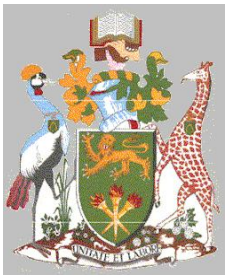

UNIVERSITY OF NAIROBI

PHYTOCHEMICAL INVESTIGATION OF *ZANTHOXYLUM GILLETII* (RUTACEAE) FOR ANTIPLASMODIAL BIOMOLECULES

BY

WAFULA ROBERT G. MASINDE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE
OF MASTER OF SCIENCE IN CHEMISTRY OF THE
UNIVERSITY OF NAIROBI

2014

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. The findings of other researchers has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements.

6/11/2014

WAFULA ROBERT G.

MASINDE

I56/70558/2011

This thesis is submitted for examination with our approval as the University supervisors

6/11/2014

DR. LEONIDAH K. OMOSA.
DEPARTMENT OF CHEMISTRY
UNIVERSTY OF NAIROBI

11/11/2014

PROF. JACOB O. MIDIWO
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

12/11/2014

PROF. ABIY YENESEW
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

DEDICATION

THIS THESIS IS DEDICATED TO MY DEAR WIFE PAMELA WAFULA, MY DAUGHTER NICOLE NELIMA, MY SON RODNEY SIMIYU AND MY SIBLINGS

ACKNOWLEDGMENTS

I am indebted to my supervisors Dr. Leonidah K. Omosa, Professor Jacob O. Midiwo and Professor Abiy Yenesew for their tireless guidance, moral support and for their motherly and fatherly touch during my entire research period.

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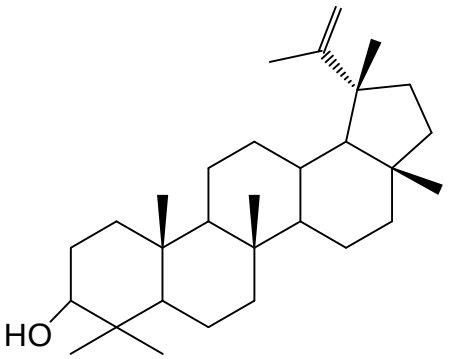
ABSTRACT

Currently, the problem of resistance posed by the malaria causing parasites to the available drugs, demands for collective scientific research to counter the recurrent problem. In addition to this, the malaria vector has also become resistant to the current pesticides of choice putting the lives of people especially those living in Saharan and sub-Saharan Africa highly at risk. In this study the stem bark of *Zanthoxylum gilletii* was air dried and pulverized into fine powder. The plant material was extracted using 50% methanol in dichloromethane and the extract subsequently subjected to column chromatography (CC) using silica gel as the stationary matrix and different solvents systems of varying polarities. The fractions obtained from the main column were purified by further CC using both silica gel and Sephadex LH 20 and crystallization yielding a total of six compounds including; three benzophenanthridine alkaloids; dihydrochelerythrine **(64),** 8-acetonyldihydrochelerythrine **(66)**, norchelerythrine **(67)**, one terpenoid, lupeol **(63),** one lignin, seasamine **(65)** and an amide, fagaramide (**68).** The pure compounds obtained were characterized using spectroscopic techniques.

The crude extract and the isolated compounds from this plant were evaluated for anti-plasmodial activities against three strains of *Plasmodium falciparum* namely; chloroquine-sensitive Sierra Leone (D6) and chloroquine-resistant Indochina (W2) and artemisinin resistant strain (3D7). The crude extract exhibited good anti-plasmodial activities with IC_{50} values of 2.52, 1.48 and 1.43 µg/ml against W2, D6 and 3D7, respectively. Three pure compounds tested which were isolated in sufficient yields also exhibited interesting activities against the three strains of *Plasmodium falciparum*. Seasamine **(65)** showed good activities with IC_{50} values of 1.92, 3.23 and 2.94 µg/ml while 8-acetonyldihydrochelerythrine (**66**) showed moderate activities with IC_{50} values of 4.02, 4.06 and 3.37 µg/ ml against the W2, D6 and 3D7 strains, respectively. Fagaramide (**68**) was inactive, exhibiting IC_{50} values of 15.15, 7.73 and 7.72 µg/ml against W2, D6 and 3D7, *Plasmodium* strains, respectively.



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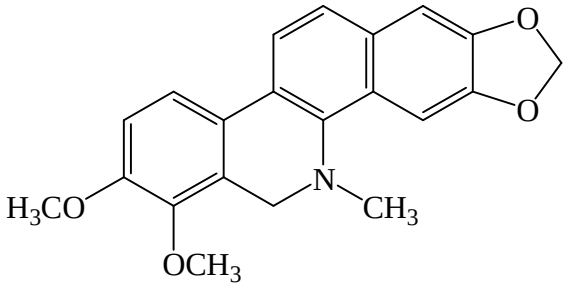

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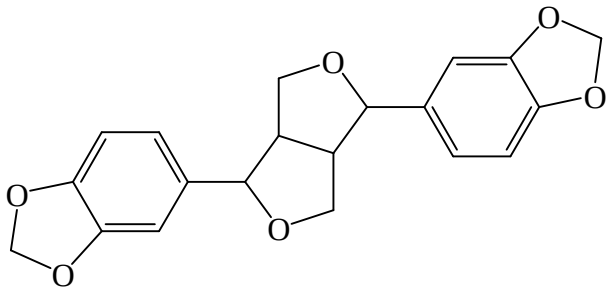

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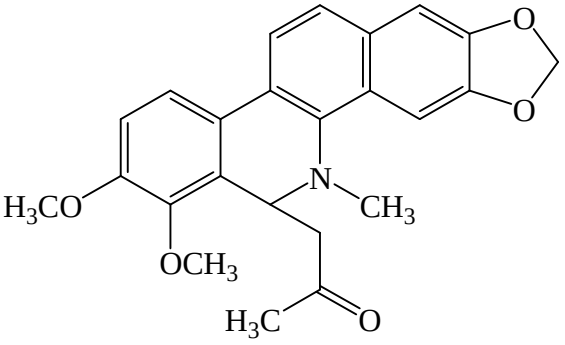

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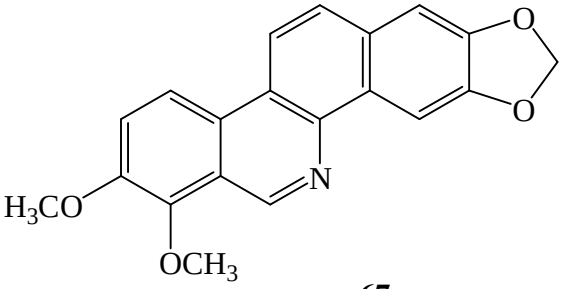

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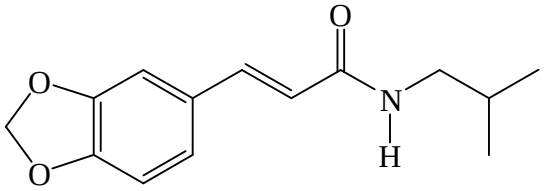


68

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

CC	Column Chromatography
$CDCl_3$	Deutrated trichloromethane
COSY	Correlation Spectroscopy
DMSO	Dimethylsulfoxide
DNP	Dictionary of Natural Product
GC/MS	Gas Chromatography/Mass Spectrometry
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HBV	Hepatitis B Virus
HSQC	Heteronuclear Single Quantum Correlation
MIC	Minimum Inhibition Concentration
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
PTLC	Preparative Thin Layer Chromatography

LIST OF APPENDICES

CHAPTER ONE
INTRODUCTION

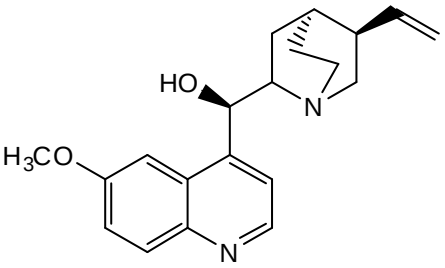
1.1 Background Information

In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are urgently required today for the treatment of malaria. Nature remains an ever evolving source for compounds of medicinal importance. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times and this emanates from the fact that some of these natural products are biologically synthesized as defense agents against plant pathogens (Kaur *et al*., 2009).

For example, use of *Cinchona succiruba* (Rubiaceae) and the Chinese herb, *Artemisia annua* for the treatment of malaria infection are known for centuries. In many tropical countries, the majority of the populations rely on traditional medical remedies especially from plants for management of different ailments mainly due to limited accessibility and/or affordability of pharmaceutical medicines (Muthaura *et al*., 2007; Gessler *et al*., 1995; Kvist, *et al*., 2006). Furthermore, most of the antimalarial drugs in use today such as quinine (**1**) and artemisinin (**2**) were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Basco *et al*., 1994). The paradigm shift to plant products is attributed partly to their safety and affordability as compared to synthetic ones (Arruda, *et al*., 1992).

Several compounds isolated from nature form a rich source of diverse structures for optimization to obtain improved therapeutics preferably with novel modes of action or chemically different from the drugs in current use (Phillipson and Wright, 1991).

Due to development of resistance of the malarial causitive parasite to most current drugs, many compounds with significant antiplasmodial activities have been isolated from plants and efforts are being made by scientists to develop some of them into future drugs speculated to be more effective and affordable (Kaur *et al*., 2009).



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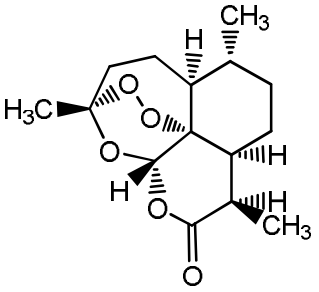

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The plant in study, *Zanthoxylum gilletti* belongs to the genus *Zanthoxylum,* distributed in the tropical and temperate region of the world with history of use for traditional herbal medicine. Previous studies have revealed interesting anti-malarial activities of some plants from this genus including; *Z. acutifolium,* Z. *chabyleum* and Z. *rhoifolium* (Arruda *et al*., 1992; Gessler, *et al*., 1994; Jullian *et al*., 2006). In Kenya there are seven *Zanthoxylum* species, which have been extensively studied for their phytochemistry and various bioactivities. This genus has shown to elaborate mainly; benzophenanthridines, lignans, terpenoids and amides. There is no scientific report on the phytochemistry and the anti-plasmodial activities of the stem bark of *Z. gilletii* and hence the interest to carry out this study.

1.2 Problem Statement

Malaria is a major impediment to socio-economic development in poor countries (Sachs and Malaney, 2002). It is documented that 300 to 660 million clinical malarial attacks occur in the world (Geissbuhler *et al*., 2007), resulting in over a million deaths (Hetzel *et al*., 2007) annually. Close to over 80 % of these deaths occur in the saharan and sub-saharan Africa (Geissbuhler *et al*., 2007). The growing resistance of the malarial parasites to the readily available drugs is pausing a threat to human lives and therefore, the urgent need for continuous efforts in the search for compounds that are active against these parasites, that can be developed into new and more effective drugs (Price and Nonsten, 2001). Economically, malaria is known to be both a disease and a cause of poverty responsible for stunting economic growth with a depreciation of 1.3 % Gross Domestic Product (GDP) per year in some African countries (Sachs and Malaney, 2002). This makes it difficult for people especially in the rural set up living in abject poverty to afford current conventional anti-malarial drugs.

1.3 Objectives

1.3.1 Main Objective

To isolate and identify biologically active compounds against the malarial causing parasite, *P. falciparum* from the stem bark of *Zanthoxylum gilletii.*

1.3.2 Specific objectives

i. To extract, isolate and characterize secondary metabolites from the stem bark of *Z. gilletii*;
ii. To determine the *in vitro* antiplasmodial activities of the stem bark of *Z. gilletii* and
iii. To determine the *in vitro* antiplasmodial activities of the isolated compounds.

1.4 Justification

The genus *Zanthoxylum* has been used traditionally by many communities in Africa to treat a number of ailments including malarial symptom such as fever, venereal infections, stomachache and wound washing. The malarial vector and parasite have shown substantial resistance to the available pesticides and antiplasmodial drugs leading to rigorous scientific search for new templates for development into active pesticides and antiplasmodial drugs respectively. Previous phytochemical studies on different parts of genus *Zanthoxylum* led to the isolation of alkaloids which exhibited strong anti-protozoal activities and hence the motivation to evaluate the anti-plasmodial potential of the stem barks of *Z. gilletii* for diverse structures which could demonstrate interesting activities. There is no phytochemical report of the stem bark of this plant from previous studies.

CHAPTER TWO
LITERATURE REVIEW

2.1 Malaria Problem

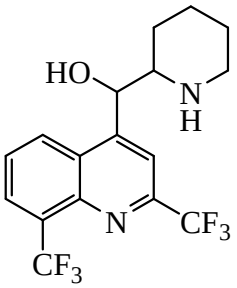
Malaria is a life threatening disease that is predominant in the tropic and the sub-tropic regions. It is caused by blood parasites *Plasmodium falciparum*, *P. ovale*, *P. malariae* and *P. vivax* (Odugbemi, *et al*., 2007) with *P. knowlesi* believed to cause malaria in monkeys in Asia. The parasites are transmitted from one person to another by female *Anopheles* mosquito. The onset of this disease is manifested by a range of symptoms such as fever, vomiting, joint pain and convulsions (Nkuo-Akenji & Menang, 2005). Besides contributing to over a million deaths yearly, malaria is known associated to anemia and its various complications including; miscarriages, brain damage, decreased cognitive abilities and irreversible disabilities (Rugemalila, *et al*., 2006). Economically, malaria has devoured countries and individuals a lot of money and other resources causing abject poverty. In some African countries, it is estimated that a countryôs Gross Domestic Product (GDP) can be reduced by up to 1.3 % due to malaria (Sachs and Malaney, 2002).

Efforts to overcome the malaria problem have been thwarted by two pertinent issues. The first one is the growing resistance of the malaria parasite *P. falciparum* to chloroquine and other commonly available synthetic drugs, as well as the growing resistance of the vector *Anopheles* to DDT and other insecticides (Bilia, 2006). The second obstacle is the limited availability of resources, coupled with higher cost and greater toxicity of alternative drugs (Saidu *et al*., 2000). Kenya, like other tropical countries, has faced the consequences of resistance of malaria parasites especially *P. falciparum* to readily available drugs like chloroquine, mefloquine and amodiaquine (WHO, 2012). Plants through their natural products have had a great contribution in the fight against malaria for along time. In Kenya and other developing countries, evidence of the use of herbal remedies in the treatment of malaria as well as other infectious diseases is available in a number of literature data (Krungkrai *et al*., 2010).

2.2 Plants and Malarial Chemotherapy

Most of the anti-malarial drugs (80 %) originate from plants. Many communities in Kenya use traditional approach in the treatment of malaria and other diseases (Koch *et al*., 2005; Kirira *et al*., 2006; Muthaura *et al*., 2007; Muthaura, *et al*., 2007; Muregi *et al*., 2003). Natural products have

been used either directly or as templates for the development of synthetic drugs. Almost all anti-malarial drugs are derived from plants including: quinine (**1**) and artemisinin (**2**) together with their analogues; mefloquine (**3**), pamaquine (**4**), pentaquine (**5**), halofantrine (**6**) and artemether (**7**) (Krungkrai *et al*., 2010).



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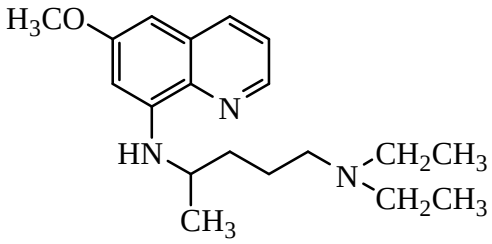

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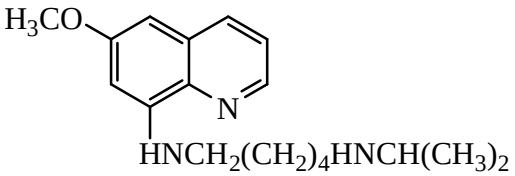

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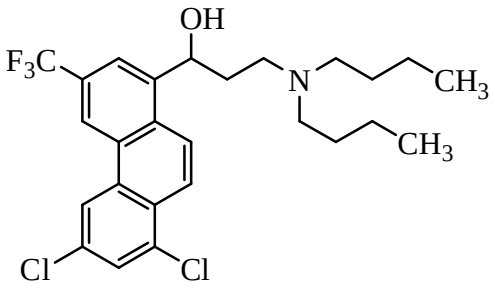

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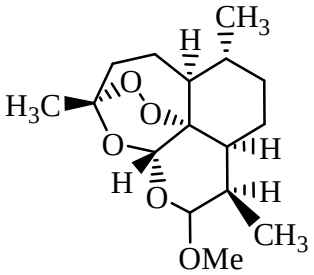

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2.3 The Genus *Zanthoxylum*

The genus *Zanthoxylum* belongs to the family Rutaceae, subfamily Rutoideae in the tribe Zanthoxyleae containing only two taxa namely; *Fagara* L and *Zanthoxylum.* The genus *Fagara* L encompasses over 240 species, while *Zanthoxylum* has only 15 species (Fish and Waterman, 1973). *Fagara* and *Zanthoxylum* have been merged into *Zanthoxylum* after thorough research by Brizicky in 1962 (Fish and Waterman, 1973).

2.3.1 Distribution of the genus *Zanthoxylum*

The genus *Zanthoxylum* consists of over 250 species, growing as shrubs or trees and distributed in the tropics, sub tropics and the temperate regions of the world (Negi *et al*., 2011). Kenya is endowed with 7 species found in moist or dry forests or in the thickets near the sea. These species include; *Z. holstzianum (*Engl.) Waterman, *Z. usamarense* (Engl.) Kokwaro, *Z. chalybeum* (Engl.) var, *chalybeum*, *Z. gilletii* (De wild) Waterman, *Z. mildbraedii* (Engl.) Waterman, *Z. paracantum* (mildbr) Kokwaro and *Z. rubescens* Hooks.f (Beentje, 1994).

2.3.2 Ethno botanical importance of the genus *Zanthoxylum*

There are several traditional uses reported for members of the genus *Zanthoxylum*. Some have even served as raw materials in the pharmaceutical and cosmetic industries (Bafi-Yeboa *et al*., 2005). In Africa members of the family Rubiaceae including *Zanthoxylum* species have been used to manage malaria in different countries (Iwu, 1994). Furthermore, the leaves and the root bark of *Zanthoxylum* species have also been used for the treatment of other diseases including; infections causing stomach-aches, tooth-aches, coughs, urinary infections rheumatism, leprous ulcerations and venereal diseases (Negi *et al*., 2011). A summary of some traditional uses of some species in this genus are tabulated in Table 2.1 below.

Table 0.1: Ethno botanical uses of genus *Zanthoxylum*

Species	Plant part used	Medicinal value	References
Z. gilletii	bark	Stomachache, joint pain, toothache, fever, rheumatism, venereal infections and washing wounds.	Kokwaro, 2009
Z. limonela	Fruit oil	Anthelminthic and gastro intestinal stimulant effects. Treatment of wounds and digestion enhancement.	Sati *et al*., 2011; Setzer *et al*., 2005
Z. capense		Pesticide and protozoal activity	Setzer *et al*., 2005;
Z. chalybeum	Leaves Bark	Snake bite and oedema in kwashiorkor. Malaria, colds, coughs and dizziness from decoctions. Chewed for toothaches, asthma, and tuberculosis	Kokwaro, 2009
Z. ailantoides	Leaves	Common cold Rheumatics, arthalgia, stasis, snake bites and blood circulation stimulant.	Cheng *et al*., 2004
Z. armatum	Fruit and seed	Aromatic tonic in fever dyspepsia and cholera	Sati *et al*., 2011
Z. chiloperone	Root bark	Anti-malaria and anti-rheumatism.	Ferreira, 2002
Z. rhoifolium	Bark	Anti-malaria, toothache, venereal chancre, ecto-parasite and digestive properties	Juliana *et al*., 2006
Z. pistaciiflorum	Leaves Bark and fruit	Headache relieve Poisoning fish	Chen *et al*., 2004
Z. usambarense	Leaves (in soup) Fruits, leaves, bark or root	Colds and flu Rheumatism, Malaria	Kokwaro, 2009

	decoction, Bark or fruit (in milk) Roots and bark	Fever, sore throat, tonsillitis and chest pain, coughs	
Z. elephantiasis		Used in the treatment of diarrhea, chest diseases, intermittent fever, ear-aches and tooth diseases	Diequez-Hurtado *et al*., 2003
Z. leprieurii		Used in the treatment of gonorrhea, kidney pain and sterility	Tatsadjieu *et al*., 2003

The above summary of the traditional uses of the genus *Zanthoxylum* requires concerted efforts by natural products scientists towards validating their traditional uses including the use of these plants to manage malaria. This could be achieved by subjecting the constituent compounds of different extracts of *Zanthoxylum* to antiplasmodial assays to establish their anti-malarial potential thus linking modern drug discovery to traditional medicine. Such research shall offer the basis for the discovery of pharmaceutical lead compounds from natural products.

2.3.3 Biological activities of the genus *Zanthoxylum*

Previous studies have shown that plants in the genus *Zanthoxylum* have good biological activities including; larvicidal, analgesics, anthelminthic, anti-viral, antioxidant anti-fungal, antibiotic, and anti-inflammatory and cytotoxicity (Table 2.2).

Table 0.2: Summary of Biological Activities of *Zanthoxylum* species

Species	**Plant part**	**Compound/Extract**	**Activity**	**Reference**
Z. usambarense	Stem bark	Ethanolic/Methanolic extract	Anti-plasmodial	Negi *et al*., 2011; Tatsadjieu *et al*., 2003
Z. tingoassuiba	Not specified	Essential oil	Anti-microbial	,,
Z. tetraspernum	Stem bark	Benzophenanthridine alkaloids	Anti-bacterial	,,
Z. chiloperone	Stem bark	Alkaloid extract	Anti-fungal	,,
Z. americana	Whole plant	extracts	Anti-fungal	,,
Z. rhoifolium	Leaves	Alkyl amides	Anti-tumor	,,

2.4 Phytochemical information of *Zanthoxylum* species

Phytochemical studies carried out on some *Zanthoxylum* species have revealed the presence of alkaloids of various skeletal types, lignans, coumarins amides as common secondary metabolites which also have chemotaxonomic importance to the genus. Other metabolites such as flavonoids, sterols and terpenes have also been isolated from plants from this genus (Waterman and Grundon, 1983; Adesina, 2005).

2.4.1 Alkaloids from the genus *Zanthoxylum*

Different classes of alkaloids, which are nitrogen containing compounds with low molecular weight, have previously been isolated from different plant parts of *Zanthoxylu*m species. Approximately 600 different alkaloids have been reported from this genus according to Chapman and Hall (2002) mainly belonging to the following classes; benzophenanthridines, protoberberines, aporphine and tetrahydroprotoberberine, tryptophan-derivatives (canthin-6-one and indoloquinazoline alkaloids) and alkaloids based on anthranilic acid (Waterman, 1990).

2.4.1.1 Benzophenanthridine Alkaloids from the genus *Zanthoxylum*

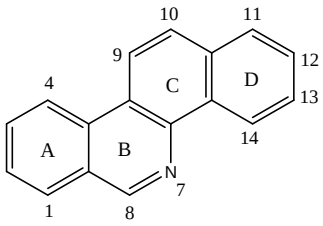
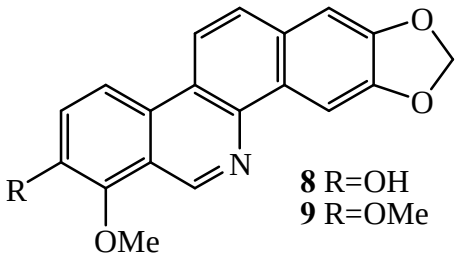


Figure 0.1: Basic structure of Benzophenanthridine

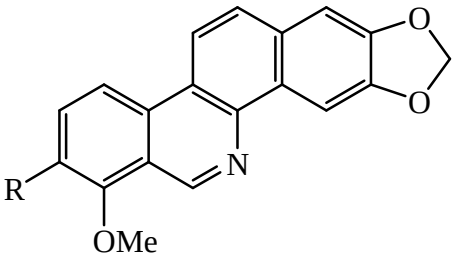
Figure 1 above represents the basic structure of benzophenanthridines. The derivatives of this structure can be oxygenated more often at either C-1 and C-2 or at C-3 and C-4 positions on ring A and rarely at C-8 position, while the formation of a methylenedioxy is prevalent on C-12 and C-13 on ring D as well as C-1, 2, 3 and C-4 positions on ring A. *N*-methyl is common at C-7 position. The Table below summarizes some benzophenanthridines alkaloids that have been isolated from some species from the genus *Zanthoxylum*.

Table 0.3: Some Benzophenanthridine alkaloids from the genus *Zanthoxylum*

Compound	**Plant part**	**Plant source**	**Reference**
Decarine **(8)**	Roots	*Z. tetraspernum*	Mansoor *et al*., 2013
Norchelerythrine **(9)**			
Dihydrochelerythrine **(10)**			
6-acetonyldihydrochelerythrine **(11)**			
Tridecanochelerythrine (**12)**			
8-acetonyldihydronitidine (**13)**	Stem bark		Nissanka *et al*., 2001
8-acetonyldihydroavicine **(14)**		*Z. buesgenii*	,,
Buesgeniine **(15)**		*Z. rhoifolium*	Tane and Connolly, 2005
Zanthoxyline **(16)**			De Moura *et al*., 1997



8 R=OH
9 R=OMe



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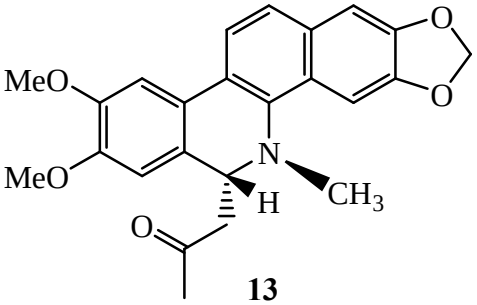
11 $R=CH_2COCH_3$
12 $R=CH_2CO(CH_2)_{10}CH_3$



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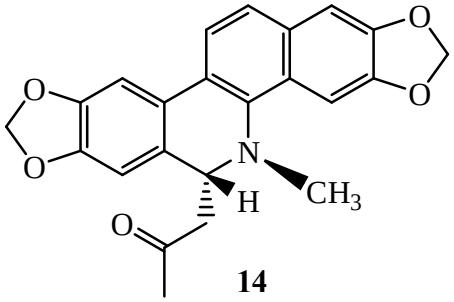

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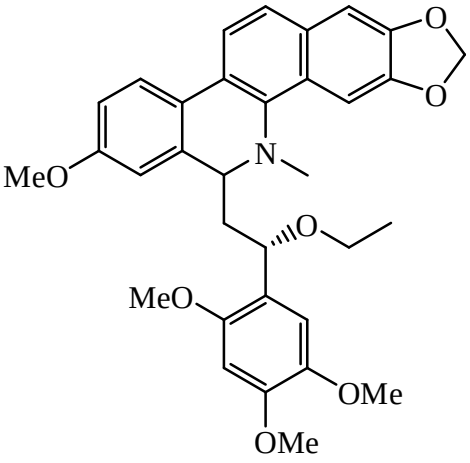

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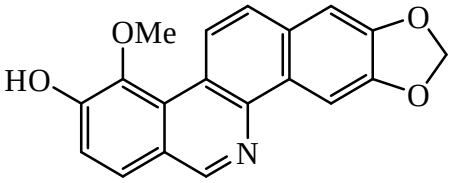

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2.4.1.2 Protoberberine alkaloids from *Zanthoxylum*

Protoberberine alkaloids are biogenetically derived from the tyrosine pathway. 5,6-Dihydrodibenzo (Ŭ,ƀ) quinolizinuim ($C_{17}H_{14}N^+$) is the basic skeleton of the quaternary protoberberine alkaloids (Liscombe *et al*., 2005; Grycová *et al*., 2007). The basic structure of protoberberine alkaloids is shown below in Figure 2.2.

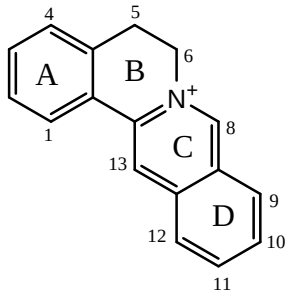
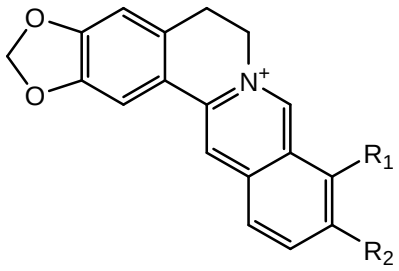


Figure 0.2: The basic structure of Protoberberines

These compounds are distributed in such plant families as Papaveraceae, Berberidaceae, Fumariaceae, Menispermaceae, Ranunculaceae, Rutaceae, Annonaceae, as well as a few examples in Magnoliaceae and Convolvulaceae (Bentley, 1997, 1998 a, b, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006). Berberine is the main alkaloid from these families which can easily be converted to many other protoberberine derivatives. These alkaloids contain a methylenedioxy at C-2, C-3 on ring A and oxygenation usually takes place at C-9, C-10 or at C-10 and C-11 on ring D. Some examples of protoberberine alkaloids are indicated in Table 2.4 below.

Table 0.4: Some Protoberberine alkaloids from the genus *Zanthoxylum*

Compound	**Plant part**	**Plant source**	**Reference**
Berberine **(17)**		*Z. chiloperone*	Ferreira *et al*., 2002
Berberrubine **(18)**		*Z. nitidine*	(Jiang, 2007)
Coptisine**(19)**		,,	,,



17 R_1, R_2 = OCH_3

18 R_1, R_2 = OCH_2O

19 R_1 = OH; R_2 = OCH_3

2.4.1.3 Bishordeninyl terpene from *Zanthoxylum*

Bishordeninyl terpenes have been isolated from this genus as racemates (Thuy *et al*., 1999). A monoterpene constitutes their basic structure and substitution on these compounds takes place at C-3 and C-5.

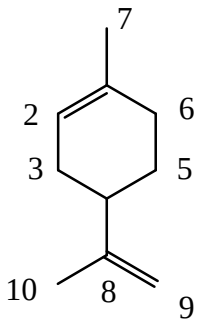
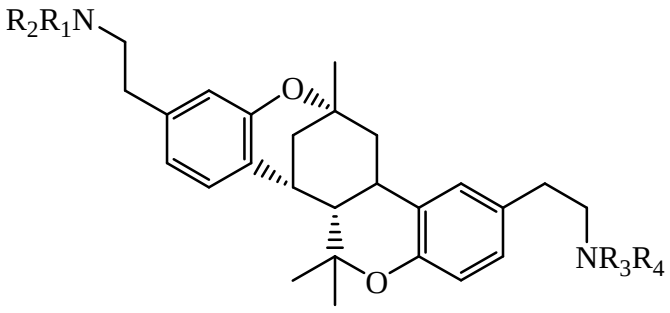


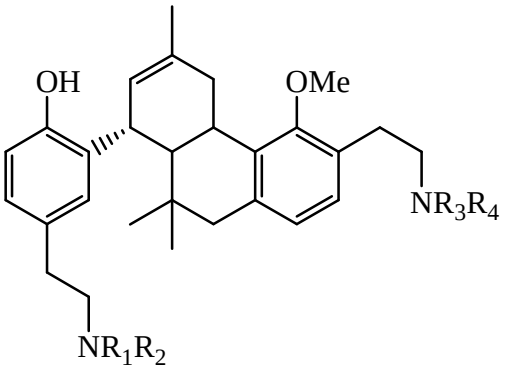
Figure 0.3: The basic structure of bishordeninyl terpene

Table 0.5: Bishordeninyl alkaloids

Compound	**Plant source**	**reference**
N,N´-Demethylisoalfileramine **(20)**	*Z. coriaceum*	Manuel, 1990
Isoalfileramine **(21)**		
N´-Demethylalfileramine **(22)**		
N,N´-Demethylalfileramine **(23)**		
N,N´-Demethylculantraramine **(24)**		
Culantraramine **(25)**		

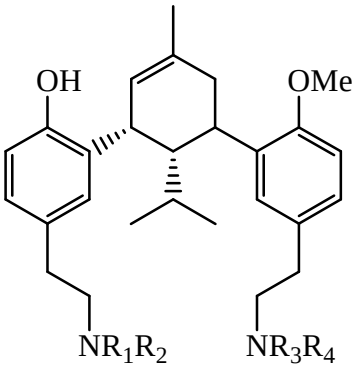


20 R_1, R_3 = H R_2, R_4 = CH_3
21 R_1, R_2, R_3, R_4 = CH_3



22 R_1, R_2, $R_3 = CH_3$; $R_4 = H$

23 R_1, $R_3 = H$, R_2; $R_4 = CH_3$



24 R_1, $R_3 = H$; R_2, $R_4 = CH_3$

25 R_1, R_2, R_3, $R_4 = CH_3$

2.4.1.4 Aporphine alkaloids from the genus *Zanthoxylum*

Aporphine alkaloids belong to the isoquinoline type of alkaloids (Kuo *et al*., 2012). Basically aporphine alkaloids are derived from L-tyrosine (Fig. 5). There were seven aporphine alkaloids isolated and characterized from the genus *Zanthoxylum* by the year 2010 (DNP, 2011). Substitution on the basic structure takes place mainly at C-1 and C-2; positions 3, 8, 9, 10 and 11 can also have substituents which are mainly OCH_3 and OH (Kuo *et al*., 2012). Some aporphine alkaloids isolated from the genus *Zanthoxylum* are summarized below in Table 2.6

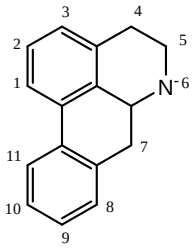
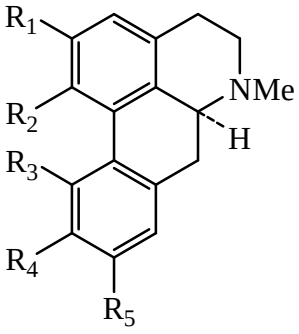


Figure 0.4: The basic skeleton of aporphine

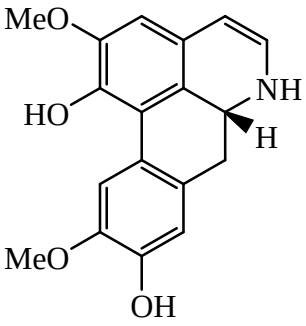
Table 0.6: Aporphine alkaloids of the genus *Zanthoxylum*

Compound	**Plant source and part**	**Reference**
Xanthoplanine (**26**)	*Z. planispium*	(Ishii, 1961)
Laurifoline (**27**)	*Z. elephantiasis*	(Hufford, 1976)
Methylcorydine (**28**)	*Z. nigrescens*	,,
Liriodenin**e** (**29**)	*Z. simulans*	,,
N-acetyldehyderoanonaine (**30**)	*Z. simulans*	(Ih-Sheng *et al*., 1996)
N-Acetylanonaine (**31**)	*Z. bungeanum*	,,



26 R_1, R_4, $R_5 = OCH_3$; $R_2 = OH$; $R_3 = H$

28 R_1, R_3, $R_4 = OCH_3$; $R_2 = OH$; $R_5 = H$



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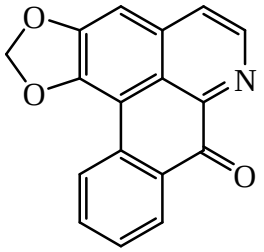

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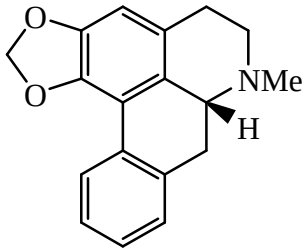

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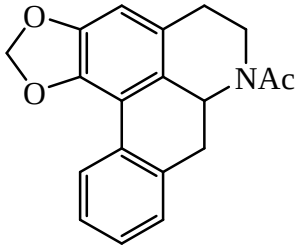


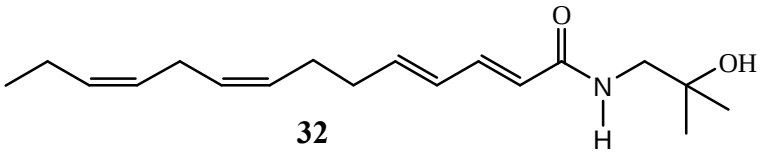
31

2.4.1.5 Amide alkaloids from the genus *Zanthoxylum*

Organic amides are secondary metabolites that are mostly aliphatic and in rare occasions aromatic ones can be isolated. They are characterized by their pungent taste and fragrance (Kashiwanda *et al*., 1997). Alkamides are a broad and expanding group of bioactive natural compounds found in at least 33 plant families. Despite the relatively simple molecular architecture of alkamides, these natural products show broad structural variability and a range of important biological activities, such as antimicrobial, antiviral, larvicidal, insecticidal, diuretic, analgesic and antioxidant activities. Many plant species containing alkamides have been used in traditional medicine by different civilizations around the world (Rios-Chavez *et al*., 2003). The table below summarizes some of the amides characterized from the genus *Zanthoxylum* in Table 2.7.

