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DEPARTMENT OF CHEMISTRY

PHYTOCHEMICAL INVESTIGATION OF FOUR ASPHODELACEAE PLANTS FOR ANTIPLASMODIAL PRINCIPLES

BY

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DECLARATION

This is my original work, except where reference is made, and has never been submitted anywhere for award of any degree or diploma in any University.

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DEDICATION

This PhD thesis is dedicated to my brother, Zelalem Tefera Ayana, whose advice, support and love helped me all along the way and made me who I am today. You will always be in my heart!

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ABSTRACT

In the era of resistance to the available antimalarial drugs, the discovery of antiplasmodial compounds with novel mechanism of action is needed. In this regard, plants with documented traditional uses remain an important source for such compounds. Some members of the family Asphodelaceae especially the genera: *Kniphofia, Bulbine* and *Aloe*, are used in treatment of a wide range of ailments including malaria. In this research, four plants in the Asphodelaceae family: *Kniphofia foliosa*, *Bulbine frutescens*, *Aloe dawei* and *Aloe lateritia* subspecies *graminicola* were investigated for antiplasmodial principles.

A total of fourty (40) compounds were isolated from the four plant species by using a combination of chromatographic methods (column chromatography on oxalic acid impregnated silica gel, Sephadex LH-20, preparative TLC) and crystallization. Characterization of the isolated pure compounds was done using spectroscopic techniques: Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), Ultra Violet Spectroscopy (UV), Infra Red Spectroscopy (IR) and Circular Dichroism (CD). Determination of the enantiomeric purity of the two compounds was performed on chiralcel OD-H HPLC column.

From the roots of *K. foliosa,* eleven compounds; a pre-anthraquinone, a monomeric anthraquinone, three dimeric anthraquinones, four phenylanthraquinones and two naphthalene derivatives were isolated and characterized. Of these, the anthraquinone-anthrone dimer, 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) and the phenylanthraquinone, knipholone cyclooxanthrone (**134**) are new compounds. Similarly, two new compounds: 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**) and 6',8-*O*-dimethylknipholone (**150**) along with seven known compounds were isolated from the roots of *B. frutescens*. One of the known compounds, 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**) where the octaketide chain is folded in an unusual way as in aloesaponarin II, was reported for the first time from the sub-family Asphodeloideae. Investigation of the roots of *A. dawei* has resulted in the isolation of seven naphthoquinones and ten anthraquinones. Of these, one of the naphthoquinones, 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**) is a new natural product. Furthermore, this is the second report of the occurrence of naphthoquinones in Asphodelaceae family. From the leaves of *A. lateritia* subspecies *graminicola* nine anthraquinones, three

furancarboxaldehydes, and a chromone were isolated. This is the first report of the occurence of the furancarboxaldehyde derivatives in the family Asphodelaceae.

The crude extracts and the isolated compounds were tested for *in vitro* antiplasmodial activity against the chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* and showed *in vitro* antiplasmodial activity with the highest activity being exhibited by the dimeric anthraquinones 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**) [IC_{50} 0.76 μg/mL against W2 and, 1.72 against D6], 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) [IC_{50} 1.17 μg/mL against W2 and, 4.07 against D6] and chryslandicin (**13**) [IC_{50} 1.53 μg/mL against W2 and, 2.14 against D6 strains].

The isolates from the roots of *A. dawei* were evaluated for cytotoxicity against MDA-MB-231 (ER Negative) and MCF-7 (ER Positive) breast cancer cell lines. Two of the quinones, 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) and chrysophanol-8-methyl ether (**156**), showed strong cytotoxicity (IC_{50} 1.15 and 4.85 μM, respectively) against MCF-7 breast cancer cells. The isolates from *K. foliosa*, *B. frutescens* and *A. lateritia* subspecies *graminicola* were also evaluated for cytotoxicity against human cervix carcinoma KB-3-1 cell lines, and the phenylanthraquinone, knipholone (**14**) demonstrated strong activity with an IC_{50} value of 0.43 μM. Two semi-synthetic knipholone derivatives, knipholone Mannich base (**163**) and knipholone-1,3-oxazine (**164**), were prepared and tested for antiplasmodial activity against the W2 and D6 strains of *Plasmodium falciparum* and cytotoxicity against human cervix carcinoma KB-3-1 cell; both showed improved in the antiplasmodial activity and lower cytotoxicity compared to the parent molecule, knipholone (**14**).

Overall, the four plants investigated here produced a wide variety of polyketide derivatives (mainly anthraquinones, naphthoquinones, phenylanthraquinones, xanthones and furancarboxaldehydes), some of which showed antiplasmodial and cytotoxic activities.

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Journal Publications

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Abdissa, N., Heydenreich, M., Midiwo, J.O., Ndakala, A., Majer, Z. Neumann, B., Stammler, H., Sewald, N., Yenesew, A. (2014a). A xanthone and a phenylanthraquinone from the roots of *Bulbine frutescens,* and the revision of six *seco*-anthraquinones into xanthones. *Phytochemistry Letters,* 9, 67-73.

Abdissa, **N**., Induli, M., Fitzpatrick, P., Alao, J.P., Sunnerhagen, P., Landberg, G., Yenesew, A., Erdélyi, M. (2014b). Cytotoxic quinones from the roots of *Aloe dawei*. *Molecules,* 19, 3264-3273.

Induli, M., Gebru, M., **Abdissa**, **N**., Akala, H., Wekesa, I., Byamukama, R., Heydenreich, M., Murunga, S., Dagne, E., Yenesew, A. (2013). Antiplasmodial quinones from the rhizomes of *Kniphofia foliosa*. *Natural Product Communications*, 8, 1261-1264.

Paper Presentations

Abdissa, **N**., Heydenreich, M., Midiwo, J.O., Ndakala, A., Yenesew, A. (2012). Antiplasmodial anthraquinones from Eastern African plants of Asphodelaceae family. DAAD workshop, Nairobi, Kenya.

Abdissa, **N**., Heydenreich, M., Midiwo, J.O., Ndakala, A., Sewald, N., Yenesew, A. (2013). Quinones from the roots of *Bulbine frutescens* and semi-synthesis of knipholone derivatives. University of Bielefeld, Bielefeld, Germany.

Abdissa, **N**., Induli, M., Akala, H.M., Heydenreich, M., Midiwo, J.O., Ndakala, A., Yenesew, A. (2013). Antiplasmodial quinones from the roots of *Kniphofia foliosa.* 15th NAPRECA Symposium, Khartoum, Sudan**.**

LIST OF ABBREVIATIONS AND SYMBOLS

ACT	Artemisinin-based Combination Therapy
CD	Circular Dichroism
COSY	Correlated Spectroscopy
CQ	Chloroquine
d	Doublet
dd	Doublet of a doublet
DEPT	Distortionless Enhancement by Polarization Transfer
ESIMS	Electron Spray Ionization Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	Concentration of 50% Inhibition
J	Coupling constant
MHz	Mega Hertz
MS	Mass Spectrometry
nm	nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
PTLC	Preparative Thin Layer Chromatography
s	singlet
t	triplet
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization
λ_{max}	Maximum wavelength of absorption
1D NMR	One Dimensional Nuclear Magnetic Resonance
2D NMR	Two Dimensional Nuclear Magnetic Resonance

CHAPTER ONE

INTRODUCTION

1.1 General

The history of medicine is an account of mankind's effort to deal with human illnesses. In prehistoric times, people obtained medicine for their ailments from their environments, particularly from plants (Schulz *et al*., 2001; Robert and Halberstein, 2005). There is comprhensive evidence that medicinal plants represent the oldest and most widespread form of medication (Kaur *et al*., 2009; Robert and Halberstein, 2005). It is not surprising that plants remain an indispensable sources of novel and effective pharmaceuticals. The advent of phytochemistry and pharmaceutical chemistry has enhanced the ability to utilize active compounds isolated from the plants, or their synthetic equivalents in medicine. This is because the degree of chemical diversity and novelty that medicinal plants contain is broader than that from any other source (Cragg *et al*., 1997; Harvey, 2001; Chin *et al*., 2006).

Africa has an immensely rich biodiversity and knowledge in the use of plants to treat various ailments. In fact, WHO estimates that 80% of the population in sub-Saharan Africa depend solely on traditional medicine from plants for their primary healthcare needs because of their ready availability, cheapness and socio-cultural background (Jayaweera, 1982). However, these resources have hardly been investigated scientifically. Efforts have been made to document African medicinal plants including their mode of traditional uses as exemplified by Kokwaro (2009) and Sofowora (1996), who have documented some traditional medicinal plants used in East and West Africa, respectively. In Kenya, the practice of traditional medicine has gained official recognition since WHO's Alma Ata declaration of 1978 (WHO and UNICEF, 1978).

Since then, traditional medicine has gradually curved itself a niche in the provision of healthcare services. It is estimated that up to 75% of the people in Kenya have used traditional medicines at one time or another (Maneno and Mwaniza, 1991). Due to the ability of plants to bio-synthesize a plethora of compounds, whose structural diversity is often beyond the dreams of even the most imaginative chemists, plants remain important in the treatment of various diseases, including malaria.

Malaria is one of the most prevalent and potentially fatal parasitic infections in tropical and subtropical regions of the world. Despite malaria being treatable and preventable, it is estimated that 207 million cases and 627, 000 deaths were reported in 2012 alone. Most cases (80%) and deaths (90%) occurred in Africa with 77% of deaths being on children under five years of age (WHO, 2013). In Kenya, it is an endemic disease affecting more than four million people with the highest incidence being recorded in Rift valley, Centeral, Western and Eastern provinces (Pascaline *et al*., 2011).

One of the major factors for the increase in the global burden of malaria is the emergence of drug resistant strains of the malaria parasite, *Plasmodium falciparum*, to many of the currently available antimalarial drugs (Hyde, 2007; Ginsburg and Deharo, 2011). Although the resistance varies with geographical distribution, *P. falciparium* has shown resistance to almost all of the currently used antimalarial drugs including the Artemisinin-based Combination Therapy (ACT) (Sibley *et al.*, 2001). In addition, this mosquito-borne disease has a serious global economic impact that affects the productivity of individuals, families and the society at large, since it causes energy loss, debilitation and loss of work capacity; consequently the economic damage due to malaria is more than any other human parasitic disease, predominantly in poor countries

within tropical and subtropical climates (Sachs and Malaney, 2002). Consequently, there is an urgent need for new, affordable and accessible antimalarial agents from nature with novel mechanism of action.

Natural products have served as lead structures for the development of antimalarial drugs. This is in fact a pointer that new antimalarial leads may emerge from plants , especially from those with recognized traditional uses. In this regard, some members of the family Asphodelaceae including the genera *Kniphofia*, *Bulbin*e and *Aloe* are widely used in traditional medicine in East Africa with some showing *in vitro* antiplasmodial activity. In a preliminary testing, the extract of the roots of *Kniphofia foliosa*, *Bulbine frutescens*, *Aloe dawei* and leaves of *Aloe lateritia* subspecies *graminicola* showed good antiplasmodial activities with IC_{50} values between 10 and 30 µg/mL. In this research, therefore, the phytochemistry of these plants was investigated and the isolated compounds tested for antiplasmodial and cytotoxic activities.

1.2 Statement of the Problem

The World Health Organization (WHO) estimates that there are 200–500 million malaria cases annually, directly causing more than half a million deaths (WHO, 2013). Today, Africa alone accounts for 90% of malaria mortality because the malaria vector mosquito, *Anopheles gambiae*, is widespread in this part of the world.

Beyond the human toll, malaria has significant economic impact in endemic countries-costing Africa $12 billion in lost GDP every year and consuming 40% of all public health spending (Sachs and Malaney, 2002). The emergence and potential spread of strains of malaria parasites which are resistant to currently available antimalarial drugs, including artemisinin-based drugs,

has prompted the search for new drugs through the use of high-throughput screening and combinatorial chemistry, genomics, and vaccine development. However, this effort has yet to deliver a single drug despite the enormous resources expended during the past fifteen years. Currently, there are no promising candidates in the pipeline, signifying the importance of diversifying the search for new lead structures in antimalarial drug development.

1.3 Objectives

1.3.1 General Objective

The overall objective of this study was to identify antiplasmodial compounds from *Kniphofia foliosa*, *Bulbine frutescens* and *Aloe dawei* and *Aloe lateritia* subspecies *graminicola* species.

1.3.2 Specific Objectives

The specific objectives of this study were:

1) To establish the antiplasmodial activity of the crude extracts of *Kniphofia foliosa*, *Bulbine frutescens*, *Aloe dawei* and *Aloe lateritia* subspecies *graminicola*;
2) To isolate compounds from the four plant species;
3) To elucidate the structures of the isolated compounds;
4) To establish antiplasmodial and cytotoxic activities of the isolated compounds;
5) To modify the active compounds so as to improve their activity.

1.4 Justification

The use of indigenous medicinal plants for the treatment of parasitic diseases is well known since ancient times. Based on the historical success of the existing first line antimalarial drugs, quinine and artemisinin (Balint, 2001) from herbal medicine, there is still potential of isolating

lead antimalarial compounds from plants which have documented traditional uses to treat malaria.

In East Africa, some members of the Asphodelaceae family are widely used in traditional medicine for the treatment of malaria. In this regard, a decoction of roots of *A. lateritia* subspecies *graminicola* has been widely used in Kenya for the induction of vomiting to cure malaria (Kokwaro, 1976), while in Rwanda the root extracts of *A. dawei* has commonly been drunk to treat malaria (Schemelzer and Gurib-Fakim, 2008).

The family is also characterized by the presence of aromatic polyketides mainly dimeric anthraquinones and phenylanthraquinones, some of which exhibited remarkable activities against *P. falciparum*. The dimeric anthraquinones from the root of *K. foliosa* showed high inhibition of the growth of the malaria parasite (Wube *et al*., 2005). The phenylanthraquinones, knipholone and knipholone-type dimeric phenylanthraquinones from *K. foliosa* and *B. frutescens*, possess antiplasmodial activities slightly lower than that of choroquine itself (Bringmann *et al*., 1999a; 2008a). However, information on the chemistry of these plants and related compounds responsible for antiplasmodial activity is limited to only few species. The present study was therefore focused on phytochemical studies and antiplasmodial activities of the compounds from *K. foliosa*, *B. frutescens, A. dawei* and *A. lateritia* subspecies *graminicola*.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Malaria Problem

Malaria is the most devastating parasitic diseases among the tropical diseases. It is endemic in many tropical and sub-tropical areas of the world, with the major effect being felt in sub-Saharan Africa, where about 90% of cases and deaths occur (WHO, 2013; Casteel, 2003). The burden is inclined towards children (Snow *et al.,* 2005) and pregnant women in whom the malaria parasites easily sequesters in the placenta (Rowe and Kyes, 2004; WHO, 2013).

Since malaria affects human health, it also directily or indirectly affects economic growth and development. It reduces national income by inhibiting investment, savings and tourism (Sachs and Malaney, 2002). It not only increases medical costs on treatment and prevention, but also causes those inflicted with the disease to forego income and employment opportunities due to prolonged illness. Episodes of the disease during childhood can result in impaired cognitive development, retarded learning ability and can encourage school absenteeism, which may result in inadequate human capital formation. Furthermore, malaria provokes households to migrate from high malaria risk regions in the rural areas to low risk regions (Sachs and Malaney, 2002).

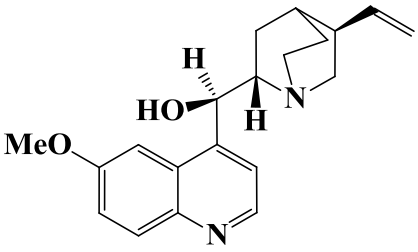
There are approximately one hundred *Plasmodium* species, of which four; *P. falciparum*, *P. malariae, P. ovale* and *P. vivax* can infect humans. Among these, *P. falciparium* is the most fatal, as it rapidly develops to the cerebral stage of malaria and is responsible for most of the mortalities in Africa (Casteel, 2003). *Plasmodium falciparum* is transmitted *via* the bites of infected female *Anopheles* mosquitoes. In the human body, the parasite multiplies in the liver

and then infects red blood cells. Transmission intensity depends on the prevalence and infectiousness of gametocytes that circulate in peripheral blood and the number of *Anopheles* mosquitoes in the area (Sauerwein, 2007).

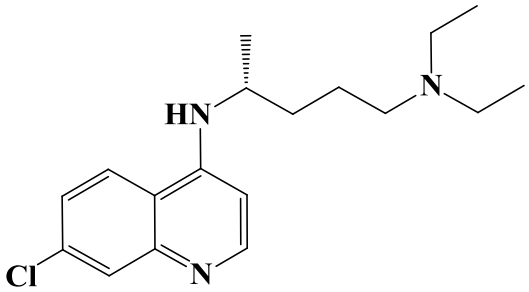
The major challenge in the treatment of malaria at the moment is the emergence of drug resistant strains of the malaria parasite *P. falciparum* to many of the currently available antimalarial drugs (Winstanley *et al*., 2002; Hyde, 2002; 2007). Although the resistance varies with geographical distribution, *P. falciparium* has shown resistance to almost all antimalarial drugs including, the artemisinin based combination therapy (Trape *et al*., 2002; Sibley *et al*, 2001). Widespread resistance of *P. falciparum* has been documented in many areas of the world, including sub-Saharan Africa, Southeast Asia, South Asia, Oceania, and the Amazon basin (Ryan, 1999; WHO, 2012).

The main reason for this serious situation is the limitation in both number and structural variety of antimalarial drugs in clinical use. Therefore, there is need to develop efficacious and structurally diverse antimalarial drugs with new modes of action to replace the current drugs that are becoming ineffective. In this regard, the plant kingdom remains a major source of structurally diverse antimalarial drugs. The search for antimalarial compounds from plants can even be expedited by focusing on plants that are known to be used traditionally in the management of the disease. This approach has been used in the past and led to the development of some antimalarial drugs. One of the most important antimalarial compound to come from the plant kingdom is quinine (**1**), which was isolated from the bark of *Cinchona succuriba* (Rubiaceae), and it served as a template for the synthesis of chloroquine (**2**) and mefloquine (**3**) (Balint 2001; Price 2002). The most recent drug, artemisinin (**4**) from the Chinese antimalarial herb *Artemisia annua*

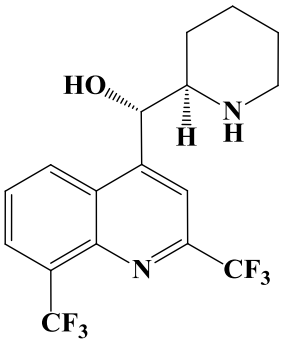
(Astraceae) (Balint, 2001; Price, 2002) has been used successfully against chloroquine-resistant parasites. Artemisini derivatives (**5-7**) have been used for enhance efficiency in malaria treatment (Klayman, 1985; Butler and Wu, 1992).



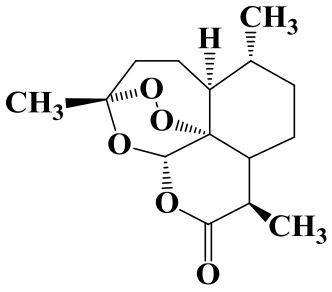
Quinine (**1**)



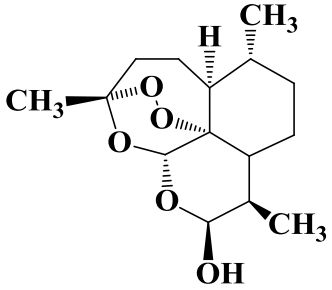
Chloroquine (**2**)



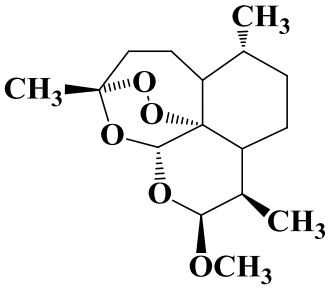
Mefloquine (**3**)



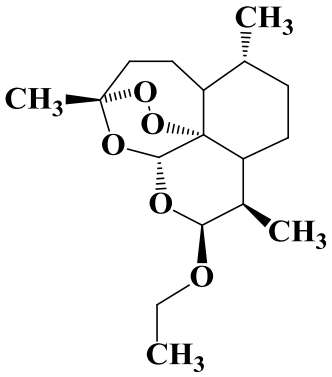
Artemisinin (**4**)



Dihydroartemisinin (**5**)



Artemether (**6**)



Arteether (**7**)

2.2 Malaria Control Strategies

Malaria is a disease that differs in its characteristics from country to country and even within the same country. Consequently, no single strategy is applicable for all situations. There are four approaches to malaria control: biological control, vaccine development, insecticide and chemotherapy.

2.2.1 Biological Control

Biological control refers to utilization of natural enemies or predators of mosquitoes such as fish, bacteria and fungi, that attack mosquitoes (Das & Amalraj, 1997). Although this method is more difficult to employ, it has the advantage of being safe to humans and non-target organisms. Furthermore, the low cost of production in some cases, and the lower risk of resistance development compared to chemical control methods makes this method attractive (Yap, 1985). The two most common methods of biological controls are the use of larvivorous fish and bacterial larvicides (Meisch, 1985).

Fish in the family of *Cyprinodontidae* have been investigated for control of mosquitoe for many years (Meisch, 1985). The use of larvivorous fish in controlling malaria vectors has been successful; especially in the areas associated with rice fields (Lacey & Lacey, 1990). However, larval control using fish may take long (1-2 months to establish) and can only be effective when large a number of the predator fish is bred. In ponds and marshes where there is dense aquatic vegetation, mosquito control by fish is not very effective as this ecology is less conducive for their breeding (Fletcher *et al*., 1992).

Bacterial larvicides are used mostly outdoors in irrigation ditches, standing ponds, tidal water, and storm water retention areas (Lacey and Lacey, 1990; Rishikesh *et al*., 1988). The effectiveness of this method depends primarily on the environmental conditions, the formulation of the product, and water quality. The most used microbial larvicides for mosquito control are *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs). Bti is a naturally occurring soil bacterium first registered by U.S. Environmental Protection Agency as an insecticide (for control of mosquito larvae) in 1983 (Karch *et al*., 1992; Walker, 2002; Becker, 1998). Bs was initially registered in 1991 for use against various kinds of mosquito larvae (Chavasse and Yap, 1997). Mosquito larvae eat the dormant spore form of the bacterium and associated toxin produced by the bacteria. The toxins bind to receptor cells present in the mosquito thereby disrupting the gut in the insects (Shukla *et al*., 1997).

2.2.2 Vaccine Development

Malaria vaccine development initiative was started in the middle of the 19th century (Baird *et al*., 1991). However, the lack of reliable and predictive animal models, antigenic diversity and variability of the parasite hampered the development of a malaria vaccine (Marc *et al*., 2007). The first success in malaria vaccine development was registered in 1973, when it was reported that immunization with sporozoites attenuated by irradiation can protect healthy volunteers against infection (Clyde *et al*., 1973). However, the vaccination consisted of bites of about a thousand mosquitoes infected with malaria parasites that had been irradiated. This was unlikely to be a practical means of mass vaccination and hence it was limited to experimental models and was not approved for human vaccine trials. Another milestone in vaccine development was Spf66, a hybrid polymer vaccine combining four different antigens from *P. falciparum*. However, it failed to show efficacy in phase III field trials (Graves *et al*., 2006). Currently, there

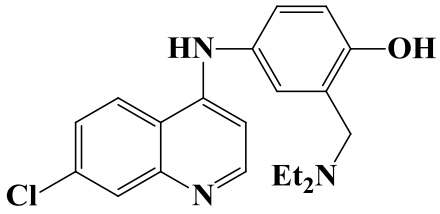
is no licensed malaria vaccine with the most advanced and promising vaccine RTS,S/AS0 against *Plasmodium falciparum* still currently in Phase III clinical trials (WHO, 2013).

2.2.3 Insecticides

Vector control is an important part of the global malaria control strategy. The use of insecticides involve eradicating the adult mosquitoes directly by spraying insecticides at the sites in which they hide. Organochlorines, like dichlorodiphenyltrichloroethane (DDT) and dieldrin, organophosphates such as malathion and temephos constitute groups of insecticides (Casida and Quistad, 1998) that have been applied to control mosquitoes. The environmental concerns on the use of insecticides are well documented especially on organochlorines like dichlorodiphenyltrichloroethane (DDT) and it has worsened with time and use. Vector-insecticide resistance and environmental concerns over the use of such insecticides has increased tremendously and has limited the choice of insecticides for such applications (Ronald, 2007; WHO, 2008).

2.2.4 Chemotherapy

The malaria chemotherapy has largely relied upon drugs that have been derived from two traditional medicinal plants. Quinine (**1**), extracted from *Cinchona* plants, has provided the basis for the development of synthetic quinoline-containing drugs such as chloroquine (**2**), mefloquine (**3**) and amodiaquine (**8**) (Foley and Tilley, 1998). The other drug, artemisinin (**4**), extracted from the plant *Artemisia annua,* served as a template for the design of structuraly related compounds such as dihydroartemisinin (**5**), artemether (**6**) and arteether (**7**) (Klayman, 1985; Butler and Wu, 1992).



Amodiaquine (**8**)

2.2.4.1 Quinolines

For decades, quinoline-based antimalarial drugs have been the mainstay for antimalarial chemotherapy. Members of this category contain a quinoline ring as their basic pharmacophore with substituents at various positions on this ring. The parent drug quinine (**1**) was the first antimalarial drug isolated from the bark of *Cinchona* species (Rubiaceae) in 1820 (Christian *et al*., 2005). The discovery of quinine was recognised as the most unexpected medical discovery of the 17th century. Furthermore, malaria treatment with quinine **(1)** marked the first successful use of a chemical compound to treat an infectious disease (Achan *et al*., 2011). Quinine **(1)** acts by inhibiting the heme polymerase enzyme, thus facilitating an aggregation of cytotoxic heme (Slater *et al*., 1992). Resistance to quinine was first noted from Thai-Cambodian border in mid 1960s (Pickard, 2002). Since then clinical resistance to quinine therapy has been observed sporadically in all parts of the world where malaria is endemic. Even though it is the oldest antimalarial drug, it is still used in combination with other drugs to reduce the effects of resistance (Ekland *et al*., 2008).

Another class of antimalarial drug formed through structural modification of quinine are the 4-aminoquinolines such as chloroquine (**2**) and mefloquine (**3**), of which the former was the more effective, cheaper, safer, and more commonly available drug. Unfortunately, after early success, the malarial parasite (especially *P. falciparum*) became resistant to chloroquine (Tinto *et al*.,

2006). Treatment of chloroquine-resistant malaria was then performed with alternative drugs or drug combinations, which were rather expensive and sometimes toxic. Mefloquine (**3**) was introduced to the market following the Vietnam War to protect American troops against multi-drug resistant *P. falciparum*. It has been widely used for chemoprophylaxis in areas where chloroquine-resistant *P. falciparum* is prevalent; unfortunately, its use was limited due to its toxicity and high cost (Muckenhaupt, 1995; Wongsrichanalai *et al*., 2001). Interestingly, it has never been approved for treatment of malaria in the United States because of the neurotoxicity at the doses required for the treatment (Muckenhaupt, 1995).

Amodiaquine (**8**) is another 4-aminoquinoline having a similar structure to chloroquine; it is assumed to have similar mechanism of action as chloroquine. It has been frequently used in combination with chloroquine and it is believed to be more effective in clearing out parasites in uncomplicated malaria than chloroquine. However, it is associated with some side effects such as itching, nausea, vomiting and abdominal pain, which hampered its wider application (Foley and Tilley, 1998).

2.2.4.2 Artemisinins

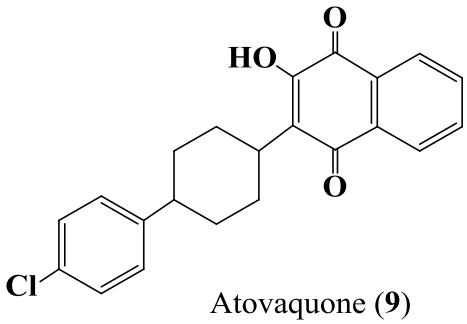
Artemisia annua is a Chinese herb that has been used over 1000 years for the treatment of fever, thus predating the use of quinine in the Western world (Bray *et al.,* 2005). The active compound was first isolated from the plant in 1971 and named artemsinin (**4**) (*qinghaosu* in Chinese). It has been proven that it is effective against all forms of multi-drug resistant *P. falciparum* and also has the fastest clearance rate from the blood system of all antimalarials currently used (Butler and Wu, 1992).

The artemisinins (also known as the sesquiterpene endoperoxides) possess an endoperoxide bridge that is believed to be the center of antiplasmodial activity. The drug acts primarily at the trophozite phase, thus preventing further progression of the disease. The proposed modes of action for these antimalarials includes activation of the peroxide bridge by Fe^{2+} (from heme or free Fe^{2+}) which then alkylates heme inhibiting crystal growth or, through alkylation of essential proteins of the parasite (Meshnick *et al*., 1991; Posner, 1992). Although artemisinin is the most preferred drug to treat malaria, its therapeutic value is limited by its low solubility in both oil and water. Consequently, in the search for more effective and soluble drugs, Chinese researchers prepared a number of derivatives of the parent drug (Klayman, 1985; Butler and Wu, 1992).

Dihydroartemisinin (**5**) was produced by reduction of artemisinin and then modified to give a series of analogues including artemether (**6**) and arteether (**7**) (Butler and Wu, 1992). Although these compounds are more potent than artemisinin, they have short plasma half-lives and produce fatal central nervous system (CNS) toxicity at high dose (Brewer *et al*., 1994).

2.2.4.3 Naphthoquinones

Atovaquone (**9**) was identified as an antimalarial in the early 1980s. It has been proved to be highly effective against the multi-drug resistant parasites and is known to selectively inhibit the electron transport in the parasites' mitochondrion without interfering with that of the host (Fry and Pudney, 1992; Srivastava *et al*., 1997). However, insolubility in water limits the application of this drug (Srivastava *et al*., 1997).



Atovaquone (**9**)

2.2.4.3 Combination Therapy

Combination therapy involving the use of two or more antimalarial drugs with independent modes of action and different biochemical targets in the parasite to improve therapeutic efficacy and also delay the development of resistance to the individual components of the combination. The combination of drugs is currently divided into two categories. The non-artemisinin based and artemisinin based combinations (Ernest and Mokuolu, 2005).

2.2.4.3.1 Non-artemsinin Based Combinations

The sulphadoxine-pyrimethamine (SP) combination was used as a drug of choice to treat chloroquine-resistant malaria in the early 1960s; however, resistance to SP was first reported from the Thai-Cambodian border in the same year (Bjorkman *et al*., 1990). Since then SP resistance has been reported from large parts of malaria endemic countries (Aramburu *et al*., 1999; Vasconcelos *et al*., 2000); however, it is still in the market as it is easily affordable to the majority of African patients (Mbugi *et al*., 2006).

The antibiotic tetracycline and its derivatives, such as doxycycline, are consistently active against all *Plasmodium* species in combination with quinine. They are particularly useful for the treatment of severe falciparum malaria (Schlitzer, 2008). However, the use of these drugs is

associated with side effects including nausea, dysphoria, tinnitus, and deafness of cinchonism (Wichmann *et al*., 2004). Amodiaquine combination with sulfadoxine-pyrimethamine showed excellent antimalarial efficacy while fairly high levels of resistance to each individual agent has been observed. However, serious adverse reactions associated with the use of this combination has limited its uses (Dorsey *et al*., 2002). The combination of atovaquone (**9**) and proguanil (Malarone) has been effective against multidrug-resistant falciparum malaria (Musset *et al*., 2006; Radloff *et al*., 1996), but its use has been limited by the associated side effects (Radloff *et al*., 1996).

2.2.4.3.2 Artemsinin Based Combinations

The advantages of artemisinin-based combination therapy (ACT) are associated with the mode of action of artemisinin derivatives (potent and fast acting) and that the partner drug in ACT has long half-life, which allows killing the parasites that may have escaped the artemisinin inhibition (Brian *et al*., 2011; WHO, 2008). WHO recommend the use of ACTs for treating *falciparum* malaria in all countries where resistance to monotherapies or non-artemisinin combination therapies is prevalent. Indeed many countries in Africa including Kenya have adopted the artemisinin combination therapy as first line or second line drug. Unfortunately, the reports on drug resistance to artemisinin-derivatives (Jambou *et al*., 2005; Dondorp *et al*., 2009) including ACT have been reported (Wichmann *et al*., 2004; White, 2010).

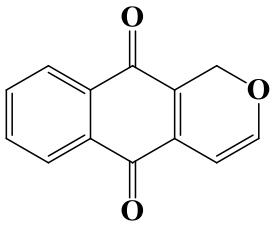
2.3 The Use of Natural Products in Malaria Chemotherapy

Medicinal plants have served as a source of lead compounds for the most common antimalarial drugs currently on the market. In light of the historic success of anti-malarial drugs, quinine and artemisinin, both from natural origin, a number of studies have adopted an ethnobotanical

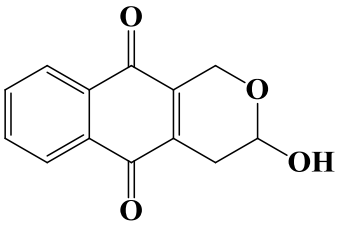
approach through investigation of plants from malaria endemic areas in the search for novel antimalarial lead compounds. Indeedantiplasmodial activities have been observed among the natural products comprised of alkaloids (Oliveira *et al*., 2009; Frederich *et al*., 2008), terpenes (Saxena *et al*., 2003; Pedersen *et al*., 2009; Efange *et al*., 2009; Murata *et al*., 2008), quassinoids (Saxena *et al*., 2003), flavonoids (Saxena *et al*., 2003; Muiva *et al*., 2009; Kunert *et al*., 2008), xanthones (Zelefack *et al*., 2009), and quinones (Saxena *et al*., 2003; Hou *et al*., 2009; Endale *et al*., 2012; Bringmann *et al*., 1999a).

2.4 Antimalarial Quinones

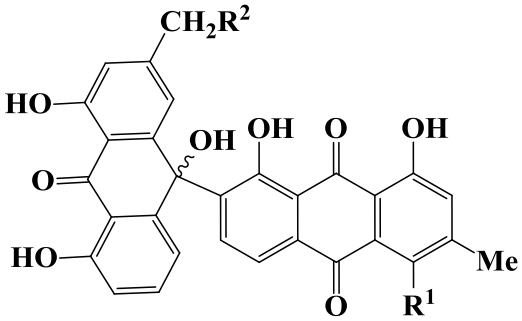
Among quinones that have shown antimalarial activity, the naphthaquinone derivative, atovaquone, was reported as an effective antimalarial drug against the multidrug resistant parasite with a different mechanism of action from that of the other antimalarial drugs (Fry and Pudney, 1992). The quinones pentalongin (**10**) (IC_{50} 0.27 µg/mL) and psychorubrin (**11**) (IC_{50} 0.91 µg/mL) recently isolated from the roots of *Pentas longiflora* showed promising *in vitro* antiplamodial activities (Endale *et al*., 2012). The bianthraquinones, chrysalodin (**12**) (IC_{50} 0.26 µg/mL) and chryslandicin (**13**) (IC_{50} 0.54 µg/mL), isolated from the root of *K. foliosa,* showed high inhibition of the growth of the malaria parasite (Wube *et al.,* 2005). The phenylanthraquinones, knipholone (**14**) (IC_{50} 0.67µg/mL) and knipholone anthrone (**15**) (IC_{50} 0.34 µg/mL) isolated from the roots of *K. foliosa* and *B. frutescens*, showed antiplasmodial activity (Bringmann *et al*., 1999a).



Pentalongin (**10**)

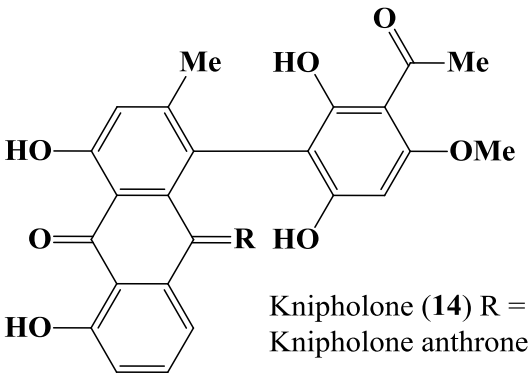


Psychorubrin (**11**)



Chrysalodin (**12**) R^1 = H, R^2 = OH

Chryslandicin (**13**) R^1 = OH, R^2 = H



Knipholone (**14**) R = O

Knipholone anthrone (**15**) R = H, H

2.5 Botanical Information

The plant family Liliaceae was conceived in 1789 with its recoginized 300 genera and 4500 species, widely distributed in tropical and sub-tropical areas of the world (Hutchinson, 1934). It was later noted that it embraced a heterogeneous assortment of genera. Consequently, revision was made by Dahlgren and Clifford (1982), it was subdivided into six homogeneous families; Asparagaceae, Alliaceae, Asphodelaceae, Dracaenaceae, Eriospermaceae and Hyacinthaceae. According to the new classification, the family Asphodelaceae comprised three sub-families; namely Astelioideae, Anthericodeae and Asphodeloideae with the genera placed in these families as shown below:

Astelioideae: *Astelia*, *Cohnia*, *Cordyline* and *Milligania*

Anthericodeae: *Agrostocrinum*, *Alania*, *Anthericum*, *Arnocrinum*, D*asystachys*, *Debesia*, *Dichopogon*, D*iuranthera*, *Paradisia*, *Simethis*, *Tricoryne* and *Verdickia*.

Asphodeloideae: *Aloe*, *Alectorurus*, *Aprica*, *Asphodeline*, *Asphodelus*, *Kniphofia*, *Bilbine*, *Bulbinella*, *Bulbinopsis*, *Chamaealoe*, *Chortolirion*, *Eremurus*, *Gasteria*, *Glyphosperma*, *Haworthia*, *Arachyandra* and *verinea*.

In 1985, the plant family Asphodelaceae was again revised and the sub-families Astelioideae and Anthericodeae were raised to families refered to as Asteliaceae and Anthericaceae respectively (Dahlgren *et al*., 1985). Whereas, the sub-family Asphodeloideae was kept in the Asphodelaceae family with two defined new sub-families Alooideae (with the genera *Aloe, Astroloba, Chamaealoe, Gasteria, Haworthia, Lomathophyllum,* and *Poellnitzia*) and the Asphodeloideae (with the genera: *Asphodeline, Asphodelus, Bulbine, Bulbinella, Jodrellia, Kniphofia, Smiethis*, and *Trachyandra*) (Dahlgren *et al*., 1985). Although the above classification seems to enjoy universal acceptance; however, there are still different views on the relationship of the various genera within the family and even on the circumscription of the family. For example, Cronquist, (1981; 1988) placed the genus *Kniphofia* in the Alooideae sub-family based on the similarities of floral morphology and inflorescence. Whereas Dahlgren *et al*. (1985) placed it in the Asphodeloideae sub-family based on differences in the anatomical construction of the leaves. The chloroplast DNA (CP DNA) sequence (Chase *et al*., 2000) and phytochemical evidence (van Wyk *et al*., 1995a) supported the placement of *Kniphofia* in Asphodeloideae along with *Bulbine*, *Bulbinella, Asphodeline, Asphodelus, Jodrellia, Smiethis*, and *Trachyandra*. Within such uncertainty, the importance of more in depth investigations on the metabolic profiles of plants of this family cannot be over-emphasized in order to get better insight into their intra-family relationships.

2.5.2 The Genus *Kniphofia*

The genus *Kniphofia* Moench, commonly known as 'red hot pokers', comprises about 71 species (Bosch, 2008). The genus is evergreen characterized by a perennial, rhizomatous, usually acaulescent and herbaceous habit. Leaves are linear and usually keeled grass like (Smith and van Wyk, 1998). Florescences are sub-spicate racemes with a bract at the base of pedicels. Flowers are tubular rising above the foliage with different colours ranging from white, yellow to various shades of red which are more conspicuous at the apex of the inflorescence. Fruits are globose to ovoid capsules that house seeds that are usually flattened (Whitehouse, 2002; Ramdhani, 2006).

The genus is almost entirely African with two species (*Kniphofia pallidiflora* and *Kniphofia ankaratrensis*) from Madagascar and one (*Kniphofia sumarae*) from Yemen. Most *Kniphofia* species (about 47 species) are found in Eastern area of South Africa, preferring altitude more than 1500 m (Codd, 1968; 2005). About seven *Kniphofia* species occur in Ethiopia, of which five; including *Kniphofia foliosa*, *Kniphofia hildebrandtii*, *Kniphofia isoetifolia*, *Kniphofia insignis* and *Kniphofia schimperi* are endemic (Whitehouse 2002; Demissew and Nordal, 2010). Only one species, *Kniphofia thomsonii* is found in Kenya (Chase *et al*., 1995).

Although the genus *Kniphofia* is widely recognized for its ornamental value owing to colorful flowers, their use in traditional medicine is limited to few species. Infusions made from rhizomes of *K. laxiflora* and *K. rooperi* are used to treat chest ailments, while crushed roots and rhizomes of *K. uvaria* are included in enemas administered for painful menstruation. *K. caulescens* is frequently planted around Basuto huts as a charm against lightenning (Codd, 1968). Infusions of *K. uvaria* are taken orally in South African by Xhosa women to treat infertility. Furthermore,

infusions of *K. buchananii* and *K. parviflora* are used as snake deterrents (Matsiliza and Barker, 2001).

2.5.2.1 *Kniphofia foliosa*

Kniphofia foliosa Hochst is a perennial herb which grows on road sides, overgrazed areas with scattered trees and is commonly distributed in the mountainous regions of Central and Northern Ethiopia (Marias, 1974). The specific name '*foliosa*' refers to the many crowded rosulate leaves at the base. The flowers opening upwards from the base and usually appear from June to October (Demissew and Nordal, 2010). The roots of *K. foliosa* have been used for the treatment of abdominal cramps and for wound healing (Abate, 1989).

Figure 2.1: Photograph of *Kniphofia foliosa* (by Negera Abdissa, Ethiopia, 2011)

2.5.3 The Genus *Bulbine*

The genus *Bulbine* (Asphodelaceae) comprises 40 species. It is mainly distributed in Southern Africa (Reid, 1993). *Bulbine abyssinica* and *Bulbine frutescens* are the only *Bulbine* species found in Eastern Africa (van Wyk *et al*., 1995a). *Bulbine* is an interesting, lush-looking plant even when not in bloom and they are succulent with largely branched, rhizomatous, and solitary geophytes (Barnes *et al*., 1994). With few exceptions, all have yellow flowers and the filaments are bearded with yellow pointed hairs. Some are ornamental plants and are sold in nurseries and garden shops, frequently as plant hybrids. Although the plant is widely recognized for its ornamental value owing to colorful flowers and succulent leaves, it is also known for its use in traditional medicinal practice. The medicinal uses of the *Bulbine* species (Table 2.1) have been known for centuries (Coopoosamy *et al*., 2000; van Wyk and Gericke, 2000).

Table 2.1: Ethnomedicinal uses of *Bulbine* species (Coopoosamy *et al*., 2000; van Wyk and Gericke, 2000)

Species	Treatment	Part used
B. latifolia	Stomach ailments, rheumatism	Dried bases
B. longifolia	Stomach ailments, burns, sunburn	Dried bases and leaves
B. narcissifolia	Burns, wounds, rashes	Roots
B. natalensis	Diarrhoea, burns, rashes, sunburn, corns, warts	Leaves
B. frutescens	Diarrhoea, burns, rashes, blisters, insect bites, cracked lips	Dried bases and Leaves

2.5.3.1 *Bulbine frutescens*

Bulbine frutescens is called a home garden plant as it is widely used as an ornamental plant. It's yellow coloured flowers combines attractively with the orange coloured petals, which contributed towards the beauty of the bi-colour look of the plant. The leaf exudate of *B. frutescens* is used for the treatment of various ailments, particularly for wound healing and aesthetic purposes (Rabe and van Staden 1997; Abegaz *et al*., 2002). The gel extract of the same plant has been patented as a promoter of wound healing (Lusunzi and Karuso, 2001a).

Figure 2.2: Photograph of *Bulbine frutescens* (by Negera Abdissa, Nairobi, 2013)

2.5.4 The Genus *Aloe*

Aloe is the largest genus in the family and comprises of over 500 species, ranging from diminutive shrubs to large tree-like forms (Smith *et al*., 1995). The name *Aloe* is presumed to come from the Greek ‘alsos’ or Arabic ‘alloh’ both words referning to the bitter substance from the leaves of the plant. *Aloes* are perennial evergreen shrubs with succulent leaves having

flowers of an elongated tubular form varying in color from orange to bright red mainly distributed in Africa and Madagascar with only a few species found in the Arabian Peninsula (Reynolds, 1950). *Aloe* in East Africa is represented by 83 species, of which 26 grow naturally in Kenya including six endemic species: *A. kilifiensis*, *A. nyeriensis*, *A. ngobitensis*, *A. elgonicaa*, *A. classenii* and *A. ukambensis* (Carter, 1994; Reynolds, 1966).

Reynolds has divided *Aloe* species into 20 diffirent groups (group 1 to 20) (Table 2.2) depending on similarities in morphology (Reynolds, 1966). This morphology-based grouping has advantages; however, does not necessarily reflect genetic relationships among the species. For instance, group 19 (shrubby *Aloes*) encompassing *Aloes* with prolonged stems share similarities with group 5 (*Aloes* with striped perianth) in inflorescence and leaf characters, even though they differ with respect to caulescence and branching. Moreover, *A. dawei* of group 19 and *A. secundiflora* of group 14 have the morphological similarity of spotted perianth shape. Chemotaxonomic studies by Viljoen and van Wyk (2000) revealed a close relation of the secondary metabolite profiles of the *Aloe* groups 5, 14, 16 and 19. With the uncertainty of the conventional morphology-based classification, the importance of more-in-depth investigations on the metabolic profiles of *Aloe* species cannot be over-emphasized in order to establish their intergeneric relationships.

Table 2.2: Reynold's grouping of tropical African *Aloes* (Reynolds, 1966)

Group	Description	Representative speceis
Group 1	grass *Aloes*	*A. myriacontha*
Group 2	lepto *Aloes*	*A. nuttii*
Group 3	bulbous species	*A. bkettneri*
Group 4	perianth striped	*A. peckii*
Group 5	----------	*A. Dorotheae*
Group 6	Sapanariae	*A. lateritia*
Group 7	Hereroenses	*A. hereroensis*
Group 8	perianth trigonously indented above the overy	*A. chabaudii*
Group 9	Verae	*A. barbadensis*
Group 10	pendet series	*A. veseyi*
Group 11	Laterbracteatae	*A. cryptopoda*
Group 12	----------	*A. christianii*
Group 13	perianths clavate	*A. camperi*
Group 14	flowers second	*A. secundiflora*
Group 15	racemes bottle brush like	*A. aculeata*
Group 16	large compact rosettes	*A. percrassa*
Group 17	leaves spreading, canaliculate	*A. magalacantha*
Group 18	tall stemed species	*A. volkensii*
Group 19	Shrubs	*A. dawei*
Group 20	Trees	*A. eminens*

The genus *Aloe* has a long medicinal history since 1500 BC. *Aloe* gel, which is found in the interior of the leaves, has been used for treatment of a variety of skin ailments (Morton, 1961). The gel works by hydrating and protecting the wound until the body can repair itself. It is important to note that some *Aloe* species are poisonous, so correct identification of the species must be made before treatment. *Aloe vera* and some others, such as *A. ferox* have been used for a

long time in folk medicine for the treatment of constipation, burns and dermatitis (Man *et al*., 1996).

2.5.4.1 *Aloe dawei*

Aloe dawei A. Berger, is widely distributed in Kenya, Uganda, Tanzania and Rwanda (Reynolds, 1966). The plant is named after Mr. Dawe, the former curator of the Botanical Gardens at Entebbe, Uganda, who first described *Aloes* as "armed with pungent reddish-brown teeth" in the early 1900s (Berger, 1959). In Rwanda the roots extract of *A. dawei* is drunk to cure malaria and its leaf sap to treat inflammation of the ear (Schemelzer and Gurib-Fakim, 2008). In Kenya, the leaf infusion is drunk in small quantities for skin diseases while warm decoction is used for bathing. Hot leaves are used as a poultice for treating kidney ailments (Kokwaro, 2009).

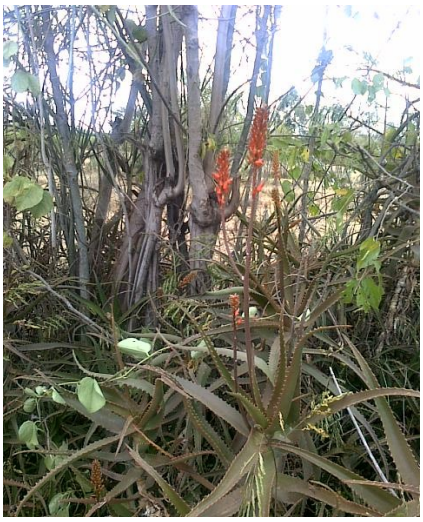


Figure 2.3: Photograph of *Aloe dawei* (by Martha Induli, Awendo, Kenya, 2012)

2.5.4.2 *Aloe lateritia* Subspecies *graminicola*

Aloe lateritia subspecies *graminicola* is widely distributed in East Africa. In Kenya, it is found in the Rift Valley and Central Province (Reynold, 1966). The slow growing stemless to short stemmed rosettes with the dark green leaves are the distinict morphological features of the plant

(Reynold, 1966). It is documented that the decoction of *A. lateritia* subspecies *graminicola* is used for inducing vomiting in order to cure stomach diseases and malaria (Kokwaro 1976).

Figure 2.4: Photograph of *Aloe lateritia* subspecies *graminicola* (by Negera Abdissa, Nairobi, Kenya, 2013)

2.6 Phytochemistry of the Family Asphodelaceae

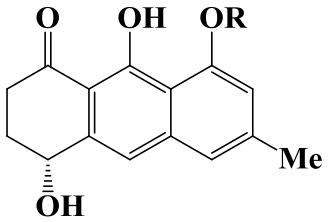
Only a few genera of the family Asphodelaceae have so far been investigated for secondary metabolites and biological activity. By far, the most extensively investigated genus in this family is the genus *Aloe*, while only limited information is available on the genera *Bulbine* and *Kniphofia.* Most of the compounds so far reported from this family are polyketide derivatives, mainly anthraquinones and their pre-cursurs. Other classes of natural products found in this family include isofuranonaphthoquinones, naphthalene and phloroglucinol derivatives, and other polyphenols. The distribution and occurrence of these is presented here.

2.6.1 Phytochemistry of the Genus *Kniphofia*

The genus *Kniphofia* is rich in anthraquinones including monomeric and dimeric anthraquinones and phenylanthraquinones.

2.6.1.1 Pre-anthraquinones

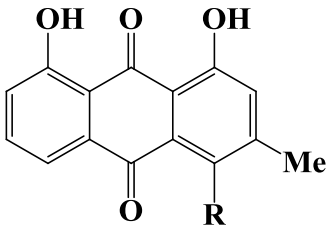
Pre-anthraquinones are considered to be precursors leading to anthraquinones; *in vitro*, they readily convert to the corresponding anthraquinones by treatment with a base (Yenesew *et al*., 1994) showing the close relationships between these two subclasses. Only two related pre-anthraquinones; aloesaponol III (**16**) and aloesaponol III-8-methyl ether (**17**) have been reported from the stem of *K. foliosa* (Yenesew *et al*., 1994).



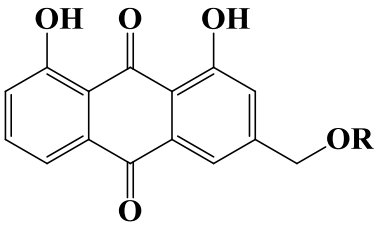
Aloesaponol III (**16**) R = H
Aloesaponol III-8-methyl ether (**17**) R = Me

2.6.1.2 Monomeric Anthraquinones

Monomeric anthraquinones are commonly found in the genus *Kniphofia*. In a chemotaxonomic survey, the 1,8-dihydroxyanthraquinones, chrysophanol (**18**) and islandicin (**19**) were reported from most *Kniphofia* species analysed with few exception (Berhanu *et al*., 1986; Yenesew *et al*., 1988; van Wyk, 1995a). Two additional anthraquinones, aloe-emodin (**20**) and aloe-emodin acetate (**21**) in which the methyl at C-3 is oxidized have been reported from *K. foliosa* (Berhanu and Dagne, 1984).



Chrysophanol (**18**) R = H
Islandicin (**19**) R = OH

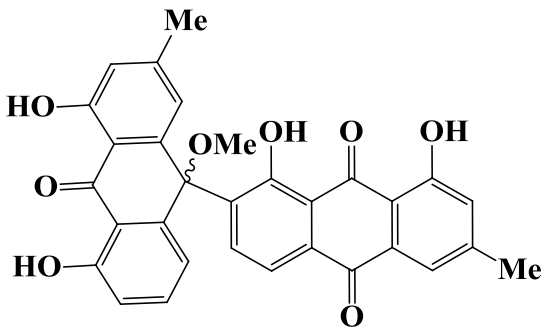


Aloe-emodin (**20**) R = H
Aloe-emodin acetate (**21**) R = Ac

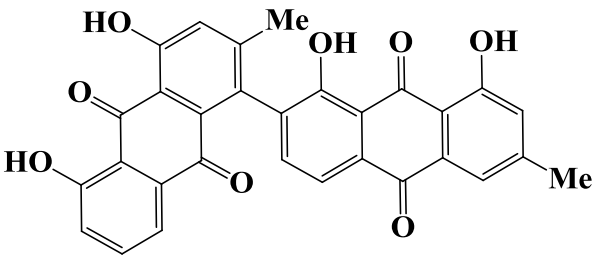
2.6.1.3 Dimeric Anthraquinones

The genus *Kniphofia* has proved to be a good source of dimeric anthraquinones (van Wyk *et al*., 1995a). Dimerization of two identical anthraquinones by phenol-oxidative coupling and mixed dimerization have been observed in this genus. The occurrence of dimers of the 1,8-dihydroxy-3-methylanthraquinone, chrysophanol and the corresponding anthrone (chrysophanol anthrone) has been reported from *Kniphofia* species (van Wyk *et al*., 1995a).

The first two unsymmetrical bianthraquinone pigments composed of an anthraquinone and an anthrone dimer of this genus are, chrysalodin (**12**) (Dagne *et al*., 1987) and chryslandicin (**13**) both isolated from *K. foliosa* (Yenesew *et al*., 1988). Recently a dimer of chrysophanol oxanthrone and islandicin moieties with a methoxy group at C-10, chryslandicin 10-methyl ether (**22**) has been reported from the rhizomes of *K. foliosa* (Induli *et al*., 2013). Asphodeline (**23**), a dimer comprised of two chrysophanol moieties, was also reported from the root of *K. tysonii* (van Wyk *et al*., 1995a).



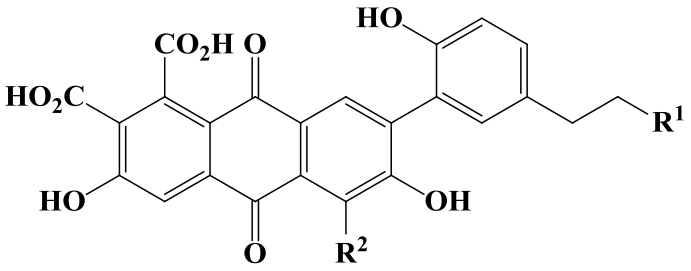
Chryslandicin 10-methyl ether (**22**)



Asphodeline (**23**)

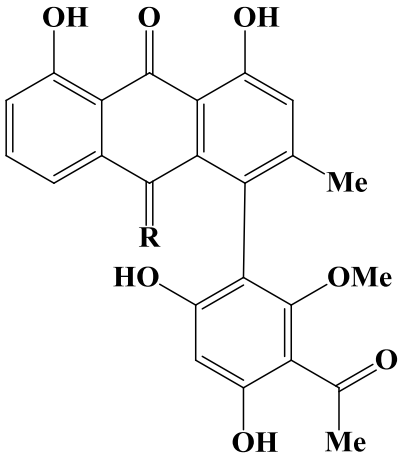
2.6.1.4 Phenylanthraquinones

Another interesting and emerging class of secondary metabolites that are produced by the sub-family Asphodeloideae are the phenylanthraquinones, consisting of a 1,8-dihydroxyanthraquinone and an acetylphloroglucinol portion linked through a biaryl axis. For many years, only a few phenylanthraquinones were known from insect sources with the phenyl moiety attached to the 2-position of the anthracene ring. The first natural phenylanthraquinones, laccaic acids A (**24**), B (**25**), C (**26**), and E (**27**) were isolated from insect (*Laccifer lacca*) heavily infested trees as the major constituents of the water-soluble lac-dye, a commercial source of dye for textiles and art work used as far back as the 16th century (Bringmann *et al*., 2008b; Burwood *et al*., 1965; 1967; Harley, 1982).

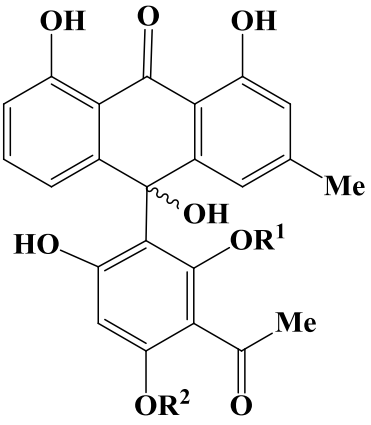


Laccaic acid A (**24**) R^1 = NHCOMe, R^2 = OH
Laccaic acid B (**25**) R^1 = R^2 = OH
Laccaic acid C (**26**) R^1 = NH_2COOH, R^2 = OH
Laccaic acid E (**27**) R^1 = NH_2, R^2 = OH

The first plant-derived phenylanthraquinone with acetylphloroglucinol moiety attached to C-4 of chrysophanol, trivial name knipholone (**14**), was reported from the roots of *K. foliosa* in 1984 (Dagne and Steglich, 1984). Comparative studies on the roots of fourteen *Kniphofia* species showed knipholone (**14**) to be the major pigment in these taxa and it was suggested that it may be a taxonomic marker for the genus *Kniphofia* (Yenesew *et al*., 1988). Since then, the closely related phenylanthraquinones, knipholone anthrone (**15**), isoknipholone (**28**) and isoknipholone anthrone (**29**) (Dagne and Yenesew, 1993; Yenesew *et al*., 1994), as well as those where the acetylphloroglucinol is attached at C-10 of 10-oxochrysophanol anthrone, *vis* foliosone (**30**) and isofoliosone (**31**) were reported from *K. foliosa* (Yenesew *et al*., 1994).



Isoknipholone (**28**) R = O
Isoknipholone anthrone (**29**) R = H, H

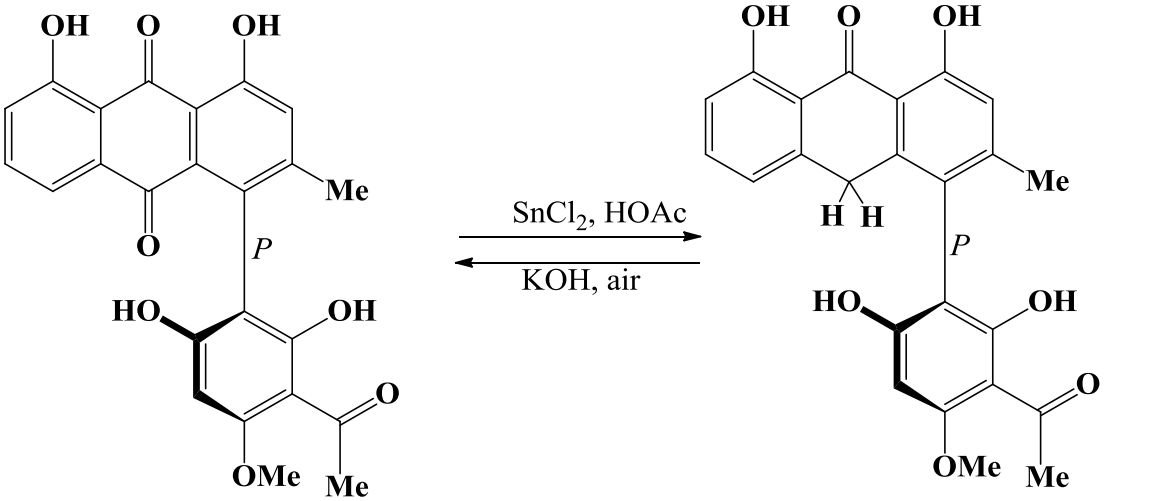


Foliosone (**30**) R^1 = H, R^2 = Me
Isofoliosone (**31**) R^1 = Me, R^2 = H

Phenylanthraquinones become axially chiral, when the biaryl axis is equipped with large *ortho* substituents restricting rotation about that axis. For this reason, natural knipholone (**14**) and knipholone anthrone (**15**) are optically active. The optical rotations for these two compounds are reported as $+80^0$ (Dagne and Steglich, 1984) and $+200^0$ (Dagne and Yenesew, 1993) for knipholone and knipholone anthrone respectively. The absolute configuration of these two

phenylanthraquinones at the biaryl axis was first assigned to be *M*-configured based on CD-quantum calculations (Bringmann *et al.,* 1999b). The configuration of other phenylanthraquinones were then assigned through comparision of CD spectra with **14** and **15** of these compounds (Bringmann *et al*., 1999b).

The stereochemical assignment of **14** and **15** was latter revised (Bringmann *et al*., 2007a) as *P* on the basis of synthetic work and high-level quantum chemical CD calculations. The computational result that showed both have identical configuration is in agreement with the fact that these two compounds can be interconverted in a stereospecific reaction (Scheme 2.1).



Knipholone (**14**) Knipholone anthrone (**15**)

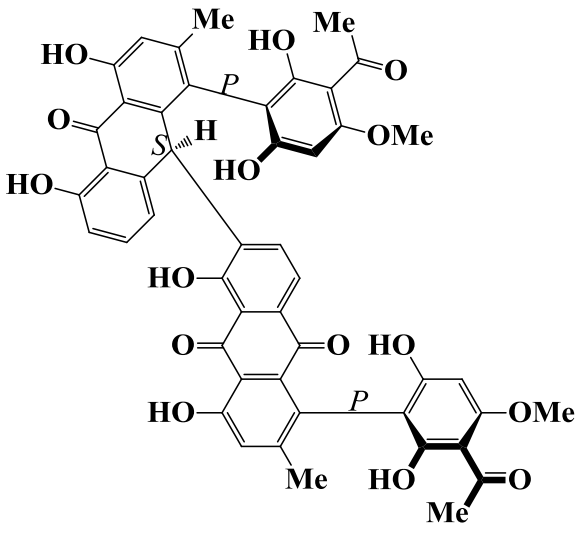
Scheme 2.1: Knipholone-knipholone anthrone interconversion

2.4.1.5 Dimeric Phenylanthraquinones

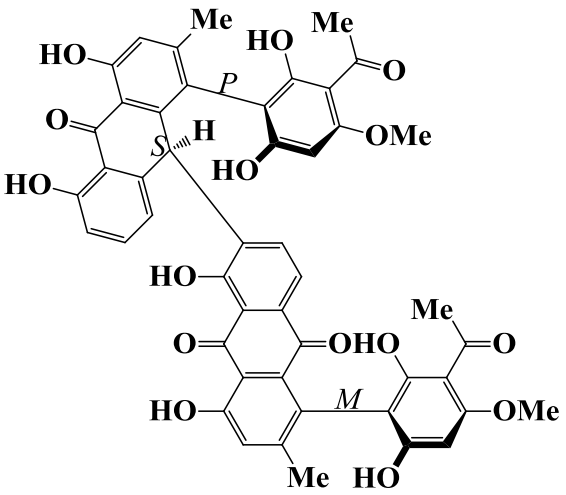
Knipholone anthrone (**15**) has been reported to be the major compound in the roots and stem of *K. foliosa* (Dagne and Yenesew, 1993). One reason for this could be that the plant stores compound **15** for subsequent synthesis of more complex, and in ecological terms, possibly more

useful dimeric and tetrameric products. Indeed joziknipholones A (**32**) and B (**33**), the first dimeric phenylanthraquinones have been reported from the stem of *K. foliosa* (Induli, *et al*., 2013) for the second time after being reported from *Bulbine frutescens* (Bringmann *et al*., 2008a).

These dimeric phenylanthraquinones possess two configurationally stable biaryl axes, in addition to central sp^2–sp^3 axis between the two phenylanthraquinone portions. The absolute configuration of **32** and **33** were determined by reductive cleavage of the central sp^2–sp^3 linkage between the knipholone (**14**) and knipholone anthrone (**15**) portions with sodium dithionite under inert condition, and by quantum chemical CD calculations. Cleavage of the central bond was performed using an optimized protocol with workup under oxygen-free conditions thus avoiding subsequent oxidation of the anthrone product **15**. Based on the re-established absolute axial configuration of **14** and **15** (Bringmann *et al*., 2007a), compounds **32** and **33** were assigned as 4*P*,4'*P*,10'*S* and 4*M*,4'*P*,10'*S*, respectively (Bringmann *et al*., 2008a).



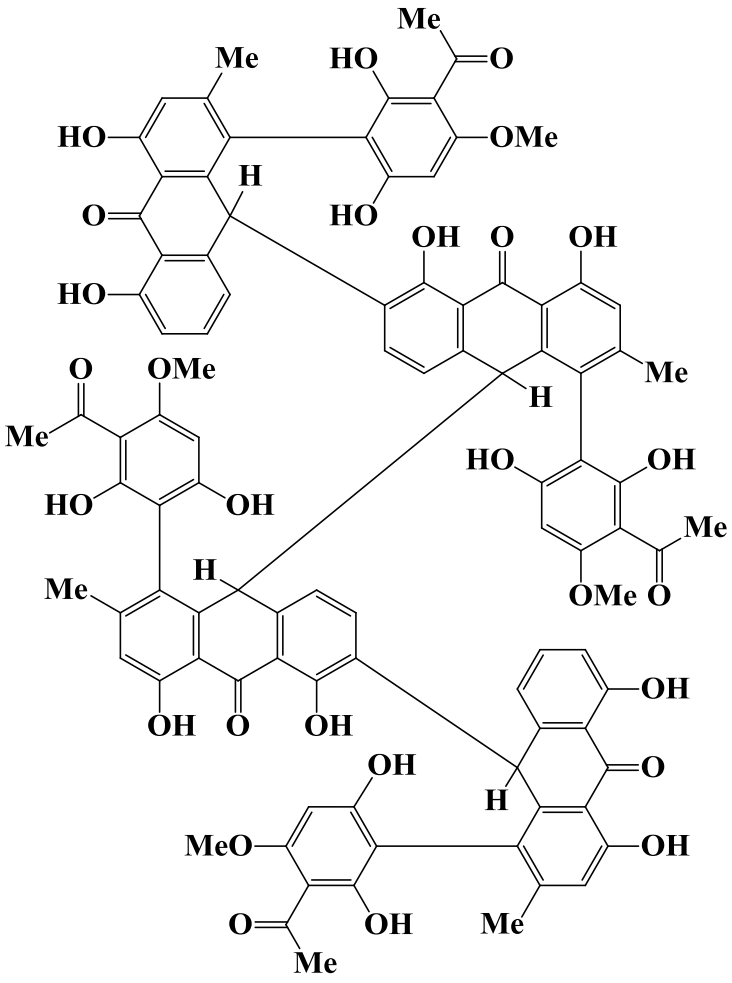
Joziknipholones A (**32**)



Joziknipholones B (**33**)

2.4.1.6 Tetrameric Phenylanthraquinones

The tendency of knipholone anthrone to undergo fascinating free-radical oxidative coupling reactions has most recently become even more apparent from the isolation of jozijoziknipholone (**34**), a tetrameric phenylanthraquinone was isolated from *K. foliosa* (Gebru, 2010). The biological activity and absolute configuration of this fascinating molecule with four stereogenic centers, four biaryl axes and two sp^2-sp^3 and one sp^3-sp^3 axes has not yet been evaluated.

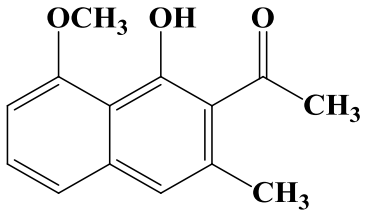


Jozijoziknipholone (**34**)

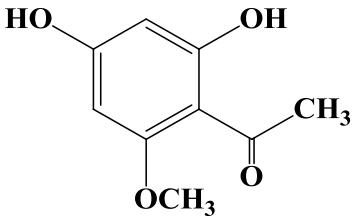
The occurrence of the knipholone dimers and tetramers just discovered in these plant species shows the metabolic potential of these plants and makes them attractive subjects for further phytochemical and biological investigations.

2.4.1.7 Miscellaneous Compounds

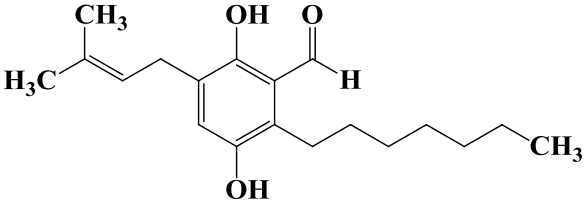
A naphthalene derivative; 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene (**35**) (Wube *et al*., 2005) and an acetophenone derivative; 4,6-dihydroxy-2-methoxyacetophenone (**36**) (Yenesew *et al*., 1994) were also isolated from the roots and stem of *K. foliosa* respectively. Whereas, flavoglucin (**37**) and 3'",4'"-dehydroflavoglucin (**38**) were reported from the roots of *K. thomsonii* (Achieng, 2009).



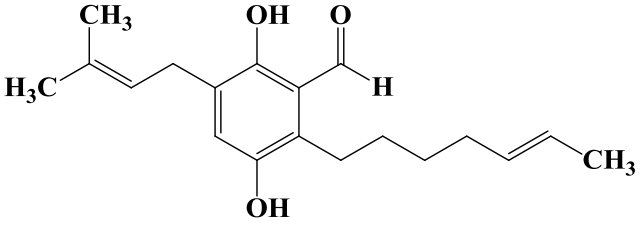
2-Acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene (**35**)



4,6-Dihydroxy-2-methoxyacetophenone (**36**)



Flavoglucin (**37**)

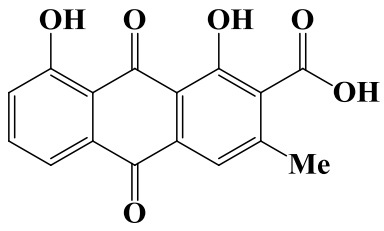


5'",6'"-Dehydroflavoglaucin (**38**)

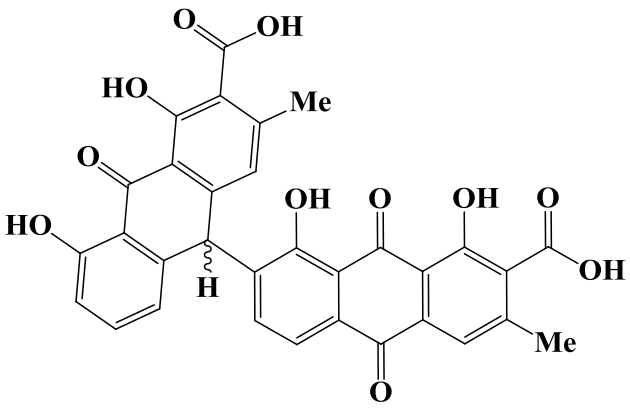
2.4.2 Phytochemistry of the Genus *Bulbine*

Only a few *Bulbine* species have been investigated for secondary metabolites, which show the presence of a variety of polyketide derivatives mainly anthraquinones (monomeric and dimeric), phenylanthraquinones and isofuranonaphthoquinones.

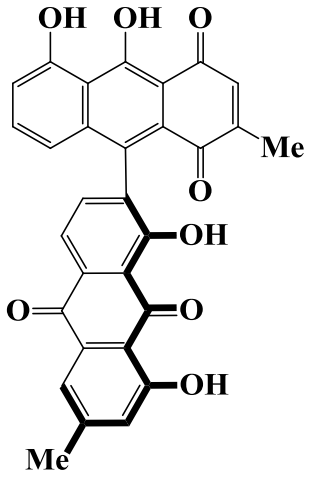
The monomeric anthraquinones; aloe-emodin (**20**) and chrysophanoic acid (**39**) were reported from the roots of *B. annua* (van Rheede *et al*.,1964). Whereas, the anthraquinone dimer, 7'-10-bichrysophanoic acid (**40**) was reported from the roots of *B. capitata* (Lusunzi and Karuso, 2001b). A study on the fruits of *B. abyssinica* resulted in the isolation and characterization of three new dimeric anthracene derivatives named abyquinones A (**41**), B (**42**), and C (**43**), by a combination of spectroscopic methods, chemical transformation, and quantum chemical CD calculations (Wanjohi *et al*., 2005).



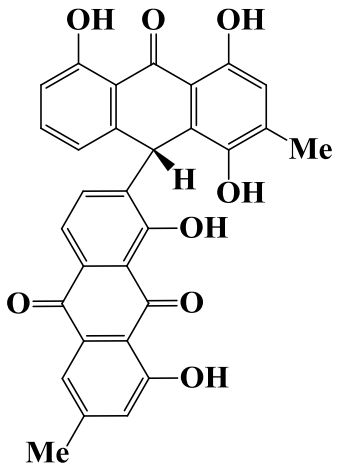
Chrysophanoic acid (**39**)



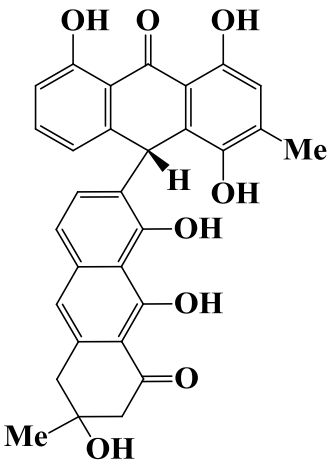
7'-10-Bichrysophanoic acid (**40**)



Abyquinone A (**41**)

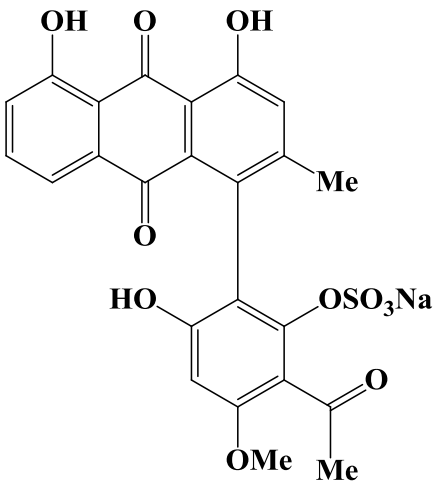


Abyquinone B (**42**)

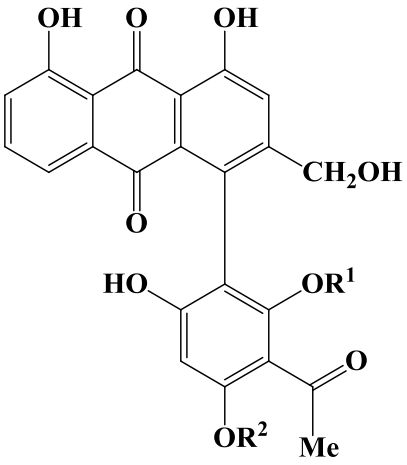


Abyquinone C (**43**)

The genus *Bulbine* also produces a variety of knipholone-type phenylanthraquinones, diferring in the substitution pattern at the hydroxyl groups of the acetylphloroglucinol part and/or the oxidation state of the anthraquinone moiety. Knipholone related phenylanthraquinones with antiplasmodial activity have been isolated from *B. capitata* and *B. abyssinica* (Bezabih *et al*., 1997; Bezabih and Abegaz, 1998; Bringmann *et al*., 2002a). Phytochemical studies on *B. frutescens* resulted in the isolation of knipholone (**14**), 6'-*O*-sulfate knipholone (**44**), gaboroquinones A (**45**) and B (**46**), 4'-*O*-demethylknipholone (**47**), 4'-*O*-demethylknipholone-4'-*β*-D-glucopyranoside (**48**) and isoknipholone (**28**) (Abegaz *et al*., 2002; Mutanyatta *et al*., 2005) with the latter two compounds showing remarkable antiplasmodial activities. Bulbine-knipholone (**49**) has been reported from the roots of *B. abyssinica* (Bringmann *et al*., 2002a).

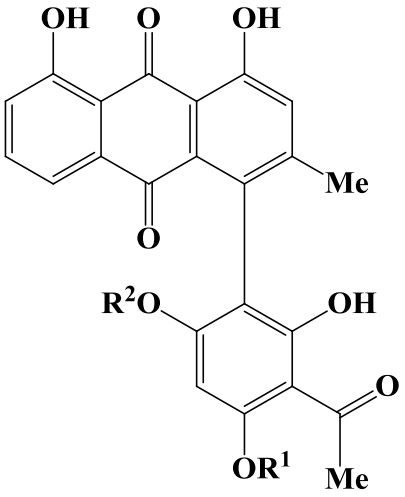


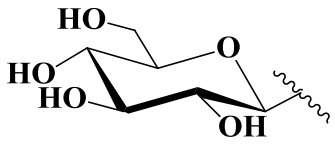
6'-*O*-Sulfate knipholone (**44**)



Gaboroquinone A (**45**) $R^1 = CH_3$, $R^2 = H$

Gaboroquinone B (**46**) $R^1 = H$, $R^2 = CH_3$



Glc = 

4'-*O*-demethylknipholone (**47**) $R^1 = R^2 = H$

4'-*O*-demethylknipholone-4'-β-D-glucopyranoside (**48**) R^1 = Glc, $R^2 = H$

Bulbine knipholone (**49**) $R^1 = H$, $R^2 = Me$

Mutanyatta *et al*. (2005) showed that phenylanthraquinones do not occur in enantiomerically pure form in *B. frutescens*, but rather as scalmic mixture with varing ratios (Table 2.3).

Table 2.3: Enantiomeric analysis of phenylanthraquinones in *B. frutescens* (Mutanyatta *et al*., 2005)

Compounds	Enantiomeric ratios	
	M (%)	P (%)
Knipholone (**14**)	60	40
Isoknipholone (**28**)	52	48
4'-*O*-Demethyl knipholone (**47**)	93	7
4'-*O*-Demethylknipholone-4'-β-D-glucopyranoside (**48**)	95	5

From *B. capitata* four isofuranonaphthoquinones (**50-53**) were reported (Bezabih *et al*., 1997), of **50** and **51** have also been reported from *B. frutescens* (Bringmann *et al*., 2008a). The related compounds, 4-*O*-methyleleutherol (**54**) and isoeleutherol (**55**), have been reported from the roots of *B. capitata* (Bezabih and Abegaz, 1998). The distribution of secondary metabolites in *Bulbine* species is summarized in Table 2.4.

Table 2.4: Compounds isolated from the genus *Bulbine*

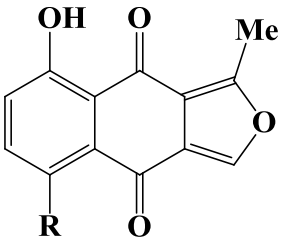
Compounds	**Species (plant part)**	**Reference**
Anthraquinones		
Aloe-emodin (**20**)	*B. annua* (L, R)	van Rheede *et al*., 1964
Chrysophanoic acid (**39**)	*B.asphodeloides* (R)	van Rheede *et al*., 1964
	B. capitata (R)	Lusunzi and Karuso, 2001b
7'-10-Bichrysophanoic acid (**40**)	*B. capitata* (R)	Lusunzi and Karuso, 2001b
Phenylanthraquinones		
Knipholone (**14**)	*B. abyssinica* (R)	Bringmann *et al*., 2002a
	B. capitata (R)	Lusunzi and karuso, 2001b
	B. capitata (Ap)	Bezabih and Abegaz, 1998
Knipholone anthrone (**15**)	*B. abyssinica* (R)	Bringmann *et al*., 2002a
4'-*O*-Dimethylknipholone (**47**)	*B. abyssinica* (R)	Bringmann *et al*., 2002a
Foliosone (**30**)	*B. capitata* (Fr)	Bezabih and Abegaz, 1998

Table 2.4: Compounds isolated from the genus *Bulbine*

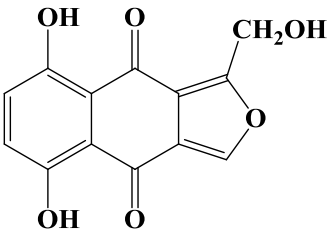
Compounds	**Species (plant part)**	**Reference**
4'-Demethylknipholone-4'-*O*-*β*-glucopyranoside (**48**)	*B. capitata* (R)	Lusunzi and karuso, 2001b
Bulbine knipholone (**49**)	*B. abyssinica* (R)	Bringmann *et al*., 2002a
Isoknipholone (**28**)	*B. capitata* (R)	Bezabih and Abegaz, 1998
Gaboroquinone A (**45**)	*B. capitata* (R)	Bezabih *et al*., 2001
Gaboroquinone B (**46**)	*B. capitata* (R)	Bezabih *et al*., 2001
Joziknipholone A (**32**)	*B. frutescence* (R)	Bringmann *et al.*, 2008a
Joziknipholone B (**33**)	*B. frutescence* (R)	Bringmann *et al.*, 2008a
Abyquinone A (**41**)	*B. abyssinica* (Fr)	Wanjohi *et al*., 2005
Abyquinone B (**42**)	*B. abyssinica* (Fr)	Wanjohi *et al*., 2005
Abyquinone C (**43**)	*B. abyssinica* (Fr)	Wanjohi *et al*., 2005
Furanonaphthanones		
1-Methyl-5-hydroxy-8-methoxynaphtho [2-3-C]-furan-4,9-dione (**50**)	*B. capitata* (R)	Bezabih *et al*., 2001
1-Methyl-8-hydroxy-5-methoxy naphtha[2-3-C]-furan-4,9-dione(**51**)	*B. capitata* (R)	Bezabih *et al*., 2001
5,8-Dihydroxy-1-hydroxy-methylnaphtho[2-3-C]-furan-4,9-dione(**52**)	*B. capitata* (R)	Bezabih *et al*., 2001
5,8-Dihydroxy-1-methylnaphtho[2-3-C]-furan-4,9-dione(**53**)	*B. capitata* (R)	Bezabih *et al*., 2001
Miscellaneous		
4-*O*-methyleleutherol (**54**)	*B. capitata* (R)	Bezabih and Abegaz, 1998
Isoeleutherol (**55**)	*B. capitata* (R)	Bezabih and Abegaz, 1998
Luteolin (**56**)	*B. capitata* (R)	Bezabih and Abegaz, 1998
Apigenin (**57**)	*B. capitata* (R)	Bezabih *et al*., 1997
5-Allyl-3-methoxybenzene-1,2-diol (**58**)	*B. capitata* (R)	Bezabih *et al*., 1997

(R = Root, L = Leaf, Ap = Arial part, Fr = Fruit)

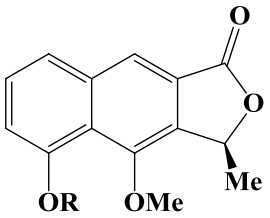
The flavonoids (**56** and **57**) along with the phenyl propanoid derivatives, **58** are among the minor compounds of *Bulbine* (Table 2.4).



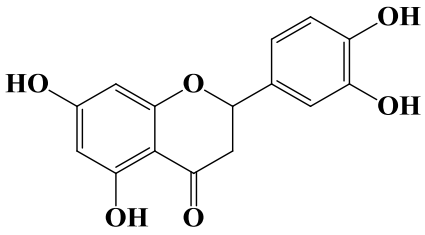
8-Hydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**50**) R = H
5,8-Dihydroxy-l-methylnaphtho-[2,3-c]furan-4,9-dione (**51**) R = OH
8-Hydroxy-5-methoxy-l-methylnaphtho-[2,3-c]furan-4,9-dione (**52**) R = OMe



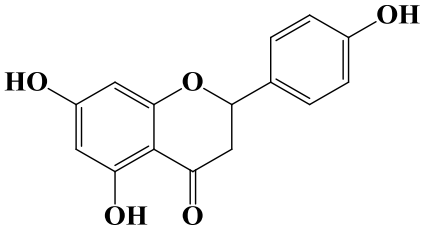
5,8-Dihydroxy-l-hydroxymethylnaphtho-[2,3-c]furan-4,9 dione (**53**)



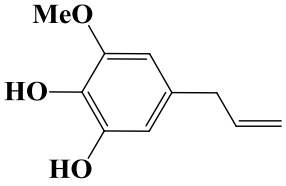
Isoeleutherol (**54**) R = H
4-*O*-methyleleutherol (**55**) R = Me



Luteolin (**56**)



Apigenin (**57**)



5-Allyl-3-methoxybenzene-1,2-diol (**58**)

2.4.3 Phytochemistry of the Genus *Aloe*

The genus *Aloe* is the most extensively investigated genus among the 17 genera of the family Asphodelaceae. This may be due to its well documented medicinal values and chemotaxonomic interests. Most of the compounds so far reported from *Aloe* are quinone derivatives, mainly pre-anthraquinones, anthraquinones, anthrones and naphthoquinones. Other compounds including chromones, phenylpyrones, flavonoids, alkaloids and steroids have also been reported.

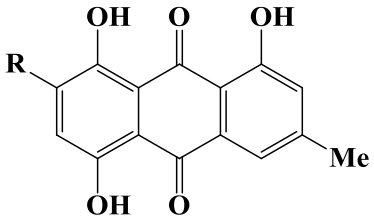
2.4.3.1 Anthraquinones

The anthraquinone derivatives found in *Aloe* are generally of two types, 1,8-dihydroxy-3-methyl anthraquinone and 3,8-dihydroxy-1-methyl anthraquinone derivatives. These two groups of anthraquinone derivatives appear to have been formed by two parallel biosynthetic routes (Scheme 2.2) differing by the way the octaketide chain is folded (Leisterner, 1971; Manito, 1981).

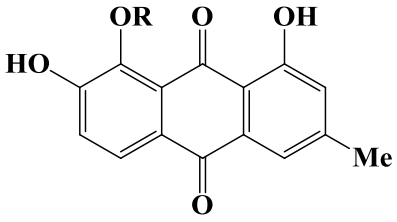
The 1,8-dihydroxy-3-methyl anthraquinone derivatives are chrysophanol-based where the cyclization of an octaketide chain has folded in the common way. Anthraquinones of this-type are known to occur both in leaves and roots of *Aloe* species. Chrysophanol (**18**) and related anthraquinone derivatives such as helminthosporin (**59**) and isoxanthorin (**60**) are detected in both roots and leaves of most of the *Aloe* species surveyed (Yagi *et al*., 1977a; Dagne and Alemu, 1991; Thomson, 1987). Whereas, aloe-emodin (**20**), nataloe-emodin (**61**), nataloe-emodin-8-methyl ether (**62**), including its *O*-glycosides, *O*-α-L-rhamnopyranosylaloe-emodin (**63**) and nataloe-emodin-2-*O*-β-D-glucopyranoside (**64**), are the main constituents of the leaves of *Aloe* species (van Wyk *et al*., 1995b; Conner *et al*., 1987; 1989). A dimeric anthraquionone, asphodelin (**23**) has been reported from subterranean stem of *A. saponaria* (Yagi *et al*., 1978a).

On the other hand, the rare 3,8-dihydroxy-1-methylanthraquinone derivatives where the cyclization of an octaketide chain has folded in an unusual way, as in aloesaponarin II (**65**), are confined to roots of these plants (van Wyk *et al*., 1995b). Aloesaponarin I (**66**), laccaic acid D methyl ester (**67**) and deoxyerythrolaccin (**68**) were reported to have anti-microbial activities (Yagi *et al*., 1973) and occur in the roots of many *Aloe* species including *A. saponaria* (Dagne *et al*., 2000). It is worth noting that, apart from genus *Aloe* and the related genus *Lomathophyllum*

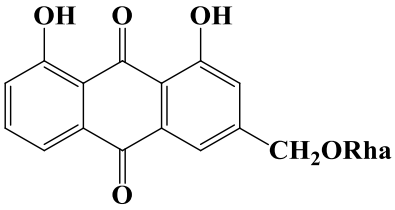
(van Wyk *et al*., 1995c), there is no report of such anthraquinone derivatives from any other sources.



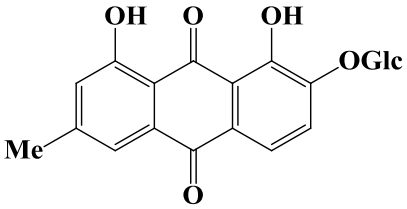
Helminthosporin (**59**) R = H
Isoxanthorin (**60**) R = OMe



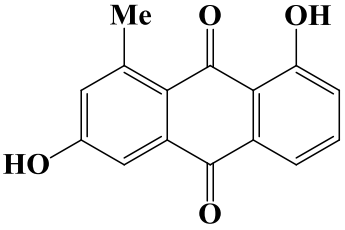
Nataloe-emodin (**61**) R = H
Nataloe-emodin-8-methyl ether (**62**) R = Me



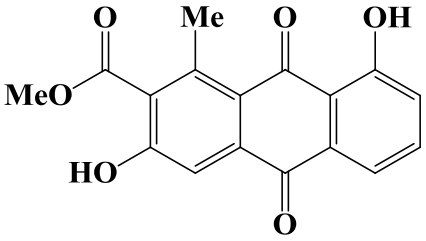
O-α-L-Rhamnopyranosylaloe-emodin (**63**)



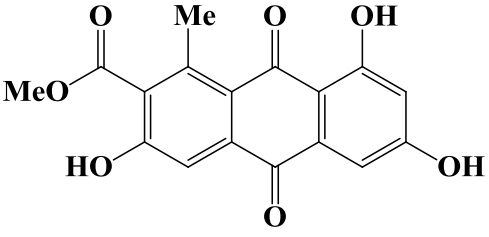
Nataloe-emodin-2-*O*-β-D-Glucopyranoside (**64**)



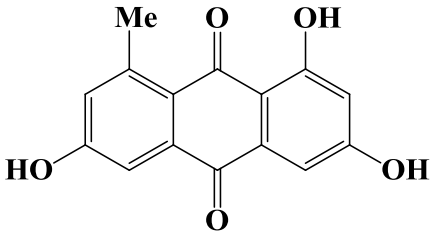
Aloesaponarin II (**65**)



Aloesaponarin I (**66**)



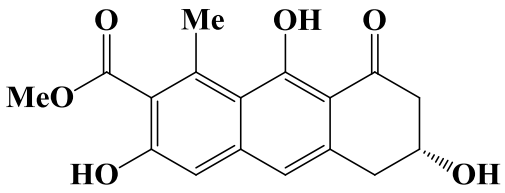
Laccaic acid D methyl ester (**67**)



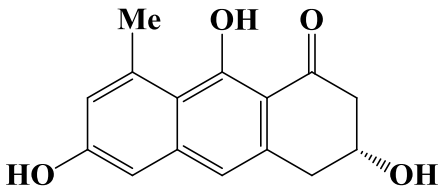
Deoxyerythrolaccin (**68**)

2.4.3.2 Pre-anthraquinones

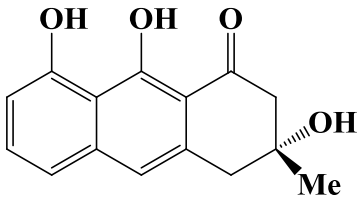
Pre-anthraquinones are considerded to be the progenitors (pre-stage of the anthraquinone derivatives) and they readily convert to the corresponding anthraquinones upon treatment with bases (Yenesew *et al*., 1993). Aloesaponol I (**69**) and aloesaponol II (**70**) occur in the roots and subterranean parts of many *Aloe* species (Dagne *et al*., 2000; Dagne *et al*., 1996). The precursor of chrysophanol, pre-chrysophanol (**71**) was reported from the subterranean parts of *Aloe graminicola* (Yenesew *et al*., 1993). Whereas aloesaponol III (**16**), aloesaponol IV (**72**), aloesaponol I-6-*O*-glucopyranoside (**73**), aloesaponol II-6-*O*-glucopyranoside (**74**), aloesaponol III-8-*O*-glucopyranoside (**75**) were isolated from subterranean parts of *A. saponaria* (Yagi *et al*., 1977a; b); aloesaponol III (**16**) has also been reported from the roots of *K. foliosa* (Yenesew *et al*., 1993). From the dark brown callus tissue of *A. barbadensis* leaves, two pre-anthraquinone glucosides; aloesaponol III-4-*O*-glucopyranoside (**76**) and aloesaponol IV-4-*O*-glucopyranoside (**77**) were isolated (Dagne *et al*., 2000). The wide presence of pre-anthraquinone derivatives in particular, aloesaponol I (**69**) and aloesaponol II (**70**) only in the genus *Aloe*, indicated that these compounds are characteristic constituents of the genus. Chemotaxonomic studies have also shown that this type of compounds are conspicously absent from the roots of closely related genera including *Kniphofia* and *Bulbine* (Dagne and Yenesew, 1994).



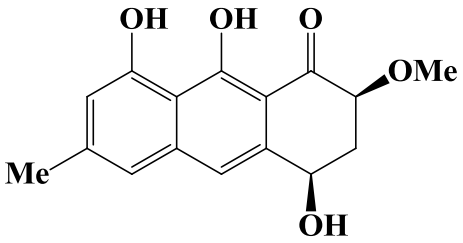
Aloesaponol I (**69**)



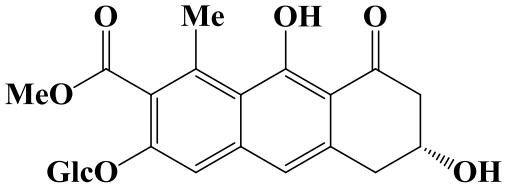
Aloesaponol II (**70**)



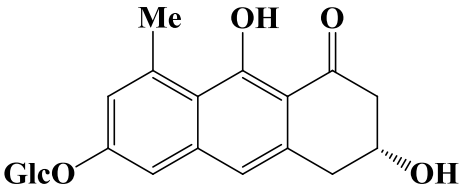
Pre-chrysophanol (**71**)



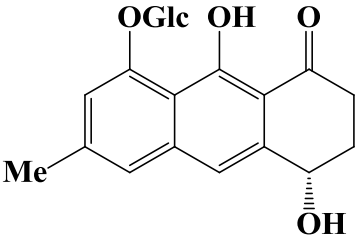
Aloesaponol IV (**72**)



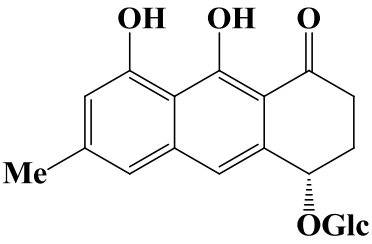
Aloesaponol I-6-*O*-glucopyranoside (**73**)



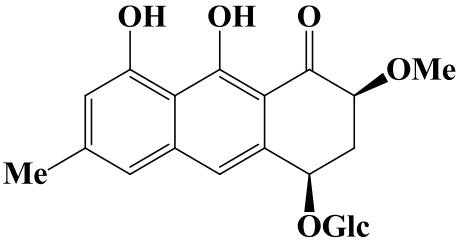
Aloesaponol II-6-*O*-glucopyranoside (**74**)



Aloesaponol III-8-*O*-glucopyranoside (**75**)



Aloesaponol III-4-*O*-glucopyranoside (**76**)

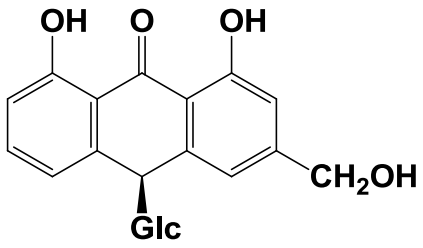


Aloesaponol IV-4-*O*-glucopyranoside (**77**)

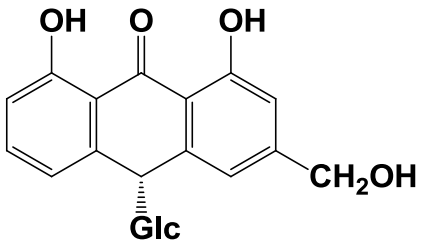
2.4.3.3 Anthrones

Anthrones are considered to be the biosynthetic precursors of anthraquinones *via* simple oxidation (Yagi *et al*., 1973). The *C*-glycosylated anthrones (e.g. barbaloin) are the most important classes of compounds found in *Aloe* species and are believed to be mainly responsible

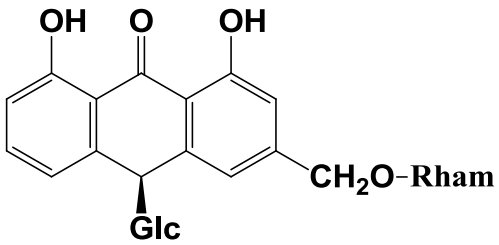
for the bitter and purgative properties of the well known commercial *Aloe* drug. Barbaloin was first isolated from *A. barbadensis* (Reynolds, 1985; Dagne *et al*., 2000), and is a collective name of aloin A (**78**) and B (**79**), two diastereomeric *C*-glycosides that differ in the configuration at C-10 of the anthrone moeity. The study on *A. aborescens*, showed that barbaloin is concentrated on the top of each leaf (near the apex) than those at the lower parts of the branch (Chauser-Volfson and Gutteman, 1996). In general, the anthrones with *C*-glycosides were reported as the major constituents of the leaves of *Aloe* species (Reynolds, 1985; McCarthy, 1969). Aloinoside A (**80**), aloinoside B (**81**), 10-hydroxyaloin A (**82**), 10-hydroxyaloin B (**83**), chrysophanolanthrone (**84**), aloe-emodinanthrone (**85**), nataloin (**86**), homonataloin (**87**), 8-*O*-methyl-7-hydroxyaloin A (**88**) and 8-*O*-methyl-7-hydroxyaloin B (**89**) are among some of the anthrones reported from *Aloe* species (Dagne *et al*., 2000; Chen *et al*., 2012). The rare anthraquinone-anthrone dimer, bianthracene II (**90**) and bianthracene IV (**91**) are also reported from subterranean parts of *A. saponaria* (Yagi *et al.,* 1978a). The glycosylated anthraquinone/anthrone derivatives, elgonicardine (**92**) were reported from the roots of *A. elgonica* (Conner *et al*., 1990).



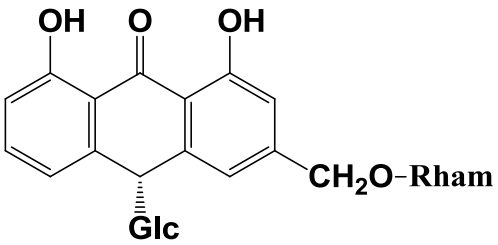
Aloin A (**78**)



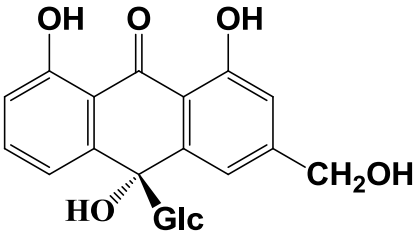
Aloin B (**79**)



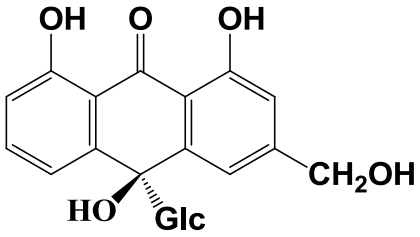
Aloinoside A (**80**)



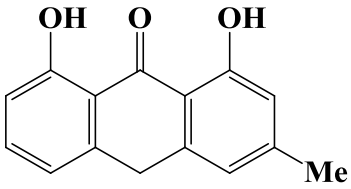
Aloinoside B (**81**)



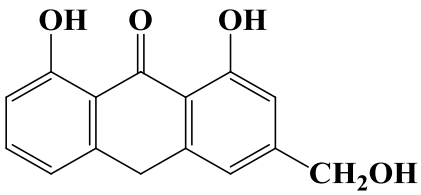
10-Hydroxyaloin A (**82**)



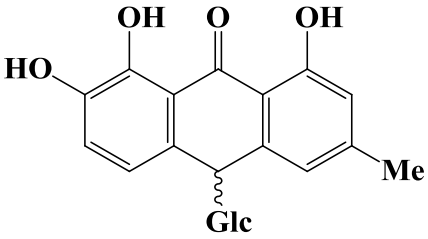
10-Hydroxyaloin B (**83**)



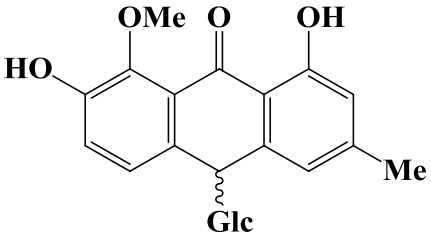
Chrysophanolanthrone (**84**)



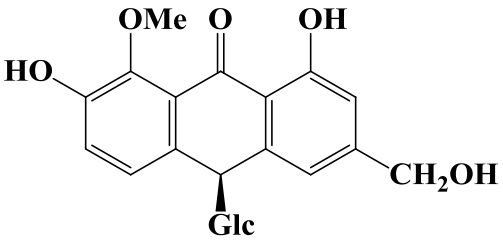
Aloe-emodinanthrone (**85**)



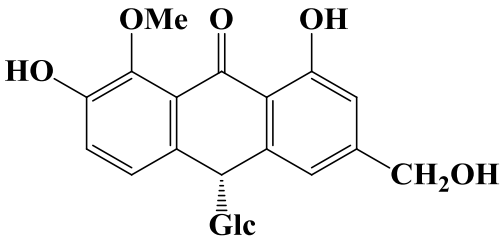
Nataloin (**86**)



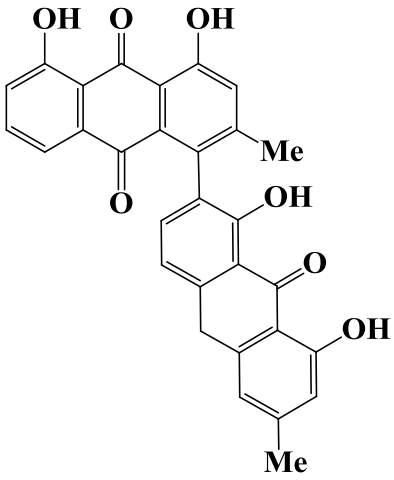
Homonataloin (**87**)



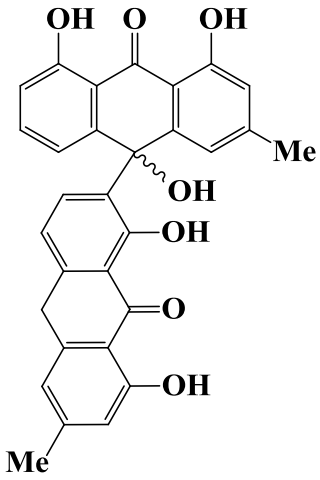
8-*O*-Methyl-7-hydroxyaloin A (**88**)



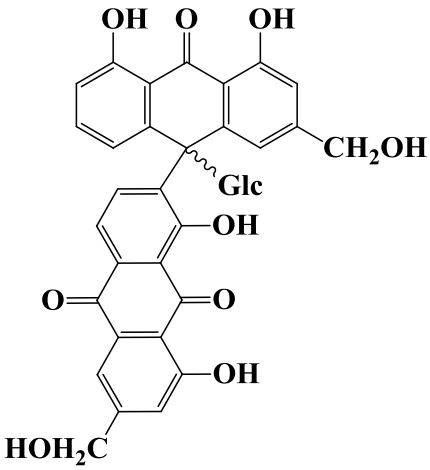
8-*O*-Methyl-7-hydroxyaloin B (**89**)



Bianthracene II (**90**)



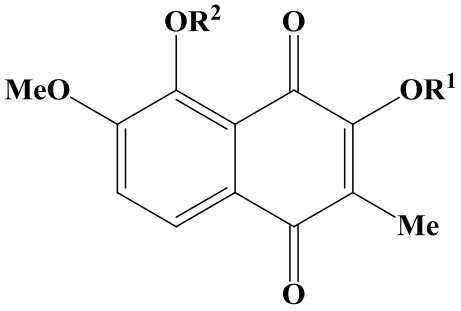
Bianthracene IV (**91**)



Elgonicardine (**92**)

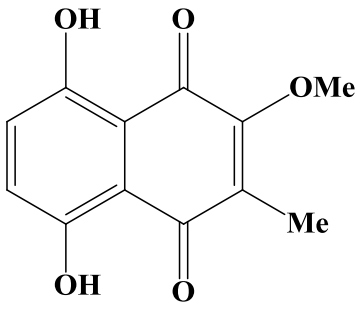
2.4.3.4 Napththoquinones

The plant families well known for producing naphthoquinones are Droseraceae (Zenk *et al*., 1969; Culham and Gornall, 1994; Sidhu *et al.,* 1974; Kreher *et al*., 1990), Bignoniaceae (Itokawa *et al*., 1992), Ebenaceae (Zhong *et al*., 1984; Zakaria *et al*., 1984), Juglandaceae (Binder *et al*., 1989), Nepenthaceae (Likhitwitayawuid *et al*., 1998) and Plumbagnaceae (Dinda *et al*., 1995; 1998). Induli *et al*. (2012) reported three 1,4-naphthoquinone derivatives; 5-hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione (**93**), 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) and ancistroquinone C (**95**) from the roots of *Aloe secundiflora*. This is the only report on the occurrence of such naphthoquinones in the genus *Aloe* and probably from the family Asphodelaceae.



5-Hydroxy-3,6-dimethoxy-2-methylnaphthalene-1,4-dione (**93**) R^1 = Me, R^2 = H

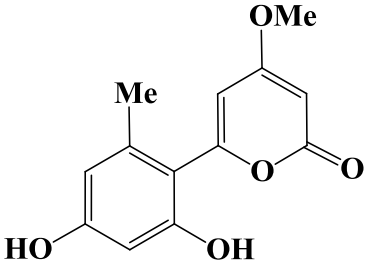
5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) R^1 = H, R^2 = Me



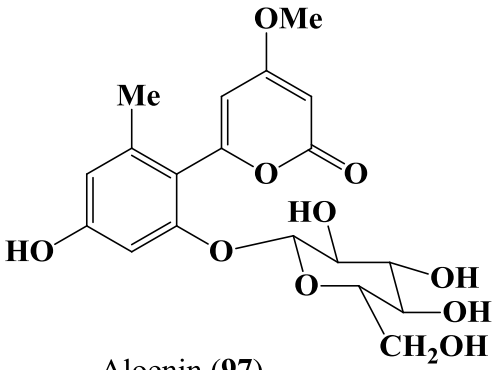
Ancistroquinone C (**95**)

2.4.3.5 Other Secondary Metabolies Isolated from *Aloe*

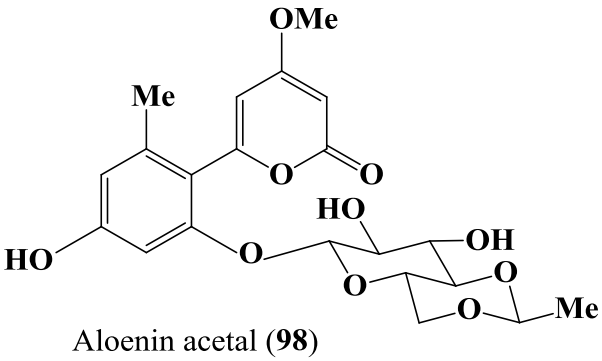
Some pyrones (**96-100)**, chromones (**101-108**), flavonoids (**109-112**), steroids (**113-116**) and alkaloids (**117-120**) are known to occur in the genus *Aloe*.



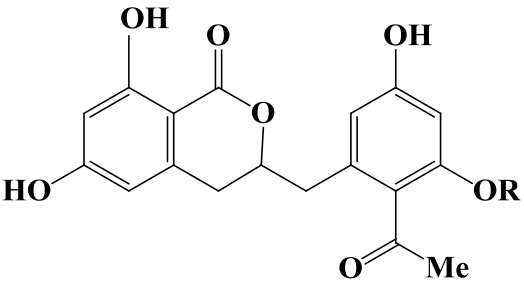
Aloenin aglycone (**96**)



Aloenin (**97**)

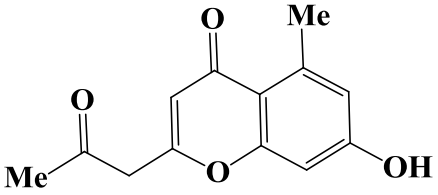


Aloenin acetal (**98**)

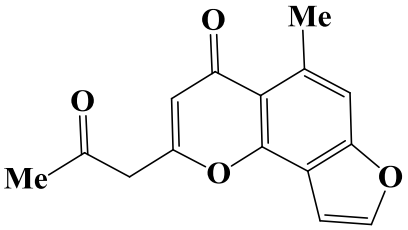


Feralolide (**99**) R = H

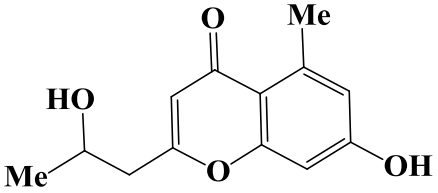
Dihydroisocoumarin glucoside (**100**) R = Glc



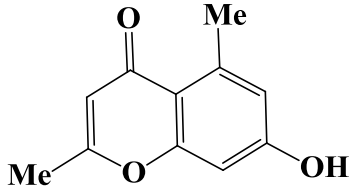
Aloesone (**101**)



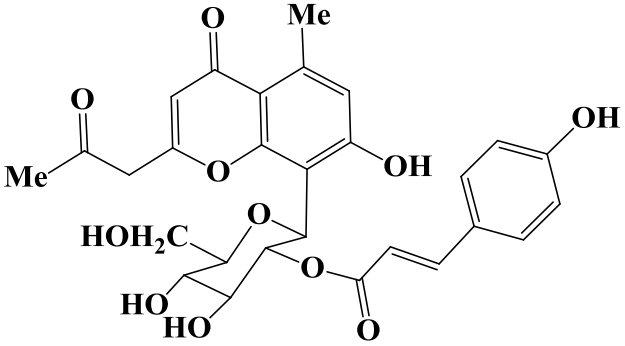
Furoaloesone (**102**)



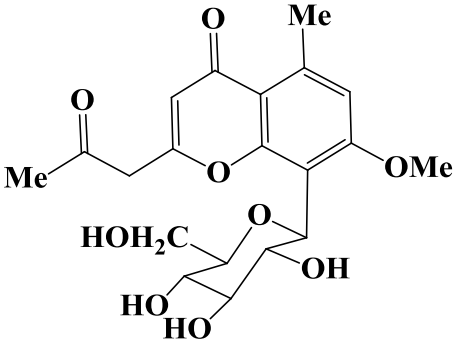
Aloesol (**103**)



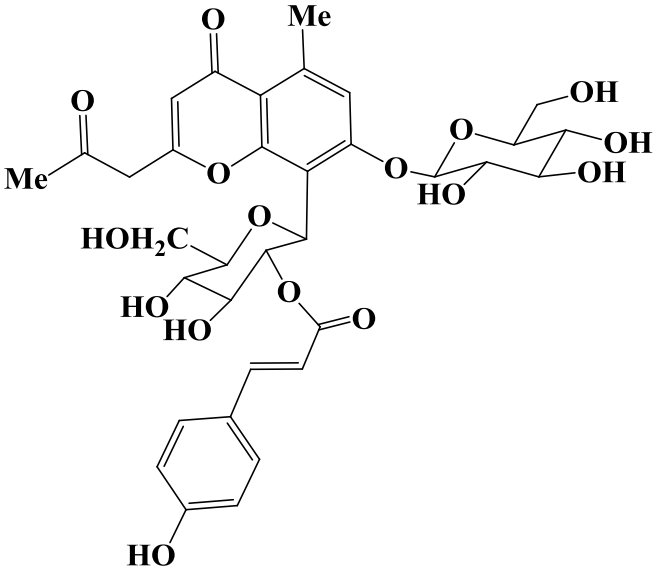
7-Hydroxy-2,5-dimethylchromone (**104**)



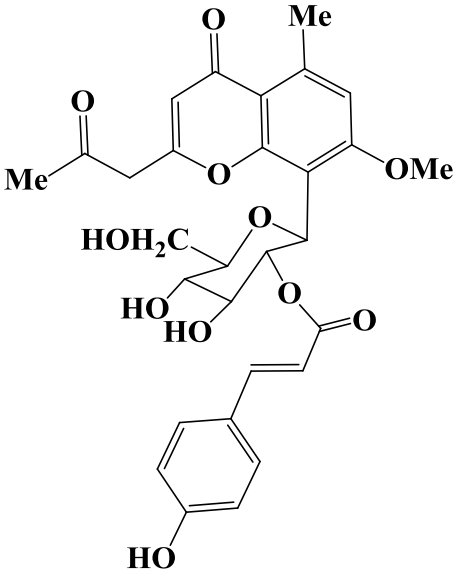
Aloeresin A (**105**)



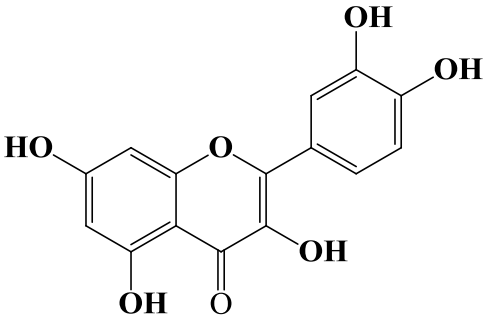
Aloeresin B (**106**)



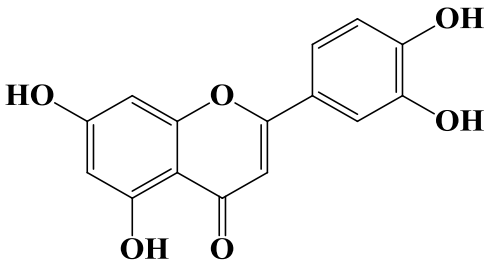
Aloeresin C (**107**)



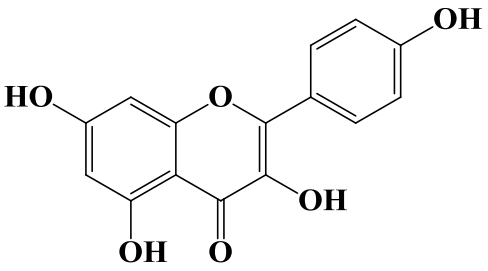
Aloeresin D (**108**)



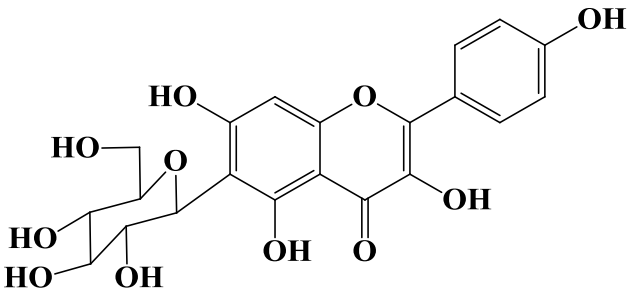
Quercetin (**109**)



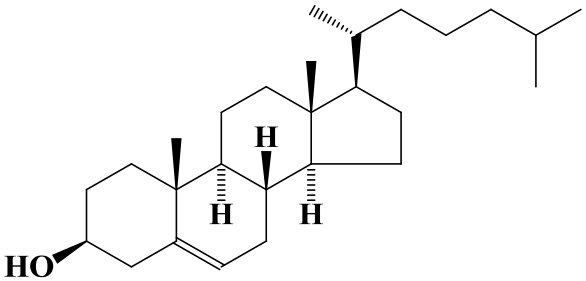
Luteolin (**110**)



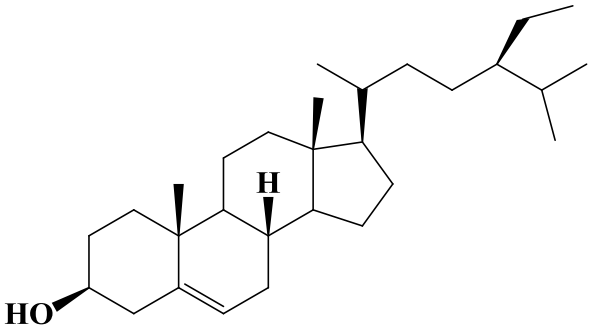
Kaempferol (**111**)



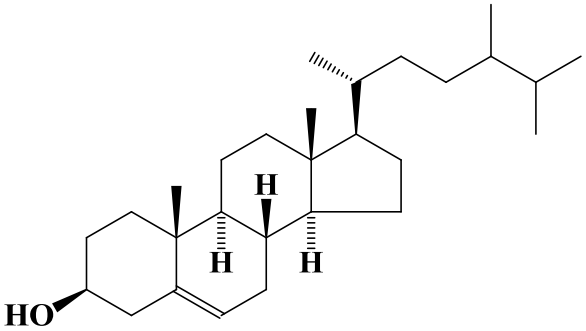
Isovitexin (**112**)



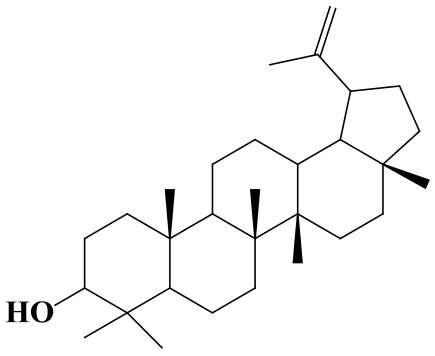
Cholesterol (**113**)



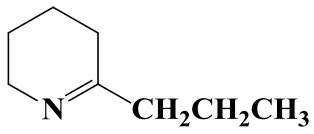
β-sitosterol (**114**)



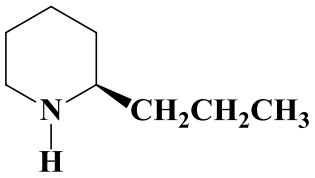
Campesterol (**115**)



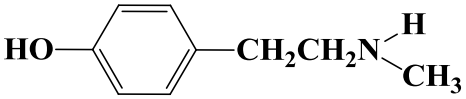
Lupeol (**116**)



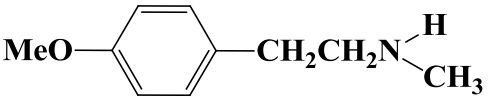
Coniceine (**117**)



Coniine (**118**)



N-Methyltyramine (**119**)



O, *N*-Dimethyltyramine (**120**)

2.5 Biosynthesis of Quinones of the Asphodelaceae

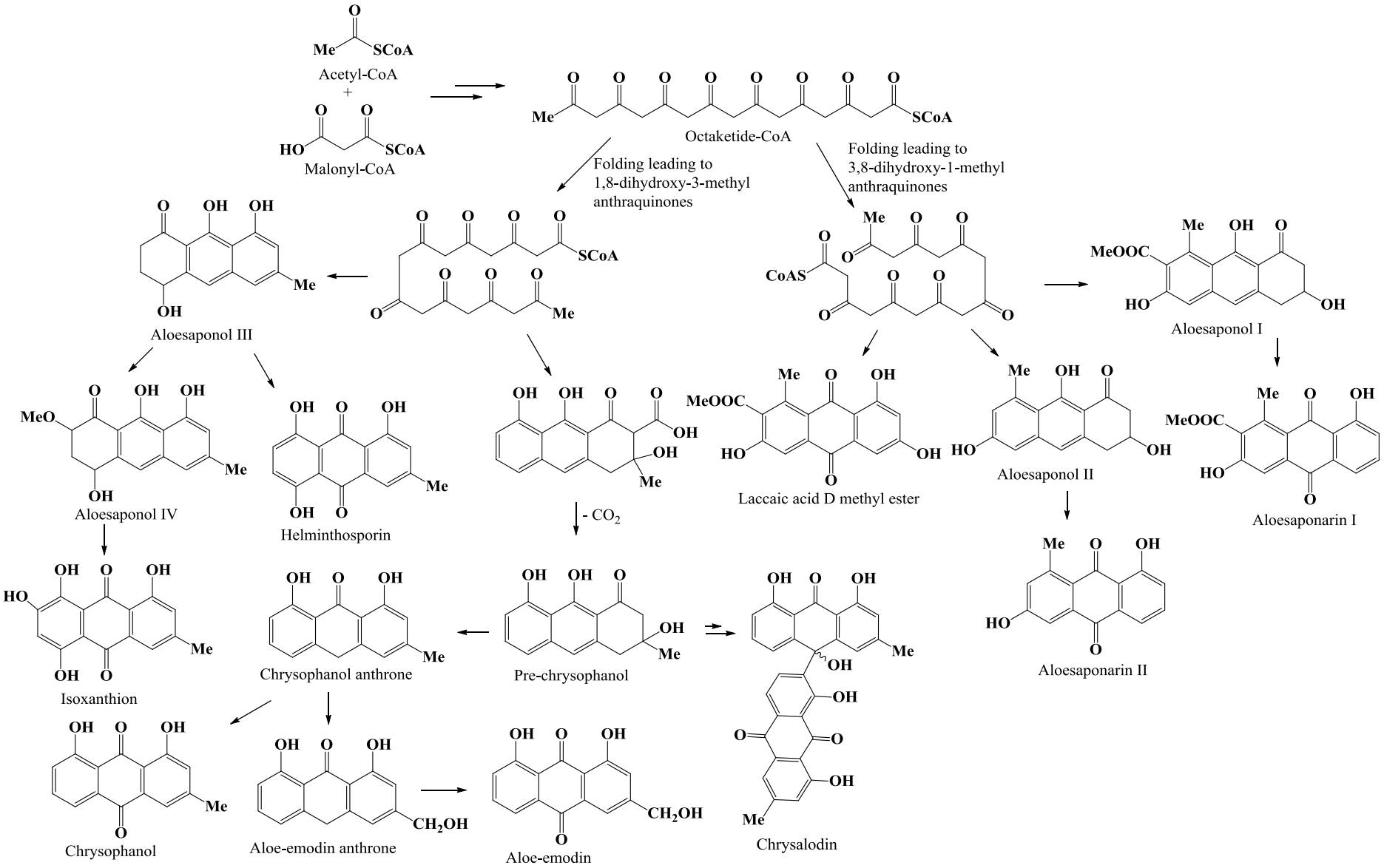
The quinones of this family including anthraquinones, phenylanthraquinones and naphthoquinones are all derived through the polyketide pathway.

2.5.1 Biosynthesis of Anthraquinones in Asphodelaceae

In a living cell, transformation and interconversion of a number of organic compounds is needed to enable organisms to live, reproduce and grow. For this purpose, systematic metabolic pathways involving an integrated network of enzyme-mediated and carefully regulated chemical reactions are used. Thus, different metabolic pathways are required to synthesize specialized molecules from the basic compounds. One of the metabolic pathways leading to the synthesis of large classes of natural products, mainly anthraquinones is the acetate pathway, also refered to as the polyketide pathway (Leistner, 1971). This pathway, governed by the polyketide synthase enzyme, generates an amazing array of natural products by catalyzing condensation of a number of acetyl units from malonyl-CoA into a growing polyketide chain (Dewick, 2002; Austin and Noel, 2003). The large number of natural products produced by this pathway may be due to the

number of acetyl units used, variation in the folding of the polyketide chain and mechanism of ring formation.

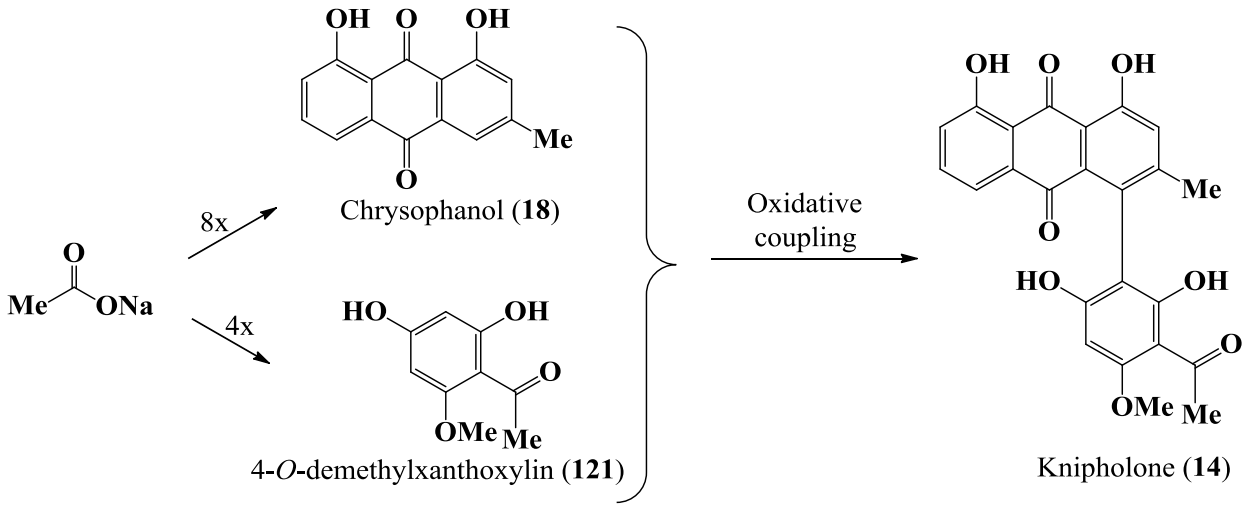
The acetyl unit (starter group) condenses stepwise with molecules of malonyl-CoA (extension units) to give a linear octaketide intermediate which can be folded (Scheme 2.2) in at least two different ways to provide either 1,8-dihydroxy-3-methylanthraquinone or 3,8-dihydroxy-1-methylanthraquinone derivatives (van den Berg and Labadie, 1989). In both pathways, after the polyketide chain has folded, the ring at the centre of the fold is formed first, followed by the next two rings. Decarboxylation appear to occur before complete aromatization take place leading to the formation of pre-anthraquinone intermediates such as atrochrysone carboxylic acid and aloesaponols. The pre-anthraquinones dehydrate to form the anthrones which subsequently undergo extra oxygenation to produce anthraquinones *via* oxidation. The biosynthetic studies by Yagi *et al*. (1978b) have shown that aloesaponarin I is derived from the pre-anthraquinone, aloesaponol I, while laccaic acid-D methyl ester is derived through a parallel route.



