Adama Science and Technology University

School of Applied Natural Science

Department of Applied Chemistry

Final Project Report

‘Phytochemical Analysis and Biological Activity of Selected Medicinal Plants of Ethiopia’

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List of abbreviations

DEPT	Distortionless Enhancement by Polarization Transfer
IC_{50}	50 % Inhibition Concentration
IR	Infra Red
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
UV	Ultra Violet

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Acknowledgment

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Summary

Interest in obtaining biologically active compounds from natural sources has recently spiked due to their low toxicity, complete biodegradability, availability from renewable sources, and in most cases, low cost. According to the WHO, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals and are responsible for protection of the plant against microbial infections or infestations by pests.

The report comprises phytochemical analysis, biological activity and molecular docking analysis work done on the roots of *Tephrosia villosa*, *Balanites egyptica*, *Zanthoxylum chalybeum*, *Euphorbia schimperiana*, *Teclea nobilis, Clausena anisata* and *Kniphofia schimperiana*. Over all, the work resulted in isolation and full spectroscopic charachterization of **27** compounds (**1**-**27**). Essential oils extracts of fruits of *Zanthoxylum chalybeum* were analyzed by GC-MS and a total of **19** compounds were identified. Detailed of isolation procedures, and comprehensive spectroscopic (UV-Vis, IR, NMR; 1D and 2D) characterization and antibacterial activity of the isolated compounds and crude extracts are included in this report. The antibacterial activity the crude extracts as well as isolated compounds were conducted using agar disk diffusion method. Docking studies of four compounds (**17**-**20**) was performed with DNA-Gyrase (6F86) and LasR binding domain (2UVO) employing flexible ligand docking approach by using AutoDock Vina. So far the work resulted in two publications (Tesfaye Nuru et al., 2018 and Dandena et al., 2019), two international conference paper presentations by PI and three more manuscripts from the results of *Tehphrosia villosa*, *Euphorbia schimperiana* and *Z. chalybeum* are on preparation.

Students/researchers involved in the project directly

1. Mathewos Anza (3rd year PhD student, he is currently working on *Zanthoxylum chalybeum* and *Euphorbia schimperiana* as part of his PhD project along with other four selected plants from Ethiopian flora which are not part of this project).

2. Tesfaye Nuru (completed his MSc in Medicinal Chemistry). He worked on *Teclea nobilis* as MSc thesis project topic.
3. Kebede Shenkute (completed his MSc in medicinal chemistry). He worked on *Kniphofia schimperiana* as MSc thesis project topic and supported the work on polar fractions of *Balanites egyptica*.
4. Dandena Tamene (Completed his MSc in Chemistry (NPs). He worked on *Clausena anisata* as MSc thesis project topic.
5. Abdane W. and Mekdes D. (Completed their BSc in Applied Chemistry). They worked on *Balanites egyptica* as BSc project topic.
6. Dagne Addisu and Mathewos Anza (collaborated on *Tephrosia vogelii* plant work).

1. Introduction

1.1. Background

Plants have been known and used since time immemorial to treat most of the diseases affecting human kind and animals, therefore scientists have found them to be a better choice for bioactive compounds (Jeyaseelan et al., 2010, Khan et al., 2011). The introduction of synthetic drugs, however, changed the trend and attracted many to turn to use them on the expense of botanical drugs; a trend which according to researchers is changing and many people are using medicinal herbs. According to Ngule et al., (2013), about 80% of the individuals from developing countries use traditional medicinal plants as medicine.

According to the WHO report, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical semi synthesis (Hamilton, 2003). Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals and are responsible for protection of the plant against microbial infections or infestations by pests (Doughari et al., 2009).

The medicinal values of plants are attributed to pharmacologically active compounds that have no direct impact on the plants main processes but research has proved these compounds to have great medicinal values. These compounds are used by plants for ecological interaction and are called secondary metabolites or phytochemicals. The secondary metabolites are often referred to as "natural products" which can be subdivided into terpenoids, alkaloids, shikimates polyketides and etc. The classification is based on the means by which they are biosynthesized. Advent, introduction and development of several new and highly specific *in vitro* bioassay techniques, chromatographic methods and spectroscopic techniques, have made it much easier to screen, isolate and identify potential drugs lead compounds quickly and precisely. Over the years medicinal plants have been tested extensively and found to have broad pharmacological uses such as anti-inflammatory, antibacterial, anti-diabetic, anti-fungal, anti-cancer, antioxidant, hepatoprotective, haemolytic, larvicidal, antihelmintic, pain relief, central nervous system, sexual impotence and erectile dysfunction activities (Farook and Atlee, 2011, Hosahally et al., 2012).

Ethiopians have used traditional medicines for many centuries, the use of which has become an integral part of the different cultures where 80% of the people use medicinal plants and plant remedies in one way or another. Moreover, medicinal plants remain the most important and sometimes the only source of therapeutics (Hamilton, 2003). The indigenous peoples of different localities in the country have developed their own specific knowledge of plant resource uses, management and conservation (Teferi and Hahn, 2003).The dependence on medicinal plants is due to the low proportion of medical doctors to patients and furthermore the expensive cost of modern medicine, to treat various infections and the acquisition of drug resistance by pathogens, particularly in third world countries necessitates the search for an alternative medicine from natural products. Many previous studies conducted in Ethiopia have shown the antimicrobial activities of many indigenous plants used in ethnomedicine (Tesfaye et al. 2006).

1.2 Statement of the problem

Demands of traditional herbal medicines are increasing day by day not only by the developing countries but also by the developed countries throughout the world. The demand is due to the increased acceptance of traditional herbal medicines, because of having their safe therapeutic effect with affordable cost. There is also much biomedical knowledge on causation, prevention,

treatment and control of various infectious diseases, nevertheless, it remains a public health problem particularly because of the rapid development of resistance to the available first line drugs. All over the world scientific research is getting momentum to evaluate the pharmacological activities and medicinal properties of different plant species. According to different reports, the secondary metabolites which are found in these plants are used to cure and protect various diseases of human and animals. Even if there are reports from different countries about the uses of specific herbal plants in curing many diseases caused by bacterial infection, there are only few scientific works reported on the genus *Balanites*, *Euphorbia*, *Teclea*, *Kniphofia* and *Tephrosia* species from Ethiopian flora. This inhibits the standardization and internationally recognized system of traditional medicine in Ethiopia. The main thing is all species in Ethiopia are not well discovered, identified and studied. The other important concern is also the chemical constituents are not known to use appropriately and difficult to regulate the dose and concentration. These poses to the use of traditional medicine is a risk of toxicity and deaths to users.

Most of these selected plants are reported to have various traditional uses such as treatment of abdominal pains, emetic, eye infections, fevers, food meal, gastro intestinal illnesses, intestinal parasites, wound, treatment of malaria, bacterial infections etc. However, until today the chemical constituents and biological activity of most of these plants from Ethiopian flora is not documented. Thus, this project is intended to isolate and characterize the chemical components from various plant parts, and conduct the biological assay on selected strains of mircoorganisms. To the best of our knowledge, there is no prior publication that has come out from the phytochemistry and biological point of view on the genus *Balanites*, *Tephrosia*, *Euphorbia*, *Teclea*, and *Merremia* from Ethiopian flora which makes this study reasonable.

1.3 Significance of the study

The studies on antimicrobial activities of Ethiopian medicinal plants are still scarce, and there is need for further investigations along this line to strengthen the pharmacological study profile, evaluate the effectiveness of these plants against disease causing agents and their ultimate utilization in drug development. Clinical observations on traditional remedies are possible and useful. Some herbal remedies may be safe and effective for the treatment of microbial infection.

Nevertheless, better evidence from randomized clinical trials and cytoxicity assay is required before herbal remedies can be recommended on a large scale. In order to prioritize remedies, there is need for preliminary clinical observational studies based on existing data from the ethnobotanical and ethnopharmacological study of Traditional Ethiopian plants. This research can provide an evidence base for the traditional use of the plants by identifying their chemical constituents and validate through biological screening. This study also aims to engender further pharmacological studies on the active ingredients of the plants.

1.4 Objectives

1.4.1 General objective

The overall objective of this study is to isolate, characterize and test the biological activity of secondary metabolites from selected medicinal plants of Ethiopian flora.

1.4.2 Specific objectives

- To extract various plant parts of selected medicinal plants of Ethiopian flora
- To isolate secondary metabolites using silica gel column chromatographic techniques
- To elucidate the chemical structures of isolated compounds using spectroscopic methods
- To evaluate antibacterial activity of the crude extracts and isolated compounds against selected strains of microorganisms (*E. coli*, *S. aureus*, and *S. epidermidis*).
- To analyze antioxidant activity of the plant extracts and isolated compounds using DPPH and Ferric thiocyanate
- To analyze antifungal activity of the plant extracts and isolated compounds against three fungi strains (*Fusarium oxysporum, Fusarium solani,* and *Aspergillus niger*)
- To conduct qualitative analysis of total phenolics, tannins, alkaloids, anthraquinones, and other secondary metabolites present in crude extracts

2. Literature review

2.1 Overview of the geographical distribution and ethnobotanical uses

2.1.1 The genus *Balanites*

Balanites is a genus of flowering plants belongs to the family *Balanitaceae* or *Zygophyllaceae*. It is one of the most wide-spread woody plants of the African continent. It is distributed through much of Africa from costal Mauritania and Senegal to Somalia and Egypt, southwards to Zambia and Zimbabwe, as well as in the Middle East from Yemen to Jordan and Israel, Benin, Burkina Faso, Cameroon, Chad, Djibouti, Ethiopia, Gambia, Ghana, and Zambia are the primary African countries where *Balanites* are grown (Sands, 2001). *Balanites* are not only grown throughout the African continent but also in the Middle East, the Arabian Peninsula, and Southern Asia. Israel, Jordan, Saudi Arabia, North and South Yemen, India, and Myanmar are the countries beyond Africa where *Balanites* naturally grow.

The genus *Balanites* is also one of those traditionally used medicinal plants in Ethiopia. The *Balanites* tree has a long history of use as a resource, especially in the African continent where it is the most wide-spread. The tree also has biblical connections. It is believed that *Balanites* was the source of one of the ingredients of the perfume 'spikenard' as used by the Egyptian royalty (Hall, 1991). Today this plant plays a diverse cultural and traditional role in different societies. Chips of the wood placed in elephant dung are used to prevent elephants from attacking people or property in East Africa. The mistletoe grown in *B. aegyptiaca* is taken as concoctions to enhance scholastic ability. The fruit is used to hang around the neck of a potential victim to ward off blood-sucking sorcerers in Saharan Morocco (Burkhill, 1985). If, as is generally believed, humanity began in Africa, then the bittersweet, *Balanites* fruit is likely among the oldest of all foods. Certainly this resilient evergreen tree has been helping mankind for thousands of years. Its fruits have been found in Pharaohs' tombs dating back to at least the 12th dynasty in ancient Egypt; thus even royalty has appreciated it for 4,000 years (Ladipo, 1989).

Several species of *Balanites* are also grown as ornamentals. The wood is hard, durable, worked easily and made into yokes, wooden spoons, pestles, mortars, handles, stools and combs. It shows no serious seasoning defects and no tendency towards surface checking or splitting. It

glues firmly and takes a clear varnish. *Balanites rotundifolia* (Van Tiegh) Blatter, which belongs to the family *Balanitaceae* or *Zygophyllaceae* is one of traditionally used medicinal plants in genus *Balanites*. *Balanites* is named in different parts of Ethiopia with different names such as 'kudkuda' (Amaharic), 'Baddana' (Sidamigna and Afaan Oromoo), and 'Badano'o' (Haddiyigna). *Balanites rotundifolia* is shrub or tree which is widely distributed in the flora of low lands in Ethiopia.

Balanites rotndifolia (Fig 1) is multi branched, spiny shrub or tree up to l0 m tall. Trunk short and often branching from near the base. Bark is dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long. Leaves with two separate leaflets; leaflets obviate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young as shown in Figure 1. Flowers in fascicles in the leaf axils, and are fragrant, yellowish-green. Fruit is a rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. Pulp is bitter-sweet and edible. Seed is the pyrene (stone), 1.5 to 3 cm long, light brown, fibrous, and extremely hard.

Fig 1. *Balanites rotundifolia* (Badano) (Photo taken by Milkyas Endale, August, 2016)

2.1.2 The genus *Zanthoxylum*

The *Zanthoxylum* genus that belongs to the family Rutaceae, comprises about 200 species of aromatic trees and shrubs native to the middle latitudes of North and South America, Africa, Asia, and Australia (Talapatra et al., 1973). Many species of the *Zanthoxylum* genus have been used in different parts of the world especially in Asia, Africa and America to treat a number of

diseases in humans and animals (Diéguez et al., 2004). The bark of *Z. liebmannianum*, is used in Mexico for the treatment of stomach pains, amebiasis, intestinal parasites and as a local anesthetic agent (Ross et al., 2004). Some species are used for the treatment of malaria; such is the case of *Z. rhoifolium* and *Z. chalybeum* (Jullian et al., 2006).

Stem bark decoctions or root bark decoctions of *Zanthoxylum chalybeum* is widely taken to treat malaria, fevers and headache, sickle cell disease, respiratory tract ailments including colds and tuberculosis, skin diseases including ulcers, urticaria, tumours and measles, intestinal problems including abdominal pain, diarrhea, intestinal worms, bilharzia, amoebas, colic, general body pain and vomiting. A root infusion is drunk to treat bacterial muscle infections, female sterility, venereal diseases, uterine fibroids and, together with chicken meat, as an aphrodisiac (Engeu et al., 2008). In Ethiopia it used as a traditional medicinal plant for humans and animals, root bark and stem bark is taken to treat toothache and the leaves are taken to treat the breast cancer of livestock's (Reta, 2013).

Fig 2. *Z. chalybeum* (Ga'da) (picture taken by Mathewos Anza, Dec, 2014)

2.1.3 The genus *Tephrosia*

The genus *Tephrosia* belongs to the family *Leguminosae* and subfamily *Papilionaceae*. There are approximately 400 species included in this genus. The genus is widely distributed in tropical, sub-tropical and arid regions of the world (Willis, 1972). The plants are prostate or erect herbs or in the form of soft or woody shrubs. Many plants from this genus have been used traditionally for the treatment of diseases like rheumatic pains, syphilis, dropsy, stomach ache, diarrhea,

asthma, abortifacient, respiratory disorderrs, laxative, diuretic, and inflammation (Qureshi et al., 2010). *Tephrosia purpurea*, an important plant of the genus is used as tonic, laxative, antivenom, antiulcer, antidiarhheal and in leprosy (Virupanagouda et al., 2011). *Tephrosia vogelli*, and *Tephrosia purpurea* (Fig.2), are the two mainly used species of the genus used as tonic, laxative, anti-venom, antiulcer, antidiarrheal and treatment of leprosy. Ethanol (80%) extract of the dried fruit showed weak antibacterial activity against *Staphylococcus aureus* on agar plate, weak antiviral activity against measles virus on cell culture, strong antifungal activity against *Microsporum canis* (Vlietinck 1995).

Fig 3. Picture of *Tephrosia vogelii* **(**Picture taken by Milkyas Endale, March 12, 2014)

2.1.4 The genus *Euphorbia*

Despite its actual and potential importance, traditional medicine, or more precisely herbal remedies, were overlooked and discounted. One of the important reasons for this was the unavailability of published data on ethnobotanical, ethnomedicine and phytoconstituents of most medicinal plants. Of these, the genus *Euphorbia* is among the list of medicinal plants that needs to be explored although there is some supportive information with regard to the ethnobotanical uses in various formulations. Moreover, to the best of our knowledge the phytoconstituents and medicinal uses of Ethiopian *Euphorbia* species has not been well studied. Thus, identifying the secondary metabolites in some selected species from the genus will not only mitigate but also help bridge the gap between traditional and scientific medicine. There is information indicating that in rural southern and eastern parts of Ethiopia the local peoples still use the plants to heal various diseases by making the plant formulations in various forms. In southern part of Ethiopia, peoples use the stem and root of the plant for the treatment of diarrhea and skin diseases-the

juice from the apical parts of the plant is applied on the affected area. The ethnobotanical information of Ethiopian *Euphorbia* species is summarized in Table 1. This summary is based on the available literatures at the Ethiopian National Herbarium and Institute of Biodiversity Conservation and Research (IBCR).

Table 1. List of *Euphorbia* species documented at the National Herbarium of Ethiopia

Plant species	Ethnobotanical information	Place of Availability
Euphorbia jatrophoides	-	• About 35 km S.E. of Negelle, on the road to Filtu, along the edge of the road (5^{O} 15'N, 39^{o} 55'E)
Euphorbia doloensis	-	• 24 km from Dolo on the road to Melka Suftu
Euphorbia uniglans	-	• 42 km from Negele on the road to Melka Guba
Euphorbia pirottae	Somali name: Buryo Sap harmful for skin and eyes. Untouched by the livestock but, if goats and cattle eat it accidentally, they die over time	• Eastern Hararge region 5km from Harar to Gosololey (8^{O} 13'N, 43^{o} 31'E) • 95 km from Negele to Fitu road (5^{O} 00N, 40^{o} 12'E)
Euphorbia schimperiana	Amharic name: Anterfa Oromifa name: Guri or aeno It has white latex Sidamigna: Binjale Farmers use the water extracts for insecticidal property	• Sidamo, near Yigalem hospital • 26 km SE of Shashemneen, along Wadossa region • Zekuwala 74 km of SE of A.A (8^{O} 32'N, 38^{o} 51'E) • Bale region: bale mountains national park between Goda and the border of the BMNP. 5km of Goba, (6^{O} 57'N, 39^{o} 57'E)
Euphorbia hirata	Vernacular name: deefa-ka-gie (Oromo) Local medicinal uses	• NW shore of lake Hawassa 40 m from the water edge • Tepi institute of Agricultural Research (7^{O} 11.02N, 35^{o} 25.58E) • Metehara Sugar cane plantation and sugar factory, in clinic compound (8^{O} 31'N, 39^{o} 12'E) • 16 km from Chencha (6^{O} 22N, 37^{o} 36'E)
Euphorbia pulcherima		• Bihere Tsige public recreation
Euphorbia indica	Anti fungal Yequaqucha medhanit (Ahmaric)	• Gamo Gofa zone • Abader (upper awash)
Euphorbia hypericifolia	Anti fungal Yequaqucha medhanit (Ahmaric)	• Abadir (upper awash) • Gamo Gofa. 7km on Chencha road (6^{O} 08'N, 37^{o} 35'E)

Euphorbia crotonoides	Vernacular name: Robii (oromifa) Guri (oromiya)-near Zeway Used as traditional uses for skin diseases During circumscription Of male children It has milky exudates Near Zeway peoples use the plant for stomach problems	• 17 km south of Dolo Mena on the roa to the river Welmel (6^O 18'N, 39^o 49'E) • Sidamo: West of Arero, on the western slope of the Arero mountain (4^O 44'N, 38^o 48'E) • Oromiya region: Bochesa village, 7 km southeast of Zeway town
Euphorbia polycenemoides		• 10 km N of Dolo Menna, on the road to Goba (6^O 25'N, 39^o 44'E) • Omo national park
Euphorbia lathyris	Seeds are eaten for headache (sometimes with honey or sugar)	• Haromaya college area 15 km NW of Harar (9^O 24'N, 42^o 1'E)

2.1.5 The genus *Teclea*

Teclea is a genus in the subfamily toddaliea of the family Rutaceae. There are about 30 species in Africa (Victor, 2000), some Kenyan species include; *Teclea ameniensis, T. grand [folia, T. kanangesis, T. nobilis, T. trichocarpa* and *T. simplicifolia* (Beentje 1994).

2.1.5.1 Botanical information on *Teclea nobilis*

Teclea nobilis is a shrub or understory tree 4-18 m high, which is evergreen, with a grey brown bark and finely grooved leaves. It is widely distributed in tropical Eastern Africa, namely Ethiopia, Sudan, Somalia, Kenya, Uganda, Tanzania and also in Arabia. This plant is used in folk medicine of many African societies (Beentje 1994).

2.1.5.2 Ethnomedical information on the genus *Teclea*

Teclea nobilis is a plant used in folk medicine as an analgesic and antipyretic agent. In South Africa, the bark of *T. nobilis* is reported to be a gonorrhoea remedy while in Tanzania, the leaves are used as cure for fever (Watt and Breyer-Brandwijk, 1962). Similarly in Ethiopian folk medicine the leaves are

used to control pain (Mascolo *et al.,* 1988). *T. trichocarpa* is used by traditional healers belonging to the Akamba tribe of East Africa for malaria treatment, as an anti-helmintic and the vapour is inhaled as a cure for fever (Watt and Breyer-Brandwijk, 1962). The various parts of the plant including leaves and stem bark are said to be a remedy for gonorrhea and pain (Watt & Breyer-Brandwijk, 1962). *T. ouabanguiensis* is used as a remedy for coughs and asthma in Cameroon (Watt and Breyer-Brandwijk, 1962).

2.1.6 The genus *Kniphofia*

The genus *Kniphofia* named after the German botanist Johann Hieronymus Kniphof belongs to the subfamily Asphodeloidea [Armitage, 2000]. The genus is common among horticulturists and is entirely African (main land) with only three species occuring outside Africa (Ramdhani, 2007) i.e. *Kniphofia pallidiflora, Kniphofia ankaratrensis* and *Kniphofia sumarae,* where the first two are indigenous to Madagascar and the later to Yemen (Bringmann *el al.,* 2008). Due to their tall scarlet or red flowers the genus is commonly known as 'red hot pokers' and encompasses about 70 species [Bosch, 2008; Bringmann *et al.,* 2008a]. It is widely distributed in the Eastern and Southern part of Africa, of which about 47 of them occur in Southern Africa (Droop, 1986; Bringmann *et al.,* 2008).

2.1.6.1 Ethnobotanical uses of the genus *Kniphofia*

Although the genus is widely recognized for its ornamental value owing to their colourful flowers, the use of the genus in traditional medicine is limited to few species which is summarized in Table 2.

Table 2. Summary of the ethnobotanical uses of the genus *Kniphofia*

Species	Plant part	Used for/as	References
K.buchananii	Plant infusion	Snake deterrents, Chest ailments	Ramdhani, 2007 Bringmann et al., 2008
K.caulescens	Part not specified	Charm against lightening	Ramdhani, 2007
K.foliosa	Roots	Abdominal cramp and ache Wound healing	Wube et al., 2005 Bringmann et al., 2008
K.isoetifolia	Roots	Gonorea, hepatitis B	Yineger et al., 2008
K.laxiflora	Plant infusion	Snake deterrents, Chest ailments	Bringmann et al., 2008
K.linearifolia	Roots	To treat infertility	Bosch, 2008

K.parvifolia	Plant infusion	Snake deterrents, Chest ailments	Bringmann et al., 2008 Ramdhani, 2007
K.ritualis	Part not specified	Shoulder pains	Ramdhani, 2007
K.rooperi	Plant infusion	Chest ailments and sanke detterrent	Bringmann et al., 2008 Ramdhani, 2007

2.1.7 The genus *Clausena*

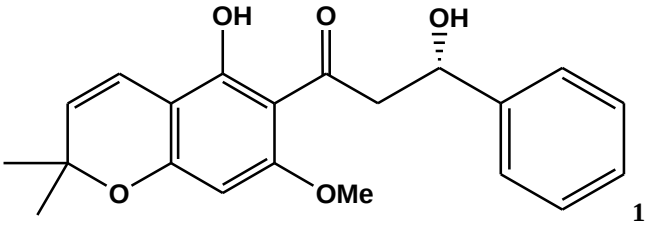
The genus *Clausena* belongs to the family Rutaceae and distributed throughout tropics and subtropics. *Clausena* is a genus of about 14 species of evergreen trees, occurring mostly in India and tropical Asia (Burkill, 1966). One of the most advantageous features of the species of this genus is their availability in the different parts of the world, mainly in India Tropical Asia and South Africa. They are being easy to grow and free of pests and diseases as well as can withstand heavy pruning (Sofowora, 1993). The most distinctive morphological character of the genus *Clausena* is the gynophore, which in the typical species is a large, well-developed, hourglass-shaped structure supporting the ovary (Ayisi et al., 2003). Nevertheless, it is present in all species of *Clausena* and separates them from the species of other related genera. The numerous species of this genus, still only imperfectly studied with respect to the minute flower characters, cannot be arranged now in natural sections or subgenera.

2.2 Phytochemistry of the selected plants

2.2.1 Previous chemical studies on the genus of *Tephrosia*

2.2.1.1 *Tephrosia elata*

Recently, naturally occurring elatadihydrochalcone bearing pyranodihydrochalcone moiety was isolated from seedpods of *Tephrosia elata*, which was distributed in East Africa. This plant is also used as traditional medicine to treat infectious diseases. It is the first report on the occurrence of a β-hydroxydihydrochalcone in the genus *Tephrosia* species. The potent antiplasmodial activity has stimulated interest in the synthesis of naturally occurring elatadihydrochalcone A new β-hydroxydihydrochalcone named (S)-elatadihydrochalcone (**1**) was isolated from the seedpods of *Tephrosia elata*. This compound along with other flavonoids are responsible for the antiplasmodial activity of this plant (Muiva et al., 2009).



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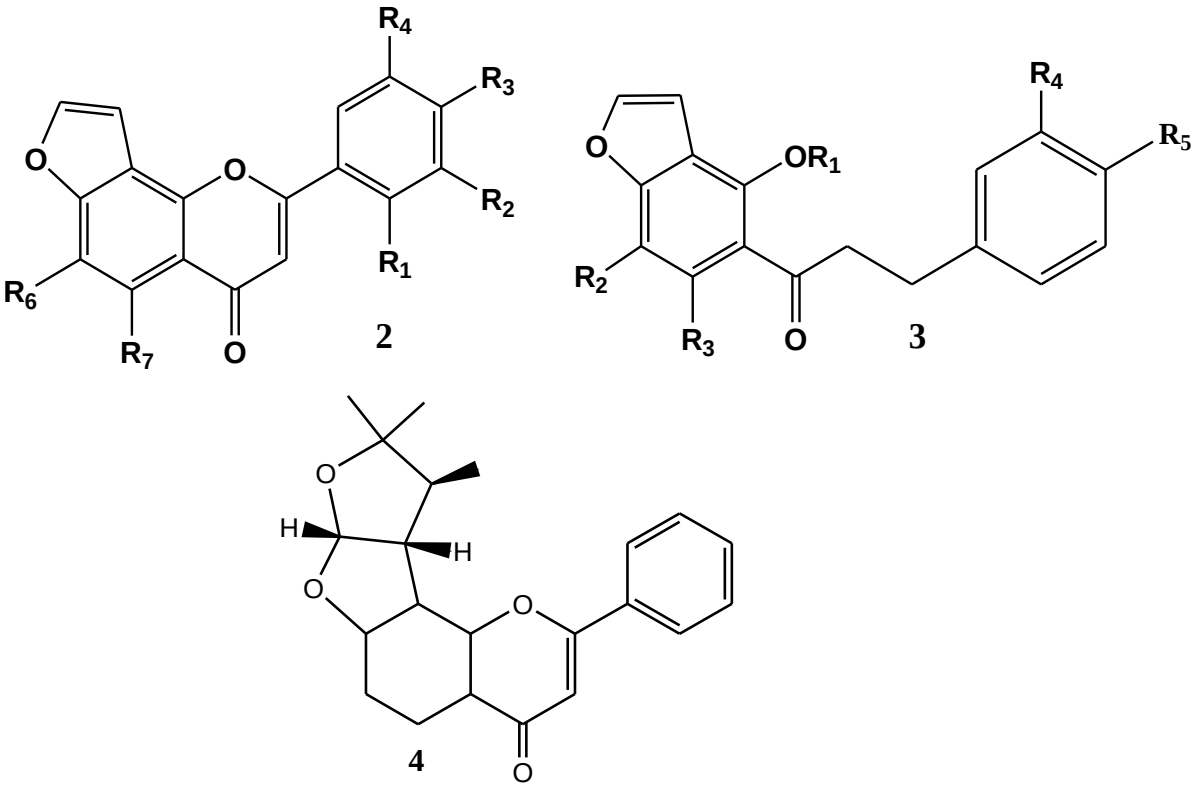
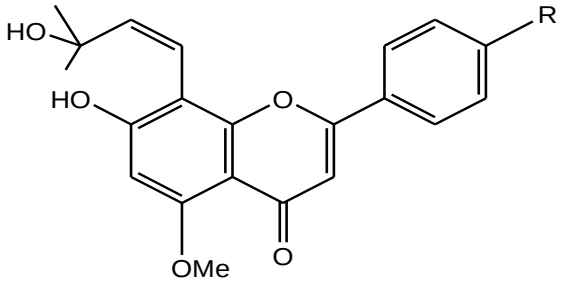
2.2.1.2 *Tephrosia purpurea* (Linn.) Pers.

The ethanolic extract of this plant has been reported to have anticancer activity against KB cells in culture (Ahmed, 1999). The aqueous extract of seeds has shown significant *in vivo* hypoglycaemic activity in diabetic rabbits. The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity. The phytochemical investigations on *Tephrosia purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, and sterols. Ahmad et al isolated and reported the NMR spectra of pongaglabol (**2**), purpuritenin (**3**) and semiglabrin (**4**) from *Tephrosia purpurea* aerial parts (Ahmad, 1999).



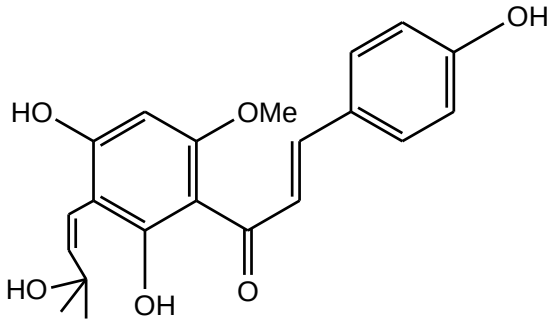
2.2.1.3 *Tephrosia candida*

In a continued investigation of medicinal plants from the genus *Tephrosia*, phytochemical analysis of a methylene chloride/methanol (1:1) extract of the air-dried aerial parts of *Tephrosia candida* afforded two new 8-prenylated flavonoids, namely, tephrocandidins **5** and **6**, a new prenylated chalcone, candidachalcone (**7**), a new sesquiterpene (**8**), and a previously reported pea flavonoid phytoalexin, pisatin (**9**). There is a current interest in naturally occurring phytoestrogens as potential alternatives to hormonal replacement therapy (HRT). With the observation that *Tephrosia candida* can produce unusual prenylated flavonoids, whether or not such modified flavonoids can act as estrogen mimics (Hegazy et al., 2011).



5 R = H

6 R = OH



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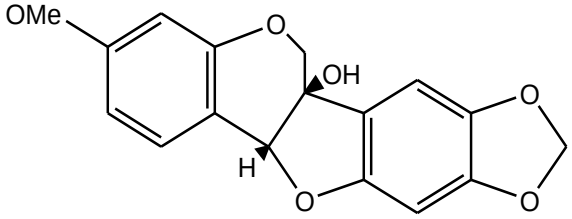

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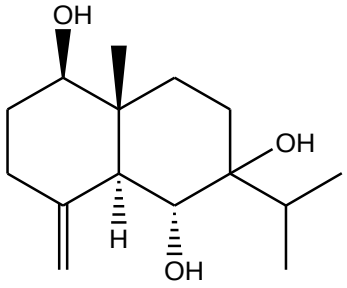

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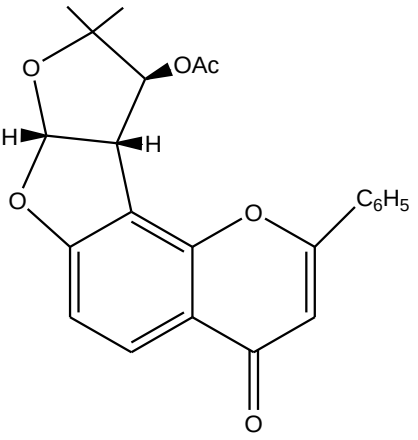
2.2.1.4 *Tephrosia nubica*

Four 7-oxygenated -8- prenyl flavones, semiglabrin (**10**) pseudosemiglabrin (**11**), apollinine (**12**) and lanceolatin A (**13**) were isolated from *Tephrosia nubica* herb (Ammar and Diwany, 1988).



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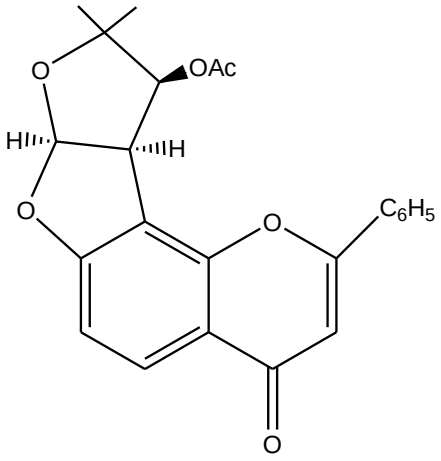

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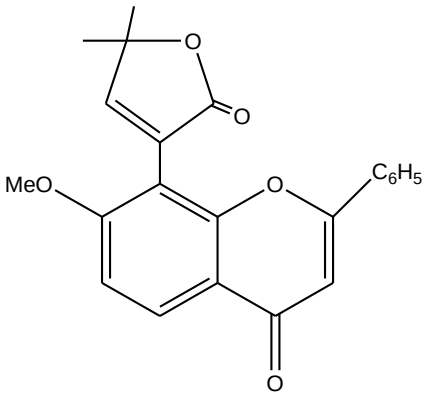

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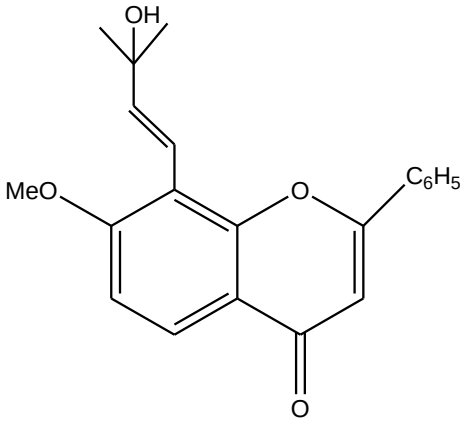

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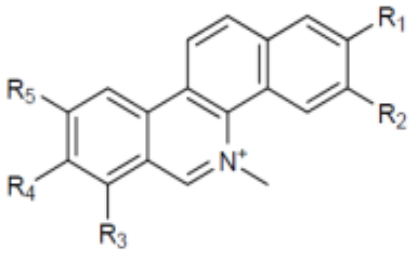
2.2.2 Chemical constituents of the genus *Zanthoxylum*

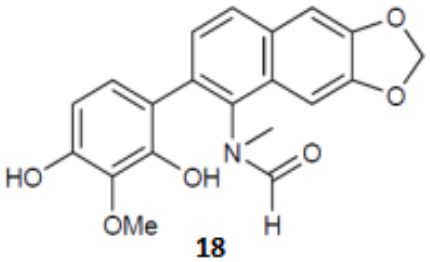
Several members of this genus are used in traditional medicine around the world. In terms of phytochemistry, more than 90 species have been studied and among secondary metabolites that appear most frequently are alkaloids (Ahmad et al., 2003; Jiang et al., 2007; Huang et al., 2008), coumarins (Mester, 1983; Simanek, 1985) and lignans (Chen et al., 1999; Fiorentino et al., 2007; Chen et al., 2008). The main isolated alkaloids from the genus are of two types: isoquinolines and quinolones.

The benzophenanthridines are the most frequently reported type of alkaloid in the genus *Zanthoxylum* and have great interest due to important and varied biological activity including antimalarial (Neuwinger, 1996; Rahman et al., 2005), antileukemic (Eun and Koh, 2004), antioxidant (Pérez et al., 2003), antibacterial (Gonzaga et al., 2003), antimicrobial (Nissanka et al., 2004) and antifungal activities (Queiroz et al., 2006), among others. The main representatives of this type of alkaloids are

fagaronine (**14**), nitidine (**15**), chelerythrine (**16**) and sanguinarine (**17**). Compounds with similar chemical structure to iwamide (**18**) and integriamide (**19**), isolated from various species of the genus *Zanthoxylum*, have been classified by different authors within benzophenanthridine alkaloids (Krane et al., 1984).



14 R_1 = OH; R_2 = OMe; R_3 = H; R_4 = R_5 = OMe
15 $R_1 + R_2 = OCH_2O$; R_3 = H; R_4 = R_5 = OMe
16 $R_1 + R_2 = OCH_2O$; R_3 = R_4 = OMe; R_5 = H
17 $R_1 + R_2 = R_3 + R_4 = OCH_2O$; R_5 = H



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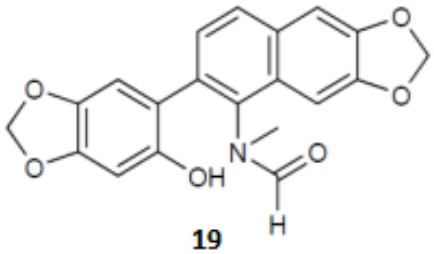

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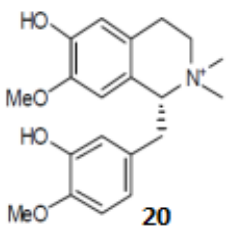
Berberine (**20**) and protoberberine alkaloids have been reported in several species of the genus *Zanthoxylum*, for example tetrahydroberberines such as *N*-methyltetrahydrocolumbamine (**21**) and *N*-methyltetrahydropalmatine (**22**), have been isolated from the bark of *Z. quinduense* (Patiño and Cuca, 2010). Berberine (**20**) is characterized by its significant leishmanicidal and antimicrobial activities and is usually the responsible for the yellowing observed in wood and bark of some species of this genus, as in the case of *Z. monophyllum* that is used as a dye (Patiño and Cuca, 2011). In the genus *Zanthoxylum*, aporphine alkaloids there are not the most representative, but they have been isolated from various species and are of great importance because several have antitumoral activity. For example, N,N-dimethyllindicarpine (**23**), obtained from the root bark of *Z. zanthoxyloides* (Queiroz et al., 2006).



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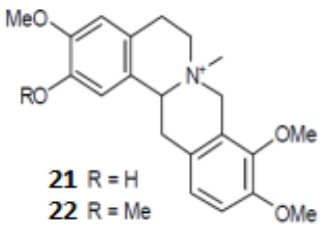
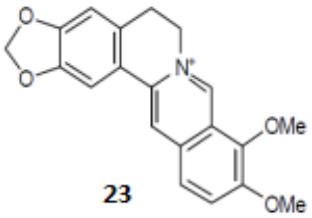


21 R = H
22 R = Me



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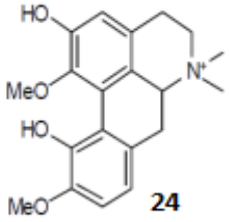
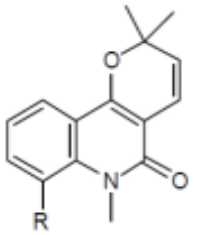
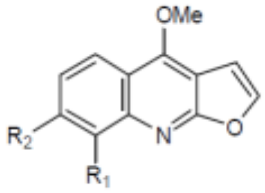


24

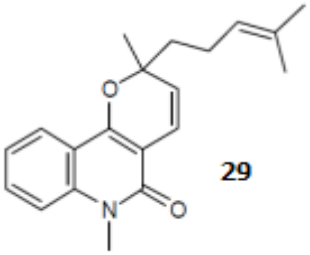
Quinoline alkaloids have been isolated from the bark of *Z.budrunga* founding two pyranoquinoline: N-methylflindersine (**25**) and zanthobungeanine (**26**), together with two furoquinolines dictamine (**27**) and skimmianine (**28**) (Rahman et al., 2005). From *Z. simulans* also have been isolated pyranoquinoline alkaloids as zhantosimulin (**29**) and huajiaosimulim (**30**), with cytotoxic activity (Chen et al., 1997).



25 R = H
26 R = OMe



27 $R_1 = R_2 = H$
28 $R_1 = R_2 = OMe$



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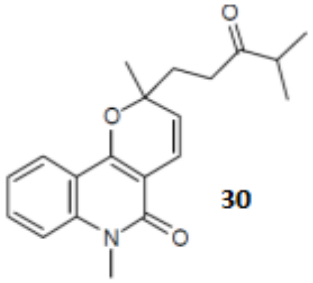
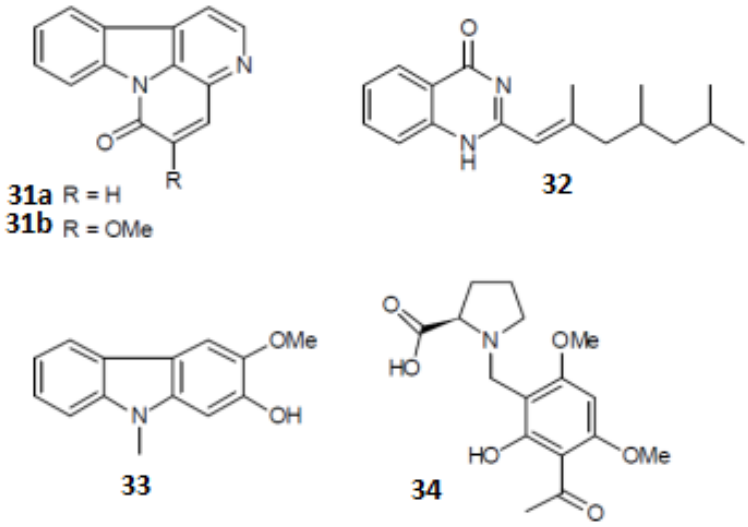


30

Canthin-6-one alkaloids of importance for its leishmanicidal activity are rare in the family Rutaceae, are found in a few genders including *Zanthoxylum*. From *Z. chiloperone* (Chen et al., 199) and *Z. budrunga* (Rahman et al., 2005) have been isolated canthin-6-one (**31a**) and 5-methoxycanthin-6-one (**31b**).Quinazoline alkaloids have been isolated from *Z. budrunga*, as is the case lunacridina (**32**) (Bastos et al., 1999). Carbazole alkaloids such as 3-methoxy-9-methyl-9H-carbazol-2-ol (**33**) were obtained from the wood of *Z. rhoifolium* (Andersson et al., 1996). Recently, from the bark of *Z.*

monophyllum was isolated an alkaloid derived from proline, called monophyllidin (**34**) with antibacterial activity against *Enterococcus faecalis* (Patiño and Cuca, 2011).

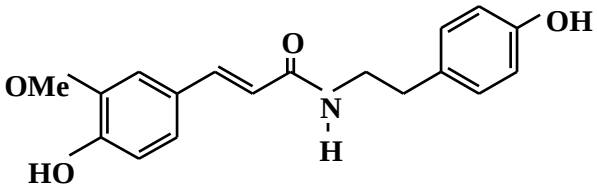


2.2.3 Chemical constituents of the genus *Balanites*

Balanites is an ethnomedicinally important genus used for the treatment of various diseases. A number of researches have been performed to identify biologically active compounds from different species of *Balanites*. Phytochemical reports on family Balanitaceae revealed the presence of alkaloids, steroidal and saponins (Saharan et al., 2008), furanocoumarin, and flavonoids namely quercetin 3-glucoside, quercetin-3-rutinoside; 3-glucoside, 3-rutinoside, 3,7-diglucoside and 3-rhamnogalactoside of isorhamnetin (Salwa et al., 1988).

2.2.3.1 Chemical constituents of *Balanites aegyptiaca*

High performance liquid chromatographic HPLC analysis of a dichloromethane extract of the stem-barks of *Balanites aegyptiaca* has yielded two known alkaloids, N-trans-feruloyl-tyramine **(35)** and N-cis-feruloyltyramine **(36)**, and three common metabolites, vanillic acid, syringic acid and 3-hydroxy-1--4-hydroxy-3-methoxyphenyl.-1-propanone (Sarker and Nash, 2000).



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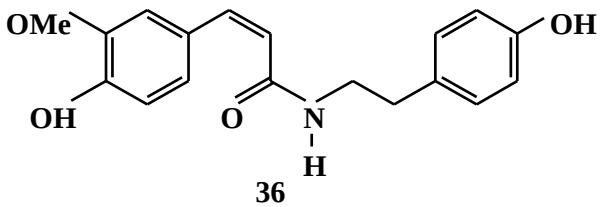

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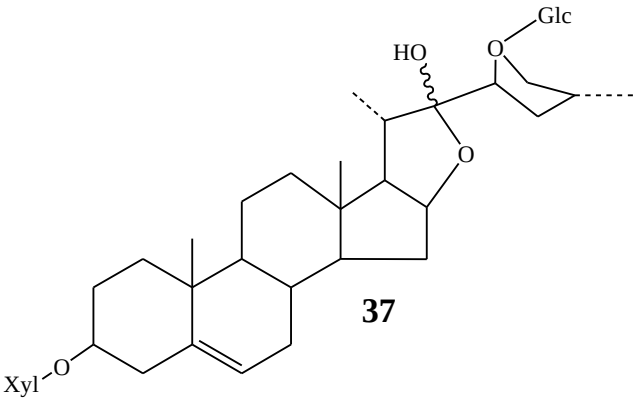
A related study conducted on *Balanites aegyptiaca* revealed compounds such as a furostanol saponin, balanitesin (**37**) and balagyptin (**38**).from the mesocarp of the fruits (Kamel, 1998).



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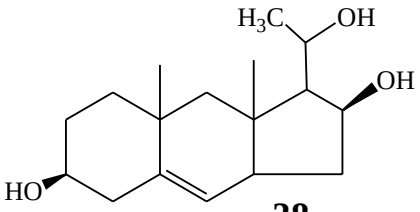

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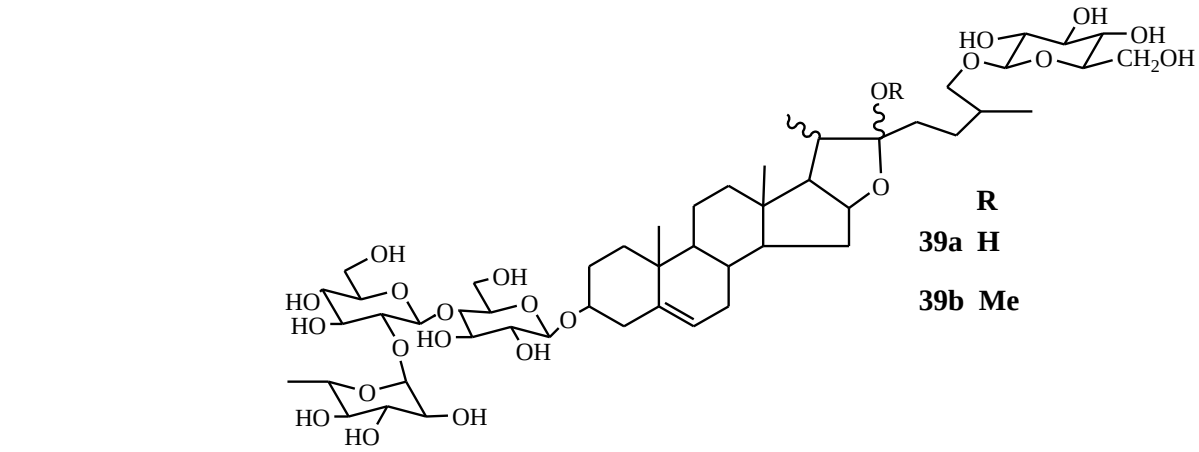
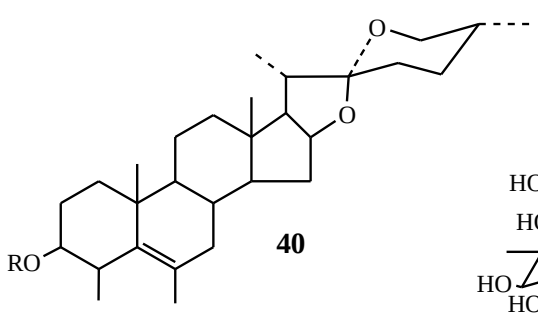
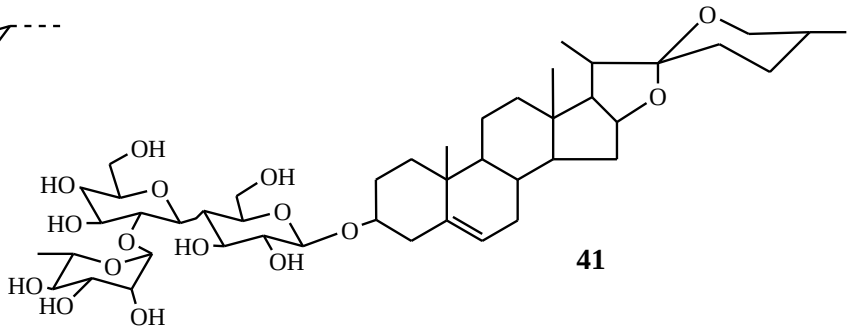
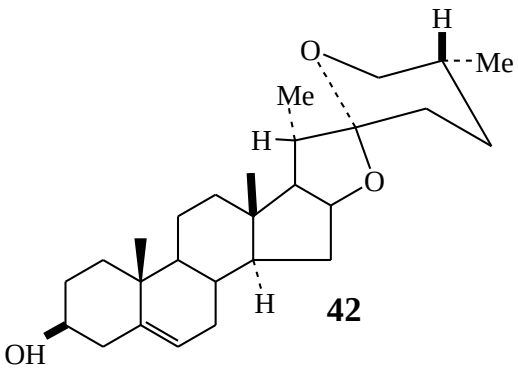
In addition to a known spirostanol glycoside, balanitin-3, and a new sapogenol, 6-methyldiosgenin (**39a,b**), a new furostanol saponin (**40**), and balanitoside (**41**) has been isolated from the fruits (mesocarp) of *Balanites aegyptiaca* (Kamel, 1998).







Related studies on the various plant parts of *Balanites aegyptiaca* revealed that the major sapogenins are Diosgenin (**42**), Yamogenin (**43**), Sarsapogenin (**44**) and Hecognin (**45**) .



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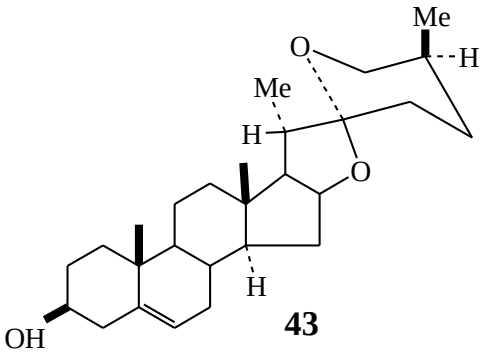

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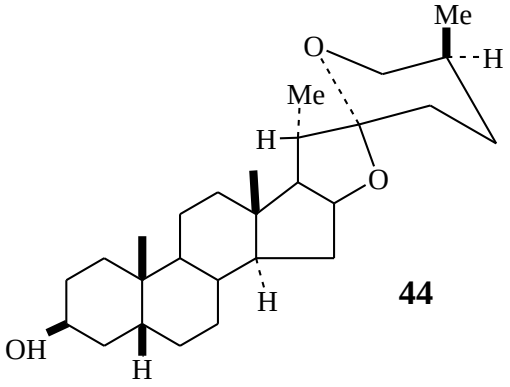

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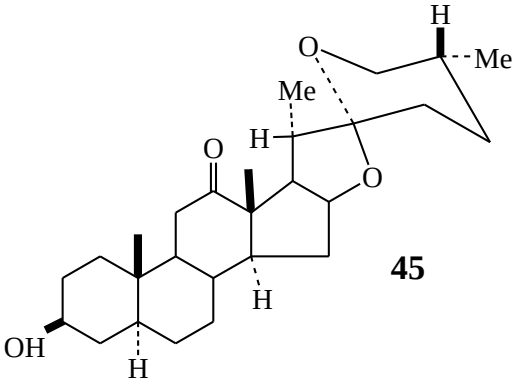

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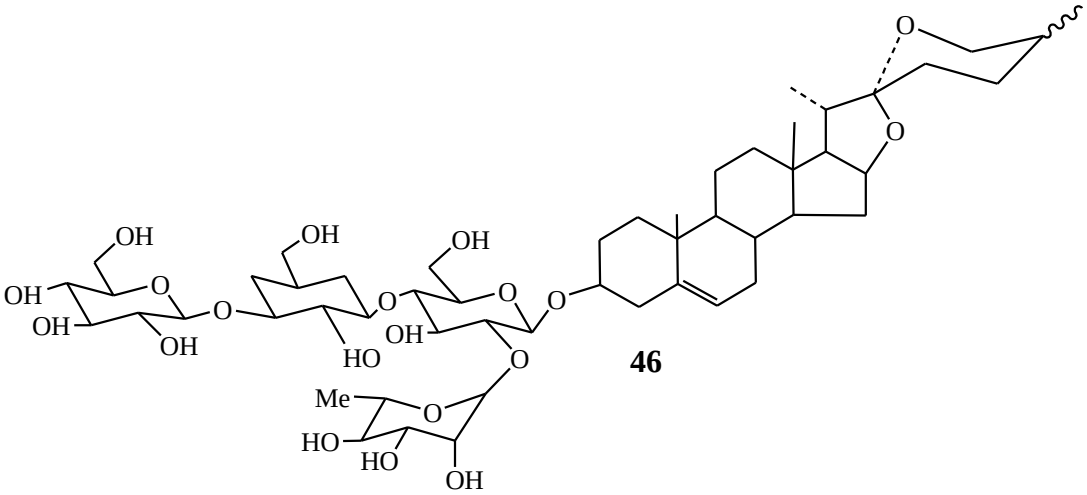
2.2.3.2 Chemical constituents of *Balanites roxburghii*

Phytochemical analysis of fruits, roots and barks contain of *Balanites roxburghii* revealed that the plant is rich in steroidal saponins (**46**) and exhibit spermicidal, cardiovascular and moluscicidal activities (Banerji et al., 1981).



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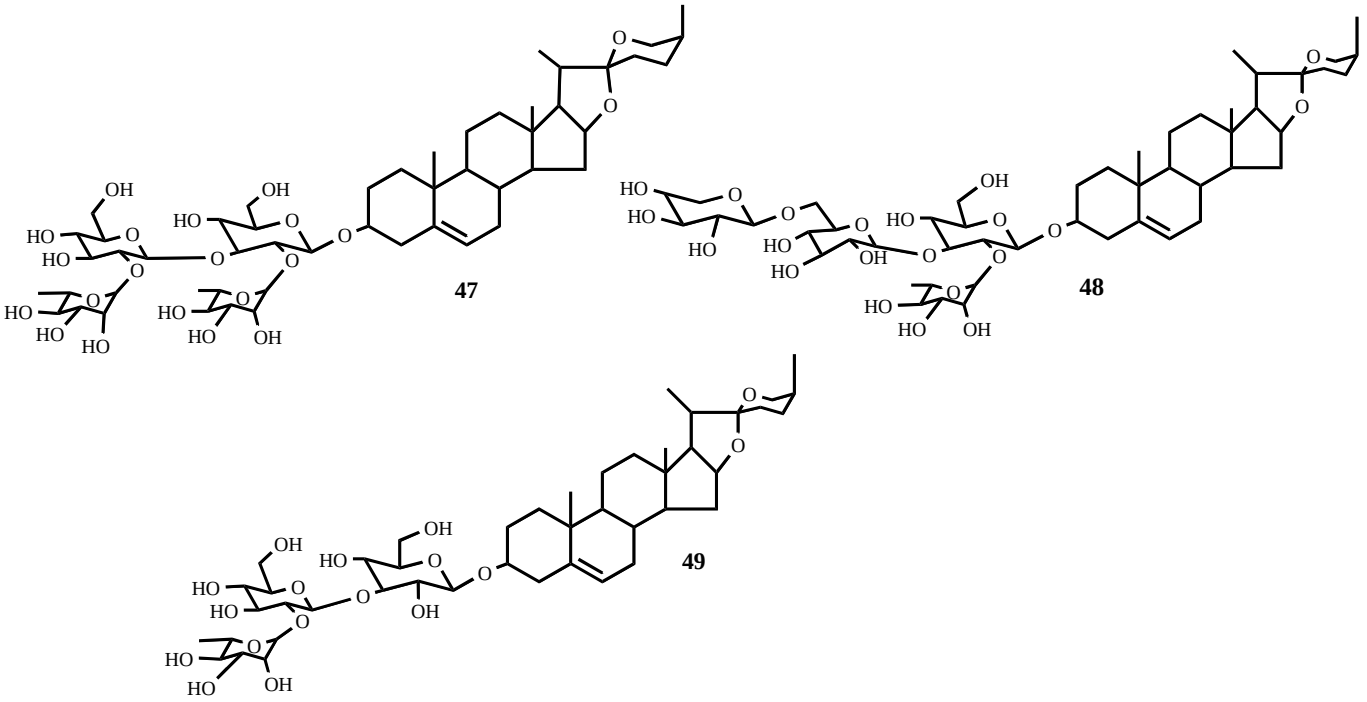
2.2.3.3 Chemical constituents of *Balanites maughamii*

Phytochemical analysis of the fruit of *Balanites maughamii* revealed the presence of balanitin-l (**47)**, balanitin-2 (**48**) and balanitin-3 (**49**). The fruit is used to control snail intermediate hosts of schistosomiasis as it possesses properties lethal to molluscs and *Bilharzial miracidia* and cercaria (Koko et al., 2005).



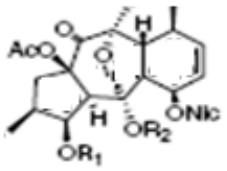
2.2.4 The chemical constituents of the genus *Euphorbia*

Several species of the genus *Euphorbia* have been tested for their efficiency as anti- viral and anti tumor agents, partly based on information concerning plants that have traditionally been used medication to treat various human diseases (Bernal & Correa 1990). So far various skin irritating and tumor-promoting diterpenoids were isolated from the genus having tigliane, ingenane, and daphnane skeletons (Ahmad et al, 2002). The latex contained euphorbic acid, euphorbine, proteins, tannins, volatile oils, alkaloids, resins, and other substances. Drying did not remove its toxicity. Euphorbic acid anhydride was the main toxic component of the latex, and had a locally irritant effect on the skin and on the epithelium of the alimentary, sometimes diarrhea with blood in the faeces, feeble heart-beat, and depression of the central nervous system. Poisoning may occur in almost all animal species (Degli Esposti et al. 1994).

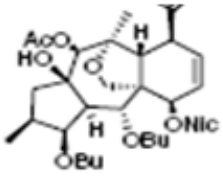
Research on inhibitors of the respiratory chain has revealed the existence of compounds with a high potential for basic biomedical research as possible antitumor agents (Degli Esposti *et al*. 1994, Zafra-Polo *et al*. 1996). Isodecipidone (**50**) and isodecipinone (**51**) inhibited (IC_{50} 21.8l M) prolyl endopeptidase (PEP). Alterations in the enzyme's levels cause different diseases such as Alzheimer's, depression, mania, thrombosis, HIV, and cancer (Ahmad et. al 2002). Diterpenoids with myrsinane skelton, ingenol-type and other related diterpenoids (**52-85**, table 3) has also been reported from the genus. In addition to diterpenoids, flavonoids (**86**), phenolics (**87, 88**), coumarins (**89, 90**), triterpenoids and steroids are also reported by several authors from the genus; see table 3 (Jassbi et.al, 2006), Ahmad et al., 2002).

