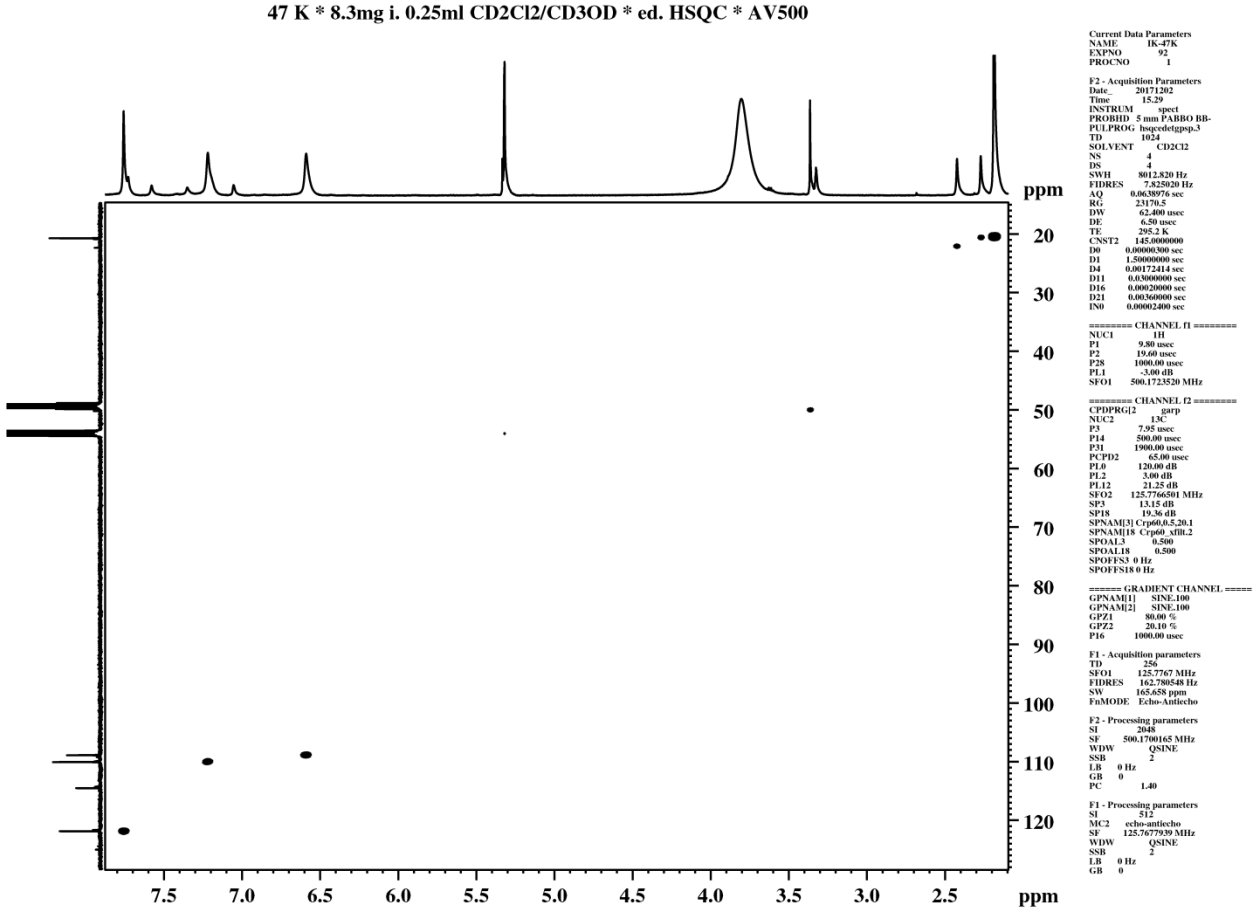
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 500.1735 MHz
FIDRES 101.725258 Hz
SW 13.016 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 500.1700547 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 500.1700547 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

HSQC SPECTRUM FOR COMPOUND 111 (CD_2Cl_2/CD_3OD, 500 MHz)



Current Data Parameters
NAME IK-47K
EXPNO 92
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171202
Time 15.29
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT CD2Cl2
NS 4
DS 4
SWH 8012.820 Hz
FIDRES 7.825920 Hz
AQ 0.0638976 sec
RG 23170.5
DW 62.400 usec
DE 6.50 usec
TE 295.2 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
P31 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfil0.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