Table 0.7: Amides Alkaloids from the genus *Zanthoxylum*

Compound	**Plant source**	**Reference**
Bungeanool (32)	*Z. bungeanum* (pericarp)	Quanbo *et al*., 1997
Tetrahydrobungeanool (33)		
Dehydro-ɔ-sanshool (34)		
Isobungeanool (35)		
Dihydrobungeanool (36)		
Herclvine (37)	*Zanthoxylum ssp* (stem bark)	Kashiwanda *et al*., 1997
Hydroxy-sanshool (38)	*Zanthoxylum ssp* (fruit)	
(2E,4E,8E,10E,12E)-N-isobutyl-2,4,810,12-tetradecapentaenamide (39)		
Ŭ-Sanshool (40)		
Lanyuamide (41)	*Z. integrifoliolum* (stem bark)	Sheng *et al*., 1999
3-Methoxyaegeline (42)	*Z. syncarpum* (leaves)	Ross *et al*., 2005
O-Methyl tembamide (43)	*Z. ailantoides* (root bark)	Cheng *et al*., 2005
(+)-Tembamide (44)		



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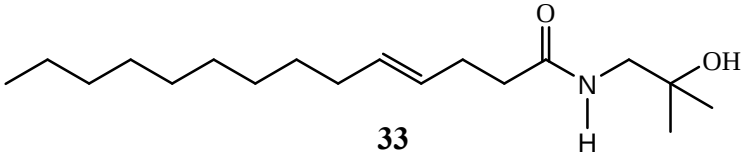

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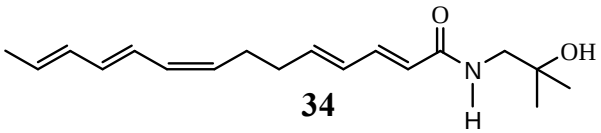

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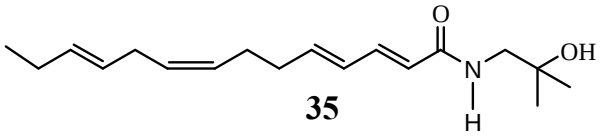

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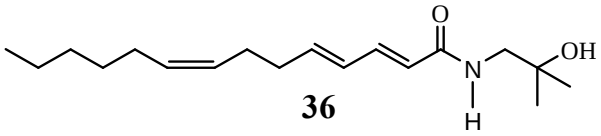

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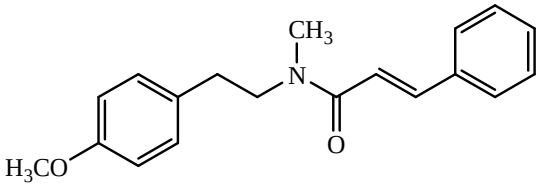

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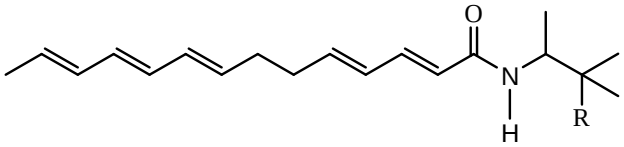
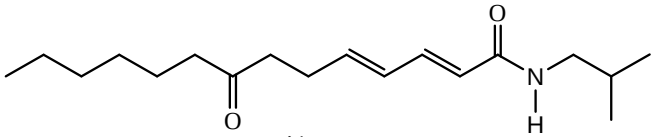


38 R = OH, 2E,4E,8E,10E,12E

39 R = OH, 2E,4E,8Z,10E,12E



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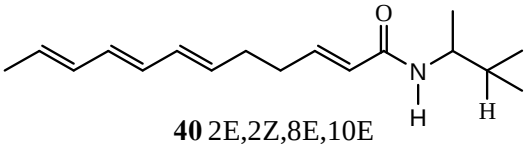
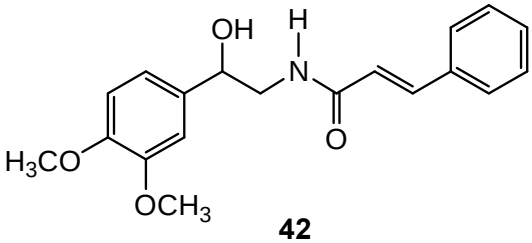


40 2E,2Z,8E,10E



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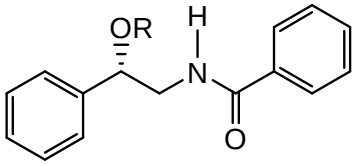


43 R = CH_3

44 R = H

2.4.2 Coumarins from the genus *Zanthoxylum*

Coumarins have a C_6-C_3 skeleton (Fig. 2.5). The basic structure of coumarins contains a lactone attached to a benzene ring. Oxygenation usually takes place at C-7 and C-8 and rarely at C-5 and C-6. Bioassays have been carried out on some coumarins and various activities have been reported, the most interesting of these being against the HIV virus (Cheng *et al*., 2005) . Over two hundred coumarins have been reported (Chapman and Hall, 2002).

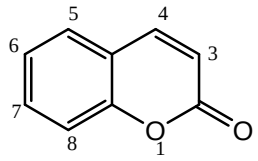
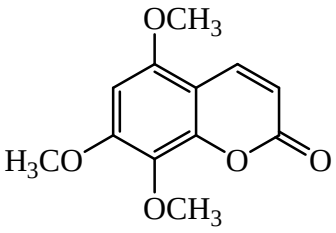


Figure 0.5: The basic structure of Coumarins

Table 0.8: Coumarins from the genus *Zanthoxylum*

Coumarin	**Plant source and part**	**Reference**
5,7,8-Trimethoxycoumarin (**45**)	*Z. ailantoides*	Cheng *et al*., 2005
Psoralen (**46**)	*Z. americanum*	Bafi-Yeboa *et al*., 2003
Aurapten (**47**)	*Z. coco*	Muñoz *et al*., 1982
6,7,8-Trimethoxycoumarin (**48**)	*Z. procerum*	Boulware and Stermitz, 1981
Collinin (**49**)	*Z. schinifolium*	Chang *et al*., 1997
Schininallylol (**50**)	„	„



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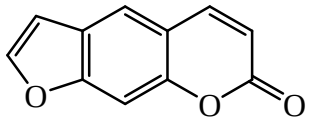

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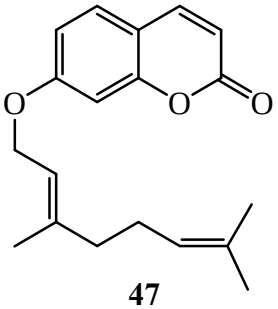

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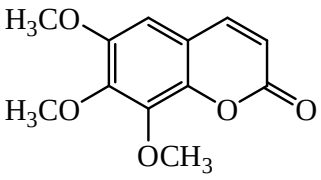

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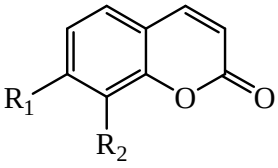
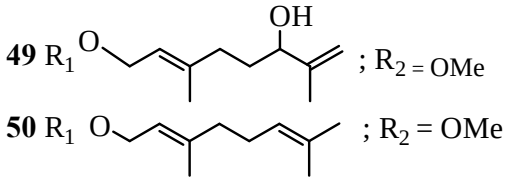




2.4.3 Flavonoids from the genus *Zanthoxylum*

Flavonoids are secondary metabolites that have a C_6-C_3-C_6 moiety existing as flavans, flavones, flavanones, chalcones and dihydrochalcones (Fig. 2.6). The shikimic pathway remains fundamental in their biosynthetic pathway utilizing cinnamoyl-CoA as a starter unit whose chain is extended by three molecules of malonyl-CoA (Dewick, 2009).

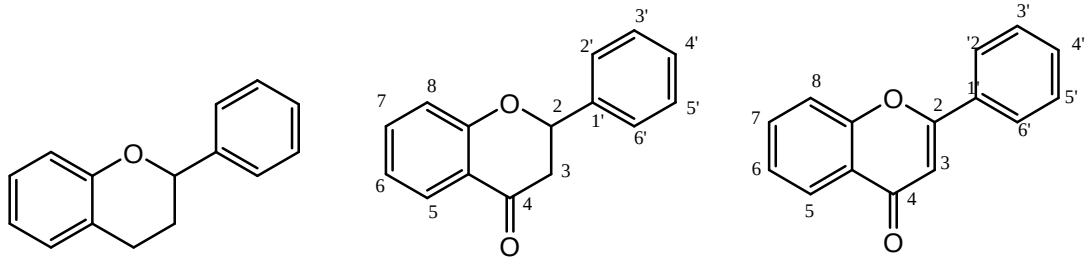
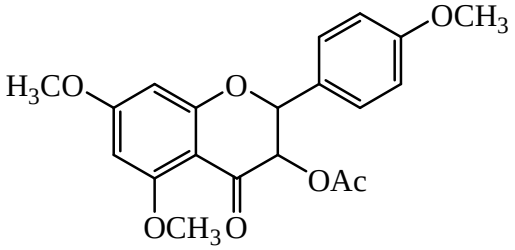


Figure 0.6: The skeletons of Flavans, Flavanones and Flavones.

The flavonoids summarized in Table 2.9 have also been isolated from the genus *Zanthoxylum*.

Table 0.9: Flavonoids from the genus *Zanthoxylum*

Compound	**Plant source and part**	**Reference**
3,5-Diacetyl tambulin (**51**)	*Z. integrifoliolum* (stem bark)	Sati *et al*., 2011; Sheng *et al*., 1999
Diosmetin (**52**)	*Z. avicennae*/leaves	Cho *et al*., 2012
Apigenin(**53**)	,,	,,
Quercetin (**54**)	*Z. bungeanum* (stem bark)	Xiong *et al*., 1994
5,2´-Dihydroxy-3-methoxy-7-O-ɓ-D glycopyranoiside (**55**)	*Z. armatum* (roots)	Sati *et al*., 2011



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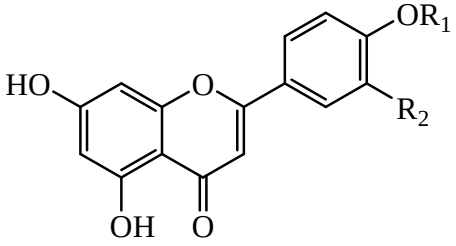
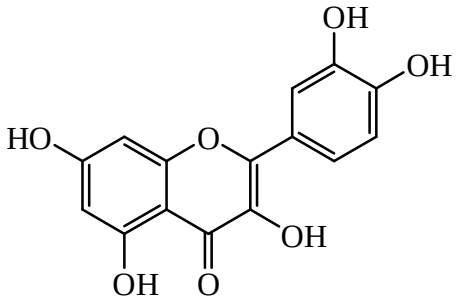


52 R_1 = Me; R_2 = OH
53 R_1, R_2 = H



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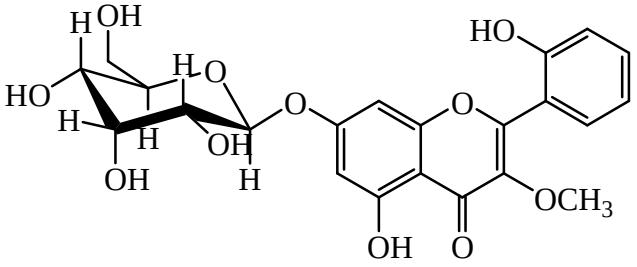

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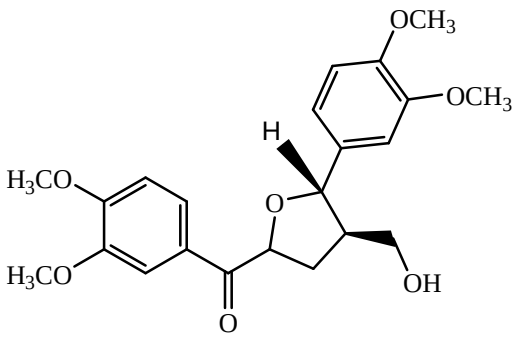
2.4.4 Lignans from the genus *Zanthoxylum*

Lignans are phenolic dimmers of cinnamic acid building up from the shikimic pathway (Fish and Waterman, 1973). Lignans possess a C_6-C_3-C_3-C_6 structural moiety. Some of the compounds isolated from *Zanthoxylum* species are summarized in Table 2.10 below.

Table 0.10: Lignans from the genus *Zanthoxylum*

Compound	**Plant source**	**Reference**
Magnone A **(56)**	*Z. podocarpum*	Niu *et al*., 2011
(-)-Syringaresinol **(57)**	*Z. budranga*	Mukhlesur *et al*., 2005
(-)-Seasamine **(58)**	,,	,,
(-)-Simulanol **(59)**	*Z. simulans*	Yang *et al*., 2002
Zanthpodocarpin **(60)**	*Z. podocarpum*	Zhou *et al*., 2011
7,9′-Epoxylignan **(61)**	*Z. culantrillo*	Cuca S *et al*., 1998
Eudesmin **(62)**	*Z. armatum*	Guo *et al*., 2011



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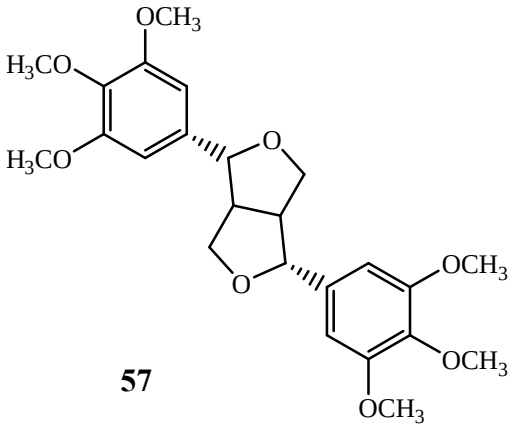

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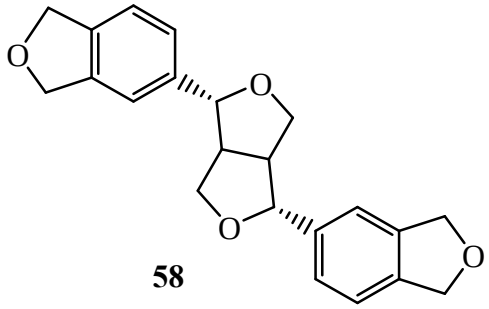

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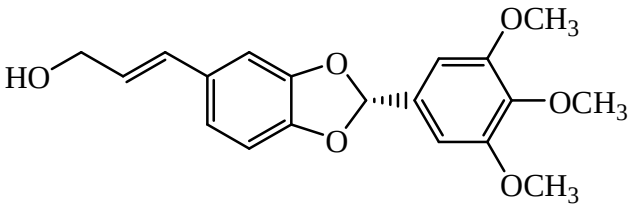

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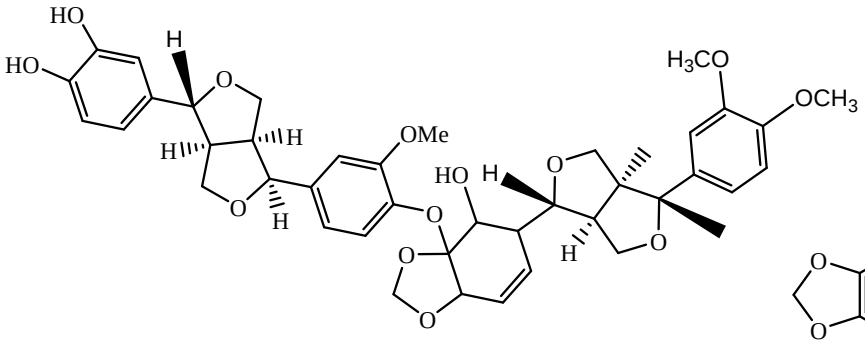

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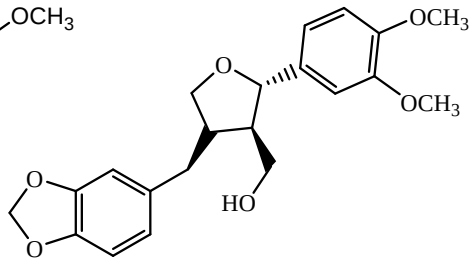

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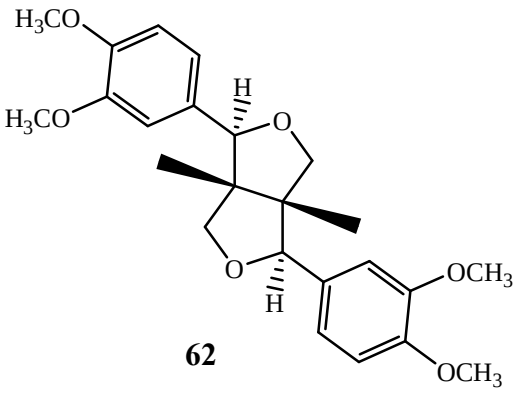


62

CHAPTER THREE
MATERIALS AND METHODS

3.1 General

Merck silica gel 60 (70-230 mesh) and Sephadex LH-20 were used as stationary phases for column chromatography (CC). Plates for preparative thin layer chromatography (PTLC) (1.0 mm, 20 x 20 cm) were prepared using Merck silica gel 60 ($PF_{254+366}$) for purification; factory made analytical aluminium TLC plates (silica gel 60 $F_{254,}$ Merck) were used to monitor the purity of the fractions from the column by visualizing the spots under UV light at 254 or 366 nm, followed by spraying with iodine and Dragendorffôs reagent for both the non UV active and alkaloid tests respectively. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz and Bruker-Avance 500 and 600 MHz spectrometers. The Homo Nuclear Correlation Spectroscopy (COSY), Hetero Nuclear Single Quantum Coherence (HSQC) and Hetero nuclear Multiple Bond Connectivity (HMBC) spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm relative to the internal standard tetra methyl silane (TMS). The major solvents used for chromatography were *n*-hexane and ethyl acetate

3.2 Plant Material

The stem bark of *Zanthoxylum gilletii* was collected from Kakamega Forest in Kakamega County, Western region of Kenya in February, 2013. The plant was identified by Mr. Patrick Mutiso of the University of Nairobi Herbarium, School of Biological Sciences (SBS), where a voucher specimen is deposited. The plant material was air dried under shade and pulverized into fine powder using a Willy mill at the Department of Chemistry, University of Nairobi.

3.3 Extraction and Isolation of compounds from *Zanthoxylum gilletii*

The stem bark of *Zanthoxylum gilletii* (3.8 kg) was air dried under shade, pulverized into fine powder and exhaustively extracted by cold percolation at room temperature using a mixture of 3 litres of methanol (MeOH) and 3 litres of dichloromethane (CH_2Cl_2) for a period of 72 hrs. The filtrate was concentrated *in vacuo* on a rotary evaporator and combined to give 300 g of yellowish and partly oily extract which translated to 7.8 % of the pulverized material. The extract obtained using 50 % MeOH in CH_2Cl_2 (100 g) was adsorbed onto an equal amount of silica gel (100 g) and loaded onto 500 g of silica gel column packed using 100 % *n*-hexane. The column was eluted

serially with solvent systems of increasing polarity, initially with 2 % and then 4 %, 6 %, 8 %, 12 %, 18 %, 30 %, 50 % up to 100 % EtOAc in hexane resulting to 160 fractions of 200 ml each.

The fractions were concentrated *in vacuo* on a rotatory evaporator and spotted on analytical TLC plates. The fractions with similar TLC profiles were combined based on their TLC profiles into fourteen fractions. The fraction that eluted with 2 % EtOAc in *n*-hexane yielded white amorphous solid of lupeol (**63,** 1.4 g). The fractions that eluted with 3 % EtOAc in *n*-hexane crystallized in the conical flask. The crystals were filtered out *in vacuo* using a Buchner funnel and washed severally with 90 % CH_2Cl_2 in *n*-hexane and dried in open air yielding 20 mg of dihydrochelerythrine.

The fractions of the major column eluted with 4 % EtOAc in *n*-hexane were combined; solvent removed *in vacuo* using a rotatory evaporator and re-crystallized using 60 % CH_2Cl_2 in *n*-hexane to produce a white amorphous solid of the lignin, seasamine (**65,** 3.2 g). The mother liquor was recrystallized from 80 % CH_2Cl_2 in *n*-hexane, filtered and dried yielding, 8-acetonyldihydrochelerythrine (**66,** 4.25 mg). The fractions of the main column eluted with 5-8 % EtOAc in *n*-hexane were combined and solvent removed *in vacuo* on a rotatory evaporator and loaded on a Sephadex LH 20 column leading to isolation of a white amorphous solid of norchelerythrine (**67**, 5.15 mg**)** and an aromatic amide fagaramide (**68,** 6.3 g)

3.4 Biological activities

The crude and the pure compounds were assayed using a non-radioactive assay technique developed by Smilkstein *et al* (2004), with modifications according to (Johnson *et al* (2007) to determine 50 % growth inhibition of the cultured parasites. Two *Plasmodium falciparum* parasite strains, chloroquine-sensitive Sierra Leone (D6) and chloroquine-resistant Indochina (W2) and artemisinin resistant strain (3D7) were grown as described by Johnson *et al* (2007). The crude extract, pure compounds and the reference drug were dissolved in 99.5 % DMSO and diluted by complete Roswell Park Memorial Institute 1640 series of cell culture medium (RPMI 1640) prepared from RPMI 1640 powder. The complete RPMI 1640 media was then incubated at 4 ^{o}C and used within 2 weeks.

Serial dilutions of chloroquine and test samples were prepared on a 96-well plate, making sure that the amount of DMSO was equal or less than 0.0875 %. The culture-adapted *P. falciparum* at 2 % hematocrit and 1 % parastemia, were added on the plate containing a range of drug doses and incubated in gas mixture (5 % CO_2, 5 % O_2 and 90 % N_2) at 37 ^oC. The termination of the assay was done 72 hours later by freezing at -80 ^oC. The parasite growth quantified as mean ± standard deviation (Mean IC_{50} ± SD) as described by (Johnson *et al*., 2007).

CHAPTER FOUR
RESULTS AND DISCUSSION

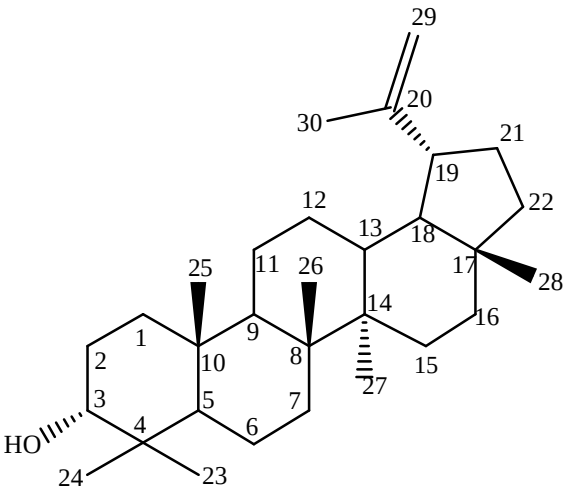
4.1 Secondary metabolites isolated from *Zanthoxylum gilletii*

The air dried and ground stem bark of *Z. gilletii* was extracted using methanol/dichloromethane (1:1). The resultant extract was subjected to isolation using column chromatography in silica gel and Sephadex LH-20 yielding six compounds characterized as a steroid, lupeol (63), a benzophenanthridine alkaloid, dihydrochelerythrine (64), one lignin, seasamine (65), two benzophenanthridine alkaloids, 8-acetonyldihydrochelerythrine (66) and norchelerythrine (67), and an amide, fagaramide (68). The detailed spectroscopic characterization of these compounds is discussed below.

4.1.1 Lupeol (63)

Compound **63** was isolated as white amorphous solids with melting point of 191-192 ęC. The spot on TLC was UV_{254} inactive and therefore was located by exposure to iodine vapor. The ^{1}H-NMR (Table 4.1) spectrum clearly showed seven singlets for methyl groups at ŭ 0.96 (Me-23), 0.88 (Me-24), 0.82 (Me-25), 1.03 (Me-26), 0.94 (Me-27), 0.79 (Me-28), 0.68 (Me-30). The olefinic protons for the methylene group (CH_2) appeared at ŭ 4.68 and 4.56 as broad singlets. The ^{13}C NMR spectrum (Table 4.1) showed 30 carbon atoms, characteristic of triterpenoids. Furthermore one of these carbons was oxygenated and therefore appeared downfield shifted at ŭ 79.2 and was assigned to C-3. The resonance at ŭ 151.2 represented a quaternary carbon for C-20 and that appearing at ŭ 109.6 was assigned to one of the 191 were assigned to C-5, C-8, C-10, C-14 and C-17 respectively.

The ^{13}C-NMR spectrum also showed peaks at ŭ 18.5, 21.2, 25.3, 27.6, 27.7, 30.1, 34.5, 35.8, 38.9 and 40.2 assigned to methylene carbons at C-6, C-11, C-12, C-2, C-15, C-21, C-7, C-16, and C-1 and C-22 respectively. These translated to ten carbons. Subsequently, there were seven characteristic peaks for methyl carbon atoms resonating at ŭ 14.8, 15.6, 16.2, 16.4, 18.2, 19.5 and 28.2 for C-27, C-24, C-25, C-26, C-28, C-30 and C-23. Based on this spectroscopic data and comparison with literature values this compound was identified as lupeol (**63**) previously isolated from many plant species including Z. *rhoifolium* (Reynolds *et al*., 1986).



63

Table 0.1: NMR Data f or lupeol (63)

C-Position	**δ_H (H, *m*,)**	**δ_C NMR**	**HMBC (^2J, ^3J)**
1	1.67	38.9	
2	1.52	27.6	
3	3. 20 (1H, *m*)	79.2	C-1, 2, 24
4		39.1	
5	0.66 (1H, *s*)	55.5	
6	1.38	18.5	
7	1.38	34.5	
8		41.0	
9	1.28	50.6	
10		37.4	
11	1.25	21.2	
12	1.43	25.3	
13	1.62	38.3	
14		43.0	
15	1.68	27.7	C-17

16	1.52	35.8	C-14,C-18,C-22
17		43.2	
18	1.35	48.5	
19	2.45 (1H, *s*)	48.2	C-3, 17, 20, 29, 30
20		151.2	
21	1.97	30.1	C-18, 20, 22
22	1.41	40.2	C-16, 18,
23	0.96 (3H, *s*)	28.2	
24	0.88 (3H, *s*)	15.6	
25	0.82 (3H, *s*)	16.4	
26	1.03 (3H, *s*)	16.2	
27	0.94 (3H, *s*)	14.8	
28	0.79 (3H, *s*)	18.2	
29	4.73 (1H, *s*)	109.6	C-20, 19, 30
30	1.68 (3H, s)	19.5	C-20,C-29

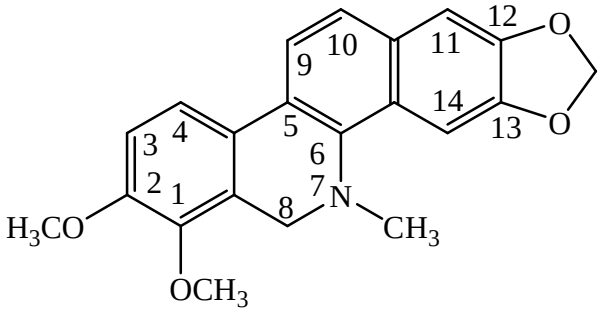
4.1.2 Dihydrochelerythrine (64)

Compound **64** was isolated as colorless crystals which gave a positive alkaloid test (orange coloration) with Dragendorffôs spray reagent and a melting point of 113-115 °C. The spot on TLC fluoresced to blue under UV (366 and 254 nm), and on prolonged exposure to air/light, the spot turned yellow, typical of alkaloids with a benzophenanthridine skeleton (Ming *et al*., 1987). The ^{13}C-NMR spectral data showed 21 peaks, 16 of these were sp^2 hydridized, (6) constituting a 4-ring system that is in agreement with a benzophenanthridine alkaloid skeleton (Nissanka *et al*., 2001).

^{1}H-NMR spectra showed characteristics peaks for two methoxyl (ŭ 3.93 and 3.88; ŭ 61.0 and 55.9), methylenedioxy (ŭ 6.05; ŭ 101.7) and *N*-methyl (ŭ 2.58, ŭ 41.4) groups. The ^{1}H-NMR

spectrum further showed six peaks in the aromatic region; one set of *ortho*-coupled doublets at ŭ 6.96 and 7.51 (J = 8.4 Hz) which were assigned to H-3 and H-4, respectively. A second set of doublets at ŭ 7.73 and 7.48 (J = 9.0 Hz) were respectively assigned to H-9 and H-10. The singlet at ŭ 7.12 was assigned to H-11 and that at ŭ 7.66 to H-14. This aromatic pattern suggested oxygenation at C-1, 2, 12 and 13 corresponding to two methoxyl and methylenedioxy groups. The ^{3}J correlations of both H-11 and OCH_2O protons with C-13 and between a ŭ 6.05 (OCH_2O) and the quaternary carbons C-12 and C-13 established the substitution pattern in this ring. A singlet at ŭ 2.58 (3H) showed the presence of an *N*-methyl (*N*-CH_3) based on the ^{3}J correlations of these identical protons with both C-8 and C-6.

The NMR data and HMBC correlations were used to confirm the structure. Subsequently the ^{13}C-NMR data had 21 clear peaks, of which 16 were for sp^2 carbons (ŭ 100.8-152.7) depicting a 4-ring system which agrees with benzophenanthridine alkaloid skeleton (Nissanka *et al*., 2001). There were two methoxy groups assigned to position C-1 and C-2 with chemical shifts at ŭ 56.8 and 61.7, respectively. Comparing these values with literature ones this compound was identified as dihydrochelerythrine, an alkaloid that was previously reported from *Z. rubescens* (Waterman, 1976).



64

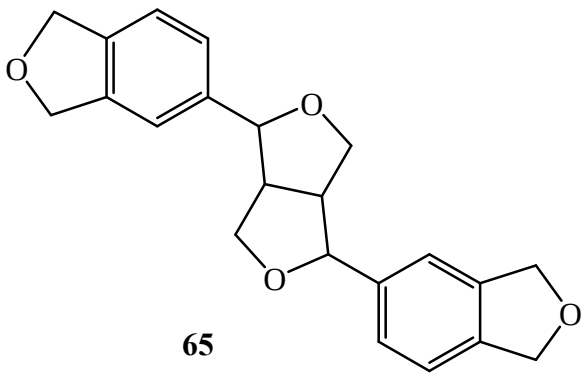
Table 0.2: NMR data for dihydrochelerythrine (64)

C-Position	**δ_H (H, *m*)**	**δ_C NMR**	**HMBC (^2J, ^3J)**
1	-	146.0	
2	-	152.7	
3	6.96 (1H, *d*, *J* = 8.4)	111.4	C-4a, 1
4	7.51 (1H, *d*, *J* = 8.4)	118.8	C-8a, 5, 2
4a	-	126.4	
5	-	126.3	
6	-	145.0	
7	-	-	
8	4.30 (2H, *s*)	48.9	C-6, 4a, 1, N-CH3
8a		124.6	
9	7.73 (1H, *d*, *J* = 9.0)	120.4	C-10a, 6, 4a
10	7.48 (1H, *d*, *J* = 9.0)	123.9	C-10a, 14a, 11, 5
10a	-	131.1	
11	7.12 (1H, *s*)	104.4	C-14a, 13, 10
12		148.4	
13		148.7	
14	7.66 (1H, *s*)	100.0	C-12, 10a, 6
14a		126.7	
OCH_3	3.93 (3H, *s*)	56.8	C-2
OCH_3	3.88 (3H, *s*)	61.7	C-1
N-CH_3	2.58 (3H, *s*)	41.4	C-8, 6
OCH_2O	6.05 (2H, *s*)	101.7	C-13, 12

4.1.3 Seasamine (65)

Compound **3** was isolated as white amorphous solids which were UV active, with melting point of 121-122 °C. The ^{1}H-NMR (Table 4.3) showed two sets of doublets at ŭ 3.88 (*J* = 9.2 Hz) and 4.24 (*J* = 9.2 Hz) assigned to methylene protons at C-4 and C-8 respectively. The ^{1}H-NMR further displayed a multiplet at ŭ 3.05 for the methyne protons at C-1 and C-5. There was an additional doublet at ŭ 4.74 (*J* = 3.6 Hz) for H-2 and H-6. The downfield peak appearing at ŭ 5.99 was characteristic of methylenedioxy protons. Furthermore, the ^{1}H-NMR displayed signals for aromatic protons at ŭ 6.90 (*d*, *J* = 8.0 Hz) for H-2´and H-2 ´´; at ŭ 6.82 for H- 5´ and H-5´´ at ŭ 6.86 (*dd*, *J* = 8.0 Hz) assigned to H-6´and H-6´´.