Scheme 2.2: Proposed biogenetic pathway leading to some anthraquinones (Dewick, 2002)

2.5.2 Biosynthesis of Phenylanthraquinones

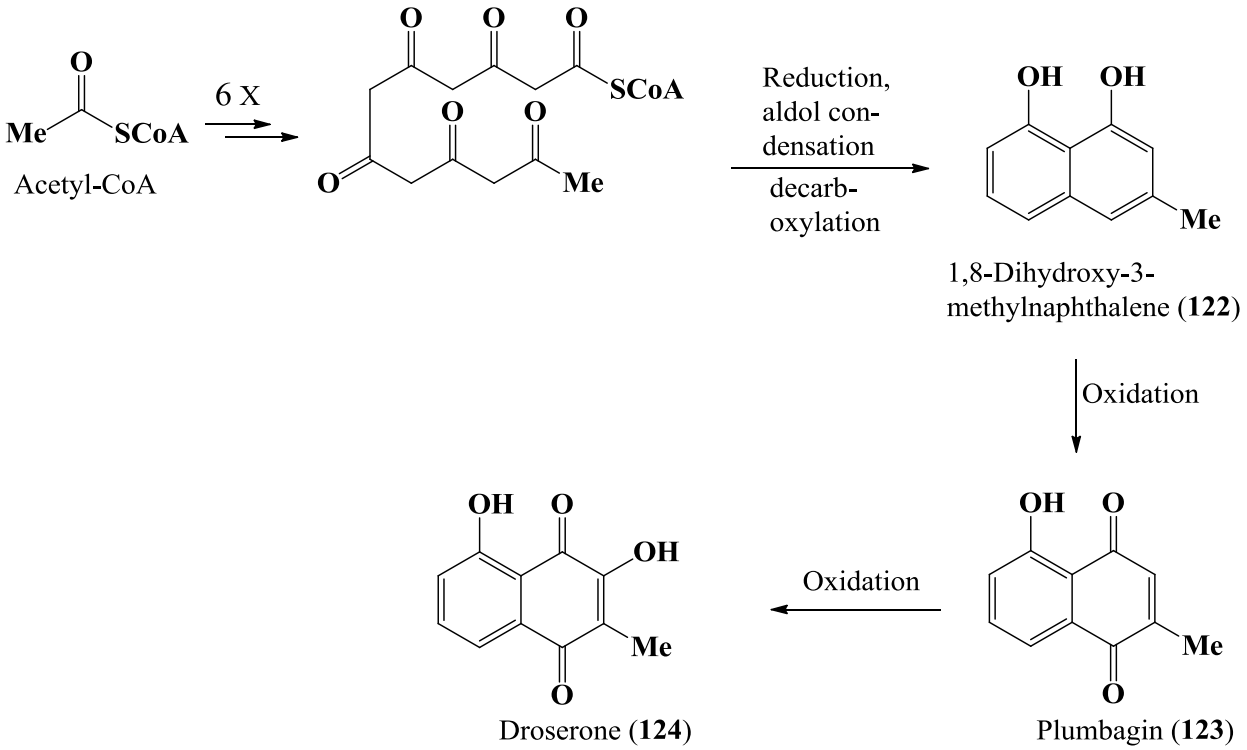
Phenylanthraquinones consist of two different aromatic portions; both biosynthetically postulated to be formed *via* apparently divergent polyketidic pathways. The biosynthesis of the most abundant phenylanthraquinones, knipholone (**14**), was investigated through feeding experiments with labeled precursors to sterile plant cultures (Bringmann *et al*., 2007b). This study showed that both halves of the phenylanthraquinones are built from polyketide chains and are coupled at a late stage to give knipholone (Bringmann *et al*., 2007b; 2008d). The acetophenone part of the molecule originated from four acetate units whereas the chrysophanol portion is drived from eight acetate units (Scheme 2.3). It has also been shown that *O*-methylation *ortho* to the acetyl group occurred before the phenol-oxidative coupling, which indicates that 4,6-dihydroxy-2-methoxyacetophenone (but not 2,4,6-trihydroxyacetophenone) is the substrate that leads towards the formation of knipholone and related phenylanthraquinone derivatives (Bringmann *et al*., 2007b).



Scheme 2.3: Biosynthesis of the phenylanthraquinone, knipholone (Bringmann *et al*., 2007b)

2.5.3 Biosynthesis of Naphthoquinones

Naphthoquinones of the Asphodelaceae appear to have been formed through the polyketide pathway through condensation of six acetyl-CoA units. The biosynthesis of droserone was determined by feeding experiments with labeled precursors to sterile plant cultures which unambiguously showed its acetogenic origin (Scheme 2.4) (Bringmann *et al*., 2000).



Scheme 2.4: Biosynthesis of the naphthoquinone, droserone (Bringmann *et al*., 2000)

2.6 Antiplasmodial Activity of Compounds from the Asphodelaceae

Several plants from the family Asphodelaceae are widely used in traditional medicine in East Africa and in some other parts of the world. Many of them are used in treatment of a range of ailments but only a few species from this family have been phytochemically studied. The antiplasmodial properties of anthraquinones of this family are most notable (Table 2.5). The dimeric anthraquinones, chrysalodin (1**2**) (IC_{50} 0.26 µg/mL) and chryslandicin (**13**) (IC_{50} 0.54

μg/mL), isolated from the root of *K. foliosa,* showed high inhibition of the growth of the malaria parasite, *P. falciparum* (Wube *et al*., 2005). Phenylanthraquinones exhibit a wide range of pharmacological activity including antiplasmodial properties (Table 2.4). Knipholone (IC_{50} 0.65 μg/mL) and knipholone anthrone (IC_{50} 0.38 μg/mL), first isolated from the roots of *K. foliosa*, showed considerable *in vitro* antiplasmodial activity against the chloroquine resistant K1 and the chloroquine-sensitive NF54 strains of *Plasmodium falciparum* (Bringmann *et al*., 1999a). Interestingly the monomeric units, 4-*O*-demethylxanthoxylin (**121**) and chrysophanol (**18**) that constitute the phenylanthraquinones exhibit no activity against malaria parasites individually (Bringmann *et al*., 2002a). Moreover, the dimeric phenylanthraquinones, joziknipholone A (IC_{50} 0.14 μg/mL) and B (IC_{50} 0.23 μg/mL) have also showed remarkable antiplasmodial activity against the K1 (chloroquine-resistant) and NF54 (chloroquine-sensitive) strains of *P. falciparum* (Bringmann *et al*., 2008a).

Phenylanthraquinones have also showed activity against human tumor cell lines (Reid, 1993). Habtemariam, (2010) reported that knipholone (**14**) induces a rapid onset of cytotoxicity (IC_{50} 0.5 - 3.3 μM). Knipholone and its derivatives are potentially a new class of natural anticancer agents with a wide range of toxicological and pharmacological implications. It is also important to point out that knipholone anthrone (**15**) has antioxidant activity by preventing deoxyribose degradation *via* hydroxyl radicals (Habtemariam, 2007; 2010).

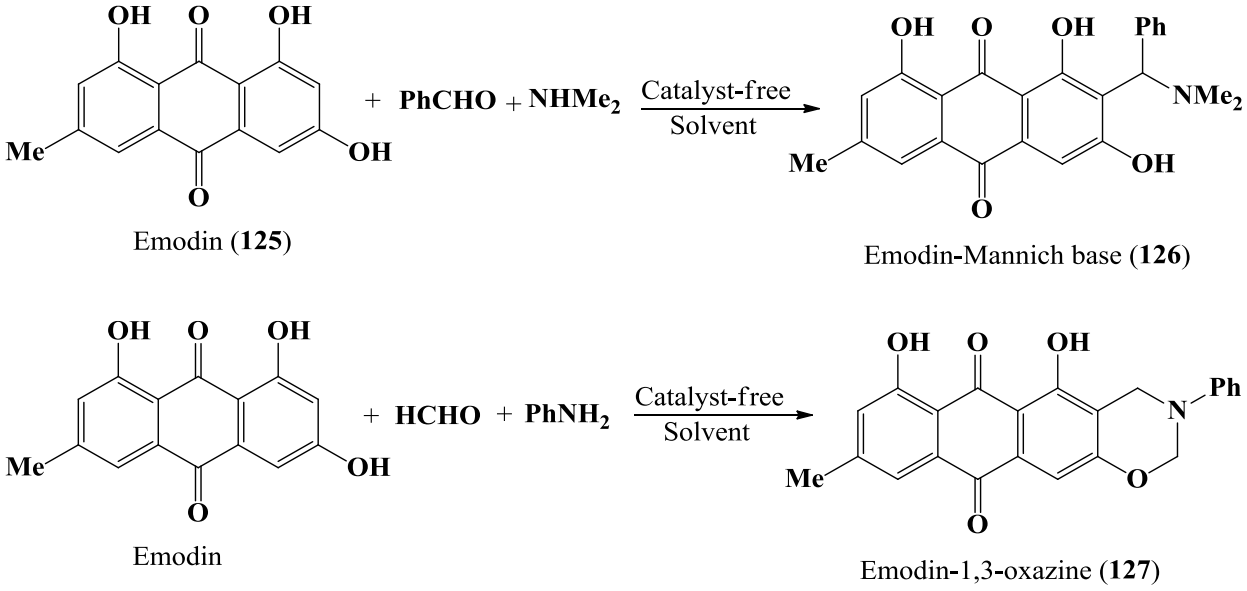
Table 2.5: Antiplasmodial activity of natural anthraquinones against the K1 and NF54 strains of *P. falciparum*

Compounds	IC_{50} µg mL^{-1} (K1)	IC_{50} µg mL^{-1} (NF54)	Reference
Chrysalodin (**12**)	0.26	-	Wube *et al*., 2005
Chryslandicin (**13**)	0.54	-	Wube *et al*., 2005
Knipholone (**14**)	0.67	0.91	Abegaz *et al*., 2002
Knipholone anthrone (**15**)	0.38	0.42	Bringmann *et al*., 1999a
Chrysophanol (**18**)	>5.00	-	Bringmann *et al*., 2002a
Aloe-emodin (**20**)	>5.00	-	Bringmann *et al*., 2002a
Isoknipholone (**28**)	0.12	-	Mutanyatta *et al*., 2005
Joziknipholone A (**32**)	0.14	-	Bringmann *et al*., 2008a
Joziknipholone B (**33**)	0.23	-	Bringmann *et al*., 2008a
6'-*O*-sulfate knipholone (**44**)	>5.00	-	Mutanyatta *et al*., 2005
Gaboroquinone A (**45**)	4.80	4.20	Abegaz *et al*., 2002
Gaboroquinone B (**46**)	>5.00	>5.00	Abegaz *et al*., 2002
4-*O*-Demethylknipholone (**47**)	1.80	1.55	Bringmann *et al*., 1999a
4-*O*-Demethylknipholone-4-*O*-*ß*- D-glucopyranoside (**48**)	0.41	0.43	Abegaz *et al*., 2002
Bulbine knipholone (**58**)	0.71	-	Bringmann *et al*., 2002a
4-*O*-demethylxanthoxylin (**121**)	>5.00	-	Bringmann *et al*., 2002a

- Not tested

2.7 Synthesis of Mannich Bases

Synthesis using natural products as starting scaffolds is a key component in drug discovery because of the rich structural diversity nature provides (Balthaser *et al*., 2011). It is also postulated that, the modification of existing active molecules offers cost effective and convenient strategy to improve bioactivity (Subramaniapillai, 2013). Mannich reaction offers a convenient method for introducing an amino-alkyl chain, which is believed to alter the biological profile of drugs (Tramontini, 1973). This reaction is among the most useful carbon-carbon bond formation reactions in organic synthesis, mainly focusing on the condensation of enolizable ketones with formaldehyde and amine derivatives. Mannich-type reactions of hydroxyanthraquinones have also been reported recently (Scheme 2.5) (Zhao *et al*., 2013) showing that such reactions on phenylanthraquinones my result in more active derivatives.



Emodin (**125**)

Emodin-Mannich base (**126**)

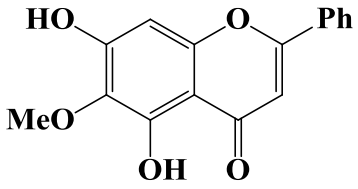
Emodin

Emodin-1,3-oxazine (**127**)

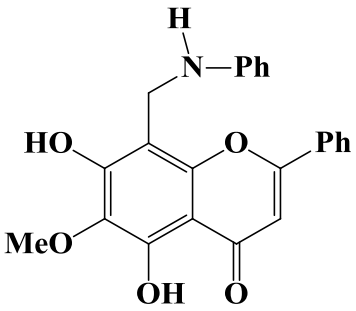
Scheme 2.5: Preparation of emodin Mannich base and 1,3-oxazine

Mannich reaction has played a significant role in synthesizing bioactive compounds. In several instance; the Mannich derivatives showed better activity, solubility and bioavailability than the

parent molecules (Subramaniapillai, 2013). For example, the 8-aminophenyl derivatives of oroxylin-A (**129**), synthesized by Mannich reaction, showed more *α*-glucosidase inhibitory activity than the parent molecule, oroxylin-A (**128**) (Babu *et al*., 2008).

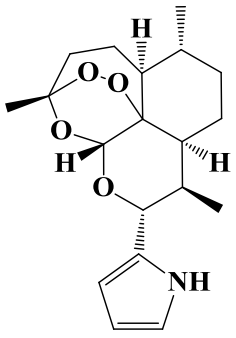


Oroxylin-A (**128**)

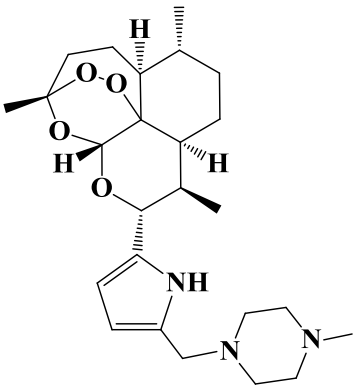


8-Aminophenyl oroxylin-A (**129**)

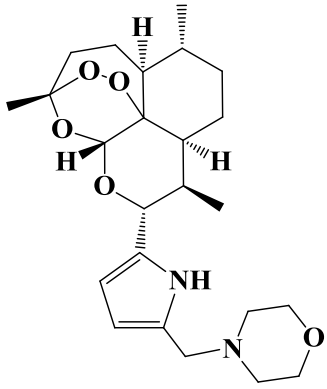
The chemotherapeutic application of the antimalarial drug, artemisinin (**4**) is also limited due to its poor water solubility. Although artemisinin derivatives (such as dihydroartemisinin, artemether, arteether and sodium artesunate) exhibited significant improvement in solubility, they are easily removed from the blood stream and hence lead to the resurgence of the parasite. Pacorel *et al*. (2010) synthesized artemisinin Mannich derivatives **130** and **131**, from C-10-*α*-pyrroleartemisinin **129**. The Mannich derivatives showed better solubility and so does bioavailability when compared to the non-basic derivatives.



Pyrroleartemisinin (**129**)



Pyrroleartemisinin derivative **130**



Pyrroleartemisinin derivative **131**

CHAPTER THREE

MATERIAL AND METHODS

3.1 General

Melting points were recorded on Butch Melting point B-540, Switzerland. Column chromatography (CC) and flash chromatography were carried out on silica gel (0.06-0.2 mm, Merck) impregnated with 3% aq. oxalic acid. Analytical TLC was performed on Merck pre-coated silica gel 60 F_{254} plates. Gel filtration was performed on Sephadex LH-20. UV spectra were recorded on a Specord S600, Analytik Jena AG, Germany. High Resolution EI-MS was done on Micromass GC-TOF Micro Mass Spectrometer (Micromass, Wythenshawe, Waters Inc., UK). CD spectra were measured on JASCO J-810 Spectropolarimeter. IR were recorded on a JASCO Fourier Transform IR-460 Spectrometer. ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, and NOESY NMR spectra were recorded on a Bruker Avance 500 and 600 Spectrometer using the residual solvent peaks as a reference. Optical purity was determined using Chiralcel OD-H HPLC. Optical rotations were taken on a JASCO P-1020 polarimeter.

3.2 Plant Materials

The roots of *Kniphofia foliosa* were collected from Gedo, Ethiopia, 193 km from Addis Ababa on the way to Fincha Sugar Factory in September, 2011. The plant material was identified by Prof. Sebsebe Demissew, Department of Biology, Addis Ababa University, Ethiopia where a voucher specimen (voucher number NA-03) has been deposited. The roots of *Bulbine frutescens* were collected from the University of Nairobi, Chiromo Campus Garden in May 2012. The plant material was identified at University of Nairobi, School of Biological Sciences Herbarium where a voucher specimen (voucher number SGM-AYT-2004–27) has been deposited. The roots of *Aloe dawei* were collected from Nyalenda primary school, Awendo, Kenya in February 2012.

The plant was identified by Mr Patrick Chalo Mutiso of the University of Nairobi, School of Biological Sciences Herbarium where a voucher specimen (voucher number NAA2012/01) has been deposited. The leaves of *Aloe lateritia* subspecies *graminicola* were collected from University of Nairobi, Chiromo Campus Garden in September 2012. For authentication, refer to Yenesew *et al*. (1993).

3.3 Extraction and Isolation

3.3.1 Extraction and Isolation of Compounds from the Roots of *Kniphofia foliosa*

The air-dried roots (1.1 kg) of *K*. *foliosa* were extracted initially with ethyl acetate by cold exhaustive percolation and then with methanol (3 x 3 L) for 24 hrs. The methanol extract was partitioned between ethyl acetate and water. Based on TLC profile, the organic (ethyl acetate) layer was combined with the initial ethyl acetate extract and the combined extract was concentrated on a rotary evaporator to give 60 g (5.5%) of a residue. The crude extract (58 g) was adsorbed on silica gel and subjected to column chromatography on oxalic acid impregnated silica gel (450 g), which was then eluted with *n*-hexane containing increasing amounts of ethyl acetate. Fractions eluted with 5% ethyl acetate in *n*-hexane gave chrysophanol (**18**, 23 mg). Fractions eluted with 10-15% of ethyl acetate in *n*-hexane was applied on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) followed by purification using column chromatography (eluent: increasing gradient of ethyl acetate in *n*-hexane) and to give 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**, 21.0 mg), 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**, 4.2 mg), knipholone cyclooxanthrone (**134**, 3.6 mg) and nepodin (**135**, 26.1 mg) . Fractions eluted with 20-25% ethyl acetate in *n*-hexane gave chryslandicine (**13**, 6.3 mg), knipholone (**14**, 24.2 mg), knipholone anthrone (**15**, 14.3 mg) and isoknipholone (**28**, 6.5 mg) after purification by preparative thin layer chromatography (silica gel, 30% ethyl acetate in *n*-

hexane). The fractions eluted with 30-40% ethyl acetate in *n*-hexane were further purified by column chromatography on Sephadex LH-20 (eluent: CH_2Cl_2/MeOH, 1:1) to give aloesaponol III (**16**, 13.0 mg) and dianellin (**136**, 56.0 mg).

3.3.2 Extraction and Isolation of Compounds from the Roots of *Bulbine frutescens*

The air-dried roots (940 g) of *B. frutescens* were extracted using CH_2Cl_2/MeOH (1:1) three times for 24 hrs by cold percolation. The extract was then concentrated under vacum using a rotary evaporator to yield a dark brown residue (56 g, 5.9%). A 50 g portion of the extract was subjected to flash column chromatography (column size: 80 X 9 cm) on oxalic acid impregnated silica gel (600 g) eluting as follows: CH_2Cl_2 (fractions 1-8), CH_2Cl_2-EtOAc (1:1) (fractions 9-16) and EtOAc-MeOH (9:1) (fractions 17-22) each of *ca.* 250 mL. Fractions 3-6 were combined based on their TLC profile and then loaded on Sephadex LH-20 column (60 X 3; eluent: CH_2Cl_2/MeOH, 1:1, 200 mL) followed by purification on prep. TLC (silica gel; petroleum ether/EtOAc, 4:1) to give chrysophanol (**18**,12.3 mg), 6',8-*O*-dimethylknipholone (**150**, 4.5 mg), 4-*O*-methyleleutherol (**53**, 5.4 mg) and 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-C]furan-4,9-dione (**50**, 3.7 mg). Fractions 9-15 were combined and subsequently subjected to column chromatography (column size: 60 X 4) on silica gel (300 g) impregnated with oxalic acid (eluent: increasing gradient of EtOAc in petroleum ether; 0:10, 1:9, 1:4, 2:3, 1:1, 3:2, 0:10 each 700 mL) followed by further purification of the fractions on Sephadex LH-20 (60 X 3; eluent: CH_2Cl_2/MeOH, 1:1, 200 mL) yielding knipholone (**14**, 124.2 mg), isoknipholone (**28**, 6.5 mg), 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**, 12.2 mg) and 2,6-dimethoxy-4-hydroxyacetophenone (**151**, 7.4 mg). Similar treatment of fractions 17-20 resulted in the isolation of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**, 6.8 mg).

3.3.3 Extraction and Isolation of Compounds from the Roots of *Aloe dawei*

The air-dried and ground roots (1.4 kg) of *A. dawei* were exhaustively extracted with MeOH/CH_2Cl_2 (1:1) by cold percolation at room temperature. The extract was evaporated under reduced pressure to yield a reddish-brown crude extract (31.0 g). The extract was subjected to column chromatography (column size: 80 X 4) on oxalic acid impregnated silica gel (300 g) eluting with *n*-hexane containing increasing amounts of ethyl acetate to afford 34 major fractions *ca*. 200 mL each. The fraction eluted with 1% EtOAc in *n*-hexane gave 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**, 9.2 mg) and helminthosporin (**59**, 12.3 mg); while the fraction eluted with 3% EtOAc in *n*-hexane after purification by column chromatography on Sephadex LH-20 (eluting with MeOH/CH_2Cl_2; 1:1) afforded hydroxydroserone (**153**, 9.4 mg) and chrysophanol (**18**, 11.8 mg). Fractions eluted with 5-8% EtOAc in *n*-hexane were combined and purified by CC (column size: 60 X 3) on oxalic acid impregnated silica gel (eluent: increasing gradient of EtOAc in *n*-hexane) and gave four compounds: droserone (**123**, 8.8 mg), chrysophanol-8-methyl ether (**156**, 32.7 mg), malvone A (**154**, 26.4 mg), droserone-5-methyl ether (**152**, 13.5 mg) and asphodeline (**23**, 9.2 mg). Fractions eluted with 10-18% EtOAc in *n*-hexane gave aloesaponarin I (**66**, 14.3 mg) and aloesaponarin II (**65**, 17.8 mg) after the combined fractions was purified by column chromatography on Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1). Fractions eluted with 20-30% of ethyl acetate in *n*-hexane were combined and applied on Sephadex LH-20 (eluent: MeOH/CH_2Cl_2; 1:1) followed by purification using column chromatography (column size: 60 X 3) on oxalic acid impregnated silica gel (eluent: increasing gradient of EtOAc in *n*-hexane) which gave 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**, 9.8 mg), ancistroquinone C (**95**, 7.5 mg), deoxyerythrolaccin (**68**, 7.7 mg), aloesaponol I (**69**, 4.2 mg), laccaic acid D-methyl ester (**67**, 11.9 mg) and aloesaponol II-6-methyl ether (**157**, 15.7 mg).

3.3.4 Extraction and Isolation of Compounds from the Leaves of *Aloe lateritia* Subspecies *graminicola*

The air-dried leaves of *A. lateritia* subspecies *graminicola* (1.7 kg) were exhaustively extracted by cold percolation at room temperature with MeOH/CH_2Cl_2; 1:1 (4.5 L) four times for 24 hrs in each case. The extract was concentrated under reduced pressure to yield a brown crude extract (62.0 g, 3.6%). A 60 g portion of the extract was subjected to column chromatography (column size: 90 X 6) on oxalic acid impregnated silica gel (400 g) eluting with *n*-hexane containing increasing amounts of EtOAc to afford 52 major fractions (*ca*. 200 mL each). Fractions 8-10 (2% EtOAc in *n*-hexane) were combined and purified on Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give chrysophanol (**18**, 21.3 mg) and helminthosporin (**59**, 4.6 mg); while fractions 12-17 (5% EtOAc in *n*-hexane) were combined and purified on column chromatography (column size: 60 X 3) with increasing gradient of EtOAc in *n*-hexane to give aloesaponarin II (**65**, 34.0 mg) and asphodeline (**23**, 17.1 mg). Fractions 20-32 (10-20% EtOAc in *n*-hexane) contained mixtures of five compounds which were combined and subsequently subjected to column chromatography (column size: 80 X 4) on silica gel (300 g) impregnated with oxalic acid (eluent: increasing gradient of EtOAc in petroleum ether) followed by further purification of the fractions on Sephadex LH-20 (60 X 3; eluent: CH_2Cl_2/MeOH, 1:1) to yield noreugenin (**162**, 4.8 mg), laccaic acid D methyl ester (**67**, 9.0 mg), aloesaponol I (**69**, 13.5 mg), norobtusifolin (**158**, 6.3 mg) and nataloe-emodin (**61**, 5.8 mg). Fractions 35-40 (30% EtOAc in *n*-hexane) showed three blue fluorescing compounds when viewed under UV light at 366 nm. The purifications of these fractions using Sephadex LH-20 (60 X 3; eluent: CH_2Cl_3/MeOH, 1:1) followed by PTLC (30 % EtOAc in *n*-hexane) yielded three furancarboxaldehydes; 5-hydroxymethyl-2-furancarboxaldehyde (**159**, 6.3 mg), 5-acetyloxymethyl-2-furancarboxaldehyde (**160**, 6.8 mg) and

cirsiumaldehyde (**161**, 4.6 mg). The fractions eluted with 32-50% EtOAc in *n*-hexane were purified on Sephadex LH-20 (60 X 3; eluent: CH_2Cl_2/MeOH, 1:1) to give aloe-emodin (**20**, 11.0 mg).

3.4 Experimental Procedures for Modification Reactions

3.4.1 Hydrolysis of Dianellin

Compound **136** (6.0 mg) was treated with 5% HCl (2 mL) under reflux for 6 hrs. The mixture was then diluted with water and extracted with ethyl acetate. The ethyl acetate layer was concentrated on a rotary evaporator. The residue (5.6 mg) was subjected to column chromatography (column size: 60 cm length and 2 cm diameter) on silica gel (100 g) impregnated with oxalic acid (eluent: increasing gradient of ethyl acetate in *n*-hexane) to afford nepodin (**135**, 4.1 mg, 68.3% yield).

3.4.2 Methylation of 8-Hydroxy-6-methylxanthone-1-carboxylic Acid (138)

To a solution of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**) (6.0 mg, 11.8 mmol), in acetone (20 mL), $NaHCO_3$ (150 mg) and dimethylsulfate (0.3 mL) were added. The mixture was stirred for 6 hrs at room temperature. TLC was done using 20% ethyl acetate in petroleum ether to monitor the completion of the reaction. The mixture was dried using a rotary evaporator and then partitioned between ethyl acetate and water. The organic layer was then concentrated and purified by CC on silica gel (eluent: increasing gradient of ethyl acetate in *n*-hexane) to afford 8-methoxy-6-methylxanthone-1-carboxylic acid methyl ester (**138a**, 5.3 mg, 88.3% yield).

3.4.3 Preparation Knipholone Mannich Base (163)

In a 250 mL round bottom flask, 11.5 mg (0.027 mmol) of knipholone (**14**) was dissolved in 50 mL of dioxane, and 2.0 mL of diethyl amine and 0.8 mL of formaldehyde (aq) were added. The mixture was then stirred for 48 hrs at 65 ℃ and the reaction progress was monitored by TLC using 20% ethyl acetate in petroleum ether. The solvent was evaporated and the reaction mixture was purified by column chromatography (60 cm length and 4 cm diameter) on oxalic acid impregnated silica gel (increasing gradient of ethyl acetate in petroleum ether as eluting solvent) to give **163** (6.3 mg, 54.7% yield).

3.4.4 Preparation of Knipholone-1,3-oxazine (164)

Compound **164** was also prepared following the procedure described in section 3.4.3 where knipholone (16.0 mg; 0.037 mmol), formaldehyde (2.2 mL) and aniline (2.7 mL) were dissolved in 50 mL of dioxane. The mixture was refluxed at 65 ℃ for 24 hrs, and then purified by column chromatography (60 cm length and 4 cm diameter) on oxalic acid impregnated silica gel (increasing gradient of ethyl acetate in petroleum ether as eluting solvent) to give 7.6 mg (47.5% yield) of **164**.

3.5 X-ray Single Crystal Analysis of 8-Hydroxy-6-methylxanthone-1-carboxylic Acid (138)

Single crystals of **138** were coated with a layer of hydrocarbon oil and attached to a MicroMount^{TM}. A suitable crystal was selected and mounted on a SuperNova, Dual Atlas diffractometer. The crystal was kept at 100(1) K during data collection. The structure was solved using OLEX2 (Sheldrick, 2008) and refined with SHELX-97 (Dolomanov *et al*., 2009). Crystal Data: monoclinic, a = 15.6907(12) Å, b = 4.9175(4) Å, c = 15.3304(11) Å, β = 92.895(6)°, V =

1181.36(16) $Å^3$, space group $P2_1/c$ (no. 14), $Z = 4$, μ(Cu Kα) = 0.973, 3854 reflections measured, 2053 unique (R_{int} = 0.0242) which were used in all calculations. The final wR_2 was 0.0984 (all data) and R_1 was 0.0365 (I > 2σ(I)). Further details can be obtained *via* CCDC. CCDC 968696 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

3.6 Physical and Spectroscopic Data

Chrysophanol (18)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) [nm]: 275, 324, 405. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 12.13 (*s*, 1H, OH-1), 12.02 (*s*, 1H, OH-8), 7.80 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.70 (*d*, *J* = 7.6 Hz, 1H, H-5), 7.55 (*brs*, 1H, H-4), 7.37 (*d*, *J* = 8.4 Hz, 1H, H-7), 7.21 (*brs*, 1H, H-2), 2.44 (*s*, 3H, 3-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 194.4 (C-9), 184.6 (C-10), 164.8 (C-1), 164.6 (C-8), 152.2 (C-3), 140.4 (C-6), 136.4 (C-5a), 136.2 (C-4a), 127.6 (C-7), 127.3 (C-2), 123.6 (C-4), 122.4 (C-5), 119.0 (C-8a), 116.9 (C-1a), 24.7 (C-CH_3). EIMS m/z (70 eV, rel. int.): 254 (72, $[M]^+$).

Aloesaponol III (16)

Colorless crystalline solid. UV λ_{max} ($CHCl_3$) [nm]: 280, 293, 312, 377. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 16.32 (*s*, 1H, OH-9), 9.68 (*s*, 1H, OH-8), 7.29 (s, 1H, H-10), 7.11 (*bd*, *J* = 1.2, 1H, H-5), 6.70 (*bd*, *J* = 1.2, 1H, H-7), 4.95 (*dd*, *J* = 5.2, 1.5, 1H, H-4), 4.68 (*bs*, 1H, OH-4), 2.75 - 2.95 (m, 2H, H-2), 2.40 (*s*, 3H, 6-CH_3), 2.15 - 2.33 (m, 2H, H-3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 205.5 (C-1), 166.6 (C-9), 158.7 (C-8), 144.6 (C-6), 141.9 (C-4a),

140.7 (C-1a), 119.5 (C-5), 116.7 (C-10), 113.4 (C-7), 111.8 (C-5a), 109.3 (C-8a), 67.8 (C-4), 34.7 (C-2), 32.1 (C-3), 22.0 (C-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 258 (23, $[M]^+$).

10-Hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (132)

Yellow amorphous powder. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.26 (*s*, 1H, OH-1), 12.18 (*s*, 1H, OH-1'), 12.06 (*s*, 1H, OH-8), 11.62 (*s*, 1H, OH-8'), 8.66 (*d*, *J* = 8.0 Hz, 1H, H-6'), 7.85 (*d*, *J* = 8.0 Hz, 1H, H-5'), 7.51 (*t*, *J* = 8.4 Hz, 1H, H-6), 7.37 (*d*, *J* = 1.4 Hz, 1H, H-4'), 7.04 (*d*, *J* = 1.4 Hz, 1H, H-2'), 6.92 (*d*, *J* = 8.4 Hz, 1H, H-7), 6. 79 (*d*, *J* = 1.2 Hz, 1H, H-2), 6.76 (*d*, *J* = 8.4 Hz, 1H, H-5), 6.64 (*d*, *J* = 1.2 Hz, 1H, H-4), 2.32 (*s*, 3H, 3'-CH_3), 2.23 (*s*, 3H, 3-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 193.0 (C-9), 192.1(C-9'), 181.5 (C-10'), 161.9 (C-1'), 161.8 (C-1), 161.5 (C-8), 159.1(C-8'), 149.6 (C-3'), 148.9 (C-3), 148.4 (C-4a), 148.3 (C-5a), 142.2 (C-7'), 137.3 (C-6), 133.3 (C-4'a), 132.8 (C-5'a), 132.2 (C-6'), 124.3 (C-2'), 120.9 (C-4), 120.9 (C-4'), 119.9 (C-5), 119.3 (C-5'), 117.2 (C-2), 116.9 (C-7), 115.9 (C-8'a), 114.7 (C-8a), 114.0 (C-1'a), 112.6 (C-1a), 70.0 (C-10), 22.2 (3-CH_3), 22.0 (3'-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 491.1 (42, $[M\text{-}OH]^+$).

10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (133)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 284, 354, 425. CD ($CHCl_3$): $\Delta\varepsilon_{272}$ = +4.1 cm^2 mol^{-1}. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 12.37 (*s*, 1H, OH-1), 12.27 (*s*, 1H, OH-1'), 12.06 (*s*, 1H, OH-8), 11.72 (*s*, 1H, OH-8'), 8.66 (*d*, *J* = 8.0 Hz, 1H, H-6'), 7.95 (*d*, *J* = 8.0 Hz, 1H, H-5'), 7.61 (*d*, *J* = 1.2 Hz, 1H, H-4'), 7.43 (*t*, *J* = 8.4 Hz, 1H, H-6), 7.04 (*d*, *J* = 1.2 Hz, 1H, H-2'), 6.94 (*dd*, *J* = 8.4, 1.2 Hz, 1H, H-7), 6.78 (*dd*, *J* = 8.4, 1.2 Hz, 1H, H-5), 6.77 (*d*, *J* = 1.2 Hz, 1H, H-2), 6.62 (*d*, *J* = 1.2 Hz, 1H, H-4), 2.86 (*s*, 10-OCH_3), 2.35 (*s*, 3H, 3'-CH_3), 2.26 (*s*, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm): 193.1 (C-9), 191.4 (C-9'), 182.1 (C-10'),

162.9 (C-1), 162.6 (C-8), 162.4 (C-8'), 158.9 (C-1'), 149.9 (C-3'), 149.0 (C-3), 148.1(C-4a), 143.6 (C-4'a), 143.2 (C-5a), 136.8 (C-6), 133.1(C-6'), 132.2 (C-7'), 131.4 (C-5'a), 124.6 (C-2'), 121.7 (C-4'), 120.9 (C-4), 119.6 (C-5'), 119.3 (C-2), 118.1 (C-5), 117.7 (C-7), 116.2 (C-8'a), 116.1 (C-8a), 115.7 (C-1'a), 114.3 (C-1a), 75.6 (C-10), 50.7 (10-OCH_3), 22.4 (3'-CH_3), 22.3 (3-CH_3). EIMS *m/z* (70 eV, rel. int.): 491 (43, $[M\text{-}OMe]^+$), 490 (29), 392 (42), 391 (53), 353 (43), 294 (59), 265 (72), 196 (100). TOF HRMS *m/z* = 491.0353, $[M\text{-}OMe]^+$ $C_{31}H_{22}O_8$.

Chryslandicin (13)

Red crystalline solid. UV λ_{max} ($CHCl_3$) nm: 272, 295, 386, 496. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 13.48 (*s*, OH-4')12.42 (*s*, 1H, OH-1), 12.32 (*s*, 1H, OH-1'), 12.30 (*s*, 1H, OH-8), 12.07 (*s*, 1H, OH-8'), 8.63 (*d*, *J* = 8.0 Hz, 1H, H-6'), 8.06 (*d*, *J* = 8.0 Hz, 1H, H-5'), 7.40 (*t*, *J* = 8.2Hz, 1H, H-6), 7.08 (*brs*, 1H, H-2'), 6.93 (*d*, *J* = 8.2 Hz, 1H, H-5), 6.81 (*d*, *J* = 8.2 Hz, 1H, H-7), 6.77 (*d*, *J* = 1.4 Hz, 1H, H-2), 6.61 (*d*, *J* = 1.4 Hz, 1H, H-4), 2.35 (*s*, 3H, 3'-CH_3), 2.25 (*s*, 3H,3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm): 192.4 (C-9), 190.3 (C-9'), 186.4 (C-10'), 162.5 (C-8), 162.3 (C-1), 158.6 (C-8'), 157.9 (C-1'), 157.7 (-4'), 148.8 (C-3), 146.2 (C-3), 146.2 (C-4a), 146.0 (C-5a), 141.9 (C-7'), 141.1 (C-3'), 136.2 (C-6), 133.0 (C-5'a), 132.2 (C-6'), 129.0 (C-2'), 120.1 (C-4), 118.8 (C-5), 118.8 (C-5'), 118.1 (C-2), 117.8 (C-7), 116.2 (C-4'a), 114.4 (C-1'a), 112.2 (C-8'a), 111.2 (C-8a), 110.7 (C-1a), 71.0 (C-10), 22.3 (3'-CH_3), 22.1 (3-CH_3).

Knipholone (14)

Deep red solid. UV λ_{max} ($CHCl_3$) nm: 284, 354, 425. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 14.22 (*s*, 1H, OH-1'), 12.52 (*s*, 1H, OH-1), 11.99 (*s*, 1H, OH-8), 7.76 (*dd*, *J* = 8.4, 7.5 Hz, 1H, H-6), 7.56 (*dd*, *J* = 7.6, 1.2 Hz, 1H, H-5), 7.33 (*s*, 1H, H-2), 7.31 (*dd*, *J* = 8.4, 1.2 Hz, 1H, H-

7), 6.24 (*s*, 1H, H-6'), 3.99 (*s*, 3H, OCH_3), 2.64 (*s*, 3H, CO-CH_3), 2.18 (*s*, 3H, CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 203.7 (CO), 194.1 (C-9), 182.7 (C-10), 164.7 (C-2'), 163.7 (C-1), 163.4 (C-4'), 162.7 (C-8), 162.0 (C-6'), 153.0 (C-3), 138.2 (C-6), 135.6 (C-5a), 133.1 (C-4a), 128.9 (C-4), 125.4 (C-2), 124.1 (C-7), 120.1 (C-5), 116.4 (C-8a), 115.7 (C-1a), 108.5 (C-1'), 106.2 (C-3'), 91.8 (C-5'), 56.1 (C-OCH_3), 33.1 (C-$COCH_3$), 21.0 (C-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 434 (100, $[M]^+$); $C_{24}H_{18}O_8$. EI-MS (70 eV, rel. int.) *m/z* = 434 (100, $[M]^+$).

Knipholone Anthrone (15)

Bluish solid. ^1H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 14.15 (*s*, 1H, OH-2'), 12.36 (*s*, 1H, OH-1), 12.05 (*s*, 1H, OH-8), 7.31(*dd*, *J* = 8.2, 7.2 Hz, 1H, H-6), 6.82 (*s*, 1H, H-2), 6.72 (*dd*, *J* = 7.2, 1.2 Hz, 1H, H-5), 6.64 (*dd*, *J* = 8.2, 1.2 Hz, 1H, H-7), 6.11 (*s*, 1H, H-5'), 4.00 (*brs*, 2H, H-10), 3.82 (*s*, 3H, 4'-OCH_3), 2.59 (*s*, 3H, $COCH_3$), 2.03 (*s*, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm): 204.2 (CO), 194.4 (C-9), 164.7 (C-1), 164.2 (C-2'), 163.8 (C-4'), 163.1 (C-6'), 160.6 (C-8), 150.4 (C-3), 143.8 (C-4a), 142.9 (C-5a), 136.7 (C-6), 120.8 (C-4), 119.5 (C-2), 117.7 (C-7), 116.0 (C-1a), 115.5 (C-5), 115.1 (C-8a), 106.7 (C-1'), 105.2 (C-3'), 91.1 (C-5'), 54.5 (4'-OCH_3), 33.5 (CO*Me*), 31.9 (C-10), 21.1 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 420.1 (42, $[M]^+$).

Knipholone Cyclooxanthrone (134)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 284, 354, 425. CD ($CHCl_3$): $\Delta\varepsilon_{273}$ = -3.8 cm^2 mol^{-1}. ^1H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 15.02 (*s*, 1H, OH-2'), 12.25 (*s*, 1H, OH-1), 11.66 (*s*, 1H, OH-8), 7.63 (*t*, *J* = 7.9 Hz, 1H, H-6), 7.46 (*d*, *J* = 7.6 Hz, 1H, H-5), 7.06 (*d*, *J* = 8.3, Hz, 1H, H-7), 7.00 (*s*, 1H, H-2), 6.38 (*s*, 1H, H-5'), 5.57 (*s*, 1H, H-10), 3.99 (*s*, 3H, 4'-OCH_3), 2.72 (*s*, 3H, $COCH_3$), 2.42 (*s*, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm):

204.2 (CO), 192.2 (C-9), 163.2 (C-4'), 163.0 (C-2'), 162.8 (C-8), 162.5 (C-6'), 160.8 (C-1), 147.4 (C-3), 138.6 (C-5a), 136.5 (C-6), 134.8 (C-4a), 120.5 (C-2), 120.1 (C-4), 118.7 (C-5), 118.2 (C-7), 114.1 (C-8a), 110.2 (C-1a), 107.3 (C-3'), 106.1 (C-1'), 91.3 (C-5'), 72.2 (C-10), 56.0 (4'-OCH_3), 33.3 (CO*Me*), 31.9 (C-10), 23.7 (3-CH_3). EIMS *m/z* (70 eV, rel. int.): 418 (29, $[M]^+$), 403 (20), 139 (33), 125 (50), 111 (73), 97 (100), 83 (80), 71 (81), 57 (97), 43 (94). TOF HRMS *m/z* = 418.1055, $[M]^+$ $C_{24}H_{18}O_7$ (calculated for 418.1053).

Isoknipholone (28)

Red amorphous solid. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 13.60 (*s*, 1H, OH-4'), 12.56 (*s*, 1H, OH-1), 11.95 (*s*, 1H, OH-8), 7.78 (*dd*, *J* = 8.4, 7.8 Hz, 1H, H-6), 7.58 (*dd*, *J* = 7.8, 1.2 Hz, 1H, H-5), 7.37 (*s*, 1H, H-2), 7.32 (*dd*, *J* = 8.4, 1.2 Hz, 1H, H-7), 6.30 (*s*, 1H, H-6'), 3.41 (*s*, 3H, 2'-OCH_3), 2.64 (*s*, 3H, CO-CH_3), 2.22 (*s*, 3H, CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 204.8 (CO), 194.8 (C-9), 184.0 (C-10), 167.2 (C-4'), 164.5 (C-2'), 163.1 (C-6'), 161.8 (C-1), 161.4 (C-8), 153.2 (C-3), 138.9 (C-6), 136.4 (C-5a), 134.0 (C-4a), 129.6 (C-4), 126.4 (C-2), 124.9 (C-7), 120.9 (C-5), 117.2 (C-8a), 116.5 (C-1a), 115.5 (C-1'), 110.4 (C-3'), 101.0 (C-5'), 61.7 (2'-OCH_3), 32.3 (CO*Me*), 21.9 (C-10), 23.7 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 434.1 (42, $[M]^+$).

Nepodin (135)

^{1}H NMR ($CDCl_3$, 25 °C) δ (200 MHz, ppm): 17.33 (*s*, 1H, OH-1), 10.23 (*s*, 1H, OH-8), 7.46 (*J* = 8.0 Hz, 1H, H-6), 7.06 (*d*, *J* = 7.4 Hz, 1H, H-5), 6.98 (*s*, 1H, H-4), 6.83 (*d*, *J* = 8.0 Hz, 1H, H-7), 2.75 (*s*, 3H, CO-CH_3), 2.42 (*s*, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (50 MHz, ppm): 205.2(CO), 168.8 (C-1), 158.5 (C-8), 138.6 (C-3), 133.5 (C-4a), 133.0 (C-6), 122.1 (C-5), 117.7 (C-4), 113.9 (C-8a), 113.2 (C-2), 111.4 (C-7), 32.4 (C-$COCH_3$), 25.5 (3-CH_3).

Dianellin (136)

Viscous yellow oil. $[\alpha]_D^{25}$ -104.5^0 (*c* 0.25, MeOH). UV λ_{max} ($CHCl_3$) nm: 266, 340. ^{1}H NMR (acetone-d_6, 25 °C) δ (600 MHz, ppm): 9.63 (1H, 1-OH), 7.45 (1H, *dd*, *J* = 1.2, 8.0 Hz, H-5), 7.42 (1H, *dd*, *J* = 8.0, 8.2 Hz, H-6), 7.42 (1H, *dd*, *J* = 1.2, 8.2 Hz, H-7), 7.18 (1H, *s*, H-4), 5.17 (1H, *d*, *J* = 7.8 Hz, H-1'), 4.80 (1H, *d*, *J* = 1.4 Hz, H-1"), 4.12 (1H, *dd*, *J* = 2.0, 11.1 Hz, H-6'a), 3.91 (1H, *dd*, 3.4, 1.5 Hz , H-2"), 3.78 (1H, *ddd, 9.6,* 7.3, 2.1Hz, H-5'),3.74 (1H, *dd*, *J* = 3.4, 9.3 Hz, H-3"), 3.68 (3H, *m*, H-6'b, H-5"and H-2'), 3.63 (1H, *t*, *J* = 8.9 Hz, H-3'), 3.49 (1H, *dd*, *J* = 8.8, 9.6 Hz, H-4'), 3.43 (1H, *t*, *J* = 9.4 Hz, H-4"), 2.55 (3H, *s*, H-12), 2.30 (3H, *s*, H-13), 1.21 (3H, *d*, *J* = 6.2 Hz, H-6"). ^{13}C NMR (acetone-d_6, 25 °C) δ (150 MHz, ppm): 204.8 (C-11), 156.4 (C-8), 152.8 (C-1), 138.1 (C-10), 135.0 (C-3), 128.9 (C-6), 127.0 (C-2), 124.2 (C-5), 121.2 (C-4), 115.3 (C-9), 112.5 (C-7), 104.7 (C-1'), 102.5 (C-1"), 78.7 (C-3'), 77.9 (C-5'), 75.4 (C-2'), 74.4 (C-4"), 73.2 (C-3"), 72.6 (C-2"), 72.1 (C-4'), 70.0 (C-5"), 68.5 (C-6'), 32.9 (C-12), 20.5 (C-13), 18.8 (C-6"). EIMS *m/z* (70 eV, rel. int.): 525 (5, $[M+1]^+$), 216 (35), 201 (34), 198 (12), 91 (15), 73 (16), 64 (40), 46 (100). TOF HRMS *m/z* = 525.1970, $[M+1]^+$ $C_{25}H_{33}O_{12}$ (calculated for 525.1972).

3,8-Dihydroxy-1-methylanthraquinone-2-carboxylic Acid (137)

Yellow amorphous powder. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 13.00 (*s*, 1H, OH-8), 7.74 (*s*, 1H, H-4), 7.74 (*dd*, *J* = 8.3, 7.5 Hz, 1H, H-6), 7.72 (*dd*, *J* = 7.5, 1.2 Hz, 1H, H-5), 7.33 (*dd*, *J* = 8.3, 12 Hz, 1H, H-7), 2.85 (*s*, 3H, 1-CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 191.5 (C-9), 183.4 (C-10), 169.2 (COOH), 163.6 (C-8), 160.5 (C-3) 143.7 (C-1), 138.7 (C-4a), 137.5 (C-6), 134.4 (C-5a), 131.6 (C-2), 125.8 (C-7), 125.1 (C-1a), 119.9 (C-5), 118.5 (C-8a), 113.8 (C-4), 21.1 (C-CH_3). EIMS m/z (70 eV, rel. int.): 298 (38, $[M]^+$).

8-Hydroxy-6-methylxanthone-1-carboxylic Acid (138)

Yellow crystalline solid. mp. 267–268 °C. UV λ_{max} (acetonitrile) [nm]: 225, 254, 362, 386. IR (KBr) ν_{max} cm^{-1} 3486-3142, 1650, 1612, 1589, 1476, 1450. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 12.25 (*s*, 1H, OH-8), 7.94 (*dd*, *J* = 8.5, 7.3 Hz, 1H, H-3), 7.68 (*dd*, *J* = 8.5, 1.1 Hz, 1H, H-2), 7.45 (*dd*, *J* = 7.3, 1.1 Hz, 1H, H-4), 6.89 (*br d*, *J* = 1.5 Hz, 1H, H-5), 6.66 (*br d*, *J* = 1.5 Hz, 1H, H-7), 2.45 (*d*, *J* = 0.6 Hz, 3H, 6-CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 181.6 (C-9), 169.8 (COO), 162.2 (C-8), 157.1 (C-4a), 156.8 (C-5a), 150.7 (C-6), 136.6 (C-3), 135.8 (C-1), 123.8 (C-4), 120.1 (C-2), 117.8 (C-1a), 112.2 (C-7), 108.3 (C-5), 107.7 (C-8a), 22.6 (6-CH_3). EI-MS (70 eV, rel. int.): *m/z* = 270 (100, $[M]^+$), 252 (75), 226 (62), 223 (25), 197 (17), 130 (18). HR-EI-MS *m/z* = 270.0520, $[M]^+$ (calculated for $C_{15}H_{10}O_5$, 270.528).

8-Methoxy-6-methylxanthone-1-carboxylic Acid methyl ester (138a)

Yellow amorphous solid. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 7.66 (*dd*, *J* = 8.5, 7.3 Hz, 1H, H-3), 7.47 (*dd*, *J* = 8.5, 1.1 Hz, 1H, H-2), 7.26 (*dd*, *J* = 7.3, 1.1 Hz, 1H, H-4), 6.87 (*d*, *J* = 1.5 Hz, 1H, H-5), 6.61 (*d*, *J* = 1.5 Hz, 1H, H-7), 4.05 (*s*, 3H, $COOCH_3$), 3.99 (*s*, 3H, 8-OCH_3), 2.47 (*s*, 3H, 6-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm): 174.9 (C-9), 170.3 (COO),, 160.5 (C-8), 157.6 (C-5a), 154.9 (C-4a), 146.9 (C-6), 134.2 (C-1), 133.5 (C-3), 122.4 (C-4), 120.1 (C-1a), 118.8 (C-2), 110.2 (C-8a), 109.9 (C-5), 107.0 (C-7), 56.5 (8-OCH_3), 53.1 (OCH_3), 22.5 (6-CH_3).

6',8-*O*-Dimethylknipholone (150)

Yellow amorphous solid. mp. 263–264 °C. UV λ_{max} (acetonitrile) [nm]: 220, 256, 286, 415. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 13.91 (*s*, 1H, OH-2'), 13.39 (*s*, 1H, OH-1), 7.74 (*dd*, *J* = 7.8, 1.1 Hz, 1H, H-5), 7.64 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.29 (*dd*, *J* = 7.8, 1.1 Hz, 1H, H-7), 7.25 (*s*,

1H, H-2), 6.14 (*s*, 1H, H-5'), 4.08 (*s*, 3H, 8-OCH_3), 4.02 (*s*, 3H, 4'-OCH_3), 3.79 (*s*, 3H, 6'-OCH_3), 2.69 (*s*, 3H, $COCH_3$), 2.10 (*s*, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (126 MHz, ppm): 203.5 (CO), 189.0 (C-9), 183.5 (C-10), 162.9 (C-4'), 162.6 (C-6'), 162.4 (C-2'), 162.3 (C-1), 160.2 (C-8), 149.4 (C-3), 136.9 (C-5a), 135.3 (C-6), 130.9 (C-4a), 126.6 (C-4), 125.3 (C-2), 120.6 (C-8a), 120.0 (C-5), 117.2 (C-7), 116.2 (C-1a), 109.3 (C-1'), 106.2 (C-3'), 86.2 (C-5'), 56.6 (8-OCH3), 55.7 (6'-OCH_3), 55.4 (4'-OCH_3), 33.3 ($COCH_3$), 20.7 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 462 (15, $[M]^+$), 447 (14), 431 (9), 357 (17), 342 (24), 105 (35), 100 (100),72 (37). HR-EI-MS *m/z* = 462.13142, $[M]^+$ (calculated for $C_{26}H_{22}O_8$, 462.13147).

4-*O*-Methyleleutherol (53)

Colourless amorphous powder. UV λ_{max} ($CHCl_3$) nm: 254, 312, 340, 374. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 8.05 (*s*, 1H, H-9), 7.53 (*d*, *J* = 7.8 Hz, 1H, H-8), 7.41 (*t*, *J* = 7.8 Hz, 1H, H-7), 6.96 (*d*, *J* = 7.8 Hz, 1H, H-6), 5.70 (*q*, *J* = 6.7 Hz, 1H, H-3), 3.95 (*s*, 3H, 4-OCH_3), 3.82 (*s*, 3H, 5-OCH_3), 1.67 (*d*, *J* = 6.7 Hz, 3H, 3-CH_3). ^{13}C NMR ($CDCl_3$, 25 °C) δ (125 MHz, ppm): 170.1 (C-1), 156.6 (C-4), 151.9 (C-5), 138.4 (C-8a), 136.2 (C-1a), 127.5 (C-7), 125.4 (C-3a), 123.5 (C-5a), 122.8 (C-8), 122.3 (C-9), 108.3 (C-6), 77.5 (C-3), 62.7 (8-OCH_3), 56.3 (5-OCH_3).

5,8-Dihydroxy-1-hydroxymethynaphtho[2,3-C]furan-4,9-dione (50)

Colourless amorphous powder. UV λ_{max} ($CHCl_3$) nm: 242, 303, 460. ^{1}H NMR ($CDCl_3$, 25 °C) δ (500 MHz, ppm): 12.82 (*s*, 1H, OH-8), 12.63 (*s*, 1H, OH-5), 8.18 (*s*, 1H, H-3), 7.29 (*d*, *J* = 8.2, 2H, H-6 /H-7) 4.96 (*s*, 2H, CH_2OH).

2,6-Dimethoxy-4-hydroxyacetophenone (151)

Pale yellow solid. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 6.14 (*s*, 2H, H-3/H-5), 3.73 (*s*, 6H, OCH_3-2/OCH_3-6), 2.30 (*s*, 3H, CH_3. ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 200.3 (CO), 160.9 (C-4), 159.0 (C-2/C-6), 113.8 (C-1), 92.8 (C-3/C-5), 56.0 (OCH_3-2/OCH_3-6), 32.6 (CH_3); EIMS m/z (70 eV, rel. int.): 196 (29, $[M]^+$).

Droserone (123)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 224, 260, 286 and 350. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 11.38 (*s*, 1H, OH-5), 7.69 (*t*, *J* = 7.9 Hz, 1H, H-7), 7.49 (*d*, *J* = 7.4 Hz, 1H, H-8), 7.25 (*d*, *J* = 8.4 Hz, 1H, H-6), 1.92 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 184.9 (C-1), 184.5 (C-4), 160.4 (C-5), 155.7 (C-3), 137.3 (C-7), 132.8 (C-9), 123.3 (C-6), 120.9 (C-2), 118.7 (C-8), 114.1 (C-10), 9.1 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 205.1 (46, $[M+H]^+$).

Droserone 5-methyl ether (152)

Yellow amorphous solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 7.75 (*t*, *J* = 8.0 Hz, 1H, H-7), 7.60 (*d*, *J* = 7.5 Hz, 1H, H-8), 7.46 (*d*, *J* = 8.5 Hz, 1H, H-6), 3.92 (*s*, 3H, 5-OCH_3), 1.88 (*s*, 3H, 2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 219.4 (16, $[M+H]^+$). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 184.5 (C-1), 178.9 (C-4), 159.4 (C-5), 155.9 (C-3), 135.8 (C-7), 134.2 (C-2), 118.2 (C-8), 117.8 (C-6), 117.1 (C-10), 117.0 (C-9), 56.4 (5-OCH_3), 8.3 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 219 (16, $[M+H]^+$).

Hydroxydroserone (153)

Yellow crystals. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.90 (*s*, 1H, OH-5), 11.72 (*s*, 1H, OH-8), 7.34 (*d*, *J* = 9.4 Hz, 1H, H-7), 7.28 (*d*, *J* = 9.2 Hz, 1H, H-6), 1.93 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 189.1 (C-1), 182.9 (C-4), 156.9 (C-3), 156.2 (C-5), 155.5 (C-8), 130.1 (C-6), 127.5 (C-7), 120.5 (C-2), 111.0 (C-10), 110.9 (C-9), 8.2 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 221 (27, $[M+H]^+$).

5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (94)

Yellow amorphous solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.52 (*s*, 1H, OH-5), 11.99 (*s*, 1H, OH-8), 7.33 (*d*, *J* = 8.5 Hz, 1H, H-6), 7.33 (*d*, *J* = 8.5 Hz, 1H, H-7), 4.04 (*s*, 3H, 3-OCH_3), 1.98 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 191.7 (C-1), 186.3 (C-4), 161.2 (C-3), 159.8 (C-5), 159.2 (C-8), 147.6 (C-10), 147.2 (C-9), 135.0 (C-2), 132.6 (C-6), 131.8 (C-7), 64.3 (3-OCH_3), 11.9 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 235 (23, $[M+H]^+$).

Malvone A (154)

Red crystals. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 9.76 (*s*, 1H, OH-5), 7.41 (*d*, *J* = 8.2 Hz, 1H, H-8), 7.09 (*d*, *J* = 8.2 Hz, 1H, H-7), 3.97 (*s*, 3H, 3-OCH_3), 1.95 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 186.0 (C-1), 183.4 (C-4), 157.0 (C-3), 152.4 (C-5), 150.0 (C-6), 133.1 (C-2), 122.4 (C-9), 120.2 (C-8), 119.8 (C-7), 114.8 (C-10), 60.4 (3-OCH_3), 9.4 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 235 (23, $[M+H]^+$).

6-Hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (155)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 225, 260, 285 and 350. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 7.65 (*d*, *J* = 8.4 Hz, 1H, H-8), 7.19 (*d*, *J* = 8.4 Hz, 1H, H-7), 3.95 (*s*, 3H,

3-OCH_3), 3.77 (*s,* 3H, 5-OCH_3), 1.91 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 187.2 (C-1), 183.0 (C-4), 161.4 (C-3), 160.0 (C-6), 150.2 (C-5), 131.5 (C-2), 127.9 (C-10), 127.4 (C-10), 126.5 (C-8), 123.6 (C-7), 63.6 (5-OCH_3), 63.5 (3-OCH_3), 12.0 (2-CH_3). HRESIMS *m/z* = 247.0643, $[M - H]^-$ (calculated for $C_{13}H_{12}O_5$, 247.0684).

Ancistroquinone C (95)

Yellow needle solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 7.78 (*d*, *J* = 8.6 Hz, 1H, H-8), 7.43 (*d*, *J* = 8.6 Hz, 1H, H-7), 3.91 (*s*, 3H, 6-OCH_3), 3.78 (*s*, 3H, 5-OCH_3), 1.88 (*s*, 3H, 2-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 183.8 (C-1), 179.6 (C-4), 157.5 (C-6), 155.8 (C-3), 148.6 (C-5), 125.6 (C-9), 123.3 (C-8), 122.9 (C-10), 117.5 (C-2), 116.8 (C-7), 60.5 (5-OCH_3), 56.3 (6-OCH_3), 8.4 (2-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 249 (17, $[M+H]^+$).

Chrysophanol-8-methyl ether (156)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 272, 324, 406 and 434. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.85 (*s*, 1H, OH-1), 7.86 (*t*, *J* = 8.0 Hz, 1H, H-6), 7.79 (*d*, *J* = 7.6 Hz, 1H, H-5), 7.61 (*d*, *J* = 8.4 Hz, 1H, H-7), 7.46 (*d*, *J* = 1.8 Hz, 1H, H-4), 7.15 (*d*, *J* = 1.8 Hz, 1H, H-2), 3.96 (*s*, 3H, 8-OCH_3), 2.40 (*s*, 3H, 3-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 187.7 (C-9), 182.3 (C-10), 161.6 (C-1), 160.6 (C-8), 147.5 (C-3), 136.2 (C-6), 134.9 (C-5a), 132.1 (C-4a), 124.1 (C-2), 119.8 (C-8a), 119.4 (C-7), 119.3 (C-5), 119.3 (C-4), 114.7 (C-1a), 56.6 (8-OCH_3), 21.5 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 269 (17, $[M+H]^+$).

Helminthosporin (59)

Red amorphous soild. UV λ_{max} ($CHCl_3$) nm: 493, 431, 401 and 282. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.94 (*s*, 1H, OH-5), 12.25 (*s*, 1H, OH-8), 12.07 (*s*, 1H, OH-1), 7.63 (*d*, *J* = 1.6 Hz, 1H, H-4), 7.23 (*d*, *J* = 8.0 Hz, 1H, H-6), 7.23 (*d*, *J* = 8.0 Hz, 1H, H-7), 7.05 (*dd*, *J* = 1.8,

0.9 Hz, 1H, H-2), 2.43 (*s*, 3H, 3-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 190.6 (C-9), 186.6 (C-10), 162.8 (C-1), 158.2 (C-5), 157.6 (C-8), 149.1 (C-3), 133.2 (C-4a), 129.6 (C-7), 129.5 (C-6), 124.6 (C-2), 120.8 (C-4), 114.0 (C-1a), 112.8 (C-8a), 112.5 (C-5a), 22.3 (3-CH_3).

Aloesaponarin II (65)

Orange amorphous solid. UV λ_{max} ($CHCl_3$) nm: 282, 316, 386 and 412. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.97 (*s*, 1H, OH-1), 7.71 (*t*, *J* = 7.9 Hz, 1H, H-3), 7.61 (*d*, *J* = 7.6 Hz, 1H, H-4), 7.44 (*d*, *J* = 2.1 Hz, 1H, H-5), 7.31 (*d*, *J* = 8.3 Hz, 1H, H-2), 7.03 (*d*, *J* = 2.1 Hz, 1H, H-7), 2.70 (*s*, 3H, 8-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 189.4 (C-9), 182.3 (C-10), 162.4 (C-6), 161.5 (C-1), 145.5 (C-8), 137.0 (C-5a), 136.1 (C-3), 132.6 (C-4a), 124.6 (C-7), 124.3 (C-2), 122.5 (C-8a), 118.3 (C-4), 116.5 (C-1a), 112.1 (C-5), 23.6 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 255 (31, $[M+H]^+$).

Deoxyerythrolaccin (68)

Yellow amorphous solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 13.24 (*s*, 1H, OH-1), 7.44 (*d*, *J* = 2.6 Hz, 1H, H-5), 7.05 (*d*, *J* = 2.5 Hz, 1H, H-4), 7.04 (*d*, *J* = 2.6 Hz, 1H, H-7), 6.57 (*d*, *J* = 2.5 Hz, 1H, H-2), 2.70 (*s*, 3H, 8-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 188.2 (C-9), 182.5 (C-10), 164.6 (C-1), 164.2 (C-3), 162.0 (C-6), 145.1 (C-8), 136.9 (C-4a), 134.5 (C-5a), 124.8 (C-7), 122.6 (C-8a), 112.3 (C-5), 110.1 (C-1a), 108.4 (C-2), 107.3 (C-4), 23.8 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 271 (15, $[M+H]^+$).

Aloesaponarin I (66)

Yellow crystal solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 12.77 (*s*, 1H, OH-1), 7.68 (*d*, *J* = 7.6 Hz, 1H, H-4), 7.58 (*t*, *J* = 7.6 Hz, 1H, H-3), 7.56 (*s*, 1H, H-5), 7.29 (*d*, *J* = 8.2 Hz, 1H, H-2), 3.88 (*s*, 3H, $COOCH_3$), 2.58 (*s*, 3H, 8-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 189.2 (C-9), 181.8 (C-10), 167.2 (COO), 161.4 (C-1), 158.9 (C-6), 141.0 (C-8), 136.7 (C-5a), 136.1 (C-3), 132.3 (C-4a), 129.6 (C-7), 124.4 (C-2), 122.5 (C-8a), 118.3 (C-4), 116.7 (C-1a), 112.0 (C-5), 52.5 (OCH_3), 19.9 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 313 (26, $[M+H]^+$).

Laccaic Acid-D Methyl ester (67)

Red crystalline solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 13.01 (*s*, 1H, OH-1), 7.53 (*s*, 1H, H-5), 6.99 (*d*, *J* = 2.4 Hz, 1H, H-4), 6.52 (*d*, *J* = 2.4 Hz, 1H, H-2), 3.84 (*s*, 3H, $COOCH_3$), 2.54 (*s*, 3H, 8-CH_3). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 188.0 (C-9), 182.2 (C-10), 167.8 (COO), 164.8 (C-1), 164.4 (C-3), 158.5 (C-6), 141.0 (C-8), 136.8 (C-5a), 134.3 (C-4a), 130.0 (C-8a), 122.8 (C-7), 112.3 (C-5), 110.4 (C-1a), 108.7 (C-2), 107.6 (C-4), 52.8 (OCH_3), 20.1 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 329 (18, $[M+H]^+$).

Aloesaponol II-6-methyl ether (157)

Colourless crystalline solid. UV λ_{max} ($CHCl_3$) nm: 280, 293, 312 and 377. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 7.21 (*d*, *J* = 1.1 Hz, 1H, H-10), 7.17 (*d*, *J* = 1.5 Hz, 1H, H-5), 6.81 (*d*, *J* = 1.5 Hz, 1H, H-7), 4.80 – 4.71 (*m*, 1H, H-3), 3.89 (*s*, 3H, 6-OCH_3), 2.81 – 2.74 (*m*, 2H, H-2), 2.43 (*s*, 3H, 8-CH_3), 2.18 – 2.15 (*m*, 1H, H-4), 1.98 (*dd*, *J* = 8.4, 4.6 Hz,1H, H-4). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 204.2 (C-1), 164.9 (C-9), 159.2 (C-6), 142.1 (C-4a), 141.9 (C-8), 139.5 (C-5a), 119.4 (C-5), 114.6 (C-10), 112.5 (C-8a), 109.3 (C-1a), 108.2 (C-7),

66.3 (C-3), 55.8 (6-OCH_3), 34.9 (C-2), 31.1 (C-4), 21.8 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 273 (36, $[M+H]^+$).

Aloesaponol I (69)

Colourless solid. UV λ_{max} ($CHCl_3$) nm: 281, 286, 314 and 383. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 6.95 (*s*, 1H, H-10), 6.92 (s, 1H, H-5), 4.24 (*m*, 1H, H-3), 3.83 (*s*, 3H, $COOCH_3$), 2.89 (*m*, 1H, H-4), 3.12 (*dd*, *J* = 8.4, 4.6 Hz, 1H, H-4), 2.69 (*s*, 3H, 8-CH_3), 2.68 (*m*, 1H, H-2), 2.94 (*dd*, *J* = 8.4, 4.6 Hz, 1H, H-2). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 203.8 (C-1), 170.4 (COO), 166.0 (C-9), 155.0 (C-6), 141.0 (C-4a), 140.8 (C-8), 136.4 (C-7), 125.5 (C-5a), 116.5 (C-10), 115.4 (C-8a), 110.3 (C-1a), 107.5 (C-5), 64.4 (C-3), 52.3 (OCH_3), 46.4 (C-2), 37.5 (C-4), 20.6 (8-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 317 (36, $[M+H]^+$).

Asphodelin (23)

Yellow amorphous solid. UV λ_{max} ($CHCl_3$) nm: 256, 294, 371, 382, 516, 527, and 543. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 12.60 (*s*, 1H, OH-1),12.40 (s, 1H, OH-1'), 12.05 (*s*, 1H, OH-8), 12.03 (s, 1H, OH-8'), 7.98 (*d*, *J* = 8.0 Hz, 1H, H-5'), 7.73 (*d*, *J* = 1.4 Hz, 1H, H-4'), 7.62 (*dd*, *J* = 7.4, 1.2 Hz, 1H, H-5), 7.60 (*t*, *J* = 7.4 Hz, 1H, H-6), 7.40 (*d*, *J* = 8.0 Hz, 1H, H-6'), 7.32 (*s*, 1H, H-2), 7.28 (*dd*, *J* = 7.4, 1.2 Hz, 1H, H-7), 7.14 (*d*, *J* = 1.4 Hz, 1H, H-2'), 2.52 (*s*, 3H, 3'-CH_3), 2.17 (*s*, 3H, 3-CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 192.8 (C-9), 192.8 (C-9'), 182.4 (C-10), 181.9 (C-10'), 162.9 (C-1), 162.8 (C-8), 162.2 (C-1'), 159.7 (C-8'), 149.4 (C-3), 149.2 (C-3'), 137.3 (C-4), 137.2 (C-6), 135.0 (C-6'), 134.0 (C-4'a), 133.5 (C-7'), 132.8 (C-4a), 131.1 (C-5'a), 130.4 (C-5a), 125.6 (C-2), 124.3 (C-5), 124.2 (C-7), 121.4 (C-2'), 120.4 (C-4'), 120.0 (C-5'), 115.7 (C-8a), 115.5 (C-8'a), 114.8 (C-1a), 113.9 (C-1'a), 22.3 (3-CH_3), 21.5 (3'-CH_3).

Norobtusifolin (158)

Red amorphous solid. UV λ_{max} ($CHCl_3$) nm: 213, 287, 323 and 396. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 13.18 (s, 1H, OH-1), 12.03 (s, 1H, OH-8), 7.83 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.79 (*d*, *J* = 7.6 Hz, 1H, H-5), 7.68 (*s*, 1H, H-4), 7.33 (*d*, *J* = 8.4 Hz, 1H, H-7), 2.40 (*s*, 3H, 3-CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 194.3 (C-9), 181.1 (C-10), 163.2 (C-8), 150.8 (C-2), 150.3 (C-1), 138.4 (C-6), 135.2 (C-5a), 133.3 (C-3), 125.0 (C-4a), 124.5 (C-7), 123.9 (C-4), 120.1 (C-5), 117.0 (C-8a), 115.4 (C-1a), 16.5 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 270 (53, $[M+H]^+$).

Nataloe-emodin (61)

Red amorphous solid. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 12.92 (s, 1H, OH-1), 11.93 (s, 1H, OH-8), 7.75 (*d*, *J* = 8.1 Hz, 1H, H-5), 7.59 (*d*, *J* = 1.2 Hz, 1H, H-4), 7.31 (*d*, *J* = 8.2 Hz, 1H, H-6), 7.14 (*d*, *J* = 1.2 Hz, 1H, H-2) 2.49 (*s*, 3H, 3-CH_3). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 193.2 (C-9), 180.1 (C-10), 162.6 (C-1), 152.3 (C-7), 150.3 (C-8), 149.8 (C-3), 134.0 (C-4a), 124.9 C-5a), 123.3 (C-2), 121.4 (C-5), 121.0 (C-6), 120.5 (C-4), 116.2 (C-8a), 113.9 (C-1a), 21.3 (3-CH_3). EI-MS (70 eV, rel. int.) *m/z* = 270 (57, $[M+H]^+$).

Aloe-emodin (20)

Yellow amorphous solid. ^{1}H NMR (DMSO-d_6, 25 °C) δ (500 MHz, ppm): 11.93 (s, 1H, OH-1), 11.92 (s, 1H, OH-8), 7.83 (*t*, *J* = 8.4 Hz, 1H, H-6), 7.71 (*d*, *J* = 8.4 Hz, 1.2, 1H, H-5), 7.68 (*d*, *J* = 1.5 Hz, 1H, H-4), 7.38 (*d*, *J* = 8.4, 1.2 Hz, 1H, H-7), 7.28 (*d*, *J* = 1.5 Hz, 1H, H-2), 4.62 (*s*, 2H, 3-CH_2OH). ^{13}C NMR (DMSO-d_6, 25 °C) δ (126 MHz, ppm): 193.6 (C-9), 181.4 (C-10), 161.6 (C-1), 161.3 (C-8), 153.7 (C-3), 137.4 (C-6), 133.3 (C-4a), 133.1 (C-5a), 124.4 (C-7), 120.7 (C-2), 119.4 (C-5), 117.1 (C-4), 115.9 (C-8a), 114.5 (C-1a), 62.1 (3-CH_2OH).

5-Hydroxymethyl-2-furancarboxaldehyde (159)

Coulorless oil. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 9.60 (*s*, 1H, H-1), 7.39 (*d*, *J* = 3.5 Hz, 1H, H-3), 6.59 (*d*, *J* = 3.5 Hz, 1H, H-4), 4.65 (s, 2H, H-6). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 177.2 (C-1), 162.0 (C-5), 152.5 (C-2), 123.0 (C-3), 109.3 (C-4), 56.5 (C-6). EI-MS (70 eV, rel. int.) *m/z* = 126 (76, $[M+H]^+$).

5-Acetyloxymethyl-2-furancarboxaldehyde (160)

Coulorless oil. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 9.60 (*s*, 1H, H-1), 7.43 (*d*, *J* = 3.6 Hz, 1H, H-3), 6.76 (*d*, *J* = 3.6 Hz, 1H, H-4), 5.12 (*s*, 2H, H-6), 2.88 (*s*, 3H, $COCH_3$). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 178.5 (C-1), 170.5 (COO), 156.6 (C-2), 154.0 (C-5), 123.2 (C-3), 113.3 (C-4), 58.3 (C-6), 20.5 (CH_3). EI-MS (70 eV, rel. int.) *m/z* = 168 (76, $[M+H]^+$).

Cirsiumaldehyde (161)

Coulorless oil. ^{1}H NMR (acetone-d_6, 25 °C) δ (500 MHz, ppm): 9.65 (*s*, 1H, H-1/1'), 7.41 (*d*, *J* = 3.5 Hz, 1H, H-3/3'), 6.73 (*d*, *J* = 3.6 Hz, 1H, H-4/4'), 4.69 (*s*, 2H, H-6/6'). ^{13}C NMR (acetone-d_6, 25 °C) δ (126 MHz, ppm): 178.4 (C-1/1'), 123.2 (C-3/3'), 112.7 (C-4/4'), 64.9 (C-6/6'), 154.0 (C-2/2'), 158.4 (C-5/5').

Noreugenin (162)

Colorless solid. ^{1}H NMR (acetone-d_6, 25 °C) δ (200 MHz, ppm): 12.89 (*s*, 1H, OH-7), 6.36 (*d*, *J* = 2.2 Hz, 1H, H-4), 6.22 (*d*, *J* = 2.2 Hz, 1H, H-6), 6.07 (*s*, 1H, H-2), 2.37 (*s*, 3H, CH_3); ^{13}C NMR (acetone-d_6, 25 °C) δ (50 MHz, ppm): 182.5 (C-1), 167.7 C-3), 164.1 (C-7), 162.7 (C-5), 158.6 (C-4a), 108.3 (C-6), 104.4 (C-7a), 98.9 (C-4), 93.8 (C-2), 19.6 (C-CH_3).

Knipholone Mannich Base (**163**)

Red amorphous soild, mp. 192–193 °C. UV λ_{max} (acetonitrile): 219 nm, 252 nm, 284 nm, 423 nm. ^{1}H NMR (500 MHz, acetone-d_6): δ_H 13.80 (s, 1H, 2'-OH), 7.72 (*t*, *J* = 7.8 Hz, 1H, H-6), 7.53 (*dd*, *J* = 7.8, 1.1 Hz, 1H, H-5), 7.31(*s*, 1H, H-2), 7.24 (*dd*, *J* = 7.8, 1.1 Hz, 1H, H-7), 4.01 (*d*, *J* = 1.9 Hz, 2H, N-*CH*$_2$), 3.86 (*s*, 3H, H-OCH_3), 2.73 (*m*, 4H, CH_2), 2.69 (*s*, 3H, $COCH_3$), 2.16 (*s*, 3H, 3-CH_3), 1.10 (*t*, *J* = 7.1 Hz, 6H, CH_3). ^{13}C NMR (126 MHz, acetone-d_6): δ_H 203.4 (CO), 194.1 (C-9), 182.7 (C-10), 166.0 (C-6'), 163.2 (C-4'), 163.1 (C-1), 162.6 (C-8), 161.7 (C-2'), 152.6 (C-3), 138.1 (C-6), 135.7 (C-5a), 132.5 (C-4a), 130.2 (C-4), 125.3 (C-2), 124.0 (C-7), 120.0 (C-5), 116.5 (C-8a), 115.6 (C-1a), 112.5 (C-5'), 108.2 (C-1'), 108.0 (C-3'), 63.1 (4'-OCH_3), 50.9 (N-CH_2), 46.6 (C-CH_2), 31.2 ($COCH_3$), 21.0 (3-CH_3), 11.3 (CH_3). EIMS (70 eV) *m/z* 519 $[M]^+$).

Knipholone-1,3-oxazine (**164**)

Red amorphous soild, mp. 197 – 198 °C. UV λ_{max} (acetonitrile): 217 nm, 242 nm, 290 nm, 419 nm. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 13.35 (*s*, 2'-1H, OH), 12.58 (*s*, 1H, 8-OH), 12.07 (*s*, 1H, 1-OH), 7.59 (*t*, *J* = 7.9 Hz, 1H, H-6), 7.52 (*dd*, *J* = 7.5, 1.2 Hz, 1H, H-5), 7.30 – 7.21 (*m*, 3H, H-2/3"/5"), 7.08 – 7.01 (*m*, 2H, H-2"/6"), 7.01 – 6.94 (*m*, 1H, H-4"), 5.34 (*d*, *J* = 10.5 Hz, 1H, H-NCH_2O), 5.25 (*d*, *J* = 10.5 Hz, 1H, H- NCH_2O), 4.72 (*s*, 2H, NCH_2), 3.93 (*s*, 3H, 4- OCH_3), 2.75 (*s*, 3H, $COCH_3$), 1.97 (*s*, 3H, 3-CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ_H 203.5 (CO), 192.9 (C-9), 182.3 (C-10), 162.6 (C-1), 161.8 (C-8), 160.4 (C-2'), 159.3 (C-4'), 157.4 (C-6'), 151.2 (C-3), 147.5 (C-1"), 136.8 (C-6), 134.5 (C-5a), 131.4 (C-4a), 129.3 (C-3"/5"), 127.5 (C-4), 125.2 (C-2), 123.6 (C-7), 122.2 (C-4"), 119.7 (C-5), 118.9 (C-2"/6"), 115.6 (C-8a), 114.9 (C-1a), 112.3 (C-5'), 109.3 (C-3'), 106.1 (C-1'), 80.6 (N-CH_2O), 62.0 (4'-OCH_3), 46.6 (N-CH_2), 31.3 ($COCH_3$), 20.9 (3-CH_3). EI-MS (70 eV) *m/z* 551 $[M]^+$).

3.7 Biological Tests

3.7.1 *In vitro* Antiplasmodial Assay

Two strains of *Plasmodium falciparum*, the Indochina W2 (chloroquine-resistant) and the Sierra Leone D6 (chloroquine-sensitive), donated by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC, were maintained in continuous culture to attain replication robustness prior to assays. Drug susceptibility was tested by the malaria SYBR Green I-based *in vitro* assay technique (Juma *et al*., 2011). The reference antimalarial drugs chloroquine and mefloquine were tested along with test compounds.

3.7.2 Cytotoxicity Assay

3.7.2.1 Cytotoxicity Assay Against MCF-7 and MDA-MB-231 Human Breast Cancer Cells

MCF-7 and MDA-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 (10000 cells per well) to ensure exponential growth for the duration of the assay. After a 24 hrs preincubation 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 hrs 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.

3.7.2.2 Cytotoxicity Assay Against Human Cervix Carcinoma KB-3-1 Cell

Cytotoxicity of the compounds was evaluated as described in previous report (Sammet *et al*., 2010). The KB-3-1 cells were cultivated as a monolayer in DMEM (Dulbecco's modified Eagle medium) with glucose (4.5 g/L), L-glutamine, sodium pyruvate and phenol red, supplemented with 10% (KB-3-1) fatal bovine serum (FBS). The cells were maintained at 37 °C and 5.3% CO_2 in humidified air. On the day before the test, the cells (70% confluence) were detached with trypsin-ethylenediamine tetraacetic acid solution (0.05%; 0.02% in DPBS) and placed in sterile 96-well plates in a density of 10000 cells in 100 µL medium per well. The dilution series of the compounds were prepared from stock solutions in DMSO of concentrations of 100 mM, 50 mM or 25 mM. The stock solutions were diluted with culture medium (10% FBS [KB-3-1]) down to pM range. The dilution prepared from stock solution was added to the wells. Each concentration was tested in six replicates. The control contained the same concentration of DMSO as the first dilution. After incubation for 72 hrs at 37 °C and 5.3% CO_2-humidified air, 30 µL of an aqueous resazurin solution (175 µM) was added to each well. The cells were incubated at the same condition for 5 h. Subsequently, the fluorescence was recorded at a wavelength of 588 nm. The IC_{50} values were calculated as a sigmoidal dose response curve using GRAPHPAD PRISM 4.03.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Preliminary Screening

The crude extracts of the roots of *K. foliosa*, *B. frutescens*, *A. dawei* and the leaves of *A. lateritia* subspecies *graminicola* of the Asphodelaceae family were evaluated for antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The extracts showed antiplasmodial activity (Table 4.1) with IC_{50} between 16 and 26 µg/mL. This indicated a moderate antiplasmodial activity according to the *in vitro* assay as described by Kebenei *et al*. (2011): high when IC_{50} less than 10 µg/mL, moderate when between 10 – 50 µg/mL, low between 50 - 100 µg/mL, and inactive when greater than 100 µg/mL.

Table 4.1: Antiplasmodial activity for crude extracts of four plants

Plant species	Antiplasmodial activity IC_{50} (µg/mL)	
	D6 strain	W2 strain
K. foliosa (Roots)	18.92 ± 3.50	11.28 ± 0.01
B. frutescens (Roots)	19.53 ± 2.15	10.90 ± 2.05
A. dawei (Roots)	16.17 ± 2.46	25.75 ± 3.52
A. graminicola (Leaves)	25.49 ± 3.26	14.64 ± 2.23
Chloroquine	0.01 ± 0.001	0.22 ± 0.03
Mefloquine	0.03 ± 0.02	0.003 ± 0.001

From chemotaxonomic point of view, plants of the Asphodelaceae family are known to produce polyketide derivatives, mainly anthraquinones. The crude extract of the four plants of this family

including *K. foliosa*, *B. frutescens*, *A. dawei* and *A. lateritia* subspecies *graminicola* were subjected to chromatographic separations and resulted in the isolation of anthraquinones, phenylanthraquinones, naphthoquinones and furancarboxaldehydes. The characterization, antiplasmodial activity and cytotoxicity of these isolates are discussed in this chapter.

4.2 Secondary Metabolites from *Kniphofia foliosa*

The air-dried roots of *K. foliosa* were exhaustively extracted with ethyl acetate by cold percolation at room temperature. Chromatographic separation of the extract resulted in the isolation of the monomeric anthraquinone; chrysophanol (**18**), the pre-anthraquinone; aloesaponol III (**16**), dimeric anthraquinones; 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**), and chryslandicine (**13**), phenylanthraquinones; knipholone (**14**), knipholone anthrone (**15**), knipholone cyclooxanthrone (**134**), and isoknipholone (**28**) and naphthalene derivatives; nepodin (**135**) and dianellin (**136**). Of these, the anthraquinone-anthrone dimer, 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) and phenylanthraquinone, knipholone cyclooxanthrone (**134**) are new natural products (Abdissa *et al*., 2013). The rare naphthalene glycoside, dianellin (**136**) is reported here for the first time from the family Asphodelaceae.

4.2.1 Anthraquinones

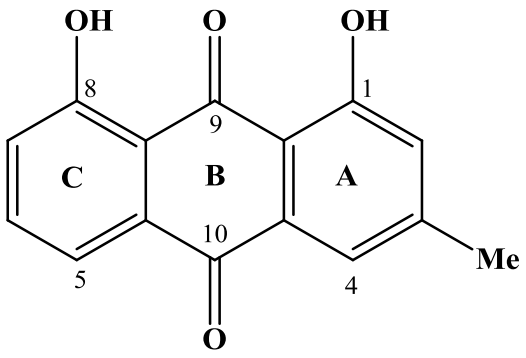
4.2.1.1 Monomeric Anthraquinone

4.2.1.1.1 Chrysophanol (18)

Compound **18** was isolated as a yellow amorphous powder with an R_f value of 0.57 (5% ethyl acetate in *n*-hexane). Upon exposure to ammonia, the yellow spot on TLC changed to red, an indication of the presence of chelated hydroxyl in a quinone moiety. The two highly downfield

shifted signals at δ_H 12.13 and 12.02 were due to chelated hydroxyl protons in ^{1}H NMR spectrum and the downfield shifted carbonyl signals at δ_C 194.4 and 184.6 in ^{13}C NMR spectrum showed that it is a 1,8-dihydroxyanthraquinone derivative (Schripsema and Dagnino, 1996).

The ^{1}H NMR spectral data (Table 4.2) further showed three mutually coupled aromatic protons at δ_H 7.70 (*d*, *J* = 7.6 Hz), 7.80 (*t*, *J* = 7.8 Hz) and 7.37 (*d*, *J* = 8.2 Hz) with ABX spin system which were assigned to H-5, H-6 and H-7 respectively of mono-substituted ring C of a 1,8-dihydroxyanthraquinone. The presence of two *meta*-coupled aromatic protons resonating at δ_H 7.21 (*d*, *J* = 2.4 Hz) and 7.55 (*d*, *J* = 2.4 Hz) assigned to H-2 and H-4, respectively with the biogenetically expected aromatic methyl (δ_H 2.46) group being at C-3 of the di-substituted ring A. The ^{1}H NMR data therefore, suggested that **18** is 1,8-dihydroxy-3-methyl anthraquinone. In consistent with the aforementioned spectral data, the ^{13}C NMR spectrum revealed 15 carbon siginals; five aromatic methines (δ_C 123.6, 122.4, 127.3, 127.6 and 140.4), seven aromatic quaternary carbons (δ_C 116.9, 119.0, 136.2, 136.4, 152.2, 164.6 and 164.8), two carbonyl carbons (δ_C 194.4 and 184.6) and a methyl (δ_C 24.7) group. Based on these and comparison with the data in the literature (Dewick, 2002), compound **18** was identified as 1,8-dihydroxy-3-methylanthracene-9,10-dione trivial name chrysophanol (**18**). Compound **18** is widely distributed in plants mainly in the families of Asphodelaceae (van Wyk *et al*., 1995a), Rhamnaceae, Rubiaceae and Polygonaceae (Dagne *et al*., 2000; Dewick, 2002). Apart from higher plants, it has also been reported as fungal metabolite (Takeda *et al*., 1973).



18

4.2.1.2 Pre-anthraquinone

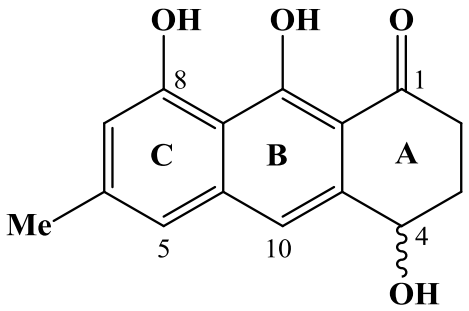
4.2.1.2.1 Aloesaponol III (16)

Compound **16** was isolated as a colorless crystalline solid. The colorless spot on TLC showed blue fluorescence under UV light (366 nm). From the MS which showed a molecular ion peak at *m/z* 258 $[M]^+$ and ^{13}C NMR spectrum displaying 15 signals, from which the molecular formula of $C_{15}H_{14}O_4$ was suggested. The UV spectrum (λ_{max} 280, 293, 312, 377 nm) was consistent with a pre-anthraquinone skeleton (Yagi *et al*., 1974).

The ^1H NMR spectrum showed a pair of *meta*-coupled aromatic protons (resonating at δ_H 7.11 (*bd*, J = 1.2 Hz) and 6.70 (*bd*, J = 1.2 Hz) assigned to H-5 and H-7, respectively) and an isolated aromatic proton (at δ_H 7.29 for H-10). A methyl group (δ_H 2.40; δ_C 22.0) was placed at C-6 (δ_C 144.6) due to the long-range coupling (Table 4.2) and the NOE interaction with H-5 and H-7 of ring C. In ring A, two mutually coupled methylene protons (at δ_H 2.75 - 2.95 and 2.15 - 2.33) were assigned to protons at C-2 (δ_C 34.7) and C-3 (δ_C 32.1), respectively. An oxymethine signal (δ_H 4.95 (*dd*, J = 5.2, 1.5 Hz); δ_C 67.8) with a broad singlet (δ_H 4.68, exchangable, for OH) showing an HMBC correlation with neighbouring carbons (C-3, C-4, C-4a). The presence of carbonyl (δ_C 205.5) at C-1 was evident from ^{13}C NMR that ring A was 4-hydroxycycohexanone

of this pre-antharquinone. The chelated hydroxyl signals at δ_H 9.68 and 16.32 were assigned to OH-8 and OH-9, respectively.

Comparison of these spectral data with the literature (Yagi, *et al*., 1977a) revealed that the compound is a 3,4-dihydro-4,8,9-trihydroxy-6-methyl-1(2*H*)-anthracenone, trivial name aloesaponol III (**16**), which had previously been isolated from *Aloe saponaria* (Yagi, *et al*., 1977a) and *Kniphofia foliosa* (Yenesew *et al.,* 1994). However, this is the first report of its ^{13}C NMR data. The absolute configuration of C-4 hydroxyl group in this compound was established to be '*S*-configured' by the application of the extended benzoate chirality rule followed by Circular Dichroism (CD) (Yagi *et al*., 1977a).



16

Table 4.2: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of chrysophanol (**18**; $CDCl_3$) and alosaponol III (**16**; acetone-d_6)

Position	18		16	
	δ_H (*m*, *J* in Hz)	δ_C	δ_H (*m*, *J* in Hz)	δ_C
1		164.8		205.5
1a		116.9		140.7
2	7.21 (*s*, 1H)	127.3	2.75 - 2.95 (m, 2H)	34.7
3		152.2	2.15 - 2.33 (m, 2H)	32.1
4	7.55 (*s*, 1H)	123.6	4.95 (*dd*, 5.2, 1.5, 1H)	67.8
4a		136.2		141.9
5	7.70 (*d*, 7.6, 1H)	122.4	7.11 (*bd*, 1.2, 1H)	119.5
5a		136.4		111.8
6	7.80 (*t*, 7.8, 1H)	140.4		144.6
7	7.37 (*d*, 8.4, 1H)	127.6	6.70 (*bd*, 1.2, 1H)	113.4
8		164.6		158.7
8a		119.0		109.3
9		194.4		166.6
10		184.6	7.29 (s, 1H)	116.7
CH_3	2.44 (*s*, 3H)	24.7	2.40 (*s*, 3H)	22.0
1-OH	12.13 (s, 1H)			
4-OH			4.68 (*bs*, 1H)	
8-OH	12.02 (s, 1H)		9.68 (*s*, 1H)	
9-OH			16.32 (*s*, 1H)	

4.2.1.3 Dimeric Anthraquinones

4.2.1.3.1 10-Hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (132)

Compound **132** was obtained as a yellow amorphous powder. The EI-MS, did not show the $[M]^+$ peak, instead $[M\text{-}OH]^+$ peak (*m/z* 491) was observed. Its UV–Vis spectrum showed characteristic absorptions (λ_{max} 265, 295, 393 and 440 nm) consistent with an anthrone-anthraquinone dimer

(Yagi *et al*., 1978a). The presence of four highly deshielded singlets (resonating at δ_H 12.26, 12.18, 12.06 and 11.72 due to the presence of four chelated hydroxyl groups), two aromatic methyl groups (δ_H 2.23; δ_C 22.2 and 2.32; 22.0) and three carbonyl groups (δ_C 193.0, 192.1 and 181.5) supported the fact that the compound was a 1,8-dihydroxyanthraquinone/anthrone derivative dimer.

The ^{1}H NMR spectrum showed that one-half of the molecule constitutes chrysophanol anthrone moiety where in ring A, two *meta*-coupled protons resonating at δ_H 6.79 (*d*, *J* = 1.2 Hz, H-2) and 6.64 (*d*, *J* = 1.2 Hz, H-4), and the biosynthetically expected methyl group (δ_H 2.23; δ_C 22.2 at C-3) were observed. In ring C, an AMX spin system for three mutually coupled aromatic protons at δ_H 6.76 (*dd*, *J* = 8.4, 1.2 Hz), 7.51 (*t*, *J* = 8.4 Hz) and 6.92 (*dd*, *J* = 8.4, 1.2 Hz) which were assigned to H-5, H-6 and H-7, respectively. The up-field shifted chemical shift values of H-4 (δ_H 6.64) and H-5 (δ_H 6.76) in the ^{1}H NMR when compared to those for protons in chrysophanol (**18**) in combination with the presence of an sp^3 hybridized oxygenated quaternary carbon (δ_C 70.0) indicated the oxanthrone nature of this half of the molecule where C-10 (δ_C 70.0) is the point of attachment to the other half of the molecule.

The ^{1}H NMR spectral pattern of the other half of this molecule showed the presence of chrysophanol moiety where the ABX pattern in chrysophanol was replaced by a pair of de-shielded *ortho*-coupled protons resulting in AB pattern resonating at δ_H 7.85 (*d*, *J* = 8.0 Hz) and δ_H 8.66 (*d*, *J* = 8.0 Hz) which were readily assigned to H-5' and H-6', respectively. This indicated that the point of attachment in this half of the molecule is at C-7' (δ_C 142.2). Therefore, this compound was composed of chrysophanol and chrysophanol oxanthrone, *vis* 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), a compound previously isolated from *Kniphofia*

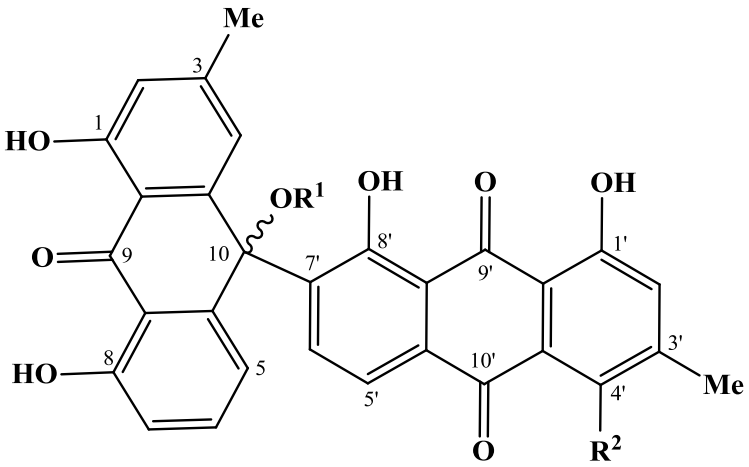
foliosa (Wube *et al*., 2005), *Senna longiracemosa* (Alemayehu *et al*., 1993), *Aloe saponaria* (Yagi *et al*., 1978a) and *Asphodelus ramosus* (Lanzetta *et al.*, 1990). It has been reported that this compound showed high inhibition of the growth of the malaria parasite, *P. falciparum* (IC_{50} 0.26 µg/mL) and weak cytotoxicity against human epidermoid carcinoma (KB) cell line (ED_{50} 104 µg) (Wube *et al*., 2005).

4.2.1.3.2 10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (133)

Compound **133** was obtained as a yellow amorphous solid. The TOF-HREIMS, did not show the $[M]^+$ peak, instead $[M\text{-}OCH_3]^+$ peak (*m/z* 491.0353) was observed. Its UV–Vis spectrum showed characteristic absorptions (λ_{max} 265, 295, 393 and 440 nm) of an anthrone-anthraquinone dimer (Alemayehu *et al*., 1993). In agreement with such moiety, the ^1H NMR spectrum showed the presence of four highly deshielded singlets resonating at δ_H 12.37, 12.27, 12.07 and 11.73 due to the presence of four chelated hydroxyl groups as in 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**).

In addition, the presence of two aromatic methyl protons resonating at δ_H 2.26 (C-3) and 2.35 (C-3') supported the fact that the compound is an anthraquinone/anthrone dimer. One-half of the molecule showed *meta*-coupled protons at δ_H 6.77 (*d*, *J* = 1.2 Hz) and 6.62 (*d*, *J* = 1.2 Hz). These signals were assigned to H-2 and H-4, respectively, with the biosynthetically expected methyl group (δ_H 2.26; δ_C 22.3) being at C-3. In addition, an ABX spin system was observed for three aromatic protons centered at δ_H 6.78 (*dd*, *J* = 8.4, 1.2 Hz), 7.43 (*t*, *J* = 8.4 Hz) and 6.94 (*dd*, *J* = 8.4, 1.2 Hz) and were assigned to H-5, H-6 and H-7, respectively of the chrysophanol anthrone moiety, leaving C-10 (δ_C 75.6) as the point of attachment to the other half of the molecule.

The ^{1}H NMR spectral pattern of the other half of the molecule showed that it was that of a chrysophanol moiety where the ABX pattern in chrysophanol is replaced by a pair of de-shielded *ortho*-coupled protons with AB pattern at δ_H 7.95 (*d*, *J* = 8.0 Hz) and δ_H 8.66 (*d*, *J* = 8.0 Hz) and these were readily assigned to H-5' and H-6' respectively. This indicated that the point of attachment in this half of the molecule is at C-7' (δ_C 132.2). Therefore, this compound was composed of chrysophanol and chrysophanol oxanthrone units similar to compound **132.** The only difference between compounds **133** and **132** was that, the 10-hydroxyl group had been replaced with methoxyl group (δ_H 2.86) in **133**. This was confirmed by ^{1}H-^{13}C HMBC experiment (Table 4.3) where the methoxyl protons correlated with C-10. The upfield chemical shift of the methoxy group (δ_H 2.86) (due to anisotropic effect) was an indication of its attachment to the sp^3 carbon, C-10 (δ_C 75.6). The compound was therefore characterized as 10-methoxy-10,7'-(chrysophanolanthrone)-chrysophanol (**133**). Although the absolute configuration of this compound has not been determined, it is worth reporting that the CD spectrum showed a positive Cotton effect at 272 nm. This is the first report of the occurrence in nature of the compound having previously been reported as a synthetic derivative (Yagi *et al*., 1978a).



132 $R^1 = R^2 = H$
133 R^1 = Me, $R^2 = H$
13 $R^1 = H$, R^2 = OH

4.2.1.3.3 Chryslandicin (13)

Compound **13** was isolated as a red crystalline solid. The EIMS showed an $[M]^+$ peak at *m/z* 524 corresponding to the molecular formula of $C_{30}H_{20}O_9$. Its UV-Vis spectrum (λ_{max} 272, 295, 386, and 496 nm) indicated a dimeric anthraquinone similar to compound **132**. Furthermore, the NMR spectra (Table 4.3) showed the presence of five highly deshielded proton signals resonating at δ_H 12.07, 12.30, 12.32, 12.42 and 13.48 due to the presence of chelated hydroxyl groups, two aromatic methyl groups (δ_H 2.26; δ_C 22.3 and 2.35; 22.1) and three carbonyl carbons (δ_C 186.4, 190.3 and 192.4) supported the suggestion that the compound is a dimeric anthraquinone/anthrone units.

One-half of the molecule constitutes chrysophanol oxanthrone skeleton consisting of two *meta*-coupled protons resonating at δ_H 6.77 (*d*, *J* = 1.4 Hz, H-2) and 6.61 (*d*, *J* = 1.4 Hz, H-4) with the biogenetically expected methyl (δ_H 2.26; δ_C 22.3) being at C-3 together with an ABX spin system for three aromatic protons which resonated at δ_H 6.93 (*d*, *J* = 8.2 Hz, H-5), 7.40 (*t*, *J* = 8.2 Hz, H-6) and 6.81 (*d*, *J* = 8.2 Hz, H-7) leaving C-10 (71.0) as the point of attachment to the other half of the molecule as in compound **132**.

In the other half of the molecule, similar ^{1}H NMR spectral pattern as in **132** was observed except that C-4' was substituted with hydroxyl group as in islandicin (Berhanu *et al*., 1986). The molecular formula of **13** was higher by 16 amu than that of **132** supporting that **13** has an additional oxygen atom. Therefore, compound **13** was 10-hydroxy-10-(islandicin-7'-yl)-chrysophanol anthrone, trivial name chryslandicin (**13**), previously isolated from the roots of *K. foliosa* and *K. thomsonii* (Dagne *et al*., 1987; Achieng, 2009). Potent antiplasmodial activity (IC_{50} 0.54 µg/mL) has been reported for this compound (Wube *et al*., 2005).

Table 4.3: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **132** (in DMSO-d_6), **133** (in $CDCl_3$) and **13** (in $CDCl_3$): δ (ppm), *J* (Hz)

Position	**132**		**133**		**13**	
	δ_H (*m, J*)	δ_C	δ_H (*m, J*)	δ_C	δ_H (*m, J*)	δ_C
1		161.8		162.9		162.3
1a		112.6		114.3		110.7
2	6. 79 (*d*, 1.2)	117.2	6.77 (*d,* 1.2)	119.3	6.77 (*d,* 1.4)	118.1
3		148.9		149.0		148.8
4	6.64 (*d*, 1.2)	120.9	6.62 (*d,* 1.2)	120.9	6.61 (*d,* 1.4)	120.1
4a		148.4		148.1		146.2
5	6.76 (*d*, 8.4)	119.9	6.78 (*dd,* 1.2, 8.4)	118.1	6.93 (*d,* 8.2)	118.8
5a		148.3		143.2		146.0
6	7.51 (*t*, 8.4)	137.3	7.43 (*t,* 8.4)	136.8	7.40 (*t,* 8.2)	136.2
7	6.92 (*d*, 8.4)	116.9	6.94 (*dd,* 1.2, 8.4)	117.7	6.81 (*d,* 8.2)	117.8
8		161.5		162.6		162.5
8a		114.7		116.1		111.2
9		193.0		193.1		192.4
10		70.0		75.6		71.0
1'		161.9		158.9		157.9
1'a		114.0		115.7		114.4
2'	7.04 (*d*, 1.4)	124.3	7.04 (*d,* 1.2)	124.6	7.08 (*s*)	129.0
3'		149.6		149.9		141.1
4'	7.37 (*d*, 1.4)	120.9	7.61 (*d,* 1.2)	121.7		157.7
4'a		133.3		143.6		116.2
5'	7.85 (*d*, 8.2)	119.3	7.95 (*d,* 8.0)	119.6	8.06 (*d,* 8.0)	118.8
5'a		132.8		131.4		133.0
6'	8.66 (*d*, 8.2)	132.2	8.66 (*d,* 8.0)	133.1	8.63 (*d,* 8.0)	132.2
7'		142.2		132.2		141.9
8'		159.1		162.4		158.6
8'a		115.9		116.2		112.2
9'		192.1		191.4		190.3
10'		181.5		182.1		186.4
3-CH_3	2.23 (*s*)	22.2	2.26 (*s*)	22.3	2.25 (*s*)	22.3
3'-CH_3	2.32 (*s*)	22.0	2.35 (*s*)	22.4	2.35 (*s*)	22.1
10-OMe			2.86 (*s*)	50.7		
1-OH	11.62 (*s*)		12.37 (*s*)		12.42 (*s*)	
1'-OH	12.26 (*s*)		12.27 (*s*)		12.32 (*s*)	
8-OH	12.18 (*s*)		12.07 (*s*)		12.30 (*s*)	
8'-OH	12.06 (*s*)		11.72 (*s*)		12.07 (*s*)	
4'-OH					13.48 (*s*)	

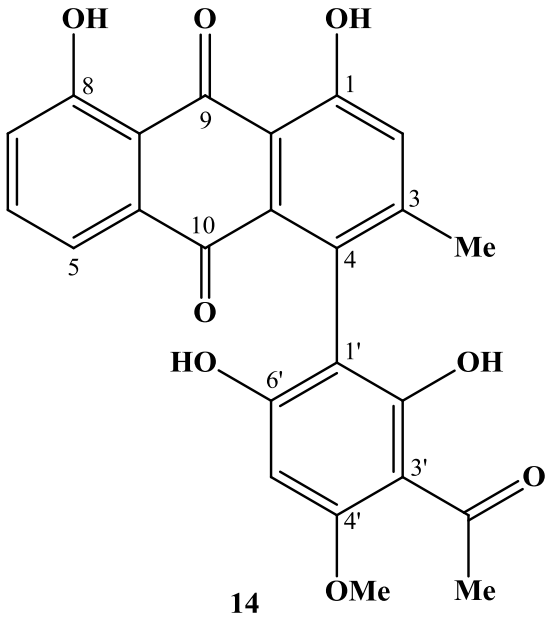
4.2.1.4 Phenylanthraquinones

4.2.1.4. 1 Knipholone (14)

Compound **14** was isolated as a deep red amorphous solid with an R_f value of 0.54 (15% ethyl acetate in *n*-hexane). The UV-Vis spectrum (λ_{max} 284, 354, and 425 nm) suggested the presence of a phenylanthraquinone moiety (Dagne and Staglish, 1984). The ^{1}H NMR spectrum was similar to that of chrysophanol with two highly deshielded singlets at δ_H 12.52, and 11.98, indicating two intramolecularly hydrogen bonded hydroxyl groups, and were assigned to 1-OH and 8-OH of a chrysophanol moiety. In agreement with this, the ^{1}H NMR spectrum showed three mutually coupled aromatic protons with an ABX spin system at δ_H 7.56 (*dd, J* = 7.6, 1.2 Hz), 7.76 (*dd, J* = 7.8, 7.6 Hz) and 7.31 (*d, J* = 7.8, 1.2 Hz) which were assigned to H-5, H-6 and H-7 respectively in ring C. However; in ring A, only a singlet at δ_H 7.33 which was assigned to H-2 with the biosynthetically expected aromatic methyl group (δ_H 2.41; δ_C 21.0) being at C-3. This implies that C-4 (δ_C 128.9) was the point of attachment with the acetylphloroglucinol methyl ether unit, whose presence was evident from NMR spectra (Table 4.4). Thus a downfield shifted singlet at δ_H 14.22 (due to the chelated hydroxyl proton), upfield shifted singlet aromatic proton (δ_H 6.24 for H-5'), an acetyl group (δ_H 2.64, δ_C 33.1, 203.7 at C-3'), and methoxy group (δ_H 3.99, δ_C 56.1 at C-4'). Therefore, compound **14** was identified as 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methylanthraquinone, trivial name knipholone (Dagne and Steglich, 1984).

The absolute configuration of this compound at the biaryl axis was established to be *P*-configured by using time dependent DFT and DFT/MRCI Circular Dichromism calculations (Bringmann *et al*., 2007a). Compound **14** has been reported from the three genera *Kniphofia*, *Bulbine*, and *Bulbinella* in Asphodelaceae family (Dagne and Steglich, 1984; Bringmann *et al*.,

2008b). Apart from the Asphodelaceae family, it has also been reported from the pods of *Senna didymobotrya* (Leguminosae) (Alemayehu *et al*., 1996). It has been reported that compound **14** showed an antiplasmodial activity against K1 (chloroquine-resistant) and NF54 (chloroquine-sensitive) strain of *Plasmodium falciparium* and was also found to be a good inhibitor of leukotriene formation (Wube *et al*., 2006). It has also been reported that knipholone induces rapid onset of cytotoxicity (IC_{50} 0.5 - 3.3 μM) (Habtemariam, 2010).



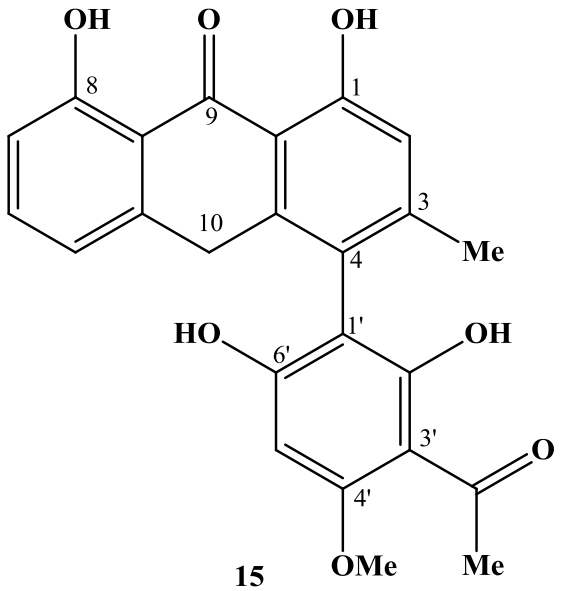
14

4.2.1.4. 2 Knipholone Anthrone (15)

Knipholone anthrone (coulorless solid), showed bluish-yellow fluorescence when viewed under UV light (366 nm). The EIMS showed the molecular ion peak at *m/z* 420, suggesting a molecular formula of $C_{24}H_{20}O_7$. The NMR spectral data pattern was similar to that of knipholone (**14**) which showed the presence of two singlets for the chelated hydroxyl groups (δ_H 12.36 and 12.05), the typical ABX pattern for the protons of H-5, H-6 and H-7, a singlet at δ_H 6.82 for H-2, a three-proton-singlet at δ_H 2.03 for the aromatic methyl group and acetylphloroglucinol methyl ether unit (whose presence was evident from the downfield shifted singlet at δ_H 14.15 due to the

chelated hydroxyl proton, the singlet aromatic proton shifted upfield to δ_H 6.11 for H-5', an acetyl proton, δ_H 2.59 at C-3' and a methoxy group, δ_H 3.82; δ_C 54.5) at C-4. The only difference was the presence of additional broad singlet at δ_H 4.00 in ^{1}H NMR spectrum integrated for two protons and a methylene carbon signal at δ_C 32.4 in the ^{13}C NMR spectrum instead of a carbonyl at C-10, suggesting that the compound is 4-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-1,8-dihydroxy-10H-anthracen-9-one, trivial name knipholone anthrone (**15**), previously reported from the stem of *K. foliosa* (Dagne and Yenesew, 1993). The absolute configuration of **15** at the axially chiral biaryl axis was established to be *P*-configured by using time dependent DFT and DFT/MRCI circular dichromism calculations (Bringmann *et al*., 2007a).

Knipholone anthrone showed considerable *in vitro* antiplasmodial activity with the IC_{50} values of 0.38 and 0.42 μg/mL against the chloroquine resistant (K1) and the chloroquine-sensitive (NF54) strains of *Plasmodium falciparum*, respectively (Bringmann *et al*., 1999a). It also showed antioxidant activity [even better than the positive control (-)-epicatechin (EC)] in scavenging superoxide anions and preventing deoxyribose degradation (Habtemariam, 2007; 2010).



15

4.2.1.4. 3 Knipholone Cyclooxanthrone (134)

Compound **134** was obtained as a yellow amorphous solid. Its molecular formula was established as $C_{24}H_{18}O_7$ from HRMS analysis (*m/z* 418.1055, calculated for 418.1053). The UV spectrum (λ_{max} 284, 354 and 425 nm) suggested the presence of a phenylanthrone moiety as in knipholone anthrone (Dagne and Yenesew, 1993). In fact the ^{1}H NMR spectrum was similar to that of knipholone anthrone (**15**) with two highly deshielded singlets at δ_H 12.25 and 11.66, indicating two intramolecular hydrogen bonded hydroxyl groups, and were assigned to 1-OH and 8-OH of a chrysophanol anthrone moiety. In agreement with this, the ^{1}H NMR spectrum showed three mutually coupled aromatic protons with an ABX spin system at δ_H 7.46 (*d, J* = 7.6 Hz), 7.63 (*t, J* = 7.9 Hz) and 7.06 (*d, J* = 8.3 Hz) which were assigned to H-5, H-6 and H-7 respectively of ring C of the chrysophanol anthrone part of the molecule. Ring A only showed a singlet at δ_H 7.00 for H-2 with the biogenetically expected aromatic methyl (δ_H 2.42; δ_C 23.7) being at C-3. As in knipholone anthrone, C-4 (δ_C 120.1) was the point of attachment of the acetylphloroglucinol methyl ether unit, whose presence was evident from NMR spectra (Table 4.4) which showed a downfield shifted singlet at δ_H 15.02 due to the chelated hydroxyl proton, upfield shifted singlet aromatic proton (δ_H 6.38 for H-5'), an acetyl group (δ_H 2.72, δ_C 33.3, 204.2) at C-3', and methoxy group (δ_H 3.99, δ_C 56.0) at C-4'.

From the ^{13}C NMR spectrum (Table 4.4) of compound **134**, the presence of only two carbonyl carbon signals (at δ_C 192.2 for C-9 and 204.2 for the acetyl carbonyl group) was evident which clearly signified the lack of a carbonyl at C-10 as in knipholone anthrone. The difference between compounds **134** and **15** (knipholone anthrone) was that the methylene (CH_2-10) signal (δ_H 4.00, 2H, *bs*; δ_C 32.4) in the latter was replaced by an oxymethine signal (δ_H 5.57, 1H, *s*; δ_C

72.2), which clearly indicates that compound **134** was knipholone oxanthrone. The HMBC spectrum (Table 4.4) showed correlation between H-10 and C-6' which suggested the presence of an unprecedented cyclic ring involving C-10 and C-6' and allowed the assignment of structure **134** for which the trivial name knipholone cyclooxanthrone was suggested (Abdissa *et al*., 2013). The Cotton effect observed in the CD spectrum (Fig. 4.1) was in agreement with the compound being chiral having a center of chirality at C-10 with an axis of chirality at C4-C1'.

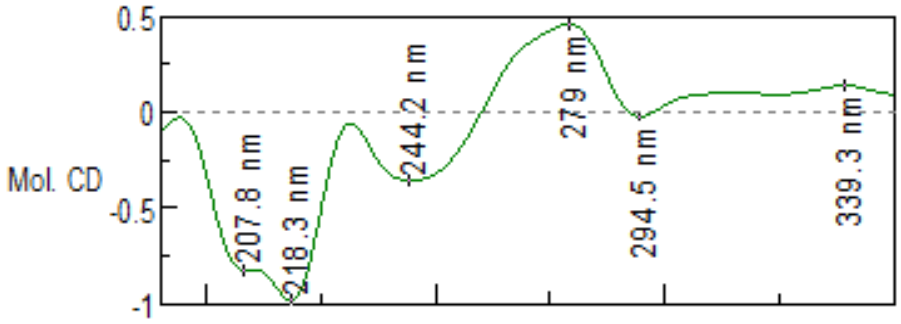
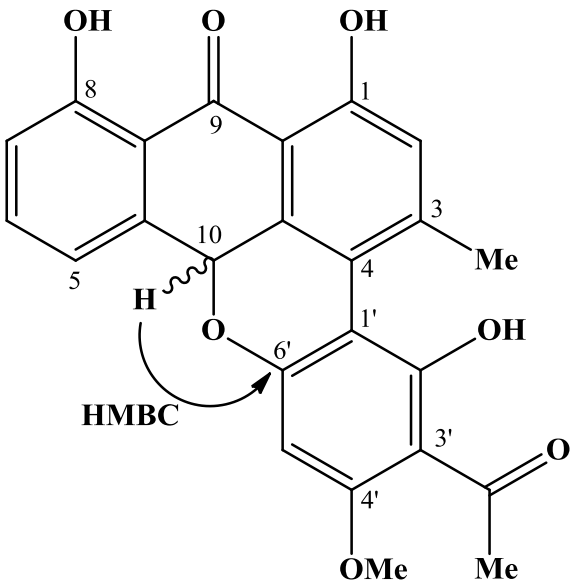
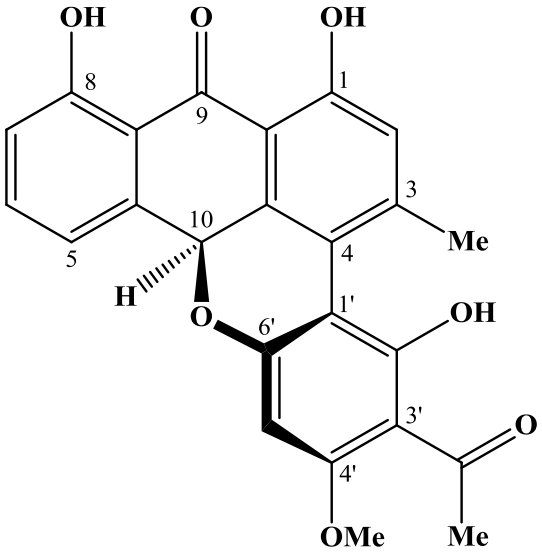


Figure 4.1: CD spectrum of compound **134**

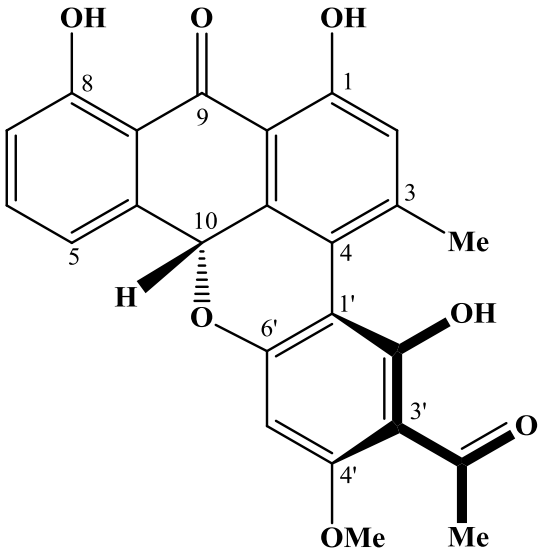
Due to the geometry of the cyclization involving C-10 and C-6', there are two possible isomers for this compound *vis-á-vis* **134a** (4*M*, 10*S*) or **134b** (4*P*, 10*R*). To decide which of these is the absolute configuration for the compound, advanced quantum chemical CD calculations based on time-dependent DFT (TDDFT) and multi-reference configurational interaction (DFT/MRCI) approaches as described by Bringmann *et al*. (2007a) may be used.



134



134a

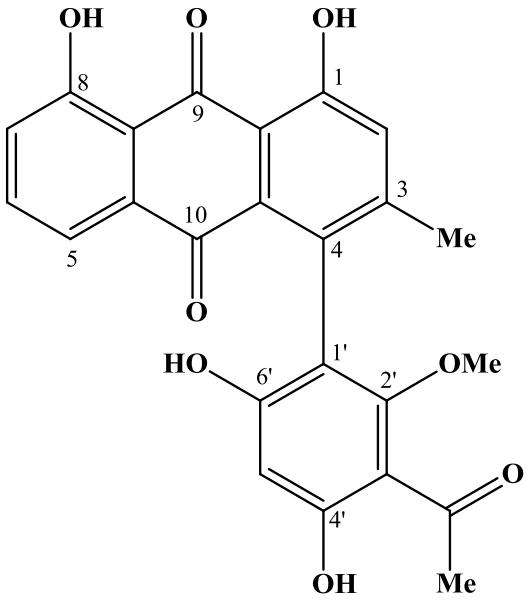


134b

4.2.1.4. 4 Isoknipholone (28)

Compound **28** was isolated as an orange amorphous solid. The EIMS showed $[M]^+$ at *m/z* 434 suggesting a molecular formula of $C_{24}H_{18}O_8$. The molecular formula and its NMR spectra indicated that it could be an isomer of knipholone (**14**). The NMR spectral pattern of **28** (Table

4.4) appears to possess a knipholone skeleton with the same chrysophanol and acetylphloroglucinol methyl ether moieties. The only difference noted was the chemical shift value for methoxyl and hydroxyl groups in the acetylphloroglucinol methyl ether unit (Table 4.4). The up-field shifted methoxyl group resonates at δ_H 3.41 (due to its proximity to the shielding region of the chrysophanol moiety) in compound **28** as opposed to δ_H 3.99 in compound **14** suggesting its placement at C-2′. Therefore compound **28** was identified as 4-(3-acetyl-4,6-dihydroxy-2-methoxyphenyl)-4,5-dihydroxy-2-methylanthraquinone, trivial name isoknipholone (**28**), which had previously been reported from the root of *Kniphofia foliosa* (Yenesew *et al*., 1994) and *Bulbine capitata* (Bezabih *et al*., 1997). This compound also showed potent antiplasmodial activity (IC_{50} 0.12 µg/mL) against the K1 (chloroquine-resistant) strain of *P. falciparum* (Mutanyatta *et al*., 2005).



28

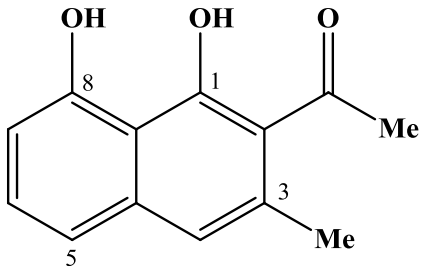
Table 4.4: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **14** (acetone-d_6), **15**, **134** ($CDCl_3$) and **28** (acetone-d_6): δ (ppm), *J* (Hz)

Position	Compound **14**		Compound **15**		Compound **134**		Compound **28**	
	δ_H (*m,* J)	δ_C	δ_H (*m,* J)	δ_C	δ_H (*m,* J)	δ_C	δ_H (*m,* J)	δ_C
1		163.7		164.7		160.8		161.8
1a		115.7		116.0		110.2		116.5
2	7.33 (*s*)	125.4	6.82 (*s*)	119.5	7.00 (*s*)	120.5	7.37 (*s*)	126.4
3		153.0		150.4		147.4		153.2
4		128.9		120.8		120.1		129.6
4a		133.1		143.8		134.8		134.0
5	7.56 (*dd*, 7.6, 1.2)	120.1	6.72 (*dd*, 7.2, 1.2)	115.5	7.46 (*d*, 7.6)	118.7	7.58 (*dd*, 7.8, 1.2)	120.9
5a		135.6		142.9		138.6		136.4
6	7.76 (*dd*, 7.8, 7.6)	138.2	7.31(*dd*, 8.2, 7.2)	136.7	7.63 (*t*, 7.9)	136.5	7.78 (*dd*, 7.8, 8.4)	138.9
7	7.31 (*dd*, 7.8, 1.2)	124.1	6.64 (*dd*, 8.2, 1.2)	117.7	7.06 (*d*, 8.3)	118.2	7.32 (*dd*, 8.4, 1.2)	124.9
8		162.7		160.6		162.8		161.4
8a		116.4		115.1		114.1		117.2
9		194.1		194.4		192.2		194.8
10		182.7	4.00 (*brs*)	31.9	5.57 (*s*)	72.2		184.0
1'		108.5		106.7		106.1		115.5
2'		164.7		164.2		163.0		164.5
3'		106.2		105.2		107.3		110.4
4'		163.4		163.8		163.2		167.2
5'	6.24 (*s*)	91.8	6.11 (*s*)	91.1	6.38 (*s*)	91.3	6.30 (*s*)	101.0
6'		162.0		163.1		162.5		163.1
*C*O		203.7		204.2		204.2		204.8
3-CH_3	2.41 (*s*)	21.0	2.03 (*s*)	21.1	2.42 (*s*)	23.7	2.22 (*s*)	21.9
CO*Me*	2.64 (*s*)	33.1	2.59 (*s*)	33.5	2.72 (*s*)	33.3	2.64 (*s*)	32.3
4'-OMe	3.99 (*s*)	56.1	3.82 (*s*)	54.5	3.99 (*s*)	56.0		
2'-OMe							3.41 (*s*)	61.7
1-OH	12.52 (*s*)		12.36 (*s*)		12.25 (*s*)		12.56 (*s*)	
8-OH	11.98 (*s*)		12.05 (*s*)		11.66 (*s*)		11.95 (*s*)	
2'-OH	14.22 (*s*)		14.15 (*s*)		15.02 (*s*)			
4'-OH							13.60 (*s*)	

4.2.1.5 Naphthalene Derivatives

4.2.1.5.1 Nepodin (135)

Compound **135** was identified as a naphthalene derivative, based on its NMR spectral data. The ^{1}H NMR spectrum exhibited signals for two chelated hydroxyl groups resonating at δ_H 10.23 and 17.33, two methyl groups (δ_H 2.24, 2.64) and four aromatic protons of which three are mutually coupled (showing ABX spin pattern) centered at δ_H 7.06 (*d, J* = 7.4 Hz), 7.46 (*t, J* = 8.0 Hz) and 6.83 (*d, J* = 8.0 Hz) and a singlet proton at δ_H 6.98. The ^{13}C NMR spectrum revealed thirteen signals; one carbonyl, six quaternary, four methine and two methyl carbons, consistent with the compound being naphthalene derivative 2-acetyl-1,8-dihydroxy-3-methylnaphthalene, trivial name nepodin (**135**), which had previously been isolated from the root of *K. foliosa* (Wube *et al*., 2005).



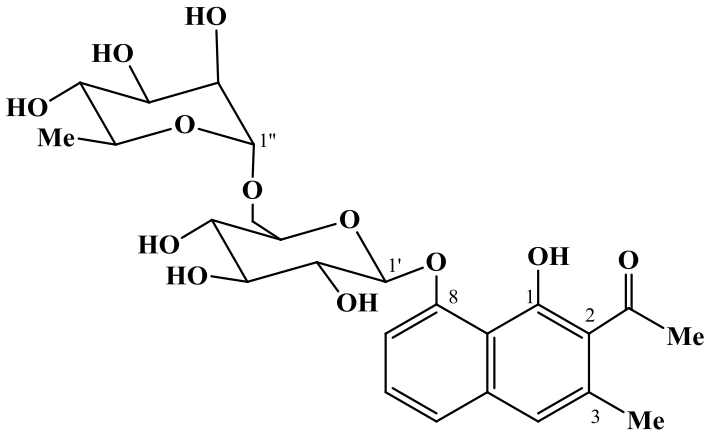
135

4.2.1.5.2 Dianellin (136)

Dianellin was found as a yellowish oil (R_f 0.45; 5% MeOH in CH_2Cl_2). The HREIMS provided a molecular ion peak at *m/z* 524.1970 $[M]^+$, corresponding to the molecular formula of $C_{25}H_{32}O_{12}$. The NMR spectra showed that **136** was a glycoside of nepodin (**135**). This was confirmed by acid hydrolysis of **136** to give **135**. The two doublet signals at 5.18 and 4.81 in the ^{1}HNMR spectrum are typical signals for anomeric protons. In addition, the presence of eleven sp^3 carbon signals between 60 ppm and 105 ppm in ^{13}C NMR indicating that the compound has a

disaccharide group containing glucose and rhamanose units (Dias *et al*., 2009). The presence of ten sp^2 carbon signals between 110 and 155 ppm along with three additional carbon signals that represented an aromatic methyl (20.5) and an acetyl moiety (32.9 and 204.8) in ^{13}C NMR, was in agreement with a nepodin glycoside.

The sugar moiety was placed at C-8 on the basis of a three-bond HMBC correlation of the anomeric proton at δ_H 5.17 (1H, *d*, *J* = 7.8 Hz, H-1') with C-8 (δ_C 156.4) of the aglycone unit. The HMBC correlations between the CH_2-6′ signals (δ_H 4.12,1H, *dd*, *J* = 2.0, 11.0 Hz, H-6′a; δ_H 3.68, 1H, *dd*, *J* = 7.2, 11.0 Hz, H-6′b) and C-1″ (δ_C 102.5), and also between H-1″ (δ_H 4.80, 1H, *d*, J = 1.4 Hz) and C-6′ (δ_C 68.5) established a 1→6 linkage between the D-glucopyranosyl and L-rhamnopyranosyl moieties. The configuration at the anomeric center of the D-glucopyranosyl group (C-1′) was determined to be β from the big coupling constant (*J* = 7.8 Hz) as the result of 1,2-diaxial relationship between H-1′ and H-2″; whereas the configuration at the anomeric centre (C-1″) of L-rhamnopyranosyl moiety, having the more stable $^1C_4$ conformation, was deduced to be *α* (alpha) from the big $^1J_{C,H}$ (168 Hz) coupling constant between H-1" and C-1", with the expected value for the β-anomer being in the range 152-160 Hz (Bock and Pedersen, 1974; El Ashry *et al*., 2008). Therefore, this compound was identified as 2-acetyl-3-methyl-1-hydroxy-8-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-naphthalene, trivial name dianellin (**136**). This is the first report of the isolation of compound **136** from the family Asphodelaceae having been reported from *Dianella* (Batterham *et al*., 1961; Dias *et al*., 2009) and *Hemerocallis* species (Cichewicz *et al*., 2002). It is worth to point out that, in the structure given for dianellin by Cichewicz *et al*. (2002), the rhamnopyranoside was drawn as α-D-rhamnopyranoside in the $^4C_1$ conformation instead of α-L-rhamnopyranoside in the $^1C_4$ form, whereas acid hydrolysis gave L-rhamnose as one of the products as reported in the same manuscript.



