PHYTOCHEMICAL INVESTIGATION OF *BRIDELIA MICRANTHA* AND *TABERNAEMONTANA VENTIRCOSA* FOR CYTOTOXIC PRINCIPLES AGAINST DRUG SENSITIVE LEUKEMIA CELL LINES

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DECLARATION

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

This research thesis is dedicated to my parents Mr. Calvin Munayi and Mrs. Judy Munayi for their support, encouragement and inspiration.

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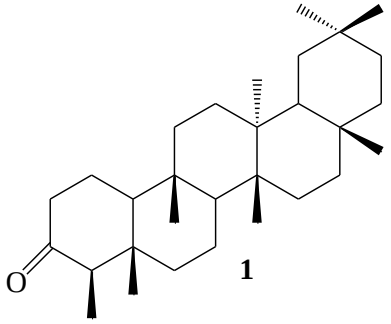
ABSTRACT

There are various treatment options that are available for the treatment of cancer. However, development of multidrug resistant cancer has become a global health challenge in the fight against cancer. *Bridelia micrantha* (Euphorbiaceae) has been used traditionally by people within the vicinity of Kakamega Forest for the treatment of tumors and related ailments. Despite its widespread use, its cytotoxicity has not been fully established. *Tabernaemontana ventricosa* (Apocynaceae) is known to contain indole and *bis*-indole alkaloids that have shown to elaborate anticancer principles. However, these cytotoxic principles have not been fully documented. This study therefore sought to investigate the phytochemical and anticancer principles of both *B. micrantha* and *T. ventricosa* to produce lead compounds to combat drug sensitive and multidrug resistant (MDR) cancer as well as validate the traditional uses of *B. micrantha* so as to improve the existing traditional knowledge. The stem bark of *Bridelia micrantha* and root bark of *Tabernaemontana ventricosa* were subjected to extraction by cold percolation and the crude extracts underwent chromatographic separation leading to the isolation of six compounds. The compounds were characterized using spectroscopic methods and identified as friedelin (**1**), *trans*-triacontyl-4-hydroxy-3-methoxycinnamate (**2**), betulinic acid (**3**), catechin (**4**), stigmasterol (**5a**) and *β*-sitosterol (**5b**). The crude extract and isolated compounds from *B. micrantha* were tested for their cytotoxicity and anticancer activity towards drug sensitive leukemia cell lines. The crude extract of *B. micrantha* showed a cell viability of 31.5% at the tested concentration (10µg/ml) with an IC_{50} value of 9.43µg/ml and thus showed good activity towards the drug sensitive leukemia cell lines. The compound, *trans*-triacontyl-4-hydroxy-3-methoxycinnamate (**2**) showed an interesting cell viability of 31.13% at 1 µg/mL. However, friedelin (**1**), betulinic acid (**3**) and catechin (**4**) showed cell viability of <30% (cell inhibition of <70%) at the tested concentration of 1µg/ml and were thus considered inactive. From this study, the stem bark of *B.* mainly used for the management of cancer by people within Kakamega Forest, showed good anticancer activity and should therefore be subjected to efficacy trials for possible anticancer use



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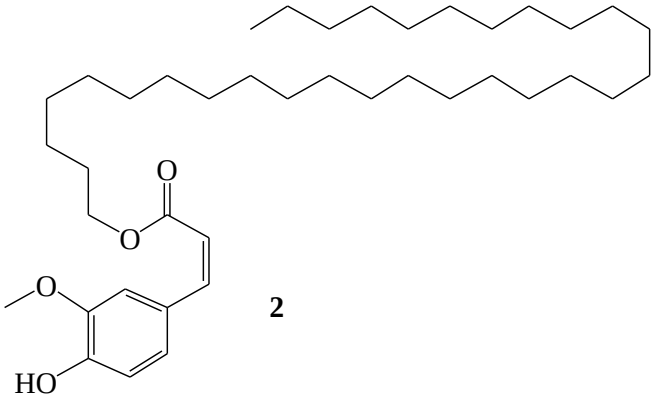

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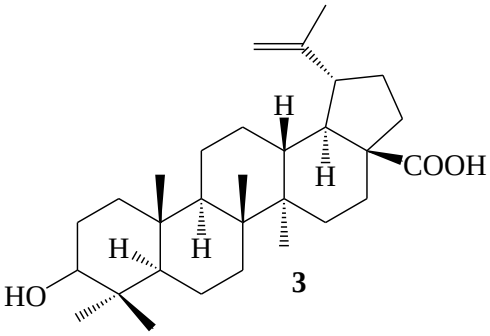

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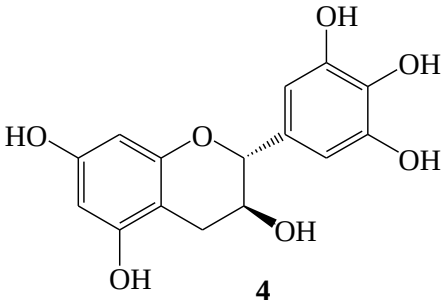

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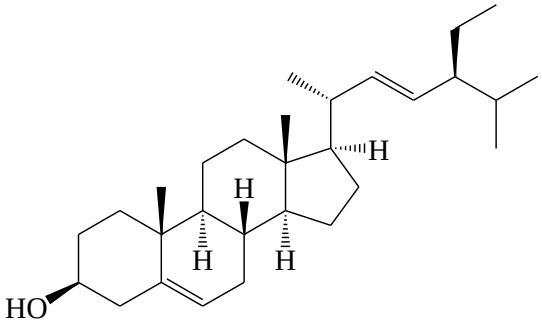

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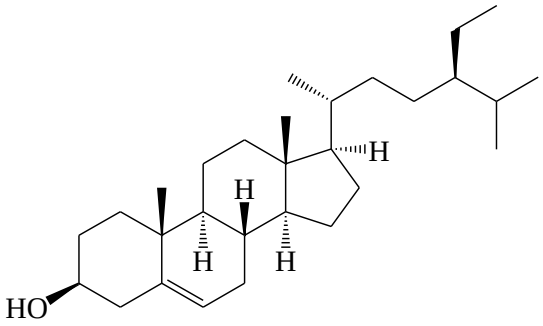

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LIST OF ABBREVIATIONS AND ACRONYMNS

ABC	Adenosine triphosphate binding cassette
AIDS	Acquired immune deficiency virus
CC	Column chromatography
CCRF-CEM	Human T cell lymphoblast-like cell line
$CDCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
COSY	Correlated spectroscopy
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EtOAc	Ethyl acetate
FCS	Fetal calf serum
HIV	Human immuno-deficiency virus
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
IC_{50}	Inhibition concentration, concentration of substance that produce 50% inhibition of certain process
MeOH	Methanol
MDR	Multidrug resistant
NMR	Nuclear magnetic resonance
P-gp	P-glycoprotein
PTLC	Preparative thin layer chromatography
RPMI	Roswell Park Memorial Institute
SBS	School of Biological Sciences
TB	Tuberculosis
TLC	Thin layer chromatography
TMS	Tetramethylsilane
UV	Ultra violet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Man has relied on nature for millennia for most of their basic needs including food, shelter, transport, fragrances and flavor (Gurib-Fakim, 2006). Apart from these ethno botanical uses, plants have also been used due to their medicinal value (Cragg and Newman, 2013) forming a sophisticated traditional medicinal system (Khazir *et al.*, 2014). In Africa, 80% of the general population depends on traditional medicine for their health care needs (WHO, 2002). Although there is widespread use of these herbal medicines in the management of various ailments including malaria, microbial infections and cancer and related ailments, their efficacy and cytotoxicity has not been established for their formulation to modern pharmaceuticals (Tamokou and Kuete, 2014).

Traditionally, there is no clear definition of cancer as it was mostly referred to as hard swellings, tumors, calluses, warts, corns or polyps. (Cragg and Newman, 2005). Despite this, Plants have still been used historically for the treatment and management of cancer (Khazir *et al*., 2014). Some of these plants that have been used traditionally include *Zingiber officinale* Roscoe, whose decoction is used together with other plants for the treatment of breast cancer in Thailand (Itharat, 2009) and *Allium saticum* (garlic) used among Asian communities to treat and prevent cancer (Thomson and Ali, 2003). However, due to the unclear definition of cancer, the efficacy of these plants remain unknown and is often treated with criticism.

Despite this, many of the anticancer drugs in clinical use today have been derived from plants either as natural products or their synthetic analogues (Gurib-Fakim, 2006). Some of the plant based anticancer drugs include vinblastine (**6**) and vincristine (**7**) isolated from *Catharanthus roseus G.Don*, a Mexican merigold tree and used traditionally for management of diabetes (Gueritte and Fahy, 2005). Others include; etoposide (**8**) isolated from *Podophyllum peltatum* (Imbert, 1998), paclitaxel (**10**) isolated from *Taxus brevifolia* (Wall and Wani, 1996), and topotecan (**12**) isolated from *Camptotheca acuminata* (Wall and Wani, 1996). Although these

anticancer agents have recorded tremendous success in management of cancer, their adverse side effects as well as the development of multidrug resistance cancer has led to continual research in new anticancer drugs from plants that is mainly focused on screening of traditionally used plants (Fernando and Rupasinghe, 2013).

In Kenya, different communities use traditional medicine to treat a number of diseases including tumors and related ailments referred to as cancer (Kareru *et al.*, 2008). However, most of these ethnomedicinal plants including *Bridelia micrantha* and *Tabernaemontana ventricosa* do not have scientific evidence to support their claimed uses. Furthermore, their safety profiles have not been tested, hence the motivation to carry out this current study. In this study, the anticancer profiles of the extracts and constituent compounds of *B. micrantha* used traditionally by the Abaluhyia people of Western Kenya to manage tumor and related diseases (Ochwang'i *et al*., 2014), and *T. ventricosa* that is rich in indole alkaloids known for their anticancer properties (van Beek *et al.*, 1984), was carried out against drug sensitive human leukemia cell lines.

1.2 Problem Statement

Cancer conditions are among the most serious health problems in the world affecting both the developed and developing countries (Fernando and Rupasinghe, 2013). However, developing countries have borne the brunt of this problem due to high costs in treatment and management of cancer as well as development of multidrug resistant (MDR) cancer that leads to recurrence of the condition even after successful treatment (Fernando and Rupasinghe, 2013). These challenges coupled with the serious side effects associated with chemotherapy and radiotherapy have resulted to many cancer patients seeking for complementary and alternative treatments (Shah *et al*., 2013) including traditional herbal medicines/remedies methods of treatment. Although these herbal medicines are beneficial to cancer patients, it is imperative to establish the cytotoxicity of the constituent principles against drug sensitive and multi-drug resistant (MDR) cancer cell lines.

1.3 Objectives

1.3.1 Main objective

To determine the cytotoxicity of the stem bark extract and constituent compounds of *Bridelia micrantha* (Hochst) Baill stem bark and *Tabernaemontana ventricosa* Hochst root bark towards drug sensitive leukemia cell lines.

1.3.2 Specific objectives

i) To extract the stem bark of *B. micrantha* (Hochst) Baill and root bark of *T. ventricosa* Hochst using 50% MeOH in CH_2Cl_2.
ii) To determine isolates from the above extracts and characterize them using different spectroscopic techniques.
iii) To evaluate the extracts and isolated compounds for anticancer potential by subjecting them to cytotoxicity test against drug sensitive leukemia cell lines

1.4 Justification

There have been great strides made in the management of cancer in recent years due to effective prevention and early detection. However, available treatment options have been found to be ineffective mainly due to the ability of the cancerous cells to develop resistance to available chemotherapy (Prakash *et al*., 2013). This resistance has been a major setback in effective management of cancer as most patients have recurrence of cancer even after successful treatment using chemotherapy (Saraswathy and Gong, 2013; Prakash *et al*., 2013). Due to the structural diversity of natural products, they have been taunted as leads to overcome MDR in cancer cells (Kuete *et al*., 2015).

CHAPTER 2

LITERATURE REVIEW

2.1 Background Information on Cancer

Cancer can be defined as a complex heterogeous disease in which abnormal cells divide without control and are able to invade other tissues and metasize to distant sites (Bodduluru *et al.*, 2015). This condition is characterized by uncontrollable formation of abnormal cells which may mass together to form a growth or tumor, or proliferate throughout the body indicating abnormal growth at other sites (Tamokou and Kuete, 2014).

Majority of cancer cases are caused in three main ways including; incorrect diet, genetic disposition and environmental factors (Reddy *et al*., 2003). Incorrect diet as well as lifestyle changes account for 30% of all cancer cases and these include tobacco smoking, inadequate uptake of fruits and vegetables, physical inactivity and obesity (Fernando and Rupasinghe, 2013). Environmental risk factors include exposure to carcinogens as well as viruses whereas genetic disposition include chromosomal rearrangement, tumor suppressor genes and spontaneous transformation (Reddy *et al*., 2003). Treatment options that are available for cancer include; surgery, radiation therapy, chemotherapy and antibody based immunotherapy (Bodduluru *et al.*, 2015; Kuete *et al*., 2015).

Globally cancer accounts for more deaths than malaria, human immune deficiency virus (HIV), acquired immuno-deficiency syndrome (AIDS) and tuberculosis (TB) with 70% of all cancer occurring in low and middle income countries (Fernando and Rupasinghe, 2013). This burden is expected to increase in developing countries, with 15.5 million people being diagnosed with cancer and 12 million people dying of cancer by 2030 (Cancer Incidence Report, 2006).

In Kenya, cancer deaths are at third place after infectious and cardiovascular diseases causing 7% of the total national mortality yearly (Cancer Incidence Report, 2006). Although there is no population based data on cancer in Kenya, it is estimated that there are about 28,000 new incidences of cancer yearly with an annual mortality of 22,000 (Cancer Incidence Report, 2006). With these statistics, it has been estimated that 60% of those affected by cancer are below the age of 70 and those at risk of getting cancer below the age of 75 years is 14% while dying from

cancer below that age is 12% (Cancer Incidence Report, 2006). The leading cancers in Kenya are breast, cervical and oesophagus in women and prostate, oesophagus and Kaposi sarcoma in men (Cancer Incidence Report, 2006).

2.2 Carcinogenesis

Although cancer is a multifaceted disease, the mechanism of metastasis is similar for all solid tumors. Metastasis is defined as the process by which a tumor cell leaves the primary tumor and travels to a secondary site through the circulatory system thereafter establishing a secondary tumor (Woodhouse *et al*., 1997). This mechanism involves a series of steps through which normal cells acquire abnormal features that cause them to divide uncontrollably and metastasize (Reddy *et al*., 2003). These conversion steps of normal cells to cancerous ones, lies in their ability to be self-sufficient to growth signals and insensitive to growth inhibitory signals (Hanahan and Weinberg, 2000). The steps involved in carcinogenesis include: initiation, propagation, growth and progression.

2.2.1 Initiation

In this stage, carcinogens react with nucleic acid which is the DNA of tissue cells causing them to be cancerous. However, this stage may remain passive and a patient may only develop cancer after a long period of time (Reddy *et al*., 2003). Although this stage mainly involves reaction with carcinogens, it may also be due to genetic susceptibility in the cells increasing their risk of becoming cancerous. This occurs through oxidative damage to DNA whereby a cell containing damaged DNA divides before the DNA can be repaired resulting into a permanent genetic alteration (Reddy *et al*., 2003).

2.2.2 Propagation

This stage is the rate determining step and can occur over a period of time. However, one may not develop cancer in their lifestyle if they adopt lifestyle and diet changes at this stage of carcinogenesis (Reddy *et al*., 2003).

2.2.3 Growth and Progression

This is the final stage of cancer development whereby cancer can invade tissues surrounding it or spread to tissues and organs that are distant (Woodhouse *et al*., 1997). Growth mainly occurs through growth of blood vessels through a process known as angiogenesis. Here, the blood vessels allow the tumor to reach blood streams and colonize secondary sites resulting in the progression of cancer (Woodhouse *et al*., 1997).

2.3 Drug Sensitive and Multidrug Resistance in Cancer Cells

There are various treatment methods that are used for the treatment and management of cancer. These include; radiotherapy, surgery, antibody based immunotherapy and chemotherapy (Kuete *et al*., 2015). Among these methods, the most commonly used for solid and hematoptoic tumors is chemotherapy (Kuete *et al*., 2015) and it includes use of antimetabolites, antitumor, antibiotics, alkylating agents, platinum analogues and natural anticancer agents (Fernando and Rupasinghe, 2013).

Although there have been great strides in treatment of cancer due to early detection and diagnosis, there has been some challenges such as poor outcomes, lack of specificity of the cancer drugs and recurrence of the cancer (Kuete *et al*., 2015). This is mainly because the drugs are usually given simultaneously and are subject to a number of factors such as metabolism, rate of absorption and delivery to target tissues that is specific to a patient (Szakács *et al*., 2006). In addition to all these factors, chemotherapy has been found to injure rapidly dividing normal cells that are drug sensitive leaving behind a large proportions of drug resistant cells (Persidis, 1999) and this results into severe side effects and drug resistance (Ma and Wang, 2009).

Drug resistance in cancer can either be intrinsic or acquired (Choi, 2005). Intrinsic resistance is also referred to as natural or primary resistance that is mostly found in lung cancer and rectal cancer. In intrinsic resistance, the cancerous tumors fail to respond initially to the standard chemotherapeutic drugs (Choi, 2005). Acquired resistance occurs when tumors respond to chemotherapy but there is relapse later (Choi, 2005). Acquired drug resistance is usually in the form of multidrug resistance, MDR. This is a phenotype that is exhibited by malignant cells (Videira *et al*., 2014) whereby the malignant cells experience decreased sensitivity to

antineoplastic agents as well as unrelated cytotoxic drugs that differ in structure and functionality (Santini *et al*., 2001) such as the anthracyclines, doxorubicin and daunorubicin, the vinca alkaloids, vincristine and vinblastine, the antibiotics actinomycin D and mitomycin among others (Saeed *et al*., 2014).

There are numerous biological mechanism that have been linked with development of MDR in cancer cells but it has been mainly associated with protein transporters known as adenosine triphosphate binding cassette (ABC) (Lage, 2008), whose main role in the body is detoxification (Szakács *et al*., 2006). MDR develops due to over-expression of an energy defendant efflux pump known as P-glycoprotein (P-gp) which is a member of the larger ABC transporters (Hyde *et al*., 1990; Gottesman and Pastan, 1993).

These P-gp transporters are conserved proteins that transport solutes across cellular membranes (Higgins *et al.,* 1997). Some of the solutes transported include cytotoxic drugs, carcinogens in food and some biological compounds (Szakács *et al*., 2006). They have been associated with development of MDR as they have shown strong resistance to various compounds including vinca alkaloids commonly used as chemotherapeutic agents against cancer (Szakács *et al*., 2006).

After appearance of MDR, using high dosage of drugs has proved to be ineffective as it leads to toxic side effects and results into further stimulation of the resistance (Ozben, 2006) thus there has been need to find alternative solutions to MDR. Based on the mechanism of MDR, two strategies have been employed to develop treatment on MDR cancer. One method is through the use of high dosage of cytotoxic drugs to overcome the effect of cell extrusion transporter proteins. However, this methods has a major drawback due to toxic side effects caused by the high dosage of the anticancer drugs (Teodori *et al*., 2002).

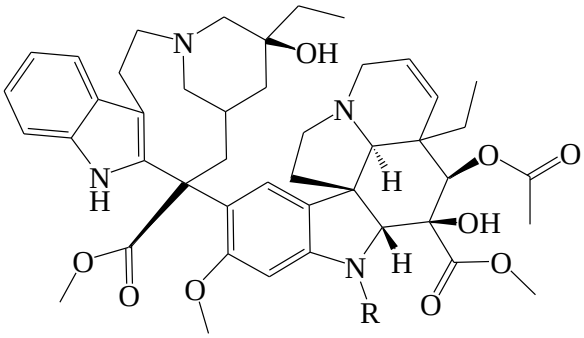
Another method is through using drugs that are not good substrate for the P-gp transporting proteins as they will not be transported out of the cells (Teodori *et al*., 2002). The third method is through use of ABC transporters inhibitors simultaneously with anticancer drugs. These inhibitors are referred to as chemosensitizers, MDR modulators or MDR reversers (Choi, 2005). These chemosensitizers sensitize resistant cells to the action of cytotoxic drugs and they include cyclic peptides, steroids, calmodulin antagonists, calcium channel blockers and drug analogues (Ambudkar *et al*., 1999).

2.4 Natural Products Based Anticancer Agents

Natural products are a rich source of pharmacological agents with half of medicinal drugs in use today being either natural products or their synthetic analogues (Newman and Cragg, 2007). In the case of cancer, over 60% of anticancer used are from plant origin (Cragg and Newman, 2005). There are five major structural classification of plant derived anticancer compounds (Newman and Cragg, 2007; Khazir *et al.,* 2014). These are vinca alkaloids from *Catharanthus roseus* (Da Rocha *et al.*, 2001), combrestatin from *Combetum caffrum* kuntze (Khazir *et al*., 2014), taxanes from *Taxus brevifolia* (Wall and Wani, 1996), camptothecin from *Camptotheca acuminata* (Wall and Wani, 1996) and epipodophyllotoxin from *Podophyllum peltatum* (Imbert, 1998 and Khazir *et al*., 2014). These classifications with their examples are summarized in Table 2.1 with corresponding structures given in Figure 2.1.

Table 2.1: Plant based anticancer agents

Vinca alkaloids			
Anticancer agent	**Mechanism of action**	**Cancer use**	**References**
Vinblastine (**6**)	Interferes with microtubule assembly	Breast, lymphoma, germ cells and renal	Jordan *et al.*, 1991; Cragg and Newman, 2005; Bhanot *et al.*, 2011
Vincristine (**7**)	Interferes with microtubule assembly	Leukaemia, lymphoma, breast and lung	
Epipodophyllotoxins			
Etoposide (**8**)	Topoisomerase II inhibitor	Lung, testicular and lymphomas	Nobili *et al*., 2009; Ma and Wang, 2009; Srivastava *et al.*, 2005
Teniposide (**9**)	Topoisomerase II inhibitor	Central nervous system tumors, malignant lymphoma and bladder	
Taxanes			
Paclitaxel **(10)**	Disrupts de-polymerization of microtubule	Advanced ovarian cancer, breast, bladder, lung and neck	Bhanot *et al.*, 2011; Ma and Wang, 2009
Docetaxel **(11)**	Disrupts de-polymerization of microtubule	Breast and lung	
Camptothecin			
Topotecan **(12)**	Inhibits DNA topoisomerase I	Ovarian, lung and pediatric	Bhanot *et al.*, 2011; Nobili *et al*., 2009
Irinotecan **(13)**	Inhibits DNA topoisomerase I	Colorectal and lung	
Combretastatin			
Combretastatin A_4 **(14)**	Inhibits tubulin polymerization	Leukemia, colon and lung	Khazir *et al*., 2014



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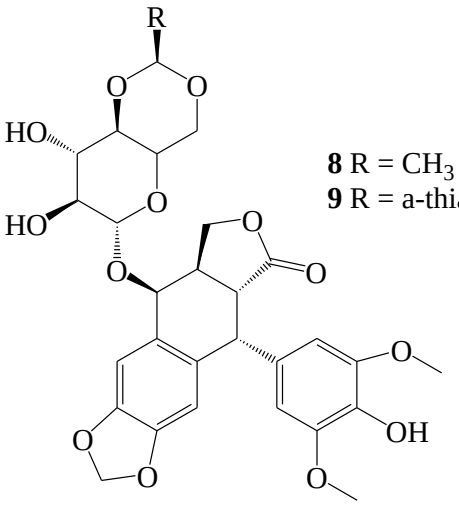
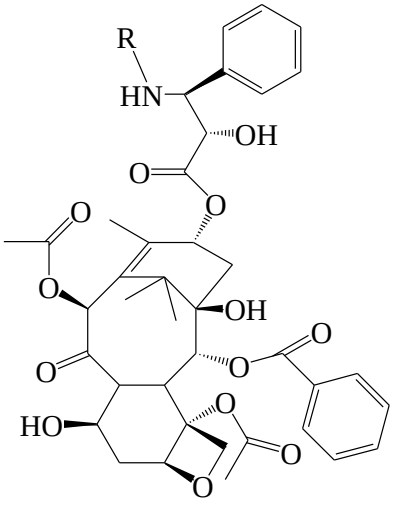
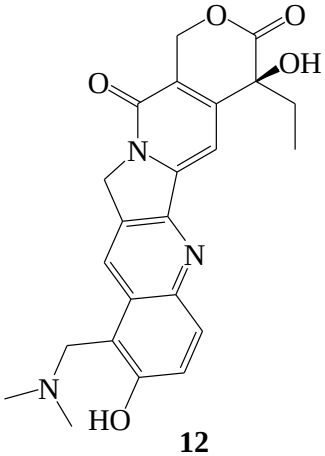
7 R = CHO



8 R = CH_3
9 R = a-thiazoe



10 R = acetophenone
11 R = *tert*-butyl acetone



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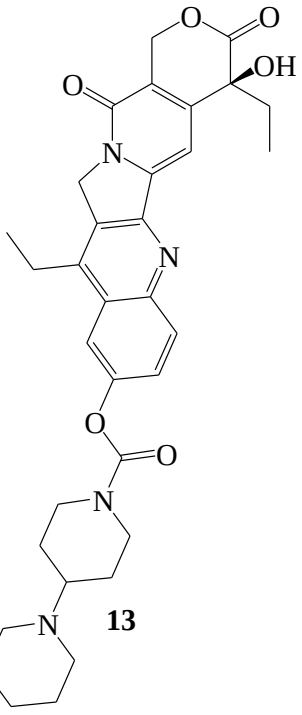

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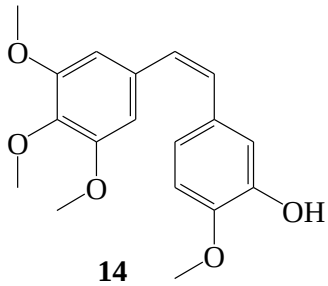

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Figure 2.1: Plant based anticancer agents

2.5 Botanical Information

2.5.1 Family Euphorbiaceae

The family Euphorbiaceae consists of 280 genera and 8000 species occurring in tropical and temperate regions worldwide. The plants in this family range from herbs and shrubs to trees and cactus (Hecker, 1968).

2.5.2 The Genus *Bridelia*

The genus *Bridelia* belongs to the family Euphorbiaceae consisting of 60–70 species, distributed around Africa and Asia, with 50 of these species distributed in Madagascar, tropical Africa, Yemen and Asia (Ngueyem *at al.*, 2009). In Kenya, this genus is represented by three species; *B. cathartica, B. micrantha* and *B. taitensis* distributed in various parts of the country (Beentje, 1994).

B. micrantha
(Photo by Bart Wursten)

B. taitensis
(https://plants.jstor.org)

B. cathartica
(Bart Wursten)

Figure 2.2: Bridelia species in Kenya

B. micrantha, also known as Coast gold leaf (Okeleye *et al.*, 2011), is a medium to tall tree up to 20m with dense widely spreading crown with alternate, large and simple leaves (Radcliffe-Smith, 1996). It mainly grows in the coastal forests, riverine and swamp forest. (Radcliffe-Smith 1996). In Kenya it is mainly found in the Eastern and Western parts of the country. Locally it is

known as *Mukwego* in Eastern Kenya (Gakuya *et al*., 2013) and S*hikagania* or *Kamulanda-ngombe* in Western Kenya (Ochwang'i *et al*., 2014).

B. cathartica is a small tree with long branches and leaves. The flowers are greenish-yellow in color. The fruits are edible and are red when raw and black when ripe (Radcliffe-Smith, 1996). *B. cathartica* mainly grows in the coastal areas and locally is known as *Mkarakara* in Swahili and *Mkalakala* in Giriama (Maundu *et al.*, 1999). *B. taitensis* is a shrub about 2 to 3 meters high with broad leaves. Its flowers are greenish yellow and appear in clusters. Its fruits are small and green when raw and black when ripe. They are edible with a sweet sour taste (Maundu *et al*., 1999). It is mainly found in the eastern, northern and coastal parts of Kenya. Locally, it is known as *Karo* (Borana), *Muce* (Mbeere), *Mwaanzia* (Akamba), *Muyee* (Tharaka) and *Lapironit* (Samburu) (Maundu *et al*., 1999).

2.6 Ethnomedicinal Uses of Plants from the Genus *Bridelia*

The roots, barks and leaves of 10 species of *Bridelia* are used for local and traditional medicines in Asia and Africa for treatment of various ailments such as bronchitis, anemia, sexually transmitted diseases among others (Ngueyem *et al*., 2009). Ethnomedicinal uses of different species of *Bridelia* are summarized in Table 2.2 below.

Table 2.2: Ethnomedicinal uses of *Bridelia Species*

Species	Plant part	Use	References
B. atroviridis	Sb, L	As an aphrodisiac and as purgative and diuretic. For the treatment of venereal diseases	Ngueyem *et al*., 2009
B. balansae	L	For the treatment of bronchitis	Ngueyem *et al*., 2009
B. brideliifolia	T, Rb, Sb, L	Used to stimulate digestion as emetic, purgative for treating vaginal prolapsed, elephantiasis, oxytocic, insanity, gastrointestinal problems and migraine	Neuwinger, 2000
B. cathartica	Rb, L, Sb	For treating anemia, asthma, constipation, anorexia, fever, cardiac pains, amoebic dysentery, hemorrhoids, female and male infertility, coughs, aphrodisiac, epigastric pain, malaria, rectal prolapsed, headache, epilepsy, kidney pain and is used as purgative	Watt and Breyer-Brandwijk, 1962; Ouma *et al*., 1997
B. crenulata	Sb	For treatment of infertility and for stopping menorrhagia	Ngueyem *et al*., 2009
Bridelia ferruginea	F, L, Sb, Rb	For treatment of dysentery, diabetes, coughs, diuretic, toothache, thrush, skin diseases and eruption. Has antimicrobial activity, anti-inflammatory properties and is used as antidote for arrow poison.	Bakoma *et al*., 2013; Olajide *et al*., 2003; Cimanga *et al*., 1999; 2001; Talla *et al*., 2002
B. grandis	Sb, L	Used in the treatment of oral cavity	Ngueyem *et al*., 2009
B. micrantha	Sb, L, Rb	For the treatment of gastro-intestinal ailments, paralysis, painful joints, retained placenta, diabetes mellitus, syphilis, jaundice, tape worm abdominal pain, conjunctivitis, headache, coughs, threadworms, sore eyes, epigastric pain, anemia, pneumonia and is used as tonic for children.	Lin *et al.*, 2002; Ngueyem *et al*., 2009; Steenkamp, 2003; Gbolade, 2009; Ssegawa and Kasenene, 2007; Gakuya *et al*., 2013; Peter *et al.*, 2014; Samie *et al.*, 2005

Sb-stem bark, Rb-root bark, L-leaves, T-twigs, F-fruits

2.7 Phytochemistry of the Genus *Bridelia*

Phytochemical analysis of different species of the genus *Bridelia* has yielded various important secondary metabolites including saponins, lignans, flavonoids and triterpenes (Ngueyem *et al*., 2009).