Table 3. Summary of the chemical constituents of different *Euphorbia* species

Euphorbia species	Flavonoids and coumarins	Triterpenoids and steroids	Other constituents
E. larica	Kaempferol-3-O-glucoside (**50**), quercetin-3-O-glucoside (**51**), kaempferol-3-rutinoside (**52**), rutin (**53**), 6-methoxyapigenin (Ulubelen et al., 1983)	β-Amyrin acetate, lupeol, lupeol acetate, ginnone, ambrein, lupeone (Ulubelen et al., 1986)	Nonacosane (**58**), octacosyl behenate (**59**) (Ulubelen et al., 1986)
E. virgata	**54**, **55**, **56**, **57**, kaempferol (**58**) (Ulubelen et al., 1983)		
E. chamaesyce	**59**, **55** (Ulubelen et al., 1983)		
E. magalanta	**54**, **55**, **56**, **57**, **60** (Ulubelen et al., 1983)		
E. petiolata		Cycloartenol,24-methylenecycloartanol (Rustaiyan et al., 1982)	
E. falcata L.		Obtusifoldienol, c-euphorbol (**61**), bamyrin (**62**) (Aynehchi and Hakimzadeh, 1978)	**58**, octadecan-2-one, eicosan-2-one (Aynehchi and Hakimzadeh, 1978)
E. lanata	Kaempferol-7-O-rhamnoside, kaempferol-3-O-galactoside, quercetin-7-O-digalactoside, esculetin (Aynehchi et al., 1978)	Sitosteryl-3-b-D-glucoside (**63**) (Aynehchi et al., 1978)	Octacosanol (**64**), 48 (Aynehchi et al., 1978)
E. tinctoria	46, quercetin, quercetin-7-glucoside, kaempferol rhamnoside (Aynehchi and Ulubelen, 1974)	**61**, **63**, euphorbol (Aynehchi and Kiumehr, 1972, 1974, 1977)	**58**, **59**, **64** (Aynehchi and Kiumehr, 1972, 1974, 1977)
E. myrsinites		**62**, taraxerol (Aynehchi et al., 1972)	**58**, **64** (Aynehchi et al., 1972)



	R_1	R_2
50	Pr	Bu
51	Bu	Bu



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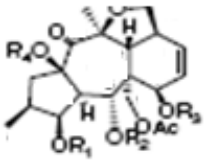
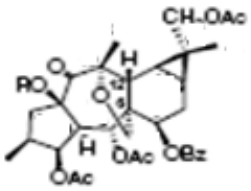
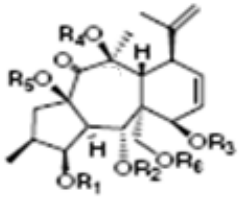


Compound	R_1	R_2	R_3	R_4
24	Ac	Bz	Ac	H
25	Ac	Bz	H	H
26	H	Bz	Ac	H
27	Ac	Bz	Ac	Ac
28	Bz	Ac	Ac	H
29	Ac	Ac	Bz	H
30	Ac	Ac	Bz	Ac
31	Ac	Ac	Nic	Ac

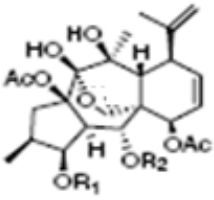


Compound	R
32	Ac
33	H



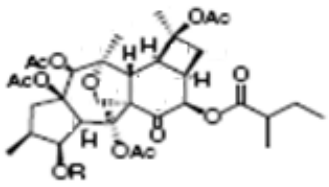
54-65

R_1	R_2	R_3	R_4	R_5	R_6
Ac	Bz	Ac	Ac	H	Ac
Ac	Bu	Ac	Ac	H	Ac
Ac	Bu	Ac	Ac	H	Nic
Ac	Bu	Ac	H	Ac	H
Ac	Bz	Ac	H	Ac	H
Ac	Bu	Ac	Ac	Ac	H
Ac	Bu	Ac	H	Ac	Ac
Ac	Bz	Ac	H	Ac	Ac
Ac	Ac	Bz	Ac	H	Ac
Ac	Ac	Bu	H	Ac	Ac
Ac	Bz	Ac	H	H	Ac
Ac	Bu	Ac	H	H	Ac

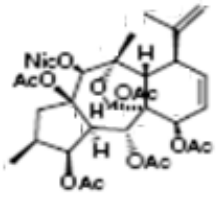


66-70

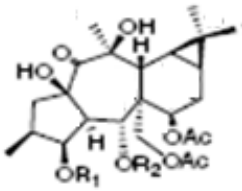
R_1	R_2
Ac	Bz
Ac	Bu
Bu	Nic
Bu	Bz
Ac	Nic



73-74

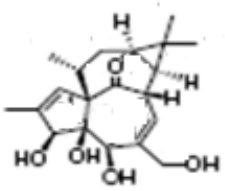


75

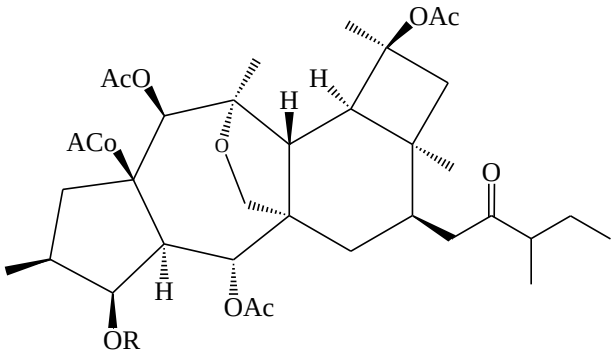


71-72

R_1	R_2
Ac	Bz
Ac	Bu

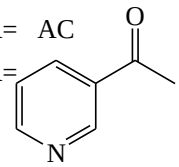


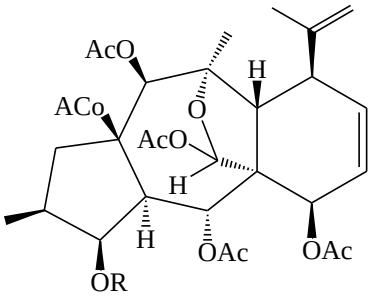
76



77 R= AC

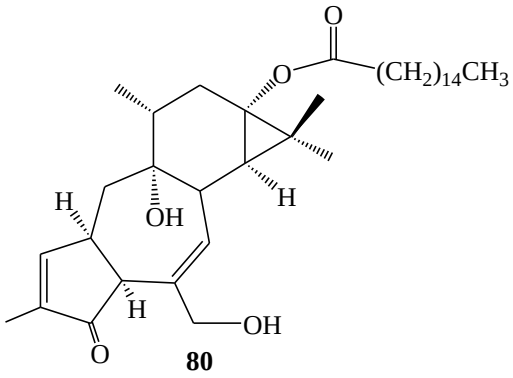
78 R=





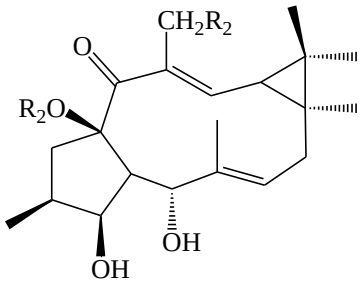
79

(Ahmed et al., 1999)



80

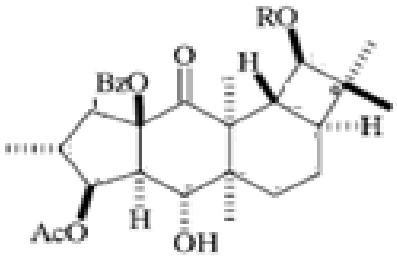
(Hamada et al., 2007)



81 R1= Cinnamoyl R2= H

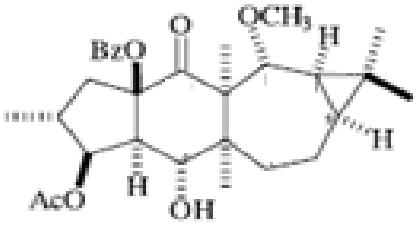
82 R1= Cinnamoyl R2= OH

(Huan et al, 2008)

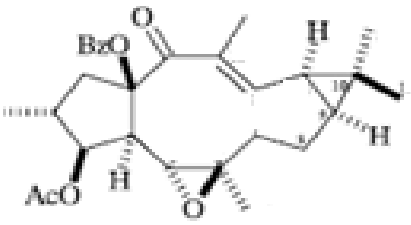


83a R = CH_3

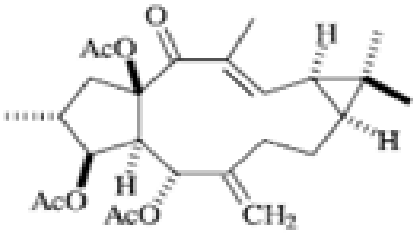
83b R = H



84

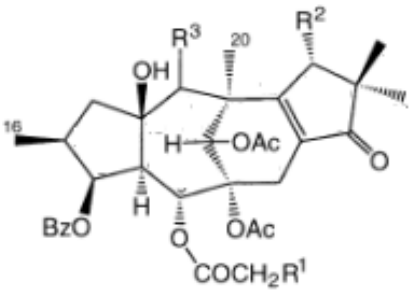


85

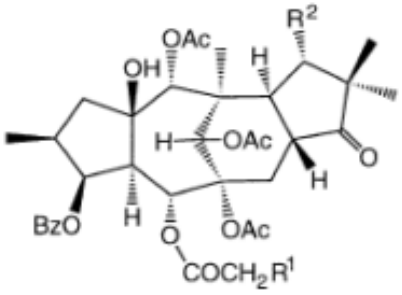


86

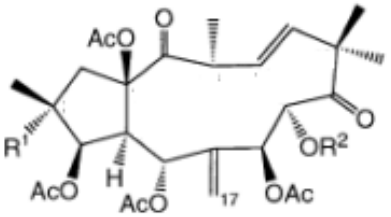
(Andrea et al., 2004)



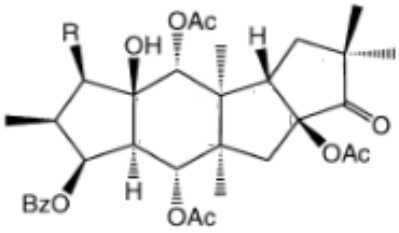
	87	88
R^1	H	OAc
R^2	OAc	H
R^3	β-OAc	α-OAc



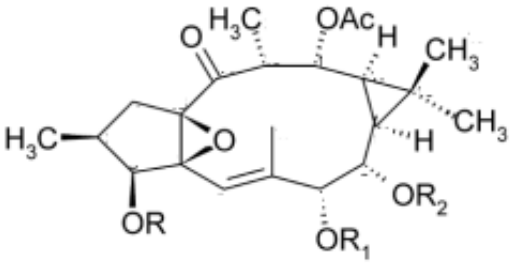
	89	90	91	92
R^1	H	OAc	OAc	OH
R^2	OAc	H	OAc	H



	93	94	95	96	97
R^1	OAc	OAc	H	OAc	OAc
R^2	iBu	Ang	iBu	MeBu	Bz



	98	99
R	OAc	H



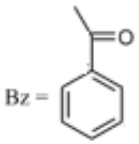
100 R=R_1=Ac; R_2=Bz

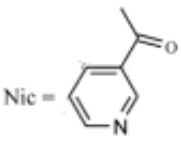
101 R=Ac; R_1=Ang; R_2=Me

102 R=H; R_1=Ang; R_2=Me;

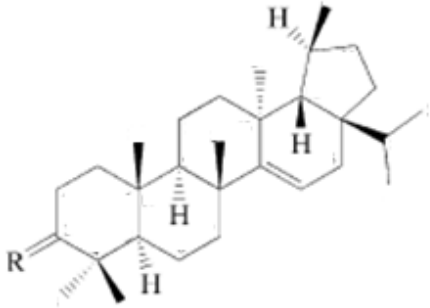
103 R=Ac; R_1=H; R_2=Bz

104 R=Ac; R_1=Bz; R_2=Nic

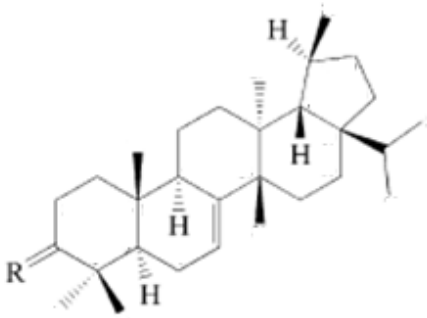




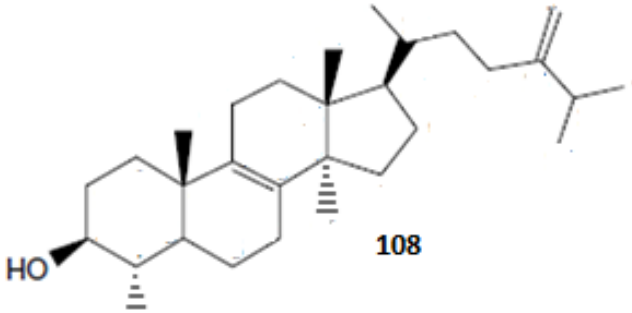
(Ravikantha et al., 2002)



106a R = α-H, β-OAc
106b R = O

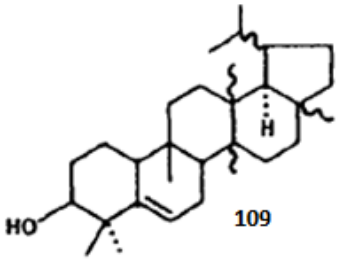


107a R = α-H, β-OAc
107b R = O

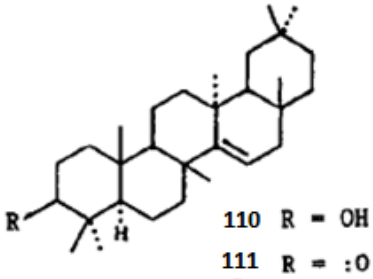


108

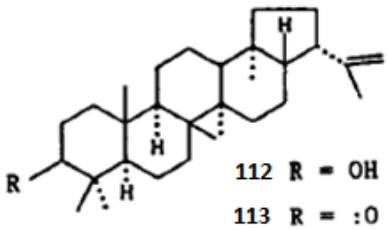
(Noureddine et al., 2008)



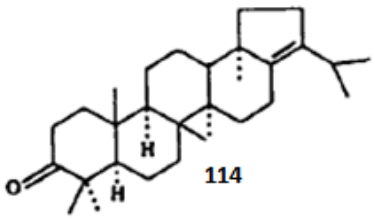
109



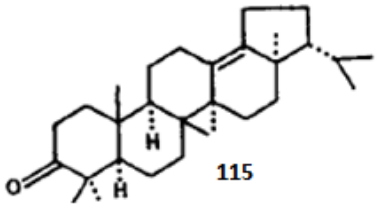
110 R = OH
111 R = :O



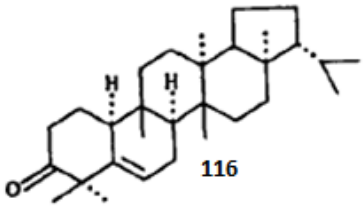
112 R = OH
113 R = :O



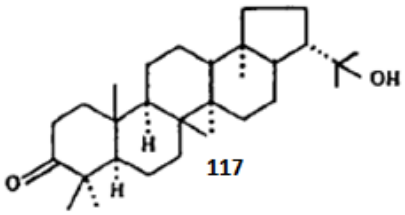
114



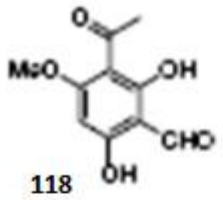
115



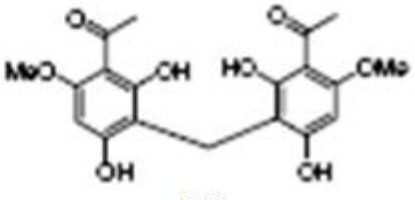
116



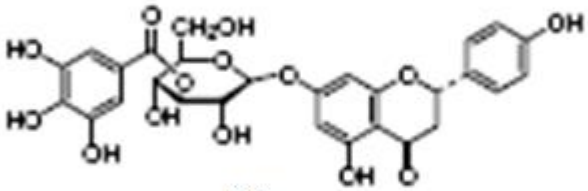
117



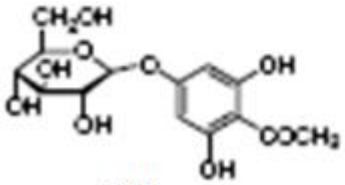
118



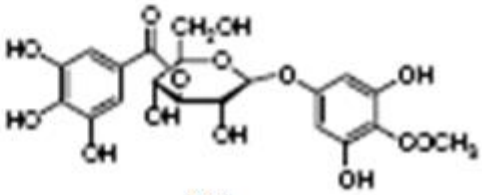
119



120



121



122

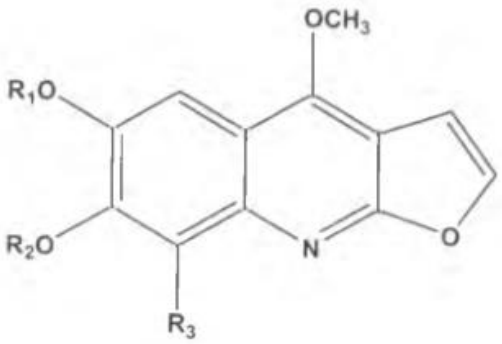
2.2.5 Chemical constituents of the genus *Teclea*

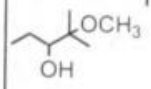
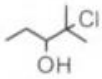
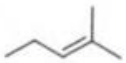
Phytochemical investigations on this genus have revealed the presence of quinoline and furoquinoline alkaloids, limonoids, tetranortriterpenes, triterpenes, alkaloids, and flavonoid glucosides from various species of the genus (Dagne et al., 1988). Some chlorinated compounds such as Chlorodesnkolbisine (**123**) have also been isolated from the aerial parts of *Teclea nobilis* (Al-Rehaily et al., 2002). These chlorinated compounds have not been reported from any other source, hence there is a possibility that the plant was not correctly identified or chlorination could be as the result of environmental factors.

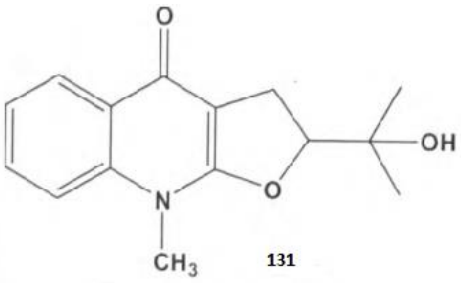
The genus *Teclea* is well known for alkaloids derived from anthranilic acid, limonoids, coumarins and triterpene derivative such as lupeol (**124**) (Waterman, 1973). The alkaloids found in this genus are classified into two: acridone and furoquinoline alkaloids. Furoquinolin**e** alkaloids are the most widespread type of alkaloid in the genus *Teclea* as well as the family Rutaceae. The list of Furoquinolin**e** alkaloids compounds isolated from *Teclea* and their respective reference is listed in Table 4 below.

Table 4. List of furanoalkaloids isolated from *Teclea nobilis*

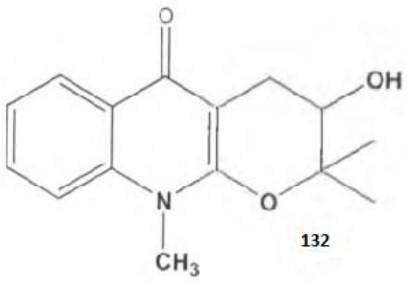
Alkaloid	**Plant source**	**Reference**
Tecleaoxine (**125**)	*T. nobilis* (Aerial part)	Al-Rehaily et al., 2002
Isotecleaoxine (**126**)	*T. nobilis* (Aerial part)	Al-Rehaily et al., 2003
Methylnkolbisine (**127**)	*T. nobilis* (Aerial part)	Al-Rehaily et al., 2003
Chlorodesnkolbisine (**128**)	*T. nobilis* (Aerial part)	Al-Rehaily et al., 2003
Nobiline (**129**)	*T. nobilis* (Aerial part)	Yenesew and Dagne, 1988
Kukusaginine (**130**)	*T. nobilis* (Aerial part)	Pusset et al., 1991
Isoplatydesmine (**131**)	*T. nobilis* (Leaf)	Yenesew and Dagne, 1988
Ribalinine (**132**)	*T. nobilis* (Leaf)	Yenesew and Dagne, 1988
Edulinine (**133**)	*T. nobilis* (Leaf)	Higa et al., 1974
Haplopine-3,3'-dimethylallyl ether (**134**)	*T. nobilis* (Aerial part)	Bessonova et al., 1974
Anhydroevoxine (**135**)	*T. nobilis* (Aerial part)	Bessonova et al., 1982
8-methoxyflindersine (**136**)	*T. nobilis* (Aerial part)	Campbell et al., 1990
Montrifoline (**137**)	*T. nobilis* (Leaf, Fruit)	Ayafor et al., 1982
Skimmianine (**138**)	*T. nobilis* (Leaf, Fruit)	Al-shama et al., 1979
Flindersiamine (**139**)	*T. nobilis* (Leaf, Fruit)	Ayafor et al., 1982
Maculine (**140**)	*T. nobilis* (Leaf, Fruit)	Fish et al., 1976



	R_1	R_2	R_3
125		CH_3	H
126	CH_3		H
127		CH_3	H
128		CH_3	H
129	CH_3		H
130	CH_3	CH_3	H



131



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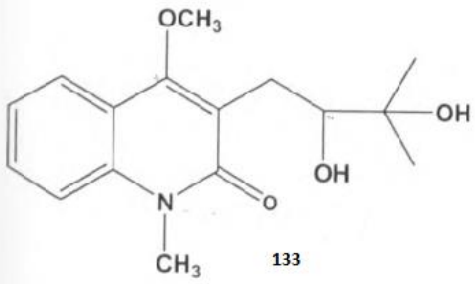
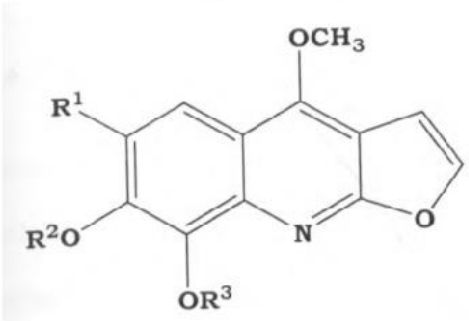
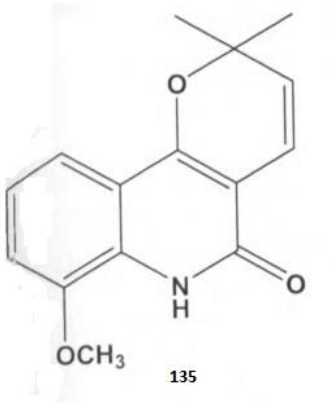


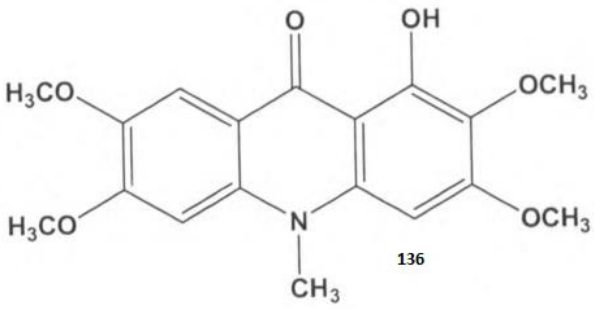
133



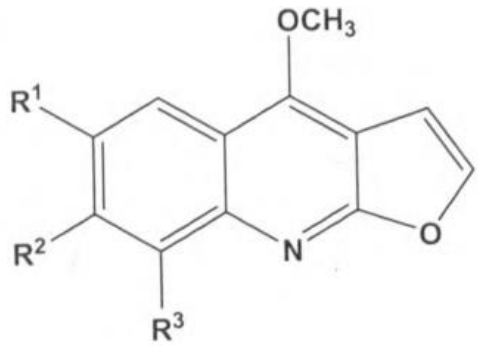
	R^1	R^2	R^3
133	H	Prenyl	CH_3
134	H		CH_3



135



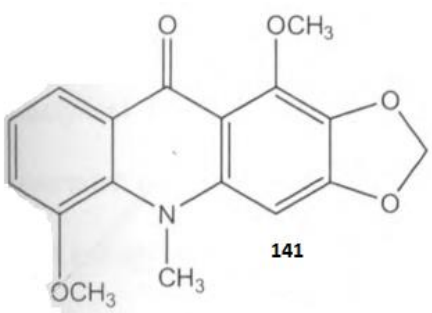
136



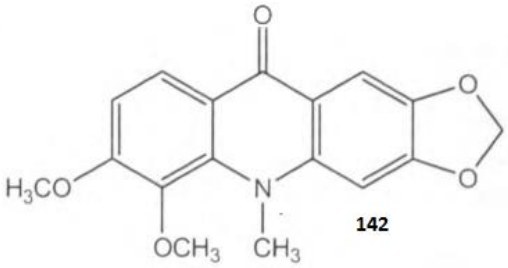
	R^1	R^2	R^3
137	$(Me)_2COHCHOHCH_2O$-	OMe	H
138	H	OMe	OMe
139	H OCH_2O	OMe	OMe
140	H OCH_2O	OMe	H

Acridone alkaloids of the genus *Teclea*

Acridone alkaloids (**141**-**144**) are found in natural plant sources, particularly plants belonging to Rutaceae, including *Teclea bolvinione* and *Teclea nobilis* species (Bergenthal et al., 1979; Vaquette 1978).



141



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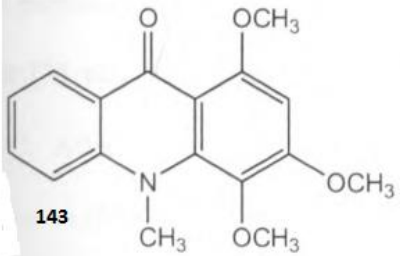

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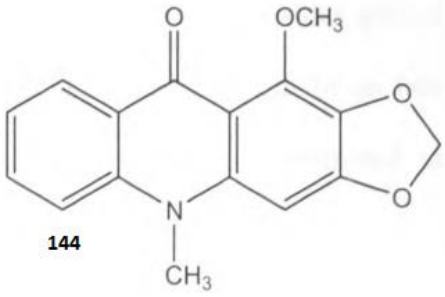

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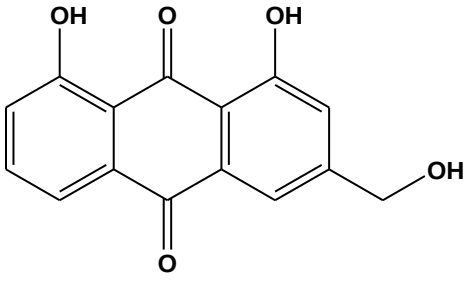
2.2.6 Chemical constituents of the genus *Kniphofia*

The genus *Kniphofia* is a rich source of monomeric and dimeric anthraquinones, anthrones, phenylanthraquinones and oxanthrones. The sources of these compounds are summarized in Table 5 below.

Table 5. List of compound isolated from the genus *Kniphofia*

Compounds	**Species**	**Reference**
Monomeric anthraquinones		
Aloe-Emodin **(145)**	*K. foliosa (leaves, flowers, fruits)*	Berhanu et al., 1986
	K. insignis (flowers)	
	K. isoetifolia (flowers)	
	K. schimperi (Flowers)	
	K. thomsonii (root)	Achieng, 2009
Aloe-Emodin acetate **(146)**	*K. foliosa (leaves)*	Berhanu et al., 1986
	K. foliosa (leaves, flowers, fruits)	
	K. isoetifolia (flowers)	
	K. thomsonii (root)	Achiene, 2009
Chrysopanic acid **(147)**	*K. foliosa* (Leaves)	Berhanu and Dagne, 1984; Berhanu *etal.*, 1986
	K. isoetifolia (Flowers, Leaves, Rhizomes)	Berhanu *et al.*, 1986
Dimeric anthraquinones		
Asphodelin **(148)**	K. albescens (Root)	Van Wyk *et al.*, 1995
Chrysalodin **(149)**	*K. foliosa* (Leaves)	Dagne *et al.*, 1987
Knipholone **(150)**	*K. ins ignis* (Rhizomes) *K. isoetifolia* (Rhizomes) *K. pumila* (Rhizomes) *K. schimperi* (Rhizomes	Berhanu *et al.*, 1985

	K foliosa (Rhizomes)	
10-Hydroxy-10-(chrysophanol-7'-yl)-chrysophanol anthrone **(151)**	*K.foliosa* (Root) *K. thomsonii* (Root)	Wube *et al.*, 2005 Achieng', 2009
10-Bichrysophanolanthrone **(152)**	*K. thomsonii* (Root)	Achieng', 2009
10-Hydroxy-10-(chrysophanol-7'-yl)-aloe-emodin anthrone **(153)**	*K thomsonii* (Root)	Achieng', 2009
10-hydroxy-10-(islandicin-7'-yl)-aloe-emodin anthrone **(154)**	*K. thomsonii* (Root)	Achieng', 2009
Phenyl anthraquinones and anthrones		
Isoknipholone **(155)**	*K.foliosa* (Stem)	Yenesew *et al.*, 1994
Isoknipholone anthrone **(156)**	*K.foliosa* (Stem)	Yenesew *et al.*, 1994
Knipholone anthrone **(157)**	*K. foliosa* (Stem)	Dagne and Yenesew (1993); Yenesew *et al.*, 1994
Oxanthrones		
Foliosone **(158)**	*K.foliosa* (Stem)	Yenesew *et al.*, 1994
Isofoliosone **(159)**		



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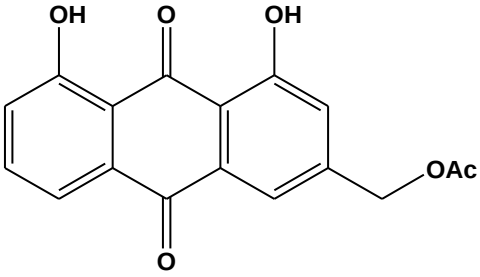

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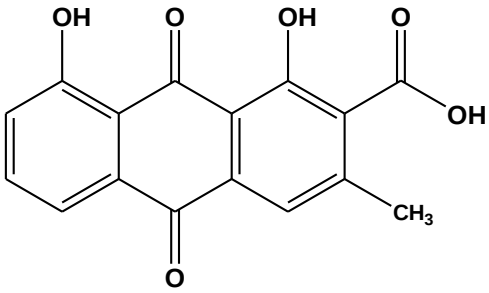

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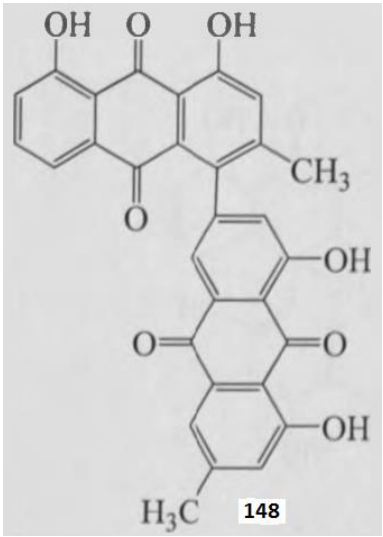

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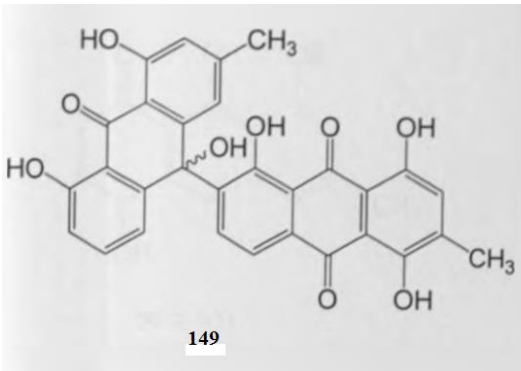

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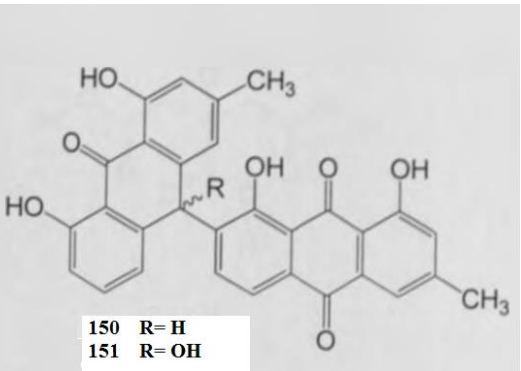
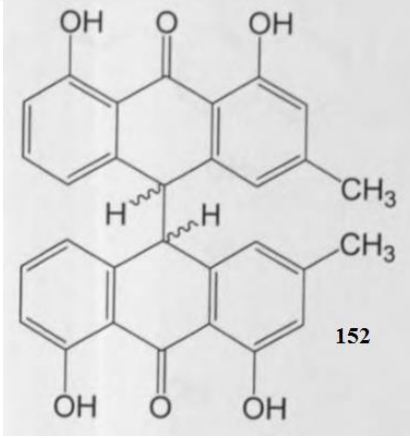


150 R= H
151 R= OH



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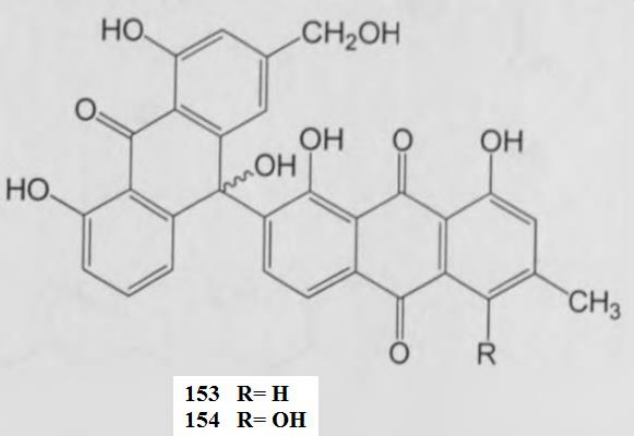
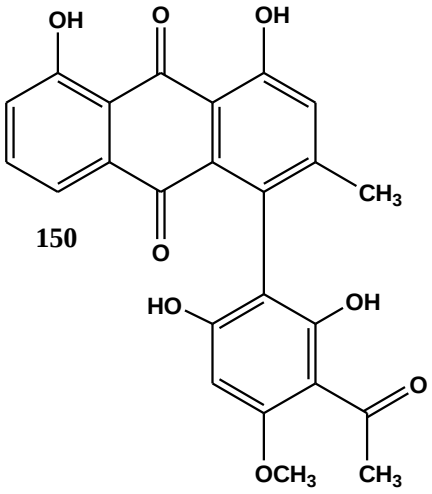


153 R= H
154 R= OH



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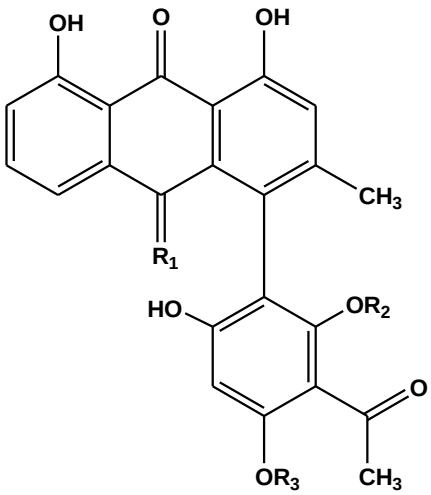
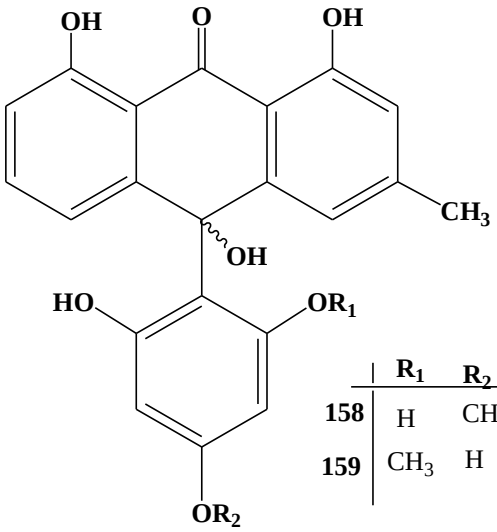
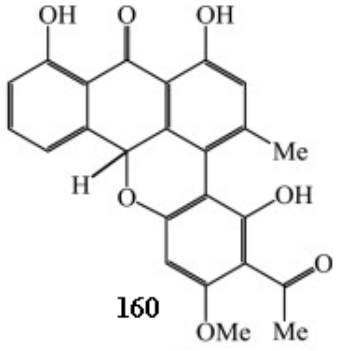


	R1	R2	R3
155	O	CH_3	H
156	H,H	CH_3	H
157	H,H	H	CH_3



	R1	R2
158	H	CH_3
159	CH_3	H

Extracts of the rhizomes of *Kniphofia foliosa* exhibited antiplasmodial activities against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of Plasmodium falciparum with IC_{50} values of 3-5 µg/mL. A phenyloxanthrone, named 10-acetonylknipholone cyclooxanthrone (**160**) was isolated from the rhizomes, along with known quinones, including the rare phenylanthraquinone dimers, joziknipholones A and B (Induli et al., 2013).



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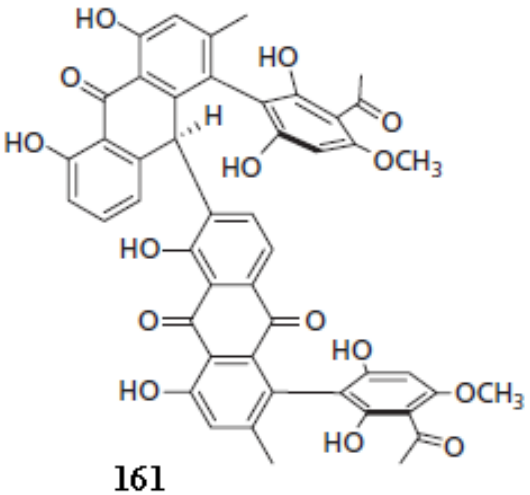

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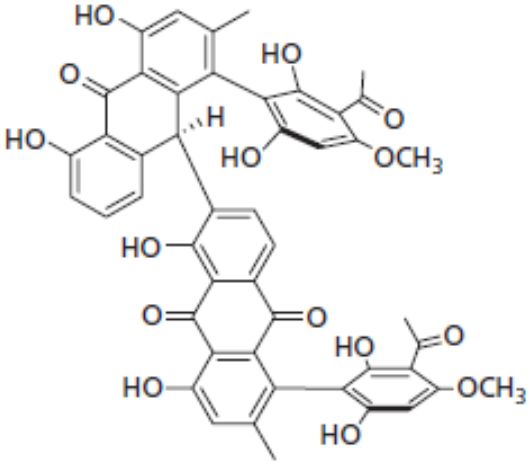

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2.2.7 Chemical constituents of *Clausena anisata*

The plant has been reported to contain coumarins, limonoids, carbazole alkaloids, monoterpenoids furanocoumarin lactones and essential oils (Hutchings et al., 1996). 1-methyl-3, 4-dimethoxy-2-quinolone, 3-formyl-1-hydroxy carbazole, heptaphylline, girinimbine, and ekeberginine were isolated from essential of clausena anisata (Hutchings et al., 1996).

2.3 Biological activity of selected medicinal plants

2.3.1 Biological activity of the genus *Kniphofia*

Some of the compounds isolated from *Kniphofia foliosa* have shown promising antimalarial activity. The diverse biological activity of the compounds isolated is summarized in Table 6.

Table 6. Biological activity of the genus *Kniphofia*

Biological activity	**Compound**	**References**
Antioxidant activity	Knipholone anthrone (**157**)	Habtemariam, 2007; Bringmann et al., 2008
Antiprotozoal Activity and radical Scavenging activity	Knipholone anthrone (**157**)	Habtemariam, 2007
Antitumor Activity	Knipholone (**150**) and Isoknipholone (**155**)	Bringmann et al., 2008
Antimalarial	Isoknipholone (**155**), Knipholone anthrone (**157**)	
Inhibition of leukotriene (Treatment of Asthema and Inflamatory Diseases)	Knipholone (**150**)	Wube et al., 2006

2.3.2 Biological activity of the genus *Teclea*

Antipyretic and analgesic activities of the ethanol extract of *Teclea nobilis* have been reported (Mascolo *et al*, 1988). Further pharmacological studies indicated that quinoline alkaloids are responsible for the observed analgesic and antipyretic activities of this plant (Yenesew and Dagne, 1988). Antifungal, antibacterial and *in vitro* anti-plasmodial activities of *Teclea trichocarpa* have also been reported (Lwande *at al.*, 1983).

2.3.3 Biological activity of the genus *Balanites*

Balanites aegyptiaca possess antioxidant, antimicrobial, anticancer, diuretic, hypocholesterolemic, wound-healing, antiviral, antidiabetic, hepatoprotective, mosquito larvicidal, anti-inflammatory and analgesic, antivenin, anthelmintic, cardioprotective, antioxidant activity, and antinociceptive properties. Bark, fruits, seeds, seed oil, and leaves of this plant are widely used in folk medicine. Emphasis of research has been on utilizing traditional medicines that have long and proven history of treating various ailments. So, further studies need to be carried out to explore *Balanites aegyptiaca* for its potential in curing and treating disease (Banerji et al., 1981).

2.3.4 Biological activity of the genus *Tephrosia*

Tephorsia genus have been reported to have hepatoprotective activity (Shah et al., 2011), antidiabetic activity (Bhadada and Goyat, 2016), antiinflammatory activity (Hassen et al., 2016), wound healing activity (Lodhi et al., 2016), antioxidant activity (Mani et al., 2017), antiulcer activity (Divya et al., 2011), anticancer activity (Hassen et al., 2017), antifungal activity (Luo et al., 2015) and antibacterial activity (Ramadevi et al., 2014).

2.3.5 Biological activity of the genus *Zanthoxylum*

Zanthoxylum genus have been reported to have larvicidal activity (Trongtokit et al., 2011), antimicrobial activity (Tangjitjaroenkun et al., 2012a), antioxidant activity (Tangjitjaroenkun et al., 2012b), and antitumor activity (Murakami et al., 1995).

2.3.5 Biological activity of the genus *Clausenea*

The genus *Clausenea* have been used widely in various parts of Africa for the treatment of bacterial and fungal infections of the skin including boils, ringworm, oral thrush and eczema (Hamza et al., 2006) and malaria (Uwaifo et al., 1984).

2.4 Significance and Beneficiaries

We expect this work to have vast impact for several reasons

(**a**) No detailed, systematic characterization of the chemical constituents selected medicinal plants has been performed. Since these plants are still being used by the local peoples, our

study will preserve and systematize the experience collected from elders in the society and also validate the traditional uses of the plants through biological assay screening

(**b**) Following the identification of efficient traditional medicines, protocols for their preparation and local validation was developed. Such protocols are necessary as the relative concentration of the chemical constituents of herbs is dependent on numerous factors, such as the season, altitude and geography of plant collection.

(**c**) Classification of efficient remedies and establishment of their standard preparation will support the local cottage industry and thereby assist the economic advancement of the area.

(**d**) The thorough analysis of active traditional medicine preparations and their chemical constituents is expected to provide novel lead compounds for rational development of new antioxidant and antibacterial drugs.

2.5 Expected output and beneficiaries

The medicinal property of the plants is due to the presence of substances such as alkaloids, glycosides, resins, terpenoids, tannins, etc. The expected outcome of this research is identifying the chemical constituents of the selected medicinal plants and conduct screening tests of the crudes as well as the isolated compounds which has not been documented yet for the selected plants. Therefore, it is expected that the information and knowledge gained from this research studies will increase the awareness of using this traditional plant, identify their constituents and validate their traditional use. Moreover, as part of the project at least three MSc students and one PhD student were sponsored as co-researchers.

3. Methodology

3.1 Instruments

Column chromatography was performed on normal and deactivated (by oxalic acid impregnation) silica gel. Thin Layer Chromatography (TLC) was done using silica gel 60 F254 (Merck) precoated plates. NMR analysis was carried out on Bruker avance 400 MHz spectrometer. Structural assignment was performed based on COSY, gTOCSY, gNOESY, gHSQC, and gHMBC. UV-Vis spectra was obtained in CH_3OH using a Hewlett-Packard 8453 spectrophotometer.

3.2 Plant materials collection and identification

The roots of *Teclea nobilis* were collected in February 2018 from Sidama zone around yirgalem town in manche kebele, Godimo place 60 km away from Hawassa town. Roots of *C. anisata* were collected from the Oromia region, west Wollega, March 2018. Roots of *K. schimperiana* and *E. shimperiana* were collected from Oromia region, Bale Zone around Goba town specifically Fasil Angaso kebele, Nov., 2017. Roots of *Tephorosia vogelli* were collected from Welayita sodo near, March, 2018. The plant was identified by Mr. Shambel Alemu and a voucher specimen was kept at the National Herbarium, Addis Ababa University, Ethiopia.

3.3 Extraction and isolation

3.3.1 Extraction and isolation from roots of *Teclea nobilis*

The air-dried grounded roots (500 g) of *Teclea nobilis* was soaked in dichloromethane/methanol (1:1, 2L) for 72hrs at room temperature. The mixture was filtered and concentrated under reduced pressure at a temperature of 40°C using rotary evaporator to give 11.8 g crude extract. The marc left was further extracted with methanol (2 L) for 72hr, filtered and concentrated in rotary evaporator to furnish 10.6 g crude extract. The dichloromethane/methanol (1:1) extract (6 g) was subjected to silica gel column chromatography and eluted with increasing gradient of ethyl acetate in *n*-hexane. A total of 51 fractions were collected. Fraction 18 (eluted with 40% EtOAc) showed single spot which was repeatedly washed with *n*-hexane to give compound **163** (18 mg). Fraction 6 (eluted with 20 % EtOAc) afforded compound **164** 5 (13 mg). Similarly, the methanol extract (5 g) was

subjected to silica gel column chromatography and eluted with increasing gradient of ethyl acetate in *n*-hexane. A total of 35 fractions were collected. Fraction 6 of methanol extract (eluted with 20 % EtOAc) yielded compound **165** (8 mg).

3.3.2. Extraction and isolation of compounds from roots of *K. schimperiana*

The grounded roots of *K. schimperiana* (500 g) was soaked for 72hr in dichloromethane/methanol (1:1), filtered and concentrated by using rotary evaporator to yield 40.52g (8.104%) crude extract. The mark left was further extracted by methanol, filtered and concentrated by rotary evaporator and yielded 46.87g (9.374%). The dichloromethane/methanol (1:1) roots extract (12 g) of *K. schmperiana* was subjected to silica gel column chromatography on oxalic acid impregnated silica gel (150 g), 250 g silica gel deactivated with 0.75 g of oxalic acid in one liter distilled water, Merck 60 H 230-400 Mesh) and eluted with increasing gradient of ethyl acetate in *n*-hexane followed by increasing gradient of methanol in dichloromethane. A total of 136 fractions (100 mL each) were collected. Fractions 99-100 (two spots) were combined, dried and washed successively with *n*-hexane to give compound **166** and knipholone derivative (**167**, 15 mg). Methanol crude extract (12 g) of *K. schmperiana* was subjected to silica gel column chromatography on oxalic acid impregnated silica gel (150 g) and a total 106 fractions (100 mL each) were collected. Fractions 64-69 were combined and repurified by silica gel column chromatography. A total of 15 fractions (15mL each) were collected of which fractions 7-9 were combined to afford compound (**168**, 18 mg).

3.3.3. Extraction and isolation of compounds from roots of *Balanite aegyptiaca*

The dichloromethane/methanol (1:1) extracts of the roots *Balanite aegyptiaca* afforded 30 g (6 %). Dichlormethane/methanol (1:1) crude extract (10 g) was subjected to silica column chromatography separation of (250 g silica gel, 230-400 mesh) and elution was carried out with increasing gradient of ethyl acetate in n-hexane. Fraction 60-65 (10 % methanol in dichloromethane) were combined, dried and washed with n-hexane to afford compound **176** (12 mg).

3.3.4 Extraction and isolation of compounds from roots of *Zanthoxylum chalybeum*

The root powder (400 g) *Z. chalybeum* was extracted by maceration with ethanol (EtOH, 1.5 L). The extract was concentrated under reduced pressure to obtain a crude residue (29g, 7.3 %). The dried ethanol extract defated by *n*-hexane. The alkaloidal constituents were extracted by acid-base extraction as previously described by Alexander (2005). The defatted Ethanolic crude extract was suspended in 5 % HCl to pH 5. Then pre-saturate with distilled water and partition by adding chloroform, the extract was separated in to two layers, aqua solution layer and organic solution layer. Then aqua and organic layers were separated by using separatory funnel and repeat for three times (extract 1). The acidic aqueous phases was basified by adding 5% NH_3 to pH 11, and then partitioned by chloroform. Then aqua and organic layers were separated by using separatory funnel and repeated for three times. Then chloroform extracts were combined, and concentrated the solvent under vacuum rotary evaporator to obtain alkaloid extract(extract 2). About 5.6g of extract 1 was subjected to silica gel column chromatography (60–120 mesh, 200g silica gel), and eluted with increasing gradient of methanol in chloroform (9:1, 8:2, 7:3, 6:4, 1:1, 4:6, 3:7, 7:1, 2:8, 1:9 and MeOH) to afford 22 fractions (each 50 mL). Fr.3-4 which showed two spots were combined on the basis of TLC profile and washed with *n*-hexane to afford pale yellow powder **177** (22 mg), **178** (28 mg).

Essential oil extraction

Air dried fruits of *Z. chalybeum* (250 g) in 2500 mL were distilled in water using hydro distillation in a Clevenger apparatus for two hours. On heating the flask, essential oil glands present in the plant material get ruptured. The steam and essential oil were vapours generated in the flask and passed through a condenser which finally converted the vapours into liquid. The condensate (mixture of essential oil and water) were collected in a 100 mL separatory funnel. Since, the water and essential oil have different densities, essential oil floats on the surface of the water in the 100 mL separators funnel. Then essential oils were separated from aqueous layer using separatory funnel. The separated essential oils for further was dried by anhydrous magnesium sulphate and filtered the solution, then the essential oil transferred into small vials, labeled and kept in the refrigerator until GC-MS analysis.

3.3.5 Extraction and isolation of compounds from of roots of *Clausenea anisata*

The pulverized powders (500 g) were soaked with dichloromethane/methanol (1:1) for 72 hr with occasional shaking. The extract was filtered and concentrated using rotary evaporator at 40^{o}C to give black crude 8.7 g (1.74 %, yield). Crude extract (8.5 g) was adsorbed on equal amount of silica gel and subjected to silica gel column chromatographic separation (150 g silica gel) and eluted with increasing gradient of ethyl acetate in *n*-hexane. A total of 65 fractions were collected. Fractions that showed similar R_f values and the same characteristic colour on TLC were combined. Fractions 11-12 showed yellow spot under UV light having the R_f value of 0.56 in *n*-hexane/ethyl acetate (8:2) solvent system. After concentrating, the solid material left was repeatedly washed with *n*-hexane to yield compound **179**. Fractions 19-25 were combined since all fractions showed single spot and similar R_f values. After concentrating, the solid was washed repeatedly with *n*-hexane to afford compound **180**.Fraction 29-34(16 mg) were combined on the basis of TLC profile and showed a single red coloured spot on TLC using *n*-hexane: EtOAc (6:4) as a mobile phase. After concentrating, the solid was washed repeatedly with *n*-hexane to afford compound **181**. Fractions 46-50 (27 mg) were combined on the basis of TLC profile and showed one spot on TLC using *n*-hexane: EtOAc (5:5) as eluent. After concentrating, the solid was washed repeatedly with *n*-hexane to afford compound **182**.

3.3.6 Extraction and isolation of compounds from roots of *Euphorbia schimperiana*

3.3.6.1 General extraction

The grounded roots of *E. schimperiana* (800 g) was soaked for 72 hr in dichloromethane/methanol (1:1), filtered and concentrated by using rotary evaporator to yield 16.52 g (5.51 %) crude extract. The mark left was further extracted by methanol, filtered and concentrated by rotary evaporator and yielded 6.87 g (1.374 %).

3.3.6.2 Acid-base extraction for alkaloid constituents

Alkaloid constituents was selectively extracted in acid-base extraction approach as previously described by Sarker, et al. 2006. The marc of CH_2Cl_2/CH_3OH (1:1) root extract of *E. shemperiana* (300 g) were extracted with1.5 L of 99.7 % ethanol at room temperature for three times while shaking by electronic shaker at speed of 3000 r/min at room temperature. The solution was filtered by suction filtration, and then solvent wasevaporated by rotary evaporator to yield crude alkaloids mixtures. The ethanol extract was further defatted by *n*-

hexane for three times. The defatted ethanolic crude extracts were suspended in 5 % HCl to pH 5. Then pre-saturate with distilled water and partition by adding chloroform separately. The extract was separated in two layers, aqueous solution layer and organic solution layer (chloroform extract). Then aqueous and organic layers were separated by separatory funnel and repeat it for three times. Then chloroform extracts was combined, and concentrated by using rotary evaporator to obtain 3 g extracts.

3.3.6.3 Isolation of compounds from root extract of *E. shimperiana*

About 15 g of chloroform/methanol (1:1) extract was subjected to column chromatography. The elution of fractions was started from 100 % *n*-hexane, and increasing polarity by adding EtOAc in 5 % ratio, followed by increasing gradient of chloroform in EtOAc. Finally, increasing gradient ratio of methanol in chlorofoem was used (99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10). Totally 51 fractions (5 mL) were collected. The fractions were checked TLC profile.

Table 11: Fractionations of chloroform/methanol (1:1) extract *E. shimperiana*

Fraction number	**Eluting solvent system**	**TLC mobile phase**	**remark**	
1.	100% n-hexane			
2.	95:5(n-hexane in Ethyl acetate)			
3.	90:10	7:3 (hex: EtoAc)		
4.	85:15		MAE-01	
5.	80:20	1:1(hex:EtoAC)		
6.	75:25			
7.	70:30			
8.	65:35		MAE-2	
9.	60:40			
10.	55:45			
11.	1:1		MAE-3	
12.	45:55			
13.	40:60			
14.	35:65			
15.	30:70			
16.	25:75			
17.	20:80			
18.	15:85			
19.	10:90		1 95:5(n-hexane in Ethyl acetate)	
			2 90:10	
20.	05:95		3 85:15	
			4 80:20	MAE-4
21.	100% Ethyl acetate		5 75:25	
			6 70:30	
22.	95:05 Ethyl acetate: Chloroform		7 65:35	
			8 60:40	
23.	90:10		9 55:45	
			10 1:1	

24.	85:15		11 45:55	
			12 40:60	
			13 35:65	
25.	80:20		14 30:70	
			15 25:75	MAE-5
26.	75:25		16 20:80	
			17 95:5(*n*-hexane in Ethyl acetate)	
27.	70:30			
28.	65:35			
29.	60:40			
30.	55:45			
31.	1:1			
32.	100% Chloroform			
33.	99:1 Chloroform : MeOH			
34.	98:2			
35.	97:3			
36.	96:4			
37.	95:5			
38.	94:6			
39.	93:7			
40.	92:8			
41.	91:9			
42.	90:10		Compound **185**	
43.	80:20			
44.	70:30			
45.	60:40			
46.	1:1			
47.	40:60			
48.	30:70			
49.	20:80			
50.	10:90			
51.	100 % MeOH			

Fraction 42-44 showed singlet spot on TLC (40:60, *n*-hexane in EtOAc mobile phase) and labeled as compound **185**.

3.3.6.4 Isolation of alkaloids from acid base crude extract of *E. shimperiana*

About 3 g of crude extract one which was explained under section of extraction (acid base extraction) above was subjected to silica gel (60-120) mesh column chromatography. The elution was started in 100 % chloroform by increasing polarity by 5 % up to 100% of MeOH. Totally 21 fractions (50 mL) were collected. Fraction 4-7 and fractions 16-17 were shown single spots while checking TLC profile, and then the fractions were combined and labeled as compound **183** and **184** (table 12). Finally they were put in the deep freezer at 4 ^{0}C until spectroscopic study. The compounds were tested by Mayers' and Wagners' reagents, and showed positive resut suggesting an alkaloid secondary metabolite.

Table 12: Fractionations extract one of root of *E. shimperiana*

Fractions number	Eluting solvent system	TLC mobile phase	remark
1.	100% chloroform	99.5:0.5 ($CHCl_3$:MeOH)	
2.	95:5(chloroform MeOH)	‘’	
3.	90:10 »	‘’	
4.	85:15 »	‘’	Compound **183**
5.	80:20 »	‘’	
6.	75:25 »	‘’	
7.	70:30	‘’	
8.	65:35 »	‘’	
9.	60:40	‘’	
10.	55:45	‘’	
11.	1:1	‘’	
12.	45:55	‘’	
13.	40:60 »	‘’	
14.	35:65	‘’	
15.	30:70	‘’	Compound **184**
16.	25:75	‘’	
17.	20:80	‘’	
18.	15:85	‘’	
19.	10:90	‘’	
20.	5:95 »	‘’	
21.	100 (MeOH)	‘’	

3.3.7 Extraction and isolation of compounds from *Tephrosia vogelii*

The grounded roots of *T. vogelii* (500 g) was soaked for 72hr in dichloromethane/methanol (1:1), filtered and concentrated by using rotary evaporator to yield 14.53 g (2.9%) crude extract. The mark left was further extracted by methanol, filtered and concentrated by rotary evaporator and yielded 6.51 g (1.3 %). Silica gel column chromatographic separation using increasing gradient of ethyl acetate in *n*-hexane followed by methanol in dichloromethane afforded seven compounds (**169-175**).

3.4 Qualitative analysis of the chemical constituents of crude extracts

Phytochemical examinations were carried out for all the extracts as per the following standard methods.

3.4.1 Detection of alkaloids: Extracts (0.1 g) dissolved individually in dilute Hydrochloric acid and filtered.

Dragendroff’s Test: Filtrate (20 mL) was treated with Dragendroff’s reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate confirms the presence of alkaloids.

3.4.2 Detection of glycosides: The extract (100 mg) was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified borntrager's test: The extracts (20 mg) was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer confirms the presence of anthranol glycosides.

3.4.3 Legal's test

The extract (20 mg) was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour confirms the presence of cardiac glycosides.

3.5.3.1 Detection of saponins

Froth Test: The **e**xtracts (20 mg) were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam confirms the presence of saponins.

3.4.4 Detection of phytosterols

Salkowski's test: The **e**xtracts (50 mg) was treated with chloroform and filtered. The filtrate was treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

3.4.5 Detection of phenols

Ferric Chloride test: The extracts (20 mg) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour confirms the presence of phenols.

3.4.6 Detection of tannins

Gelatin Test: To the extract (20 mg), 1% gelatin solution containing sodium chloride was added. Formation of white precipitate confirms the presence of tannins.

3.4.6 Detection of flavonoids

Alkaline reagent test: The extract (20 mg) was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, confirms the presence of flavonoids.

3.4.7 Detection of diterpenes

Copper acetate Test: The extract (20 mg) was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour confirms the presence of diterpenes.

3.5 Antioxidant activity

Standard protocol was used to check the antioxidant activity of the plant (Ohnishi et al., 1994). Standard solutions (140 µg/ml, 70 µg/ml, 35 µg/ml, 17.5 µg/ml, 8.75 µg/ml and 4.375 µg/ml) of the test samples were prepared in double-distilled methanol. From each concentration, 0.5 ml of the test compound was added to 3 ml of 0.1mM DPPH that will have been dissolved in methanol. The mixture was allowed to stand at room temperature for 30 minutes and the absorbance of the remaining DPPH measured at 517 nm. The radical scavenging activity was measured as the decrease in absorbance due to DPPH radical expressed as a percentage of the control solution (consisting of 0.5 ml of methanol plus 3 ml of 0.1 mM DPPH solution). The activity will then be expressed as EC_{50}, which is the concentration of the test compound required to give a 50% decrease of the absorbance from that of the control solution.

3.6 Antibacterial testing

3.6.1 Preparation of discs containing extracts

Different concentrations of 224, 168, 84 and 2.8 mg/ml (80, 60, 30 and 1% respectively) were prepared from each extract. The concentrations were incorporated into sterile blank paper discs and dried at 37^{o}C. The paper discs wwere weighed carefully for confirming exact amount of the extract being incorporated (compared to pre-weighed blank discs).

3.6.2 Bacterial culture

Escherichia coli and *Shigella flexneri* which was isolated from stool specimens in the clinic was identified according to routine cultural properties and biochemical tests. Five strains of each was included in the study. A few colonies from the overnight culture of Eosin Methylene Blue (EMB) agar was transferred into approximately 4-5ml Tripticase soy broth (TSB) medium. The broth was incubated at 37 ^{o}C for 3-4 hr and the turbidity of suspension was adjusted to that of a 0.5 McFarland barium sulfate standard. The standard suspension was used for both qualitative and quantitative antimicrobial assays.

3.6.3 Bacterial susceptibility testing

A standardized inoculum (1-2 × 10^7 cfu/ml 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. A sterile paper disc previously soaked in a known concentration of extract (20 mcg/ml per disc) was carefully placed at the centre of the labeled seeded plate. The same procedure was used for all the MRSA strains used. The plates was incubated aerobically at 37°C and examined for zones of inhibition after 24 hr. The inhibition zones was measured with a ruler and compared with the control disc (disc containing only physiological saline). All the tests were repeated 3 times for precise results and all the three repeat results showed no significant differences. SPSS soft ware and T test was used for data interpretation included calculation of the mean values, standard deviation and investigation of significant differences in results.

3.6.4 Agar-solid diffusion method

Suspension of microorganisms (1 ml) was prepared in physiological saline solution (0.9 %) and it was adjusted to 90 % of transmittance (530 nm) in a spectrophotometer. The antimicrobial spectrum of the extracts was determined qualitatively for the bacterial and fungal species in terms of zone sizes around wheels, cut in plates of agar Sabouraud and Müller–Hilton (supplemented as necessary) surface-inoculate with approximately 106 CFU of various microbial species containing 50 ml of the tested material dissolved in DMSO (equivalent to 5 mg of the dried extracts).

3.6.5 Microorganisms tested

Strains of human pathogen microorganisms used in this study was as follows. Three Gram-negative bacteria, *Escherichia coli* (LM-209), two Gram-positive bacteria, *Staphylococcus aureus* (ATTCC-6538) and *Staphylococcus epidermidis*. Antimicrobial activity of the various organic and aqueous extracts from leaves was determined by the agar-solid diffusion method (24-28). Bacteria strains was cultured overnight at 37^{o}C in Müller–Hinton agar, the filamentous and levéduriform yeasts in Sabouraud dextrose agar at 25 ^{o}C overnight and at room temperature for a period of 14 days.

The agar-solid diffusion method was used to determine antibacterial activity. The agar was melted (50 ^{o}C) and the microorganism cultures was then added aseptically to the agar medium at 45 ^{o}C in plates and poured into sterile Petri dishes to give a solid plate. All these experiments was performed in duplicate. The plates were incubated for 24-48 hr, at 37 ^{o}C for bacteria, and 10-14 days. The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotics: chloramphenicol (30 mg) for bacteria. It was used as positive control and the solvent DMSO as negative control. The minimal inhibitory concentration (MIC) was applied to the aqueous extract that had proved to be highly effective against microorganisms by the agar-diffusion method. The aqueous extract was diluted in DMSO with decreasing concentrations (from 10 000 to 625 mg/l). The strains were designated arbitrarily as sensitive or resistant and the zones was measured at the end of the incubation time. An inhibition zone of 10 mm or greater was considered to indicate good antibacterial activities.

3.7 Molecular docking analysis

Molecular docking studies were performed in order to predict the interaction of synthesized compounds with the binding sites of DNA-gyrase. Topoisomerases are interesting antibacterial drug targets, since bacteria express two isoforms, DNA gyrase and topoisomerase IV, which were both required for maintenance of proper DNA topology during transcription and replication (Duraipandiyan et al. 2014).

In this project, some of botanical isolated compounds with promising *in vitro* antibacterial activity were subjected to molecular docking studies using the ADT version 1.5.2 and AutoDock version 4.2 docking program to investigate the potential binding mode. The crystal

structure of the enzyme (PDB code 1KZN) with resolution 2.3 Å was chosen as the protein model. The structures of ligands were optimized using the HyperChem 7.0 software. Auto Dock version 4.2 was used to prepare the molecules and parameters before submitting it for docking analysis with Auto Dock. Polar hydrogen atoms were added while non-polar hydrogen atoms will be merged and then, Gasteiger partial atomic charges were assigned to the ligands. All rotatable bonds of ligands, defined by default of the program, was allowed to rotate during the automated docking process and then prepared protein and ligand structures were saved in the PDBQT format suitable for calculating energy grid maps. A grid box size of 46×46×46 Å points with a grid spacing of 0.375 Å was considered. Lamarckian genetic algorithm (LGA) program with an adaptive whole method search in the Auto Dock was chosen to calculate the different ligand conformers. After 200 independent docking runs for each ligand, a cluster analysis was done. In according to the root mean square deviation (RMSD) tolerance of 2.0 Å conformations were clustered and ranked by energy of which the conformation with the best scored pose with the lowest binding energy will be selected for these ligands (Mansourian et al., 2015; Morris et al., 1998).

4. Results and Discussion

4.1 Phytochemical screening

4.1.1 Phytochemical screening of roots extracts of *Teclea nobilis*

The phytochemical screening test conducted on root extracts of *Teclea nobilis* revealed the presence of alkaloid, tannins, flavonoids, steroids, saponins and cardiac glycoside. These phytochemical compounds are known with wide spectrum of biological activities (table 13).

Table 13: Phytochemical screening test on the root extracts of *Teclea nobilis*

Secondary metabolites	**Observation**
Alkaloid	+
Tannins	+
Saponins	+
Steroid	+
Cardiac glcoside	+
Flavonoid	+

+ = presence, - = absence

4.1.2 Phytochemical screening of *Kniphofia schimperiana*

The phytochemical screening of the root of *Kniphofia schimperiana* revealed the presence of glycosides, saponins, sterols, tannins, phenols, flavonoids and absence of alkaloids (table 14).

Table 14. Phytochemical components of crude extract of root of *Kniphofia schimperiana.*

Phytochemical compounds	MeOH Extract	DCM: MeOH Extract
Alkaloid	-	-
Glycoside	+	+
Saponin	+	+
Sterol	+	+
Tannins	+	+
Flavonoid	+	+
Phenol	+	+

MeOH=Methanol, DCM=dichloromethane, (-) absent, (+) = present

4.1.3 Phytochemical screening of roots extracts of *Balanite aegyptiaca*

The dichloromethane/methanol (1:1) extracts of the roots *Balanite aegyptiaca* was analyzed to check for the presence and absence of secondary metabolites. The qualitative phytochemical results showed the presence of alkaloids, glycosides, saponin, flavonoids and

phytostereols. The qualitative phytochemical result also showed absence of phenols (table 15).

Table 15. Phytochemical components of crude extract of root of *Balanite aegyptiaca*.

Secondary Metabolites	Methods/Reagents	CH_3OH: CH_2Cl_2 (1:1) extracts of *Balanitea egyptiaca* roots
Alkaloids	Wagner's	+
Glycosides	Modified Borntrager's	+
Saponin	Froth	+
Phytosterols	Salkowski's	+
Phenols	Ferric chloride	_
Flavanoids	Alkaline	+

Key: + presence, - absent

4.1.4 Phytochemical screening of root extracts of *Clausena anisata*

Phytochemical screening test of dichloromethane/methanol (1:1) and methanol roots extracts revealed the presence of flavonoids, phytosterols, coumarins, phenols, alkaloids, tannins, terpenoids and free reducing sugars whereas saponins were absent (table 16).

Table 16. Phytochemical screening test results.

Secondary metabolite	**CH_2Cl_2/CH_3OH (1:1) extract**	**MeOH extract**
Coumarins	+	+
Saponins	-	-
Terpenoids	+	+
Phytosterols	+	+
Flavonoides	+	+
Alkaloides	+	+
Phenols	+	+
Tannins	+	+
Free reducing sugars	+	+

+ indicates presence, − indicates absence

4.2 Characterization of compounds

4.2.1 Characterization of compounds from roots of *Teclea nobilis*

Compound **163** was isolated as white solid with R_f value of 0.6 (50% EtOAc in *n*-hexane as eluent). The spot turned orange when sprayed with dragendorff reagent, which is an indication of an alkaloid. The ^{1}H NMR spectrum showed the presence of a pair of mutually coupled doublets with AB multiplicity pattern appearing at δ_H 7.56 (*d*, H-2, *J*=2.8) and 7.01 (*d*, *J*=2.8 Hz, H-3) which are characteristic of a furan ring in furoquinoline alkaloids. A

downfield methoxy signal was observed resonating at δ_H 4.44 (δ_C 58.9). The ^{1}H NMR spectrum further revealed two aromatic signals at δ_H 7.48 (*s*, H-5) and 7.29 (*s*, H-8) suggesting *para*-oriented aromatic protons (H-5 and H-8). A downfield singlet signal observed at δ_H 6.09 (2H, δ_C 101.6) is characteristic of a methylenedioxy substituent (also confirmed from DEPT-135 spectrum pointing down). The above spectral data pattern suggest that the compound have furoquinoline alkaloid skeleton (table 17, appendix 1-3).

The ^{13}C NMR spectrum showed signals at δc 155.9 (C-4), 163.1 (C-9a) and 143.8 (C-8a). In agreement with furoquinoline alkaloid skeleton, the chemical shift positions observed for C-8a and C-9a suggest sp^2 aromatic quaternary carbons attached to nitrogen atom [6]. Olefinic carbons of the furan moiety were observed at δ_C 150.8 (C-6) and δ_C 146.1 (C-7). Due to the presence of two para oriented singlets resonating at δ_H 7.48 and 7.29, assigned to H-5 and H-8, respectively, the only possible position of the methylenedioxy moiety is at C-6/C-7 position. The corresponding carbon signals for C-5 and C-8 were observed at δ_C 102.6 and δ_C 98.2, respectively, whereas that of two vicinal oxygenated sp^2 aromatic quaternary carbons, attached to methylenedioxy moiety, appeared at δ_C 150.8 (C-6) and δ_C 146.1 (C-7). Thus, based on the above spectra feature this compound was found to be identical with furoquinoline alkaloid reported in literature with trivial name masculine (**163**), previously isolated from the leaves of *Teclea nobilis* (Dagne, 1994). However, this is the first report from the roots of the plant.

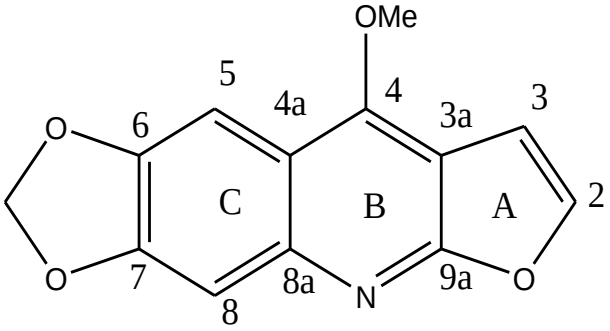

Table 17: ^{1}H ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **163**

Position	δ_H (δ in ppm, *J* in Hz)	δ_C	Masculine **(1)** Dagne (1994)	
			δ_H	**δ_C**
2	7.56 (1H, *d*, *J*=2.8 Hz)	142.6	7.55 (1H, *d*, *J*=3 Hz)	142.8
3	7.01 (1H, *d*, *J*=2.4 Hz)	104.5	7.01 (1H, *d*, *J*=3 Hz)	104.7
3a	-	102.5	-	102.7
4	-	155.9	-	156.2
4a	-	114.3	-	114.5

5	7.48 (1H, *s*)	104.5	7.50 (1H, *s*)	104.7
6	-	150.8	-	150.9
7	-	146.1	-	146.3
8	7.29 (1H, s)	98.0	7.23 (1H, *s*)	98.2
8a	-	143.8	-	144.1
9a	-	163.1	-	163.4
MeO-4	4.44 (3H, s)	58.9	4.39 (3H, *s*)	59.2
$-OCH_2O-$	6.09(2H, s)	101.6	6.07(2H, *s*)	101.8

Compound **164** was obtained as colorless amorphous solid with R_f value of 0.62 (20% EtOAc in *n*-hexane as eluent) from methanol extract. The ^{1}H NMR spectrum (table 12) revealed the presence of seven methyl singlet signals at δ_H 0.98 (Me-23), 0.77(Me-24), 0.83 (Me-25), 1.04 (Me-26), 0.95(Me-27), 0.80 (Me-28) and 1.69 (Me- 30). The presence of terminal olefinic protons was observed at δ 4.55 (H-29a) and 4.68 (H-29b). Oxygenated sp^3 methine proton was observed at δ 3.17 (m, 1H, H-3) which is a characteristic of most triterpenoids with hydroxyl group at C-3 position.

The ^{13}C NMR spectrum (table 18, appendix 4-6) revealed the presence of thirty carbon signals which is a characteristic feature of triterpenes (table 18). The ^{13}C NMR and DEPT-135 spectra displayed the presence of seven methyl carbons signals at δ_C 14.6, 16.0, 15.4, 18.0, 16.2, 19.4 and 28.0. The ^{13}C and DEPT-135 spectrum also showed ten methylene carbon signals at δ_C 18.4, 20.9, 25.2, 27.5, 29.7, 29.9, 34.3, 35.6, 38.7 and 40.0. Presence of five methine carbons (δ_C 48.0, 48.3, 50.5, 55.3 and 38.1), two olefinic carbons (δ_C 150.9 and 109.4), of which one is quaternary, and additional quaternary carbon peaks (at δ_C 38.9, 37.2, 40.9, 42.9 and 43.0) were also confirmed (appendix 4-6). Oxygenated sp^3 methine at C-3 was observed at δ_C 78.9. Based on the above spectral data and comparison with literature, compound **164** was identified as lupeol (**164**), a compound widely occurs in plants (Jain and Bari, 2010).

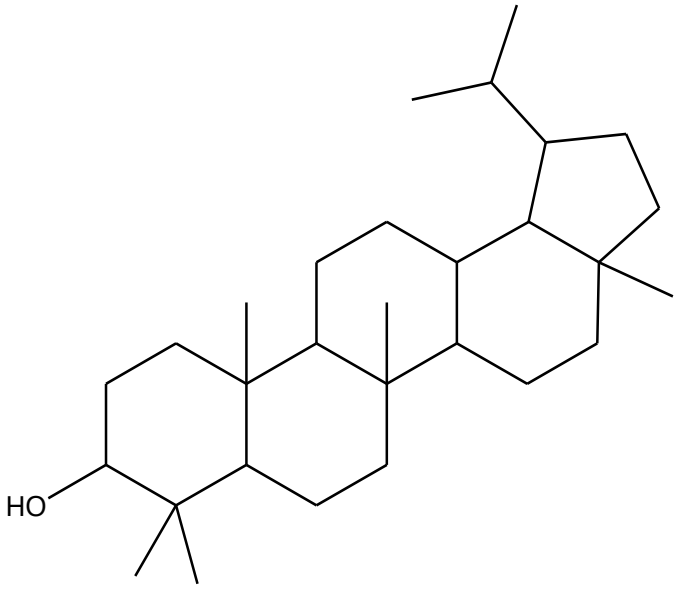

Table 18: ^{1}H ($CDCl_3$, 400MHz) and ^{13}C NMR ($CDCl_3$, 150MHz) spectral data of compound **164**

Position	δ_H (δ in ppm, *J* in Hz)	δ_C	Jain and Bari, 2010	
			δ_H	**δ_C**
1		38.7		38.9
2		27.5		27.6
3	3.17(m,1H)	78.9	3.17(m,1H)	79.2
4		38.9		39.1
5		55.3		55.5
6		18.4		18.5
7		34.3		34.5
8		40.9		41.0
9		50.5		50.6
10		37.2		37.4
11		20.9		21.1
12		25.2		25.3
13		38.1		38.3
14		42.9		43.0
15		27.5		27.6
16		35.6		35.8
17		43.0		43.2
18		48.3		48.5
19		48.0		48.2
20		150.9		151.2
21		29.9		29.9
22		40.0		40.0
23	0.98	28.0	0.95	28.2
24	0.77	15.4	0.77	15.6
25	0.83	16.2	0.81	16.3
26	1.04	16.0	1.01	16.1

27	0.95	14.6	0.93	14.7
28	0.80	18.0	0.77	18.2
29a	4.55 (m)	109.4	4.55(m)	109.5
29b	4.68 (d,2.2)		4.68(d,2.2)	
30		19.4		19.5

Compound **165** was isolated as colorless amorphous solid with R_f value of 0.7 (20% EtOAc in *n*-hexane as eluent). The ^{1}H NMR spectrum (400 MHz, $CDCl_3$, table 9, appendix 6-8) revealed the presence of seven methyl singlet signals at δ_H 0.99 (Me-23), 0.76 (Me-24), 0.83 (Me-25), 1.03 (Me-26), 0.95 (Me-27), 0.79 (Me-28) and 1.68 (Me-30). The presence of terminal olefinic protons was observed at δ 4.56 (H-29a) and 4.68 (H-29b). Oxygenated sp^3 methine proton was observed at δ 3.16 (m, 1H, H-3) which is a characteristic of most steroids with hydroxyl group at C-3 position.