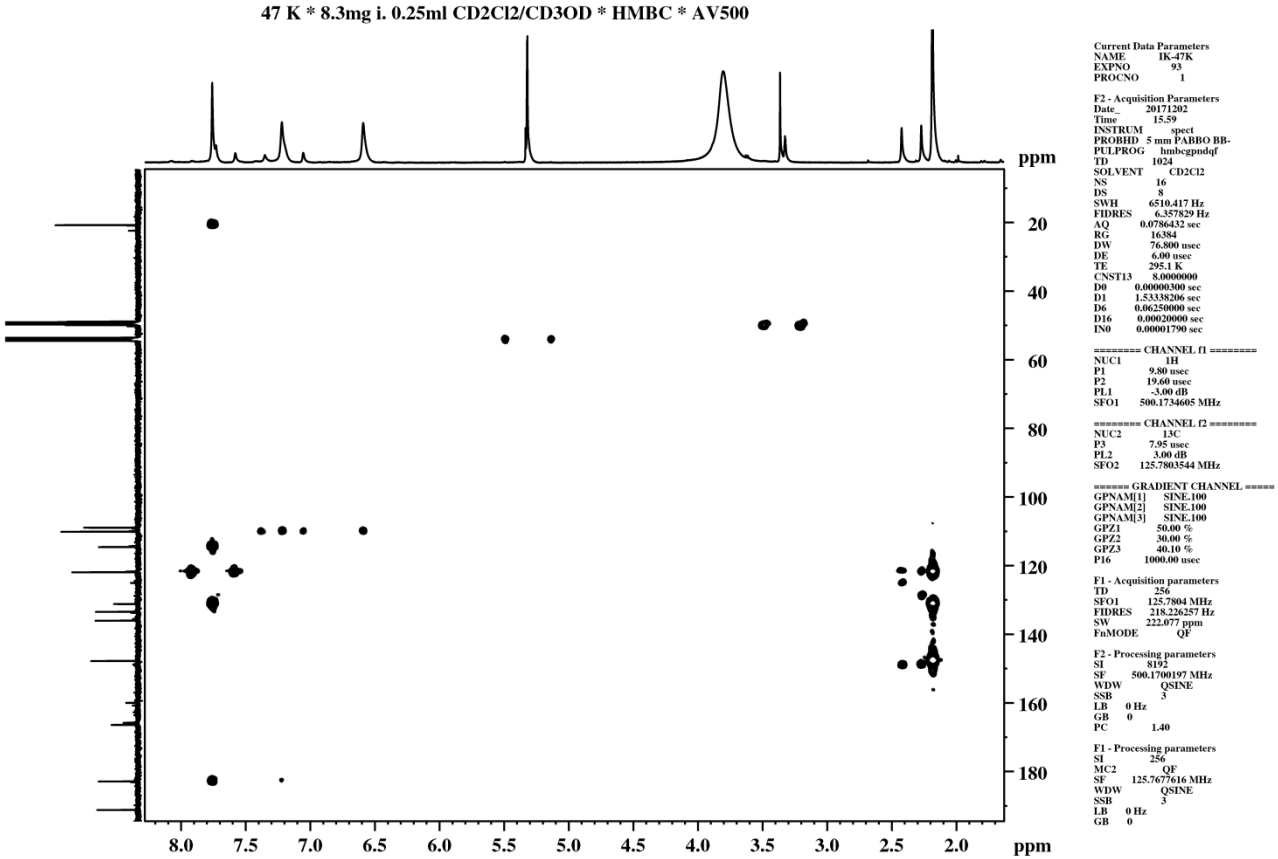
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1700165 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677939 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

HMBC SPECTRUM FOR COMPOUND 98 (CD_2Cl_2/CD_3OD, 500 MHz)



Current Data Parameters
NAME IK-47K
EXPNO 93
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171202
Time 15.59
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hmbcgpndqf
TD 1024
SOLVENT CD2Cl2
NS 16
DS 8
SWH 6510.417 Hz
FIDRES 6.357829 Hz
AQ 0.0786432 sec
RG 16384
DW 76.800 usec
DE 6.00 usec
TE 295.1 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.53338206 sec
D6 0.06250000 sec
D16 0.00020000 sec
IN0 0.00001790 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
PL1 -3.00 dB
SFO1 500.1734605 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 7.95 usec
PL2 3.00 dB
SFO2 125.7803544 MHz

====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7804 MHz
FIDRES 218.226257 Hz
SW 222.077 ppm
FnMODE QF

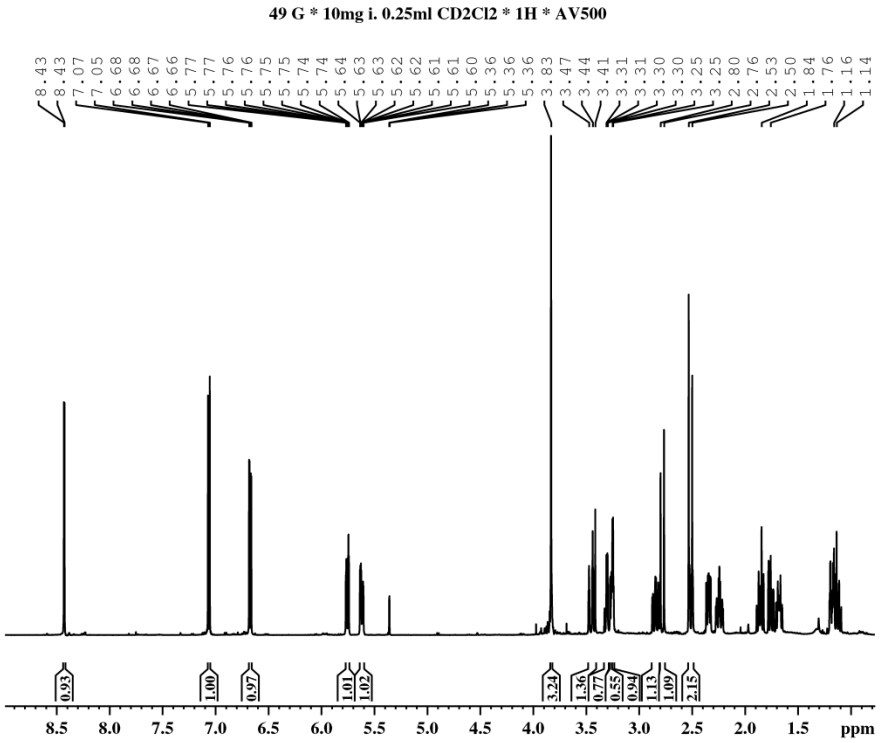
F2 - Processing parameters
SI 8192
SF 500.1700197 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 125.7677616 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0