2.7.1 Flavonoids

Flavonoids are low molecular weight polyphenolic compounds based on the flavan nucleus (Coulatate, 1990), consisting of three phenolic rings normally referred to as A, B and C pyrane rings (Figure 2.2). Flavonoids are generally classified according to their structure into flavanols, flavones, flavanones, anthocyanidin, isoflavones, dihydroflavones and chalcones (Cook and Samman, 1995).

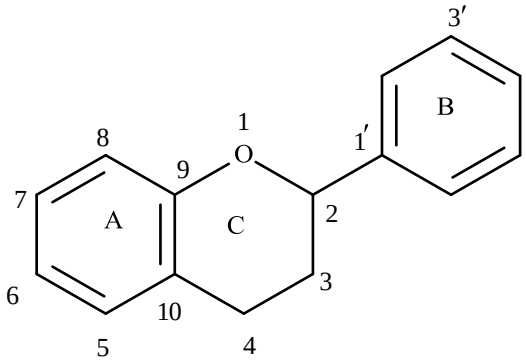
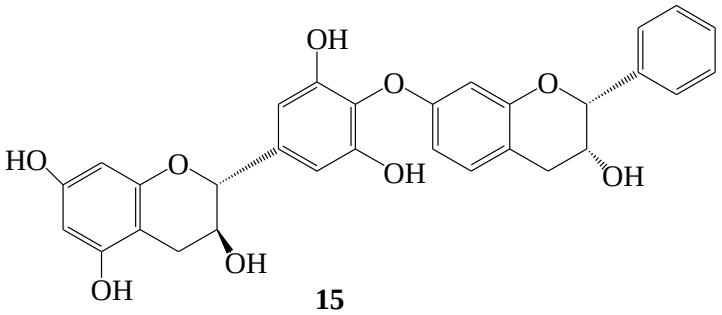


Figure 2.3: Basic structure of flavonoids

Some flavonoids from the genus *Bridelia* are summarized in Table 2.3 with corresponding structures in Figure 2.3.

Table 2.3: Flavonoids isolated from genus *Bridelia*

Compound	Plant	Plant part	Reference
Epigallocatechin (7→4´)-gallocatechin (**15**)	*B. ferruginea*	Stem bark	Bruyne *et al.*, 1997
3,3´,4´,5,5´,7-Hexahydroxyflavone (**16**)	*B. ferruginea*	Stem bark	Cimanga *et al.*,1999
3-O-α-L-Ribopyranoside (Quercetin 4´-methyl ether) (**17**)	*B. tomentosa*	Leaves	Shu *et al.*, 2006
Delphinidin (**18**)	*B. micrantha*	Stem bark and leaves	Pegel and Rogers, 1968
Caffeic acid (**19**)	*B. micrantha*	Leaves	Pegel and Roberts, 1968
Luteoforol (3,4,4,5,7-pentahydroxyflavone) (**20**)	*B. crenulata*	Stem bark	Ramesh *et al.*, 2001



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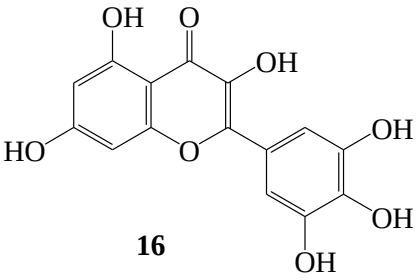
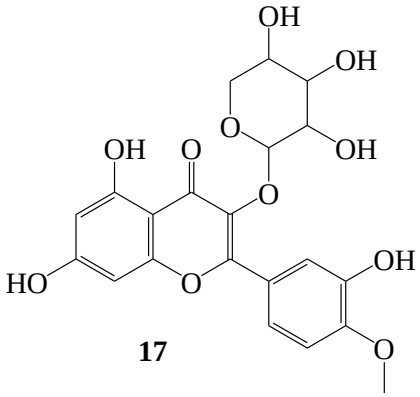


16



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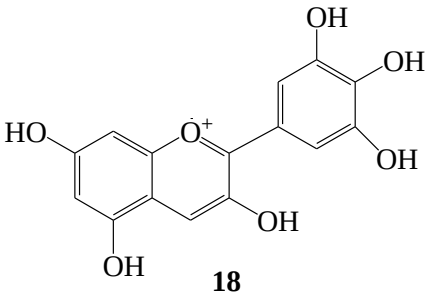

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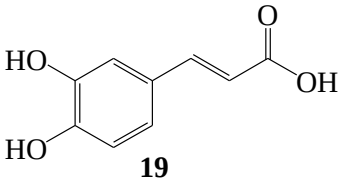

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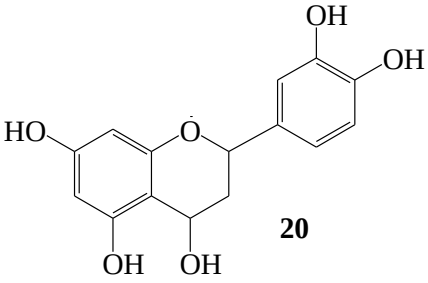

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Figure 2.4: Flavonoids isolated from the genus *Bridelia*

2.7.2 Triterpenes and saponins

2.7.2.1 Triterpenes

Triterpenes are a class of organic compounds including steroids and sterols with C_{30} carbon skeleton having six isoprene units (Sandjo and Kuete, 2013). Triterpenes can either be tricyclic or pentacyclic. Tricyclic triterpenes contain three, six membered rings and one five membered ring whereas pentacyclic triterpenes contain four six membered ring and one five membered ring or five six membered rings (Sandjo and Kuete, 2013). The basic structure of terpenes is given in Figure 2.4. The genus *Bridelia* elaborates both tricyclic and pentacyclic triterpenoids. In this study tricyclic triterpenoids were characterized from *B. micrantha.*

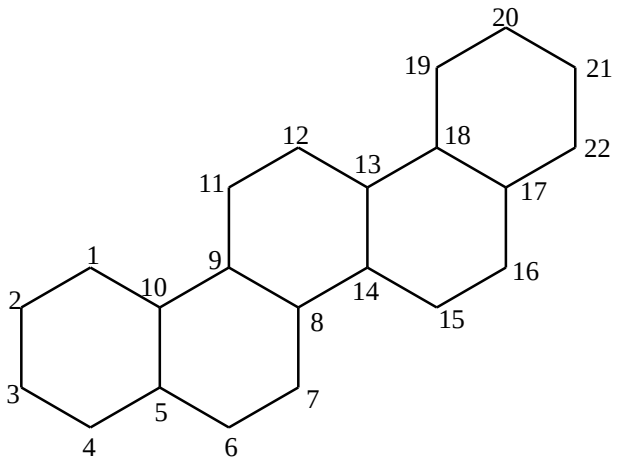


Figure 2.5: Basic structure of triterpenes

2.7.2.2 Saponins

Saponins are naturally occurring surface active glycosides consisting of a sugar moiety glycosidically linked to a hydrophobic aglycone usually a triterpenoid (Fig 2.4) or a steroid as shown in Figure 2.5.

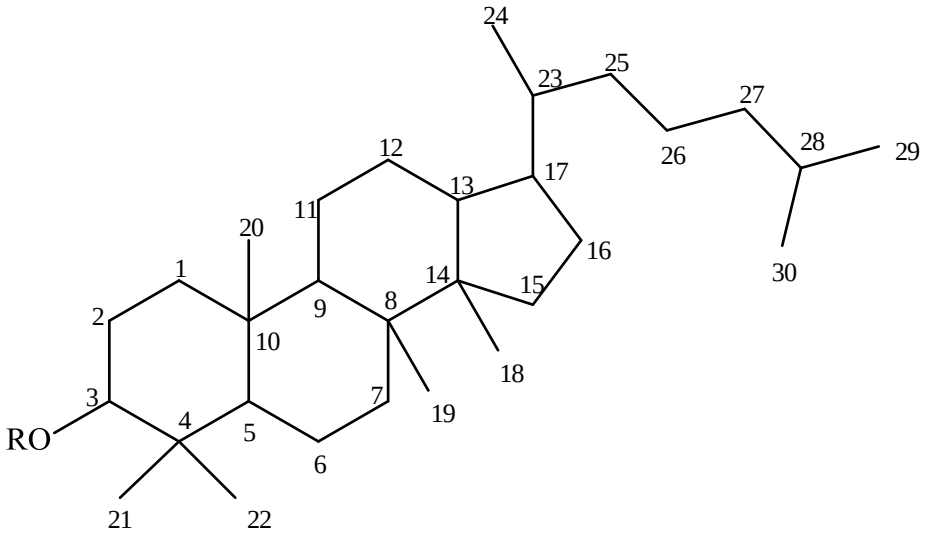
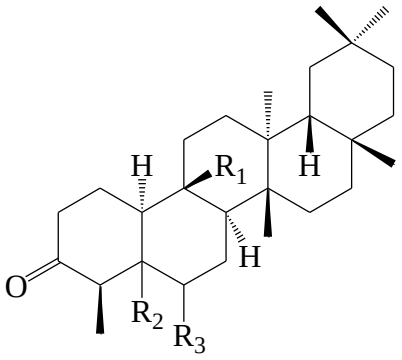


Figure 2.6: Basic structure of saponins

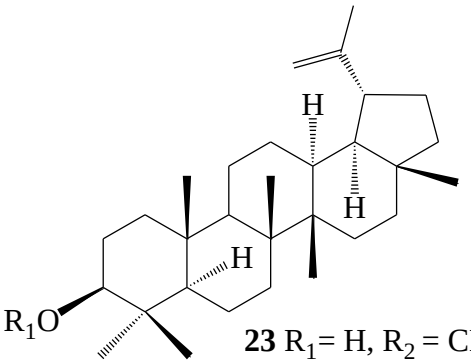
Triterpenes and saponins isolated from the genus *Bridelia* are given in Table 2.4 with corresponding structures given in figure 2.6.

Table 2.4: Triterpenes and saponins isolated from the genus *Bridelia*

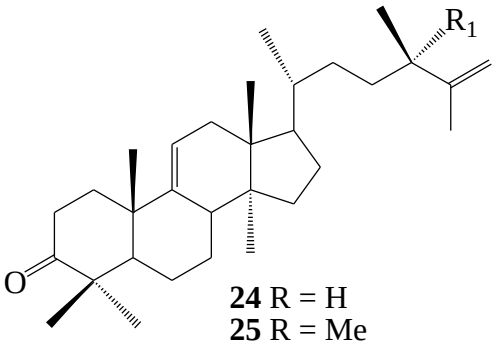
Compound	**Plant**	**Plant part**	**References**
Friedelin (**21**)	*B. monoica*	Leaves	Hui and Fung, 1968
friedelan-3-beta-ol (**22**)	*B. ovata*	Branches	Boonyaratavej and Petsom 1991
Betulin (**23**)	*B. ferruginea*	Stem bark	Lasisi and Kareem, 2011
24-Methyl-lanosta-9(11)-25-dien-3-one (**24**)	*B. tomentosa*	Roots	Boonyaratavej and Petsom, 1991
24,24-dimethyllanosta-9(11)-25-dien-3-one (**25**)	*B. ovata*	Branches	Boonyaratavej *et al.,* 1992
Taraxerone (**26**)	*B. micrantha*	Stem bark	Pegel and Rogers, 1968
Taraxerole (**27**)	*B. micrantha*	Stem bark	Pegel and Rogers, 1968
Glucoside of betulin (**28**)	*B. ferruginea*	Stem bark	Lasisi and Kareem, 2011



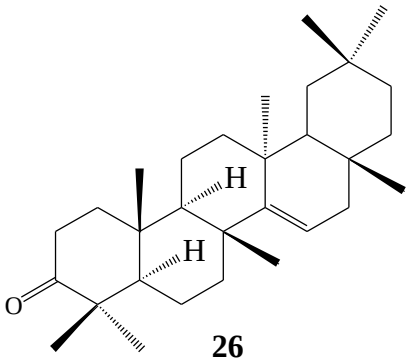
21 R_1=H, R_2 = CH_3, R_3 = H
22 R_1 = CH_2OH, R_2 = H, R_3 = CH_3



23 R_1= H, R_2 = CH_2OH
28 R_1 = Glu, R_2 = COOH



24 R = H
25 R = Me



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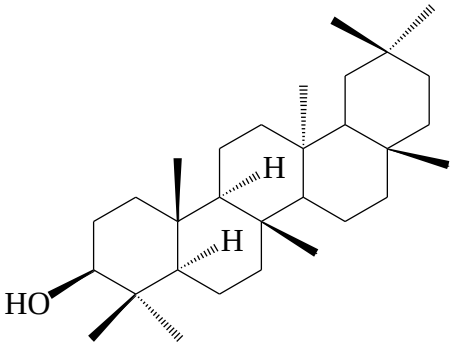

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Figure 2.7: Triterpenes and saponins isolated from the genus *Bridelia*

2.7.3 Lignans

Lignans are a group of dimeric phenypropanoids where two C_6 and C_3 are attached by its central carbon atoms. Their basic structure is given in Figure 2.7.

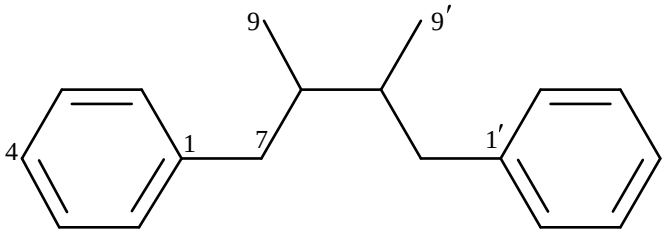
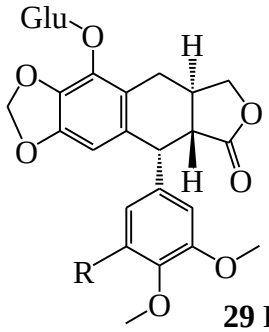


Figure 2.8: Basic structure of lignans

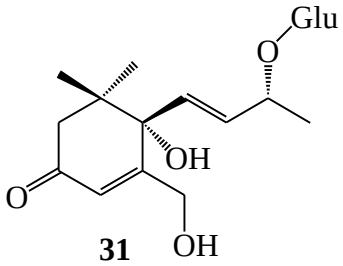
Lignans that have been isolated from the genus *Bridelia* are given in Table 2.5 with corresponding structures given in Figure 2.8.

Table 2.5: Lignans isolated from the genus *Bridelia*

Compound	**Plant**	**Plant part**	**References**
5-demethoxy-beta-peltatin-5-O-β-d-glucopyranoside (**31**)	*B. ferruginea*	Roots	Rashid *et al*., 2000
β-peltatin-5-O-β-d-glucopyranoside (**32**)	*B. ferruginea*	Roots	Rashid *et al*., 2000
Bridelioniside A (**33**)	*B. glauca*	Leaves	Sueyoshi *et al*., 2006
Bridelioniside B (**34**)			
Bridelioniside C (**35**)			
Bridelioniside D (**36**)			
Bridelioniside E (**37**)			
Bridelioniside F (**38**)			



29 R = H
30 R = OMe



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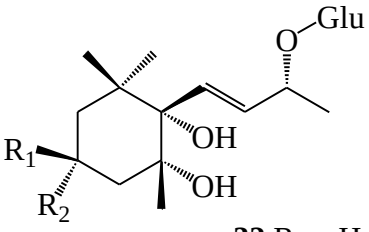
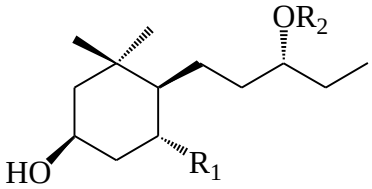
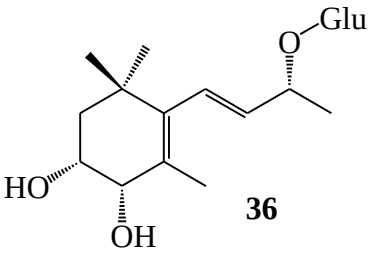


32 R_1 = H, R_2 = OH
33 R_1 = OH, R_2 = H



34 R_1 = CH_2OH, R_2= Glu
35 R_1 = COOGlu, R_2 = H



36

Figure 2.9: Lignans isolated from genus *Bridelia*

2.7.4 Tannins

Tannins are polyphenolic secondary metabolites that are found in higher plants (Karamali and Teunis, 2001) with molecular weights ranging from 500 to 3000 (Bate-smith, 1973). Tannins isolated from the genus *Bridelia* are hydrolizable tannins (Figure 2.9) that are polymers of gallic or ellagic acids that have been esterified to a core molecule (Reed, 1995). Tannins that have been isolated from the genus *Bridelia* are summarized in Table 2.6 with corresponding structures in Figure 2.10.

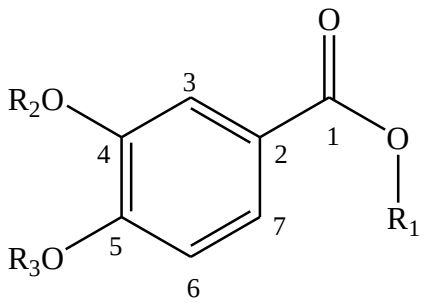
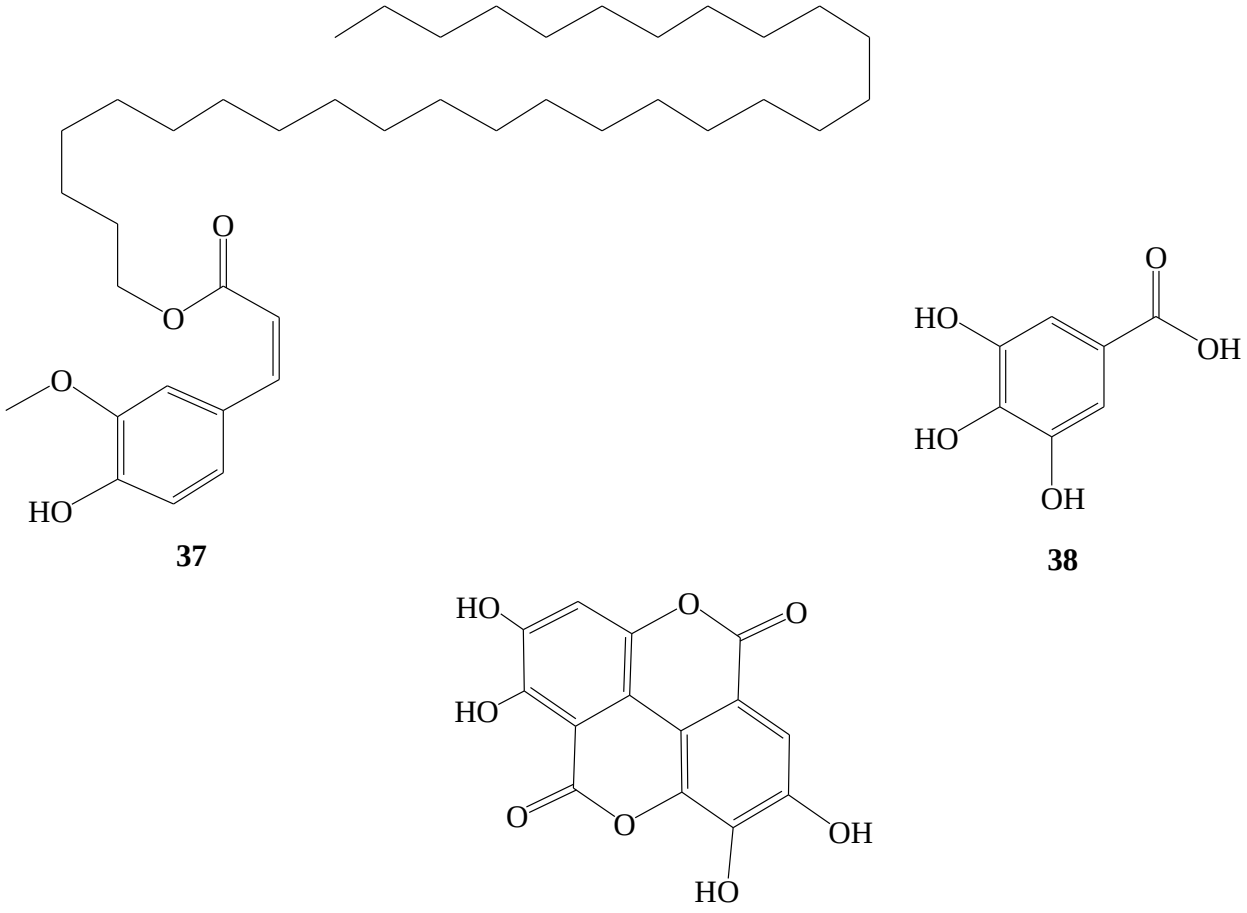


Figure 2.10: Basic structure of tannins

Table 2.6: Tannins isolated from the genus *Bridelia*

Compound	**Plant**	**Plant part**	**References**
Triacontyl ester (**37**)	*B. Ovata*	Branches	Boonyaratavej *et al.*, 1992; Ngueyem *et al.*, 2009
Gallic acid (**38**)	*B. micrantha*	Stem bark	Pegel and Rogers, 1968
Ellagic acid (**39**)	*B. micrantha*	Stem bark	Pegel and Rogers, 1968



37 **38** **39**

Figure 2.11: Tannins isolated from genus *Bridelia*

2.8 Biological Activities of *Bridelia* Extracts and Compounds

Most *Bridelia* species have been used traditionally for their antimicrobial and anti-inflammatory properties that have been investigated in order to validate these traditional uses (Ngueyem *et al*., 2009). Table 2.6 gives a summary of the biological activities of extracts and compounds of various *Bridelia* species.

Table 2.7: Biological activities of compounds and extracts in the *Bridelia* Species

Species	Part	Extract/compound	Activity	References
B. ferruginea	Sb	Aqueous	Anti-inflammatory	Olajide *et al*., 2003
	L	Methanol	Anti-diabetic	Ngueyem *et al*., 2009
	Sb, F	Ethanolic, aqueous, MeOH	Anti-microbial	Irobi *et al*., 1994
	Sb	Epigallocatechin (7→4´)-gallocatechin (**18**)	Anti-inflammatory	Bruyne *et al*., 1997.
	L	Methanolic, EtOAc, hexane	Anti-bacterial	Ngueyem *et al*., 2009
	Rb	5-Demethoxy-β-peltatin-5-O-β-D-glucopyranoside (**34**)	Antitumor	Rashid *et al*., 2000
	Rb	β-Peltatin-5-O-β-d-glucopyranoside (**35**)	Antitumor	Rashid *et al*., 2000
B. atroviridis	L	Methanolic	Antimicrobial	Ngueyem *et al*., 2009.
B. crenulata	Sb	Aqueous	Antibacterial	Ramesh *et al*., 2001; Ngueyem *et al*., 2009
		Luteoforol		
B. grandis	Sb	Methanolic, aqueous and MeOH/H_2O.	Antimicrobial, antibacterial	Ngueyem *et al*., 2009
	Rb	Not specified.	Antitrypanosomal, antiplasmodial	Atindehou *et al*., 2004
B. ndellensis	Sb	EtOH/CH_2Cl_2	Antidiabetic (type 2 diabetes)	Sokeng *et al*., 2005
B. micrantha	L	Methanolic	Antidiabetic	Adika *et al*., 2012.
	Sb	Not specified	Anti-convulsant, sedative effects	Ngo Bum *et al*., 2012
		H_2O/MeOH	Antinocicptive.	Onoja *et al*., 2014
		Ethyl acetate	Hepato-protective, anti-oxidant	Nwaehujor and Udeh 2011
		Acetone	Cytotoxicity for MDR-TB	Green *et al*., 2010
		Aqueous, MeOH in CH_2Cl_2	Anthelmintic	Waterman *et al*., 2010
	Sb	MeOH	Antibacterial	Samie *et al*., 2005; Steenkamp *et al*., 2007
	T	Not specified	Antiplasmodial	Ngueyem *et al*., 2009
	Rb	*n*-Butanol	Anti-HIV	Bessong *et al*., 2006

Rb-root bark, Sb-stem bark, T-twigs, L-leaves, F-fruit

2.9 Botanical Information on *Tabernaemontana ventricosa*

2.9.1 The Family Apocynaceae

The family Apocynaceae is mainly found in the tropics with majority being in Africa. It consists of 366 genera (Endress *et al.*, 2014). The plants in this family include shrubs, vines, trees and herbs (Endress and Bruyns, 2000).

2.9.2 The Genus *Tabernaemontana*

The genus *Tabernaemontana* belongs to the family Apocynaceae and consists of 100 species that are distributed in the tropics and sub tropics part of the world (van Beek *et al.*, 1984). In Kenya, this genus is represented by 4 species: *T. elegans, T. pachysiphon, T. stapfiana* and *T. ventricosa* (Beentje, 1994).

T. stapfiana

(Photo by Bart Wursten)

T. ventricosa

(Bart Wursten)

T. elegans

(Colin Wenham)

T. pachysiphon

(Photo by Michael Kurschner)

Figure 2.12: *Tabernaemontana* species in Kenya

T. elegans is a shrub or tree, 4-15 meters long. The stem bark is pale brown while the leaves are shiny and large (Beentje, 1994). The flowers are white or pale yellow with a nice fragrance. The fruits are grey with pale warts that resembles a toad but they are edible (Neuwinger, 2000). Locally, it is known as *Mbombo* (Swahili), *Chibombo chereru* (Digo) and *Yamoozi* (Boni) (Omino and Kokwaro, 1993).

T. ventricosa also known as forest toad tree is a small to medium tree ranging from 2.5 to 10 meters long with a greyish brown stem bark (Schmidt *et al.,* 2002). The leaves are narrowly elliptic with a round base. The flowers are white or pale yellow (Beentje, 1994). The fruits are pods that are usually joined at the base (Schmidt *et al.,* 2002). Locally, it is known as *Kitondo* (Abaluhyia), *Kiracha* (Taita) and *Mwerere* (Agikuyu) (Omino and Kokwaro, 1993).

T. stapfiana, also known as soccer ball fruit, is a medium sized tree ranging from 4 to 21 meters high with a brown bark (Beentje, 1994). The leaves are long, hairless and dark green in color with pale undersides (http://www.zimbabweflora.co.zw/speciesdata). The flowers are white with a yellow throat while the fruits are large up to 20 cm in diameter. Locally it is known as *Terendet* (Kipsigis), *Mwerere* (Agikuyu), *Mrimbo* (Taita) and *Omobondo* (Abagusii) (Omino and Kokwaro, 1993).

T. pachysiphon is a medium sized tree ranging from 4 to 10 meters with a dark brown bark. The leaves are leathery with random black spots beneath. The flowers are white while the fruits are pale green with white spots (Beentje, 1994). Locally it is known as *Mukendi* (Taita), *Mwerere* (Agikuyu), *Kiracha* (Taveta), *Kibombo* (Digo) and *Muerere* (Ameru). (Omino and Kokwaro, 1993).

2.10 Ethnomedicinal uses of *Tabernaemontana*

Plants in this species have wide medicinal uses ranging from decoctions to steam baths that are used to cure venereal diseases such as syphilis (van Beek *et al*., 1984). A summary of these ethnomedicinal uses is given in Table 2.8.

Table 2.8: Ethnomedicinal uses of *Tabernaemontana* species

Plant	Plant part	Medicinal use	Reference
T. ventricosa	Lx	Heal wounds, treat fever	van Beek *et al*., 1984; Kokwaro, 1976
T. pachysiphon	Lx, Fs, L, Rb	Sore eyes, treat wounds, headache, stomach ache, constipation, hypnotic	van Beek *et al*., 1984; Kokwaro, 1976; Omino and Kokwaro, 1993
T. stapfiana	Rb, Sb	Pneumonia, chest problems	Jeruto *et al.*, 2008
T. elegans	Rb	Pulmonary disease	van Beek *et al*., 1984; Palgrave and Palgrave, 1957; Watt and Breyer-Brandwijk, 1962
T. crassa	Lx, L, Sb, F	Ringworms, tonic, treatment of mental illness, wound disinfectant, severe headaches, leprosy, rheumatism, stomach ache, sinusitis, diarrhea, anthelmintic	van Beek *et al*., 1984; Irvine, 1961; Iwu, 1982
T. corymbosa	Ls, Sb, Rb	Treat sores, orchitis, hydrocele	van Beek *et al*., 1984
T. divaricata	Lx, Rb, Sb, Fl	Prevent wound inflammation, eye sore, toothache, headache, intestinal worms, dysentery, cornea inflammation, coughs, asthma, catarrh, fevers, mania, ulceration, morbid secretion of urine, leprosy, hiccough, vomiting, suppression of urine, disorders of semen and womb, purgative, paralysis, jungle fever, edema, rabies	van Beek *et al*., 1984; Sajem and Gosai, 2006; Jain *et al*., 2013
T. dichotoma	S, Sb, Rb, Lx, F	Purgative, snake bites, scorpion stings, ulcers, fistulae, treat wounds, toothache, eye infections	van Beek *et al*., 1984
T. orientalis	Rb, Lx, L, Sb	Stomach ache, sore nose, induce abortions, ulcers and sores, reduce swellings, headaches, toothache	van Beek *et al*., 1984; Holdsworth, 1977
T. catharinensis	Rb, Sb, L	Treat snake bites, eliminate warts, relieve toothache, anti-inflammatory	Rizo *et al*., 2013; Gomes *et al*., 2009; Boligon and Athayde, 2012

Rb-root bark, Sb-stem bark, S-seeds, Ls-leaf sap, Lx-latex, L-leaves, F-fruits, Fl-flower, Fs-fruit sap

2.11 Phytochemistry of genus *Tabernaemontana*

Phytochemical analysis of different species of the genus *Tabernaemontana* has yielded mostly indole alkaloids and a few triterpenes (van Beek *et al*., 1984).

2.11.1 Alkaloids

Alkaloids are organic nitrogenous compound with nitrogen being either in its primary, secondary or tertiary form. Alkaloids that have been isolated from genus *Tabernaemontana* are the indole and bis-indole alkaloids (van Beek *et al*., 1984).

2.11.1.1Indole alkaloids

These are alkaloids that are derived from tryptophan (tryptamine) with their structure having the indole structure (O'Connor and Maresh, 2006).

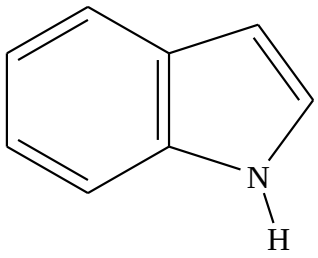


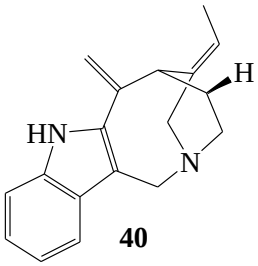
Figure 2.13: Basic structure of indole

Bis-indole alkaloids are compound that have two indole alkaloids attached to each other (van Beek *et al.*, 1984). Indole and *bis*-indole alkaloids that have been isolated from genus *Tabernaemontana* are given in Table 2.9 with corresponding structures in figure 2.12.