The ^{13}C NMR spectrum (table 19, appendix 7-9) revealed the presence of thirty carbon signals which is a characteristic feature of triterpenes and five additional carbons attributed to a benzoyl moiety (table 9). The ^{13}C NMR and DEPT-135 spectra displayed the presence of seven methyl carbons which resonated at δ_C 14.6, 16.0, 15.4, 18.0, 16.2, 19.4 and 28.0. Ten methylene carbon signals were observed resonating at δ_C 18.4, 21.0, 25.2, 27.5, 29.7, 29.9, 34.3, 35.6, 38.8 and 40.0. Presence of five methine carbons (δ_C 48.0, 48.30, 50.5, 55.3 and 38.1), two olefinic carbons (δ_C 150.8 and 109.4), of which one is quaternary, and additional quaternary carbon peaks (at δ_C 38.9, 37.2, 40.8, 42.8 and 43.0) were also confirmed. Oxygenated sp^3 methine at C-3 was observed at δ_C 78.9. The methyl signal which appeared at δ_C 18.0 (C-28), in case of compound **164**, appeared at δ_C 71.8 (C-28) in case of compound **165** suggesting the methyl (C-28) is oxidized to oxymethylene, also confirmed from DEPT-135 spectrum. This suggests that the main skeleton is a known triterpenoid betulin. However, In addition to betulin skeleton, the ^{1}H NMR spectrum revealed a benzoyl moiety with characteristic peaks of a monosubstituted aromatic ring resonating at δ_H 7.7 (2H, H-2',6'), 7.2 (2H, H-3',5') and 7.5 (H-4') coupled with the observation in ^{13}C NMR spectrum and DEPT-135 spectra with characteristic peaks resonating at δ_C 167.0, 128.8 (attributed to C-2',6'), 130.9 (C-3',5'), 132.4 (C-1') and 128.6 (C-4'). There are two possible positions in which the benzoyl moiety can be attached to main betulin skeleton (C-3 and C-28) forming ester linkage. On the basis of spectral feature, the benzoyl moiety is suggested to be attached to oxymethylelne (C-28) forming ester linkage considering the observation of downfield chemical shift value of C-28 and no change in the chemical shift value of C-3 compared to

compound **164**,. Thus, based on the above spectral data and comparison with literature, the structure of the compound was identified as benzoylbetulin (**165**). The results of this work are published in 2018 (Tesfaye Nuru et al., 2018)

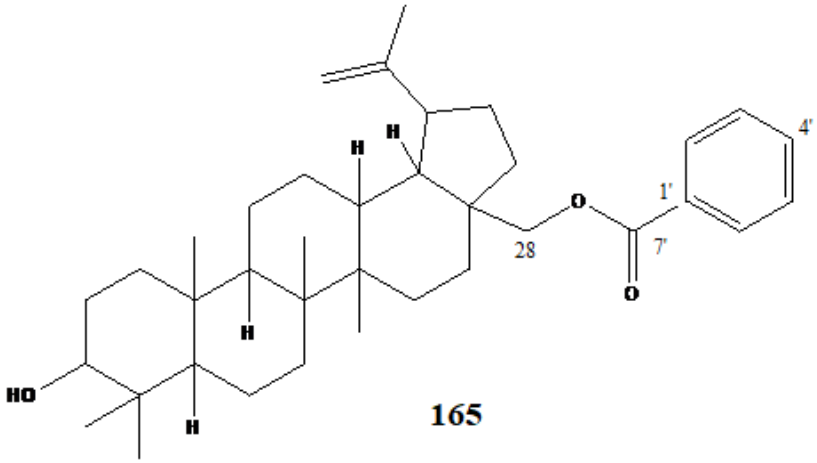

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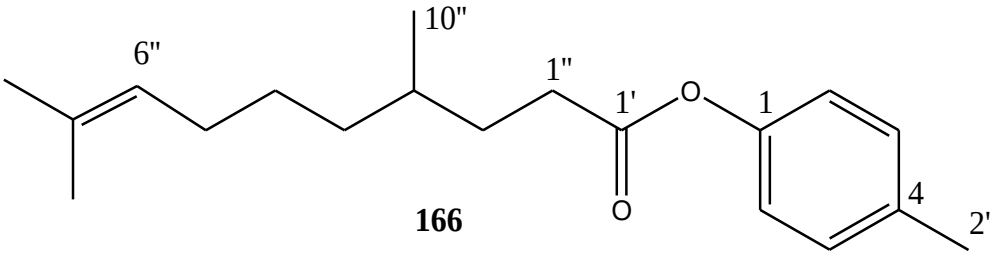
Table 19: ^{1}H ($CDCl_3$, 400MHz), ^{13}C NMR ($CDCl_3$, 150MHz) and DEPT-135 spectral data of benzoylbetulin (**165**)

Position	**δ_H (δ in ppm, *J* in Hz)**	**δ_C**	**DEPT-135**
1		38.8	38.8
2		27.5	27.5
3	3.17(m,1H)	78.9	78.9
4		38.9	
5		55.3	55.3
6		18.4	18.4
7		34.3	34.3
8		40.8	
9		50. 5	50.5
10		37.2	
11		21.0	21.0
12		25.2	25.2
13		38.1	38.1
14		42.8	
15		27.5	27.5
16		35.6	35.6
17		43.0	
18		48.3	48.3
19		48.0	48.0
20		150.8	
21		29. 9	29.9
22		40.0	40.0
23	0.99	28.0	28.0
24	0.76	15.4	15.4

25	0.83	16.2	16.2
26	1.03	16.0	16.0
27	0.95	14.6	14.6
28	4.0	71.8	71.8
29a	4.56(m)	109.4	109.4
29b	4.68(d,2.2)		
30		19.4	19.4
1’		132.4	
2’	7.7	130.9	130.9
3’	7.2	128.8	128.8
4’	7.5	130.0	
5’	7.2	128.8	128.8
6’	7.7	130.9	130.9
7’	-	167.0	

4.2.2 Characterization of compounds 4-6 from *Kniphofia schimperiana*

Compound **166** was isolated as a yellow powder with R_f value of (7.1) in (1:1 ethyl acetate: *n-hexane*). A broad IR spectrum revealed absorption band at 1690 cm^{-1}, 1450 cm^{-1} and 1250cm^{-1} are attributed to carbonyl group of an ester moiety, olefinic system and C-O part of ester moiety, respectively. The absorption peaks at 700 cm^{-1}, 2922.92 cm^{-1} and 3010cm^{-1} suggest CH_2 rocking, sp^3 C-H stretching and sp^2 C-H stretching vibrations, respectively. The ^{1}H NMR spectrum (table 20) revealed the existence of AA’XX’ spin system suggesting a 1,4 di-substituted artomatic ring (δ 6.87 (2H, *dd, J= 8,2 Hz*) and 7.03 (2H, *dd, J= 8,2 Hz*)). The peaks at δ 4.35(3H, s) suggest the presence of a methyl group attached to phenyl ring (C-1’’). The presence of an olefinic proton at δ5.2 (1H, t, H-6’’) coupled with two symmetrical methyls groups at δ1.71 (3H, s, H-8’’, 9’’), four methylenes (C-1’’, 2’’, 4’’, 5’’) suggest a geranyl moiety. The ^{13}C NMR spectrum (table 20) revealed a total of 16 carbon peaks of these the presence of ester moiety at δ176, a 1, 4-disubstitued phenyl ring (δ130.4, 130.9, 128.8 and 161.0) are all clearly evident suggesting that the methyl is located at C-4 position of phenyl ring whereas the ester geranyl moiety is located at para position to the methyl (C-1) (table 14, appendix 10-12). Moreover, the 1D NMR spectral patterns is a good agreement that the geranyl moiety is directly connected to the ester. Thus, based on the above spectral data the compound **166** was proposed as shown below.



166

Table 20: NMR data of compound **166** (400MHz, DMSO-d_6)

Position	**^{1}H NMR (δ in ppm)**	**^{13}C NMR (δ in ppm)**	**DEPT-135 (δ in ppm)**
1	-	128.9	-
2	7.03(2H, dd)	132.4	-
2'	2.0 (3H, s)	13.9	
3	6.87(2H,dd)	130.9	-
4	-	155.0	-
5	6.87(2H, dd)	130.9	-
6	7. 03(2H,d*d)*	132.4	-
1'	-	167.0	-
1''	2.71 (2H,*t,)*	65.6	
2''	1.24(2H)	30.6	30.6
3''	1.69(1H)	31.9	-
4''	1.70(2H)	29.7	29.7
5''	1.79(2H)	38.0	-
6''	5.2(2H)	128.7	-
7''	-	132.5	-
8''	1.71(3H, s)	22.5	-
9''	-	27.27	-
10''	1.06(3H,nd)	19.7	-

Compound **167** was isolated as yellow amorphous powered with R_f value of 0.62.The IR spectrum revealed absorption bands at 3100-3750cm^{-1}, 1690cm^{-1} and 1250cm^{-1}, 700cm^{-1}, 2922.92cm^{-1} and 3010cm^{-1} attributed to hydroxyl group, C-O ester moiety, CH_2 rocking, sp^3 C-H stretching and sp^2 C-H stretching vibrations, respectively.

The ^{1}H NMR spectrum (table 21) displayed the presence of three adjacent and mutually coupled aromatic protons at δ_H 7.20(*dd*, *J* = 7.8, 1.1 Hz, H-5), 7.56(t) and 7.77 (*dd*, J = 7.8, 1.1 Hz, H-7) suggesting ABX spin system for ring C. The presence of peak at δ12.98 (s, 1H, 1-OH) suggest that there is a peri hydroxyl group at C-8 position. The ^{1}H and ^{13}C NMR chemical shift pattern showing three aromatic protons having an ABX spin system, for ring C, coupled with a singlet at δ 7.25 (H-2) and a methyl peak δ 2.10 (C-3, δ_C 19.0), as expected biogenetically, are all in good agreement with the knipholoneanthrone derivatives. Signals of

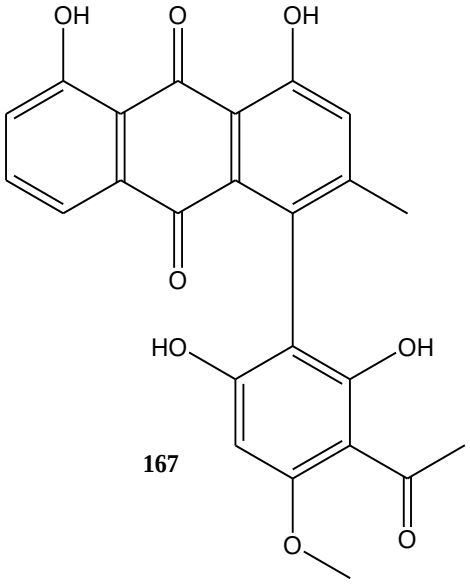
one chelated hydroxyl groups (C-1), a methyl group (C-3) as well as two isolated aromatic proton peaks (H-2 and H-5') coupled with three ABC aromatic protons peaks (H-5,6,7) indicate the chrysophanolanthrone part of the molecule is substituted at C-4 of ring A. The presence of a methoxy group at C-4' was evident from the peaks at δ_H3.1 (3H, s). The acetonyl group in acetylphloroglucinol moiety at C-3' is from a methyl at δ_H 2,0 (s, 3H).

The ^{13}C NMR spectrum revealed a total of 26 peaks in good agreement with the knipholone skeleton. Of these, the two carbonyls are evident at δ192.96 (C-9) and 182.79 (C-10). The difference of more than 5ppm, between the two peaks suggests that one of the carbonyl (C-9) is peri to the hydroxyl group (C-8) (table 15, appendix 13-15). In agreement with literature, the chemical shift value of methoxyl group (C-4') is up field, in those knipholone derivatives having an acetonyl group at C-3' with value of δ_H 3.35 and δ 59 which might be due to anisotropy effect of the acetonylmoiety at C-3'. Moreover, the upfeild chemical shift of methyl at C-3' appeared at δ_C 31.3 in agreement with literature (Abdissa et al., 2014).Thus, the above spectral data suggest that the difference between compound **167** and that of knipholone is the absence of methoxy group at C-6' of the acetylphloroglucinol moiety. Consequently, based on the above spectral data the compound was proposed as shown below in (table 21).

Table 21: NMR data of compound **167** (400MHz, DMSO-d_6) (δ in ppm)

Position	**Compound 167**			**6,8-O-dimethylknipholone (**Abdissa **et al., 2014**)	
	^{1}H NMR	**^{13}C NMR**	**DEPT-135**	**^{1}H NMR**	**^{13}C NMR**
1	-	131.6	-	-	162.3
1**a**	-	117.5	-	-	116.2
2	7.79(s, 1H,H-7)	125.0	-	7.25(s,1H, H-2	125.3
3 **3-CH$_3$**	- 1.4 (s,3H,3-CH$_3$)	147.8 21.2	- -	- 2.10(s, 3H, 3-CH3)	149.4 20.7
4	-	127.5	-	-	126.6
4a **5a**	- -	130.3 138.7	- -	- -	130.9 136.9
5	7.20 (dd,1H,*H-5*)	124.7	-	7.74 (dd,1H, H-5)	120
6	7.56(t,3H, H-6)	135.8	-	7.64(t,1H, H-6)	135.3
7	7.77(dd,1H,H-2)	118.9	-	7.29(dd,1H,H-2)	117.2
8	12.98(s,1H, 1-OH)	162.7	-	12.07(s,1H,1-OH)	160.2
			-	-	-

			-	-	-
8a	-	121.4	-	-	120.6
9	-	192.9	-		189
10	-	182.8	-	-	183.5
1’	-	108.5	-		109.3
2’	-	163.3	-	-	162.4
3’	-	104.0	-		106.2
3’C=O	-	203.3	-	-	203.5
3’ $CH_2\underline{\mathbf{CH_3}}$	0.96 (t,3H,)	14.1	-	-	33.3
4’	-	157.8	-	-	162.9
4’O$\underline{\mathbf{CH_3}}$	3.19 (s,3H, 4-OCH_3)	53.2			55.3
5’	-	114.0		6.14 (s,1H, H-5’)	86.2
6’	-	162.5	-	-	162.6



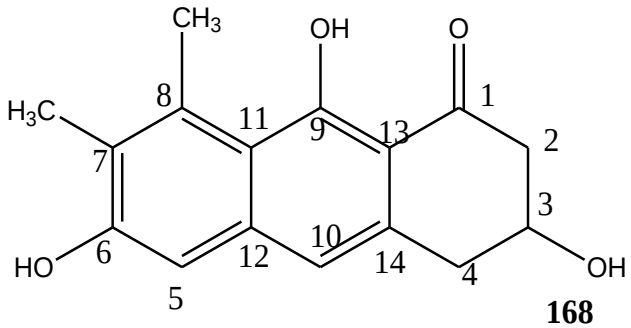
167

Compound **168** was obtained as colorless precipitate with R_f value of 0.35 in ethyl acetate: *n*-hexane (1:1). The ^{1}H NMR spectrum (DMSO-d_6,400 MHz, table 22) revealed a deshielded singlet proton at δ 15.25 (1H,s) due to the proton of the hydroxyl group at C-9 and two singlet aromatic protons at δ 6.90 (1H, s, H-10) and δ 6.95 (1H, s, H-5). It also showed that at δ 4.25 (oxygenated methane at C-3), a singlet at δ 2.51(methyl group at C-8) and another singlet at δ3.85 (methyl ester and peaks at δ 2.70 (1H, dd, H-2), δ 2.95 (1H, dd, H-2); and δ 3.15 (1H dd, H-4), δ 2.88 (1H, dd, H-4) indicate the protons of methylenes. The ^{13}C NMR spectrum (DMSO-d_6,400 MHz) showed a total of seventeen different carbons; oxygenated quaternary carbons at δ 156.2 (C-6) two carbonyl carbons at δ 204.1(C-1) and 168.7 (ester carbonyl carbon) methyl at δ 21.1, two methylenes at 38.1 (C-4) and 46.9 (C-2), methyl of an ester at δ 52.6 and oxygenated methine at δ 64.9 (C-3) (table 22, appendix 16-18). Moreover, the DEPT-135 spectrum displayed five upward peaks at δ 21.1, 52.6, 64.9 and

117.1 and two downward peaks at δ 38.1 and δ 46.9 attributed to two methylenes. Based on spectroscopic evidence compound **168** in a good agreement with compound reported in literature as 3,6,9-trimethyl-1oxo-5,6,7,8-tetrahydroanthracene-carboxyic acid methyl ester known by trivial name aloesaponol I (figure 3) where the only difference observed between compound **168** and that of Aloesaponol is the acetyl group at C-7 position is in reduced form (-CH_3). Aloesaponol was previously reported from *Aloe saponaria*, *Aloe turkanersis* and *Aloe secondiflora* and *Aloe Elegan* (Abdissa et al., 2014).

Table 22.· ^{1}H and ^{13}C NMR (DMSO-d_6, 400 MHz) data of compound **168**

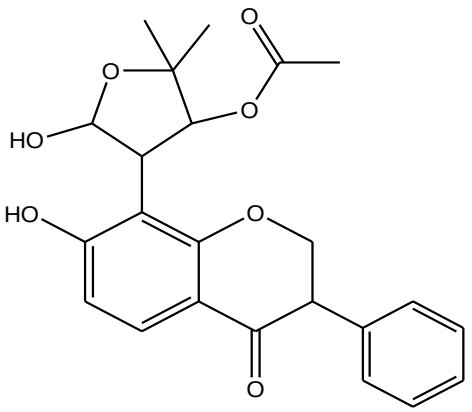
Position	Compound **168**			Abdissa et al., 2014	
	δ_H	δ_C	DEPT-135	δ_H	δ_C
1		202.4			203.7
2	2.70 (*dd* 5.5 Hz) 2.80 (*dd*,17.3Hz)	46.9		2.70 (*dd*, 5.4 Hz) 2.96 (*dd*,15.6)	46.6
3	4.25 (*m*)	64.9	CH (64.9)	4.24 (*m*)	64.4
4	2.88 (*dd*, 15.4 Hz) 3.15 (*dd*, 15.5Hz)	38.1	CH_2 (38.1)	2.90 (*dd*, 15.6 Hz) 3.14 (*dd*, 15.8 Hz)	37.5
5	6.95 (*s)*	117.1	CH (117.1)	6.95 (*s*)	116.6
6		156.2			155.1
7		141.4			140.8
8		137.7			137.2
9		166.5			165.9
10	6.90 (*s*)	108.1	CH (108.1)	6.92 (*s*)	107.5
11		126.1			125.4
12		115.8			
13		137.1			136.6
14		110.8			110.2
_COO		-			168.2
_OCH_3	-	-	-	3.83 (*s*)	52.1
8-CH_3	2.51 (*s*)	22.0	CH_3 (21.1)	2.70 (*s*)	20.8
9-OH	15.86 (*s*)			15.27 (*s*)	
7-CH_3	2.2 (s)	19	CH_3 (19)	-	-



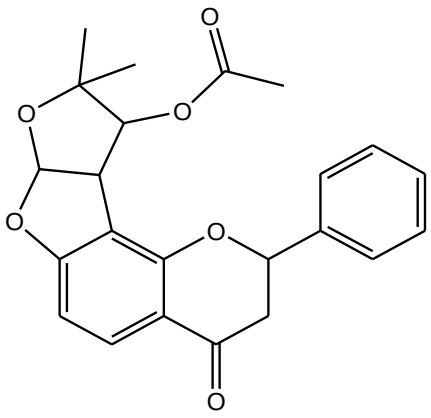
168

4.2.3 Isolated compound from roots of *Tephrosia vogelli*

The following seven (**169-175**) compounds were isolated and charachterized from dichloromethane/methanol (1:1) and methanol extracts of roots extracts *Tephrosia vogelli*. Detailes of NMR spectra characherization approaches, antibacterial assay and manuscript from this work is ongoing (appendix 19-37).



169



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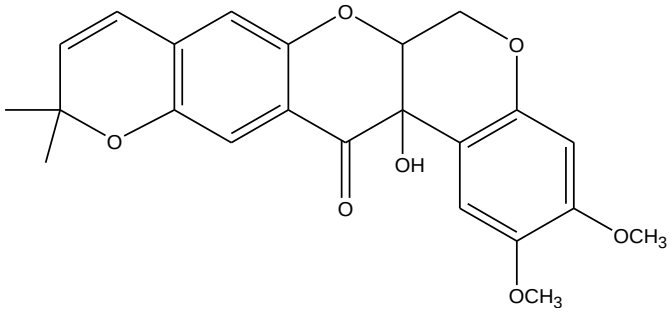

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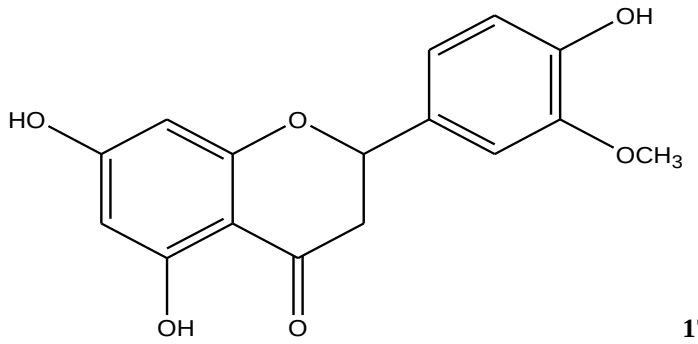

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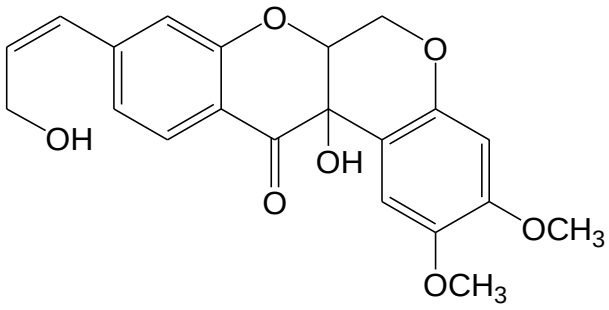

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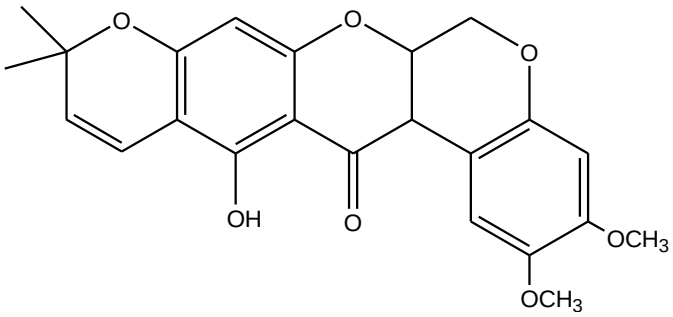
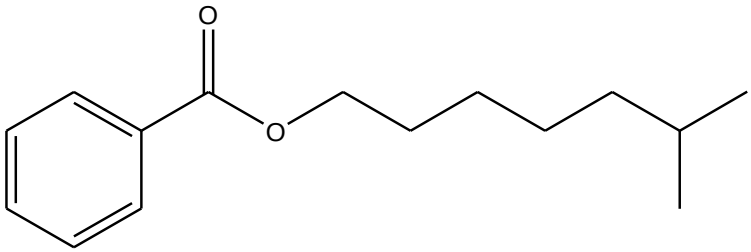


174



175

4.2.3.1 Phytochemical screening of roots of *Tephrosia vogelii*

The results from the phytochemical screening methanol extract of leaf shows the presence of tannins, saponins, flavanoids and absence of alkaloid, steroid and anthraquinones. Methanol extract of the stem bark of *Tephrosia vogelii* shows the presence of terpenoids, flavonoids, tannins, and saponnins and absence of anthraquinones, alkaloids and steroids. Whereas Dichloromethane:Methanol (1:1) root extract shows tannins, saponins, terpenes, flavonoids, steroids and absence of alkaloids and anthraquinones (table 23).

Table 23: Results of phytochemical screening tests of the DCM:MeOH root extract, methanolic extract of leaf and steam bark of *Tephrosia vogelii*

Plant constituent	Reagent used	leaf	Steam bark	Root
Alkaloids	Mayer's reagent + dragendroff's reagent	-	-	-
Tannins	1%gelatin + sodium chloride	+	+	+
Anthraquinones	HCl + $CHCl_3$ + NH_3	-	-	-
Saponins	Warming in water bath	+	+	+
Terpenes	Chloroform+ conc.sulphuric acid		+	+
Flavonoids	Dilute ammonia solution + dilute hydrochloric acid	+	+	+
Steroids	Acetic anhydride + chloroform + concentrated sulphuric acid.	-	-	+

Present (+) /Absent(-)

4.2.3.2 Charachterization of compounds from *Tephrosia vogelii*

Compound **169** named as 8-(2,5-dihydro-5,5-dimethyl-2-oxofuran-3-yl)-5-hydroxy-7-methoxy-2-phenyl-4H-chromen-4-one (Coded as A-5) was isolated as deep yellow solid melting point 128 ^oC with R_f value of 0.625 in *n*- hexane/ ethyl acetate (7:3).The UV-Vis spectrum showed absorption maxima at λ max at 343 nm that attributed to flavonoid skeleton having bath chromic shift indicating the presence of n-π* transition of carbonyl. In the IR spectrum showed broad absorption band at 3397.5 cm^{-1} attributed to hydroxyl group and absorption band at 1743 cm^{-1} indicated the presence of carbonyl and stretching of unsaturated carbonyl group, respectively. Absorption peaks at 2965cm^{-1} and 2918 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at1618 and 1516 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1138 cm^{-1} and 1092 cm^{-1} indicated C-O stretching vibrations.

From ^{1}H-NMR (400 MHz,DMSO-d_6, table 24)(Appendix 19) spectrum revealed the presence of doublet peaks at δ 7.6 and 7.3 shows the presence of B-ring flavone protons at H-2′ &6', and 3′&5'. The signals at δ 1.55 (6H, s) and at δ 3.96 (3H, s), corresponding to a gem-dimethyl group and a methoxy group, respectively. Also, it shows a singlet signal at δ 6.4 (1H, s, H-3).

The ^{13}C-NMR and DEPT-135 spectra (Appendix 20, table 24) in showed a total of eighteen carbon signals, for three methyl's at δ 24.8(C-6''&5'') and δ 56.2 (C-7), six methines at δ 133 (C-4"), δ 128.8 (C-3'&5'), δ 128.7 (C-4'), δ 126.3 (C-2'&6'), δ 110.9 (C-6), δ 103.1

(C-3). Nine quaternary carbons at δ 190.6 (C-4), δ 174.9 (C-2’’), δ 165.9 (C-7), δ 163.6 (C-2), δ 158 (C-5), δ 147.9 (C-8a), δ 130.3 (C-1’), δ 118.5 (C-4a) and δ 113.9 (C-8).

From DEPT-135 (Appendix 21), there are eight carbon signals. The signals δ at δ 133 (C-4”), δ 128.8 (C-3’&5’), δ 128.7 (C-4’), δ 126.3 (C-2’&6’), δ 110.9 (C-6), δ 103.1 (C-3) shows a methine carbons. The two peaks at δ 24.8 (C-6’’&5’’) and δ 56.2 (C-7) shows the presence of methyl and methoxy carbons respectively.

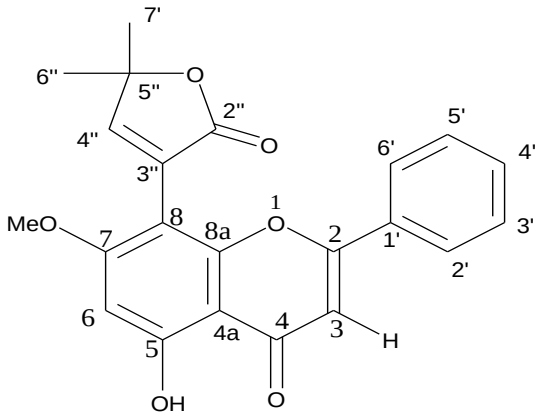


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Table 24. The ^{1}H-NMR ^{13}C-NMR and DEPT-135 spectra of compound **169** (4,5-dihydro-5,5-dimethyl-4-oxofuran-3yl)-5 hydoxy- 7-methoxy-2-phenyl-4H-chrome-4-one **(**coded as A-5)

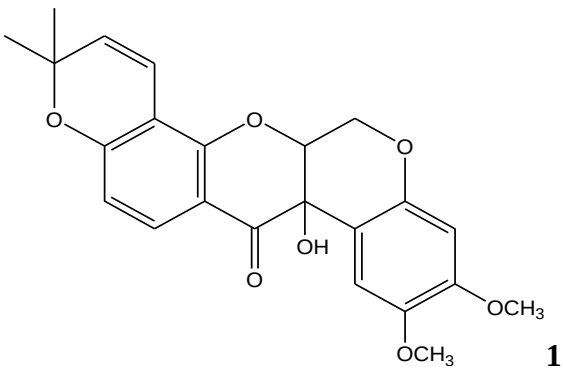
Position	^{13}C-NMR (δ in ppm)	^{1}H-NMR (δ in ppm)	DEPT	Ahmed et al., 1999	
				δ ^{1}H	δ ^{13}C
1	-	-	-	-	-
2	163.6	-	Quaternary		161.69
3	103.1	6.4 (1H, s)	CH	6.74 (s)	107.35
4	190	-	Quaternary	-	177.72
4a	118.5	-	Quaternary	-	118.04
5	158.0	-	Quaternary	-	128.13
6	110.9	7.3(1H, d)	CH	7.08 (d)	109.36
7	165.9	-	-	-	163.18
8	113.9	-	Quaternary	-	109.7
8a	147.9	-	Quaternary	-	154.87
1’	130.3	-	Quaternary	-	131.92
2’6’	126.3	7.63(1H, d)	CH	7.43 (m)	126.2
3’5’	128.8	7.3(1H, d)	CH	7.74 (m)	128.99
4’	128.7	6.9(1H, d)	CH	7.43 (m)	131.5
Germinal Me_2	(24.8)	1.55(6H, s)		1.65 (6H,s)	25.83
OMe	56.1	3.96 (3H ,s)	-	3.94 (3H,s)	56.6
2’’	174.9	-	-		170.62
3’’	-	-	Quaternary		124.24
4’’	133.0	7.1(1H, s)	CH	7.52 (s)	159.89
5’’	-	-	Quaternary	-	84.92

Compound **170** named as Tephrosin (Coded as ST-2) was isolated as yellow solid melting point 198ºC with R_f valueof 0.42 in *n*-hexane/ethyl acetate (7:3). In the IR spectrum (Appendix 6), a broad absorption band at 3487.5 cm^{-1} attributed to hydroxyl group and absorption band at 1643 cm^{-1} indicated the presence of carbonyl and stretching of unsaturated carbonyl group, respectively. Absorption peaks at 2965cm^{-1} and 2918 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at 1618 and 1516 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1138$cm^{-1}$and indicated C-O stretching.

From^{1}H-NMR (400MHz, DMSO-d_6) (Appendix 22): δ 1.34 (3H, s, H-2a''), 1.44 (3H, s, H-2''b), 3.71 (3H, s, H-5'a), 3.80 (3H, s, H-4'a), 4.46 (1H, dd, J=12,H-2a), 4.55 (1H, dd, J=0.9) H-2), 4.63 (1H, dd J=12, H-2b), 5.53 (1H, d, J=10.1, H-3''), 6.44 (1H, d, J=8.7,H-6), 6.47 (1H,S,H-3'), 6.55 (1H, s, H-6'), 6.57 (1H, d, J=10.1, H-4''), and 7.7 (1H, d, J=8.7, H-5).

The ^{13}C-NMR (Appendix 23a) showed a total of twenty three carbon signals, 28 (C-2''a, C-2''b), 55.9 (C-4'a), 56.5 (C-5'a), 63.9 (C-2a), 67.6 (C-3), 76.4 (C-2), 78.2 (C-2''), 101.5 (C-3'), 108.8 (C-1'), 108.9 (C-8), 111.5 (C-6), 111.6 (C-10), 112.7 (C-6'), 115.0 (C-4''), 128.5 (C-5), 130.0 (C-3''), 143.4 (C-5'), 148.7 (C-2'), 151.2 (C-4'), 156.0 (C-9), 159.5 (C-7), 191.3 (C-4).

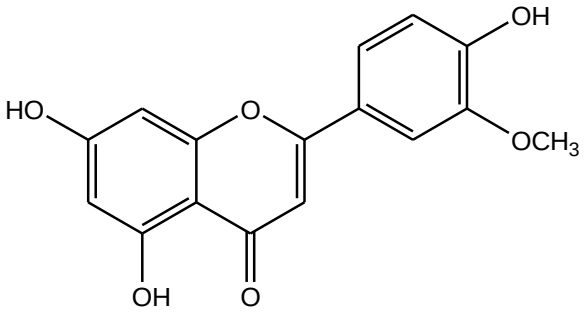
From DEPT-135 (Appendix 23b), there are twelve signals. The signals at δ, 28 (C-2''a, C-2''b), 55.9 (C-4'a), 56.5 (C-5'a), 63.9 (C-2a), 76.4 (C-2), 101.5 (C-3'), 111.5 (C-10), 111.6 (C-6), 115.0 (C-4''), 128.5 (C-5), 130.0 (C-3''), The two peaks at δ 28.5, 28.2 (C-2a''& 2b'') and δ 63.9 (C-2a) shows the presence of methyl and methylene carbons respectively (Ahmad et al, 1999).



170

Compound **171** named as 2,3-dihydro-5,7-dihydroxy-2-(4-hydroxy-3 methoxyphenyl)chromen-4-one (Coded as ST-3) was isolated as deep yellow solid with R_f valueof 0.625 in *n*-hexane/ ethyl acetate (7:3). In the IR spectrum (Appendix 24), a broad absorption band at 3397.5 cm^{-1} attributed to hydroxyl group and absorption band at 1743 cm^{-1} indicated the presence of carbonyl and stretching of unsaturated carbonyl group, respectively. Absorption peaks at 2965 cm^{-1} and 2918 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at1618 and 1516 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1290cm^{-1} indicated C-O stretching vibrations. From ^{1}H-NMR (400 MHz, table 25, DMSO-d_6) (Appendix 23) spectrum revealed the presence of peaks at δ 7.5 (S, H-2'), 7.3(d, H-6') and 6.8(d, H-5') shows the presence of B-ring flavone. The signals at δ 7.8 (1H, s, H-3) and at δ 6.2 (1H, d, H-5&8), correspond to the ring A. The signal at δ 3.8 (s, 3H,) shows methoxy group.

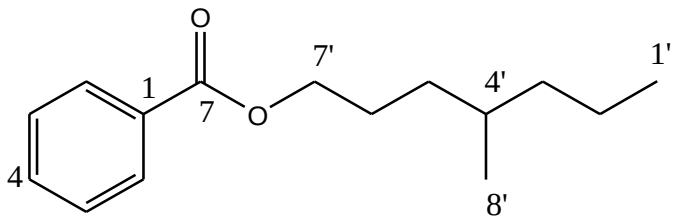
The ^{13}C-NMR spectrum (Appendix 24) showed a total of sixteen carbon signals, the quarternary carbons at δ 192 (C-4), 166.4 (C-7), 165.1 (C-2), 161.5 (C-5), 150.35 (C-3'), 144.5 (C-4'), 124 (C-1'), and δ 113 (C-4a), six methines at δ 124 (C-6'), δ 116.3 (C-5'), δ 115.5 (C-8), δ 112.1(C-2'), δ 108.5 (C-6), and δ 103.0 (C-3). The carbon at δ 56.2 shows a methoxy carbon. From DEPT-135 (Appendix 25), there are six carbon signals. The signal δ at δ 124 (C-6'), δ 116.3 (C-5'), δ 115.5 (C-8), δ 112.1(C-2'), δ 108.5 (C-6) and δ 103.0 (C-3),shows methines. The carbon at δ 56.2 shows the presence of a methoxy carbon.



171

Compound **172** named as 6-methylheptyl benzoate (coded as A2) was isolated as deep yellow solid with R_f value of 0.85 in *n*-hexane/ethyl acetate (7:3). Its IR spectrum showed absorption band at 1643 cm^{-1} suggesting the presence of carbonyl and stretching of ester carbonyl group. Absorption peaks at 2965 cm^{-1} and 2918 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at1618 and 1516 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1092 cm^{-1} indicated C-O stretching vibrations and the band at 782 shows CH_2 rocking. From ^{1}H-NMR (Appendix 26), ^{13}C-NMR (Appendix

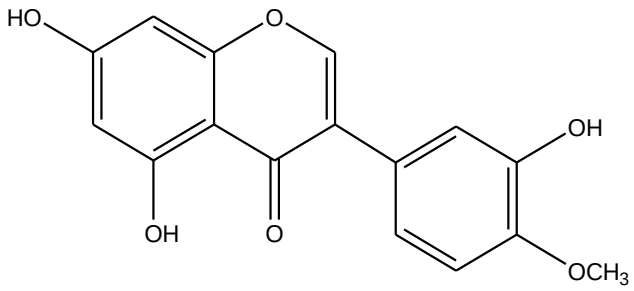
27), and DEPT-135 (Appendix 28), there are quaternary carbons at 167.4, (C-7) and 132.18 (C-1), aromatic protons at 7.9 (dd, H-2,6) and methine carbons at 132.0(C-4) and 129.9(C-2&6), CH-4′ (*δ*H 1.62; *δ*C 38.5), methyl groups CH_3-1′ (δ_H 0.87; δ_C 14.3) and CH_3-8′ (δ_H 0.86; δ_C 11.2),oxy-methylene OCH_2-7′ (δ_H 4.13; δ_C 67.8), and methylene groups CH_2-2′ (δ_H 1.26; δ_C 22.8),CH_2-3′ (δ_H 1.25; δ_C 28.8), CH_2-5′ (δ_H 1.30; δ_C 30.2) and CH_2-6′ (δ_H 1.35; δ_C 23.7). Thus, the above spectral data is in a good agreement with 4′-methylheptyl benzoate (**172**) (Hawas et al, 2018).



172

Compound **173** named as 5,7-dihydroxy-3-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one (coded as ST-6) was isolated as yellow solid with R_f value of 0.63 in *n*-hexane/ethyl acetate (7:3). Its IR spectrum showed broad absorption band at 3290 cm^{-1} attributed to hydroxyl group and absorption band at 1656 cm^{-1} indicated the presence of carbonyl and stretching of unsaturated carbonyl group, respectively. Absorption peaks at 2965 cm^{-1} and 2918 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at1618 and 1516 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1210 cm^{-1} indicated C-O stretching vibrations. From ^{1}H-NMR (400 MHz, DMSO-d_6)(Appendix 29) spectrum revealed the presence of chelated hydroxyl peri to the hydroxyl group at δ 10.8(s, 1H, 5-OH), 9.0 (s, 1H, 7-OH), and aromatic protons at δ 8.3 (s, 1H, H-2), 7.95 (d, 1H, H-6'), 7.1 (s, 1H, H-2'), 6.9 (dd,1H, H-6 & 8) and 6.8 (d, 1H, H-5') shows the presence of flavone skeleton. The signal at δ 3.8 (s, 3H,) shows methoxy protons.

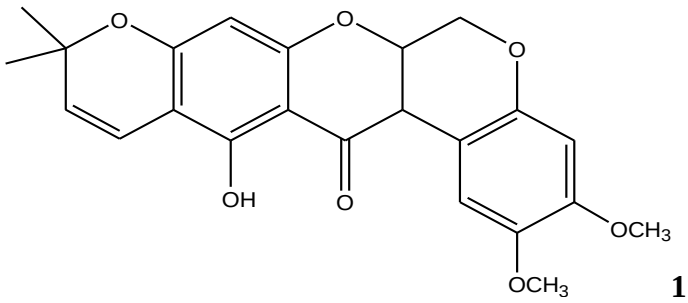
The ^{13}C-NMR spectrum (Appendix 30) showed a total of sixteen carbon signals, the quarternary carbons at δ 175.1 (C-4), 162.9 (C-7), 157.8 (C-5), 147.6 (C-3'), 146.9 (C-4'), 123.9 (C-1'), 123.4 (C-3), and δ 117.1 (C-4a), seven methines at δ 153.5 (C-2), δ 127.7 (C-6'), δ 121.9 (C-2'), δ 115.6 (C-5'), δ 113.7 (C-6), and δ 102.8 (C-8). The carbon at δ 56.1 shows a methoxy carbon. From DEPT-135 (Appendix 31), there are seven carbon signals. The signal δ at δ 153.5 (C-2), δ 127.7 (C-6'), δ 121.9 (C-2'), δ 115.6 (C-5'), δ 113.7 (C-6), and δ 102.8 (C-8). The carbon at δ 56.1 shows a methoxy carbon (Oliveria, et al 2017).



173

Compound **174** named as 12a-hydroxy-*β*-toxicarol (coded as Fr-12) was isolated as yellow solid with R_f value of 0.36 in *n*-hexane/ ethyl acetate (7:3).Its IR spectrum showed broad absorption band at 3350 cm^{-1} attributed to hydroxyl group and absorption band at 1693 cm^{-1} indicated the presence of carbonyl and stretching of unsaturated carbonyl group, respectively. Absorption peaks at 2925cm^{-1} and 2908 cm^{-1} shows the presence of sp^3 C-H stretching and sp^2 C-H stretching vibration medium peaks at1612 cm^{-1} correspond to aromatic ring C=C bond stretch. The absorption band at 1320 $cm^{-1}$and 1134 cm^{-1} indicated C-O stretching vibrations.

From^{1}H-NMR (400MHz, DMSO-d_6) (Appendix 32) spectrum revealed the presence of one hydroxyl at δ 8.3. Two methoxy groups at δ 3.6 and 3.7. Two methyl of pyran ring δ 1.2 and 1.3. diastereotopic protons of methylene group at δ 4.2 and 4.6 and pyranolefinic protons at δ 5.6 and 7.5. Methines at δ 5.1. The ^{13}C-NMR (Appendix 33) and DEPT-135 (Appendix 34) of compound methylene at δ 62, two methyls of pyran moieties, at δ 28ppm. Two methoxy at 55.6 and 56.5, methine at δ 72, pyran sp^3 quaternary carbon, carbonyl at 189, five oxygenated sp^2 quaternary carbon at δ 159.5, 156.9, 149.8, 148.8 and 143.7 and two CH of pyran ring at 129.9 and 128.5 (Chen et al., 2014).



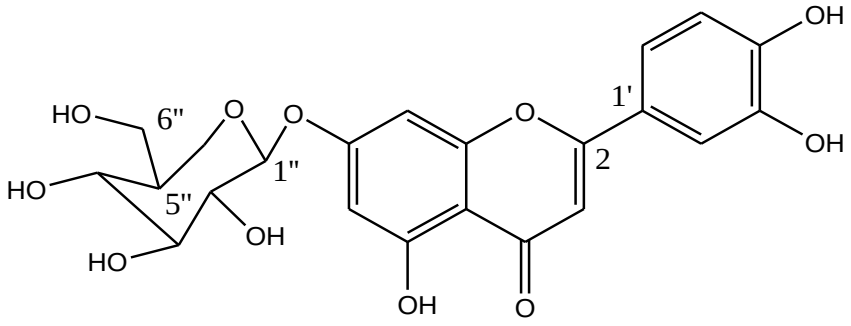
174

Compound **175** named as apigenin-7-*O*-β-D-glycoside (coded as TL7) was isolated as brown solid with R_f value of 0.27 in *n*-hexane/ethyl acetate (7:3). Its IR spectrum (KBr) exhibited the occurrence of hydroxyl (3423 cm^{-1}), a weak peak attributed to a stretching vibration of

aromatic C–H (3120 cm^{-1}), saturated hydrocarbon (2915 cm^{-1}), γ-pyrone carbonyl (1650 cm^{-1}), aromatic C=C (1609, 1590, 1510 cm^{-1}), aromatic hydroxyl (1371, 1276 cm^{-1}), aromatic C–H (1452 cm^{-1}), aromatic rings and glycoside C–O (1100, 1082, cm^{-1}) (Berashvili et al., 2005). An IR spectrum (KBr) at 3423, 1650, and 1590 cm^{-1} had the characteristic bands of apigenin.

^{1}H NMR spectra (Appendix 35, table 25) showed an apigenin skeleton on the basis of hydroxyl δ_H at 12.70 (s, OH-5), two doublets δ_H at 6.45(1H,d, J=2.0 Hz)and δ_H at 7.70 (d, J=2.10 Hz, H-8) on the A-ring; A_2B_2-type aromatic δ_H at 8.15 (1H, d, H-6', J=8.9 Hz)) and δ_H at 6.93 (1H, d, H-3', H-5', J=8.9 Hz)); together with an olefinicδ_H at 6.55 (s, H-3) on a flavone C-ring. ^{1}H NMR data fit exactly with previously reported data in the literature (Berashvili et al., 2005). Besides this, glycosidicδ_H at 5.33 (d, J=7.30 Hz, H-1") and δ_C at 99.1 (C-1") were evident in the ^{1}H and ^{13}C NMR spectra. The multipletδ_H at 3.25-3.5 (5H, m, H-3"–H-6") was assignable to the coupling between protons and methylene protons of the glucosyl ring. Proton δ_H at 3.8 (d, H-2"), hydroxyl δ_H at 5.33-5.50(1H, m) were assigned in the glycosidic ring.

Analyses of the ^{13}C NMR spectrum (Appendix 36, table 25) and DEPT-135 (Appendix 38) revealed the existence of 19 carbons, including a hexose moiety at δ_C (99.1, 72.1, 77.5, 66.5, and 64.5). The ^{13}CNMR spectrum also exhibited the presence of δ_C (149.5, 101.8, 164.6, and 93.9) for the A-ring, δ_C (104.3, 177.9, 161.6, and 115.8) for the C-ring, and δ_C (121.3, 131.4, 116.2, and 156.7) for the B-ring of the flavone. The glycosidic linkage at C-7 of the aglycon was viewed from the upfield shift of δ_C (C-7) as compared with apigenin, which can be further deduced from the existence of δ_C at 99.9 (C-1"). The large H-1" coupling constant of 6.8 Hz between H-1" and H-2" indicated a β-coupled glucose. Therefore, the structure of the purified compound was identified as apigenin-7-*O*-β-D-glycoside, in accordance with the reported data in the literature (Liu et al., 2012).



175

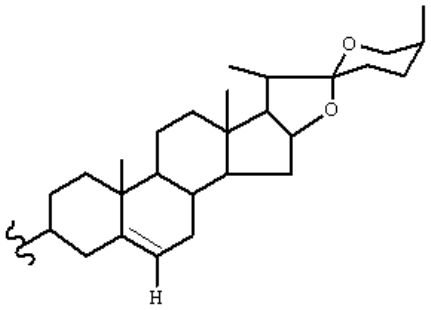
Table 25. The ^{1}H and ^{13}C spectra of Apigenin-7-*O*-*β*-D-glycoside (coded as TL7)

SN	Compound **175**		Liu et al., 2012	
2	-			163.4
3	104.3	6.55(1H,s)	6.87 (1H, s)	103.5
4	177.9			182.5
5	149.1			157.4
6	101.8	6.45(1H,d, *J*=2.0 Hz)	6.43 (1H, d, *J*=2.2 Hz)	100.3
7	164.6			164.7
8	93.9	-	6.82 (1H, d, *J*=2.2 Hz)	95.3
9	161.6			161.9
10	115.8			105.8
1’	121.3			121.4
2’	131.4	8.15 (1H, d, *J*=8.9 Hz)	7.95 (1H, d, *J*=8.9 Hz)	129.1
3’	116.2	6.93 (1H, d, *J*=8.9 Hz)	6.93 (1H, d, *J*=8.9 Hz)	116.5
4’	156.7			161.6
5’	116.2	6.93 (1H, d, *J*=8.9 Hz)	6.93 (1H, d, *J*=8.9 Hz)	116.5
6’	131.4	8.15 (1H, d, *J*=8.9 Hz)	7.95 (1H, d, *J*=8.9 Hz)	129.1
5-OH		12.7(1H, s, OH-5)	12.97 (1H, s, OH-5)	
4-OH		-	10.51 (1H, s, OH-4')	
1’’	99.1	5.33(1H,d, 7.34Hz)	5.44 (1H, d, *J*=7.4 Hz)	99.9
2’’	72.1	3.8(1H,d,J=10.1Hz)	3.71 (1H, d, *J*=10.3 Hz, H-2")	73.5
3’’	77.5 very short	3.25-3.5(5H,m)	3.27–3.47 (5H, m, *J*=6.8 Hz, glucose H-3", -4", -5", -6")	77.6
4’’	66.5	5.33-5.50(1H, m, Hydroxyl of glucose moieties)	5.12 (1H, s, OH-2"), 5.07 (1H, s, OH-3"), 5.05 (1H, s, OH-4"), 4.65 (1H, s, OH-6")	69.9
5’’	-			76.9
6’’	64.7		4.6(2H, d, *J*=8.9 Hz)	63.5

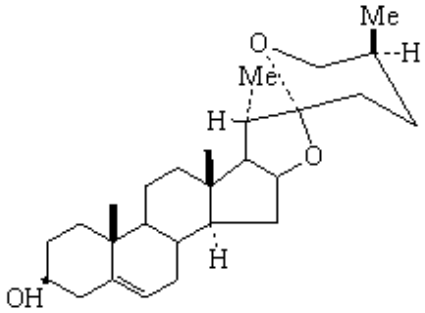
4.2.4 Characterization of isolated compound from roots of *Balanites egyptica*

Compound **176** was obtained as a yellowish powder with R_f value of 0.46 under 70/30 *n*-hexane/ethyl acetate solvent system. The ^{1}H NMR spectrum (DMSO-d_6) didn’t come out well resolved and most peaks are in aliphatic region. However, the ^{13}C NMR spectrum

(appendix 39) revealed some peaks but yet it requires more scanning hours. Methylene carbon resonance at δ 68, the sp^3 oxygenated methine at δ79.1 (C-3) and the fully substituted carbon signal at δ 100.07 (C-22) were observed. The later is in good agreement with spirocyclic carbon which has two oxygen substituents. A methane carbon was observed at δ 71 suggesting it is connected to oxygen. A methine signal (C-20) was also shifted downfield to δ 42.0 due to being in close proximity to an oxygen atom. Signals are observed at δ56.22 (C-9), δ 50.00 (C-14) and δ 59.99 (C-17) are for sp^3 quaternary carbons. The presence of a tri-substituted double bond was observed at δ 121.8 (C-6) and a fully substituted carbon resonance at δ 140.6 (C-5). Thus, based on the above spectral data and comparison with the literature value, partial structure (**176**) of the compound has been suggested.



176



8

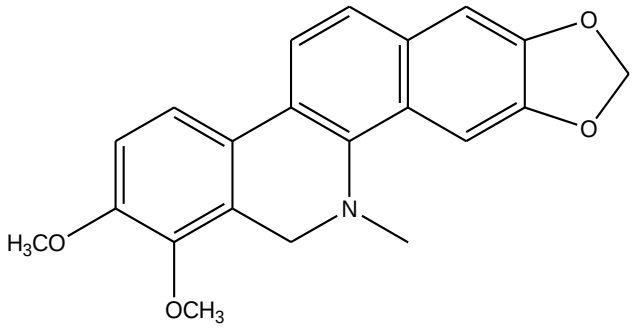
Comparison with literature report and class of compounds isolated from the genus *Balanites*, the isolated compound is closer to Yamogenin (**8**). However, since the ^{13}C NMR spectra did not pick well and all carbons peaks are not seen perfectly. Thus, we couldn't be able to assign the chemical shifts to the proposed partial structure.

4.2.4 Characterization of compounds from roots of *Zanthoxylum chalybeum*

The following three alkaloids (**177-178a**) were isolated and charachterized from alkaloids roots extracts of *Zanthoxylum chalybeum* (appendix 40-44).

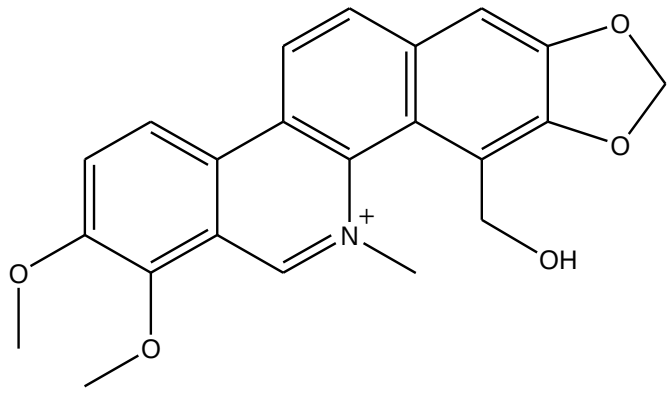
Compound **177** was obstained as a white solid with a positive dragendorff's reagent and 2,4-dinitrophenylhydrazine. ^1H NMR spectrum (appendix 40) has two signlets each integrating to three protons at δ 3.93 and 3.96 corrpsponding to two methoxy groups. Two singlet prorotns observed at δ 6.0, charachteristic of methylene dioxy group. The methylene adjacent to carbonyl group appear as doublet of doublet at δ 2.26 and 2.59, diasterotopic protons suggesting adjacent to chiral center. The proton at C-6 appears at δ 5.05 as doublet of doublet

(*J*= 11.3 and 3.8 Hz). Two pairs of ortho coupled doublets at δ 6.95, 7.7 and 7.48 and 7.53 and two singlets at δ 7.1 and 7.52 appear in aromatic region. ^{13}C NMR spectrum (appendix 41) showed δ 31.0 (CH_3), 42.8 (NCH_3), 46.8 (CH_2). 54.9 (CH), 55.8 (OCH_3), 60.9 (OCH_3), 110.6 (CH), 101.2 (CH_2), 104.3 (CH), 116 (CH), 118.8 (CH), 119.7 (CH), 123.3, 124.8, 127.3, 128.1, 131.0, 139.2, 145.5, 147.6, 148.1, 152.1, and 207.4. From the aobe spectral data, the compound was found to be dihydrochelerythrin (**177**).



177

Spectral features of compound **178** are comparable to that of dihydrochelethrin (**177**), except that additional methylene peak was observed at δ 60 ppm (appendix 43), also supported by DEPT-135 (appendix 44) pointing down. A singlet peak observed at δ 7.52 in case of **177** (appendix 42), now disappeared suppesting that the possible position of this oxymethyele is at C-4 position. Thus, based on the above spectral features the following structure (**178**) was proposed.

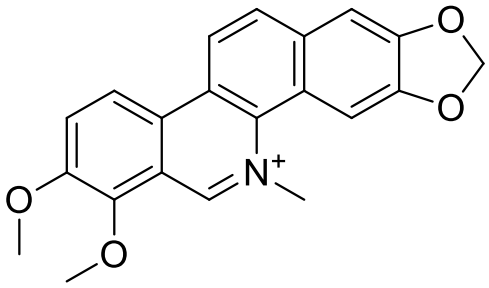


178

Compound **179** is a yellow crystalline obtained as eluted with 100% dichloromethane of acid-base extraction of the root of Z. chalybeum. The TLC fluoresced yellow under UV (365 nm). The ^{1}H NMR (300 MHz, $CDCl_3$, appendix 44a) spectrum revealed two singlet peaks at δ6.35 (2H), δ5.00 (3H) and suggesting the presence of a methyl and methylene protons attached to

an electronegative atom. Two singlet peaks observed at δ 4.23 (3H), 4.18 (3H) belong to two methoxy groups. Protons at δ6.35 (2H) is a characteristic of methylenedioxy group, also supported by DEPT-135 pointing dawn at δ103.2 in agreement with a methylene connected to hetroatom. A pair of ortho- para coupled aromatic protons at signals at δ 8.85 and δ 8.83 (dd, 2H, *J*= 10 Hz) coupled with a Protons at δ8.32and δ 8.30 (dd, 2H, J=10 Hz) and a singlet peak at δ7.78 indicated that the presence of methine aromatic singlet protons.

The ^{13}C NMR spectrum (appendix 44b) with the aid of DEPT-135 spectrum (appendix 44c) showed six quaternary carbons at δ 119.7, 120.6, 125.7, 128.5, 132.1, and 132.7 five oxygenated quaternary carbons at δ 145.9 149.2, 149.1, and 151.0 are well resolved. And also six methine carbon signals were observed δ 103.2, 104.7, 106.2, 119.2, 119.8, 126.5, 131.5 and 151.2 in the aromatic system. Two methylene carbon signals were observed at δ 101.1 which indicated the presence of methylenedioxy group and at δ 151.2 indicated that the methine carbon attached with the electronegative nitrogen atom. And three signals at δ 52.7, 57.5 and 62.7 corresponding to methyl group attached with heteroatom nitrogen and two methoxy group existence in the compound respectively. Based on the above spectroscopic evidences as well as comparison with literature the structure of the compound was found to be identical with chelerythrine (**179**) (Marek and Dommisse, 1999).

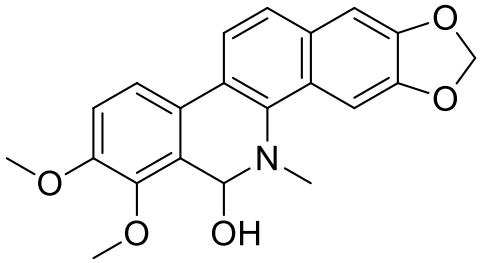


179

Compound **180** is a yellow crystalline obtained as eluted with 98:2 dichloromethane in methanol of acid-base extraction of the root of *Z. chalybeum*. The TLC fluoresced yellow under UV (365 nm). The ^{1}H NMR (300 MHz, $CDCl_3$, appendix 44d) spectrum revealed two singlet's peaks at δ 7.93, δ 7.17 and suggesting the presence of a methane aromatic and two singlet's at δ 6.61 (2H), δ5.30 (1H) and for aliphatic methine protone linked with electronegative atom and probably with aromatic ring, and O-H protons. At δ 6.12 (d, J=1.2 Hz) and at 6.11 (d, J=1.2 Hz) two protons assigned for methylene attached to an electronegative atom. Three singlet peaks observed at δ 3.77 (3H) and 3.05 (3H) belong to

two methoxy groups and 2.41 (3H) for N-CH_3. Protons at δ 6.11 and 6.12 (2H) is a characteristic of methylenedioxy group, also supported by DEPT-135 pointing dawn at δ102.2 in agreement with a methylene connected to hetroatom. A pair of ortho- para coupled aromatic protons at signals at δ 8.85 and δ 8.83 (dd, 2H, *J*= 10 Hz) coupled with a Protons at δ 8.32 and δ 8.30 (dd, 2H, J=10 Hz) and a singlet peak at δ 7.78 indicated that the Presence of methine aromatic singlet protons.

The ^{13}C NMR spectrum (appendix 44d) with the aid of DEPT-135 spectrum (appendix 44e) showed six quaternary carbons at δ 125.0, 125.7, 126.4, 130.7, and 137.79 four oxygenated quaternary carbons at δ 145.9 147.0, 147.6, and 151.0 are well resolved. And also six methine carbon signals were observed δ 100.4, 100.6, 111.8, 118.2, 119.3, and 122.8 in the aromatic system. Two methylene carbon signals were observed at δ 104.0 which indicated the presence of methylenedioxy group and at δ 77.4 indicated that the methine carbon attached with the electronegative nitrogen atom. And three signals at δ 40.8, 55.2 and 59.9 corresponding to methyl group attached with heteroatom nitrogen and two methoxy group existence in the compound respectively. Based on the above spectroscopic evidences as well as comparison with literature the structure of the compound was found to be identical with 6-hydroxydihydrochelerythrine (**180**) (Sandjo et al, 2014).

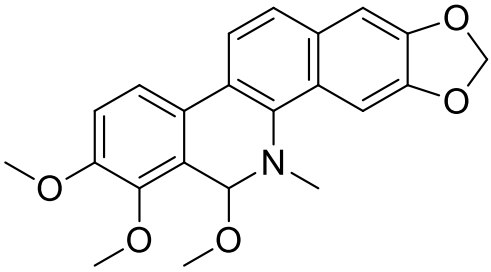


180

Compound **181** is a yellow crystalline needles obtained as eluted with 99:1 dichloromethane in methanol of acid-base extraction of the root of *Z. chalybeum*. The TLC fluoresced yellow under UV (365 nm). The ^{1}H NMR (300 MHz, $CDCl_3$, appendix 44g) spectrum revealed two singlet's peaks at δ 7.70 and δ 7.713 suggesting the presence of a methane aromatic. At δ 6.02 (d, J=1.2 Hz) and at 6.02 (d, J=1.2 Hz) two protons assigned for methylene attached to an electronegative atom, and peak at δ 5.30 (*s*) (1H) for aliphatic methine proton linked with electronegative atom and probably with aromatic ring. Three singlet peaks observed at δ 3.96 (3H), 3.93 (3H) and 3.93(3H) belong to two methoxy groups and 2.77 (3H) for N-CH_3.

Protons at δ 6.02 and 6.02 (2H) is a characteristic of methylenedioxy group, also supported by DEPT-135 pointing dawn at δ 102.2 in agreement with a methylene connected to hetroatom. A pair of ortho- para coupled aromatic protons at signals at δ 7.78 (d, 1H), δ 7.76 (dd, 1H) coupled with a Protons at δ7.48 (dd, 1H) and δ 7.05 (d, 1H).

The ^{13}C NMR spectrum (appendix 44h) analyzed with the aid of DEPT-135 spectrum (appendix 44i) showed six quaternary carbons at δ 124.8, 125.7, 126.7, 131.0, and 138.3 four oxygenated quaternary carbons at δ 146.6 147.3, 147.9, and 152.0 are well resolved. And also six methine carbon signals were observed δ 100.4, 100.6, 112.9, 118.9, 120.0, and 123.4 in the aromatic system. one methylene carbon signals were observed at δ 101.0 which indicated the presence of methylenedioxy group and one carbon at δ86.0 indicated that the methine carbon attached with the electronegative nitrogen atom. And four signals at δ 40.6, 53.9, 55.9 and 61.6 corresponding to methyl group attached with heteroatom nitrogen and three methoxy group existence in the compound respectively. Based on the above spectroscopic evidences as well as comparison with literature the structure of the compound was found to be 6-hydroxydihydrochelerythrine (**181**) (Sandjo et al, 2014).



181

Essential oil analysis of *Z. chalybeum*

About 19 chemical components of the dried fruit of *Z. chalybeum* essential oils were identified and quantified by GC-MS (table 20). The major chemical constituent is Tricyclo [5.3.0.0(3,9)]decane (82.8 %).

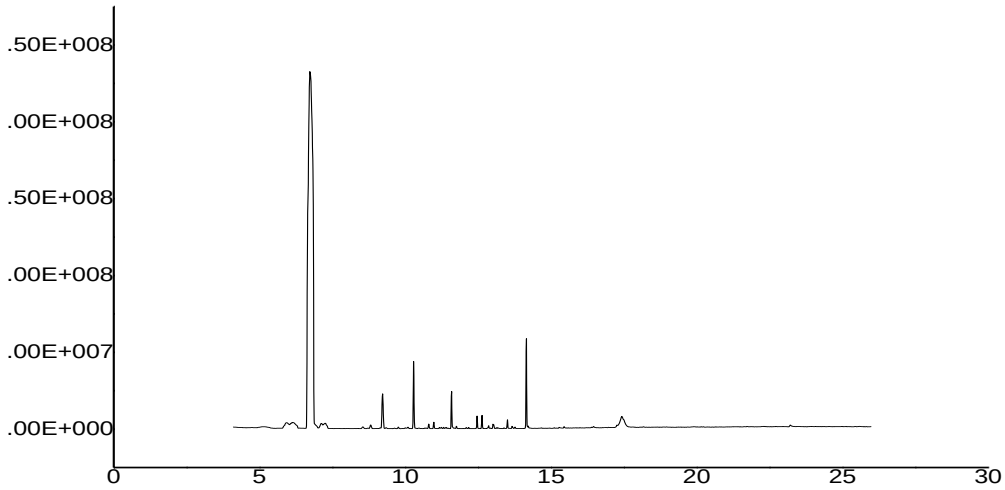
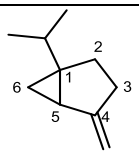
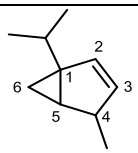
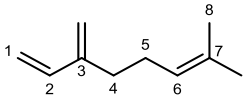
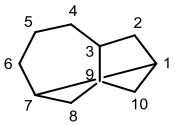
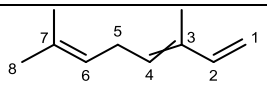
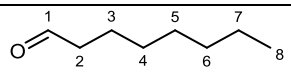
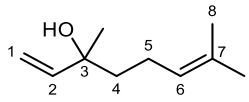
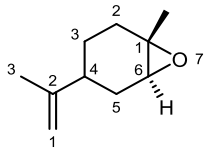
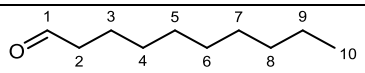
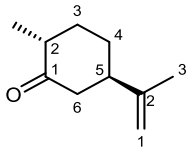
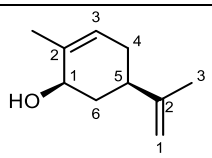
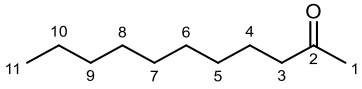
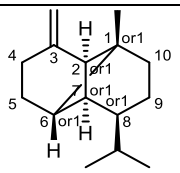
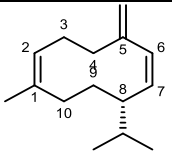
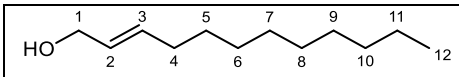
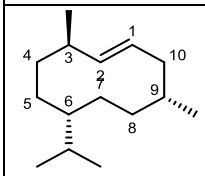
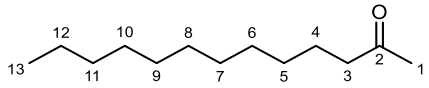


Table 26. Composition of essential oils from fruits of *Z. chalybeum*

Name of chemical constitutes	**RT**	**Percent**
 Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)	5.94	1.13
 Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)	6.11	0.49
 ß-Myrcene	6.15	1.03
 Tricyclo[5.3.0.0(3,9)]decane	6.74	82.40
 ß-Ocimene	7.12	0.59

 Octanal	7.25	0.83
 Linalool	8.82	0.25
 Limonene oxide, trans	9.22	2.17
 Decanal	10.2914	2.6206
 Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans	10.81	0.19
 2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis	10.98	0.23
 2-Undecanone	11.59	1.29
β-copaene	12.47	0.44
 (1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel	12.64	0.47
 (S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	12.87	0.11

 trans-2-Dodecen-1-ol	13.02	0.25
 Germacrene D	13.51	0.29
 2-Tridecanone	14.16	2.78
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	17.43	2.40

4.2.5 Characterization of isolated compounds from roots of *Clausenea anisata*

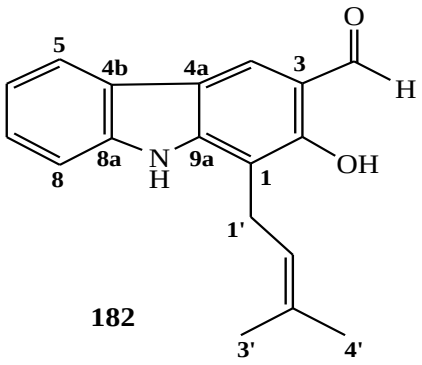
Compound **182** was obtained as a brown powder (mp: 227-228 °C) with R_f value of 0.52 in *n*-hexane/EtoAc (8:2) as eluent. The IR (KBr disk, appendix 45) spectrum showed broad vibration at 3290 cm^{-1} attributed to hydroxyl (OH), and sharp absorption at 1608 cm^{-1} attributed to a carbonyl moiety. The ^1H NMR spectrum ($CDCl_3$, 400 MHz, table 27, appendix 46) showed signals for a singlet proton at δ 11.68 (1H, *s*, OH) indicative of hydroxyl (OH) group. The downfield chemical shift of the hydroxyl group suggests the presence of intermolecular hydrogen bonding (*Peri* effect).The presence of singlet peak at δ 9.94 (1H, *s*, CHO) aldehyde proton. The presence of four aromatic protons observed at δ 7.28 (1H, *dd*, H-6), 7.43 (1H, *dd*, H-7), 7.95 (1H, *dd*, H-5, *J*=7.75Hz) and 7.99 (1H, *dd*, H-8) indicates the presence of disubstituted aromatic ring. The presence of two aliphatic methyl protons at δ 1.91 (3H, *s*, H-4') and δ 1.80 (3H, *s*, H-5'), olefinic proton at δ 5.35 (1H, *t*, J=5.96 Hz) and benzylic methylene protons at δ 3.66 (*d*, H-1', *J*=6.90 Hz) suggest the presence of a prenyl group in the compound. Moreover, the presence of a downfield singlet aromatic proton at δ 8.07 suggests that this proton is experiencing anisotropic effect of the aldehyde carbonyl. The above chemical shift positions for the aromatic singlet proton (H-4), and downfield chemical shift of hydroxyl moiety allow for unequivocal assignment of aldehyde moiety at C-3 between two carbons bearing proton H-4 (C-4) and that of C-2 bearing hydroxyl group.