The ^{13}C NMR spectrum showed peaks ŭ 135.5 and 86.0 attributed to two sets of equivalent carbons at C-1´and C-1´´ and C-2 and C-6 respectively. These NMR values enabled the placement of the methylenedioxy on the aromatic rings. The ^{13}C-NMR spectrum revealed ten non-equivalent carbon resonances, two of which were oxygenated at ŭ 147.0 and 147.9 for C-3´, 3´´ and C-4´, 4´´ respectively (each belonging to chemically equivalent carbon atoms). From the above structural elucidation and comparison with literature values, compound **65** was identified as Seasamine (Pelter and Ward, 1976) previously isolated from Z. *budrunga* (Mukhlesur *et al*., 2005)



65

Table 0.3: NMR Data for seasamine (65)

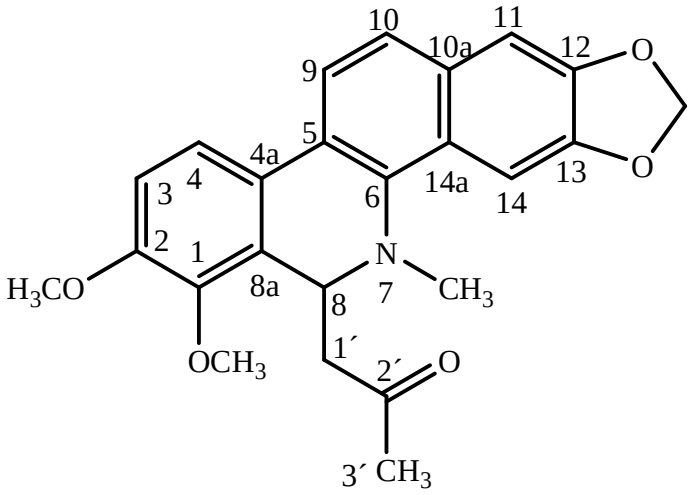
C-Position	ŭ$_H$ (H, *m, J,* in Hz)	ŭ$_C$	HMBC (^2J, ^3J)
1	3.05(1H, *m*)	54.4	C- 2, 4a, 1´
2	4.74(1H, *d*)	85.7	
4a	3.88 (1H, *d, J* = 9.2)	71.7	C- 5, 6
4e	4.24 (1H, *d*, *J* = 9.2)		C-5, 6
5	3.05 (1H, *m*)	54.4	C-6, 8a, 1´´
6	4.74 (1H, *dd*)	85.7	C-5, 4a, 2´´, 6´´
8a	3.88 (1H, *d, J* = 3.6)	71.7	C-1, 2
8e	4.24 (1H, *d*, *J* = 3.6)		C-1, 2
1´	-	135.5	
2´	6.90 (1H, *d*, *J* = 1.4)	106.4	C-3´, 4´, 6´, 2
3´	-	147.0	
4´	-	147.9	
5´	6.82 (1H, *dd*, *J* = 8.0)	108.0	C-1´, 3´
6´	6.86 (1H, *d*)	119.3	C- 4´, 2´, 2
1´´		135.5	
2´´	6.90 (1H, *d*)	106.4	C-3´´, 4´´, 6´´, 6
3´´		147.0	
4´´		147.9	
5´´	6.82 (1H, *dd*, *J* = 8.0)	108.0	C-3´´, 1´´,
6´´	6.86 (1H, *d*)	119.3	C-2´´, 4´´, 6
OCH_2O	5.99 (4H, *s*)	101.3	

4.1.4 Acetonyldihydrochelerythrine (66)

Compound **66** was isolated as colorless crystals with a melting point of 165-170 ^{o}C and was active to Dragendorffôs classification test for alkaloids. The compound on TLC plate showed blue fluorescence under UV (366 and 254 nm) light and turned yellow on prolonged exposure to air and light. The ^{1}H and ^{13}C-NMR of this compound was similar to that of compound **2** typical of alkaloids with benzophenanthridine skeleton (Ming *et al*., 1987).

The ^{13}C-NMR spectrum (Table 4.4) showed sixteen sp^2 hybridized carbons (δ_C = 75-158) constituting a four-ring system of a benzophenanthridine alkaloid. This compound is substituted at C-1 and C-2 with methoxyl (ŭ 3.96, ŭ 61.3; ŭ 3.93, ŭ 56.0) and at C-12 and C-13 with methylenedioxy group (ŭ 6.04, ŭ 101.3). The ^{3}J HMBC correlations between both H-14 and OCH_2O protons with C-12, and H-11 and OCH_2O protons with C-13 helped to fix the methylenedioxy group. The ^{1}H NMR revealed six aromatic protons two of which were singlets at ŭ 7.49 and 7.11 (1H, *s*), assigned to H-14 and H-11, respectively. Furthermore, the set of doublet at ŭ7.64 (1H, *d*, *J* = 8.4 Hz) and ŭ 6.88 (1H, *d*, *J* = 9.0 Hz) were respectively assigned to H-4 and H-3, whereas ŭ 7.45 (1H, *d*, *J* = 9.0) and ŭ 7.41 (1H, *d*, *J* = 8.4) to H-9 and H-10.

Additional peaks were observed in the ^{13}C-NMR spectrum at ŭ 43.1 for (*N*-CH_3), a carbonyl ŭ 208.0 for (-C=O), acetonide methylene carbon (CH_2C=O) at ŭ 47.3 and acetonide methyl (O=C-CH_3) at ŭ 30.7. The acetonide group was fixed at C-8 on the basis of the ^{3}J HMBC interaction of H-8 with C-1´, C-1, C-4a, C-6 and *N*-CH_3. Confirmation of all the assignments was based on HSQC and HMBC correlations, leading to the conclusion that compound **67** was 8-acetonyldihydrochelerythrine. From the above spectroscopic data coupled with literature values, the acetonide is a known natural alkaloid isolated previously from the genus *Zanthoxylum* (Negi *et al*., 2011) as acetonyldihydrochelerythrine (**66**). This acetonide is more stable than chelerythrine (Nissanka *et al*., 2001).



66

Table 0.4: NMR data for Acetonydihydrochelerythrine (66)

C-Position	**δ_H (H, *m*, *J*, in Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1		145.7	
2		152.4	
3	6.96 (1H, *d*, *J* = 90)	111.7	C-2, 1, 4a,
4	7.55 (1H, *d*, *J* = 8.4)	119.1	C-2, 5, 8a
4a		127.6	
5		131.3	
6		139.5	
7		*N*	
8	5.04 (1H, *dd*, *J* = 3.6)	55.1	C-1´, 8a, 1, 2´, 4a, 6, *N*-CH_3
8a		128.4	
9	7.49 (1H, *d*, *J* = 9.0)	120.0	C-4a, 10a
10	7.71 (1H, *d*, *J* = 8.4)	124.1	C-10a, 5, 11, 14a
10a		123.5	
11	7.11 (1H, *s*)	104.6	C-10, 13, 14a
12		147.8	

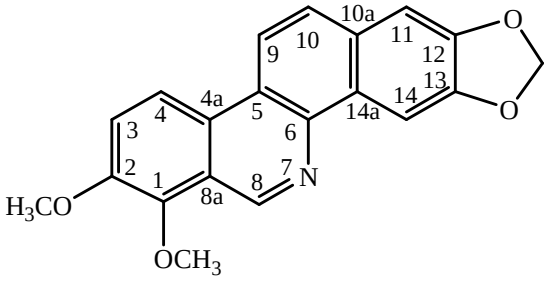
13		148.4	
14	7.49 (1H, *s*)	100.4	C-14a, 6, 10a, 12
14a		127.6	
1´	2.54, 2.29 (2H, *dd*, *J* = 11.4, 3.8)	47.3	C-2´, 8, 8a
2´		208.0	
3´	2.06 (3H, *s*)	31.5	C-2´, 1´
OCH_2O	6.04 (2H, *s*,)	101.3	C-12, 13
OCH_3	3.96 (3H, *s*)	61.3	C-1
OCH_3	3.93 (3H, *s*)	56.0	C-2
N-CH_3	2.51 (3H, *s*)	43.1	C-6, 8

4.1.5 Norchelerythrine (67)

Compound **5** was isolated as colorless crystals with a melting point of 210-212 ęC. Like compound **66**, it showed a blue fluorescence on TLC plate under UV (254 and 366 nm) light. The ^{13}C-NMR spectrum (table 4.5) revealed presence of 17 sp^2 hybridized carbons, a methylenedioxy group (ŭ 101.6) and two methoxy (ŭ 61.7 and 56.8) peaks. This compound did not have an *N*-CH_3 peak unlike **66** but ^{1}H-NMR displayed a peak for a highly downfield shifted proton for H-8 (ŭ 9.70, ŭc 146.6) consistent with an amine group. Furthermore, in the ^{1}H-NMR spectrum of the aromatic region, two additional singlets at ŭ 7.28, and 8.68 were assigned to H-11 and H-14, respectively where C-12 and C-13 were substituted with methylenedioxy group.

The ^{1}H-^{1}H COSY spectrum showed a pair of *ortho*-coupled protons (ŭ 8.36 and 7.87, *J* = 10 Hz). The two protons at ŭ 7.87 showed long range coupling with singlets at ŭ 7.28 (H-11) and at ŭ 8.68 (H-14). A second pair of *ortho*-coupled protons (Table 4.5) was assigned to H-3 and H-4 where C-1 and C-2 are substituted with methoxyl groups (Table 4.5). Based on HMBC and HSQC spectra all the atoms were assigned. From the above data and comparison with literature

compound **67** was elucidated to be norchelerythrine, a benzophenanthridine alkaloid previously reported form *Z. capense* (Mansoor *et al*., 2013).



67

Table 0.5: NMR data for norchelerythrine (5)

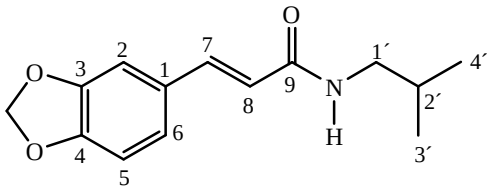
C-Position	**δ_H (H, *m*, *J*, in Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1		145.6	
2		149.6	
3	7.63 (1H, *d*, *J* = 10Hz)	118.9	C-8a, C-1, C-2
4	8.36 (1H, *d*, *J* = 10Hz)	118.2	C-8a, C-2, C-4a
4a	-	121.9	-
5	-	129.8	-
6	-	139.2	-
8	9.70 (1H, *s*)	146.6	C-8a, C-4a
8a	-	127.9	-
9	8.38 (1H, *d*, *J* = 10Hz)	118.4	C-6, C-4a, C-5
10	7.87 (1H, *d*, *J* = 10Hz)	126.9	C-11, C-10a
10a	-	120.0	-
11	7.28 (1H, *s*)	104.3	C-13, C-10, C-14a
12	-	148.4	-
13	-	148.6	-
14	8.68 (1H, *s*)	101.9	C-6, C-12

14a	-	129.2	-
-OCH_2O-	6.14 (2H, *s*)	101.7	C-13, C-12
OCH_3	4.11 (3H, *s*)	61.7	C-1
OCH_3	4.11 (3H, *s*)	56.8	C-2

4.1.6 Fagaramide (68)

Compound **68** was isolated as creamish crystals with a melting point of 113-115 °C which were UV active. The ^{1}H-NMR spectrum, exhibited an AX spin system of two olefinic protons at ŭ 6.22 (*d*, *J* = 15.0 Hz, 1H, H-8) and 7.57 (*d*, *J* = 15.0 Hz, 1H, H-7). The large coupling constant (*J* = 15.4 Hz) exhibited between H-7 and H-8 was consistent with *trans* configuration of the olefinic bond. Furthermore, the ^{1}H-NMR displayed signals at ŭ 3.2 (-CH_2, *m*), 1.87 (1H, *m*), 0.98 $(CH_3)_2$, *d*, *J* = 7.2 Hz) indicating the presence of an isobutyl amide functional group in the compound.
The analysis of this spectrum also made it possible to highlight the presence of three mutually coupled aromatic proton at ŭ 6.97 (*d*, *J* = 2.0 Hz, H-2), 6.94 (*dd*, *J* = 8.2, 2.0 Hz, H-6), and ŭ 6.78 (*d*, *J* = 8.2 Hz, H-5) of a tri-substituted benzene ring. A two proton singlet appeared at ŭ 6.0 characteristic of the methylenedioxy group and was placed at C-3/C-4.

The analysis of ^{13}C-NMR spectra (Table 4.6) confirmed the presence of 14 carbon peaks. These peaks revealed the presence of six aromatic carbons of which 3 were CH appearing at 119.5, 124.0 and 129.5, C-2, C-5 and C-6 respectively, 3 quaternary carbons at ŭ140.8, 148.4 and 149.2 assigned to C-1, C-3and C-4 respectively. There was a signal with ŭ 101.2 corresponding to the methylene-dioxy group, a signal at ŭ 166.4, ascribable to the carbonyl group of the function amide, signals at ŭ 140.2 and ŭ 119.0 ppm, ascribable to the olefinic hydrocarbons, lowfield CH_2 at ŭ 47.0 nearer to the nitrogen atom. There were also methyls observed at ŭ 20.4 and ŭ 20.4 assigned to C-3´ and C-4´. On the basis of the above ^{1}H and ^{13}C-NMR spectral data and comparison with literature values, compound **68** was characterized as fagaramide. This compound has been previously isolated from a number of plants including *Z. schinifolium* (Mbaze *et al.,* 2009).



68

Table 0.6: NMR Data for fagaramide (68)

C-Position	**δ_H (H, *m, J*, in Hz)**	**δ_C**	**HMBC (^2J, ^3J)**
1	-	140.8	
2	6.78 (1H, *s*)	119.5	C-4
3	-	148.4	
4	-	149.2	
5	6.97 (1H, *d, J* = 8.0)	129.5	C-1, C-3, C-4
6	6.94 (1H, *d, J* = 8.0)	124.0	
7	6.22 (1H, *d, J* = 15.0)	106.5	C-2, C-6, C-9
8	7.57 (1H, *d, J* = 15.0)	108.7	C-1
9	-	166.4	
1´	3.22 (2H, *m*)	47.3	C-4´, C-3´
2´	1.87 (1H, *m*)	28.9	
3´	0.99 (3H, *d*)	20.4	
4´	0.97 (3H, *d*)	20.4	
OCH_2O	6.01 (2H, *s*)	101.6	
N-H	3.85 1H, *brs*)		

4.2 Spectroscopic Data of Isolated Compounds

4.2.1 Lupeol (63)

The compound was isolated as a white amorphous solid which was not UV active but iodine active. The melting point was (191-192 °C). ^{1}H-NMR (500 MHz, $CDCl_3$) ŭ, 0.91 (H-1a), 1.67 (H-1e), 1.61 (H-2a), 1.54 (H-2e), 3.19 (H-3), 0.66 (H-5), 1.38 (H-6a), 1.51 (H-6e), 1.38 (2H-7), 1.28 (H-9), 1.25 (H-11a), 1.41 (H-11e), 1.07 (H-12a), 1.43 (H-12e), 1.62 (H-13), 1.03 (H-15a), 1.68 (H-15e), 1.38 (H-16a), 1.52 (H-16e), 1.35 (H-18), 2.37 (H-19), 1.32 (H-21a), 1.92 (H-21e), 1.03 (H-22a), 1.41 (H-22e), 0.96 (3H, *s*, H-23) 0.88 (3H, *s*, H-24) 0.82 (3H, *s*, H-25), 1.03 (3H, *s*, H-26), 0.94 (3H, *s*, H-27), 0.79 (3H, *s*, H-28), 4.56 (H-29a), 4.68 (1H-29e), 1.68 (3H, *s*, H-30).

^{13}C- NMR (125 MHz, CD_2Cl_2): ŭ 38.9 (C-1), 27.6 (C-2), 79.2 (C-3), 39.1 (C-4), 55.5 (C-5), 18.5 (C-6), 34.5 (C-7), 41.0 (C-8), 50.6 (C-9), 37.4 (C-10), 21.2 (C-11), 25.3 (C-12), 38.3 (C-13), 43.0 (C-14), 27.7 (C-15), 35.8 (C-16), 43.2 (C-17), 48.5 (C-18), 48.2 (C-19), 151.2 (C-20), 30.1 (C-21), 40.2 (C-22), 28.2 (C-23), 15.6 (C-24), 16.4 (C-25), 16.2 (C-26), 14.8 (C-27), 18.2 (C-28), 109.6 (C-29), 19.5 (C-30).

4.2.2 Dihydrochelerythrine (64)

Colorless cubes with a melting point of 113-115ę C; UV active; R_f = 0.4 (CH_2Cl_2/*n*-hexane 1:1 v/v), R_f = 0.82 (25% EtOAc in *n*-hexane). ^{1}H-NMR (600 MHz, CD_2Cl_2): ŭ 6.96 (1H, *d, J* = 10 Hz, H-3), 7.51 (1H, *d, J* = 10 Hz H-4), 4.27 (2H, *s*, H-8), 7.71 (1H, *d*, *J* =10 Hz, H-9), 7.48 (1H, *d*, *J* = 10 Hz, H-10), 7.12 (1H, *s*, H-11), 7.66 (1H, *s*, H-14), 6.14 (2H, *s*, OCH_2O), 4.11 (3H, *s*, MeO-1), 4.04 (3H, *s*, MeO-2), 2.58 (3H, *s*, CH_3-7).

^{13}C-NMR (125 MHz CD_2Cl_2): ŭ, 146.0, (C-1), 152.7 (C-2), 111.4 (C-3), 118.8 (C-4), 126.4 (C-4a), 126.3 (H-5), 145.0 (C-6), 48.9 (C-8), 124.6 (C-8a), 120.4 (C-9), 123.9 (C-10), 131.1 (C-10a). ^{1}H and ^{13}C NMR data is shown in table

4.2.3 Seasamine (65)

This compound was extracted as colorless crystals with melting point of 121-122°C. Its ^{1}H-NMR (600 MHz, CD_2Cl_2): had the following chemical shifts ŭ 3.05 (*m* H-1), 4.71 (*d*, *J* = 3.7 Hz, H-2), 3.86, (*dd, J* = 9.2, *J* = 3.6 Hz, H-4a), 4.24(*dd*, *J* = 9.2, *J* = 3.6, H-4e). 3.05(*m* ,H-5), 4.71(*d*, *J* = 3.7 Hz , H-6), 3.86(*dd*, *J* = 9.2 Hz, *J* = 3.6 Hz , H-8a), 4.24 (*dd*, *J* = 9.2 Hz, *J* = 3.6 Hz ,H-8e), 6.86 (H-2´and H-2´´), 6.78 (*dd*, *J* = 8.0 Hz, H-5´ and H-5´´), 6.86(*d*, *J* = 1.4 Hz, H-6´ and H-6´´).

^{13}C-NMR ($CDCl_3$, 50 MHz) ŭ 54.6(C-1), 86.0 (C-2), 71.9 (C-4), 54.6 (C-5), 86.0 (C-6), 71.9 (C-8), 135.3 (C-1´), 106.7 (C-2´), 147.3 (C-3´), 148.2 (C-4´), 108.4 (H-5´), 119.6 (C-6´), 135.3 (C-1´´), 106.7 (C-2´´), 147.3 (C-3´´), 148.2 (C-4´´), 108.4 (H-5´´), 119.6 (C-6´´), 101.3 (OCH_2O).

4.2.4 8-Acetonyldihydrochelerythrine (66)

Colorless crystals with a melting point of (165-170 ^{o}C); UV active; R_f = 0.49 (25% EtOAc in *n*-hexane). ^{1}H-NMR (600 MHz, CD_2Cl_2): ŭ 6.98 (1H, *d*, *J* = 9.0 Hz H-3), 7.55 (1H, *d*, *J* = 9.0 Hz, H-4), 5.04 (2H, *dd*, *J* = 3.6, 11 Hz, H-8), 7.73 (1H, *d*, *J* = 8.4 Hz, H-9), 7.50 (1H, *d*, *J* = 8.4 Hz, H-10), 7.12 (1H, *s*, H-11), 7.50 (1H, *s* H-14), 2.57-2.21 (2H, *dd*, *J* = 3.6, 11, 3.2 Hz, H-1ô), 2.09 (3H, *s*, $COCH_3$), 6.05 (2H, *s*, -OCH_2O-), 3.93 (3H, *s*, OMe), 3.93 (3H, *s*, OMe), 2.61 (3H, *s*, *N*-CH_3).

^{13}C-NMR (150 MHz, CD_2Cl_2): ŭ 145.6 (C-1), 152.3 (C-2), 111.6 (C-3), 118.8 (C-4), 127.6 (C-4a), 131.3 (C-5), 139.5 (C-6), 55.0 (C-8), 128.5 (C-8a), 118.8 (C-9), 123.9 (C-10), 123.1 (C-10a), 104.2 (C-11), 147.7 (C-12), 148.2 (C-13), 100.4 (C-14), 127.6 (C-14a), 47 (C-1´), 206.9 (C-2´), 30.5 (H-3´), 101.4 (-OCH_2O-), 60.8/55.7 (MeO-1/2), 42.7 (*N*-CH_3)

4.2.5 Norchelerythrine (67)

Colourles crystals with a melting point of 210-212 ^{o}C soluble in CH_2Cl_2. ^{1}H-NMR (600 MHz, CD_2Cl_2): ŭ 7.63 (1H, *d*, *J* = 10 Hz, H-3), 8.36 (1H, *d*, *J* =10 Hz, H-4), 9.70 (1H, *s*, H-8), 8.38 (1H, *d*, *J* = 10 Hz, H-9), 7.87 (1H, *d*, *J* =10 Hz, H-10), 7.28 (1H, *s*, H-11), 8.68 (1H, *s*, H-14), 6.14

(2H, *s*, OCH_2O), 4.11/4.04 (6H, *s*, 2(MeO). ^{13}C-NMR (125 MHz, CD_2Cl_2): ŭ 145.6 (C-1), 159.6 (C-2), 118.9 (C-3), 118.2 (C-4), 121.9 (C-4a), 129.8 (H-5) 139.2 (C-6), 146.6 (C-8), 127.9 (C-8a), 118 (C-9), 126.9 (C-10), 120.0 (C-10a), 104.3 (C-11), 148.4 (C-12), 148.6 (C-13), 101.9 (C-14), 129.2 (C-14a), 101.7 (OCH_2O), 61.7/58.8 (MeO-1/2)

4.2.6 Fagaramide (68)

Colorless solid with a melting point of 113-115 ^{o}C; UV active; R_f = 0.47 (25 % EtOAc in *n*-hexane). ^{1}H-NMR(600 MHz, CD_2Cl_2): ŭ 6.94, *s*, (H-2), 6.97, *d,* (H-5), 6.78, *d,* (H-6), 6.22, *d,* (H-7), 7.57, *d* (H-8), 3.22, *m* (1´), 1.87, *m,* (2´), 0.99, *d,* (H-3´), 0.97, *d,* (H-4´), 6.0, *s,* (OCH_2O), 5.85, *bm,* (*N*-H).

^{13}C- NMR (125 MHz, CD_2Cl_2)**,** 140.8 (C-1), 119.5 (C-2), 148.4 (C-3), 149.2 (C-4), 129.5 (C-5), 124.0 (C-6), 106.5 (C-7), 108.7 (C-8), 166.4 (C-9), 47.3 (C-1´), 28.9 (C-2´), 20.4 (C-3´), 20.4 (C-4´), 101.6 (OCH_2O).

4.3 Biological activity

The crude and some pure compounds obtained from the stem bark of *Z. gilletii* were tested for their anti-plasmodial activities. The MeOH/CH_2Cl_2 (1:1) extract of the stem bark of *Z. gilletii* showed potent anti-plasmodial activities against the chloroquine resistant (W2), chloroquine sensitive (D6) and artemisinin resistant (3D7) strains of *P. falciparum* respectively, with IC_{50} values of 2.52, 1.48 and 1.43 µg/ml, respectively. The compounds tested showed good to moderate activities with seasamine (**65**) exhibiting activities with IC_{50} values of 1.92, 3.23 and 2.94 µg/ml against W2, D6 and 3D7 strains of *P. falciparum* respectively. Compound **66** was also active against the three strains of the malaria parasite tested with IC_{50} values of 4.02, 4.06 and 3.37 µg/ml against W2, D6 and 3D7 strains, respectively. Fagaramide (**68**) showed moderate activity with IC_{50} values of 7.73 and 7.72 µg/ml against D6 and 3D7 but poor activity against W2 strain of *P. falciparum* with an IC_{50} value of 15.15 µg/ml. Surprisingly, lupeol (**63**.) exhibited incomparable antiplasmodial activities against W2 with an IC_{50} value of 32.95 and against 3D7 strain an IC_{50} value of 4.25 µg/ml (Table 4.7) below.

Table 0.7: *In-vitro* IC_{50} values of the crude and alkaloids of *Zanthoxylum gilletii* against W2 D6 and 3D7 strains of *P. falcipurum*

Samples Tested	**IC_{50} in µg/ml**		
	W2	**D6**	**3D7**
Crude extract (stem bark)	2.52	1.48	1.43
Lupeol (**63**)	32.95	-	4.52
Seasamine (**65**)	1.92	3.23	2.94
8-Acetonyldihydrochelerythrine (**66**)	4.02	4.06	3.37
Fagaramide (**68**)	15.15	7.73	7.72
Chloroquine	0. 04	0.006	0.004
Mefloquine	0.001	-	0.01

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Six pure compounds were isolated and characterized from the stem bark of *Z. gilletii* including; lupeol (**63**), dihydrochelerythrine (**64**), seasamine (**65**), 8-acetonyldihydrochelerythrine (**66**), norchelerythrine (**67**) and fagaramide (**68**). Most of the isolated compounds had a benzophenanthridine alkaloid skeleton typical of alkaloids from the genus *Zanthoxylum* (Rutaceae). The extract from the stem bark of *Z. gilletii* showed good anti-plasmodial activities against the chloroquine resistant (W2), chloroquine sensitive (D6) and artemisinin resistant (3D7) strains of *P. falciparum* with IC_{50} values of 2.52, 1.48 and 1.43 µg/ml, respectively. Most of the isolated compounds also showed good anti-plasmodial activities against the three strains of *P. falcipurum* tested with seasamine (**65**) being the most potent with IC_{50} values of 1.92, 3.23 and 2.94 µg/ml against W2, D6 and 3D7 strains of *P. falciparum*, respectively.

5.2 Recommendations

1. The stem and root barks of *Zanthoxylum gilleti* should be investigated further using modern separation techniques such as HPLC to exhaustively isolate most of the minor constituents.
2. These minor compounds should then be evaluated for their anti-malarial potential against the three strains of *P. falciparum* namely; W2, D6 and 3D7.
3. Structural diversification of the potent compounds; seasamine (**65**) and 8-acetonyldihydrochelerythrine (**66**) resulting to analogues, probably with improved anti-plasmodial activities should be undertaken.

4. Based on previous studies, which have shown good antimicrobial activities for extracts of *Zanthoxylum* species, the isolated compounds should be subjected to an array of antimicrobial assays to determine their potential as antibiotics.

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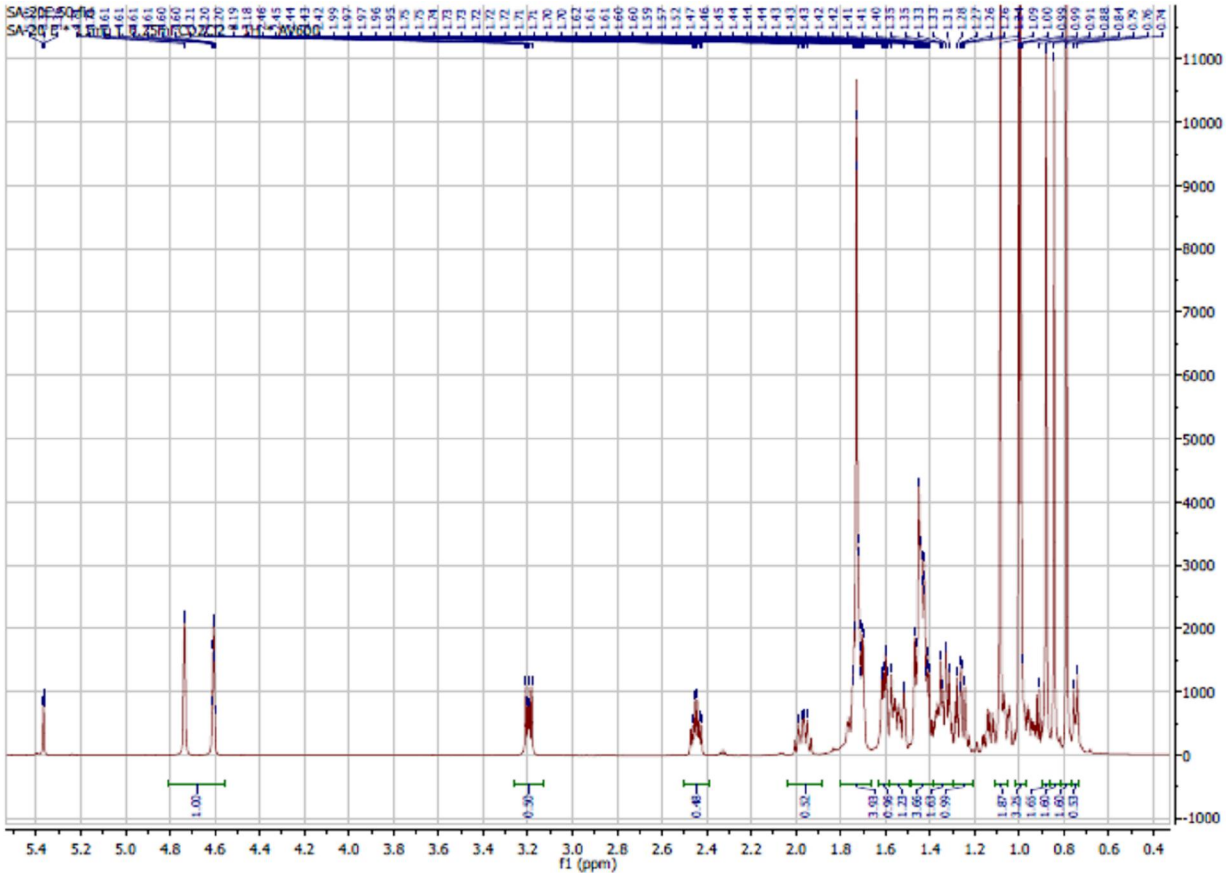
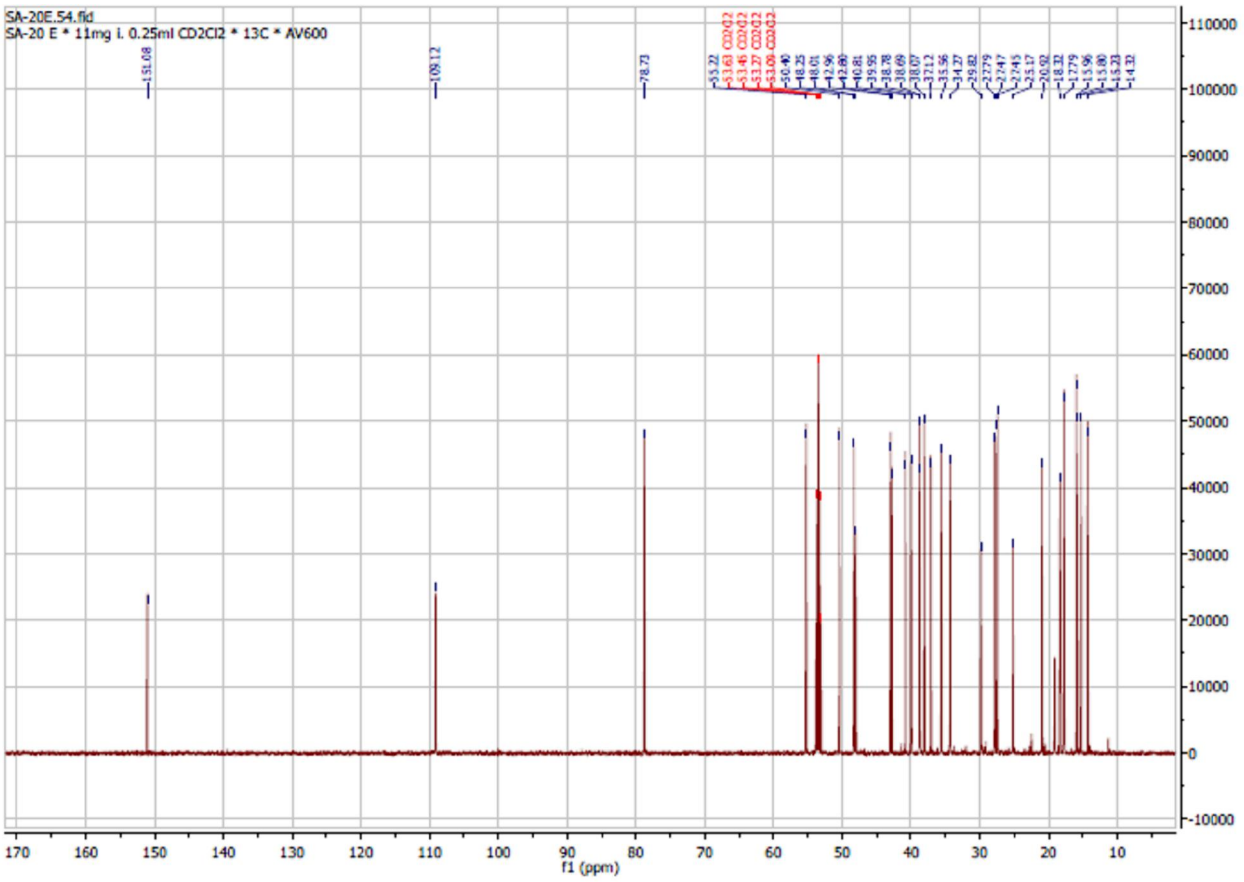
APPENDIX I: NMR Spectra for compound 63