Table 2.9: Alkaloids isolated from the genus *Tabernaemontana*

Compound	Plant	Plant part	Reference
Apparicine (**40**)	*T. elegans*	Wp	van der Heijden *et al*., 1986
16,22-Dihydro-16-hydroxyapparicine (**41**)	*T. dichotoma*	L	Perera *et al*., 1985
Conoliferine (**42**)	*T. corymbosa*	Sb	Lim and Kam, 2009
Conodiparine A (**43**)	*T. corymbosa*	L	Kam and Sim, 2003a
Conodiparine C (**44**)	*T. corymbosa*	L	Kam and Sim, 2003a
Conoduramine (**45**)	*T. crassa*	rb	Cava *et al*., 1965
Conodurine (**46**)	*T. pachysiphon*	Rb	Kingston *et al*., 1977
Conofoline (**47**)	*T. divaricata*	L	Kam and Anuradha, 1995
19-Hydroxyconopharyngine (**48**)	*T. crassa*	F, L, T, Sb	van Beek *et al*., 1985
3-Hydroxyconopharyngine (**49**)	*T. pachysiphon*	Sb, Rb	van Beek *et al*., 1984
Conophyllidine (**50**)	*T. divaricata*	L	Kam and Anuradha, 1995
3S-Cyanocoronaridine (**51**)	*T. divaricata*	Sb	Rastogi *et al*., 1980
5-Hydroxy-6-oxocoronaridine (**52**)	*T. divaricata*	Sb	Rastogi *et al*., 1980
5-Oxocoronaridine (**53**)	*T. divaricata*	Rb	Rastogi *et al*., 1980
6-Oxocoronaridine (**54**)	*T. divaricata*	Rb	Rastogi *et al*., 1980
Crassanine (**55**)	*T. crassa*	Wp	Cava *et al*., 1968
Dichomine (**56**)	*T. dichotoma*	L	Perera *et al*., 1983d
Dregamine (**57**)	*T. elegans*	Sb, Rb	van der Heijden *et al*., 1986
Ibogamine (**58**)	*T. crassa* *T. divaricata* *T. stapfiana*	Wp, Rb, Sb	Cava *et al*., 1965; Rastogi *et al*., 1980; Kingston *et al*., 1977
16-Epiisositsirikine (**59**)	*T. pachysiphon*	Sb, Rb	van Beek *et al*., 1984
Tronoharine (**60**)	*T corymbosa*	Sb	Kam *et al*., 1999
Vallesamine (**61**)	*T. dichotoma*	Sb.	Perera *et al*., 1985
Voacamine (**62**)	*T. dichotoma*	Sb	Perera *et al*., 1985
Voacangine (**63**)	*T. divaricata*	Rb, L, Fl	Arambewela and Ranatunge, 1991
3-Ethoxyvoacangine (**64**)	*T. divaricata*	Sb	Kam *et al*., 2004
Voaharine (**65**)	*T. divaricata*	L	Kam *et al*., 1992
Voastrictine (**66**)	*T. corymbosa*	Sb, Rb.	Kam *et al*., 2001
Voalenine (**67**)	*T. divaricata*	L	Kam *et al*., 2003c
Vobatricine (**68**)	*T. corymbosa*	Sb	Kam and Sim, 2002
Conodiparine E (**69**)	*T. corymbosa*	L	Kam and Sim, 2003a
Conodirinine A (**70**)	*T. corymbosa*	L	Kam and Sim, 2003b
Conodirinine B (**71**)	*T. corymbosa*	L	Kam and Sim, 2003b

Rb–root bark, Sb-stem bark, Wp-whole plant, L-leaves, F-fruit, T-twigs, Fl-flower



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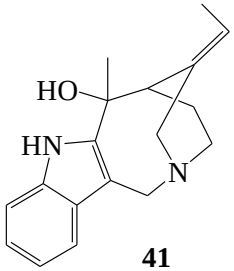

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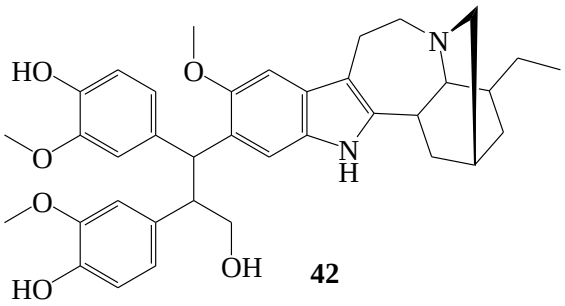

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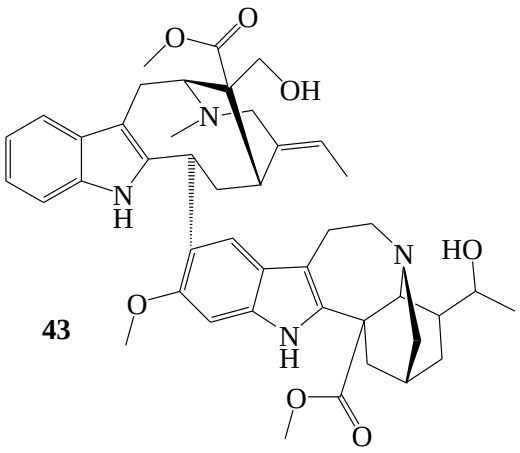

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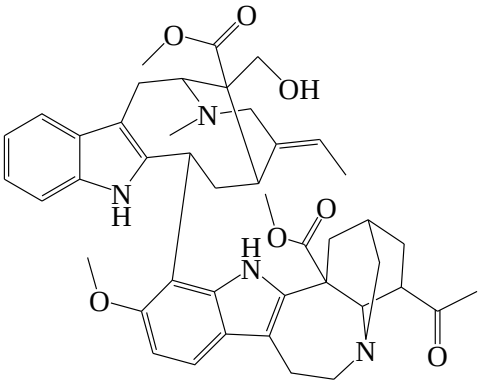

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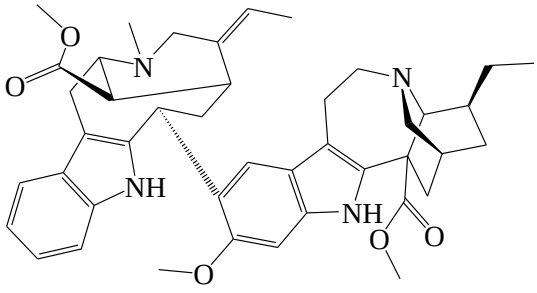

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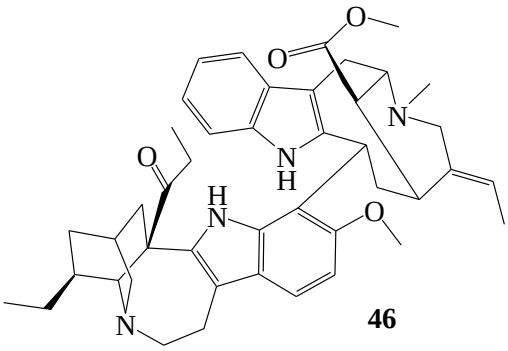

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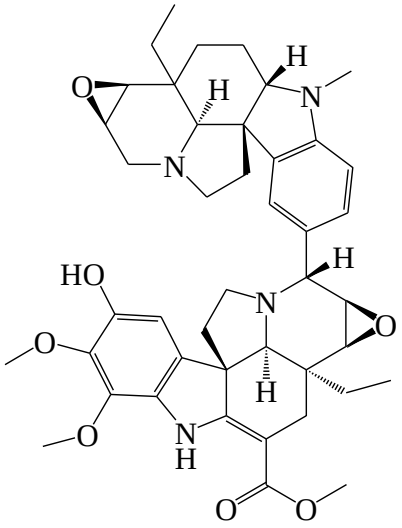

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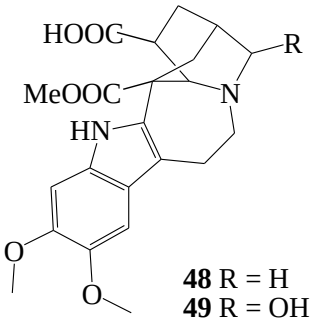

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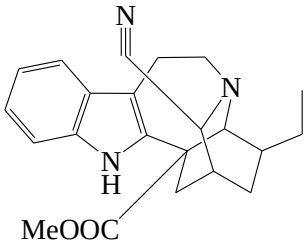
49 R = OH



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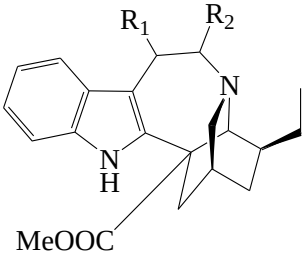
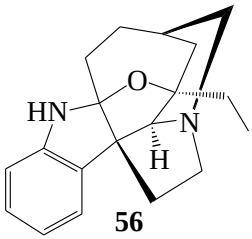


52 R_1=O R_2=OH
53 R_1-=H R_2=O
54 R_1=O R_2=H



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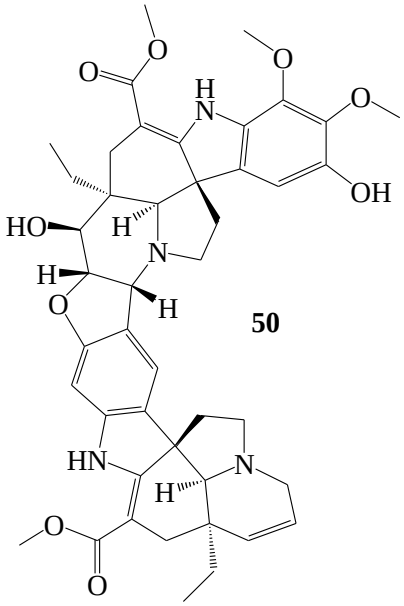

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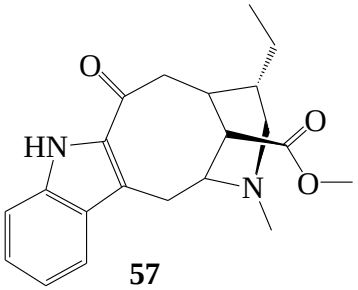

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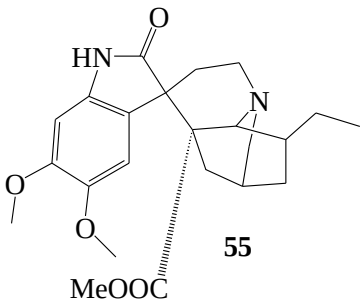

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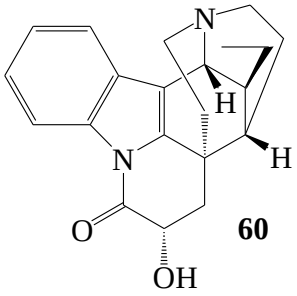

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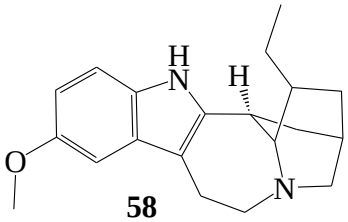

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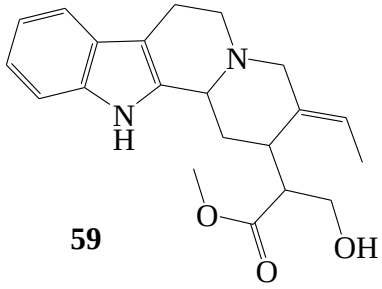

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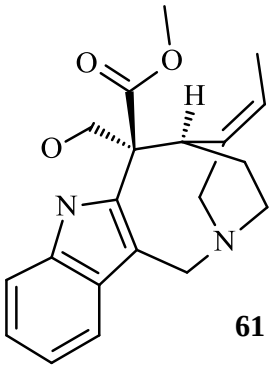

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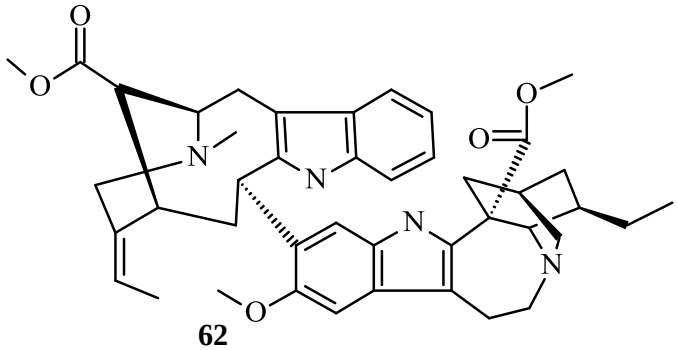

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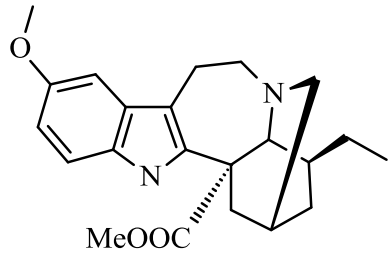

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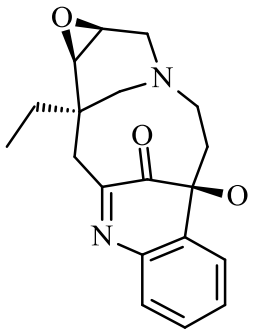

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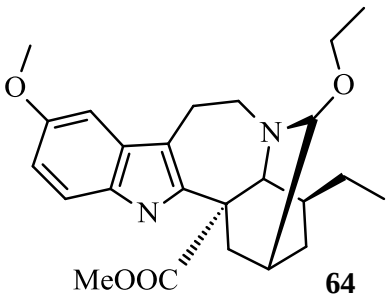

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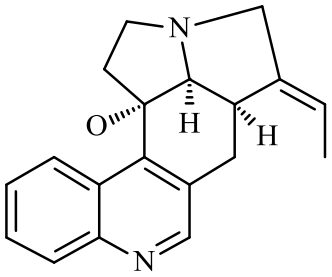

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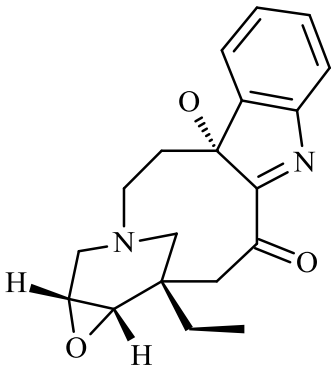

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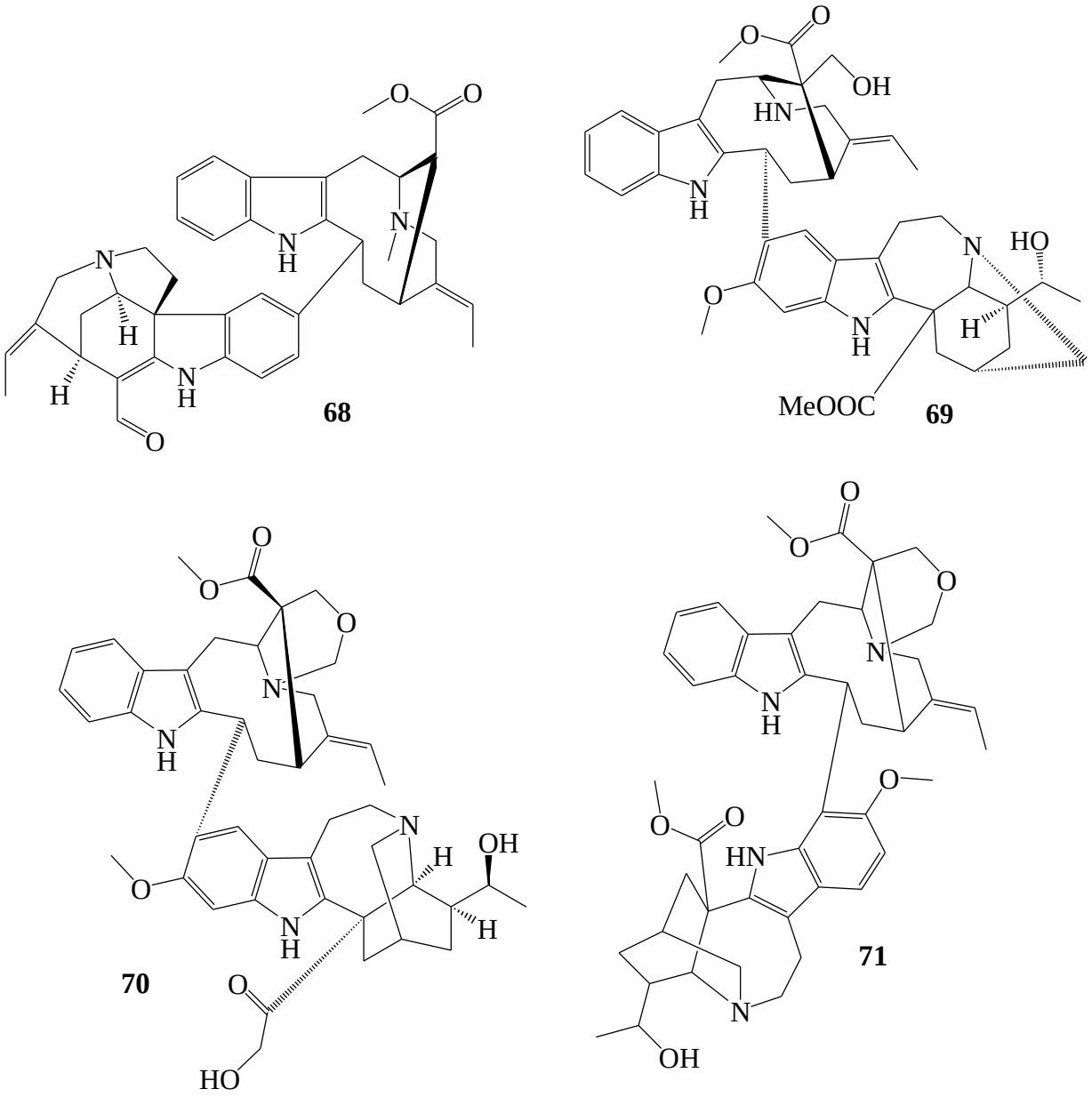


Figure 2.14: Indole and bis-indole alkaloids from *Tabernaemontana*

2.12 Biological activities of the genus *Tabernaemontana*

The genus *Tabernaemontana* has been found to possess various biological activities mostly based on their alkaloid constituents. Table 2.10 gives a summary of the biological activities of *Tabernaemontana* extracts and compounds.

Table 2.10: Biological activities of compounds and extracts in the *Tabernaemontana* species

Species	Plant part	Extract/ compound	Activity	Reference
T. dichotoma	Wp	Not specified	Hypersensitivity, Sedative activity	Perera *et al*., 1984b
	Sb, Rb	Ethanolic Aqueous	Antimicrobial	Perera *et al*., 1985
T. dichotoma	Sb	Voacamine (**64**)	Antibacterial, antiplasmodial	Federici *et al*., 2000; van Beek *et al*., 1984
T. crassa	N/S	Ethanolic	Sedative activity	Sandberg and Cronlund, 1982
T. divaricata	L	CCl_3 MeOH, EtOH	Anticancer	Thind *et al.*, 2008
	L	Aqueous Methanolic	Anti-inflammatory	Jain *et al.*, 2013
	Rb	Alkaloid fraction	Hypotensive action. CNS activity	Chattipakorn *et al*., 2007
	L	MeOH	Antioxidant	Rumzhum *et al*.,, 2012
	Rb	Dregamine (**59**)	Anti-inflammatory, antibacterial	Pallant *et al*., 2012
T. pachysiphon	Rb, Sb	Ethanolic	Antimicrobial	van Beek *et al*., 1984b
T. elegans	Cell suspension callous	Apparicine (**42**)	Antineoplastic agent, antibacterial	Gogoi *et al.*, 2014

Sb-stem bark, Rb-root bark, Wp-whole plant, N/S-not specified, L-leaves

CHAPTER 3

MATERIALS AND METHODS

3.1 General

The major stationery phase that was used for column chromatography (CC) was Merck silica gel 60 (70-230 mesh). Purification was achieved using preparative thin layer chromatography (PTLC). The PTLC plates (20 x 20 cm) were prepared using Merck silica gel 60 ($PF_{254+366}$) by making a suspension with 80 g of preparative silica gel in 200 ml of water for 6 glass plates. The resultant slurry (40 ml) was poured onto each plate and spread uniformly using a flat spatula and left overnight to dry resulting to plates of 2 mm thickness. The plates were then activated in an oven set at 110 °C for 1 hour and allowed to cool before use. Factory made analytical aluminum 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 and exposure to iodine. 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), Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectra were obtained using standard Bruker software. Chemical shifts were measured in ppm relative to the internal standard tetramethylsilane (TMS). The major solvents used for chromatography were *n*-hexane and ethyl acetate

3.2 Plant material

The stem bark of *Bridelia micrantha* was collected from Kakamega Forest, Kakamega County (approximately 50 km from Kisumu town center) in July 2014 whereas the root bark of *Tabernaemontana ventricosa* was collected from Kibwezi, Makueni County in December 2014. The plants were identified by Mr. Patrick Mutiso of the University of Nairobi Herbarium, School of Biological Sciences (SBS), where voucher specimens; RRM 2014/01 and RRM 2015/02 respectively, are deposited. The plant materials were then air dried under shade and pulverized into fine powder using a Willy mill at the Department of Chemistry, University of Nairobi.

3.3 Extraction of stem bark of *Bridelia micrantha*

The ground stem bark (4025g) was extracted exhaustively with 1:1 MeOH in CH_2Cl_2 by cold percolation for 72 hours at room temperature. The resultant filtrate was then concentrated *in vacuo* on a rotary evaporator and combined to give 350g of reddish extract that translated to about 8.69 % of the ground material.

3.3.1 Isolation of compounds from stem bark of *B. micrantha*

The crude extract (150g) of the stem bark of *B. micrantha* obtained using 1:1 MeOH in CH_2Cl_2 was adsorbed onto an equal amount (150g) of silica gel, dried *in vacuo*, ground into fine powder and loaded onto a column packed with 1500g of silica gel under 1% EtOAc in *n*-hexane. The column was then eluted sequentially with solvent systems of increasing polarity from 1% EtOAc in *n*-hexane until 100% EtOAc resulting into 344 fractions of 250 ml each. The fractions were then concentrated *in vacuo* on a rotary evaporator and spotted on analytical TLC plates with combination of fractions that had similar profiles into 14 fractions. Those that formed crystals were filtered and washed successfully in 100% *n*-hexane and the rest subjected to further purification.

Fraction 3 that crystallized out of 10% EtOAc in *n*-hexane yielded two sets of crystals labeled 3a and 3b. 3a were white needle-like crystals that was labelled compound **1** (50 mg (0.033%), R_f 0.52 (10% EtOAc in *n*-hexane)) while 3b were white amorphous solids, UV inactive that was labelled compound **2** (30.6 mg (0.024%), R_f 0.43, 20% EtOAc in *n*-hexane).

Fraction 5 that eluted with 15% EtOAc in *n*- hexane crystallized out of this solvent forming white flaky and UV active crystals that were labelled compound **3** (30 mg (0.02%), R_f 0.33 (20% EtOAc in *n*-hexane)). Fraction 10 (90% EtOAc in *n*-hexane) crystallized forming orange UV active crystals labelled compound **4**. (26 mg 0.02%), R_f 0.33 (80% EtOAc in *n*-hexane)).

3.4 Extraction of root bark of *T. ventricosa*

The ground stem bark (2 Kg) was extracted with 1:1 MeOH in CH_2Cl_2 by cold percolation for 72 hours at room temperature and the filtrate was concentrated *in vacuo* to give 106.39g of brownish extract that translated to about 5.32% of the ground material.

3.4.1 Isolation of compounds from stem bark of *T. ventricosa*

100g of the crude extract was adsorbed onto an equal amount (100g) of silica gel, dried *in vacuo*, ground into fine powder and loaded onto a column packed with 1000g of silica gel under 1% EtOAc in *n*-hexane. The column was then eluted sequentially with solvent systems of increasing polarity from 1% EtOAc in *n*-hexane until 100% EtOAc resulting into 233 fractions of 500 ml each that were concentrated *in vacuo* on a rotary evaporator and spotted on analytical TLC plates. Those that showed similar TLC profiles were combined and this resulted into fourteen fractions. Fraction 5 that eluted with 9% EtOAc in *n*-hexane crystallized out of this solvent and yielded white amorphous solids that were UV active. These crystals were found to be a mixture and were labelled **5a** and **5b**. (32mg, R_f 0.31 (10% EtOAc in *n*-hexane)).

3.5 Biological activities

3.5.1 Cell culture preparation and maintenance

The human CCRF-CEM leukemia cell lines were maintained at Roswell Park Memorial Institute media (RPMI 1640) supplemented with 10% fetal calf serum (FCS) in humidified 7% CO_2 atmosphere at 37 ^{o} C. All the experiments were done with cells that were in the logarithmic phase.

3.5.2 Resazurin growth inhibition assay

The *in vitro* response to drugs was evaluated by means of growth inhibition resazurin reduction assay (O'Brien *et al*., 2000) to assess the cytotoxicity of the test samples towards the human drug sensitive cancer cell lines (CCRF-CEM). The assay is based on reduction of the indicator dye,

resazurin, to the highly fluorescent resorufin by viable cells. Non-viable cells rapidly lose the metabolic capacity to reduce resazurin and thus produce no fluorescent signal.

Briefly, the crude extracts of *B. micrantha* and the isolated compounds from *B. micrantha* were dissolved in dimethyl sulfoxide (DMSO) and diluted with RPMI medium to give an initial concentration of 20 µg/ml for extracts and 2 µg/ml for the pure compounds. To determine the 50% inhibition from the dose response curves for the extracts and isolates that exhibited >70 cell inhibition after initial screening, various concentrations of the extracts and compounds ranging from 0.0025-50 µg/ml were prepared.

The cells were plated at a density of 1×10^4 cells/well in a 96-well plate, in a total volume of 100 µl. The extracts and isolates at a concentration of 20 µg/ml and 2 µg/ml, respectively, were then added immediately in an additional 100 µl of culture medium to obtain a total volume of 200 µL, therefore reducing the initial concentration of each sample by half to only 10 µg/ml for extracts and 1 µg/ml for compounds. After 72h incubation at 37 °C, 5% CO_2 and 95% relative humidity, 20µL resazurin (Sigma-Aldrich, Schnelldorf, Germany) 0.01% w/v in double-distilled water (ddH_2O) was added to each well and the plates incubated for a further 4 h. Fluorescence was measured on an Infinite M2000 Pro™ plate reader (Tecan, Crailsheim, Germany) using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. Each assay was done at least twice and replicated six times. The viability was evaluated based on a comparison with untreated cells to give IC_{50} values representing the samples concentrations required to inhibit 50% of cell proliferation.

3.6 Data analysis

A combination of techniques together with comparison with literature was used in the identification of isolated compounds. These include ultraviolet (UV), nuclear magnetic resonance (NMR) spectroscopic methods and melting points. Proton NMR was used to give a measure of absorbance of the different protons from each of the isolated compounds, ^{13}C-NMR for the determination of the resonation of the carbon atoms and Distortionless Enhancement by Polarization Transfer (DEPT) technique was used to ascertain the number of protons attached to each carbon. Two dimensional spectroscopy was also used for the determination of connectivity and stereochemistry of the compounds.

CHAPTER FOUR

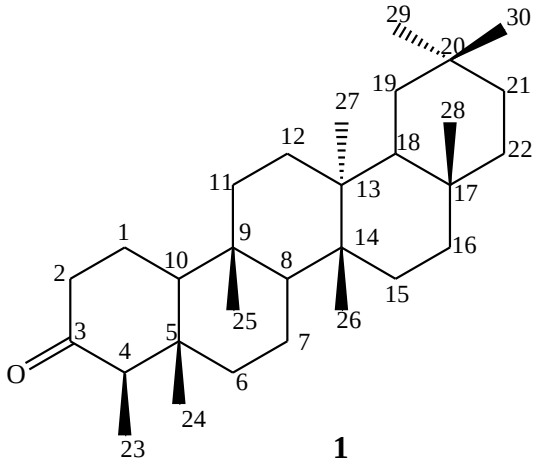
RESULTS AND DISCUSSION

4.1 Compounds isolated from *Bridelia micrantha*

The stem bark extract of *B. micrantha* yielded four compounds that were characterized as friedelin (**1**), *trans*-triacontyl-4-hydroxy-3-methoxyxinnamte (**2**), betulinic acid (**3**) and catechin (**4**).

4.1.1 Friedelin (1)

Compound **1** was isolated as a white needle-like crystals with an R_f of 0.52 10% EtOAc in *n*-hexane. The ^1H spectral data (Table 4.1) showed the presence downfield shifted protons at δ_H 2.35, 2.32 and 2.28. There were also characteristic methyl proton peaks at δ_H 0.91(H-23, *d, J*=6Hz), 0.75 (H=24, *s*), 0.87 (H=25, *s*), 1.00 (H=26, *s*), 1.03 (H=27, *s*), 1.19 (H=28, *s*), 0.97 (H=29, *s*), 1.02 (H=30, *s*). The ^{13}C spectral data (Table 4.1) exhibited a carbonyl peak at δ 212.4 (C-3) and methyl carbon peaks at δ 34.7 (C-30), δ 31.9 (C-28), δ 34.7 (C-29), δ 18.5 (C-27), δ 20.0 (C-26), δ 17.7 (C-25), δ 14.4 (C-24) and δ 6.6 (C-23). Based on the above spectroscopic evidence together with comparison with literature (Queiroga *et al.*, 2000; El Deeb *et al.*, 2003; Oliveira *et al*., 2012) the compound **1** was identified as friedelin, previously isolated from the leaves of *B. monoica* (Hui and Fung, 1968).