The ^{13}C NMR spectrum ($CDCl_3$, 100 MHz, table 27, appendix 47) in combination with DEPT-135 (appendix 48) showed the presence of 18 carbons. Among these, six signals are

due to methine carbons, eight quaternary, one benzylic methylene, two methyls and one carbonyl. The ^{13}C NMR spectrum showed peak at δ 195.4 due to carbonyl group of aldehyde moiety. Oxygenated sp^2 quaternary carbon was observed at δ 157.9 (C-2). The remaining carbons of the aromatic methine carbons were observed at δ 125.9 (C-4), 119.8 (C-5), 123.7 (C-6), 120.9 (C-7) and 110.9 (C-8). Furthermore the spectrum displayed signals due to quaternary carbons at δ 109.1 (C-1), 115.5 (C-3), 117.4 (C-4a), 125.3 (C-4b), 134.2 (C-3'), 140.2 (C-8a) and 145.1 (C-9a). The prenyl moiety appeared at δ 22.8 (C-1'), 121.3 (C-2'), 134.2 (C-3'), δ 25.9 (C-4') and 18.2 (C-5'). Thus, based on the above spectral data and comparison with literature the structure of compound **1** was found to be a derivative of carbazole alkaloid (**182**) known by trivial name heptaphyline (Chaichantipyuth et al., 1988) where the later have a hydroxyl group at C-7 position.

Table 27.^{1}H-NMR ($CDCl_3$, 400 MHz), ^{13}C-NMR and DEPT-135 (100 MHz) spectral data of compound **182**

Position	**^{1}H NMR**	**^{13}C NMR**	**DEPT-135**	Chaichantipyuth et al., 1988	
				^{1}H NMR	**^{13}C NMR**
CHO	9.94, *s*	195.4	195.4	9.90, *s*	196
2(-OH)	11.68, *s*	157.9	-	-	156.3
NH	8.19, *s*	-	-	-	-
8a	-	140.2	-	8.25, *s*	142.3
9a	-	145.1	-	-	144.8
3	-	115.5	-	-	114.7
4	8.05, *s*	125.9	125.9	-	124.3
5	7.95,1H (*d* ,*J*=7.75,2.1Hz)	119.8	119.8	8.00, *d*	120.5
4b	-	125.3	-	-	116.3
6	7.41, *s*	123.7	123.7	-	108.5
7	7.43 (1H ,*dd*, *J*=7.75, 2.1Hz)	120.9	120.9	-	158.5
8	7.99 (1H, *dd*, *J*=8, 2.1Hz)	110.9	110.9	-	95.6
4a	-	117.4	-	-	117.0
1	-	109.1	-	-	108.9
1'	3.66, (1H ,*d* ,*J*=6.90Hz)	22.9	22.9	3.65, *d* ,*J*=7 Hz	22.6
2'	5.35, 1H (*t*)	121.3	121.3	5.35, *t*, *J*=6 Hz	121.6
3'	-	134.2	-	-	131.7
4'	1.91, *s*	25.7	25.8	1.66, *s*	25.7
5'	1.80, *s*	18.2	18.2	1.82, *s*	17.9



182

Compound **183** was obtained as a brown crystalline powder with R_f value of 0.56 in *n*-hexane/EtoAc (7:3) solvent system. The IR (KBr disk, appendix 49) spectrum showed broad vibration at 3419 cm^{-1} due to the presence of the hydroxyl moiety. The strong sharp vibrations at 1617 cm^{-1} and 1100 cm^{-1} suggest the presence of olefinic C=C and carbon-oxygen (C-O), respectively. Moreover, intense vibrations at 2849 $cm^{-1}$and 2930 cm^{-1} indicate C-H vibrations of methylene (sp^2) and methyls (sp^3), respectively.

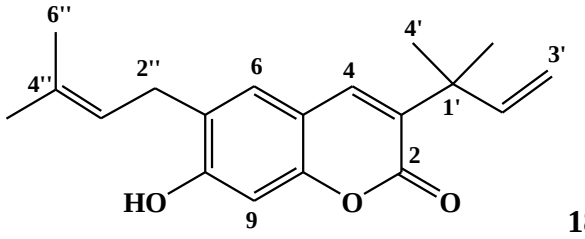
The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, table 28, appendix 50) revealed peaks at δ 7.54 (1H, *s*, H-4), δ 7.193 (1H, s, H-6) and δ 6.955 (1H, s, H-9) attributed to aromatic protons. The presence of prenyl moiety was confirmed on the basis of methylene protons (H-2'') adjacent to olefinic carbon, olefinic proton (H-3'') and two methyl groups were observed at δ 3.38, 5.34 (1H, *t*, H-3'') and δ 1.78 (3H, *s*, H-5'') and 1.81(3H, *s*, H-6''), respectively. Peak at δ 6.18 (1H, *dd*, J=10.8, 17.6 Hz, H-2') attributed to olefinic proton adjacent to terminal olefinic methylene protons at δ 5.09 (1H, H-3'') and δ5.07 (1H, H-3''). Symmetrical methyl protons were observed at δ1.48 (3H, s, H-4', 5'). The presence of two singlet aromatic proton at δ 7.19 (1H, s, H-6) and δ 6.95 (1H, s, H-9) coupled with a downfield proton at δ 7.54 (1H, *s*) are in good agreement with a chromene moiety where later (H-4) is located at β-position of the lactone moiety whereas H-6 and H-9 are located at 1,4-positions of the aromatic ring of chromene skeleton.

The ^{13}C NMR ($CDCl_3$ 100 MHz, table 28, appendix 51) spectrum in combination with DEPT-135 showed a resonance for 18 carbon atoms. Among these, five signals are due to methine carbons, eight quaternary, three methyl and two methylene carbons. The most downfield signals appearing at δ 160.7 attributed to the ester carbonyl whereas the quaternary carbons appearing at δ 157.3 (C-8) and δ 153.3 (C-10) were assigned to sp^2 oxygenated quaternary carbons. Methine aromatic carbons were observed at δ 138.3 (C-4), 102.5 (C-9), 128.2 (C-6), δ 121.2 (C-3'') and 145.6 (C-2'). The methyl signals due to C-5''

and C-6'' were observed at δ 25.8 and 17.9, respectively.Symmetrical carbons signals were also observed for C-4' and C- 5'at δ 26.2, while the methylene signals were observed at 28.6 (C-2'') and 112.8 (C-3'). Furthermore the spectrum displayed signals due to quaternary carbons at δ 131.2, 112.0, 124.9, 135.0 and 40.3 assigned to C-3, C-5, C-7, C-4'' and C-1', respectively. Thus, based on the above spectral data and comparison with literature (Kumar et al., 1995) the structure of compound **183** was proposed to be a chromene skeleton shown below.

Table 28. The ^{1}H NMR ($CDCl_3$, 400 MHz), ^{13}C NMR ($CDCl_3$, 100 MHz) and DEPT-135 spectral data of compound **183**

Position	**^{1}H NMR**	**^{13}C NMR**	**DEPT-135**	Kumar et al., 1995	
				^{1}H NMR	**^{13}C NMR**
2	-	160.7		-	161.1
3	-	131.3		-	131.9
4	7.54,1H ,*s*	138.3	138.3	7.54,1H,*s*	138.5
5	-	112.0		-	112.1
6	7.19,1H,*s*	128.2	128.2	7.17,1H,*s*	128.2
7	-	124.9		-	125.4
8	-	157.3		-	157.5
9	6.95,1H,*s*	102.5		7.04,1H,*s*	102.5
10	-	153.3		-	153.2
1'	-	40.3		-	40.31
2'	6,16, (1H, *dd*, *J*=10.8,17.6)	145.6	145.6	6.16,1H,dd,J=10.2,18Hz	145.6
3'	5.09, 5.07 (2H,dd. *J*=10.2,18Hz)	112.8	112.8	5.1 (2H, *dd, J*=10.2,18Hz)	112.7
4' ,5'	1.48, 6H, *s*	26.2		1.48 (6H, *s*)	26.1
2''	3.38 (2H, *d, J*=7.2Hz)	28.6	28.6	3.38 (2H, *d, J*=7.2Hz)	28.4
3''	5.33 (1H, *t*)	121.2		5.33,1H, *m*	121.3
4''	-	135.0	25.8	-	134.6
5''	1.80 (3H, *s*)	25.8		1.80 ,3H, *s*	25.8
6''	1.78 (3H, *s*)	17.9	17.9	1.75 ,3H, *s*	17.9



183

Compound **184** was isolated as orange powder with R_f value of 0.59 in *n*-hexane/EtoAc (6:4) as solvent system. The IR (KBr disk, appendix 52) spectrum showed broad absorption band at 1723 cm^{-1}, 1297 cm^{-1} and 2917 cm^{-1} attributed to C=C stretching of aromatic ring, C-O

stretching, and C-H stretching of methyl group, respectively. The ^{1}H NMR ($CDCl_3$, 400 MHz, table 29, appendix 53) spectrum showed two proton doublets at δ 6.36 (1H, *d*, J=9.6 Hz) and 7.77 (1H, *d*, J=9.6 Hz) attributed to olefinic protons of which one of them is downfield due to β-position of lactone moiety. Two oleifnic protons were observed at δ 7.69 (1H, *d*, H-2'', *J*=2.4 Hz) and 6.82 (1H, *d*, H-3'', *J*=2.4 Hz) coupling to each other suggesting the presence of a furan ring attached to the aromatic ring. The presence of singlet aromatic proton was observed at δ 7.36 (1H, *s*, H-6). The presence of prenyl group was confirmed based on peaks of two methyl signals at δ 1.72 (3H, *s*, H-4') and δ 1.74 (3H, *s*, H-5'), olefinic proton at δ 5.65 (1H, *t*, H-2') and oxygenated methylene at δ 5.0 (2H, *t*). The later suggests that the prenyl group is attached to oxygen. Moreover, the above ^{1}H NMR pattern suggests the compound has chromene skeleton with a furan ring fused to it.

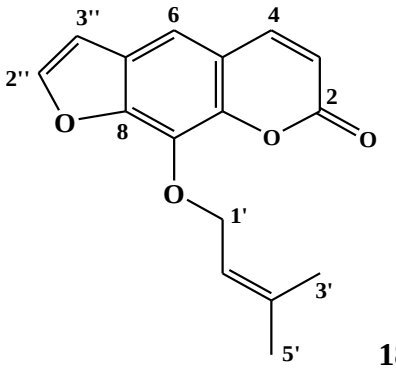
The ^{13}C NMR ($CDCl_3$, 100 MHz, table 29, appendix 54) and DEPT-135 (Appendix 55) spectrum showed a total of sixteen carbon atoms. The downfield chemical shift signal that appeared at δ 160.5 coupled with signals at δ 114.6 and 143.8 suggest α,β-conjugated lactone moiety. The other five quaternary carbons at δ 116.5, 125.9, 131.6, 144.5 and 148.6 were assigned to C-5, C-7, C-9, C-10 and C-8, respectively. Of these, three of the carbons are sp^2 oxygenated quaternary carbons i.e. C-8, C-9 and C-10 of aromatic ring.

The methine carbons of the furan moiety were observed at δ 146.6 (C-2'') and 106.7 (C-3'') of which the downfield chemical shift value of the former in agreement with its attachment to the oxygen atom. The aromatic methane at C-6 appeared at δ 113.2. Oxygenated methylene of the prenyl moiety appeared at δ 70.2 (also confirmed by DEPT-135 pointing downwards) whereas the remaining carbons of prenyl moiety group appeared at δ 119.8 (C-2'), 139.7 (C-3'), 25.8 and 18.2 (C-4' and C-5'), respectively. Thus, based on the above spectral features compound **184** was found to be in good agreement with a chromene skeleton known by trivial name imperatorin (**184**) (Muller et al., 2004).

Table 29.^{1}H NMR ($CDCl_3$, 400 MHz), ^{13}C NMR and DEPT-135 (100 MHz) spectral data of compound **184**

Position	**^{1}H NMR**	**^{13}C NMR**	**DEPT-135**	**Muller et al., 2004**	
				^{1}H NMR	**^{13}C NMR**
2	-	160.5	-		160.6
3	6.36, 1H, *d* (J=9.6 Hz)	114.6	114.6	6.36, *d*	113.0
4	7.77, 1H, d (J=9.4 Hz)	143.8	143.8	7.75, *d*	144.0
5	-	116.5			116.4

6	7.36, 1H, *s*	113.2	113.2	7.35, *s*	114.8
7	--	125.8	-	-	126.0
8	-	148.6	-	-	148.6
9	-	131.6	-	-	132.0
10	-	144.4	-	-	143.8
2''	7.69, 1H, *d* (j=2.4)	146.6	146.7	7.68, *d*	146.6
3''	6.82, 1H, *d* (J=2.05)	106.7	106.7	6.82, *d*	106.7
1'	5.00, 2H, *d* (J=7.10)	70.2	70.2	4.95, *d*	69.9
2'	5.61, 1H, *t* (7.35)	119.8	119.8	5.61, *t*	119.6
3'	-	139.7	-	-	139.7
4'	1.72, 3H, *s*	25.8	25.8	1.68, *s*	25.9
5'	1.74, 3H, *s*	18.1	18.1	1.73, *s*	18.2



184

Compound **185** was obtained as a yellowish amorphous powder (mp 175-176 °C) with R_f value of 0.53 in *n*-hexane/ethyl acetate (4:6) solvent system. The IR (KBr disk, appendix 56) spectrum showed broad vibration at 3385 cm^{-1}, sharp absorptions at 1600 cm^{-1} and 1255 cm^{-1} attributed to hydroxyl moiety (OH), aromatic benzene ring and C-O stretching, respectively. The strong absorption band at 2925 cm^{-1} showed the presence of the C-H stretching of sp^3 aliphatic moiety. The absorption band at 1730 cm^{-1} showed the presence of the C=O stretching of carboxyl moiety.

The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz, table 30, appendix 57) revealed the presence of aromatic protons at 7.178 (1H, *s*, H-5), and 6.69 (1H, *s*, H-8) suggesting two para oriented aromatic protons whereas downfield chemical shift of proton at δ 7.48 (1H, *s*, H-4) suggest the β-position of the α,β-conjuagated system. The peaks at δ 3.19 (1H, *s*, H-3') and δ 3.17 (H, *s*, H-3'*)* suggest the presence of distereotopic methyelene protons adjacent to asymmetric carbon (C-2'). This coupled with the presence of oxygenated methine signal at δ 4.73 (1H, *t*) suggest the presence of furan ring. Methyl signals were observed at δ 1.47 (6H, *s*, H-4'', 5''), δ1.37 (3H, *s*, H-5') and δ 1.27 (3H, *s*, 6'). The presence of terminal olefinic protons at δ 5.09

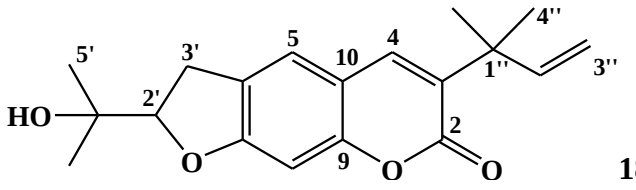
(2H, *dd*, H-3'') coupled with olefinic proton at δ 6.17 suggest the presence of rearranged prenyl moiety in the compound. The above ^{1}H NMR pattern suggests that the compound has coumarin skeleton where a reduced furan ring moiety is fused to the aromatic ring and α,β-conjugated lactone ring bearing the rearranged prenyl group.

The ^{13}C NMR spectrum (table 30, appendix 58) and DEPT-135 (appendix 59) revealed a total of eighteen carbon signals of which the downfield peak at δ 162.3 is attributed to ester carbonyl group whereas the sp^2 oxygenated quaternary aromatic carbons appeared at δ 160.3 (C-7) and δ 154.7 (C-9). The signal at δ 71.7 (C-4') assigned to the oxygenated sp^3 quaternary carbon. The signal for remaining quaternary carbons were observed at δ 130.8 (C-3), 124.3 (C-6), 113.1 (C-10) and 40.3 (C-1''). Methine carbons appeared at δ 138.1, 123.4, 97.2, 90.0 and 145.8 were assigned to C-4, C-5, C-8, C-2'and C-2'', respectively. Furthermore, the spectrum displayed signals due to methylene carbons at δ 29.7 and δ 112.1 assigned to C-3' and C-3'', also confirmed by DEPT-135 pointing downwards. Symmetrical carbons signals were observed for C-4'' and 5''at δ 26.1 and remaining methyl signals were also observed at δ 26.0 for (C-5') and 24.3 (C-6'). Thus, based on the above spectral features compound **185** was found to be in good agreement with a chromene skeleton known by the trivial name chalepin (**185**) (Orlita et al., 2008).

Table 30. ^{1}H NMR ($CDCl_3$, 400 MHz), ^{13}C NMR and DEPT-135 (100 MHz) spectral data of compound **185**

Position	**^{1}H NMR**	**^{13}C NMR**	**DEPT-135**	Orlita et al., 2008	
				^{1}H NMR	**^{13}C NMR**
2	-	162.3	-	-	162.3
3	-	130.8	-	-	130.9
4	7.48 (1H, *s*, H-4)	138.1	138.1	7.48, 1H, *s*	138.1
5	7.17 (1H, *s*, H-5)	123.3	123.25	7.20, 1H, *s*	123.3
6	-	124.6	-	-	124.6
7	-	160.3	-	-	160.2
8	6.68 (1H, *s*, H-8)	97.1	97.1	6.71, 1H, *s*	97.1
9	-	154.6	-	-	154.6
10	-	113.1	-	-	113.1
2'	4.73 (1H, *dd*, *J*= 10, 6 Hz))	90.9	90.9	4.72 (1H, *t*, *J*=12,6Hz)	90.9
3'	3.19 (2H, *dd*, *J*=18,8Hz)	29.7	29.7	3.21 (2H, *dd*)	29.6
4'	-	71.7	-	-	71.7
5'	1.37 (3H, *s*)	26.0	26.02	1.37 (3H, *s*)	26.0
6'	1.27 (3H, *s*)	24.21	24.21	1.27 (3H, *s*)	24.2
1''	-	40.3	-	-	40.3
2''	6.17 (1H, *dd*, *J*=10.8, 6.4Hz)	145.6	145.6	6.17,1H, *dd*, *J*=18,12Hz)	145.6

3''	5.10 (2H, *m*, H-3'')	112.1	112.1	5.09 (2H, *dd*)	112.1
4'', 5''	1.47 (6H, *s*, H-4'',5'')	26.2	26.2	1.47 (6H, *s*)	26.11



185

4.2.6 Charachterization of compounds from roots of *Euphorbia schimperiana*

The following three acridone alkaloids were isolated from roots of *Euphorbia schimperiana*. Details of chrachterization are presented here below.

Compound **186** was isolated as yellow powder. It's ^{1}H NMR spectrum (table 31, appendix 60) revealed a singet peak at δ 6.0 suggesting the presence of oxymethylene. Methoxy group and methyl attached to nitrogen are observed at δ 3.8 and 4.5, respectively. Aromatic peaks are observed at δ 8.5 (dd, J= 8, 2), 7.7 (*dd*, J= 7.8,2), 7.4 (dd, J= 7.8,2) and 7.2 (dd, J= 8,2) suggesting a disubstituted aromatic ring with AA'BB' spin system. The downfield chemical shift value of one of the aromatic peak at 8.5 suggests a peri effect to carbonyl carbon. In addition, peak at δ 6.5 (s) suggest a singlet aromatic peak. In agreement with the ^{1}H NMR spectrum, the ^{13}C NMR spectrum (table 31, appendix 61) revealed peaks at δ 35.0 (methyl attached to nitrogen), δ 61.0 (methoxy), and aromatic methine at δ 95 and twelve aromatic peaks. Carbonyl peak was observed at δ 178.0.Two vicinal oxygenated aromatic sp^2 quaternary carbons appeared at δ 141.9 and 142.1 confirming oxymethylene substituent. Thus, based on the above spectra and comparison with literature the structure of the compound was suggested to be an acridone alkaloid with trivial name evoxanthine (**186**) previously isolated from *Teclea amanuensis* (Joseph et al., 2008).

Table 31. ^{1}H NMR ($CDCl_3$, 400 MHz), ^{13}C NMR and DEPT-135 (100 MHz) spectral data of compound **186**

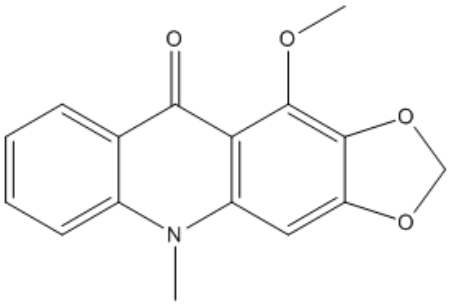
Position	**186**		**187a** (Joseph et al., 2008)	
1		153.6	-	152.3
2		142.3	-	157.1
3		143.	-	151.8
4	δ 6.5 (s)	89.7	6.29, s	86.7
4a	-	142.3	-	138.7
5	7.47 (dd, J= 7.8,2)	114.5	7.51, d (8.8)	114.6
5a	-	141.5		138.4
6		121.3	-	121.4

7	7.2 (dd, J= 8,2)	132.7	7.30, t (7.9)	133.9
8	7.7 (*dd*, J= 7.8,2)	127.5	7.72, t (8.7)	126.7
8a	δ 8.5 (dd, J= 8,2)	123.8	8.47, d(8.0)	122.2
9	-	176.8	-	180.9
9a		-	-	-
1’		-	-	-
2’		-	-	-
3’		-	-	-
4’		-	-	-
5’		-	-	-
1-OCH_3	3.8, s	60.9	3.92, s	60.8
2-OH	-O-CH_2-O (6.0, s)	101.7	-	-
3-OCH_3			4.01, s	56.0
4-OCH_3	-	-	-	-
6-OCH_3	-	-	-	-
7-OCH_3	-	-	-	-
N-CH_3	4.5, s	35.1	4.85, s	34.2

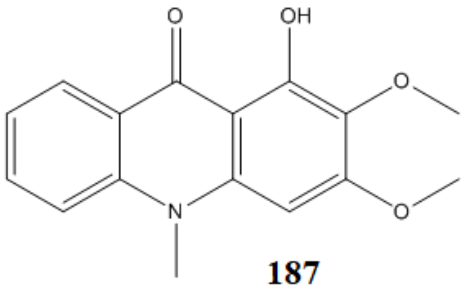
Compound **187** was isolated as yellow powder. All spectral data of compound 22 is comparable with that of evoxanthine (**186**) except the methoxy peak at δ 3.8 (s) and oxymetheyle at δ 6.0 are not seen. Instead, two additional methoxy peak are observed at δ 3.9 and 3.83. In agreement with the ^{1}H NMR spectrum (appendix 62), methoxy peaks appeared at δ 60.4 and 55.6 suggesting that the oxymethylene is likely to be two methoxy groups at C-2 and C-3 positions (appendix 63). Thus, based on the above spectra and comparison with literature the structure of the compound was suggested to be an acridone alkaloid closer to amaniensine (**187a**) previously isolated from *Teclea amanuensis* (Joseph et al., 2008).

Compound **188** was isolated as yellow powder. All spectral data of this compound is comparable with that of evoxanthine (**187**) except the methoxy peak at δ 3.8 (s) and oxymetheyle at δ 6.0 are not seen in ^{1}H NMR spectrum of compound **188** (appendix 64). Instead, two additional methoxy peask are observed at δ 3.97 and 3.66. In agreement with the ^{1}H NMR spectrum, its ^{13}C NMR spectrum (appendix 65) showed methoxy peaks at δ 60.4 and 55.6 suggesting that the oxymethylene in case of compound **187** is now present as two methoxy groups at C-2 and C-3 positions in compound **188**. In addition, the aromatic singlet peak at δ 7.1 (s) coupled with the disappearance of one of the oxygenated sp^2 quaternary peask at δ 157.3 suggest that the hydroxyl group at C-1 psotion is not there. Thus, based on the above spectra and comparison with literature the structure of the compound was

suggested to be an acridone alkaloid (**188**) closer to amaniensine (**187a**) previously isolated from *Teclea amanuensis* (Joseph et al., 2008).



186



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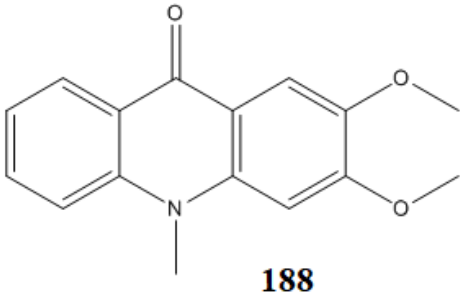
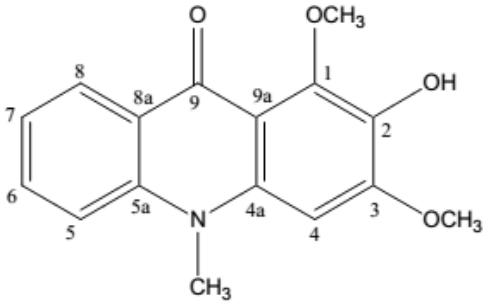


188



187a

4.3 Antibacterial activity

4.3.1 Antibacterial activity of roots extracts of *Teclea nobilis*

The antibacterial tests showed considerable antibacterial activity against the bacterial species used in the study. Methanol extract showed promising activity against the tested strains except for *S.aureus* and *P.aeroginosa* .The methanol extract was found to inhibit *E. coli* and *K. pneumonia* but extract of 1:1 ratio of dichloromethane with methanol have not any activity against bacterial species used in study. From the compounds isolated compound **163** showed promising activity except for *P.aeroginosa* while compound 2 has no any activity against bacterial species used in the study (table 32).

Table 32: Zone of bacterial growth inhibition diameter (mm).

Sample	Types of bacteria with mean inhibition diameter (mm)			
	S.aureus	*E. coli*	*K.pneumonia*	P.*aeroginosa*
Methanol extract	-	8	7	-
Methanol/dichloromethane extract	-	-	-	-
Compound **163**	9	8	8	-
Compound **164**	-	-	-	-
Ceftraxone (PC)	19	21	18	19

4.3.2 Antibacterial activity of roots of *Kniphofia schimperiana*

The two crude extracts (dichloromethane/methanol (1:1) ratio and 100% methanol) and three compounds (**166-168**) were evaluated for *in vitro* antibacterial activity using disk diffusion method (Table 22), which showed varying degrees of responses against the bacterial strains. The two crude extracts showed a little activity on Gram-positive (*S .aurous*) with zone of inhibition (7 and 8) mm and both Gram negative and one gram positive bacterial strains were not showed activity with zone of inhibition 6 mm with no different activity was observed between the two crude extracts. Out of the isolated compounds, compound **167** showed better activity aginst *S.aureus* with zone of inhibition of 11 mm (table 33).

Table 33**.** Diameter of zone of growth inhibition (in mm) of crude extracts and isolated compounds from the roots of *K.schimperiana.*

S.No	**Sample code**	***S. aureus***	***P.aeroginosa***	***E.coli***	***K.pneumonia***
1	KSC-DM:ME	8	6	6	6
2	KSC-ME	7	6	6	6
3	Compound**166**	6	6	6	6
4	compound **168**	6	6	6	6
6	Compound **167**	11	6	6	6
7	Ceftraxone (PC)	20	22	15	17
8	DMSO(NC)	6	6	6	6

ME=methanol extract, PC=positive control, NC=negative control, DMSO=dimethyl sulf

4.3.3 Antibacterial activity of extract and isolated compounds of *C. anisata*

The antibacterial activity of the extract and isolated compounds of *C. anisata* were examined at a concentration of 20 µg/mL against four pathogenic bacterial strains: two Gram-positive *S. aureus, B. substilis* and two Gram-negative *E. coli,* and *P. aeruginosa* (table 34). The results revealed that derivative of heptaphyline (**183**) and imperatorin **(184**) exhibited

comparable antibacterial activity against *S. aureus* and *B.substilis*, 14 mm zone of inhibition for both strains, compared to that of ciprofloxacin (15 mm). Chalepin (**185**) also exhibited promising antibacterial activity against *S. aureus,* and *P. aeruginosa* with 14 and 12 mm zone of inhibition, respectively, compared to that of ciprofloxacin (15 mm) whereas chalepin (**185**) revealed more antibacterial activity (16 mm zone of inhibition) against *B. substilis* compared to that of ciprofloxacin (15 mm). Generally crude extracts and pure isolated compounds were ineffective against *E. coli* pathogen at this concentration (table 34).

Table 34. Zone of bacterial growth inhibition (mm) for crude extract and isolated compounds

Sample	***E. coli***	***S. aureus***	***B. substilis***	***P. aeruginosa***
CH_2Cl_2/MeOH extract	9 ± 0.1	11± 0.1	10± 0.1	8± 0.1
MeOH extract	n	12± 0.2	13± 0.3	9± 0.2
Compound **182**	n	14± 0.1	12± 0.1	12± 0.1
Compound **184**	n	13± 0.1	14± 0.1	14± 0.1
Compound **185**	n	14± 0.2	16± 0.3	12± 0.2
Ciprofloxacin	14± 0.1	15± 0.3	15± 0.3	15± 0.3

n ≤ 6 is null, and n > 6 is sensitive

4.3.4 Antibacterial activity of extract and isolated compounds of *Z. chalybeum*

The antibacterial activity of the extract and isolated compounds of *Z. chalybeum* were examined at a concentration of 20μg/mL against four pathogenic bacterial strains: two Gram-positive *S. aureus, B. substilis* and two Gram-negative *E. coli*, and *P. aeruginosa* (table 35). The results revealed that dihydrochelerythrin (**15**) and essential oil of fruits of *Z. chalybeum* exhibited comparable antibacterial activity against *S. aureus* and *E. coli with* 14 mm zone of inhibition of essential oil for both strains and 11 and 13 zone of hinhibition for dihydrochelerythrin (**15**), respectively for the two strains, compared to that of ciprofloxacin (16 and 15 mm, respectively). Essential oil of fruits of *Z. chalybeum* exhibited promising antibacterial activity against *B. substilis* and *P. aeruginosa* with 13 and 12 mm zone of inhibition, respectively. Methanol crude extract demonstrated promising antibacterial activity against three starins i.e *E. coli*, *S. aureus* and *B. substilis* (table 36).

Table 36. Zone of bacterial growth inhibition (mm) for crude extract and isolated compounds

Sample	***E. coli***	***S. aureus***	***B. substilis***	***P. aeruginosa***
CH_2Cl_2/MeOH extract	9 ± 0.2	10± 0.1	10± 0.1	11± 0.1
MeOH extract	12± 0.1	13± 0.2	13± 0.3	10± 0.2
Essential oil of fruits	14± 0.2	14± 0.1	13± 0.1	12± 0.1

dihydrochelerythrin (**177**)	13± 0.3	11± 0.1	12± 0.1	11± 0.1
Compound **179**	11± 0.1	10± 0.2	12± 0.3	11± 0.2
Ciprofloxacin	15± 0.1	16± 0.3	18± 0.3	18± 0.3

n ≤ 6 is null, and n > 6 is sensitive

4.3.5 Antibacterial activity of extract and isolated compounds of *T. vogelii* and *B. aegyptica*

The antibacterial activity of the extract and isolated compounds of *T. vogelii* and *B. aegyptica* were examined at a concentration of 30 µg/mL against four pathogenic bacterial strains: two Gram-positive *S. aureus*, *B. substilis* and two Gram-negative *E. coli*, and *P. aeruginosa* (table 30). The results revealed that compounds **169**, **171**, **174** and **175** showed promising antibacterial activity against *E.coli* with 14, 13, 13 and 12 zone of inhibition, respectively, compared to positive control with zone of inhibition of 19mm. Compound **174** and **175** showed promising antibacterial activity against *B. substilis* with zone of inhibition of 12 and 13mm, respectively, compared to positive control (zone of inhibition of 18 mm). Methanol crude extract demonstrated promising antibacterial activity against three starins i.e *E. coli*, and *S. aureus* with zone of inhibition of 13 mm against both strains (table 37).

Table 37. Zone of bacterial growth inhibition (mm) for crude extract and isolated compounds

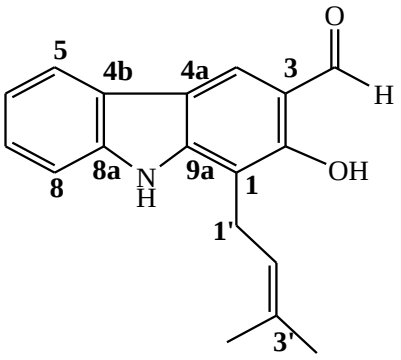
Sample	***E. coli***	***S. aureus***	***B. substilis***	***P. aeruginosa***
CH_2Cl_2/MeOH extract of *T. vogelii*	11 ± 0.2	10± 0.1	9± 0.1	9± 0.1
MeOH extract of *T. vogelii*	13± 0.1	13± 0.2	11± 0.3	9± 0.2
CH_2Cl_2/MeOH extract of *B. aegyptica*	9± 0.2	10± 0.3	10± 0.2	11± 0.3
Compound **169**	14± 0.3	9± 0.2	10± 0.1	10± 0.2
Compound **171**	13± 0.3	10± 0.1	12± 0.2	10± 0.1
Compound **174**	13± 0.3	9± 0.1	12± 0.2	9± 0.1
Compound **175**	12± 0.2	9± 0.1	13± 0.1	8± 0.1
Compound **176**	9± 0.1	9± 0.2	8± 0.3	9± 0.2
Ciprofloxacin	19± 0.1	20± 0.3	18± 0.3	19± 0.3

n ≤ 6 is null, and n > 6 is sensitive

4.4 Molecular docking analysis

Molecular docking study of *C. anisata* isolated compounds displayed moderate to better docking score within binding pocket toward *E. coli* (6F86). Coumarins **183** and **184** demonstrated better docked score (-5.9 and -5.8 kcal/mol, respectively) and formation

hydrophobic interaction with amino acid residues Glu185 and THR84 within the binding pocket whereas carbazole alkaloid (**182**) and chalepin (**184**) have moderate docked score (-5.6 and -5.5 kcal/mol) and formation hydrophobic interaction with amino acid residue PHE41, ILE186, LYS189 and ARG190 within the binding pocket (table 38).



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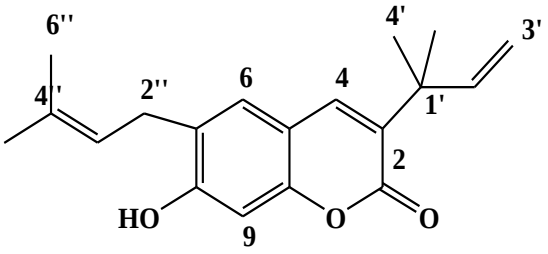

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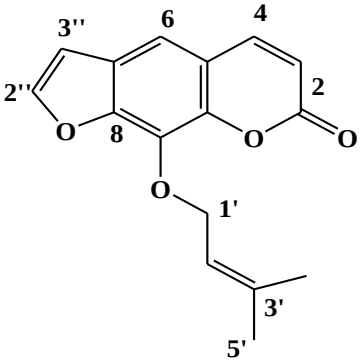

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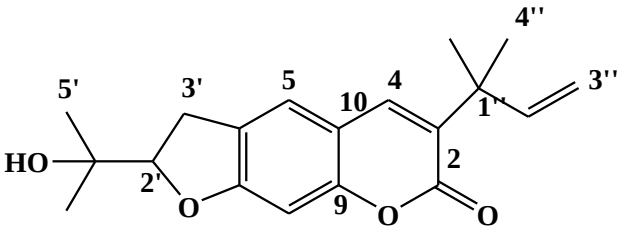

185

Table 38: The binding interactions of compounds against DNA gyrase (PDB ID. 6F86)

compounds	**Affinity (kcal/mol)**	**Interacting Amino acids**
182	-5.6	PHE41, ILE186, ARG 190, LYS189
183	-5.9	PHE41, ILE186, GLU185, ARG 190, LYS189
184	-5.8	PHE41, ARG190, ILE186, LYS189, THR84
185	-5.5	PHE41, ILE186, LYS189

3.2 Molecular docking against protein LasR binding domain

LasR as a key regulator of *P. aeruginosa* pathogenesis. Molecular docking of compounds against LasR binding domain displayed better docking score toward *P. aeruginosa* (2UVO) compared to the DNA gyrase docking results. Binding mode of active compound **182** and

184 demonstrated docked score (- 6.6 to -7.9 kcal/mol) and formation of hydrogen bonding with amino acid residue HIS119 and hydrophobic interaction with amino acid residue LEU125, GLY123, GLN45, ARG122, ASP43, and LYS42 whereas compound **183** and **185** have better docked score (-7.9 and -7.5 kcal/mol) and formation hydrophobic interaction with amino acid residues GLY 123, ALA121, GLY12, ARG122, LYS42, ASP43, and LEU125 within the binding pocket (table 39). Our previous *in vitro* antibacterial analysis demonstrated that chalepin (**185**) displayed better anti-bacterial activity compared to other compounds (16 mm zone of inhibition) (3) which is in good agreement with docking results.

Table 39: The binding interactions of LasR binding domain

Compounds	**Affinity (kcal/mol)**	**Hydrogen bonding**	**Interacting Amino acids**
182	-6.9	HIS119	LEU125, GLY 123, GLN45, ARG122, ASP43, LYS42
183	-7.9	HIS119	HIS119 (H), GLY 123, ALA121, GLY12, ARG122, LYS42, ASP43, LEU125
184	-6.6	--	LEU125, HIS119, ASP43, ARG122, LYS42
185	-7.5	--	HIS119, GLY123, ALA121, LEU125, THR80, TYR47

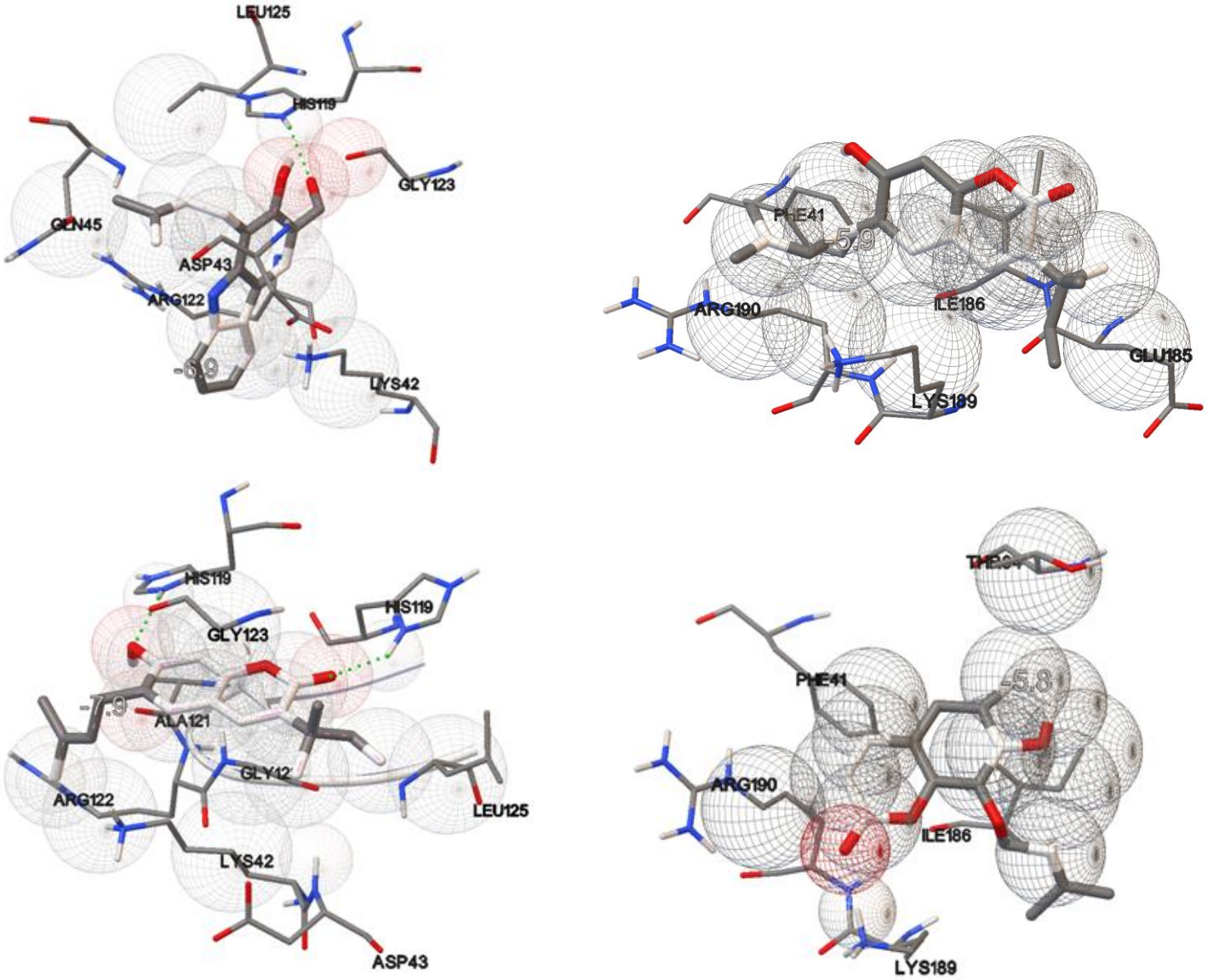

Fig 4. The binding interactions of compounds (**182**-**185**) against DNA gyrase (PDB ID. 6F86).

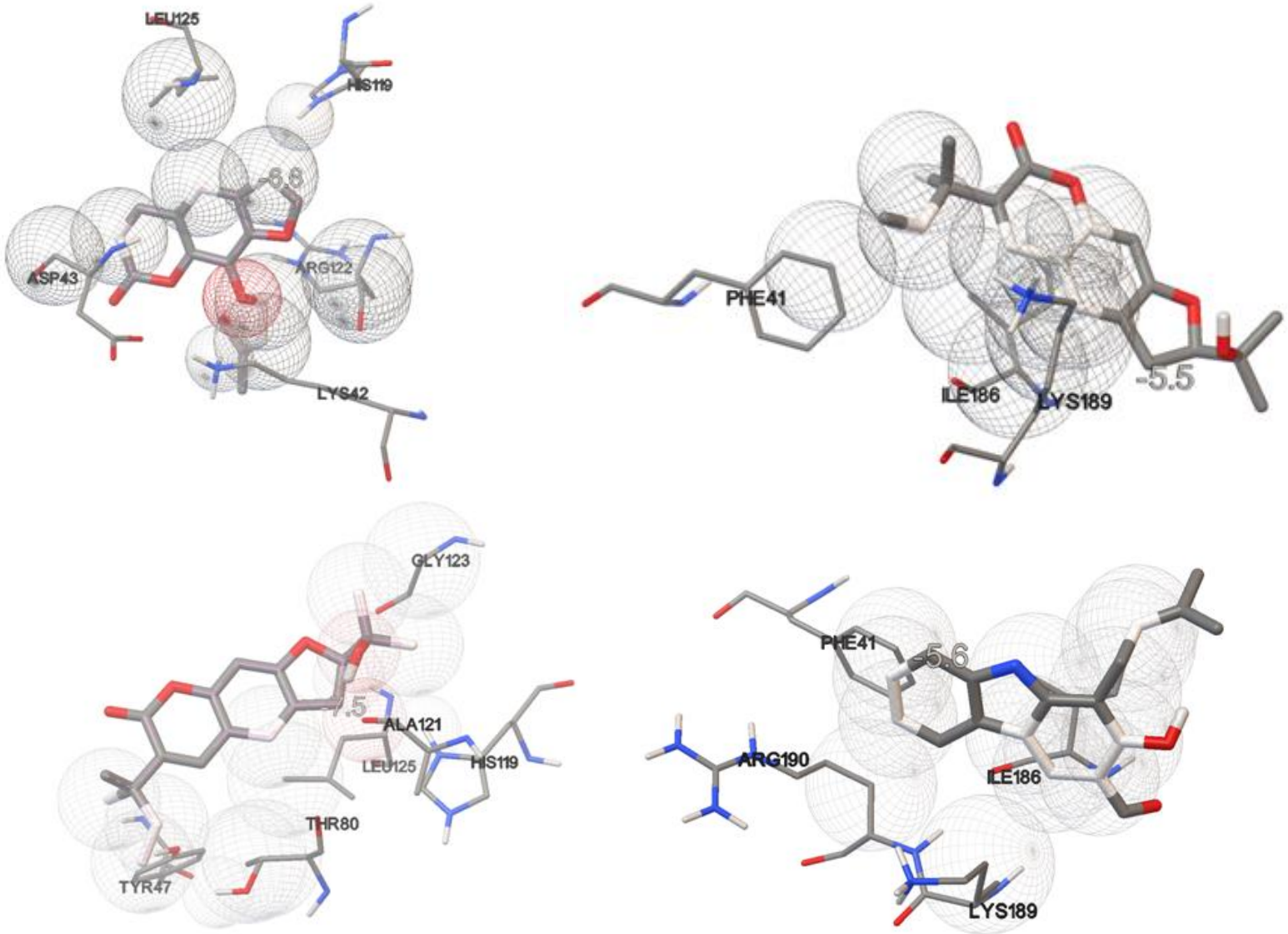

Fig 5. The binding interactions of compounds (**182**-**185**) against LasR binding domain.

5. Conclusion and Recommendation

Interest in obtaining biologically active compounds from natural sources has recently spiked due to their low toxicity, complete biodegradability, availability from renewable sources, and in most cases, low cost. This report comprises a comprehensive phytochemical analysis, biological activity and molecular docking analysis work done on the roots of *Tephrosia villosa*, *Balanites egyptica*, *Zanthoxylum chalybeum*, *Euphorbia schimperiana*, *Teclea nobilis*, *Clausena anisata* and *Kniphofia schimperiana*. Over all, the work resulted in isolation and full spectroscopic charachterization of **26** compounds (**163-188**). Essential oils extracts of fruits of *Zanthoxylum chalybeum* were analyzed by GC-MS and a total of **19** compounds were identified. Detailed of isolation procedures and comprehensive spectroscopic (UV-Vis, IR, NMR; 1D and 2D) characterization and antibacterial activity of the isolated compounds and crude extracts are included in this report. The antibacterial activity the crude extracts as well as isolated compounds were conducted using agar disk diffusion method. Docking studies of four compounds (**182-185**) was performed with DNA-Gyrase (6F86) and LasR binding domain (2UVO) employing flexible ligand docking approach by using AutoDock Vina. So far the work resulted in two publications (Tesfaye et al., 2018 and Dandena et al., 2019), two international conference paper presentations by PI and three more manuscripts from the results of *Tehphrosia vogelli*, *Euphorbia schimperiana* and *Z. chalybeum* are on preparation.

The findings of this project suggest that the traditional uses of the plants of infectious disease have been supported with detailed scientific study and in some cases active ingrideint and cruds have been identified. These findings promote further research work on formulations of crudes extracts such as methanol extracts of *Z. chalebeyum*, methanol extract of *T. vogelii* and essential oils of the fruits of *Z. chalybeum* in various doses and examine in treating infections by *E. coli* and *S. aureus*. Similarly, compounds **182-185** demonstrated promissing antibacterial activity against three strains *S. aureus*, *B. substilis*, and *P. aeruginosa* which is also supported by molecular docking analysis study. These findings promote further research work with formulations of crudes extracts of *C. anisata* and compounds **182-185** in various doses and examine their structure activity relashionship (SAR) and cytotoxicity assay so as to use them as a remedy to treat infections originated from aforementioned strains.

Last but not least, anticancer and cytoxicity assay work is also recommended for the crude extracts and isolated compounds.

6. Budget utilization

Table 40. Budget utilization summary

S.No	Items	Budget allocated	Budget utilized	Percentage
1.	Chemicals and materials purchase by the University	538,600	255,000	47.34%
2.	Instrument purchase	255,750	130,000	50.83%
3.	Project adminstrative budget	366,600	295,000	80.6%
	Total	1,160,950	680,000	
	Contingency	116,095	-	
	Grand total	**1,277,045**	**680,000**	**53.24%**

7. References

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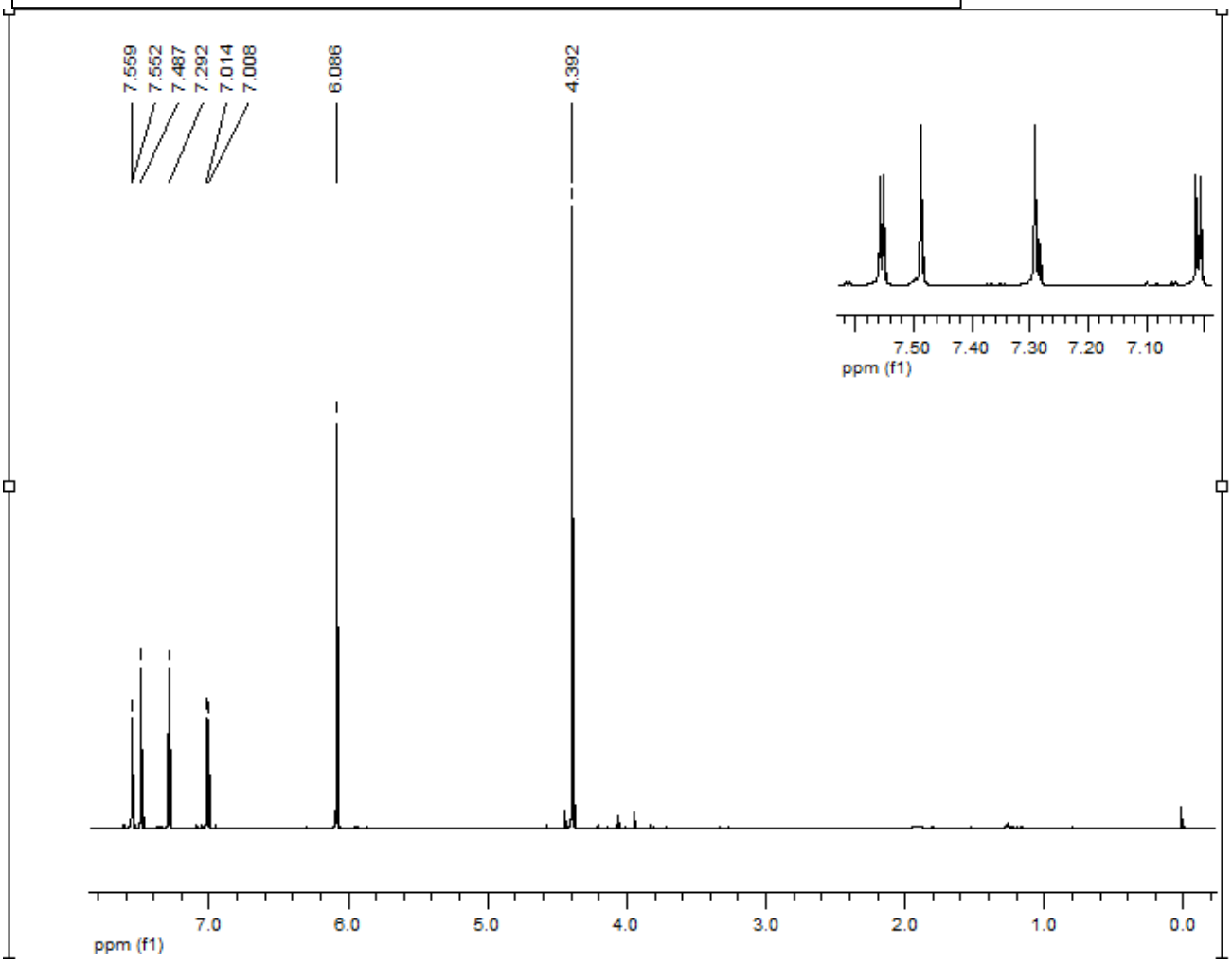
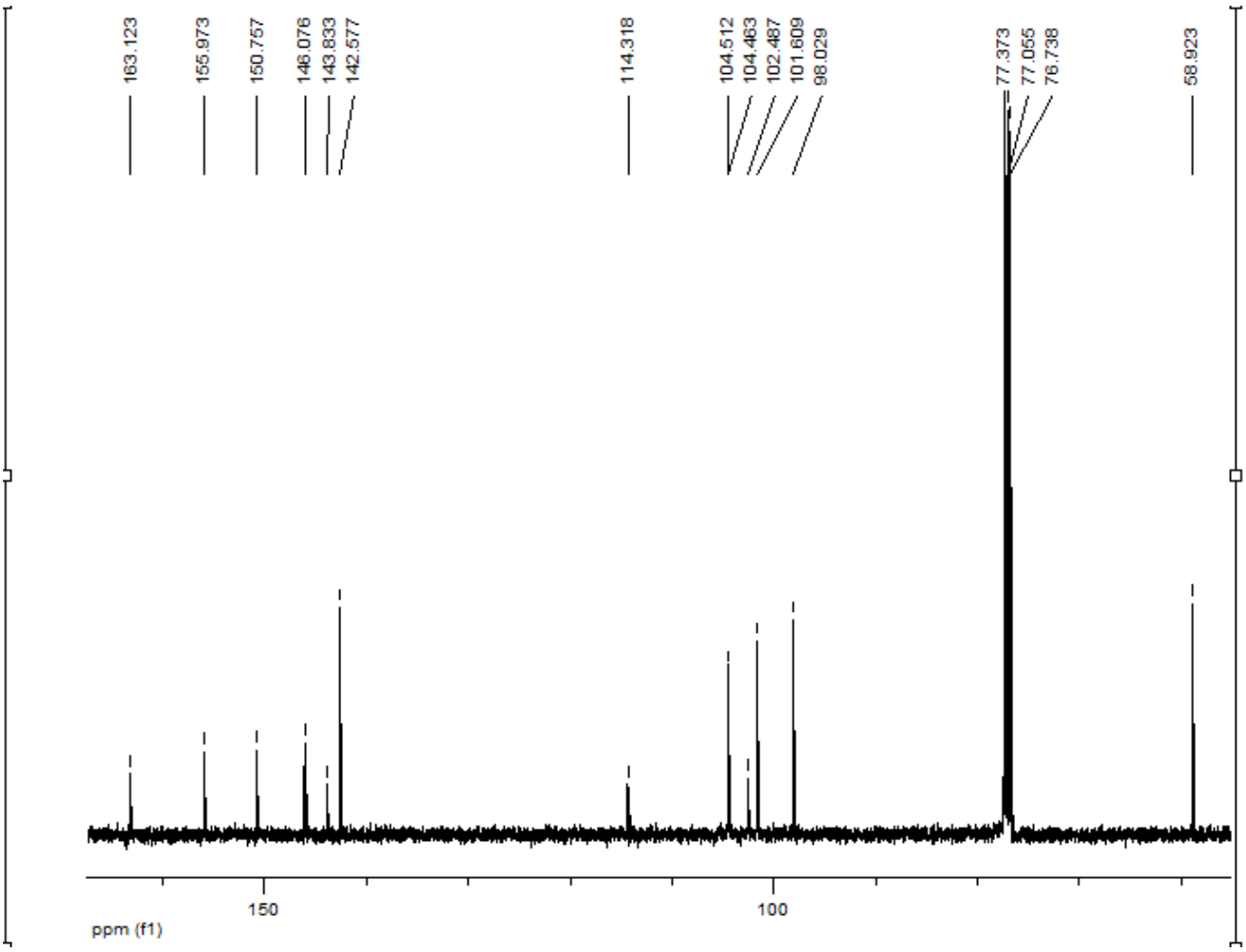
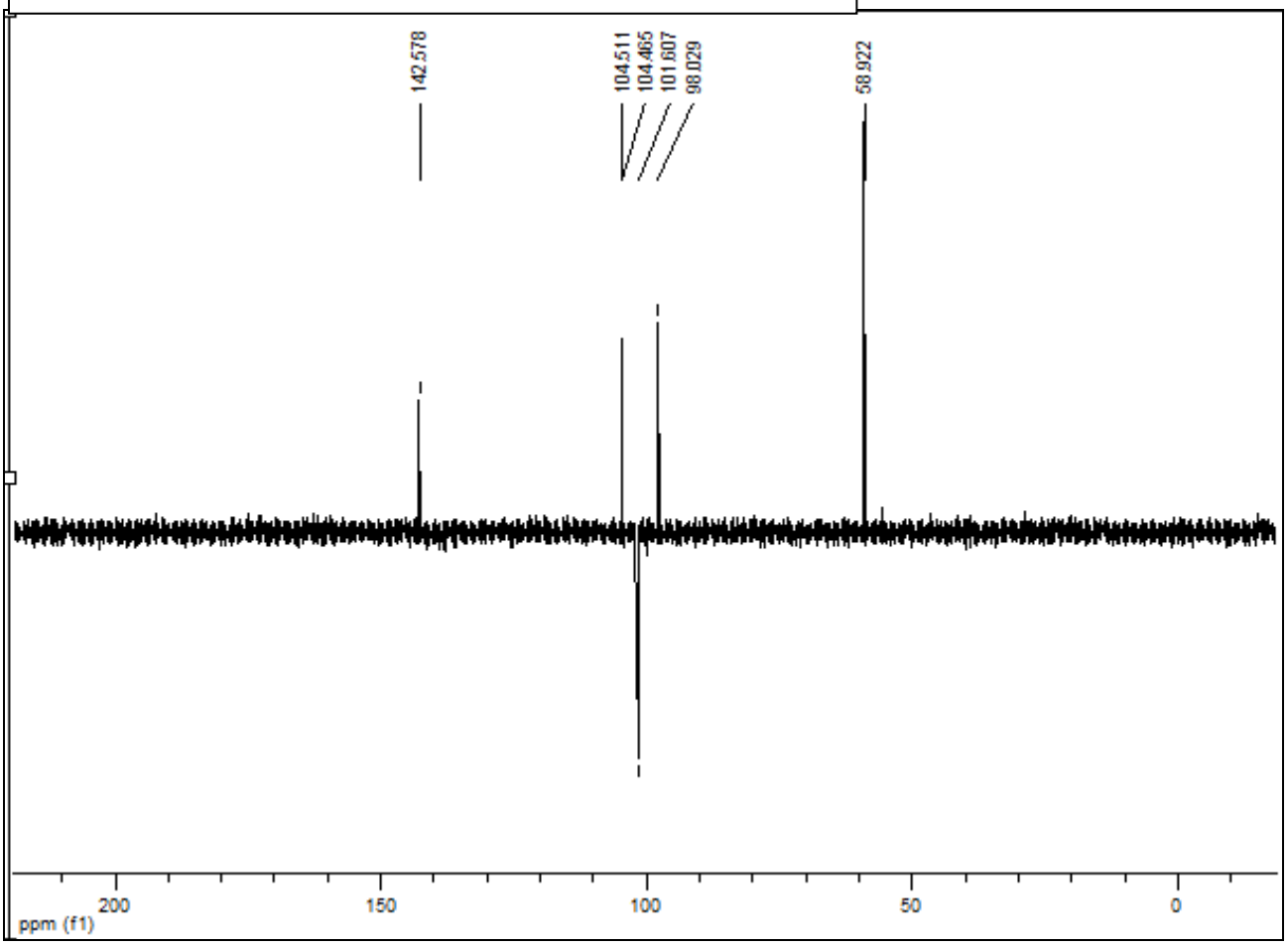
Appendix 1: **^{1}H NMR SPECTRUM FOR COMPOUND 1**



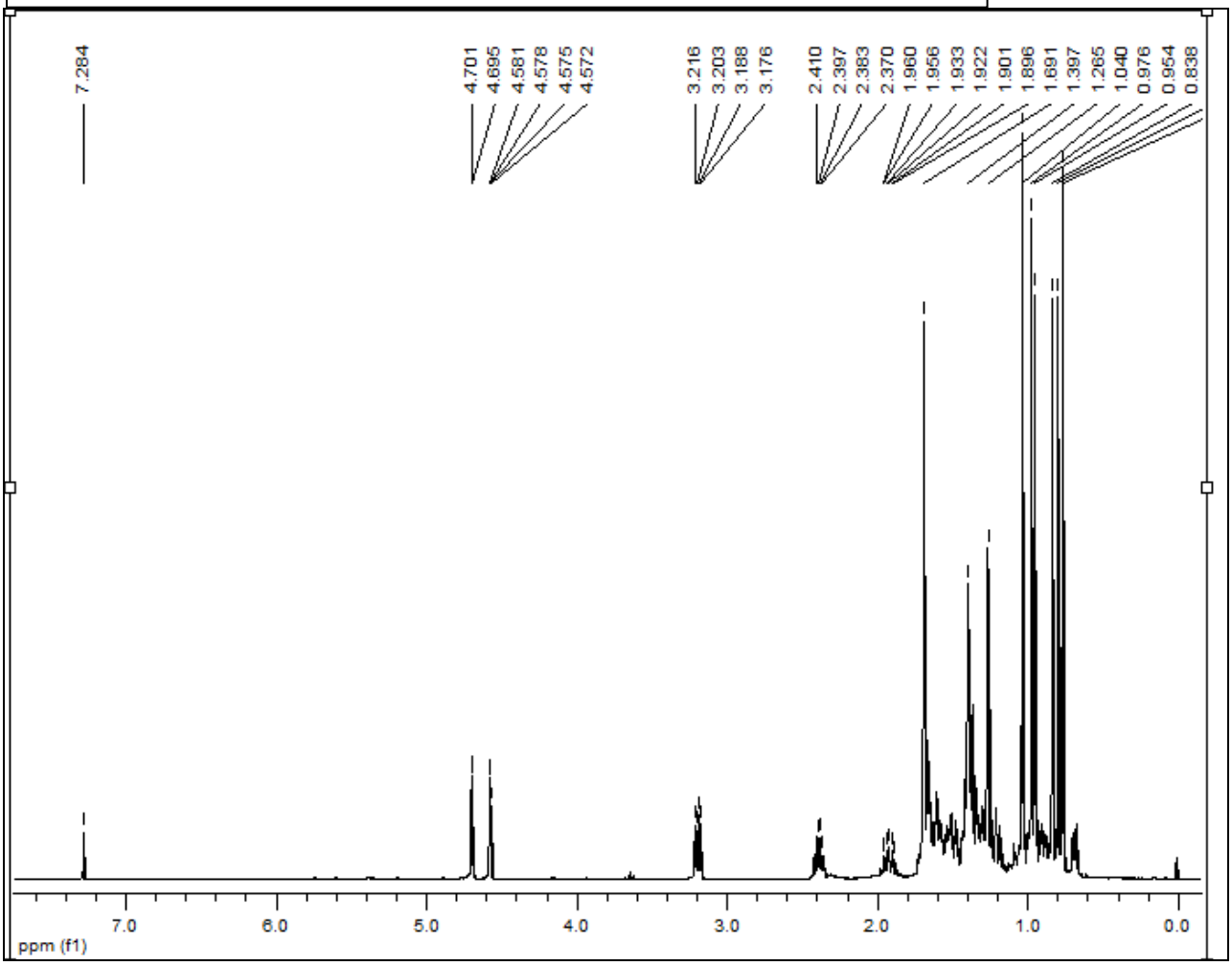
Appendix 2: ^{13}C NMR SPECTRUM FOR COMPOUND 1



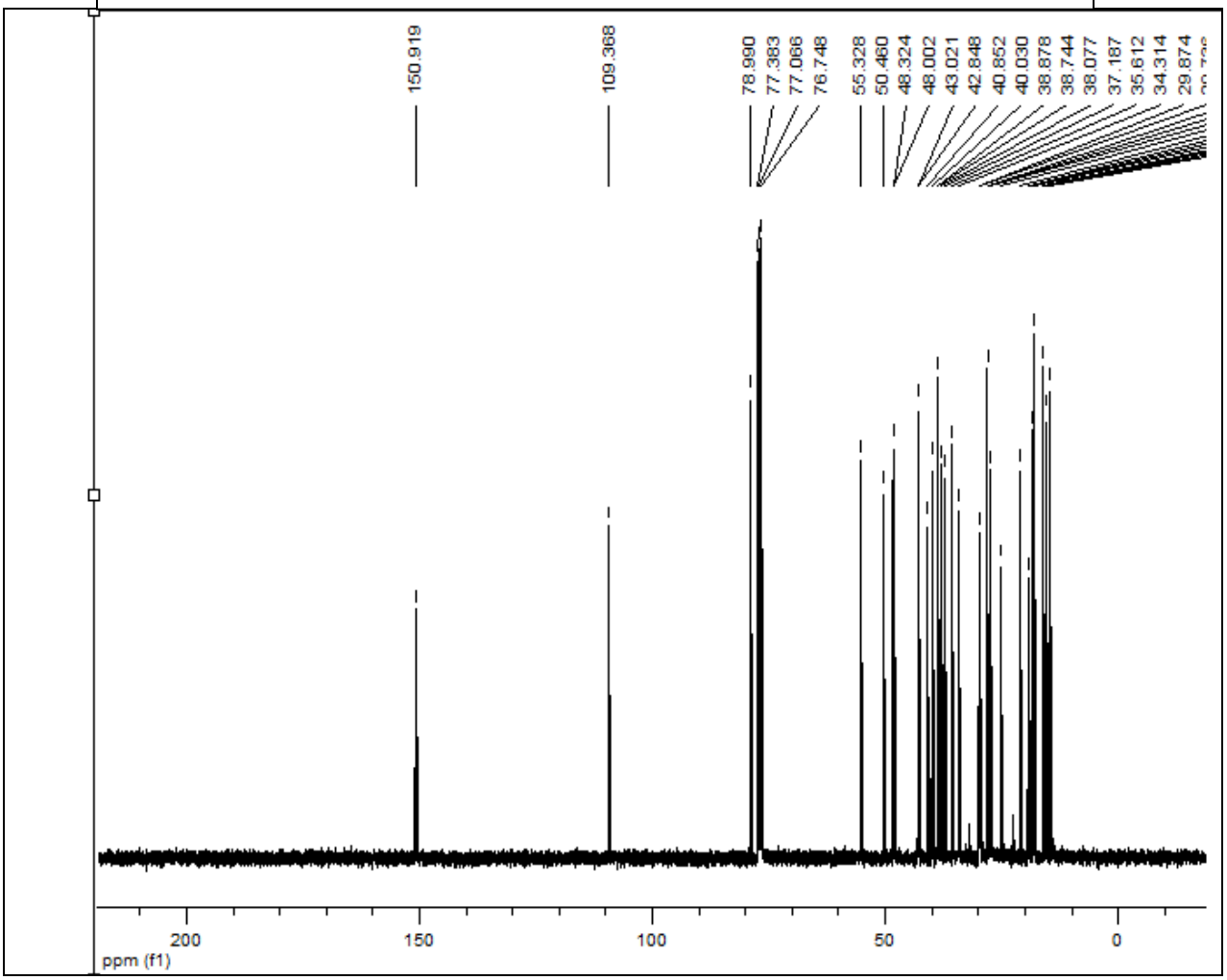
Appendix 3: **DEPT-135 SPECTRUM FOR COMPOUND 1**