136

4.3 Secondary Metabolites from the Roots of *Bulbine frutescens*

The air-dried and ground roots of *B. frutescens* were extracted using CH_2Cl_2/CH_3OH (1:1). Chromatographic separation of the extract led to the isolation and characterization of two anthraquinones: chrysophanol (**18**) and 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), a xanthone: 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), three phenyl anthraquinones: knipholone (**14**), isoknipholone (**28**) and 6',8-*O*-dimethylknipholone (**150**), two furanonaphthoquinones: 4-*O*-methyleleutherol (**53**) and 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-C]furan-4,9-dione (**50**) and an acetophenone: 2,6-dimethoxy-4-hydroxyacetophenone (**151**). Of these compounds xanthone, **138** and phenylanthraquinone, **150** are new natural products. Compounds **18**, **14** and **28** have been identified from the roots of *K. foliosa* as discussed earlier in section 4.2. The characterization of the other compounds are discussed below.

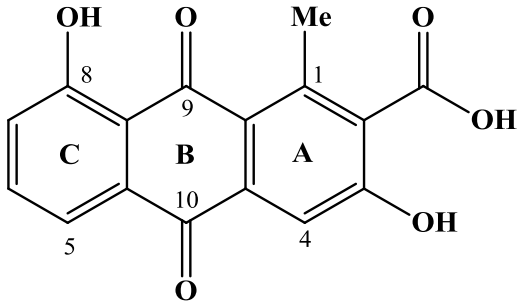
4.3.1 Anthraquinones

4.3.1.1 3,8-Dihydroxy-1-methylanthraquinone-2-carboxylic Acid (137)

Compound **137** was isolated as a yellow amorphous powder (R_f 0.65; 30% EtOAc in *n*-hexane). EIMS provided a molecular ion peak at *m/z* 298, corresponding to the molecular formula of $C_{16}H_{10}O_6$. The UV-Vis (λ_{max} 274, 310, 382, 418 nm) and ^{13}C NMR spectral data (Table 4.5) suggested an anthraquinone skeleton. In the ^{1}H NMR spectrum (Table 4.5), a downfield shifted signal at δ_H 12.95 was due to chelated hydroxyl proton at C-8. In ring C, three mutually coupled aromatic protons at δ_H 7.72 (*dd*, *J* = 7.5, 1.2 Hz), 7.74 (*dd*, *J* = 8.3, 7.5 Hz) and 7.33 (*dd*, *J* = 8.3, 1.2 Hz) were assigned to H-5, H-6 and H-7, respectively. In ring A, a singlet at δ_H 7.74 (*s*) was assigned to H-4 which otherwise is fully substituted with the deshielded methyl group (δ_H 2.85 due to the deshielding effect of the neighboring carbonyl group being at C-1). These assignments were in agreement with the HMBC correlation observed between methyl signals with C-1a and C-2 (Table 4.5).

The ^{13}C NMR spectrum (Table 4.5) of **137** showed sixteen carbon signals, which accounted for four sp^2 methines, one methyl, eight sp^2 quaternary carbon atoms and three carbonyl signals at δ_C 191.5, 183.4 and 169.2. The first two were attributed to a quinone system and the latter to a carboxylic acid at C-2 (from biogenetic considerations) of ring A, which also contains a hydroxyl group at C-3 (δ_C 160.5). The data was consistent with the compound being 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), which had previously been reported from the fungus *Streptomyces* species (Krupa *et al*., 1989). The biosynthesis of the anthraquinones of this plant including the phenylanthraquinones is similar to the chrysophanol-based compounds where the octaketide chain has folded in the usual way. However, the octaketide chain in 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**) appears to

have been folded in an unusual way as in aloesaponarin II (Dagne *et al*., 1994). This is the first report on the isolation of such anthraquinone from the sub-family Asphodeloideae.



137

Table 4.5: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data along with HMBC of Compound **137**: δ (ppm), *J* (Hz) in acetone-d_6

Position	δ_H (*m,* J)	δ_C	HMBC
1		143.7	
1a		125.1	
2		131.6	
3		160.5	
4	7.74 (*s*)	113.8	C-1a, 2, 10
4a		138.7	
5	7.72 (*dd,* 7.5, 1.2)	119.9	C-7, 8a, 10
5a		134.4	
6	7.74 (*dd*, 8.3, 7.5)	137.5	C-5a, 8
7	7.33 (*dd*, 8.3, 1.2)	125.8	C-5, 8a
8		163.6	
8a		118.7	
9		191.5	
10		183.4	
COO		169.2	
1-CH_3	2.85 (*s*)	21.1	C-1, 1a, 2
6-CH_3			
8-OH	13.0 (*s*)		

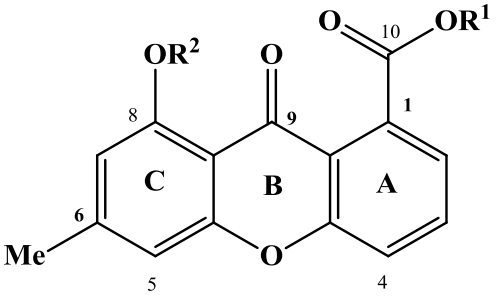
4.3.2 Xanthone

4.3.2.1 8-Hydroxy-6-methylxanthone-1-carboxylic Acid (138)

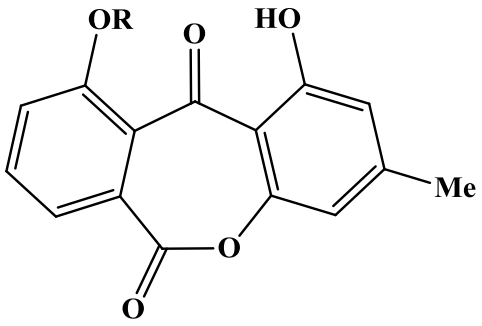
Compound **138** was obtained as a yellow crystalline solid. Its molecular formula was established as $C_{15}H_{10}O_5$ on the basis of its HREIMS (*m/z* 270.0520; $[M]^+$, calcd. 270.0528) and ^{13}C NMR spectral data which was consistent with eleven degrees of unsaturation. The UV-Vis absorption at λ_{max} 225, 254, 362 and 386 nm and the NMR spectra (Table 4.7) suggested a xanthone skeleton (Yang *et al*., 2001; Shao *et al*., 2008). The presence of methyl (δ_H 2.45, δ_C 22.6), chelated hydroxyl (12.25, *s*) and carboxylic acid (δ_C 169.8 for C=O, ν_{max} 3142-3486 cm^{-1} for OH) substituents were also evident.

The ^1H NMR spectrum (Table 4.6) revealed three mutually coupled aromatic protons with ABX spin pattern centered at δ_H 7.68 (*dd*, *J* = 8.5, 1.1 Hz, H-2), 7.94 (*dd*, *J* = 8.5, 7.3 Hz, H-3) and 7.45 (*dd*, *J* = 7.3, 1.1 Hz, H-4) of a monosubstituted (at C-1) ring-A. The substituent at C-1 was established to be carboxylic acid from the $^3J_{C,H}$ HMBC correlation of H-2 (δ_H 7.68) with the acid carbonyl carbon at δ_C 169.8 (C-10). In ring C, two *meta*-coupled aromatic protons that resonated at δ_H 6.89 (*br d*, *J* = 1.5 Hz, H-5) and 6.66 (*br d*, *J* = 1.5 Hz, H-7) were also observed. These protons displayed long-range correlation (in the ^1H-^1H COSY spectrum) and NOE (in the NOESY spectrum) interaction with methyl protons at δ_H 2.45 (δ_C 22.6) placing the methyl group at C-6 and the chelated hydroxyl at C-8. Thus, based on the above spectroscopic evidence, the compound was characterized as 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**). An alternative structure where the chrysophanol nucleus of the molecule (a metabolite which occurs widely in the family Asphodelaceae, van Wyk *et al*., 1995a; Bezabih *et al*., 1997) has been converted into *seco*-anthraquinone (**139**), as in janthinone (**140**) (Marinho *et al*., 2005), was also considered. This was because the NMR data of **138** is comparable to what has been reported for

janthinone, except for the presence of chelated OH in **138** rather than methoxyl in janthinone (**140**).



138 $R^1 = R^2 = H$
138a $R^1 = R^2 = Me$
14aa $R^1 = H$, $R^2 = Me$



139 R = H
140 R = Me

Interestingly, Shao *et al*. (2008) isolated a compound with the same spectral data as those reported for janthinone (**140**) (Marinho *et al*., 2005) and proposed the compound to be xanthone, methyl 8-hydroxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylate (**140a**), instead of the aromatic lactone janthinone. The other structural possibility was ruled out using single-crystal X-ray crystallography, and it was further suggested that the structure of janthinone (**140**) be revised (Shao *et al*., 2008) to xanthone, **140a**. With such conflicting literature information, the single-crystal X-ray structure analysis of compound **138** was perfomed (Fig.4. 2) and was found that the compound is indeed the xanthone, 8-hydroxy-6-methylxanthone-1-carboxylic acid rather than aromatic lactone (**139**). The single-crystal X-ray analysis of **138** is comparable to the previously reported crystal structure for 8-hydroxy-6-methyl-9-oxo-9*H*-xanthene-1-carboxylic acid methyl ester (Macias *et al*., 2001) with the acid group being above the tricyclic moiety with the torsional angle of 113.5^0. This is the first report on the occurrence of compound **138** in nature (Abdissa *et al*., 2014a) having previously been reported as a synthetic intermediate (Kudav *et al*, 1976). It is

worth mentioning that this is the first report on the isolation of a xanthone from the family Asphodeleaceae.

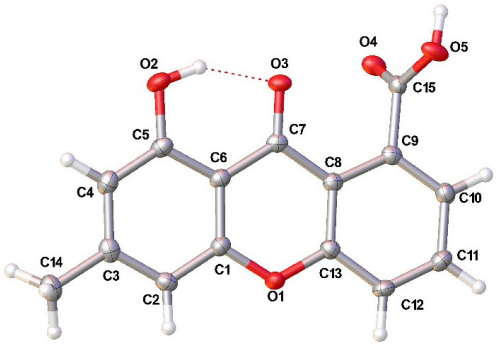
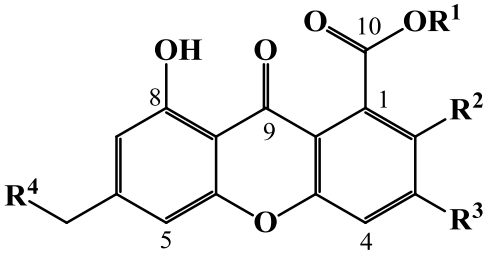


Figure 4.2: ORTEP-like view of the molecular structure of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**)

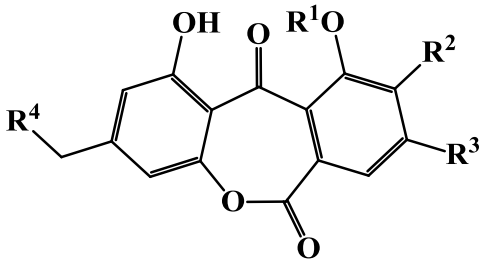
Methylation of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**) was conducted using dimethyl sulphate (Me_2SO_4) as described in section 3.4.2. The NMR spectral data of the modified compound (Table 4.6 and 4.7) were identical with the starting material **138**, except for the presence of two methoxyl groups (δ_H 3.99; δ_C 56.5 and 4.05; 53.1). The HMBC experiment showed a long-range coupling between protons of one of the methoxyl at δ_H 4.05 with the esteric carbonyl (δ_C 170.3) further confirming the structure to be 8-methoxy-6-methylxanthone-1-carboxylic acid methyl ester (**138a**).

In addition to janthinone (**140**) (Marinho *et al*., 2005), some related structures having the same *seco*-anthraquinone skeleton *vis* wentiquinones A (**141**) and B (**142**) (Sun *et al*., 2013), 1,8-dihydroxy-10-methoxy-3-methyldibenzo[b,e]oxepine-6,11-dione (**143**) (Pana *et al*., 2010), and wentiquinone C (**144)** (Li *et al*., 2014), along with **145** and **146** (Carvalho *et al*., 2001) have been reported. In line with the above discussion, the NMR data reported for these compounds were compared to those of compound **138** (Tables 4.6 and 4.7) and related xanthones (Shao *et al*.,

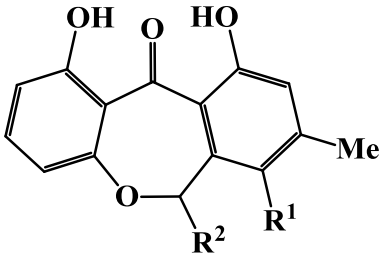
2008; Song *et al*., 2013). From this comparison then it appears that the *seco*-anthraquinone structures **141**, **142**, **143**, **144**, **145** and **146** should be revised to the xanthones **141a**, **142a**, **143a**, **144a**, **145a** and **146a**, respectively. The NMR assignments for the revised structures are shown in tables 4.6 and 4.7.



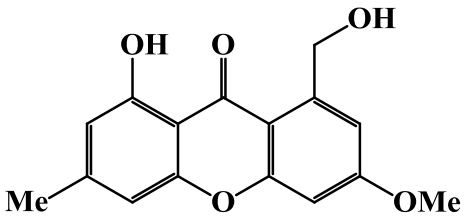
141a R^1 = Me, R^2 = R^4 = H, R^3 = OH
142a R^1 = Me, R^3 = OMe, R^2 = R^4 = H
143a R^1 = R^2 = R^4 = H, R^3 = OMe
144a R^1 = R^2 = H, R^3 = OMe, R^4 = OH
145a R^1 = Me, R^2 = R^3 = H, R^4 = OH
146a R^1 = Me, R^3 = H, R^2 = R^4 = OH



141 R^1 = R^2 = R^4 = H, R^3 = OMe
142 R^1 = Me, R^3 = OMe, R^2 = R^4 = H
143 R^1 = Me, R^2 = R^4 = H, R^3= OH
144 R^1 = R^2 = H, R^3 = OMe, R^4= OH
145 R^1 = Me, R^2 = R^3 = H, R^4 = OH
146 R^1 = Me, R^3 = H, R^2 = R^4 = OH



147 R^1 = OH, R^2 = H
148 R^1 = H, R^2 = OH



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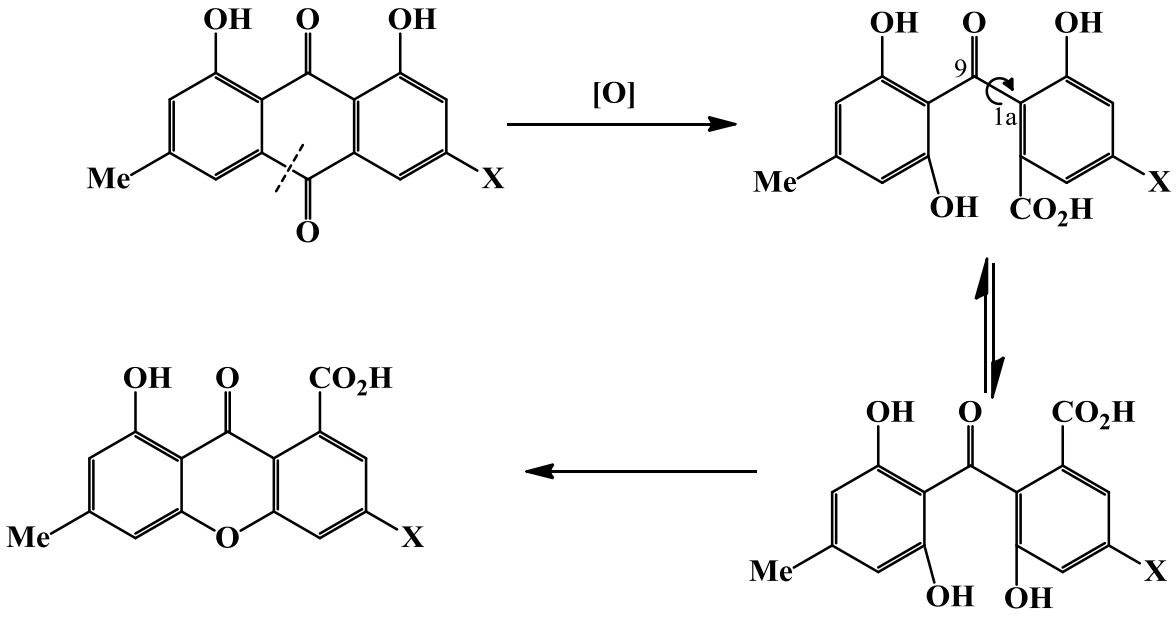
Besides comparison of the NMR data of these compounds with compounds with proven structures (**138** and **140a**) through X-ray chrystallography, the principal argument in this revision is that the chemical shift value δ_C 52-53 ppm assigned for aromatic methoxyl in the *seco*-anthraquinone structures **141**, **142**, **145** and **146** correspond to methyl ester [as the related xanthones reported by Shao *et al*. (2008) and Song *et al*. (2013)] rather than methoxyl group

attached to aromatic ring (typical value *ca* 55-56 ppm). In the case of **146** the methoxyl signal should even be more down field shifted (above 59 ppm) since it would be di-*ortho*-substituted and hence could only be the methyl ester **146a**. Methylation of **138** gave **138a** whose NMR spectral data showed typical methyl ester (4.05; 53.1) signal, which not only confirmed the structure of **138** as xanthon (8-methoxy-6-methylxanthone-1-carboxylic acid methyl ester), but also put the basis for the revision of the *seco*-anthraquinone structures **141**, **142**, **145** and **146** to xanthone methyl esters (**141a**, **142a**, **145a** and **146a**). In the case of structure **144**, if this was the correct structure it should have showed two chelated hydroxyl groups as in leptosphaerin D (**147**) (Lin *et al*., 2010) and arugosin F (**148**) (Hein *et al*., 1998) (only one is reported for **144**, Table 4.6) in the ^{1}H NMR spectrum, and this is only possible in the xanthone structure **144a**. The C-9 carbonyl in these compounds (except for **138a** which lacks chelation and C-9 appear up-field at δ_C 174.9 ppm) appeared in the narrow range of 179.2 to 181.6 ppm which is consistent with mono-chelated xanthone structures. If the *seco*-anthraqunone structures **141-146** were correct the C-9 carbonyl should have been as de-shilded as in leptosphaerin D (**147**, δ_C 198.5) (Lin *et al*., 2010) and arugosin F (**148**, δ_C 197.2 (Hein *et al*., 1998). In the case of compound **143,** two xanthones, with hydroxymethylene at C-1 have been reported along with it (Pana *et al*., 2010). The structure of one of these xanthones (**149**), which appears to be derived from **143a** through reduction of the ester carbonyl, has been confirmed by X-ray diffraction analysis; it is then reasonable to propose the revision of **143** to the xanthone **143a,** whose NMR data is similar to the other xanthones (Tables 4.6 and 4.7). The methoxyl group which appears at δ_C 57.1 in this compound is in agreement with its placement at C-8 as in **142a**.

Furthermore, that compounds **141a**–**146a** are xanthones appear to be agreeable from biogenetic point of view as these compounds co-occur with the corresponding anthraquinone precursors. Oxidative cleavage of ring B of anthraquinoid precursor(s) forms a benzophenone intermediate(s); rotation of ring A along C_{1a}-C_9 axis of the benzophenone(s) and cyclization of ring B produces the xanthones as shown in scheme 4.1. The occurrence of the benzophenone intermediates, moniliphenone (Song *et al*., 2013), 3,4-dihydro-3,9-dihydroxy-6,8-dimethoxy-3-methylanthracen-1(2H)-one and 2-(2,6-dihydroxy-4-methylbenzoyl)-3,5-dimethoxybenzoic acid (Sun *et al*., 2013) along with the corresponding anthraquinones and xanthones in *Aspergillus species* supports this biogenetic proposal. The role of moniliphenone as a biosynthetic intermediate between anthraquinones and xanthones in culture filtrate of *Monilinia fructicola* has already been reported (Kachi and Sasa, 1986).



Scheme 4.1: Biogenesis of xanthones from anthraquinones (X = H, OH, or OMe) (Kachi and Sasa, 1986)

Table 4.6: ^{1}H NMR spectral data ($\delta_{H,}$ m, *J* in Hz) of xanthones.

Position	**138**	**138a**	**141a**	**142a**	**143a**	**144a**	**145a**	**146a**
2	7.68 (*dd*, 8.5, 1.1)	7.47 *dd*, 8.5, 1.1)	7.18 (*d*, 2.2)	6.89 (*d*, 2.5)	7.18 (*d*, 2.4)	7.16 (*d*, 2.0)	7.45 (*dd*, 7.3,1.1)	
3	7.94 (*dd*, 8.5, 7.3)	7.66 (*dd*, 8.5,7.3)					7.93 (*dd*, 8.3,7.3)	7.48 (*d*, 8.0)
4	7.45 (*dd*, 7.3, 1.1)	7.26 (*dd*,7.3, 1.1)	6.98 (*d*, 2.2)	6.87 (*d*, 2.5)	6.98 (*d*, 2.4)	6.93 (*d*, 2.0)	7.77 (*dd*, 8.3,1.1)	7.62 (*d*, 8.0)
5	6.89 (*d*, 1.5)	6.87 (*d*, 1.5)	6.86 (*d*, 1.0)	6.69 (*d*, 1.1)	6.87 (*d*, 1.2)	6.95, (*br. s*)	7.00 (*d*, 1.1)	7.00 (*d*, 1.1)
7	6.66 (*d*, 1.5)	6.61 (*d*, 1.5)	6.66 (*d*, 1.1)	6.60 (*d*, 1.1)	6.67 (*d*, 1.2)	6.75, (*br. s*)	6.80 (*d*, 1.1)	6.78 (*d*, 1.1)
Me/CH_2OH	2.45 (*d*, 0.6)	2.47 (*s*)	2.40 (*s*)	2.41 (*s*)	2.41 (*s*)	4.59 (*s*)	4.60 (*d*, 5.4)	4.60 (*br. s*)
COMe		4.05 (*s*)	3.95 (*s*)	4.01 (*s*)			3.90 (*s*)	3.89 (*s*)
8-OMe		3.99 (*s*)						
3-OMe				3.97 (*s*)	3.96 (*s*)	3.94 (*s*)		
8-OH	12.25 (*s*)		12.32 (*br. s*)	12.27 (*br. s*)	12.32 (*s*)	12.41 (*s*)	12.06 (*br. s*)	12.25 (*br. s*)
2-OH								10.5 (*br. s)*
6-OH							5.40 (*t*, 5.4)	5.55 (*br. s*)

138 in acetone-d_6; **138a**, **142a** in $CDCl_3$; **141a**, **143a**, **144a**, **145a**, **146a** in DMSO-d_6. For compounds **141a-146a** NMR data extracted from literature and reassigned on the revised structure: **141a** and **142a** (Sun *et al*., 2013), **143a** (Pana *et al*., 2010), **144a** (*Li et al*., 2014), **145a** and **146a** (Carvalho *et al*., 2001).

Table 4.7: ^{13}C NMR spectral data (δ_C) of xanthones.

Position	**138**	**138a**	**141a**	**142a**	**143a**	**144a**	**145a**	**146a**
1	135.8	134.2	136.6	135.1	137.1	137.0	133.6	117.4
1a	117.8	120.1	105.8	106.7	106.4	106.5	116.6	117.4
2	120.1	118.8	101.1	101.5	101.7	101.0	122.9	149.2
3	136.6	133.5	164.8	164.7	165.4	164.8	136.0	125.7
4	123.8	122.4	111.7	112.1	112.3	111.7	119.7	120.5
4a	157.1	154.9	157.6	158.1	155.7	157.7	155.5	151.1
5	108.3	109.9	107.1	107.1	107.7	103.8	104.2	104.2
5a	156.8	157.6	155.1	155.8	155.7	155.3	155.5	155.8
6	150.7	146.9	148.7	148.6	149.3	153.6	154.5	154.4
7	112.2	107.0	111.2	111.7	111.8	107.6	107.0	106.9
8	162.2	160.5	160.5	161.5	161.0	160.6	160.5	160.8
8a	107.7	110.2	109.7	111.4	110.2	109.7	107.7	107.5
9	181.6	174.9	179.2	179.7	179.8	179.3	180.1	180.1
10	169.8	170.3	168.8	169.2	169.4	168.9	168.6	167.2
Me/CH_2OH	22.6	22.5	21.8	22.5	22.4	62.3	62.3	62.7
CO*Me*		53.1	52.5	53.1			52.7	52.6
8-OMe		56.5						
3-OMe				56.1	57.1	56.5		

138 in acetone-d_6; **138a**, **142a** in $CDCl_3$; **141a**, **143a**, **144a**, **145a**, **146a** in DMSO-d_6. For compounds **141a-146a** NMR data extracted from literature and reassigned on the revised structure: **141a** and **142a** (Sun *et al*., 2013), **143a** (Pana *et al*., 2010), **144a** (*Li et al*., 2014), **145a** and **146a** (Carvalho *et al*., 2001).

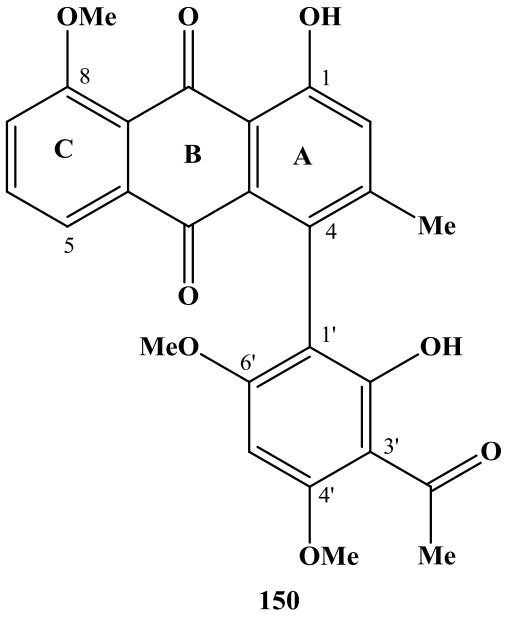
4.3.3 Phenylanthraquinone

4.3.3.1 6',8-*O*-Dimethylknipholone (150)

Compound **150** was isolated as yellow amorphous solid. HREIMS showed a molecular ion peak at *m/z* 462.1314 corresponding to molecular formula of $C_{26}H_{22}O_8$. The UV-Vis spectrum (λ_{max} 220, 256, 286, and 415 nm) was similar to that of knipholone (**14**) suggesting a phenylanthraquinone chromophore (Dagne and Steglich, 1984).

Similar to compound **14**, the ^{1}H NMR spectrum of **150** (Table 4.8) displayed the presence of three adjacent and mutually coupled aromatic protons at δ_H 7.74 (*dd*, *J* = 7.8, 1.1 Hz, H-5), 7.64 (*t*, *J* = 7.8 Hz, H-6) and 7.29 (*dd*, *J* = 7.8, 1.1 Hz, H-7) with ABX spin system for ring-C protons. In ring-A, a singlet at δ_H 7.25 was assigned to H-2 with the biosynthetically expected aromatic methyl (δ_H 2.10; δ_C 20.7) being at C-3 and the acetylphloroglucinol moiety (whose presence was evident from the downfield shifted singlet at δ_H 13.91 due to the chelated hydroxyl proton, the singlet aromatic proton shifted upfield to δ_H 6.14 for H-5', an acetyl proton, δ_H 2.69 at C-3' and a methoxy group, δ_H 4.02; δ_C 55.4) at C-4. The only difference between **150** and knipholone (Dagne and Steglich, 1984) is the presence of two additional methoxyl signals at δ_H 4.08 (δ_C 56.6) and 3.79 (δ_C 55.7). Consequently, compound **150** was confirmed as a dimethyl ether derivative of knipholone. The positions of methoxyl groups were established on the bases of HMBC and NOE experiments (Table 4.8). Consequently, the methoxyl groups resonating at δ_H 4.02 (δ_C 55.4) and 3.79 (δ_C 55.7) were placed at C-4' and C-6' respectively based on the NOE interaction between these methoxyl protons and H-5'. Whereas the other methoxyl group, δ_H 4.08 (δ_C 56.6), was placed at C-8 based on its NOE correlation with H-7. Therefore, compound **150** was characterized and named as 6',8-*O*-dimethylknipholone (**150**) (Abdissa *et al*., 2014a).

In the CD spectrum of **150**, no significant Cotton effect was observed, indicating that the compound occurs as a scalemic or racemic mixture. In agreement with this, HPLC analysis (Fig 4.3) on a chiral stationary phase column (Chiralcel OD-H) revealed that the compound is nearly 1:1 mixture (52:48) of atropisomers. This is in line with the previous assertion that phenylanthraquinones are found as scalemic or nearly racemic mixtures in *B. frutescens* (Mutanyatta *et al*., 2005).



150

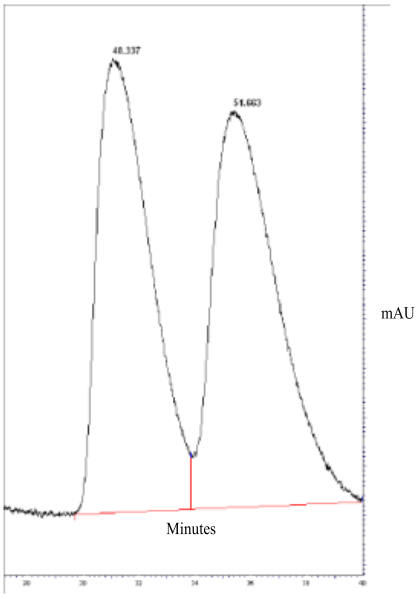


Figure 4.3: Enantiomeric analysis of 6',8-O-dimethylknipholone (**150**)

Interestingly, the structural isomer of the 'southern' part of compound **150**, 4-hydroxy-2,6-dimethoxyacetophenone (**151**) (Youssef *et al*., 1998) had also been isolated from this plant along with chrysophanol (Bezabih *et al*., 1997). 6'-*O*-methyl knipholone, with the 2-hydroxy-4,6-dimethoxyacetophenone moiety has been reported from *B. capitata* (Bezabih *et al*., 1997). It is then possible that the oxidative coupling leading to the phenyl anthraquinones **150** could have occurred between chrysophanol (chrysophanol-8-methyl ether) and 4-hydroxy-2,6-

dimethoxyacetophenone. It has already been shown that 2,4-dihydroxy-6-methoxyacetophenone (but not 2,4,6-trihydroxyacetophenone) is the substrate towards the formation of knipholone and related phenylanthraquinone derivatives (Bringmann *et al*., 2007b).

Table 4.8: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data along HMBC and NOE of Compound **150**: δ (ppm), *J* (Hz) in $CDCl_3$

Position	δ_H (*m,* J)	δ_C	HMBC	NOE
1		162.3		
1a		116.2		
2	7.25 (s)	125.3	C-1, 1a, 4, CH_3	CH_3
3		149.4		
4		126.6		
4a		130.9		
5	7.74 (*dd*, 7.8, 1.1)	120.0	C-7, 8a, 10	
5a		136.9		
6	7.64 (*t*, 7.8)	135.3	C-5a, 8	
7	7.29 (*dd*, 7.8, 1.1)	117.2	C-5, 8, 8a	8-OCH_3
8		160.2		
8a		120.6		
9		189.0		
10		183.5		
1'		109.3		
2'		162.4		
3'		106.2		
4'		162.9		
5'	6.14 (*s*)	86.2	C-1', 3', 6'	4'-OCH_3, 6'-OCH_3
6'		162.6		
CO		203.5		
3-CH_3	2.10 (*s*)	20.7	C-2, 3, 4	H-2
CO*Me*	2.69 (*s*)	33.3	C-3', CO	
8-OMe	4.08 (*s*)	56.6	C-8	H-7
4'-OMe	4.02 (*s*)	55.4	C-4'	H-5'
6'-OMe	3.79 (*s*)	55.7	C-6'	H-5'
1-OH	13.39 (*s*)		C-1, 1a, 2	
2'-OH	13.91 (*s*)		C-1', 2', 3'	

4.3.4 Furanonaphthoquinones

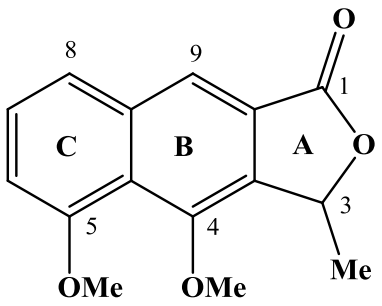
4.3.4.1 4-*O*-Methyleleutherol (53)

Compound **53** was isolated as a colourless amorphous powder with blue fluorescence in solution. The UV-Vis spectrum (254, 312, 340 and 374 nm) indicated furanonaphthoquinone nature of the compound (Dagne *et al*., 1994). The ^{1}H NMR spectrum (Table 4.9) showed a down-field shifted singlet at δ_H 8.05 (H-9) indicating its *peri*-position to carbonyl group; three mutually coupled aromatic protons with an AMX spin pattern resonating at δ_H 6.96 (*d*, *J* = 7.8 Hz, H-6), δ_H 7.41 (*t*, *J* = 7.8 Hz, H-7) and δ_H 7.53 (*d*, *J* = 7.8 Hz, H-8) which was consistent with the ring C substituted at C-5. The ^{1}H NMR spectrum further showed the presence of two signals at δ_H 3.95 and 3.82 which were assigned to aromatic methoxyl groups (OMe-4, OMe-5), a three proton doublet at δ_H 1.67 (*d*, J = 6.7 Hz, CH_3-3) coupled with an oxymethine proton quartet at δ_H 5.70 (*q*, J = 6.7 Hz, H-3). Based on these spectral data and comparison with information in the chemical literature (Bringmann *et al*., 2008a), the compound was identified as 4-*O*-methyl eleutherol (**53**).

4.3.4.2 5,8-Dihydroxy-1-hydroxymethylnaphtho[2,3-C]furan-4,9-dione (50)

Compound **50** was isolated as a colourless amorphous powder. TLC analysis showed a blue fluorescent spot under UV_{366} light. The UV-Vis absorption peaks at λ_{max} 242, 303 and 460 nm suggested a isofuranonaphthoquinone chromophore (Bezabih *et al*., 1997). The ^{1}H NMR spectrum (Table 4.9) showed two singlets at δ_H 12.63 and 12.82 for the hydroxyl groups at C-5 and C-8 which were H-bonded to a carbonyl, and two aromatic protons resonating at 7.29 (*J* = 8.2 Hz) were assigned to H-6 and H-7 of ring C. A down-field shifted aromatic singlet at δ_H 8.18 was assigned to H-3 of the furan ring (ring A). The presence of a two proton singlets at δ_H 4.96 indicated an oxymethylene substituent at C-1 of the furan ring. Therefore, this compound was

identified as 5,8-dihydroxy-1-hydroxymethynaphtho[2,3-C]furan-4,9-dione (**50**), which has been reported from *Bulbine capitata* (Bezabih *et al*., 1997).



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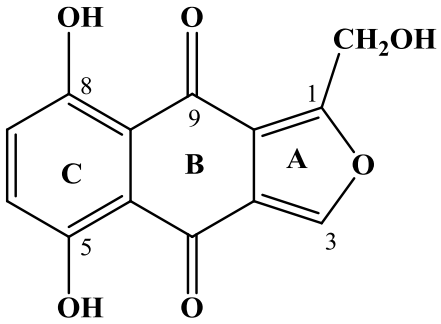


50

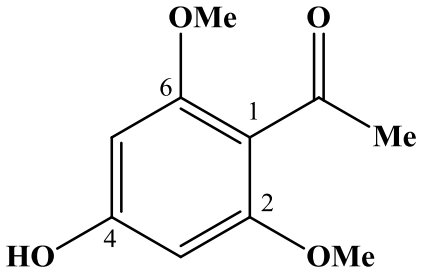
Table 4.9: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **53** and **50**: δ (ppm), in CD_2Cl_2

Position	Compound **53**		Compound **50**
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)
1		170.1	
1a		136.2	
3	5.70 (*q*, 6.7)	77.5	8.18 (*s*)
3a		125.4	
4		156.6	
5		151.9	
5a		123.5	
6	6.96 (*d*, 7.8)	108.3	7.29 (*d*, 8.2)
7	7.41 (*t*, 7.8)	127.5	7.29 (*d*, 8.2)
8	7.53 (*d*, 7.8)	122.8	
8a		138.4	
9	8.05 (*s*)	122.3	
3-CH_3	1.67 (*d*, 6.7)	20.1	
CH_2-OH			4.96 (*s*)
4-OCH_3	3.95 (*s*)	56.3	
5-OCH_3	3.82 (*s*)	62.7	

4.3.5 Acetophenone

4.3.5.1 2,6-Dimethoxy-4-hydroxyacetophenone (151)

Compound **151** was isolated as a pale yellow solid. The ^{1}H NMR spectrum showed a signal for two aromatic protons resonating at δ_H 6.14 for H-3 and H-5, a singlet at δ_H 3.73 (6H) for two methoxyl groups and a three-proton singlet at δ_H 2.30 for a methyl group indicating that the compound was 2,4,6-trisubstituted acetophenone. In agreement with this, the ^{13}C NMR spectrum revealed resonances for a methyl at δ_C 32.6, two methoxyl at δ_C 56.0, two methine at δ_C 92.8, an aromatic quaternary carbon at δ_C 113.8, three oxygenated quaternary carbon (at δ_C 160.9, 159.0, 159.0) and a carbonyl at δ_C 200.3. The compound was readily identified as 2,6-dimethoxy-4-hydroxyacetophenone (**151**) by comparing the spectral data with what has been reported in the literature (Youssef *et al*., 1998). This is the first report of the isolation of the compound from the family Asphodeleaceae.



151

4.4 Secondary Metabolites from the Roots of *Aloe dawei*

Chromatographic separation of the MeOH/CH_2Cl_2 (1:1) extract of the roots of *Aloe dawei* resulted in the isolation of seven naphthoquinones: droserone (**123**), droserone 5-methyl ether (**152**), hydroxydroserone (**153**), 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), malvone A (**154**), 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**), ancistroquinone C (**95**), along with ten anthraquinones. The naphthoquinone 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**) is a new natural product. Compounds **123**, **152**, **94**, and **154** are reported for the first in the Asphodelaceae family. The identification of one of the anthraquinone, chrysophanol (**18**), has already been discussed earlier in section 4.2. The characterization of the other compounds is discussed below.

4.4 .1 Naphthoquinones

4.4.1.1 Droserone (123)

Compound **123** was obtained as a yellow amorphous solid. The ion-positive EIMS spectrum showed a molecular ion peak of *m/z* 205 $[M+H]^+$ corresponding to the molecular formula $C_{11}H_8O_4$. The UV absorption (λ_{max} 224, 260, 286 and 350 nm) suggested a naphthoquinone chromophore (Kreher *et al*., 1990). In agreement with this, the 1,4-naphthoquinone nature of the compound was further confirmed from its molecular formula and ^{13}C NMR spectrum (Table 4.10) which displayed two down-field shifted signals (δ_C 184.5 and 184.9) for two carbonyl carbons and eight sp^2 carbon signals (Bringmann *et al*., 2008c) of the naphthoquinone skeleton.

In the ^1H NMR (in DMSO-d_6) spectrum, the three mutually coupled aromatic protons resonating at 7.49 (*d*, *J* = 7.4 Hz, 1H), 7.69 (*t*, *J* = 7.9 Hz, 1H) and 7.25 (*d*, *J* = 8.4 Hz, 1H) of an ABX spin pattern which were assigned to H-8, H-7 and H-6, respectively. The downfield shifted signal at

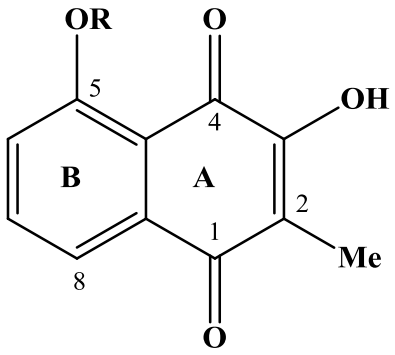
δ_H 11.38 was assigned to the hydrogen bonded phenolic hydroxyl proton placed *peri* position at C-5 of the mono-substuted ring B. The only other peak observed in the ^{1}H NMR spectrum was a three-proton *singlet* for methyl group resonating at δ_H 1.92. The up-field shifted resonance (δ_H 1.92) in ^{1}H NMR spectrum of the methyl group showed that it was attached to a quinoid ring (Bringmann *et al*., 2008c). The methyl protons (δ_H 1.92) showed HMBC correlation with the carbonyl carbon C-1 (δ_C 184.9) and oxygenated quaternary carbon (δ_C 155.7), indicating that ring A was substituted with methyl and hydroxyl groups at C-2 and C-3, respectvely, in the 1,4-naphthoquinone system. The compound was therefore identified as 3,5-dihydroxy-2-methyl-1,4-naphthoquinone, trivial name droserone (**123**), which had previously been isolated from *Diospyros melanoxylon* (Sidhu *et al*., 1974) and *Dionaea muscipula* (Kreher *et al*., 1990).

4.4.1.2 Droserone 5-methyl ether (152)

Compound **152** was obtained as a yellow amorphous solid. The EIMS provided *pseudo*-molecular ion peak at *m/z* 219 $[M+H]^+$ suggesting a molecular formula of $C_{12}H_{10}O_4$. Compound **152** also showed spectral features of a 1,4-naphthoquinone in which its ^{1}H and ^{13}C NMR spectra were similar to those of compound **123** (Table 4.10). The ^{1}H NMR spectrum (in DMSO-d_6) showed highly shielded signal for methyl protons (at δ_H 1.88) and three mutually coupled protons with ABX spin system at δ_H 7.60 (*d*, *J* = 7.5 Hz, H-5), 7.75 (*t*, *J* = 8.0 Hz, H-6) and 7.46 (*d*, *J* = 8.5 Hz, H-7). The presence of methoxyl group (δ_H 3.92) was also evident in the ^{1}H NMR spectrum.

The ^{13}C NMR spectral data reveald the presence of two carbonyl (δ_C 178.9 and 184.5), five quaternary (δ_C 155.9, 134.2, 159.4, 117.1 and 117.0), three methine (δ_C 123.3, 116.8 and 117.8) carbon signals constituting the naphthoquinone skeleton, and a methyl (δ_C 8.3) and methoxyl (δ_C

56.4) substituents. The substitution pattern in this compound was found to be the same as in **123**. The only notable difference was the presence of a methoxyl group (δ_H 3.92; δ_C 56.4) in compound **152** as revealed by the NMR spectral data (Table 4.10). The position of methoxyl group was established to be at C-5 (δ_C 159.4) following the NOE interaction observed between methoxyl protons (δ_H 3.92) and aromatic proton H-6 (δ_H 7.46). Therefore, compound **152** was identified as 3-hydroxy-5-methoxy-2-methyl-1,4-naphthoquinone, trivial name droserone 5-methyl ether (**152**), which had previously been isolated from *Nepenthes thorelii* (Likhitwitayawuid *et al*., 1998).



123 R = H
152 R = Me

Table 4.10: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **123** and **152**: δ (ppm), in DMSO-d_6

Position	Compound **123**		Compound **152**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		184.9		184.5
2		120.9		134.2
3		155.7		155.9
4		184.5		178.9
5		160.4		159.4
6	7.25 (*d*, 8.4, 1H)	123.3	7.46 (*d*, 8.5, 1H)	117.8
7	7.69 (*t*, 7.9, 1H)	137.3	7.75 (*t*, 8.0, 1H)	135.8
8	7.49 (*d*, 7.4, 1H)	118.7	7.60 (*d*, 7.5, 1H)	118.2
9		132.8		117.0
10		114.1		117.1
2-CH_3	1.92 (*s*, 3H)	9.1	1.88 (*s*, 3H)	8.3
5-OH	11.38 (*s*, 1H)			
5-OCH_3			3.92 (*s*, 3H)	56.4

4.4.1.3 Hydroxydroserone (153)

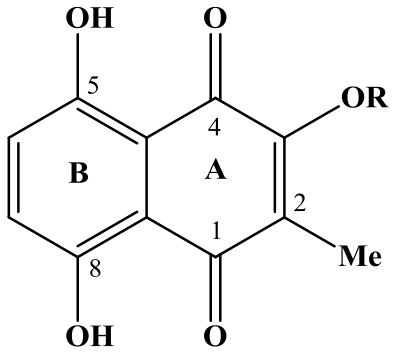
Compound **153** was isolated as yellow crystals. The EIMS showed *quasi*-molecular ion $[M+H]^+$ at *m/z* 221, consistent with the molecular formula of $C_{11}H_8O_5$. The 1,4-naphthoquinone nature of the compound was also deduced from the UV-Vis (λ_{max} 226, 272, 346, 464 nm) and ^{13}C NMR spectrum (Table 4.11) which displayed eleven non-equivalent carbon atoms, of which two were down-field shifted (δ_C 182.9 and 189.1) and therefore assigned to the two carbonyl carbons (C-1 and C-4).

In the ^{1}H NMR spectrum (Table 4.11), two singlets at δ_H 12.90 (*s*, 1H), 11.72 (*s*, 1H) were assigned to hydrogen bonded phenolic hydroxyl protons at C-5 and C-8 respectively; a pair of

ortho-coupled aromatic protons at δ_H 7.34 (J = 9.4 Hz, 1H, H-6) and 7.28 (J = 9.2 Hz, 1H, H-7) constitute the di-substituted ring B. In ring A, the presence of a methyl group at C-2 (δ_H 1.93; δ_C 8.2) and hydroxyl at C-3 (δ_C 156.9) were evident from the NMR as in compound **123** (Bringmann *et al*., 2008c). The ^{13}C NMR spectrum was also consistent with the presence of eleven carbon signals including eight quaternary carbons, two methines and a methyl carbon. From the above data and comparison with literature, compound **153** was identified as 3,5,8-trihydroxy-2-methyl-1,4-naphthoquinone, trivial name hydroxydroserone, which had previously been isolated from *Nepenthes thorelii* (Likhitwitayawuid *et al*., 1998). However, this is the first report of the isolation of hydroxydroserone from the plant family Asphodelaceae.

4.4.1.4 5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (94)

Compound **94** was obtained as a red amorphous solid. The EIMS of this compound showed $[M+H]^+$ at *m/z* 235 consistent with $C_{12}H_{10}O_5$. The ^1H and ^{13}C NMR spectra (Table 4.11) were similar to that of compound **153** except for the presence of a methoxyl group (at δ_H 4.04; δ_C 64.3). Furthermore, the two down-field shifted proton signals at δ_H 11.99 and 12.52 were assigned to hydroxyl groups at C-5 and C-8 as in compound **153**, allowing the placement of the methoxyl group at C-3 (δ_C 161.2) of the quinone ring and was supported by HMBC experiment showing correlation of both methyl and methoxyl protons to C-3. Consequently, compound **94** was identified as 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione, which was recently reported from the roots of *Aloe secundiflora* (Induli *et al*., 2012). This was only the second report of the isolation of compound **94** in nature having previously been reported as a synthetic product (Kuroda, 1943).



153 R = H
94 R = Me

Table 4.11: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **153** and **94** (in DMSO-d_6)

Position	Compound **153**		Compound **94**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		189.1		191.7
2		120.5		135.0
3		156.9		161.2
4		182.9		186.3
5		156.2		159.8
6	7.28 (*d*, 9.2, 1H)	130.1	7.33 (*d*, 3.5, 1H)	132.6
7	7.34 (*d*, 9.4, 1H)	127.5	7.33 (*d*, 3.5, 1H)	131.8
8		155.5		159.2
9		110.9		147.2
10		111.0		147.6
2-CH_3	1.93 (*s*, 3H)	8.2	1.98 (*s*, 3H)	11.9
3-OCH_3			4.04 (*s*, 3H)	64.3
5-OH	12.90 (*s*, 1H)		12.52 (*s*, 1H)	
8-OH	11.72 (*s*, 1H)		11.99 (*s*, 1H)	

4.4.1.5 Malvone A (154)

Compound **154** was isolated as a red crystals. The EIMS showed a *pseudo*-molecular ion peak at *m/z* 235 $[M+H]^+$ suggesting a molecular formula of $C_{12}H_{10}O_5$. The ^1H NMR spectrum (Table 4.12) showed two *ortho*-coupled (J = 8.2 Hz) aromatic protons at δ_H 7.41 and 7.09 which were assigned to H-8 and H-7 of a disubstituted ring B of naphthoquinone. In support that the compound was a naphthoquinone, the ^{13}C NMR spectrum showed signals for twelve carbons of which three were oxygenated (δ_C 157.0, 152.4 and 150.0). The ^{13}C NMR spectrum has also showed the presence of a methoxyl signal at δ_C 60.4 (δ_H 3.97) indicating that of the three oxygenation, one was methoxyl while the other two can be assumed to be hydroxyl groups (δ_C 152.4 and 150.0).

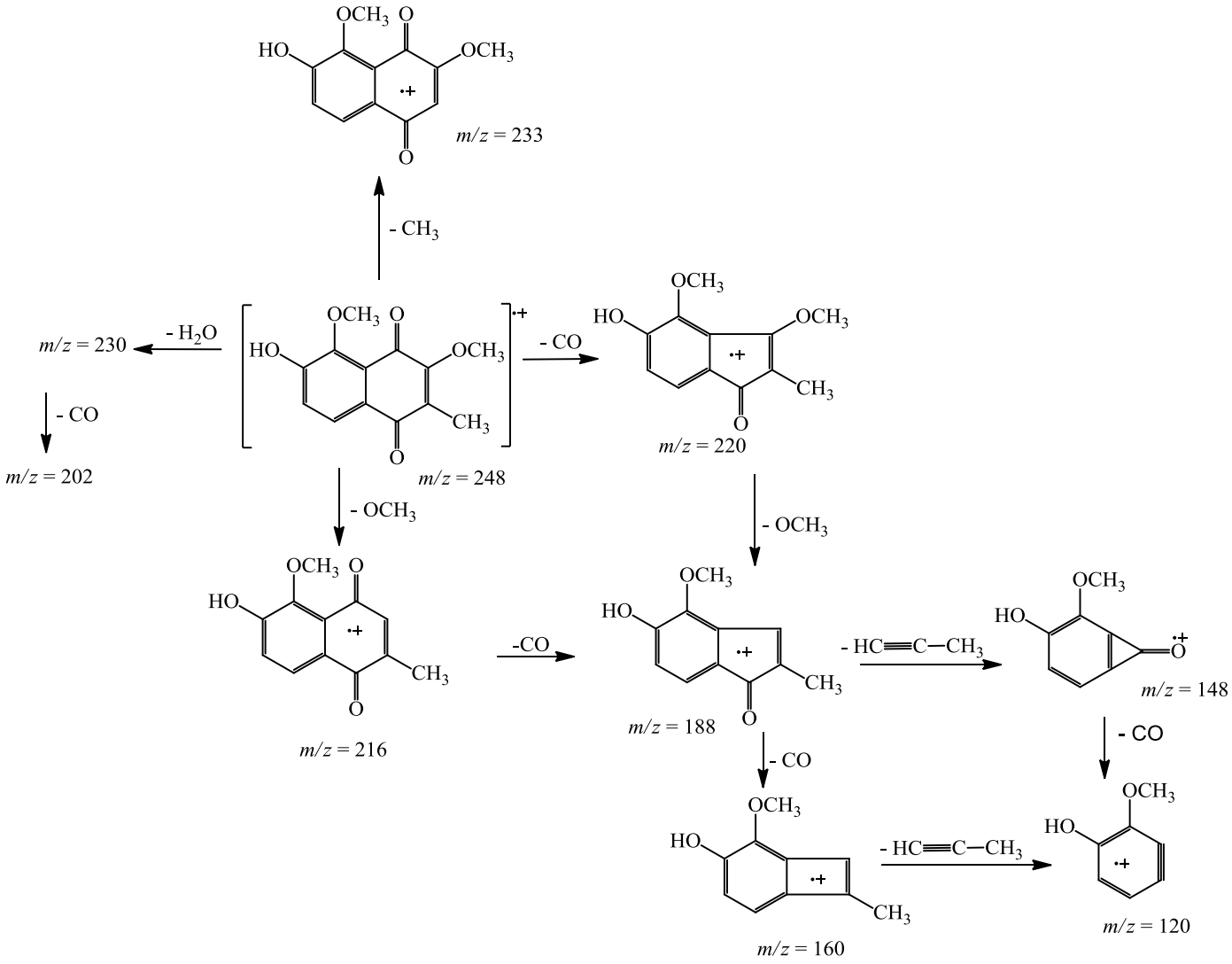
The HMBC correlation observed between the downfield shifted proton (δ_H 7.41) with a carbonyl carbon C-1 (δ_C 186.0) in ring B was consistent with this proton being *peri* to the carbonyl carbon at C-1. It follows then that C-5 (δ_C 152.4) and C-6 (δ_C 150.0) were oxygenated. Ring A of **154** was substituted with a methyl (δ_H 1.95; δ_C 9.4) and a methoxyl (δ_H 3.97; δ_C 60.4) group at C-2 (δ_C 133.1) and C-3 (δ_C 157.0), respectively. These assignments were supported by the observed HMBC correlation of both methoxyl and methyl protons to C-3 (δ_C 157.0) and NOESY interactions between methoxyl and methyl protons. Thus based on the above evidence, the compound was found to be 2-methyl-3-methoxy-5,6-dihydroxy-1,4-naphthoquinone, trivial name malvone A (**154**), which had previosly been isolated from *Malva sylvestris* (Veshkurova *et al*., 2006). This appears to be the first report of the isolation of **154** in the family Asphodelaceae.

4.4.1.6 6-Hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (155)

Compound **155** was obtained as a yellow amorphous solid. The molecular ion peak at *m/z* 247.0643 $[M-H]^-$ (expected mass = 247.0684) in HRMS and the number of signals in ^{13}C NMR spectrum suggested a molecular formula of $C_{13}H_{12}O_5$. The 1,4-naphthoquinone nature of the compound was deduced from the UV-Vis (λ_{max} 225, 260, 285 and 350 nm) and ^{13}C NMR spectra which displayed two down-field shifted signals at δ_C 183.0 and 187.2 for carbonyl carbons and eight sp^2 carbon signals (Bringmann *et al*., 2008c). Furthermore, the presence of three singlets each integrating for three protons in the ^1H NMR spectrum, resonating at δ_H 3.95, 3.77 and 1.91 (showing HSQC correlation with the carbon signals at δ_C 63.5, 63.6, and 12.0 respectively), along with signals for three oxygenated quaternary carbons (δ_C 150.2, 160.0 and 161.4) in ^{13}C NMR spectrum (Table 4.12) was in agreement with the naphthoquinone being substituted with two methoxyl, methyl and a hydroxyl groups.

The ^1H NMR spectrum further showed two *ortho*-coupled (J = 8.4 Hz) aromatic protons at δ_H 7.19 (H-7) and 7.65 (H-8) of a 5,6-disubstituted ring-B. In ring-A, the up-field shifted resonance of methyl proton (δ_H 1.91) in the ^1H NMR spectrum showed that it was attached to a quinoid ring at C-2 (δ_C 131.5) (Sankarama and Srinivа, 1979; Bringmann *et al.,* 2008c). This was further confirmed by the presence of intense fragment ions at *m/z* 120 and 148 in its mass spectrum (Scheme 4.2) indicating that a substituent was not on the benzenoid ring. In the HMBC spectrum a $^3J_{C,H}$ correlation between H-8 (δ_H 7.65) and δ_C 187.2 allowed the assignment of this carbonyl signal to C-1. That the methyl protons (δ_H 1.91) correlated with C-1 (δ_C 187.2) was consistent with the methyl group being at C-2 (δ_C 131.5) as expected biogenetically; C-3 (δ_C 161.4) was then substituted with methoxyl or hydroxyl group. Interestingly, the down-field shifted signals (at δ_C 63.5 and 63.6) for the two methoxyl carbons in ^{13}C NMR spectrum was consistent with

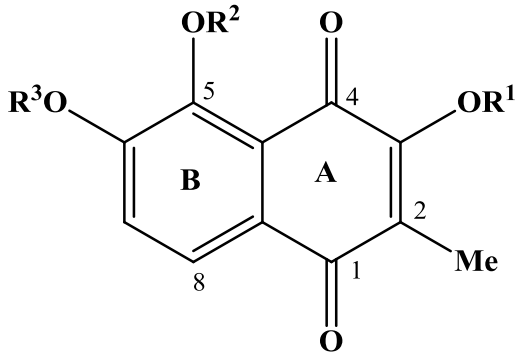
these groups being di-*ortho*-substituted and hence should be placed at C-3 and C-5 with the hydroxyl group being at C-6 (δ_C 160.0). This was further supported by HMBC correlations of 3-OMe and 2-Me protons with C-3 (δ_C 161.4); while 5-OMe and H-7 interact with C-5 (δ_C 150.2). Thus based on the above spectroscopic evidence the compound was characterized as 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**) (Abdissa *et al*., 2014b) and is a new compound.



Scheme 4.2: Proposed mass spectral fragmentation of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4 naphthoquinone (**155**)

4.4.1.7 Ancistroquinone C (95)

Compound **95** was isolated as a yellow needle-like solid. The EIMS provided a *pseudo*-molecular ion *m/z* 249 $[M+H]^+$ suggesting a molecular formula $C_{13}H_{12}O_5$. The NMR spectral data (Table 4.12) indicated that compound **95** was a 1,4-naphthoquinone with oxygenation at C-5 and C-6 of ring B, analogous to **155**. The two protons in this ring appeared at δ_H 7.78 and 7.43 (J = 8.6 Hz). As in **155**, compound **95** was trioxygenated with two methoxyl and a hydroxyl groups; whereas the two methoxyl groups in **155** were di-*ortho*-substituted (δ_C 63.5 and 63.6), in **95** only one was di-*ortho*-substituted (δ_C 60.5) while the other was normal (δ_C 56.3) the latter could only be placed at C-6. This assignment was supported by the observed NOE correlation between the methoxyl group resonating at δ_H 3.91 (δ_C 56.3) and H-7 (δ_H 7.43). The other methoxyl group was then placed at C-5 based on HMBC correlations of 5-OMe and H-7 with C-5 (δ_C 148.6). Thus, based on the above spectroscopic data, the compound was identified as 3-hydroxy-5,6-dimethoxy-2-methyl-1,4-naphthoquinone, trivial name ancistroquinone C (**95**), which had previously been isolated from stress-induced cell cultures of *Ancistrocladus abbreviates*, (Bringmann *et al.,* 2008c) and the roots of *Aloe secundiflora* (Induli *et al*., 2012).



154 $R^1 = CH_3$, $R^2 = R^3 = H$
155 $R^1 = R^2 = CH_3$, $R^3 = H$
95 $R^1 = H$, $R^2 = R^3 = CH_3$

Table 4.12: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **154**, **155** and **95** (in DMSO-d_6)

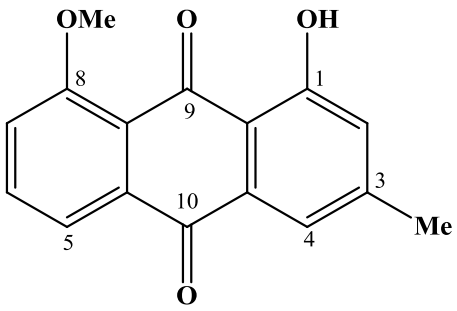
Position	Compound **154**		Compound **155**		Compound **95**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		186.0		187.2		183.8
2		133.1		131.5		117.5
3		157.0		161.4		155.8
4		183.4		183.0		179.6
5		152.4		150.2		148.6
6		150.0		160.0		157.5
7	7.09 (*d*, 8.2, 1H)	119.8	7.19 (*d*, 8.4, 1H)	123.6	7.43 (*d*, 8.6, 1H)	116.8
8	7.41 (*d*, 8.2, 1H)	120.2	7.65 (*d*, 8.4, 1H)	126.5	7.78 (*d*, 8.6, 1H)	123.3
9		122.4		127.4		125.6
10		114.8		127.9		122.9
2-CH_3	1.95 (*s*, 3H)	9.4	1.91 (*s*, 3H)	12.0	1.88 (*s*, 3H)	8.4
3-OCH_3	3.97 (*s*, 3H)	60.4	3.95 (*s*, 3H)	63.5		
5-OH	9.76 (*s*, 1H)					
5-OCH_3			3.77 (*s,* 3H)	63.6	3.78 (*s*, 3H)	60.5
6-OCH_3					3.91 (*s*, 3H)	56.3

4.4 .2 Anthraquinones

4.4.2.1 Chrysophanol-8-methyl ether (156)

Compound **156** was isolated as a yellow amorphous solid (R_f 0.62; 5% EtOAc in *n*-hexane). Upon exposure to ammonia, the yellow spot on TLC changed to red. The UV-Vis absorption maxima at 272, 324, 406 and 434 nm suggested the presence of an anthraquinone chromophore (Dagne *et al*., 1994). The EIMS showed a positive ion peak at *m/z* 269 $[M+H]^+$, corresponding to molecular formula $C_{16}H_{12}O_4$.

The NMR spectral data (Table 4.13) of **156** resembled that of chrysophanol (**18**) showing three mutually coupled aromatic protons with ABX spin pattern centered at δ_H 7.79 (*d*, *J* = 7.6 Hz, H-5), 7.86 (*t*, *J* = 8.0 Hz, H-6) and 7.61 (*d*, *J* = 8.4 Hz, H-7) in ring C, and two *meta*-coupled aromatic protons at δ_H 7.15 (*d*, *J* = 1.8 Hz, 1H, H-2) and 7.46 (*d*, *J* = 1.8 Hz, 1H, H-4) in ^{1}H NMR spectrum, with the biosynthetically expected methyl group (δ_H 2.40; δ_C 24.6) being at C-3 (δ_C 150.6) in ring A. The only notable difference between chrysophanol (**18**) and **156** was that one of the chelated OH in **18** was replaced with a three-proton singlet for a methoxyl group (δ_H 3.96; δ_C 59.7) in the later. Therefore, compound **156** was a methyl ether of chrysophanol (**18**). The position of the methoxyl group was fixed from NOE correlation of methoxyl protons with aromatic proton resonating at δ_H 7.61 (H-7). Thus, the compound was therefore identified as chrysophanol-8-methyl ether (**156**), previously reported from hairy root cultures of *Cassia obtusifolia* (Guo *et al*., 1998). It has also been reported from the roots of *Aloe berhana* (Dagne *et al*., 1992).



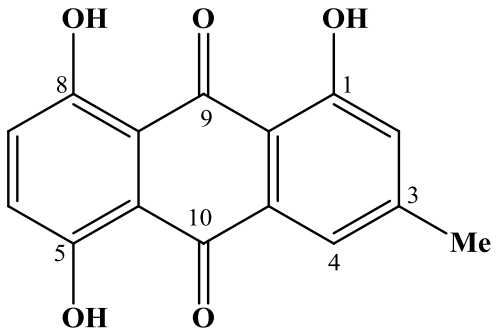
156

4.4.2.2 Helminthosporin (59)

Compound **59** was isolated as a red amorphous soild. The UV-Vis spectrum showed absorption maxima at 493, 431, 401 and 282 nm which was a characteristic of an anthraquinone with three chelated hydroxyl groups (Dagne *et al*., 1994). In agreement with this, the ^{1}H NMR spectrum

(Table 4.13) revealed the presence of three chelated hydroxyl protons at δ_H 12.94, 12.25 and 12.07 which were attributable to C-1, C-5 (or C-4) and C-8 of an anthraquinone skeleton.

In ring A, two *meta*-coupled aromatic protons at δ_H 7.05 (*dd*, *J* = 1.8 Hz, 1H, H-2) and 7.63 (*d*, *J* = 1.6 Hz, 1H, H-4) were observed in addition to the biosynthetically expected methyl group (δ_H 2.43; δ_C 22.3) at C-3 (δ_C 149.1). Whereas in ring C, two *ortho*-coupled aromatic protons resonating at δ_H 7.23 (*d*, *J* = 8.0 Hz, 1H, H-6) and 7.23 (*d*, *J* = 8.0 Hz, 1H, H-7) with the chelated hydroxyl group (δ_H 12.94) being at C-5 (δ_C 158.2). Therefore compound **59** was identified as 1,5,8-trihydroxy-3-methylanthraquinone, trivial name helminthosporin (**59**), which has been isolated from *Helminthosporium gramineum* (Yagi *et al*., 1977b) and *Drechslera holmii* (van Eijk and Roeymans, 1981). Its isolation from the roots and subterranean stem of some *Aloe* species has been reported (Yagi *et al*., 1977a; Yenesew *et al*., 1993; Induli *et al*., 2012).



59

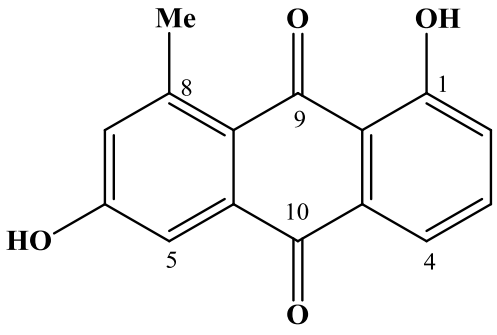
Table 4.13: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **156** and **59 (**in DMSO-d_6)

Position	Compound **156**		Compound **59**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		161.6		162.8
1a		114.7		114.0
2	7.15 (*d*, 1.8, 1H)	124.1	7.05 (*dd*, 1.8, 0.9, 1H)	124.6
3		147.5		149.1
4	7.46 (*d*, 1.8, 1H)	119.3	7.63 (*d*, 1.6, 1H)	120.8
4a		132.1		133.2
5	7.79 (*d*, 7.6, 1H)	119.3		158.2
5a		134.9		112.5
6	7.86 (*t*, 8.0, 1H)	136.2	7.23 (*d*, 8.0, 1H)	129.5
7	7.61 (*d*, 8.4, 1H)	119.4	7.23 (*d*, 8.0, 1H)	129.6
8		160.6		157.6
8a		119.8		112.8
9		187.7		190.6
10		182.3		186.6
3-CH_3	2.40 (*s*, 3H)	21.5	2.43 (*s*, 3H)	22.3
8-OCH_3	3.96 (*s*, 3H)	56.6		
1-OH	12.85 (*s*, 1H)		12.07 (*s*, 1H)	
5-OH			12.94 (*s*, 1H)	
8-OH			12.25 (*s*, 1H)	

4.4.2.3 Aloesaponarin II (65)

Compound **65** was isolated as an orange amorphous solid. The UV-Vis spectrum (λ_{max} 282, 316, 386, 412 nm) was consistent with the anthraquinone nature of the compound. The EIMS displayed the molecular ion peak at *m/z* 255 $[M+H]^+$, corresponding to molecular formula $C_{15}H_{10}O_4$ and suggesting that it was isomeric to chrysophanol.

The ^{13}C NMR spectrum showed fifteen carbon signals (Table 4.14): five aromatic methines, seven aromatic quaternary carbons, two carbonyl carbons and a methyl group. The ^{1}H NMR spectrum showed the presence of three mutually coupled aromatic protons of ABX spin pattern centered at δ_H 7.31 (*d*, *J* = 8.3 Hz), 7.71 (*t*, *J* = 7.9 Hz) and 7.61 (*d*, *J* = 7.6 Hz) and two *meta*-coupled protons resonating at δ_H 7.44 (*d*, *J* = 2.1 Hz, 1H) and 7.03 (*d*, *J* = 2.1 Hz, 1H) as in chrysophanol. One noteable difference was that of a methyl group that shifted down-field (δ_H 2.70) in compound **65** indicating its *peri*-position to carbonyl group. Furthermore, the presence of only one chelated hydroxyl group (at δ_H 12.97), allowed the placement of the methyl group at C-8. The HMBC $^{4}J_{C,H}$ correlation observed between methyl protons with the carbonyl carbon, C-9 (δ_C 189.4), further confirmed the assignment. Therefore, the structure of compound **65** was characterized as 1,6-dihydroxy-8-methylanthracene-9, 10-dione, trivial name aloesaponarin II (**65**), which has previously been reported from the roots of several *Aloe* species (van Wyk *et al*., 1995b; Yagi *et al*., 1974).

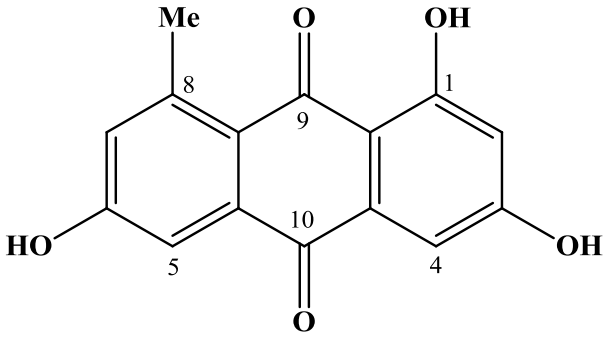


65

4.4.2.4 Deoxyerythrolaccin (68)

Compound **68** was isolated as a yellow amorphous solid. The ESIMS provided a molecular ion peak at *m/z* 271.1 $[M+H]^+$, which was attributed to the molecular formula of $C_{15}H_{10}O_5$. The ^1H NMR spectral data (Table 4.14) revealed similar spectral features to that of **65**: a three-proton singlet at δ_H 2.70 for a methyl group at C-8, and two *meta*-coupled protons centered at δ_H 7.44 (*d*, *J* = 2.6 Hz, H-5) and 7.03 (*d*, *J* = 2.6 Hz, H-7), suggesting an identical ring C. In ring A, the ABX system in aloesaponarin II was replaced by a pair of upfield shifted *meta*-coupled protons resonating at δ_H 6.57 (*d*, *J* = 2.5 Hz, H-2) and δ_H 7.06 (*d*, *J* = 2.5 Hz, H-4) suggesting that C-3 (δ_C 164.1) was substituted with a hydroxyl group and the de-shielded singlet proton at δ_H 13.24 can then be assigned to OH at C-1 (δ_C 164.5).

The ^{13}C NMR spectral data (Table 4.14) revealed the presence of fifteen carbons comprising of two carbonyls (δ_C 188.0 and δ_C 182.4); four aromatic methine carbons (δ_C 107.1, 108.2, 112.1 and 124.6), three oxygenated aromatic quaternary carbons (δ_C 161.8, 164.1 and 164.5), five aromatic quaternary carbons (δ_C 110.0, 122.4, 134.3, 136.7 and 145.0) and a methyl carbon (δ_C 23.6). The compound was therefore, identified as 1,3,6-trihydroxy-8-methylanthracene-9,10-dione, trivial name deoxyerythrolaccin (**68**), which had previously been reported from *Aloe saponaria* (Yagi *et al*., 1974).



68

Table 4.14: ^{1}H (500 MHz) and ^{13}C (125 MHz,) NMR data of Compound **65** and **68** (in DMSO-d_6)

Position	Compound **65**		Compound **68**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		161.5		164.5
1a		116.5		110.0
2	7.31 (*d*, 8.3, 1H)	124.3	6.57 (*d*, 2.5, 1H)	108.2
3	7.71 (*t*, 7.9, 1H)	136.1		164.1
4	7.61 (*d*, 7.6, 1H)	118.3	7.06 (*d*, 2.5, 1H)	107.1
4a		132.6		136.7
5	7.44 (*d*, 2.1, 1H)	112.1	7.44 (*d*, 2.6, 1H)	112.1
5a		137.0		134.3
6		162.4		161.8
7	7.03 (*d*, 2.1, 1H)	124.6	7.03 (*d*, 2.6, 1H)	124.6
8		145.5		145.0
8a		122.5		122.4
9		189.4		188.0
10		182.3		182.4
8-CH_3	2.70 (*s*, 3H)	23.6	2.70 (*s*, 3H)	23.6
1-OH	12.97 (*s*, 1H)		13.24 (*s*, 1H)	

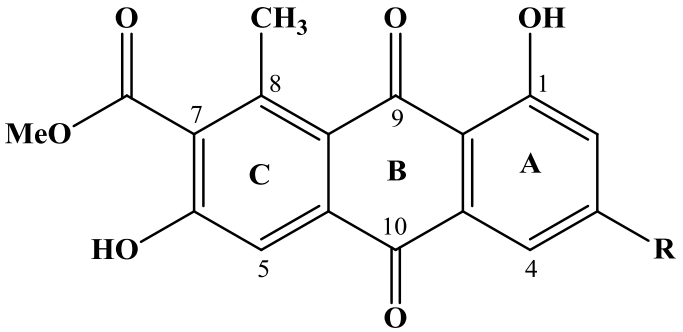
4.4.2.5 Aloesaponarin I (66)

Compound **66** was isolated as yellow crystals (R_f 0.50; 20% EtOAc in *n*-hexane). Its molecular formula was established as $C_{17}H_{12}O_6$ on the basis of its ESIMS (*m/z* 313 $[M+H]^+$) and ^{13}C NMR spectral data (Table 4.15) which was consistent with twelve degrees of unsaturation. The ^1H NMR (500 MHz) showed highly deshielded signal at δ_H 12.77 due to chelated hydroxyl proton at C-1 (δ_C 161.4), a three-proton singlet at δ_H 3.88 (δ_C 52.5) and a shielded carbonyl (δ_C 167.2), indicating the presence of a methyl ester group. The downfield shifted methyl signal observed at δ_H 2.58 (δ_C 19.9) due to the *peri*-effect allowing its placement at C-8 (δ_C 141.0) as in aloesaponarin II. In addition, the ^1H NMR spectrum further showed the presence of three mutually coupled aromatic protons with an ABX pattern centered at δ_H 7.29 (*d*, *J* = 8.2 Hz, H-2), 7.58 (*t*, *J* = 7.6 Hz, H-3) and 7.68 (*d*, *J* = 7.6 Hz ,H-4) of ring A as in aloesaponarin II . In ring C, the singlet aromatic proton at δ_H 7.56 showed HMBC correlation with C-6, C-7, C-8a and C-10 which was in agreement with its placement at C-5 of trisubstituted (C-6, C-7, C-8) ring C. The substituents in this ring, the methyl group (δ_H 2.58 at C-8), a hydroxyl group (at C-6) and methyl ester (at C-7) were evident from the NMR spectra (Table 4.15).

The ^{13}C NMR spectral data (Table 4.15) revealed the presence of seventeen carbon atom; three carbonyls (δ_C 189.2, 181.8 and 167.2), four aromatic methine carbons (δ_C 136.1, 124.4, 118.3 and 112.0), two oxygenated aromatic quaternary carbons (δ_C 161.4 and 158.9), six non-oxygenated aromatic quaternary carbons (δ_C 141.0, 136.7, 132.3, 129.6, 122.5 and 116.7), a methoxyl group (δ_C 52.5) and a methyl carbon (δ_C 19.9). Based on the above spectral data, compound **66** was identified as 3,8-dihydroxy-1-methyl-2-anthraquinoic acid methyl ester, trivial name alosaponarin I (**66**) which had previously been isolated from the stem of *Aloe saponaria* (Yagi *et al*., 1974).

4.4.2.6 Laccaic acid D methyl ester (67)

Compound **67** was isolated as a red crystalline solid. The EIMS of this compound displayed a molecular ion peak at *m/z* 329 $[M+H]^+$, consistent with the molecular formula of $C_{17}H_{12}O_7$. The ^1H NMR spectral data (Table 4.15) showed a signal at δ_H 13.01 for chelated hydroxyl group at C-1 and three-proton singlet at δ_H 3.84 (δ_C 52.8) for a methoxy group and downfield shifted methyl signal (at δ_H 2.54; δ_C 20.1) due to the deshielding effect of the neighboring carbonyl group similar to aloesaponarin I. The trisubstituted ring C, which was evident from the singlet aromatic proton at δ_H 7.53 showed HMBC correlation with C-6, C-7, C-8a and C-10. A methyl group (δ_H 2.54) and a methyl ester (δ_H 3.84; δ_C 52.8 and δ_C 167.8 for the ester carbonyl) was also consistent with the alosaponarin I skeleton. In the ^1H NMR spectrum, the presence of two downfield shifted *meta*-coupled aromatic protons at δ_H 6.52 (*d*, *J* = 2.4 Hz, 1H) and 6.99 (*d*, *J* = 2.4 Hz, 1H) corresponding to protons at C-2 and C-4 respectively in ring A suggest that C-3 (164.3) of aloesaponarin I was attached to hydroxyl group. Therefore, the structure of compound **67** was identified as 3,6,8-trihydroxy-1-methyl-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid methyl ester, trivial name laccaic acid D methyl ester (**67**). This compound is a typical compound found in many *Aloe* species roots (Dagne *et al*., 1992; Yenesew *et al*., 1993; van Wyk *et al*., 1995b).



66 R = H
67 R = OH

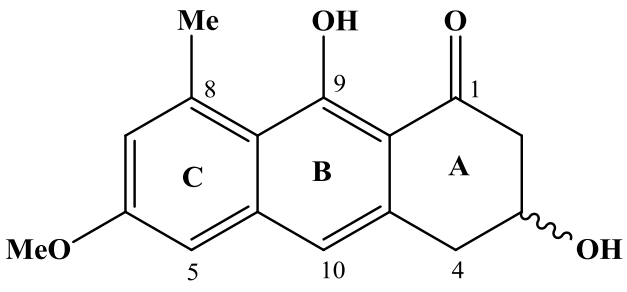
Table 4.15: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **66** and **67** (in DMSO-d_6)

Position	Compound **66**		Compound **67**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		161.4		164.8
1a		116.7		110.4
2	7.29 (*d*, 8.2, 1H)	124.4	6.52 (*d*, 2.4, 1H)	108.7
3	7.58 (*t*, 7.6, 1H)	136.1		164.4
4	7.68 (*d*, 7.6, 1H)	118.3	6.99 (*d*, 2.4, 1H)	107.6
4a		132.3		134.3
5	7.56 (*s*, 1H)	112.0	7.53 (*s*, 1H)	112.3
5a		136.7		136.8
6		158.9		158.5
7		129.6		122.8
8		141.0		141.0
8a		122.5		130.0
9		189.2		188.0
10		181.8		182.2
8-CH_3	2.58 (*s*, 3H)	19.9	2.54 (*s*, 3H)	20.1
OCH_3	3.88 (*s*, 3H)	52.5	3.84 (*s*, 3H)	52.8
CO		167.2		167.8
1-OH	12.77 (*s*, 1H)		13.01 (*s*, 1H)	

4.4.2.7 Aloesaponol II-6-methyl ether (157)

Compound **157** was isolated as a colourless crystalline solid. The colourless spot on TLC showed blueish fluorescence when viewed at 366 nm under UV light. The molecular ion peak at *m/z* 273 $[M+H]^+$ and the ^{13}C NMR spectrum showing the presence of 16 carbon atoms suggested the molecular formula $C_{16}H_{16}O_4$. The UV-Vis spectrum (λ_{max} 280, 293, 312, 377 nm) was typical of a pre-anthraquinone (Yagi *et al*., 1974).

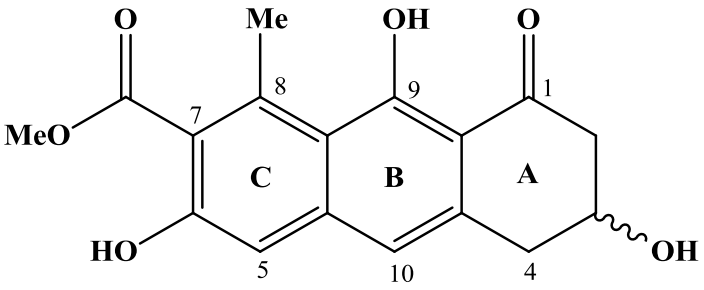
The ^{1}H NMR (Table 4.16) showed three aromatic protons, two of which are *meta*-coupled resonating at δ_H 7.17 (*d*, *J* = 1.5 Hz) and 6.81 (*d*, *J* = 1.5 Hz) assigned to H-5 and H-7 respectively of ring C which was substituted at C-6 (methoxyl) and C-8 (methyl). An isolated aromatic proton at δ_H 6.81 was assigned to H-10 of ring B which also contains a highly deshielded hydroxyl group (δ_H 16.32) at C-9. Three sets of mutually coupled aliphatic protons at δ_H 1.98 & 2.17 (2H, *m*), 2.76 (2H, *m*) and 4.75 (1H, *m*) were assigned to ring A protons which was substituted with carbonyl (δ_C 204.2, C-1) and hydroxyl (δ_C 66.3, C-3). Three-proton singlets at δ_H 3.89 and 2.43 indicated the presence of methoxyl (at C-6) and a methyl (at C-8) groups respectively. The ^{13}C NMR spectrum gave rise to sixteen carbon signals; assignable to a carbonyl (δ_C 204.2), three aromatic and one aliphatic methines (119.4, 108.2, 114.6, 66.3), two methylenes (34.9, 31.1), two oxygenated aromatic quaternary carbons (159.2, 164.9), five quaternary aromatic carbons (109.3, 142.1, 139.5, 112.5, 141.9), a methyl (21.8) and a methoxyl (55.8) carbons. The position of a methoxyl group was established at C-6 by NOE experiment showing correlation between the methoxyl protons with the two aromatic (H-5 and H-7) protons. Comparison of the spectral data with information in the literature (Yagi *et al*., 1974) revealed that the compound is 6-methoxy-3,9-dihydroxy-8-methyl-3,4-dihydro-2H-anthracen-1-one, trivial name aloesaponol II-6-methyl ether (**157**), which had previously been isolated from *A. saponaria* (Yagi *et al*., 1974).



157

4.4.2.8 Aloesaponol I (69)

Compound **69** was isolated as a colourless solid. The colourless spot on TLC showed a blueish fluorescence under 366 nm UV light. The EIMS displayed a molecular ion peak at *m/z* 317 $[M+H]^+$ suggeting a molecular formula of $C_{17}H_{12}O_7$. The UV spectrum (λ_{max} 281, 286, 314 and 383 nm) was in agreement with the presence of a pre-anthraquinone skeleton (Yagi *et al*., 1974). This was further confirmed by NMR spectral data (Table 4.16) that revealed signals for a carbonyl at C-1 (δ_C 203.8), an oxymethine at C-3 (δ_H 4.24; δ_C 64.4) and two methylenes at C-2 (δ_H 2.68 and 2.94; δ_C 46.4) and C-4 (δ_H 2.89 & 3.12; δ_C 37.5) of ring A. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **69** (Table 4.16) with that of **157** showed the two compounds are closely related with the only notable difference being the replacement of the two *meta*-coupled protons of ring C in **157** with a singlet proton at δ_H 6.92 (H-5) indicating that C-7 was substituted with a methyl ester (δ_H 3.83, δ_C 52.3 & 170.4) group as in aloesaponarin I. The NMR spectra further showed the presence of a singlet aromatic proton centered at δ_H 6.95 assigned to H-10, with a methyl group (δ_H 2.69, δ_C 20.6) being at C-8 (δ_C 140.8). The structure of compound **69** was therefore characterized as 3,6,9-trihydroxy-8-methyl-1-oxo-5,6,7,8-tetrahydro-anthracene-2-carboxylic acid, trivial name aloesaponol I (**69**), previously reported from *A. saponaria* (Yagi *et al*., 1974). However, the absolute configuration at the stereocenter (C-3) has not been determined to date.



69

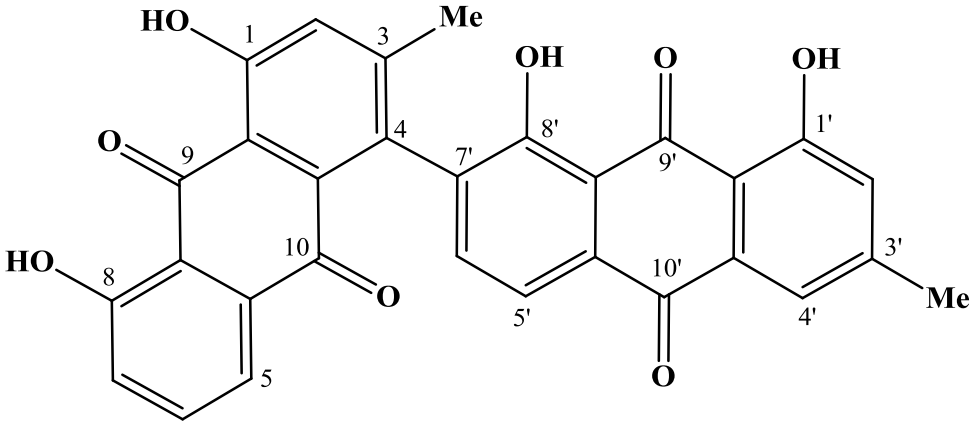
Table 4.16: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **157** and **69** (in DMSO-d_6)

Position	Compound **157**		Compound **69**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		204.2		203.8
1a		109.3		110.3
2	2.76 (*m*, 2H)	34.9	2.68 & 2.94 (*m*, 1H)	46.4
3	4.75 (*m*, 1H)	66.3	4.24 (*m*, 1H)	64.4
4	2.17 & 1.98 (*m*, 1H)	31.1	2.89 & 3.12 (*m*, 1H)	37.5
4a		142.1		141.0
5	7.17 (*d*, 1.5, 1H)	119.4	6.92 (s, 1H)	107.5
5a		139.5		125.5
6		159.2		155.0
7	6.81 (*d*, 1.5, 1H)	108.2		136.4
8		141.9		140.8
8a		112.5		115.4
9		164.9		166.0
10	7.21 (*d*, 1.1, 1H)	114.6	6.95 (*s*, 1H)	116.5
CO				170.4
8-CH_3	2.43 (*s*, 3H)	21.8	2.69(*s*, 3H)	20.6
6-OCH_3	3.89 (*s*, 3H)	55.8		
COO*CH_3*			3.83 (*s*, 3H)	52.3

4.4.2.9 Asphodelin (23)

Compound **23** was isolated as a yellow amorphous solid. The UV spectrum (λ_{max} 256, 294, 371, 382, 516, 527 and 543 nm) and the NMR spectral data showing four chelated hydroxyl (δ_H 12.60, 12.40, 12.05, 12.03), two aromatic methyl (δ_H 2.52, 2.17) and four carbonyl groups (192.8, 192.8, 182.4, 181.9) was consistent with this compound being an anthraquinone dimer (Yagi *et al*., 1978a).

In one-half of the molecule, an ABX spin system was observed for three aromatic protons which resonated at δ_H 7.62 (*dd*, *J* = 7.4, 1.2 Hz), 7.60 (*t*, *J* = 7.4 Hz) and 7.28 (*dd*, *J* = 7.4, 1.2 Hz) and were respectively assigned to H-5, H-6 and H-7 of the chrysophanol moiety. In addition, a singlet aromatic proton at δ_H 7.32 was observed for H-2 indicating C-4 was the point of attachment to the other half of the molecule. The up-field shifted biosynthetically expected methyl (δ_H 2.17; δ_C 22.3) group at C-3 due to anisotropic effect from the other half of the molecule supporting C-4 (δ_C 137.3) linkage to the other half of the molecule. For the other half of the molecule, a similar pattern was observed in the ^{1}H NMR spectrum except that the ABX pattern in chrysophanol was replaced by a pair of de-shielded *ortho*-coupled protons with AX pattern at δ_H 7.98 (*d*, *J* = 8.0 Hz) and δ_H 7.40 (*d*, *J* = 8.0 Hz) which were readily assigned to H-5' and H-6' respectively. This indicated that the point of attachment in this half of the molecule was at C-7' (δ_C 133.5). Therefore, this compound was characterized as 4,-7'-bichrysophanol, trivial name asphodeline (**23**) (Yagi *et al*., 1978a) which had been isolated from *Aloe saponaria* (Yagi *et al*., 1978a) and *Asphodelus ramosus* (Adinolei *et al*., 1991). However, the configuration at the biaryl linkage has not be established to date.



23

4.5 Secondary Metabolites from the Leaves of *Aloe lateritia* Subspecies *graminicola*

Chromatographic separation of the extracts of the leaves of *Aloe lateritia* subspecies *graminicola* resulted in the isolation of thirteen compounds including nine anthraquinones, three furancarboxaldehydes, and a chromone. Of which six anthraquinones; chrysophanol (**18**), helminthosporin (**59**) , aloesaponarin II (**65**), laccaic acid D methyl ester (**67**), aloesaponol I (**69**) and asphodelin (**23**) have been also idetified from the roots of *Aloe dawei*. Their identification has already been discussed in section 4.4. The furancarboxaldehyde derivatives; 5-hydroxymethyl-2-furancarboxaldehyde (**159**), 5-acetyloxymethyl-2-furancarboxaldehyde (**160**) and cirsiumaldehyde (**161**) are the first report of their isolation from the family Asphodelaceae.

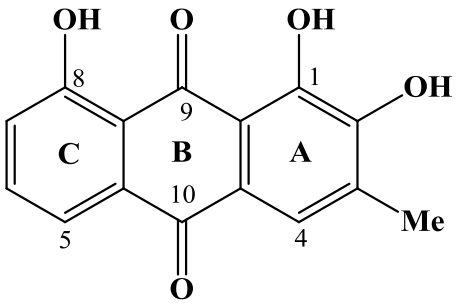
4.5.1 Anthraquinones

4.5.1.1 Norobtusifolin (158)

Compound **158** was isolated as a red solid. The EIMS showed a molecular ion peak at *m/z* 270 corresponding to the molecular formula $C_{15}H_{10}O_5$. The UV-Vis absorption peak at λ_{max} 213, 287, 323, 396 was consistent with an anthraquinone skeleton.

The ^{1}H NMR spectrum displayed signals for two chelated hydroxyl groups (δ_H 13.18 and 12.03), three mutually coupled aromatic protons of ABX spin pattern centered at δ_H 7.79 (*d*, *J* = 7.6 Hz), 7.83 (*t*, *J* = 7.8 Hz) and 7.33 (*d*, *J* = 8.4 Hz) which were assigned to H-5, H-6 and H-7 respectively of ring C. In ring A, the only aromatic proton signal observed was for H-4 (δ_H 7.68), with C-3 substituted with biogenetically expected methyl group (δ_H 2.40) similar to chrysophanol. C-1 was substituted with the chelated hydroxyl group (δ_H 13.18) with the corresponding carbon (C-1) resonating at δ_C 150.3, which was upfield-shited. The chemical shift of C-2 (δ_C 150.8) suggested that it was also oxygenated. Overall, the ^{13}C NMR spectrum (Table

4.17) showed fifteen carbon signals; four aromatic methines, eight aromatic quaternary carbons, two carbonyl carbons and a methyl group. The molecular mass of **158** was found to be higher by 16 amu than that of chrysophanol (**18**) supporting that **158** was 2-hydroxychrysophanol, trivial name norobtusifolin (**158**), which had previously been isolated from the roots of *Hemerocallis fulva* (Takido, 1958). This appears to be the first report on the isolation of this compound in the family Asphodelaceae.

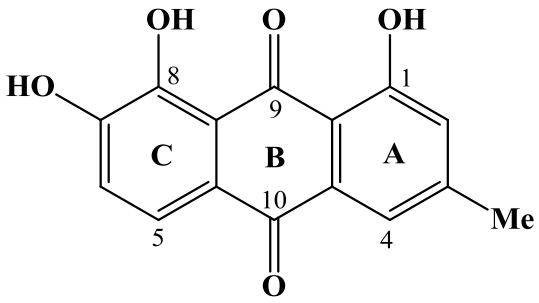


158

4.5.1.2 Nataloe-emodin (61)

Compound **61** was obtained as a red amorphous solid. The EIMS showed a molecular ion peak at *m/z* 270 corresponding to the molecular formula $C_{15}H_{10}O_5$ which suggested that **61** was an isomer of **158**. The ^{1}H NMR spectral data (Table 4.17) revealed signals for a pair of *meta*-coupled aromatic protons at δ_H 7.14 (*d*, *J* = 1.2 Hz, 1H, H-2) and 7.59 (*d*, *J* = 1.2 Hz, 1H, H-4) of ring A, which was substituted at C-1 (with OH, δ_H 12.92) and C-3 (methyl, δ_H 2.49). In ring C, two *ortho*-coupled aromatic protons at δ_H 7.75 (*d*, *J* = 8.1 Hz) and 7.31 (*d*, *J* = 8.2 Hz) which were assigned to H-5 and H-6, respectively, required substitution at C-7 and C-8 which could only be hydroxyl groups due to the shielding reflected in the chemical shift values (δ_C 152.3 and δ_C 150.3) for the two *ortho*-oxygenated aromatic carbon atoms. This was in agreement with the structure of 1,2,8-trihydroxy-6-methyl-9,10-anthracenedione, trivial name nataloe-emodin (**61**),

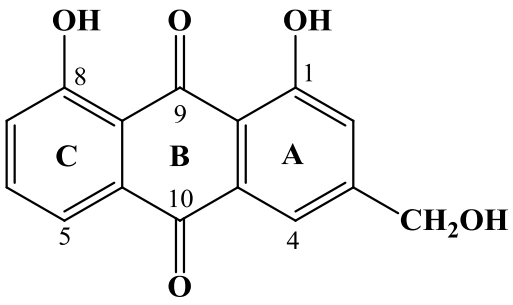
which had previously been reported from *Aloe nyeriensis* (Conner *et al*., 1987) and *Senna longiracemosa* (Alemayehu *et al*., 1993).



61

4.5.1.3 Aloe-emodin (20)

Aloe-emodin (**20**) was isolated as a yellow solid. The ^{1}H NMR spectrum (Table 4.17) resembled that of chrysophanol and revealed two chelated hydroxyl protons (δ_H 11.93 and 11.92), three mutually coupled protons with ABX pattern resonating at δ_H 7.71 (*dd*, *J* = 8.4, 1.2 Hz, H-5), 7.83 (*t*, *J* = 8.4 Hz, H-6) and 7.37 (*dd*, *J* = 8.4, 1.2 Hz, H-7) of ring C and, two *meta*-coupled protons centered at δ_H 7.28 (*d*, *J* = 1.5 Hz, H-2) and 7.68 (*d*, *J* = 1.5 Hz, H-4) of the disubstituted ring A. The only difference was that the aromatic methyl signal in chrysophanol was replaced with oxymethylene group (δ_H 4.62; δ_C 62.1), which could only be placed at C-3 on the basis of biosynthetic consideration (that the methyl group in chrysophanol was oxidized to oxymethylene group giving rise to **20**). This assignment was supported by HMBC correlations of the oxymethylene protons with C-2, C-3 and C-4. The ^{13}C NMR spectrum also revealed the presence of fifteen non-equivalent carbon atoms (two carbonyls, five aromatic methines, seven quaterinary and one oxymethylene carbons). Therefore, the compound was identified as 1,8-dihydroxy-3-hydroxymethylene anthraquinone, trivial name aloe-emodin (**20**). The compound is a common metabolite in the leaves of *Aloe* species (Dagne *et al*., 2000).



20

Table 4.17: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **158** (in acetone-d_6), **61** (in acetone-d_6) and **20** (in DMSO-d_6)

Position	**158**		**61**		**20**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C
1		150.3		162.6		161.6
1a		115.4		113.9		114.5
2		150.8	7.14 (*d*, 1.2, 1H)	123.3	7.28 (*d*, 1.5, 1H)	120.7
3		133.3		149.8		153.7
4	7.68 (*s*, 1H)	123.9	7.59 (*d*, 1.2, 1H)	120.5	7.68 (*d*, 1.5, 1H)	117.1
4a		125.0		134.0		133.3
5	7.79 (*d*, 7.6, 1H)	120.1	7.75 (*d*, 8.1, 1H)	121.4	7.71 (*d*, 8.4, 1.2, 1H)	119.4
5a		135.2		124.9		133.1
6	7.83 (*t*, 7.8, 1H)	138.4	7.31 (*d*, 8.2, 1H)	121.0	7.83 (*t*, 8.4, 1H)	137.4
7	7.33 (*d*, 8.4, 1H)	124.5		152.3	7.38 (*d*, 8.4, 1.2, 1H)	124.4
8		163.2		150.3		161.3
8a		117.0		116.2		115.9
9		194.3		193.2		193.6
10		181.1		180.1		181.4
3-CH_3	2.40 (*s*, 3H)	16.5	2.49 (*s*, 3H)	21.3		
3-CH_2OH					4.62 (*s*, 2H)	62.1
1-OH	13.18 (s, 1H)		12.92 (s, 1H)		11.93 (s, 1H)	
8-OH	12.03 (s, 1H)		11.93 (s, 1H)		11.92 (s, 1H)	

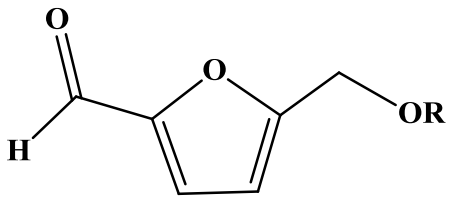
4.5.2 Furancarboxaldehydes

4.5.2.1 5-Hydroxymethyl-2-furancarboxaldehyde (159)

Compound **159** was isolated as a coulorless oil. Its EIMS spectrum exhibited a molecular ion peak at *m/z* 126, suggesting a molecular formula of $C_6H_6O_3$, which was consistent with four degrees of unsaturation. The presence of only two protons resonating at δ_H 7.39 (*d*, *J* = 3.5 Hz) and 6.59 (*d*, *J* = 3.5 Hz) in ^{1}H NMR spectrum (Table 4.18) and four sp^2 hybridized carbons at δ_C 109.3, 123.0, 152.5 and 162.0 in the ^{13}C NMR spectrum indicated the compound was a 5-membered heterocyclic furan ring which was substituted at C-2 and C-5 with the substituents being proposed as formyl (δ_H 9.49, δ_C 177.2) and hydroxymethylene (δ_H 4.60, δ_C 56.5) groups. Consequently, the structure of **159** was established as 5-hydroxymethyl-2-furancarboxaldehyde, which had previously been isolated from the roots of *Hydrangea chinensis* (Khalil *et al*., 2003). However, this is the first report on the occurence of the compound in the family Asphodelaceae.

4.5.2.2 5-Acetyloxymethyl-2-furancarboxaldehyde (160)

5-Acetyloxymethyl-2-furancarboxaldehyde (**160**) was also isolated as a coulorless oil. The EIMS spectrum exhibited a molecular ion peak at *m/z* 168, consistent with the molecular formula $C_8H_8O_4$. The NMR spectral data (Table 4.18) of compound **160** was similar to **159** except for the presence of an additional three-proton singlet signal at δ_H 2.88 in ^{1}H NMR, and two carbon signals at δ_C 20.5 and 170.5 in ^{13}C NMR spectrum attributed to the acetyl group in the former. That compound **160** was thus an acetate of **159** and supported by EIMS which showed a fragment ion at *m/z* 126 for the aglycone in **160** which is basically **159**. Consequently, compound **160** was identified as 5-acetyloxymethyl-2-furancarboxaldehyde, which had previously been isolated from the roots of *Hydrangea chinensis* (Khalil *et al*., 2003). This is also the first report on the occurence of the compound in the family Asphodelaceae.

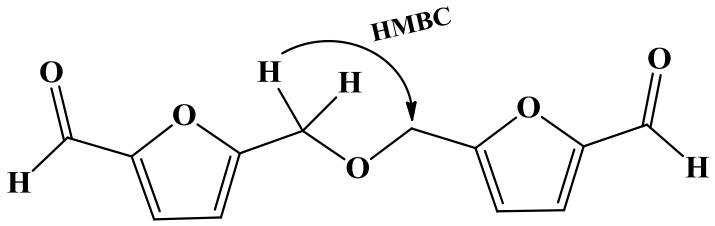


159 R = H
160 R = Ac

4.5.2.3 Cirsiumaldehyde (161)

Cirsiumaldehyde (**161**) was isolated as a colourless oil. Its NMR spectral data (Table 4.18) was similar to that of compound **159**, showing the presence of two mutually coupled protons resonating at δ_H 7.41 (*d*, *J* = 3.5 Hz) and 6.73 (*d*, *J* = 3.6 Hz) in ^{1}H NMR spectrum and four sp^2 carbons at δ_C 112.7, 123.2, 154.0 and 158.4 in the ^{13}C NMR spectrum suggesting the presence of a furan ring was noted. The presence of a formyl (δ_H 9.65; δ_C 178.4) and oxymethylene (δ_H 4.69; δ_C 64.9) groups were also evident for a 5-oxymethylene-2-furancarboxaldehyde skeleton. The only noticeable difference was the chemical shift value of oxymethylene carbon differing by *ca*. 8 ppm (Table 4.18) indicating that **159** may be a dimer of compound **159**. Unfortunately the EIMS did not show the molecular ion peak, instead the fragment ion peak at *m/z* 126 corresponding to the monomeric ion produced by cleavage of **161** was observed as the highest peak. Despite this, the dimeric nature was established from the HMBC spectrum which showed unusual correlation between the oxymethylene protons δ_H 4.69 (H-6) and δ_C 64.9 (C-6) indicated that there must be a carbon atom which is three bonds away and yet chemically equivalent. This is only possible in symmetrical dimers where the methylene proton in one of the monomers showing a ^3J correlation to the methylene carbon of the other monomer. Therefore, compound **161** was identified as a dimer of 5-hydroxymethyl-2-furancarboxaldehyde (**159)** and named cirsiumaldehyde (**161**), which had previously been isolated from the roots of *Cirsium chlorolepis*

(Shen and Mu, 1990). However, this is the first report on the isolation of the compound in the family Asphodelaceae.



161

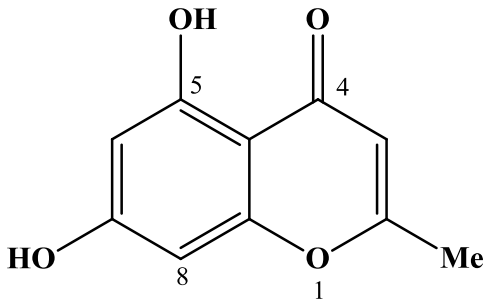
Table 4.18: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of Compound **159**, **160** and **161** (acetone-d_6)

Position	**159**		**160**			**161**	
	δ_H (*m, J* in Hz)	δ_C	δ_H (*m, J* in Hz)	δ_C	Position	δ_H (*m, J* in Hz)	δ_C
1	9.60 (*s*, 1H)	177.2	9.60 (*s*, 1H)	178.5	1/1'	9.65 (*s*, 1H)	178.4
2		152.5		156.6	2/2'		154.0
3	7.39 (*d*, 3.5, 1H)	123.0	7.43 (*d*, 3.6, 1H)	123.2	3/3'	7.41 (*d*, 3.5, 1H)	123.2
4	6.59 (*d*, 3.5, 1H)	109.3	6.76 (*d*, 3.6, 1H)	113.3	4/4'	6.73 (*d*, 3.6, 1H)	112.7
5		162.0		154.0	5/5'		158.4
6	4.65 (s, 2H)	56.5	5.12 (*s*, 2H)	58.3	6/6'	4.69 (*s*, 2H)	64.9
COO				170.5			
CH_3			2.88 (*s*, 3H)	20.5			

4.5.3.1 Noreugenin (162)

Noreugenin (**162**) was isolated as a colourless solid. The ^{1}H and ^{13}C NMR spectra were typical of a chromone derivative (Tane *et al*., 1990). A highly deshielded singlet at δ_H 12.89 was for hydroxyl proton, two *meta*-coupled aromatic protons resonating at δ_H 6.22 and 6.36 (J = 2.2 Hz) for H-6 and H-8, respectively, an olefinic proton at δ_H 6.07 (H-3) and a three-proton singlet at δ_H

2.37 for an aromatic methyl group at C-2 were observed in ^{1}H NMR spectrum. The ^{13}C NMR revealed carbon signals for ten carbon atoms; three methine (93.8, 98.9 and 108.3), five quaternary (104.4, 158.6, 162.7, 164.1 and 167.7) one carbonyl (182.5) and a methyl (19.6) carbon. From the above spectral data and comparison with literature (Badawi *et al*., 1976), the compound was identified as 2-methyl-5,7-dihydroxychromone, trivial name noreugenin (**162**), which had previously been isolated from the roots of *Schumanniophyton magnificum* (Tane *et al*., 1990) and *Adina rubescens* (Badawi *et al*., 1976).



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4.6 Chemotaxonomic Significance of the Isolated Compounds

The plant family Asphodelaceae (with 16 genera and 780 species) is divided into two sub-families: The Alooideae (with the genera *Aloe, Astroloba, Chamaealoe, Gasteria, Haworthia, Lomathophyllum,* and *Poellnitzia*) and the Asphodeloideae (with the genera *Asphodeline, Kniphofia, Bulbine, Bulbinella, Asphodelus, Jodrellia, Smiethis*, and *Trachyandra*) (Dahlgren *et al*., 1985). Despite the various attempts that have been made to provide stable classification for these two sub-families of the Asphodelaceae (Hutchinson, 1934; Cronquist, 1981; Dahlgren *et al*., 1985), the inter relationships amongst some genera are still unresolved.

The taxonomic studies made by Hutchinson (1934) and Cronquist (1981; 1988) classified the genus *Kniphofia* within Alooideae sub-family based on inflorescence and floral morphology. On the other hand, Dahlgren *et al*. (1985) placed this genus in the Asphodeloideae sub-family based on differences in the anatomical construction of the leaves. Through chloroplast DNA (CP DNA) sequence data, Chase *et al*. (2000) supported the placement of *Kniphofia* in Asphodeloideae along with *Bulbine*. The occurrence of anthraquinone derivatives have already been recognized to serve as valuable chemotaxonomic markers of some taxa within this family (Smith and van Wyk, 1998; van Wyk *et al*., 1995a). The chemotaxonomic investigation by van Wyk *et al*. (1995a) on the roots of several plant of the Asphodeloideae has showed that the genera *Bulbine*, *Bulbinella* and *Kniphofia* form a monophyletic unit within the sub-family Asphodeloideae.

The anthraquinones isolated here from the roots of *K. foliosa* are all 1,8-dihydroxy-3-methyl anthraquinone derivatives and lack oxygenation at C-6 which is typical in the sub-family Asphodeloideae. The occurence of chrysophanol (1**8**), aloesaponol III (**16**) and 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**) in *K. foliosa* and *A. saponaria* (Yagi *et al*., 1974; 1977a; 1978a) showed the presence of relationships between the two sub-families within the Asphodelaceae family. On the other hand, phenylanthraquinones have only been reported in the Asphodeloideae sub-family (to genera *Kniphofia*, *Bulbine*, and *Bulbinella*) (Dagne and Steglich, 1984; Bringmann *et al*., 2008b; Yenesew *et al*., 1994; Bezabih *et al*., 1997).

In the present study, knipholone (**14**) and isoknipholone (**28**) have been isolated from the roots of *K. foliosa* and *B. frutescens*, which supports the close relationship between the genera *Kniphofia* and *Bulbine*. Furthermore, the isolation of two new phenylanthraquinones, knipholone cyclooxanthrone (**134**) and 6',8-*O*-dimethylknipholone (**150**) from the roots of *K. foliosa* and *B.*

frutescens respectively (Abdissa *et al*., 2013; 2014a) support that the two genera remain good source of a variety of phenylanthraquinones. The dimeric phenylanthraquinones, jozi-knipholone A and B isolated from the *B. frutescence* (Bringmann *et al*., 2008a) and *K. foliosa* (Induli *et al*., 2013) is further proof that the two genera are closely related. Apart from the Asphodelaceae family, the only report of the occurence of a minor amount of the phenylanthraquinone, knipholone (**14**) is from the pods of *Senna didymobotrya* (Leguminosae) (Alemayehu *et al*., 1996). The 3,8-dihydroxy-1-methylanthraquinone derivative isolated here from *B. frutescens* (Asphodeloideae) is 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**). This is the first report on the occurence of such anthraquinone from the sub-family Asphodeloideae.

The genus *Aloe* (Alooideae sub-family) that consists of more than 500 species (Okamura *et al*., 1996; Mabberley, 1987) also suffer from heterogeneity among the species. Comparative studies of morphology, anatomy and phytochemistry at the infrageneric levels did not fully resolve the taxonomic instability within *Aloes*. Reynolds has divided *Aloes* into 20 sub-generic groups ranging from grass to tree *Aloe*s depending on similarities in morphology (Reynolds, 1966). This morphology-based grouping; however, does not necessarily reflect genetic relationships. For instance, group 19 (shrubby *Aloes*), encompassing *Aloes* with prolonged stems, shares similarities with group 5 (*Aloes* with striped perianth) in inflorescence and leaf characters, even though they differ with respect to caulescence and branching. Moreover, *A. dawei* of group 19 and *A. secundiflora* of group 14 have the morphological similarity of spotted perianth shape (Viljoen and van Wyk, 2000). The chemotaxonomic study by Viljoen and van Wyk (2000) has showed that the secondary metabolite profiles of the *Aloe* groups 5, 14, 16 and 19 are closely related.

The anthraquinone derivatives isolated from both *A. dawei* and *A. graminicola* are of two kinds; 1,8-dihydroxy-3-methyl anthraquinones (eg. chrysophanol) and 3,8-dihydroxy-1-methyl anthraquinones (eg. aloesaponarin II), which appear to have been formed by two parallel routes differing by the way the octaketide chain was folded (Scheme-2.2). The 1,8-dihydroxy-3-methylanthraquinone derivatives such as chrysophanol (**18**), aloe-emodine (**20**) and asphodelin (**23**) reported here from *A. dawei* and *A. graminicola* are the common denominators of Asphodeloideae (van Rheede and van Oudtshoorn, 1964; Berhanu and Dagne, 1984) indicating chemical affinity between Alooideae and Asphodeloideae. Whereas the 3,8-dihydroxy-1-methyl anthraquinone derivatives, aloesaponarin II (**65**), alosaponarin I (**66**), laccaic acid D methyl ester (**67**), deoxyerythrolaccin (**68**), aloesaponol I (**69**) and aloesaponol II-6-methyl ether (**157**) are the main constituents of the Alooideae. In particular the 7-methyl ester anthraquinone derivatives **66**, **67** and **69** appear to be restricted to the genus *Aloe* and hence these compounds could be the markers of the taxa. However, these compounds being found in almost all *Aloe* species, have limited chemotaxonomic importance at intra-generic level.

Naphthoquinones have a restricted distribution in *Aloe* and only three compounds belonging to this chemical class had been isolated from *Aloe secundiflora* (Induli *et al*., 2013). In this work, seven 1,4-naphthoquinones; droserone (**123**), droserone-5-methyl ether (**152**), hydroxydroserone (**153**), 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), malvone A (**154**), 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**) and ancistroquinone C (**95**) all with similar oxygenation pattern at position C-3 and C-5 were isolated from the roots of *A. dawei* (Abdissa *et al*., 2014b). This was the second report of the isolation of naphthoquinones from the genus *Aloe* following the report from *A. secundiflora* (Induli *et al*., 2013). The isolation of these naphthoquinones from *A. dawei* of group 19 (Abdissa *et al*., 2014b) and *A. secundiflora* of group

14 (Induli *et al*., 2013) is in support of the previous morphological and chemotaxonomic report that the two groups are related (Viljoen and van Wyk, 2000). It is worthwhile then to conduct directed chemotaxonomic survey of members of groups 5, 14, 16 and 19 for the presence of naphthoquinones since affinities have been observed among members of these taxa (Viljoen and van Wyk, 2000).

4.7 Biological Assay

Quinones including anthraquinones and naphthoquinones have gained great toxicological and pharmacological importance, due to the fact that several chemotherapeutic agents contain the quinone nucleus. This is related to their chemical structure (the quinone nucleus), allowing them to involve in multiple biological oxidative processes (Monks and Jones, 2002). Consequently, the crude extracts and isolated compounds were tested for their antiplasmodial activity (against W2 and D6 strains of *P. falciparum*) and cytotoxicty (aginst MCF-7 breast cancer and human cervix carcinoma KB-3-1 cells).

4.7.1 *In vitro* Antiplasmodial Assay

The major isolated compounds together with the crude extracts were assayed for *in vitro* antiplasmodial activity (Table 4.19). The dimeric anthraquinones including 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) and chryslandicin (**13**) exhibited potent activity against both chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* with low cytotoxicity against the human cervix carcinoma KB-3-1 cell lines. This supports the assertion that this class of compounds may constitute a new class of antimalarial lead structures (Wube *et al*., 2005). The *in vitro* antiplasmodial activity of knipholone-type phenylanthraquinones;

knipholone, isoknipholone and knipholone anthrone have been reported to have considerable activity against the chloroquine-resistant (K1) and the chloroquine-sensitive (NF54) strains of *Plasmodium falciparum* (Bringmann *et al*., 1999a). However, lower activity was observed against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) strains of *Plasmodium falciparum* for the phenylanthraquinones; knipholone, knipholone antharone, and isoknipholone (Table 4.19). This may be because of the phenylanthraquinones isolated were not enantiomerically enriched forms since they usually occur as scalemic or nearly as racemic mixtures in nature (Muttanyata *et al.*, 2005). High inhibition of growth of malaria parasite against D6 and W2 strains was also observed for dianellin (**136**).

Table 4.19: *In vitro* antiplasmodial activity (against W2 and D6 strains of *P. falciparum*) and cytotoxicity (against KB-3-1 cancer cells) of the isolated compounds.

Isolates	IC_{50} (µg mL^{-1})		IC_{50} (µM)
	Chloroquine-resistant (W2)	Chloroquine-sensitive (D6)	Human cervix carcinoma (KB-3-1)
10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**)	1.17 ± 0.12	4.07 ± 1.54	> 6.50
Chryslandicin (**13**)	1.53 ± 0.32	2.14 ± 0.50	> 6.24
Knipholone (**14**)	8.01 ± 0.50	10.1 ± 0.20	0.43
Knipholone anthrone (**15**)	3.60 ± 0.90	4.10 ± 0.80	-
knipholone cyclooxanthrone (**134**)	6.13 ± 1.59	3.96 ± 0.70	6.07
6',8-*O*-dimethylknipholone (**150**)	2.16 ± 0.29	4.69 ± 1.18	-
Isoknipholone (**28**)	7.91 ± 1.20	8.62 ± 1.65	-
Dianellin (**136**)	3.28 ± 0.19	5.47 ± 1.20	Not active
Asphodelin (**23**)	6.00 ± 0.25	8.00 ± 0.72	> 6.3
Aloesaponarin II (**65**)	5.00 ± 0.36	18.60 ± 7.10	0.98
Laccaic acid D methyl ester (**67**)	12.84 ± 2.89	13.13 ± 1.46	-
Aloesaponol I (**69**)	8.00 ± 1.21	20.13 ± 8.68	> 7.1
Knipholone Mannich base (**163**)	1.86 ± 0.19	5.08 ± 0.69	1.89
knipholone-1,3-oxazine **(164)**	4.15 ± 1.18	6.97 ± 1.39	2.50
Chloroquine	0.01 ± 0.001	0.22 ± 0.03	-
Mefloquine	0.03 ± 0.02	0.003 ± 0.001	-

" - " Not tested

4.7.2. Cytotoxicity Assay

Quinones, in particular naphthoquinones, have raised distinct toxicological and pharmacological interest. The quinone core, presumably capable of modulating oxidative biochemical processes (Monks and Jones, 2002), is a common structural element in cancer chemotherapeutic agents such as doxorubicin, mitomycin C, and mitoxantrone. Herbal quinone metabolites have been reported to possess cytotoxic activity (Huang *et al*., 2007; Endale *et al*., 2012; Queiroz *et al*.,

2008; Lim *et al.*, 2007; Lu *et al*., 2013). In line with this, knipholone (**14**) showed high cytotoxicity (IC_{50} 0.43 µM) (Fig 4.4) among the tested compounds against human cervix carcinoma KB-3-1 cell lines (Table 4.19). This supports the realization that knipholone and its derivatives could be potentially a new class of natural anticancer agents with a wide range of toxicological and pharmacological implications as knipholone induces a rapid onset of cytotoxicity with IC_{50} values ranging from 0.5 to 3.3 µM (Habtemariam, 2010).

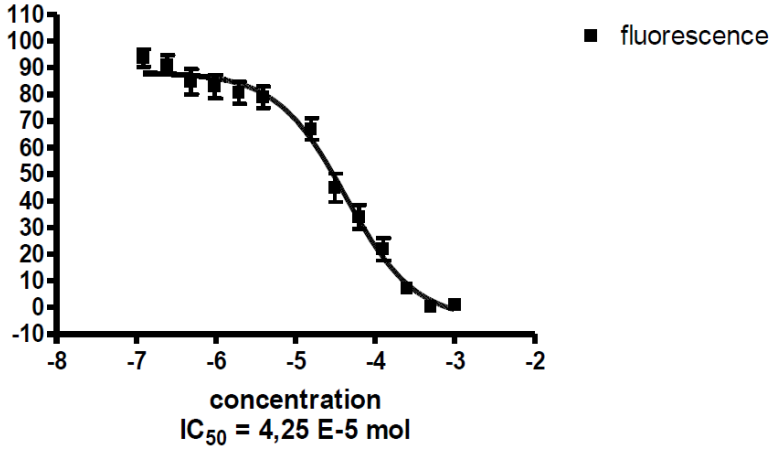


Figure 4.4: Fluorescence spectrum of the cytotoxicity of knipholone

Compounds from the roots of *A. dawei* were therefore assayed for activity against the MDA-MB-231 (ER negative) and MCF-7 (ER positive) breast cancer cell lines (Table 4.20). Of the isolated compounds, 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) and chrysophanol-8-methyl ether (**156**) showed strong cytotoxicity against MCF-7 cells. Compounds **65**, **68**, **69**, **154** and **157** possessed medium cytotoxicity, at least on one of the two studied cancer cell lines; whereas most constituents had low cytotoxicity. The potent and selective activity of compounds **94** and **156** is worth noting (Table 4.20). In this regard, it is worth while to test these compounds on a variety of cell-lines including normal cells.

Table 4.20: Cytotoxicity of the constituents of the roots extract of *A. dawei*

Compound	IC_{50} (μM)	
	MCF-7	MDA-MB-231
6-Hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**)	>403	>403
Ancistroquinone C (**95**)	>370	330
5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**)	1.15	408
Malvone A (**154**)	222	65
Droserone (**123**)	>490	>490
Droserone-5-methyl ether (**152**)	>459	>459
Hydroxydroserone (**153**)	432	>455
Chrysophanol (**18**)	>394	>394
Helminthosporin (**59**)	>370	>370
Aloesaponarin I (**66**)	211	>357
Aloesaponarin II (**65**)	157	72
Laccaic acid D-methyl ester (**67**)	>305	277
Deoxyerythrolaccin (**68**)	178	140
Chrysophanol-8-methyl ether (**156**)	4.85	>100
Aloesaponol I (**69**)	>352	125
Aloesaponol II-6-methyl ether (**157**)	261	131
1-Isopropyl-3-(pyridin-4-ylethynyl)-1H-Pyrazolo[3,4-d]pyrimidin-4-amine	0.05	0.17

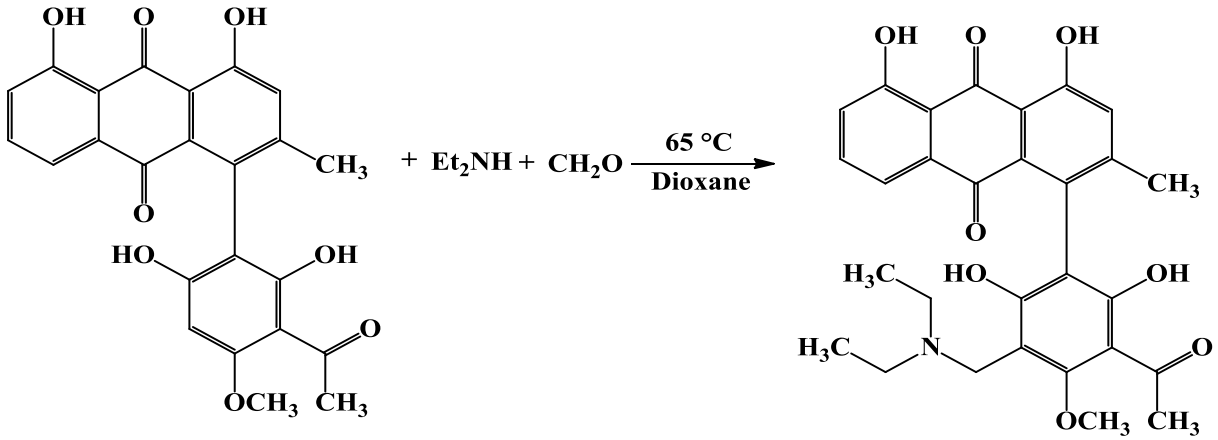
4.8 Structural Modification of Knipholone

The axialy chiral biaryl natural compounds, particularly phenylanthraquinones, which are composed of an anthraquinone and an acetylphloroglucinol moiety, have gained considerable attention due to their antiplasmodial (Abegaz *et al.,* 2002; Bringmann *et al*., 1999a) and anticancer (Reid, 1993; Habtemariam, 2007; 2010) activities. The activity of these compounds is associated with the entire molecular array of the molecules including the stereogenic axes (Bringmann *et al*., 1999a).

Knipholone (**14**), the major constituent of the roots of *K. foliosa* and *B. frutescens* showed considerable antiplasmodial activity with IC_{50} values of 0.65 and 0.91 μg/mL against the K1 and NF54 strains of *P. falciparum* (Abegaz *et al*., 2002). It is also reported that it induces a rapid onset of cytotoxicity with IC_{50} values ranging from 0.5 to 3.3 μM (Habtemariam, 2010). In this regard, knipholone and knipholone-type phenylanthraquinones could be a promising candidate for new drug development with a wide range of toxicological and pharmacological implications. However, knipholone derivatives have not been synthesized in order to obtain less toxic and more potent antimalarial agents; the only synthetic information available is the atropo-enantioselective total synthesis of knipholone using 'lactone concept' (Bringmann *et al*., 2002b).

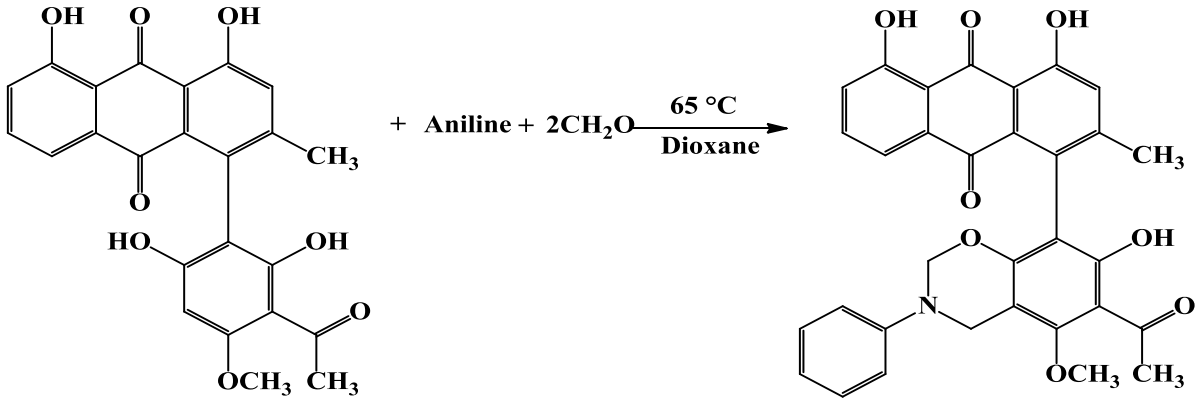
Following the high cytotoxicity (IC_{50} 0.43 μM against human cervix carcinoma KB-3-1 cells) and marginal antiplasmodial activity (IC_{50} = 8.01 μg/mL against chloroquine-resistant (W2) strain) of knipholone, two new knipholone derivatives (**163** and **164)** were prepared using catalyst-free Mannich-type reaction (Scheme 4.3 and 4.4) (Zhao *et al*., 2013). This is because Mannich derivatives commonly show better activity, solubility and bioavailability than the corresponding parent molecules (Subramaniapillai, 2013). The reaction involves a C–C bond formation where knipholone as the active hydrogen atom-containing molecule reacts with formaldehyde and an amine derivative with elimination of water. The use of the secondary amine, diethylamine, allowed the modification of acetylphloroglucinol part of knipholone at C-5' (Scheme 4.3) into knipholone Mannich base (**163**). In another reaction, the presence of the primary amine, aniline, the Mannich base initially formed condensed, involving the vicinal hydroxyl group at C-6' with a second formaldehyde molecule through an imidazole cyclization reaction, forming knipholone-1,3-oxazine (**164**, Scheme 4.4). All the spectroscopic data (refer to

section 3.6) of these compounds were in agreement with the proposed structures (Abdissa *et al*., 2014a).



Knipholone (14) **163**

Scheme 4.3: Partial synthesis of knipholone Mannich base (**163**)



Knipholone (14) **164**

Scheme 4.4: Partial synthesis of knipholone-1,3-oxazine (**164**)

When tested for *in vitro* antiplasmodial activity and cytotoxicity, an improvement in the *in vitro* antiplasmodial activity (Table 4.19) against the chloroquine-resistant (W2) strain and remarkable decrease in the cytotoxicity (Table 4.19) against human cervix carcinoma KB-3-1 cell was observed for these knipholone derivatives (**163** and **164**) as compared to the parent phenylanthraquinone

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5. 1 Conclusions

From the roots of *Kniphofia foliosa* two new compounds, 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) and knipholone cyclooxanthrone (**134**) along with dianellin (**136**) and eight other known compounds were isolated. The isolation of the rare naphthalene glycoside, dianellin (**136**) is the first report of its occurrence from the family Asphodelaceae. The isolation of phenylanthraquinones including the unprecedented knipholone cyclooxanthrone supports the placement of *Kniphofia* in the Asphodeloideae sub-family along with *Bulbine*.

Phytochemical investigation of *Bulbine frutescens* led to the isolation of nine compounds. Two of these: 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**) and 6',8-*O*-dimethylknipholone (**150**) are new compounds and one, 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**) with the octaketide chain folded in the unusual way as in aloesaponarin II, is new to the sub-family Asphodeloideae.

From the roots of *Aloe dawei* seventeen (seven naphthoquinones and ten anthraquinones) compounds were isolated. One of these, the naphthoquinone, 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**) is a new compound. This is the second report on the occurrence of a naphthoquinones from the genus *Aloe*.

The leaves of *Aloe graminicola* led to the isolation of thirteen compounds (nine anthraquinones, three furancarboxaldehydes, and a chromone). The occurance of furancarboxaldehydes; 5-hydroxymethyl-2-furancarboxaldehyde **(159**), 5-acetyloxymethyl-2-furancarboxaldehyde **(160)** and cirsiumaldehyde **(161**) is the first report from the family Asphodelaceae.

In-vitro antiplasmodial evaluation reveal that the dimeric anthraquinones; 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) and chryslandicin (**13**) are exellent antiplasmodial agents against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum.* This supports the assertion that dimeric anthraquinones may constitute a new class of antimalarial lead structures.

The isolates from the roots of *A. dawei* were also assayed for activity against the MDA-MB-231 (ER negative) and MCF-7 (ER positive) breast cancer cell lines. Two of the quinones, 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) and chrysophanol-8-methyl ether (**156**), showed strong and selective cytotoxicity against MCF-7 breast cancer cells.

The two semi-synthetic knipholone derivatives (**163** and **164**) showed improvement in the *in vitro* antiplasmodial activity against the D6 and W2 strains and lower cytotoxicity against human cervix carcinoma KB-3-1 cell compared to the parent molecule.