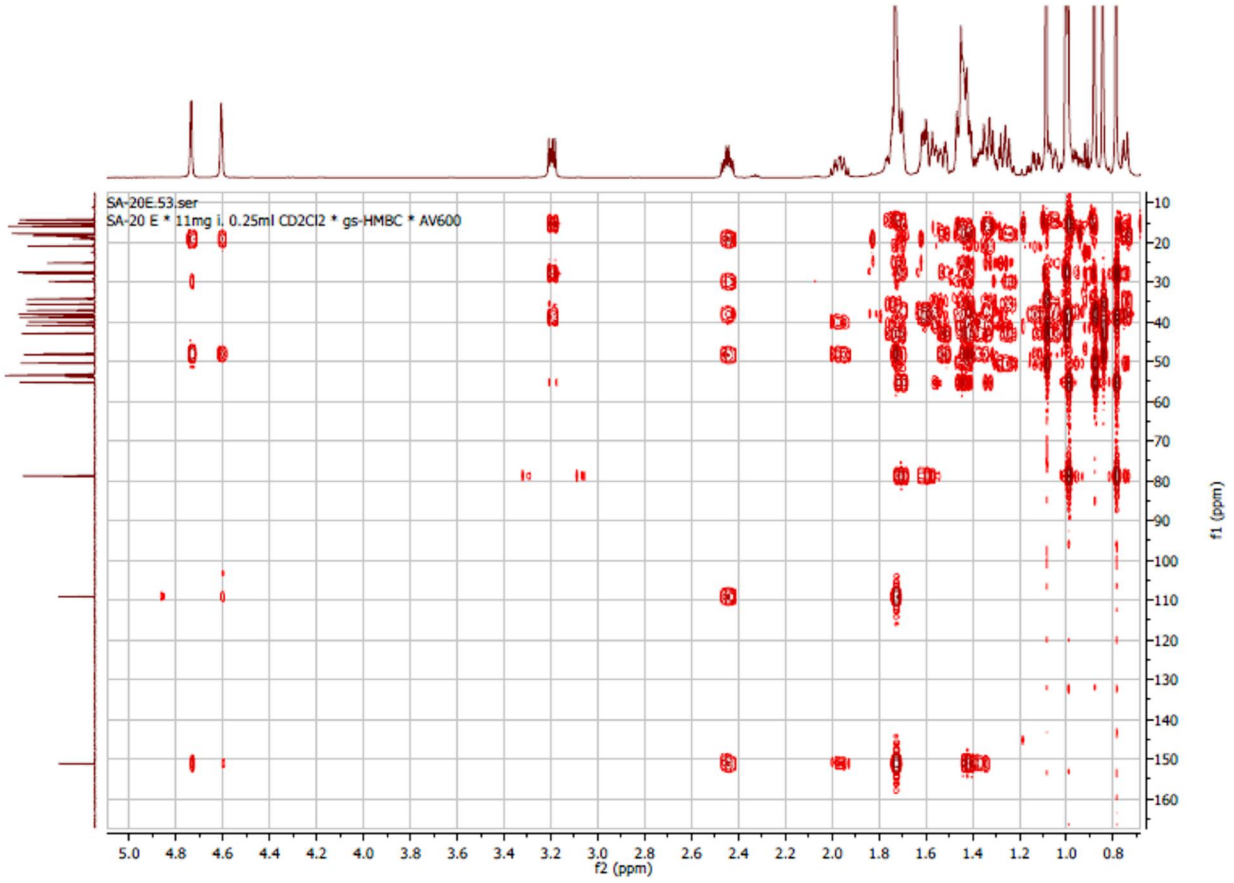
APPENDIX 1



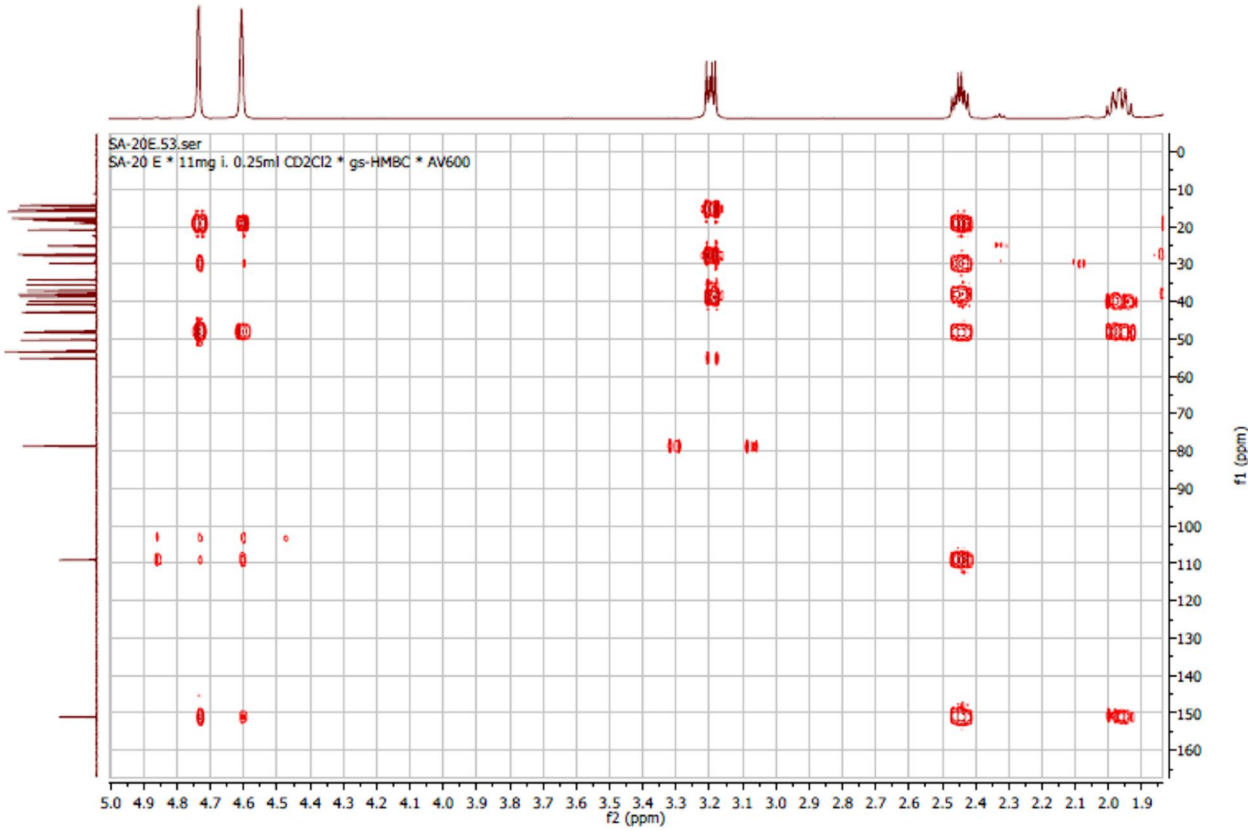
^{1}H NMR Spectrum for Lupeol (63)



^{13}C NMR Spectrum for Lupeol (63)



HMBC spectra for Lupeol (63)



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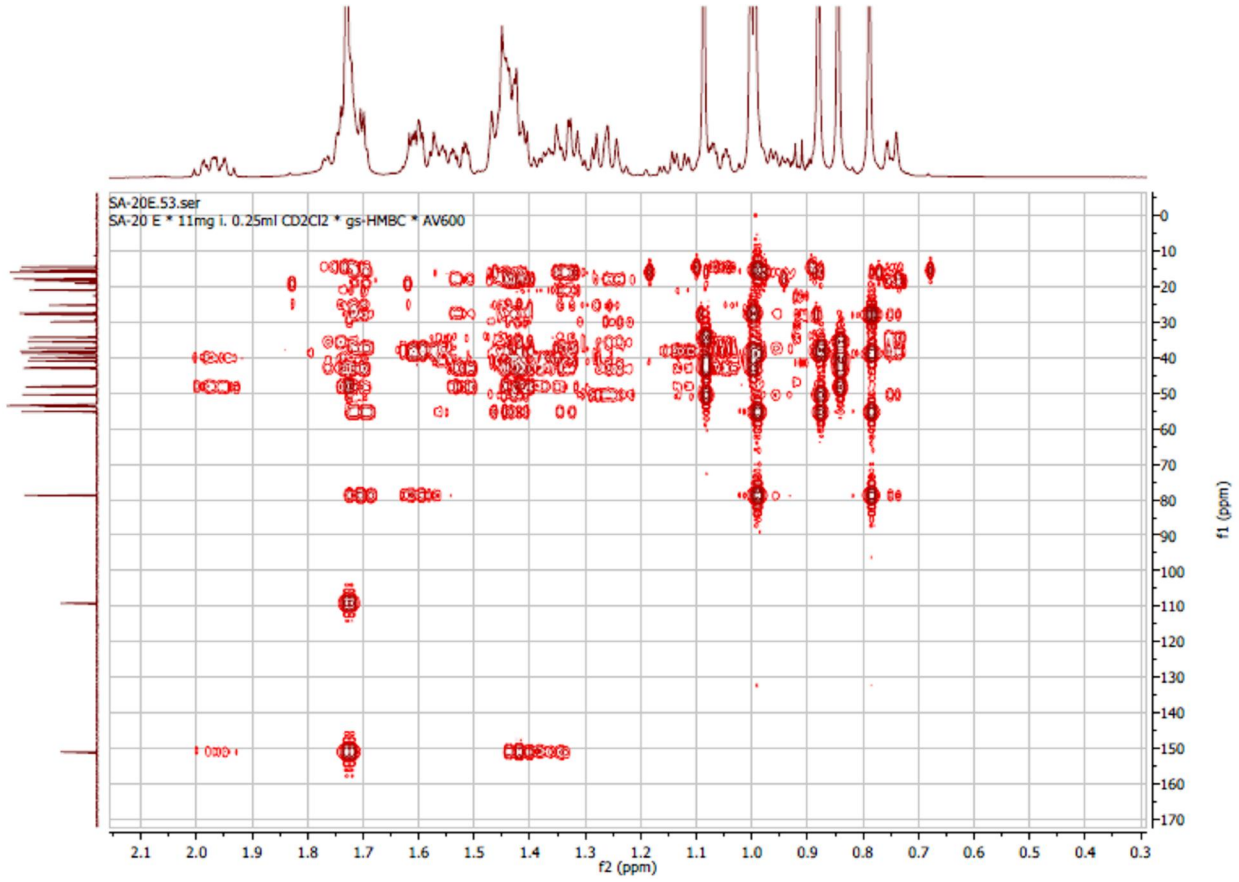
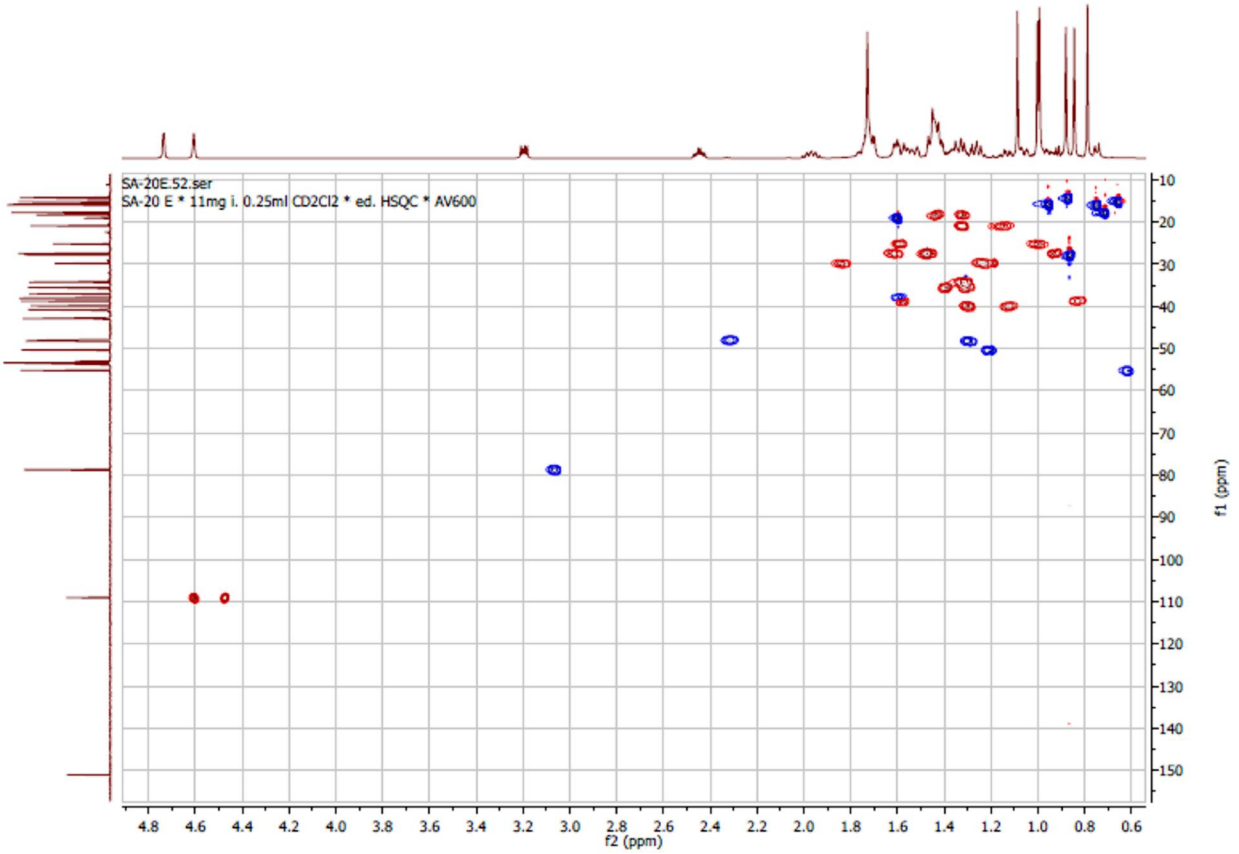
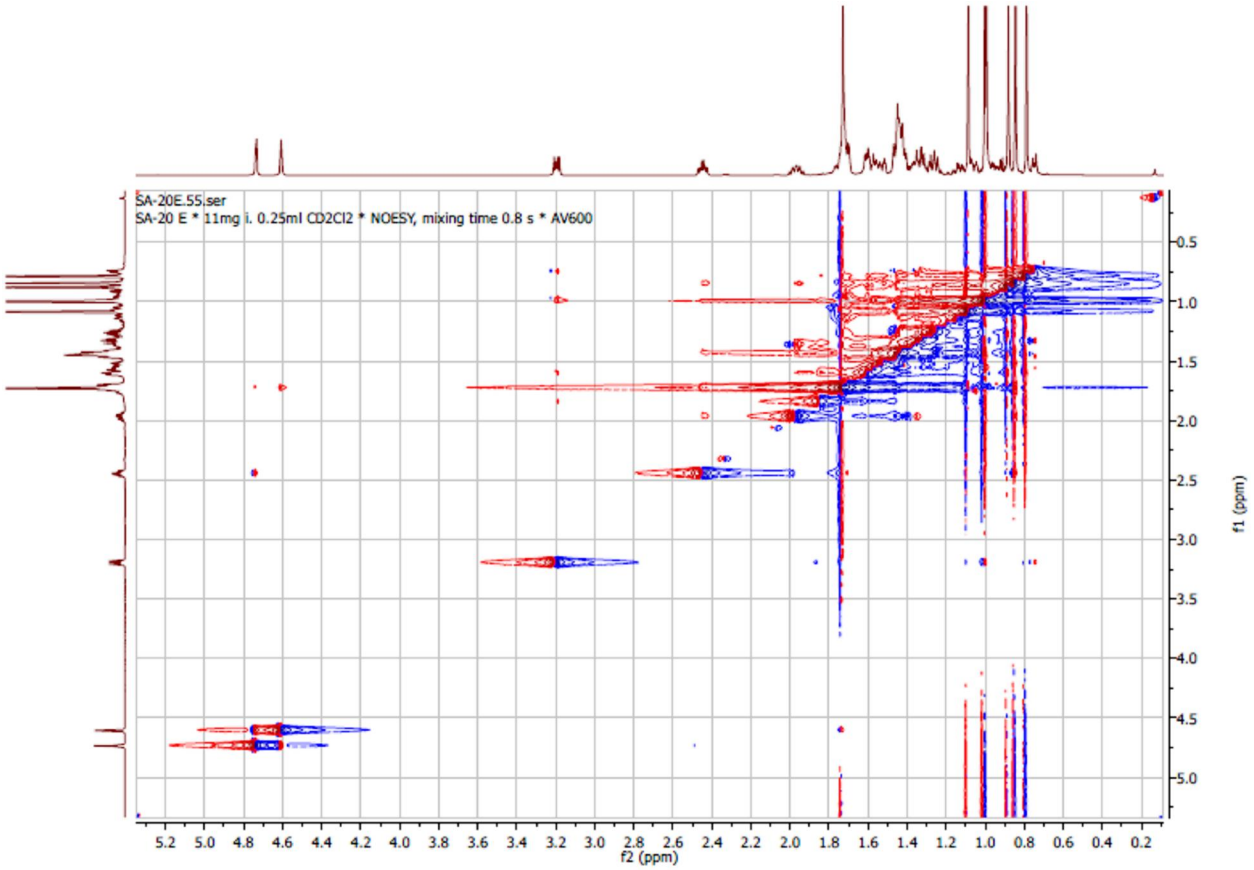


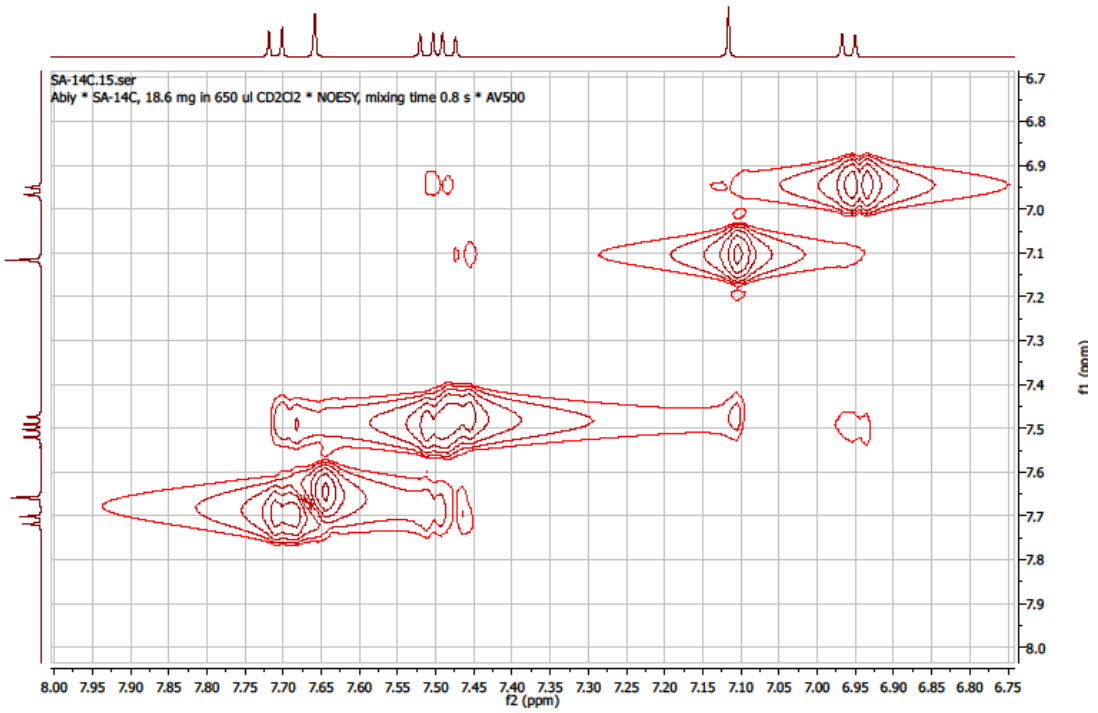
HMBC Spectrum for Lupeol (63)



HSQC Spectrum for Lupeol (63)



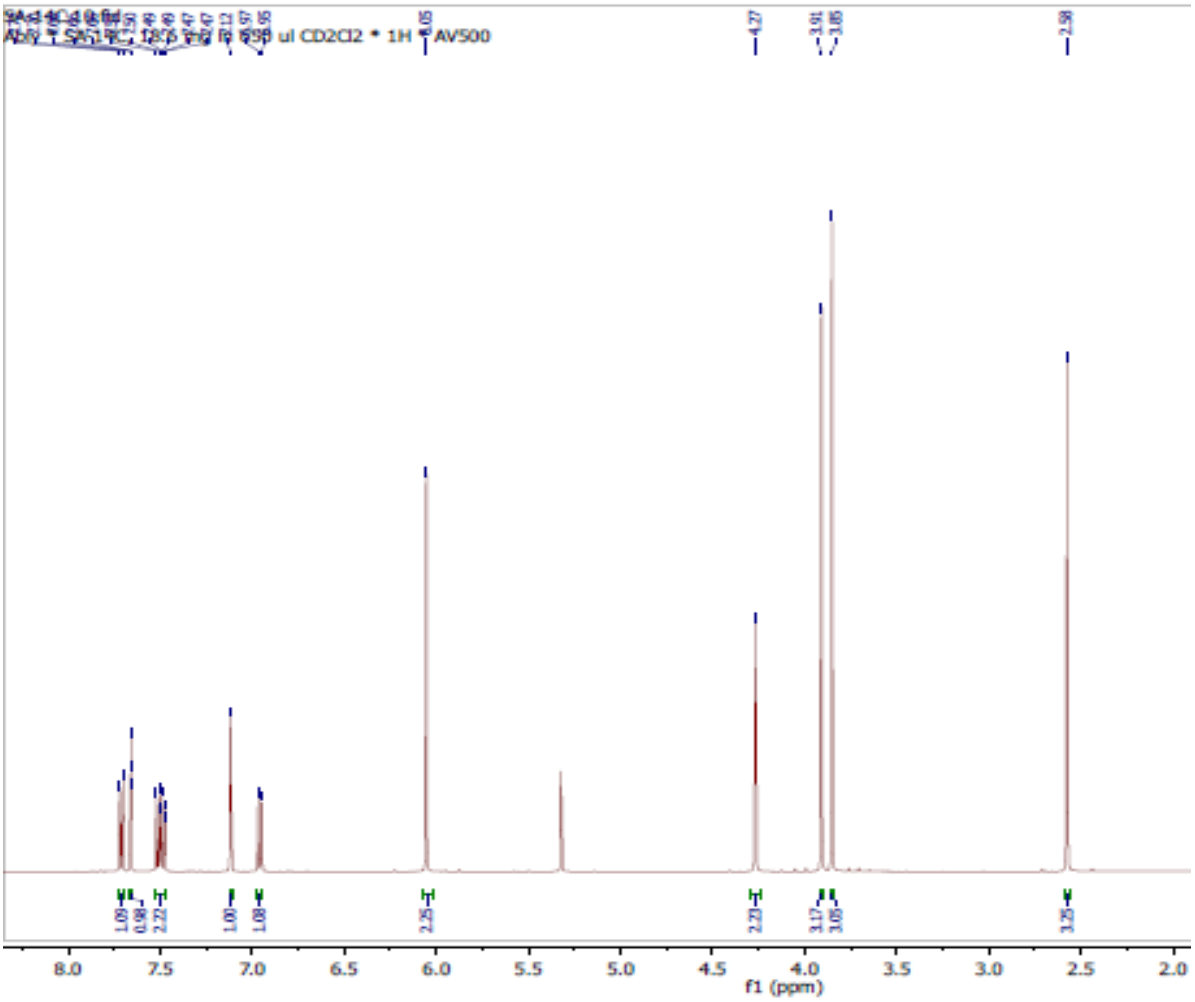
NOESY Spectrum for Lupeol (63)



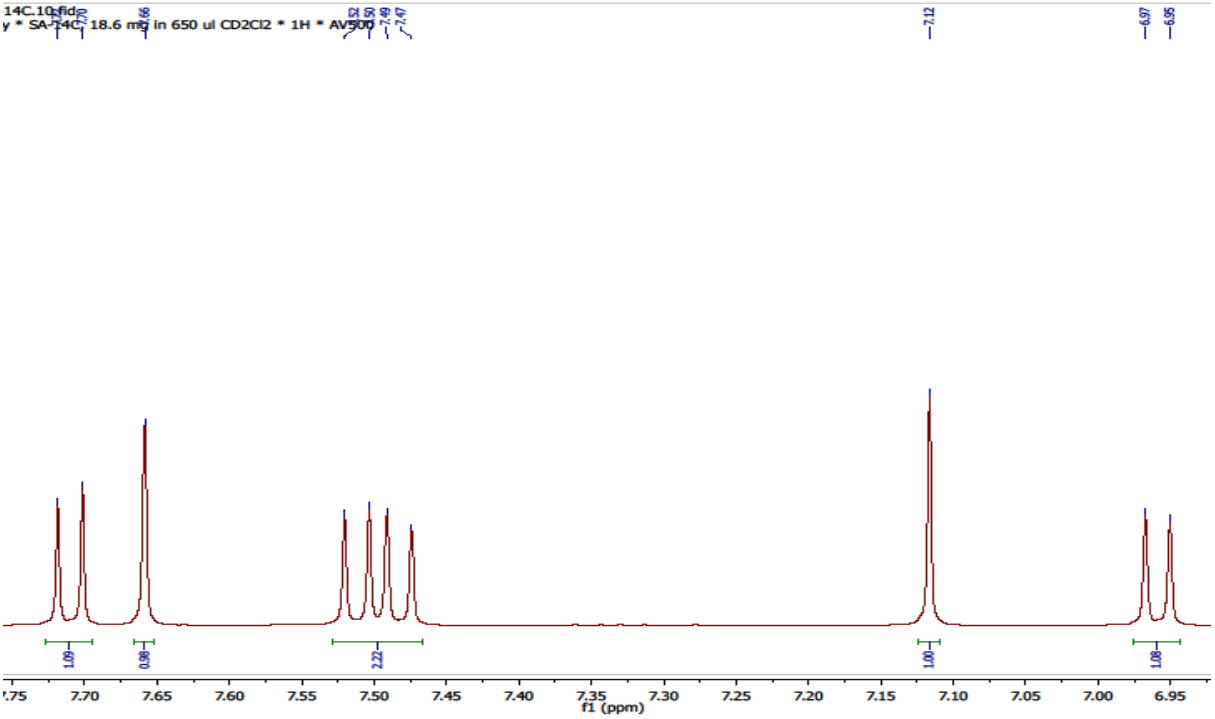
NOESY Spectrum for Lupeol (63)

APPENDIX II NMR Spectra for compound 64

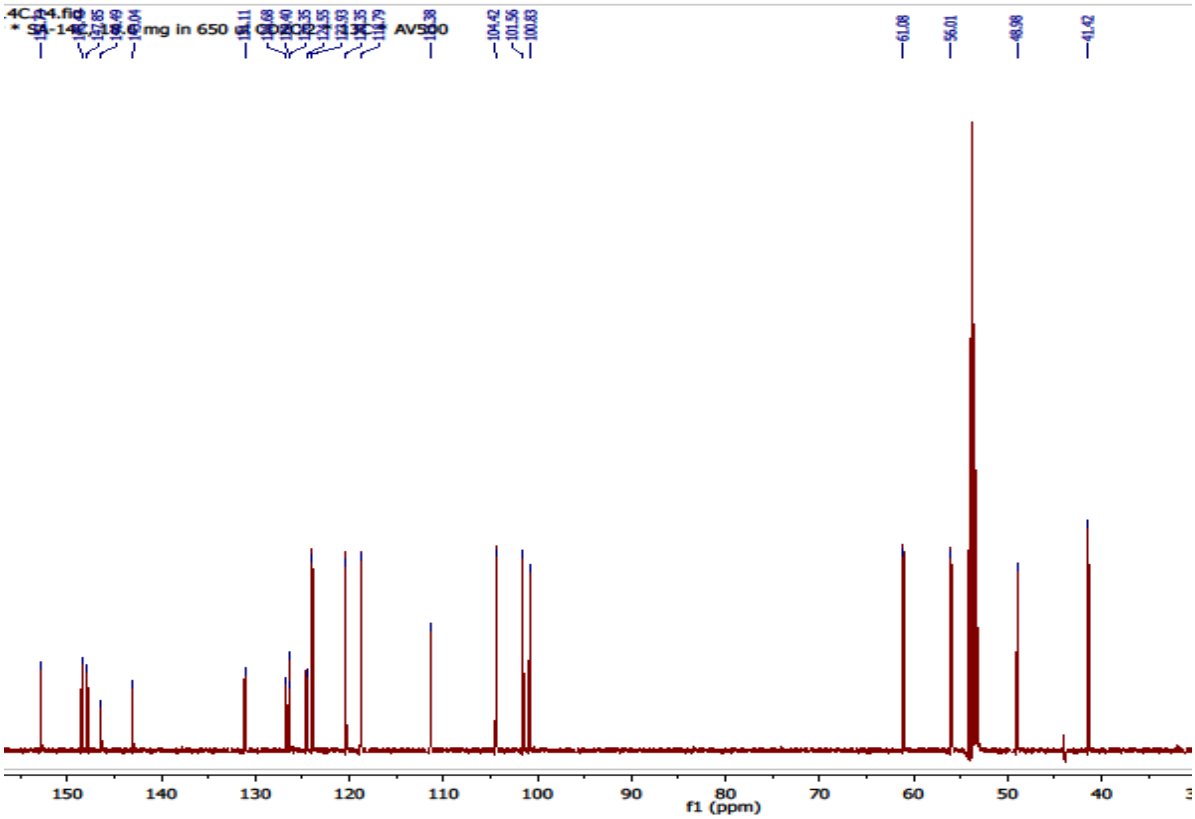
APPENDIX 64



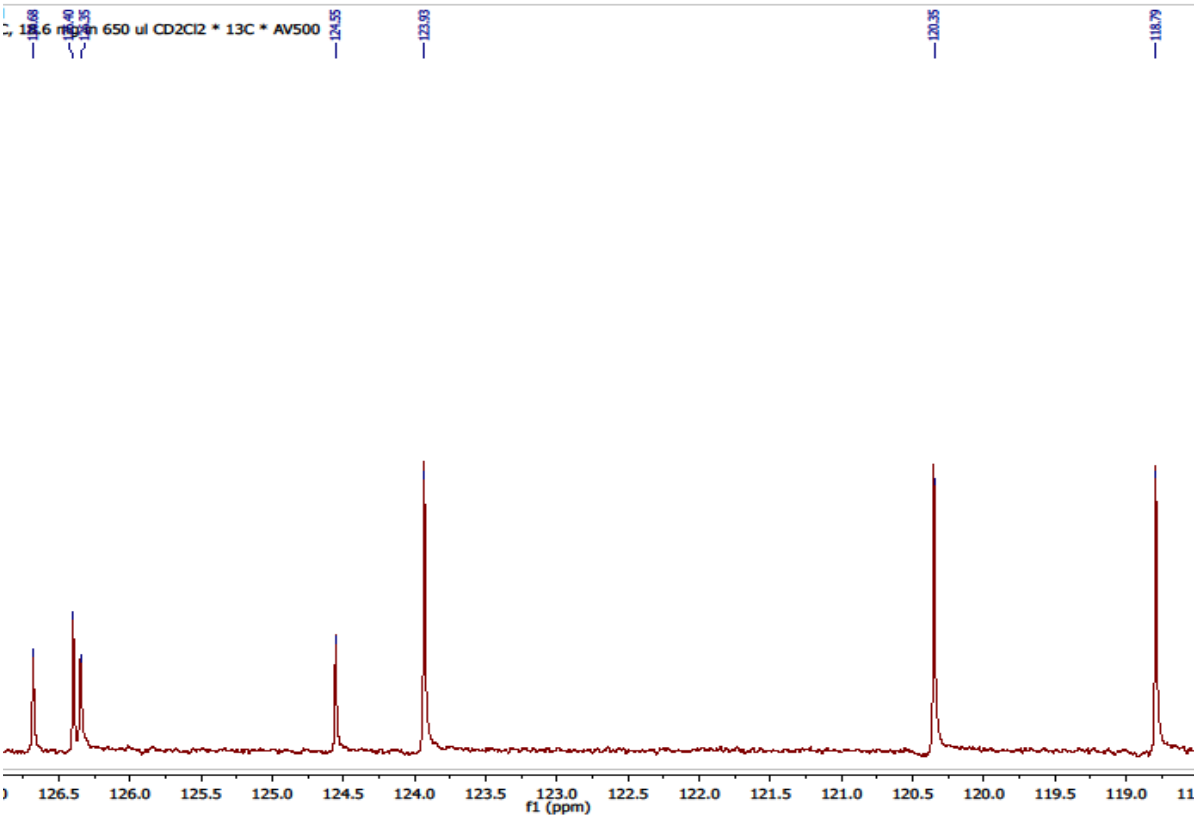
^{1}H NMR Spectrum for Dihydrochelerythrine (64)



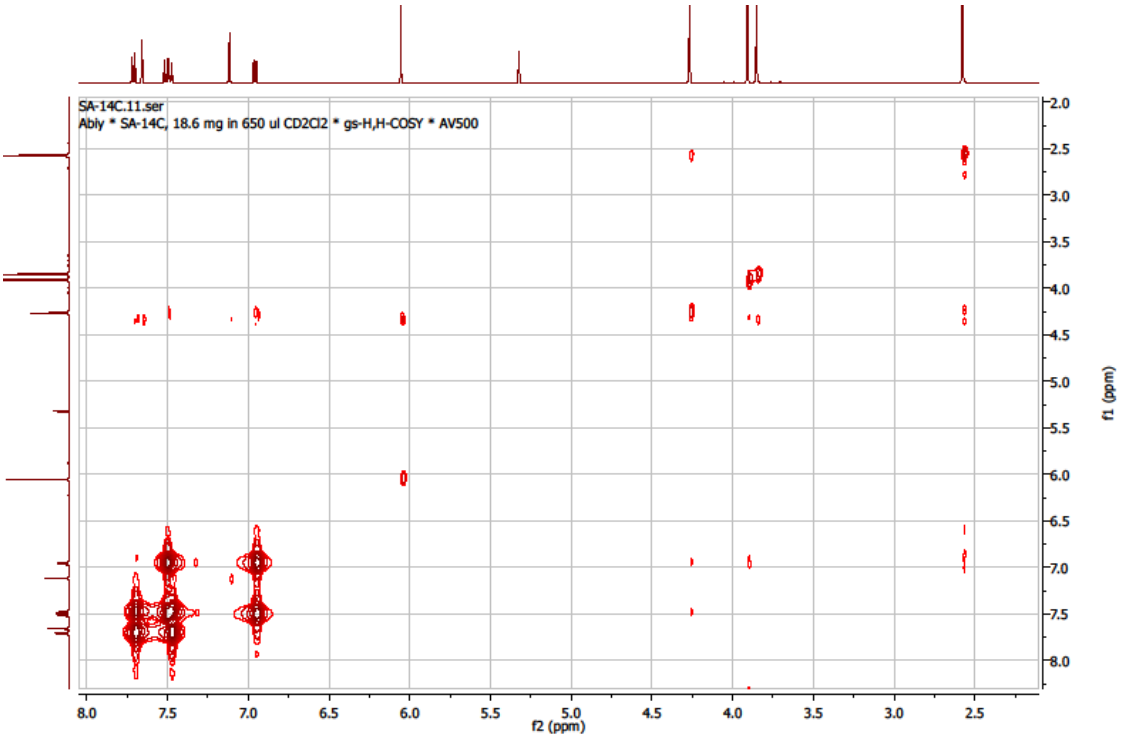
^{1}H NMR Spectrum for Dihydrochelerythrine (64)



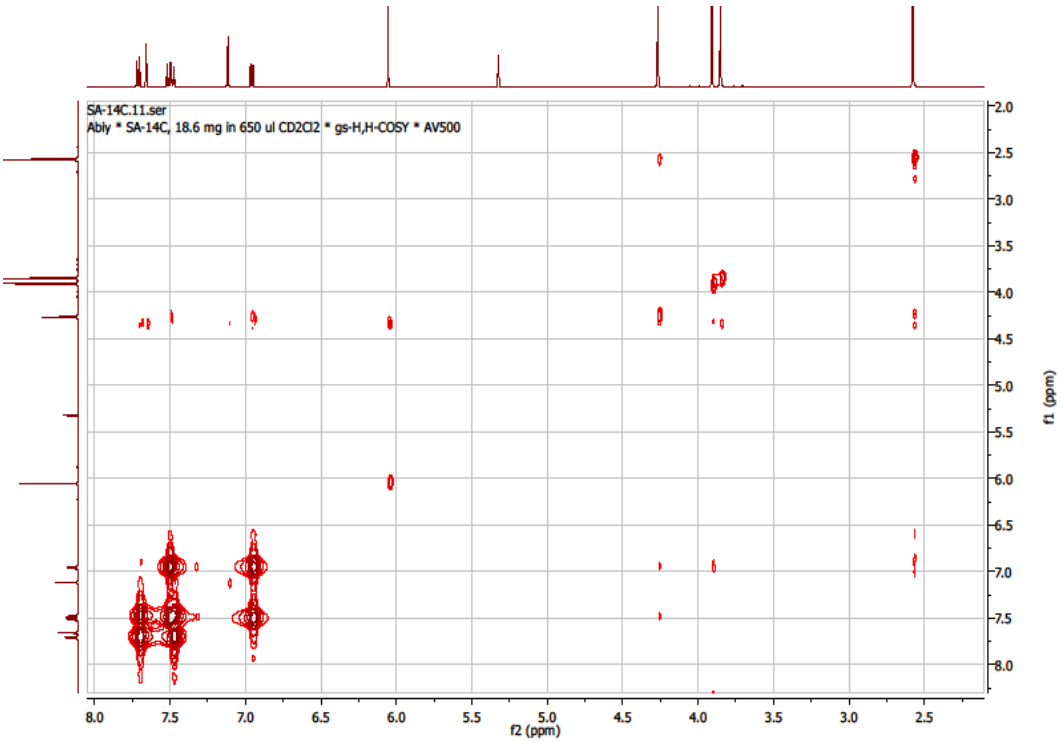
^{13}C NMR Spectrum for Dihydrochelerythrine (64)