APPENDIX O

SPECTRUM FOR COMPOUND 99

^{1}H NMR SPECTRUM FOR COMPOUND 99 (CD_2Cl_2, 500 MHz)



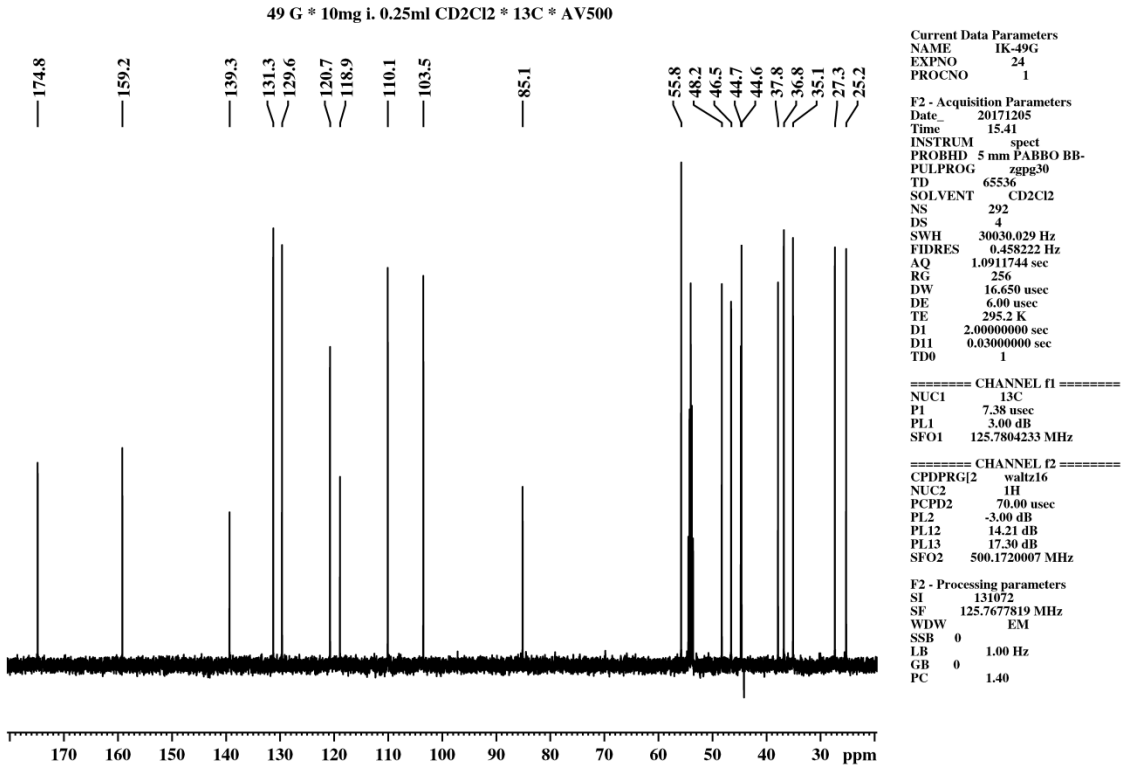
Current Data Parameters
NAME IK-49G
EXPNO 20
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171205
Time 11.10
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CD2Cl2
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 90.5
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

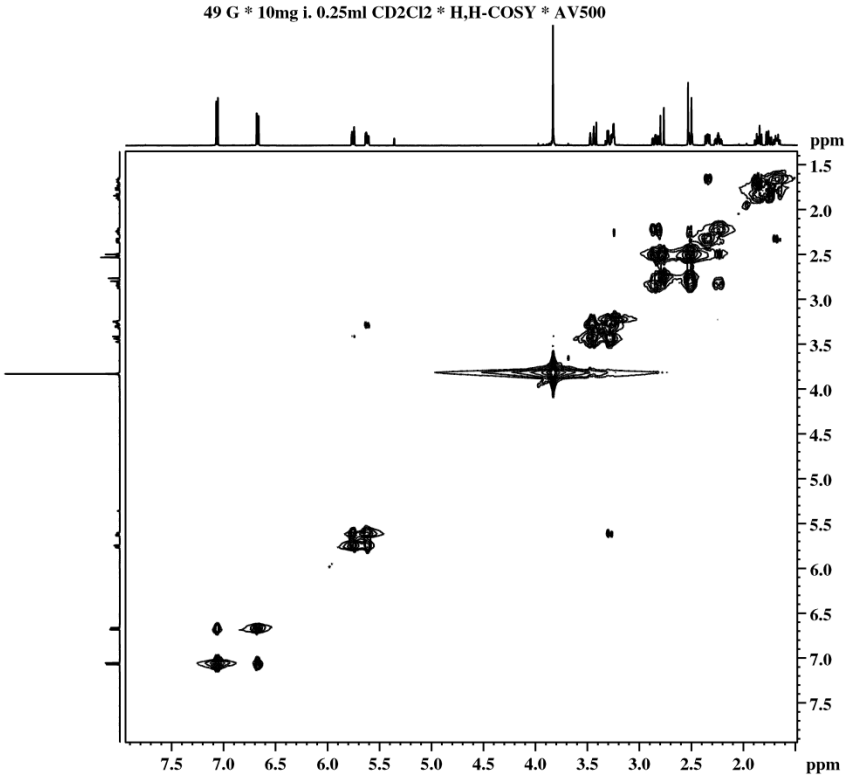
======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