5.2 Recommendations

1) Further phytochemical investigation of the roots of *B. frutescens* and *K. foliosa* should be done under inert condition to isolate unstable knipholone anthrone-type compounds. This may result in the identification of novel and more active compounds.
2) The absolute configuration of the two new compounds, 10-methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**) at stereogenic center (C-10) and knipholone cyclooxanthrone (**134**) with an axis of chirality at C4-C1' and a center chirality at C-10 should be resolved. Due to the cyclization involving C-10 and C-6', there are only two possible isomers for **134**, *vis-á-vis* (4*M*, 10*S*) or (4*P*, 10*R*); to decide which of these is the absolute configuration for the compound. Advanced quantum chemical CD calculations based on time-dependent DFT (TDDFT) and multireference configurational interaction (DFT/MRCI) approaches may be used.
3) Considering that the dimeric anthraquinones showed good antiplasmodial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum,* it will be interesting to work on the combination of these compounds with standard first line drugs. It is also important to point out that the antiplasmodial and anticancer activities of phenylanthraquinones are related to the entire molecular array of the molecule including the biaryl axis. It will be worthwhile to test the pure enantiomeric forms of the compounds to determine the relationship between configuration and activity.
4) Considering that the genus *Jodrellia*, named after the Jodrell Laboratory in Kew, England was carved out from the genus *Bulbine* in 1978 (Baijnath, 1978) only based on the observation that the floral morphology of some of its species did not agree with those of *Bulbine* genus. The taxonomy of the species within the genus is still slightly confused

and phytochemical information on the genus is not available. It would be interesting to investigate the phytochemistry (particularly knipholone-type phenylanthraquinones) of the genus *Jodrellia* to establish its chemotaxonomic relationship with *Bulbine*.

5) The study has resulted in the isolation of seven 1,4-naphthoquinones from the roots of *A. dawei* (Abdissa *et al*., 2014b) and it is the second report of the occurrence of naphthoquinones from the genus *Aloe* following the report from *Aloe secundiflora* (Induli *et al*., 2013). The occurrence of these compounds in *A. dawei* of group 19 and *A. secundiflora* of group 14 is in support of the previous morphological and chemotaxonomic report that the two groups are related. It is worthwhile then to conduct directed chemotaxonomic survey of members of groups 5, 14, 16 and 19 for the presence of naphthoquinones since affinities have been observed among members of these taxa.

6) It is worth to test the naphthoquinone, 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**) and anthraquinone, chrysophanol-8-methyl ether (**156**) against different cell-lines including normal cell to determine the potential of these compounds as anticancer drug.

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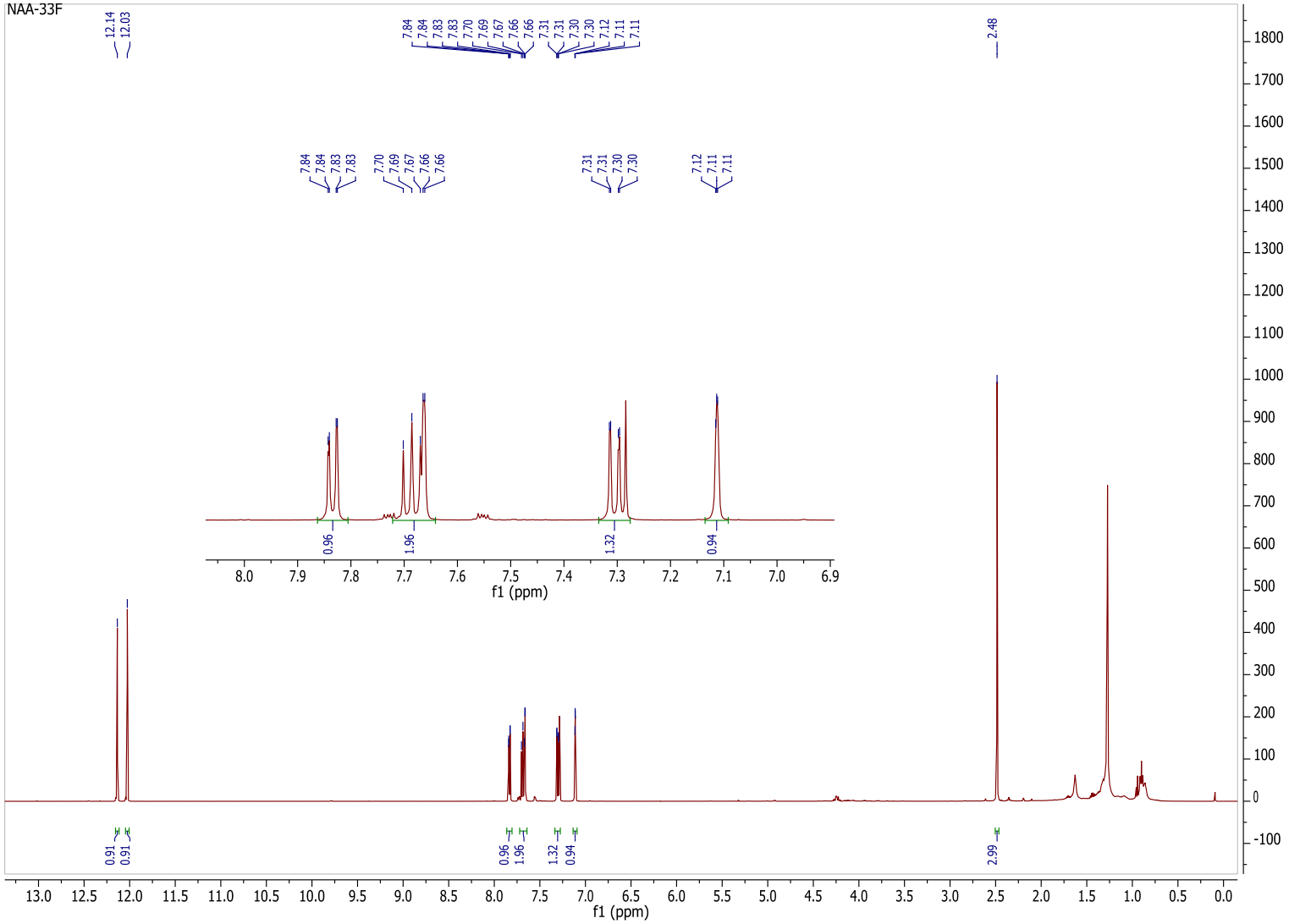
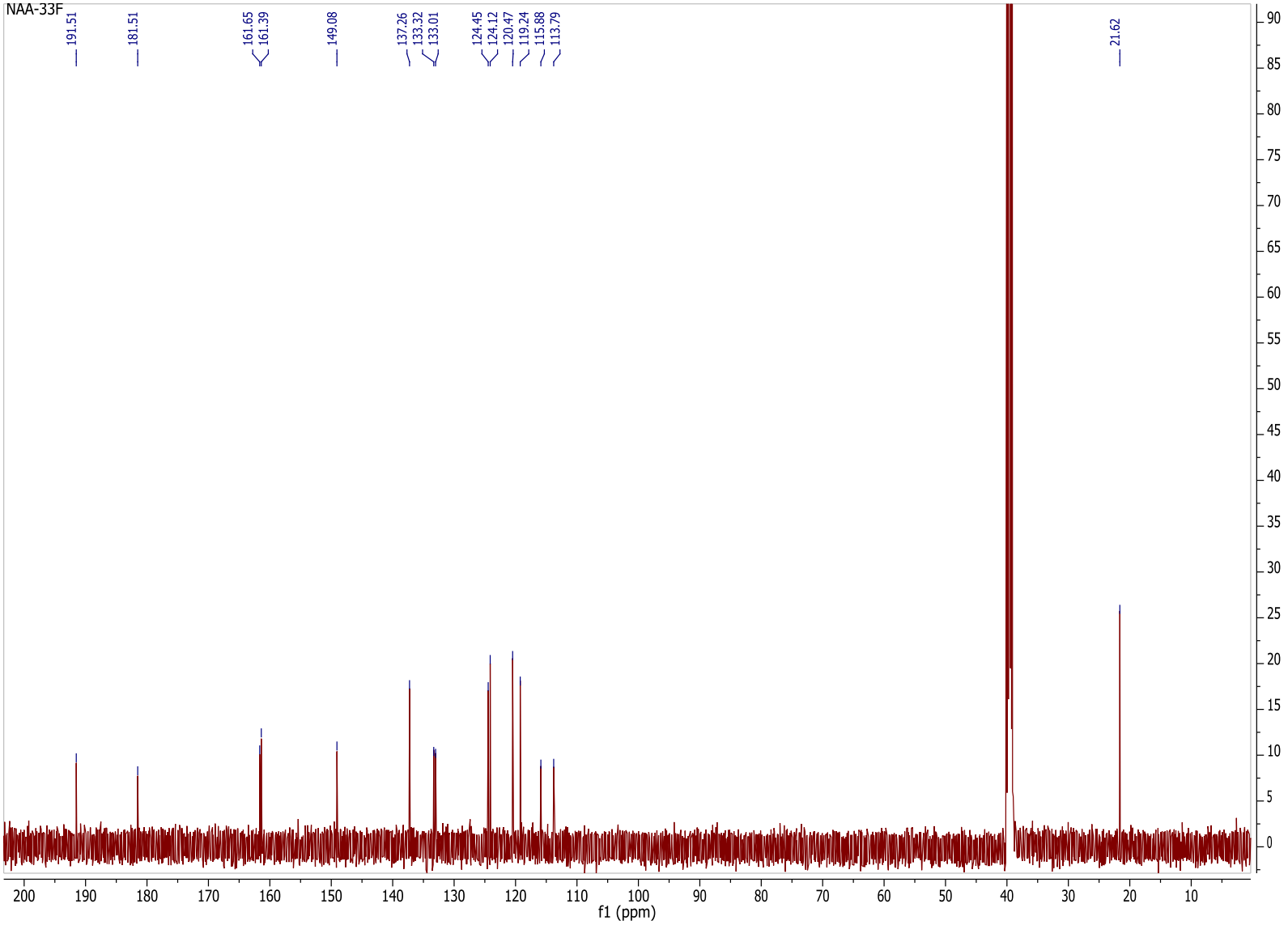
Appendixes

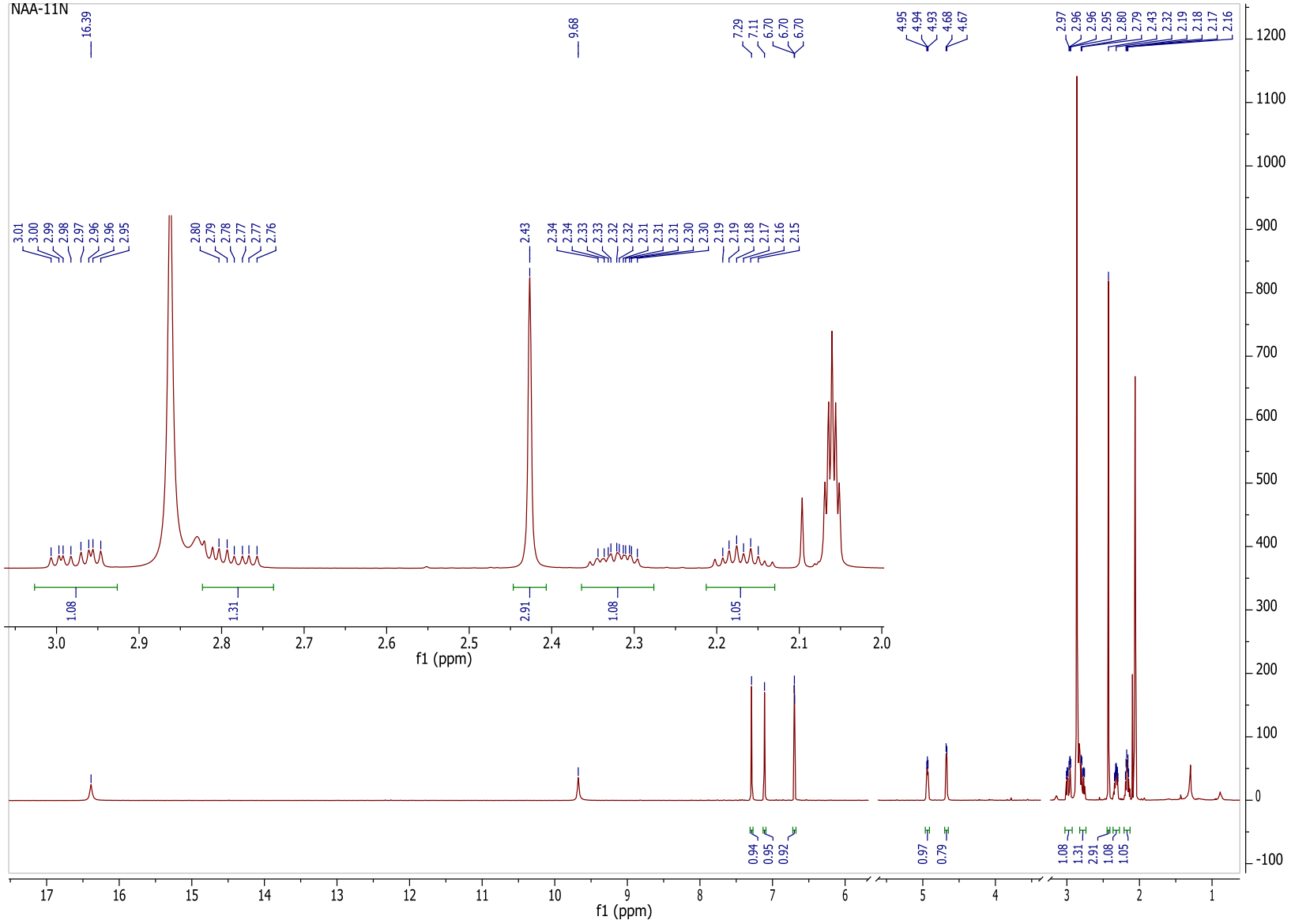
Appendix 1A: ^{1}H NMR spectrum of chrysophanol (**18**), $CDCl_3$, 500 MHz



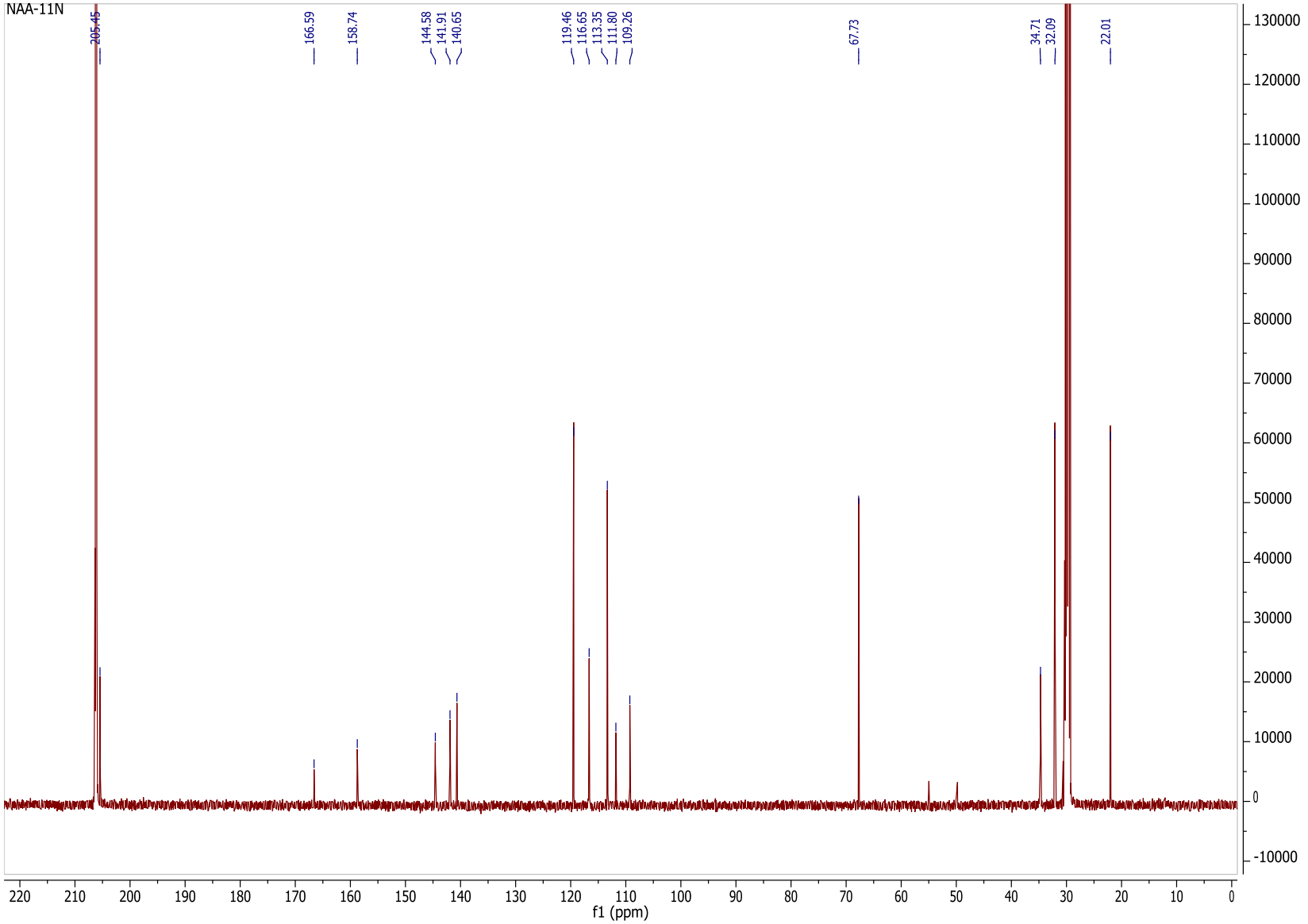
Appendix 1B: ^{13}C NMR spectrum of chrysophanol (**18**), DMSO-d_6, 126 MHz



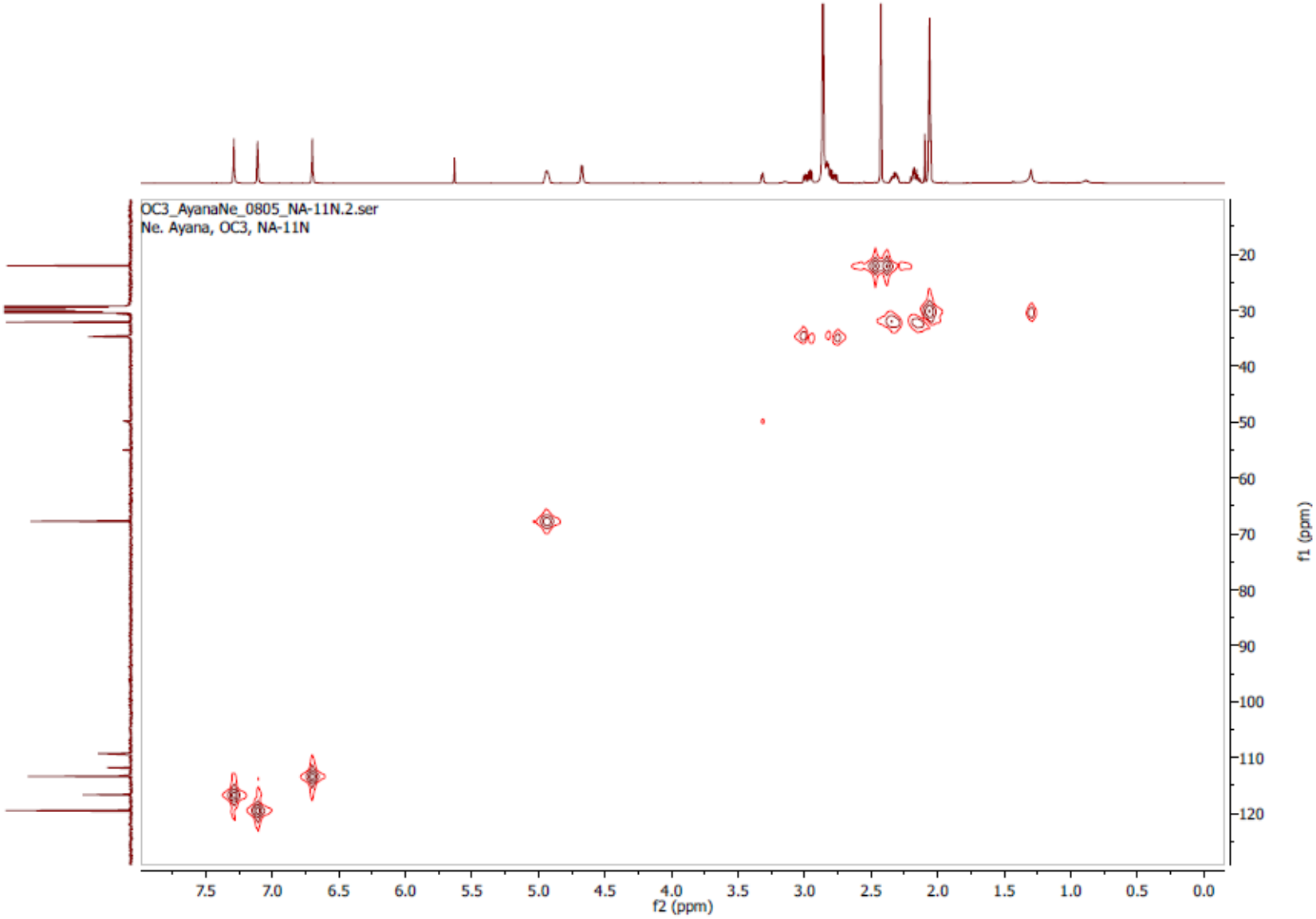
Appendix 2A: ^{1}H NMR spectrum of aloesaponol III (**16**), acetone-d_6, 500 MHz



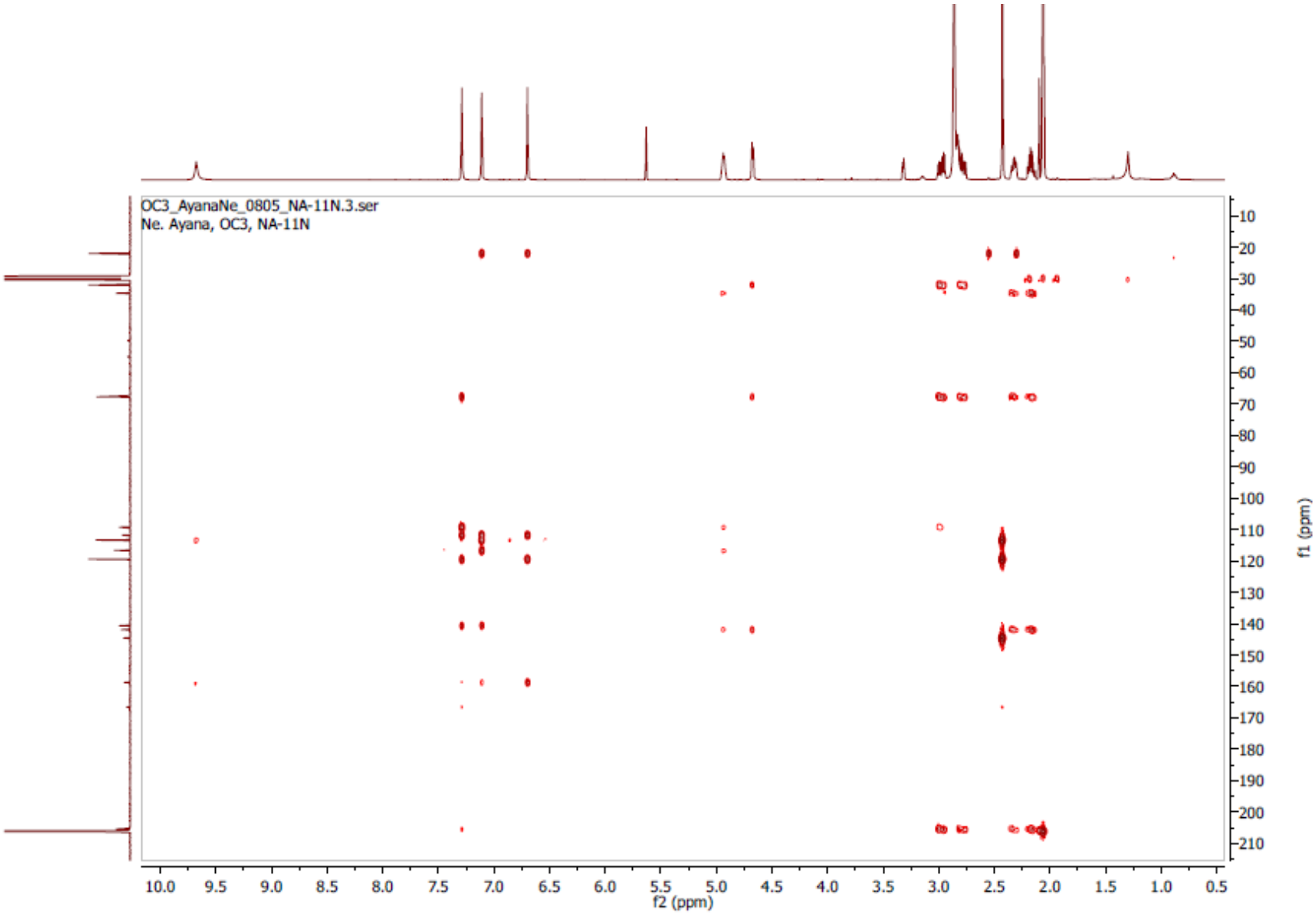
Appendix 2B: ^{13}C NMR spectrum of Aloesaponol III (**16**), acetone-d_6, 126 MHz



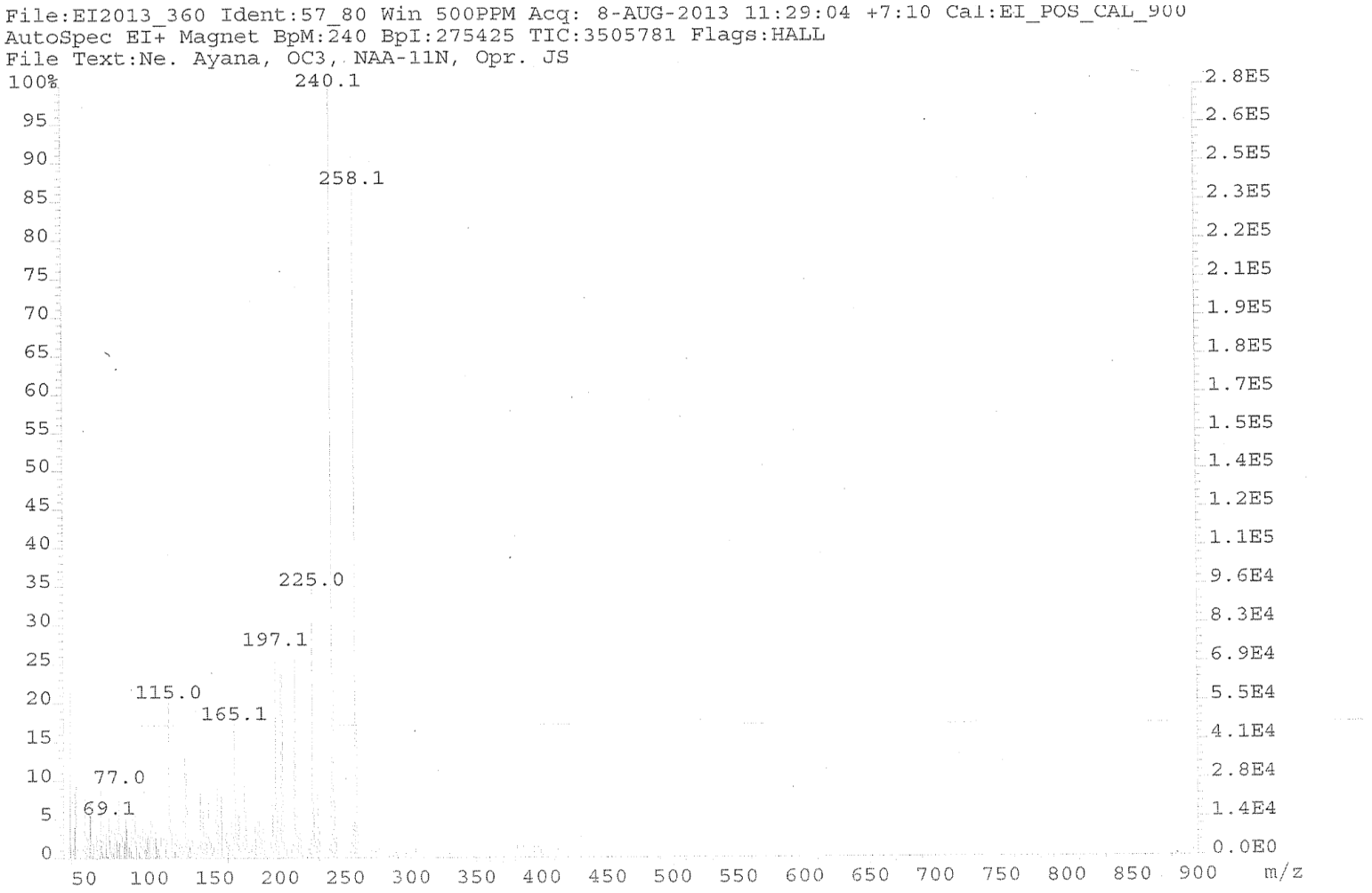
Appendix 2C: HSQC spectrum of Aloesaponol III (**16**), acetone-d_6, 500 MHz



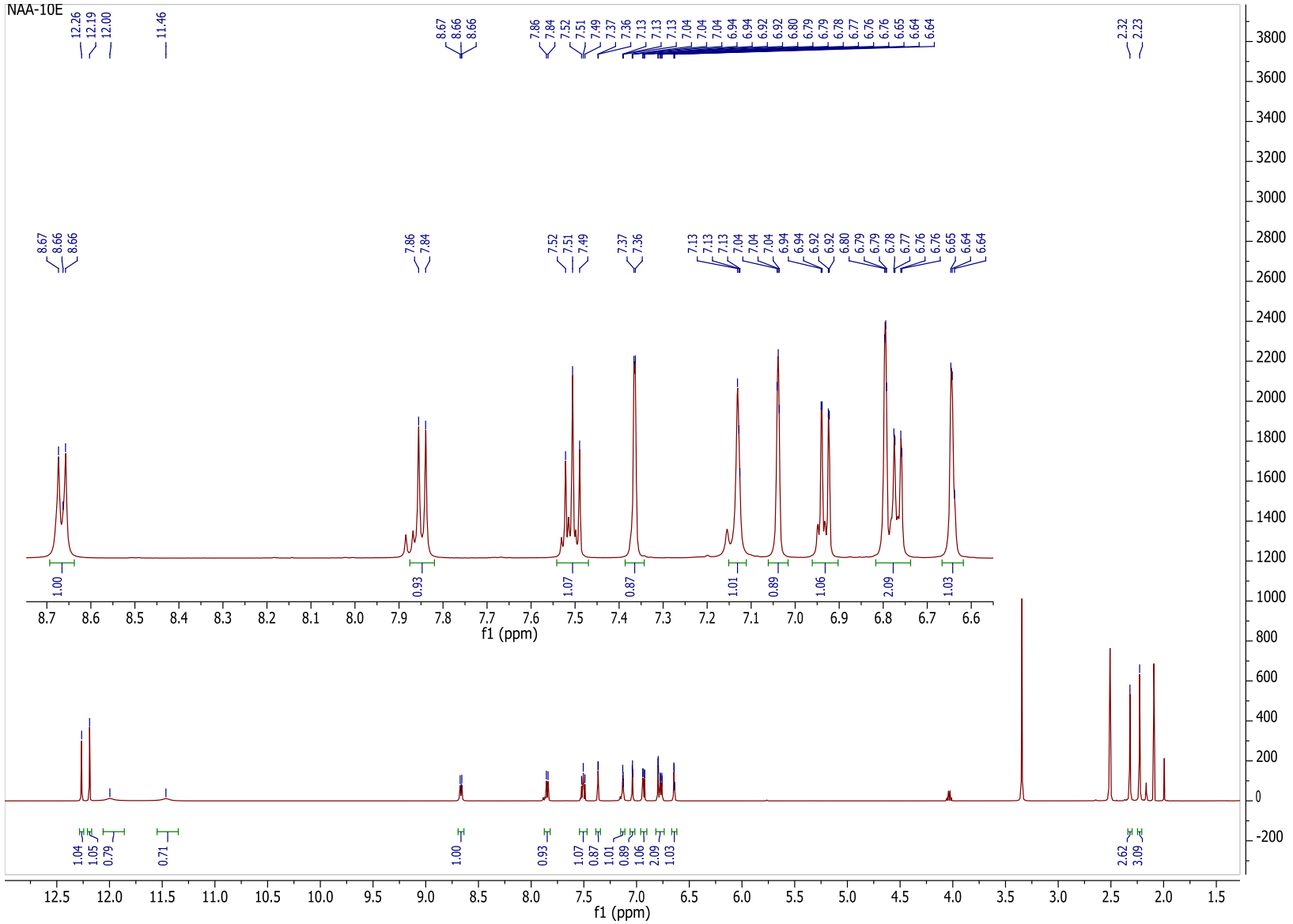
Appendix 2D: HMBC spectrum of aloesaponol III (**16**), acetone-d_6, 500 MHz



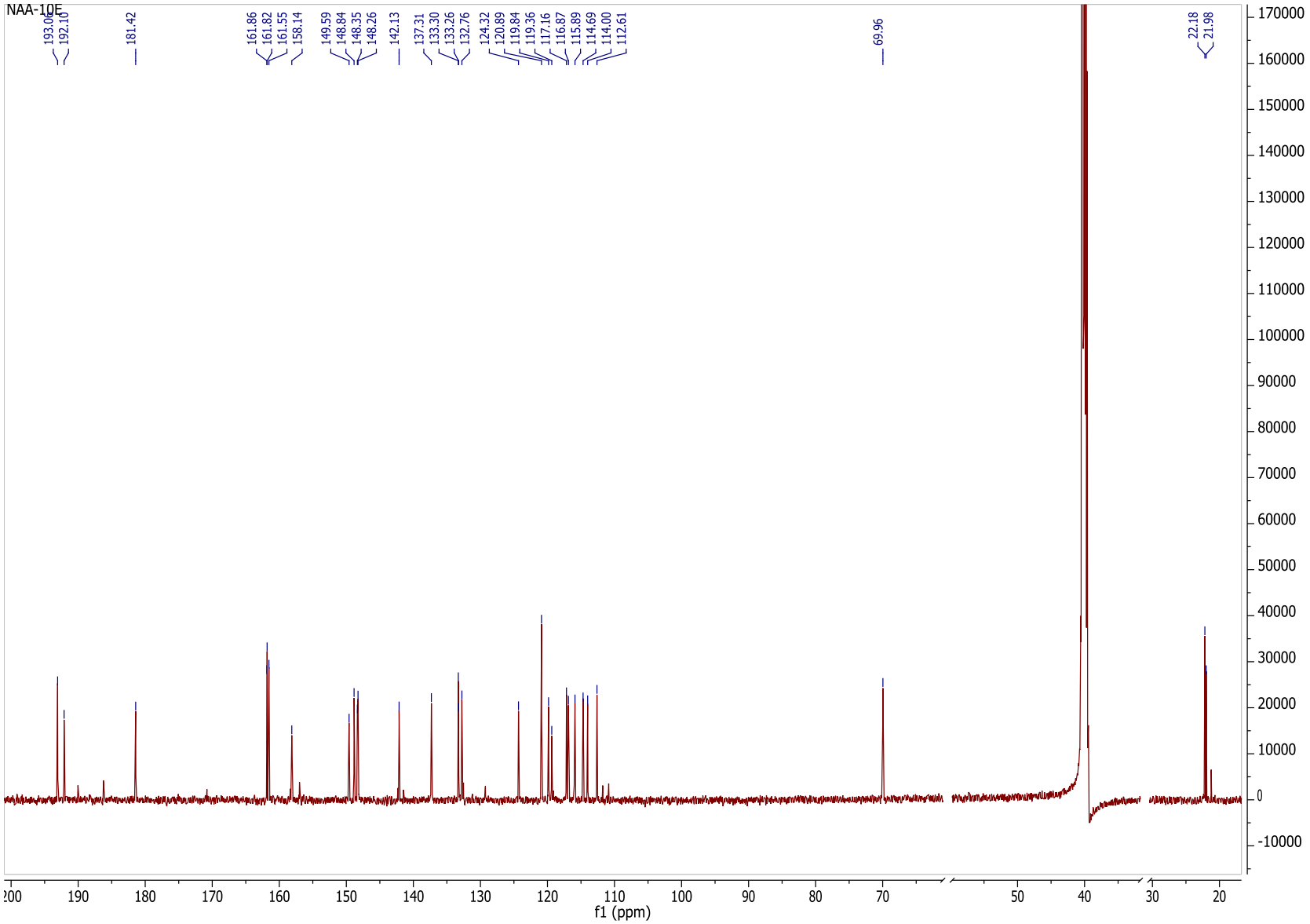
Appendix 2E: ESIMS spectrum of of aloesaponol III (**16**)



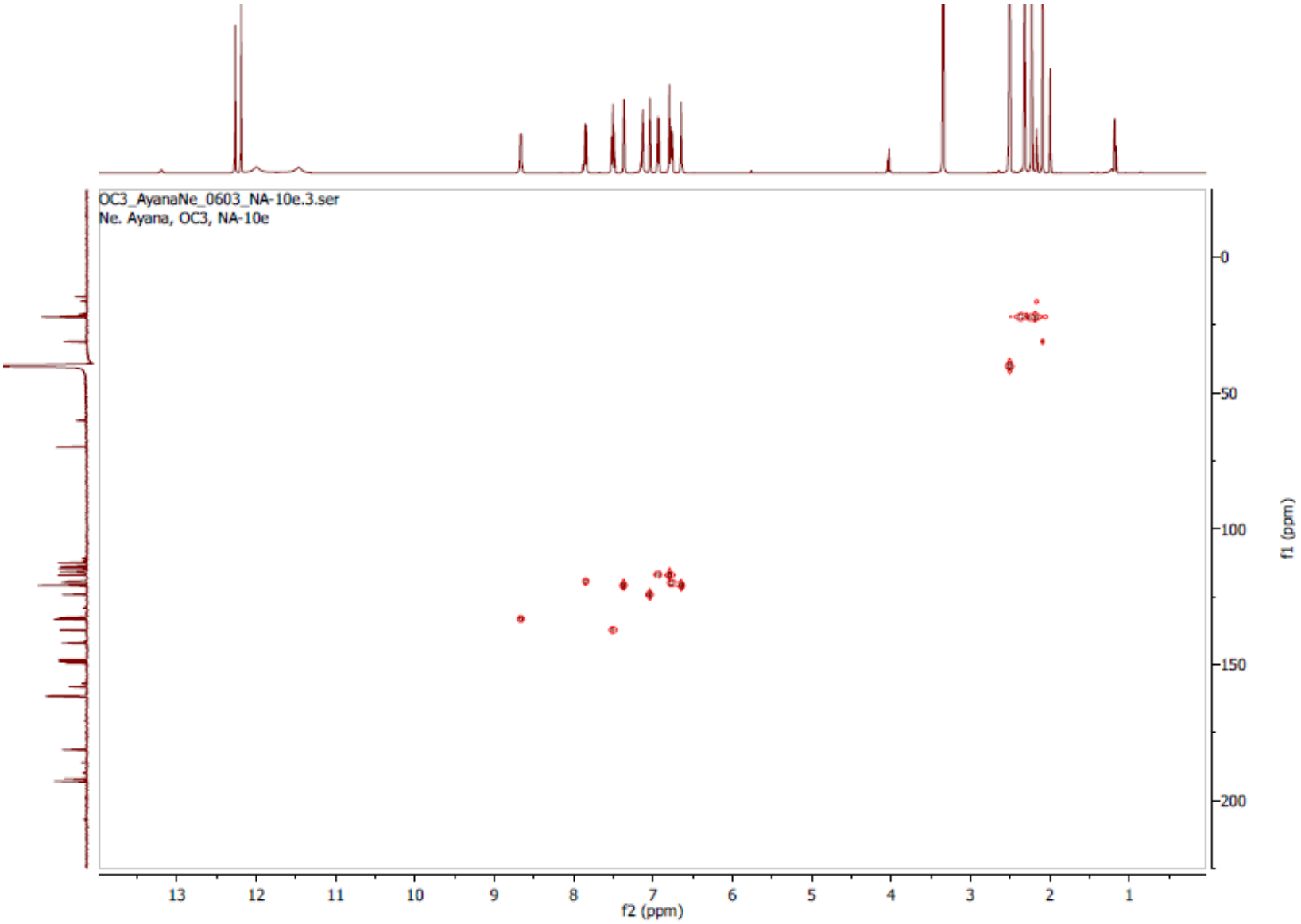
Appendix 3A: ^{1}H NMR spectrum of 10-hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), DMSO-d_6, 500 MHz



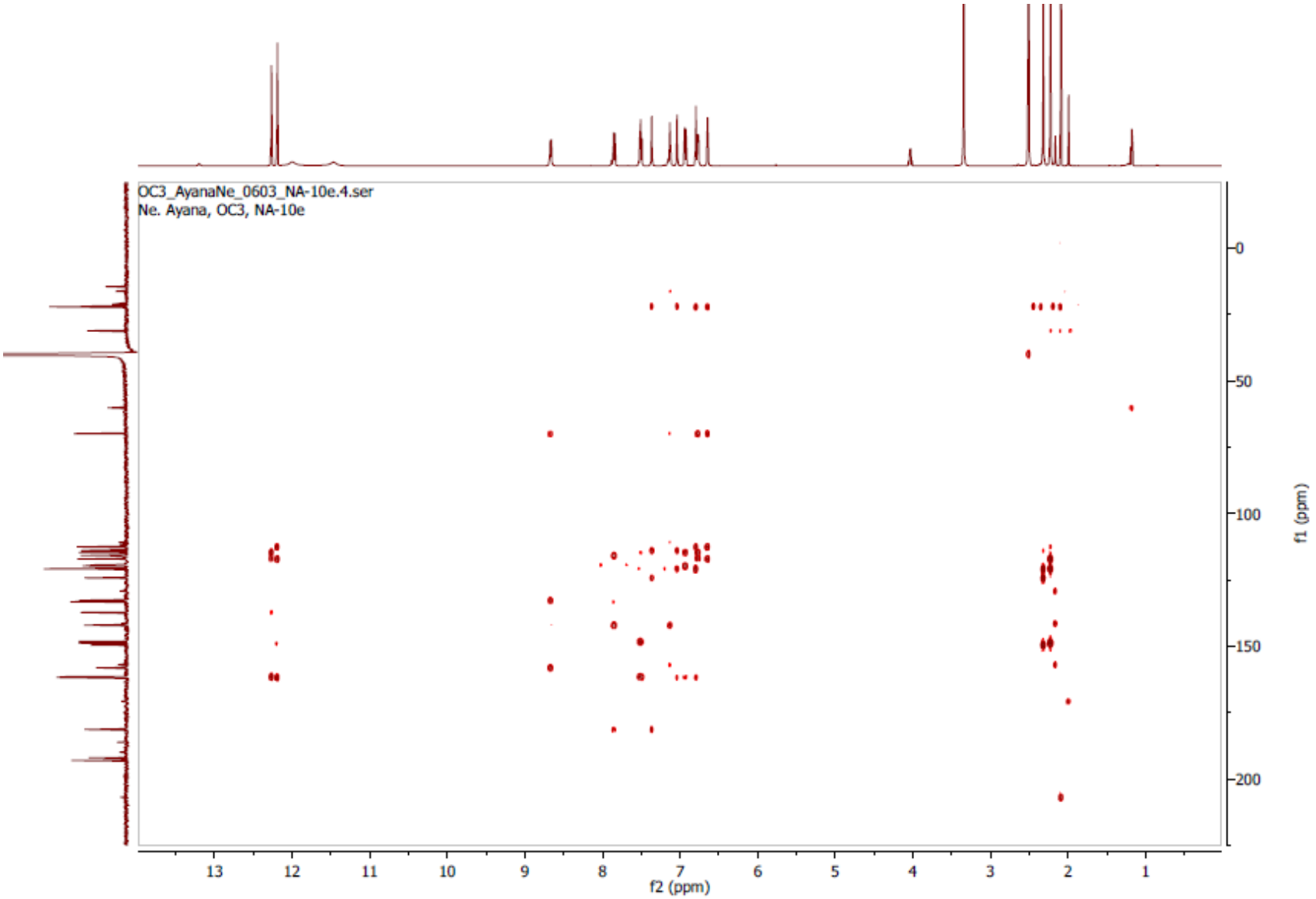
Appendix 3B: ^{13}C NMR spectrum of 10-Hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), DMSO-d_6, 126 MHz



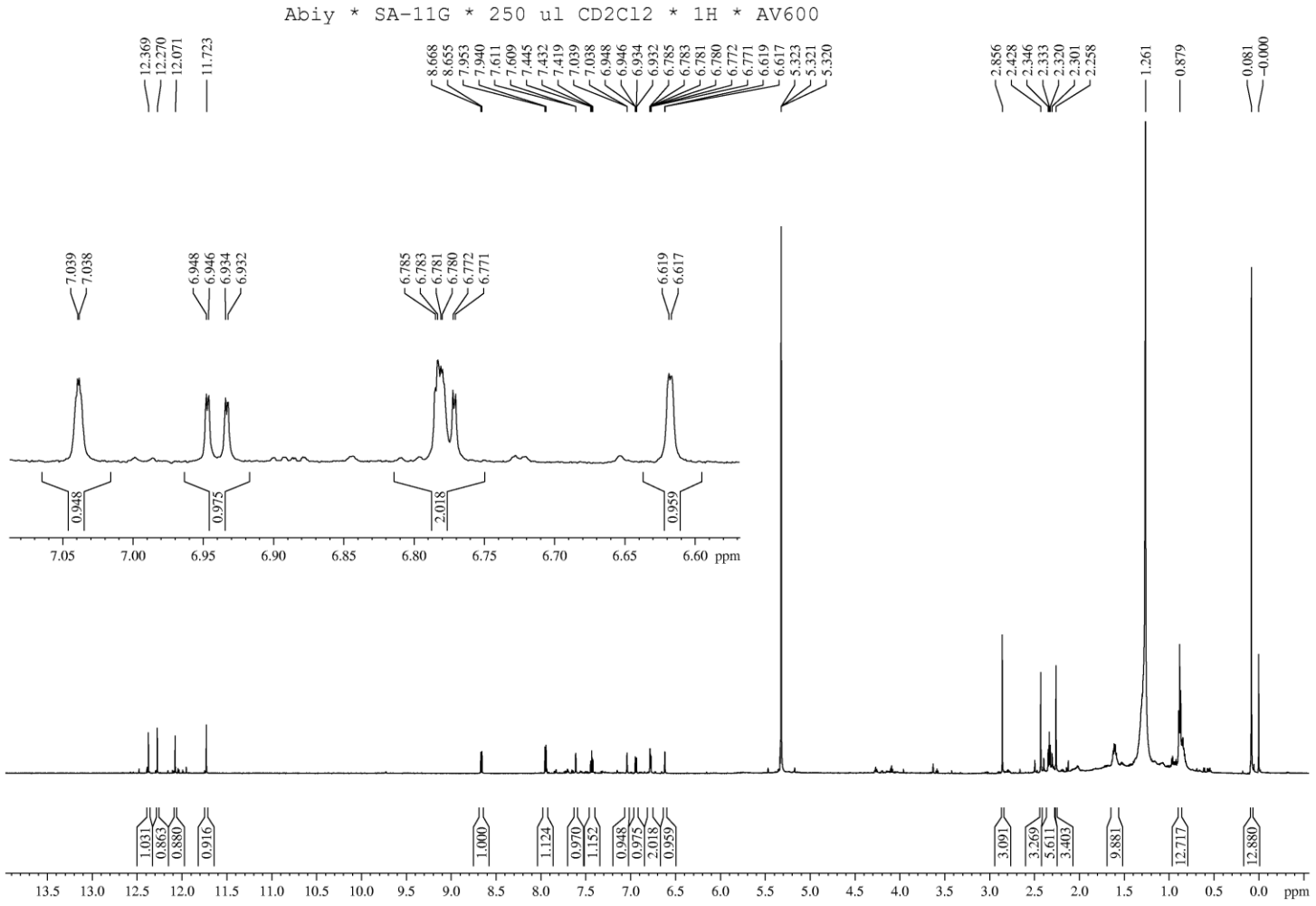
Appendix 3C: HSQC spectrum of 10-Hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), DMSO-d_6, 500 MHz



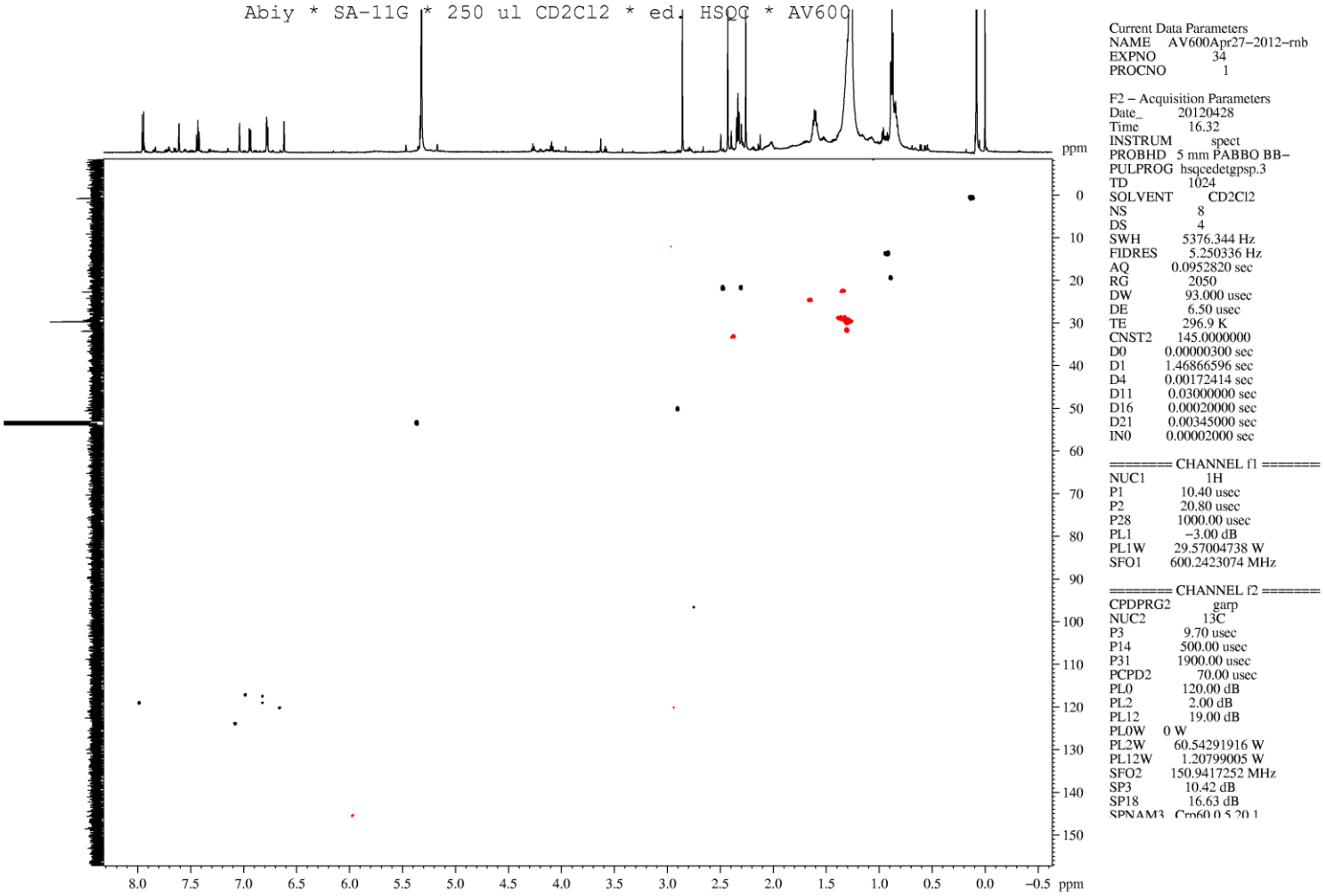
Appendix 3D: HMBC spectrum of 10-Hydroxy-10,7'-(chrysophanol anthrone)-chrysophanol (**132**), DMSO-d_6, 500 MHz



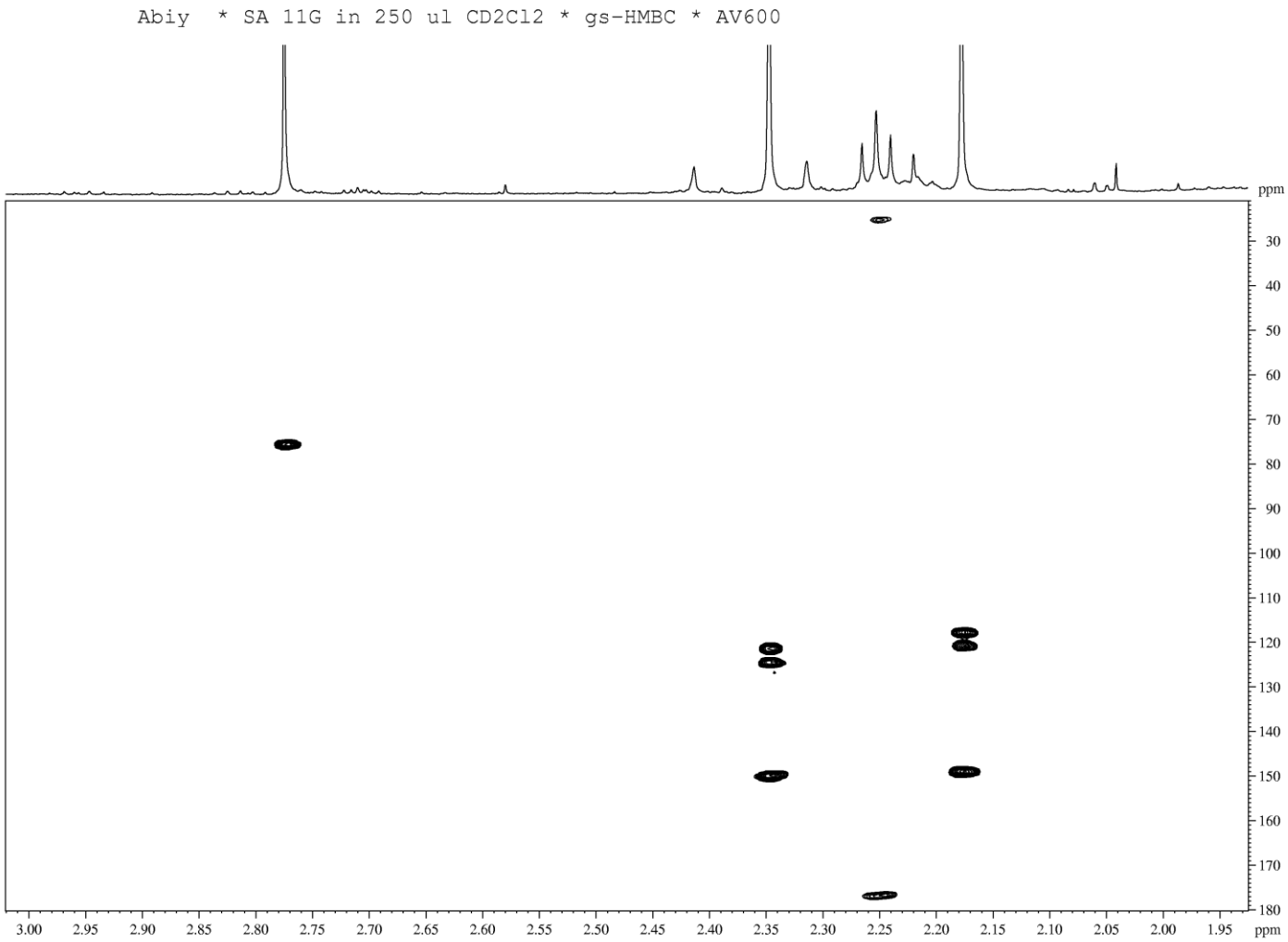
Appendix 4A: ^{1}H NMR spectrum of 10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**), CD_2Cl_2, 600 MHz



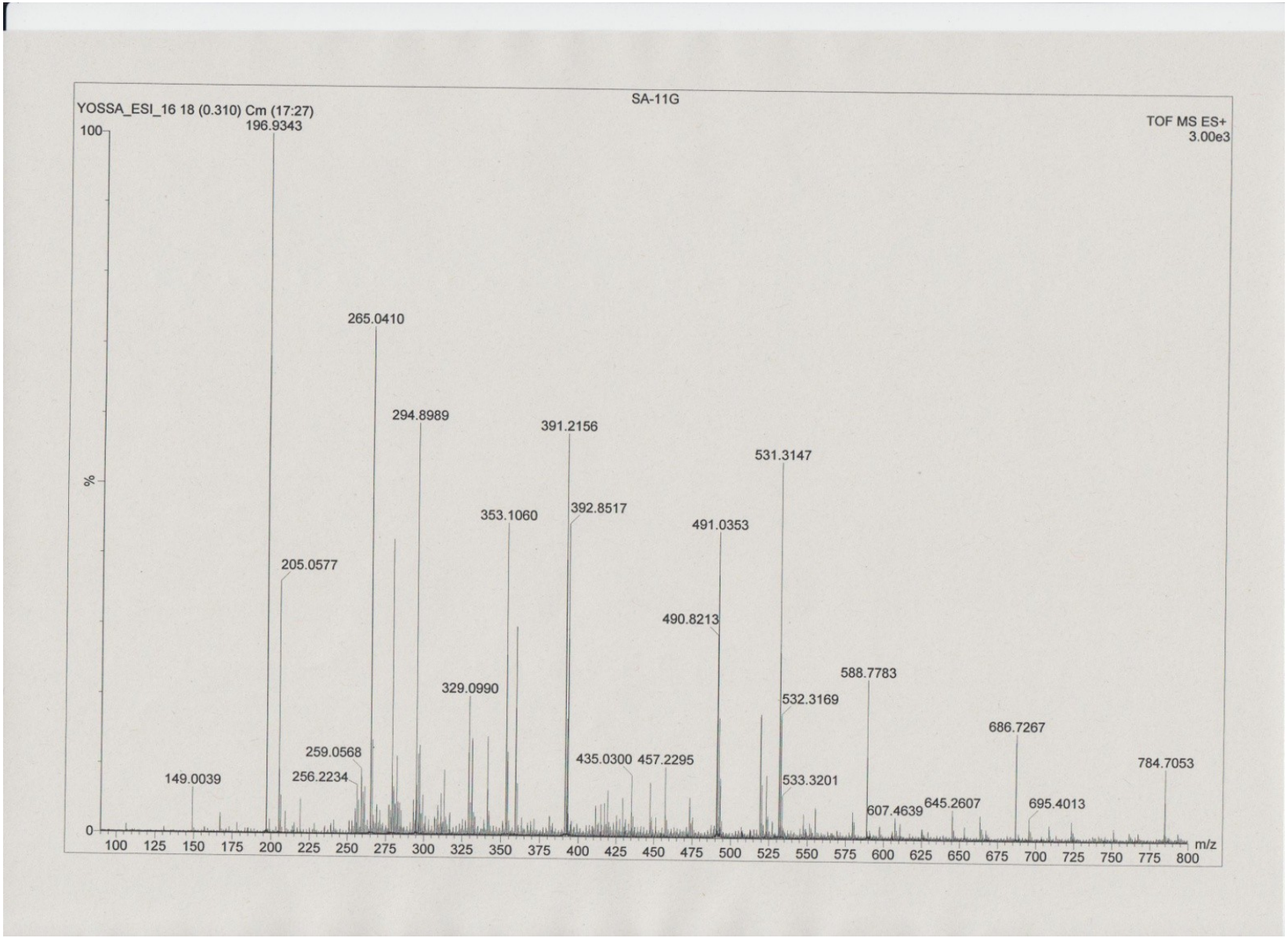
Appendix 4B: HSQC spectrum of 10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**), CD_2Cl_2, 600 MHz



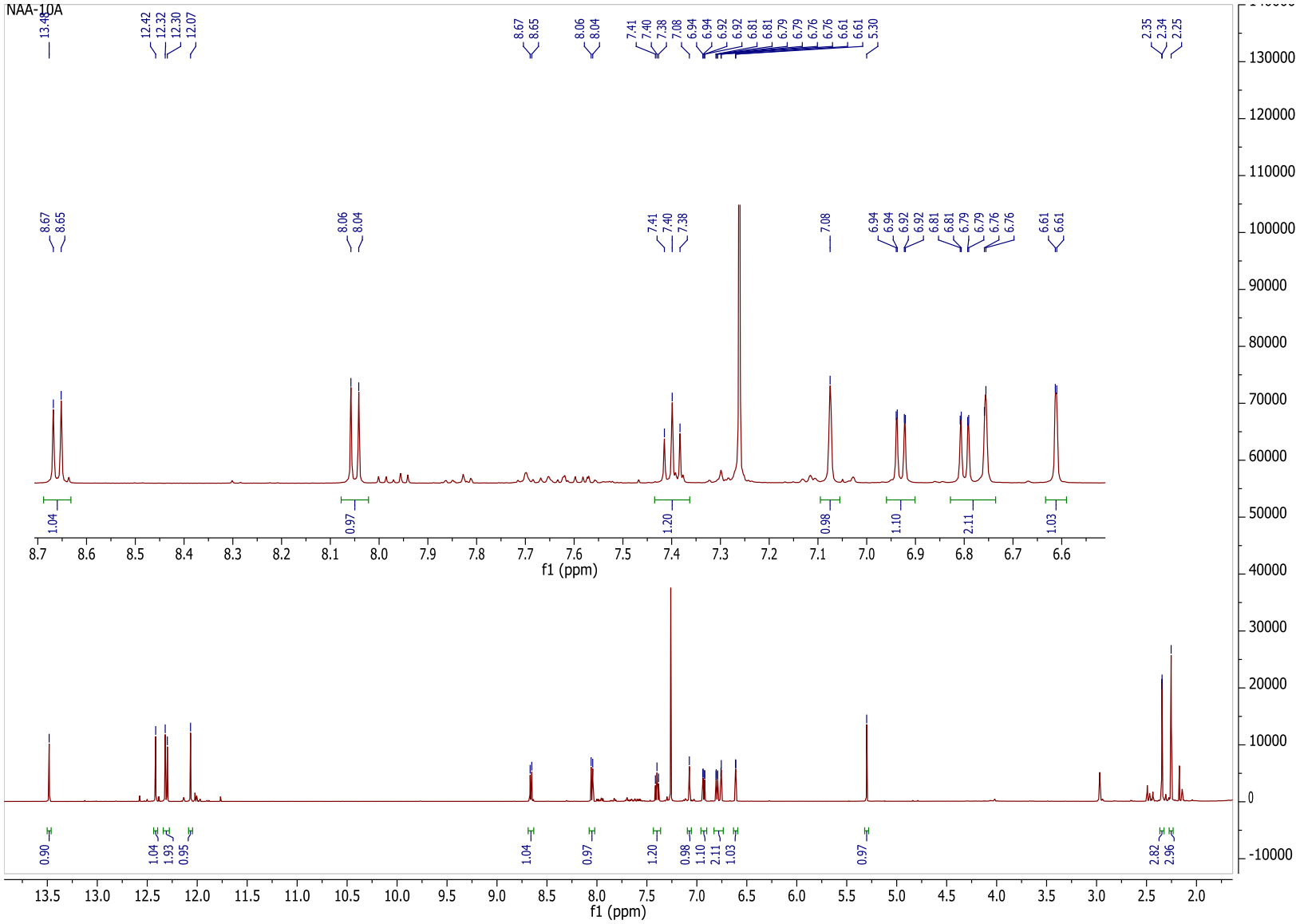
Appendix 4C: HMBC spectrum of 10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**), CD_2Cl_2, 600 MHz



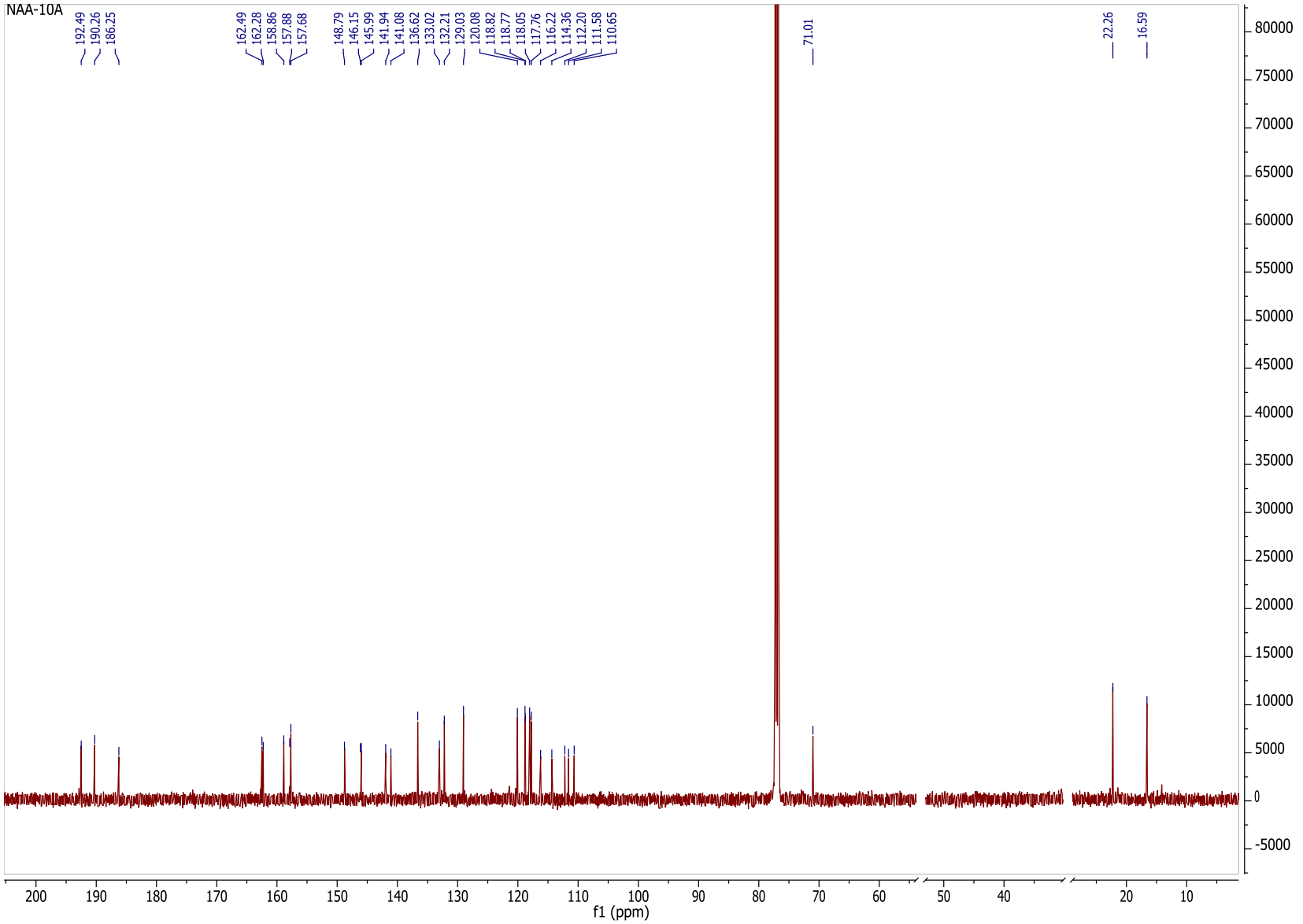
Appendix 4D: ESIMS spectrum of of 10-Methoxy-10,7'-(chrysophanol anthrone)-chrysophanol (**133**)



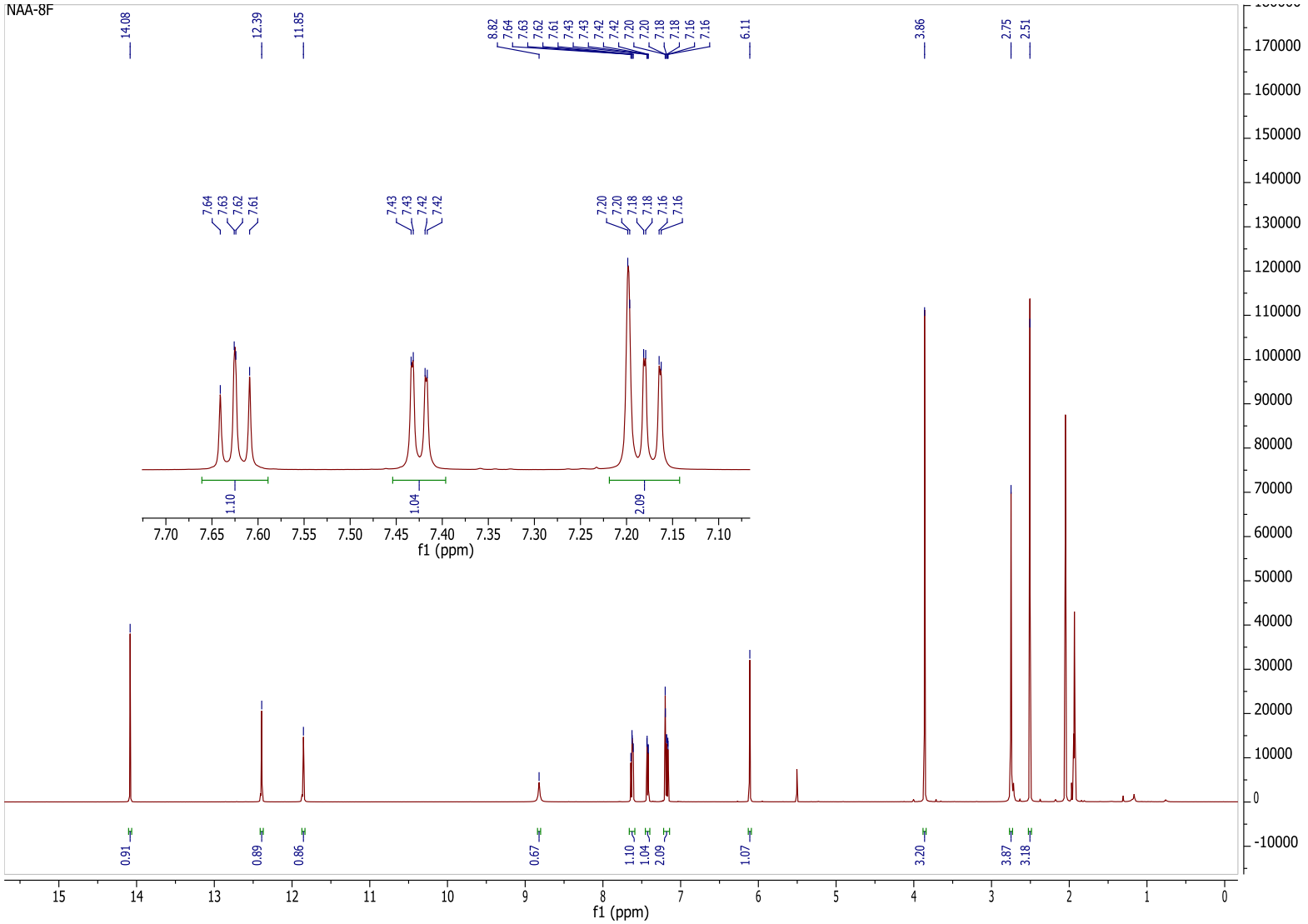
Appendix 5A: ^{1}H NMR spectrum of chryslandicin (**13**), $CDCl_3$, 500 MHz



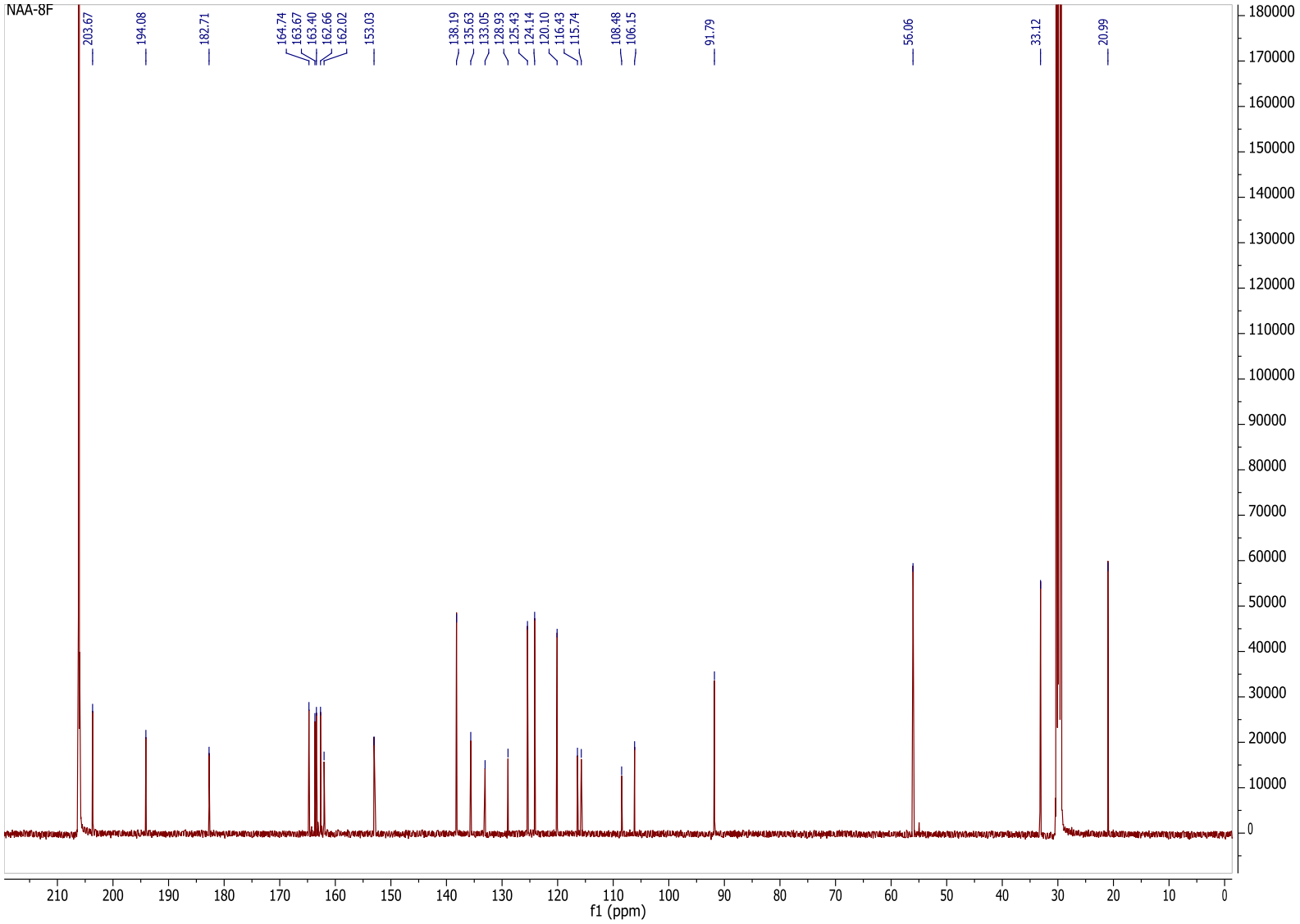
Appendix 5B: ^{13}C NMR spectrum of chryslandicin (**13**), $CDCl_3$, 126 MHz



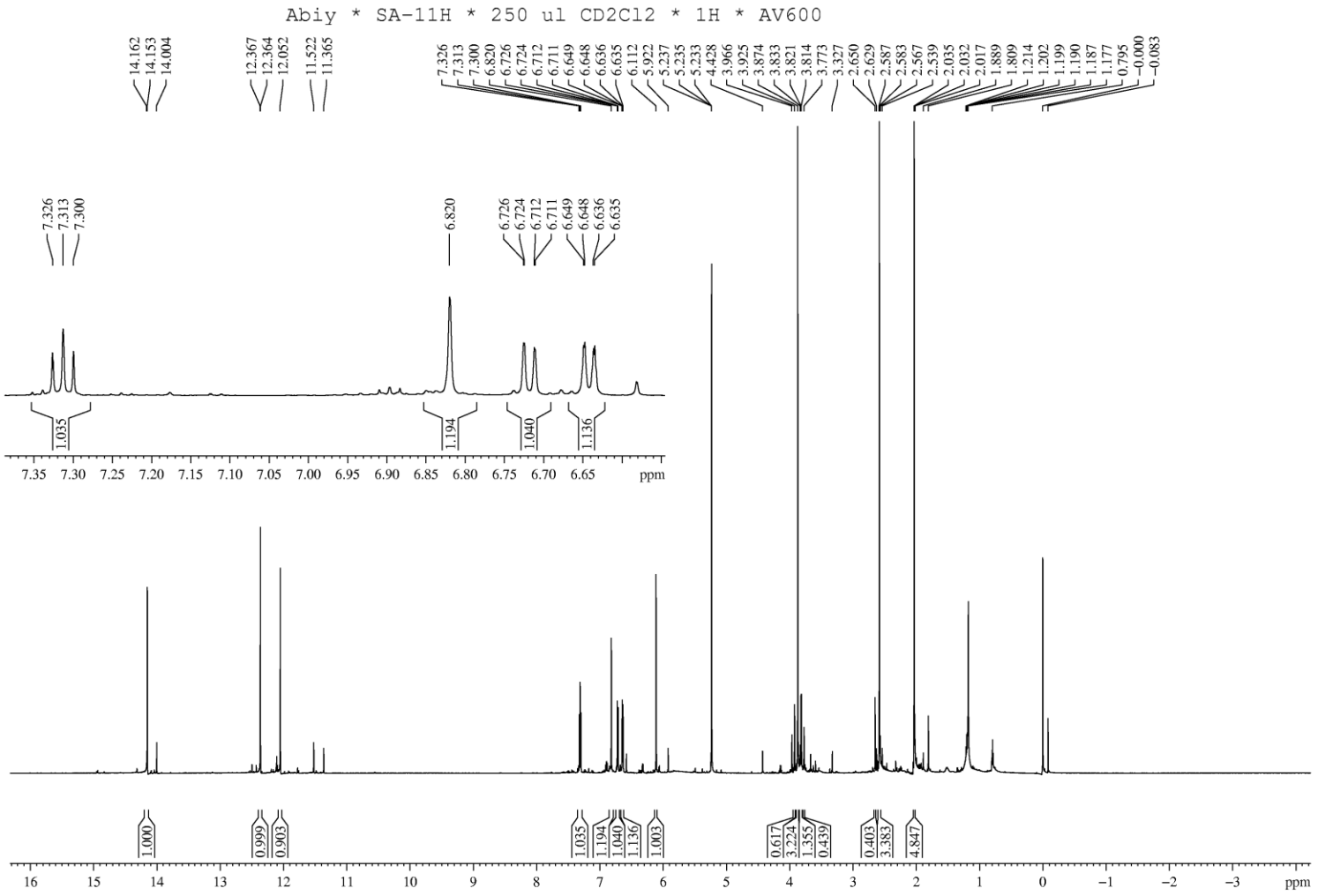
Appendix 6A: ^{1}H NMR spectrum of knipholone (**14**), acetone-d_6, 500 MHz



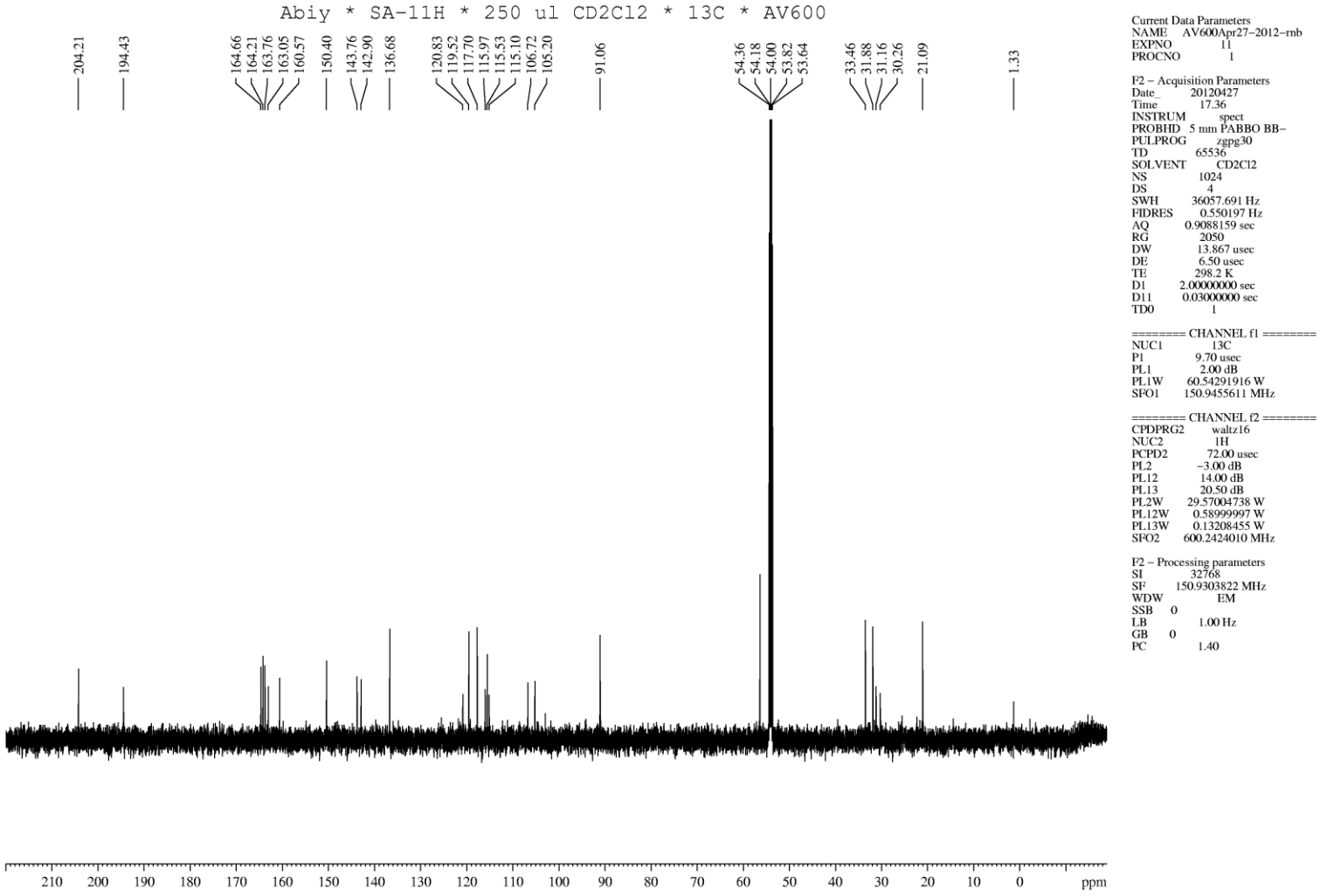
Appendix 6B: ^{13}C NMR spectrum of knipholone (**14**), acetone-d_6, 126 MHz



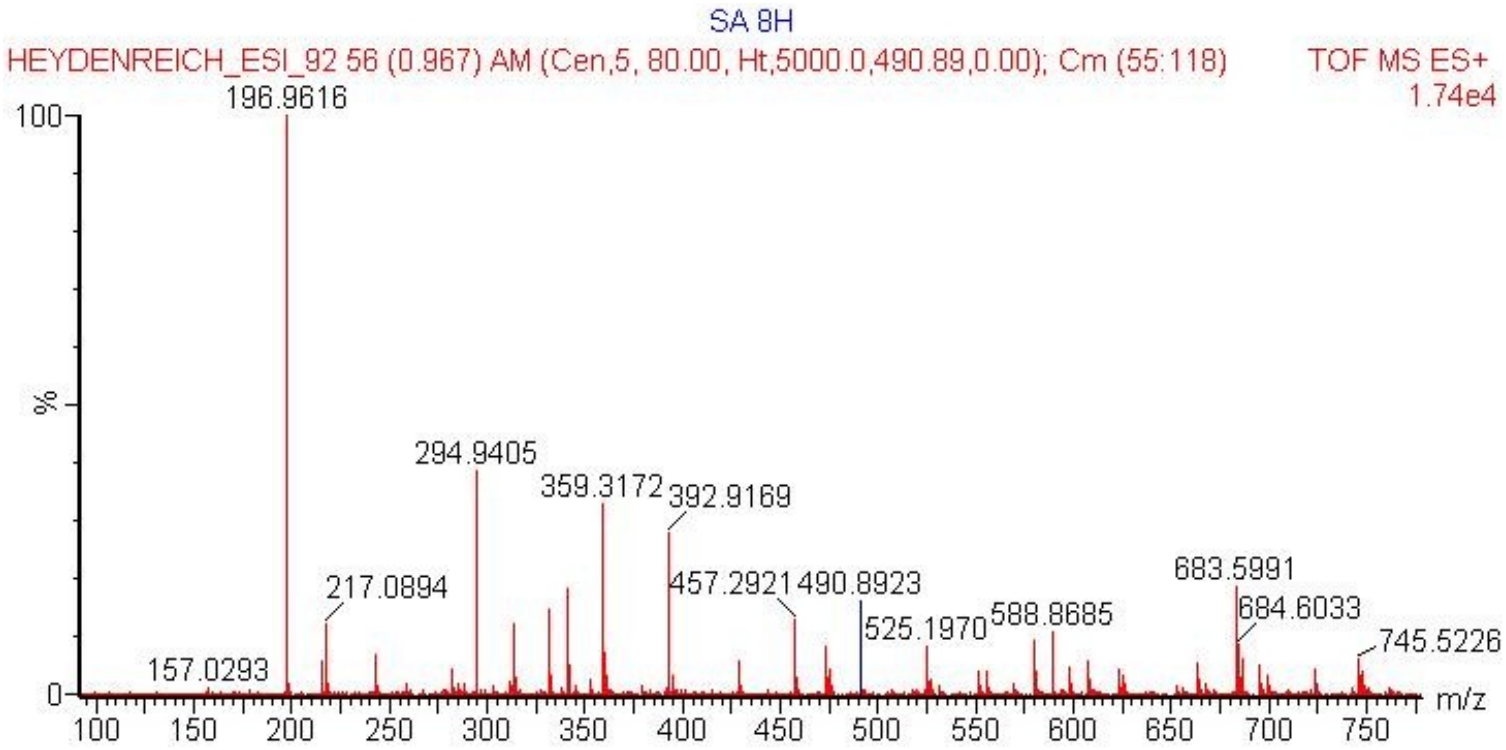
Appendix 7A: ^{1}H NMR spectrum of knipholone anthrone (**15**), CD_2Cl_2, 600 MHz



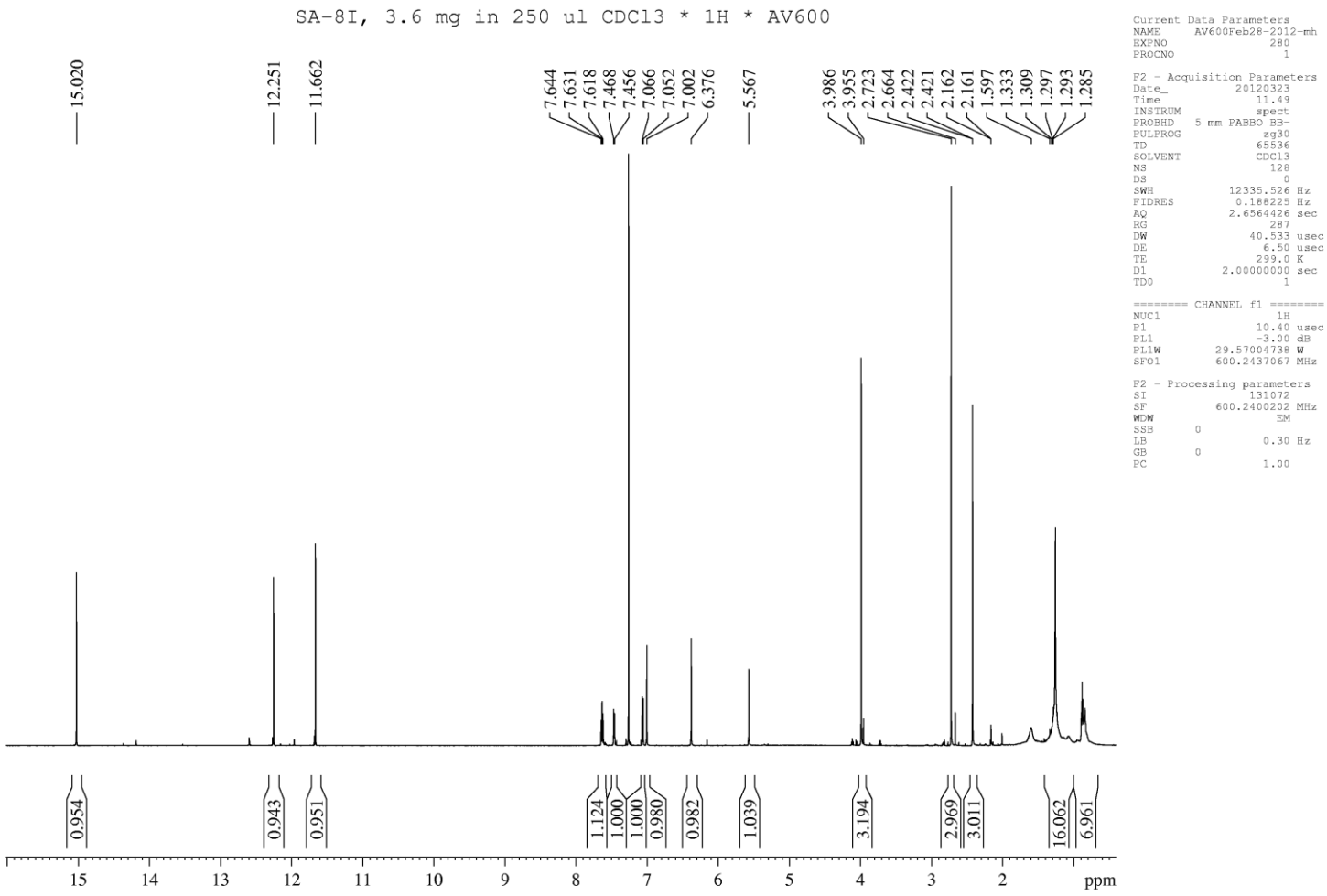
Appendix 7B: ^{13}C NMR spectrum of knipholone anthrone (**15**), CD_2Cl_2, 200 MHz



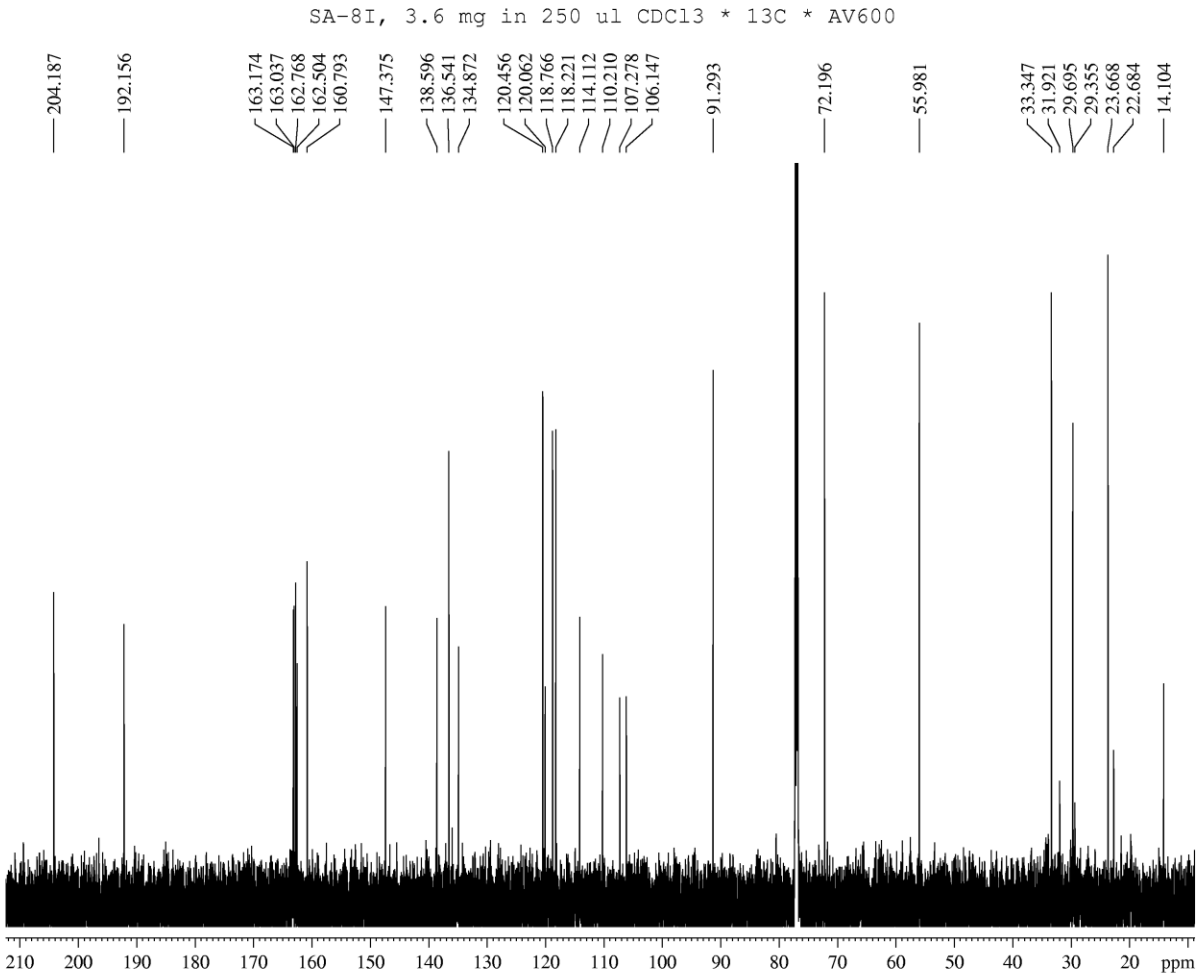
Appendix 7C: ESIMS spectrum of knipholone anthrone (**15**)



Appendix 8A: ^{1}H NMR spectrum of knipholone cyclooxanthrone (**134**), $CDCl_3$, 600 MHz



Appendix 8B: ^{13}C NMR spectrum of knipholone cyclooxanthrone (**134**), $CDCl_3$, 200 MHz

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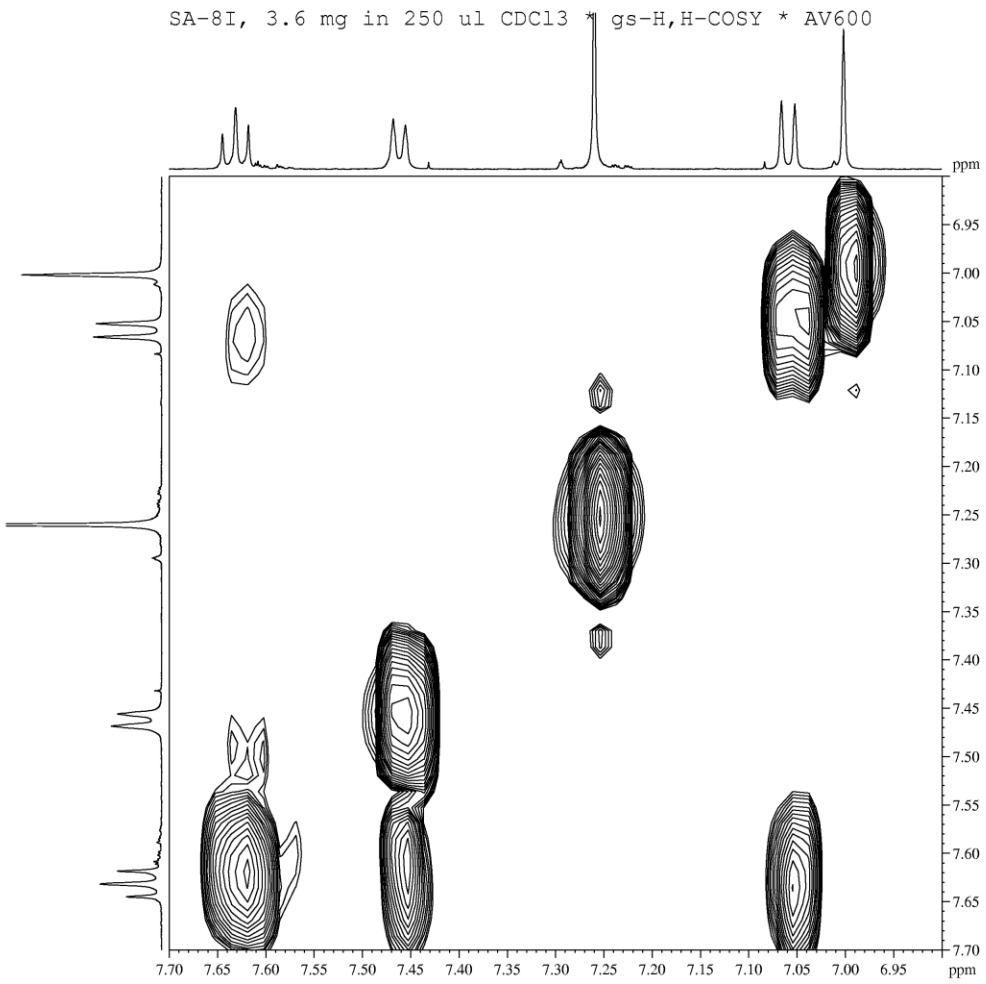
Appendix 8C: H,H-COSY spectrum of knipholone cyclooxanthrone (**134**), $CDCl_3$, 600 MHz

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SOLVENT              CDCl3
NS                         4
DS                         8
SWH               10204.082 Hz
FIDRES             4.982462 Hz
AQ                0.1004020 sec
RG                       203
DW                   49.000 usec
DE                    6.50 usec
TE                   299.0 K
D0              0.00000300 sec
D1              1.51433504 sec
D13             0.00000400 sec
D16             0.00020000 sec
IN0             0.00009800 sec

======== CHANNEL f1 ========
NUC1                     1H
P0                    10.40 usec
P1                    10.40 usec
PL1                   -3.00 dB
PL1W           29.57004738 W
SFO1          600.2445283 MHz

====== GRADIENT CHANNEL =====
GPNAM1             SINE.100
GPZ1                  10.00 %
P16                 1000.00 usec

F1 - Acquisition parameters
TD                       128
SFO1              600.2445 MHz
FIDRES           79.719391 Hz
SW                  17.000 ppm
FnMODE                   QF

F2 - Processing parameters
SI                      1024
SF             600.2400202 MHz
WDW                     SINE
SSB       0
LB        0 Hz
GB        0
PC                      1.40

F1 - Processing parameters
SI                      1024
MC2                       QF
SF             600.2400202 MHz
WDW
SSB       0
LB        0 Hz
GB        0
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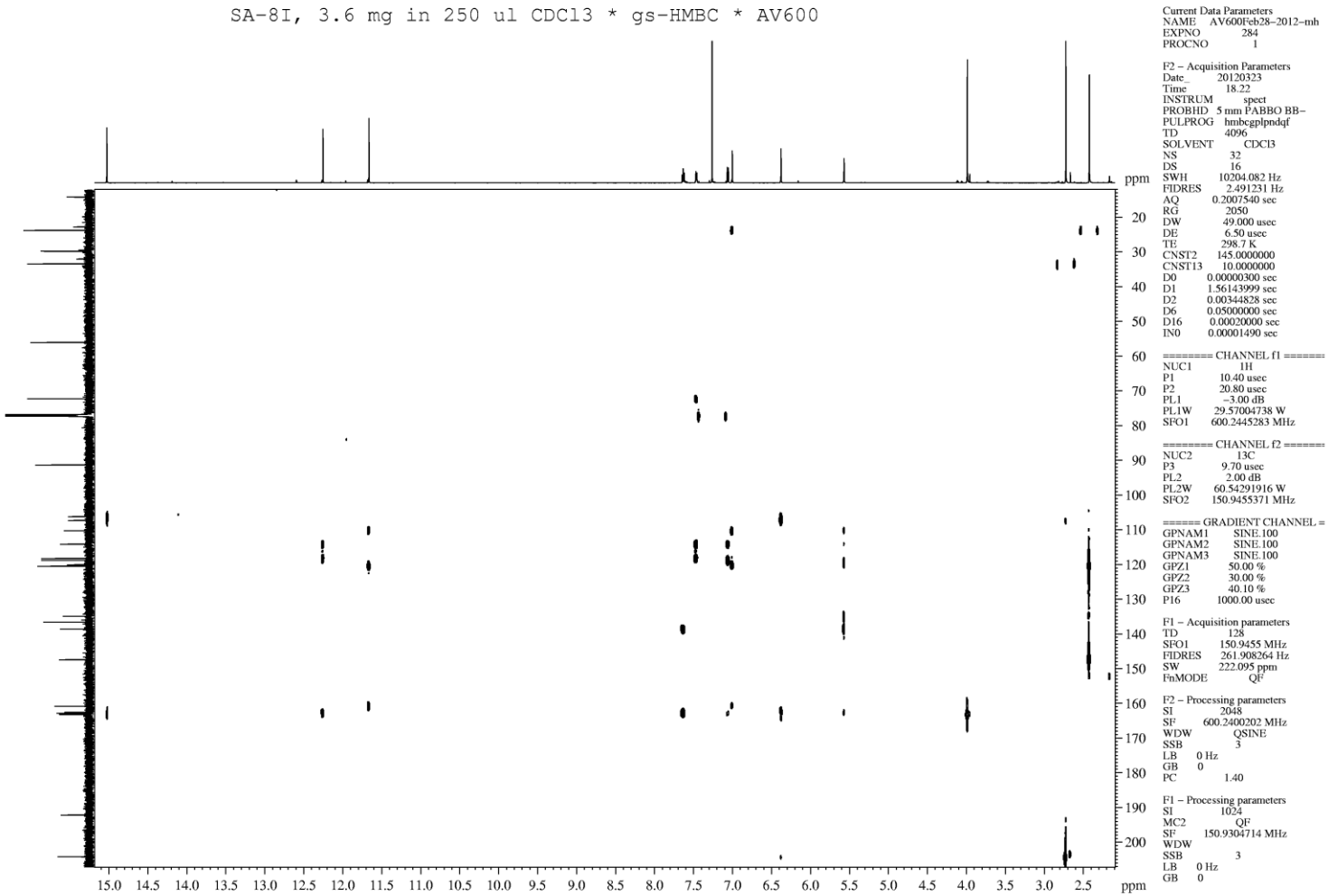
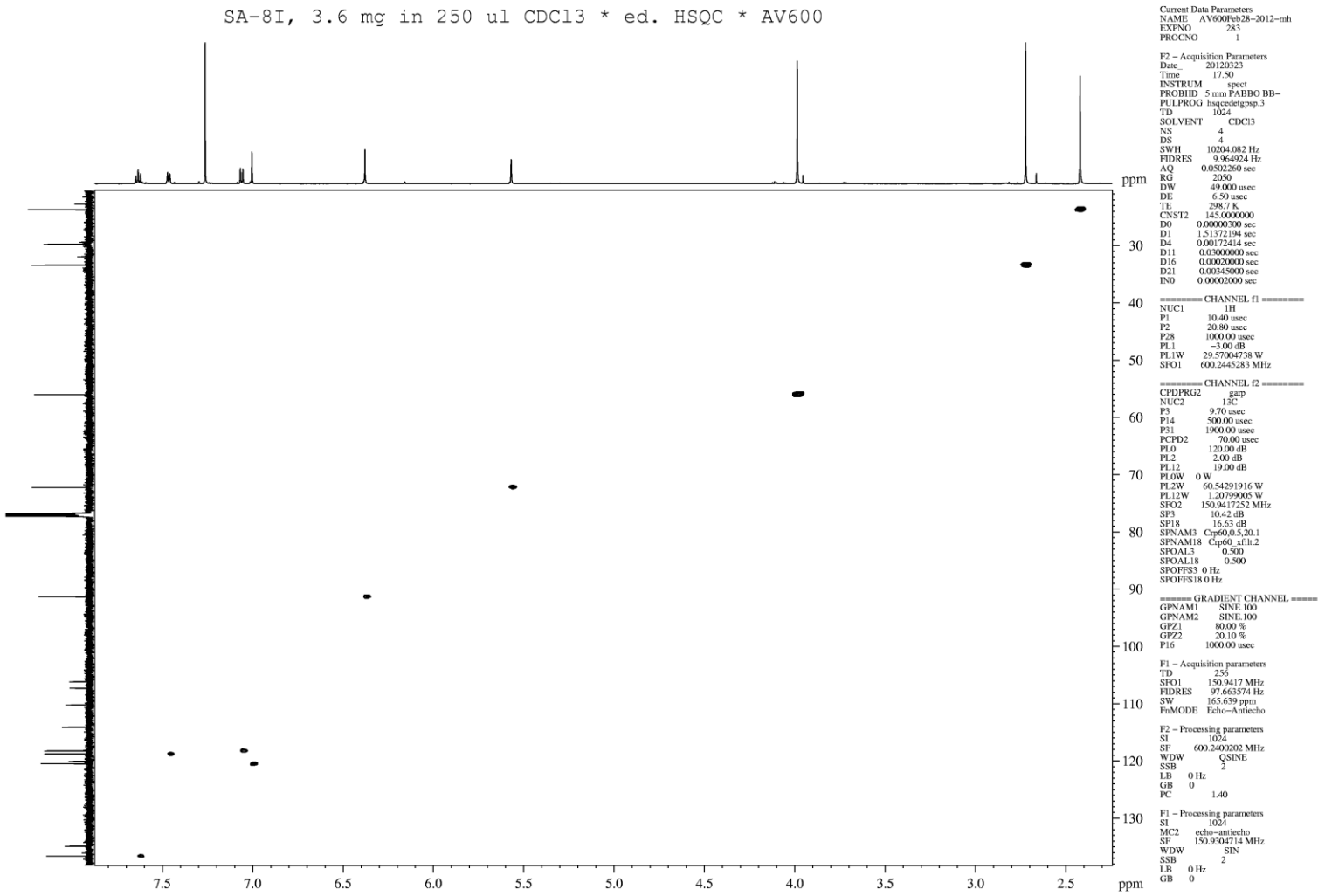
Appendix 8D: HMBC spectrum of knipholone cyclooxanthrone (**134**), $CDCl_3$, 600 MHz



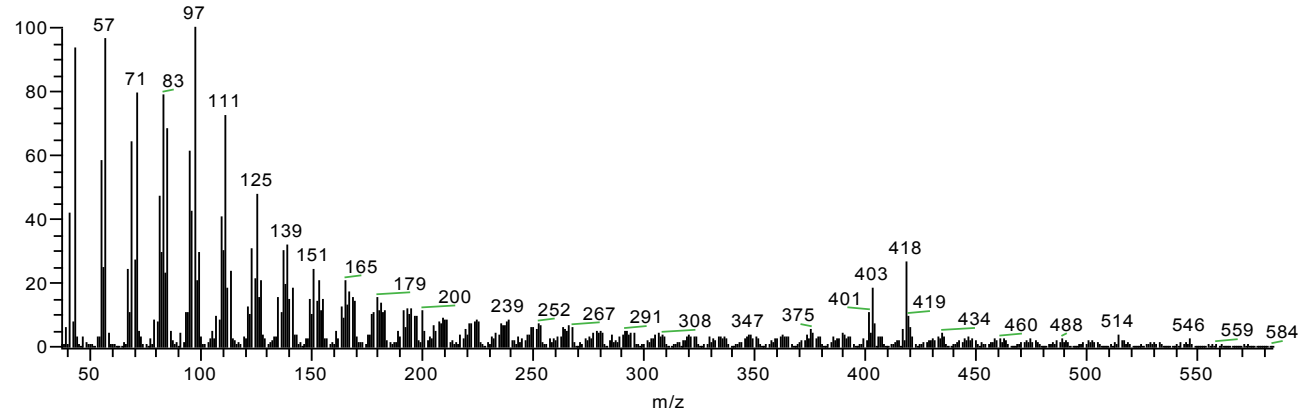
Appendix 8E: HSQC spectrum of knipholone cyclooxanthrone (**134**), $CDCl_3$, 600 MHz



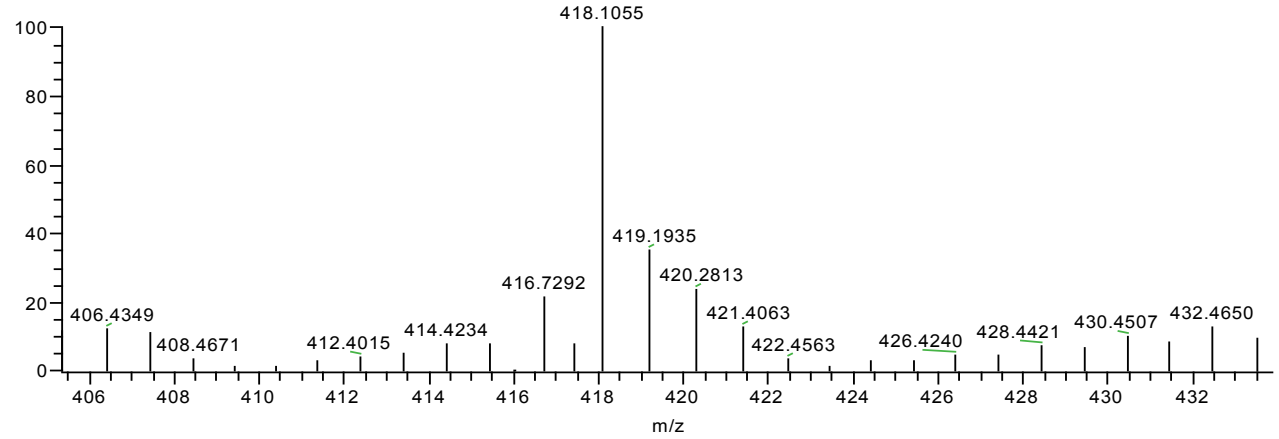
Appendix 8F: HRMS spectrum of knipholone cyclooxanthrone (**134**)

8I, MW=418

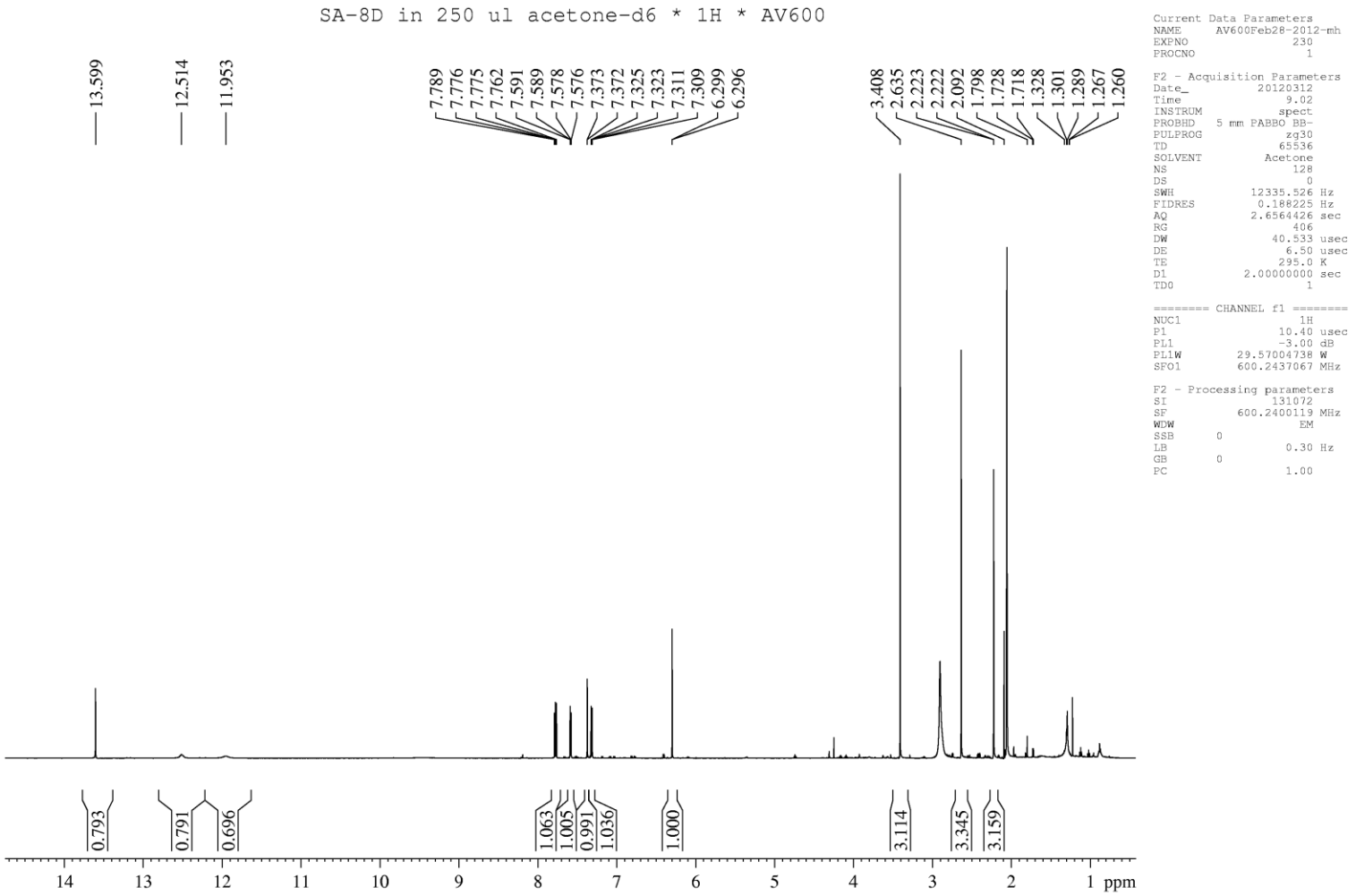
Heydenreich_84 #28-242 RT: 0.29-1.06
T: + c Full ms [35.00-650.00]



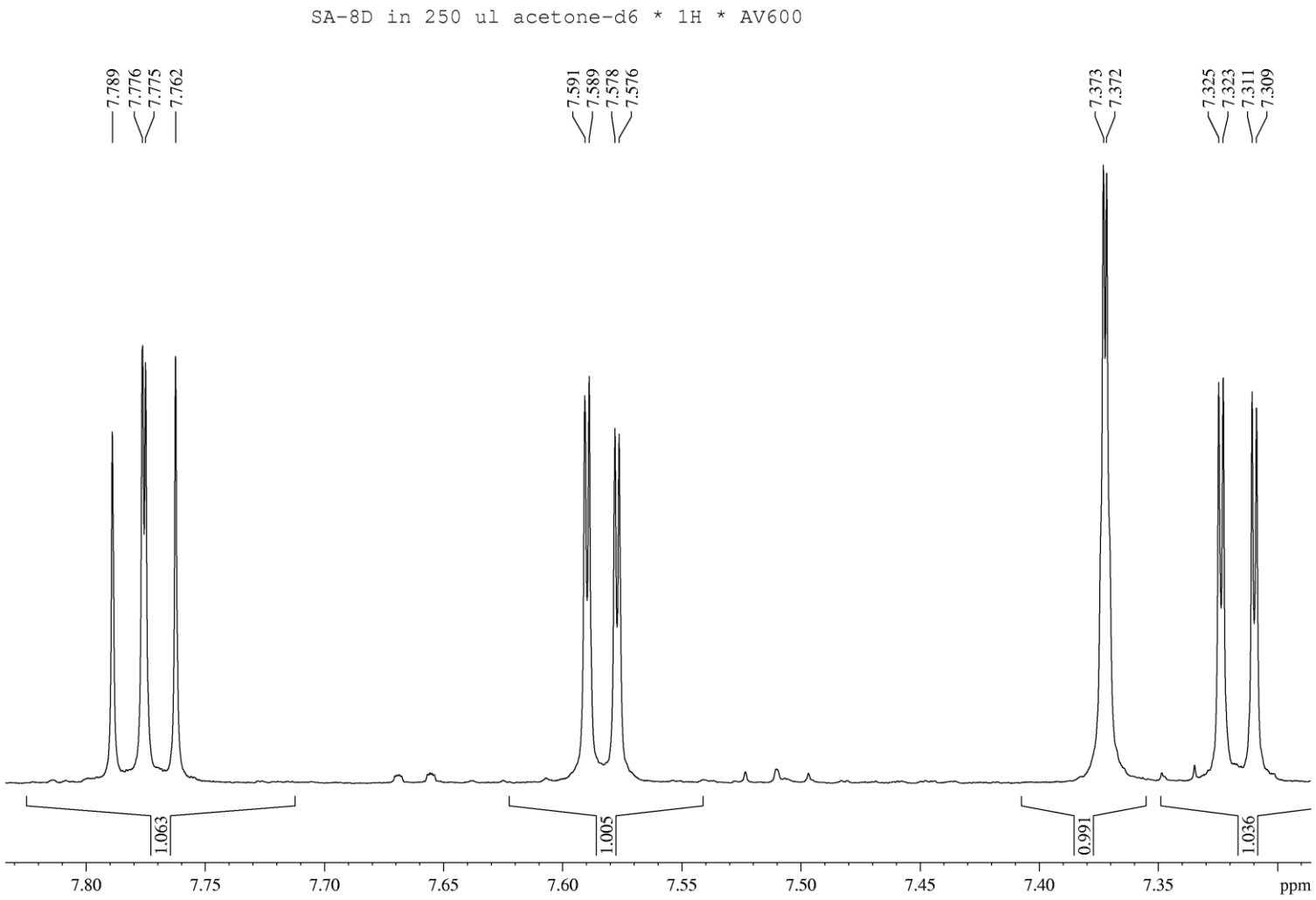
Heydenreich_84 #28-242 RT: 0.29-1.06 AV: 215 NL: 2.72E5
T: + c Full ms [35.00-650.00]



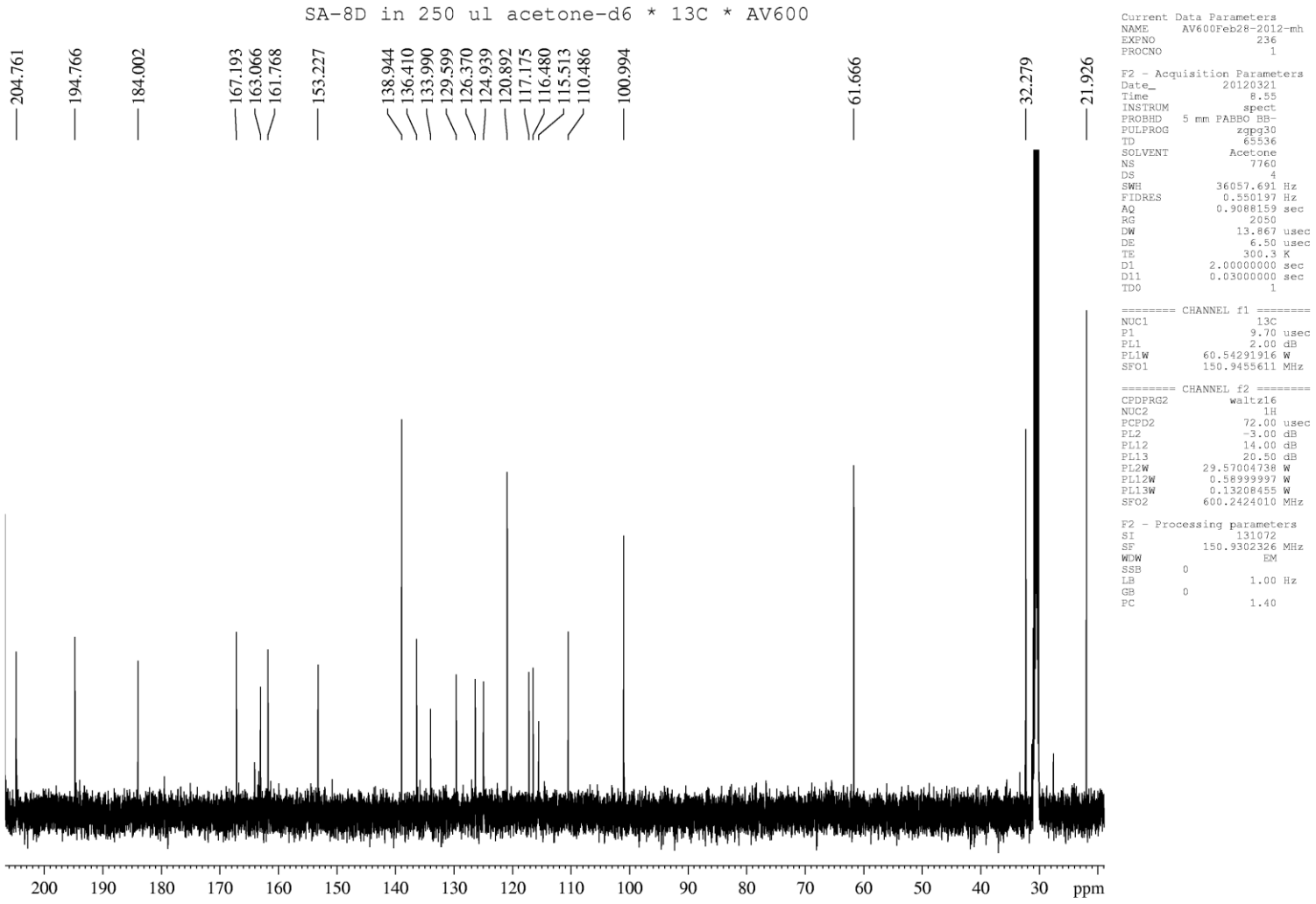
Appendix 9A: ^{1}H NMR spectrum of isoknipholone (**28**), acetone-d_6, 600 MHz



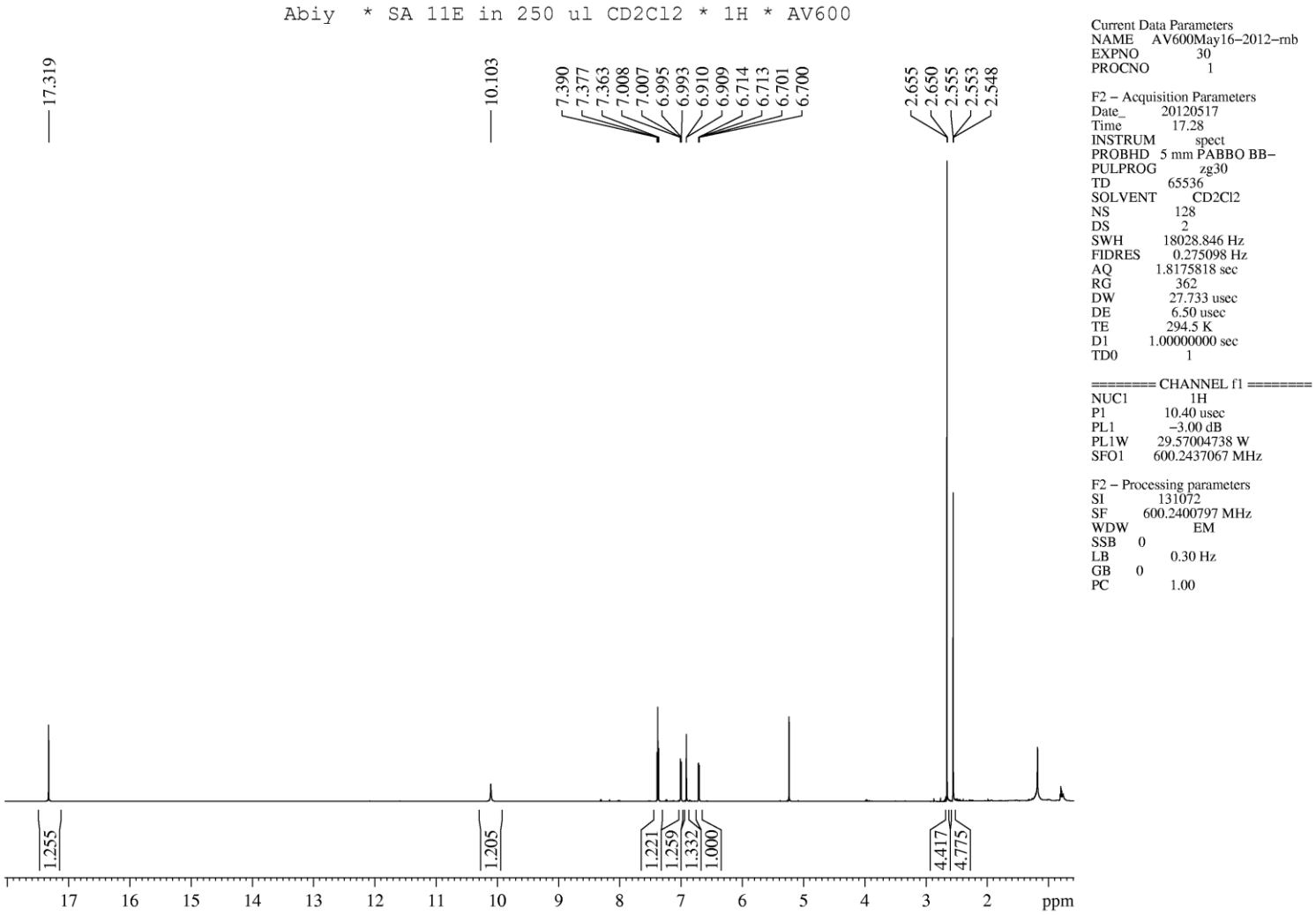
Appendix 9B: Expanded ^{1}H NMR spectrum of isoknipholone (**28**), acetone-d_6, 600 MHz



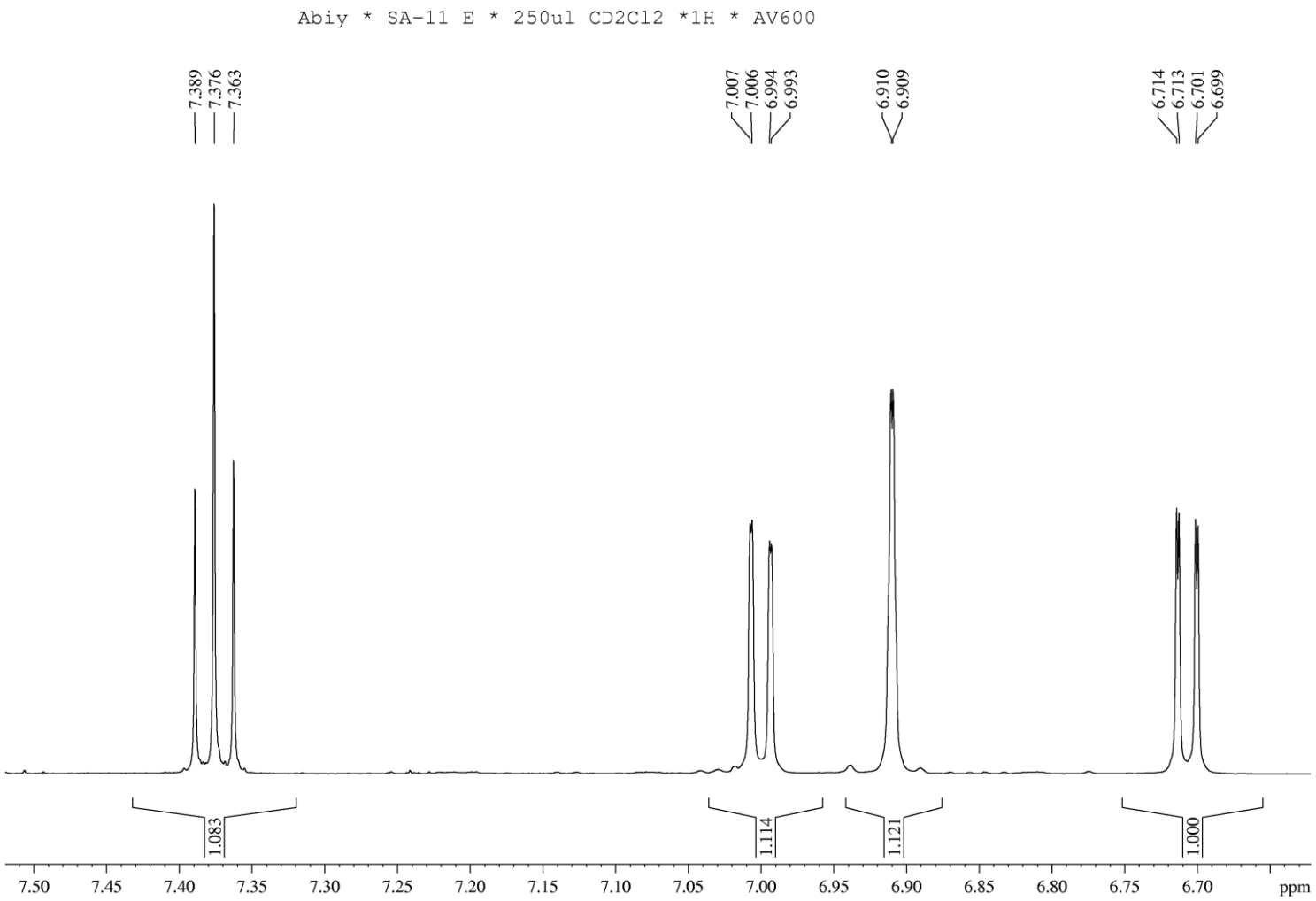
Appendix 8C: ^{13}C NMR spectrum of isoknipholone (**28**), acetone-d_6, 200 MHz