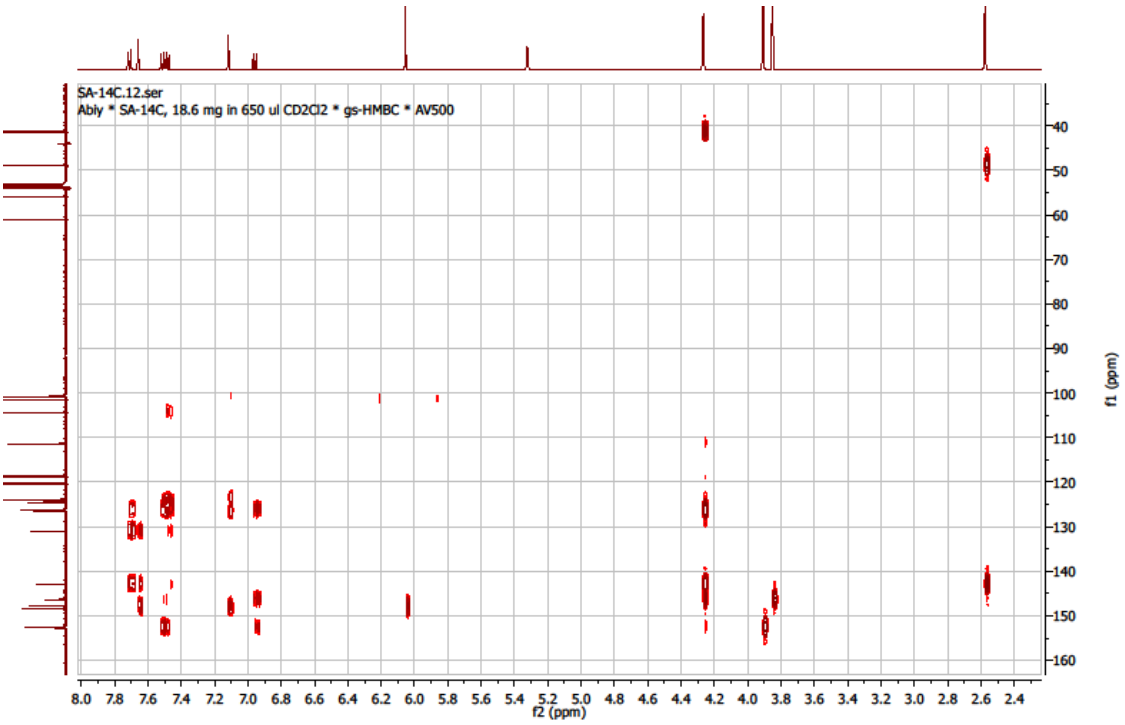
^{13}C NMR Spectrum for Dihydrochelerythrine (64)



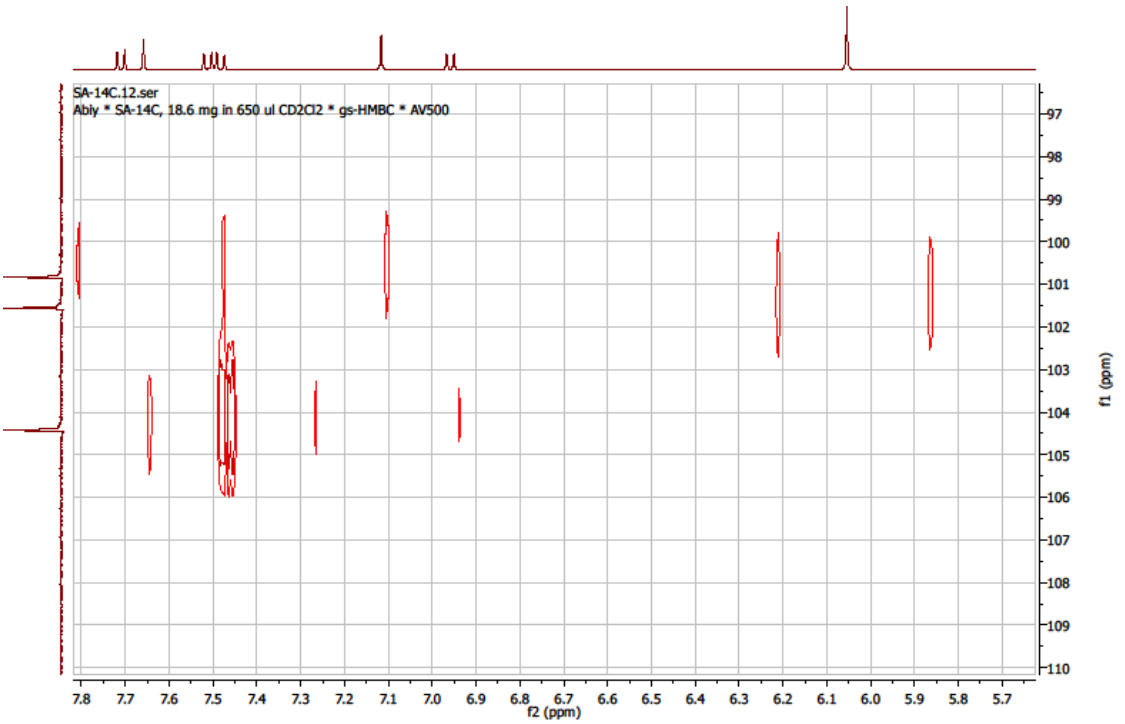
HMBC Spectrum for Dihydrochelerythrine (64)



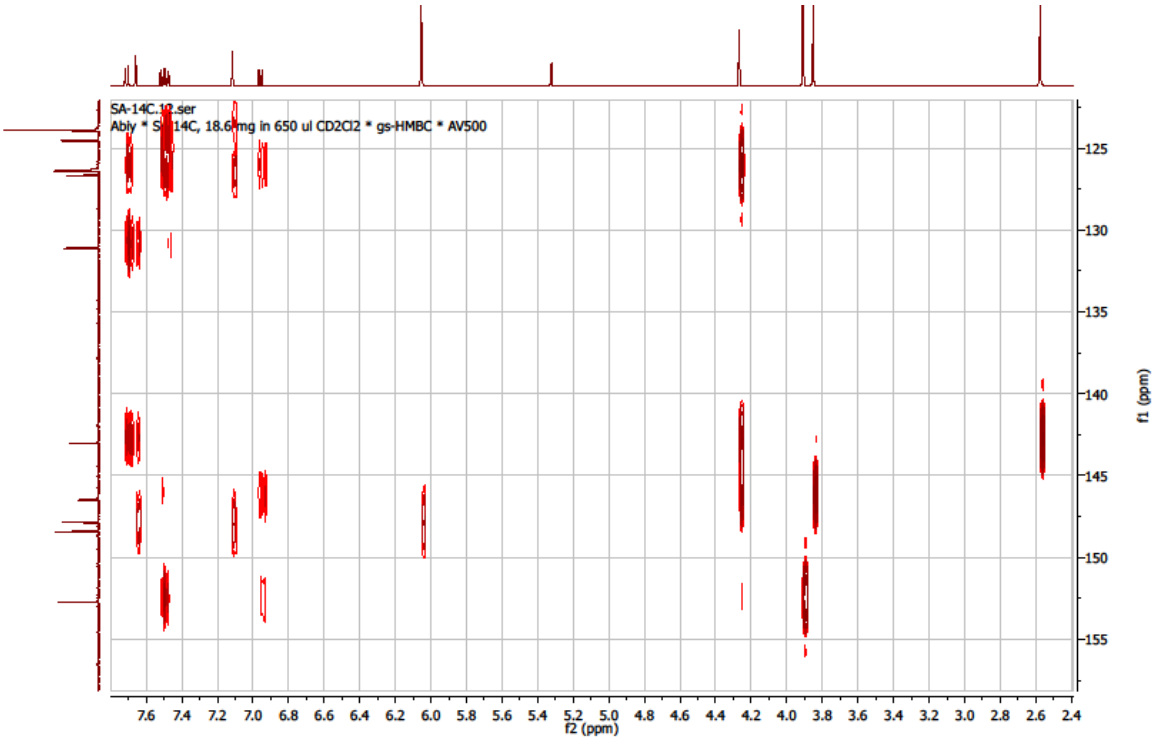
HMBC Spectrum for Dihydrochelerythrine (64)



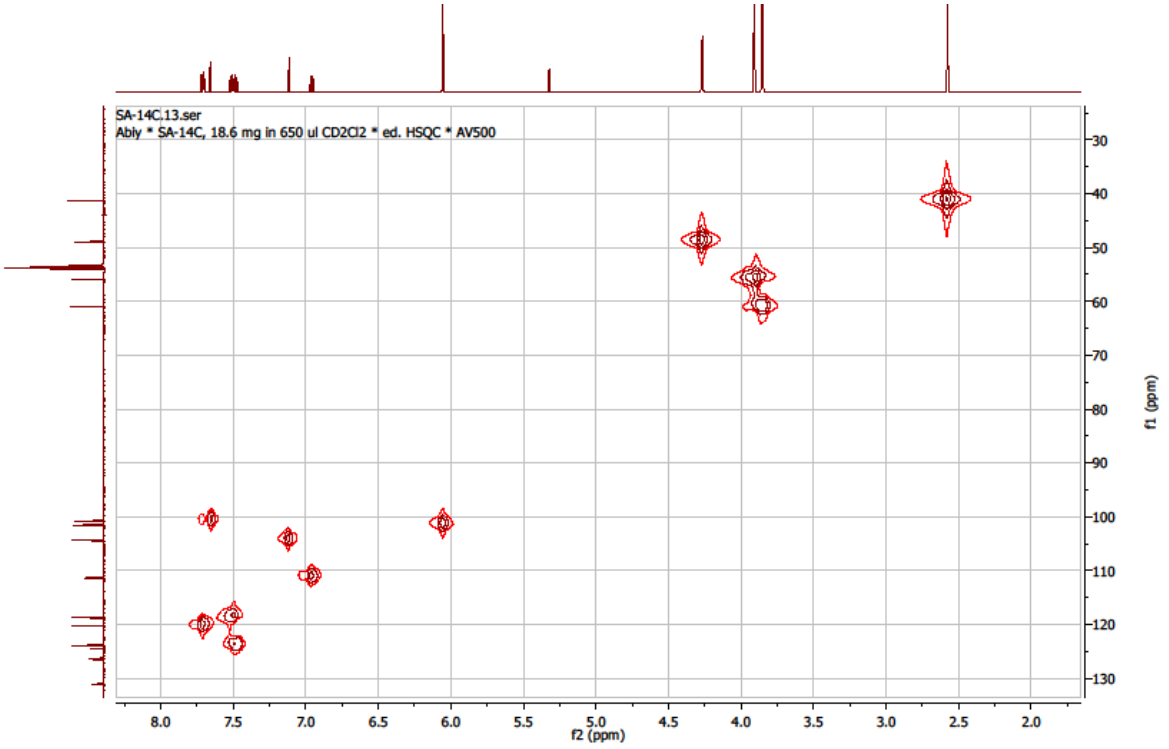
HMBC Spectrum for Dihydrochelerythrine (64)



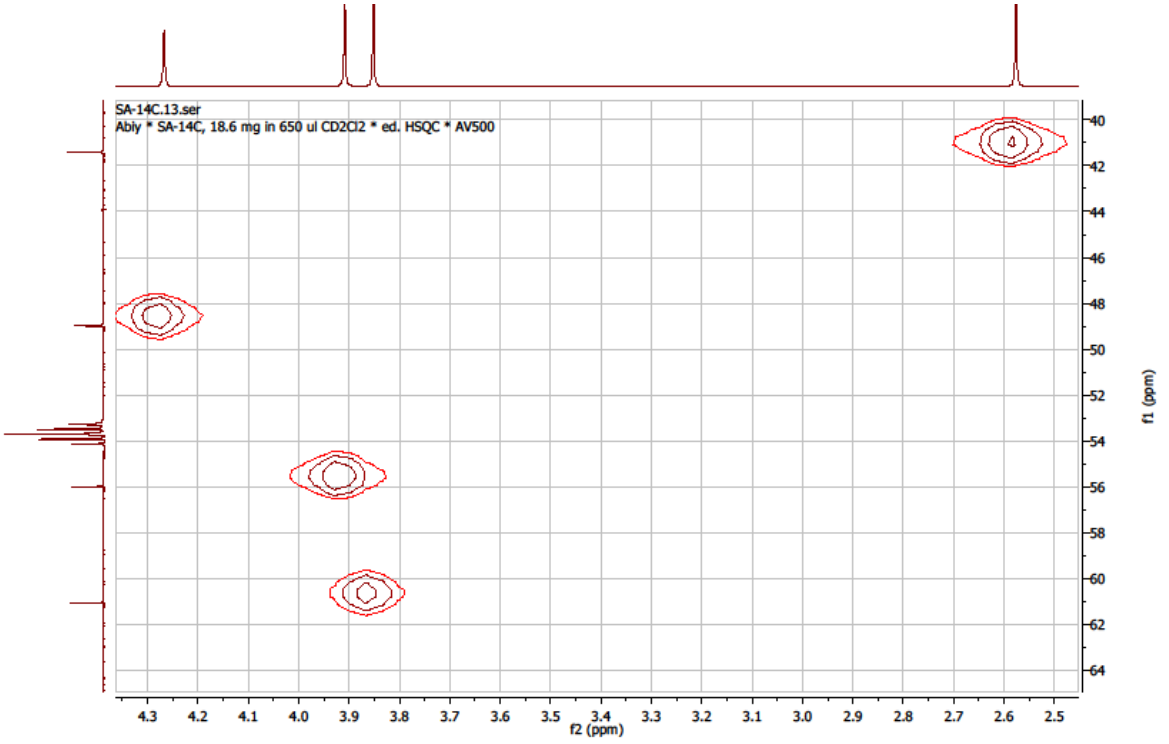
HMBC Spectrum for Dihydrochelerythrine (64)



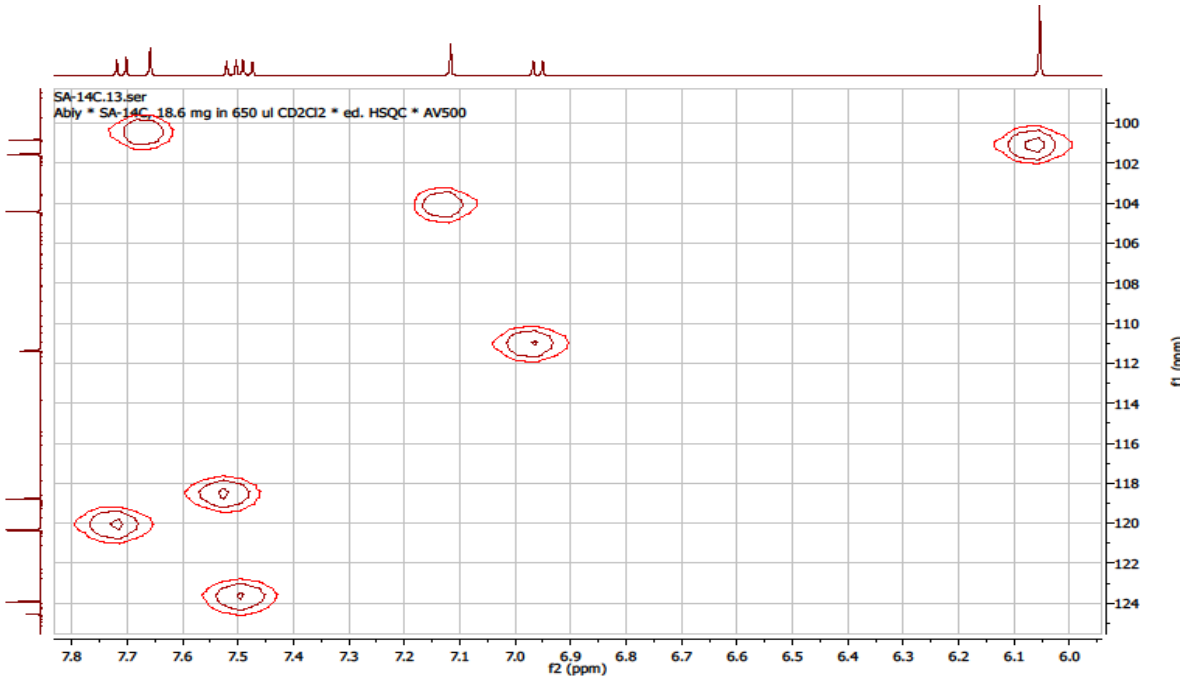
HMBC Spectrum for Dihydrochelerythrine (64)



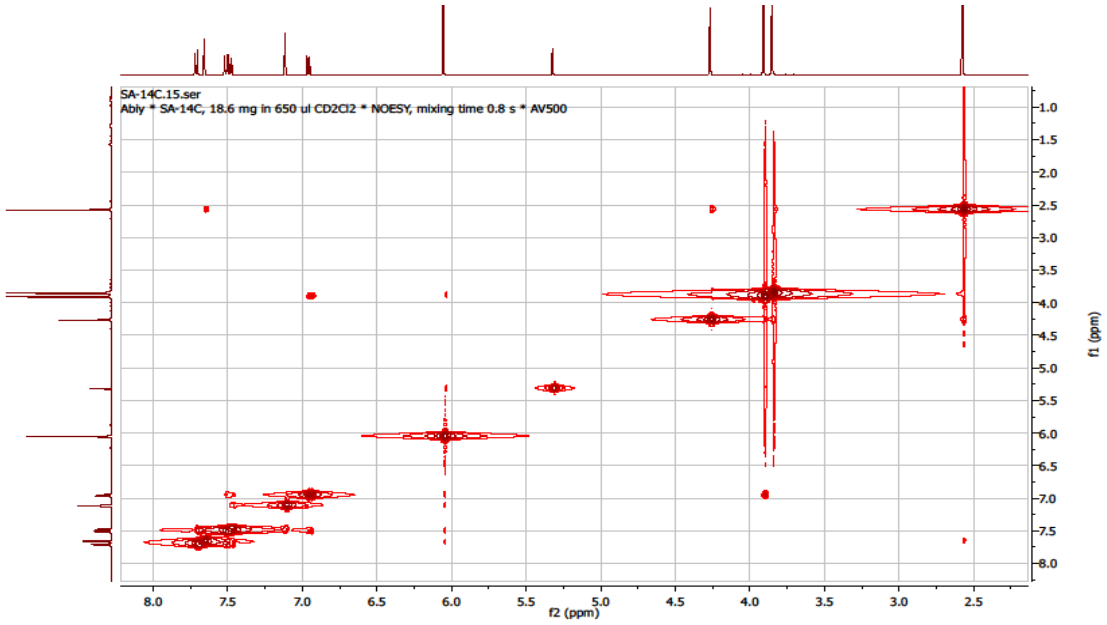
HSQC Spectrum for Dihydrochelerythrine (64)



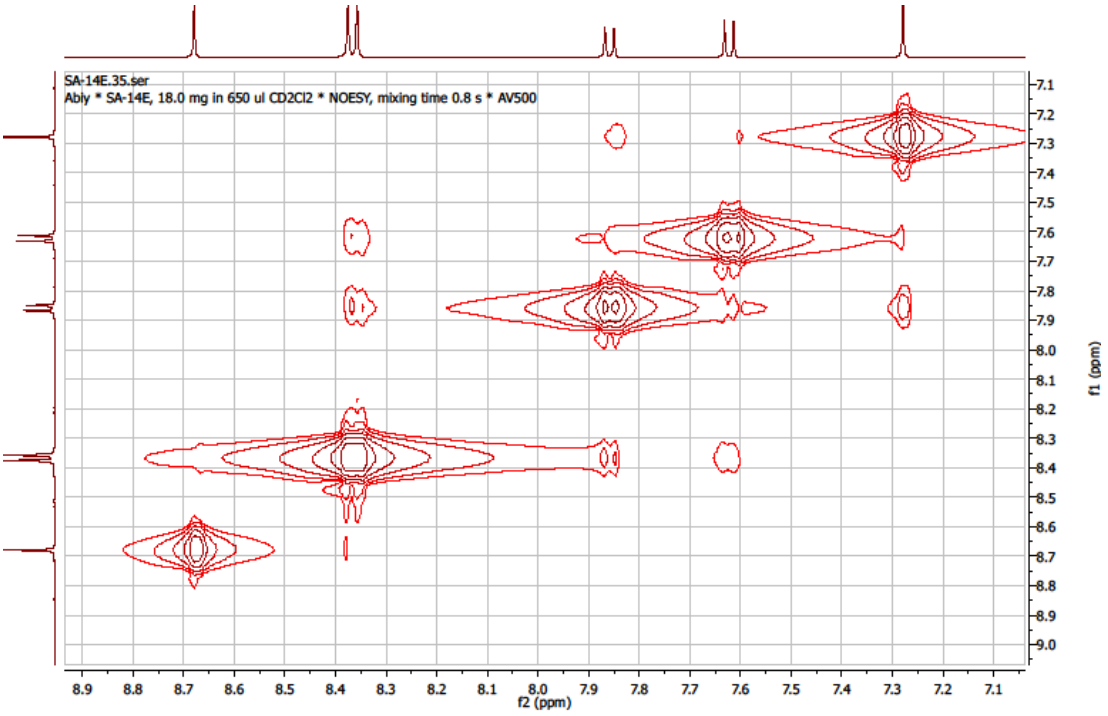
HSQC for dihydrochelerythrine (64)



NOESY for dihydrochelerythrine (64)



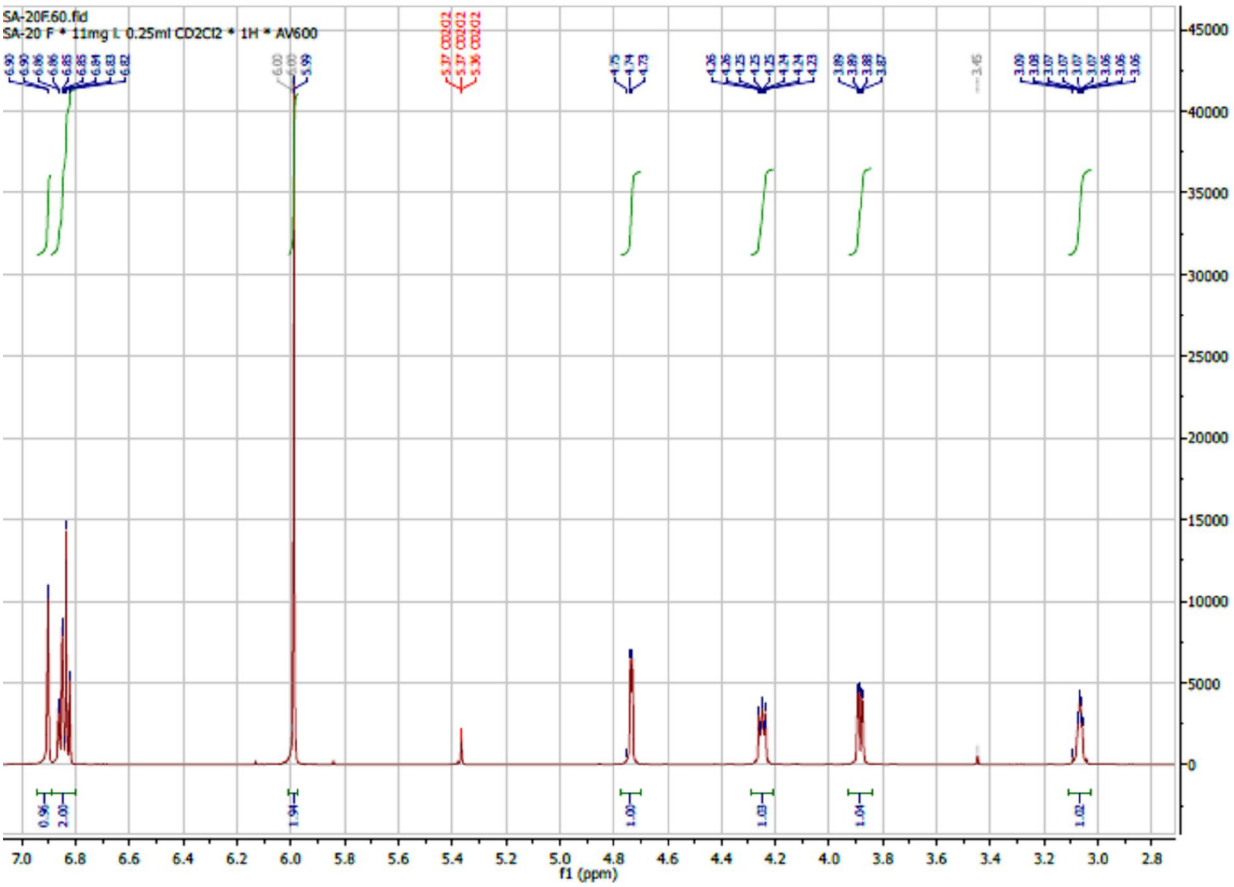
- **NOESY for dihydrochelerythrine (64)**



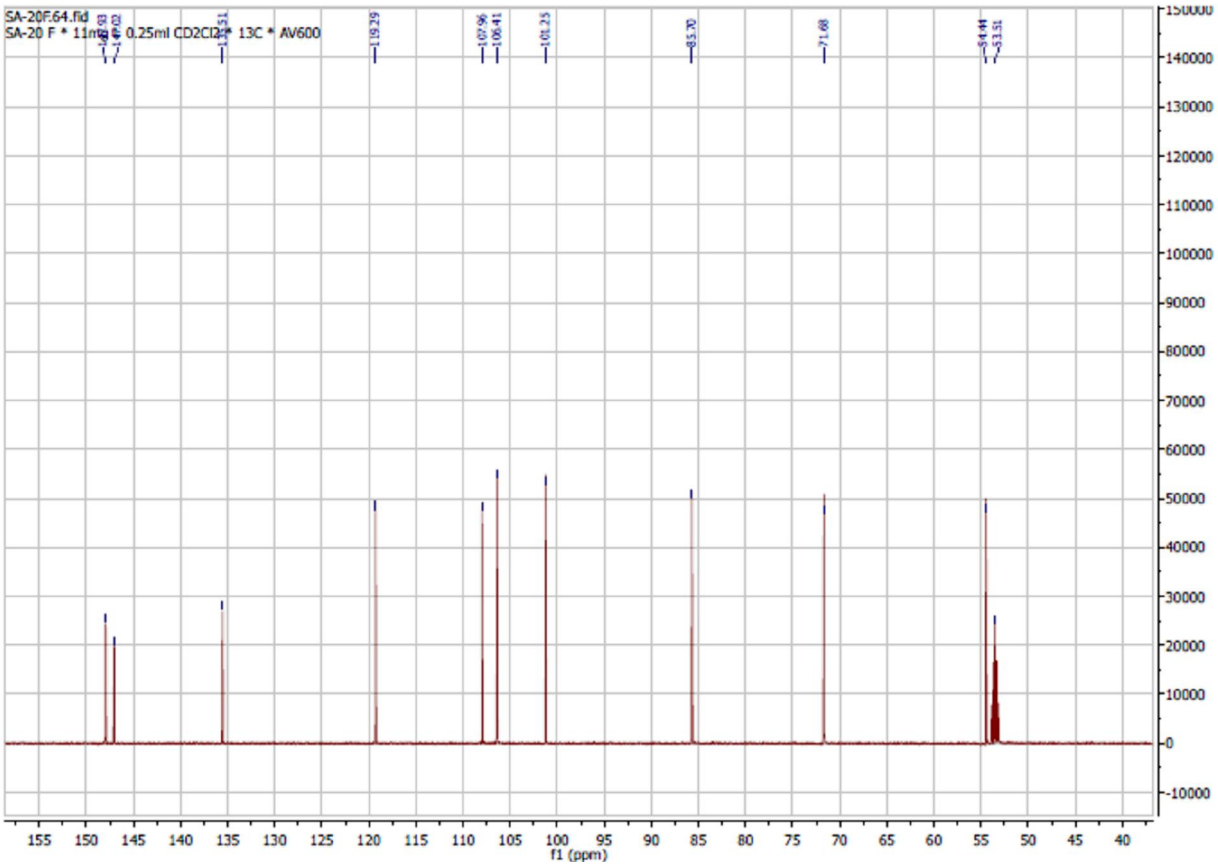
NOESY for dihydrochelerythrine (64)

APPENDIX III: NMR Spectra for compound 65

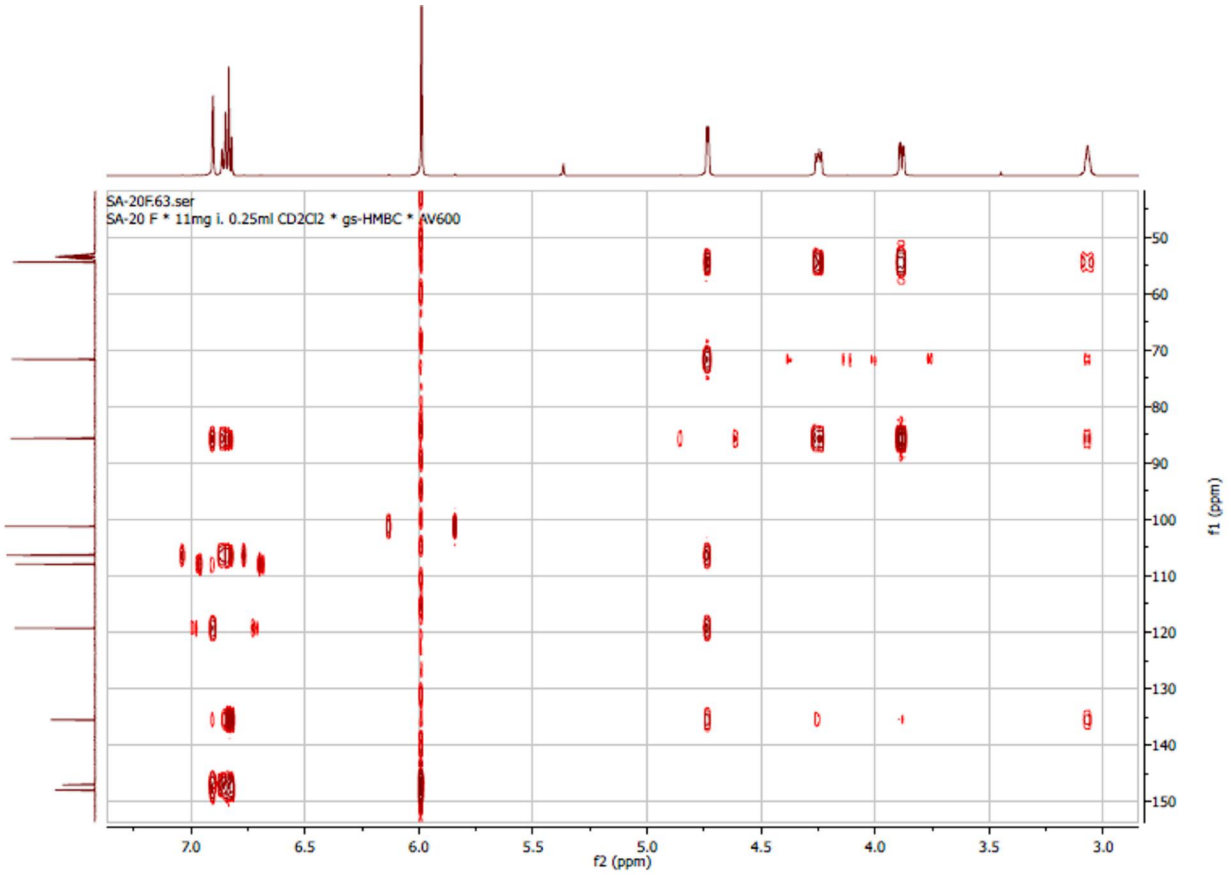
APPENDIX 65



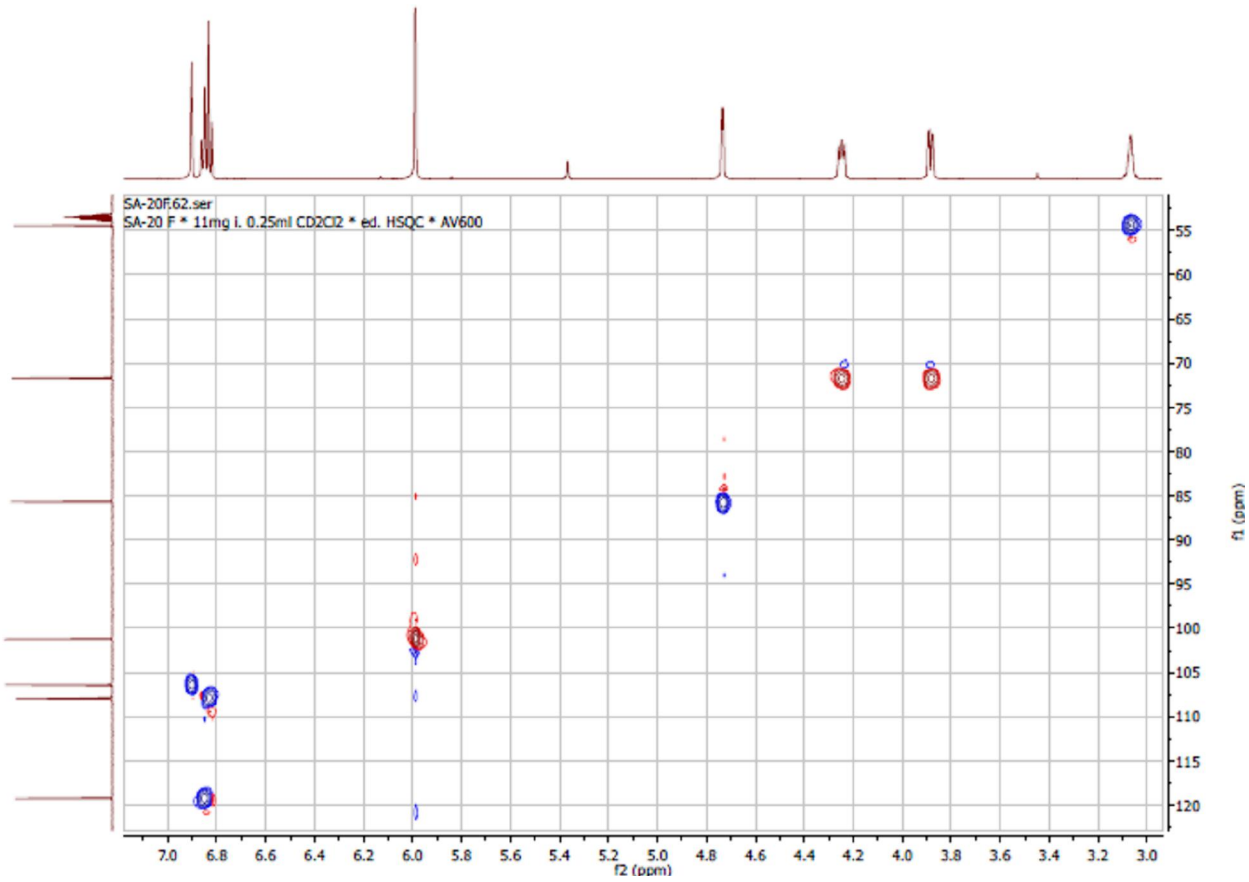
^{1}H NMR for seasamine (65)



