UNIVERSITY OF NAIROBI

COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES

DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF SELECTED *MILLETTIA* (LEGUMINOSAE) AND *OCHNA* (OCHNACEAE) SPECIES FOR ANTICANCER ACTIVITIES

BY

TSEGAYE DEYOU WOLDEMEDHIN

(I80/85101/2012)

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DECLARATION

I hereby declare that this is my own original work and has never been submitted anywhere else for award of any degree or diploma. All sources I have used have been acknowledged by means of completed references according to University of Nairobi guidelines.

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DEDICATION

This work is dedicated to my beloved family.

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ABSTRACT

Despite the availability of well established cancer therapies, death from cancer is common and is predicted to rise. There is evidence that natural products play a significant role in cancer therapy and prevention; with considerable number of anticancer agents in use are either natural products or their derivatives. Flavonoids are among classes of natural products gaining a lot of interest as potential anticancer and cancer chemopreventive agents. In this regard, plants from two flavonoid rich genera, *Millettia* (*Millettia oblata* ssp. *teitensis*, *Millettia dura* and *Millettia usaramensis* ssp. *usaramensis*) of the Leguminosae family and *Ochna* (*Ochna holstii* and *Ochna ovata*) of the Ochnaceae family were investigated.

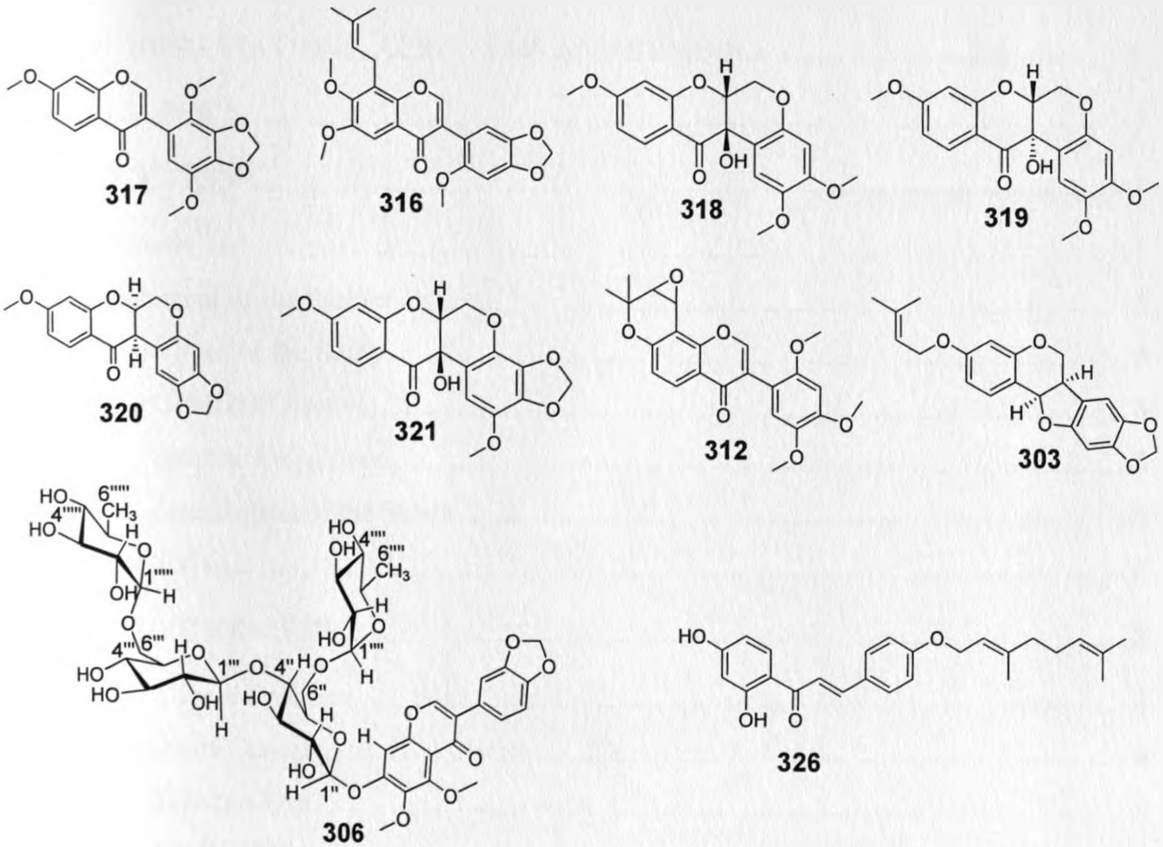
Chromatographic (column chromatography on silica gel, Sephadex LH-20, preparative TLC and HPLC) separation of the extracts from the five plants led to the identification of a total of sixty six compounds, out of which ten are new. Four derivatives of the isolated compounds were also prepared. The structural elucidation of the compounds was performed using spectroscopic and spectrometric analyses: Nuclear Magnetic Resonance (NMR), Ultra Violet spectroscopy (UV), Circular Dichroism (CD), X-ray crystallography, Polarimetry and Mass Spectrometry (MS).

The crude extract of the leaves *of Millettia oblata* ssp. *teitensis* yielded two new isoflavones (**316** and **317**) and four new structurally related rotenoids (**318-321**) along with eight known compounds. Similarly, the leaves of *Millettia usaramensis* ssp. *usaramensis* led to the identification of five rotenoids, three isoflavones and one triterpene, of which the isoflavone (**312**) is new. One of the known rotenoid (**313**) is reported here for the first time from the genus *Millettia*. The root bark extract of *Millettia usaramensis* ssp. *usaramensis* gave thirteen compounds (chalcones, rotenoids, flavanoids and cinnamyl alcohol) of which the chalcone (**326**) is a new compound. From the roots of *Millettia oblata* ssp. *teitensis*, thirteen compounds were identified. Among these, the tetraglycoside isoflavone (**306**) is a new compound. Similar work on the root bark of *Millettia dura* yielded six isoflavones, one chalcone and a pterocarpan, named 3-*O*-prenylmaakiain (**303**) is new compound. Similarly, investigation of the stem bark and leaves of *Ochna holstii* yielded dimeric and monomeric flavonoids along with dasycarponin (**332**) and 2,4-dihydroxyphenylmethyl acetate (**335**). Furthermore, the root bark of *Ochna ovata* also gave seven compounds some of which were also obtained from the stem and leaves of *Ochna holstii*. Four alkaloids (**336-339**) obtained from root bark of *Ochna ovata* are reported here for the first time from the family Ochnaceae. This is the first report on the phytochemistry of the two *Ochna* species.

The crude extracts and some of their constituents were evaluated for anticancer activities. The crude extract of the roots of *M. oblata* ssp. *teitensis* showed strong activity (4.5 µg/mL) against ER-negative MDB-MB-231 human breast cancer cell-line followed by crude extract of root bark of *M. usaramensis* ssp *usaramensis* (11.6 µg/mL). The pure compounds were also found cytotoxic aganist ER-negative MDB-MB-231 human breast cancer cell-line (IC_{50} 10.5-88.1 µg/mL) among which the highest activity was recorded for usararotenoid C (**154**, 10.5 µg/mL) followed by maximaisoflavone J (**325**, 11.2 µg/mL). The activity of **154** is almost four times higher than that of epimillettosin (**137**, 39.7 µg/mL) with the only structural difference between the two is that **154** has a prenyl group at C-8 and a methoxyl group at C-9 while in 137 the prenyl has cyclized into 2,2-dimethylchromene. Similarly, the activity of maximaisoflavone J (**325**) is almost five times higher than maximaisoflavone B (**304**, 53.8

μg/mL); while the only difference between the two compounds is the replacement of the methoxyl group at C-4' in **325** by a methylenedioxy (C-3'/C-4') in maximaisoflavone B (**304**). The strong activities observed for the crude extracts; roots of *Millettia oblata* ssp. *teitensis* and root bark of *Millettia usaramensis* ssp. *usaramensis* could be due to their active component; maximaisoflavone J (**325**) and usararotenoid C (**154**), respectively. Some compounds were also evaluated for cytotoxicity against Vero cells (IC_{50} 6.7-67.4 μg/mL). Strong activity was recorded for the dimeric flavonoid, calodenone (**253**). This compound is ten times more active than the related compound, lophirone A (**252**), a compound which only differ from **253** by lack of a methoxyl group at C-15. The isolated constituents were also tested in Krebs-2 *in vitro* for translation inhibitory, but none of the compounds showed translation inhibitory activity.

Overall, the investigation of the five plants yielded a wide range of new and known compounds as monomeric and dimeric flavonoids, rotenoids, isoflavonoids, chalcones, alkaloids, triterpene and two simple molecules (**331** and **335**), some of which showed moderate to low cytotoxicity on the ER-negative MDB-MB-231 human breast cancer cell-line and Vero cells.



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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLs

1D NMR: One Dimensional Nuclear Magnetic Resonance
2D NMR: Two Dimensional Nuclear Magnetic Resonance
4CL: 4-coumaric acid CoA ligase;
ACC: acetyl-CoA carboxylase,
AIHW Australian Institute of Health and Welfare
AACR: Australasian Association of Cancer Registries
ANS: anthocyanidin synthase
brd: broad doublet
C4H: cinnamic acid 4-hydroxylase
CD: Circular Dichroism
CHS: Chalcone synthase
CHI: chalcone isomerase
COSY: Correlated Spectroscopy
d: doublet
dd: doublet of a doublet
DFR: Dihydroflavonol 4-reductase,
DMEM: Dulbecco's Modified Eagle Medium
DMSO: Dimethyl sulphoxide
ESIMS: Electron Spray
F3H: Flavanone 3-hydroxylase,
F3'H: Flavonoid 3'-hydroxylase,
FLS: Flavonol synthase;
GLOBOCAN: Global Burden of Cancer Study
HMBC: Heteronuclear Multiple Bond Correlation
HREIMS: High Resolution Electron Ionization Mass Spectrometry
IARC: The International Agency for Research on Cancer
IC50: Concentration of 50% Inhibition
J: Coupling constant
LC_{50} Latal Concentration
LC-MS: Liquid Chromatography Mass Spectrometry
MALDI: Matrix Assisted Laser Desorption Ionization
MHz: Mega Hertz
MS: Mass Spectrometry
NGCMK- National Guidelines for Cancer Management Kenya
NMR: Nuclear Magnetic Resonance
NOE: Nuclear Overhauser Effect
NR: anthocyanidin reductase.
OMT1: *O*-methyltransferase 1,
PAL: phenylalanine ammonia-lyase,
Prep-HPLC: Preparative High Performance Liquid Chromatography
PTLC: Preparative Thin Layer Chromatography
s: singlet
t: triplet
TLC: Thin Layer Chromatography
UV: Ultra Violet
WCMC: World Conservation Monitoring Centre
WHO: World Health Organization
λ_{max}: Maximum wavelength of absorption

CHAPTER ONE

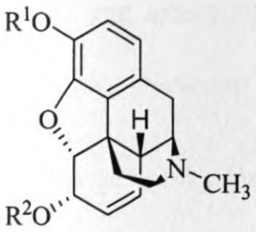
INTRODUCTION

1.1 General

The healing power of plants and their products is an ancient knowledge and is as old as the history of mankind (Cowan, 1999; Petrovska 2012). Plants have been used by humans for the treatment of many diseases and illnesses over the thousands of years (Dias *et al.*, 2012; Petrovska 2012). The earliest record on the use of plants was described on a clay tablet in cuneiform from Mesopotamia, 2600 B.C. which documented about 1,000 plant products derived from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh), *Cedrus* species (cedar), *Glycyrrhiza glabra* (licorice), and *Papaver somniferum* (poppy juice) among others; these were used for the treatment of cough, cold, parasitic infections and inflammation and are still used nowadays (Cragg *et al*, 1997; Fakim, 2006; Ji *et al.*, 2009; Dias *et al.*, 2012). The Ebers Papyrus (2900 B.C.), an Egyptian pharmaceutical record, documented over 700 plant-based drugs ranging from gargles, pills, infusions, to ointments. The Chinese Materia Medica (1100 B.C., Wu Shi Er Bing Fang, contains 52 prescriptions), Shennong Herbal (~100 B.C., 365 drugs) and the Tang Herbal (659 A.D., 850 drugs) are among the old records of the historical uses of plants (Cragg *et al*, 1997; Fakim, 2006; Ji *et al.*, 2009).

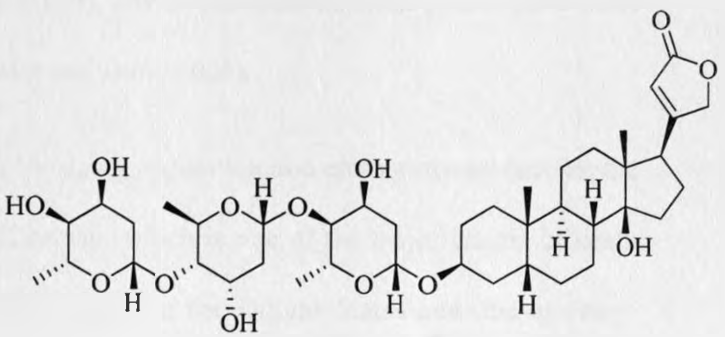
Traditional medicine practices have laid the foundation of modern medicine, and plants used in such practice have served as the basis from which most of the contemporary medicines are derived - through clinical, pharmacological and chemical investigations (Butler, 2004; Dias *et al.*, 2012). The earliest historical examples (in 1803) of natural products used as medicine include the alkaloid morphine (**1**), from *Papaver somniferum* L (opium poppy). Heroin (**2**),

the diacetyl derivative of morphine; was obtained (in the 1870s) from a crude extract of *P. somniferum* containing morphine through boiling of the extract in acetic anhydride. Similarly, methylation of the extract containing morphine readily formed codeine (**3**), a painkiller (Dias *et al.*, 2012). *Digitalis purpurea* L (foxglove) is another old medicinal plant which was familiar in the 10th century, whilst its active cardiotonic glycoside constituent, digitoxin (**4**), was not known until the 18th century as an enhancer of cardiac conduction (Dias *et al.*, 2012). Digitoxin (**4**) and its analogues have long been used in the management of congestive heart failure; however, they are being replaced due to possible long term detrimental effects (Dias *et al.*, 2012; Elbaz *et al.*, 2012).



1: $R^1 = R^2 = H$

2: $R^1 = R^2 = COCH_3$; **3**: $R^1 = CH_3$, $R^2 = H$



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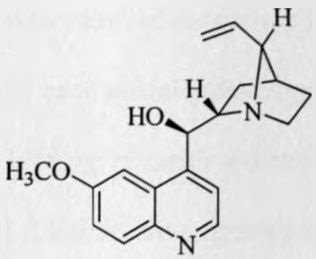
The alkaloid quinine (**5**), isolated from *Cinchona* species (in 1820), is an old anti-malarial drug which is still used in modern world (Achan *et al.*, 2011). Pilocarpine (**6**), an alkaloid from *Pilocarpus jaborandi* (Rutaceae), is an old medicine (for more than 100 years) used in the treatment of chronic open-angle glaucoma and acute angle-closure glaucoma (Dias *et al.*, 2012).



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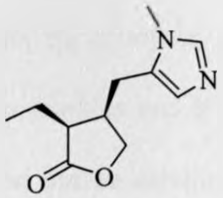

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Plants based drugs continue to play a vital role in healthcare and about 80% of the world population still rely on traditional medicinal plants for their primary healthcare requirements (Ekor, 2013). Plant based treatment is not limited to traditional applications, they also play a key role in contemporary drug discovery; for example over 60 and 75% of the current drugs for cancer and infectious diseases, respectively have been derived from plants (and other natural origin) (Newman *et al.*, 2003; Butler and Buss, 2006).

Due to change in lifestyle, the increase in the aging population and environmental factors, the majority of world population is at risk of cancer, which is one of the major causes of death worldwide. For example, one-in-every-four deaths in the United States and one-in-every-eight worldwide, is due to cancer (Kinghorn *et al.*, 2009; American Cancer Society, 2011). The global burden of cancer continues to increase largely because of the aging population with an increasing adoption of cancer causing lifestyles, particularly smoking contributes significantly to this (Jemal *et al.*, 2011). In the year 2000, there were an estimated 10.1 million new cancer cases, of which 6.2 million deaths were reported (Parkin, 2004). Moreover, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared to 12.7 million new cancer cases and 7.6 million deaths, in 2008; indicating an increasing trend in incidences and mortalities due to cancer (American Cancer Society, 2011; Ferlay *et al.*, 2015).

The most common new cases of cancer are lung, breast, prostate, colon-rectum, stomach and liver cancers. In both economically developed and developing countries, the most frequently diagnosed cancers leading to death are breast cancer in females and lung cancer in males (Jemal *et al.*, 2011). Despite the extensive studies carried out on carcinogenesis for the last fifty years, the rate of cancer incidences and morbidities are ascending worldwide. In fact cancer kills more people globally than malaria, HIV/Aids and tuberculosis put together (Garcia *et al.*, 2007; American Cancer Society, 2011).

The rich chemical diversity of secondary metabolites make plants, as one of the most important sources of pharmacologically active principles used as drugs, including anticancer agents. The genus *Millettia* (Leguminoseae) with the major constituents; flavonoids and isoflavonoids (including rotenoids) has an important place in the pharmacopoeias with potential therapeutic uses; it displayed wide variety of activities; such as antitumoral (Ito *et al.*, 2004), anti-inflammatory (Ye *et al.*, 2014), cytotoxicity against different cancer cell-lines (Rayanil *et al.*, 2011; Ye *et al.*, 2012), antiviral (Banzouzi e*t al.*, 2008), insecticidal (Yenesew, 1997), trypanocidal (Rajemiarimiraho *et al.*, 2013) and antiplasmodial (Yenesew *et al.*, 2003; Rajemiarimiraho *et al.*, 2013; Derese *et al.*, 2014) activity. Many species of Ochnaceae family are also known to be rich in dimeric and monomeric flavonoids. These plants are used in traditional medicine for the treatment of different aliments. Biflavonoids from this family and also from other plants are known to have different activities including anticancer and anti-tumor promoters (Murakami *et al.*, 1992; Daniel *et al.*, 2007; Fidelis *et al.*, 2012); antimalarial (Ichino *et al.*, 2006), anti-inflammatory, anti-viral and anti-tuberculosis (Lee *et al.*, 2006) activities.

In this research, phytochemical investigation of three *Millettia* species (*M. oblata* ssp. *teitensis*, *M. dura* and *M. usaramensis* ssp. *usaramensis*) and two *Ochna* species (*O. holstii* and *O. ovata*) were undertaken. The isolated compounds of these plants were evaluated for anticancer activity against human MDB-MB-231 breast cancer cell-line, Vero cells and translation inhibitory activity. Some of the isolated compounds were also evaluated for antiplasmodial and larvicidal activity.

1.2 Statement of the Problem

Cancer remains a major public health burden in both developed and developing countries. Despite the fact that, multidisciplinary scientific investigations have been intensified to combat this disease, the risk of cancer is on the rise. In 2012, there were about 14.1 million new cancer cases and 8.2 million cancer-related deaths that occurred which is a significant increase from the 2008 figures (12.7 million cases and 7.6 million deaths). By 2025, an estimated 19.3 million new cancer cases and 11.4 million of cancer deaths are projected to occur worldwide. This projection of growth of cancer cases appears to be due to aging of the global population and also due to rapid development of resistance to chemotherapuetic drugs by cancer cells (http://www.bibliotecapleyades.net/salud/salud_defeatcancer176.htm). Some cancer chemotherapuetic drugs are usually associated with high toxicity and undesirable side effects, which again amplifies the demand for alternative drugs with fewer side effects and greater therapeutic efficacies. Thus, an urgent search for new and effective anticancer agents to curb the cancer threat is required.

1.3 Objectives of the Study

1.3.1 General Objective

The general objective of this study was to identify anticancer principles from three *Millettia* and two *Ochna* species.

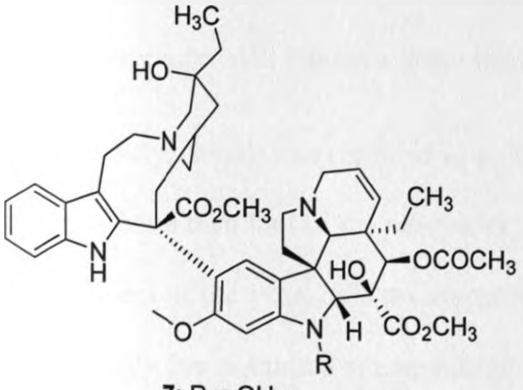
1.3.2 Specific Objectives

1. To isolate and characterize secondary metabolites from *Millettia dura*, *Millettia oblata* ssp. *teitensis*, *Millettia usaramensis* ssp. *usaramensis*, *Ochna holstii* and *Ochna ovata*;
2. To establish the anticancer activities of the crude extracts and isolated compounds;
3. To modify some of the isolated compounds inorder to enhance their activities.

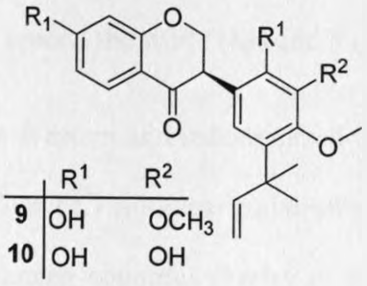
1.3.3 Justification of the Study

The discovery of vinca alkaloids; vinblastine (**7**) and vincristine (**8**) from the Madagascar periwinkle, *Catharanthus roseus* as anticancer drugs laid the foundation that nature is potential source of anticancer drugs. Flavonoids are among classes of natural products gaining a lot of interest as potential cancer chemotheraphy and chemopreventive agents. In line with this, flavonoids from the seeds of *Millettia pachcarpa* showed a strong cytotoxicity and apoptotic effect against different cell-lines such as HepG2, C26, LL2 and B16 (Ye *et al.*, 2012). Similarly, the isoflavone of *Millettia dielsiana* with 2,2-dimethylchromene and methoxyl groups exhibited a potent anti-inflammatory effect by decreasing NO (nitric oxide) production and TNF-α secretion (Ye *et al.*, 2014). Moreover, two prenylated isoflavanone; pervilleanone (**9**) and 3'-*O*-demethylpervilleanone (**10**), isolated from *Millettia pervilleana* showed mild growth inhibition on different human cancer cell-lines of lung, breast and central nervous system (CNS) (Palazzino *et al.*, 2003). In the same light, a biflavone,

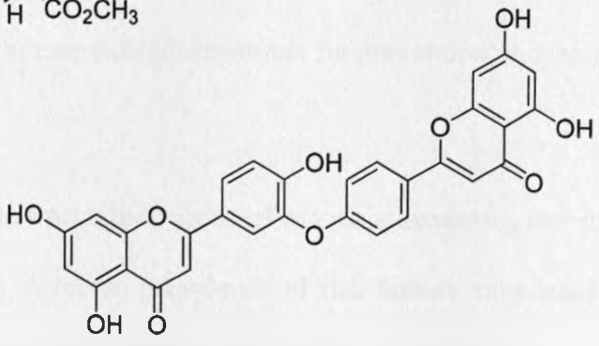
ochnaflavone (**11**) the the taxonomic marker of the genus, *Ochna* species exhibited a notable growth inhibitory effect against human colon cancer cell HCT-15 with IC_{50} value of 4.1 µM (Kang *et al.*, 2009). Among the estimated 200 *Millettia* and 85 *Ochna* species, the phytochemical and biological investigation is only limited to small percentage which has led to inadequate information on the chemistry of these plants and those compounds responsible for certain biological activity such as for cancer chemoprevention and other forms of diseases. The current study, was therefore, focused on investigation of *Millettia* and *Ochna* species for anticancer effect of the isolates, characterization of the constituents which might serve as hits in cancer drug development.



7: R = CH_3
8: R = CHO



	R^1	R^2
9	OH	OCH_3
10	OH	OH



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CHAPTER TWO

LITERATURE REVIEW

2.1 The Cancer Problem

The word cancer has originated from a Greek term, *carcinos*, for a crab to describe the physical similarity of malignant tumors that spread over the human body and was ascribed to Hippocrates (460-370 BC), who is considered as the father of medicine (Sudhakar, 2009). Cancer is generally characterized by uncontrolled growth and spread of abnormal cells which lack the ability to communicate with neighboring cells. Globally, tens of millions of people are diagnosed with cancer each year and ultimately more than half of the patients die from it. Furthermore, old people are most vulnerable to cancer and with continuous growth in aging population; cancer will remain a major health problem around the world (Ma and Yu, 2006).

Until recently, cancer was regarded as a disease of the Western and industrialized countries, however, more than half of the new cancer cases (56% of 12.7 millions) and deaths (63% of 7.6 millions) in the year 2008 were reported in developing countries (Ferlay *et al.*, 2010). This is partly due to limited or non-existent resources for prevention, diagnosis and treatment of cancer (WHO, 2008).

In Africa, as in the rest of the world, the burden of cancer is increasing due to growing of the aging population as well as increased prevalence of risk factors associated with economic transition. The overall population of Africa is projected to increase by about 50% between 2010 and 2030 (Jemal *et al.*, 2012); and approximately by 90% for those aged above 60 years, the age at which cancer most frequently arises. The risk of cancer will even be higher because of lifestyle changes associated with urbanization and economic development (Jemal

et al., 2012). Due to limited resources coupled with other imperative public health problems, including HIV/AIDS, malaria and tuberculosis; cancer has been under-recognized in Africa and consequently received low health priority, despite its growing burden (American Cancer Society, 2011a). In Kenya, it ranks third as a cause of death after infectious and cardiovascular diseases, each year accounting for 7% of the total national mortality (NGCMK, 2013). Breast, cervical and oesophagus cancers are the leading cases in women; whereas, prostate, oesophagus and Kaposi's sarcoma are the most common cancer cases in men in this country (NGCMK, 2013).

2.2 Causes of Cancer

The causes for most of the cancers are not clearly understood; however, some factors that increase individuals' risk of developing cancer are well known (AIHW and AACR, 2012). The risk factors responsible for the process of carcinogenesis include genetic factors in which its effect is only observed in a relatively small percentage (5-10%) of all human cancers while the remaining percentage (90-95%) is due to cancer causing lifestyle among others (Anand *et al.*, 2008). The risk of developing cancer is generally categorized as behavioral (smoking, drinking heavy alcohol, eating unhealthy foods, being overweight/obese and physical inactivity, certain infectious), environmental (UV radiation, secondhand smoke, pollution, pesticides), biological (gender, race, age and skin type) and hereditary (inheriting mutated genes from parents) (http://www.familycancercenter.com).

2.2.1 Tobacco Use

Smoking tobacco is highly linked with the development of certain types of cancer which can cause death (Kuper *et al.*, 2002). Tobacco smoke is a very complex matrix of over 7,000 chemical compounds (Table 2.1), of which some 70 are known for their carcinogenicity

(http://www.cdc.gov/tobacco/data_statistics/sgr/2010/consumer_booklet/chemicals_smoke/). It accounts for 25-30% of all cancer deaths, more than any other single cause of cancer (Anand *et al.*, 2008). In addition to lung cancer, which is mostly caused by tobacco use, it also amplifies the risks for cancers of the mouth, lips, nose and sinuses, larynx, pharynx, esophagus, stomach, pancreas, kidney, bladder, uterus, cervix, colon-rectum, ovary and acute myeloid leukemia (American Cancer Society, 2014).

Table 2.1: Some of the tobacco smoke chemicals with carcinogenic risks to humans

Classification	Carcinogen in the tobacco smoke
Group 1*	Arsenic, benzene, benzo[α]pyrene, cadmium Chromium (hexavalent), formaladehyde, nickel 4-(N-Nitrosoamino)-1-(3-pyridyl)-1-butanone (NNK) N-Nitrosononicotine (NNN)
Group 2A**	Lead
Group 2B ***	Acetaldehyde, acrylonitrile, isoprene, styrene

*carcinogenic to humans ** probably carcinogenic to humans *** possibly carcinogenic to humans according to IARC classification (http://www.hc-sc.gc.ca/hc-ps/pubs/tobac-tabac/carcinogens-cancerogenes/index-eng.php#t2)

2.2.2 Alcohol Use

Heavy alcohol consumption is regarded as one of the top ten risks of disease burden, including cancer. The International Agency for Research on Cancer (IARC) has established the carcinogenicity of ethanol in animals, and classified alcohol consumption as carcinogenic to humans (Testino *et al.*, 2012); it is associated to the risks of oral cavity, pharynx, larynx, oesophagus, colorectum, liver, pancreas and breast cancers. Heavy alcohol consumption accounts for 3.5% of all cancer deaths (Boffetta and Hashibe, 2006). The mechanisms of alcohol-mediated carcinogenesis have not been well established, even though possible body events including a genotoxic effect of acetaldehyde from oxidation of alcohol, increasing of oestrogen concentration which is important for breast carcinogenesis, generation of reactive

oxygen and nitrogen species and changes in folate metabolism take place; and are proposed to be causes of carcinogenesis (Boffetta and Hashibe, 2006). Alcohol blocks the release of folate from hepatocyte and consequently disrupts the folate supply to tissue and rapidly causing a defect in cell replication (Hwang *et al.*, 2012).

2.2.3 Dietary Factors and Obesity

Diet plays a big role in contributing to cancer risks, which appears to account for about 30% and 20% of cancer cases in Western and developing countries, respectively (Key *et al.*, 2004). Obesity which is both dietary and genetic based, is a growing health problem worldwide and is associated with cancers of the colon, rectum, breast, endometrium, kidney and oesophagus. It is also one of the risk factors causing cancer of the pancreas, liver, gall bladder, the gastric cardia and Non-Hodgkin Lymphomas (Warren and Devine, 2007; Vucenik *et al.*, 2012). The mechanism by which obesity drives cancer is complex and not well understood. However, it has been well-known that the most abundant hormones; leptin and adiponectin, produced by fat tissue are involved in carcinogenic process (Hursting and Dunlap, 2012).

2.2.4 Chronic Infections

Certain viral, bacterial, and parasitic chronic infections have also been considered as risk factors for several types of cancer in humans and account for about 15-20% of cancers worldwide; the higher share of this figure is in developing countries since certain infections are more prevalent in these countries (Fontham, 2009). *Aspergillus* is the only fungus known so far causing cancer in humans. This fungus is capable of producing aflatoxins, and is associated with increased risk of developing liver cancer, especially in people with hepatitis B (Campbell, 2007). Other infectious associated with cancer include the *Helicobacter pylori*

bacterium which is a risk factor to stomach cancer; the human papilloma viruses (HPV) for cervical cancer; the hepatitis B and C viruses (HBV and HCV) for hepatocellular cancer; Epstein-Barr virus-a risk factor for Burkitt's lymphoma and non-Hodgkin lymphoma (Parkin, 2006; Campbell, 2007). The human immunodeficiency virus (HIV) also increases incidences of several cancers through immunosuppression, which together with the human herpes virus for Nasopharynx cancer 8, accounts for about 0.9% of all cancers.

Other relatively less important causes of cancer include the schistosomes associated with bladder cancer, liver human T-cell, lymphotropic virus type I (0.03%) for acute T-cell leukaemia/lymphoma and the liver flukes (0.02%) for Bile duct (Parkin, 2006; Campbell, 2007). The mechanisms by which viruses cause a viral-associated cancer is through insertion of their viral DNA into host genome cell and promote carcinogenesis by damaging the host DNA. On the other hand, there are no specific ways by which bacteria and protozoa undergo DNA integration and alter the DNA of a host cell directly. However, many bacterial metabolic products are potentially mutagenic to cause changes in cellular DNA's (Campbell, 2007).

2.2.5 Hereditary Factors

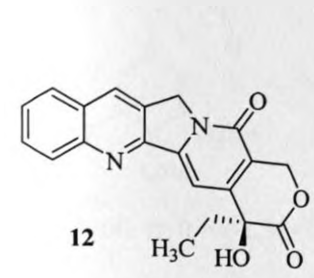
Cancer is not usually inherited, but certain cancers, especially the breast, ovarian, colorectal and prostate cancers may occur due to inherited faulty genes from the family. For example, two genes, BRCA1 and BRCA2 have been identified to increase the risk of breast and ovarian cancers by *ca.* 5 and 10%, respectively (Frank, 1998). Recently, new genes, including the rare mutations and high penetrant genes (TP53 and PTEN) and the frequent mutations and moderate penetrant (CHEK2, ATM and PALB2), have been identified as breast cancer susceptibility genes (Economopoulou *et al.*, 2015).

2.3 Natural Products in Cancer Treatment

Secondary metabolites from plants and other sources (including microbes and marine organisms) continue to play an important role in anticancer drug discovery (Pan *et al.*, 2010). There are several examples of such products of natural origin that are used as chemotherapeutic or chemopreventive agents. *Catharanthus roseus* G. Don. (Apocynaceae), a Madagascar periwinkle, is an important dicotyledonous medicinal plant used by various traditions for the treatment of diabetes; it was later noted that the plant also possesses many other therapeutic effects, including reduction of white blood cell counts, bone marrow depression in rats and activity against lymphocytic leukemia in mice. Subsequently, the vinca alkaloids, vinblastine (VLB, **7**) and vincristine (VCR, **8**), were isolated from this plant as the first anticancer agents in clinical use (Cragg and Newman, 2005; Prakash *et al.*, 2013). VLB (7) and VCR (**8**) are structurally related and displaying similar kind of action; inhibiting the cell division at metaphase; primarily targeting tubulin and microtubules (Aslam *et al.*, 2009). At high concentrations, these alkaloids depolymerize the microtubules and destroy mitotic spindles, leaving the dividing cancer cells with condensed chromosomes and blocked mitosis (Jordan and Wilson, 2004). These alkaloids are used for the treatment of a variety of cancers, including leukemia, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma in combination with other cancer chemotherapeutic drugs (Cragg and Newman, 2005)

Camptothecin (a modified monoterpene indole alkaloid, **12**) and paclitaxel (a diterpene alkaloid, **13**) found in the stem bark of *Camptotheca acuminata* (Nyssaceae), a native of China, and *Taxus brevifolia* found in the northwest region of the USA, respectively, are also examples of antitumor secondary metabolites. Camptothecin (**12**) was also found in other

unrelated plants families, including Icacinaceae, Rubiaceae, Apocynaceae and Gelsemiaceae (Lorence and Nessler, 2004). Taxol (paclitaxel) (**13**) was later found to be a fungal metabolite from *Taxomyces adreanae, Pestalotiopsis microspora*, *Tubercularia* sp. and *Phyllosticta citricarpa* (Demain and Vaishnav, 2011). Camptothecin (**12**) exclusively inhibits topoisomerase I, an enzyme involved in DNA duplication; while paclitaxel, which has been approved for breast and ovarian cancer, binds to a protein, tubulin, consequently inhibiting cell division (Wall and Wani, 1996). In spite of its promising anti-tumor activity, camptothecin exhibited severe and unpredictable toxicity; because of which its semisynthetic analogues irinotecan (**14**) and topotecan (**15**) were approved for the treatment of colorectal and ovarian cancer, respectively (Malonne and Atassi, 1997).



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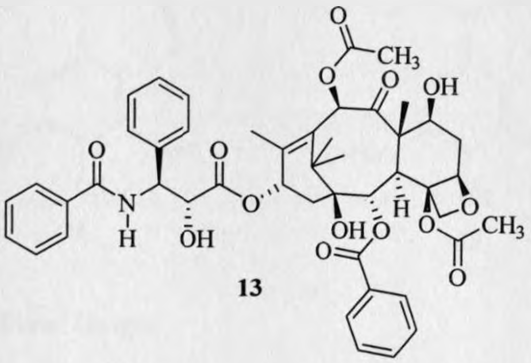

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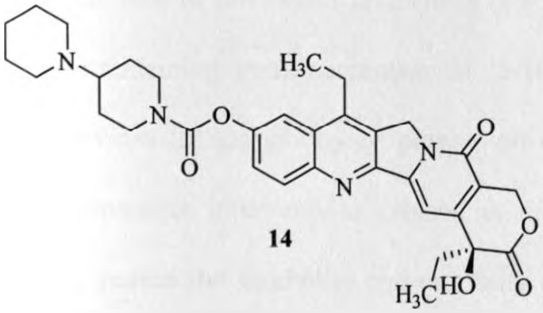

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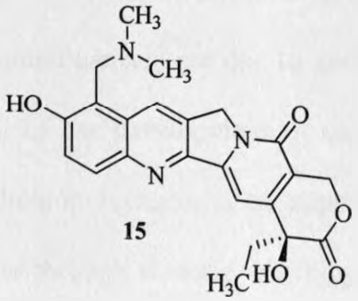

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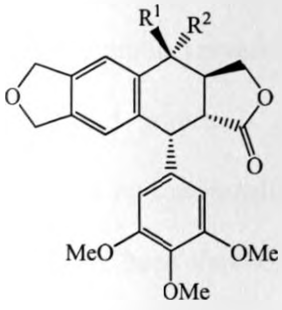
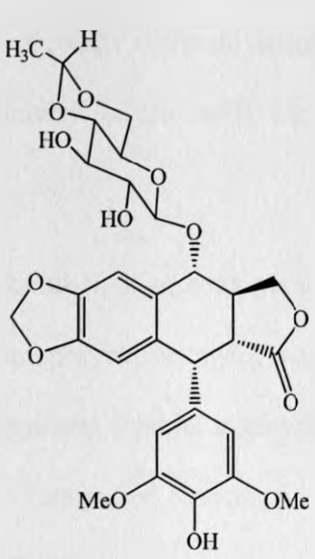
Podophyllotoxin (**16**), also called podofilox, obtained from *Podophyllum peltatum* (mayapple) and *Podophyllum hexandrum* is used as an inhibitor of the enzyme topoisomerase II in cancer treatment (Jordan and Wilson, 2004). Epipodophyllotoxin (**17**), an epimer of **16**,

has been isolated as an active antitumor agent from the roots of *Podophyllum* species, *Podophyllum peltatum* Linnaeus and *Podophyllum emodi* Wallich (Berberidaceae), and its two semi-synthetic derivatives, etoposide (**18**) and teniposide (**19**) are used in the treatment of lymphomas, bronchial and testicular cancers by targeting topoisomerase II (Björkholm, 1990; Cragg and Newman, 2005).



16: R^1= H, R^2= OH; **17**: R^1= OH, R^2= H



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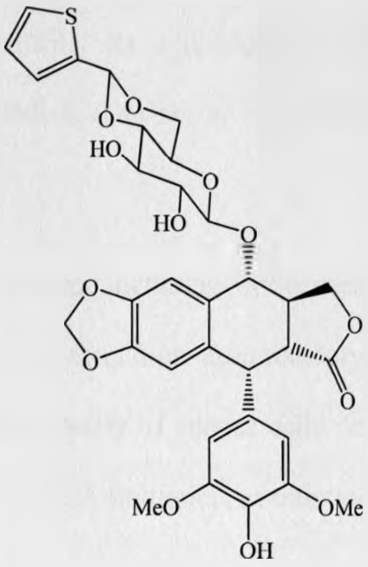

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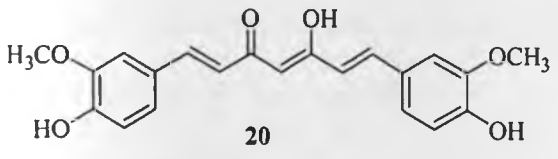
2.4 Cancer Chemopreventive Agents of Plant Origin

The fact that most of the cancer incidences (90-95%) are due to environmental and lifestyle factors (the remaining small percentage of 5-10% of all human cancers are due to genetic factors) provides the possibility of prevention or reduction of the development of cancer through appropriate interventions (Anand *et al.*, 2008). Chemoprevention is an approach designed to reduce the morbidity and mortality due to cancer through slowing, blocking, or reversing the process of carcinogenesis before malignancy by using natural or synthetic compounds (Johnson and Mukthar, 2007; Nobili *et al.*, 2009; Rahman *et al.*, 2010). It is considered as a promising approach for cancer control, and has become increasingly popular

in recent years. There are several natural products of plant origin that are known to be chemopreventive agents against commonly occurring cancers (Johnson and Mukthar, 2007; Nobili *et al.*, 2009). Some of the examples include curcumin (**20**) and reeveratrol (**21**).

Curcumin (**20**), a polyphenol and the most studied cancer chemopreventive agent; extracted from the rhizome of *Curcuma longa* L. (turmeric) causes suppression, retardation or inversion of carcinogenic process through different mechanisms. Its antioxidant, anti-tumoural and anti-inflammatory activity is also well established (Duvoix *et al.*, 2005; Johnson and Mukthar, 2007).

The polyphenol, resveratrol (**21**), found in numerous plant species, including mulberries, peanuts and grapes is also a chemopreventive agent with a potent anti-inflammatory, antioxidant and antiproliferative agent that inhibits a growth of variety of cancer cells. Its activity has been demonstrated at all stages of carcinogenic process (initiation, promotion and progression) (Athar *et al.*, 2007; 2009).



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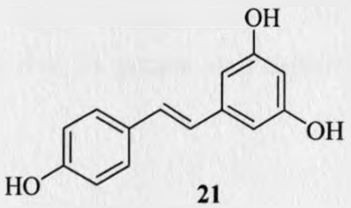

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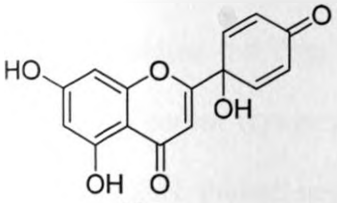
2.4.1 Flavonoids in Cancer Chemoprevention

Another group of compounds with potential anticancer activities are flavonoids found in vegetables, fruits, plant extracts, and herbs, having the ability to interfere with the initiation, promotion and progression of cancer. Protoapigenone (**22**), a flavonoid isolated from *Thelypteris torresiana* (Gaud) exhibited a significant cytotoxic effect on various gynecological cancer cells, including ovarian, breast and cervical cancer cells, with the

highest cytotoxicity being on ovarian cancer cells, with IC_{50} values of 0.69 ± 0.92 μM for MDAH-2774 and 0.78 ± 0.28 μM for SKOV3 of human ovarian cancer cells through cell arrest at S and G2/M (Chang *et al.*, 2008). Many of the pharmacological properties of flavonoids, including anticancer activities, are linked to their functions as antioxidants, free-radical scavengers, hydrogen-donating ability and metal chelating properties (Nijveldt *et al.*, 2001). The anticarcinogenic effect of flavonoids is considerably infuenced by their chemical structures, including the position/number of the phenolic/methoxyl functionality and the ring C saturation/oxidation levels (Harborne and Williams, 2000).

In line with this, the antiproliferative effects of methoxylated and hydroxylated flavones against SCC-9 human oral squamous carcinoma cells were established. The 5,7,4′-trimethoxylflavone (**23**) was found to be about eight times more potent (IC_{50} value of 5 μM) than the unmethylated flavone, 5,7,4′-trihydroxyflavone (apigenin, **24**). A similar trend of activity was observed again between the 5,7-dimethoxylflavone (**25**) and its unmethylated analog, 5,7-dihydroxyflavone (chrysin, **26**). The greater potency of the two methoxylated versus the two hydroxylated flavones could possibly be due to greater cell uptake of the methoxylated flavones (Walle *et al.*, 2007).



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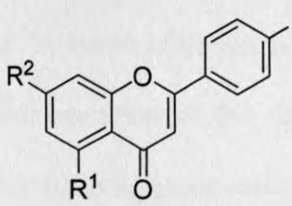
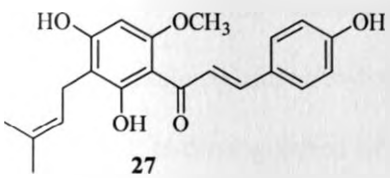


23: $R^1 = R^2 = R^3 = OCH^3$
24: $R^1 = R^2 = R^3 = OH$
25: $R^1 = R^2 = OCH_3$; $R^3 = H$
26: $R^1 = R^2 = OH$, $R^3 = H$

The antiproliferative activity of two chalcones, xanthohumol (**27**), dehydrocycloxanthohumol (**28**), and the flavanone, isoxanthohumol (**29**) from, *Humulus lupulus* was also studied. They showed a dose-dependent (0.1 to 100 μM) decrease in growth of human breast cancer (MCF-

7), colon cancer (HT-29) and ovarian cancer (A-2780) cells *in vitro*, with **27** being the most effective antiproliferative agent. Importantly, the chalcone xanthohumol (**27**) was highly antiproliferative against A-2780 cells, with IC_{50} values of 0.52 and 5.2 μM after 2 and 4 days, respectively (Miranda *et al.*, 1999).



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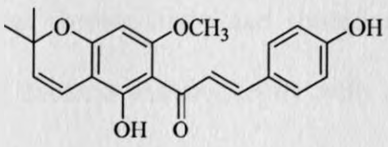

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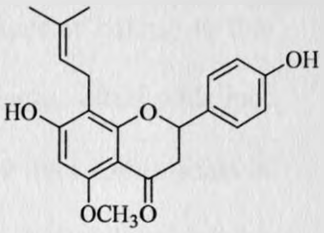

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2.5 The Family Leguminosae

The Leguminosae, also called the Legume family is the third largest family of angiosperms/flowering plants, exceeded only by Asteraceae (Compositae) and Orchidaceae families. It comprises over 19,000 species classified in more than 700 genera and widely distributed throughout the temperate and tropical regions of the world (Wojciechowski *et al.*, 2004; de la Estralla, 2010). Plants of this family are usually trees, shrubs, woody or perennial or annual herbs with the roots producing tinny nodules containing bacteria for nitrogen fixation (Gaur, 1993). It is subdivided into three subfamilies distinguished as Mimosoideae, Caesalpinioideae and Papilionoideae. In terms of economic importance, Legumes are only second to Poaceae (grasses), and some members of this family are cultivated as crops, for oils, fiber, fuel, timber, tannins, gums, fodders, green manures and forages (Wojciechowski *et al.*, 2004; Zarnowaski *et al.*, 2001; http://www.ildis.org/Leguminosae/). *Copaifera* (Leguminoseae) species are also known to yield valuable resins, used in varnishes, paints and lacquers and *Indigofera* speies are cultivated for the blue dye. More importantly, several

species in the genera *Lonchocarpus* and *Derris* are known as sources of rotenone, which is used as an insecticide, fish poison or molluscicide (http://www.ildis.org/Leguminosae/).

2.5.1 The Subfamily Papilionoideae

The Papilionoideae is the largest and most widespread of the three Legume subfamilies in which more than 50% (*ca.* 476 genera and 13,860 species) of the species belong to this subfamily. It includes mostly trees, shrubs, herbs and sometimes lianas, often with root nodules containing nitrogen-fixing bacteria and frequently with non-protein amino-acids in the seeds. It is distinguished from the other two subfamilies by vegetative, floral and fruiting nature including floral development (Wojciechowski *et al.*, 2004). Papilionoideae species are extremely important and its members yield nutritious food, fiber, shelter, valuable medicines and also potent poisons (Perveen and Qaiser, 1998; Wojciechowski *et al.*, 2004; de la Estralla *et al.*, 2010; Sharma and Kumar, 2013). The genus *Millettia* belongs to this subfamily.

2.5.2 The Genus *Millettia*

The genus *Millettia* (family Leguminoseae, subfamily Papilionoideae) contains over 200 species that are distributed in tropical and subtropical regions of Africa (139 species), Asia and Australia (Geesink, 1981; Banzouzi *et al.*, 2008). It is a tree, shrub or liana, or rarely semi-herbaceous plants with a woody rootstock. In Kenya, this genus is represented by six species namely *Millettia dura, M. lasiantha, M. leucantha, M. oblata* ssp. *teitensis, M. tanaensis* and *M. usaramensis* ssp. usaramensis, distributed in different regions of the country (Beentje, 1994).

2.5.2.1 Millettia oblata subspecies teitensis (Dunn)

M. oblata Dunn ssp. *teitensis*, is a vulnerable species (Beentje, 1994) due to illegal exploitation of its wood (WCMC, 1998). It is a tree growing up to 20 m with brown and corrugated bark. The leaves usually possess 9-21 leaflets with the lowermost being smallest. The stipels usually extend to 5 mm and the leaflets being elliptic-ovate or slightly obovate having a 4-11 by 1.5-3 cm dimensions. Flowers are purple blue in color, petals 1.5-2 cm long and fruit being oblong with downwards curved tip up to 10 by 2 cm, glabrescent (Beentje, 1994).

Figure 2.1: Picture of *Millettia oblata* ssp. *teitensis* taken by P. C. Mutiso (July, 2014)

2.5.2.2 Millettia dura (Dunn)

M. dura Dunn is also widely distributed in tropical and subtropical regions. This plant is used as a food to livestock, as a source of firewood and charcoal. The wood is tough and resistant to termites, and consequently used for a timber (Orwa *et al.*, 2009). It is a tree or shrub of light grey and scaly bark. The leaves usually carry 15-19 leaflets which are elliptic or ovate, 3-9 by 1.5-3 cm, pubescent beneath; and the stipels 1mm narrow. Flowers are blue or purplish, petals are *ca* 20-28 mm long and fruit are oblong, 14-20 cm with *ca* 2 cm glabrescent (Beentje, 1994).

Figure 2.2. Picture of *Millettia dura* taken by Tsegaye (April; 2014)

2.5.2.3 ***Millettia usaramensis subspecies usaramensis* (Taub)**

M. usaramensis Taub is a shrub or tree growing up to 10 m whose bark is grey and leaves with 7-17 leaflets which are elliptical shape (lowermost) to broadly ovate with dimension 2-7 x 1.5-3.5 cm and glabrous or nearly so. Flowers are mauve or purple-blue, petals elongate up to 11-14 cm and fruits are oblong to 11 by 1 cm that are pubscent, dehiscent and spiralling when open (Beentje, 1994). Two subspecies are known; the subspecies *usrasamensis* found in Kenya and Tanzania while the other subspecies, *australis*, occurs in Zimbabwe, Malawi and Mozambique (Yenesew *et al.*, 1998).

Figure 2.3. Picture of *Millettia usaramensis* (http://www.mozambiqueflora.com)

2.5.3 Traditional Uses of the Genus *Millettia*

The genus *Millettia* has been used in traditional medicinal practices in alleviating several aliments, with about 60% of the species are known for their medicinal uses for the treatment of different conditions (Banzouzi *et al.*, 2008). The insecticidal, piscicidal activity and fish poisoning effects of the seeds of *Millettia* species are among the old practices. This genus is also used as agents for killing of worms and snails, and for a cure against intestinal parasites as well (Singhal *et al.*, 1982; Ngamga *et al.*, 1993).

In Chinese folk medicine, for example, *Millettia nitida* var. *hirsutissima* and *M. speciosa* are used to treat menstrual conditions (pain and irregularity), rheumatic pain, aching pain, and paralysis (Cheng *et al.*, 2005; Yin *et al.*, 2010). It was also reported that *M. nitida* has been used for the treatments of blood stasis condition such as anemia, inflammation of peripheral blood vessels and thrombotic changes in vessels through promotion of the blood circulation (Liao *et al.*, 2013). The roots and leaves of *Millettia leptobotrya* Dunn have been used for the treatment of fracture, traumatic injury and rheumatoid arthritis by Chinese (Na *et al.*, 2013).

M. pachycarpa is widely used as an antihelminthic, a medication capable of expelling parasitic intestinal worms (Ye *et al.*, 2012). There is also evidence that a concoction of the root and stem bark of *M. griffoniana* Baill. is employed in Cameroon as an oral treatment for boils (also called furuncle), insects bits, inflammatory conditions (such as pneumonia and asthma), amenorrhea (an abnormal absence of menstruation), sterility and menopausal disorders (Yankep *et al.*, 2003; Wanda *et al.*, 2006). In Thai folkloric medicine, the stem and leaves of *M. caerulea* (Graham) Baker are applied to wounds to reduce further infection (Perez, 2014), while *M. erythrocalyx* has been used for treating stomach pain (Sritularak *et al.*, 2002). In West Africa, *M. thonningii* has been used in traditional medical practice in

treating several ailments; the bark infusion is used to treat constipation in children while in Nigeria the leaf extracts of this plant cures diarrheal symptoms and dysentery (Harrison *et al.*, 2011).

The juice of the leaves of *M. thonningii* is also reported to be a poison to *Bulinus* snail, vector for schistosomiasis (Asomaning, 1995). According to Kokwaro (1993), the root of *M. usaramensis* is reported to be used as antidote against snake bite. Furthermore, *M. oblata*, which is found in Kenya and Tanzania, is used to treat stomachaches, and as a remedy for cough, swollen part of the body and bladder problems (Banzouzi *et al.*, 2008). *M. lasiantha* is used as an aphrodisiac by either chewing the roots or drinking aqueous decoction. Gurgling the roots extracts of *M. makodensis* Harms gives relief from toothache (Harrison *et al.*, 2011). *M. dura* Dunn is also well known in Africa pharmacopeia for being used to treat hemias, diarrheas, menstruation complications and for healing wounds (Banzouzi *et al.*, 2008).

There is also a report regarding the medicinal properties of *M. aboensis* that its leaf is used for ulcer healing and as laxatives, whereas the roots are used to treat gastro-intestinal disturbances and liver diseases (Ugwueze *et al.*, 2013). It is also crucial to note that the root of *M. pulchra* is used for postpartum women and people with certain health conditions, apparently to stock up blood to treat postpartum frail and malnutrition whereas its aerial parts is used to eliminate inflammation, alleviate pain, increase blood circulation, and relax and activate tendons in rheumatic arthralgia (Wang *et al.*, 2015).

2.6 The Ochnaceae Family

The family Ochnaceae is a flowering plant represented by about 500 species and over 27 genera, recently divided into three subfamilies [Ochnoideae (Burnett), Quiinoideae (Luerss), and Medusagynoideae (Reveal)]. It is distributed in the tropical and subtropical regions of Africa, Asia and America (Schneider *et al.*, 2014). The Ochnaceae is most represented in the neotropics regions, while the tropical Africa is the second center of diversity. Members of the family are mostly trees or shrubs with few being herbaceous plants. All members of the family are evergreen and the leaves are mostly alternate and generally simple parallel lateral veins (Schneider *et al.*, 2014; http://www.britannica.com/plant/Ochnaceae).

2.6.1 The Genus *Ochna*

The genus *Ochna* L. (Greek, Ochne: wild pear) belongs to Ochnaceae family and comprises *ca.* 85 species of evergreen trees, shrubs and shrublets in Africa, Asia and Madagascar, while some species are also found in tropical and subtropical zones of the world. Members of this genus are usually called *Ochnas* or Mickey Mouse plants, a name originating from the shape of their drupelets fruit. Some of the species, especially *Ochna serullata*, *O. kirkii*, *O. mossambicensis*, *O. schweinfurthiana* and *O. thomasiana* are widely cultivated for decorative purposes due to their colorful and attracting flowers (Riley, 1963; Cullen, 1997; Pegnyemb *et al*, 2003; Bandi *et al.*, 2012). In Kenya, this genus is represented by *O. holstii*, *O. holtzii*, *O. thomasiana*, *O. mossambicensis*, *O. apetala*, *O. insculpta*, *O. ineremis*, *O. kirkii* and *O. ovata* (Beentje, 1994).

Figure 2.4. Ochna - the Mickey Mouse plant.
(https://en.wikipedia.org/wiki/Ochna#/media/File:Mickey_Mouse_bush.jpg)

2.6.2 Traditional Uses of the Genus *Ochna*

The genus *Ochna* has a long history of use as herbal remedies in Asia and Africa for cure of different aliments. Some representative examples are presented in Table 2.2.

Table 2.2: Traditional uses of some *Ochna* species

***Ochna* species**	**Traditional uses**	**References**
O. afzelii	treatment of jaundice, toothache, female sterility, menstrual complaints, lumbago, dysentery	(Pegnyemb *et al.*, 2001)
O. integerrima	digestive tonic, antihelmentic, antidsyenteric, antipyretic properties	(Perry, 1980)
O. lanceoleta	used as abortifacient, treating gastric complaints, menstrual disorder	(Muthukumarasamy *et al.*, 2003)
O. obtusata	treatment for ulcer, asthma, bronchitis	(Karimulla and Kumar, 2012)
O. pumbila	antidote to snake bites, menstrual complaints, asthma	(Kamil, *et al.*, 1987)
O. shweinfurthina	antimalarial, antihelmentic, dressing wounds, treatment of measles, typhoid fever and skin infections	(Abdullahi *et al.*, 2010)
O. squarrosa	digestive tonic, curative effect against asthma, ulcer, lumbago, menstrual complaints	(Reddy *et al.*, 1983)
O. serrulata	treatment of osseous illnesses and hemorrhoids	(Colla *et al.*, 2011)

2.7 Compounds of the Leguminosae

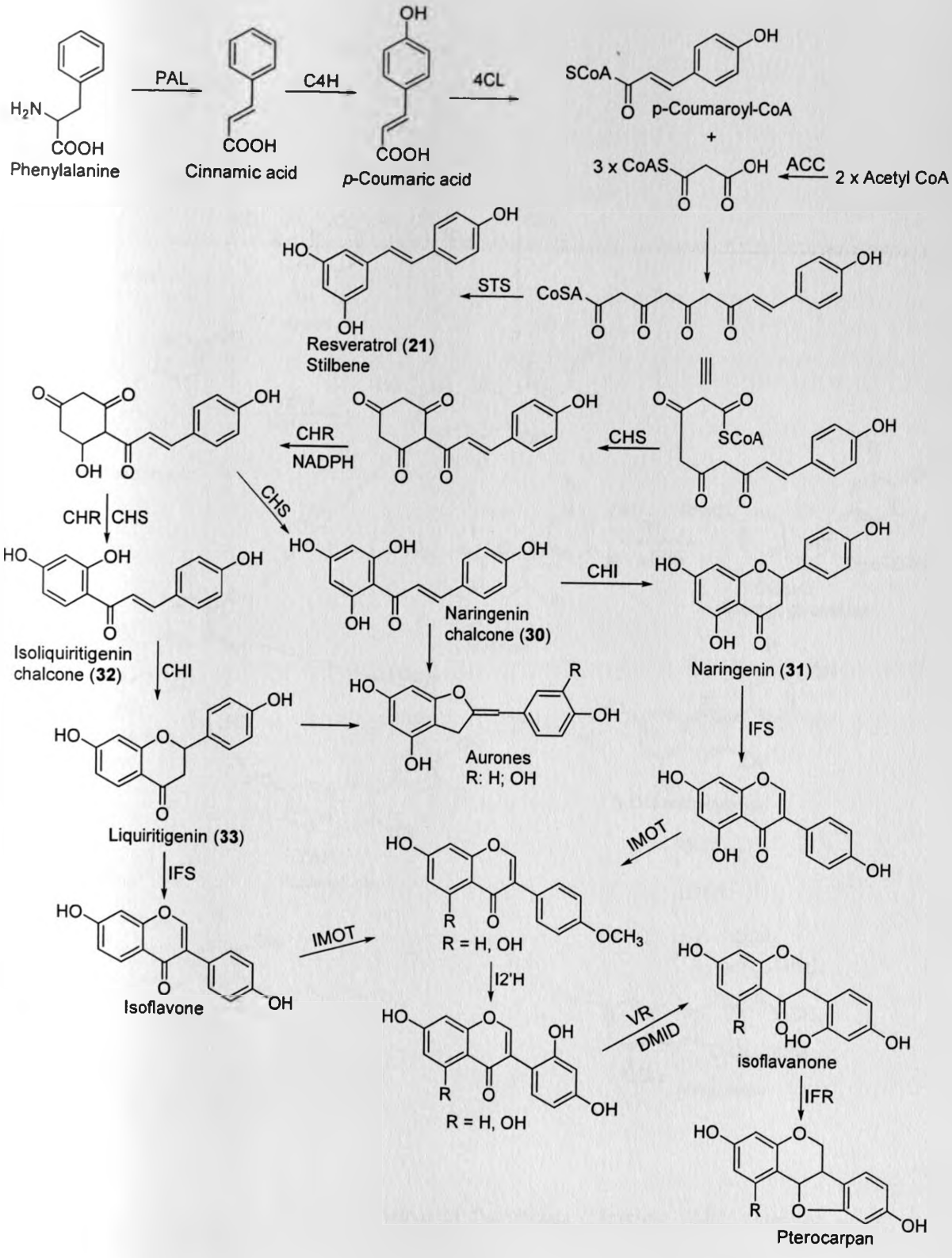
The isoflavonoids, a subclass of flavonoid, are more or less unique to the subfamily Papilionoideae of the Leguminosae family (*ca.* 90%) (Yazaki *et al.*, 2009). Rotenoids, an isoflavonoid subclass, are also typically found in several members of Leguminosae namely; *Derris*, *Loncocarpus*, *Millettia*, *Tephrosia and Mundulea* species. Alkaloids have also been recorded in some plants of the Leguminosae such as in *Erythrina*, *Genista* and *Virgilia* (Greinwald *et al.*, 1989; García-Mateos *et al.*, 2005; Kacem *et al.*, 2014).

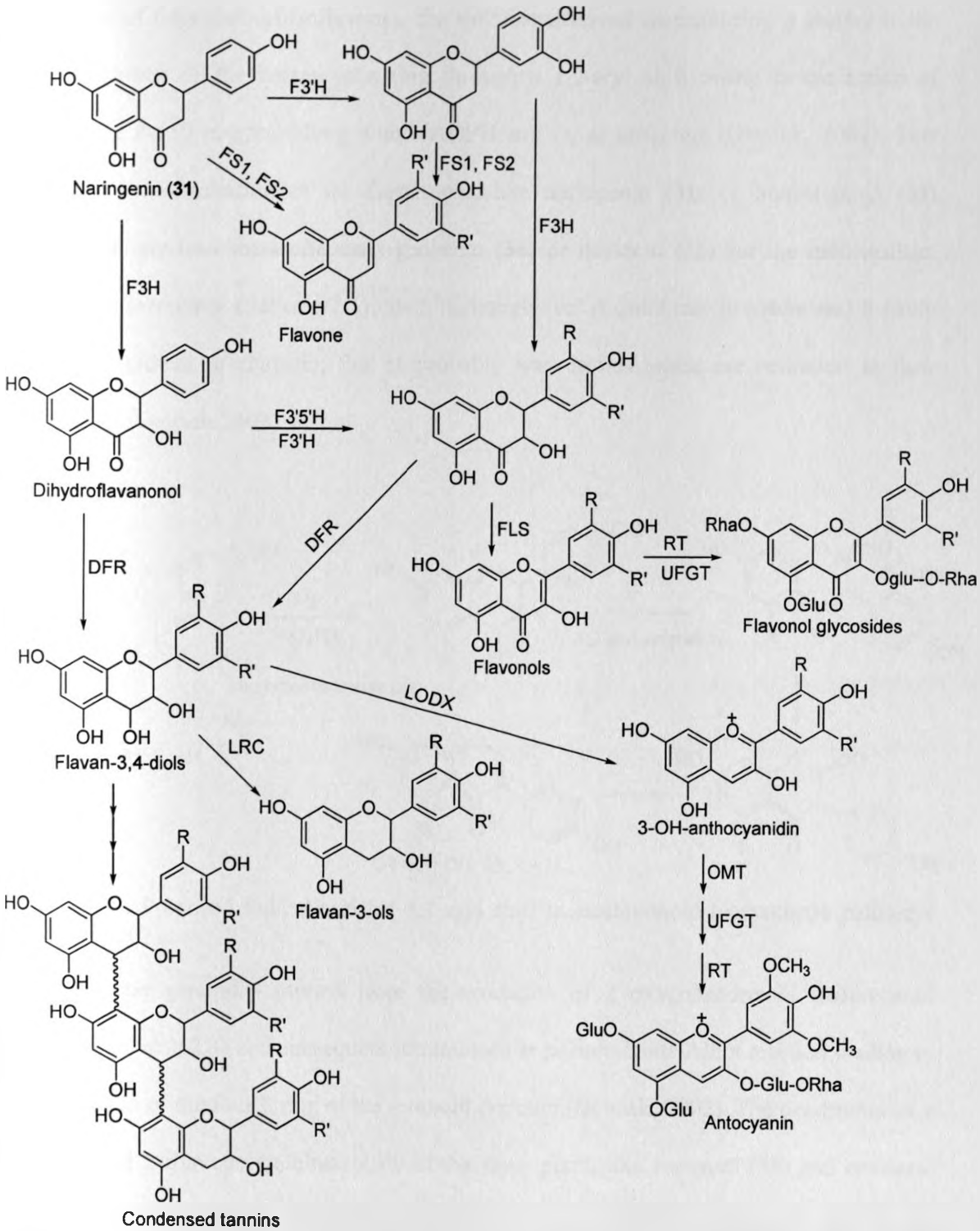
2.7.1 Biosynthesis of Flavonoids

Biosynthetically, flavonoids are an outcome of the combination of the phenylpropanoid and polyketide pathways which generally involves condensation, isomerization, oxidation and reduction reactions (Saito *et al.*, 2013). The *p*-coumaroyl-CoA is offered by phenylpropanoid pathway which in turn is initiated from the aromatic amino acids (phenylalanine and tyrosine) through shikimate pathway. The polyketide pathway is in charge of the two carbon atoms, chain enlongation by consuming malonyl-CoA units (Saito *et al.*, 2013).

The polyketide is true backbone of flavonoids and stilbenes which is generated from a C_6C_3, cinnamoyl-CoA starter unit and three molecules of malonyl-CoA (chain extender) (Scheme 2.1). Based on the types of the enzyme involved at this stage (stilbene synthase or chalcone synthase), the polyketide can be folded in two ways to give stilbenes [resveratrol (**21**)] or chalcones [naringenin chalcone (**30**)] through an aldol or Claisen-type reaction, respectively (Dewick, 2002). As this stage is key to all flavanoids biosynthesis, the formed chalcones subsequently serve as precursors for the formation of a series of flavonoid derivatives. A stereospecific six-membered heterocyclic ring, the flavanone [(naringenin, **31**), Schem 2.1] is formed by Michael-type nucleophilic attack of a phenol group on the unsaturated ketone

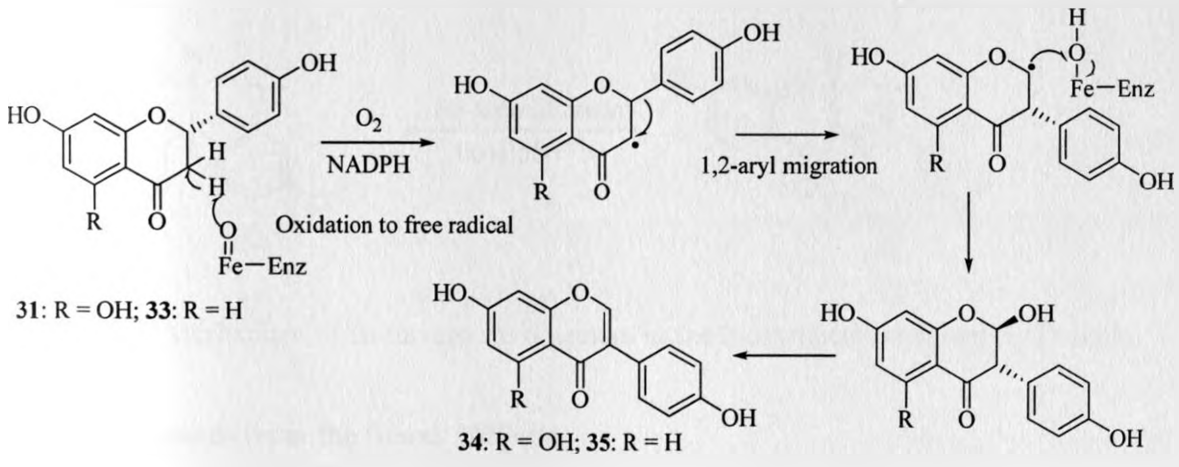
system as it appears in most of the flavanoid structure. One of the hydroxyl groups in many flavonoid structures, (in liquiritigenin, **33**), formed from isoliquiritigenin chalcone (**32**), is lost owing to the action of the reductase enzyme (CHR) assisted by the chalcone synthase (CHS) causing isoliquiritigenin to be formed rather than the naringenin-chalcone (**30**), containing all the hydroxyl groups. Attributable to different oxidation and substitution on the flavanone basic skeleton (naringenin, **31**), many structural variants including flavones, flavonols, anthocyanidins, and catechins can be formed (Dewick, 2002).


Phenylalanine
PAL
Cinnamic acid
C4H
p-Coumaric acid
4CL
p-Coumaroyl-CoA
+
3 x CoAS
ACC
2 x Acetyl CoA
STS
Resveratrol (21)
Stilbene
CoSA
CHR
NADPH
CHS
CHR CHS
CHS
Isoliquiritigenin chalcone (32)
CHI
Naringenin chalcone (30)
CHI
Naringenin (31)
IFS
Liquiritigenin (33)
Aurones
R: H; OH
IMOT
IFS
IMOT
R = H, OH
I2'H
R = H, OH
Isoflavone
VR
DMID
isoflavanone
IFR
Pterocarpan



Scheme 2.1. General biosynthetic pathways of flavonoids (Dewick, 2002; Harrison *et al.*, 2011; Saito *et al.*, 2013).

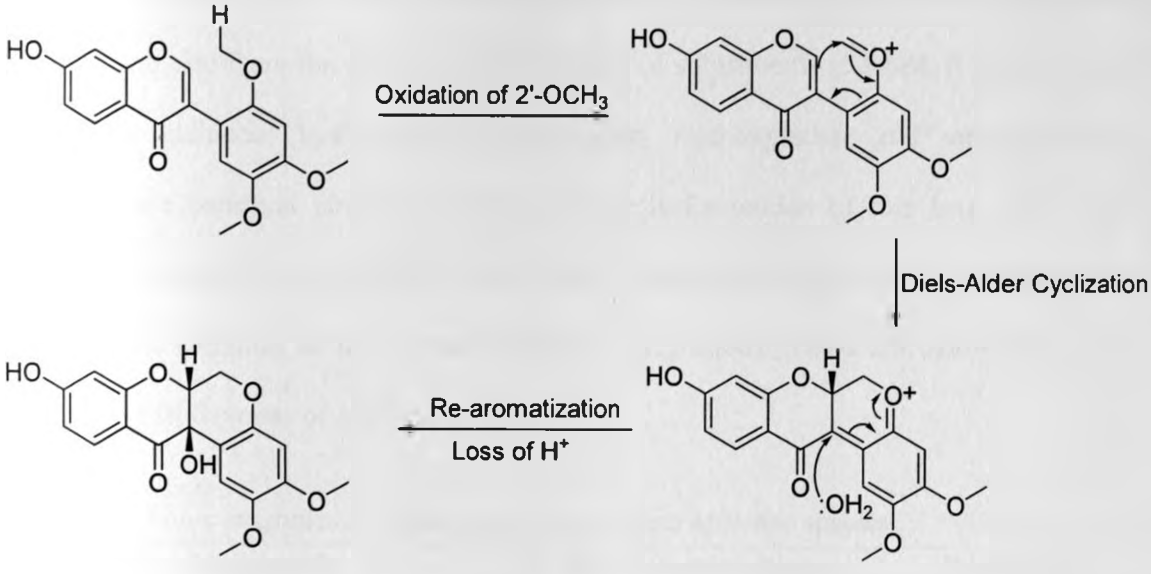
In the case of formation of isoflavones, the shikimate-derived aromatic ring is shifted to the adjacent carbon of the heterocyclic ring through a 1,2-aryl shift owing to the action of cytochrome P-450 enzyme along with NADPH and O_2 as cofactors (Dewick, 2002). This brings the transformation of the flavanones, like naringenin (**31**) or liquiritigenin (**33**) correspondingly into the isoflavones genistein (**34**) or daidzein (**35**) *via* the intermediate, hydroxyisoflavanones (Scheme 2.1). Such rearrangement is quite rare in nature and it likely involves a radical mechanism; that is probably why isoflavonoids are restricted in their distribution (Dewick 2002).



Scheme 2.2. Proposed radical assisted 1,2-aryl shift in isoflavonoid biosynthetic pathways (Dewick, 2002).

Rotenoids are generally formed from the oxidation of 2'-oxygenations in isoflavonoid skeleton (Scheme 2.3) and subsequent intramolecular *pseudo*-Diels-Alder reaction leading to the formation of the fourth ring of the rotenoid skeleton (Dewick, 2002). The occurrence of a rotenoid and isoflavone simultaneously in the same plant, like toxicarol (**36**) and toxicarol isoflavone (**37**) in *Millettia brandisiana* (Pancharoen *et al.*, 2008) or dolineone (**38**) and the isoflavanone, neotenone (**39**) (the corresponding isoflavone also occurs), in the roots of *Neorautanenia pseudopachyrrhiza* (Crombie and Whiting, 1963) and in many others

suggests the biosynthetic connection of rotenoids and isoflavones. In addition, the low frequency of occurrence of free 2'-oxygenated natural isoflavones as compared with that of the flavones also supports the fact that isoflavones are building blocks of rotenoids (Crombie and Thomas, 1967).



Scheme 2.3. Mechanism of isoflavone involvement in the biosynthesis of rotenoid (Dewick, 2002).

2.8 Compounds from the Genus *Millettia*

The genus *Millettia* is known to be a rich source of flavonoids. The occurrence of rare secondary metabolites including coumarins (Asomaning *et al.*, 1999; Rajemiarimirahoa *et al.*, 2013), alkaloids (Kamnaing *et al.*, 1994; Ngamga *et al.*, 2007) and terpenoids (Ongoka *et al.*, 2008; Kamto *et al.*, 2012) has been also reported in some *Millettia* species.

2.8.1 Isoflavonoids

Several isoflavonoids have been reported from different *Millettia* species (Table 2.3). The majority of these isoflavonoids contain one or two prenyl group(s) as a side chain, either in acyclic or in cyclic forms, or as a combination of the two forms (Sritularak *et al.*, 2002). Structural diversity occurs as the result of different oxidation levels of the isoflavonoid core skeleton, and also from the number and complexity of substituents attached. It has also been noted that additional hydroxylation, geranylation, methoxylation, and methylenedioxy formation are common structural features of the isoflavonoids of this taxa, which has eventually enhances the structural diversification. Among the isoflavonoids, the isoflavones are the most common in this genus (Máximo *et al.*, 2000). Table 2.3 below lists some examples of isoflavones of *Millettia* species.

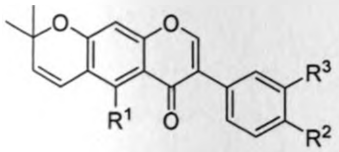
Table 2.3: Some examples of isoflavones isolated from *Millettia* species

Compounds	Plants species (parts)	References
Alpinumisoflavone (**40**)	*M. thonningii* (root bark, seeds, seed pods)	Olivares *et al.*, 1982; Asomaning *et al.*, 1999
O,*O*-Dimethylalpinumisoflavone (**41**)	*M. thonningii* (seeds, root bark, seed pods)	Olivares *et al.*, 1982; Asomaning *et al.*, 1999
4'-Methylalpinumisoflauone (**42**)	*M. thonningii* (seeds, root bark, seed pods)	Olivares *et al.*, 1982; Asomaning *et al.*, 1995; 1999
3', 5-Dihydroxy-4'-methoxyl-2", 2"-dimethylpyrano-(5", 6": 6, 7)isoflauone (**43**)	*M. thonningii* (seeds, root bark, seed pods)	Olivares *et al.*, 1982; Asomaning *et al.*, 1995; 1999
Robustone (**44**)	*M. thonningii* (root bark, seed pods root wood)	Asomaning *et al.*, 1995; 1999
5-*O*-Methyl-4'-*O*-(3-methyl-2-butenyl) alpinumisoflavone (**45**)	*M. thonningii* (root bark, root wood)	Asomaning *et al.*, 1995; 1999
5-*O*-Methylalpinumisofavone (**46**)	*M. thonningii* (root wood)	Asomaning *et al.*, 1999
Thonninginisoflavone (**47**)	*M. thonningii* (root bark)	Asomaning *et al.*, 1995

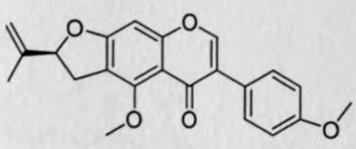
Compounds	Plants species (parts)	References
7-*O*-Geranylpseudobaptigenin (**48**)	*M. grifoniana* (root bark)	Yankep *et al.*, 1997
7-*O*-Geranylformononetin (**49**)	*M. grifoniana* (root bark)	Yankep *et al.*, 1997
Calopogonium isoflavone B (**50**)	*M. grifoniana* (root bark)	Yankep *et al.*, 1997
7,2'-Dimethoxy-4'5'-methylenedioxyisoflavone (**51**)	*Millettia grifoniana* (root bark), *Millettia dura*(stem bark)	Yankep *et al.*, 1997 (Dagne *et al.*, 1991)
2',4',5'-Trimethoxy-2",2"-dimethylpyrano[5",6":6,7]isoflavone (**52**)	*M. ichthyochtona* (leaves)	Kamperdick *et al.*, 1998
Aurmillone(**53**)	*M. auriculata* (seeds)	Acharya *et al.*, 1986
Odorantin (**54**)	*M. grifoniana* (root bark)	Yankep *et al.*, 1997
Barbigerone (**55**)	*M. Pachycarpa* (seeds)	Ye *et al.*, 2012
	M. usaramensis ssp. *usaramensis* (stem bark)	Yenesew *et al.*, 2003
4'5'-Dimethoxy-6",6"-dimethylpyranoisoflavone (**56**)	*M. Pachycarpa* (seeds)	Ye *et al.*, 2012
5-Hydroxy-3',4'-dimethoxy- 2",2"-dimethylpyrano[5",6":6,7]isoflavone (**57**)	*M. Pachycarpa* (seeds)	Ye *et al.*, 2012
5,4'-Dihydroxy-3'- methoxy- 2",2"-dimethylpyrano[5",6":6,7]isoflavone (**58**)	*M. Pachycarpa* (seeds)	Ye *et al.*, 2012
Millewanin G (**59**)	*M. pachycarpa* (leaves)	Ito *et al.*, 2006
Millewanin H (**60**)	*M. pachycarpa* (leaves)	Ito *et al.*, 2006
Furowanin B (**61**)	*M. pachycarpa* (leaves)	Ito *et al.*, 2006
Isoauriculatin (**62**)	*M. auriculata* (roots)	Shabbw and Zaman, 1970
Auriculin (**63**)	*M. auriculata* (roots)	Shabbw and Zaman, 1970
Isoaurmillone (**64**)	*M. auriculata* (seed pods)	Gupta *et al.*, 1983
2'-Deoxgisoaunculutin (**65**)	*M. auriculata* (roots)	Rao *et al.*,1992
2'-*O*-Methytisoauriculatln (**66**)	*M. auriculata* (roots)	Rao *et al.*, 1992
Scandenone (**67**)	*M. auriculata* (roots)	Rao *et al.*, 1992
Auricularin (**68**)	*M. auriculata* (roots)	Rao *et al.*, 1992
Auriculasin (**69**)	*M. auriculata* (leaves)	Minhaj *et al.*, 1976; Ito *et al.*, 2004
Isoauriculasin (**70**)	*M. auriculata* (leaves)	Minhaj *et al.*, 1976
Hirsutissimiside A (**71**)	*M. nitida* var. *hirsutissima* (stem twigs)	Cheng *et al.*, 2005

Compounds	Plants species (parts)	References
Formononetin 7-*O*-β -D-apiofuranosyl-(1→6)-β -D-glucopyranoside (**72**)	*M. nitida* var. *hirsutissima* (stem twigs)	Cheng *et al.*, 2005
Hirsutissimiside B (**73**)	*M. nitida* var. *hirsutissima* (stem twigs)	Cheng *et al.*, 2005
Hirsutissimiside C (**74**)	*M. nitida* var. *hirsutissima* (stem twigs)	Cheng *et al.*, 2005
Conrauinone A (**75**)	*M. conraui* (stem bark)	Fuendjiep, 1998
5-Methoxyldurmillone (**76**)	*M. conraui* (stem bark)	Fuendjiep, 1998; Tchinda *et al*, 2007
Conrauinone B (**77**)	*M. conraui* (stem bark)	Fuendjiep, 1998
Brandisianin A (**78**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Brandisianin B(**79**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Brandisianin C (**80**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Brandisianin D (**81**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Brandisianin E (**82**)	*M. brandisiana* (leaves)	Kikuchi et al., 2007
4'-Demethyltoxicarolisoflavone (**83**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Viridiflorin (**84**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007; Pancharoen *et al.*, 2008
Olibergin (**85**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
Ferugone (**86**)	*M. ferruginea* ssp. *darassana* (root bark)	Dagne *et al.*, 1990
7-*O*-Geranyl-6-methoxypseudobaptigenin (**87**)	*M. conraui* (stem bark)	Tchinda *et al*, 2007
Millesianin F (**88**)	*M. dielsiana* (stem twigs)	Gong *et al.*, 2014
Millesianin G (**89**)	*M. dielsiana* (stem twigs)	Gong *et al.*, 2014
Durallone (**90**)	*M. dura* (seed pods)	Yenesew *et al.*, 1996
Durmillone (**91**)	*M. dura* (stem bark)	Yenesew *et al.*, 1996
	M. grifoniana (RB)	Yankep *et al.*, 1997
6-Demethyldurallone (**92**)	*M. dura* (seed pods)	Yenesew *et al.*, 1996
Perdurallone (**93**)	*M. dura* (seed pods)	Yenesew *et al.*, 1996
Isoerythrin-A-4'-[3-methylbut-2-enyl]ether (**94**)	*M. dura* (seed pods)	Yenesew *et al.*, 1996
Calopogonium isoflavone A (**95**)	*M. dura* (stem bark)	Yenesew *et al.*, 1996
Maximaisoflavone D (**96**)	*M. dura* (stem bark)	Yenesew *et al.*, 1996
Maximaisoflavone H (**97**)	*M. dura* (stem bark)	Yenesew *et al.*, 1996
6-Methoxycalpogonium A (**98**)	*M. dura* (seed pods)	Yenesew *et* al., 1997a
Griffonianone B (**99**)	*M. griffoniana* (root bark)	Yankep *et al.*, 2001

Compounds	Plants species (parts)	References
Griffonianone B methylether (**100**)	*M. griffoniana* (root bark)	Yankep *et al.*, 2001
7-Hydroxy-6-methoxyl-3',4'-methylenedioxyisofavone (**101**)	*M. griffoniana* (root bark)	Yankep *et al.*, 2001
Griffonianone C (**102**)	*M. griffoniana* (root bark)	Yankep *et al.*, 2001
Gliricidin (**103**)	*M. laurentii* (heart wood)	Kamnaing *et al.*, 1999
Conrauinone C (**104**)	*M. couraui* (stem bark)	Fuendjiep *et al.*, 1998a
Nordurlettone (**105**)	*M. ferruginea* ssp. *darassana* (seeds)	Dagne *et al.*, 1990
Millesianin C (**106**)	*M. brandisiana* (stem twigs)	Ye *et al.*, 2014
7,4'-Di-*O*-prenylgenistein (**107**)	*M. brandisiana* (Leaves)	Pancharoen *et al.*, 2008
Robustigenin (**108**)	*M. brandisiana* (Leaves)	Pancharoen *et al.*, 2008
Toxicarolisoflavone (**37**)	*M. brandisiana* (Leaves)	Pancharoen *et al.*, 2008
Millewanin A (**109**)	*M. taiwaniana* (stems)	Ito *et al.*, 2004
Millewanin B (**110**)	*M. taiwaniana* (stems)	Ito *et al.*, 2004
Millewanin C (**111**)	*M. taiwaniana* (stems)	Ito *et al.*., 2004
Millewanin D (**112**)	*M. taiwaniana*(stems)	Ito *et al.*, 2004
Millewanin E (**113**)	*M. taiwaniana* (stems)	Ito *et al.*, 2004
Norisojamaicin (**114**)	*M. usaramensis* ssp. *usaramensis* (stem bark)	Yenesew *et al.*, 1998
Maximaisoflavone G (**115**)	*M. usaramensis ssp. usaramensis* (stem bark)	Yenesew *et al.*, 1998
Courauinone D (**116**)	*M. couraui* (stem bark)	Fuendjiep *et al.*, 1998a
3',4'-Dihydroxy-7-*O*-[(E)-3,7-dimethyl-2,6-octadienyl]isoflavone (**117**)	*M. griffoniana*	Wanda *et al.*, 2006
4'-*O*-Geranylisoliquiritigenin (**118**)	*M. griffoniana*	Wanda *et al.*, 2006
4'-Prenyloxyderrone (**119**)	*M. oblata* ssp. *teitensis* (stem bark)	Derese *et al.*, 2014



	R^1	R^2	R^3
40	OH	OH	H
41	OCH_3	OCH_3	H
42	OH	OCH_3	H
43	OH	OCH_3	OH
44	OH	$-OCH_2O-$	
45	OCH_3	O-Prenyl	H
46	OCH_3	OH	H



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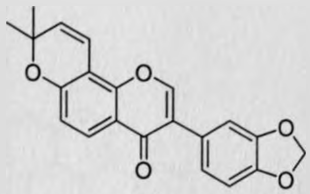

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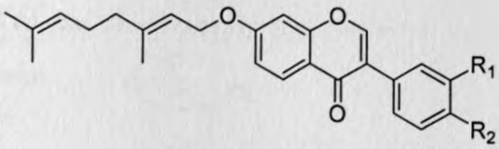
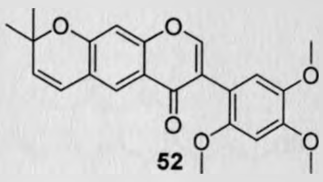


	R^1	R^2
48	$-OCH_2O-$	
49	H	OCH_3



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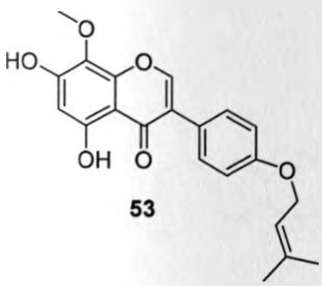

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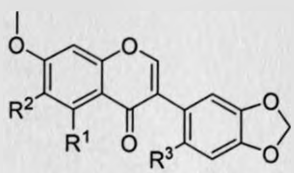
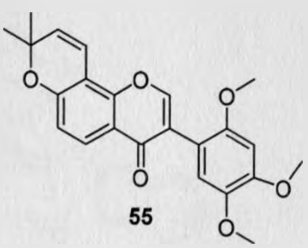


	R^1	R^2	R^3
51	H	H	OCH_3
54	OCH_3	OCH_3	H



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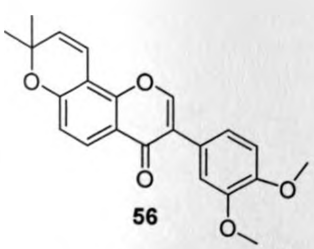

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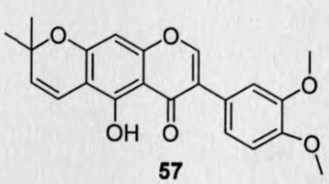

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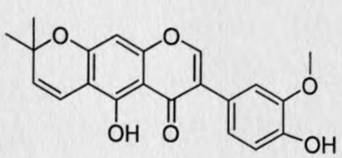

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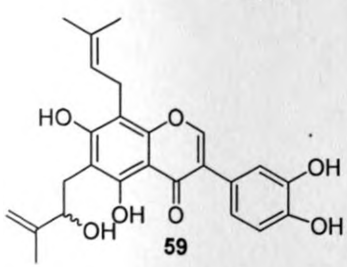

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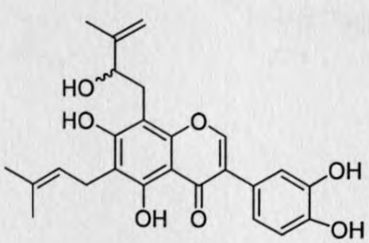

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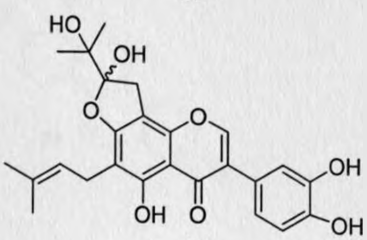

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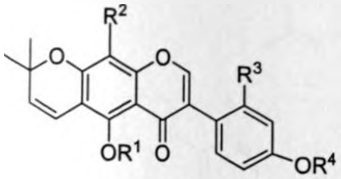
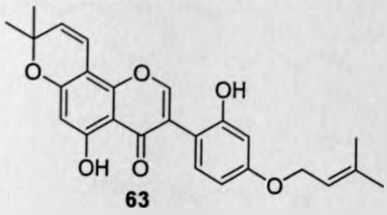


	R^1	R^2	R^3	R^4
62	H	H	OH	Prenyl
65	H	H	H	Prenyl
66	H	H	OCH_3	Prenyl
67	H	Prenyl	H	H



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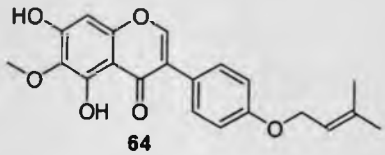

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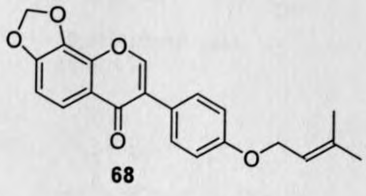

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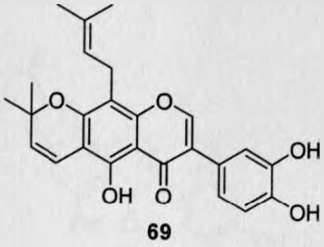

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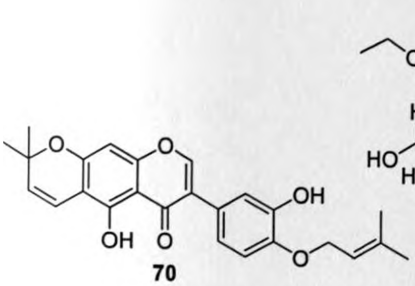

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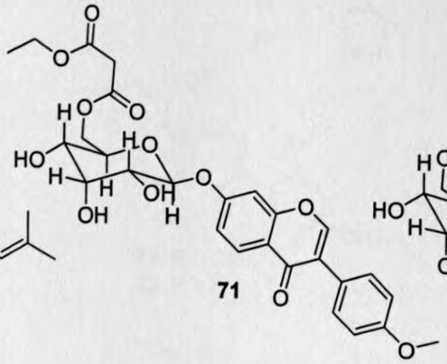

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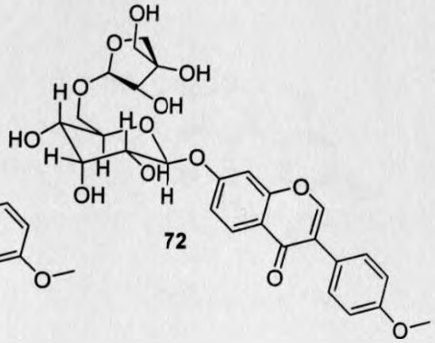

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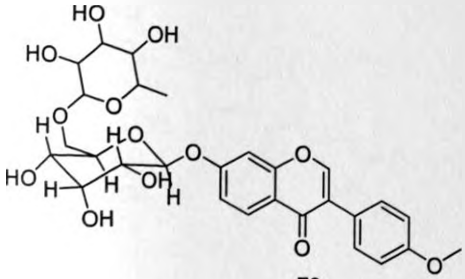

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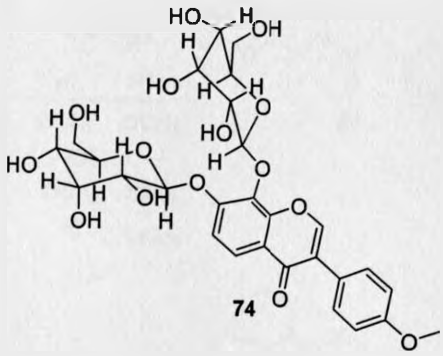

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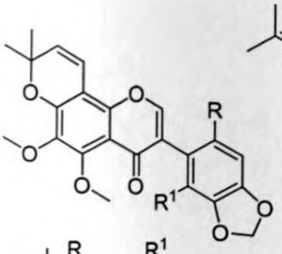
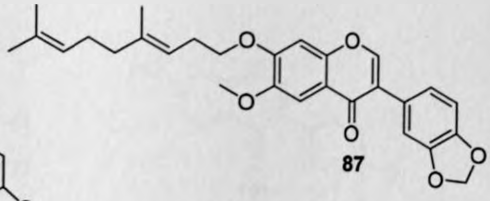


	R	R^1
75	OCH_3	H
76	H	H



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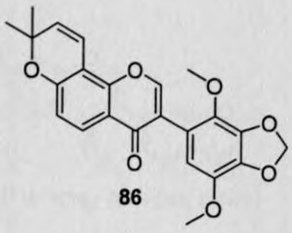

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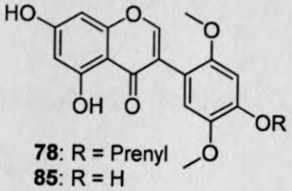
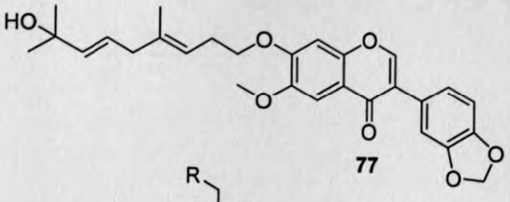


78: R = Prenyl
85: R = H



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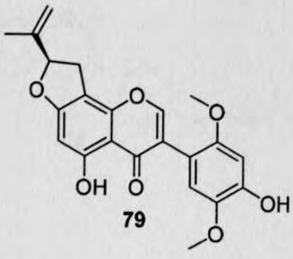

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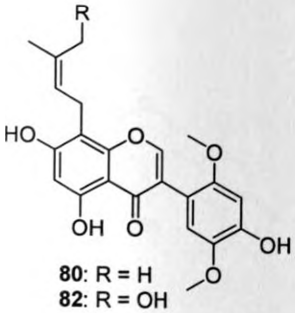
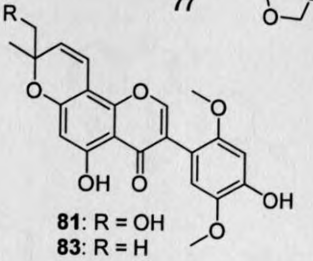
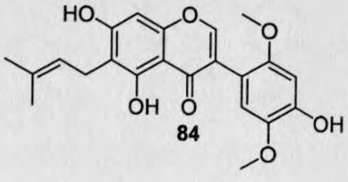


80: R = H
82: R = OH



81: R = OH
83: R = H



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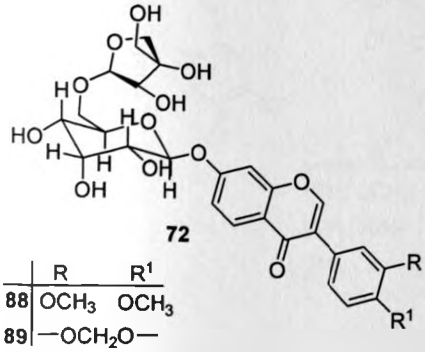
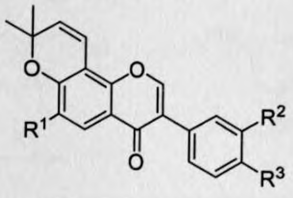
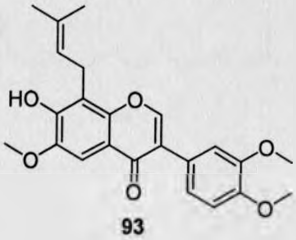


72

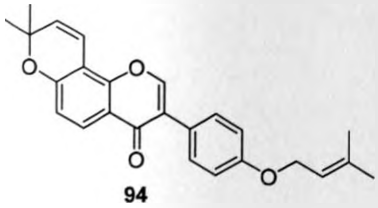
	R	R^1
88	OCH_3	OCH_3
89	$-OCH_2O-$	



	R^1	R^2	R^3
90	OCH_3	OCH_3	OCH_3
91	OCH_3	$-OCH_2O-$	
92	OH	OCH_3	OCH_3
95	H	H	OCH_3



93



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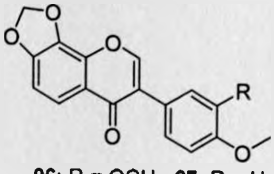
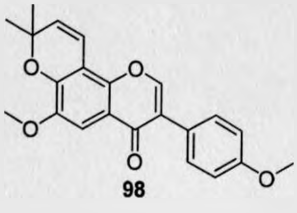


96: R = OCH_3; **97**: R = H



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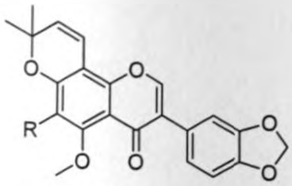
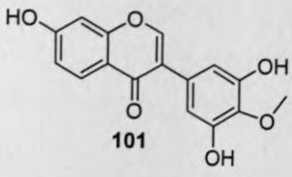


99: R = OH; **100:** OCH_3



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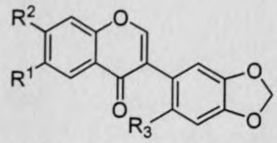
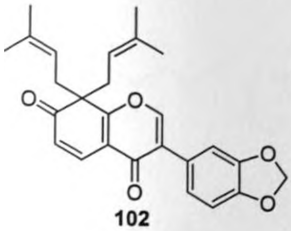


102: $R^1 = OCH_3$; $R^2 = OH$; $R^3 = H$



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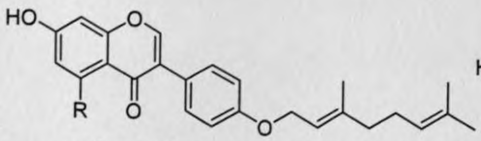
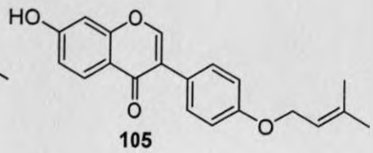


R	104	116
	H	OH



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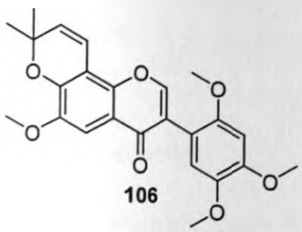

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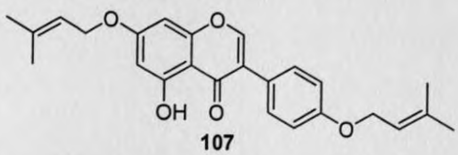

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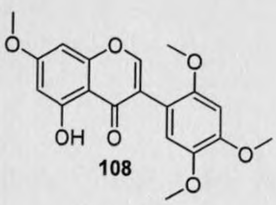

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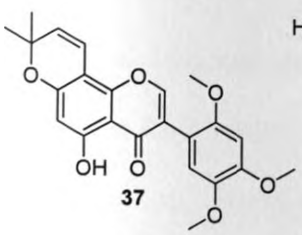
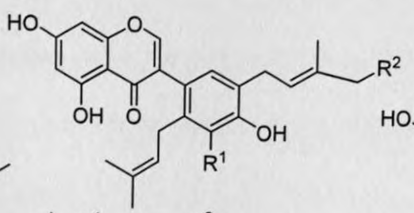
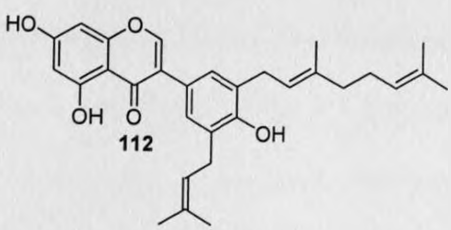


37



	R^1	R^2
109	OCH_3	H
110	OCH_3	Prenyl
111	H	Prenyl



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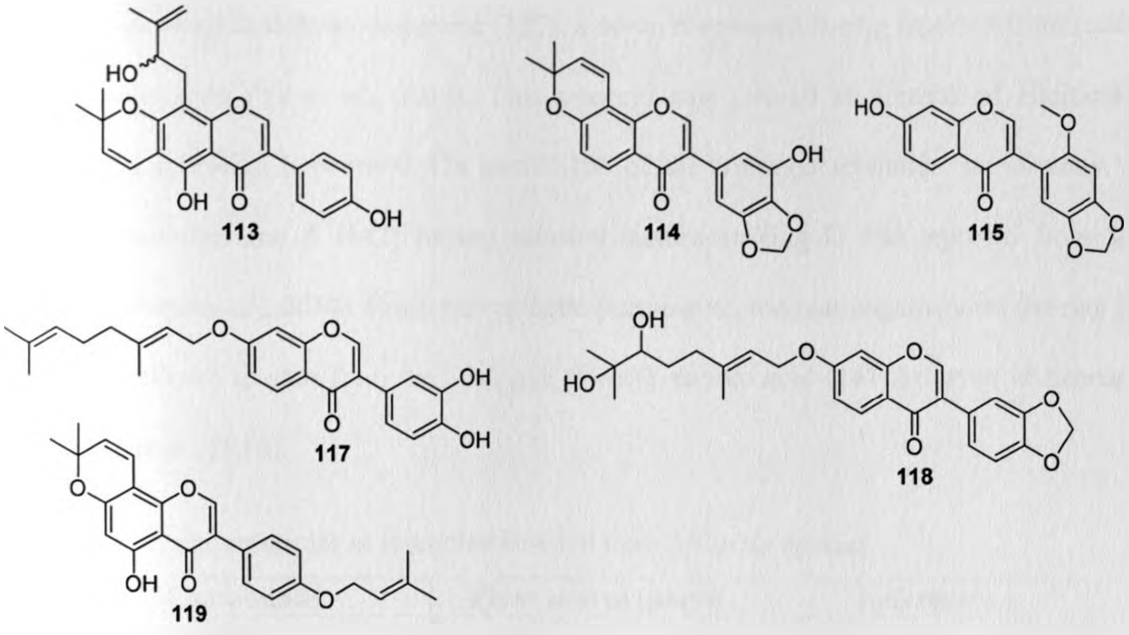


2.8.2 Rotenoids

Several rotenoids have been isolated from different *Millettia* species (Table 2.4). The rotenoids of *Millettia* species are characterized by regular methoxylation/methylenedioxy formation at the 2,3-positions in ring A. This is exemplified by the rotenoids isolated from *M. usaramensis* ssp. *usaramensis* (**139, 140, 141**) having methylenedioxy groups at C-2/3 of ring A. A prenyl group at C-8 is also commonly observed either in a cyclized form into five-/six-membered ring to form ring E as observed in several rotenoids (**121-126**, **132-135** and **149-153**) or as open chain as in **154**. A series of rotenoids with hydroxylation at C-11 of ring D were also common metabolites of this plant as in the case of **128, 129** and **131**.

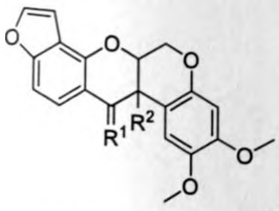
Owing to additional oxidative elaborations of the B/C ring junction, rotenoids with C-12a hydroxylation/alkoxylation (12a-hydroxy/alkoxyrotenoid), C-6a/C-12a dehydrogenation (dehydrorotenoids), C-6 hydroxylation/alkoxylation or combinations of these are common to the genus *Millettia* (Table 2.4) (Crombie, 1982). Unlike the common rotenoids, expansion of the B-ring from six to seven membered ring system was also noted. An example of this is 13-

homo-13-oxa-6a,12a-dehydrodegueline (**127**), a seven membered B-ring reported from seeds of *M. pachycarpa* (Ye *et al.*, 2012). This rotenoid was formed as a result of additional oxygen incorporation between C-12a and C-12b of the common rotenoids. Importantly, a rotenoid, caeruleanone A (**142**) having unusual feature on ring D was reported from *M. caerulea* (Pérez *et al.*, 2014). From biosynthetic perspective, the rearrangement on the ring D in **142** is believed to start from the analogue of rot-2'-enonic acid (**147**) as given in Scheme 2.6 (Pérez *et al.*, 2014).

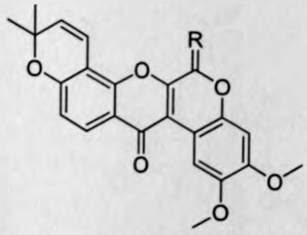
Table 2.4: Some examples of rotenoids isolated from *Millettia* species

Compounds	Plant source (parts)	References
12-Deoxo-12a-methoxylelliptone (**120**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
6-Methoxy-6a,12a-dehydrodeguelin (**121**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
6-Hydroxy-6a,12a-dehydrodeguelin (**122**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
6-Oxo-6a,12a-dehydrodeguelin (**123**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
12a-Hydroxyelliptone (**124**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
Elliptone (**125**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
6a,12a-Dehydrodeguelin (**126**)	*M. duchesnei* (twigs)	*Ngandeu et al., 2008*
13-Homo-13-oxa-6a,12a-dehydrodeguelin (**127**)	*M. pachycarpa* (seeds)	Ye *et al.*, 2012
Sermundone (**128**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
6-Deoxyclitoriacetal (**129**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
Elliptol (**130**)	*M. duchesnei* (twigs)	Ngandeu *et al.*, 2008
Stemonal (**131**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
α-Toxicarol (**36**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
6a,12a-Dehydrosermundone (**132**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
12a-Hydroxy-α-toxicarol (**133**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
6a,12a-Dehydro-α-toxicarol (**134**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
6-Hydroxy-6a,12a-dehydro-α-toxicarol (**135**)	*M. brandisiana* (leaves)	Pancharoen *et al.*, 2008
Griffonianone A (**136**)	*M. griffoniana* (root bark)	Yankep *et al.*, 2001

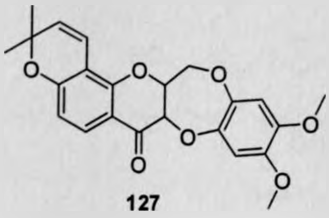
Compounds	Plants species (parts)	References
12a-Epimillettosin (**137**)	*M. usaramensis ssp. usaramensis* (stem bark)	Yenesew *et al.*, 1998
Millettosin (**138**)	*M. usaramensis ssp. usaramensis* (stem bark)	Yenesew *et al.*, 1998
Usararotenoid A (**139**)	*M. usaramensis ssp. usaramensis* (stem bark)	Yenesew *et al.*, 1998
12α-Hydroxy-12-dihydrousararotenoid A (**140**)	*M. usaramensis* ssp. *usaramensis* (stem bark)	Yenesew *et al.*, 1998
Usararotenoid B (**141**)	*M. usaramensis* ssp. *usaramensis* (stem bark)	Yenesew *et al.*, 1998
Caeruleanone A (**142**)	*M. caerulea* (fruits)	Pérez *et al.*, 2014
Caeruleanone B (**143**)	*M. caerulea* (fruits)	Pérez *et al.*, 2014
Caeruleanone C (**144**)	*M. caerulea* (fruits)	Pérez *et al.*, 2014
Retenone (**145**)	*M. pachycarpa*	Singhal *et al.*, 1982
	M. dura (seeds)	Ollis *et al.*, 1967
12a-Hydroxyrotenone (**146**)	*M. pachycarpa*	Singhal *et al.*, 1982
Rot-2'-enonic acid (**147**)	*M. pachycarpa*	Singhal *et al.*, 1982
12a-Hydroxyrot-2-enonic acid (**148**)	*M. pachycarpa*	Singhal *et al.*, 1982
Sumatrol (**149**)	*M. auriculata* (seeds)	Raju and Srimannarayana, 1978
Millettone (**150**)	*M. dura* (seeds)	Ollis *et al.*, 1967
6a, 12a-Dehydromillettone (**151**)	*M. usaramensis* ssp. *usaramensis* (stem bark)	Yenesew *et al.*, 2003
Deguelin (**152**)	*M. dura* (seeds) *M. ferruginea* (seeds)	Ollis *et al.*, 1967 Dagne *et al.*, 1991
Tephrosin (**153**)	*M. dura* (seeds) *M. ferruginea* (seeds)	Ollis *et al.*, 1967 Dagne *et al.*, 1991
Usararotenoid C (**154**)	*M. usaramensis* spp. *usaramensis* (stem bark)	Yenesew *et al.*, 2003



124: R^1 = O, R^2 = OH
125: R^1 = O, R^2 = H



	121	122	123	126
R	H, OCH_3	H, OH	O	H, H



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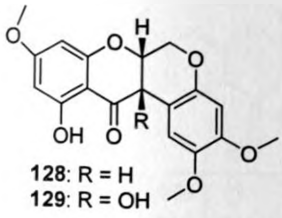
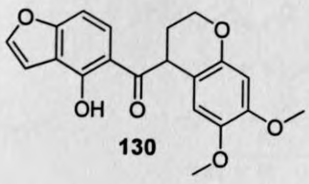


128: R = H
129: R = OH



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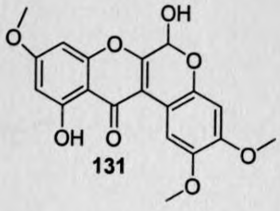

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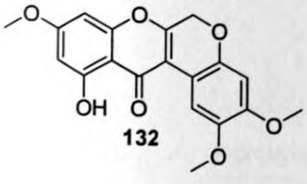

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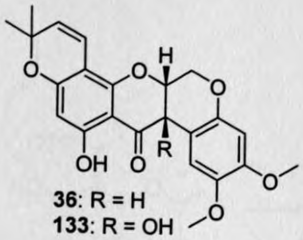
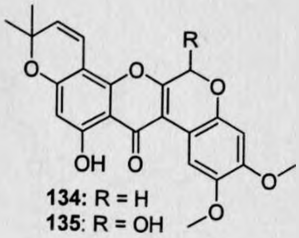
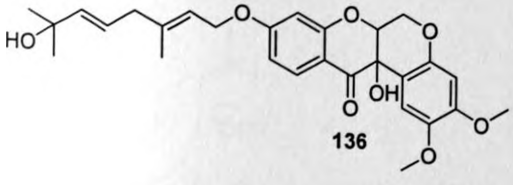


36: R = H
133: R = OH



134: R = H
135: R = OH



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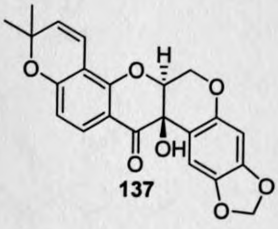

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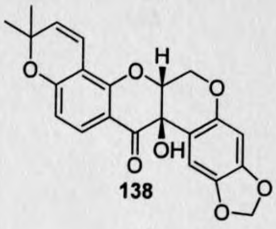

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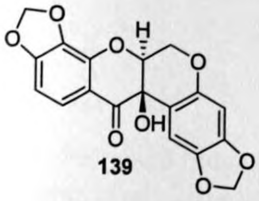

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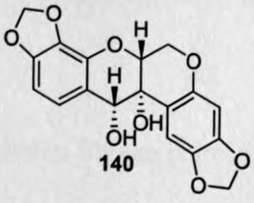

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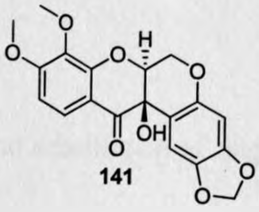

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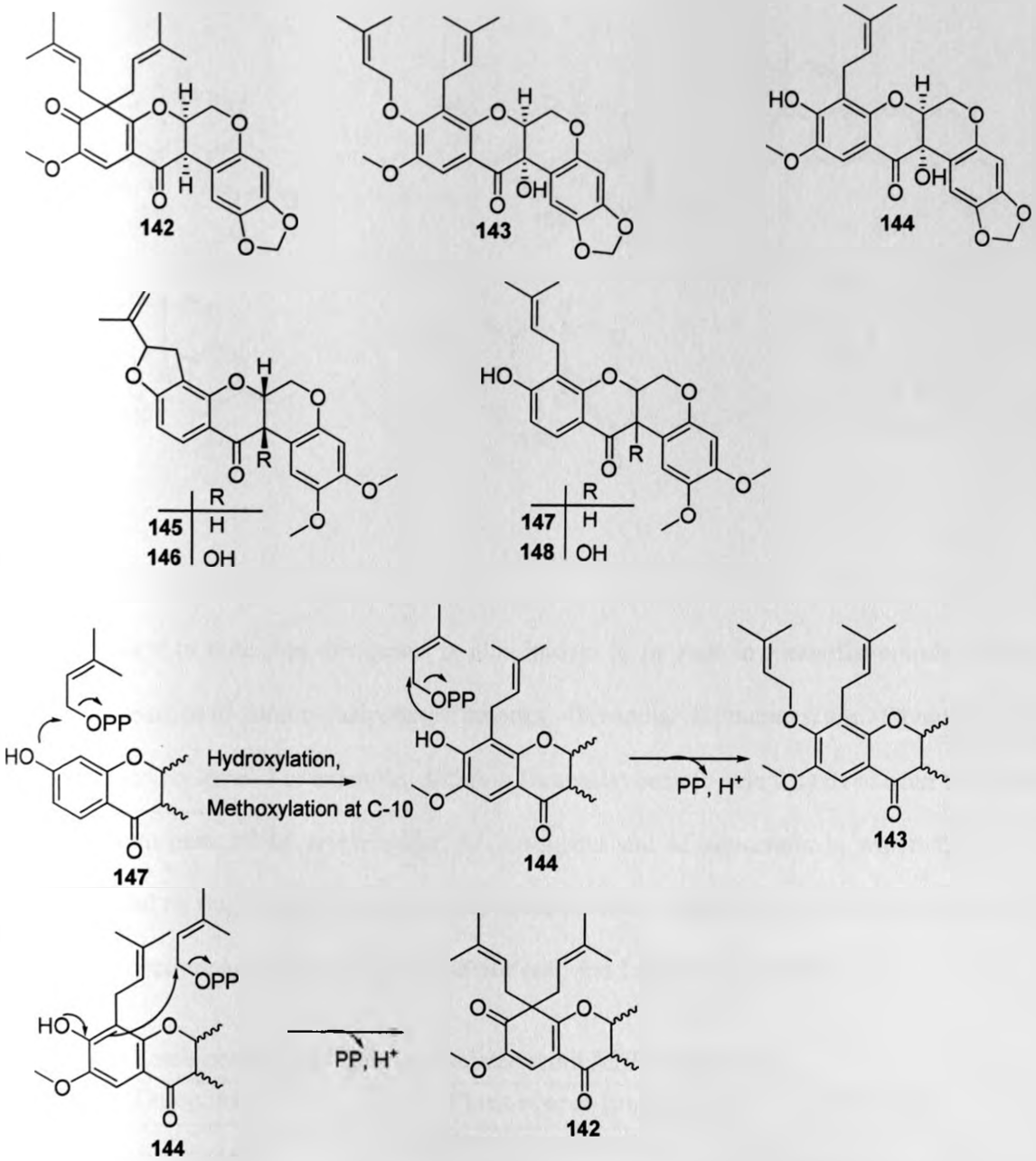
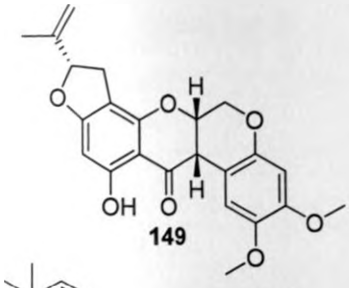
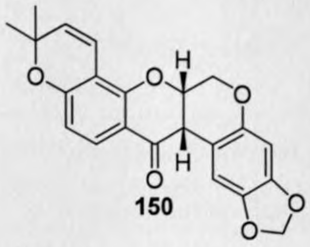
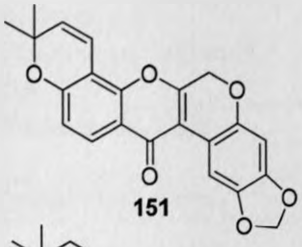
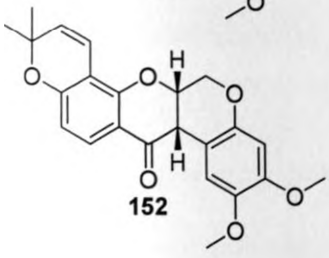


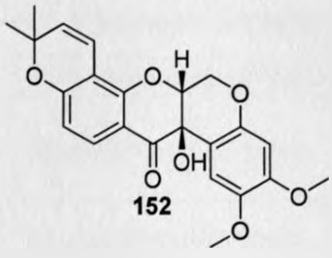
Scheme 2.4. Proposed biogenetic pathway for the rearrangement and substitution of ring D in **142-144** (Perez *et al.*, 2014).

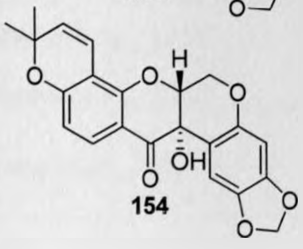












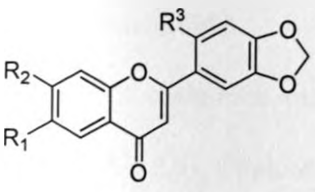
2.8.3 Flavones

It is important to note that this genus is also known to be rich in furanoflavonoids, which could be classified as furano-chalcones, -flavones, -flavonols, -flavanones, and -flavans, based on their basic skeleton. For example, different furanoflavones (Table 2.5) have been reported from different parts of *M. erythrocalyx*, *M. sanagana* and *M. leucantha* in which the furan unit is located on ring A at C-7/C-8 as in **157-164, 170, 174** (Mbafor *et al.*, 1995; Sritularak *et al.*, 2002; Phrutivorapongkul *et al.*, 2003; Sritularak and Likhitwitayawuid, 2006).

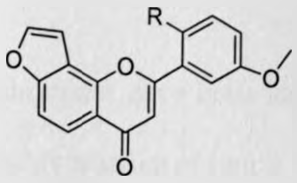
Table 2.5: Some examples of flavones isolated from *Millettia* species

Compounds	Plant source (parts)	References
Millettocalyxin A (**155**)	*Millettia erythrocalyx* (stem bark)	Sritularak *et al.*, 2002
Millettocalyxin B (**156**)	*Millettia erythrocalyx* (stem bark)	Sritularak *et al.*, 2002
Millettocalyxin C (**157**)	*Millettia erythrocalyx* (stem bark)	Sritularak *et al.*, 2002
Pongol methyl ether (**158**)	*M. erythrocalyx* (seed pods)	Sritularak *et al.*, 2002
3',4'-Methylenedioxy-[2",3":7,8]-furanoflavonol (**159**)	*M. erythrocalyx* (seed pods)	Sritularak and Likhitwitayawuid *et al.*, 2006

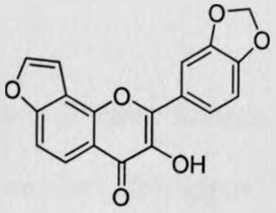
Compounds	Plants species (parts)	References
6,3'-Dimethoxy-[2",3":7,8]-furanoflavone (**160**)	*M. erythrocalyx* (seed pods)	Sritularak and Likhitwitayawuid *et al.*, 2006
Lanceolatin (**161**)	*M. sanagana* (root bark)	Mbafor *et al.*, 1995
Kanjone (**162**)	*M. sanagana* (root bark)	Mbafor *et al.*, 1995
5-Methoxyfurano[7,8:4",5"]flavone (**163**)	*M. sanagana* (root bark)	Mbafor *et al.*, 1995
Sanaganone (**164**)	*M. sanagana* (root bark)	Mbafor *et al.*, 1995
[2]Benzopyrano[4,3-b] furo[2,3h][1]benzopyran-6(8H)-one (**165**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
2",2"-Dimethylchromene-[5",6":7,8]-flavone (**166**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
6-Methoxyl-2",2"-dimethylpyrano-[5",6":8,7]flavone (**167**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
5-Methoxyl-2,2-dimethylpyrano-[5,6:8,7]flavone (**168**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
6-Hydroxy-2,2-dimethylpyrano-[5",6":8,7]flavone (**169**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
3',5'-Dimethoxy-[2",3" : 7,8]-furanoflavone (**170**)	*M. erythrocalyx* (leaves)	Likhitwitayawui *et al.*, 2005
Desmethoxykanugin (**171**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
3',4'-Methylenedioxy-7-methoxyflavone (**172**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
3',4'-Methylenedioxy-5,7-dimethoxyflavone (**173**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
Karanjin (**174**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
3', 4'-Methylenedioxy-7-methoxyxyflaone (**175**)	*M. hemsleyana* (stem bark)	Mahmoud and Waterman, 1985



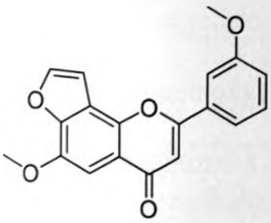
	R^1	R^2	R^3
155	H	OCH_3	OCH_3
156	*O*-Prenyl	OCH_3	H



157: R = OCH_3
158: R = H



159



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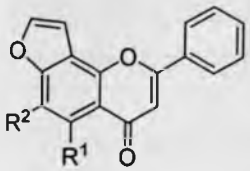
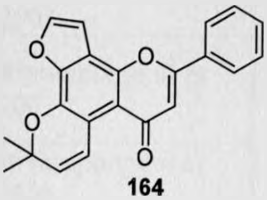
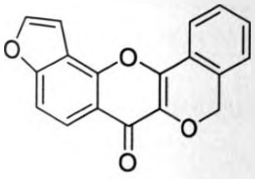


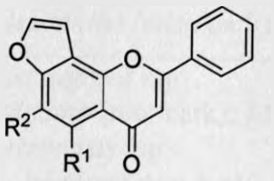
	R^1	R^2
161	H	H
162	H	OCH_3
163	OCH_3	H



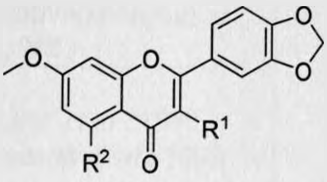
164



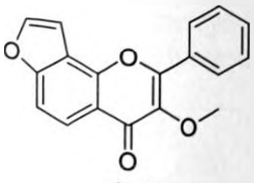
165



	R^1	R^2
166	H	H
167	H	OCH_3
168	OCH_3	H
169	H	OH



	R^1	R^2
171	OCH_3	H
172	H	H
173	H	OCH_3



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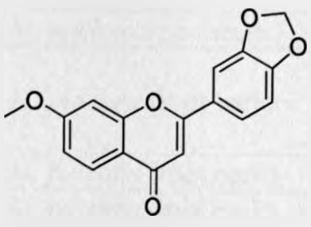


175

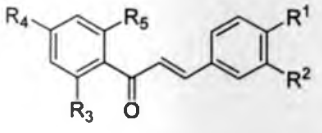
2.8.4 Chalconoids

A number of chalcones and dihydrochalcones have been identified from different *Millettia* species (Table 2.6). Chalcones without oxygenation of ring A are also common to this genus.

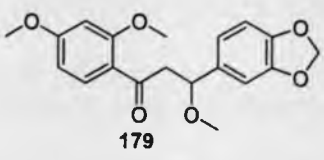
Table 2.6: Some examples of chalconoids isolated from *Millettia* species

Compounds	Plant source (parts)	References
2',4'-Dimethoxy-3,4-methylenedioxychalcone (**176**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
2'-Hydroxy-3,4,4',6'tetramethoxychalcone (**177**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
2',4',6'-Trimethoxy-3,4-methylenedioxychalcone (**178**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
dihydromilletenone methyl ether (**179**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
2,4,6,β -Tetramethoxy-3',4'-methylenedioxychalcone (**180**)	*M. leucantha* (stem bark)	Phrutivorapongkul *et al.*, 2003
4'-*O*-Geranylisoliquiritegenin (**181**)	*M. ferruginea* ssp *darassana* (root bark); *M. usaramensis* ssp *usaramensis* (stem bark)	Dagne *et al.*, 1990 Yenesew *et al.*, 1998
4-Hydroxylonchocarpin (**182**)	*M. dura* (stem bark) *M. pachycarpa* (seeds)	Dagne *et al.*, 1991; Ye *et al.*, 2012
4-Methoxylonchocarpin (**183**)	*M. pachycarpa* (seeds)	Ye *et al.*, 2012
(*E*)-1-(5-methoxyl-2, 2-dimethyl-2H-chromen-6-yl)-3-(3-methoxylphenyl)propenone (**184**)	*M. pachycarpa* (seeds)	Ye *et al.*, 2012
Millepachine (**185**)	*M. pachycarpa* (seeds)	Ye *et al.*, 2012
(*E*)-1-(4-Hydroxybenzofuran-5-yl)-3-phenylprop-2-en-1-one (**186**)	*M. pulchra* (root barks)	Wang *et al.*, 2015
Pongamol (**187**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
Ovalitenin A (**188**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
(*R*)-Ovalitenin B (**189**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
Lonchocarpin (**190**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
Pongachalconel (**191**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
Purpurenone (**192**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
2-Hydroxy-3,4-dimethoxy-[2",3":4',3']-furanochalcone (**193**)	*M. erythrocalyx* (seed pods)	Sritularak and Likhitwitayawuid, 2006

Compounds	Plants species (parts)	References
2',3-Dihydroxy-4-methoxy-4'-γ,γ-dimethylallyloxychalcone (**194**)	*M. erythrocalyx* (seed pods)	Sritularak and Likhitwitayawuid, 2006
Butein (**195**)	*M. nitida* var. *hirsutissima* (stem)	Liao *et al.*, 2013
Isoliquiritigenin (**196**)	*M. nitida* var. *hirsutissima* (stem)	Liao *et al.*, 2013
Pongamol (**197**)	*M. sanagana* (root bark)	Mbafor *et al.*, 1995



	R^1	R^2	R^3	R^4	R^5
176	—OCH_2O—		H	OCH_3	OCH_3
177	OCH_3	OCH_3	OCH_3	OCH_3	OH
178	—OCH_2O—		OCH_3	OCH_3	OCH_3



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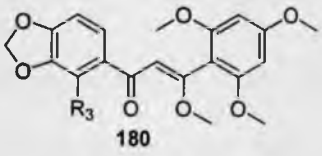

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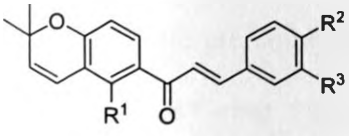
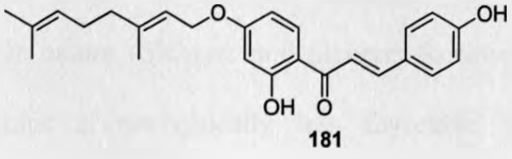


	R^1	R^2	R^3
182	OH	OH	H
183	OH	OCH_3	H
184	OCH_3	OCH_3	H



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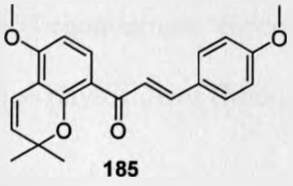

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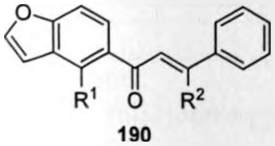
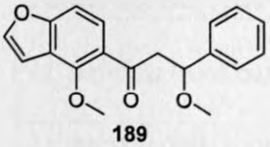


190

	R^1	R^2
186	OH	H
187	OCH_3	OH
188	OCH_3	H



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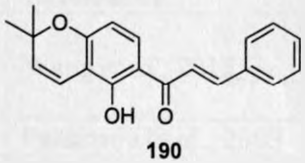

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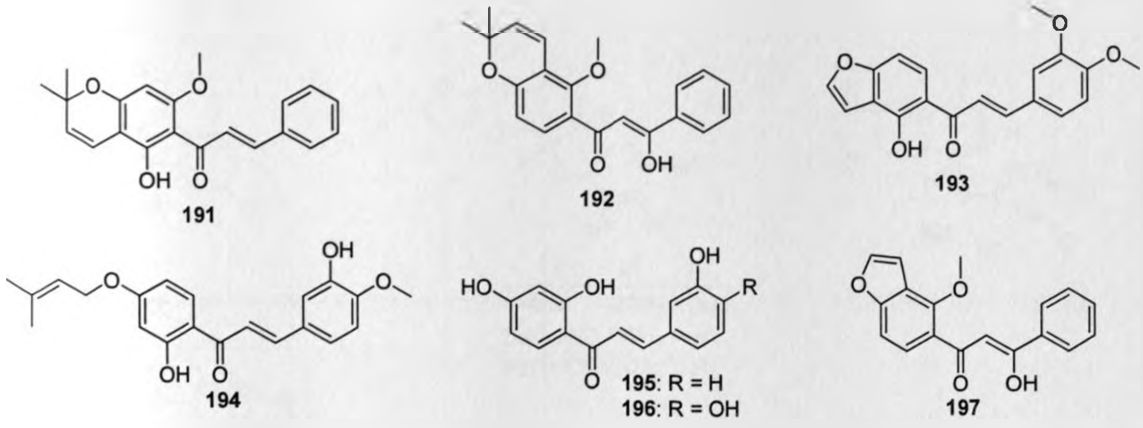
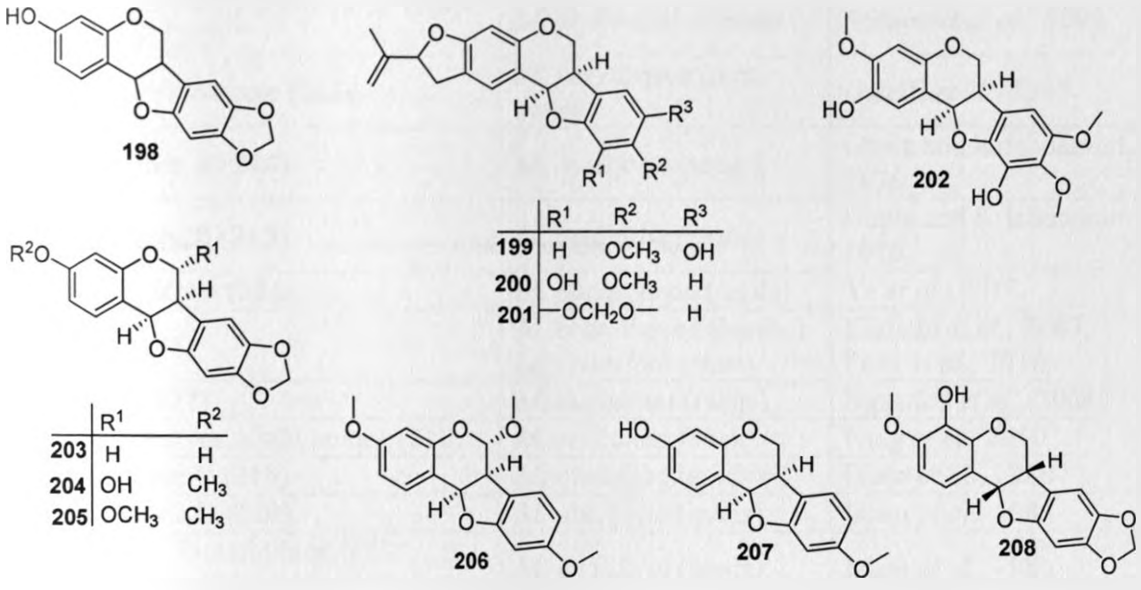


2.8.5 Pterocarpans

Pterocarpans are isoflavoid derivatives with additional heterocyclic ring formed by coupling of the B ring to the C-4 oxygen atom. These compounds have two stereocenters and hence there are four possible stereoisomers. However, the *cis*-configurations [(6a*R*,11a*R*) and (6a*S*,11a*S*)] are preferably found in nature (Sicherer and Sicherer-Roetman, 1980) than the *trans*-fused B/C-ring system which is energetically less favorable. Like isoflavones, pterocarpans are also widely distributed in Leguminosae especially in Paplionoidae subfamily, and play an important ecological role as phytoalexins (Máximo *et al.*, 2000).

Table 2.7: Some examples of pterocarpans isolated from *Millettia* species

Compounds	**Plants species (parts)**	**References**
[2]benzopyrano[4,3-b]furo[2,3h][1]benzopyran-6(8H)-one (**198**)	*M. pulchra* (root bark)	Wang *et al.*, 2015
Pervilline (**199**)	*M. pervilleana* (root bark)	Palazzino *et al.*, 2003
Pervillinine (**200**)	*M. pervilleana* (root bark)	Palazzino *et al.*, 2003
Emoroidocarpan (**201**)	*M. pervilleana* (root bark)	Palazzino *et al.*, 2003
Brandisianin F (**202**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007
(-)-Maackiain (**203**)	*M. pulchra*	Baruah *et al.*, 1984
(-)-Pterocarpin (**204**)	*M. pulchra*	Baruah *et al.*, 1984
6α-Methoxypterocarpin (**205**)	*M. pulchra*	Baruah *et al.*, 1984
6α-Methoxyhomopteocarpin (**206**)	*M. pulchra*	Baruah *et al.*, 1984
Medicarpin (**207**)	*M. leucantha* (wood)	Rayanil *et al.*, 2011
4-Hydroxy-3-methoxy-8,9-methylenedioxypterocarpan (**208**)	*M. leucantha* (wood)	Rayanil *et al.*, 2011



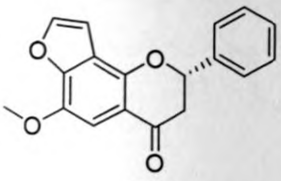
2.8.6 Flavanones, Isoflavanones, Flavans and Isoflavans of Millettia Species

The level of oxidation of hetrocyclic ring -C of flavonoids determines the structural variation. When ring-C of iso/flavones is oxidized at C-3, hydroxyisoflavanones/hydroxyisoflavanones are formed, while complete loss of oxygenation at C-4 results in the formation of isoflavans (or flavans). There are a quite number of such compounds (Table 2.8) identified in the genus *Millettia*.

Table 2.8: Some examples of flavanones, isoflavanones and flavans isolated from *Millettia* species

Compounds	Plant species (parts)	References
Pervilleanone (**9**)	*M. pervilleana* (root bark)	Galeffi *et al.*, 1997
3'-*O*-Demethylpervilleanone (**10**)	*M. pervilleana* (root bark)	Galeffi *et al.*, 1997
(-)-(2*S*)-6-Methoxy-[2",3":7,8]furanoflavanone (**209**)	*M. erythrocalyx* (root)	Sritularak *et al.*, 2002
(2R, 3R)-5,4'-Dihydroxy-8-prenyl-6",6"-dimethylpyrano[2",3":7,6]-dihydroflavonol (**210**)	*M. nitida* var. *hirsutissima* (stem) *M. reticulata* (stem)	Liao *et al.*, 2013; Fang *et al.*, 2010
(-)-(2S)-6,3',4'-Trimethoxy-[2",3":7,8]furanoflavanone (**211**)	*M. erythrocalyx* (seed pods)	Sritularak and Likhitwitayawuid, 2006

Compounds	Plants species (parts)	References
2,5-Dimethoxy-4-hydroxy-[2",3":7,8]-furanoflavan (**212**)	*M. erythrocalyx* (root)	Sritularak *et al.*, 2002
Dimethylpervilleanone (**213**)	*M. pervilleana* (root bark)	Galeffi *et al.*, 1997
Ovalichromene A (**214**)	*M. ovalifolia* (seeds)	Gupta and Krishnamurt, 1976
Ovalichromene B (**215**)	*M. ovalifolia* (seeds)	Gupta and Krishnamurt, 1976
Dorspoinsettifolin (**216**)	*M. pachycarpa* (seeds)	Ye *et al.*, 2012
Naringenin (**31**)	*M. brandisiana* (leaves) *M. reticulata* (stem)	Kikuchi *et al.*, 2007; Fang *et al.*, 2010
Eriodictyol (**217**)	*M. duchesnei* (twigs)	Ngandeu *et al.*, 2008
5,7,3',5'-Tetrahydroxyflavanone (**218**)	*M. reticulata* (stem)	Fang *et al.*, 2010
Ovaliflavanone C (**219**)	*M. ovalifolia* (seeds)	Islam *et al.*, 1980
Ovaliflavanone D (**220**)	*M. ovalifolia* (seeds)	Islam *et al.*, 1980
7-Hydroxy-3',4'-methylene-6-*C*-prenylflavanone (**221**)	*M. ovalifolia* (seeds)	Islam *et al.*, 1980
7-(γ,γ-Dimethyallyloxy)3',4'-methylenedioxyflavanone (**222**)	*M. ovalifolia* (seeds)	Islam *et al.*, 1980
(-) isolonchocarpin (**223**)	*M. pulchra* (root)	Wang *et al.*, 2015
Milletenin A (**224**)	*M. ovalifolia* (leaves)	Khan and Zaman, 1974
Milletenin B (**225**)	*M. ovalifolia* (leaves)	Khan and Zaman, 1974
(2R)-7, 3'-dihydroxy-6,4'-methoxylflavan (**226**)	*M. nitida* var. *hirsutissima* (stem)	Liao *et al.*, 2013
(3R)-7, 4'-dihydroxy-2'-methoxyisoflavan (**227**)	*M. nitida* var. *hirsutissima* (stem)	Liao *et al.*, 2013
(3R)-Vestitol (**228**)	*M. nitida* var. *hirsutissima* (stem)	Liao *et al.*, 2013
Neomillinol (**229**)	*M. racemosa* (debarked stem)	Rao *et al.*, 1996
Millinolol (**230**)	*M. racemosa* (debarked stem)	Rao *et al.*, 1996
3R-(-)-Isomillinol-B (**231**)	*M. racemosa* (debarked stem)	Rao and Krupadanam, 1994
Millinol-B (**232**)	*M. racemosa* (debarked stem)	Kumar *et al.*, 1989
Millinol (**233**)	*M. racemosa* (debarked stem)	Kumar *et al.*, 1989
3R-(-)-Laxifloran (**234**)	*M. racemosa* (debarked stem)	Rao and Krupadanam, 1994
Epicatechin (**235**)	*M. brandisiana* (leaves)	Kikuchi *et al.*, 2007



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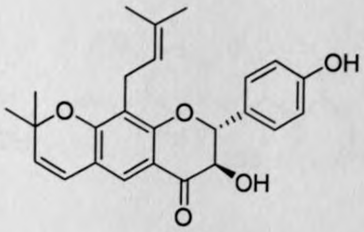

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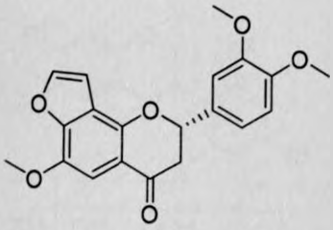

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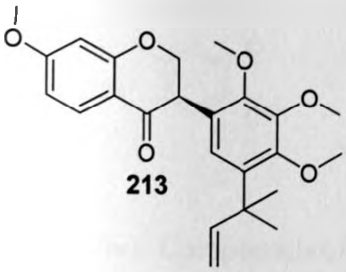

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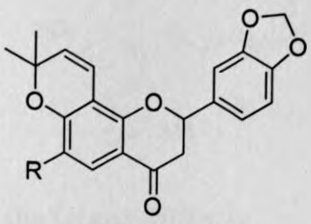
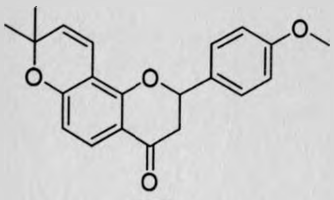


214: R = OCH_3
215: R = H



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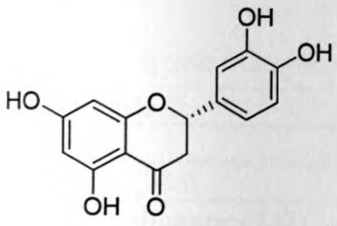

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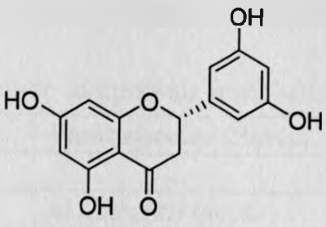

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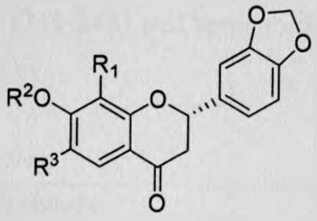
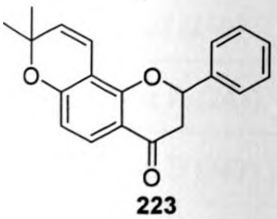


	R^1	R^2	R^3
219	Prenyl	H	H
220	Prenyl	H	Prenyl
221	H	Prenyl	H
222	H	H	Prenyl



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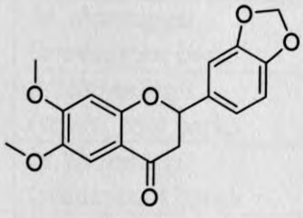

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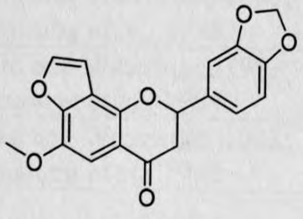

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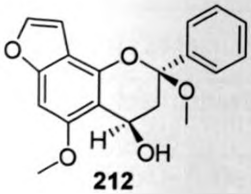

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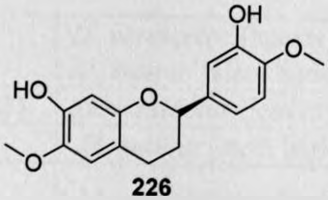

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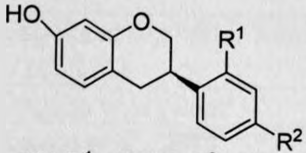
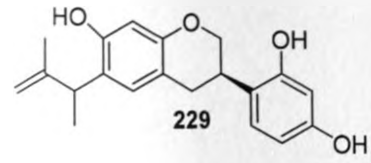
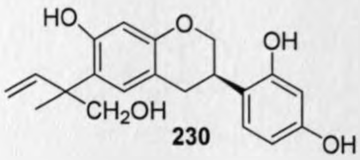
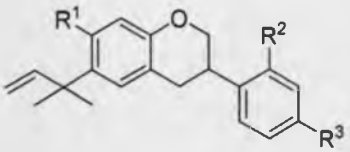


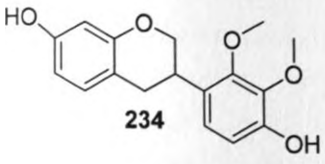
227: R^1 = OCH_3, R^2 = OH
228: R^1 = OH, R^2 = OCH_3

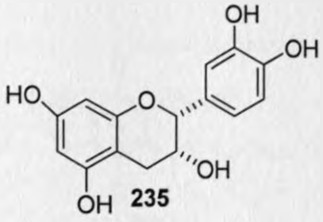






	R^1	R^2	R^3
231	OH	OCH_3	OCH_3
232	OH	OCH_3	OH
233	OH	OH	OH



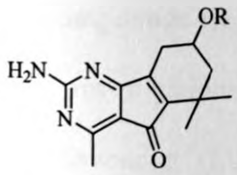


2.8.7 Minor Compounds of the Genus *Millettia*

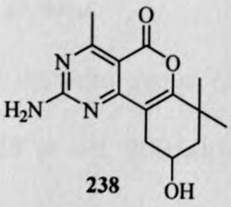
Some minor compounds including alkaloids (**236-239**) coumarins (**240-243**) and terpenoids (**244-249**) have been also reported from the genus *Millettia* (Table 2.9).

Table 2.9: Some examples of minor compounds from *Millettia* species

Compounds	Plant species (Parts)	References
Alkaloids		
Millaurine (**236**)	*M.laurentii* (seeds)	Ngamga *et al.*, 1993
O-Acetylmillaurine (**237**)	*M. laurentii* (seeds)	Ngamga *et al.*, 1993
Millettonine (**238**)	*M. laurentii* (stem bark)	Kamnaing, *et al.*, 1994
Millaurine A (**239**)	*M. laurentii* (seeds)	Ngamga *et al.*, 2007
Coumarins		
Robustic acid (**240**)	*M. thonningii* (seeds; root bark)	Khalid and Waterman, 1983; Asomaning *et al.*, 1999
Thonningine A (**241**)	*M. thonningii* (seeds; root bark)	Khalid and Waterman, 1983; Asomaning *et al.*, 1999
Thonningine B (**242**)	*M. thonningii* (seeds; root bark)	Khalid and Waterman, 1983; Asomaning *et al.*, 1999
Pervilleanine (**243**)	*M. pervilleana* (root bark)	Palazzino *et al.*, 2003
Terpenoids		
Stigmasterol (**244**)	*M. versicolor* (leaves); *M. mannii* (stem bark)	Ongoka *et al.*, 2008; Kamto *et al.*, 2012
22,23-Dihydrostigmasterol (**245**)	*M. versicolor* (leaves)	Ongoka *et al.*, 2008
β-sitosterol (**246**)	*M. mannii* (stem bark)	Kamto *et al.*, 2012
β -sitosterol 3-O- β-D-glucopyranoside (**247**)	*M. mannii* (stem bark)	Kamto *et al.*, 2012
Lupeol (**248**)	*M. mannii* (stem bark)	Kamto *et al.*, 2012
Lupenone (**249**)	*M. mannii* (stem bark)	Kamto *et al.*, 2012



236: R = H, **237**: R = Ac



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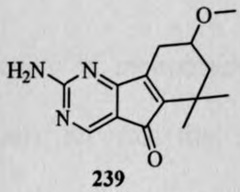

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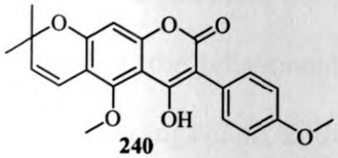

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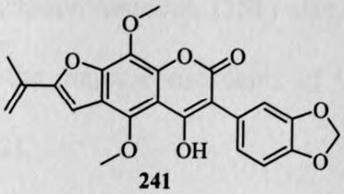

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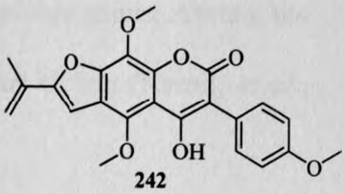

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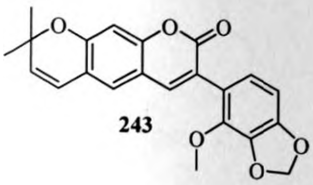

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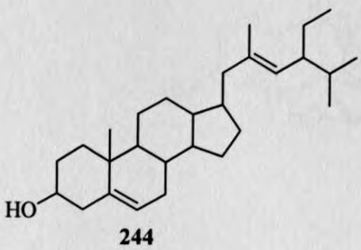

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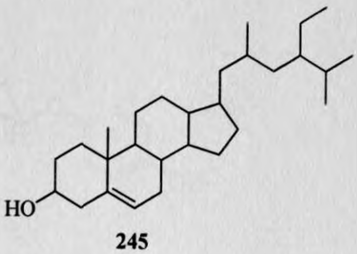

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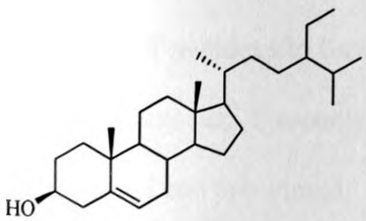
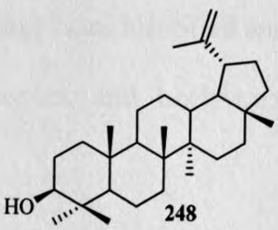


246: R = H

247: R = Glucose



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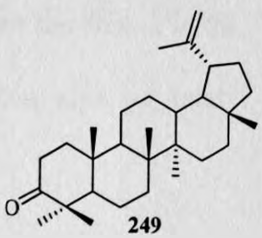
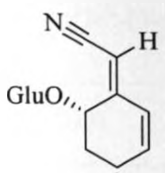


249

2.9 Compounds from the *Genus Ochna*

Previous investigations have shown that the genus *Ochna* is a source of monomeric and dimeric flavonoids (Likhitwitayawuid *et al.*, 2005a). It has also been reported that minor compounds: anthranoids, triterpenes, steroids, fatty acids and cyanoglucoside, menisdaurine (**250**) and a glucoside derivative, lanceoloside A (**251**) also occur in this genus. Among the flavonoids, the biflavonoids are the major constituents of the genus *Ochna* (Kamil, *et al.*, 1987; Messanga *et al.*, 2001; 2002).



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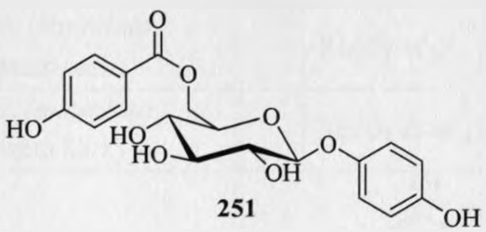


251

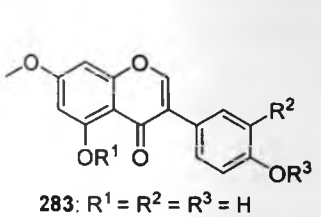
2.9.1 Monoflavonoids

A number of monomeric flavonoids have been identified and reported from the *Ochna* in the form of flavones, flavonols, flavononols, and isoflavonoids, which can also be rarely glycosidated and prenylated.

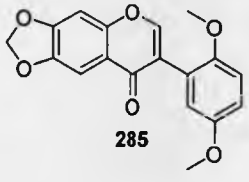
Table 2.10: Some examples of monoflavonoids isolated from *Ochna* species

Compounds	Plant species (parts)	References
Irilone (**283**)	*O. calodendron* (leaves)	Messanga *et al.*, 2002
3'-Methoxylirilone (**284**)	*O. calodendron* (leaves)	Messanga *et al.*, 2002
Hemerocallone (**285**)	*O. schweinfurthiana* (stem bark)	Ndongo *et al.*, 2015
6-(γ,γ-Dimethylallyl)taxifolin 7-*O*-β-D-glucoside (**286**)	*O. integerrima* (leaves)	Likhitwitayawuid *et al.*, 2001
6-(γ,γ-Dimethylallyl)kaempferol 7-*O*-β-D-glucoside (**287**)	*O. integerrima* (leaves, twigs)	Reutrakul *et al.*, 2007
6-(γ,γ-Dimethylallyl)quercetin 7-*O*-β-D-glucoside (**288**)	*O. integerrima* (leaves)	Reutrakul *et al.*, 2007

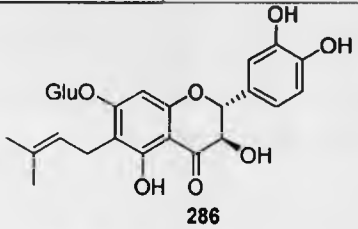
Compounds	Plants species (parts)	References
6-(3-Hydroxymethylbuthyl) quercetin 7-*O*-*β*-D-glucoside (**289**)	*O. integerrima* (leaves)	Reutrakul *et al.*, 2007
6-(γ,γ-Dimethylallyl)dihydrokaempferol 7-*O*-*β*-D-glucoside (**290**)	*O. integerrima* (leaves)	Reutrakul *et al.*, 2007
6-(3-Hydroxy-3-methylbuthyl)taxifolin 7-*O*-*β*-D-glucoside (**291**)	*O. integerrima* (leaves)	Reutrakul *et al.*, 2007
4'-Hydroxy-3'-methoxyfurano[2",3",6,7]flavone (**292**)	*O. squarrosa* (root bark)	Anuradha *et al.*, 2006
3',4'-Dihydroxyfurano[2",3",6,7]flavones (**293**)	*O. squarrosa* (root bark)	Anuradha *et al.*, 2006
5-Methoxyfurano[4",5",6,7]flavone (**294**)	*O. squarrosa* (root bark)	Anuradha *et al.*, 2006
5-*O*-methylsquarrosin (**295**)	*O. squarrosa* (root bark)	Anuradha *et al.*, 2006
Kaempferol 3-*O*-β-D-glucopyranoside (**296**)	*O. lanceolata* (stem bark)	Reddy *et al.*, 2008
Afrormosin (**297**)	*O. lanceolata* (stem bark)	Reddy *et al.*, 2008



283: $R^1 = R^2 = R^3 = H$
284: $R^1 = R^3 = H$, $R^2 = OCH3$



285



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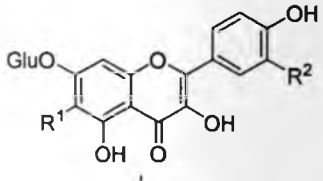
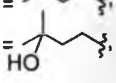
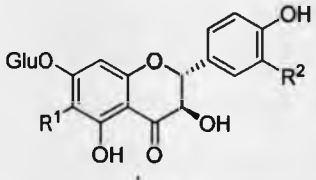
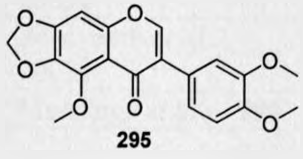


287: R^1=  , R^2 = H
288: R^1=  , R^2 = OH
289: R^1=  , R^2 = OH



290: R^1=  , R^2 = H
291: R^1=  , R^2 = OH



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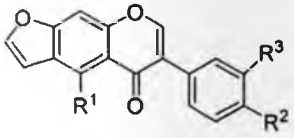
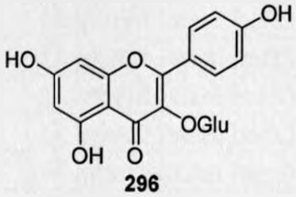


	R^1	R^2	R^3
292	OCH_3	OCH_3	OCH_3
293	H	OH	OH
294	OCH_3	H	H



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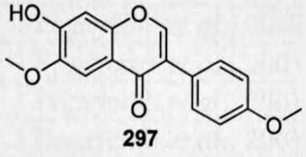


297

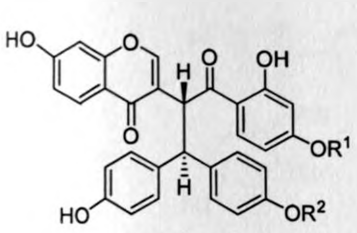
2.9.2 Biflavonoids

Biflavonoids are common in the family Ochnaceae including *Ochna* species (Fidelis *et al.*, 2014). Structurally, biflavonoids are defined as a polyphenolic molecules containing two identical (flavone/flavone, flavanone/flavanone) or non-identical (flavanone/flavone) monomeric units. They are structurally diversified as a result of different modes of oxidative coupling (symmetrically or unsymmetrically) between the monomeric units through carbon-carbon or carbon-oxygen linkages. These different possibilities together with the positions and the number/type of functionality of the flavonoid unit introduce considerable structural diversities among the biflavanoids (Lin *et al.*, 2001; Jiang *et al.*, 2002).

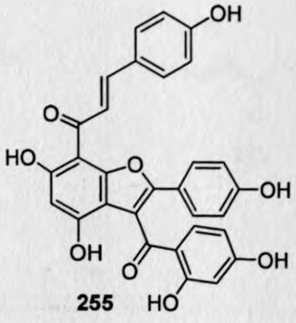
Table 2.11: Some examples of dimeric flavonoids isolated from *Ochna* species

Compounds	Plant species (parts)	References
Lophirone A (**252**)	*O. calodendron* (stem bark); *O. squarrosa* (root bark); *O. afzelii* (stem bark)	Messanga *et al.*, 1992; Anuradha *et al.*, 2006; Pegnyemb *et al.*, 2003a
Calodenone (**253**)	*O. calodendron* (stem bark), *O. squarrosa* (root bark); *O. afzelii* (stem bark)	Messanga *et al.*, 1992; Anuradha *et al.*, 2006; Pegnyemb *et al.*, 2003a
Afzelone D (**254**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2003a
Calodenin A (**255**)	*O. calodendron* (stem bark)	Messanga *et al.*, 1994
Calodenin B (**256**)	*O. calodendron* (stem bark); *O. afzelii* (stem bark)	Messanga *et al.*, 1994; Pegnyemb *et al.*, 2003
Lophirone K (**257**)	*O. calodendron* (stem bark)	Messanga *et al.*, 1994
Calodenin C (**258**)	*O. calodendron* (stem bark)	Messanga *et al.*, 1998
Afzelone A (**259**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2003
Afzelone B (**260**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2003
Afzelone C (**261**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2003
Isolophirone C (**262**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2001
Dihydrolophirone C (**263**)	*O. afzelii* (stem bark)	Pegnyemb *et al.*, 2001
Isocampylospermone A (**264**)	*O. integerrima* (stem bark)	Ichino *et al.*, 2006
Campylospermone A (**265**)	*O. integerrima* (stem bark)	Ichino *et al.*, 2006
Amentoflavone (**266**)	*Ochna schweinfurthiana* (stem bark)	Ndongo *et al.*, 2015

Compounds	Plants species (parts)	References
Agathisflavone (**267**)	*Ochna schweinfurthiana* (stem bark)	Ndongo *et al.*, 2015
6'''-Hydroxylophirone B (**268**)	*O. integerrima* (stem bark)	Kaewamamatawong *et al.*, 2002
6'''-Hydroxylophirone B 4'''-*O*-*β*-glucoside (**269**)	*O. integerrima* (stem bark)	Kaewamamatawong *et al.*, 2002
3-(2,4-Dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl)ethenyl ketone (**270**)	*O. integerrima* (stem bark)	Kaewamamatawong *et al.*, 2002
3-(2,4-Dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-ydroxyphenyl)ethyl ketone (**271**)	*O. integerrima* (stem bark)	Kaewamamatawong *et al.*, 2002
7-*O*-Methyltetrahydroochnaflavone (**272**)	*O. beddomei* (leaves)	Jayaprakasam *et al.*, 2000
Ochnaflavone (**11**)	*O. squarrosa* (leaves) *O. lanceolata* (stem bark)	Okigawa *et al.*, 1976 Reddy *et al.*, 2008
Ochnaflavone 4'-*O*-methyl ether (**273**)	*O. squarrosa* (leaves)	Okigawa *et al.*, 1976
Ochnaflavone 7,4'-*O*-methyl ether (**274**)	*O. squarrosa* (leaves)	Okigawa *et al.*, 1976
2,3-Dihydroochnaflavone 7''-*O*-methyl ether (**275**)	*O. lanceolata* (stem bark)	Reddy *et al.*, 2008
2,3-Dihydroochnaflavone (**276**)	*O. lanceolata* (stem bark)	Reddy *et al.*, 2008
7,4',7'',4'''-Tetramethylisochamaejasmin (**277**)	*O. lanceolata* (stem bark)	Reddy *et al.*, 2008
Lophirone H (**278**)	*O. squarrosa* (stem bark)	Anuradha *et al.*, 2006
Lophirone L (**279**)	*O. squarrosa* (stem bark)	Anuradha *et al.*, 2006



252: $R^1 = R^2 = H$
253: $R^1 = H$, $R^2 = CH_3$
254: $R^1 = R^2 = CH_3$



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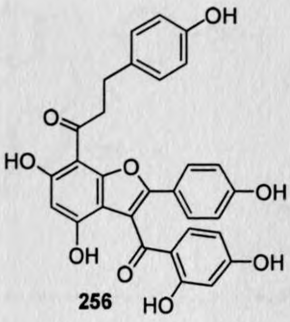

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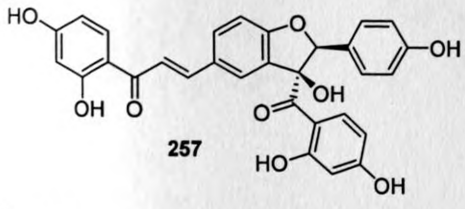

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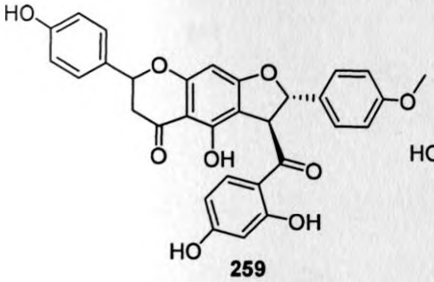

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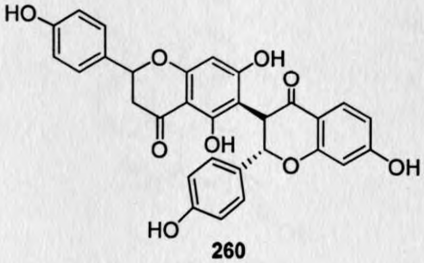

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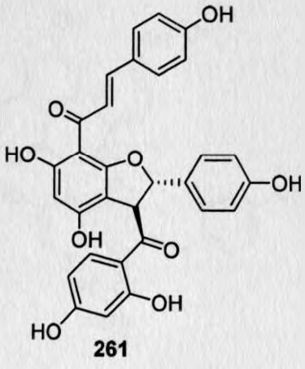

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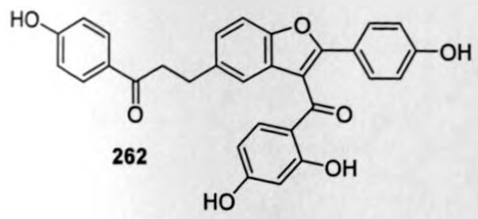

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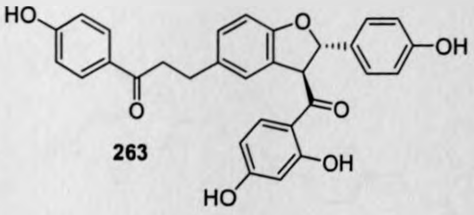

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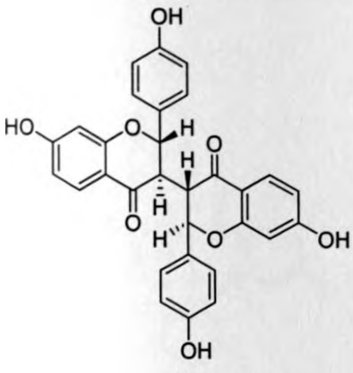

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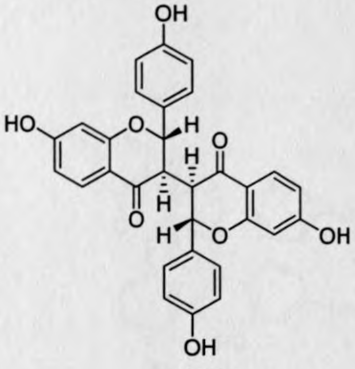

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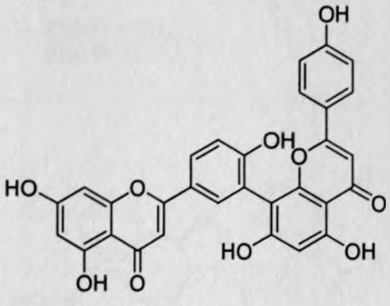

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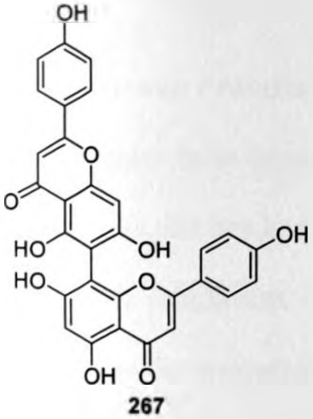

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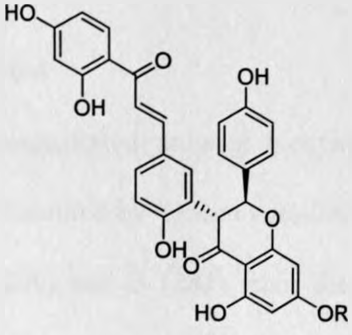
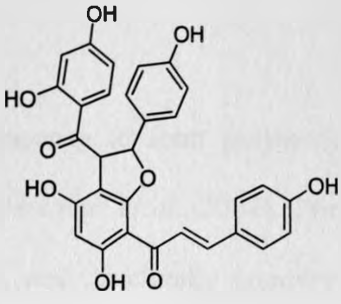


268: R = H
269: R = β-Glu



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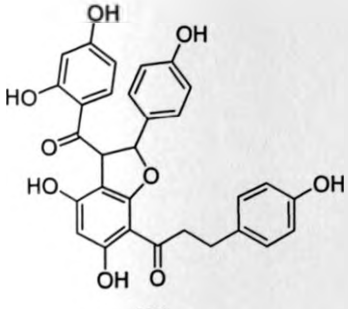

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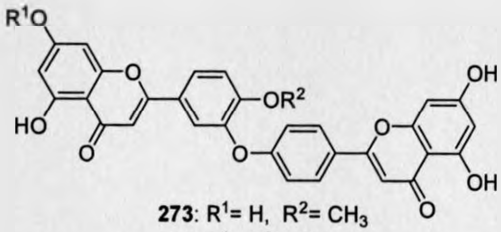
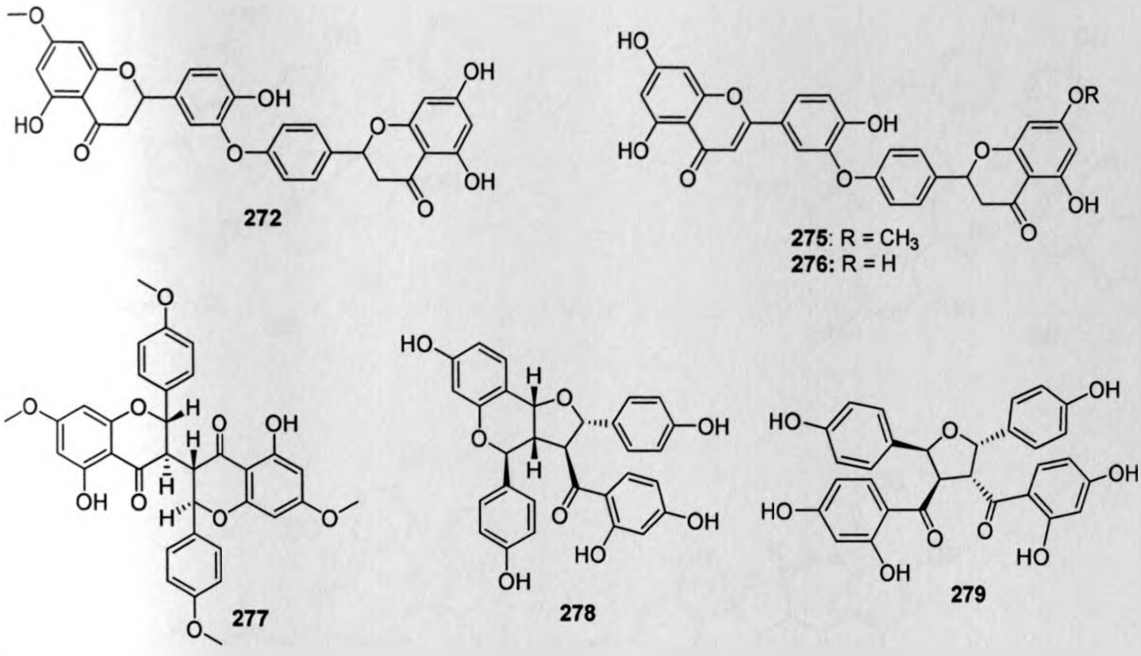
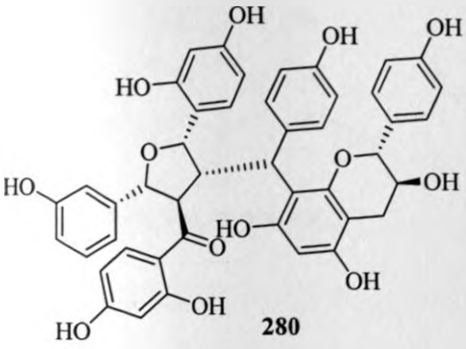


273: R^1= H, R^2= CH_3
274: R^1= R^2 = CH_3

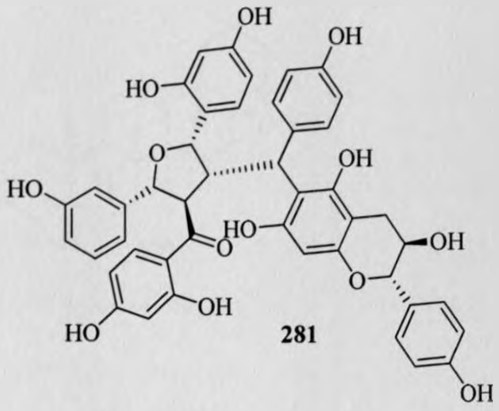


2.9.3 Tri- and Pentaflavonoid

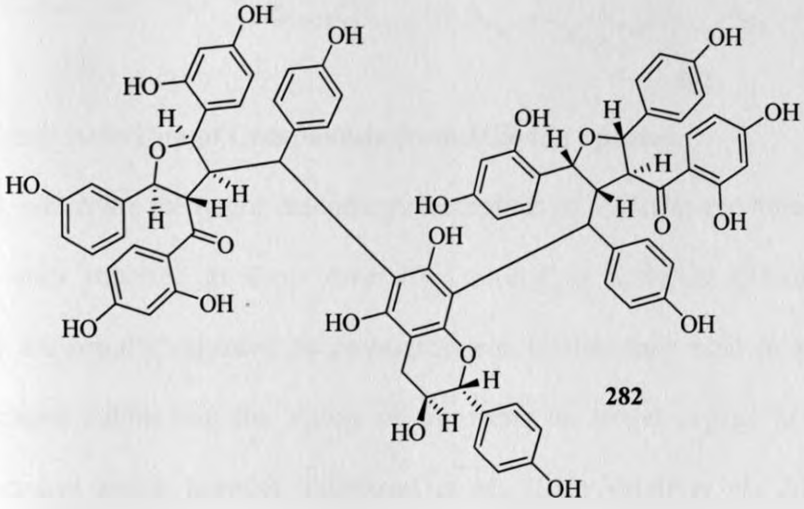
Ochna species have also demonstrated unusual biosynthetic capacity to form polymeric flavonoids as this has been illustrated by *Ochna calodendron* (Messanga *et al.*, 2002). Two triflavonoid; caloflavans A (**280**) and B (**281**) from the leaves, and structurally complex, pentaflavonoids; ochnachalcone (**282**) from the stem bark of *Ochna calodendron* have been identified and reported (Messanga *et al.*, 2001).