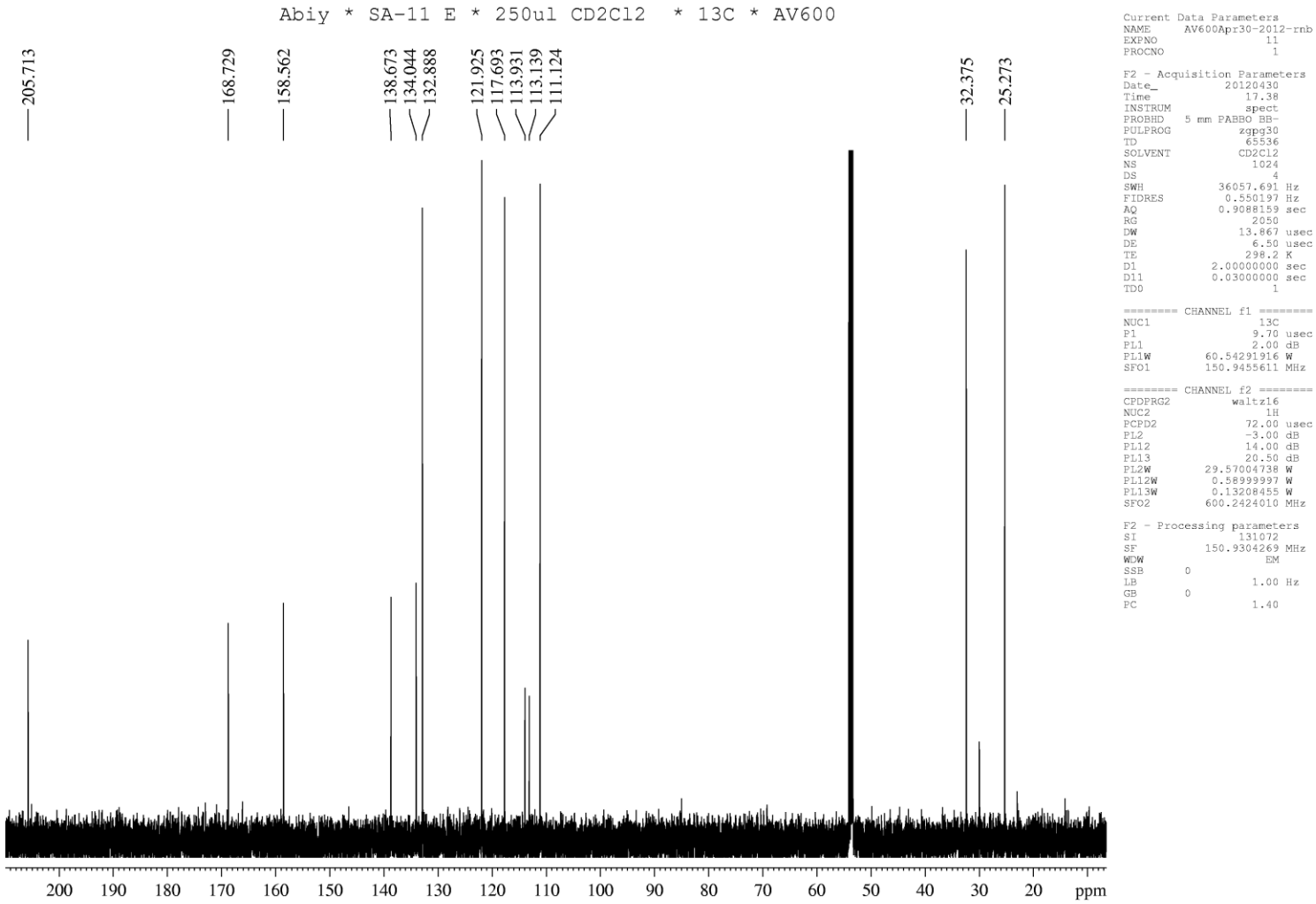
Appendix 10A: ^{1}H NMR spectrum of nepodin (**135**), CD_2Cl_2, 600 MHz



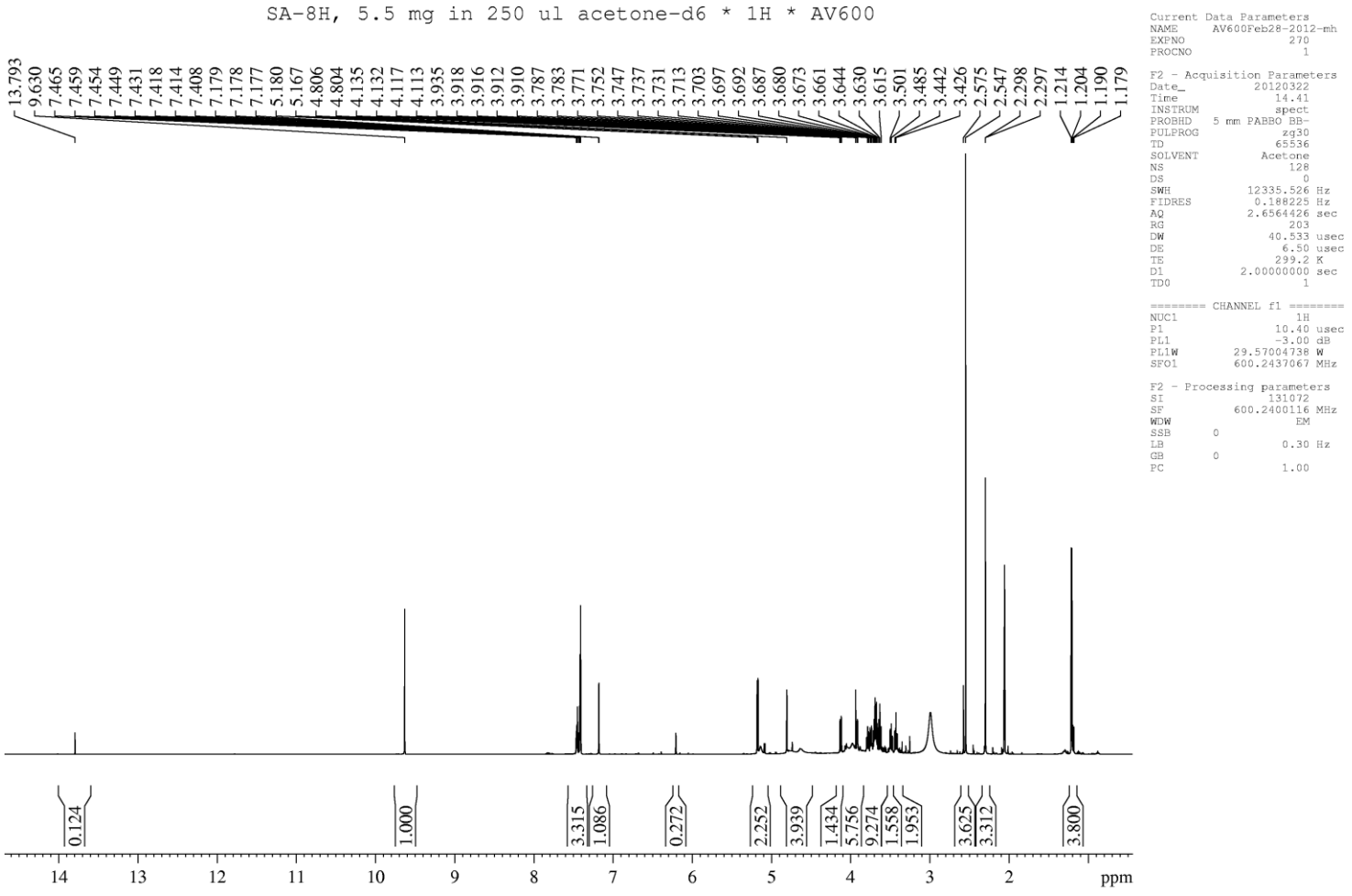
Appendix 10B: Expanded ^{1}H NMR spectrum of nepodin (**135**), CD_2Cl_2, 600 MHz



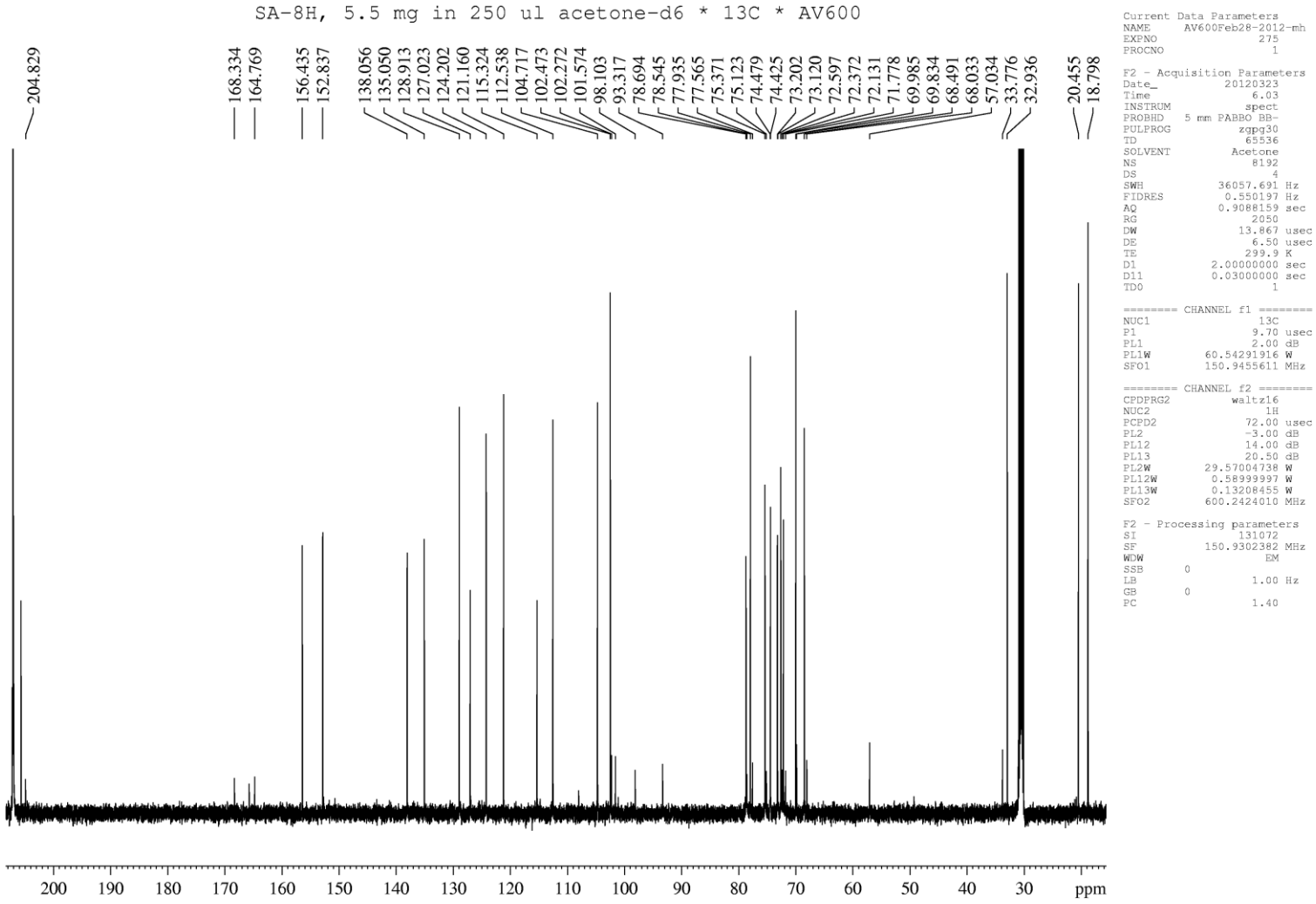
Appendix 10C: ^{13}C NMR spectrum of nepodin (**135**), CD_2Cl_2, 200 MHz



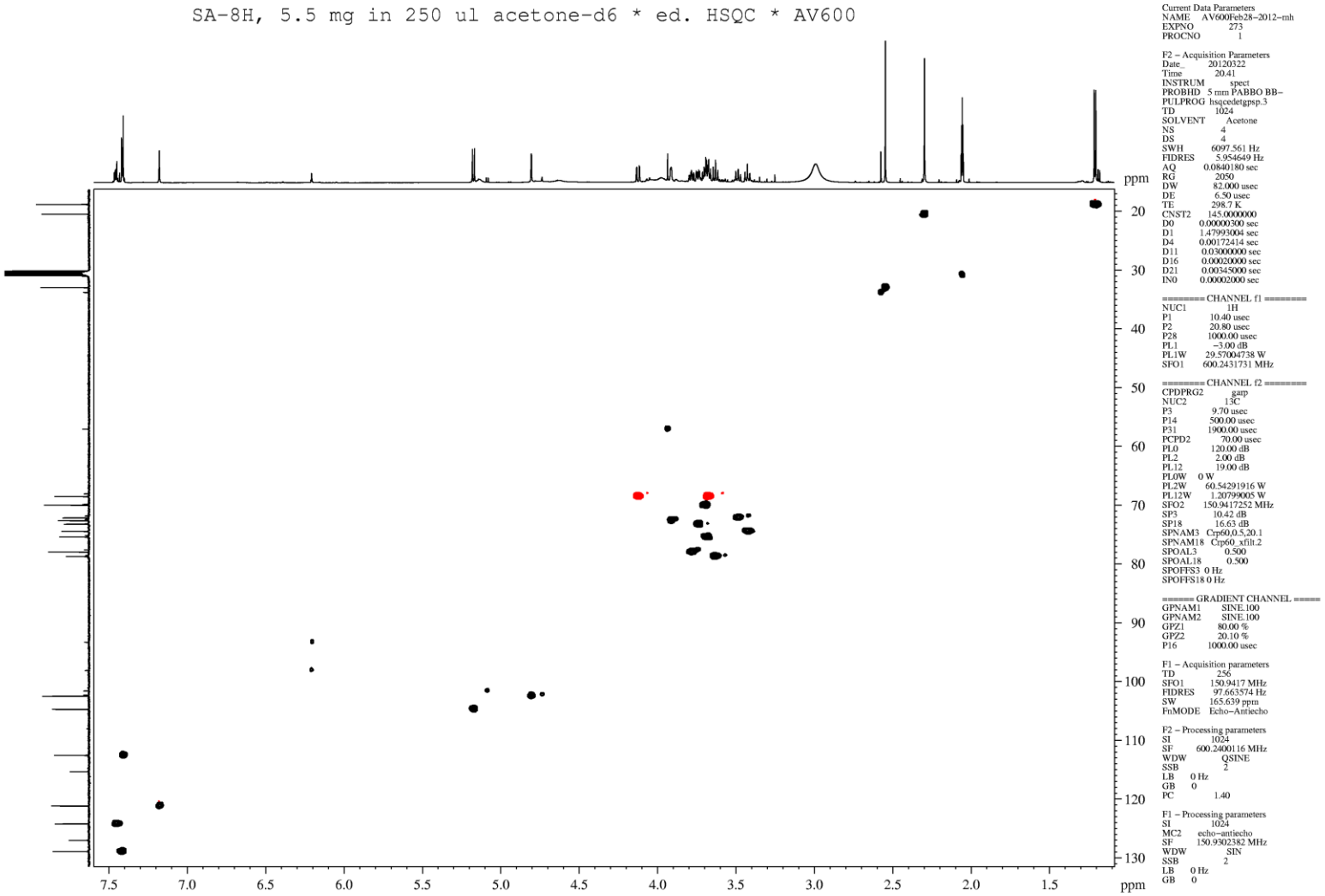
Appendix 11A: ^{1}H NMR spectrum of dianellin (**136**), acetone-d_6, 600 MHz



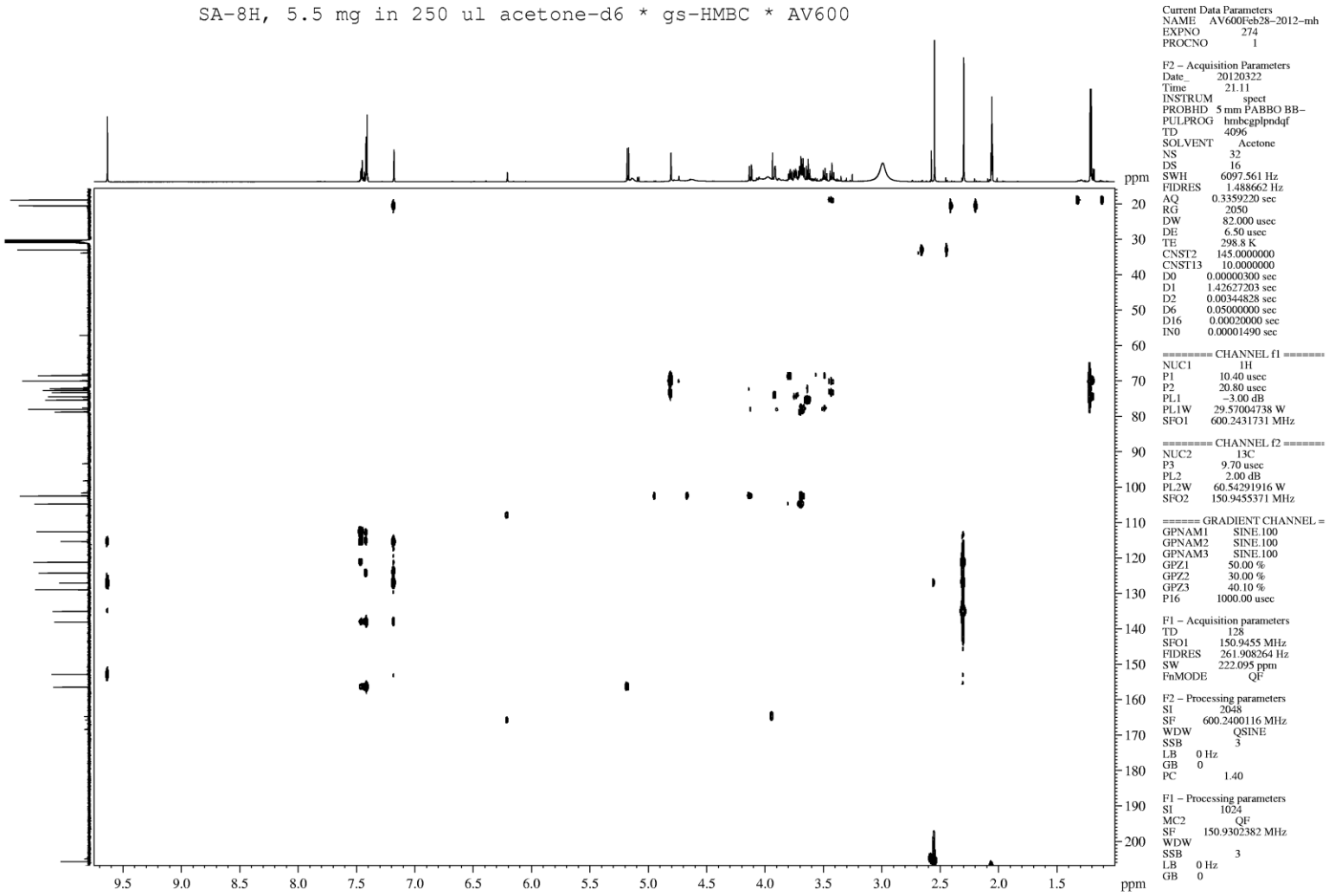
Appendix 11B: ^{13}C NMR spectrum of of dianellin (**136**), acetone-d_6, 200 MHz



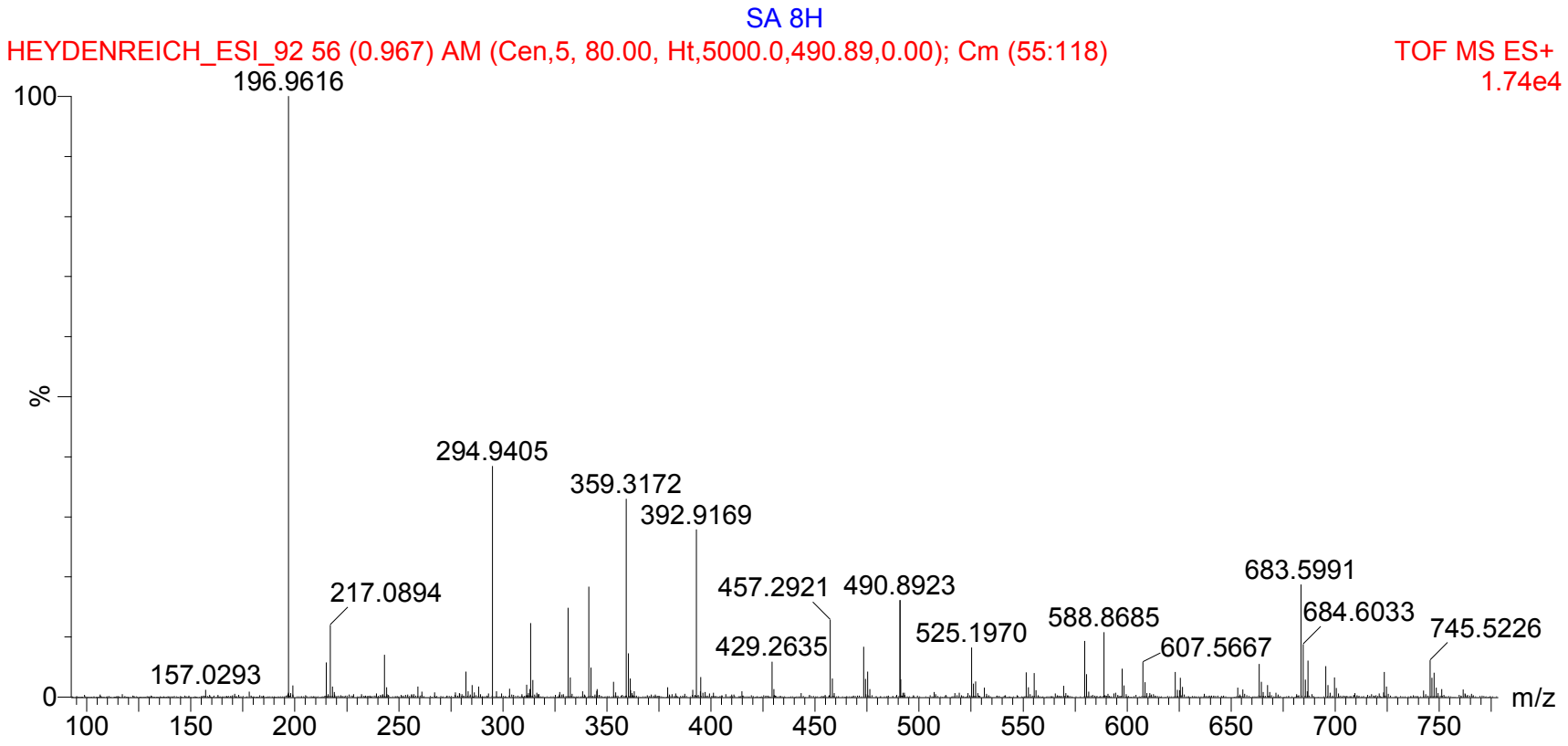
Appendix 11C: HSQC spectrum of dianellin (**136**), acetone-d_6, 600 MHz



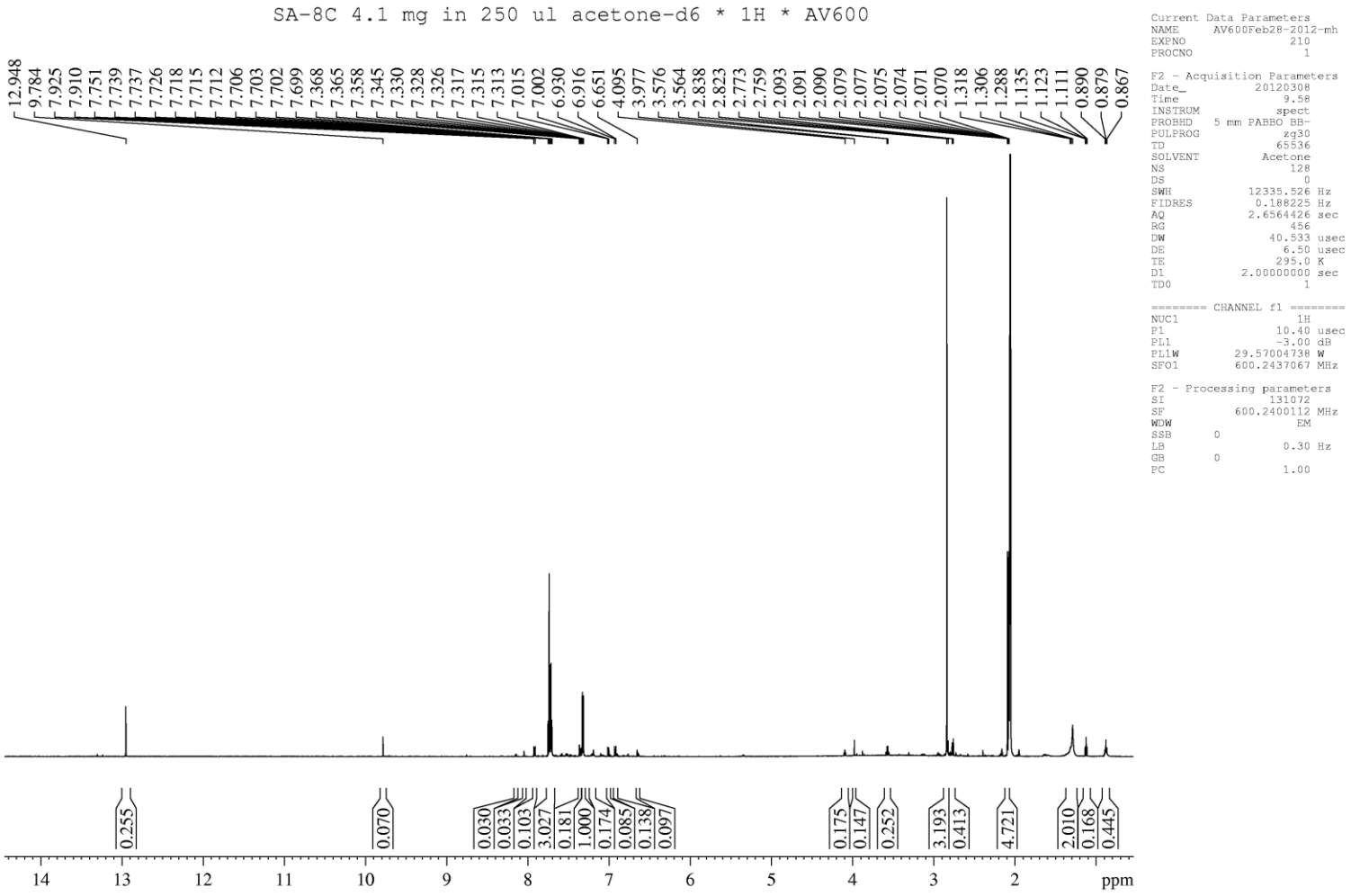
Appendix 11D: HMBC spectrum of dianellin (**136**), acetone-d_6, 600 MHz



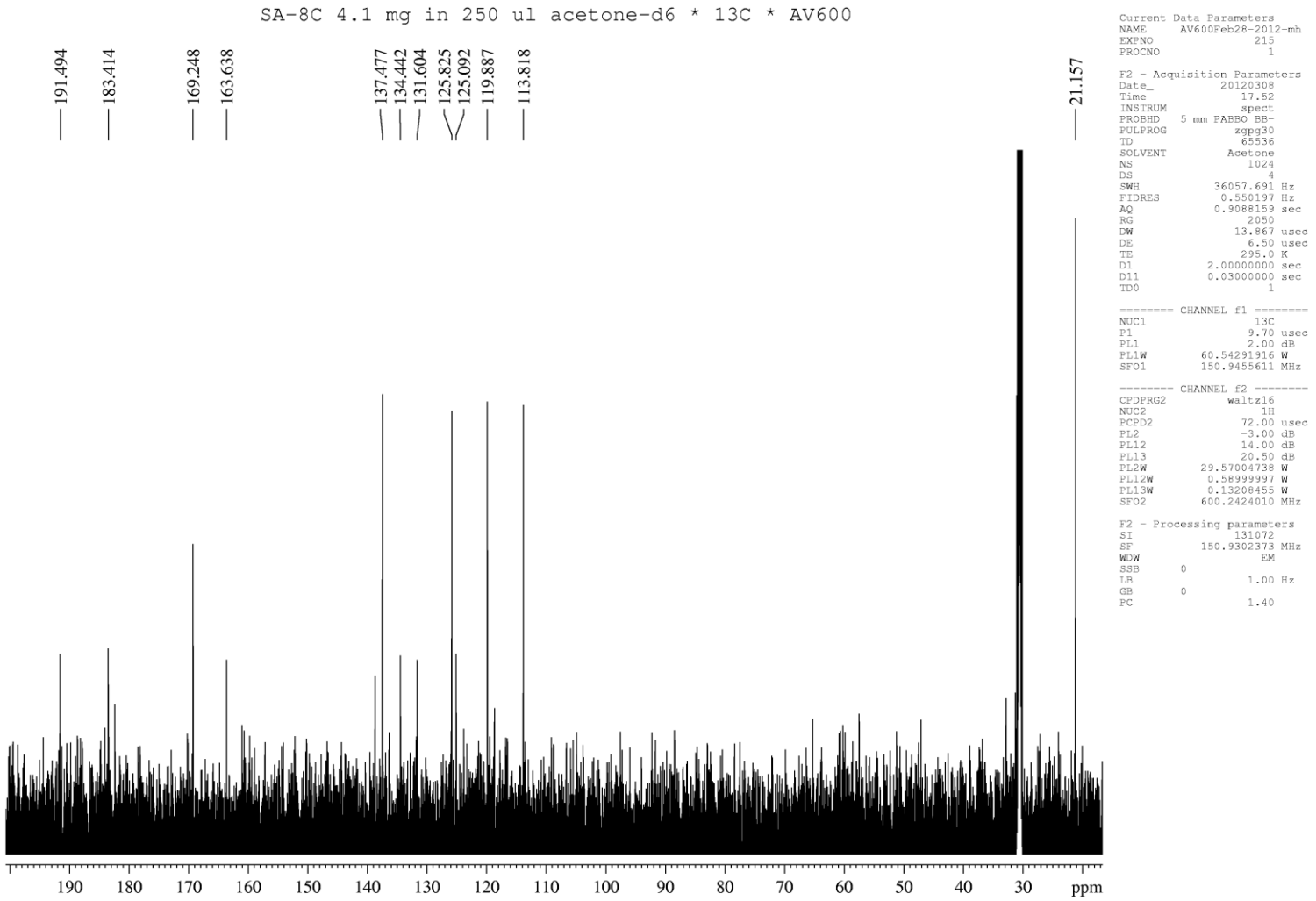
Appendix 11E: ESIMS spectrum of dianellin (**136**)



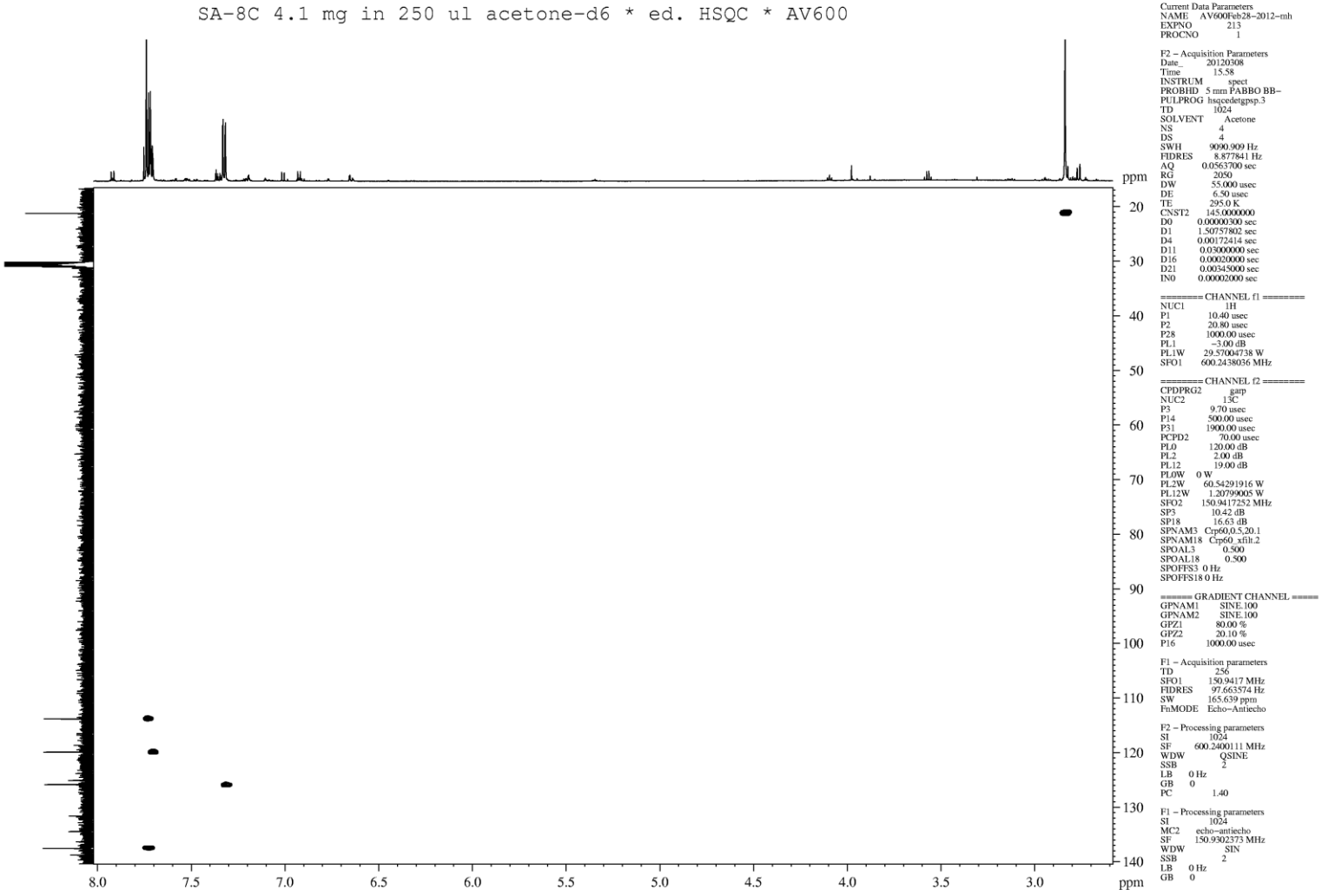
Appendix 12A: ^{1}H NMR spectrum of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), acetone-d_6, 600 MHz



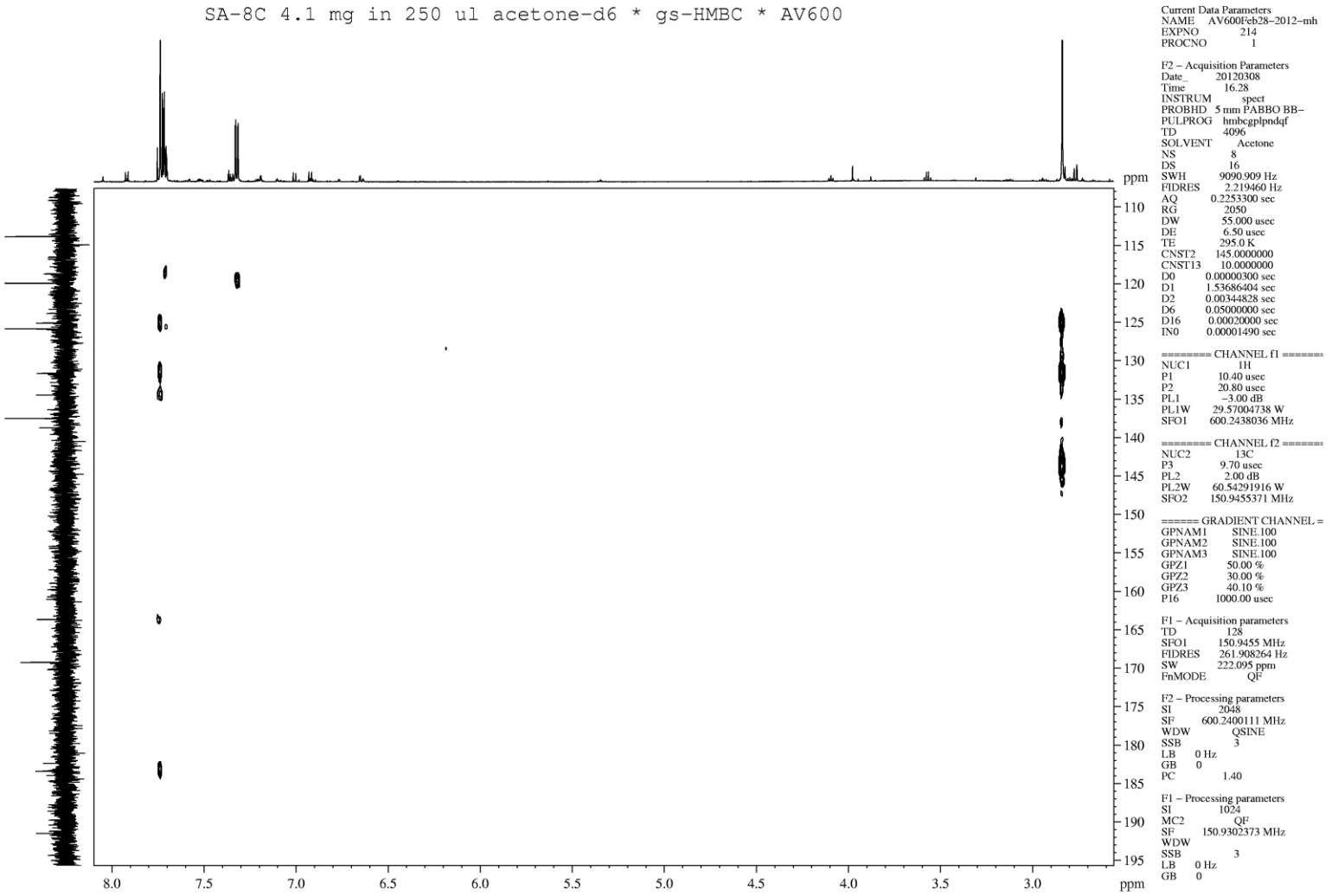
Appendix 12B: ^{13}C NMR spectrum of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), acetone-d_6, 200 MHz



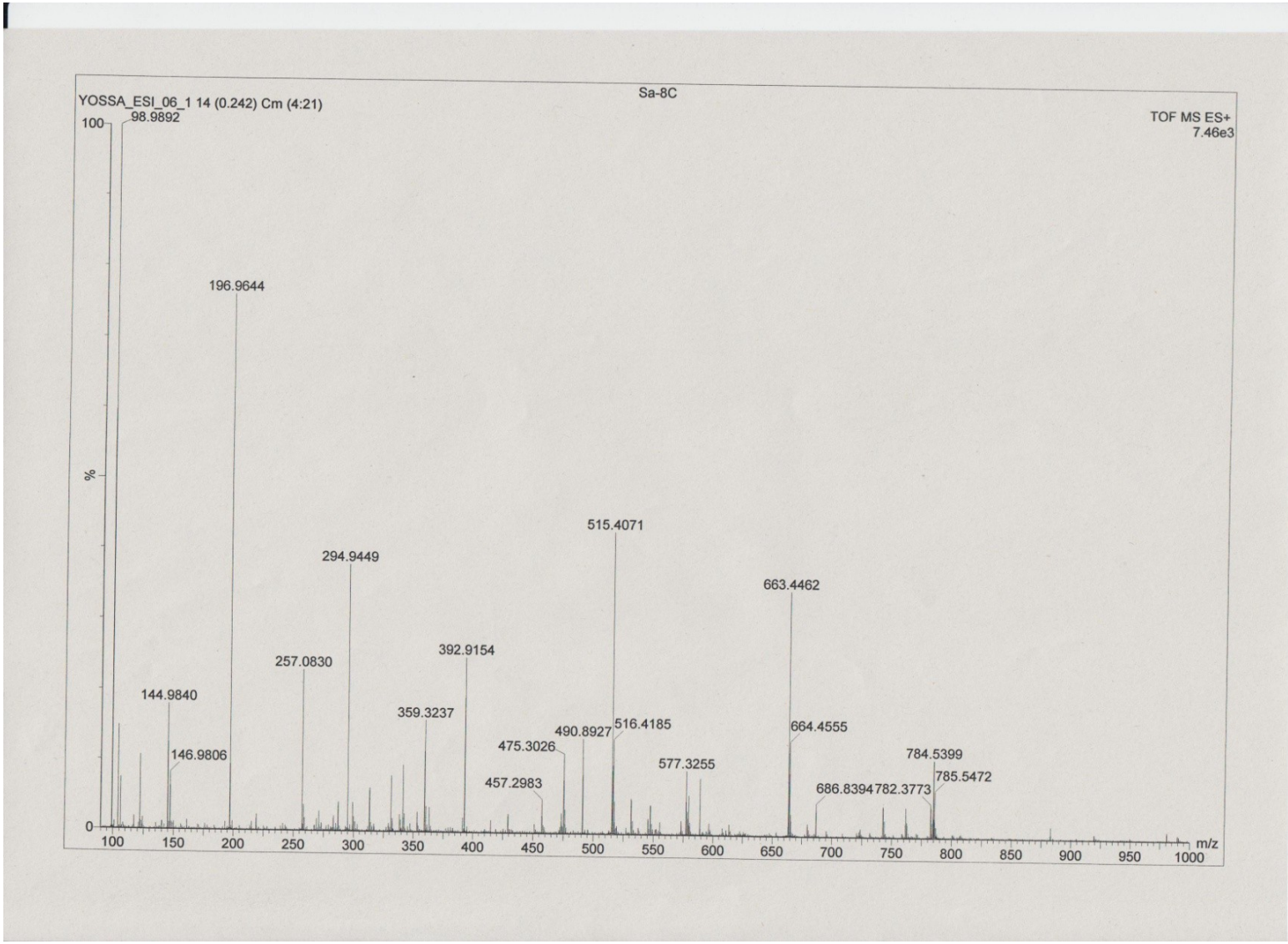
Appendix 12C: HSQC spectrum of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), acetone-d_6, 600 MHz



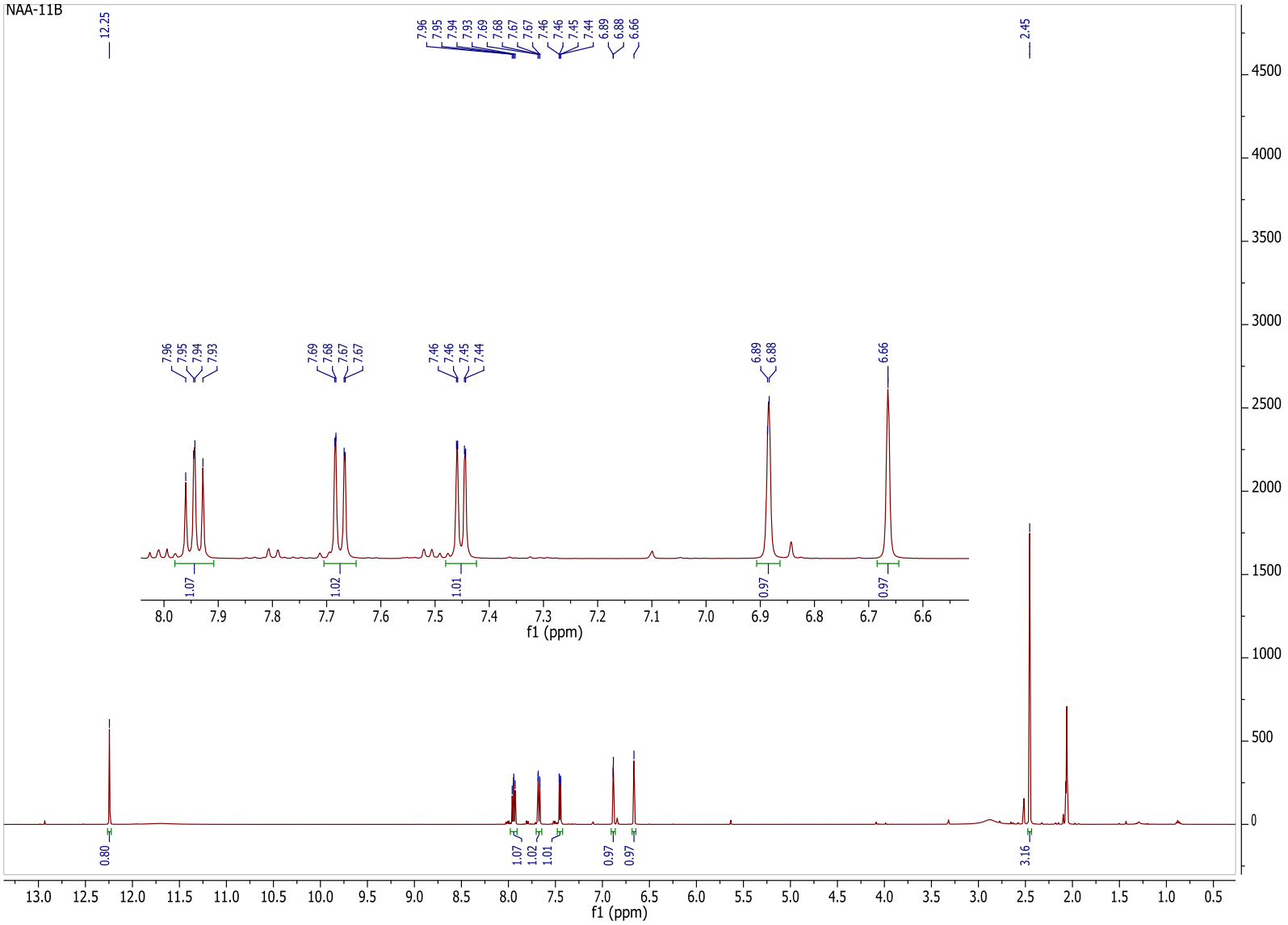
Appendix 12D: HMBC spectrum of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**), acetone-d_6, 600 MHz



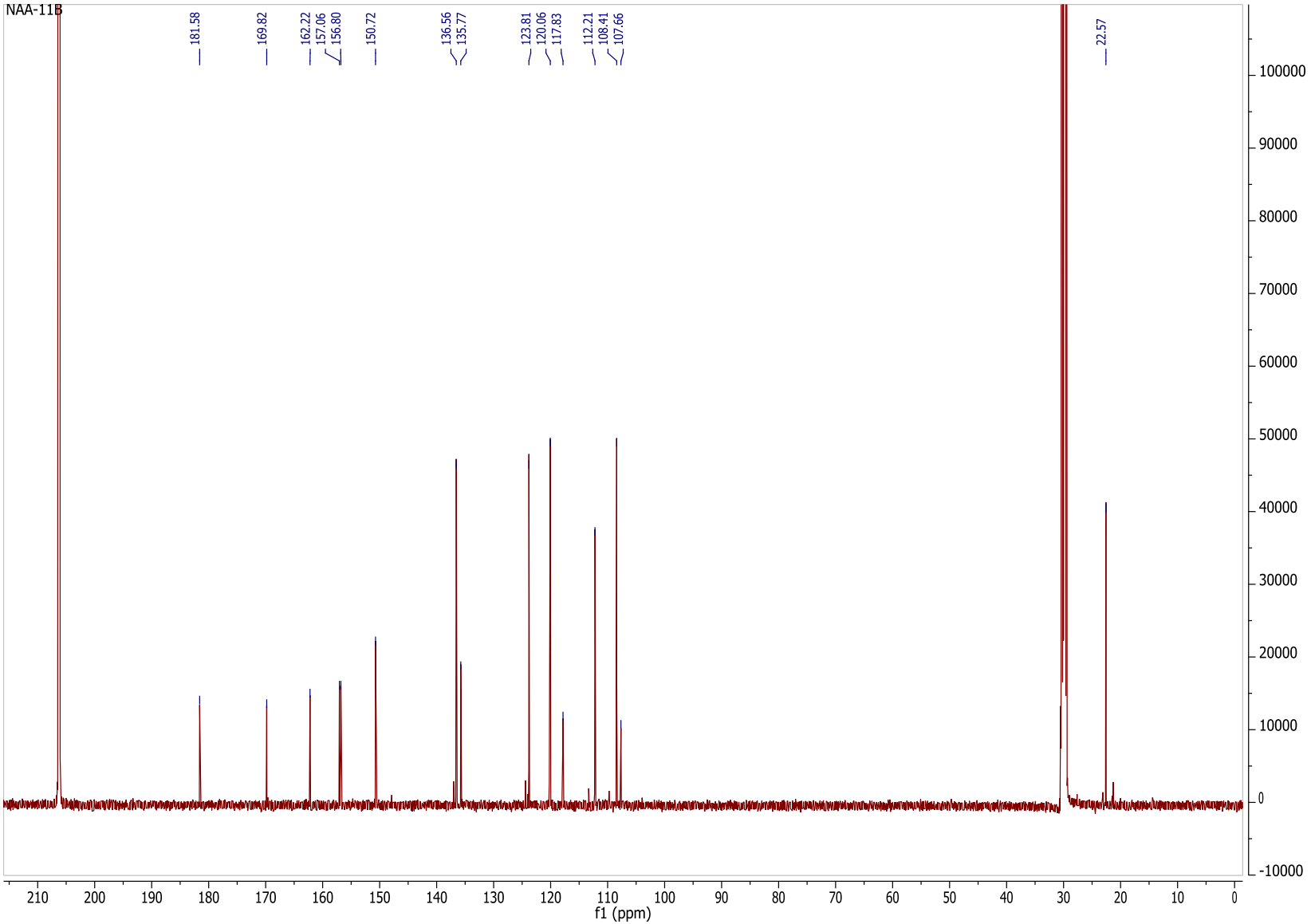
Appendix 12E: ESIMS spectrum of 3,8-dihydroxy-1-methylanthraquinone-2-carboxylic acid (**137**)



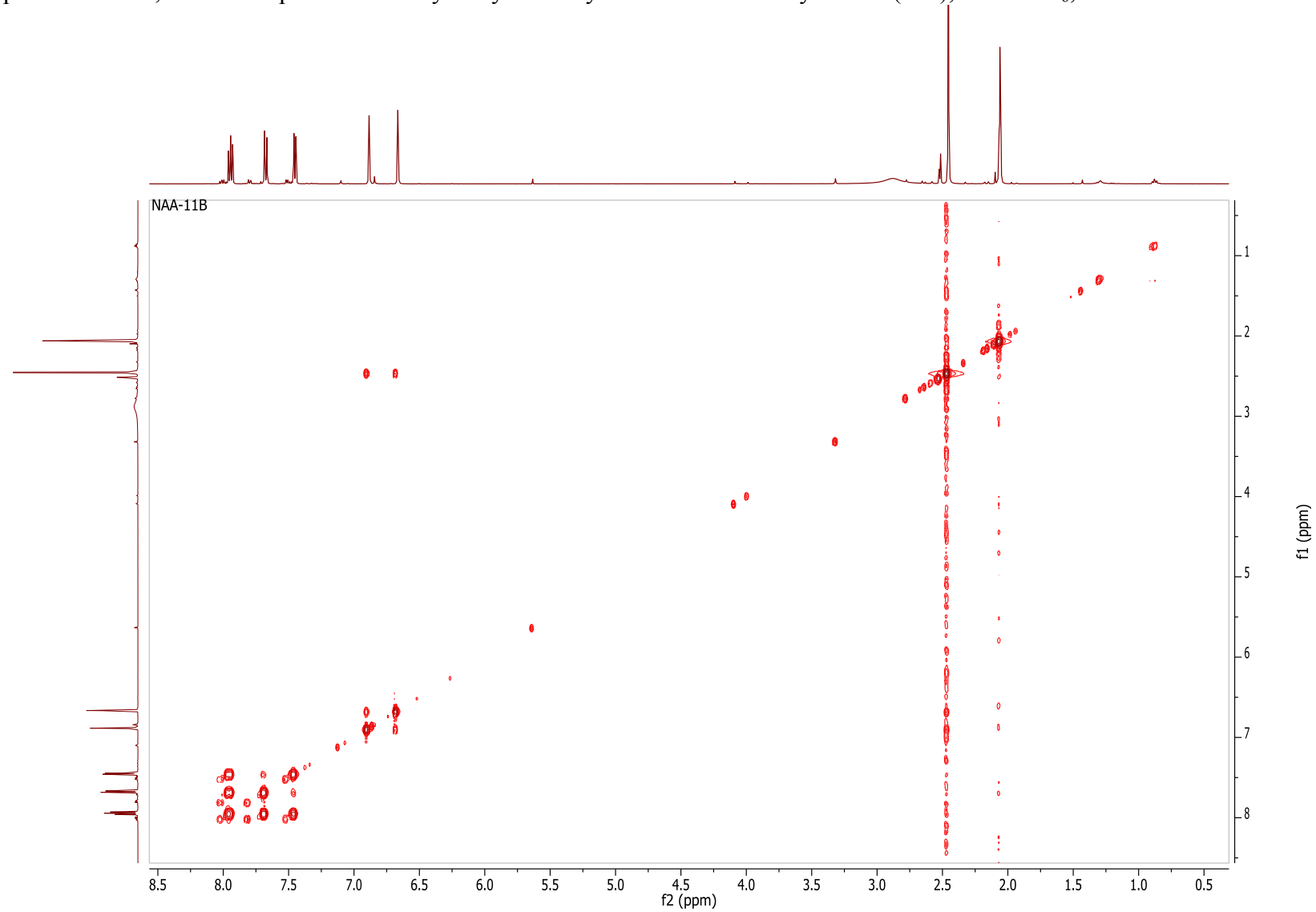
Appendix 13A: ^{1}H NMR spectrum of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), acetone-d_6, 500 MHz



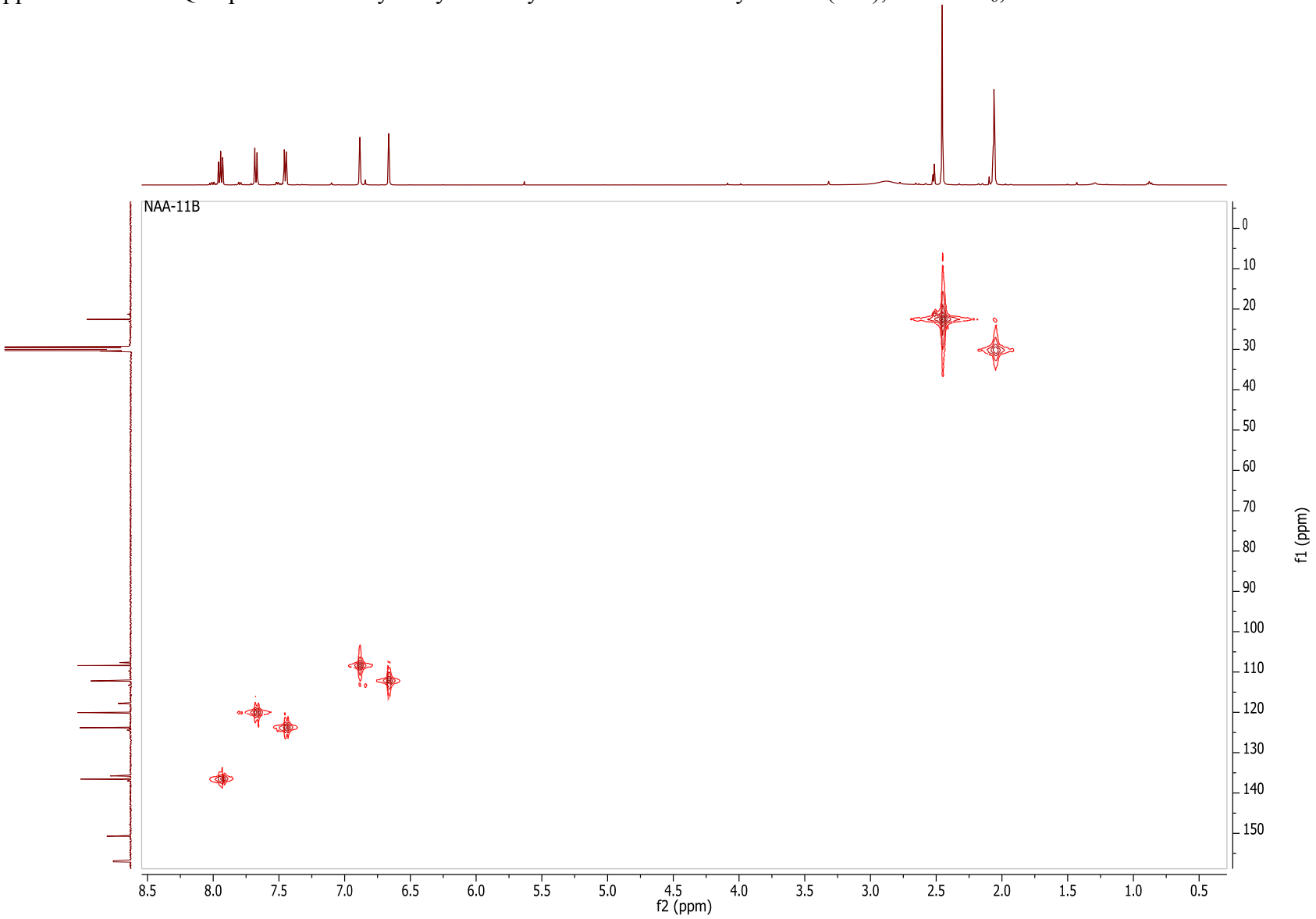
Appendix 13B: ^{13}C NMR spectrum of of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), acetone-d_6, 126 MHz



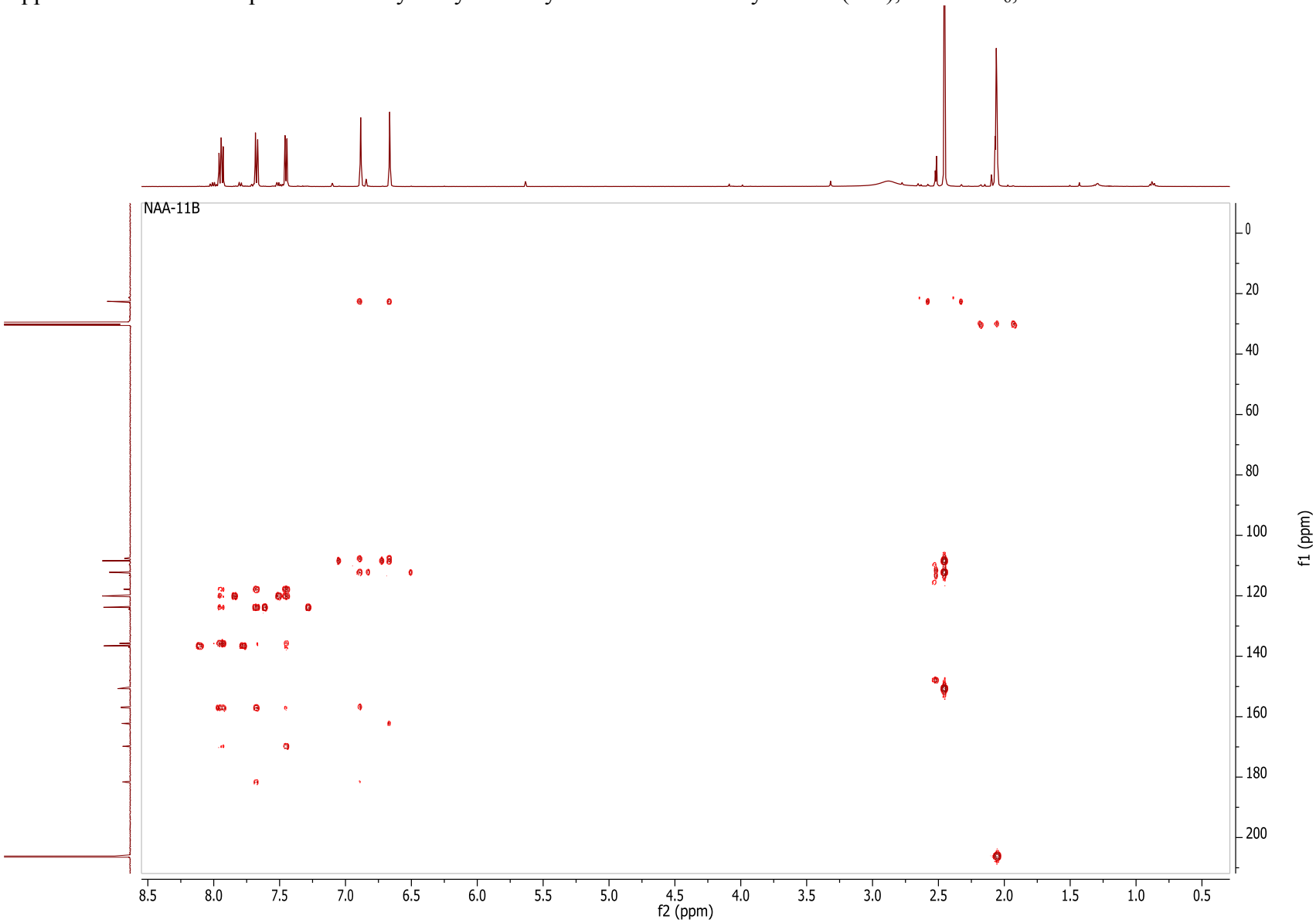
Appendix 13C: H, H-COSY spectrum of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), acetone-d_6, 500 MHz



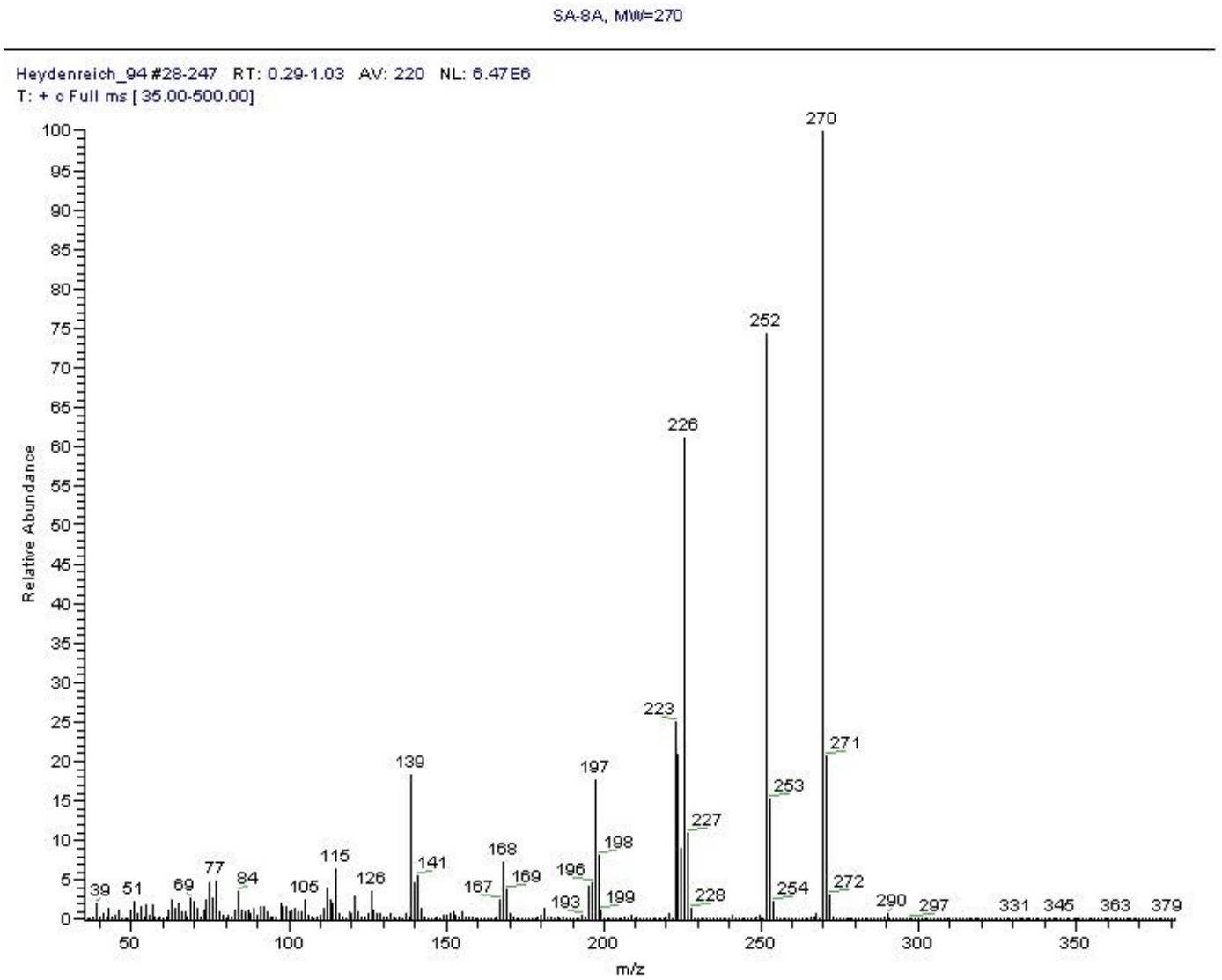
Appendix 13D: HSQC spectrum of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), acetone-d_6, 500 MHz



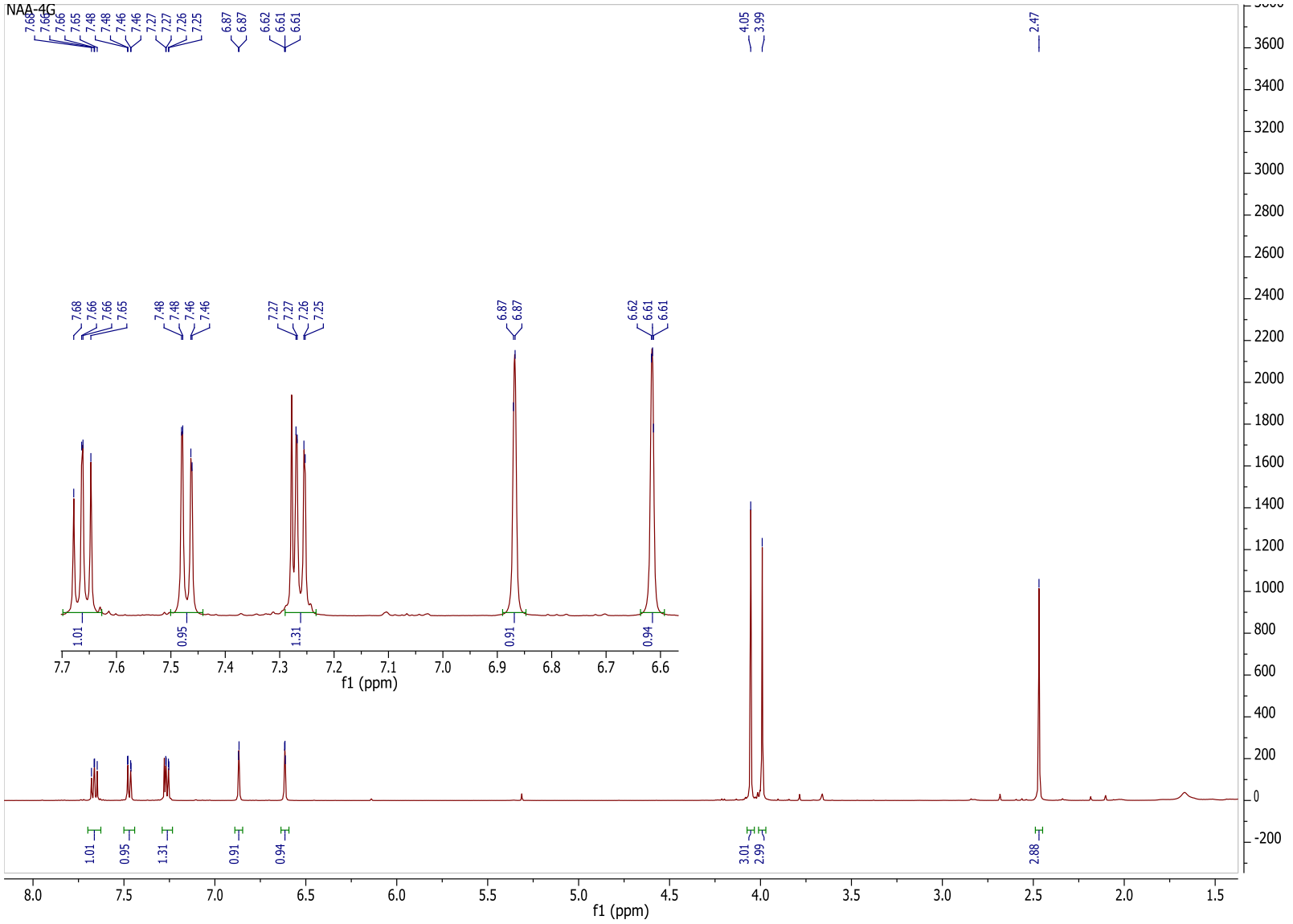
Appendix 13E: HMBC spectrum of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**), acetone-d_6, 500 MHz



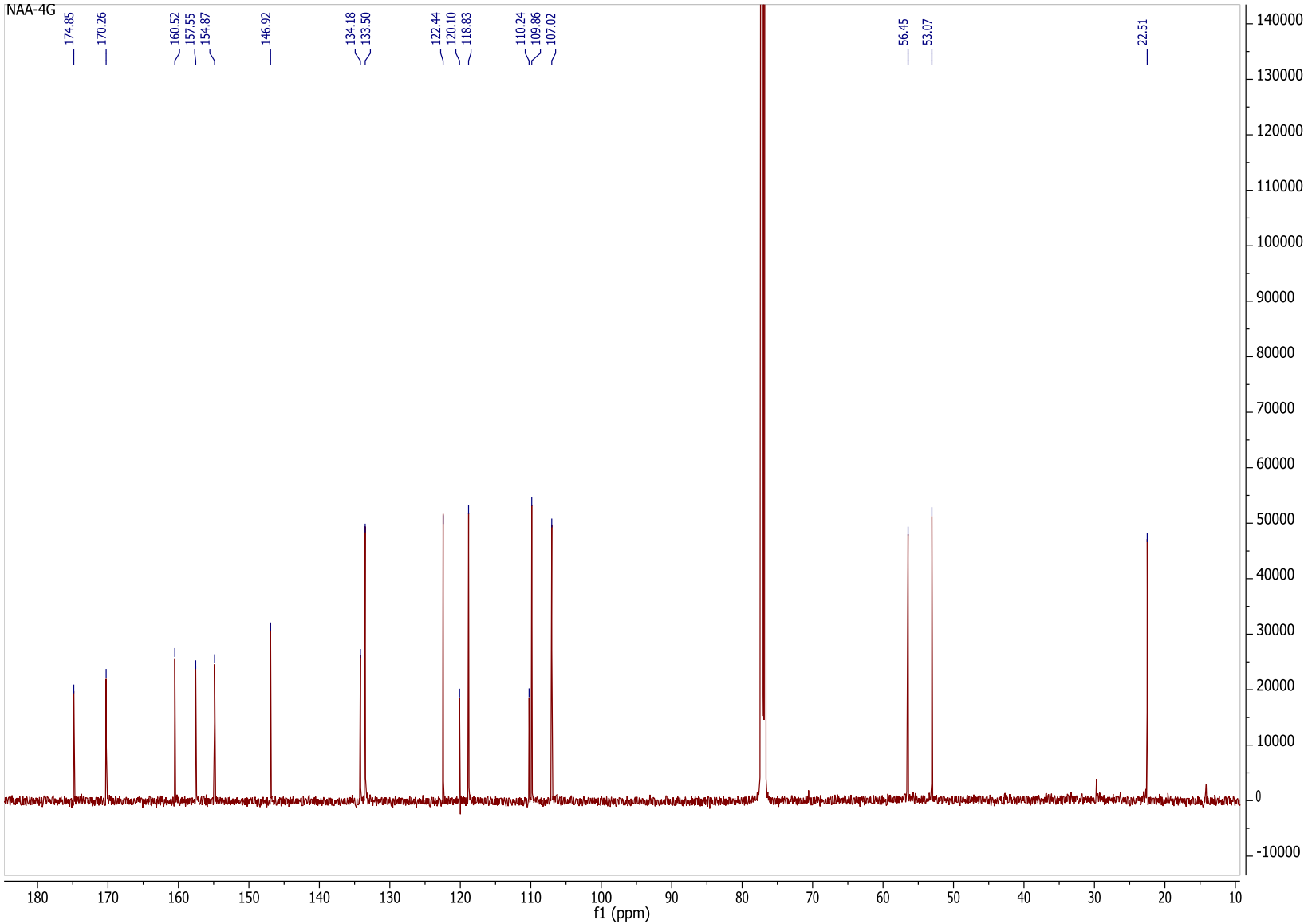
Appendix 13F: ESIMS spectrum of 8-hydroxy-6-methylxanthone-1-carboxylic acid (**138**)



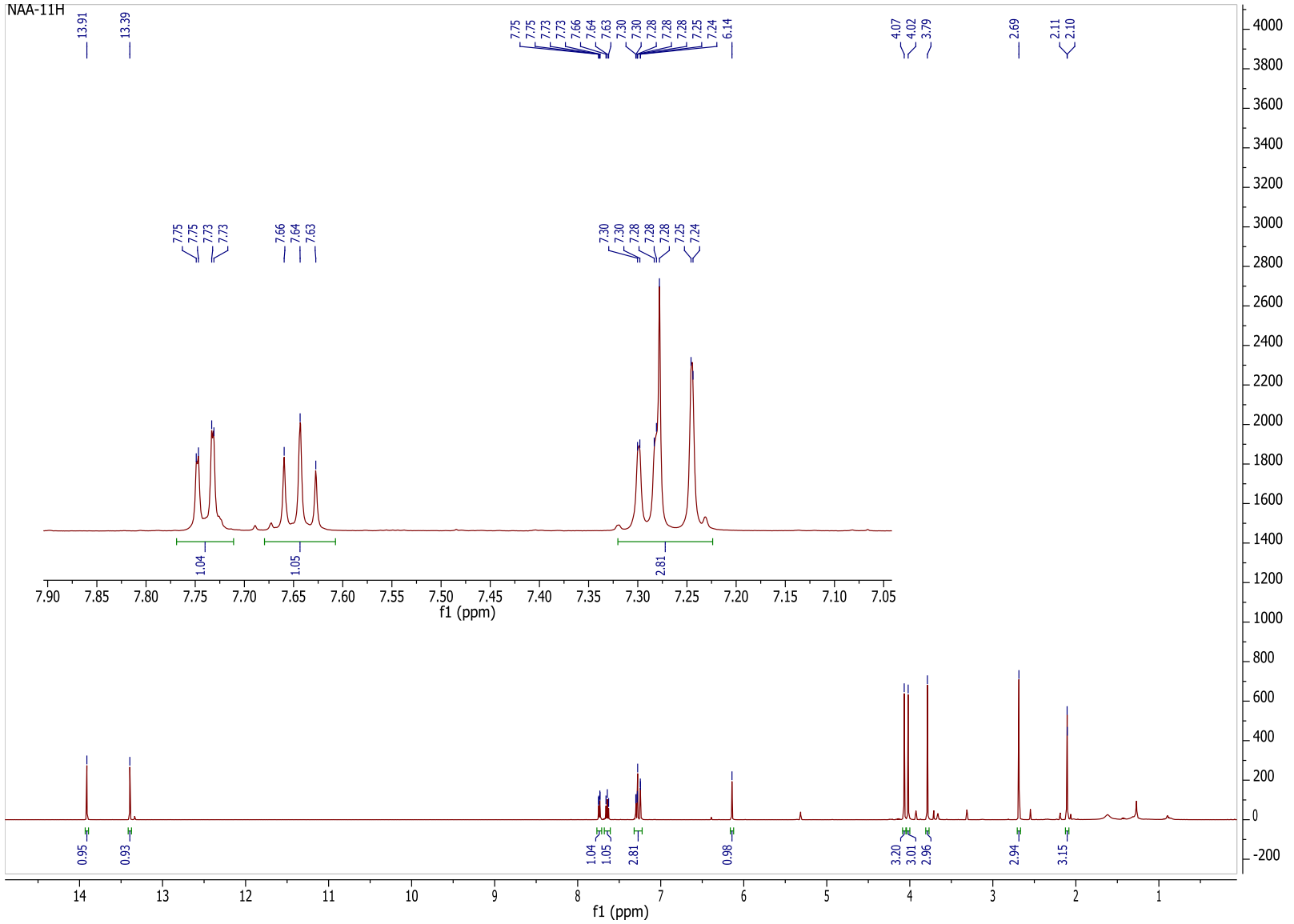
Appendix 14A: ^{1}H NMR spectrum of 8-methoxy-6-methylxanthone-1-carboxylic acid methyl ester (**138a**), $CDCl_3$, 500 MHz



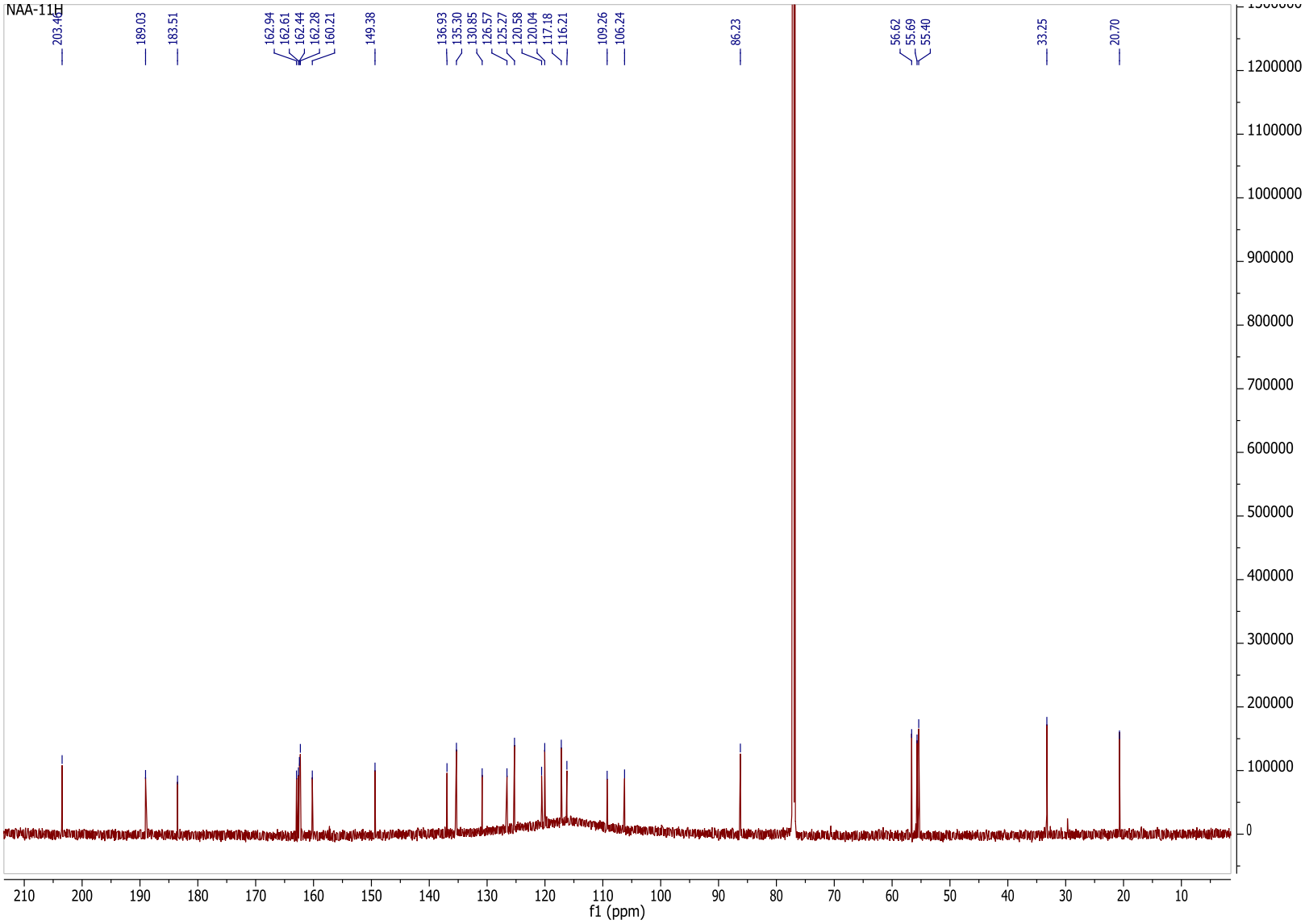
Appendix 14B: ^{13}C NMR spectrum of 8-methoxy-6-methylxanthone-1-carboxylic acid methyl ester (**138a**), $CDCl_3$, 126 MHz



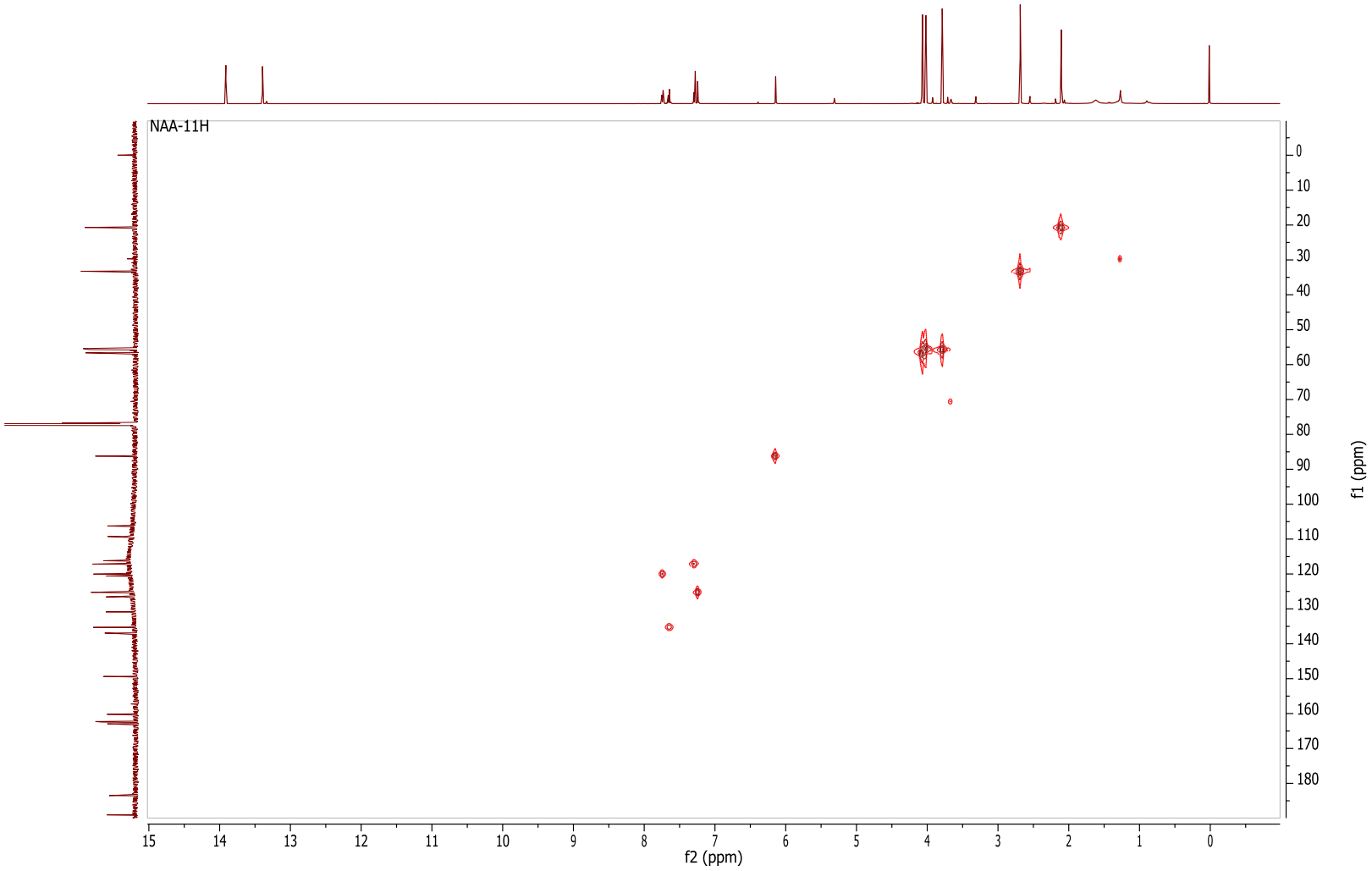
Appendix 15A: ^{1}H NMR spectrum of 8, 6'-dimethoxyknipholone (**150**), $CDCl_3$, 500 MHz



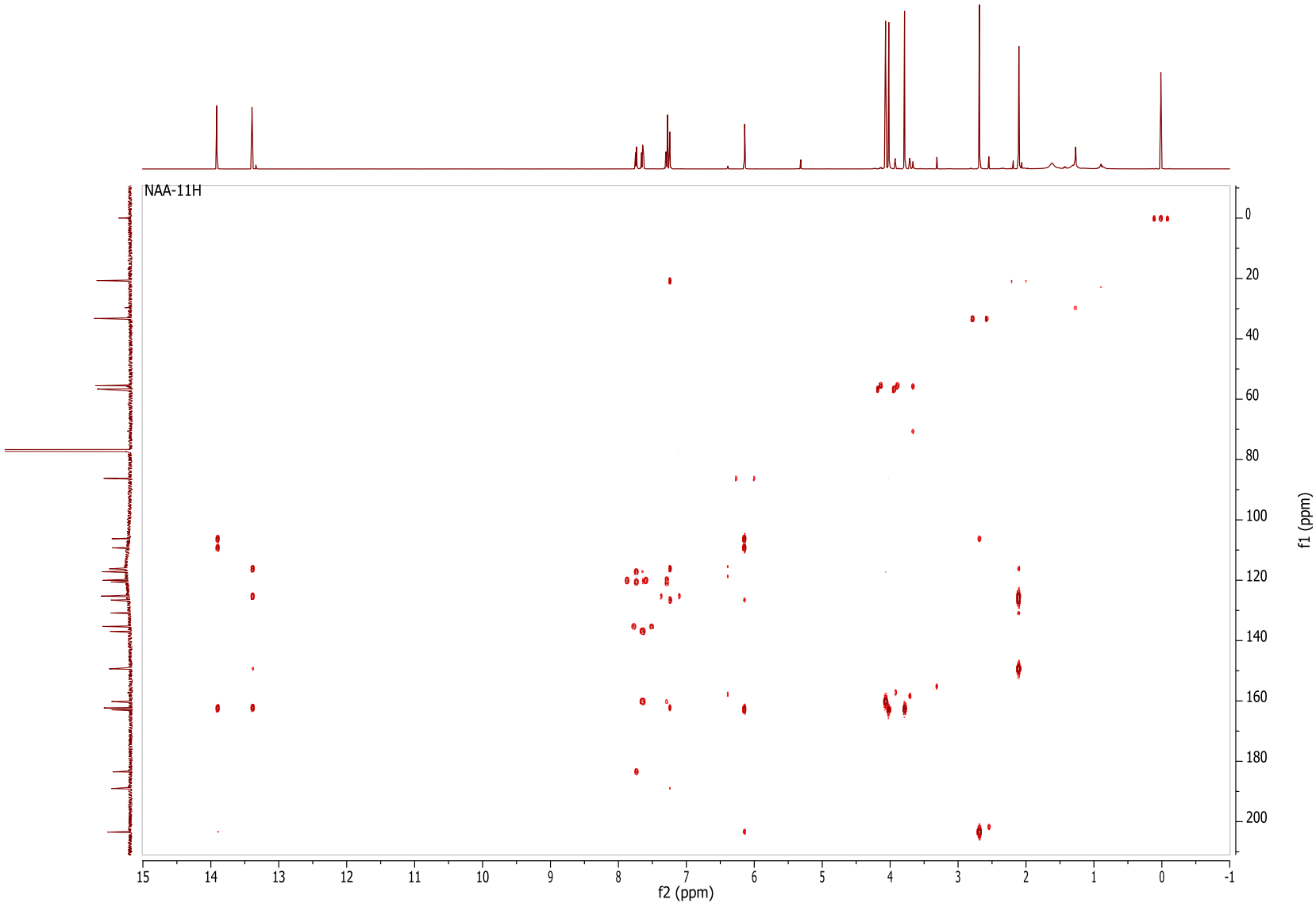
Appendix 15B: ^{13}C NMR spectrum of 8, 6'-dimethoxyknipholone (**150**), $CDCl_3$, 126 MHz



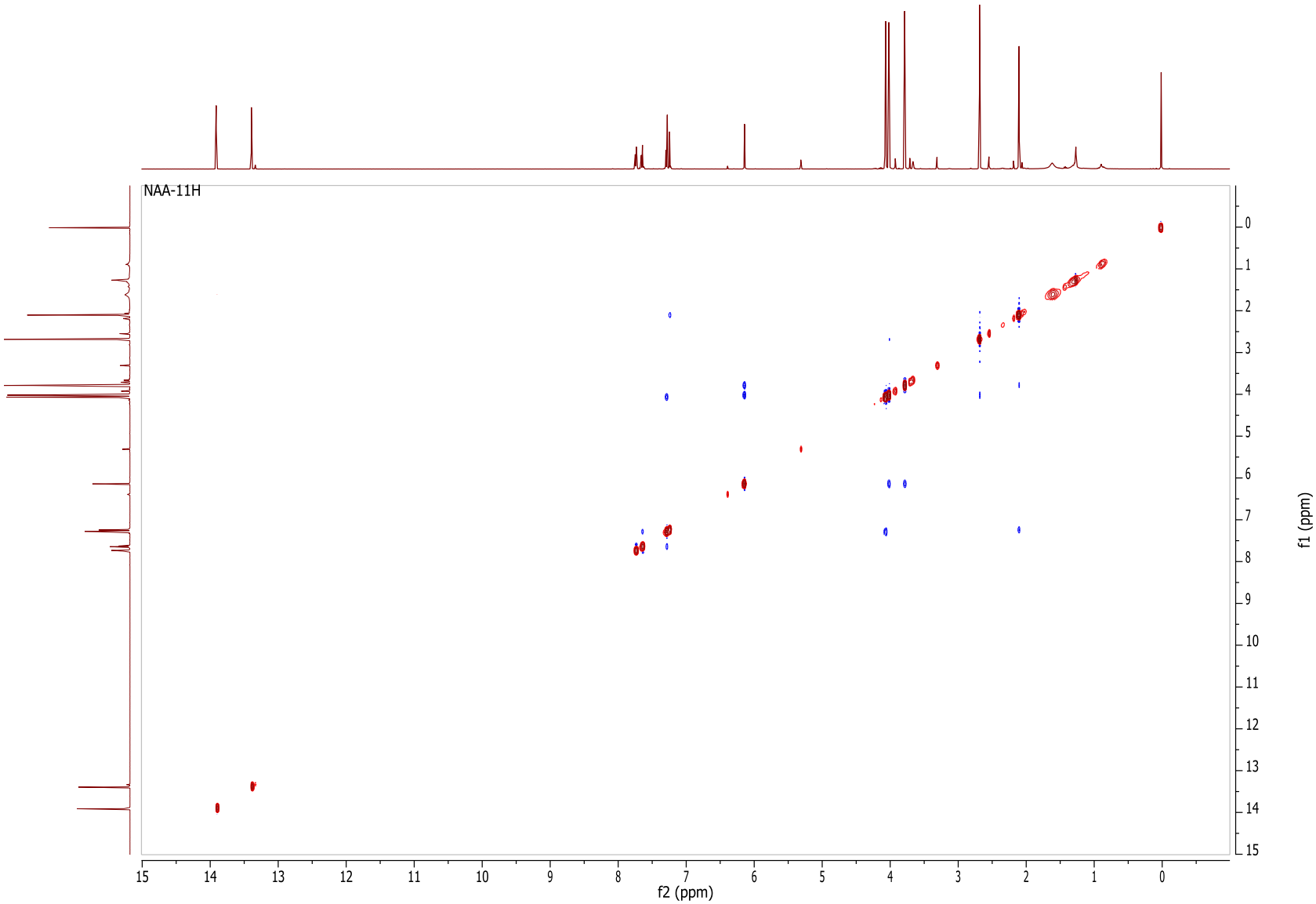
Appendix 15C: HSQC spectrum of 8, 6'-dimethoxyknipholone (**150**), $CDCl_3$, 500 MHz



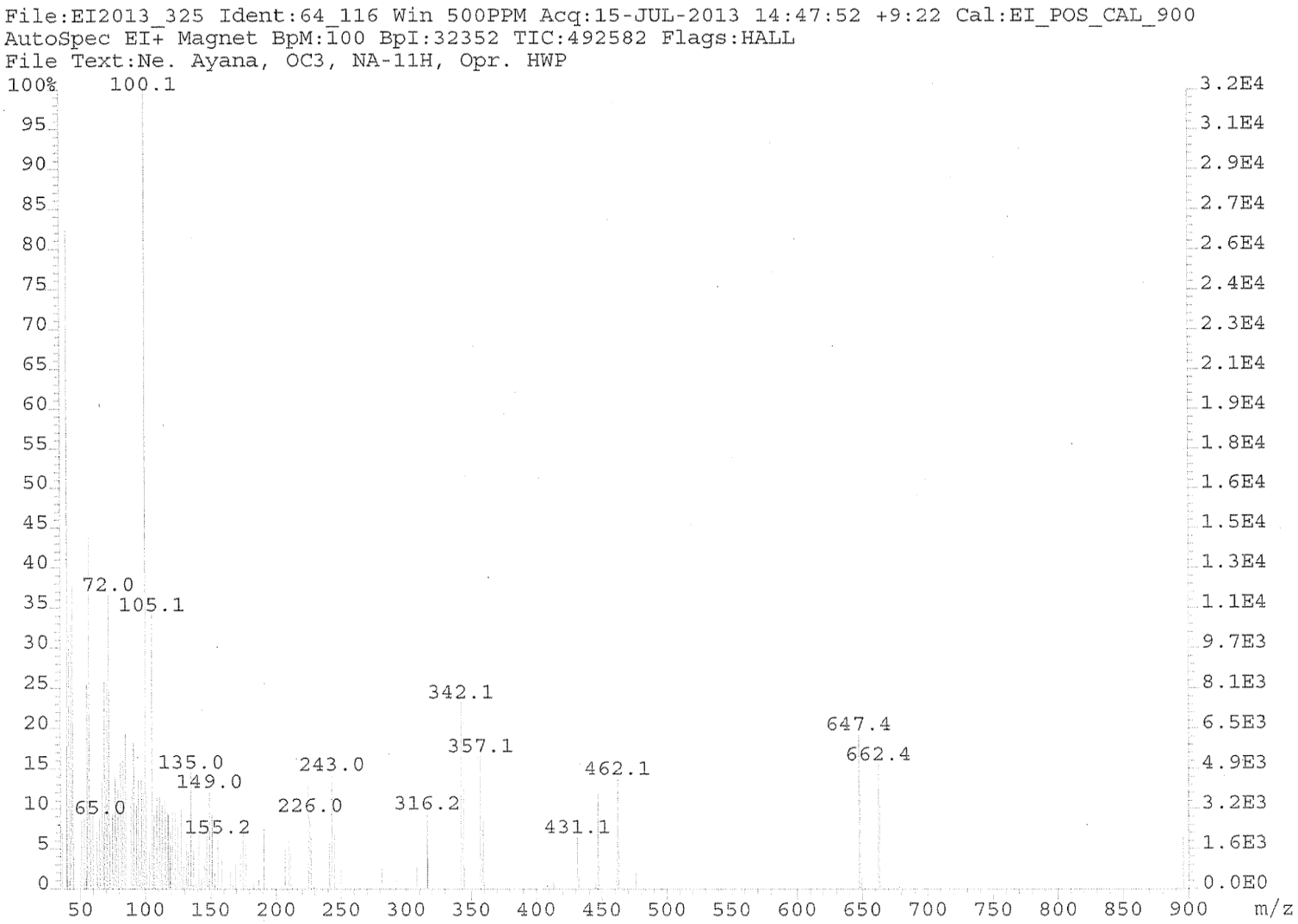
Appendix 15D: HMBC spectrum of 8, 6'-dimethoxyknipholone (**150**), $CDCl_3$, 500 MHz



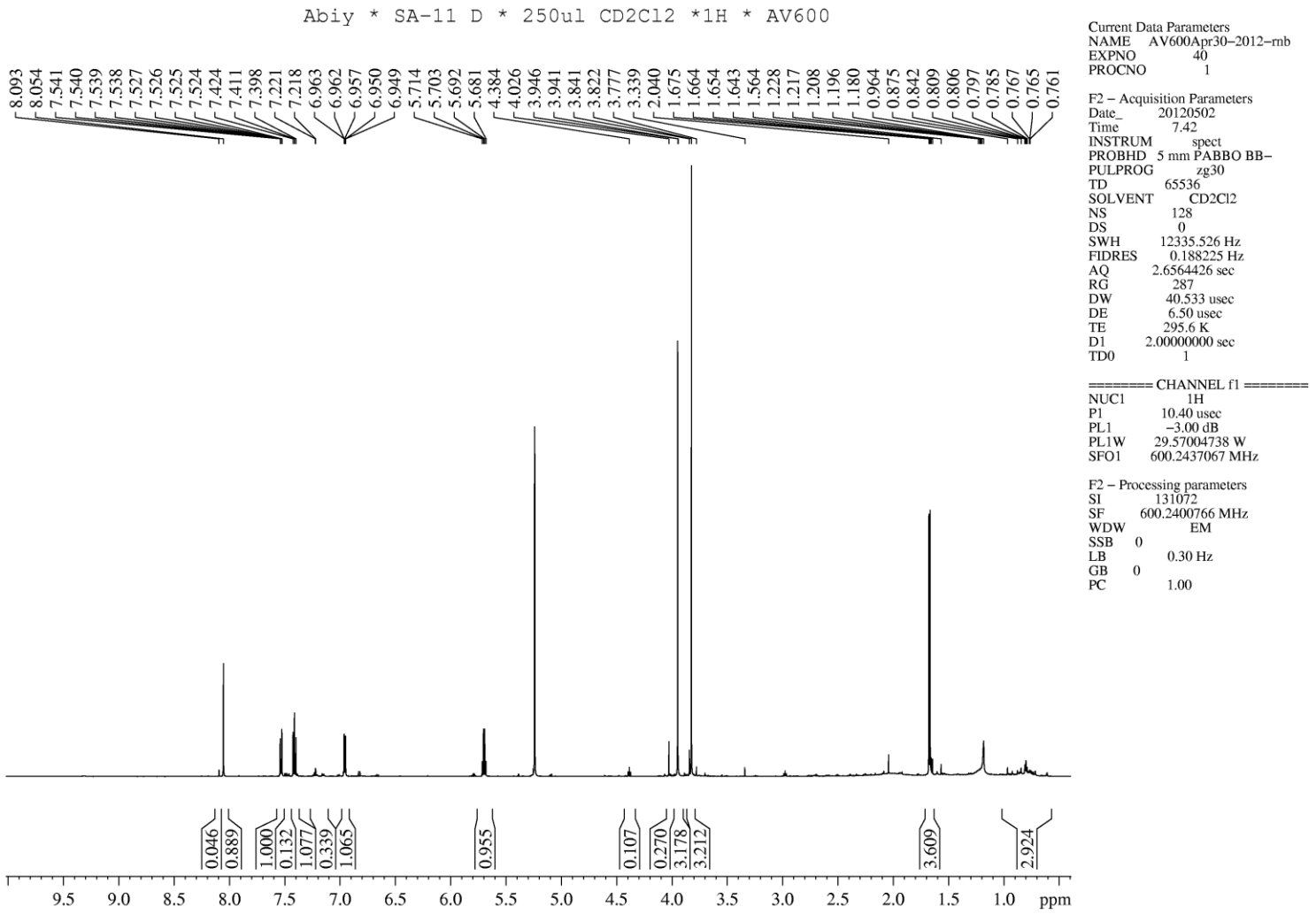
Appendix 15E: NOE spectrum of 8, 6'-dimethoxyknipholone (**150**), $CDCl_3$, 500 MHz



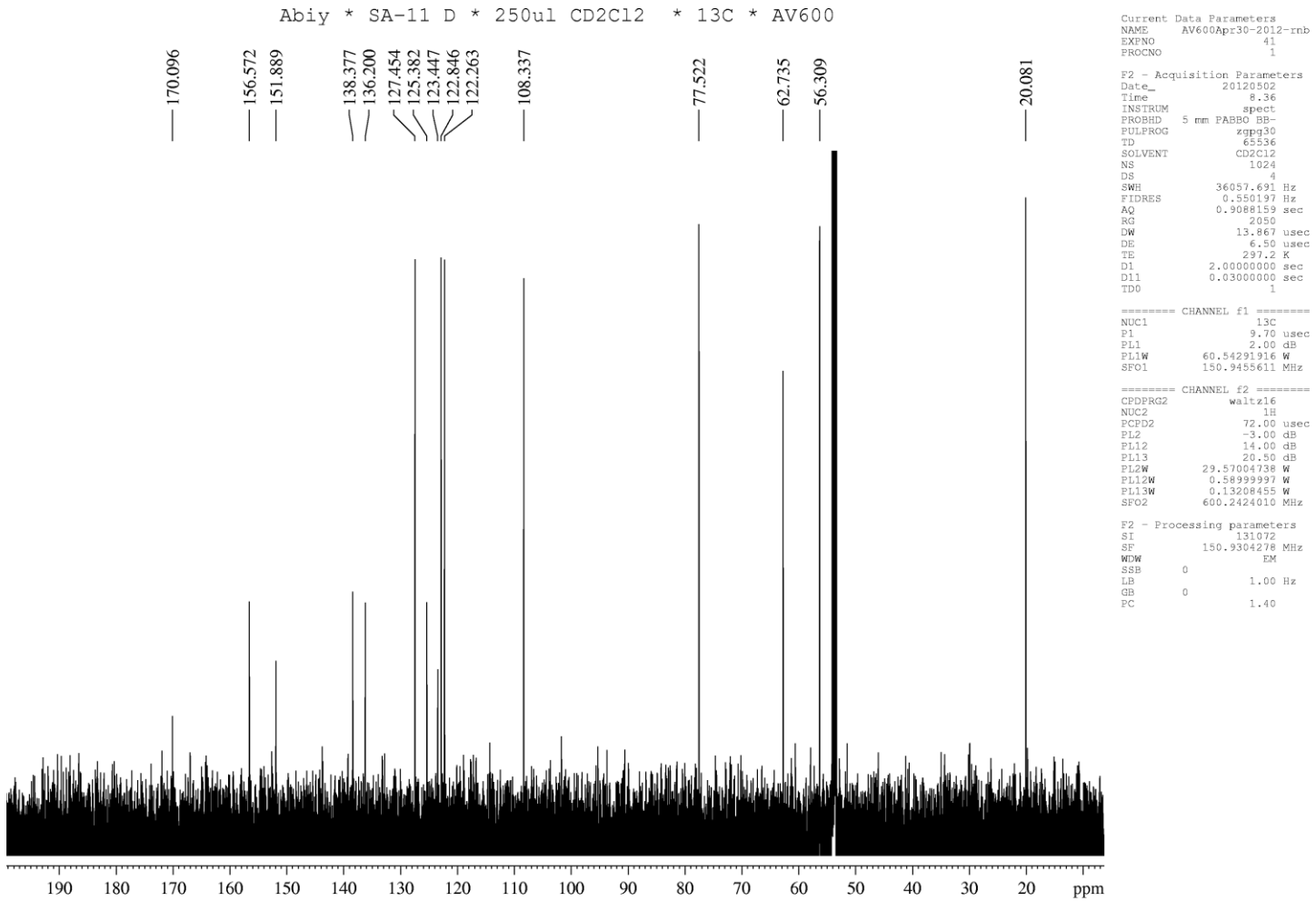
Appendix 15F: ESIMS spectrum of 8, 6'-dimethoxyknipholone (**150**)



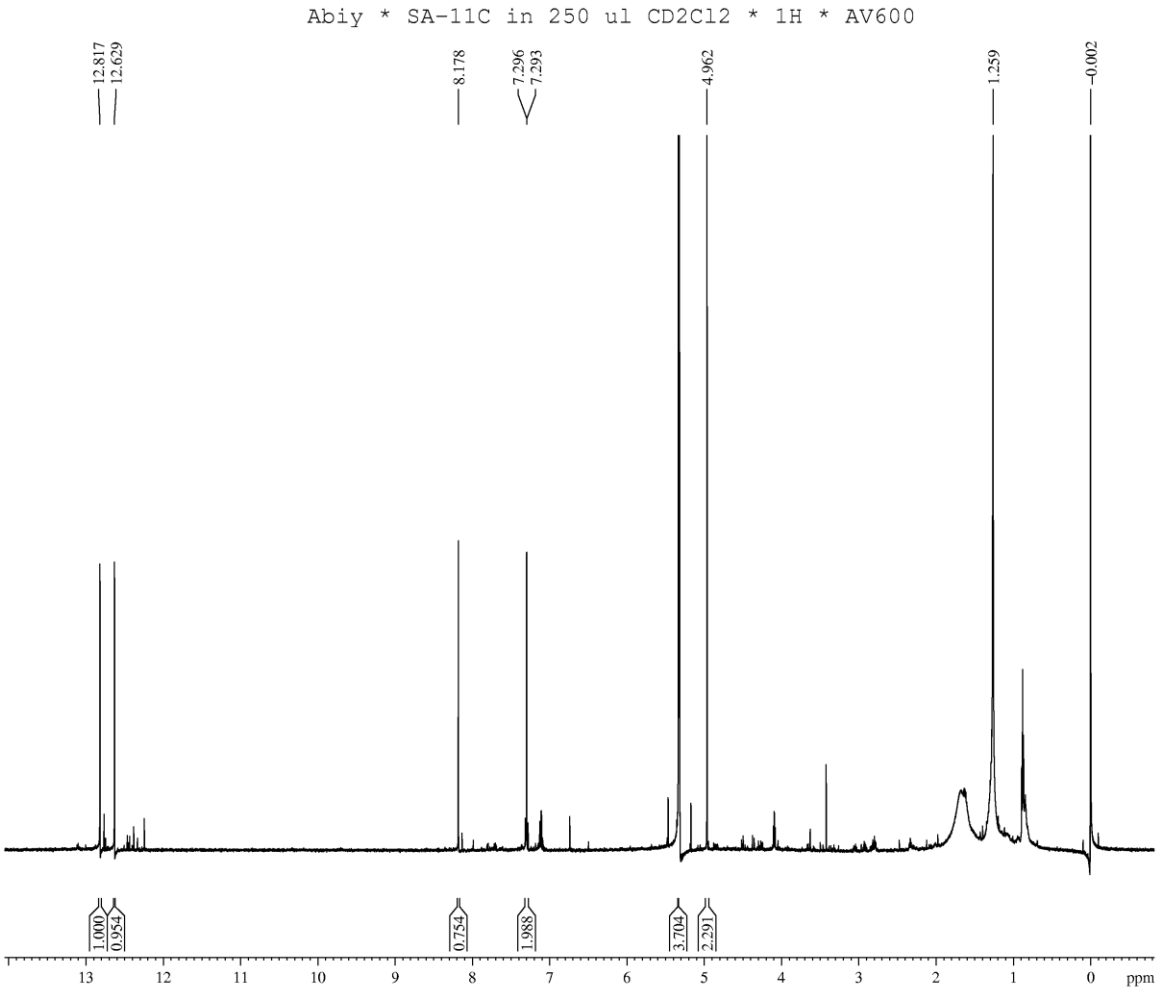
Appendix 16A: ^{1}H NMR spectrum of 4-*O*-methyleleutherol (**53**), CD_2Cl_2, 600 MHz



Appendix 16B: ^{13}C NMR spectrum of of 4-*O*-methyleleutherol (**53**), CD_2Cl_2, 200 MHz



Appendix 17A: ^{1}H NMR spectrum of 5,8-dihydroxy-1-hydroxymethynaphtho[2,3-C]furan-4,9-dione (**50**), CD_2Cl_2, 600 MHz



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PROCNO 1

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Time 16.23
INSTRUM spect
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TD 65536
SOLVENT CD2Cl2
NS 128
DS 0
SWH 12335.526 Hz
FIDRES 0.188225 Hz
AQ 2.6564426 sec
RG 512
DW 40.533 usec
DE 6.50 usec
TE 296.2 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 =======
NUC1 1H
P1 10.40 usec
PL1 –3.00 dB
PL1W 29.57004738 W
SFO1 600.2437067 MHz

F2 – Processing parameters
SI 131072
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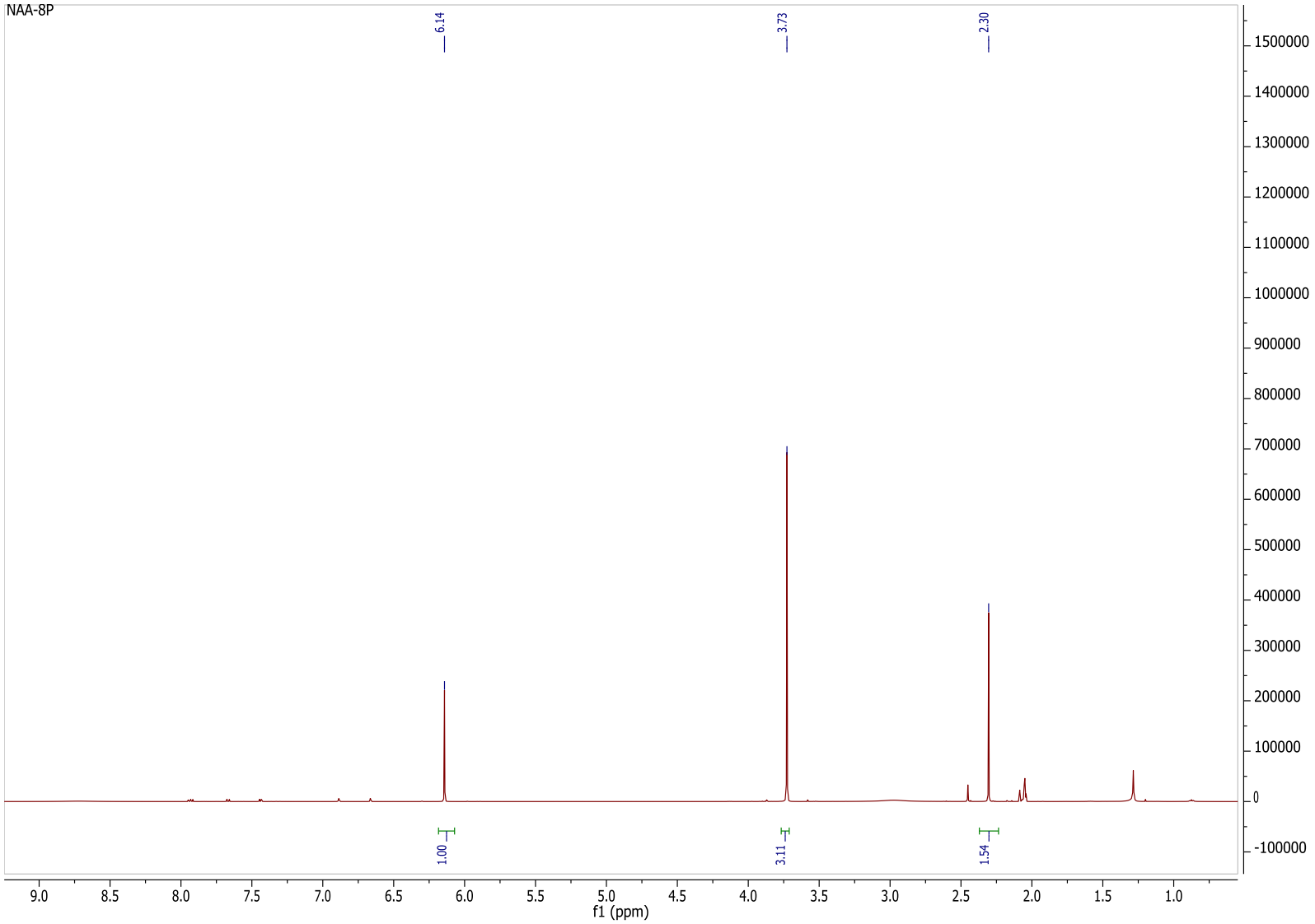
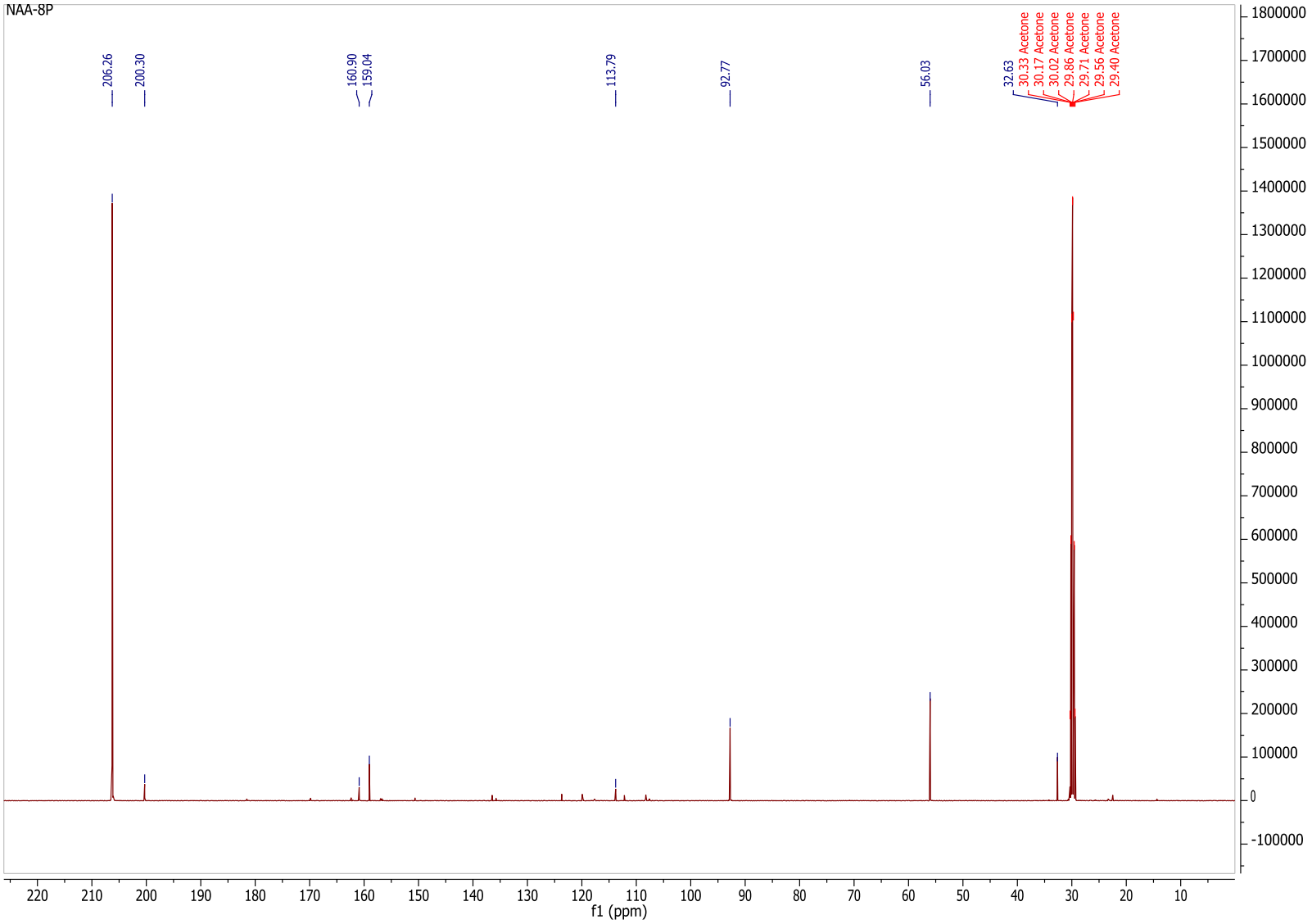
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

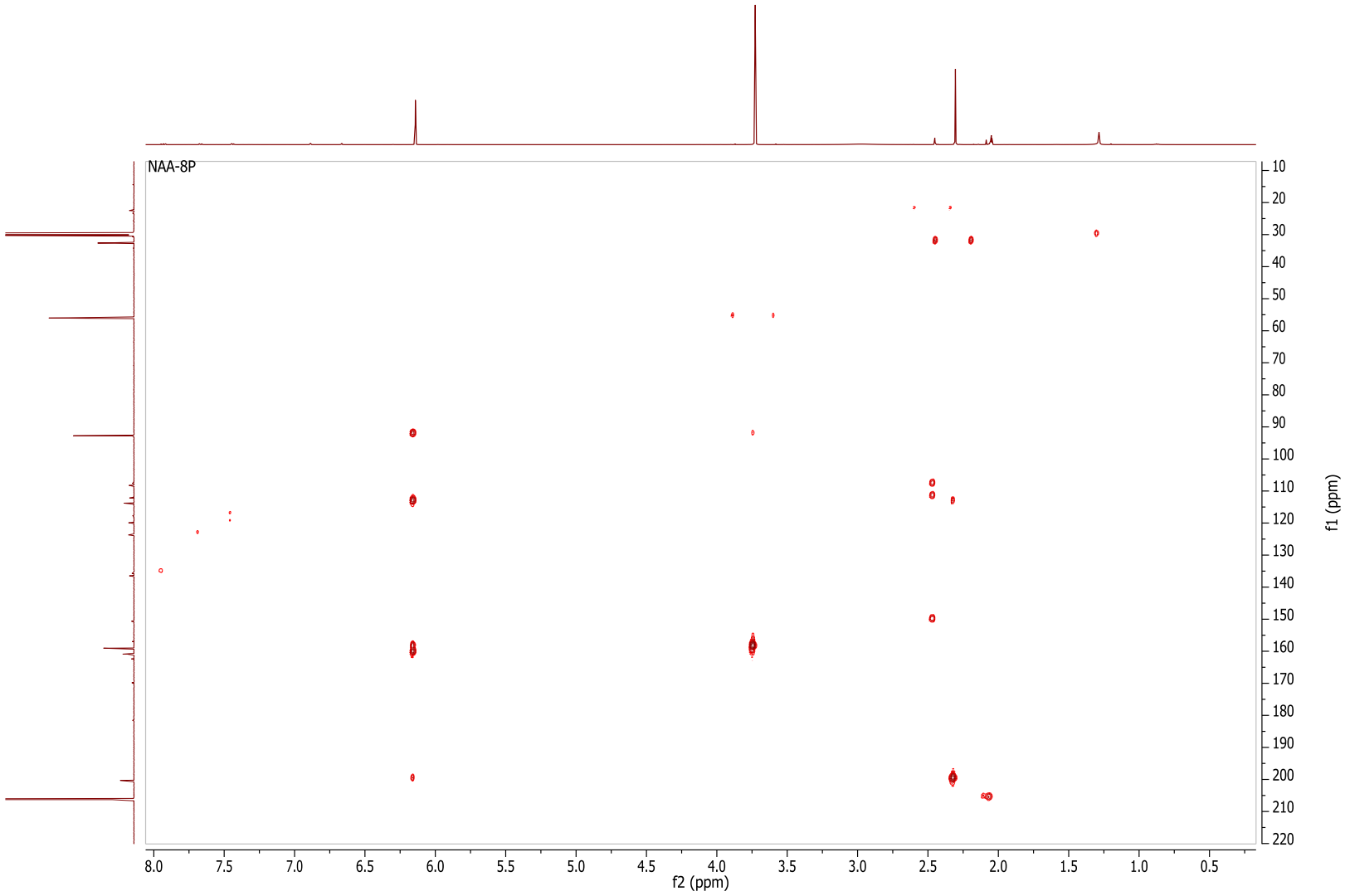
Appendix 18A: ^{1}H NMR spectrum of 2,6-dimethoxy-4-hydroxyacetophenone (**151**), acetone-d_6, 500 MHz



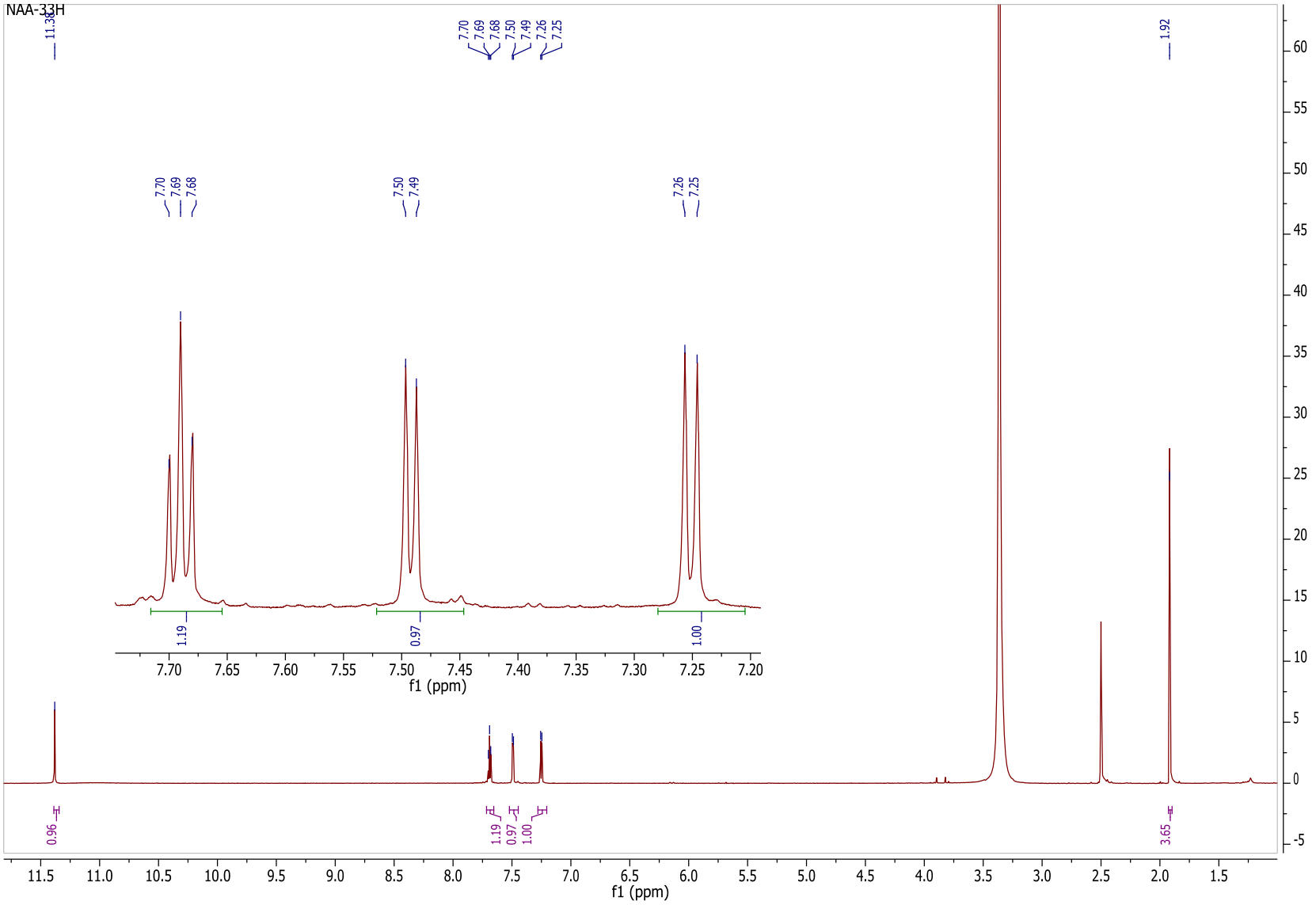
Appendix 18B: ^{13}C NMR spectrum of 2,6-dimethoxy-4-hydroxyacetophenone (**151**), acetone-d_6, 125 MHz



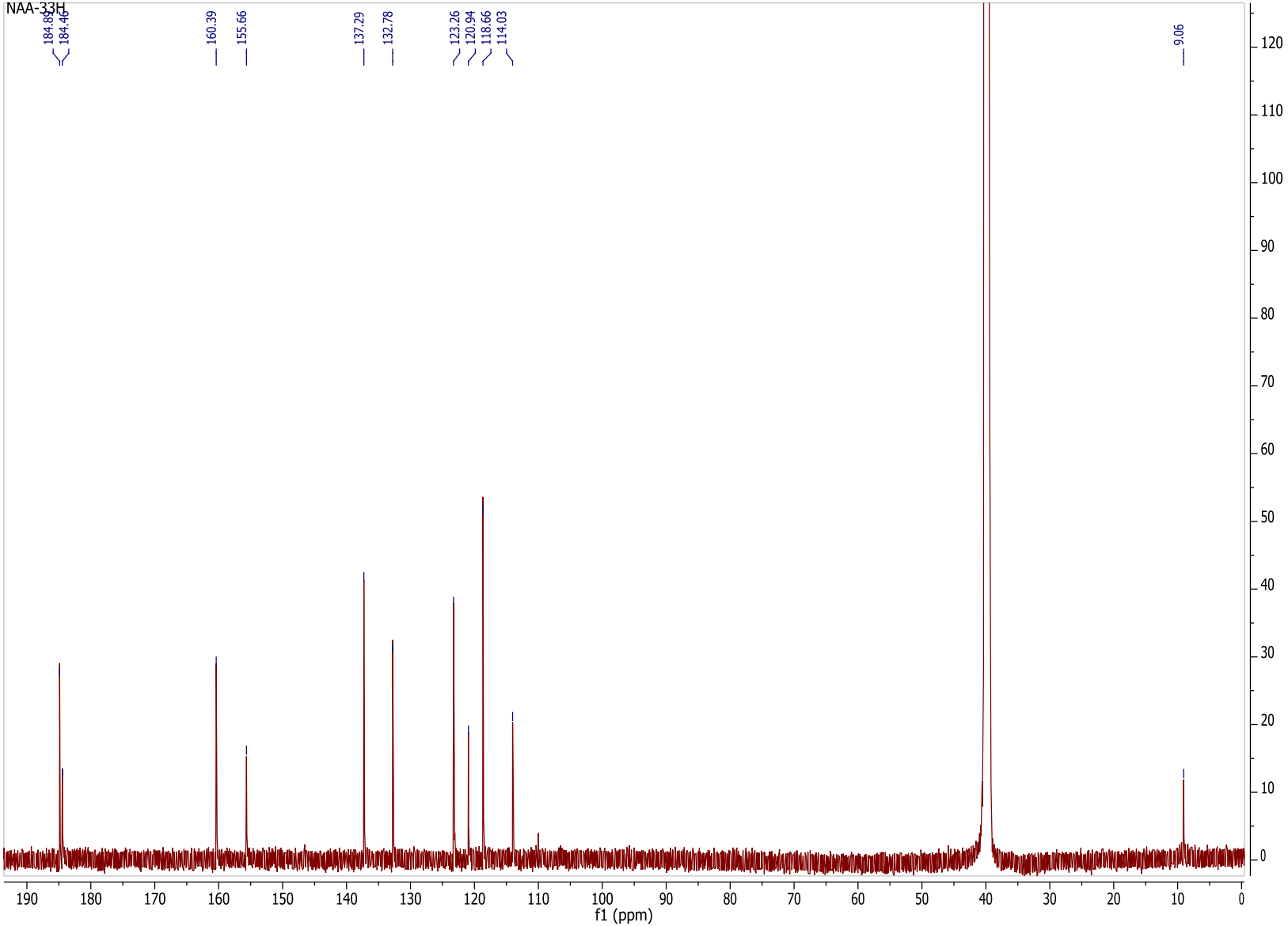
Appendix 18C: HMBC spectrum of 2,6-dimethoxy-4-hydroxyacetophenone (**151**), acetone-d_6, 500 MHz