1

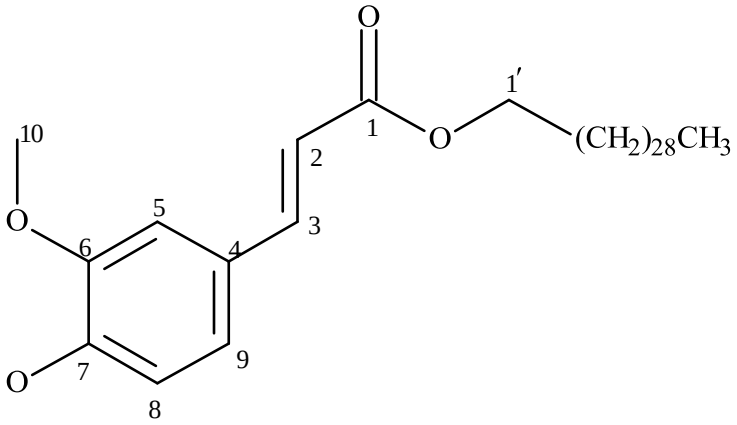
Table 4.1: ^{1}H (CD_2Cl_2 at 600 MHz) and ^{13}C (CD_2Cl_2 at 200 MHz) NMR chemical shift data for compound 1 (Queiroga *et al.*, 2000; El Deeb *et al.*, 2003; Oliveira *et al*., 2012)

C, position	^{1}H NMR, δ, ppm (Experimental)	^{1}H NMR, δ, ppm (Literature)	^{13}C NMR, δ, ppm (Experimental)	^{13}C NMR, δ, ppm (Literature)
1	2.00, 1.13, *m*	1.95, 1.17, *m*	22.2	22.3
2	2.35, 2.32, *m*	2.37,2.27, *m*	41.5	41.5
3			212.4	213.2
4	2.28, *m*	2.25	58.0	58.2
5			42.	42.1
6	1.72, 1.28	1.73, 1.28, *d*,8 Hz	41.2	41.3
7	1.53, 1.35, *m*	1.49, 1.36, *m*	18.2	18.2
8	1.40, *m*	1.38, *m*	53	53.1
9			37.4	37.4
10		1.53, *m*	59.3	59.4
11	1.45, 1.23, *m*	1.45, 1.26, *m*	35.6	35.6
12		1.33, 1.33, *m*	30.5	30.5
13			39.7	39.7
14			38.3	38.3
15	1.46, 1.28, *m*	1.47, 1.27, *m*	32.3	32.4
16	1.58, 1.35, *m*	1.58, 1.35, *m*	36.0	36.0
17			29.9	30.0
18	1.57, *m*	1.56, *m*	42.8	42.8
19	1.39, 1.23, *m*	1.37, 1.21, *m*	35.2	35.3
20			28.0	28.1
21	1.53, 1.31, *m*	1.50, 1.31, *m*	32.3	32.7
22	1.53, 0.96, *m*	1.50, 0.94, *m*	39.2	39.2
23	0.91, *d*, 6 Hz	0.88, *d*	6.6	7.2
24	0.75, *s*	0.71, *s*	14.4	15.0
25	0.87, *s*	0.86, *s*	17.7	17.9
26	1.00, *s*	1.00, s	20.0	20.2
27	1.03, *s*	1.04, *s*	18.5	18.6
28	1.19, *s*	1.17, *s*	31.9	32.1
29	0.97, *s*	0.99, *s*	34.7	35.7
30	1.02, *s*	0.94, *s*	31.6	32.8

4.1.2 *trans*-Triacontyl-4-hydroxy-3-methoxycinnamate (2)

Compound **2** was isolated as a white amorphous solid with a melting point of 59-61 ºC and an R_f value of 0.43 (20% EtOAc in *n*-hexane). ^{1}H NMR spectrum (Table 4.2) exhibited three aromatic protons at δ_H 6.94 (H-5) while the other two at δ 7.11 (*d*, H-8, *J* = 6Hz) and 7.13 (*d*, H-9, *J* = 6Hz) that were coupling with each other. There was also a characteristic methoxy peak at δ_H 3.97 (H-10) and two olefinic protons at δ_H 6.36 (*d*, H-2, *J* = 12Hz) and 7.63 (*d*, H-3, *J* = 12Hz). Furthermore, there was a hydroxymethine proton at δ_H 3.95 (H-7), two deshieled protons at δ_H 4.20 (H-1′, *m*) attributed to a methylene attached to an oxygen that is attached to a carbonyl and methylene protons between δ_H 1.73–0.91 (H-2′-30′) that were assigned to a long chain hydrocarbon.

The presence of these groups were confirmed by ^{13}C NMR spectrum that showed peaks corresponding to δ_C 144.3 (C-3) and 115.7 (C-2) for the olefinic carbons, δ_C 56 (C-10) for methoxy carbon and δ_C146.9 (C-7) and δ_C 147.9 (C-6) for oxygenated aromatic carbon. The aromatic carbons appeared at δ_C 127.1 (C-4), δ_C 114.5 (C-5), δ_C 122.9 (C-8) and 109.3 (C-9). The ^{13}C NMR spectrum (Table 4.2) also exhibited a characteristic peaks of an ester carbon at δ_C 167, a methyl attached to an oxygen at δ_C 64.5 (C-1′) and a series of signals between δ_C 13.9–28.8 (C-2´–C-30´) indicating presence of long chain carbon atoms. Based upon the above spectroscopic evidence (1D and 2D) together with comparison with literature, the compound **2** was identified as *trans*-triacontyl-4-hydroxy-3-methoxycinnamate, previously isolated from the branches of *B. ovata* (Boonyaratavej *et al*., 1992).



2

Table 4.2: ^{1}H (CD_2Cl_2 at 600 MHz) and ^{13}C (CD_2Cl_2 at 200 MHz) NMR chemical shift data together with HMBC and HMQC correlations for compound 2

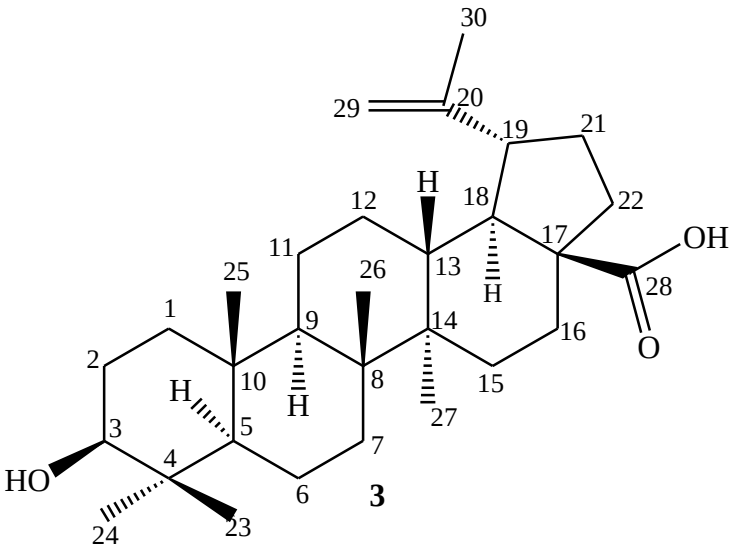
C, position	^{1}H NMR, δ, ppm	^{13}C NMR, δ, ppm	HMQC, δ, ppm	HMBC, ^{2}J	HMBC, ^{3}J
1		167			
2	6.63, *d*, *J* = 12 Hz	115.7	115.7	C_1	C_3, C_4
3	7.63, *d*, *J* = 12 Hz	144.3	144.3	C_2, C_4	C_1, C_5, C_9
4		127.1			
5	6.94, *d*, *J* = 12 Hz	114.5	114.5	C_4, C_6	C_7, C_9
6		147.9			
7	3.95, *s*	146.9			
8	7.11, *d*, *J* = 6 Hz	122.9	122.9	C_7, C_9	C_4, C_6
9	7.13, *d*, *J* = 6 Hz	109.3	109.3	C_4, C_8	C_3, C_7
10	3.97, *t*,3 H	56	56	C_8	C_7
1′	4.20, *m*	64.5	64.5	$C_{2'}$	C_1
2′	1.73	28.8	28.8	$C_{3'}$	C_1
3′	1.42	26	26		
28′	1.30	31.9	31.9	$C_{27'}$, $C_{29'}$	$C_{30'}$
29′	1.35	22.7	22.7	$C_{28'}$	$C_{30'}$
30′	0.91, *t*, 3H	13.9	13.9	$C_{29'}$	$C_{28'}$

4.1.3 Betulinic acid (3)

Compound **3** was isolated as a white amorphous powder with an R_f value of 0.33 (20% EtOAc in *n*-hexane).

^{1}H NMR (Table 4.3) showed presence of a hydroxymethine proton δ_H 3.16, *m*, (H-3), olefinic proton at δ_H 4.72/4.59, *d*, *J* = 6Hz, (H-29), methyl protons at δ_H 1.96, *s* (H-30), 0.91, *m* (H-26), 0.94, *s* (H-24), 0.91, *m* (H-23), methyne protons δ_H 1.59, *m* (H-18) and 2.9, *m* (H-19). The ^{13}C NMR spectra data (Table 4.3), carbonyl peak was observed at δ_C 180.0 (C-28), sp^3 hybridized oxygenated carbon at δ_C79.2 (C-3), two olefinic carbons at δ_C 150.6 (C-20) and 109.9 (C-29). Methyl carbon peaks were also evident at δ_C 15.6 (C-25), 14.9 (C-27), 19.6 (C-30), 28.2 (C-24) and 16.2 (C-23/26). From the spectroscopic evidence (1D and 2D) given above and comparison

with literature, the compound **3** was identified as betulinic acid, previously isolated from the stem bark of *B. ferruginea* (Lasisi and Kareem, 2011).



3

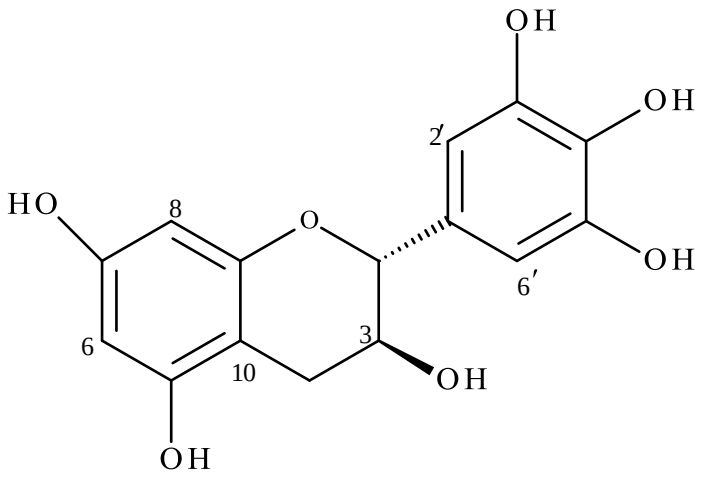
Table 4.3: ^{1}H ($CDCl_3$ and MeOD at 600 MHz) and ^{13}C ($CDCl_3$ at 200 MHz) NMR chemical shift data together with HMQC and HMBC correlations for compound 3

C, position	^{1}H NMR, δ, ppm	^{13}C NMR, δ, ppm	HMQC, δ, ppm	HMBC, ^{2}J	HMBC, ^{3}J
1	1.69, *m*	38.9	1.69		C-5, C-3
2	1.69, *m*	27.6	1.69	C-3	C-4
3	3.16, *s*	79.2	3.16		
4		39.9			
5	0.67, *s*	55.6	0.67	C-10	C-1, C-25, C-23
6	1.36, *m*	18.5	1.36	C-5	
7	1.36, *m*	34.5	1.36		C-5, C-9
8		40.9			
9	1.24, *s*	50.7	1.24	C-8	C-25, C-26
10		37.4			
11	1.24, *m*	30.8	1.24		C-8, C-13
12	1.69, *m*	25.7	1.69	C-13, C-11	
13	2.18, *m*	38.6	2.18		
14		42.7			
15	1.24, *m*	29.9	1.24		C-8, C-13
16	2.23, *m*	32.4	2.23		C-14, C-18
17		56.5			
18	1.59, *m*	49.5	1.59	C-19, C-17	C-20, C-28
19	2.9, *m*	47.1	2.9		
20		150.6			
21	1.42, *m*	21.1	1.42		C-18
22	1.96, *m*	37.2	1.96		C-18
23	0.91, *s*	16.2	0.91		C-5
24	0.94, *s*	28.2	0.94	C-4	C-5
25	0.73, *m*	15.6	0.73		C-5
26	0.91, *s*	16.2	0.91	C-8	C-9, C-7,C-14
27	0.94, *m*	14.9	0.94		C-8, C-15, C-13
28		180			
29	α- 4.72 β- 4.59	109.9	4.72, 4.59		C-30, C-19
30	1.96	19.6	1.69		

4.1.3 Catechin (4)

Compound **5**, was isolated as an orange amorphous solid with melting point of 160.5-163 ºC and an R_f value of 0.33 (80% EtOAc in *n*-hexane). The ^{1}H NMR spectrum exhibited three aromatic protons one appearing at δ_H 6.29, *s* (H-2′/ H-6′) while the others were upfield shifted at δ_H 5.82, *s* (H-6) and 5.75, *s* (H-8) as they were both flanked between two hydroxyl groups. There was a doublet resonating at δ_H 4.42 (H-2) due to methylene proton (*J* = 6 Hz) and three sets of multiplets at δ_H 4.36 (H-3), 2.40 (H-4) and 2.70 (H-4).

The ^{13}C NMR spectrum confirmed the presence of oxygenated aromatic carbon atoms at δ_C 157.7 (C-5), 157.9 (C-7), 147 (C-3′), 134.2 (C-4′) and 147 (C-5′). There was an additional oxygenated carbon signal at δ_C 68.9 (C-3) and oxygenated carbons of an epoxide nature at δ_C 83 (C-2) and 157 (C-9). Based on the above spectroscopic evidence and comparison with literature, compound **5** was identified as catechin, previously isolated from *Croton lechleri* (Cai *et al*., 1991).



4

Table 4.4: ^{1}H (MeOD at 600 MHz) and ^{13}C (MeOD 200 MHz) NMR chemical shift data together with HMQC and HMBC correlations for compound 4

C, position	^{1}H NMR, δ, ppm	^{13}C NMR, δ, ppm	HMQC, δ, ppm	HMBC, ^{2}J	HMBC, ^{3}J
2	4.42, *d*, *J* = 6 Hz	83.0	83.0	C-3, C-1′	C-4, C-2′, C-6′, C-9
3	4.36	68.9			
4	α-2.40 β-2.70	28.3	28.3	α –C-3, C-10 β –C-3, C-10	α-C-2, C-5 β-C-5, C-2
5		157.7			
6	5.82, *s*	96.4	96.4	C-7, C-5	C-10, C-8
7		157.9			
8	5.75, *s*	95.7	95.7	C-9	C-10, C-6
9		157.0			
10		100.9			
1′		131.7			
2′	6.29, *s*	107.4	107.4	C-3′, C-1′	C-$_4$′, C-6′, C-2
3′		147			
4′		134.2			
5′		147			
6′	6.29, *s*	107.4	6.29	C-5′, C-1′	C-4′, C-2′, C-2

4.2 Compounds isolated from *Tabernaemontana ventricosa*

The root bark extract of *T. ventricosa* yielded two compounds that were isolated as a mixture in the ratio of 1:1 which were characterized as stigmasterol (**5a**) and *β*-sitosterol (**5b**).

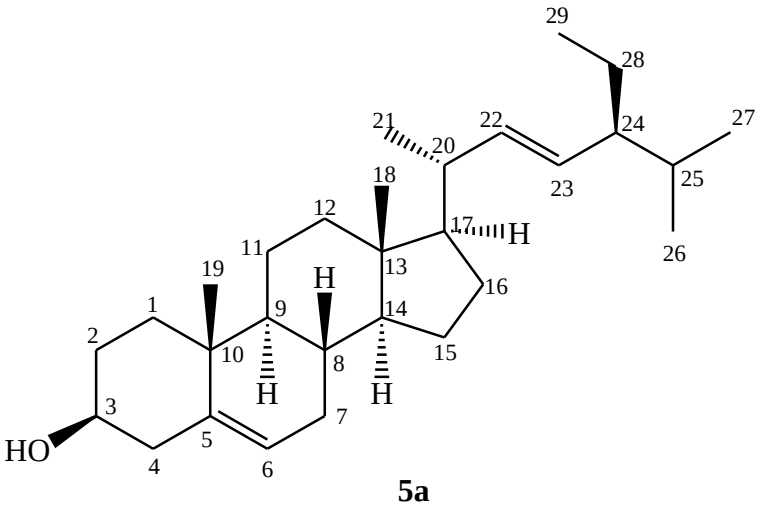
4.2.1 Stigmasterol (5a)

Compound **5a** was isolated as a white amorphous solid with an R_f value of 0.31 (10% EtOAc in *n*-hexane).

The ^{1}H NMR (Table 4.5) spectrum exhibited the characteristic steroidal type compounds with proton peaks between δ_H 0.67 and 2.25. There was the presence of three olefinic methine protons at δ_H 5.33 (H-6), 5.12 (H-23) and 4.98 (H-22) with an oxymethine proton at δ_H 3.51 (H-3). The spectrum also showed presence of two angular methyl protons appearing as singlets at δ_H 1.05, *s* (H-18) and 0.67, *s* (H-19) and four other methyl appearing at δ_H 0.82, *d, J* = 6Hz (H-26), 0.80, *d, J* = 6Hz (H-27), 0.91, *d, J* = 6Hz (H-28), 0.83, *m* (H-29).

The ^{13}C NMR spectrum (Table 4.5) confirmed the presence of four olefinic carbons at δ_C 140.7 (C-5), 121.7 (C-6), 138.3 (C-22) and 129.3 (C-23). There was also presence of an oxygenated carbon atom at δ_C 71.8 (C-3) and six methyl carbons at δ_C12.0 (C-18/29), 19.4 (C-19), 21.1 (C-21) 21.2 (C-26) and 20.2 (C-27).

The spectral data shows that compound **5a** has a sterol structure and comparison with literature (Jamaluddin *et al.,* 1994; Cayme and Ragasa, 2004; Subhadhirasakul and Pechpongs, 2005; Chaturvedula and Prakash, 2012) shows that it is stigmasterol previously isolated from *Tabernaemontana hilariana* (Cardoso *et al.*, 1998).



5a

Table 4.5: ^{1}H ($CDCl_3$ at 600 MHz) and ^{13}C ($CDCl_3$ at 200 MHz) NMR chemical shift data for compound 5a (Jamaluddin *et al.,* 1994; Cayme and Ragasa, 2003; Subhadhirasakul and Pechpongs; 2005, and Chaturvedula and Prakash, 2012)

C, position	^{1}H NMR, δ, ppm (Experimental)	^{1}H NMR, δ, ppm (Literature)	^{13}C NMR, δ, ppm (Experimental)	^{13}C NMR, δ, ppm (Literature)
1			37.3	37.23
2			31.7	31.67
3	3.51, *m*	3.51, *m*	71.8	71.60
4			42.3	42.28
5			140.7	140.73
6	5.33	5.31	121.7	121.70
7			31.9	31.67
8			31.9	31.67
9			50.2	50.11
10			36.5	36.45
11			21.1	21.06
12			39.7	39.67
13			42.3	42.28
14			56.9	56.74
15			24.3	24.34
16			28.9	28.91
17			56.2	56.03
18	1.05, *s*	1.03, *s*	12.0	11.96
19	0.67, *s*	0.71, *s*	19.4	19.39
20			40.4	40.49
21			21.1	21.06
22	4.98, *m*	4.98, *m*	138.3	138.30
23	5.12, *m*	5.14, *m*	129.3	129.25
24			51.2	51.22
25			32.4	31.63
26	0.82, *d*, *J*= 6Hz, 3H	0.82, *d*, *J*=6.6 Hz, 3H	21.2	21.19
27	0.80, *d*, *J*= 6Hz 3H	0.80, *d*, *J*=6.6 Hz 3H	20.2	18.87
28	0.91, *d*, *J*= 6Hz, 3H	0.91, *d*, *J*=6.6 Hz, 3H	25.4	25.40
29	0.83, *m*	0.83, *t*, *J*=7.1 Hz, 3H	12.0	12.03

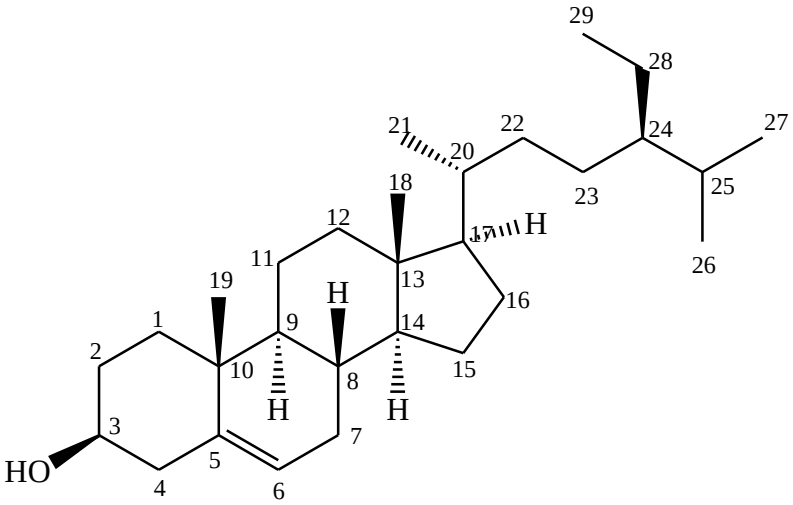
4.2.2 *β*-sitosterol (5b)

Compound **5b** was isolated as a white amorphous solid with an R_f value of 0.31 (10% EtOAc in *n*-hexane).

The ^{1}H NMR (Table 4.6) spectrum exhibited the characteristic steroidal type compounds with proton peaks between δ_H 0.67 and 2.25. There was presence of one olefinic methine protons at δ_H 5.33 (H-6) with an oxymethine proton at δ_H 3.51 (H-3). The spectrum also showed presence of six methyl protons at δ_H 0.67, *s* (H-19), 1.02, *s* (H-20), 0.94, *d, J* = 6Hz (H-21), 0.82, *d, J* = 6Hz (H-26), 0.80, *d, J* = 6Hz (H-27), and 0.83, *m* (H-29).

The ^{13}C NMR spectrum (Table 4.6) confirmed the presence of two olefinic carbons at δ_C 140.7 (C-5) and 121.7 (C-6). There was also presence of an oxygenated carbon atom at δ_C 71.8 (C-3) and six methyl carbons at δ_C 11.8 (C-18), 19.4 (C-19), 18.8 (C-21), 19.8 (C-26), (C-27) and 12.2 (C-29).

The spectral data shows that compound 5a has a sterol structure and comparison with literature (Jamaluddin *et al.,* 1994; Cayme and Ragasa, 2003; Subhadhirasakul and Pechpongs, 2005; Chaturvedula and Prakash, 2012) shows that it is *β*-sitosterol previously isolated from *Tabernaemontana hilariana* (Cardoso *et al.*, 1998).



5b

Table 4.6: ^{1}H ($CDCl_3$ at 600 MHz) and ^{13}C ($CDCl_3$ at 200 MHz) NMR chemical shift data for compound 5b (Jamaluddin *et al.,* 1994; Cayme and Ragasa, 2003; Subhadhirasakul and Pechpongs, 2005 and Chaturvedula and Prakash, 2012)

C, position	^{1}H NMR, δ, ppm (Experimental)	^{1}H NMR, δ, ppm (Literature)	^{13}C NMR, δ, ppm (Experimental)	^{13}C NMR, δ, ppm (Literature)
1			37.3	37.36
2			31.9	31.90
3	3.51, *m*	3.53, *m*	71.8	71.77
4			42.2	42.29
5			140.7	140.75
6	5.33, 1H	5.36, *t*, 1H	121.7	121.69
7			31.7	31.65
8			31.9	31.93
9			50.2	50.13
10			36.5	36.50
11			21.1	21.09
12			39.8	39.78
13			42.2	42.32
14			56.8	56.77
15			24.3	24.31
16			28.2	28.26
17			56.1	56.06
18			11.8	11.86
19	0.67, *s*, 3H	0.68, *s* 3H	19.4	19.40
20	1.02, *s*, 3H	1.01, 3H	36.1	36.15
21	0.94, *d*, 6Hz	0.93, *d J*=6.4Hz, *3H*	18.8	18.79
22			34.0	33.95
23			26.1	26.07
24			45.9	45.83
25			29.2	29.16
26	0.82, *d*, 6Hz	0.83, *d*, *J*=6.4Hz 3H	19.8	19.83
27	0.80, *d*, 6Hz	0.81, *d*, *J*=6.4Hz 3H	20.2	19.04
28			23.1	23.07
29	0.83, *m*.	0.84, *t*, *J*=7.2Hz 3H	12.2	11.99

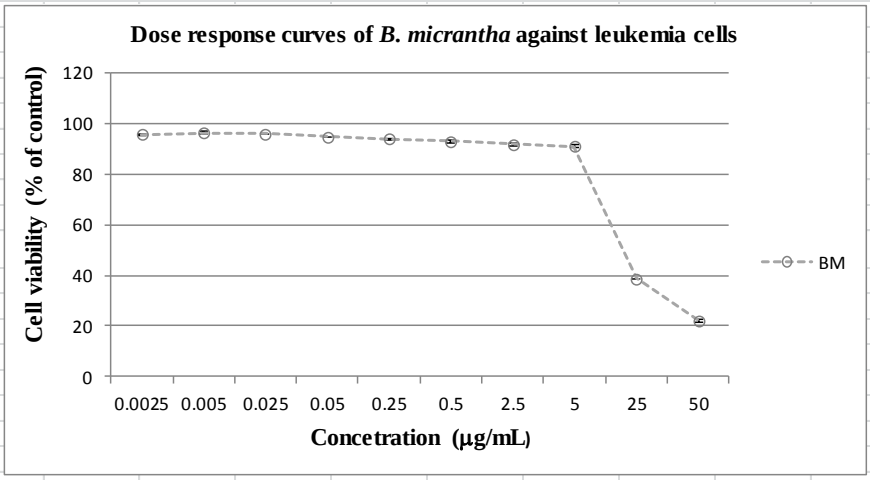
4.3 Biological activities

The stem bark extract and isolated compounds from *B. micrantha* were tested at a fixed concentration of 10 µg/mL and 1 µg/mL, respectively to determine the cell viabilities (Table 4.7). The established drug doxorubicin was used as a control. The extract and isolated compounds that showed a cell viability of more than 50% were considered to be cytotoxic (Boik, 2001).

Table 4.7: Cytotoxicity of *B. micrantha* extract and isolated compounds at a fixed concentration towards CCRF-CEM cells as determined by the resazurin assay

Plant Name/compound	**% Cell viability at 10 µg/ml for extract and 1 g/ml for compound and doxorubicin)**
B. micrantha (50% MeOH in CH_2Cl_2, stem bark)	31.5
B. micrantha (5% H_2O in MeOH, stem bark)	45.8
Doxorubicin (standard drug)	6.5
Friedelin (**1**)	79.53
Trans-triacontyl-4-hydroxy-3-methoxycinammate (**2**)	31.13
Betulinic acid (**3**)	<30
Catechin (**4**)	<30

B. micrantha extract showed interesting cell viability and was then tested at different concentrations to give a dose response curve (Figure 4.1) that was used to calculate the IC_{50} value.



IC_{50} = 9.43597

Figure 4.1: Dose response curve of *B. micrantha* stem bark against the drug sensitive leukemia cell lines.

This showed that the extract had an IC_{50} value of 9.43 µg/mL and thus had good anticancer activity against the drug sensitive leukemia cell lines. However, friedelin (**1**), betulinic acid (**3**) and catechin (**4**) were inactive at 1 µg/mL as they inhibited <70% of the proliferation of the drug sensitive CCRF-CEM.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1) The stem bark of *B. micrantha* yielded four compounds that were characterized as friedelin (**1**), *trans*-triacontyl-4-hydroxy-3-methoxycinnamate (**2**), betulinic acid (**3**) and catechin (**4**). This is the first report of the compound **4** in *Bridelia micrantha.*
2) The root bark of *T. ventricosa* yielded two compounds, stigmasterol (**5a**) and *β*-sitosterol (**5b**) isolated as a mixture in the ratio of 1:1 based on the integration of the olefinic protons.
3) The extracts of *B. micrantha* (50% MeOH in CH_2Cl_2) showed good anticancer activity with an IC_{50} value of 9.43 µg/mL while most of the isolated compounds showed poor activity at the tested concentration of 1 µg/mL against the drug sensitive leukemia cells. The high activity of the extract could be due to the synergetic effects of the compounds.