280



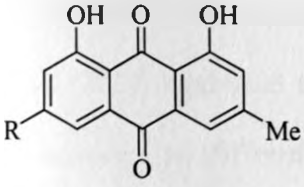
281



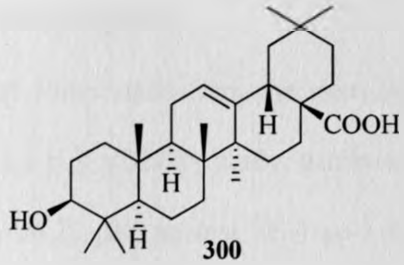
282

Table 2.12. Minor compounds from *Ochna* species (anthranoid, terpenoid and fatty acid)

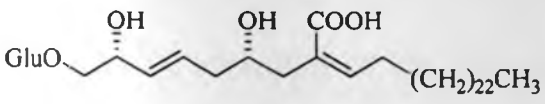
Compounds	Plant species (parts)	References
Chrysophanol (**298**)	*O. squarrosa* (root bark)	Anuradha *et al.*, 2006
Przewalskinone B (**299**)	*O. obtusata* (stem bark)	Sivaprakasam *et al.*, 1997
Oleanolic acid (**300**)	*O. squarrosa* (heartwood)	Messanga *et al.*, 1998
Calodendroside B (**301**)	*O. calodendron* (root bark)	Messanga *et al.*, 2001
Calodendroside C (**302**)	*O. calodendron* (root bark)	Messanga *et al.*, 2001



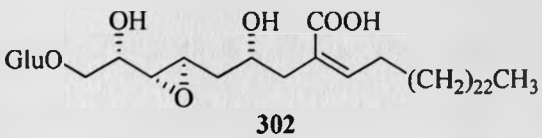
298: R = H, **299**: R OMe



300



301



302

2.10 Biological Activities of Compounds from *Millettia* Species

Isoflavonoids, which are the major secondary metabolites of *Millettia* and other Leguminosae plants, have been reported to show diversified biological activities (Miadokova, 2009). Isoflavonoids are usually regarded as phytoestrogens in that they bind to both α- and β-estrogen receptors mimicking the action of estrogens on target organs to cause various hormone-dependent health benefits (Pilšáková *et al.*, 2010; Vitale *et al.*, 2012). The anti-oxidant property of flavonoids has been the most studied and, the level of activity depends upon the arrangement of functional groups on the nuclear structure. In general, the configuration, substitution, and position/number of hydroxyl groups considerably infuence the mechanisms (radical scavenging and metal ion chelation) of anti-oxidant activity of flavonoids (Kumar *et al.*, 2013). The anti-oxidant property of flavonoids offers an additional important mechanism through which they protect against chronic diseases (Miadokova, 2009). Flavonoids have been also known for their antibacterial, hepatoprotective, anti-inflammatory, anticancer, and antiviral activities (Kumar et al., 2013).

2.10.1 Anticancer Activities of Compounds from *Milletti* Species

Fang *et al.* (2010) evaluated the anticancer activity of compounds from the stem bark of *Millettia reticulata* on different human cancer cells. In a cell viability study, genistein (**34**) showed the strongest inhibitory activity with an IC_{50} 16.23 μM against SK-Hep-1 human hepatocellular carcinoma cells. That showed observed apoptotic induction in SK-Hep-1 cells by genistein (**34**), could be due to loss of mitochondrial membrane potential and an activation in the protein expression of Fas, FasL, and p53 (Fang *et al.*, 2010). The isoflavone, brandisianin D (**81**) with a C-7/8 pyran ring isolated from the leaves of *M. brandisiana* exhibited death-receptor 5 expression enhancement activity in a luciferase assay based in DLD-1/*Sac*I cells suggesting that compound **81** might overcome tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-resistance by an increase in DR5 expression (Kikuchi *et al.*, 2007).

In the search for cancer chemopreventive agents, the inhibitory effect of auriculasin (**69**) isolated from *M. taiwaniana* and *M. auriculata* (Minhaj *et al.*, 1976) was investigated, and the compound exhibited significant inhibitory effect on mouse skin tumor promotion in an in *vivo* two-stage carcinogenesis test (Ito *et al.*, 2004). The cytotoxic and apoptotic effects of the constituents of the seeds of *M. pachycarpa* were also reported (Ye *et al.*, 2012). The studies of the cytotoxic effects against some cancer cell lines (HepG2, C26, LL2 and B16) and apoptotic effects against HeLa-C3 revealed that the isoflavonoid derivatives barbigerone (**55**), deguelin (**152**), tephrosin (**153**) and millepachine (**185**) demonstrated significant cytotoxic and apoptotic effects against cancer cells (Ye *et al.*, 2012).

Furthermore, pervilleanone (**9**) and 3'-*O*-demethylpervilleanone (**10**), isolated from *M. pervilleana* showed mild growth inhibitory activities against human lung (NCI-H460), breast

(MCF7) and CNS (SF-268) cancer cell-lines (Palazzino *et al.*, 2003). Phrutivorapongkul *et al.* (2003) also determined the cytotoxic activity of two chalcones (**176** and **178**) from the stem bark of *M. leucantha* against human lung cancer cell line, NCI-H460, and a moderate activity was observed for both chalcones; (**178**) showed better toxicity suggesting the importance of the 2'-OCH_3 of the ring A.

2.10.2 Anti-inflamatory Activity of Compounds from *Millettia* Species

Chronic inflammation is associated with increase in risk factors for different cancers; and importantly, eliminating inflammation may indirectly mean preventing and treating a cancer (Rayburn *et al.*, 2009). In search for anti-inflammatory agents from *Millettia*, Phrutivorapongkul *et al.* (2003) evaluated the anti-inflamatory activity of chalcones and flavones of *M. leucantha*. Among the compounds evaluated, the flavone **171** showed a significant inhibition of both cyclo-oxygenases, COX-1 and COX-2, which are mediators of inflammation with a preferential effect on COX-2 over COX-1.

The constituents of *M. dielsiana* were also evaluated for their potential to inhibit production of NO and TNF-α (Ye *et al.*, 2014). That is, the tetra-methoxylated and prenylated isoflavone, millesianin C (**106**) showed the most potent anti-inflammatory effect, decreasing nitric oxide (NO) production (1.37 ± 0.10 μM), similar to that of the positive control, dexamethasone and decreasing TNF-α secretion (9.33 ± 1.08 ng/ml) better than that the control. The other tested compounds by Ye *et al.* (2014) for anti-inflammatory activity showed moderate to no activities with the results revealing the importance of 2,2-dimethylchromene moiety in increasing the inhibitory activity of NO production and TNF-α secretion. The C-2′/C-6 methoxyl groups were also observed to be an additional requirement for the enhancement of inhibitory activity of NO production and TNF-α secretion; while the formation

methylenedioxy at C-4′ and C-5′ is only responsible for the decreasing of NO production inhibitory activity (Ye *et al.*, 2014).

2.10.3 Antiplasmodial Activities of Compounds from *Millettia* Species

In Yenesew *et al.* (2003), the antiplasmodial activity of the chemical constituents of *M. usaramensis* ssp. *usaramensis* [chalcone, rotenoids and isoflavones] has been reported with the chalcone, 4'-*O*-geranylisoliquiritegin (**181**) being the most active (IC_{50}: 8.7 and 10.6 µM against chloroquine-resistant (W2) and chloroquine sensitive (D6) strains of *Plasmodium falciparum*, respectively. Moreover, moderate activities were exhibited for rotenoids; usararotenoid C (**154**), usararotenoid A (**139**), dihydrousararotenoid A (**140**), 12a-epimillettosin (**137**) and dehydromillettone (**151**). Compounds having a prenyl or 2,2-dimethylpyrano substitution were observed to be the most active. The complete loss of activity in compound **140** compared to **139** (with 12-carbonyl group) could suggest the importance of the 12a-carbonyl functionality. Similarly, Derese *et al.* (2014) evaluated the antiplasmodial activity of some isoflavone derivatives isolated from stem bark of *M. oblata* ssp. *teitensis* and *M. dura*, in which the isoflavone 4'-prenyloxyderrone (**119**) from *M. oblata* ssp. *teitensi*, showed moderate activity at IC_{50} 14.9 and 13.3 µM against chloroquine-resistant (W2) and chloroquine sensitive (D6) strains of *Plasmodium falciparum*, respectively.

2.10.4 Insectcidal and Larvicidal Activities of Compounds from *Millettia* Species

Rotenoids are well known for their larvicidal and insectcidal activity (Fang and Casida, 1998). In recent study, Bosire *et al.* (2014) revealed that usararotenoid A (**139**), a rotenoid from *M. usaramensis* ssp. *usaramensis* with *trans*-B/C ring junction and methylenedioxy group at C-2/C-3 showed high larvicidal activity (LC_{50} 4.3 ± 0.8 µg/mL, at 48 h) against the 4th instar *A. aegyptii*. This observation is to the contrary to the earlier finding that rotenoids with *trans*-

B/C ring junction are less insecticidal (Joseph and Casida, 1992) than the *cis*-rotenoid. This suggests that the mechanism of action of usararotenoid A (**139**) as larvicidal could be different from the other *cis*-fused rotenoids.

2.10.5 Estrogenic Activity of Compounds from *Millettia* Species

The prenylated isoflavonoids millewanin G (**59**), millewanin H (**60**) and furowanin B (**61**) from the leaves of *M. pachycarpa* were tested for their anti-estrogenic activity (under non-cytotoxic conditions to the cells) based on their inhibition of β-galactosidase activity induced by 17β-estradiol (E2) in the yeast two-hybrid assay (Ito *et al.*, 2006). These isoflavones exhibited potent and dose-dependent inhibitory effects on β-galactosidase activity induced by E2 with IC_{50} values of 29, 18, and 13 μM, respectively, with furowanin B (**61**) exhibiting the most significant inhibition (Ito *et al.*, 2006). Okamoto *et al.* (2006) also evaluated several prenylated isoflavones from *M. pachycarpa* for their anti-estrogenic activity and only genistein (**34**) that exhibited estrogenic activity.

2.11 Biological Activities of Compounds isolated from *Ochna* Species

The bioactivity of the crude extracts from different *Ochna* species have been evaluated to reveal a wide range of interesting biological activities. The promising analgesic and anti-inflammatory activities of the root bark extract of *O. squarrosa* (Anuradha *et al.*, 2006); antibacterial activity of the acetone and methanol extracts of leaves of *O. schweinfurthiana* (Abdulahi *et al.*, 2010); antiviral, antiparasitic and anti-plasmodial activities of of stem bark extracts of *O. integerrimma* (Ichino *et al.*, 2006); antioxidant and allelopathic activity of hydroalcoholic extracts of leaves and stems of *O. serrulata* (Colla *et al.*, 2011) have been reported, justifying the uses of this plant in the traditional medicine.

The biflavone, ochnaflavone (**11**) isolated from different *Ochna* species was evaluated against growth inhibitory activity in cultured human colon cancer cell line HCT-15 and showed the inhibitory at IC_{50} 4.1 μM (Kang *et al.*, 2009). Another dimeric flavonoid, calodenin B (**256**), identified in the stem bark of *O. calodendron*, was analyzed for cytotoxic activity against the human MCF-7 breast cancer cells and showed IC_{50} value of 56±7μM. Moreover, the biflavones from stem bark of *Ochna schweinfurthiana*, amentoflavone (**266**) and agathisflavone (**267**) also showed good cytotoxic activity, IC_{50} values of 20.7 and 10.0 μM, respectively against cervical adenocarcinoma (HeLa) cells (Ndongo *et al.*, 2015).

Ichino *et al.* (2006) isolated two antiplasmodial principles as isocampylospermone A (**264**) and campylospermone A (**265**) from *O. integerrima* with **264** showing a potent activity against the multidrug resistant strain of *Plasmodium falciparum* (K1, IC_{50} 0.08μg/ml) and against the sensitive strain (FCR3, IC_{50} 0.26 μg/ml). Campylospermone A (**265**) which is a steroisomer of **264** showed significantly lower activity than **264**, IC_{50} 5.2 and 4.5 μg/ml for K1 and FCR3, respectively demonstrating the effects of configrution in this antiplasmodial activity. Prenylated and glycosylated monomeric flavonoids from *O. integerrima* (**288**, **289**, **290** and **291**) were also examined for anti-HIV-1 activity and showed significant activities, with EC_{50} values ranging from 14.0 to 102.4 μg/ml. The biflavonoid, 2",3"-dihydroochnaflavone 7"-*O*-methyl ether (**267**) was also tested and found to be very active in the syncytium assay (EC_{50} 0.9 μg/ml) while a potent inhibition of HIV-1 in reverse transcriptase (RT) assay with IC_{50} 2.4 μg/ml (Reutrakul *et al.*, 2007). Moreover, two monomeric (**292** and **293**) and a dimeric (**279**) flavonoids from the root bark of *O. squarrosa* exhibited significant analgesic (tail-flick technique) and anti-inflammatory activities (carrageenan induced paw oedema method) (Anuradha *et al.*, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1 General

Melting points were obtained on a Büchi Melting point B-545 Switzerland apparatus, optical rotations were measured on Perkin Elmer 341-LC and CD experiments were run on a Jasco J-715 spectropolarimeter. UV spectra were recorded on a Specord S600 (Analytik Jena AG) and Molecular Devices SpectraMax M2 spectrophotometers. NMR spectra were acquired on Varian M-200, Varian MR-400, Varian VNMR-S 500, Bruker Avance II 600 and Bruker Avance III HD 800 spectrometers, using the residual solvent peaks as reference. The spectra were processed using MestReNova 10.0 software. LC-ESIMS were acquired on a Perkin-Elmer PE SCIEX API 150EX instrument equipped with a Turbolon spray ion source connected to a Gemini 5 mm RPC_{18} 110 Å column, and applying a H_2O/MeCN 80:20-20:80 gradient with a separation time of 8 minutes. HRMS (EI) spectra were obtained on a Micromass GC-TOFmicro mass spectrometer (Micromass, Wythenshawe, Waters Inc., UK), using direct inlet and 70 eV ionization voltage. TLC analyses were carried out on Merck pre-coated silica gel 60 F_{254} plates. Prep-TLC was done on a glass plates of 20 x 20 cm dimension, pre-coated with silica gel $60F_{254}$ having 0.25 to 1 mm thickness. Column chromatography was run on silica gel 60 Å (70-230 mesh). Gel filtration was performed on Sephadex LH-20. Preparative HPLC was carried out on a Waters 600E instrument using the Chromulan (Pikron Ltd) software and an RP C_8 Kromasil® (250 mm x 55 mm) column eluting with a H_2O/CH_3OH mixtures. Enantiomeric purity analyses of oblarotenoid A (**318**) and 9-methoxy-2,3-methylenedioxyrotenoid (**320**) were performed using Chiral HPLC chromatography on Varian 9012Q coupled with Varian 9050 UV-Vis detector (eluent: HPLC

grade *n*-hexane and isopropanol, 19:1). X-ray data were acquired using an Agilent SuperNova Dual diffractometer with Atlas detector at T = 123.0(1) K using mirror-monochromatized Cu-Kα radiation (λ = 1.54184 Å).

3.2 Plant Materials

All study plant materials used in this study were collected from Kenya. The root bark and leaves of *Millettia usaramensis* ssp. *usaramensis* (Voucher No. Mathenge 2008/374); (Voucher No. TD-04/2014) were collected from Jadini forest, Coastal region in February 2008 and July 2014, respectively. The root bark of *Millettia dura* (Voucher No. TD-03/2013) was collected from Mulathankari, Meru County (GPS: Latitude N 0, 3 min, 17.5S, longitude E37, 40 mn, 32.4S and altitude 1492.5 m) in 2013 whereas the roots and leaves of *Millettia oblata* ssp *teitensis* (Voucher No. TD-04/2014) were collected from Ngaongao forest, Taita Hill, Taita County in July, 2014. The stem bark of *Ochna ovata* (Voucher No.TD-01/2012), stem bark and leaves of *Ochna holstii* (Voucher No. TD-02/2013) were collected from Ngong forest, Nairobi County in November, 2012 and June 2013, respectively. All plants were identified by Mr. S.G. Mathenge or Mr. Patrick Chalo Mutiso of the School of Biological Sciences, University of Nairobi, Kenya where specimen were deposited.

3.3 Extraction and Isolation

3.3.1 Extraction and Isolation of Compounds from the Root Bark of *M. dura*

The air-dried and ground root bark (1.5 kg) of *M. dura* was extracted with CH_2Cl_2/CH_3OH (1:1), four times each for 24 hrs by cold percolation at room temperature. The solvent was removed on a rotary evaporator to give 100 g of a light brown crude extract. A portion of the crude extract (90 g) was subjected to column chromatography on silica gel eluted with *n*-hexane containing increasing amounts of EtOAc. The fractions eluted with 2% EtOAc in *n*-

hexane were combined and further separated by column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2:CH_3OH, 1:1) to give 3-*O*-prenylmaakiain (**303**, 1.5 mg) which was further purified on reverse-phase preparative HPLC using CH_3OH:H_2O (70:30 to 90:10) in gradient decreasing of polarity. The fractions eluted with 2% EtOAc in *n*-hexane were combined and further separated by column chromatography on silica gel using *n*-hexane and EtOAc (4:1) to give calopogonium isoflavone B (**50**, 140.9 mg). maximaisoflavone B (**304**, 23.6 mg) and impure isoerythrin A-4'-(3-methylbut-2-enyl) ether (**94**). The purification of (**94**, 6.8 mg) was achieved by reverse-phase preparative HPLC using a CH_3OH:H_2O (60:40→90:10) in gradient decreasing of polarity.

Durmillone (**91**, 7.4 mg) was obtained from 6% EtOAc in *n*-hexane elution through crystallization in CH_3OH. The fractions eluted with 8-10% EtOAc were combined and subjected to column chromatography on silica gel using *n*-hexane/EtOAc (4:1) to give 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone (**51**, 156.6 mg). Similarly, 7-hydroxy-3',4',8-trimethoxyisoflavone (**305**, 7.1 mg) was obtained as colorless solid from the fractions eluted with 12-15% EtOAc in *n*-hexane of the original column. The fractions eluted with 25-30% EtOAc in *n*-hexane were combined and applied on Sepahedex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1 mixture) to give butein (**195**, 2.7 mg).

3.3.2 Extraction and Isolation of Compounds from the Roots of *M. oblata* ssp. *teitensis*

The air-dried roots (1.5 kg) of the roots of *M. oblata* ssp. *teitensis* was crushed and extracted as described in section 3.3.1 to give 100 g of the a light brown crude extract. A portion of the crude extract (95 g) was subjected to column chromatography on silica gel (500 g) using *n*-hexane containing increasing percentages of EtOAc to afford 142 eluents, each *ca.* 250 mL.

The 142 eluents were analysed by TLC and combined into nine major fractions (labeled as Fr. I-IX).

Fr.I (2-3%, EtOAc in *n*-hexane) gave 4'-prenyloxyderrone (**119**, 4.8 mg) and isoerythrin A - 4'-(3-methylbut-2-enyl) ether (**94**, 5.1 mg) after purification by column chromatography on silica gel (eluent: *n*-hexane/EtOAc; 9:1) and subsequently by reverse phase preparative HPLC (CH_3OH/ H_2O, 70:30→90:10) through gradient elution of decreasing polarity. Fr. II (3% EtOAc/*n*-hexane) showed five major spots on TLC and these were separated using column chromatography on silica gel eluting with *n*-hexane/EtOAc (90:10→60:40%) to give calopogonium isoflavone B (**50**, 9.0 mg), pseudobaptigenin (**308**, 6.4 mg), 2',7,-dimethoxy-4',5'-methylenedioxyisoflavone (**51**, 7.6 mg), durmillone (**91**, 15.1 mg) and additional amount of **94** (3.2 mg). Four compounds; 4'-hydroxyisoloncocarpin (**309**, 4.3 mg), 4-hydroxyloncocarpin (**182**, 11.2 mg) and 4-hydroxyderricin (**311**, 14.1 mg) and further amount of **91** (4.0 mg) were obtained from Fr. V by repetitive reverse phase preparative HPLC (gradient elution with CH_3OH/H_2O (60:40→90:10)).

Fraction VII (6-8% EtOAc in *n*-hexane) was a mixture of two compounds which were purified using column chromatography on silica gel eluting at 10%, 20%, 30% and 40% of EtOAc in *n*-hexane. The fraction at the 20% of this elution gave maximaisoflavone A (**307**, 10.9 mg) and the one at 30-40% EtOAc afforded additional amount of durmillone (**91**, 8.2 mg). The fraction eluted at 15-20% EtOAc in the original column (Fracion VIII) was subjected to column chromatography on Sephadex LH-20 using CH_2Cl_2/CH_3OH (1:1) to give butein (**195**, 5.5 mg) and an impure (+)-(2*R*,3*R*)-fustin (**310**, 9.0 mg), which was purified on reverse phase preparative HPLC using CH_3OH/H_2O (50:50→90:10) in a gradient elution of decreasing polarity. Similarly, Fr.IX, (100% EtOAc elution) was purified to give obloneside

(**306**, 102.1 mg) by Prep-HPLC (CH_3OH/H_2O, 30:70) and Sephadex LH-20 eluting with 100% CH_3OH.

3.3.3 Extraction and Isolation of Compounds from the Leaves of *M. usaramensis* ssp. *usaramensis*

The air-dried and powdered leaves of *M. usaramensis* ssp. *usaramensis* (2.5 kg) was extracted with (CH_2Cl_2/CH_3OH, 1:1) four times for 24 hours each, to yield a dark greenish extract (220 g) after removal of the solvent by rotary evaporator. A portion (120 g) of the crude extract was initially subjected to column chromatography on a silica gel (500 g) with gradient elution of EtOAc in *n*-hexane to give a total of 156 eluents, each *ca.* 250 mL. These eluents were analysed by TLC and combined to give ten major fractions (Fr. I-X). Lupenone (**315**, 8.7 mg) was obtained from 2% EtOAc in *n*-hexane (Fr. II) by chromatography over Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1) followed by crystallization from CH_3OH. The fractions collected at 4% EtOAc in *n*-hexane (Fr. IV) gave barbigerone (**55**, 270.0 mg) which was purified by crystallization (from 10% EtOAc in *n*-hexane).

The subsequent fraction, Fr. V (5% EtOAc in *n*-hexane) was subjected to column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1) and yielded toxicarol isoflavone (**37**, 5.3 mg) and a mixture of two other compounds. This mixture was separated by reverse phase preparative HPLC (gradient elution with CH_3OH/H_2O mixture, 50:50→90:10) to give deguelin (**152**, 6.0 mg) and tephrosin (**153**, 4.7 mg). Similarly, sarcolobin (**313**, 5.0 mg) was obtained from Fr VII (6% EtOAc/*n*-hexane) by reverse phase preparative HPLC using 40:60 ratio of H_2O:CH_3OH solvent system. Fr. VIII (8% EtOAc in *n*-hexane) contained two compounds which were separated by column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1) into 6-hydroxydeguelin (**314**, 3.4 mg) and 6-hydroxy-6a,12a-dehydrodeguelin (**122**, 2.1 mg). The fractions, eluted with 15% EtOAc in *n*-

hexane (Fr. IX) gave 3",4"-epoxybarbigerone (**312**, 8.5 mg) which precipitated as an amorphous solid from 20% EtOAc in *n*-hexane.

3.3.4 Extraction and Isolation of Compounds from the Leaves of *M. oblata* ssp. *teitensis*

The air-dried and ground leaves of *M. oblata* ssp *teitensis* (1.6 kg) was extracted using CH_2Cl_2/CH_3OH, 1:1 (4 x 3L) for 24 hours in each case and yielded 120 g of a dark green crude extract after the solvents were removed on rotary evaporator. A portion of the crude extract (110 g) was subjected to column chromatography (silica gel, 500 g) using *n*-hexane containing increasing amounts of EtOAc to give a total of 125 fractions each *ca.* 250 mL. The fractions 31-35, eluted with 3% EtOAc in *n*-hexane, were combined and subjected to column chromatography on Sephadex LH-20, (eluent: CH_2Cl_2/CH_3OH, 1:1) and yielded maximaisoflavone B (**304**, 4.2 mg) as amorphous colorless solid and impure form of maximaisoflavone J (**325**) which was combined with the subsequent fractions (38-44, eluted with 3% EtOAc in *n*-hexane) and purified by column chromatography on Sephadex LH-20, (CH_3OH/CH_2Cl_2, 1:1) to yield maximaisoflavone J (**325**, 8.4 mg).

Similarly, fractions 46-54, eluted with 5% EtOAc in *n*- hexane) were combined and subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1) to give needle-like crystals (from methanol) of 9-methoxy-2,3-dimethylenedioxyrotenoid (**320**, 33.4 mg) together with a mixture of **320** and oblarotenoid A (**318**). This mixture was purified on reverse phase preparative HPLC using CH_3OH/ H_2O (60:40→90:10) to give oblarotenoid A (**318,** 6.3 mg). The combined fractions (Fr. 55-62, 5% EtOAc in *n*-hexane) was subjected to column chromatography on Sephadex LH-20 using CH_2Cl_2/CH_3OH (1:1) to yield 6a,12a-dehydrodeguelin (**126**, 53.1 mg) as a yellowish solid (from methanol). The fractions eluted with 7% EtOAc in *n*-hexane (Fr. 67-71) showed the presence of six compounds on TLC

analysis. These fractions were combined and separted by column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1) to give a colorless solid of 8-prenylmilldurone (**316**, 17.9 mg) and two major sub-fractions labeled as 'sfr1' and 'sfr2'. The 'sfr1' was subjected to reverse phase preparative HPLC to give two compounds; munduserone (**322**, 5.4 mg) and tephrosin (**153**, 9.1 mg) (CH_3OH/H_2O, 60:40→90:10, decreasing polarity).

Similarly, sub-fractions ('sfr2') was separated on reverse phase preparative HPLC (CH_3OH/H_2O, 60:40→90:10, decreasing polarity) to afford oblarotenoid B (**319**, 4.0 mg), additional amount of oblarotenoid A (**318**, 7.6 mg) and oblarotenoid C (**321,** 1.4 mg). The fractions eluted with 9% ethyl acetate in *n*-hexane (Fr.74-77) were combined and applied on Sephadex LH-20 and eluted with CH_2Cl_2/CH_3OH, 1:1 to give additional amount of 8-prenylmilldurone (**316,** 3.0 mg) and a mixture of two other compounds (**317** and **323**). The two compounds; 12a-hydroxymunduserone (**323**, 4.3 mg, a colorless solid) and 7,2',5'-trimethoxy-3',4'-methylenedioxyisoflavone (**317** , 23.5 mg, a crystal in CH_3OH) were separated using column chromatography on silica gel using *n*-hexane:EtOAc (4:1)→(7:3). Purification of 2',6,7-trimethoxy-4',5'-methylenedioxyisoflavone (**324**, 7.0 mg), as a colorless amorphous solid (from CH_3OH) was done from the combined fractions (Fr. 85-90, 10-12% EtOAc in *n*-hexane) using Sephadex LH-20 with CH_2Cl_2/CH_3OH, 1:1 as eluent. Maximaisoflavone G (**115**, 98.7 mg), a colorless solid was precipitated from fractions 95-104 eluted with 20% EtOAc in *n*-hexane.

3.3.5 Extraction and Isolation of Compounds from Root Bark of *M. usaramensis* ssp. *usaramensis*

The dried and ground root bark of *M. usaramensis* ssp. *usaramensis* (1 kg) was extracted using CH_2Cl_2/CH_3OH, 1:1 mixture (3 × 3 L), for 24 hours in each case, yielding 110 g of a brown orange crude extract following concentration by rotary evaporator. Approximately 100 g of the crude extract was subjected to column chromatography on silica gel (500 g) eluting with *n*-hexane containing increasing percentage of EtOAc. The fractions eluting with 2% EtOAc in *n*-hexane gave colenemol (**330**, 110 mg) and millettosin (**138**, 12.1 mg). The fractions eluted with 3% EtOAc in *n*-hexane were combined and purified by crystallization from methanol to yield 12a-epimillettosin (**137**, 140.4 mg).

The combined fractions eluted with 4% EtOAc in *n*-hexane were further purified by crystallization from methanol, giving usararotenoid A (**139**, 86.0 mg). The mother liquid of this fraction was further separated by MPLC (elueting with *n*-hexane containing increasing amounts of CH_2Cl_2) to give 4'-*O*-geranylisoliquiritigenin (**181**, 95.0 mg), 4-*O*-geranylisoliquiritigenin (**326**, 94.7 mg) and a mixture of two compounds. The mixture was separated by reverse phase preparative HPLC, using the CH_3OH/H_2O (60:40→90:10) gradient elution to give usararotenoid C (**154**, 9.4 mg) and 12a-epimillettosin (**137**, 6.0 mg). Another portion of this fraction (4% EtOAc in *n*-hexane) was purified on Sephadex, using CH_2Cl_2/CH_3OH 1:1 as an eluent and gave maximaisoflavone H (**97**, 4.0 mg) and additional amount of usararotenoid A (**139**).

The fractions eluted by 5% EtOAc in *n*-hexane were combined and applied on column chromatography, (using CH_2Cl_2/hexane 8:2) to provide three sub-fractions. One of these was purified by reverse phase preparative HPLC (60:40→90:10) to give 7-*O*-geranyl-5-

hydroxyflavanone (**329**, 2.6 mg). Tephrosin (**153**, 2.5 mg) was obtained from the fractions eluted with 6% EtOAc in *n*-hexane, after purification on Sephadex with CH_2Cl_2/CH_3OH (1:1) and subsequently by preparative RP-HPLC, using CH_3OH/H_2O gradient elution (60:40→90:10). The fractions eluted with 7% EtOAc in *n*-hexane were combined and subjected to CC on Sephadex, LH-20 using CH_2Cl_2/CH_3OH, 1:1 followed by Prep-HPLC purification (CH_3OH/H_2O, 60:40) to give (**327**, 11.4 mg), (**328**, 27.6 mg), and further amounts of (**153**, 1.8 mg). The combined fractions eluted with 11-15% EtOAc in *n*-hexane was crystallized (from methanol) to afford 12-dihydrousararotenoid A (**140**, 157.8 mg).

3.3.6 Extraction and Isolation of Compounds from the Stem Bark of *O. holstii*

The dried and milled stem bark of *Ochna holstii* (2.4 kg) was exhaustively extracted with CH_3OH and afforded a dark brown crude extract (280 g). The extract was initially partitioned between EtOAc and H_2O (4:1) three times to afford EtOAc extract (120 g) after removal of the solvent by rotary evaporator. A portion of the EtOAc extract (70 g) was subjected to column chromatography on silica gel (400 g) and fractionated using 20, 30, 40, 50, 60, 80, 100% EtOAc in *n*-hexane, each of *ca.* 500 mL.

The fractions eluted with 50% EtOAc (in the original column) were analyzed by TLC and combined to give four major fractions labeled as Fr.50-I, Fr.50-II, Fr.50-III and Fr.50-IV. Fraction (Fr.50-IV) was then subjected to column chromatography on silica gel (100 g) eluting with CH_2Cl_2/ EtOAc mixtures, to give four subfractions: (100% EtOAc, Fr.50-IVa); (4:1, Fr.50-IVb); (3:2, Fr.50-IVc) and (1:1, Fr.50-IVd) of CH_2Cl_2 in EtOAc. Purification of the sub-fraction Fr.50-IVc on Sephadex LH-20 column using CH_3OH as eluent then gave lophirone A (**253**, 38.6 mg) and calodenone (**252**, 47.1 mg). The fraction, Fr.50-II was subjected to reverse phase preparative HPLC purification using CH_3OH/ H_2O (40:60→90:10)

in gradient elution and yielded three compounds namely; 4',5-dimethoxy-6,7-methylenedioxyflavone (**333**, 6.2 mg), 3',4',5-trimethoxy-6,7-methylenedioxyflavone (**295**, 7.7 mg) and afzelone D (**254**, 4.8 mg). (±)-Catechin (**334**, 600 mg), the major compound of this plant, was obtained as amorphous crystal (from CH_3OH) from fraction (Fr-50-III), from the 50% EtOAc in the original column collected at 50% EtOAc/*n*-hexane. The purification of 2,4-dihydroxyphenylmethylacetate (**335**, 8.4 mg) was achieved after the sub-fraction, Fr-IVd was purified by preparative HPLC (CH_3OH/H_2O, 1:1) along with additional amount of lophirone A (**252**, 16.1 mg) and calodenone (**253**, 5.3 mg).

3.3.7 Extraction and Isolation of Compounds from the Leaves of *O. holstii*

The air-dried and ground leaves of *Ochna holstii* (1.5 kg) was extracted with CH_3OH to give 250 g of crude extract. A portion of this extract (70 g) was subjected to column chromatography on silica gel eluting with *n*-hexane containing increasing amounts (10, 25, 35%, 50, 65, 85 and 100%) of EtOAc to give a total of 75 eluents which were combined into seven major fractions after TLC analysis.

The fractions eluted with 25% EtOAc in *n*-hexane were combined and purified over Sephadex LH-20 column eluting with CH_2Cl_2/CH_3OH, 1:1 to give 4-hydroxyloncocarpin (**182**, 6.0 mg). The fractions eluted with 50% EtOAc in *n*-hexane (Fr. 47-52) were combined and separated as above to afford 2",3"-dihydroochnaflavone-7-*O*-methyl ether (**275**, 37.7 mg), 2",3"-dihydroochnaflavone (**276**, 20.8 mg), and ochnaflavone-7-*O*-methyl ether (**331**, 28.4 mg). Ochnaflavone (**11**, 18.3 mg) and further amount of **276** (4.1 mg) were obtained upon column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1) of the fractions (Fr. 54-57) eluted with 65% EtOAc in *n*-hexane. The fractions (Fr. 60-65) eluted at 65% were combined and a portion of this combined fractions were subjected to column chromatography

on Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1) to afford additional amount of ochnaflavone (**11**, 3.7 mg) and a mixture of **276** and lophirone A (**252**). Further purification of this mixture on preparative TLC using CH_2Cl_2/EtOAc (4:1) yielded lophirone A (**252**, 6.1 mg) and further amounts of **275** (2.5 mg). Dasycarponin (**332**, 7.3 mg) was obtained as needles upon crystallization (from CH_3OH) of the fraction eluted with 100% EtOAc of the original column.

3.3.8 Extraction and Isolation of Compounds from the Root Bark of *O. ovata*

The root bark of *O. ovata* (1 kg) was air-dried, crushed and extracted (4 x 24 hours) with CH_2Cl_2/CH_3OH (1:1) to afford 100 g of deep brown and gummy crude extract. A portion of this extract (90 g) was initially adsorbed on a silica gel and subjected to vacuum liquid chromatography (VLC) using *n*-hexane containing increasing amounts of EtOAc. The fractions (Fr. 9-11) collected with 10% EtOAc in *n*-hexane were combined, subjected to column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/CH_3OH, 1:1) and further purified on reverse phase preparative HPLC (CH_3OH/H_2O, 60:40) to give maculine (**336**, 7.5 mg) and *N*-methylflidersine (**339**, 2.1 mg). Flindersiamine (**337**, 9.0 mg) and kokusaginine (**338**, 4.6 mg) were obtained from the fractions (Fr. 16-20) collected by 20% EtOAc in *n*-hexane, through similar treatment; Sephadex LH-20 (CH_2Cl_2/CH_3OH, 1:1) and then by preparative HPLC (CH_3OH/H_2O, 60:40); while lophirone A (**252**, 60.5 mg) and calodenone (**253**, 42.8 mg) were obtained from 50% EtOAc in *n*-hexane fractions upon column chromatographic separation on Sephadex LH-20, (CH_2Cl_2/CH_3OH, 1:1). Dasycarponin (**333**, 200.6 mg) was obtained by crystallization in CH_3OH of the fraction eluted with100% EtOAc.

3.4 Procedures for Structure Modification

3.4.1 Preparation of 12a-Deoxyusararotenoid A (140a)

To a solution containing 10 mg of 12-dihydrousararotenoid A (**140**) in 50 mL acetone, four drops of concentrated HCl were added and the solution was kept overnight. Purification of the product on preparative TLC using CH_2Cl_2/*n*-hexane (4:1) yielded colorless solid of 12a-deoxyusararotenoid-A (**140a**, 6 mg).

3.4.2 Preparation of 6a,12a-Dehydrousararotenoid A (140b)

A solution of 12-dihydrousararotenoid A (**140**, 15 mg) was refluxed in CH_3OH (10 ml) containing 10 drops of concentrated HCl over water bath for 1 hour. The product was purified by column chromatography on silica gel using CH_2Cl_2/hexane as eluent to give colorless solid of 6a,12a-dehydrousararotenoid A (**140b**, 9 mg).

3.4.3 Acetylation of Maximaisoflavone G (115)

Maximaisoflavone G (**115**, 20 mg) was dissolved in a mixture of pyridine (4 mL) and acetic anhydride (4 mL), and then refluxed at 60°C in heating mantle for 3 hours. The crude reaction mixture was then adsorbed in silica gel and subjected to column chromatography on silica gel (*n*-hexane/EtOAc, 8:3, eluent) to give an amorphous solid of 7-acetyl-2'-methoxy-4',5'-methylenedioxyisoflavone (**115a**) or 7-acetylmaximaisoflavone G (**115a**, 14.8 mg).

3.4.4 Hydrolysis of Obloneside (306)

To a solution containing 20 mg of obloneside (**306**) in 10 mL methanol, five drops of concentrated H_2SO_4 were added. This mixture was then refluxed at 60°C in heating mantle for 1 hour after which the crude reaction mixture was purified by column chromatography (silica

gel, *n*-hexane/EtOAc, 1:1) to give a colorless solid of 6-hydroxy-7,8-dimethoxy-4',5'-methylenedioxyisoflavone (**306a**, 8.1 mg).

3.5 X-ray Diffraction Analysis

Single crystals were obtained by slow diffusion of tetrahydropyrane into a 3:1 mixture of dichloromethane/acetonitrile solution of the complex. X-ray crystallographic data for 12a-epimillettosin (**137**), usararotenoid A (**139**), dihydrousararotenoid A (**140**), 9-methoxy-2,3-dimethylenedioxyrotenoid (**320**) and dasycarponin (**333**) were collected on an Agilent SuperNova Dual diffractometer with Atlas detector at $T = 123.0(1)$ K using mirror-monochromatized Cu-$K\alpha$ radiation ($\lambda = 1.54184$ Å). CrysAlisPro software was used for data collection, integration and reduction as well as applying the Analytical numeric absorption correction using a multifaceted crystal model.

For 12-dihydrousararotenoid A (**140**), the X-ray data collection was performed on an Bruker-Nonius KappaCCD diffractometer with an APEX-II detector at 173.0(1) K using graphite monochromatorized Mo-$K\alpha$ radiation ($\lambda = 0.71073$ Å), where, the data collection and reduction were done using the program *COLLECT* and *HKL DENZO AND SCALEPAC*, respectively. The intensities were corrected for absorption using SADABS. The structures were solved by Direct method with SHELXS and refined by full-matrix least-squares using SHELXL-2013 within OLEX2 package. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms in usararotenoid A (**139**) and 12a-epimillettosin (**137**) were located from difference Fourier maps and their positions and thermal parameters were refined freely. All the hydrogen atoms in 12-dihydrousararotenoid A (**140**), 9-methoxy-2,3-dimethylenedioxyrotenoid (**320**) and dasycarponin (**332**) were refined using riding models

with U_{eq}(H) of 1.5U_{eq}(parent) for terminal groups and 1.2 U_{eq} (parent) for non-terminal groups.

Usararotenoid A (139): 0.44×0.25×0.18 mm, $C_{18}H_{12}O_8$, M = 356.28, orthorhombic, space group $P2_12_12_1$, a = 6.84414(7) Å, b = 14.31429(11) Å, c = 14.76660(12) Å, α = 90°, β = 90°, γ = 90°, V = 1446.67(2) Å^3, Z = 4, ρ = 1.636 g cm^{-3}, μ = 1.122 mm^{-1}, F(000) = 736, 23102 reflections (θ_{max} = 76.62°) measured (3023 unique, R_{int} = 0.0272, completeness = 99.7%), Final R indices ($I > 2\sigma(I)$): R_1= 0.0284, wR_2 = 0.0764, R indices (all data): R_1= 0.0286, wR_2 = 0.0766. GOF = 1.061 for 283 parameters and 0 restraints, largest diff. peak and hole 0.218/–0.206 eÅ^{-3}. CCDC-1409159 contains the supplementary data for this structure.

12-Dihydrousararotenoid A (140): 0.41×0.27×0.24 mm, $C_{18}H_{14}O_8$, M = 358.29, monoclinic, space group $P2_1$, a = 9.5507(19) Å, b = 7.3479(15) Å, c = 11.163(2) Å, α = 90°, β = 109.85(3)°, γ = 90°, V = 736.9(3) Å^3, Z = 2, ρ = 1.615 g cm^{-3}, μ = 0.129 mm^{-1}, F(000) = 372, 4298 reflections (θ_{max} = 28.874°) measured (3291 unique, R_{int} = 0.0247, completeness = 91.0%), Final R indices ($I > 2\sigma(I)$): R_1= 0.0398, wR_2 = 0.0873, R indices (all data): R_1= 0.0520, wR_2 = 0.0946. GOF = 1.033 for 237 parameters and 1 restraints, largest diff. peak and hole 0.241/–0.203 eÅ^{-3}. CCDC-1409160 contains the supplementary data for this structure.

12a-Epimillettosin (137): 0.328×0.204×0.130 mm, $C_{22}H_{18}O_7$, M = 394.36, monoclinic, space group $P2_1$, a = 8.31466(12) Å, b = 9.32119(15) Å, c = 12.44700(19) Å, α = 90°, β = 109.1601(16)°, γ = 90°, V = 911.24(2) Å^3, Z = 2, ρ = 1.437 g cm^{-3}, μ = 0.904 mm^{-1}, F(000) = 412, 14242 reflections (θ_{max} = 76.32°) measured (3580 unique, R_{int} = 0.022, completeness = 99.5%), Final R indices ($I > 2\sigma(I)$): R_1= 0.0260, wR_2 = 0.0685, R indices (all data): R_1=

0.0260, wR_2 = 0.0687. *GOF* = 1.033 for 334 parameters and 1 restraints, largest diff. peak and hole 0.190/–0.133 eÅ^{-3}. CCDC-1061815 contains the supplementary data for this structure.

9-Methoxy-2,3-dimethylenedioxyrotenoid (320): 0.565×0.090×0.056 mm, 2 x $C_{18}H_{14}O_6$, M = 652.58, orthorhombic, space group $P2_12_12_1$, a = 5.19667(5) Å, b = 13.40708(9) Å, c = 42.3221(3) Å, α = 90°, β = 90°, γ = 90°, V = 2948.67(4) Å^3, Z = 4, ρ = 1.470 g cm^{-3}, μ = 0.936 mm^{-1}, F(000) = 1360, 74051 reflections (θ_{max} = 76.883°) measured (6232 unique, R_{int} = 0.030, completeness = 99.9%), Final R indices ($I > 2\sigma(I)$): R_1= 0.0297, wR_2 = 0.0746, R indices (all data): R_1= 0.0441, wR_2 = 0.0753. *GOF* = 1.061 for 435 parameters and 0 restraints, largest diff. peak and hole 0.213/–0.193 eÅ^{-3}. Absolute structure parameter x = 0.0(4). CCDC-1061111 contains the supplementary data for this structure.

Dasycarponin (332): 0.55×0.16×0.13 mm, $C_{14}H_{19}NO_8$, M = 329.30, monoclinic, space group $P2_12_12_1$, a = 8.26754(10) Å, b = 10.30873(10) Å, c = 17.80239(17) Å, α = 90°, β = 90°, γ = 90°, V = 1517.26(3) Å^3, Z = 4, ρ = 1.442 g cm^{-3}, μ = 1.022 mm^{-1}, F(000) = 696, 14060 reflections (θ_{max} = 66.750°) measured (2660 unique, R_{int} = 0.0321, completeness = 98.9%), Final R indices ($I > 2\sigma(I)$): R_1= 0.0245, wR_2 = 0.0632, R indices (all data): R_1= 0.0251, wR_2 = 0.0637. Flack = 0.02(5). *GOF* = 1.053 for 232 parameters and 0 restraint, largest diff. peak and hole 0.179/–0.149 eÅ^{-3}.

3.6 Physical Constants and Spectroscopic Data

3.6.1 Spectroscopic and Physical Data of Compounds from Root Bark of *M. dura*

3-*O*-Prenylmaackiain (303)

Colorless oil. ^{1}H NMR (Table 4.2); ^{13}C NMR (Table 4.2); ESIMS, $[M+H]^+$ *m/z*: 353.6, HRMS (EI) *m/z*: obs 353.1386, calcd. 353.1311 ($C_{21}H_{20}O_5$).

Calopogonium isoflavone B (50)

Colorless solid; mp 154-156 °C; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.91 (1H, *s*, H-2), 8.05 (1H, *d*, *J* = 9.0 Hz, H-5), 6.84 (1H, *d*, *J* = 9 Hz, H-6), 7.08 (1H, *d*, *J* = 1.8 Hz, H-2'), 6.86 (1H, *d*, 7.8 Hz, H-5'), 6.96 (1H, *dd*, *J* = 1.8, 7.8 Hz, H-6'), 5.51 (1H, *d*, *J* = 10.2 Hz, H-3"), 6.79 (1H, *d*, *J* = 10.2 Hz, H-4"), 1.49 (6H, *s*, 2x CH_3), 5.98 (2H, *s*, OCH_2O); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 151.9 (C-2), 125.7 (C-3), 175.7 (C-4), 118.3 (C-4a), 126.7 (C-5), 115.2 (C-6), 157.3 (C-7), 109.2 (C-8), 152.3 (C-8a), 124.8 (C-1'), 109.8 (C-2'), 147.6 (C-3'), 147.6 (C-4'), 108.4 (C-5'), 122.4 (C-6'), 77.7 (C-2"), 130.3 (C-3"), 114.9 (C-4"), 28.2 (C-5"/6"), 101.2 (OCH_2O); ESIMS, $[M+H]^+$ *m/z*: 349.3 for $C_{21}H_{16}O_5$.

Isoerythrin A-4'-(3-methylbut-2-enyl) ether (94)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.94 (1H, *s*, H-2), 8.06 (1H, *d*, *J* = 8.8 Hz, H-5), 6.86 (1H, *d*, *J* = 8.8 Hz, H-6), 7.48 (2H, *d*, *J* = 8.8 Hz, H-2'/6'), 6.98 (2H, *d*, *J* = 8.8 Hz, H-3'/5'), 5.72 (1H, *d*, *J* = 9.6 Hz, H-3"), 6.81 (1H, *d*, *J* = 9.6 Hz, H-4"), 1.50 (6H, *s*, CH_3-5"/6"), 4.55 (2H, *d*, *J* = 7.2 Hz, OCH_2-1'"), 5.51 (1H, *t*, *J* = 7.2 Hz, H-2'"), 1.80 (3H, *s*, CH_3-4'"), 1.76 (3H, *s*, CH_3-5'"); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 151.7 (C-2), 124.8 (C-3), 175.9 (C- 4), 118.4 (C-4a), 126.8 (C-5), 115.3 (C-6), 157.3 (C-7), 109.2 (C-8), 152.4 (C-8a), 124.1 (C-1'), 130.1 (C-2'), 114.7 (C-3'), 158.9 (C-4'), 114.7 (C- 5'), 130.1 (C-6'), 77.7 (C-2"), 130.3

(C-3"), 114.9 (C-4"), 28.2 (C- 5"/6"), 64.8 (C-1'"), 119.6 (C-2'"), 138.3 (C-3'"), 25.9 (C-4'"), 18.2 (C-5'"); ESIMS, $[M+H]^+$ *m/z*: 389.4 for $C_{25}H_{24}O_6$.

Maximaisoflavone B (304)

Colorless solid; ^1H NMR ($CDCl_3$, 399.94 MHz): δ 7.89 (1H, *s*, H-2), 8.18 (1H, *d*, *J* = 8.8, H-5), 7.09 (1H, *brs*, H-2'), 6.95-6.99 (2H, *m*, H-6, H6'), 6.84-6.86 (2H, *m*, H-8, H-5') 4.61 (2H, *d*, *J* = 6.8, OCH_2-1"), 5.51 (1H, *t*, *J* = 6.8, CH-2"), 1.83 (3H , *s*, CH_3-4"), 1.78 (3H, *s*, CH_3-5"); ESIMS, $[M+H]^+$ *m/z*: 351.6 for $C_{21}O_{18}O_5$.

Durmillone (91)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.55 (1H, *s*, H-2), 7.94 (1H, *s*, H-5), 7.10 (1H, *d*, *J* = 1.8 Hz, H-2'), 6.87 (1H, *d*, *J* = 7.8 Hz, H-5'), 6.98 (1H, *dd*, *J* = 1.8,7.8 Hz, H-6'), 5.74 (1H, *d*, *J* = 10.2 Hz, H-3"), 6.81 (1H, *d*, *J* = 10.2 Hz, H-4"), 1.56 (6H, *s*, CH_3-5"/6"), 5.99 (2H, *s*, OCH_2O), 3.96 (3H, *s*, OCH_3); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 151.8 (C-2), 124.4 (C-3), 175.5 (C- 4), 117.6 (C-4a), 105.1 (C-5), 147.2-147.7 (C-6), 147.2-147.7 (C-7), 110.2 (C-8), 147.2-147.7 (C-8a), 125.9 (C-1'), 109.2 (C-2'), 147.2-147.7 (C-3'), 147.2-147.7 (C-4'), 108.4 (C- 5'), 122.4 (C-6'), 78.2 (C-2"), 130.4 (C-3"),115.2 (C-4"), 28.0 (C- 5"/6"), 101.2 (OCH_2O), 56.4 (OCH_3-6); ESIMS, $[M+H]^+$ *m/z*: 379.4 for $C_{22}H_{18}O_6$.

7,2'-Dimethoxy-4',5'-methylenedioxyisoflavone (51)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.88 (1H, *s*, H-2), 8.18 (1H, *d*, *J* = 8.4 Hz, H-5), 6.98 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-6), 6.85 (1H, *d*, *J* = 2.4 Hz, H-8), 6.61 (1H, *s*, H-3'), 6.82 (1H, *s*, H-6'), 3.91 (3H, *s*, OCH_3-7) 3.72 (3H, *s*, OCH_3-2') 5.95 (2H, *s*, OCH_2O-4'/5'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 154.1 (C-2), 122.2 (C-3), 175.7 (C-4), 127.8 (C-5), 114.4 (C-6), 163.9 (C-7), 100.2 (C-8), 157.9 (C-8a), 112.8 (C-1'), 95.4 (C-3'), 148.4 (C-4'), 141.2

(C-5'), 111.2 (C-6'), 55.8 (OCH_3-7), 56.9 (OCH_3-2'), 101.4 (OCH_2O-4'/5'); ESIMS, $[M+H]^+$ *m/z*: 327.2 for $C_{18}H_{14}O_6$.

7-Hydroxy-8,3',4'-trimethoxyisoflavone (305)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 8.01 (1H, *s*, H-2), 7.97 (1H, *d*, *J* = 8.4 Hz, H-5), 7.05 (1H, *d*, *J* = 8.4 Hz, H-6), 7.19 (1H, *d*, *J* = 2.4 HZ, H-2'), 6.93 (1H, *d*, *J* = 8.4 Hz, H-5'), 7.05 (1H, *dd*, *J* = 2.4, 8.4 Hz, H-6'), 4.08 (3H, *s*, OCH_3-8), 3.93 (3H, *s*, OCH_3-3'), 3.91 (3H, *s*, OCH_3-4'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 151.7 (C-2), 124.8 (C-3), 175.9 (C-4), 118.9 (C-4a), 122.1 (C-5), 114.0 (C-6), 153.2 (C-7), 133.9 (C-8), 150.0 (C-8a), 124.3 (C-1'), 148.8 (C-3'), 149.2 (C-4'), 111.1 (C-5'), 121.1 (C-6'), 61.9 (OCH_3-8), 55.9 (OCH_3-3'), 55.9 (OCH_3-4'). ESIMS), $[M+H]^+$ *m/z*: 329.5 for $C_{18}H_{16}O_6$.

Butein (195)

Yellow solid turned brown on exposure to air on TLC. ^1H NMR (CD_3OD, 799.87 MHz): δ 7.28 (1H, *d*, *J* = 2.4 Hz, H-2), 6.92 (1H, *d*, *J* = 8.8 Hz, H-5), 7.22 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-6), 7.64 (1H, *d*, *J* = 15.2, H-α), 7.82 (1H, *d*, *J* = 15.2 Hz, H-β), 6.39 (1H, *d*, *J* = 2.4, H-3'), 6.52 (1H, *dd*, *J* = 2.4, 9.6 Hz, H-5'), 8.06 (1H, *d*, *J* = 9.6 Hz, H -6'); ^{13}C NMR (CD_3OD, 201.15 MHz): δ 127.1 (C-1), 114.5 (C-2), 145.5 (C-3), 148.6 (C-4), 115.3 (C-5), 122.3 (C-6), 116.9 (C-α), 144.8 (C-β), 192.2 (C=O), 113.4 (C-1'), 165.1 (C-2'), 102.5 (C-3'), 166.2 (C-4'), 107.8 (C-5'), 131.9 (C-6'). ESIMS, $[M+H]^+$ *m/z*: 273.3 for $C_{15}H_{12}O_5$.

3.6.2 Spectroscopic and Physical Data of Compounds from Roots of *M. oblata* ssp. *teitensis*

Obloneside (306)

Colorless solid in methanol; mp 190-192 °C. UV (λ_{max}, CH_3OH): 290 nm; ^1H NMR (799.87, CH_3OD) see Table 4.6; ^{13}C NMR (201.15, CH_3OD) see Table 4.6; HREIMS, $[M+H]^+$ *m/z*: 959.3017; ESIMS *m/z*: 959.5 (calcd. 959.2954) for $C_{42}H_{54}O_{25}$.

Isoplatycarpanetin (306a)

Colorless solid; ^1H NMR (799.87, $CDCl_3$) see Table 4.6; ^{13}C NMR (201.15, $CDCl_3$) see Table 4.6; ESIMS, $[M+H]^+$ *m/z*: 343.1 (calcd. 343.1) for $C_{18}H_{14}O_7$.

Pseudobaptigenin methyl ether (307)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.91(1H, *s*, H-2), 8.20 (1H, *d*, *J* = 8.8 Hz, H-5), 6.99 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-6), 6.85 (1H, *d*, *J* = 2.4 Hz, H-8), 7.09 (1H, *d*, *J* = 1.6 Hz, H-2'), 6.87 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.98 (1H, *dd*, *J* = 1.6, 8.0 Hz, H-6'), 3.92 (3H, *s*, OCH_3-7), 5.99 (2H, *s*, OCH_2O-3'/4'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 154.9 (C-2), 127.7 (C-3), 178.4 (C-4), (121.0C-4a), 130.5 (C-5), 117.3 (C-6), 166.7 (C-7), 102.8 (C-8), 166.6 (C-8a), 128.4 (C-1'), 112.4 (C-2'), 150.3 (C-3'), 150.3 (C-4'), 111.0 (C-5'), 125.0 (C-6'), 58.5 (7-OCH_3), 103.8 (3'/4'-OCH_2O); ESIMS, $[M+H]^+$ *m/z*: 297.2 for $C_{17}H_{13}O_5$.

Maximaisoflavone A (308)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.89 (1H, *s*, H-2), 7.89 (1H, *d*, *J* = 8.0 Hz, H-5), 6.98 (1H, *d*, *J* = 8.0 Hz, H-6), 7.07 (1H, *d*, *J* = 1.6 Hz, H-2'), 6.87 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.95 (1H, *dd*, *J* =1.6, 8.0 Hz, H-6'), 6.21 (2H, *s*, OCH_2O-7/8), 5.99 (2H, *s*, OCH_2O-3'/4'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 151.7 (C-2), 124.8 (C-3), 175.4 (C-4), 120.5 (C-4a), 121.1 (C-5), 107.3 (C-6), 152.3 (C-7), 134.5 (C-8), 151.7 (C-8a), 125.4 (C-1'), 109.9 (C-2'), 147.8*

(C-3'), 147.7* (C-4'), 108.4 (C-5'), 122.5 (C-6'), 103.1 (OCH_2O-7/8), 101.2 (OCH_2O-3'/4'); ESIMS, $[M+H]^+$ *m/z*: 311.5 for $C_{17}H_{10}O_6$. * may be interchanged

4'-Hydroxyisoloncocarpin (309)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 5.41 (1H, *dd*, *J* = 3.2, 12.6 Hz, H-2), 2.81 (1H, *dd*, *J* = 3.2, 16.8 Hz, H-3_{eq}), 3.01 (1H, *dd*, *J* = 12.6, 16.8 Hz, H-3_{ax}), 7.74 (1H, *d*, *J* = 8.0 Hz, H-5), 6.49 (1H, *d*, *J* = 8.0 Hz, H-6), 7.36 (2H, *d*, *J* = 8.0 Hz, H-2'/6'), 6.89 (2H, *d*, *J* = 8.0 Hz, H-3'/5'), 5.56 (1H, *d*, *J* = 9.6 Hz, H-3"), 6.63 (1H, *d*, *J* = 9.6 Hz, H-4"), 1.47 (3H, *s*, CH_3-5"), 1.44 (3H, *s*, CH_3-6"); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 82.2 (C-2), 46.8 (C-3), 193.5 (C-4), 117.4 (C-4a), 130.6 (C-5), 113.8 (C-6), 162.3 (C-7), 112.1 (C-8), 160.4 (C-8a), 133.9 (C-1'), 130.5 (C-2'), 118.2 (C-3'), 158.5 (C-4'), 118.2 (C-5'), 130.5 (C-6'), 80.2 (C-2"), 131.5 (C-3"), 118.6 (C-4"), 31.1 (C-5"), 30.8 (C-6"); ESIMS, $[M+H]^+$ *m/z*: 323.5 for $C_{20}H_{18}O_4$.

(+)-(2*R*,3*R*)-Fustin (310)

Brown gum; $[\alpha]_D^{25}$ +2.8 (*c* 0.003, CH_3OH), CD (*c* 2.6 x 10^{-4}, CH_3OH): 335 (10.77), 305 (-31.00) nm. ^1H NMR ($CDCl_3$, 799.87 MHz): δ 4.96 (1H, *d*, *J* = 12.0 Hz, H-2), 5.51 (1H, *d*, *J* = 12.0 Hz, H-3), 7.74 (1H, *d*, *J* = 8.8 Hz, H-5), 6.56 (1H, *dd*, *J* = 1.6, 8.8 Hz, H-6), 6.35 (*d*, *J* = 1.6 Hz, H-8), 7.01 (*d*, *J* = 1.6 Hz, H-2'), 6.83 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.88 (1H, *dd*, *J* = 2.4, 8.0 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 86.9 (C-2), 75.8 (C-3), 195.8 (C-4), 114.7 (C-4a), 131.4* (C-5), 113.4 (C-6), 168.1 (C-7), 104.9 (C-8), 166.3 (C-8a), 131.3* (C-1'), 117.2 (C-2'), 147.5 (C-3'), 148.3 (C-4'), 117.4 (C-5'), 122.2 (C-6'); ESIMS, $[M+H]^+$ *m/z*: 289.2 for $C_{15}H_{12}O_6$. * may be interchanged

4-Hydroxyloncocarpin (182)

Yellow solid in methanol; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.56 (2H, *d*, *J* = 8.8 Hz, H-2/6), 6.88 (2H, *d*, *J* = 8.8 Hz, H-3/5), 7.43 (1H, *d*, *J* = 15.2 Hz, H-α), 7.83 (1H, *d*, *J* = 15.2 Hz, H-β), 6.38 (1H, *d*, *J* = 8.8 Hz, H-5'), 7.71 (1H, *d*, *J* = 8.8 Hz, H-6'), 5.59 (1H, *d*, *J* = 9.6 Hz, H-3"), 6.75 (1H, *d*, *J* = 9.6 Hz, H-4"), 1.47 (3H, *s*, CH_3-5"), 1.47 (3H, *s*, CH_3 6", 13.77 (1H, *s*, OH-2'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 130.4 (C-1), 133.2 (C-2/6), 118.7* (C-3/5), 160.6 (C-4), 120.6 (C-α), 146.7 (C-β), 194.7 (C=O), 112.1 (C-1'), 163.6 (C-2'), 116.8 (C-3'), 162.4 (C-4'), 110.9 (C-5'), 133.2 (C-6'), 80.5 (C-2"), 130.8 (C-3"), 118.6* (C-4"), 31.0 (C-5"), 31.0 (C-6"); ESIMS, $[M+H]^+$ *m/z*: 323.5 for $C_{20}H_{18}O_4$. * may be interchanged.

4-Hydroxyderricin (311)

Yellow solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.56 (2H, *d*, *J* = 8.8 Hz, H-2/6), 6.89 (2H, *d*, *J* = 8.8 Hz, H-3/5), 7.46 (1H, *d*, *J* = 15.2 Hz, H-α), 7.83 (1H, *d*, *J* = 15.2 Hz, H-β), 6.49 (1H, *d*, *J* = 8.8 Hz, H-5'), 7.79 (1H, *d*, *J* = 8.8 Hz, H-6'), 3.39 (2H, *d*, *J* = 6.4, H-1"), 5.23 (1H, *t*, *J* = 6.4 Hz, H-2"), 1.68 (3H, *s*, CH_3-4"), 1.79 (3H, *s*, CH_3-5"), 3.91 (3H, *s*, OCH_3-4'), 13.46 (1H, *s*, OH-2'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 130.2 (C-1), 133.2 (C-2/6), 118.7 (C-3/5), 160.9 (C-4), 120.7 (C-α), 146.7 (C-β), 194.9 (C=O) 117.3 (C-1'), 165.7 (C-2'), 120.2 (C-3'), 165.9 (C-4'), 104.7 (C-5'), 131.8 (C-6'), 24.4 (C-1"), 124.7 (C-2"), 134.6 (C-3"), 28.5 (C-4"), 20.5 (C-5"), 58.4 (OCH_3-4'); ESIMS, $[M+H]^+$ *m/z*: 339.3 for $C_{21}H_{22}O_4$.

3.6.3 Spectroscopic and Physical Data of Compounds from Leaves of *M. usaramensis* ssp. *usaramensis*

Barbigerone (55)

Colorless solid in methanol; ^{1}H NMR (799.87 MHz, CDCl3): δ 7.97 (1H, *s*, H-2), 8.05 (1H, *d*, *J* = 8.8 Hz, H-5), 6.85 (1H, *d*, *J* = 8.8 Hz, H-6), 6.62 (1H, *s*, H-3'), 6.95 (1H, *s*, H-6'), 5.71 (1H, *d*, *J* = 9.6 Hz, H-3"), 6.82 (1H, *d*, *J* = 9.6 Hz, H-4"), 1.49 (3H, *s*, CH_3-5"), 1.49 (3H, *s*,

CH_3-6", 3.77 (3H, *s*, OCH_3-2'), 3.93 (3H, *s*, OCH_3-3'), 3.85 (3H, *s*, OCH_3-5'); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 153.9 (C-2), 121.5 (C-3), 175.6 (C-4), 118.5 (C-4a), 126.7 (C-5), 115.1 (C-6), 157.2 (C-7), 109.3 (C-8), 152.4 (C-8a), 112.4 (C-1'), 151.9 (C-2'), 98.3 (C-3'), 149.8 (C-4'), 143.1 (C-5'), 115.3 (C-6'), 77.6 (C-2"), 130.2 (C-3"), 115.1 (C-4"), 28.1 (C-5"), 28.1 (C-6"), 56.9 (OCH_3-2'), 56.2 (OCH_3-4'), 56.6 (OCH_3-5'); ESIMS, $[M]^+$ *m/z*: 394.9, $[M+Na]^+$ at *m/z*: 417.6 for $C_{23}H_{22}O_6$.

Toxicarol isoflavone (37)

Colorless solid in methanol; ^1H NMR (799.87 MHz, CDCl3): δ 7.90 (1H, *s*, H-2), 6.29 (1H, *s*, H-6), 6.63 (1H, *s*, H-3'), 6.88 (1H, *s*, H-6'), 5.58 (1H, *d*, *J* = 10.4 Hz, H-3"), 6.69 (1H, *d*, *J* = 10.4 Hz, H-4"), 1.48 (3H, *s*, CH_3-5"), 1.48 (3H, *s*, CH_3-6"), 3.79 (3H, *s*, OCH_3-2'), 3.93(3H, *s*, OCH_3-4'), 3.86 (3H, *s*, OCH_3-5'), 12.94 (1H, *s*, OH-5); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 154.5 (C-2), 120.6 (C-3),180.9 (C-4), 106.4 (C-4a), 162.2 (C-5), 100.3 (C-6), 159.4 (C-7), 101.2 (C-8), 152.2 (C-8a), 110.7 (C-1'), 152.0 (C-2'), 98.2 (C-3'), 150.1 (C-4'),143.1(C-5'), 115.1 (C-6'), 78.0 (C-2"), 127.4 (C-3"), 114.7 (C-4"), 28.2 (C-5"), 28.2 (C-6"), 56.9 (OCH_3-2'), 56.2 (OCH_3-4'), 56.6 (OCH_3-5'); ESIMS, $[M+H]^+$ *m/z*: 411.3; $[M+Na]^+$ *m/z*: 433.5 for $C_{23}H_{22}O_7$.

3",4"-Epoxybarbigerone (312)

Colorless solid ; ^1H NMR (799.87 MHz, CDCl3): δ 8.06 (1H, *s*, H-2), 8.15 (1H, *d*, *J* = 8.8 Hz, H-5), 7.01 (1H, *d*, *J* = 8.8 Hz, H-6), 6.51 (1H, *s*, H-3'), 6.93 (1H, *s*, H-6'), 2.93 (1H, *d*, *J* = 7.2 Hz, H-3"), 3.58 (1H, *d*, *J* = 7.2 Hz, H-4"), 1.53 (3H, *s*, CH_3, H-5"), 1.12 (3H, *s*, CH_3, H-6"), 3.47 (3H, *s*, OCH_3-2'), 3.92 (3H, *s*, OCH_3-4'), 3.79 (3H, *s*, OCH_3-5'); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 154.1 (C-2), 121.5 (C-3), 176.4 (C-4), 119.2 (C-4a), 125.4 (C-5), 116.8 (C-6), 157.6 (C-7), 114.0 (C-8), 155.4 (C-8a), 111.7 (C-1'), 151.6 (C-2'), 97.6 (C-3'), 149.8 (C-4'),

142.9 (C-5'), 115.5 (C-6'), 75.8 (C-2"), 40.5 (C-3"), 33.9 (C-4"), 27.0 (C-5"), 23.5 (C-6"), 56.2 (OCH_3-2'), 56.2 (OCH_3-4'), 56.5 (OCH_3-5'); ESIMS, *m/z*: 395.3; MALDI-TOF, *m/z*: 394.107 (calcd., $[M+H]^+$ 410.1 for $C_{23}H_{22}O_7$).

Deguelin (152)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 6.79 (1H, *s*, H-1), 6.45 (1H, *s*, H-4), 4.57 (1H, *dd*, *J* = 1.6,12.0 Hz, 6α), 4.12 (1H, *d*, *J* = 12.0 Hz, H-β), 4.85 (1H, *m*, H-6a), 6.45 (1H, *d*, *J* = 8.8 Hz, H-10), 7.75 (1H, *d*, *J* = 8.8 Hz, H-11), 3.77 (1H, *d*, *J* = 4.0 Hz, H-12a), 5.56 (1H, *d*, *J* =10.4 Hz, H-3'), 6.65 (1H, *d*, *J* = 10.4 Hz, H-4'), 1.45 (3H, *s*, CH_3-5'), 1.37 (3H, *s*, CH_3-6'), 3.77 (3H, *s*, OCH_3-2), 3.81 (3H, *s*, OCH_3-3); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 110.4 (C-1), 147.4 (C-2), 143.9 (C-3), 100.9 (C-4), 149.5 (C-4a), 66.3 (C-6), 72.4 (C-6a), 156.9 (C-7a), 109.1 (C-8), 160.1 (C-9), 111.5 (C-10), 128.6 (C-11), 112.8 (C-11a), 189.2 (C-12), 44.4 (C-12a), 104.8 (C-12b), 77.7 (C-2'), 128.7 (C-3'), 115.8 (C-4'), 28.5 (C-5'), 28.2 (C-6'), 56.3 (OCH_3-2), 55.9 (OCH_3-3); ESIMS, $[M+H]^+$ *m/z*: 395.1; $[M+Na]^+$ *m/z*: 417.5 for $C_{23}H_{22}O_6$.

Tephrosin (153)

Colorless solid; mp 103-105 °C; ^1H NMR (see Table 4.11); ^{13}C NMR (see Table 4.11); α_D^{20}-16.9; CD (*c* 1.83 x 10^{-4}, CH_3OH): *ca.* 330 (-10.92); ESIMS, $[M+H-H_2O]^+$ *m/z*: 393.2; $[M+H]^+$ *m/z*: 411.4; $[M+Na]^+$ *m/z*: 433.6 for $C_{23}H_{22}O_7$.

Sarcolobin (313)

Colorless solid; ^1H NMR (see Table 4.11); ^{13}C NMR (see Table 4.11); α_D^{20}-38.0 (c 0.00025, CH_2Cl_2); CD (*c* 4.88 x 10^{-5}, CH_3OH): 354 (0.410), 324 (-2.67) nm; ESIMS, $[M+H-H_2O]^+$ *m/z*: 393.2; $[M+H]^+$ *m/z*: 411.3; $[M+Na]^+$ *m/z*: 433.5 for $C_{23}H_{22}O_7$.

6-Hydroxydeguelin (314)

Colorless solid from methanol; ^{1}H NMR (799.87 MHz, $CDCl_3$): δ 6.79 (lH, *s*, H-1), 6.45 (lH, *s*, H-4), 6.46 (lH, *d*, *J* = 8.8 Hz, H-10), 7.76 (lH, *d*, *J* = 8.7 Hz, H-11), 5.55 (lH, *d*, *J*= 10.4 Hz, H-3'), 6.58 (lH, *d*, *J* = 10.4 Hz, H-4'), 3.92 (lH, *d*, *J* = 4.0 Hz, H-12a), 4.81 (lH, *dd*, *J* = 2.4, 4.9, H-6a), 5.81 (lH, *dd*, *J* = 2.4, 3.2 Hz, H-6), 1.45 (3H, *s*, H-5'), 1.38 (3H, *s*, H-6'), 3.77 (3H, *s*, OCH_3-3), 3.81(3H, s, OCH_3-2); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 28.2 *(*C-6'), 28.5 *(*C-5'), 40.4 (C-12a), 55.9 (OCH_3-2), 56.3 (OMe-3), 73.3 (C-6a), 77.7 (C-2'), 90.1 (C-6), 101.4 (C-4), 104.9 (C-12b), 109.0 (C-8), 110.1(C-1), 111.6 *(d, C-* 10), 128.6 (C-11), 128.8 (C-3'), 144.2 (C-2), 144.3 (C-4a), 149.6 (C-3), 156.3 (C-7a), 160.6 (C-9), 188.9 (C-12); ESIMS, $[M+H]^+$ *m/z*: 411.3 for $C_{23}H_{22}O_7$.

6-Hydroxy-6a,12a-dehydrodeguelin (122)

Colorless solid; ^{1}H NMR (799.87 MHz, $CDCl_3$): δ 8.56 (1H, *s*, H-1), 6.66 (1H, *s*, H-4), 6.23 (1H, *d*, *J* = 8.8 Hz, H-6), 6.85 (1H, *d*, *J* = 8.8 Hz, H-10), 7.99 (1H, *d*, *J* = 8.8 Hz, H-11), 5.75 (1H, *d*, *J* = 9.6 Hz, H-3'), 6.83 (1H, *d*, *J* = 9.6 Hz, H-4'), 1.54 (3H, *s*, CH_3-5'), 1.51 (3H, *s*, CH_3-6'), 3.94 (3H, *s*, OCH_3-2), 3.88 (3H, *s*, OCH_3-3). ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 109.2 (C-1), 144.4 (C-2), 149.4 (C-3), 101.2 (C-4), 142.3 (C-4a), 89.4 (C-6), 153.6 (C-6a), 151.2 (C-7a), 108.1 (C-8), 157.7 (C-9), 115.4 (C-10), 126.4 (C-11), 118.1 (C-11a), 175.9 (C-12), 110.5 (C-12a), 108.1 (C-12b), 78.0 (C-2'), 130.5 (C-3'), 114.7 (C-4'), 28.2 (C-5'), 28.3 (C-6'), 56.2 (OCH_3-2) 55.9 (OCH_3-3); ESIMS, $[M+H]^+$ *m/z*: 409.6 for $C_{23}H_{20}O_7$.

3.6.4 Spectroscopic and Physical Data of Compounds from Leaves of *M. oblata* ssp. *teitensis*

8-prenylmilldurone (316)

Colorless solid; mp 183-185°C; UV (MeOH) λ_{max} (log ε): 305 (3.88), 280 (sh) (3.56) nm.^{1}H and ^{13}C NMR (see Table 4.13); HREIMS, $[M+H]^+$ *m/z*: 425.1600 (calcd. 425.1522) for $C_{24}H_{24}O_7$.

7,2',5'-Trimethoxy-3',4'-methylenedioxyisoflavone (317)

Colorless crystal in methanol; mp 184-186 °C; UV (MeOH) λ_{max} (log ε): 295 (4.09) and 300 (sh) (4.06) nm; ^{1}H and ^{13}C NMR (see Table 4.13); HREIMS, $[M+H]^+$ *m/z*: 357.0974 (calcd. 357.0896) for $C_{19}H_{16}O_7$.

Oblarotenoid A (318)

Colorless solid; mp 128-130 °C; $[\alpha]_D^{20}$ -38.3; UV (MeOH) λmax: 250 and 269 nm; CD (MeOH): 300 (23.0833), 327 (-15.1694) nm; ^{1}H and ^{13}C NMR (see Table 4.14); HREIMS, $[M+Na]^+$ *m/z*: 365.0645; (calcd. 365.0739) for $C_{18}H_{14}O_7$.

Oblarotenoid B (319)

Colorless solid; mp >205°C; $[\alpha]_D^{20}$ + 130.3°; UV (MeOH) λ_{max} (log ε): 285 (sh) (3.40) and 300 (3.45) nm; CD (MeOH): 340 (2.9294), 300 (-2.0102) nm); ^{1}H and ^{13}C NMR (see Table 4.14); HREIMS, $[M+H-H_2O]^+$ *m/z*: 325.0712 (calcd. 343.07395 for $[M+H]^+$) for $C_{18}H_{14}O_7$.

9-Methoxy-2,3-dimethylenedioxyrotenoid (320)

Needle crystal in methanol; mp 193-195 °C; $[\alpha]_D^{20}$+38.3 °C; UV λmax (log ε): 285 (3.49), 300(3.51) nm. CD (MeOH): 300 (31.4803), 340 (-7.8223) nm; ^{1}H and ^{13}C NMR (see Table 4.15); HREIMS, $[M+H]^+$ *m/z*: 327.0869 (calcd. 327.0790) for $C_{18}H_{14}O_6$.

Oblarotenoid C (321)

Colorless solid; mp 132-134 °C; $[\alpha]_D^{20}$ -9.04; UV λ_{max} (log ε): 245 (3.04), 260 (3.08), 280 (3.01) nm. CD (MeOH): 285 (25.29), 327 (-9.66) nm. ^{1}H and ^{13}C NMR (see Table 4.15); HREIMS, $[M+Na]^+$ *m/z*: 395.0698 and $[M+H]^+$ *m/z*: 373.0918 (calcd. 373.0845) for $C_{19}H_{16}O_8$.

Munduserone (322)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 6.74 (1H, *s*, H-1), 6.46 (1H, *s*, H-4), 4.62 (1H, *dd*, *J* = 3.2, 12.0 Hz, H-6α), 4.18 (1H, *d*, *J* = 12.0 Hz, H-6β), 4.49 (1H, *t*, *J* = 3.2 Hz, H-6a), 6.42 (1H, *d*, *J* = 2.4 Hz, H-8), 6.58 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-10), 7.87 (1H, *d*, *J* = 8.8 Hz, H-11), 3.85 (1H, *d*, *J* = 4.0 Hz, H-12a), 3.76 (3H, *s*, OCH_3-2), 3.79 (3H, *s*, OCH_3-3), 3.80 (3H, *s*, OCH_3-9); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 110.3 (C-1), 143.9 (C-2), 149.5 (C-3), 100.9 (C-4), 147.4 (C-4a), 66.3 (C-6), 72.4 (C-6a), 162.8 (C-7a), 100.7 (C-8), 166.5 (C-9), 110.7 (C-10), 129.4 (C-11), 112.3 (C-11a), 189.3 (C-12), 44.6 (C-12a), 104.7 (C-12b), 56.3 (OCH_3-2), 55.9 (OCH_3-3), 55.7 (OCH_3-9); EMSI, $[M+H]^+$ *m/z*: 343.1, $[M+Na]^+$ *m/z*: 365.6 for $C_{19}H_{18}O_6$.

(6*R*,12*S*)-12a-Hydroxymunduserone (323)

Colorless solid; $[\alpha]_D^{20}$ + 53.3 (CH_3OH); CD (*c* 2.79 x 10^{-4}, CH_3OH): 350 (+0.92), 330 (-5.62) nm; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 6.54 (1H, *s*, H-1), 6.49 (1H, *s*, H-4), 4.61 (1H, *dd*, *J* = 2.4, 12.0 Hz, H-6α), 4.49 (1H, *dd*, *J* = 0.8, 12.0 Hz, H-6β), 4.59 (1H, *dd*, *J* = 0.8, 2.4 Hz, H-6a), 6.38 (1H, *d*, *J* = 2.4 Hz, H-8), 6.59 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-10), 7.85 (1H, *d*, *J* = 8.8 Hz, H-11), 3.72 (3H, *s*, OCH_3-2), 3.81 (3H, *s*, OCH_3-3), 3.79 (3H, *s*, OCH_3-9), 4.40 (1H, *s*, OH-12a); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 109.3 (C-1), 144.0 (C-2), 151.2 (C-3), 101.1 (C-4), 148.4 (C-4a), 63.9 (C-6), 76.2 (C-6a),162.6 (C-7a), 100.7 (C-8), 167.1 (C-9), 111.2 (C-

10), 129.3 (C-11), 111.1 (C-11a), 191.4 (C-12), 67.6 (C-12a), 108.6 (C-12b), 56.4 (OCH_3-2), 55.9 (OCH_3-3), 55.7 (OCH_3-9); ESIMS, $[M+H-H_2O]^+$ *m/z*: 341.1, $[M+Na]^+$ *m/z*: 381.1 for $C_{19}H_{18}O_7$.

6a,12a-Dehydrodeguelin (126)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 8.46 (1H, *s*, H-1), 6.56 (1H, *s*, H-4), 5.03 (2H, *s*, H-6), 6.87 (1H, *d*, *J* = 8.8 Hz, H-10), 8.05 (1H, *d*, *J* = 8.8 Hz, H-11), 5.73 (1H, *d*, *J* = 9.6 Hz, H-3'), 6.77 (1H, *d*, *J* = 9.6 Hz, H-4'), 1.50 (6H, *s*, CH_3-5'/6'), 3.96 (3H, *s*, OCH_3-2), 3.88 (3H, *s*, OCH_3-3); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 110.0 (C-1), 144.0 (C-2), 148.9 (C-3), 100.4 (C-4), 146.3 (C-4a), 64.9 (C-6), 156.2 (C-6a), 151.1 (C-7a), 109.1 (C-8), 157.2 (C-9), 115.4 (C-10), 126.5 (C-11), 118.5 (C-11a), 174.4 (C-12), 111.8 (C-12a), 110.6 (C-12b), 77.8 (C-2'), 130.6 (C-3'), 114.7 (C-4'), 28.2 (C-5'/6'), 56.3 (OCH_3-2), 55.9 (OCH_3-3); ESIMS, $[M+H]^+$ *m/z*: 393.1 for $C_{23}H_{20}O_6$.

Maximaisoflavone G (115)

Colorless solid; mp>230 °C; ^1H NMR data (see Table 4.17); ^{13}C NMR data (see Table 4.17); ESIMS, $[M+H]^+$ *m/z* 313.2 for $C_{17}H_{12}O_6$.

7-Acetoxymaximaisoflavone G (115a)

Colorless solid; ^1H NMR (799.87 MHz, $CDCl_3$): δ 7.94 (1H, *s*, H-2), 8.30 (1H, *d*, *J* = 8.8 Hz, H-5), 7.28 (1H, *d*, *J* = 2.4 Hz, H-8), 7.15 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-6), 6.82 (1H, *s*, H-6'), 6.62 (1H, *s*, H-3'), 5.96 (2H, *s*, OCH_2O), 3.72 (3H, *s*, OCH_3), 2.34 (3H, *s*, $COCH_3$); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 153.6 (C-2), 155.6 (C-7), 153.3(C-8a), 145.5 (C-4'), 140.2 (C-5'), 126.8 (C-5), 124.4 (C-4a), 121.3 (C-3), 118.3 (C-6), 109.9 (C-8), 110.1 (C-6'), 94.4 (C-3'), 100.4 (OCH_2O), 55.8 (OCH_3), 20.1 ($OCOCH_3$), 167.5 ($OCOCH_3$); ESIMS, $[M+H]^+$ *m/z*: 355.5 for $C_{19}H_{14}O_7$.

Milldurone (324)

Amorphous solid; mp >230 °C; ^{1}H NMR data (see Table 4.17); ^{13}C NMR data (see Table 4.17); ESIMS, $[M+H]^+$ *m/z*: 357.3 for $C_{19}H_{16}O_7$.

Maximaisoflavone J (325)

Colorless solid; ^{1}H NMR data (see Table 4.17); ^{13}C NMR data (see Table 4.17); ESIMS, $[M+H]^+$ *m/z*: 337.2 for $C_{21}H_{20}O_4$.

3.6.5 Spectroscopic and Physical Data of Compounds from Root Bark of *M. usaramensis* ssp. *usaramensis*

4-*O*-Geranylisoliquiritigenin (326)

Yellow solid; mp 95-97 °C; UV (CH_3OH) λmax (log ε) 299 (4.2), 370 (3.5) nm; ^{1}H and ^{13}C NMR data (see Table 4.18); HREIMS, $[M+H]^+$ *m/z*: 392.1968 (calcd. 392.1988) for $C_{25}H_{28}O_4$.

(*S*)- 4'-*O*-geranyl-7-hydroxyflavanone (327)

Colorless sticky oil; $[\alpha]_D^{20}$ -27.5 (CH_3OH); UV (CH_3OH) λmax (log ε) 275 (4.2), 310 (3.7) nm; CD (MeOH): 332 (+4.3), 302 (-8.7); ^{1}H and ^{13}C NMR data (see Table 4.19); HREIMS, $[M+H]^+$ *m/z* 392.1968 (calcd. 392.1988) for $C_{25}H_{28}O_4$.

Colenemol (330)

Colorless solid; ^{1}H NMR (799.87 MHz, $CDCl_3$): δ 7.31 (2H, *d*, *J* = 8.8 Hz, H-2/6), 6.87 (2H, *d*, *J* = 8.8 Hz, H-3/ 5), 6.55 (1H, *d*, *J* = 16.0 Hz, H-α), 6.23 (1H, *td*, *J* = 16.0, 6.4 Hz, H-β), 4.29 (2H, *dd*, *J* = 1.6, 6.4 Hz, H-γ), 4.54 (2H, *d*, *J* = 7.2 Hz, H-1'), 5.49 (1H, *t*, *J* = 7.2 Hz, H-2'), 2.07-2.09 (2H, *m*, H-4'), 2.12-2.15 (2H, *m*, H-5"), 5.09 (1H, *t*, *J* = 6.4, H-6'), 1.68 (3H, *s*, H-8'), 1.61 (3H, *s*, H-9'). 1.74 (3H, *s*, H-10'); ^{13}C NMR (201.15 MHz, $CDCl_3$): δ 129.3 (C-1), 127.6 (C-2/6), 114.8 (C-3/5), 158.7 (C-4), 131.1 (C-α), 126.2 (C-β), 63.9 (C-γ), 64.9 (C-1'),

119.4 (C-2'), 141.3 (C-3'), 39.6 (C-4'), 26.3 (C-5'), 123.8 (C-6'), 131.8 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.7 (C-10'); HREIMS, $[M]^+$ *m/z* 286.1931 for $C_{19}H_{26}O_2$.

Usararotenoid A (139)

Colorless crystal in methanol; $[\alpha]_D^{20}$ + 353.3° (c 0.0015, CH_2Cl_2); CD (CH_3OH): 350 (+6.69); ^1H NMR (799.87 MHz, DMSO-d6): δ 7. 48 (1H, *s*, H-1), 6.52 (1H, *s*, H-4), 4.36 (1H, *dd*, *J* = 4.8, 9.6, H-6α), 4.33 (1H, *dd*, *J* = 9.6, 11.2, H-6β), 4.69 (1H, *dd*, *J* = 4.8, 11.2, Hz, H-6a), 6.81 (1H, *d*, *J* = 8.8 Hz, H-10), 7.49 (1H, *d*, *J* = 8.0 Hz, H-11), 6.19 (1H, *d*, 0.8 Hz, OCH_2O-8/9), 6.15 (1H, *d*, 1.6 Hz, OCH_2O-8/9), 6.00 (2H, *d*, 1.6 Hz, OCH_2O-2/3), 6.78 (1H, *s*, 12a-OH); ^{13}C NMR (201.15 MHz, DMSO-d6): δ 110.4 (C-1), 141.6 (C-2), 148.8 (C-3), 98.3 (C-4), 150.3 (C-4a), 61.4 (C-6), 76.4 (C-6a), 143.9 (C-7a), 134.1 (C-8), 153.9 (C-9), 103.9 (C-10), 123.8 (C-11), 117.4 (C-11a), 187.8 (C-12), 66.4 (C-12a), 112.2 (C-12b), 103.3(OCH_2O-8/9), 101.8 (OCH_2O-2/3); ESIMS, $[M+H-H_2O]^+$ *m/z*: 339.3] for $C_{18}H_{12}O_8$; X-ray data (see Section 3.5).

Epimillettosin (137)

Needles (MeOH); mp 256-258 °C; $[\alpha]_D^{20}$+230.4°; CD (MeOH): 324 (-10.2), 348 (+31.9); UV (MeOH) λmax (log ε) 235 (4.54), 240 (2.38), 276 (4.38), 312 (3.99); ^1H NMR ($CDCl_3$,799.87 MHz): δ 7.71 (1H, *s*, H-1), 6.41 (1H, *s*, H-4), 4.45 (1H, *dd*, *J* = 9.6, 11.2 Hz, H-6α), 4.38 (1H, *dd*, *J* = 4.8, 10.4 Hz, H-6β), 4.62 (1H, *dd*, *J* = 4.8, 12.0 Hz, H-6a), 6.57 (1H, *d*, *J* = 8.8 Hz, H-10), 7.79 (1H, *d*, *J* = 8.8 Hz, H-11), 5.63 (1H, *d*, *J* = 9.6 Hz, H-3'), 6.64 (1H, *d*, *J* = 10.4 Hz, H-4'), 5.94 (2H, *s*, OCH_2O-2/3), 1.48 (3H, *s*, CH_3-5'), 1.45 (3H, *s*, CH_3-6'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 109.3 (C-1), 142.4 (C-2), 149.5 (C-3), 98.5 (C-4), 150.7 (C-4a). 61.7 (C-6), 76.7(C-6a), 155.6 (C-7a), 108.9 (C-8), 159.7 (C-9), 112.2 (C-10), 129.7 (C-11), 113.5 (C-11a), 187.4 (C-12), 66.7 (12a), 110.6 (C-12b), 77.8 (C-2'), 129.3 (C-3'), 115.4 (C-4'), 28.5 (C-

5'), 28.1 (C-6'), 101.5 (OCH_2O-2/3); ESIMS, $[M+H-H_2O]^+$ *m/z*: 395.0 $[M]^+$ *m/z*: 377.3 for $C_{22}H_{18}O_7$; X-ray data (see Section 3.5).

12-Dihydrousararotenoid A (140)

Colorless crystal; $[\alpha]_D^{20}$+354.8° (*c* 0.00025, CH_3OH); ^1H NMR (DMSO-d6, 799.87 MHz): δ 7.86 (1H, *s*, H-1), 6.45 (1H, *s*, H-4), 4.22-426 (2H, *m*, H-6α/6β), 4.36 (1H, *dd*, *J* = 5.6, 10.4 Hz, H-6a), 6.59 (1H, *d*, *J* = 8.0 Hz, H-10), 6.99 (1H, *d*, *J* = 8.8 Hz, H-11), 4.73 (1H, *d*, *J* = 10.4 Hz, H-12), 5.61 (1H, *d*, J = 10.4, 12-OH), 5.57 (1H, *s*, 12a-OH), 5.97 (2H, OCH_2O-8/9), 5.95 (2H, OCH_2O-2/3); ^{13}C NMR (DMSO-d_6, 201.15 MHz): δ 108.2 (C-1), 141.8 (C-2), 148.3 (C-3), 98.1 (C-4), 149.2 (C-4a), 61.9 (C-6), 73.6 (C-6a), 138.3 (C-7a), 133.2 (C-8), 147.7 (C-9), 102.0 (C-10), 121.5 (C-11), 123.3 (C-11a), 70.6 (C-12), 65.5 (C-12a), 117.9 (C-12b), 101.7 (OCH_2O-8/9), 101.6 (OCH_2O-2/3); ESIMS, $[M+H-H_2O]^+$ *m/z*: 341.1 for $C_{18}H_{14}O_8$; X-ray data (see Section 3.5).

Usararotenoid C (154)

Colorless solid; ^1H NMR ($CDCl_3$, 499.88 MHz): δ 7.69 (1H, *s*, H-1), 6.41 (1H, *s*, H-4), 4.45 (1H, *t*, *J* = 10.5 Hz, H-6α), 4.36 (1H, *dd*, *J* = 4.5, 10.0 Hz, H-6β), 4.60 (1H, *dd*, *J* = 4.5, 11.5 Hz, H-6a), 6.70 (1H, *d*, *J* = 9.0 Hz, H-10), 7.88 (1H, *d*, *J* = 9.0 Hz, H-11), 3.36 (2H, *m*, H-1'), 5.17 (1H, *t*, *J* = 7.0 Hz, H-2'), 1.77 (3H, *s*, CH_3-4'), 1.68 (3H, *s*, CH_3-5'), 3.90 (3H, *s*, OCH_3-9), 5.94 (2H, *s*, OCH_2O-2/3); ^{13}C NMR ($CDCl_3$, 125.71 MHz): δ 112.1 (C-1), 152.0 (C-3), 154.4 (C-4a), C-6 (64.4), 79.1 (C-6a), C-7a (160.7), 120.0 (C-8), 165.9 (C-9), 108.6 (C-10), 130.9 (C-11), 116.7 (C-11a), 190.3 (C-12), 69.1 (C-12a), 113.2 (C-12b), 24.7 (C-1'), 124.2 (C-2'), 134.7 (C-3'), 20.5 (C-4'), 28.5 (C-5'); ESIMS, $[M+H-H_2O]^+$ *m/z*: 393.3 for $C_{23}H_{22}O_7$

_O_-Geranylisoliquiritigenin (181)

Yellow solid; ^{1}H NMR ($CDCl_3$, 499.88 MHz): δ 7.56 (2H, *d*, *J* = 8.5 Hz, H-2/6), 6.88 (2H, *d*, *J* = 8.0 Hz, H-3/5), 6.48-6.51 (2H, *d*, *m*, 3'/5'), 7.82 (1H, *d*, *J* = 9.0 Hz, H-6'), 7.45 (1H, *d*, *J* = 15.5 Hz, H-α), 7.84 (1H, *d*, *J* = 15.5 Hz, H-β), 4.59 (2H, *d*, *J* = 6.5 Hz, H-1"), 5.48 (1H, *t*, *J* = 6.5 Hz, H-2"), 2.09-2.14 (4H, *m*, H-4"/H-5"), 5.09 (1H, *t*, *J* = 6.0, H-6"), 1.61 (3H, *s*, CH_3-8"), 1.68 (3H, s, CH_3-8"), 1.75 (3H, *s*, CH_3-10"); ESIMS, $[M+H]^+$ *m/z*: 393.4 (calcd. 393.2) for $C_{25}H_{28}O_4$.

7-_O_-Geranyl-5-hydroxyflavanone (329)

Colorless solid; ^{1}H NMR ($CDCl_3$, 399.95): δ (1H, *dd*, *J* = 4.0, 12.0 Hz, H-2), 3.07 (1H, *dd*, *J* = 12.0, 16.0 Hz, H-3_{ax}), 2.77 (1H, *dd*, *J* = 4.0, 16.0 Hz, H-3_{eq}), 6.06 (1H, *d*, *J* = 2.4 Hz, H-6), 6.03 (1H, *d*, *J* = 2.4 Hz, H-8), 7.32 (2H, *d*, *J* = 8.0 Hz, H-2'/6'), 6.87 (1H, *d*, *J* = 8.0 Hz, H-3'/5'), 4.53 (2H, *d*, *J* = 4.0, H-1"), 5.42 (1H, *m*, H-2"), 2.08 (4H, *m*, H-4"/5"), 2.08 (*m*), 5.06 (1H, *m*, H-6"), 1.66 (3H, *s*, H-8"), 1.59 (3H, *s*, H-9"), 1.70 (3H, *s*, H-10"), 11.98 (1H, *s*, OH-5).

Maximaisoflavone H (97)

Colorless solid; ^{1}H NMR (DMSO-d_6, 799.87 MHz): δ 8.42 (1H, *s*, H-2), 7.71 (1H, *d*, *J* = 8.8 Hz, H-5), 7.18 (1H, *d*, *J* = 8.8 Hz, H-6), 7.50 (2H, *d*, *J* = 8.8 Hz, H-2'/6'), 7.00 (2H, *d*, *J* = 8.8 Hz, H-3'/5'), 6.32 (2H, *s*, OCH_2O-7/8), 3.79 (3H, s, OCH_3-4'); ^{13}C NMR (DMSO-d_6, 201.15 MHz): δ 153.6 (C-2), 123.6 (C-3), 174.9 (C-4), 120.3 (C-4a), 120.5 (C-5), 107.9 (C-6), 152.5 (C-7), 134.8 (C-8), 140.9 (C-8a), 124.3 (C-1'), 130.7 (C-2'/6'), 114.2 (C-3'/5'), 159.5 (C-4'), 104.2 (OCH2O-7/8), 55.6 (OCH_3-4').

Jamaicin (329)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.93 (1H, *s*, H-2), 8.06 (1H, *d*, *J* = 8.8 Hz, H-5), 6.87 (1H, *d*, *J* = 8.8 Hz, H-6), 6.84 (1H, *s*, H-6'), 6.64 (1H, *s*, H-3'), 5.73 (1H, *d*, *J* = 10.4 Hz, H-3"), 6.83 (1H, *d*, *J* = 10.4 Hz, H-4"), 1.52 (6H, *s*, CH_3-5"/6"), 5.91 (2H, *s*, OCH2O-4'/5'), 3.74 (3H, *s*, OCH_3-2'); ESIMS, $[M+H]^+$ *m/z*: 379.2 for $C_{22}H_{18}O_6$.

12a-Deoxyusararotenoid A (140a)

Colorless solid; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 6.69 (1H, *s*, H-1), 6.44 (1H, *s*, H-4), 4.18 (1H, *dd*, *J* = 3.6, 12.0 Hz, H-6α), 4.65 (1H, *dd*, *J* = 3.0, 12.0 Hz, H-6β), 4.98 (1H, *t*, *J* = 3.8 Hz, H-6a), 6.58 (1H, *d*, *J* = 8.4 Hz, H-10), 7.60 (1H, *d*, *J* = 8.4 Hz, H-11), 3.86 (1H, *d*, *J* = 3.8 Hz, H-12a), 5.83 (1H, *d*, *J* = 1.0 Hz, OCH_2O-2/3), 5.87 (1H, *d*, *J* = 1.0 Hz, OCH_2O-2/3), 6.01 (1H, *d*, *J* = 1.2 Hz, OCH_2O-8/9), 6.08 (1H, *d*, *J* = 1.2 Hz, OCH_2O-8/9); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 107.0 (C-1), 144.8 (C-2), 148.2 (C-3), 99.2 (C-4), 148.8 (C-4a), 66.3 (C-6), 72.8 (C-6a), 142.5 (C-7a), 134.6 (C-8), 154.9 (C-9), 104.0 (C-10), 123.7 (C-11), 115.7 (C-11a), 188.9 (C-12), 45.6 (C- 12a), 105.4 (C-12b), 101.4 (OCH_2O-2/3), 103.0 (OCH_2O-8/9).

6a,12a-Dehydrousararotenoid A (140b)

Colorless solid; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.27 (1H, *s*, H-1), 6.57 (1H, *s*, H-4), 7.09 (1H, *d*, *J* = 8.4 Hz, H-10), 7.80 (1H, *d*, *J* = 8.4 Hz, H-11), 5.08 (2H, *s*, H-6), 6.01 (2H, *s*, OCH_2O-2/3), 6.32 (2H, *s*, OCH_2O-8/9).

3.6.6 Spectroscopic and Physical Data of Compounds from Leaves of *O. holstii*

2",3"-Dihydroochnaflavone (275)

Yellow solid; ^{1}H (DMSO-d_6, 600.24 MHz) and ^{13}C NMR (DMSO-d_6, 150.95 MHz) data (see Table 4.20); HREIMS, $[M]^+$ *m/z* 540.1052 (calcd. 540.1056) for $C_{30}H_{20}O_{10}$.

2",3"-Dihydroochnaflavone-7"-*O*-methyl ether (276)

Yellow solid; ^{1}H (acetone-d_6, 600.24 MHz) and ^{13}C NMR (acetone-d_6, 150.95 MHz) data (see Table 4.20); HREIMS, $[M]^+$ *m/z* 554.1209 (calcd. 554.1213) for $C_{31}H_{22}O_{10}$.

Ochnaflavone (11)

Yellow solid in methanol; Yellow solid; ^{1}H (DMSO-d_6, 600.24 MHz) and ^{13}C NMR (DMSO-d_6, 150.95 MHz) data (see Table 4.21); HREIMS, $[M]^+$ *m/z*: 538.0893 (calcd. 538.0900) for $C_{30}H_{18}O_{10}$.

Ochnaflavone-7"-*O*-methyl ether (331)

Yellow solid in methanol; yellow solid; ^{1}H (acetone-d_6, 600.24 MHz) and ^{13}C NMR (acetone-d_6, 150.95 MHz) data (see Table 4.21); HREIMS, $[M]^+$ *m/z*: 552.1041 (calcd. 552.1056) for $C_{31}H_{20}O_{10}$.

Dasycarponin (332)

Needle crystal; ^{1}H NMR (DMSO-d_6, 600.24): δ 6.19 (1H, *d*, *J* = 9.6 Hz, H-2), 5.93 (1H, *d*, *J* = 9.6 Hz, H-3), 3.15 (1H, *m*, H-4), 3.15(1H, *m*, H-5), 4.57 (1H, *d*, *J* = 3.6 Hz, H-6), 5.74 (1H, *s*, H-7), 4.39 (1H, *d*, *J* = 7.8 Hz, H-1'), 4.04 (1H, *m*, H-2'), 2.88 (1H, *m*, H-3'), 4.45 (1H, *m*, H-4'), 3.09 (1H, *m*, H-5'), 3.50 (1H, *m*, H-6'), 3.66 (1H, *m*, H-6'), 4.91-5.14 (OH); ^{13}C NMR (DMSO-d_6, 150.95): δ 154.6 (C-1), 125.5 (C-2), 140.1 (C-3), 77.1 (C-4), 77.0 (C-5), 77.3 (C-6), 99.1 (C-7), 118.1 (C-8), 103.7 (C-1'), 69.0 (C-2'), 73.7 (C-3'), 70.365.9 (C-4'), (C-5'), 61.6 (C-6'); ESIMS, $[M+H]^+$ *m/z*: 330.4 for $C_{14}H_{19}NO_8$.

3.6.7 Spectroscopic and Physical Data of Compounds from Stem Bark of *O. holstii*

Lophirone A (252)

Colorless solid in methanol; ^{1}H NMR (DMSO-d_6, 799.87 MHz) (see Table 4.23) and ^{13}C NMR (acetone-d_6, 150.95 MHz) (see Table 4.23); ESIMS, $[M+H]^+$ *m/z*: 511.7 for $C_{30}H_{20}O_8$.

Calodenone (253)

Colorless solid in methanol; ^{1}H (acetone-d_6, 600.24 MHz), ^{13}C (acetone-d_6, 150.95 MHz) NMR data (see Table 4.23); ESIMS, $[M+H]^+$ *m/z*: 525.5 for $C_{31}H_{24}O_8$.

Afzelone D (254)

Colorless solid; ^{1}H ($CDCl_3$, 399.97 MHz), ^{13}C (DMSO-d_6, 201.15 MHz) NMR data (see Table 4.23); ESIMS, $[M-H]^-$ *m/z*: 537.6 for $C_{32}H_{26}O_8$.

5,4'-Dimethoxy-6,7-methylenedioxyisoflavone (333)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.78 (1H, *s*, H-2), 6.64 (1H, *s*, H-8), 7.47 (2H, *d*, *J* = 8.8 Hz, H-2'/6'), 6.95 (2H, *d*, *J* = 9.6 Hz, H-3'/5'), 6.07 (2H, *s*, OCH_2O-6/7), 4.08 (3H, *s*, OCH_3-5), 3.83 (3H, *s*, OCH_3-4'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 150.3 (C-2), 125.4 (C-3), 175.5 (C-4), 110.9 (C-4a), 141.7 (C-5), 135.6 (C-6), 152.8 (C-7), 93.3 (C-8), 154.8 (C-8a), 124.1 (C-1'), 130.4 (C-2'/6'), 113.8 (C-3'/5'), 159.5 (C-4'), 102.1 (OCH_2O-6/7), 60.9 (OCH_3-5), 54.9 (OCH_3-4'); ESIMS, $[M+H]^+$ *m/z*: 327.1 (calcd. 327.1) for $C_{18}H_{14}O_6$.

5,3',4'-Trimethoxy-6,7-methylenedioxyisoflavone (295)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.79 (1H, *s*, H-2), 6.64 (1H, *s*, H-8), 7.19 (1H, *d*, *J* = 2.4 Hz, H-2'), 6.90 (1H, *d*, *J* = 8.0 Hz, H-5'), 7.01 (1H, *dd*, *J* = 1.6, 8.0 Hz, H-6'), 6.07 (2H, *s*, OCH_2O-6/7), 4.09 (3H, *s*, OCH_3-5), 3.92 (3H, *s*, OCH_3-3'), 3.90 (3H, *s*, OCH_3-4'); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 150.4 (C-2), 125.4 (C-3), 175.2 (C-4), 113.8 (C-4a),

141.7 (C-5), 135.5 (C-6), 152.8 (C-7), 93.2 (C-8), 154.7 (C-8a), 124.6 (C-1'), 112.8 (C-2'), 148.7 (C-3'), 149.1 (C-4'), 111.0 (C-5'), 121.3 (C-6'), 102.2 (OCH_2O-6/7), 61.3 (OCH_3-5), 56.0 (OCH_3-3'), 55.9 (OCH_3-4'); ESIMS, $[M+H]^+$ *m/z*: 357.1 (calcd.357.1) for $C_{19}H_{16}O_7$.

(±)-Catechin (334)

Pinkish solid; ^{1}H NMR (DMSO-d_6, 799.87 MHz): δ 4.77 (1H, *brs*, H-2), 4.04 (1H, *m*, H-3), 2.52 (1H, *dd*, *J* = 4.0, 16.8 Hz, H-4), 2.72 (1H, *dd*, *J* = 5.6, 16.8 Hz, H-4), 5.93 (1H, *d*, *J* = 2.4 Hz, H-6), 5.77 (1H, *d*, *J* = 2.4 Hz, H-8), 6.93 (1H, *d*, *J* = 2.4 Hz, H-2'), 6.71 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.68 (1H, *dd*, *J* = 2.4, 8.0 Hz, H-6'), 4.69 (1H, *d*, *J* = 4.0 Hz, OH-3), 8.79-9.19 (4H, OH-5,7,3',4'); ^{13}C NMR (DMSO-d_6, 201.15 MHz): δ 78.5 (C-2), 65.4 (C-3), 28.7 (C-4), 99.0 (C-4a), 156.7 (C-5), 95.6 (C-6), 156.9 (C-7), 94.6 (C-8), 156.2 (C-8a), 131.1 (C-1'), 115.3 (C-2'), 144.9 (C-3'), 144.9 (C-4'), 115.3 (C-5'), 118.5 (C-6'); ESIMS, $[M-H]^-$ *m/z*: 289.2 (calcd. 289.1) for $C_{15}H_{14}O_6$.

2,4-Dihydroxyphenylmethylacetate (335)

Brown gum solid; ^{1}H NMR (DMSO-d_6, 799.87 MHz): δ 6.26 (1H, *d*, *J* = 2.4 Hz, H-3) , 6.12 (1H, *dd*, *J* = 2.4, 8.8 Hz, H-5), 6.84 (1H, *d*, *J* = 8.0 Hz, H-6), 3.56 (3H, *s*, $COOC\underline{H}_3$), 3.41 (2H, *s*, *Ph*-$C\underline{H}_2$), 9.13, 9.32 (2H, *s*, OH-2,4); ^{13}C NMR (DMSO-d_6, 201.15 MHz): δ 112.2 (C-1), 156.5 (C-2), 102.7 (C-3), 157.7 (C-4), 106.4 (C-5), 131.7 (C-6), 51.8 ($COO\underline{C}H_3$), 172.7 ($\underline{C}OOCH_3$), 34.8 (*Ph*-$\underline{C}H_2$); ESIMS, $[M+H]^+$ *m/z*: 183.2 (calcd. 183.1) for $C_9H_{10}O_4$.

3.6.8 Spectroscopic and Physical Data of Compounds from Root Bark of *O. ovata*

Maculine (336)

Colorless solid; ^{1}H NMR ($CDCl_3$, 799.87 MHz): δ 7.57 (1H, *d*, *J* = 2.4 Hz, H-2), 7.03 (1H, *d*, *J* = 2.4 Hz, H-3), 7.52 (1H, *s*, H-5), 7.30 (1H, s, H-8), 4.41 (3H, *s*, OCH_3), 6.09 (2H, *s*,

OCH_2O-6/7); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 142.6 (C-2), 104.5 (C-3), 102.5 (C-3a), 156.0 (C-4), 114.4 (C-4a), 98.0 (C-5), 146.1 (C-6), 150.8 (C-7), 104.5 (C-8), 143.9 (C-8a), 163.2 (C-9a), 58.9 (OCH_3-4), 101.6 (OCH_2O-6/7); ESIMS, $[M+H]^+$ *m/z*: 244.6 for $C_{13}H_9NO_4$.

Flindersiamine (337)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.58 (1H, *d*, *J* = 3.2 Hz, H-2), 7.02 (*d*, *J* = 2.4 Hz, H-3), 7.28 (1H, *s*, H-5), 4.40 (3H, *s*, (OCH_3-4), 4.26 (3H, *s*, OCH_3-8), 6.06 (2H, *s*, OCH_2O-6/7); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 143.1 (C-2), 104.3 (C-3), 102.9 (C-3a), 156.1 (C-4), 115.0 (C-4a), 92.4 (C-5), 146.8 (C-6), 138.0 (C-7), 137.8 (C-8), 136.0 (C-8a), 162.6 (C-9a), 58.9 (OCH_3-4), 60.6 (OCH_3-8), 101.5 (OCH_2O-6/7); ESIMS, $[M+H]^+$ *m/z*: 274.2 for $C_{14}H_{11}NO_5$.

Kokusaginine (338)

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.58 (1H, *d*, *J* = 3.2 Hz, H-2), 7.05 (1H, *d*, *J* = 2.4 Hz, H-3), 7.49 (1H, *s*, H-5), 7.39 (1H, *s*, H-8), 4.45 (3H, *s*, OCH_3-4), 4.02 (3H, *s*, OCH_3-6), 4.03 (3H, *s*, OCH3-7); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 142.5 (C-2), 104.6 (C-3), 102.3 (C-3a), 155.9 (C-4), 112.9 (C-4a), 100.2 (C-5), 147.9 (C-6), 152.7 (C-7), 106.8 (C-8), 142.6 (C-8a), 163.3 (C-9a), 58.9 (OCH_3-4), 55.9 (OCH_3-6), 55.9 (OCH_3-7); ESIMS, $[M+H]^+$ *m/z* 260.3 for $C_{14}H_{13}NO_4$.

***N*-Methylflindersine (339)**

Colorless solid; ^1H NMR ($CDCl_3$, 799.87 MHz): δ 7.97 (1H, *dd*, *J* = 1.2, 8.0 Hz, H-5), 7.23 (1H, *ddd*, *J* = 0.8, 7.2, 8.0 Hz, H-6), 7.55 (1H, *ddd*, *J* = 1.6, 7.2, 8.4 Hz, H-7), 7.32 (1H, *d*, *J* = 8.8 Hz, H-8), 6.75 (1H, *d*, *J* = 10.0 Hz, H-1'), 5.54 (1H, *d*, *J* = 10.0 Hz, H-2'), 1.52 (6H, *s*, CH_3-4'/5'), 3.69 (3H, *s*, NCH_3); ^{13}C NMR ($CDCl_3$, 201.15 MHz): δ 161.0 (C-2), 105.9 (C-3),

155.2 (C-4), 116.1 (C-4a), 123.1 (C-5), 121.7 (C-6), 130.9 (C-7), 114.0 (C-8), 139.4 (C-8a), 117.9 (C-1'), 126.3 (C-2'), 78.7 (C-3'), 28.2 (C-4'/5'), 29.3 (1-NCH_3); ESIMS, $[M+H]^+$ *m/z*: 242.6 for $C_{15}H_{15}NO_2$.

3.7 Procedure for Biological Studies

3.7.1 Cytotoxicity Assays

MDB-MB-231 human breast cancer cells were cultured in Dulbecco's modified eagle medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10^4 cells per well) to ensure exponential growth for the duration of the assay. After a 24 h pre incubation growth, the medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 72 h incubation, cell viability was measured using Alamar Blue reagent (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were performed independently at least six times. Statistical analyses were performed using a two-tailed Student's t-test. $P < 0.05$ was considered to be statistically significant. Cytotoxicity on the Vero cell was carried out according to Irungu *et al.* (2014).

To assess the cytotoxicity of compounds on HEK-293 cells in dose response, a resazurin-based viability assay was used. In brief, HEK-293 cells were grown in DMEM medium (Life Technologies), containing 10 % fetal calf serum (FCS; Gibco), trypsinised, counted and seeded at 2000 cells per well in 45 µL media into TC-treated 384-well plates (Greiner) and

left to adhere overnight at 37 °C, 5% CO_2 and 95 % humidity. Test compounds were prepared by diluting compounds 1 in 25 in sterile water and then another 1 in 10 dilution, to give a top final test concentration of 40 μM, 0.4% DMSO. Plates were incubated for 72 h at 37 °C, 5% CO_2 and 95 % humidity, and then the media was removed and replaced by 35 μL of 44 μM resazurin in DMEM without FCS. The plates were incubated for another 4-6 h at 37°C, 5% CO_2 and 95% humidity, before reading on an EnVision® Plate Reader (PerkinElmer) using fluorescence excitation/emission settings of 530 nm/595 nm. The % growth was standardized to controls (40 μM puromycin as positive and 0.4% DMSO as negative control) using Microsoft® Excel 2013. A statistical analysis including IC_{50} determination and graphical output was performed in GraphPad Prism® 6 using nonlinear regression variable slope curve fitting.

3.7.2 Translation Inhibitory Assay

A previously developed assay (Novac *et al.*, 2004) was used to measure the translation inhibitory activity of the studied compounds. The compounds were suspended at a concentration of 10 mM in DMSO and subsequently diluted to 200 μM in water. They were tested at a final concentration of 20 μM in Krebs-2 translation extracts programmed with a bicistronic Firefly-HCV IRES-Renilla luciferase mRNA construct. Translation reactions were incubated at 30°C for 60 min at which point the luciferase activities were then measured. Compounds that inhibit only FF would be considered cap-dependent translation inhibitors, compounds that inhibit expression of Ren only would be inhibitors of HCV IRES translation while compounds that inhibit both FF and Ren would likely be translation elongation inhibitors.

3.7.3 *Plasmodium falciparum* Culture

In vitro parasite culture of the *P. falciparum* strains 3D7 and Dd2 were maintained in RPMI with 10 mM Hepes (Life Technologies), 50 µg/mL hypoxanthine (Sigma) and 5% human serum from male AB plasma and 2.5 mg/mL AlbuMAX II® (Life Technologies). Human 0+ erythrocytes were obtained from the Australian Red Cross Blood Service (Agreement No: 13-04QLD-09). The parasites were maintained at 2-8 % parasitaemia (% P) at 5 % haematocrit (% H), and incubated at 37 °C, 5 % CO_2, 5 % O_2, 90 % N_2 and 95 % humidity.

3.7.4 *Plasmodium falciparum* Growth Inhibition and Lavicidal Assay

A previously developed, well-established asexual *P. falciparum* imaging assay was used to determine parasite growth inhibition according to the procedure described in Duffy and Avery (2012). Larvicidal assay was done according to Bosire *et al.* (2014).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Preliminary Cytotoxicity Evaluation of the Crude Extracts

The crude extracts of *Millettia dura* (root bark), *M. usaramensis* ssp. *usaramensis* (root bark and leaves), *M. oblata* ssp. *teitensis* (roots and leaves) and *Ochna holstii* (stem bark and leaves) were evaluated for cytotoxic activities against the MDB-MB-231 human breast cancer cell-line (Table 4.1). The crude extract of the roots of *M. oblata* ssp. *teitensis* showed strong activity followed by the crude extract of the root of *M. usaramensis* ssp. *usaramensis*. The root bark extract of *Millettia dura* exhibited moderate activity while the leaves and stem bark extract were inactive (Kebenei *et al.*, 2011).

Table 4.1: Cytotoxicity of the crude extracts against the MDB-MB-231 cell-line (in µg/mL)

Crude extract of the plant species	**IC_{50}**
Millettia oblata spp. teitensis (roots)	4.5
M. usaramensis spp.usaramensis (root bark)	11.6
M. dura (root bark)	31.7
M. oblata spp. teitensis (leaves)	>100
M. usaramensis spp.usaramensis (leaves)	>100
O. holstii (stem bark)	>100
O. holstii (leaves)	*

* Percentage viability at 100 µg/mL greater than 80%

The constituents of these plant extracts were isolated, characterized and evaluated for anticancer activities, and in some cases for antiplasmodial activities. The characterization and activities of these compounds are discused in the subsequent sections.

4.2 Characterization of Compounds from the Root Bark of *Millettia dura*

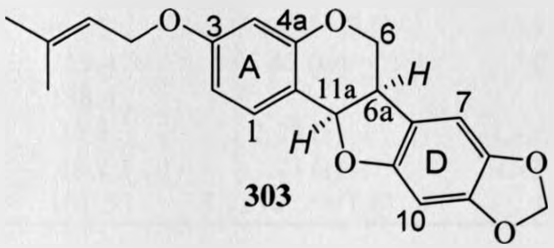
The air-dried and ground root bark of *M. dura* was exhaustively extracted with dichloromethane/methanol (1:1) to afford a gummy brown crude extract (100 g). A chromatographic separation of this crude extract led to the identification of a new pterocarpan (**303**) along with six known isoflavones and a chalcone. The structural characterization of the constituents of the root bark of *M. dura* is discussed in this section.

4.2.1 3-*O*-Prenylmaakiain (303)

Compound **303** was obtained as colorless oil. Its HREIMS revealed a *pseudo*-molecular, $[M+H]^+$ ion peak at *m/z* 353.1386 (calcd. 353.1311) corresponding to the molecular formula $C_{21}H_{20}O_5$. The NMR spectrum of compound **303** displayed signals at (δ_H 4.23, *dd*, J = 4.8, 11.2 Hz; δ_C 66.5), (δ_H 3.66, *t*, J = 11.2 Hz; δ_C 66.5), (δ_H 3.48, *ddd*, J = 1.6, 4.8, 6.4 Hz; δ_C 40.2) and (δ_H 5.49, *d*, J = 7.2 Hz; δ_C 78.6) for $\underline{CH_2}$-6, $\underline{CH}$-6a and $\underline{CH}$-11a, respectively for a pterocarpan skeleton (Tanaka *et al.*, 1998). The ^{1}H NMR spectrum further revealed the presence of three mutually coupled protons in an AXY spin system at δ_H 7.39 (*d*, J = 8.8 Hz), 6.64 (*dd*, J = 2.4, 8.8 Hz) and 6.48 (*d*, J = 2.4 Hz) with the corresponding carbon signals appearing at δ_C 131.7, 109.8 and 102.4 for the ring A. The deshielded proton at δ_H 7.39 was placed at C-1 (δ_C 131.7), based on its three bonds HMBC correlations with two aromatic carbon atoms at δ_C 160.3 for C-3 and 156.5 for C-4a, and oxygenated sp^3 carbon at δ_C 78.6 for C-11a, and consequently the two coupling partners (6.64 and 6.48) were assigned to C-2 (δ_C 109.8) and C-4 (δ_C 102.4), respectively of ring A which is oxygenated at C-3 (from biogenetic consideration).

Furthermore, the NMR data (Table 4.2) showed the presence of a prenyloxy group [δ_H (δ_C): 4.49 (64.9) for OCH_2-1', 5.47 (119.4) for CH-2', 1.79 (25.8) for CH_3-4' and 1.73 (18.2) for

CH_3-5' and a quaternary carbon at δ_C 138.4 for C-3'], a methylenedioxy group at (δ_H 5.92, 5.89; δ_C 101.3) as substituents and two singlet aromatic protons. The prenyloxy group was placed at C-3 of ring A (δ_C 160.3) based on the NOE interaction between CH_2-1' of the prenyl unit with H-2 (δ_H 6.64) and H-4 (δ_H 6.48). This was further confirmed by the HMBC correlation of the CH_2-1' protons with the C-3 (δ_C 160.3). Consequently, the methylenedioxy group could only be located at C-8/C-9 of the ring D, because of the two aromatic singlets being at δ_H 6.72 (H-7) and 6.43 (H-10). The ^3J coupling constant (J = 6.4 Hz) between H-6a and H-11a suggested a *cis* B/C ring junction and, this is supported by the NOESY spectrum, which showed NOE interaction between these protons (H-6a and H-11a). Although the absolute configurations at C-6a and C-11a was not determined, due to the paucity of the sample, this new compound was characterized as (6a*R**,11aR*)-3-*O*-prenylmaackiain (**303**).



303

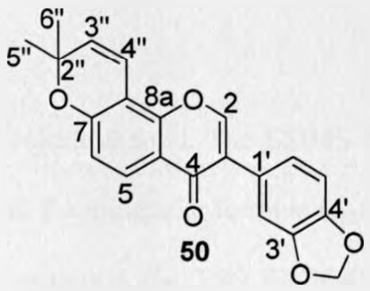
Table 4.2: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **303** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	δ_C	δ_H (*m*, *J* in Hz)	HMBC (H→C)
1	131.7	7.39 (*d*, *J* = 8.8)	C-3, C-4a, C-11a
2	109.8	6.64 (*dd*, *J* = 2.4, 8.8)	C-4, C-11b
3	160.3		
4	102.4	6.48 (*d*, *J* = 2.4)	C-2, C-11b
4a	156.5		
6	66.5	6ax: 4.23 (*dd*, *J* = 4.8, 11.2) 6eq: 3.66 (*t*, *J* = 11.2)	C-4a, C-6a, C-6b, C-11a
6a	40.2	3.48 (*ddd*, *J* = 1.6, 4.8, 6.4)	C-6, C-6b, C-10a
6b	117.9		
7	104.7	6.72 (*s*)	C-8, C-9, C-10a
8	141.7		
9	148.1		
10	93.8	6.43 (*s*)	C-8, C-9, C-6b, C-10a
10a	154.3		
11a	78.6	5.49 (*d*, *J* = 7.2)	C-1, C-6, C-4a, C-11b
11b	112.3		
1'	64.9	4.49 (*d*, *J* = 7.2)	C-3, C-2', C-3'
2'	119.4	5.47 (*m*)	C-4', C-5'
3'	138.4		
4'	25.8	1.79 (*s*)	C-2', C- 3', C-5'
5'	18.2	1.73 (*s*)	C-2', C-3', C-4'
8/9-OCH_2O	101.3	5.92 (*d*, *J* = 1.6)	C-8, C-9

4.2.2 Calopogonium isoflavone B (50)

Compound **50** was isolated as an amorphous solid from methanol. The ESIMS gave a *pseudo*-molecular ion, $[M+H]^+$ peak at *m/z* 349.3 for the molecular formula $C_{21}H_{16}O_5$. In the ^{1}H NMR spectrum (Table 4.3), the presence of a deshielded singlet peak at δ_H 7.91 together with the carbon signals at δ_C 151.9 (C-2), 125.7 (C-3) and 175.7 (C-4) is consistent with an isoflavone skeleton for **50**. The ^{1}H NMR data further revealed the presence of two *ortho*-coupled AX doublets at δ_H 8.05 and 6.84 (*J* = 9.0 Hz) for ring A. The proton at δ_H 8.05 was assigned to H-5 owing to its HMBC correlations with δ_C 157.3 (C-7), 152.3 (C-8a) and 175. 7 (C-4) and

deshielding effect of the carbonyl, consequently the doublet at δ_H 6.84 (J = 9.0 Hz) is assigned to H-6 of the ring A, which is substituted at C-7 and C-8. Moreover, in the ^{1}H NMR, signals of an AMX-type protons at δ_H 6.96 (*dd*, J = 1.8, 7.8 Hz), 6.86 (*d*, J = 7.8 Hz) and 7.08 (*d*, J = 1.8 Hz) were observed and assigned to H-6', H-5' and H-2' of ring B, respectively. The presence of a 2,2-dimethylchromene at [(δ_H 5.51, *d*, J = 10.2 Hz; δ_C 130.3), (6.79, *d*, J = 10.2 Hz; δ_C 114.9) and (1.49, *s*; δ_C 28.2)] and a methylenedioxy (δ_H 5.98; δ_C 101.2) groups was evident. These groups were placed at C-7/C-8 of ring A and C-3'/-4' of ring B, respectively, on the bais of the HMBC correlation. Compound **50** was therefore, identified as calopogonium isoflavone B, a compound previously reported from the root bark of *M. grifoniana* (Yankep *et al.*, 1997).

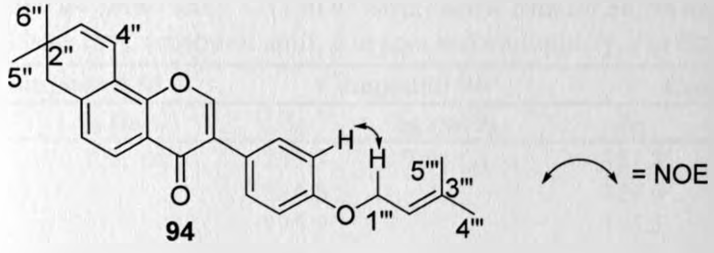


50

4.2.3 Isoerythrin A-4'-(3-methylbut-2-enyl) ether (94)

Compound **94** was obtained as shinny crystals from methanol. ESIMS analysis exhibited a $[M+H]^+$ ion peak at *m/z* 389.4 compatible with the molecular formula $C_{25}H_{24}O_6$. Similar to **50**, this compound is also an isoflavone derivative as shown from the NMR spectra [δ_H 7.94 (s, H-2); δ_C 151.7 (C-2), 124.8 (C-3) and 175.9 (C-4)]. The NMR spectra further showed that compound **94** has identical ring A as that of **50**. In ring B, the presence of an AA'XX' spin system at δ_H (7.48, *d*, J = 8.8 for H-2'/6') and (6.98, *d*, J = 8.8 for H-3'/5') is consistent with 4'-substitution; which in this case is an *O*-prenyl group [δ_H (δ_C): 4.55, *d*, J = 7.2 Hz (64.8) for

OCH_2-1'''; 5.51, *t*, *J* = 7.2 Hz (119.6) for CH-2'''; 1.80, *s*, (25.9) for CH_3-4''' and 1.76, *s*, (18.2) for CH_3-5''' and a quaternary carbon at δ_C 138.3 for C-3''']. The placement of the prenyloxy group at C-4' was confirmed from NOE interaction of the methylene protons of the prenyl group with H-3'/H-5'. Therefore, based on this spectroscopic evidence, compound **94** was identified as isoerythrin A-4'-(3-methylbut-2-enyl) ether, a compound that has been previously reported from the seed pods of this plant (Yenesew *et al.*, 1996).

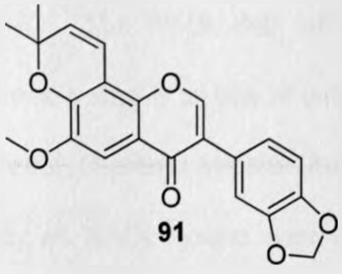


94

4.2.4 Durmillone (91)

Compound **91** was obtained as colorless solid. The ESIMS spectrum showed a $[M+H]^+$ ion peak at *m/z* 379.4 compatible with the molecular formula $C_{22}H_{18}O_6$. This compound is also an isoflavone derivative, having a methoxyl (δ_H 3.96; δ_C 56.4), a methylendioxy (δ_H 5.99; δ_C 101.2) and a 2,2-dimethylchromene groups as substituents (Table 4.3). The proton at δ_H 6.81 of 2,2-dimethychromene system showed HMBC correlations with ring A carbons (C-7, C-8a, C-8) suggesting that the 2,2-dimethychromene group is located at C-7/C-8 on ring A. The fact that there is only one aromatic singlet at δ_H 7.94 (assigned to H-5) in this ring, suggested that C-6 is substituted, which in this case should be a methoxyl group (δ_H 3.96; δ_C 56.4). This placement was confirmed from the NOE interaction of this methoxyl group with H-5. The NMR spectra data (Table 4.3) for ring B are identical to those observed for calopogonium isoflavone B (**50**), placing the methylenedioxy group at C-3'/C-4' as in **50**. Therefore, compound **91** is a 6-methoxyl derivative of calopogonium isoflavone B, trivial name

durmillone, which was earlier reported from seeds of *M. dura* (Ollis *et al.*, 1967) and root bark of *M. graffoniana* (Yankep *et al.*, 1997).



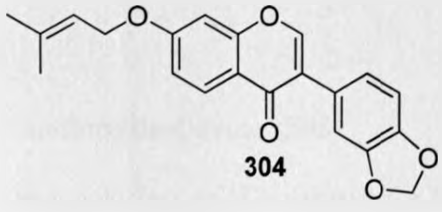
91

Table 4.3: ^{1}H (600.24 MHz) and ^{13}C (150.95 MHz) NMR data for **50**, **94** and **91** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 50		Compound 94		Compound 91	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	151.9	7.91 (*s*)	151.7	7.94 (*s*)	151.8	7.55 (*s*)
3	125.7		124.8		124.4	
4	175.7		175.9		175.5	
4a	118.3		118.4		117.6	
5	126.7	8.05 (*d*, *J* = 9)	126.8	8.06 (*d*, *J* = 8.8)	105.1	7.94 (*s*)
6	115.2	6.84 (*d*, *J* = 9)	115.3	6.86 (*d*, *J* = 8.8)	147.2-147.7	
7	157.3		157.3		147.2-147.7	
8	109.2		109.2		110.2	
8a	152.3		152.4		147.2-147.7	
1'	124.8		124.1		125.9	
2'	109.8	7.08 (*d*, *J* = 1.8)	130.1	7.48 (*d*, *J* = 8.8)	109.2	7.10 (*d*, *J* = 1.8)
3'	147.6		114.7	6.98 (*d*, *J* = 8.8)	147.2-147.7	
4'	147.6		158.9		147.2-147.7	
5'	108.4	6.86 (*d*, *J* = 7.8)	114.7	6.98 (*d*, *J* = 8.8)	108.4	6.87 (*d*, *J* = 7.8)
6'	122.4	6.96 (*dd*, *J* = 1.8, 7.8)	130.1	7.48 (*d*, *J* = 8.8)	122.4	6.98 (*dd*, *J* = 1.8,7.8)
2"	77.7		77.7		78.2	
3"	130.3	5.51 (*d*, *J* = 10.2)	130.3	5.72 (*d*, *J* = 9.6)	130.4	5.74 (*d*, *J* = 10.2)
4"	114.9	6.79 (*d*, *J* = 10.2)	114.9	6.81 (*d*, *J* = 9.6)	115.2	6.81 (*d*, *J* = 10.2)
5"/6"	28.2	1.49 (*s*)	28.2	1.5 (*s*)	28.0	1.56 (*s*)
1"'			64.8	4.55 (*d*, *J* = 7.2)		
2"'			119.6	5.51 (*t*, *J* = 7.2)		
3"'			138.3			
4"'			25.9	1.80 (*s*)		
5"'			18.2	1.76 (*s*)		
OCH_2O	101.2	5.98 (*s*)			101.2	5.99 (s)
6-OCH_3					56.4	3.96 (*s*)

4.2.5 Maximaisoflavone B (304)

The ESIMS analysis of compound **304** showed a $[M+H]^+$ ion peak at *m/z* 351.6 corresponding to the molecular formula $C_{21}O_{18}O_5$. The NMR data of **304** is also consistent with an isoflavone core skeleton, with identical ring B as that of calopogonium isoflavone B (**50**). In ring A of compound **304**, signals for *O*-prenyl system situating at C-7, and three mutually coupled aromatic protons forming an AMX system were observed for H-5, H-6 and H-7. Hence, based on the spectral data, compound **304** was characterized as 7-*O*-prenyl-3',4'-methylenedioxyisoflavone (trivial name maximaisoflavone B), a compound which was previously reported from stem bark of *M. dura* (Dagne *et al.*, 1991). The NMR data of this compound is in Section 3.6.1.

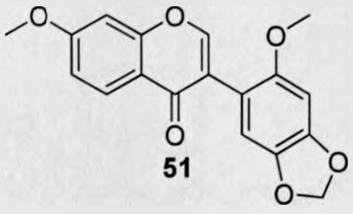


304

4.2.6 7,2'-Dimethoxy-4',5'-methylenedioxyisoflavone (51)

Compound **51**, a colorless solid from methanol, was isolated as the major compound of this plant. Its molecular formula $C_{18}H_{14}O_6$ was deduced from the ESIMS spectrum showing a $[M+H]^+$ ion peak at *m/z* 327.2. The NMR data (Table 4.4) revealed that **51** is an isoflavone derivative accommodating two methoxyl [δ_H 3.91 (δ_C 55.8) and δ_H 3.72 (δ_C 56.9)] and a methylenedioxy (δ_H 5.95; δ_C 101.4) groups as substituents. In ring A, an AMX spin system at δ_H 8.18 (*d*, *J* = 8.4 Hz), 6.98 (*dd*, *J* = 2.4, 8.4 Hz) and 6.85 (*d*, *J* = 2.4 Hz) were oberved and assigned to H-5, H-6 and H-8, respectively, with C-7 (δ_C 163.9) being substituted with methoxyl group (δ_H 3.91, δ_C 55.8) as this was established from HMBC experiment (correlation of the the methoxyl protons with C-7).

In ring B, two *para*-oriented aromatic singlets were displayed at δ_H 6.61 (H-3'), 6.82 (H-6') and showed HMBC correlation to C-2' (δ_C 152.9); the site to which the second methoxyl group (δ_H 3.72; δ_C 56.9) is attached (confirmed from HMBC correlation of the methoxyl protons with C-2'). The methylenedioxy group (δ_H 5.95; δ_C 101.4) was then placed at C-4'/C-5' accounting for the *para* orientation of the two protons, H-3' and H-6'. Compound **51** was therefore, identified as 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone, previously isolated from the stem bark of *Millettia dura* (Dagne *et al.*, 1991) and *M. graffoniana* (Yankep *et al.*, 1997).



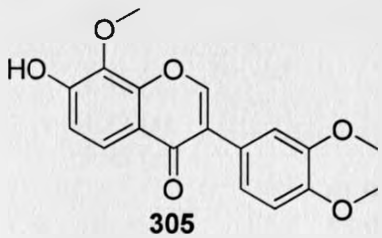
51

4.2.7 7-Hydroxy-8,3',4'-trimethoxylisoflavone (305)

Compound **305** was isolated as a colorless soild, analyzed for $[M+H]^+$ 329.5 in the ESIMS, corresponding to the molecular formula $C_{18}H_{16}O_6$. The NMR data of compound **305** (Table 4.4) is consistent with an isoflavone core skeleton on which three methoxyl groups are attached. In the ^1H NMR spectrum (Table 4.4), five aromatic protons, in two sets of spin system were observed. In one set, *ortho*-coupled AX doublets at δ_H 7.97 and 7.05 (J = 8.4 Hz) were assigned to H-5 and H-6, respectively in the ring A. The second set constituting an AMX spin system at δ_H 7.19 (*d*, J = 2.4 Hz), 7.05 (*dd*, J = 2.4, 8.4 Hz) and 6.93 (*d*, J = 8.4 Hz) were due to H-2', H-6' and H-5' of the ring B, respectively.

The presence of three methoxyl groups was also evident from the NMR spectra (Table 4.4). The methoxyl group at δ_H 3.91 showed an NOE correlation with the ring B proton at δ_H 6.93

(H-5') and, similarly the one at δ_H 3.93 showed NOE correlation with the ring B proton at δ_H 7.19 (H-2'), allowing their placement at C-4' and C-3' of the ring B, respectively. The third methoxyl group would only be placed at C-7 or C-8 of the ring A. The high carbon chemical shift of this methoxyl (δ_C 61.9) suggested that it is a di-*ortho*-substituted (Oberholz *et al.*, 1974) and consequently placed at C-8; and leaving C-7 to have a free hydroxyl group. Based on the above evidences, compound **305** was characterized as 7-hydroxy-8,3',4'-trimethoxyisoflavone, a compound which was earlier isolated from *Xanthocercis zambesiaca* (Harper *et al.*, 1976) and *Dipteryx alata (*Puebla *et al.*, 2010) of Leguminosae family. This report seems to be the first of its isoltion from the genus *Millettia.*



305

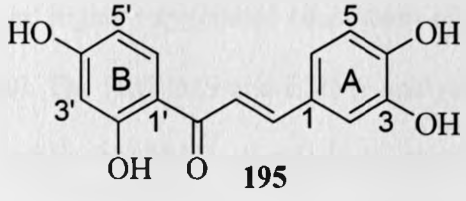
Table 4.4: ^{1}H (600.24 MHz) and ^{13}C (150.95 MHz) NMR data for **51** and **305** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 51		Compound 305	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	154.1	7.88 (*s*)	151.7	8.01 (*s*)
3	122.2		124.8	
4	175.7		175.9	
4a	118.4		118.9	
5	127.8	8.18 (*d*, *J* = 8.4)	122.1	7.97 (*d*, *J* = 8.4)
6	114.4	6.98 (*dd*, *J* = 2.4, 8.4)	114.0	7.05 (*d*, *J* = 8.4)
7	163.9		153.2	
8	100.2	6.85 (*d*, *J* = 2.4)	133.9	
8a	157.9		150.0	
1'	112.8		124.3	
2'	152.9		112.5	7.19 (*d*, *J* = 2.4)
3'	95.4	6.61 (*s*)	148.8	
4'	148.4		149.2	
5'	141.2		111.1	6.93 (*d*, *J* = 8.4)
6'	111.2	6.82 (*s*)	121.1	7.05 (*dd*, *J* = 2.4, 8.4)
7-OCH_3	55.8	3.91 (*s*)		
2'-OCH_3	56.9	3.72 (*s*)		
OCH_2O	101.4	5.95 (*s*)		
8-OCH_3			61.9	4.08 (*s*)
3'-OCH_3			55.9	3.93 (*s*)
4'-OCH_3			55.9	3.91 (*s*)

4.2.8 Butein (195)

Compound **195** was obtained as a yellow solid which turned brown upon exposure to air on TLC. The ESIMS spectrum for **195** exhibited a $[M+H]^+$ ion peak at *m/z* 273.3 suggesting the molecular formula $C_{15}H_{12}O_5$. In the ^{1}H NMR spectrum (Table 4.5), two *trans*-coupled (^{3}J = 15.2 Hz) olefinic protons at δ_H 7.64 (H-α) and 7.82 (H-β) together with the carbon signals at δ_C 192.2 (C=O), 116.9 (C-α) and 144.8 (C-β), in the ^{13}C NMR spectrum, is consistent with the compound having a chalcone skeleton (Abegaz *et al.*, 2002; Vijayakumar *et al.*, 2013). Furthermore, the ^{1}H NMR and H,H- COSY spectra displayed the presence of two sets of aromatic protons showing an AMX spins system, in which one at (*vis.* δ_H 6.92, *d*, 8.8 Hz for

H-5; 7.22, *dd*, 2.4, 8.8 Hz for H-6 and 7.28, *d*, 2.4 for H-2) and the other at (δ_H 6.39, *d*, 2.4 Hz for H-3'; 6.52, *dd*, 2.4, 9.6 Hz for H-5' and 8.06, *d*, 9.6 Hz for H-6') allocated to rings A and B, respectively. This suggested that **195** is tetra-oxygenated [at C-3 (δ_C 145.5), C-4 (δ_C 148.6), C-2' (δ_C 165.1) and C-4' (δ_C 166.2)], and characterized as 3,4,2',4'-tetrahydroxychalcone (a trival name butein), a compound which was previously reported from *Millettia nitida* var. *hirsutissima* (Liao *et al.*, 2013) and *Bidens pilosa* (Tian *et al.*, 2011).



195

Table 4.5: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **195** acquired in CD_3OD at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	δ_C	δ_H (*m*, *J*)
1	127.1	
2	114.5	7.28 (*d*, *J* = 2.4)
3	145.5	
4	148.6	
5	115.3	6.92 (*d*, *J* = 8.8)
6	122.3	7.22 (*dd*, *J* = 2.4, 8.8)
C-α	116.9	7.64 (*d*, *J* = 15.2)
C-β	144.8	7.82 (*d*, *J* = 15.2)
C=O	192.2	
1'	113.4	
2'	165.1	
3'	102.5	6.39 (*d*, *J* = 2.4)
4'	166.2	
5'	107.8	6.52 (*dd*, *J* = 2.4, 9.6)
6'	131.9	8.06 (*d*, *J* = 9.6)

4.3 Characterization of Compounds from the Roots of *Millettia oblata* ssp. *teitensis*

The roots of *M. oblata* ssp. *teitensis* was air-dried, ground and exhaustively extracted with dichloromethane/methanol (1:1) to give a brown gummy extract (100 g). The extract was then

subjected to different chromatographic separation to afford thirteen compounds. Among these constituents, the isoflavones calopogonium isoflavone B (**50**), 7,2'-dimethoxy-4',5'-methylenedioxyisoflavone (**51**), isoerythrin A-4'-(3-methylbut-2-enyl) ether (**94**), durmillone (**91**) and the chalcone; butein (**195**) were also isolated from root bark of *M. dura* and their structures characterized as in Section 4.2.

4.3.1 Obloneside (306)

Compound **306**, the major and highly oxygenated constituent of this plant, was isolated as a colorless solid from methanol. The HREIMS and ESIMS analyses gave $[M+H]^+$ ion peak at *m/z* 959.3017 and 959.5 (calcd. 959.2954), respectively corresponding to the molecular formula $C_{42}H_{54}O_{25}$. The UV maximum (λ_{max}) was observed at 290 nm and the NMR (^1H, ^{13}C: Table 4.6) spectra showed characterstic signals for isoflavone skeleton.

In NMR spectra of compound **306**, two methoxyl groups at (δ_H 3.91; δ_C 64.1) and (δ_H 3.92; δ_C 63.6) and a methylenedioxy (δ_H 5.98; δ_C 103.7) were evident as substituents on the isoflavone core skeleton. The methoxyl groups were respectively placed at C-5 (δ_C 155.3) and C-6 (δ_C 143.4) based on HMBC spectrum in the ring A. Whereas, the methylendioxy group was located at C-3'/C-4' of the ring B, which also accommodated three aromatic protons with an AMX-spin system at δ_H 7.03 (H-2'), 6.84 (H-5') and 6.97 (H-6').

The clustered proton peaks in the mid-field region (δ_H 3.34-3.83) in the ^1H NMR spectrum indicated the presence of glycoside moieties. The number of monosaccharidic units was deduced to be four; from the anomeric protons/carbons signals at δ_H/δ_C (5.08/102.9), (4.55/106.7), (4.77/103.8) and (4.74/103.7) ppm. The appearance of two methyl groups (δ_H 1.30, δ_C 19.4) and (δ_H 1.24, δ_C 19.3) in the NMR spectra suggested the two glycosides to be

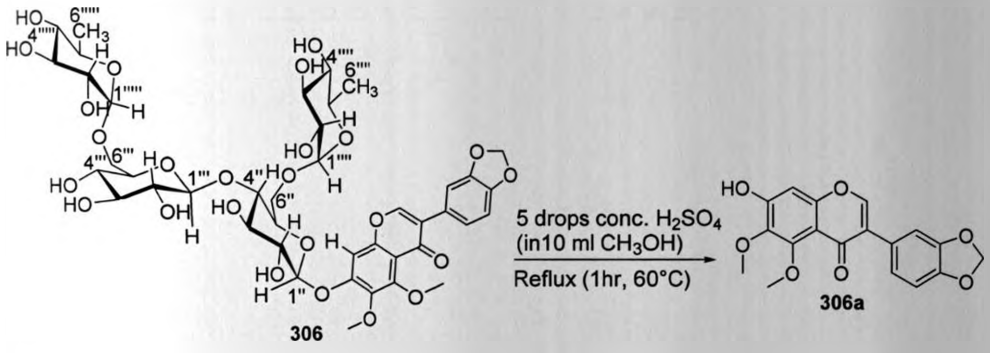
rhamnoside units and the other two being glucosides. The anomeric proton resonating at δ_H 5.08 (H-1") showed HMBC correlation with the isoflavone carbon at δ_C 158.3 (C-7) and NOE correlation with the isoflavone proton at δ_H 7.12 (H-8); indicating that the glycoside chain should be placed at C-7 through the C-1"-*O*-C-7 linkage.

Moreover, the anomeric proton (δ_H 5.08, H-1") showed coupling with the multiplet centered at δ_H 3.81 (H-2") with large coupling constant (J = 8.0 Hz), which suggested the β-linkage of the sugar end (glucoside) to the aglycone, isoflavone nucleus (Jacobsen, 2007). The H-2" (3.81) showed a three bonds HMBC correlation with the carbon at δ_C 90.7 (C-4") to which again the anomeric proton of the second sugar moiety (δ_H 4.55, *d*, J = 7.2 Hz, H-1"') showed HMBC correlaation. Moreover, the multiplet proton at δ_H 3.66, H-H4" (attached to C-4", δ_C 90.7) of the first sugar moiety showed a ^3J HMBC correlation to the anomeric carbon, C-1"' (δ_C 106.7) and NOE correlation with the anomeric proton (δ_H 4.55, H-1"') of the second sugar moiety, defining the linkage of the two sugars as C-4"-*O*-C-1"'. Hence, this observation coupled with the large coupling constant for H-1"' (J = 7.2 Hz) established the β-glucopyranosyl-(4"→1"')-β-glucopyranosyl linkage between the two glucoside moieties.

Similarily, the anomeric protons of the two rhmnosyl moieties δ_H 4.74 and 4.77, respectively showed a three bond HMBC correlations with C-6" (δ_C 69.2) and C-6"' (δ_C 69.9); and respirocally, the OCH_2-6" and OCH_2-6"' protons showed HMBC correlations to the respective anomeric carbons of the rhamnosyls to establish linkages: α-rhmnosyl-(1""→6")-β-glucosyl and α-rhmnosyl-(1""'→6"')-β-glucosyl respectively.

The aglycone of **306**, 6-hydroxy-7,8-dimethoxy-3',4'-methylenedioxyisoflavone, (trival name: isoplatycarpanetin, **306a**) was also obtained through acid hydrolysis and characterized

accordingly. The NMR data (Table 4.6) of **306a** is related to the isoflavone nucleus of **306** but signifcant changes in chemical shift were observed on cleavage of the sugar moiety (shifting of the signal for H-8 from δ_H 7.12 in **306** to δ_H 6.85 in **306a**). A complete chemical shift assignment of **306** was accomplished based on 2D (HSQC, HMBC, TOCSY, NOESY and COSY) NMR analyses. Therefore, compound **306** was characterized as isoplatycarpanetin-7-*O*-β-glucosyl-((6"→1"")-α-rhmnosyl)-(4"→1"')-β-glucosyl (6"'→1"")-α-rhmnoside which is a new compound and given a trival name obloneside.



306 **306a**

Table 4.6: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **306** (CD_3OD) and **306a** ($CDCl_3$) acquired at 25°C (chemical shift, δ in ppm and multiplicity, J in Hz).

Position	Compound 306			Compound 306a	
	δ_C	δ_H (m, J)	HMBC	δ_C	δ_H (m, J)
	Isoflavone nucleus				
2	155.5		C-4, C-8a, C-3	151.2	
3	127.3			125.1	
4	178.4			175.1	
4a	116.5			112.9	
5	155.3			151.6	
6	143.4			138.3	
7	158.3			154.6	
8	103.2	7.12 (s)	C-4a, C-6, C-8a, C-7	99.1	6.85 (s)
8a	156.8			154.4	
1'	128.2			125.4	
2'	112.1	7.03 (d, J = 1.6)	C-4', C-6'	110.0	7.09 (d, J = 1.6)
3'	150.2			147.7	
4'	150.2			147.7	
5'	110.4	6.84 (d, J = 8.0)	C-1', C-3'	108.4	6.89 (d, J = 8.0)
6'	125.1	6.97 (dd, J = 0.8, 8.0)	C-2', C-4'	122.6	6.96 (dd, J = 1.6, 8.0)
5-OCH_3	64.1	3.91(s)		62.1	3.97 (s)
6-OCH_3	63.6	3.92 (s)		61.8	4.04 (s)
3'/4'-OCH_2O	103.7	5.97 (s); 5.98 (s)		101.2	5.98 (s)
	ß-glucoside moiety-1				
1"	102.9	5.08 (d, J = 8.0)	C-7		
2"	74.9	3.79-3.83 (m)			
3"	71.5	3.45-3.49 (m)			
4"	90.7	3.64-3.67 (m)	C-1'''		
5"	77.9	3.55-3.61/3.72-3.74 (m)			
6"	69.2	3.55-3.61(m)	C-1''''		
		4.07 (d, J = 9.6)			

	β-glucoside moiety-2	
1'''	106.7	4.55 (*d*, *J* = 7.2)
2'''	76.5	3.35-3.38 (*m*)
3'''	73.0	3.34-3.38 (*m*)
4'''	78.9	3.45-3.49 (*m*)
5'''	77.9	3.55-3.61/3.72-3.74 (*m*)
6'''	69.9	3.55-3.61(*m*)
		4.11 (*d*, *J* = 9.6)
	α-rhamnoside moiety-3	
1''''	103.7	4.74 (*d*, *J* = 1.6)
2''''	73.3	3.94 (*m*)
3''''	75.4/ 75.5	3.39-3.42 (*m*)
4''''	73.5/ 73.7	3.72-3.74/3.79-3.83 (*m*)
5''''	71.3	3.64-3.68 (*m*)
6''''	19.4	1.30 (*d*, *J* = 6.4)
	α-rhamnoside moiety-4	
1'''''	103.8	4.77 (*d*, *J* = 1.6)
2'''''	72.2	3.93 (*m*)
3'''''	75.4/ 75.5	3.39-3.42 (*m*)
4'''''	73.5/ 73.7	3.72-3.74/3.79-3.83 (*m*)
5'''''	71.2	3.68-3.70 (*m*)
6'''''	19.3	1.24 (*d*, *J* = 6.4)

C-4"

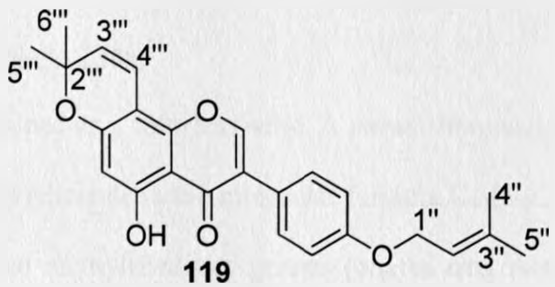
C-1""

C-6"

C-6"'

4.3.2 4'-Prenyloxyderrone (119)

The ESIMS analysis of compound **119** revealed the molecular formula $C_{25}H_{24}O_5$ from the $[M+H]^+$ ion peak at *m/z* 405.2. The signal at δ_H 7.89 (for H-2) in the ^1H NMR and the signals at δ_C 152.3 (for C-2), 123.7 (for C-3) and 180.9 (for C-4) in the ^{13}C NMR spectra (Table 4.7) suggested that compound **119** is an isoflavone derivative (Table 4.7). The NMR spectra revealed that compound **119** has identical ring B with **94** with the only substituent in this ring being a prenyloxy group at C-4'. This placement was confirmed from the NOESY spectrum which showed NOE correlation between the methylene protons of the prenyloxy group and H-3'/H-5' of ring B. Ring A of **119** also has a 2,2-dimethylchromene group as in **94** (Table 4.7); indeed the only difference between these two compounds is the hydroxyl at C-5 in **119** (δ_H 12.94). Therefore, compound **119** was characterized as 5-hydroxy-2",2"-dimethylpyrano[5",6":7,8]-4'-prenyloxyisoflavone, trivial name 4'-prenyloxyderrone. This compound was previously reported from the stem bark of *M. oblata* ssp. *teitensis* (Derese *et al.*, 2014).

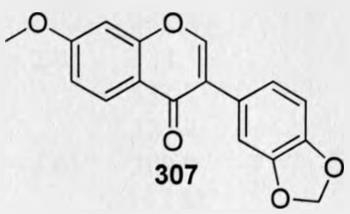


119

4.3.3 Pseudobaptigenin methyl ether (307)

The ESIMS of compound **307** showed a $[M+H]^+$ peak at *m/z* 297.2 for the molecular formula $C_{17}H_{13}O_5$. The NMR data (Table 4.7) revealed this compound to be an isoflavone derivative, substituted with a methoxyl (δ_H 3.92; δ_C 58.5) and a methylenedioxy (δ_H 5.99; δ_C 103.8) groups. The methoxyl group showed NOE correlations with H-6 and H-8, and HMBC

correlation with the carbon at δ_C 166.7 (C-7), allowing its placement at C-7 of the ring A. In the ^{1}H NMR spectrum (Table 4.7), an AMX spin system at δ_H 8.20 (*d*, *J* = 8.8 Hz), 6.99 (*dd*, *J* = 2.4, 8.8 Hz) and 6.85 (*d*, *J* = 2.4 Hz) were assigned to H-5, H-6 and H-8, respectively in the ring A. Furthermore, another three mutually coupled protons of AMX spin system were observed at δ_H 7.09 *d*, *J* = 1.6 Hz; 6.98, *dd*, *J* = 1.6, 8.0 Hz and 6.87, *d*, *J* = 8.0 Hz for H-2', H-6' and H-5', respectively in the ring B, which is substituted with the methylenedioxy group at C-3' (δ_C 150.3) and C-4' (δ_C 150.3). The coupling interaction was supported by the COSY correlation map: 6.87 ppm↔6.98 ppm ↔7.09 ppm. Therefore, this isolate was characterized as 7-methoxy-3',4'-methylenedioxyisoflavone (trivial name pseudobaptigenin methyl ether), a compound which has been previously identified from the leaves of *Ateleiaherbert-smithii* (Veitch *et al.*, 2003).

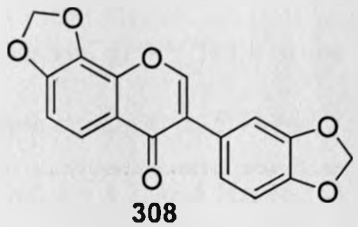


307

4.3.4 Maximaisoflavone A (308)

Compound **308** was obtained as a colorless solid. A *pseudo*-molecular ion peak *m/z* at 311.5, $[M+H]^+$ in the ESIMS corresponds to the molecular formula $C_{17}H_{10}O_6$. That the compound is an isoflavone having two methylenedioxy groups (one in ring A and one in ring B) was deduced from the NMR spectra (Table 4.7). The NMR spectrum of **308** indicated that this compound has identical ring B with that of **307** (Table 4.7). In ring A, the methoxyl group at C-7 of **307** is replaced by a methylenedioxy group (δ_H 6.21; δ δ_H 103.1) at C-7/C-8 in the case of **308**. This compound was therefore, identified as 7,8,3',4'-dimethylenedioxyisoflavone

(trivial name maximaisoflavone A), previously isolated from *Tephrosia maxima* (Rao *et al.*, 1984).



308

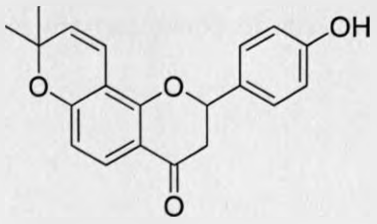
Table 4.7: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **307**, **308** and **119** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 307		Compound 308		Compound 119	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	154.9	7.91 (*s*)	151.7	7.89 (*s*)	152.3	7.89 (s)
3	127.7		124.8		123.7	
4	178.4		175.4		180.9	
4a	121.0		120.5		106.1	
5	130.5	8.20 (*d*, *J* = 8.8)	121.1	7.89 (*d*, *J* = 8.0)	162.3	
6	117.3	6.99 (*dd*, *J* = 2.4, 8.8)	107.3	6.98 (*d*, *J* = 8.0)	100.3	6.29 (*s*)
7	166.7		152.3		159.6	
8	102.8	6.85 (*d*, *J* = 2.4)	134.5		101.1	
8a	166.6		151.7		152.2	
1'	128.4		125.4		122.8	
2'	112.4	7.09 (*d*, *J* = 1.6)	109.9	7.07 (*d*, *J* = 1.6)	130.1	7.44 (*d*, *J* = 8.8)
3'	150.3		147.8*		114.9	6.99 (*d*, *J* = 8.8)
4'	150.3		147.7*		159.1	
5'	111.0	6.87 (*d*, *J* = 8.0)	108.4	6.87 (*d*, *J* = 8.0)	114.9	6.99 (*d*, *J* = 8.8)
6'	125.0	6.98 (*dd*, *J* = 1.6, 8.0)	122.5	6.95 (*dd*, *J* = 1.6, 8.0)	130.1	7.44 (*d*, *J* = 8.8)
1"					64.9	4.55 (*d*, *J* = 6.4)
2"					119.5	5.51 (*d*, *J* = 6.4)
3"					138,4	
4"					25.9	1.81 (*s*)
5"					18.2	1.76 (*s*)
2"'					78.1	
3"'					127.4	5.59 (*d*, *J* = 9.6)
4"'					114.6	6.68 (*d*, *J* = 9.6)
5"'/6"'					28.2	1.48 (*s*)
7-OCH_3	58.5	3.92 (*s*)				
7/8-OCH_2O			103.1	6.21 (*s*)		
3'/4' OCH_2O	103.8	5.99 (*s*)	101.2	5.99 (*s*)		
5-OH						12.94 (*s*)

* may be interchanged

4.3.5 4'-Hydroxyisoloncocarpin (309)

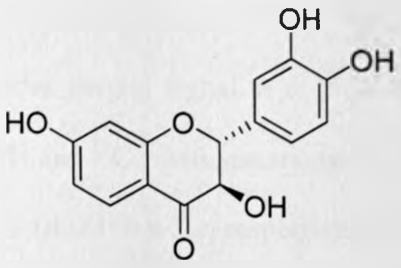
The molecular formula of compound **309** was determined as $C_{20}H_{18}O_4$ based on the *pseudo*-molecular ion, $[M+H]^+$ peak observed at *m/z* 323.5 in the ESIMS spectrum. The ^1H NMR (Table 4.8) exhibited an AMX spin system at δ_H 5.41 (*dd*, J = 3.2, 12.6 Hz, H-2; 3.01 (*dd*, J = 12.6, 16.8 Hz, H-3_{ax}) and 2.81 (*dd*, J = 3.2, 16.8 Hz, H-3_{eq}), with the carbon resonances at δ_C 82.2 (C-2), 46.8 (C-3) and 193.5 (C=O), consistent with the flavanone nature of **309.** The presence of a 2,2-dimethylchromene ring was also apparent from the NMR spectra (Table 4.8). In ring B, an AA'XX' spin system at δ_H 7.36 (*d*, J = 8.0 Hz) and 6.89 (*d*, J = 8.0 Hz) were observed and assigned to H-2'/6' and H-3'/5', respectively with C-4' substituted with hydroxyl group (δ_C 158.5). Furthermore, two mutually *ortho*-coupled AX doublets (J = 8.0 Hz) at δ_H 7.74 and 6.49 were displayed and assigned to H-5 and H-6, respectively of ring A, allowing the placement of the 2,2-dimethylchromene ring at C-7/C-8. This was further corroborated by the HMBC correlation observed between H-4" (6.63) of the 2,2-dimethylchromene ring with C-8a (δ_C 160.4) and C-7 (δ_C 162.3) of ring A. Compound **309** was therefore, characterized as 4'-hydroxy-2",2"-dimethypyrano[5",6":7,8]flavanone, (trivial name 4'-hydroxyisoloncocarpin), a compound previously isolated from the stem bark of *M. ferruginea* ssp. *ferruginea* (Dagne *et al.*, 1989).



309

4.3.6 (+)-(2*R*,3*R*)-Fustin (310)

Compound **310**, isolated as brown gum, $[\alpha]_D^{25}$ +2.8, was determined to have a molecular formula $C_{15}H_{12}O_6$ from ESIMS analysis, showing $[M+H]^+$ ion peak at *m/z* 289.2. The presence of two *trans*-oriented proton peaks at δ_H 4.51 (*d*, *J* = 12.0 Hz; δ_C: 75.8 for H-3) and 4.96 (*d*, *J* = 12.0 Hz; δ_C: 86.9 for H-2) in ^{1}H NMR spectrum together with the ^{13}C NMR signal at δ_C 195.8 (for C=O) suggested that **310** is a flavanonol. The ^{1}H NMR spectrum (Table 4.8) further displayed signals for six protons forming two sets of AMX spin systems, the connectivities of which were established by H,H-COSY spectrum as δ_H 6.35 (H-8) ↔ 6.56 (H-6) ↔ 7.74 (H-5) of the ring A which is oxygenated (with hydroxyl group) at C-7, and δ_H 6.83 (H-5') ↔ 6.88 (H-6') ↔ 7.01 (H-2') of the ring B which is oxygenated (with hydroxyl group) at C-3' and C-4'. Thus, the compound is identified as 7,3',4'-trihydroxyflavanonol. The large coupling constant (*J* = 12.0 Hz) between H-2 (δ_H 4.96) and H-3 (δ_H 4.51) of ring C containing hydroxyl group at C-3, indicated a *di-axial* relationship between the two protons. Thus two isomers, a pair of enantiomers, (2*R*,3*R*) or (2*S*,3*S*) absolute configuration are possible. However, the positive optical rotation $[\alpha]_D^{25}$ +2.8, and a positive Cotton effect at *ca.* 335 nm, and negative one at *ca.* 305 nm in the CD spectrum, are consistent with the (2*R*,3*R*) absolute configuration (Slade *et al.*, 2005). Based on the above data, compound **310** was characterized as (+)-(2*R*,3*R*)-7,3',4'-trihydroxyflavanonol, a compound earlier reported from the heartwood of *Acacia mollissima* (Roux and Paulus, 1960).



310

Table 4.8: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **309** and **310** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

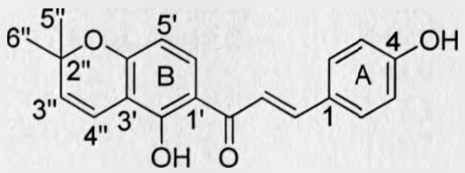
Position	Compound 309		Compound 310	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	82.2	5.41 (*dd*, *J* = 3.2, 12.6)	86.9	4.96 (*d*, *J* = 12.0)
3	46.8	2.81 (*dd*, *J* = 3.2, 16.8)	75.8	5.51 (*d*, *J* = 12.0)
		3.01 (*dd*, *J* = 12.6, 16.8)		
4	193.5		195.8	
4a	117.4		114.7	
5	130.6	7.74 (*d*, *J* = 8.0)	131.4*	7.74 (*d*, *J* = 8.8)
6	113.8	6.49 (*d*, *J* = 8.0)	113.4	6.56 (*dd*, *J* = 1.6, 8.8)
7	162.3		168.1	
8	112.1		104.9	6.35 (*d*, *J* = 1.6)
8a	160.4		166.3	
1'	133.9		131.3*	
2'	130.5	7.36 (*d*, *J* = 8.0)	117.2	7.01 (*d*, *J* = 1.6)
3'	118.2	6.89 (*d*, *J* = 8.0)	147.5	
4'	158.5		148.3	
5'	118.2	6.89 (*d*, *J* = 8.0)	117.4	6.83 (*d*, *J* = 8.0)
6'	130.5	7.36 (*d*, *J* = 8.0)	122.2	6.88 (*dd*, *J* = 2.4, 8.0)
2"	80.2			
3"	131.5	5.56 (*d*, *J* = 9.6)		
4"	118.6	6.63 (*d*, *J* = 9.6)		
5"	31.1	1.47 (*s*)		
6"	30.8	1.44 (*s*)		

* may be interchangeable

4.3.7 4-Hydroxyloncocarpin (182)

Compound **182** was isolated as a yellow solid from methanol. The ESIMS spectrum showed a *pseudo*-molecular ion, $[M+H]^+$ peak at *m/z* 323.5 for which the molecular formula $C_{20}H_{18}O_4$ was suggested. The appearance of the signals at δ_H 7.43 (*d*, *J* = 15.2 Hz, H-α) and 7.83 (*d*, *J* = 15.2 Hz, H-β) for two *trans*-oriented olefinic protons, together with the signals at

δ_C 194.7 (C=O), 120.6 (C-α) and 146.7 (C-β) in the NMR spectra revealed that **182** is a chalcone derivative. A deshielded singlet signal at δ 13.77 was due to chelated hydroxyl group at C-2' of ring B. In the ^1H and ^{13}C NMR spectra, two *cis*-oriented *vicinal* protons at δ_H 5.59 (*d*, *J* = 9.6 Hz) and δ_H 6.75 (*d*, *J* = 9.6 Hz) respectively at δ_C 130.8 and δ_C 118.6, signal for two *geminal* CH_3 groups at δ_H 1.50 (6H, s; δ_C 30.0) and a quaternary oxygenated carbon at δ_C 78.0 supported the presence of a 2,2-dimethylchromene moiety on the chalcone skeleton. The two *ortho*-coupled aromatic doublets at δ_H 7.71 and 6.38 (*J* = 8.8 Hz) were assigned to H-6' and H-5' of ring B, respectively; which is substituted with the 2,2-dimethylchromene moiety at C-3'/C-4'. Moreover, four aromatic protons with an AA'XX' spin system centering at δ_H 7.56 (H-2/H-6) and 6.88 (H-3/H-5) were observed for the ring A, substituted with hydroxyl group at C-4. Compound **182** was therefore, characterized as 2',4-dihydroxy-3',4'-(2",2"-dimethylchromene)chalcone, trival name 4-hydroxylonchocarpin which was previously reported from *Desmodium renifolium* (Li *et al.*, 2014) and *M. ferruginea* ssp. *ferruginea* (Dagne *et al.*, 1989).

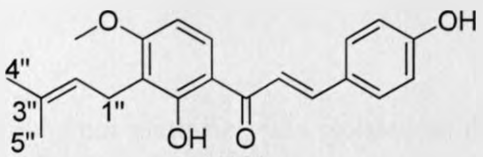


182

4.3.8 4-Hydroxyderricin (311)

The ESIMS of compound **311** showed a *pseudo*-molecular ion, $[M+H]^+$ at *m/z* 339.3 for which the molecular formula $C_{21}H_{22}O_4$ was suggested. Similar to **182**, the NMR spectra (Table 4.9) showed that compound **311** has a chalcone core skeleton with identical ring A and B. As in **182**, compound **311** is also di-substituted at C-3' and C-4', but this time the substituents are methoxyl group (δ_H 3.91; δ_C 58.4) at C-4' and prenyl (Table 4.9) at C-3'.