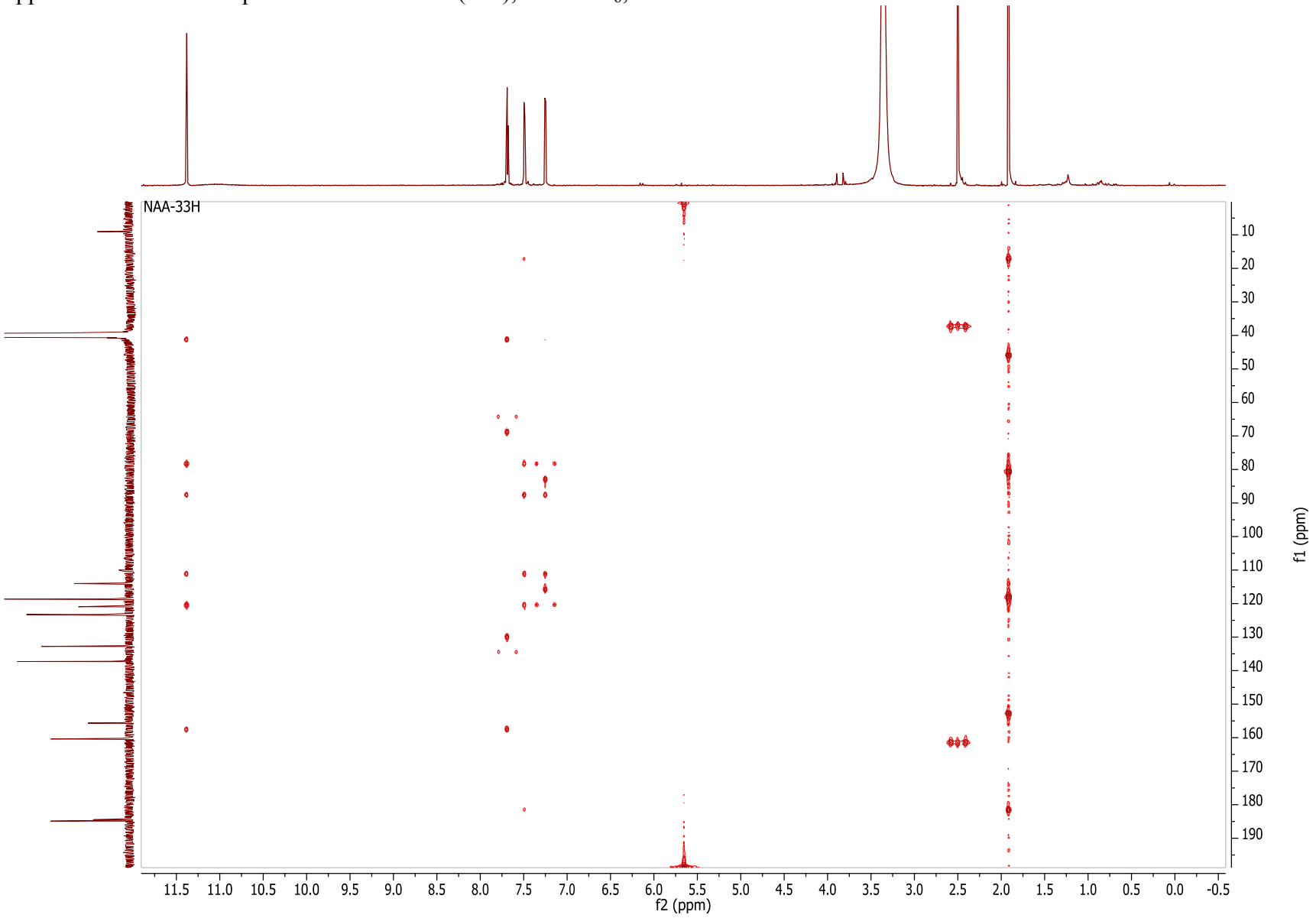
Appendix 19A: ^{1}H NMR spectrum of droserone (**123**), DMSO-d_6, 600 MHz



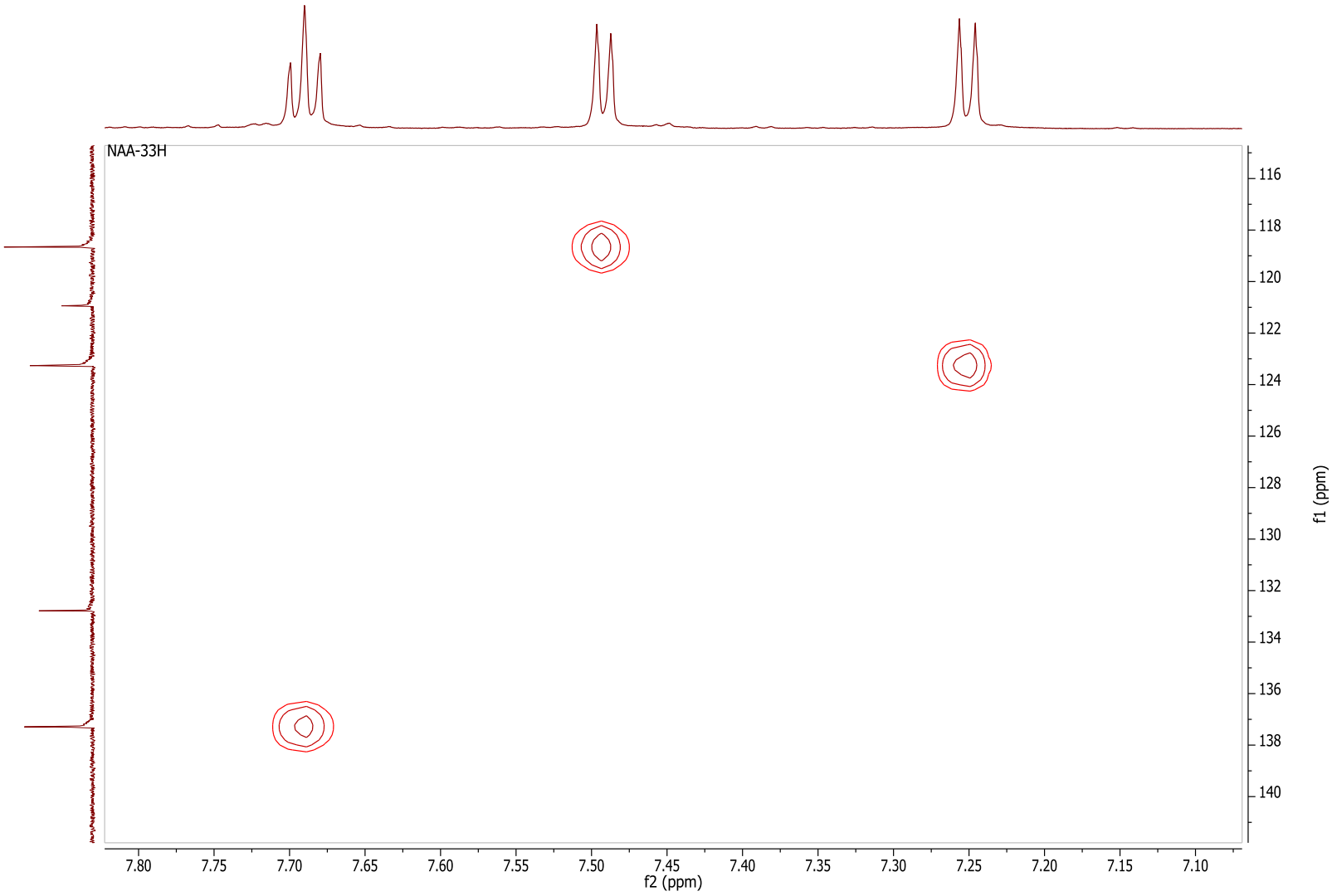
Appendix 19B: ^{13}C NMR spectrum of droserone (**123**), DMSO-d_6, 200 MHz



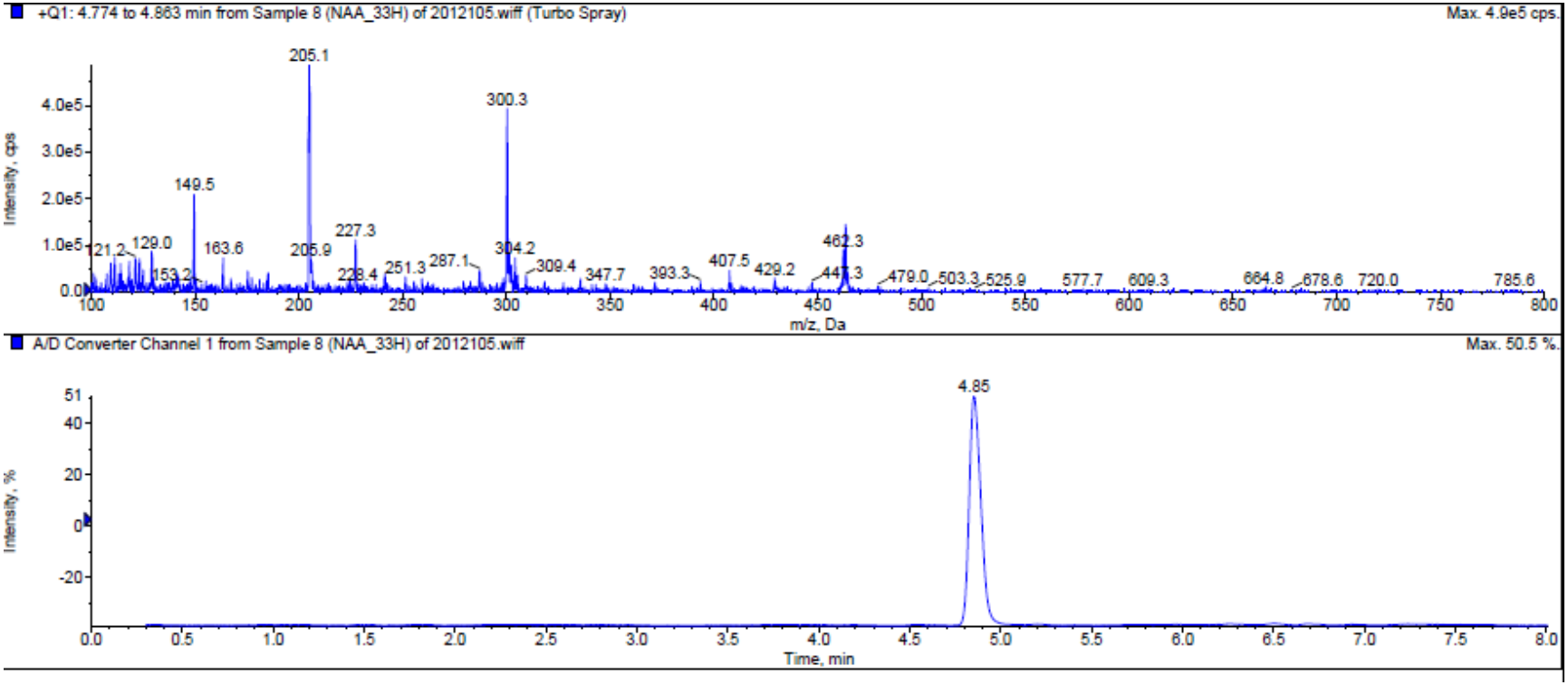
Appendix 19C: HMBC spectrum of droserone (**123**), DMSO-d_6, 600 MHz



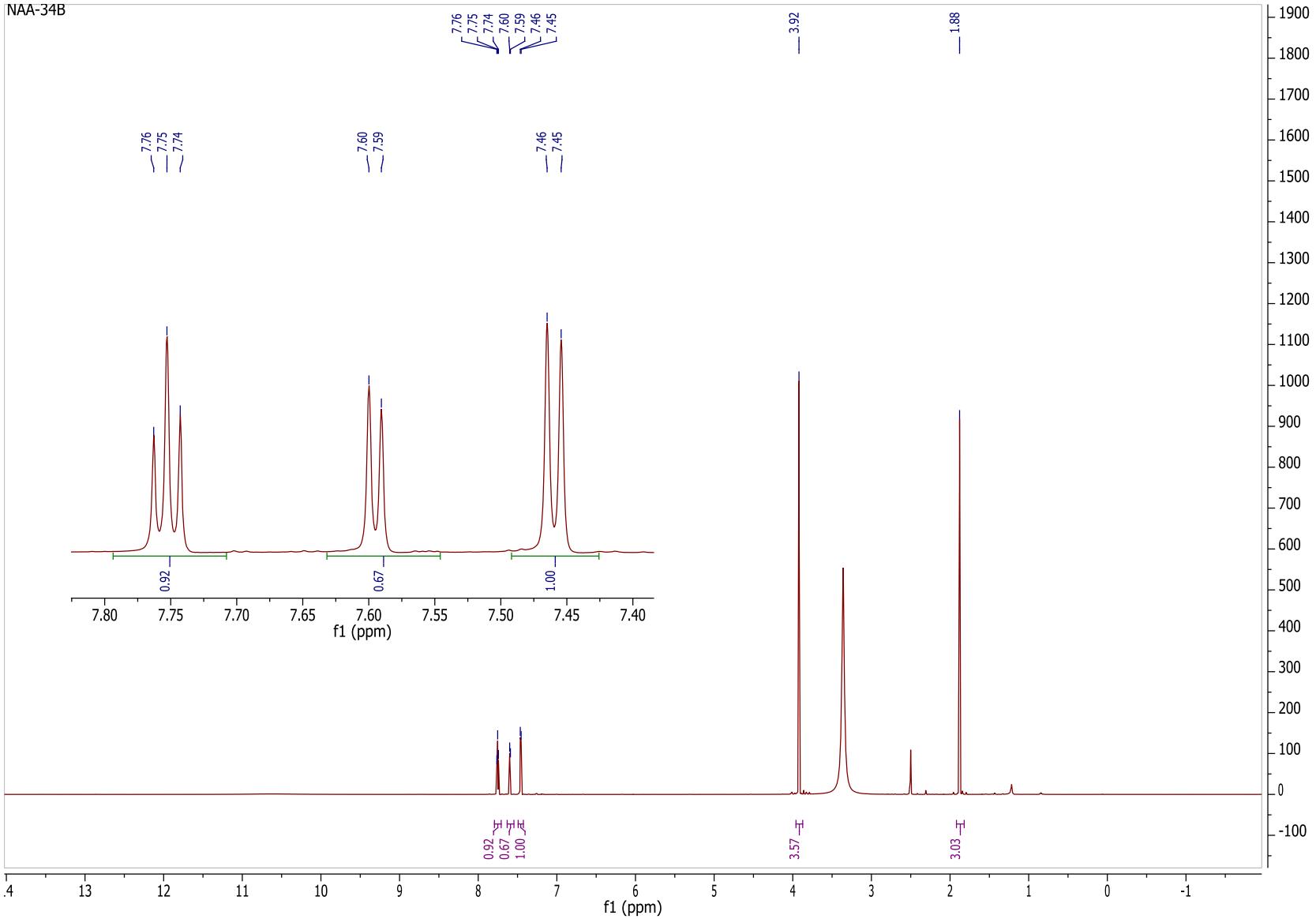
Appendix 19D: HSQC spectrum of droserone (**123**), DMSO-d_6, 600 MHz



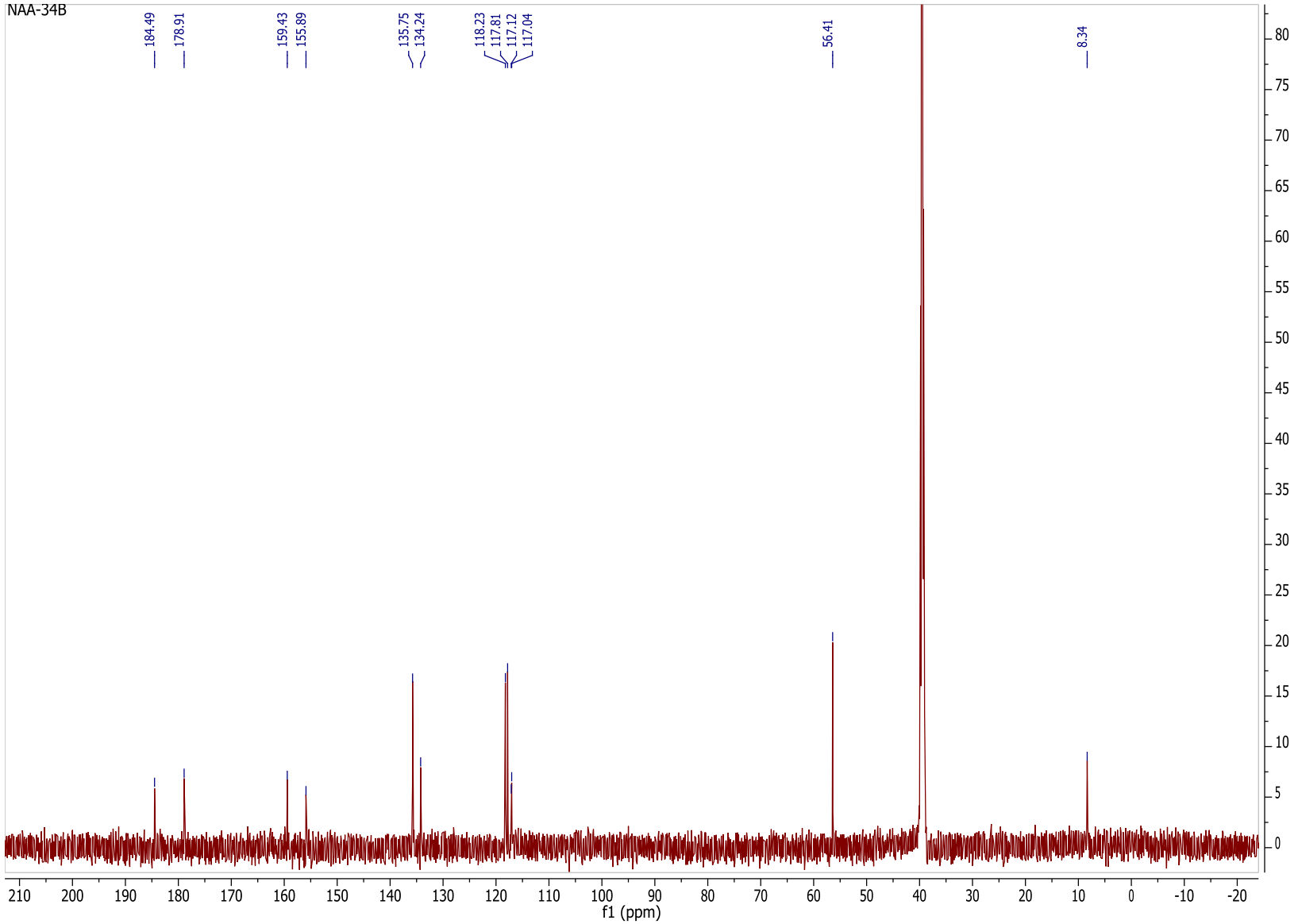
Appendix 19E: ESIMS spectrum of droserone (**123**)



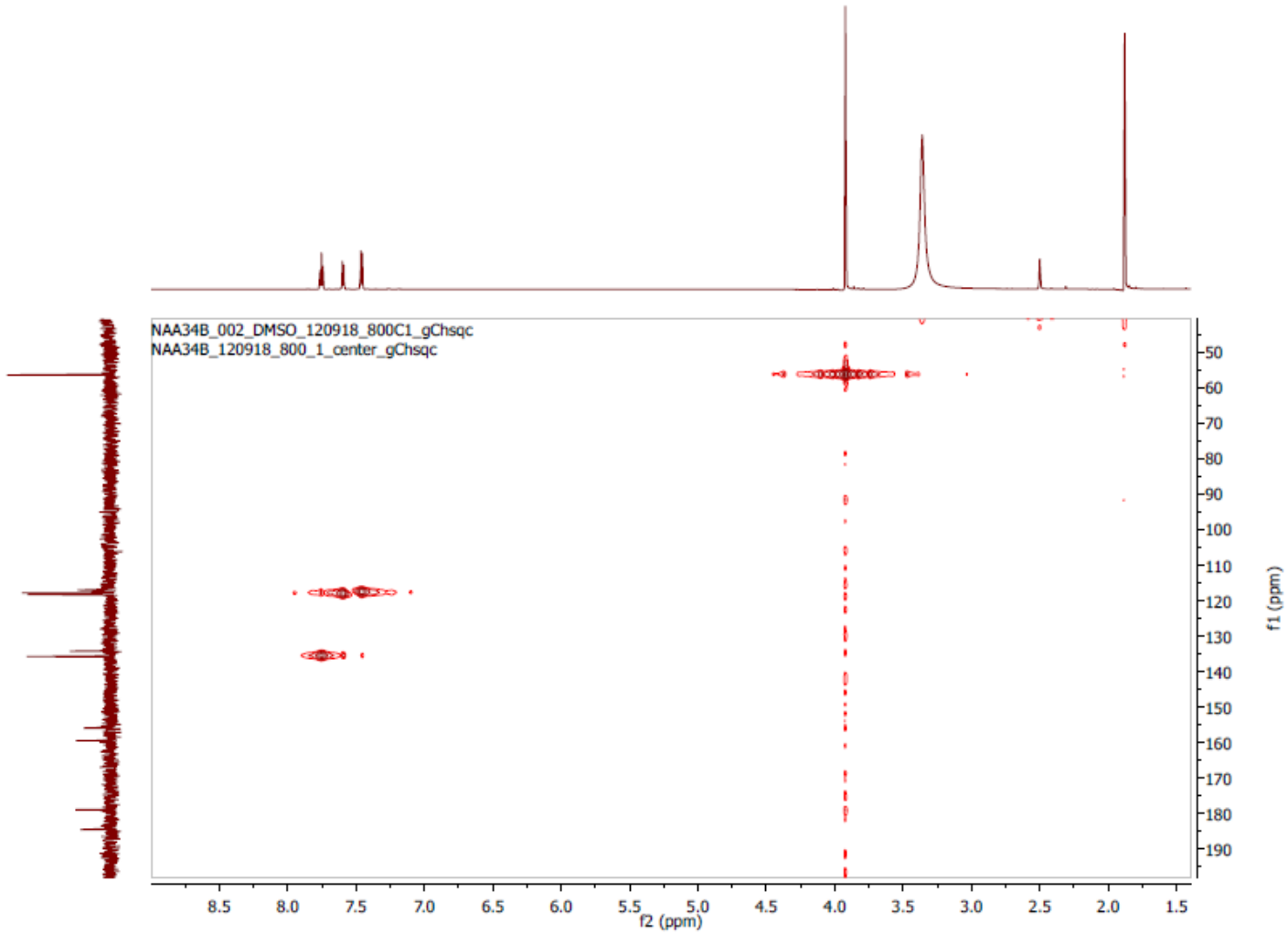
Appendix 20A: ^{1}H NMR spectrum of droserone 5-methyl ether (**152**), DMSO-d_6, 600 MHz



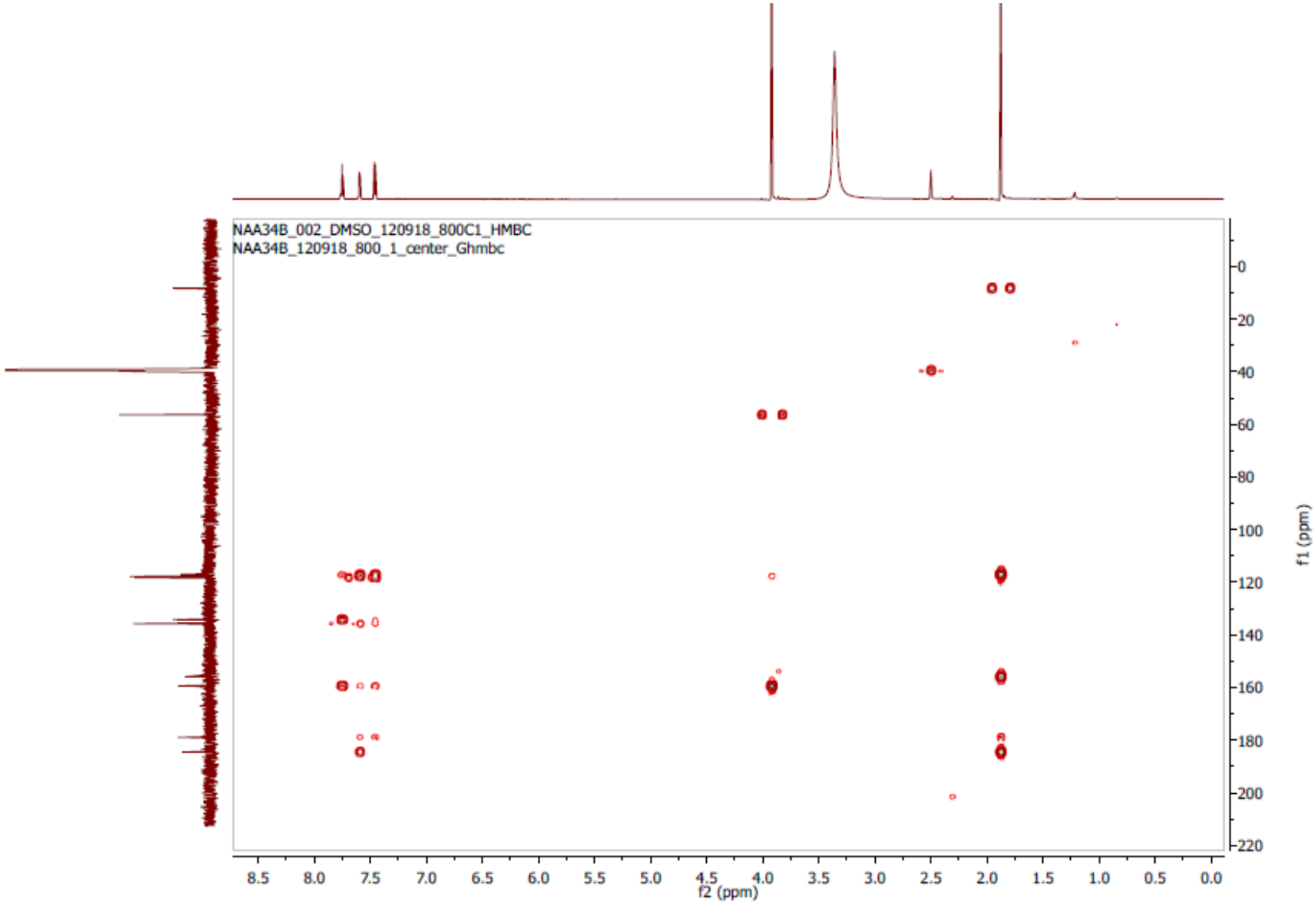
Appendix 20B: ^{13}C NMR spectrum of droserone 5-methyl ether (**152**), DMSO-d_6, 200 MHz



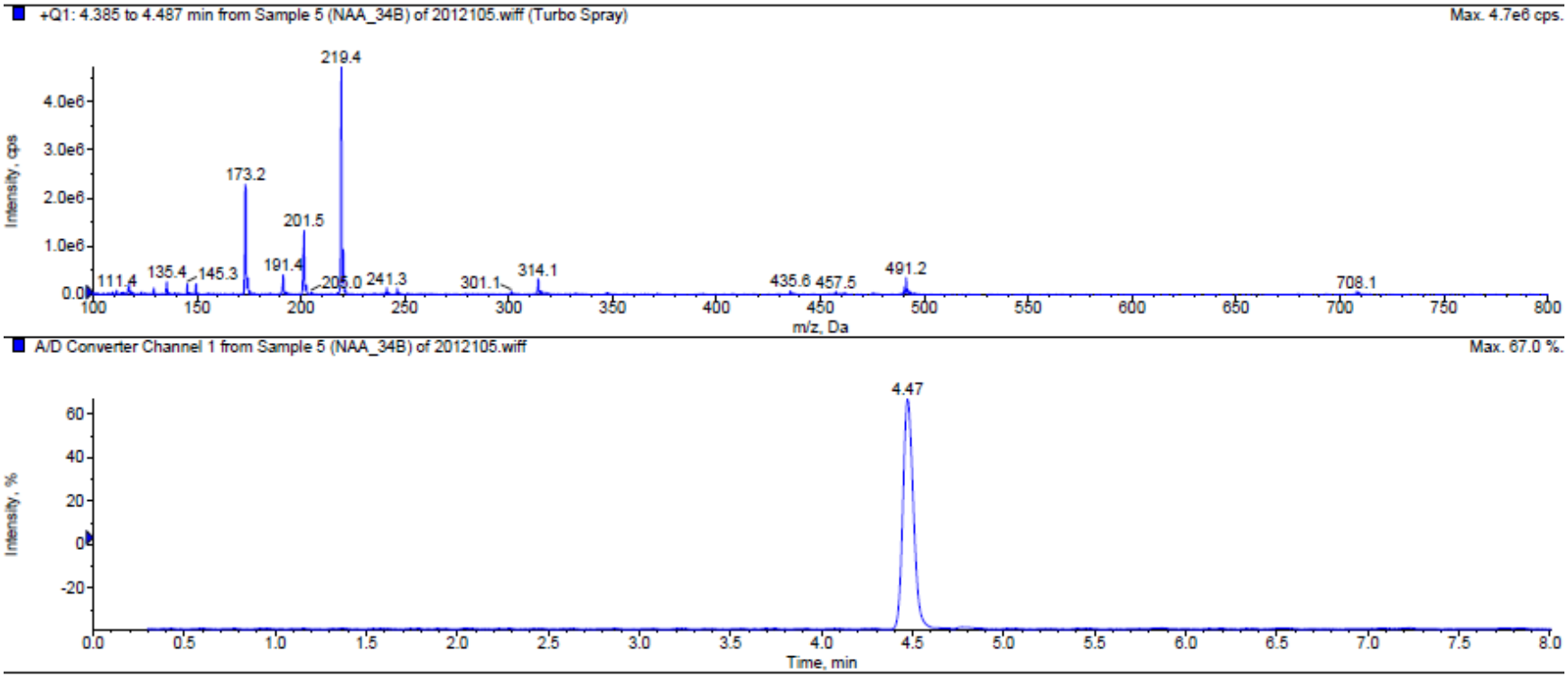
Appendix 20C: HSQC spectrum of droserone 5-methyl ether (**152**), DMSO-d_6, 600 MHz



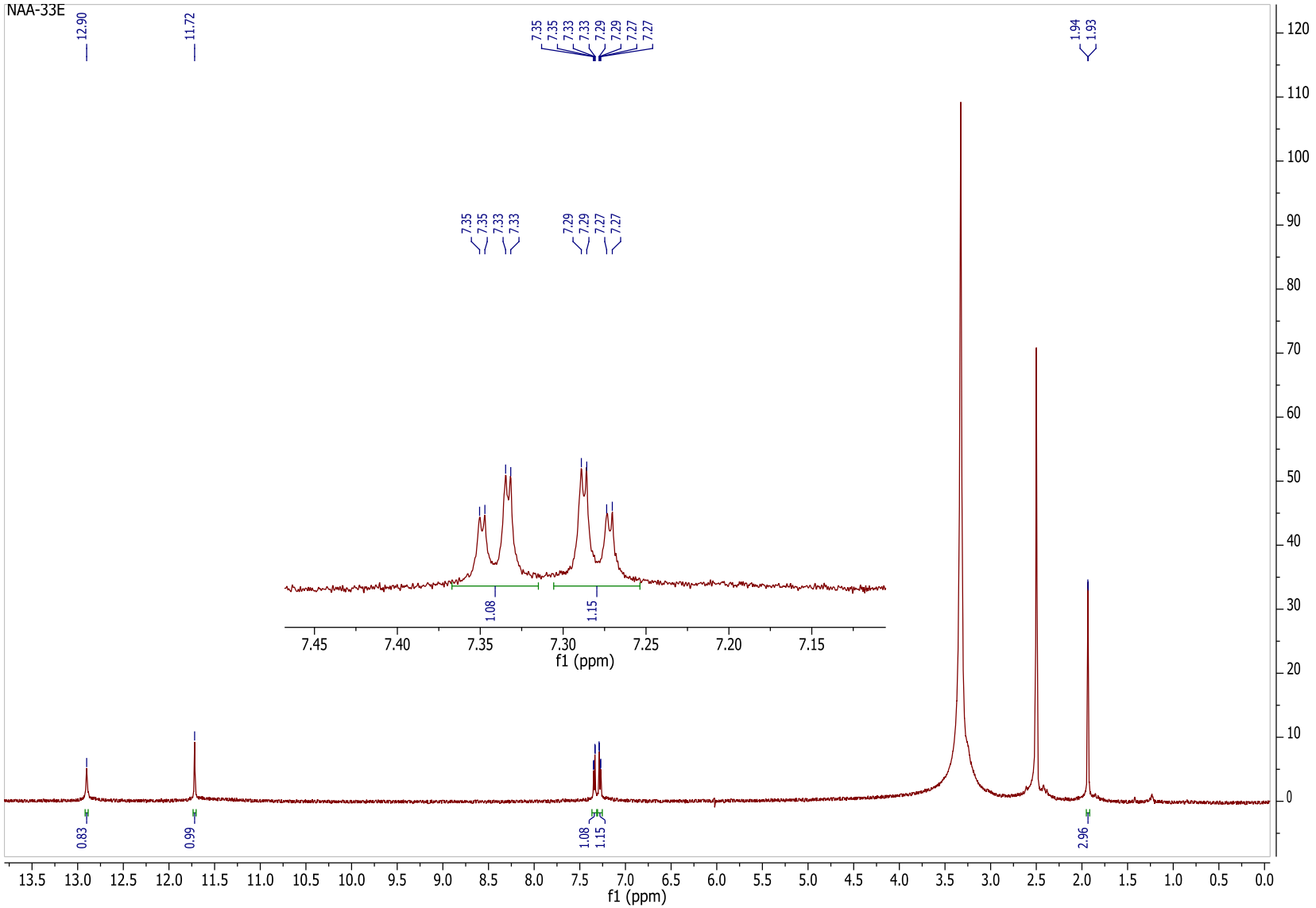
Appendix 20D: HMBC spectrum of droserone 5-methyl ether (**152**), DMSO-d_6, 600 MHz



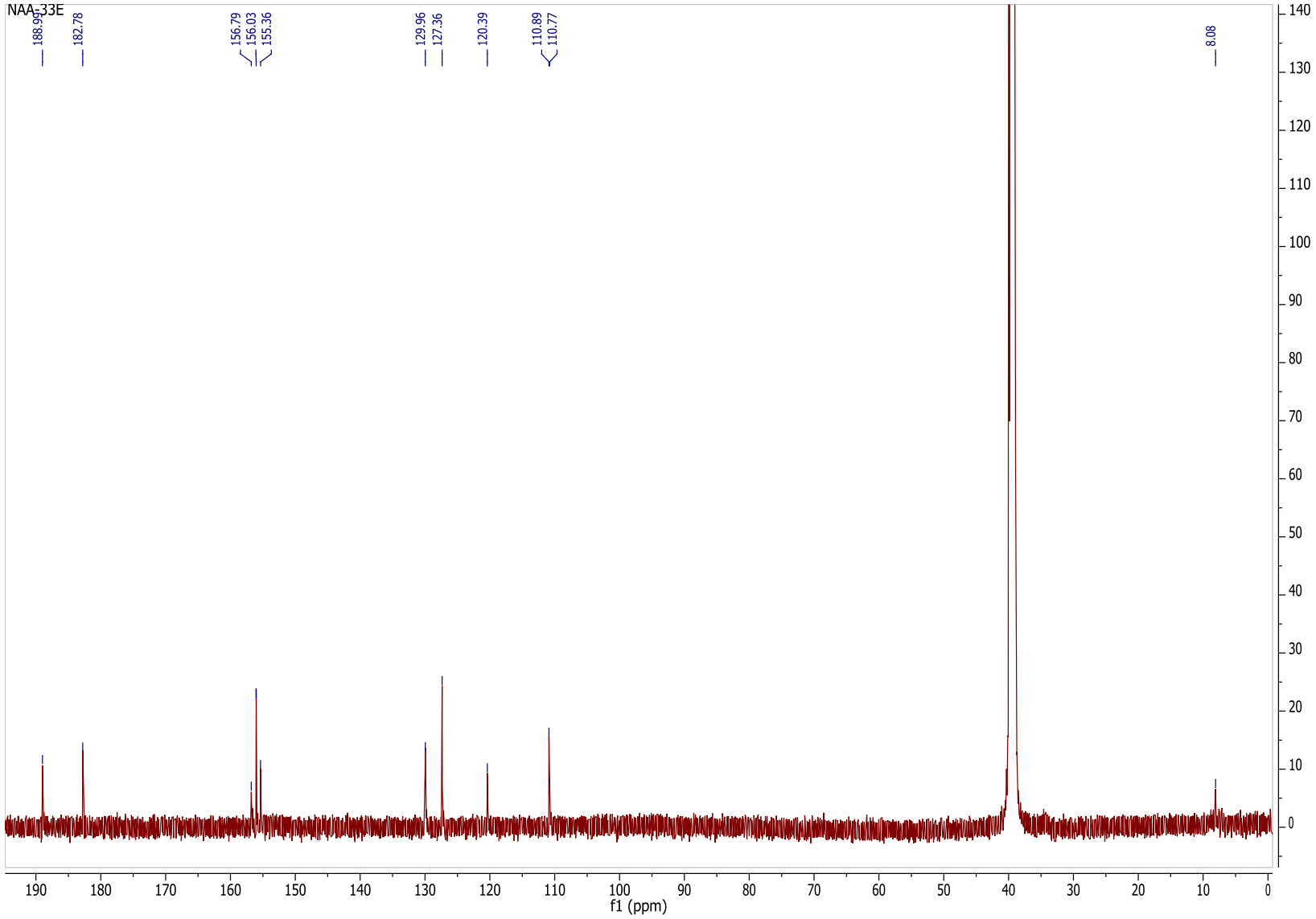
Appendix 20E: ESIMS spectrum of droserone 5-methyl ether (**152**)



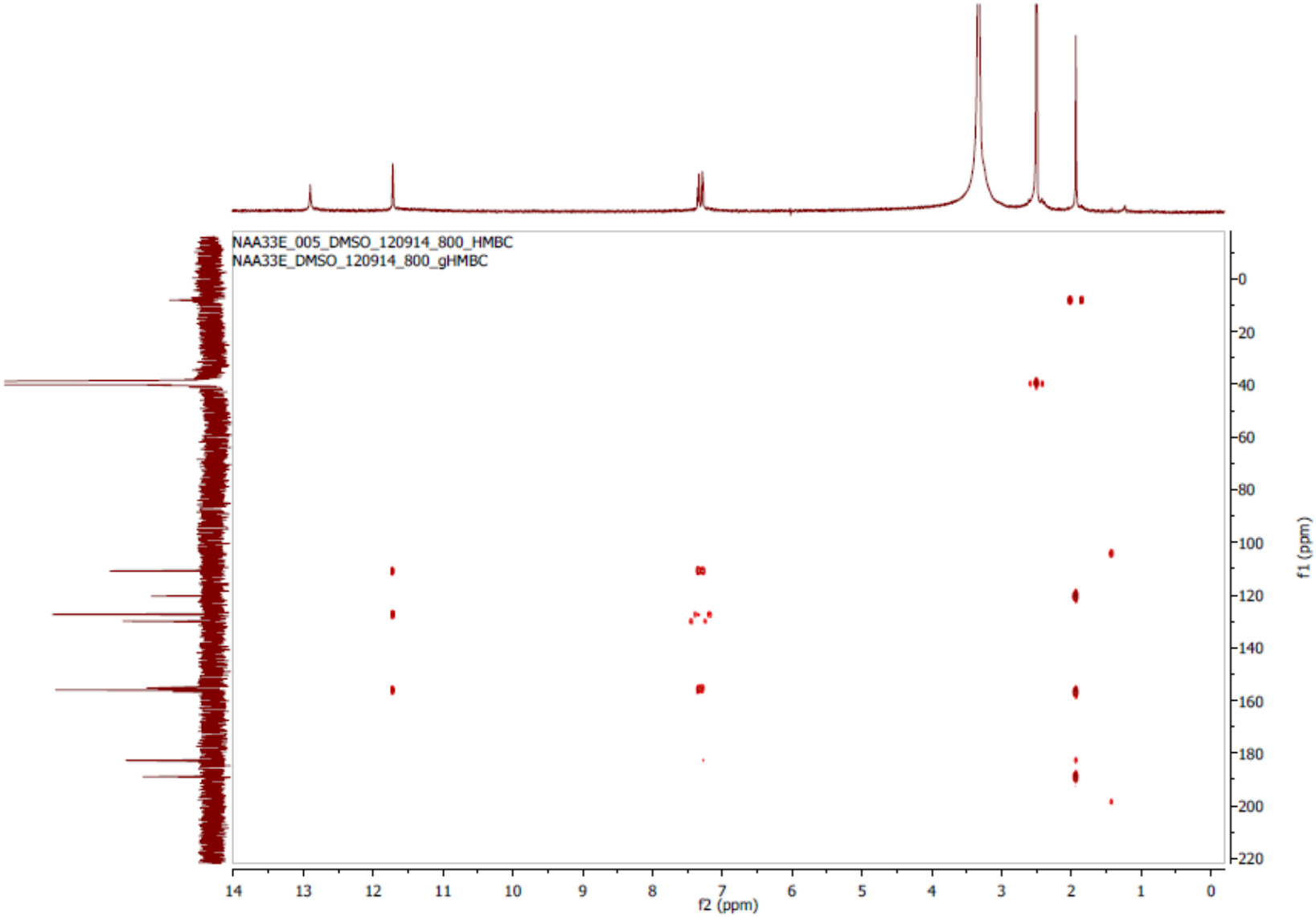
Appendix 21A: ^{1}H NMR spectrum of hydroxydroserone (**153**), DMSO-d_6, 600 MHz



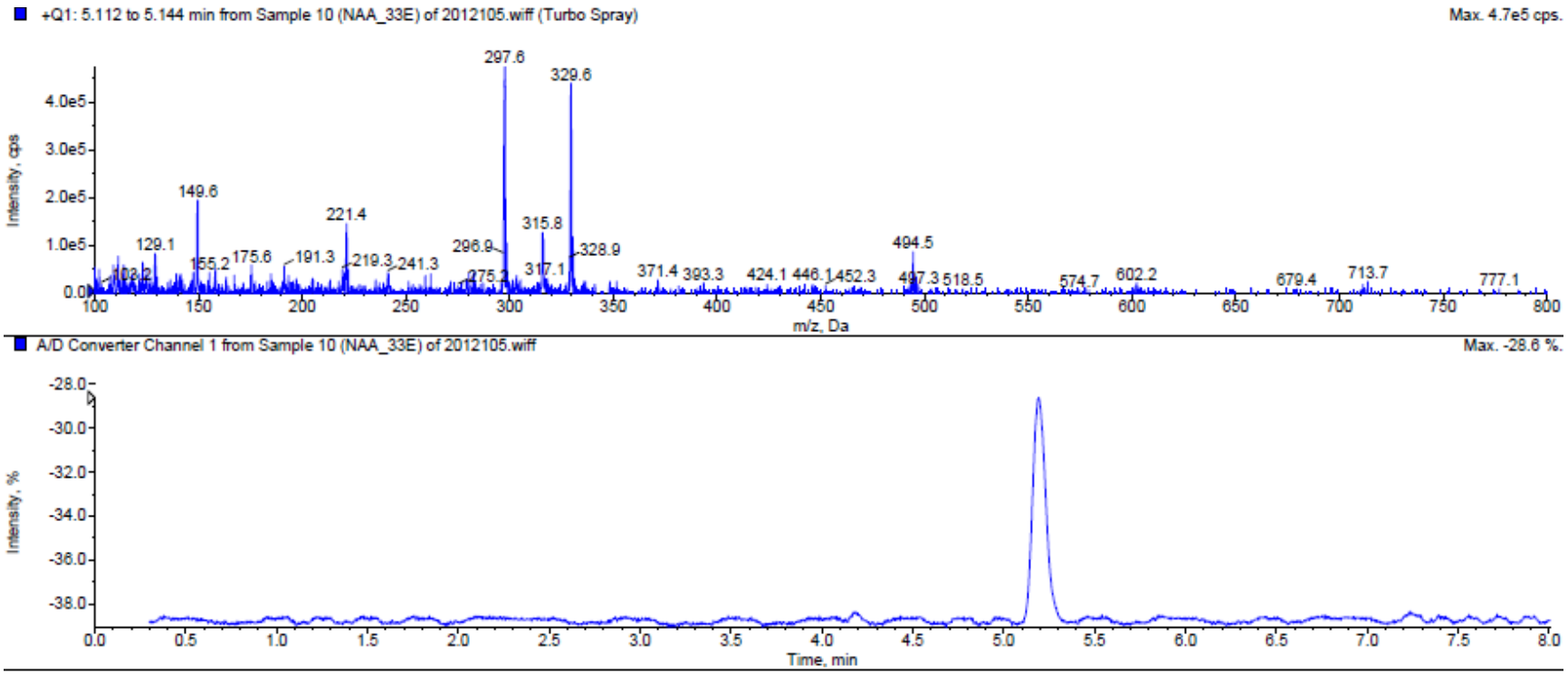
Appendix 21B: ^{13}C NMR spectrum of hydroxydroserone (**152**), DMSO-d_6, 200 MHz



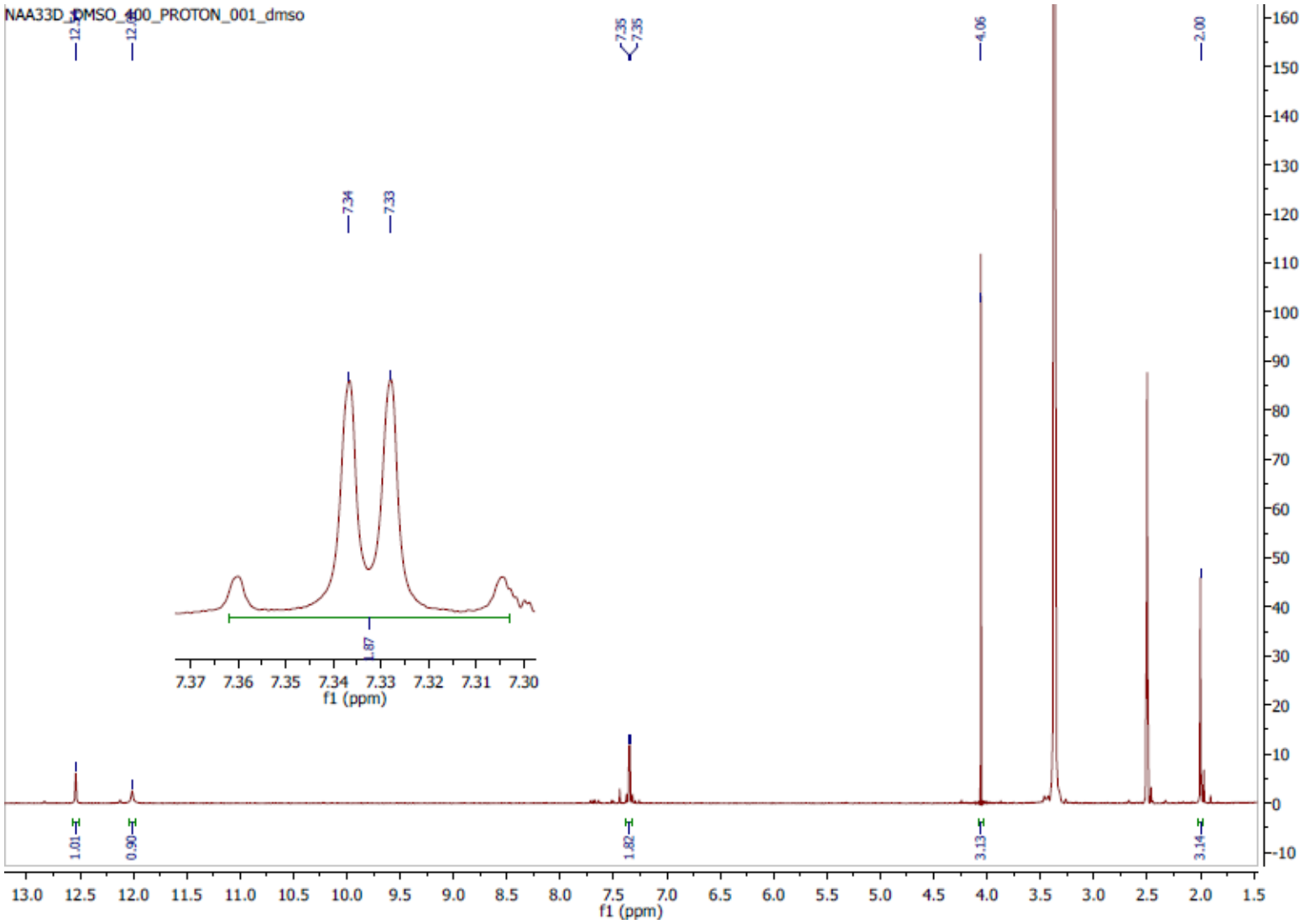
Appendix 21C: HMBC spectrum of hydroxydroserone (**153**), DMSO-d_6, 600 MHz



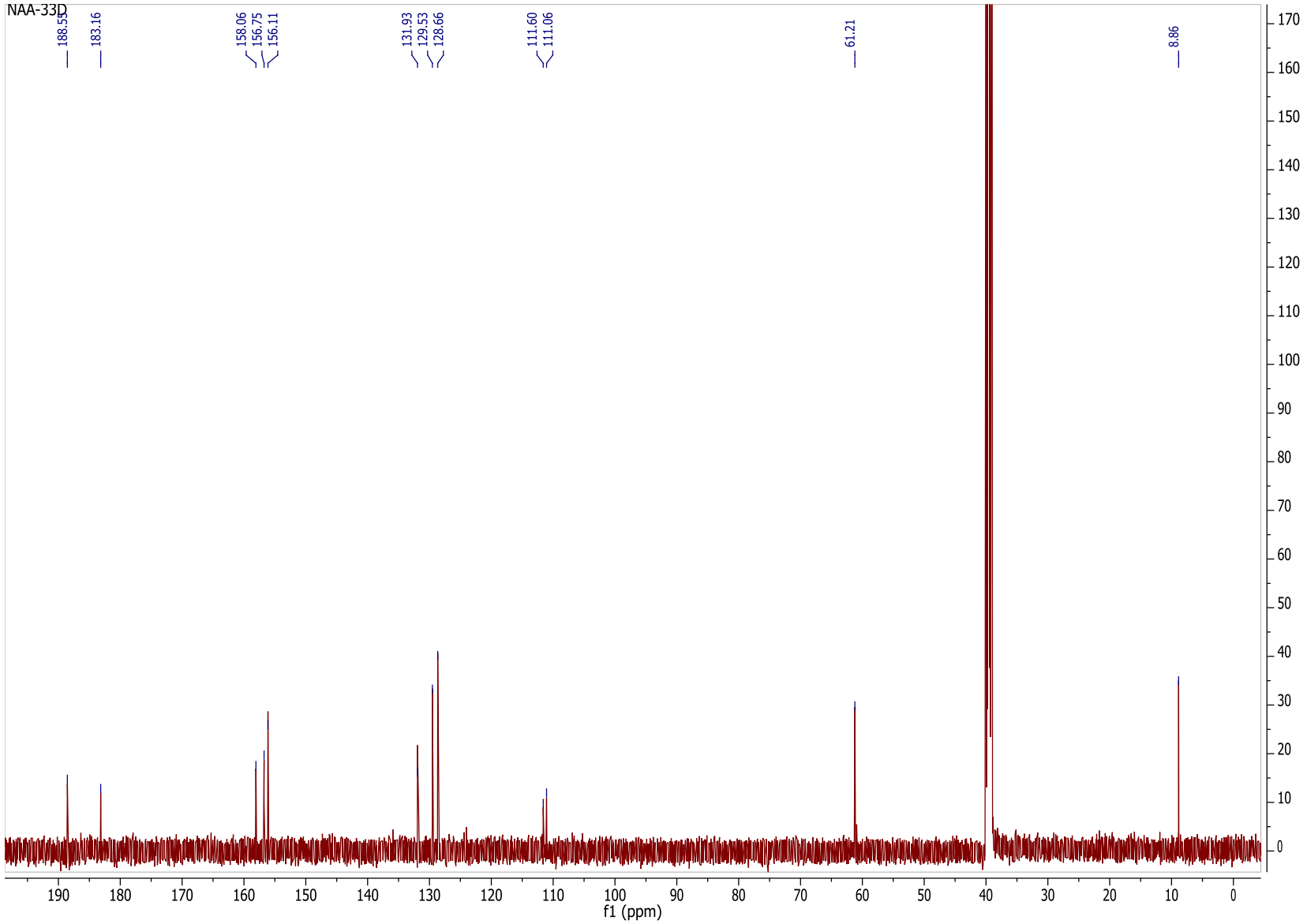
Appendix 21D: ESIMS spectrum of hydroxydroserone (**153**)



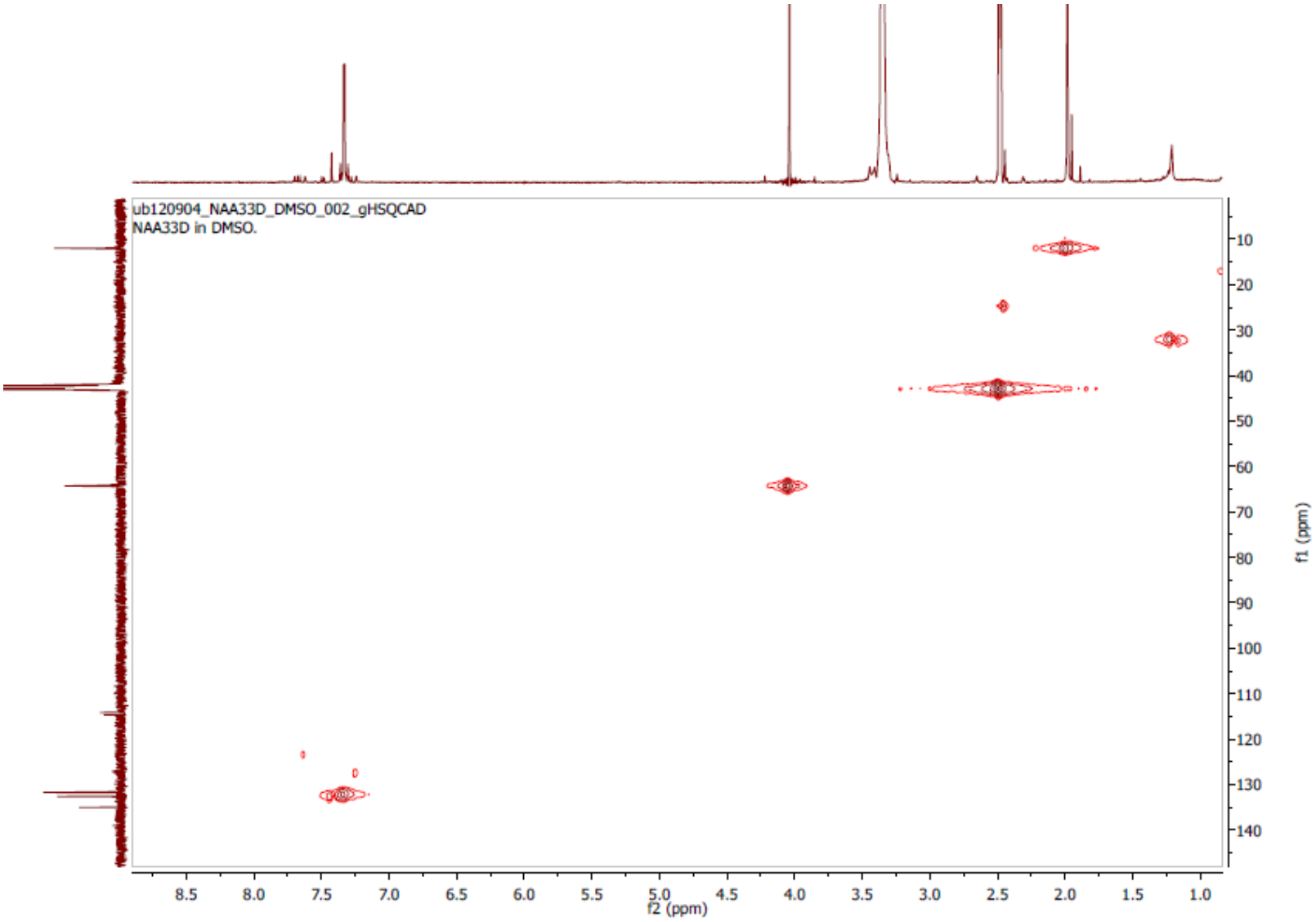
Appendix 22A: ^{1}H NMR spectrum of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), DMSO-d_6, 600 MHz



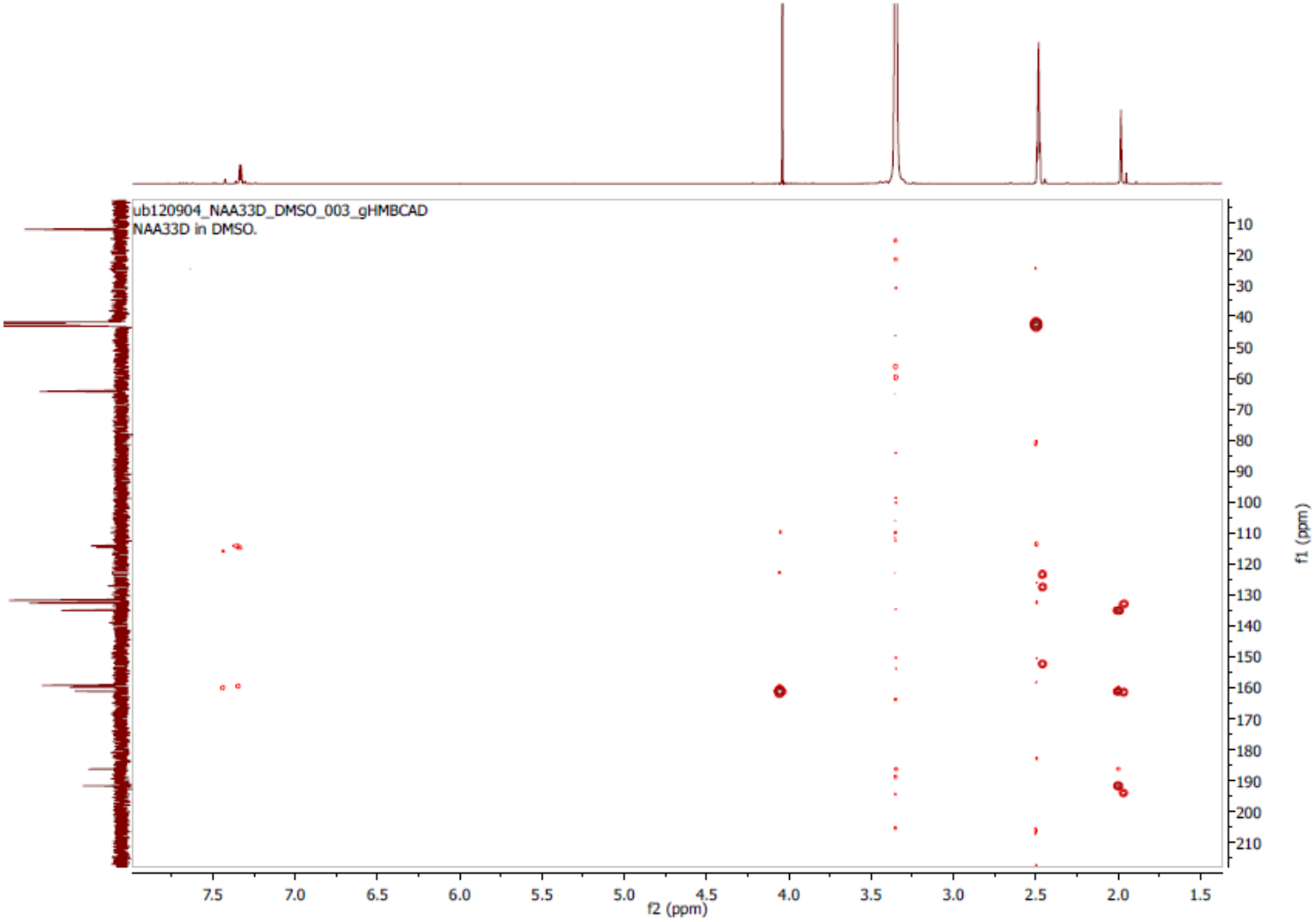
Appendix 22B: ^{13}C NMR spectrum of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), DMSO-d_6, 200 MHz



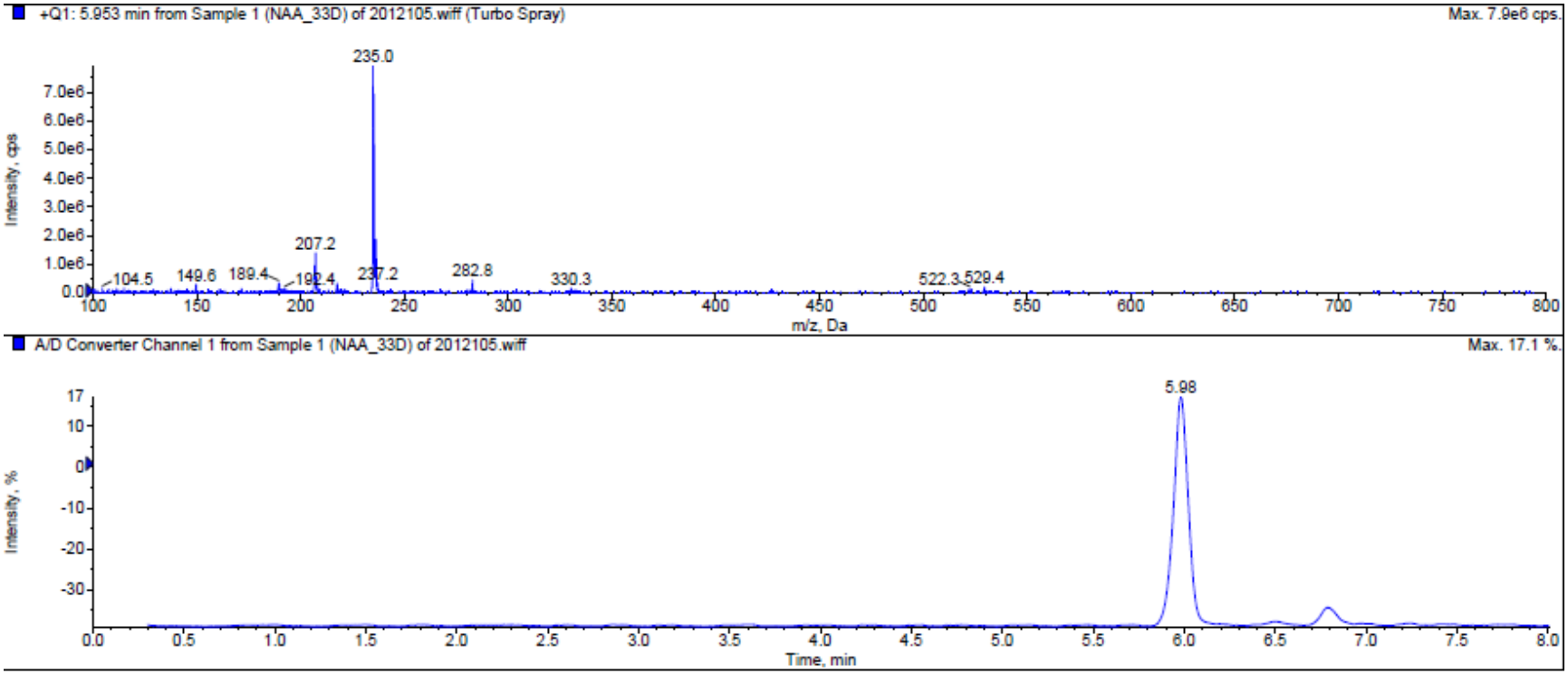
Appendix 22C: HSQC spectrum of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), DMSO-d_6, 600 MHz



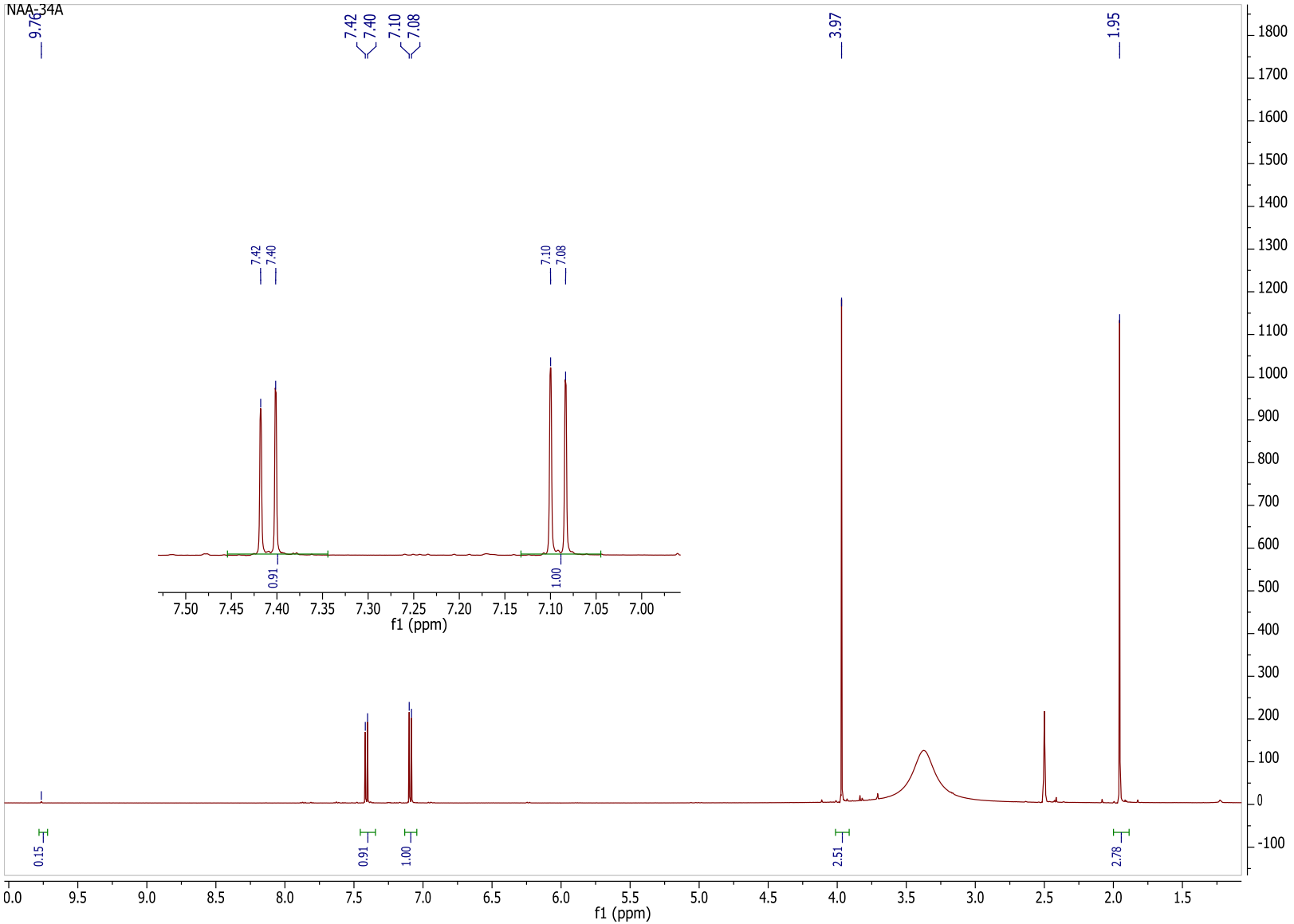
Appendix 22D: HMBC spectrum of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**), DMSO-d_6, 600 MHz



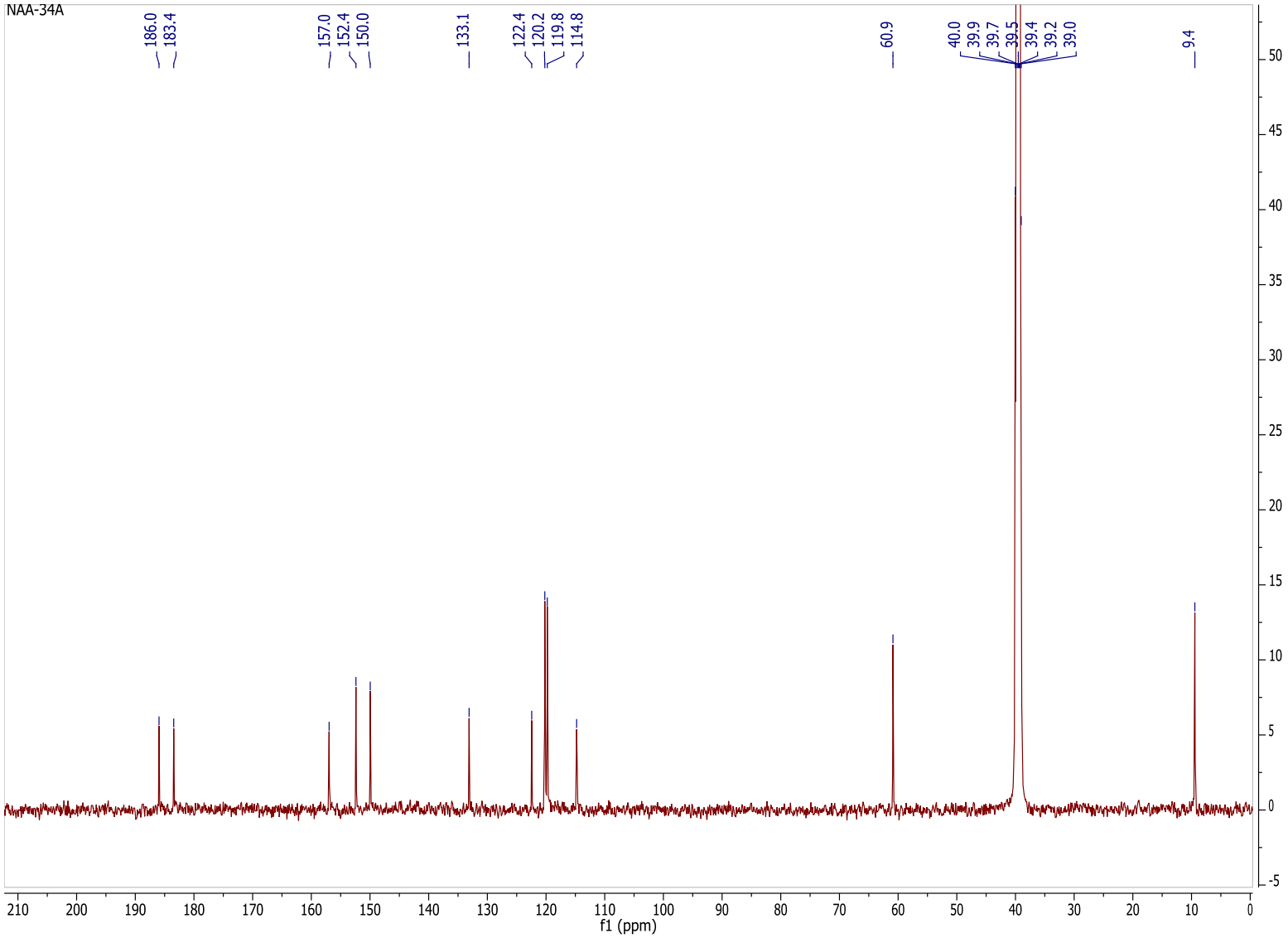
Appendix 22E: ESIMS spectrum of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (**94**)



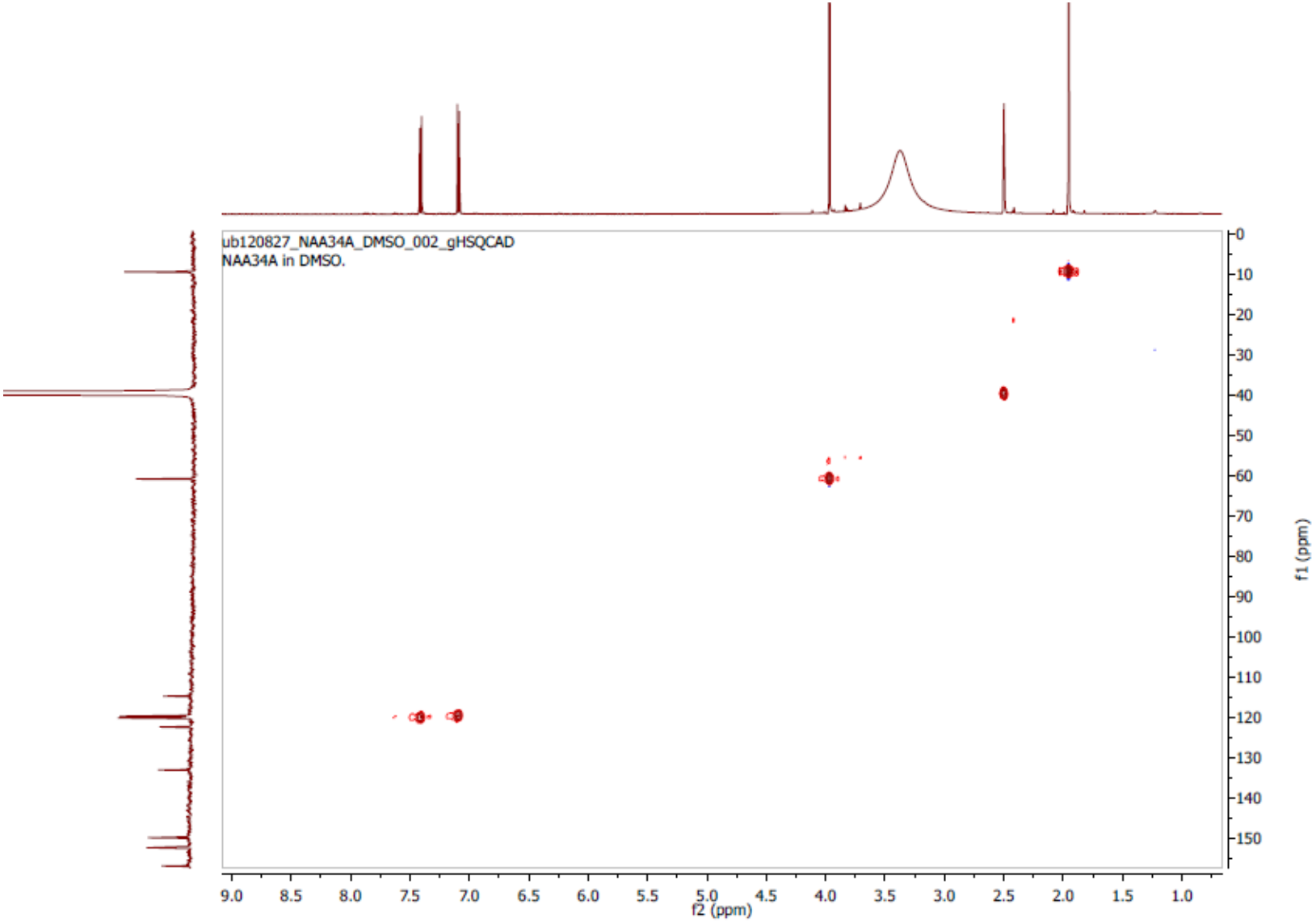
Appendix 23A: ^{1}H NMR spectrum of malvone A (**154**), DMSO-d_6, 600 MHz



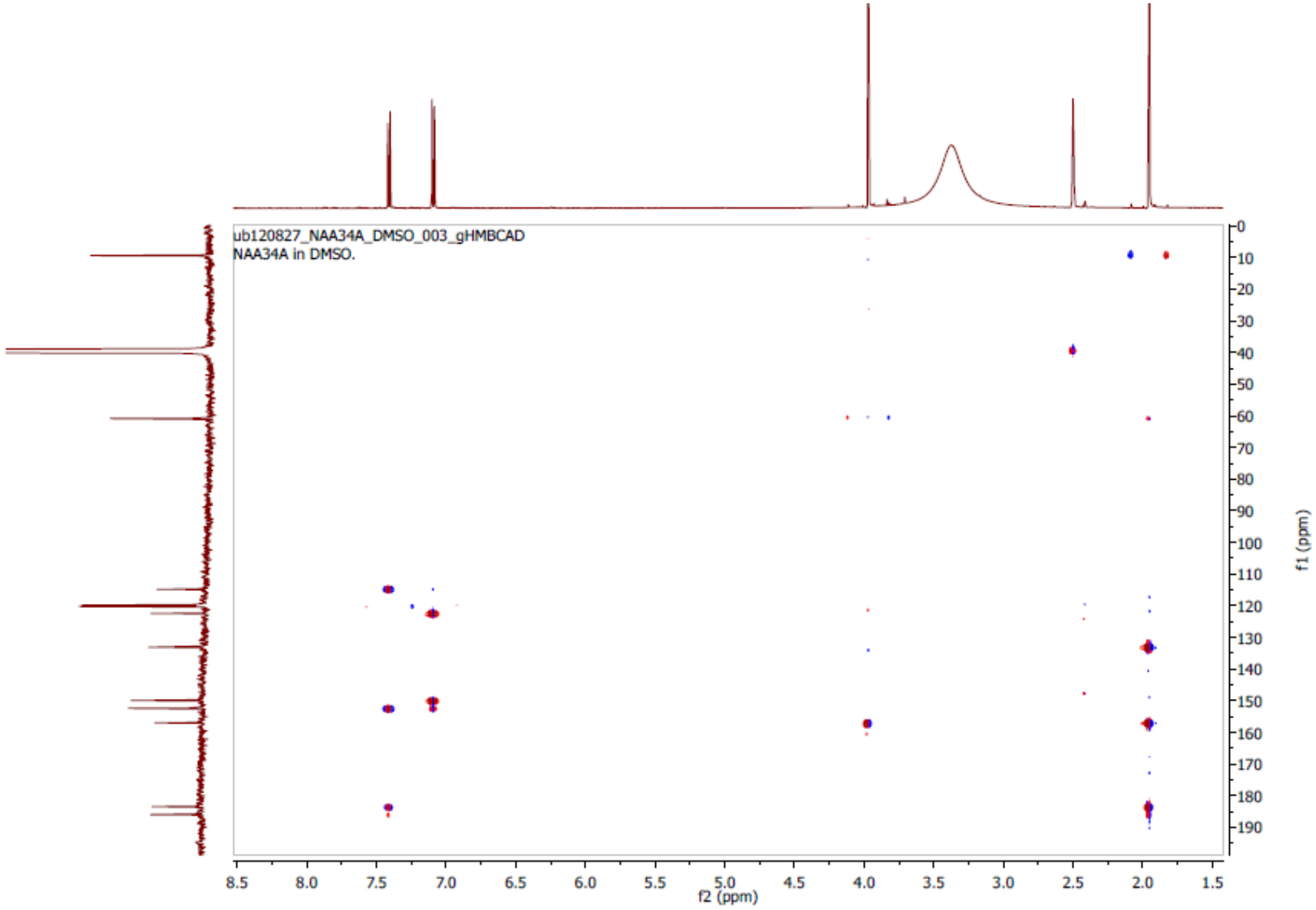
Appendix 23B: ^{13}C NMR spectrum of malvone A (**154**), DMSO-d_6, 200 MHz



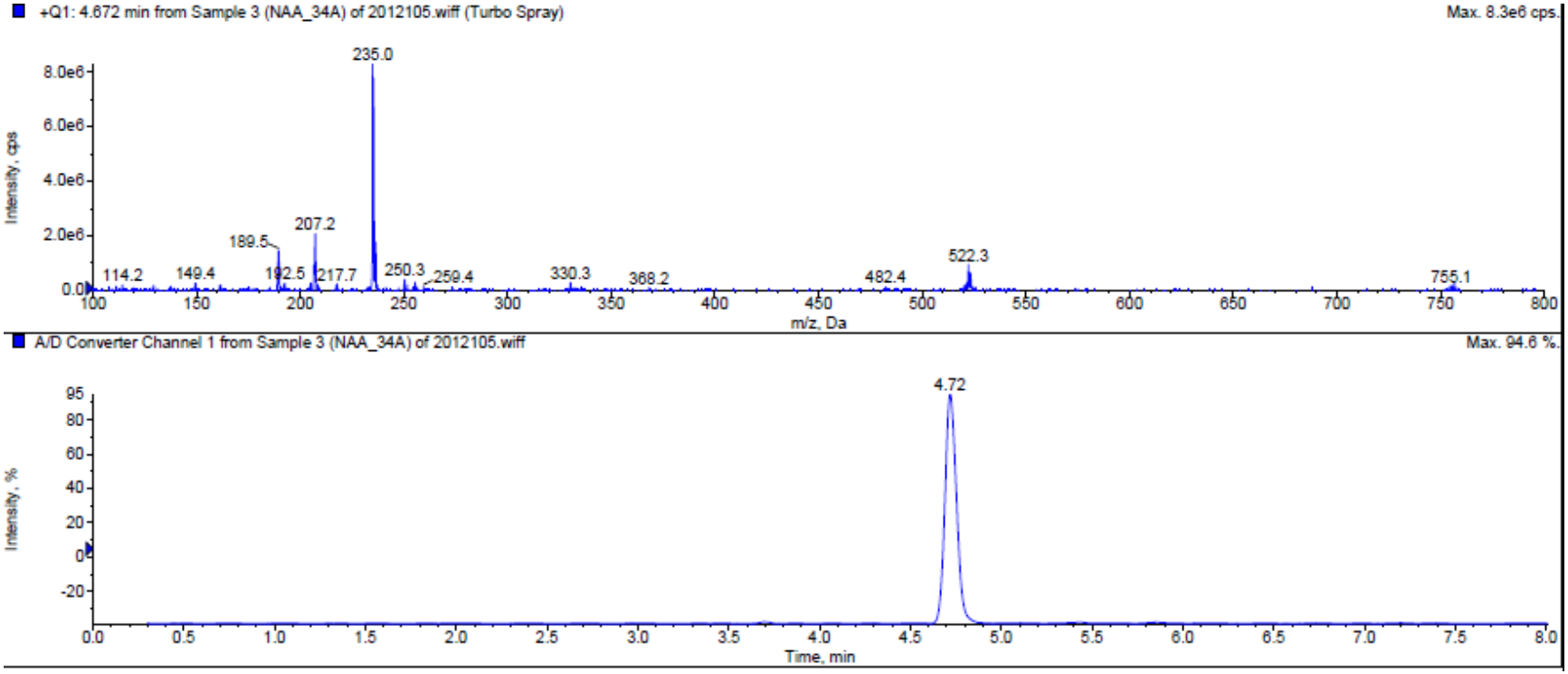
Appendix 23C: HSQC spectrum of malvone A (**154**), DMSO-d_6, 600 MHz



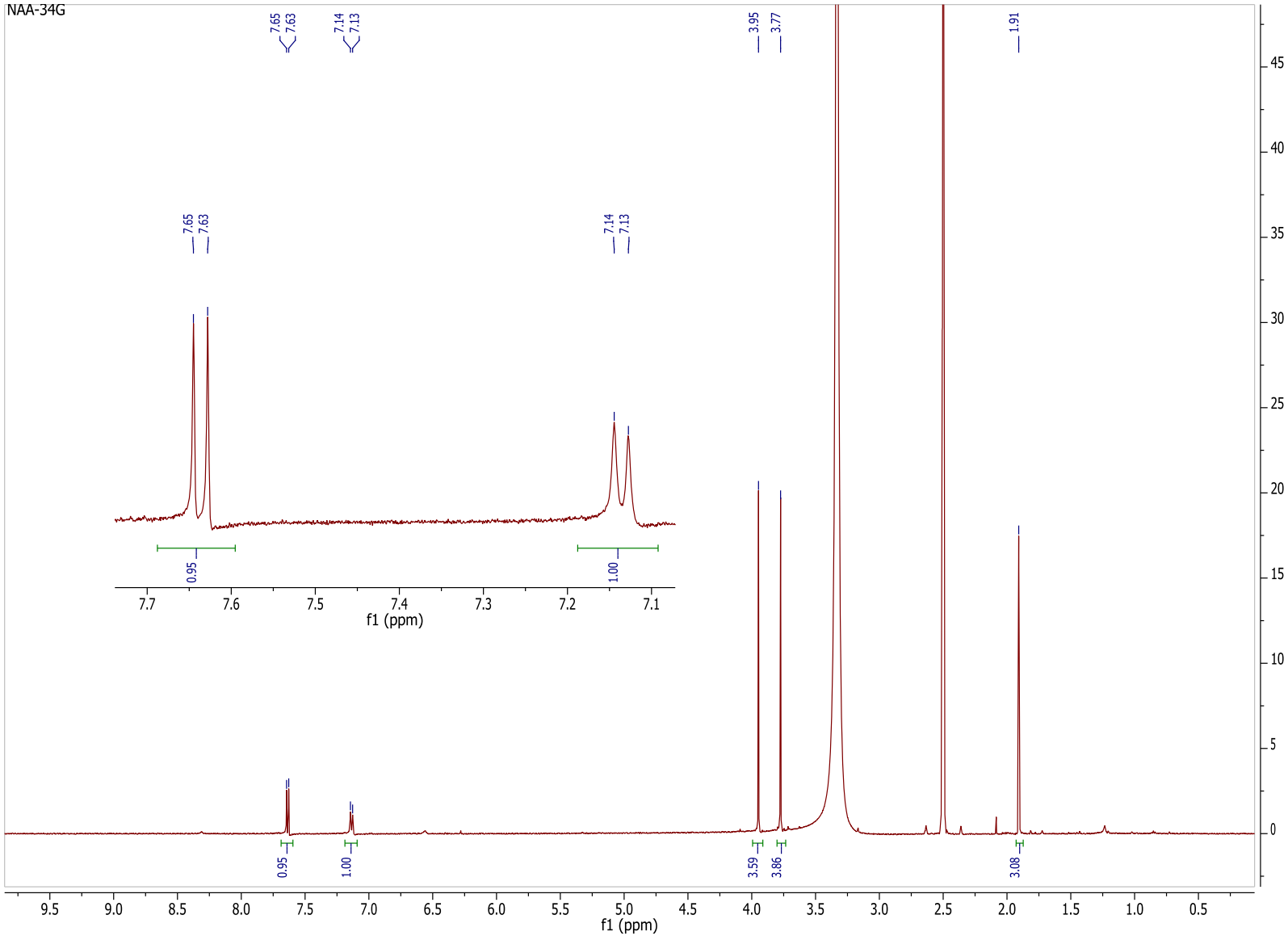
Appendix 23D: HMBC spectrum of malvone A (**154**), DMSO-d_6, 600 MHz



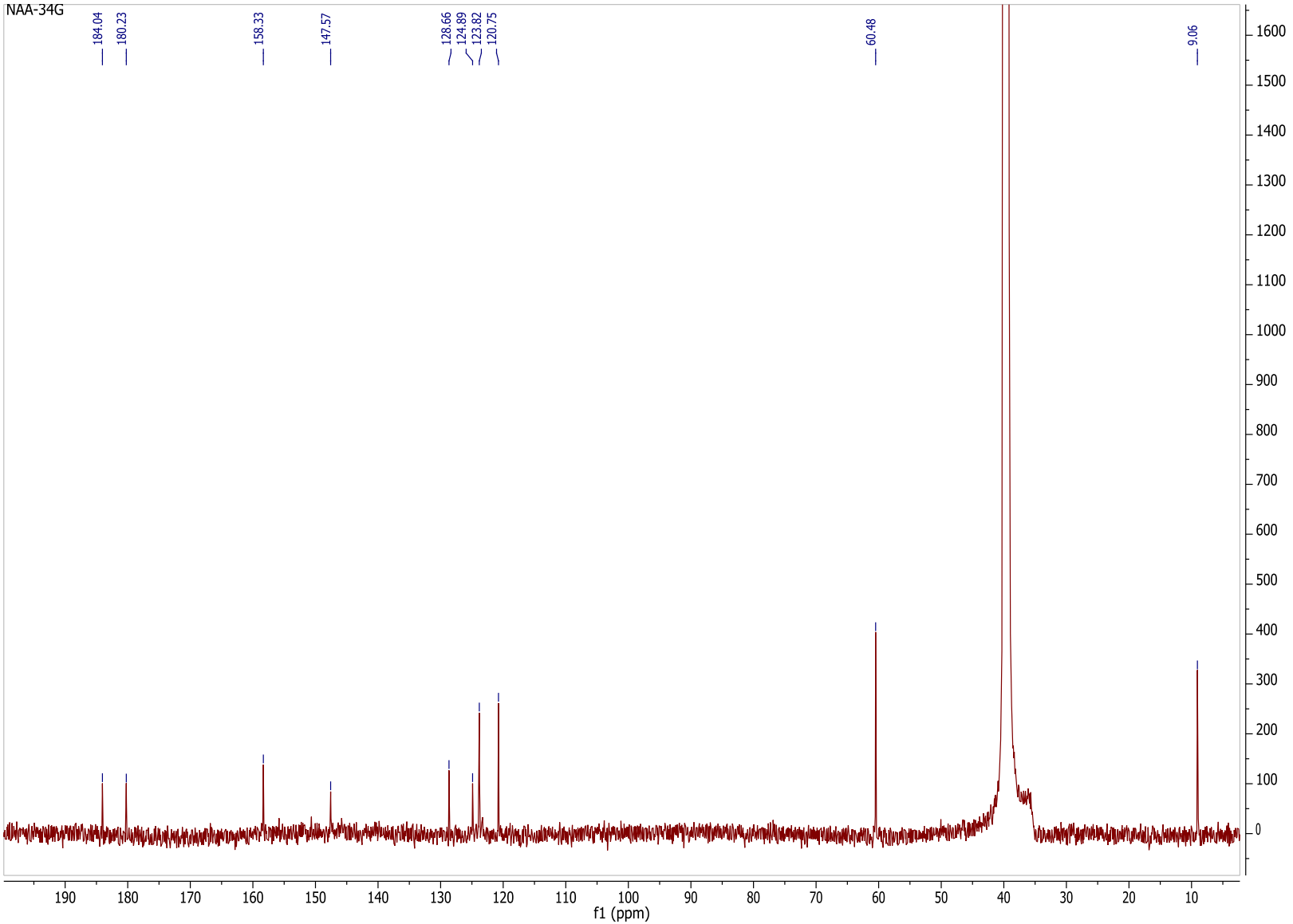
Appendix 23E: ESIMS spectrum of malvone A (**154**)



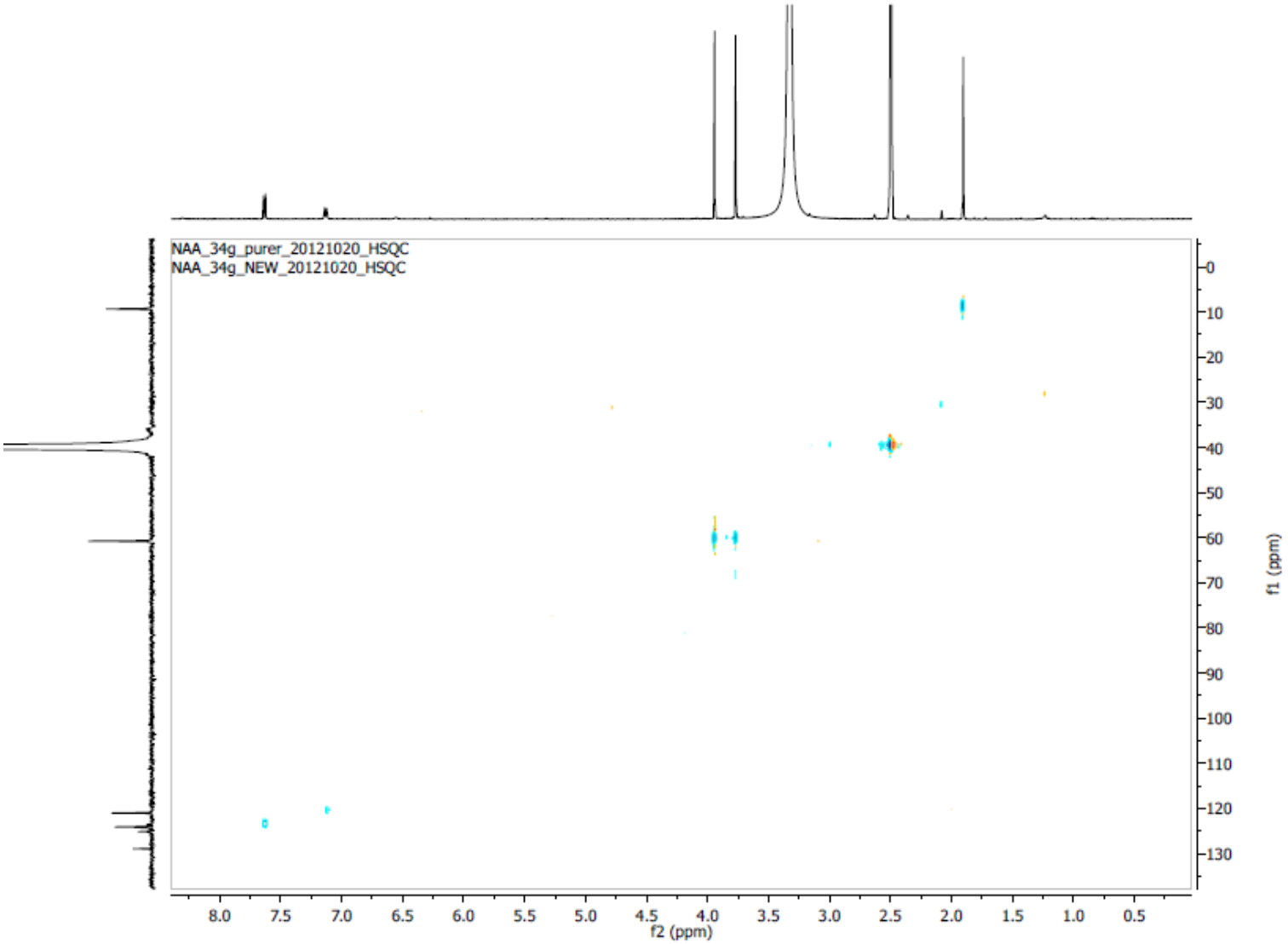
Appendix 24A: ^{1}H NMR spectrum of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**), DMSO-d_6, 600 MHz



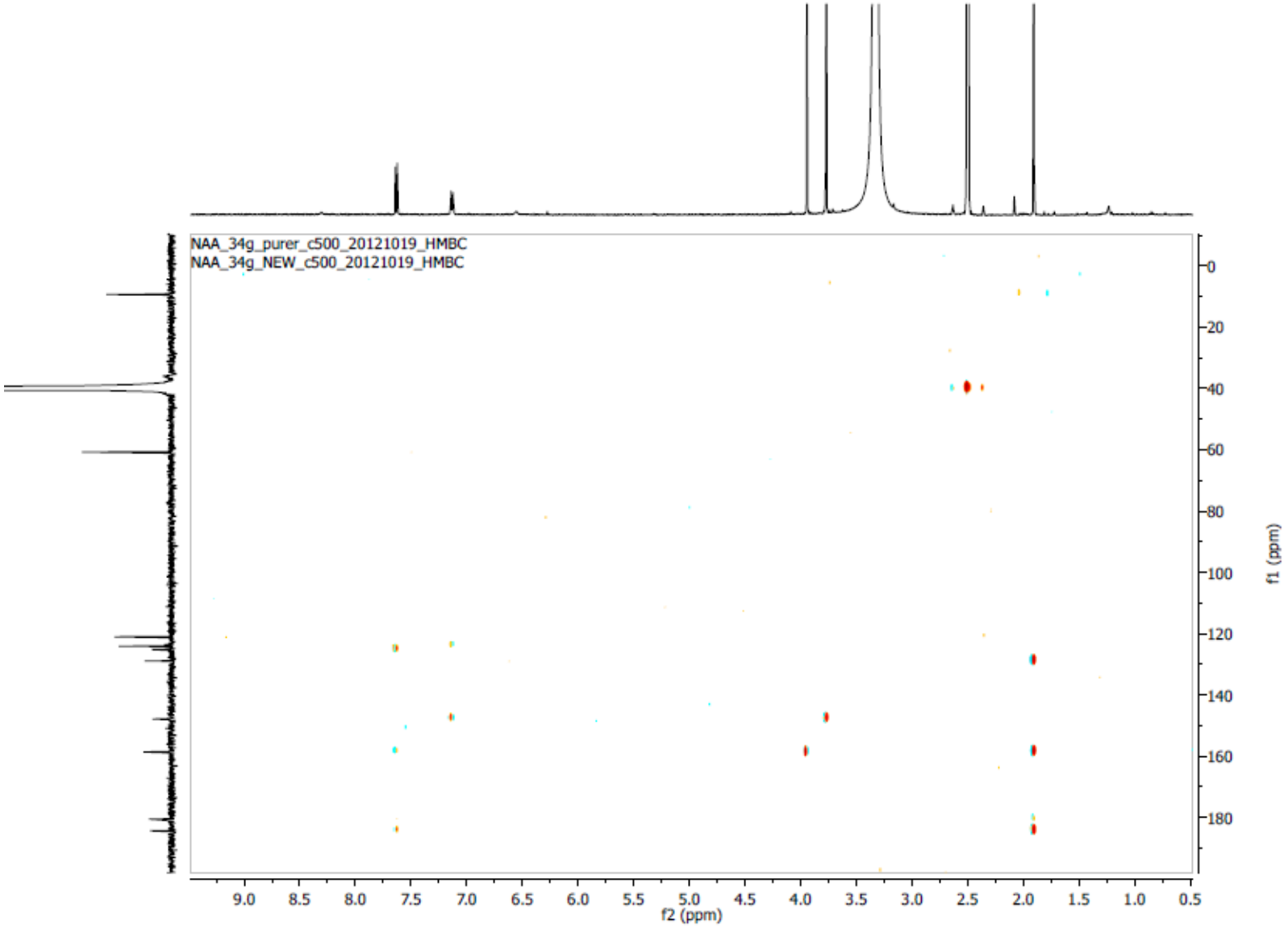
Appendix 24B: ^{13}C NMR spectrum of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**), DMSO-d_6, 200 MHz



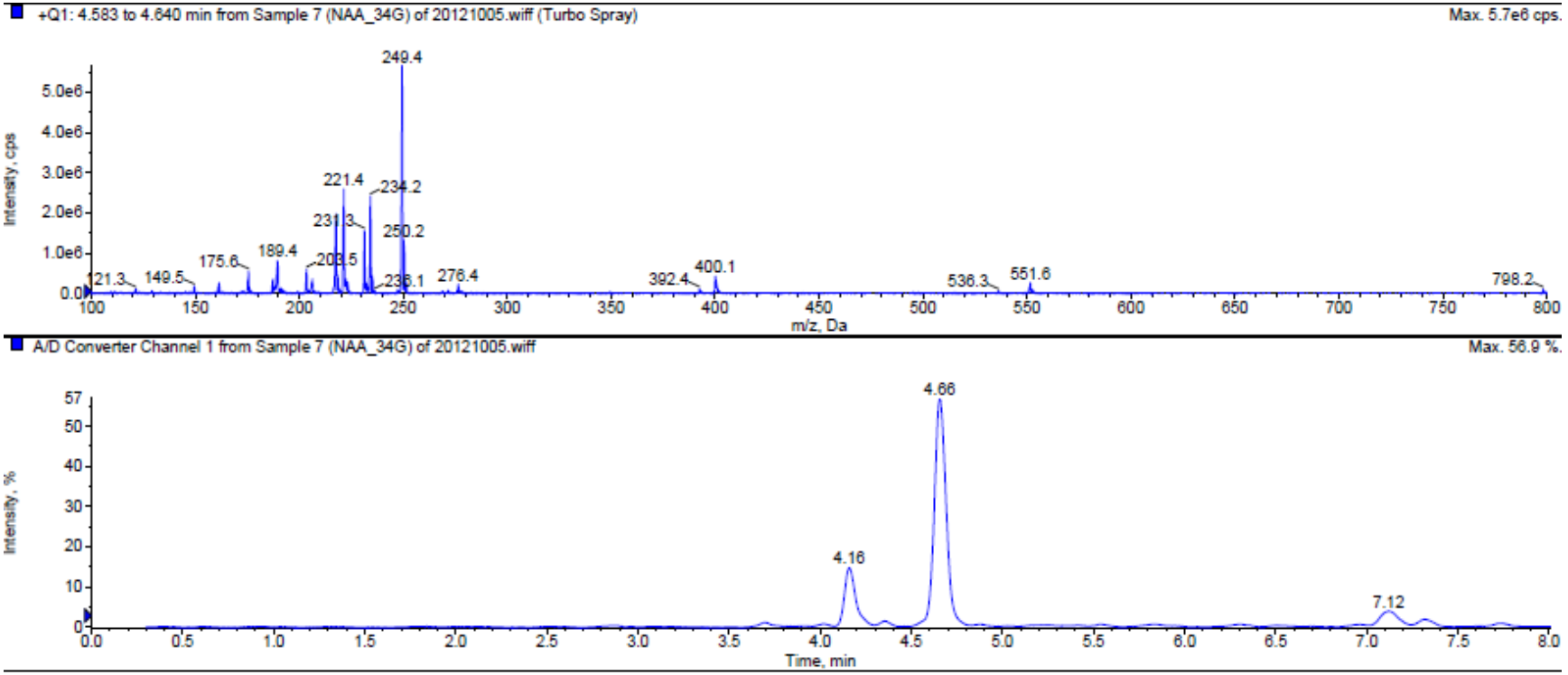
Appendix 24C: HSQC spectrum of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**), DMSO-d_6, 600 MHz



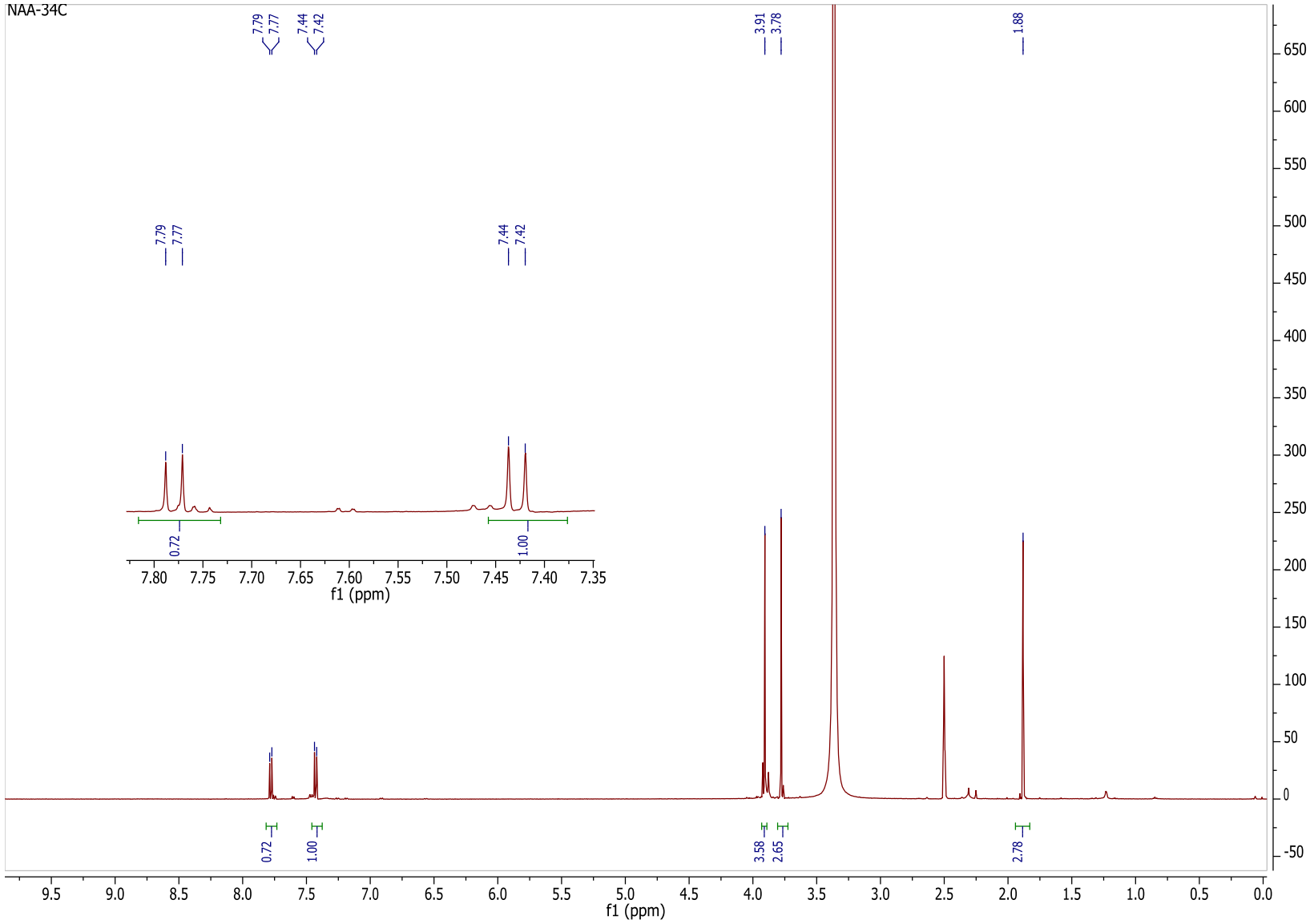
Appendix 24D: HMBC spectrum of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**), DMSO-d_6, 600 MHz



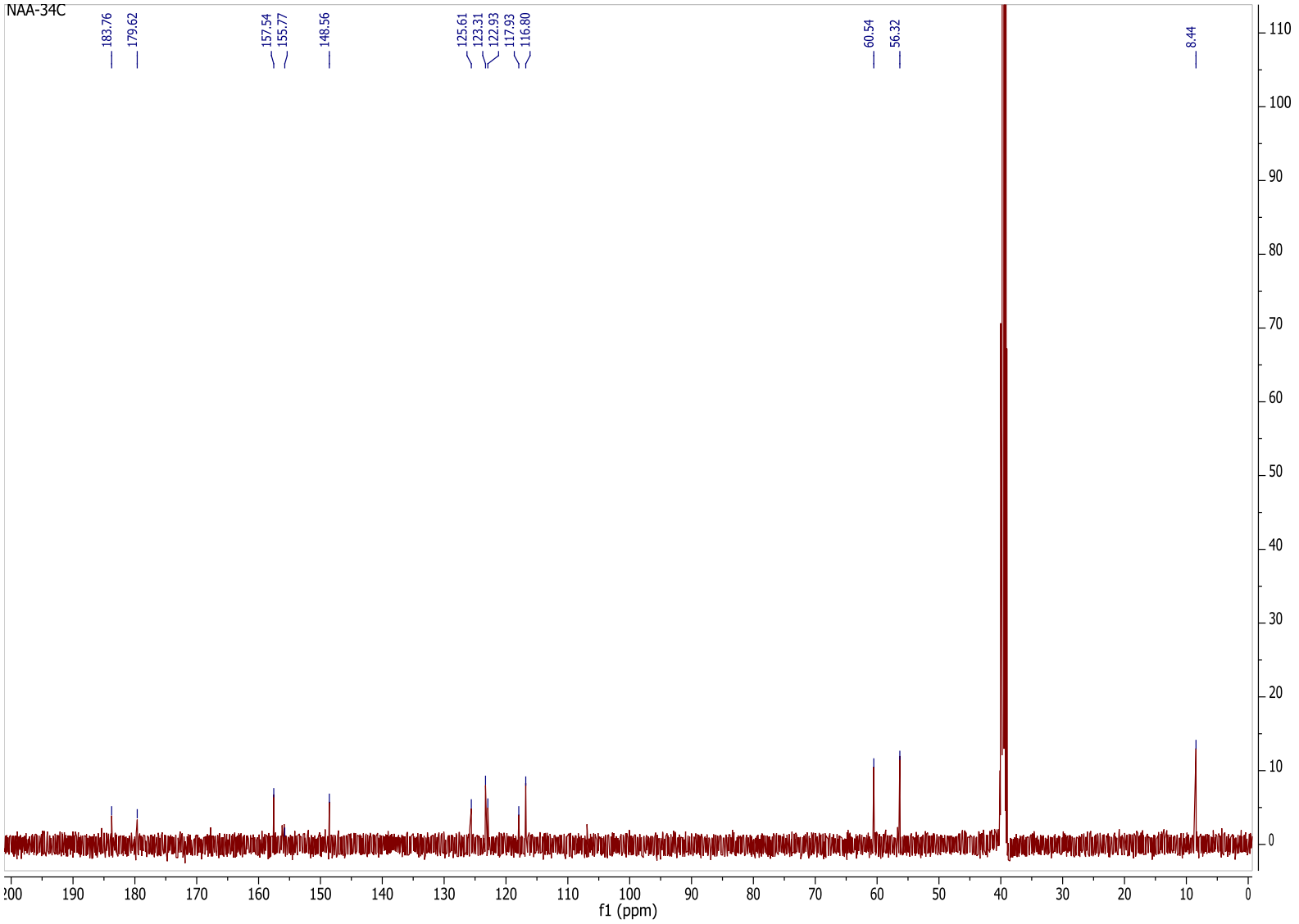
Appendix 24E: ESIMS spectrum of 6-hydroxy-3,5-dimethoxy-2-methyl-1,4-naphthoquinone (**155**)



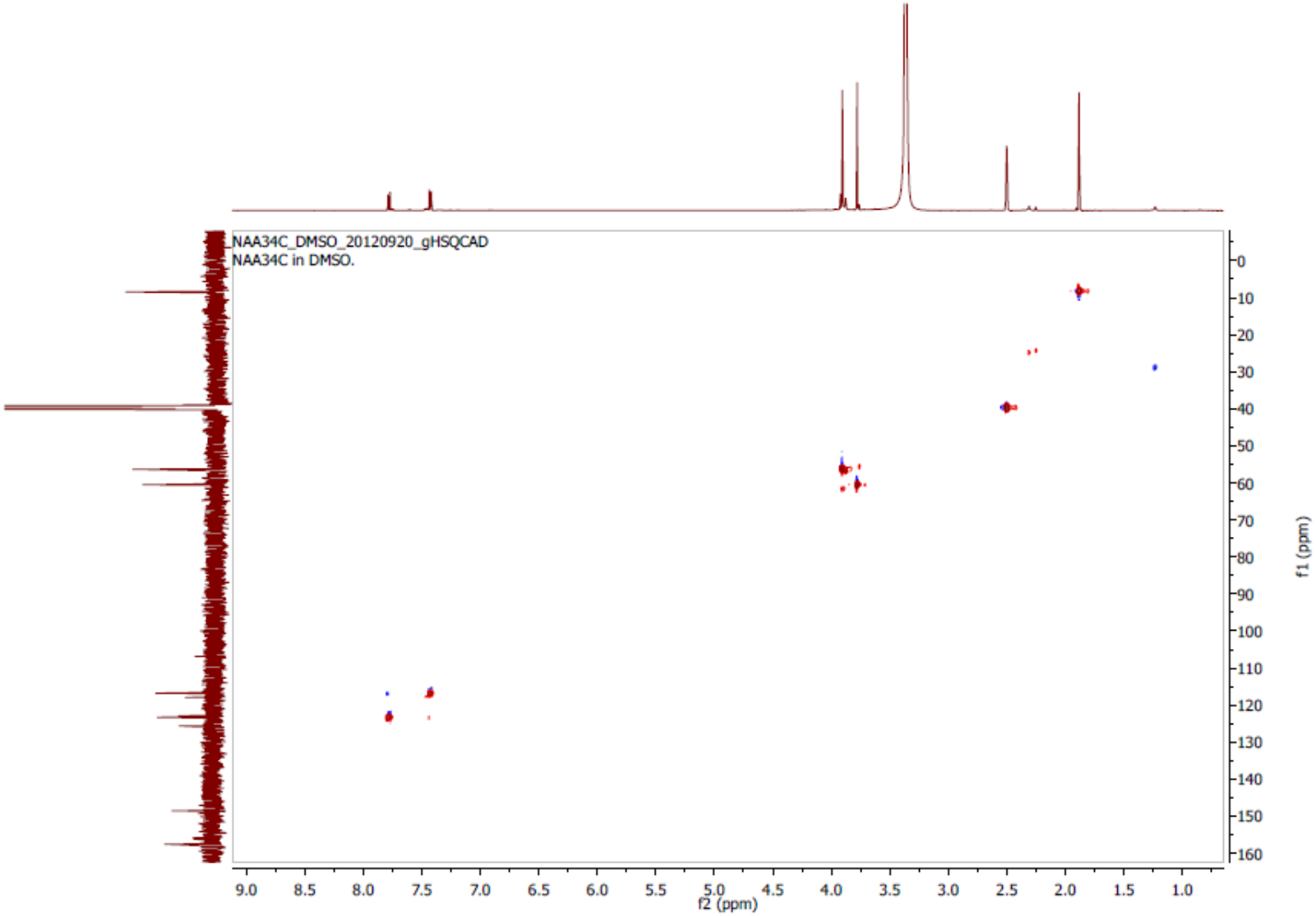
Appendix 25A: ^{1}H NMR spectrum of ancistroquinone C (**95**), DMSO-d_6, 600 MHz



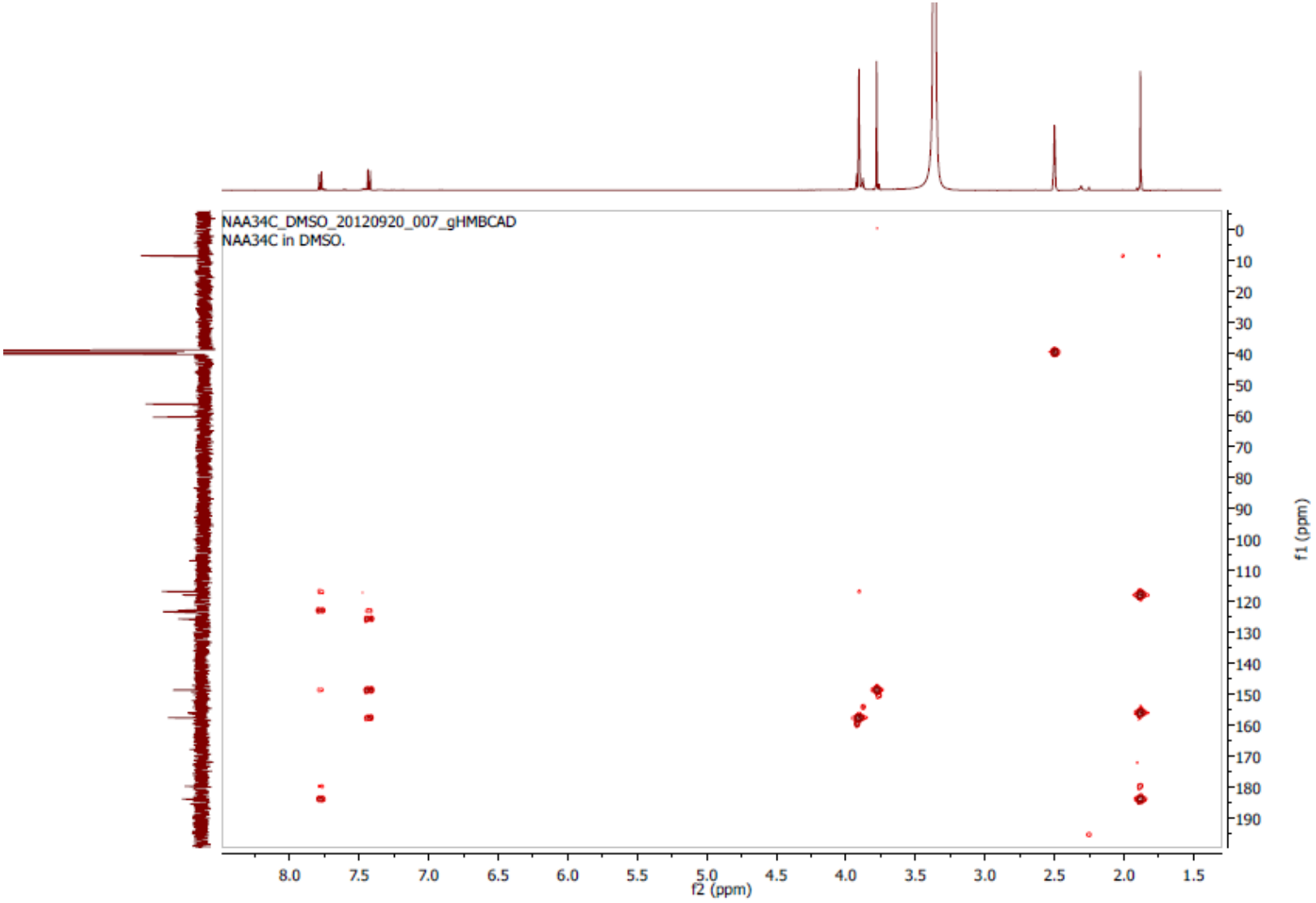
Appendix 25B: ^{13}C NMR spectrum of ancistroquinone C (**95**), DMSO-d_6, 200 MHz



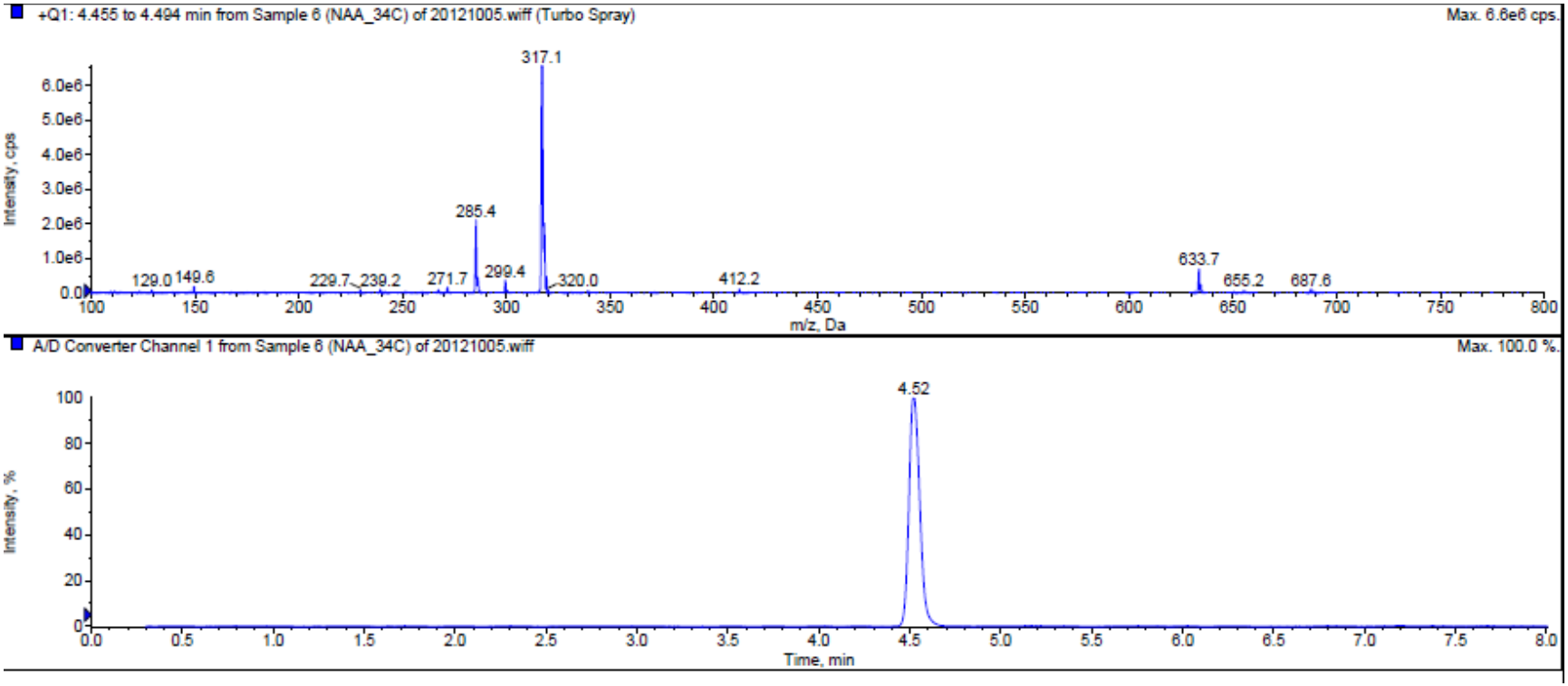
Appendix 25C: HSQC spectrum of ancistroquinone C (**95**), DMSO-d_6, 600 MHz



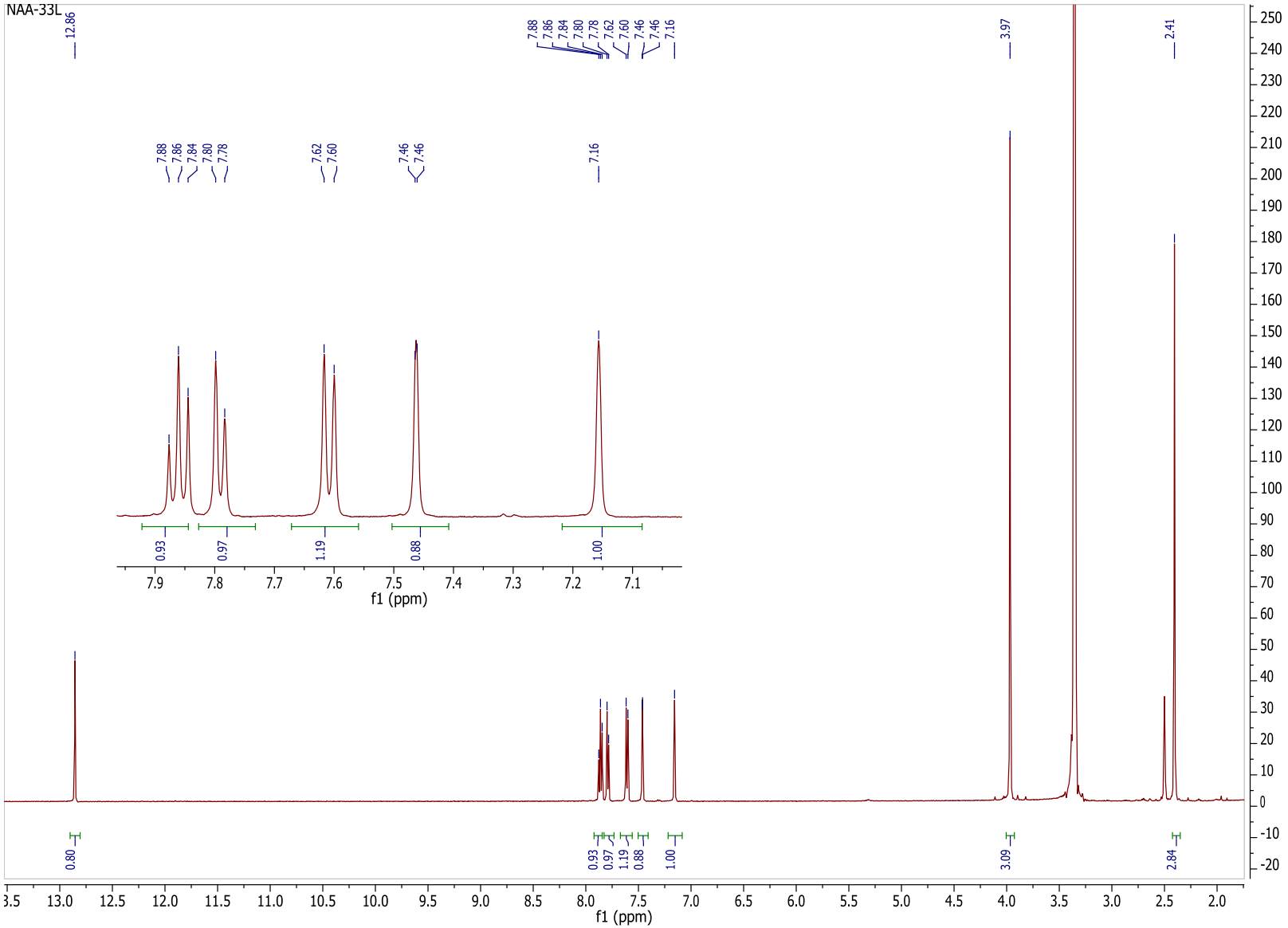
Appendix 25D: HMBC spectrum of ancistroquinone C (**95**), DMSO-d_6, 600 MHz