F2 - Processing parameters
SI 131072
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

^{13}C NMR SPECTRUM FOR COMPOUND 99 (CD_2Cl_2, 125 MHz)



HH-COSSY SPECTRUM FOR COMPOUND 99 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-49G
EXPNO 21
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171205
Time 12.48
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygpqf
TD 2048
SOLVENT CD2Cl2
NS 4
DS 8
SWH 4699.248 Hz
FIDRES 2.294555 Hz
AQ 0.2179072 sec
RG 35.9
DW 106.400 usec
DE 6.00 usec
TE 295.2 K
D0 0.00000300 sec
D1 1.42094600 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00021280 sec

======== CHANNEL f1 ========
NUC1 1H
P0 9.80 usec
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1721896 MHz

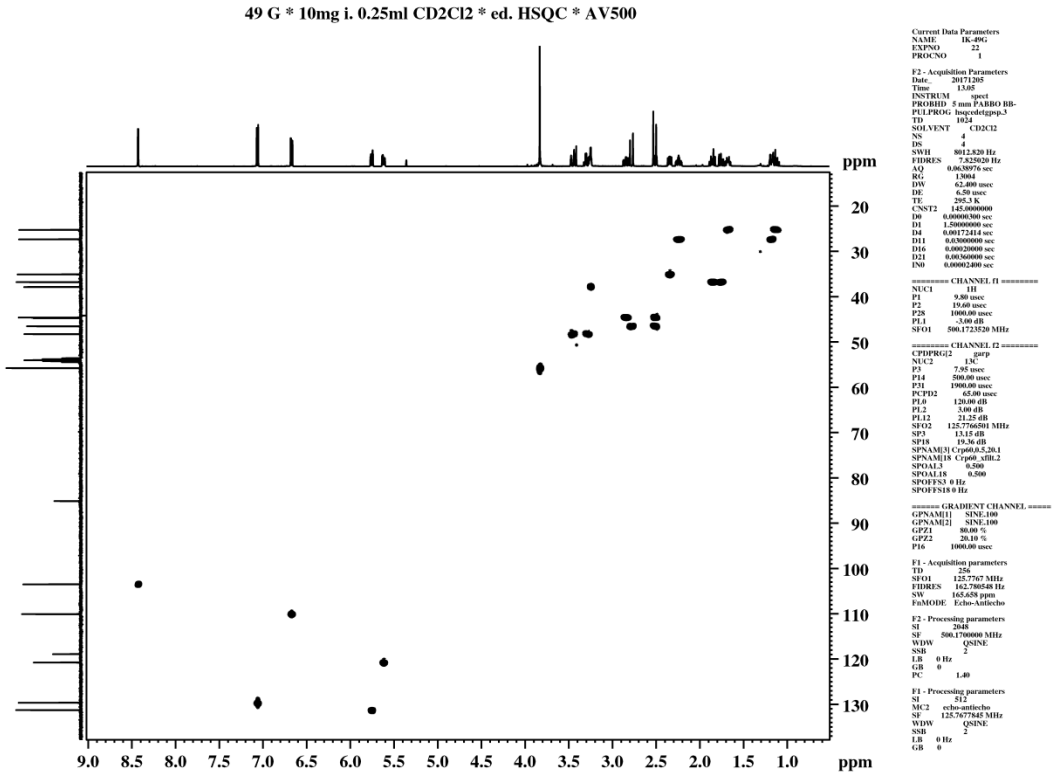
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 500.1722 MHz
FIDRES 73.425751 Hz
SW 9.395 ppm
FnMODE QF

F2 - Processing parameters
SI 2048
SF 500.1700000 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 500.1700000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

HSQC SPECTRUM FOR COMPOUND 99 (CD_2Cl_2, 500 MHz)



Current Data Parameters
NAME IK-49G
EXPNO 22
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171205
Time 13.05
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hsqcedetgpsp.3
TD 1024
SOLVENT CD2Cl2
NS 4
DS 4
SWH 8012.820 Hz
FIDRES 7.825020 Hz
AQ 0.063897 6 sec
RG 13004
DW 62.400 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
D0 0.00000300 sec
D1 1.50000000 sec
D4 0.00172414 sec
D11 0.03000000 sec
D16 0.00020000 sec
D21 0.00360000 sec
IN0 0.00002400 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
P28 1000.00 usec
PL1 -3.00 dB
SFO1 500.1723520 MHz

======== CHANNEL f2 ========
CPDPRG[2 garp
NUC2 13C
P3 7.95 usec
P14 500.00 usec
PJ1 1900.00 usec
PCPD2 65.00 usec
PL0 120.00 dB
PL2 3.00 dB
PL12 21.25 dB
SFO2 125.7766501 MHz
SP3 13.15 dB
SP18 19.36 dB
SPNAM[3] Crp60,0.5,20.1
SPNAM[18 Crp60_xfilt.2
SPOAL3 0.500
SPOAL18 0.500
SPOFFS3 0 Hz
SPOFFS18 0 Hz