The placement of the prenyl group at C-3' and methoxyl group at C-4', was established from HMBC correlation between the methoxyl protons and C-4' (δ_C 165.9), and similarly, between the methylene protons of the prenyl group with C-4' (δ_C 165.9) and C-2' (δ_C 165.7). Thus, compound **311** was identified as 4-hydroxy-3'-prenyl-4'-methoxylchalcone, trivial name 4-hydroxyderricin. This compound has been isolated previously from *Angelica keiskei* and seems to be here the first report from the genus *Millettia* (Baba *et al.*, 1990).



311

Table 4.9: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data of **182** and **311** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 182		Compound 311	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
1	130.4		130.2	
2/6	133.2	7.56 (*d*, *J* = 8.8)	133.2	7.56 (*d*, *J* = 8.8)
3/5	118.7*	6.88 (*d*, *J* = 8.8)	118.7	6.89 (*d*, *J* = 8.8)
4	160.6		160.9	
C-α	120.6	7.43 (*d*, *J* = 15.2)	120.7	7.46 (*d*, *J* = 15.2)
C-β	146.7	7.83 (*d*, *J* = 15.2)	146.7	7.83 (*d*, *J* = 15.2)
C=O	194.7		194.9	
1'	112.1		117.3	
2'	163.6		165.7	
3'	116.8		120.2	
4'	162.4		165.9	
5'	110.9	6.38 (*d*, *J* = 8.8)	104.7	6.49 (*d*, *J* = 8.8)
6'	133.2	7.71 (*d*, *J* = 8.8)	131.8	7.79 (*d*, *J* = 8.8)
1"			24.4	3.39 (*d*, *J* = 6.4)
2"	80.5		124.7	5.23 (*t*, *J* = 6.4)
3"	130.8	5.59 (*d*, *J* = 9.6)	134.6	
4"	118.6*	6.75 (*d*, *J* = 9.6)	28.5	1.68 (*s*)
5"	31.0	1.47 (*s*)	20.5	1.79 (*s*)
6"	31.0	1.47 (*s*)		
4'-OCH_3			58.4	3.91 (*s*)
2'-OH		13.77 (*s*)		13.46 (*s*)

* may be interchangeable

4.4 Characterization of Compounds from the Leaves of *Millettia usramensis* ssp. *usramensis*

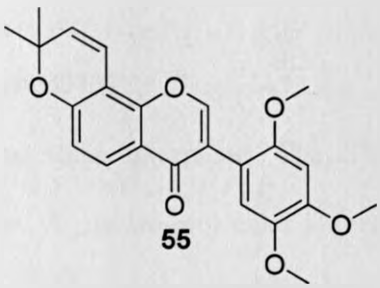
The leaves of *M. usaramensis* ssp. *usaramensis* was air-dried, ground and extracted with methanol to afford a dark green crude extract (220 g). Purification of a portion of the extract (120 g) using different chromatographic separation methods (CC on silica gel, Sephadex, LH-20, RP-HPLC) led to the identification of five rotenoids, three isoflavones and one triterpene, with one of the isoflavones is being new.

4.4.1 Barbigerone (55)

Compound **55**, colorless solid from methanol, was isolated as the major constituent of the extract. ESIMS analysis gave a molecular ion peak $[M]^+$ at *m/z* 394.9 and *pseudo*-molecular ion $[M+Na]^+$ at *m/z* 417.6 for the molecular formula $C_{23}H_{22}O_6$. A singlet olefinic peak at δ_H 7.97 in the ^1H NMR spectrum, and the corresponding carbon peak at δ_C 153.9 (oxygenated sp^2 carbon) indicated that **55** is an isoflavone derivative. Both ^1H and ^{13}C NMR spectra (Table 4.10) revealed the presence of three methoxyl and a 2,2-dimethylchromene substituents on the isoflavone skeleton. The ^1H NMR spectrum further showed two *ortho*-coupled (J = 8.8 Hz) aromatic doublets in ring A at δ_H 8.05 and 6.85 corresponding to H-5 and H-6 respectively, suggesting C-7 and C-8 are substituted.

In the HMBC spectrum, correlations of the olefinic doublets at δ_H 5.71 and 6.82 of the 2,2-dimethylchromene ring with δ_C 157.2 (C-7), 109.3 (C-8) and 152.4 (C-8a) allowed the placement of the 2,2-dimethylchromene group at C-7/C-8 of the ring A. This means all the three methoxyl groups are located in ring B; the exact placement [C-5' (δ_C 143.1), C-2' (δ_C 151.9) and C-4' (δ_C 149.8)] of which was established based on the NOE interaction (H-6'↔5'-OCH_3 and 2'-OCH_3↔H-3'↔4'-OCH_3). In agreement with this substitution pattern, two *para*-oriented aromatic singlets at δ_H 6.62 and 6.95 were assigned to H-3' and H-6',

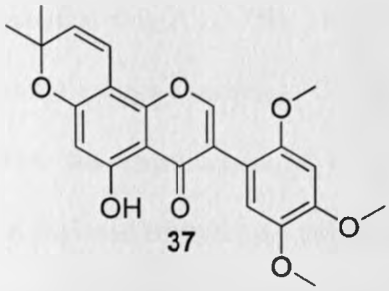
respectively. Thus, based on this data, compound **55** was characterized as 2',4',5'-trimethoxy-2",2"-dimethylpyrano[5",6":7,8]isoflavone, trivial name barbigerone, a compound previously reported from the stem bark of *M. usaramensis* ssp. *usaramensis* (Yenesew *et al.*, 2003) and seeds of *M. pachycarpa* (Ye *et al.*, 2012).



55

4.4.2 Toxicarolisoflavone (37)

The ESIMS of compound **37** exhibited a *pseduo*-molecular ions, $[M+H]^+$ peak at *m/z* 411.3 and $[M+Na]^+$ at *m/z* 433.5 corresponding to the molecular formula $C_{23}H_{22}O_7$. The proton signal at δ_H 7.90 (H-2) bonded to the carbon at δ_C 154.5 (C-2) along with the signals at δ_C 120.6 (C-3) and δ_C 180.9 (C-4) are typical of ring C of an isoflavone. The ^1H and ^{13}C NMR (Table 4.10) spectra of **37** further showed the presence of a 2,2-dimethylchromene, three methoxyl, and a chealted hydroxyl group at C-5 (δ_H 12.94) on the isoflavone core skeleton. The spectral feature of compound **37** showed that it has identical ring B as that of barbigerone (**55**), where the three methoxyl groups are located. In ring A, where aromatic singlet (H-6) is observed, contains the 2,2-dimethylchromene ring, which was placed in the same position as in **55** at C-7/C-8, in addition to the chelated hydroxyl group. Therefore, compound **37** was identified and characterized as 5-hydroxy-2',4',5'-trimethoxy-2",2"-dimethylchromene[5",6":7,8]isoflavone, trivial name toxicarol isoflavone, previously reported from *Tephrosia polyphylla* (Dagne *et al.*, 1992) and *Millettia brandisiana* (Pancharoen *et al.*, 2008).



37

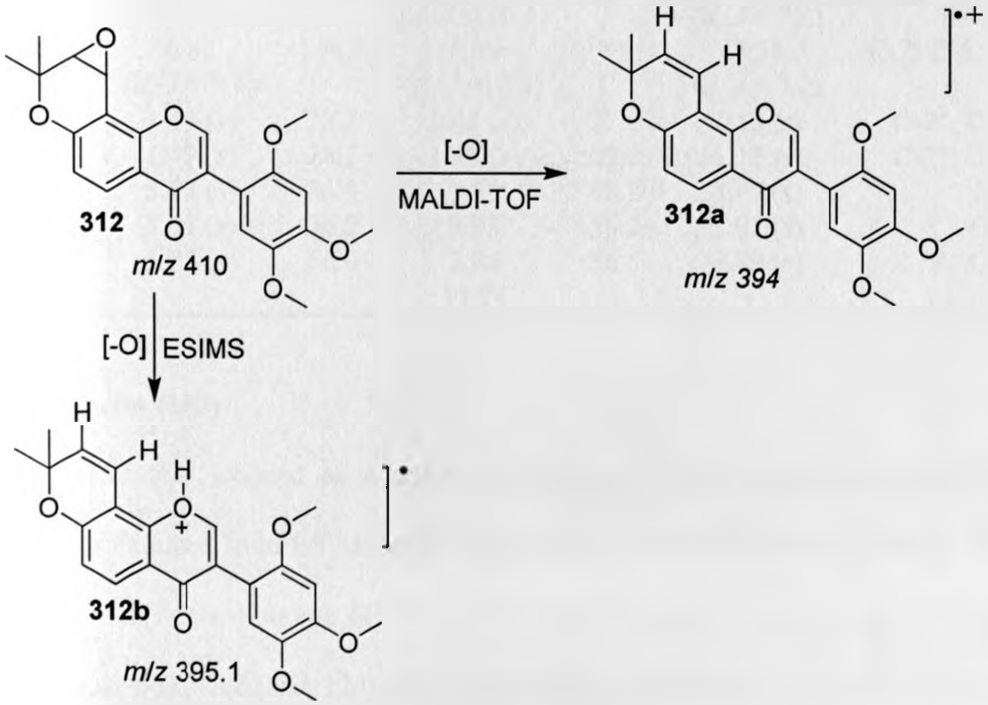
4.4.3 3",4"-Epoxybarbigerone (312)

Compound **312** was isolated as solid amorphous. The UV spectrum showed absorption maxima at 295 and 300 (sh) nm. A *pseduo*-molecular ion, peak at *m/z* 394.107 (**312a**) was observed in the MALDI-TOF analysis and *m/z* 395.3 (**312b**) in ESIMS (without the HPLC system), Scheme 4.1.

The NMR spectra of compound **312** also correspond to an isoflavone skeleton [8.06 (*s*) attached to sp^2 oxygenated carbon at δ_C 154.1]. Similar to barbigerone (**55**), in ring A of **312**, two *ortho*-coupled AX doublets were exhibited at δ_H 7.01 (H-6) and 8.15 (H-5), indicating *di*-substitution of **312** at C-7 and C-8. Furthermore, similar to **37** and **55**, three methoxyl groups and two *para*-related aromatic singlets were exhibited for ring B where their placement was established based on their NOE and HMBC correlations similar to what has been observed in **37** and **55**.

The *cis*-oriented olefinic doublets in **37** and **55**, corresponding to the 2,2,-dimethylchromene system are not observed in **312**. Instead, two mutually coupled oxymethine doublets of high order splitting (also showing long range coupling with $2xCH_3$ groups) were observed at δ_H 2.93 (1H, *d*, *J* = 7.2 Hz, H-3") and 3.58 (1H, *d*, *J* = 7.2 Hz, H-4") for the 2,2-dimethylcromane system, attached to carbon atoms at δ_C 40.5 and 33.9, respectively. These oxymethine protons exhibited HMBC correlations to C-7 and C-8 supporting the placement

of the 2,2-dimethylchromane system at ring A (C-7/8). The C-3"/C-4" oxymethine formation could be either due to two hydroxyl groups attached to C-3" and C-4" or occurrence of a C-3"-C-4"-epoxy moiety. However, the chemical shifts of C-3" and C-4" would appear, respectively at *ca.* 65 and 80 in the case of hydroxyl substituents (Fang and Casida, 1997) ruling out the possiblity of the two free hydroxyl groups. Thus, this would support the epoxy group being fixed at C-3", C-4". In fact, the observed chemical shifts for the two protons and their carbons [δ_H 2.93, δ_C 40.5 for C-3"and δ_H 3.58, δ_C 33.9 for C-4"] are slightly shielded than normally expected for oxygenated sp^3 carbon atoms and their protons (Yoon *et al.*, 2011). These shielded values in this case could be due to anisotropic effect of the strianed 3-membered ring of the epoxide system. Therefore, based on the above spectroscopic data, this new compound was characterized as 3",4"-epoxy derivative of barbigerone and hence, the name 3",4"-epoxybarbigerone is given.



Scheme 4.1. Proposed MS ionization of **312** in MALDI-TOF and ESIMS.

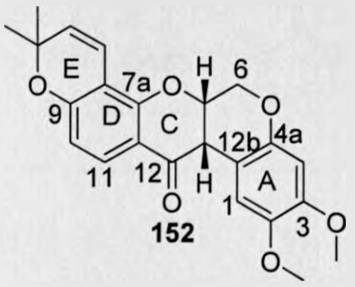
Table 4.10: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **55**, **37** and **312** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 55		Compound 37		Comopund 312		
	δ_C	δ_H (m, *J*)	δ_C	δ_H (m, *J*)	δ_C	δ_H (m, *J*)	HMBC
2	153.9	7.97 (*s*)	154.5	7.90 (*s*)	154.1	8.06 (*s*)	C-3, C-4, C- 8a, C-1'
3	121.5		120.6		121.5		
4	175.6		180.9		176.4		
4a	118.5		106.4		119.2		
5	126.7	8.05 (*d*, *J* = 8.8)	162.2		125.4	8.15 (*d*, *J* = 8.8)	C-4, C-7, C-8a
6	115.1	6.85 (*d*, *J* = 8.8)	100.3	6.29 (*s*)	116.8	7.01 (*d*, *J* = 8.8)	C-7, C-8, C-4a
7	157.2		159.4		157.6		
8	109.3		101.2		114.0		
8a	152.4		152.2		155.4		
1'	112.4		110.7		111.7		
2'	151.9		152.0		151.6		
3'	98.3	6.62 (*s*)	98.2	6.63 (*s*)	97.6	6.51 (*s*)	C-1', C-2, C-4', C-5'
4'	149.8		150.1		149.8		
5'	143.1		143.1		142.9		
6'	115.3	6.95 (*s*)	115.1	6.88 (*s*)	115.5	6.93 (*s*)	C-3, C-1', C-2, C-4', C-5'
2"	77.6		78.0		75.8		
3"	130.2	5.71 (*d*, *J* = 9.6)	127.4	5.58 (*d*, *J* = 10.4)	40.5	2.93 (*d*, *J* = 7.2)	C-8, C-2", C-3", C-4"
4"	115.1	6.82 (*d*, *J* = 9.6)	114.7	6.69 (*d*, *J* =10.4)	33.91	3.58 (*d*, *J* = 7.2)	C-7, C-8, C-3", C-4"
5"	28.1	1.49 (*s*)	28.2	1.48 (*s*)	27.0	1.53 (*s*)	C-2", C-3", C-6"
6"	28.1	1.49 (*s*)	28.2	1.48 (*s*)	23.5	1.12 (*s*)	C-2", C-3", C-5"
2'-OCH_3	56.9	3.77 (*s*)	56.9	3.79	56.2	3.47 (*s*)	C-2'
4'-OCH_3	56.2	3.93 (*s*)	56.2	3.93	56.2	3.92 (*s*)	C-4'
5'-OCH_3	56.6	3.85 (*s*)	56.6	3.86	56.5	3.79 (*s*)	C-5'
5-OH				12.94			

4.4.4 Deguelin (152)

Compound **152** was isolated as a colorless amorphous solid whose molecular formula $C_{23}H_{22}O_6$ was deduced from ESIMS analysis showing a *pseudo*-molecular ion peak, $[M+H]^+$ at *m/z* 395.1 and $[M+Na]^+$ at *m/z* 417.5. In the ^{1}H NMR spectrum, signals at δ_H 4.57 (*dd*, *J* = 1.6, 12.0 Hz, H-6α); 4.12 (*brd*, 12.0 Hz, H-6β), 4.85 (*m*, H-6a) and 3.77 (*d*, *J* = 4.0 Hz, 12a-H) together with their corresponding carbon signals at δ_C 66.3, 72.4 and 44.4, in ^{13}C NMR

spectrum, are typical of a rotenoid (Yenesew *et al.*, 1998; Vasconcelos *et al.*, 2012) having a 2,2-dimethylchromene and two methoxyl groups as a substituents. The ^{1}H NMR further depicted the presence of two *ortho*-coupled (J = 8.8 Hz) aromatic doublets at δ_H 7.75 for H-11 and δ_H 6.45 for H-10 indicating that ring D is *di*-substituted at C-8 and C-9. The substitient at this postion being the 2,2-dimethylchromene, was established from the HMBC correlation of the olefinic protons of the 2,2-dimethylchromene with C-8, C-9, and C-7a of ring D. The occurrence of two *para*-oriented aromatic protons at δ_H 6.79 (H-1) and δ_H 6.45 (H-4) in ring A, allowed the placement of the two methoxyl groups at C-2 and C-3. The chemical shift value of H-1 (δ_H 6.79, *s*) is consistent with the thermodynamically more stable *cis*-B/C ring jucntion (Oberholzer *et al.*, 1974). Therefore, compound **152** was identified as deguelin (Ollis *et al.*, 1967; Dagne *et al.*, 1991).

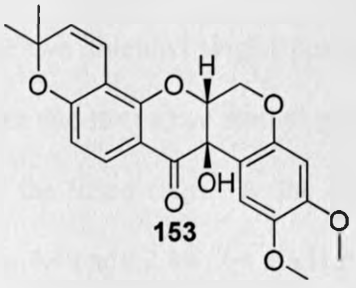


152

4.4.5 Tephrosin (153)

Compound **153** was also isolated as a colorless solid; molecular formula $C_{23}H_{22}O_7$, from ESIMS analysis showing a *pseudo*-molecular ion peak $[M+H-H_2O]^+$ at *m/z* 393.2. The ^{1}H (Table 4.11) and ^{13}C (Table 4.12) NMR spectra of **153** are compatible with 12a-hydroxyrotenoids with the C-12a carbon signal appearing at δ_C 67.5 (Yenesew *et al.*, 1998; Vasconcelos *et al.*, 2012). Similar to **152**, the NMR data of compound **153** also exhibited a 2,2-dimethylchromene and two methoxyl groups. In addition, the presence of two *ortho*-coupled and two *para*-related aromatic protons suggested that **153** has the same substitution

pattern in rings A and D as **152**. Examination of the HMBC and NOE spectra allowed the placement of the 2,2-dimethylchromene group in ring D at C-8/C-9 along with the two *ortho*-coupled protons (H-11 and H-10). Similarly, the two singlets (δ_H 6.56 and 6.48) correspond to H-1 and H-4 of ring A, respectively, with the methoxyl groups being at C-2 and C-3. Like in **152**, a *cis*-B/C ring junction was deduced from chemical shift value of H-1 (δ_H 6.56 ppm). The 6a*R*, 12a*R* absolute configuration was established from the CD spectrum (Figure 4.1) showing negative Cotton effect at *ca.* 330 nm and negative optical rotation [α_D^{20}-16.9] (Vasconcelos *et al.*, 2012). Thus, based on the spectroscopic data, this isolate was identified as (-)-(6a*R*,12a*R*)-tephrosin (Ollis *et al.*, 1967; Dagne *et al.*, 1991).



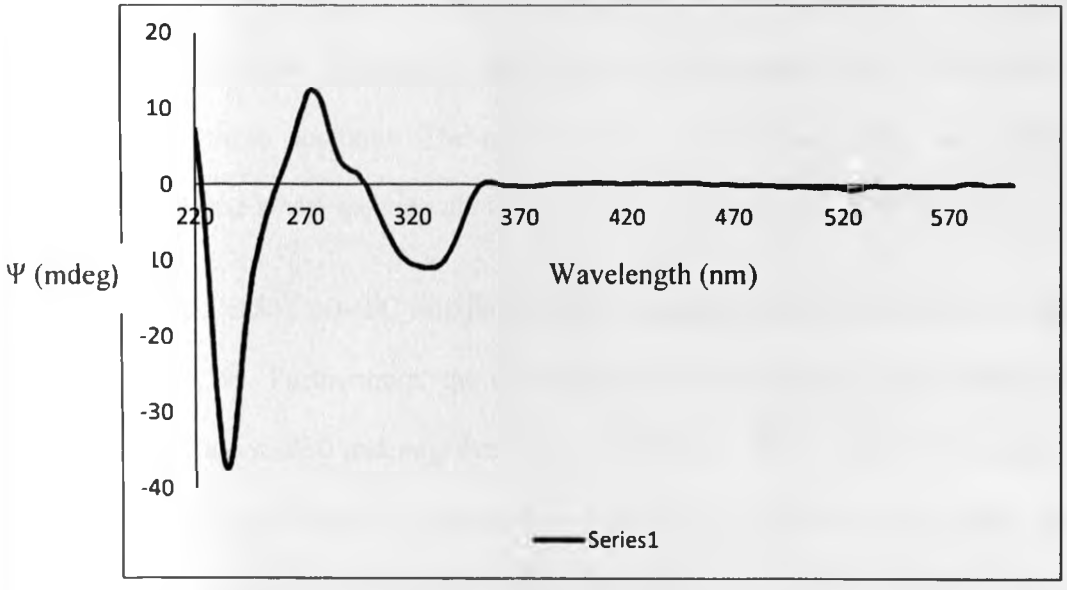


Figure 4.1. The CD spectrum of tephrosin (**153**)

4.4.6 Sarcolobin (313)

The ESIMS analysis of **313** gave a *pseudo*-molecular ion peak, $[M+H-H_2O]^+$ at *m/z* 393.2 for $C_{23}H_{22}O_7$. Compound **313** also possesses a 12a-hydroxyrotenoid skeleton having the NMR spectral data for rings A-D similar with those of tephrosin (**153**). As in **153**, the placement of the two methoxyl groups is at C-2/C-3 of ring A was based on their HMBC correlations with C-2 and C-3 as well as NOE interaction with the two aromatic singlets at δ_H 6.59 (H-1) and 6.50 (H-4).

Compound **313** differs from tephrosin (**153**) on the nature of the five carbon system of ring E. In **313**, the prenyl group was rearranged to form two fused rings, as 6,6-dimethyl-2-oxa-bicyclo[3.1.0]pentane. Thus, the two shielded singlet peaks at δ_H 1.12 and 0.63 ppm each integrating for three protons were due to the two methyl groups; CH_3-5' and CH_3-6' attached to the cyclopropane portion of the fused rings. On the other hand, two coupled doublets (from the COSY spectrum) at δ_H 4.49 and 2.49 (J = 5.6 Hz) were assigned to the protons on the bridging carbon atoms, H-2' and H-3', respectively. These protons, H-2' (4.49) and H-3' (2.49) showed HMBC interaction with C-9, C-4' and weakly with C-7a supporting their placement at these positions. The nature of this five-carbon system was confirmed by comparison of the NMR spectra with literature (Wangesteen *et al.*, 2005).

As in tephrosin (**153**), *cis*-B/C ring junction was suggested based on the chemical shift value of H-1 (δ_H 6.59). Furthermore, the CD spectrum of **313** (Figure 4.2) showed a positive Cotton effect at *ca.* 350 and negative on at *ca.* 320 nm which along with negative optical rotation ($[\alpha]_D^{20}$ - 38.0 for **313** is consistent with 6a*R*,12a*R* (Slade *et al.*, 2005). Therefore, based on the spectroscopic data, **313** was identified as sarcolobin, a compound previously isolated from *Sarcolobus globosus* (Asclepiadaceae) (Wangesteen *et al.*, 2005) and

Tephrosia vogelii (Leguminosae) (Stevenson *et al.*, 2012). This is the first report of this compound from the genus *Millettia*. Compound **313** could be biogenetically derived from **313a**

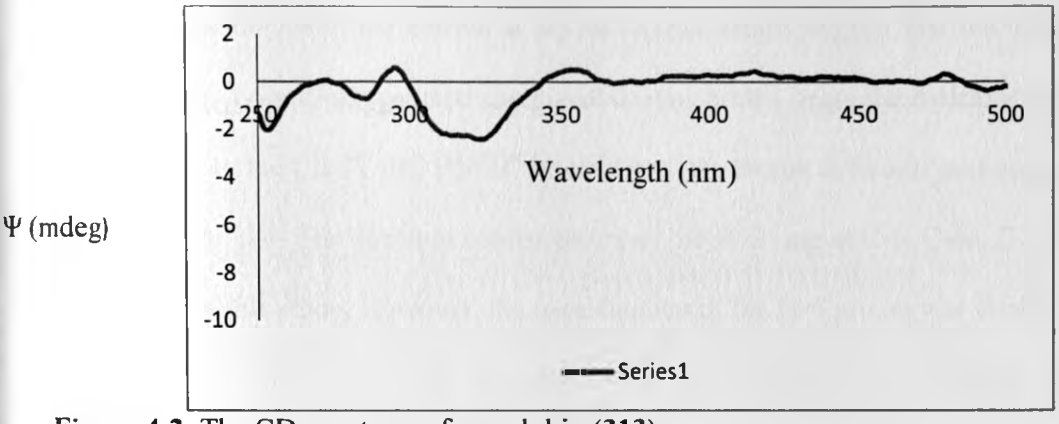
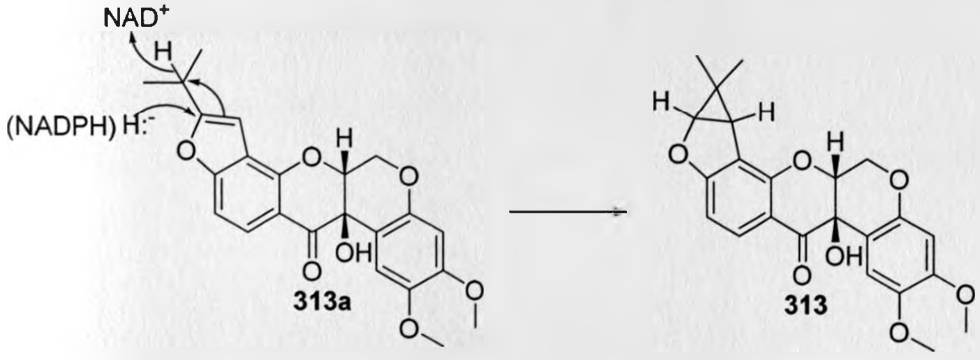


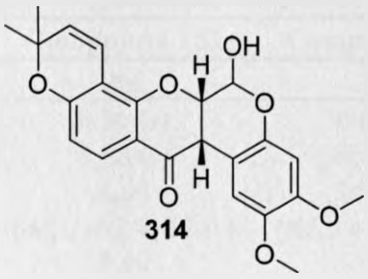
Figure 4.2. The CD spectrum of sarcolobin (**313**)



4.4.7 6-Hydroxydeguelin (314)

ESIMS analysis of **314** gave a *pseudo*-molecular ion, $[M+H]^+$ peak at *m/z* 411.3 for $C_{23}H_{22}O_7$. The ^1H NMR data particularly, the peaks at δ_H 3.92 (*d*, *J* = 4.0 Hz) for H-12a together with the one at δ_H 6.79 (s), correspond to a *cis*-B/C ring fused rotenoid skeleton. Comparison of the ^1H and ^{13}C NMR data of **314** with that of deguelin (**152**) showed similarities for the rings A, C and D. That is the presence of 2,2-dimethylchromene at C-8/C-9, two *ortho*-related AX-doublets for H-10 and H-11 of ring D, two methoxyl groups and two *para*-oriented aromatic protons of the ring A were also established in **314**. The NMR data of this compound is in Section 3.6.3.

In a COSY spectrum, a proton at δ_H 5.81(δ_C 90.1) showed a coupling with the proton at δ_H 4.81 (H-6a) and to a broad singlet at δ_H 3.31. In HMBC spectrum, this proton (δ_H 5.81) showed a weak correlation with C-4a (δ_C 144.2). In addition, proton at δ_H 3.31 showed HMBC correlation with the carbon at δ_C 90.1. This would suggest that the deshielded sp^3 carbon (δ_C 90.1) is *di*-oxygenated (hemiacetal) which also bears the hydroxyl group (O-H, 3.31). Based on the COSY and HMBC interactions, the proton at δ_H 5.81 was assigned to H-6 and δ_C 90.1 to C-6. The absolute configuration of the B/C ring at C-6, C-6a, C-12a were not established in this study. Howover, the α-orientation of the H-6 proton was established based on the $J_{H\text{-}6,6a}$ = 3.2 Hz). Thus, compound **314** was identified as 6α-hydroxydeguelin, a compound that was previously reported from *Mundulea sericea* (Luyengi *et al.*, 1994). The NMR data for compound **314** is in Section 3.6.3.

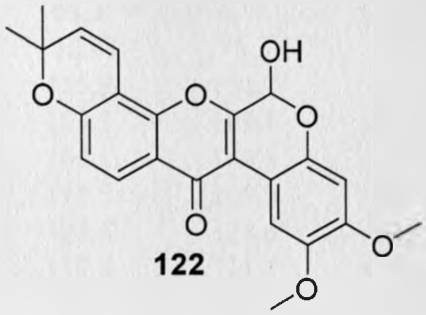


314

4.4.8 6-Hydroxy-6a,12a-dehydrodeguelin (122)

Compound **122** was assigned a molecular formula $C_{23}H_{20}O_7$ from ESIMS analysis $[M+H]^+$ ion peak at 409.6. Comparison of its ^1H and ^{13}C NMR data with that of compound **314** indicated that **122** is a dehydro derivative of **314**. The strongly de-shielded singlet proton at δ_H 8.56 due to H-1 of ring A and replacement of the sp^3 carbon signals with sp^2 signals for C-6a and C-12a in the ^{13}C NMR spectrum together with the shielded carbonyl (δ_C 175.7), further confirmed that **122** is dehydrorotenoid (Silva *et al.*, 1998). The singlet at δ_H 6.23 assigned to the proton at C-6 (δ_C 89.4) which also bears a hydroxyl group similar to **314**. As in compound **314**, the NMR spectra of **122** (Table 4.11 and Table 4.12) displayed signals for

2,2-dimethylchromene at C-9/C-8 (from HMBC spectrum), two *ortho*-coupled and AX-type doublets (H-10 and H-11) in ring D; and two methoxyl groups and two *para*-oriented aromatic protons allocated to ring A. The configrution at C-6 has not been established. Thus, compound **122** was identified as 6-hydroxy-6a,12a-dehydrodeguelin, a compound, previously isolated from *M. duchesnei* (Ngandeu *et al.*, 2008).



122

Table 4.11: ^{1}H (799.87 MHz) NMR data for **152**, **153**, **313** and **122** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 152	Compound 153	Compound 313	Compound 122
	δ_H	δ_H	δ_H	δ_H
1	6.79 (*s*)	6.56 (*s*)	6.59 (*s*)	8.56 (*s*)
4	6.45 (*s*)	6.48 (*s*)	6.50 (*s*)	6.66 (*s*)
6	4.57 (*dd*, *J* = 1.6,12.0) 4.12 (*brd*, *J* = 12.0)	4.63 (*dd*, *J* = 2.4, 12.0) 4.49 (*dd*, *J* = 0.8, 12.8)	4.63 (*dd*, *J* = 2.4, 12.0) 4.50 (*brd*, *J* = 12.8)	6.23 (*d*, *J* = 8.8)
6a	4.85 (*m*)	4.56 (*dd*, *J* = 0.8, 1.6)	4.57 (*dd*, *J* = 0, 2.4)	
10	6.45 (*d*, *J* = 8.8)	6.47 (*d*, *J* = 8.8)	6.50 (*d*, *J* = 8.0)	6.85 (*d*, *J* = 8.8)
11	7.75 (*d*, *J* = 8.8)	7.72 (*d*, *J* = 8.8)	7.77 (*d*, *J* = 8.0)	7.99 (*d*, *J* = 8.8)
12a	3.77 (*d*, *J* = 4.0)			
2'			4.48 (*d*, *J* = 5.6)	
3'	5.56 (*d*, *J* =10.4)	5.55 (*d*, *J* = 9.6)	2.49 (*d*, *J* = 5.6)	5.75 (*d*, *J* = 9.6)
4'	6.65 (*d*, *J* = 10.4)	6.60 (*d*, *J* = 9.6)		6.83 (*d*, *J* = 9.6)
5'	1.45 (*s*)	1.45 (*s*)	1.11 (*s*)	1.54 (*s*)
6'	1.37 (*s*)	1.39 (*s*)	0.63 (*s*)	1.51 (*s*)
2-OCH_3	3.77 (*s*)	3.73 (*s*)	3.74 (*s*)	3.94 (*s*)
3-OCH_3	3.81 (*s*)	3.82 (*s*)	3.83 (*s*)	3.88 (*s*)
12-OH		4.43 (*s*)	4.39 (*s*)	

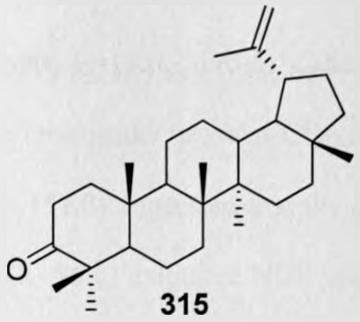
Table 4.12: ^{13}C (201.15 MHz) NMR data for **152**, **153**, **313** and **122** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm).

Position	152	153	313	122
	δ_C	δ_C	δ_C	δ_C
1	110.4	111.9	109.4	109.2
2	147.4	148.4	143.9	144.4
3	143.9	143.9	151.0	149.4
4	100.9	101.1	100.9	101.2
4a	149.5	151.1	148.4	142.3
6	66.3	67.5	63.9	89.4
6a	72.4	76.3	76.3	153.6
7a	156.9	156.7	158.4	151.2
8	109.1	109.1	115.5	108.1
9	160.1	160.8	169.6	157.7
10	111.5	109.3	105.1	115.4
11	128.6	128.6	128.6	126.4
11a	112.8	111.1	112.0	118.1
12	189.2	191.4	191.5	175.9
12a	44.4	67.5	67.6	110.5
12b	104.8	108.6	108.7	108.1
2'	77.7	78.0	73.5	78.0
3'	128.7	128.8	29.7	130.5
4'	115.8	115.4	14.3	114.7
5'	28.5	28.3	22.8	28.2
6'	28.2	28.5	12.6	28.3
2-OCH_3	56.3	56.4	56.3	56.2
3-OCH_3	55.9	55.9	55.9	55.9

4.4.9 Lupenone (315)

Compound **315** was obtained as needle-like crystal from methanol for which the NMR data suggested a tritepene skeleton. Thus, the ^1H NMR spectrum displayed a complex sets of peaks in the aliphatic shielded region (δ_H: 0.80-2.50) for methyl, methylene and methine protons, and at δ_H 4.69 (*d*, *J* = 2.4) and 4.57 (*m*) for the two *geminal* terminal olefinic protons. Similarly, the ^{13}C NMR spectrum exhibited signals for thirty carbon atoms including one at δ_C 218.2 (for C=O), 109.4 (for sp^2-CH_2) and 150.9 (for sp^2-C). The remaining twenty seven peaks (δ_C 14.5-54.9) were allocated for aliphatic quaternary, methine, methylene and methyl carbon atoms. The establishment of the structure of **315** was based on comaprision

with the spectral data in the literature. This compound was therefore, identifiied as lupenone which was also reported previously from *Tephrosia villosa* (Prashant and Krupadanam, 1993) and *Polypodium Vulgare* (Prakash and Prakash, 2012).



315

4.5 Characterization of Compounds from the Leaves of *Millettia teitensis* ssp. *teitensis*

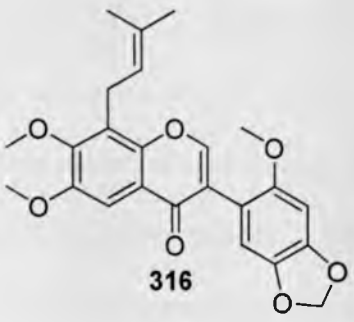
The air-dried and powdered leaves of *M. oblata* ssp. *teitensis* were extracted with dichloromethane/methanol (1:1) to give a dark green crude extract (100 g). This extract was subjected to different chromatographic separation techniques to give two new isoflavones (**316** and **317**) and four new rotenoids (**318-321**) along with the known isoflavones; maximaisoflavone B (**304**), maximaisoflavone J (**325**), maximaisoflavone G (**115**) and 2',6,7-trimethoxy-4',5'-methylenedioxyisoflavone (**324**), and the rotenoids; 6a,12a-dehydrodeguelin (**126**), munduserone (**322**), tephrosin (**153**) and 12a-hydroxymunduserone (**323**). The structural characterization of these isolates is presented in the following section.

4.5.1 8-Prenylmilldurone (316)

Compound **316** was obtained as a colorless amorphous solid from methanol, whose UV spectrum showed λ_{max} at 280 (sh) and 305 nm. The molecular formula was deduced to be $C_{24}H_{24}O_7$ from HREIMS $[M+H]^+$ ion peak at *m/z* 425.1600 (calcd. for 425.1522). In ^1H NMR (Table 4.13), a peak at δ_H 7.97 (H-2) along with the ^{13}C NMR (Table 4.13) signals at

δ_C 154.2 (C-2), 121.4 (C-3) and 175.9 (C-4) is consistent with the compound being an isoflavone derivative. The presence of three methoxyl, a *C*-prenyl and a methylenedioxy groups as substituents was also exhibited in the NMR spectra (Table 4.13). In ring A, the only aromatic proton at δ_H 7.57 (*s*) was assigned to H-5 owing to its HMBC correlations to three oxygenated sp^2 carbon atoms, C=O (δ_C 175.9), C-8a (δ_C 150.0) and C-7 (δ_C 151.8) (Table 4.13). In the HMBC, the crosspeaks between CH_2-1" (δ_H 3.59) of the prenyl group with C-8 (δ_C 124.7) and C-7 (δ_C 151.8) suggested that the prenyl group is attached to C-8. The methoxyl group at δ_H 3.95 (δ_C 56.0) exhibited NOE interaction with H-5 (7.57) of ring A, allowing its placement at C-6 (δ_C 150.9). The second methoxyl group (δ_H 3.92; δ_C 61.2) was placed at C-7 due to the HMBC correlation of the methoxyl protons with C-7 (δ_C 151.8). The deshielded chemical shift (δ_C 61.2) of this methoxyl group also requires a *di-ortho* substitution and hence, confirms its placement at C-7 of the ring A.

It then follows that the third methoxyl (δ_H 3.74, δ_C 56.9) and the methylenedioxy (δ_H 5.97; δ_C 101.4) groups are located in ring B. In agreement with this, two *para*-oriented aromatic singlets at δ_H 6.63 and 6.82 were assigned to H-3' and H-6' respectively, with the methoxyl at C-2' (δ_C 153.0) and methylenedioxy at C-4' (δ_C 148.4)/C-5' (δ_C 141.2) in this ring. The substitution pattern of this ring was confirmed from the HMBC specrum (Table 4.13) and NOE interaction 2'-OCH_3 (δ_H 3.74) ↔ H-3' (δ_H 6.63). Therefore, this new compound was characterized as 2',6,7-trimethoxy-8-(3,3-dimethylallyl)-4',5'-methylenedioxyisoflavone, for which a trivial name 8-prenylmilldurone was given by relating it to milldurone (**324**), an isoflavone isolated from seeds of *M. dura* (Ollis *et al.*, 1967) and leaves of *Ateleia glazioviana* (Yokosuka *et al.*, 2007).



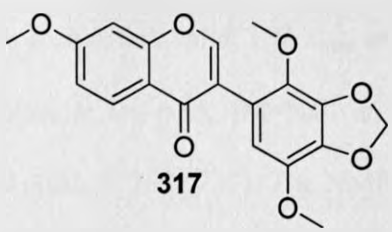
316

4.5.2 7,2',5'-Trimethoxy-3',4'-methylenedioxyisoflavone (317)

Compound **317** was obtained as colorless needles. HRESIMS analysis gave $[M+H]^+$ ion peak at *m/z* 357.0974 (calcd. 357.0896) for which the molecular formula $C_{19}H_{16}O_7$ was suggested. The UV (λ_{max} at 295 and 300, sh nm) and the NMR spectra (Table 4.13) is consistent with isoflavone skeleton for **317**. The presence of three methoxyl and a methylenedioxy groups was also shown from the NMR spectra (Table 4.13). Moreover, three mutually coupled protons at δ_H 6.86 (*d*, *J* = 2.4 Hz, H-8), 6.99 (*dd*, *J* = 2.4, 8.8 Hz, H 6) and 8.19 (*d*, *J* = 8.8 Hz, H-5) are consistent with oxygenation at C-7 (δ_C 163.9) of ring A. The substituent at C-7 was established to be a methoxyl group (δ_H 3.92; δ_C 55.8) from the NOESY experiment where this group showed interaction with H-8 (δ_H 6.86) of ring A. It then follows that the two methoxyl and the methylenedioxy groups are located in ring B. In agreement with this, only one aromatic proton (δ_H 6.52) was observed in ring B, which is tetra-substituted (with two methoxyl and a methylenedioxy groups).

The chemical shift values of the oxygenated carbon atoms (δ_C 136.8, 137.1, 138.9 and 139.2) require that this ring is oxygenated at C-2', C-3', C-4' and C-5' with the singlet proton at δ_H 6.52 assigend to H-6' (Yokosuka *et al.*, 2007). In support to this, the signal at δ_H 6.52 showed a strong three bonds HMBC crosspeak with C-3 (δ_C 122.3) of the ring C. Furthermore, this aromatic proton exhibited an NOE interaction with the methoxyl group resonating at δ_H 3.86

(δ_C 56.9) placing this methoxyl at C-5'. On the other hand, the deshielded carbon chemical shift (δ_C 60.2) value of the second methoxyl group in this ring (δ_H 3.85) requires that it is di-*ortho* substituted. It was therefore, placed at C-2' of ring B, the placement of which was supported by HMBC correlation of its protons with C-2'. The methylenedioxy group is then placed at C-3'/C-4'. Therefore, this new compound was characterized as 7,2',5'-trimethoxyl-3',4' methylenedioxyisoflavone.



317

Table 4.13: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR for **316** and **317** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 316			Compound 317		
	δ_C	δ_H (*m*, *J*)	HMBC	δ_C	δ_H (*m*, *J*)	HMBC
2	154.2	7.97 (*s*)	3, 4, 8a, 1'	153.7	7.88 (*s*)	3, 4, 8a, 1'
3	121.4			122.3		
4	175.9			175.7		
4a	120.8			118.4		
5	103.9	7.57 (*s*)	4, 6, 7, 4a, C-8a	127.8	8.19 (*d*, *J* = 8.8)	4, 7, 8a
6	150.9			114.5	6.99 (*dd*, *J* = 2.4, 8.8)	8, 4a
7	151.8			163.9		
8	124.7			100.2	6.86 (*d*, *J* = 2.4)	6, 7, 4a, 8a
8a	150.0			158.0		
1'	113.1			117.9		
2'	153.0			136.8		
3'	95.5	6.63 (*s*)	1', 2', 4', 5'	138.9		
4'	148.4			137.1		
5'	141.2			139.2		
6'	111.2	6.82 (*s*)	2, 2', 4', 5'	110.1	6.52 (*s*)	2, 2', 4', 5'
1"	23.0	3.59 (*d*, *J* = 7.2)	7, 8, 8a, 2", 3"			
2"	121.6	5.22 (*m*)	4", 5"			
3"	132.6					

4"	17.9	1.83 (*s*)	2", 3", 5"			
5"	25.8	1.69 (*s*)	2", 3", 4"			
6-OMe	56.0	3.95 (*s*)	6	-	-	-
7-OMe	61.2,	3.92 (*s*)	7	55.8	3.92 (*s*)	7
2'-OMe	56.9,	3.74 (*s*)	2'	60.2	3.85 (*s*)	2'
5'-OMe	-	-	-	56.9	3.86 (*s*)	5'
OCH_2O	101.4	5.97 (*s*)	4', 5'	101.9	6.02 (*s*)	3', 4'

4.5.3 Oblarotenoid A (318)

Compound **318** was isolated as a colorless solid; UV λ_{max} at 250 and 269 nm. The ESIMS spectrum showed a *pseudo*-molecular ion peak, $[M+Na]^+$ at *m/z* 365.0645 corresponding to the molecular formula $C_{18}H_{14}O_7$ (calcd. 365.0739). The NMR data of compound **318** (Table 4.14) suggested a 12a-hydroxyrotenoid derivative (Messana *et al.*, 1986; Yenesew *et al.*, 1998; 2003) having one methoxyl (δ_H 3.79; δ_C 55.9) and a methylenedioxy (δ_H 5.83 and 5.83; δ_C 101.3) groups as substituents. The presence of a hydroxyl group at C-12a was confirmed by HMBC spectrum which showed correlations of the hydroxyl proton with the carbon signals at δ_C 191.2 (C-12), δ_C 75.9 (C-6a) and δ_C 67.7 (C-12a).

In the ^{1}H NMR spectrum, three mutually coupled aromatic protons at δ_H 7.84 (*d*, *J* = 8.8 Hz), 6.59 (*dd*, *J* = 2.4, 8.8 Hz) and 6.38 (*d*, *J* = 2.4 Hz) were observed and assigned to H-11, H-10 and H-8 of the ring D, respectively.The HMBC correlations of H-11 with C-9 (δ_C 167.1) and C-12 (δ_C 191.2) confirmed this placement. The methoxyl group was placed at C-9 (δ_C 167.1) owing to its three bonds HMBC correlations to this carbon. Moreover, the ^{1}H NMR spectrum showed two aromatic singlets at δ_H 6.52 and 6.46, assignable to H-1 and H-4 of ring A, espectively with the methylenedioxy group being at C-2/C-3.

The equatorial orientation of H-6a was established by small coupling constant observed for H-6a with both methylene protons at C-6. Furthermore, the chemical shift of H-1 (δ_H 6.51) is consistent with *cis*-B/C ring junction (Kostova and Ognyanov, 1986; Vasconcelos *et al.*, 2012). The CD spectrum (Figure 4.3) showed a negative Cotton effect at *ca.* 325 nm which is in agreement with the 6a*R*,12a*R* absolute configuration (Slade *et al.*, 2005). The enatiomeric purity of compound **318** was established to be 100% through HPLC analysis on chiral column. This new compound was therefore, characterized as (6a*R*,12a*R*)-9-methoxyl-2,3-methylenedioxy-12a-hydroxyrotenoid for which a trivial name oblarotenoid A is suggested.

318

4.5.4 Oblarotenoid B (319)

Compound **319** was isolated as a colorless solid and showed UV λ_{max} at 285 (sh) and 300 nm. The HREIMS analysis gave a *pseudo*-molecular ion, $[M+H-H_2O]^+$ peak at *m/z* 325.0712 for $C_{18}H_{14}O_7$. The ^1H [(δ_H 4.37, *dd*, *J* = 4.0, 9.6 Hz, H-6α), (δ_H 4.44, *dd*, *J* = 9.6, 11.2 Hz, H-6β) and (δ_H 4.63, *dd*, *J* = 4.0, 11.2 Hz, H-6a)] and ^{13}C [δ_C 61.6 (C-6), 76.6 (C-6a), 66.6 (C-12a) and 187.0 (C-12)] NMR spectra of **319** are consistent with a 12a-hydroxyrotenoid skeleton (Messana *et al.*, 1986; Yenesew *et al.*, 1998; 2003) having a methoxyl (δ_H 3.86; δ_C 55.8) and a methylenedioxy (δ_H 5.95; δ_C 101.5) groups as substituents. Similar to **318**, the ^1H NMR spectrum of **319** displayed signals for three mutally coupled protons at δ_H 7.93 (*d*, *J* = 8.8 Hz, H-11), 6.69 (*dd*, *J* = 2.4, 8.8 Hz, H-10) and 6.50 (*d*, *J* = 2.4 Hz, H-8) of the ring D. The methoxyl group was located at C-9 (δ_C 166.1) based on its NOE and HMBC correlations similar to that of **318**. In ring A, two *para*-oriented aromatic singlets at δ_H 7.71 and 6.42 were

assigned to H-1 and H-4 respectively; which therefore, allowed the placement of the methylenedioxy group at C-2/C-3 (δ_C 142.4/149.5).

Importantly, the chemical shift value for H-1 (δ_H 7.71) is strongly deshielded compared to that of **318** and other rotenoids with *cis*-B/C junction. This observation and the large coupling constant between H-6a and one of the H-6 protons (J = 11.2 Hz, a 1,2-diaxial) suggested that **319** possesses a *trans*-orientation at the B/C ring junction, which could either be 6a*R*,12a*S* or 6a*S*,12a*R* configuration (Messana *et al.*, 1986; Kostova and Ognyanov, 1986; Yenesew *et al.*, 1998). The CD spectrum of **319** (Figure 4.3) showed a positive Cotton effect at *ca.* 340 nm and negative one at *ca.* 300 nm which together with the positive optical rotation corresponds to 6a*R*,12*S* of absolute configuration (Slade *et al.*, 2005). Therefore, this new compound was characterized (+)-(6a*R*,12a*S*)-9-methoxy-2,3-methylenedioxy-12a-hydroxyrotenoid for which a trivial name oblarotenoid B is suggested.

319

Table 4.14: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data for **318** and **319** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

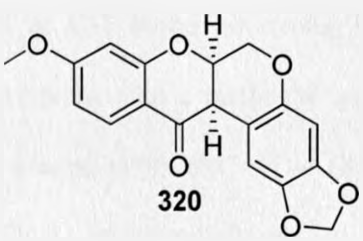
Position	Compound 319			Compound 318		
	δ_C	δ_H (*m*, *J*)	HMBC	δ_C	δ_H (*m*, *J*)	HMBC
1	109.3	7.71 (*s*)	2, 3, 4a, 12a	105.8	6.52 (*s*)	2, 12a, 3, 4a
2	142.4			142.3		
3	149.5			149.3		
4	98.5	6.42 (*s*)	2, 3, 4a, 12b	99.1	6.46 (*s*)	2, 4a, 12b
4a	150.7			149.4		
6	61.6	4.37 (*dd*, *J* = 4.0, 9.6)	4a, 6a, 12a	63.9	4.59 (*dd*, *J* = 2.4,12.0)	6a, 4a, 12a
		4.44 (*dd*, *J* = 9.6, 11.2)	6a, 12a		4.47 (*brd*, *J* = 12.0)	6a
6a	76.6	4.63 (*dd*, *J* = 4.0, 11.2)	6, 12a	75.9	4.57 (*t*, *J* = 2.4)	12a, 12b
7a	161.5			162.5		
8	100.5	6.50 (*d*, *J* = 2.4)	9, 10, 7a, 11a	100.7	6.38 (*d*, *J* = 2.4)	11a, 7a, 10
9	166.1			167.1		
10	111.1	6.69 (*dd*, *J* = 2.4, 8.8)	8, 11a	111.1	6.59 (*dd*, *J* = 8.8, 2.4)	11a, 8
11	130.6	7.93 (*d*, *J* = 8.8)	9, 12, 7a	129.4	7.84 (*d*, *J* = 8.8)	7a, 9, 12
11a	113.6			111.0		
12	187.0			191.2		
12a	66.6			67.7		
12b	110.5			109.7		
9-OCH_3	55.8	3.86 (*s*)	9	55.7	3.79 (*s*)	9
OCH_2O	101.5	5.95 (*s*)	2,3	101.3	5.85 (*d*, *J* = 1.2)	2, 3
					5.83 (*d*, *J* = 1.2)	
12a-OH	-	2.59 (*s*)	6a, 12b		4.43 (*s*)	12, 6a, 12a

4.5.5 9-Methoxyl-2,3-methylenedioxyrotenoid (320)

Compound **320** was obtained as needles with 100% enantiomeric purity, UV λ_{max} at 285 and 300 nm. Its molecular formula was found to be $C_{18}H_{14}O_6$ from HREIMS at *m/z* $[M+H]^+$ 327.0869 (calcd. 327.3002). The ^{1}H NMR spectrum of **320** displayed signals for four aliphatic protons at δ_H 4.16 (*brd*, *J* = 12.0 Hz for H-6β), 4.59 (*dd*, *J* = 3.2, 12.0 Hz for H- 6α), 4.92 (*t*, *J* = 3.2, 7.2 Hz, for H-6a) and 3.80 (*d*, *J* = 7.2 Hz for H-12a) which is consistent with rotenoid skeleton (Dagne *et al.*, 1989). The NMR data (Table 4.15) of **320** is closely related

to those of **318** and **319**. The only significant difference is the absence of oxygenation (hydroxyl group) at C-12a in **320**. Otherwise, compound **320** has identical substitents in rings A and D as **318** and **319**. The placement of the methoxyl group in ring D, the methylendedioxy at C-2/C-3 of the ring A and the assignment of two aromatic singlets (δ_H 6.73 and 6.43) to H-1 and H-4 were carried out similarly as in **318** and **319**.

The chemical shift value for H-1 (δ_H 6.73) of **320** is consistent with the *cis*-orientation at the B/C ring junction (Kostova and Ognyanov, 1986; Vasconcelos *et al.*, 2012). This *cis* orientation was further confirmed by X-ray data (www.ccdc.cam.ac.uk/data_request/cif). Miyano (1970) synthesized compound **320** without determining the absolute configrution at the B/C ring junction. In this synthetic attempt, the NMR data was not generated as the product was found insoluble; yet *cis* relative configrution was suggested based on the fact that the product was obtained by 49% which they supposed the *cis* isomer. In this study, to determine the absolute configuration, the CD and OR data were generated. Thus, the CD spectrum (Figure 4.3) of compound **320** which showed a negative CE at *ca.* 340 nm and positive one at *ca.* 300 nm and, the dextrorotatory nature ($[\alpha]_D^{25}$ = +38.3) is consistent with 6a*R*,12a*R* absolute configuration. This new compound was therefore, named as (6a*R*,12a*R*)-9-methoxyl-2,3-methylenedioxyroxyrotenoid.



320

4.5.6 Oblarotenoid C (321)

Compound **321**, the sixth new compound from this plant, was obtained as a colorless soild and showed UV λ_{max} at 245, 260 and 280 nm. The molecular formula $C_{19}H_{16}O_8$ was deduced from HREIMS analysis showing $[M+H]^+$ ion peak at *m/z* 373.0918 and $[M+Na]^+$ 395.0698. Three mutually coupled aliphatic protons at δ_H 4.67 (*dd*, *J* = 2.4, 12.0 Hz, H-6α), 4.53 (*brd*, *J* = 12.0 Hz, H-6β) and 4.59 (*dd*, *J* =1.6, 2.4 Hz, H-6a) together with the corresponding carbon signal at δ_C 63.9 (C-6), 76.1 (C-6a) and 67.4 (C-12a) are indicative of a 12a-hydroxyrotenoid skeleton for compound **321**. A broad singet at δ_H 4.40 was assigned to hydroxyl group at C-12a from its HMBC correlation to C-12, C-6a and C-12a.

Furthermore, the presence of two methoxyl and one methylenedioxy group as substituents were apparent from the NMR spectra (Table 4.15). The methoxyl group resonating at δ_H 3.80; δ_C 55.8 was placed at C-9 as in compound **318** and **319** due to its NOE interaction with H-8 (δ_H 6.38), and HMBC correlation with C-9 (δ_C 167.2) of the ring D. This methoxyl group being the only substituent in this ring, as in compound **318**, **319** and **320**, three mutually coupled aromatic protons were displayed for H-8, H-10 and H-11 (Table 4.15). In ring A of compound **321**, only one singlet proton (δ_H 6.25) was observed which is unusual for rotenoids of the family Leguminosae.

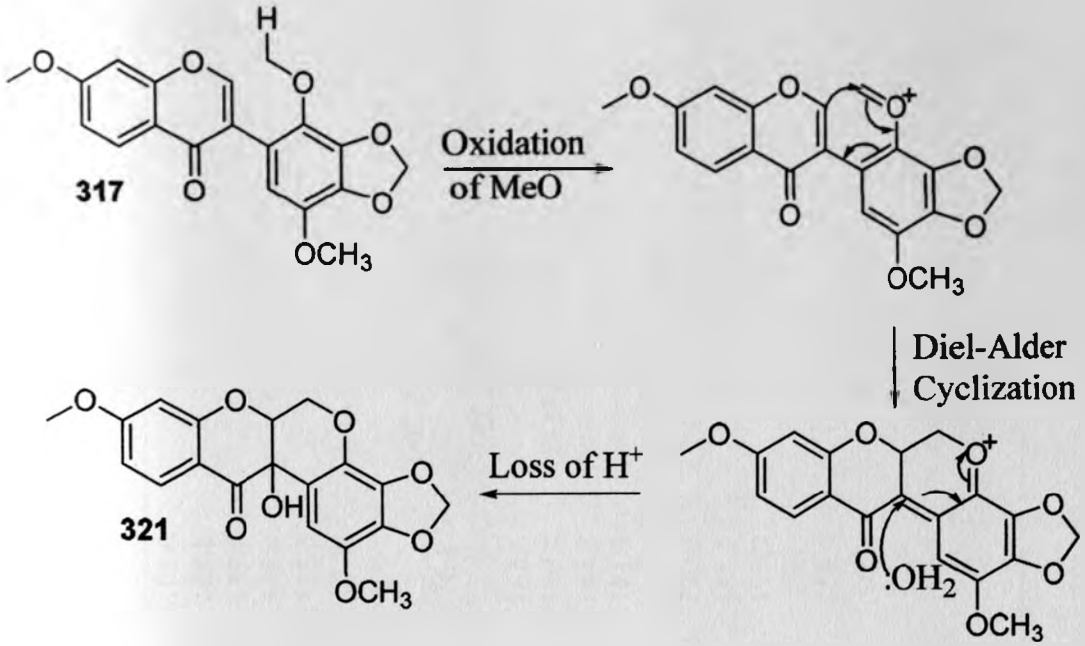
This singlet proton was placed at C-1 based on strong HMBC crosspeak with C-12a. Otherwise, the ring is fully substituted with a methoxyl and methylenedioxy groups. The methylenedioxy group could be placed either at C-3/C-4 (**321**) or C-2/C-3 (**321a**) with the second methoxyl group (δ_H 3.74; δ_C 56.9) at C-2 or C-4, respectively. The fact that the singlet proton (δ_H 6.25) showed strong HMBC correlation with the aromatic carbon to which this methoxyl is connected (δ_C 138.8) and the resonance of the carbon of the methoxyl being

at δ_C 56.9 (normal range, Oberholz *et al.*, 1974) in ^{13}C NMR, led to the placement of the methylenedioxy group at C-3/C-4 and the methoxyl at C-2 (**321**).

The chemical shift value of H-1 (δ_H 6.25) as well as the small coupling constant between H-6a/H-6 (J = 2.4, 1.6 Hz) suggested a *cis*- configrution at the B/C ring junction. The absolute configration of **321** was determined as 6a*R*,12a*R* from the CD spectrum (Figure 4.3) which showed a negative CE at *ca.* 327 nm coupled with the levorotatory nature, $[\alpha]_D$ -9.0, of this compound. Hence, compound **321** was characterized as (-)-(6a*R*,12a*R*)-2,9-dimethoxy-3,4-methylenedioxy-12a-hydroxyrotenoid for which a trival name oblarotenoid C is given. This new compound appears to be the first example of rotenoid with such oxygenation pattern in ring A. The isoflavone **317** having the same oxygenation pattern on its ring B (ring A of rotenoids) co-occurs with **321** in this plant; and it is possible that the rotenoid **321** is biogenetically derived from the isoflavone **317** as shown in Scheme 4.2.

321

321a



Scheme 4.2. Proposed biogenetic route of compound 317 into 321

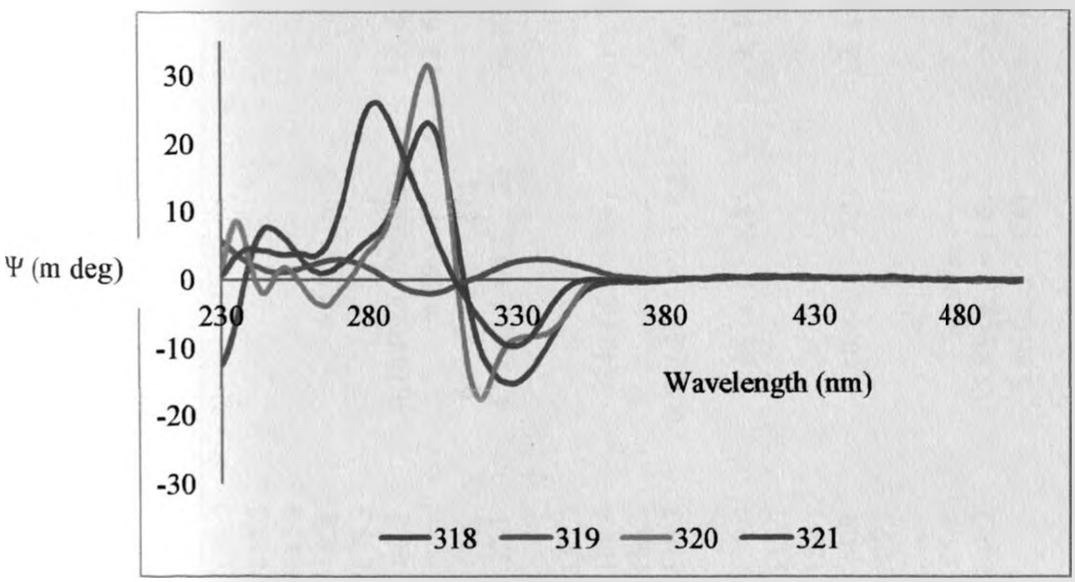


Figure 4.3. The CD spectra of the four new rotenoids **318**, **319**, **320**, **321**.

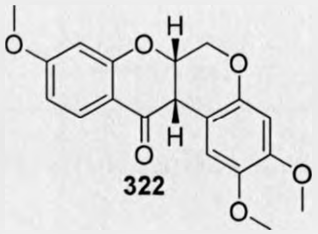
Table 4.15: ^{1}H (799.87 MHz) and ^{13}C (201.25 MHz) NMR data for **320** and **321** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 320			Compound 321		
	δ_C	δ_H (*m, J*)	HMBC	δ_C	δ_H (*m, J*)	HMBC
1	106.9	6.73, *s*	2, 3, 4a, 12a, 12b	105.6	6.25 (*s*)	2, 3, 4a, 12a
2	142.3			138.8		
3	147.8			137.9		
4	98.8	6.43, *s*	2, 3, 4a, 12b	135.9		
4a	148.3			133.6		
6	66.3	4.16 (*brd, J* = 12.0)	12, 6a, 12a	63.9	4.53 (*d, J* = 12.0)	6a
		4.59	12, 6a, 12a		4.67	6a, 12a, 4a
		(*dd, J* = 3.2, 12.0)			(*dd, J* = 2.4, 12.0)	
6a	72.1	4.92 (*t, J* = 3.2, 7.2)	6, 12, 12b	76.1	4.59	12b
					(*dd, J* =1.6, 2.4)	
7a	162.7			162.6		
8	100.6	6.42 (*d, J* = 2.4)	9, 10, 7a, 11a	100.8	6.38 (*d, J* = 2.4)	10, 7a, 11a
9	166.5			167.2		
10	110.7	6.57 (*dd, J* = 2.4, 8.8)	8, 11a	111.3	6.61	11a
					(*dd, J* = 2.4, 8.8)	
11	129.4	7.86 (*d, J* = 8.8)	9, 12, 7a	129.3	7.85 (*d, J* = 8.8)	9, 12, 7a
11a	112.7			111.1		
12	189.1			190.9		
12a	44.9	3.80, (*d, J* = 7.2)	12, 4a, 12b	67.4		
12b	105.7			113.0		
2-OMe	-	-	-	56.9	3.74 (*s*)	2
9-OMe	55.7	3.79 (*s*)	9	55.8	3.80 (*s*)	9
OCH_2O	101.2	5.81 (*d, J* = 1.6)	2, 3	102.6	6.02 (*d, J* = 1.3)	3, 4
		5.86 (*d, J* = 1.6)			5.98 (*d, J* = 1.3)	
12a-OH	-	-	-		4.40, *brs*	12, 6a, 12a

4.5.7 Munduserone (322)

The molecular formula $C_{19}H_{18}O_6$ was suggested for **322** from ESIMS analysis which showed a $[M+H]^+$ ion *m/z* at 343.2. The ^1H and ^{13}C NMR spectral data (Table 4.16) of compound **322** corresponds to a rotenoid [δ_H 4.62, *dd*, J = 3.2, 12.0 Hz and 4.18, *brd*, J = 12.0 Hz for CH_2-6; 4.94, *t*, J = 3.2 Hz for H-6a and 3.85, *d*, J = 4.0 Hz] having a *cis*-B/C fused rings (δ_H 6.74, *s*, for H-1) and three methoxyl substituents. In the ^1H NMR spectrum, three mutually coupled protons at δ_H 7.87 (*d*, J = 8.8 Hz, H-11), 6.58 (*dd*, J = 2.4, 8.8 Hz, H-10) and 6.42 (*d*, J = 2.4 Hz, H-8) were observed for ring D. This assignment was consistent with HMBC correlation of H-11 (δ_H 7.87) with carbon signals at δ_C 165.5 (C-9), 162.8 (C-7a) and 189.3 (C-12).

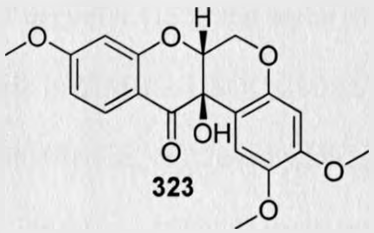
The COSY correlaton of the signals δ_H 7.87 (H-11) ↔ δ_H 6.58 (H-10) ↔ δ_H 6.42 (H-8) is consistent that these signals belong to one spin system in ring D, which is substituted at C-9 with methoxyl group (δ_H 3.80; δ_C 55.7). The placement of this methoxyl group was confirmed from the HMBC (with C-9) and NOE (with H-8 and H-10). In ring A, two aromatic singlets at δ_H 6.74 and δ_H 6.46 are characteristic of protons at C-1 and C-4 of *cis*-fused rotenoids having substituents at C-2 and C-3. The substituents in this ring are two methoxyl groups, the placement of which was established from their NOE interaction maps with the ring protons [3.76 (2-OCH_3) ↔ 6.74 (H-1) and 3.79 (3-OCH_3) ↔ 6.46 (H-4)]. Thus, this secondary metabolite, was identified as 2,3,9-trimethoxylrotenoid, trivial name munduserone, a compound which has been previously isolated from *Tephrosia fulvinervis* (Dagne *et al.*, 1989a).



322

4.5.8 12a-Hydroxymunduserone (323)

Compound **323** was analyzed for $C_{19}H_{18}O_7$ from ESIMS $[M+H-H_2O]^+$ *m/z* at 341.1. The ^1H, ^{13}C NMR data (Table 4.16) corresponds to a *cis*-12a-hydroxyrotenoid with three methoxyl substituents. The NMR spectral data showed that the substitution pattern of **323** is the same as that of **322** except for the presence of 12a-hydroxy group in the **323** (δ_C 67.6). Thus, the NMR and MS data is consistent with compound **323** being 2,3,9-trimethoxyl-12a-hydroxyrotenoid. The placement of the methoxyl groups were established on the basis of HMBC, NOE and COSY spectroscopic data as in **322**. The CD spectrum (Figure 4.4) of **323** showed a positive CE at *ca.* 350 and negative at *ca.* 330 nm which corresponds to 6a*R*,12*R*, absolute configrution. Hence, compound **323** was identified as (6*R*,12*R*)-12a-hydroxymunduserone, a compound previously isolated from *Tephrosia fulvinervis* (Dagne *et al.*, 1989a).



323

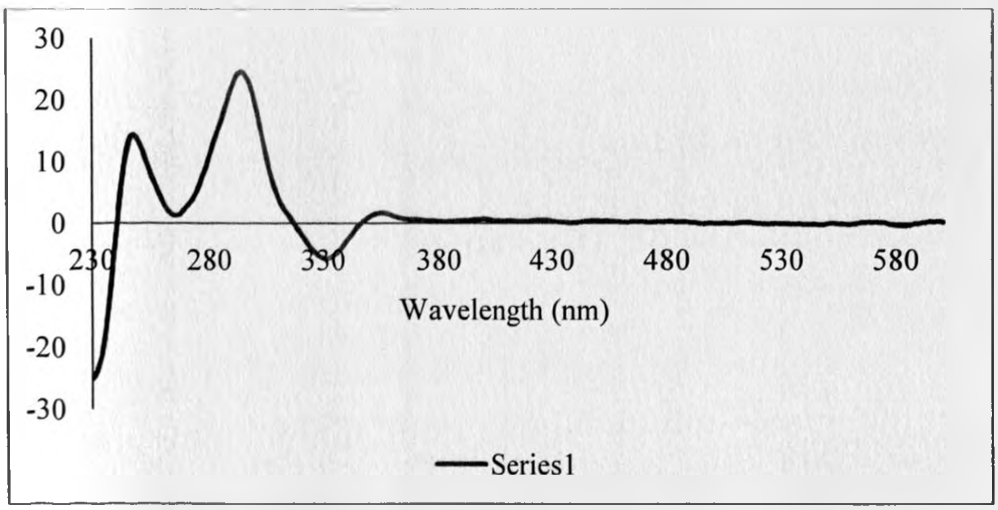
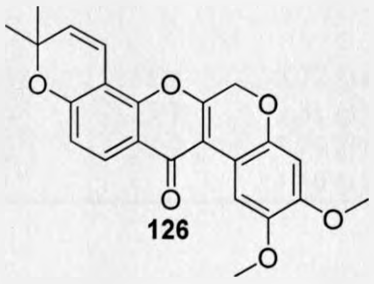


Figure 4.4. The CD spectrum of (6*R*,12*R*)-12a-hydroxymunduserone (**323**)

4.5.9 6a,12a-Dehydrodeguelin (126)

Compound **126**, the major constituent of the leaves of this plant was isolated as yellow soild. Its ESIMS gave $[M+H]^+$ ion peak at *m/z* 393.1, and the molecular formula $C_{23}H_{20}O_6$ was suggested. The spectroscopic data of **126** (Table 4.16) is consistent with this compound being 6a,12a-dehydrorotenoid derivative with a 2,2-dimethylchromene and two methoxyl groups as substituents. The absence of the signals for H-12a and H-6a, and appearance of a singlet proton peak at δ_H 5.03 (integrated for two protons) for CH_2-6 is in agreement that this compound is a 6a,12a-dehyrorotenoid. The methylene signal at δ_H 5.03 showed HMBC correlation with δ_C 111.8 (C-12a), 156.2 (C-6a) and 146.3 (C-4a), and hence unambiguously assigned to CH_2-6. The placement of the two methoxyl groups at C-2 and C-3 of ring A, and the 2,2-dimethylchromene at C-8/C-9 of ring D were established through comparison of its spectroscopic data with those of deguelin (**152**) and tephrosin (**153**) (Table 4.11). Complete assignment was done on the basis of HMBC, HSQC, NOESY and COSY spectral analyses. Hence, compound **126** was identidfied as 6a,12a-dehydrodeguelin, previously iolated from *Derris trifoliata* (Yenesew *et al.*, 2006) and *Millettia duchesnei* (Ngandeu *et al.*, 2008).



126

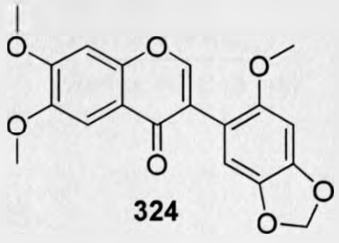
Table 4.16: ^{1}H (799.87 MHz) and ^{13}C (201.15 MHz) NMR data of **322**, **323** and **126** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Postion	Compound 322		Compound 323		Compound 126	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
1	110.3	6.74 (*s*)	109.3	6.54 (*s*)	110.0	8.46 (*s*)
2	143.9		144.0		144.0	
3	149.5		151.2		148.9	
4	100.9	6.46 (*s*)	101.1	6.49 (*s*)	100.4	6.56 (*s*)
4a	147.4		148.4		146.3	
6	66.3	4.62 (*dd*, *J* = 3.2, 12.0) 4.18 (*d*, *J* = 12.0)	63.9	4.61 (*dd*, *J* = 2.4, 12.0) 4.61 (*dd*, *J* = 0.8, 12.0)	64.9	5.03 (*s*)
6a	72.4	4.94 (*t*, *J* = 3.2)	76.2	4.59 (*dd*, *J* = 0.8, 2.4)	156.2	
7a	162.8		162.6		151.1	
8	100.7	6.42 (*d*, *J* = 2.4)	100.7	6.38 (*d*, *J* = 2.4)	109.1	
9	166.5		167.1		157.2	
10	110.7	6.58 (*dd*, *J* = 2.4, 8.8)	111.2	6.59 (*dd*, *J* = 2.4, 8.8)	115.4	6.87 (*d*, *J* = 8.8)
11	129.4	7.87 (*d*, *J* = 8.8)	129.3	7.85 (*d*, *J* = 8.8)	126.5	8.05 (*d*, *J* = 8.8)
11a	112.3		111.1		118.5	
12	189.3		191.4		174.4	
12a	44.6	3.85 (*d*, *J* = 4.0)	67.6		111.8	
12b	104.7		108.6		110.6	
2'					77.8	
3'					130.6	5.73 (*d*, *J* = 9.6)
4'					114.7	6.77 (*d*, *J* = 9.6)
5'/6'					28.2	1.50 (*s*)
2-OCH_3	56.3	3.76 (*s*)	56.4	3.72 (*s*)	56.3	3.96 (*s*)
3-OCH_3	55.9	3.79 (*s*)	55.9	3.81 (*s*)	55.9	3.88 (*s*)
9-OCH_3	55.7	3.80 (*s*)	55.7	3.79 (*s*)		
12a-OH				4.40 (*s*)		

4.5.10 Milldurone (324)

ESIMS analysis of **324** gave $[M+H]^+$ *m/z* at 357.3 corresponding to the molecular formula $C_{19}H_{16}O_7$. The ^{1}H, ^{13}C NMR (Table 4.17) data are consistent with an isoflavone derivative having three methoxyl and a methylenedioxy groups as substituents. The ^{1}H NMR displayed four singlet aromatic protons (δ_H: 7.45, 7.27, 6.94 and 6.88) in which one pair was allocated

to each ring A and ring B. Based on HMBC examination, the signals at δ_H 7.45 and 7.27 were assigned to H-5 and H-8 of ring A, respectively. In the same way, the signals at δ_H 6.94 and 6.88 were assigned to H-3' and H-6' of the ring B, respectively. The methoxyl group at δ_H 3.92 (δ_C 56.2) showed NOE interaction with H-5 while the one at δ_H 3.98 (δ_C 56.8) with H-8 and hence, placed at C-6 and C-7 of the ring A, respectively. The methylenedioxy group was located at C-4' (δ_C 148.3)/C-5' (δ_C 140.8), allowing the third methoxyl (δ_H 3.72; δ_C 57.1) being at C-2' (δ_C 153.3). Therefore, compound **324** was identified as 2',6,7-methoxyl-4',5'-methylenedioxyisoflavone, trivial name, milldurone earlier isolated from *Ateleia glazioviana* (Yokosuka *et al.*, 2007) and *Millettia leptobotrya* (Na *et al.*, 2013).

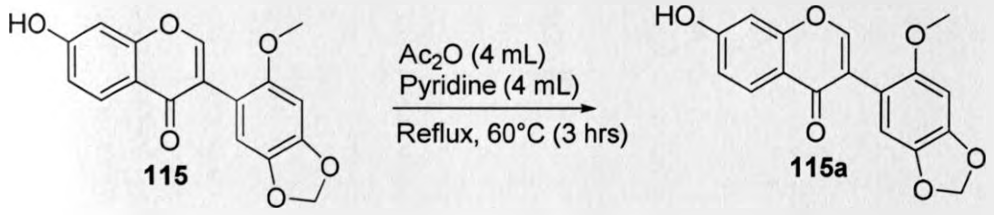


324

4.5.11 Maximaisoflavone G (115)

Compound **115**, another major constituent, was obtained as an amorphous soild for which the molecular formula $C_{17}H_{12}O_6$ was suggested from ESIMS analysis, $[M+H]^+$ ion peak at *m/z* 313.2. The ^1H and ^{13}C NMR spectra (Table 4.17) of **115** are consistent with an isoflavone skeleton possessing a methylenedioxy, a methoxyl and hydroxyl groups. Furthermore, three mutually coupled aromatic protons at δ_H 7.87 (*d*, J = 8.8 Hz, H-5), 6.88 (*dd*, J = 2.4, 8.8 Hz, H-6) and 6.81 (*d*, J = 2.4 Hz, H-8) correspond to ring A protons which is oxygenated at C-7. In agreement with this, the deshielded proton (δ_H 7.87) showed HMBC correlation with the C=O and two oxygenated aromatic carbon atoms at δ_C 162.9 (C-7) and 157.9 (C-8a). In acetylated product (**115**a), additional carbon signal at δ_C 167.5 (esteric carbonyl) was

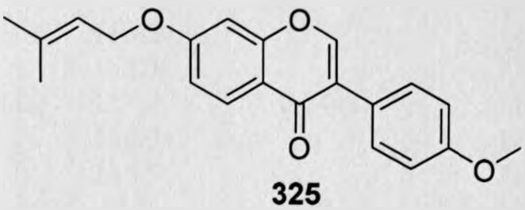
observed, to which H-6 (δ_H 6.88) showed long range HMBC correlation. This indicated that the hydroxyl group in **115** was at C-7, and consequently, the methoxyl group was placed in ring B along with the methylenedioxy group. In ring B, two isolated aromatic protons were also observed for H-3' (δ_H 6.74, *s*) and H-6' (δ_H 6.78, *s*) suggesting the methoxyl group should be placed at C-2' (δ_C 153.3) and the methylenedioxy at C-4' (δ_C 148.3) and C-5' (δ_C 140.7). Therefore, this isolate was identified as 7-hydroxy-2-methoxyl-3',4'-methylenedioxyisoflavone, trivial name maximaisoflavone G, previously reported from *M. usaramensis* ssp. *usaramensis* (Yenesew *et al.*, 1998).



4.5.12 Maximaisoflavone J (325)

Compound **325** was isolated as colorless solid, analyzed for $C_{21}H_{20}O_4$ from ESIMS $[M+H]^+$ at *m/z* 337.2. In the ^{1}H NMR spectrum, the peak at δ_H 7.91 (*s*, H-2), attached to an oxygenated sp^2-carbon (δ_C 152.0) for C-2 is a suggestive of an isoflavone skeleton for compound **325**. Furthermore, the ^{1}H NMR spectrum (Table 4.17) exhibited signals corresponding to a prenyloxy and a methoxyl groups. The sets of spin systems; AMX and AA'XX' were also observed in the ^{1}H NMR spectrum; wherein the AMX protons were allocated to ring A protons as δ_H 8.19 (*d*, J = 8.8 Hz for H-5), 6.99 (*dd*, J = 2.4, 8.8 Hz for H-6) and 6.85 (*d*, J = 2.4 Hz for H-8). On the other hand, the AA'XX' were assigned to ring B protons as δ_H 7.50 (*d*, J = 8.8 Hz for H-2'/6') and 6.97 (*d*, J = 8.8 Hz for H-3'/5'). This clearly suggested oxygenation at C-7 and C-4' which of course through prenyloxy and a methoxyl groups.

The position of the prenyloxy group was established to be at C-7 of ring A on the basis of HMBC interaction of the CH_2-1" protons with C-7 (δ_C 163.2). Similarly, the methoxyl group showed HMBC correlation with C-4' and NOE with H-3'/5' (δ_H 6.97) and hence, placed at C-4'. Based on the above data, compound **325** was identified as 4'-methoxy-7-prenyloxyisoflavone, trivial name maximaisoflavone J, previously isolated from stem bark of *Millettia oblata* ssp. *teitensis* (Derese *et al.*, 2014).



325

Table 4.17: ^{1}H (799.87 MHz) and ^{13}C (201.25 MHz) NMR data of (**324**, $CDCl_3$), **115** (DMSO-d_6) and (**325**, DMSO-d_6) at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 324		Compound 115		Compund 325	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	154.6	8.27 (*s*)	154.8	8.06 (*s*)	152.0	7.91 (*s*)
3	121.7		121.9		124.9	
4	174.5		175.0		175.9	
4a	117.3		116.9		118.3	
5	104.5	7.45 (*s*)	127.8	7.87 (*d*, *J* = 8.8)	127.7	8.19 (*d*, *J* = 8.8)
6	147.8		115.6	6.88 (*dd*, *J* = 2.4, 8.8)	114.9	6.99 (*dd*, *J* = 2.4, 8.8)
7	154.7		162.9		163.2	
8	100.8	7.27 (*s*)	102.6	6.81 (*d*, *J* = 2.4)	100.9	6.85 (*d*, *J* = 2.4)
8a	152.2		157.9		157.9	
1'	113.5		113.2		124.3	
2'	153.3		153.3		130.1	7.50 (*d*, *J* = 8.8)
3'	96.0	6.94 (*s*)	111.4	6.74 (*s*)	113.9	6.97 (*d*, *J* = 8.8)
4'	148.3		148.3		159.6	
5'	140.8		140.7		113.9	6.97 (*d*, *J* = 8.8)
6'	111.5	6.88 (*s*)	95.9	6.78 (*s*)	130.1	7.50 (*d*, *J* = 8.8)
1"					65.5	4.62 (*d*, *J* = 6.4)
2"					118.6	5.51 (*t*, *J* = 6.4)
3"					139.4	
4"					25.9	1.83 (*s*)
5"					18.3	1.78 (*s*)
6-OCH_3	56.2	3.92 (*s*)				
7-OCH_3	56.8	3.98 (*s*)				
2'-OCH_3	57.1	3.72 (*s*)	56.9	3.63 (*s*)		
4'-OCH_3					55.4	3.84 (*s*)
4'/5'-OCH_2O	101.6	6.07 (*s*)	101.6	5.93 (*s*)		

4.6 Characterization of Compounds from Root Bark of *Millettia usaramensis ssp. usaramensis*

Chromatographic separation of the dichloromethane/methanol (1:1) extract of the dried and ground root bark of *M. usaramensis* ssp. *usaramensis*, led to the isolation of new chalcone (**326**). The known compounds usararotenoid A (**139**, Yenesew *et al.*, 1998), 12-dihydrousararotenoid A (**140**, Yenesew *et al.*, 1998), millettosin (**138**, Ollis *et al.*, 1967), 12a-epimillettosin (**137**, Yenesew *et al.*, 1998), usararotenoid C (**154**, Yenesew *et al.*, 2003), jamaicin (**328**, Dagne *et al.*, 1989), 4'-*O*-geranylisoliquiritigenin (**181**, Dagne *et al.*, 1990;

Yenesew *et al.*, 1998), 7-*O*-geranyl-5-hydroxyflavanone (**329**, Madhusudhana *et al.*, 2010), tephrosin (**153**, Ollis *et al.*, 1967; Dagne *et al.*, 1991), maximaisoflavone H (**97,** Dagne *et al.*, 1991; Yenesew *et al.*,1996) and colenemol (**330,** Rader *et al.*, 1997) were previously reported from the stem bark of this plant (Yenesew *et al.*, 1998; 2003), from the seeds of *Millettia dura* (Ollis *et al.*, 1967), from the roots of *Tephrosia villosa* (Madhusudhana *et al.*, 2010) and from the root of *Coleonema pulchellum* (Rader *et al.*, 1997) and their identities were confirmed by comparison of their spectroscopic data to those previously published.

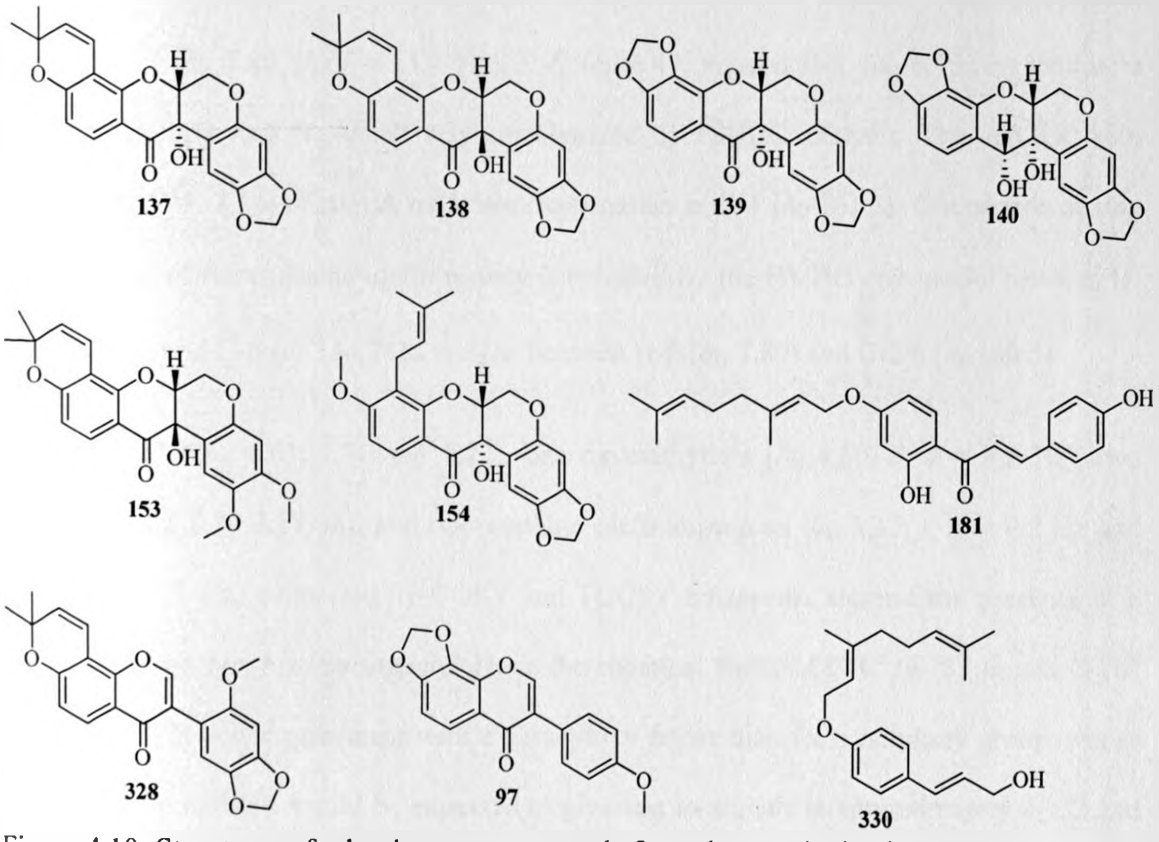


Figure 4.10. Structures of other known compounds from the root bark of *M. usaramensis* ssp *usaramensis*

4.6.1 4-*O*-Geranylisoliquiritigenin (326)

Compound **326** was isolated as a yellow solid. Its HREIMS showed a molecular ion peak at *m/z* 392.1968 consistent with the molecular formula $C_{25}H_{28}O_4$ (calcd. 392.1988). The UV absorbance (at λ_{max} 299 nm and 370 nm), ^{1}H NMR [showing the characteristic *trans*-oriented olefinic (^3J = 15.4 Hz) doublets for H-α (δ_H 7.39) and H-β (δ_H 7.80)] and the ^{13}C NMR [for C=O (δ_C 192.2), C-α (δ_C 118.9) and C-β (δ_C 144.7)] spectra (Table 4.18) are indicative of a chalcone core skeleton (Abegaz *et al.*, 2002; Vijayakumar *et al.*, 2013). A broad singlet at δ_H 13.64 suggests a chelated hydroxyl group (OH-2'), whereas three mutually coupled aromatic protons, H-6' (δ_H 7.82, *d*, ^3J = 15.4 Hz), H-5' (δ_H 6.47, *m*) and H-3' (δ_H 6.45, *m*) indicate a 2',4'-dioxygenaed ring B, which was corroborated by HMBC analysis. The AA'XX' spin system (δ_H 6.91, 7.54) of ring A indicates oxygenation at C-4 (δ_C 161.3). Connection of ring A to the C-β of the chalcone olefin moiety is revealed by the HMBC crosspeaks between H-2/6 (δ_H 7.54) and C-β (δ_C 144.7) as well as between H-β (δ_H 7.80) and C-2/6 (δ_c 130.5).

Three methyls (δ_H 1.63, 1.71 and 1.77), one oxymethylene (δ_H 4.60, *d*, *J* = 7.2 Hz), two methylene (δ_H 2.10-2.17, *m*), and two methine olefinic protons (δ_H 5.12, *t*, *J* = 7.2 Hz and 5.50, *t*, *J* = 7.2 Hz) connected by COSY and TOCSY crosspeaks suggest the presence of a geranyloxy or a neryloxy substituent. Here, the chemical shifts of C-4" (δ_C 39.6) and C-10" (δ_C 16.8) are in better agreement with a geranyloxy rather than for a neryloxy group, whose corresponding carbons would be expected to give rise to signals at approximately δ_C 32 and δ_C 23, respectively (Kozawa *et al.*, 1977). This was further corroborated by the NOE interaction between H-1" and CH_3-10". The position of geranyloxy group was fixed at C-4 of ring A from NOE interaction between the oxymethylene H-1" (δ_H 4.60) of the geranyloxy side chain and the H-3/5 (δ_H 6.91). This placement was further supported by the HMBC

correlation of H-1" (δ_H 4.60) and C-4 (δ_C 161.3). Based on the above spectroscopic data, compound **326** was characterized as (*E*)-1-(2,4-dihydroxyphenyl)-3-(4-(((*E*)-3,7-dimethylocta-2,6-dien-1-yl)oxy)phenyl)prop-2-en-1-one, and assigned the trivial name 4-*O*-geranylisoliquiritigenin.

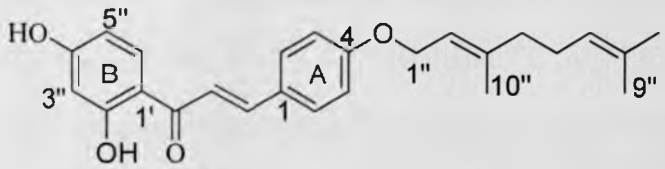


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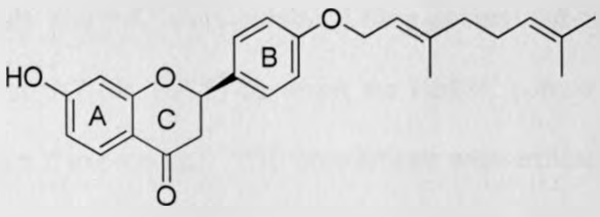
Table 4.18: ^{1}H (799.87) and ^{13}C (201.15) NMR data for compound **326** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 326		
	δ_C	δ_H (m, *J*)	HMBC
1	127.4	-	
2/6	130.5	7.54 (*d*, *J* = 8.8)	β, 4, 6
3/5	115.3	6.91 (*d*, *J* = 8.8)	1, 4
4	161.3	-	
C=O	192.2	-	
C-α	118.9	7.39 (*d*, *J* = 15.4)	1, β
C-β	144.7	7.80 (*d*, *J* = 15.4)	2, 6
1'	114.2	-	
2'	163.6	-	
OH-2'	-	13.64 (*brs*)	1′, 3′, 4′
3'	103.7	6.45 (*m*)	1', 2', 4', 5'
4'	166.2		
5'	108.3	6.47 (*m*)	1', 3'
6'	132.0	7.82 (*d*, *J* = 8.0)	2', 4', C=O
1"	65.2	4.60 (*d*, *J* = 7.2)	4, 2", 3"
2"	117.6	5.50 (*t*, *J* = 7.2)	
3"	142.0	-	
4"	39.6	2.10-2.17 (*m*)	2", 3"
5"	25.8	2.10-2.17 (*m*)	3", 6"
6"	123.8	5.12 (*t*, *J* = 7.2)	5", 8", 9"
7"	131.9	-	
8"	26.3	1.71 (*s*)	6", 7", 9"
9"	17.8	1.63 (*s*)	6", 7", 9"
10"	16.8	1.77 (*s*)	2", 3", 4"

4.6.2 (*S*)-4'-*O*-Geranyl-7-hydroxyflavanone (327)

Compound **327** was obtained as a white solid with $[\alpha]_D^{20}$ -27.5. Its molecular formula was determined to be $C_{25}H_{28}O_4$ on the basis of HRMS (EI: $[M]^+$ obs. *m/z* 392.1968, calcd. 392.1987; ESI: $[M+H]^+$ obs. *m/z* 393.2068, calcd. 393.2066). The ^1H NMR [the aliphatic ABX spin system of ring B with H-3a (δ_H 2.78, *dd*, *J* = 2.4, 16.8 Hz), H-3b (δ_H 3.06, *dd*, *J* = 13.2, 16.8 Hz) and H-2 (δ_H 5.40, *dd*, *J* = 2.4, 13.2 Hz)] and ^{13}C (δ_C 79.9 for C-2, δ_C 44.1 for C-3 and δ_C 191.9 for C-4 cabonyl) NMR spectra of **327** are consistent with flavanone skeleton (Table 4.19). The presence of an aromatic AMX system of ring A with H-5 (δ_H 7.79, *d*, *J* = 8.4 Hz), H-6 (δ_H 6.57, *dd*, *J* = 2.4, 8.4 Hz) and H-8 (δ_H 6.48, *d*, *J* = 2.4 Hz) is consistent with C-7 oxygenation (δ_C 164.2) and, which is expected biogenetically.

The AA'XX' system at δ_H 7.37 (H-2'/H-6') and δ_H 6.93 (H-3'/ H-5') is in agreement with C-4' oxygenated (δ_C 159.5) ring B. As in compound **326**, the presence of geranyloxy moiety (Dagne *et al.*, 1990) (Table 4.19) was also confirmed for **327**. The HMBC correlation of CH_2-1" to C-4' indicated the connection of the geranyloxy group to C-4' of ring C through an ether linkage, which was corroborated by the NOE correlation observed between CH_2-1" and H-3'/H-5'. The CD spectrum (Figure 4.5) of **327** displayed a positive Cotton effect at 332 nm and a negative one at 302 nm, consistent with *S*-configuration at C-2 (Slade *et al.*, 2005). This compound, was therefore, identified as (2*S*)-*E*-2-(4-((3,7-dimethylocta-2,6-dien-1-yl)oxy)phenyl)-7-hydroxychroman-4-one, and given the semisystematic name (-)-(2*S*)-4'-*O*-geranyl-7-hydroxyflavanone, previously isolated from the root bark of this plant (Ivan, 2014).



327

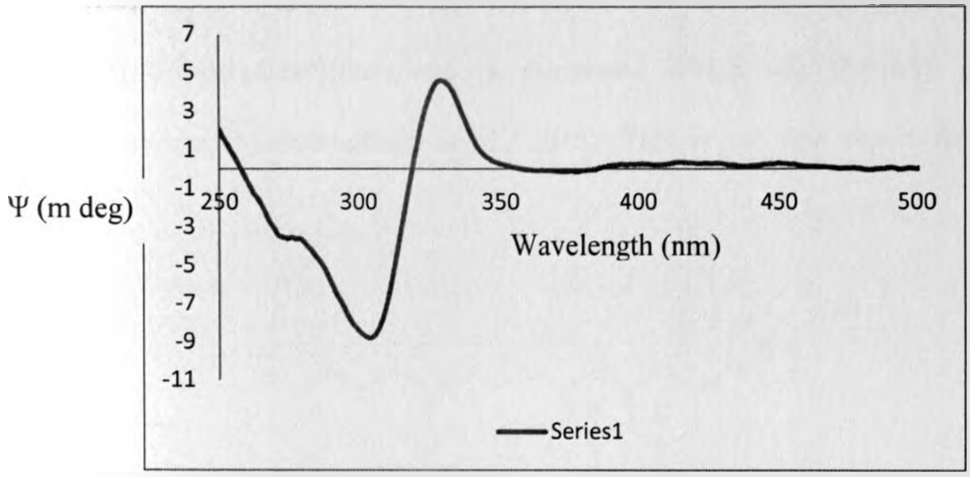
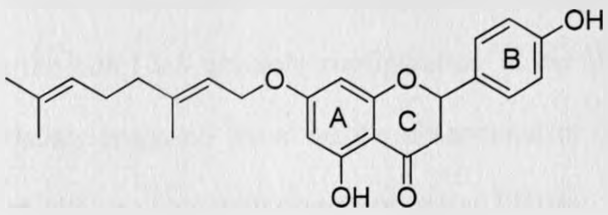


Figure 4.5. The CD spectrum of (*S*)-4'-*O*-geranyl-7-hydroxyflavanone (**327**)

4.6.3 7-*O*-Geranyl-5-hydroxyflavanone (329)

ESI-MS analysis of compound **329** revealed the molecular ion, $[M+H]^+$ peak at *m/z* 409.2 consistent with the molecular formula $C_{25}H_{28}O_5$. The ^1H NMR showed (Table 4.19), three protons at δ_H 5.34 (*dd*, *J* = 13.0, 3.0 Hz), 3.06 (*dd*, *J* = 17.2, 13.0 Hz) and 2.78 (*dd*, *J* = 17.2, 3.0 Hz) corresponding to H-2, H-3ax and H-3eq, respectively for a flavanone core skeleton (Topcu *et al.*, 1996; Yoon *et al.*, 2011). A deshielded singlet signal at δ_H 11.98 was due to chelated hydroxyl group at C-5. In ring A, two *meta* coupled (*J* = 2.4 Hz) doublets at δ_H 6.06 and 6.03 were observed and assigned to H-6 and H-8, respectively. Moreover, four protons of AA'XX'-type and *ortho*-coupled (*J* = 8.0 Hz) were displayed at δ_H 7.32 and 6.87, assignable to H-2'/6' and H-3'/5'of the ring B, indicating a 1,4-disubstitution of this ring. Similar to compound **327**, signals for three methyl singlets (δ_H 1.59, 1.69 and 1.70), two multiplet methylene signals (δ_H 2.05-2.11), a methylenoxy doublet (δ_H 4.53) and two multiplet olefinic

proton signals at δ_H 5.06 and 5.42 were exhibited for a geranyl substituent. The geranyloxy group was connected to C-7 (δ_C 164.8) based on the HMBC correlation between H-1" (δ_H 4.53) and C-7. In NOESY experiment, NOE correlations were exhibited for CH_2-1" protons with H-6 (δ_H 6.06) and H-8 (δ_H 6.03) of the ring A, supporting the connectivity of the geranyloxy group at C-7. This isolate is therefore, characterized as 5,4'-dihydroxy-7-*O*-[(E)-3,7-dimethyl-2,6-octadienyl]flavanone, a compound which was formerly isolated from *Tephrosia villosa* (Madhusudhana *et al.*, 2010). This is the first report from the genus *Millettia.*



329

Table 4.19: ^1H (600.25) and ^{13}C (150.95) data for (**327**, CD_2Cl_2) and (**329**, $CDCl_3$, ^1H, 399.95), (DMSO-d_6, ^{13}C, 201.15) NMR at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 327			Compound 329
	δ_C	δ_H (m, *J*)	HMBC	δ_H (m, *J*)
2	79.9	5.40 (*dd*, *J* = 2.4, 13.2)	3, 1', 2', 6'	5.34 (*dd*, *J* = 4.0, 12.0)
3	44.1	2.78 (*dd*, *J* = 2.4, 16.8) 3.06 (*dd*, *J* = 13.2, 16.8)	2, 1'	3.07 (*dd*, *J* = 12.0, 16.0) 2.77 (*dd*, *J* = 4.0, 16.0)
4	191.9			
4a	114.9			
5	129.4	7.79 (*d*, *J* = 8.4)	7, 8a	
6	110.9	6.57 (*dd*, *J* = 2.4, 8.4)	7, 8, 4a	6.06 (*d*, *J* = 2.4)
7	164.2			
8	103.6	6.48 (*d*, *J* = 2.4)	6, 7, 4a, 8a	6.03 (*d*, *J* = 2.4)
8a	164.0			
1'	130.9			
2'/6'	128.0	7.37 (*m*)	2, 3', 4', 6'	7.32 (*d*, *J* = 8.0)
3'/5'	115.7	6.93 (*m*)	1', 4', 5'	6.87 (*d*, *J* = 8.0)
4'	159.5			
1"	65.3	4.56 (*d*, *J* = 6.6)	4', 2", 3"	4.53 (*d*, *J* = 4.0)

2"	119.6	5.46 (*t*, *J* = 6.0)	4", 10"	5.42 (*m*)
3"	141.6			
4"	39.8	2.11 (*m*)	2", 6"	2.08 (*m*)
5"	26.5	2.11 (*m*)	4", 6", 7"	2.08 (*m*)
6"	124.0	5.10 (*m*)	8", 9"	5.06 (*m*)
7"	132.0			
8"	17.7	1.60 (*s*)		1.66 (*s*)
9"	25.7	1.67 (*s*)		1.59 (*s*)
10"	16.6	1.73 (*s*)		1.70 (*s*)
5-OH				11.98 (*s*)

As part of the structural elucidation, the X-ray structures of usararotenoid A (**139**, Figure 4.6), 12-dihydrousararotenoid A (**140**, Figure 4.7), and 12a-epimillettosin (**137**, Figure 4.8) of this plant were obtained. The solid state structure of epimillettosin (**137**) was obtained for the very first time, confirming the 6*aR*,12a*S* absolute configuration of the B/C ring junction. This configrution was previously proposed based on the observation of $[\alpha]_D$ = +230.4, and the positive Cotton effect at 348 nm along with a negative one at 324 nm.

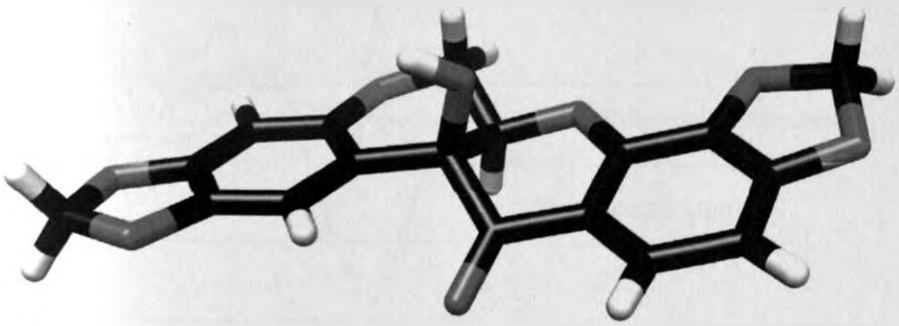


Figure 4.6. The X-ray structure of usararotenoid A (**139**).

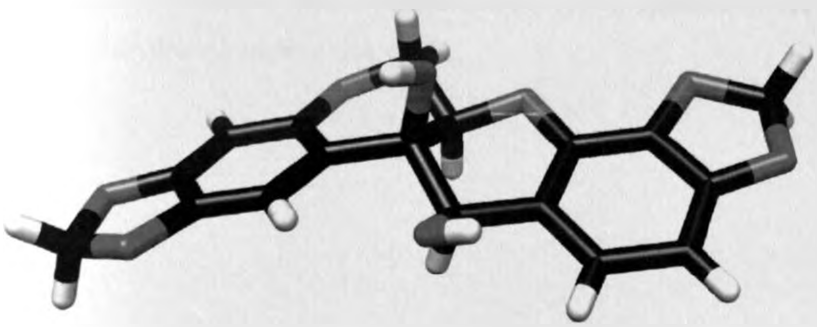


Figure 4.7. The X-ray structure of 12-dihydrousararotenoid A (**140**).

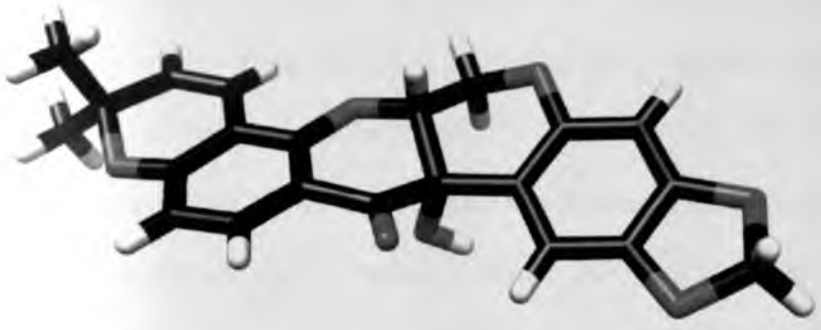


Figure 4.8. The X-ray structure of 12a-epimillettosin (**137**).

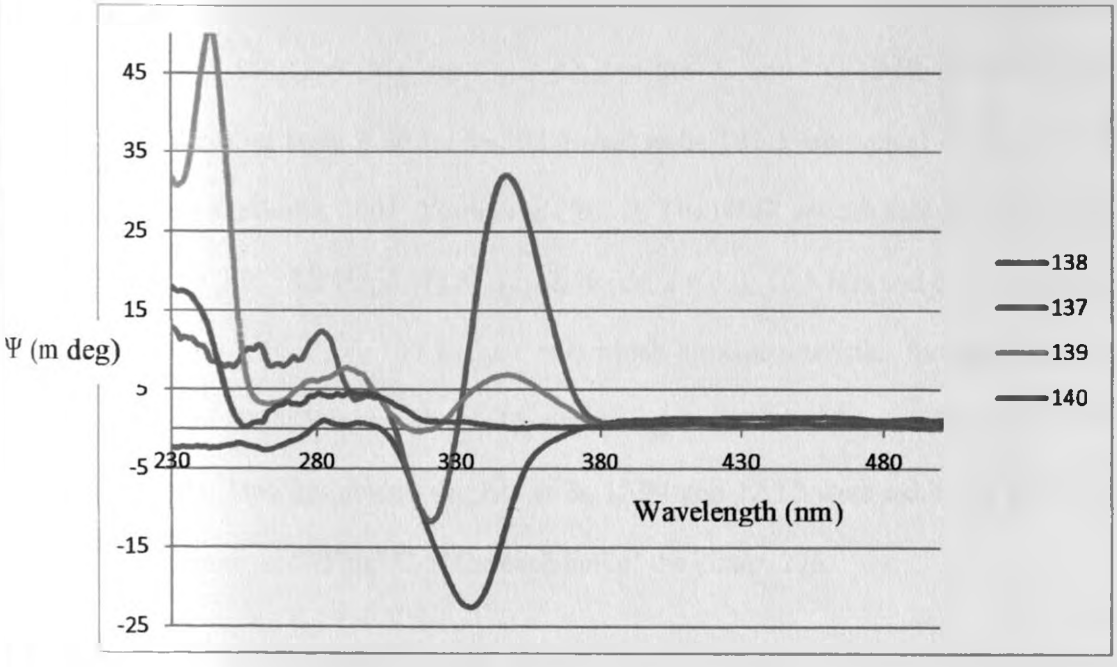


Figure 4.9. The CD spectra of 12a-epimillettosin (**137**), millettosin (**138**), usararotenoid A (**139**) and 12-dihydrousararotenoid A (**140**).

4.7 Characterization of Compounds from Leaves of *Ochna holstii*

The dried and ground leaves of *Ochna holstii* was exhaustively extracted using methanol to yield a dark green crude extract. Chromatographic separation of this extract led to the identification of five dimeric flavanoids (**11**, **252**, **275**, **276**, **332**), one chalcone (**182**) and a cyanoglucoside (**333**). Compound **182** was also isolated from the roots of *Millettia oblata* ssp. *teitensis*. The characterization of these isolates is presented in the following section.

4.7.1 2",3"-Dihydroochnaflavone-7"-*O*-methyl ether (275)

Compound **275** was obtained as a yellow solid. Its HREIMS spectrum indicated the molecular ion, at *m/z* at 554.1209 $[M]^+$ for $C_{31}H_{22}O_{10}$. In the ^1H and ^{13}C NMR spectra (Table 4.20), signals resonating at δ_H 6.86 (*s*; δ_C 103.9) and at δ_C 181.2 are typical of ring C of flavone (Wawer and Zielinska, 2001; Yoon *et al.*, 2011). The NMR spectra also showed signals at δ_H 5.69 (*dd*, J = 3.0, 13.2 Hz; δ_C 78.8), [δ_H 2.79 (*dd*, J = 3.0, 16.8 Hz) and δ_H 3.35 (*dd*, J = 13.2, 16.8 Hz); δ_C 42.2] and δ_C 197.2 (for C=O) which are characteristics for ring C of flavanone (Topcu *et al.*, 1996; Yoon *et al.*, 2011), suggesting that **275** is a flavone-flavanone dimer (Rao *et al.*, 1997). Two deshielded singlets at δ_H 12.94 and 12.12 were exhibited due to chelated hydroxyl groups at C-5 and C-5" for each half of the dimer, **275**.

Furthermore, the ^1H NMR spectrum exhibited the presence of signals of AA'XX'-spin system centering at δ_H 6.94 for H-3"'/5"' and 7.50 for H-2"'/6"'. The signal at δ_H 7.50 (Table 4.20) showed HMBC correlation with C-2" of the C-ring of the flavanone part (refer to the structure of **275** numbering system) and H-2" showed HMBC correlation with C-2"'/C-6"'. This means that H-2", H-2"'/6"' and H-3"'/5"' together with two *meta* coupled protons (from ^1H-^1H COSY spectrum) at δ_H 6.10 and 6.13 (J = 2.4 Hz) belong to the flavanone monomeric unit (naringenin, **31**) of **275**.

Similarly in the other half of the molecule, the presence of two *meta*-coupled protons resonating at δ_H 6.20 and 6.49 (J = 2.0 Hz) is consistent with oxygenation at C-5 and C-7 of ring A. Moreover, three mutually coupled protons at δ_H 7.88 (*dd*, J = 2.4, 8.4 Hz), 7.82 (*d*, J = 2.4 Hz), 7.16 (*d*, J = 9.0 Hz) were observed and assignable to B-ring of the second monomeric unit (i.e the flavone (luteolin, **275a**) of the dimer (Table 4.20).

The monomeric units; the flavanone (naringenin, **31**)) and flavone (luteolin, **275a**) were oxidatively coupled through oxygen to form the dimer, **275**. The point of linkage was established (from the HMBC analysis) to be between C-4''' of the flavanone and C-3'-of the flavone through oxygen linkage. The molecule has one methoxyl group (δ_H 3.80; δ_C 56.0) and was fixed C-7'' based on the basis of HMBC correlation of this group with the C-7'' of the flavanone side. Complete assignment of the NMR data (Table 4.20) was performed using HMBC and HSQC spectra. Based on the above evidences, compound **275** was identified as 2'',3''-dihydroochnaflavone-7''-*O*-methyl ether which was previously reported from *Ochna integerrima* (Likhitwitayawuid *et al.*, 2001).

4.7.2 2'',3''-Dihydroochnaflavone (276)

HREIMS analysis of **276** gave a molecular ion at *m/z* 540.1052 $[M]^+$ corresponding to the molecular formula $C_{30}H_{20}O_{10}$. Similar to that of **275**, the ^{1}H NMR spectrum (Table 4.20) of compound **276** displayed two sets of *meta*-coupled protons for ring A and A', one olefnic proton at δ_H 6.71 for the flavone ring C (H-3) and three aliphatic protons δ_H 5.78 (*dd*, J = 3.0, 12.6 Hz), 2.83 (*dd*, J = 3.0, 16.8 Hz) and 3.22 (*dd*, J = 12.6, 16.8 Hz); characteristics of ring C of a flavanone, suggesting that **276** is also a flavone-flavanone dimer. The point of linkage was also established from the HMBC spectrum as in compound **275**. The only marked

difference between the spectral data of compound **275** and **276** is the absence of the signal corresponding to a methoxyl group in the latter. Based on the above data and comparison with those of **275**, this compound was characterized as 2",3"-dihydroochnaflavone previously reported from *Ochna integerrima* (Likhitwitayawuid *et al.*, 2001).

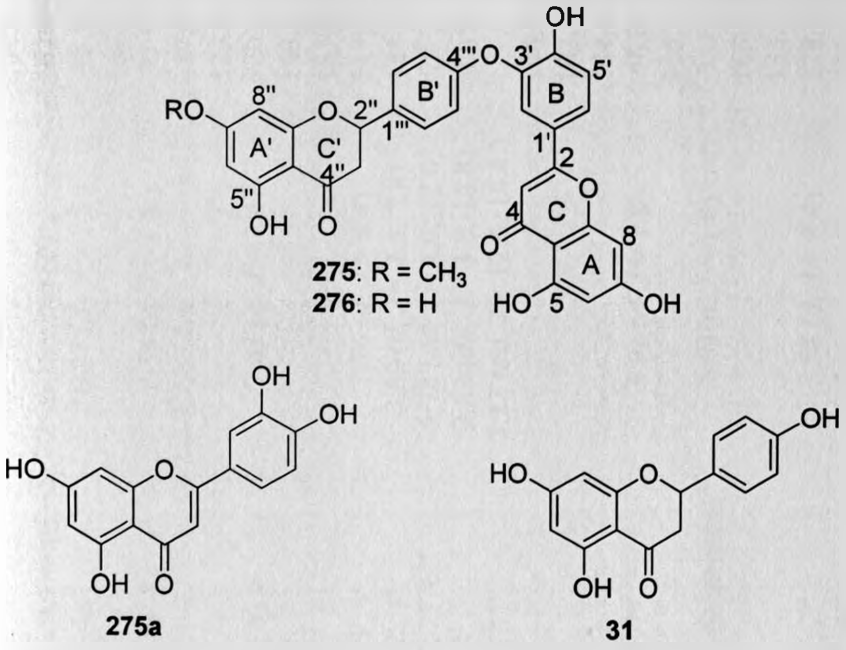


Table 4.20: ^1H(600.24) and ^{13}C NMR (150.93) data for (275, DMSO-d6) and (276, acetone-d_6) acquired at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 275			Compound 276		
	δ_H (m, *J* in Hz)	δ_C	HMBC	δ_H (m, *J* in Hz)	δ_C	HMBC
2		163.1			163.2	
3	6.86 (*s*)	103.9	2, 4	6.71(*s*)	103.9	2, 4, C-1'
4		181.2			182.1	
4a		104.1			104.4	
5		161.8			162.4	
6	6.20 (*d*, *J* = 2.4)	99.4	5, 4a	6.27 (*d*, *J* = 1.8)	98.9	4a, 8
7		164.7			164.1	
8	6.49 (*d*, *J* = 2.4)	94.5	6, 7, 4a, 8a	6.55 (*d*, *J* = 1.8)	93.9	6, 7, 4a, 8a
8a		157.7			157.9	
1'		122.5			123.4	
2'	7.82 (*d*, *J* = 2.4)	121.5	2, 3', 4', 6'	7.82 (*d*, *J* = 2.4)	120.5	2, 3', 4', 6'
3'		142.8			143.1	
4'		154.1			153.0	
5'	7.15 (*d*, *J* = 8.4)	118.3	1', 3', 4'	7.26 (*d*, *J* = 8.4)	117.8	1', 3', 4'
6'	7.87 (*dd*, *J* = 2.4, 8.4)	125.4	2, 2', 4'	7.89 (*d*, *J* = 2.4, 8.4)	124.5	2, 2', C-4'
2"	5.69 (*dd*, *J* = 3.0, 13.2)	78.8	4",1'", 2'"/6'"	5.78 (*dd*, *J* = 3.0, 12.6)	78.7	1'", 2'"/6'"
$3"_{eq}$	2.79 (*dd*, *J* = 3.0, 16.8)	42.2	4"	2.83 (*dd*, *J* = 3.0, 16.8)	42.5	4'', 4a", 1'"
$3"_{ax}$	3.35 (*dd*, *J* = 13.2, 16.8)		4", 1'"	3.22 (*dd*, *J* = 12.6, 16.8)		2", 1'", 4"
4"		197.2			196.0	
4"a		103.1			102.3	
5"		163.7			164.1	
6"	6.10 (*d*, *J* = 2.4)	95.2	5", 7"	5.98 (*d*, *J* = 1.8)	96.0	8'', 7'', 4a''
7"		167.9			166.6	
8"	6.13 (*d*, *J* = 2.4)	94.3	7", 8a"	6.01(*d*, *J* = 1.8)	95.0	6", 7", 4a"
8a"		163.2			163.3	
1'"		132.5			133.3	
2'"/6'"	7.50 (*d*, *J* = 8.4)	128.7	4'", 2"	7.58 (*d*, *J* = 8.4)	128.2	2", 4'"
3'"/5'"	6.94 (*dd*, *J* = 8.4)	116.1	1'", 4'"	7.06 (*d*, *J* = 8.4)	116.5	1'", 4'"
4'"		158.5			158.2	
OCH_3	3.80	56.0	7"			
5-OH	12.94 (*s*)		6, 4a, 5	12.98 (*s*)		6, 4a, 5
5"-OH	12.12 (*s*)			12.19 (*s*)		5", 6", 4a"

4.7.3 Ochnaflavone (11)

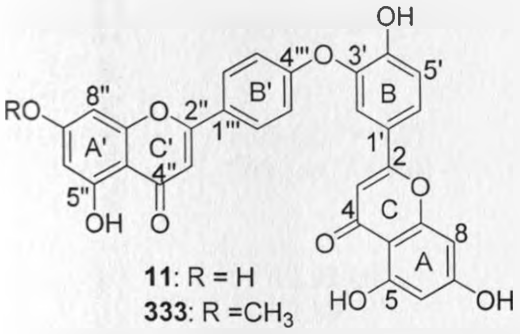
Compound **11** was obtained as yellow amorphous solid from methanol. The HREIMS analysis gave a molecular formula $C_{30}H_{18}O_{10}$ (M^+, *m/z* 538.0893). The NMR data (Table 4.21) showed high degree of similarities with those of compounds **275** and **276**. The only remarkable spectral (and hence, structural) difference between **11** and compounds **275** and **276** is the absence of the characteristic signals attributable to a flavanone nucleus in the **11**. Instead, the ^{1}H NMR of compound **11** has two closely spaced singlets at δ_H 6.88/6.89 for H-3/H-3" which showed HMBC correlation with C-1'/-1''' (δ_C 124.8/122.8) suggesting compound **11** is a flavone-flavone dimer.

The pattern of oxygenation in **11** suggested that this compound is a dimer of luteolin (**275a**) and apigenin (**24**). The complete chemical shift assignment of **11** was performed using HMBC, HSQC and COSY spectra and also through comparison with the spectral data for **275** and **276**. Therefore, compound **11** was identified as ochnaflavone, a compound previously reported from various *Ochna* species; *Ochna squarrosa* (Okigawa and Kawano, 1973; Okigawa *et al.*, 1976), *Ochna obtusata* (Rao *et al.*, 1997) and *O. lanceolata* (Reddy *et al.*, 2008).

4.7.4 Ochnaflavone-7"-*O*-methyl ether (331)

Compound **331** was isolated as yellow amorphous solid from methanol. The HREIMS analysis exhibited molecular ion, $[M]^+$ peak at *m/z* 552.1041 consistent with molecular formula $C_{31}H_{20}O_{10}$. The NMR data (Table 4.21) of compound **331** are highly similar to **11**. The only notable difference between **331** and **11** is the appearance of additional signal corresponding to a methoxyl group in **331**. Similar to **11**, two singlets δ_H at 6.89 for H-3 (HMBC with C-1', δ_C 122.8) and 6.95 for H-3" (HMBC correlation with C-1''', δ_C 124.7) were

observed for **331** suggesting that the compound is also a flavone-flavone dimer of the monomers; luteolin (**275a**) and apigenin (**24**). Hence, compound **331** was identified as ochnaflavone-7"-*O*-methyl ether (**331**), a compound previously reported from *Ochna integerrima* (Reutrakul *et al.*; 2007).



11: R = H
333: R =CH_3

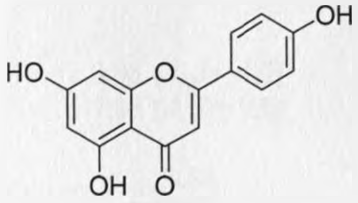


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Table 4.21: ^{1}H (600.24) and ^{13}C NMR (150.95) data for (**11**, DMSO-d_6) and (**331**, acetone-d_6) acquired at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 11		Compound 331	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
	Luteolin unit		**Luteolin unit**	
2	163.5		163.1	
3	104.3[c]	6.89 (*s*)	104.7	6.89 (*s*)
4	182.2		182.2	
4a	104.5		104.2	
5	161.9		161.6	
6	99.4	6.20 (*d*, *J* = 2.4)[a]	99.3	6.19 (*d*, *J* = 2.4)
7	164.7[d]		164.6	
8	94.5	6.49 (*d*, *J* = 2.4)[b]	94.6	6.50 (*d*, *J* = 1.8)
8a	157.8		157.7	
1'	122.8		122.8	
2'	121.8	7.91-7.93 (*m*)	121.8	7.91-7.93 (*m*)
3'	142.0		142.0	
4'	153.8		153.8	
5'	118.3	7.18 (*d*, *J* = 9.0)	118.4	7.18 (*d*, *J* = 9.0)
6'	125.8	7.91-7.93 (*m*)	125.8	7.91-7.93 (*m*)

	Apigenin unit		Apigenin-7-methyl ether unit	
2"	163.1		163.5	
3"	104.2[c]	6.88 (*s*)	104.7	6.95 (s)
4"	182.2		182.4	
4"a	104.1		105.2	
5"	161.9		161.8	
6"	99.4	6.21 (*d*, *J* = 2.4)[a]	98.5	6.39 (*d*, *J* = 2.4)
7"	164.8[d]		165.7	
8"	94.5	6.50 (*d*, *J* = 1.8)[b]	93.2	6.77 (*d*, *J* = 2.4)
8a"	157.8		157.7	
1"'	124.8		124.7	
2"'/6"'	128.9	8.05 (*d*, *J* = 9.0)	128.9	8.08 (*d*, *J* = 9.0)
3"'/5"'	116.5	7.03 (*dd*, *J* = 9.0)	116.5	7.05 (*d*, *J* = 9.0)
4"'	161.3		161.3	
OCH_3			56.5	3.88
5-OH		12.92 (*s*)[e]		12.92 (*s*)
5"-OH		12.89 (*s*)[e]		12.89 (*s*)

a, b,c,d,e: chemical shifts with the same superscript can be interchangeable

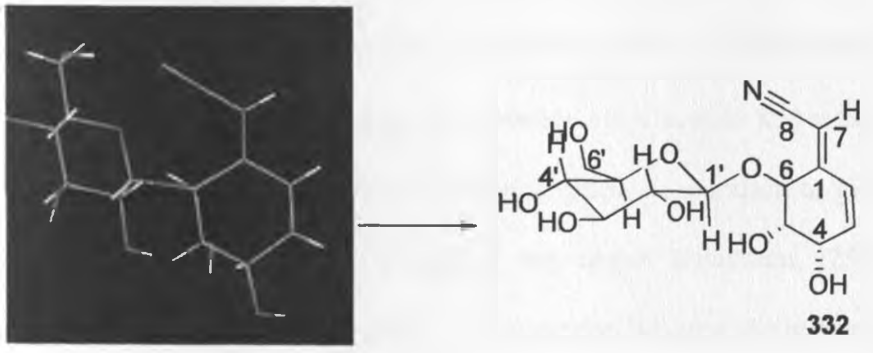
4.7.5 Dasycarponin (332)

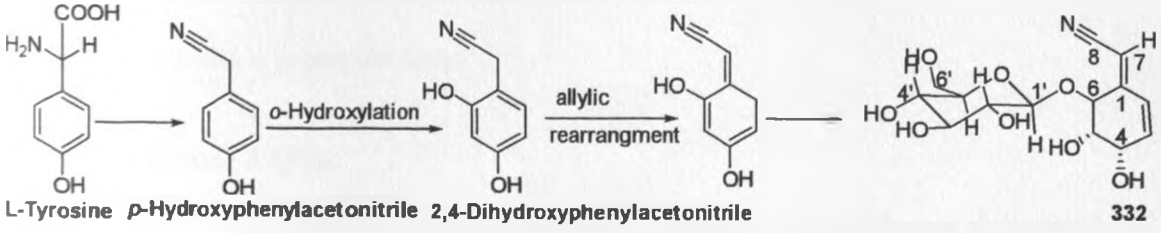
Compound **332** was obtained as a colorless needles and the molecular formula $C_{14}H_{19}NO_8$ was deduced from ESIMS analysis which gave *m/z*, $[M+H]^+$ at 330.4. The even $[M+H]^+$ molecular weight suggested the presence of one nitrogen in **332**. This compound was identified as dasycarponin (Wu *et al.*, 1979; Zinchem *et al.*, 2014) based on the following spectroscopic evidence. In the ^{1}H NMR spectrum, the chemical shifts ranging from δ_H 2.88-4.57 ppm along with the carbon signal at δ_C 103.5 is indicative of the presence of a glycoside moiety. A doublet at δ_H 4.39 showing HSQC correlation with a carbon signal at δ_C 103.7 represent the anomeric proton (and carbon) of the glycoside.

The large coupling constant (J = 7.8 Hz, 1,2-diaxial between the anomeric proton and H-2') is indicative of the β-linkage of the glycoside to the aglycone. Furthermore, the ^{1}H NMR spectrum exhibited two mutually coupled and *cis*-oriented olefinic protons at δ_H 6.19 and 5.93 (J = 9.6 Hz) assignable to H-2 and H-3 respectively. The occurrence of isolated olefinic proton at δ_H 5.72 was also evident which could be allocated to the exocyclic olefinic proton,

H-7. In the HMBC spectrum, a three bond correlation was observed between the anomeric proton, H-1' (δ_H 4.39) and the C-6 (δ_C 77.3), confirming that C-6 is the point of linkage of the glycoside to the aglycone nucleus. Complete chemical shift assignment to the quaternary carbons was carried out using HMBC and HSQC spectra.

In the X-ray crystal structure of **332**, the two hydrogen atoms at C-4 and C-5, and the glycoside unit at C-6 are in the same face with the relative configuration 4*S**,5*S**,6*S** being proposed. Biogenetically, compound **332** is assumed to be L-tyrosine-derived through p-hydroxyphenylacetonitrile as an intermediate; consequently undergoing *O*-hydroxylation, allylic rearrangement and glucosylation (Scheme 4. 3, Zinchem *et al.*, 2014). Therefore, based on the above data and X-ray structure, compound **332** was identified as (4*S**,5*S**,6*S**)-dasycarponin (Wu *et al.*, 1979; Zinchem *et al.*, 2014).





Scheme 4.3. Proposed biogenetic pathways of formation of dasycarponin (**332**) (Azinchem *et al.*, 2014).

Table 4.22: ^{1}H (600.24) and ^{13}C NMR (150.95) data for **332** acquired in DMSO- d_6 at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	δ_C	δ_H (*m*, *J*)
1	154.6	-
2	125.5	6.19 (1H, *d*, *J* = 9.6)
3	140.1	5.93 (1H, *d*, *J* = 9.6)
4	77.1	3.15 (1H, *m*)
5	77.0	3.15(1H, *m*)
6	77.3	4.57 (1H, *d*, *J* = 3.6)
7	99.1	5.74 (1H, *s*)
8	118.1	-
1'	103.7	4.39 (1H, *d*, *J* = 7.8)
2'	69.0	4.04 (1H, *m*)
3'	73.7	2.88 (1H, *m*)
4'	65.9	4.45 (1H, *m*)
5'	70.3	3.09 (1H, *m*)
6'	61.6	3.50 (1H, *m*)
		3.66 (1H, *m*)
Glu-OH		4.91-5.14

4.8 Characterization of Compounds from the Stem Bark of *Ochna holstii*

The air-dried stem bark of *Ochna holstii* was ground and extracted with methanol to give a crude extract. This crude extract was partitioned between ethyl acetate and water (4:1). The ethyl acetate extract was subjected to column chromatographic separation to give a total of seven compounds which includes three related rearranged biflavones (**252-254**), two isoflavone (**295** and **334**), one flavan (**235**) and a simple benzene derivative (**335**). The secondary metabolite **252** was also isolated from the leaves of this plant. The characterization of these compounds is presented here.

4.8.1 Lophirone A (252)

Compound **252** was isolated as colorless solid from methanol. ESIMS gave a molecular ion, $[M+H]^+$ at *m/z* at 511.7 compatible with the molecular formula $C_{30}H_{20}O_8$. The ^{13}C NMR spectrum (Table 4.23) accommodated signals for thirty carbon atom which were assigned to

two C=O (δ_C 204.3 and 174.8), twenty six sp^2 carbons (including seven oxygenated, δ_C155.9-166.5) and two methine sp^3 carbon atoms (δ_C 43.6 and 53.0). This suggested that **252** is unsubstituted dimeric flavonoid. The singlet at δ_H 8.33 showed a three bonds HMBC correlations with the C=O at δ_C 175.8 suggesting that this pair belong to an isoflavone arm of the molecule. The ^{1}H NMR and COSY spectra revealed the occurrence of two 1,4-disubstituted benzene rings (B and B') each having an AA'XX' spin system at δ_H 7.14 for 4H (H-21, H-25, H-27 and H-31) and 6.49-6.54 for 4H (H-22, H-24, H-28 and H-30), respectively.

Moreover, two AMX-type protons were also exhibited at δ_H 7.81 (*d*, *J* = 8.8 Hz, for H-5), 6.85 (*dd*, *J* = 1.6, 8.8 Hz, for H-6) and 6.73 (*d*, *J* = 2.4 Hz, for H-8) for the ring A and at δ_H 6.11 (*d*, *J* = 2.4 Hz, for H-14), 6.37 (*dd*, *J* = 2.4, 8.8 Hz, for H-15), 8.22 (*d*, *J* = 8.8 Hz, for H-17) for the ring A'. Again, the ^{1}H NMR together with COSY identified, two coupled and *trans*-oriented (*J* = 12 Hz) methine protons at δ_H 6.13 and 4.79 which are respectively connected to carbons at δ_C 43.6 and 53.0 ppm (from the HSQC spectrum). Through detailed analyses of the 1D and 2D NMR data and comparison with the literature, this compound was identified as lophirone A (**252**) previously reported from *Lophira lanceolata* (Ghogomua *et al.*, 1987), *Ochna calodendron* (Messanga *et al.*, 1992); *Ochna afzelii* (Pegnyemb *et al.*, 2003a) and *Ochna squarrosa* (Anuradha *et al.*, 2006).

4.8.2 Calodenone (253)

Compound **253** was isolated as a colorless solid. The ESIMS analysis gave a $[M+H]^+$ ion peak at *m/z* 525.5 compatible with the molecular formula $C_{31}H_{24}O_8$. The NMR data (Table 4.23) of compound **253** displayed identical oxygenation with that of **252** (seven oxygenated aromatic carbon atoms and consequently, identical proton spin system over all rings systems).

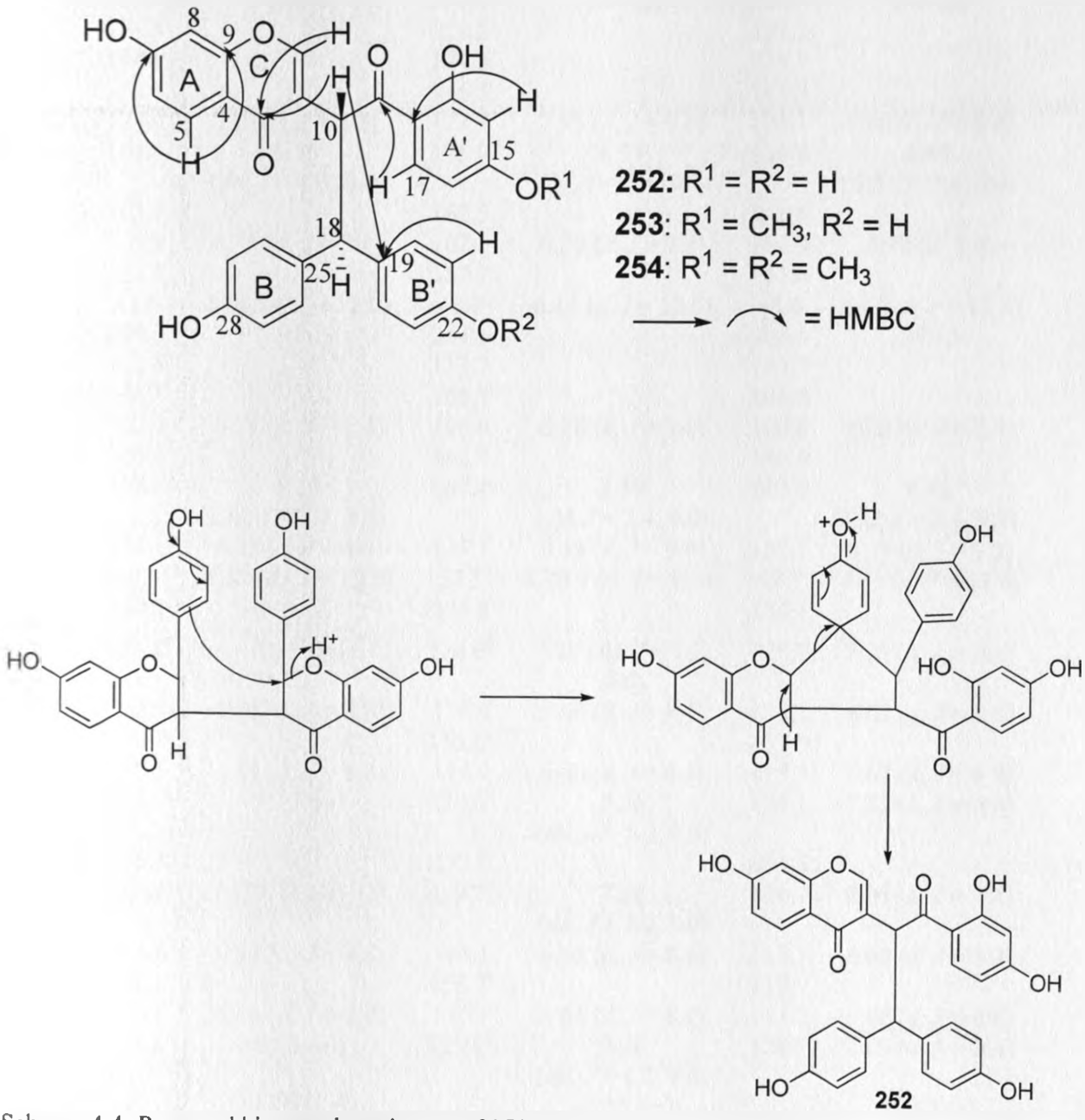
The ^{1}H and ^{13}C NMR signals for the two aliphatic systems, a singlet for a chelated hydroxyl at C-14 (δ_H 12.55) were also observed in **253**. The only difference between **252** and **253** is the appearance of signals for one methoxyl at (δ 3.81, 55.2) in the latter compound. The placement of the methoxyl was established from its NOE with H-14 (δ_H 6.28) and HMBC interactions that the C-15 position is methoxylated. Thus, based on these evidences and comparison of the data with that of lophirone A (**252**), compound **253** was identified as calodenone, previously reported from *Ochna calodendron* (Messanga *et al.*, 1992); *Ochna afzelii* (Pegnyemb *et al.*, 2003a) and *Ochna squarrosa* (Anuradha *et al.*, 2006).

4.8.3 Afzelone D (254)

Compound **254** was isolated as colorless solid with $[M-H]^-$ ion peak at *m/z* 537.6 in the ESIMS, corresponding to the molecular formula $C_{32}H_{26}O_8$. This compound **254** has spectral data (Table 4.23) related to **252** and **253**. In the NMR data of **254,** signals for two methoxyl groups at (δ_H 3.77, δ_C 55.5) and (δ_H 3.65, δ_C 55.1) were exhibited. The methoxyl group resonating at (δ_H 3.77, δ_C 55.5) was placed at C-15 similar to **253** based on its NOE with H-14 (δ_H 6.23) and HMBC interaction with C-15 (δ_C 166.5). Similarly, the second methoxyl group at (δ_H 3.65, δ_C 55.1) was placed at C-22 of the ring B'. Hence, based on these evidences and comparison of the spectral data with that of lophirone A (**252**) and calodenone (**253**), compound **254** was characterized as afzelone D, a compound which has been previously isolated from *Ochna afzeelii* (Pegnyemb *et al.*, 2003a).

The mode of dimerization involved in lophirone A (**252**); and hence in calodenone (**253**) and afzelone D (**254**) was observed to be unusual and believed to be derived biogenetically from an ordinary flavanone-flavanone dimer through enzymatic assisted 1,2-phenyl migration from one arm of the dimer and consequently, causing opening of the ring C in the hosting wing as

in Scheme 4.4 (Niwa *et al.*, 1984). The term "isobiflavonoid" was therefore, used for this class of compounds (Ghogomua *et al.*, 1987).



Scheme 4.4. Proposed biogenetic pathways of **252-254** (Niwa *et al.*, 1984).

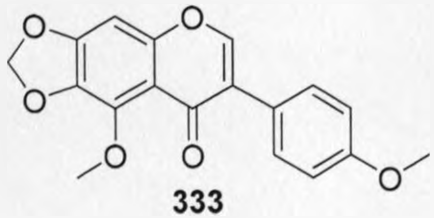
Table 4.23: (^{1}H, ^{13}C) NMR data for (**252**), (**253**) and (**254**) acquired at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

	Compound 252		**Compound 253**		**Compound 254**	
Position	δ_C	δ_H (*m, J*)	δ_C	δ_H (*m, J*)	δ_C	δ_H (*m, J*)
1	155.9	8.33 (*s*)	155.4	8.27 (*s*)	154.9	8.05 (*s*)
2	121.8		121.2		121.3	
3	174.8		174.3		174.9	
4	116.9		116.5		117.5	
5	127.9	7.81(*d*, *J* = 8.8)	127.4	7.93 (*d*, *J* = 9.0)	128.2	8.00 (*d*, *J* = 8.8)
6	115.6	6.85 (*dd*, *J* = 1.6, 8.8)	115.0	6.91 (d*d*, J = 2.4, 9.0)	114.7	6.81 (d*d*, J = 2.4, 8.8)
7	162.9		162.5		160.4	
8	102.9	6.73 (*d*, *J* = 2.4)	102.3	6.76 (*d*, *J* = 1.8)	102.9	6.72 (*d*, 2.4)
9	158.2		157.7		157.6	
10	43.6	5.93 (*d*, *J* = 12.0)	43.2	6.15 (*d*, *J* = 12.0)	43.1	6.07 (*d*, *J* = 12.4)
11	204.3		204.1		203.5	203.5
12	113.8		113.7		113.7	
13	166.5		165.9		165.8	
14	103.0	6.11 (*d*, *J* = 2.4)	100.6	6.28 (*d*, *J* = 3.0)	100.8	6.23 (*d*, *J* = 2.4)
15	165.7		166.7		166.5	
16	108.6	6.37 (*dd*, *J* = 2.4, 8.8)	107.4	6.49 (*dd*, *J* = 2.4, 9.0)	107.9	6.41 (*dd*, *J* = 2.8, 9.2)
17	134.1	8.22 (*d*, *J* = 8.8)	133.1	8.38 (*d*, *J* = 9.0)	132.7	8.17 (*d*, *J* = 9.2)
18	53.0	4.67 (*d*, *J* = 12.0)	52.5	4.79 9 (*d*, *J* = 11.4)	52.7	4.69 (*d*, *J* = 12.0)
19	134.3		134.8		134.1	
20	129.1[a]	7.13 (*m*)	128.6[b]	7.26 (*dd*, *J* = 1.2, 9.0)	129.1	7.21 (*d*, *J* = *8.8*)
21	115.5	6.49 (*d*, *J* = *8.8*)	114.9	6.60 (*d*, *J* = 8.4)	115.5	6.62 (*d*, *J* = 8.8)
22	156.1		155.6[c]		157.9	
23	115.5	6.49 (*d*, *J* = 8.8)	114.9	6.60 (*d*, *J* = 8.4)	115.5	6.62 (*d*, *J* = 8.8)
24	129.1[a]	7.13 (*m*)	128.6[b]	7.26 (*dd*, *J* = 1.2, 9.0)	129.1	7.21 (*d*, *J* = *8.8*)
25	135.4		133.7		135.5	
26	129.8[a]	7.13 (*m*)	129.2[b]	7.26 (*dd*, *J* = 1.2, 9.0)	128.7	7.19 (*d*, *J* = 8.4)
27	115.6	6.54 (*d*, *J* = 8.8)	115.1	6.64 (*d*, *J* = 8.4)	113.7	6.68 (*d*, *J* = 8.8)
28	156.2		155.7[c]		153.9	
29	115.6	6.54 (*d*, *J* = 8.8)	115.1	6.64 (*d*, *J* = 8.4)	113.7	6.68 (*d*, *J* = 8.8)
30	129.8[a]	7.13 (*m*)	129.2[b]	7.26 (*dd*, *J* = 1.2, 9.0)	128.7	7.19 (*d*, *J* = 8.4)
5-OH		12.55 (*s*)		12.55 (*s*))		12.62 (*s*)
15-OCH_3			55.2	3.81 (*s*)	55.5	3.77 (*s*)
22-OCH_3					55.1	3.65 (*s*)

[a,b,c] Chemical shift with the same superscript may be interchangeable. **252**: ^{1}H (799.87, DMSO-d_6), ^{13}C (150.95, acetone d_6) NMR; **253**: ^{1}H (600.24), ^{13}C (150.95) (acetone-d_6) NMR and **254**: ^{1}H (399.97, $CDCl_3$), ^{13}C (201.15) NMR (DMSO-d_6).

4.8.4 5,4'-Dimethoxy-6,7-methylenedioxyisoflavone (333)

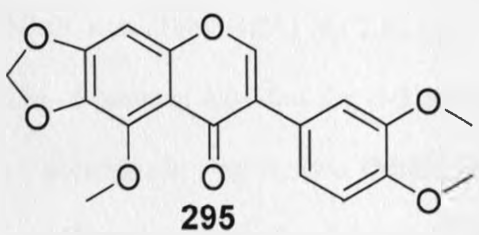
Compound **333**, $[M+H]^+$ at *m/z* 327.4; for $C_{18}H_{14}O_6$, was suggested to be an isoflavone derivative based on the ^1H NMR signal at (δ_H 7.78, *s*; attached to sp^2-oxygenated carbon (δ_C 150.3 (for C-2) having a methylenedioxy (δ_H 6.07; δ_C 102.2) and two methoxyl groups (δ_H 3.83, δ_C 55.4, and δ_H 4.08. δ_C 61.3). In ring B, the presence of an AA'XX'-spin system at δ_H 7.47 (*d*, *J* = 8.8 Hz for H-2'/H-6') and 6.95 (*d*, *J* = 9.6 Hz for H-3'/H-5') is consistent with C-4' oxygenation. The substituent at C-4' was established to be a methoxyl group (at δ_H 3.83; δ_C 55.4) due to its NOE interaction with H-3'/H-5' of ring B. The ^1H NMR spectrum further showed a singlet at δ_H 6.64 in ring A, which otherwise is substituted with methoxyl at (δ_H 4.08; δ_C 61.3) and methylenedioxy at (δ_H 6.07; δ_C 102.1) groups could be located on the ring A. The deshielded chemical shift of the carbon of the methoxyl group (δ_C 61.3) requires that this methoxyl is a *di-ortho* substituted that could be placed either at C-8 or C-5 providing that the methylenedioxy to be at C-6/C-7. In HMBC spectrum, only weak correlation was observed between the proton at δ_H 6.64 and the C=O (δ_C 175.5), which could be *W*-coupling and the chemical shift value of the proton. This would suggest the proton (δ_H 6.64) being at C-8 and the methoxyl group at C-5. Therefore, compound **333** was identified as 5,4'-dimethoxy-6,7-methylenedioxyisoflavone, earlier isolated from of *Iris tingitana* (El-emary *et al.*, 1980).



333

4.8.5 5,3',4'-Trimethoxy-6,7-methylenedioxyisoflavone (295)

ESIMS analysis of **295** gave a molecular ion peak, $[M+H]^+$ at *m/z* 357.1 for $C_{19}H_{16}O_7$. The NMR (Table 4.24) data suggested that **295** is also an isoflavone derivative with three methoxyl and methylenedioxy group as substituents. Comparison of the NMR data of **295** with **333** showed identical ring A with methylenedioxy (δ_H 6.07; δ_C 102.3) being placed at C-6 (δ_C 135.5)/C-7 (δ_C 152.8), and one methoxyl (δ_H 4.09; δ_C 61.3) group at C-5 (δ_C 141.7). In ring B, the AA'XX' spin system of **333** is replaced by AMX [δ_H 7.01 (*dd*, *J* = 1.6, 8.0 Hz), 7.19 (*d*, *J* = 2.4 Hz) and 6.90 (*d*, *J* = 8.0 Hz) and assignable to H-6', H-2' and H-5', respectively] in ring B of **295,** which is substituted with two methoxyl groups (δ_H 3.92, δ_C 56.0) and (δ_H 3.90, δ_C 55.9) at C-3' (δ_C 148.7) and C-4' (δ_C 149.1), respectively. The substitution pattern in this ring was confirmed from the HMBC spectrum. Therefore, compound **295** was identified as 3',4',5-trimethoxyl-6,7-methylenedioxyisoflavone (trivial name 5-*O*-methylsquarrosin), a compound previously isolated from *O. squarrosa* (Anuradha *et al.*, 2006)



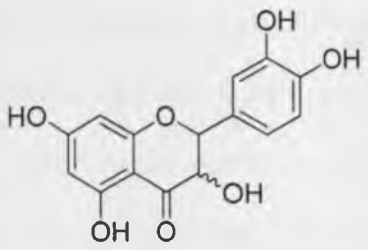
295

Table 4.24: ^{1}H (799.87) and ^{13}C NMR (201.15) data of **333** and **295** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound (333)		Compound (295)	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	150.3	7.78 (*s*)	150.4	7.79 (*s*)
3	125.4		125.4	
4	175.5		175.2	
4a	110.9		113.8	
5	141.7		141.7	
6	135.6		135.5	
7	152.8		152.8	
8	93.3	6.64 (*s*)	93.2	6.64 (*s*)
8a	154.8		154.7	
1'	124.1		124.6	
2'	130.4	7.47 (*d*, *J* = 8.8)	112.8	7.19 (*d*, *J* = 2.4)
3'	113.8	6.95 (*d*, *J* = 9.6)	148.7	
4'	159.5		149.1	
5'	113.8	6.95 (*d*, *J* = 8.8)	111.0	6.90 (*d*, *J* = 8.0)
6'	130.4	7.47 (*d*, *J* = 9.6)	121.3	7.01 (*dd*, *J* = 1.6, 8.0)
6/7-OCH_2O	102.1	6.07 (*s*)	102.2	6.07 (*s*)
5-OCH_3	60.9	4.08 (*s*)	61.3	4.09 (*s*)
3'-OCH_3			56.0	3.92 (*s*)
4'-OCH_3	54.9	3.83 (*s*)	55.9	3.90 (*s*)

4.8.6 (±)-Catechin (334)

The negative mode ESIMS analysis of **334** gave *m/z* 289.2 corresponding to the molecular formula $C_{15}H_{14}O_6$. The ^{1}H NMR data (Table 4.25) [δ_H 2.52 (*dd*, *J* = 4.0, 16.8 Hz) and 2.72 (*dd*, *J* = 5.6, 16.8 Hz) for CH_2-4 protons, 4.04 (*m*) for H-3 and 4.77 (*brs*) for H-2] is very compatible with the flavanol skeleton. In ring A, two shielded and *meta* coupled aromatic protons were observed at δ_H 5.93 and 5.77 (*d*, *J* = 2.4 Hz) for H-6 and H-8, respectively; which is consistent with C-5 and C-7 oxygenation. Furthermore, a three protons at 6.93 (*d*, *J* = 2.4 Hz, for H-2'), 6.71 (*d*, *J* = 8.0 Hz, for H-5') and 6.68 (*dd*, *J* = 2.4, 8.0 Hz, for H-6') were exhibited for the ring B with C-3' and C-4' being hydroxylated. Compound **334** was obtained as a racemic mixture, and therefore, identified as (±)- catechin.



334

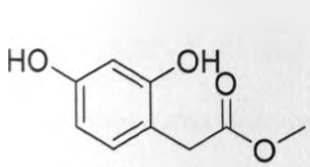
Table 4.25: ^{1}H (799.87) and ^{13}C NMR (201.15) data for **334** acquired in DMSO-d_6 at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	δ_C	δ_H (*m*, *J*)
2	78.5	4.77 (*brs*)
3	65.4	4.04 (*m*)
4	28.7	2.52 (*dd*, *J* = 4.0, 16.8)
		2.72 (*dd*, *J* = 5.6, 16.8)
4a	99.0	
5	156.7	
6	95.6	5.93 (*d*, *J* = 2.4)
7	156.9	
8	94.6	5.77 (*d*, *J* = 2.4)
8a	156.2	
1'	131.1	
2'	115.3	6.93 (*d*, *J* = 2.4)
3'	144.9	
4'	144.9	
5'	115.3	6.71 (*d*, *J* = 8.0)
6'	118.5	6.68 (*dd*, *J* = 2.4, 8.0)
3-OH		4.69 (*d*, *J* = 4.0)
4xAr-OH		8.79-9.19

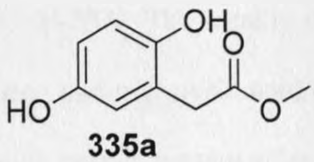
4.8.7 2,4-Dihydroxyphenylmethyl acetate (335)

Compound **335** was isolated as a brown gum. The molecular formula $C_9H_{10}O_4$ was deduced from *pseudo*-molecular ion peak $[M+H]^+$ at *m/z* 183.2 in the ESIMS spectrum. The ^{1}H NMR spectrum revealed that **335** is a trisubstituted benzene derivative. In the HSQC spectrum, three carbon atoms of the benzene ring are protonated while the remaining three are quaternary attached to two hydroxyl groups and a methylacetyl group. [(δ_H 3.41, δ_C 34.8 for Ph-$\underline{CH_2}$), (δ_H 3.56, δ_C 51.8 for COO$\underline{CH_3}$) and δ_C 172.8 for $\underline{C}OOCH_3$]. In the ^{1}H NMR spectrum, three mutually coupled protons, at δ_H 6.85 (*d*, *J* = 8.8 Hz), 6.27 (*d*, *J* = 2.4 Hz) and 6.15 (*dd*, *J* =

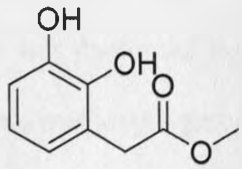
2.4, 8.8 Hz) were observed. With the methylacetyl group being at C-1on the benzene ring, the two hydroxyl groups could be either in 2,4 (*meta*, **335**), 2,5 (*para*,**335a**) or 3,4 (*ortho*,**335b**) relationship. However, the two carbon atoms attached to the hydroxyl groups appeared at (δ 156.5 and 157.6) suggesting the *meta* relationship of the two hydroxyl groups as in **335**. These two carbons are expected to be shielded in the other two alternatives (**335a** and **335b**). The absence of HMBC correlation between the proton at δ_H 6.12 (*dd*, *J* = 2.4, 8.8 Hz, H-5) and the carbon at δ_C 156.5, suggested the δ_C 156.5 to be assigned to C-2 and δ_C 157.7 for C-4. Therefore, based on the spectroscopic data, compound **335** was identified as 2,4-dihydroxyphenylmethylacetate, a compound previously isolated from *Thalictrum fortune* (Xiantao *et al.*, 2007) and obtained for the first time from *Ochna* species.



335



335a



335b

Table 4.26: ^{1}H (799.87) and ^{13}C NMR (201.15) data for compound (**335**) acquired in DMSO-d6 at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

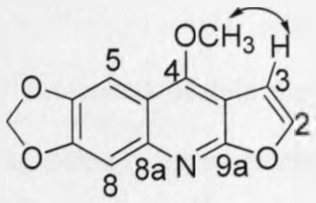
Position	δ_C	δ_H (*m*, *J*)
1	112.2	
2	156.5	
3	102.7	6.26 (*d*, *J* = 2.4)
4	157.7	
5	106.4	6.12 (*dd*, *J* = 2.4, 8.8)
6	131.7	6.84 (*d*, *J* = 8.0)
COOCH_3	51.8	3.56 (*s*)
COOCH_3	172.7	
Ph-CH2	34.8	3.41 (*s*)
2x*Ph*-OH		9.13 (*s*), 9.32 (*s*)

4.9 Characterization of Compounds from the Root Bark of *Ochna ovata*

The root bark of *O. ovata* was air-dried, ground and extracted with dichloromethane/methanol (1:1) to give a crude extract. This crude extract was subjected to vacuum liquid chromatography (VLC) using *n*-hexane containing increasing amounts of ethyl acetate. The fractions collected from VLC were subjected different chromatographic purification to afford the dimeric lophirone A (**252**) and calodenone (**253**), dasycarponin (**333**) and four minor furoquinoline alkaloids (**336-339**). The structure characterization of compounds (**336-339**) is presented here.

4.9.1 Maculine (336)

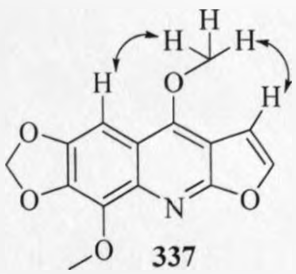
Compound **336** was isolated as a cololess solid whose *pseudo*-molecular ion peak, $[M+H]^+$ at *m/z* 244.6 in ESIMS corresponds to $C_{13}H_9NO_4$. The identity of **336** was confirmed to be a furanoquinoline alkaloid having two olefinic AX doublets, a methoxyl group, a methylenedioxy group and two aromatic singlet protons (Cao *et al.*, 2008; Sichaem *et al.*, 2014). The AX doublets (J = 2.4 Hz) at δ_H 7.57 (δ_C 142.6) and 7.03 (δ_C 104.5) are consistent with the H-2 and H-3 of the furan moiety. The methoxyl group (δ_H 4.41) showed NOE interaction with the furan proton at (δ_H 7.03, H-3) and hence, it was placed at C-4 (δ_C 156.0). The two aromatic singlets at δ_H 7.52 and 7.30 were assigned to H-5 and H-8, allowing the methylenedioxy group to be fixed at C-6/7. Therefore, this compound was identified as maculine (Paulini *et al.*, 1989; Cardoso-Lopes *et al.*, 2010) and it is the first report of this compound in family Ochnaceae.



336

4.9.2 Flindersiamine (337)

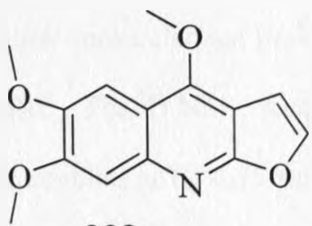
The isolate **337** was analyzed for $[M+H]^+$ at *m/z* 274.2 (for $C_{14}H_{11}NO_5$) in the ESIMS analysis. The ^1H NMR (Table 4.25) of **337** revealed that this compound is also furoquinoline alkaloid derivative similar to **336** accommodating two methoxyl groups (δ_H 4.26, 4.40), two AX doublets (δ_H 7.58, *d*, *J* = 3.2 Hz; 7.02, *d*, *J* = 2.4 Hz), a methylenedioxy group (δ_H 6.06) and an aromatic singlet (δ_H 7.28). It is evident that one of the aromatic singlets observed for 336 was replaced by a methoxyl group in **337**, as the only remarkable structural difference. The methoxyl group resonating at δ_H 4.40 (δ_C 58.9) showed NOE interactions with furan ring proton (δ_H 7.02, H-3) and the aromatic singlet (δ_H 7.28). This observation suggested that this methoxyl must be flunked between these two groups [H-3 (7.02)↔OCH_3 (4.40)↔H -5 (7.28)] and placed at C-4. This in turn allowed the second methoxyl group (δ_H 4.26) to be placed at C-8 (δ_C 137.8) and the methylenedioxy group being fixed at C-6/7. Based on this evidence, 337 was thus identified as flindersiamine (Paulini *et al.*, 1989; Cardoso-Lopes *et al.*, 2010), again as the first report of this compound in the family of Ochnaceae family.



337

4.9.3 Kokusaginine (338)

Compound **338** is another minor compound of this plant whose ESIMS spectrum gave, $[M+H]^+$ ion pek at *m/z* 260.3 corresponding to the molecular formula $C_{14}H_{13}NO_4$. Its ^1H NMR data (Table 4.27) is also consistent with the furoquinoline derivative as **336** and **337**. The ^1H NMR spectrum of **338** displayed signals for two olefinic AX doublets for furan ring, two aromatic singlets and three methoxyl groups. Compound **338** exhibited similar oxygenation pattern to **336,** however, the signals corresponding to methylenedioxy group in (**336**) was replaced by two methoxyl groups (δ_H 4.01, 4.03) in the case of **338** at C-6 and C-7. The third methoxyl group resonating at (δ_H 4.41); (δ_C 58.8) was placed at C-4, and the two singlets at δ_H 7.46 and 7.34 being assigned to H-5 and H-8. Compound **338** was consequently, identiifed as kokusaginine (Paulini *et al.*, 1989; Cardoso-Lopes *et al.*, 2010). This is its first report in the Ochnaceae family.



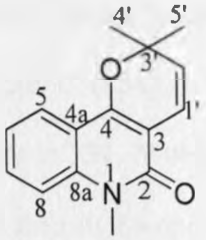
338

Table 4.27: ^{1}H (799.87) and ^{13}C NMR (201.15) data for compound **336-338** acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	Compound 336		Compound 337		Compound 338	
	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)	δ_C	δ_H (*m*, *J*)
2	142.6	7.57 (*d*, *J* = 2.4)	143.1	7.58 (*d*, *J* = 3.2)	142.5	7.58 (*d*, *J* = 3.2)
3	104.5	7.03 (*d*, *J* = 2.4)	104.3	7.02 (*d*, *J* = 2.4)	104.6	7.05 (*d*, *J* = 2.4)
3a	102.5		102.9		102.3	
4	156.0		156.1		155.9	
4a	114.4		115.0		112.9	
5	98.0	7.52 (*s*)	92.4	7.28 (*s*)	100.2	7.49 (*s*)
6	146.1		146.8		147.9	
7	150.8		138.0		152.7	
8	104.5	7.30 (*s*)	137.8		106.8	7.39 (*s*)
8a	143.9		136.0		142.6	
9a	163.2		162.6		163.3	
4- OCH_3	58.9	4.41 (*s*)	58.9	4.40 (*s*)	58.9	4.45 (*s*)
8- OCH_3			60.6	4.26 (*s*)		
6- OCH_3					55.9	4.02 (*s*)
7-OCH_3					55.9	4.03 (*s*)
6/7-OCH_2O	101.6	6.09 (*s*)	101.5	6.06 (*s*)		

4.9.4 *N*-Methylflindersine (339)

ESIMS analysis of **339** gave a *pseudo*-molecular ion peak, $[M+H]^+$ at *m/z* 242.6 compatible with the molecular formula $C_{15}H_{15}NO_2$. The ^{1}H NMR spectrum (Table 4.28) of **339** exhibited peaks for two *cis*-oriented olefinic doublets at δ_H 6.75 and 5.54 (*J* = 10.0 Hz), three methyl groups, one at δ_H 3.69 for N-CH_3 and at δ_H 1.52 for 4'/5'-CH_3, and for four aromatic protons at δ_H 7.97 (H-5), 7.23 (H-6), 7.55 (H-7) 7.32 (H-8) corresponding to angular pyranoquinoline skeleton (Brader *et al.*, 1993). Therefore, based on the spectral evidences and comparison with the literature, compound **339** was identified as *N*-methylflindersine (Brader *et al.*, 1993; Luo *et al.*, 2009), and this is its first report in the Ochnaceae family.



339

Table 4.28: ^{1}H (399.94) and ^{13}C NMR (201.15) data for compound (**339**) acquired in $CDCl_3$ at 25°C (chemical shift, δ in ppm and multiplicity, *J* in Hz).

Position	δ_C	δ_H (*m*, *J*)
2	161.0	
3	105.9	
4	155.2	
4a	116.1	
5	123.1	7.97 (*dd*, *J* = 1.2, 8.0)
6	121.7	7.23 (*ddd*, *J* = 0.8, 7.2, 8.0)
7	130.9	7.55 (*ddd*, *J* = 1.6, 7.2, 8.4)
8	114.0	7.32 (*d*, *J* = 8.8)
8a	139.4	
1'	117.9	6.75 (*d*, *J* = 10.0)
2'	126.3	5.54 (*d*, *J* = 10.0)
3'	78.7	
4'/5'	28.2	1.52 (*s*)
1-NCH_3	29.3	3.69 (*s*)

4.10 Biological Activities

4.10.1 Cytototixicty Assay

The cytotoxic activities of the crude extracts of *Millettia* (*M. dura*, *M. usaramensis* ssp. *usaramensis*, *M. oblata* ssp. *teitensis*) and *Ochna* (*O. holstii*) species (Table 4.1), and some of their constituents (Table 4.29) against the ER negative MDB-MB-231 human breast cancer cell-line were investigated. It was noted that the activities of the roots and root bark extracts are higher than those of the leaves and the stem bark extracts. Among the compounds isolated and tested from the root bark of *M. usaramensis* ssp *usaramensis*, six compounds, **137**, **138**, **139**, **154**, **181** and **330** showed moderate to low activities (IC_{50} 10.5-59.3 µg/mL) against the MDB-MB-231; the highest activity was recorded for the rotenoid usararotenoid C (**154**, 10.5

μg/mL). Epimillettosin (**137**, IC_{50} = 39.7 μg/mL) was almost four times weaker than the structurally related rotenoid, usarotenoid C (**154**), which indicated the importance of the uncyclized prenyl group for this activity in **154**. Millettosin (**138**, 24.3 μg/mL) having a *cis*-fused B/C ring junction is more active than its 6a-epimer, epimillettosin (**137**) having *trans*-B/C ring junction, showing that activity of rotenoids could also be influenced by the geometry and/or configuration at C-6a and C-12a. It is worth to note that the common rotenoids, rotenone and tephrosin, both having *cis*-fused B/C ring junction were reported to have high cytotoxicities against different cell-lines (Cao *et al.*, 2004; Matsuda *et al.*, 2007; Ye *et al.*, 2012).

Interestingly 12-dihydrousararotenoid A (**140**), which has a hydroxyl instead of a carbonyl functionality at C-12, is inactive (IC_{50} >100 μg/mL) and this probably suggested the importance of carbonyl functionality for cytotoxicity against this cell-line. On the other hand, Ivan (2014) reported the activity of a 12-dihydrorotenoid (which also lacks carbonyl functionality) on MCF-7 breast cell-line. This appears to suggest that the activities of rotenoid derivatives are variable on the type of cell-lines against which they are tested. Therefore, for establishing structure-activity-relationships, more such compounds should be tested against a varity of cancer cell-lines.

The isoflavone, maximaisoflavone J (**325**) and the rotenoid, 9-methoxyl-2,3-dimethylenedioxyrotenoid (**320**), isolated from the leaves of *M. oblata* ssp. *teitensis*, also showed moderate and low activities with IC_{50} values of 11.2 and 30.6 μg/mL, respectively, against MDB-MB-231 cell line. Maximaisoflavone J (**325**), having a methoxyl substituent at C-4', is at almost five times more active than the structurally related isoflavone, maximaisoflavone B (**304**, 53.8 μg/mL) having a methylenedioxy group at C-3'/4'.

Table 4.29: Cytotoxicity activities of some isolated compounds against the ER-negative, MDB-MB-231 human breast cell-line

Compounds	IC_{50} (µg/mL)
12a-Epimillettosin (**137**)	39.7
Millettosin (**138**)	24.3
Usararotenoid A (**139**)	31.1
Usararotenoid C (**154**)	10.5
4'-*O*-geranylisoliquiritigenin (**181**)	49.9
Colenemol (**330**)	59.3
Maximaisoflavone B (**304**)	53.8
7,2'-Dimethoxyl-4',5'-methylenedioxyisoflavone (**51**)	56.8
4-Hydroxyloncocarpin (**182**)	88.1
Maximaisoflavone A (**308**)	34.9
2",3"-Dihydroochnaflavone (**275**)	66.9
Ochnaflavone-7"-*O*-methyl ether (**332**)	25.4
Maximaisoflavone J (**325**)	11.2
9-Methoxyl-2,3-methylenedioxyrotenoid (**320**)	30.6
7,2',5'-Trimethoxyl-3',4'-methylenedioxyisoflavone (**317**)	59.7
Lophirone A (**252**)	71.1
Calodenone (**253**)	45.7
12-Dihydrousararotenoid A (**140**)	>100
3",4"-Epoxybarbigerone (**312**)	>100
2",3"-Dihydroochnaflavone-7"-*O*-methyl ether (**276**)	>100
Barbigerone (**55**)	>100
Durmillone (**91**)	>100
8-prenylmilldurone (**316**)	>100
1-isopropyl-3-(pyridin-4-yl ethynyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine*	0.05

* Reference drug/positive control

The cytotoxic activities against Vero cells of some isolated compounds and crude extracts were also determined (Table 4.30) as described in Irungu *et al.* (2014). Among the tested compounds, strong activity was recorded for the dimeric flavonoid, calodenone (**253**). This compound is ten times more active than the related compound, lophirone A (**252**), a compound which differs from **253** by lack of a methoxyl group at C-15. This indicates the importance of the methoxyl group at C-15 for the observed activity in calodeneone (**253**).

Table 4.30: Cytotoxicity of some isolated compounds and crude extracts against Vero cells

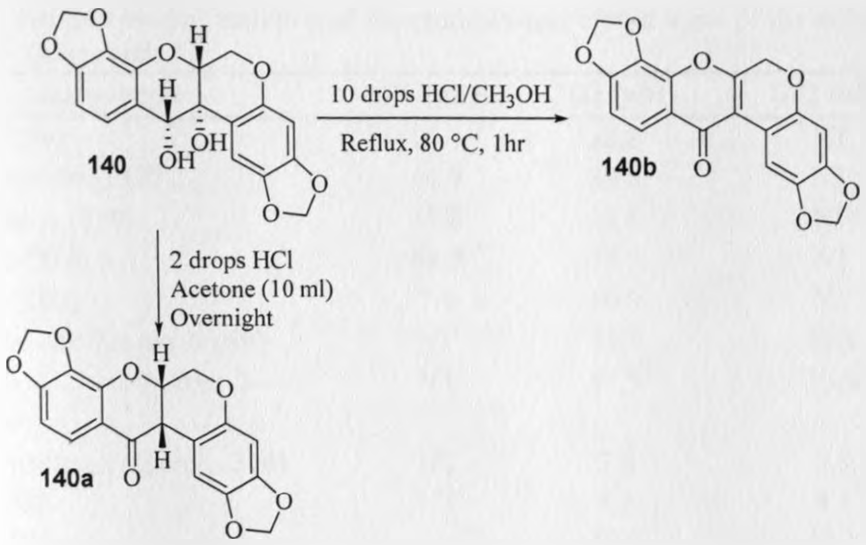
Compounds	IC_{50} (µg/mL)
Crude extract of stem bark of *O. holstii*	41.7
12a-Epimillettosin (**137**)	17.2
Calodenone (**253**)	6.8
Lophirone A (**252**)	67.4
Usararotenoid A (**139**)	30.2
Melarsoprol*	0.76

* reference drug/positive control

The isolated constituents were also tested for *in vitro* translation inhibition using Krebs-II translation extracts programmed with a bicistronic firefly-HCV IRES-Renilla mRNA construct, to monitor cap-dependent as well as cap-independent translation (Novac *et al.*, 2004). However, none of the compounds showed translation inhibitory activity.

4.10.2 Antiplasmodial and Larvicidal Activity

Flavonoids are also well known for their antiplasmodial and larvicidal activties (Yenesew *et al.*, 2009; Bosire *et al.*, 2014; Derese *et al.*, 2014). In this regard, some of the isolated compounds and the extracts were evaluated for antiplasmodial activity against two chloroquine-sensitive (D6 and 3D7) and chloroquine-resistant (Dd2) strains of *Plasmodium falciparum*. The major rotenoids and two derivatives (**140a** and **140b**) of **140** (Scheme 4.5) were also tested for larvicidal activity against the 4th instar larvae of *Aedes aegypti*.