5.2 Recommendations

1) Comprehensive phytochemical investigation should be carried out on the roots and leaves of *B. micrantha* and the stem bark and leaves of *T. ventricosa.*
2) The anticancer potential of the isolated compounds against the drug resistant and other cancer cell lines should be determined.
3) The stem bark of *Bridelia micrantha* mainly used for the management of cancer by people within Kakamega Forest, showed good anticancer activity and should be subjected to efficacy trials for possible anticancer use.
4) The isolated compounds from both the plants should be subjected to other biological tests to determine their potency.
5) Establish the mode of action of the B. micrantha extracts and compounds in controlling cancer

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APPENDICES

APPENDIX 1: SPECTRA FOR COMPOUND 1

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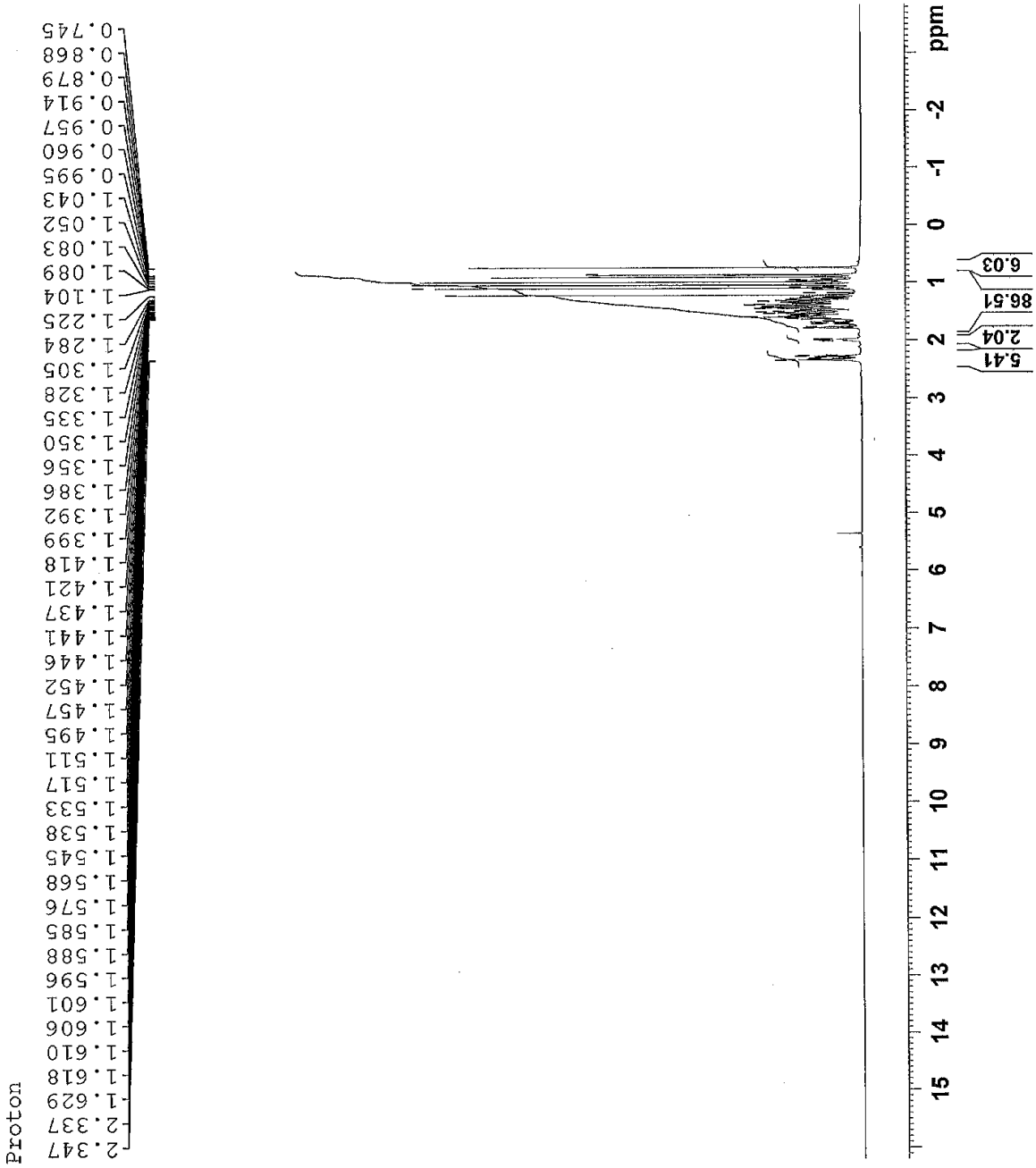

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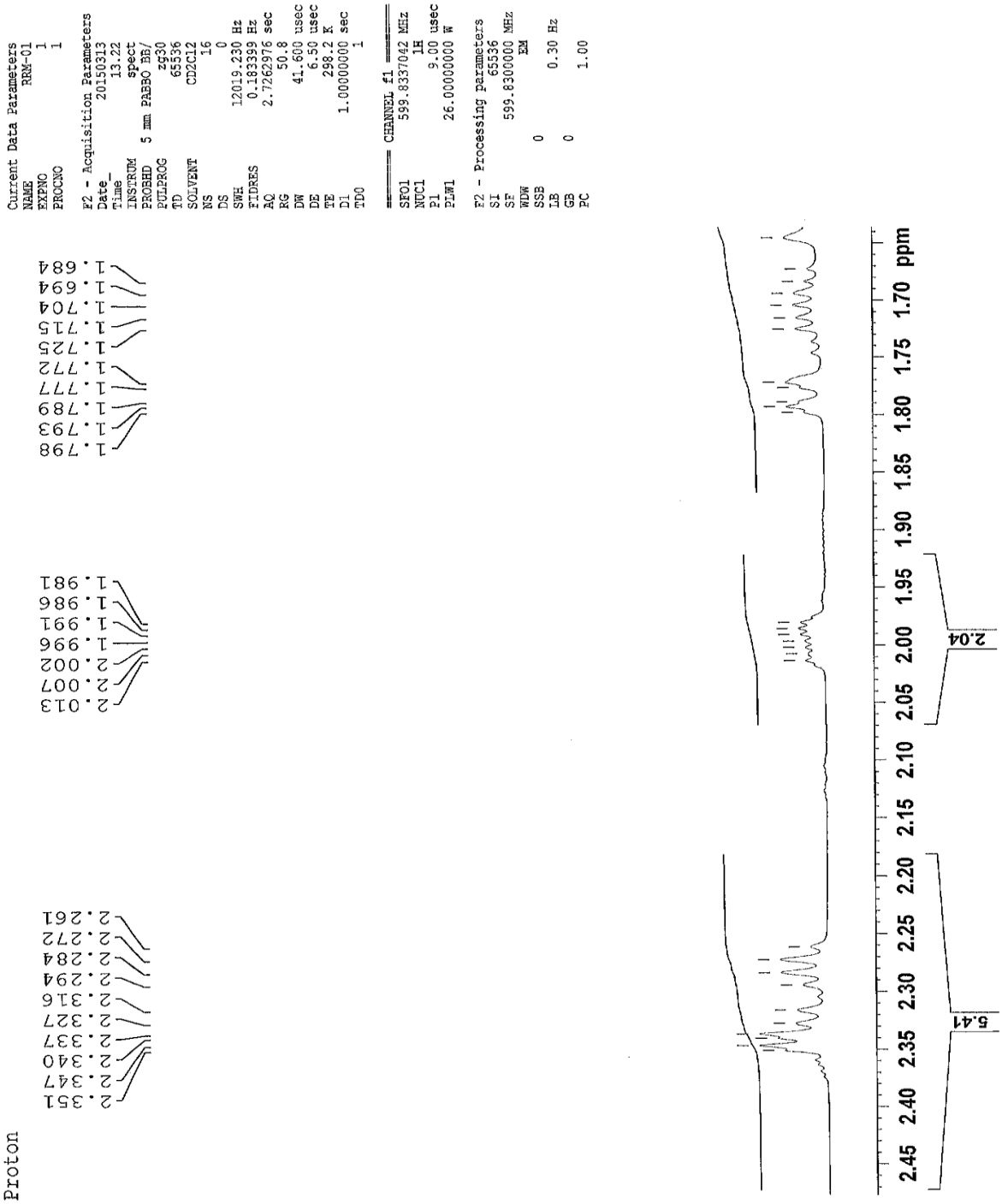

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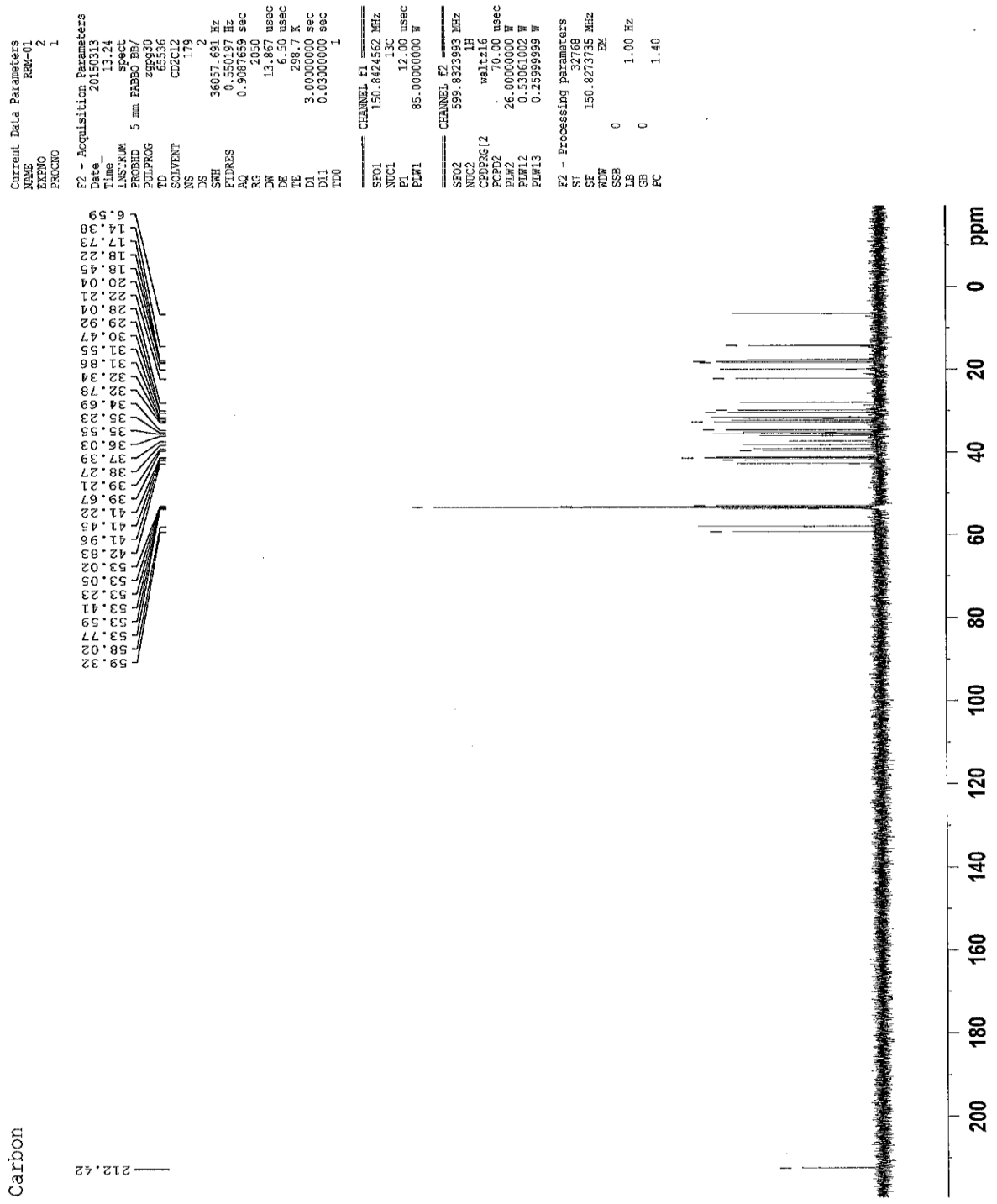

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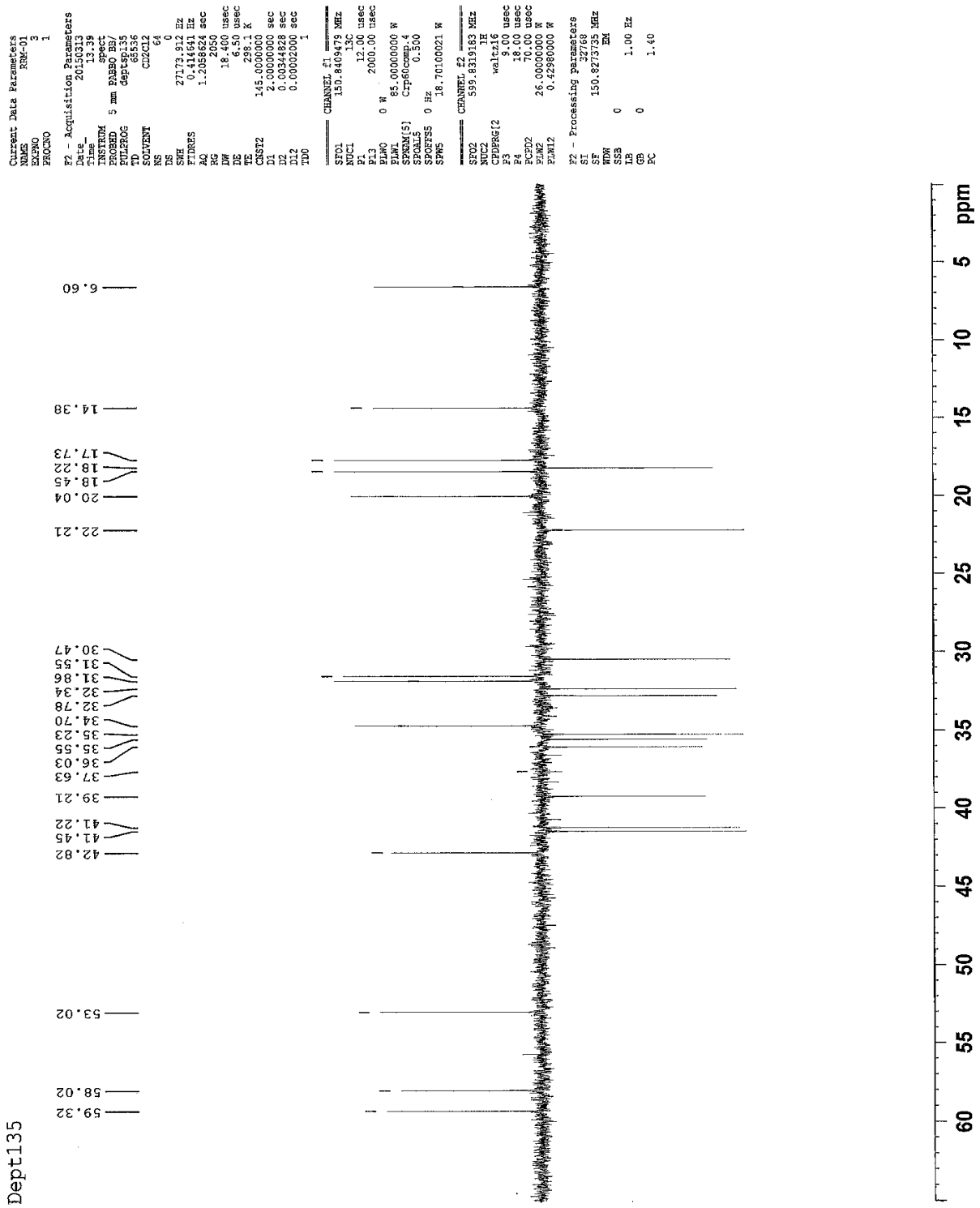

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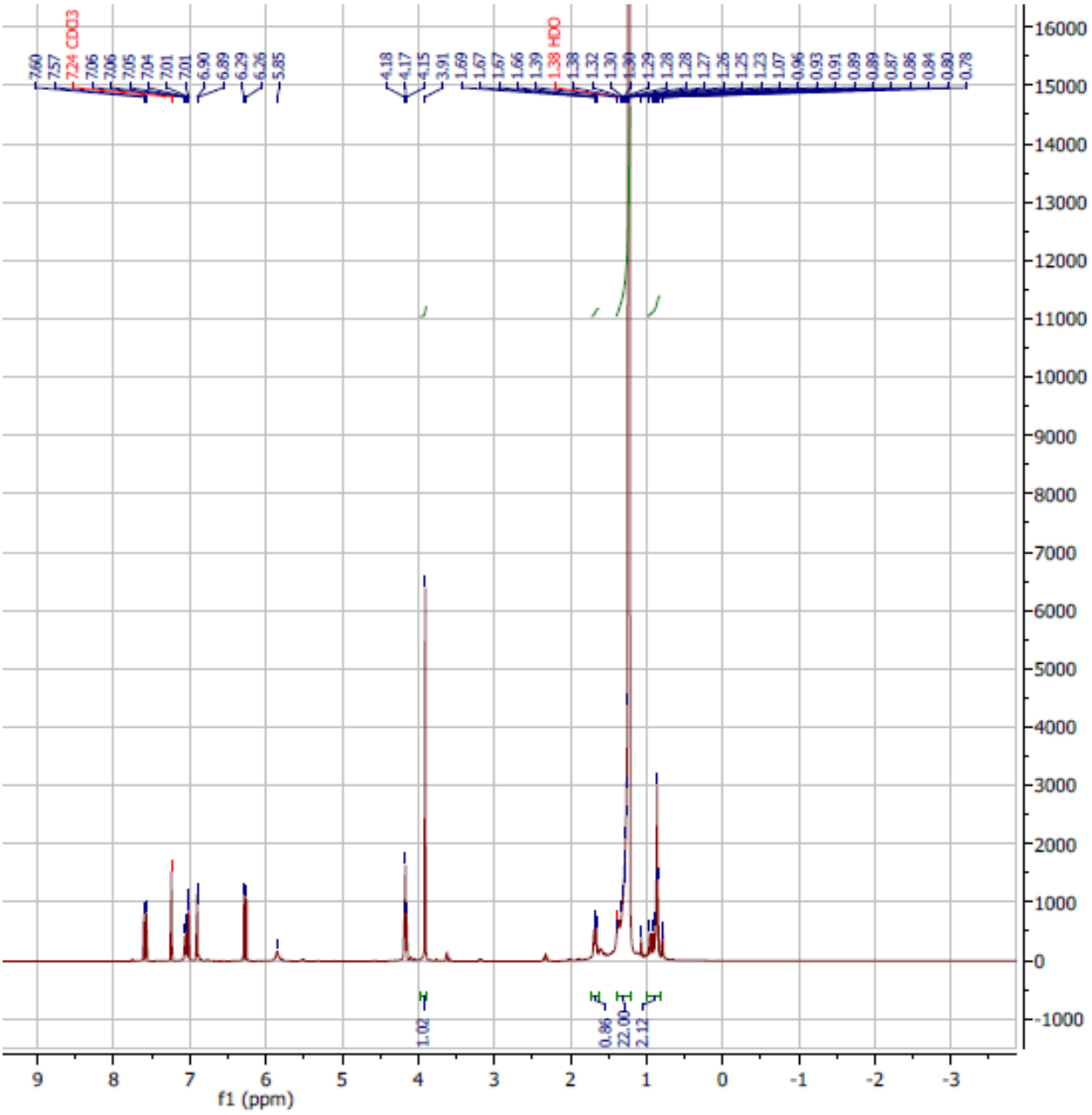

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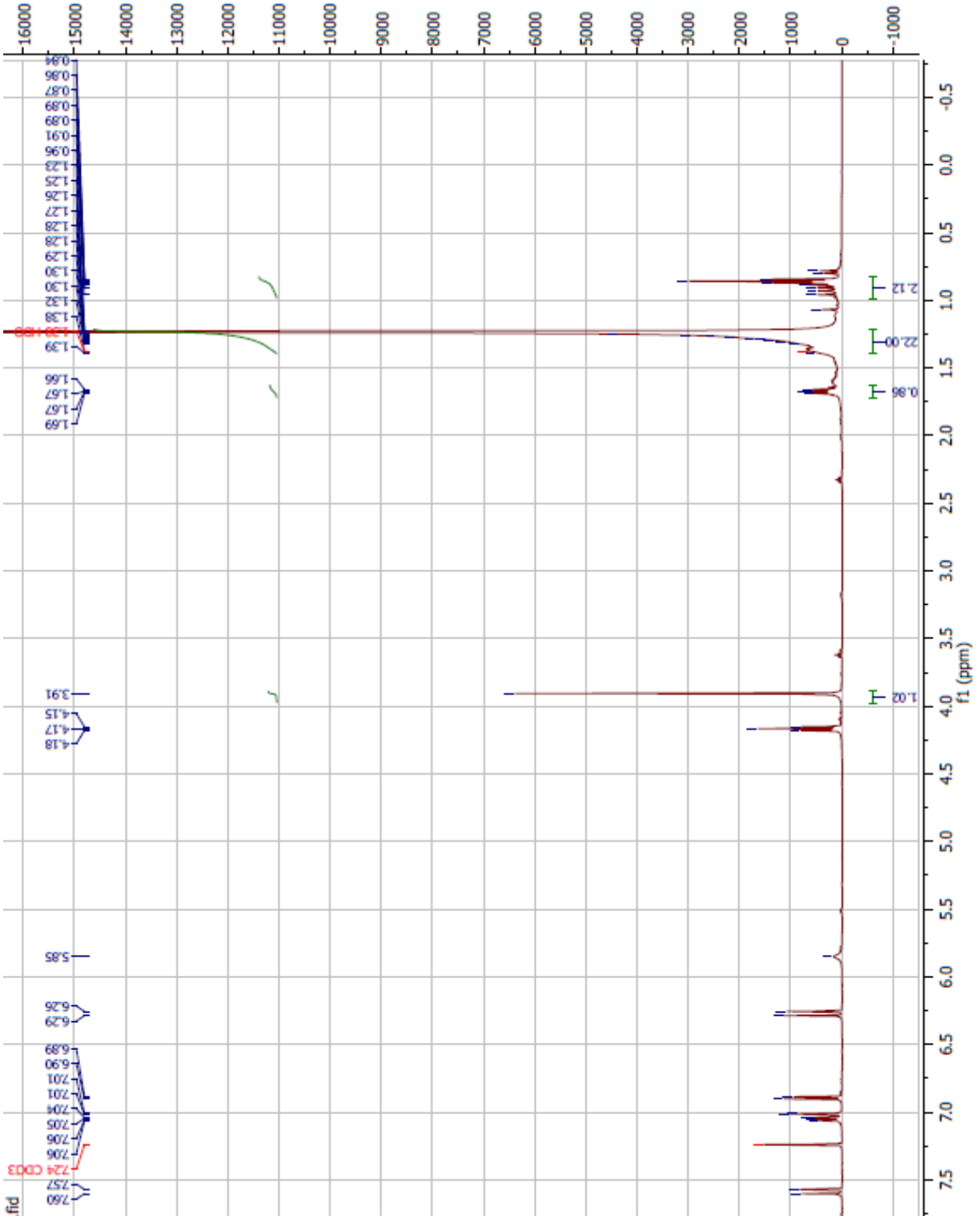

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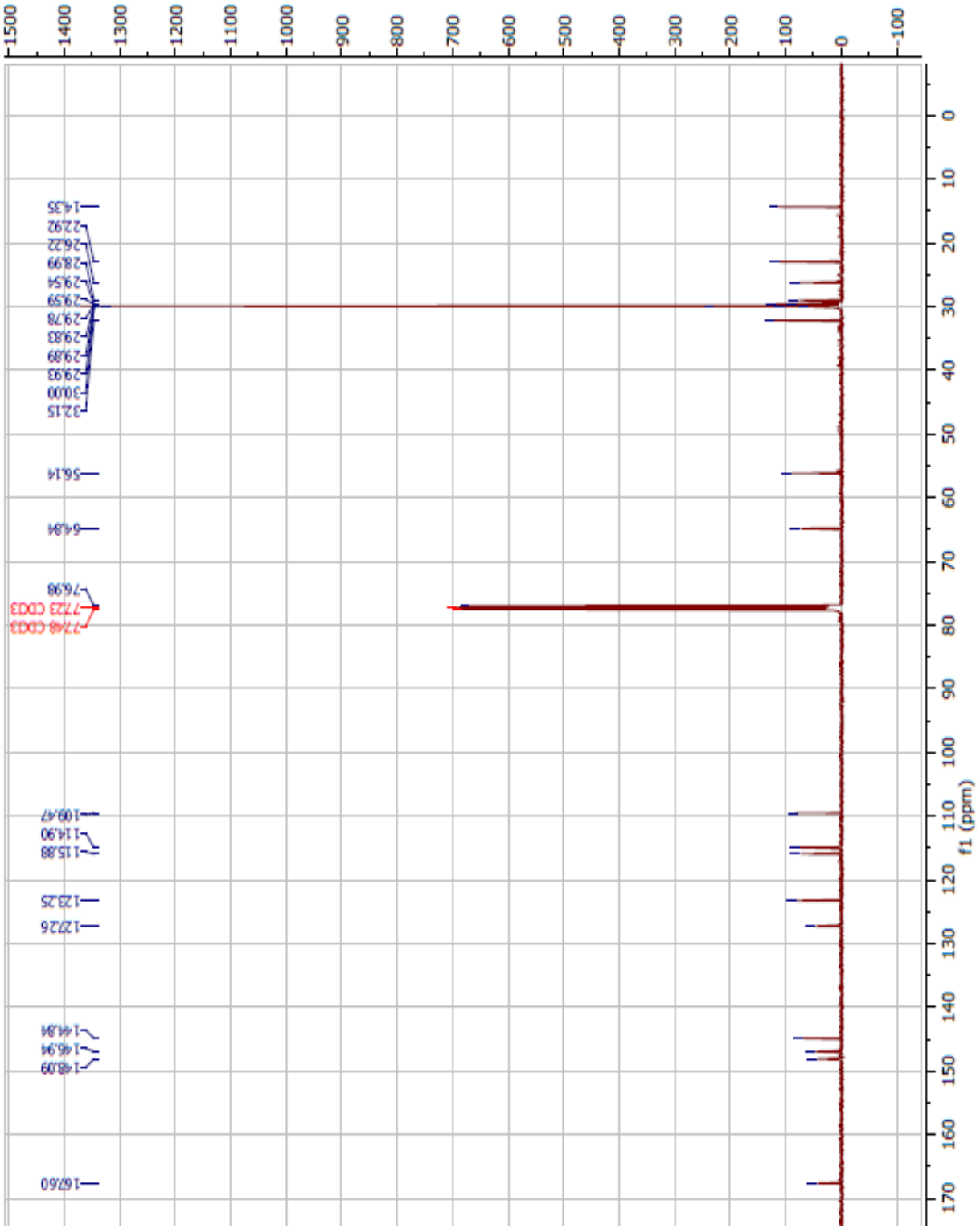

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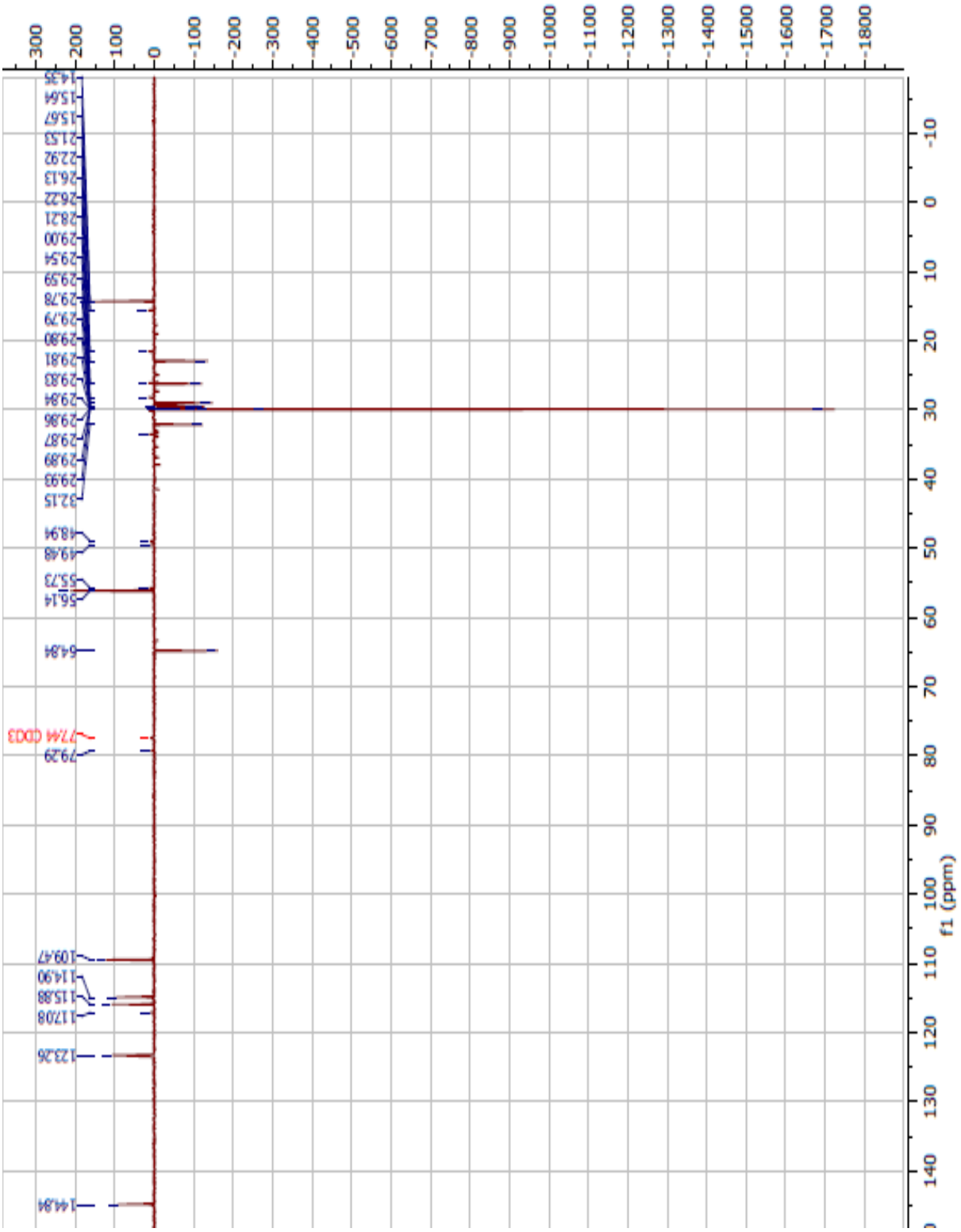
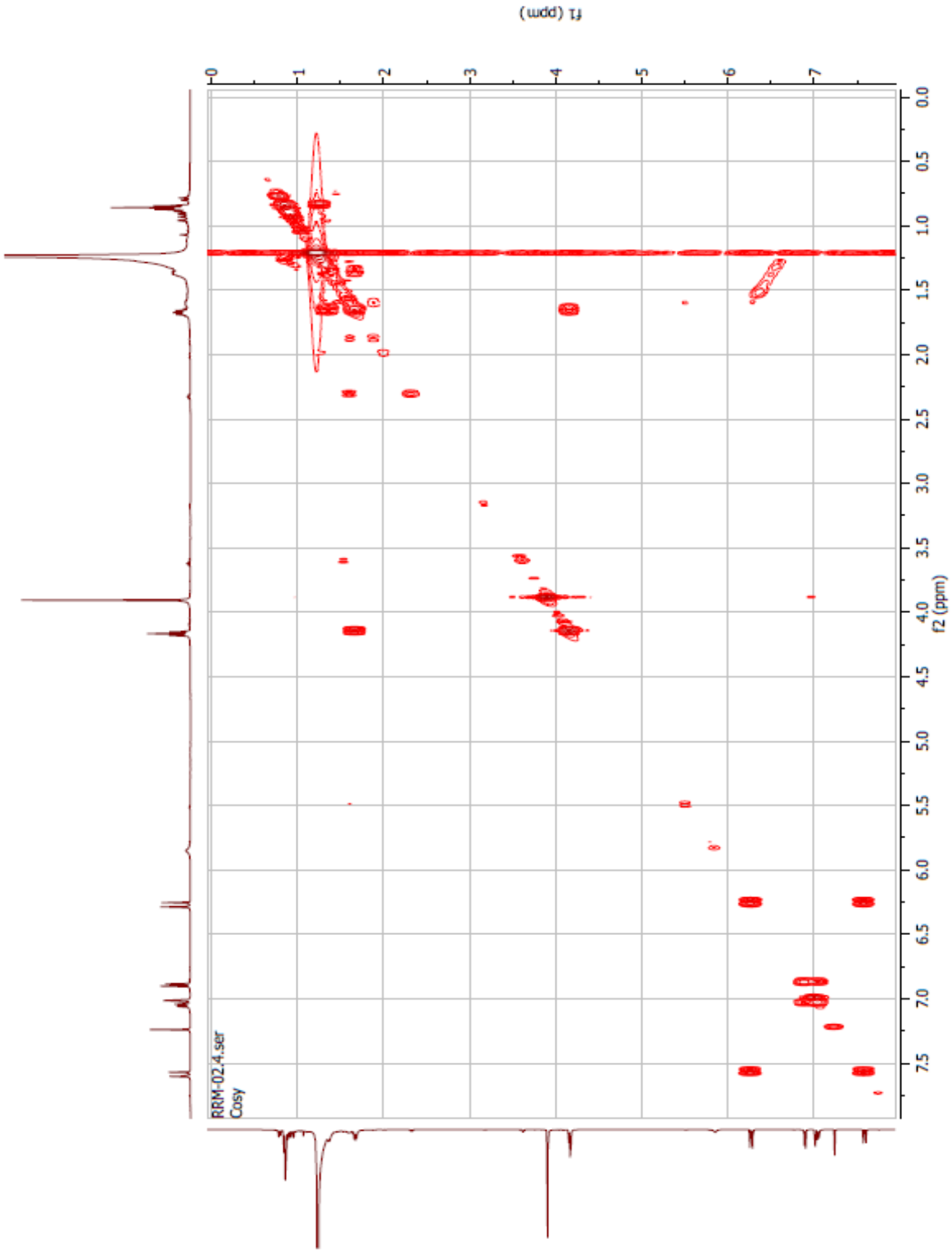