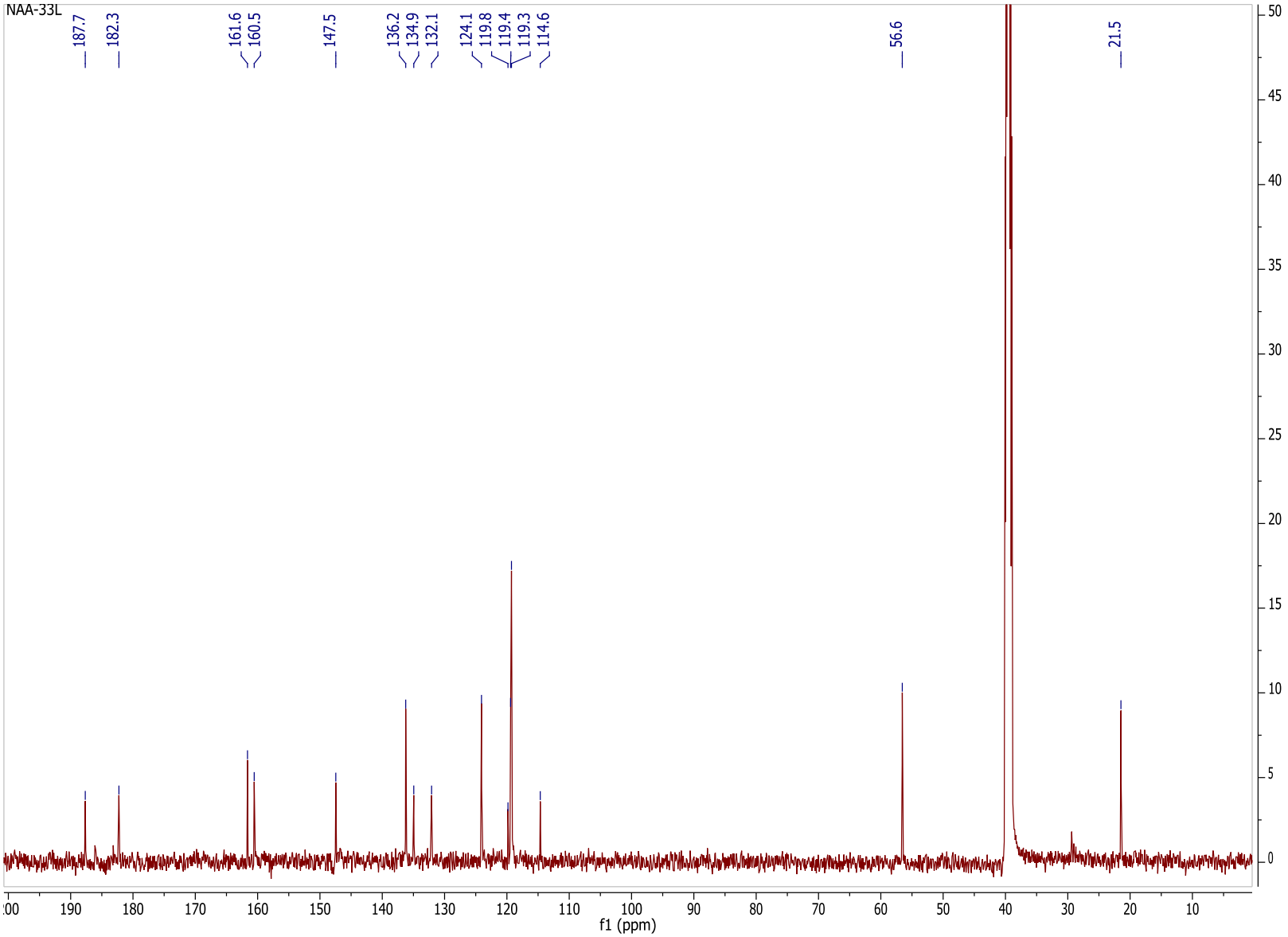
Appendix 25E: ESIMS spectrum of ancistroquinone C (**95**)



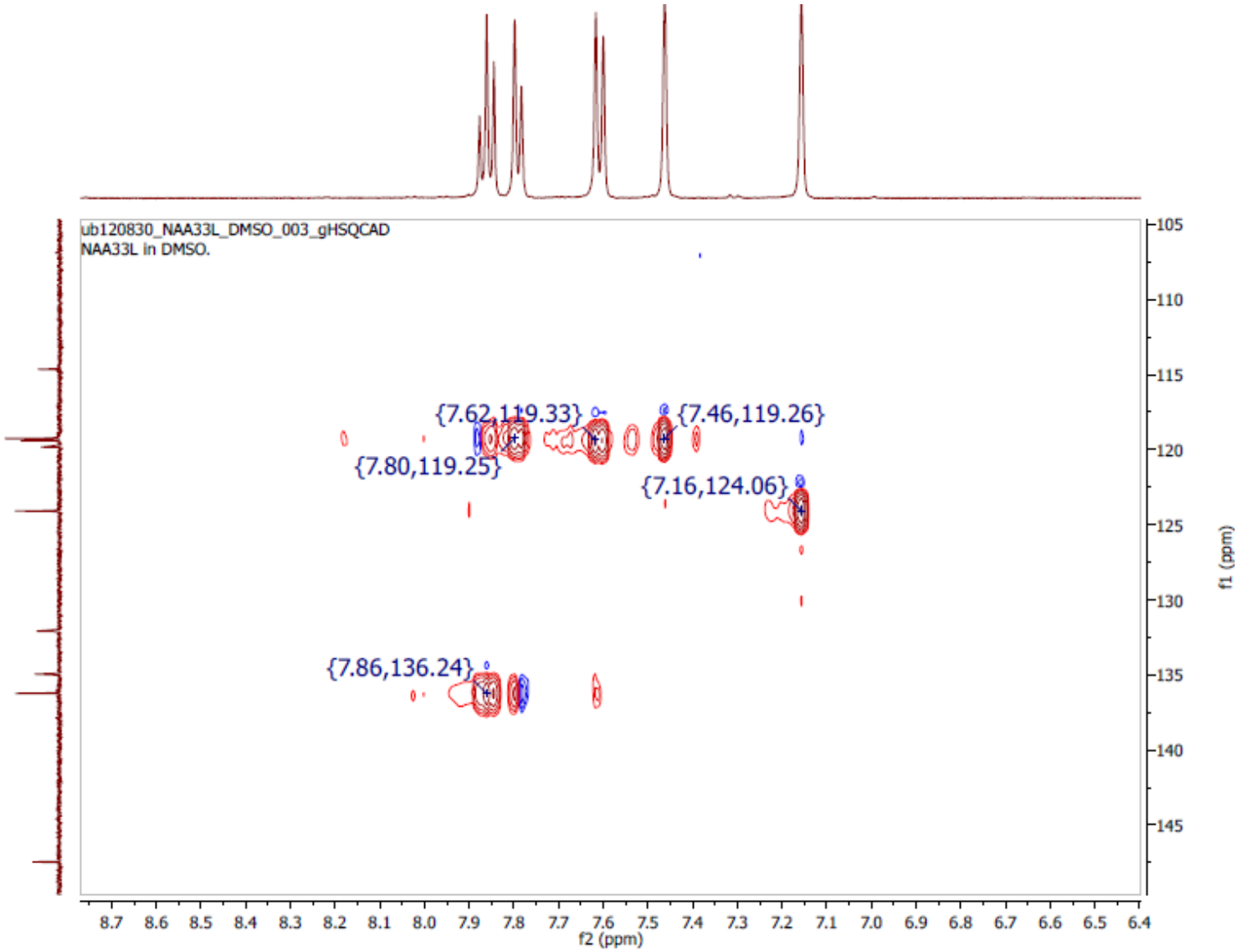
Appendix 26A: ^{1}H NMR spectrum of chrysophanol-8-methyl ether (**156**), DMSO-d_6, 600 MHz



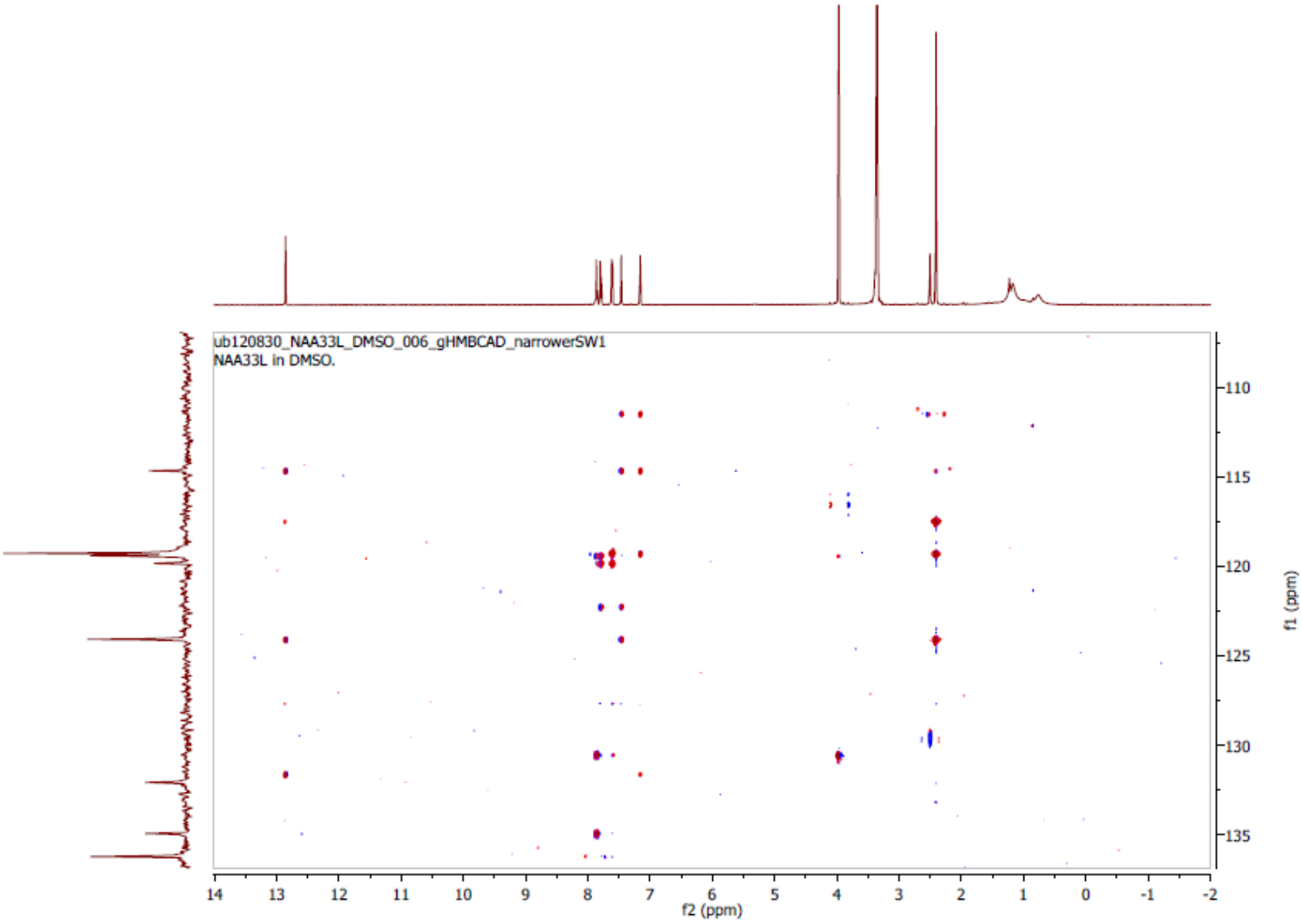
Appendix 26B: ^{13}C NMR spectrum of chrysophanol-8-methyl ether (**156**), DMSO-d_6, 200 MHz



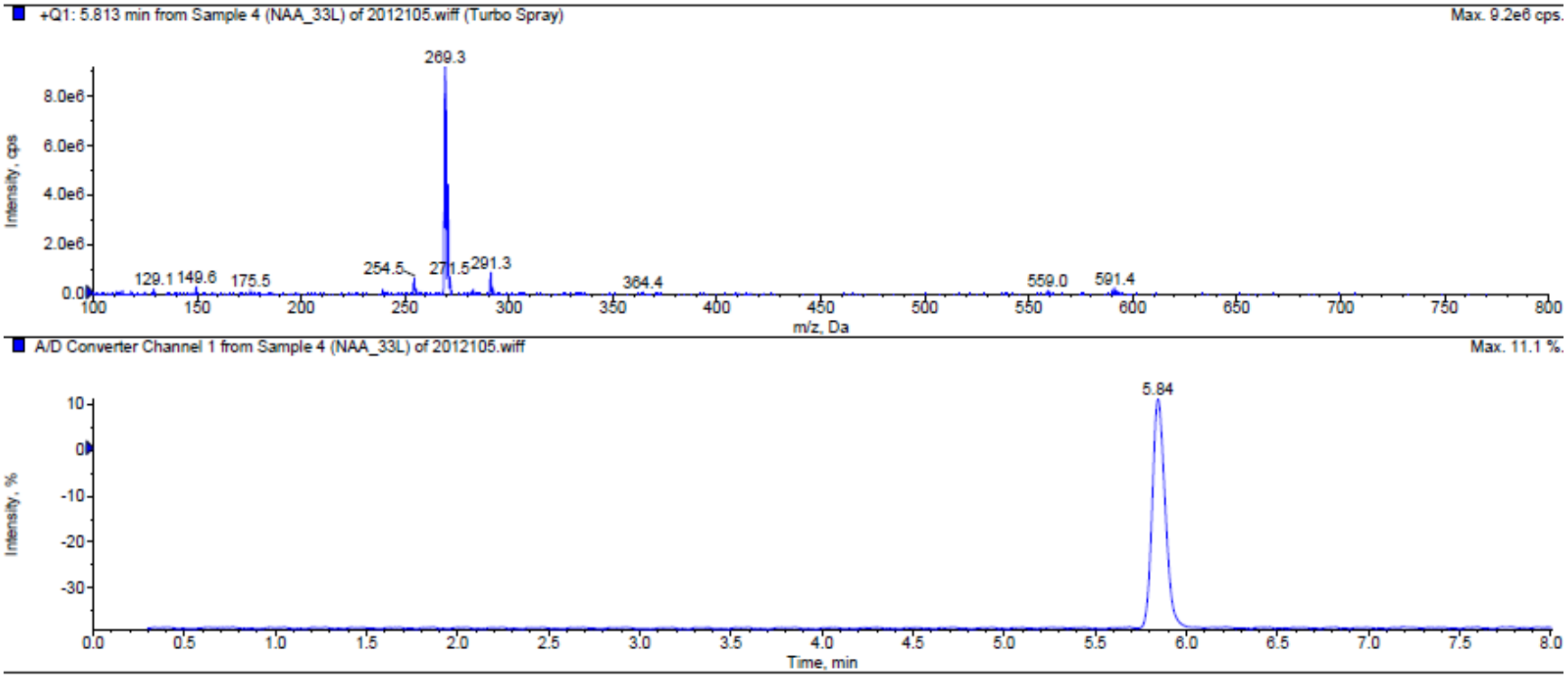
Appendix 26C: HSQC spectrum of chrysophanol-8-methyl ether (**156**), DMSO-d_6, 600 MHz



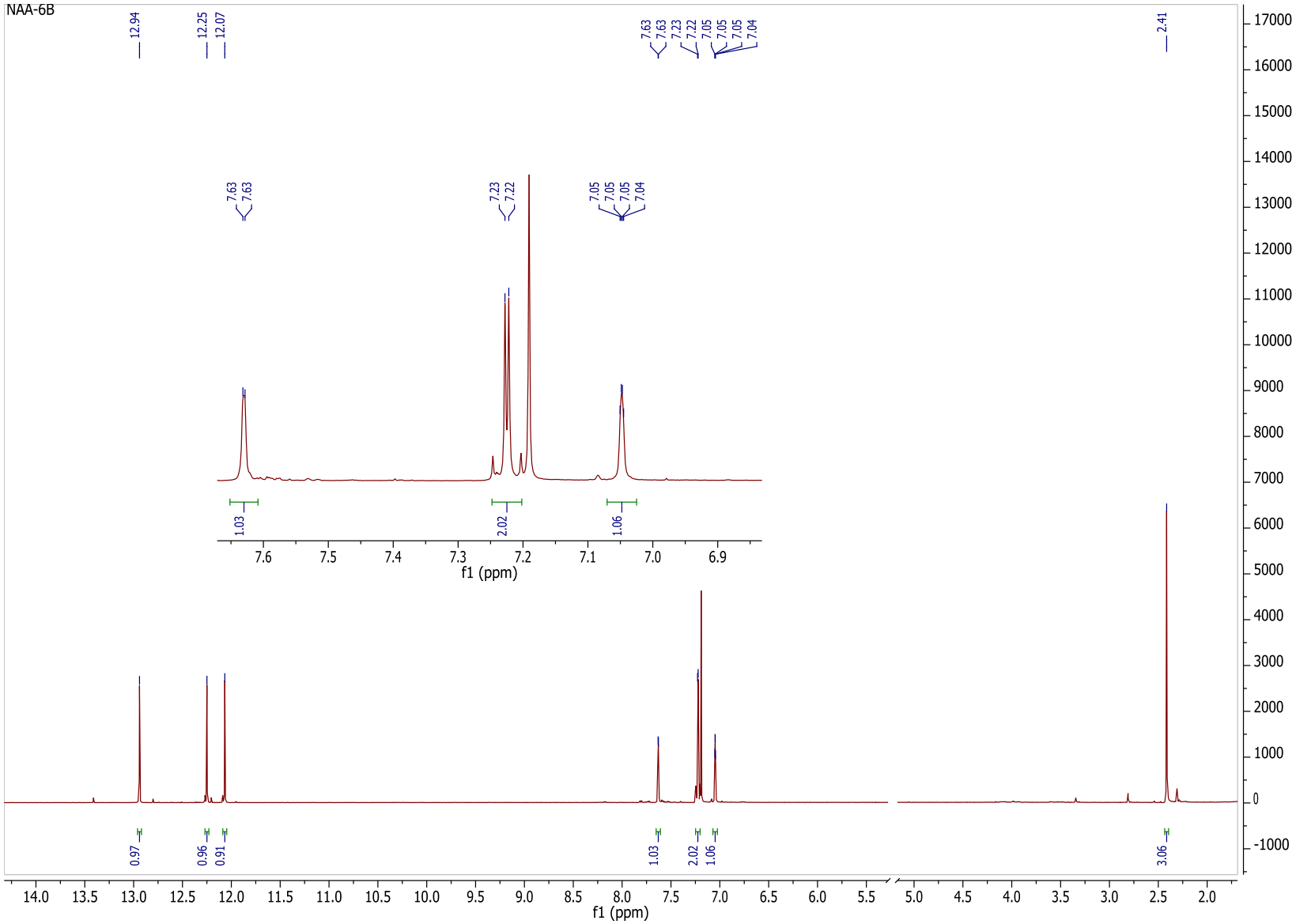
Appendix 26D: HMBC spectrum of chrysophanol-8-methyl ether (**156**), DMSO-d_6, 600 MHz



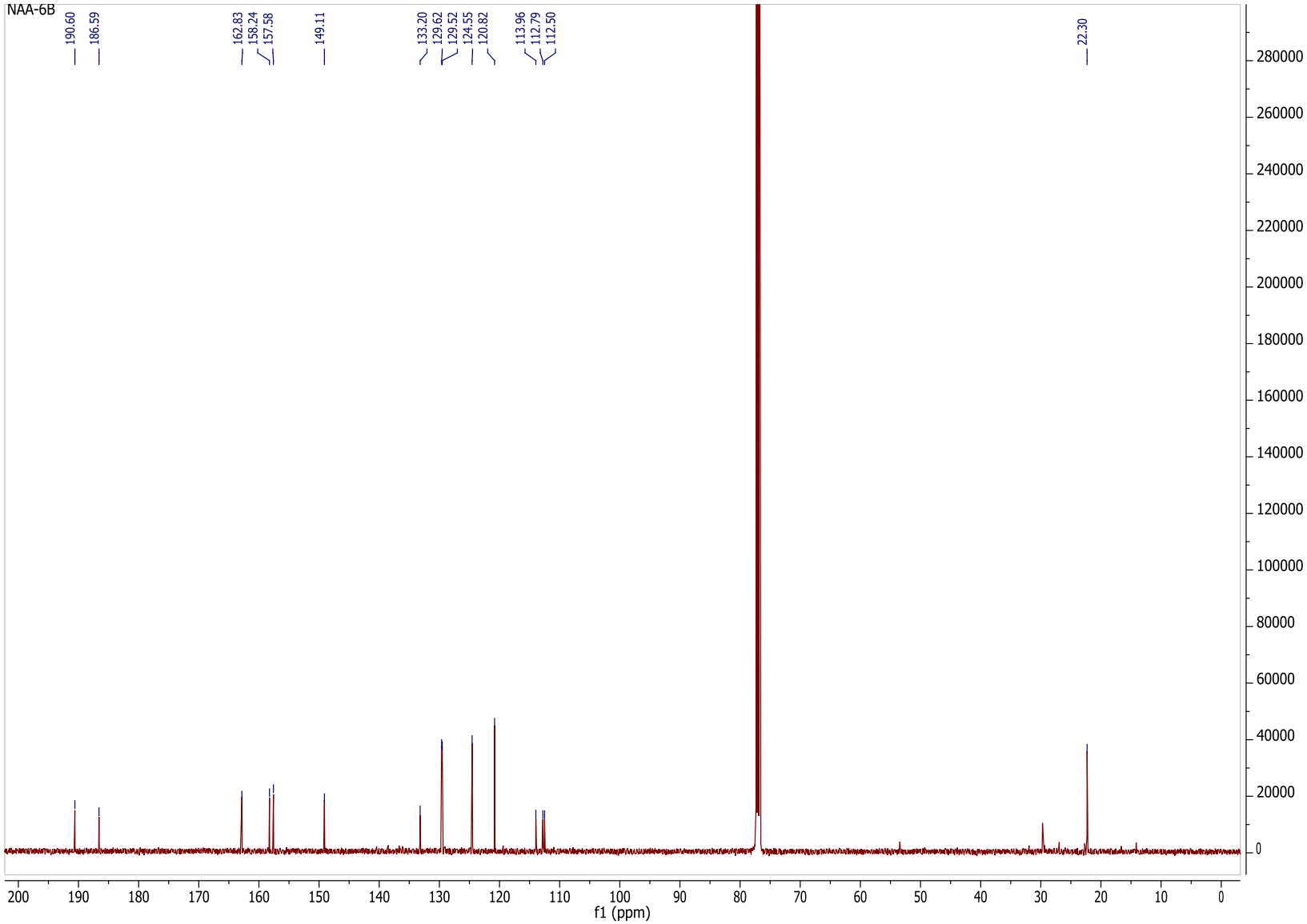
Appendix 26E: ESIMS spectrum of chrysophanol-8-methyl ether (**156**)



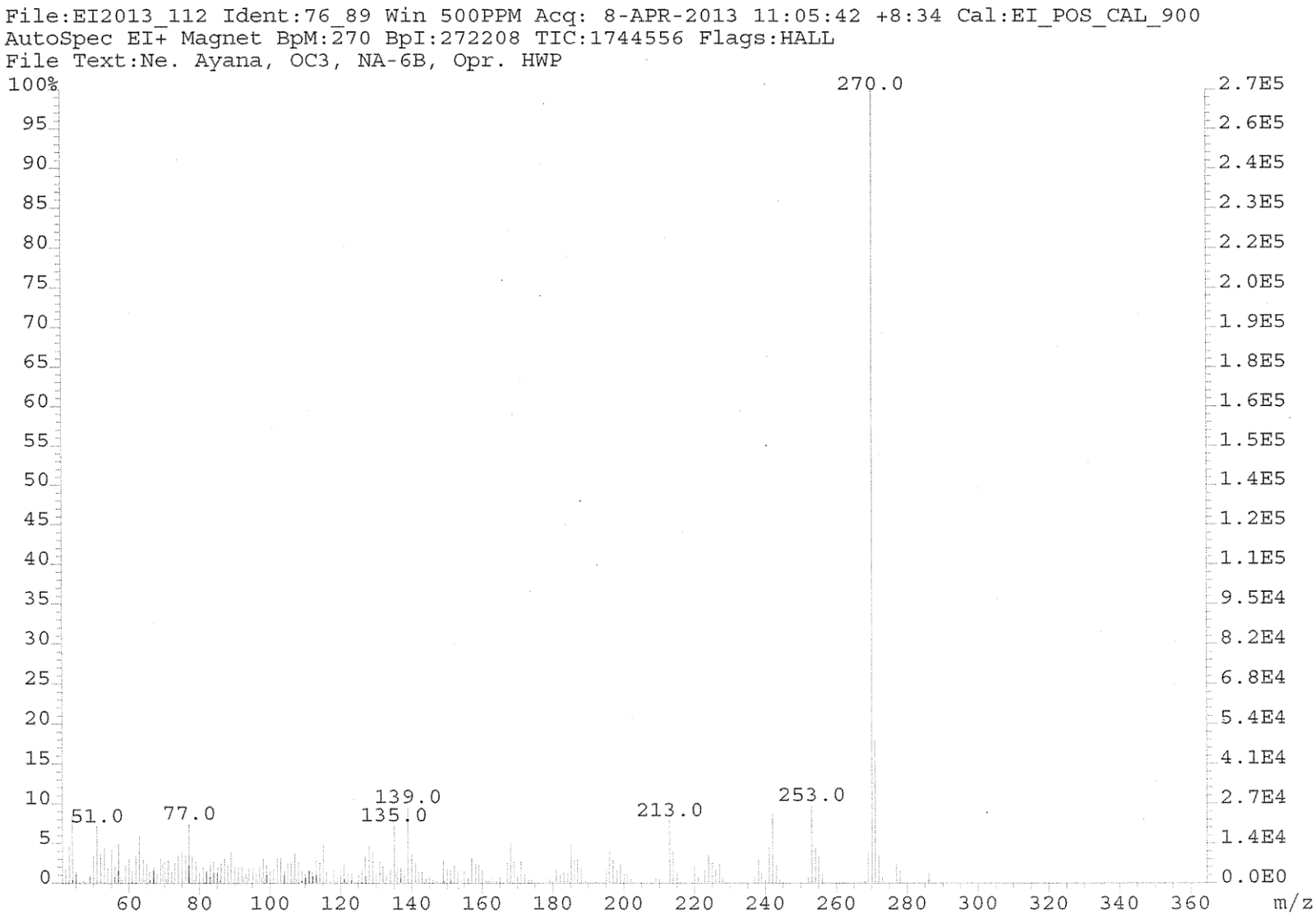
Appendix 27A: ^{1}H NMR spectrum of helminthosporin (**59**), $CDCl_3$, 600 MHz



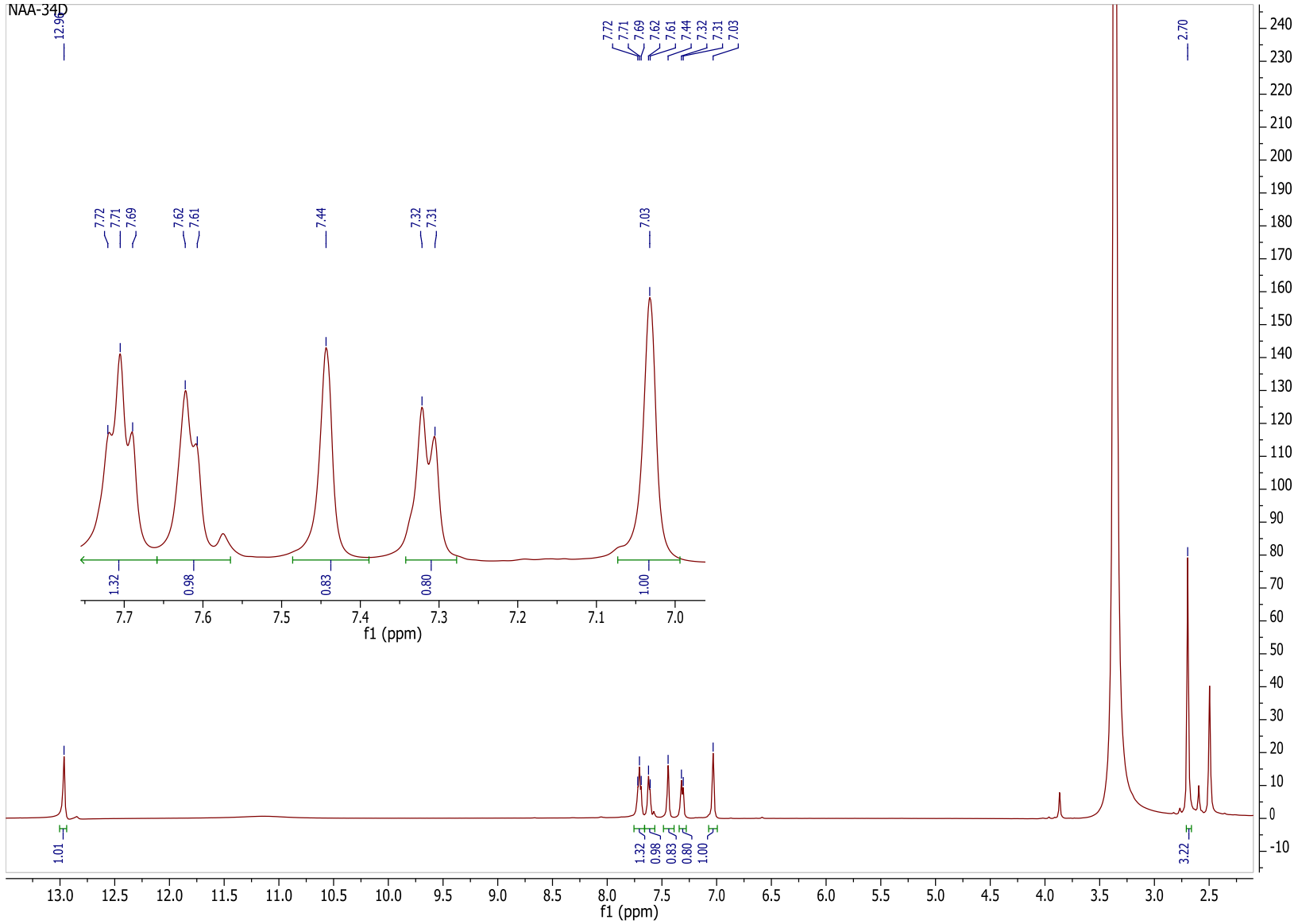
Appendix 27B: ^{13}C NMR spectrum of of helminthosporin (**59**), $CDCl_3$, 600 MHz



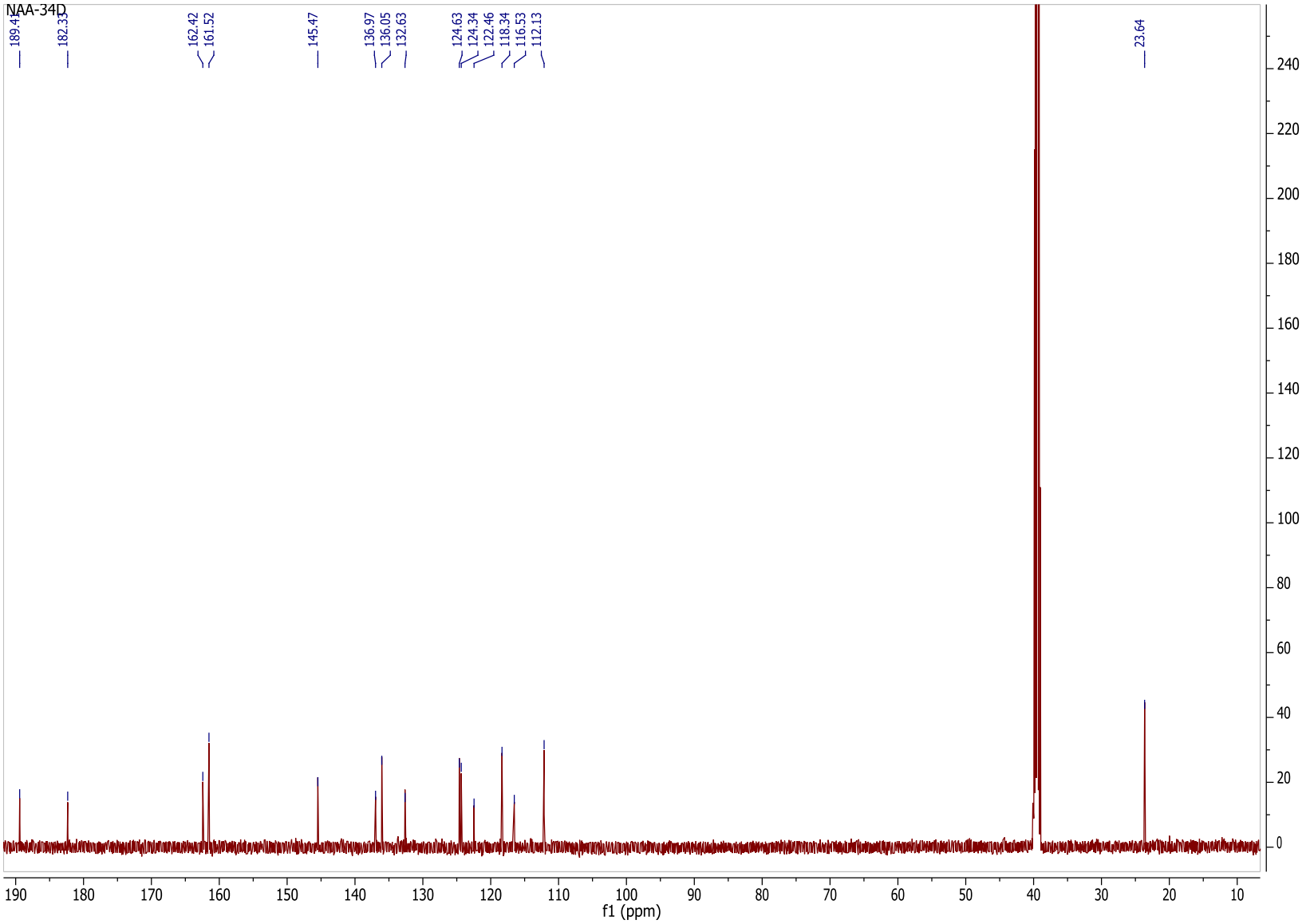
Appendix 27C: ESIMS spectrum of helminthosporin (**59**)



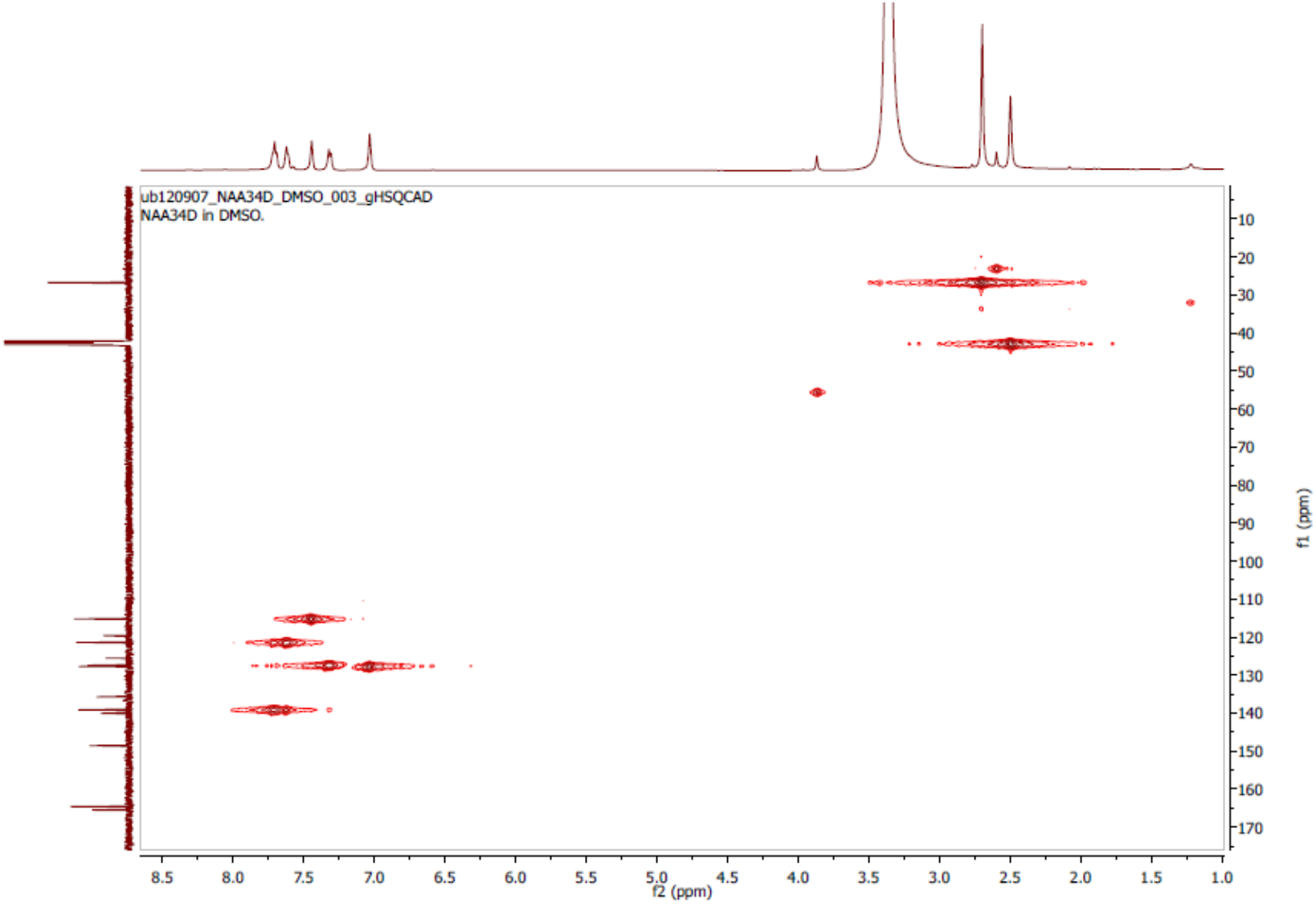
Appendix 28A: ^{1}H NMR spectrum of aloesaponarin II (**65**), DMSO-d_6, 600 MHz



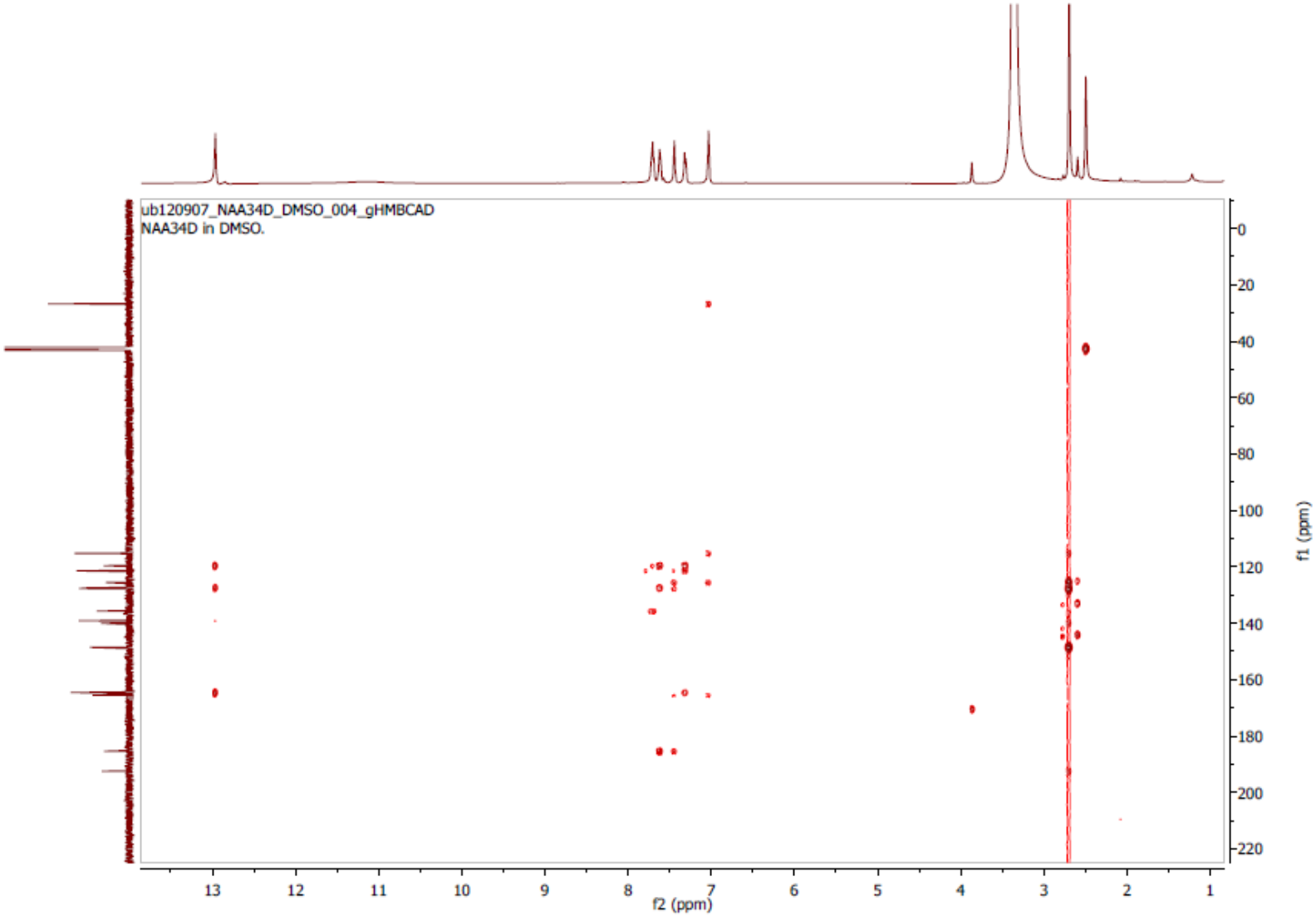
Appendix 28B: ^{13}C NMR spectrum of aloesaponarin II (**65**), DMSO-d_6, 200 MHz



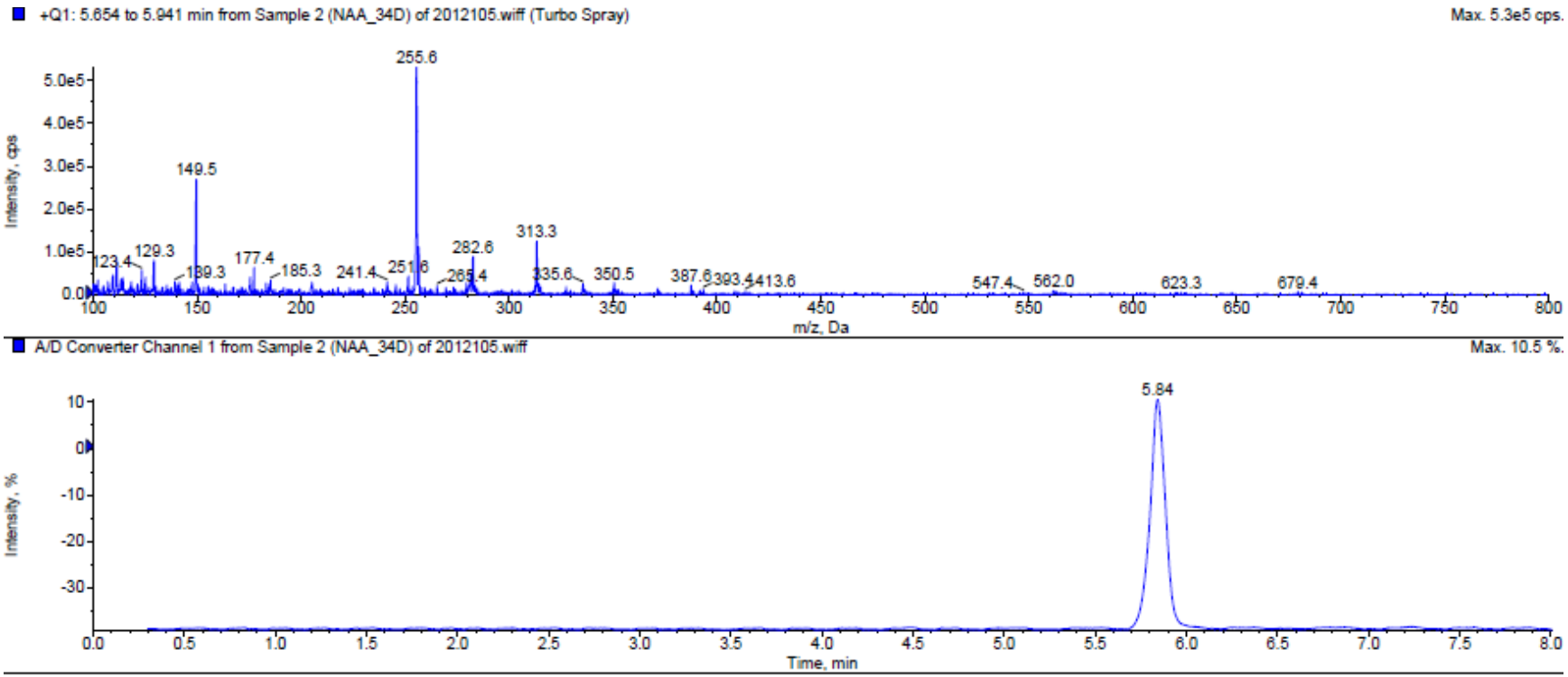
Appendix 28C: HSQC spectrum of aloesaponarin II (**65**), DMSO-d_6, 600 MHz



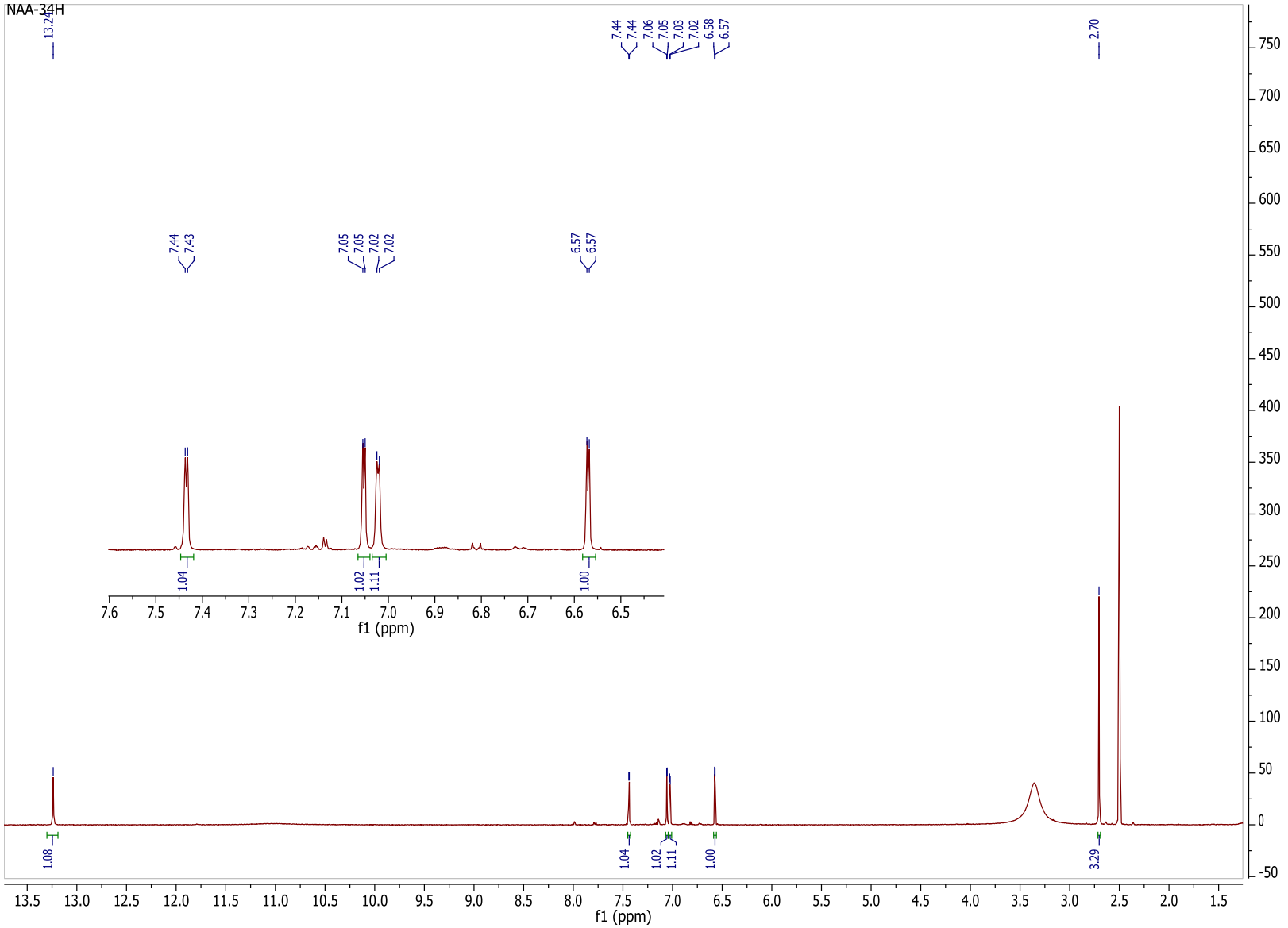
Appendix 28D: HMBC spectrum of aloesaponarin II (**65**), DMSO-d_6, 600 MHz



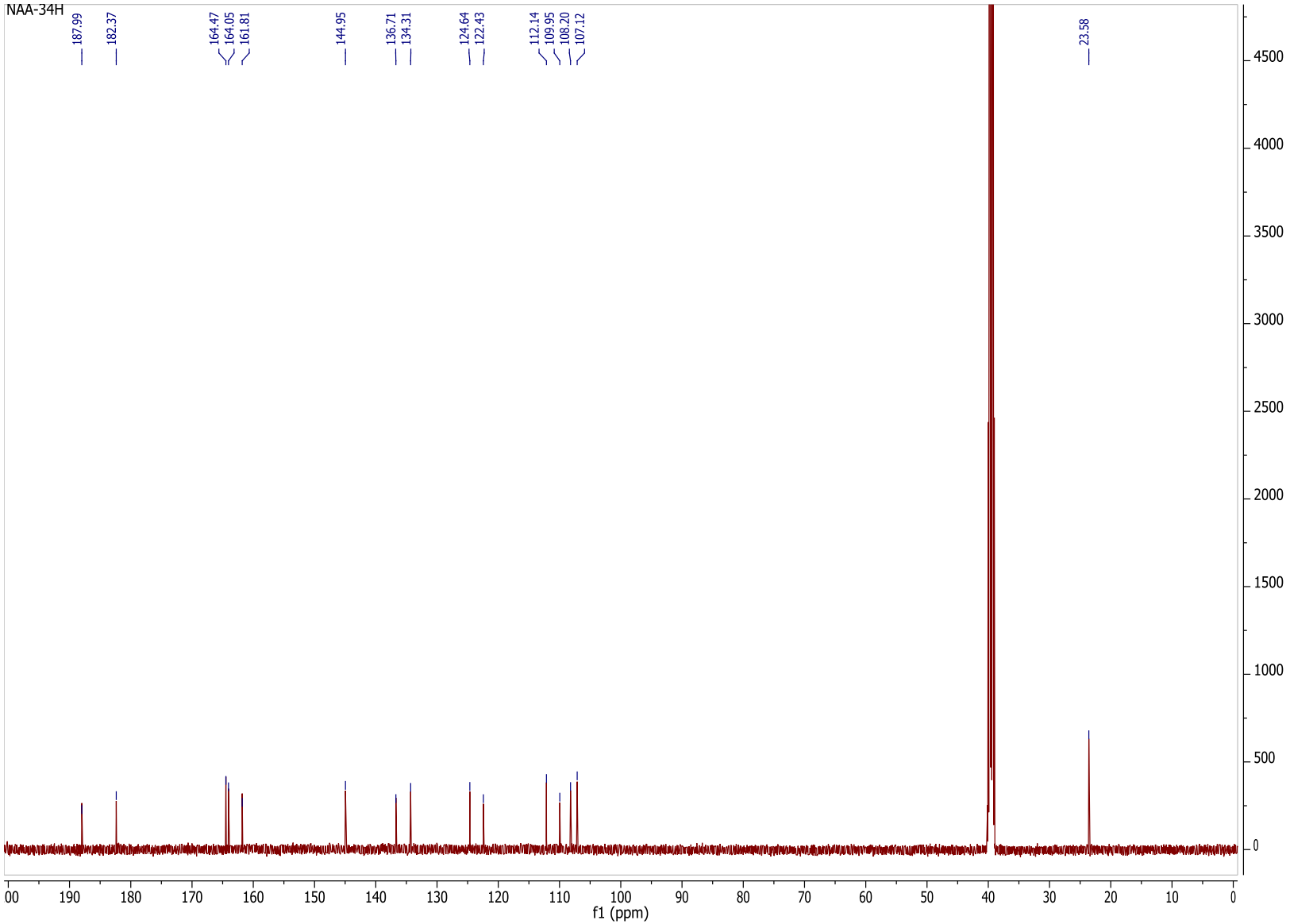
Appendix 28E: ESIMS spectrum of aloesaponarin II (**65**)



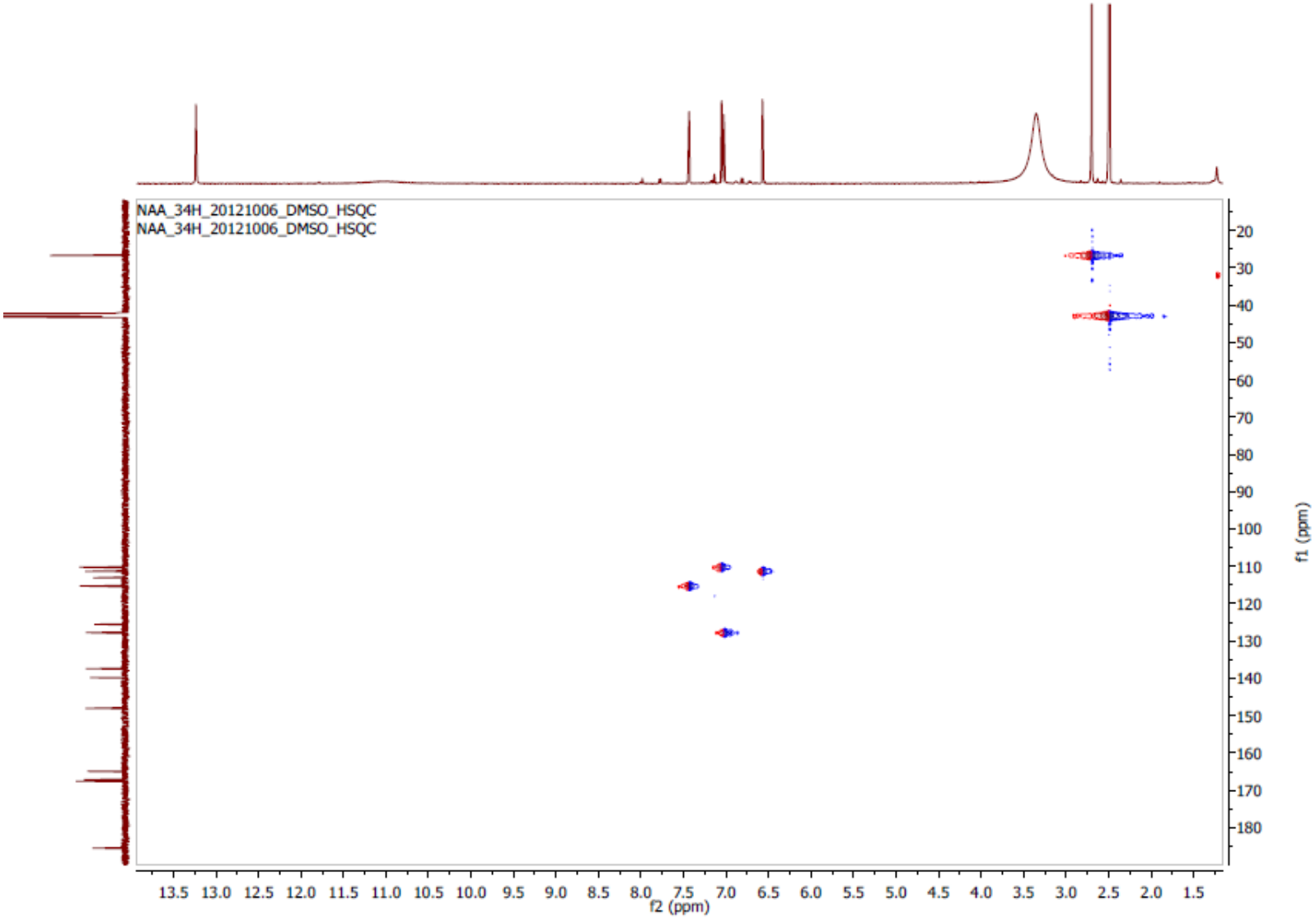
Appendix 29A: ^{1}H NMR spectrum of deoxyerythrolaccin (**68**), DMSO-d_6, 600 MHz



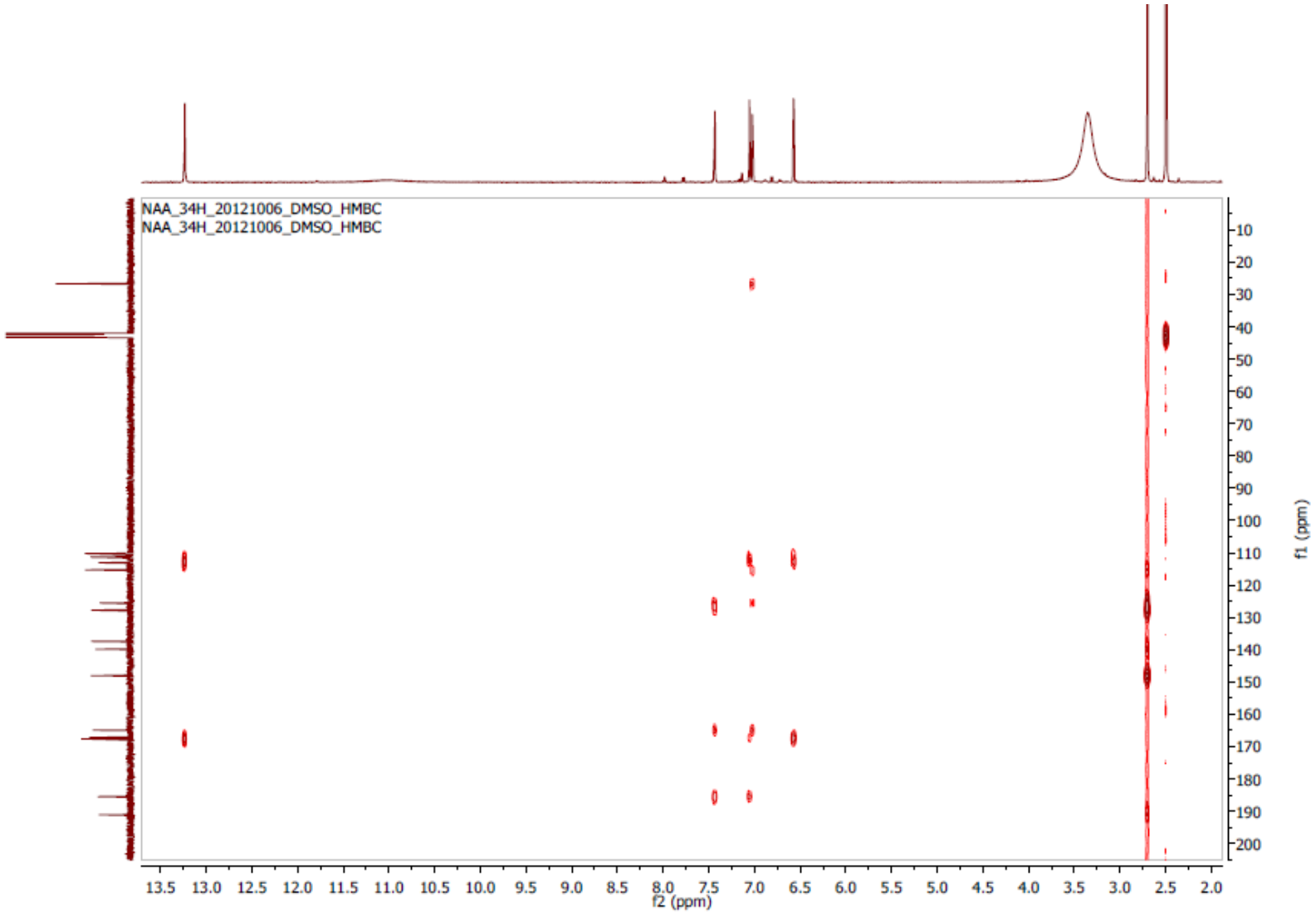
Appendix 29B: ^{13}C NMR spectrum of of deoxyerythrolaccin (**68**), DMSO-d_6, 200 MHz



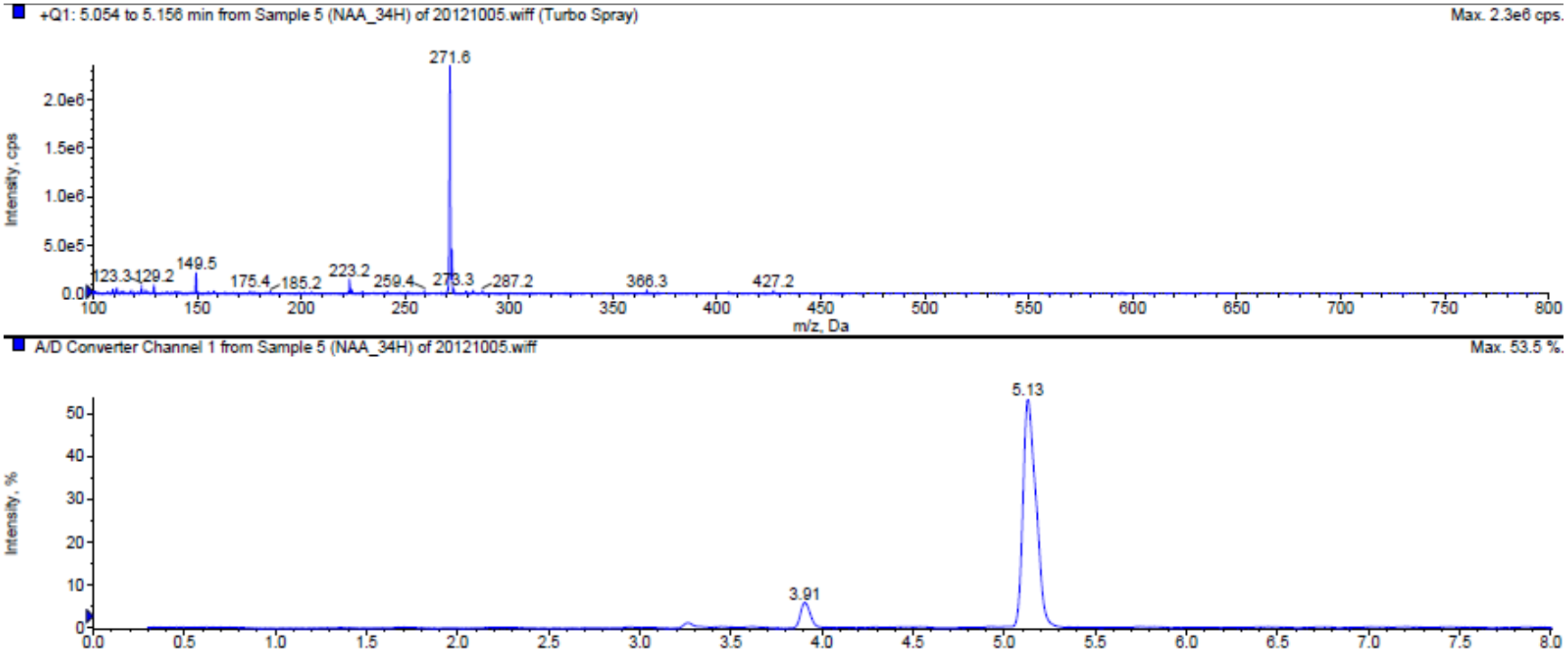
Appendix 29C: HSQC spectrum of deoxyerythrolaccin (**68**), DMSO-d_6, 600 MHz



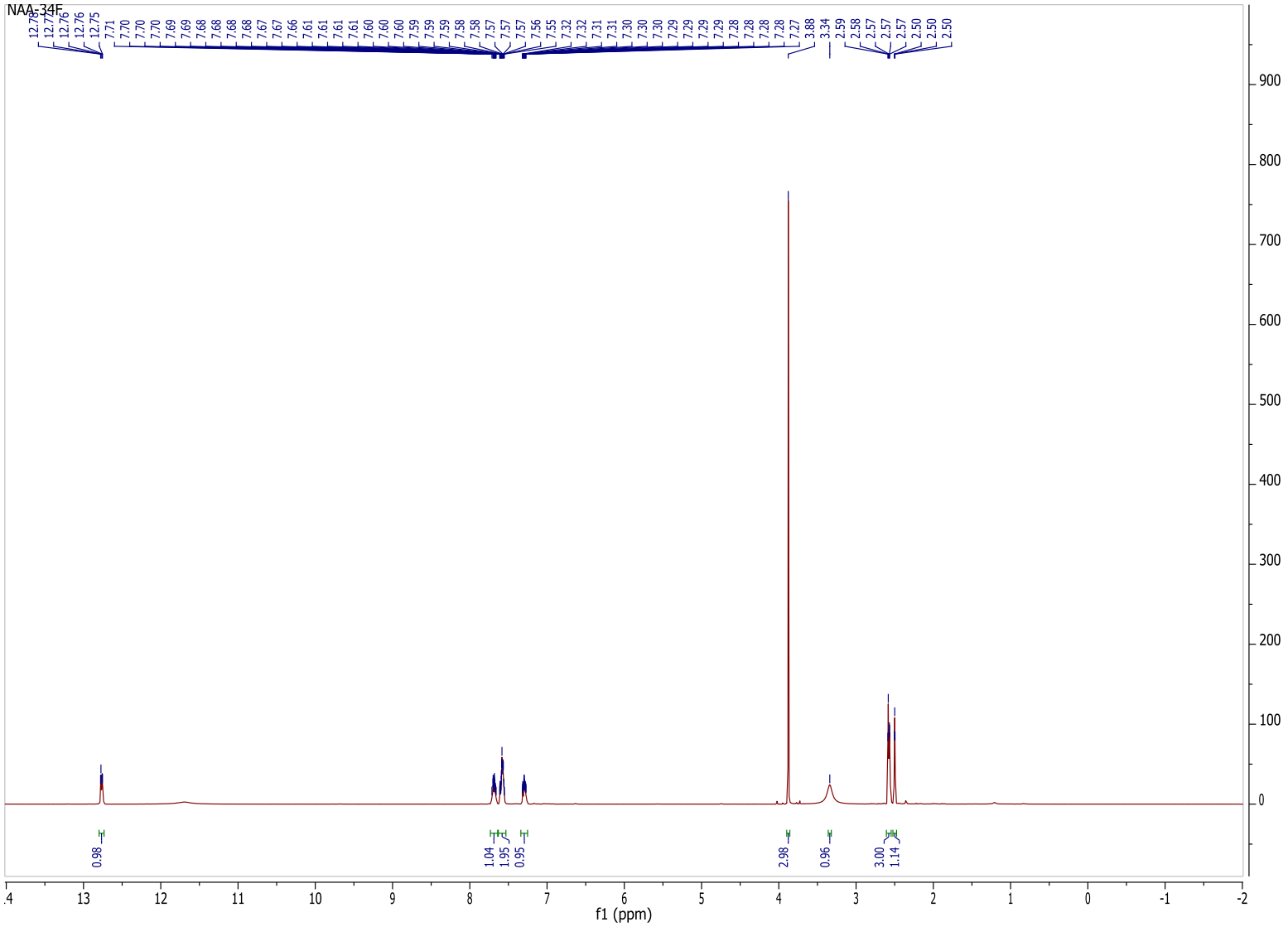
Appendix 29D: HMBC spectrum of deoxyerythrolaccin (**68**), DMSO-d_6, 600 MHz



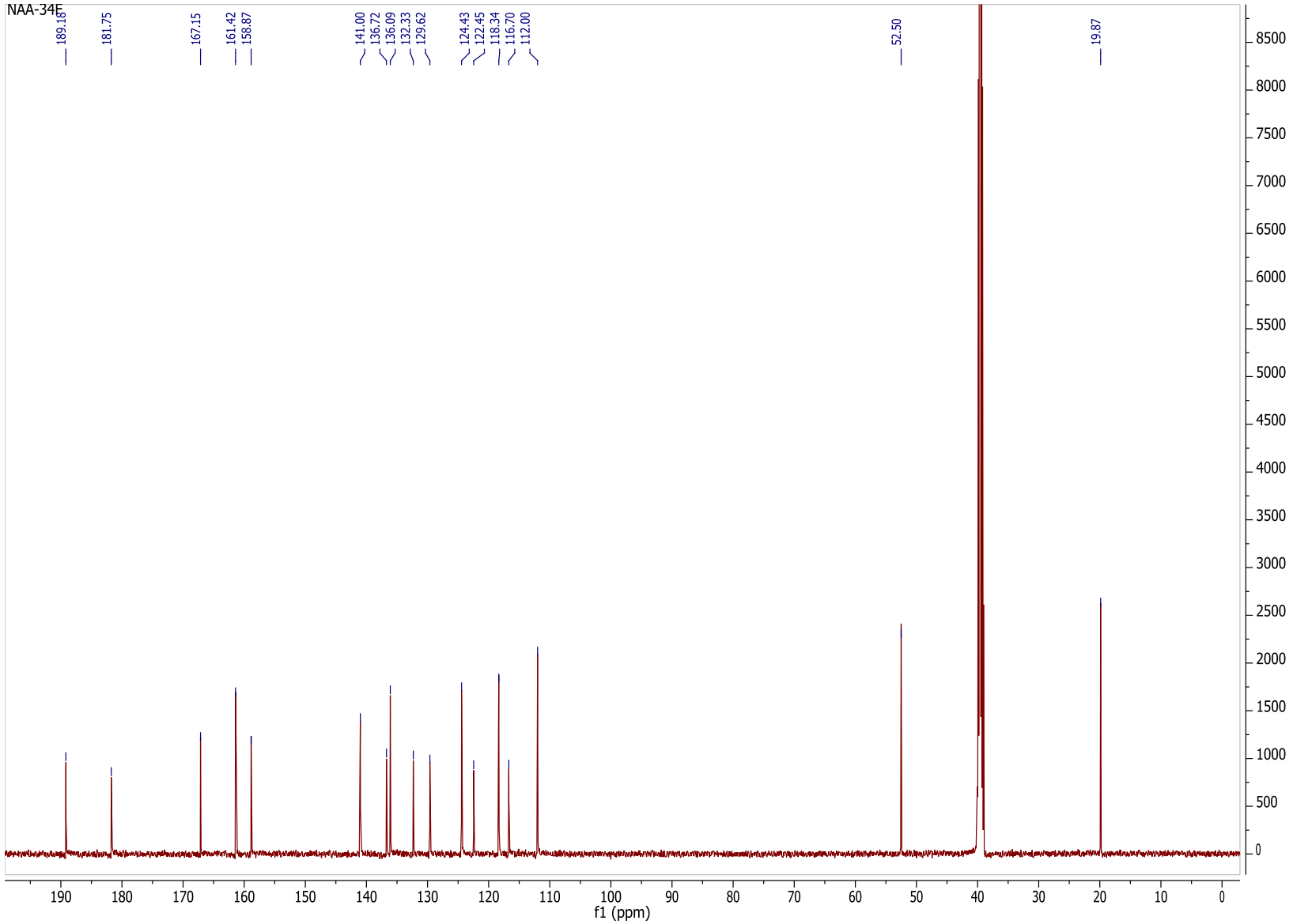
Appendix 29E: ESIMS spectrum of deoxyerythrolaccin (**68**)



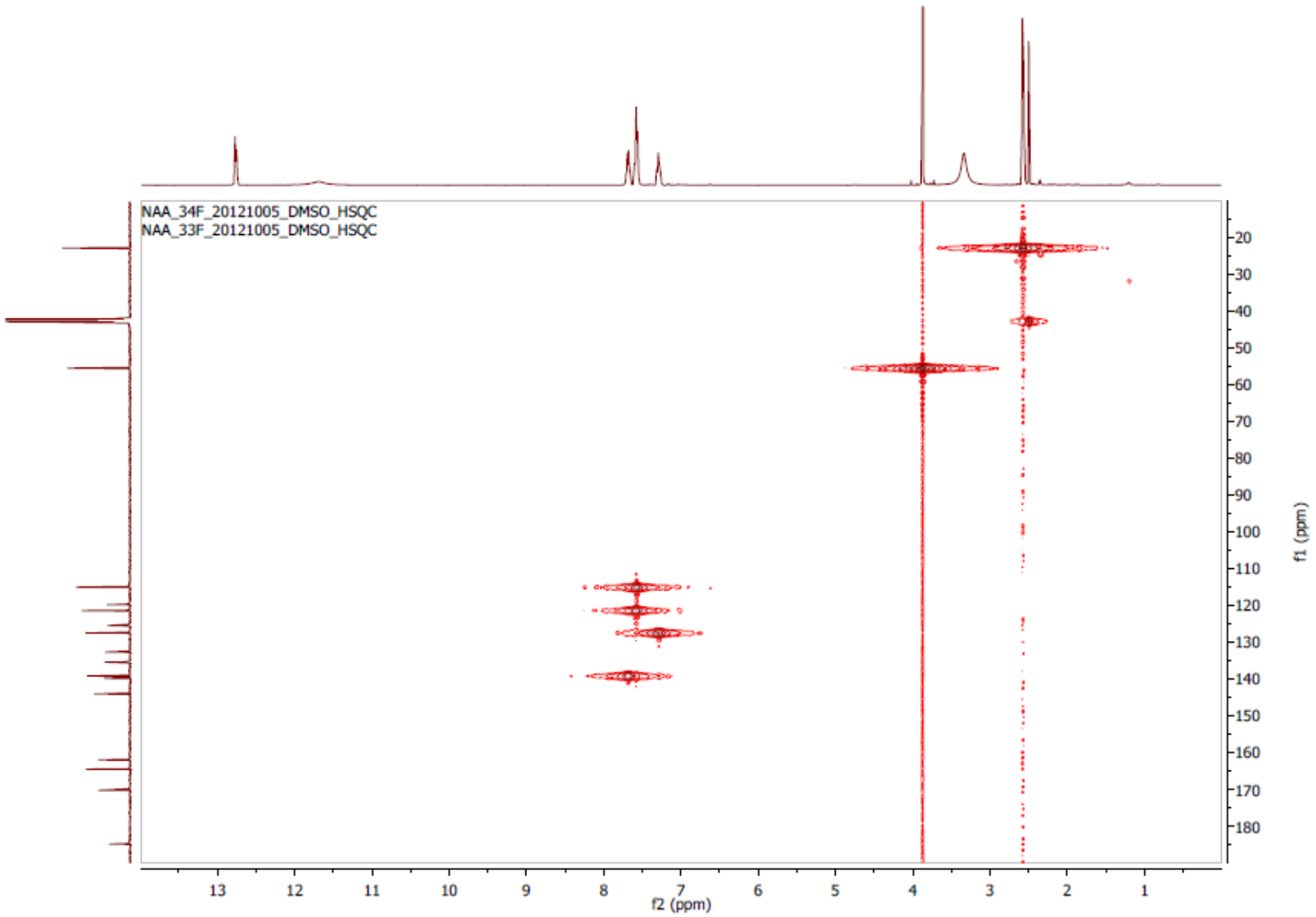
Appendix 30A: ^{1}H NMR spectrum of aloesaponarin I (**66**), DMSO-d_6, 600 MHz



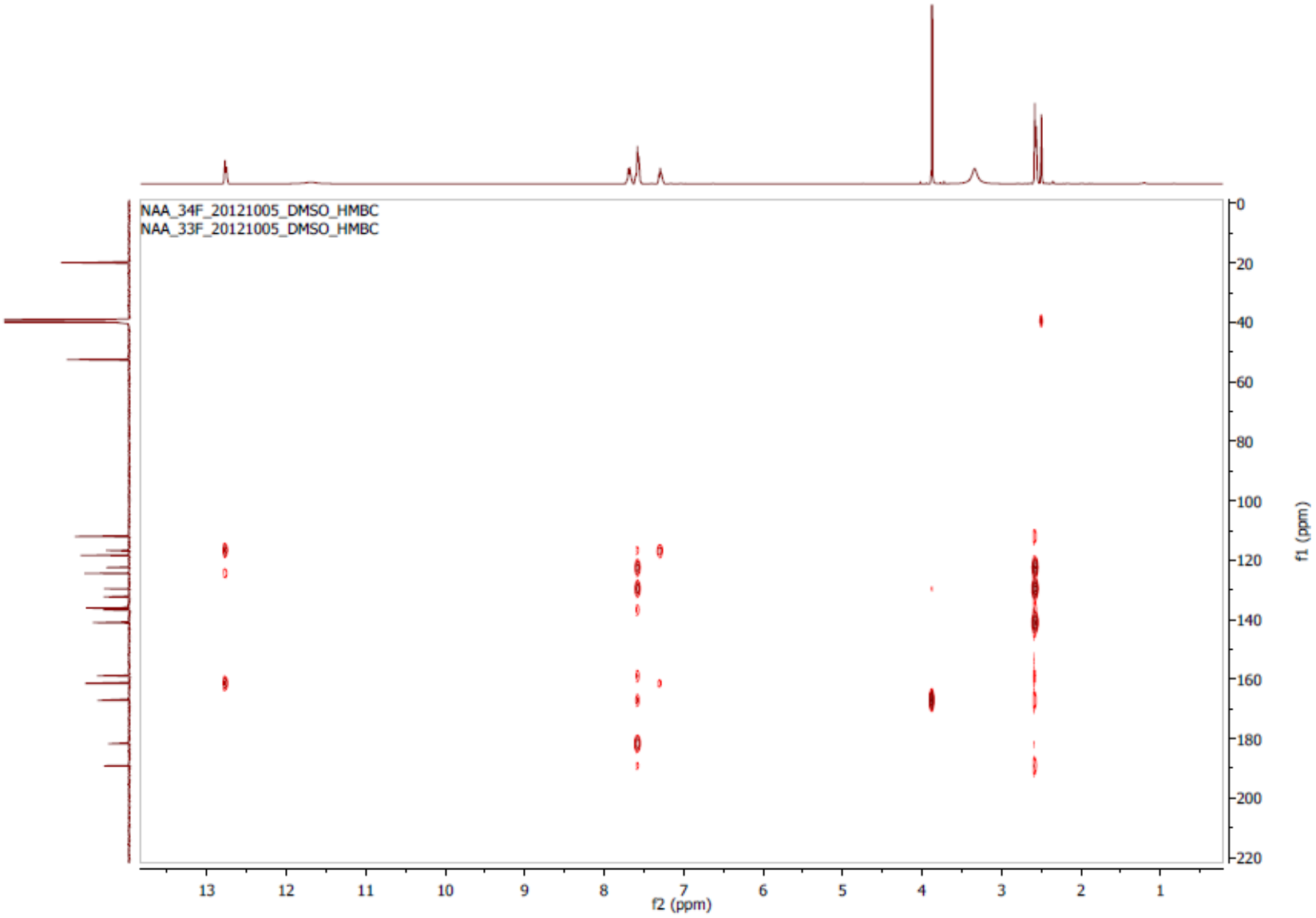
Appendix 30B: ^{13}C NMR spectrum of aloesaponarin I (**66**), DMSO-d_6, 200 MHz



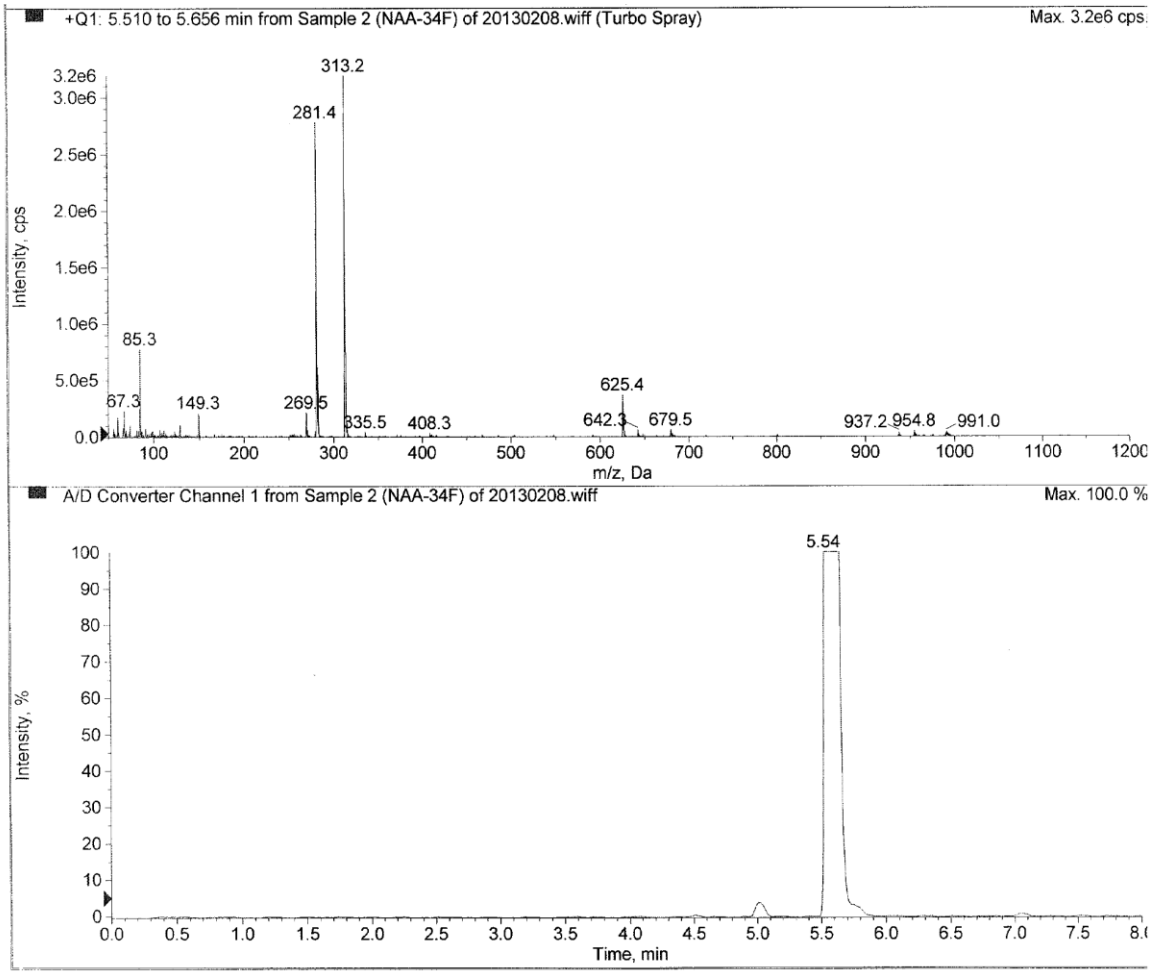
Appendix 30C: HSQC spectrum of aloesaponarin I (**66**), DMSO-d_6, 600 MHz



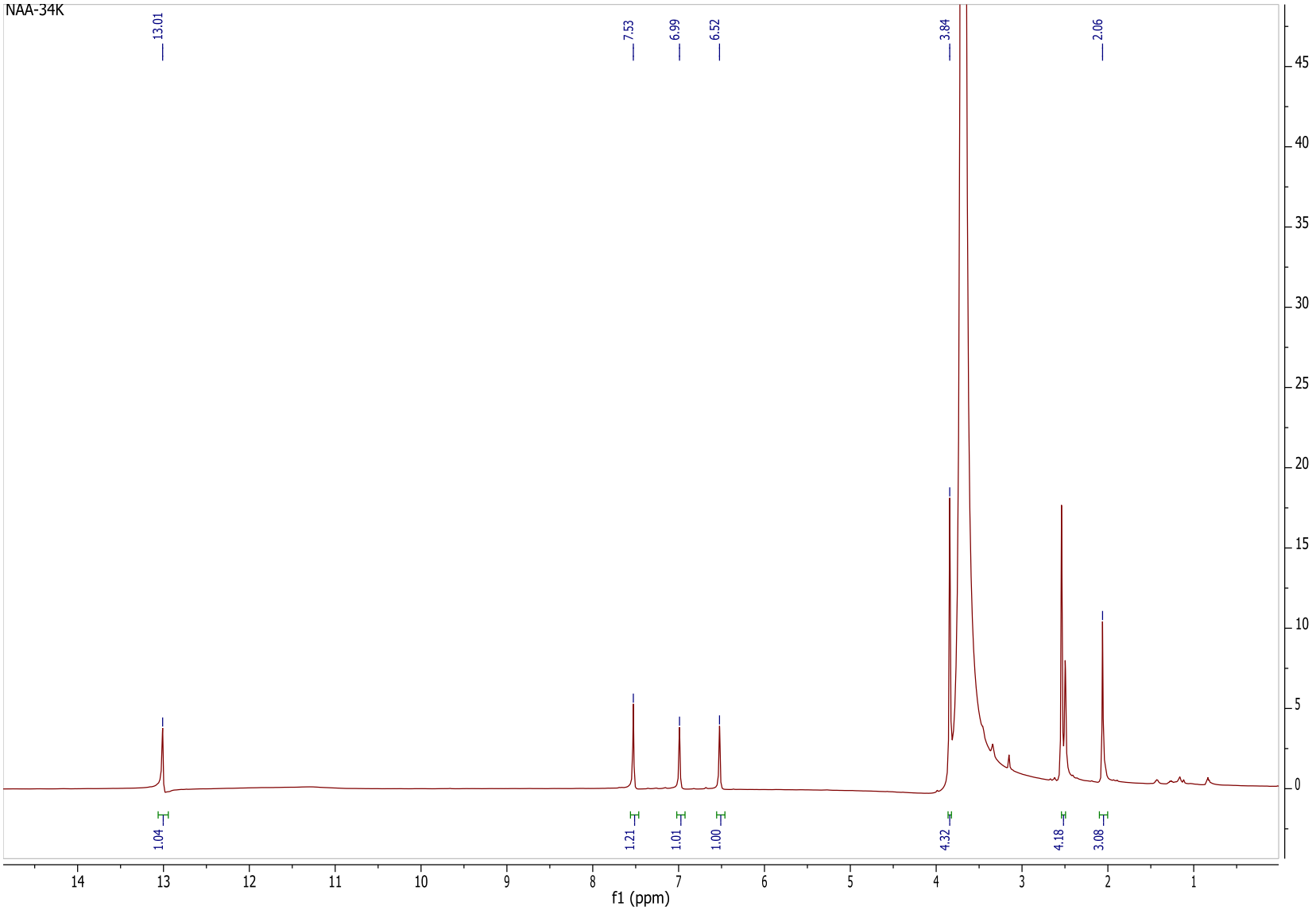
Appendix 30D: HMBC spectrum of aloesaponarin I (**66**), DMSO-d_6, 600 MHz



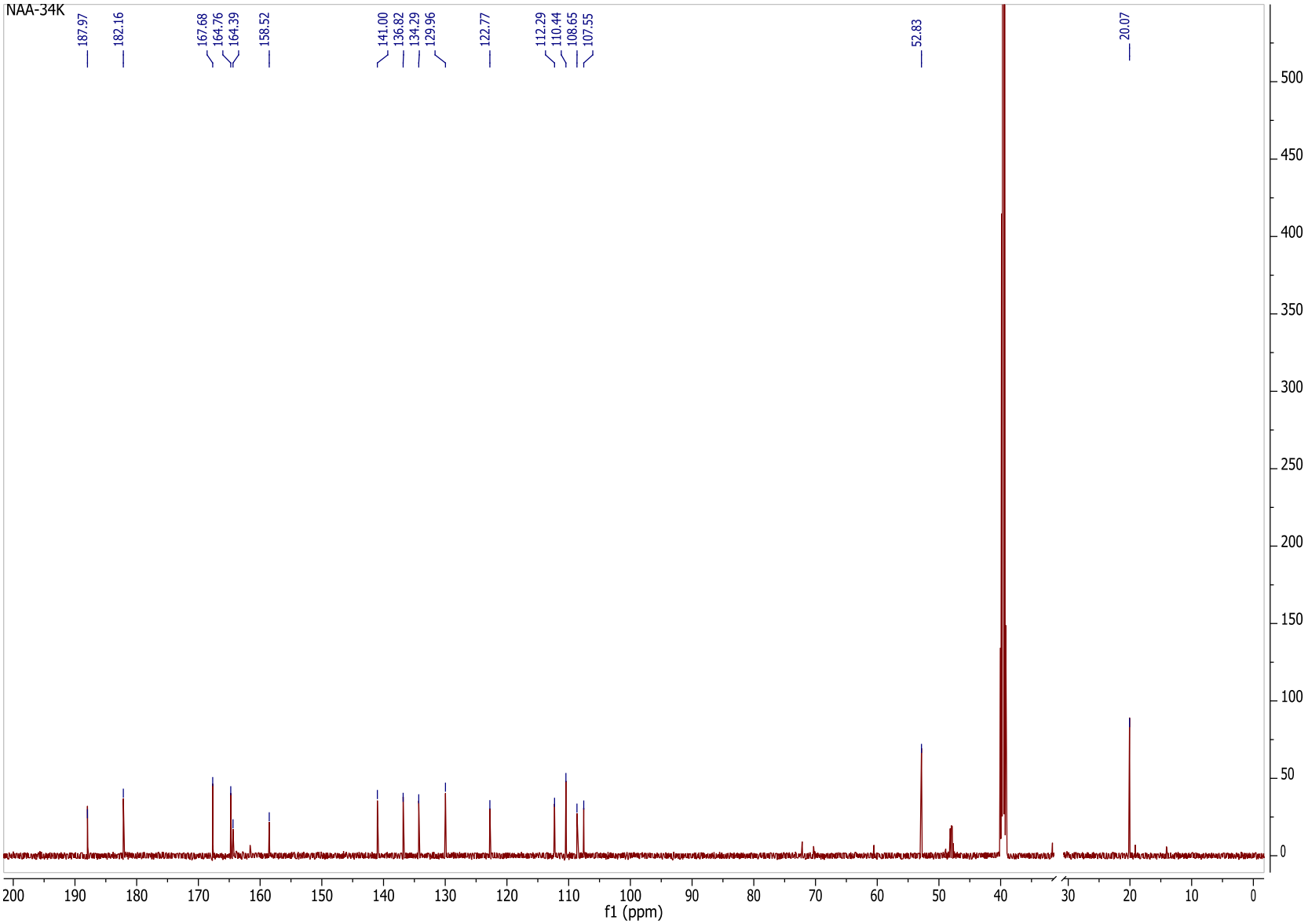
Appendix 30E: ESIMS spectrum of of aloesaponarin I (**66**)



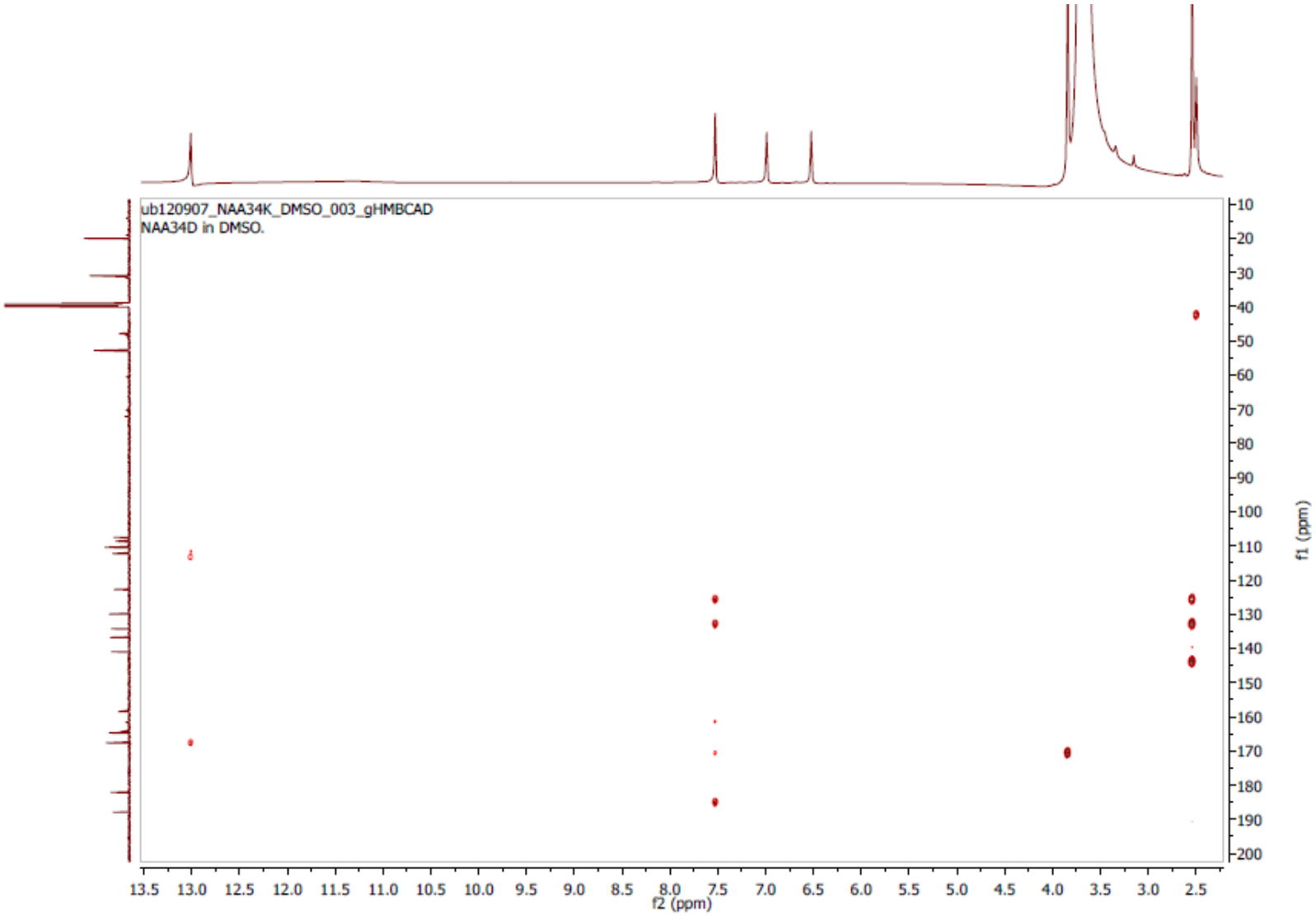
Appendix 31A: ^{1}H NMR spectrum of laccaic acid D methyl ester (**67**), DMSO-d_6, 600 MHz



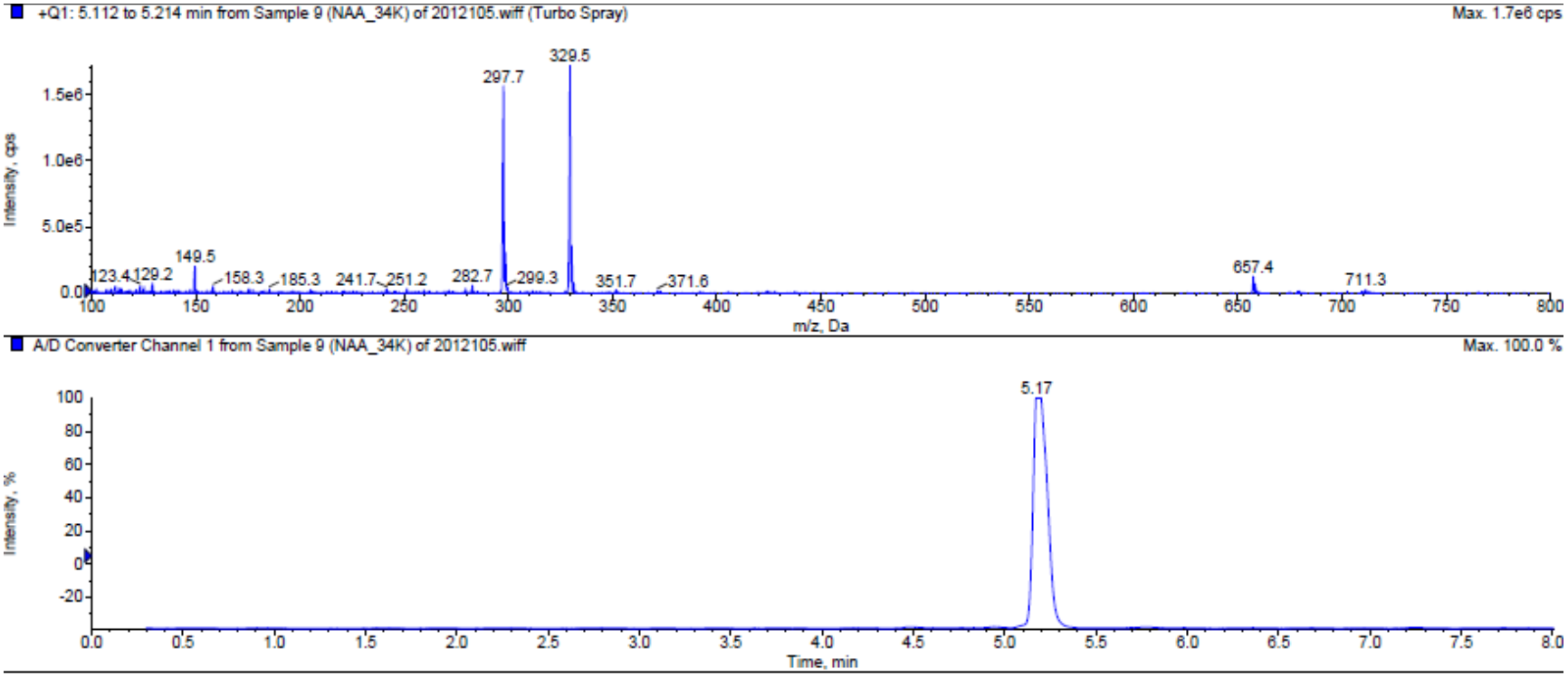
Appendix 31B: ^{13}C NMR spectrum of laccaic acid D methyl ester (**67**), DMSO-d_6, 200 MHz



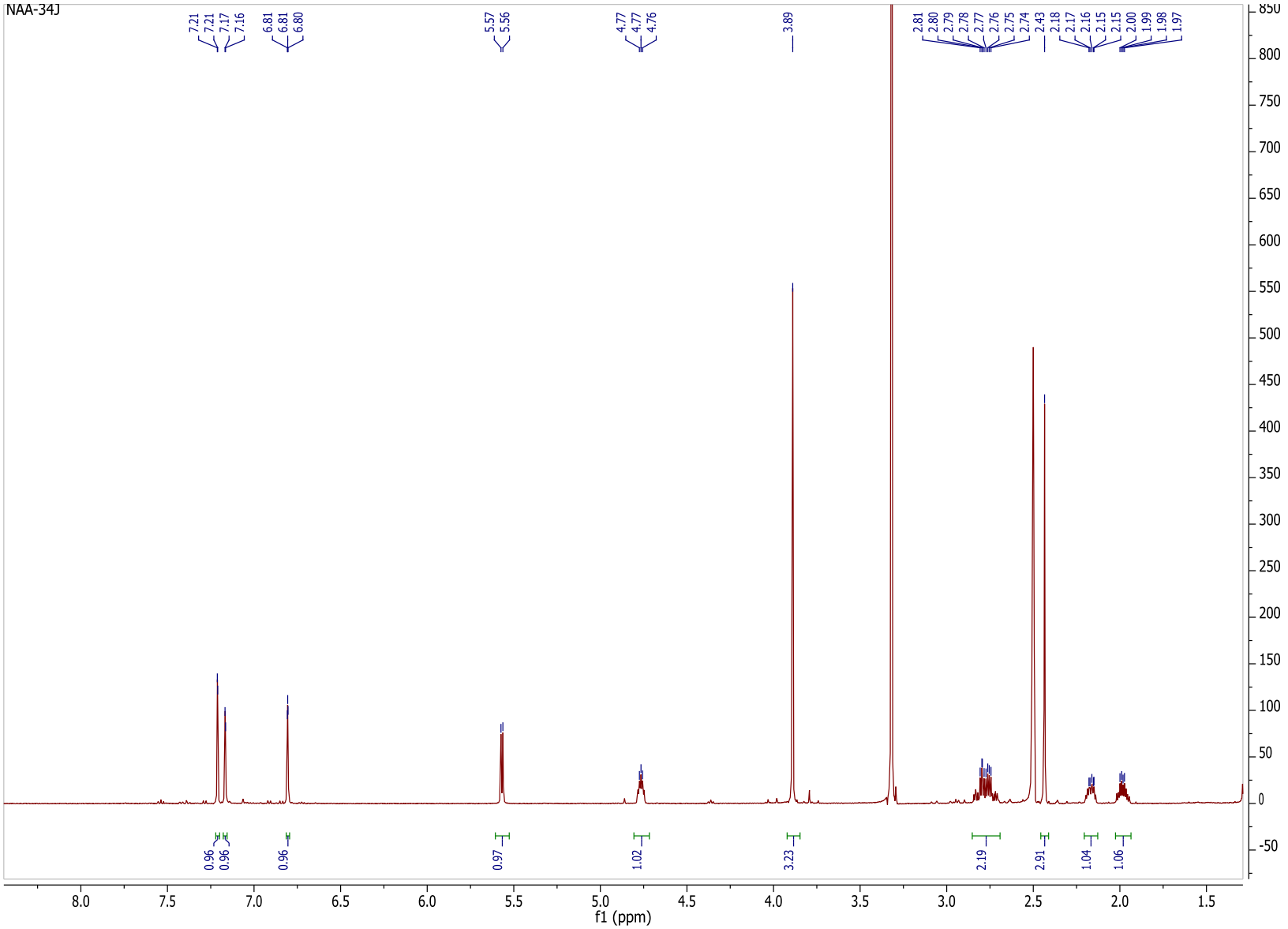
Appendix 31C: HMBC spectrum of laccaic acid D methyl ester (**67**), DMSO-d_6, 600 MHz



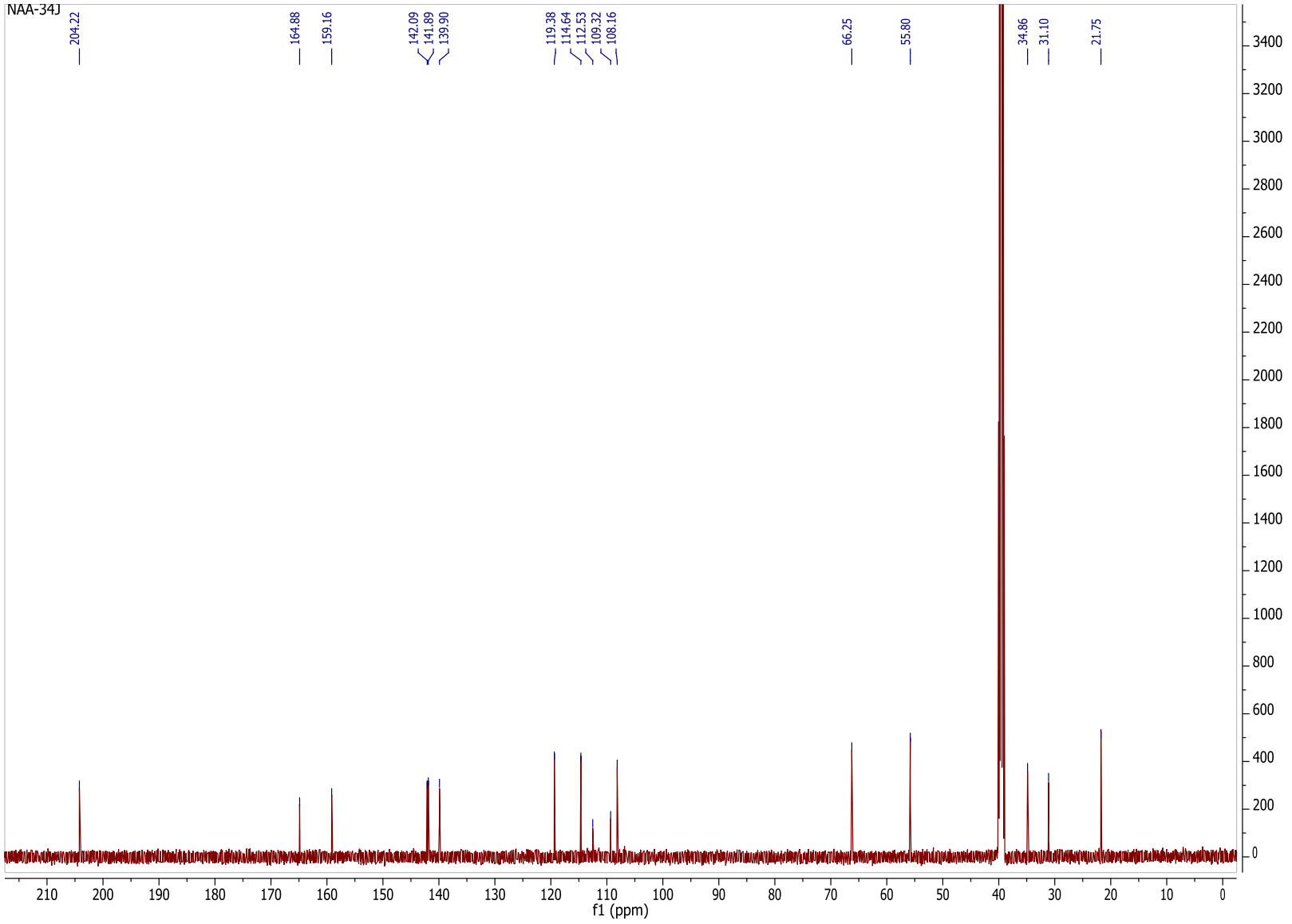
Appendix 31D: ESIMS spectrum of laccaic acid D methyl ester (**67**)



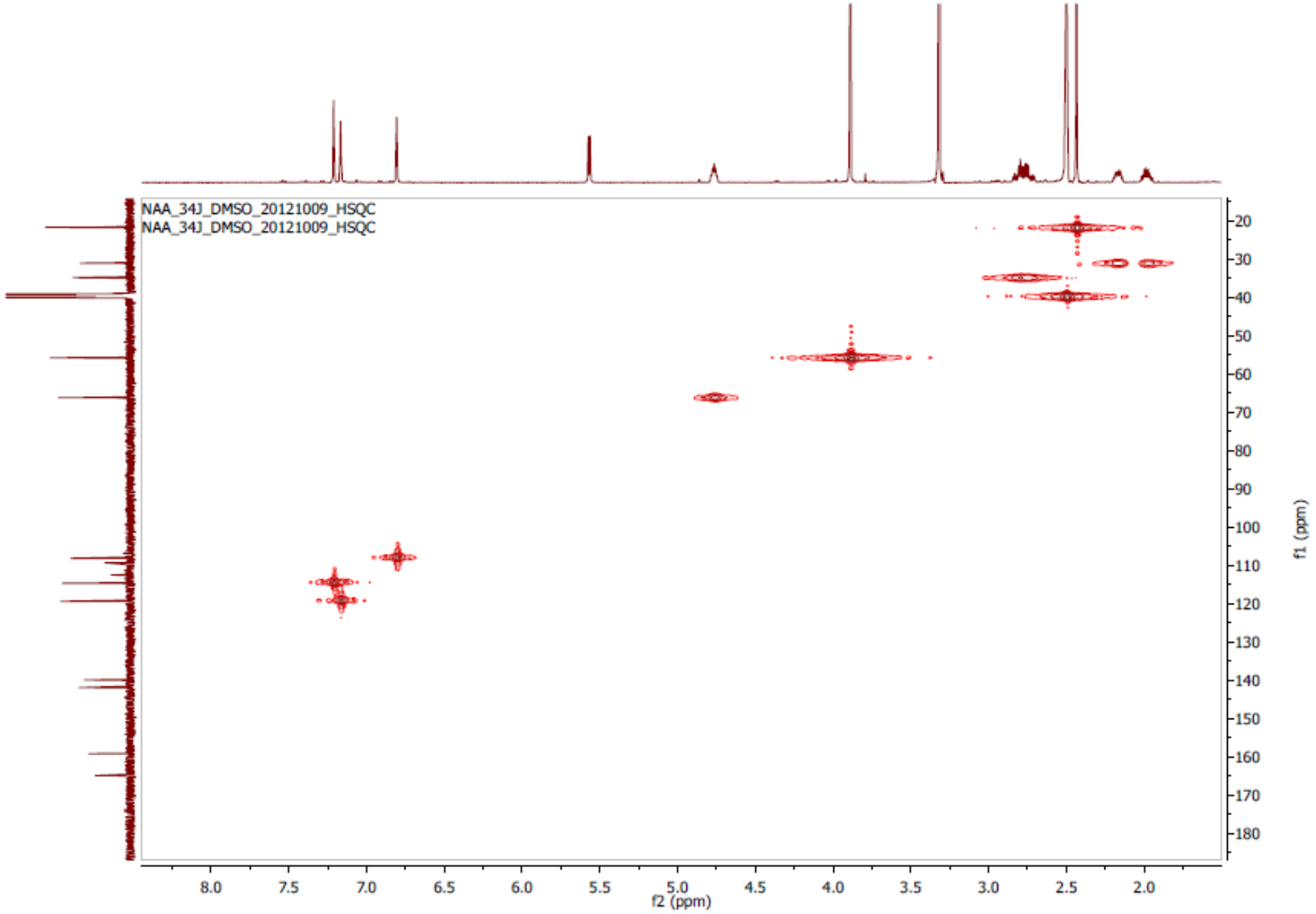
Appendix 32A: ^{1}H NMR spectrum of aloesaponol II-6-methyl ether (**157**), DMSO-d_6, 600 MHz



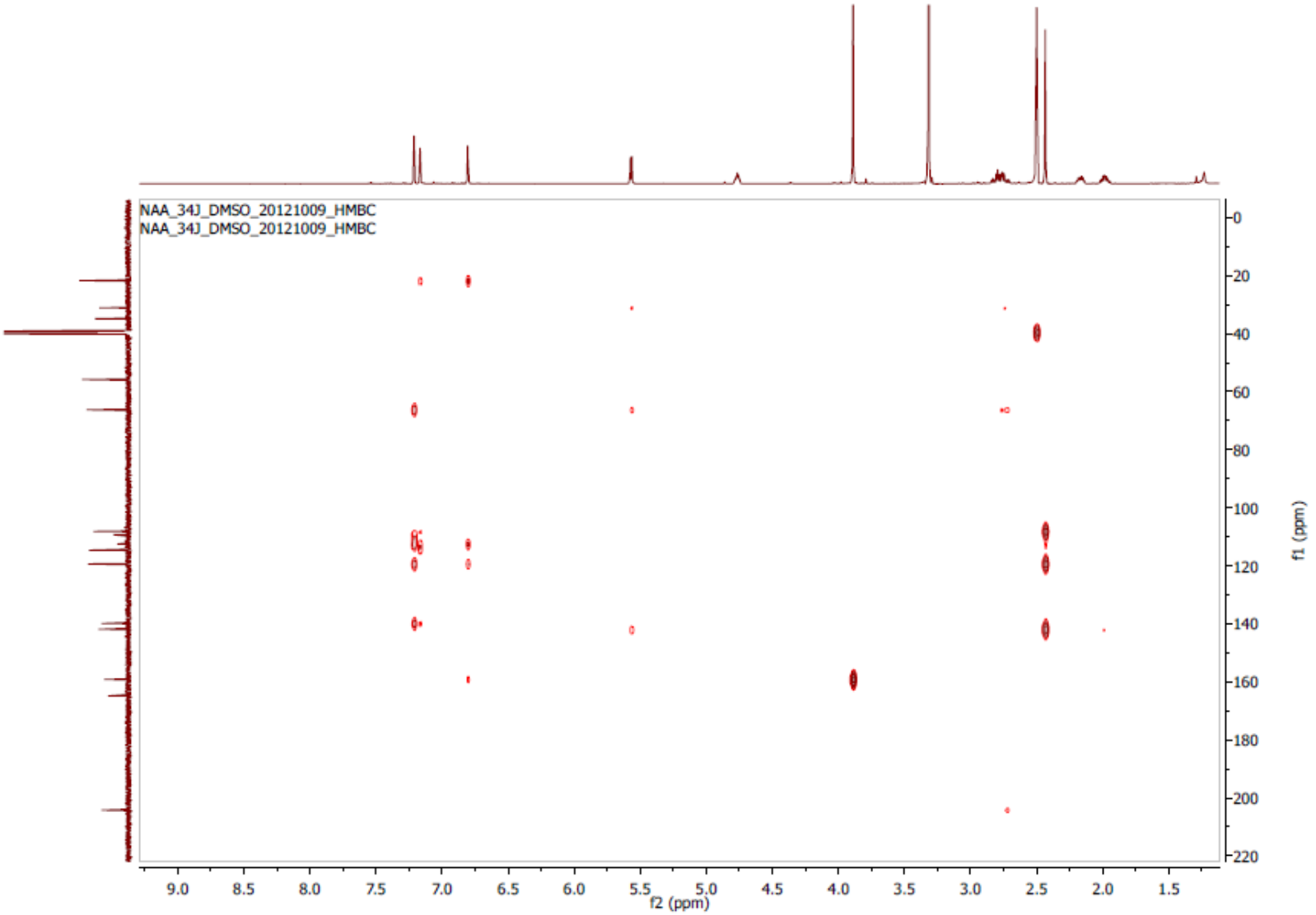
Appendix 32B: ^{13}C NMR spectrum of aloesaponol II-6-methyl ether (**157**), DMSO-d_6, 200 MHz



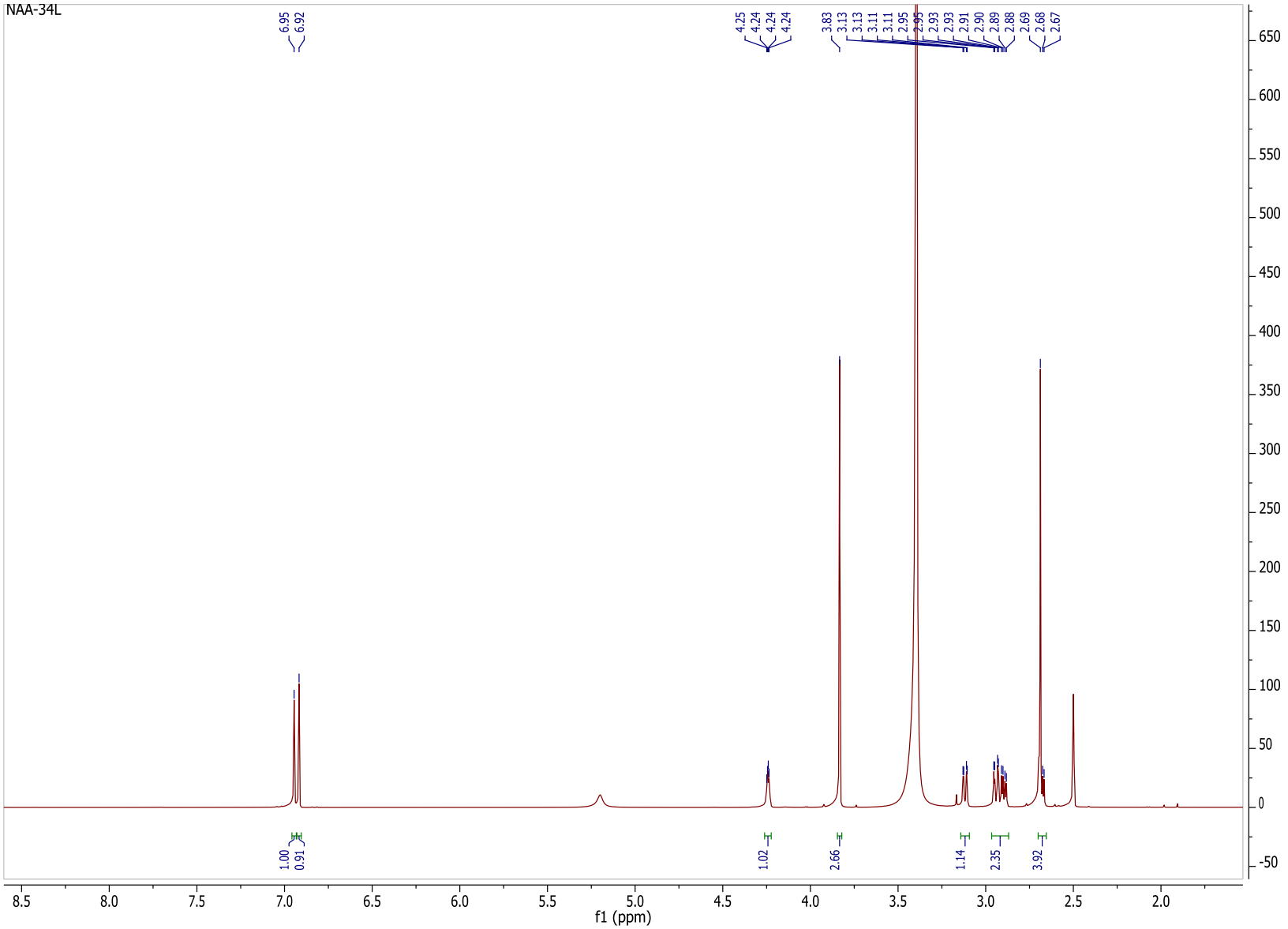
Appendix 32C: HSQC spectrum of aloesaponol II-6-methyl ether (**157**), DMSO-d_6, 600 MHz



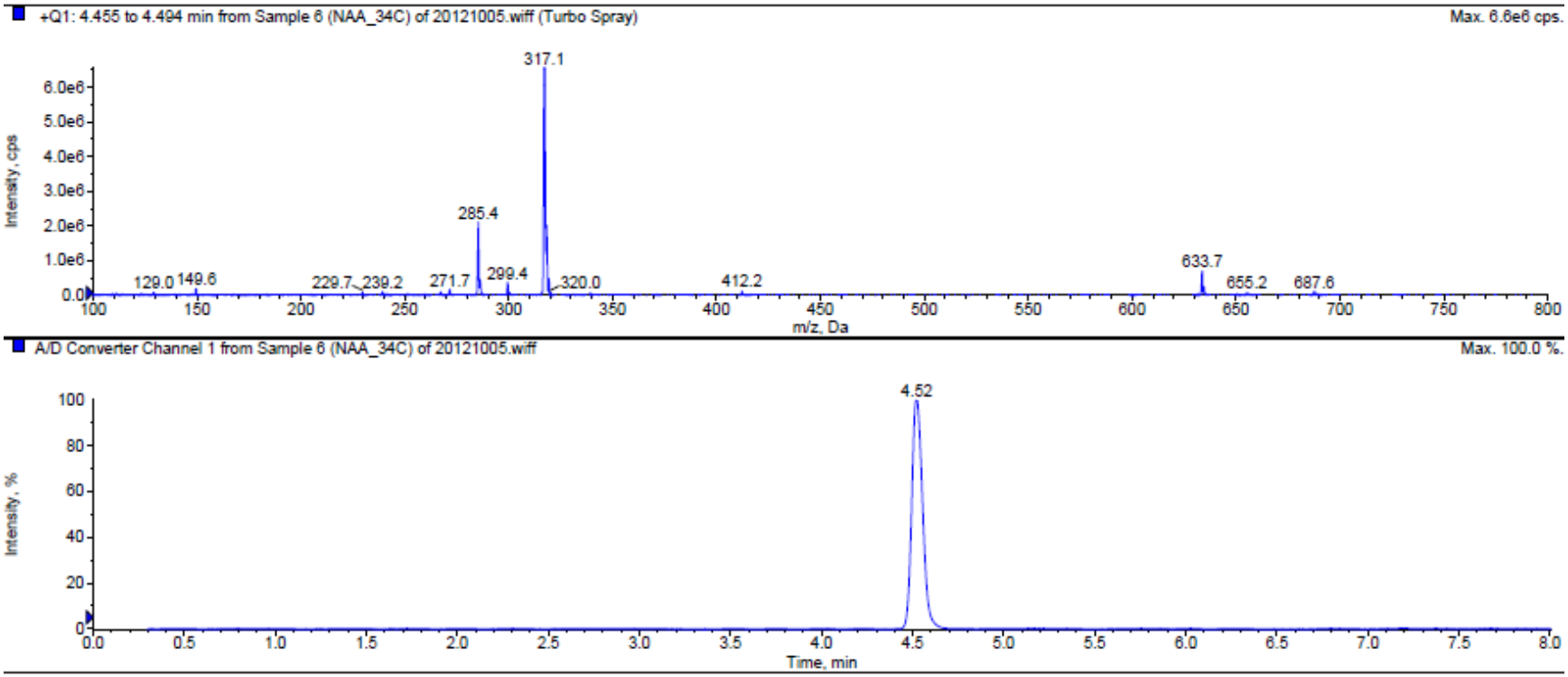
Appendix 32D: HMBC spectrum of aloesaponol II-6-methyl ether (**157**), DMSO-d_6, 600 MHz



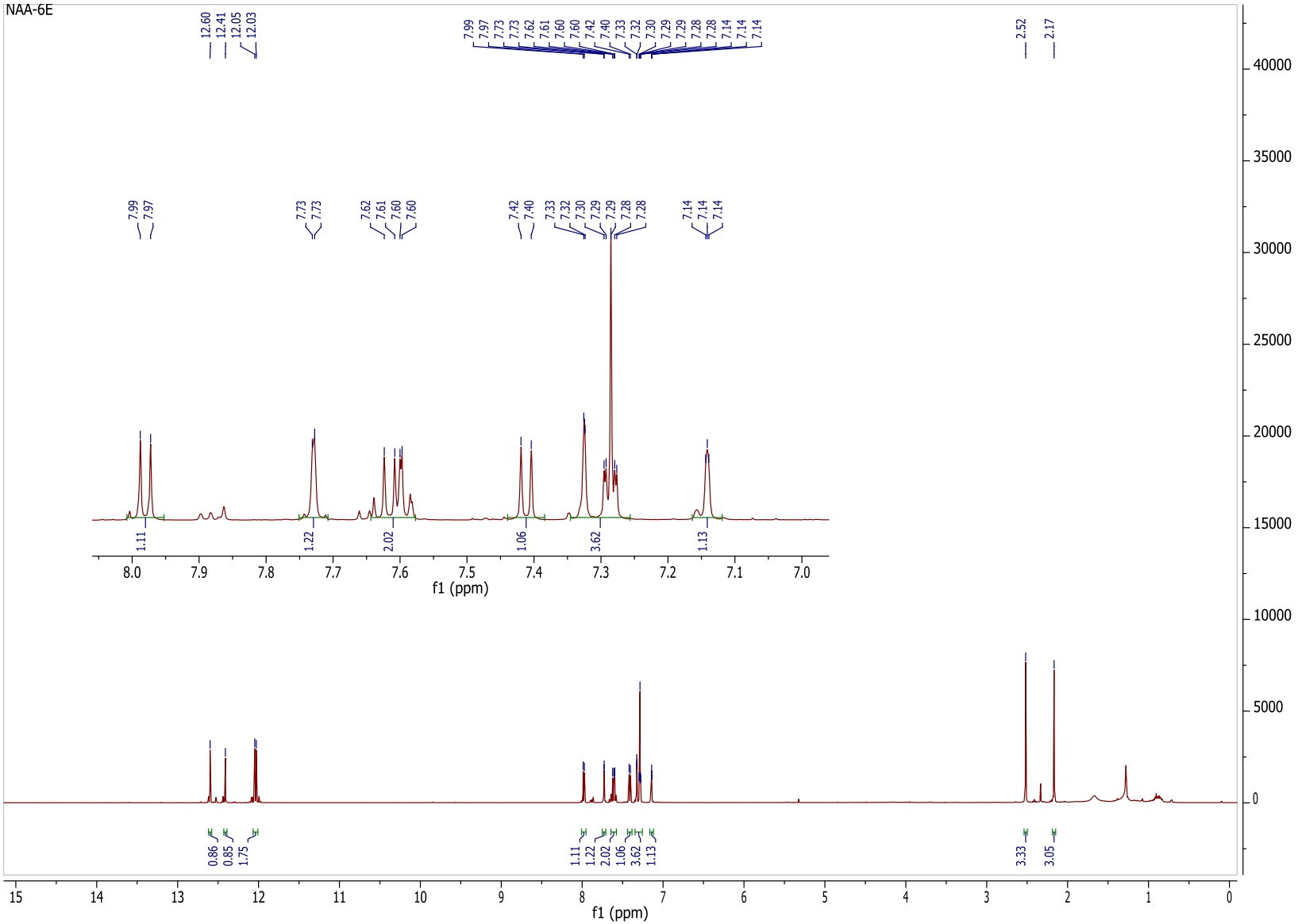
Appendix 33A: ^{1}H NMR spectrum of aloesaponol I (**69**), DMSO-d_6, 600 MHz



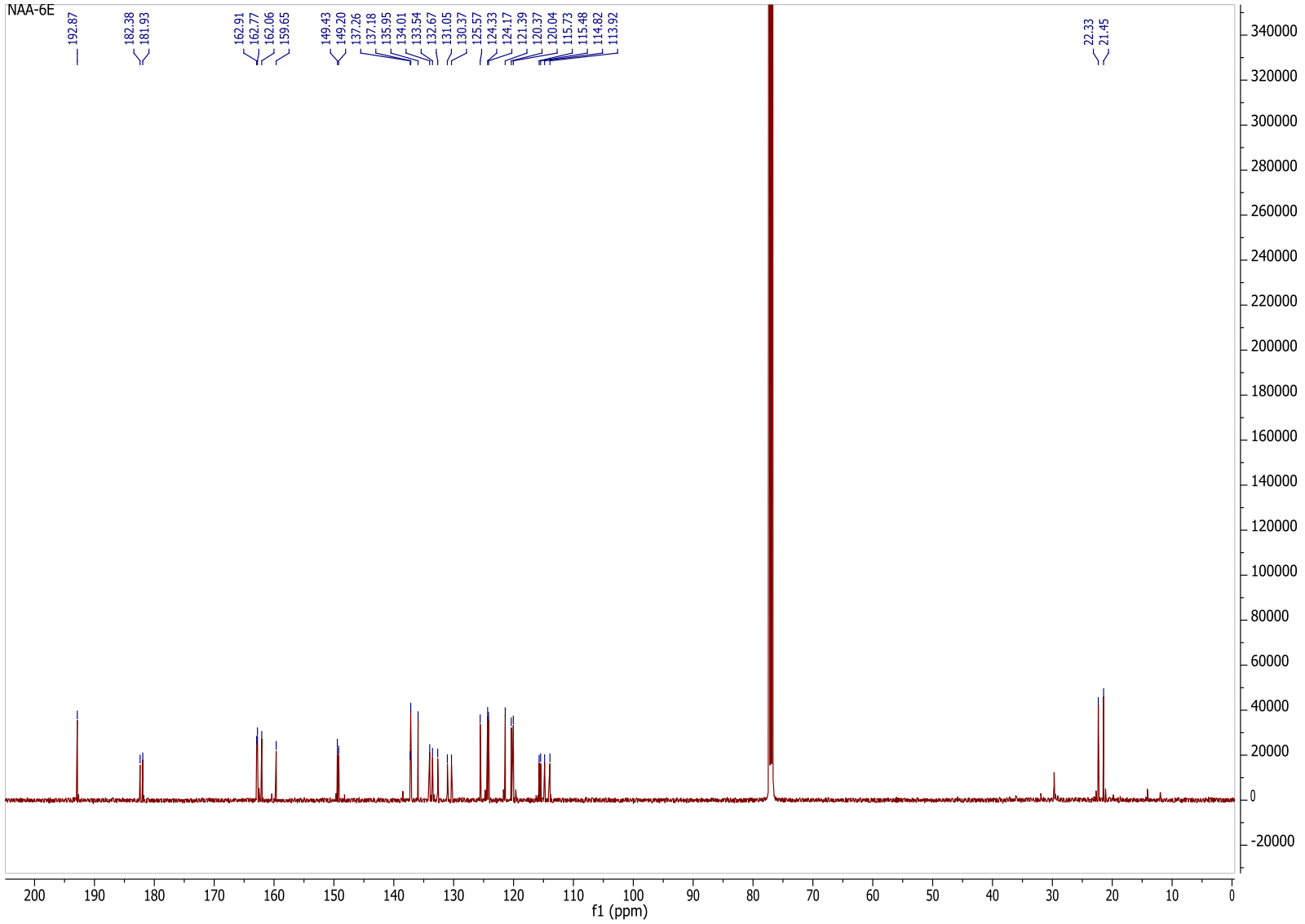
Appendix 33B: ESIMS spectrum of aloesaponol I (**69**)