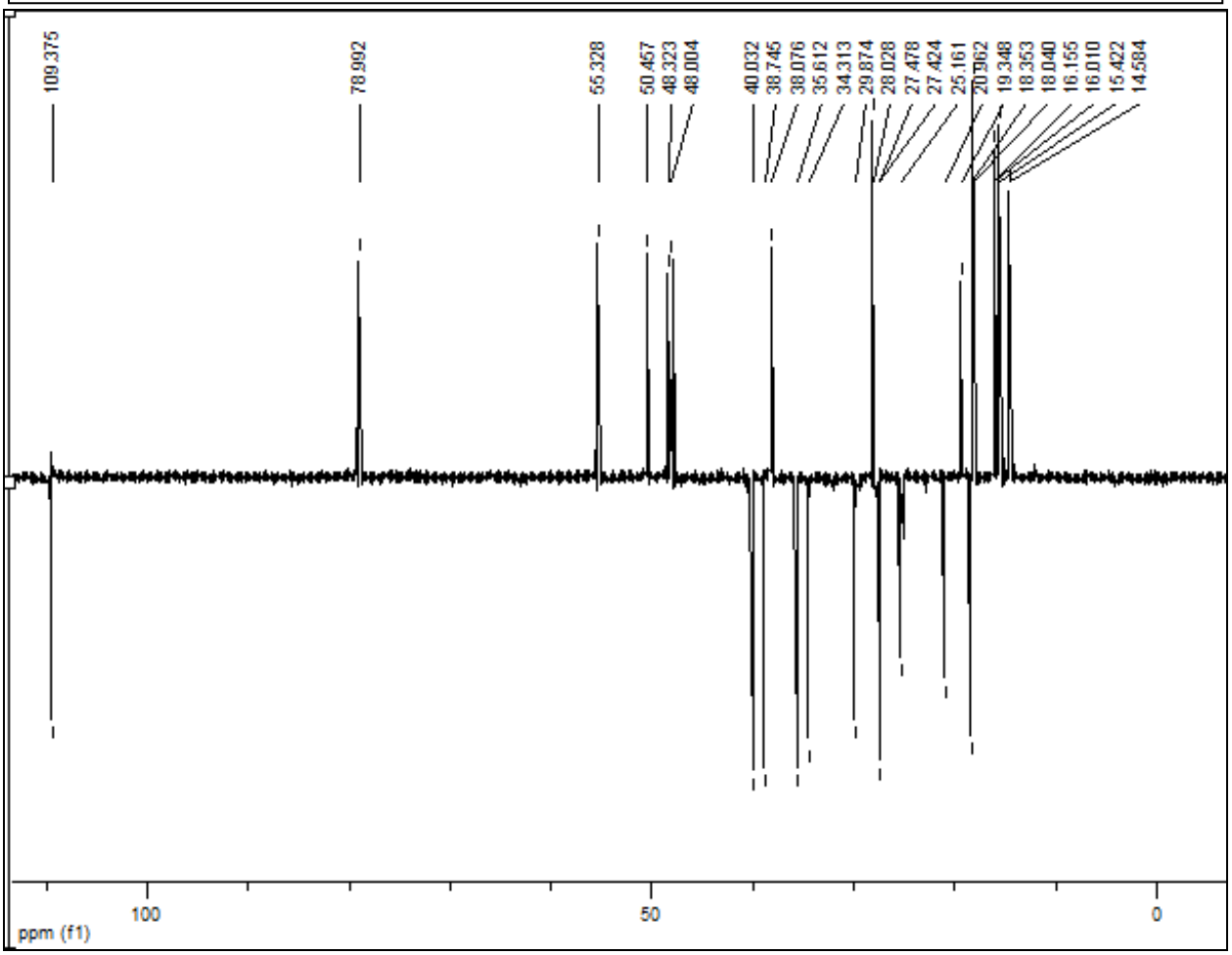
Appendix 4: **^{1}H NMR SPECTRUM FOR COMPOUND 2**



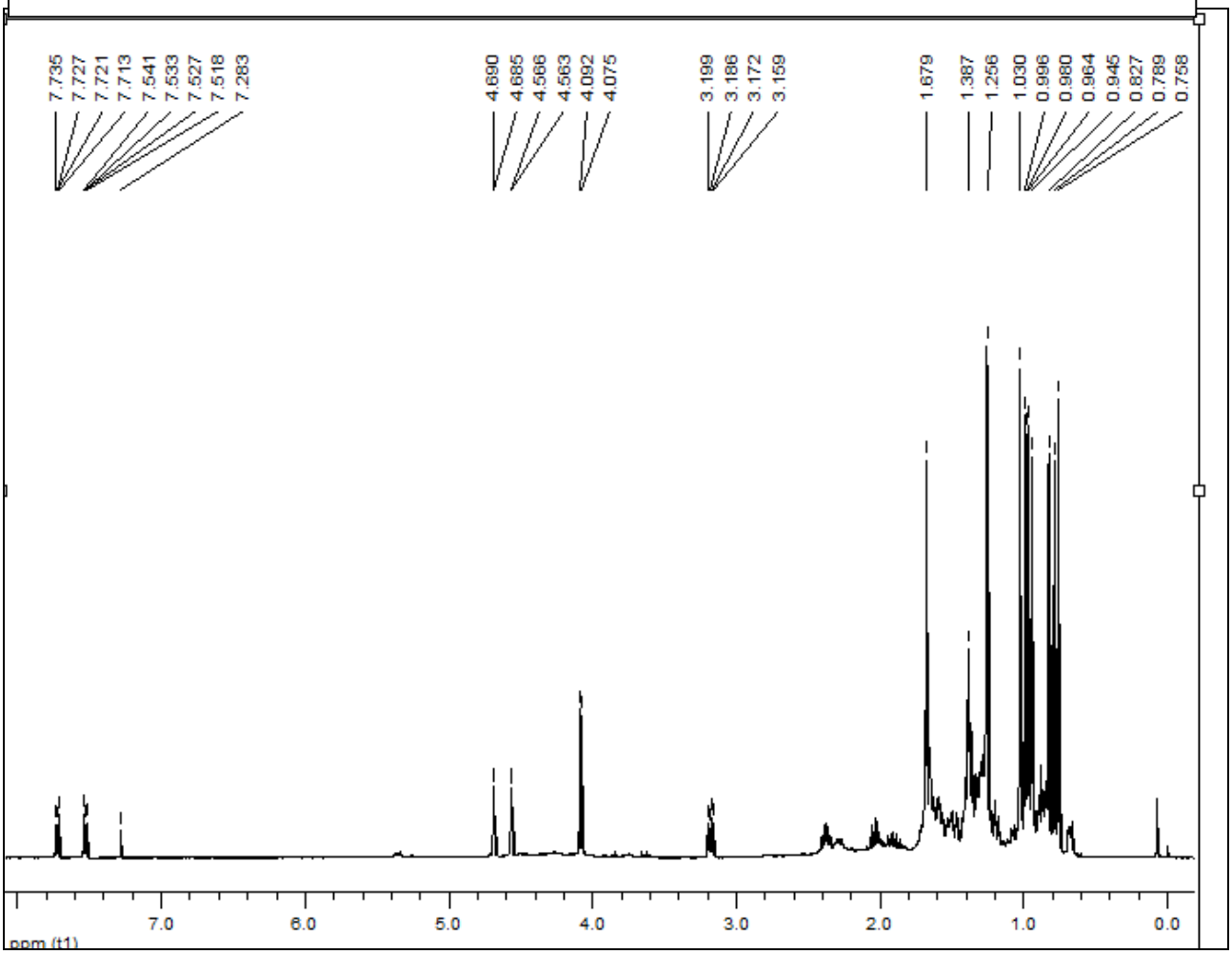
Appendix 5: **^{13}C NMR SPECTRUM FOR COMPOUND 2**



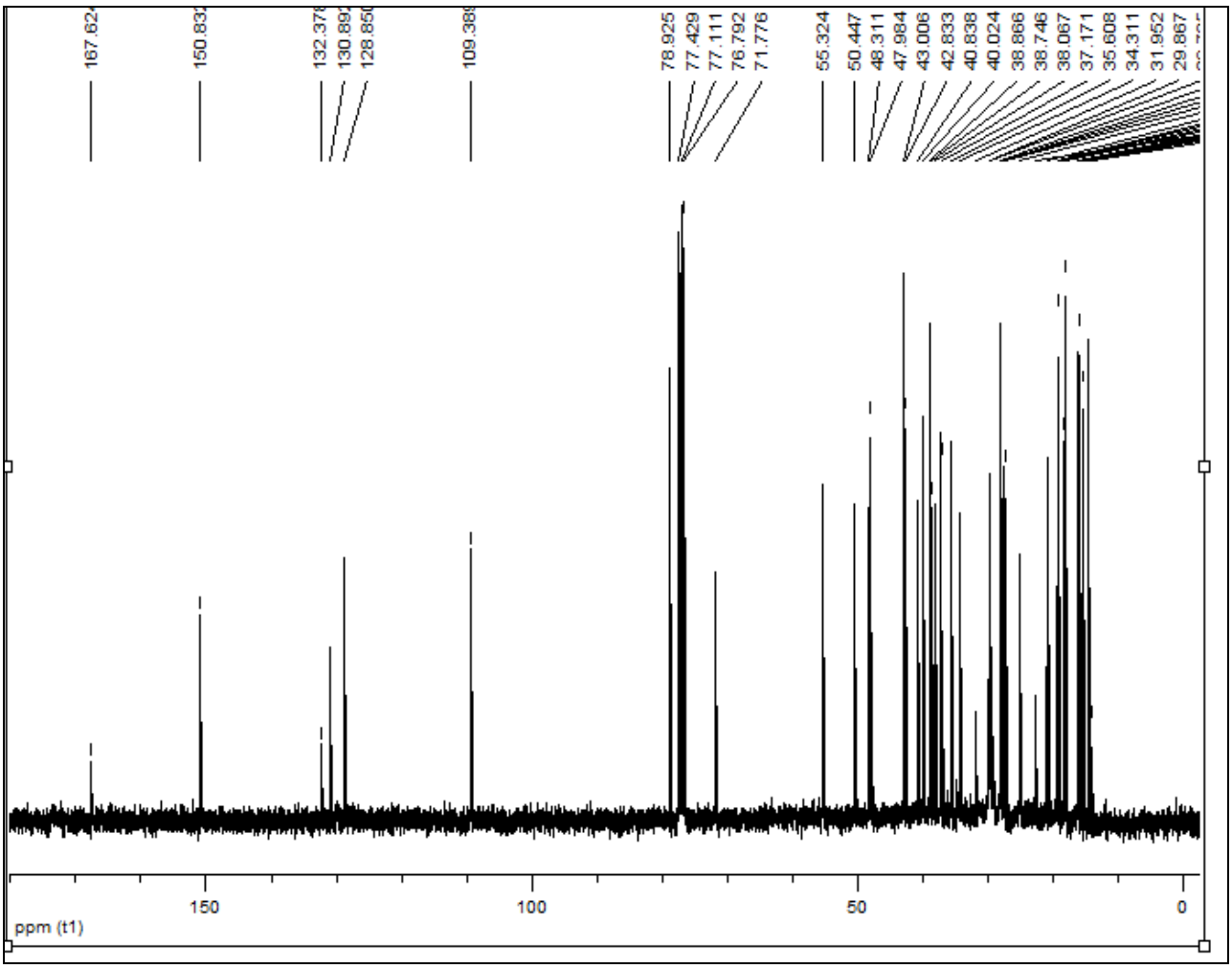
Appendix 6: **DEPT-135 SPECTRUM FOR COMPOUND 2**



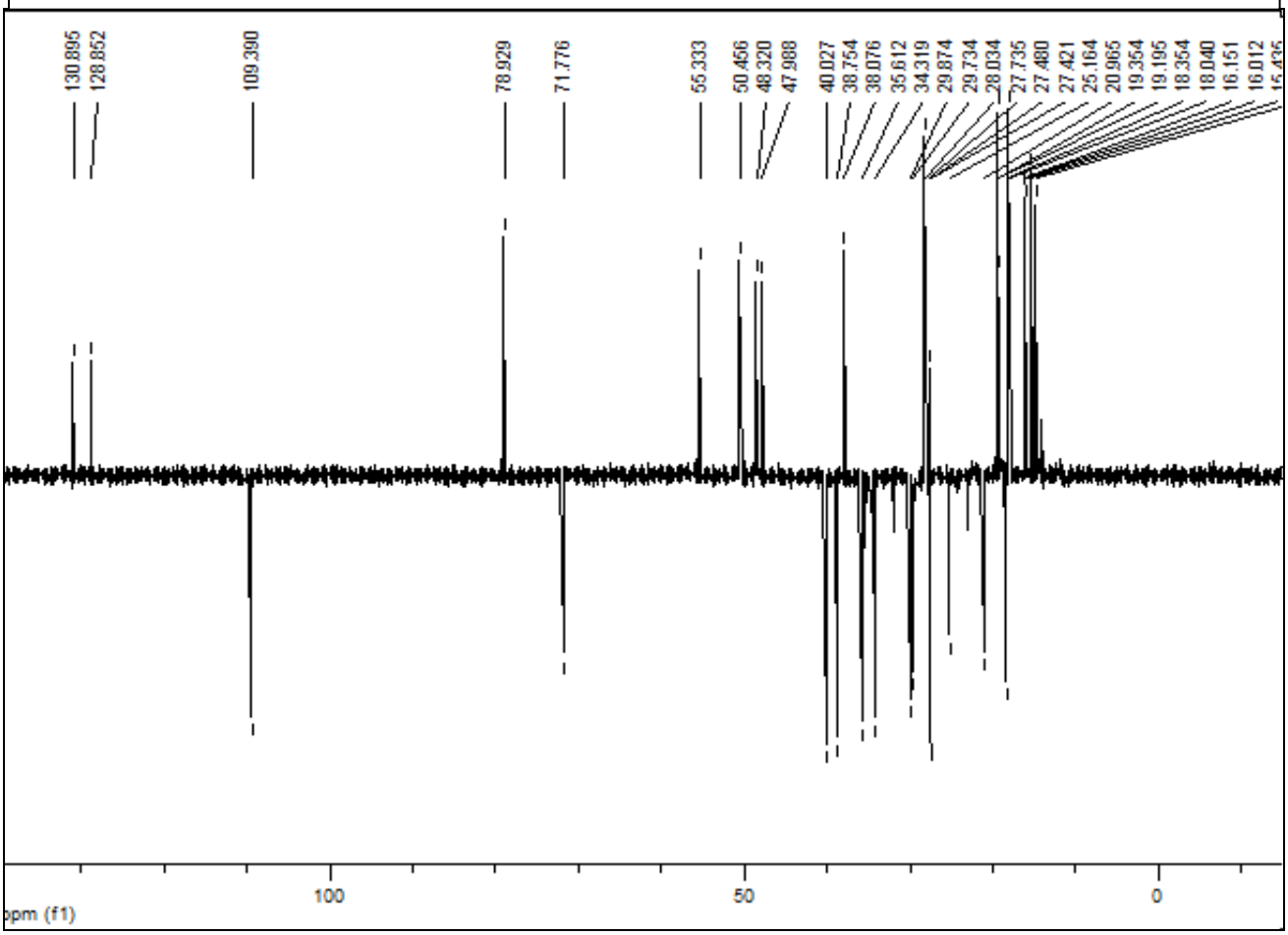
Appendix 7: **^{1}H NMR SPECTRUM FOR COMPOUND 3**



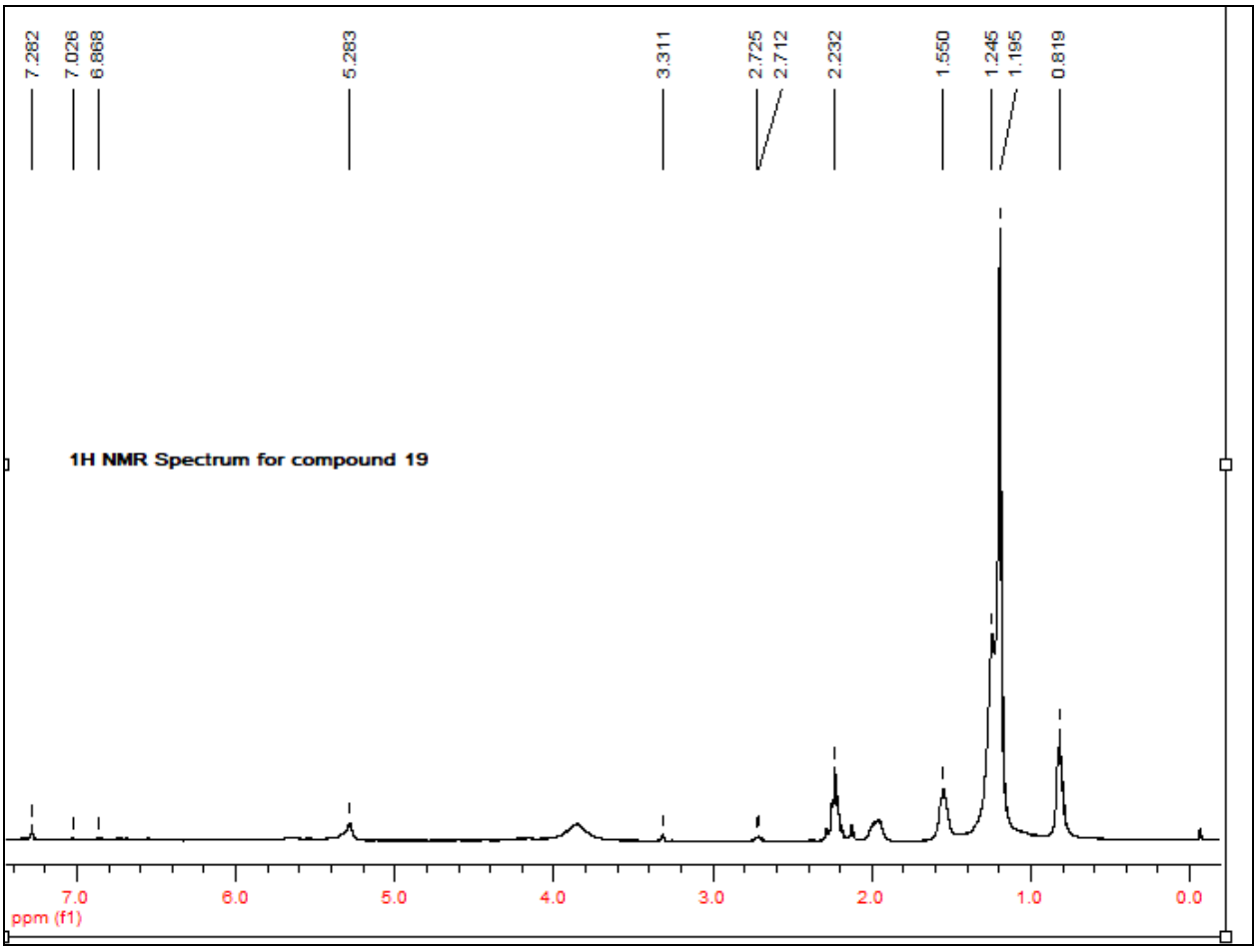
Appendix 8: **^{13}C NMR SPECTRUM FOR COMPOUND 3**



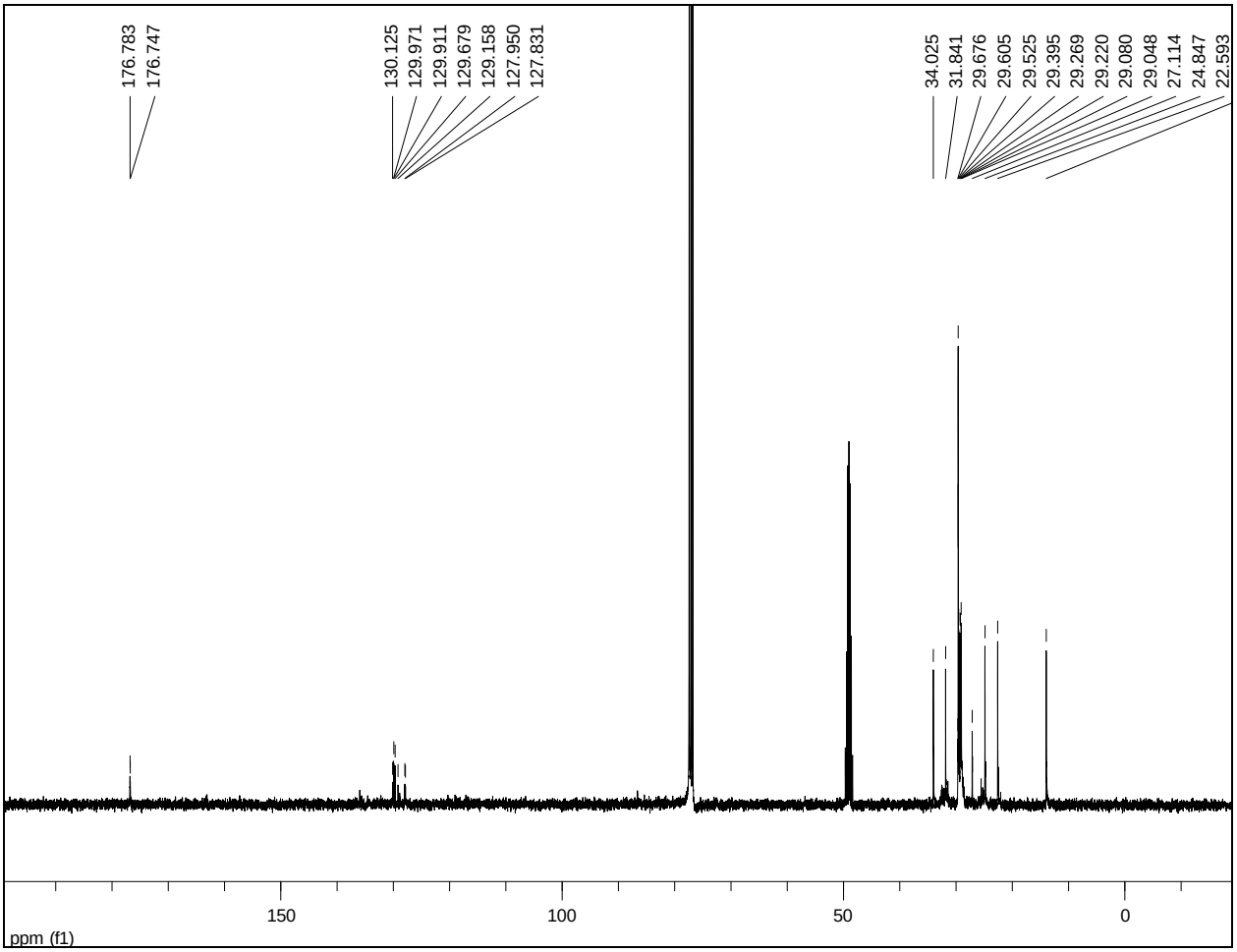
Appendix 9: **DEPT-135 SPECTRUM FOR COMPOUND 3**



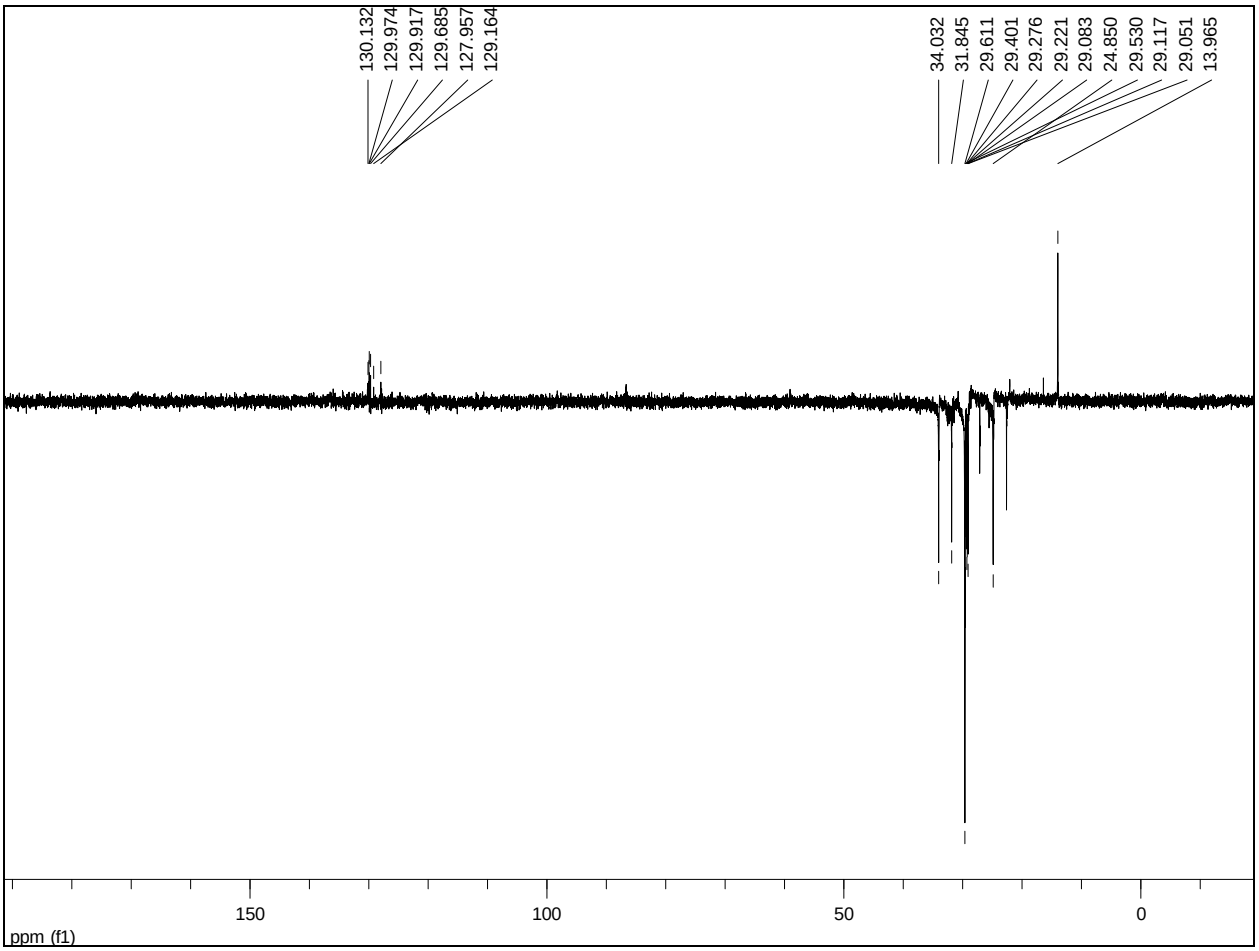
Appendix 10**:** ^{1}HNMR spectrum for compound **4**



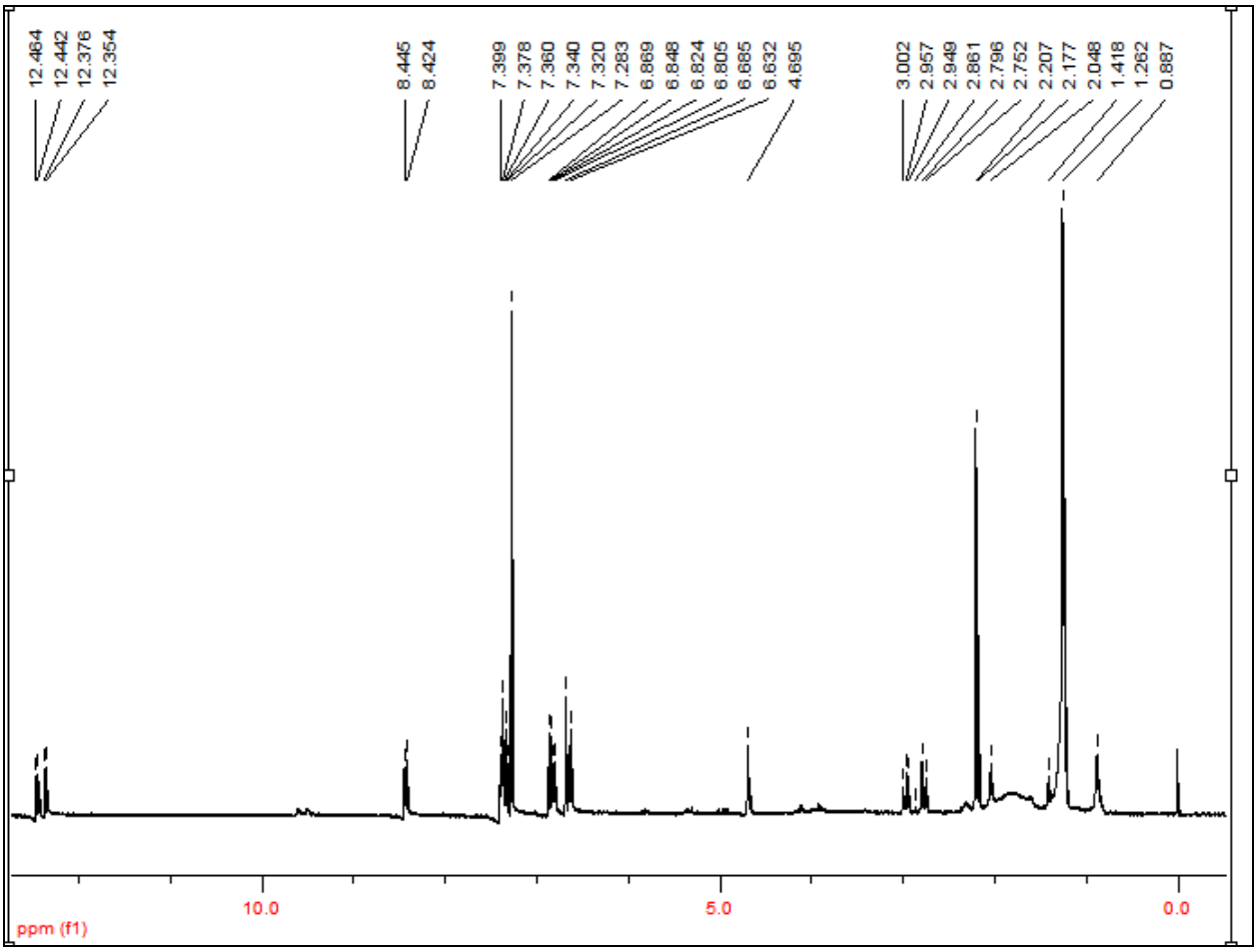
Appendix 11: ^{13}C NMR spectrum for compound **4**



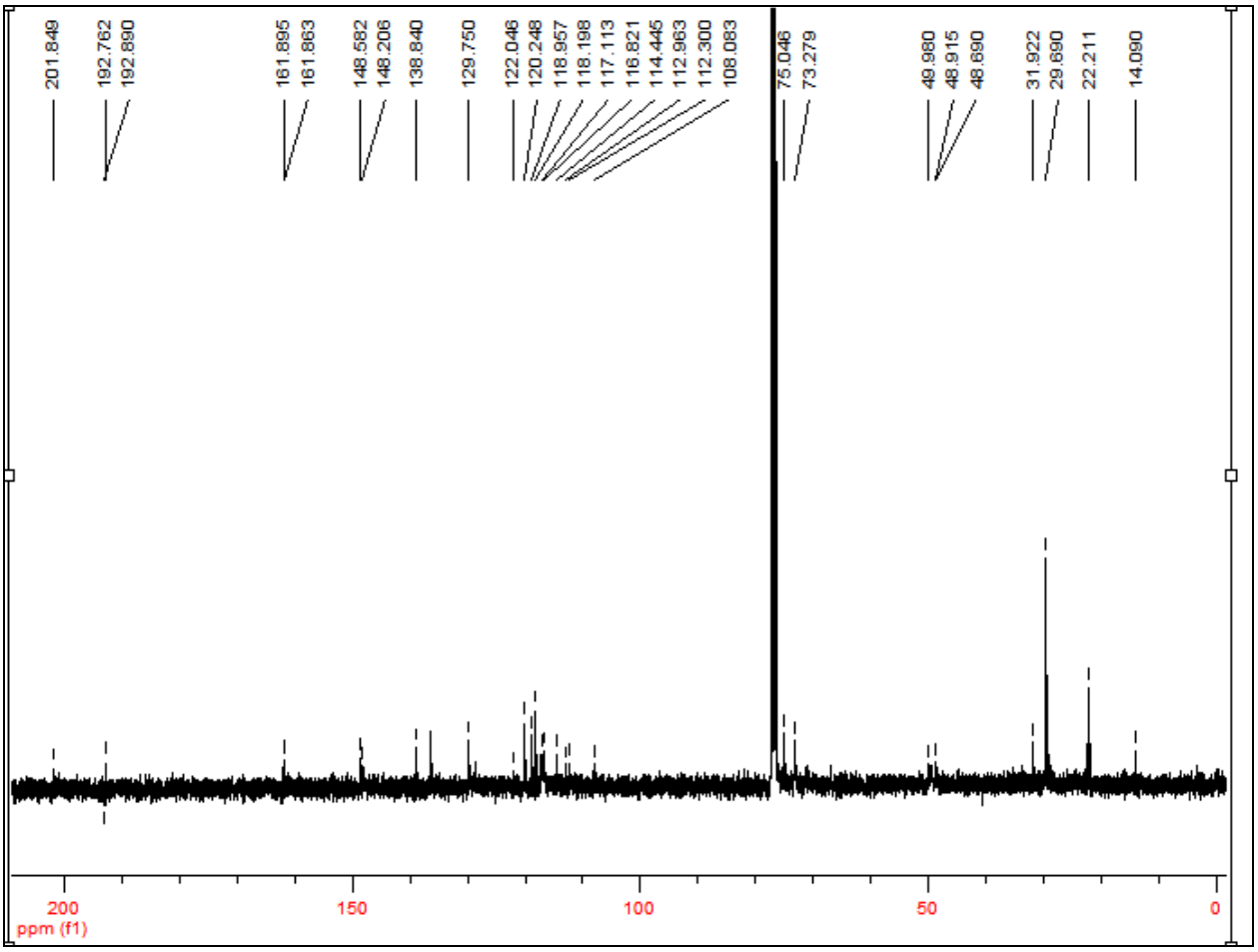
Appendix 12: DEPT-135 spectrum for compound **4**



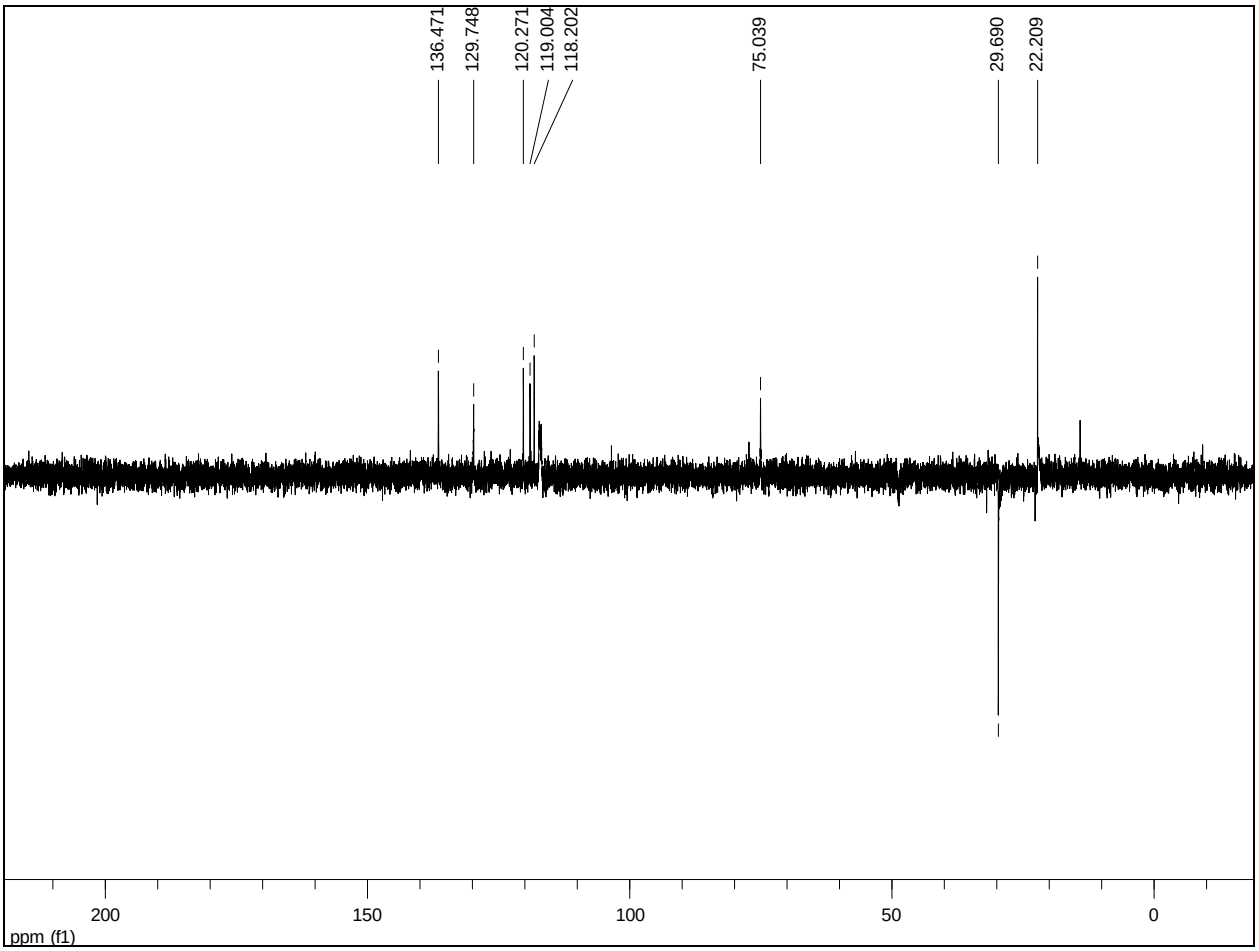
Appendix 13: ^{1}H NMR spectrum for compound **5**



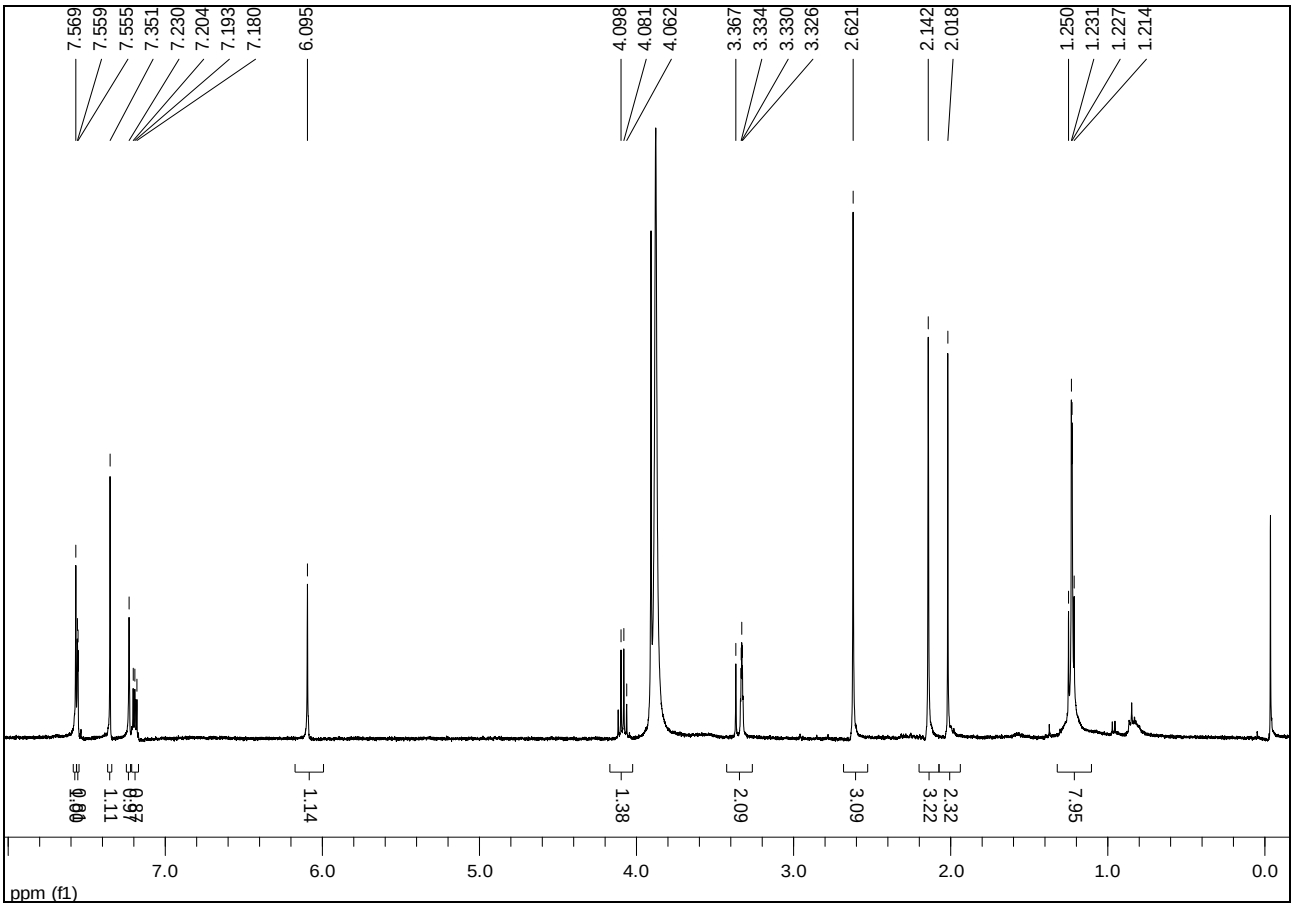
Appendix 14: ^{13}C NMR spectrum for compound **5**



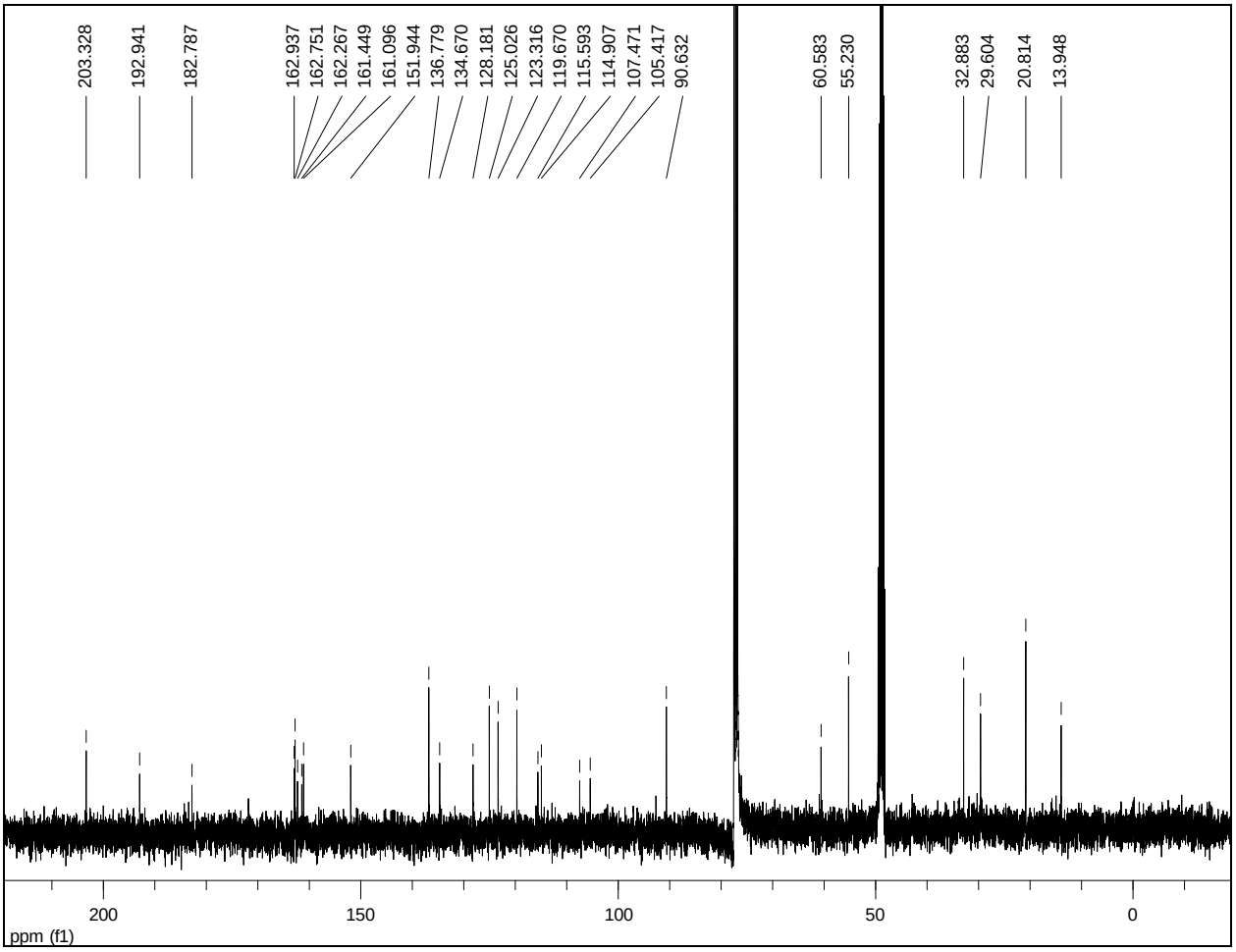
Appendix 15: DEPT-135 spectrum for compound **5**



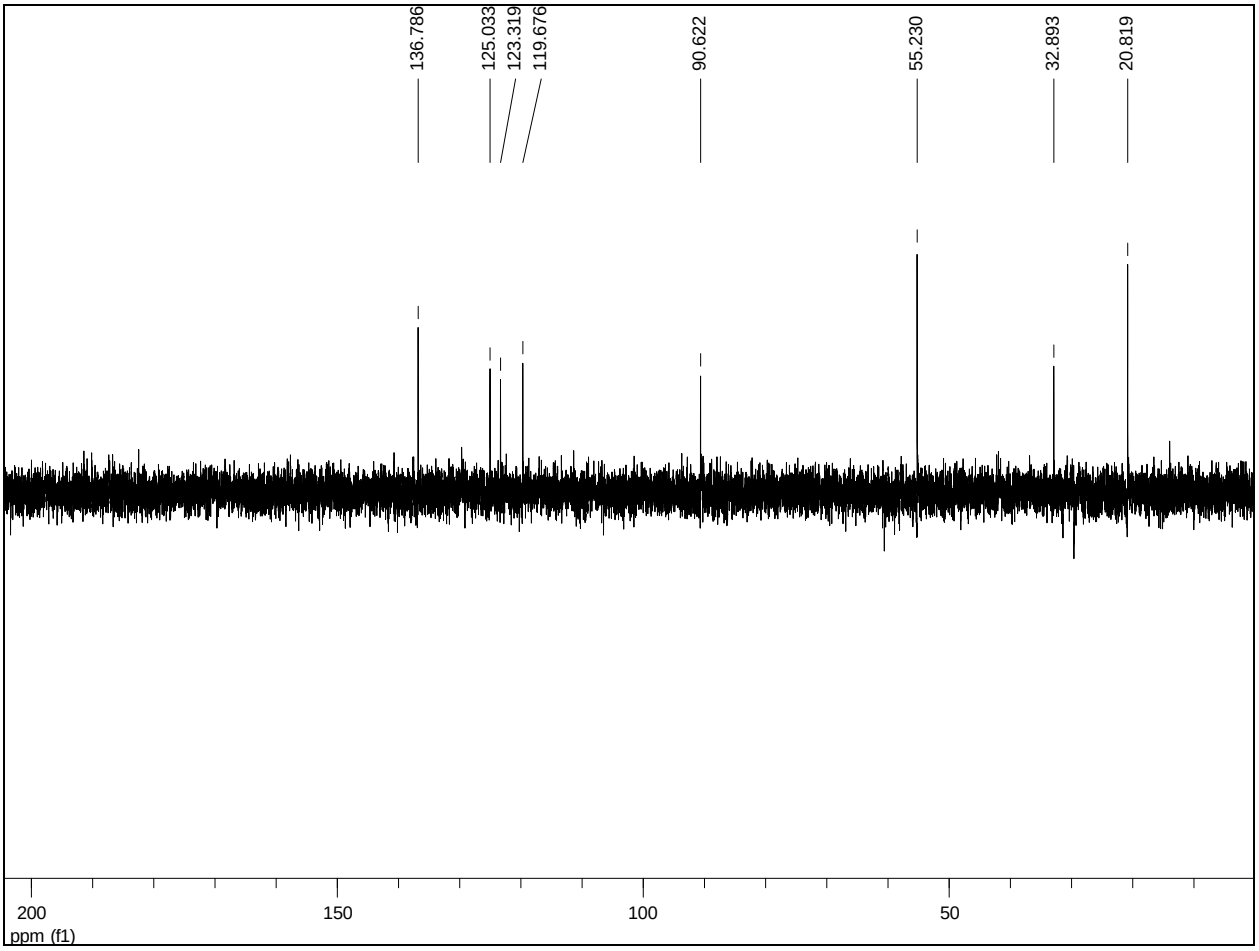
Appendix 16: ^{1}H NMR spectrum for compound **6**



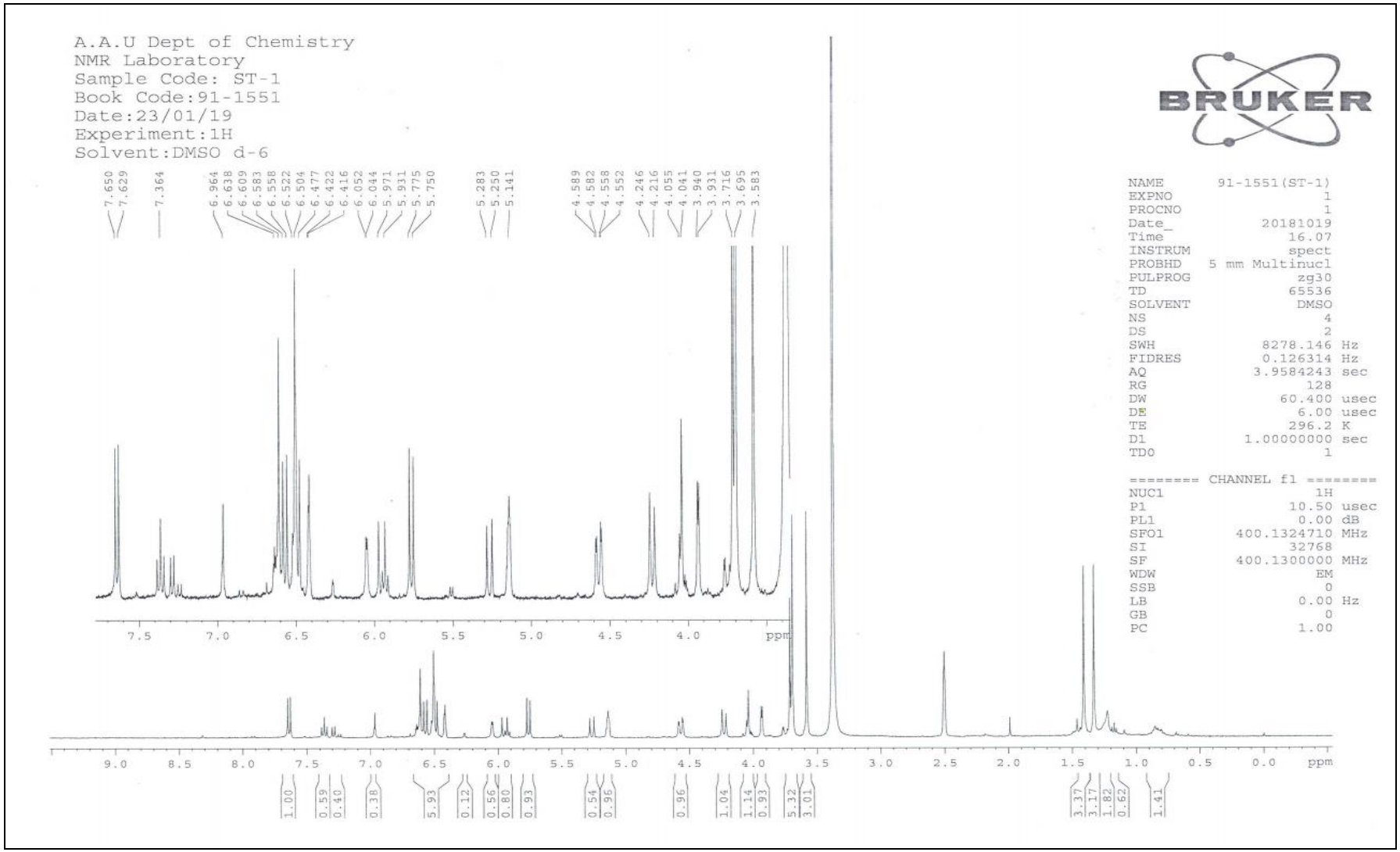
Appendix 17: ^{13}C NMR spectrum for compound **6**



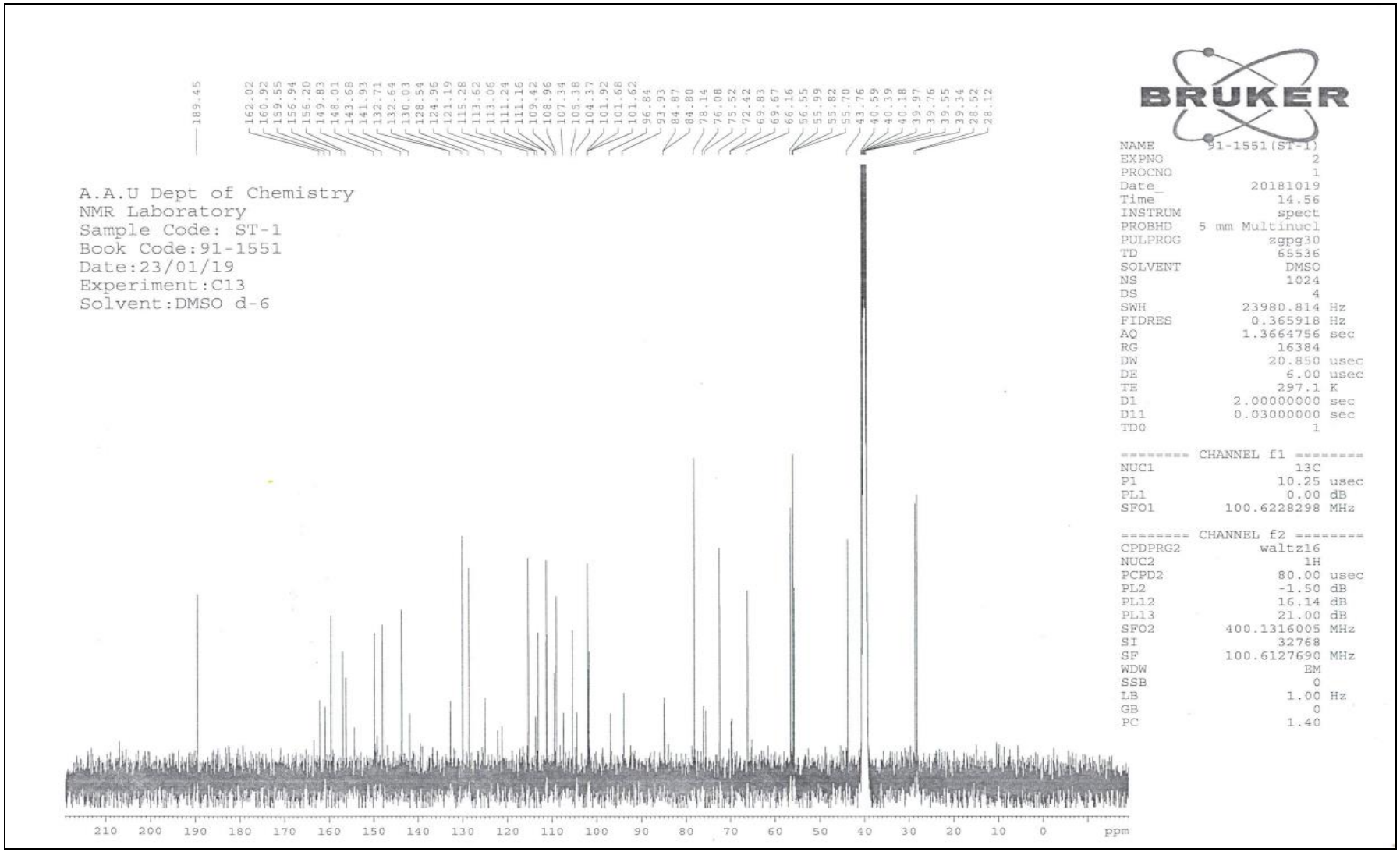
Appendix 18: DEPT-135 spectrum for compound **6**



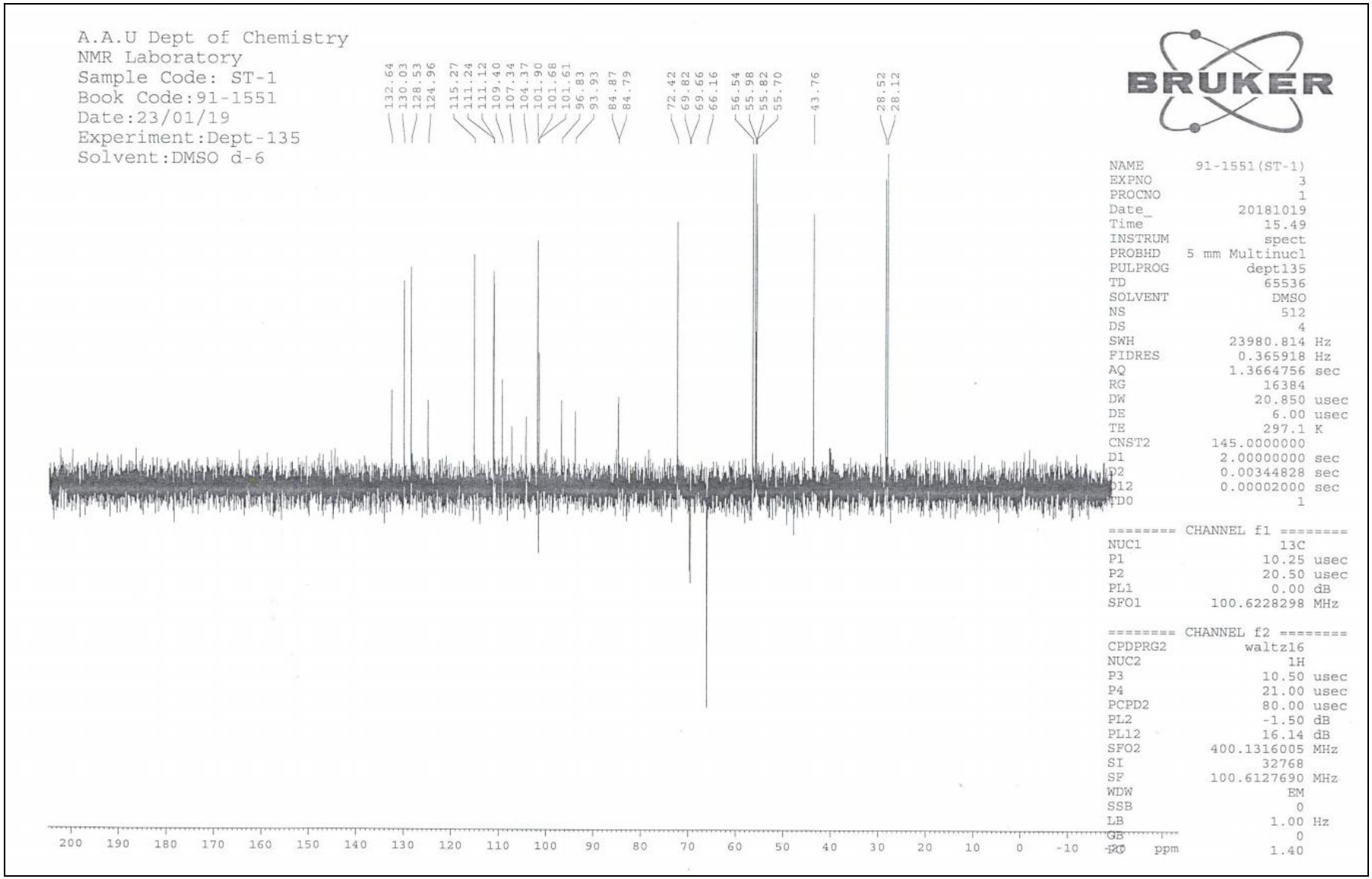
Appendix 19: ^{1}H NMR spectrum of compound 7 (ST-1)



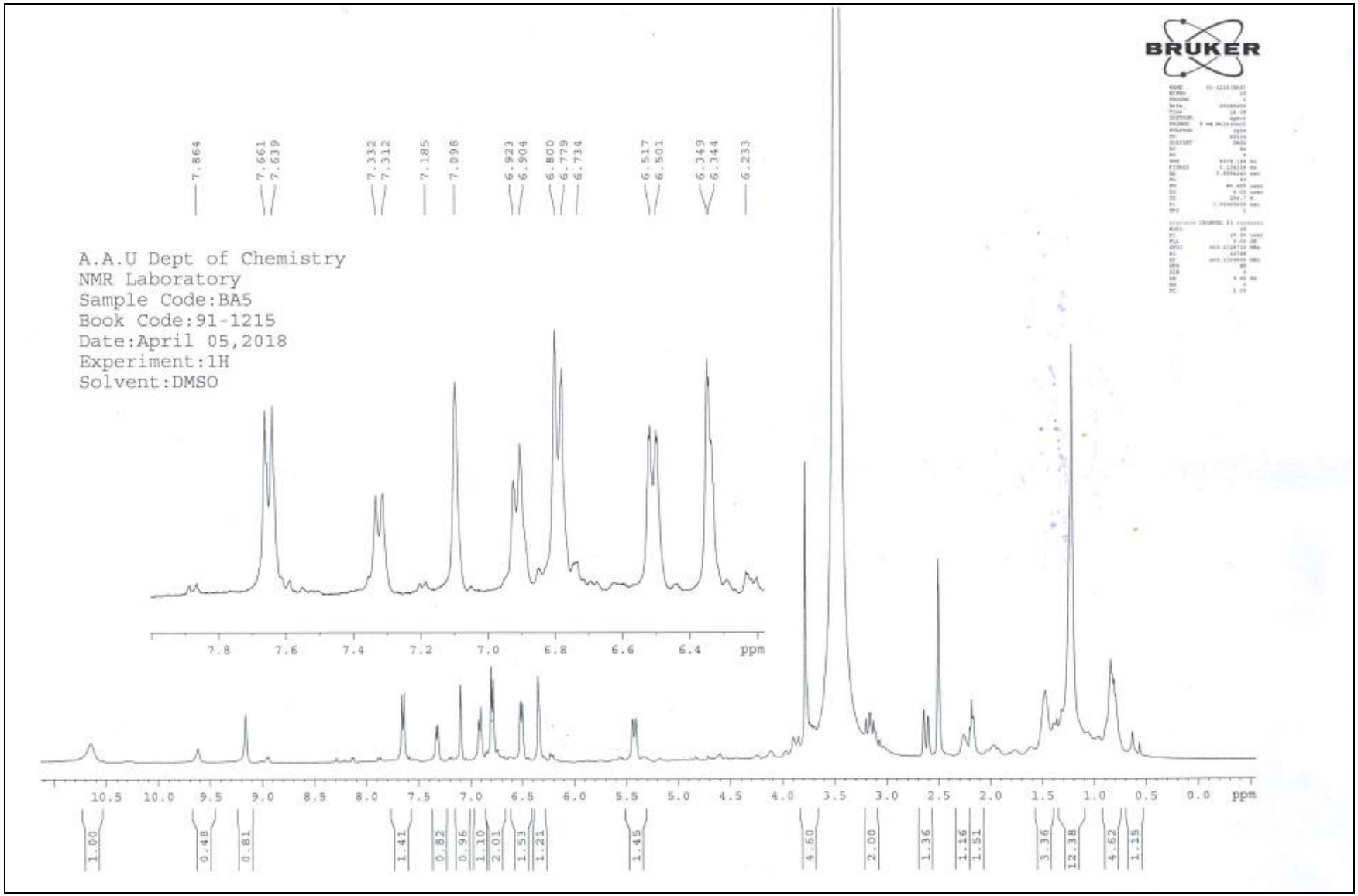
Appendix 20: ^{13}C NMR spectrum of compound 7 (ST-1)



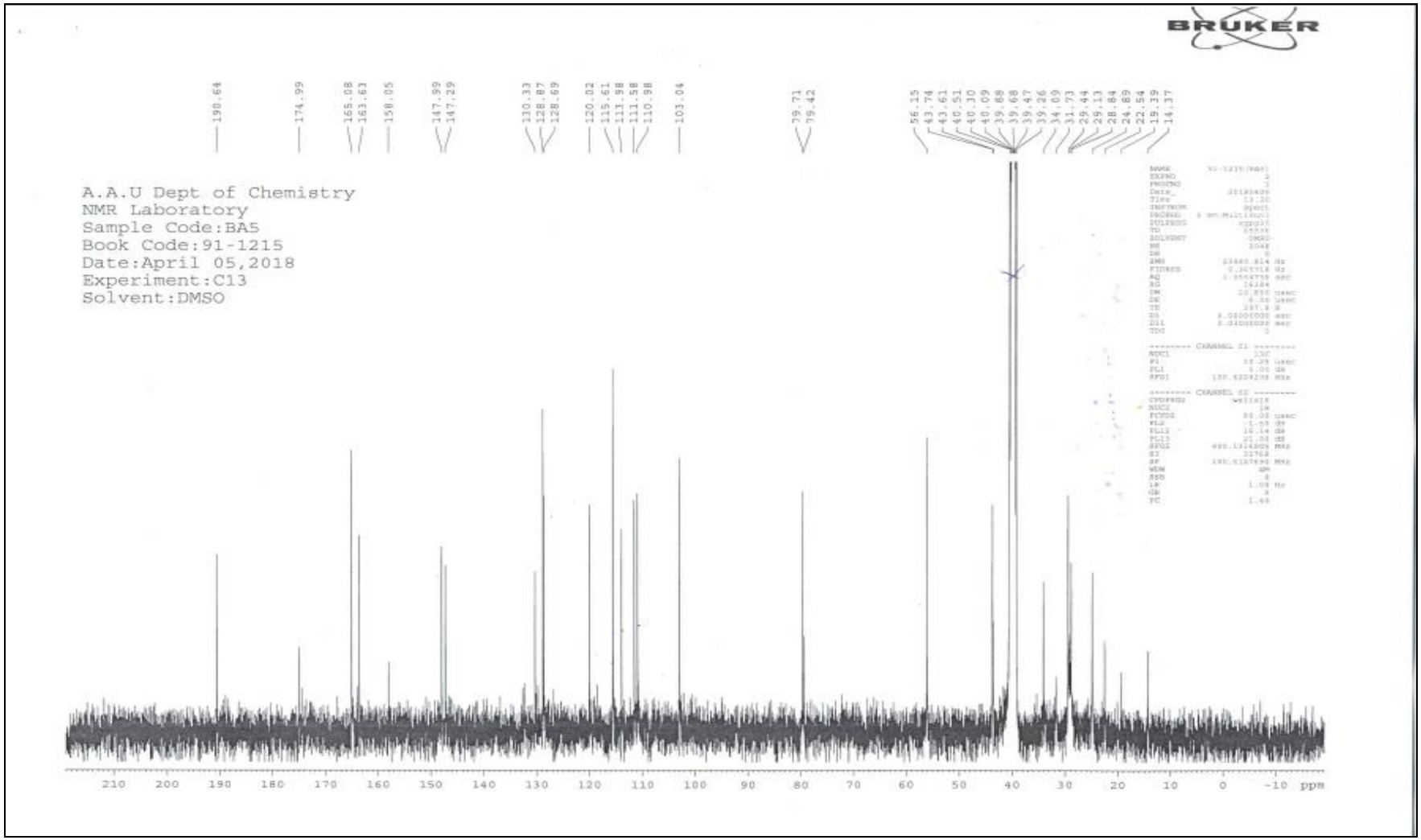
Appendix 21: DEPT-135 spectrum of compound 7 (ST-1)