COSY SPECTRUM FOR COMPOUND 2



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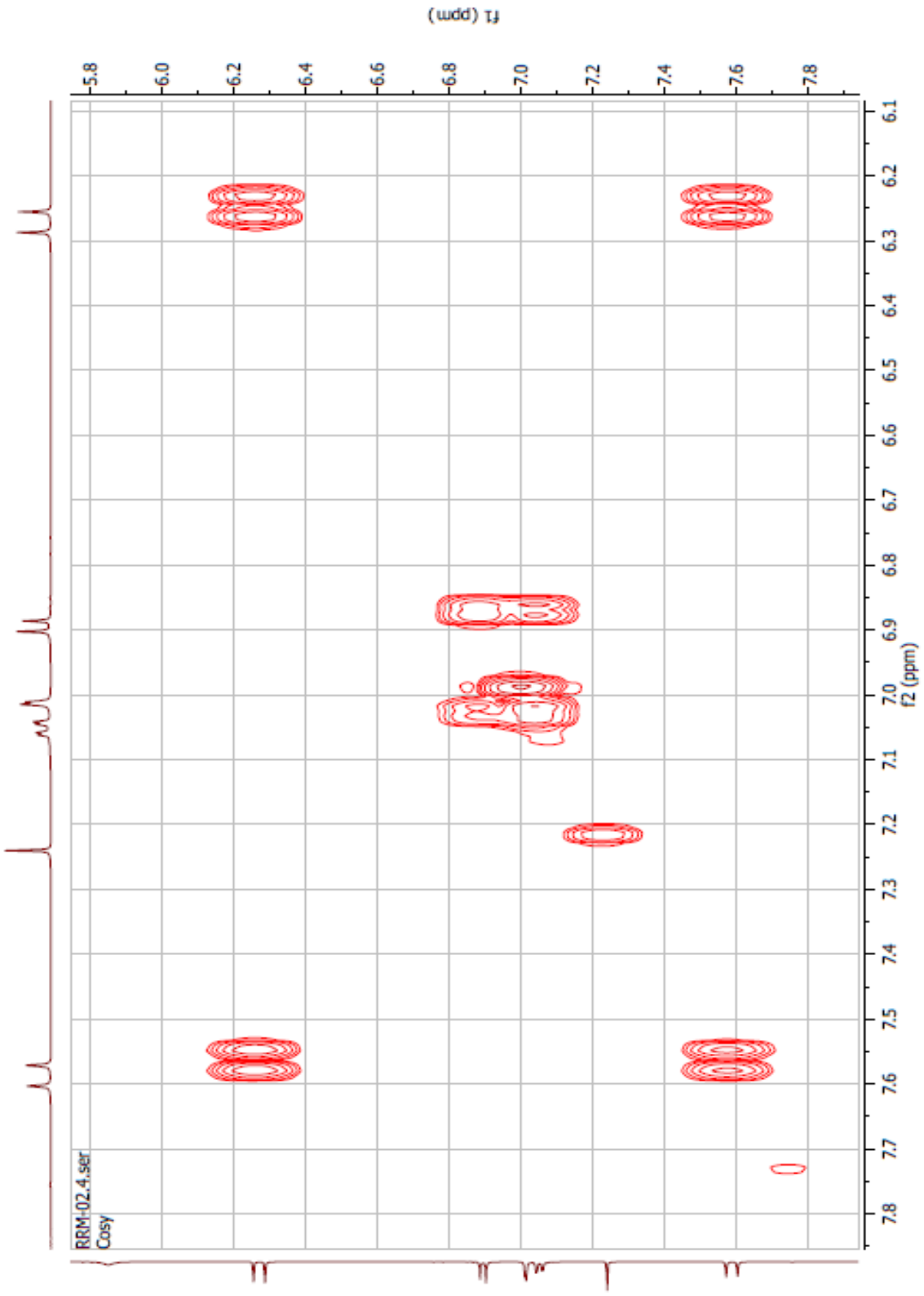

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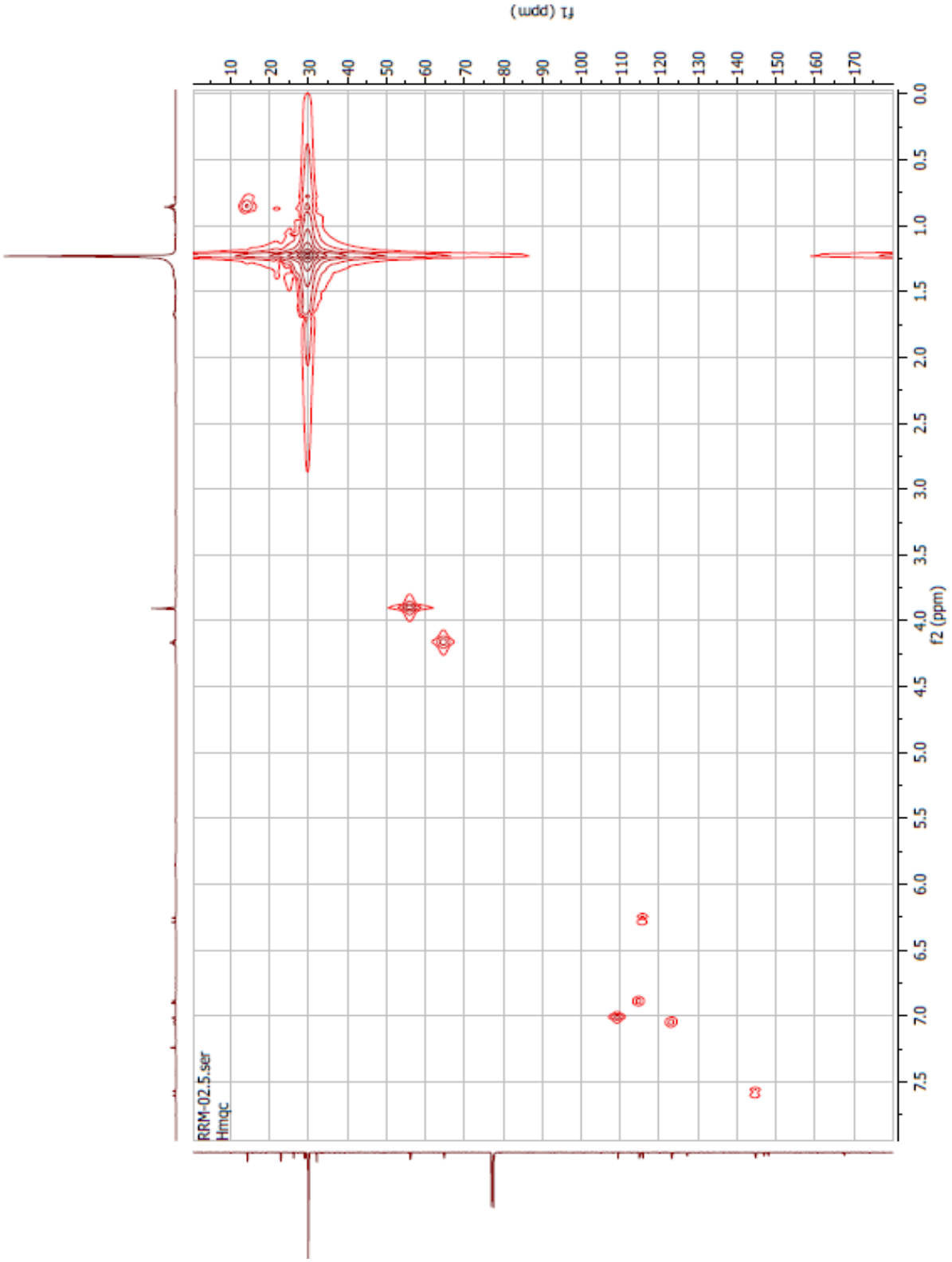

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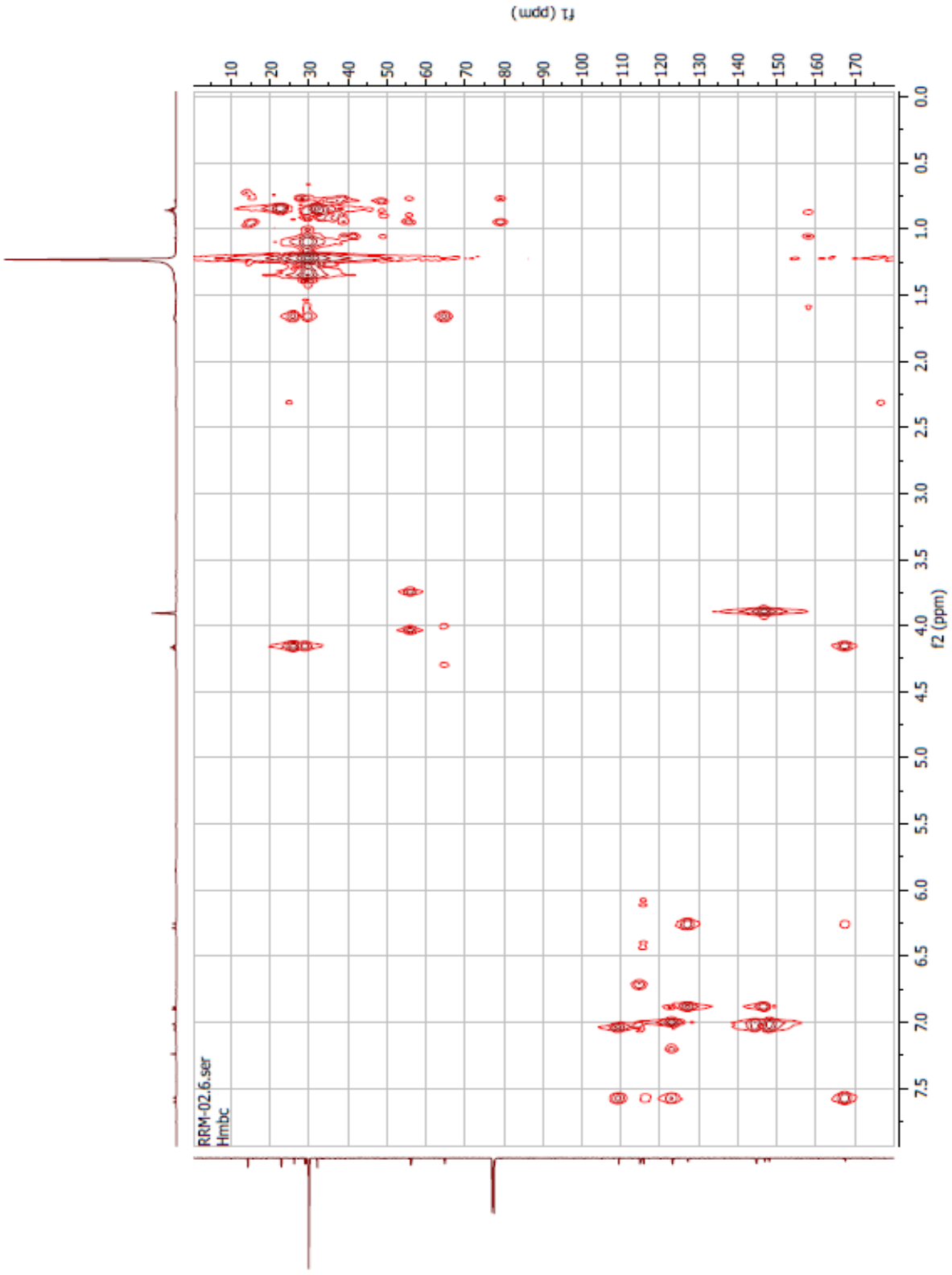

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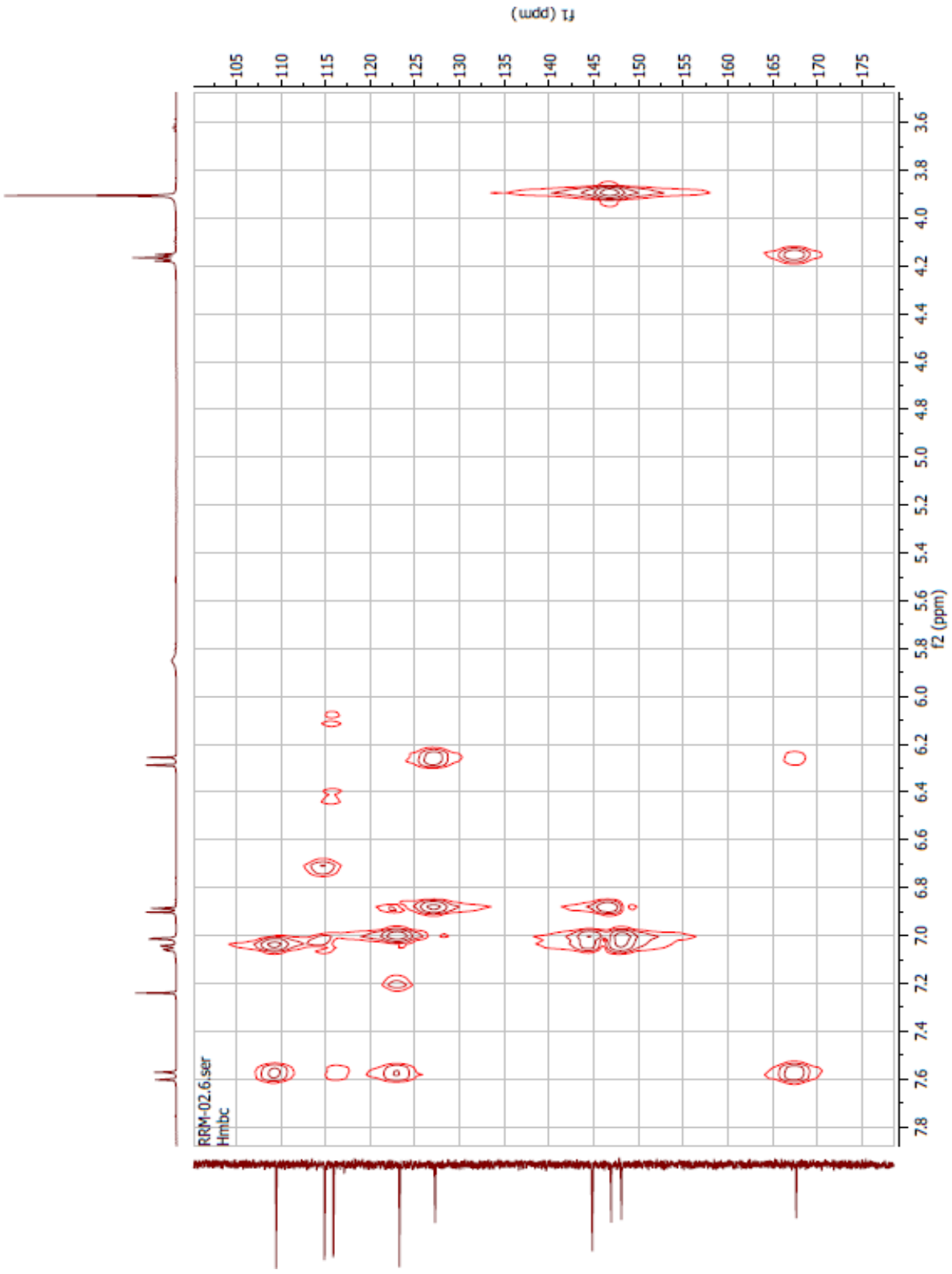

APPENDIX 3: SPECTRA FOR COMPOUND 3

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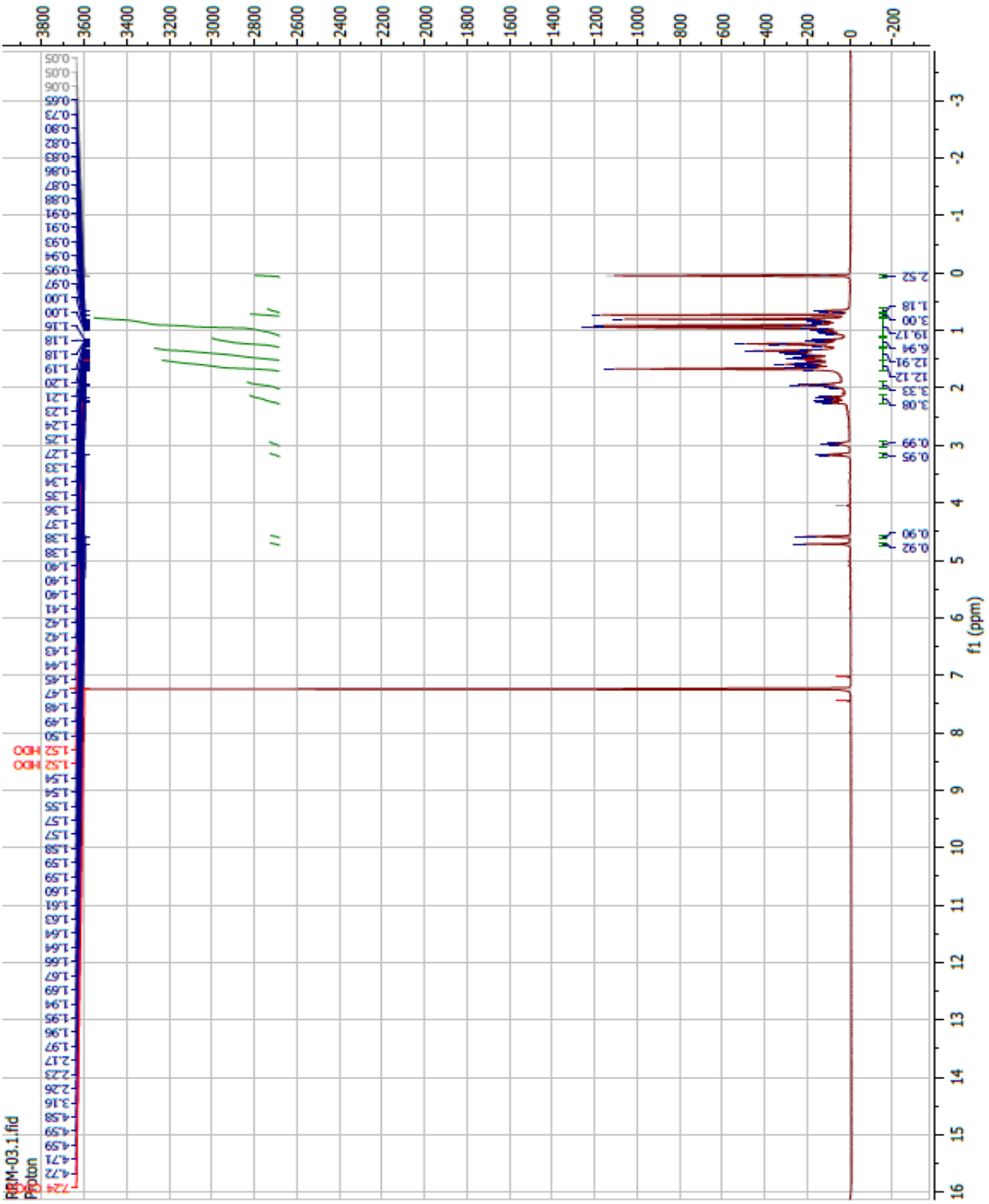

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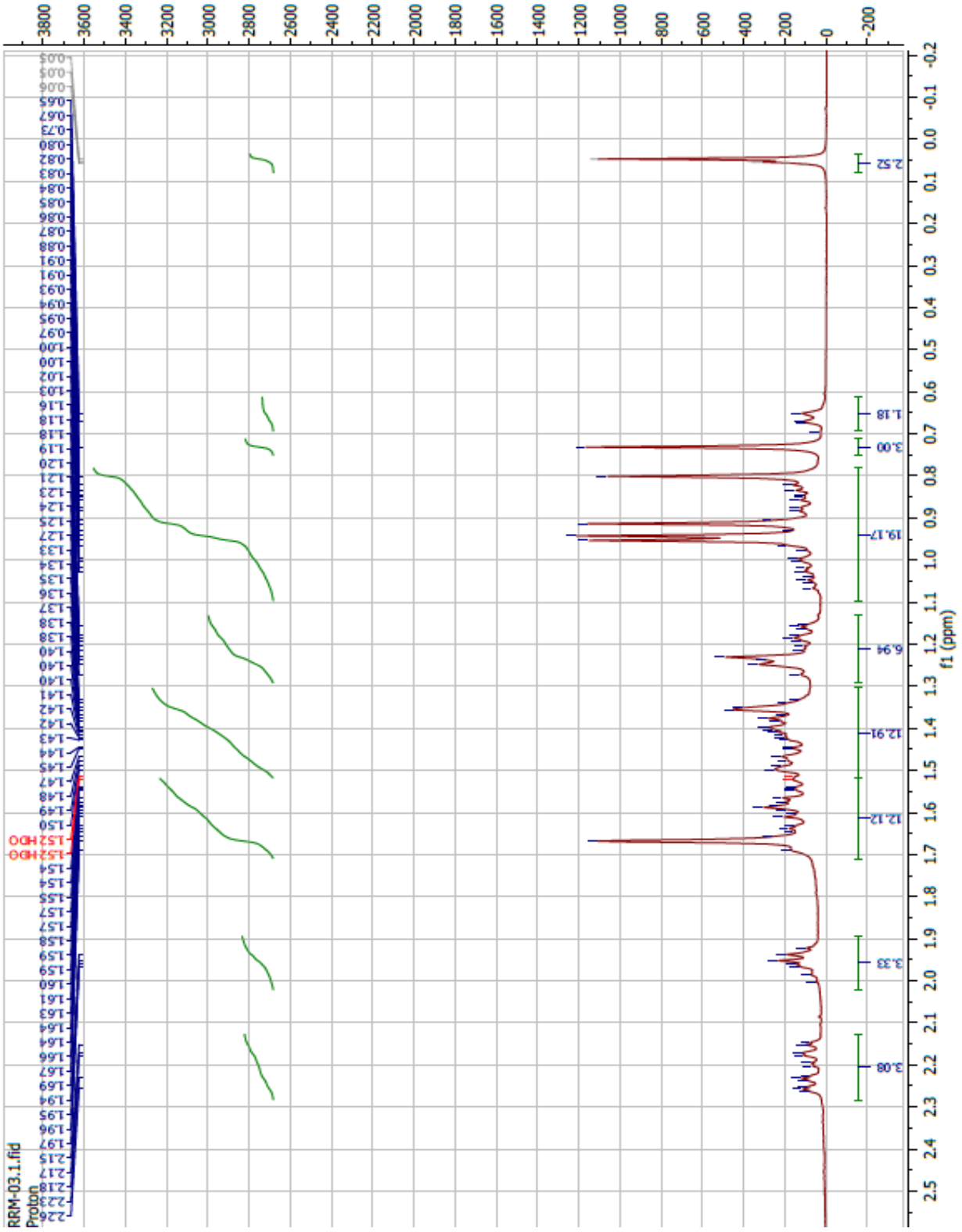

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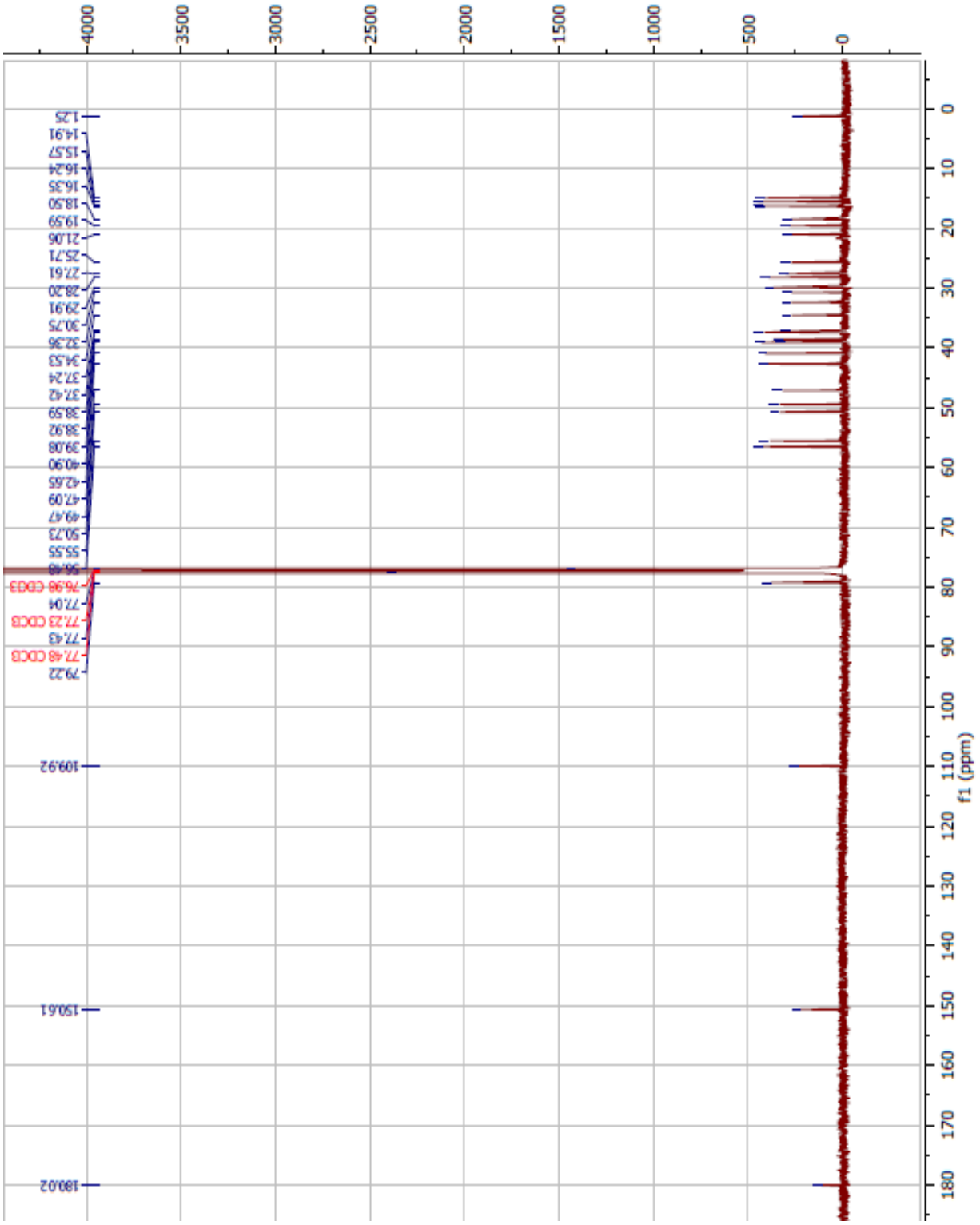

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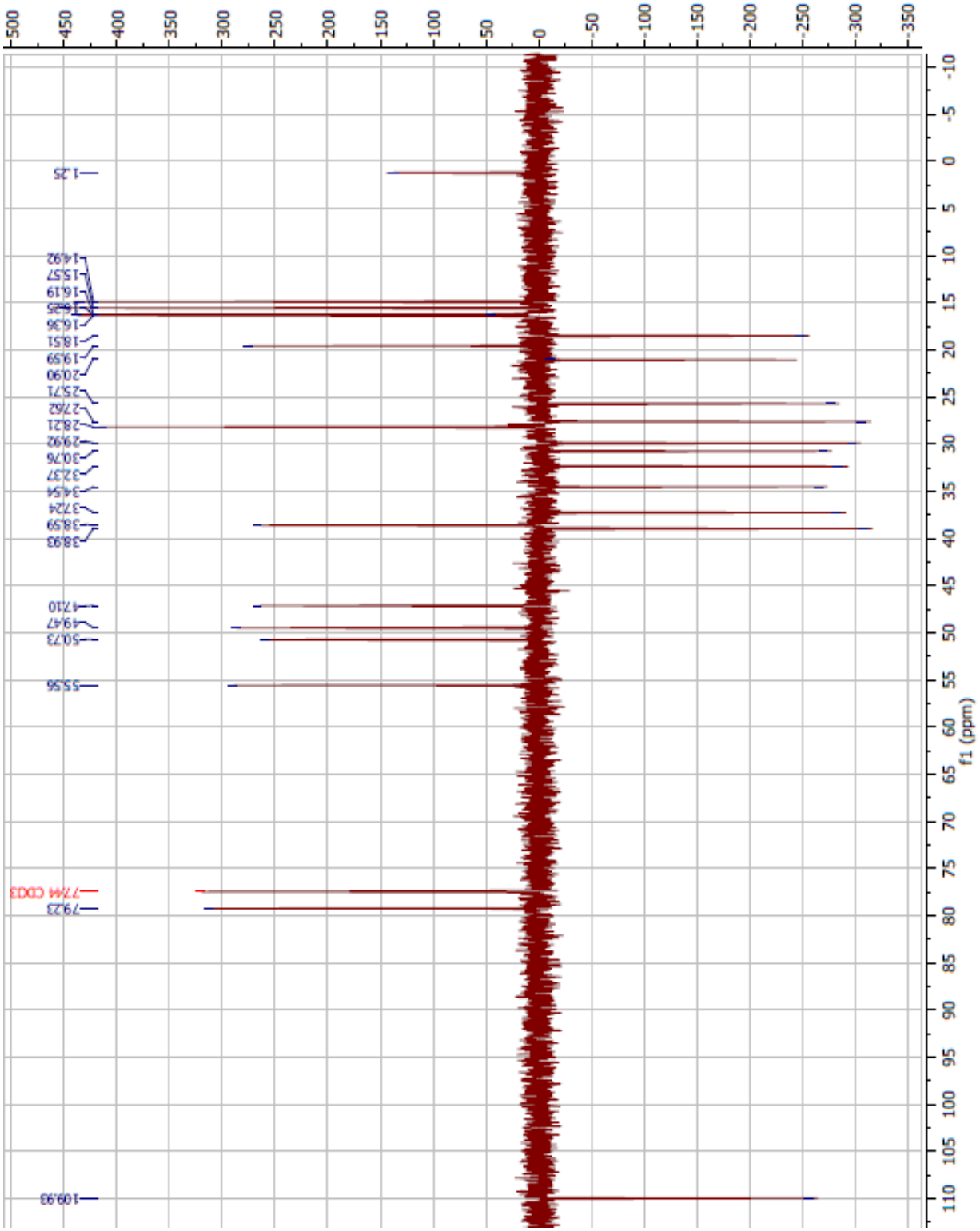
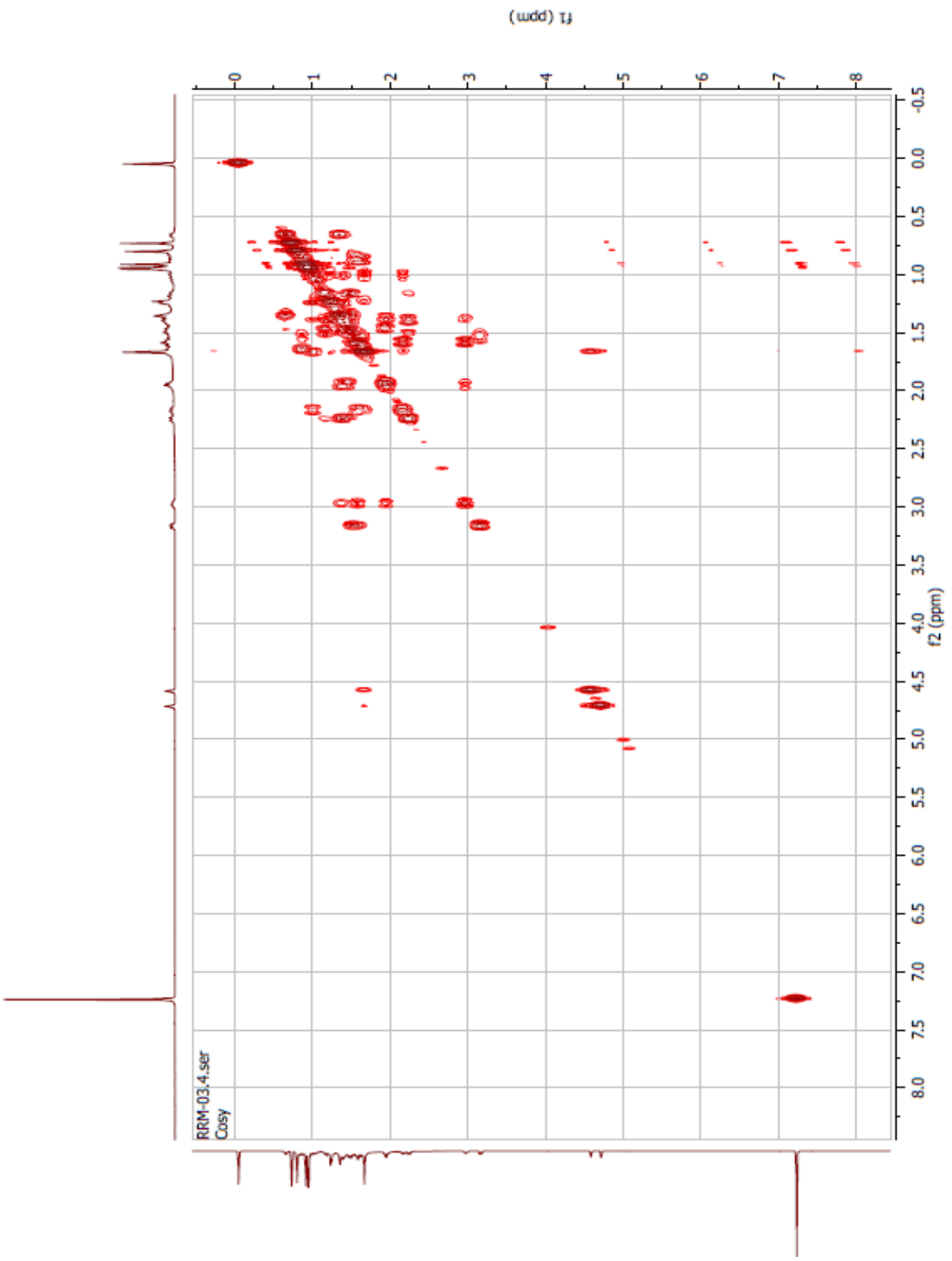