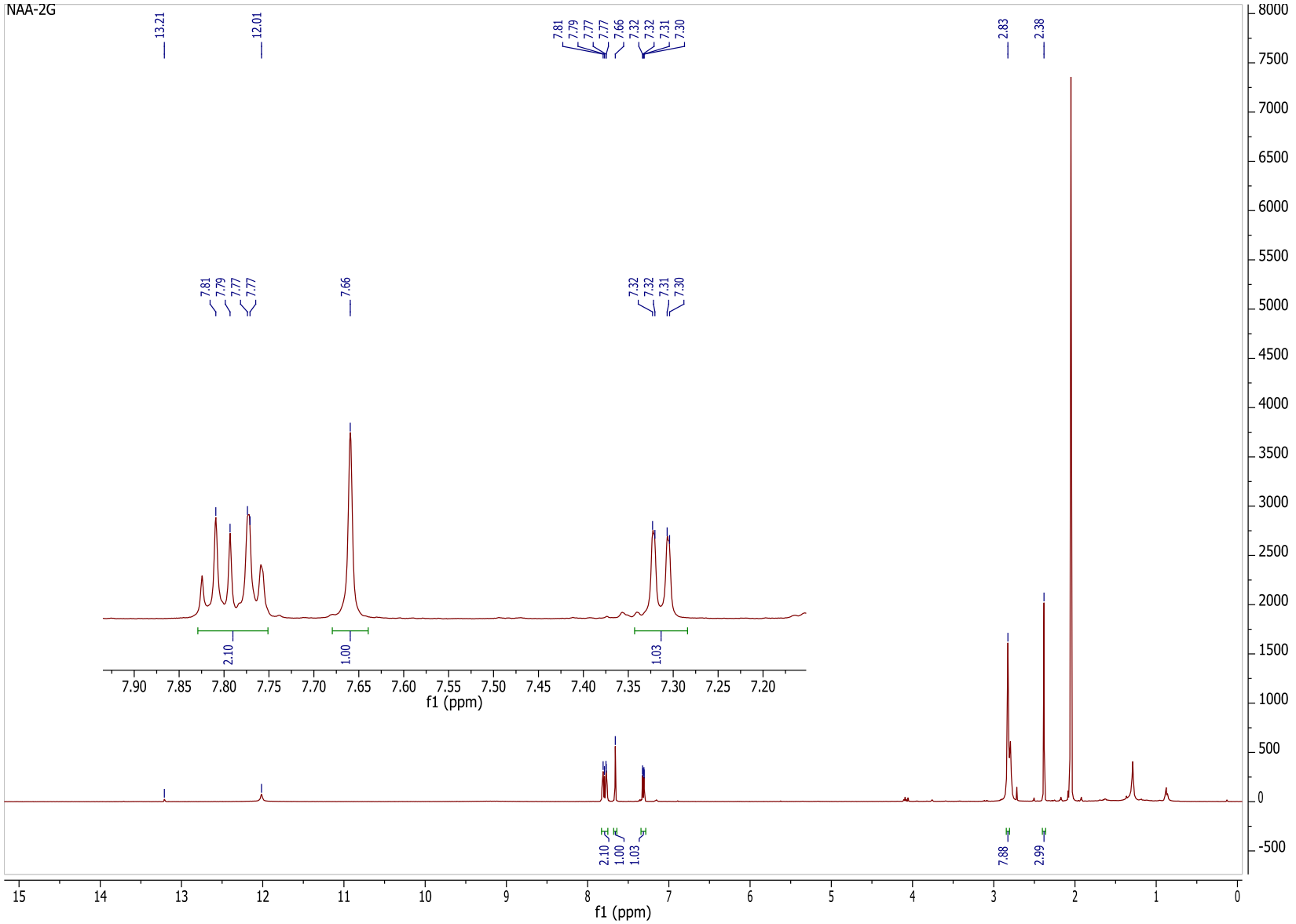
Appendix 34A: ^{1}H NMR spectrum of asphodeline (**23**), $CDCl_3$, 600 MHz



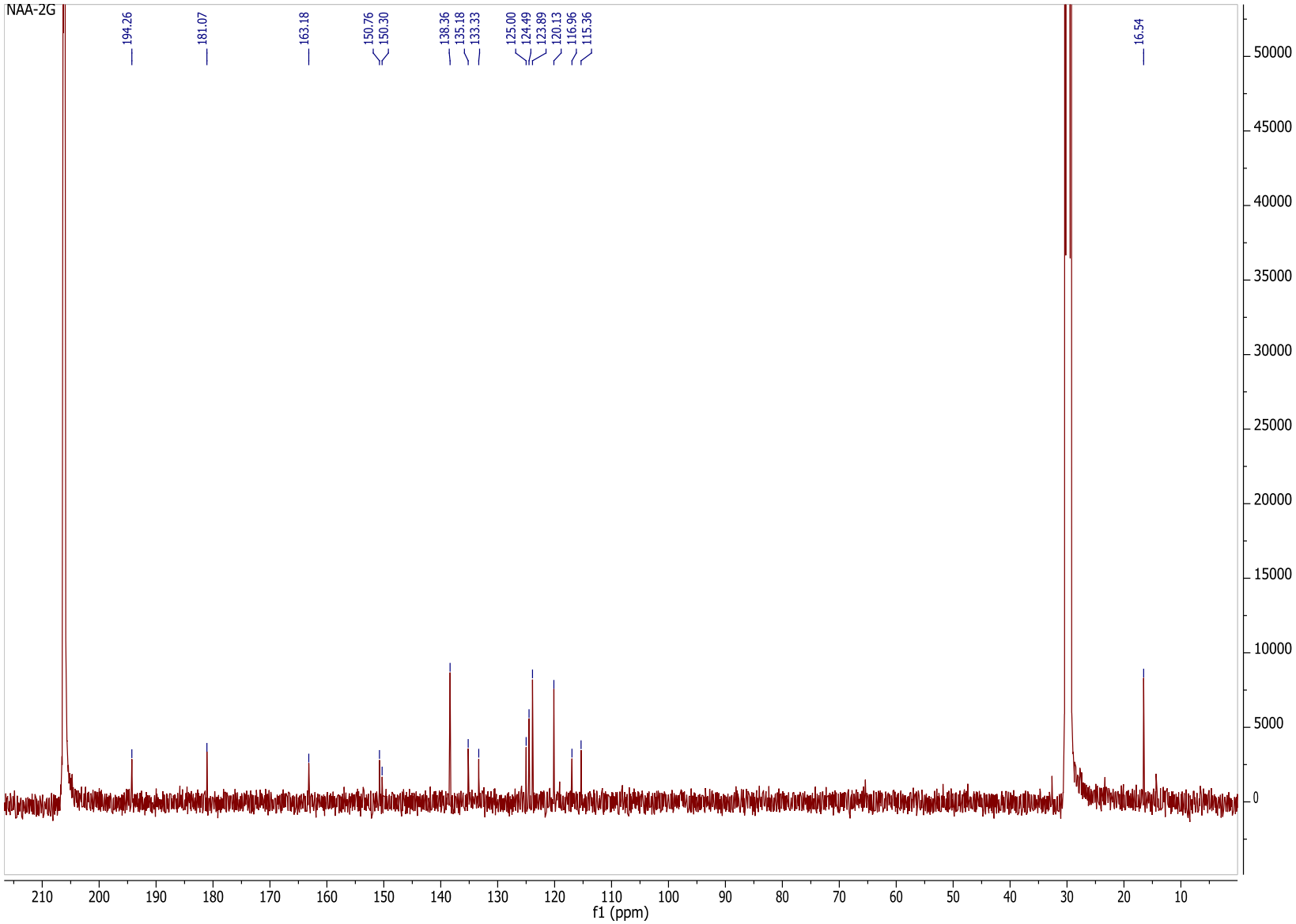
Appendix 34 B: ^{13}C NMR spectrum of of asphodeline (**23**), $CDCl_3$, 200 MHz



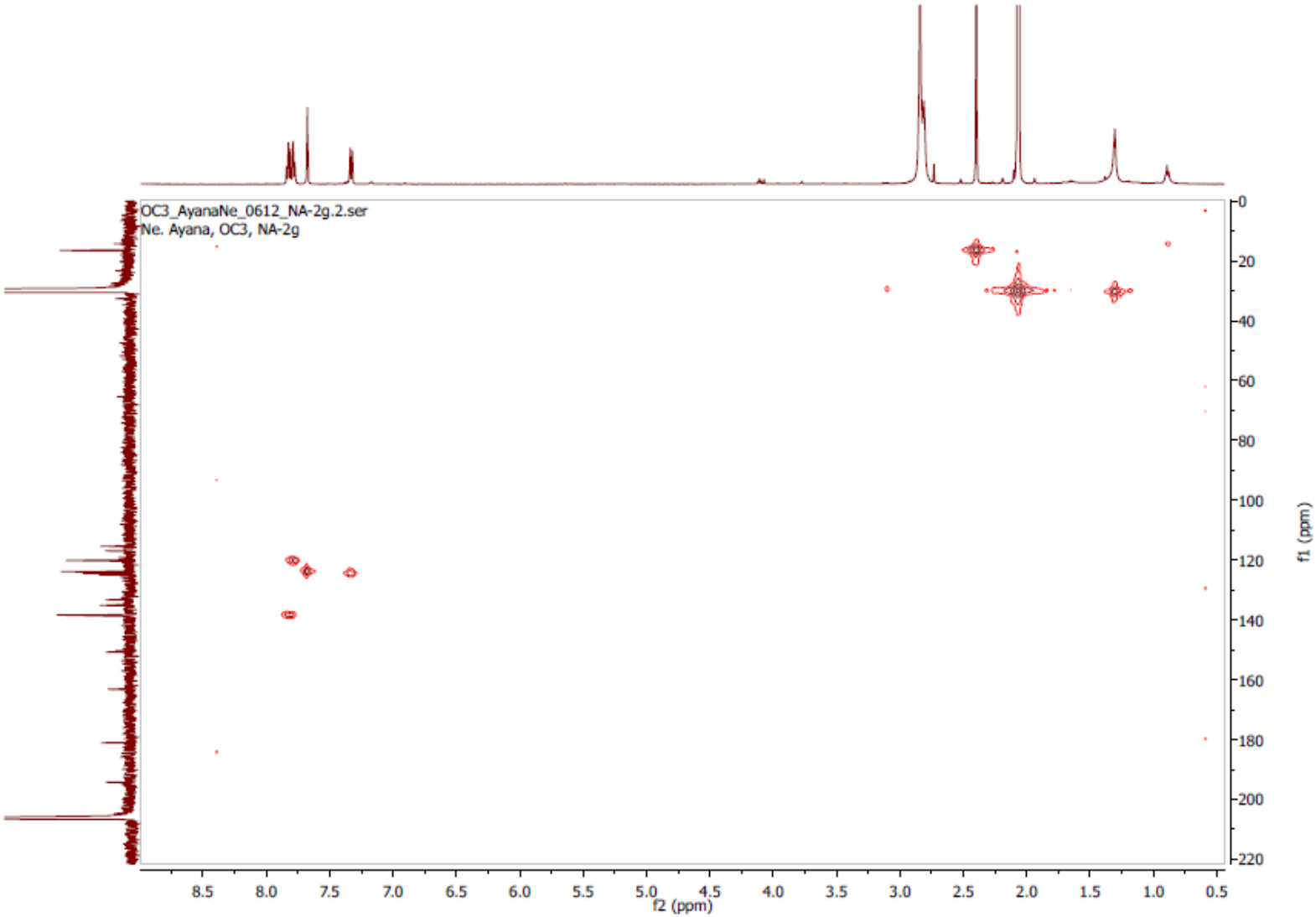
Appendix 35A: ^{1}H NMR spectrum of norobtusifolin (**158**), acetone-d_6, 500 MHz



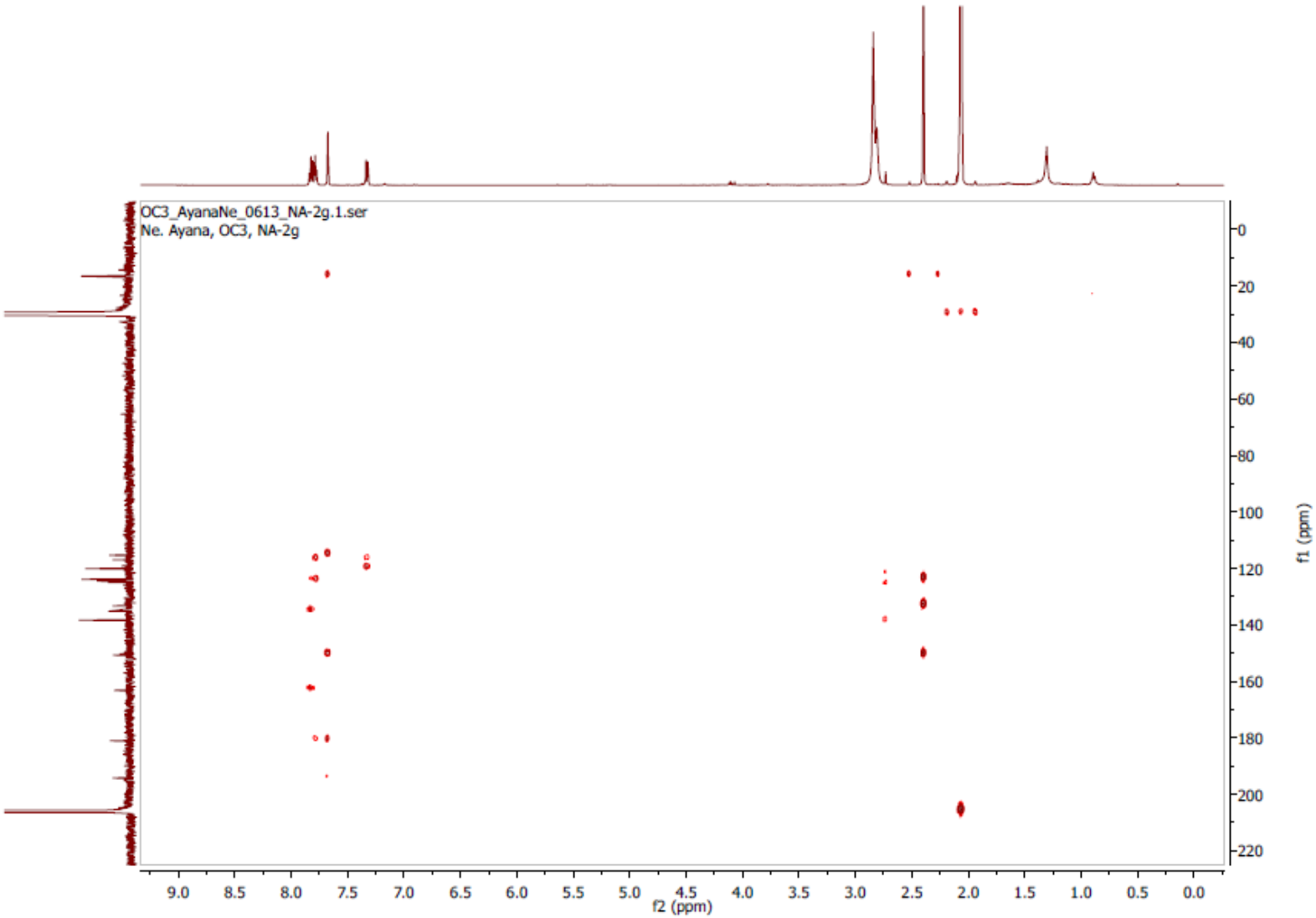
Appendix 35B: ^{13}C NMR spectrum of of norobtusifolin (**158**), acetone-d_6, 126 MHz



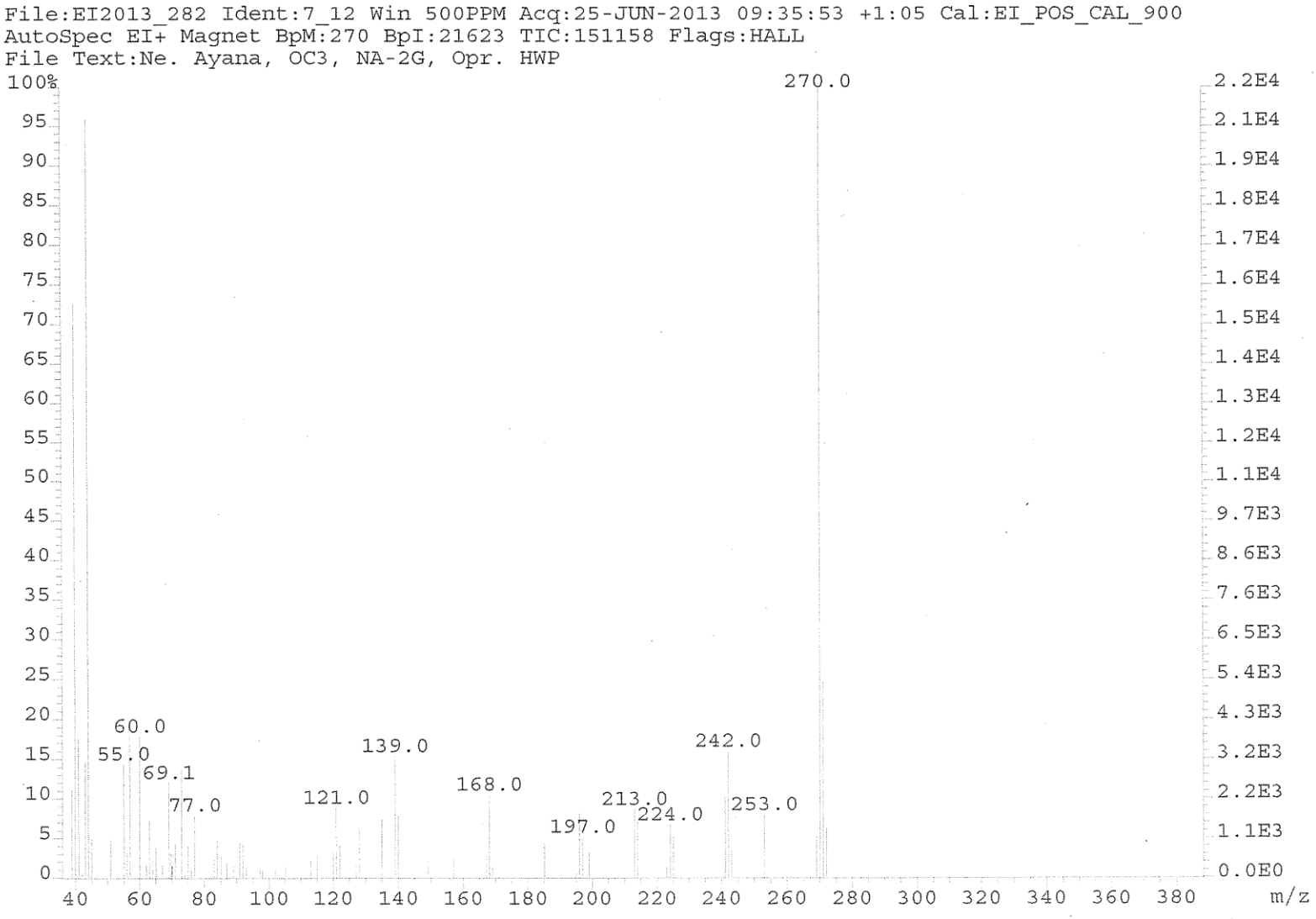
Appendix 35C: HSQC spectrum of norobtusifolin (**158**), acetone-d_6, 500 MHz



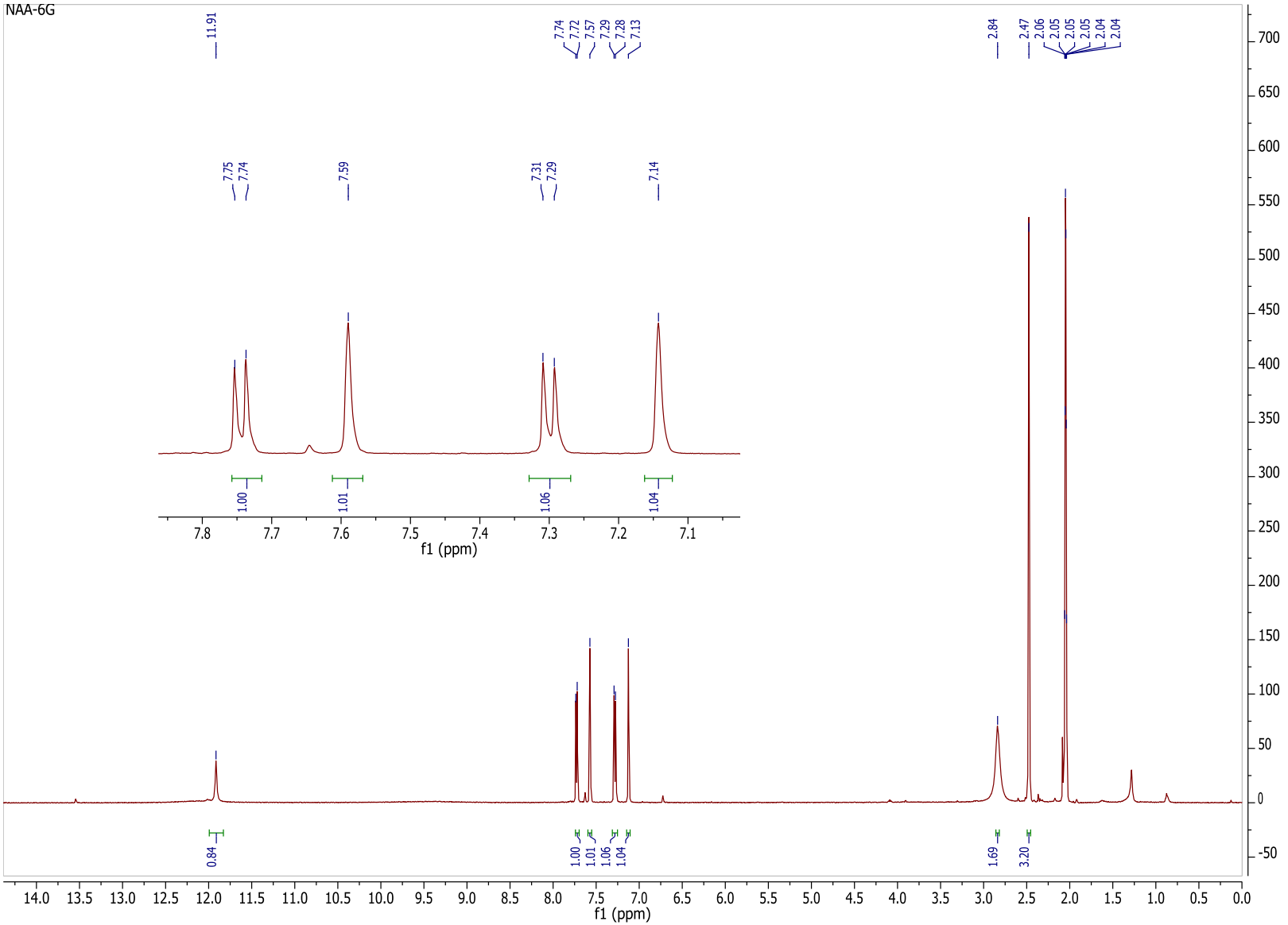
Appendix 35D: HMBC spectrum of norobtusifolin (**158**), acetone-d_6, 500 MHz



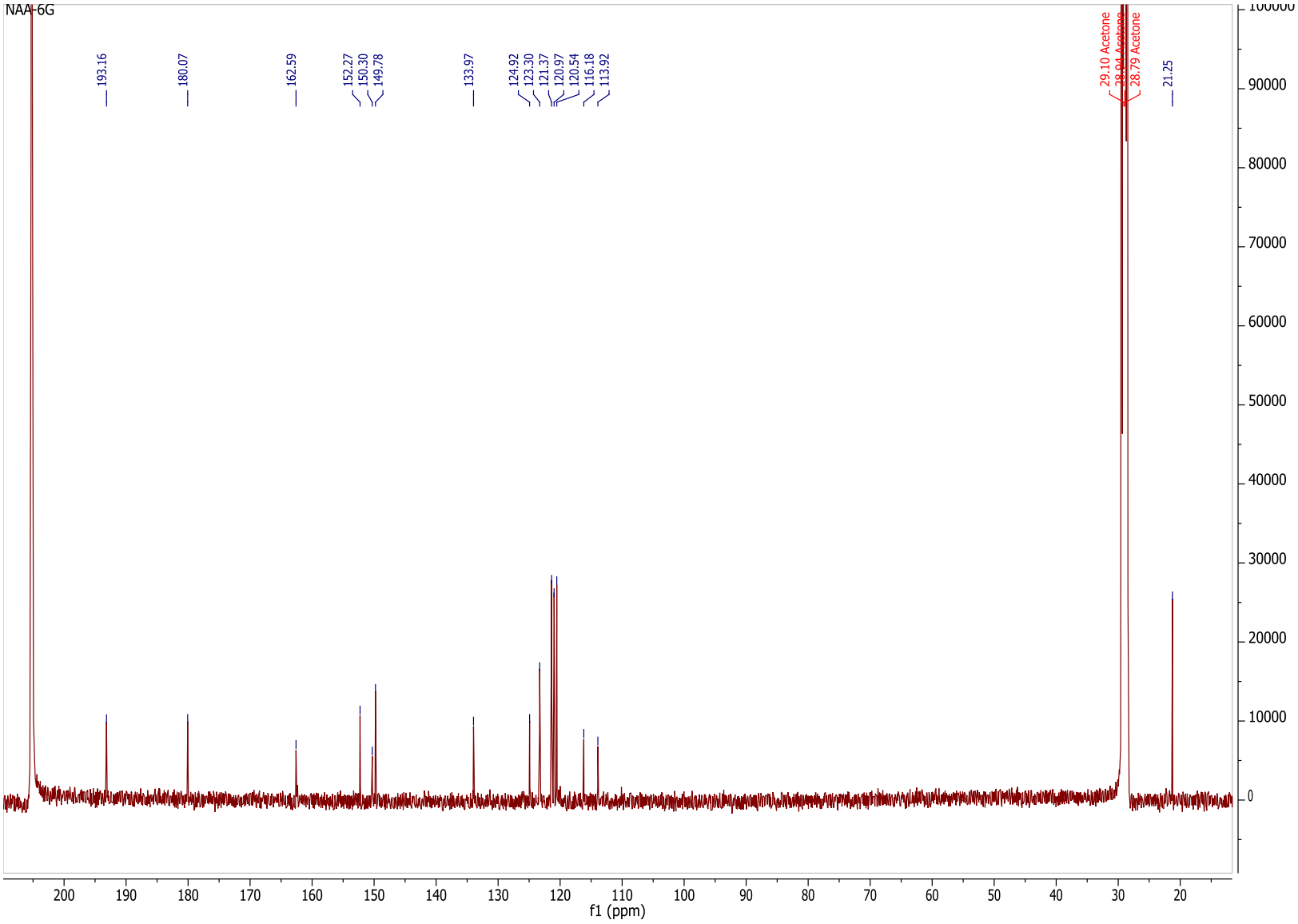
Appendix 35E: ESIMS spectrum of norobtusifolin (**158**)



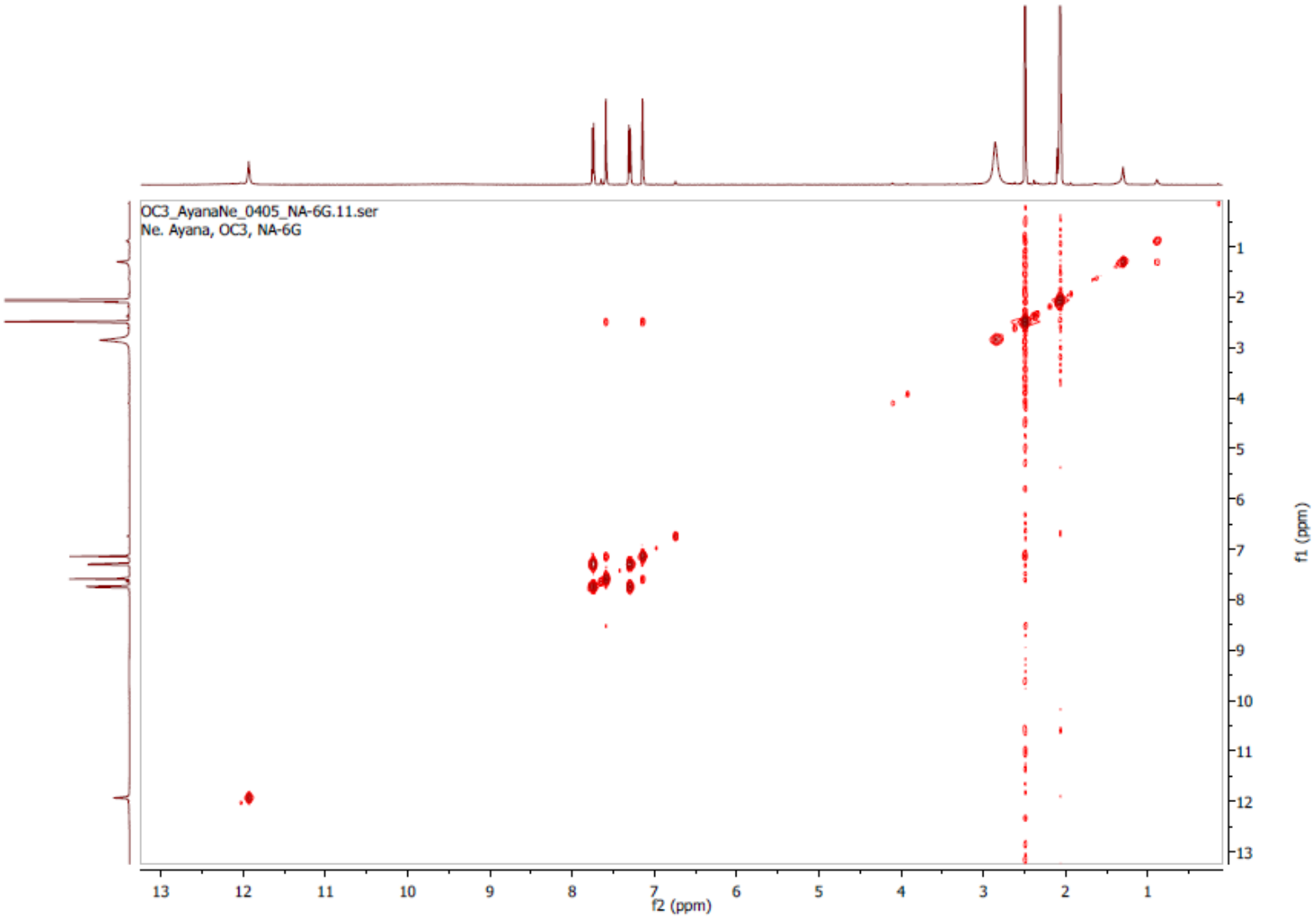
Appendix 36A: ^{1}H NMR spectrum of nataloe-emodin (**61**), acetone-d_6, 500 MHz



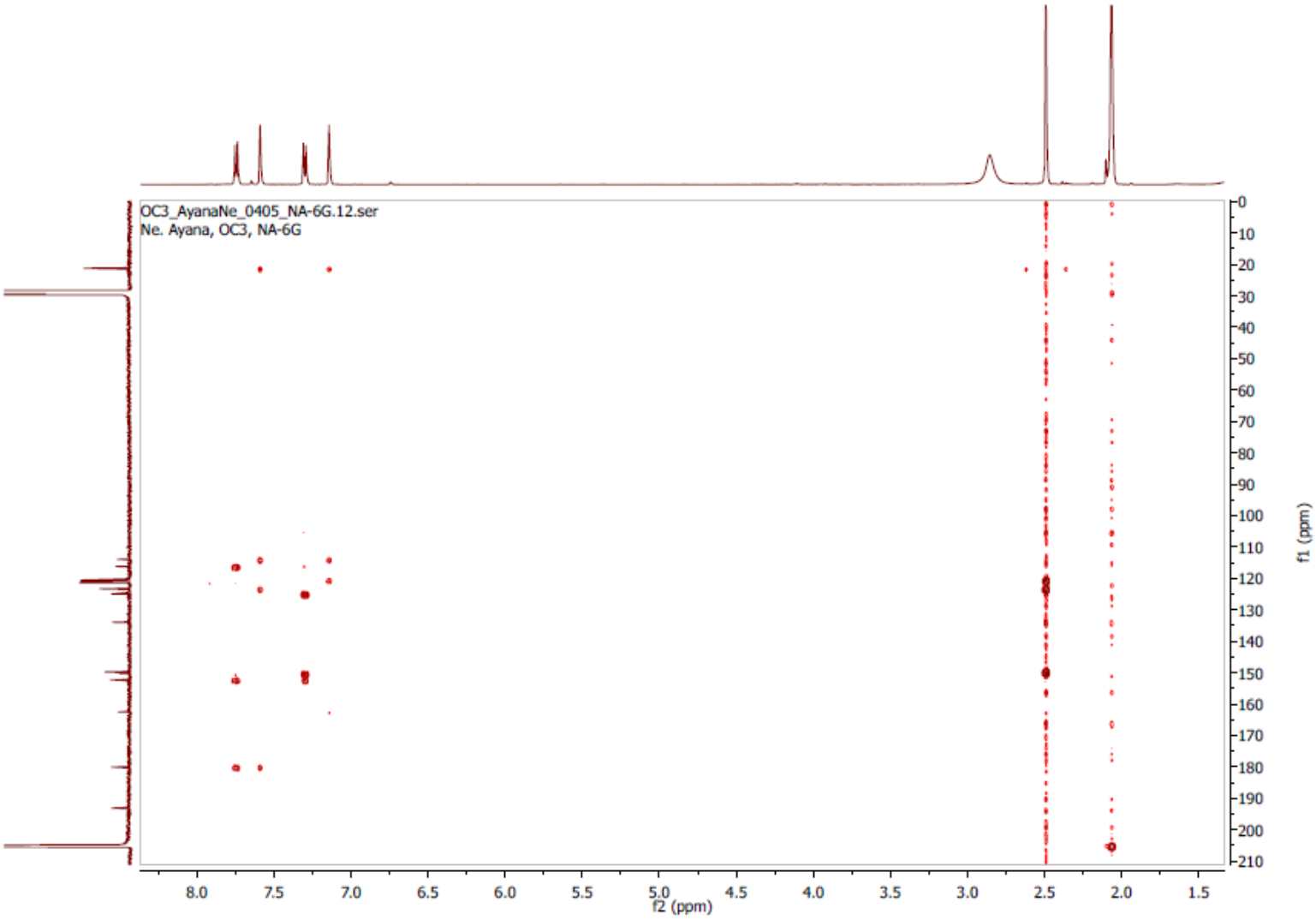
Appendix 36B: ^{13}C NMR spectrum of nataloe-emodin (**61**), acetone-d_6, 126 MHz



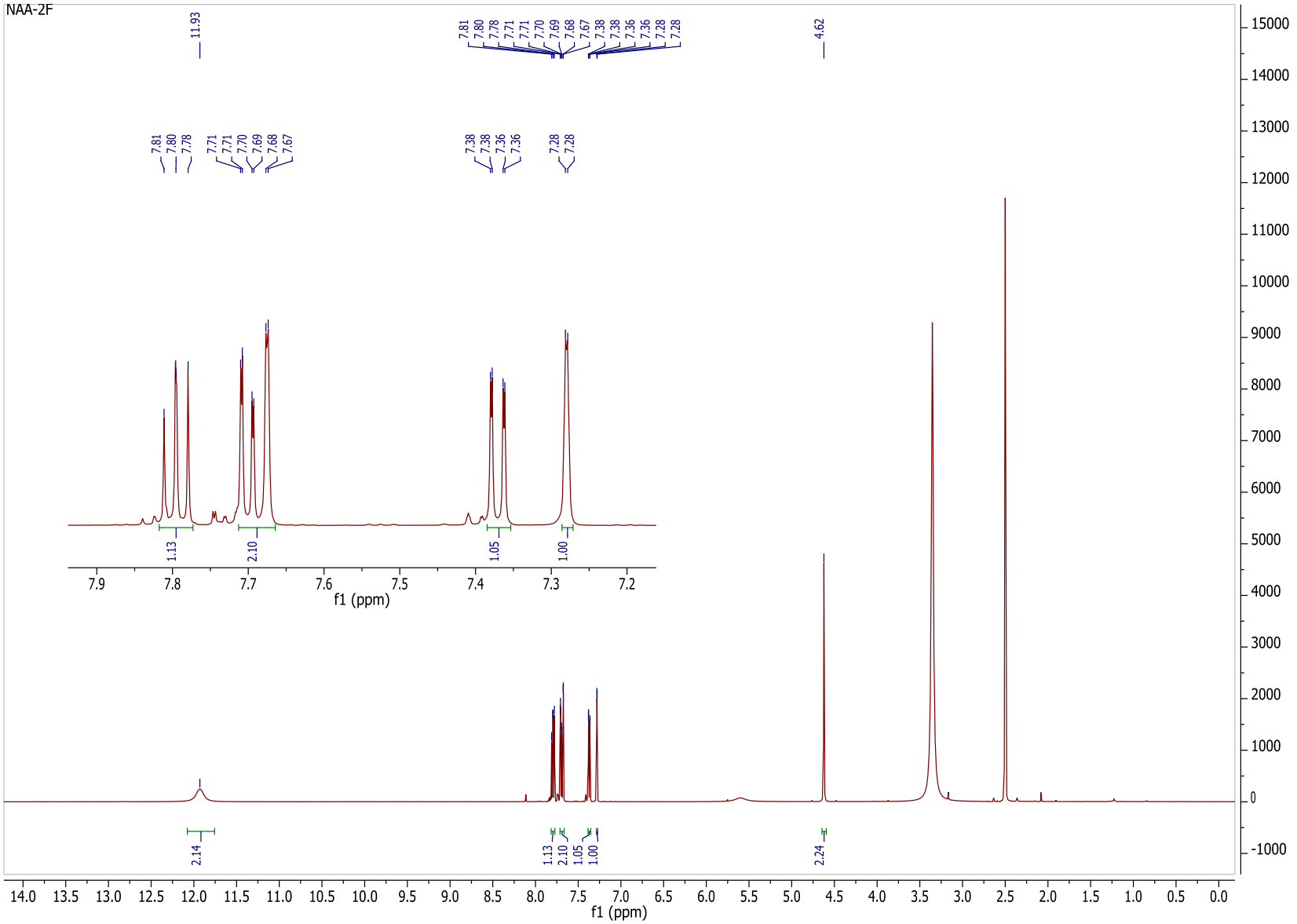
Appendix 36C: HSQC spectrum of nataloe-emodin (**61**), acetone-d_6, 500 MHz



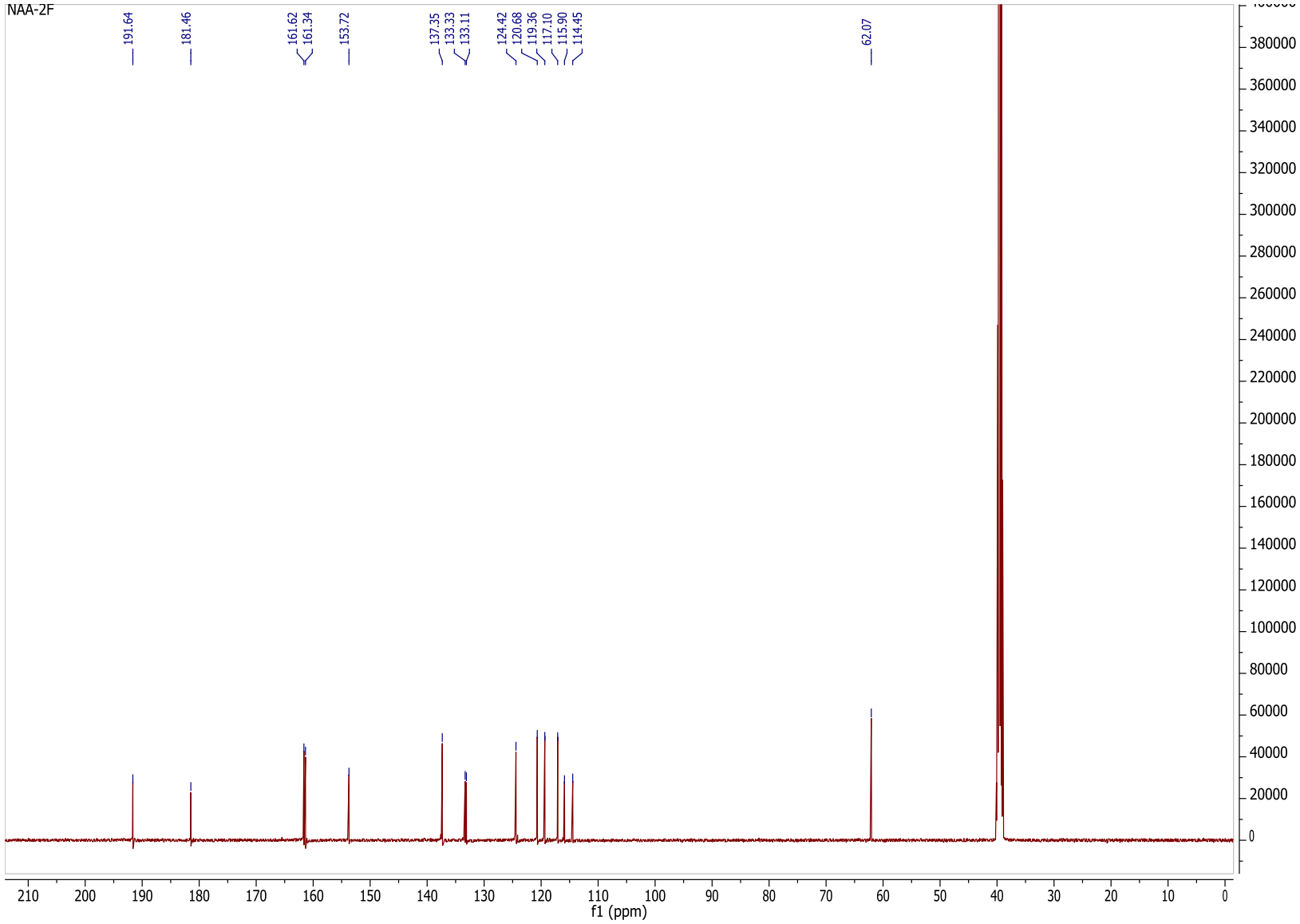
Appendix 36D: HMBC spectrum of nataloe-emodin (**61**), acetone-d_6, 500 MHz



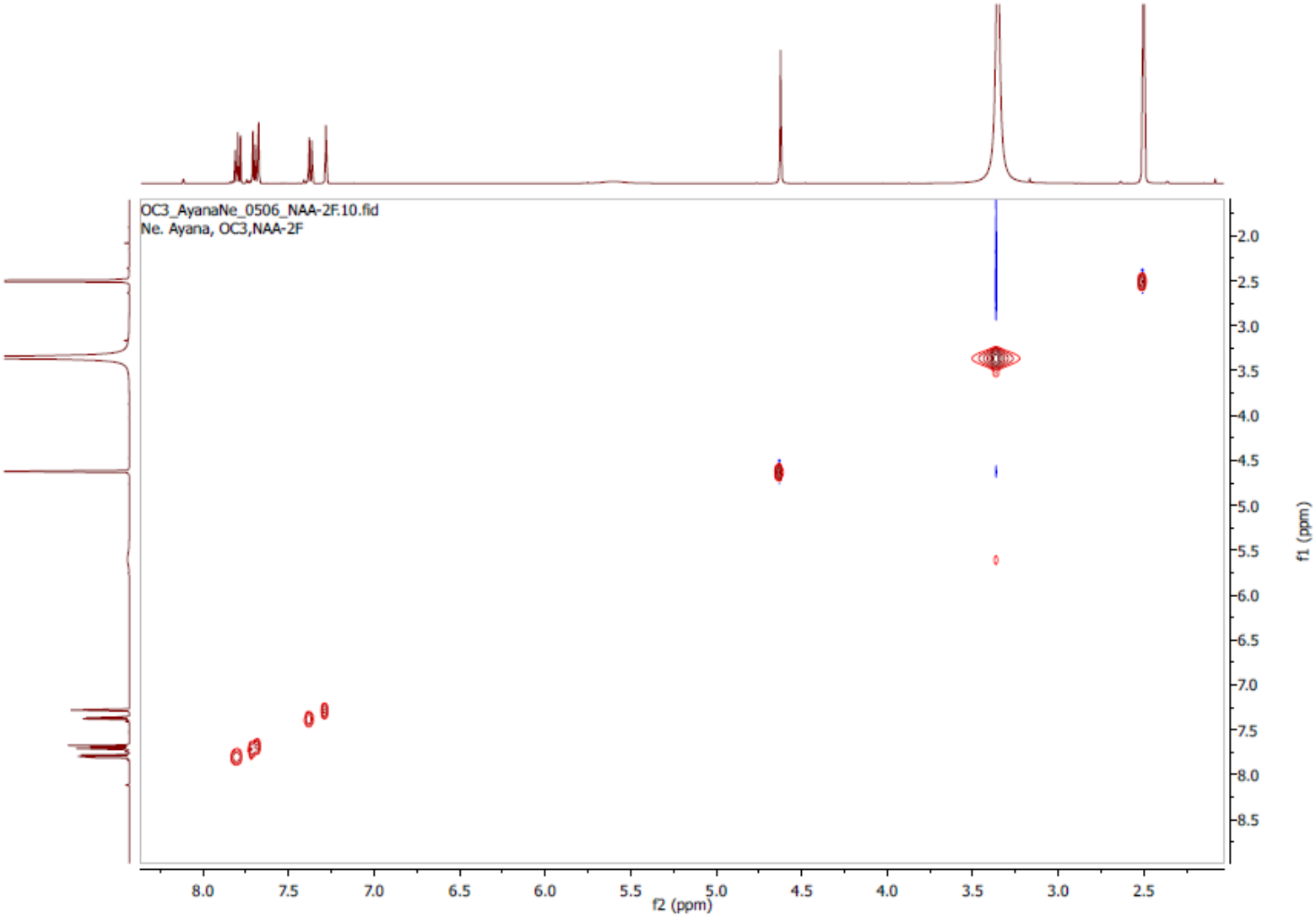
Appendix 37A: ^{1}H NMR spectrum of aloe-emodin (**20**), DMSO-d_6, 500 MHz



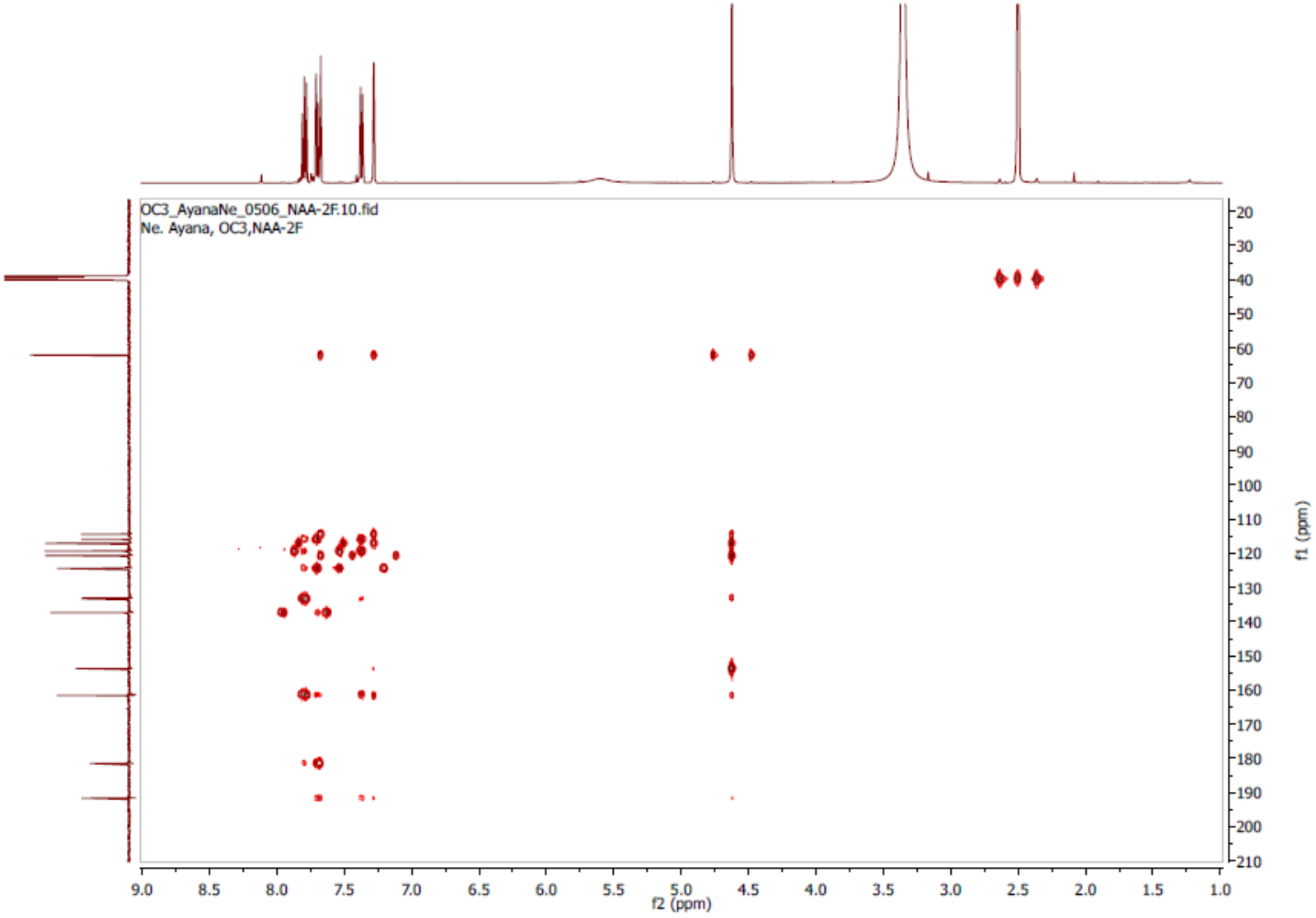
Appendix 37B: ^{13}C NMR spectrum of aloe-emodin (**20**), DMSO-d_6, 126 MHz



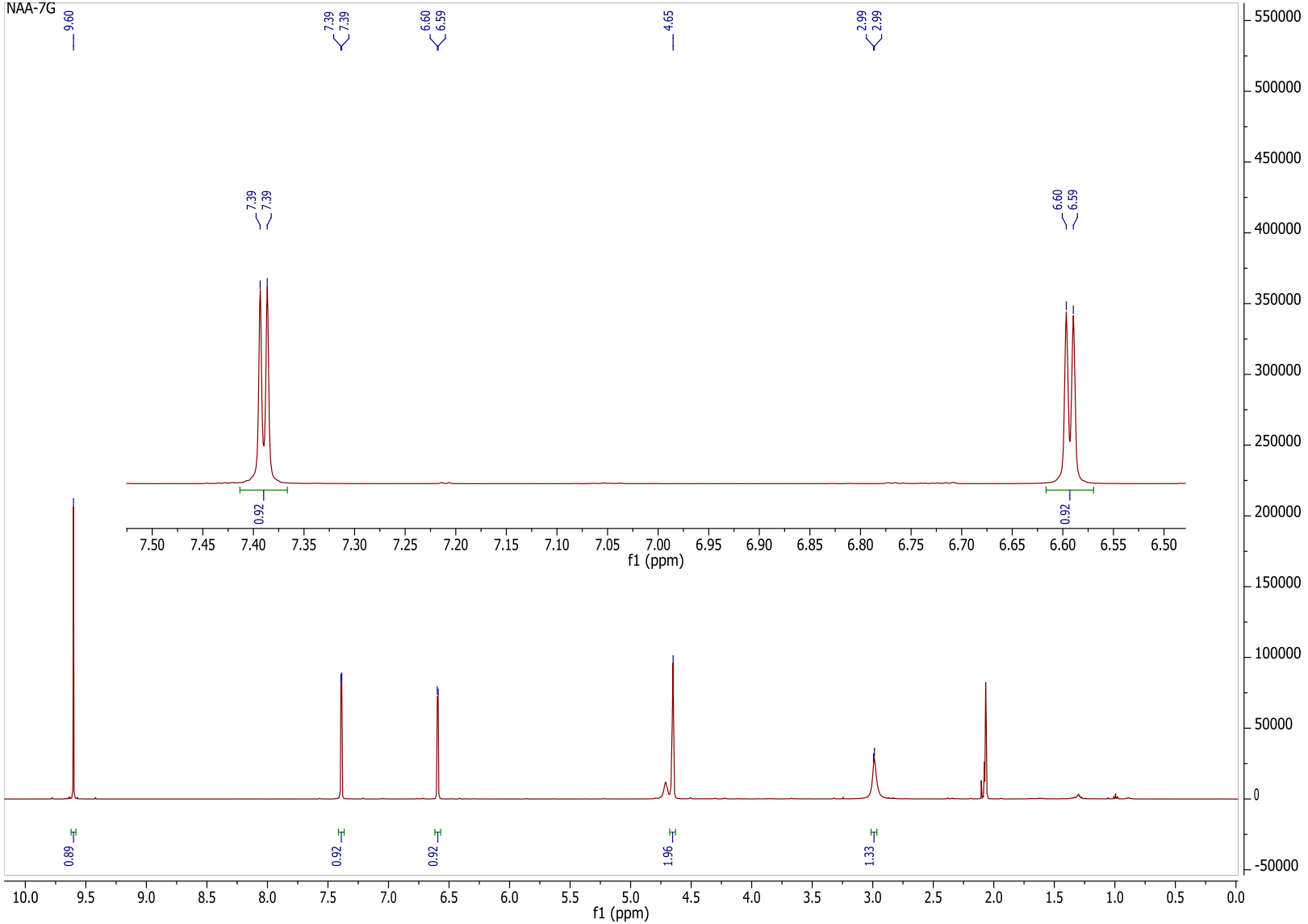
Appendix 37C: HSQC spectrum of aloe-emodin (**20**), DMSO-d_6, 500 MHz



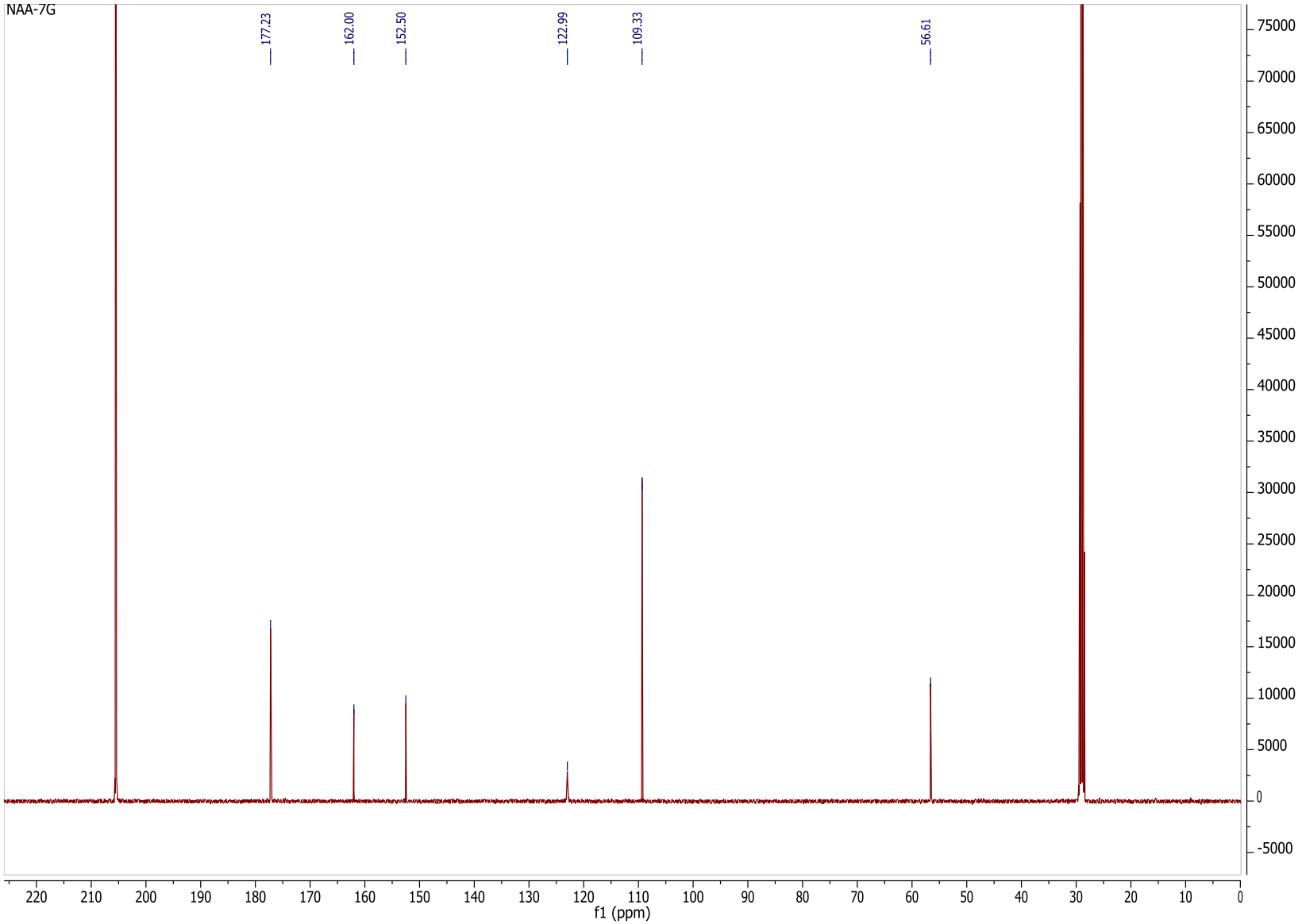
Appendix 37D: HMBC spectrum of aloe-emodin (**20**), DMSO-d_6, 500 MHz



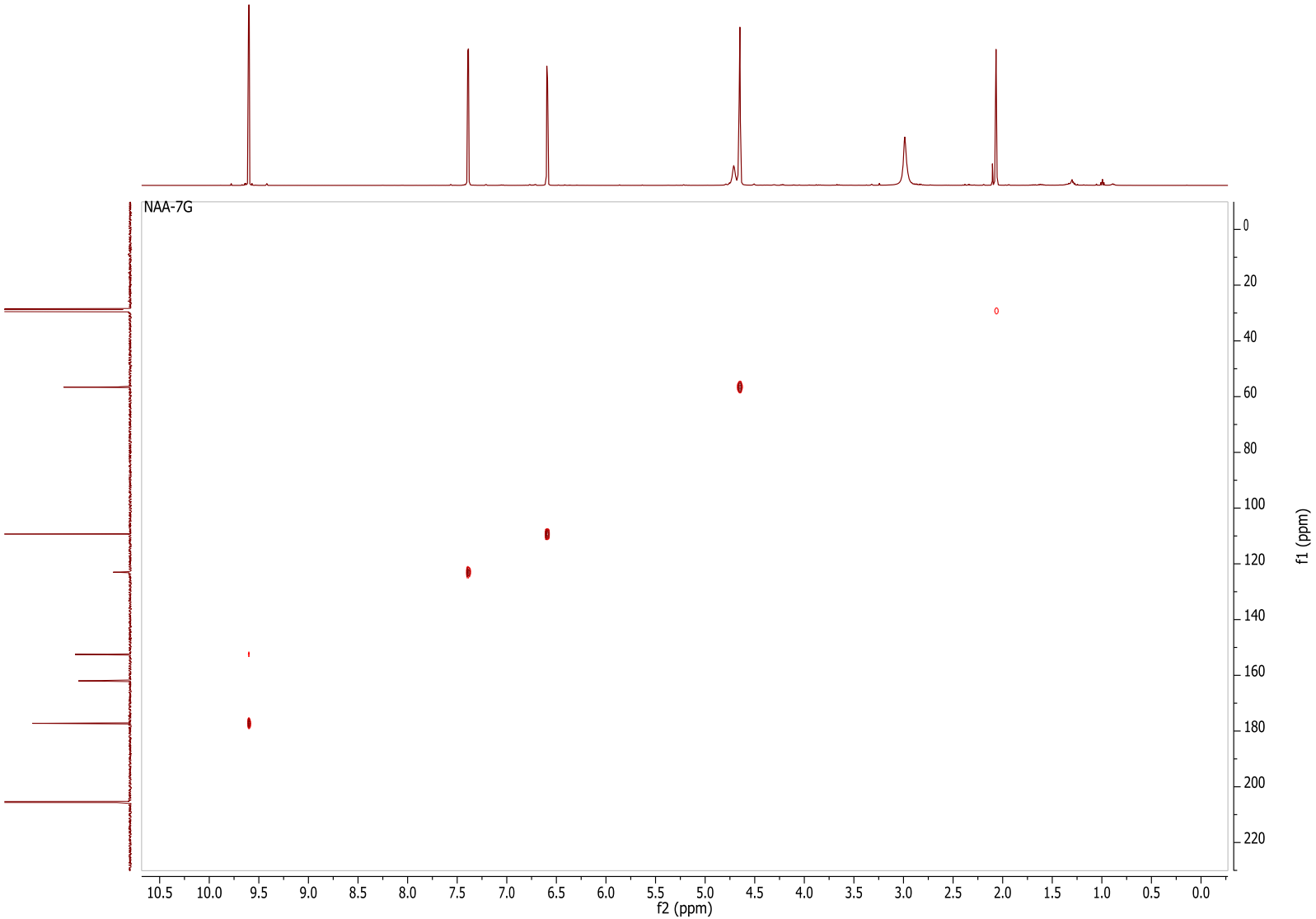
Appendix 38A: ^{1}H NMR spectrum of 5-hydroxymethyl-2-furancarboxaldehyde (**159**), acetone-d_6, 500 MHz



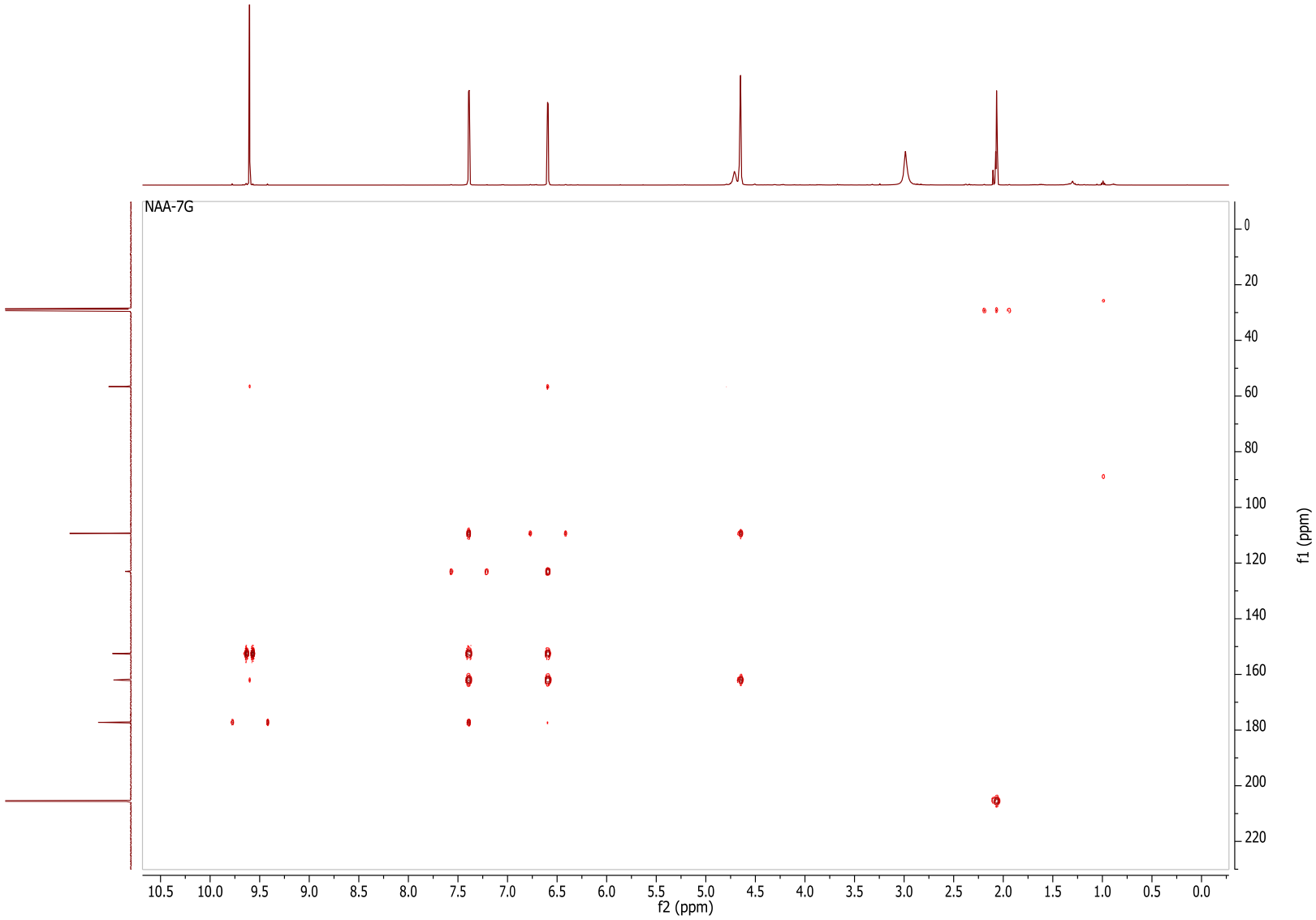
Appendix 38B: ^{13}C NMR spectrum of 5-hydroxymethyl-2-furancarboxaldehyde (**159**), acetone-d_6, 126 MHz



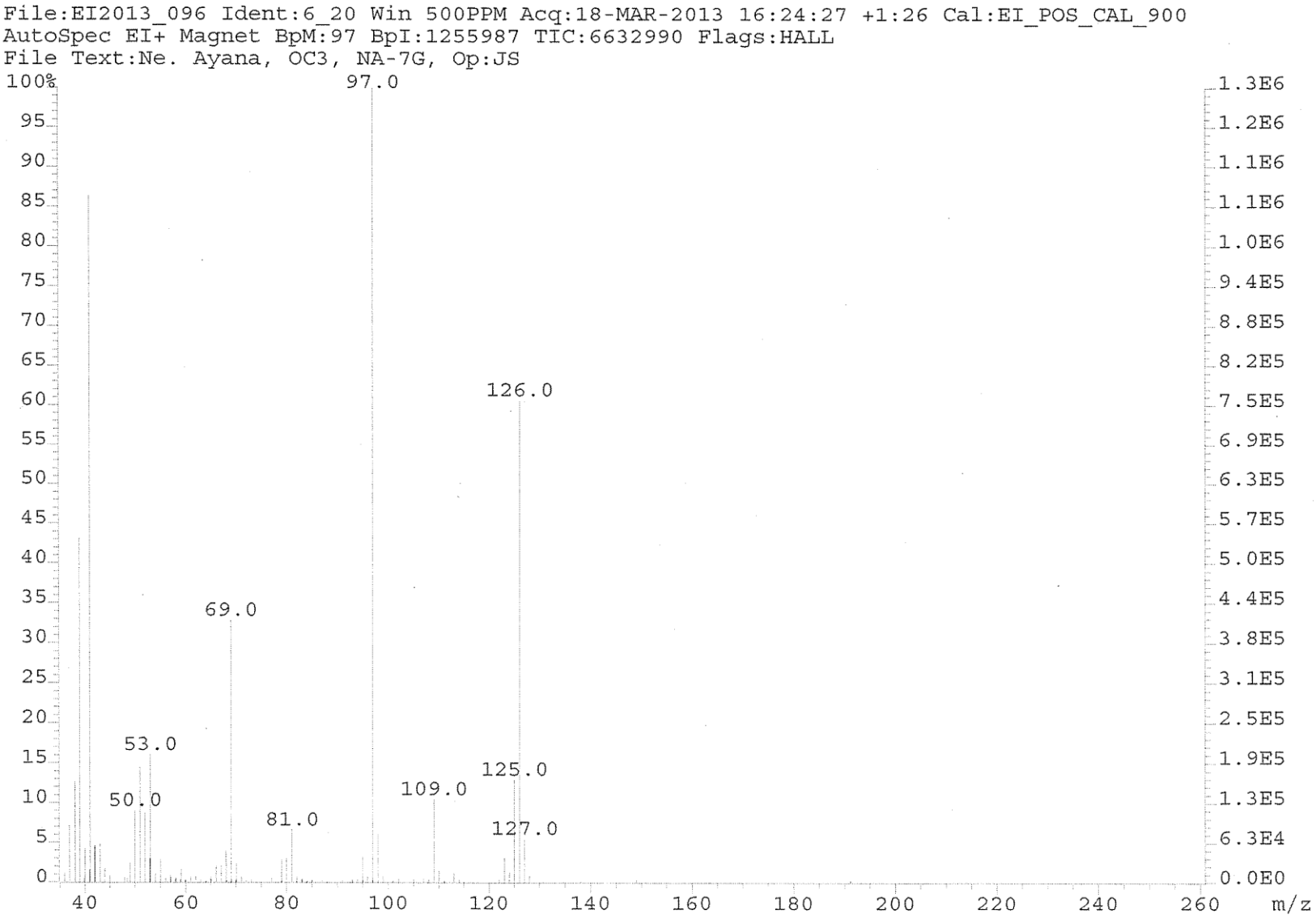
Appendix 38C: HSQC spectrum of 5-hydroxymethyl-2-furancarboxaldehyde (**159**), acetone-d_6, 500 MHz



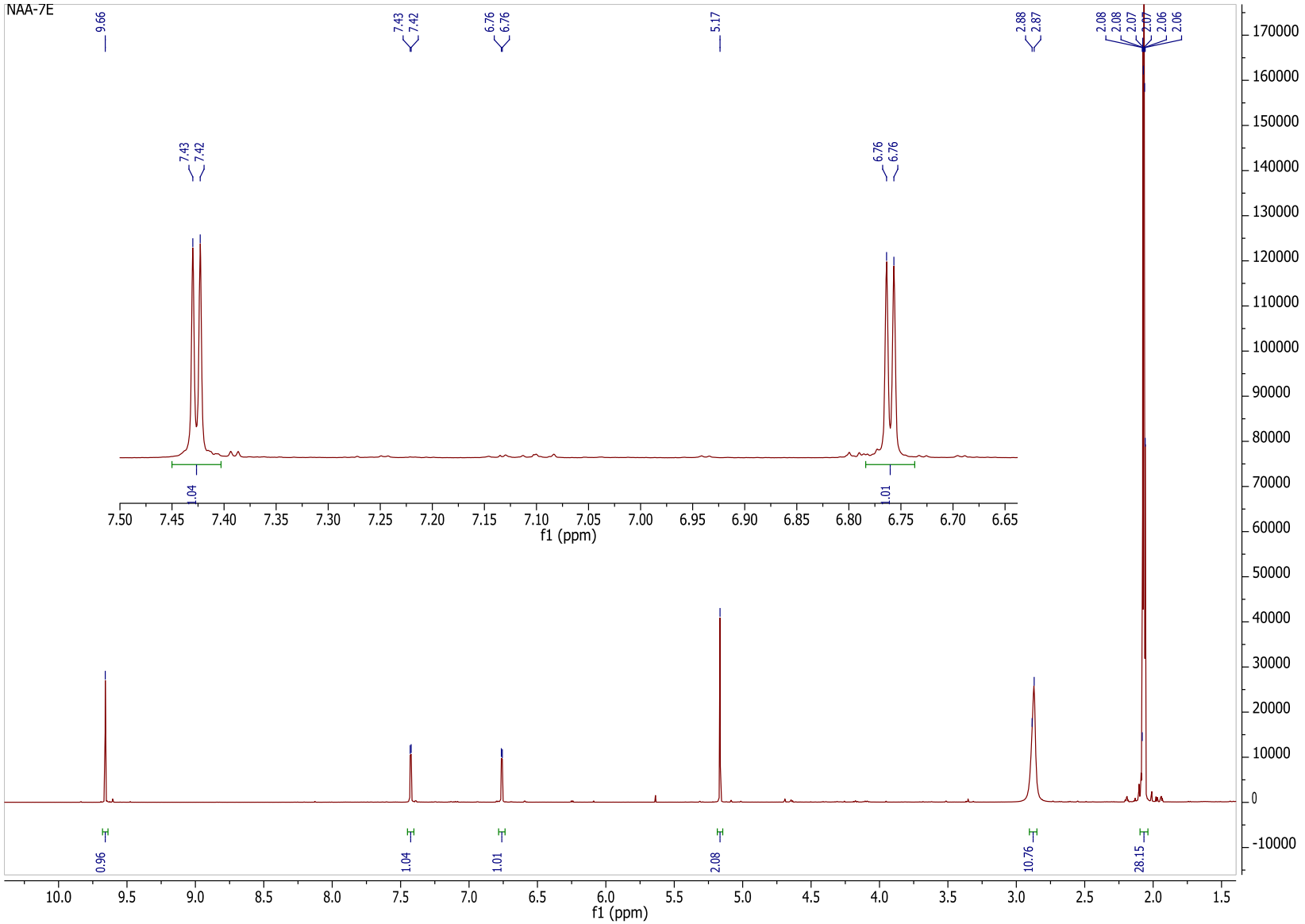
Appendix 38D: HMBC spectrum of 5-hydroxymethyl-2-furancarboxaldehyde (**159**), acetone-d_6, 500 MHz



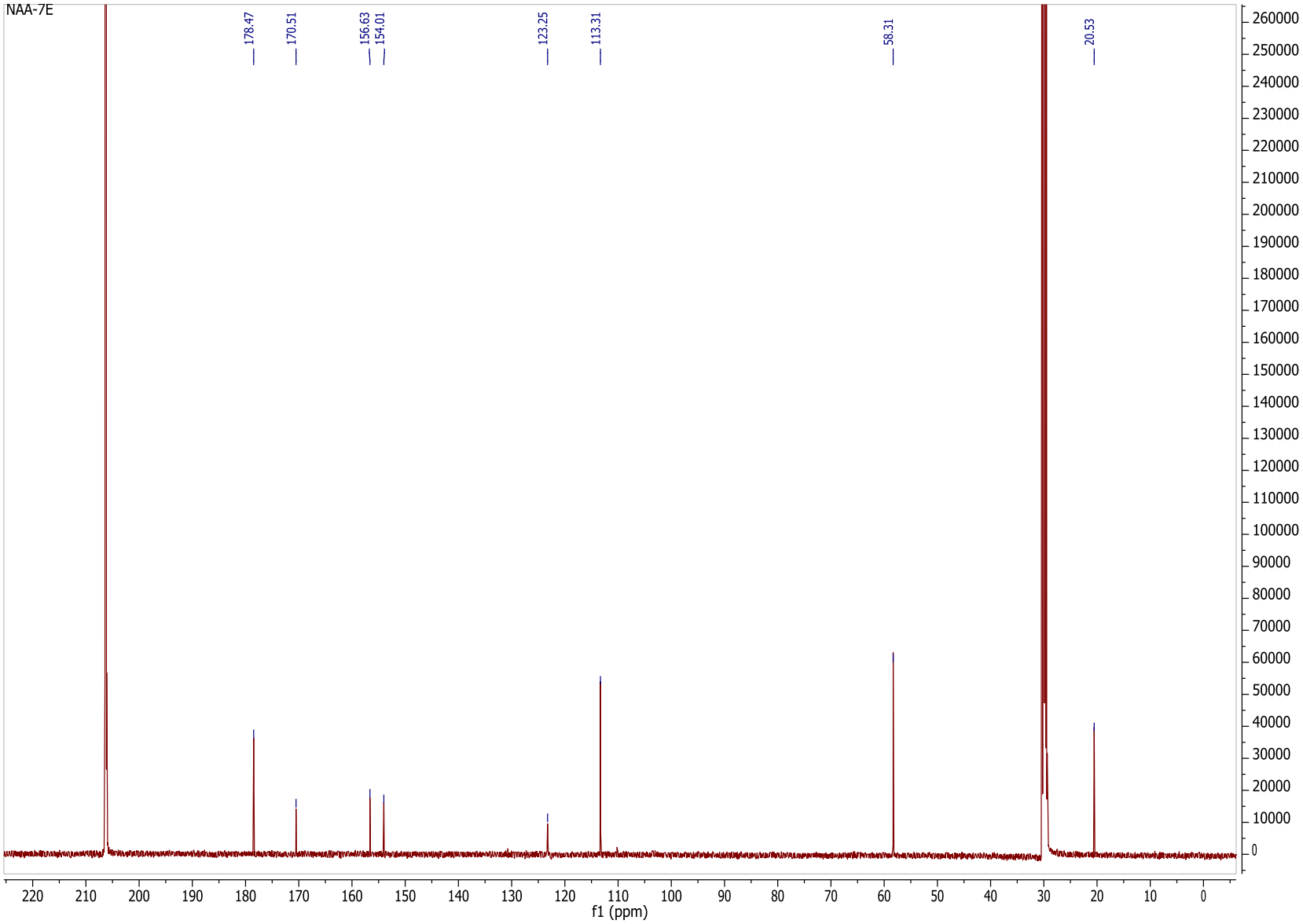
Appendix 38E: ESIMS spectrum of 5-hydroxymethyl-2-furancarboxaldehyde (**159**)



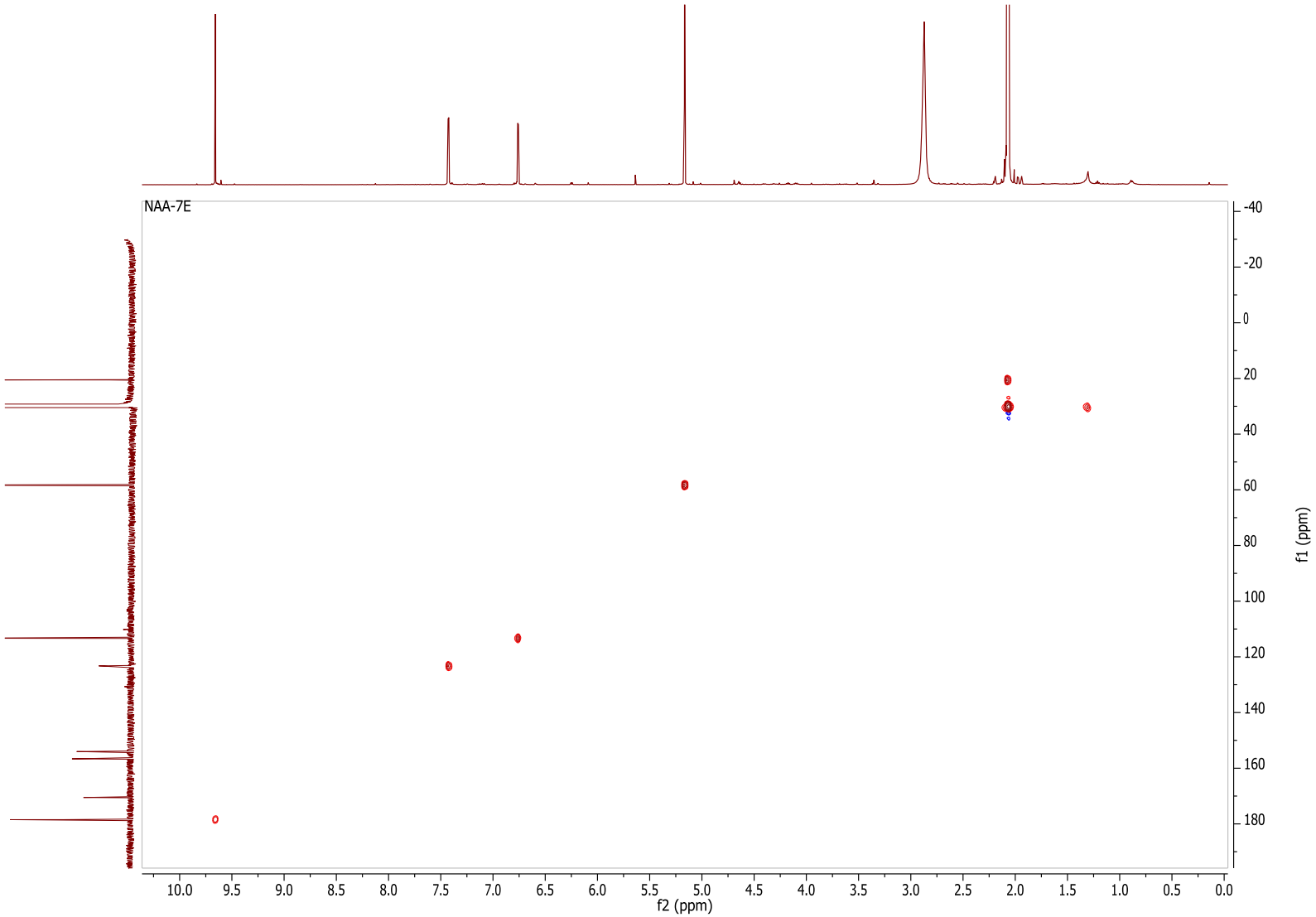
Appendix 39A: ^{1}H NMR spectrum of 5-acetyloxymethyl-2-furancarboxaldehyde (**160**), acetone-d_6, 500 MHz



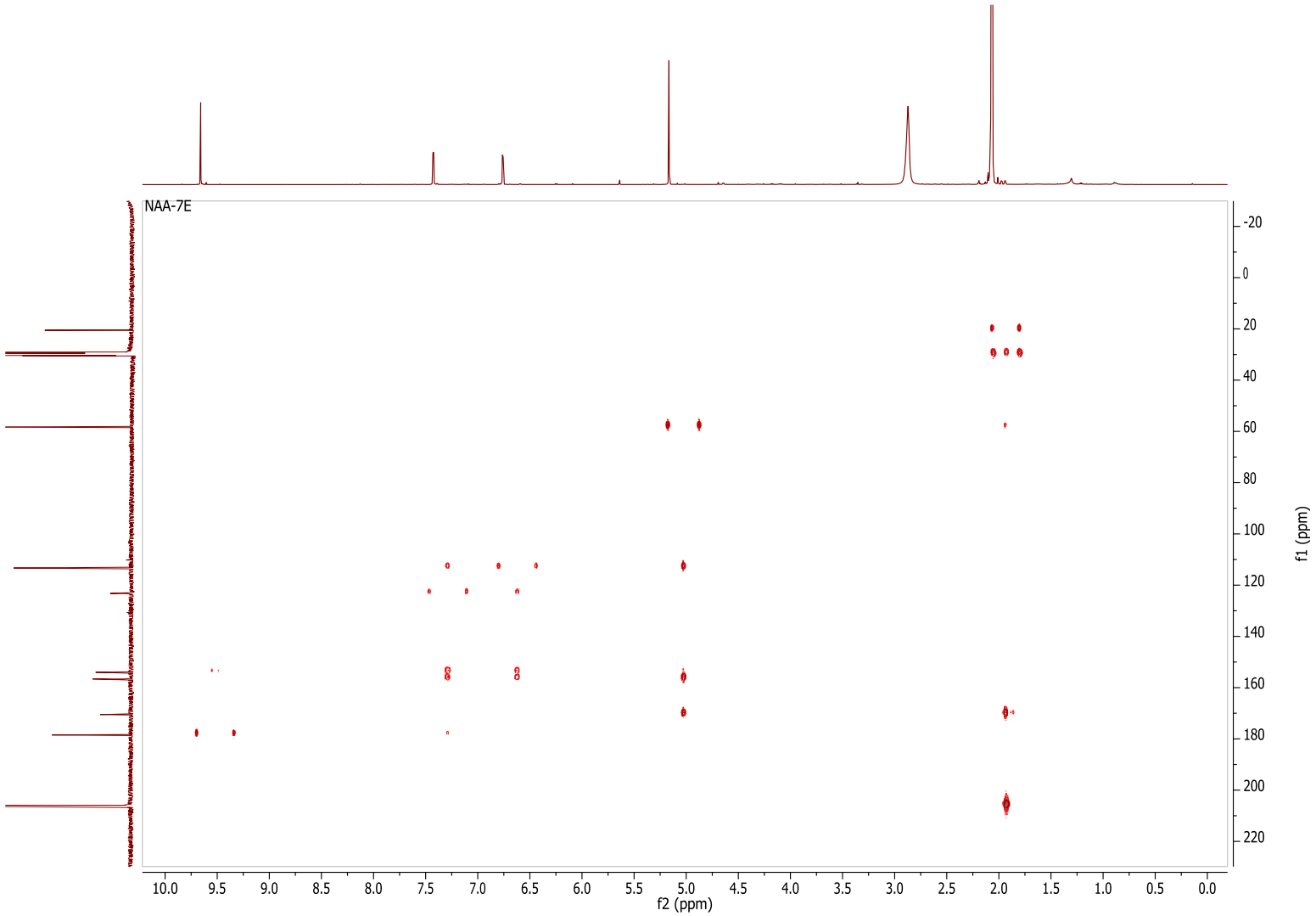
Appendix 39B: ^{13}C NMR spectrum of 5-acetyloxymethyl-2-furancarboxaldehyde (**160**), acetone-d_6, 126 MHz



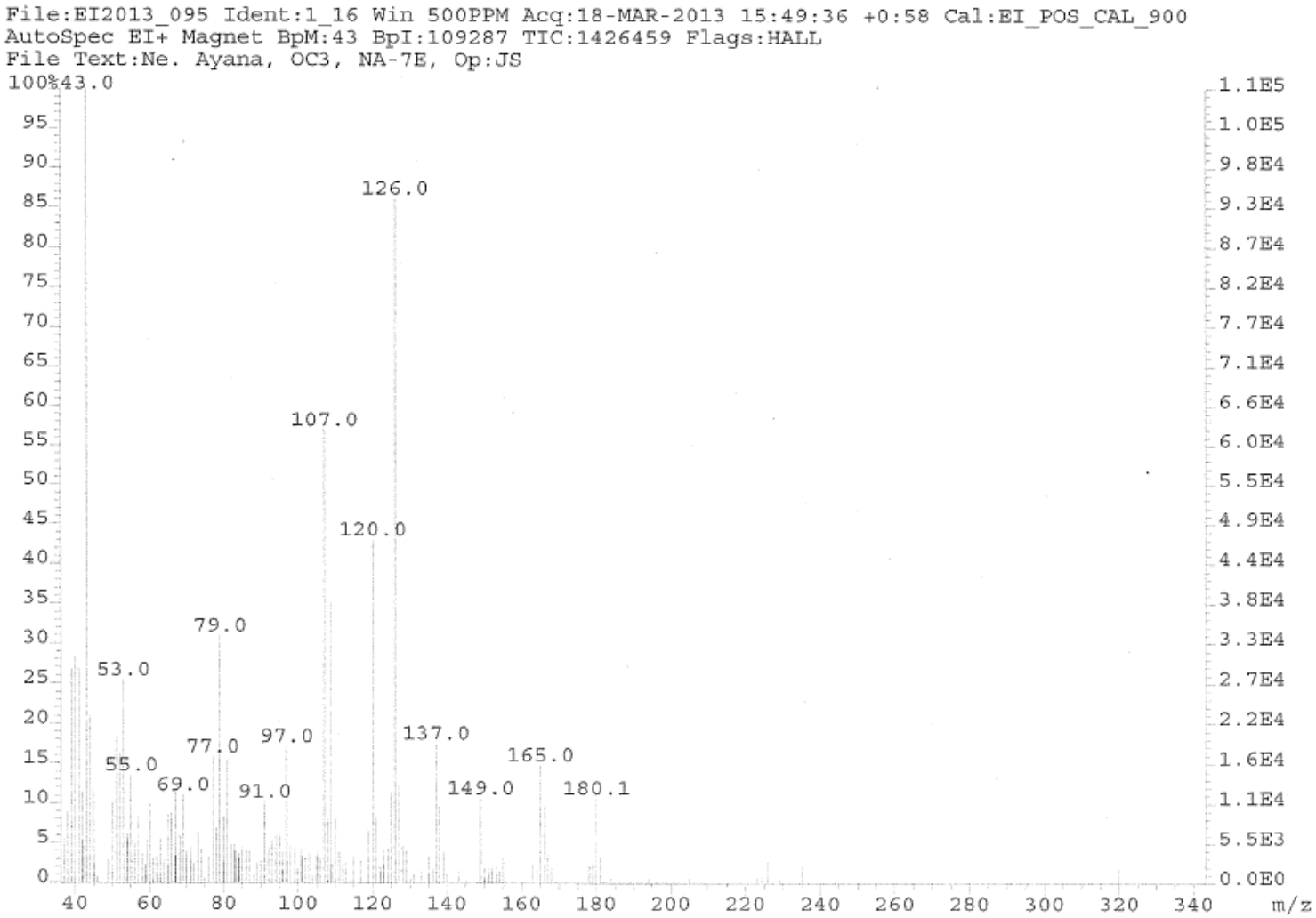
Appendix 39C: HSQC spectrum of 5-acetyloxymethyl-2-furancarboxaldehyde (**160**), acetone-d_6, 500 MHz



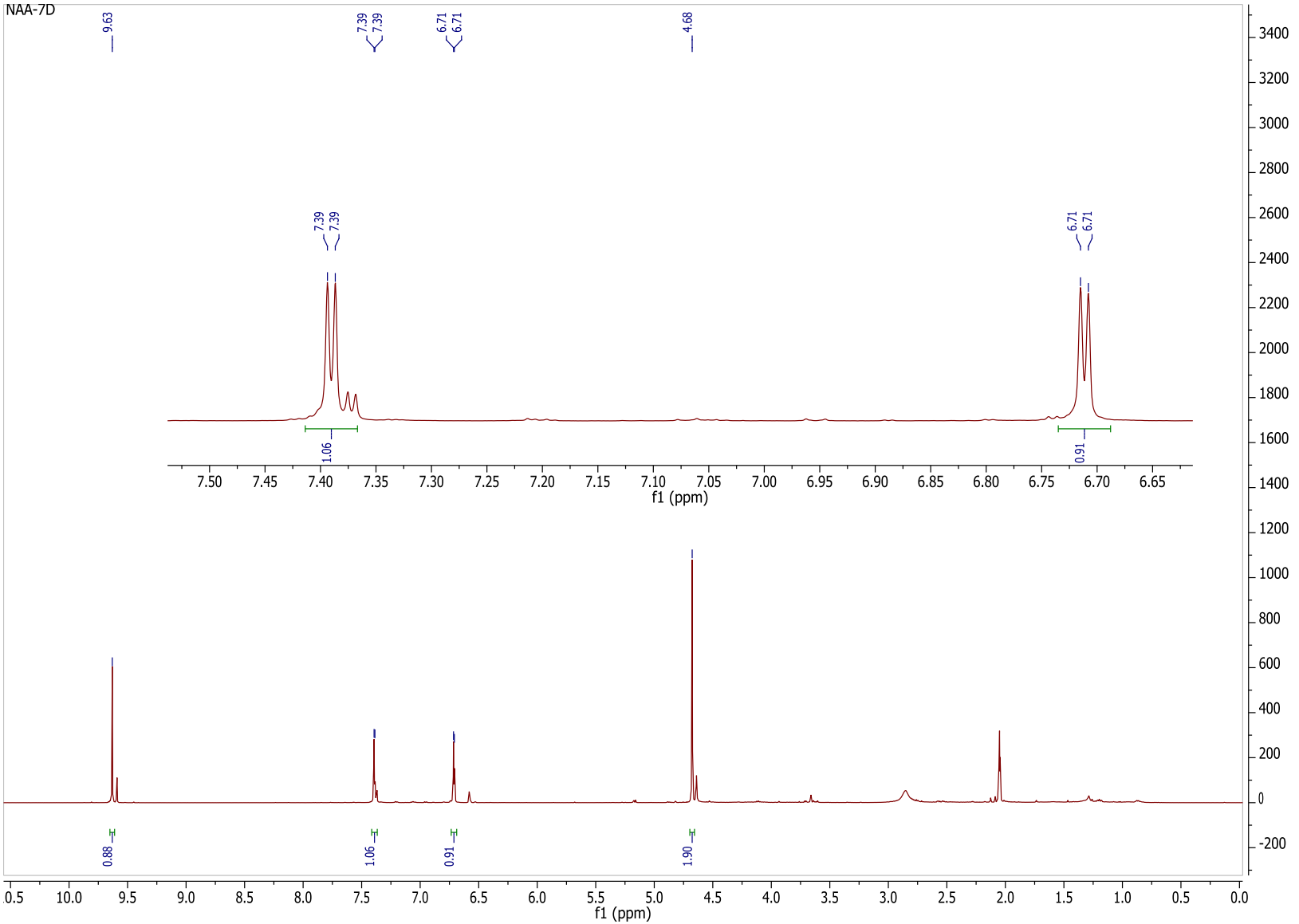
Appendix 39D: HMBC spectrum of 5-acetyloxymethyl-2-furancarboxaldehyde (**160**), acetone-d_6, 500 MHz



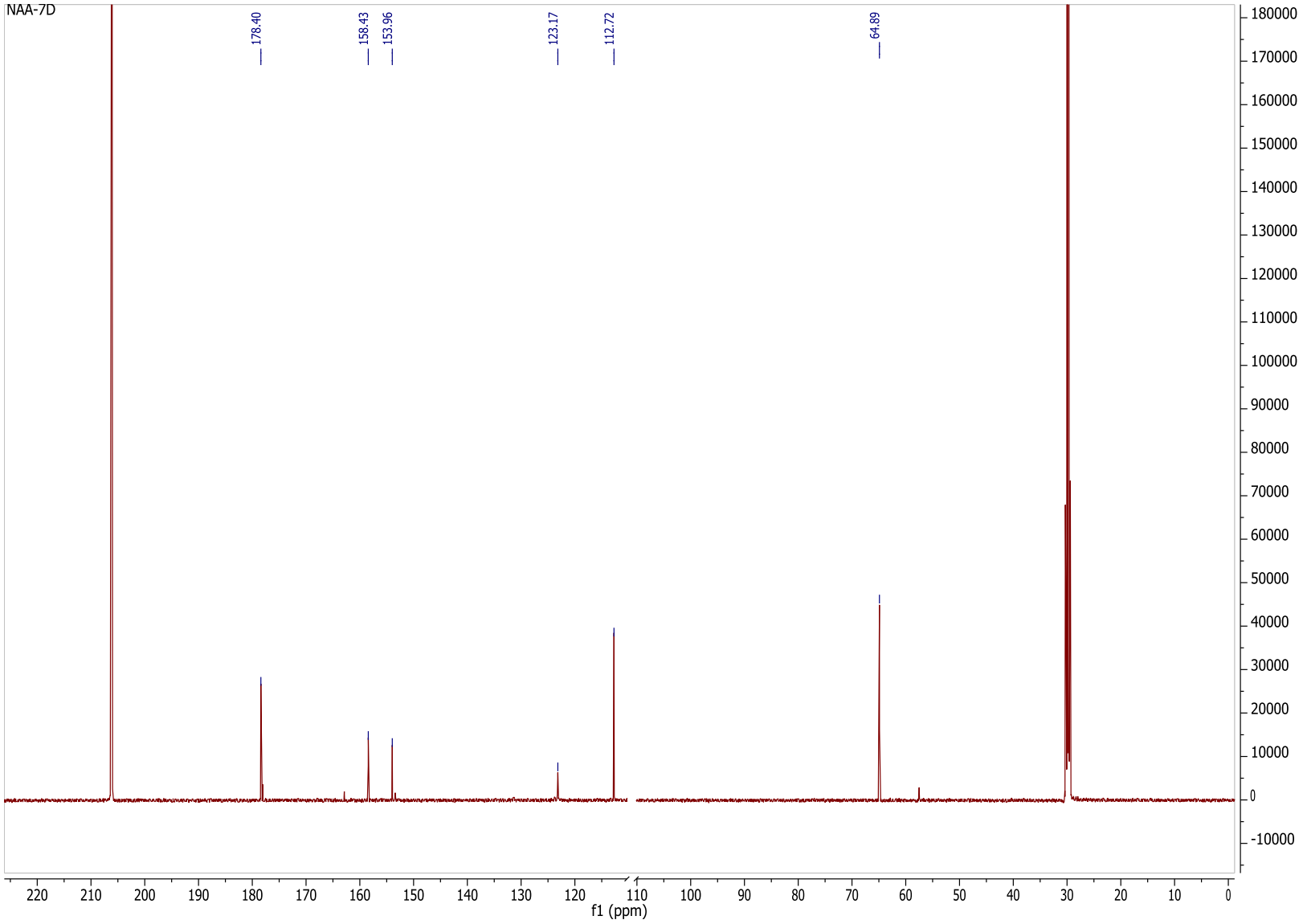
Appendix 39E: ESIMS spectrum of 5-acetyloxymethyl-2-furancarboxaldehyde (**160**)



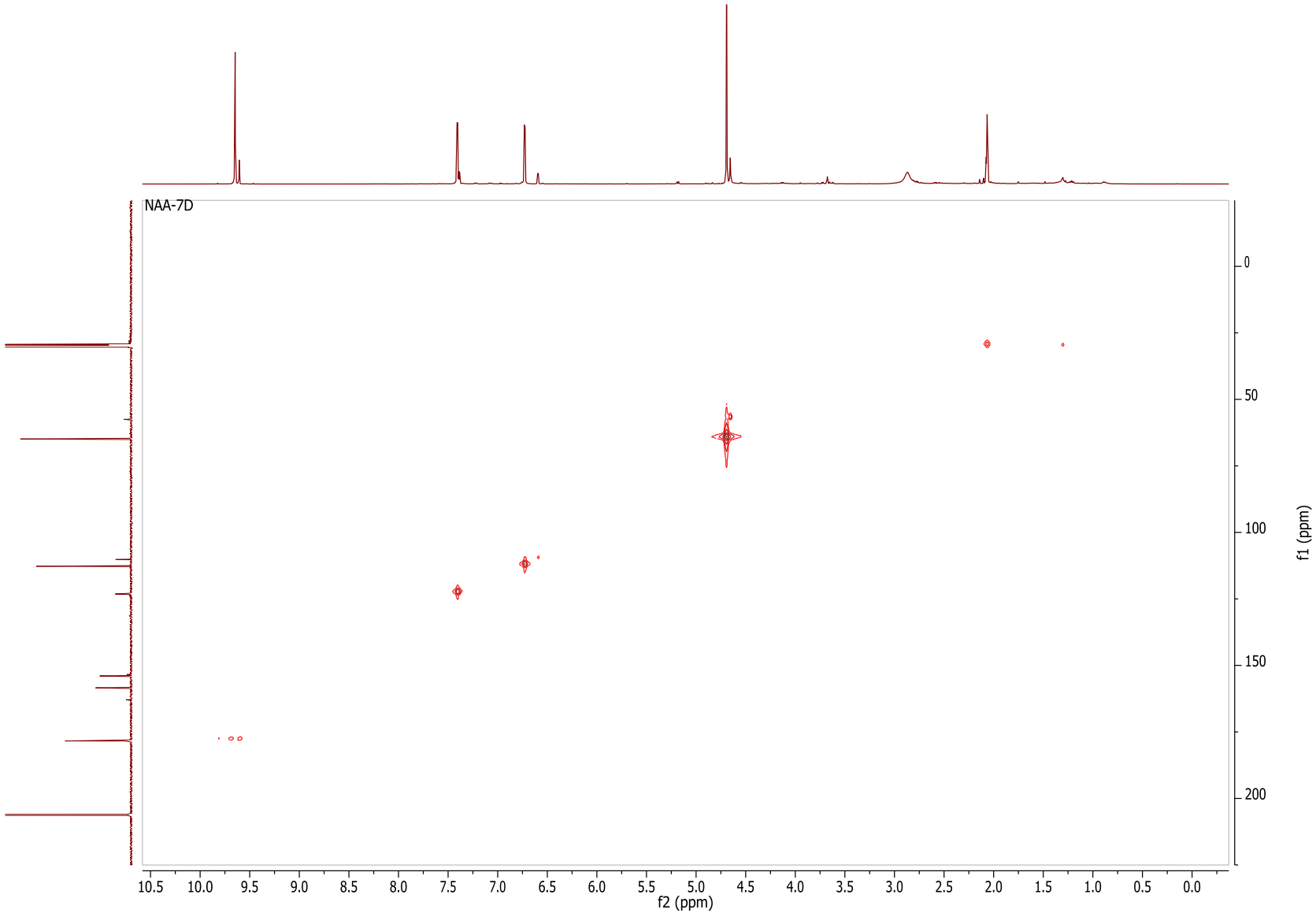
Appendix 40A: ^{1}H NMR spectrum of cirsiumaldehyde (**161**), acetone-d_6, 500 MHz



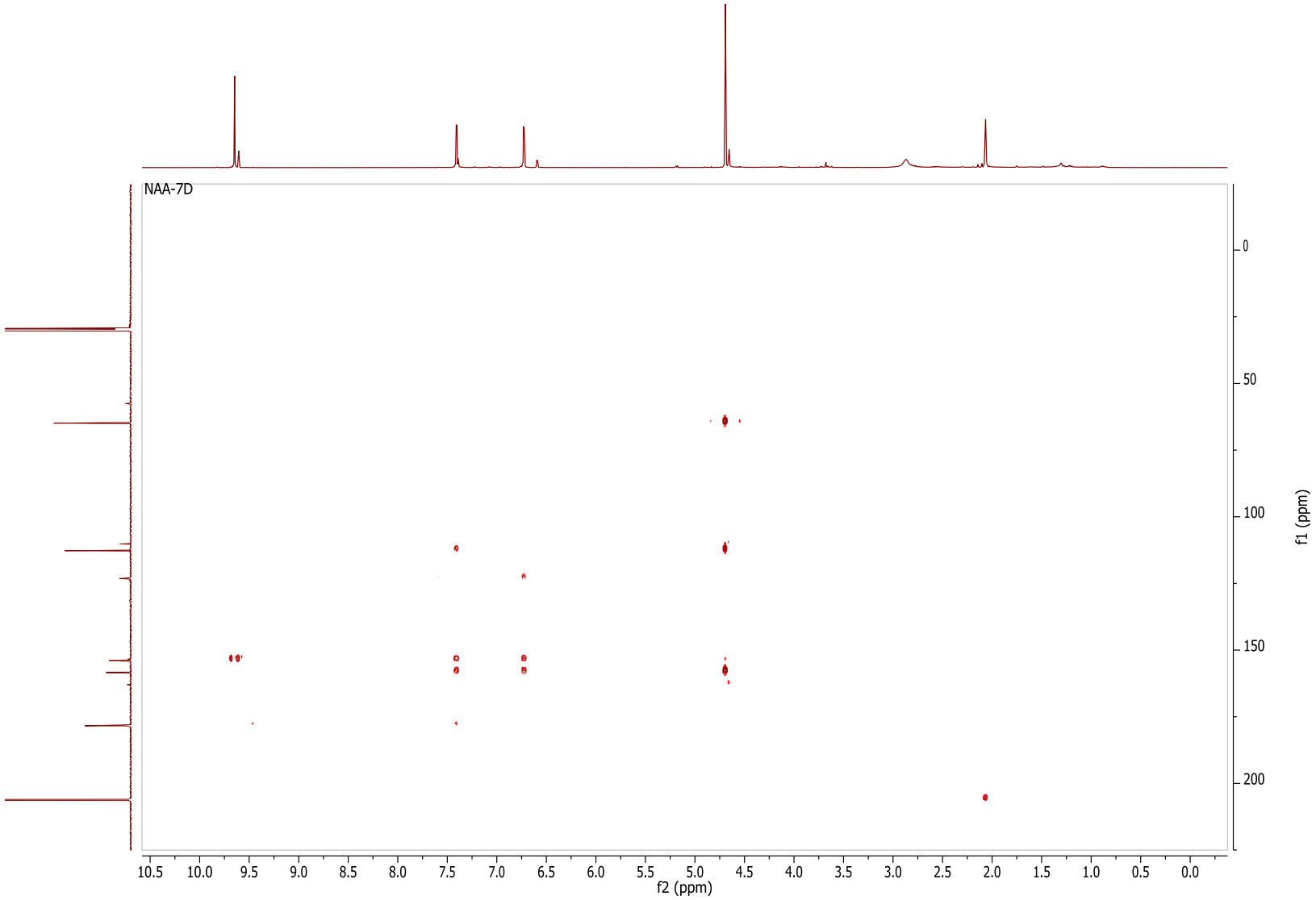
Appendix 40B: ^{13}C NMR spectrum of of cirsiumaldehyde (**161**), acetone-d_6, 126 MHz



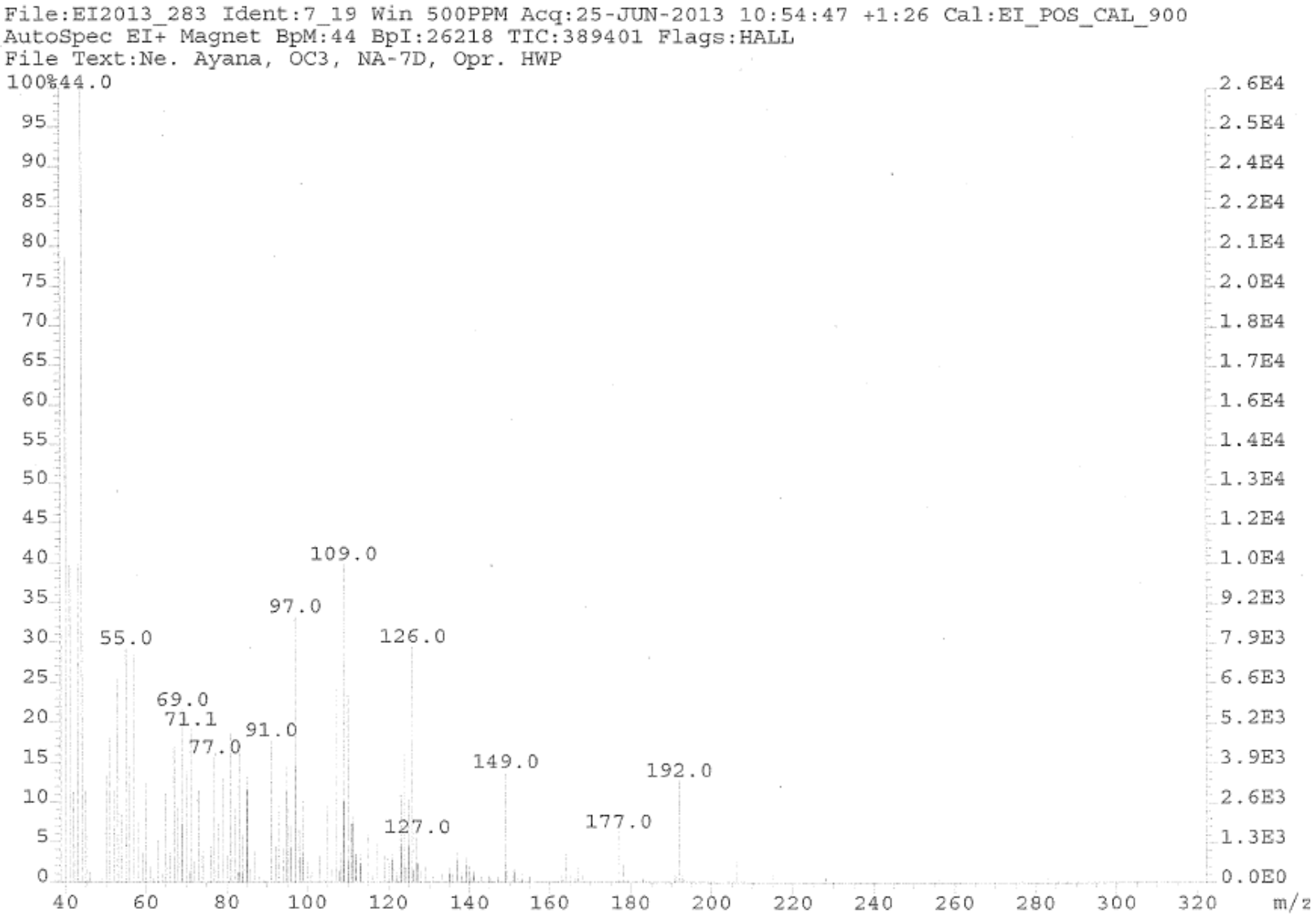
Appendix 40C: HSQC spectrum of cirsiumaldehyde (**161**), acetone-d_6, 500 MHz



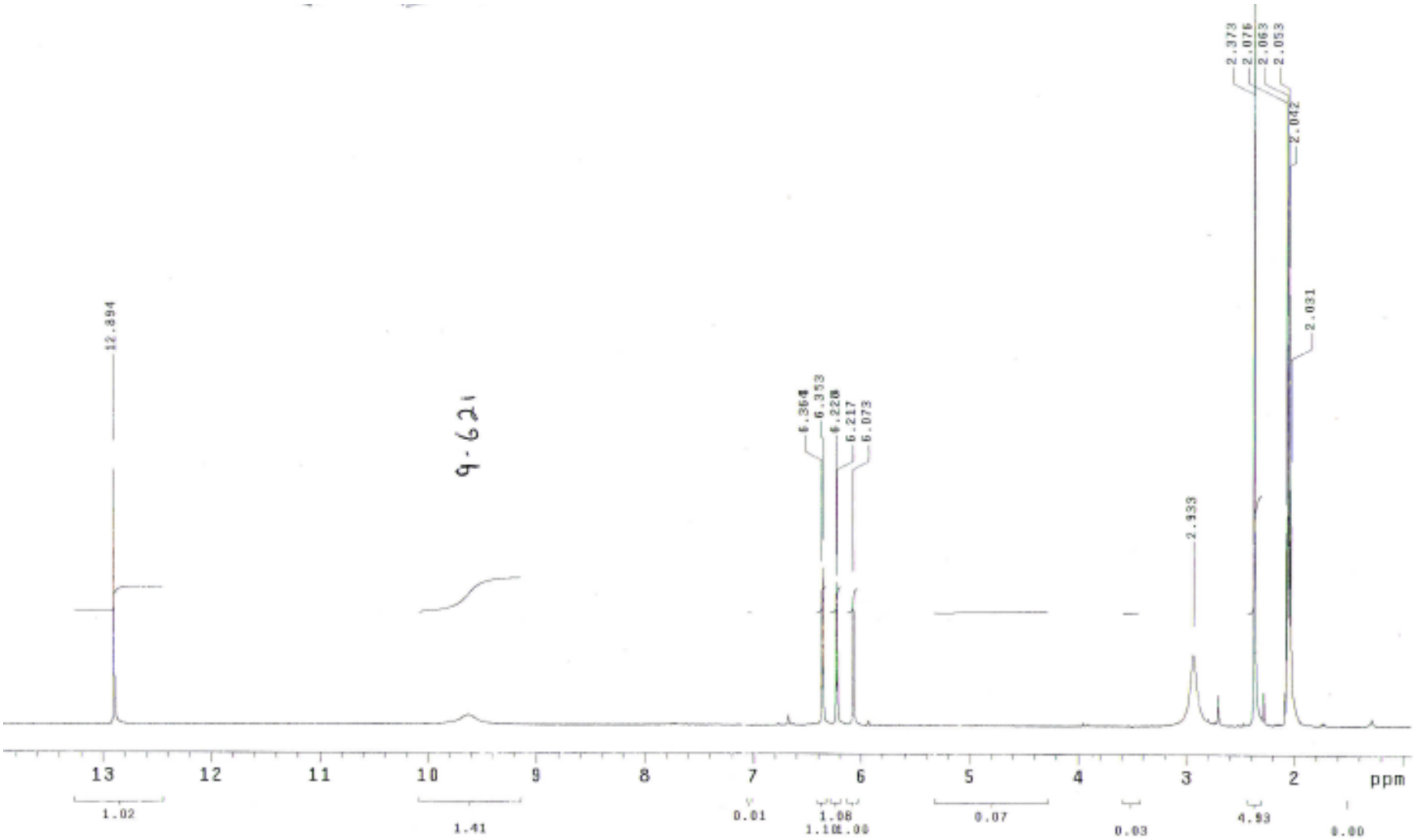
Appendix 40D: HMBC spectrum of cirsiumaldehyde (**161**), acetone-d_6, 500 MHz



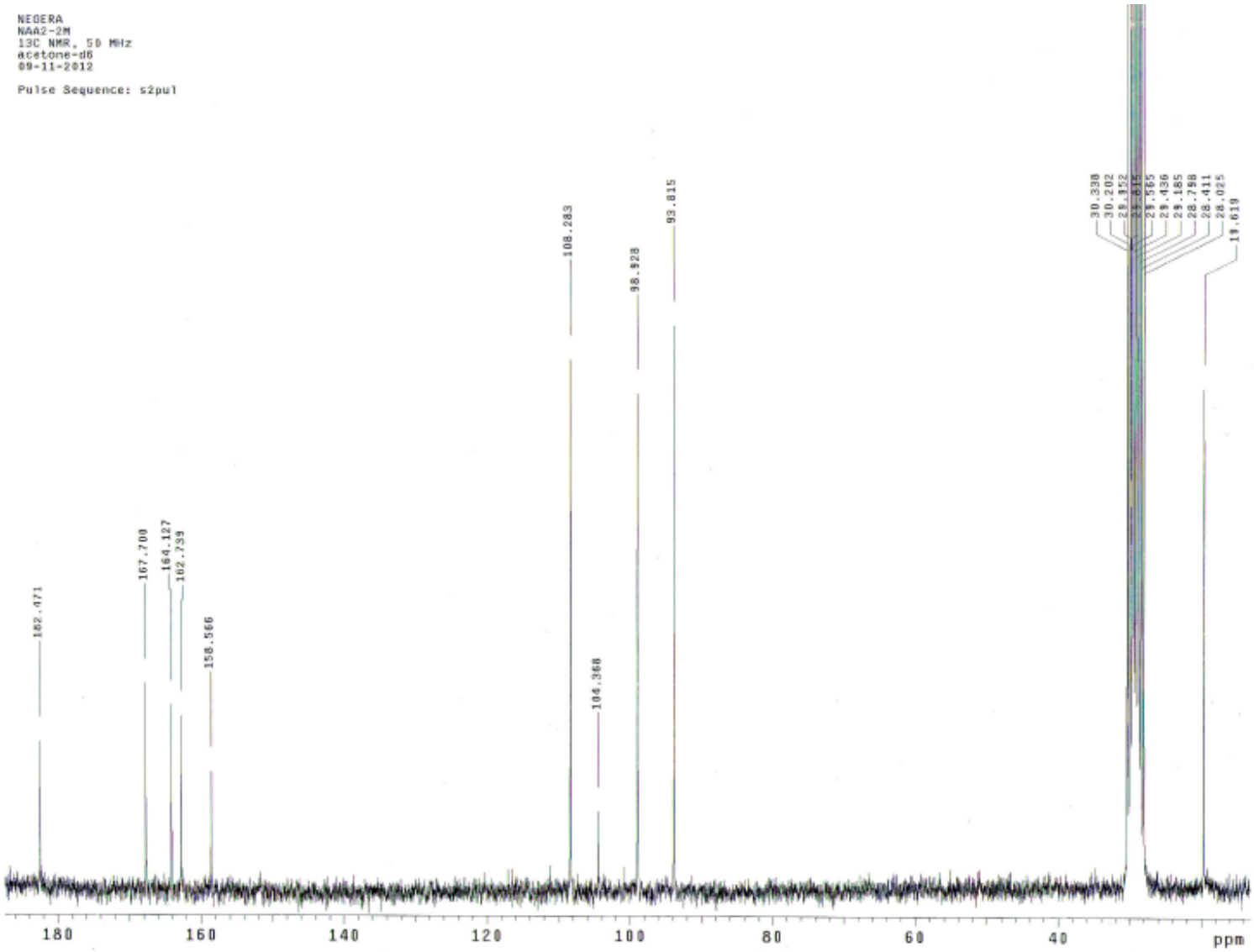
Appendix 40E: ESIMS spectrum of cirsiumaldehyde (**161**)



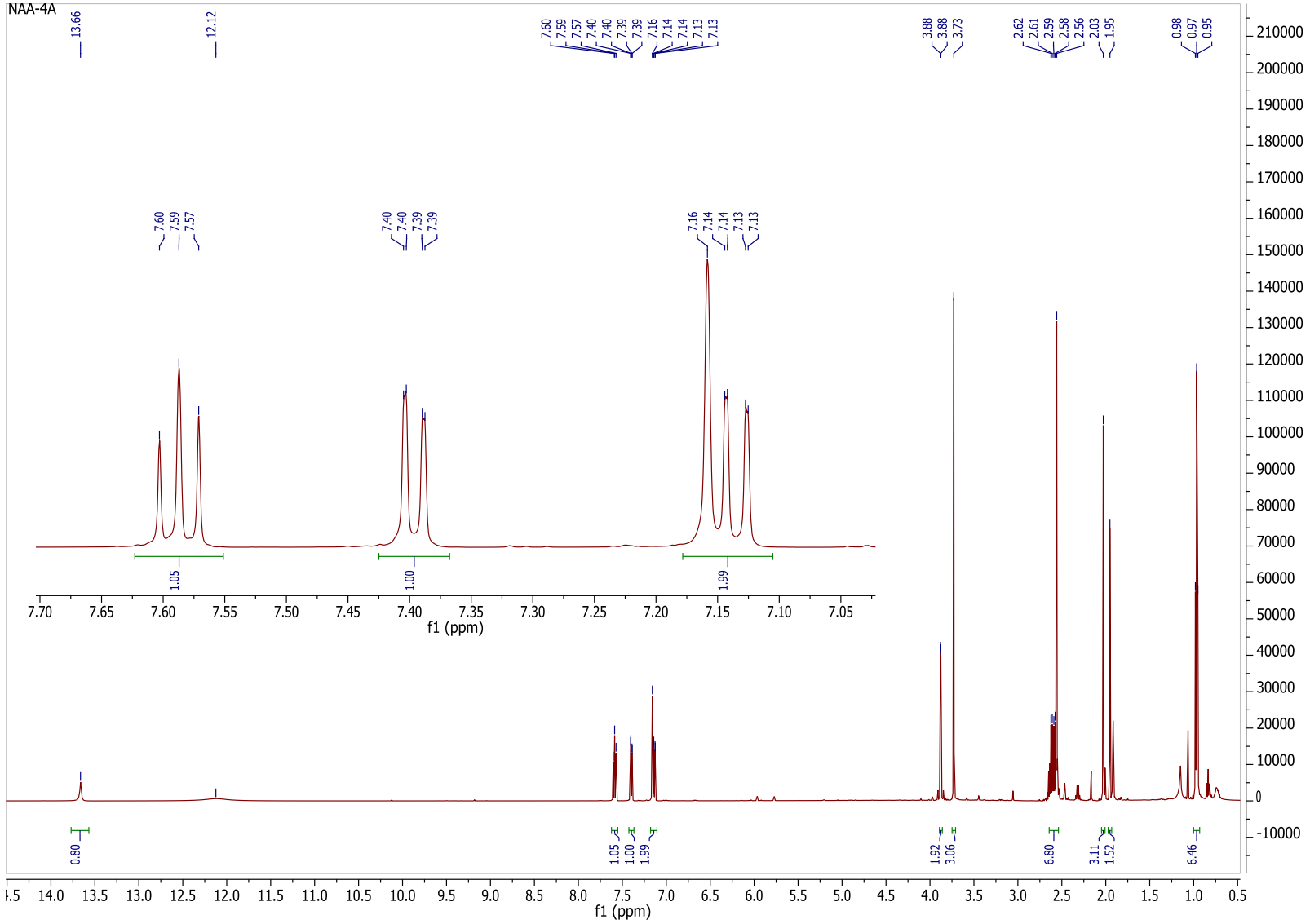
Appendix 41A: ^{1}H NMR spectrum of noreugenin (**162**), $CDCl_3$, 200 MHz



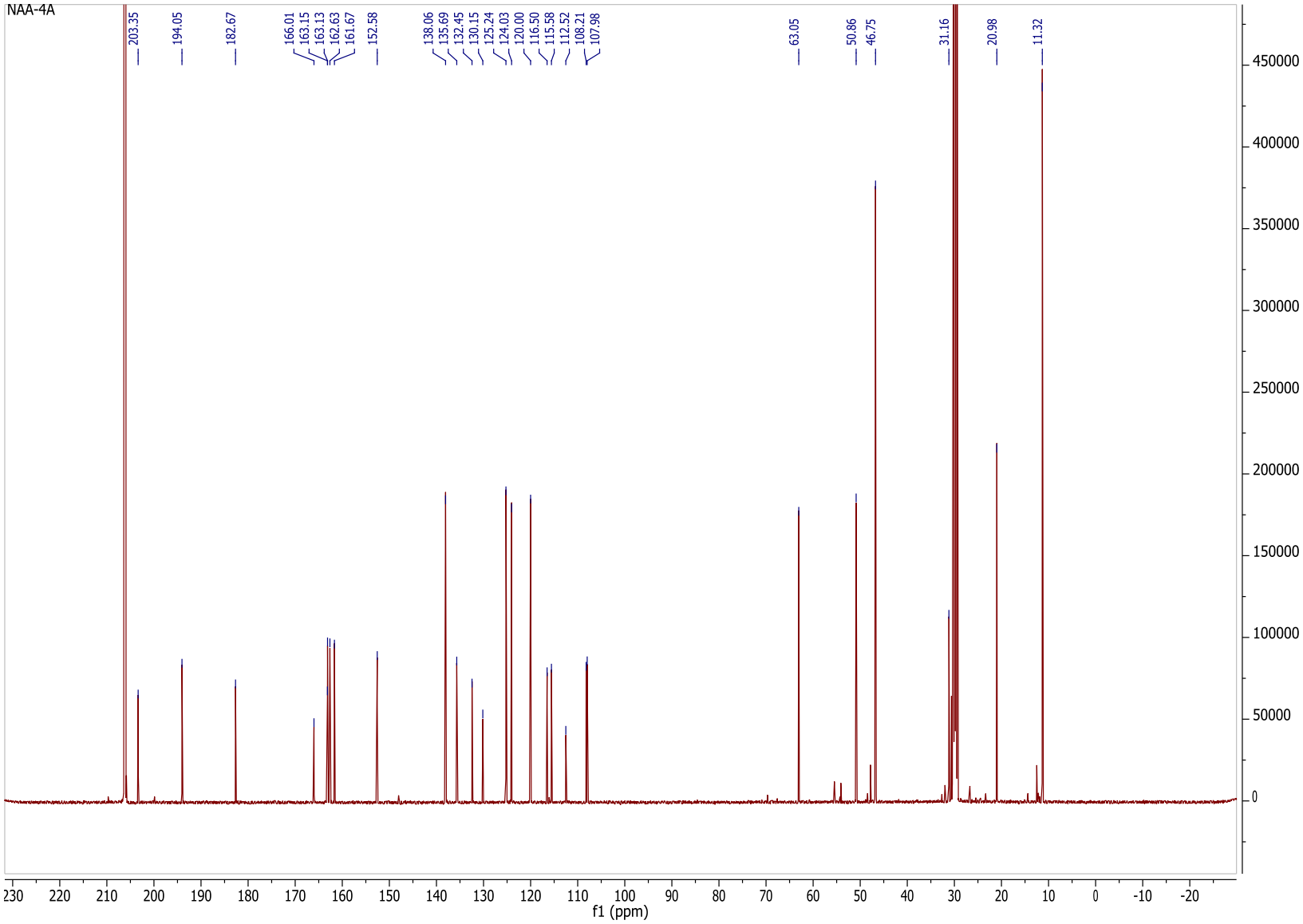
Appendix 41B: ^{13}C NMR spectrum of noreugenin (**162**), $CDCl_3$, 50 MHz



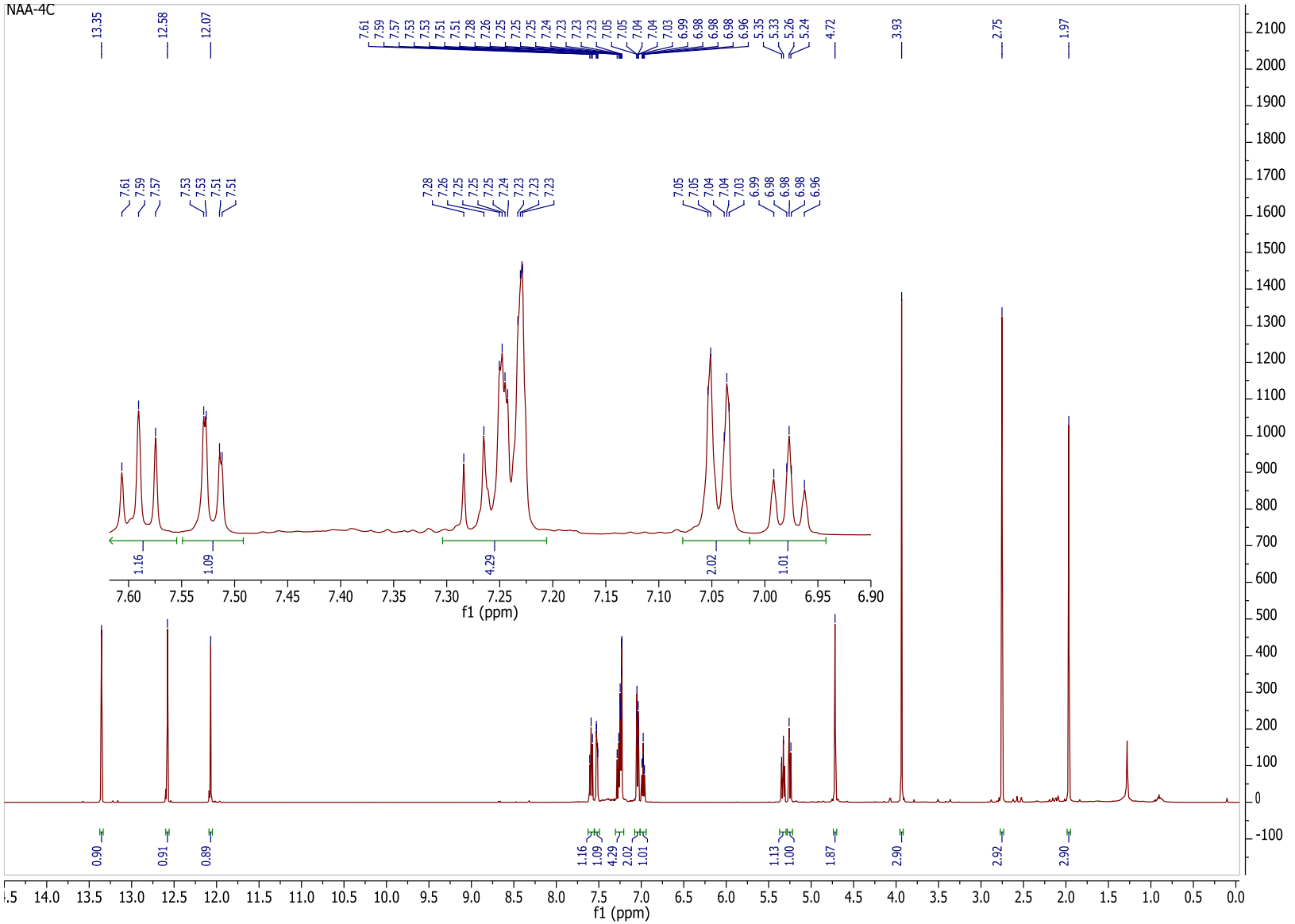
Appendix 42A: ^{1}H NMR spectrum of knipholone Mannich base (**163**), acetone-d_6, 500 MHz



Appendix 42B: ^{13}C NMR spectrum of knipholone Mannich base (**163**), acetone-d_6, 126 MHz



Appendix 43A: ^{1}H NMR spectrum of knipholone-1,3-oxazine (**164**), $CDCl_3$, 500 MHz



Appendix 43B: ^{13}C NMR spectrum of knipholone-1,3-oxazine (**164**), $CDCl_3$, 126 MHz