^{13}C NMR for seasamine (65)



HMBC for seasamine (65)

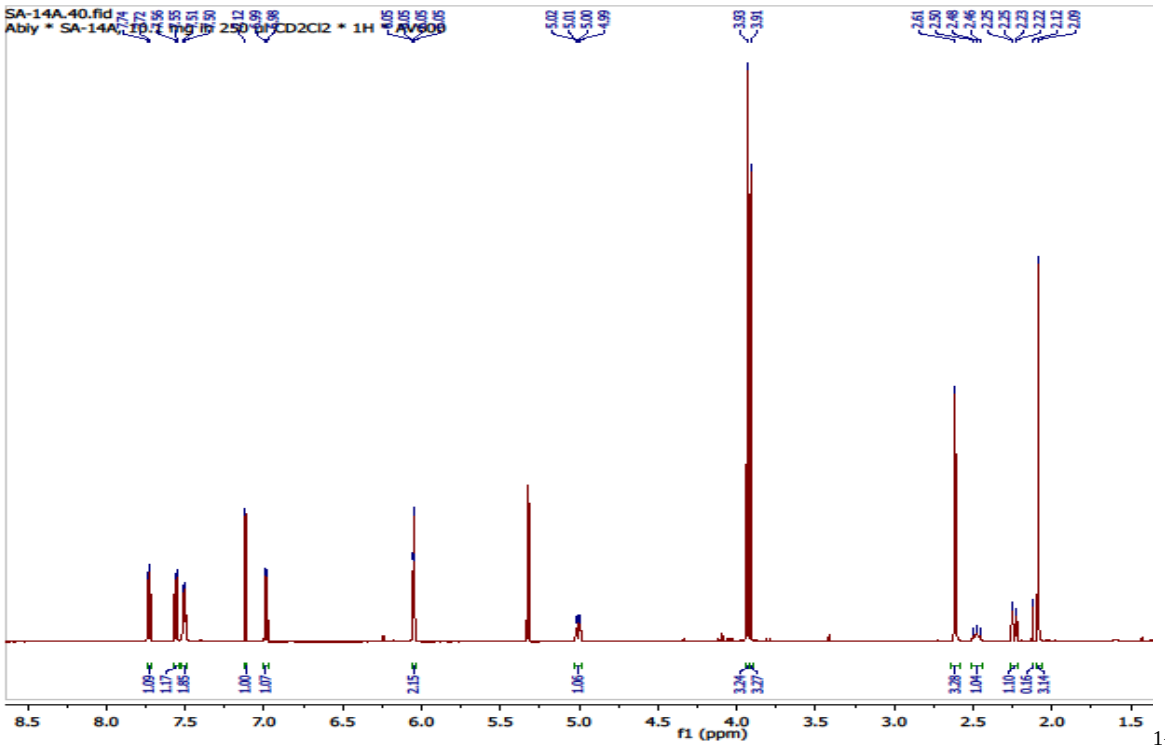


HSQC for seasamine (65)

APPENDIX IV NMR Spectra for compound 66

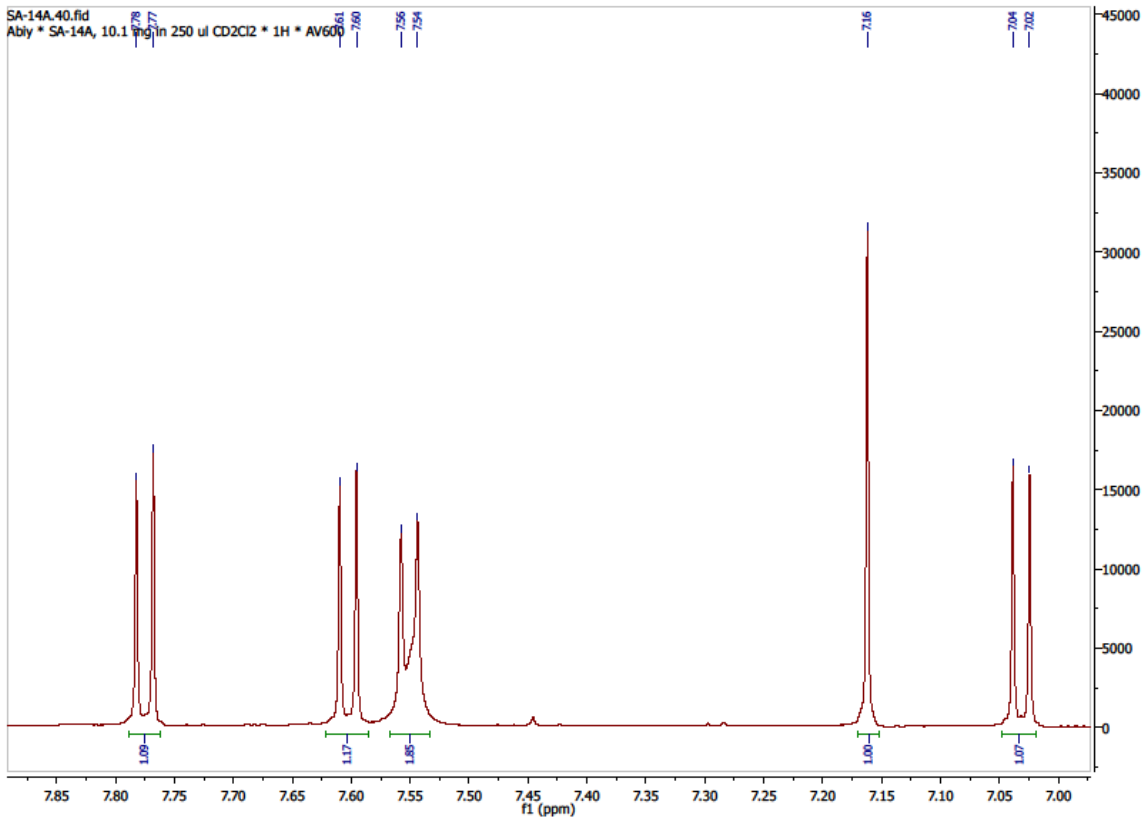
8-Acetonyldihydrochelerythrine (66)

APPENDIX 66

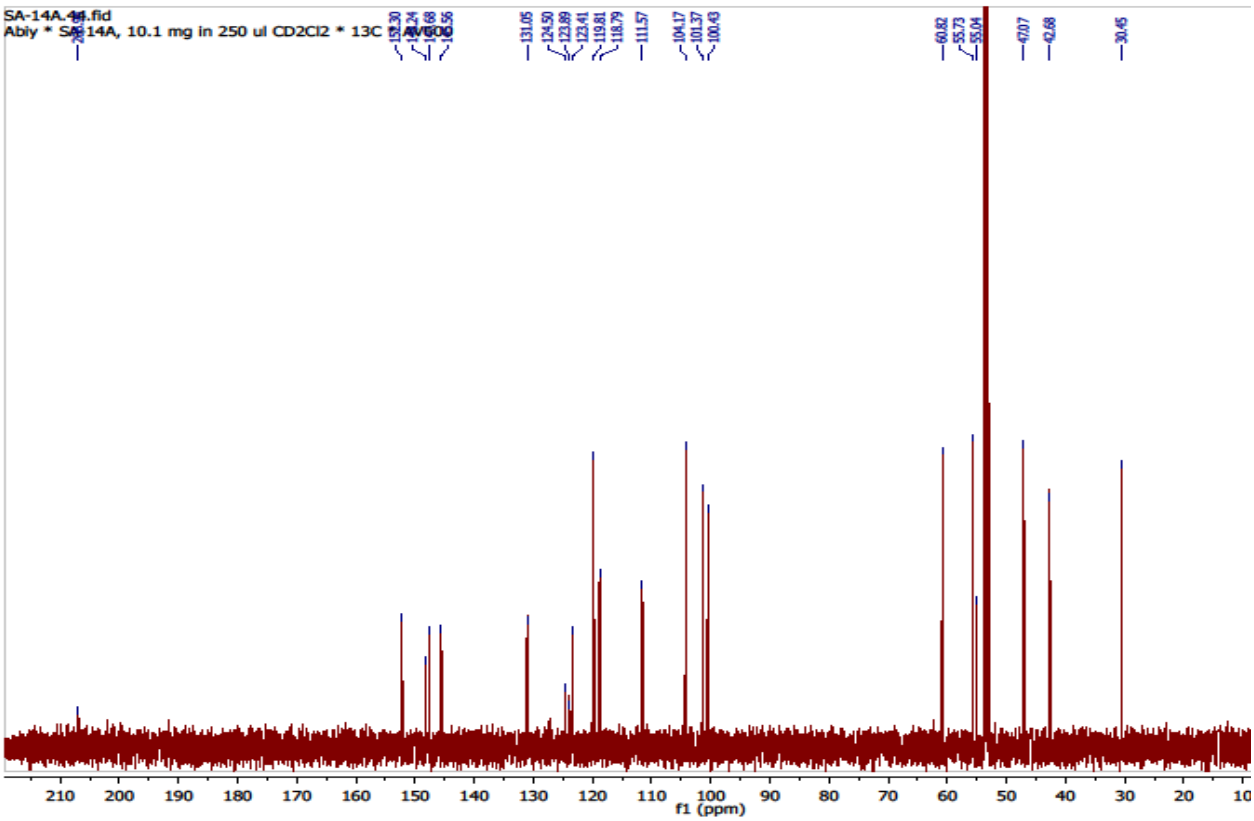


^{1}H

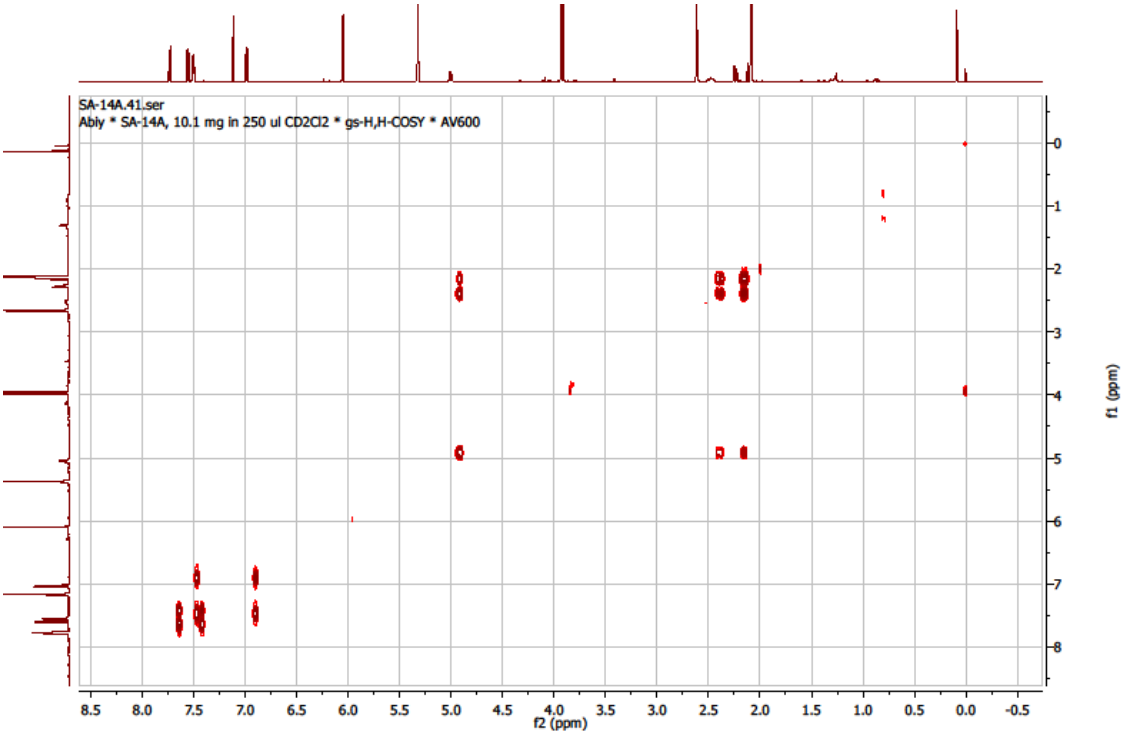
NMR spectrum for 8-acetonyldihydrochelerythrine (66)



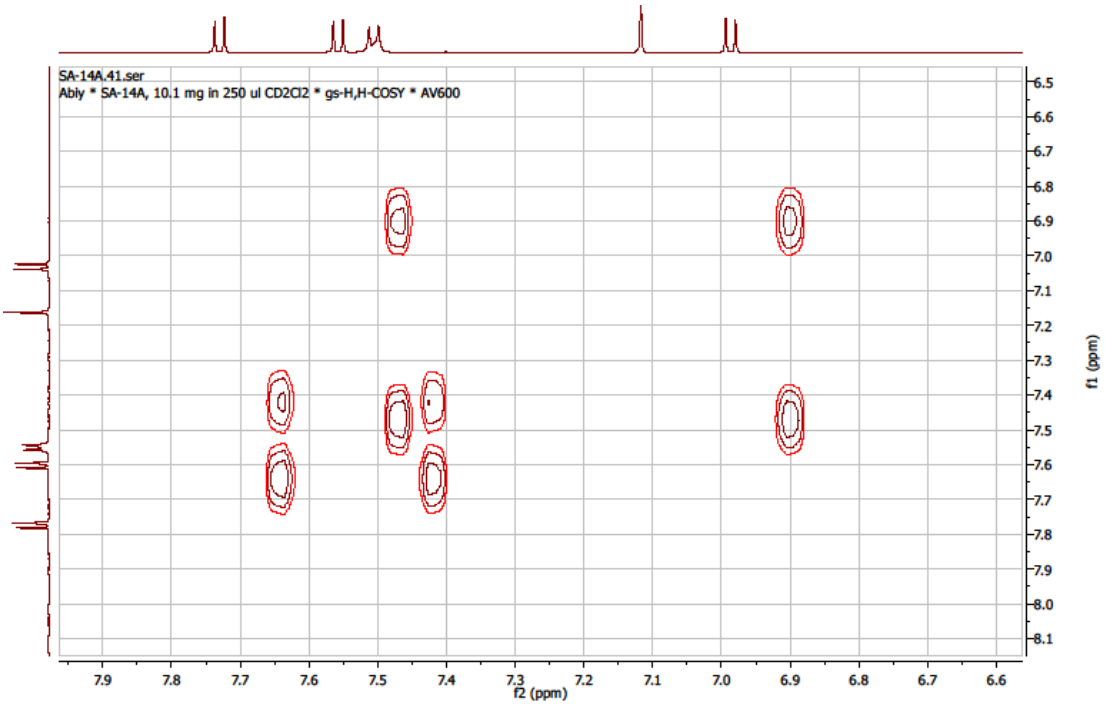
^{1}H NMR spectrum for 8-acetonyldihydrochelerythrine (66)



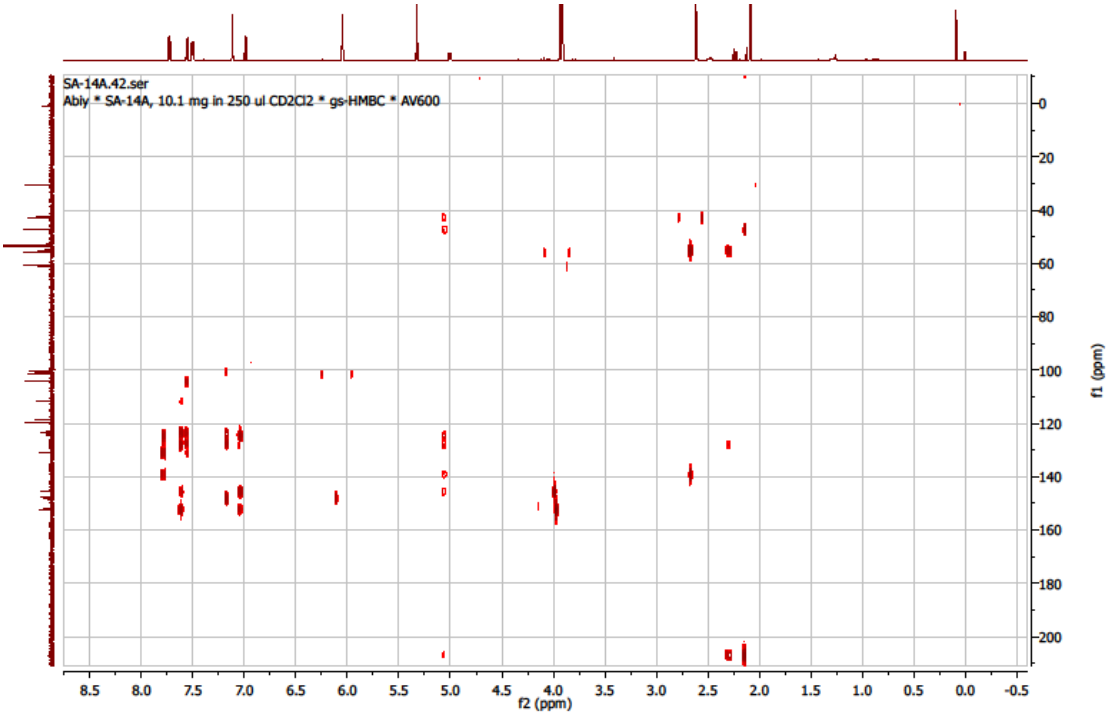
^{13}C NMR spectrum for 8-acetonyldihydrochelerythrine (66)



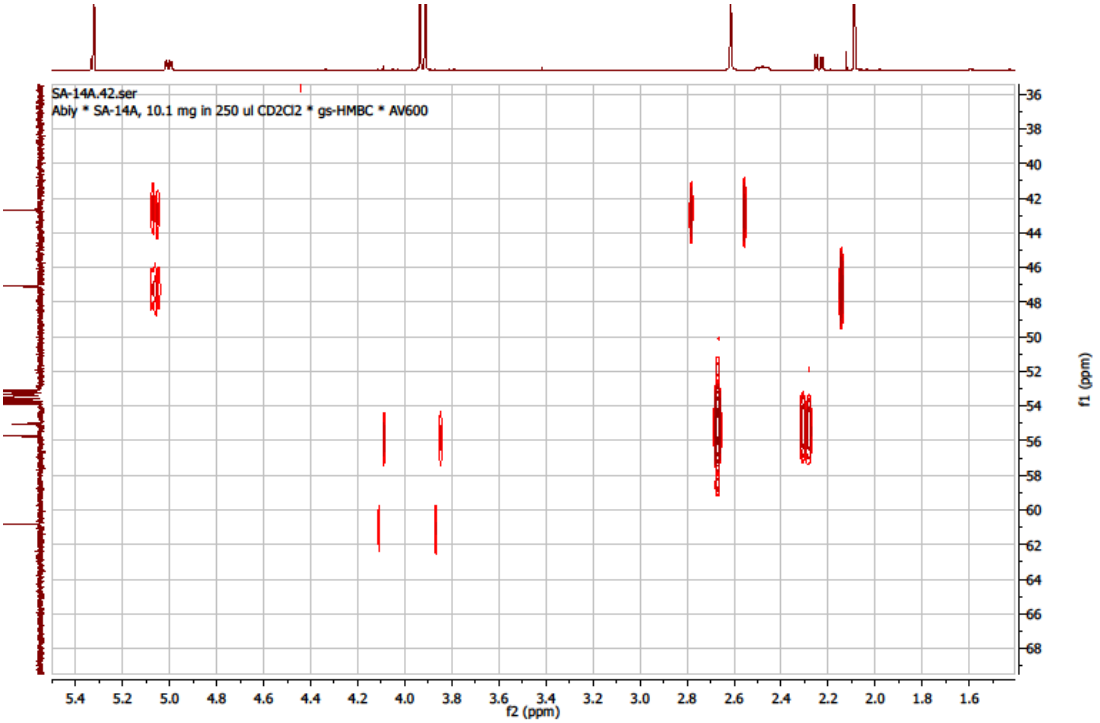
^{1}H, ^{1}H-COSY for 8-acetonyldihydrochelerythrine (66)



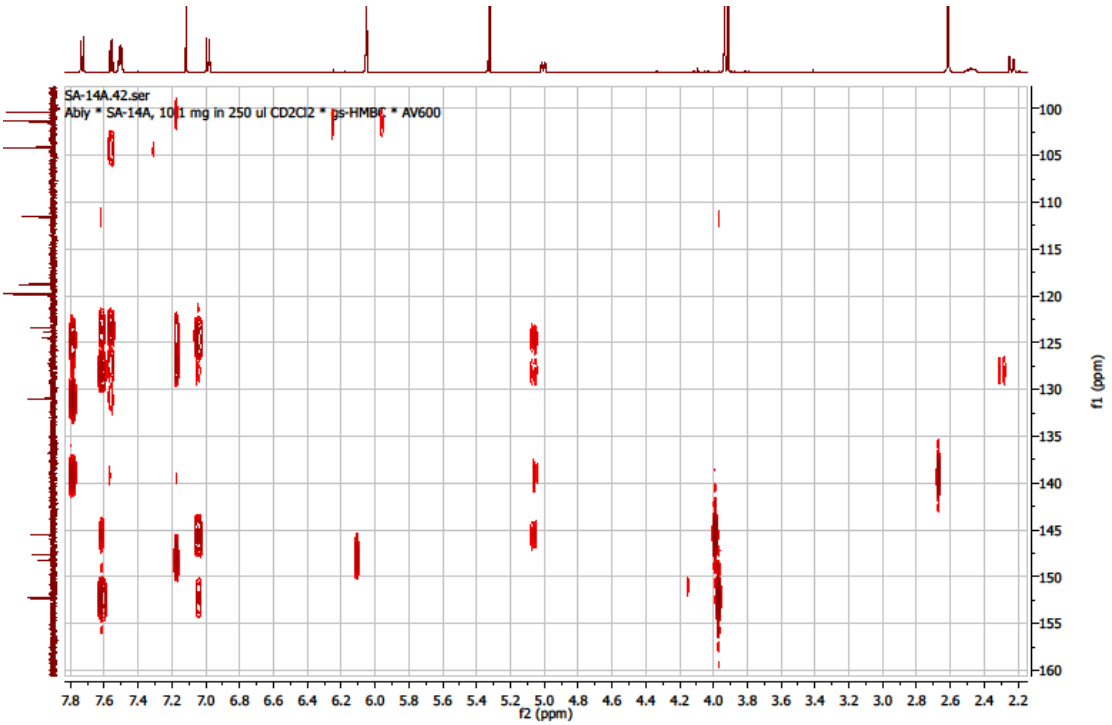
^{1}H, ^{1}H-COSY for 8-acetonyldihydrochelerythrine (expansion) (66)



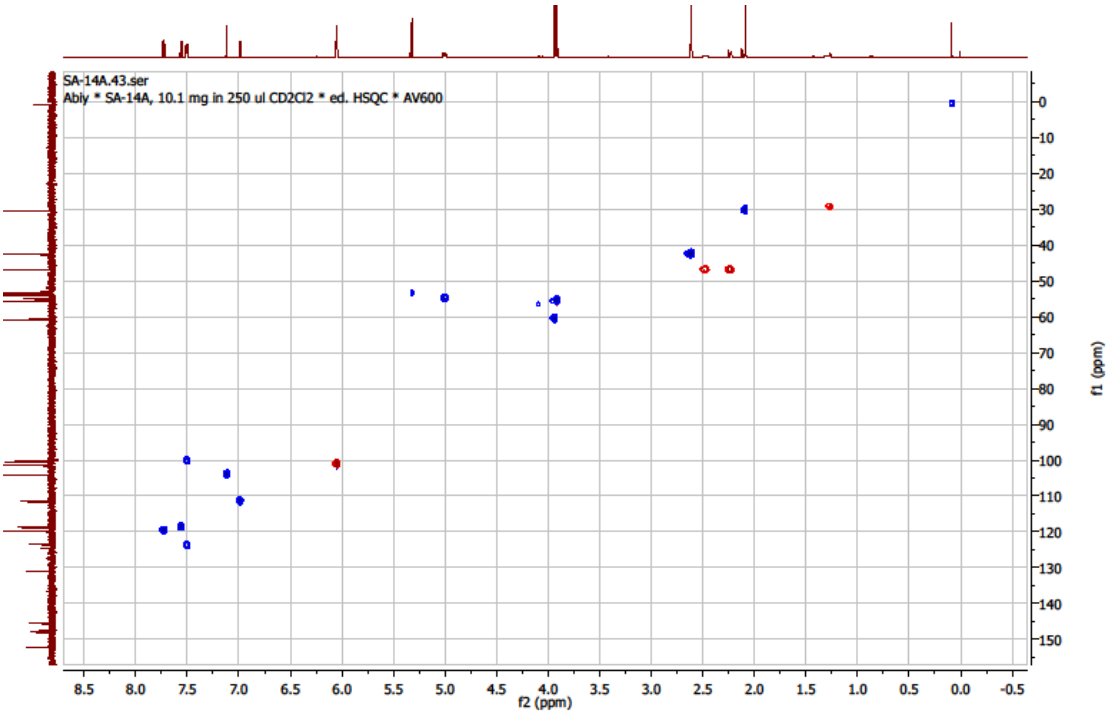
HMBC for 8-acetonyldihydrochelerythrine (66)



HMBC for 8-acetonyldihydrochelerythrine (66)

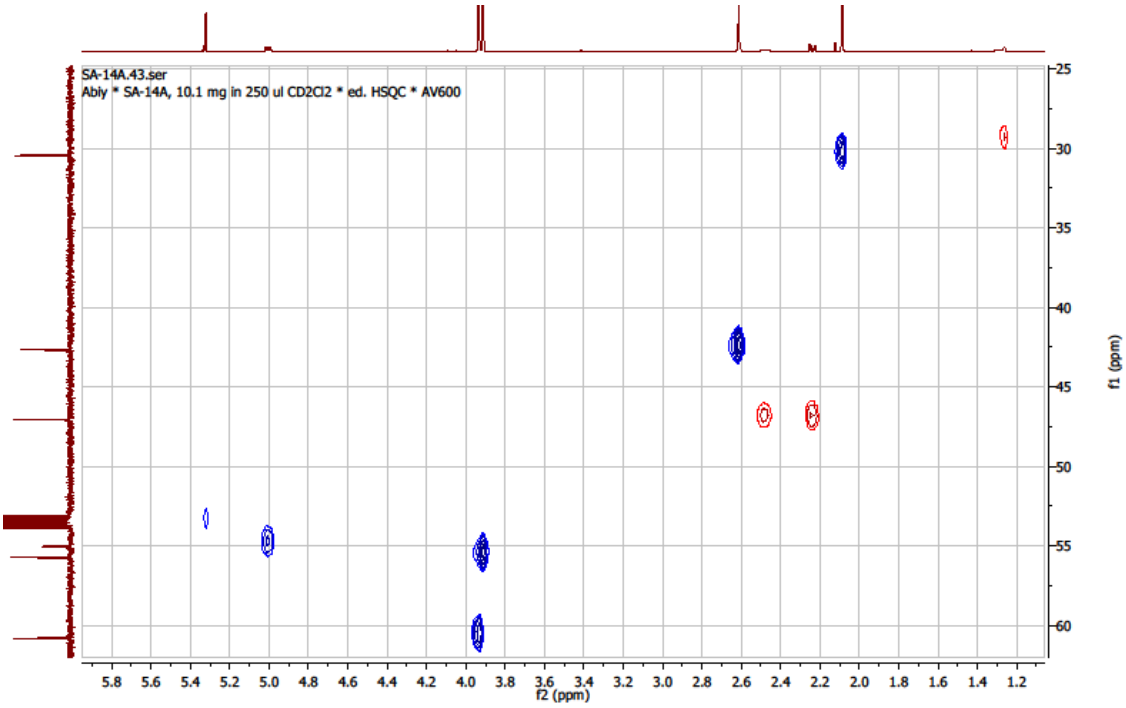


HSQC for 8-acetonyldihydrochelerythrine (66)

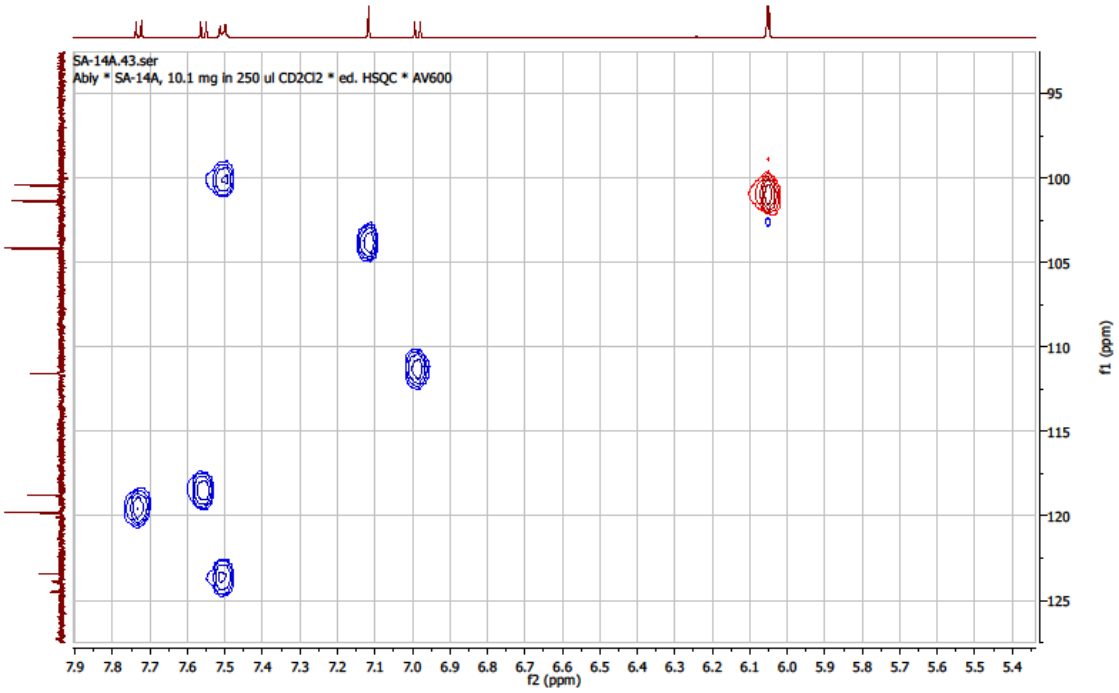


HSQC for 8-acetonyldihydrochelerythrine (66)

e



HSQC for 8-acetonyldihydrochelerythrine (66)

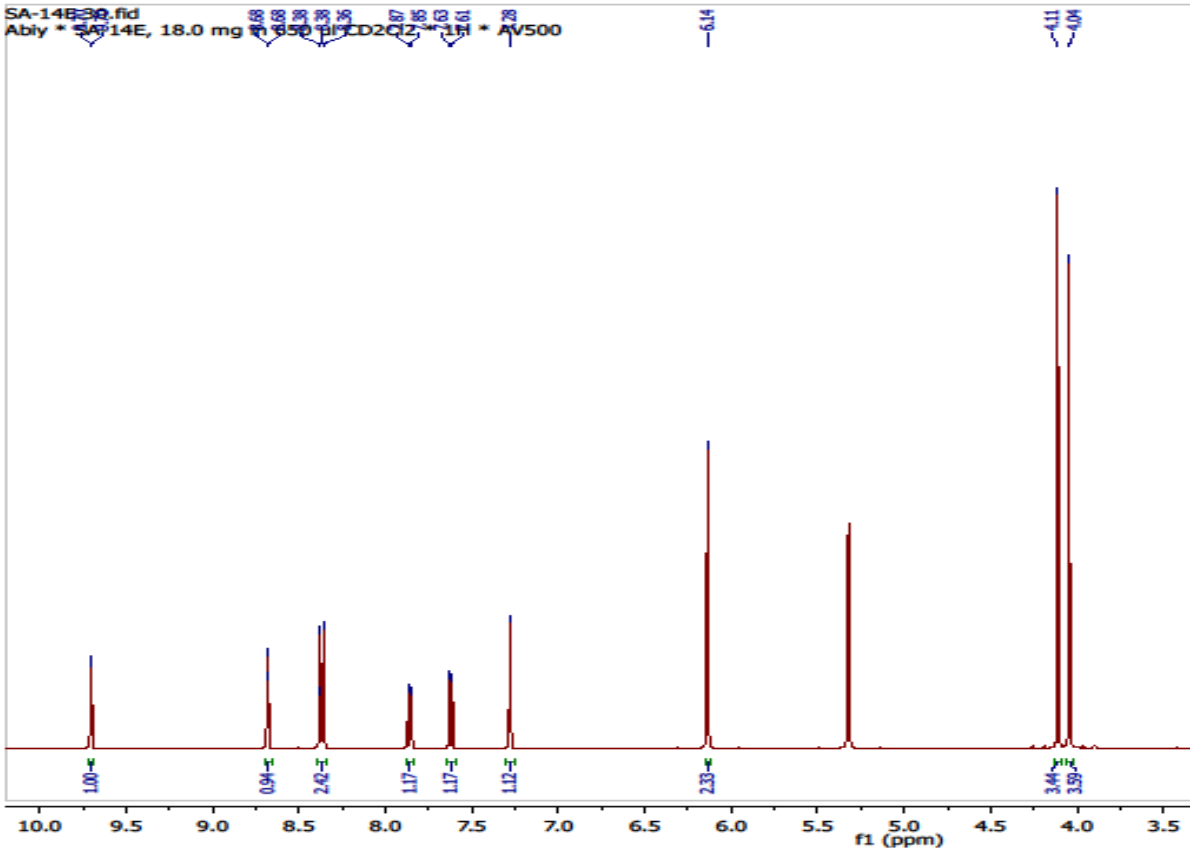


HSQC for 8-acetonyldihydrochelerythrine (66)

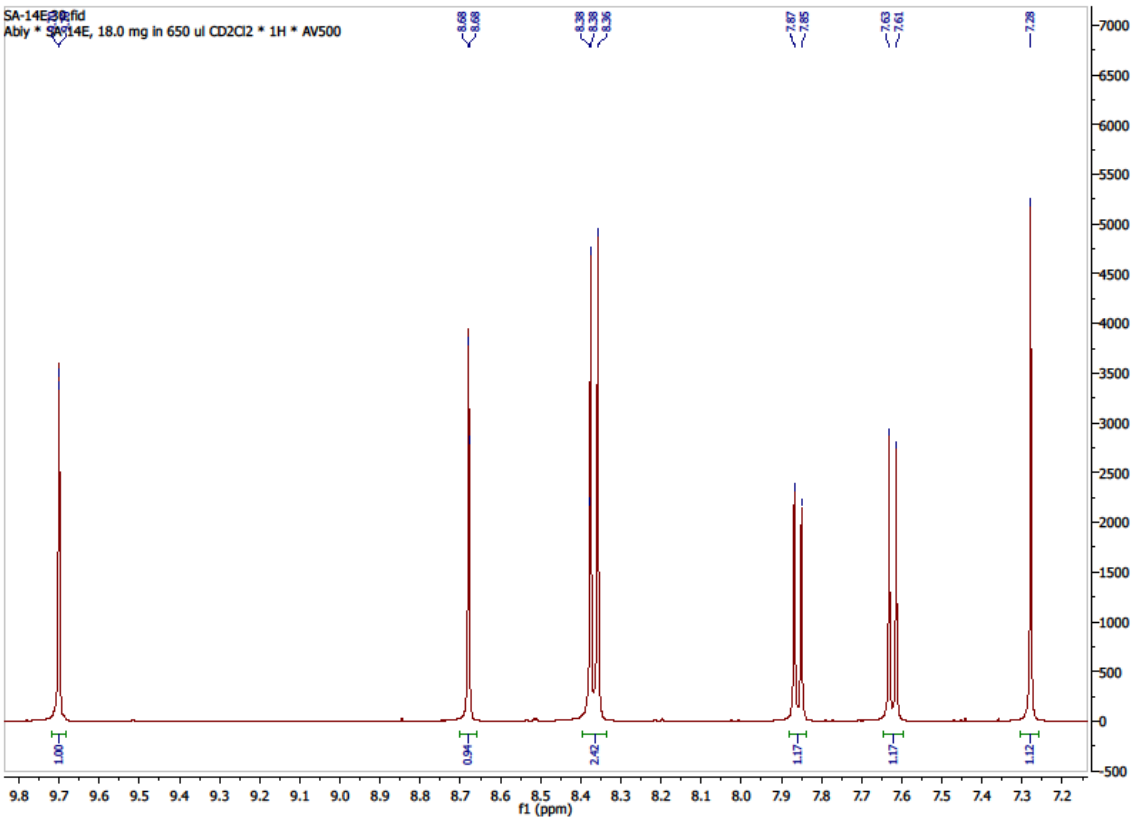
APPENDIX V NMR Spectra for compound 67

Norchelerythrine (67)