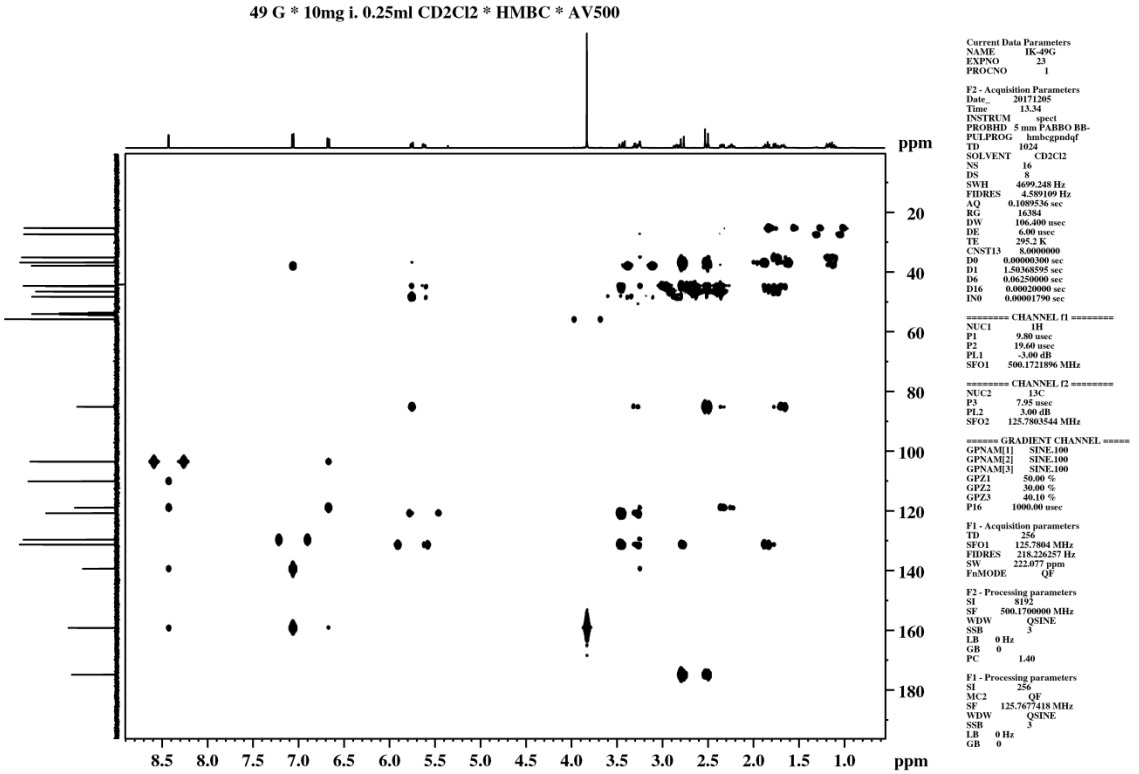
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPZ1 80.00 %
GPZ2 20.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7767 MHz
FIDRES 162.780548 Hz
SW 165.658 ppm
FnMODE Echo-Antiecho

F2 - Processing parameters
SI 2048
SF 500.1700000 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 512
MC2 echo-antiecho
SF 125.7677845 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

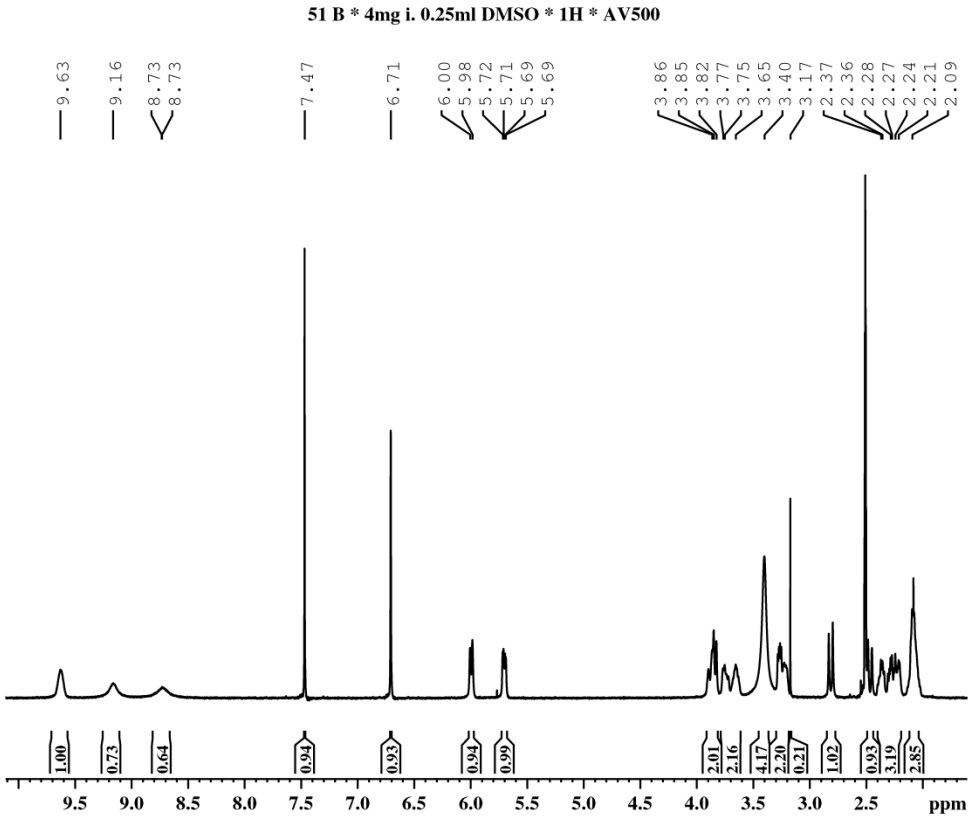
HMBC SPECTRUM FOR COMPOUND 99 (CD_2Cl_2, 500 MHz)