Scheme 4.5. Preparation of derivatives from 12-dihydrousararotenoid A (**140**).

The crude extract of the root bark of *M. usaramensis* displayed highest antiplasmodial activity against the two chloroquine-sensitive D6 (0.99 μM) and 3D7 (2.0 μM) strains of *P. falciparum*. The new chalcone (**326**, IC_{50} 3.8 μM and 5.3 μM) and the rotenoid, deguelin (**152**, 4.3 μM and 4.5 μM) exhibited strong antiplasmodial activities against the chloroquine-sensitive (3D7) and the chloroquine-resistant (Dd2) strains of *Plasmodium falciparum*, respectively. Furthermore, a dimeric flavonoid, calodenone (**253**), a rotenoid, tephrosin (**153**) and the isoflavone derivatives, calopogonium isoflavone A (**50**) and isoerythrin A-4'-[3-methylbut-2-enyl] ether (**94**) showed significant antiplasmodial activity on both resistant and sensitive strains of *Plasmodium falciparum* (Table 4.31).

Table 4.31: Antiplasmodial activities of the crude extract of and some of the isolated compounds

Compounds	D6 (μM)	3D7 (μM)	Dd2 (μM)
Colenemol (**330**)	14.3	38.8	NT
12a-Epimillettosin (**137**)	44.9	39.8	NT
Usararotenoid A (**139**)	38.8	13.8	NT
(±)-Catechin (**334**)	48.9	28.9	NT
Calodenone (**253**)	7.8	10.9	NT
Calopogonium isoflavone A (**50**)	NT	13.5	19.9
Isoerythrin A-4'-[3-methylbut-2-enyl]ether (**94**)	NT	11.9	15.9
4-*O*-Geranylisoliquiritigenin (**326**)	NT	3.8	5.3
Deguelin (**152**)	NT	4.3	4.5
Tephrosin (**153**)		13.3	12.1
4-Hydroxyloncocarpin (**182**)	NT	9.6	90% at 40 μM
Barbigerone (**55**)	NT	95% at 40 μM	95% at 40 μM
Crude extract of the root bark of *M. usaramensis* ssp. *usaramensis*	0.99	2.0	NT

* IC_{50} for the crude extract is in μg/mL. Positive controls: pyrmethamine (IC_{50} = 6.1 ± 5.1 nM (3D7), 62% at 40 mM (Dd2), chloroquine (IC_{50} = 4.3 ± 0.3 nM (3D7), IC_{50} = 69.9 ± 34.5 nM (Dd2), pyronaridine (IC_{50} = 10.7 ± 10.0 nM (3D7), IC_{50} = 12.6 ± 7.2 nM (Dd2) puromycin (IC_{50} = 43.7 ± 29.7 nM (3D7), IC_{50} = 54.3 ± 12.8 nM (Dd2), artesunate (IC_{50} = 1.6 ± 1.5 nM (3D7), IC_{50} = 0.8 ± 0.5 nM (Dd2), dihydroartemisinin (IC_{50} = 0.4 ± 0.5 nM (3D7), IC_{50} = 0.4 ± 0.3 nM (Dd2); chloroquine (IC_{50} = 0.22 ± 0.03 μM (D6), mefloquine (IC_{50} = 0.00 ± 0.001 μM (D6). NT: Not Tested

The rotenoid, usararotenoid A (**139**), despite having a *trans*-B/C ring junction showed high larvicidal activity (LC_{50} 4.3 μg/mL at 48 h) against the 4th instar *A. aegypti* larvae which is comparable with that of deguelin (**152**, 2.63 μg/mL). 12a-Deoxyusarotenoid A (**140a**) and 6a,12a-dehydrousararotenoid-A (**140b**) were prepared from 12-dihydrousararotenoid A (**140**) as shown in Scheme 4.5. Interestingly, these derivatives were completely inactive even at 100 μg/mL against the 4th instar *A. aegypti* larvae at 48 h. Usararotenoid A (**139**) appears to be the first rotenoid with *trans*-B/C ring junction to have significant larvicidal activity.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In this study, three *Millettia* species (*M. oblata* ssp. *teitensis*, *M. dura* and *M. usaramensis* ssp. *usaramensis*) and two *Ochna* species (*O. holstii* and *O. ovata*) were investigated to afford a total of sixty six compounds among which ten are new. These included monomeric and dimeric flavonoids, rotenoids, isoflavonoids, chalcones, alkaloids, terpenenoid and other simple benzene and cyclohexene derivatives. All identified rotenoids and isoflavones in *Millettia* species (except toxicarolisoflavone, **37**) are C-11 or C-5 deoxygenated.

From the leaves *of M. oblata* ssp. *teitensis* fourteen isoflavonoids were isolated and characterized. Among these, four rotenoids (**318-321**) and two isoflavones (**316**, **317**) are new. The new rotenoid oblarotenoid C (**321**) appears to have been biogenetically derived from the isoflavone **317** through oxidation of the 2'-OCH_3 and cyclization. This is the first example of rotenoid with such oxygenation pattern on the ring A. Similarly, the roots of *M. oblata* ssp. *teitensis* yielded thirteen compounds which included isoflavones, chalcones, flavanone and flavanonol. The tetraglycoside isoflavone, obloneside (**306**) is a new compound. The root bark of *M. dura* also gave six isoflavones, one chalcone and a new pterocarpan (**303**). Four of the isoflavones isolated from *M. dura*; **50**, **51**, **91**, **94** and the chalcone **195** were also identified in root bark of *M. oblata* ssp. *teitensis*. Similar work on the root bark of *M. usaramensis* ssp. *usaramensis* gave thriteen compounds as chalcones, rotenoids, flavanoids and cinnamyl alcohol, of which compound **326** is a new. Phytochemical investigation of the leaves of *M. usaramensis* ssp. *usaramensis* led to the identification of five

rotenoids, three isoflavones and one triterpene. The isoflavone **312,** containing an epoxy moiety on the 2,2-dimethylchromane ring is a new compound. The known rotenoid **313** with rearranged 2,2-dimethylchromene is reported here for the first time from the genus *Millettia.*

Similar investigations of the stem bark and leaves *O. holstii* yielded a total of twelve dimeric and monomeric flavonoids along with some simple molecules, **333** and **335**. The root bark of *O. ovata* also gave seven compounds. The major compounds; lophirone A (**252**) and calodenone (**253**) and compound **333** were also isolated from *O. holstii.* The four alkaloids (**336-339**) of the root bark of *O. ovata* were reported here for the first time from the family Ochnaceae. Four derivatives from the isolated compounds were also prepared.

Some of the isolated compounds were evaluated for their cytotoxic activities against MDB-MB-231 and Vero cells, translation inhibitory activity and antiplasmodial activities. The derivatives (**115a**, **140a**, **140b** and **306a**) did not exhibit any activity.

The constituents of *M. usaramensis* and its crude extracts showed moderate to low activities against the ER negative, MDB-MB-231 breast cancer cell-line and on the Vero cell-line, out of which, usararotenoid C (**154**) showed the highest activity. The isoflavone, maximaisoflavone J (**325**) and the new rotenoid, **320** of the leaves of *M. oblata* also showed moderate and low activities against the same cell-line, respectively. The dimeric flavonoid, calodenone (**253**) showed significant cytotoxicity against the Vero cell-line.

Compounds **326** and **152** exhibited strong antiplasmodial activity against the chloroquine sensitive (3D7) and the chloroquine resistant (Dd2) strains of *Plasmodium falciparum.* The chalcone, **326** is also cytotoxic to the HEK293 cell- line, limiting its further development as an antimalarial lead compound.

5.2 Recommendations

I. Comparative HPLC profiling of subspecies of *Millettia usaramensis* should be done to draw the exact differences in terms of their secondary metabolites.

II. The leaves of *Millettia usaramensis* ssp. *usaramensis* should be re-investigated to find out some more metabolites with modified 2,2-dimethylchromene ring.

III. *Ochna* species found in Kenya should be phytochemically and biologically investigated to establish their chemical and biological profile.

IV. Integrated absolute configuration studies such as X-ray, experimental and theoretical CD calculations should be done for the rest isolated rotenoids and compounds of *Ochna* species.

V. The mechanism of larvicidal activity by *trans*-12a-hydroxyrotenoid, **139** should be established.

VI. The cytotoxic activity should also be conducted on other cancer cell-lines.

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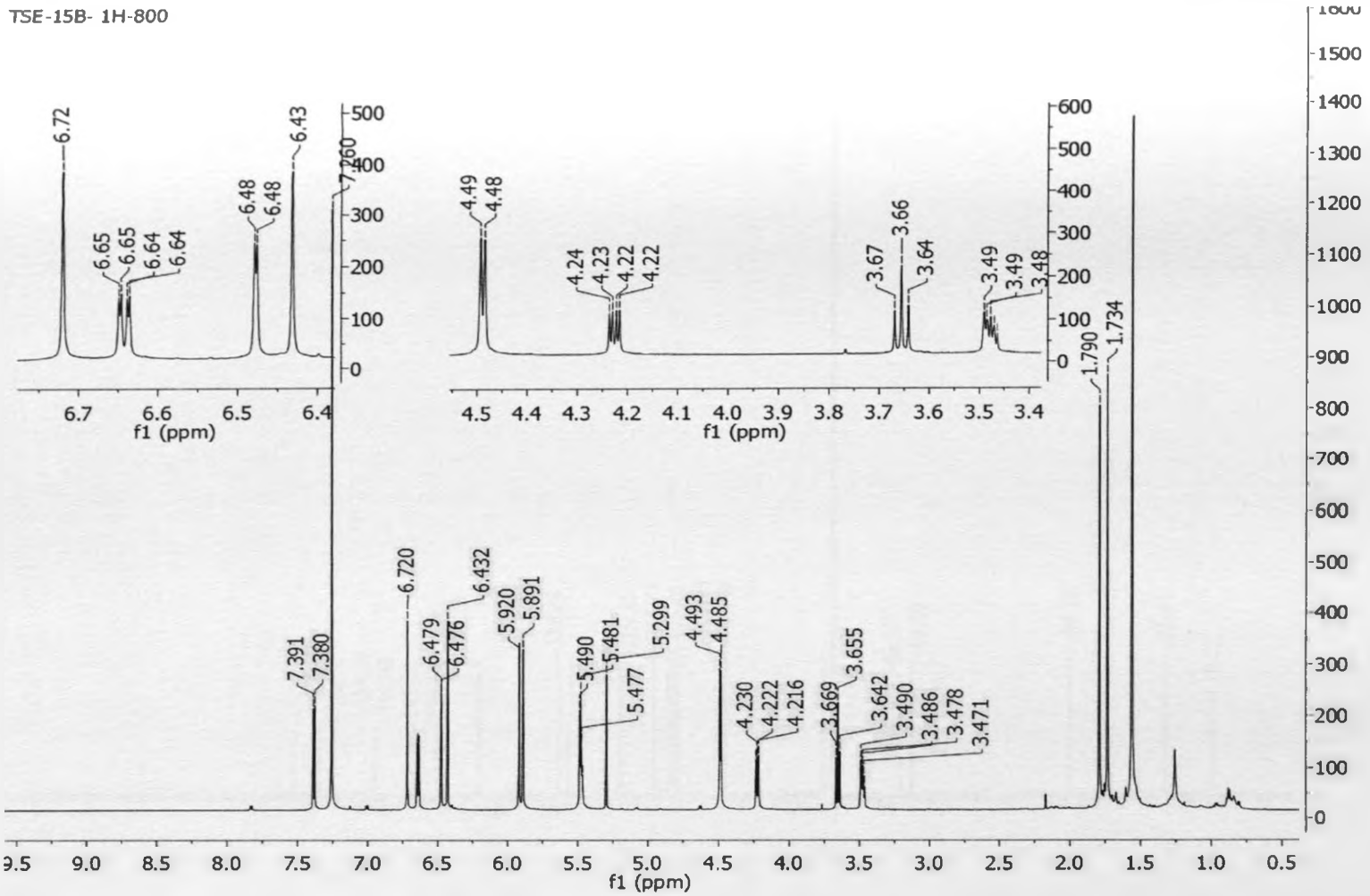
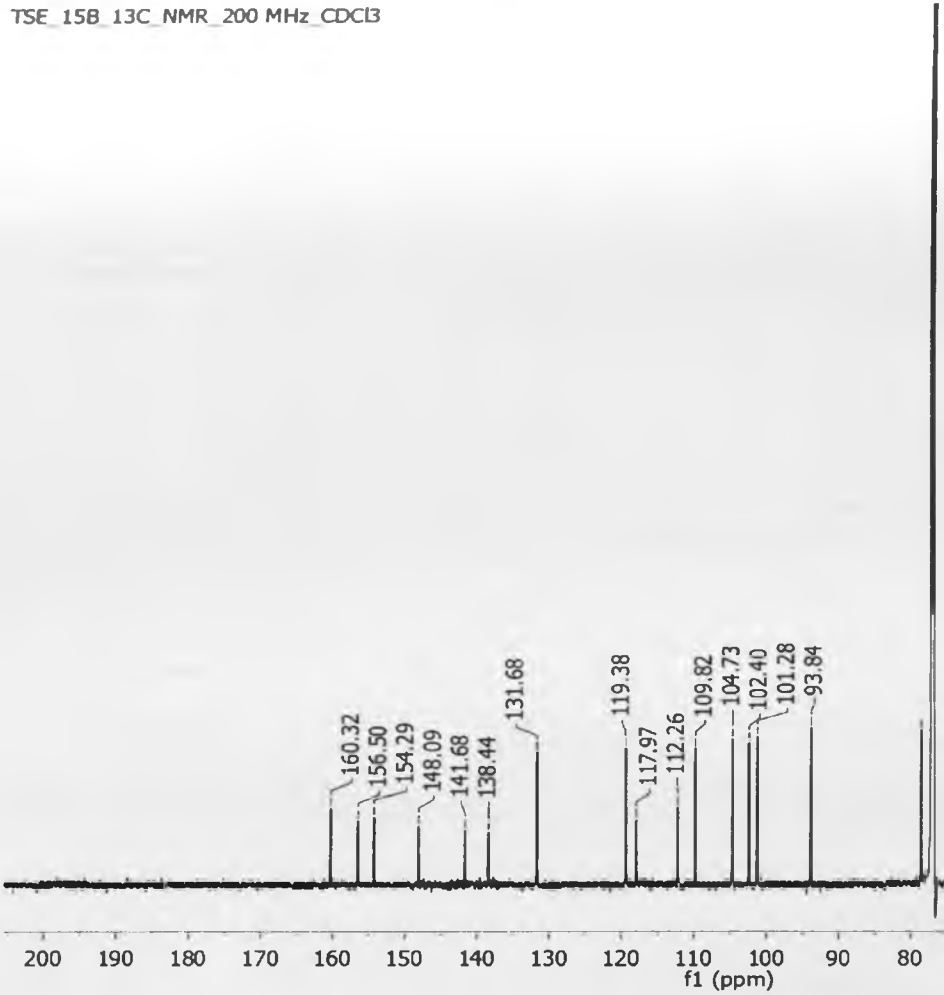
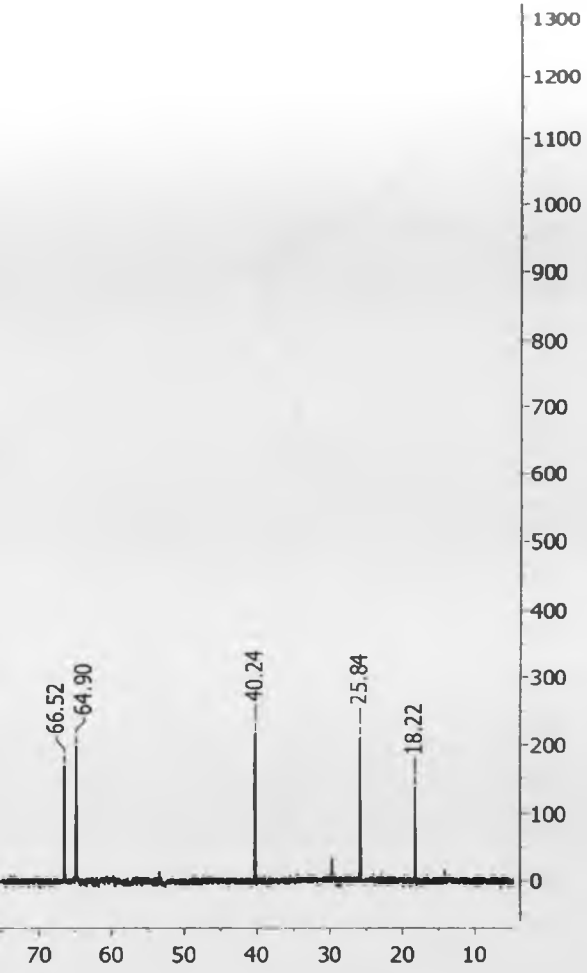
APPENDICES

Appendix1A: ^{1}H NMR (788.87 MHz) spectrum of compound 303

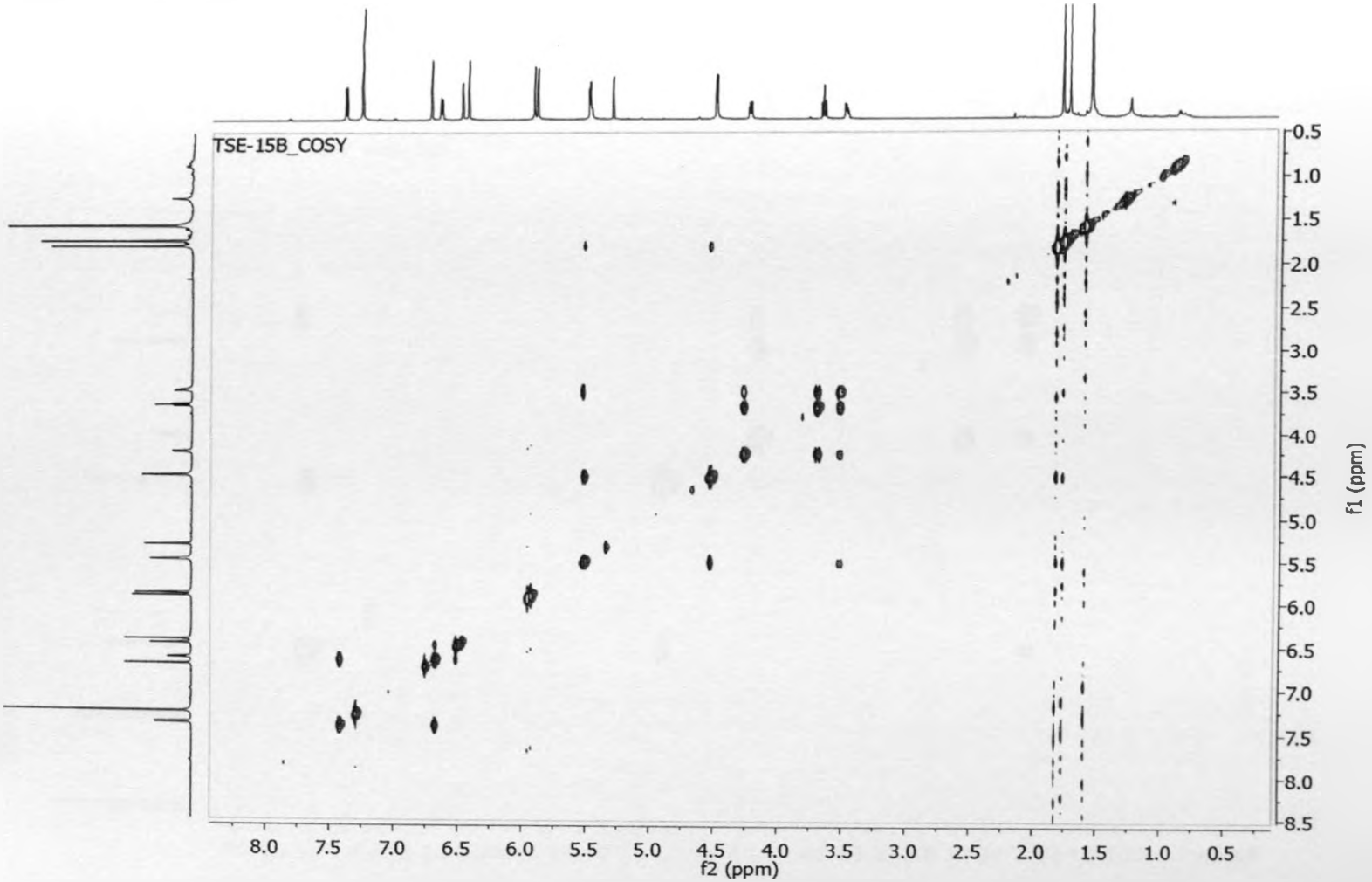


Appendix 1B: ^{13}C NMR (201.15 MHz) spectrum of compound 303

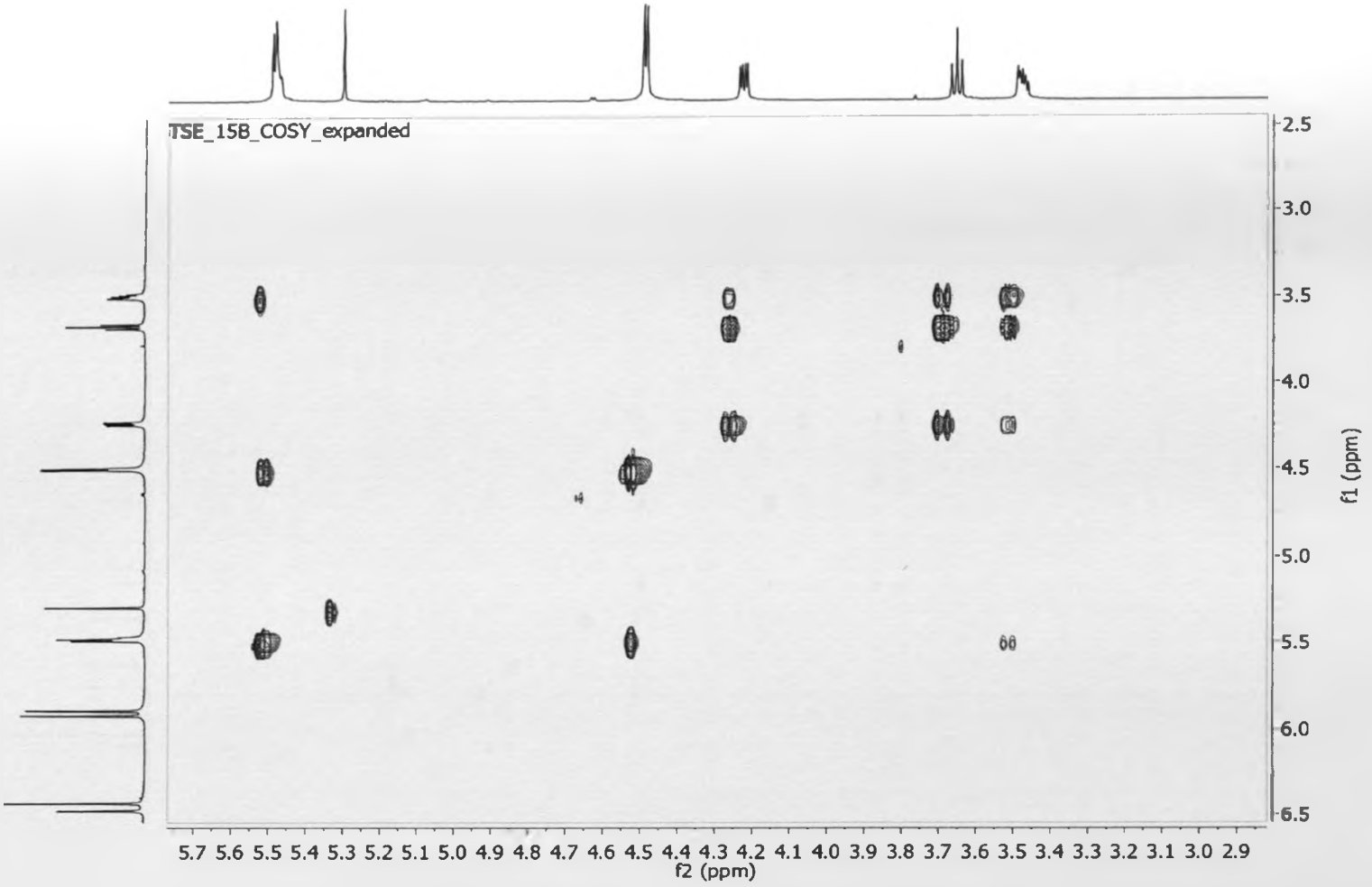



1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
66.52
64.90
40.24
25.84
18.22
70
60
50
40
30
20
10

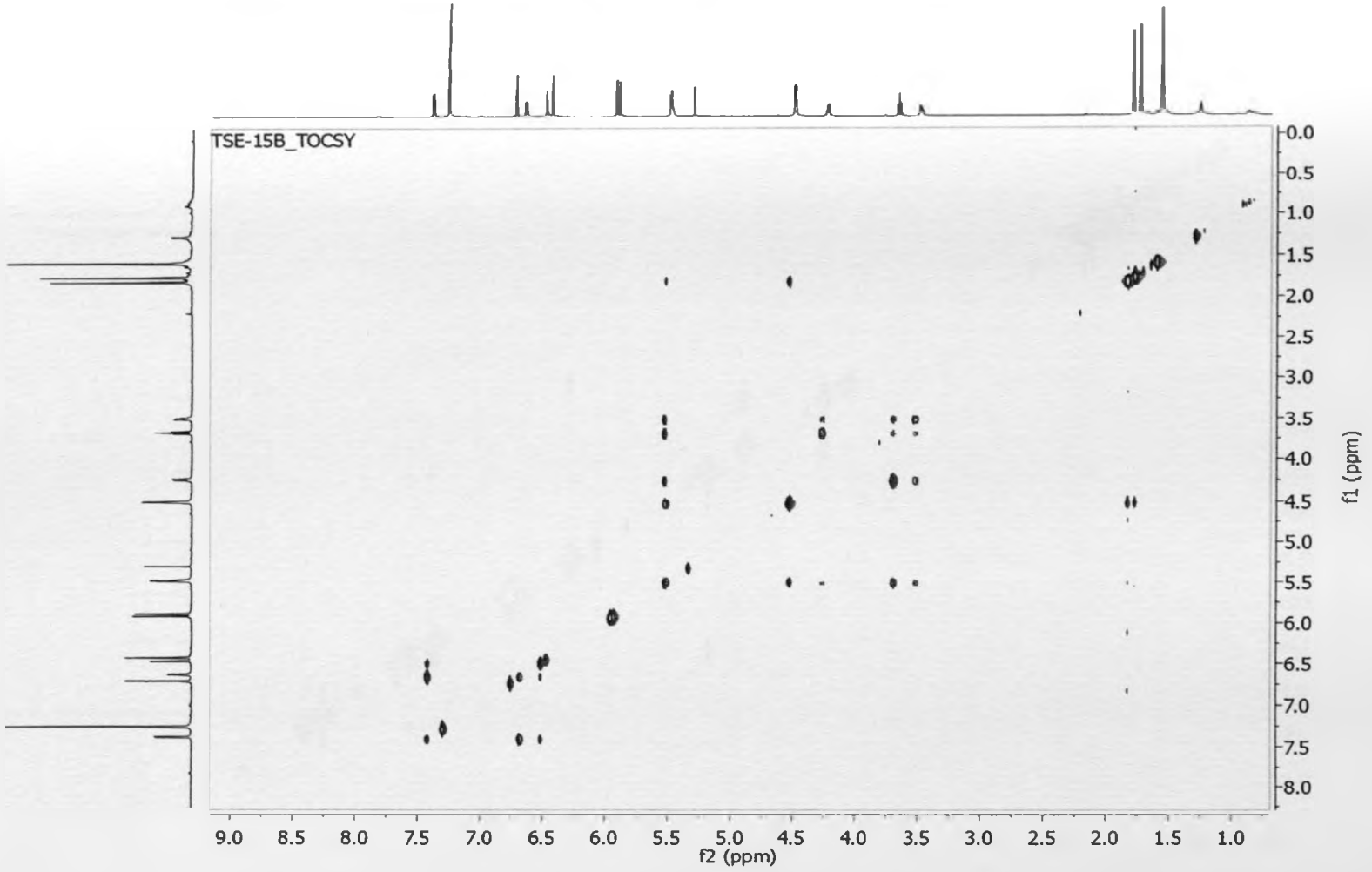
Appendix 1C: COSY (788.87 MHz) spectrum of compound 303



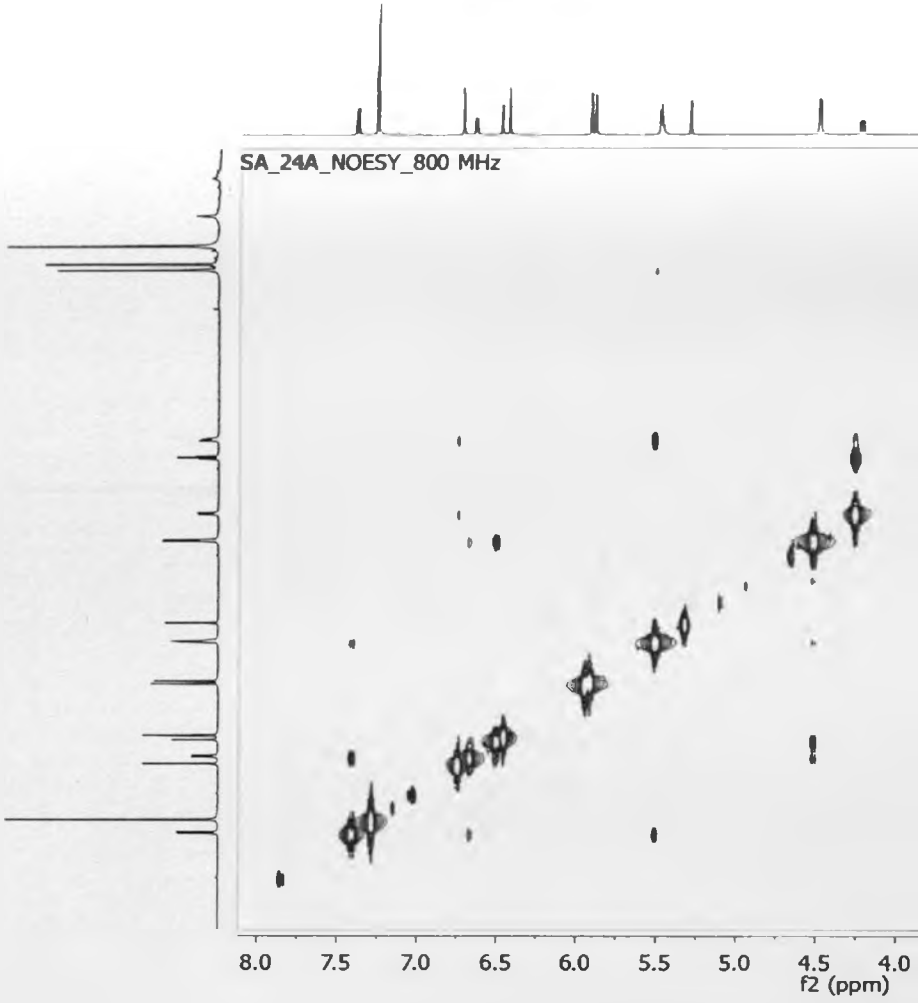
Appendix 1D: COSY expanded (788.87 MHz) spectrum of compound 303

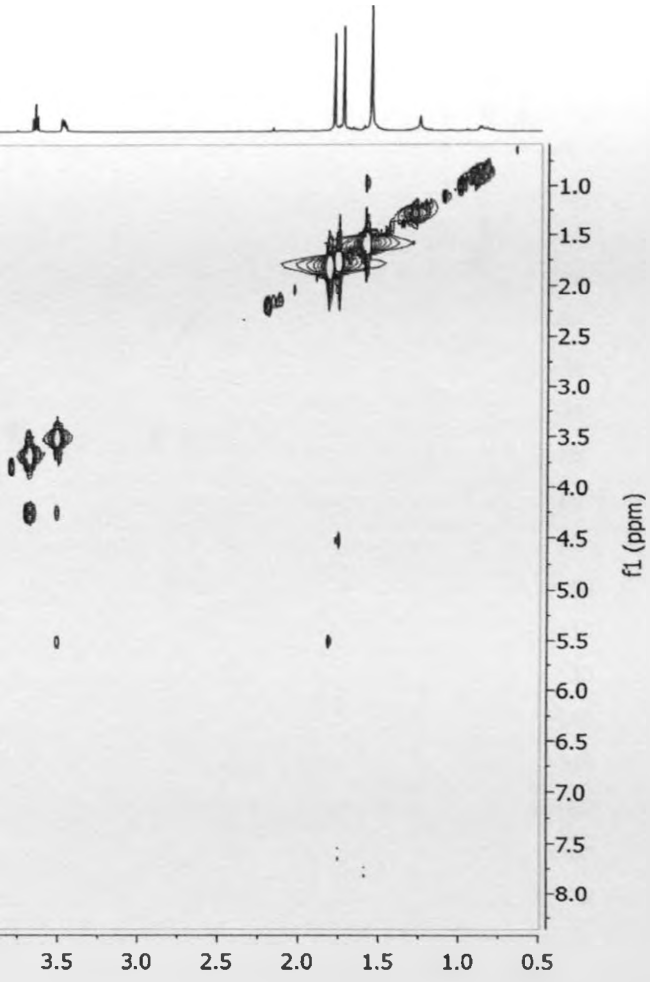


Appendix 1E: TOCSY expanded (788.87 MHz) spectrum of compound 303

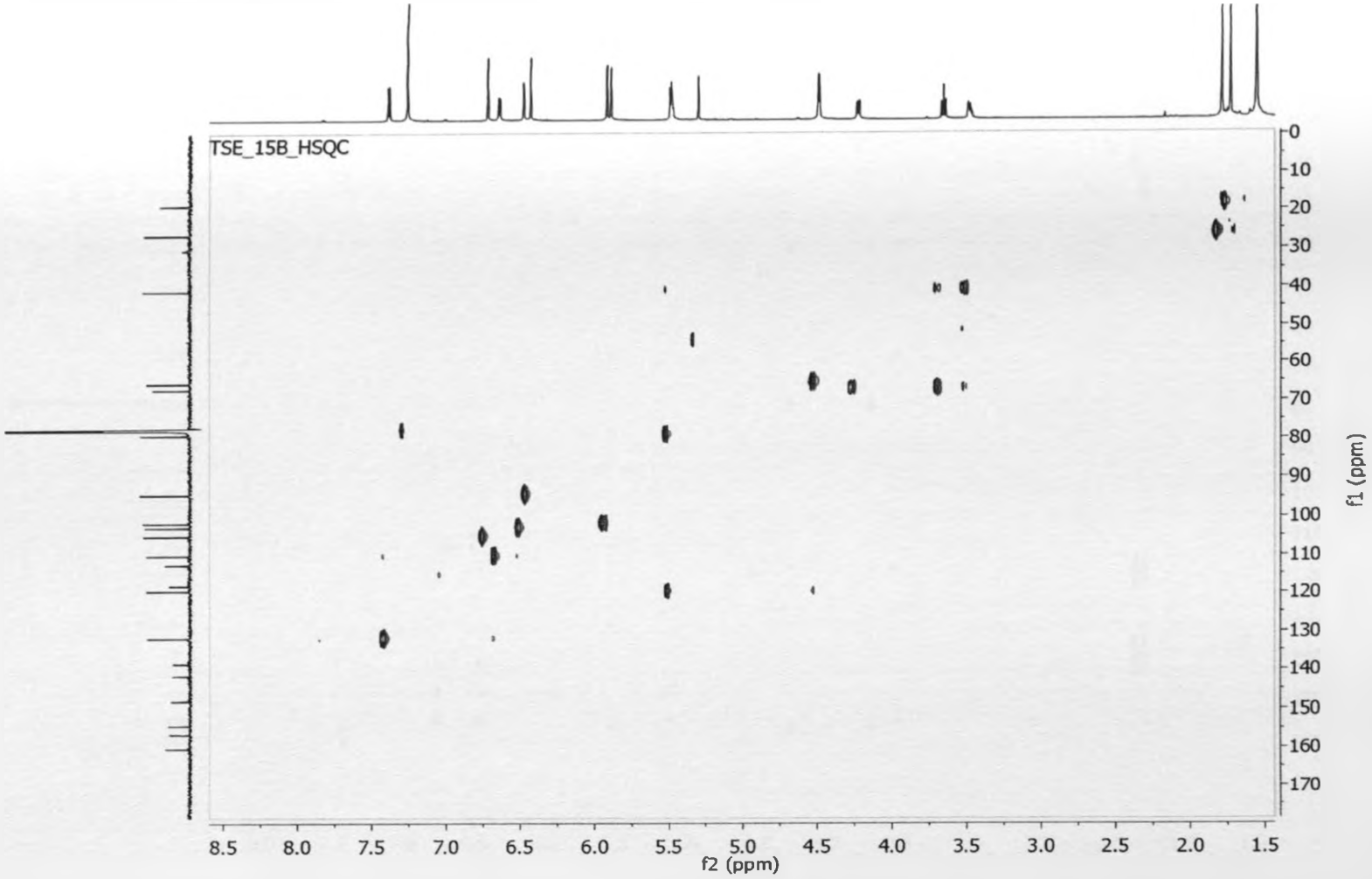


Appendix 1F: NOESY (788.87 MHz) spectrum of compound 303

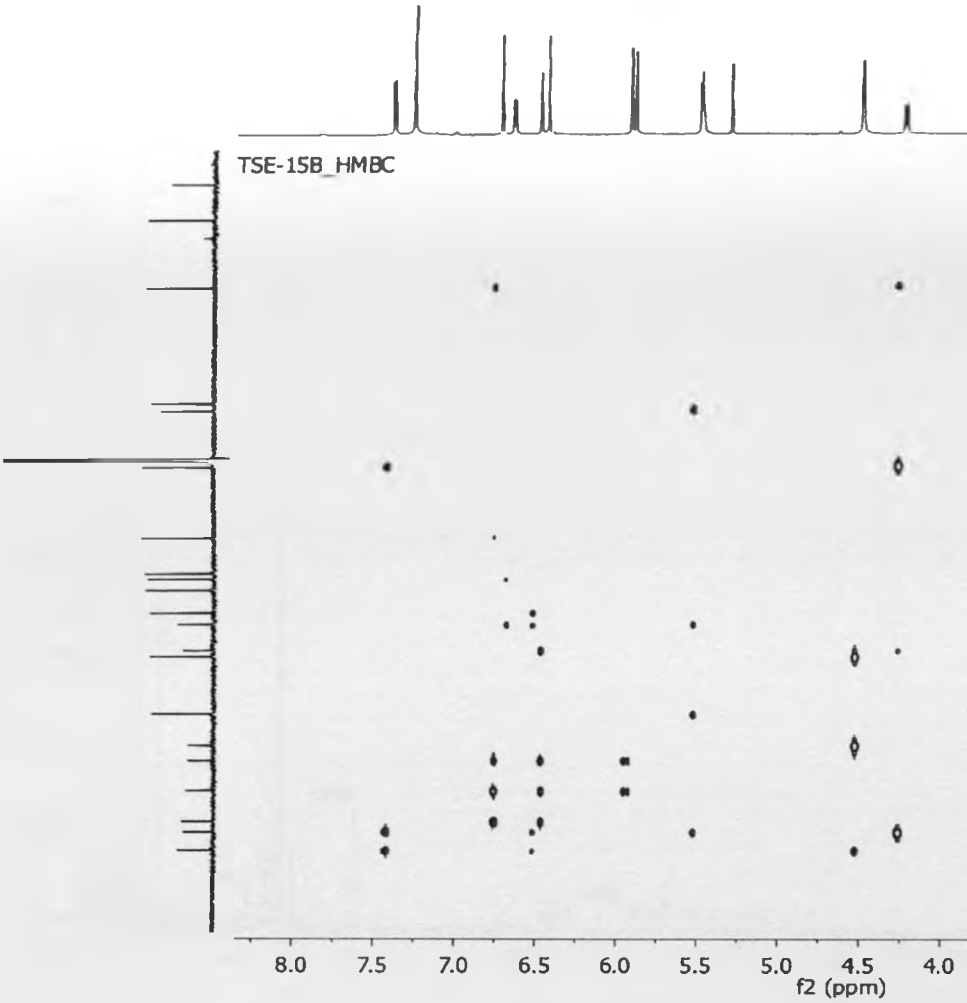


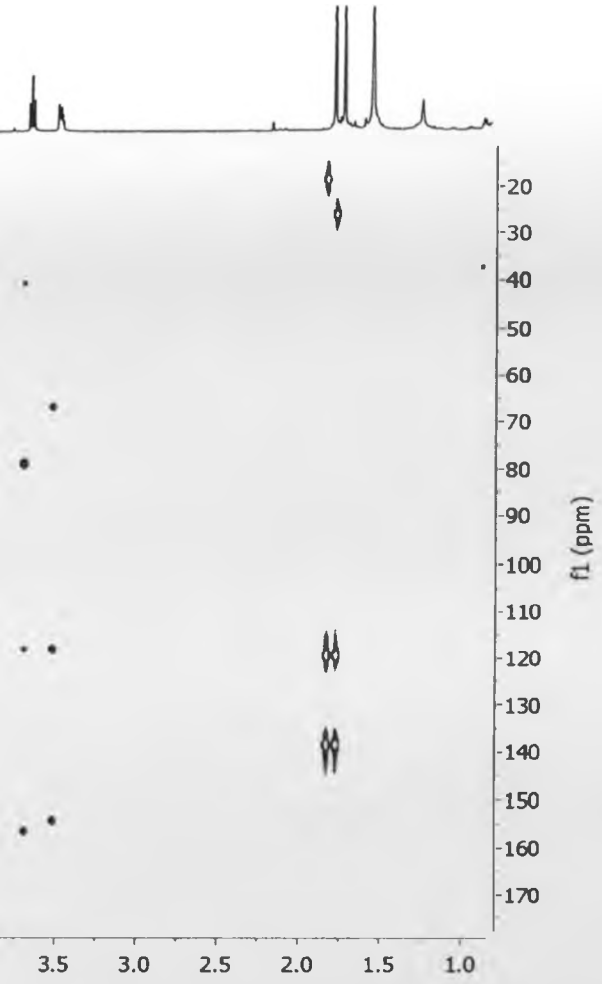

1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
f1 (ppm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5

Appendix 1G: HSQC (788.87/201.15 MHz) spectrum of compound 303

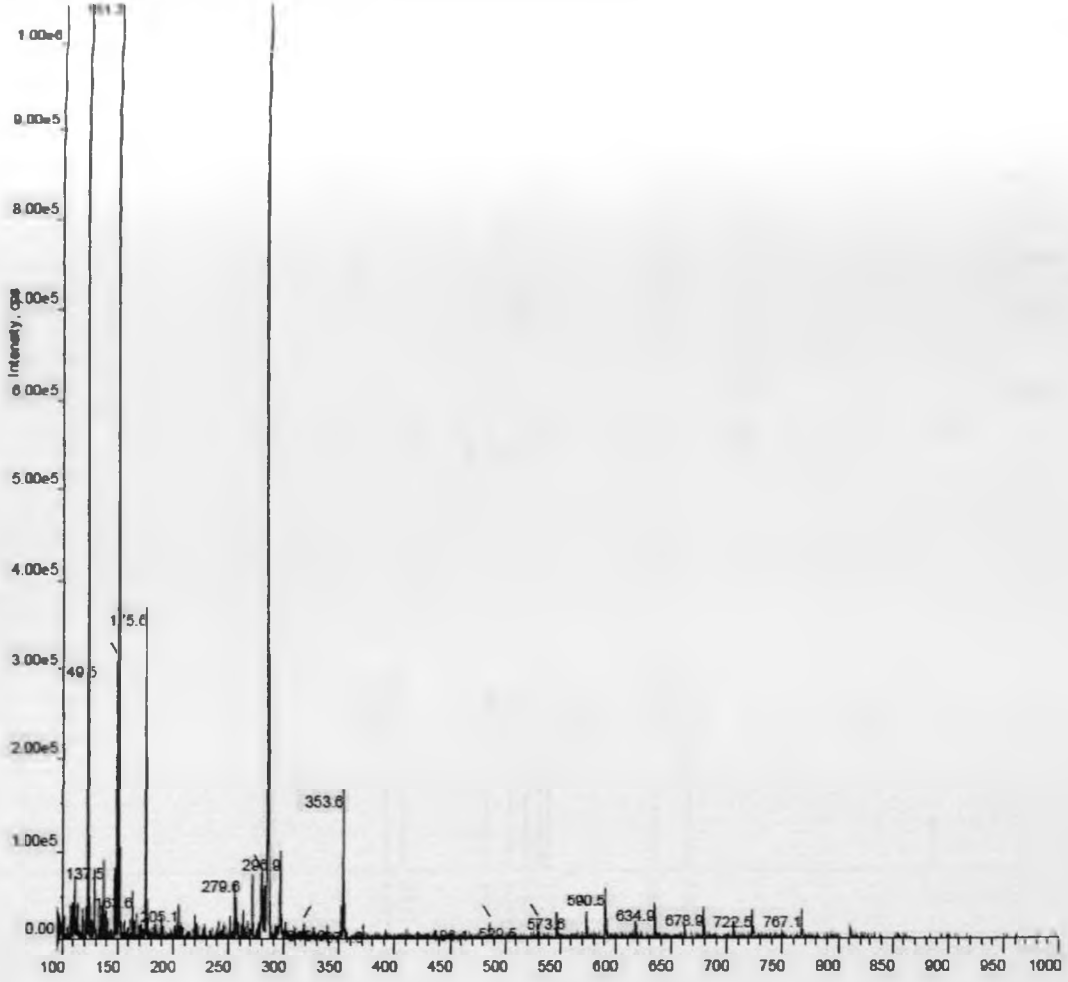


Appendix 1H: HMBC (788.87/201.15 MHz) spectrum of compound 303

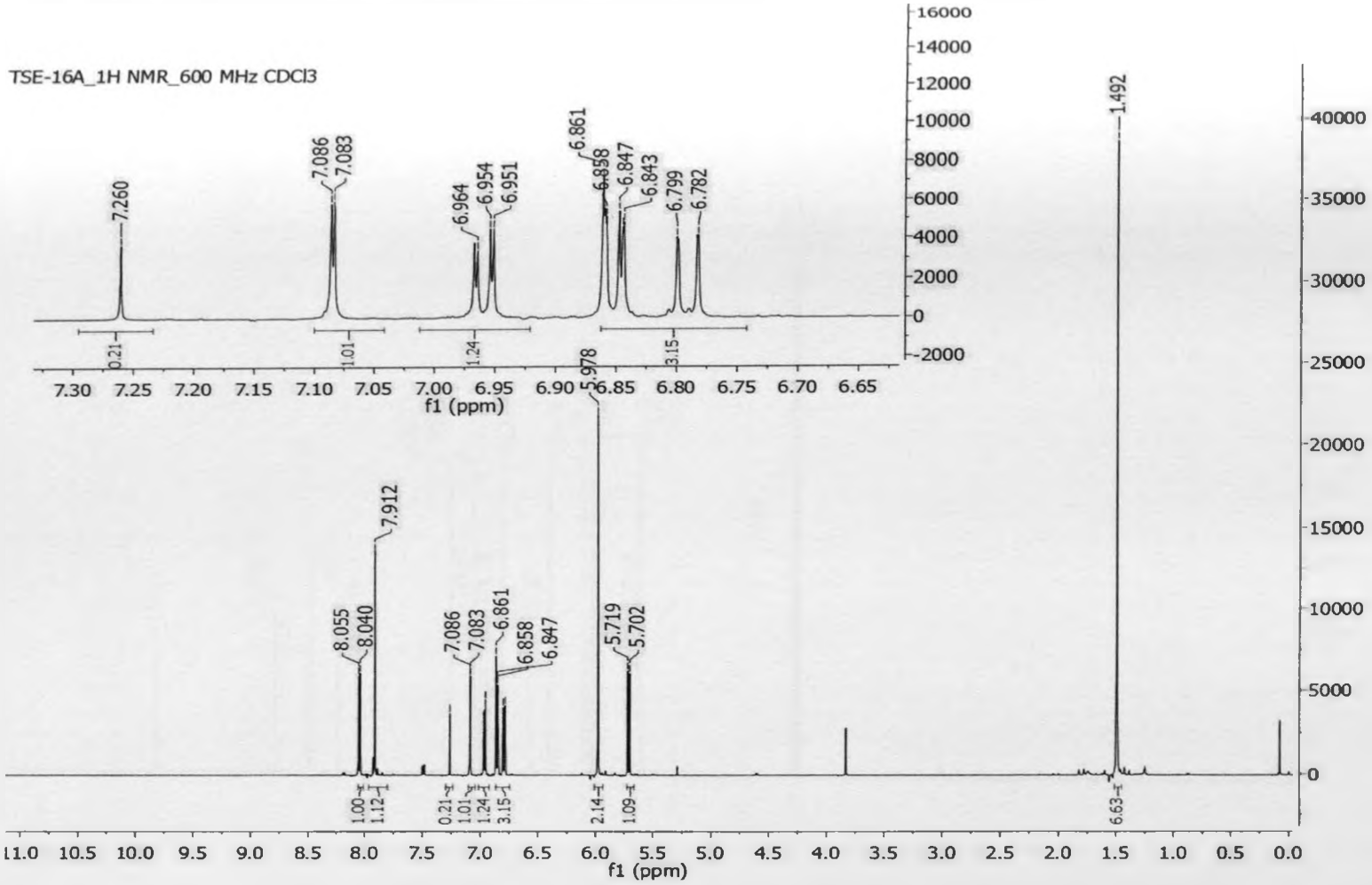



20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)
3.5
3.0
2.5
2.0
1.5
1.0

Appendix 11: ESIMS spectrum of compound 303

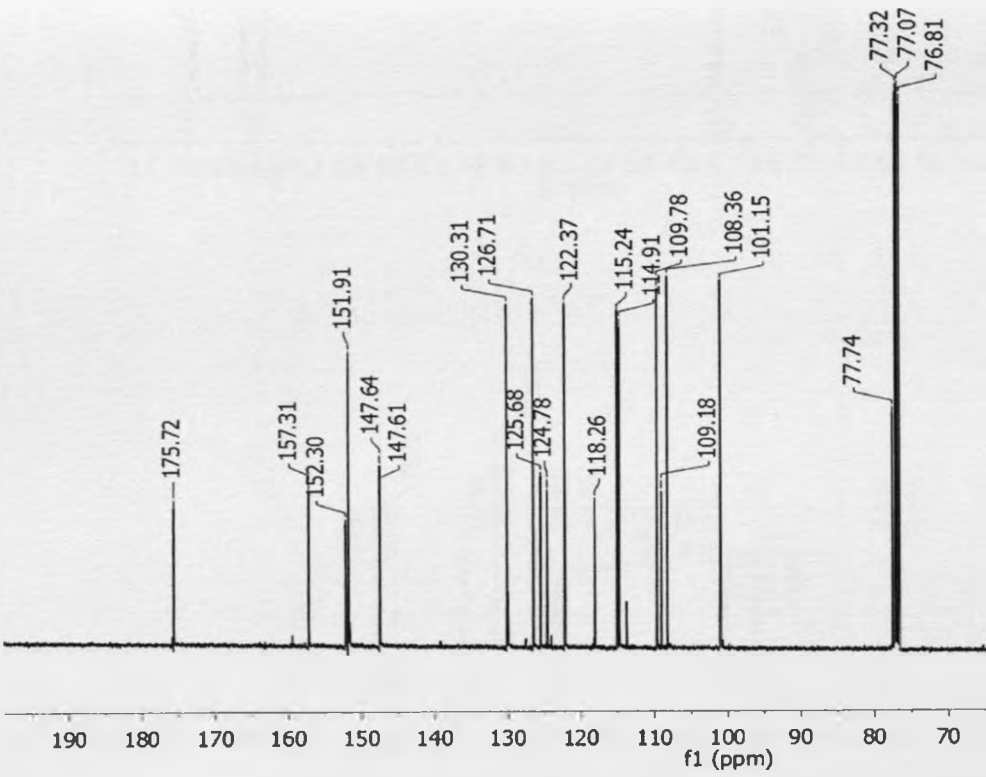


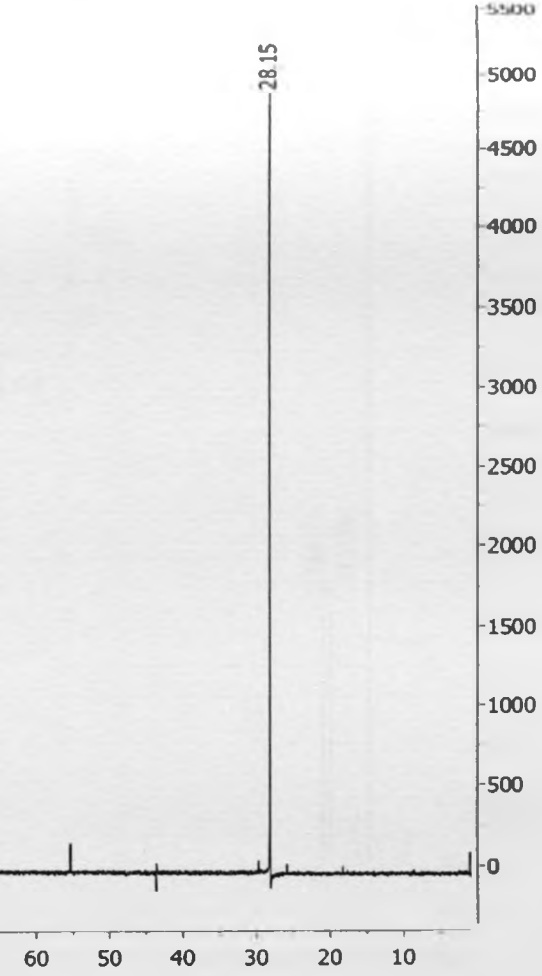
Appendix 2A: ^{1}H NMR (600.24 MHz) spectrum of compound 50



Appendix 2B: ^{13}C ***NMR (150.95 MHz) spectrum of compound 50***

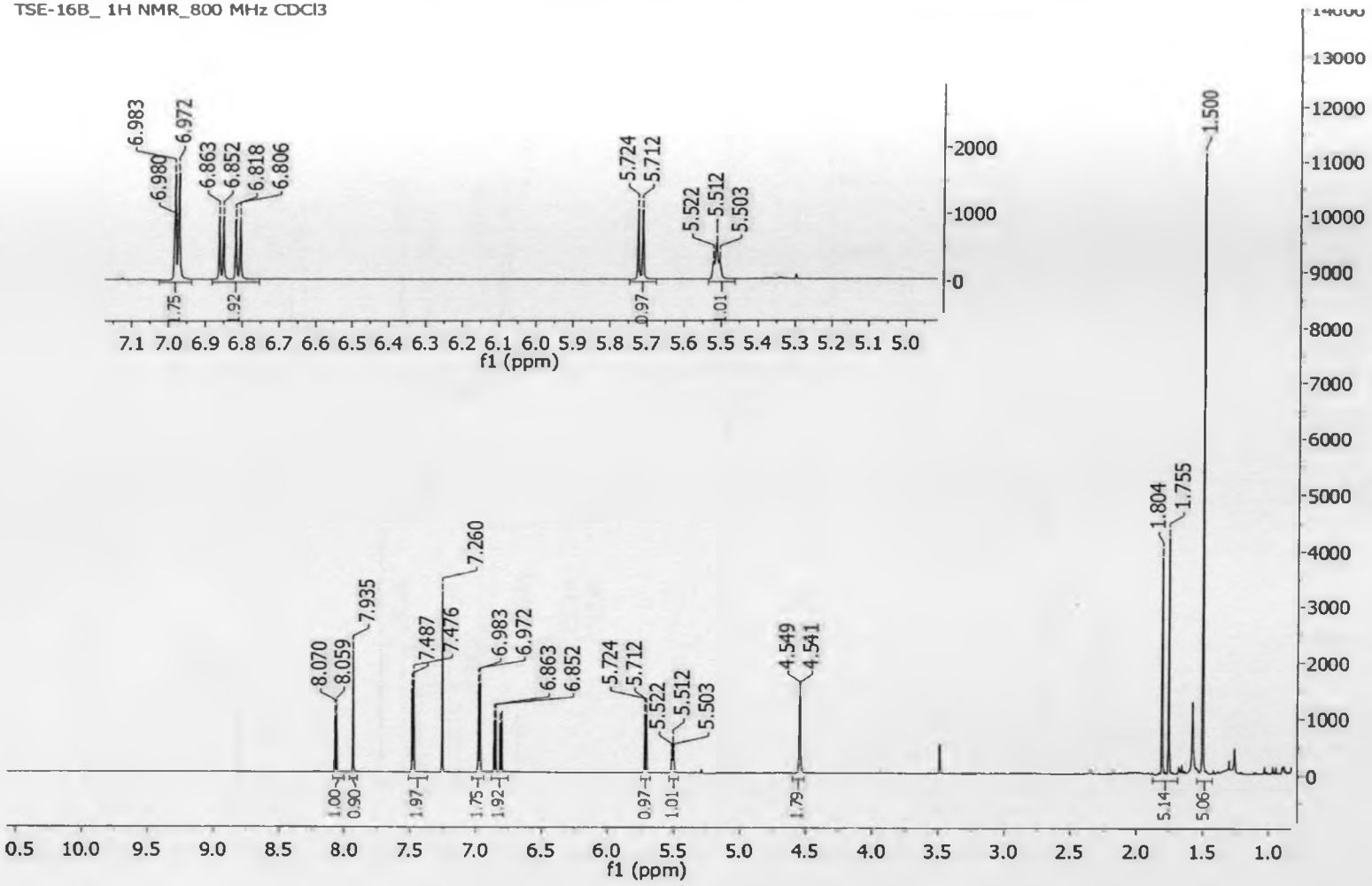
TSE-16A_13C NMR_150 MHZ CDCl3



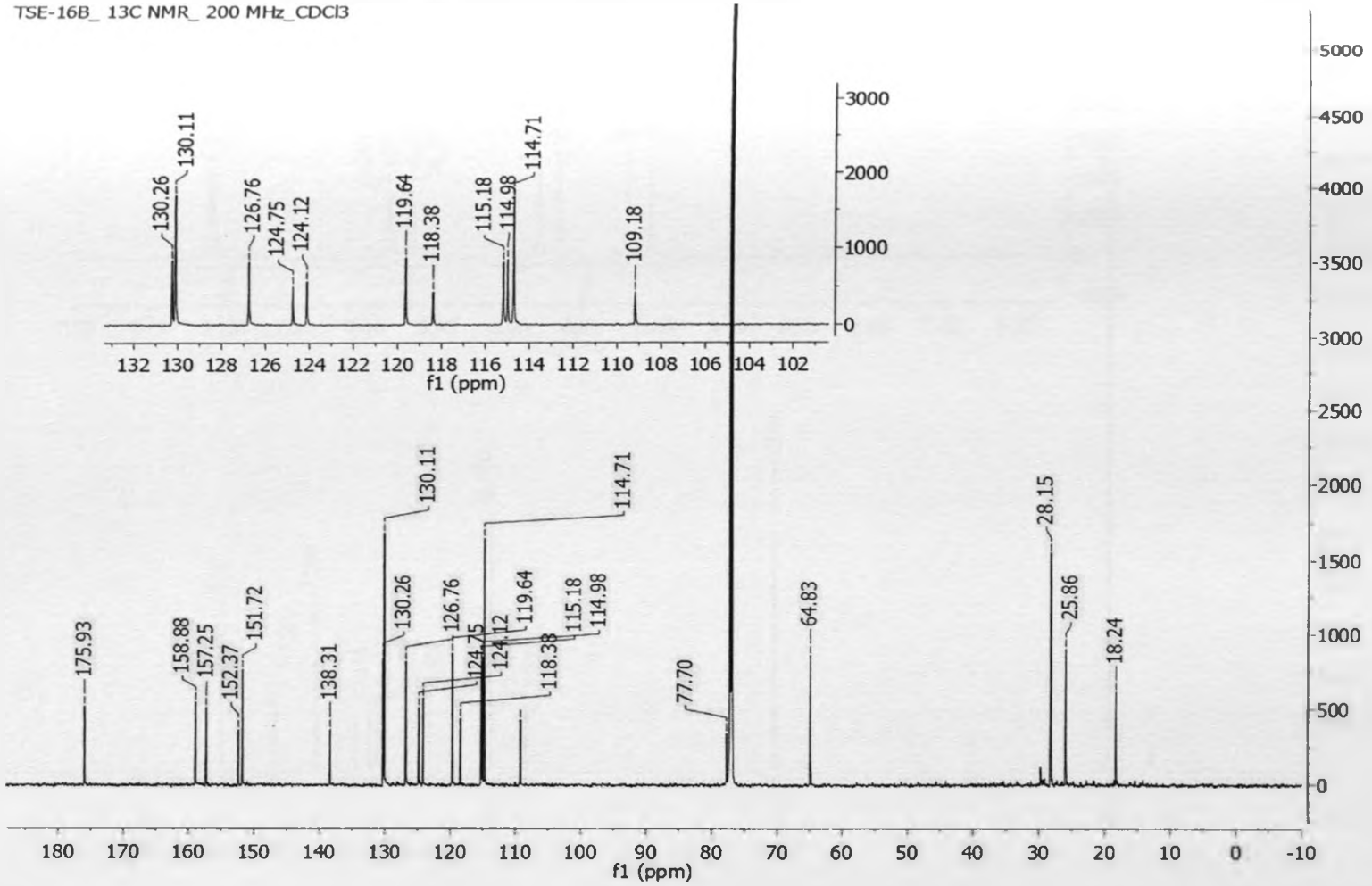

28.15
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
60
50
40
30
20
10

Appendix 3A: ^{1}H NMR (600.24 MHz) spectrum of compound 94

TSE-16B_ 1H NMR_800 MHz CDCl3

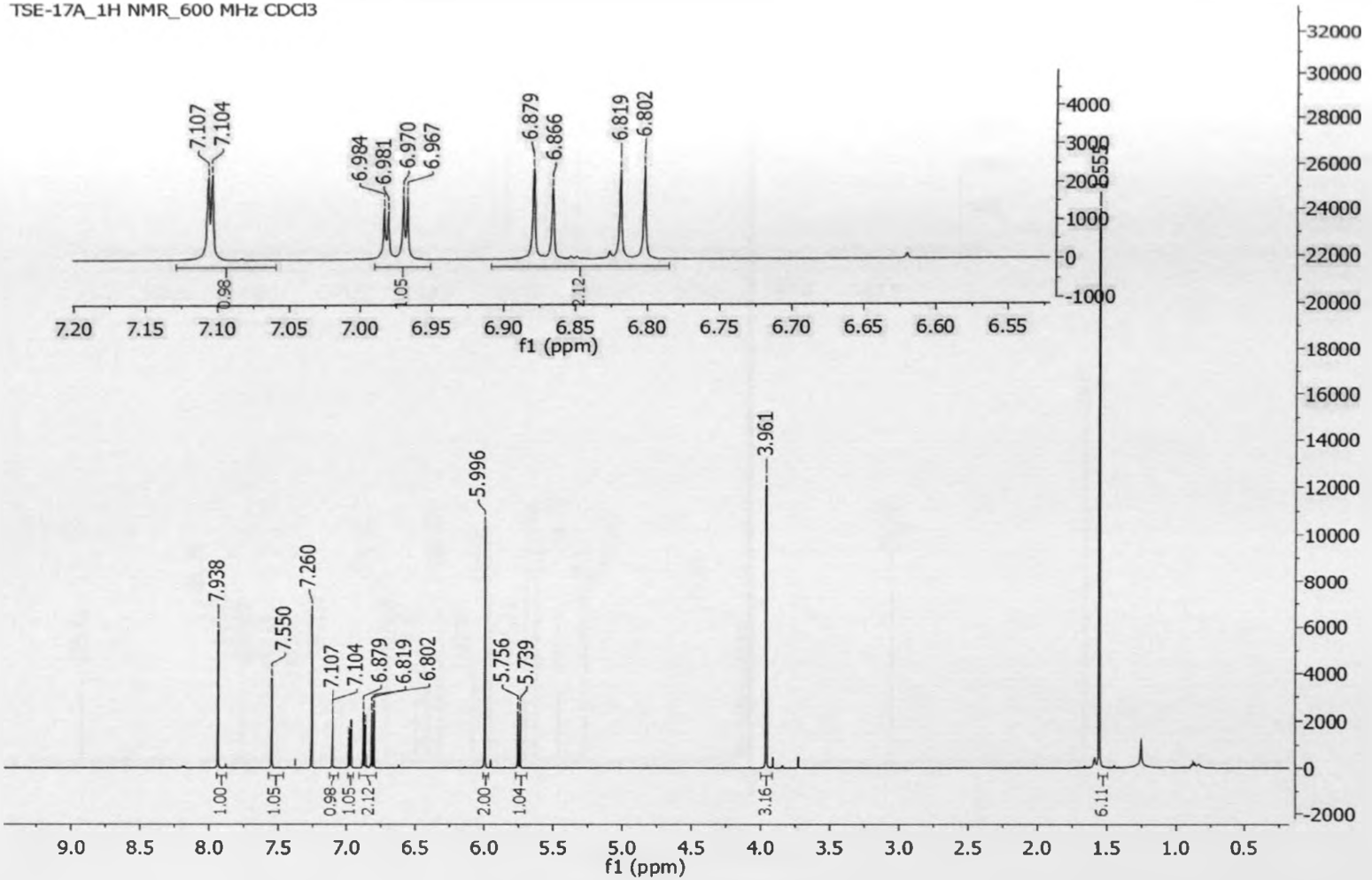


Appendix 3B: ^{13}C NMR (150.95 MHz) spectrum of of compound 94

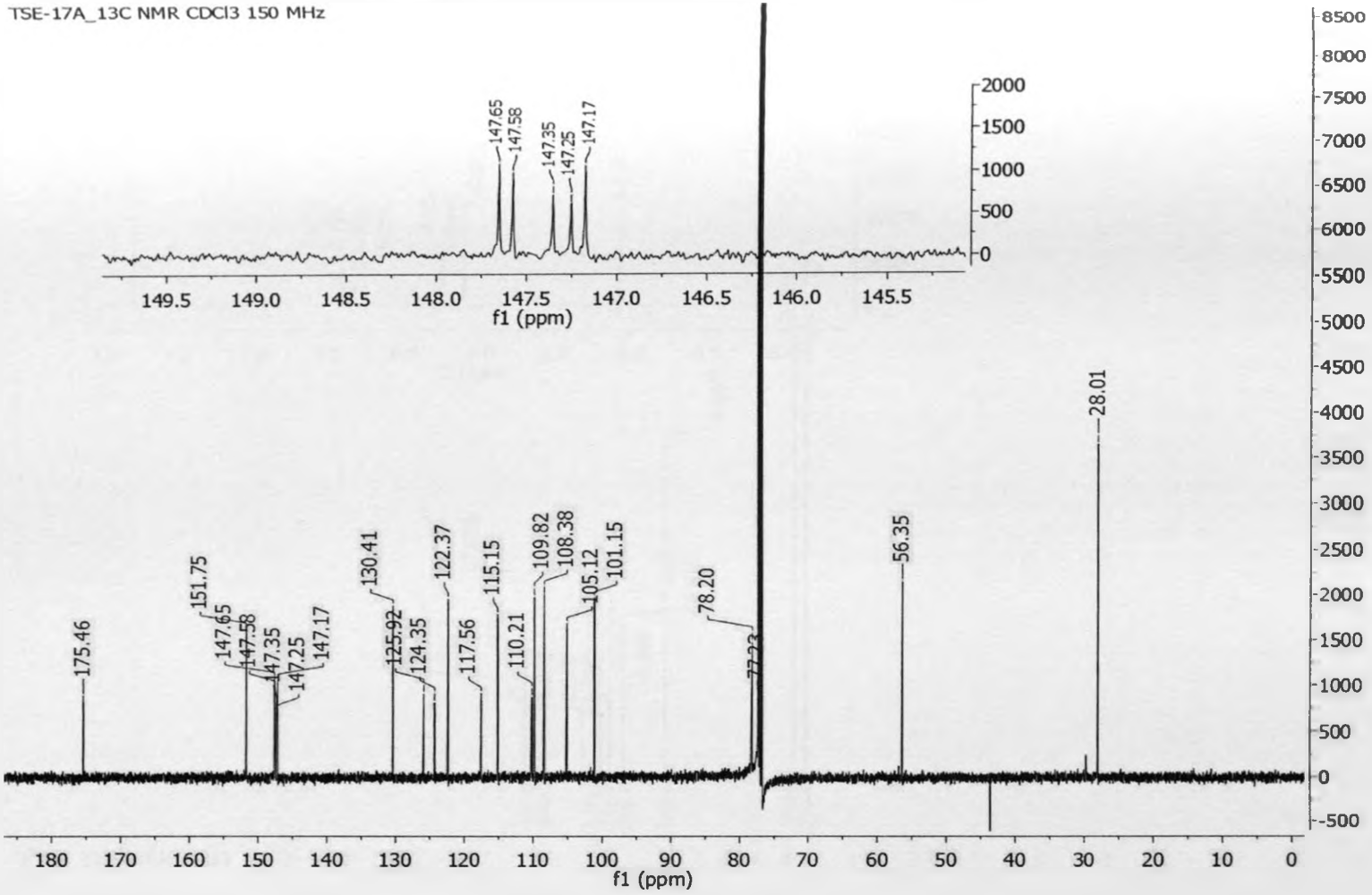


Appendix 4A: ^1H NMR (600.24 MHz) spectrum of of compound 91

TSE-17A_1H NMR_600 MHz CDCl3

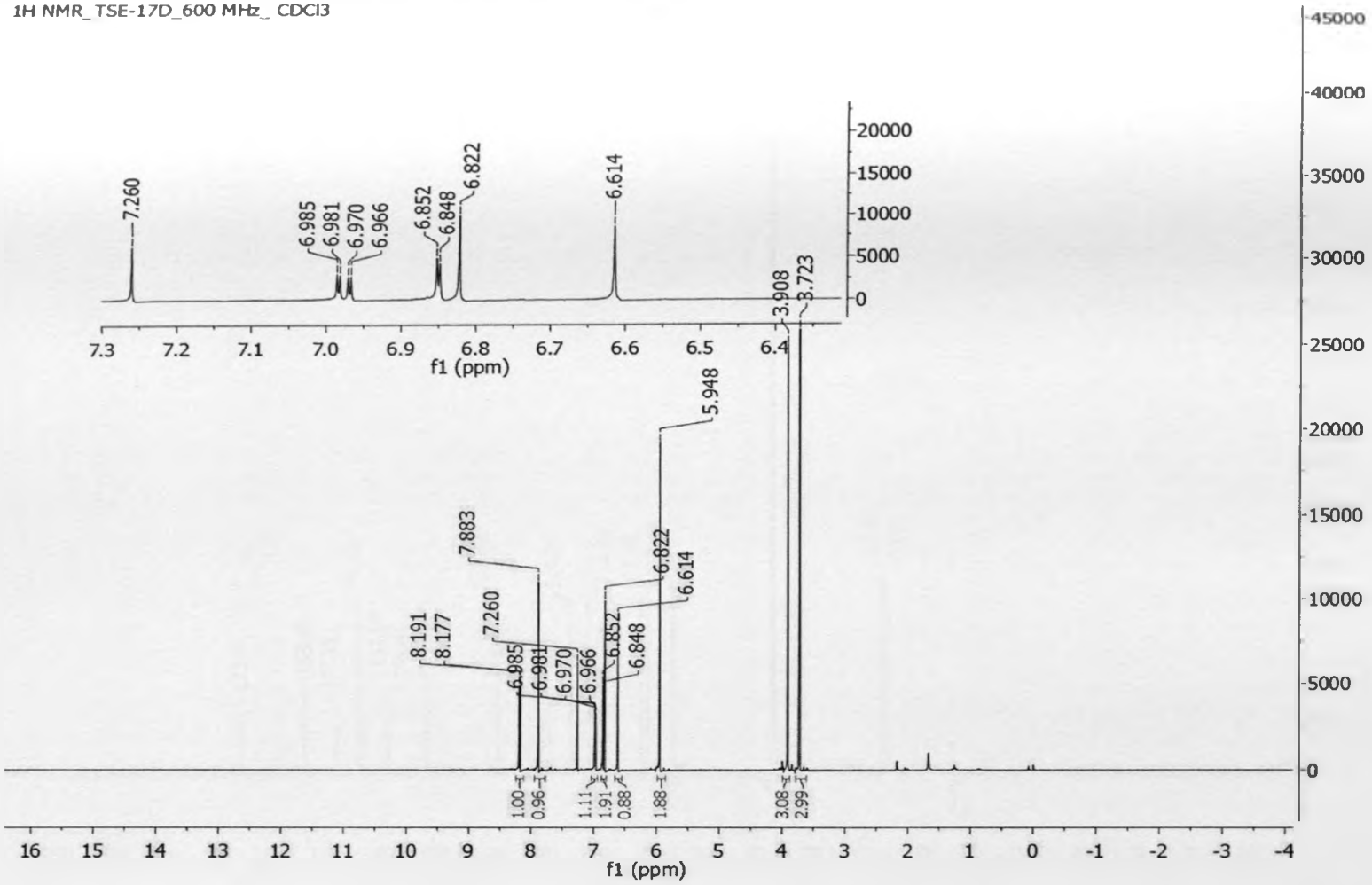


Appendix 4B: ^{13}C NMR (150.95 MHz) spectrum of compound 91



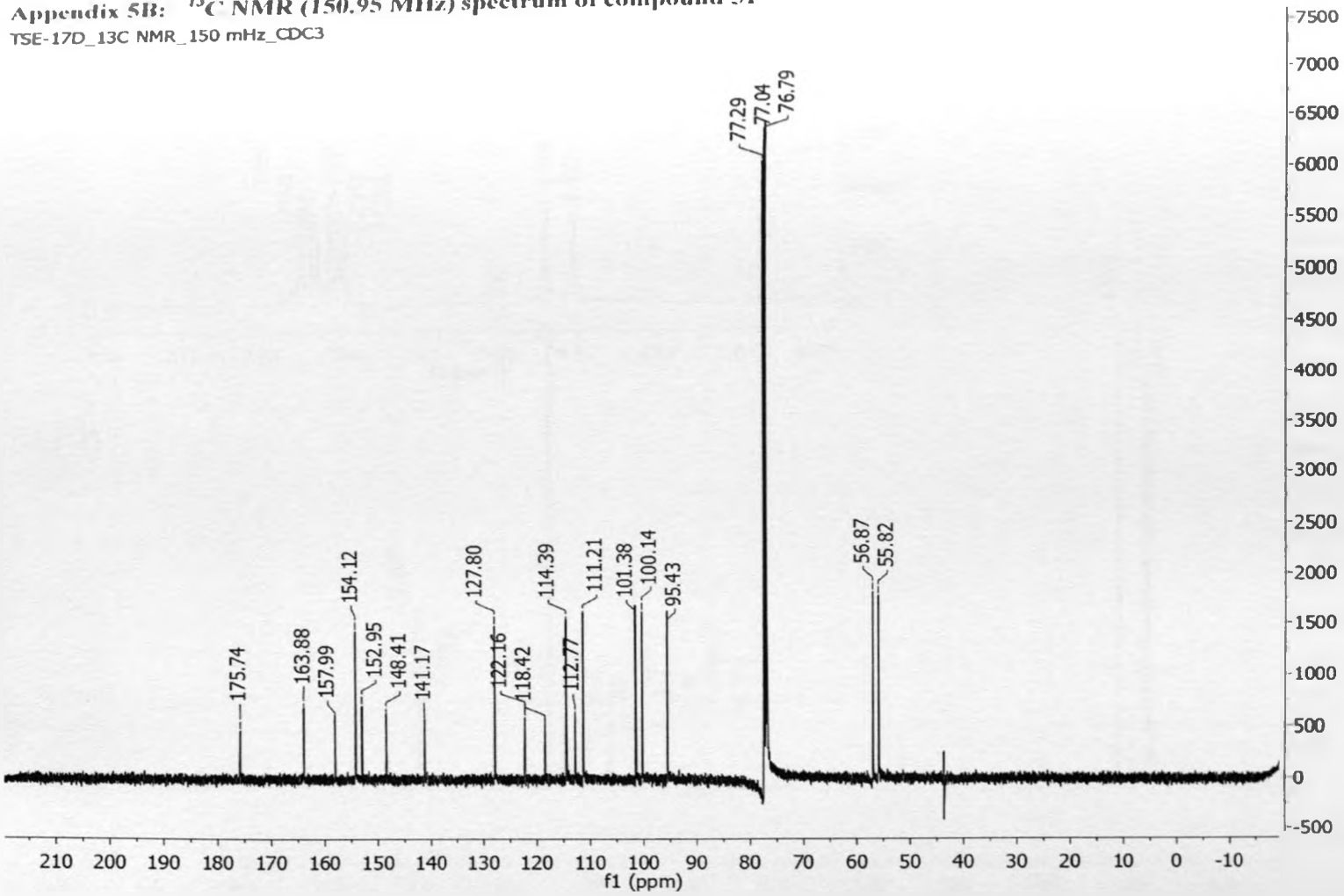
Appendix 5A: ^{1}H NMR (600.24 MHz) spectrum of compound 51

1H NMR_TSE-17D_600 MHz_ CDCl3

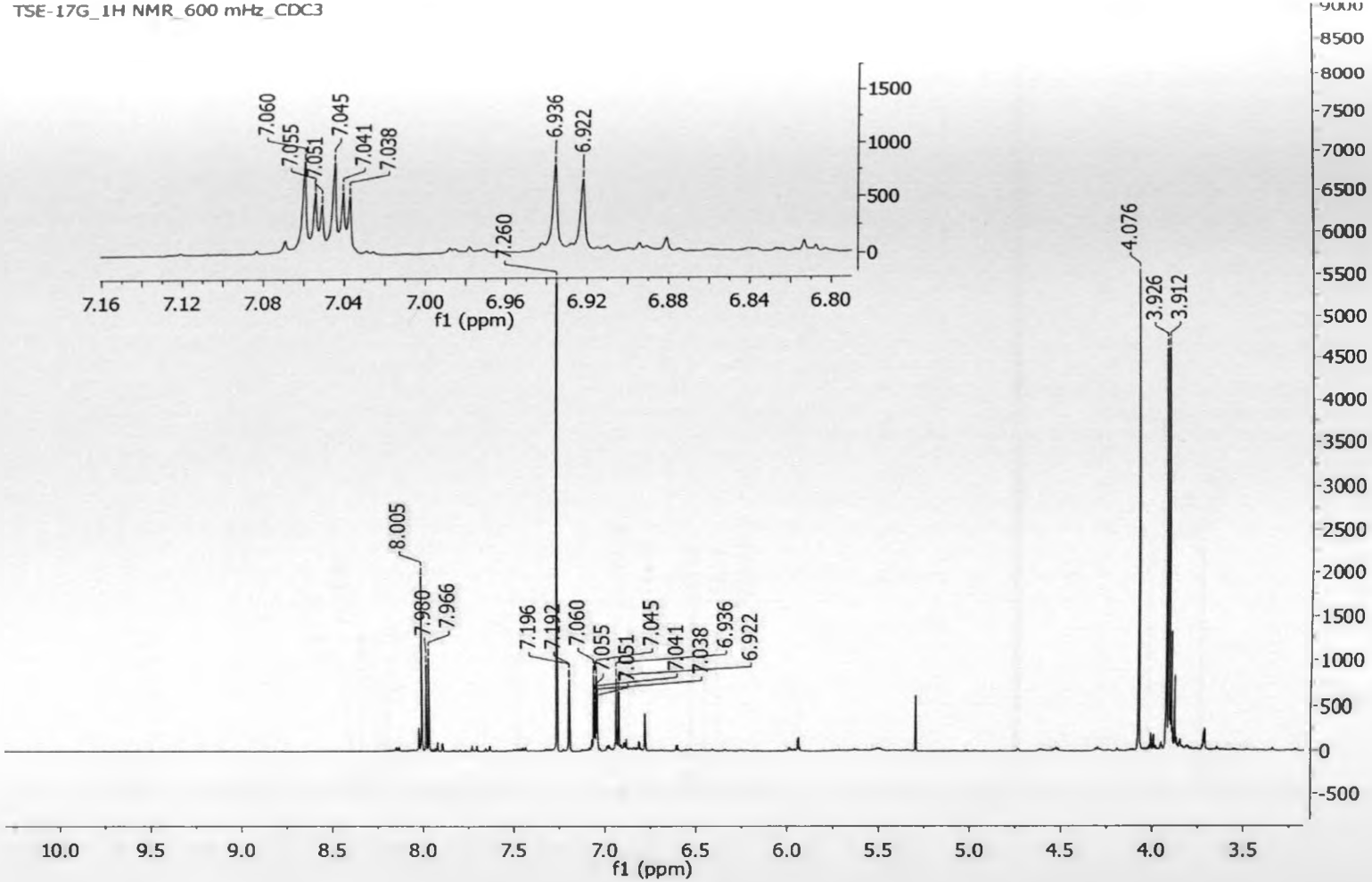


Appendix 5B: ^{13}C **NMR (150.95 MHz) spectrum of compound 51**

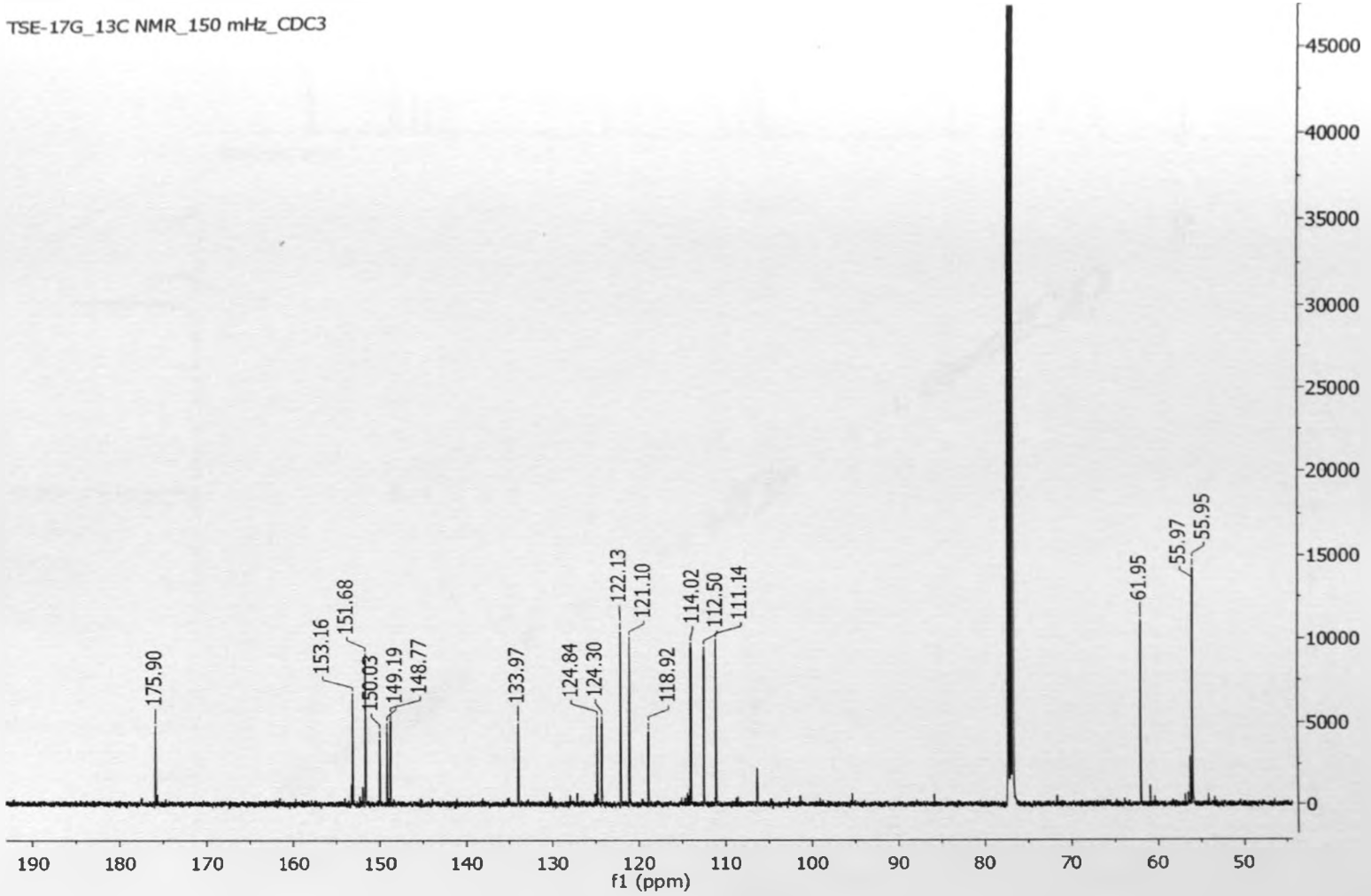
TSE-17D_13C NMR_150 mHz_CDC3



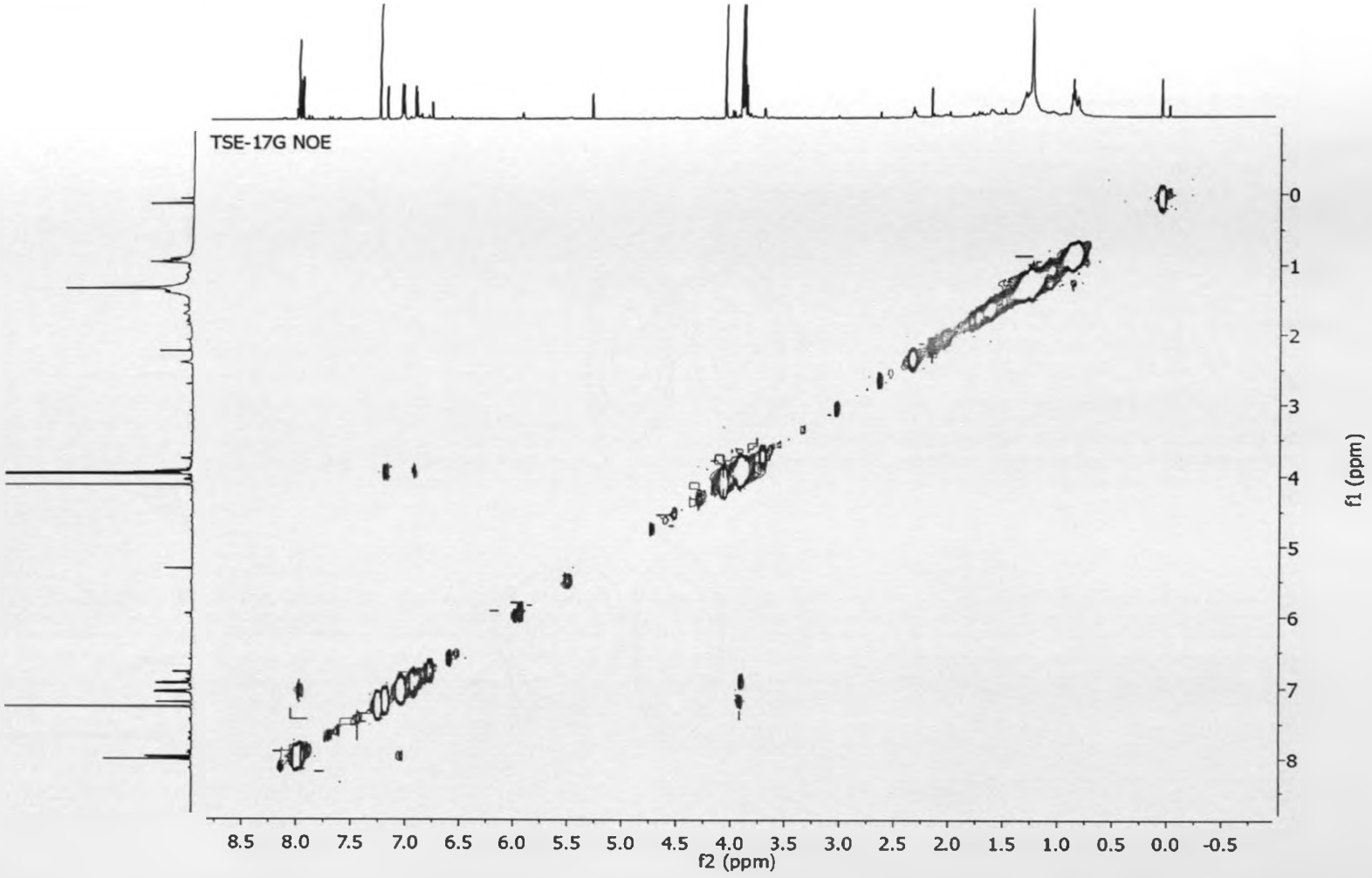
Appendix 6A: ^{1}H NMR (600.24 MHz) spectrum of compound 305



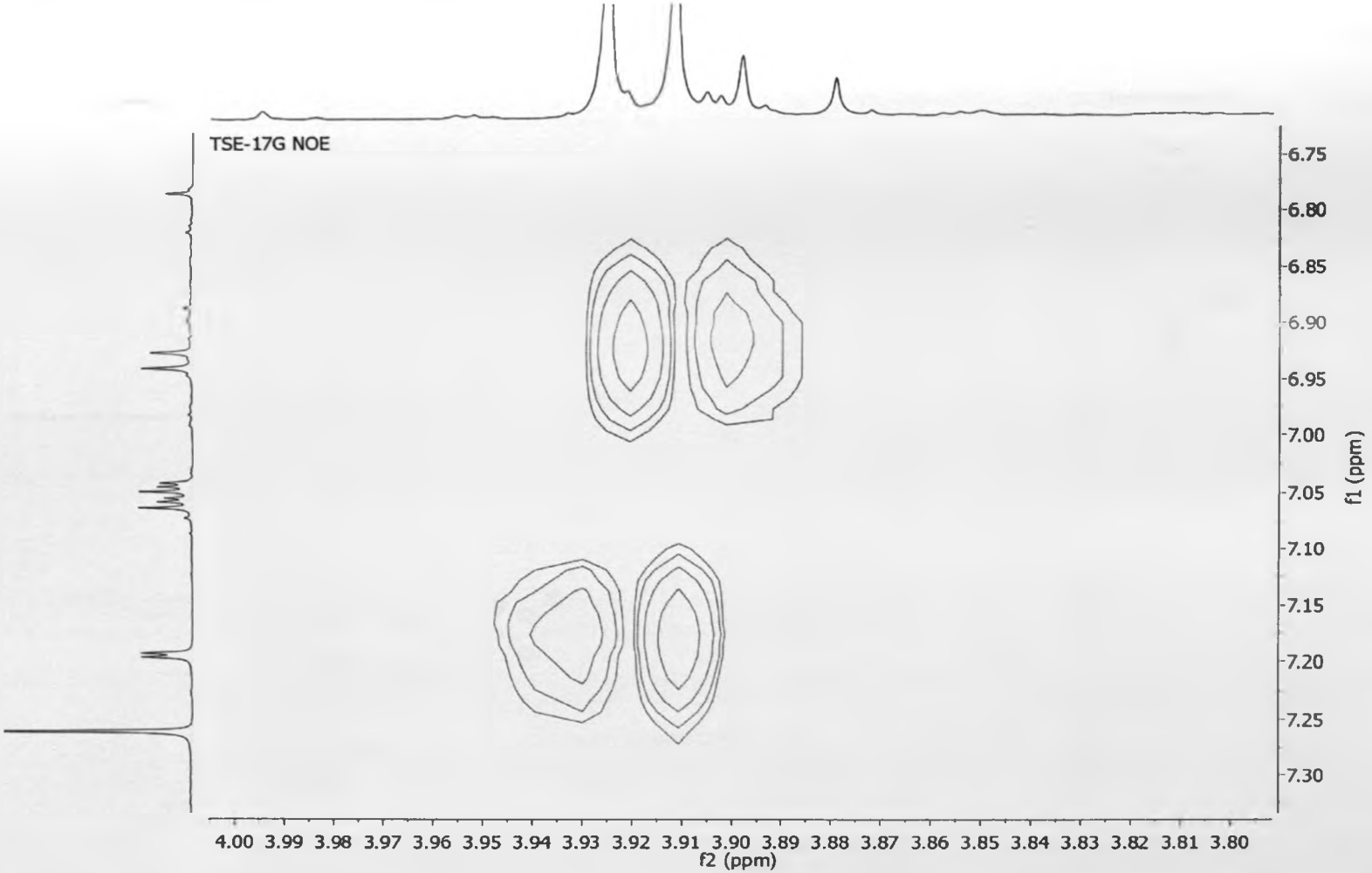
Appendix 6B: ^{13}C **NMR (150.95 MHz) spectrum of compound 305**



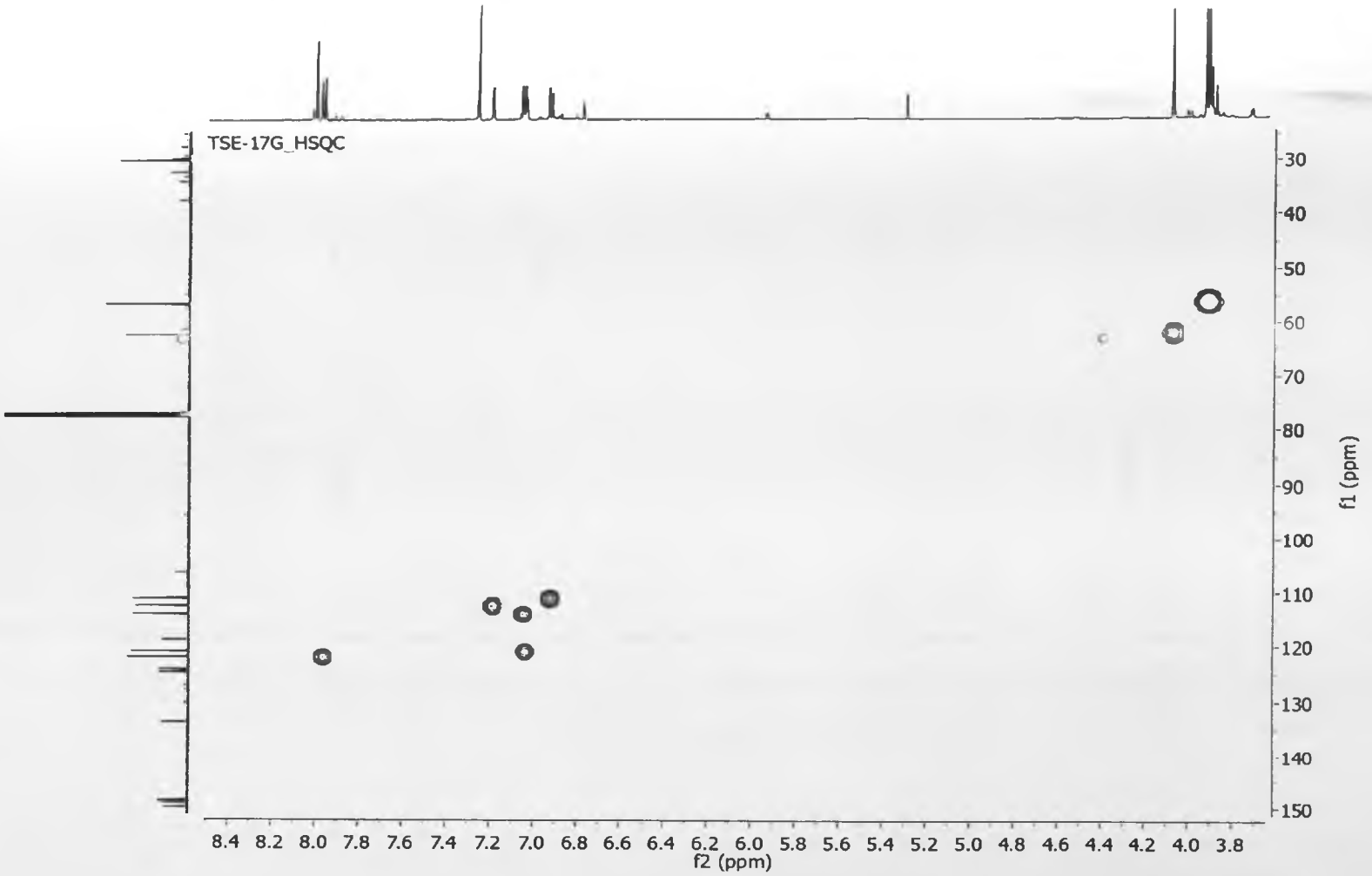
Appendix 6C: ^{1}H *NMR (600.24 MHz) spectrum of compound 305*



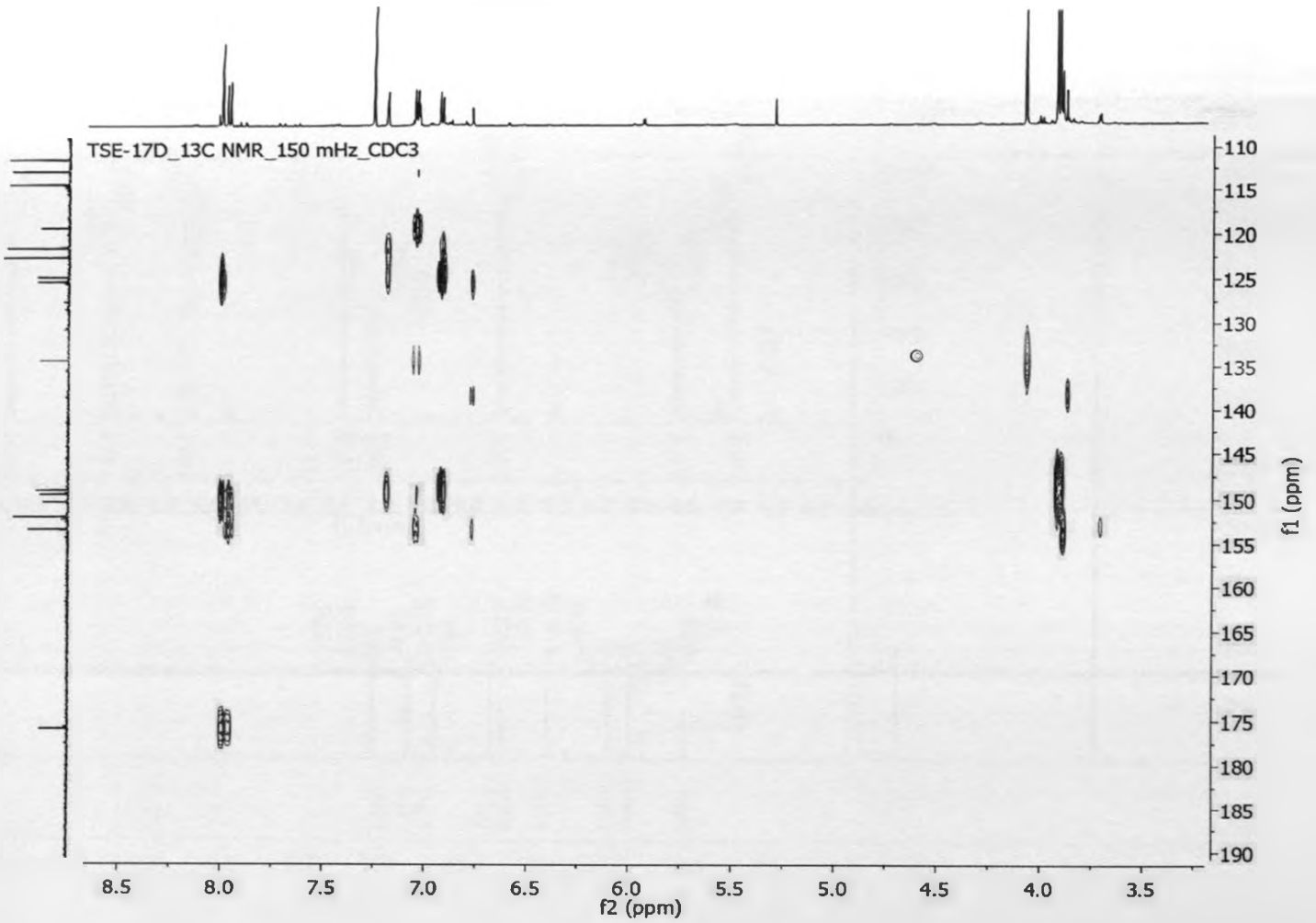
Appendix 6D: ^{1}H *NMR (600.24 MHz) spectrum of compound 305*



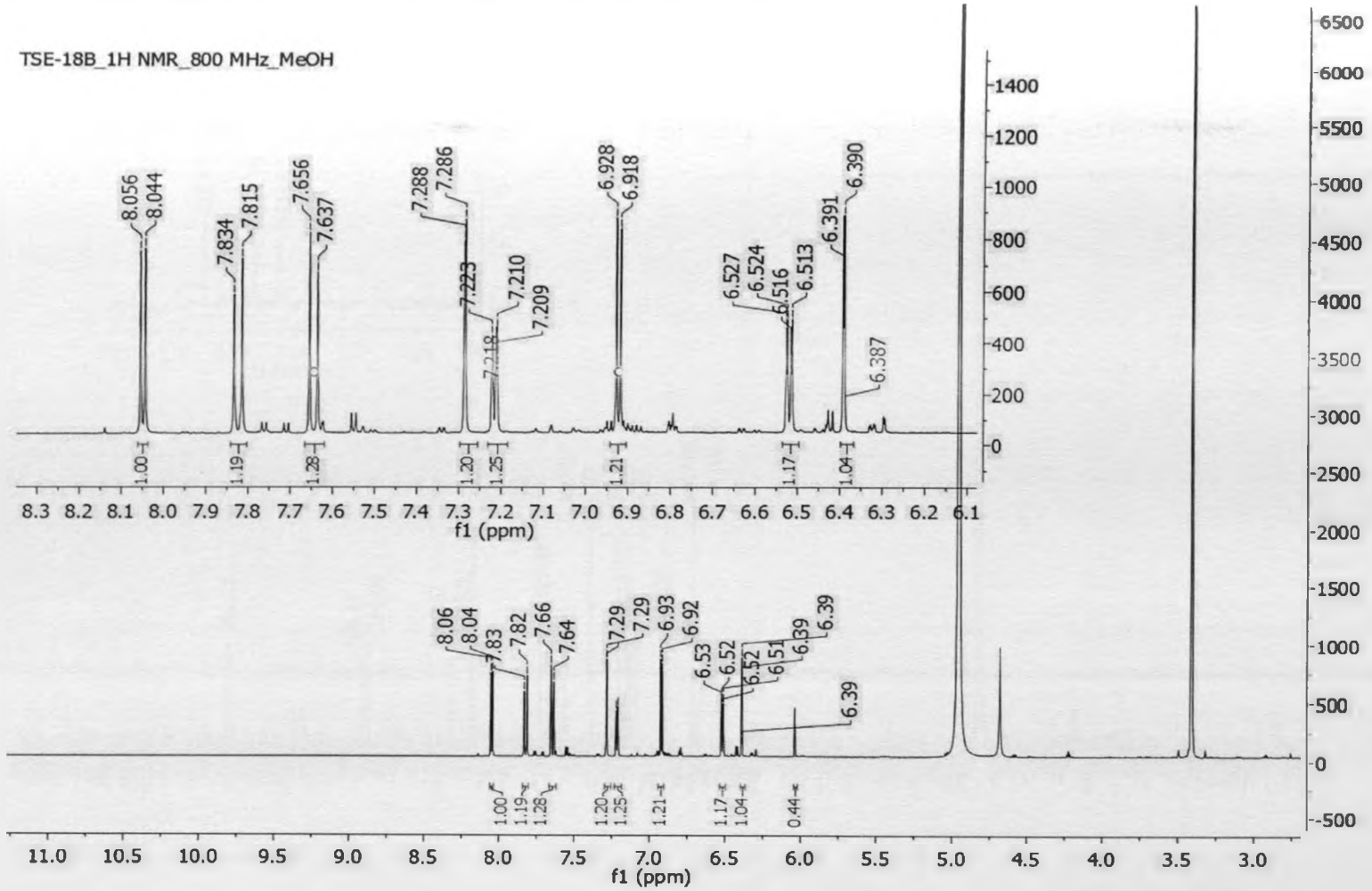
Appendix 6E: HSQC (600.24/150.95 MHz) spectrum of compound 305



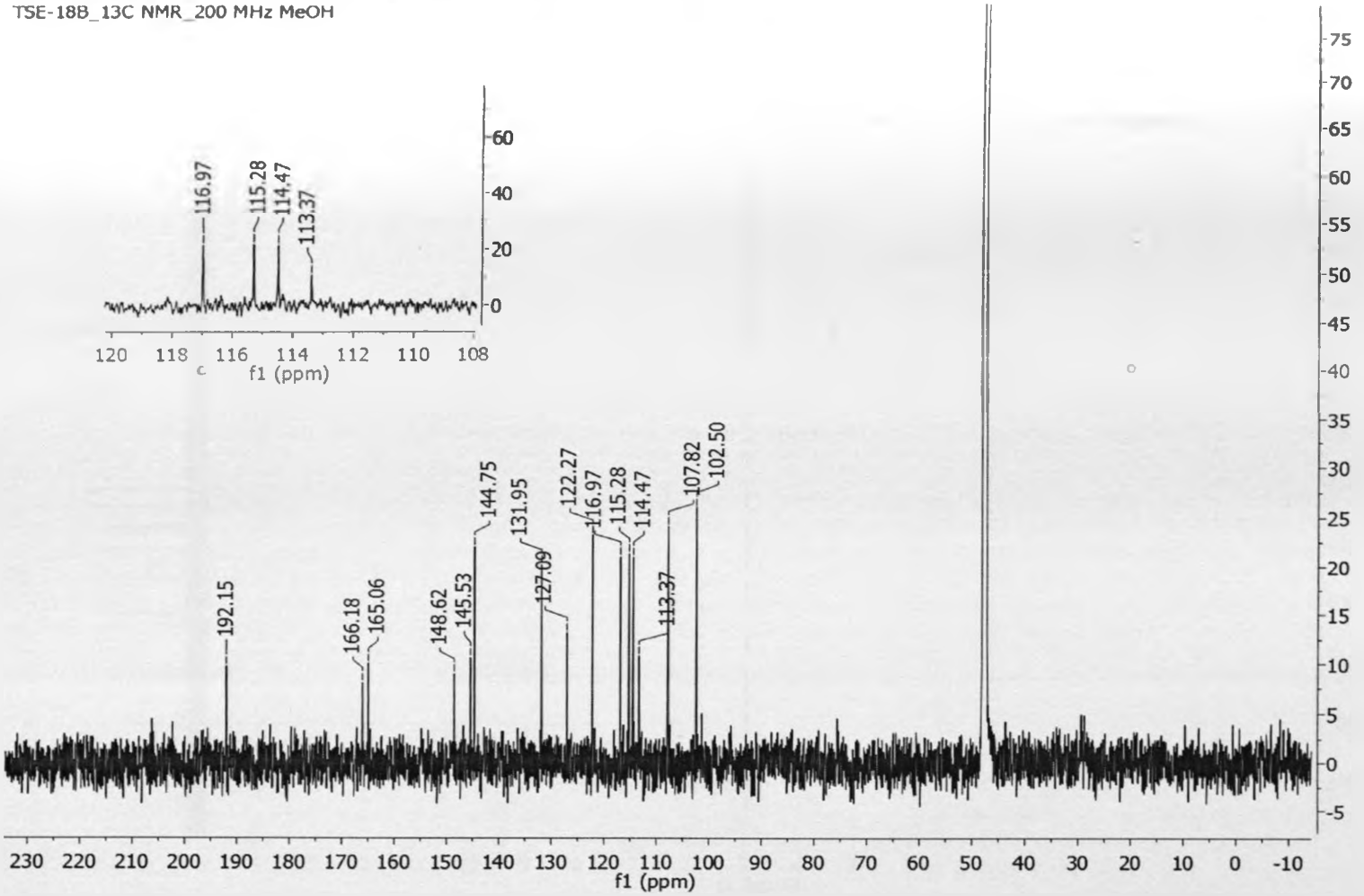
Appendix 6F: HMBC (600.24/150.95 MHz) spectrum of compound 305



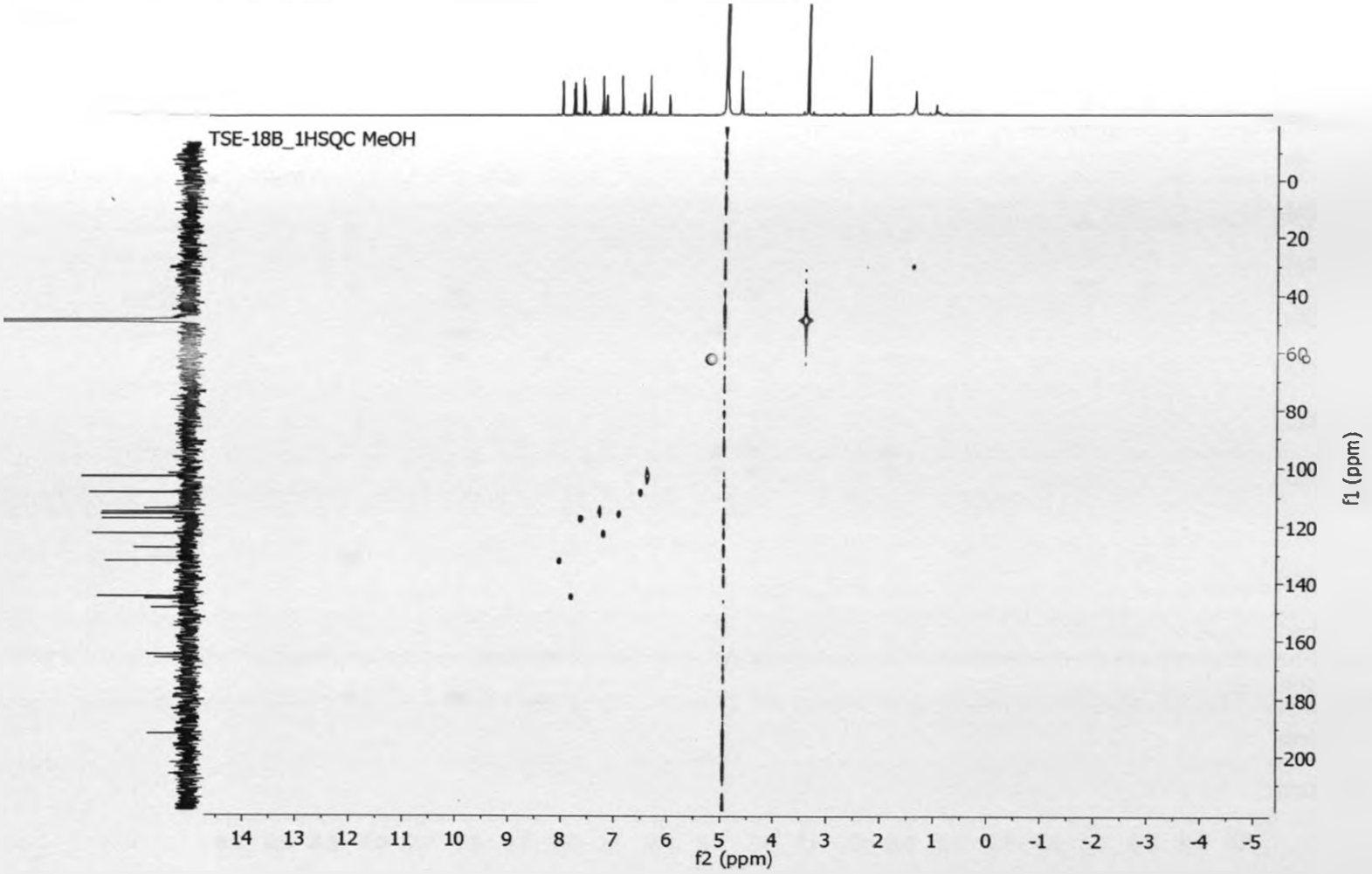
Appendix 7A: ^{1}H NMR (799.87 MHz) spectrum compound 195



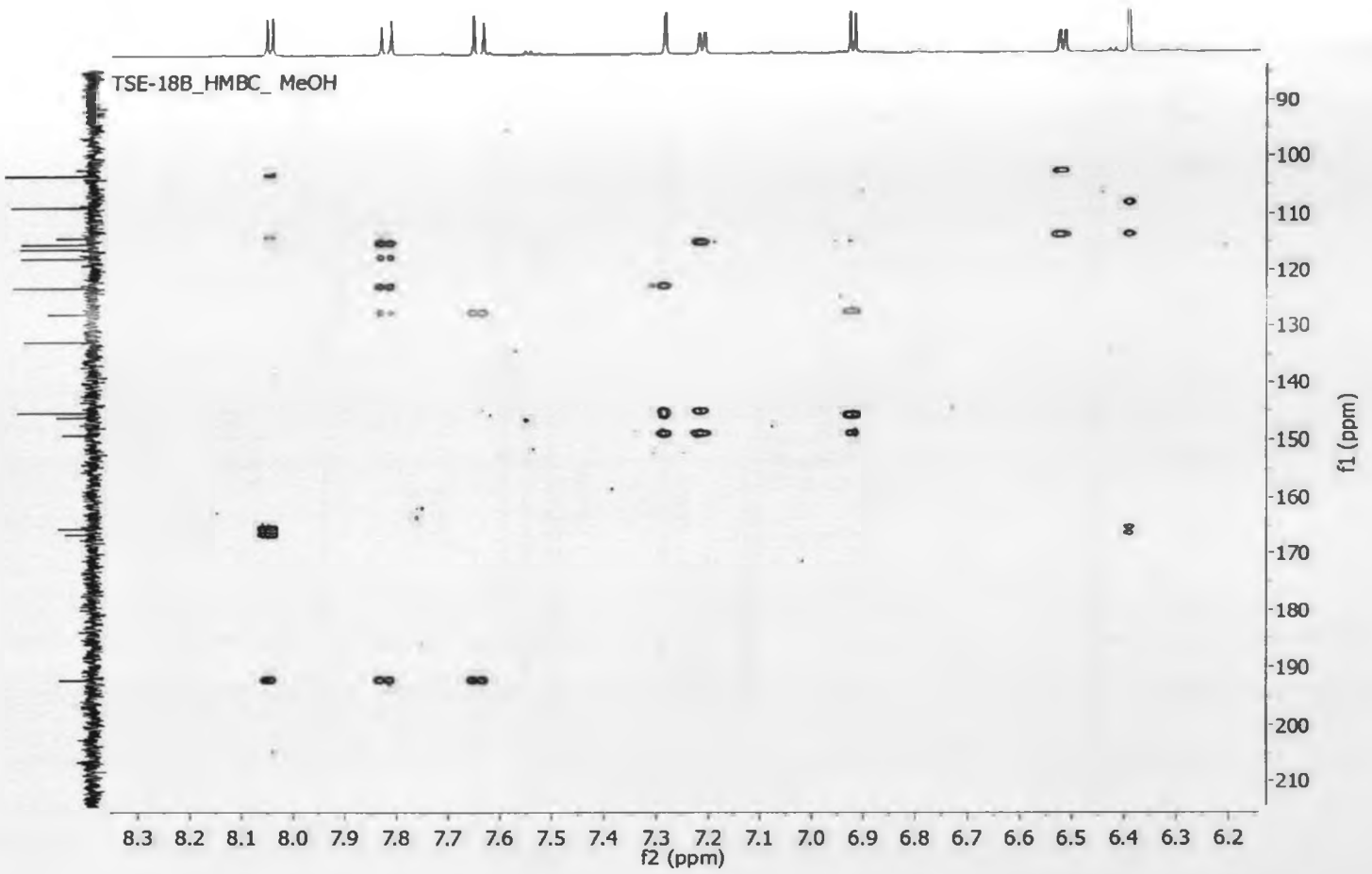
Appendix 7B: ^{13}C ***NMR (201.15 MHz) spectrum of compound 195***



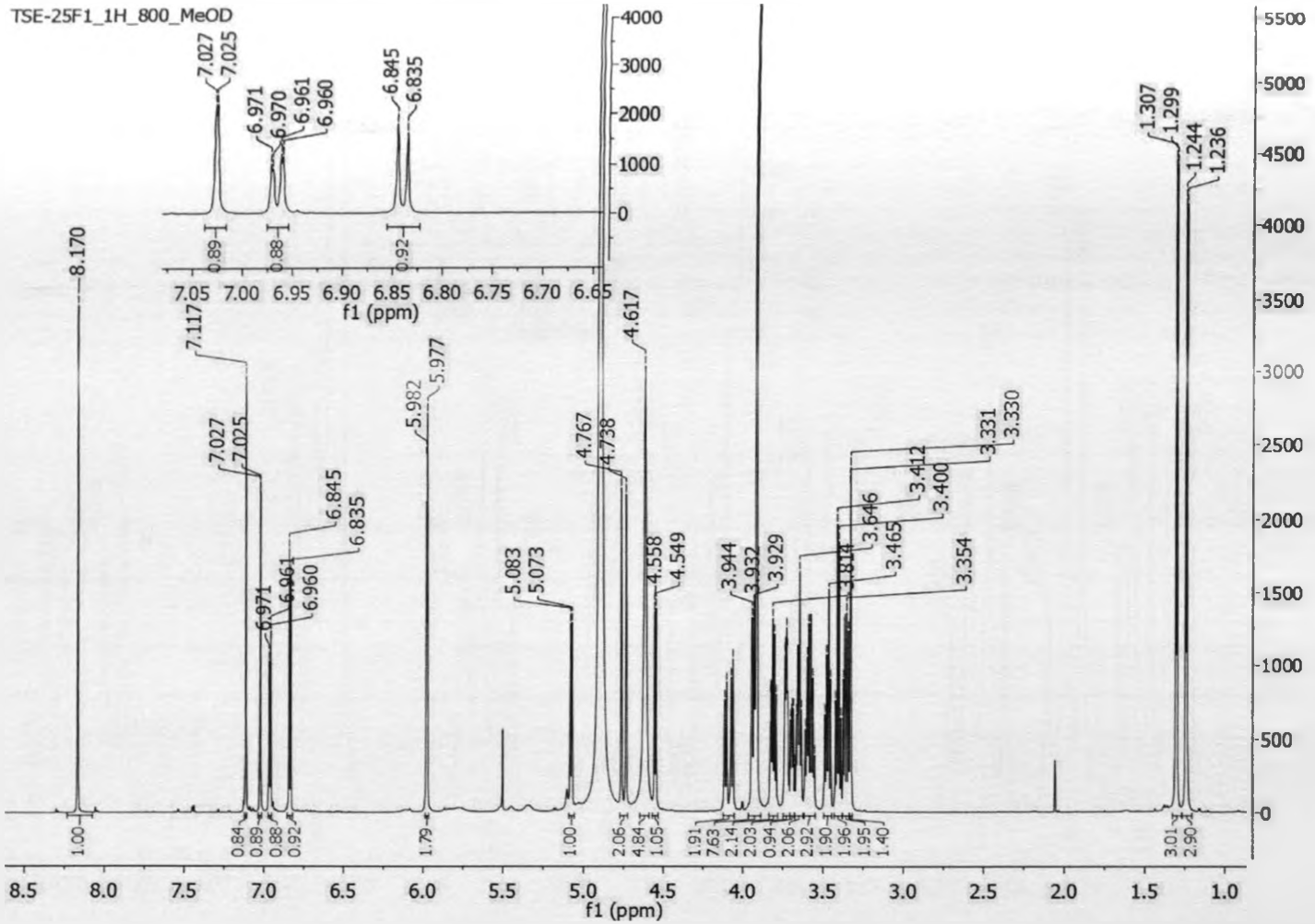
Appendix 7C: HSQC (799.87/201.15 MHz) spectrum of compound 195



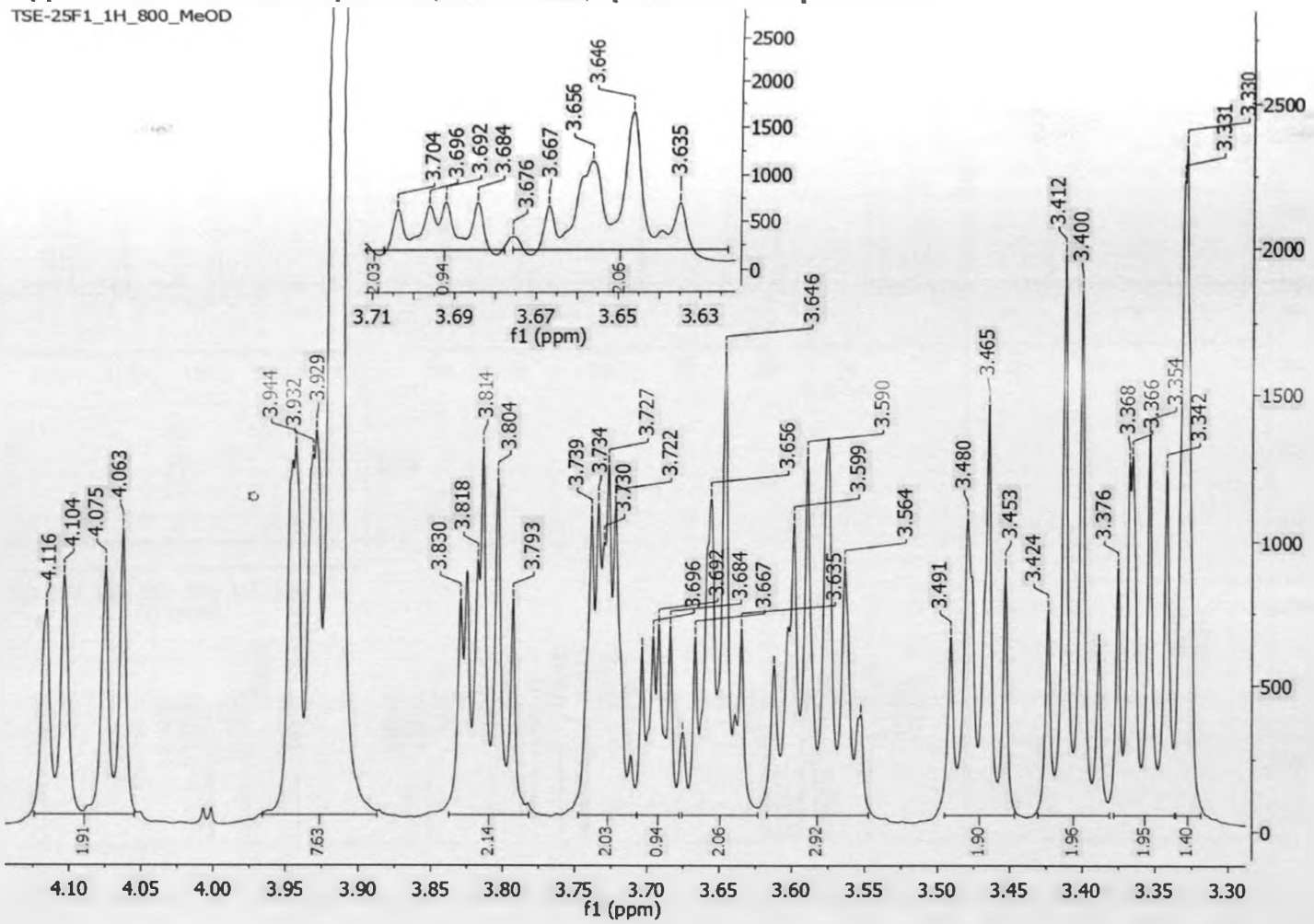
Appendix 7D: HMBC (799.87/201.15 MHz) spectrum of compound 195



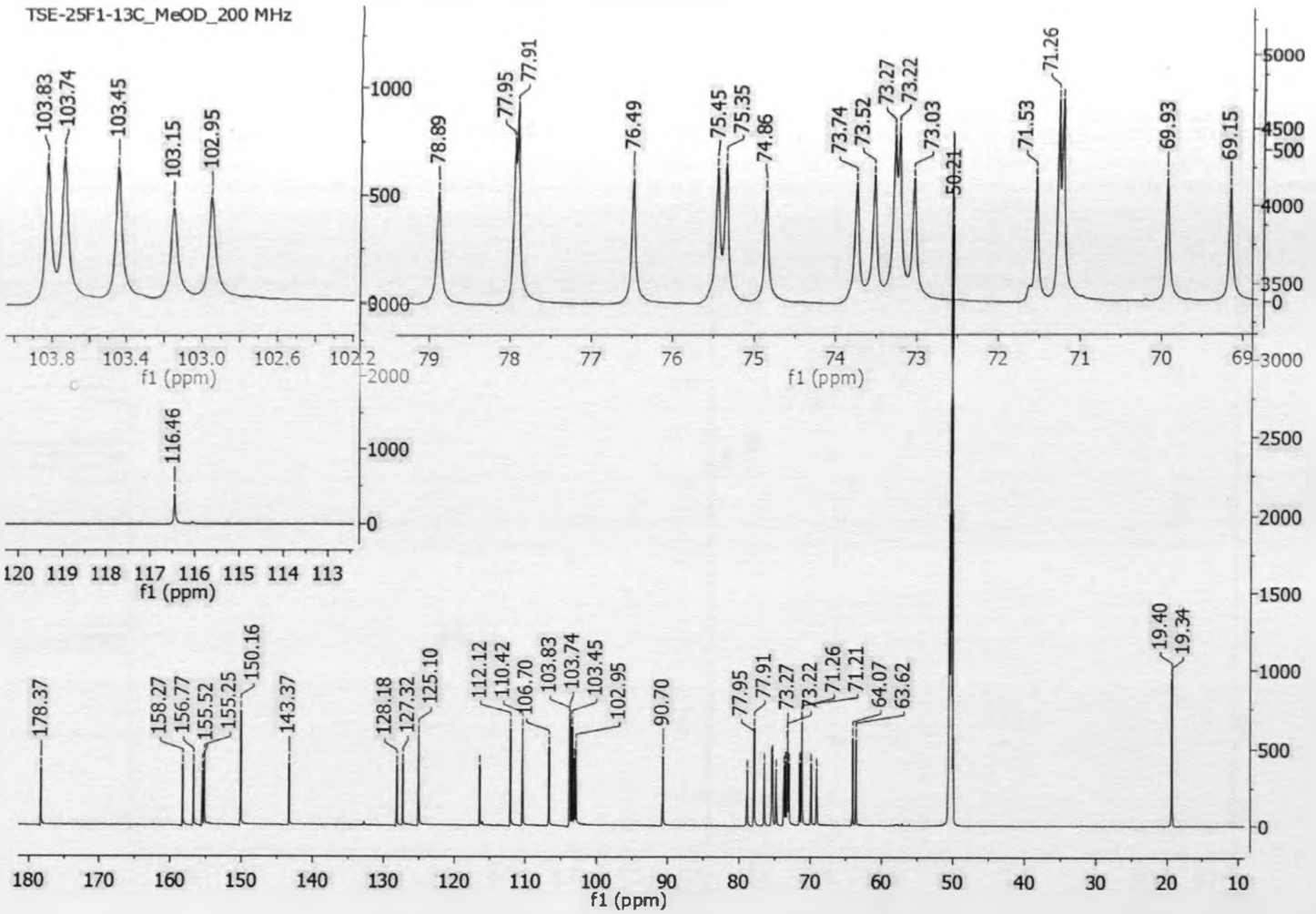
Appendix 8A: ^{1}H NMR (799.87 MHz) spectrum of compound 306



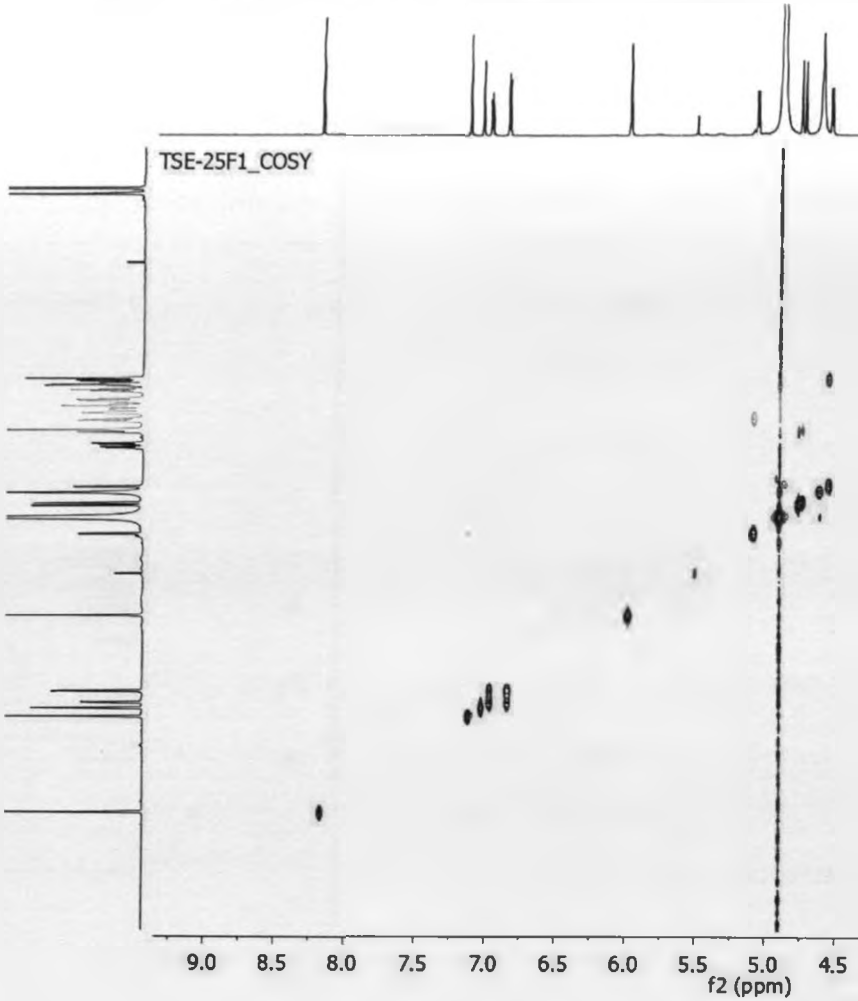
Appendix 8B: ^{1}H NMR expanded (799.87 MHz) spectrum of compound 306

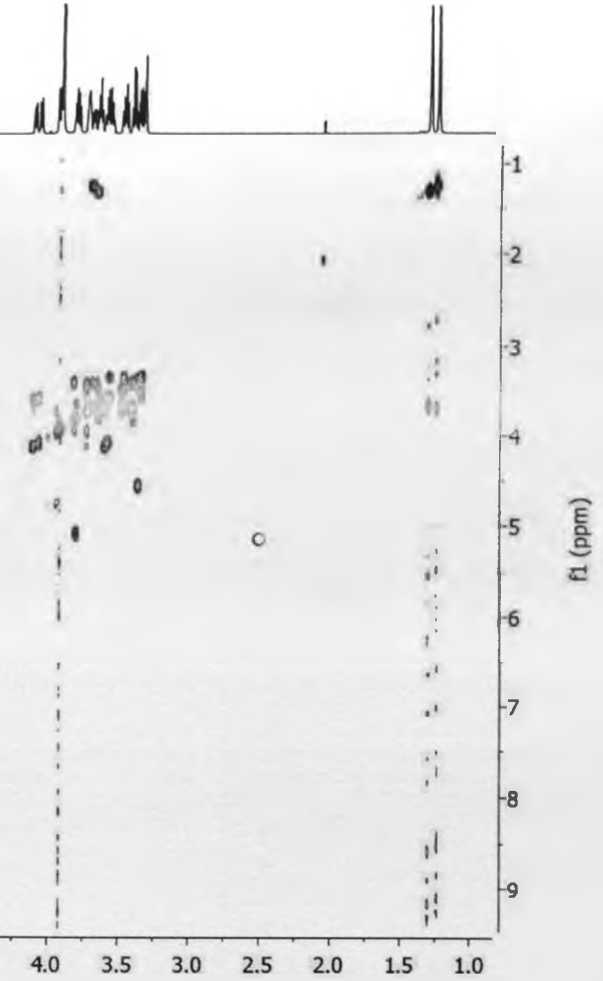


Appendix 8C: ^{13}C *NMR (201.15) spectrum of compound 306*

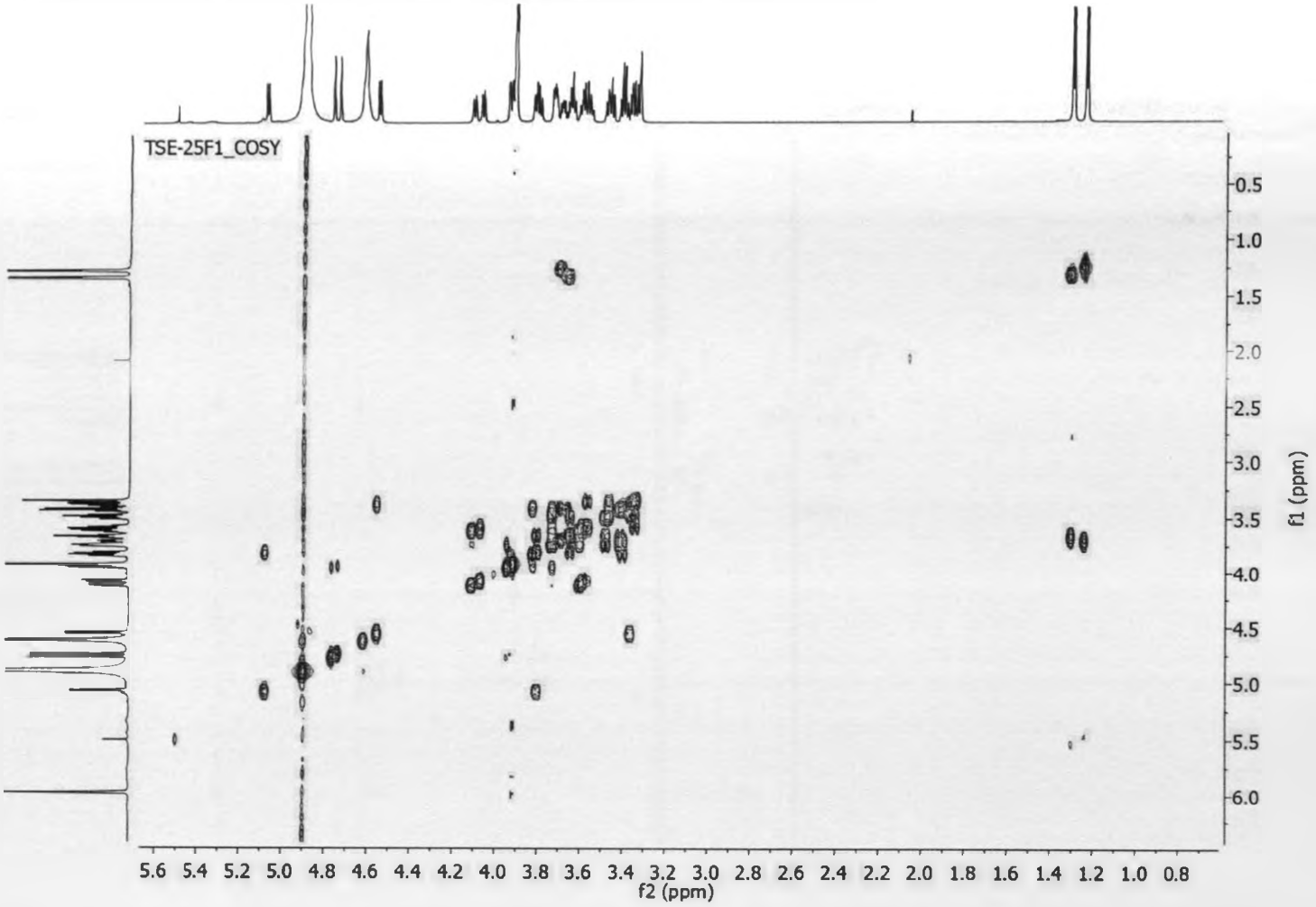


Appendix 8D: COSY (799.87 MHz) spectrum of compound 306

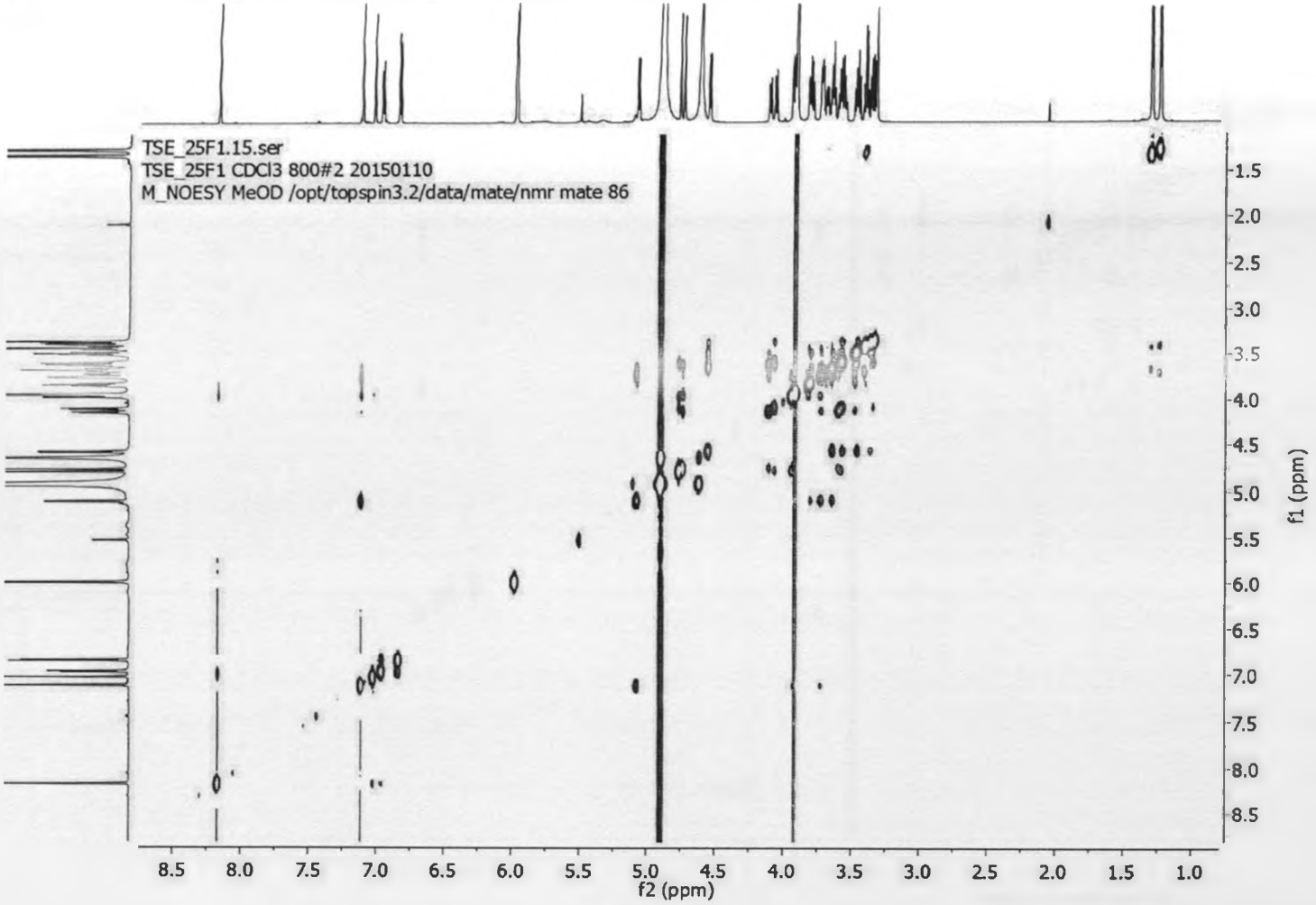



1
2
3
4
5
6
7
8
9
f1 (ppm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0

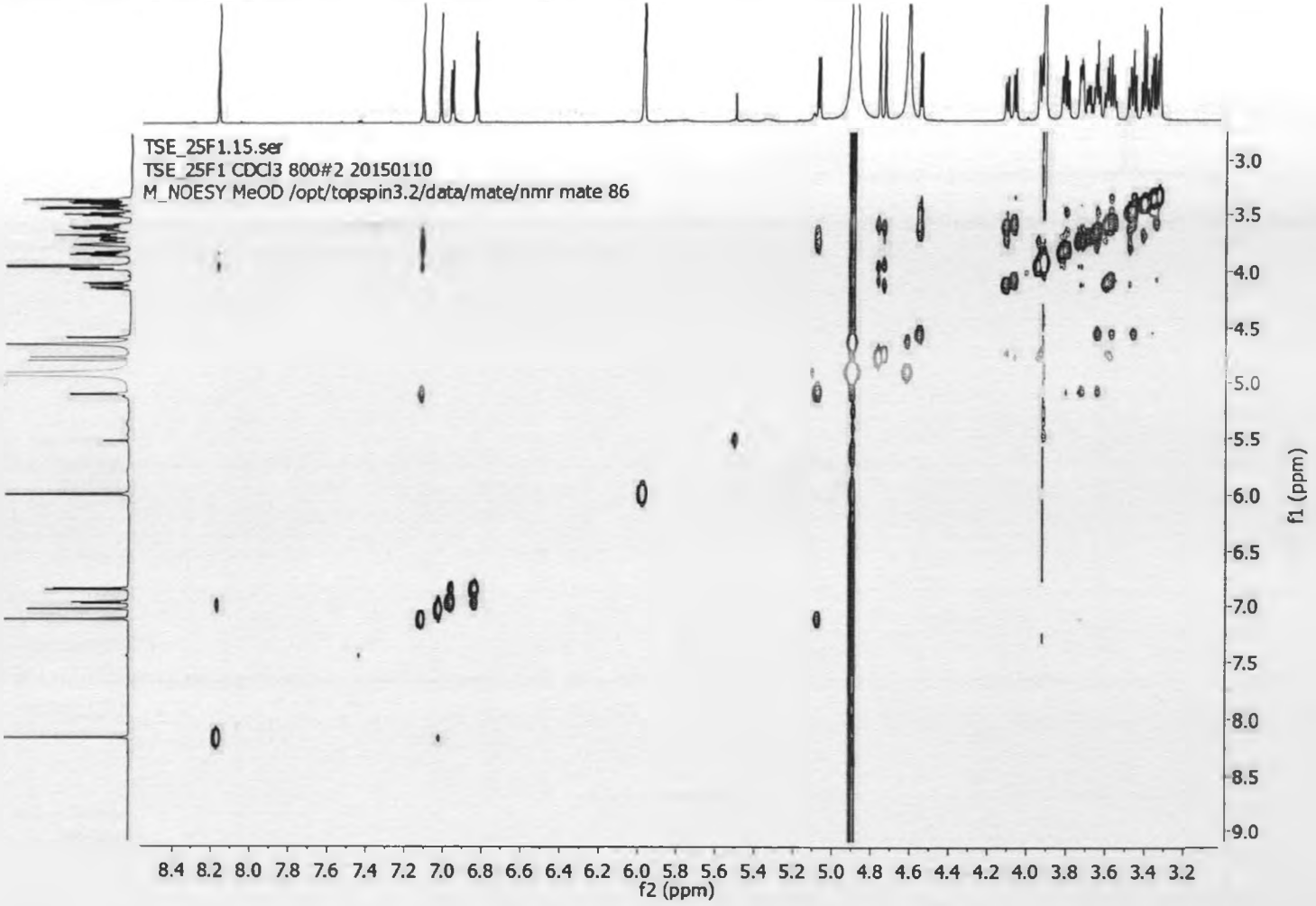
Appendix 8E: COSY expanded (799.87 MHz) spectrum of compound 306



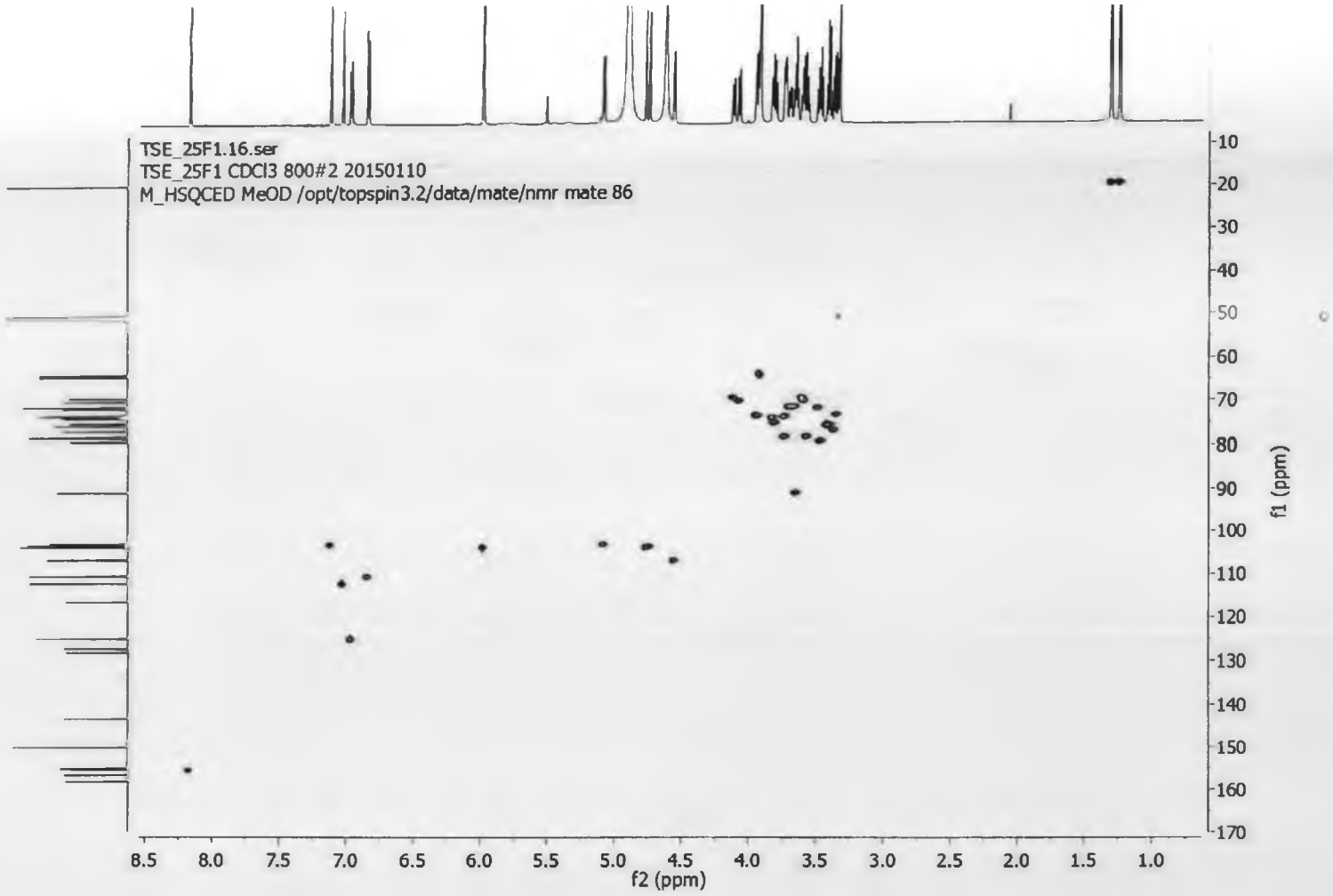
Appendix 8F: NOESY (799.87 MHz) spectrum of compound 306



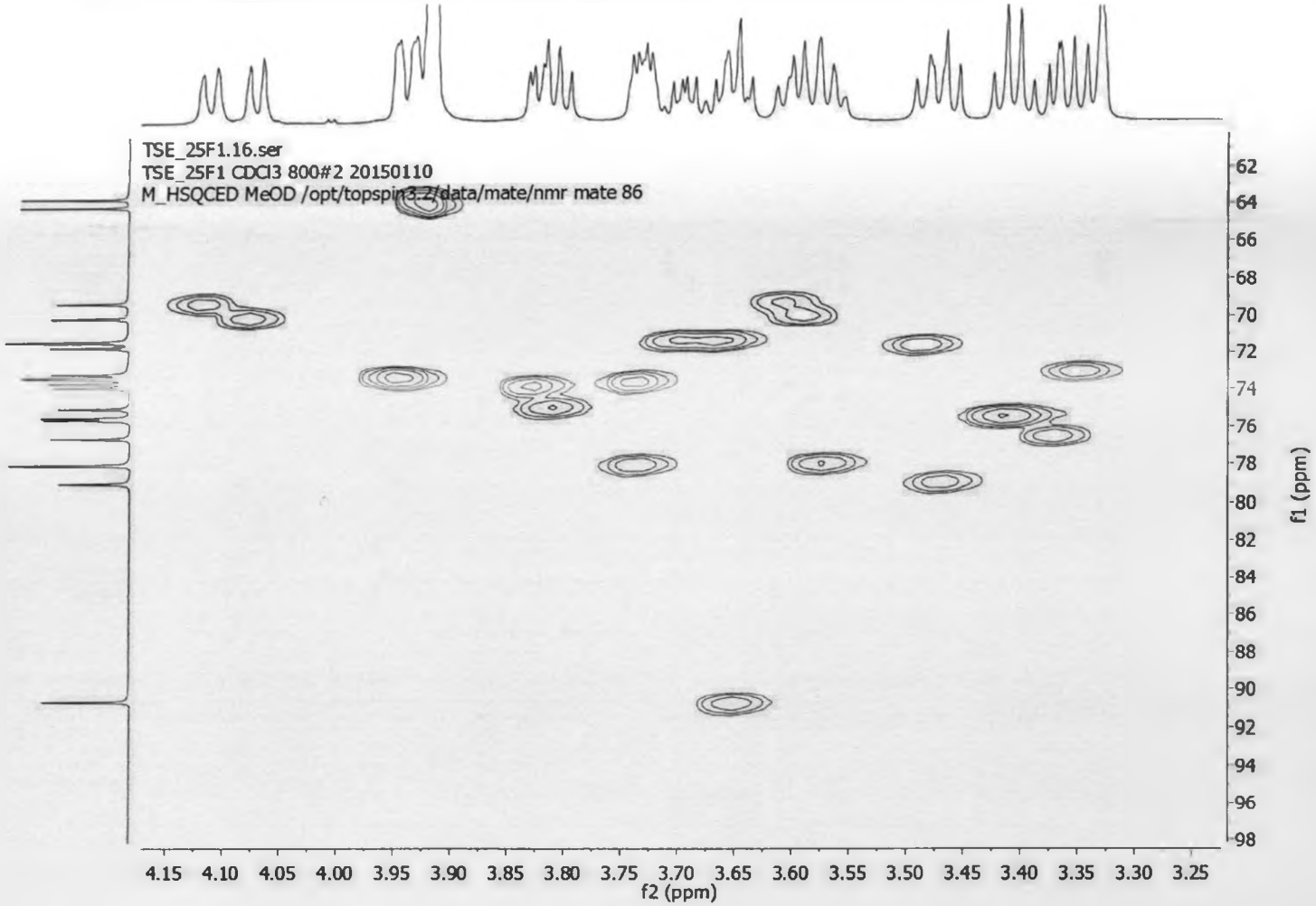
Appendix 8G: NOESY expanded (799.87 MHz) spectrum of compound 306



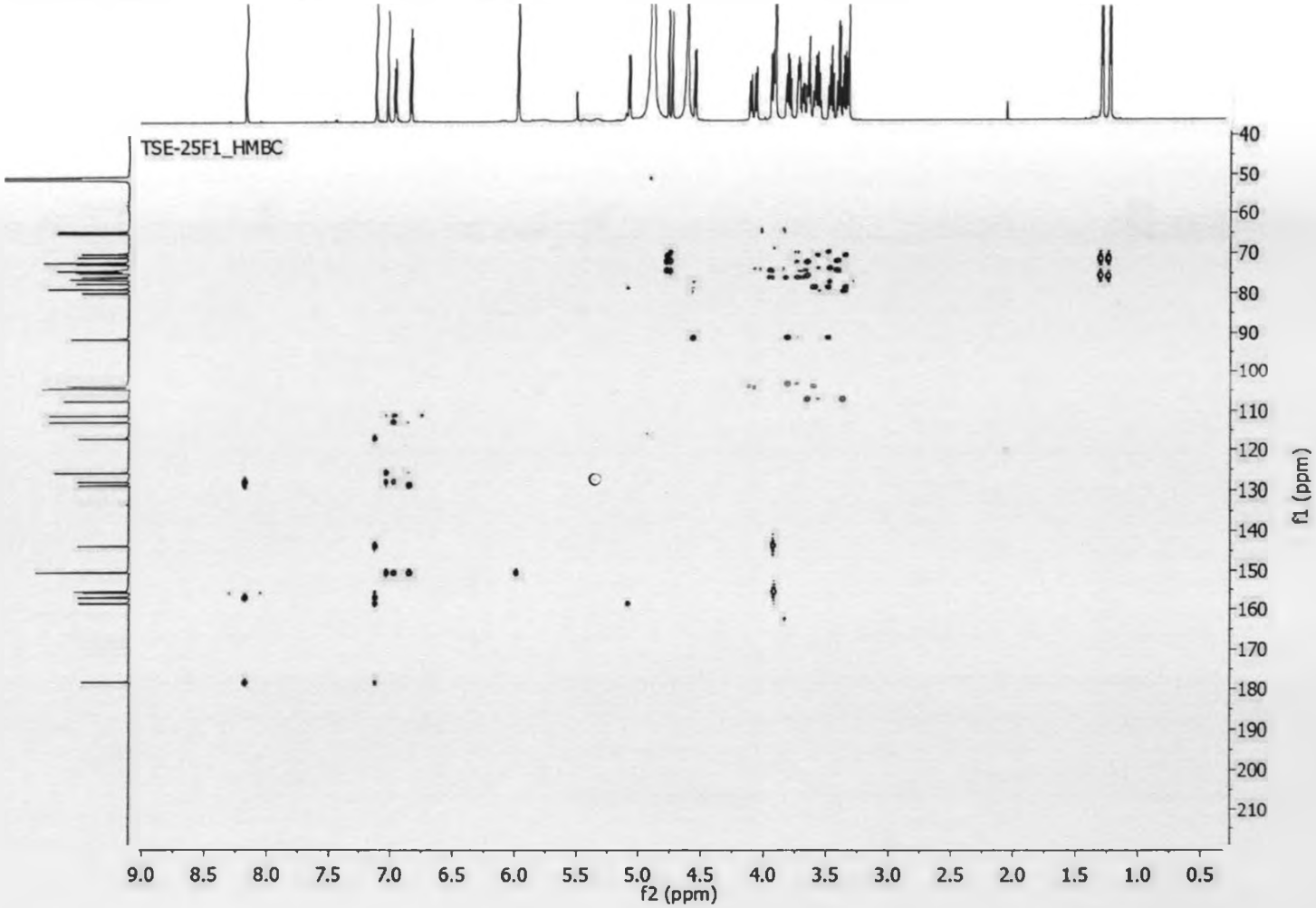
Appendix 8H: HSQC (799.87/201.15 MHz) spectrum of compound 306



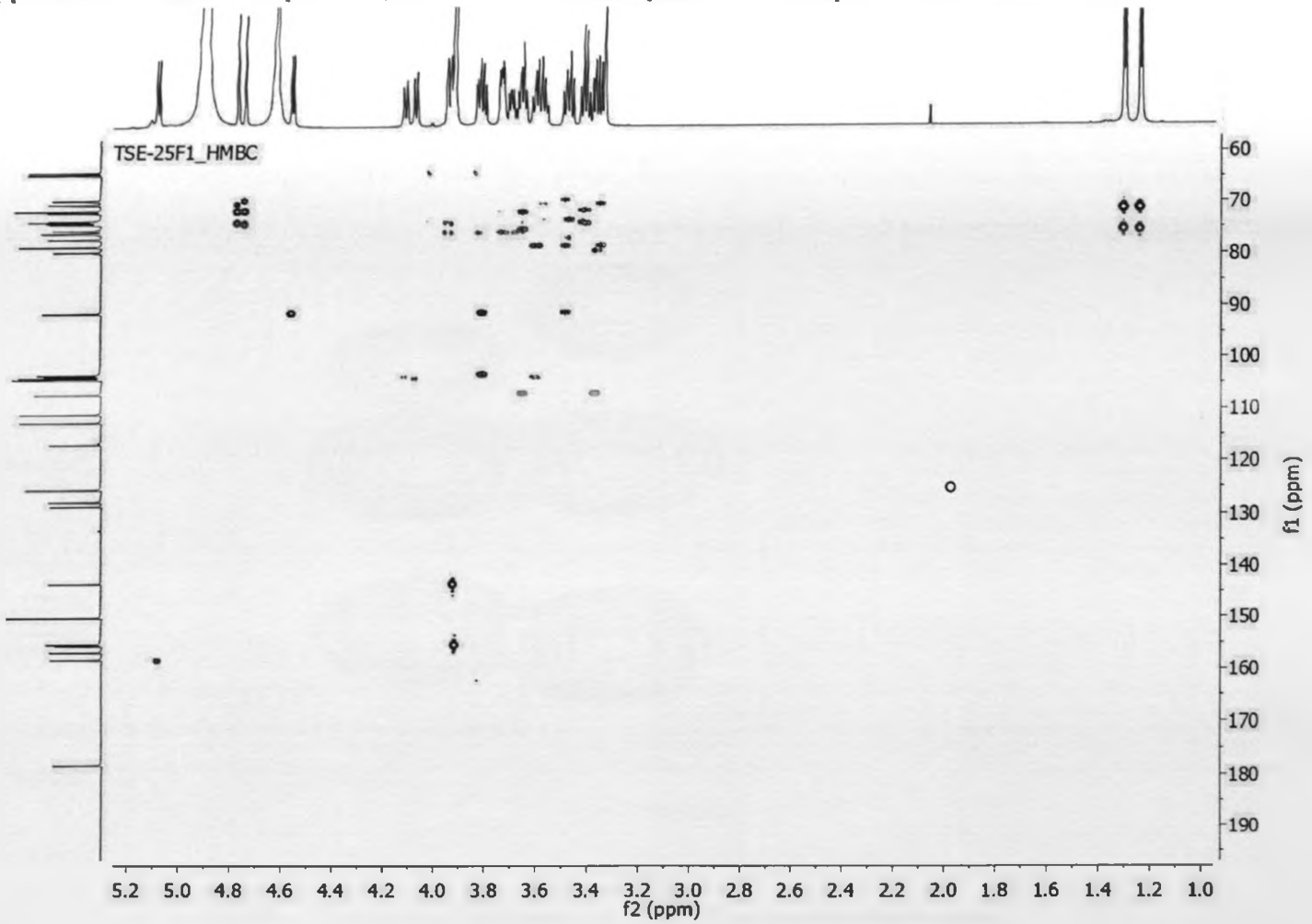
Appendix 81: HSQC expanded (799.87/201.15 MHz) spectrum of compound 306



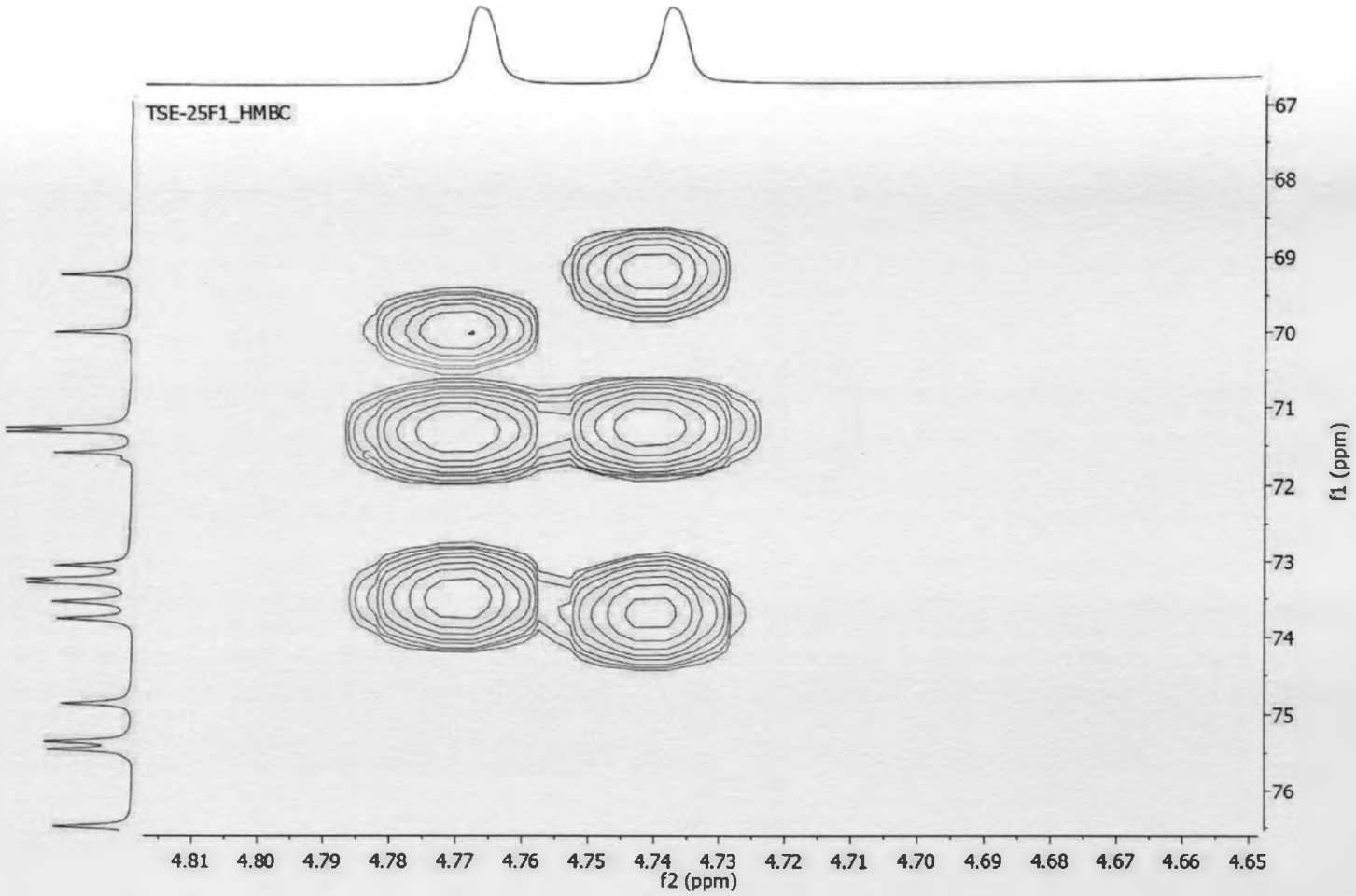
Appendix 8J: **HMBC (799.87/201.15 MHz) spectrum of compound 306**



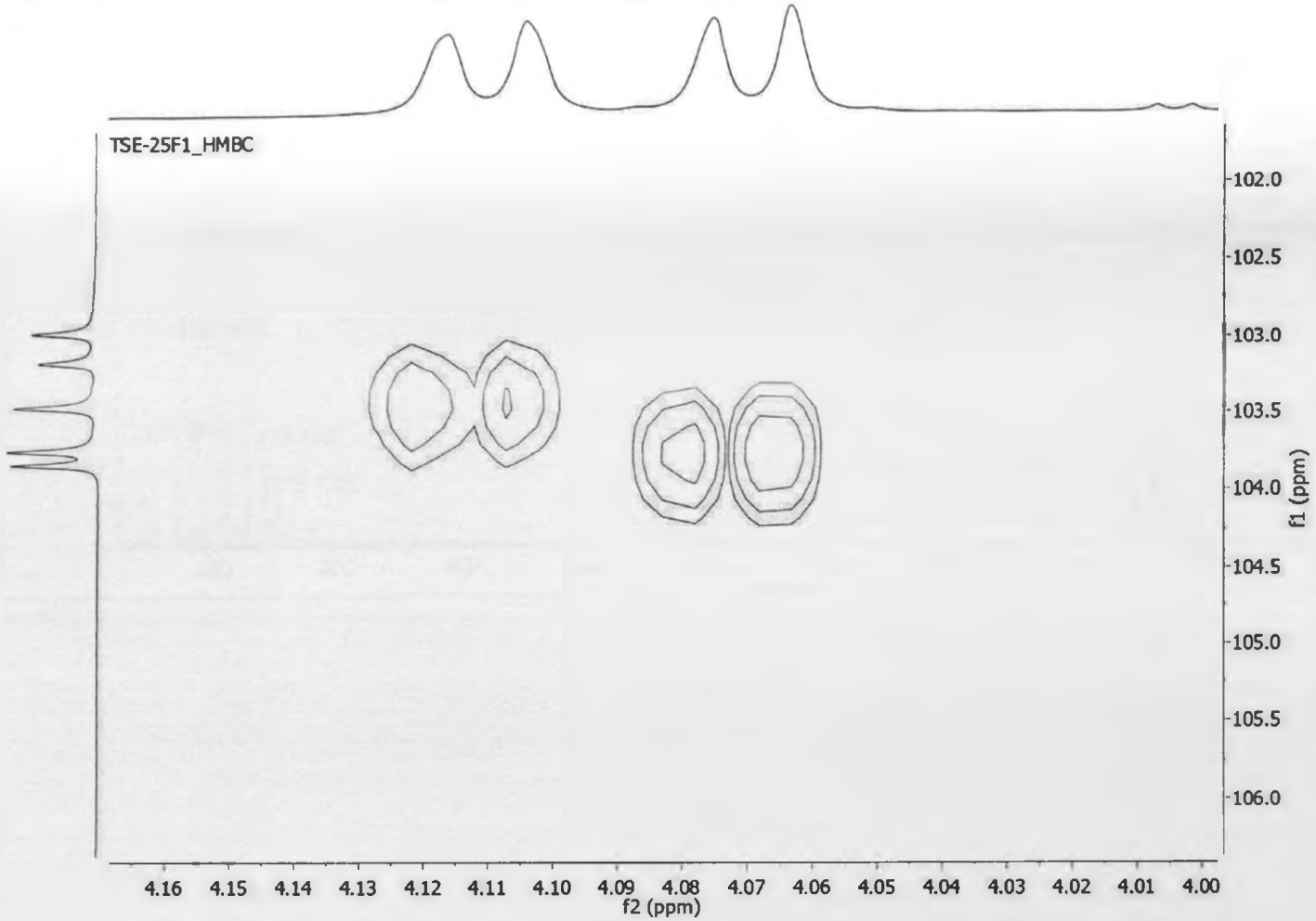
Appendix 8K: HMBC expanded (799.87/201.15 MHz) spectrum of compound 306