COSY SPECTRUM FOR COMPOUND 3



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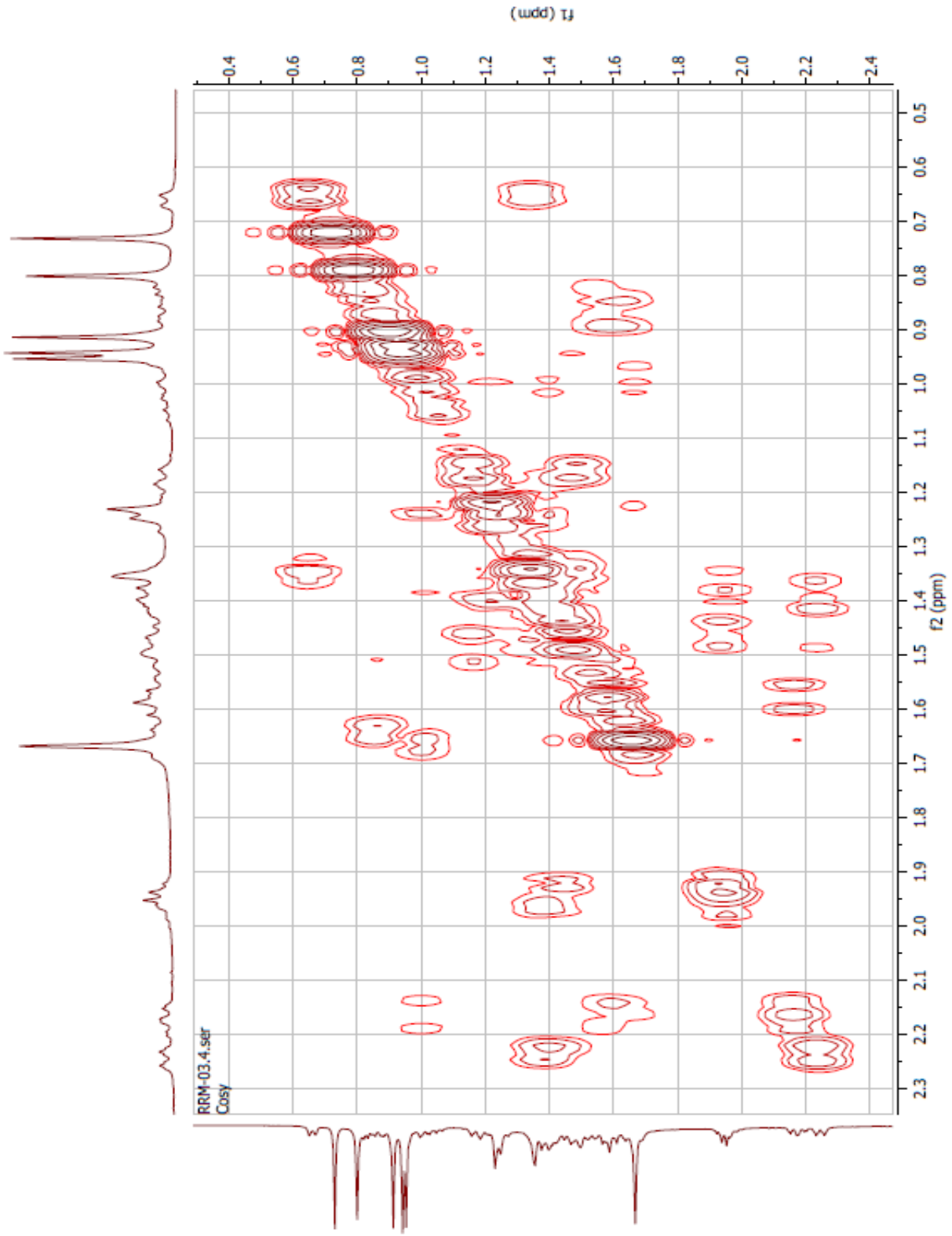

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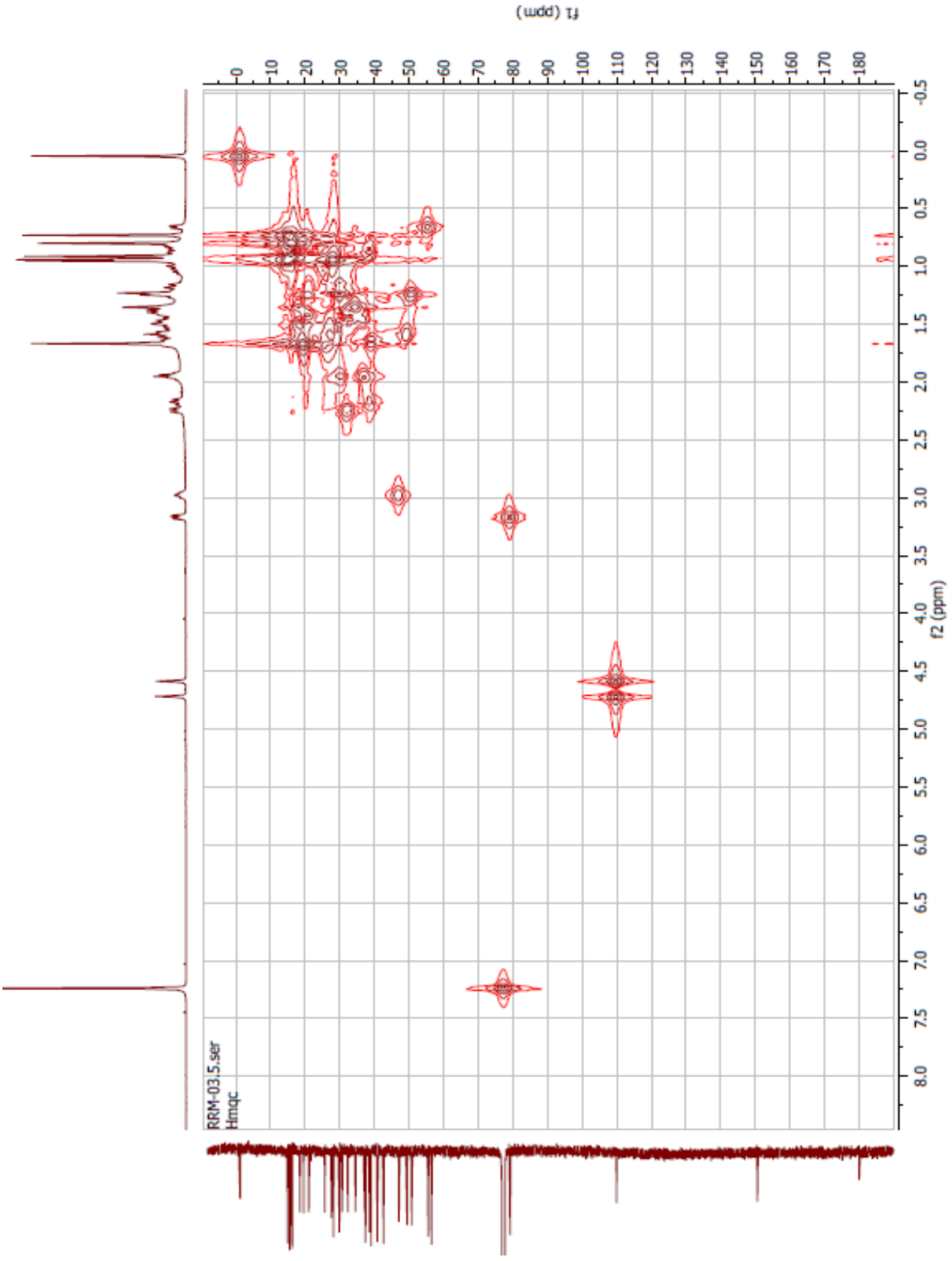

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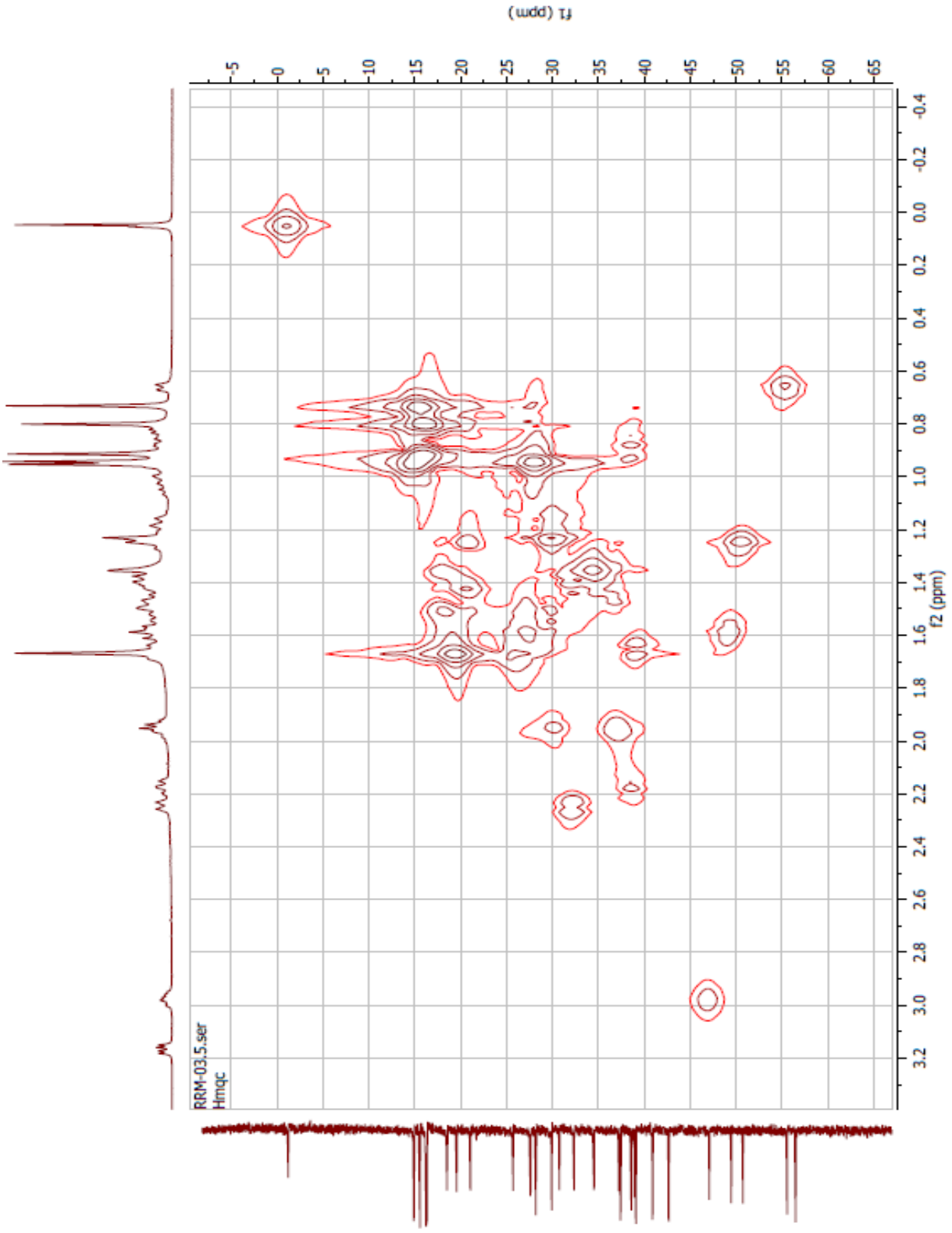

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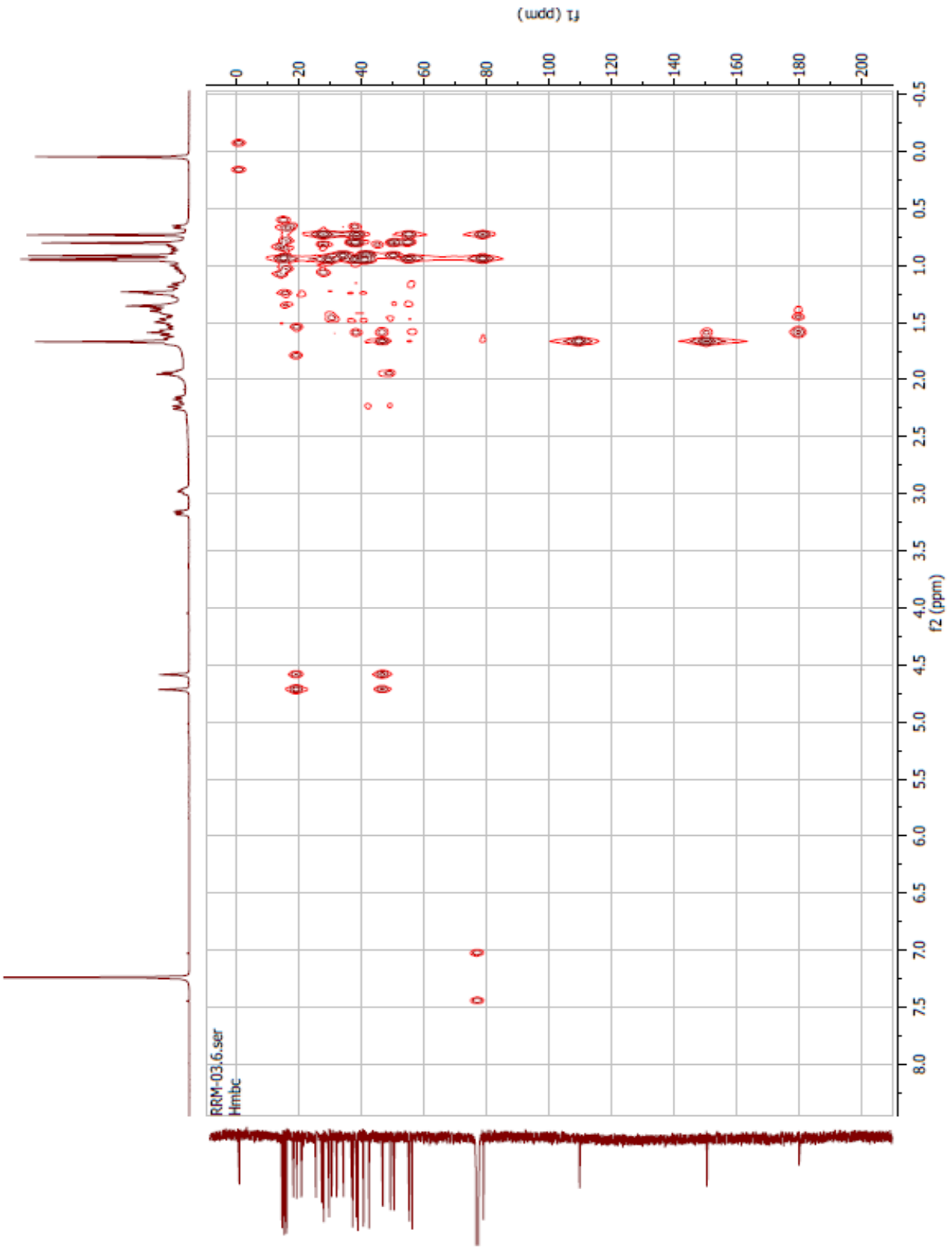

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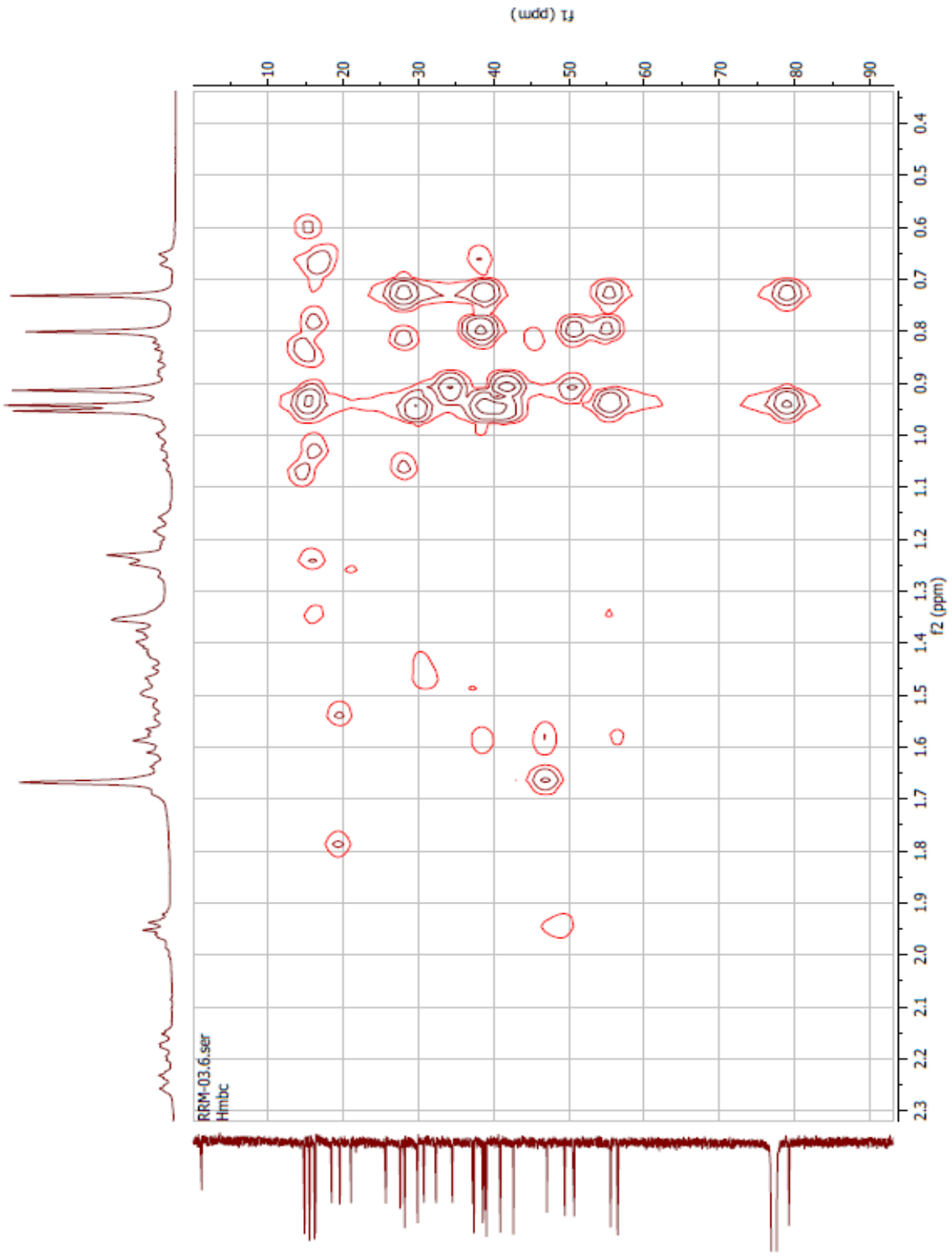

APPENDIX 4: SPECTRA FOR COMPOUND 4

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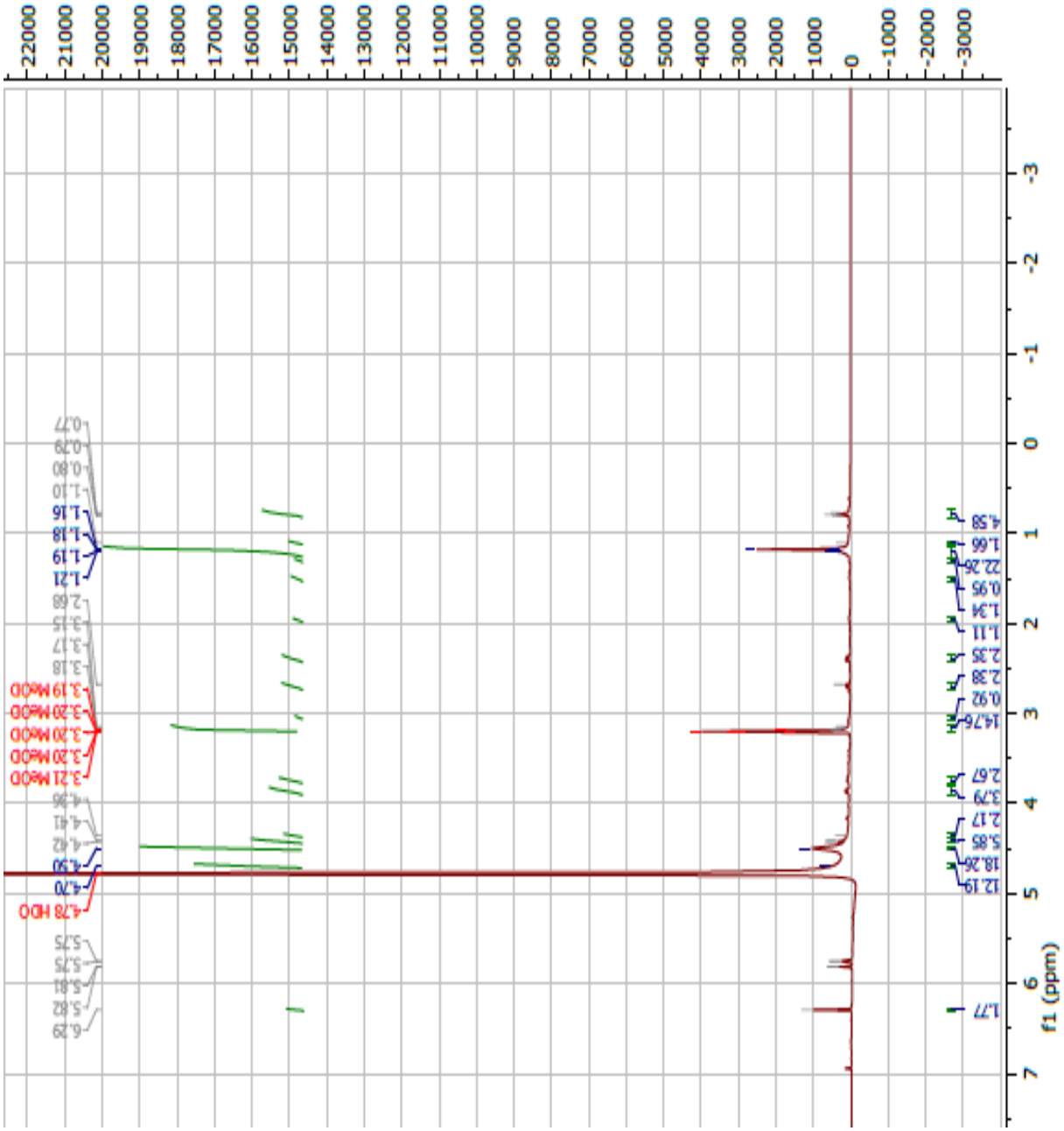

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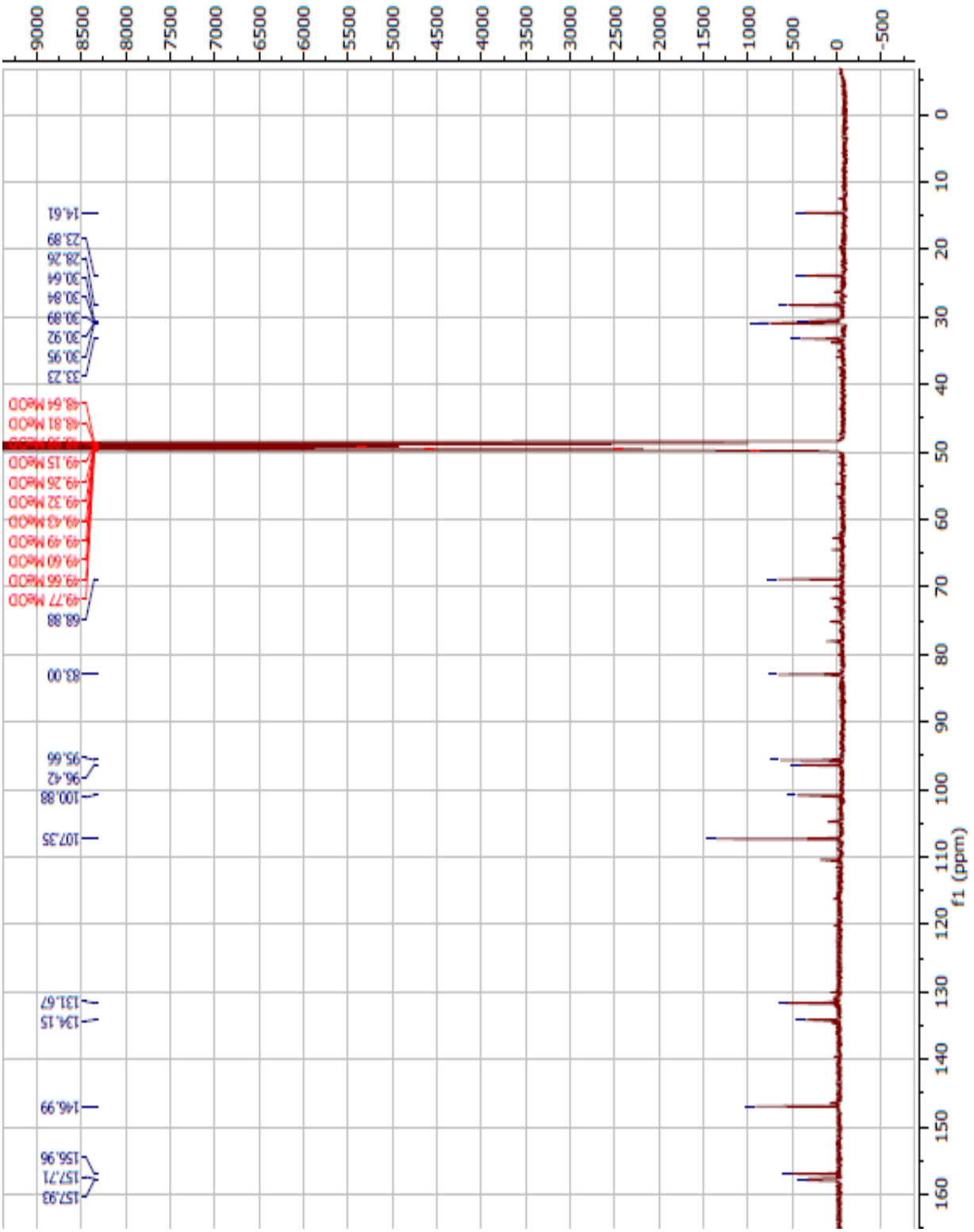

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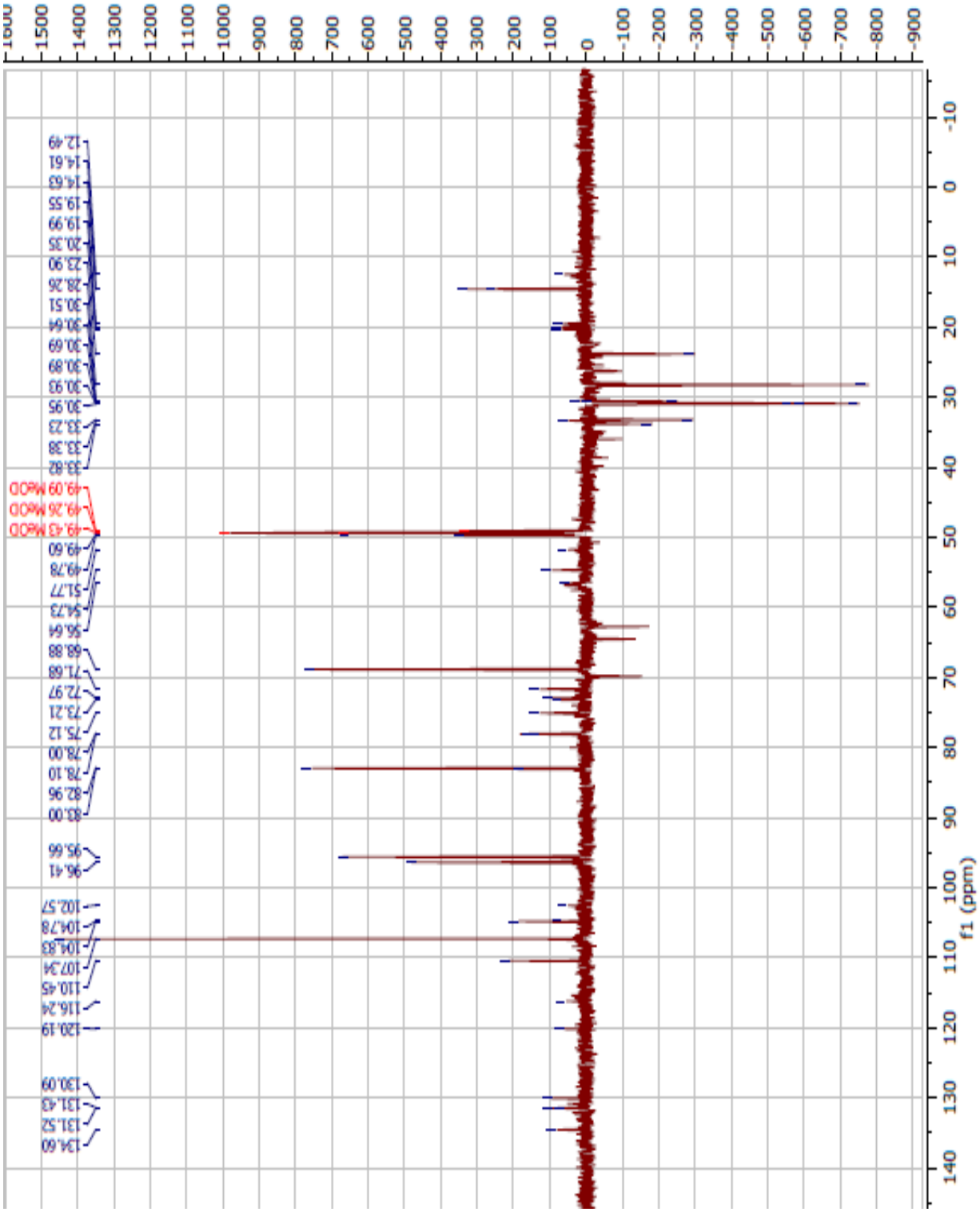