APPENDIX P

SPECTRUM FOR COMPOUND 110

^{1}H NMR SPECTRUM FOR COMPOUND 110 (DMSO, 500 MHz)



Current Data Parameters
NAME IK-51B
EXPNO 30
PROCNO 1

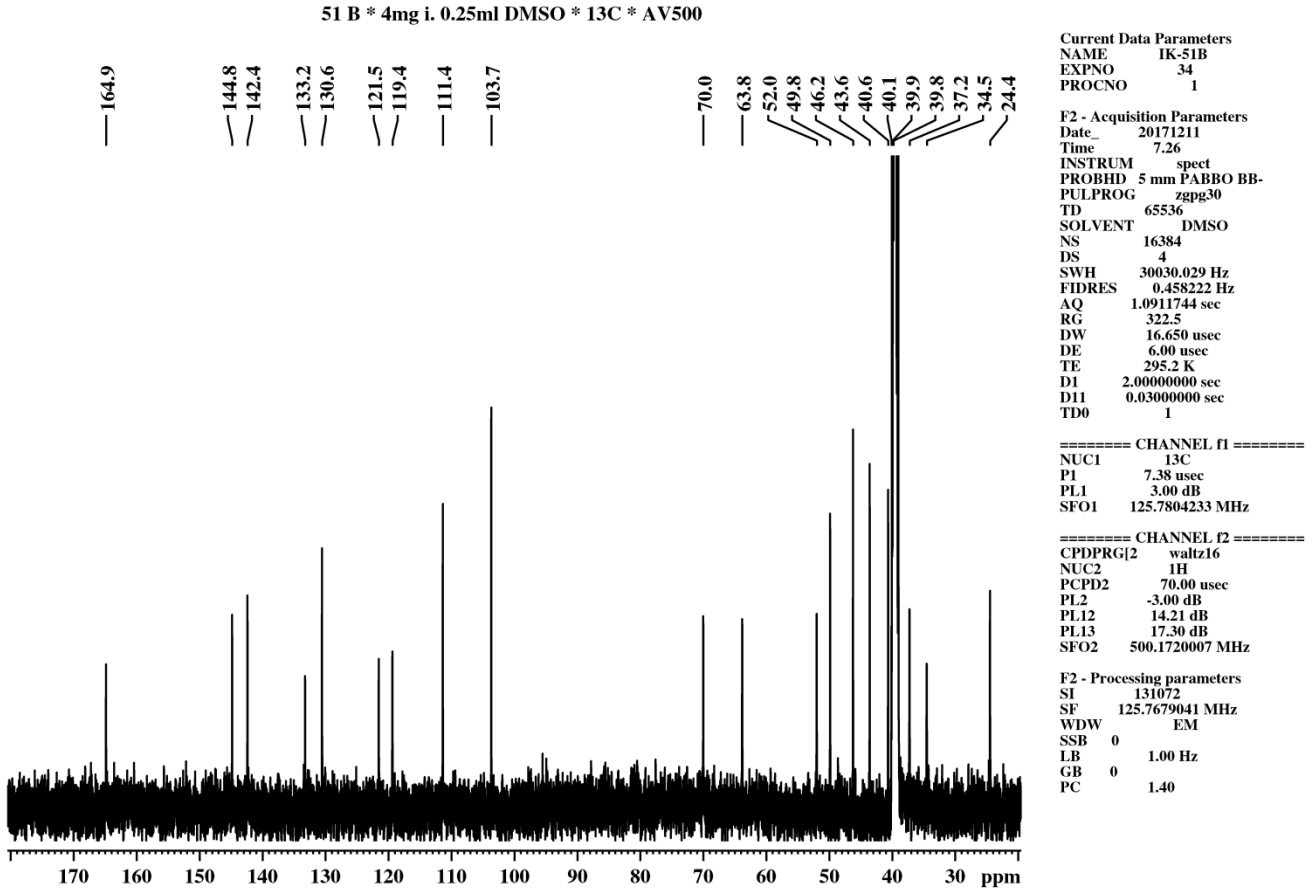
F2 - Acquisition Parameters
Date_ 20171206
Time 16.02
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 362
DW 48.400 usec
DE 6.00 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1730888 MHz