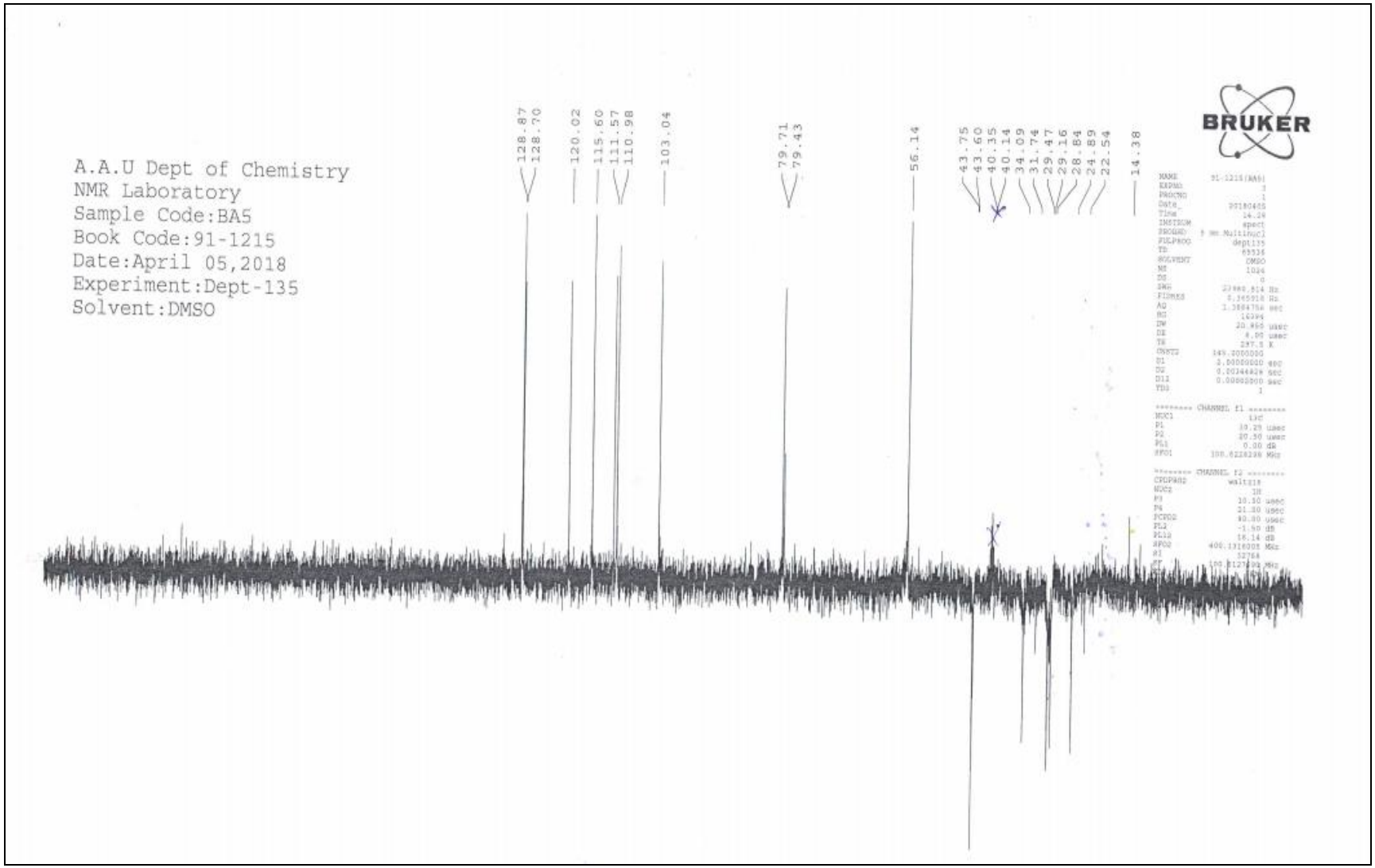
Appendix 22: ^{1}H NMR spectrum of compound 8 (BA-5)



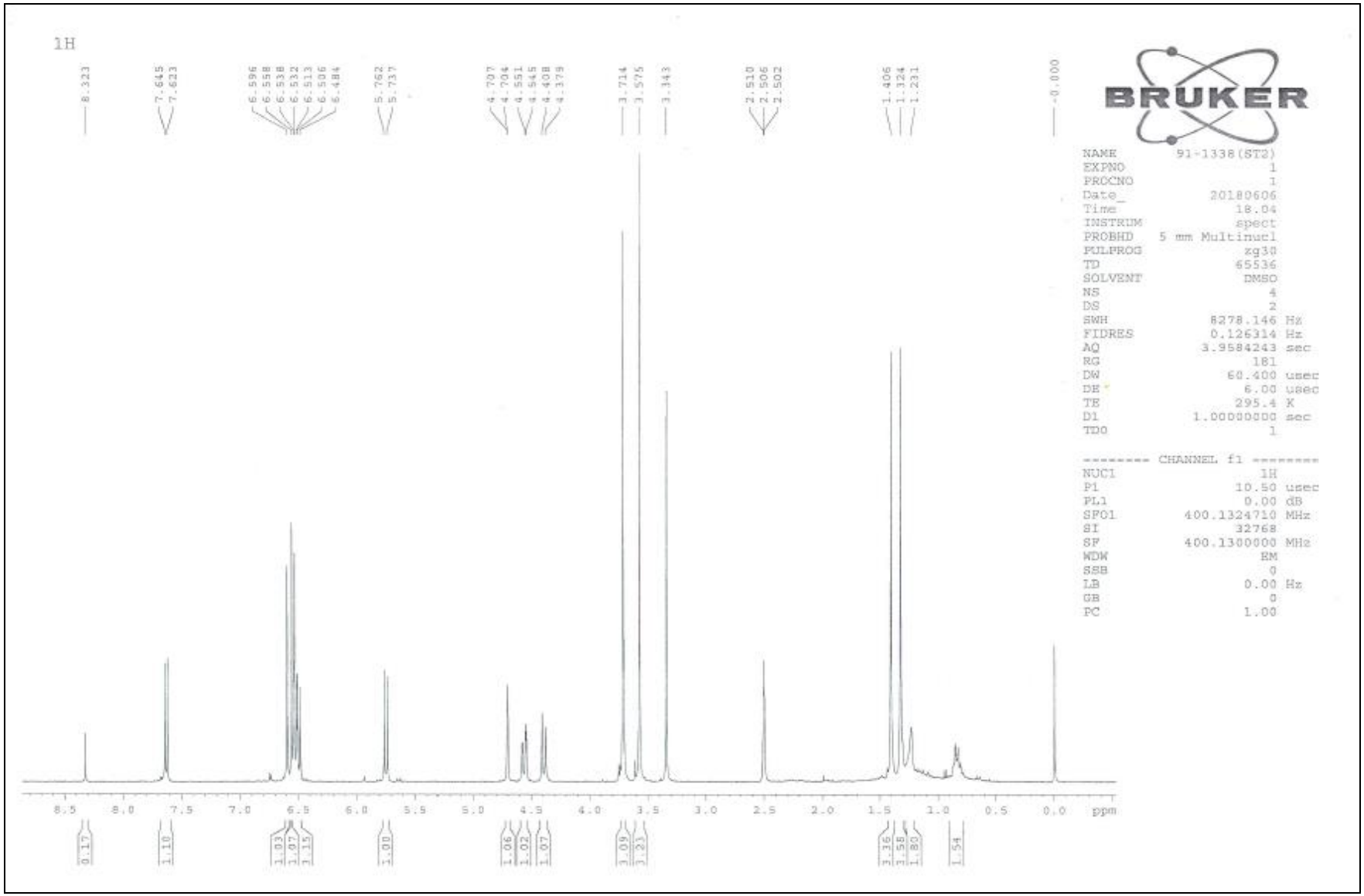
Appendix 23a: ^{13}C NMR spectrum of compound 8 (BA-5)



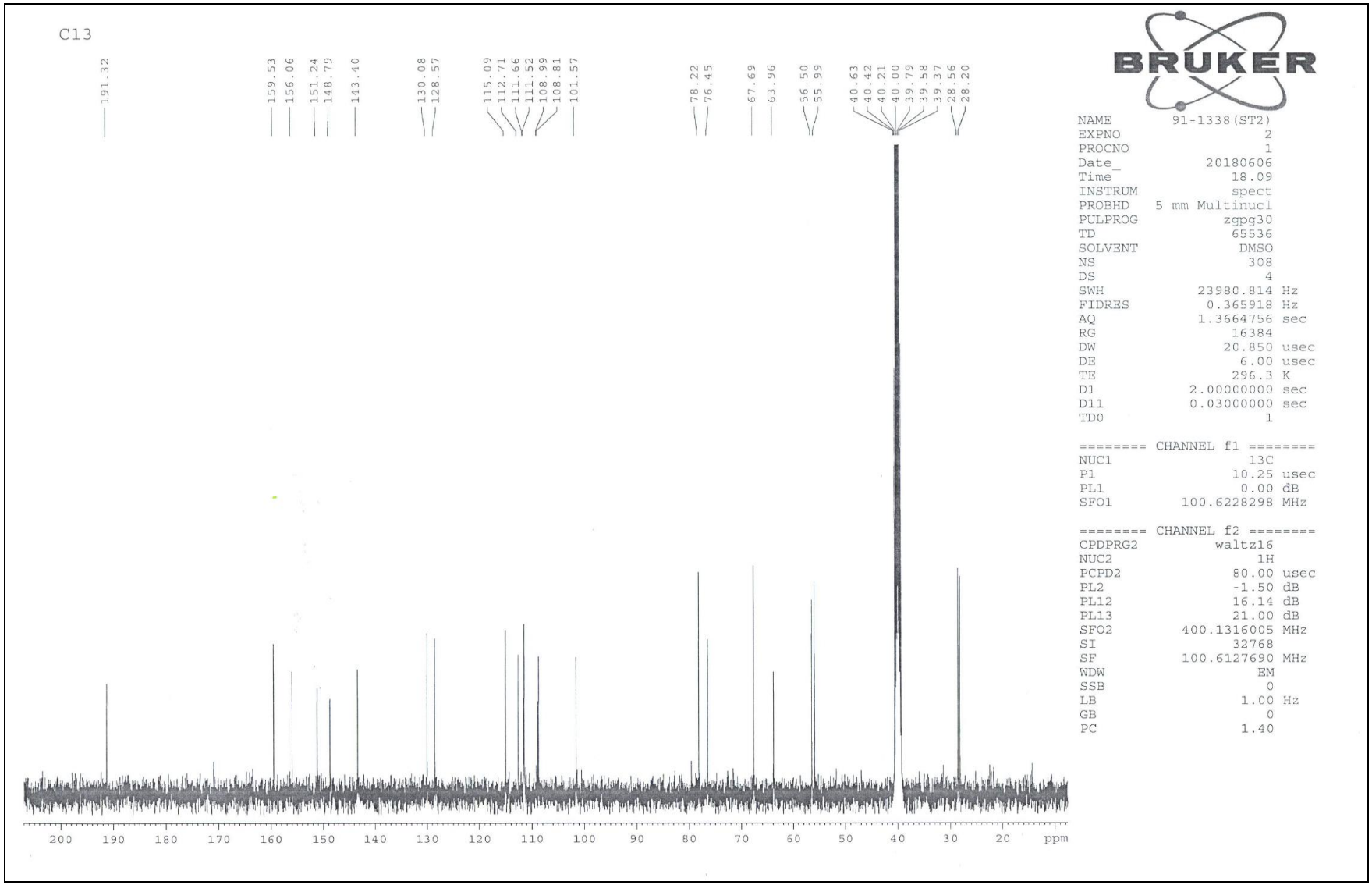
Appendix 23b: DEPT-135 sepctrum of compound 8 (BA-5)



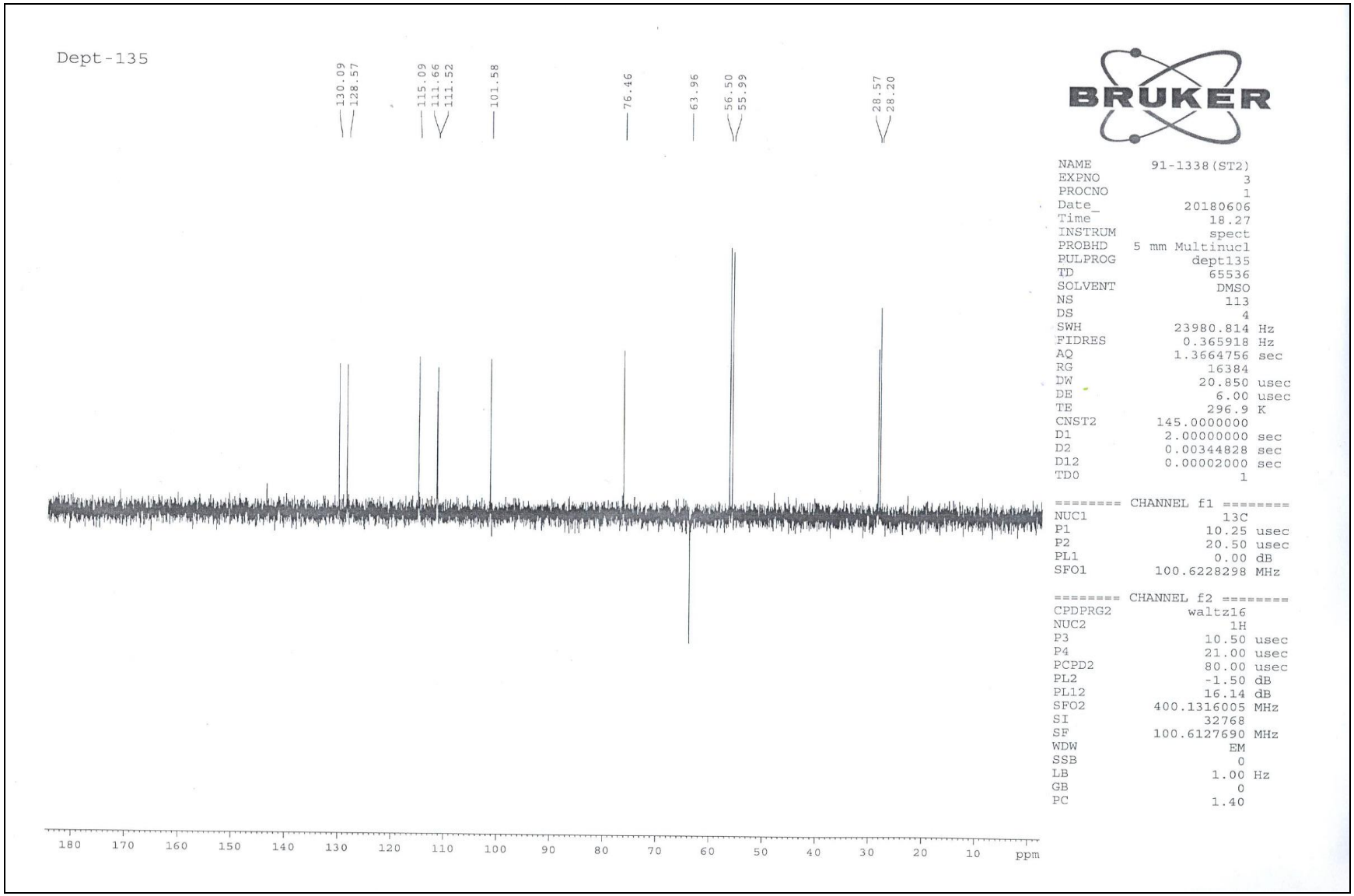
Appendix 24: ^{1}H NMR spectrum of compound 9 (ST-2)



Appendix 25: ^{13}C NMR spectrum of compound 9 (ST-2)



Appendix 25: DEPT-135 spectrum of compound 9 (ST-2)



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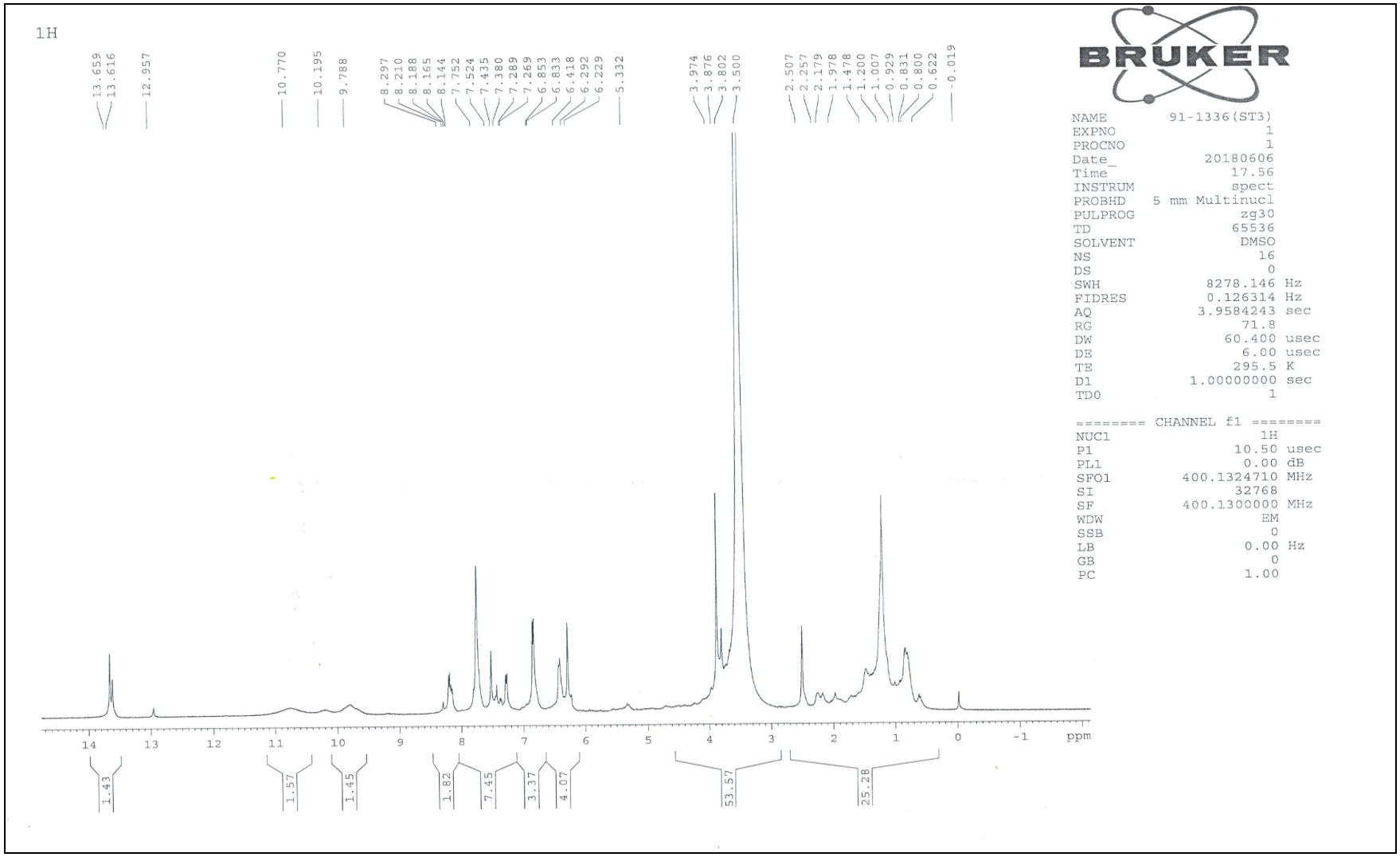
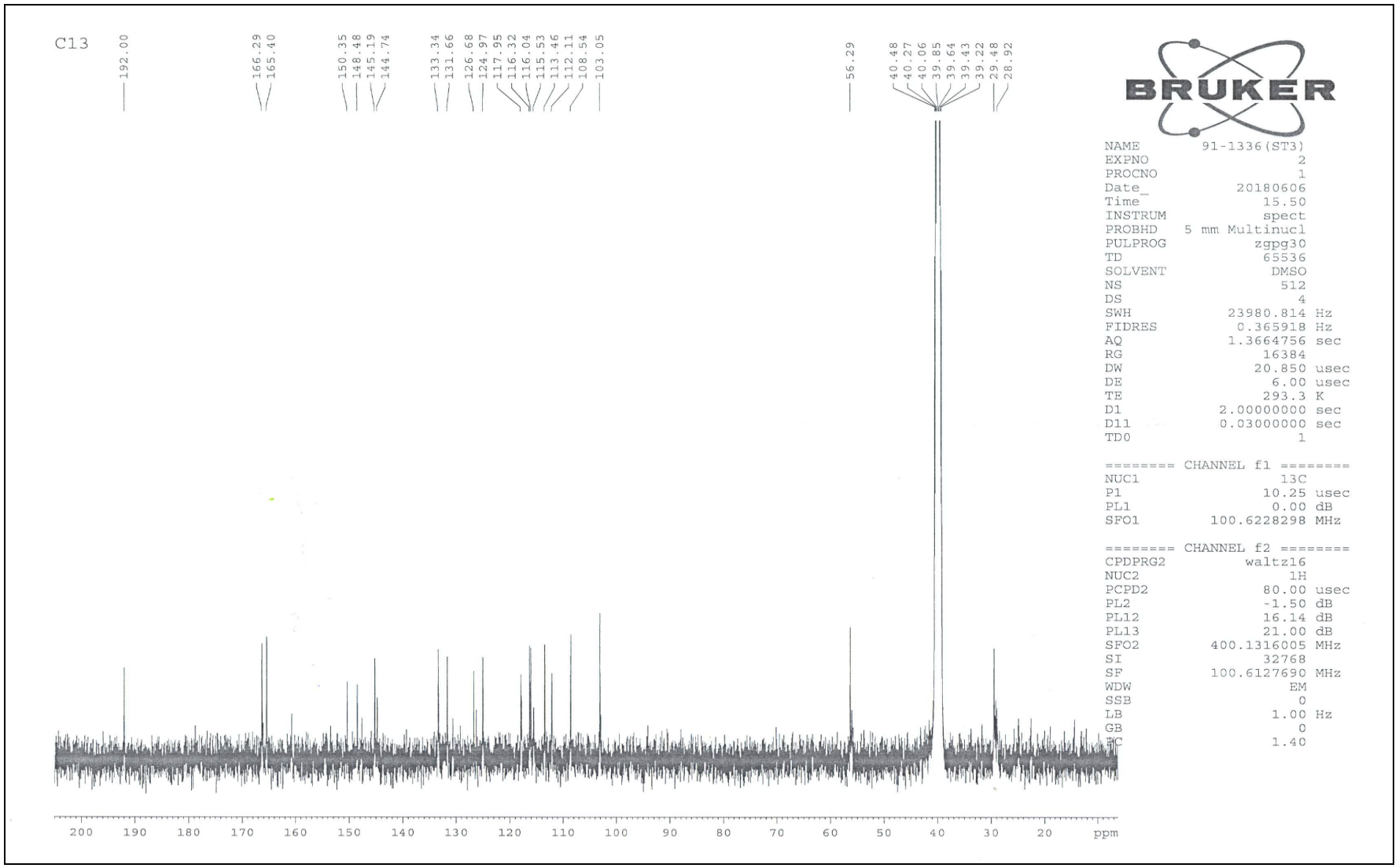
EXPNO	3	
PROCNO	1	
Date_	20180606	
Time	18.27	
INSTRUM	spect	
PROBHD	5 mm Multinucl	
PULPROG	dept135	
TD	65536	
SOLVENT	DMSO	
NS	113	
DS	4	
SWH	23980.814	Hz
FIDRES	0.365918	Hz
AQ	1.3664756	sec
RG	16384	
DW	20.850	usec
DE	6.00	usec
TE	296.9	K
CNST2	145.0000000	
D1	2.00000000	sec
D2	0.00344828	sec
D12	0.00002000	sec
TD0	1	
========	CHANNEL f1 ========	
NUC1	13C	
P1	10.25	usec
P2	20.50	usec
PL1	0.00	dB
SFO1	100.6228298	MHz
========	CHANNEL f2 ========	
CPDPRG2	waltz16	
NUC2	1H	
P3	10.50	usec
P4	21.00	usec
PCPD2	80.00	usec
PL2	-1.50	dB
PL12	16.14	dB
SFO2	400.1316005	MHz
SI	32768	
SF	100.6127690	MHz
WDW	EM	
SSB	0	
LB	1.00	Hz
GB	0	
PC	1.40	

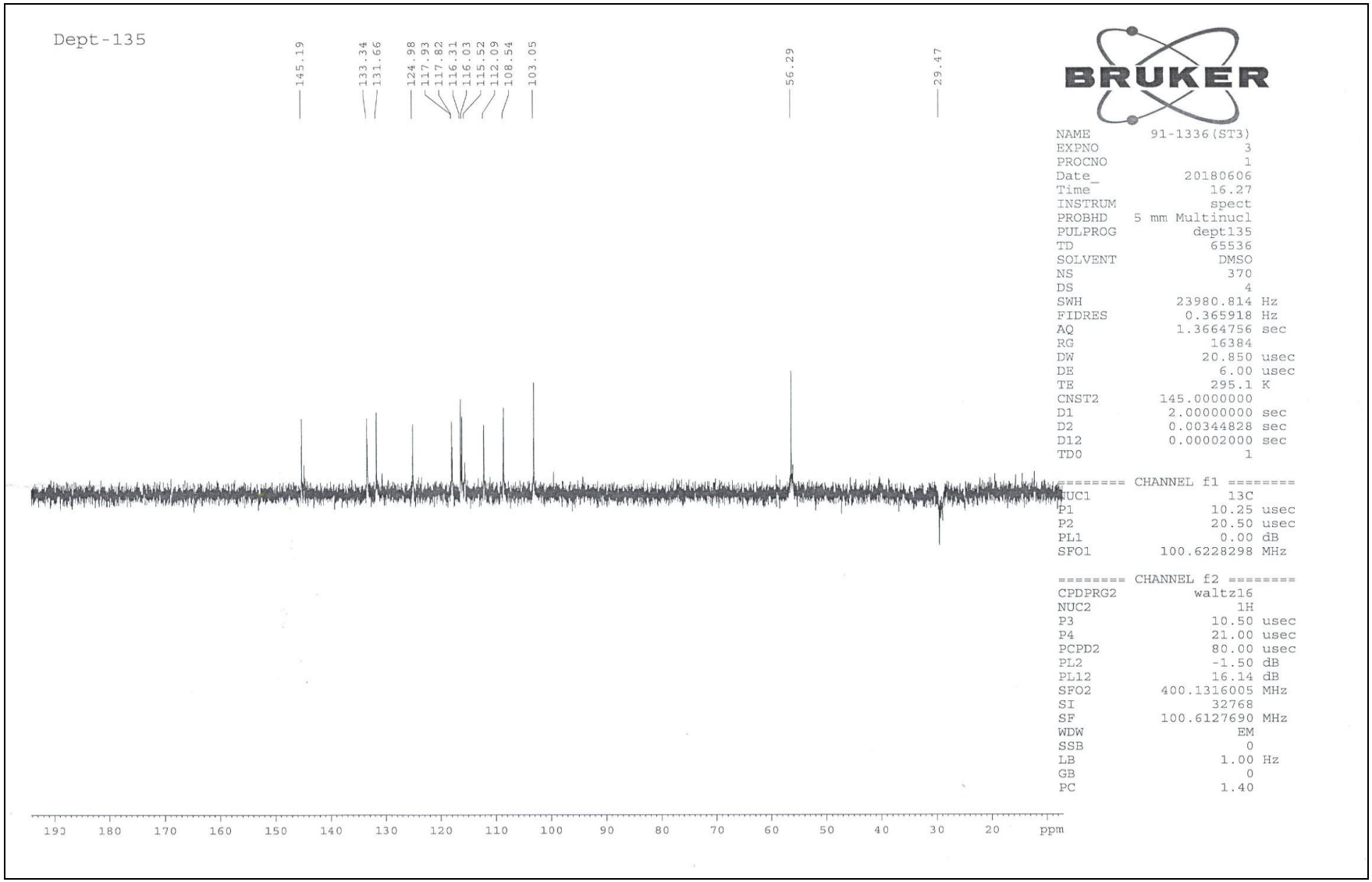
Appendix 26: ^{1}H NMR spectrum of compound 10 (ST-3/6)



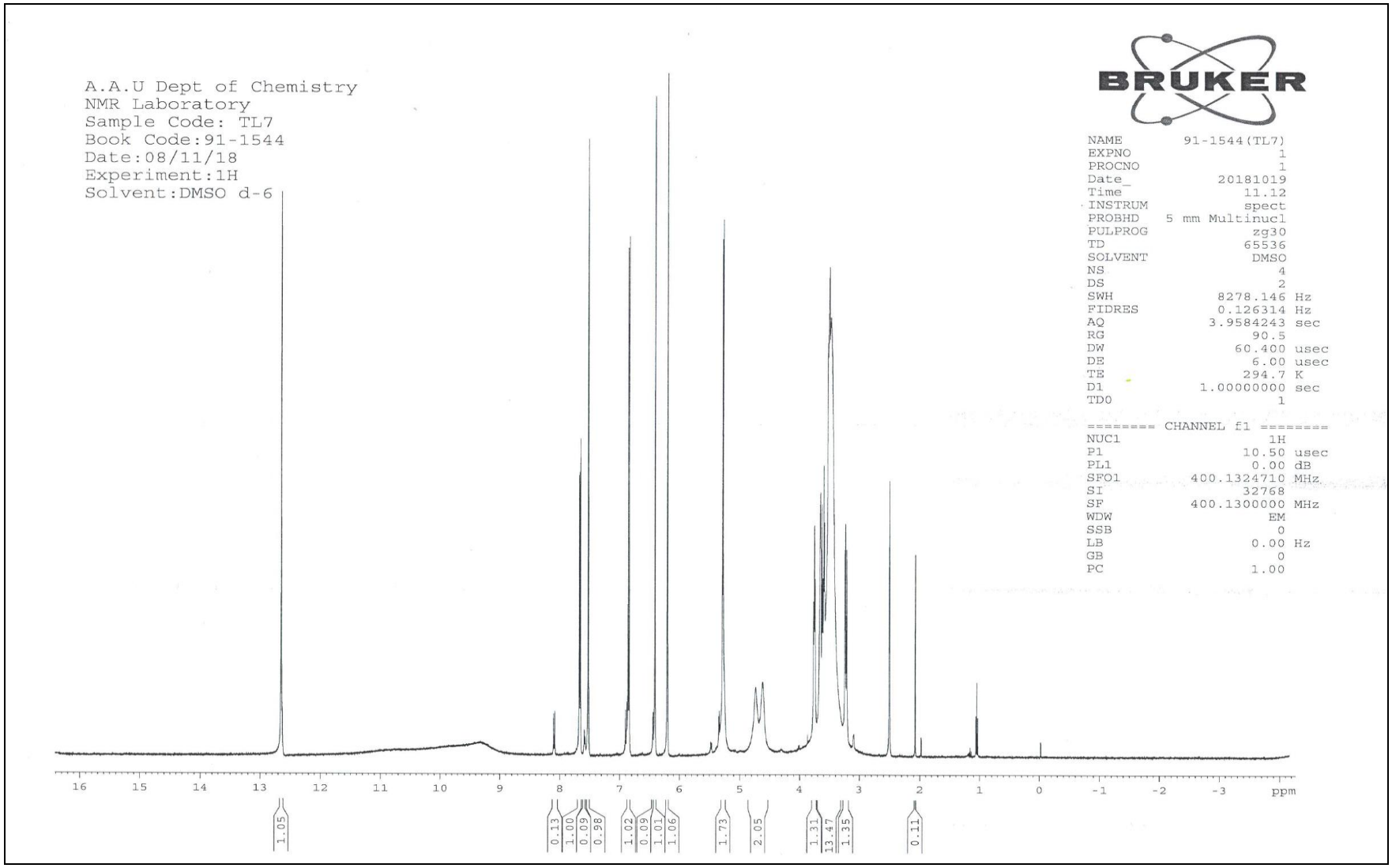
Appendix 27: ^{13}C NMR spectrum of compound 10 (ST-3/6)



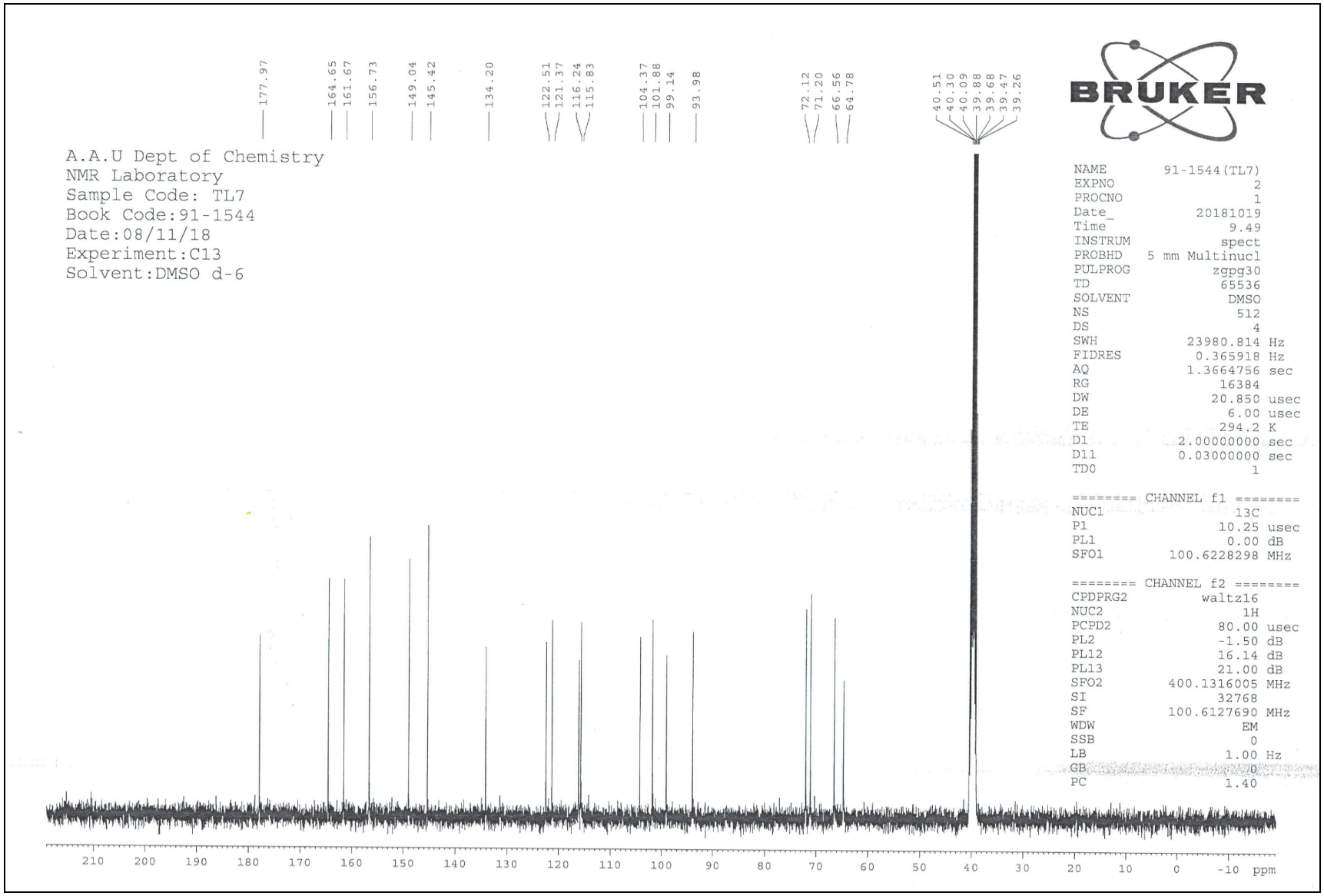
Appendix 28: DEPT-135 spectrum of compound 10 (ST-3/6)



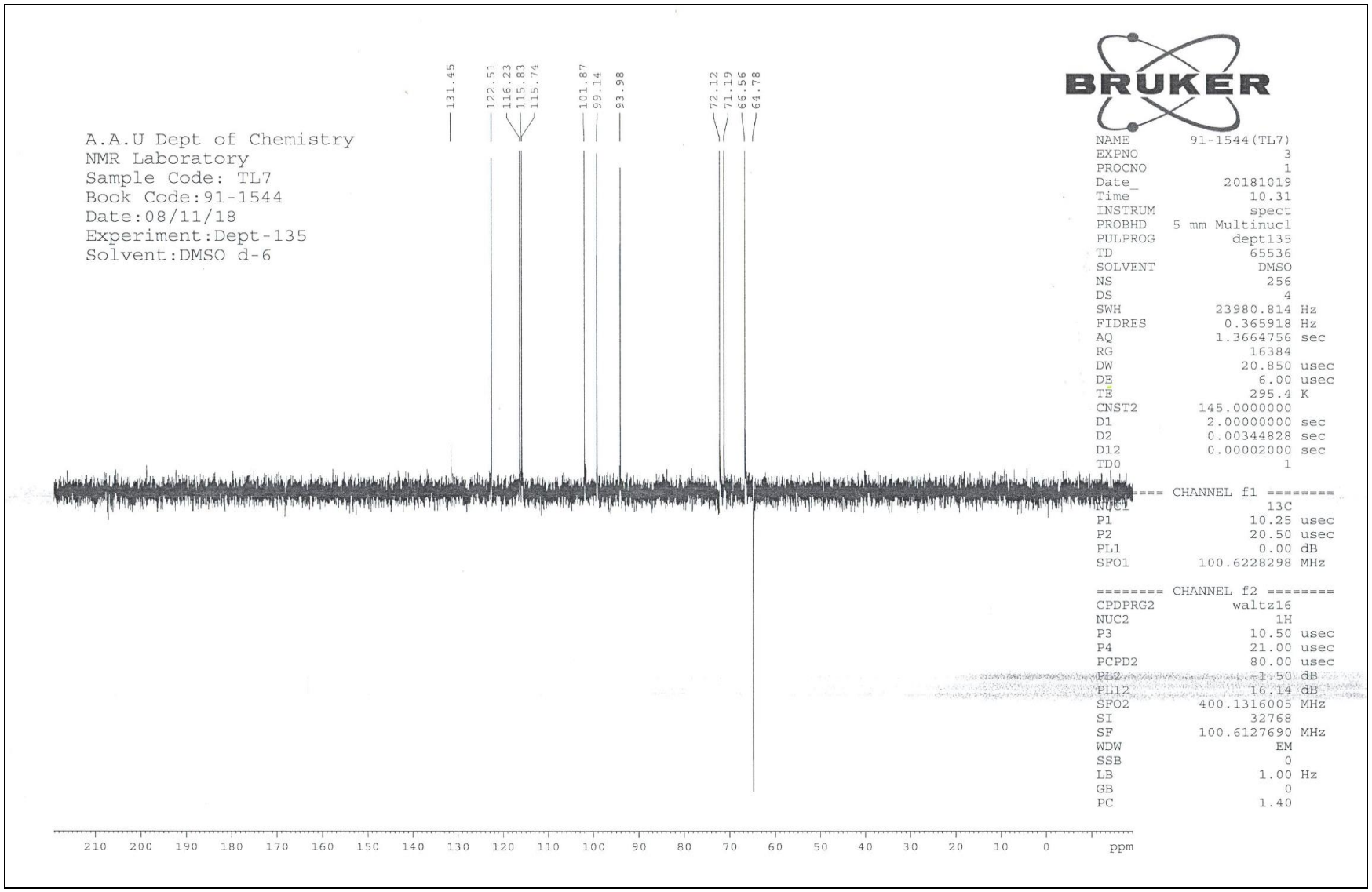
Appendix 29: ^{1}H NMR spectrum of compound 11 (TL-7)



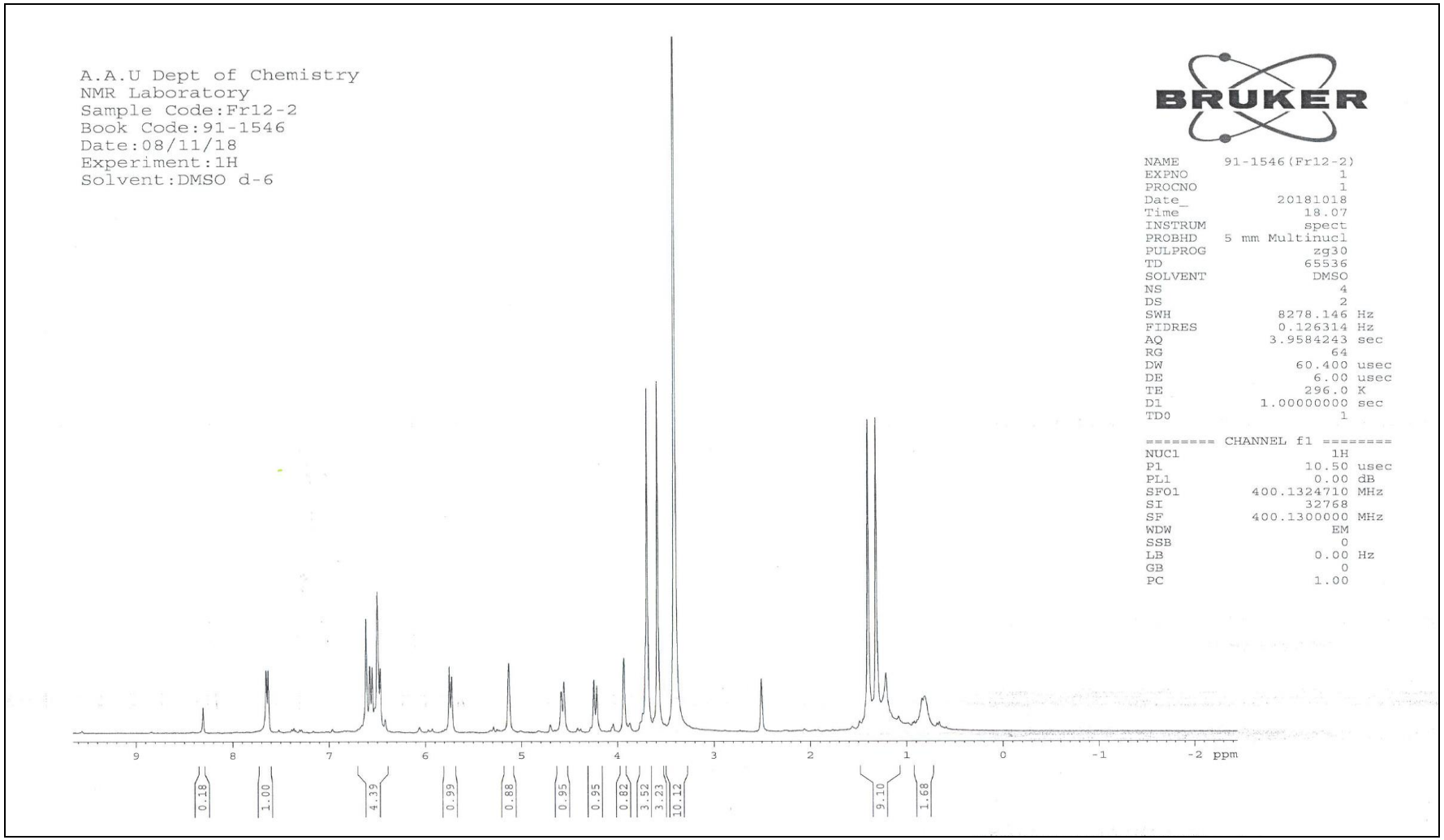
Appendix 30: ^{13}C NMR spectrum of compound 11 (TL-7)



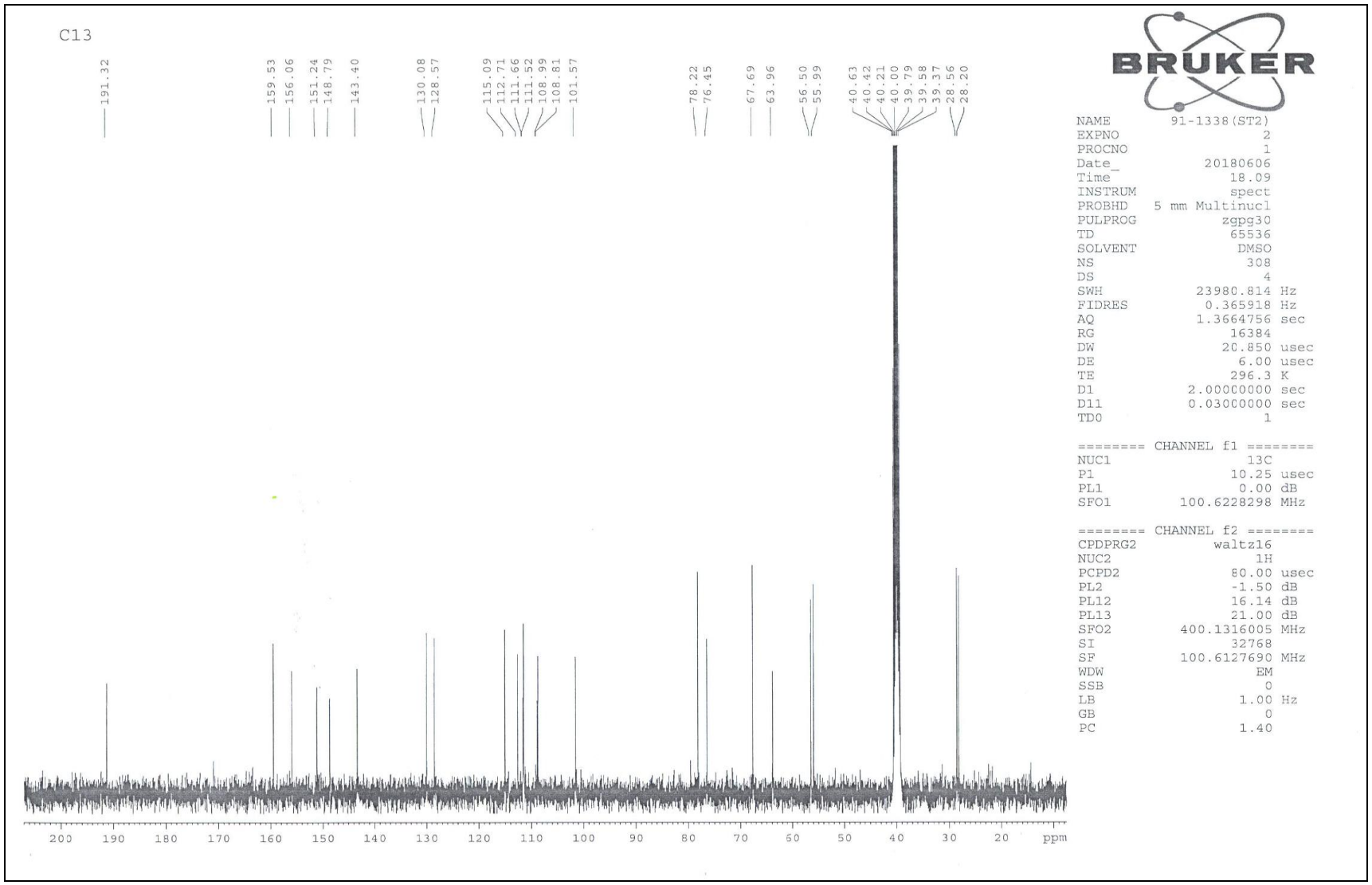
Appendix 31: DEPT-135 spectrum of compound 11 (TL-7)



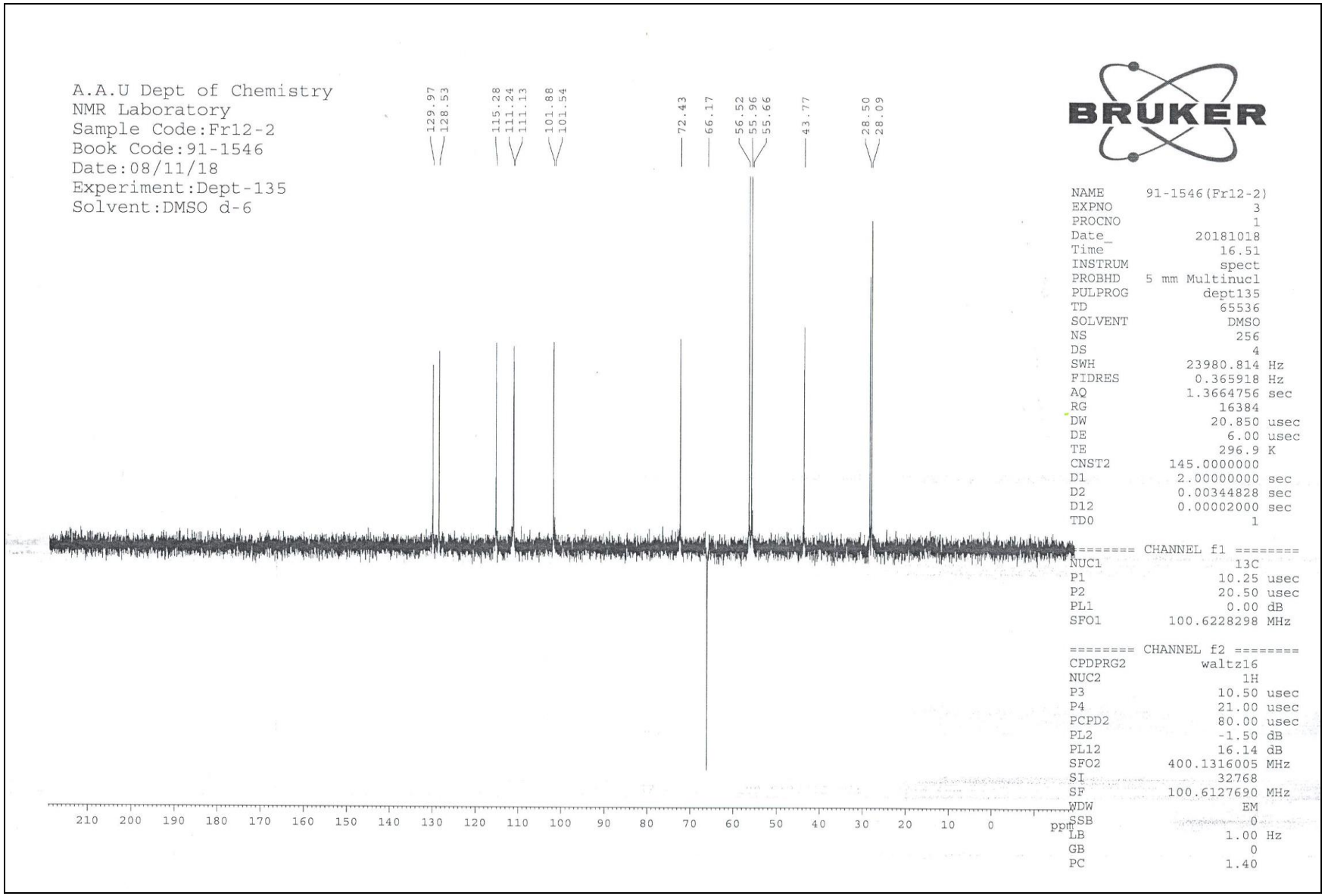
Appendix 32: ^{1}H NMR spectrum of compound 12 (FR-12)



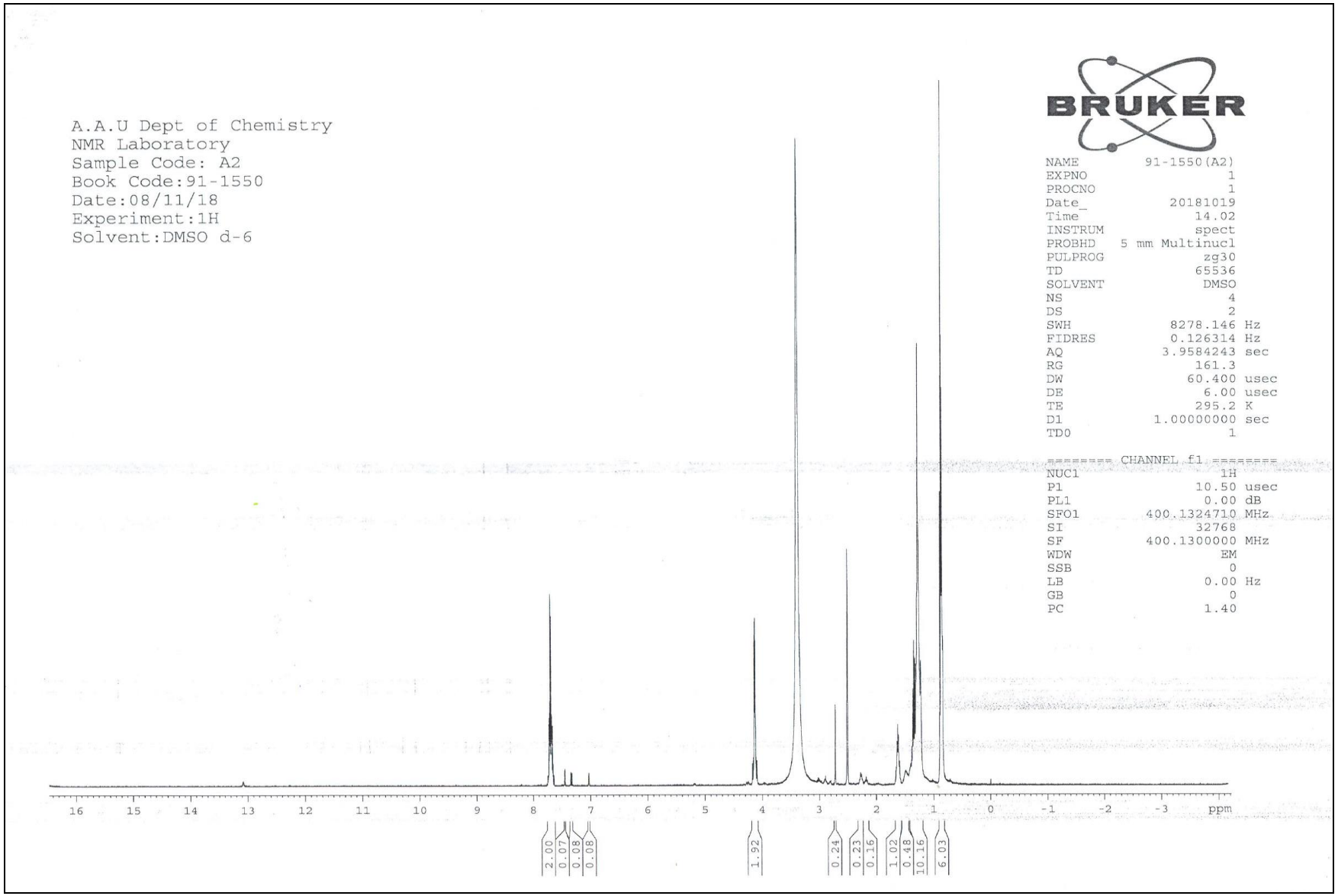
Appendix 33: ^{13}C NMR spectrum of compound 12 (FR-12)



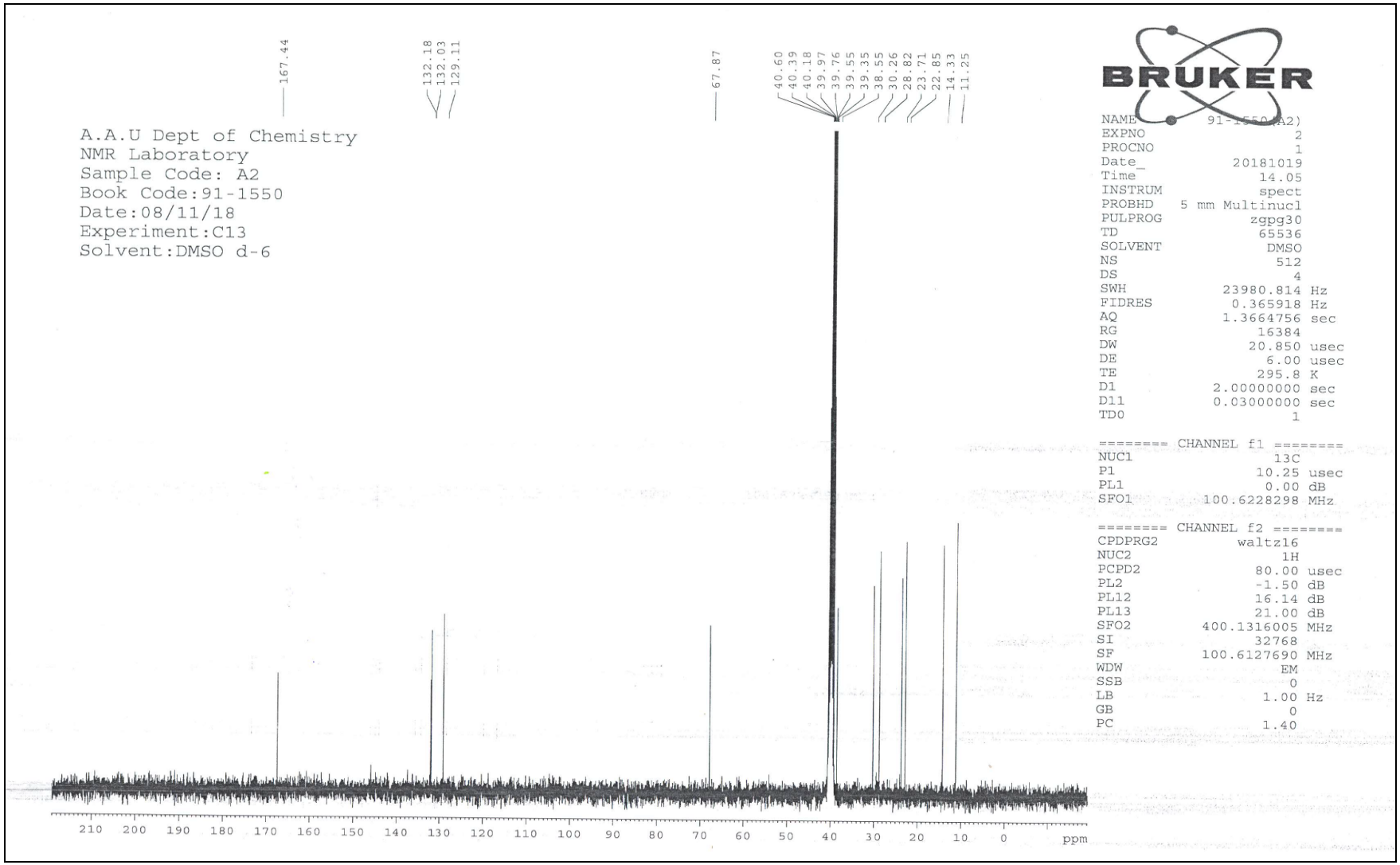
Appendix 34: DEPR-135 spectrum of compound 12 (FR-12)



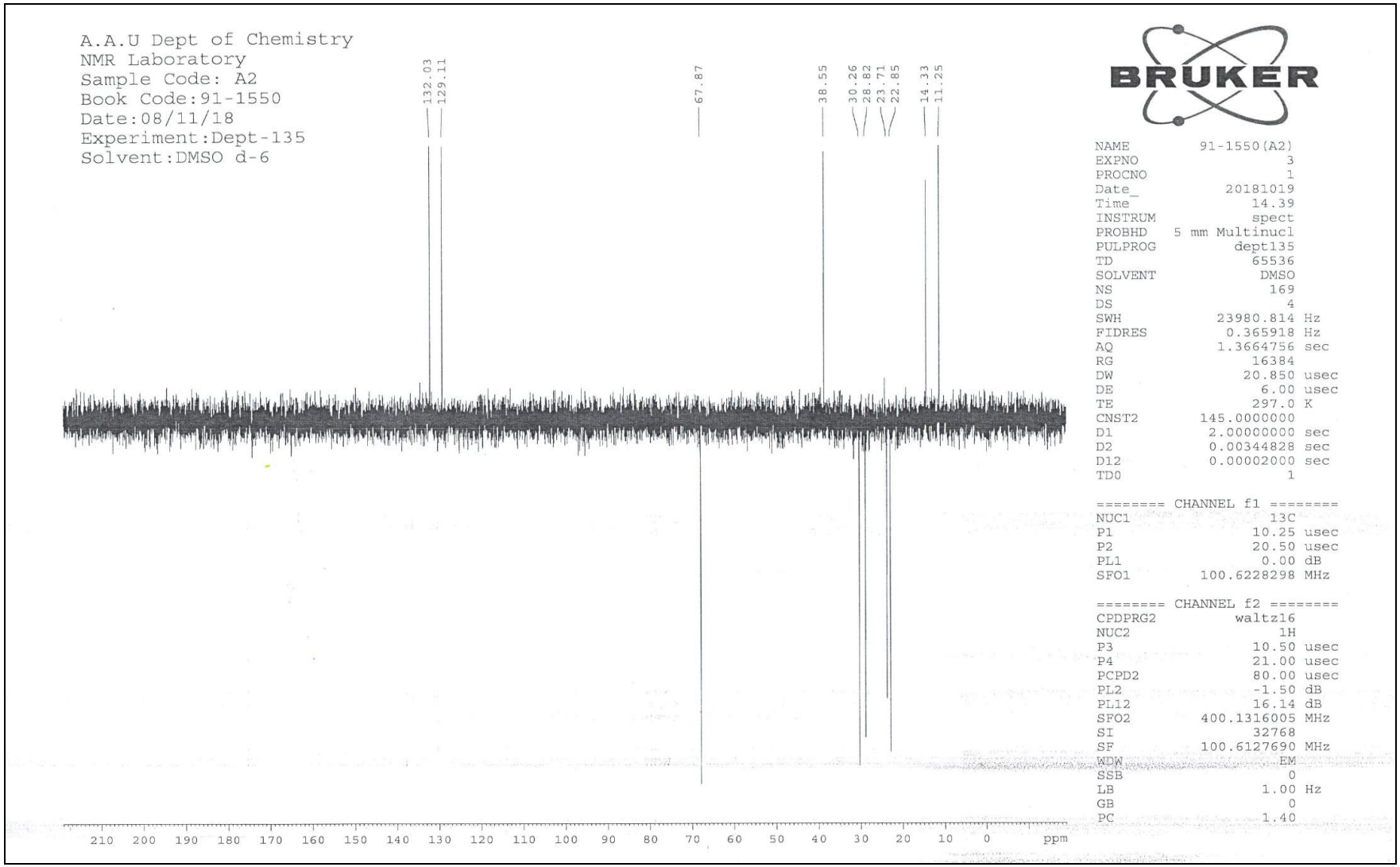
Appendix 35: ^{1}H NMR spectrum of compound 13 (A2)



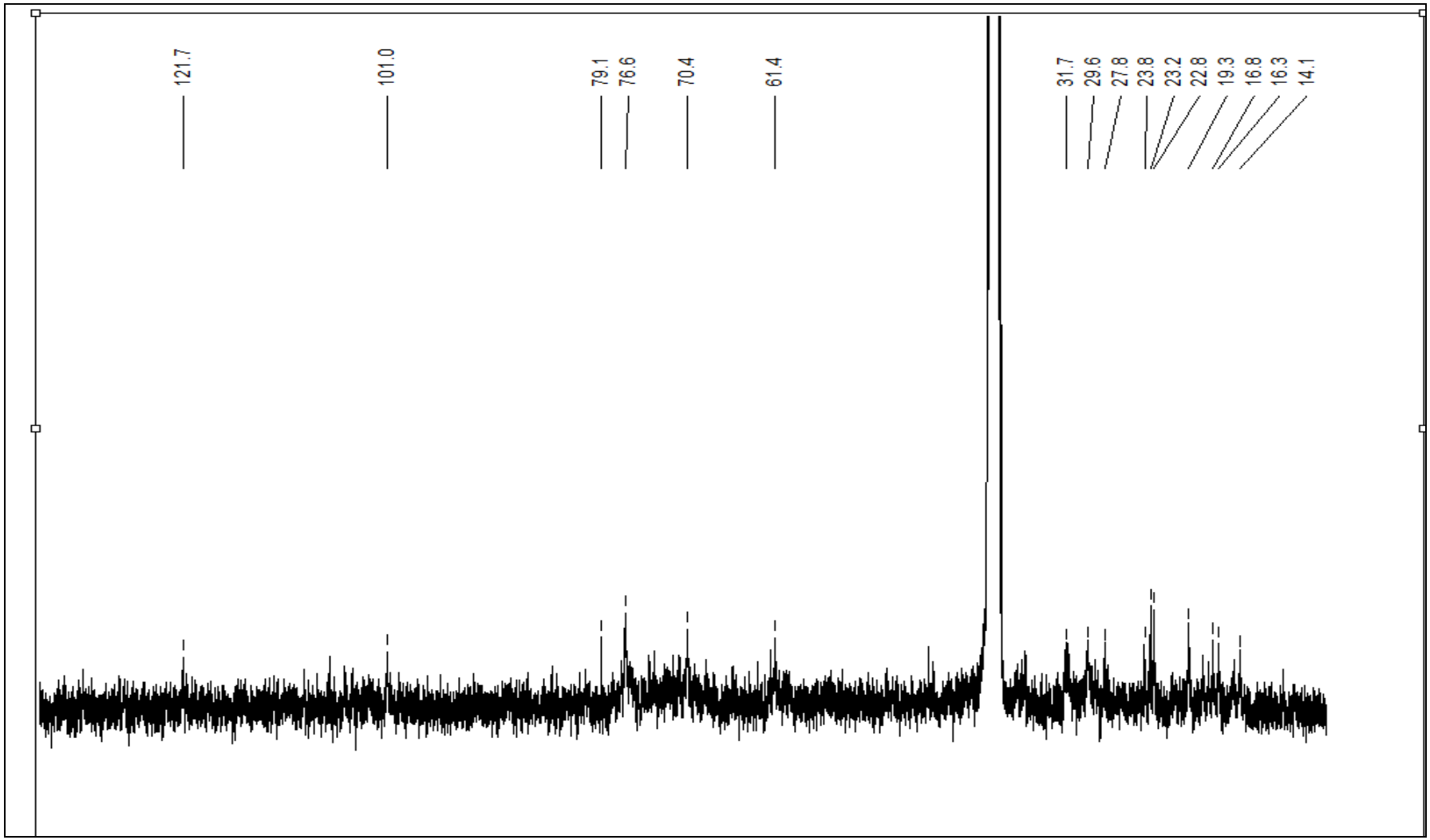
Appendix 36: ^{13}C NMR spectrum of compound 13 (A2)