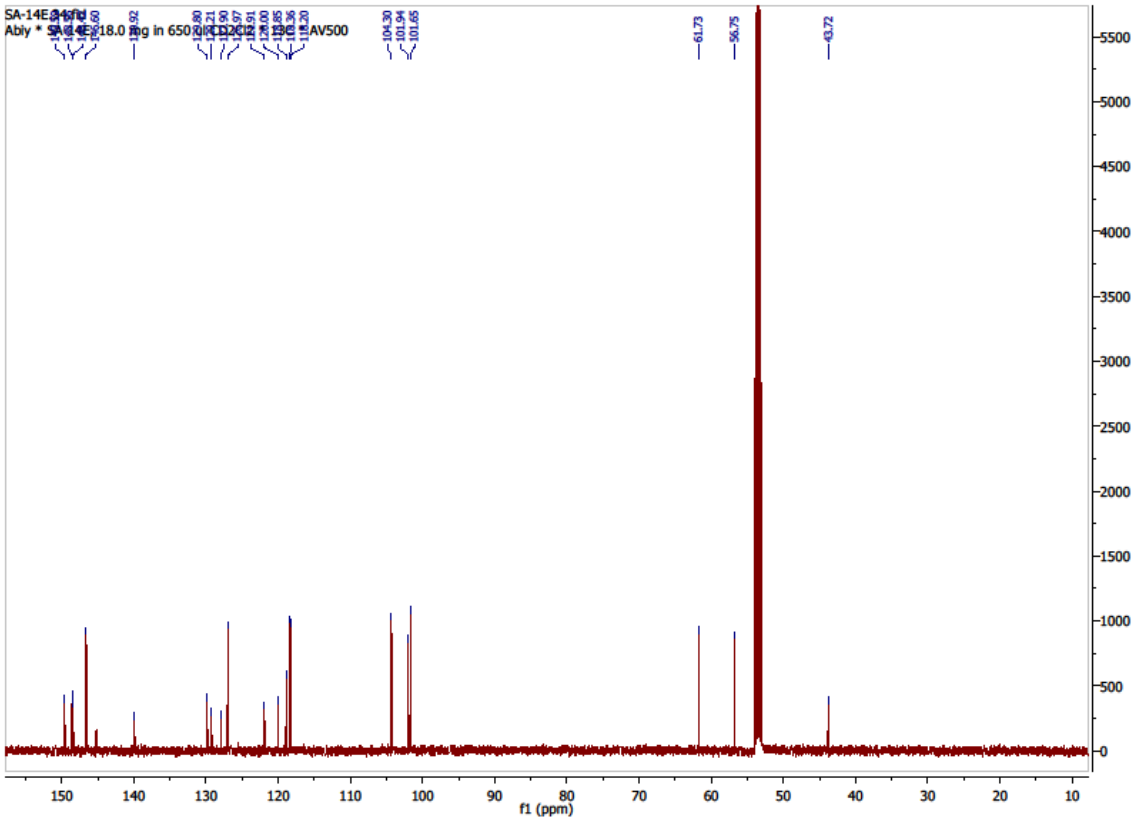
APPENDIX67



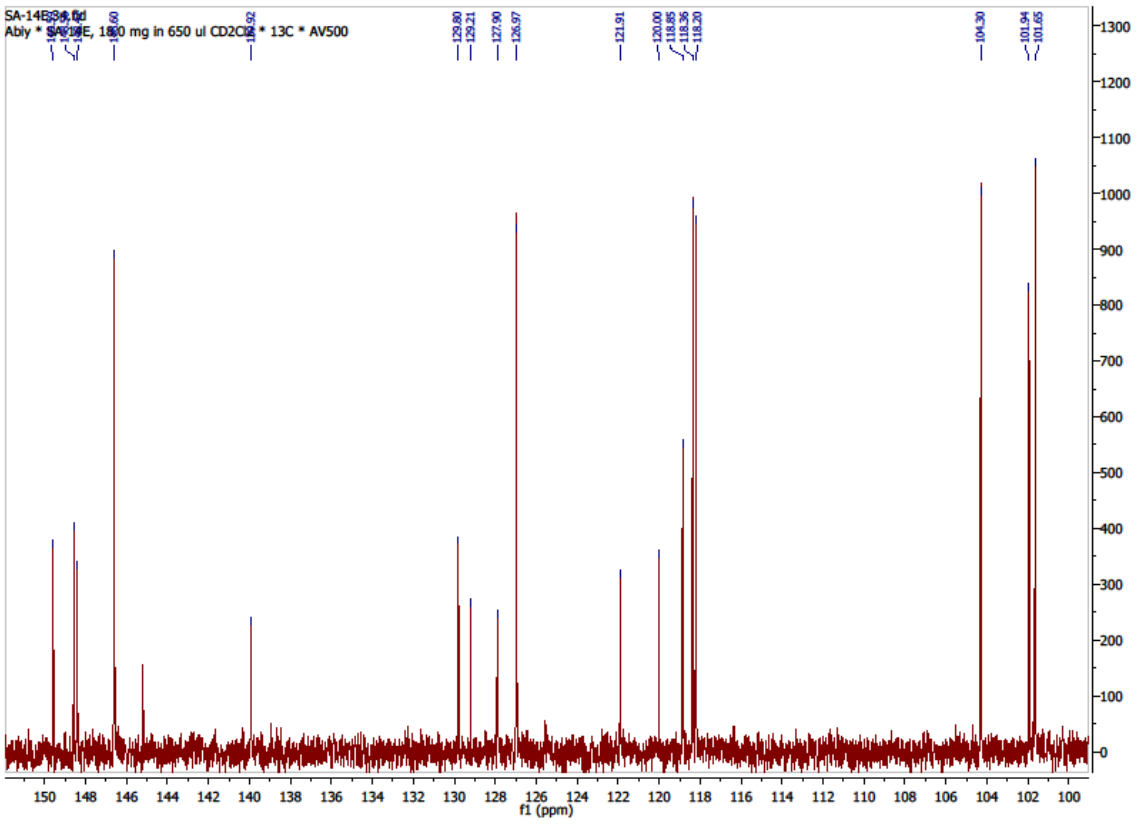
^{1}H NMR spectrum for norchelerythrine (67)



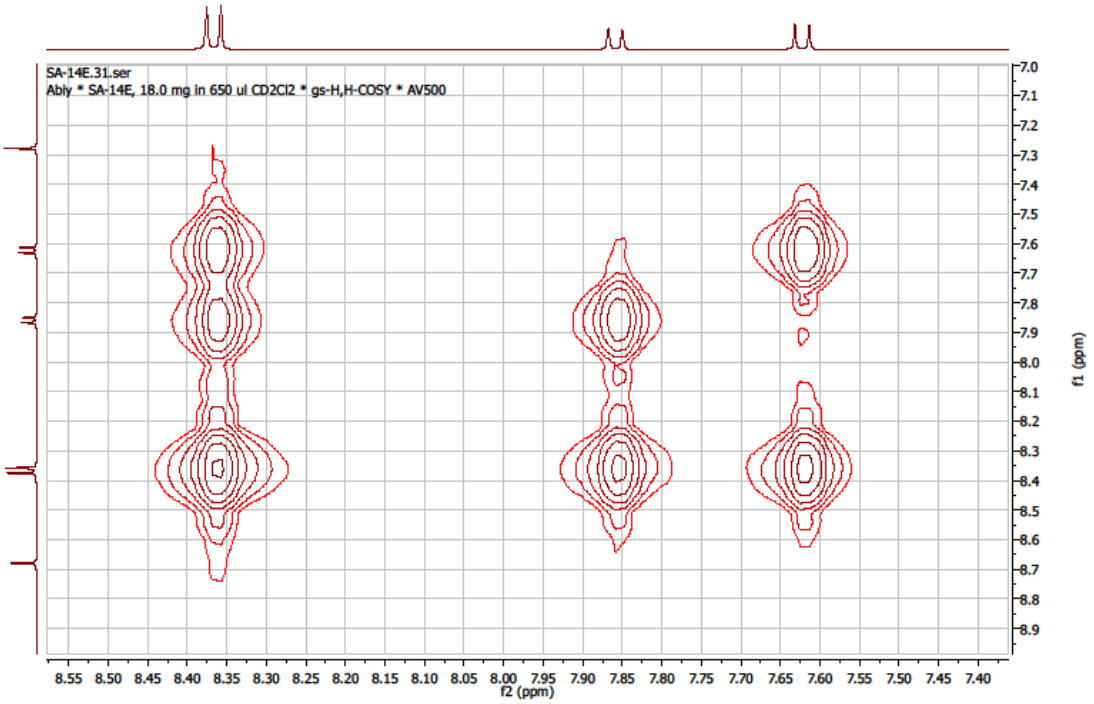
^{1}H NMR spectrum for norchelerythrine (67)



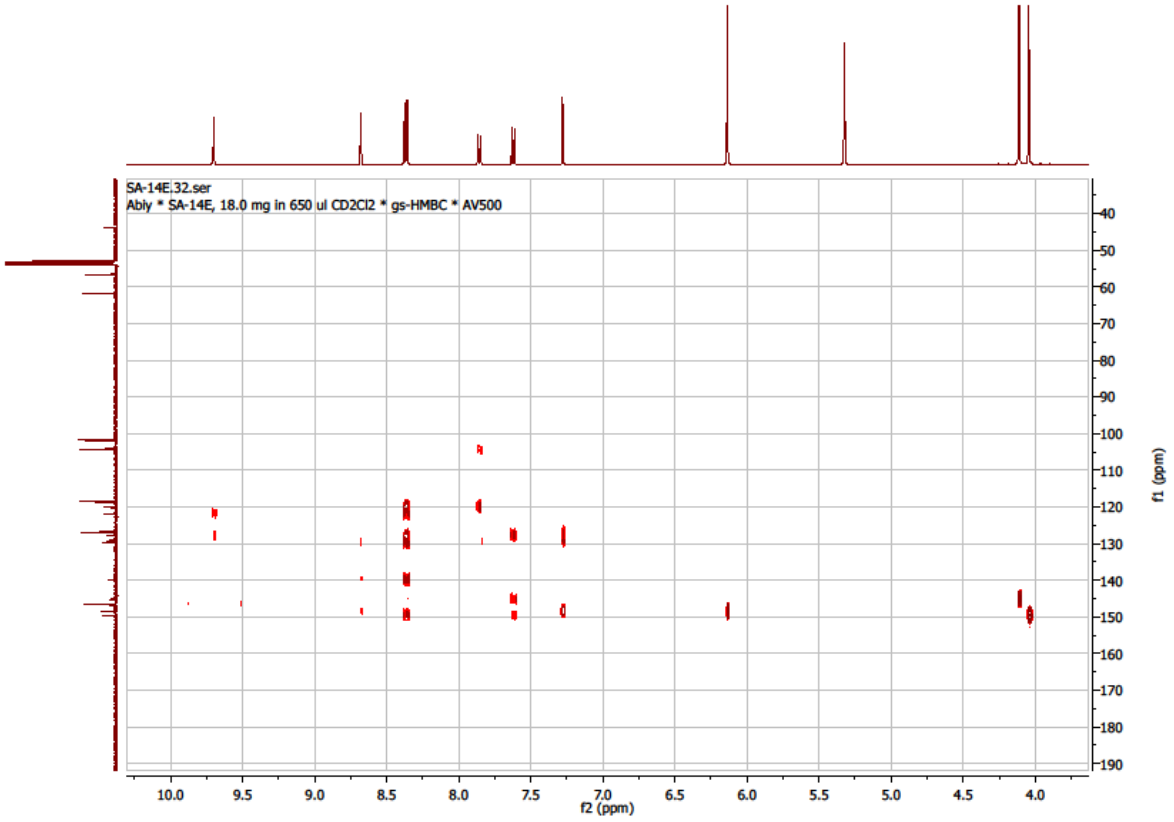
^{13}C NMR for norchelerythrine (67)



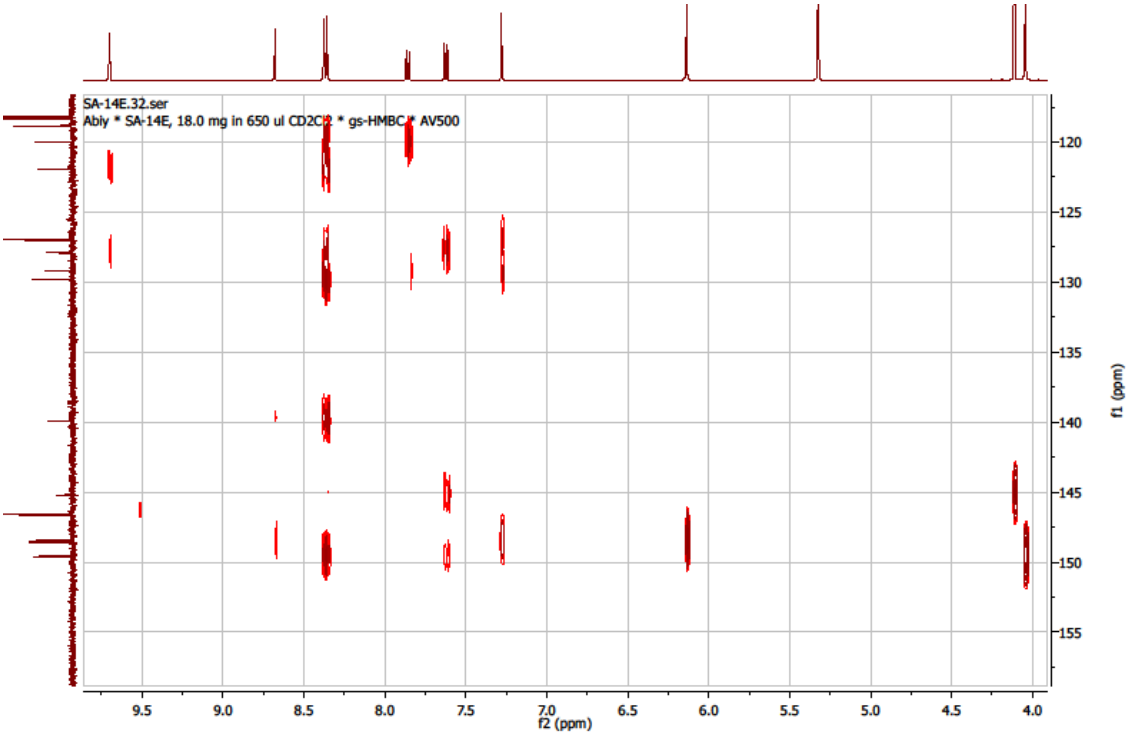
^{13}C NMR for norchelerythrine (67)



^{1}H, ^{1}H-COSY for norchelerythrine (67)

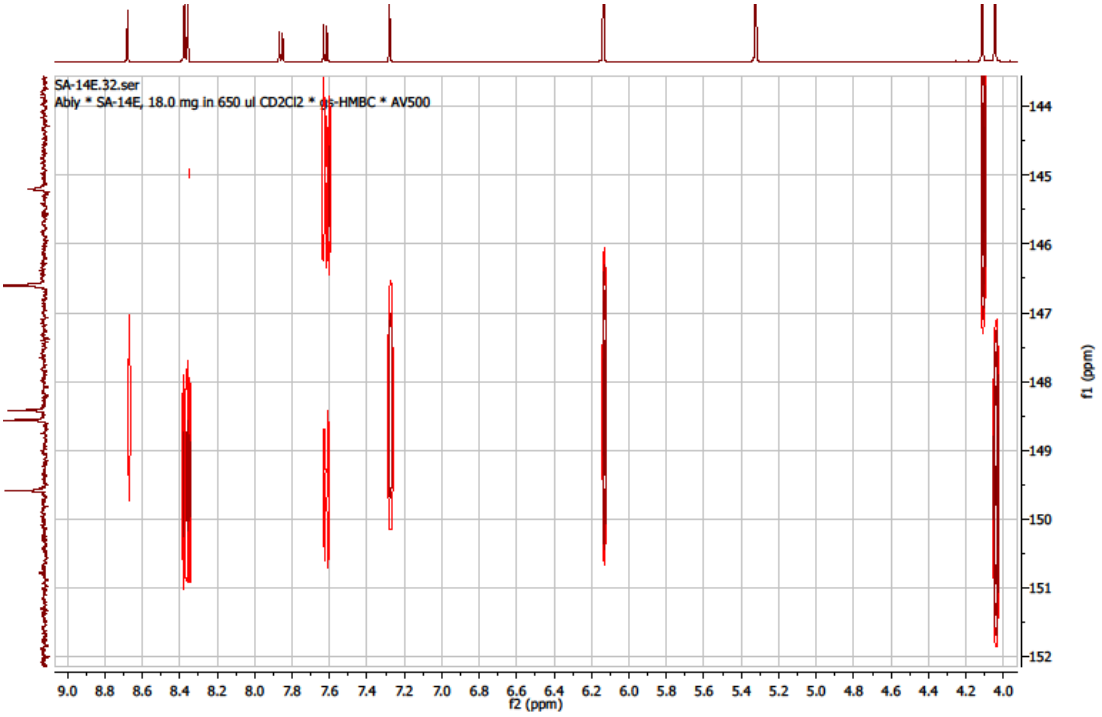


[1]HMBC for norchelerythrine (67)

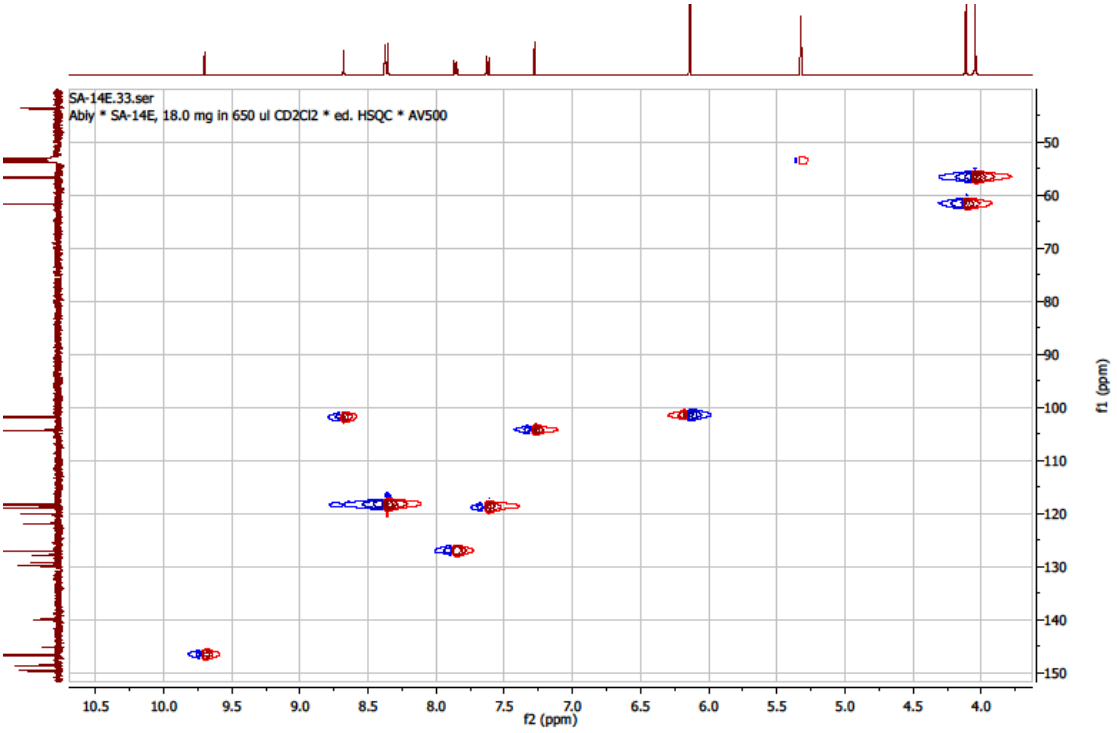


[1]HMBC Spectrum for Norchelerythrine (67)

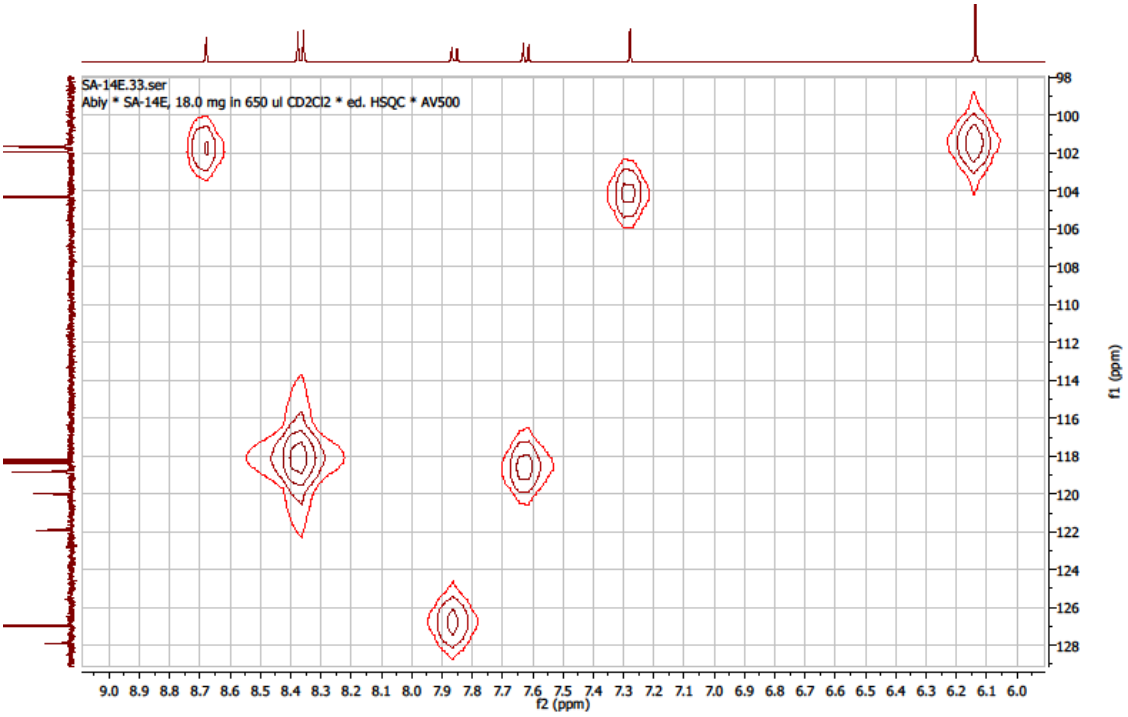
[1]HMBC for norchelerythrine (67)



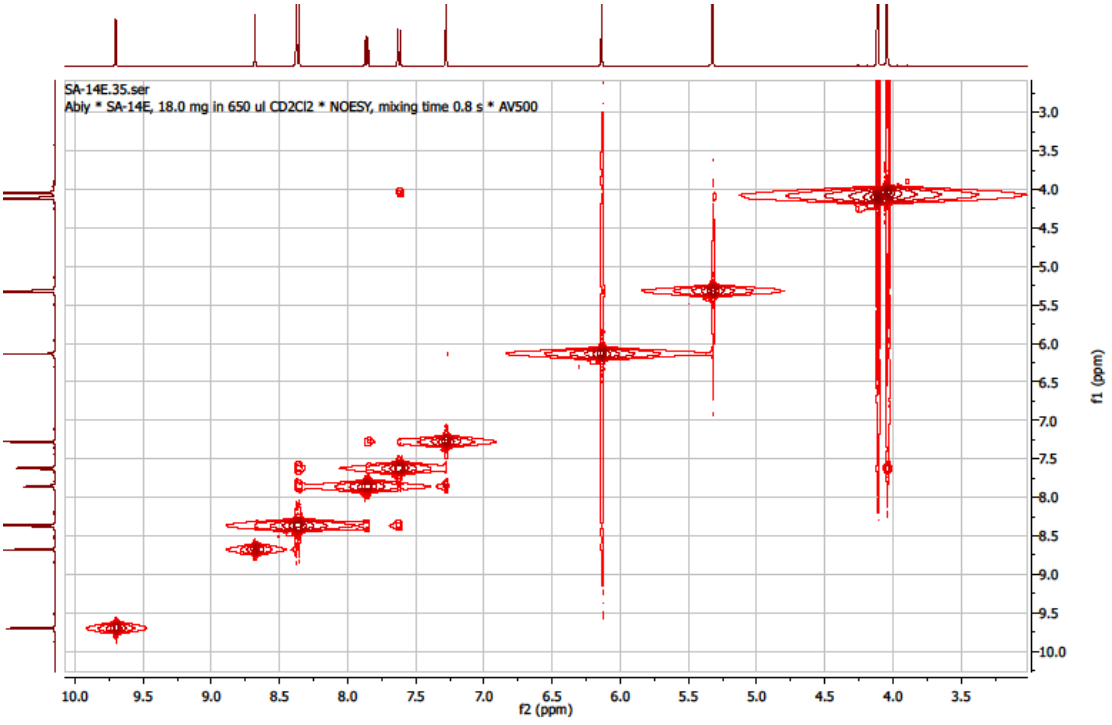
[1]HMBC for norchelerythrine (67)



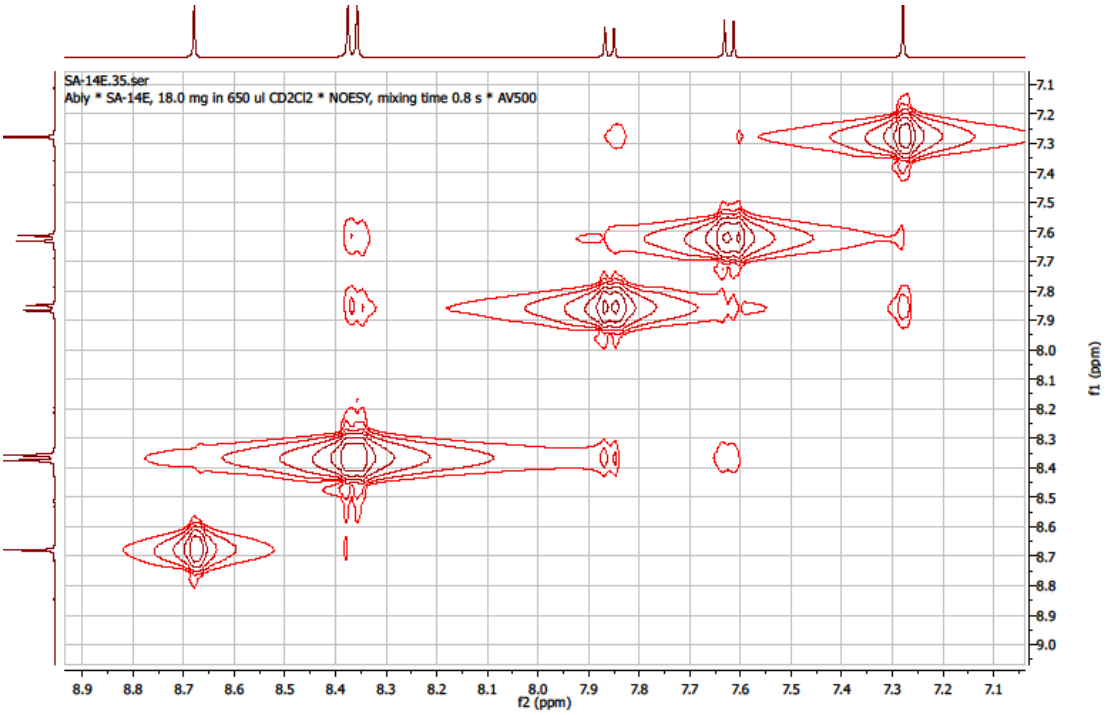
HSQC Spectrum for Norchelerythrine (67)



HSQC Spectrum for Norchelerythrine (67)



NOESY Spectrum for Norchelerythrine (67)

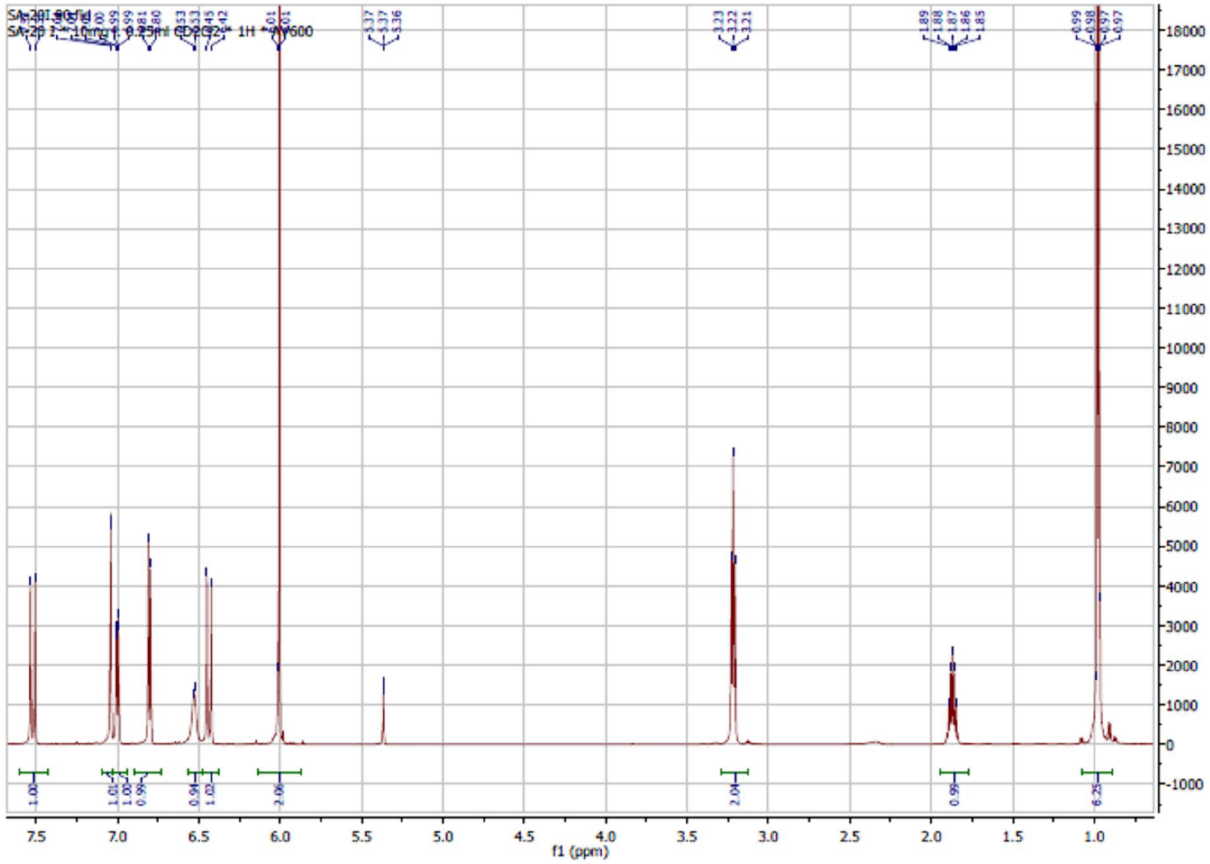


NOESY Spectrum for Norchelerythrine (67)

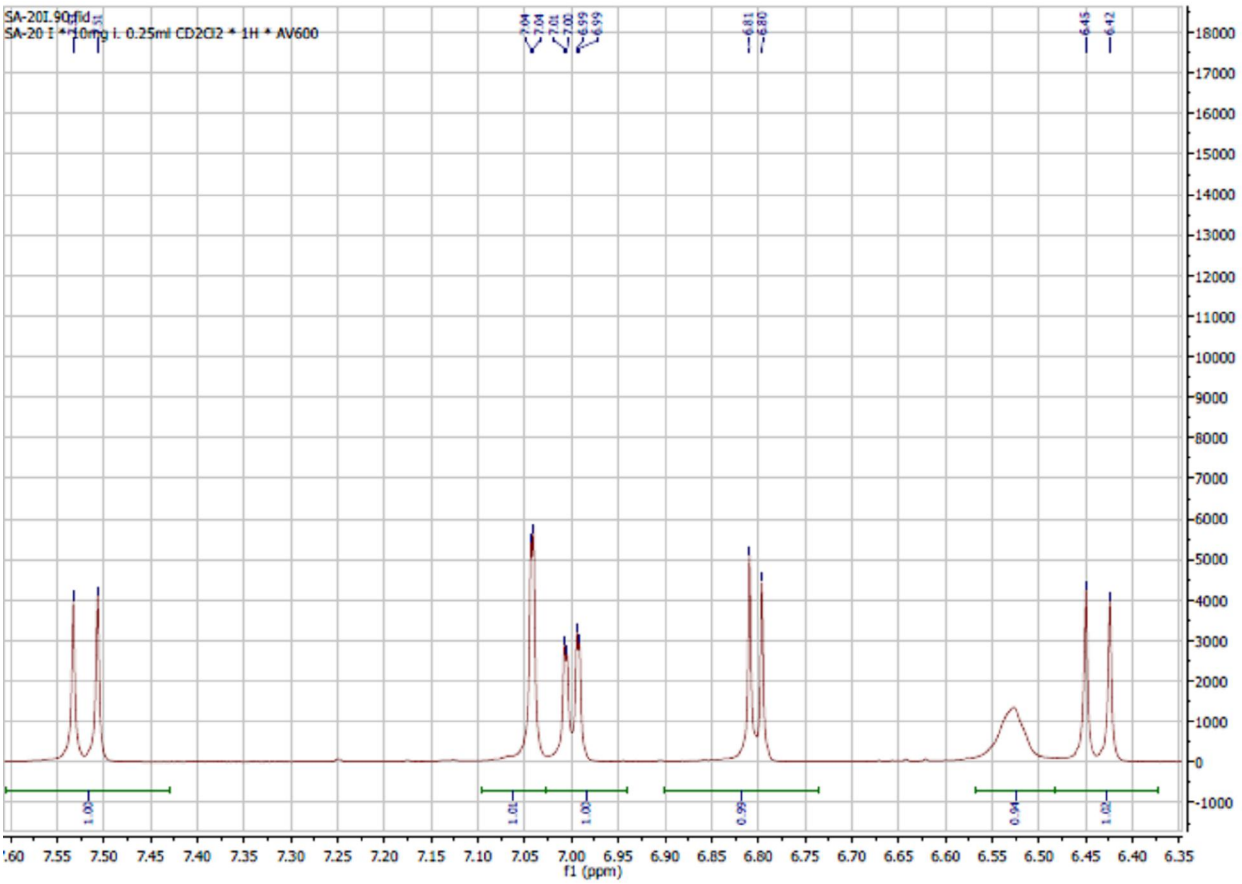
APPENDIX VI: NMR Spectra for compound 68