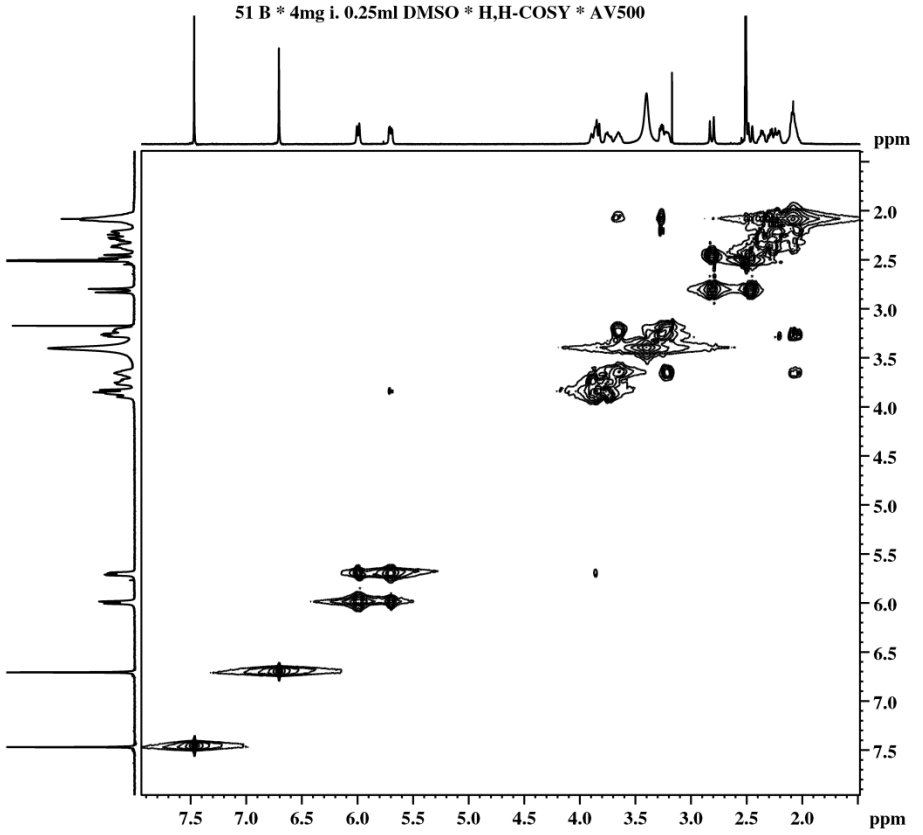
F2 - Processing parameters
SI 131072
SF 500.1700000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

\

^{13}C NMR SPECTRUM FOR COMPOUND 110 (DMSO, 500 MHz)



HH-COSSY SPECTRUM FOR COMPOUND 110 (DMSO, 500 MHz)



Current Data Parameters
NAME IK-51B
EXPNO 31
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171207
Time 7.59
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG cosygpqf
TD 2048
SOLVENT DMSO
NS 4
DS 8
SWH 4432.624 Hz
FIDRES 2.164367 Hz
AQ 0.2310144 sec
RG 128
DW 112.800 usec
DE 6.00 usec
TE 295.2 K
D0 0.00000300 sec
D1 1.40661001 sec
D13 0.00000400 sec
D16 0.00020000 sec
IN0 0.00022560 sec

======== CHANNEL f1 ========
NUC1 1H
P0 9.80 usec
P1 9.80 usec
PL1 -3.00 dB
SFO1 500.1729115 MHz

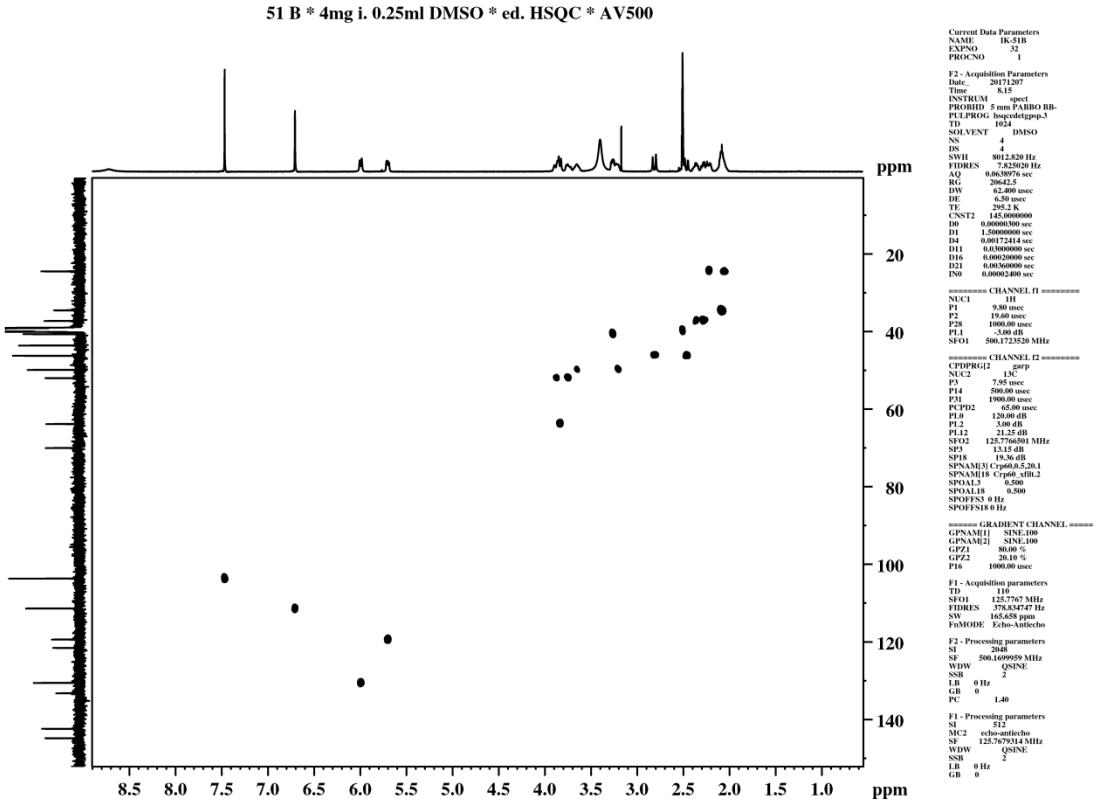
====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPZ1 10.00 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 128
SFO1 500.1729 MHz
FIDRES 69.259750 Hz
SW 8.862 ppm
FnMODE QF