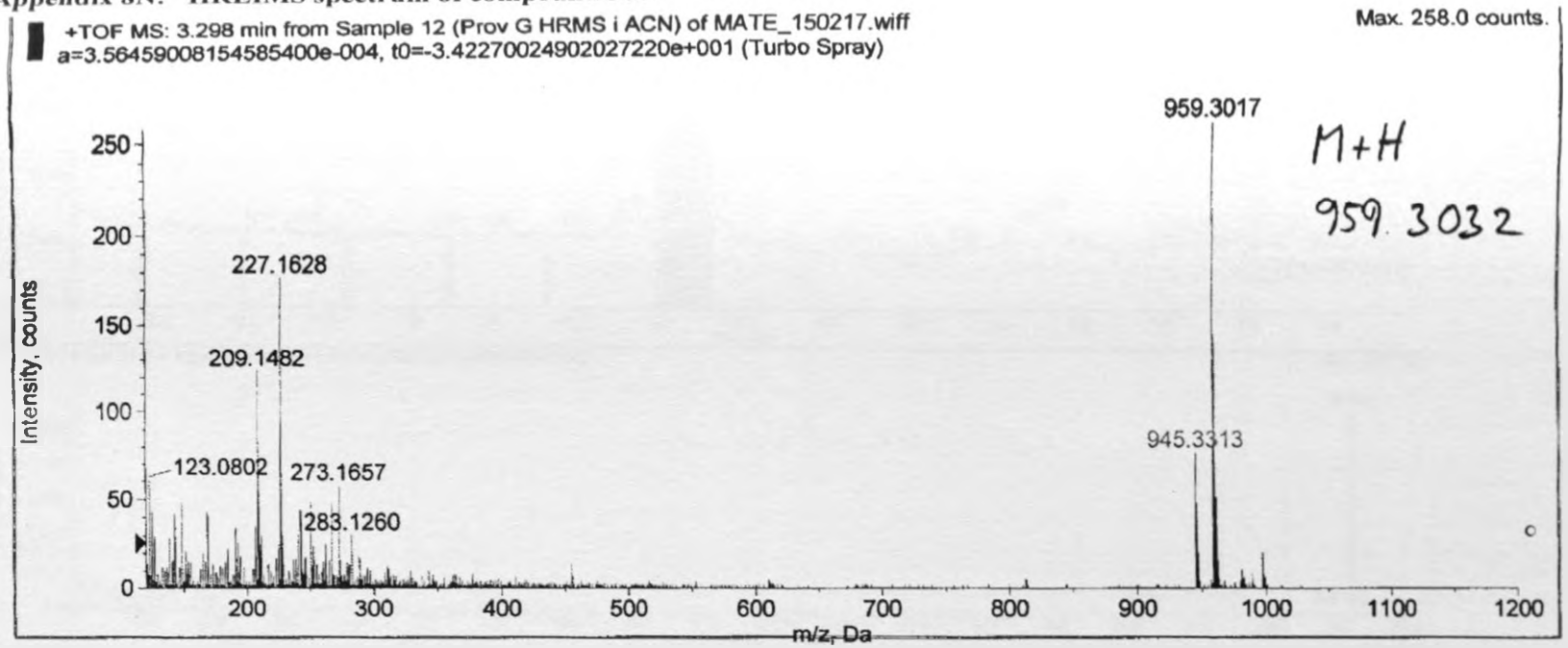
Appendix 8L: **HMBC expanded-1 (799.87/201.15 MHz) spectrum of compound 306**



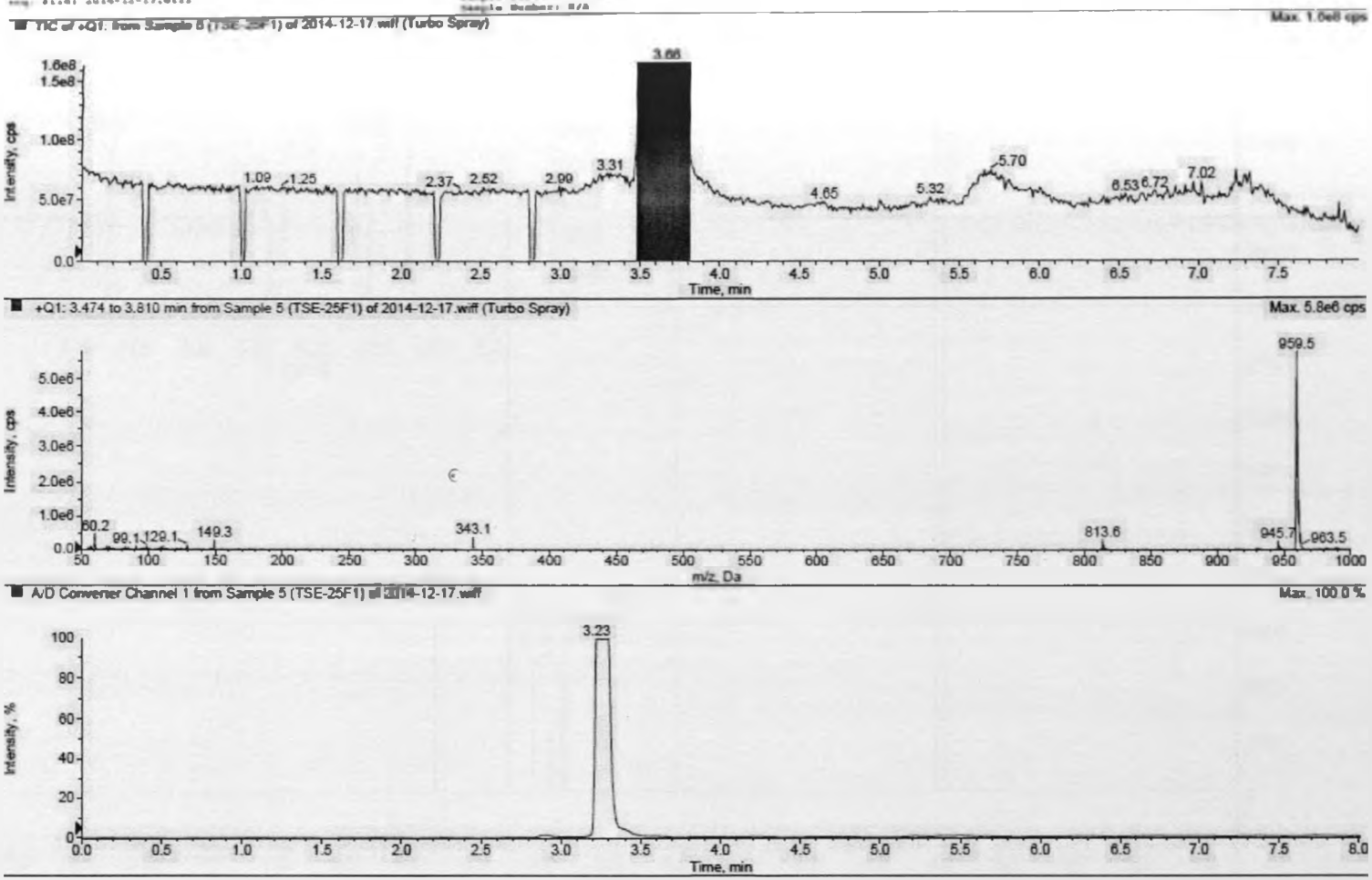
Appendix 8M: ***HMBC expanded-2 (799.87/201.15 MHz) spectrum of compound 306***



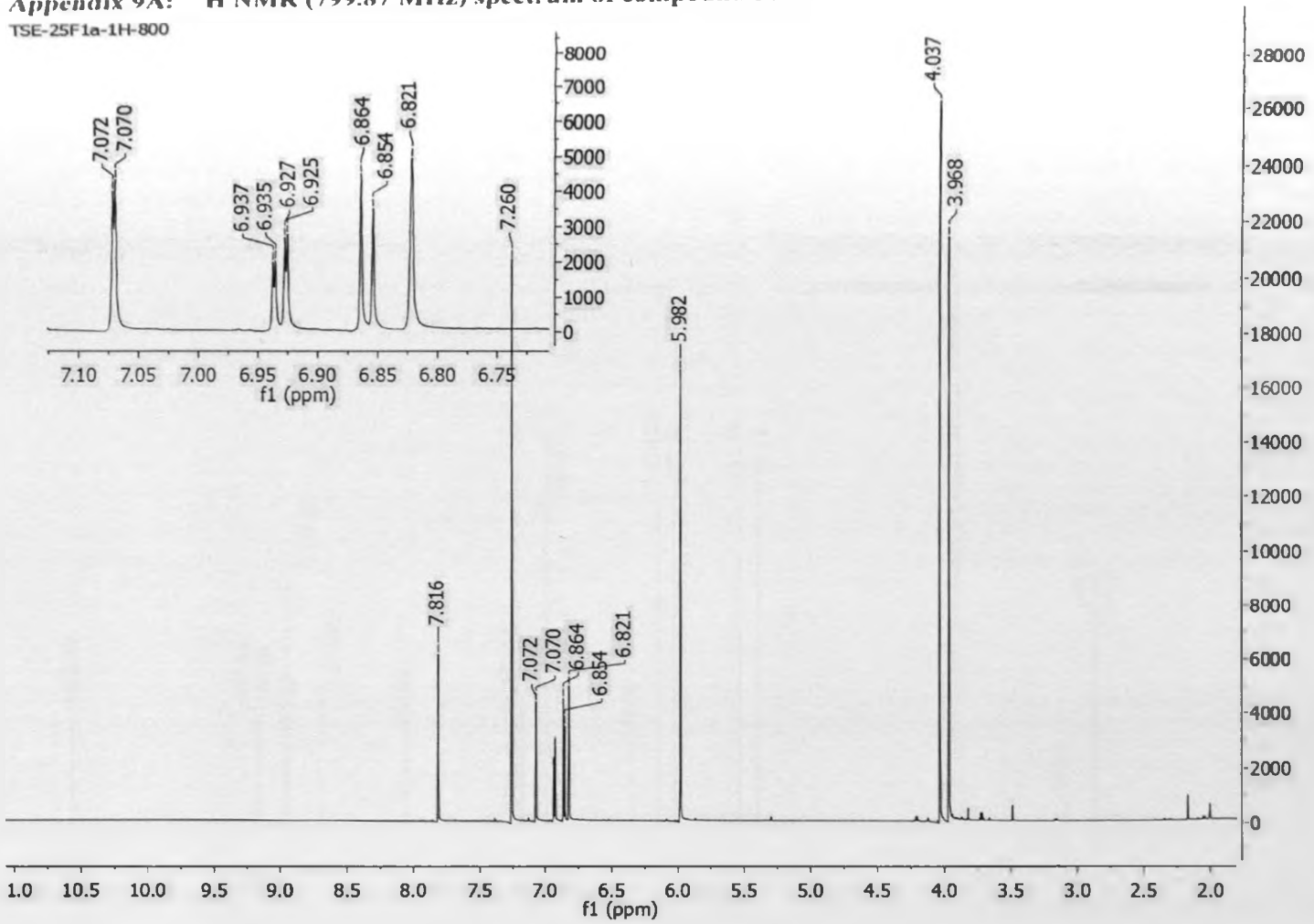
Appendix 8N: *HREIMS spectrum of compound 306*



Appendix 8O: *ESIMS spectrum of compound 306*

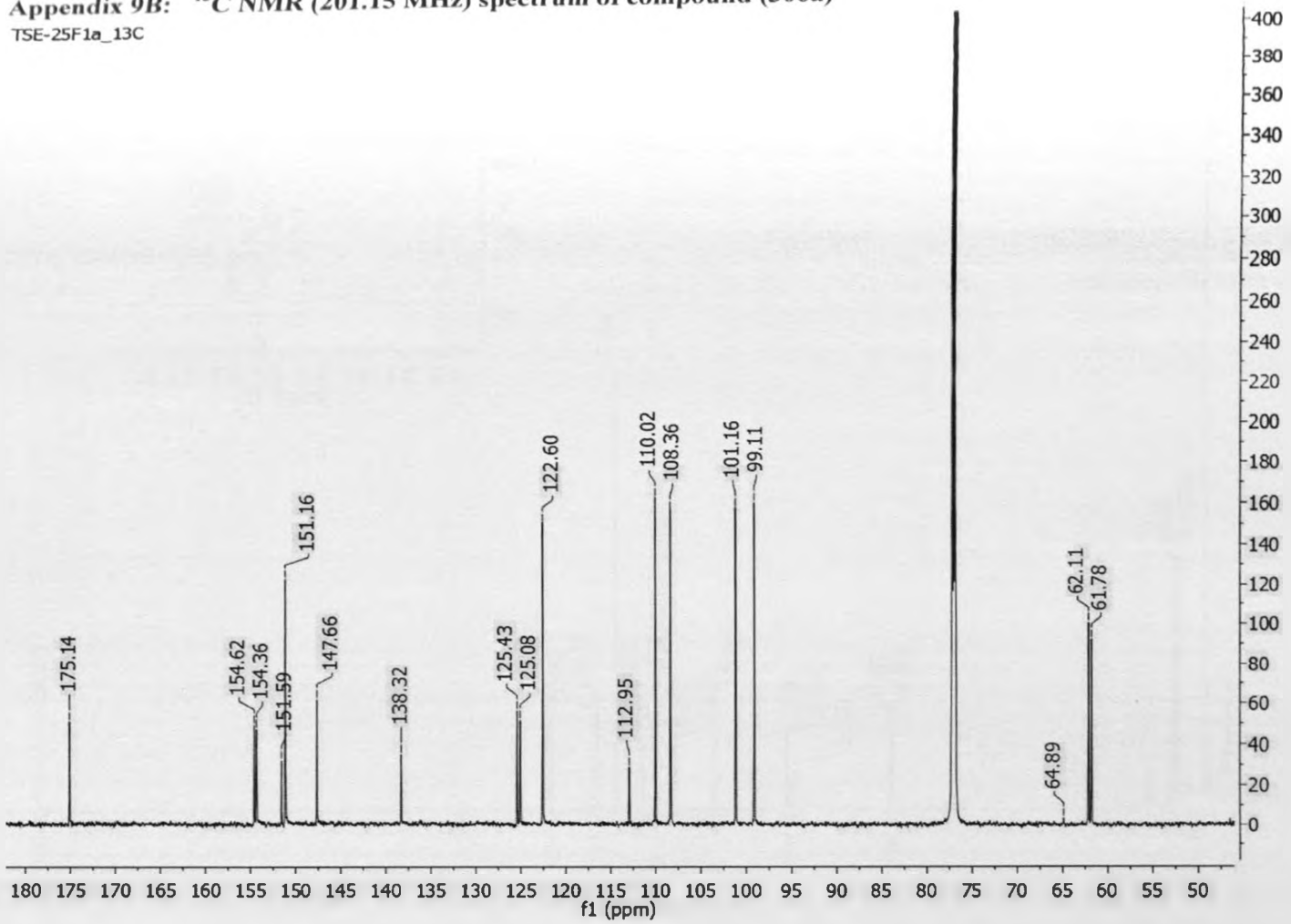


Appendix 9A: **^{1}H NMR (799.87 MHz) spectrum of compound 306a**



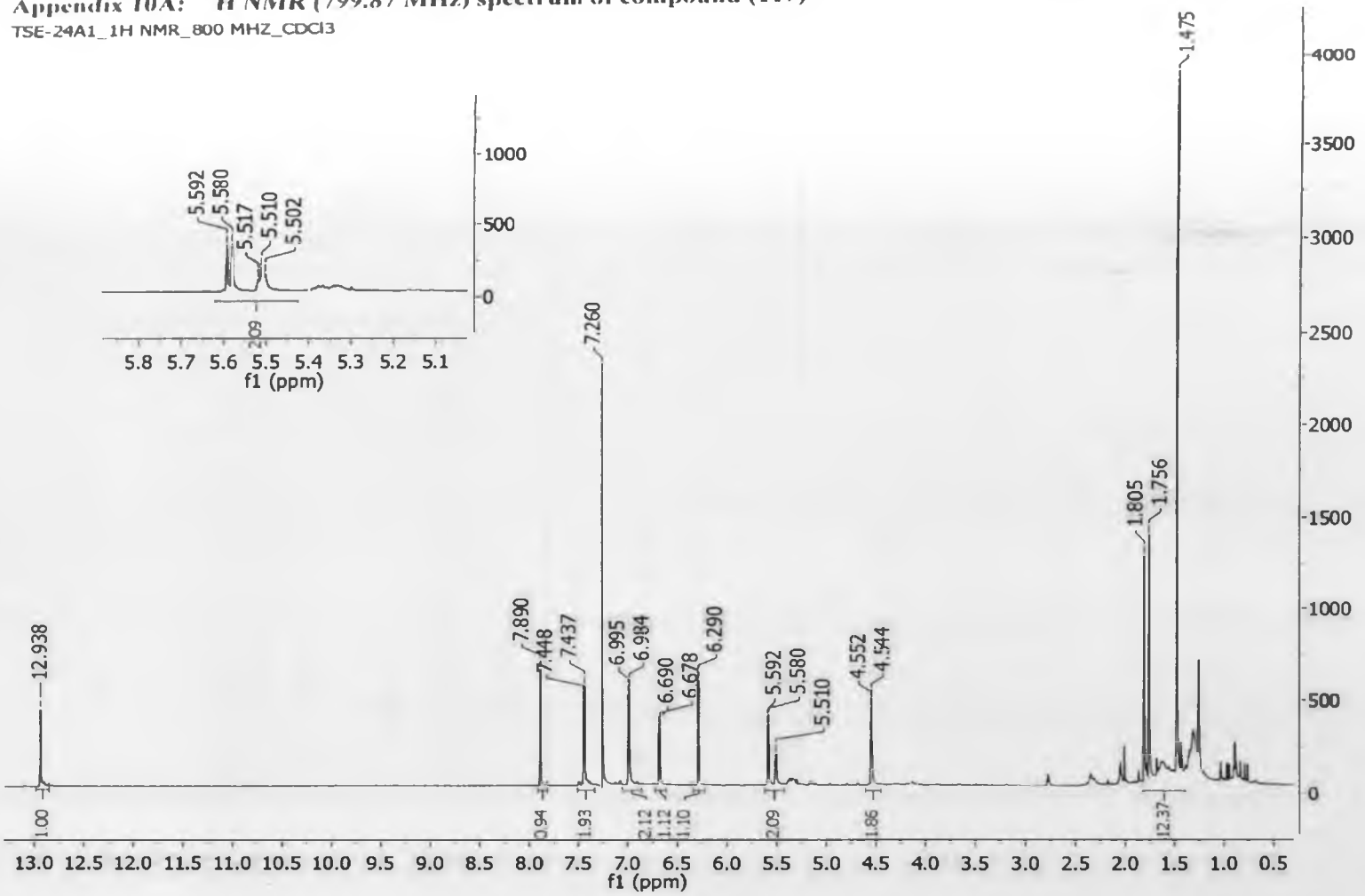
Appendix 9B: ^{13}C NMR (201.15 MHz) spectrum of compound (306a)

TSE-25F1a_13C

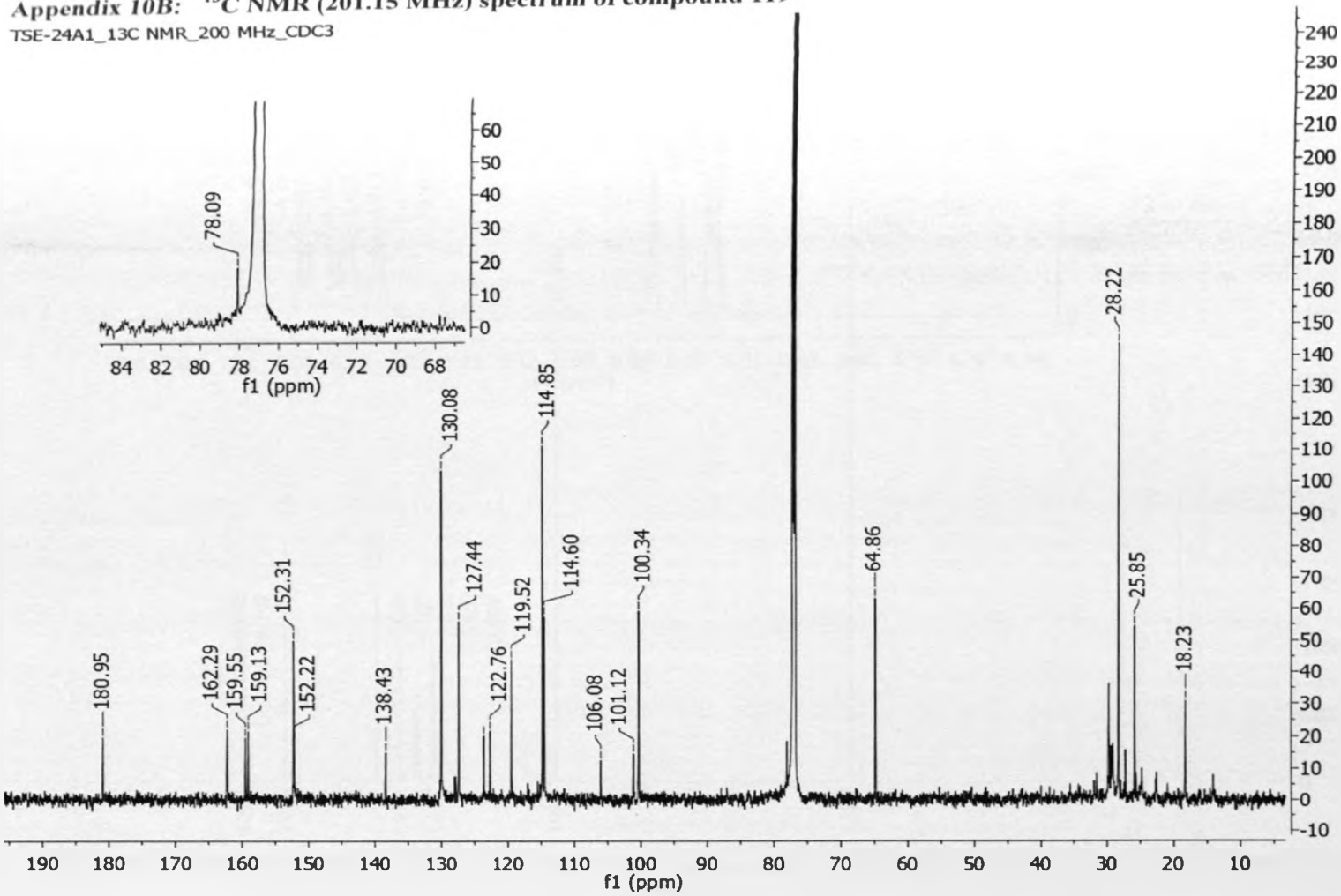


Appendix 10A: ^1H NMR (799.87 MHz) spectrum of compound (119)

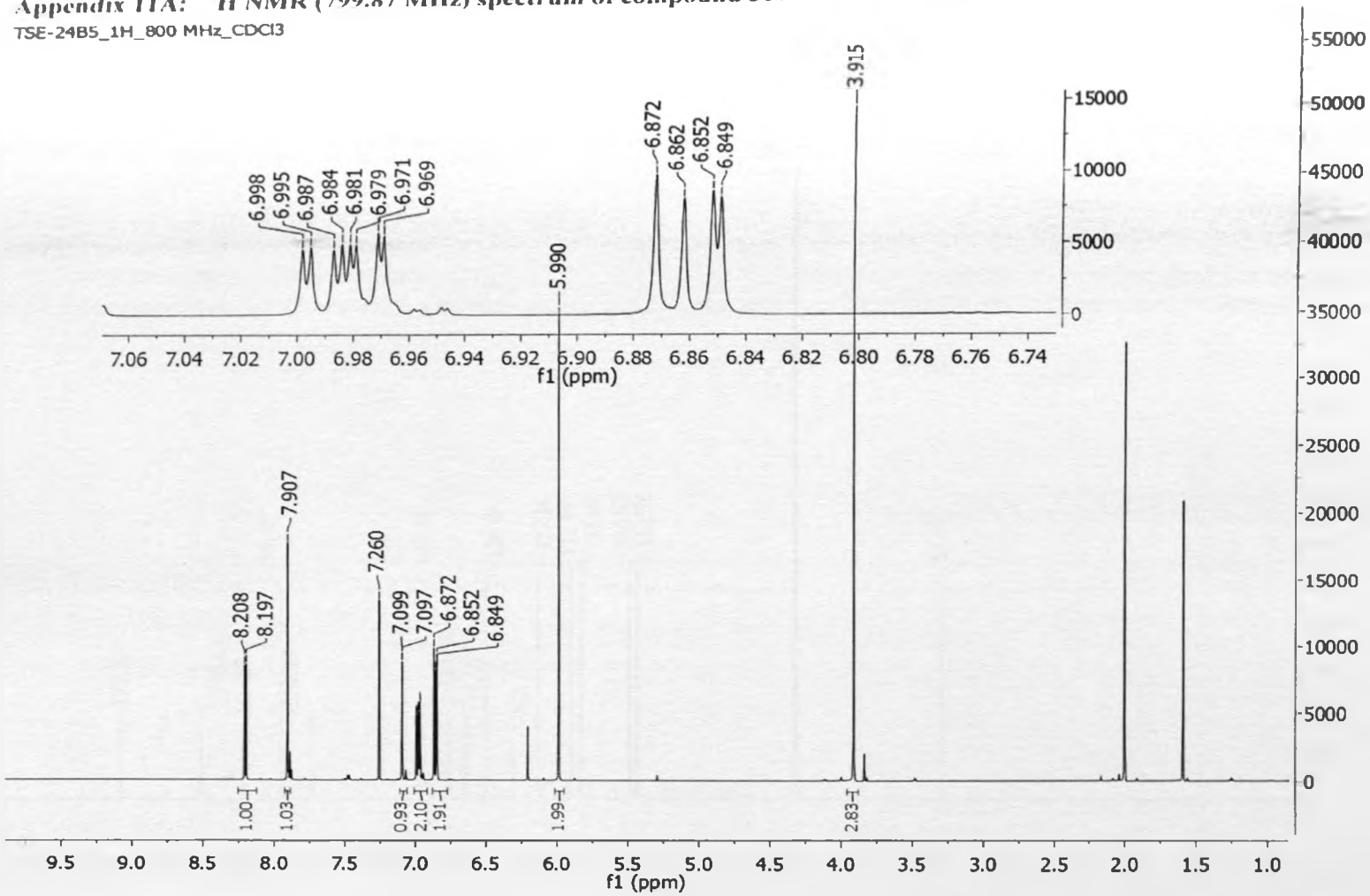
TSE-24A1_1H NMR_800 MHZ_CDCl3



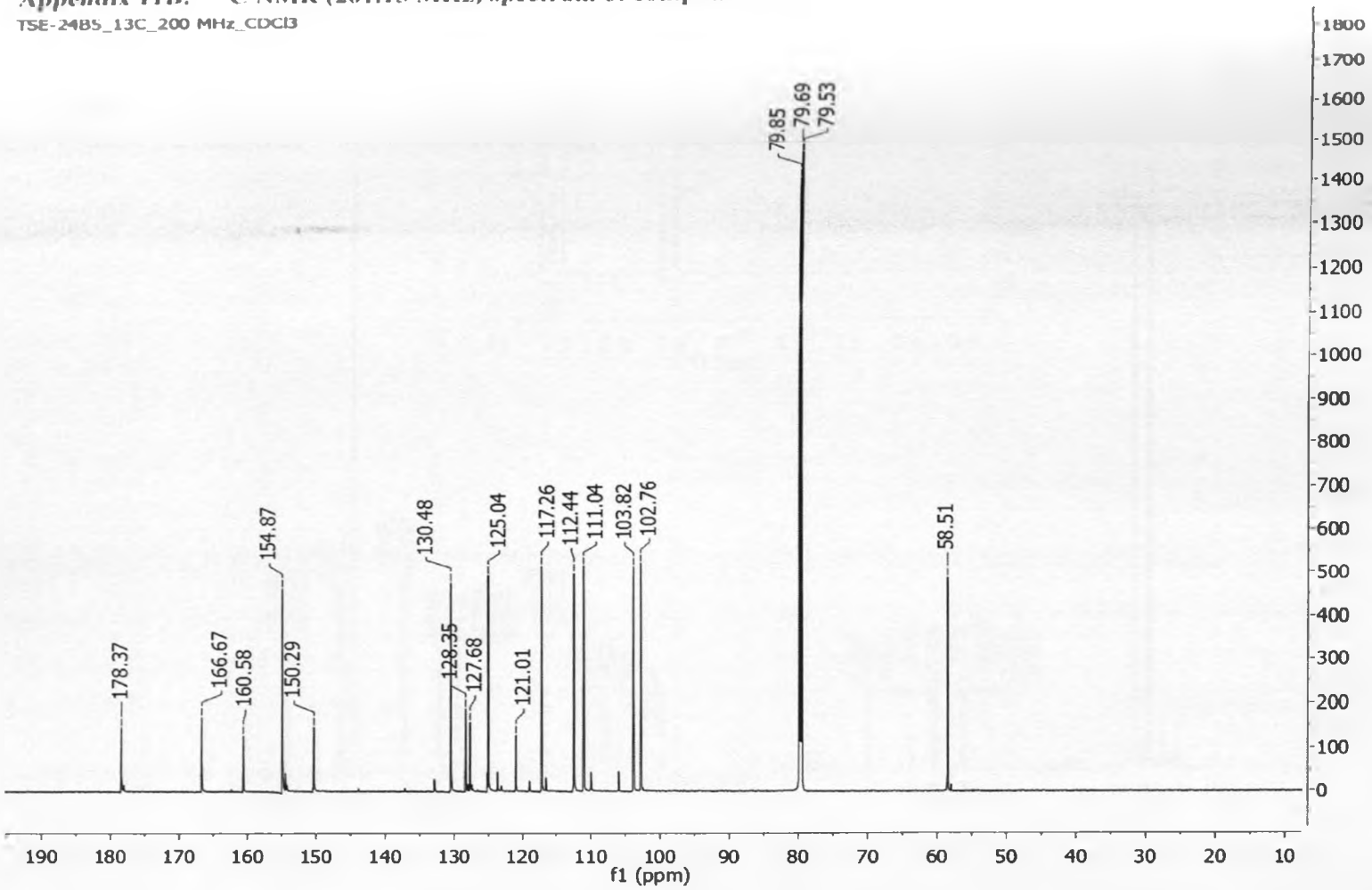
Appendix 10B: ^{13}C NMR (201.15 MHz) spectrum of compound 119



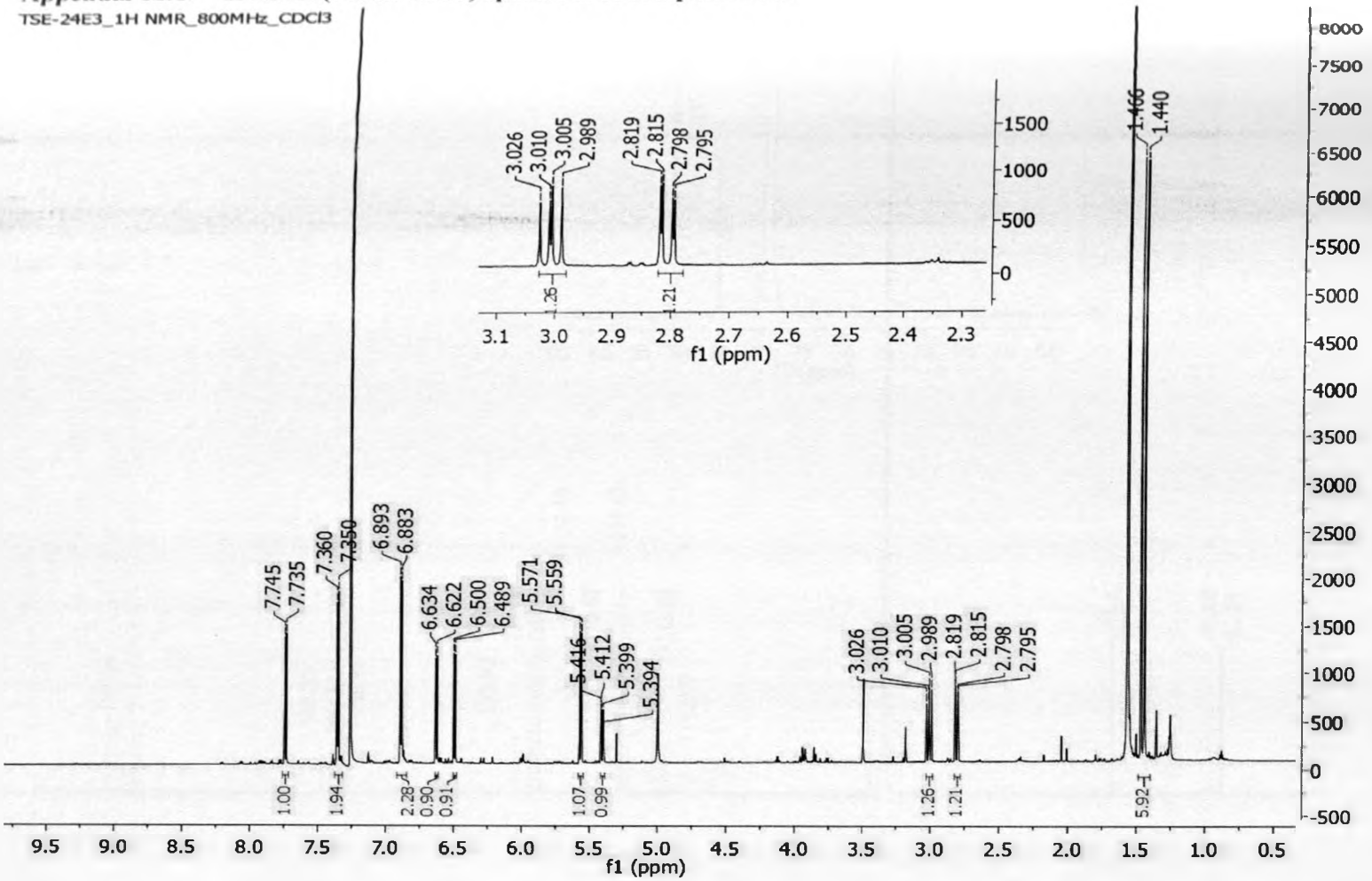
Appendix 11A: ^1H NMR (799.87 MHz) spectrum of compound 307



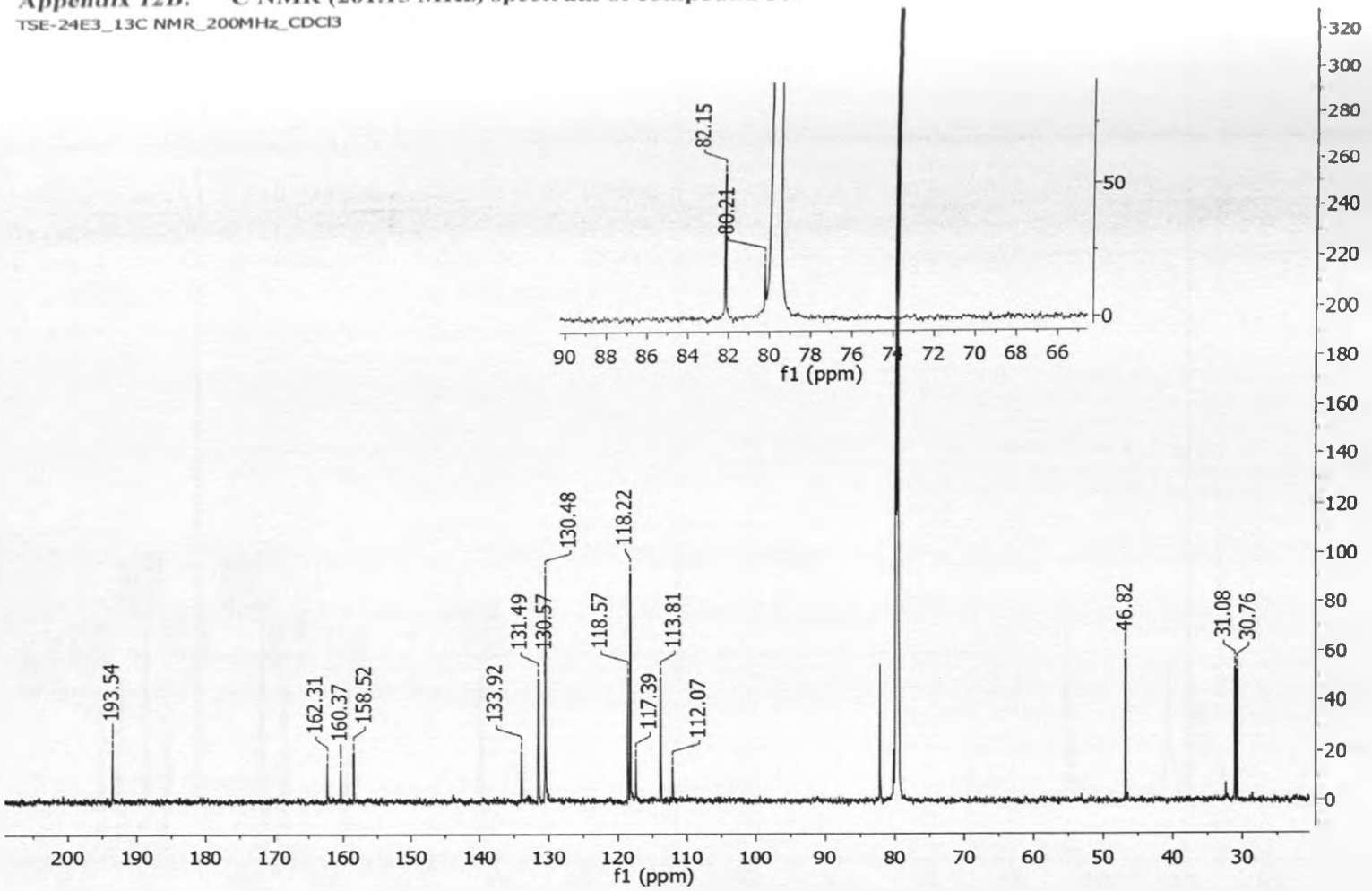
Appendix 11B: ^{13}C NMR (201.15 MHz) spectrum of compound 307



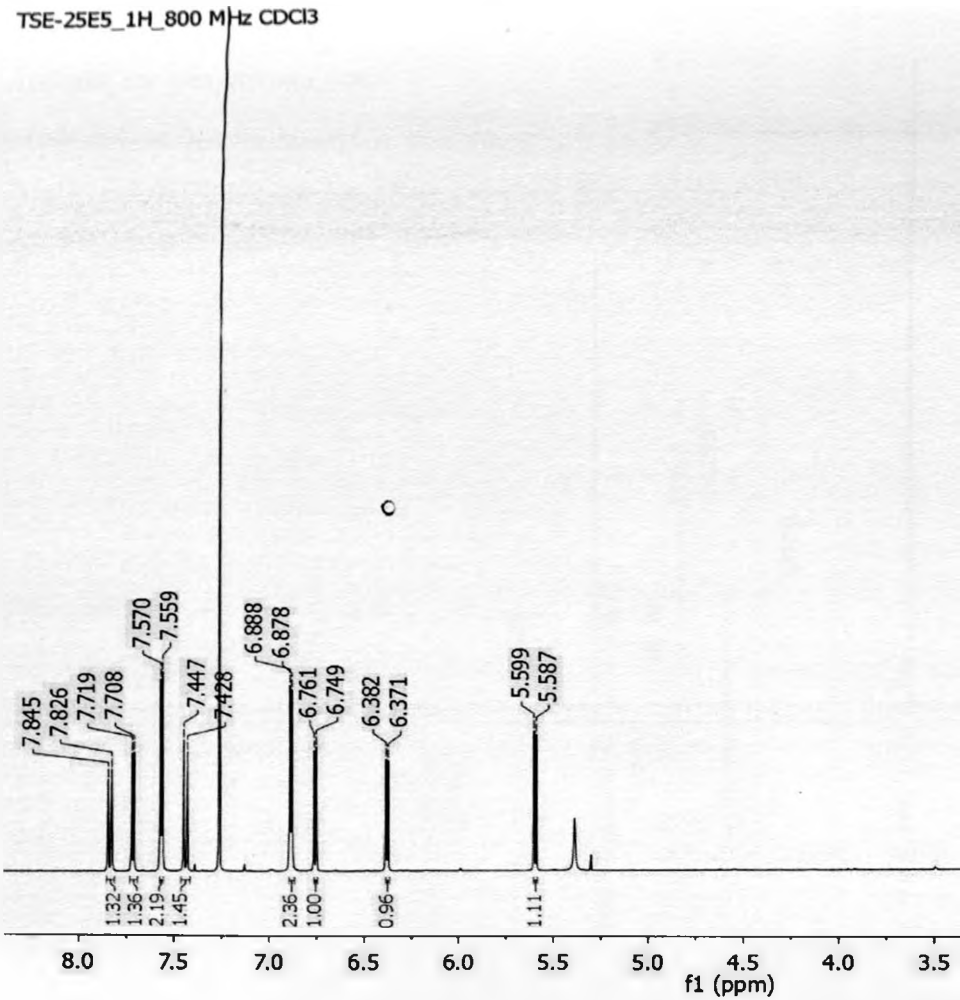
Appendix 12A: ^{1}H NMR (799.87 MHz) spectrum of compound 309

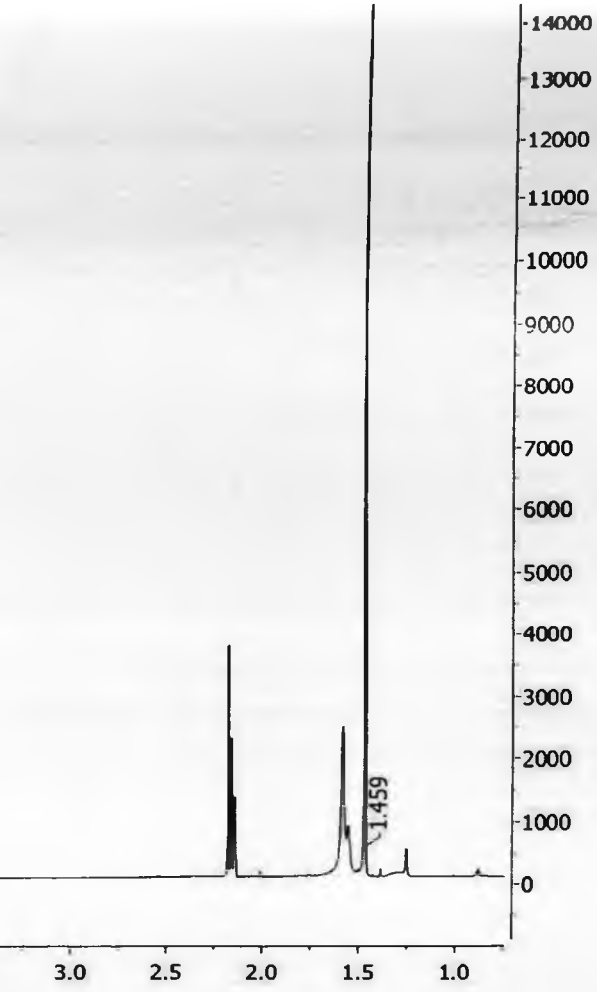


Appendix 12B: **^{13}C NMR (201.15 MHz) spectrum of compound 309**

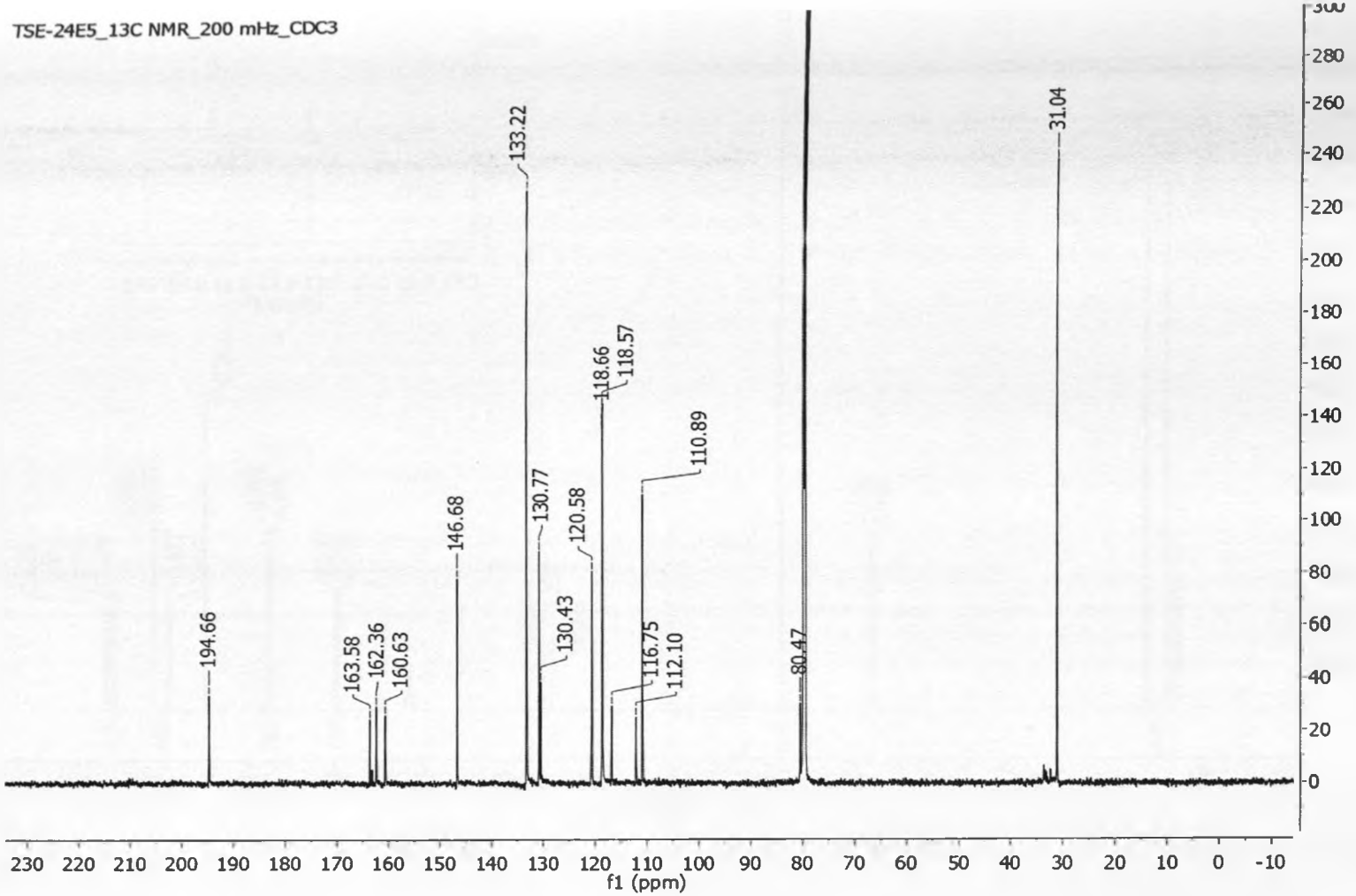


Appendix 13A: ^{1}H NMR (799.87 MHz) spectrum of compound 182



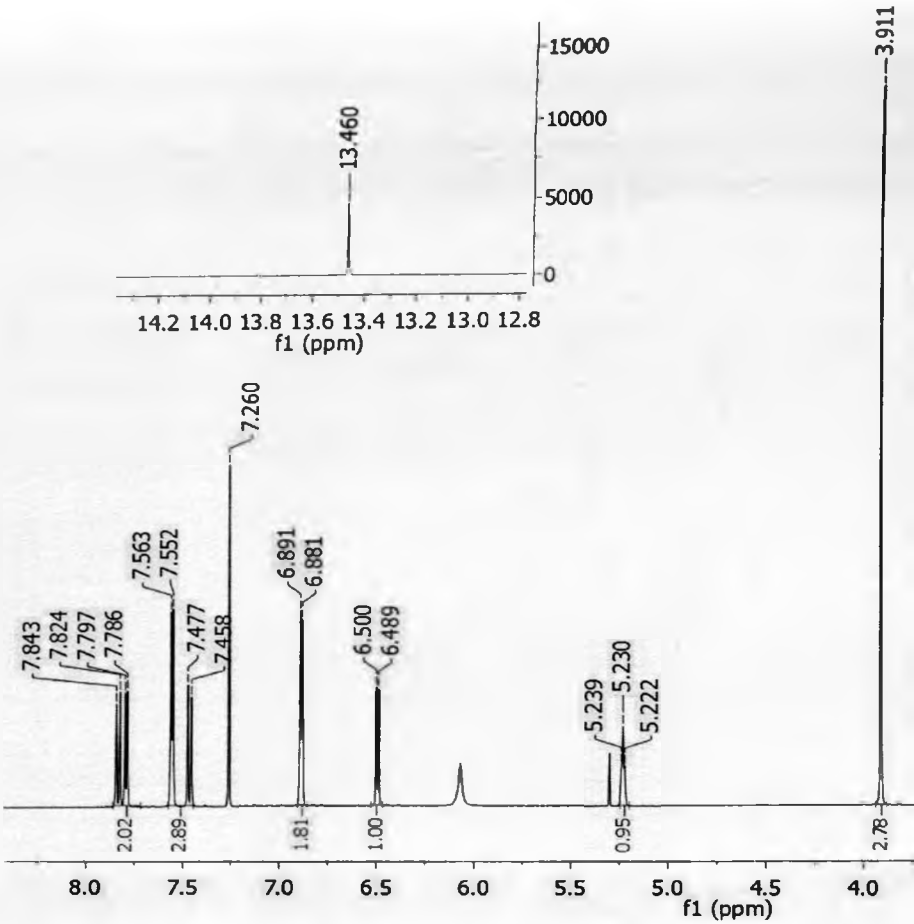

14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1.459
3.0
2.5
2.0
1.5
1.0

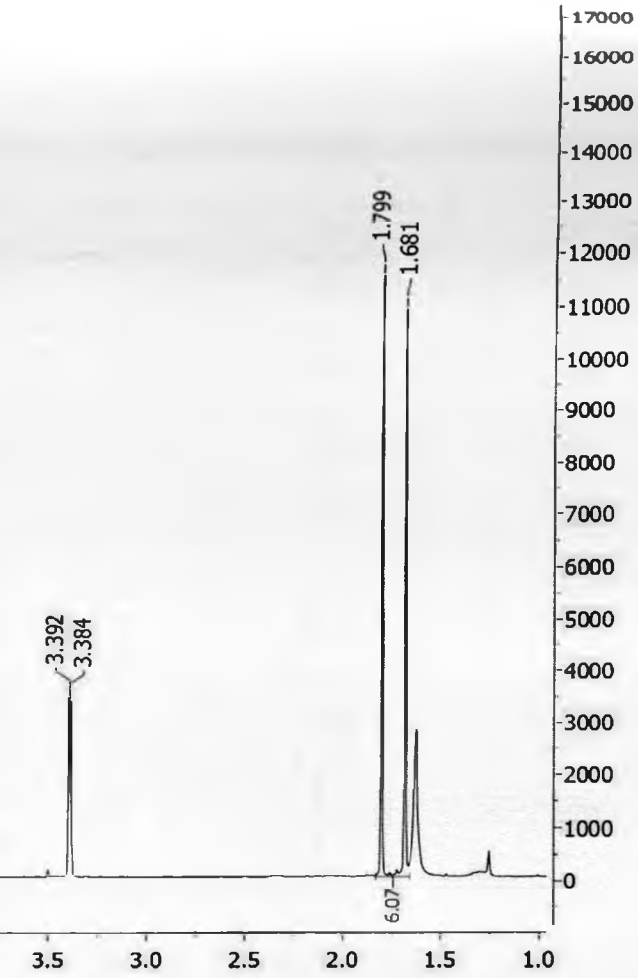
Appendix 13B: ^{13}C NMR (201.15 MHz) spectrum of compound 182



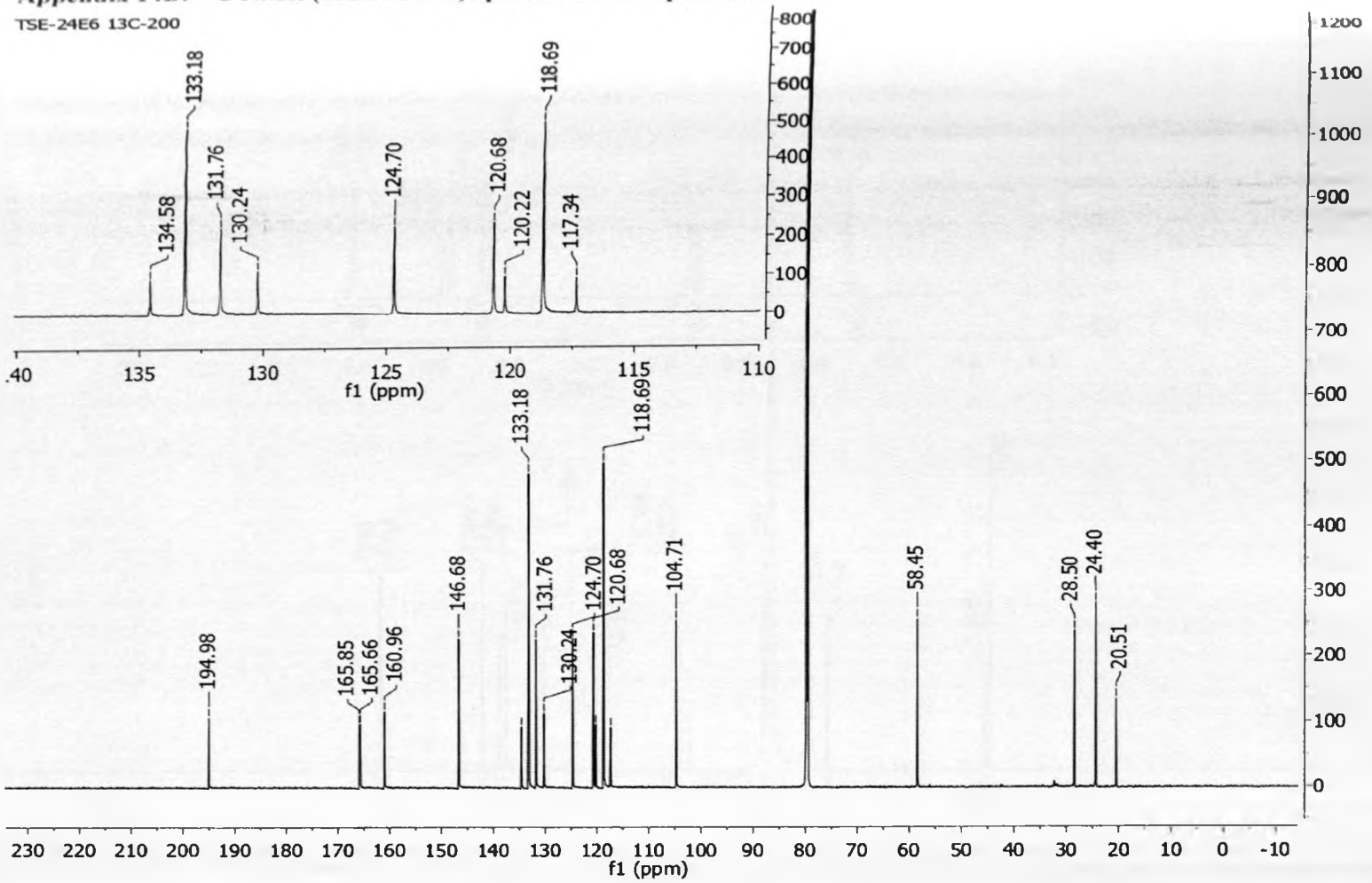
Appendix 14A: **^{1}H NMR (799.87 MHz) spectrum of compound 311**

TSE-24E6_13C NMR_800 MHz_CDC3



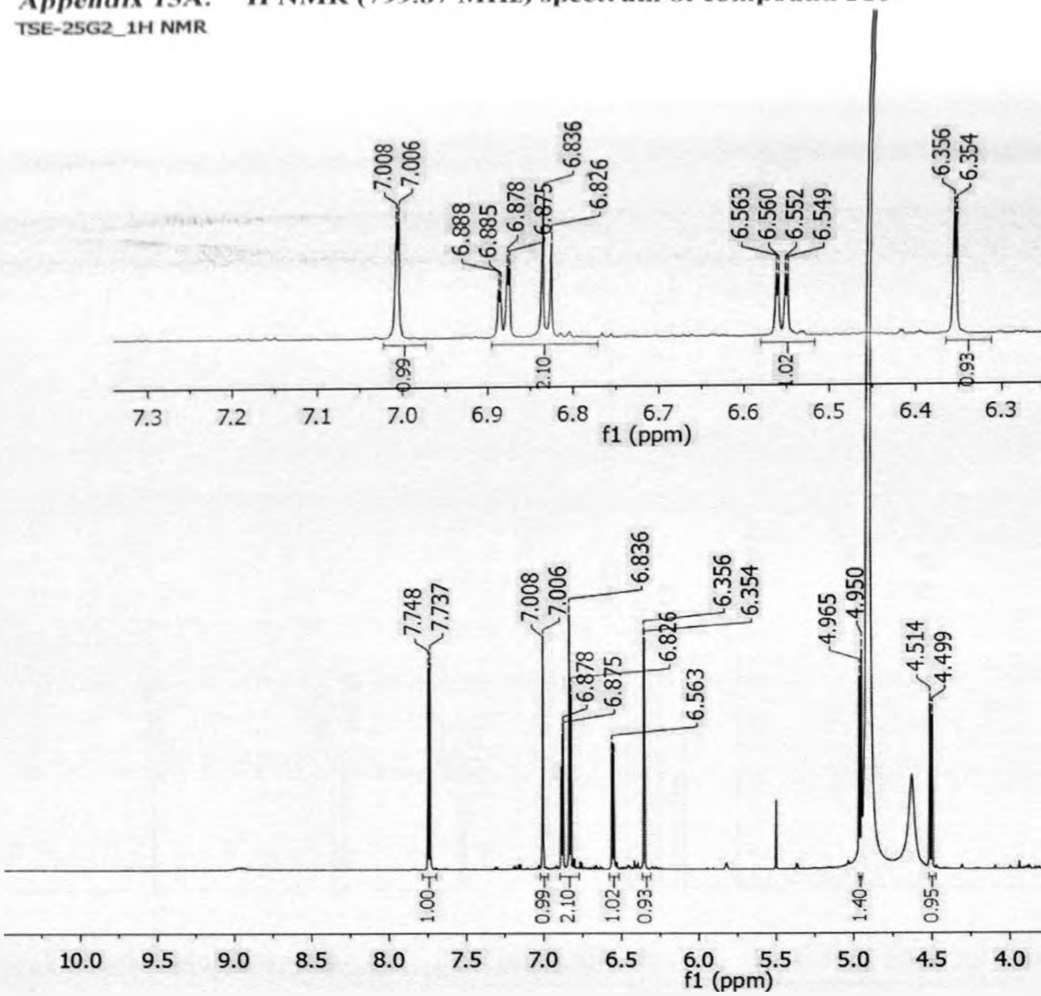

17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
1.799
1.681
3.392
3.384
6.07
3.5
3.0
2.5
2.0
1.5
1.0

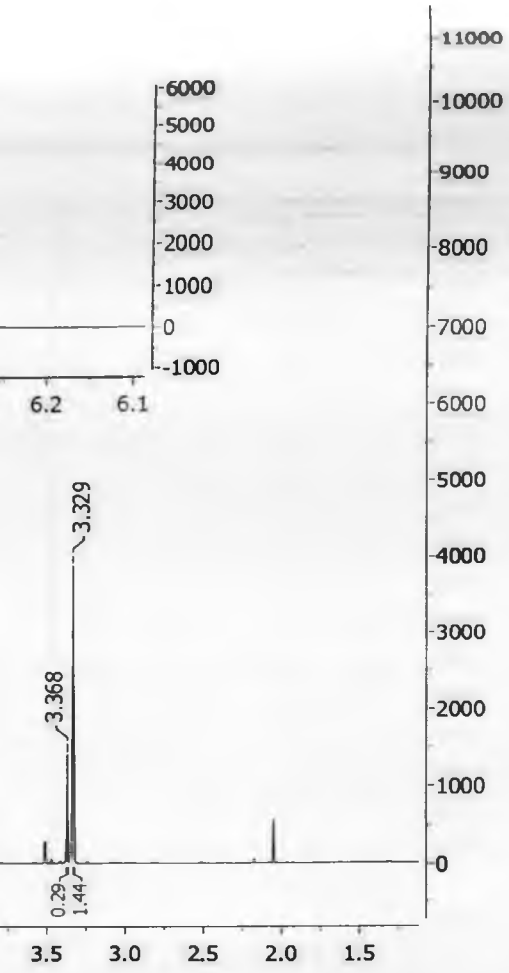
Appendix 14B: ^{13}C NMR (201.15 MHz) spectrum of compound 311



Appendix 15A: ^{1}H NMR (799.87 MHz) spectrum of compound 310

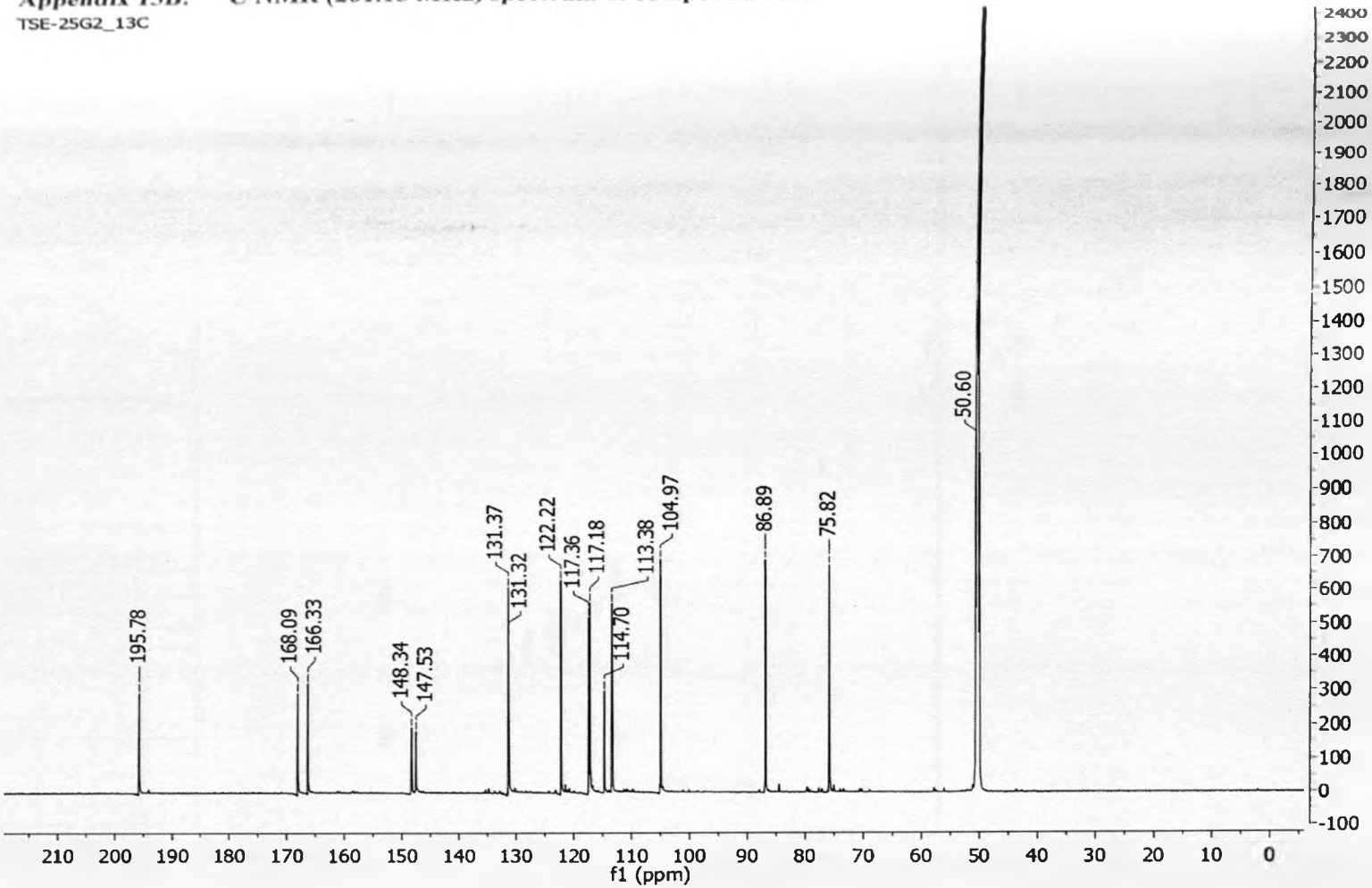
TSE-25G2_1H NMR



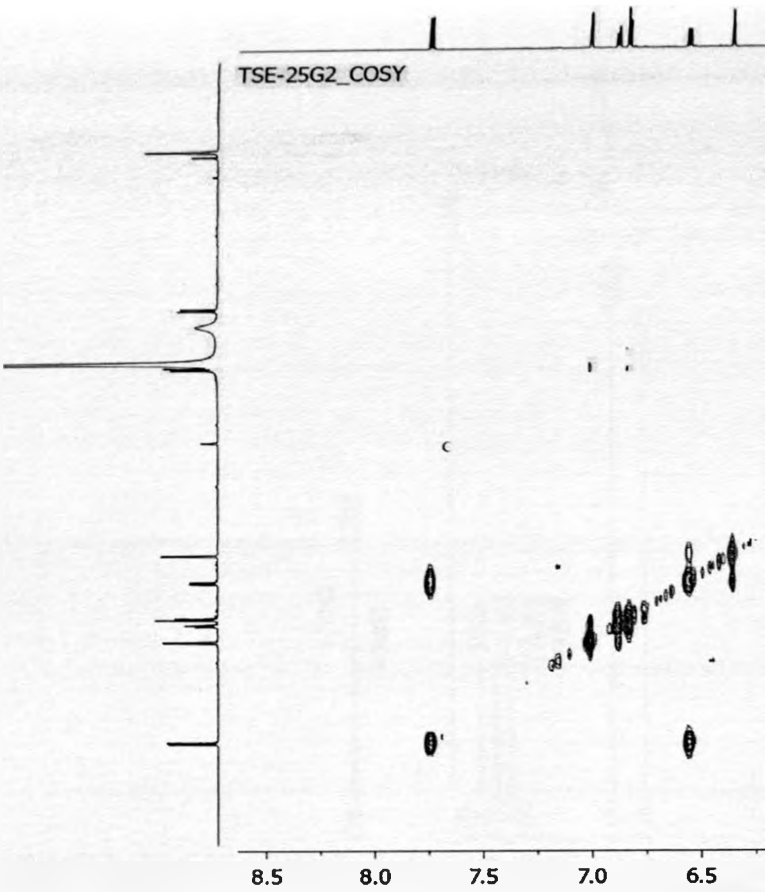

6000
5000
4000
3000
2000
1000
0
-1000
6.2
6.1
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
3.329
3.368
0.29
1.44
3.5
3.0
2.5
2.0
1.5

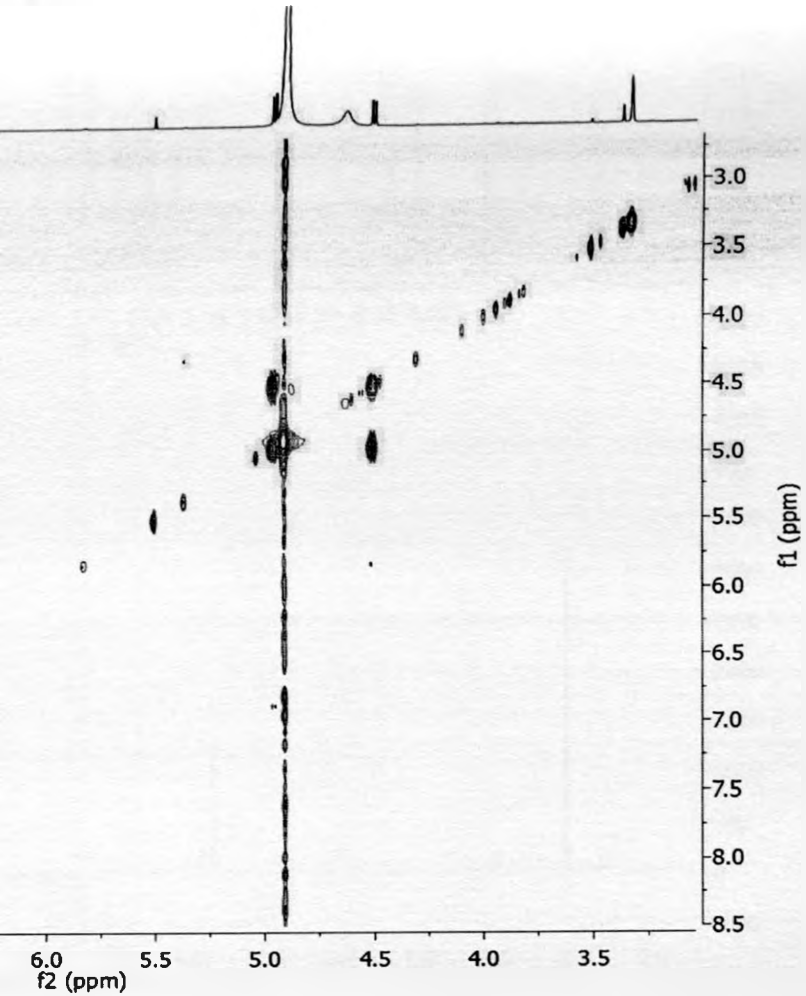
Appendix 15B: ***^{13}C NMR (201.15 MHz) spectrum of compound 310.***

TSE-25G2_13C

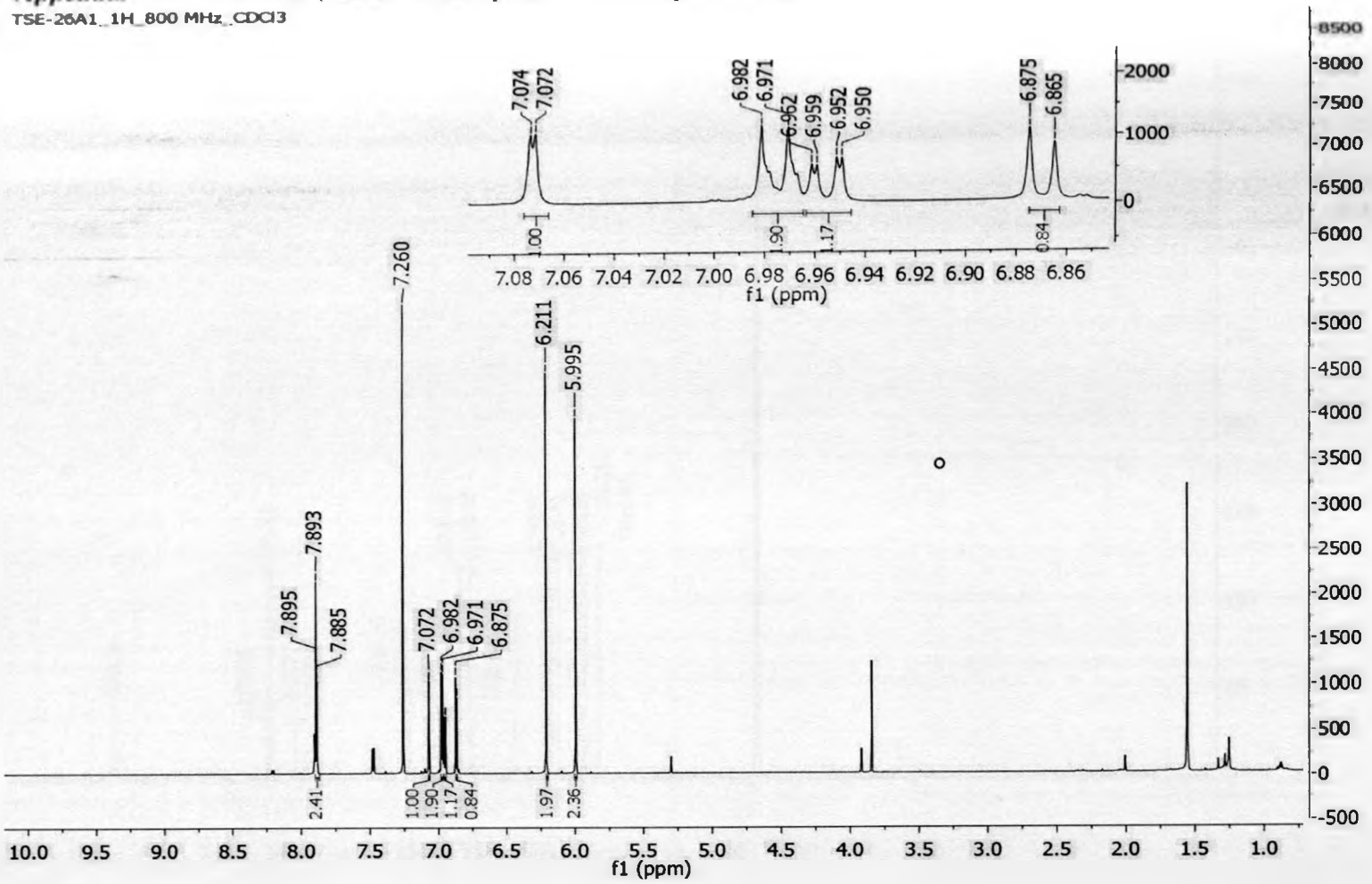


Appendix 15C: COSY (799.87 MHz) spectrum of compou

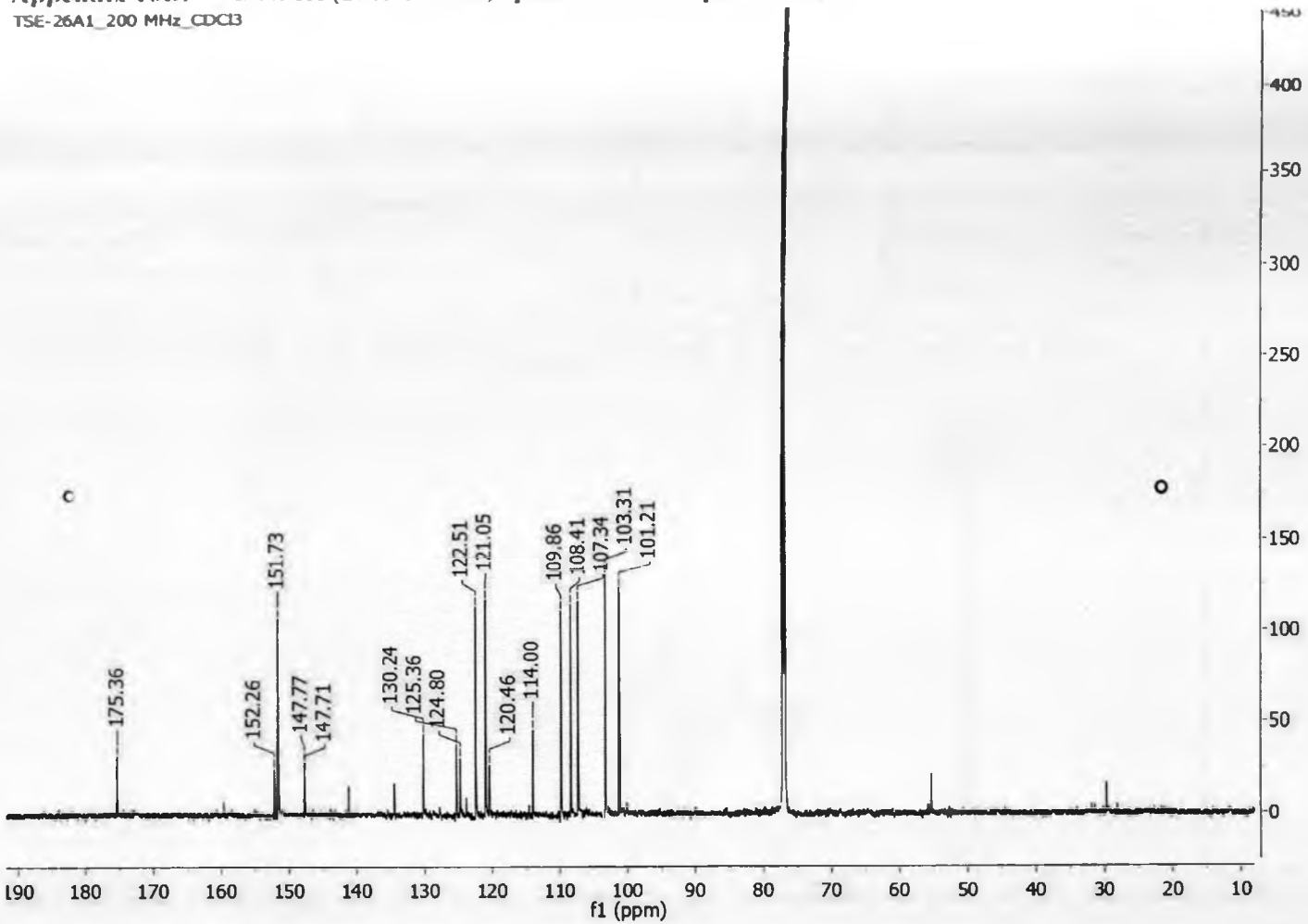



3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
f2 (ppm)

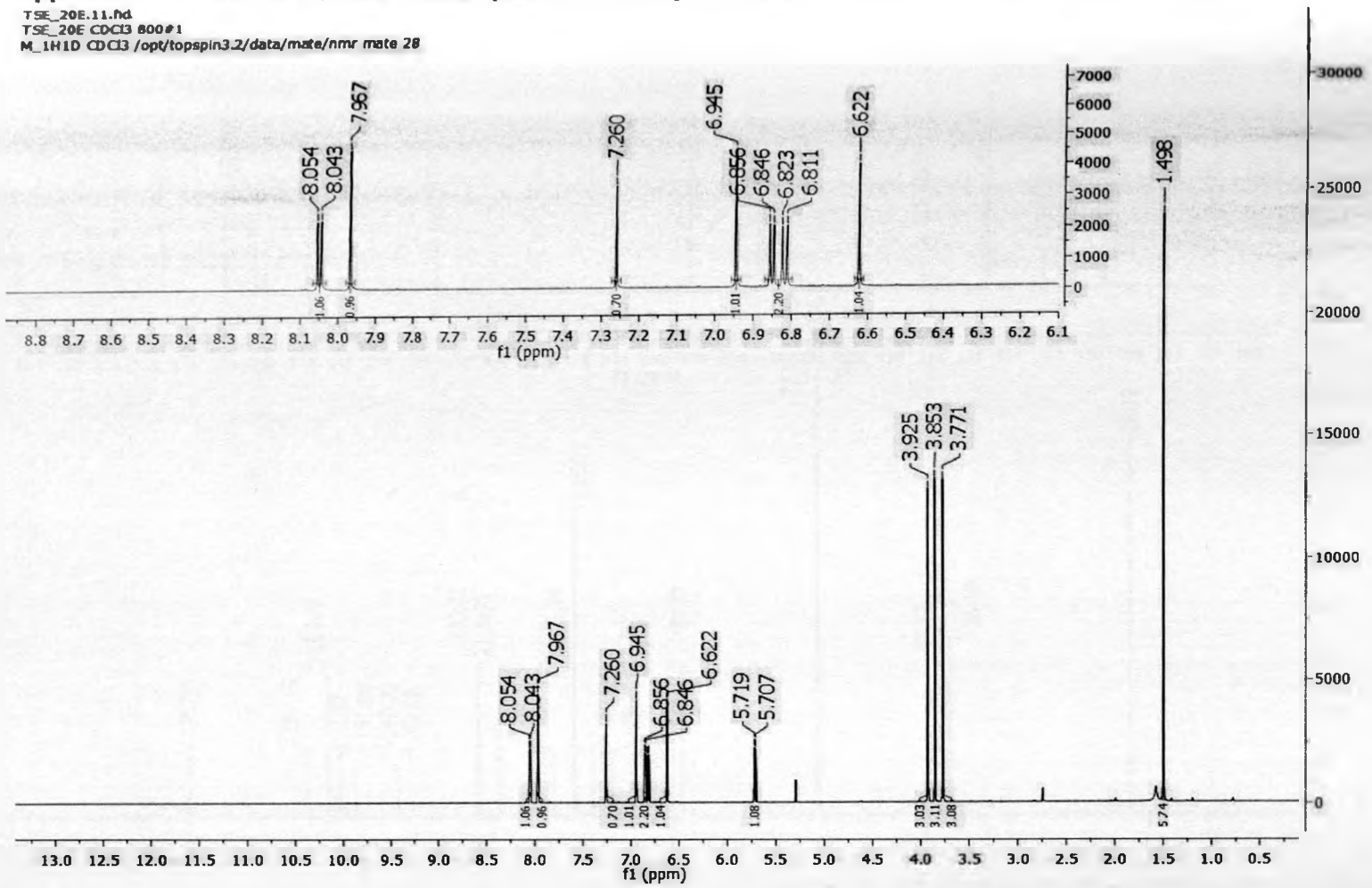
Appendix 16A: ^{1}**H NMR (799.87 MHz) spectrum of compound 308**



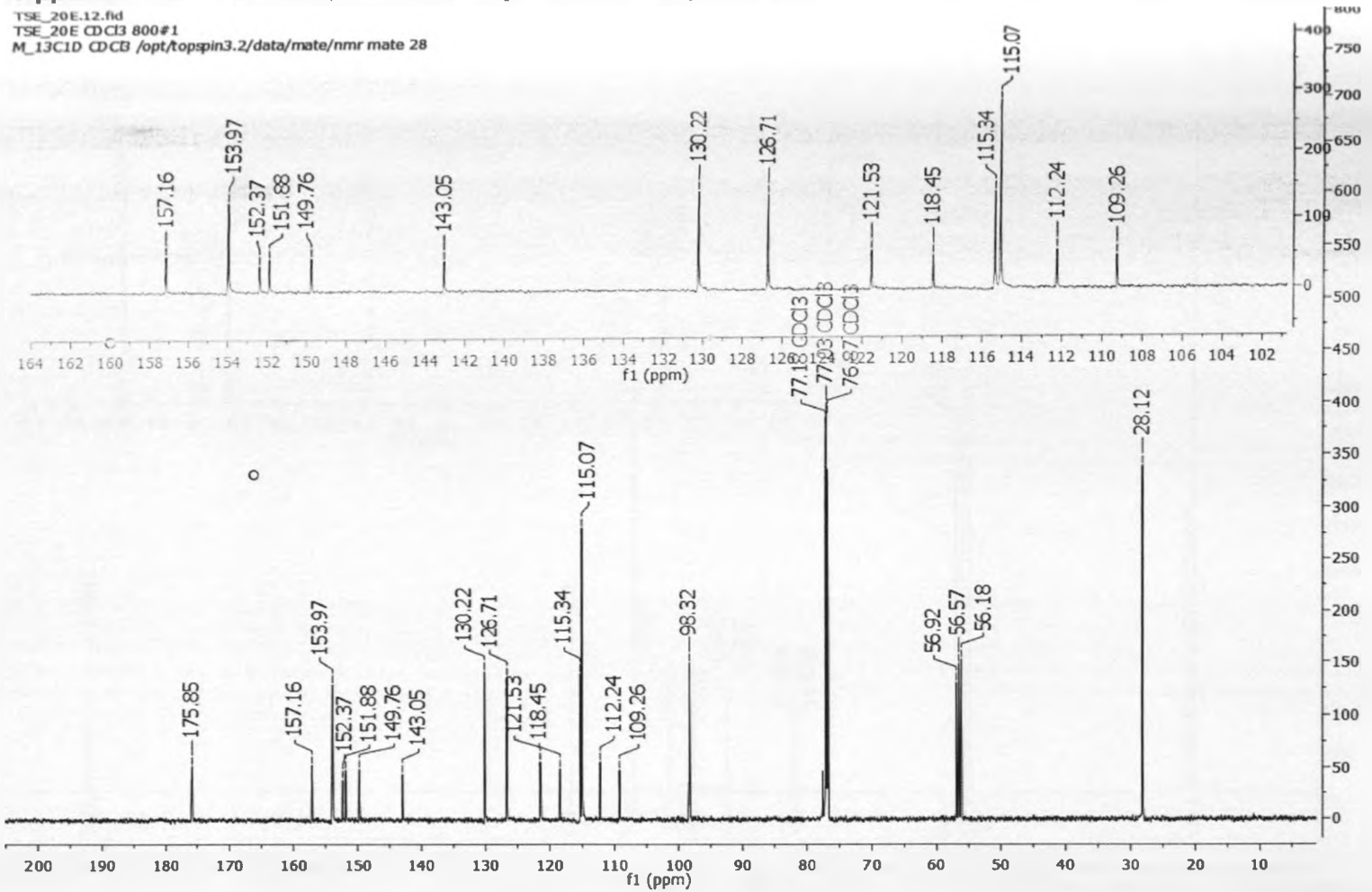
Appendix 16B: ^{13}C *NMR (201.15 MHz) spectrum of compound 308*



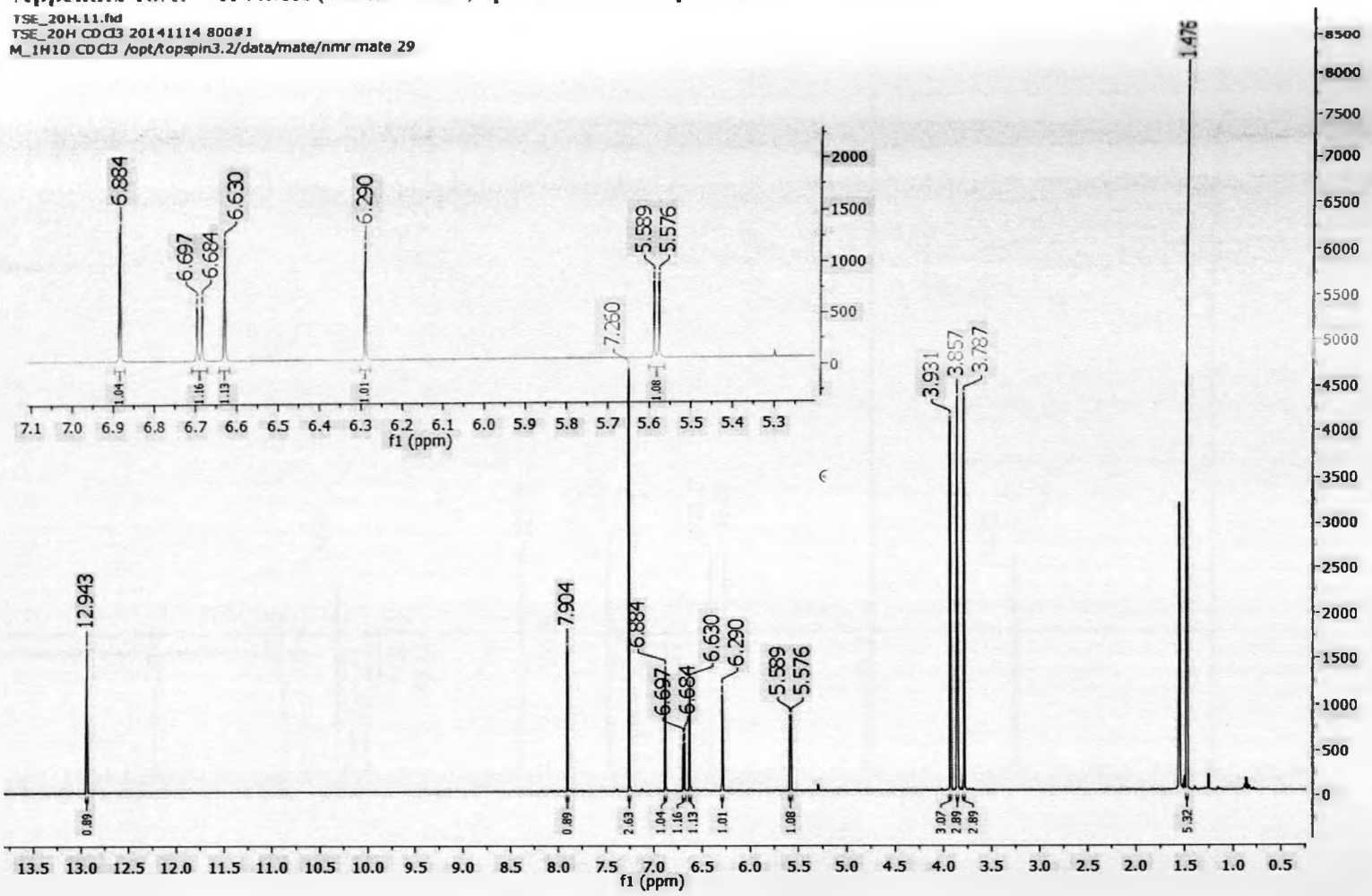
Appendix 17A: ^1H NMR (799.87 MHz) spectrum of compound 55



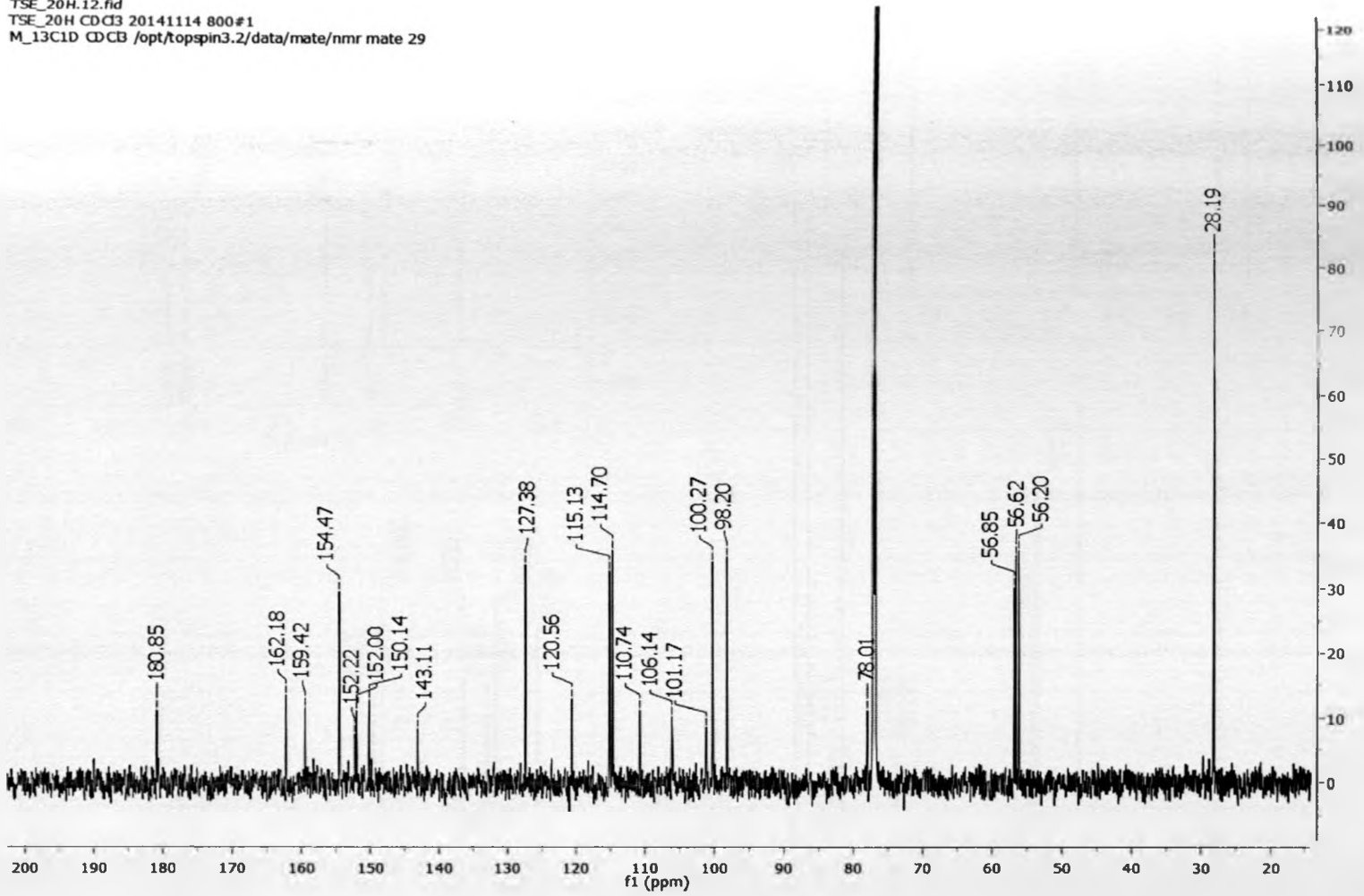
Appendix 17B: ^{13}C NMR (201.15 MHz) spectrum of compound 55



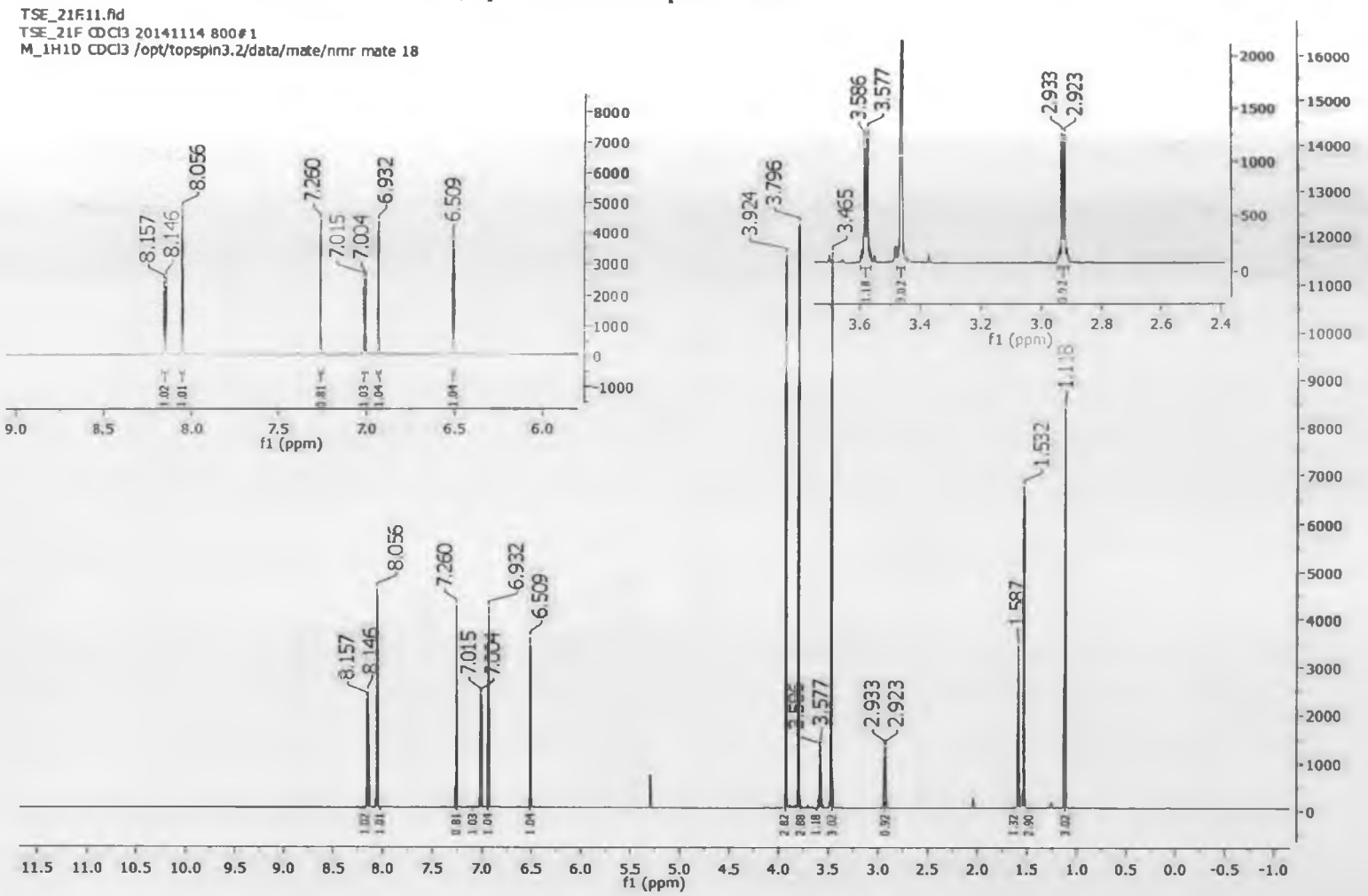
Appendix 18A: ^1H NMR (799.87 MHz) spectrum of compound 37



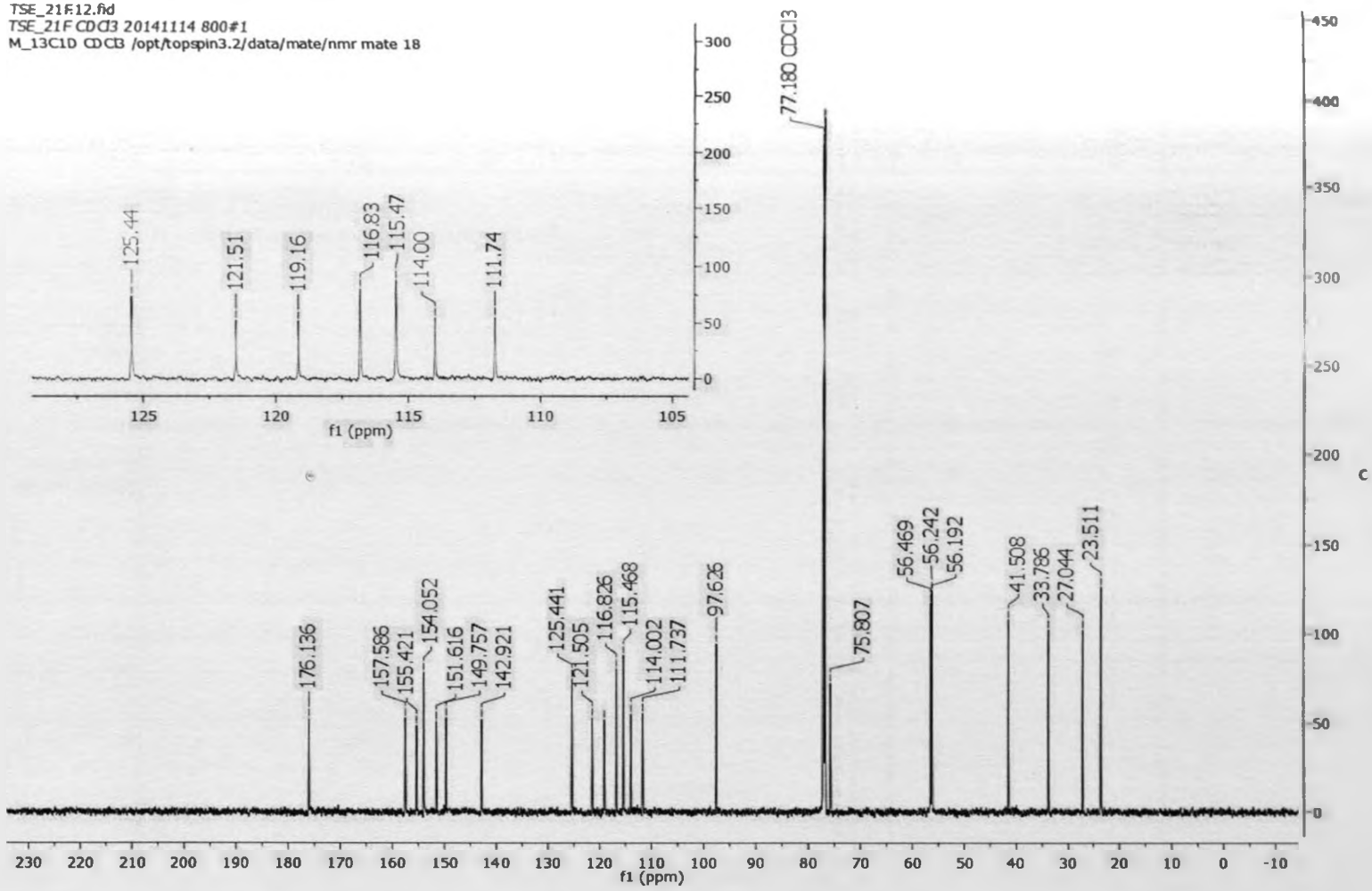
Appendix 18B: ^{13}C NMR (201.15 MHz) spectrum of compound 37



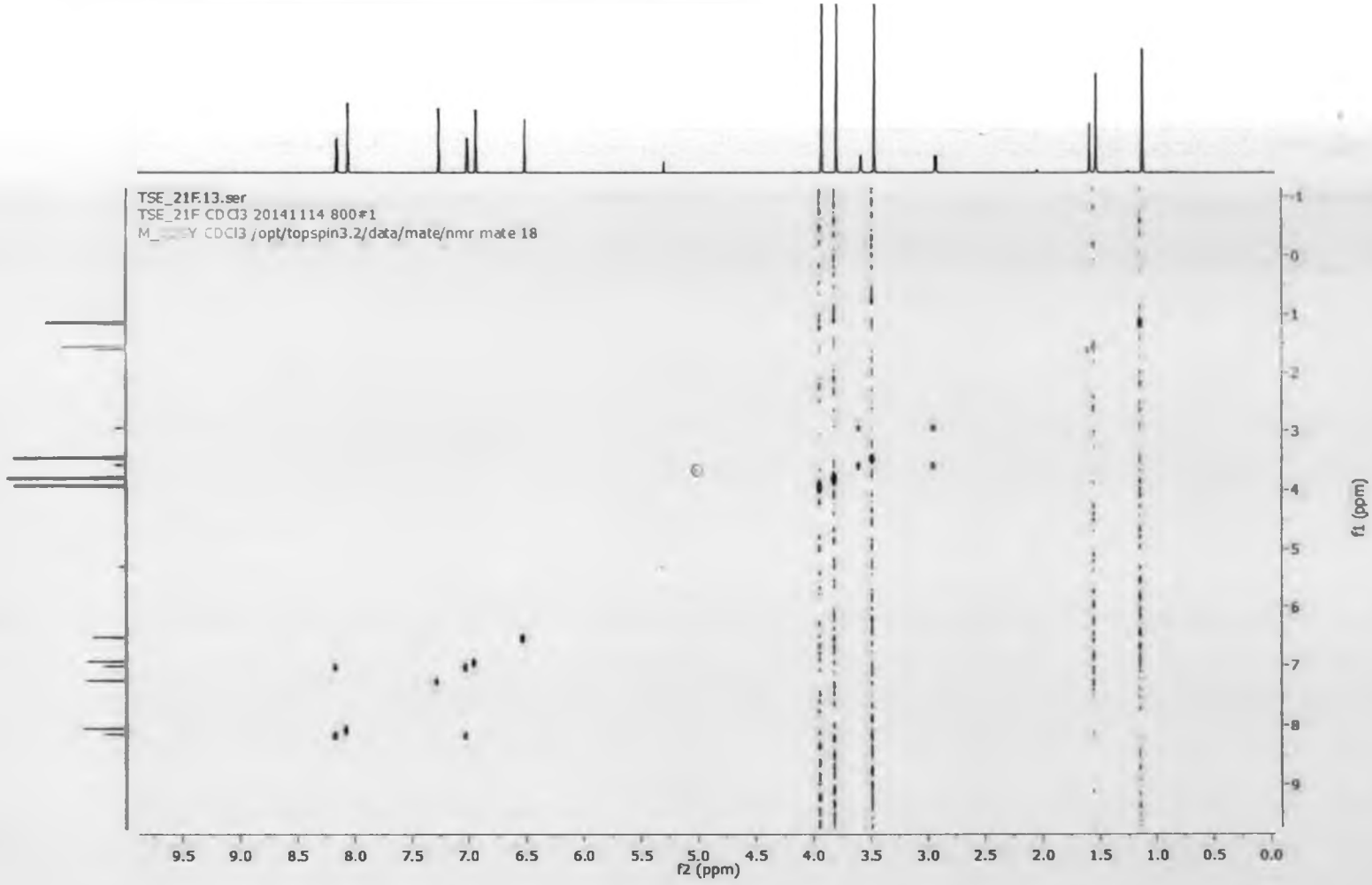
Appendix 19A: ^{1}H NMR (799.87 MHz) spectrum of compound 312



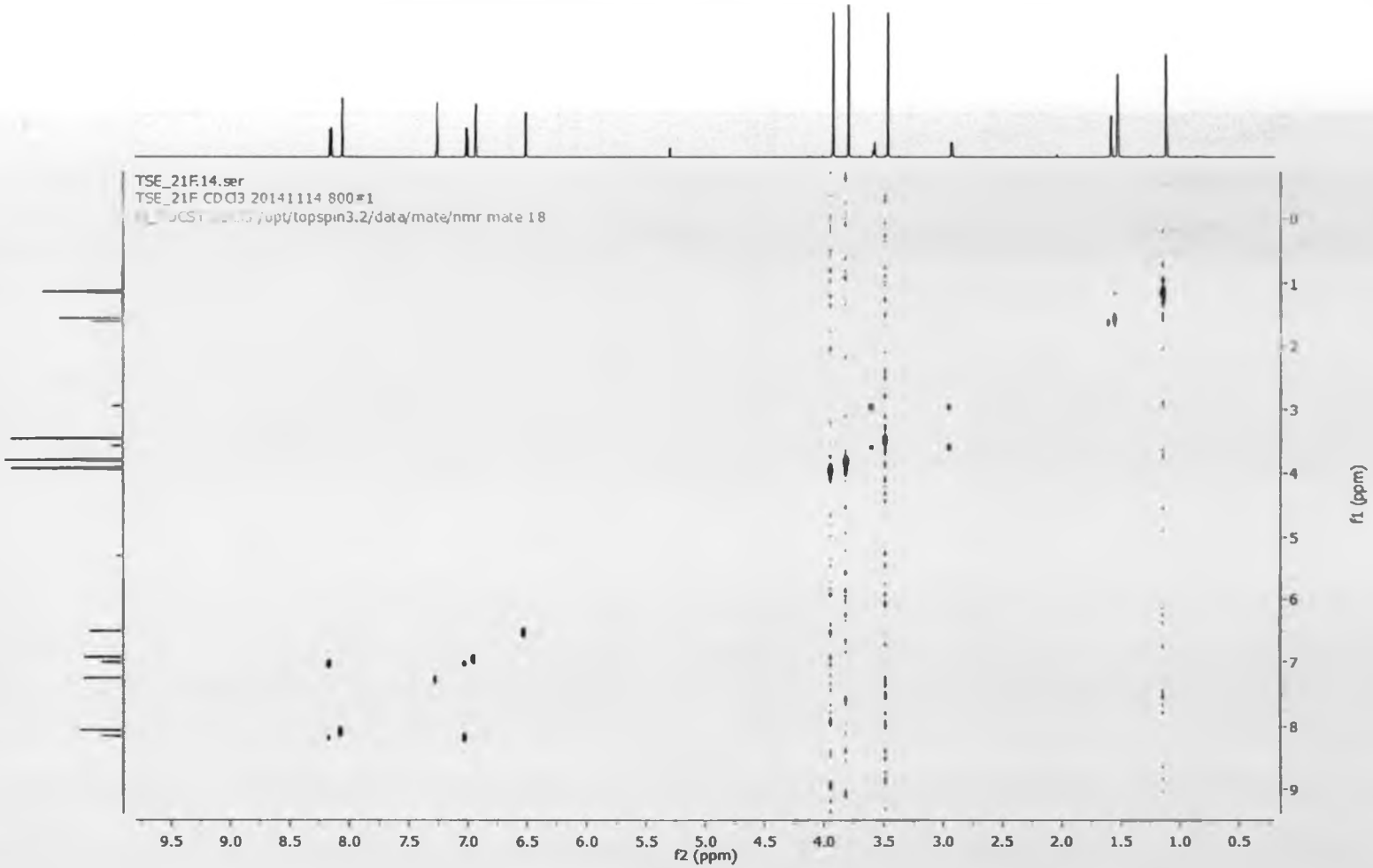
Appendix 19B: ^{13}C NMR (201.15 MHz) spectrum of compound 312



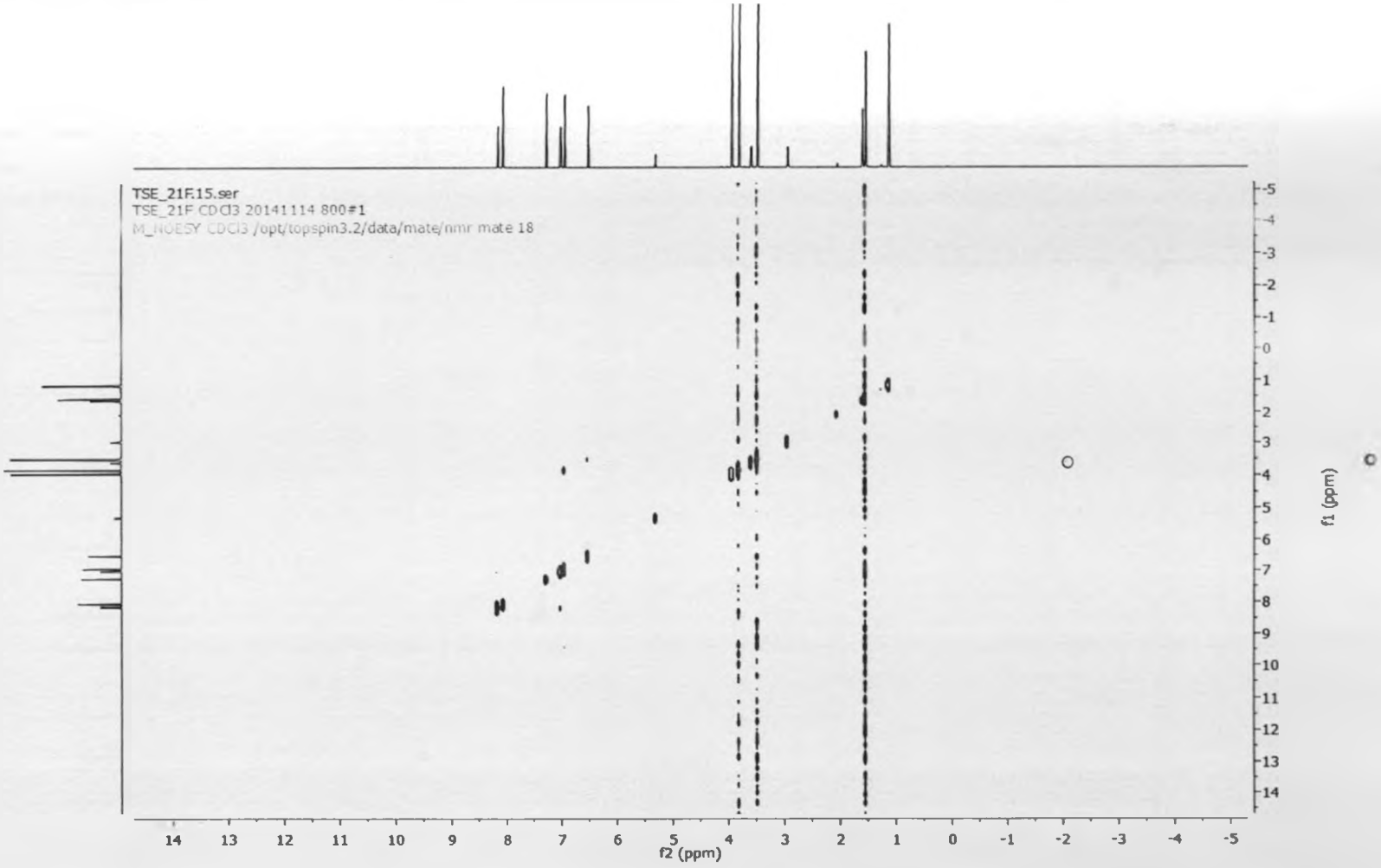
Appendix 19C: COSY (799.87 MHz) spectrum of compound 312



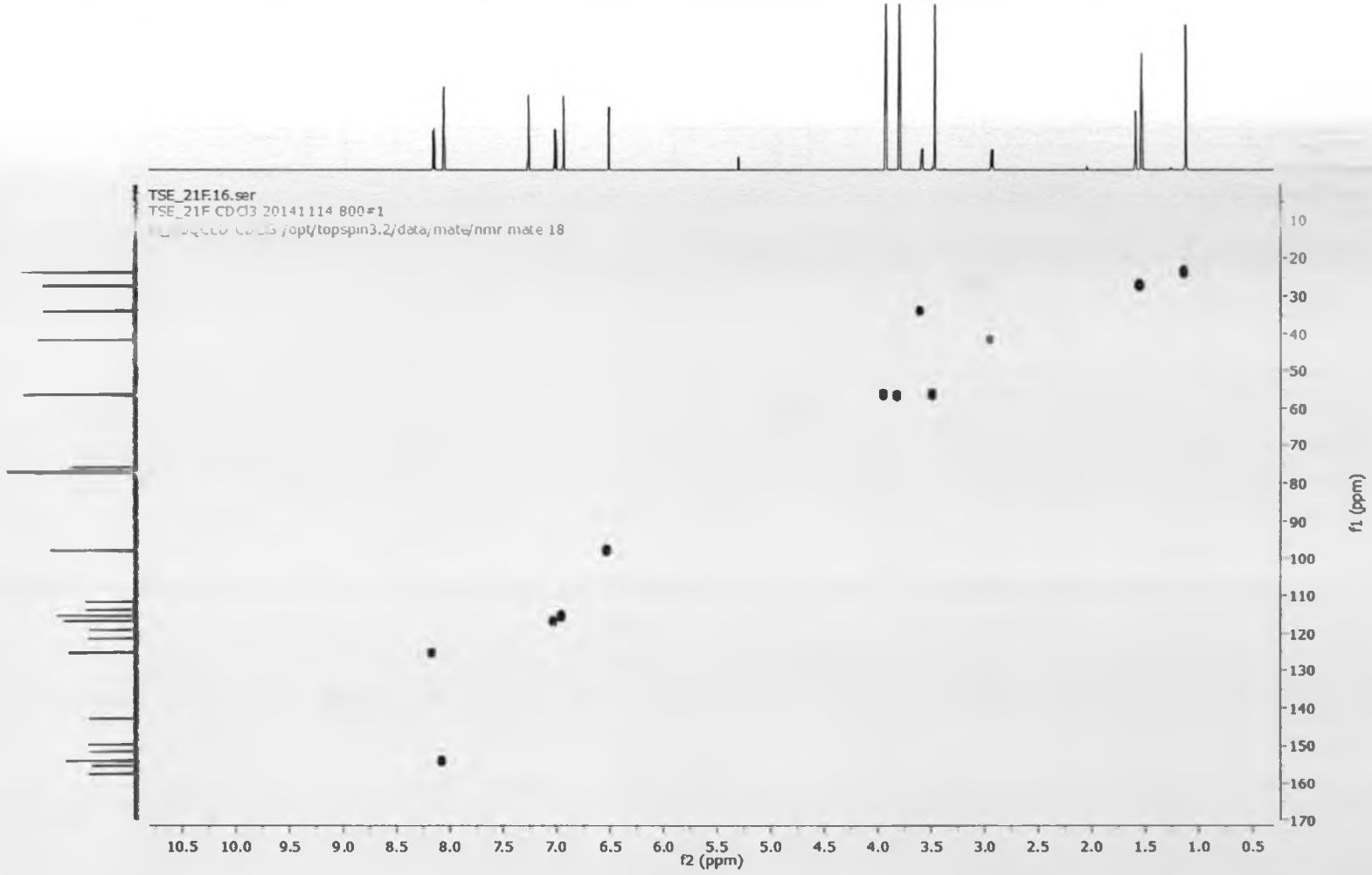
Appendix 19D: TOCSY (799.87 MHz) spectrum of compound 312



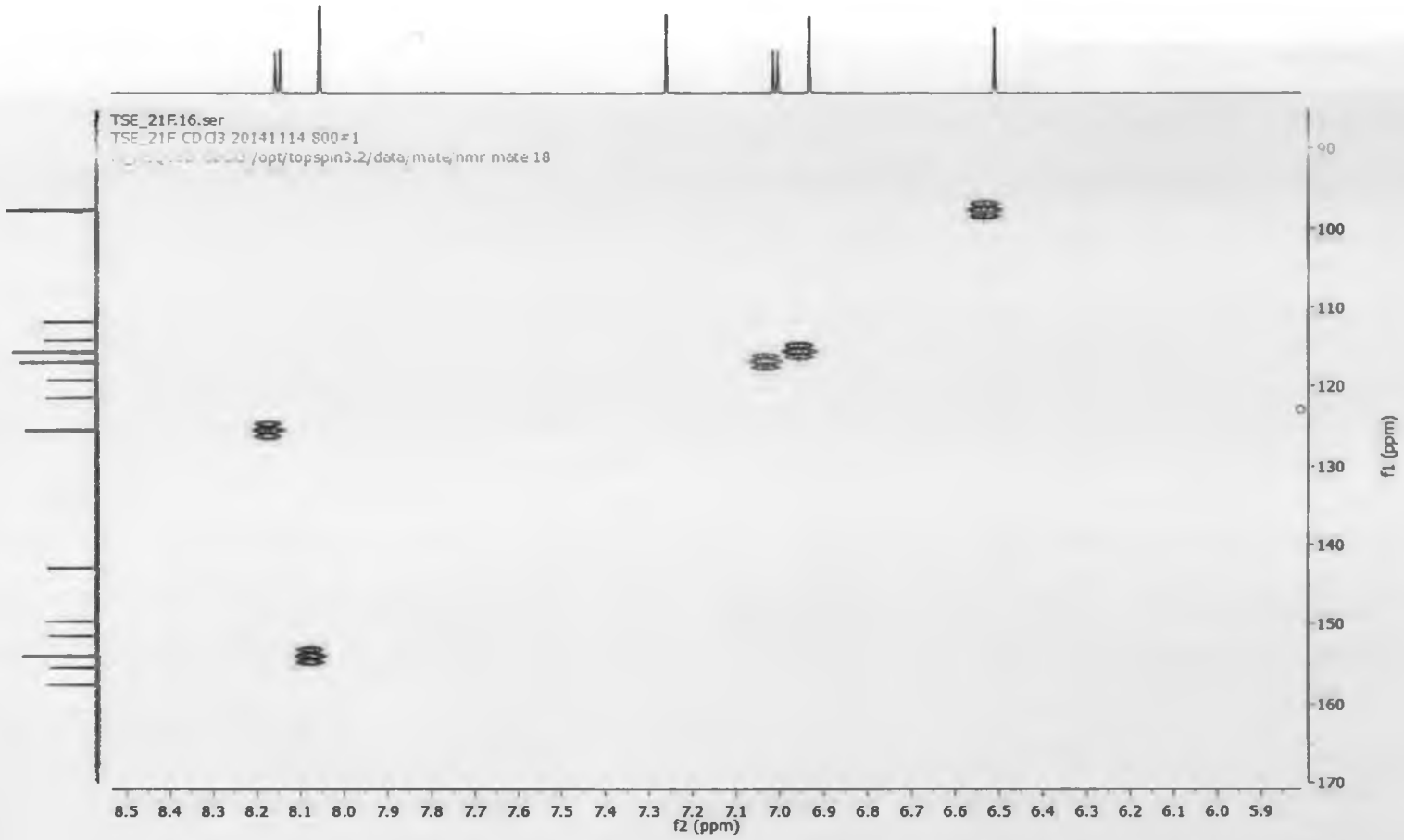
Appendix 19E: NOESY (799.87 MHz) spectrum of compound 312



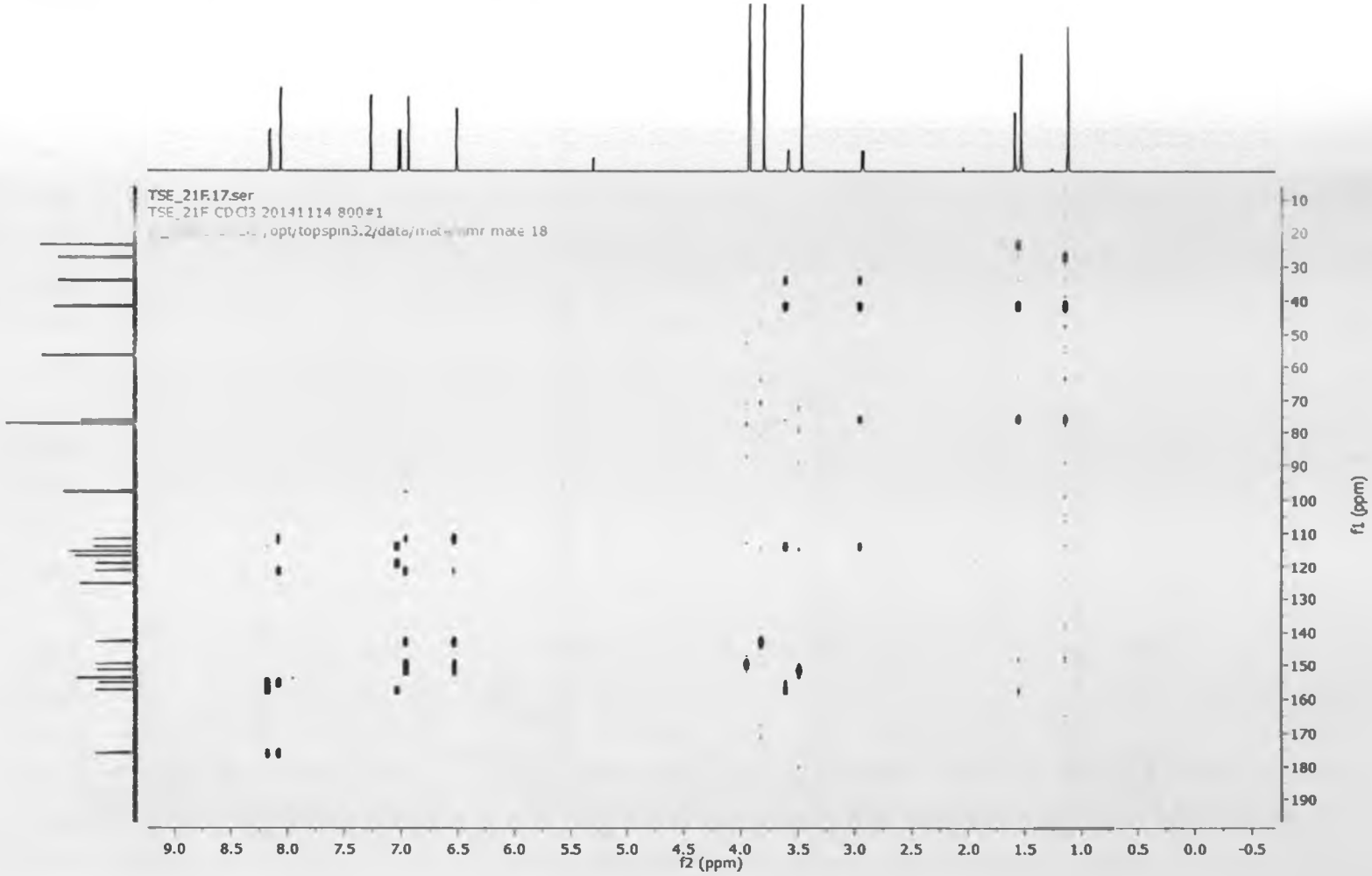
Appendix 19F: HSQC (799.87/201.15 MHz) spectrum of compound 312



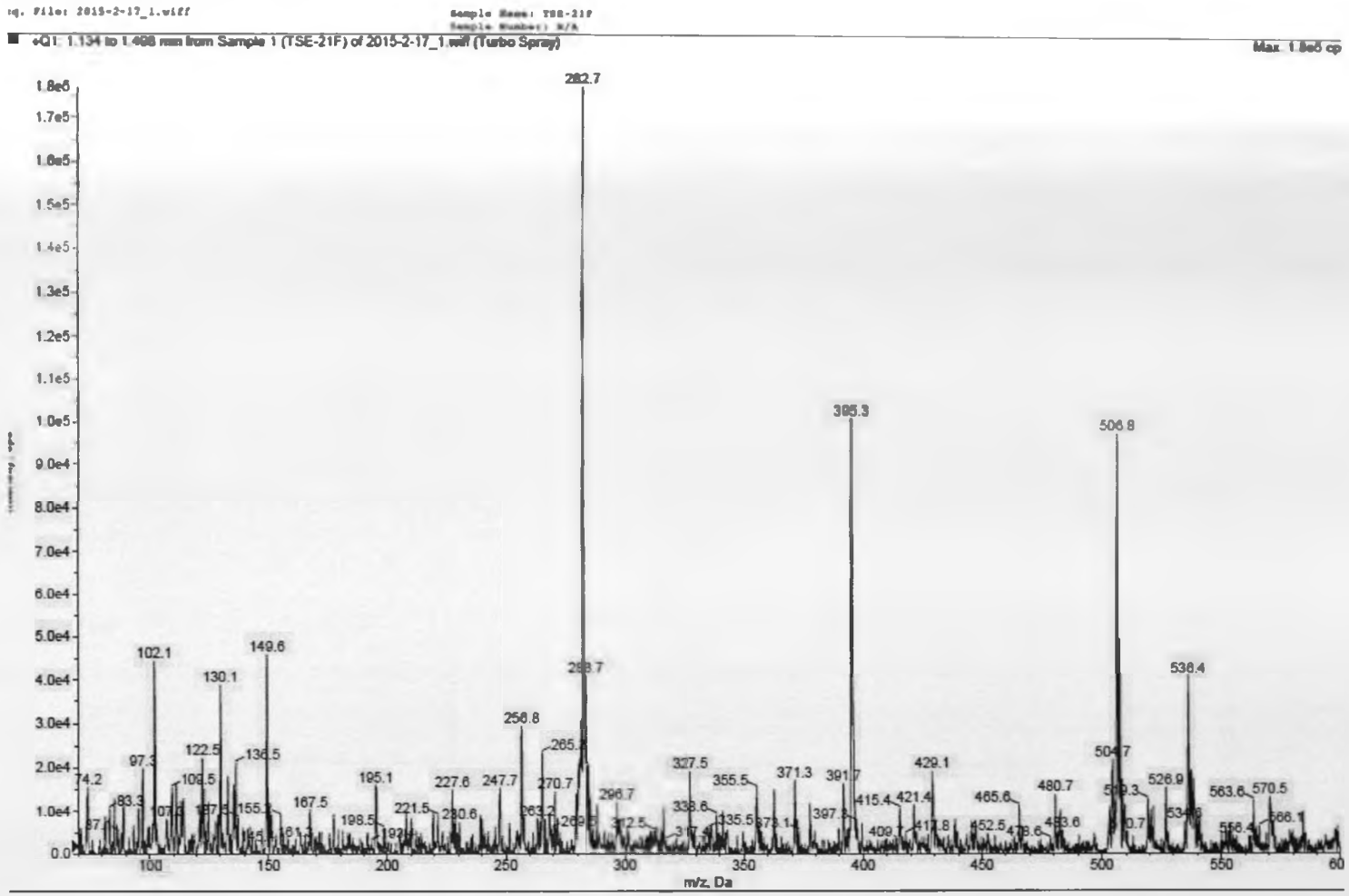
Appendix 19G: HMBC expanded (799.87/201.15 MHz) spectrum of compound 312



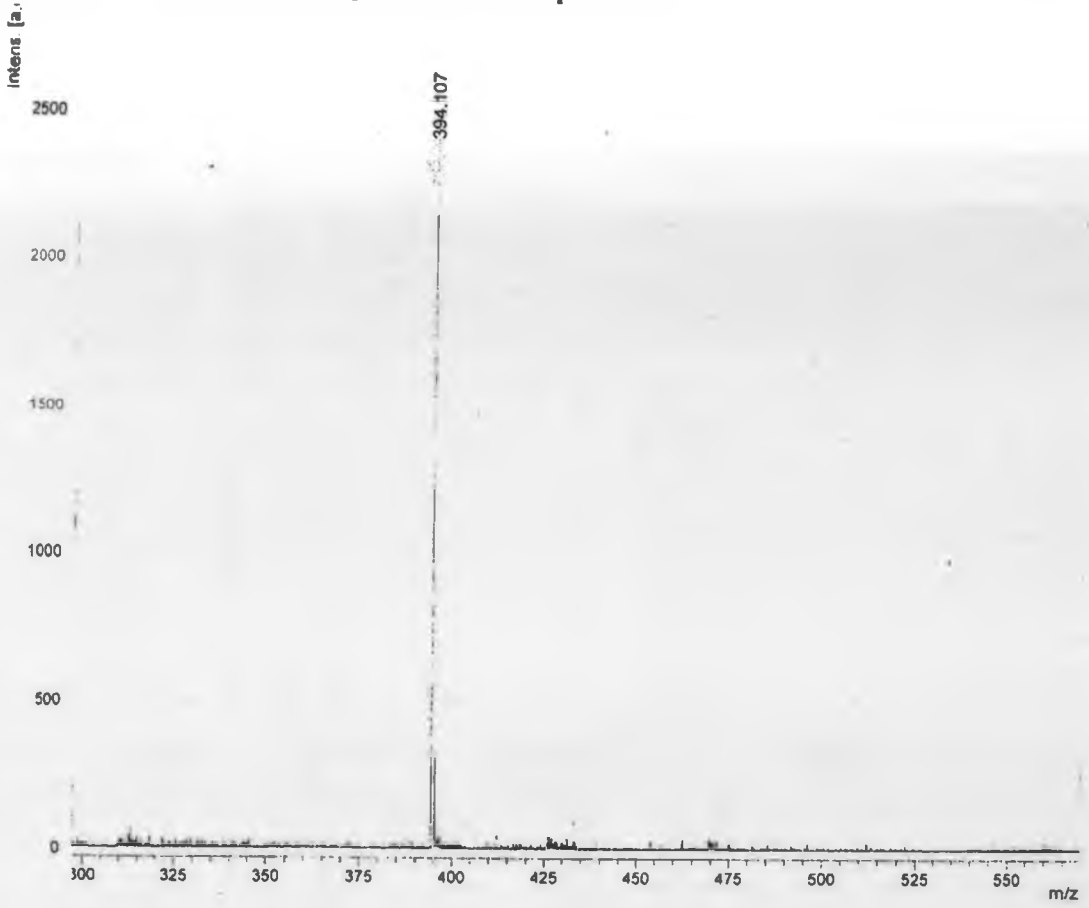
Appendix 19H: HMBC (799.87/201.15 MHz) spectrum of compound 312



Appendix 191: ESIMS spectrum of compound 312

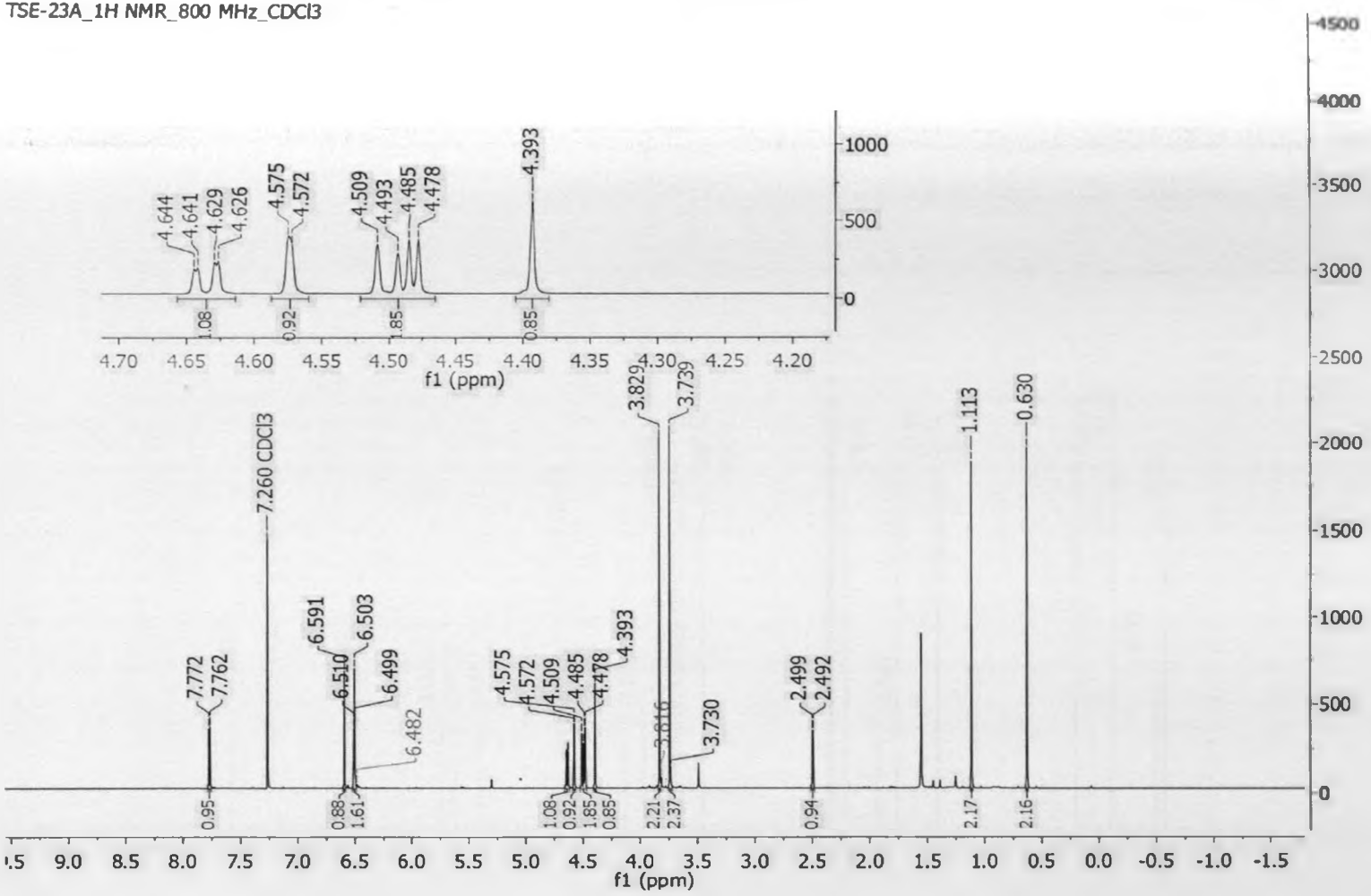


Appendix 19J: MALDI-TOF spectrum of compound 312

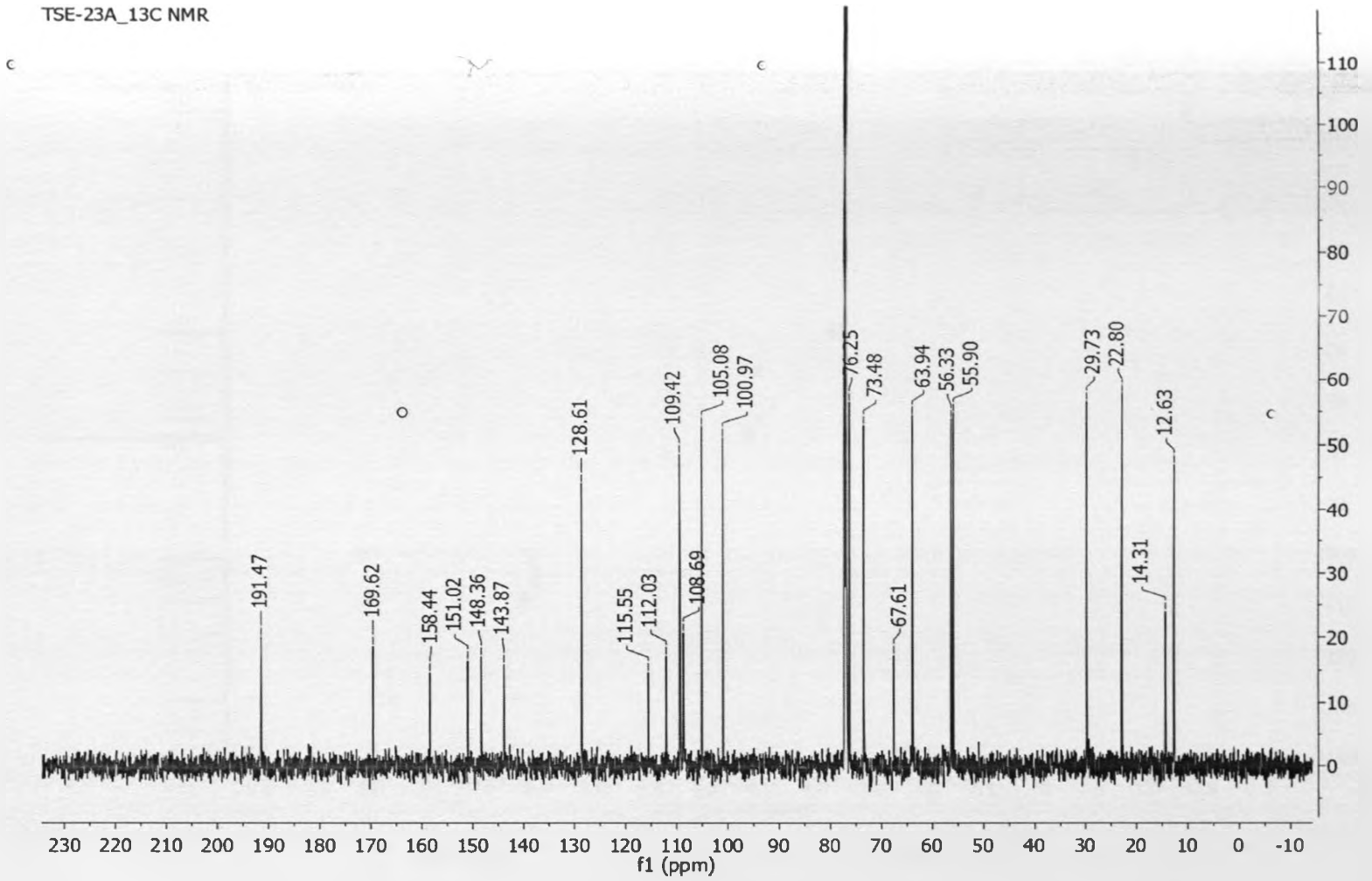


Appendix 20A: ^{1}H NMR (799.87 MHz) spectrum of compound 313

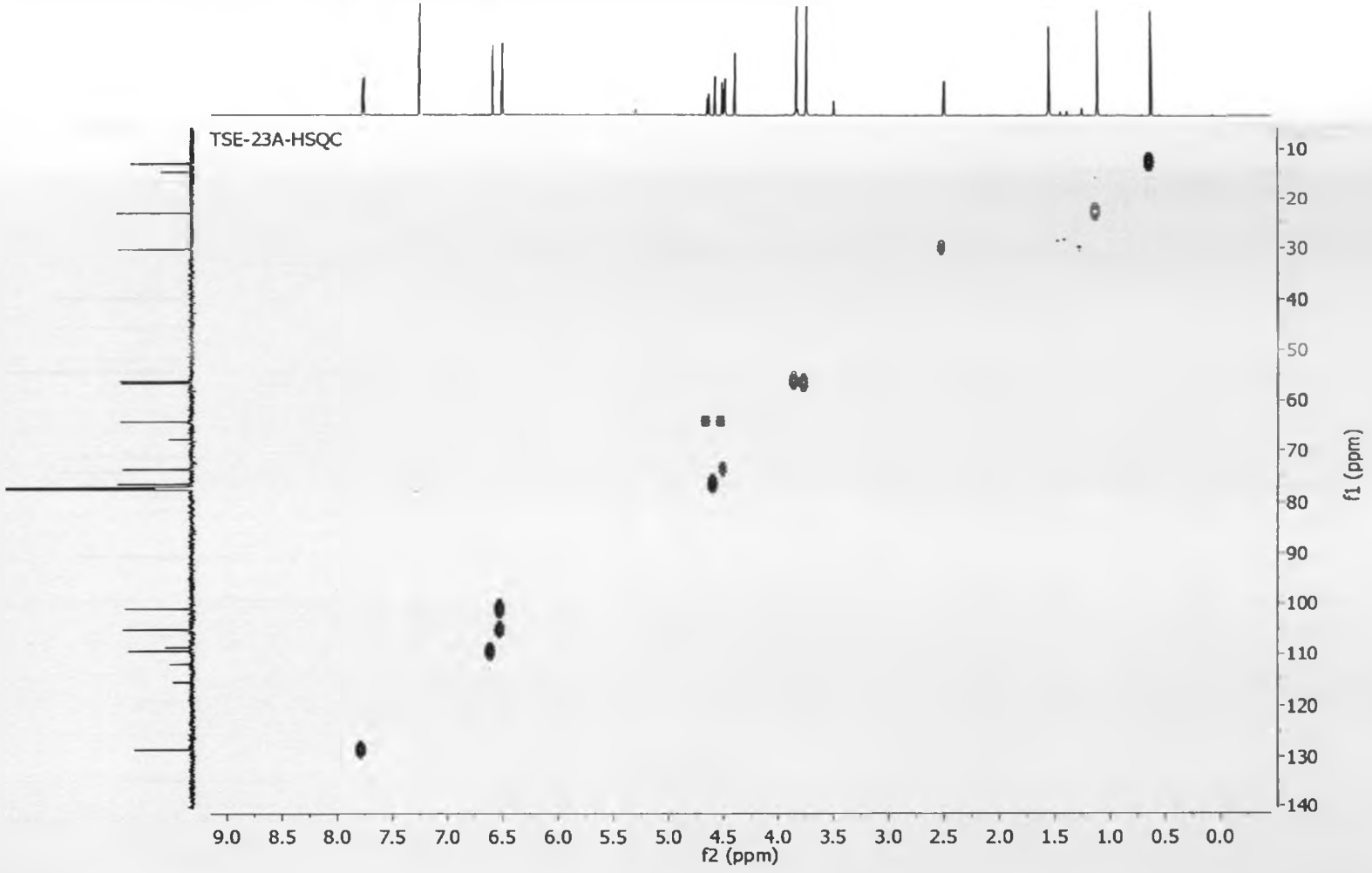
TSE-23A_1H NMR_800 MHz_CDCl3



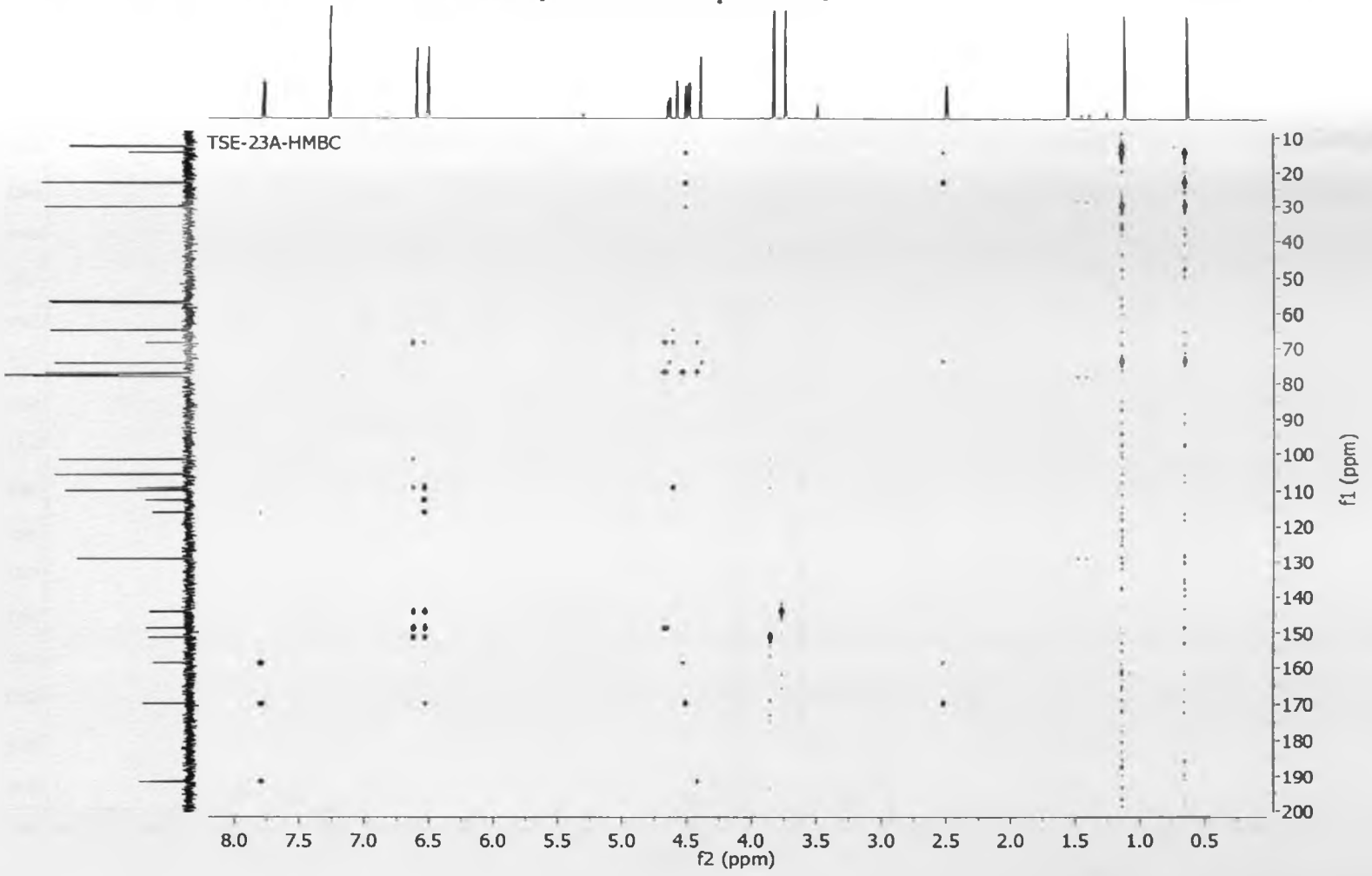
Appendix 20B: ^{13}C NMR (201.15 MHz) spectrum of compound 313



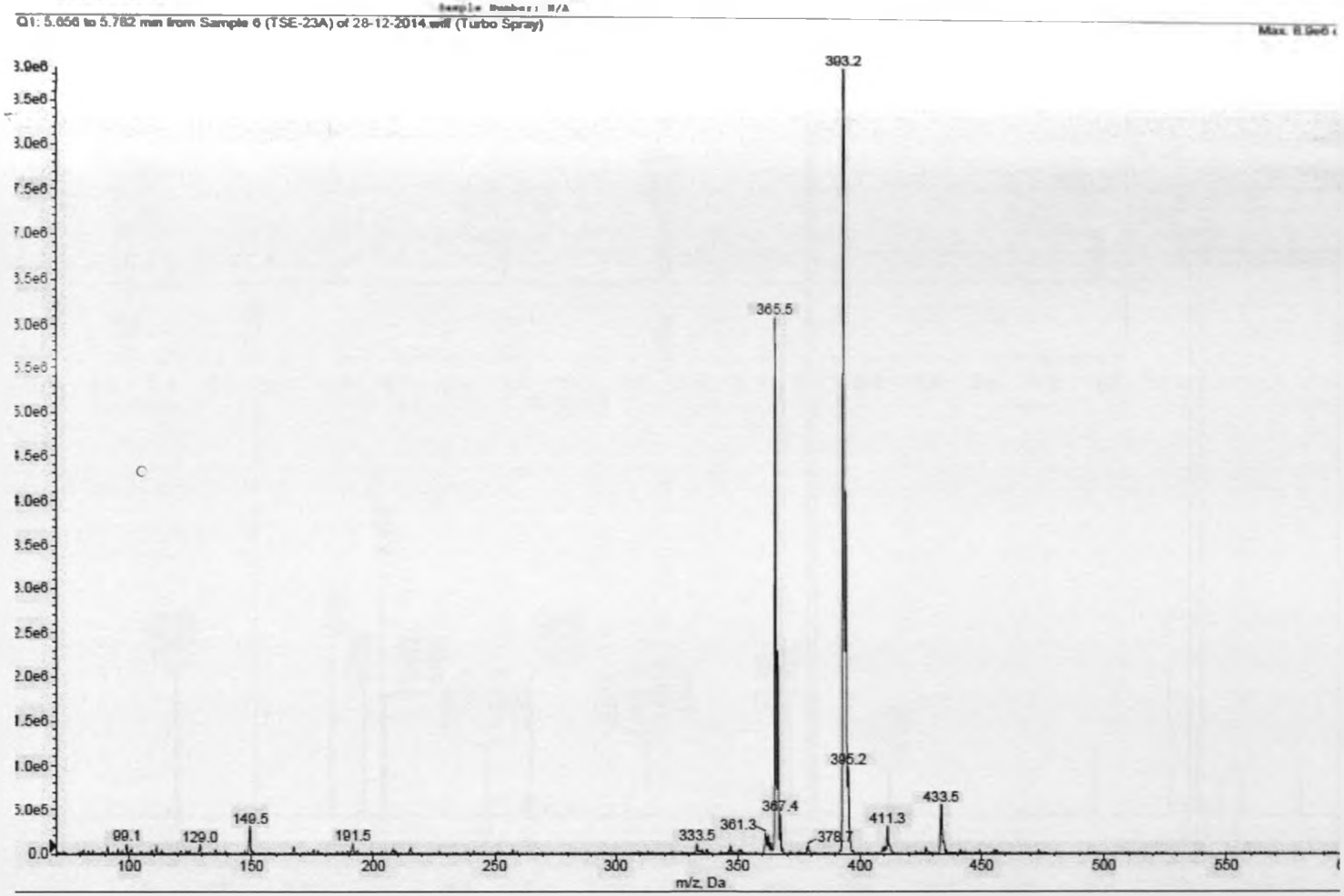
Appendix 20C: HSQC (799.87/201.15 MHz) spectrum of compound 313



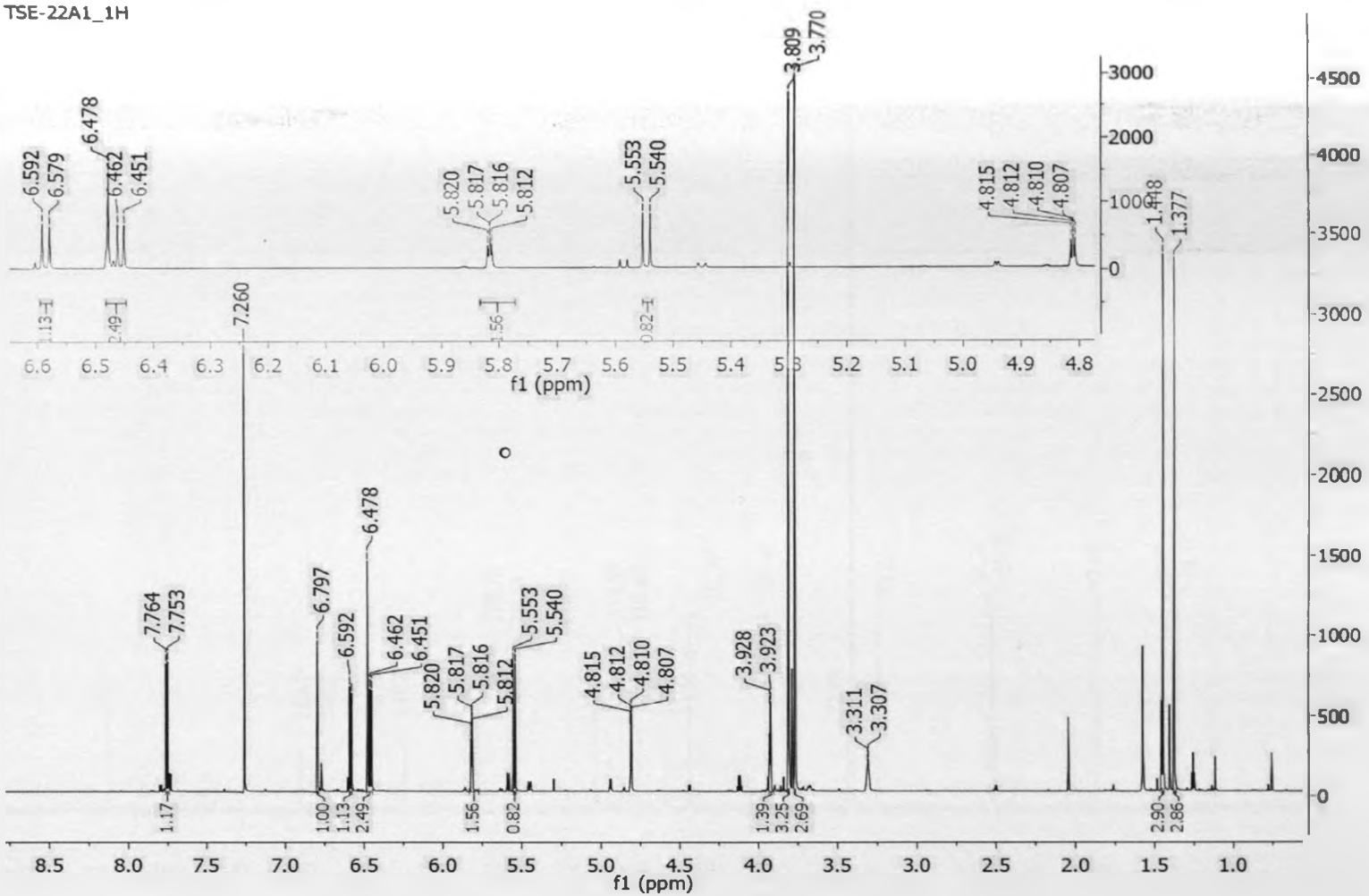
Appendix 20D: HMBC (799.87/201.15 MHz) spectrum of compound 313



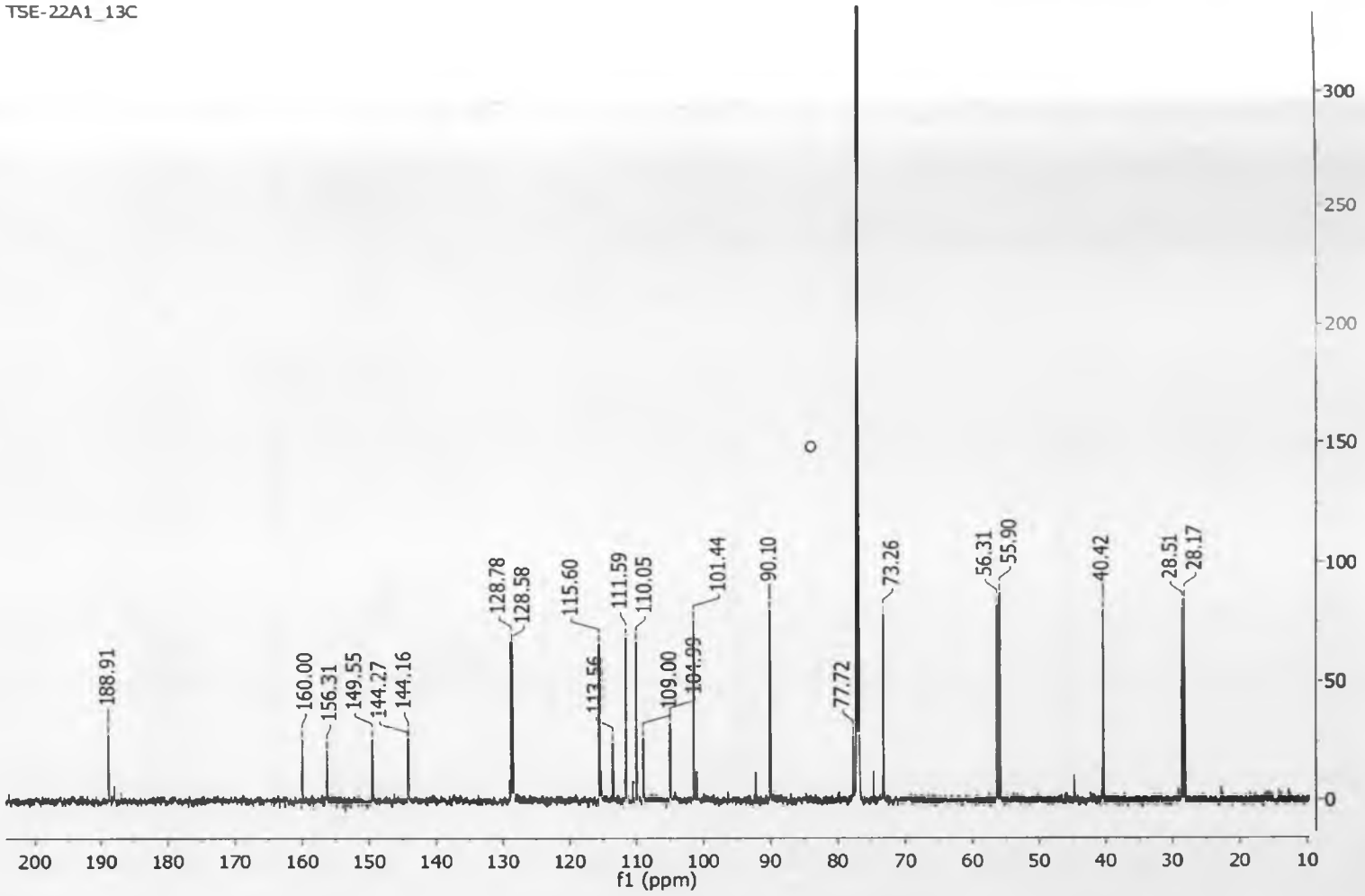
Appendix20E: ESIMS spectrum of compound 313



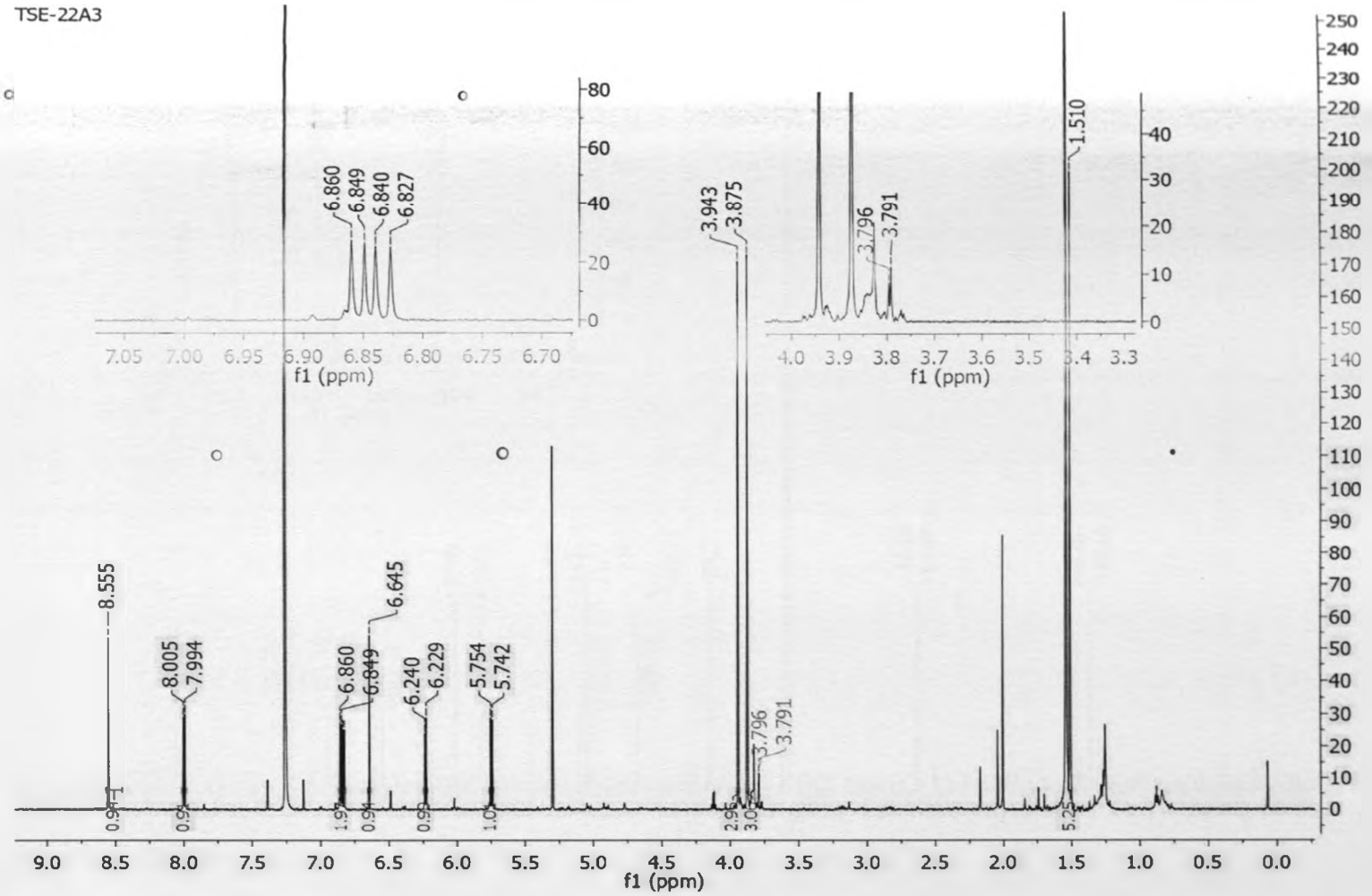
Appendix 21A: ^{1}H NMR (799.87 MHz) spectrum of compound 314



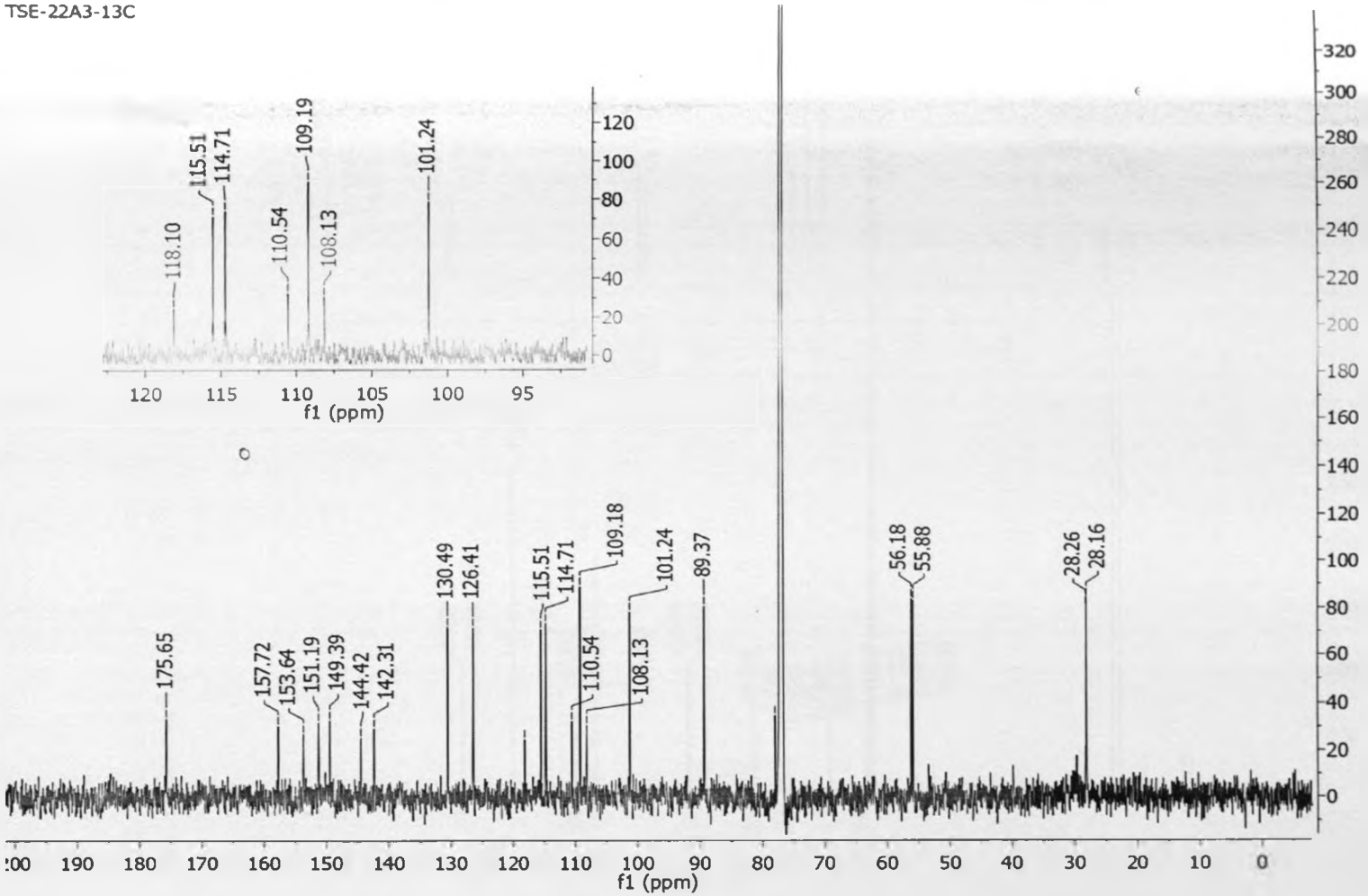
Appendix 21B: ^{1}H NMR (799.87 MHz) spectrum of compound 314



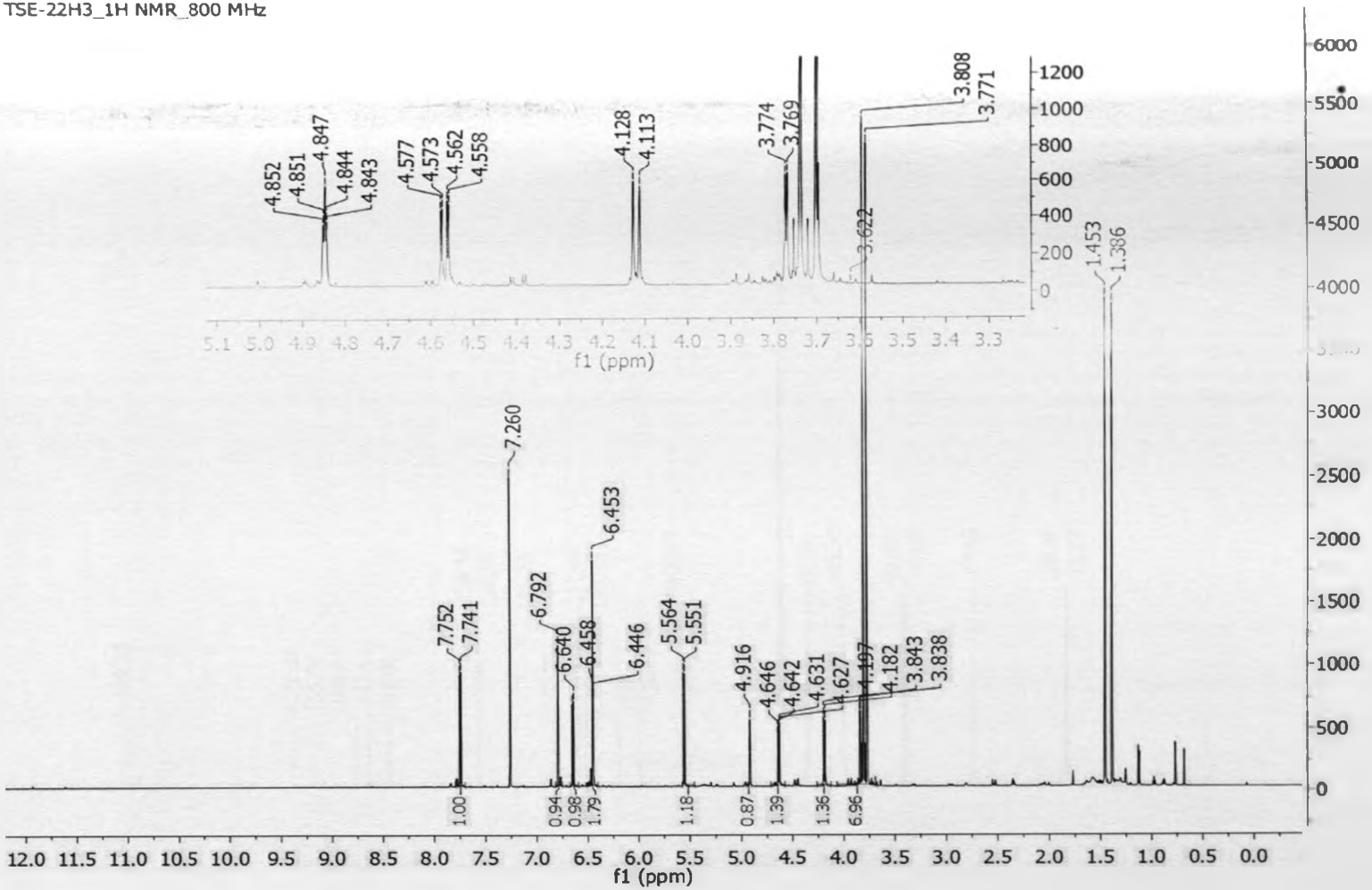
Appendix 22A: ^{1}H NMR (799.87 MHz) spectrum of compound 122