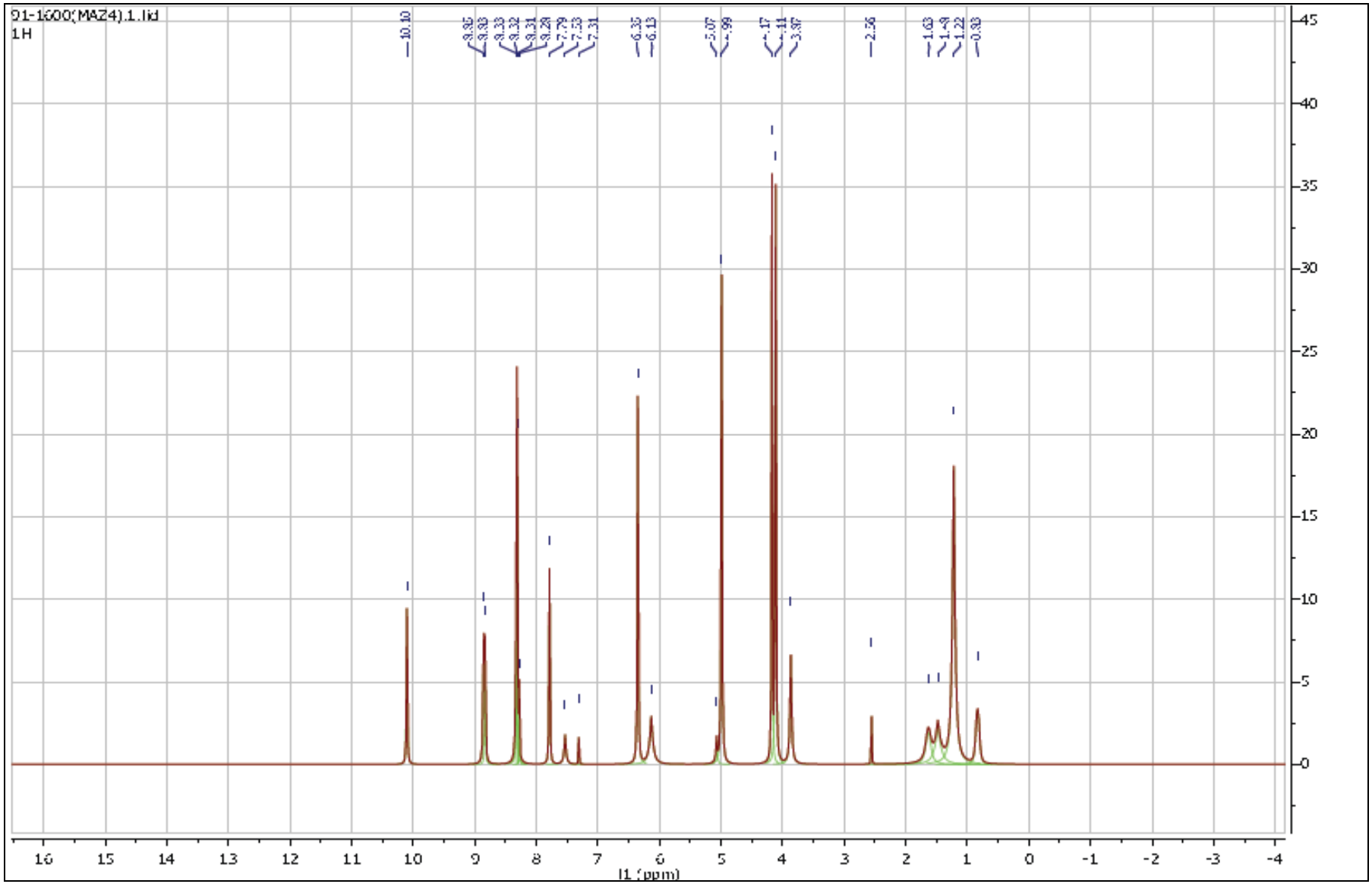
Appendix 37: DEPT-135 spectrum of compound 13 (A2)



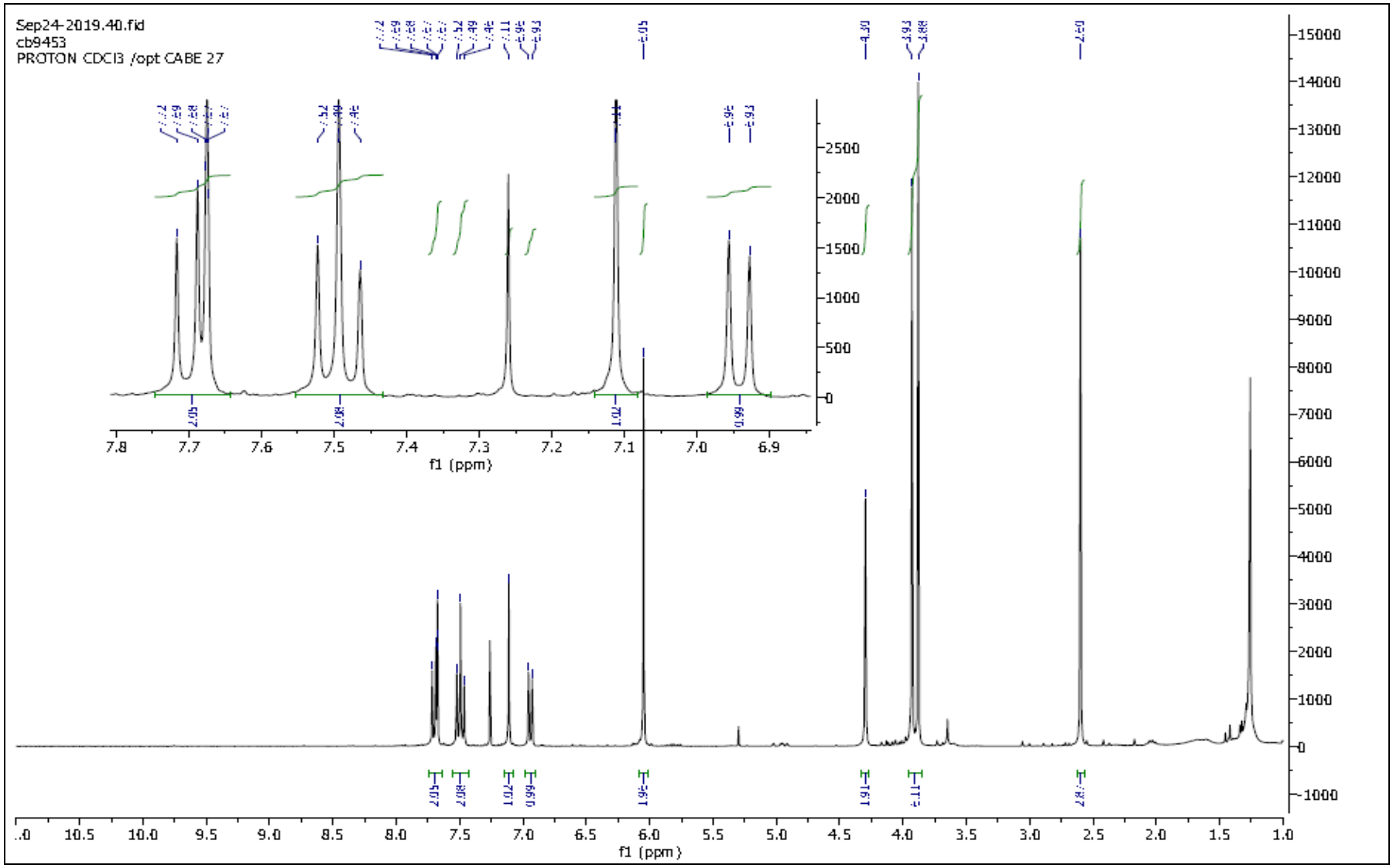
Appendix 38: ^{13}C NMR spectrum of compound 14



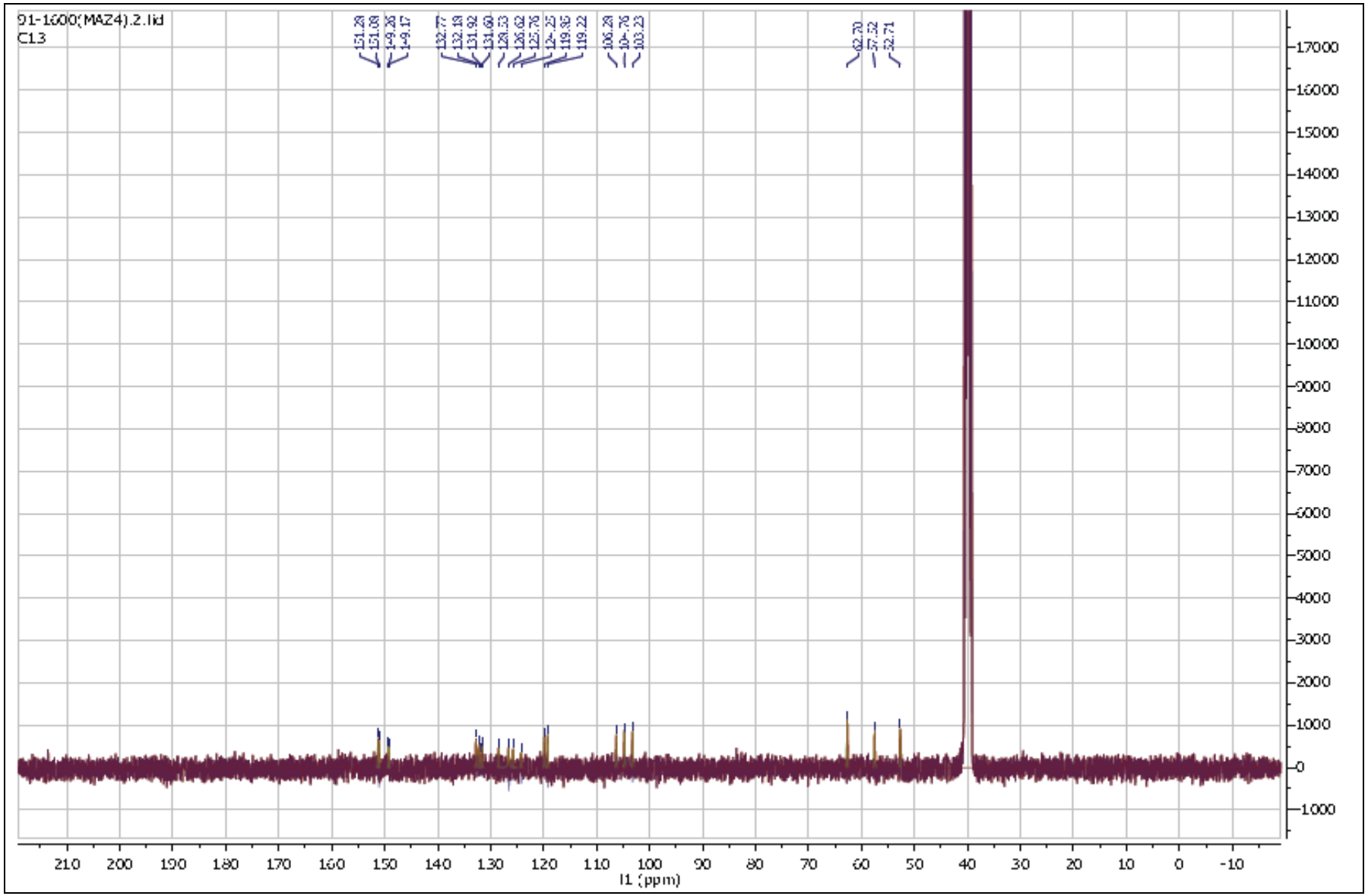
Appendix 39: ^{1}H NMR spectrum of compound 15 (MAZ-4)



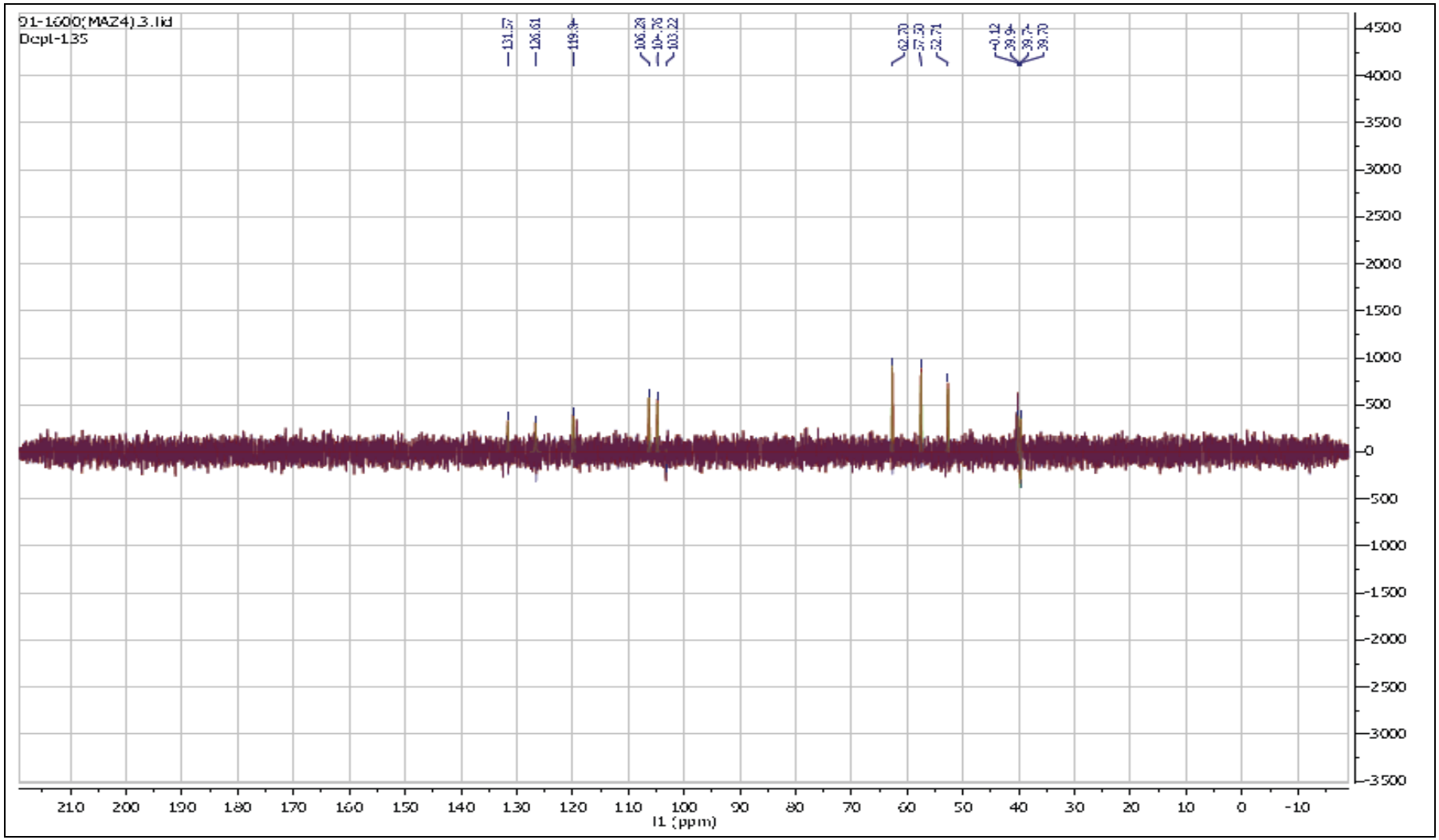
Appendix 39a: ^{1}H NMR spectrum of compound 15 (MAZ-4)



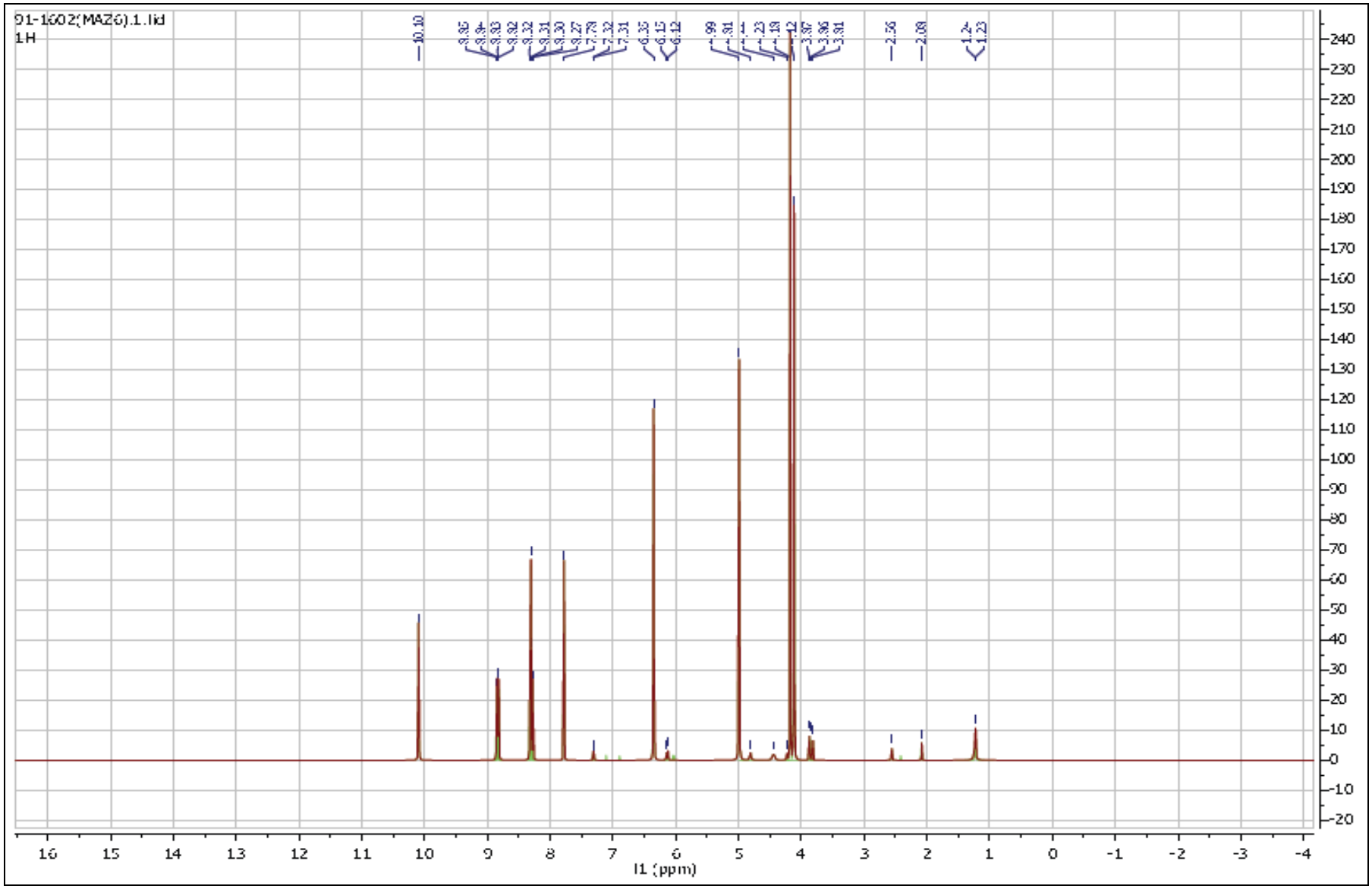
Appendix 40: ^{13}C NMR spectrum of compound 15 (MAZ-4)



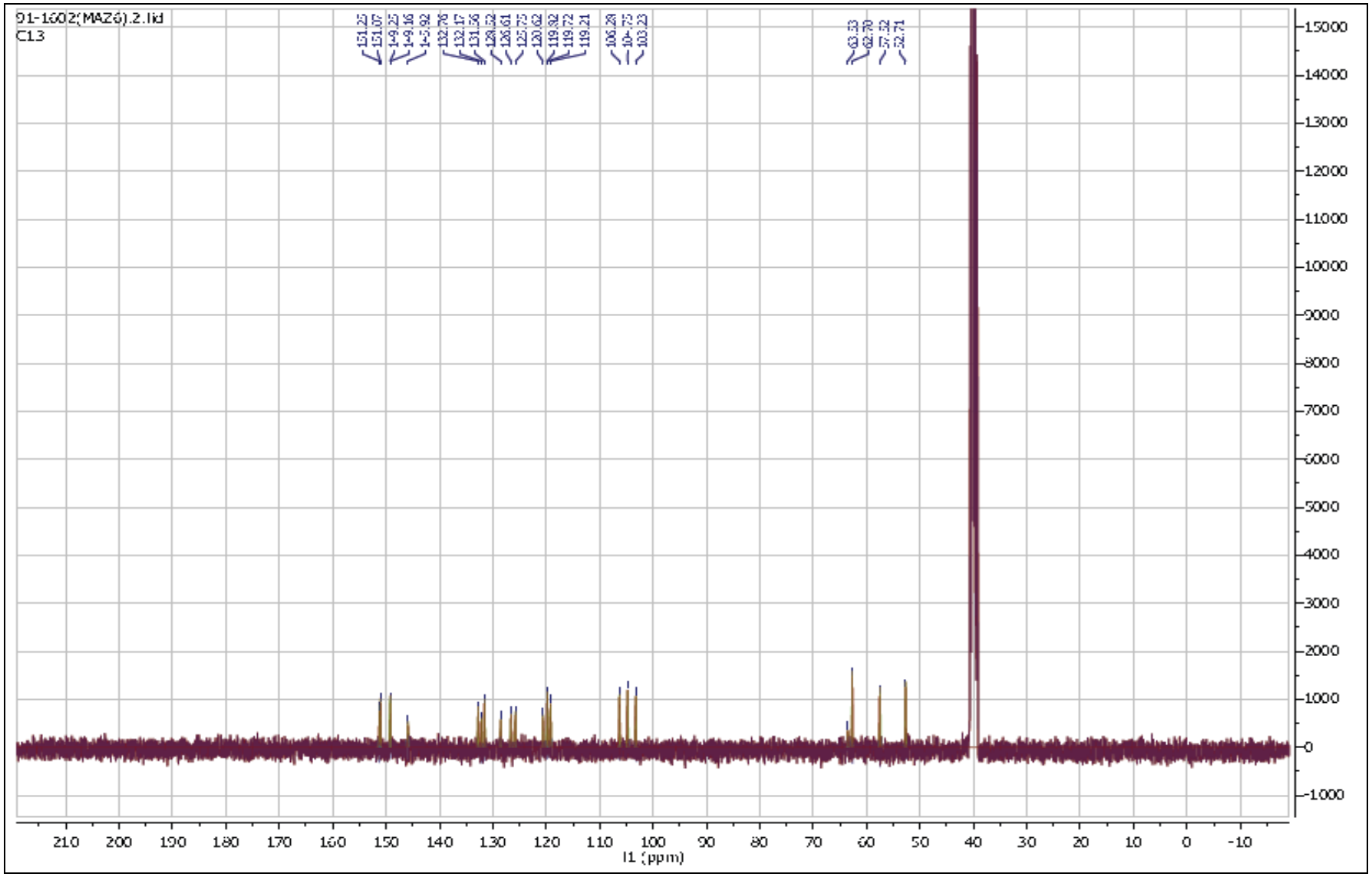
Appendix 41: DEPT-135 spectrum of compound 15 (MAZ-4)



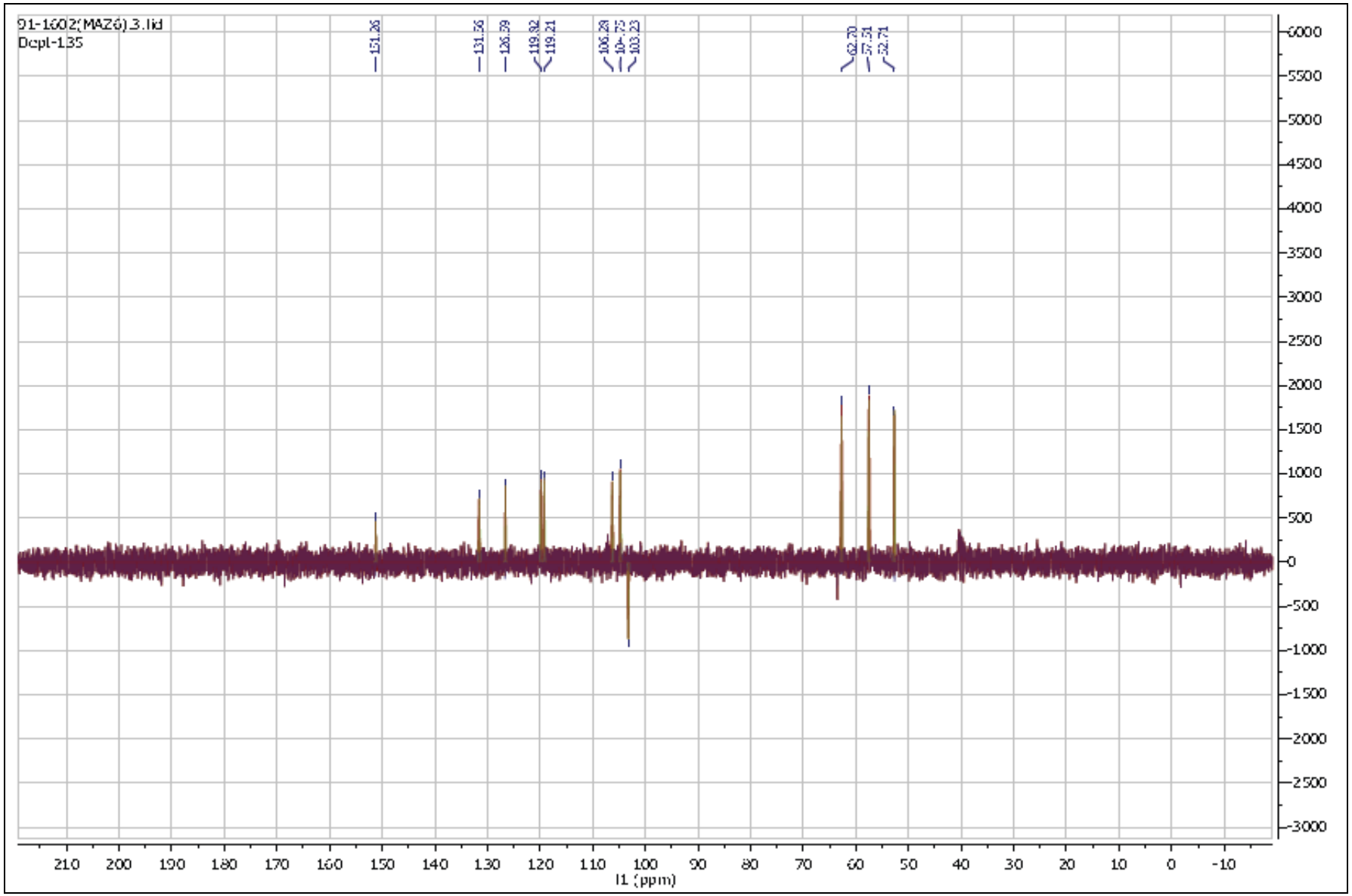
Appendix 42: ^{1}H NMR spectrum of compound 16 (MAZ-6)



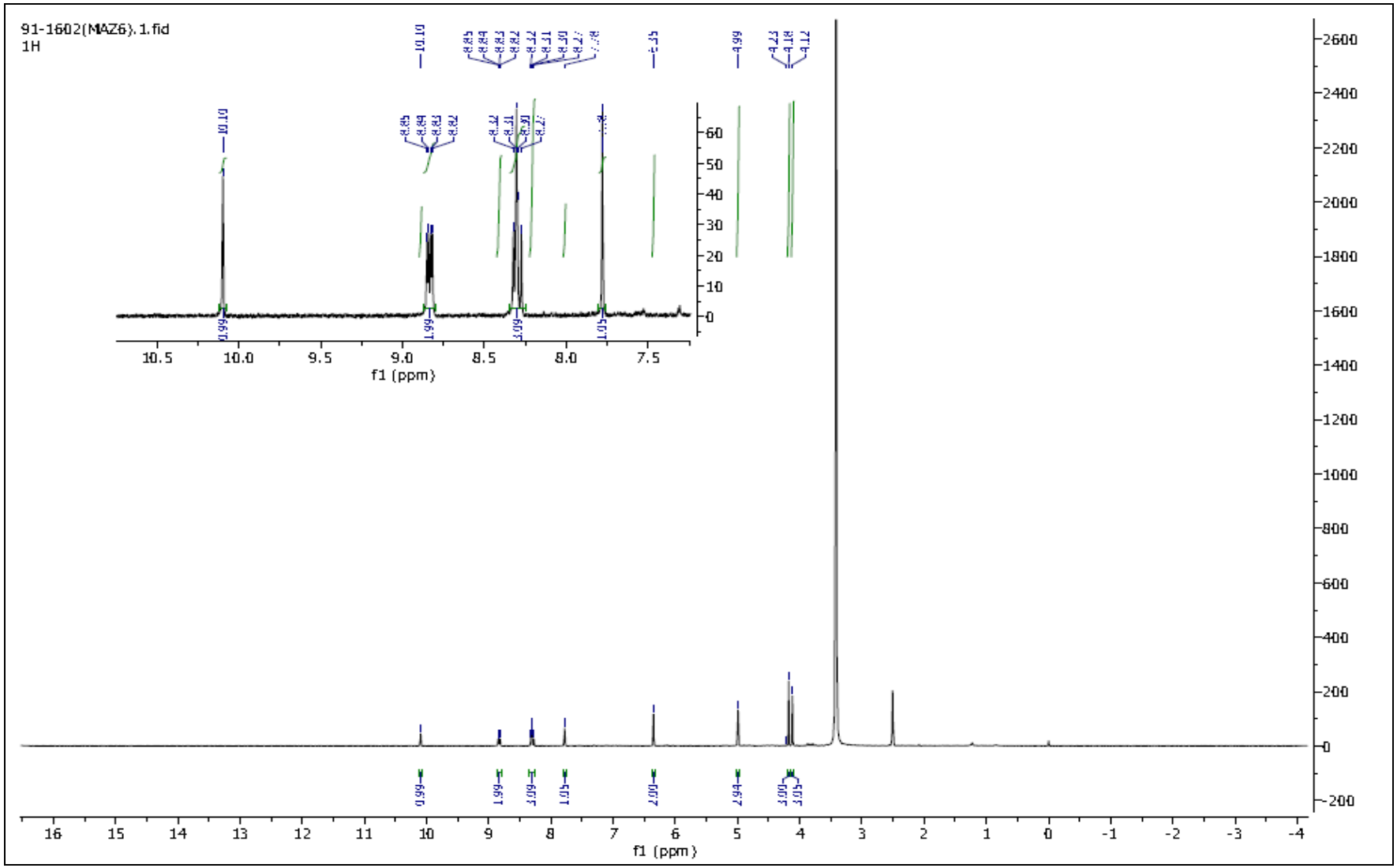
Appendix 43: ^{13}C NMR spectrum of compound 16 (MAZ-6)



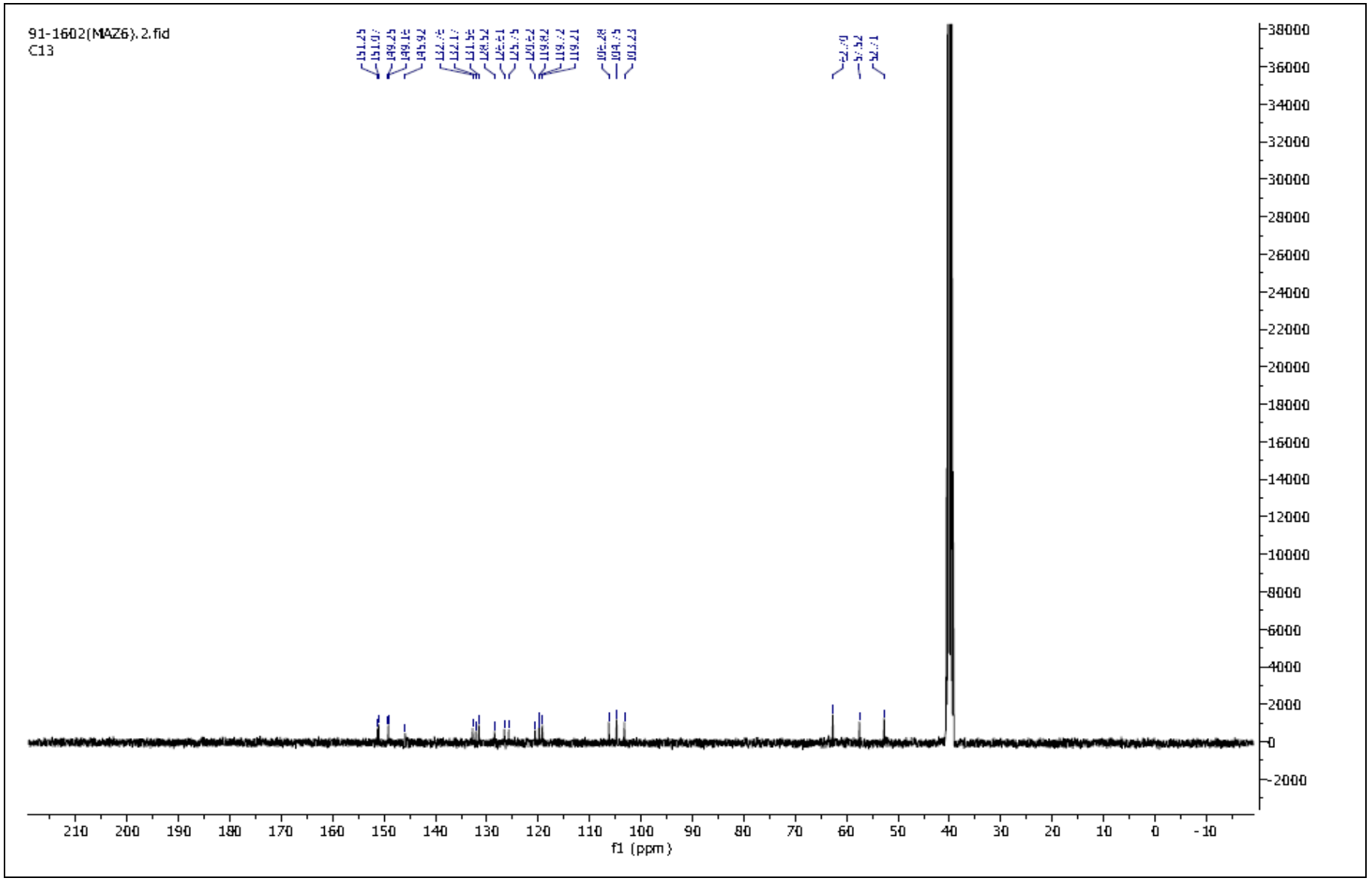
Appendix 44: DEPT-135 spectrum of compound 16 (MAZ-6)



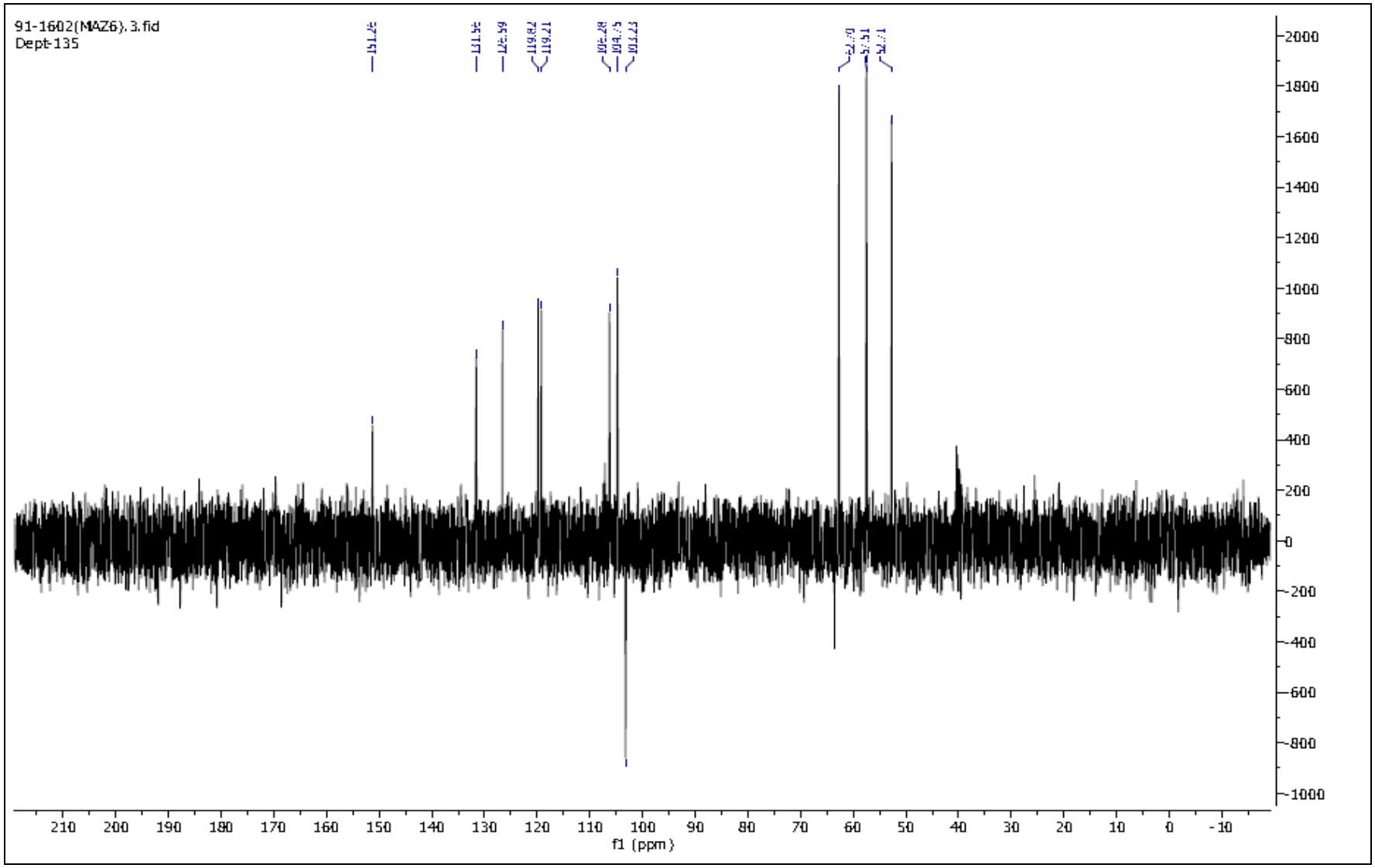
Appendix 44a: ^{1}H NMR spectrum of compound 16a



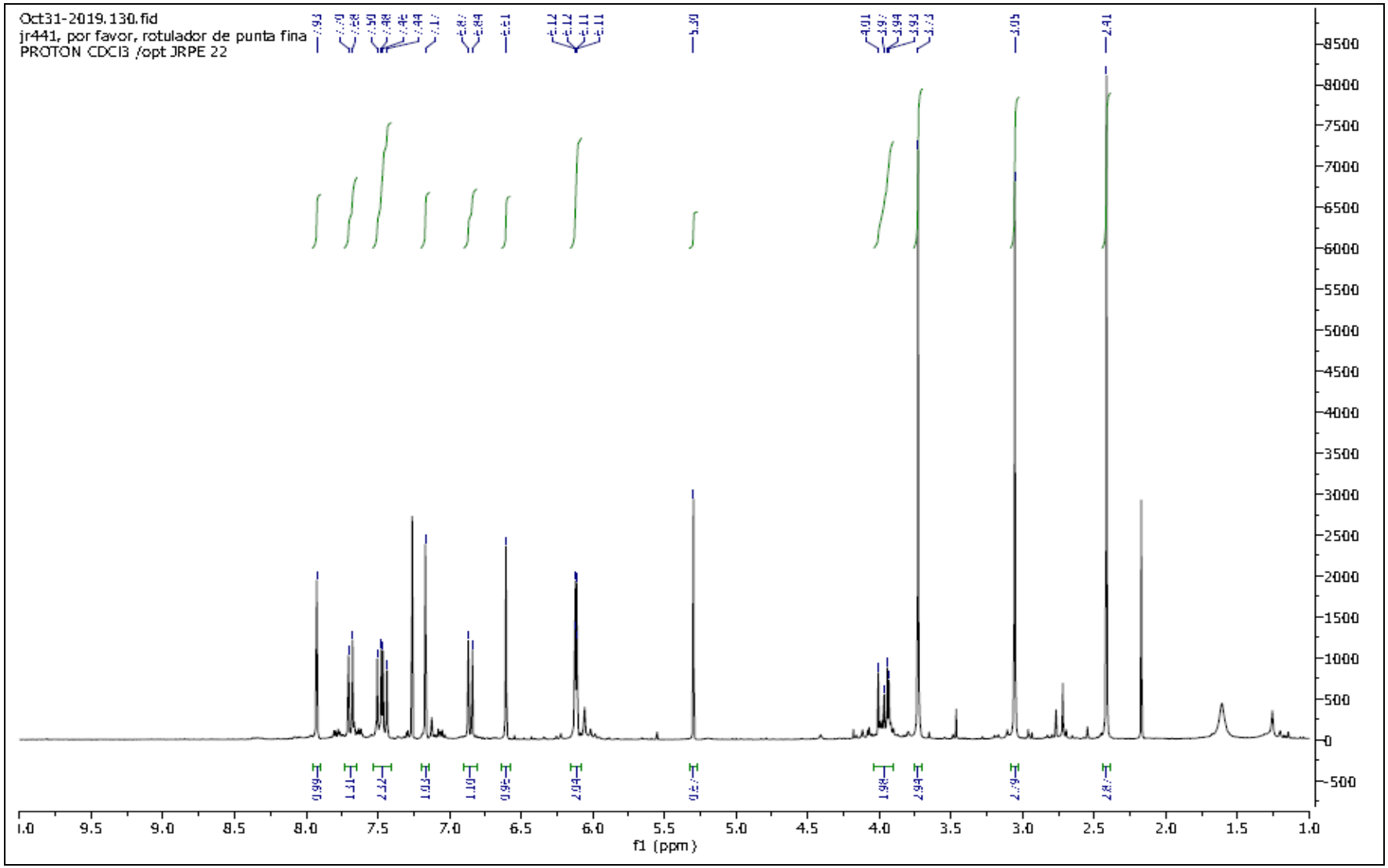
Appendix 44b: ^{13}C NMR spectrum of compound 16a



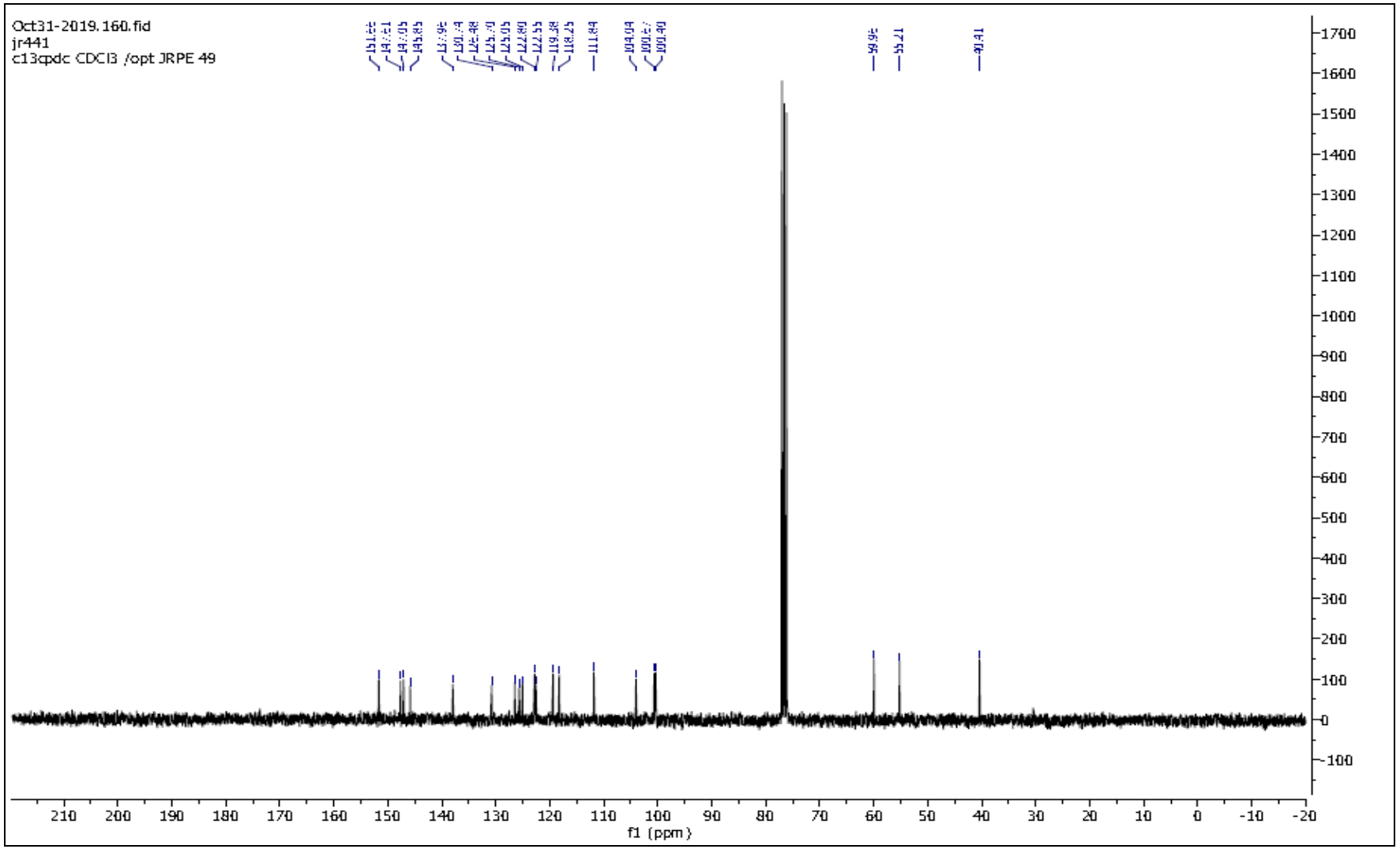
Appendix 44c: DEPT-135 spectrum of compound 16a



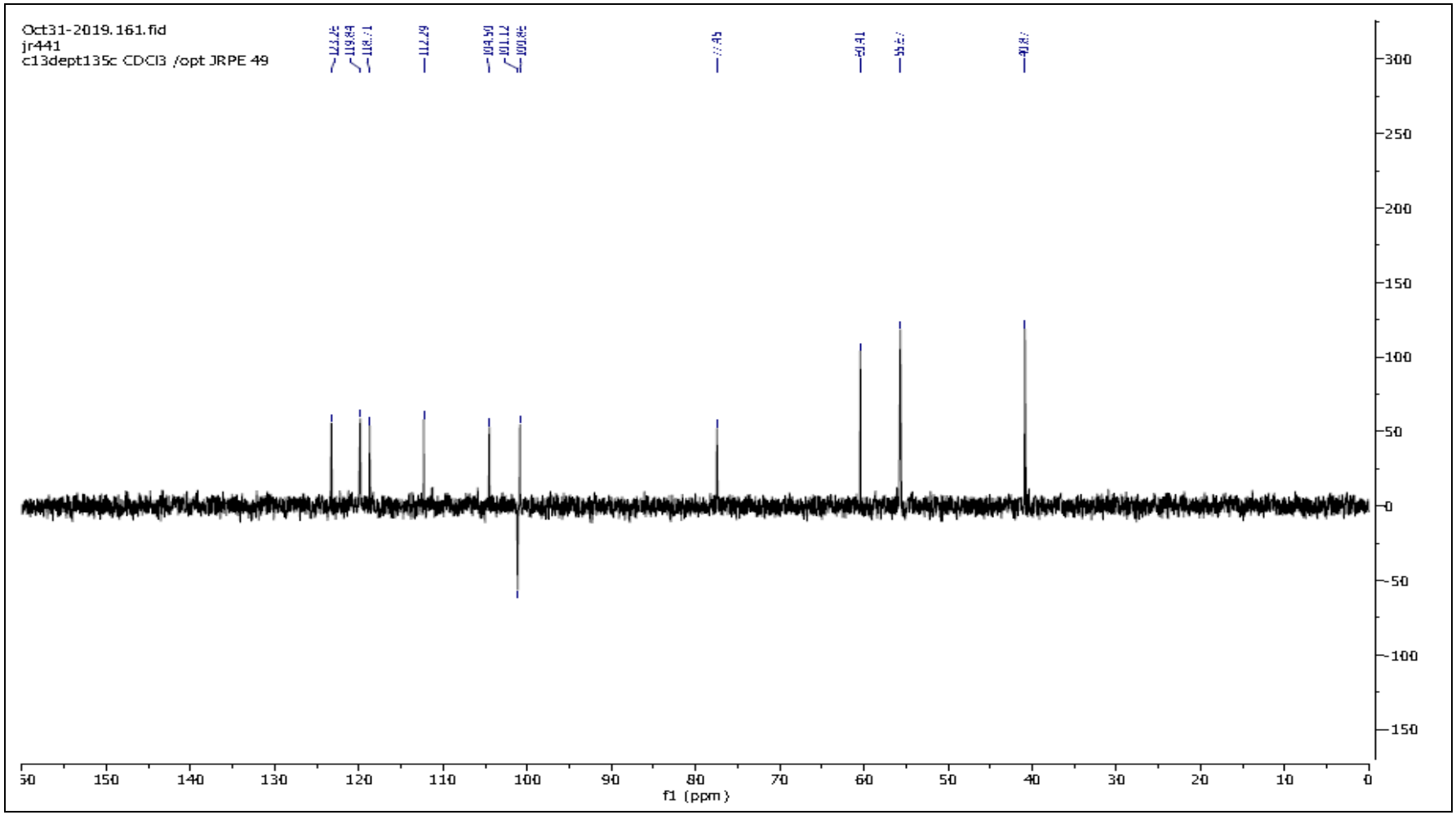
Appendix 44d: ^{1}H NMR spectrum of compound 16b



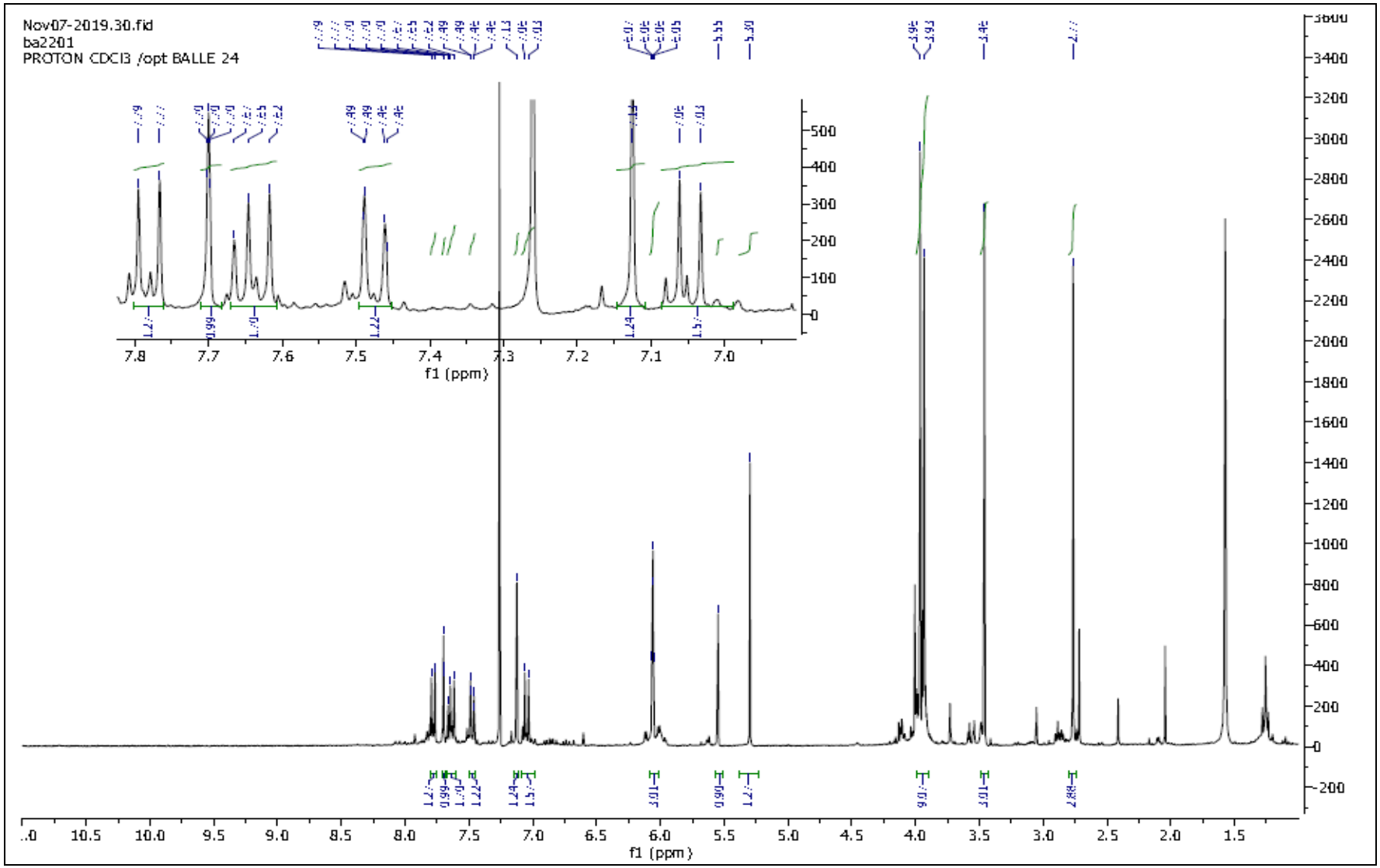
Appendix 44e: ^{13}C NMR spectrum of compound 16b



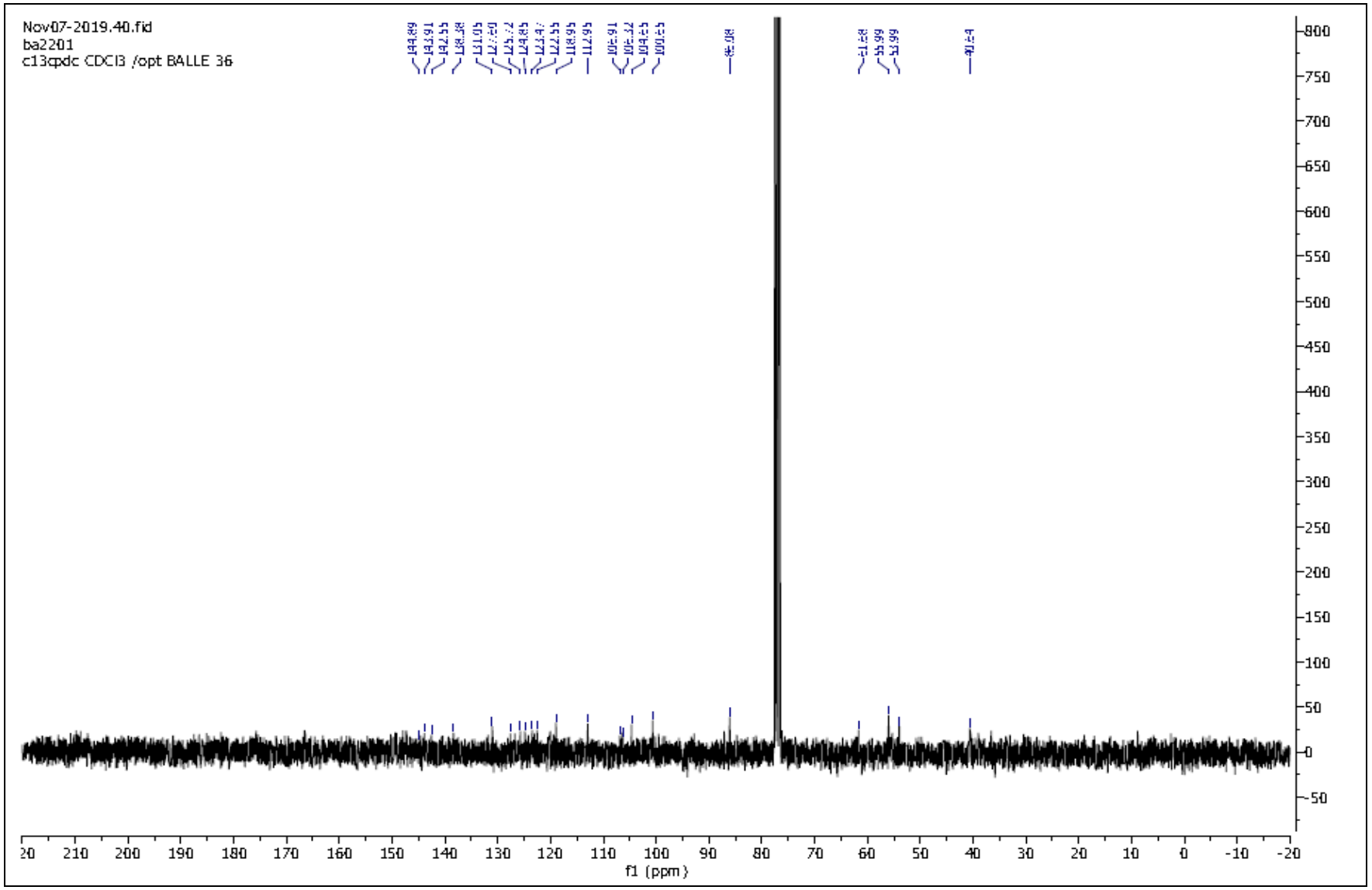
Appendix 44f: ^{13}C NMR spectrum of compound 16b



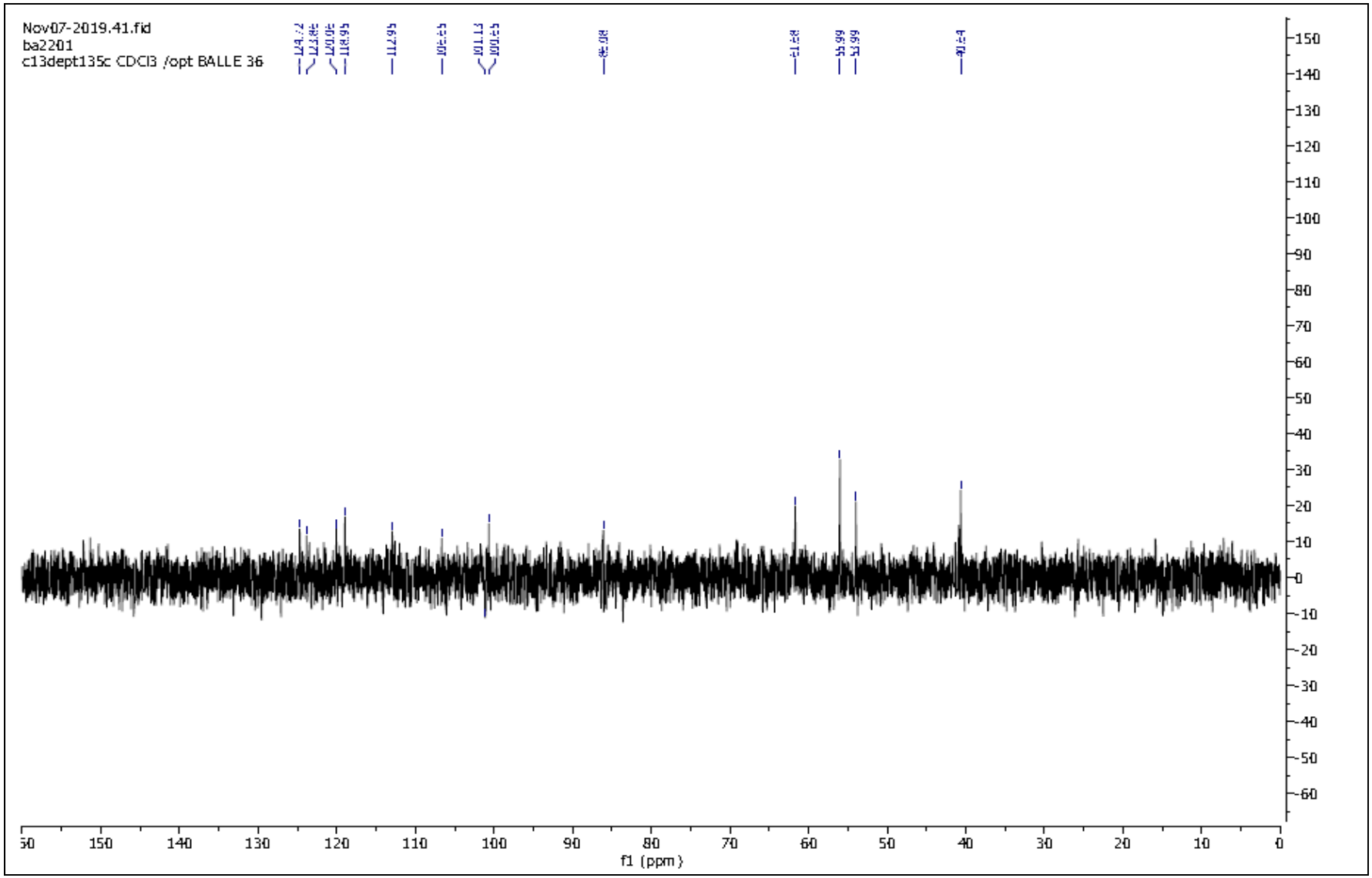
Appendix 44g: ^{1}H NMR spectrum of compound 16c



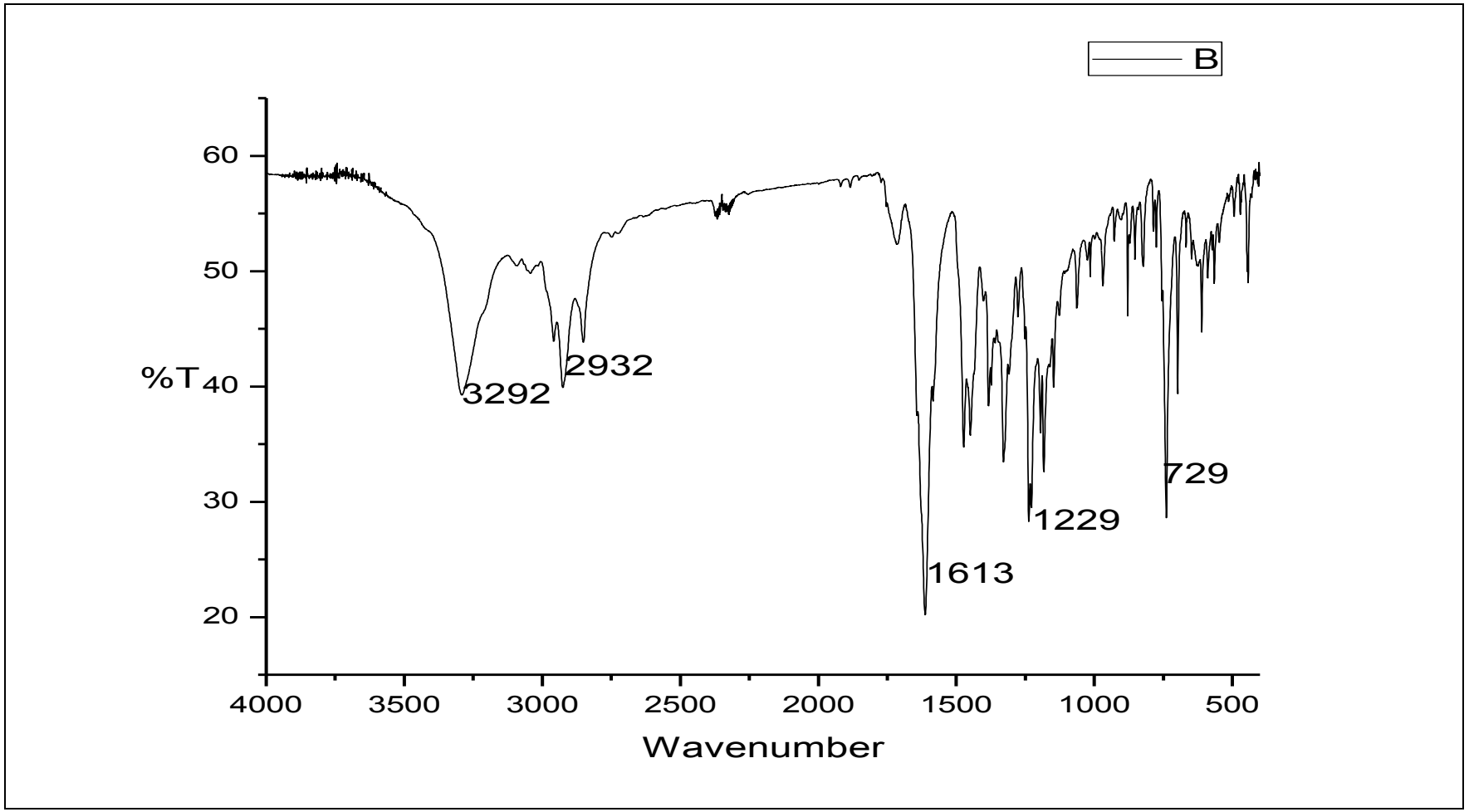
Appendix 44h: ^{13}C NMR spectrum of compound 16c



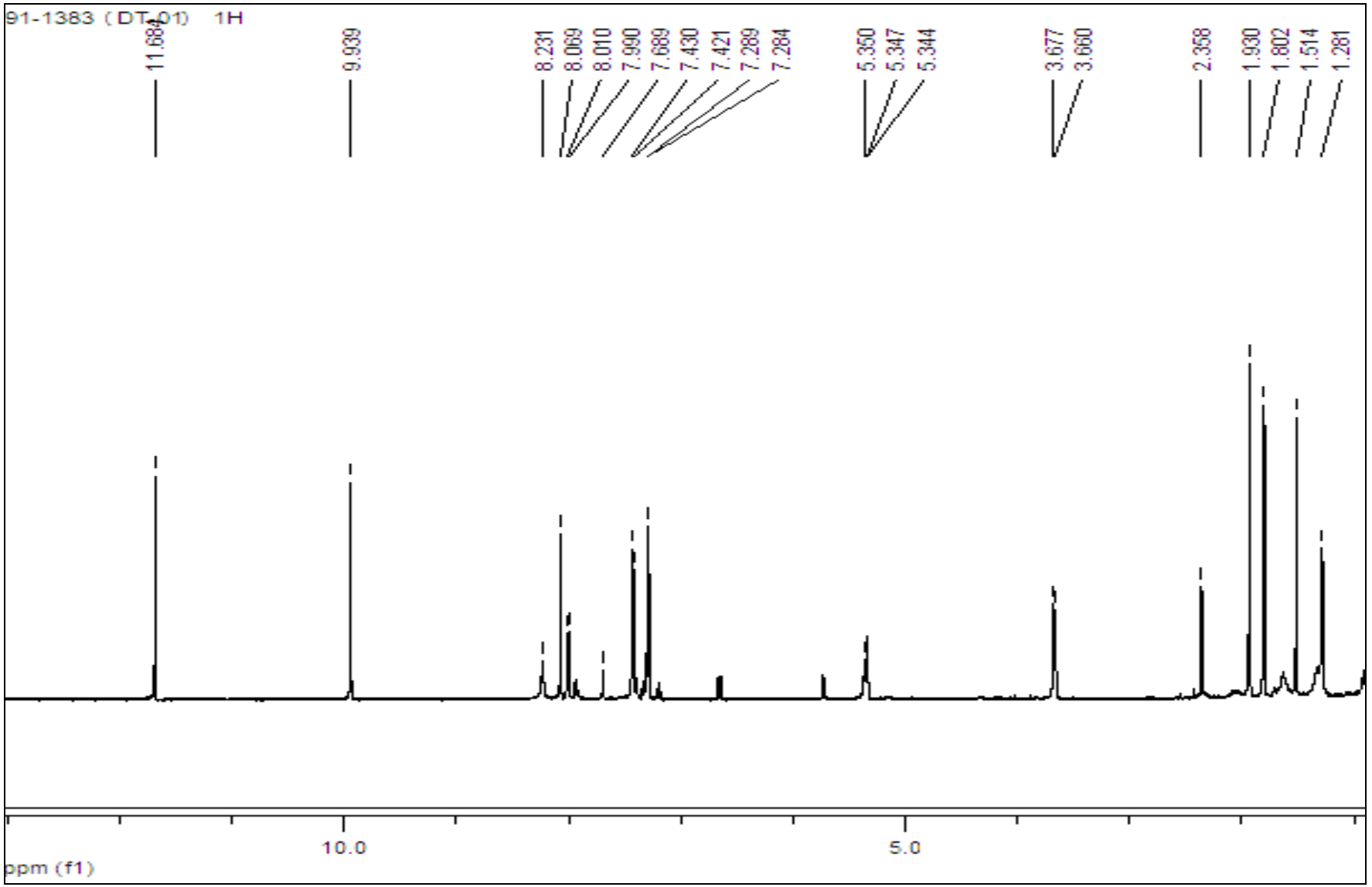
Appendix 44i: DEPT-135 spectrum of compound 16c