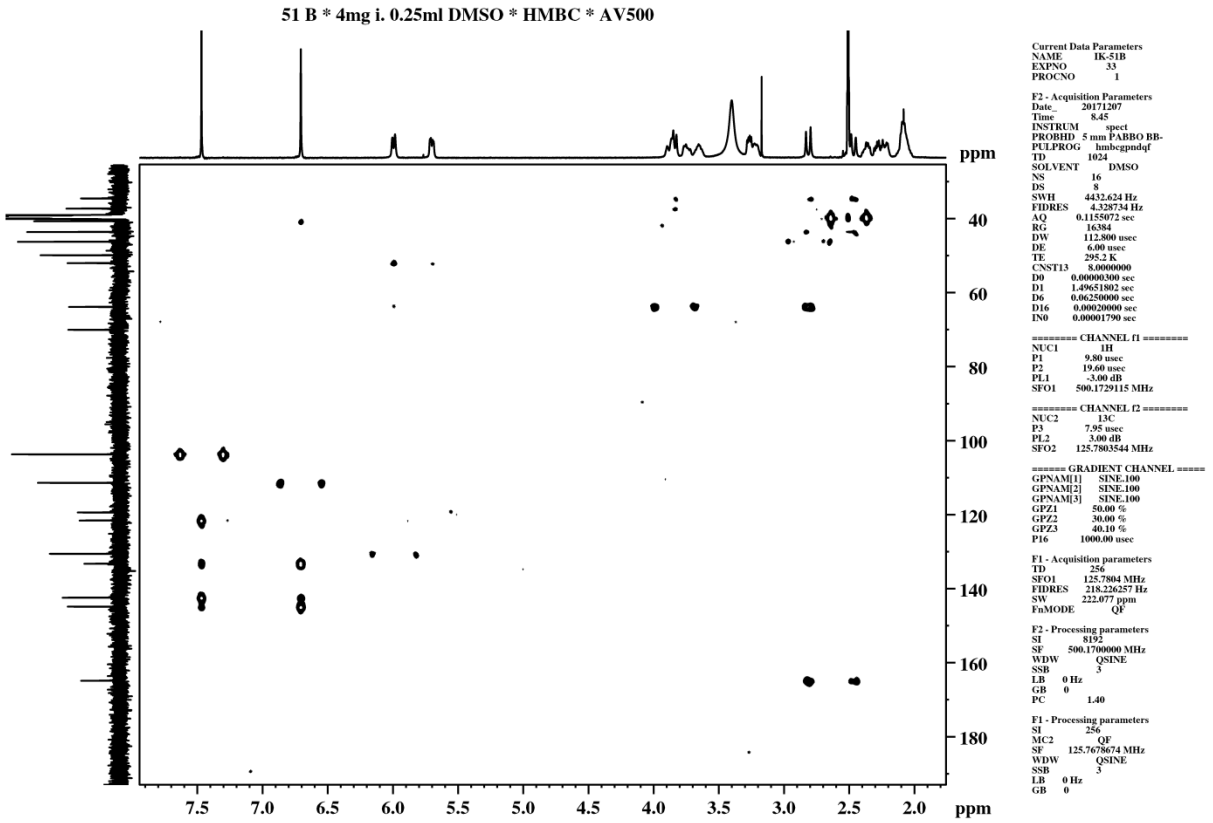
F2 - Processing parameters
SI 2048
SF 500.1700000 MHz
WDW EM
SSB 0
LB 5.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 500.1700000 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0

HSQC SPECTRUM FOR COMPOUND 110 (DMSO, 500 MHz)



HMBC SPECTRUM FOR COMPOUND 110 (DMSO, 500 MHz)



Current Data Parameters
NAME IK-51B
EXPNO 33
PROCNO 1

F2 - Acquisition Parameters
Date_ 20171207
Time 8.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG hmbcgpndqf
TD 1024
SOLVENT DMSO
NS 16
DS 8
SWH 4432.624 Hz
FIDRES 4.328734 Hz
AQ 0.1155072 sec
RG 16384
DW 112.800 usec
DE 6.00 usec
TE 295.2 K
CNST13 8.0000000
D0 0.00000300 sec
D1 1.49651802 sec
D6 0.06250000 sec
D16 0.00020000 sec
IN0 0.00001790 sec

======== CHANNEL f1 ========
NUC1 1H
P1 9.80 usec
P2 19.60 usec
PL1 3.00 dB
SFO1 500.1729115 MHz

======== CHANNEL f2 ========
NUC2 13C
P3 7.95 usec
PL2 3.00 dB
SFO2 125.7803544 MHz

====== GRADIENT CHANNEL =====
GPNAM[1] SINE.100
GPNAM[2] SINE.100
GPNAM[3] SINE.100
GPZ1 50.00 %
GPZ2 30.00 %
GPZ3 40.10 %
P16 1000.00 usec

F1 - Acquisition parameters
TD 256
SFO1 125.7804 MHz
FIDRES 218.226257 Hz
SW 222.077 ppm
FnMODE QF

F2 - Processing parameters
SI 8192
SF 500.1700000 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 256
MC2 QF
SF 125.7678674 MHz
WDW QSINE
SSB 3
LB 0 Hz
GB 0