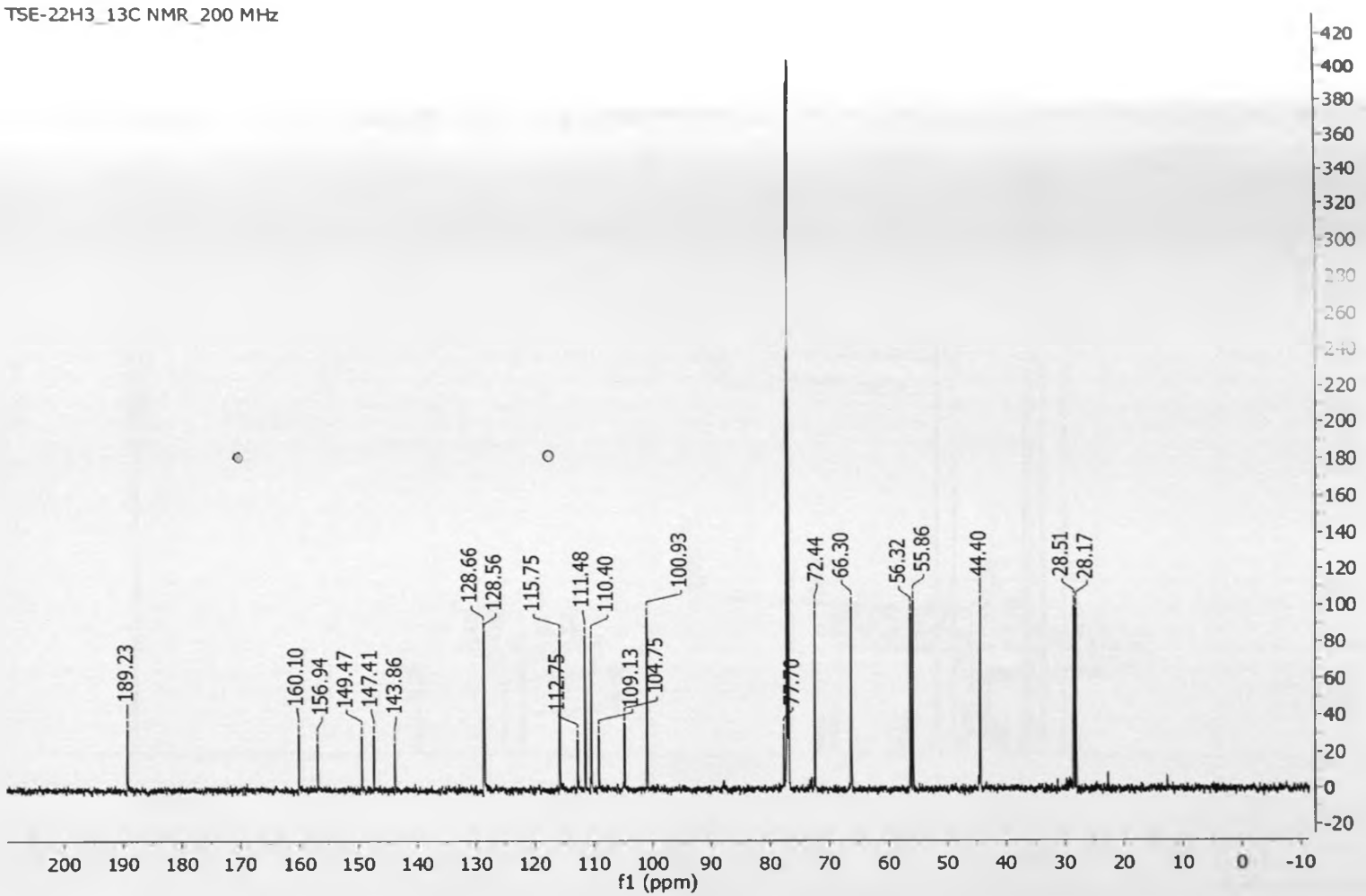
Appendix 22B: ^{13}C NMR (201.15 MHz) spectrum of compound 122



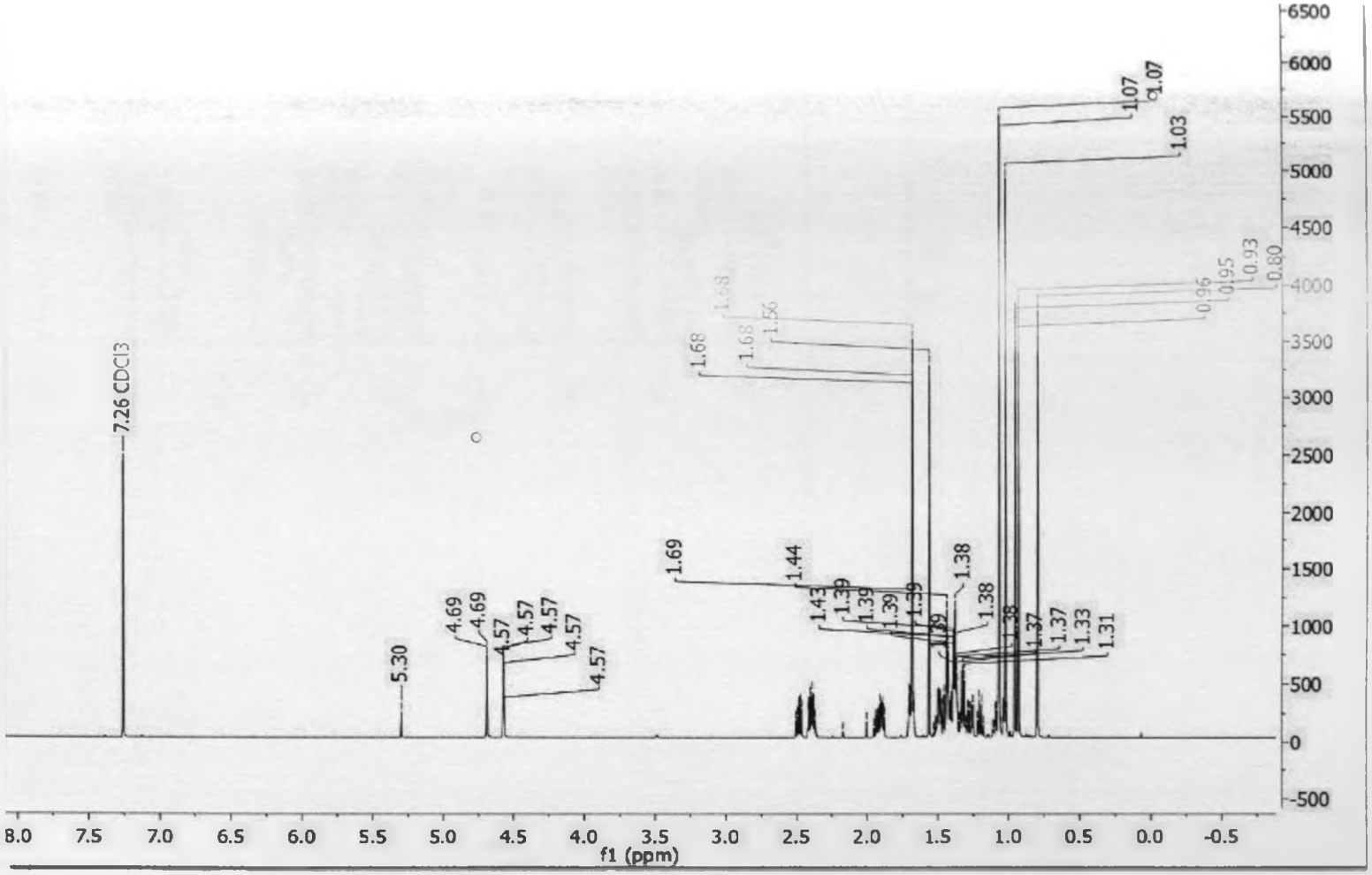
Appendix 23A: ^{1}H NMR (799.87 MHz) spectrum of compound 152



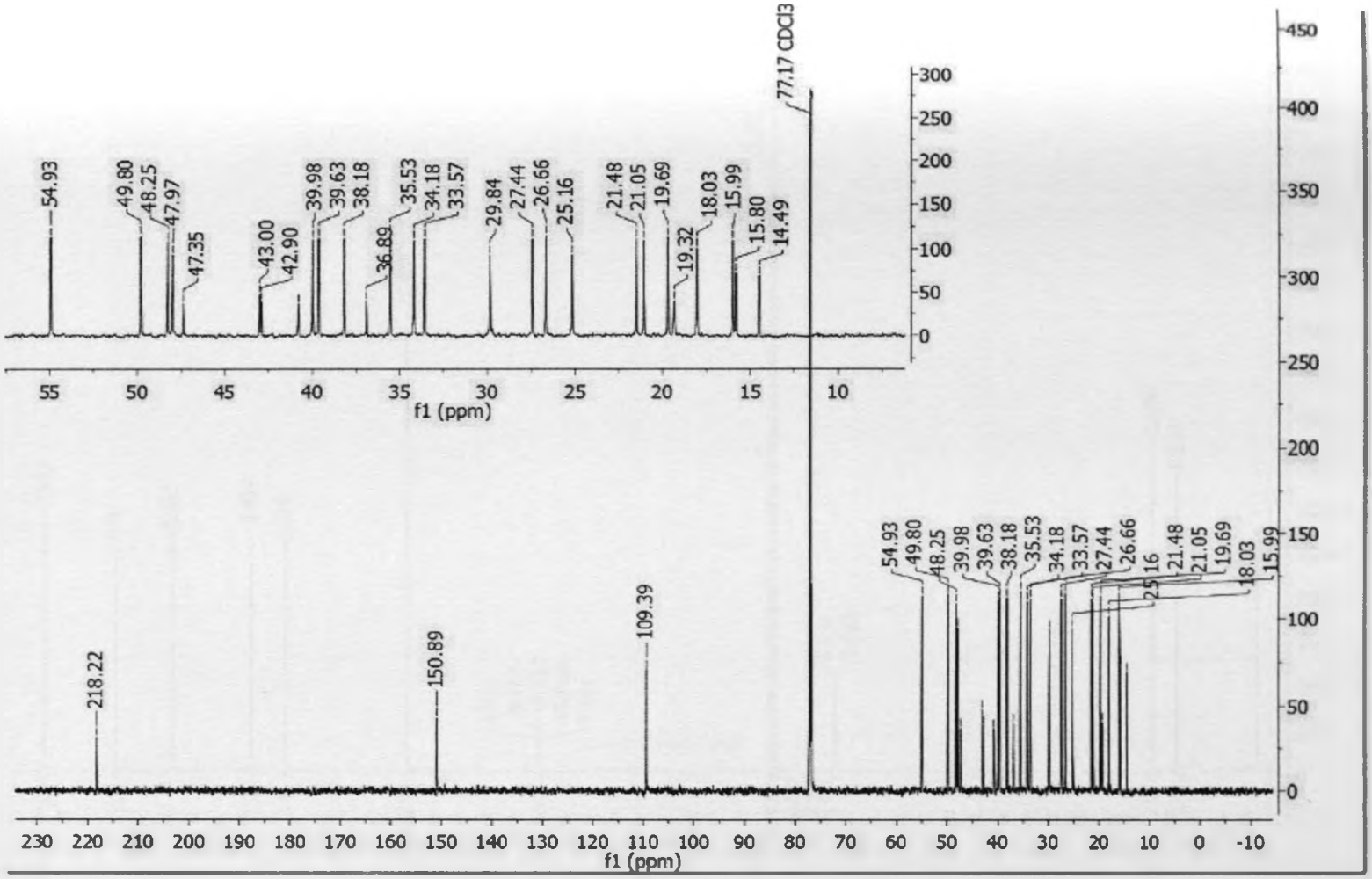
Appendix 23B: ^{13}C NMR (201.15 MHz) spectrum of compound 152



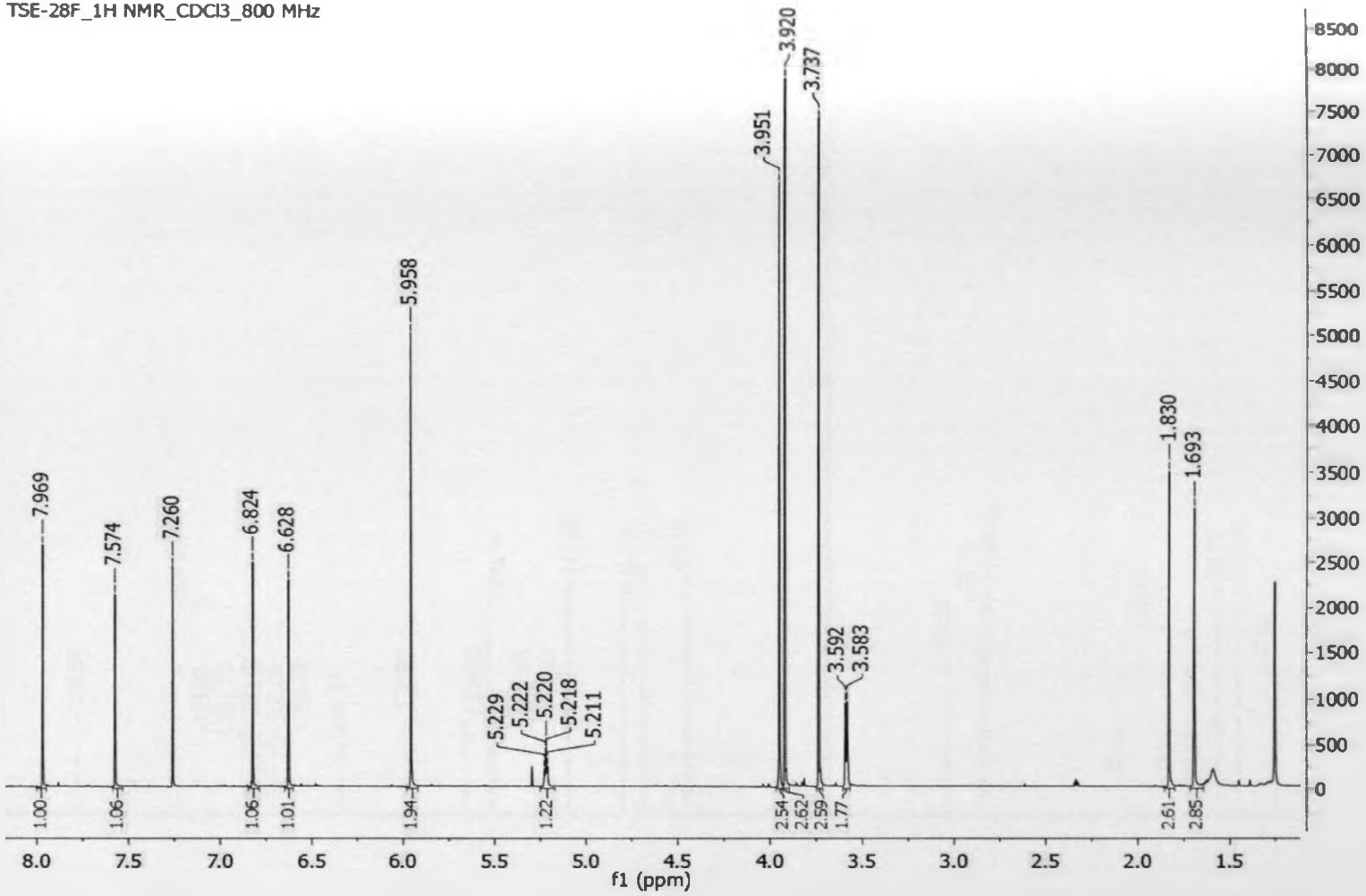
Appendix 24A: ^{1}H NMR (799.87 MHz) spectrum of compound 315



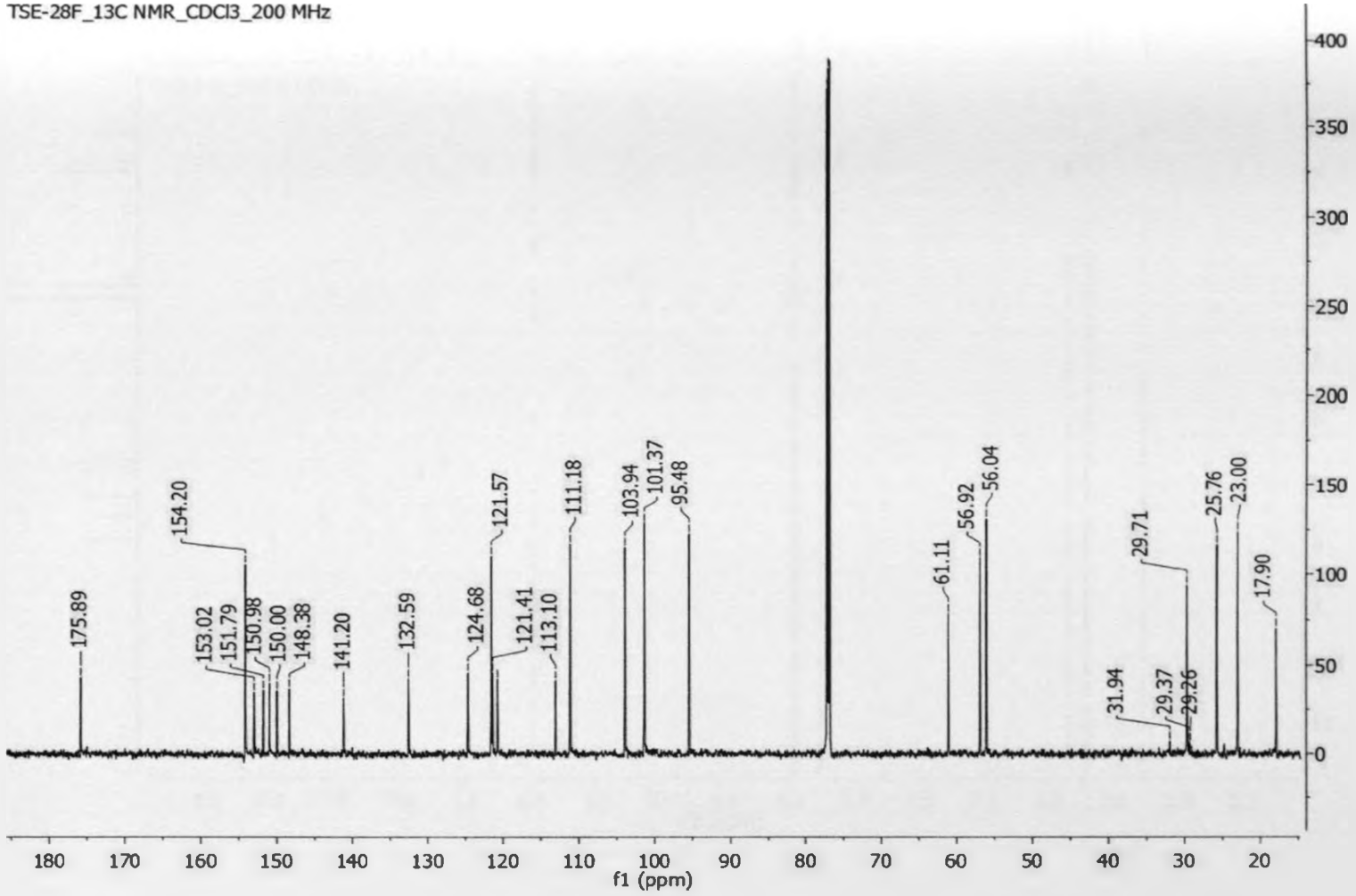
Appendix 24B: ^{13}C NMR (201.15 MHz) spectrum of compound 315



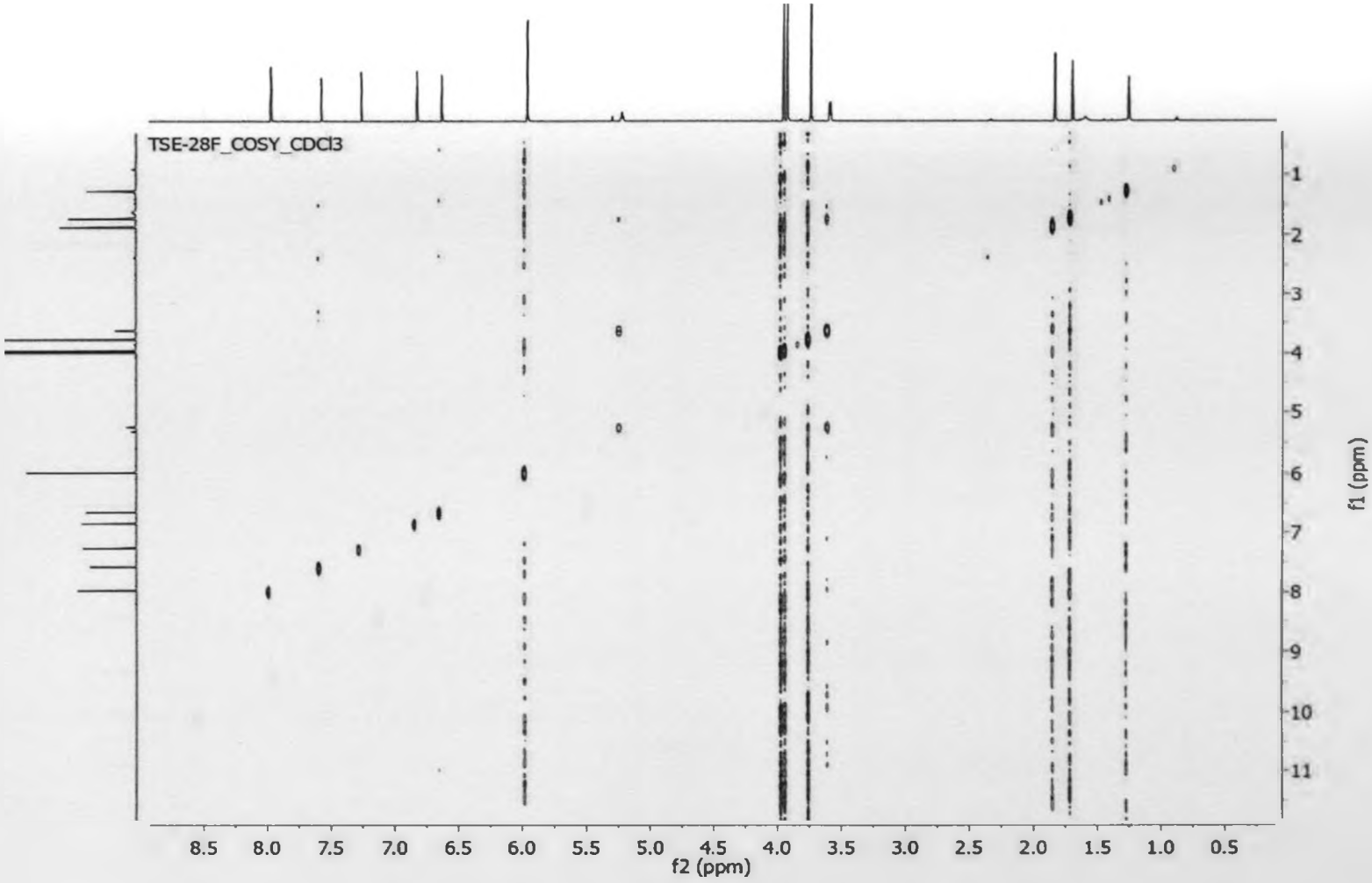
Appendix 25A: ^{1}H NMR (799.87 MHz) spectrum of compound 316



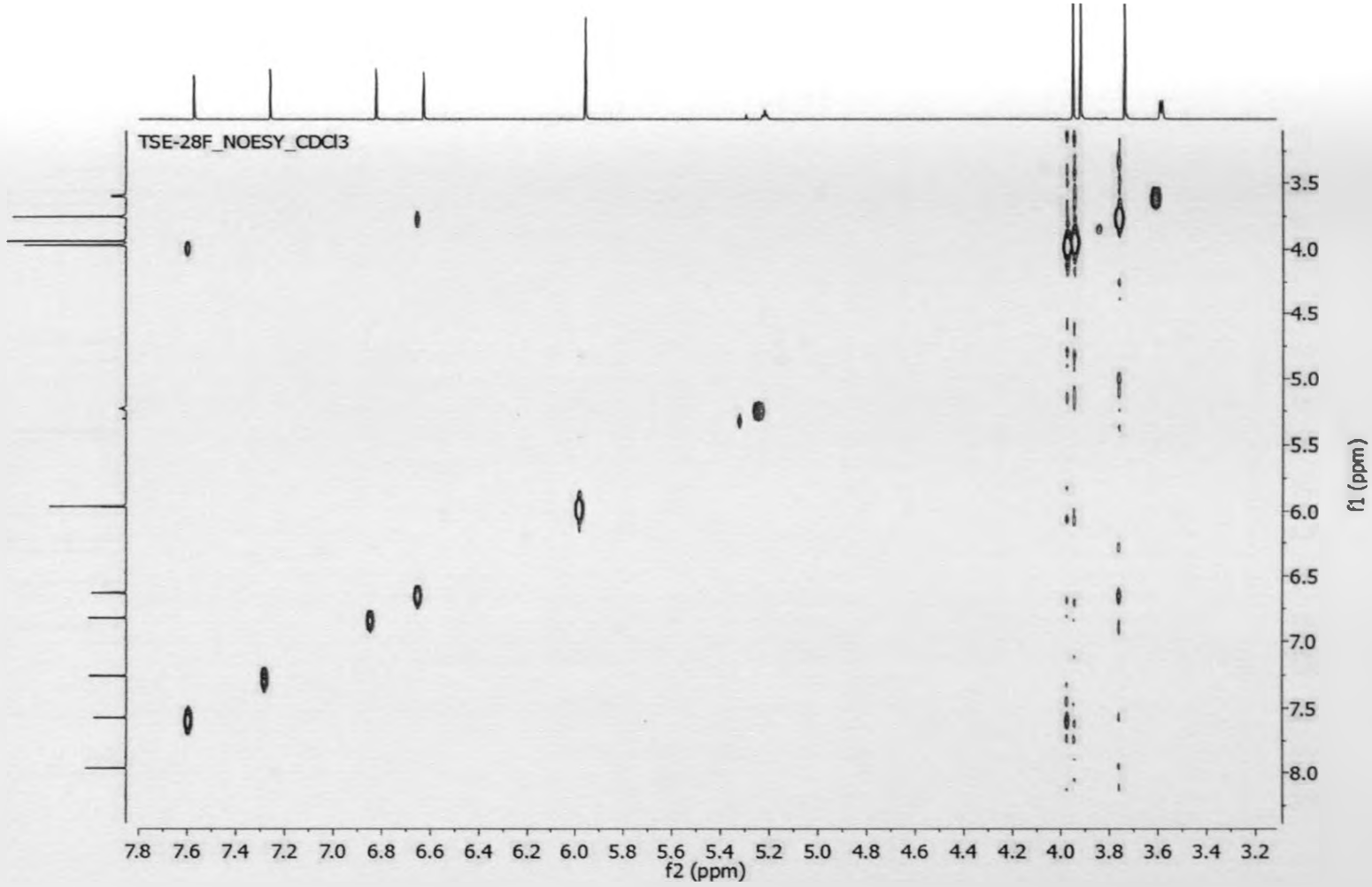
Appendix 25B: ^{13}C NMR (201.15 MHz) spectrum of compound 316



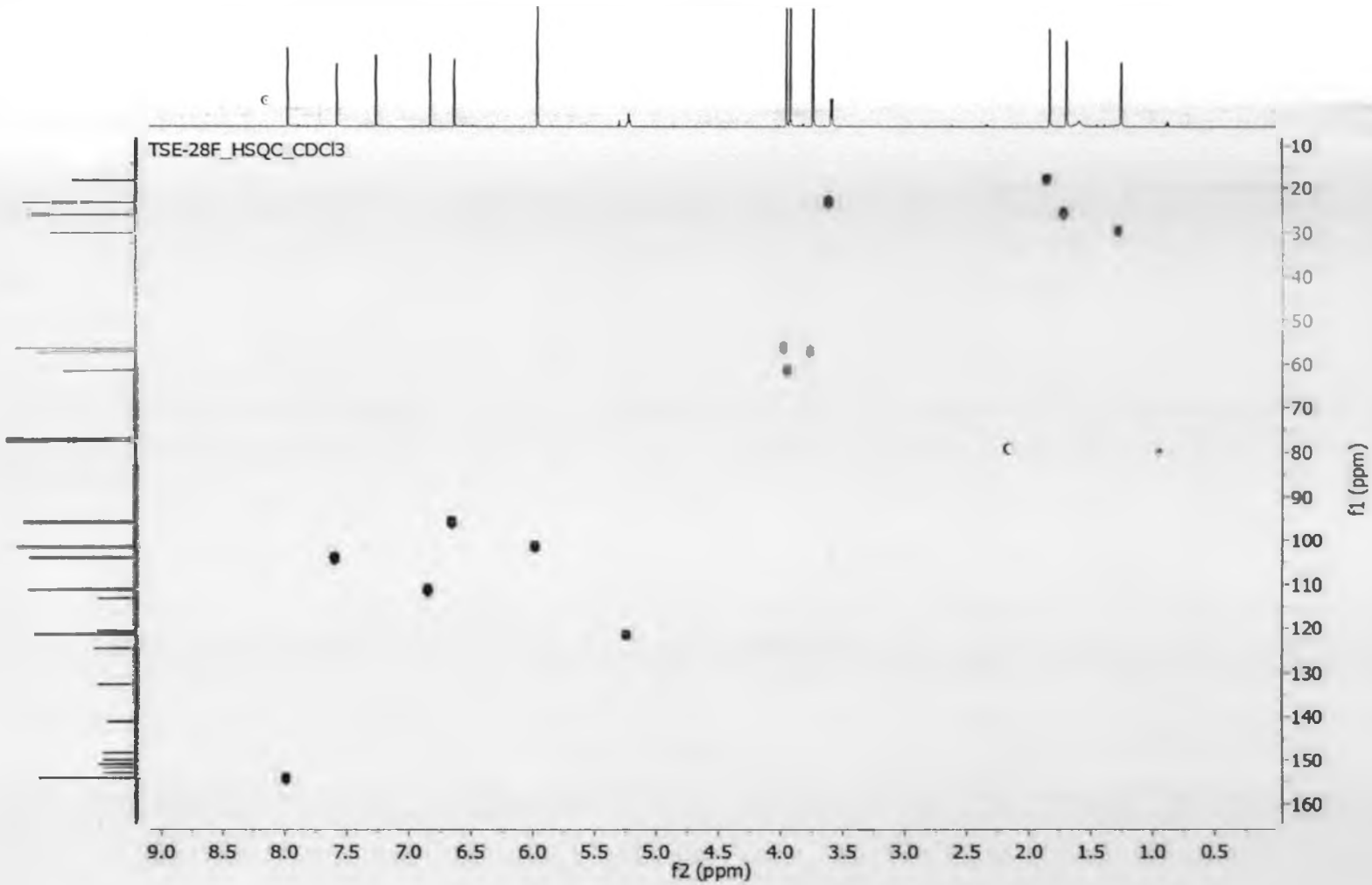
Appendix 25C: COSY (799.87 MHz) spectrum of compound 316



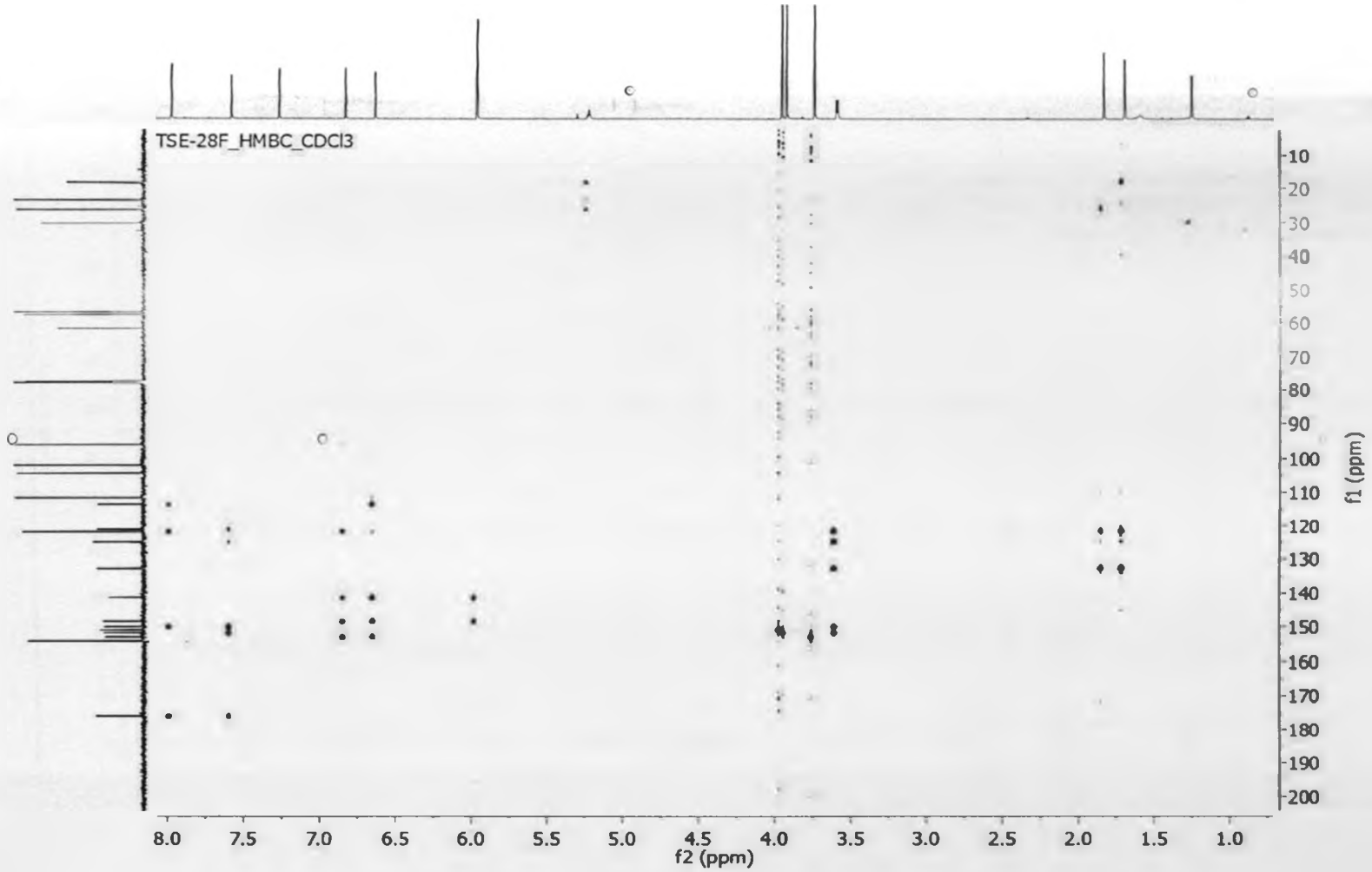
Appendix 25D: NOESY (799.87 MHz) spectrum of compound 316



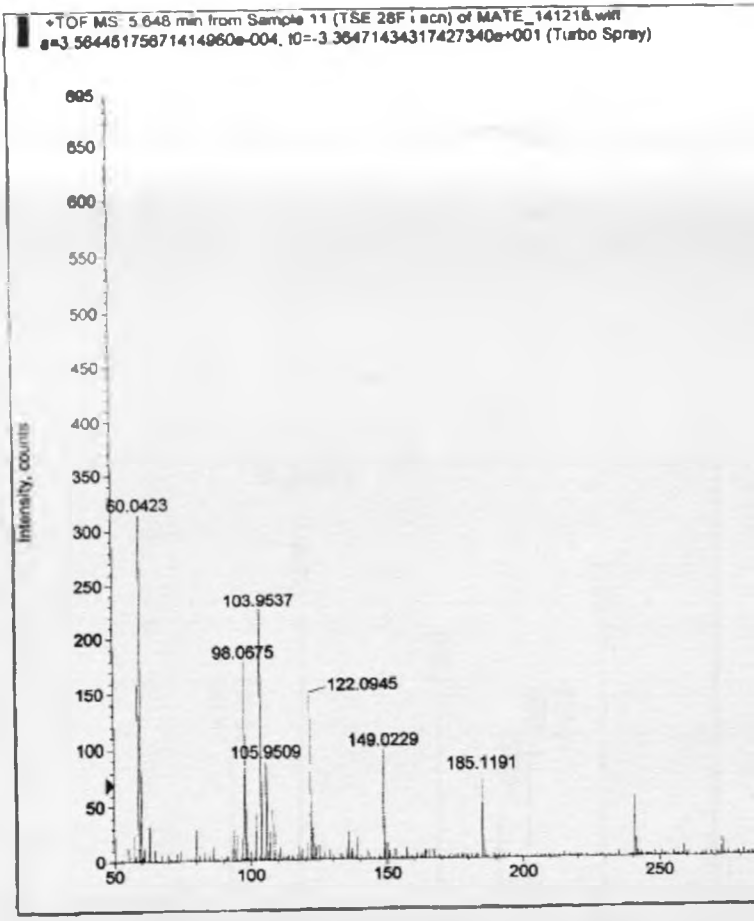
Appendix 25E: HSQC (799.87/201.15 MHz) spectrum of compound 316

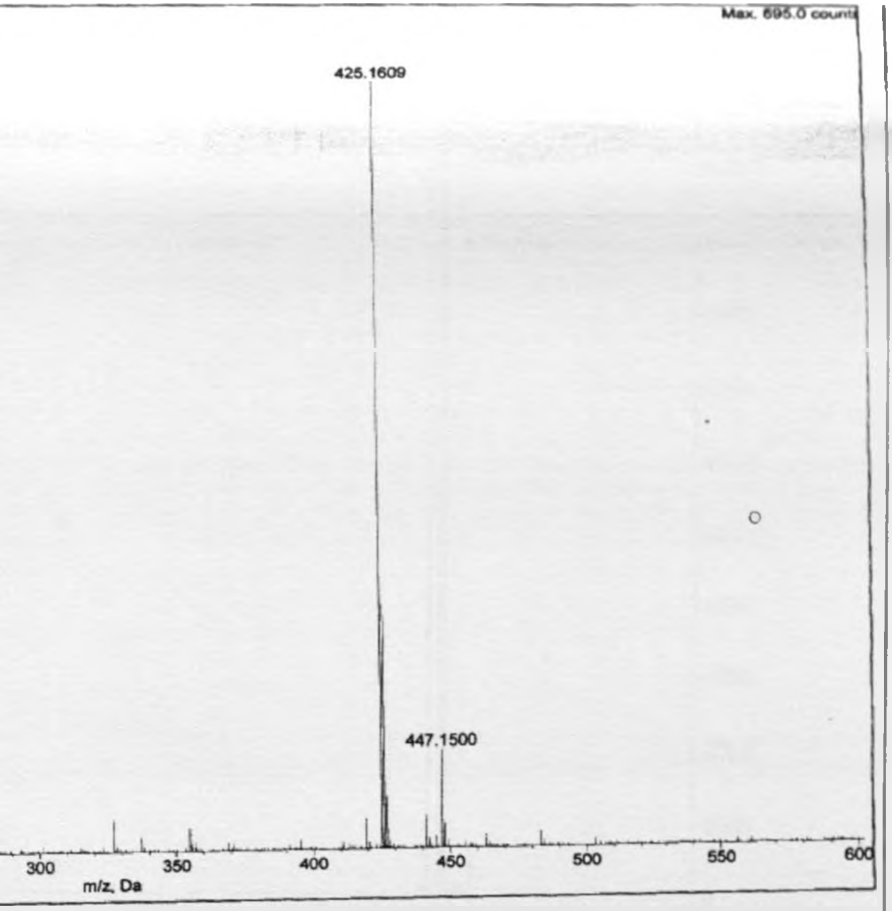


Appendix 25F: HMBC (799.87/201.15 MHz) spectrum of compound 316

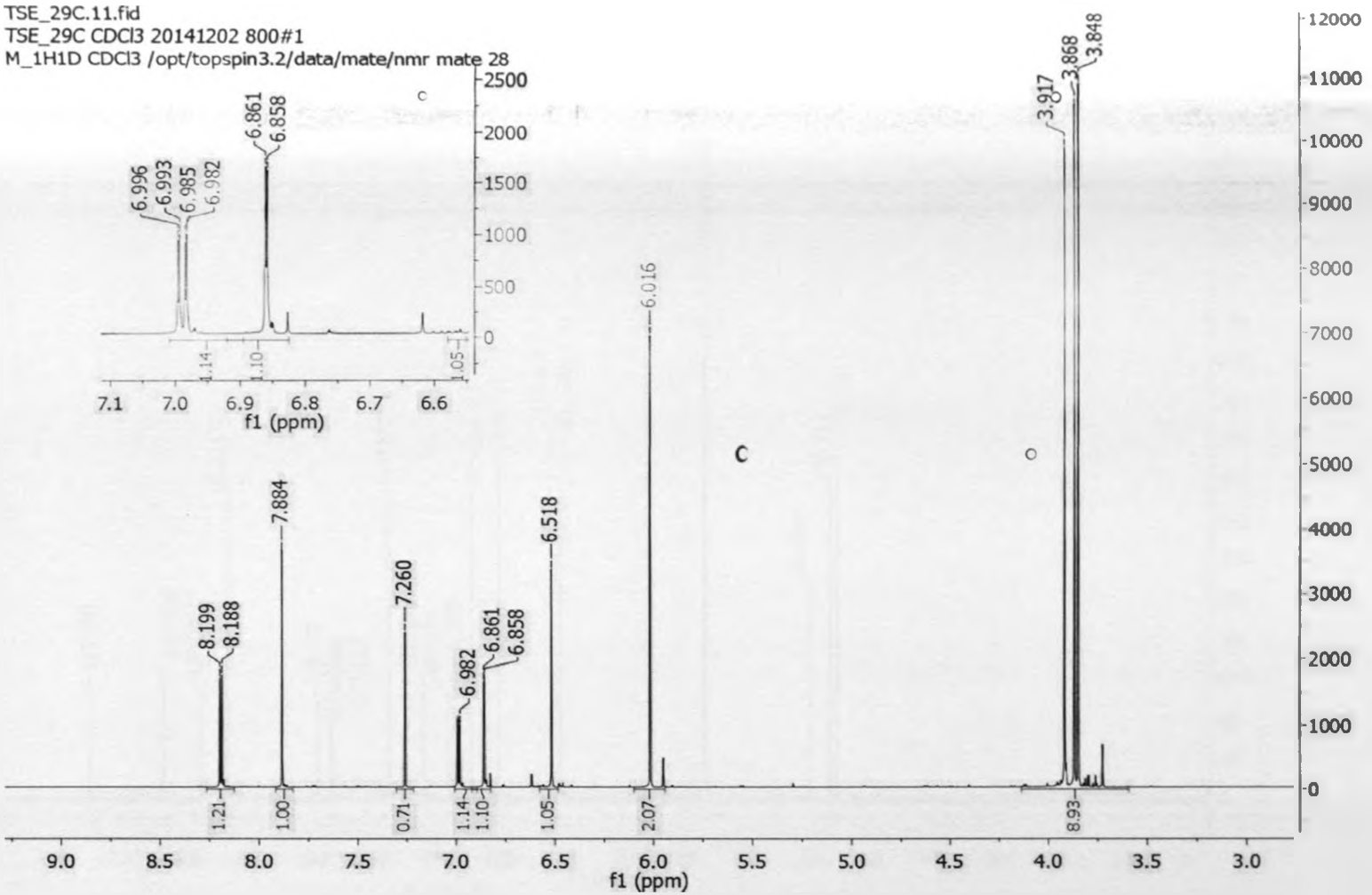


Appendix 25G: HREIMS spectrum of compound 316

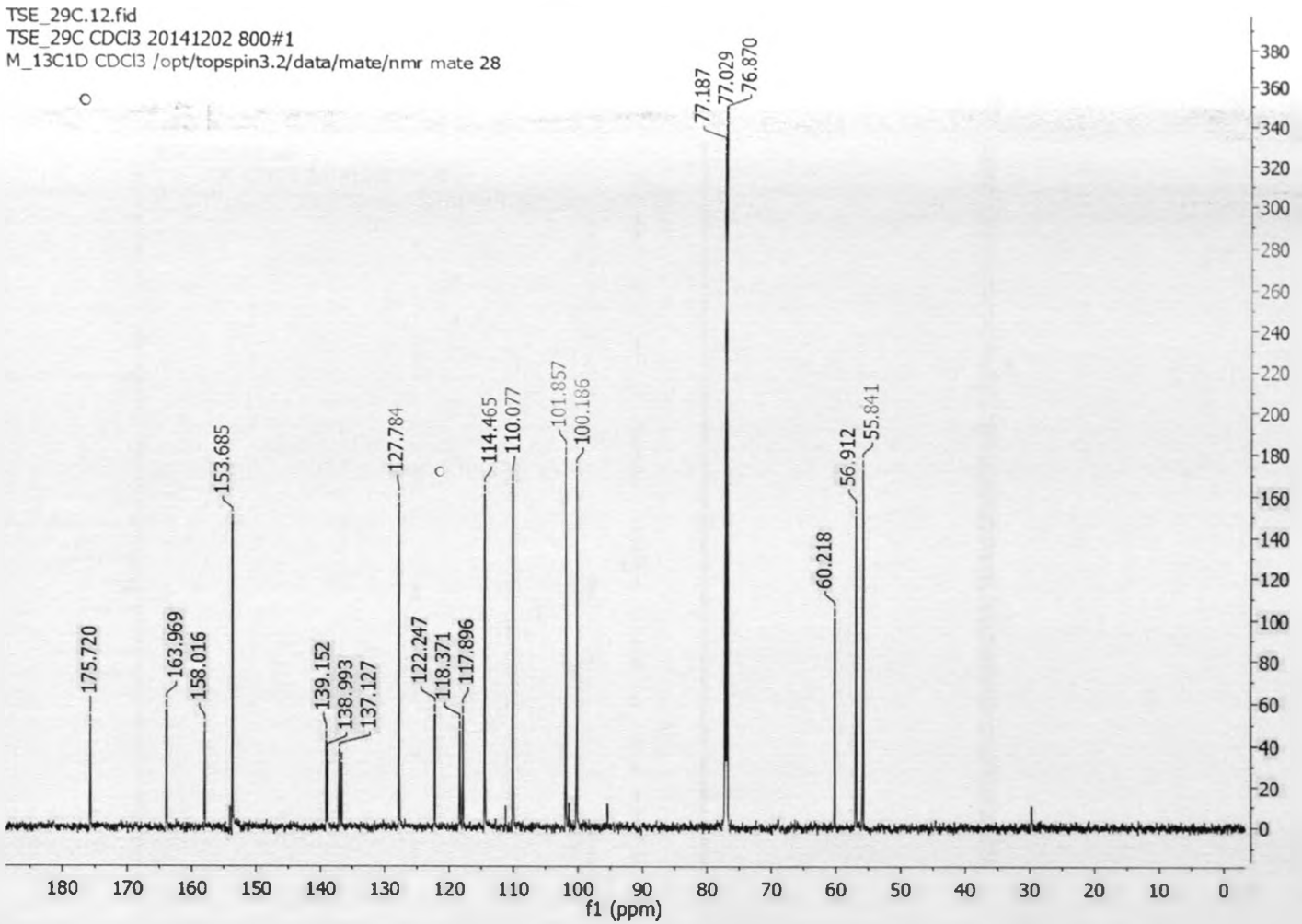



Max. 695.0 counts
425.1609
447.1500
300
350
400
450
500
550
600
m/z, Da

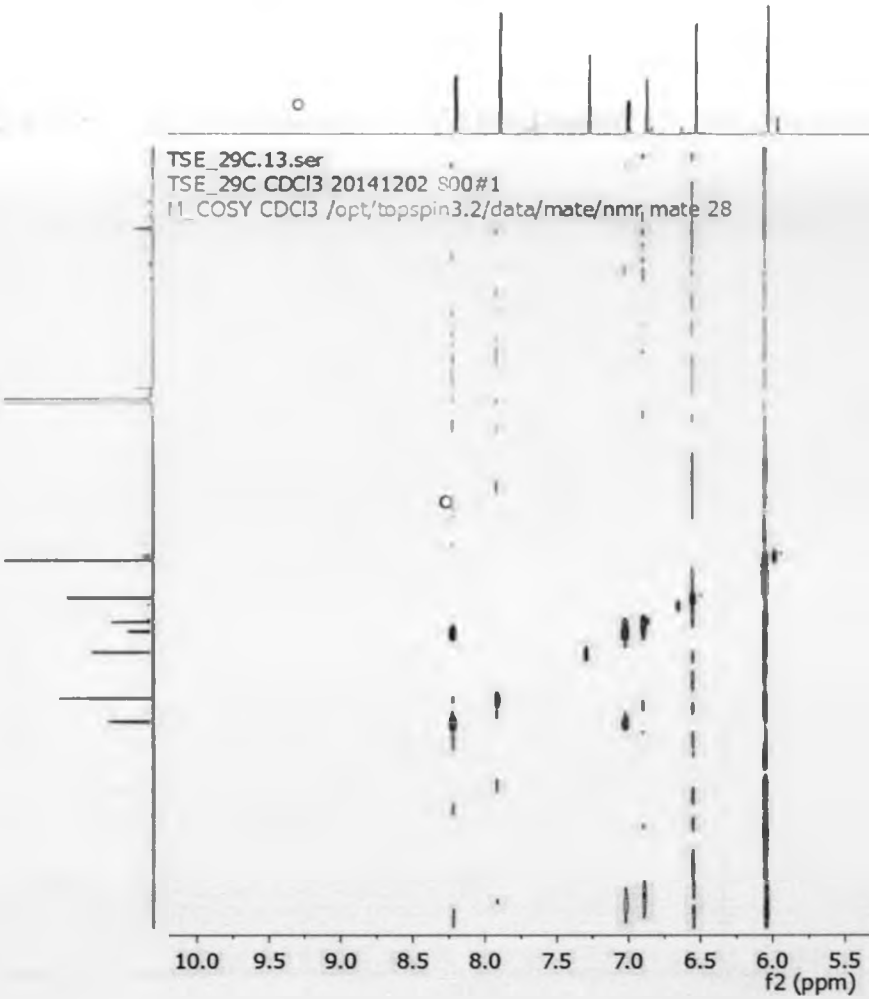
Appendix 26A: ^{1}H NMR (799.87 MHz) spectrum of compound 317

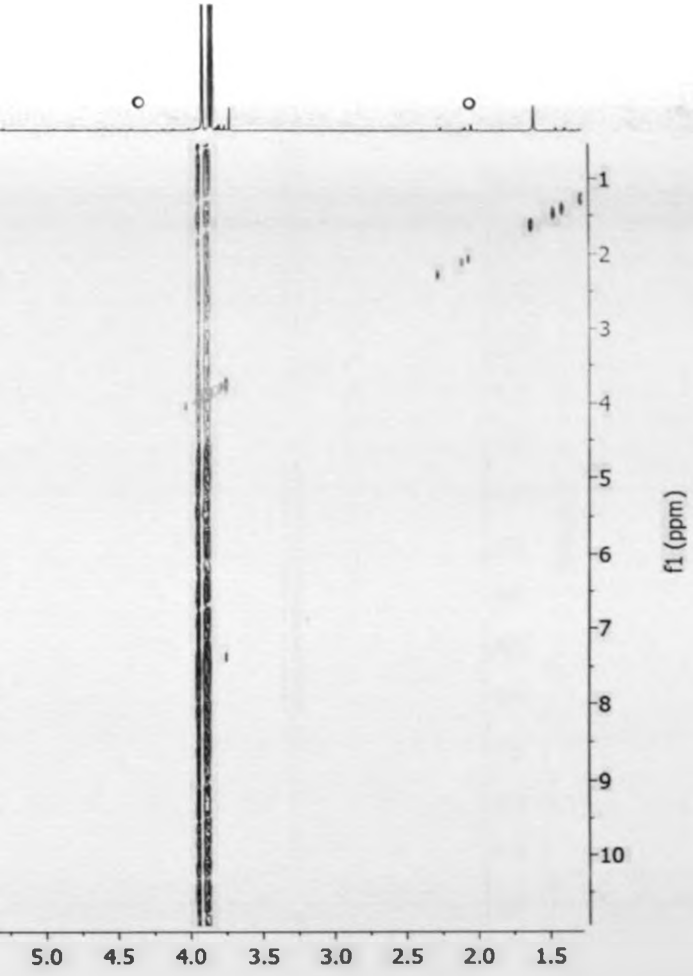


Appendix 26B: ^{13}C NMR (201.15 MHz) spectrum of compound 317

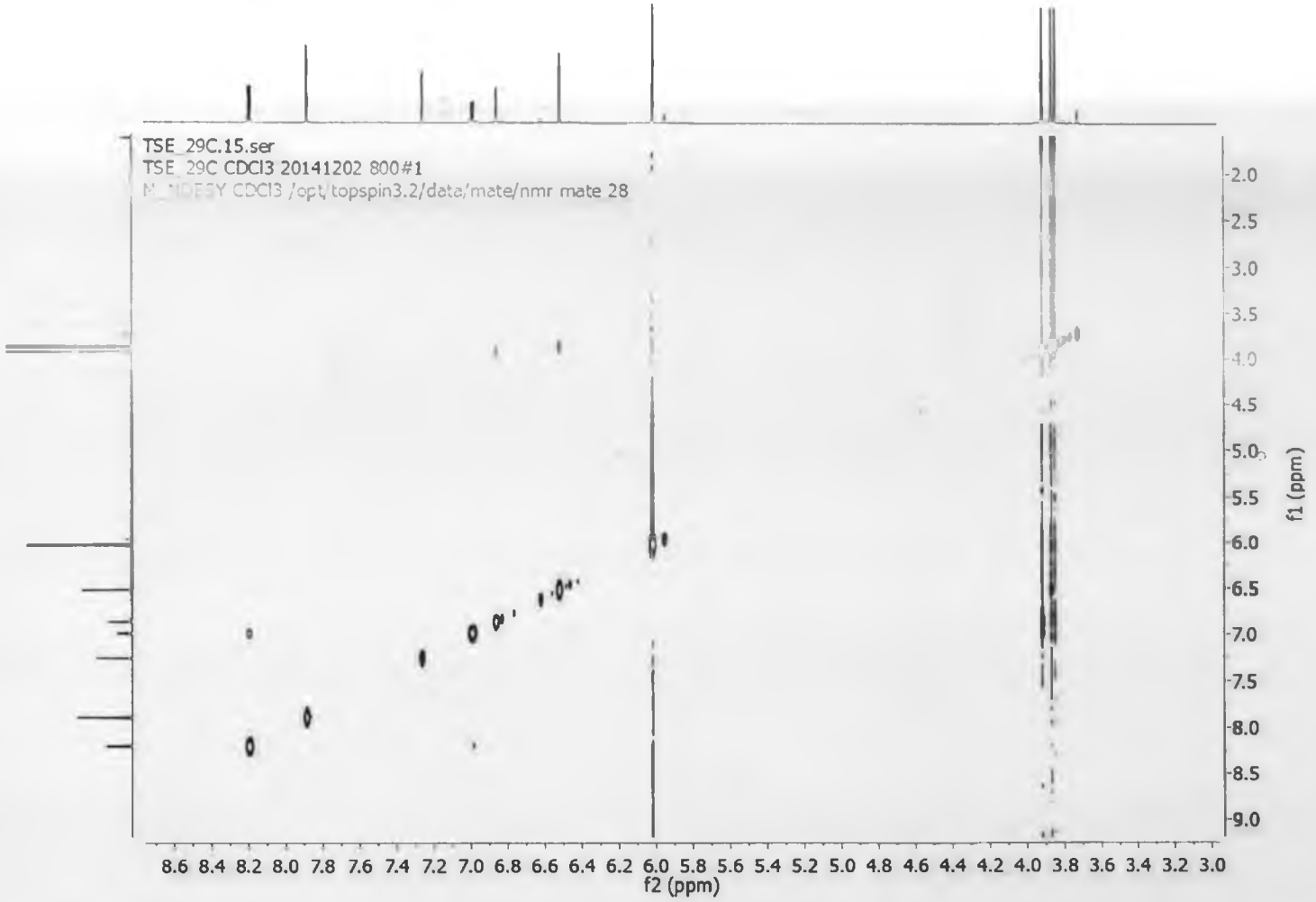


Appendix 26C: COSY (799.78 MHz) spectrum of compound 317

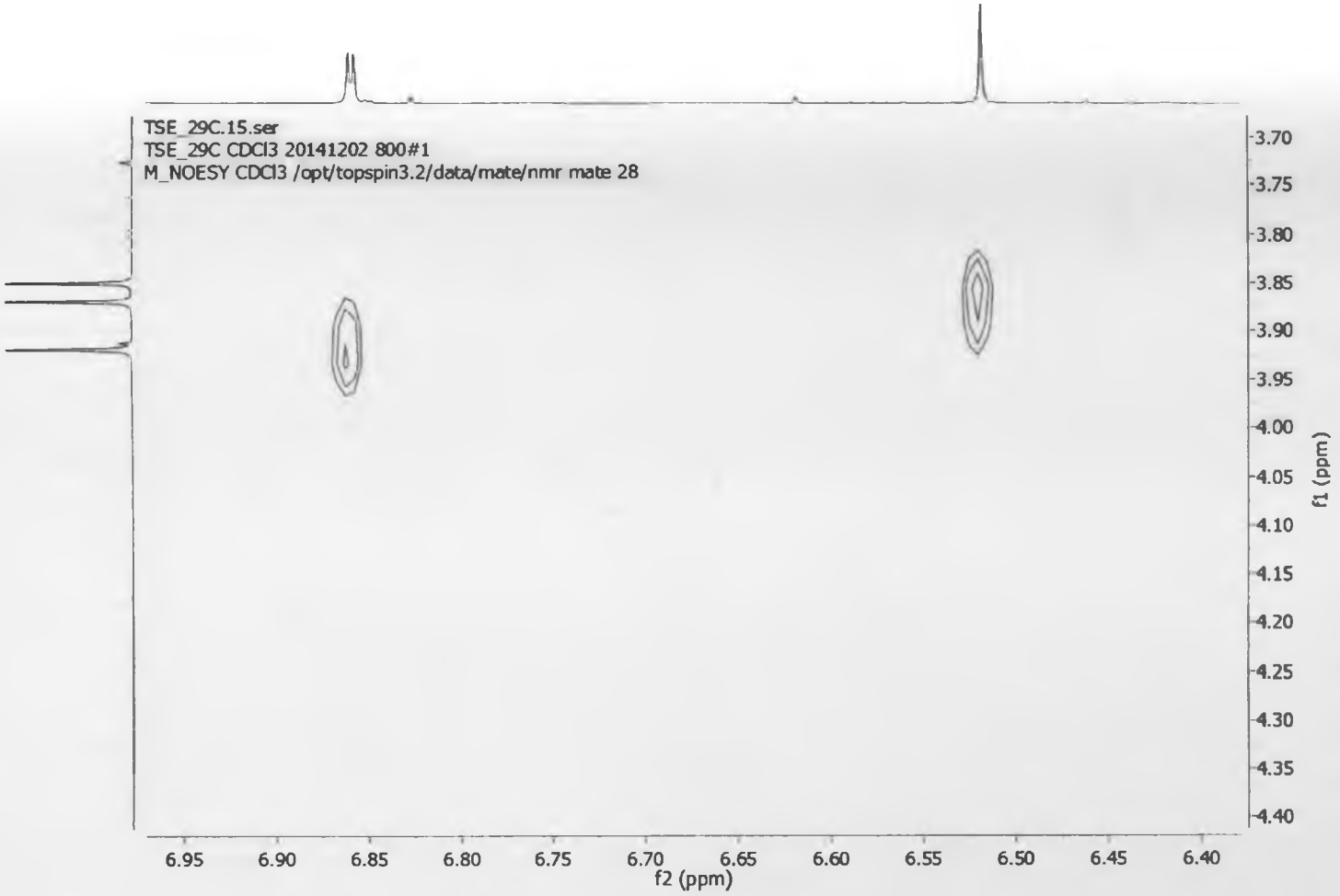



1
2
3
4
5
6
7
8
9
10
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5

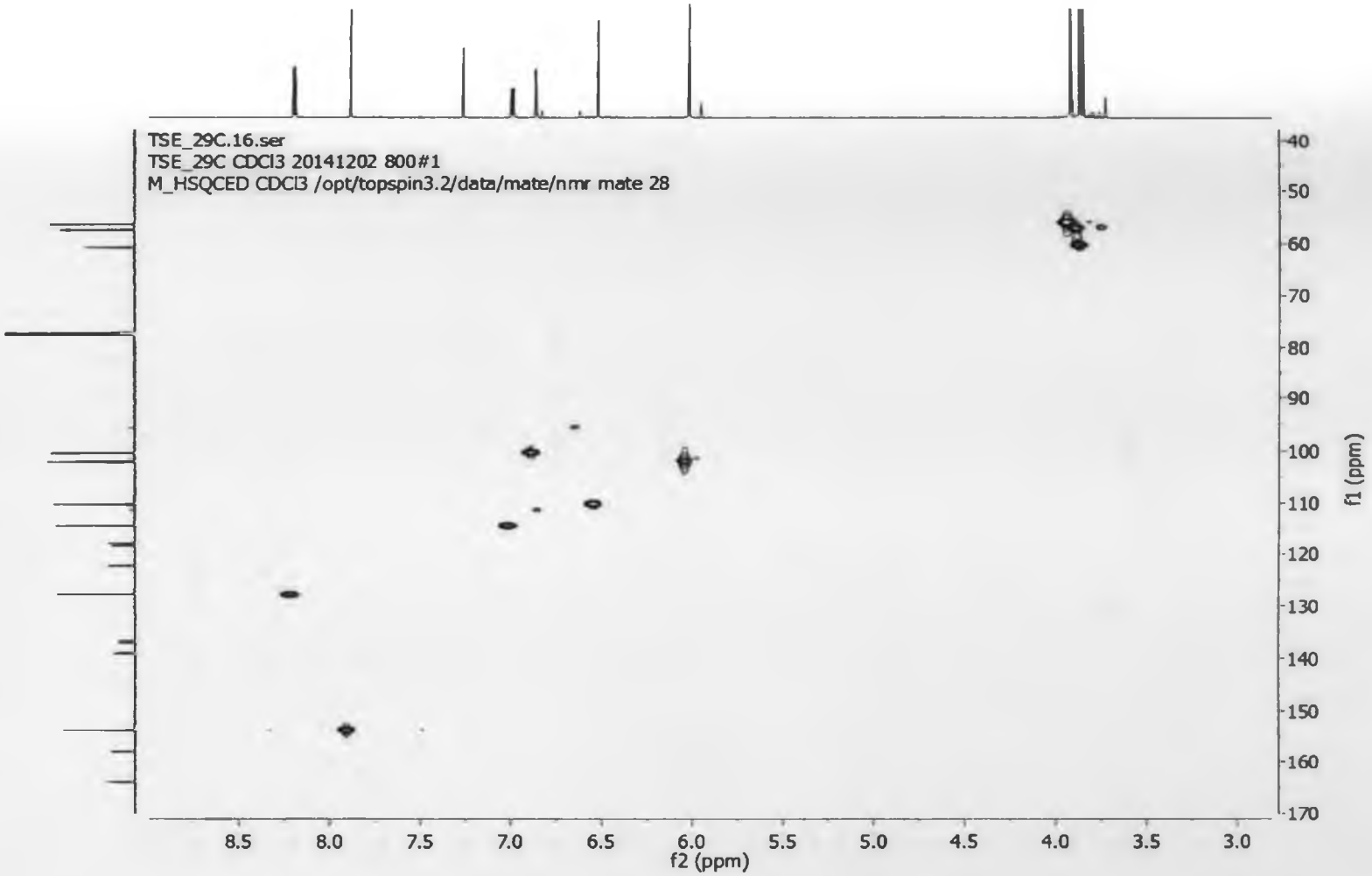
Appendix 26D: NOESY (799.78 MHz) spectrum of compound 317



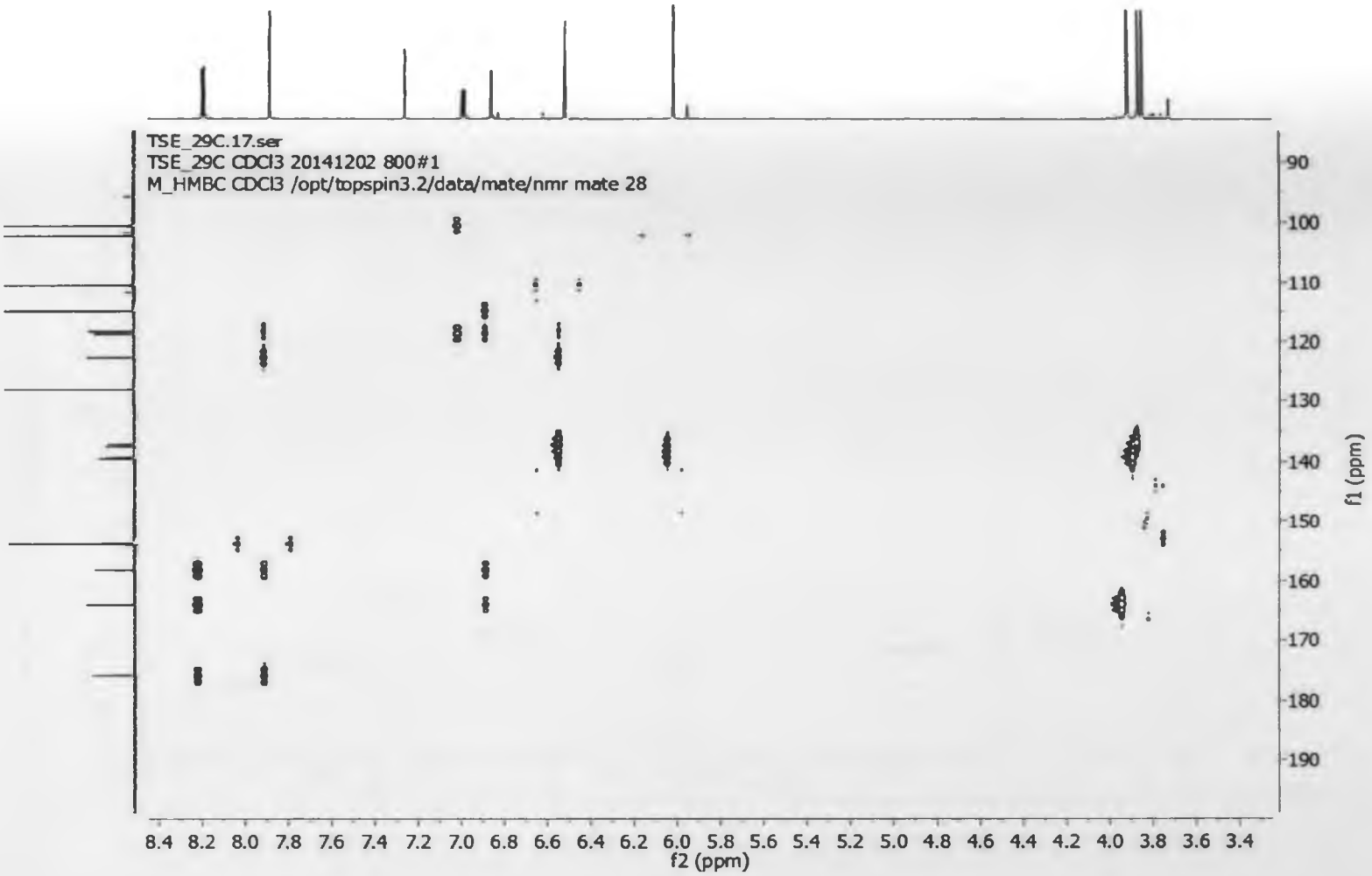
Appendix 26E: NOESY expanded (799.78 MHz) spectrum of compound 317



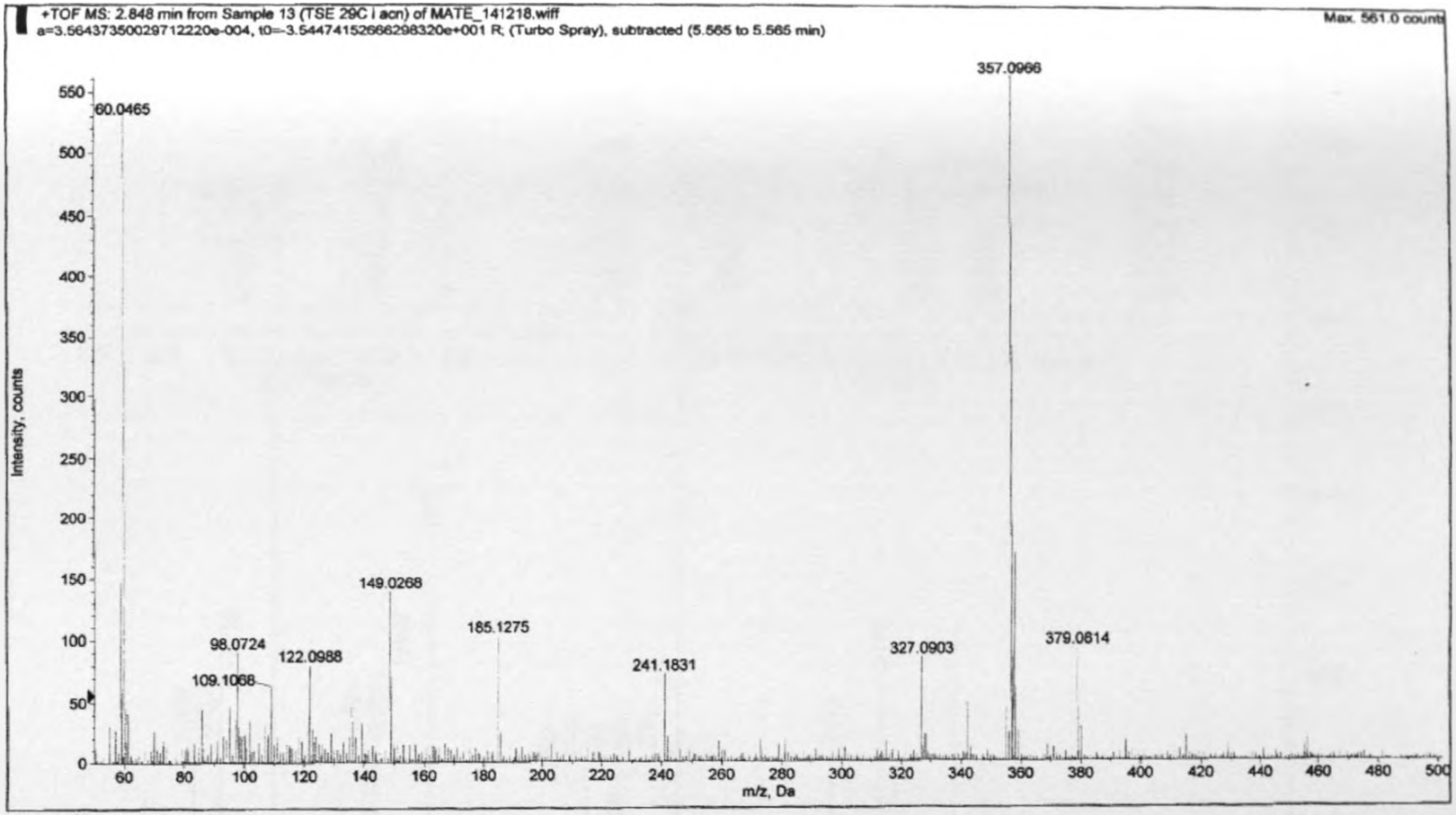
Appendix 26F: HSQC (799.78/201.15 MHz) spectrum of compound 317



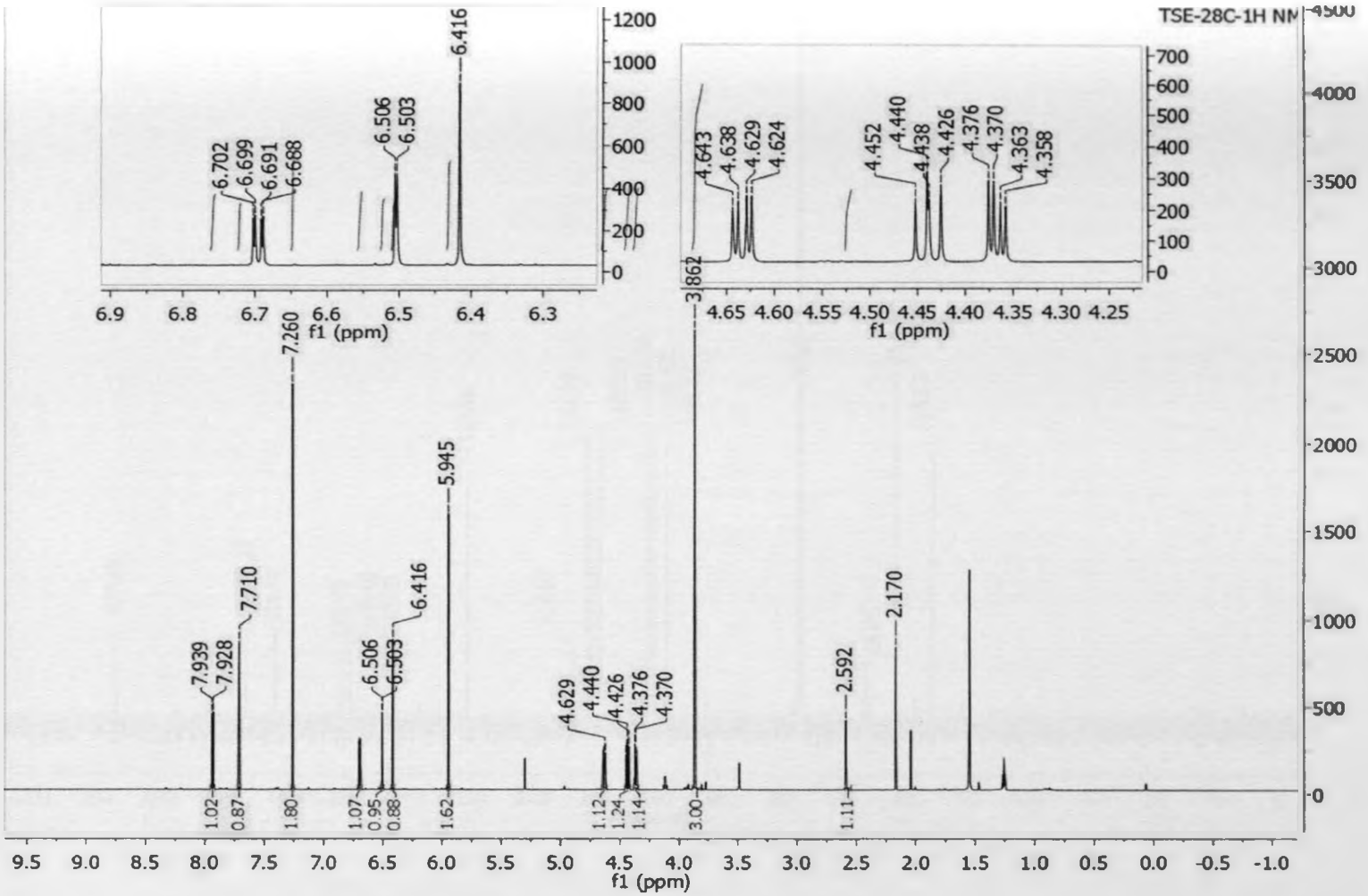
Appendix 26G: HMBC (799.78/201.15 MHz) spectrum of compound 317



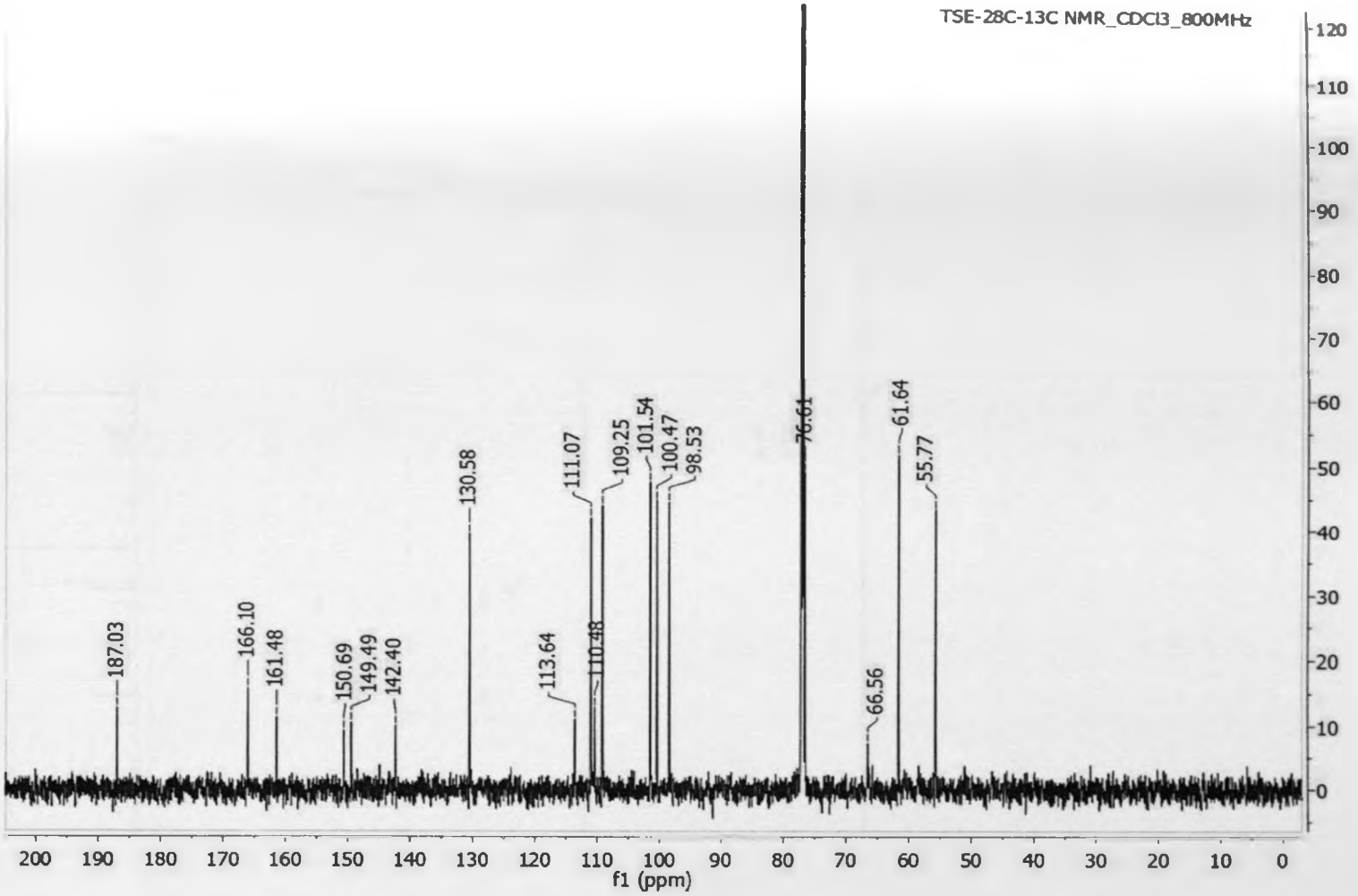
Appendix 26H: HREIMS spectrum of compound 317



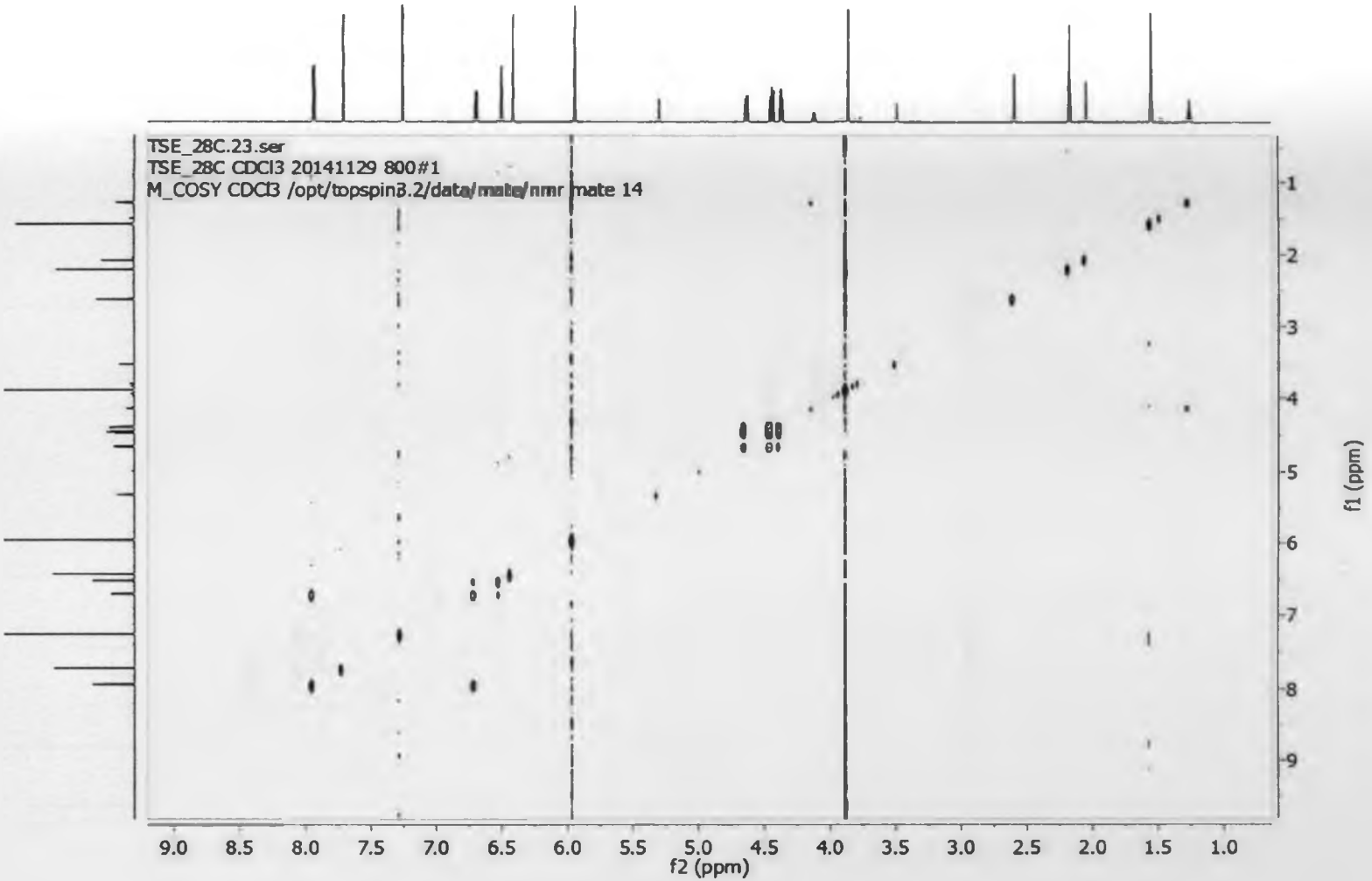
Appendix 27A: ^{1}H NMR (799.78 MHz) spectrum of compound 319



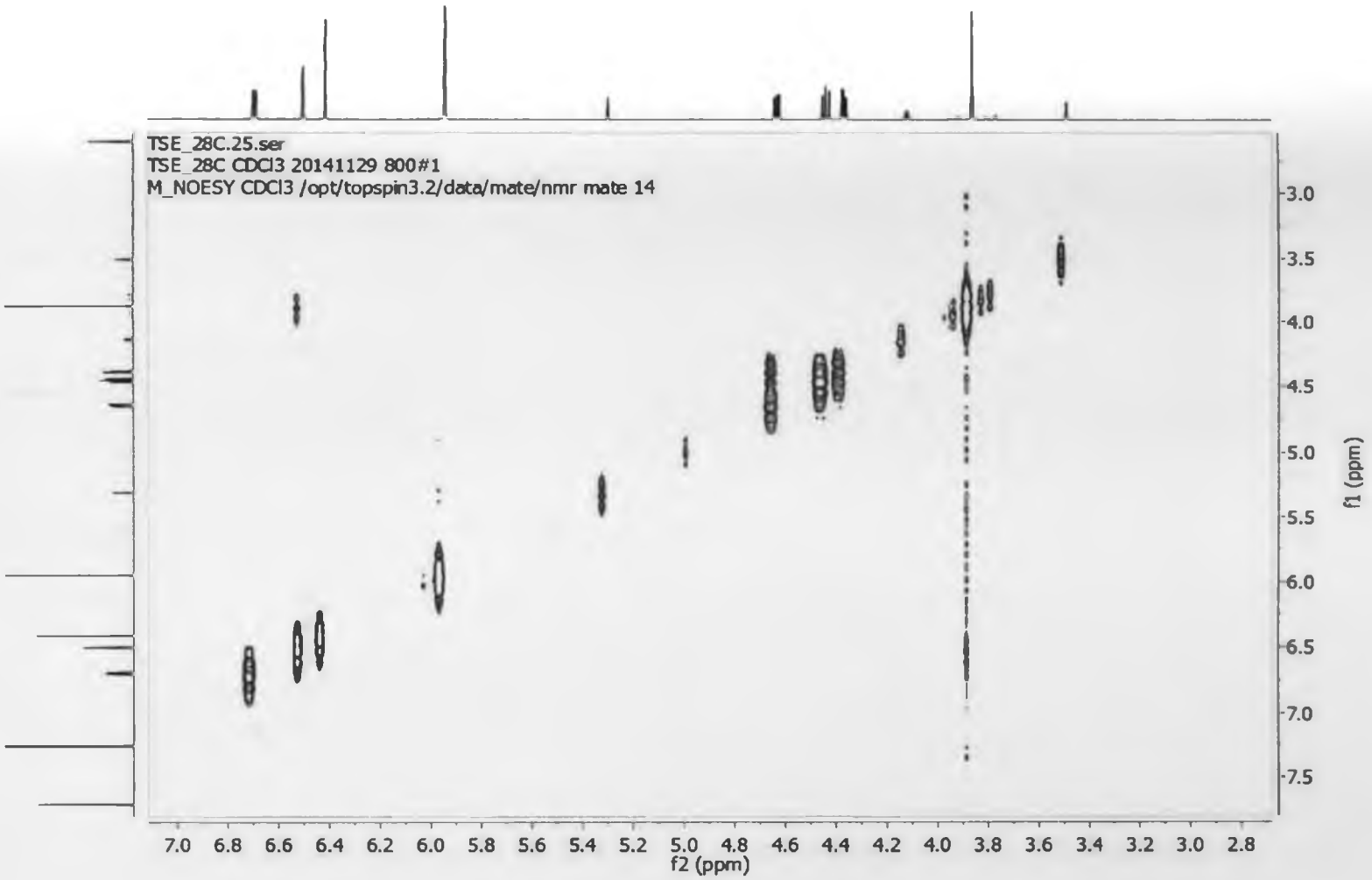
Appendix 27B: ^{13}C NMR (201.15 MHz) spectrum of compound 319



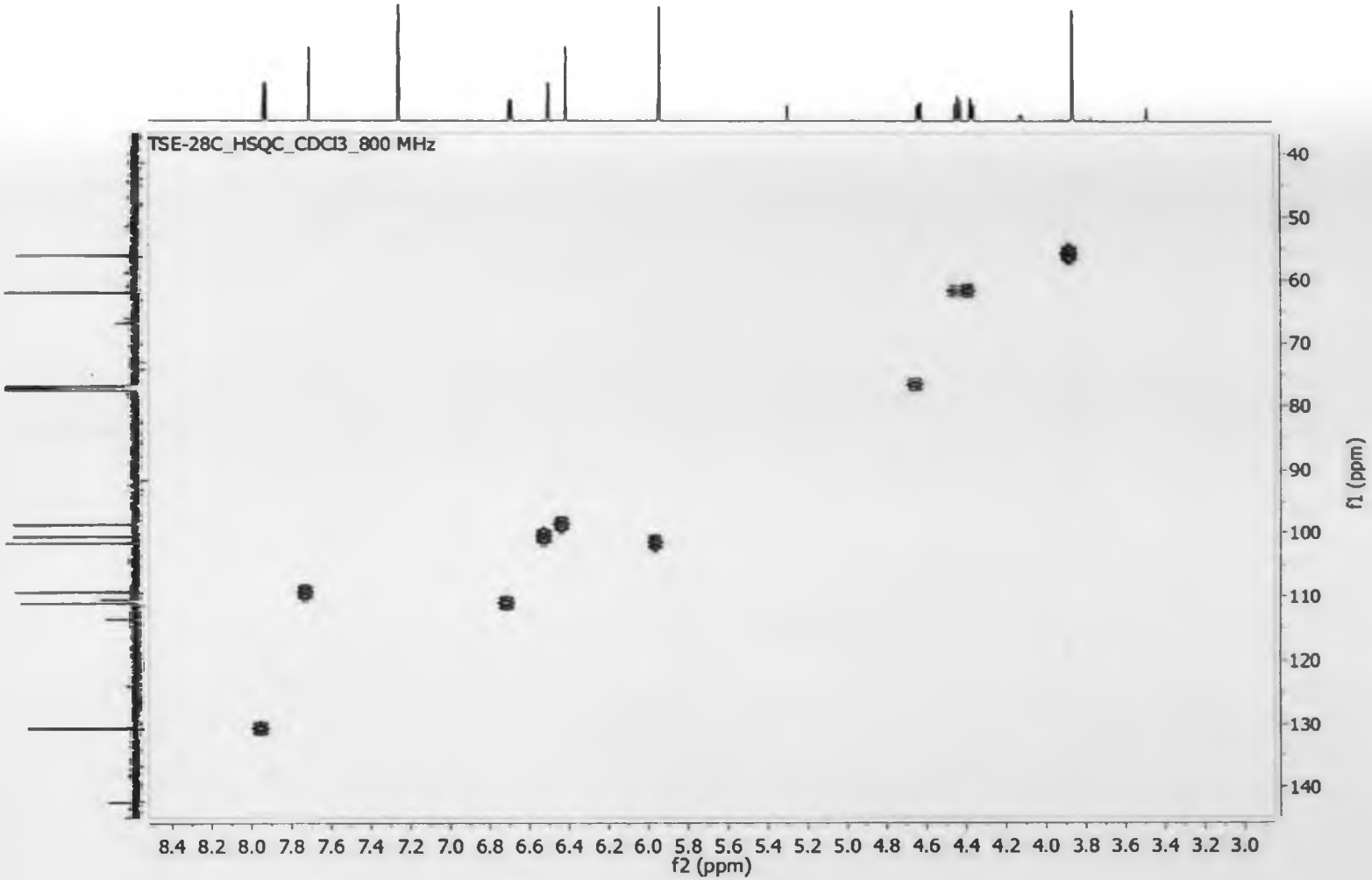
Appendix 27C: COSY (799.87 MHz) spectrum of compound 319



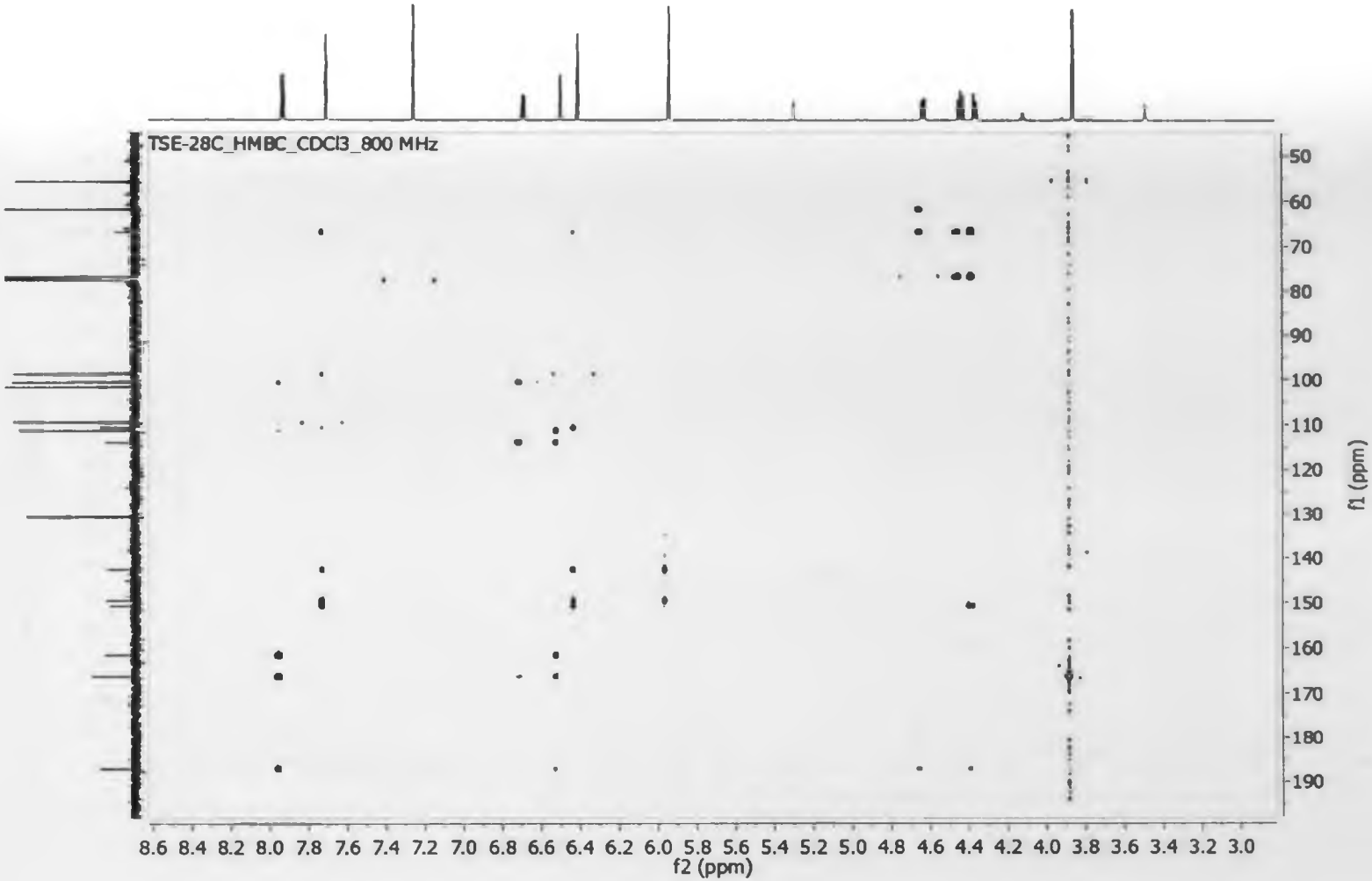
Appendix 27D: NOESY (799.87 MHz) spectrum of compound 319



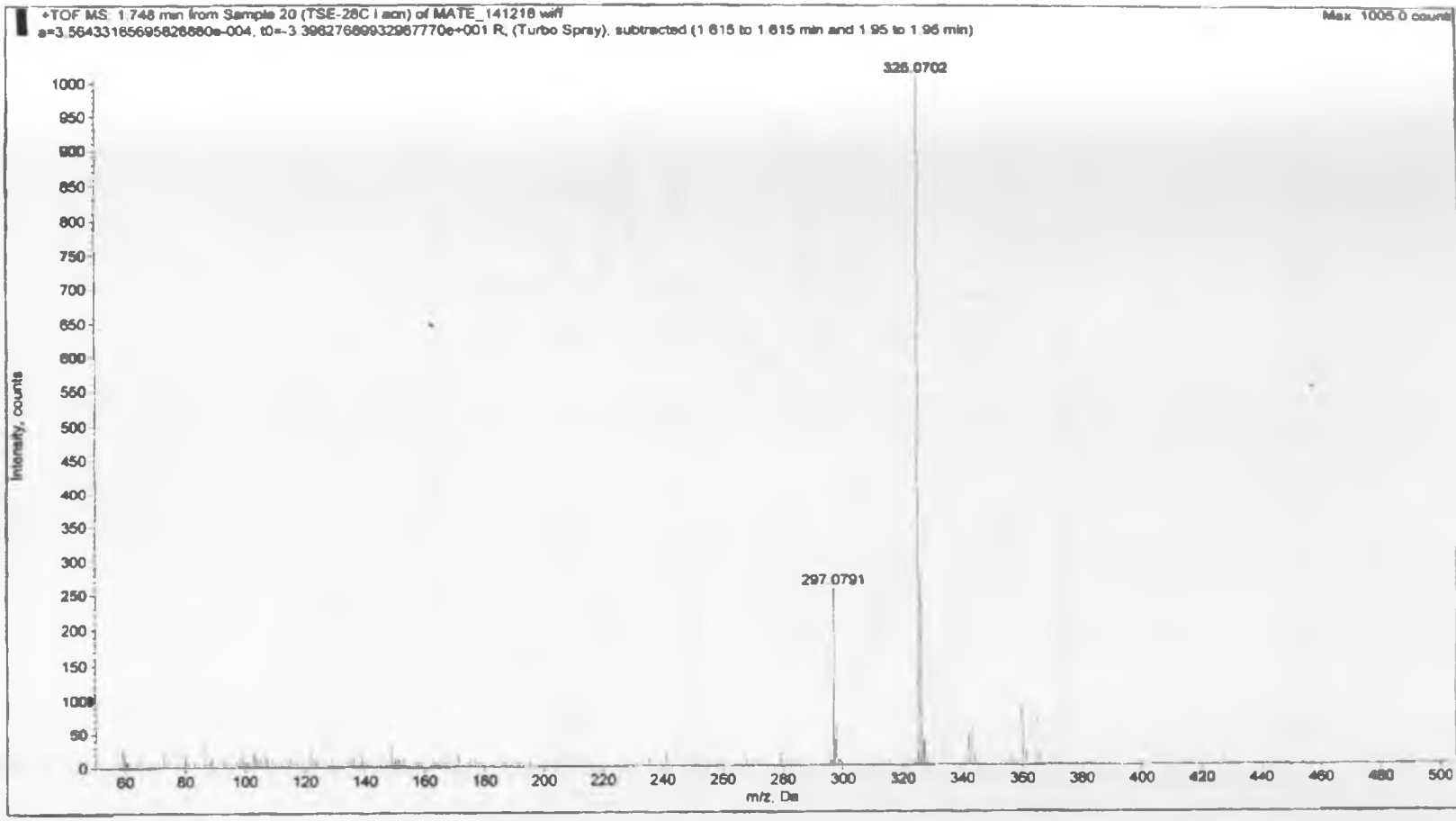
Appendix 27E: HSQC (799.87/201.15 MHz) spectrum of compound 319



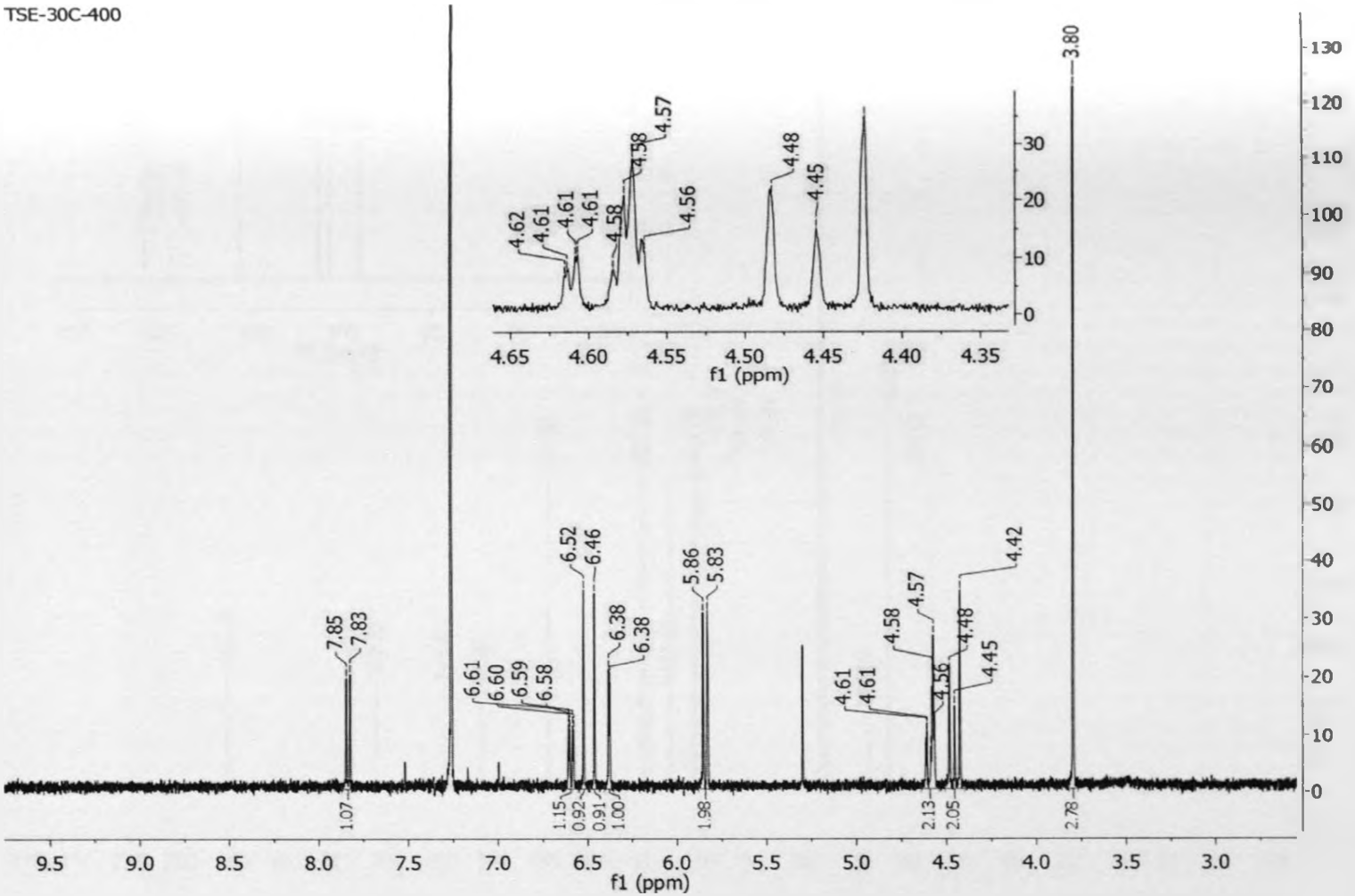
Appendix 27F: HMBC (799.87/201.15 MHz) spectrum of compound 319



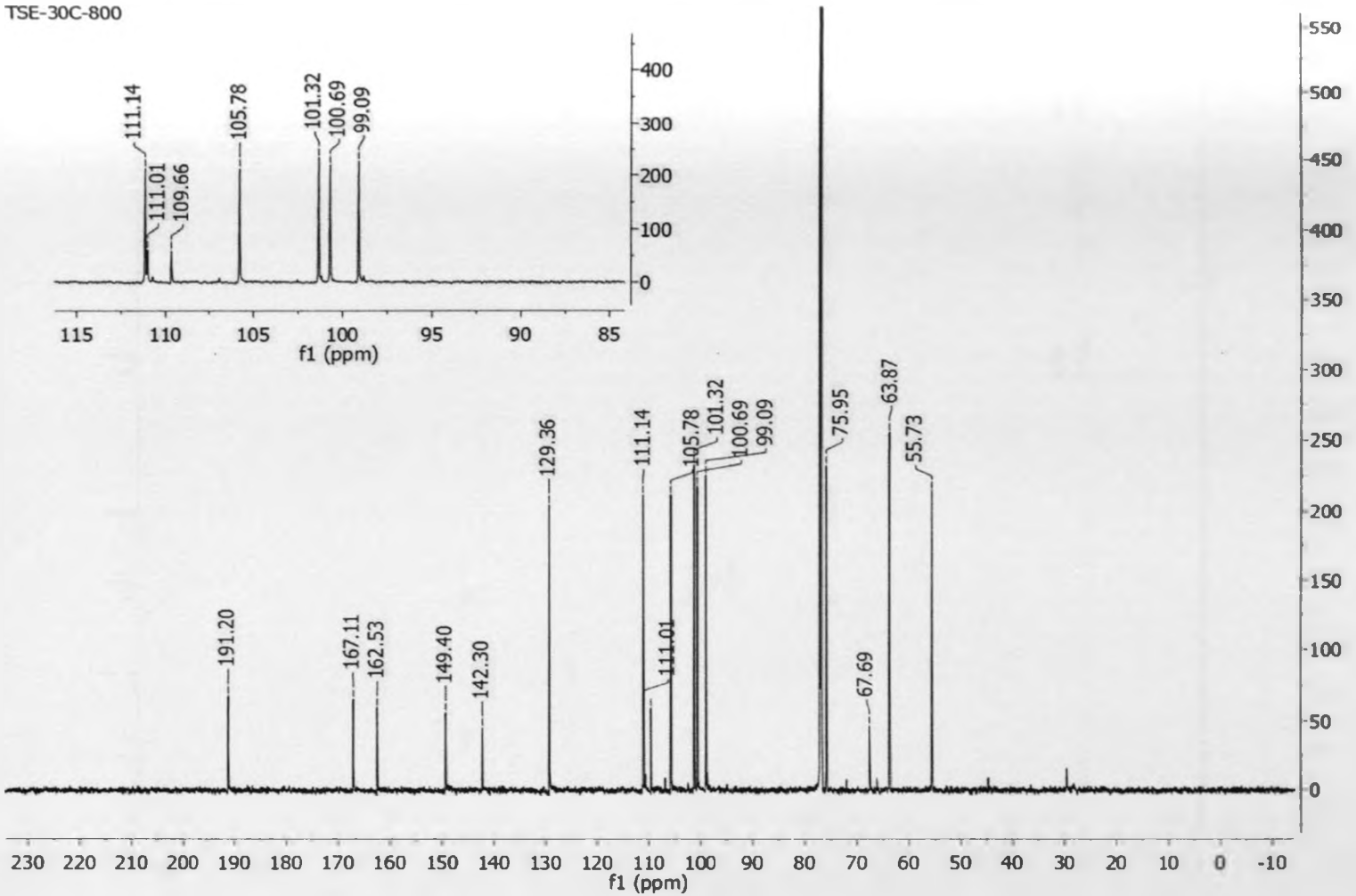
Appendix 27G: HREIMS spectrum of compound 319



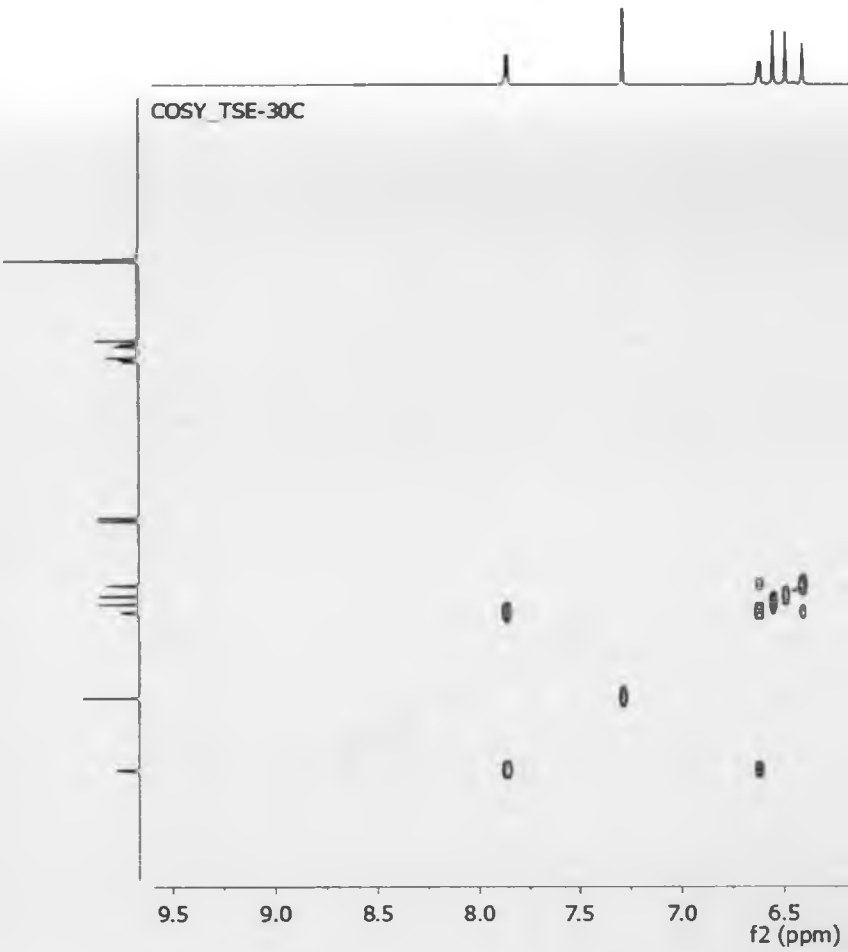
Appendix 28A: ^{1}H NMR (399.97 MHz) spectrum of compound 318



Appendix 28B: ^{13}C NMR (201.15 MHz) spectrum of compound 318

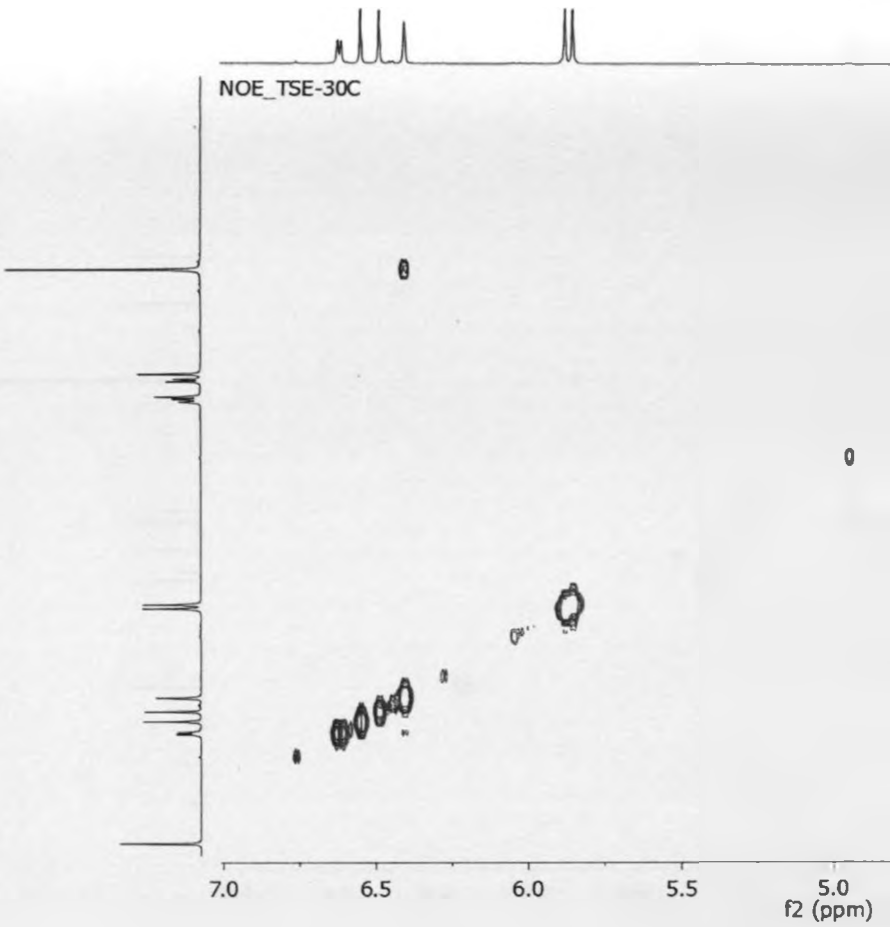


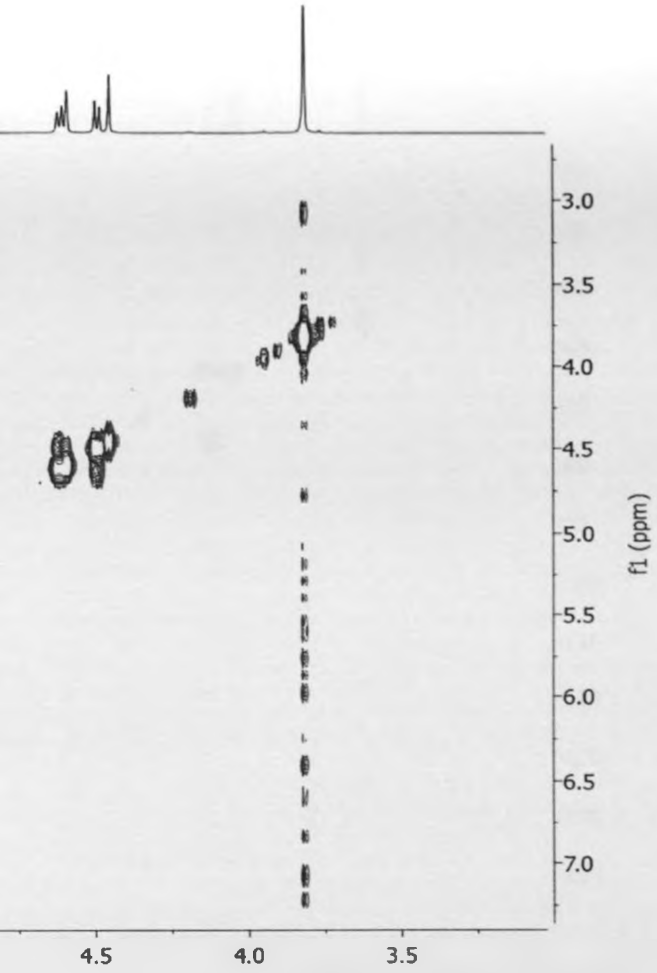
Appendix 28C: COSY (799.87 MHz) spectrum of compound 318



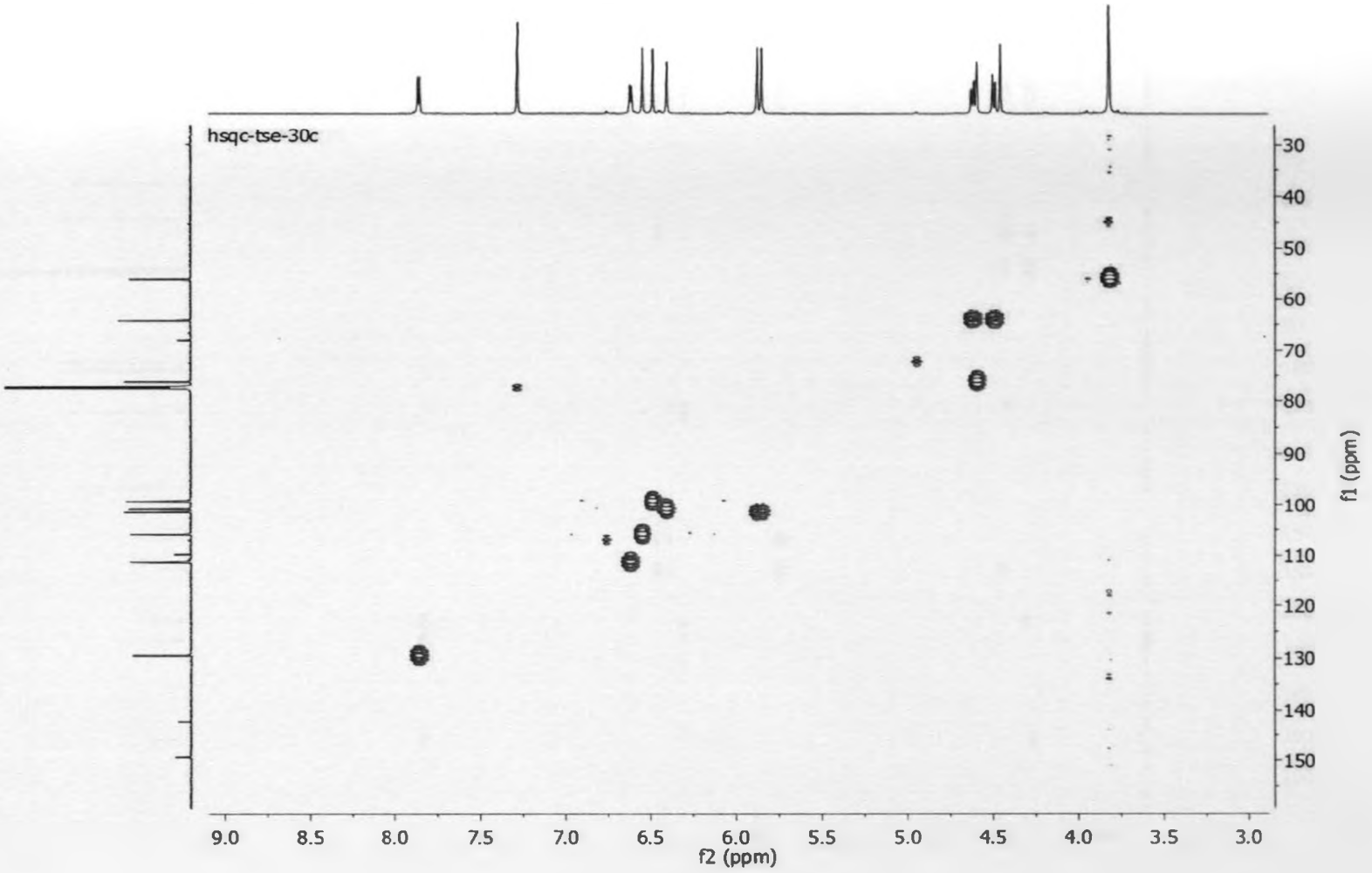
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5

Appendix 28D: NOESY (799.87 MHz) spectrum of compound 318

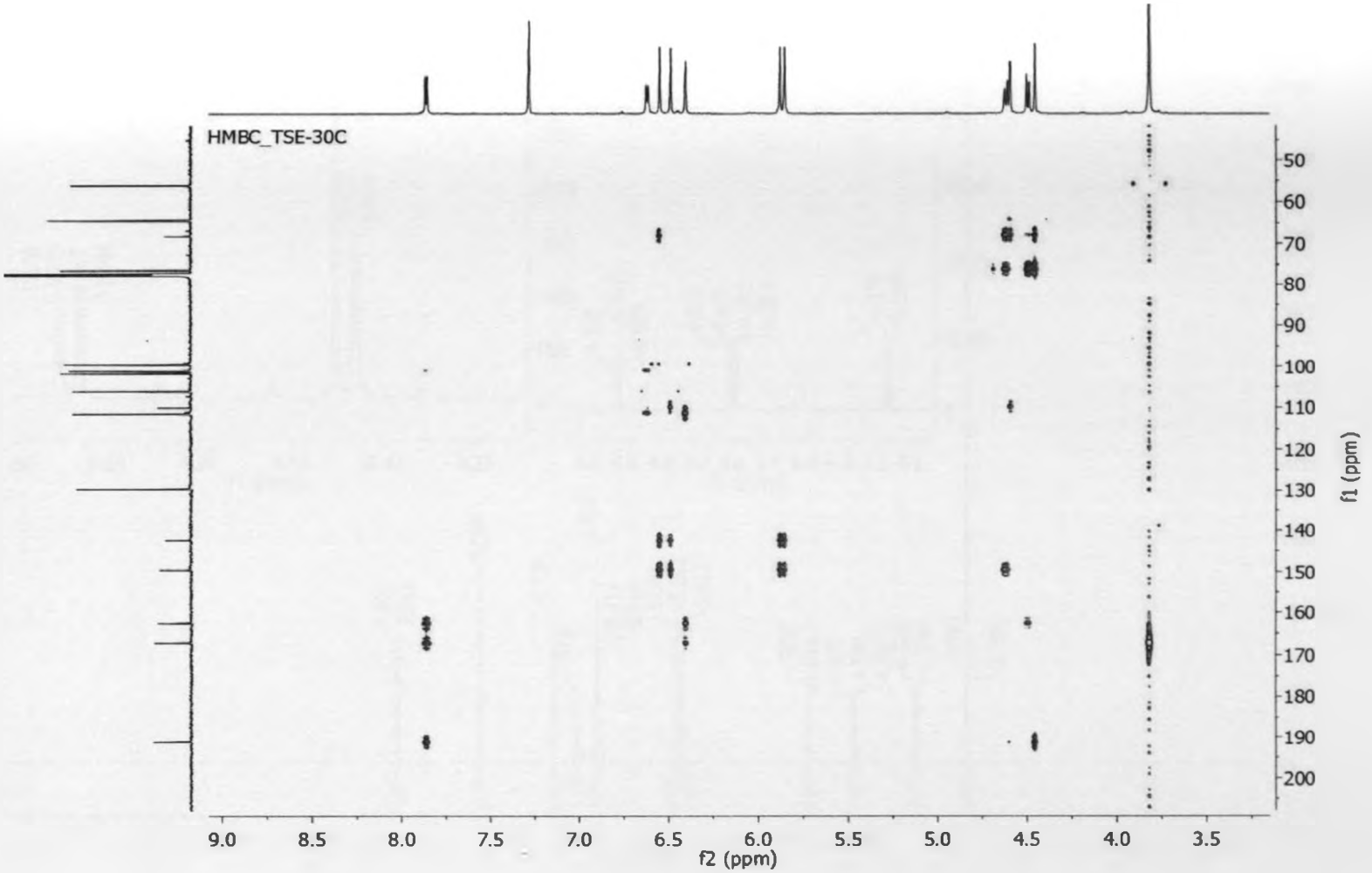



3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
f1 (ppm)
4.5
4.0
3.5

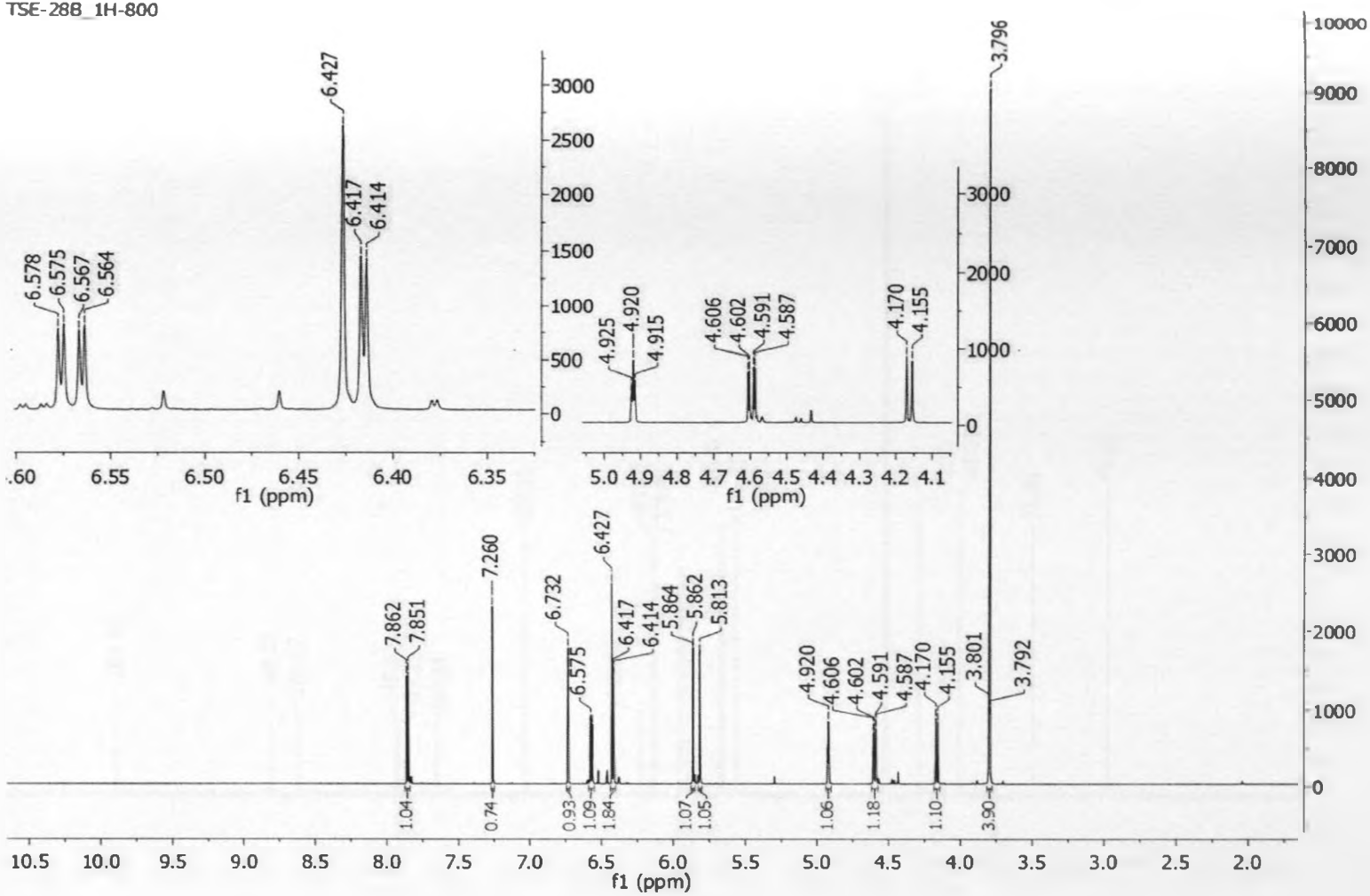
Appendix 28E: HSQC (799.87 MHz/201.15 MHz) spectrum of compound 318



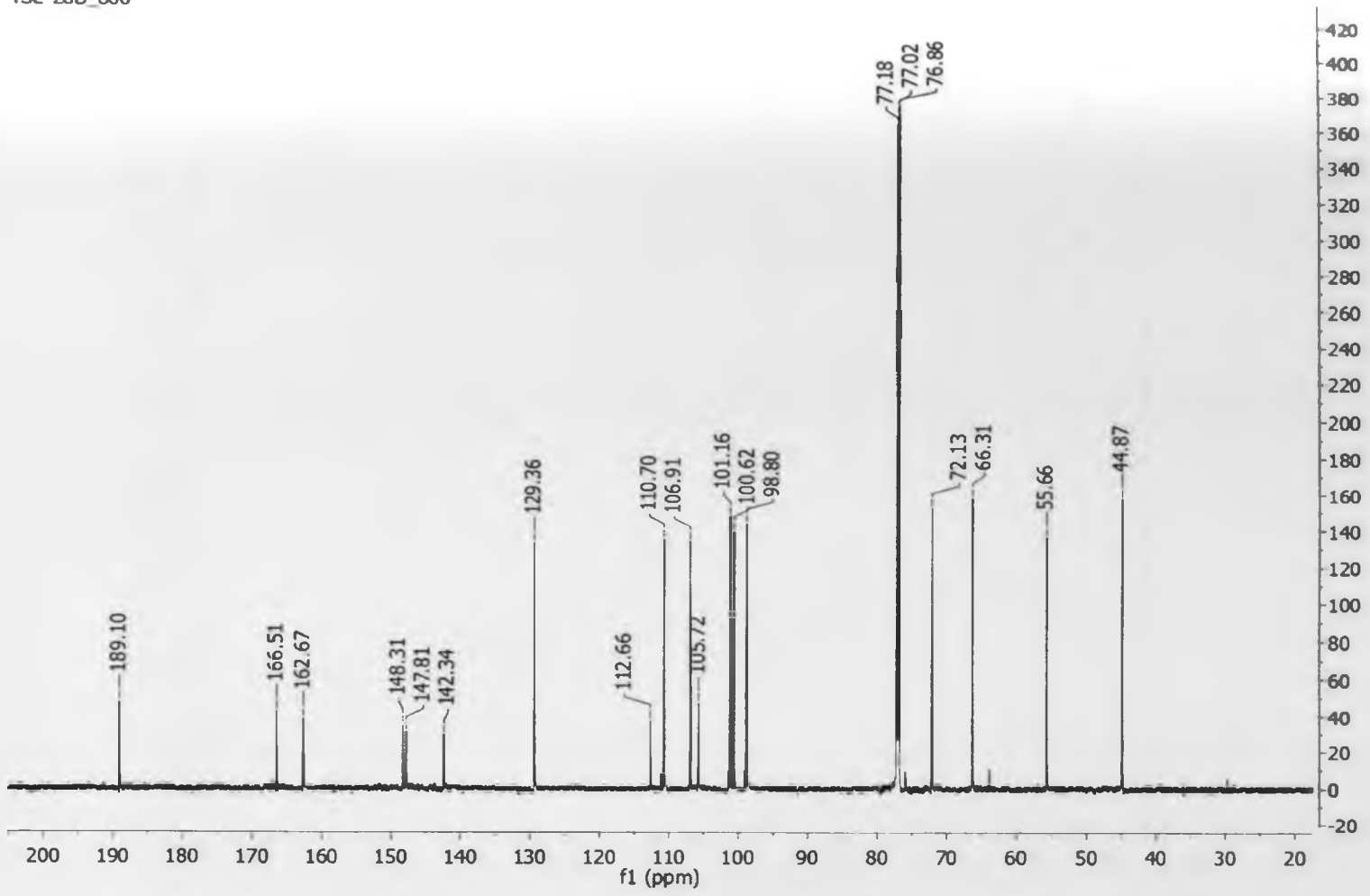
Appendix 28F: HMBC (799.87 MHz/201.15 MHz) spectrum of compound 318



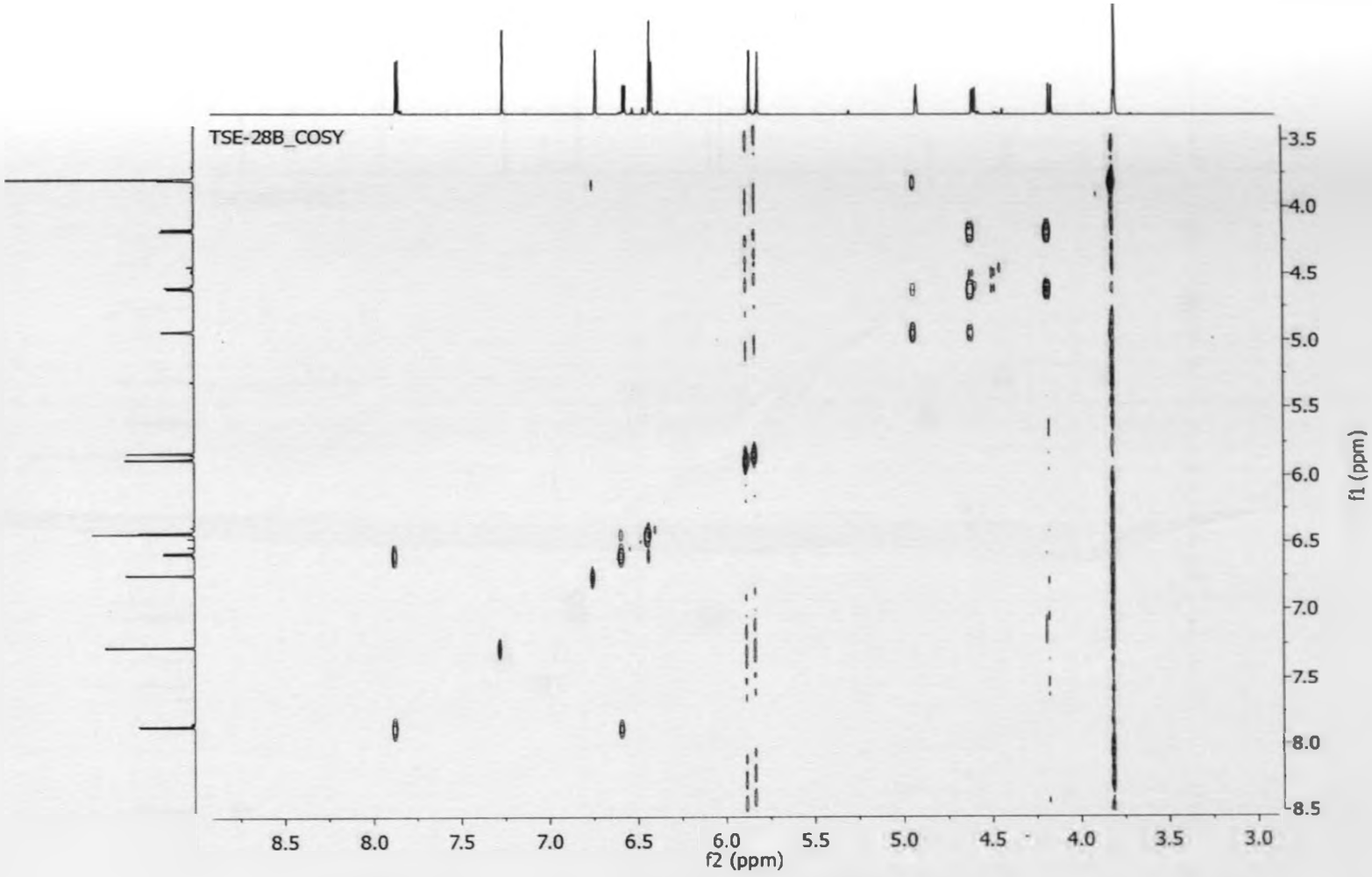
Appendix 29A: ^{1}H NMR (799.87 MHz) spectrum of compound 320



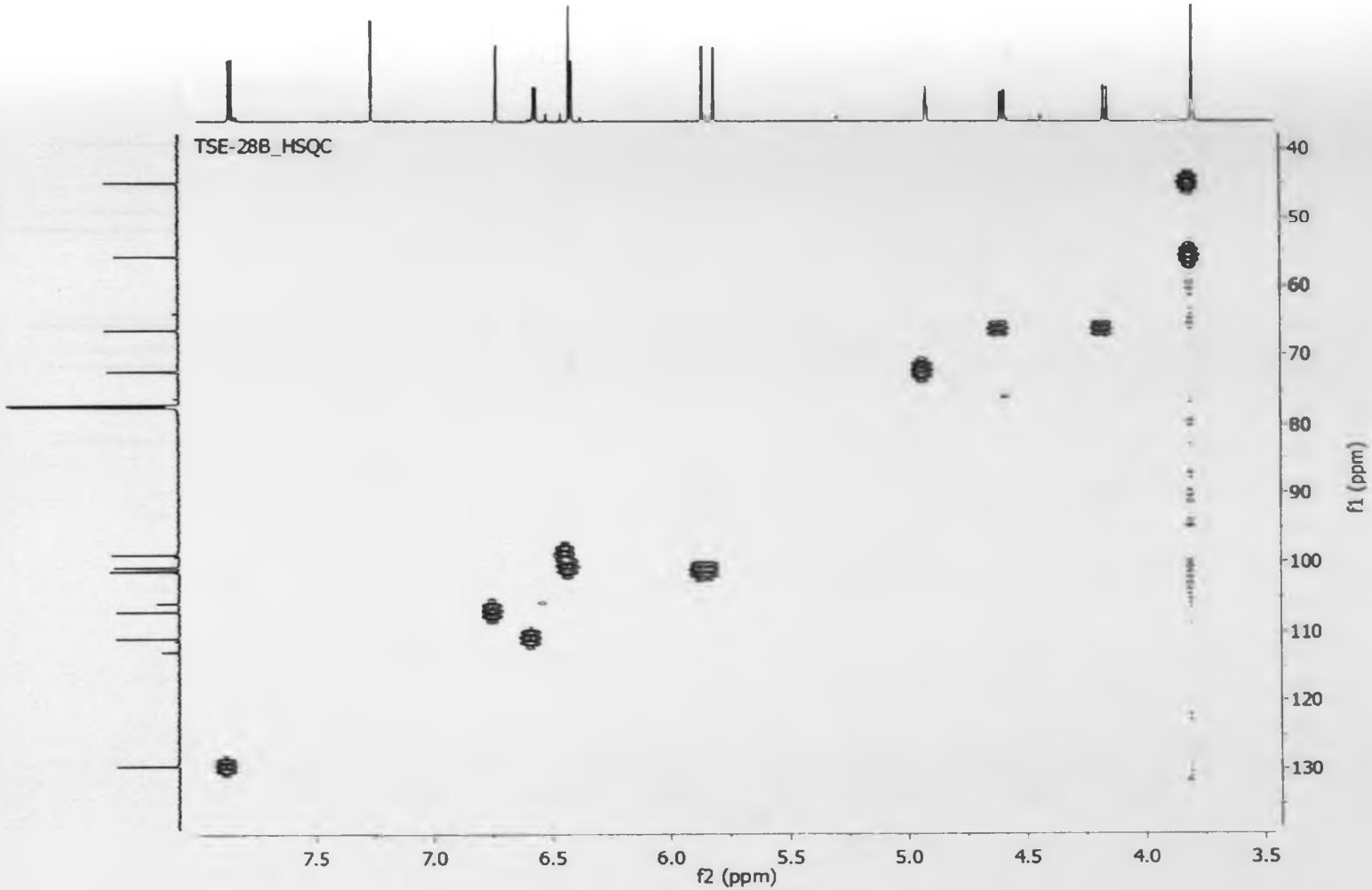
Appendix 29B: ^{1}H NMR (799.87 MHz) spectrum of compound 320



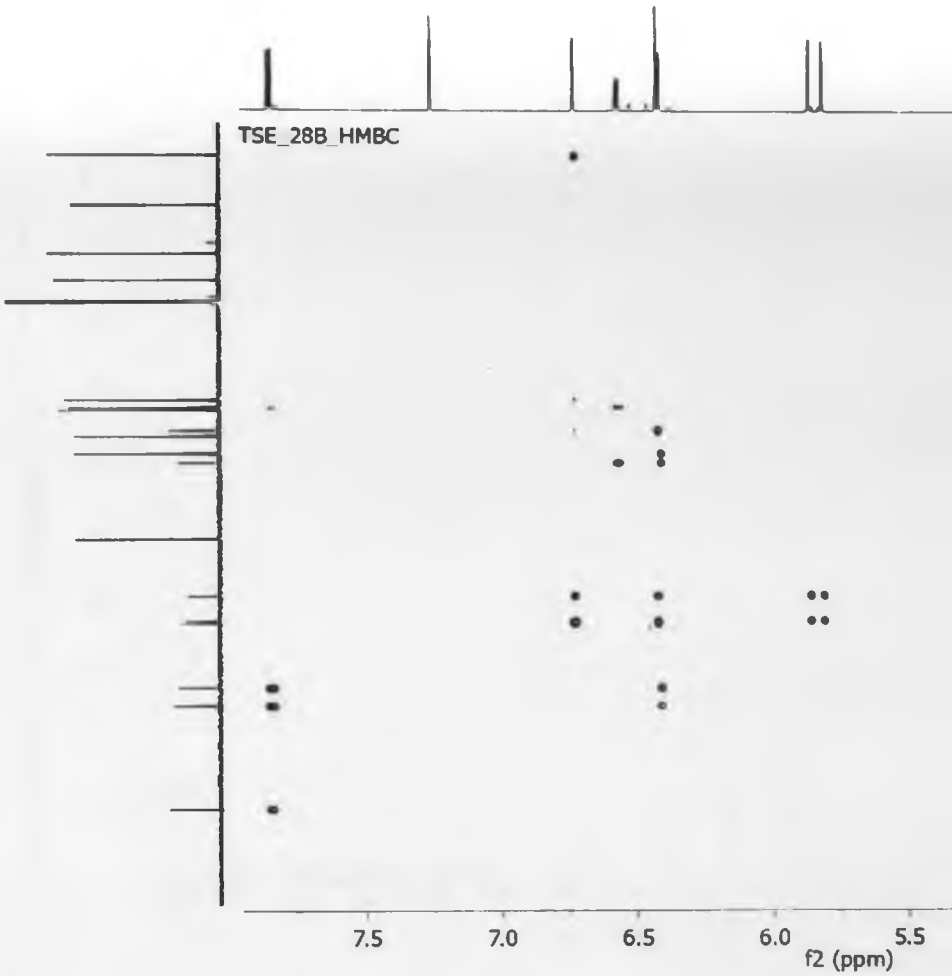
Appendix 29C: COSY (799.87 MHz) spectrum of compound 320

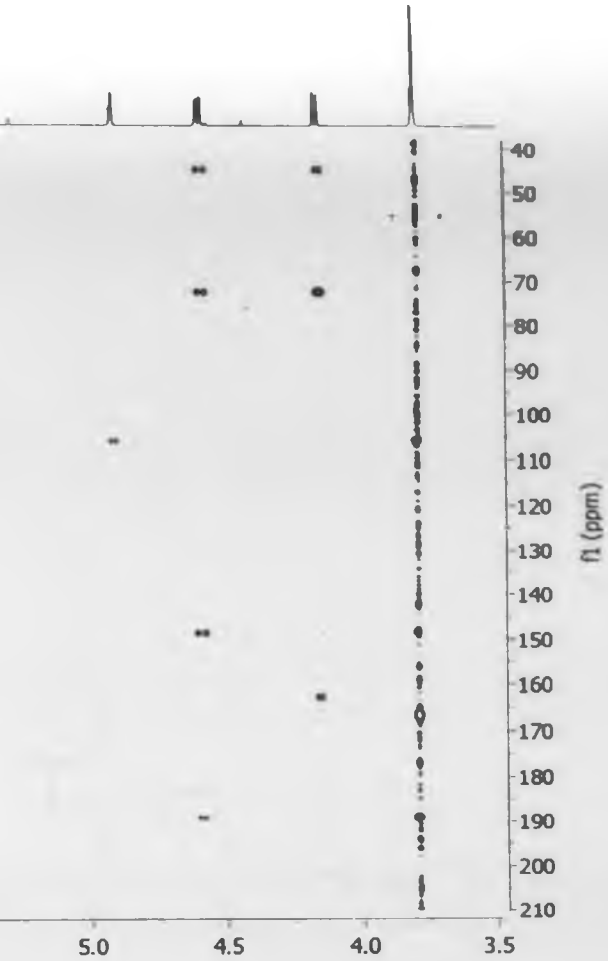


Appendix 29D: HSQC (799.87/201.15 MHz) spectrum of compound 320

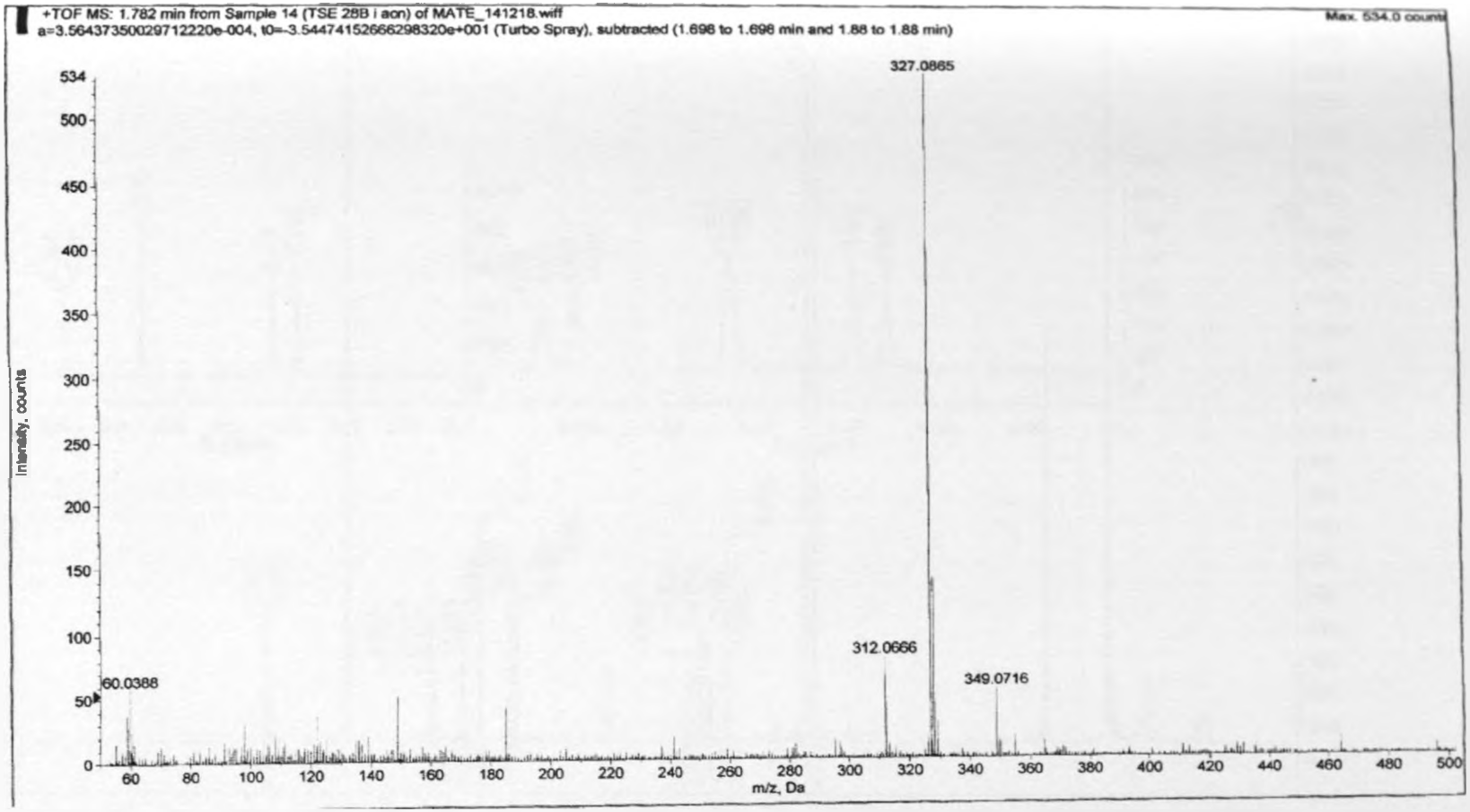


Appendix 29E: HSQC (799.87/201.15 MHz) spectrum of compound 320

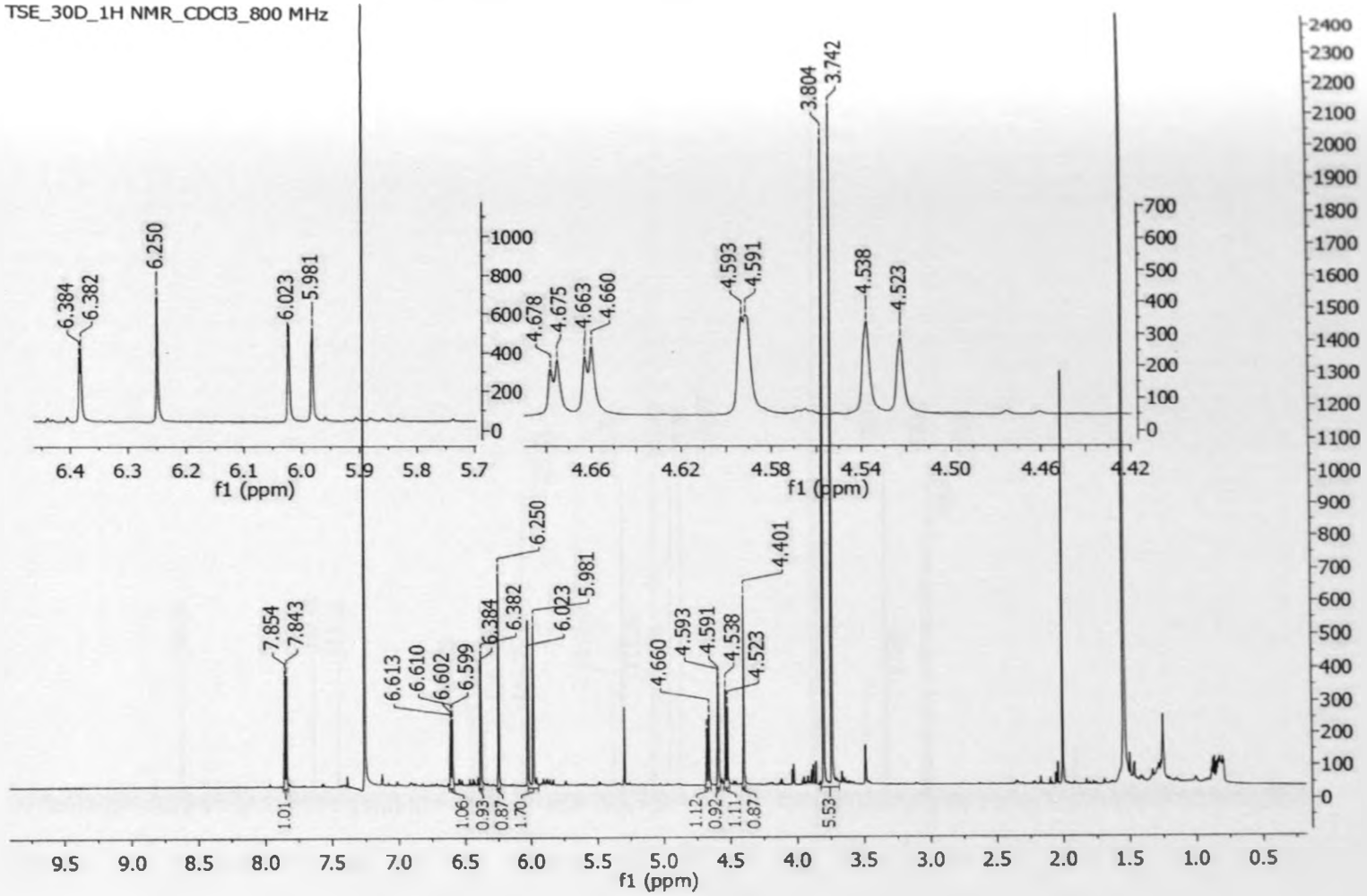


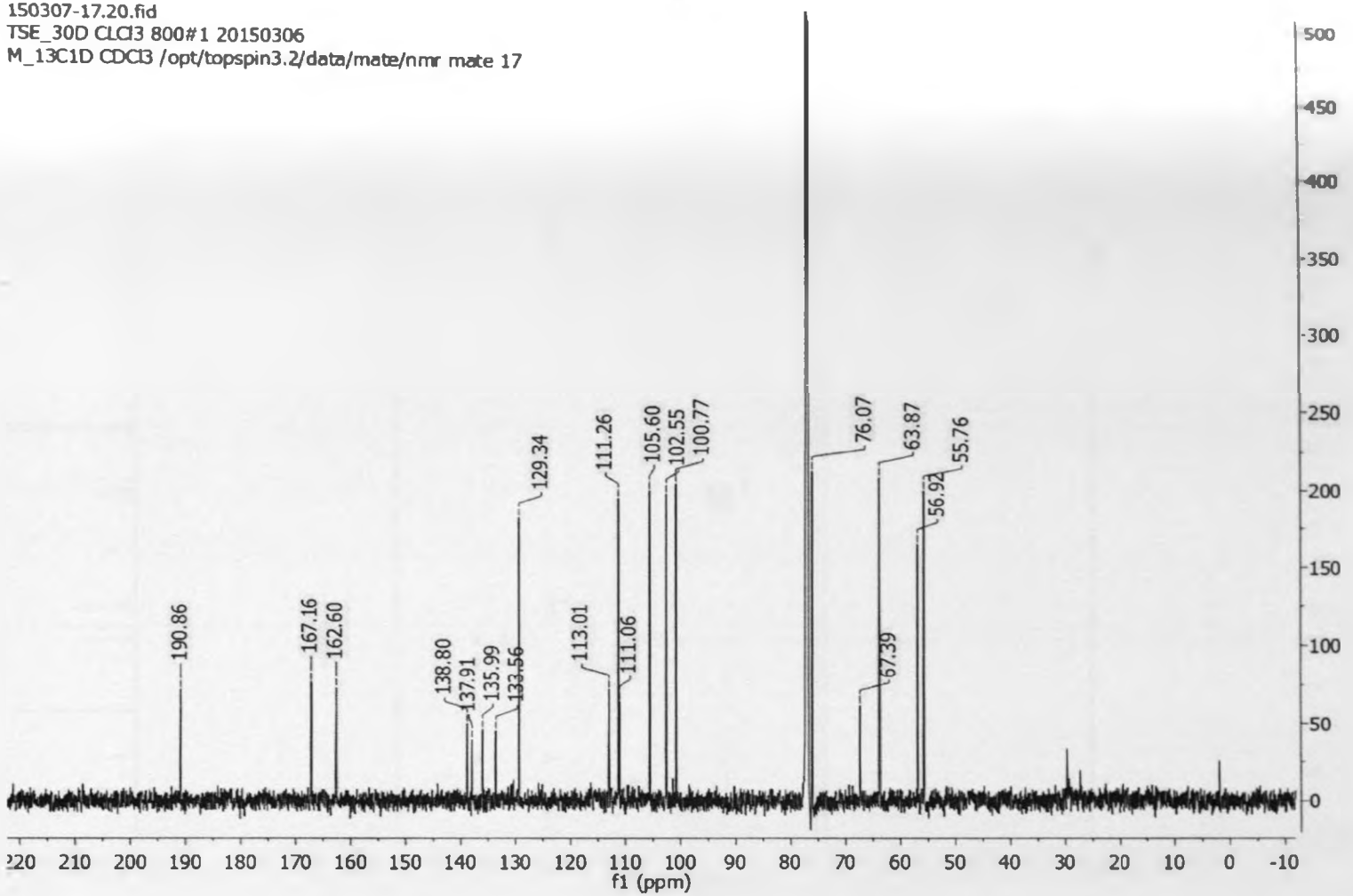

40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
f1 (ppm)
5.0
4.5
4.0
3.5

Appendix 29F: HRESMS spectrum of compound 320

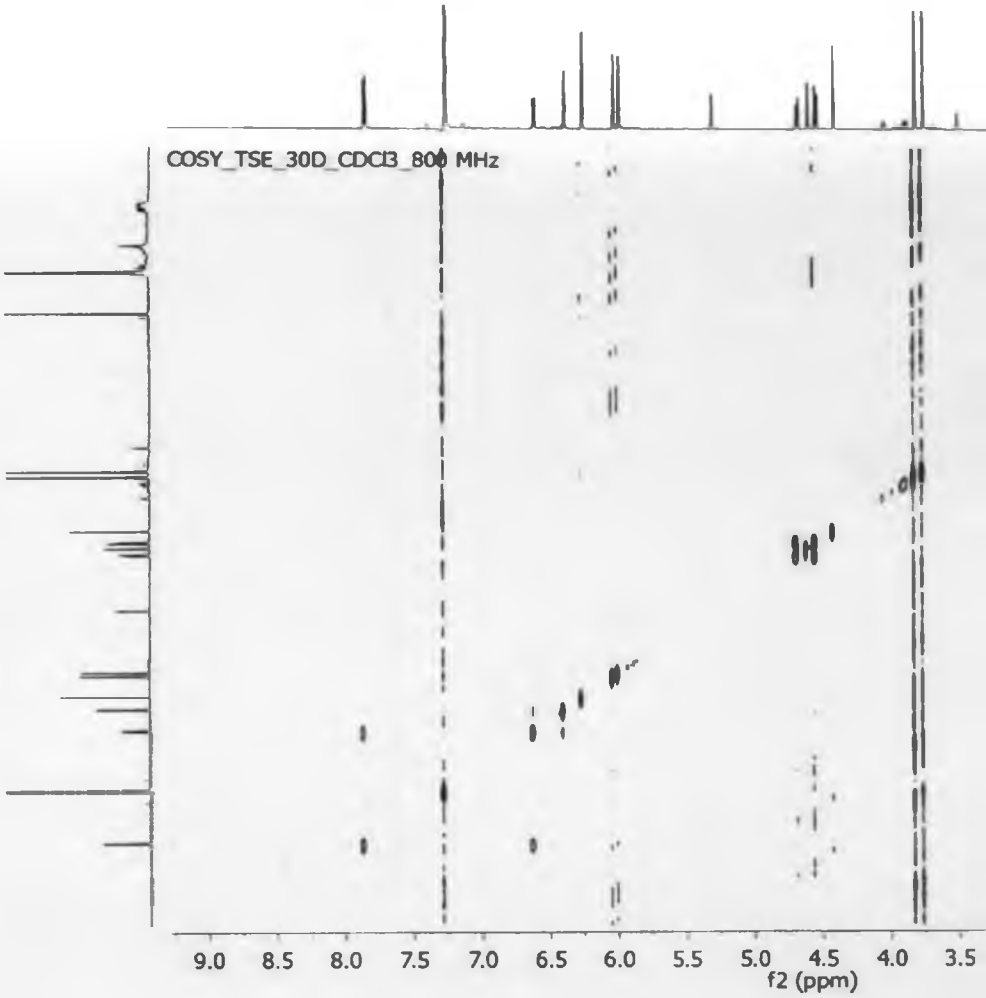


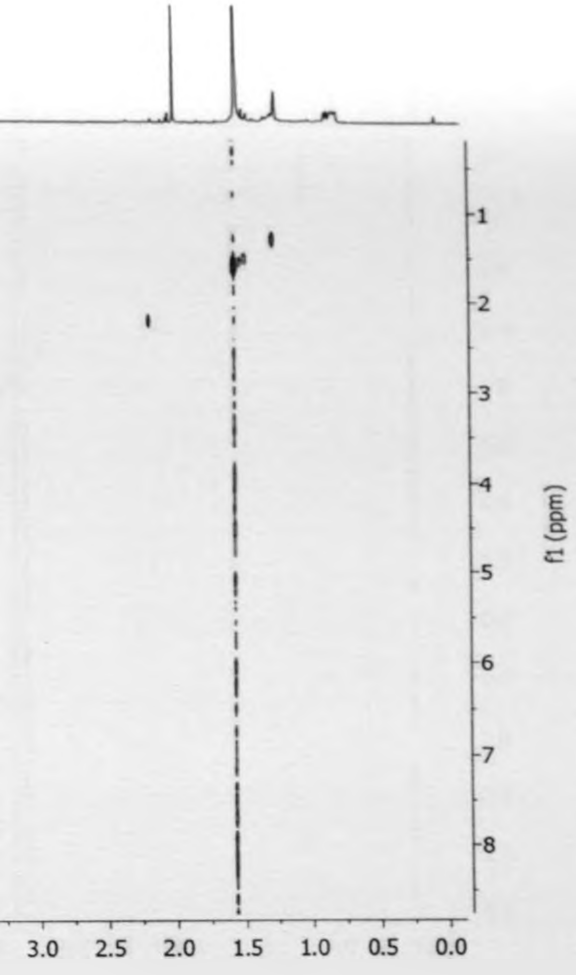
Appendix 30A: ^{1}H NMR (799.87 MHz) spectrum of compound 321



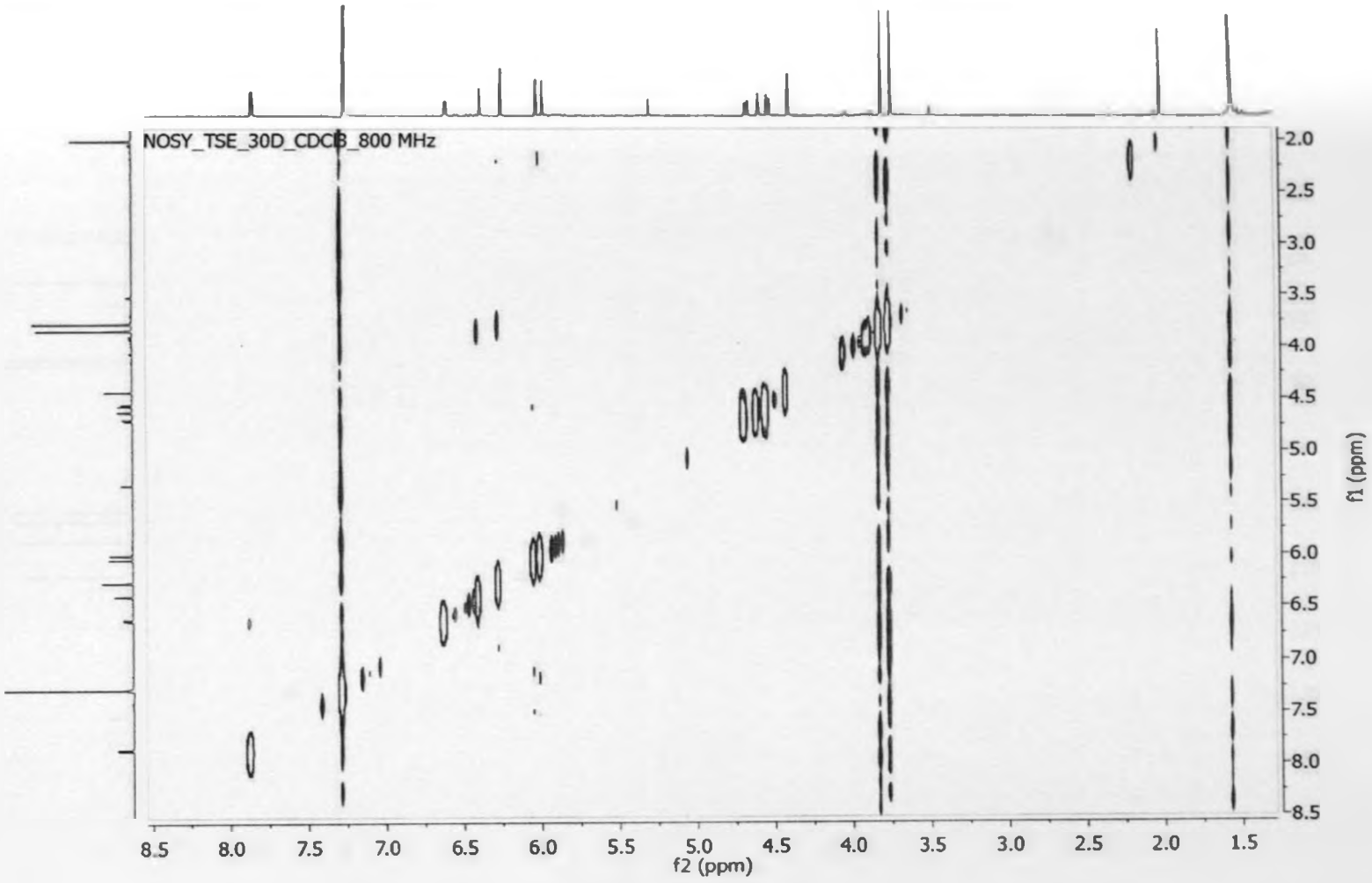

150307-17.20.fid
TSE_30D CLCl3 800#1 20150306
M_13C1D CDCl3 /opt/topspin3.2/data/mate/nmr mate 17
190.86
167.16
162.60
138.80
137.91
135.99
133.56
129.34
113.01
111.26
111.06
105.60
102.55
100.77
76.07
67.39
63.87
56.92
55.76
f1 (ppm)

Appendix 30C: COSY (799.87 MHz) spectrum of compound 321

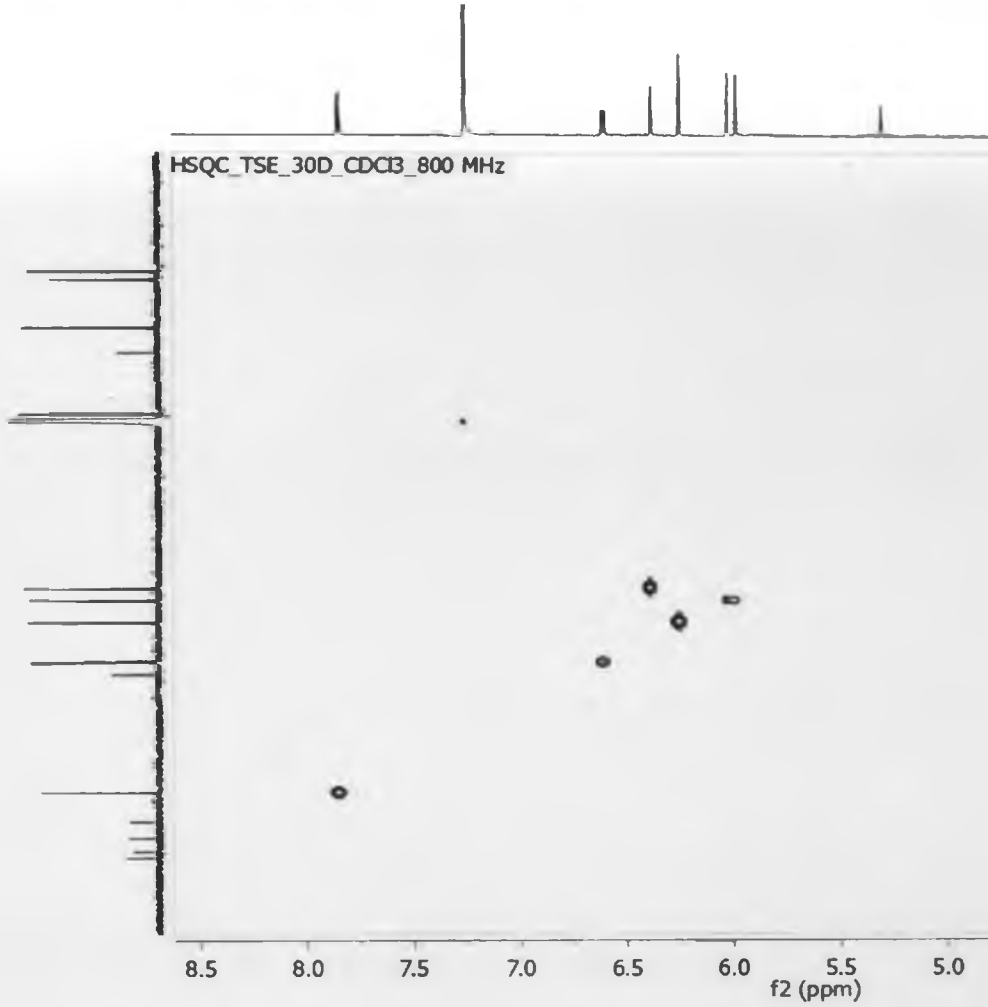


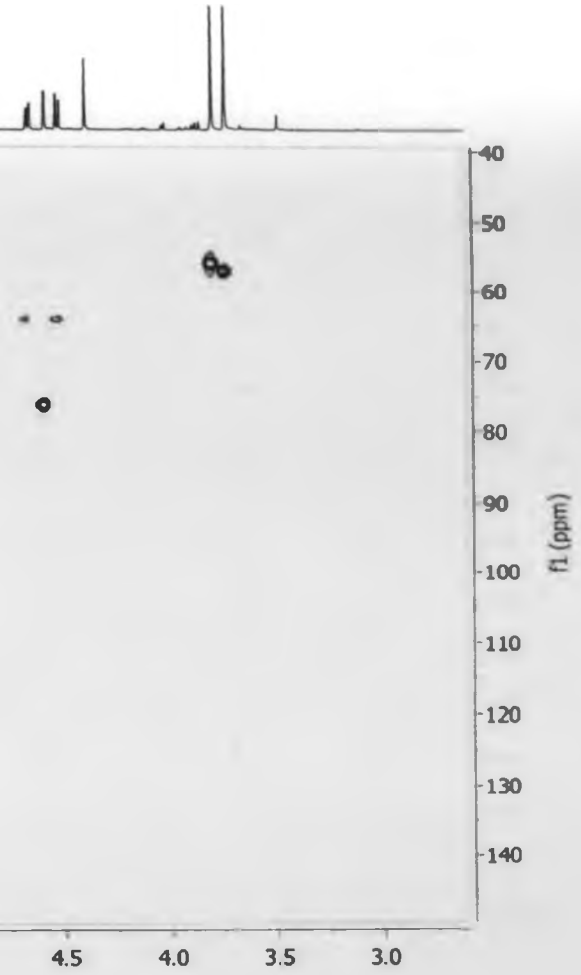

1
2
3
4
5
6
7
8
f1 (ppm)
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Appendix 30D: NOESY (799.87 MHz) spectrum of compound 321