Fagaramide (68)

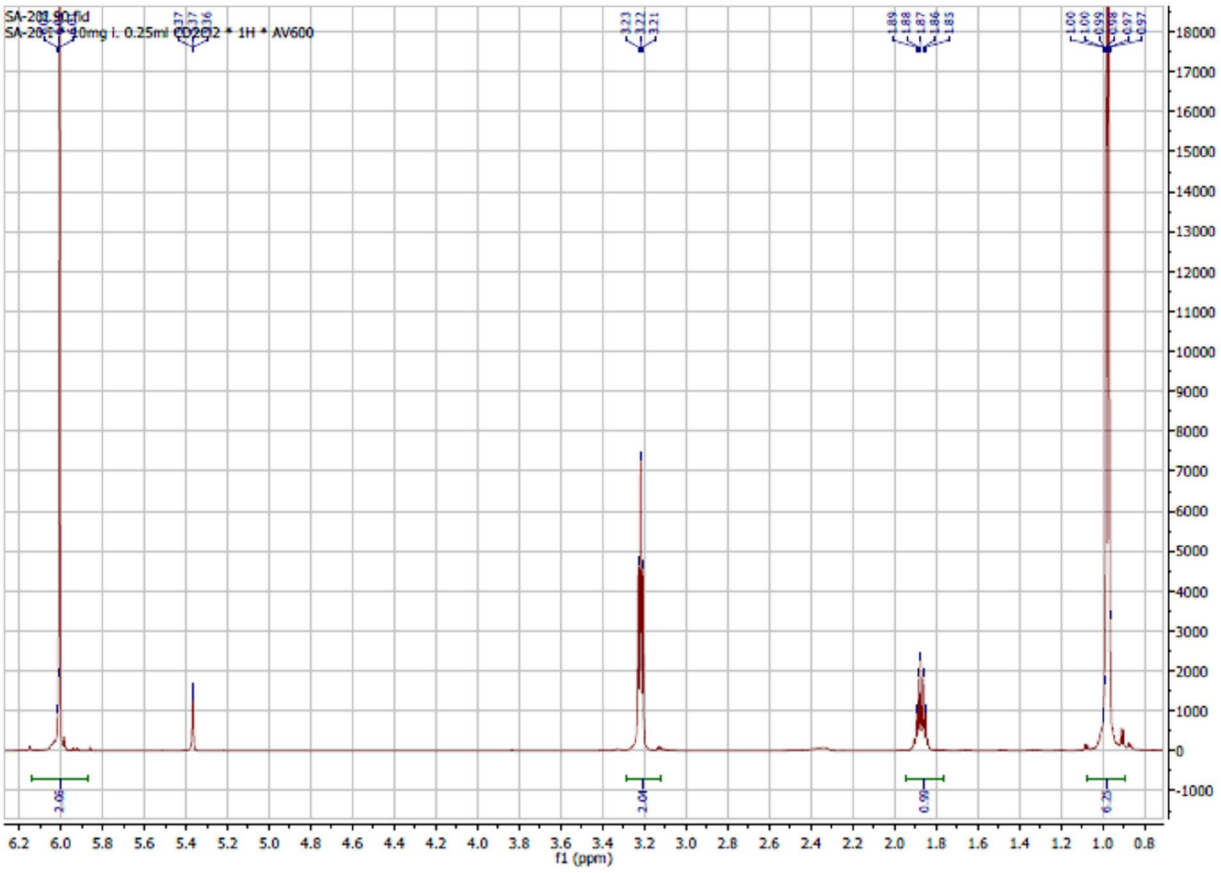
APPENDIX 28



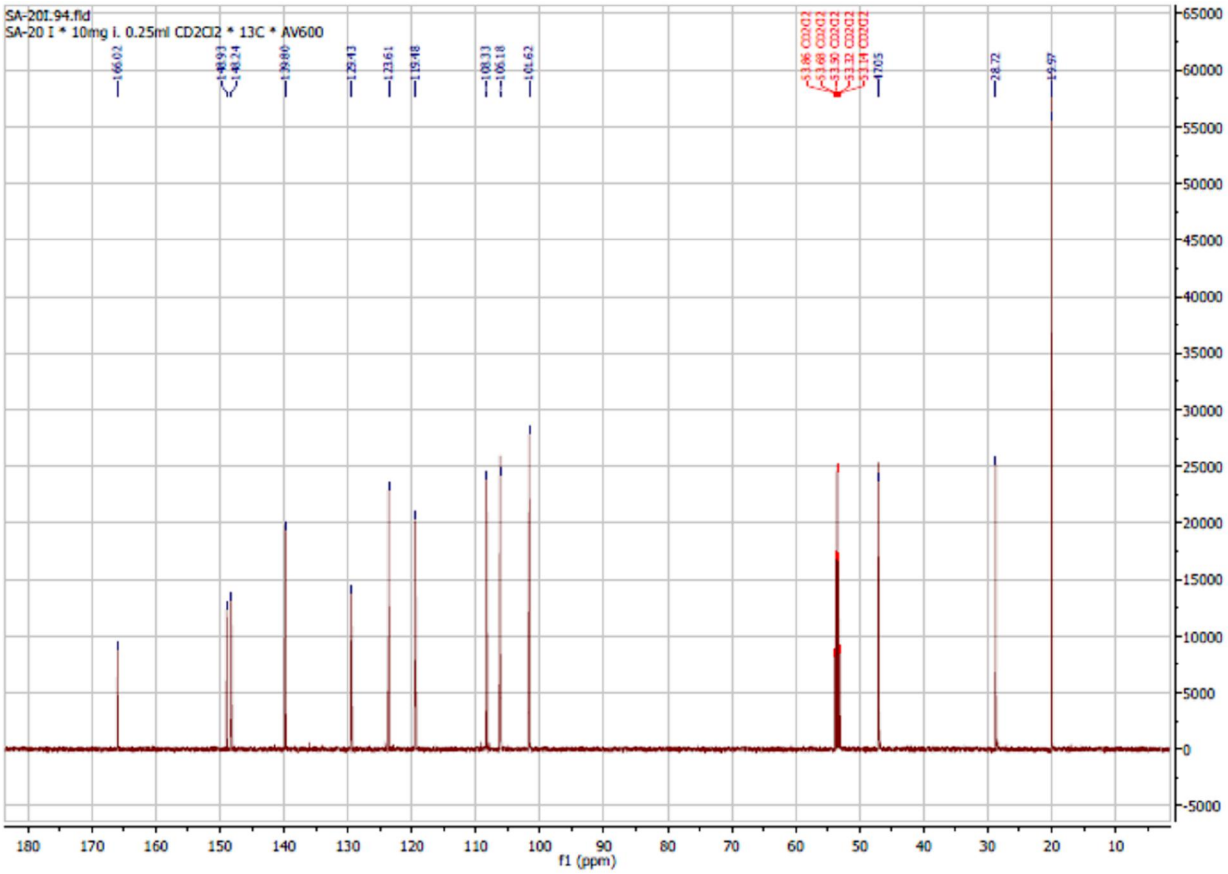
^{1}H NMR Spectrum for Fagaramide (68)



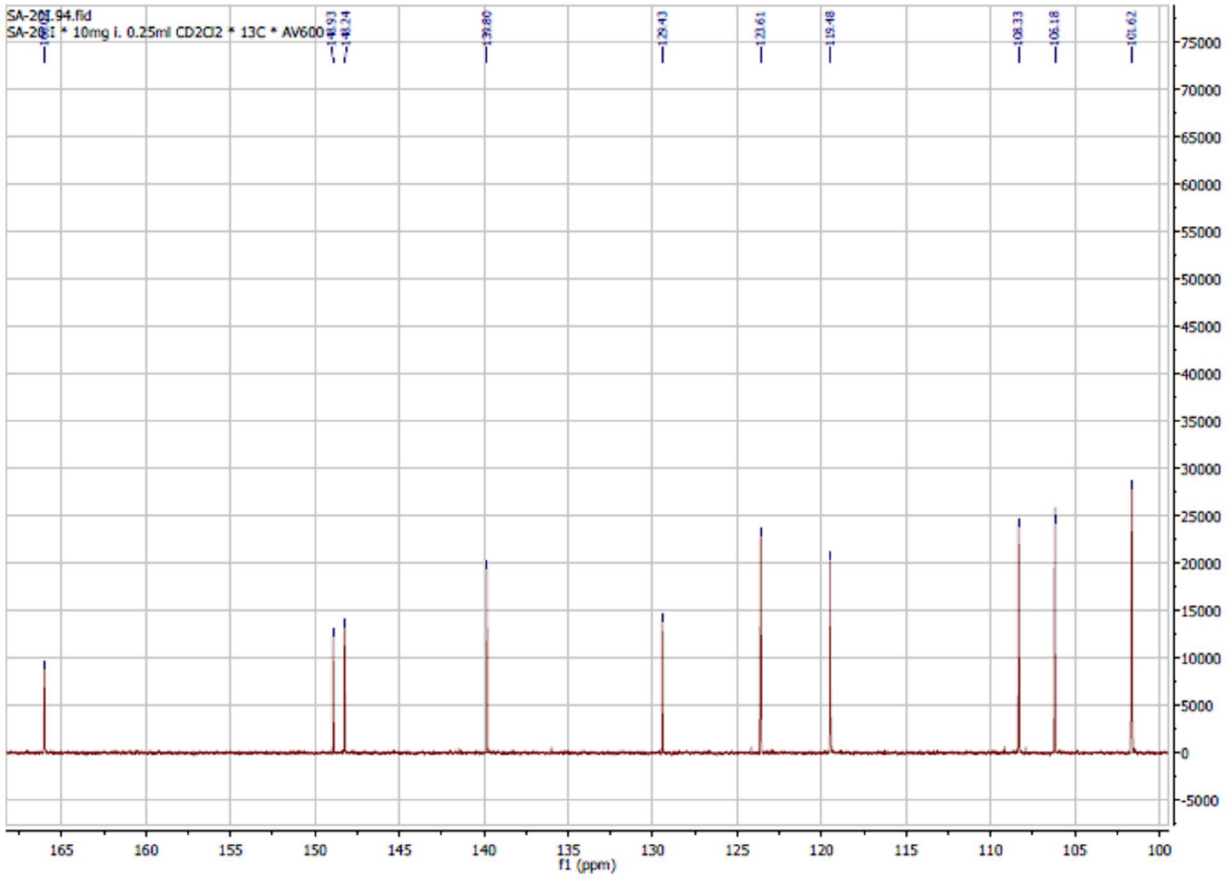
^{1}H NMR Spectrum for Fagaramide (68)



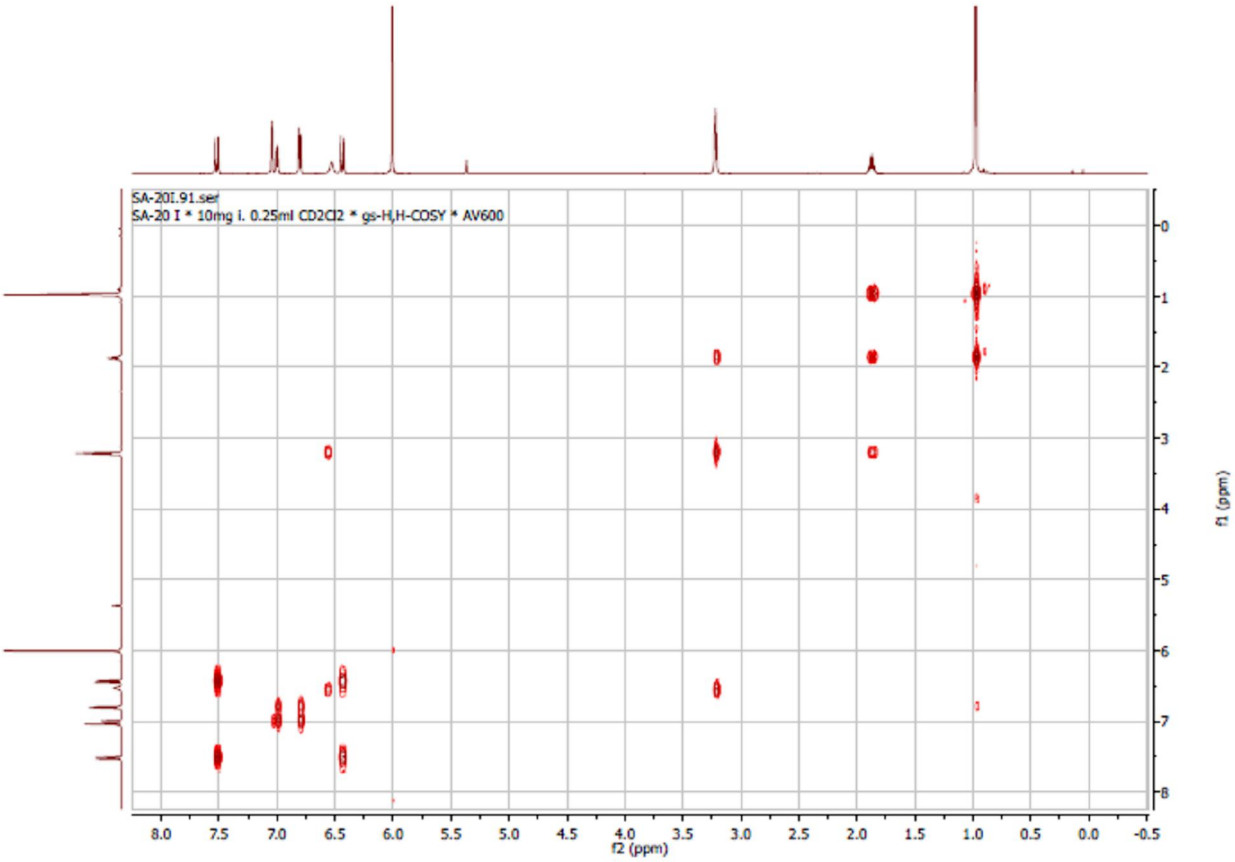
^{1}H NMR Spectrum for Fagaramide (68)



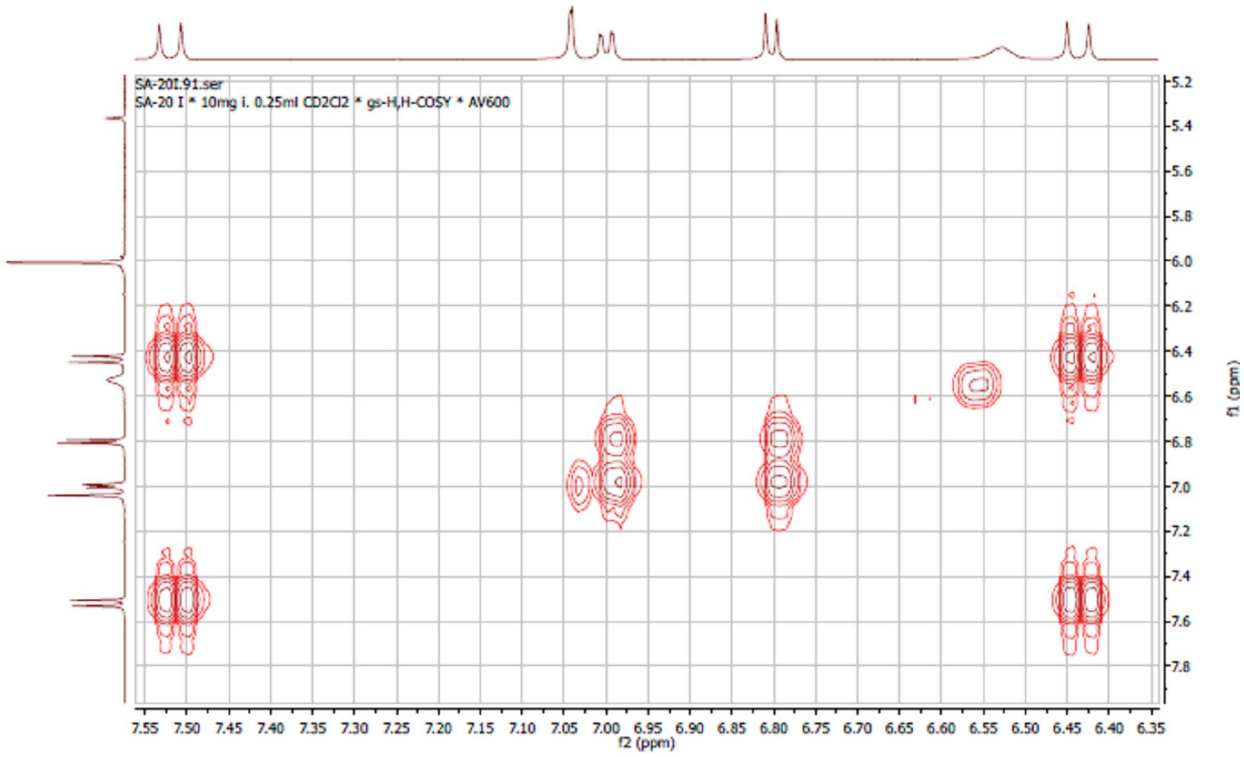
^{13}C NMR Spectrum for Fagaramide (68)



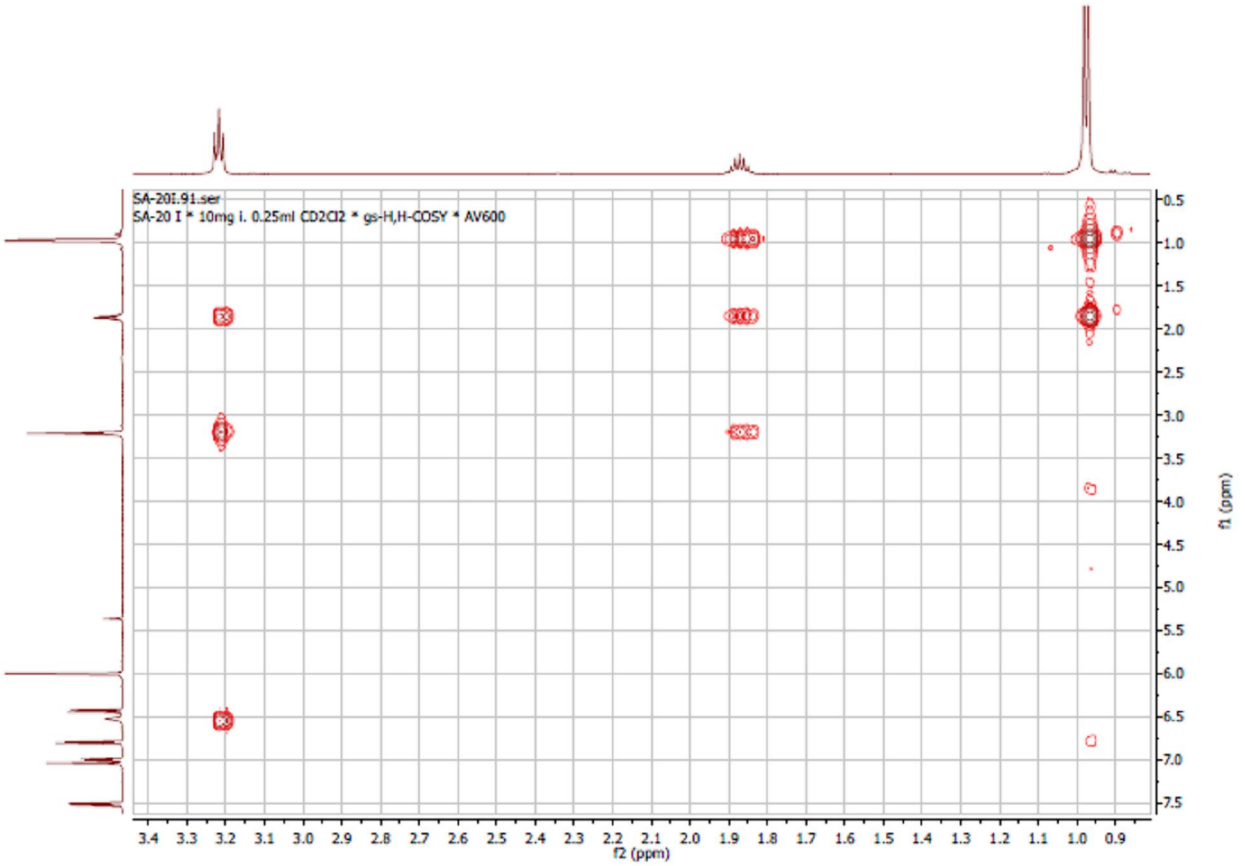
^{13}C NMR Spectrum for Fagaramide (68)



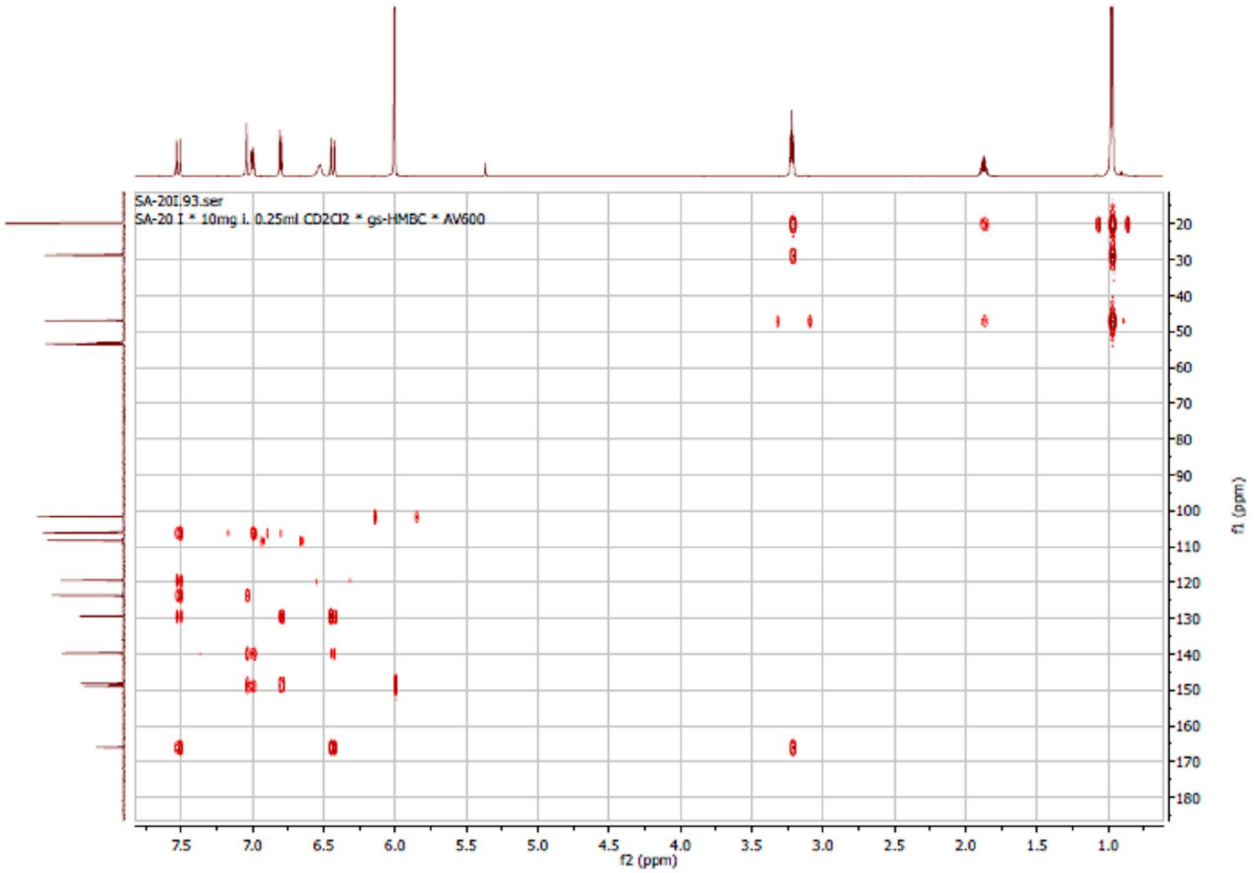
^{1}H, 1H COSY Spectrum for Fagaramide (68)



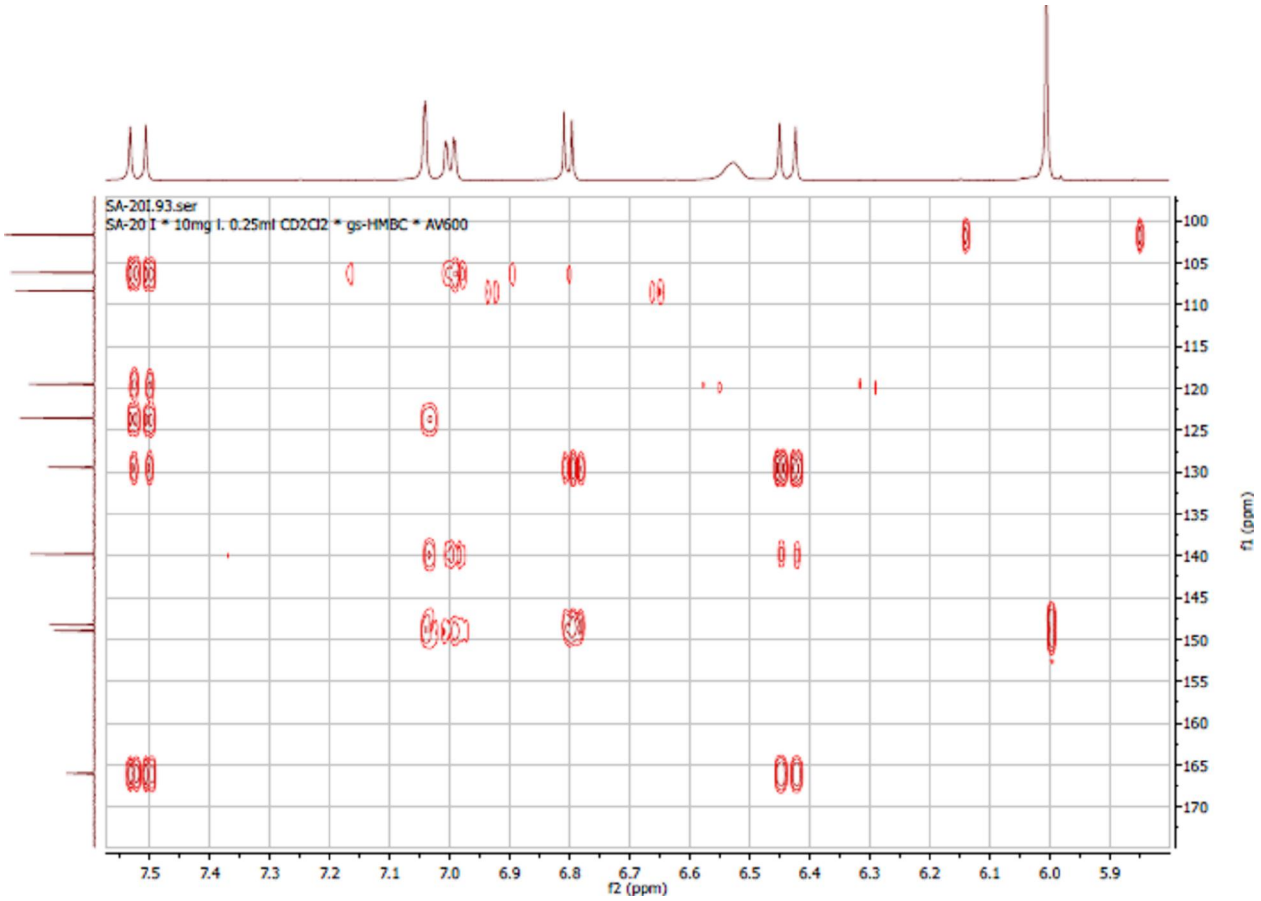
^{1}H, 1H COSY Spectrum for Fagaramide (68)



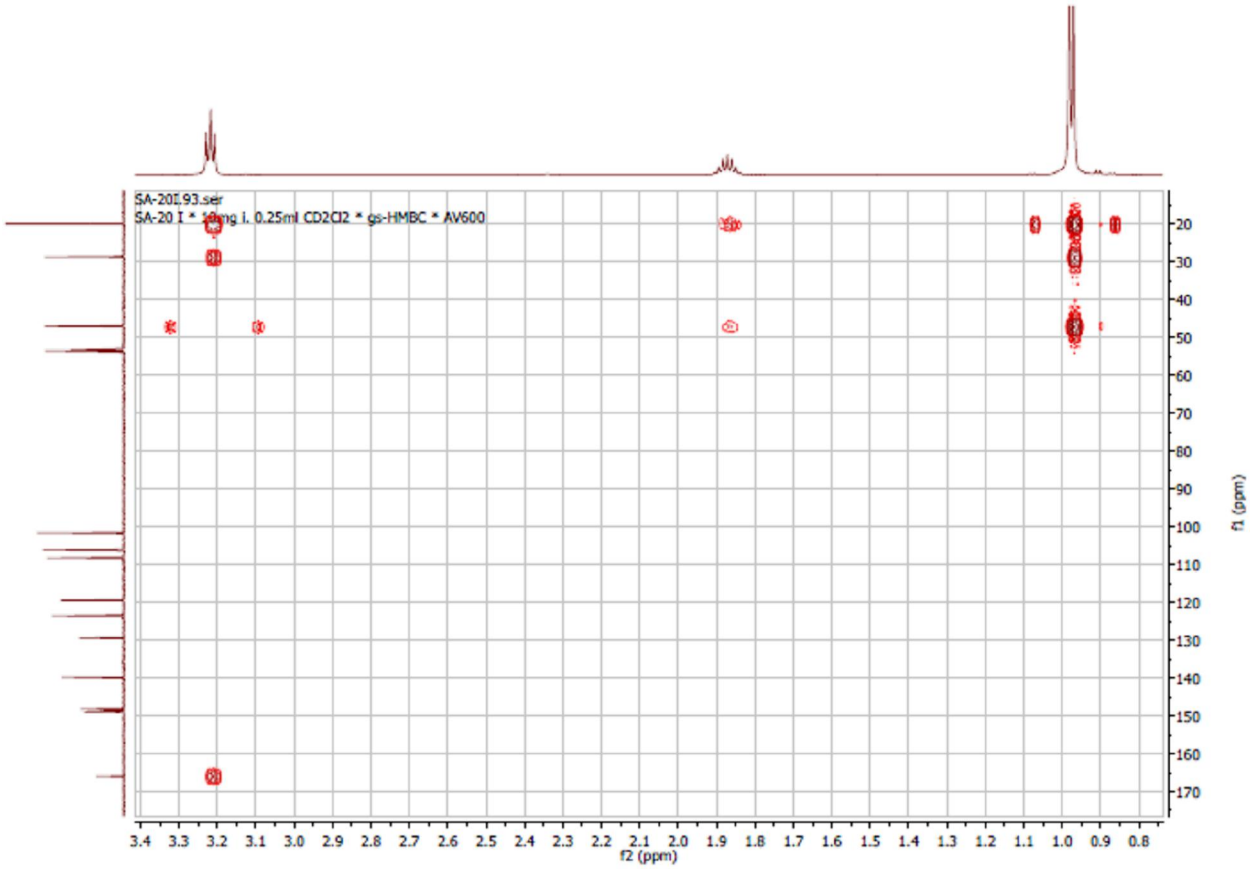
^{1}H, 1H COSY Spectrum for Fagaramide (68)



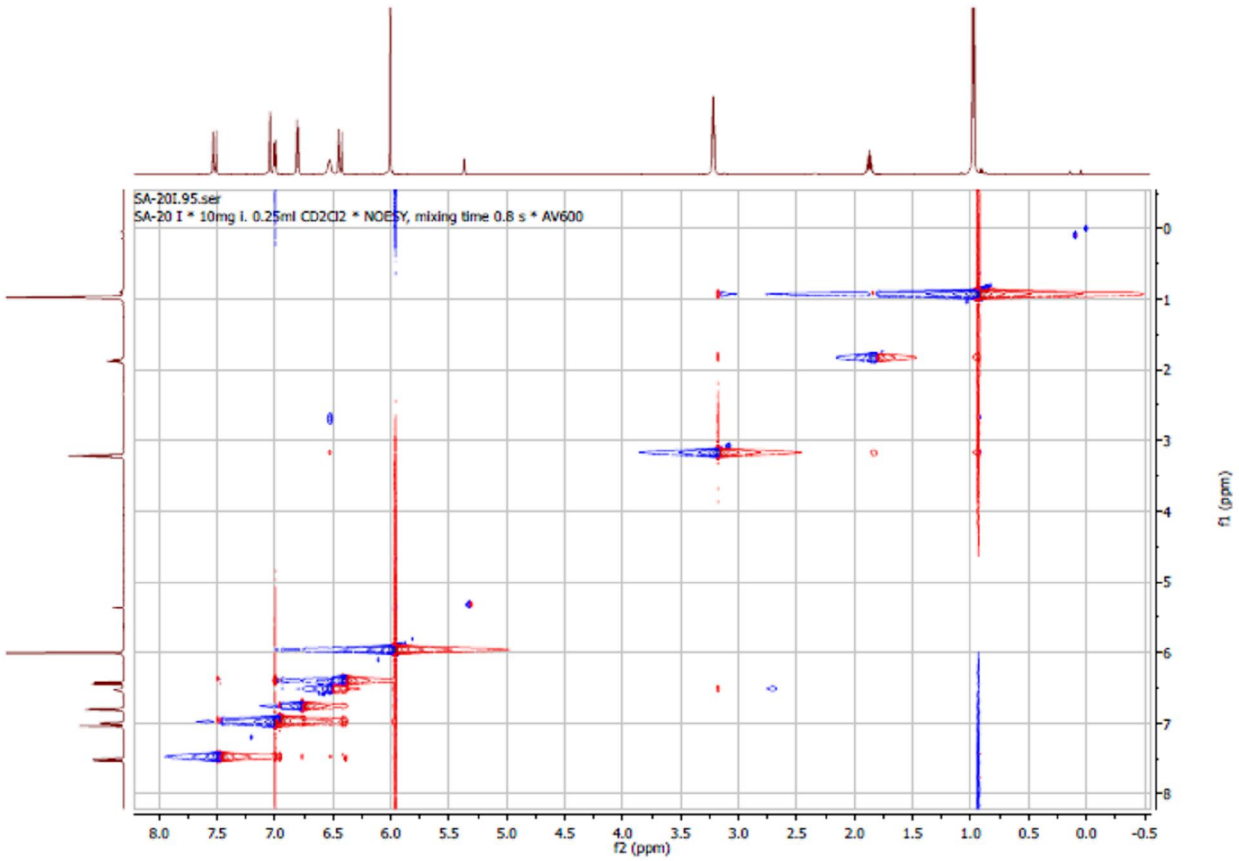
HMBC Spectrum for Fagaramide (68)



HMBC Spectrum for Fagaramide (68)



HMBC Spectrum for Fagaramide (68)



NOESY Spectrum for Fagaramide (68)