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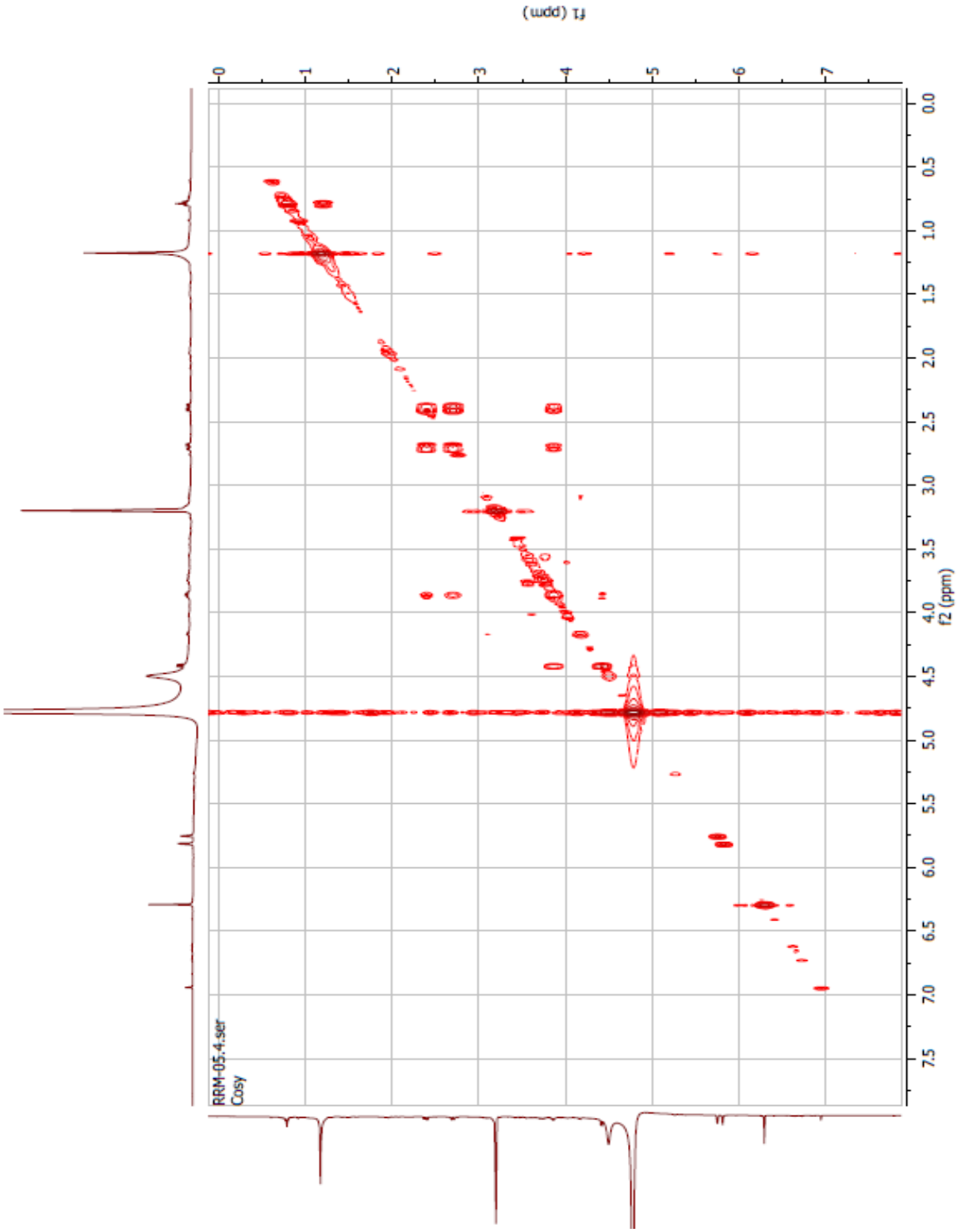

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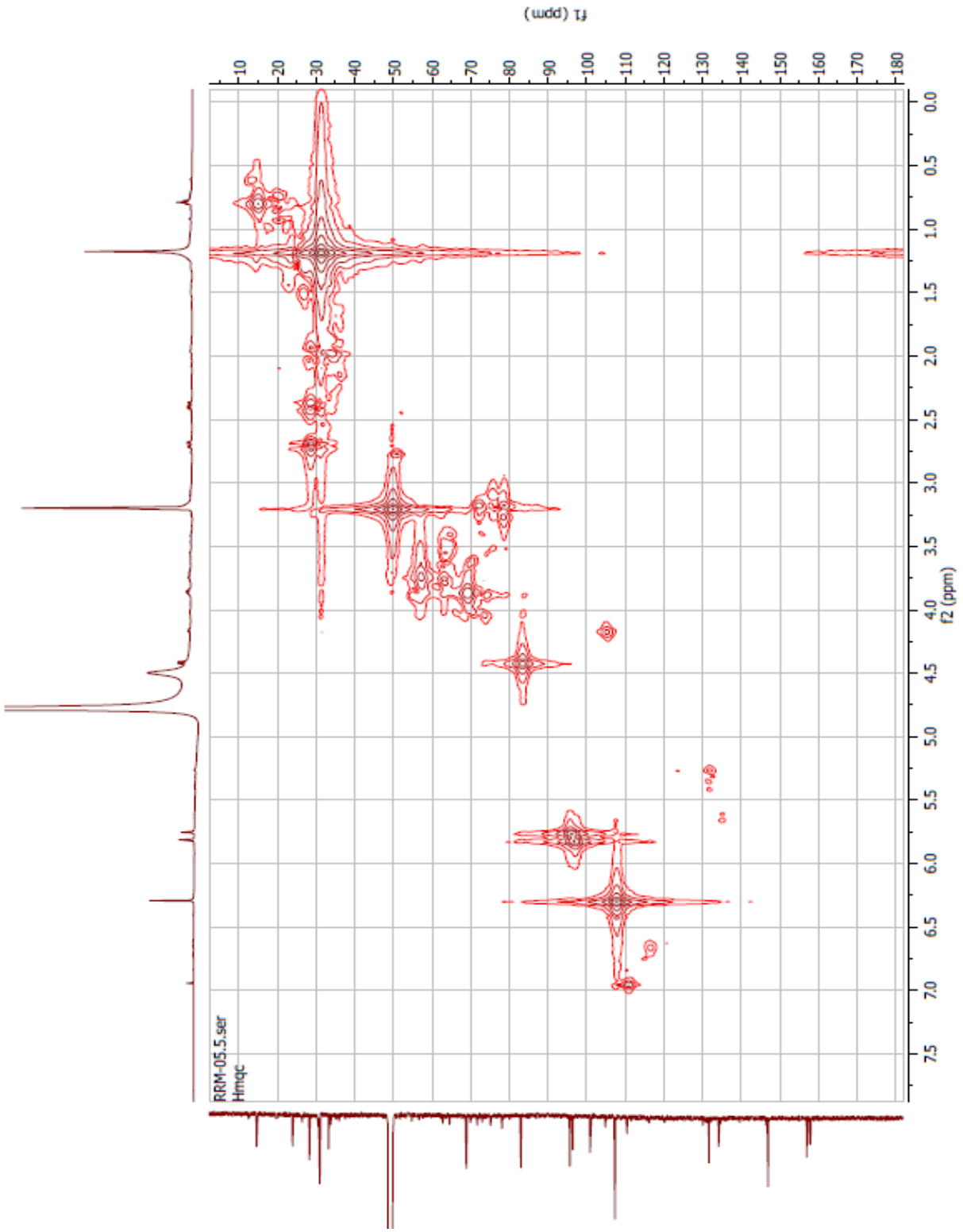

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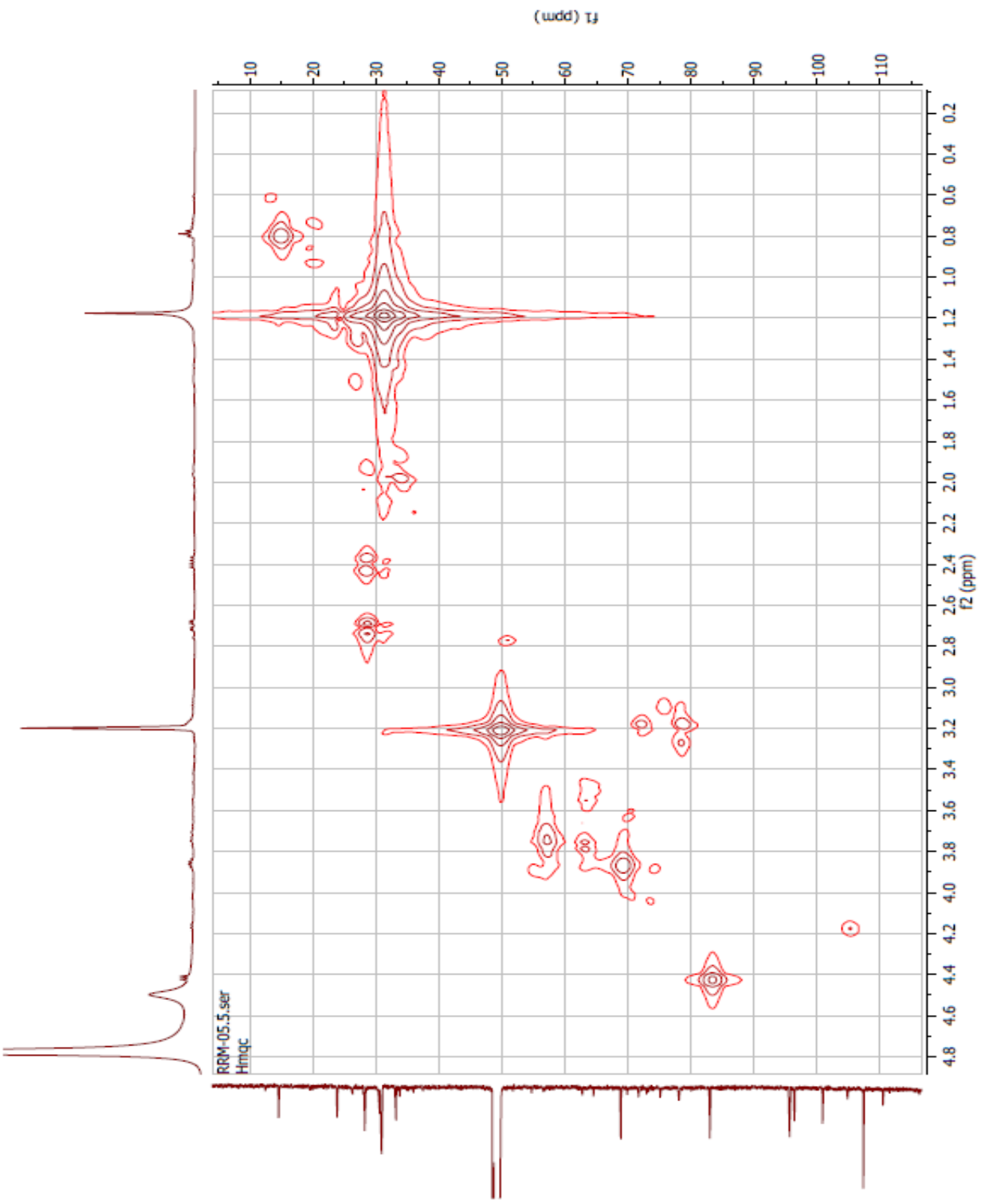

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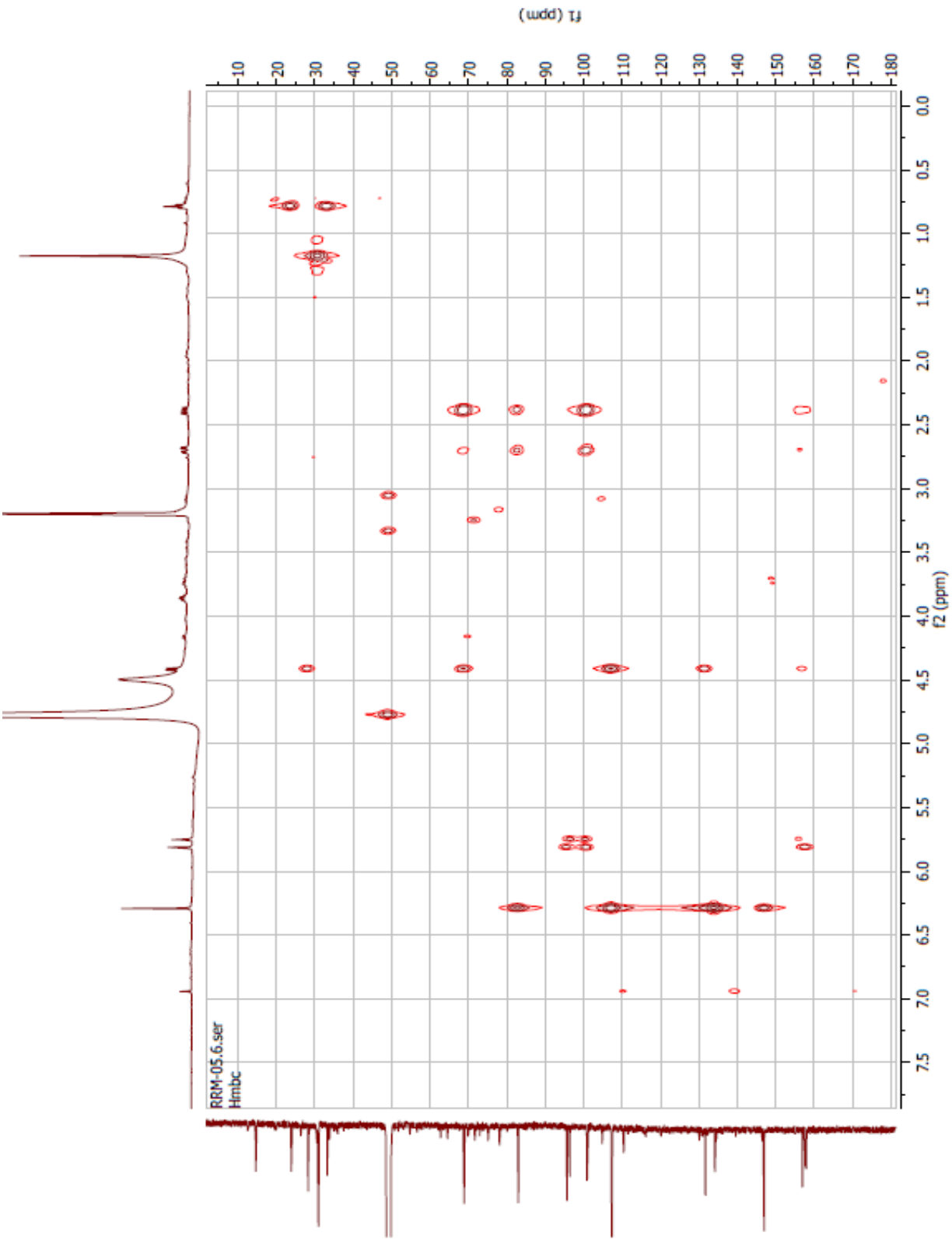
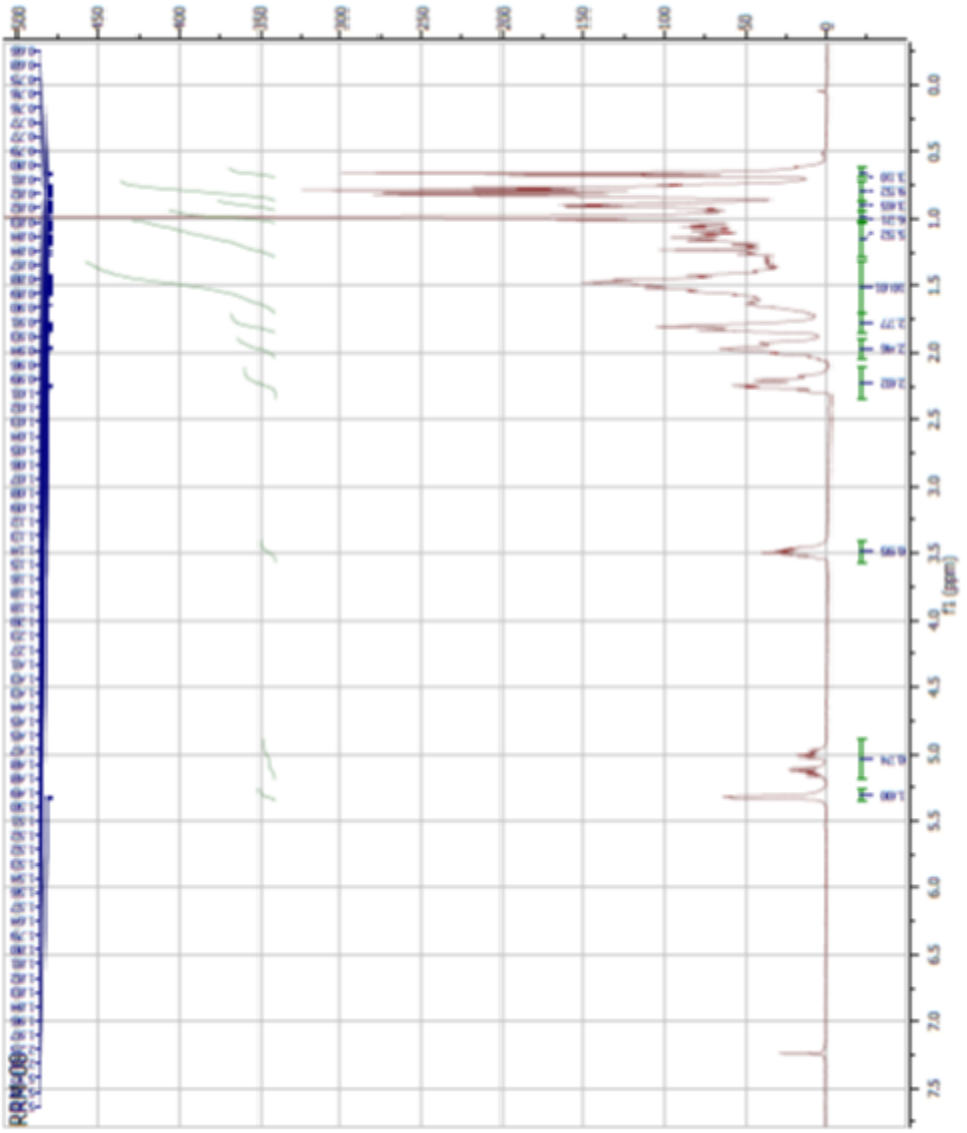
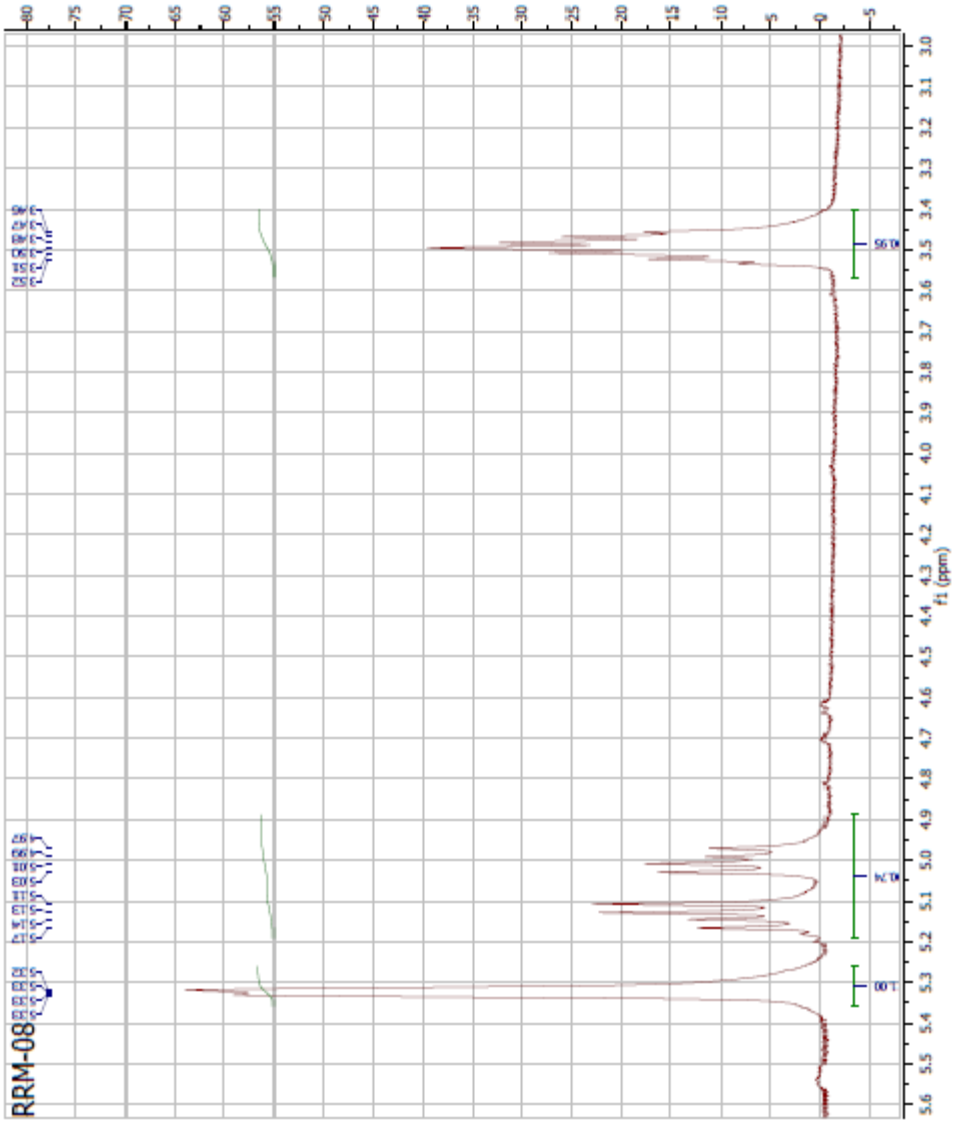


APPENDIX 5: SPECTRA FOR COMPOUND 5a AND 5b

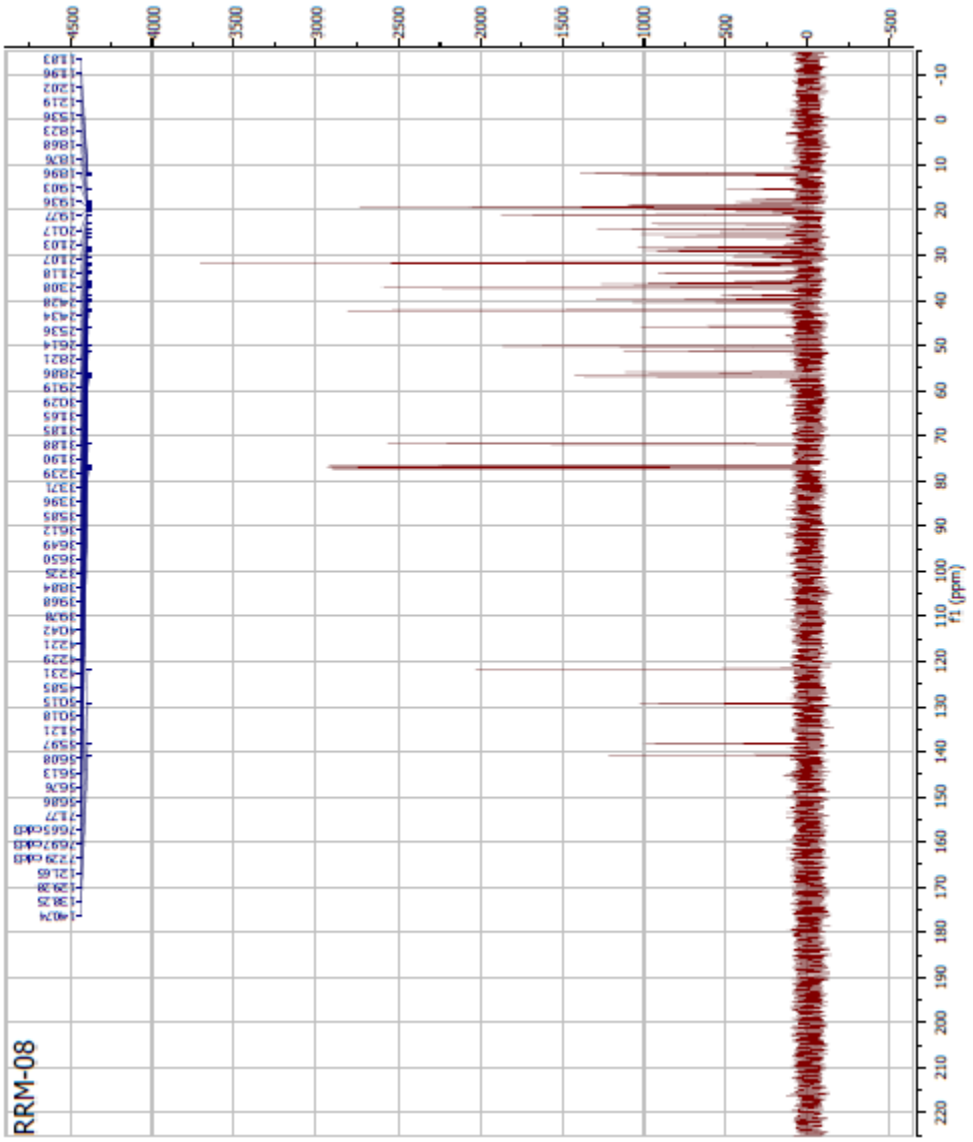
^{1}H SPECTRUMFOR COMPOUND 5



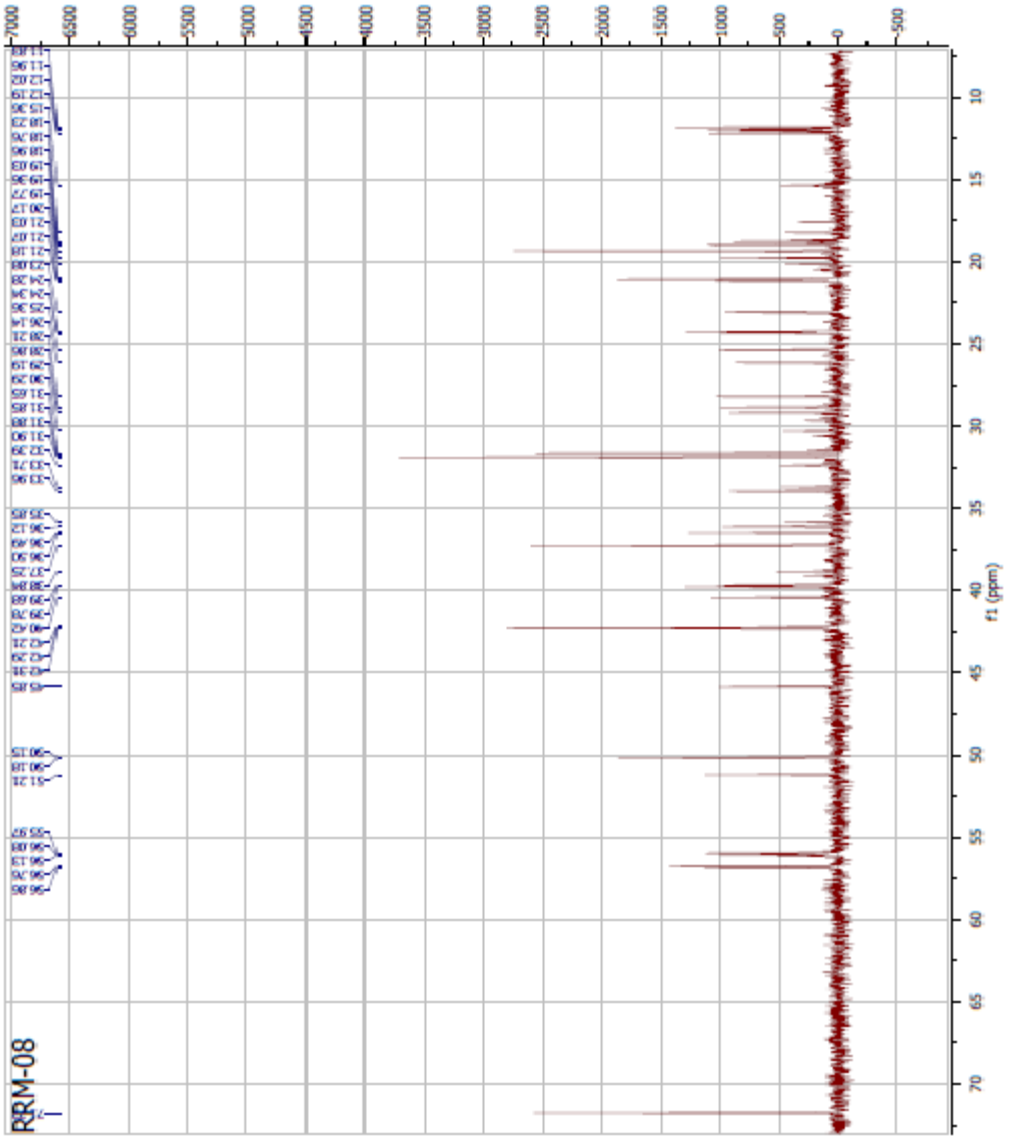
^{1}H SPECTRUM FOR COMPOUND 5



^{13}C SPECTRUM FOR COMPOUND 5



^{13}C SPECTRUM FOR COMPOUND 5



^{13}C SPECTRUM FOR COMPOUND 5