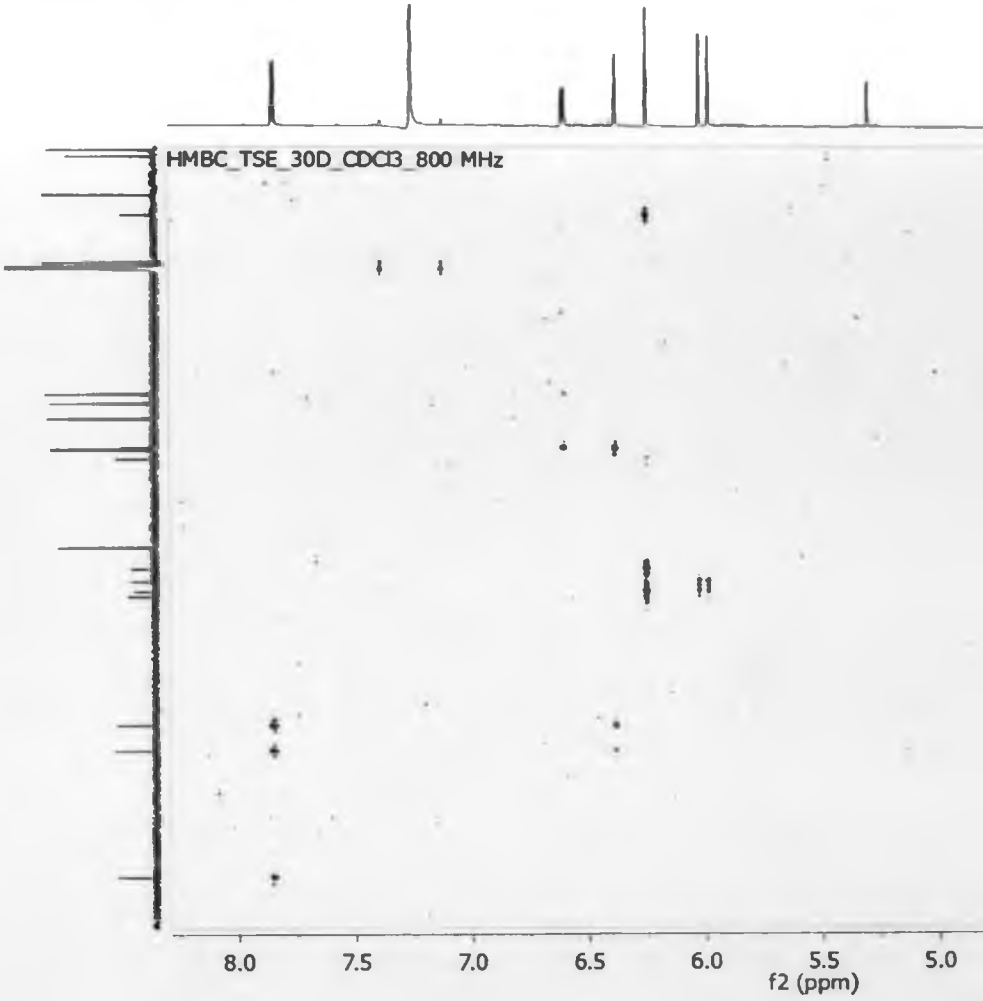


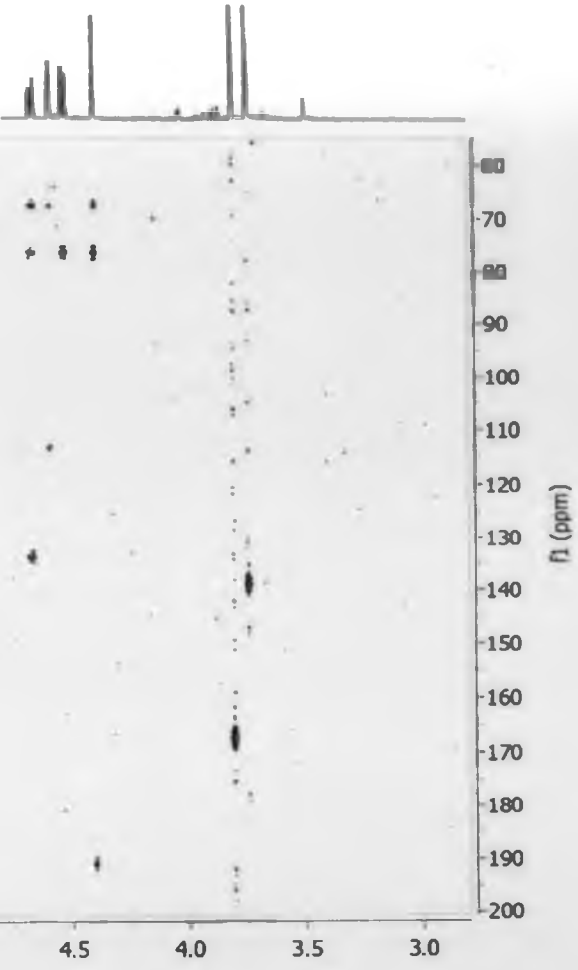
Appendix 30E: HSQC (799.87/201.15 MHz) spectrum of compound 321



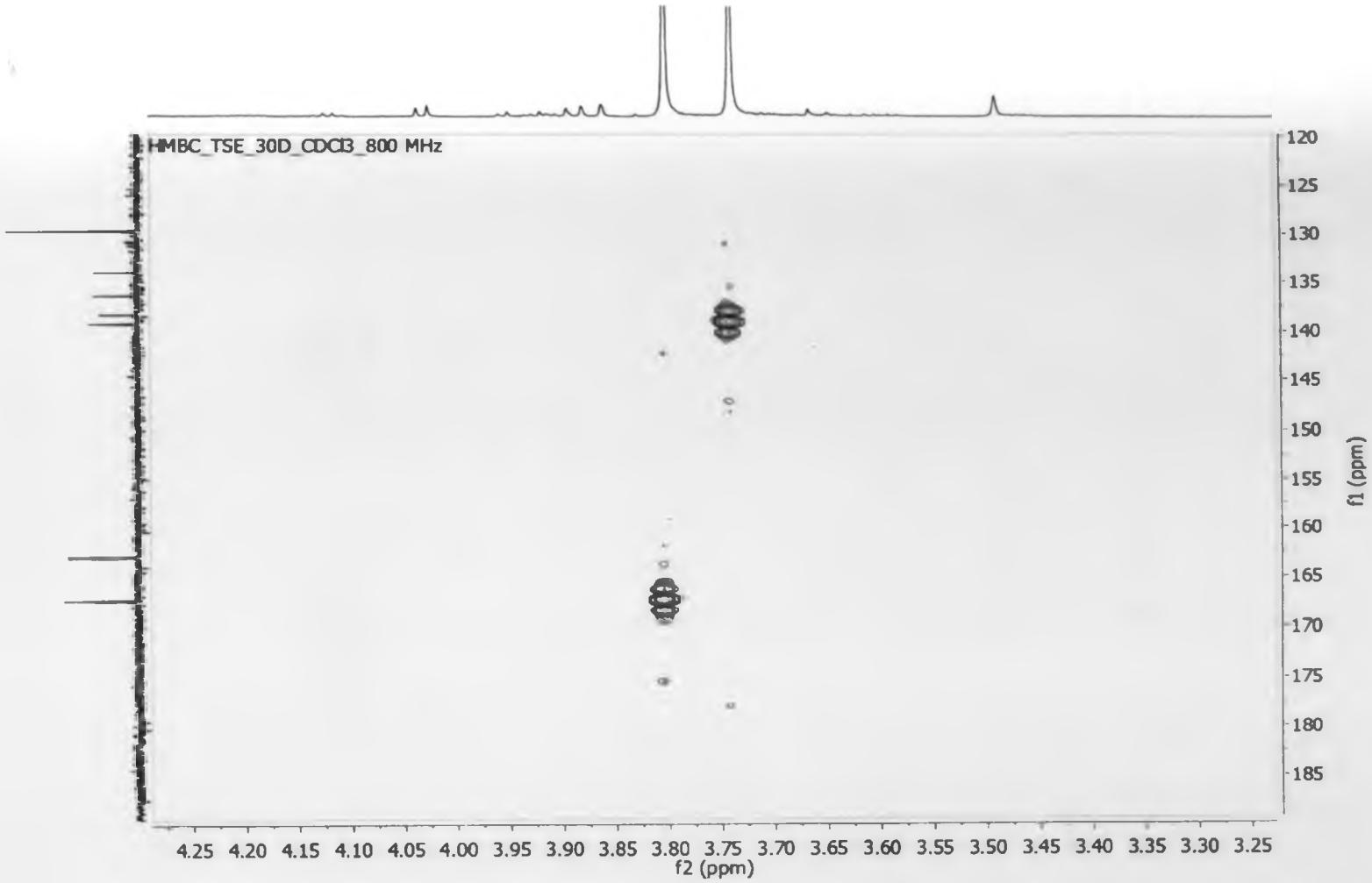

40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)
4.5
4.0
3.5
3.0

Appendix 30F: HMBC (799.87/201.15 MHz) spectrum of compound 321

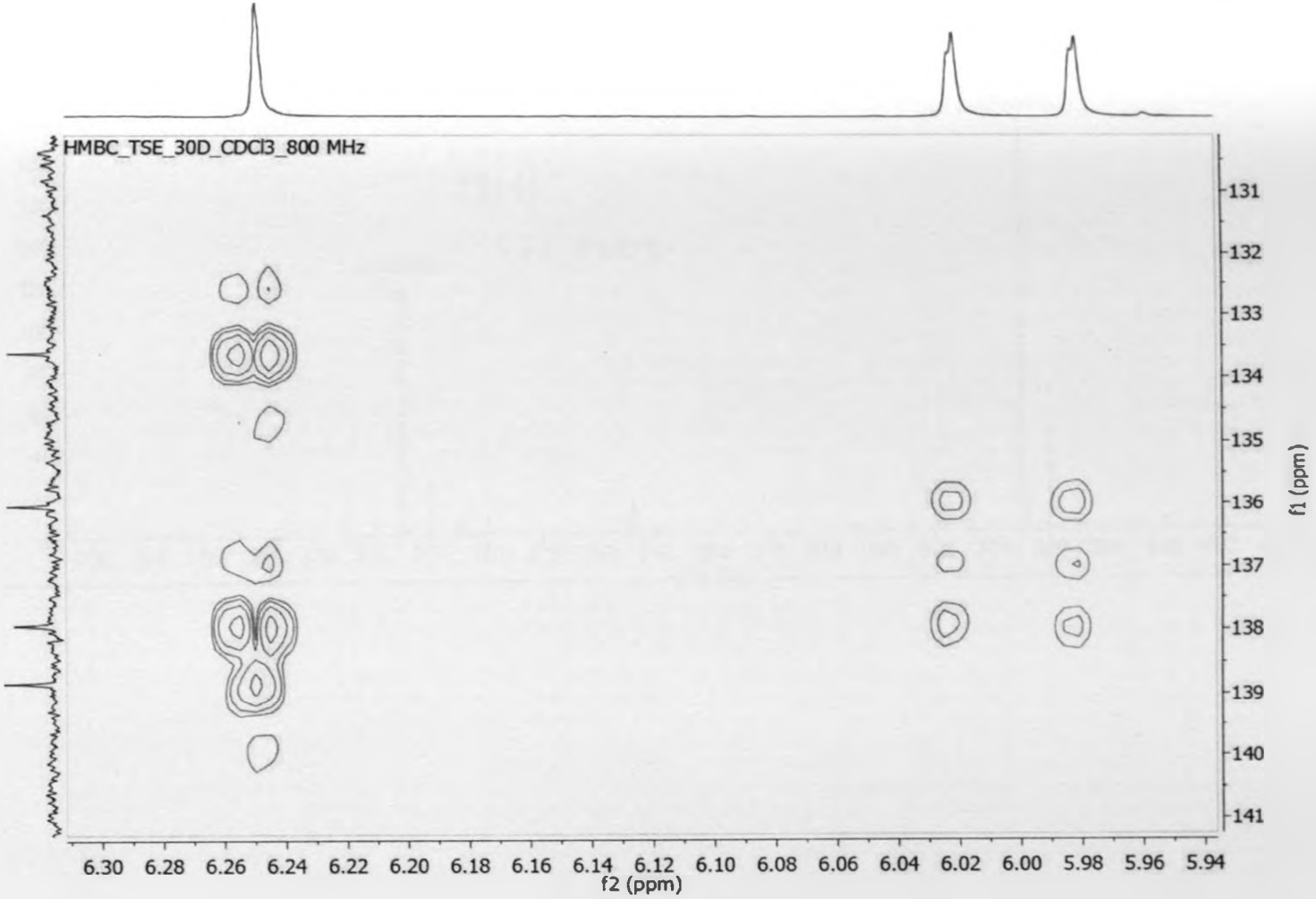



60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)
4.5
4.0
3.5
3.0

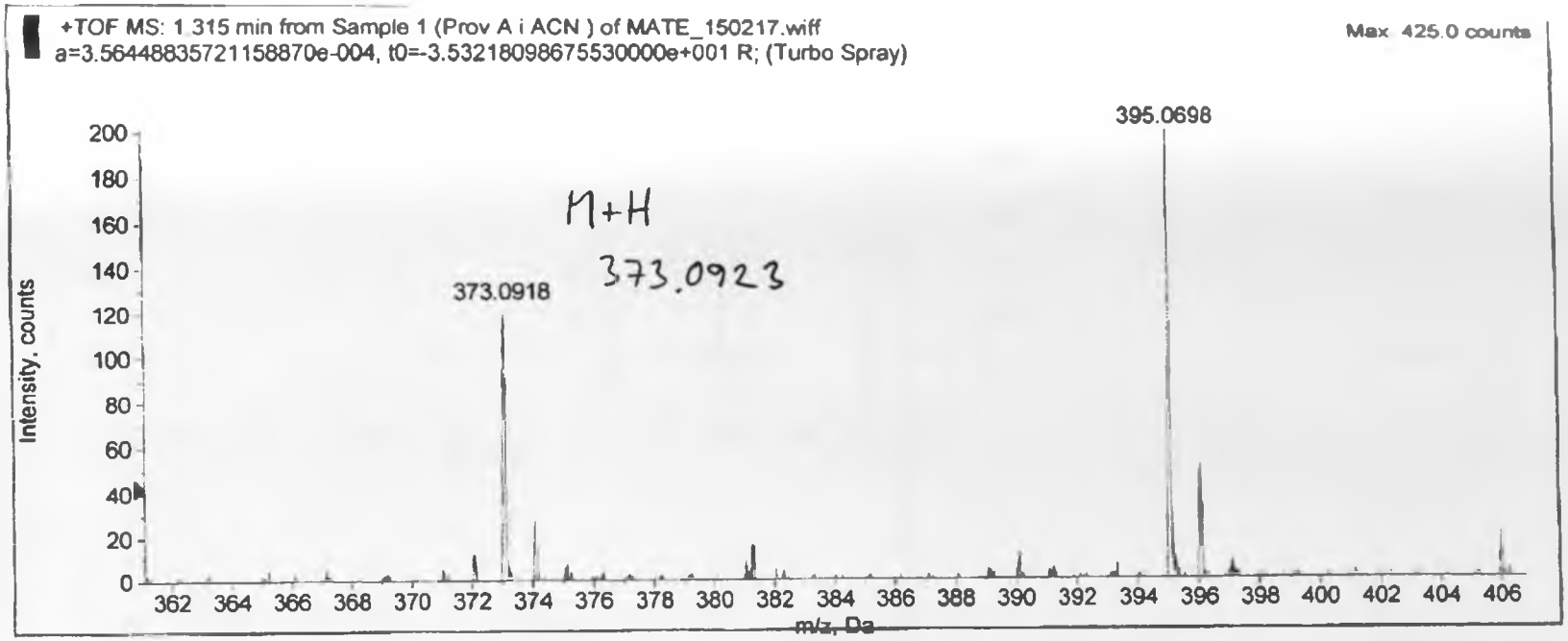
Appendix 30G: HMBC expanded (799.87/201.15 MHz) spectrum of compound 321



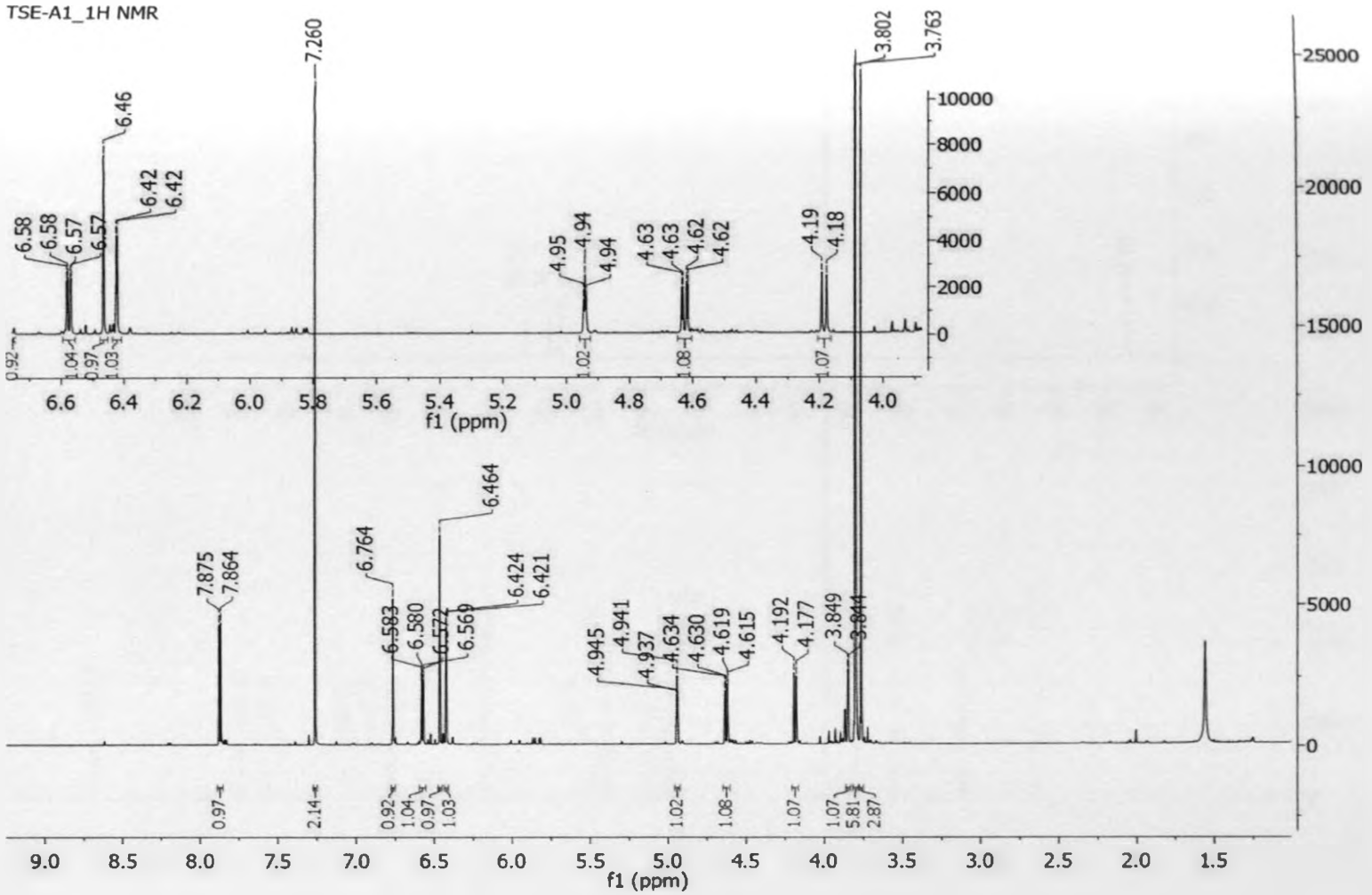
Appendix 30H: HMBC expanded-1 (799.87/201.15 MHz) spectrum of compound 321



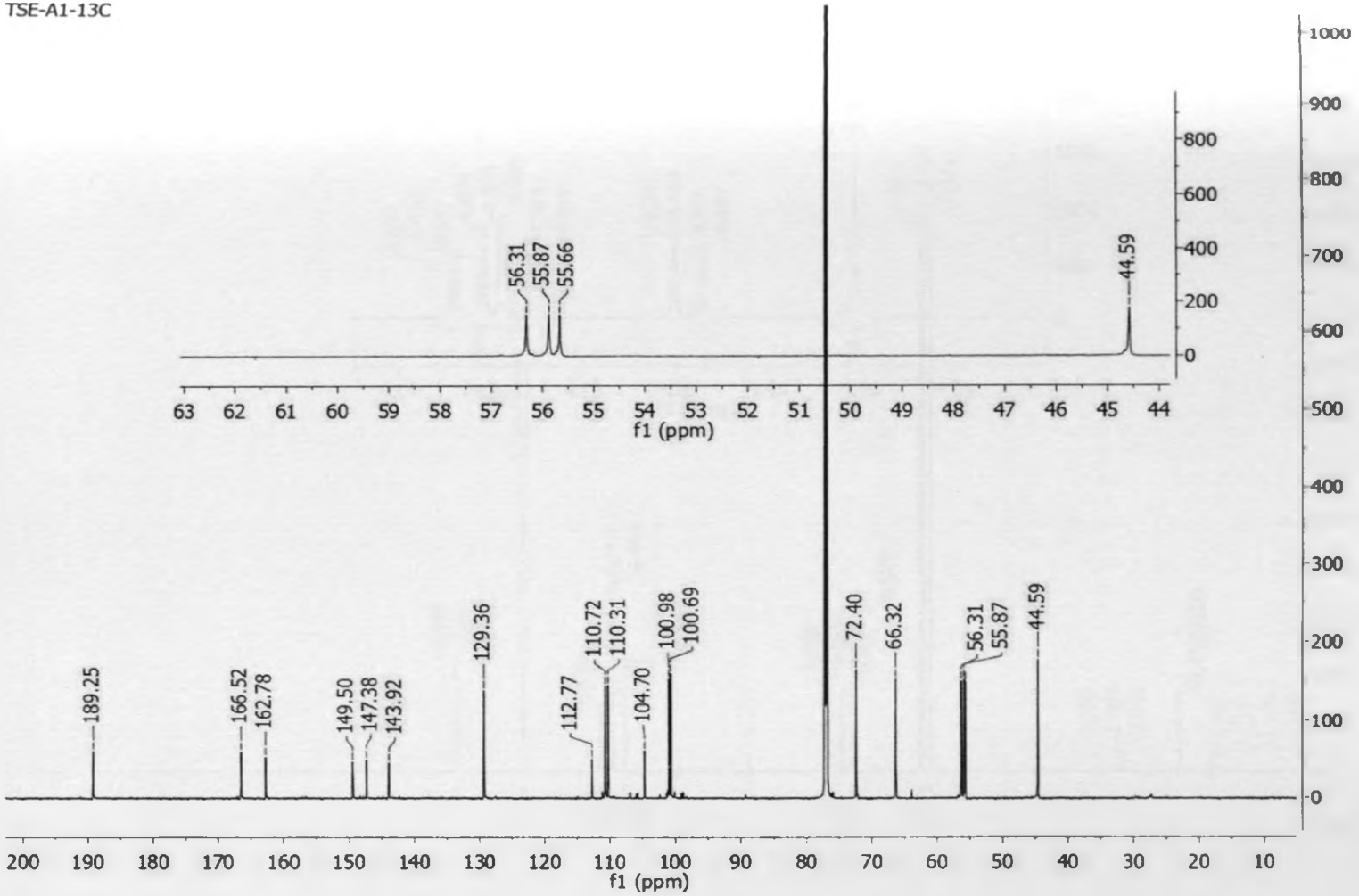
Appendix 301: HREIMS spectrum of compound 321



Appendix 31A: ^1H NMR (799.87 MHz) spectrum of compound 322

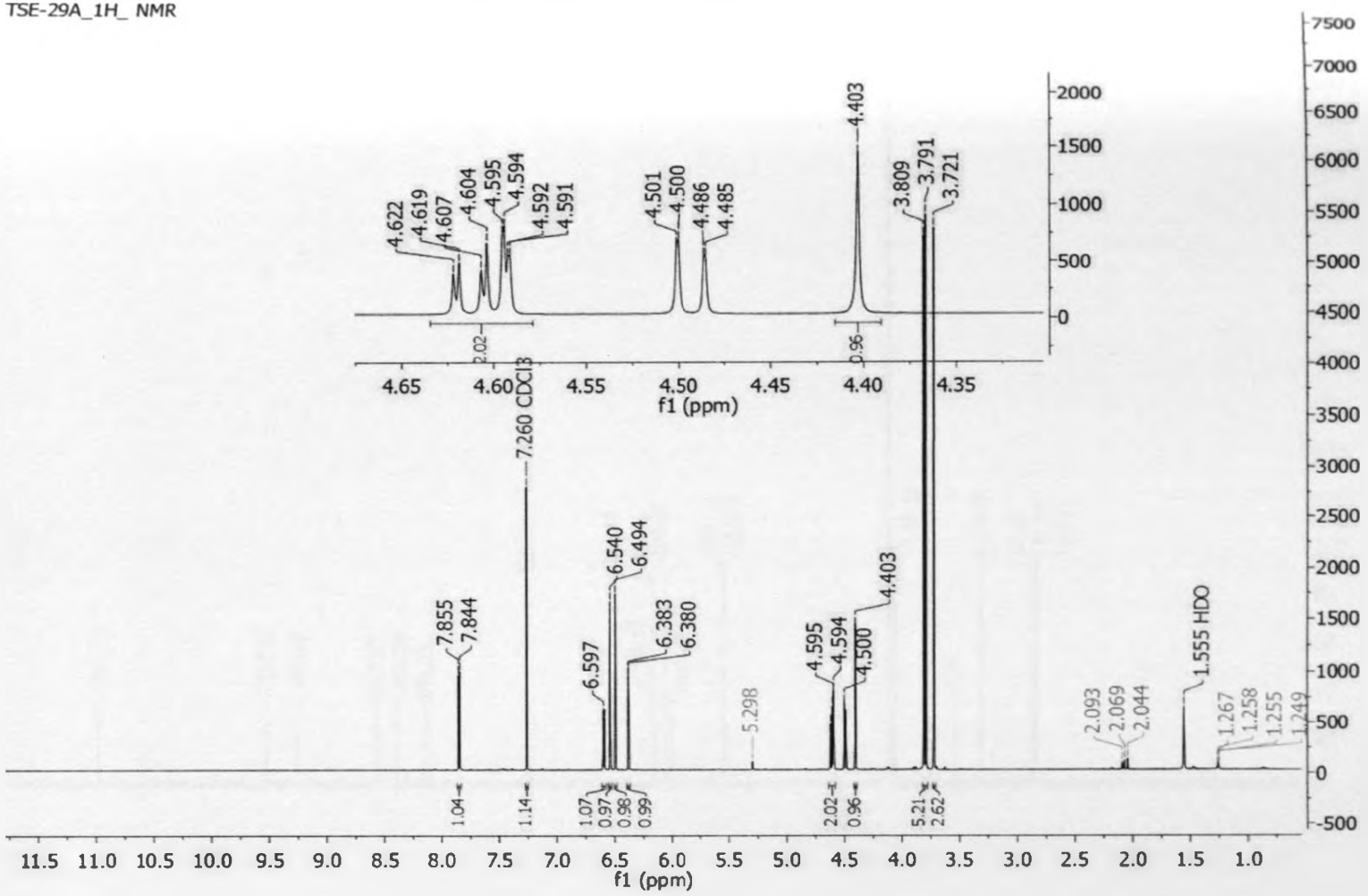


Appendix 31B: ^{13}C NMR (201.15 MHz) spectrum of compound 322

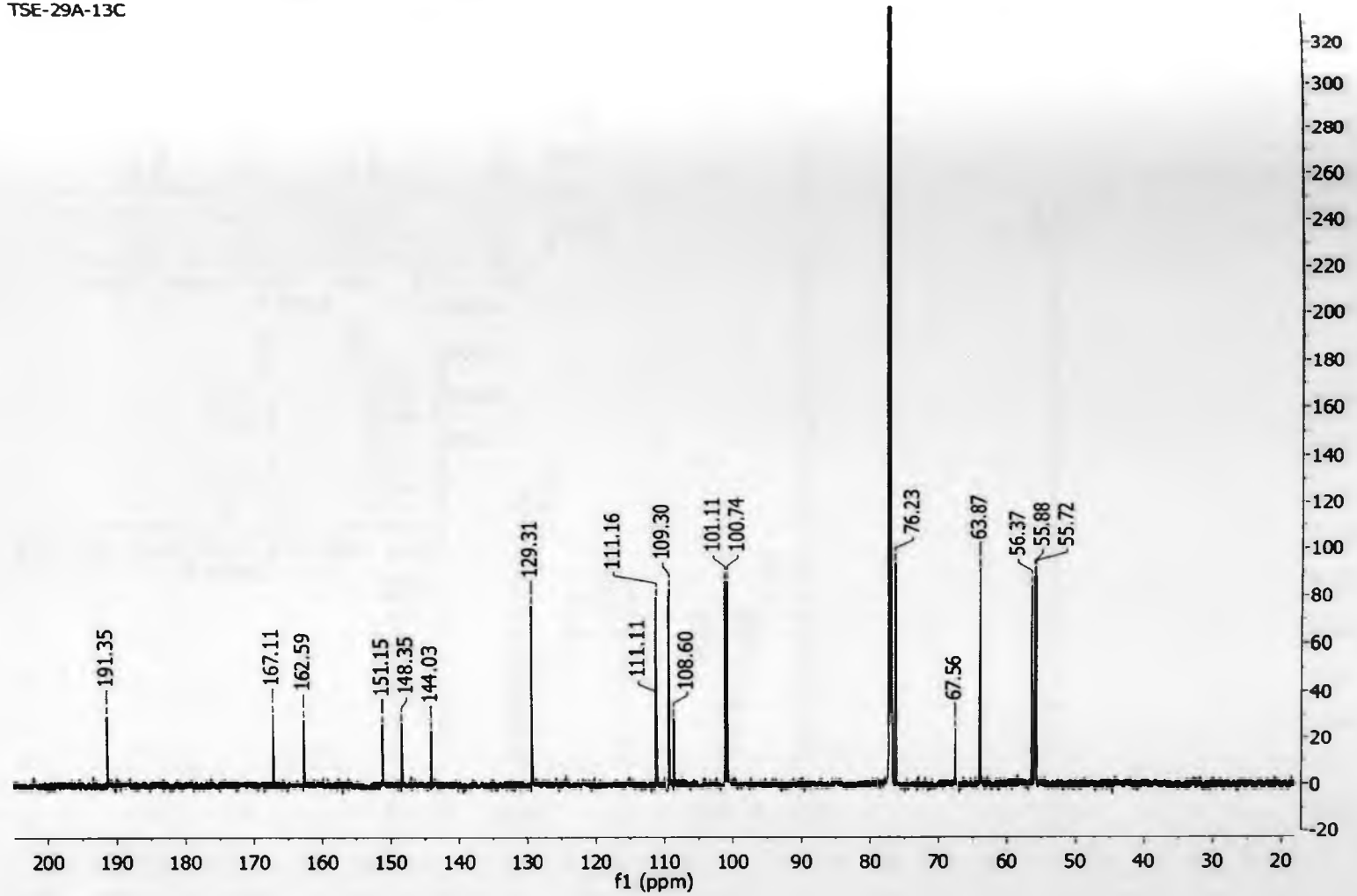


Appendix 32A: ^{1}HNMR (799.87 MHz) spectrum of compound 323

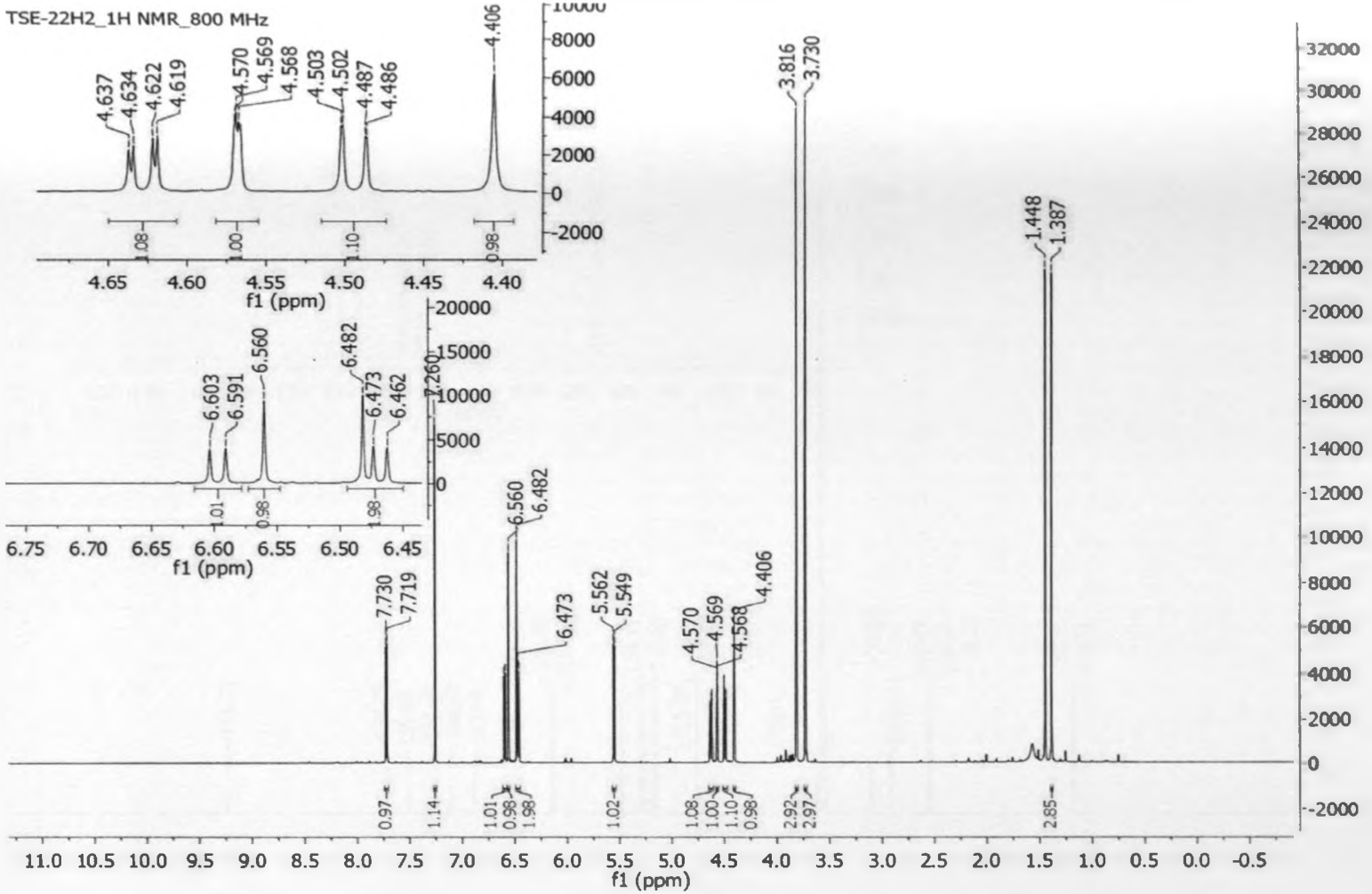
TSE-29A_1H_ NMR



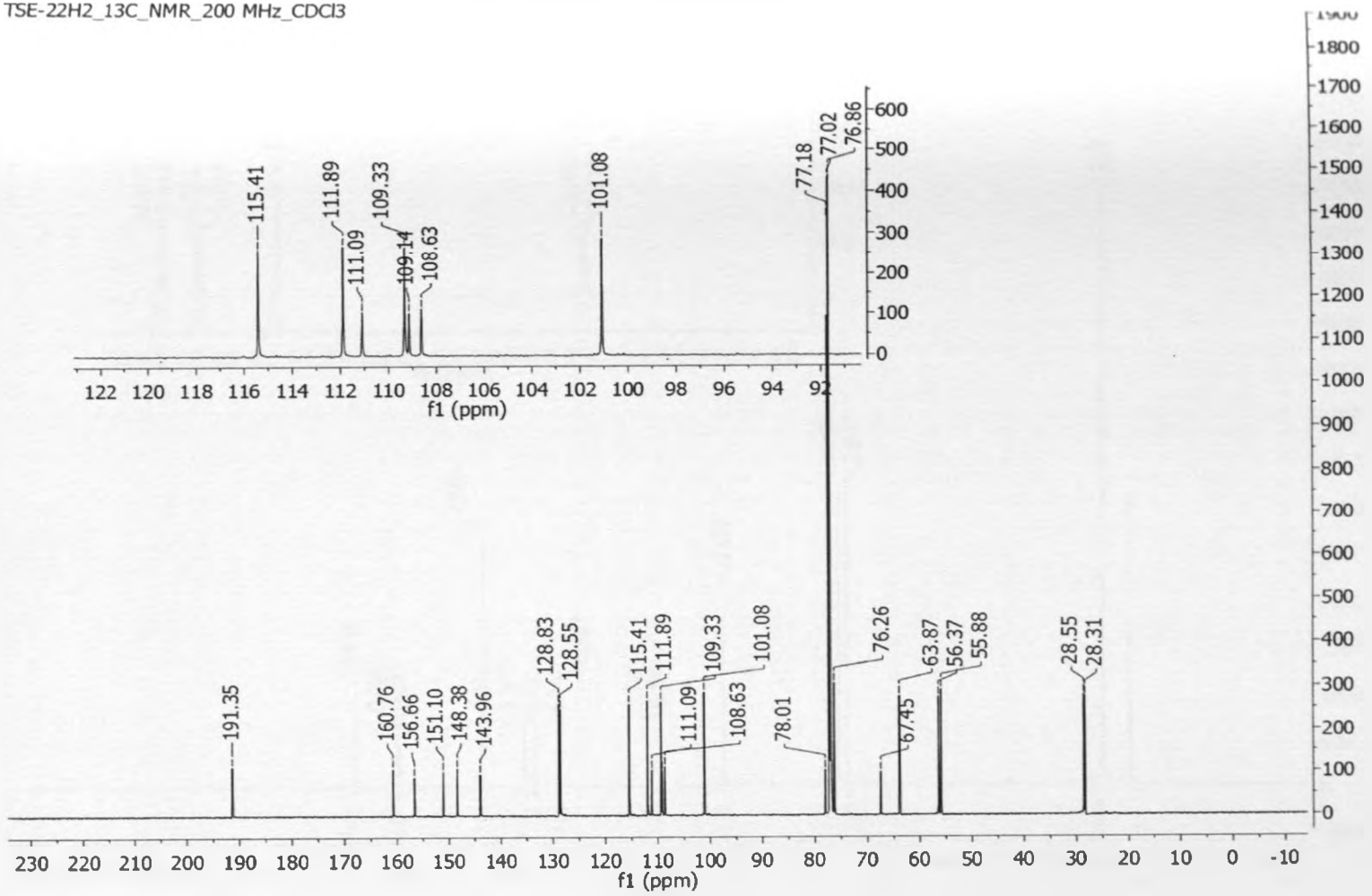
Appendix 32B: ^{13}C NMR (201.15 MHz) spectrum of compound 323



Appendix 33A: ^{1}H NMR (799.87 MHz) spectrum of compound 153

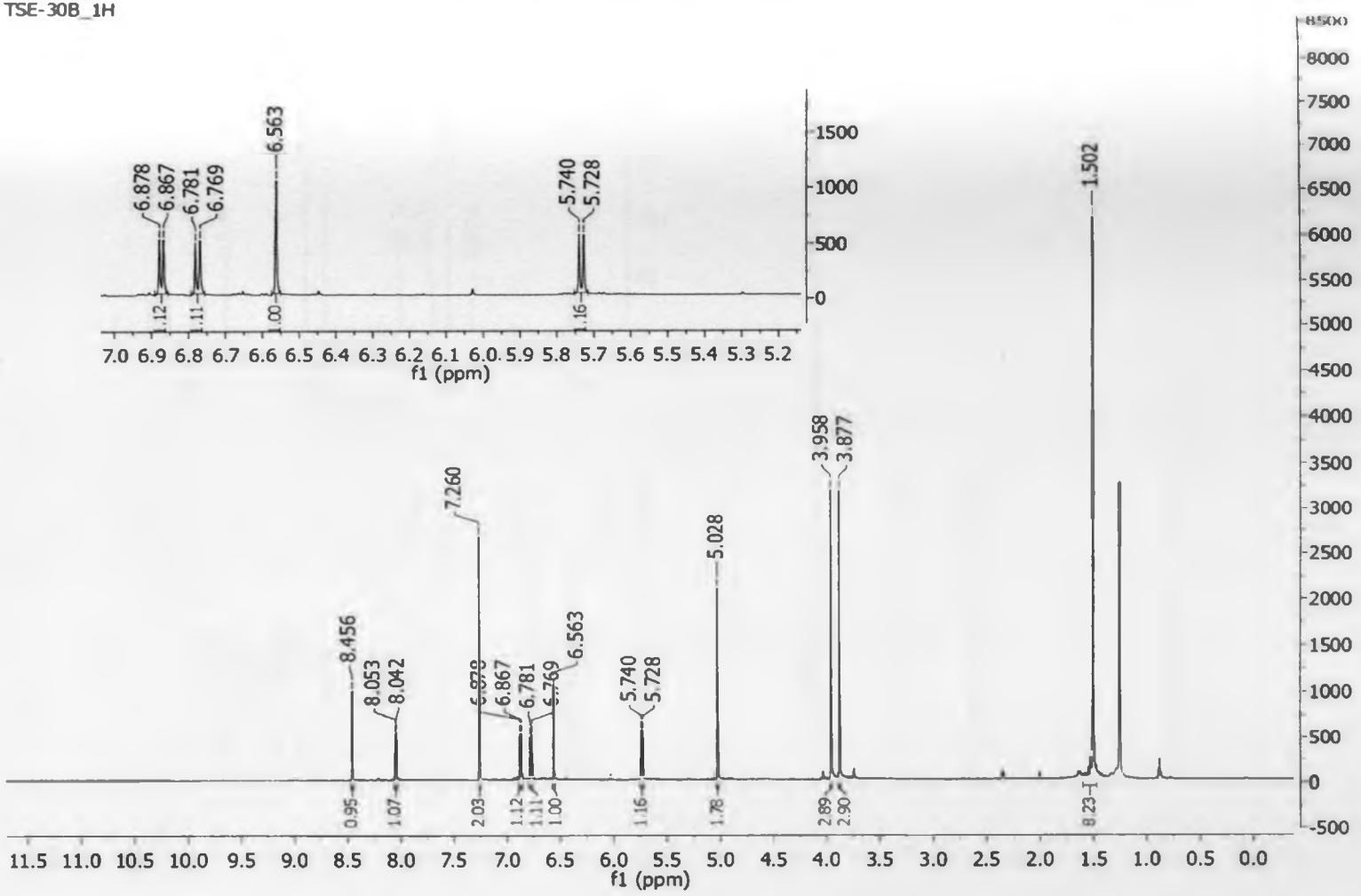


Appendix 33B: ^{13}C NMR (201.15 MHz) spectrum of compound 153

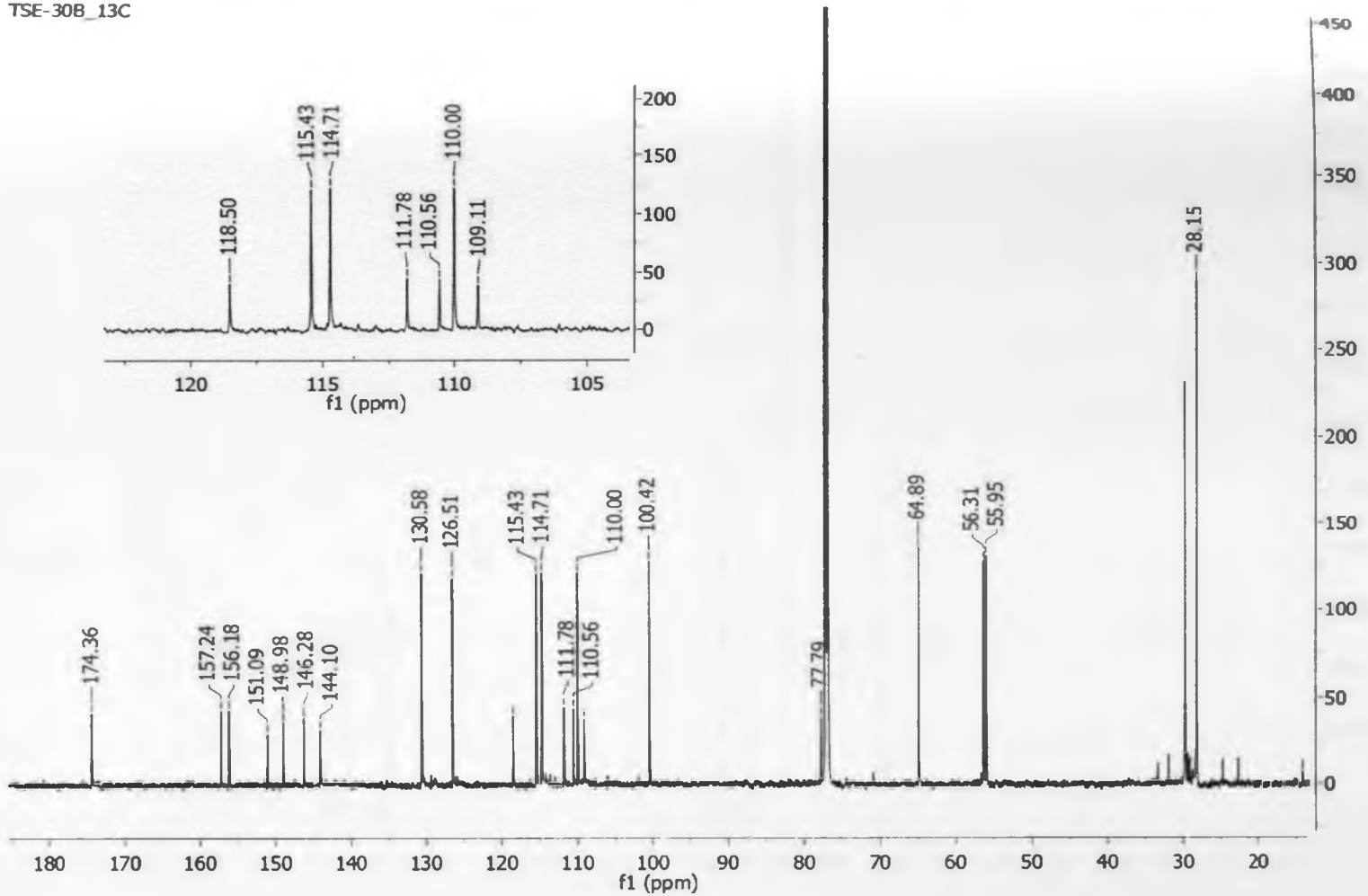


Appendix 34A: ^{1}H NMR (799.87 MHz) spectrum of compound 126

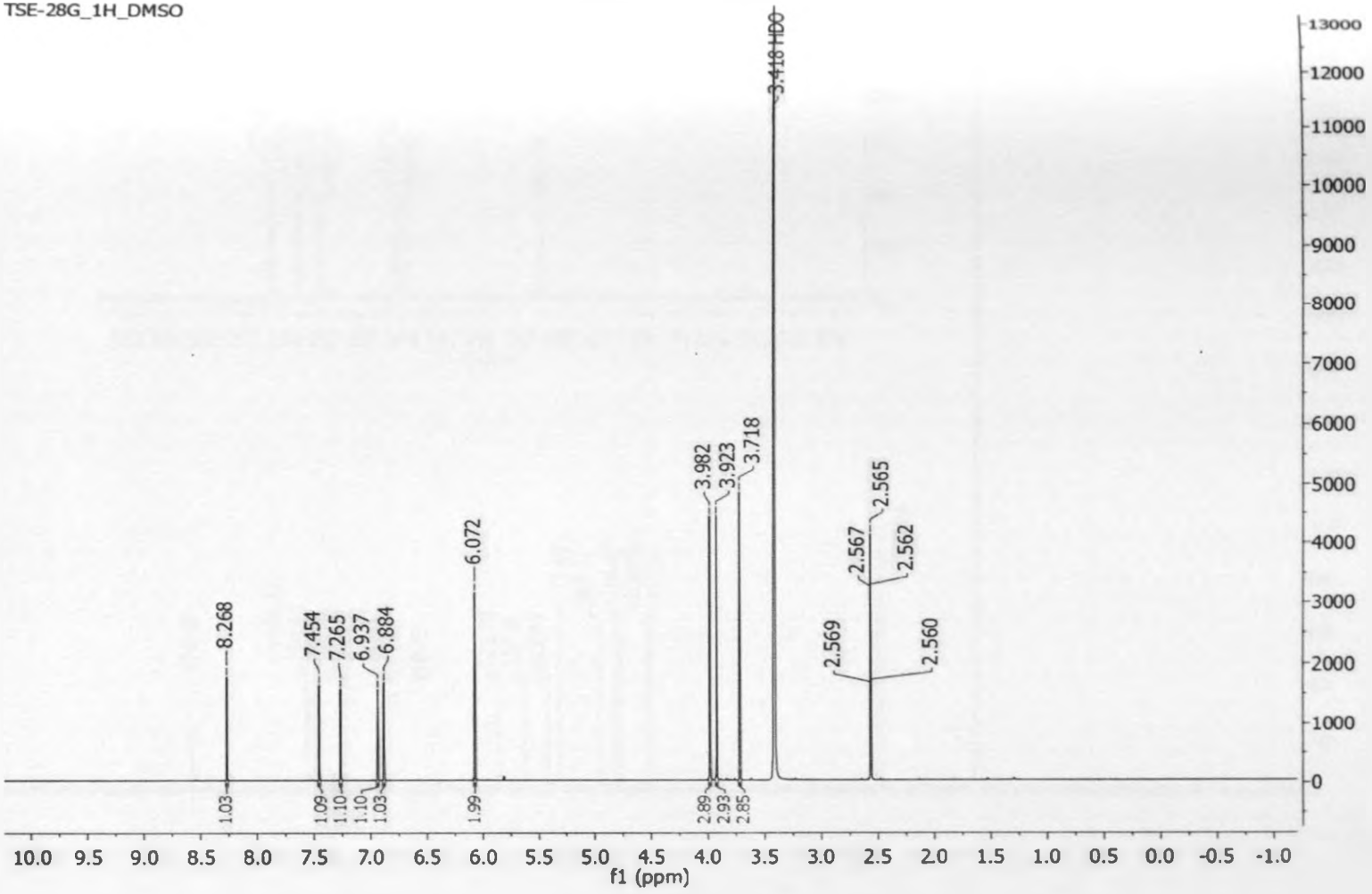
TSE-30B_1H



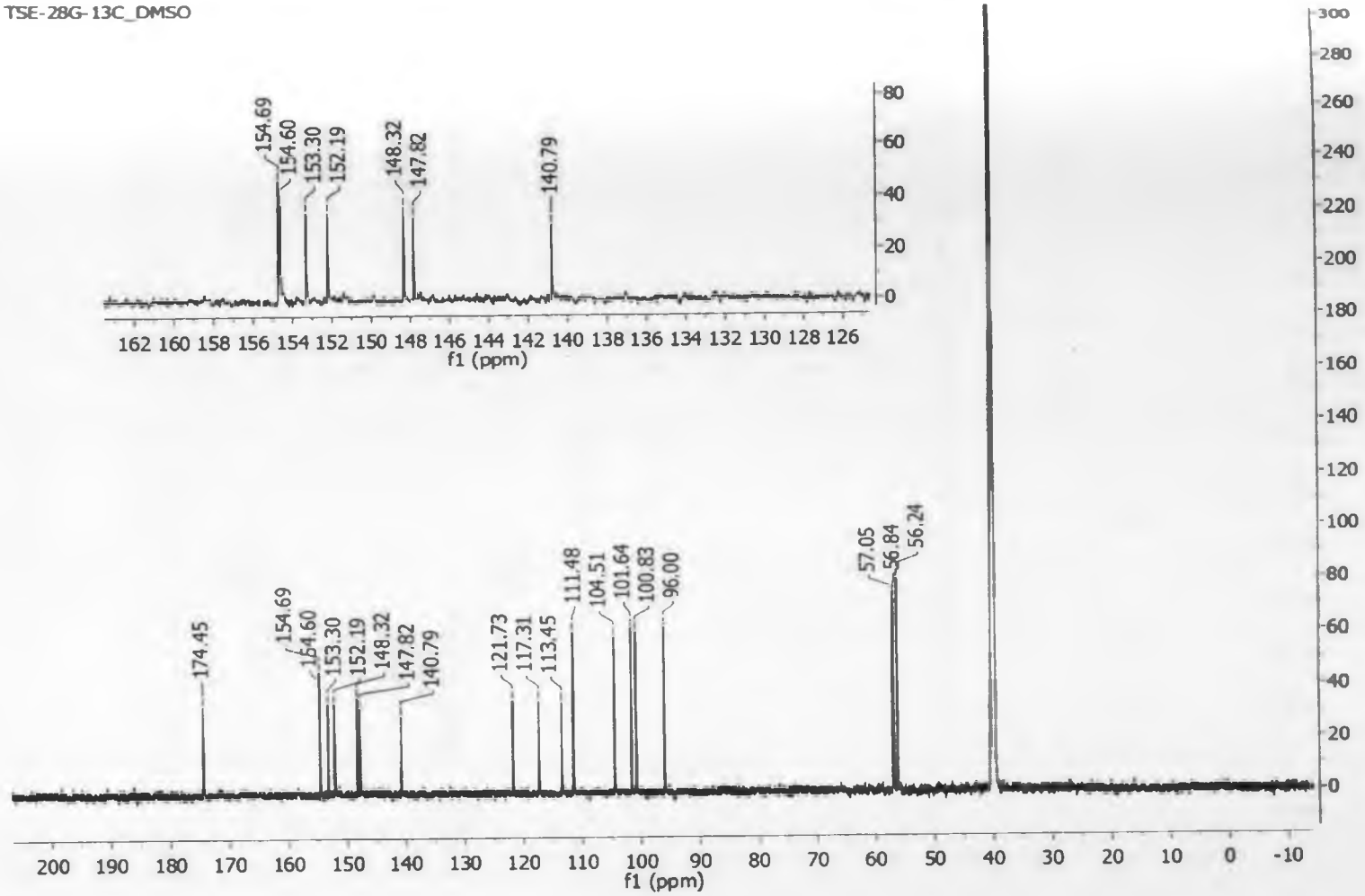
Appendix 34B: ^{13}C NMR (201.15 MHz) spectrum of compound 126



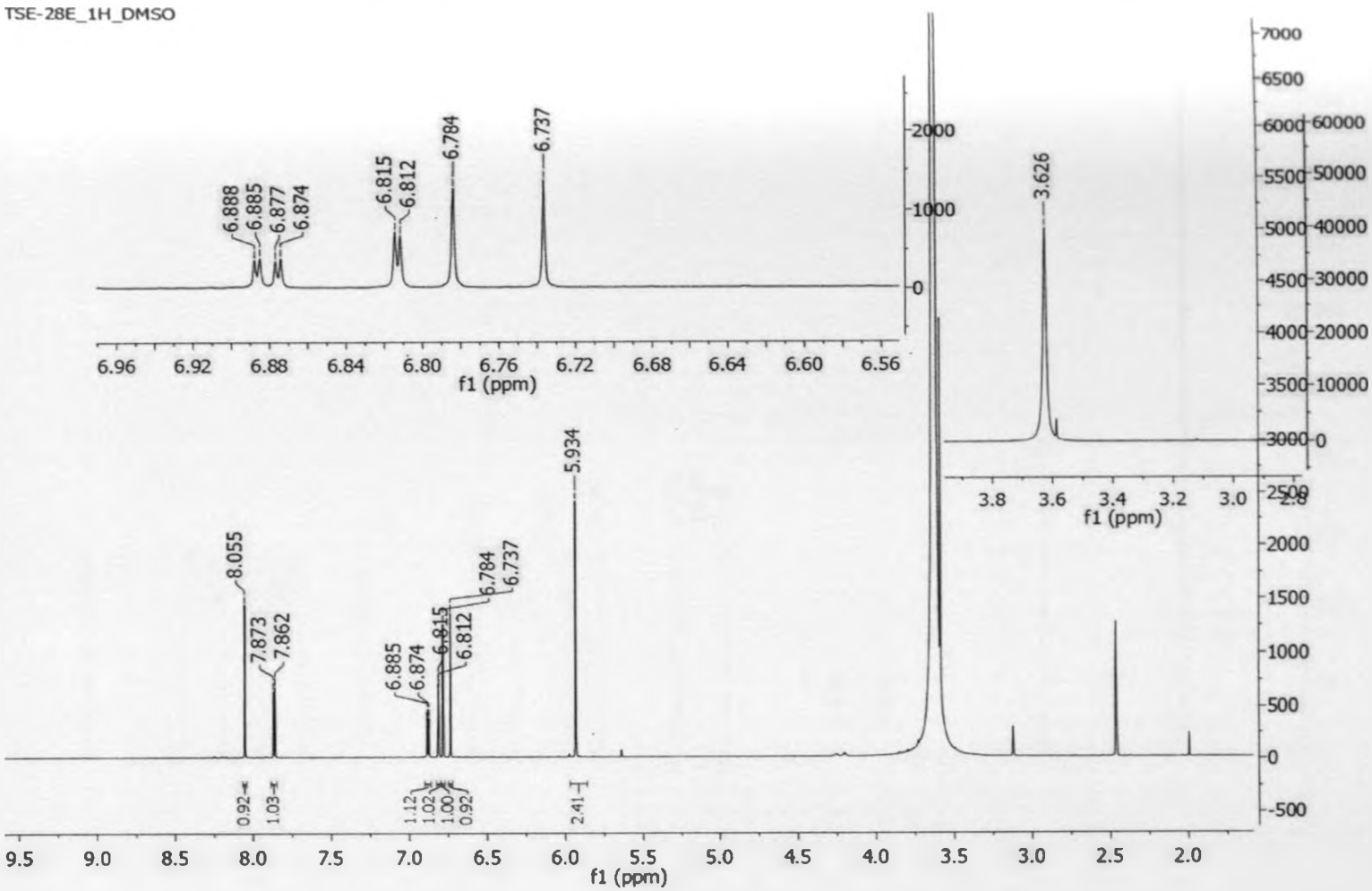
Appendix 35A: ^{1}H NMR (799.87 MHz) spectrum of compound 324



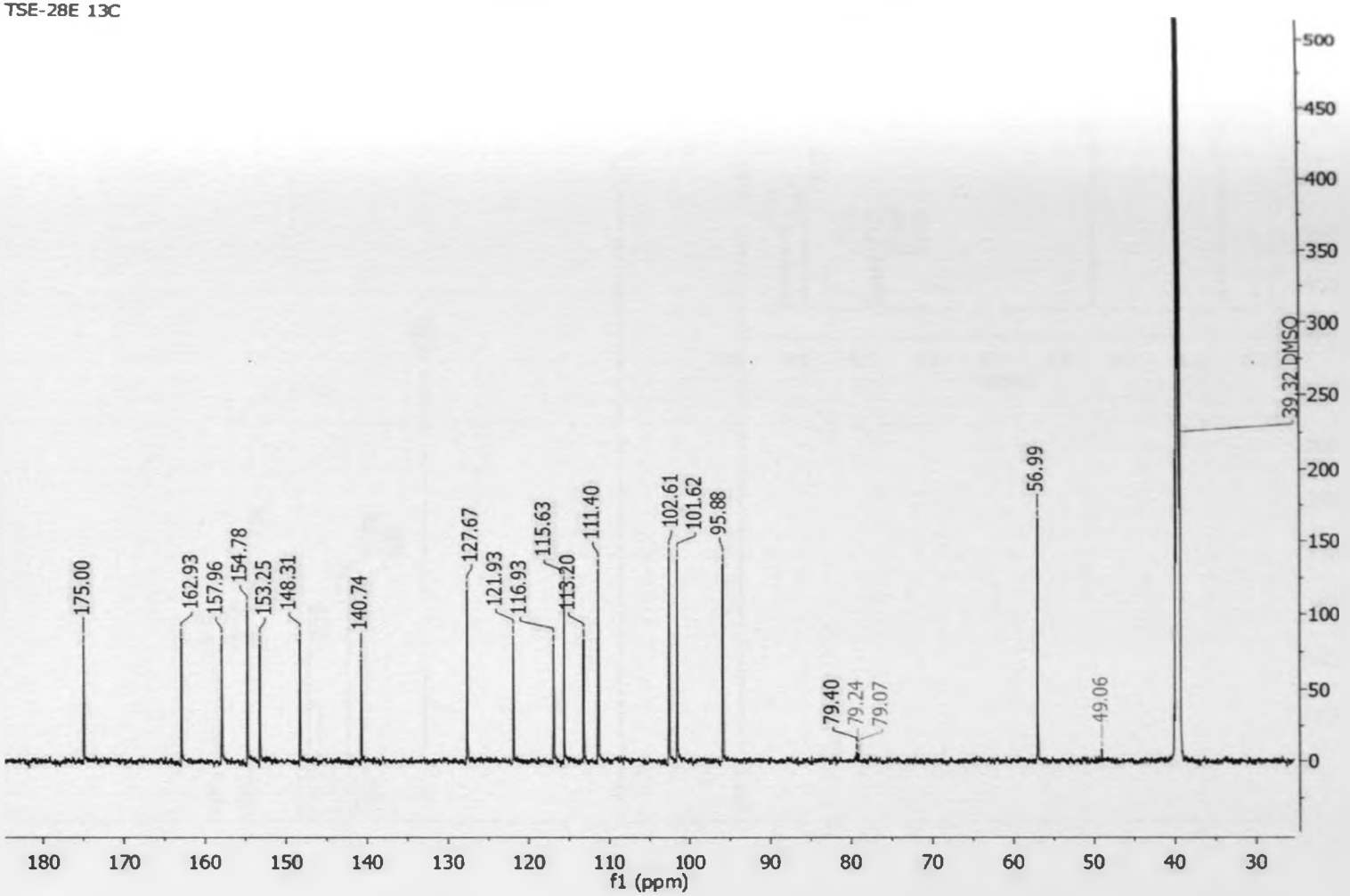
Appendix 35B: ^{13}C NMR (201.15 MHz) spectrum of compound 324



Appendix 36A: ^{1}H NMR (799.87 MHz) spectrum of compound 115

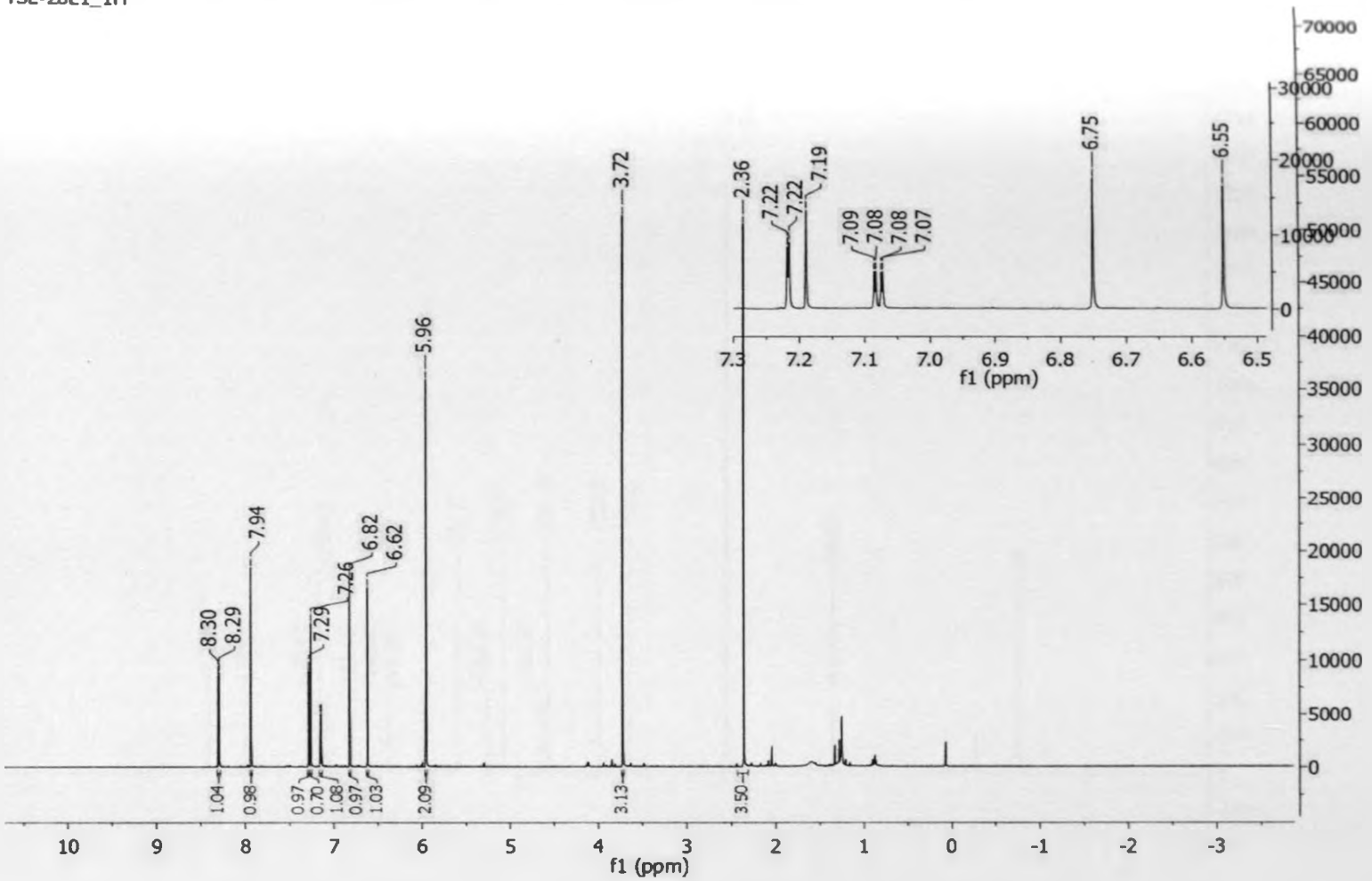


Appendix 36B: **^{13}C NMR (201.15 MHz) spectrum of compound 115**



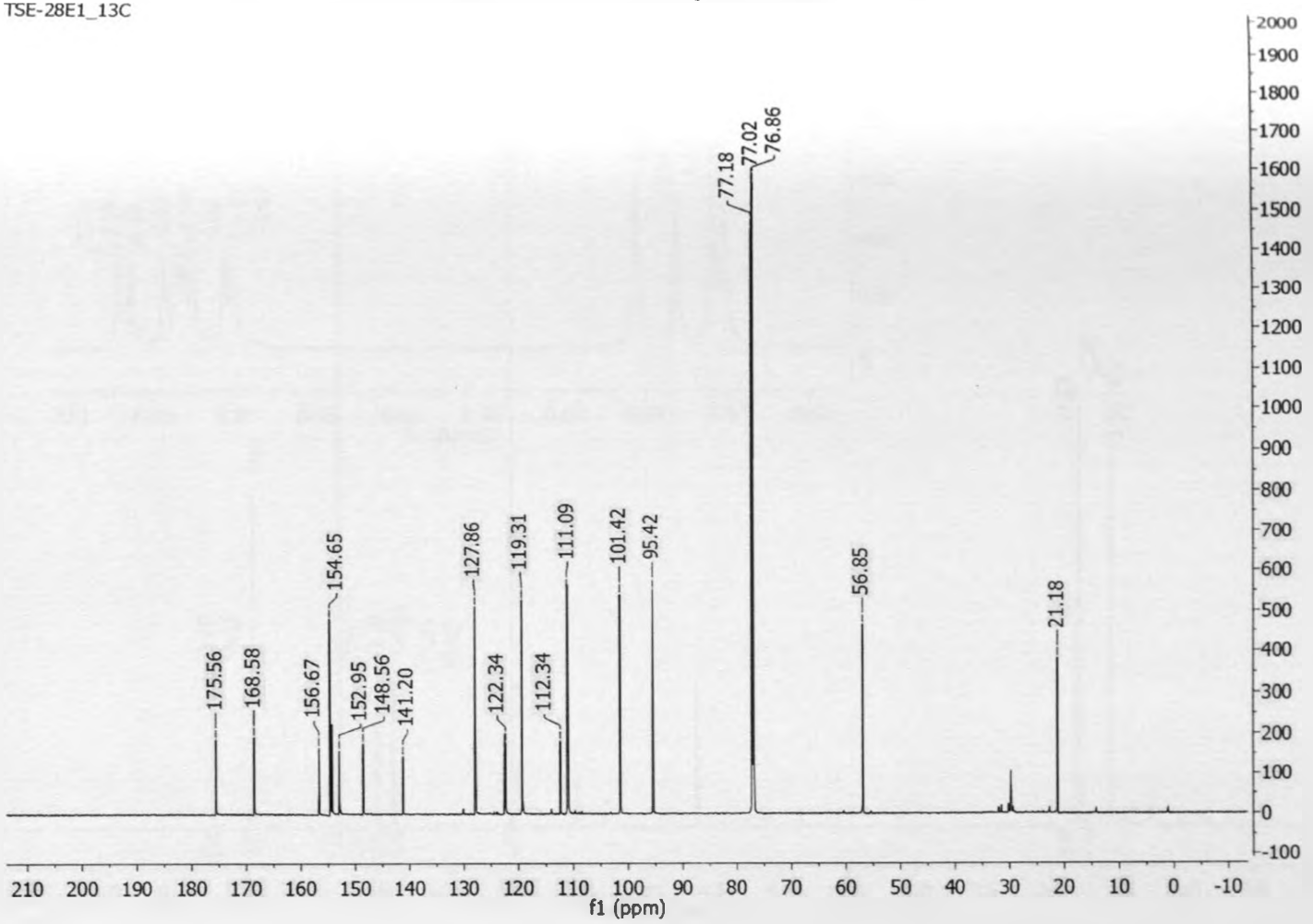
Appendix 37A: ^{1}H NMR (799.87 MHz) spectrum of compound 115a

TSE-28E1_1H



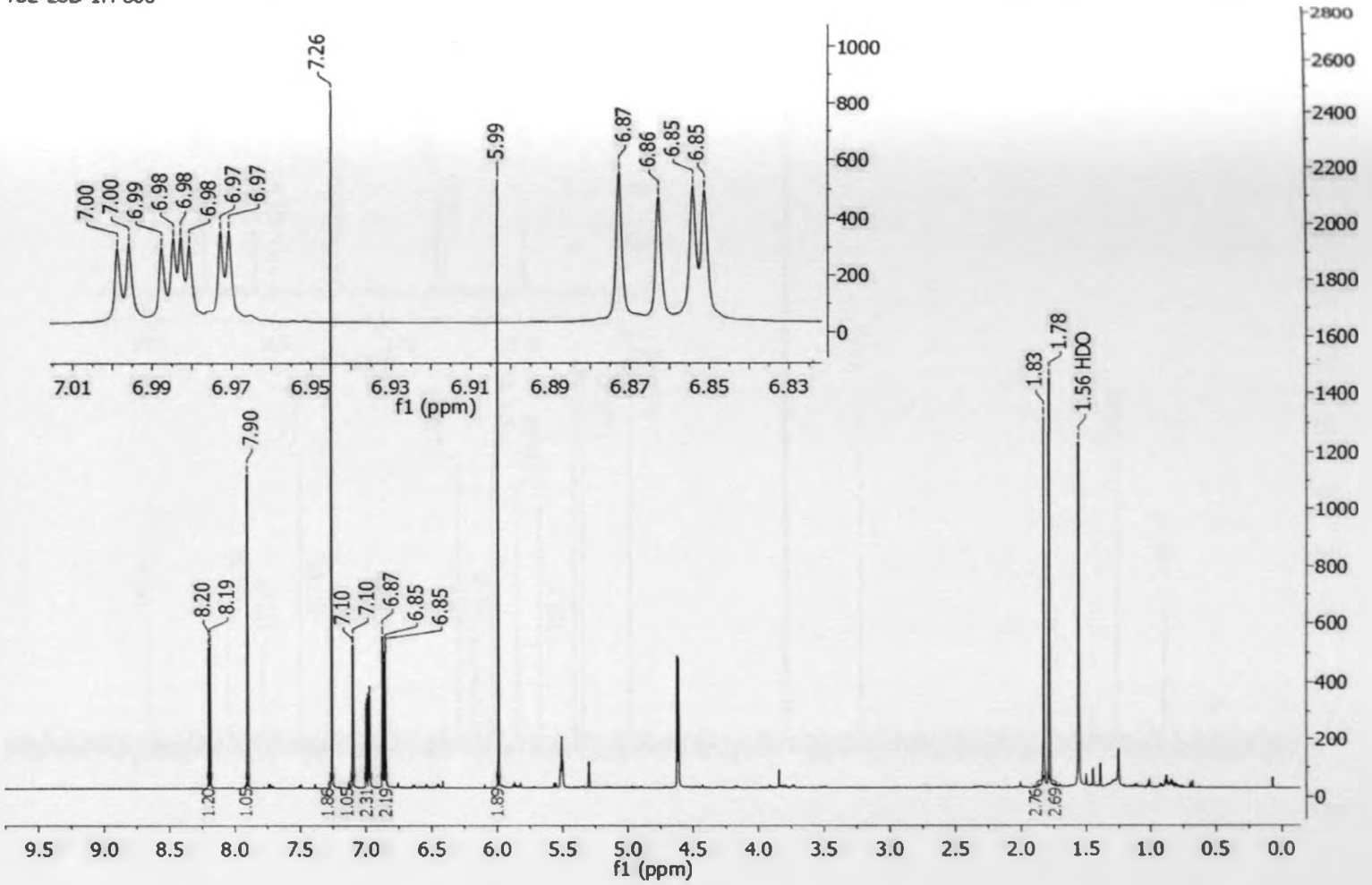
Appendix 37B: ^{13}C NMR (201.15 MHz) spectrum of compound 115a

TSE-28E1_13C



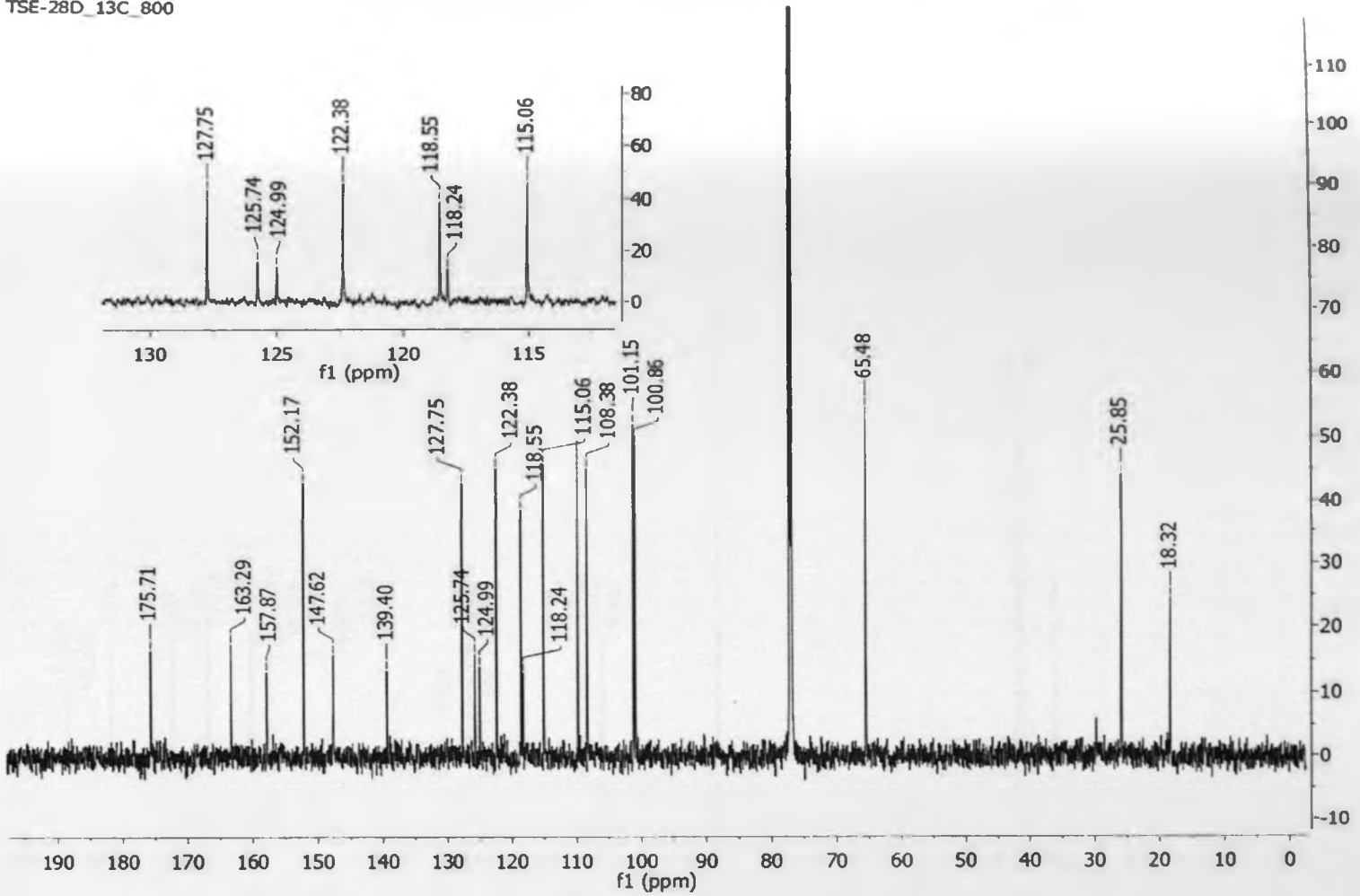
Appendix 38A: ^{1}H NMR (799.87 MHz) spectrum of compound 304

TSE-28D-1H-800



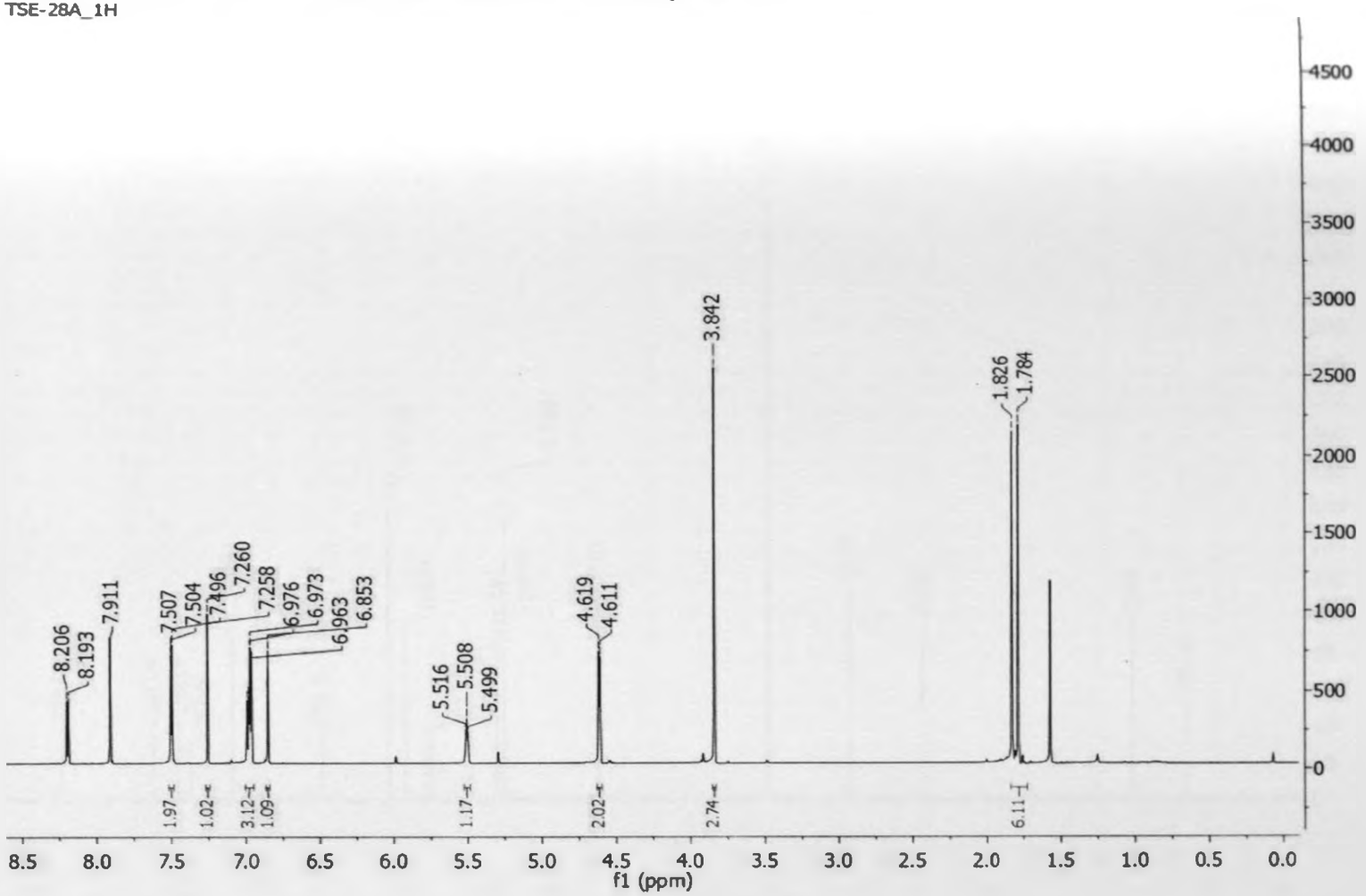
Appendix 38B: ^{13}C NMR (201.15 MHz) spectrum of compound 304

TSE-28D_13C_800



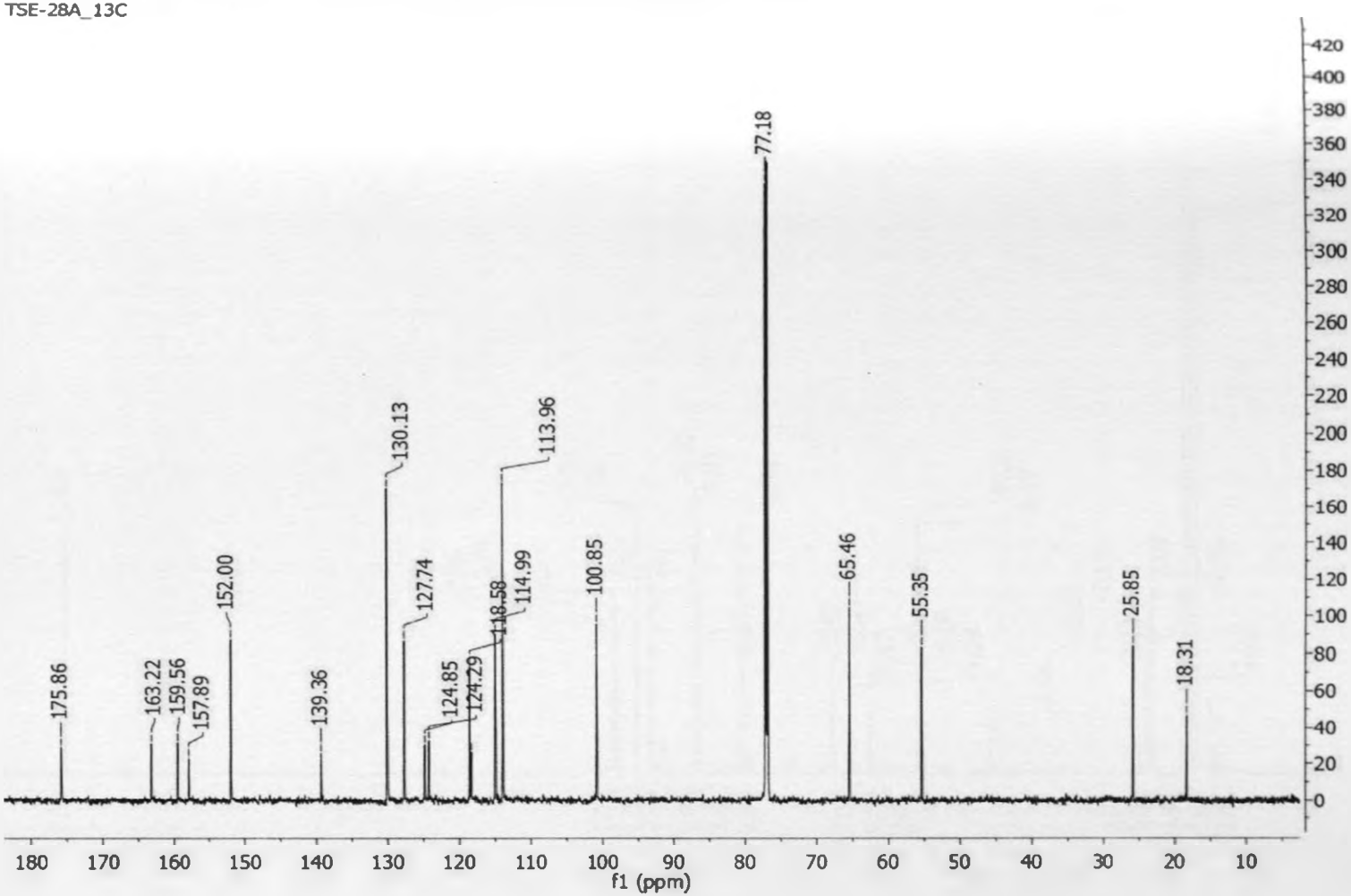
Appendix 39A: ^{1}H NMR (799.87 MHz) spectrum of compound 325

TSE-28A_1H



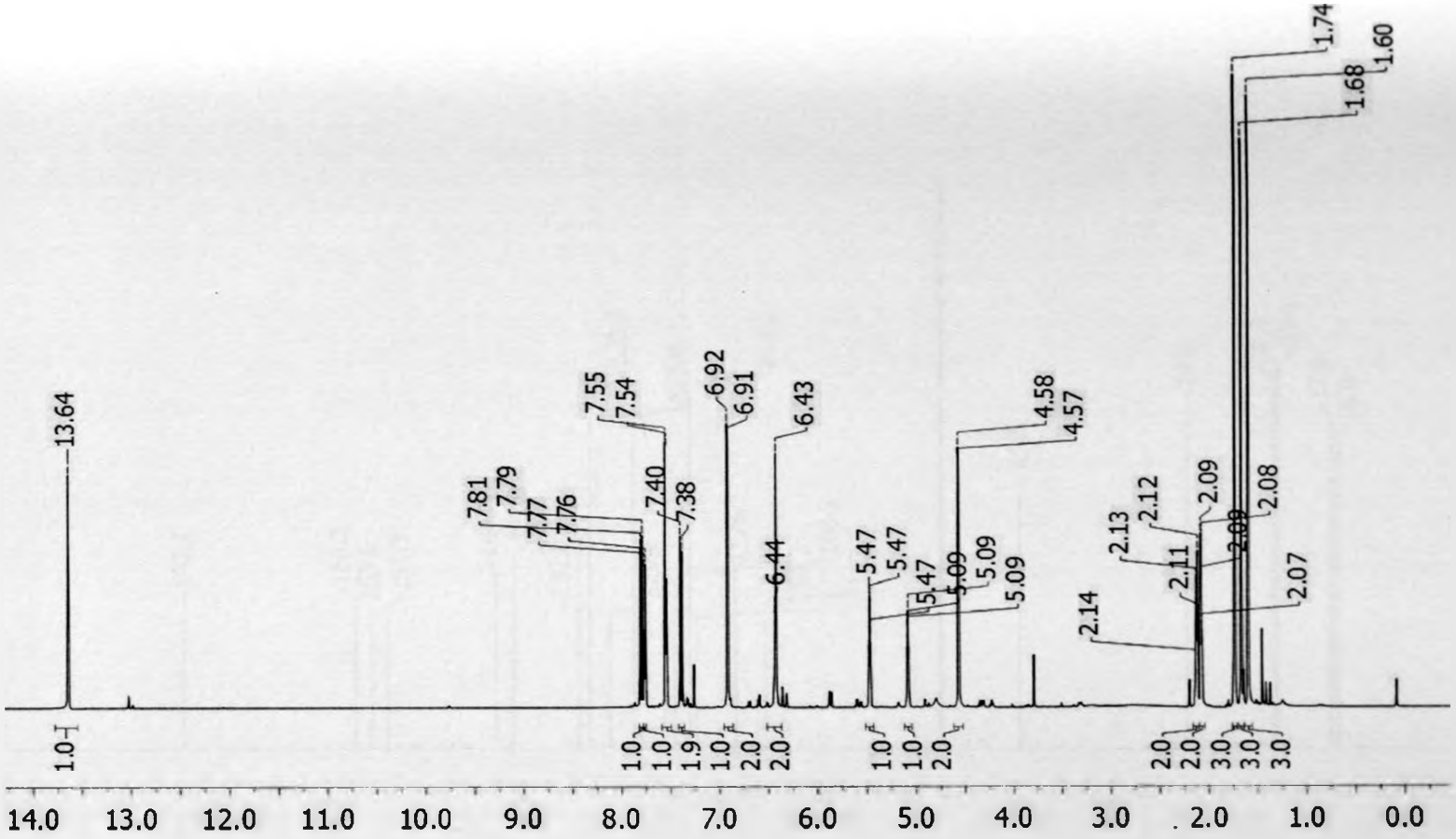
Appendix 39B: ^{13}C NMR (201.15 MHz) spectrum of compound 325

TSE-28A_13C



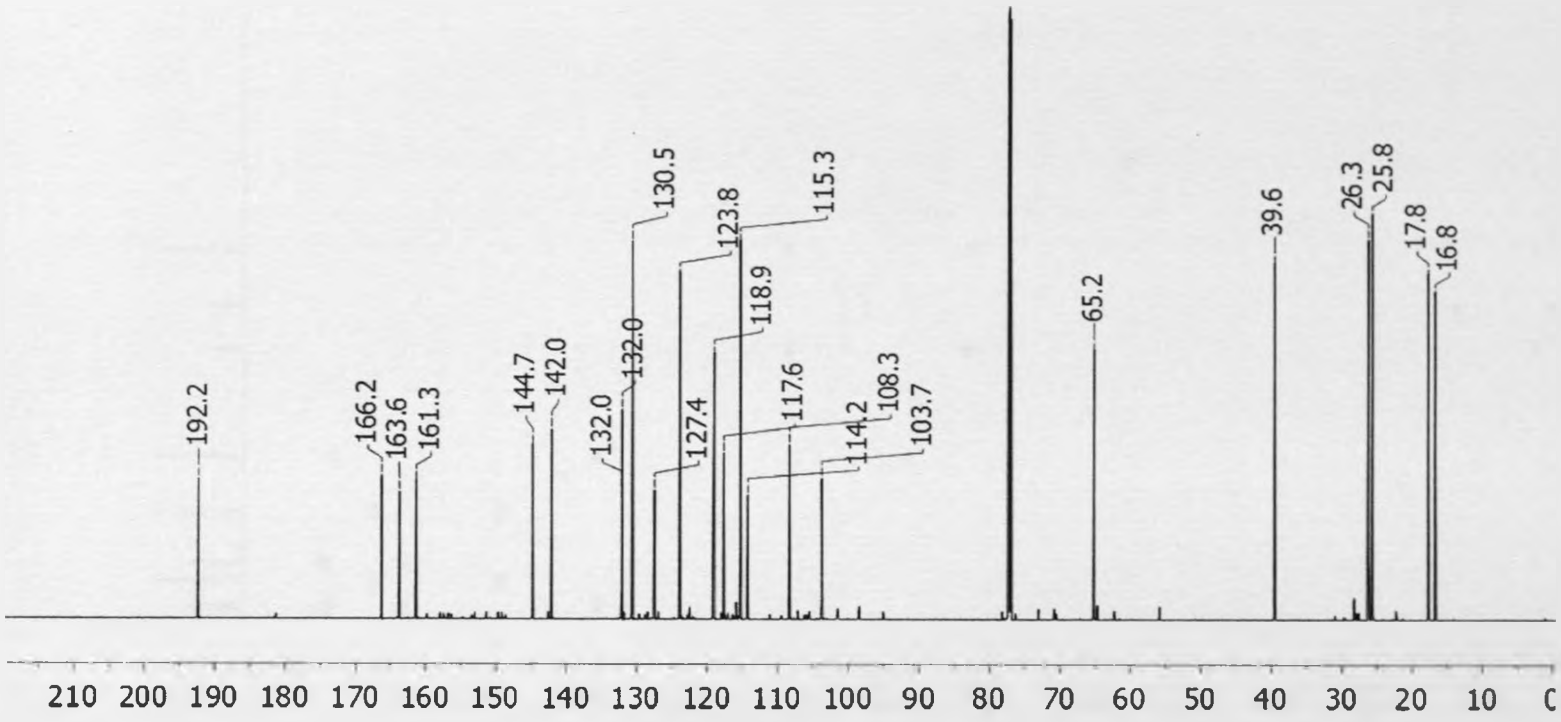
Appendix 40A: ^{1}H NMR (799.87 MHz) spectrum of compound 326

TDW_19A_1.11.fid
TDW_19A
M_1H1D CDCl3 /opt/topspin3.2/data/mate/nmr mate 17

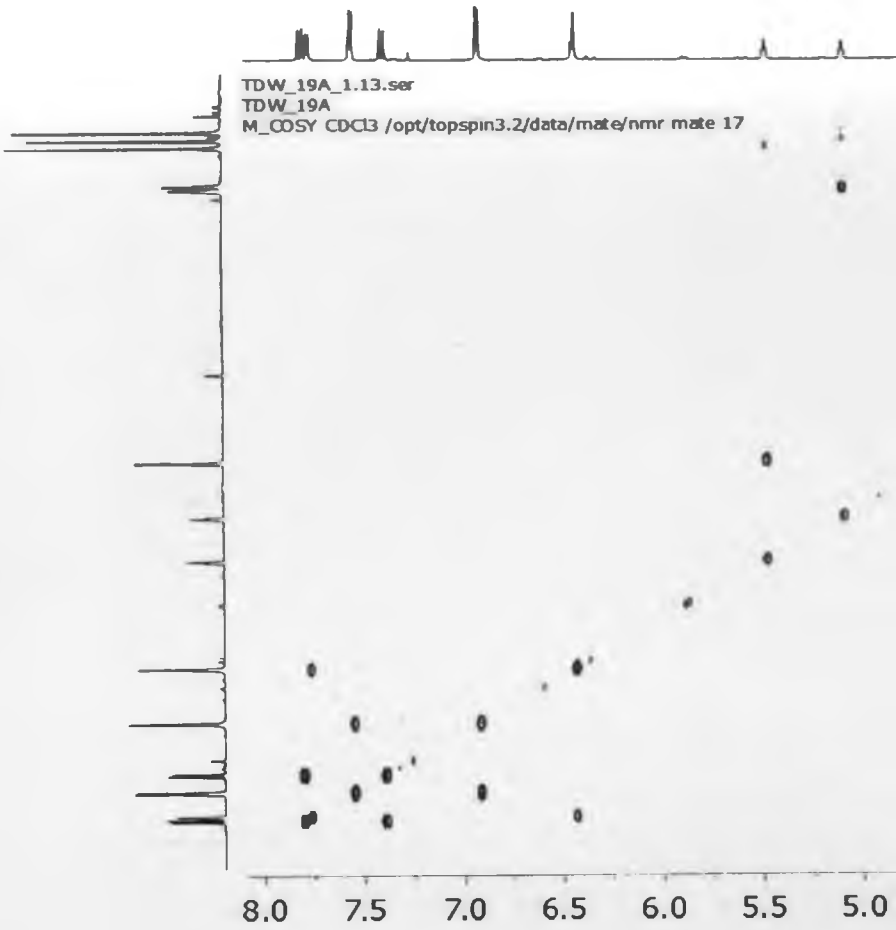


Appendix 40B: ^{13}C **NMR (201.15 MHz) spectrum of compound 326.**

TDW_19A_1.12.fid
TDW_19A
M_13C1D CDCl3 /opt/topspin3.2/data/mate/nmr mate 17

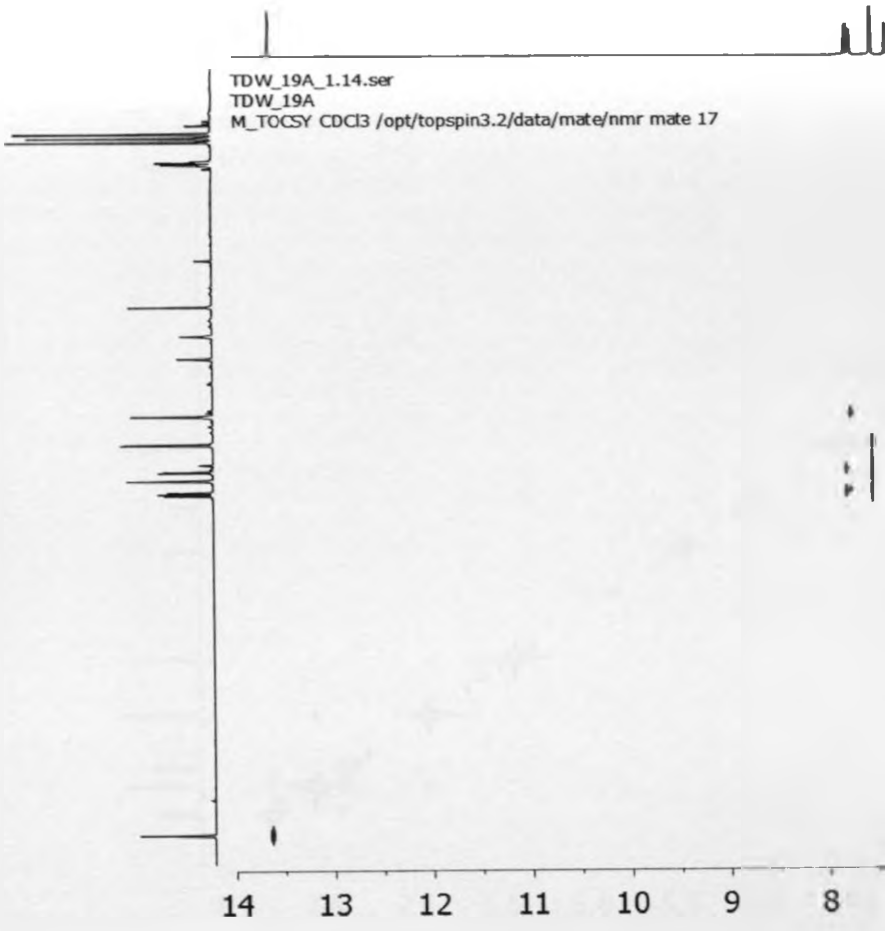


Appendix 40C: COSY (799.88 MHz) spectrum of compound 326



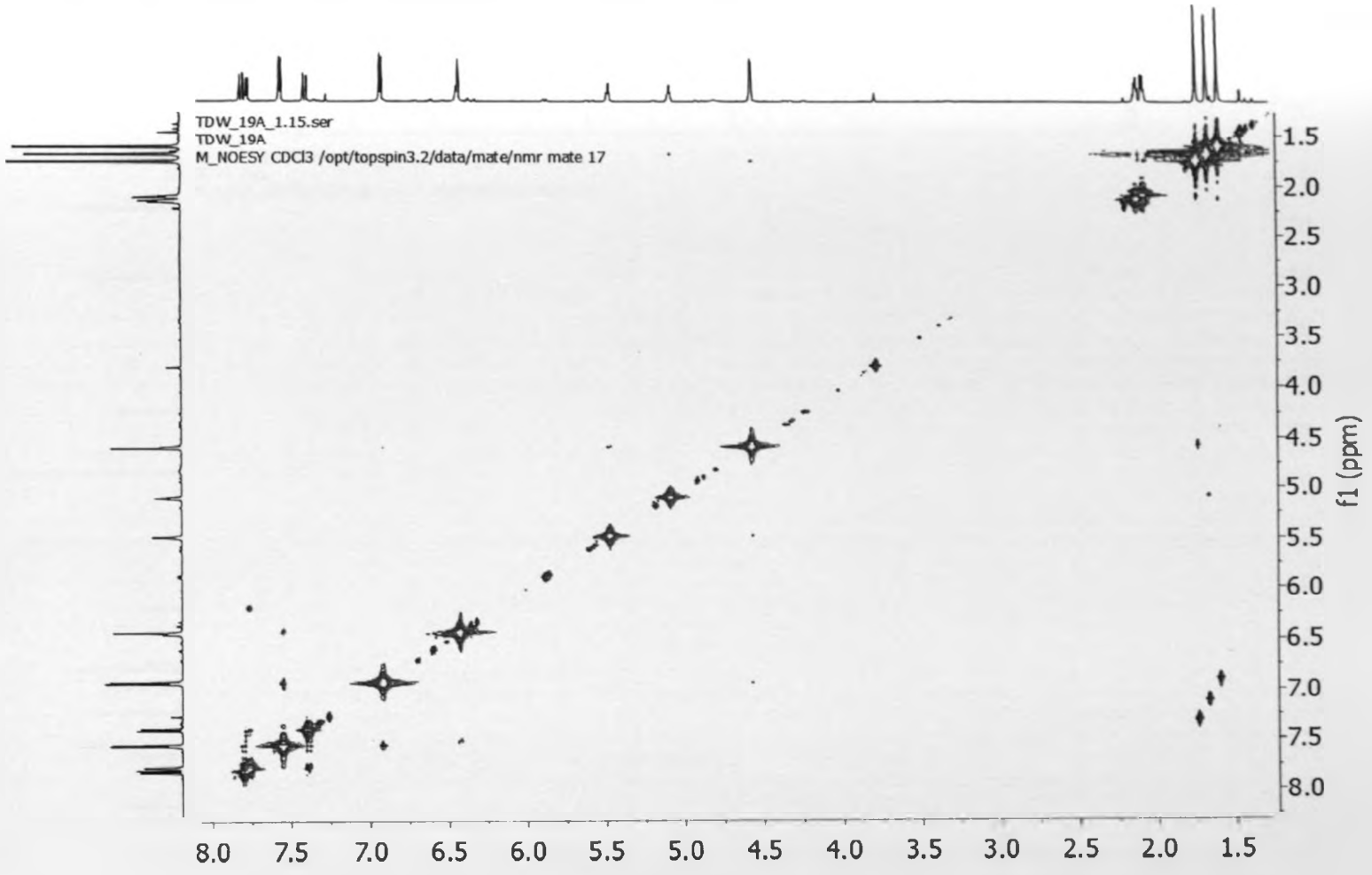
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
f1 (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5

Appendix 40D: TOCSY (799.88 MHz) spectrum of compound 326

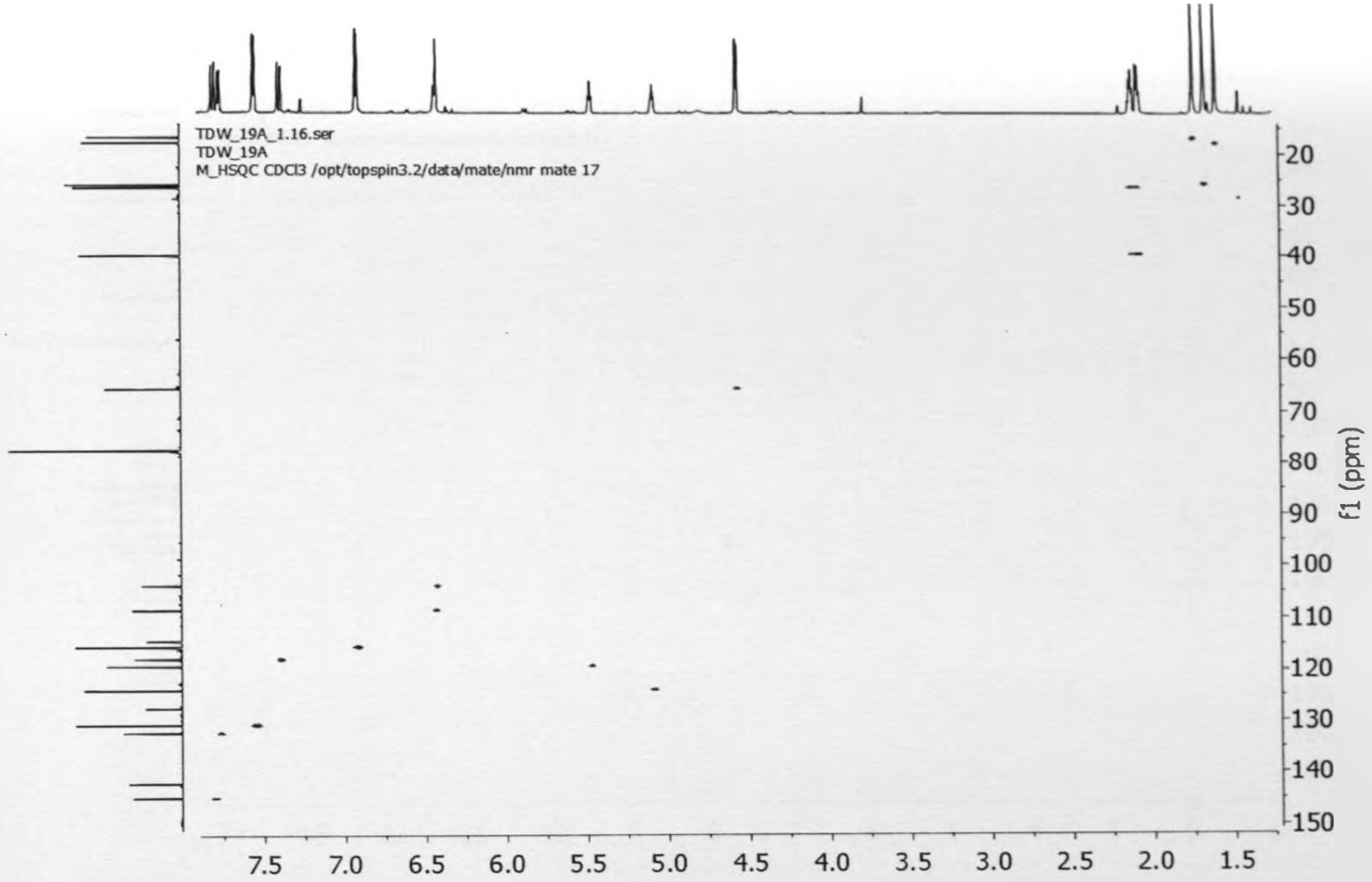


1
2
3
4
5
6
7
8
9
10
11
12
13
14
f1 (ppm)
7
6
5
4
3
2

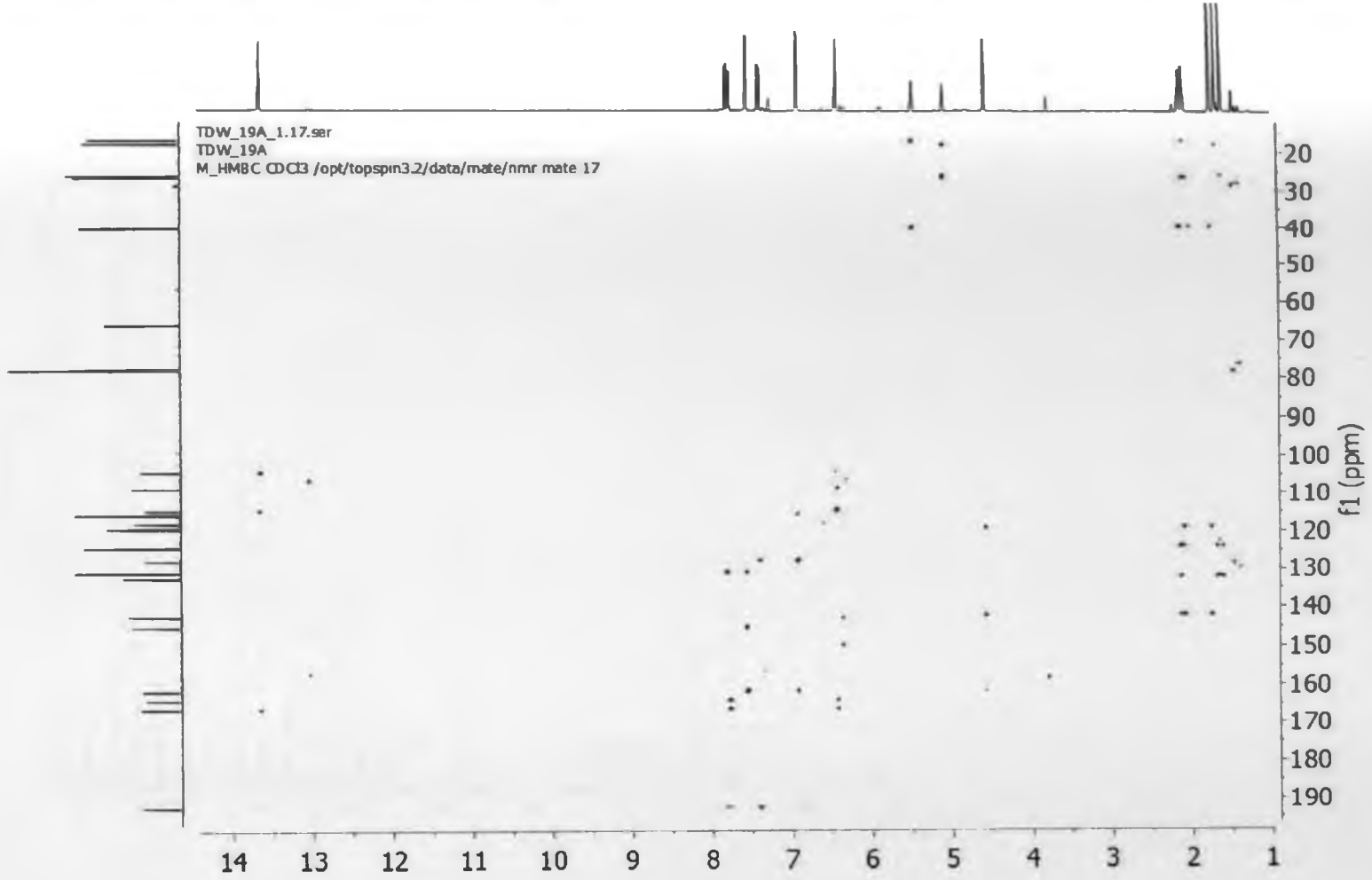
Appendix 40E: NOESY (799.88 MHz) spectrum of compound 326



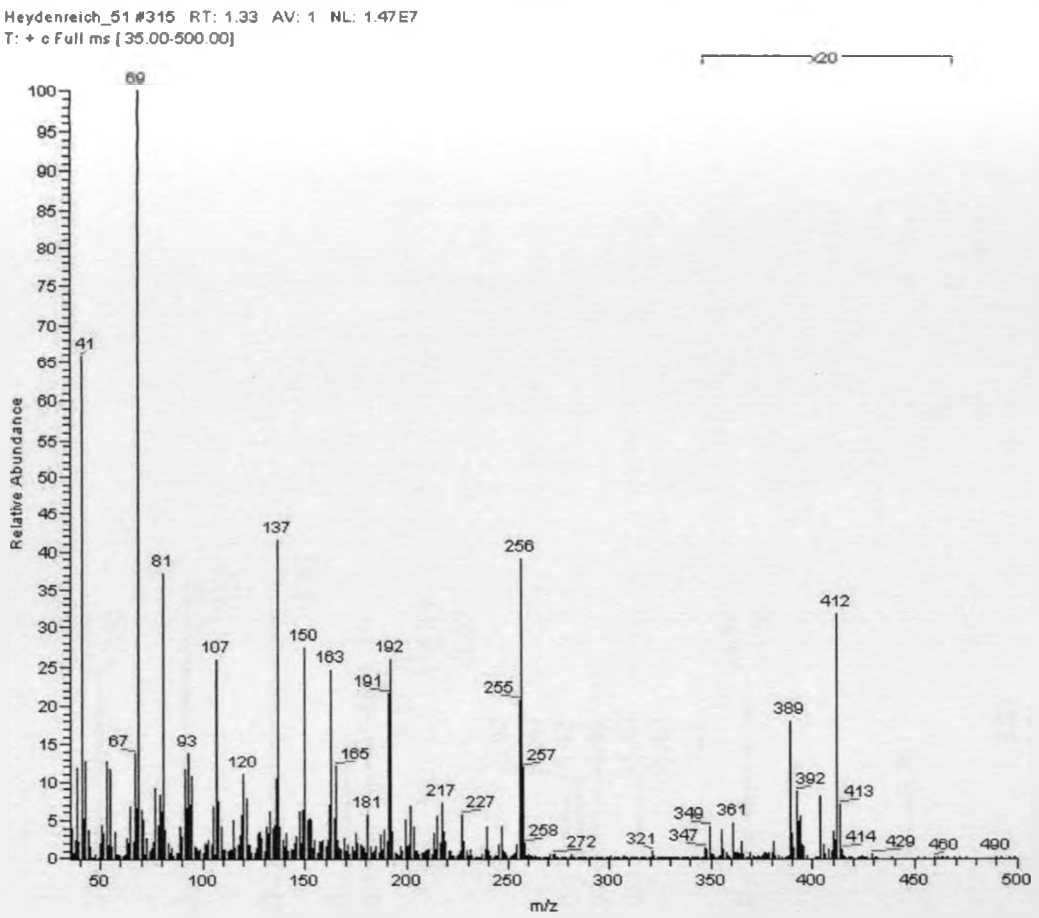
Appendix 40F: HSQC (799.88 MHz/ 201.15 MHz) spectrum of compound 326



Appendix 40G: HMBC (799.88 MHz/ 201.15 MHz) spectrum of compound 326

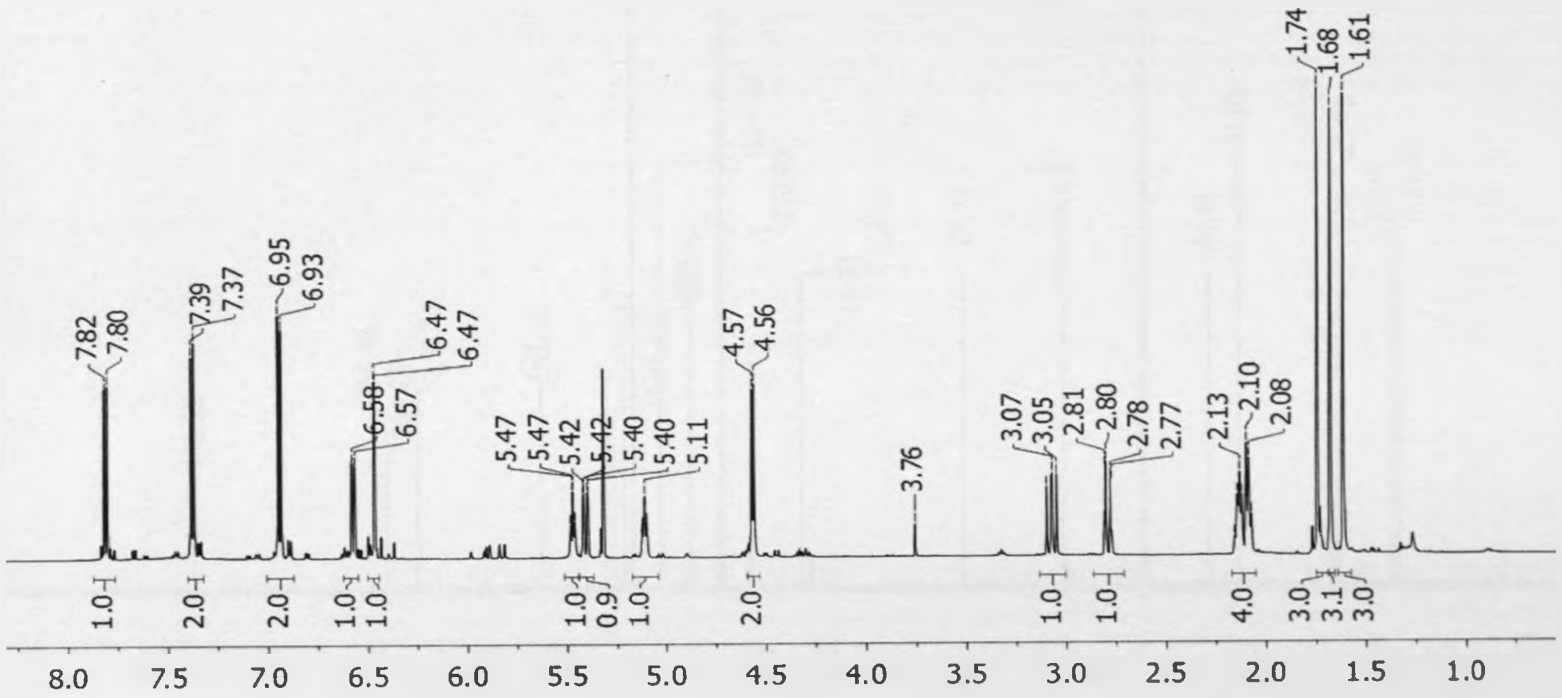


Appendix 40H: HRESIMS spectrum of compound 326

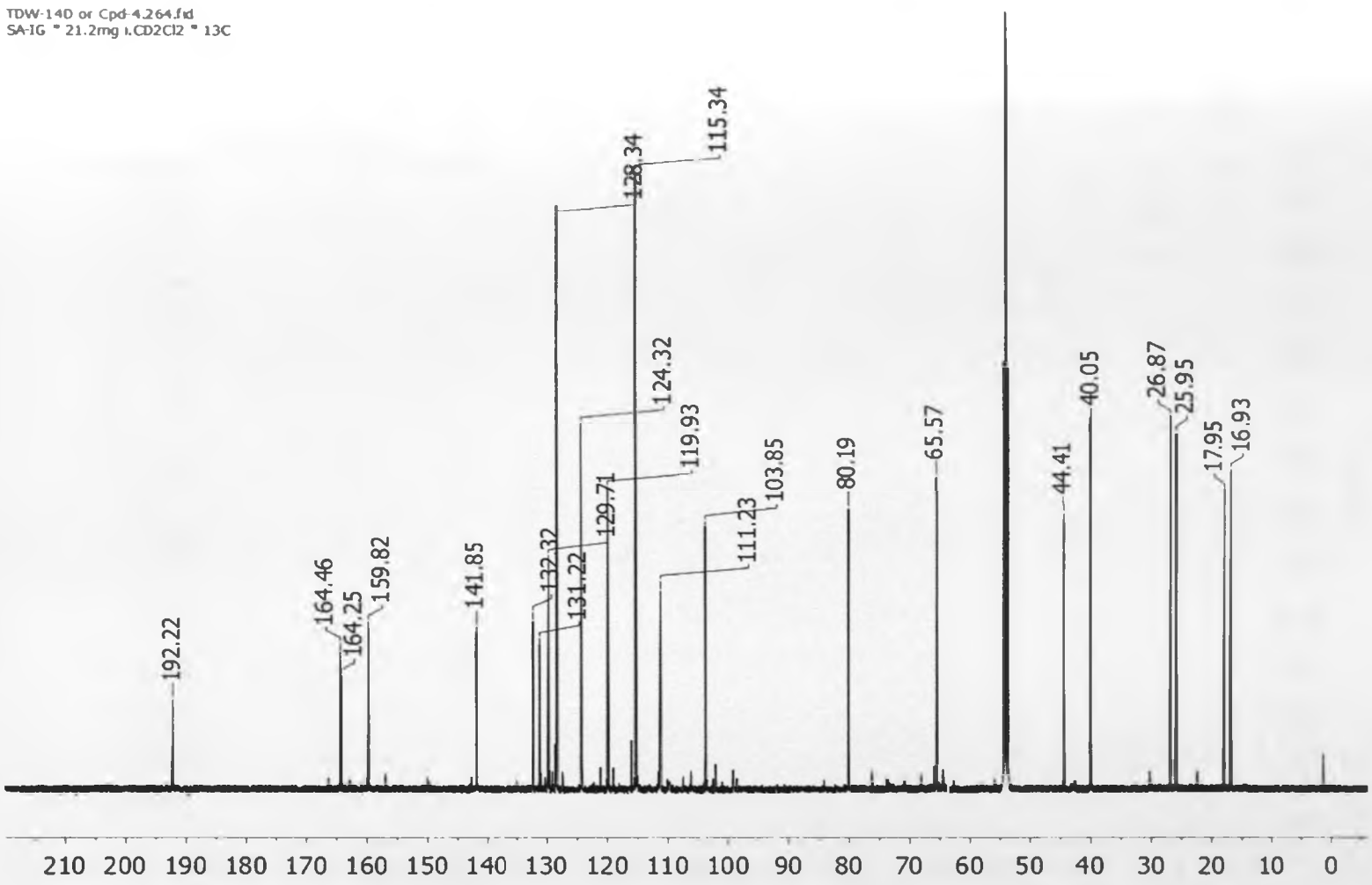


Appendix 41A: ^{1}H NMR (600.24 MHz) spectrum of compound 327

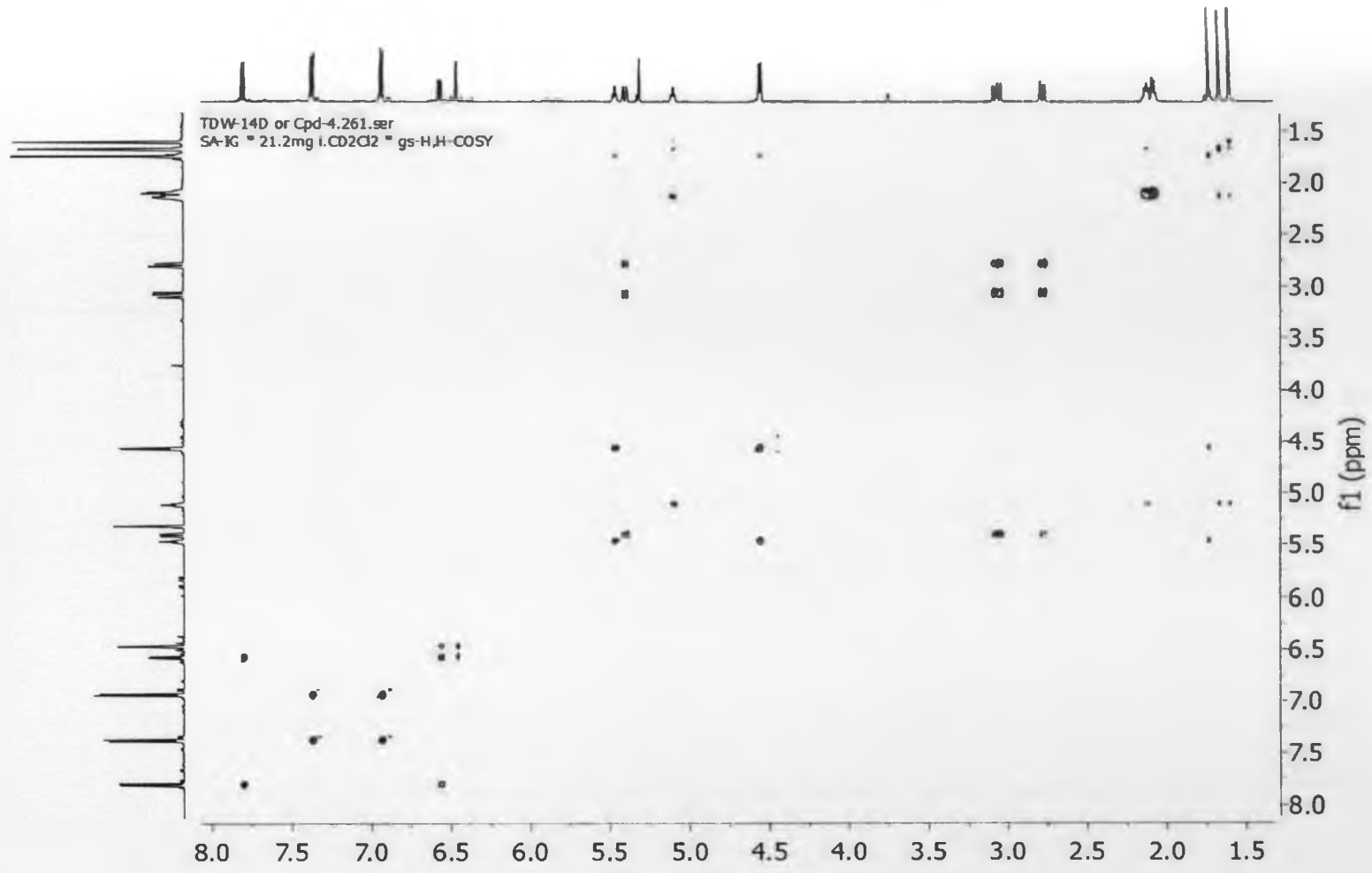
TDW-14D or Cpd-4.260.fid
SA-IG * 21.2mg i.CD2Cl2 * 1H



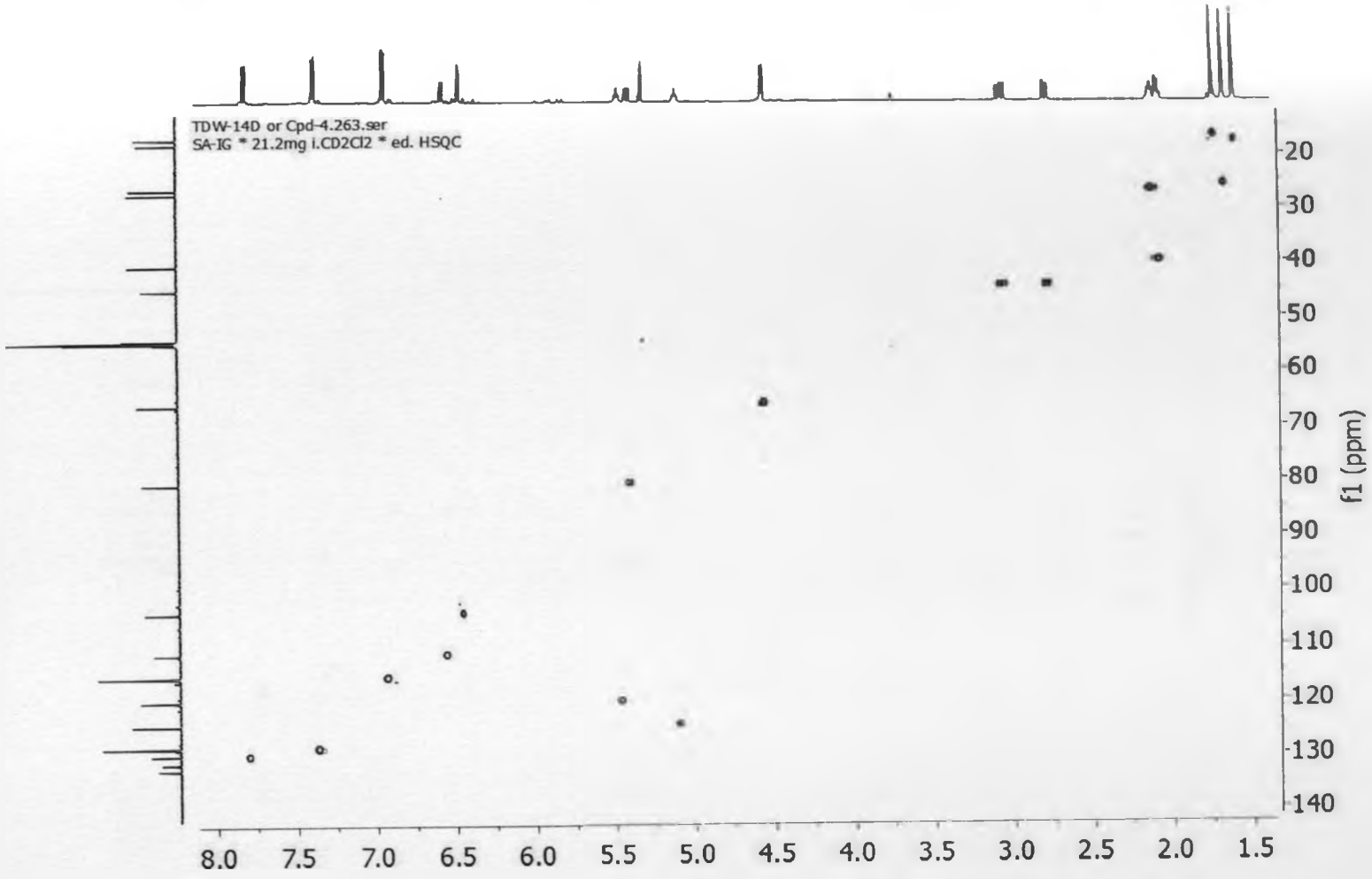
Appendix 41B:...^{13}C NMR (150.95 MHz) spectrum for compound 327



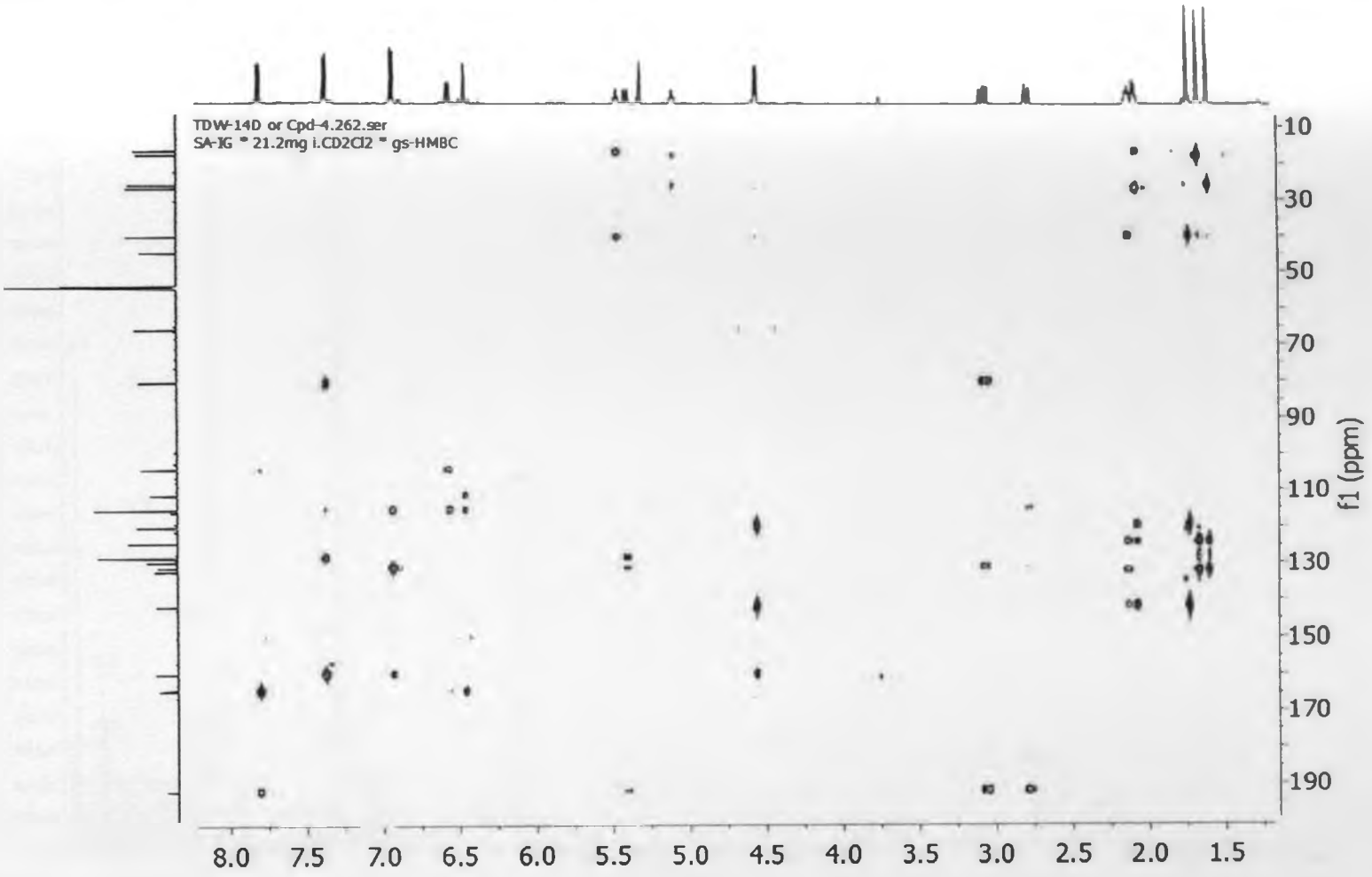
Appendix 41C: COSY (600.24 MHz) spectrum of compound 327



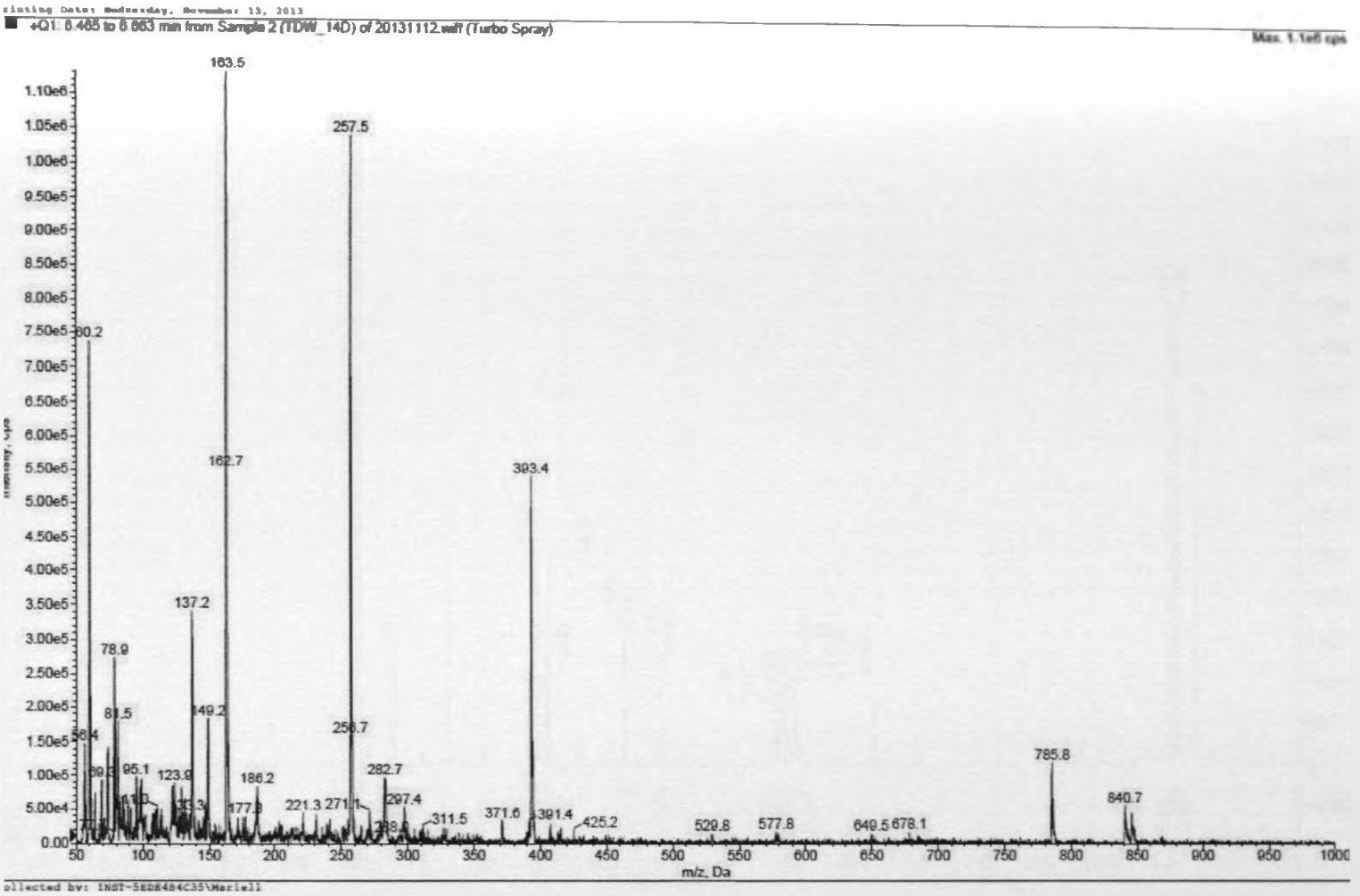
Appendix 41D: HSQC (600.24 MHz /150.95 MHz) spectrum of compound 327



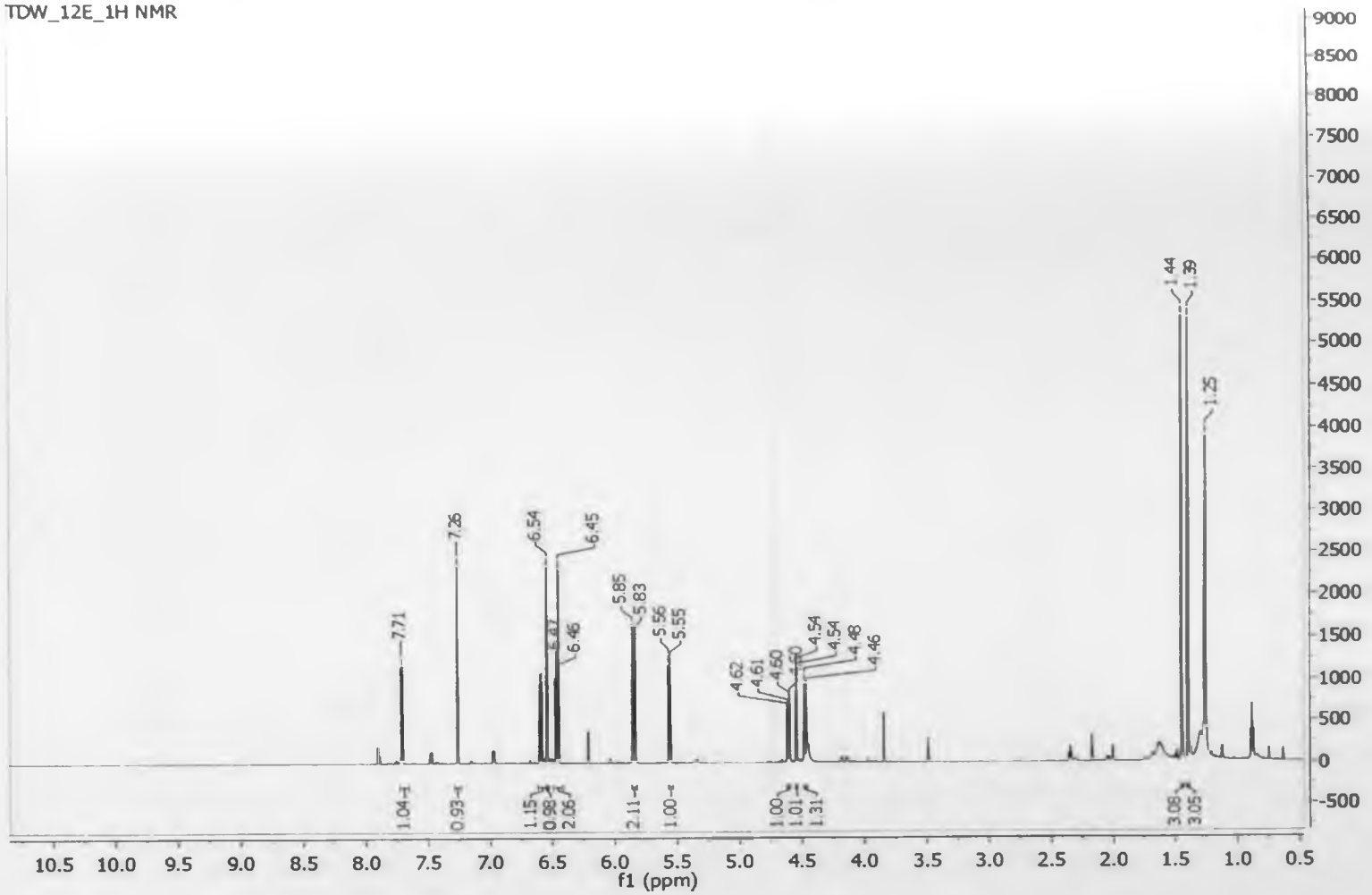
Appendix 41E: HMBC (600.24 MHz /150.95 MHz) spectrum of compound 327



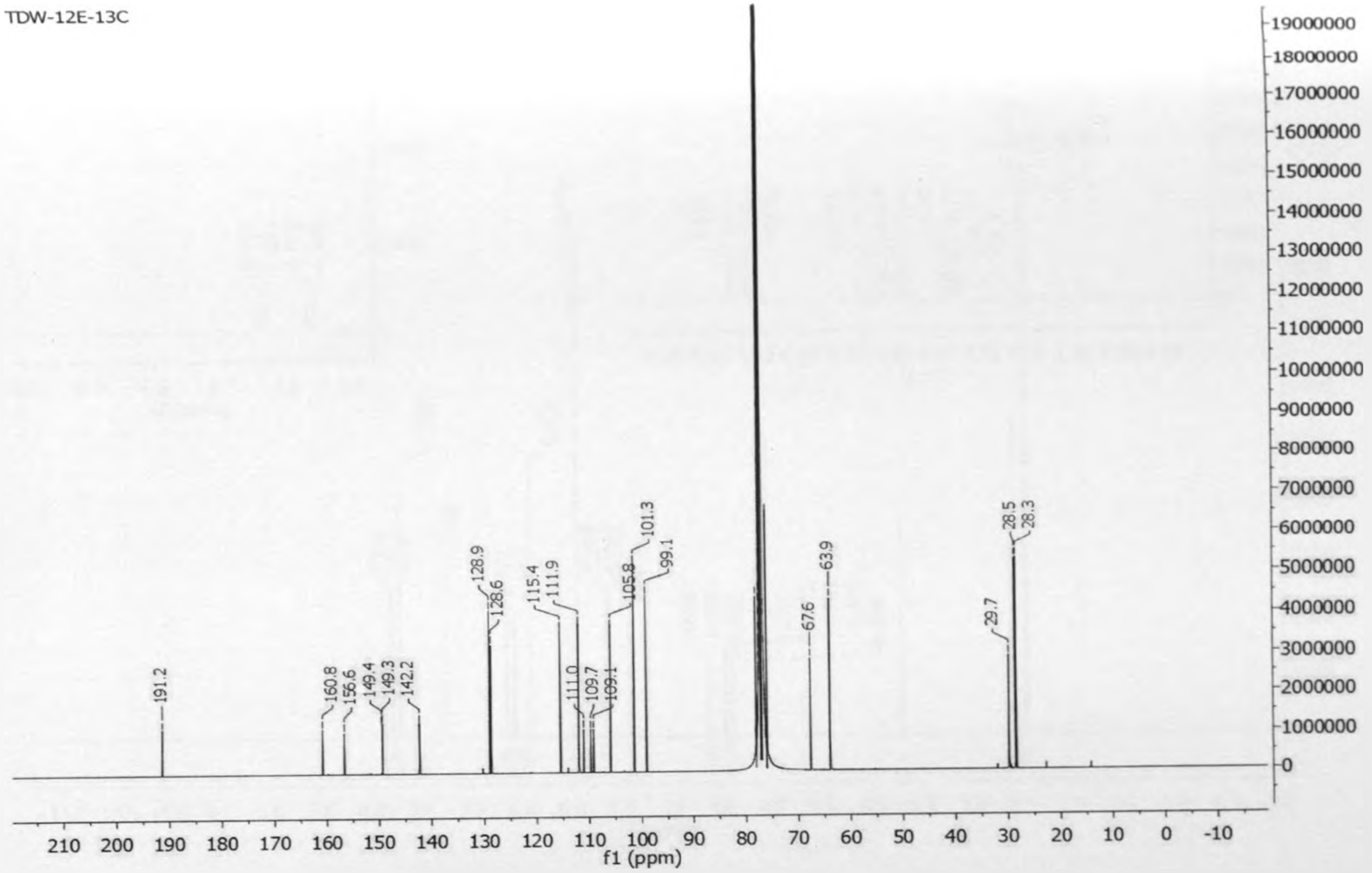
Appendix 41F: ESIMS spectrum of compound 327



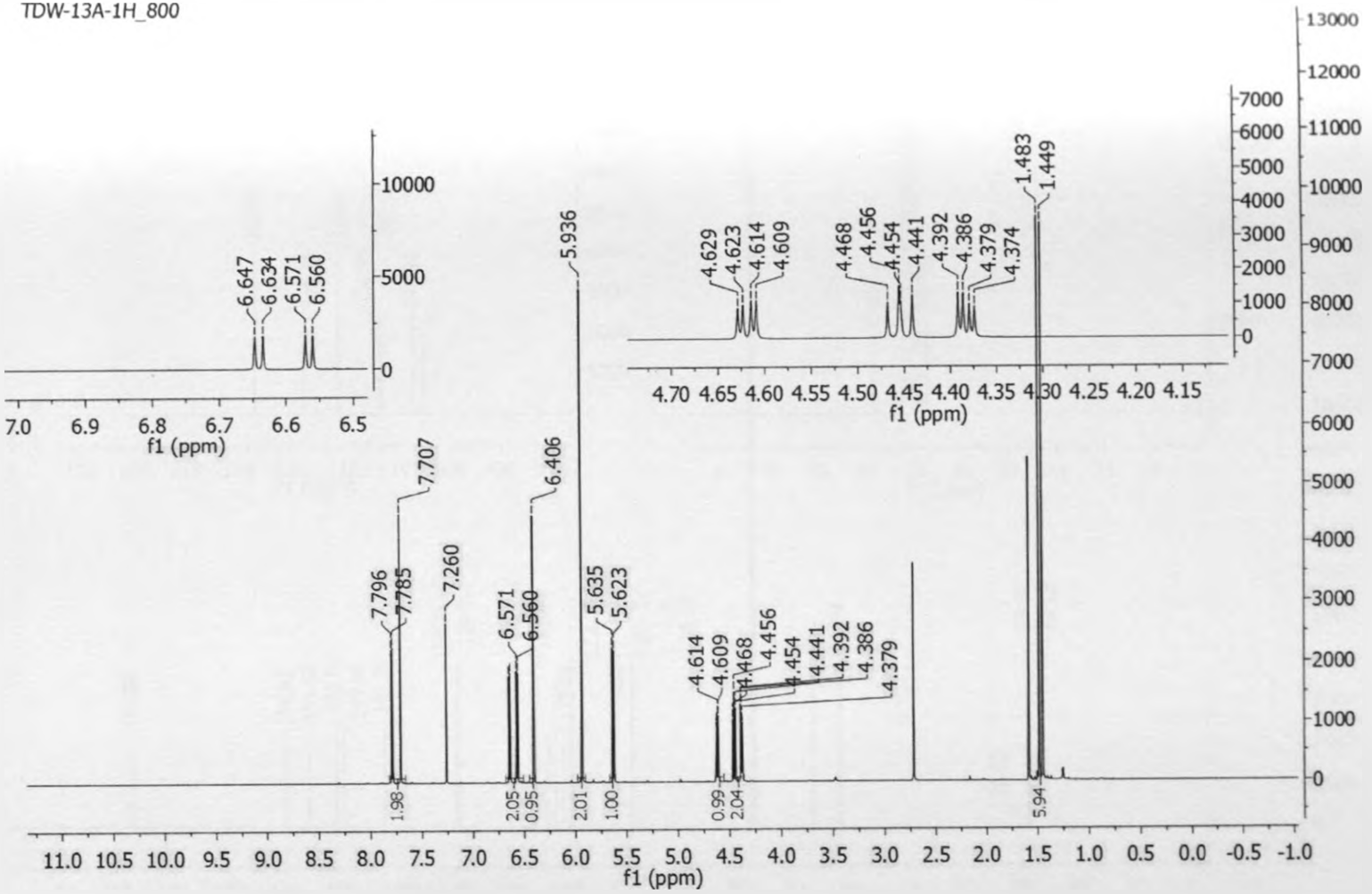
Appendix 42A: ^{1}H NMR (799.87 MHz) spectrum for compound 138



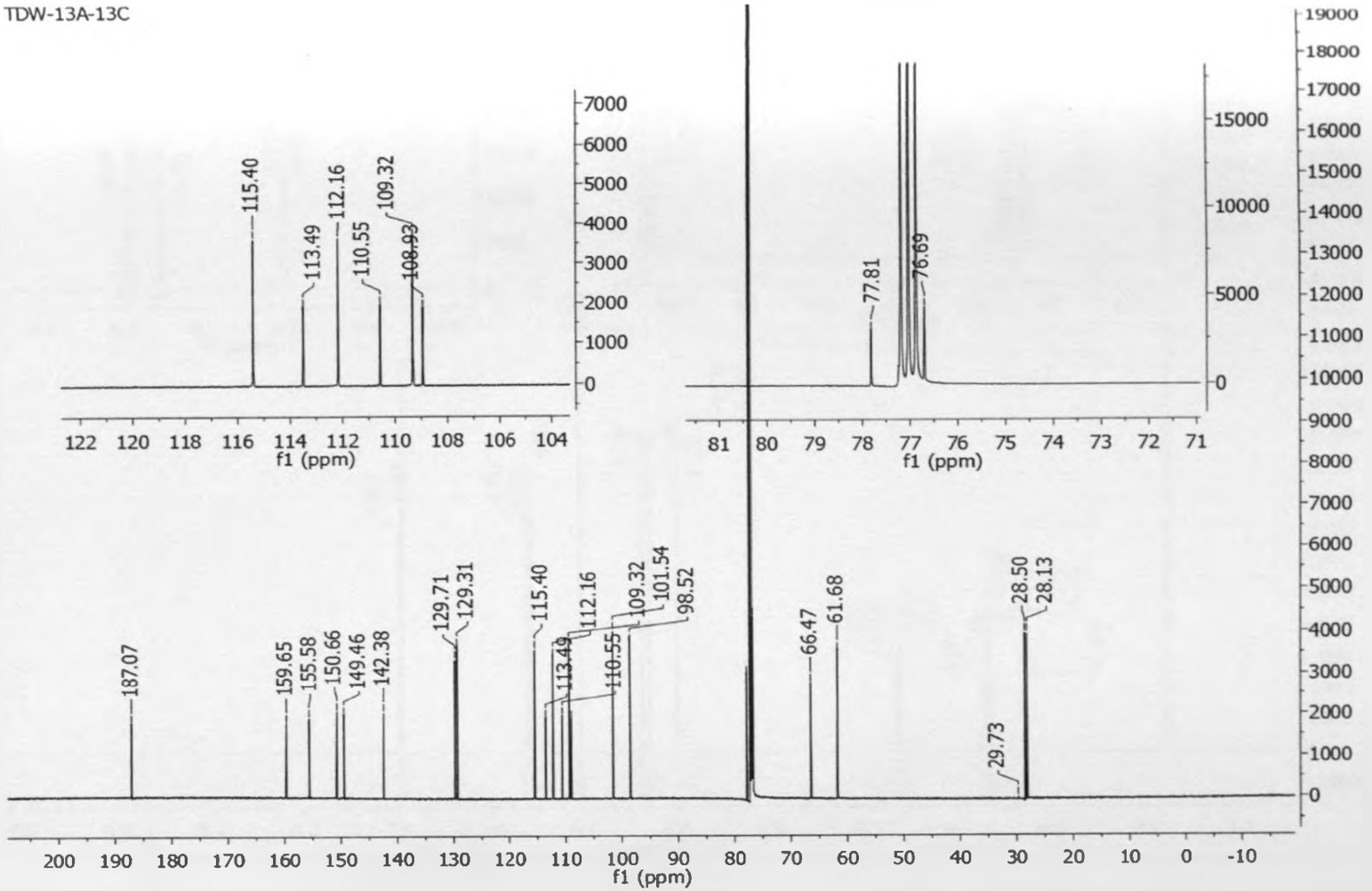
Appendix 42B: ^{13}C NMR (201.15 MHz) spectrum of compound 138



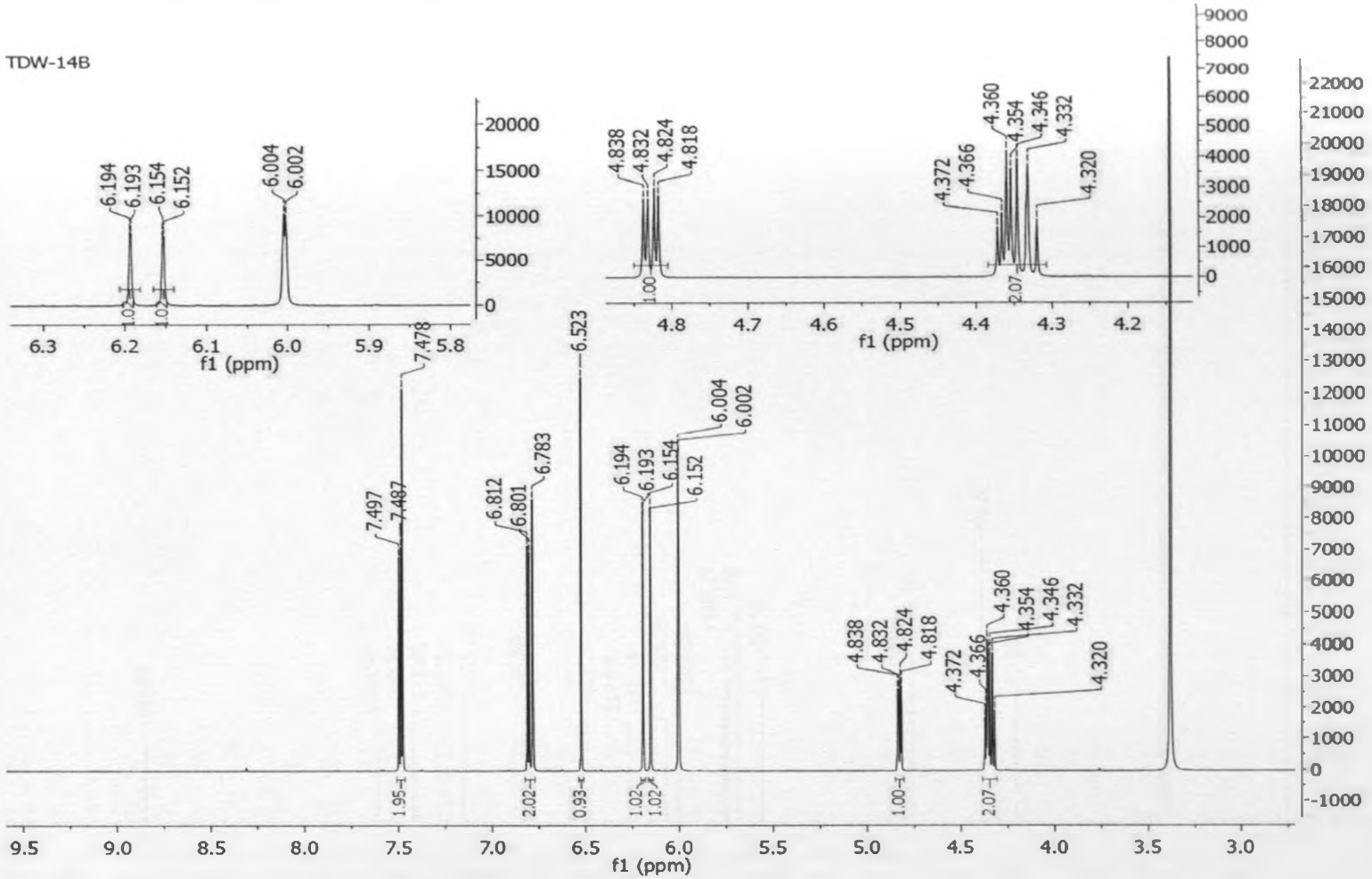
Appendix 43A: ^{1}H NMR (799.87 MHz) spectrum of compound 137



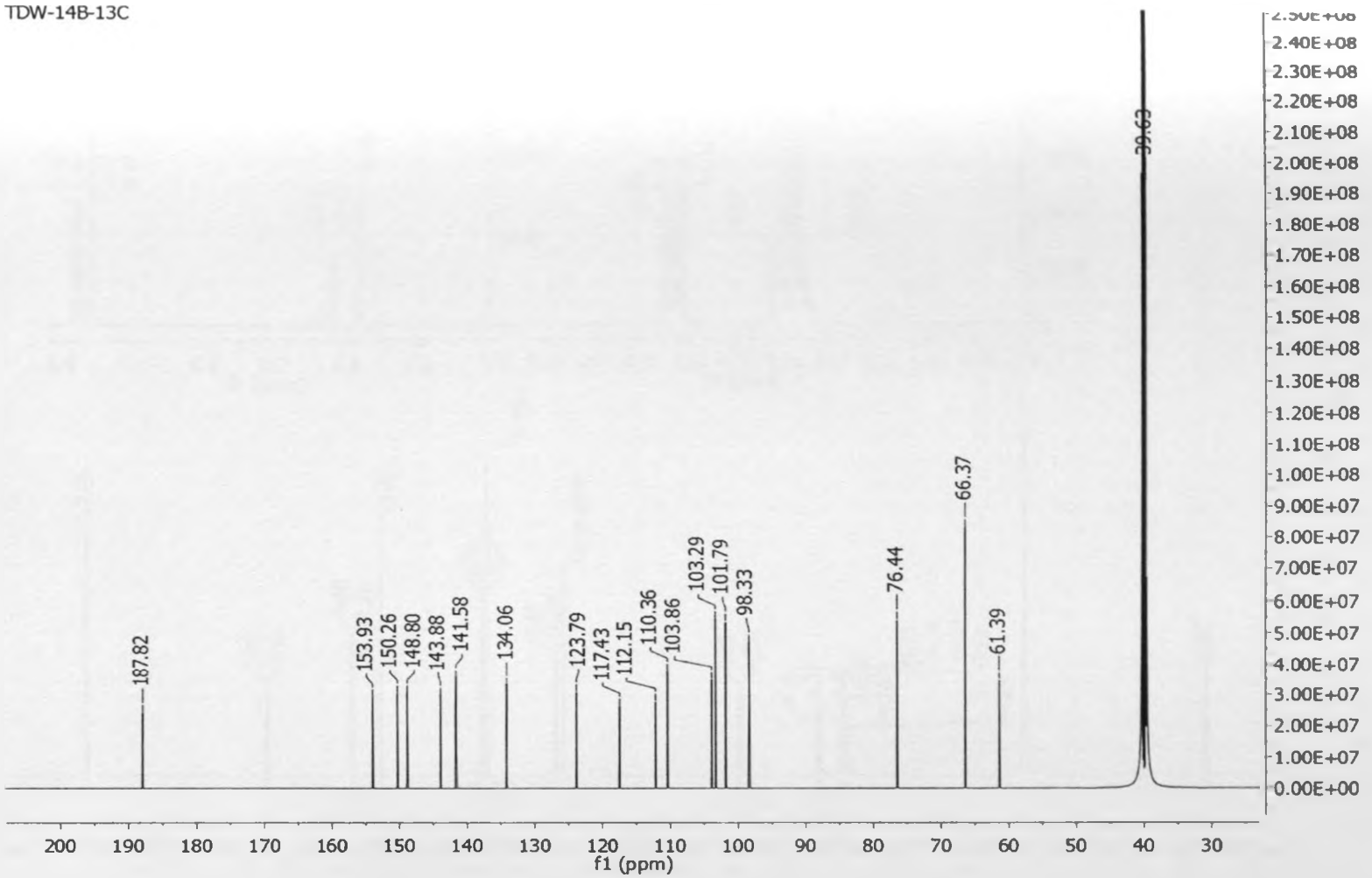
Appendix 43B: ^{13}C NMR (201.15 MHz) spectrum of compound 137



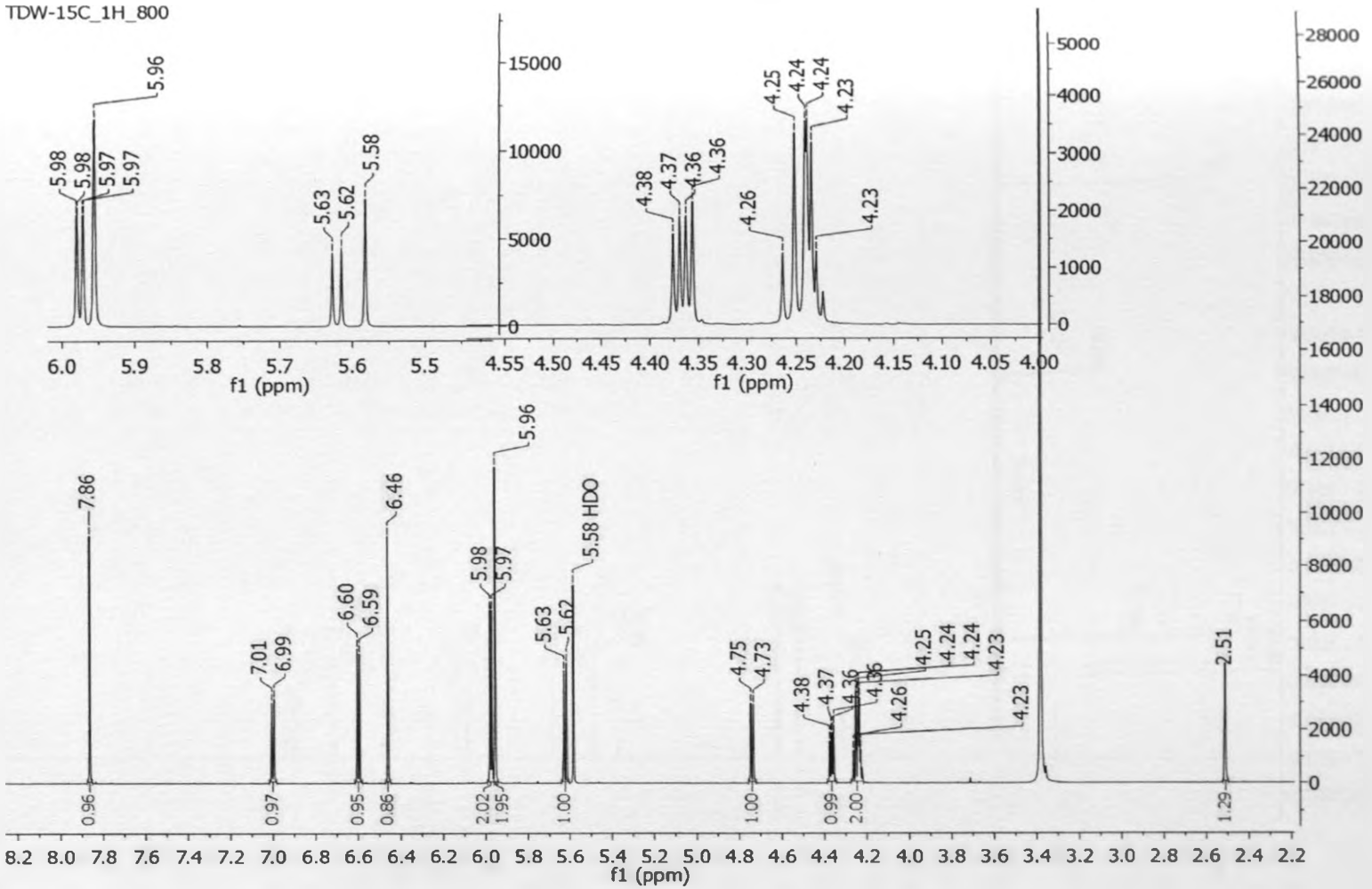
Appendix 44A: ^{1}H NMR (799.87 MHz) spectrum of compound 139



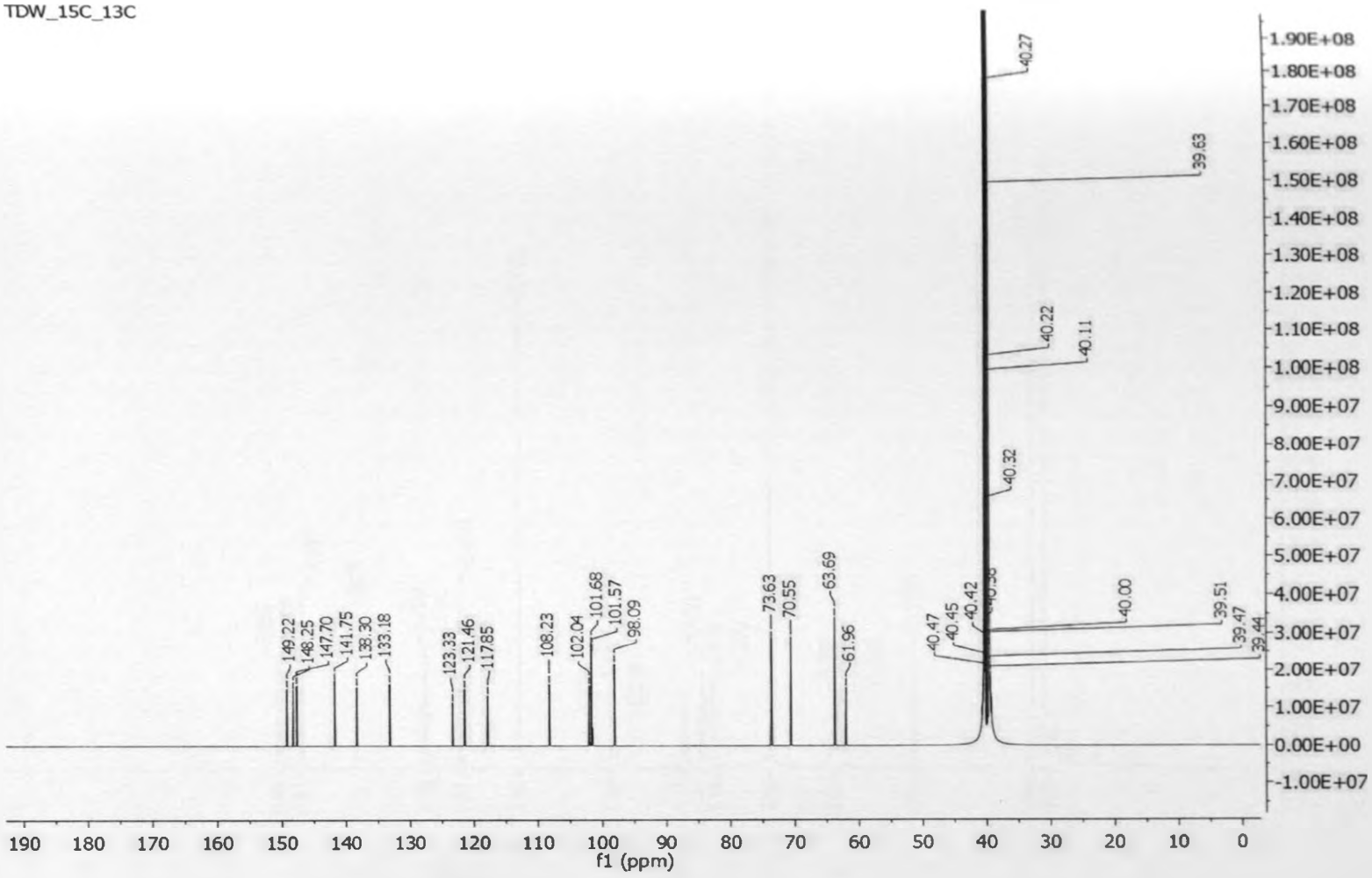
Appendix 44B: ^{13}C NMR (201.15 MHz) spectrum of compound 139