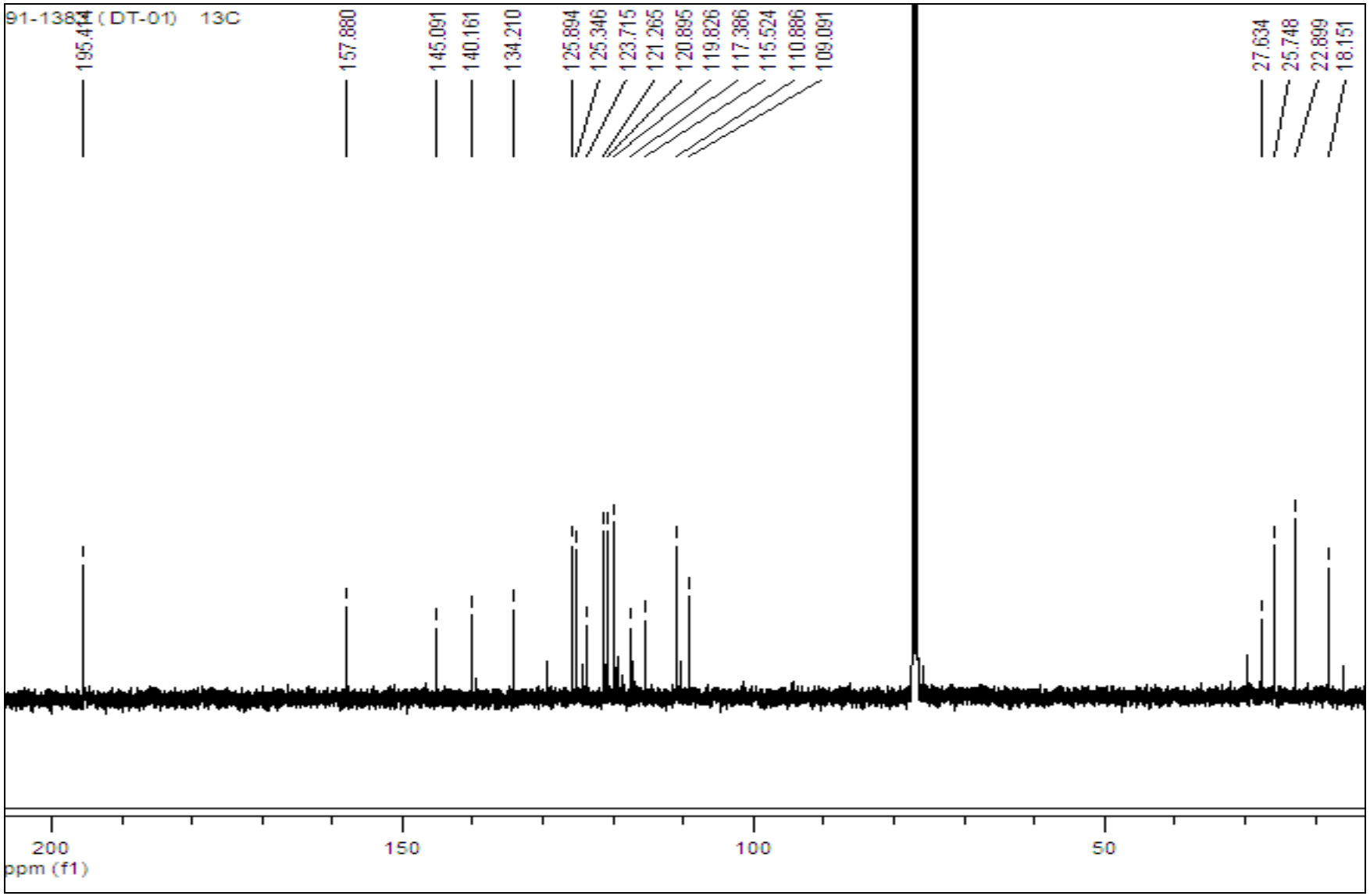
Appendix 45: IR spectrum of compound **17**



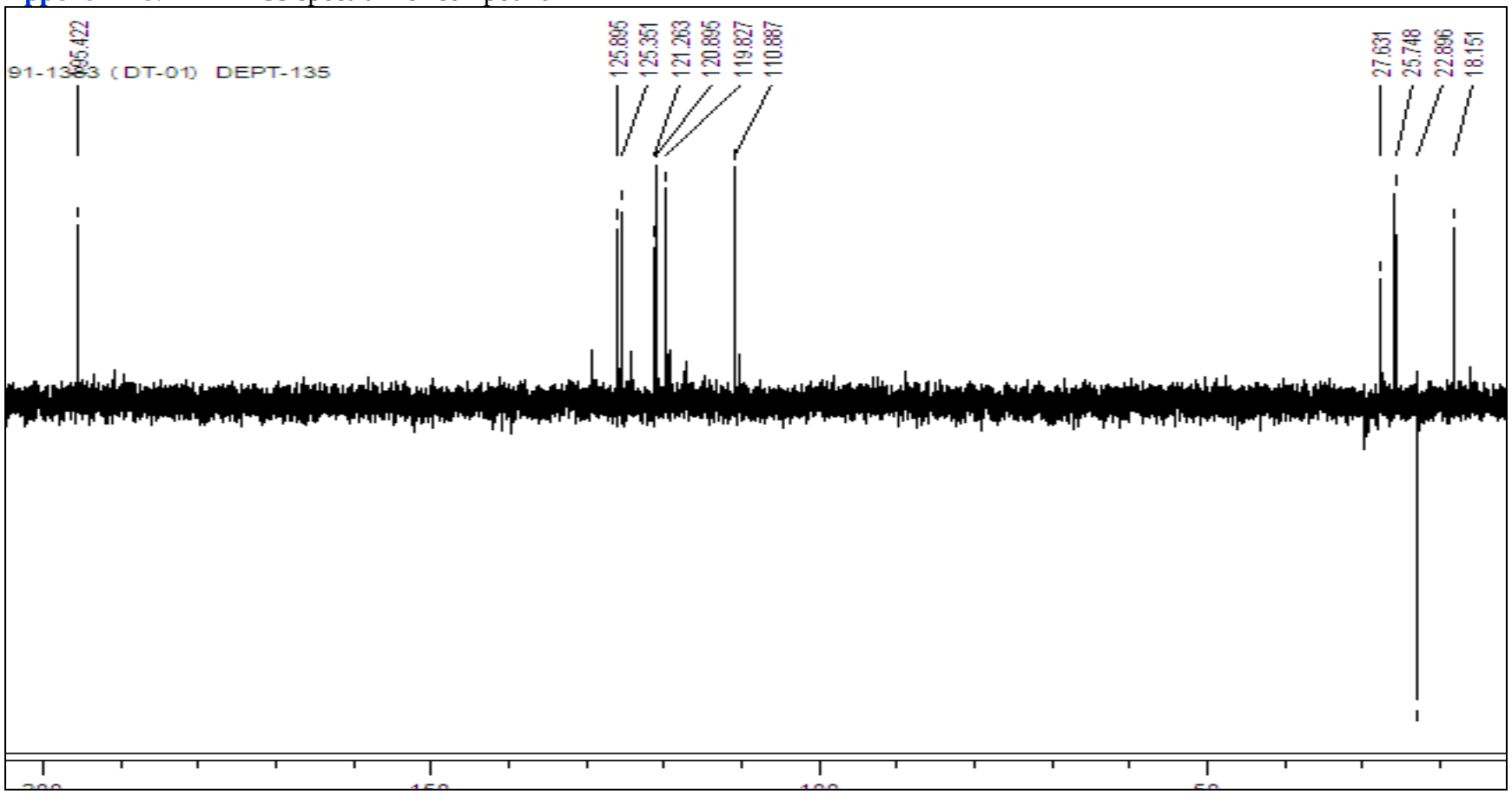
Appendix 46: ^{1}H-NMR spectrum of compound **17**



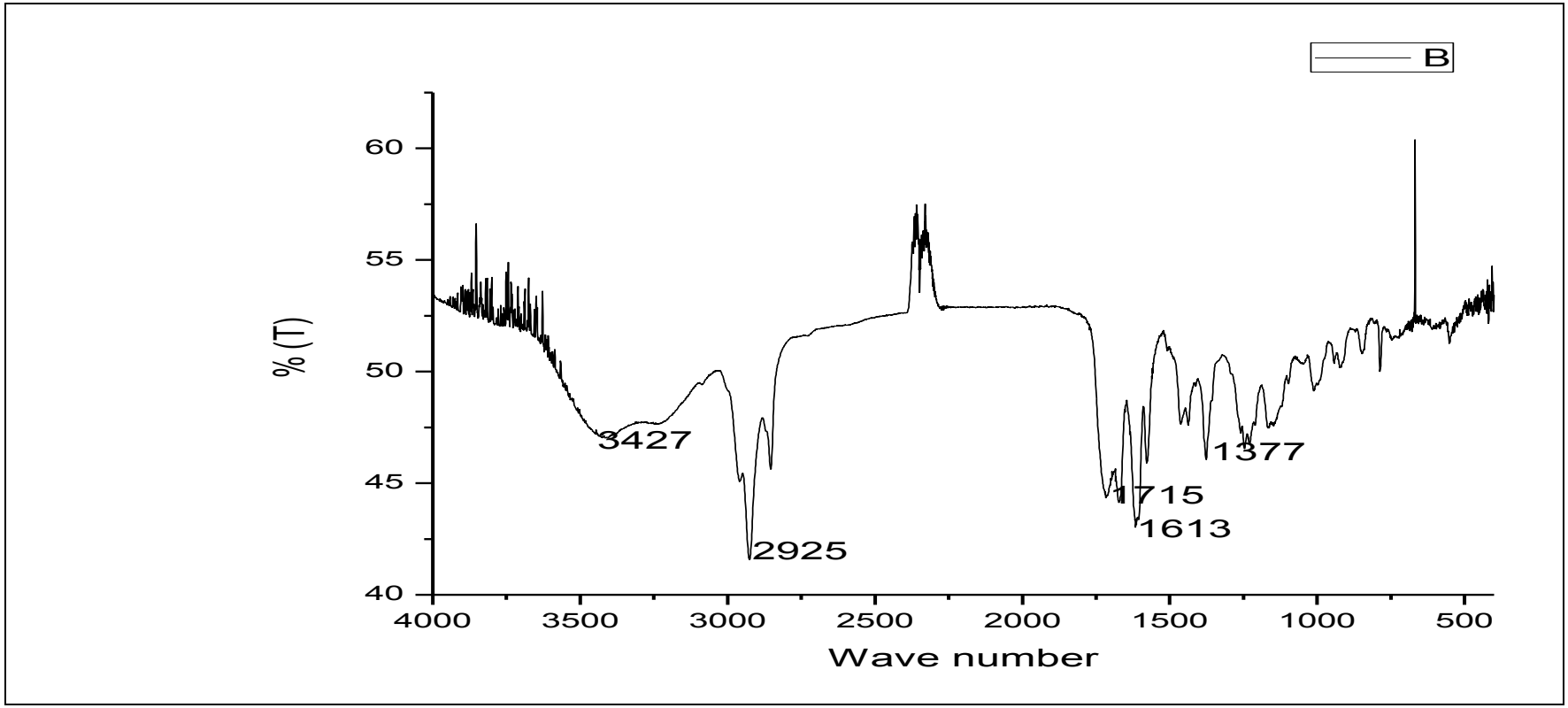
Appendix 47: ^{13}C-NMR spectrum of compound **17**



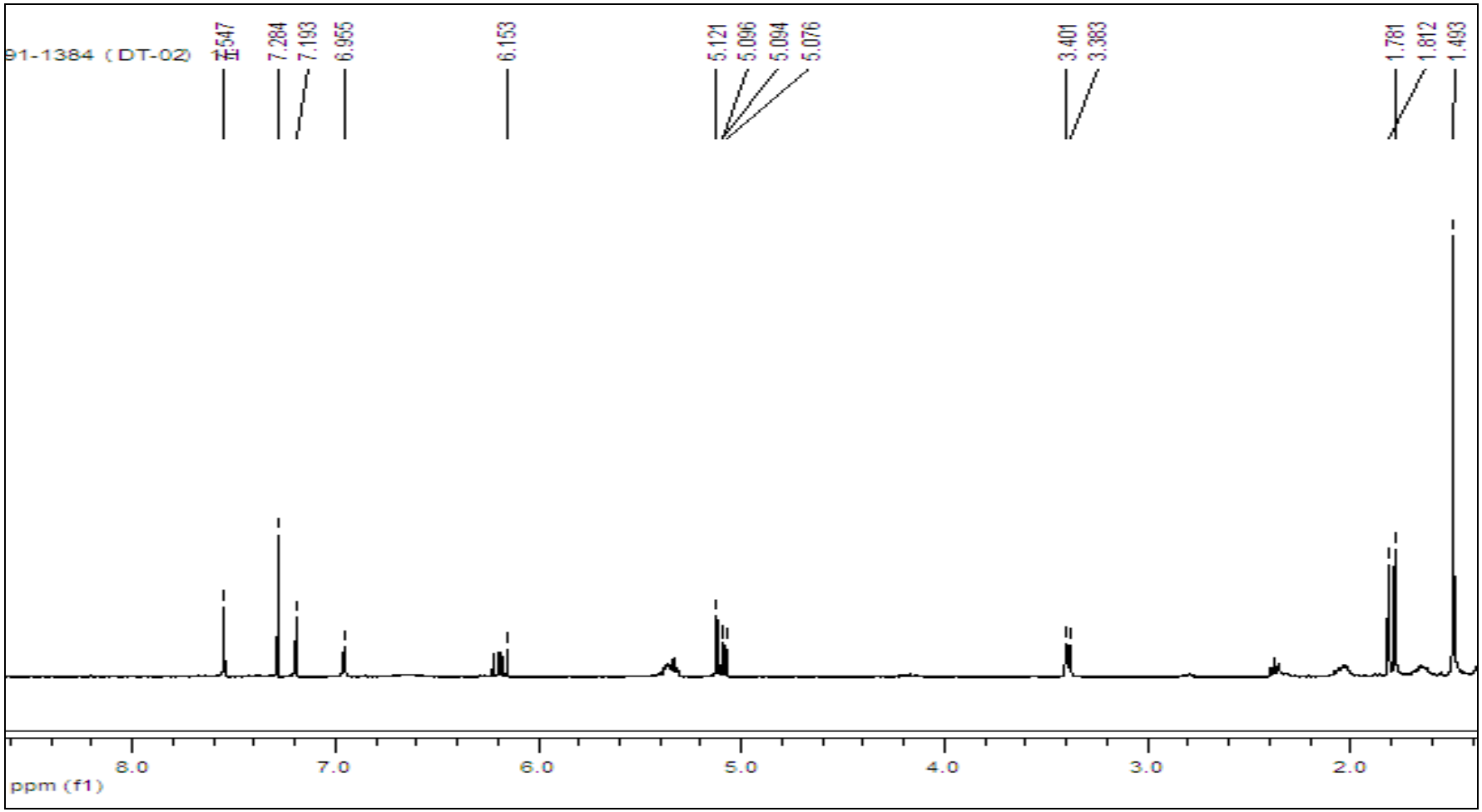
Appendix 48: DEPT-135 spectrum of compound **17**



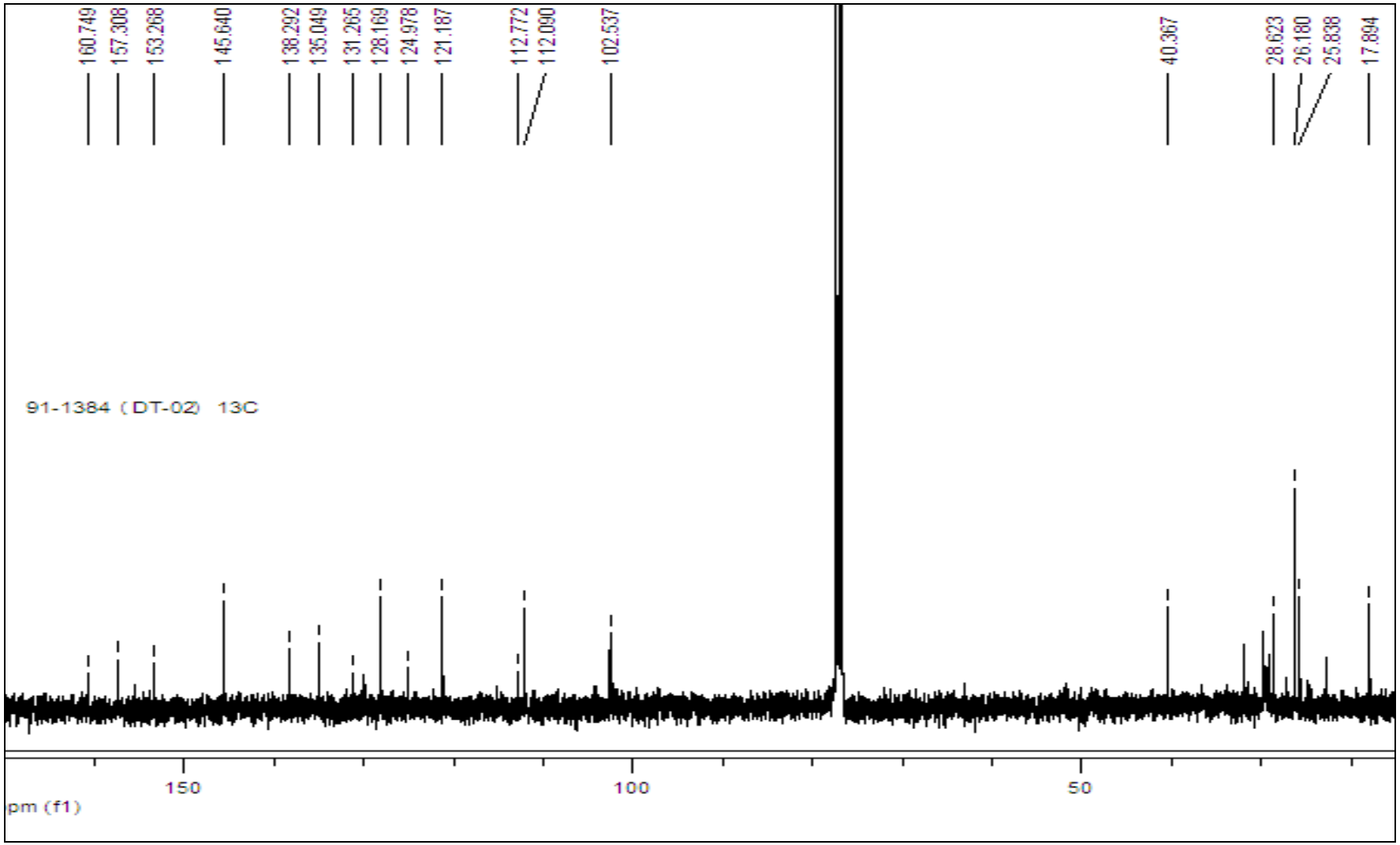
Appendix 49: IR spectrum of compound **18**



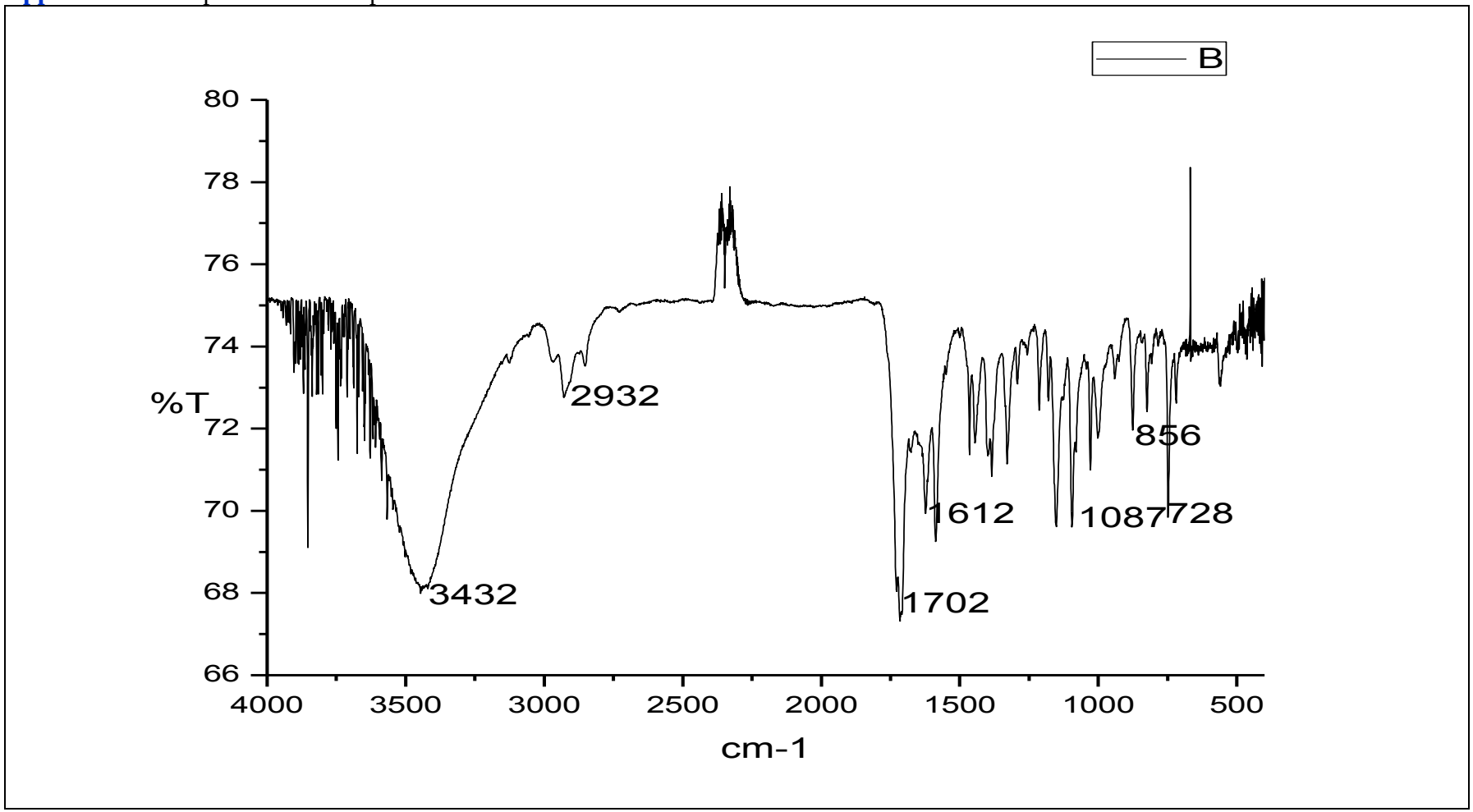
Appendix 50: ^{1}H-NMR spectrum of compound **18**



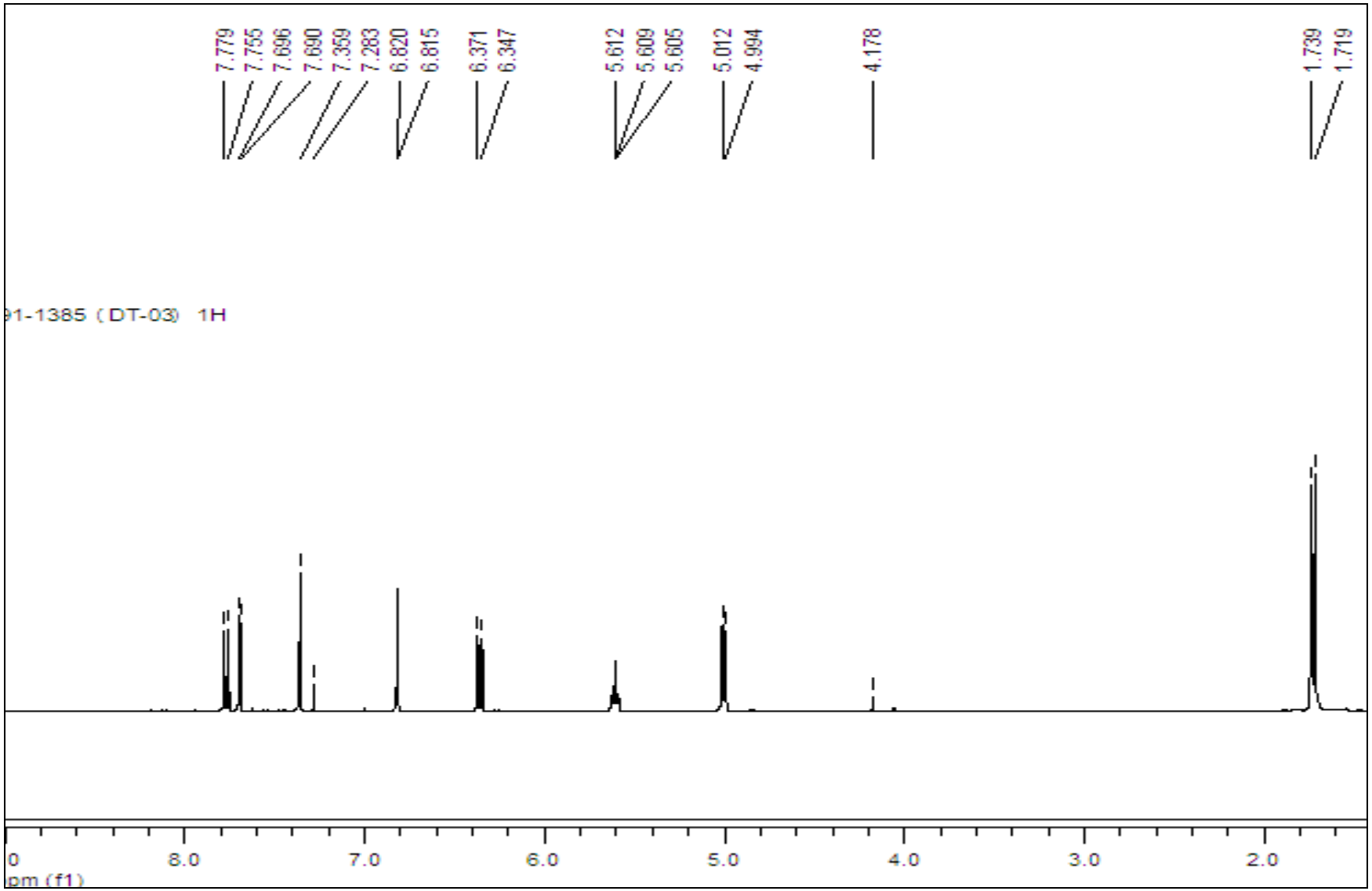
Appendix 51: ^{13}C- NMR spectrum of compound **18**



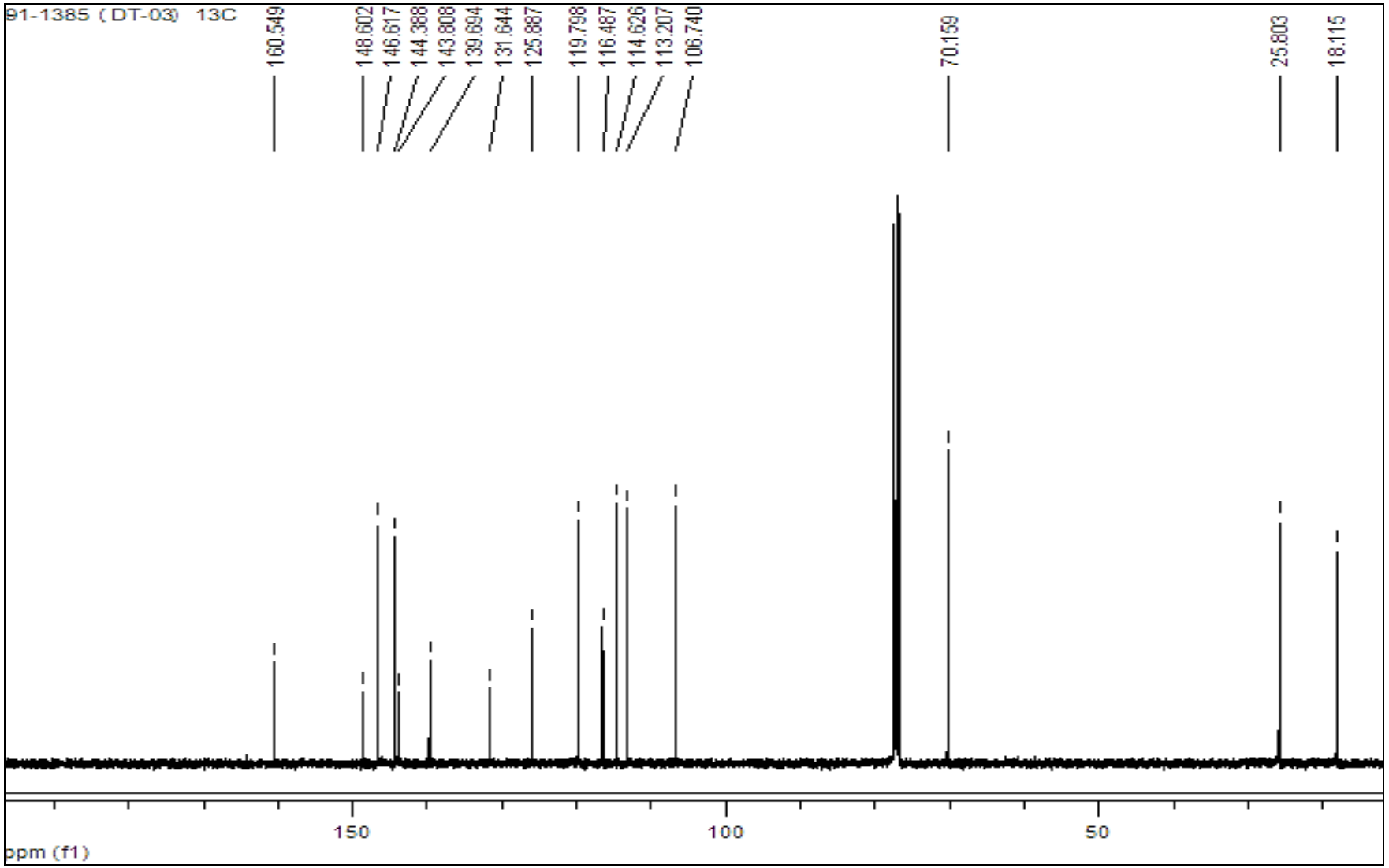
Appendix 52: IR spectrum of compound **19**



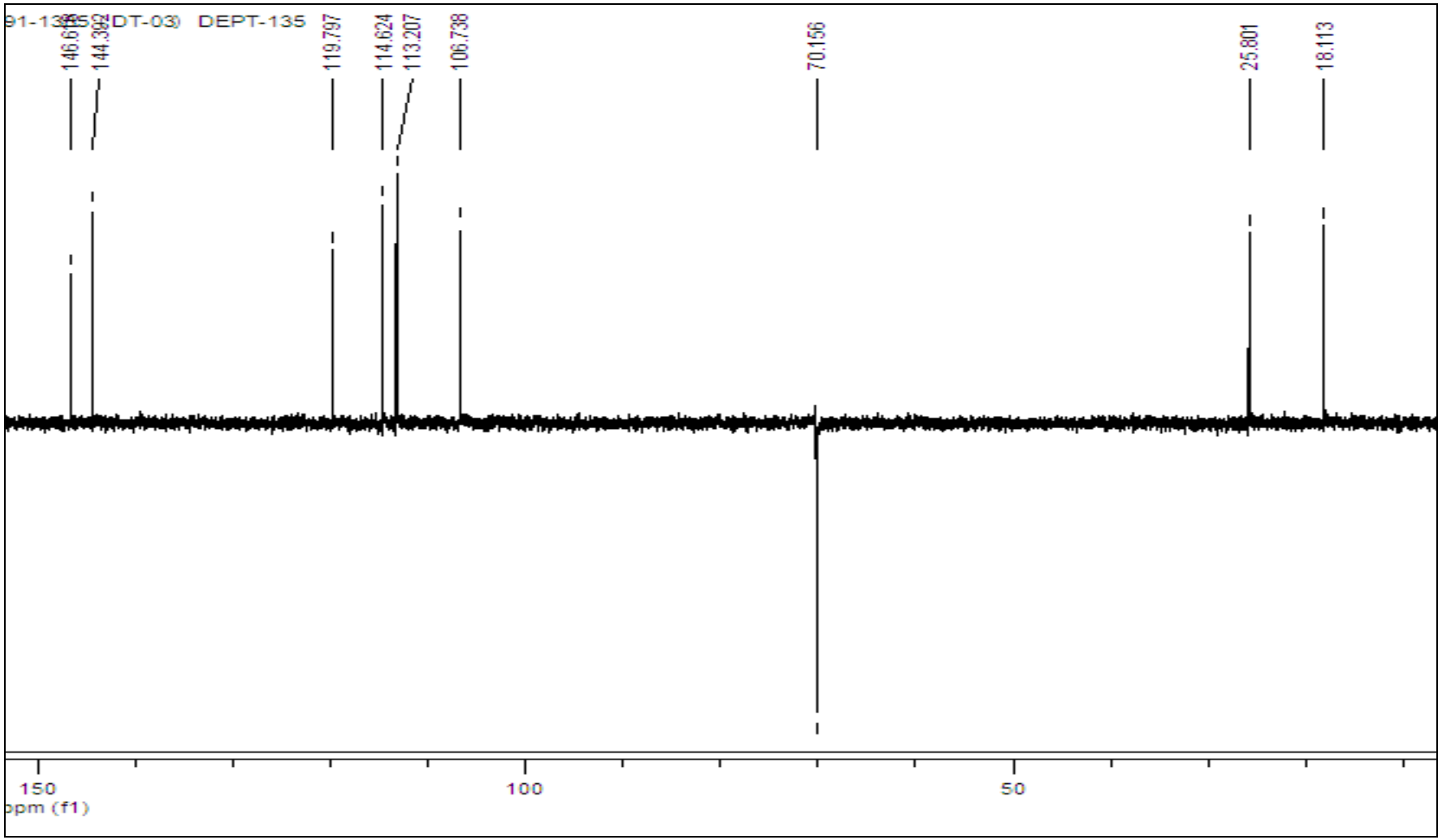
Appendix 53: ^{1}H-NMR spectrum of compound **19**



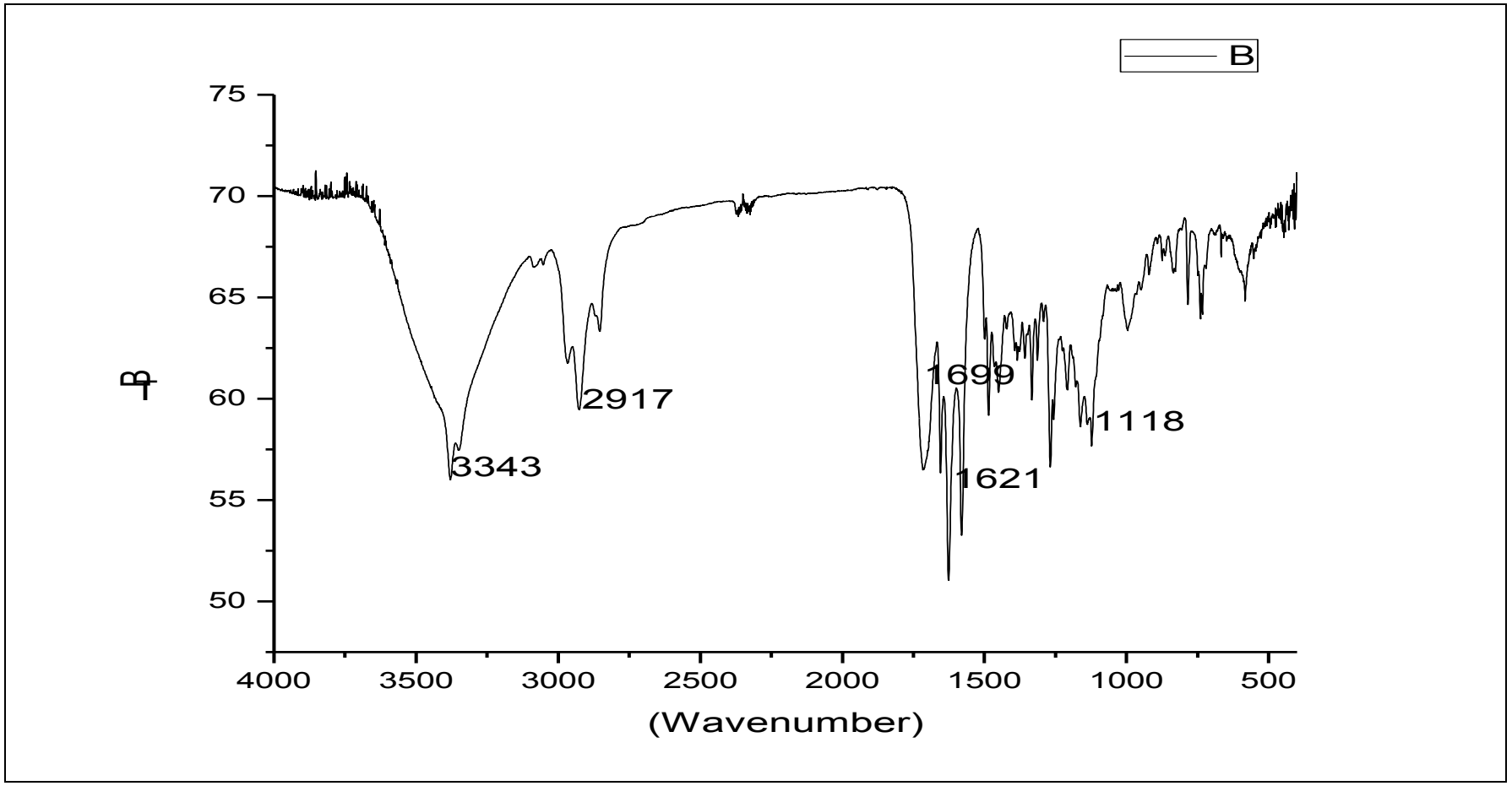
Appendix 54: ^{13}C- NMR spectrum of compound **19**



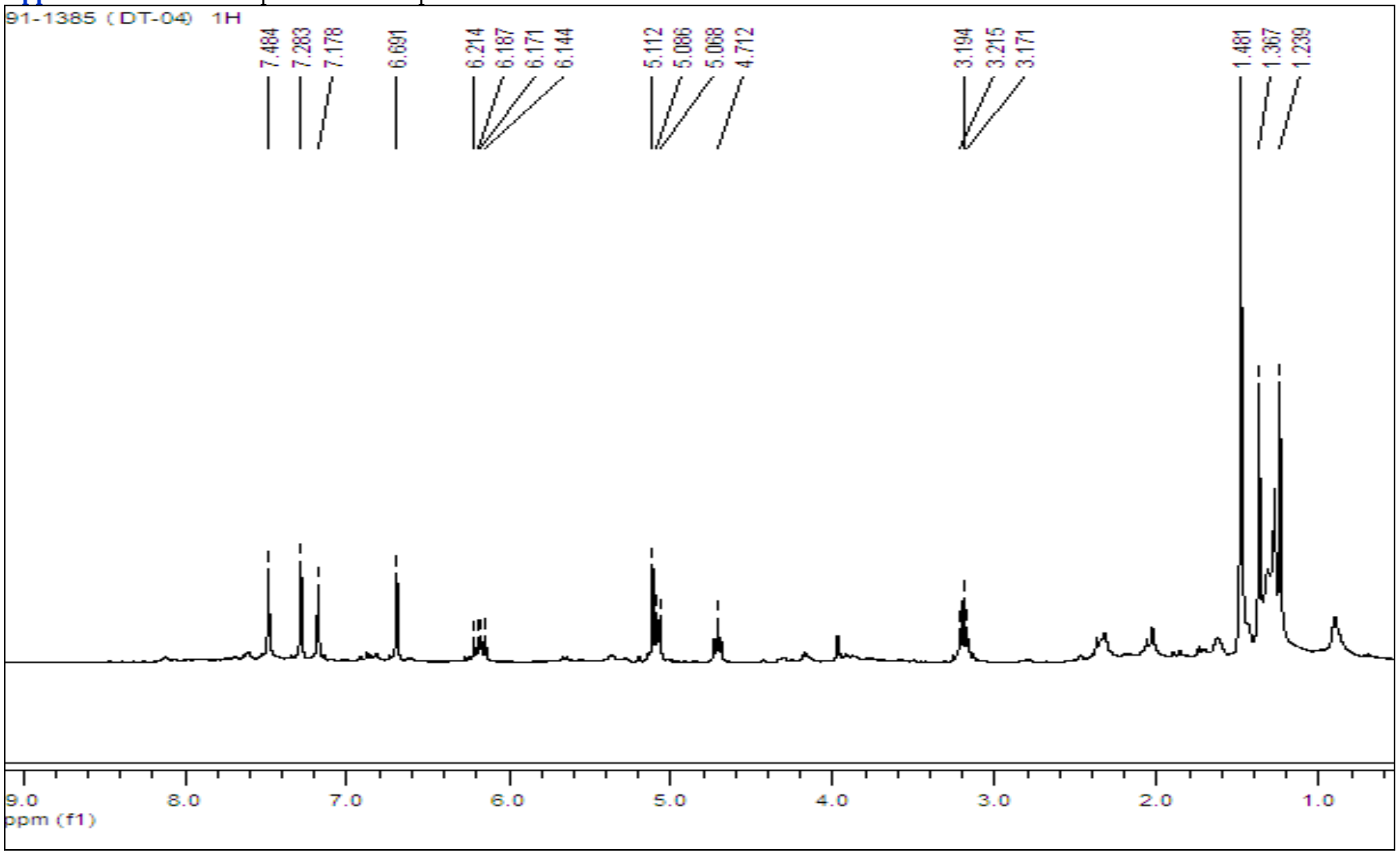
Appendix 55: DEPT-135 spectrum of compound **19**



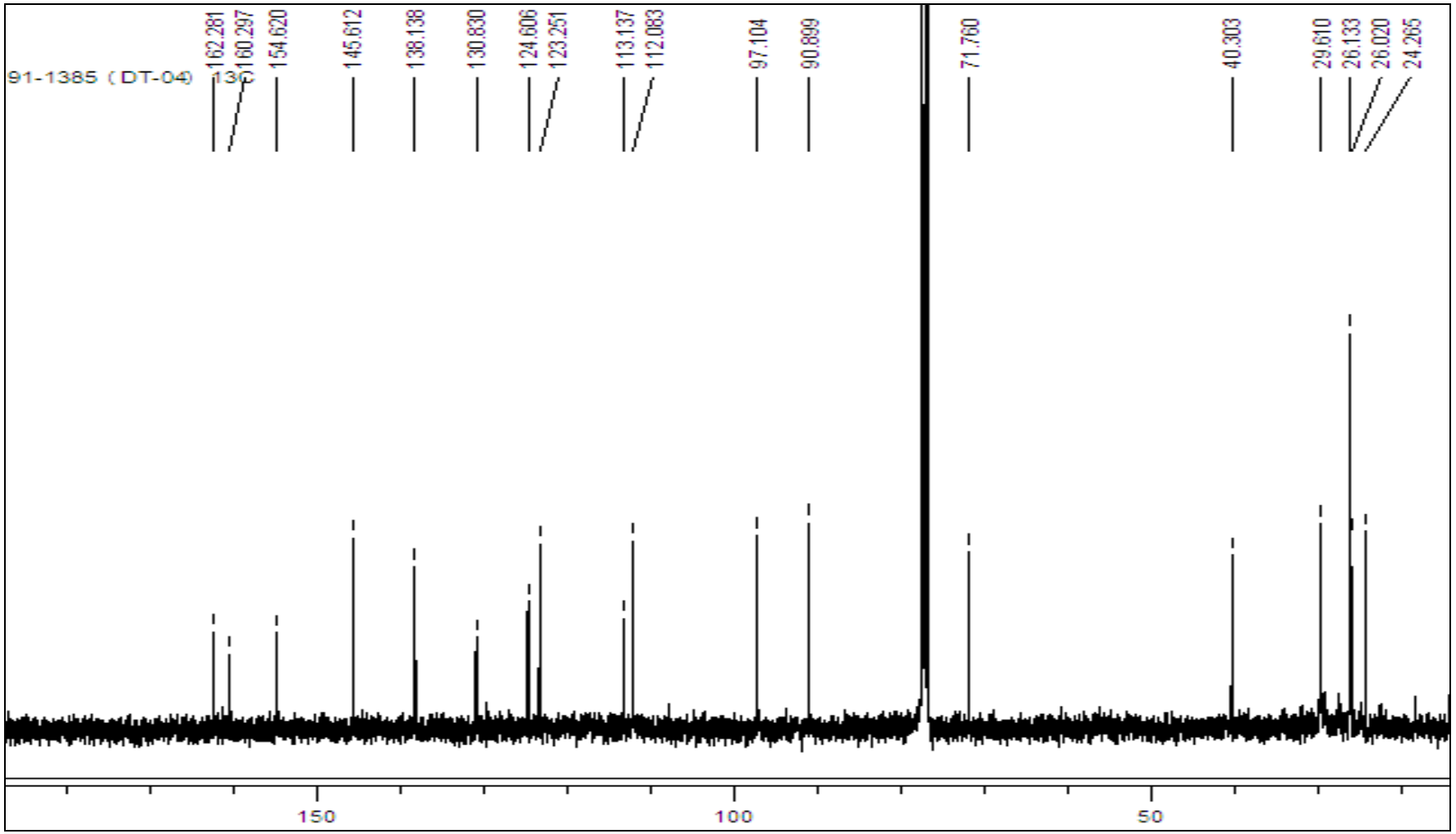
Appendix 56: IR spectrum of compound **20**



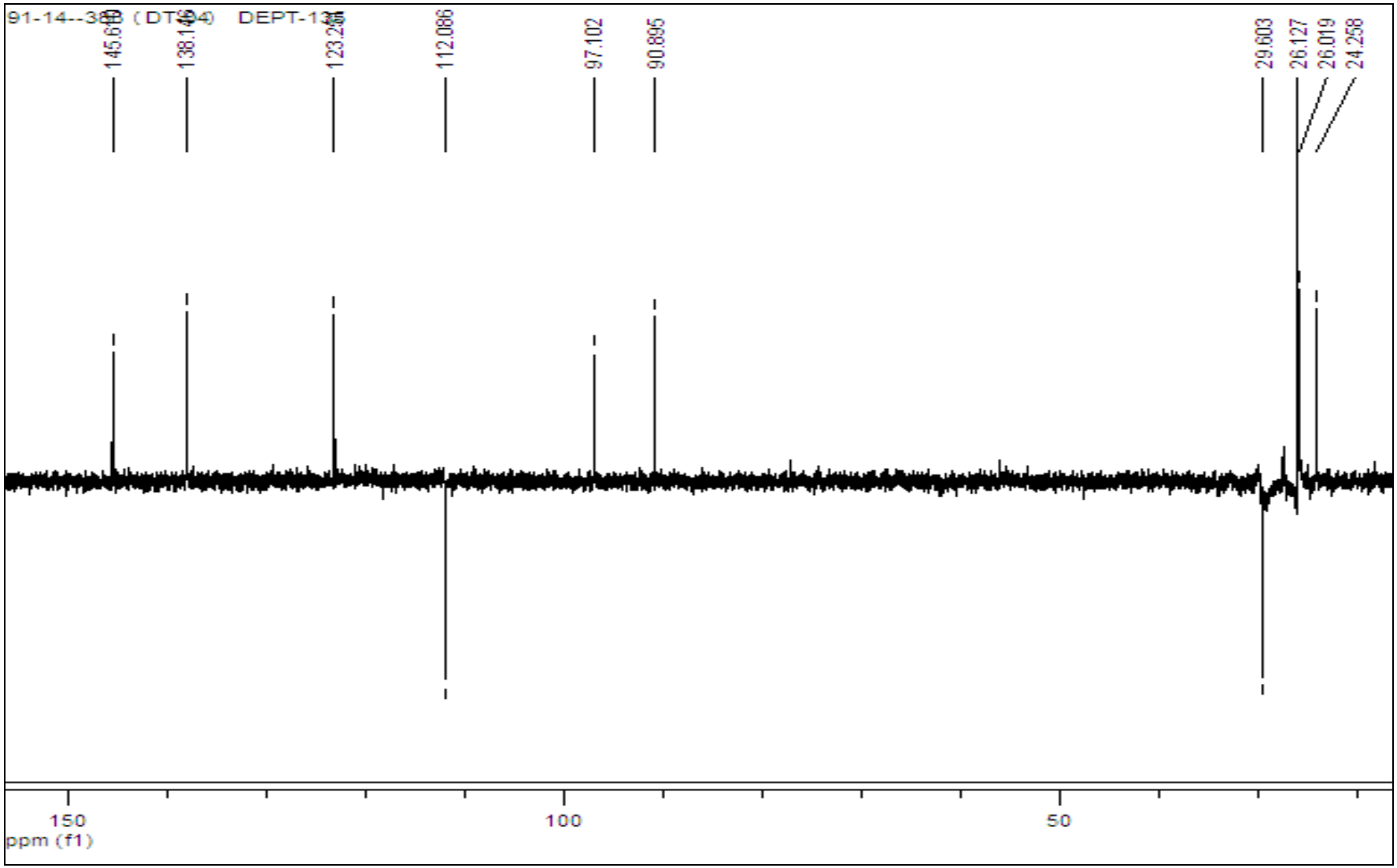
Appendix 57: ^{1}H-NMR spectrum of compound **20**



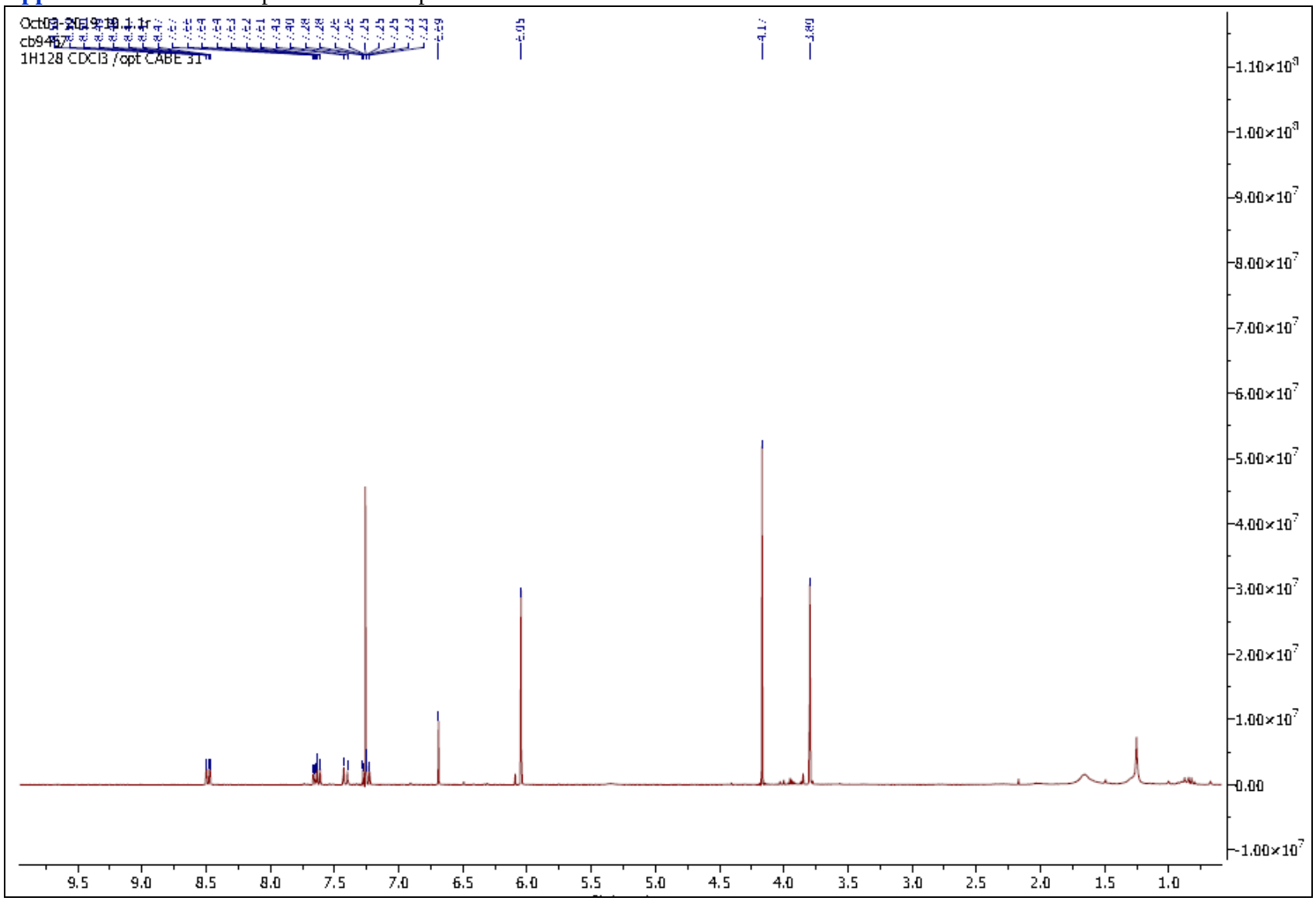
Appendix 58: ^{13}C- NMR spectrum of compound **20**



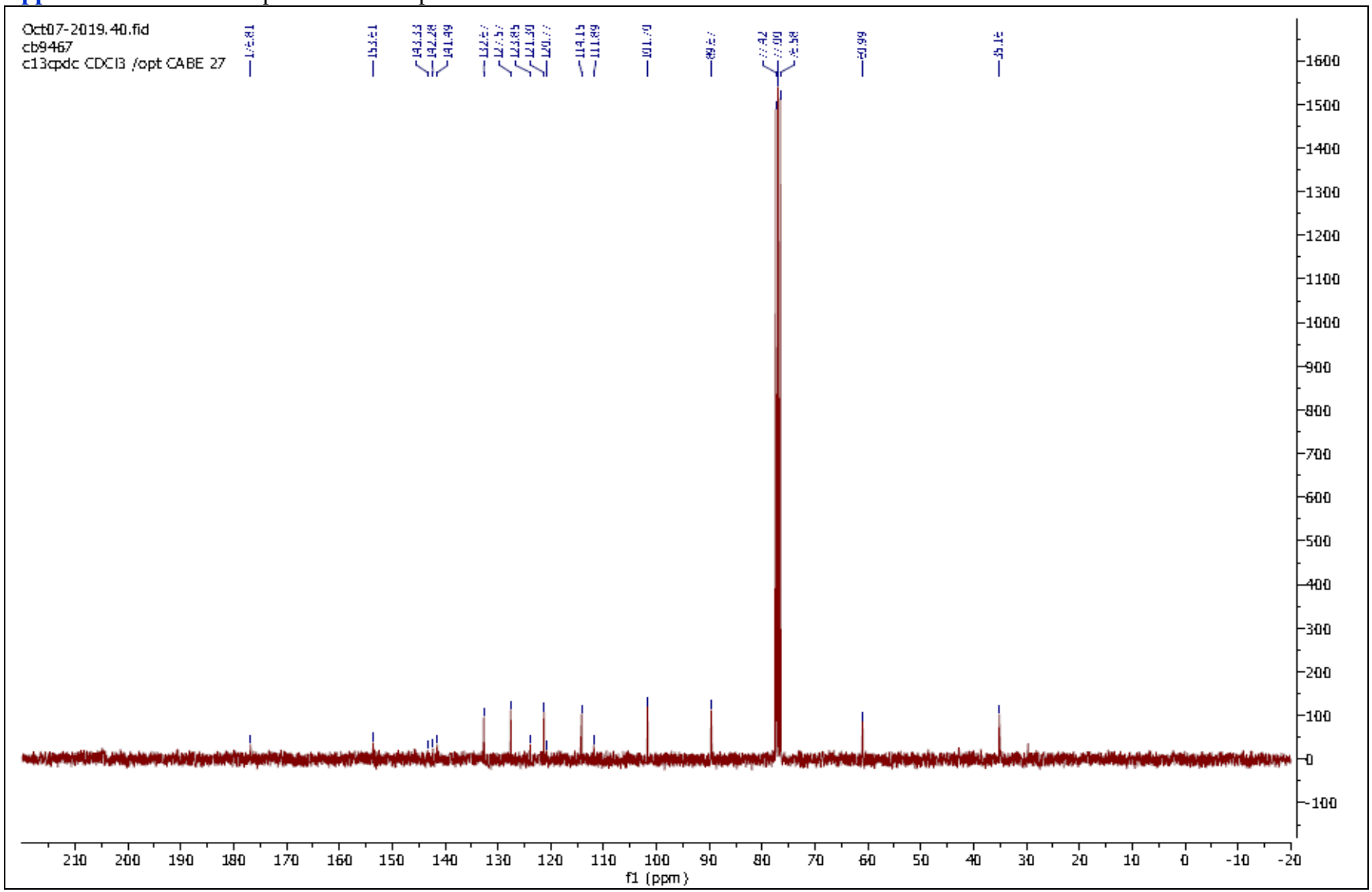
Appendix 59: DEPT-135 spectrum of compound **20**



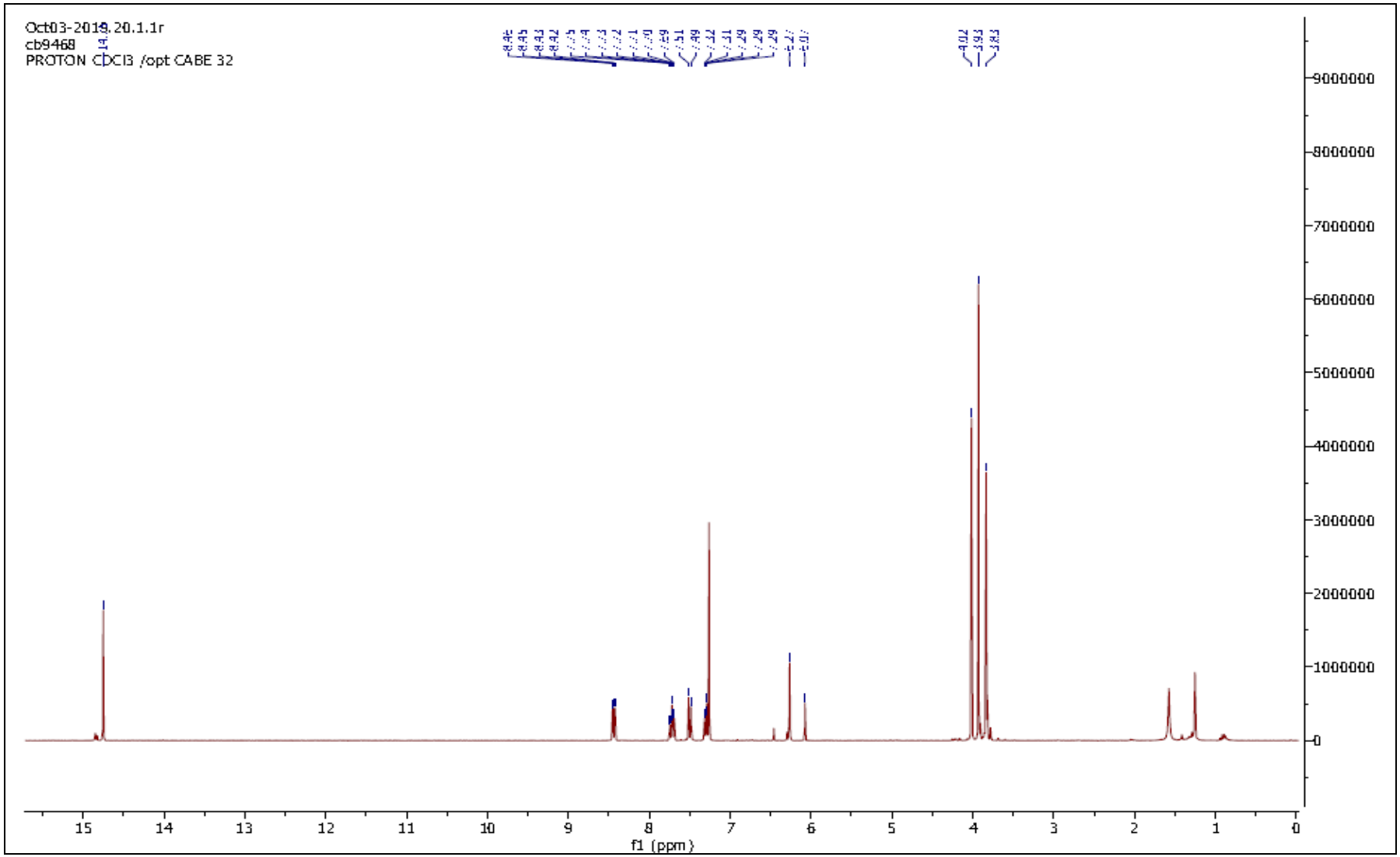
Appendix 60: 1H NMR spectrum of compound **21**



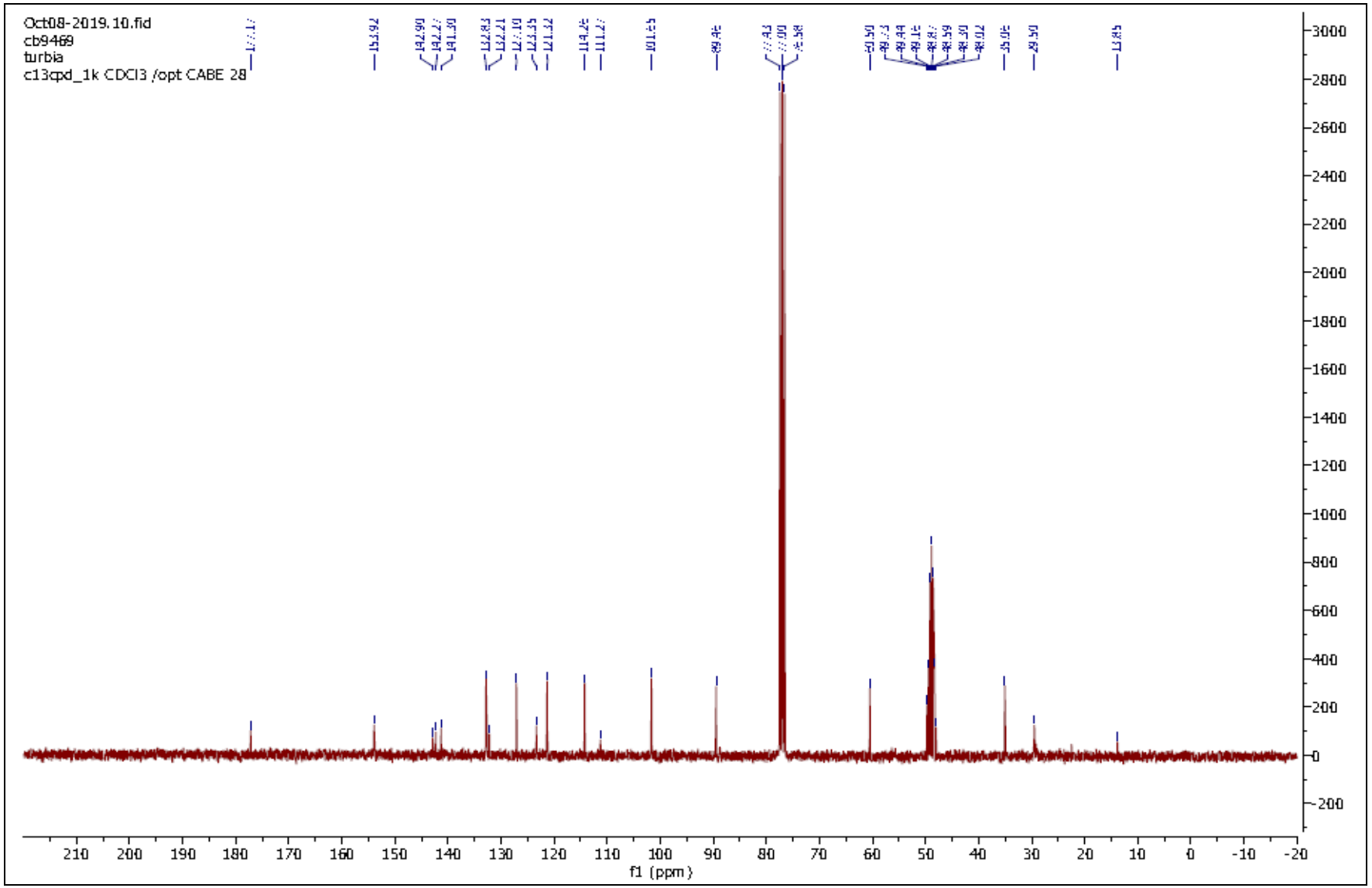
Appendix 61: ^{13}C NMR spectrum of compound **21**



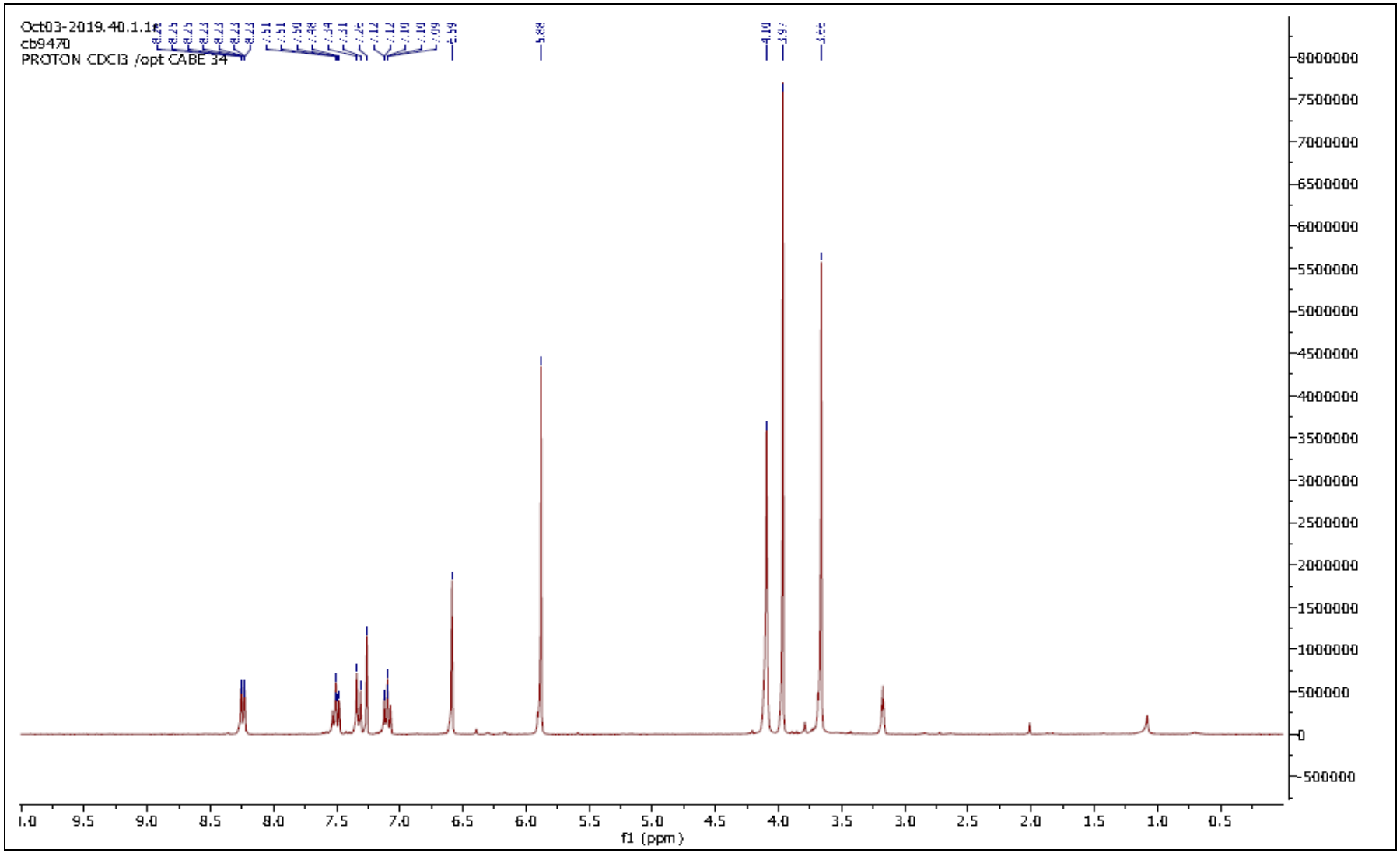
Appendix 62: ^{1}H NMR spectrum of compound **22**



Appendix 63: ^{13}C NMR spectrum of compound **22**



Appendix 64: ^{1}H NMR spectrum of compound **23**



Appendix 65: ^{13}C NMR spectrum of compound **23**