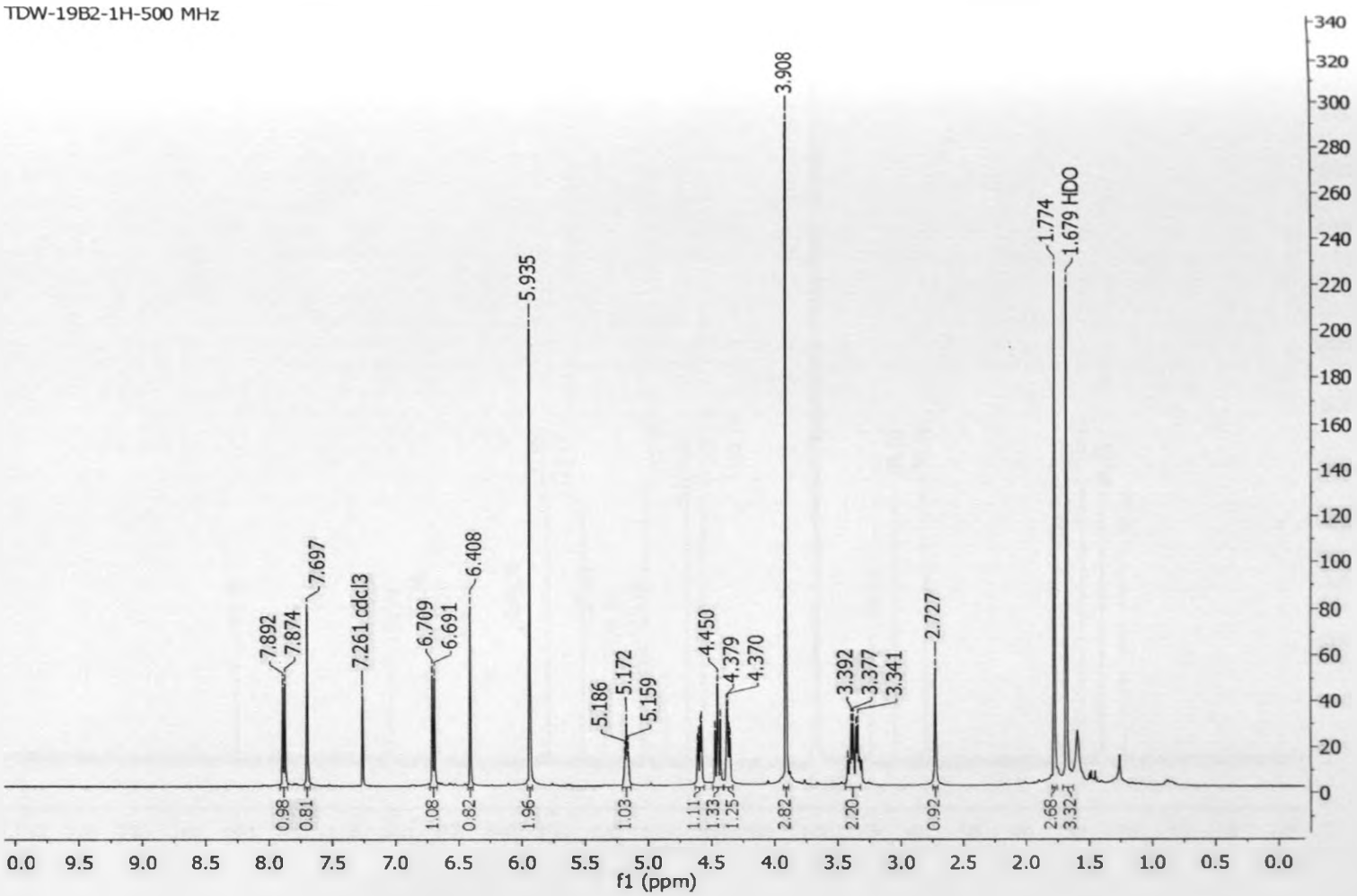
Appendix 45A: ^{1}H NMR (799.87 MHz) spectrum of compound 140



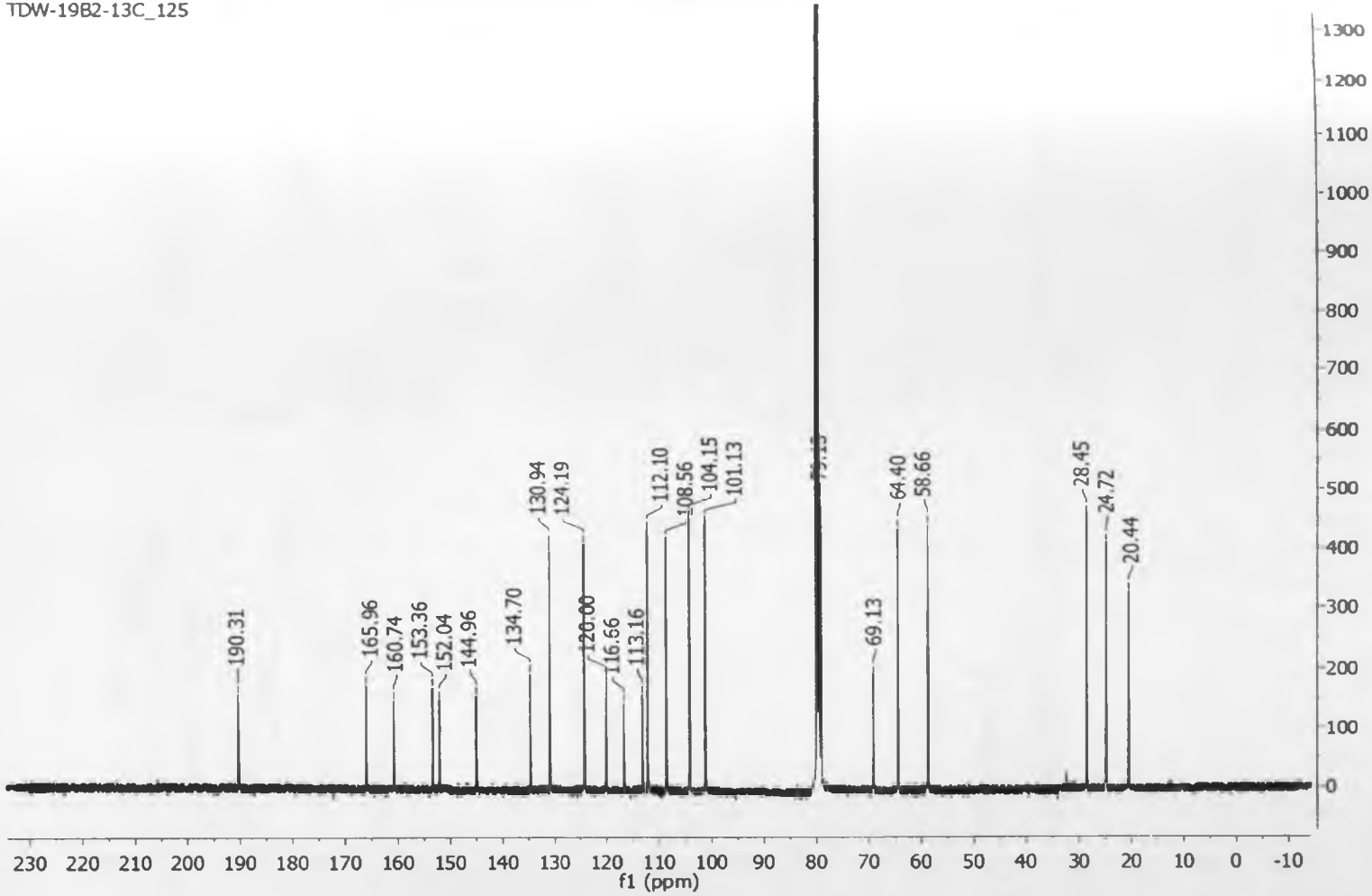
Appendix 45B: ^{13}C NMR (201.15 MHz) spectrum of compound 140



Appendix 46A: ^{1}H NMR (499.88 MHz) spectrum of compound 154

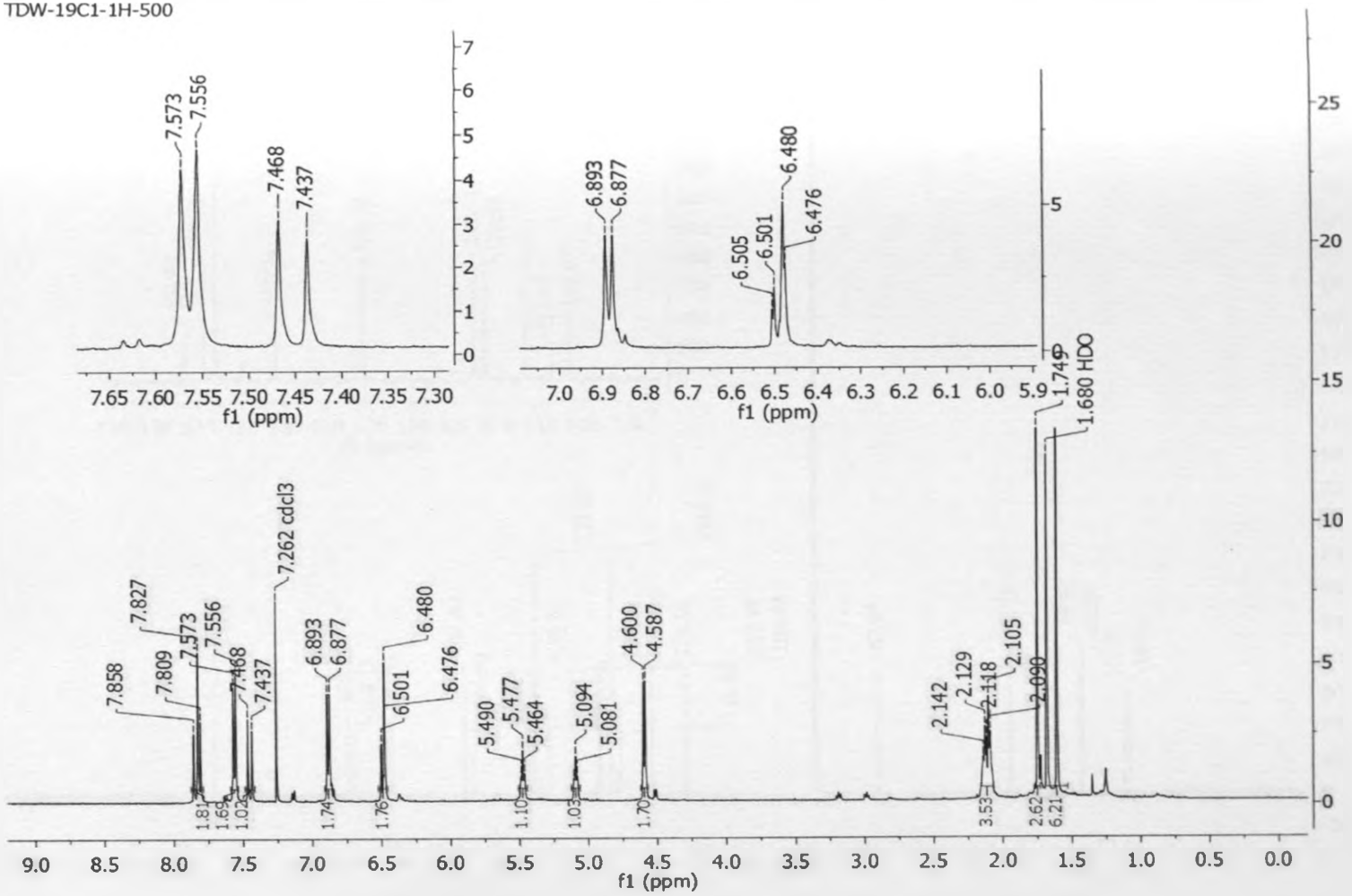


Appendix 46B: ^{13}C **NMR (125.77 MHz) spectrum of compound 154**

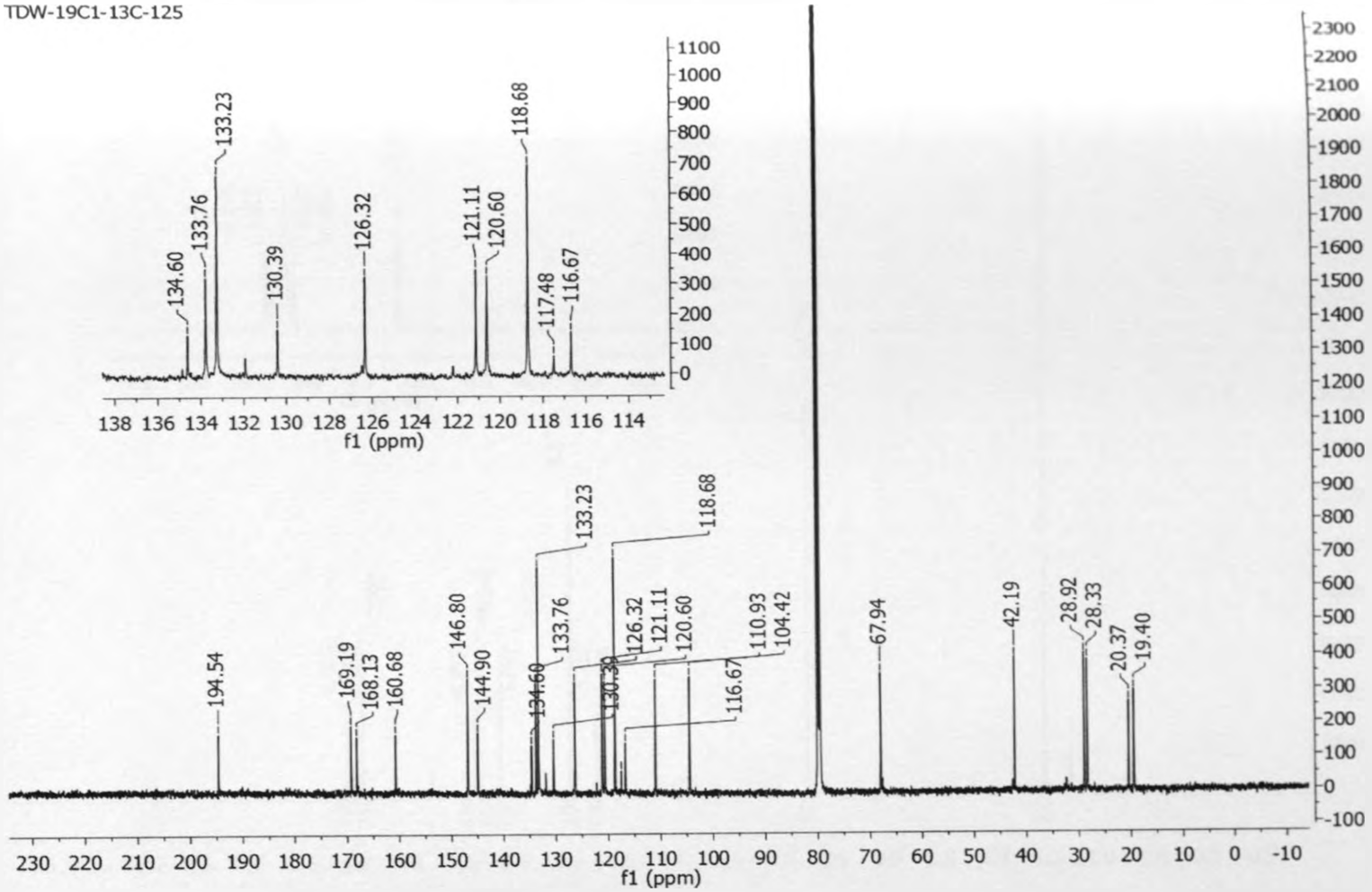


Appendix 47A: ^{1}H NMR (499.88 MHz) spectrum of compound 181

TDW-19C1-1H-500

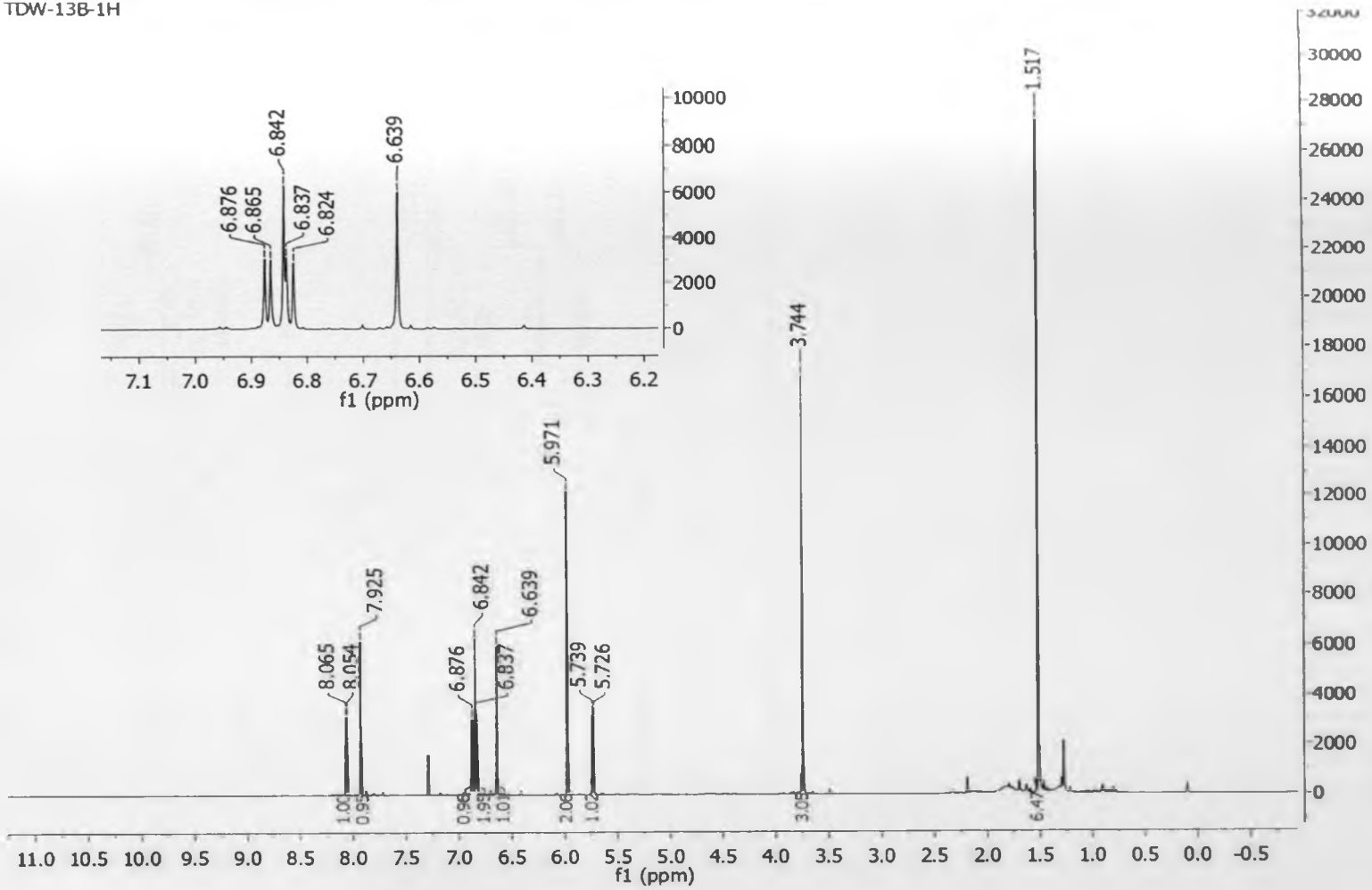


Appendix 47B: ^{13}C NMR (125.77 MHz) spectrum of compound 181



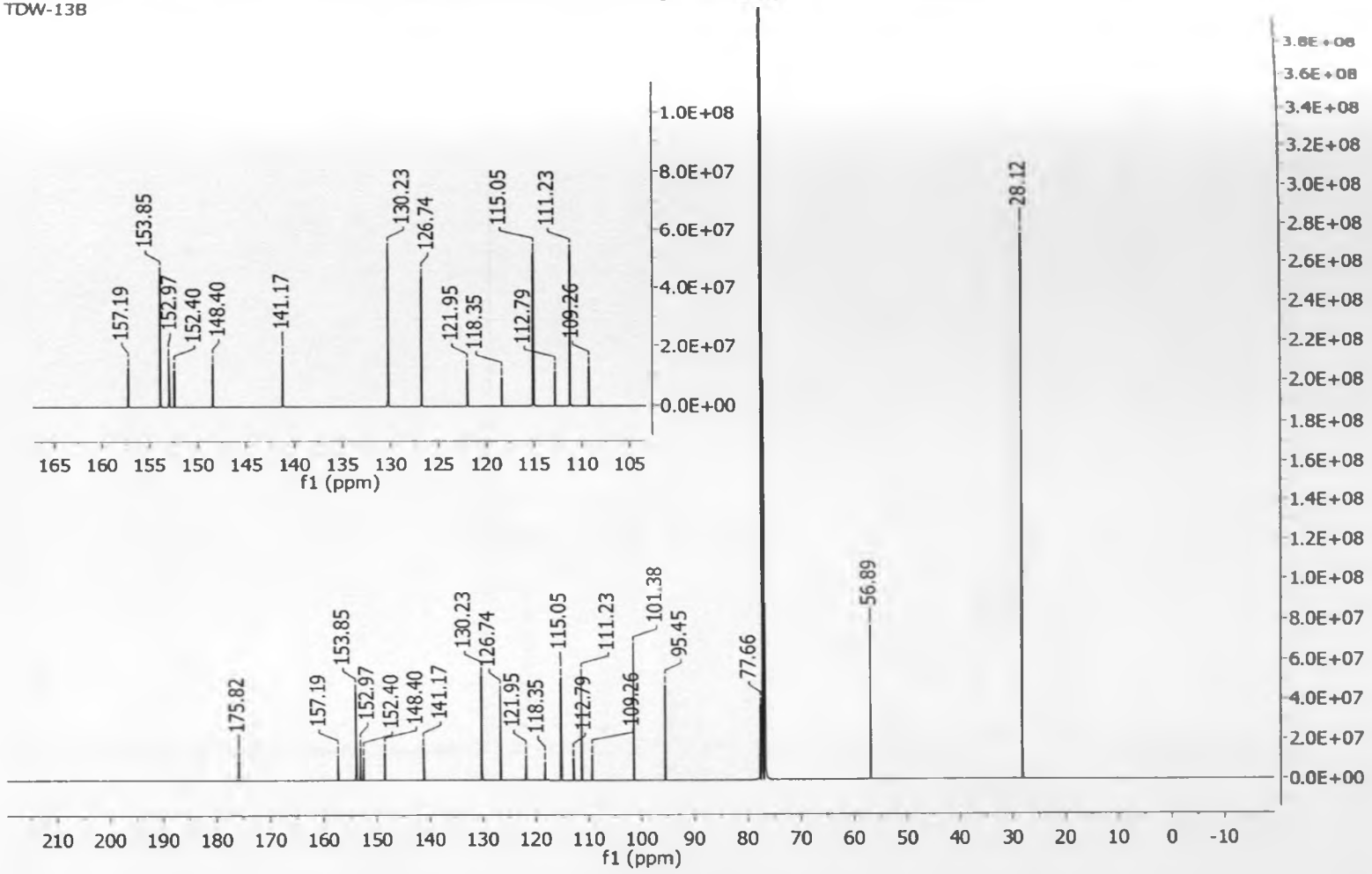
Appendix 48A: ^{1}H NMR (799.87 MHz) spectrum of compound 328

TDW-13B-1H

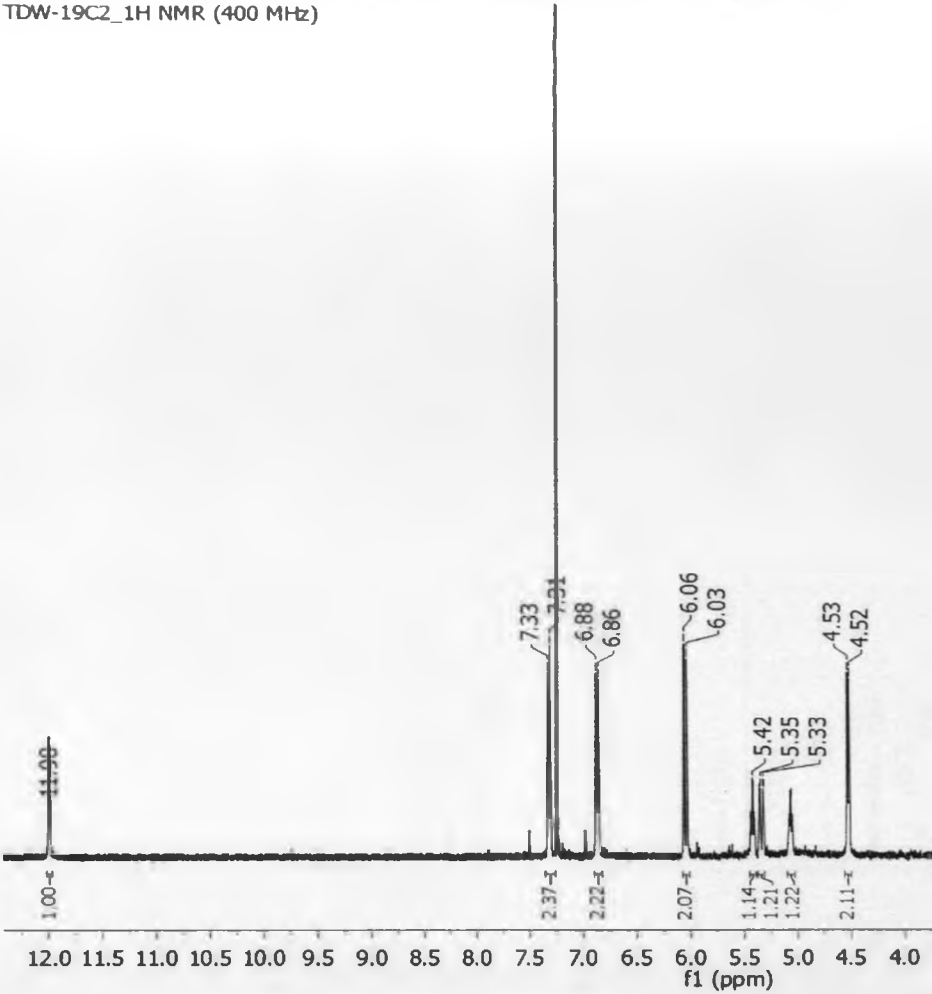


Appendix 48B: ^{13}C NMR (201.15 MHz) spectrum of compound 328

TDW-13B



Appendix 49A: ^{1}H NMR (399.94 MHz) spectrum of compound 329



3.06
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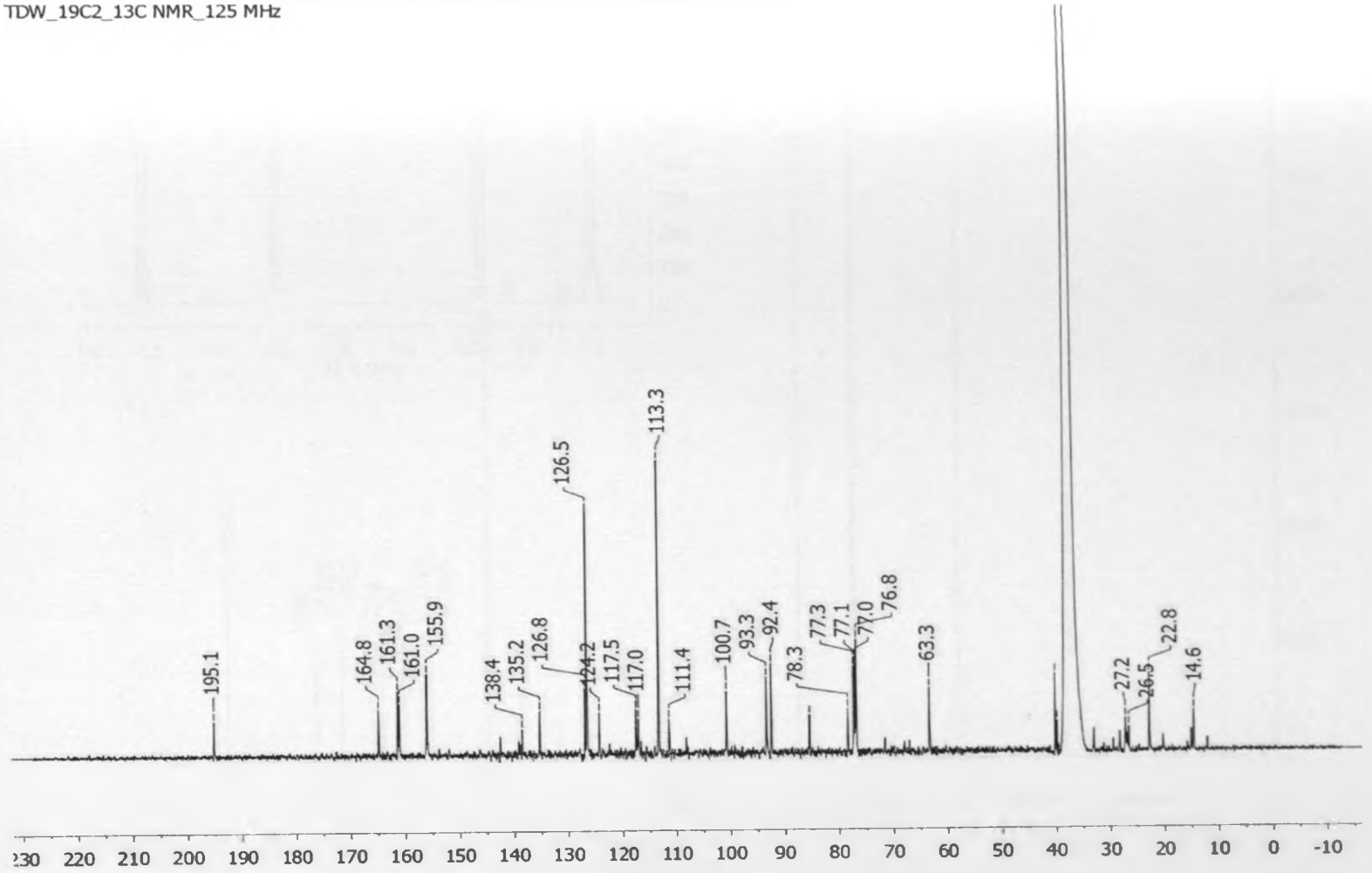
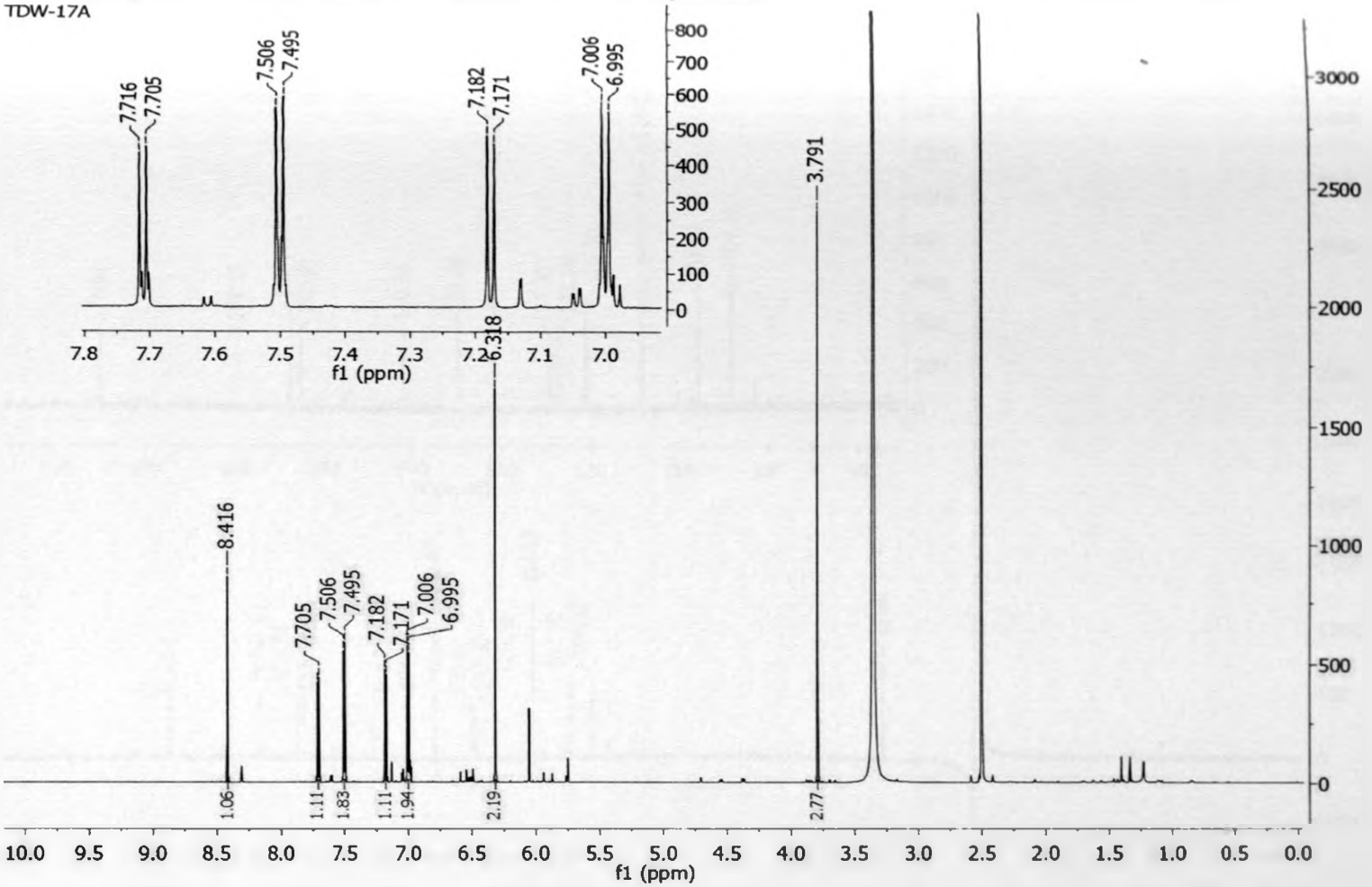
2.78
2.75
2.74
2.11
2.10
2.08
2.07
2.06
2.05
1.70
1.66
1.59
1.27
1.40
4.36
3.47
3.41
3.18
120
110
100
90
80
70
60
50
40
30
20
10
0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0

Appendix 49B: ^{13}C NMR (125.77 MHz) spectrum of compound 329

TDW_19C2_13C NMR_125 MHz

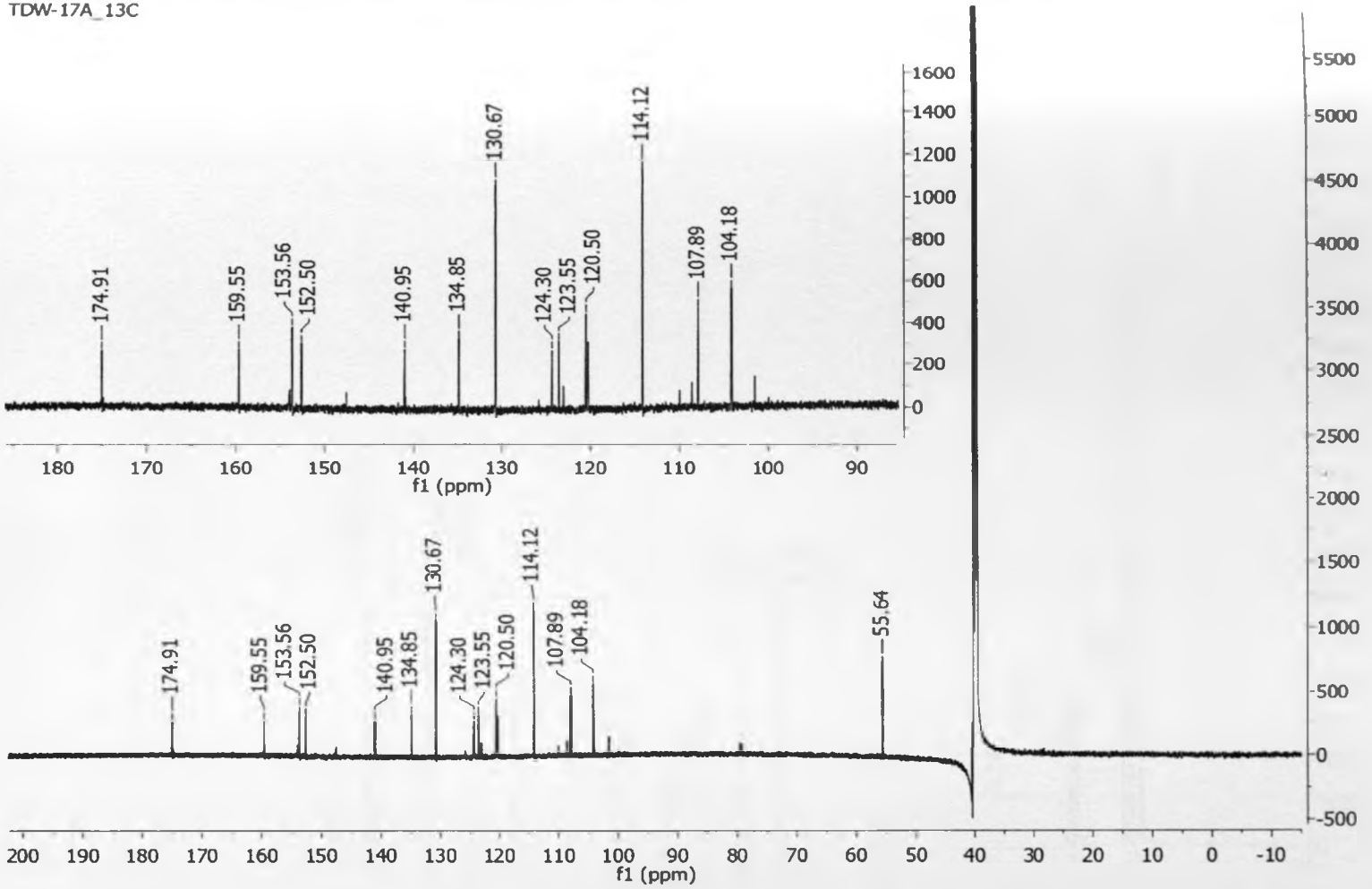


Appendix 50A: ^{1}H NMR (799.87 MHz) spectrum of compound 97

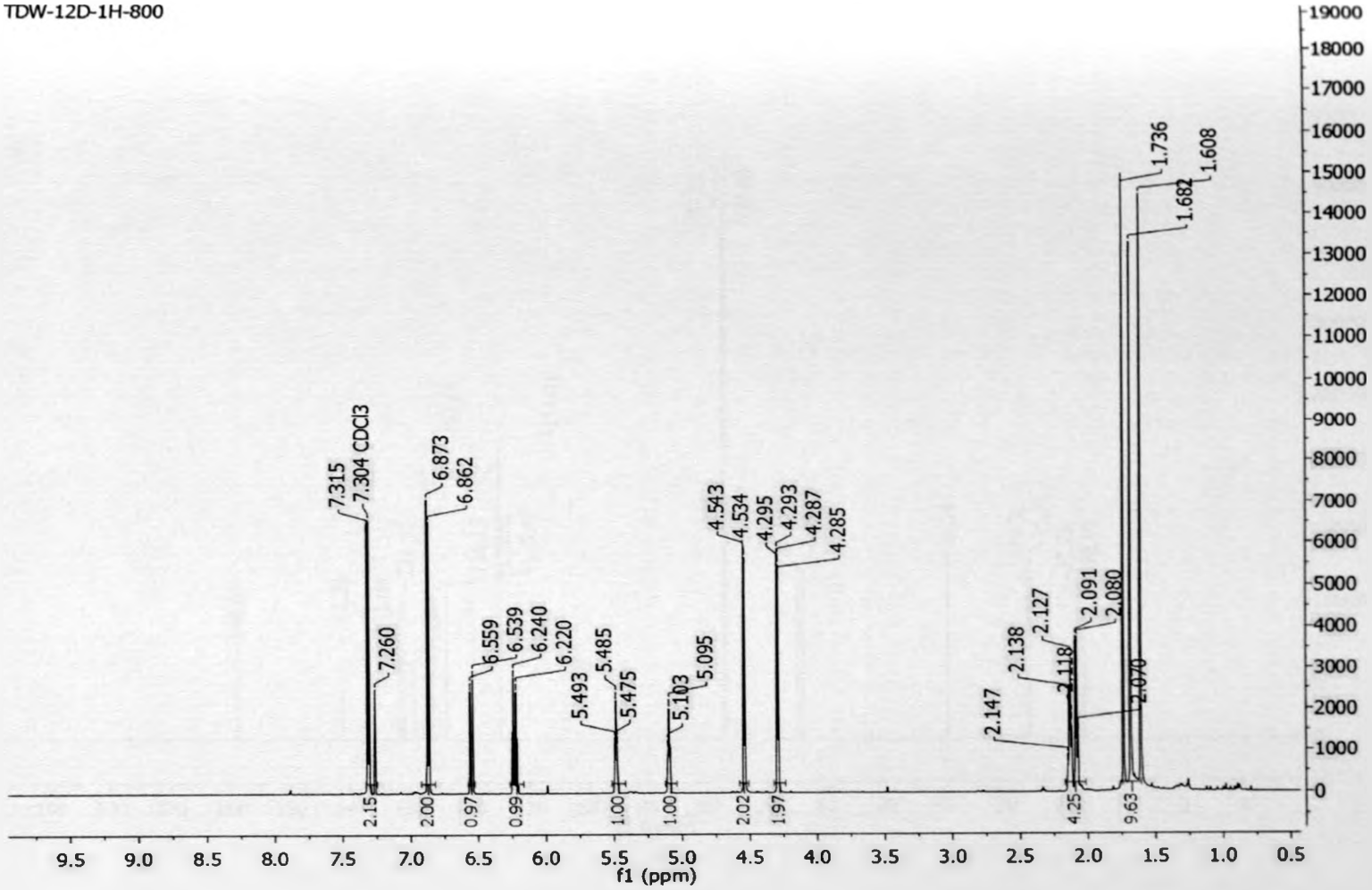


Appendix 50B: ^{13}C NMR (201.15 MHz) spectrum of compound 97

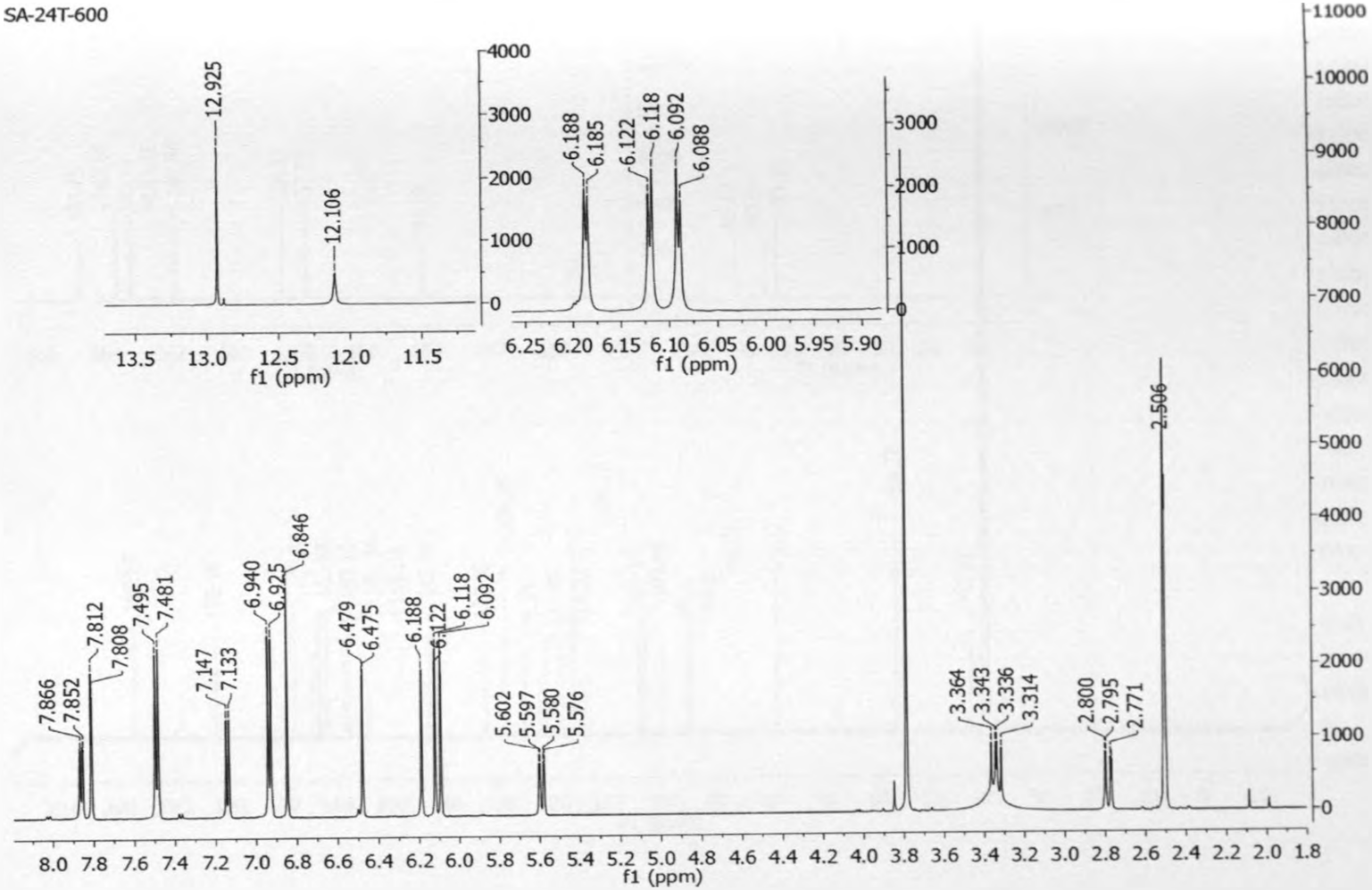
TDW-17A_13C



Appendix 51A: ^{1}H NMR (799.87 MHz) spectrum of compound 330

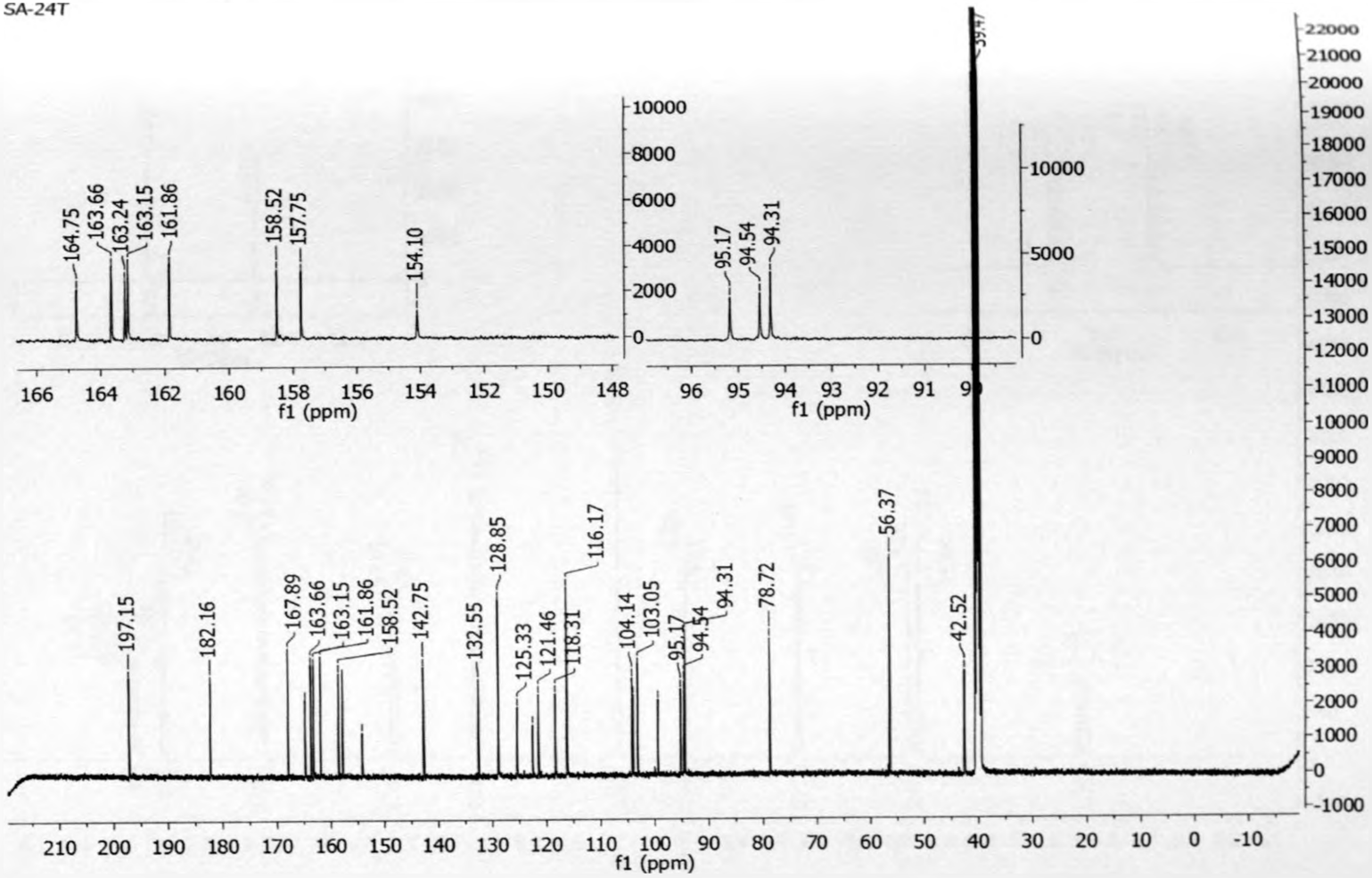


Appendix 52A: ^{1}H NMR (600.24 MHz) spectrum of compound 275



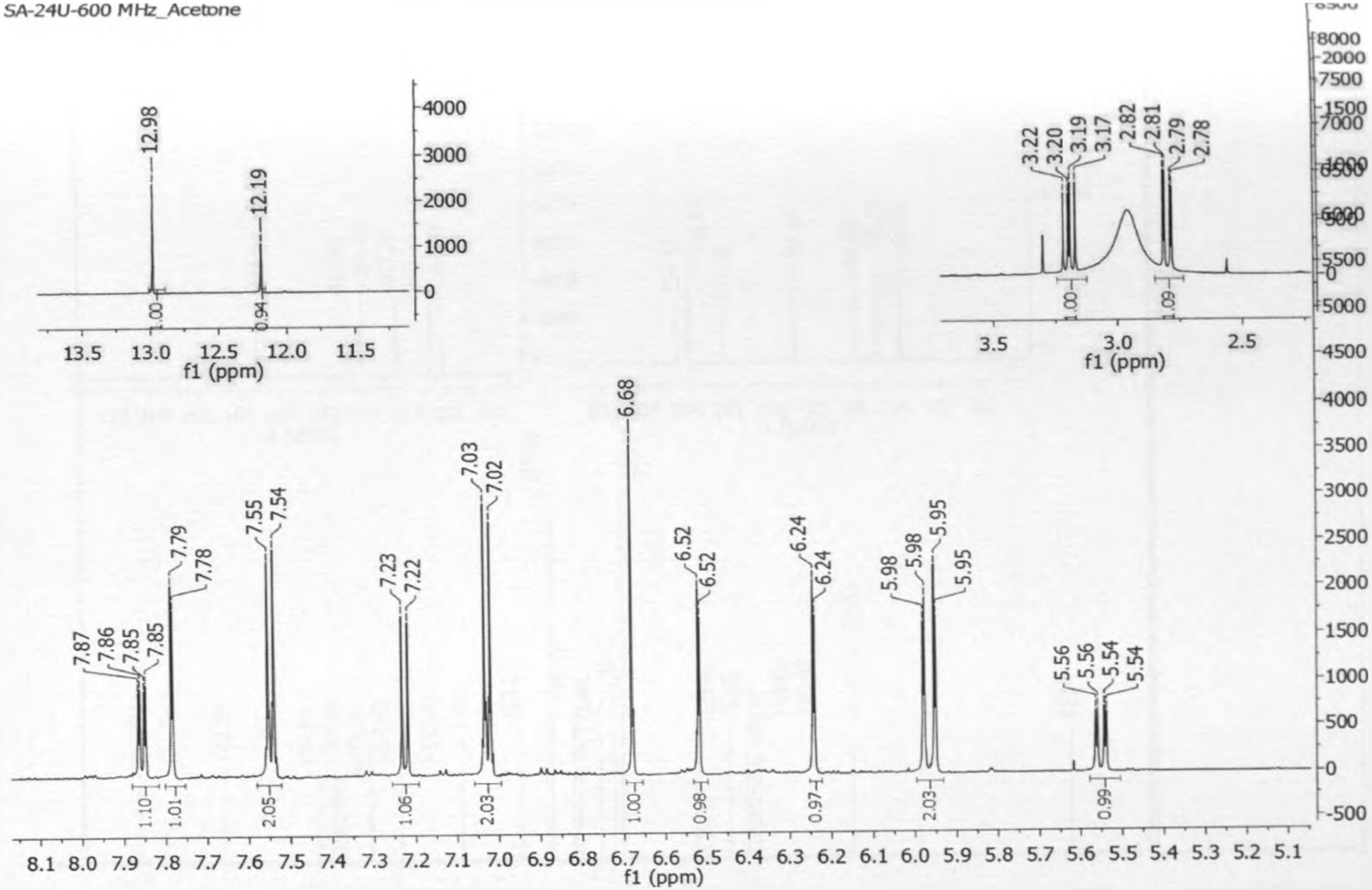
Appendix 52B: ^{13}C NMR (150.95 MHz) spectrum of compound (27S)

SA-24T

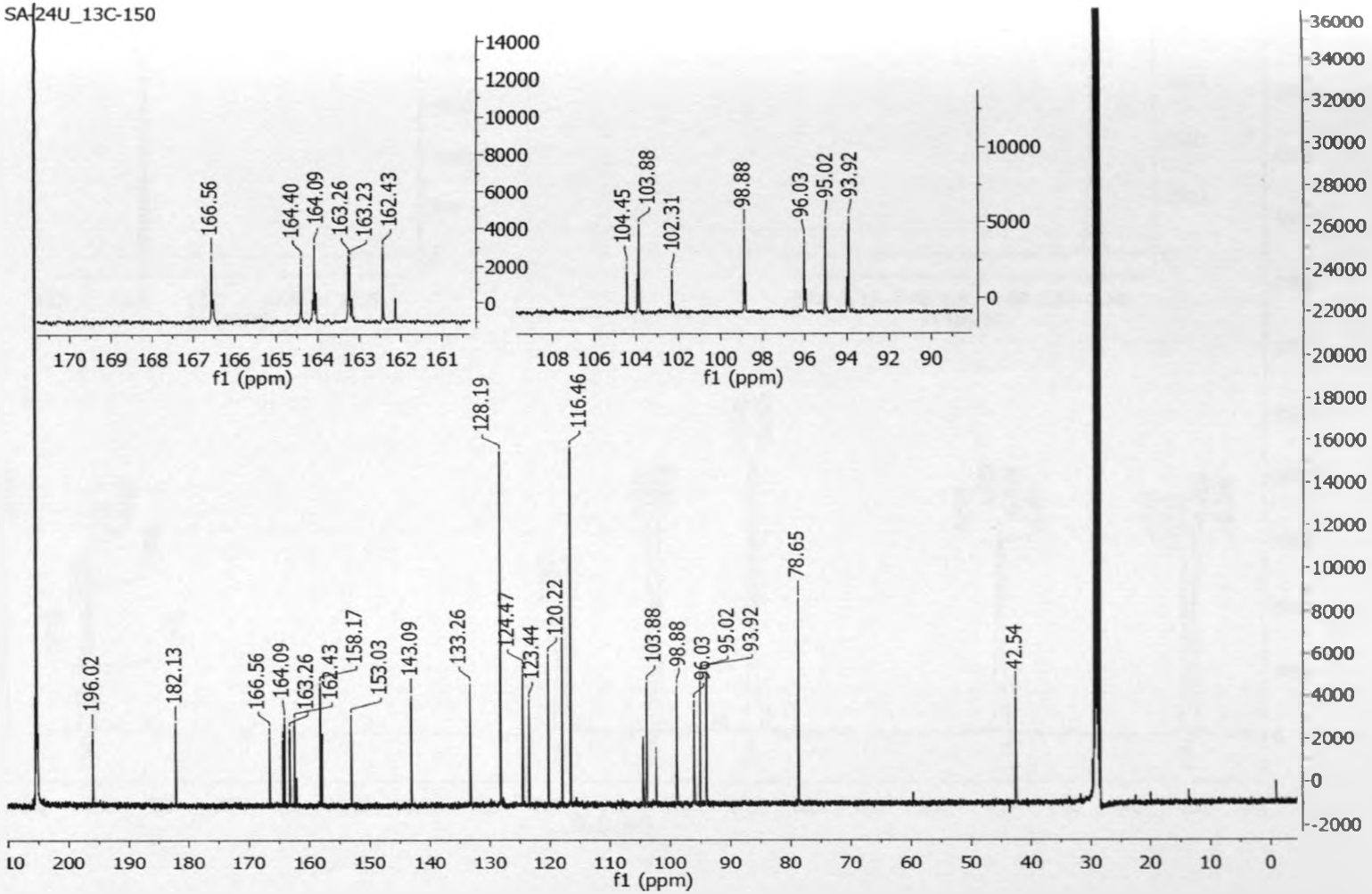


Appendix 53A: ^{1}H NMR (600.24 MHz) spectrum for compound 276

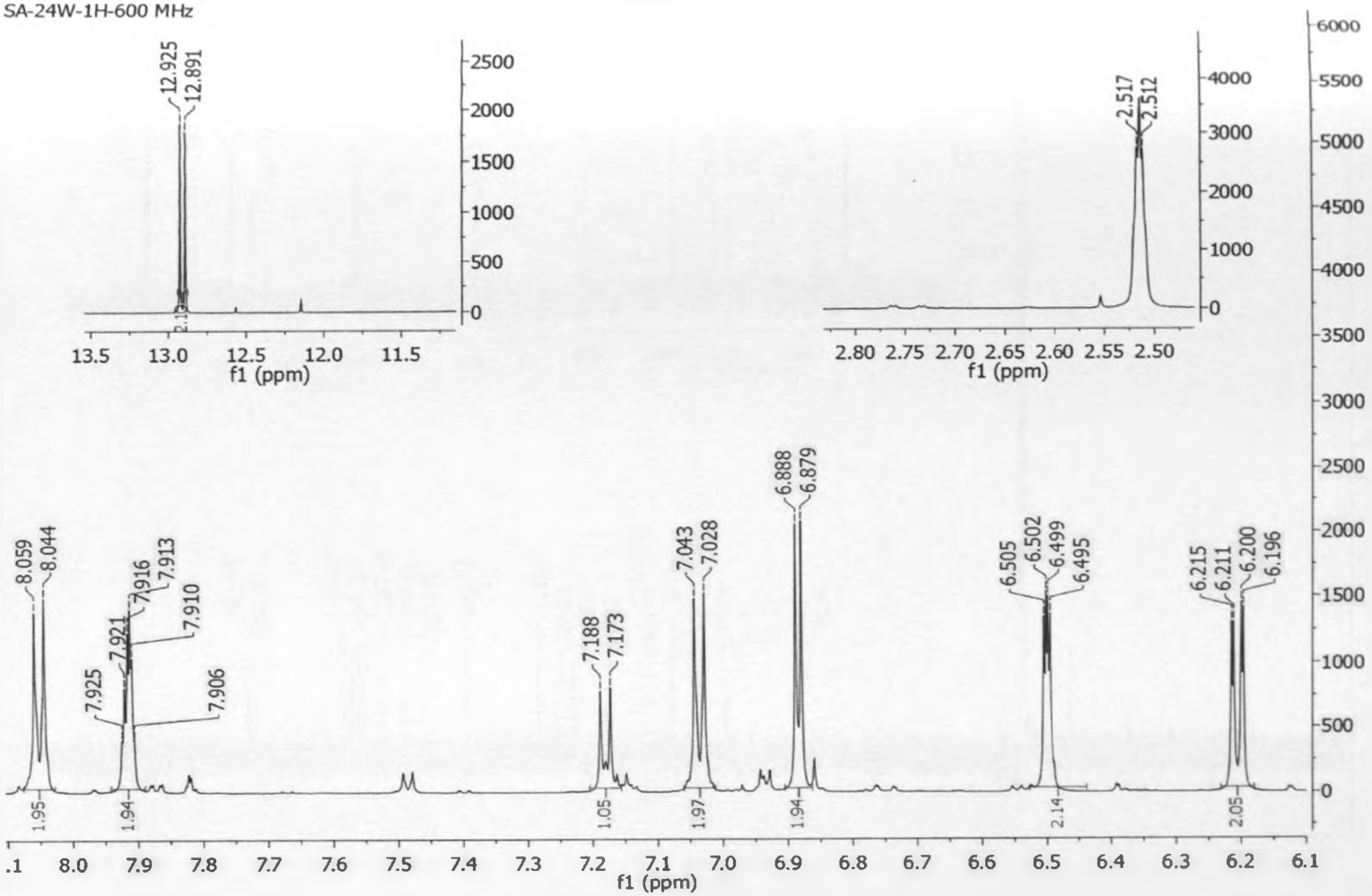
SA-24U-600 MHz_Acetone



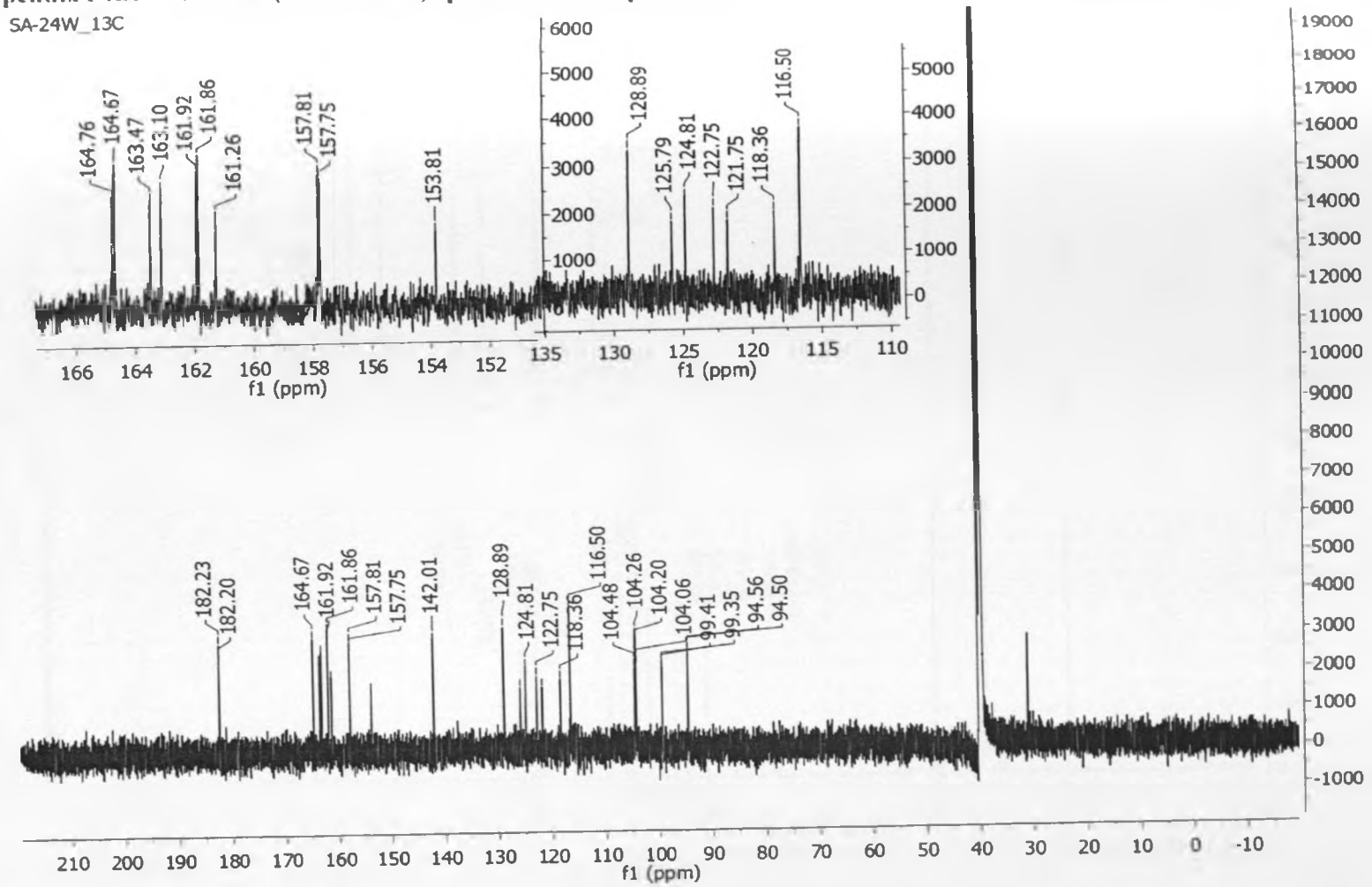
Appendix 53B: ^{13}C NMR (150.95 MHz) spectrum of compound 276



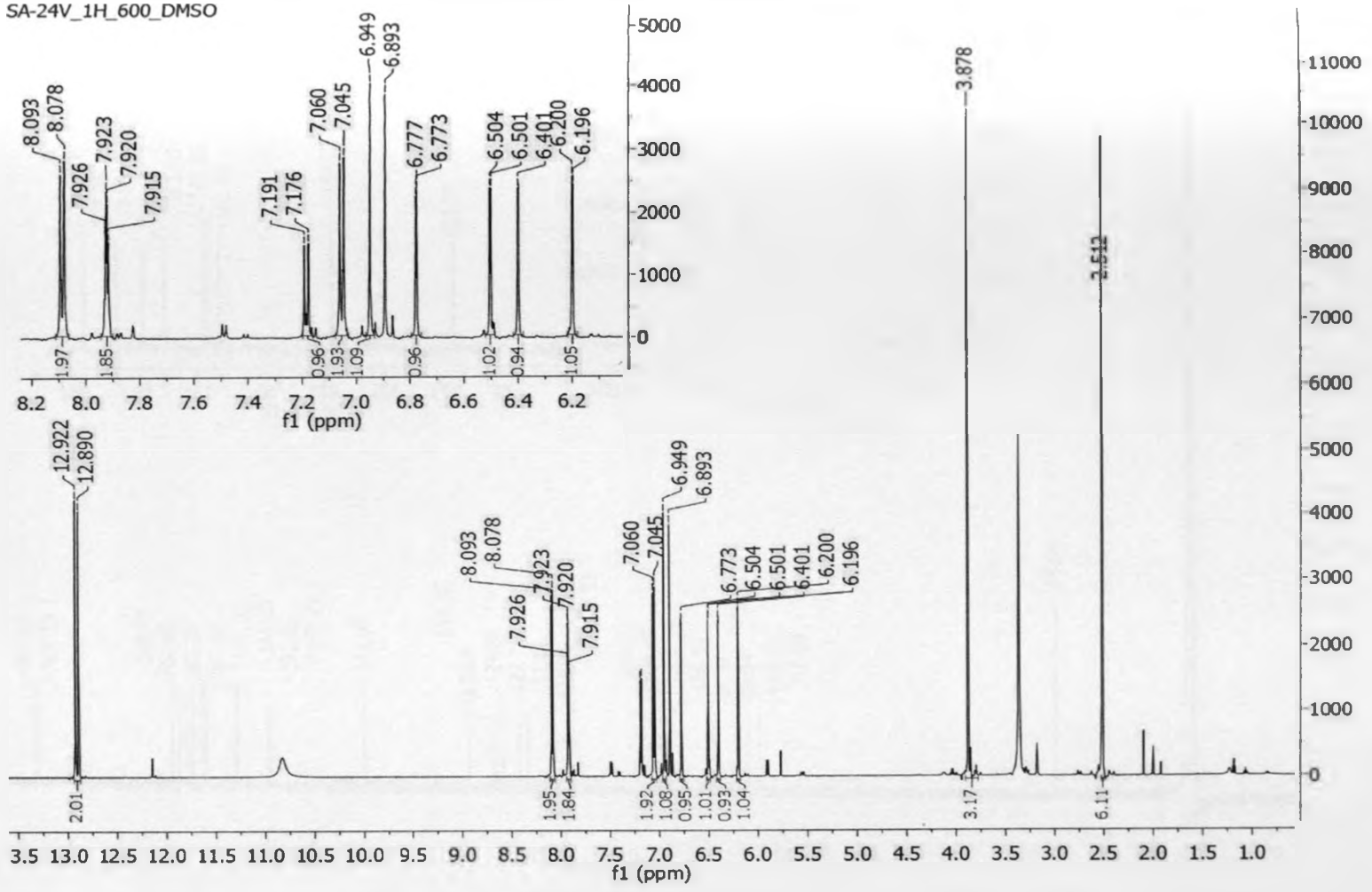
Appendix 54A: ^{1}H NMR (600.24 MHz) spectrum of compound 11



Appendix 54B: ^{13}C NMR (150.95 MHz) spectrum of compound 11

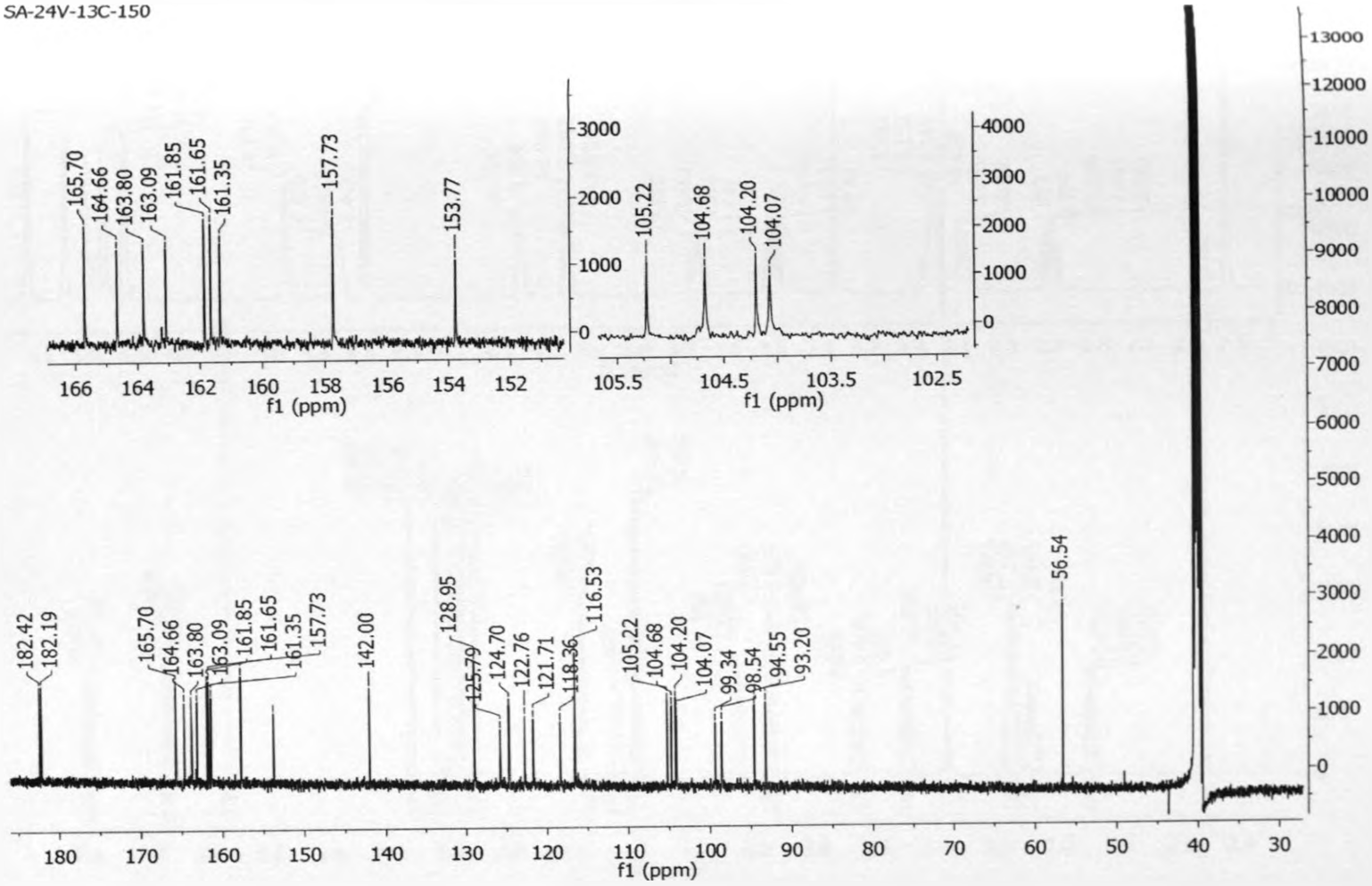


Appendix 55A: ^{1}H NMR (600.24 MHz) spectrum of compound 331

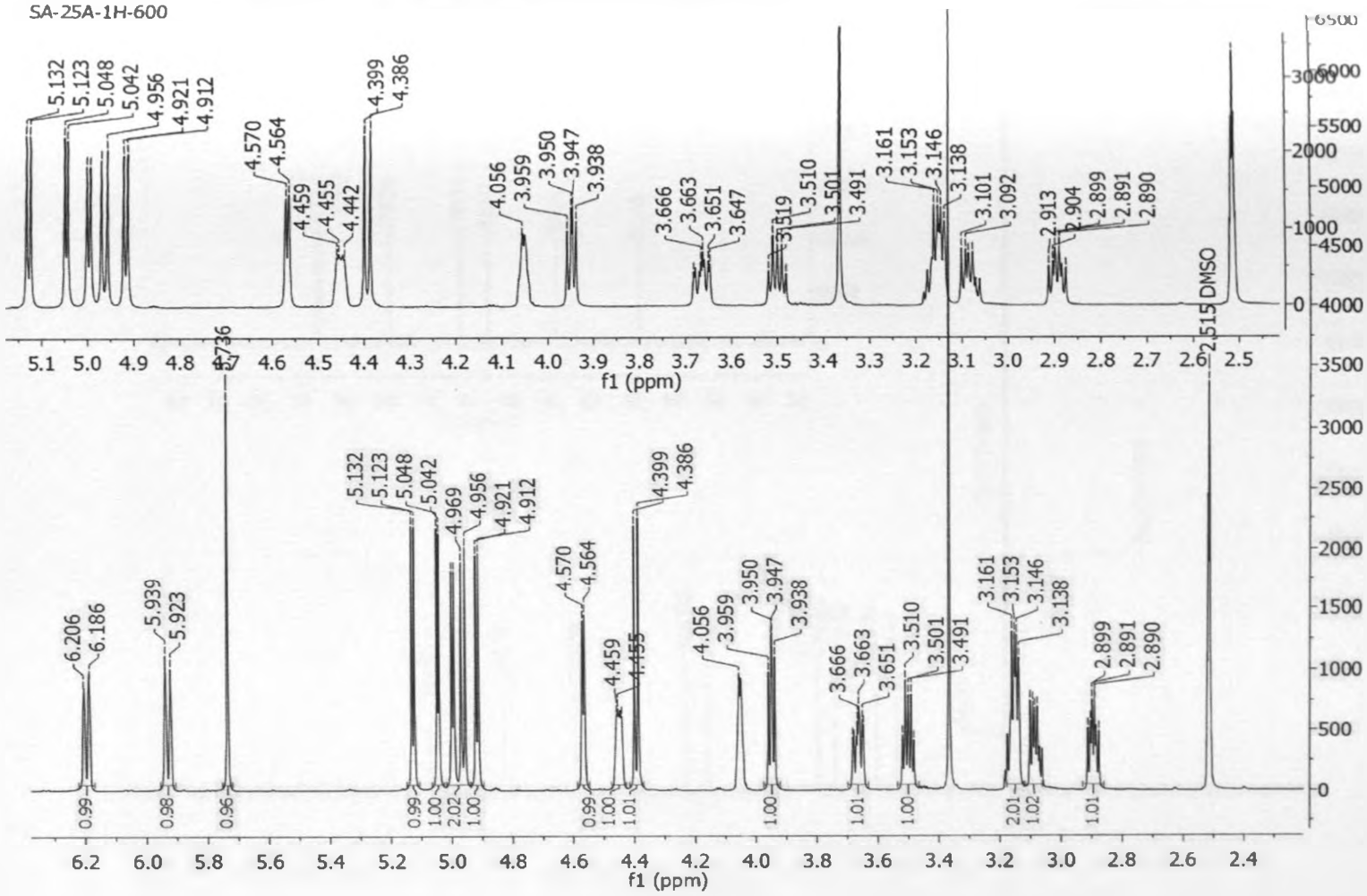


Appendix 55B: ^{13}C NMR (150.95 MHz) spectrum of compound 331

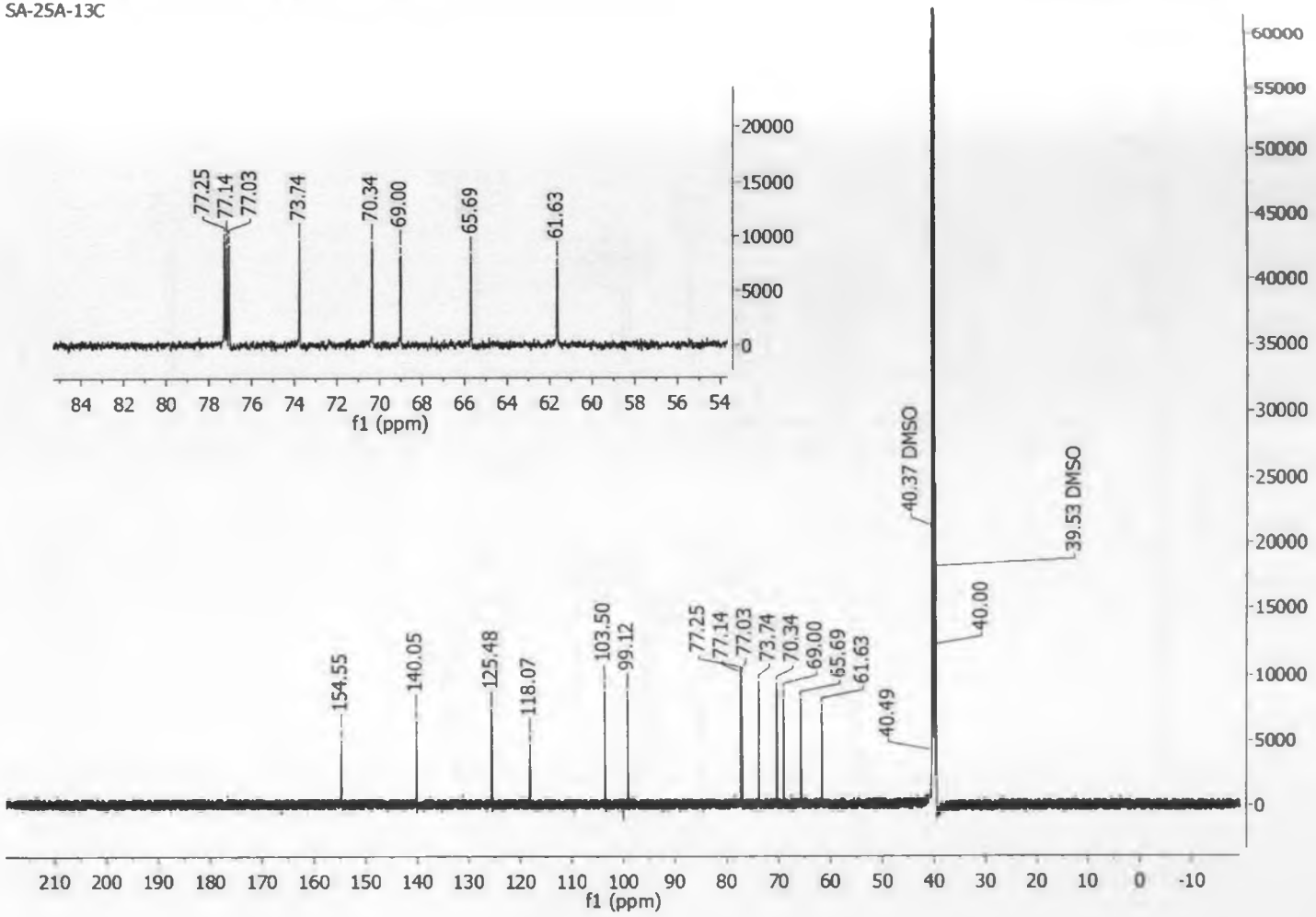
SA-24V-13C-150



Appendix 56A: ^{1}H NMR (600.24 MHz) spectrum of compound 332

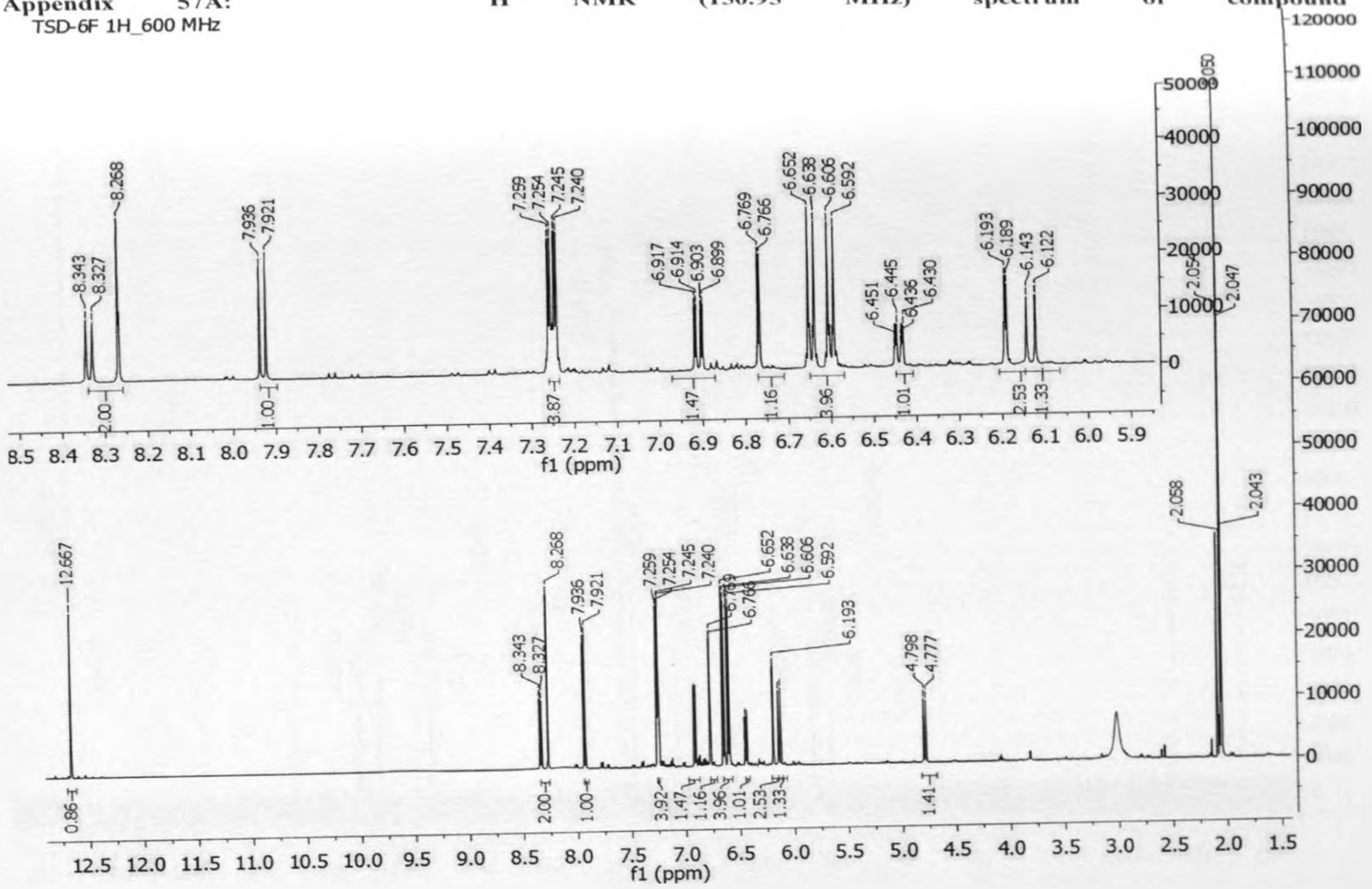


Appendix 56B: ^{13}C NMR (150.95 MHz) spectrum of compound 332

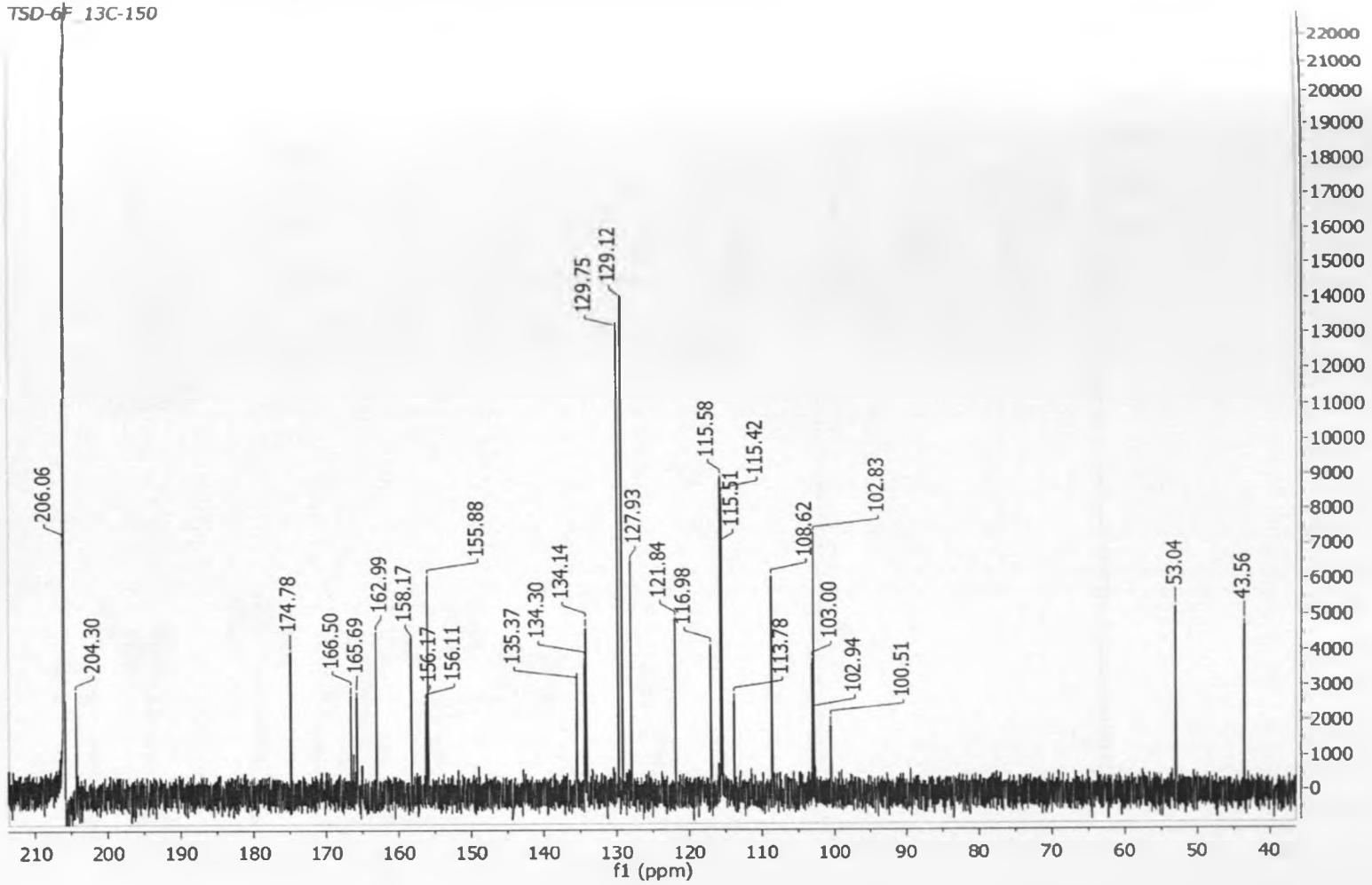


Appendix 57A: ^{1}H NMR (150.95 MHz) spectrum of compound 252

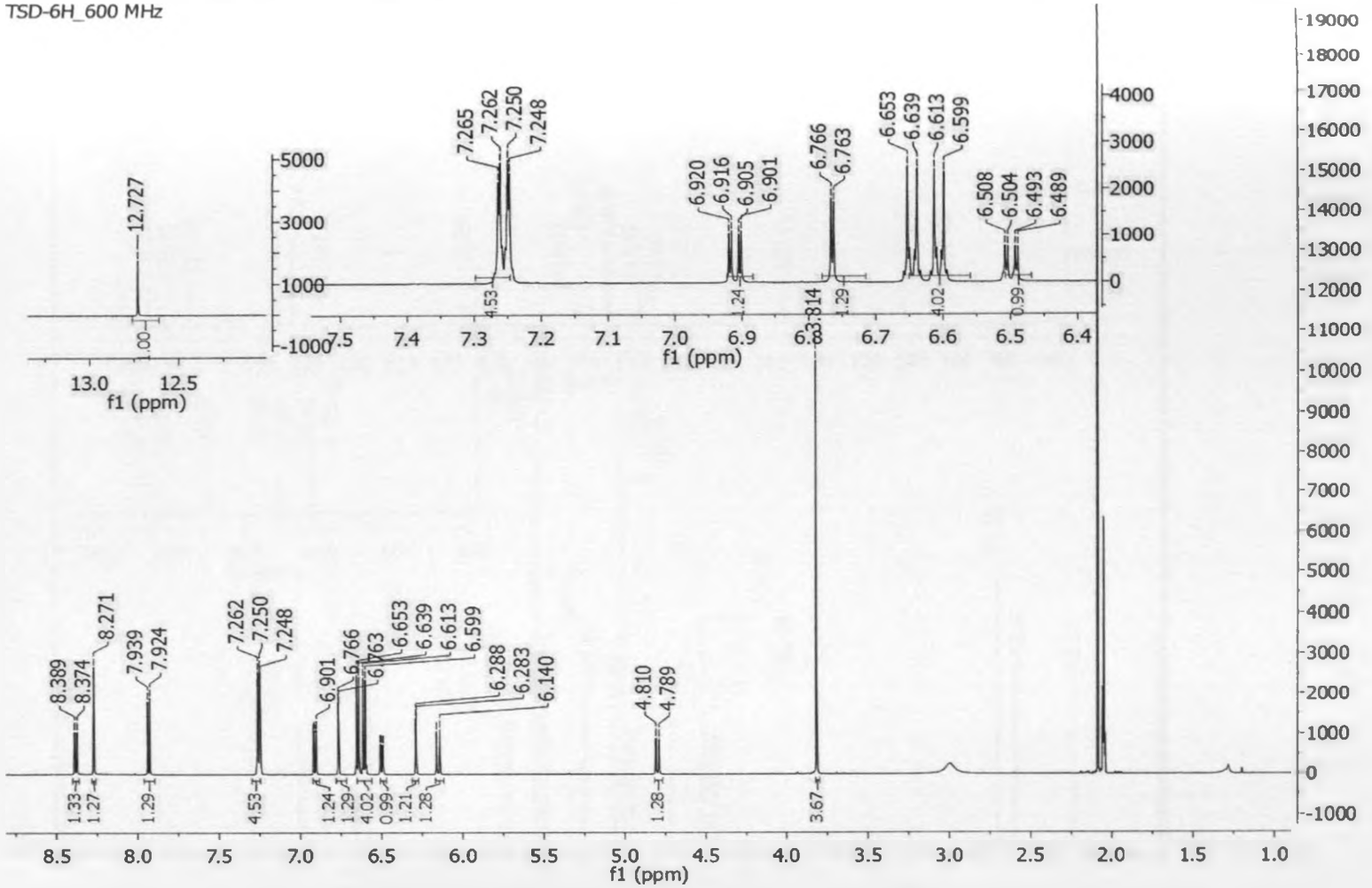
TSD-6F 1H_600 MHz



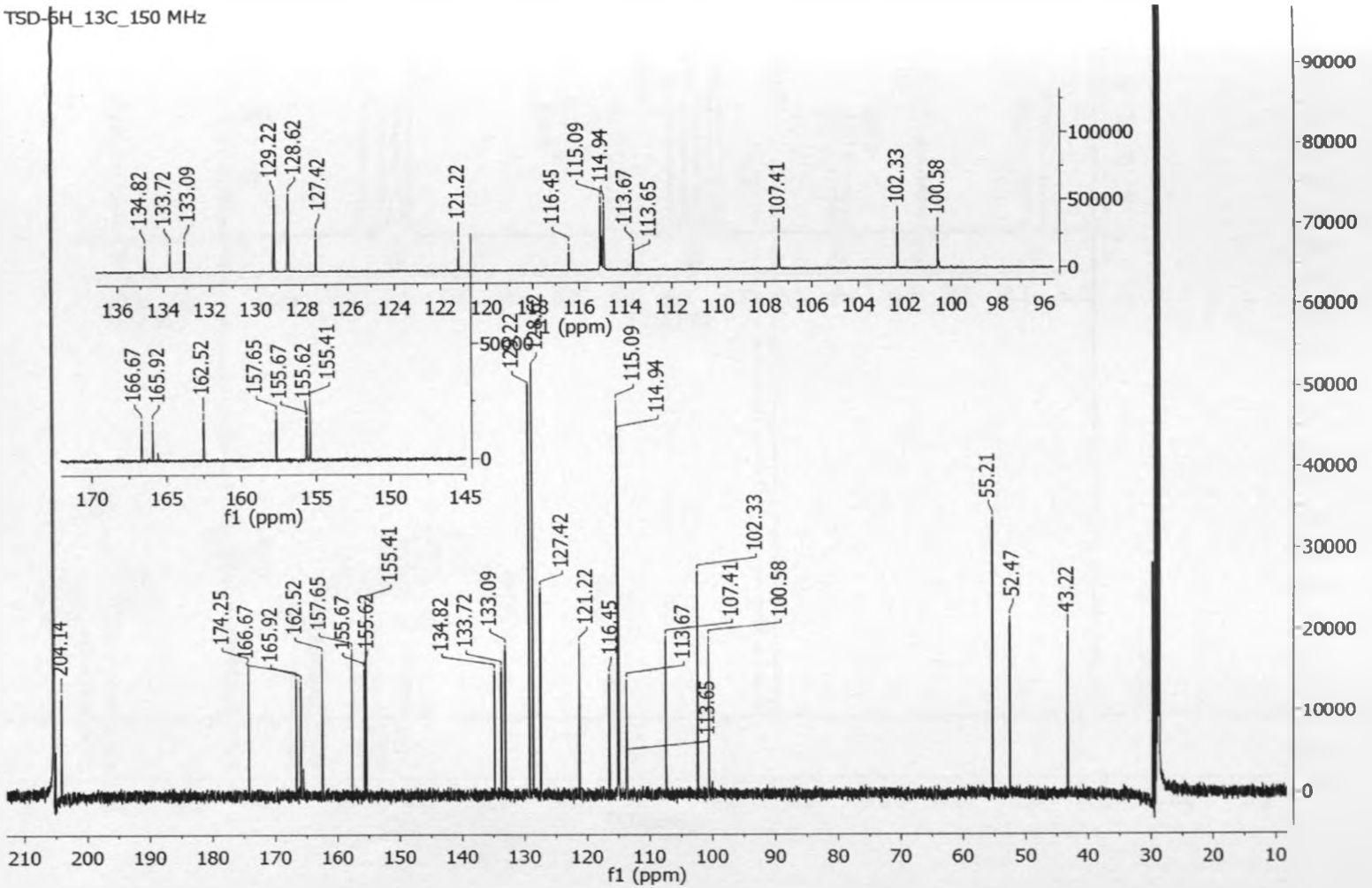
Appendix 57B: ^{13}C NMR (150.95 MHz) spectrum of compound 252



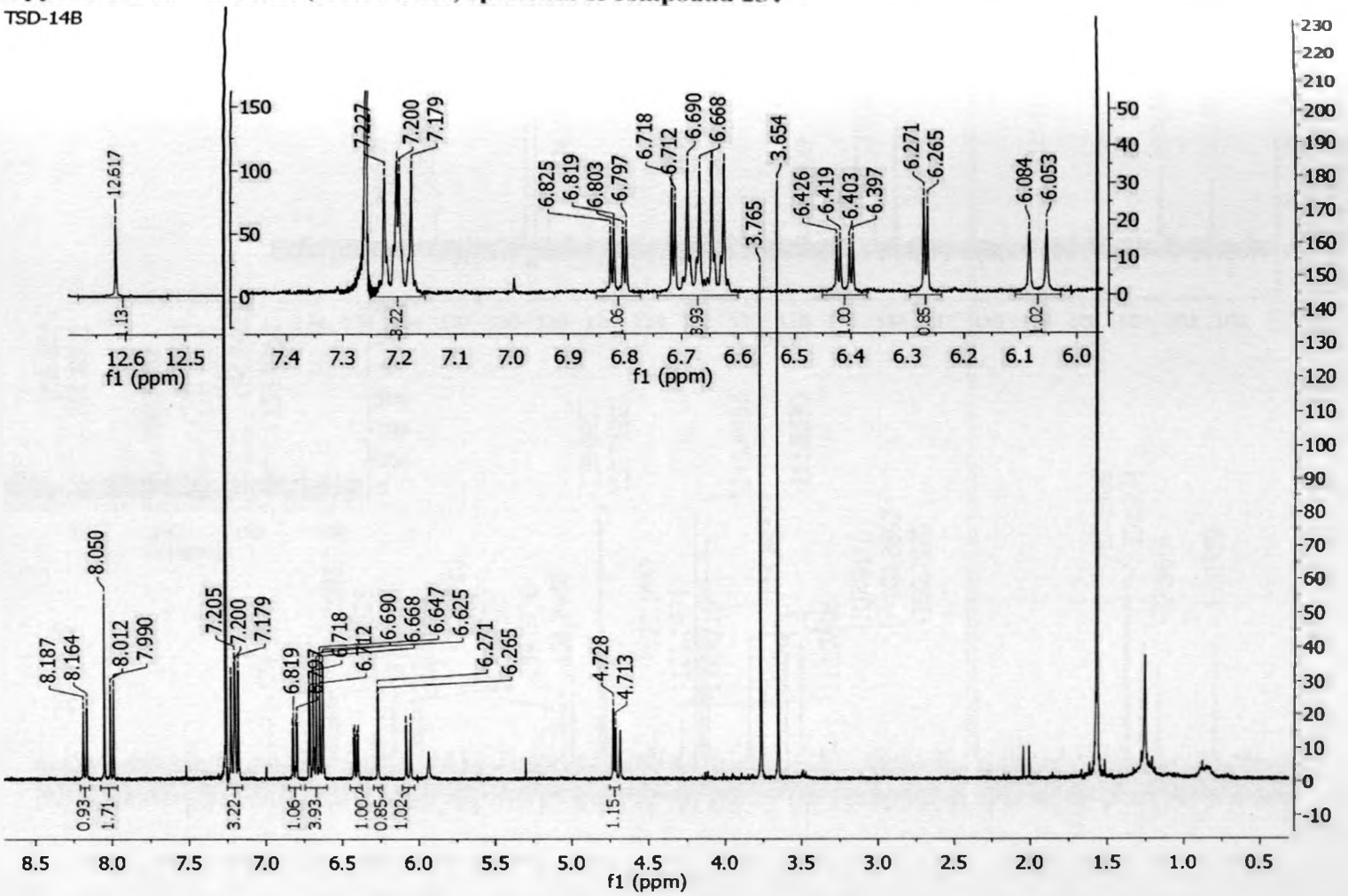
Appendix 58A: ^{1}H NMR (600.24 MHz) spectrum of compound 253



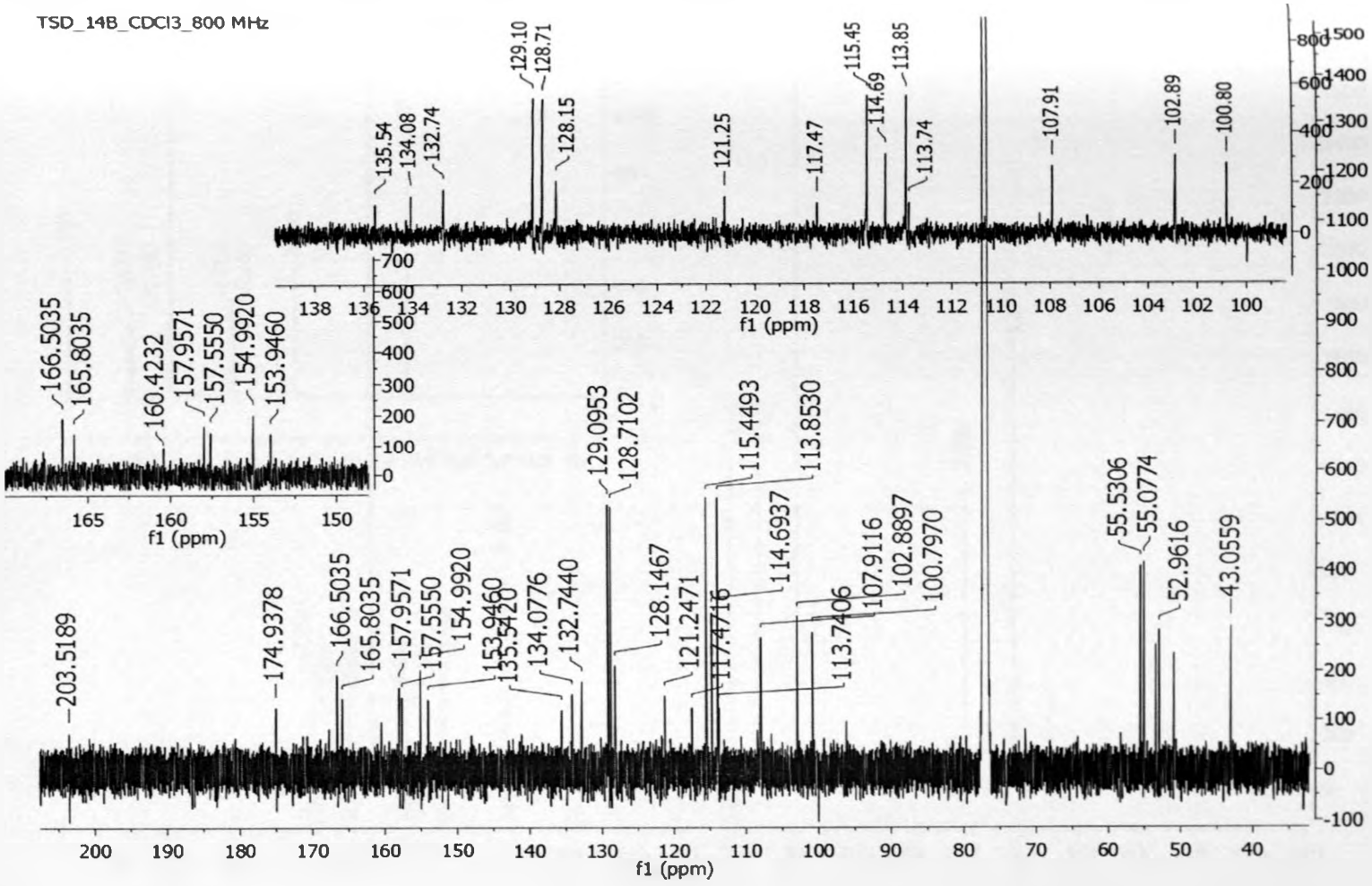
Appendix 58B: ^{1}H NMR (150.95 MHz) spectrum of compound 253



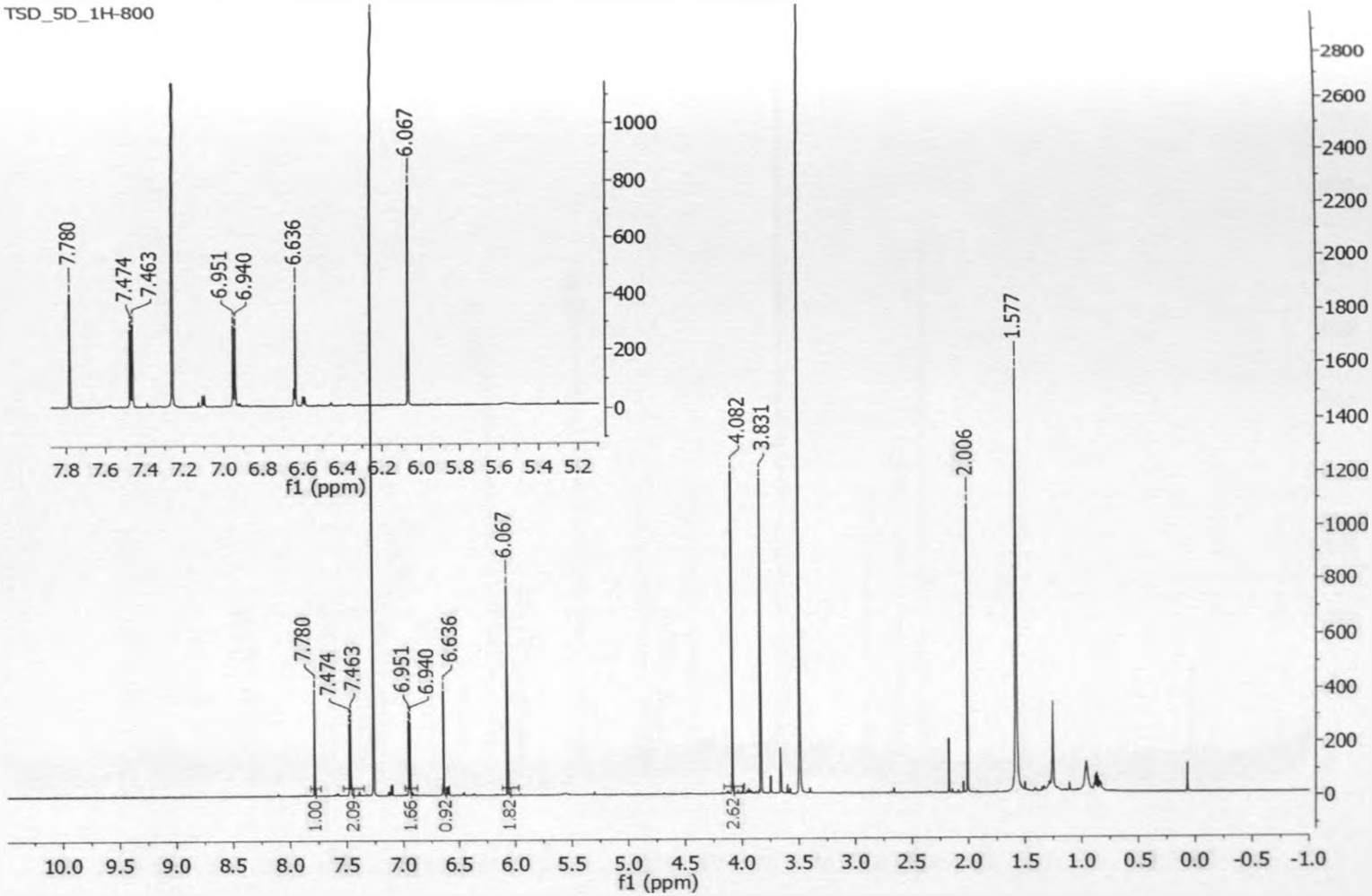
Appendix 59A: ^{1}H NMR (399.97 MHz) spectrum of compound 254



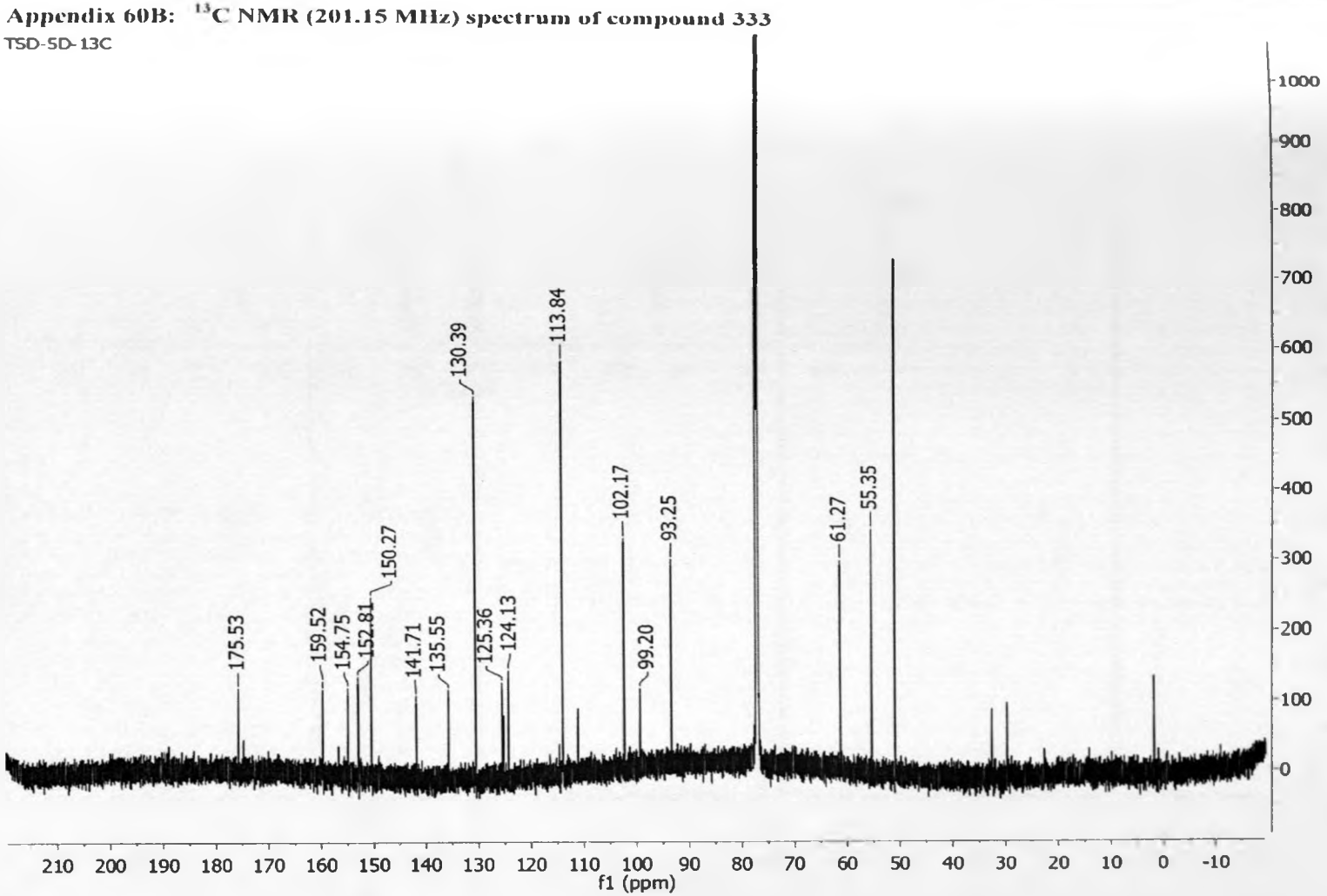
Appendix 59B: ^{13}C NMR (201.15 MHz) spectrum of compound 254



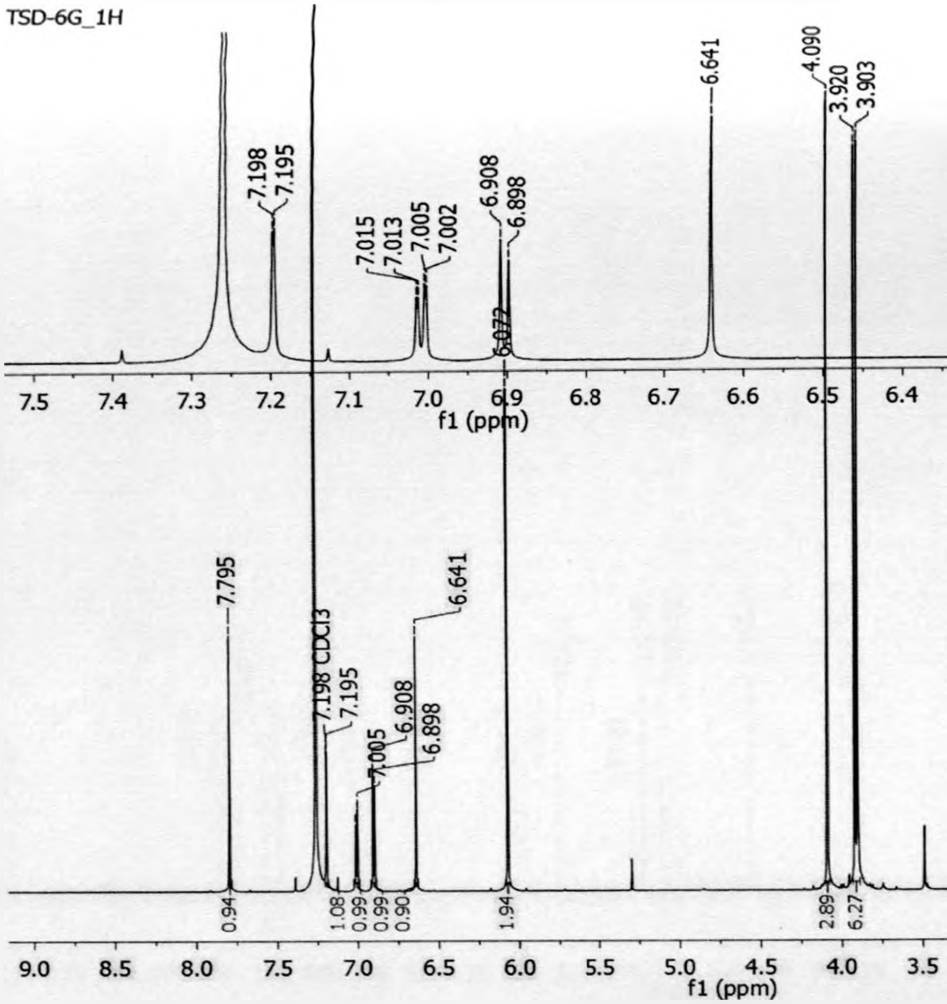
Appendix 60A: ^{1}H NMR (799.87 MHz) spectrum of compound 333



Appendix 60B: ^{13}C NMR (201.15 MHz) spectrum of compound 333



Appendix 61A: ^{1}H NMR (799.87 MHz) spectrum of compound 295

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50
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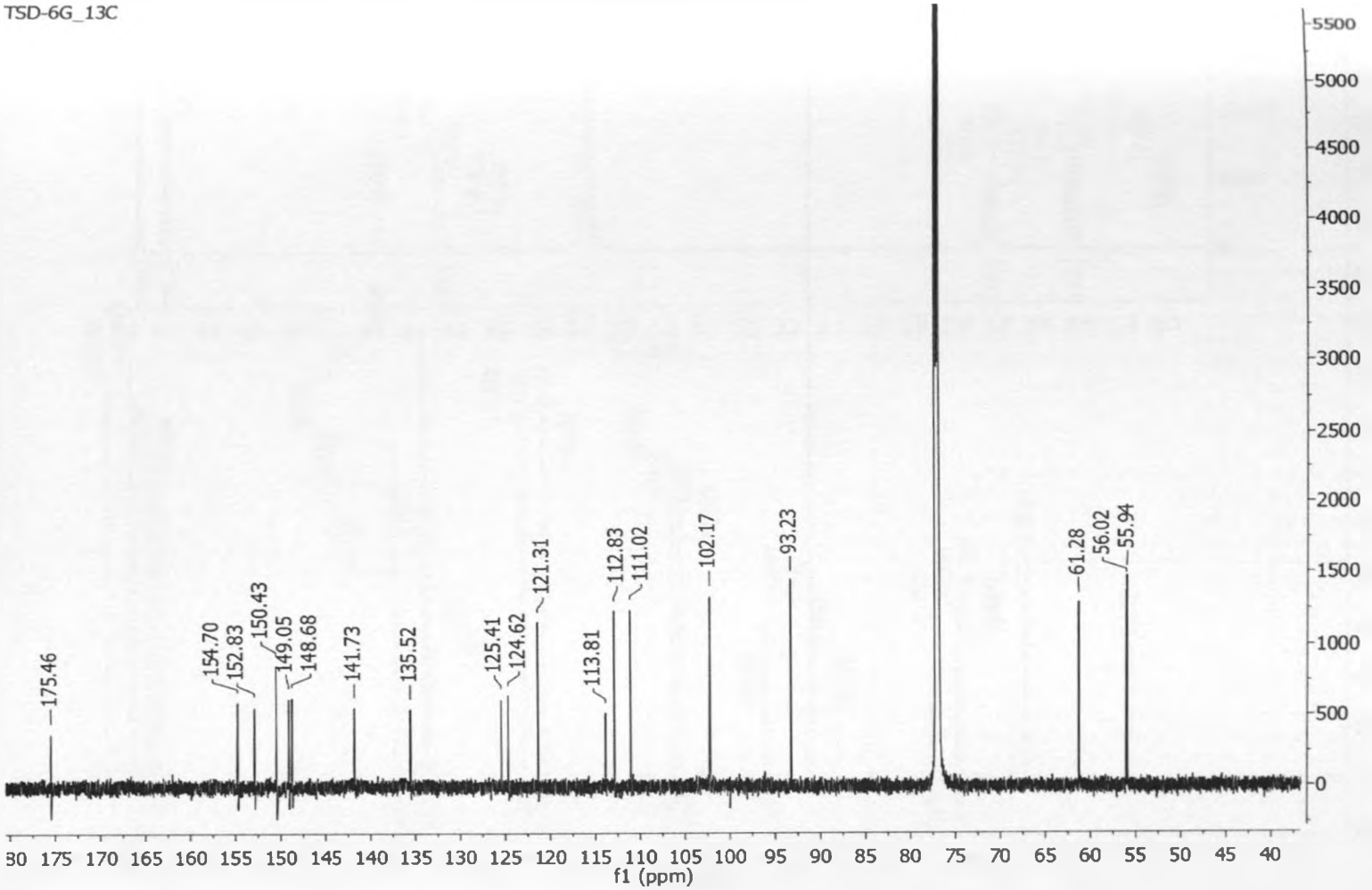
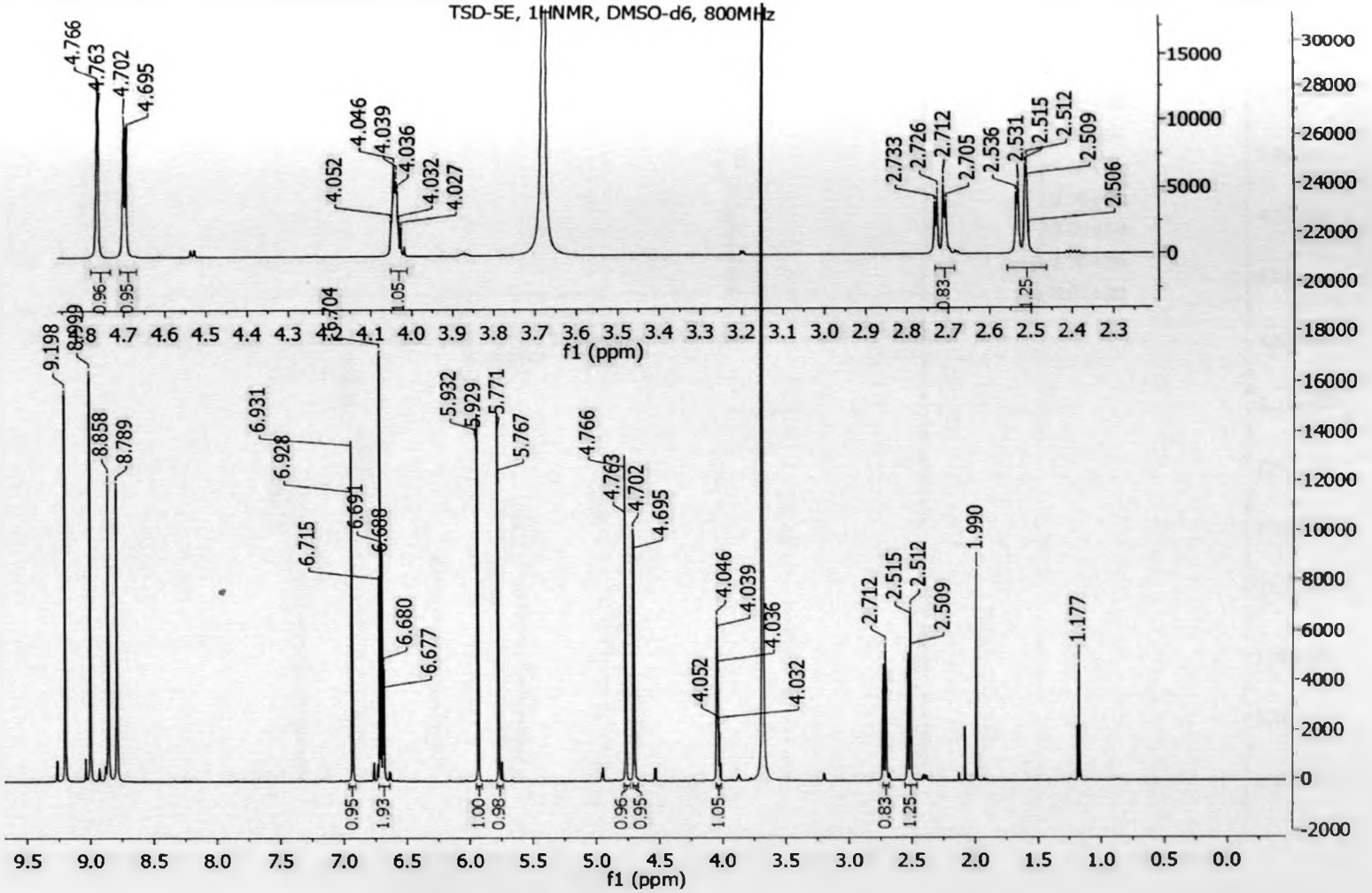
3.0
2.5
2.0
1.5
1.0
0.5

Appendix 61B: ^{13}C NMR (201.15 MHz) spectrum for compound 295

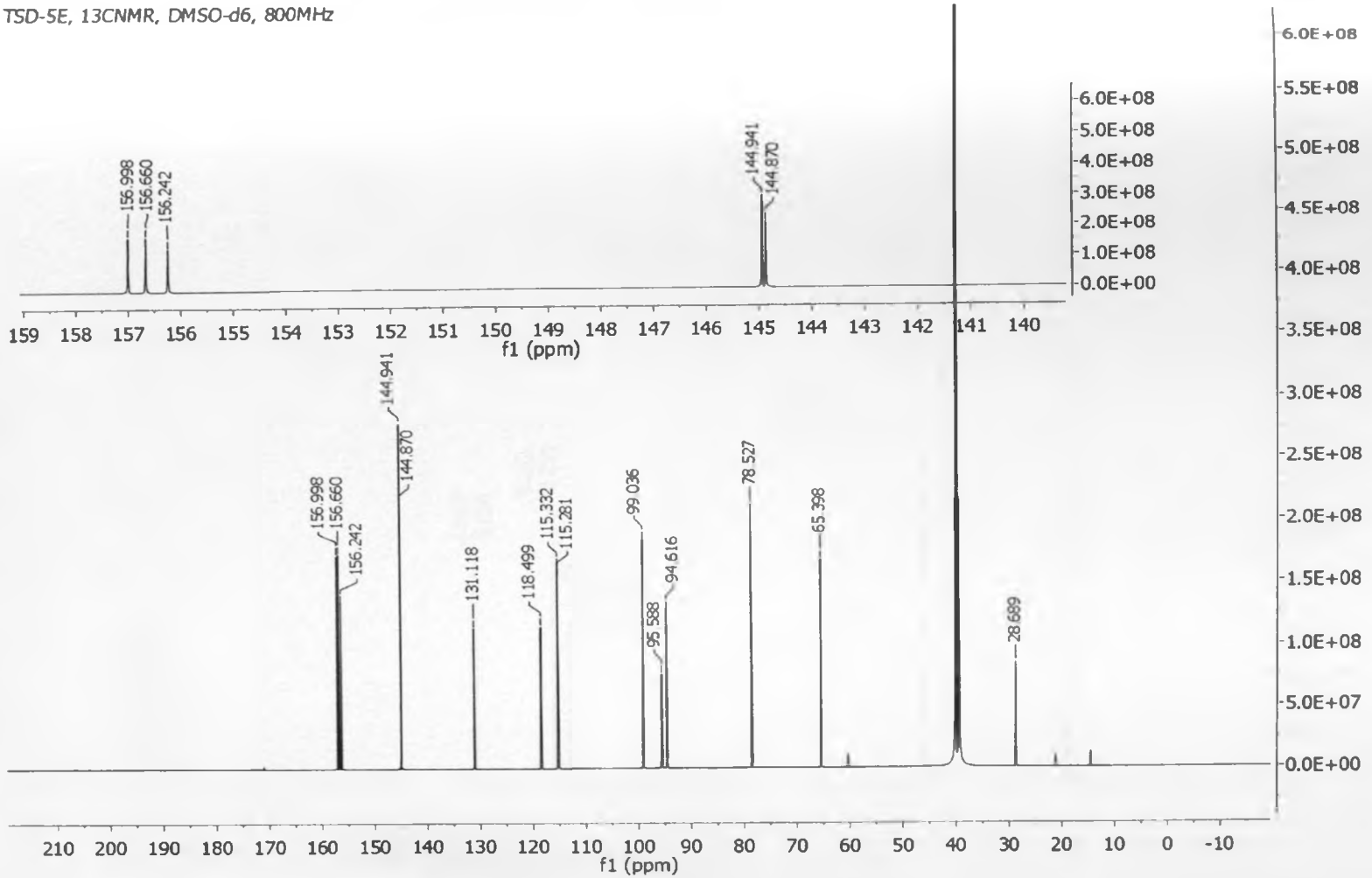


Appendix 62A: ^{1}H NMR (799.87 MHz) spectrum of compound 334

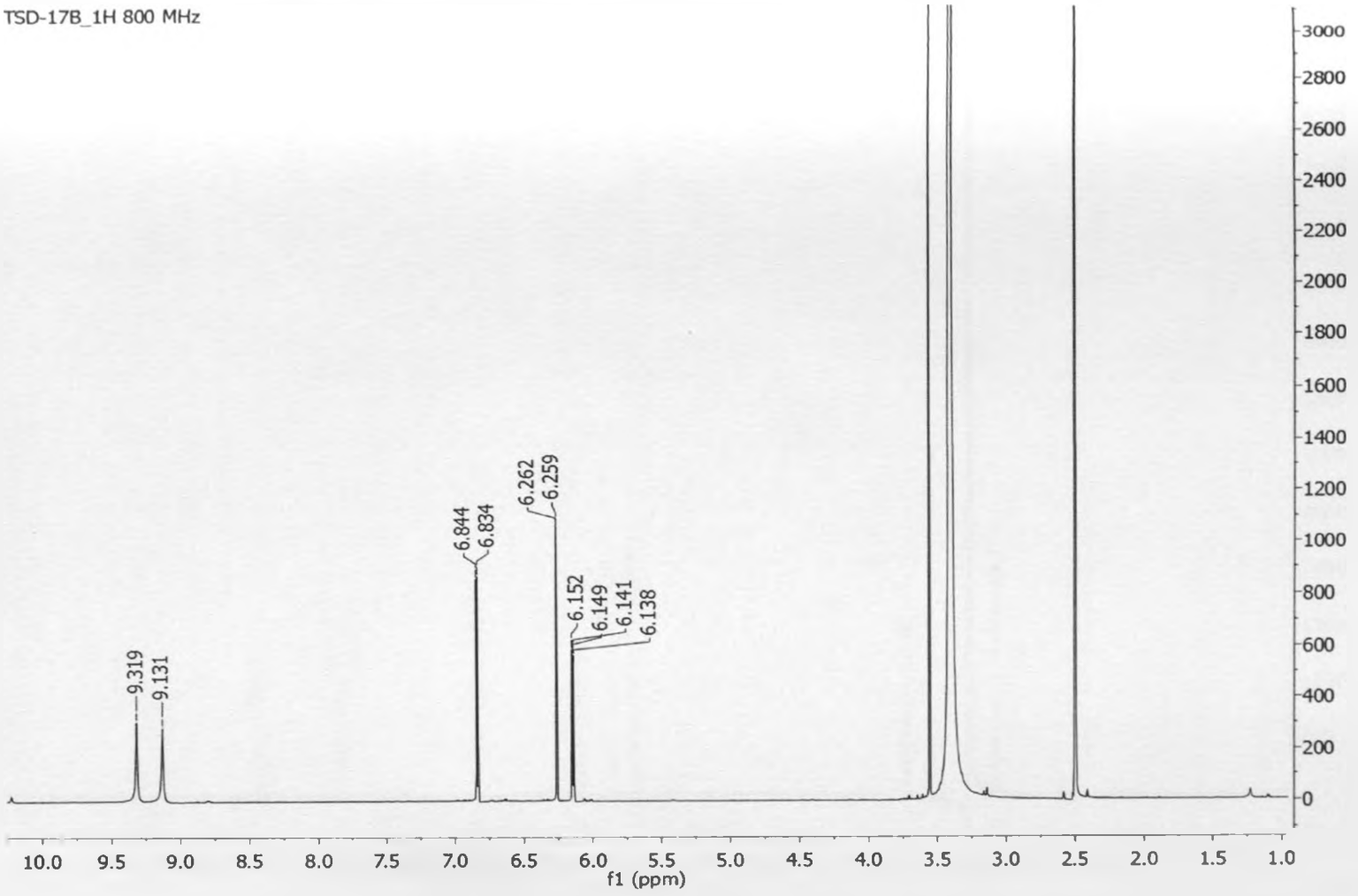


Appendix 62B: ^{13}C NMR (201.15 MHz) spectrum of compound 334

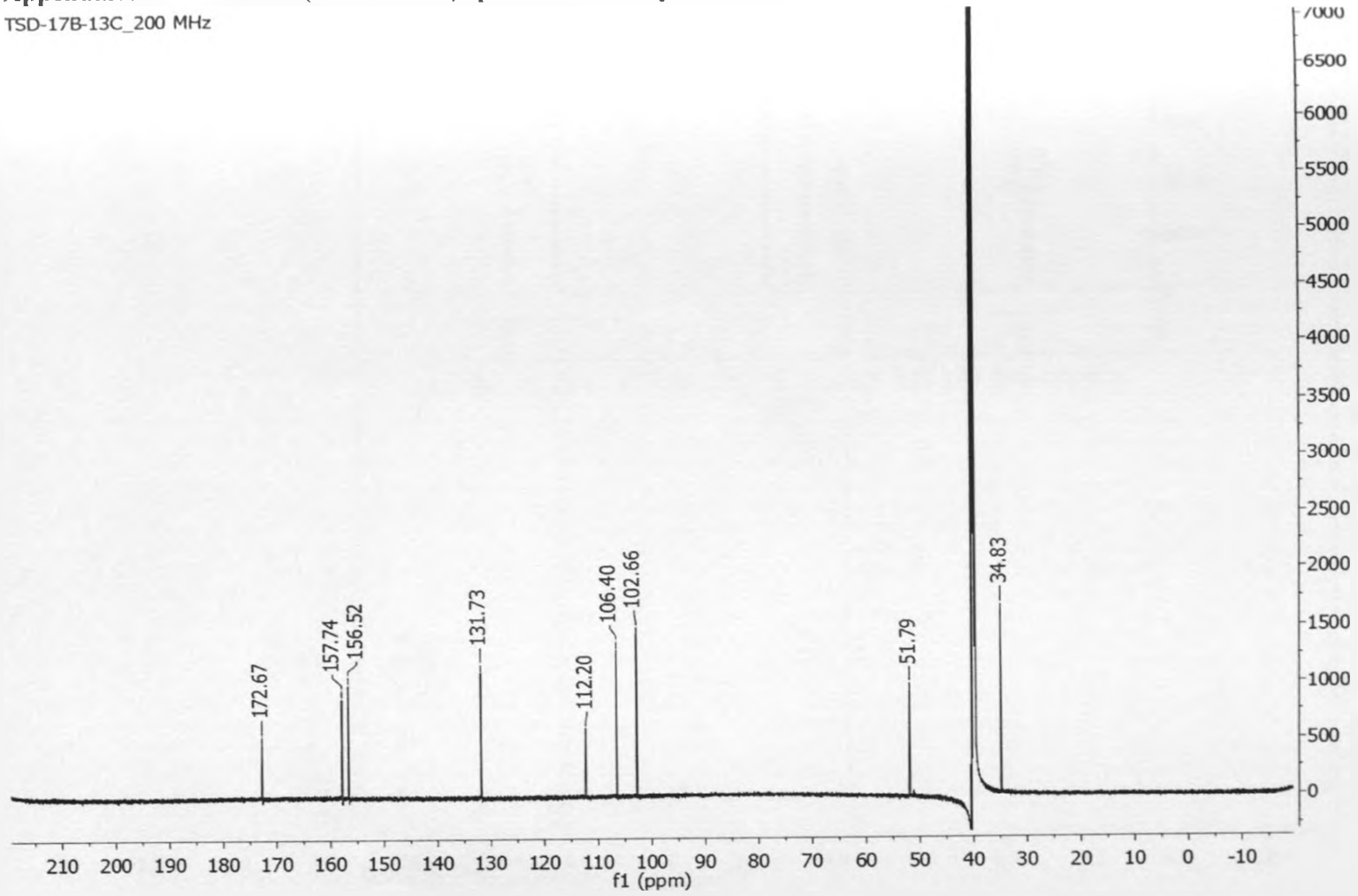
TSD-5E, 13CNMR, DMSO-d6, 800MHz



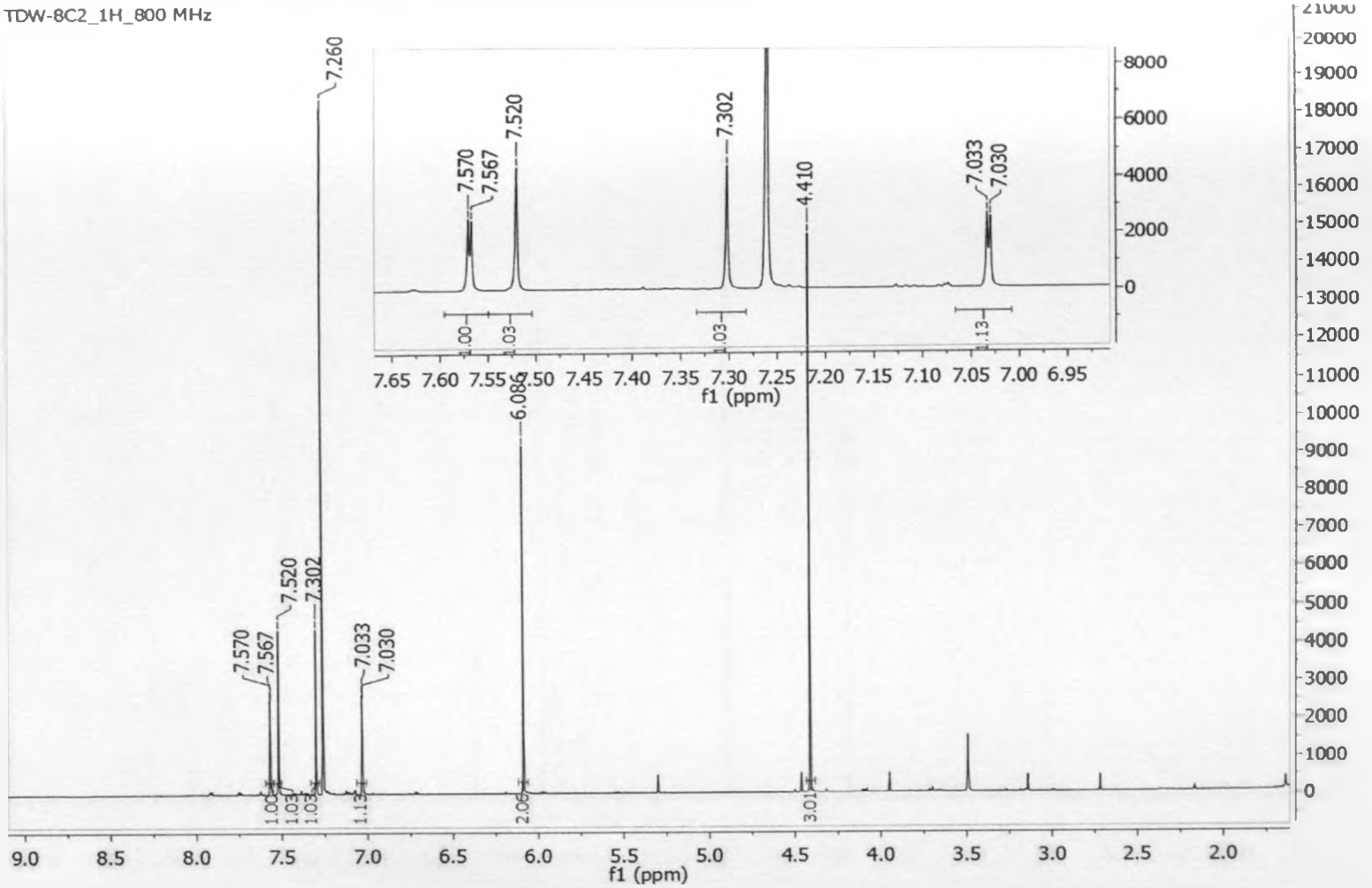
Appendix 63 A: ^{1}H NMR (799.87 MHz) spectrum of compound 335



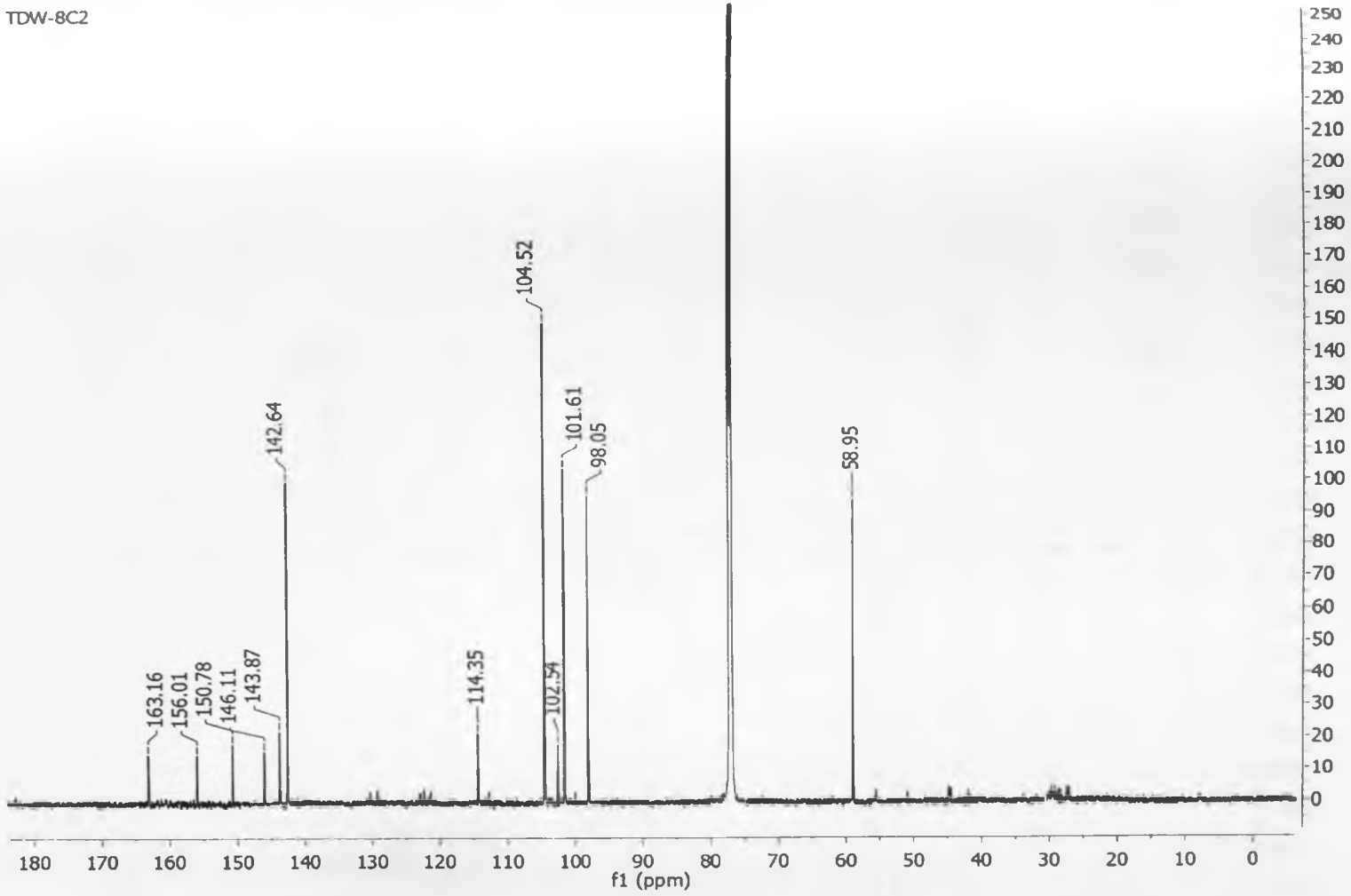
Appendix63B: ^{13}C NMR (201.15 MHz) spectrum of compound 335



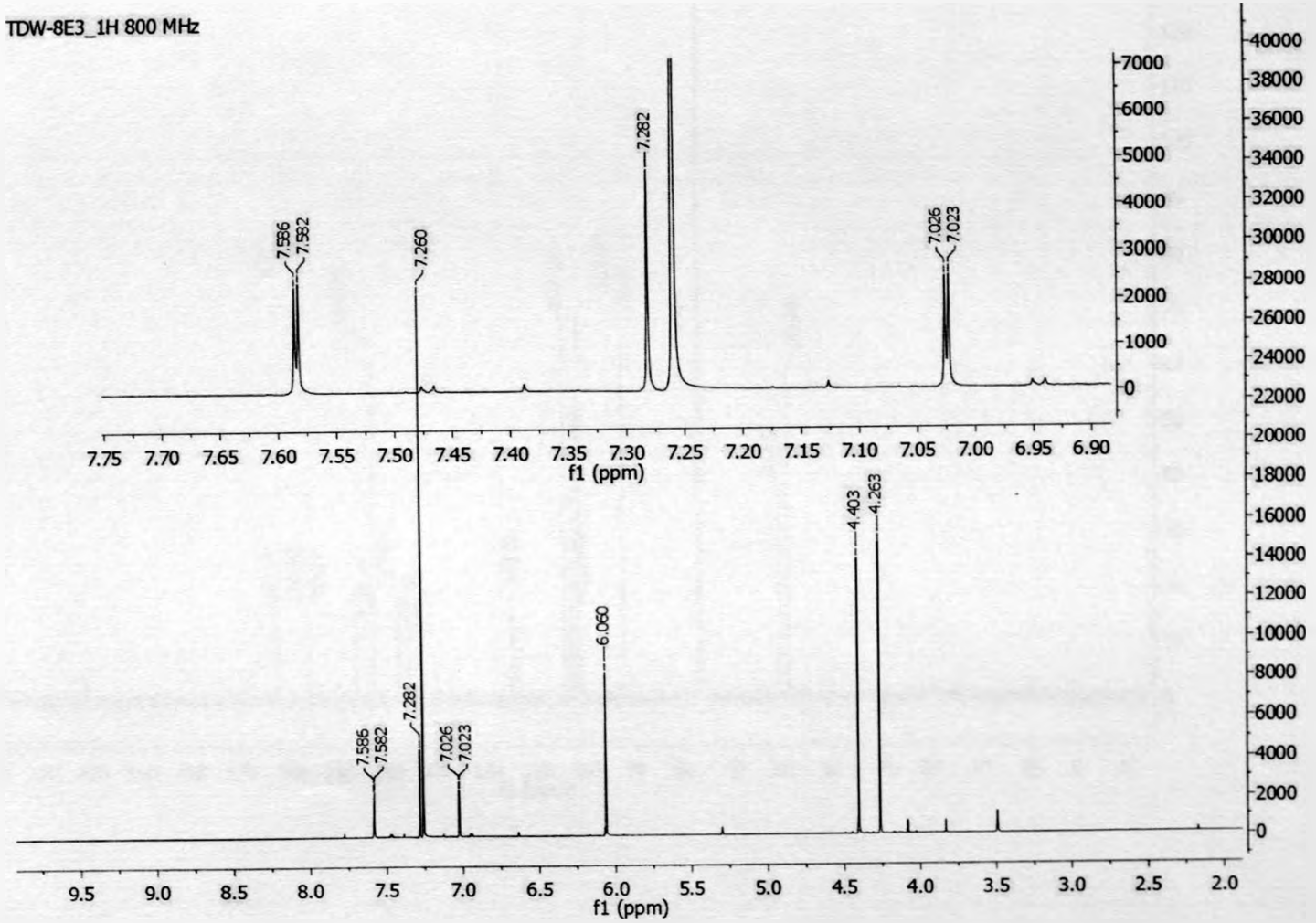
Appendix 64A: ^{1}H NMR (788.87 MHz) spectrum of compound 336



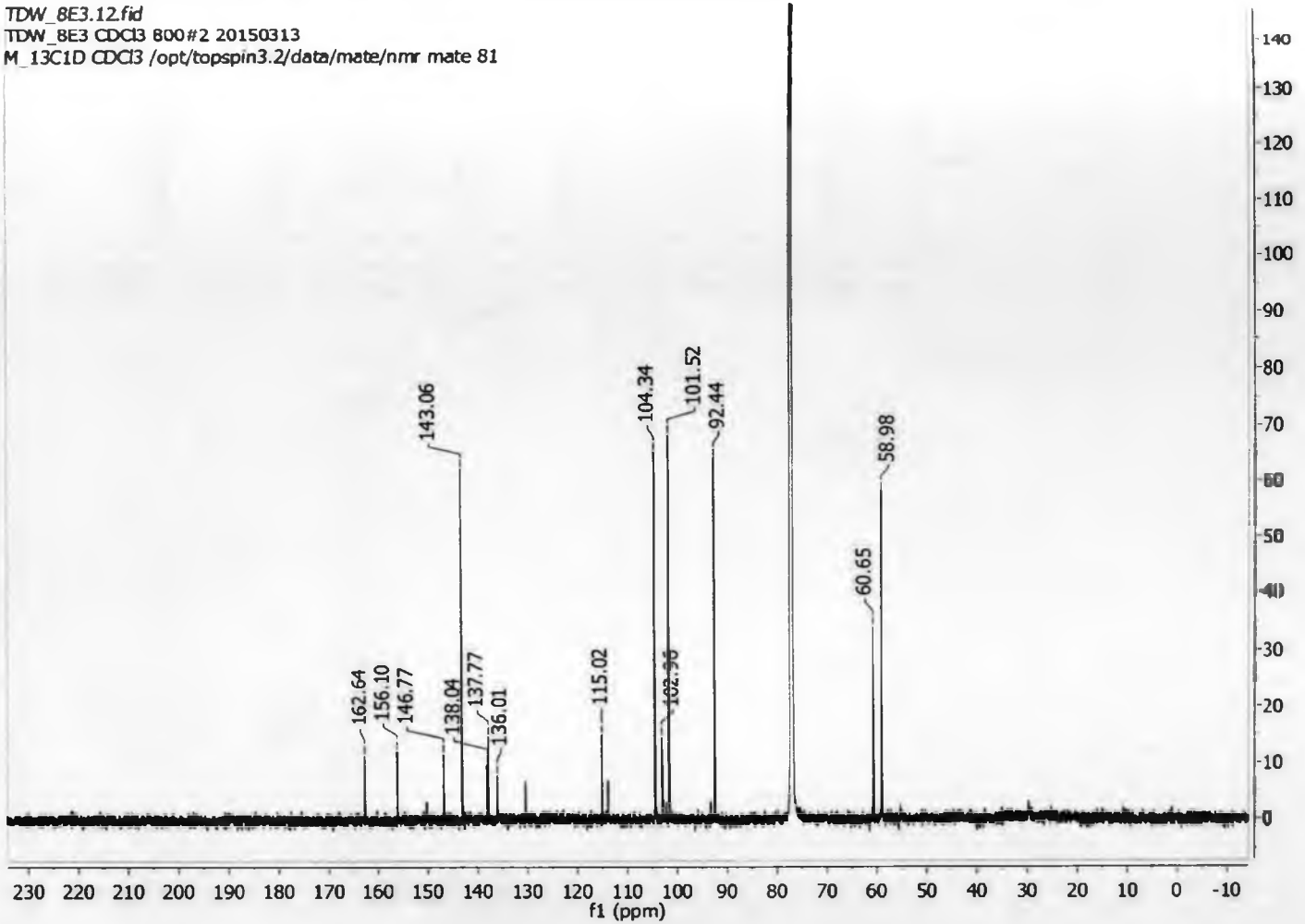
Appendix 64B: ^{13}C NMR (201.15 MHz) spectrum of compound 336



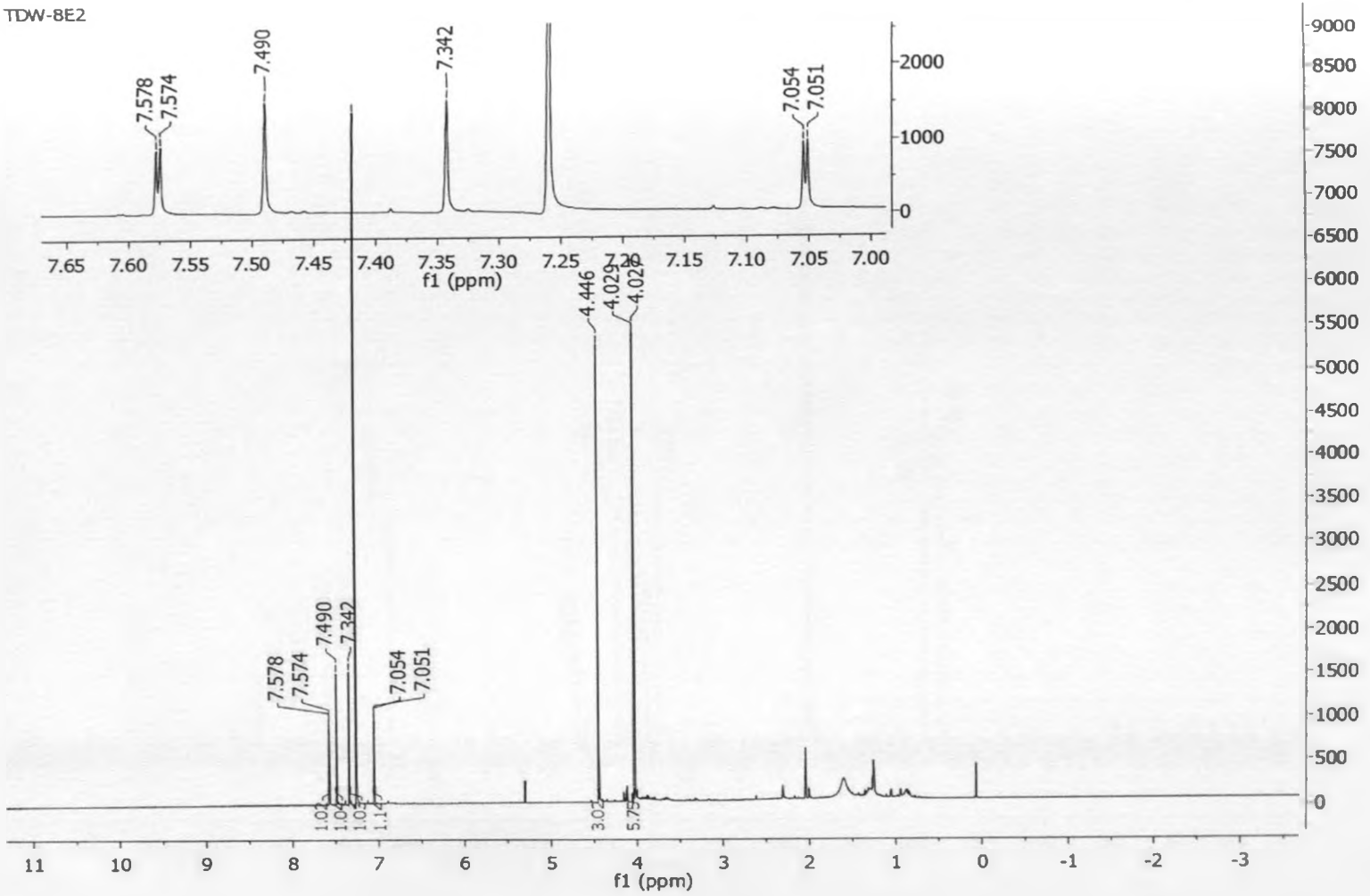
Appendix 65A: ^{1}H NMR (799.87 MHz) spectrum of compound 337



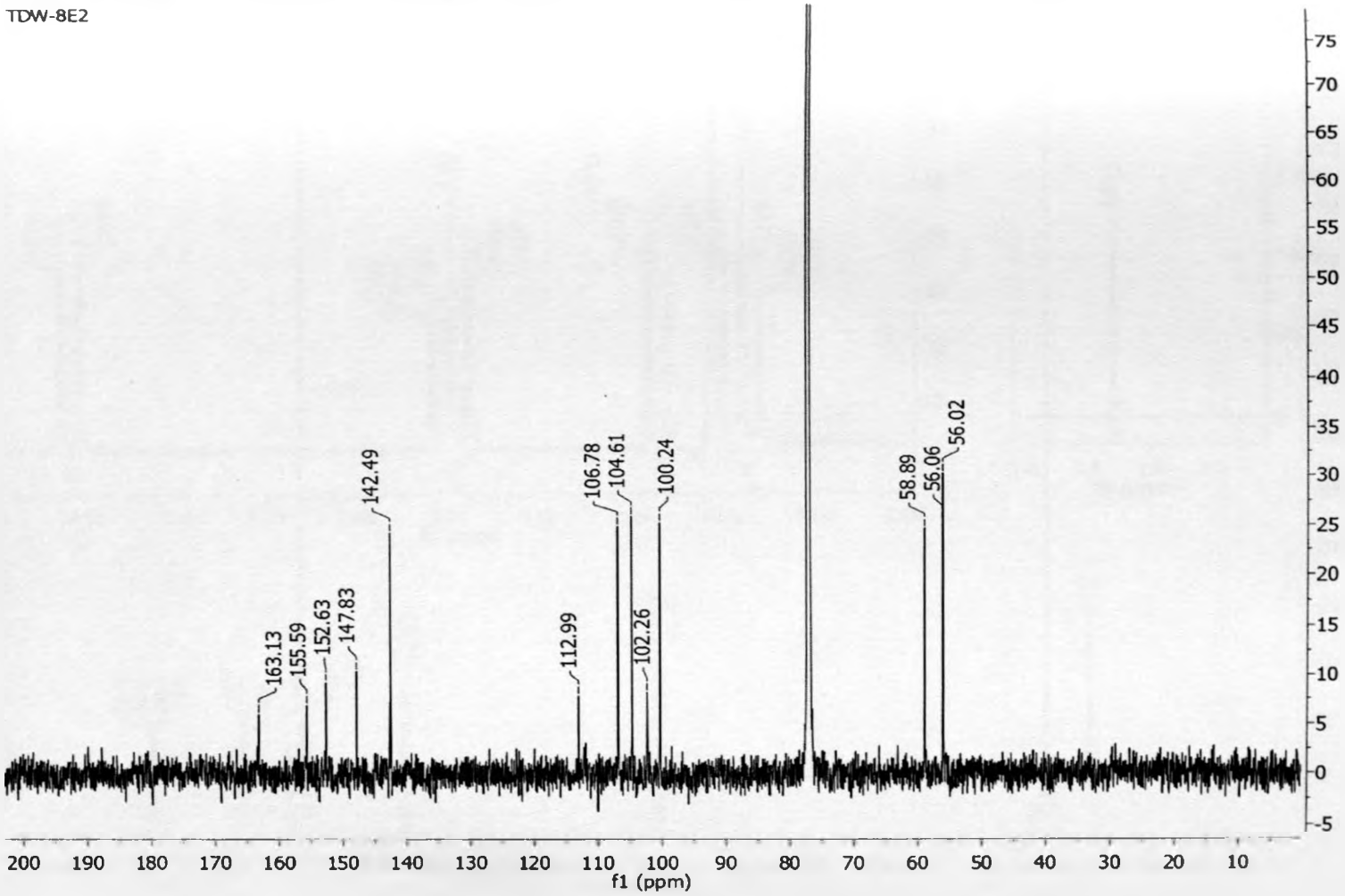
Appendix 65B: ^{1}H NMR (799.87 MHz) spectrum of compound 337



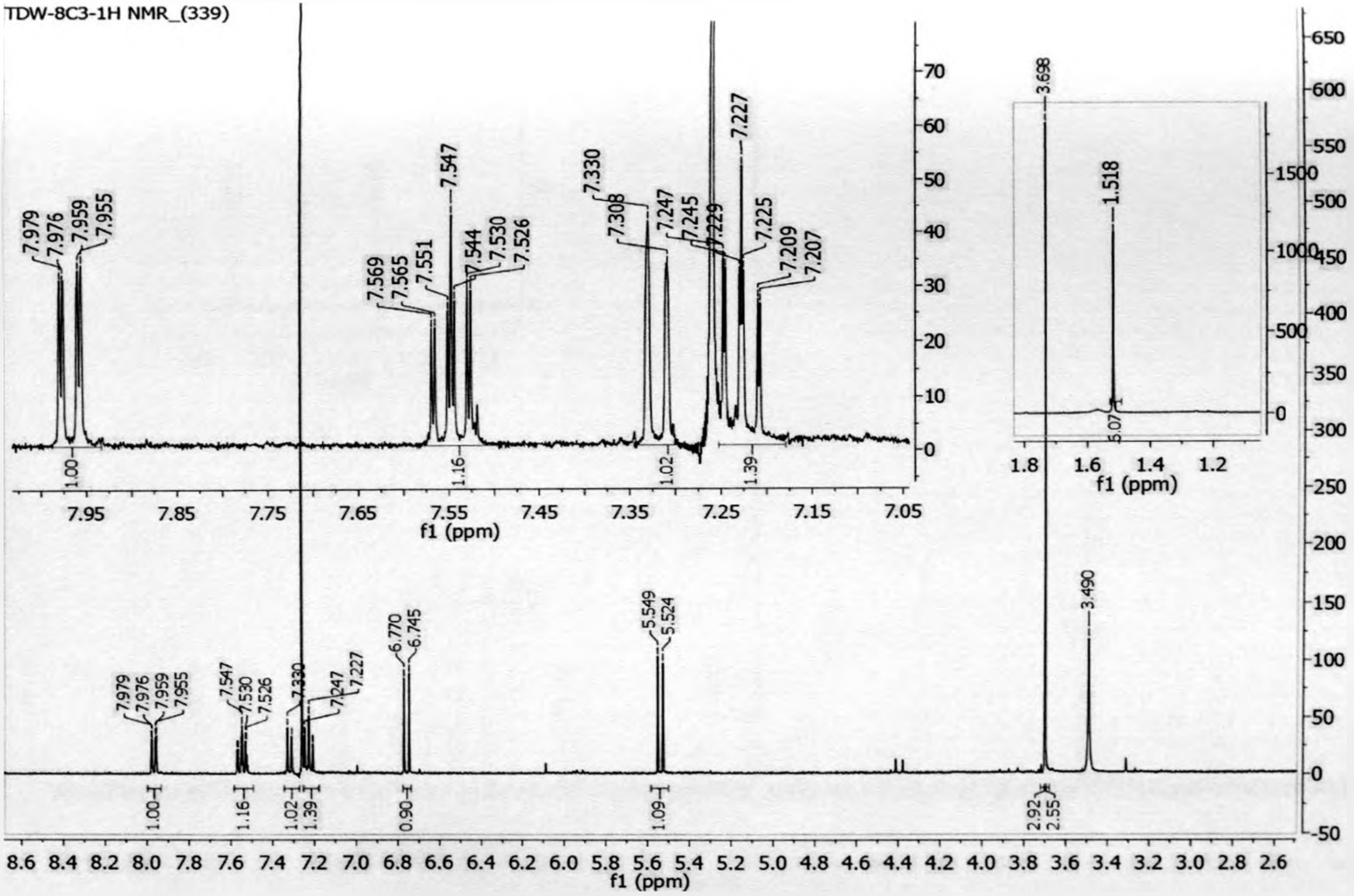
Appendix 66A: ^{1}H NMR (799.87 MHz) spectrum of compound 338



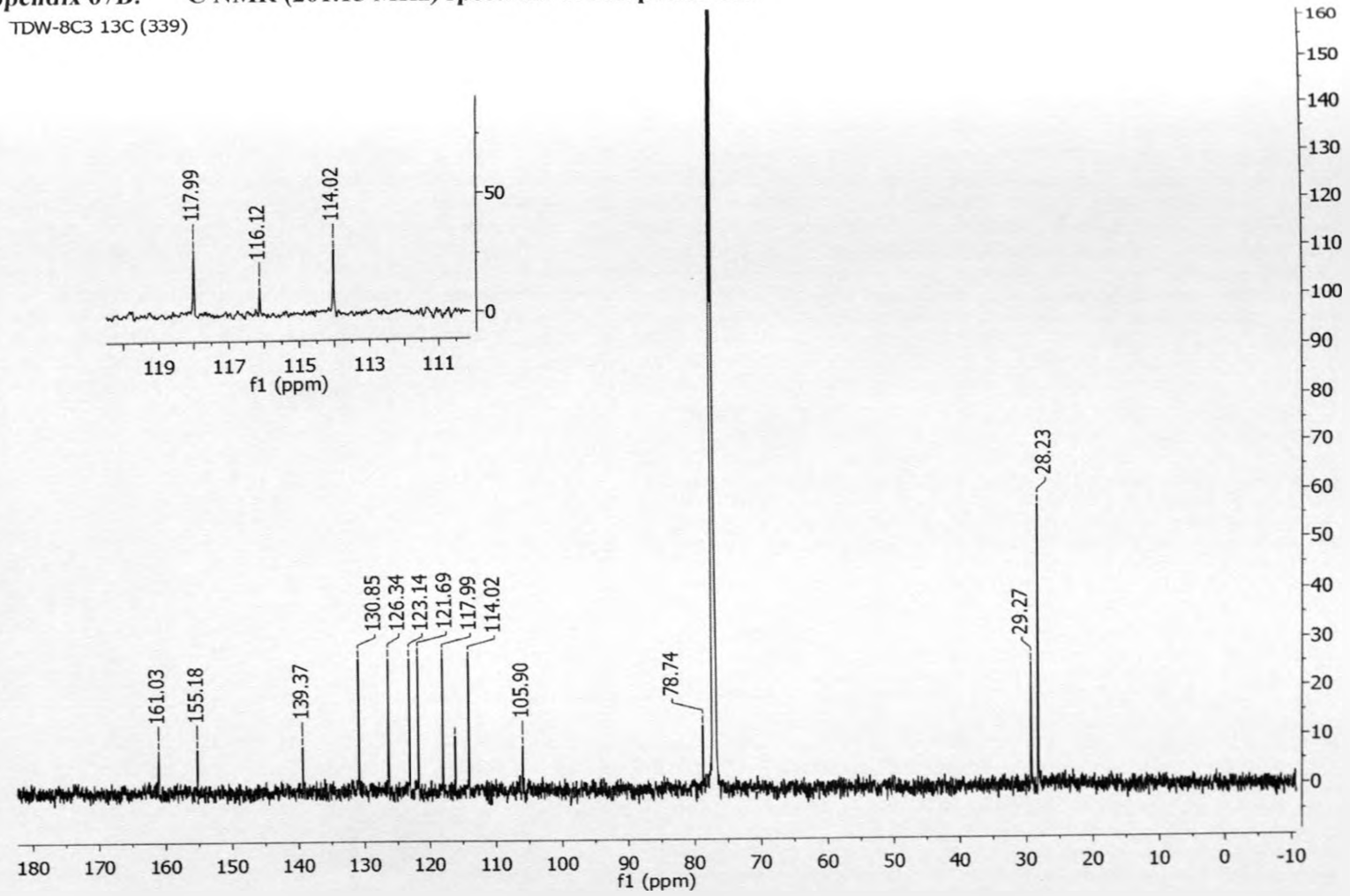
Appendix 66B: ^{13}C NMR (201.15 MHz) spectrum of compound 338



Appendix 67A: ^{1}H NMR (799.87 MHz) spectrum of compound 339



Appendix 67B: ^{13}C **NMR (201.15 MHz) spectrum of compound 339**



The X-ray data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.