ลิโมนอยด์จากเมล็ดตะบูนขาวและตะบูนดำ จังหวัดสุราษฎร์ธานี

นางสาวชุติมา จิตตนิยม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

LIMONOIDS FROM THE SEEDS OF *Xylocarpus granatum* and *Xylocarpus moluccensis*, SURATTHANI PROVINCE

Miss Chutima Jittaniyom

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2011

Thesis Title	LIMONOIDS FROM THE SEEDS OF *Xylocarpus granatum* and *Xylocarpus moluccensis*, SURATTHANI PROVINCE
By	Miss Chutima Jittaniyom
Field of Study	Chemistry
Thesis Advisor	Assistant Professor Khanitha Pudhom, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

……Chairman
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

……Thesis Advisor
(Assistant Professor Khanitha Pudhom, Ph.D.)

……... Examiner
(Assistant Professor Pattara Sawasdee, Ph.D.)

……... External Examiner
(Damrong Sommit, Ph.D.)

ชุติมา จิตตนิยม : ลิโมนอยด์จากเมล็ดตะบูนขาวและตะบูนดำ จังหวัดสุราษฎร์ธานี. **(LIMONOIDS FROM THE SEEDS OF *Xylocarpus granatum* and *Xylocarpus moluccensis*, SURATTHANI PROVINCE)** อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร. ขนิษฐา พุดหอม, 135 หน้า.

งานวิจัยนี้ได้ทำการแยกและพิสูจน์ทราบโครงสร้างของลิโมนอยด์ที่มีฤทธิ์ทางชีวภาพจากเมล็ดตะบูนขาว *Xylocarpus granatum* Koeing. และตะบูนดำ *Xylocarpus moluccensis* Roem. ที่เก็บจากจังหวัดสุราษฎร์ธานี เมื่อนำส่วนสกัดเอธิลอะซิเตทของตะบูนขาวมาทำการแยกให้บริสุทธิ์ด้วยเทคนิคทางโครมาโทรกราฟี ได้สารที่มีการรายงานมาก่อน 10 ชนิดคือ xyloccensin K (**1**), 6-acetoxycedrodorin (**2**), andirobin (**3**), methylangolensate (**4**), methyl-6-β-hydroxy angolensate (**5**), gedunin (**6**), 7-deacetylgedunin (**7**), kihadalactone A (**8**), toonaciliatavarin E (**9**) และ protoxylocarpin G (**10**) และเมื่อนำส่วนสกัดเอธิลอะซิเตทของตะบูนดำมาแยกด้วยวิธีการเดียวกัน พบว่าได้ลิโมนอยด์ประเภท mexicanolide ชนิดใหม่ 1 ชนิดซึ่งให้ชื่อว่า thaimoluccensin D (**14**) และนอกจากสาร **3**, **4**, **6** และ **7** ที่พบในเมล็ดตะบูนขาวแล้ว ยังพบสารที่มีการรายงานมาก่อนอีก 3 ชนิด ได้แก่ protoxylocarpin H (**11**), moluccensin I (**12**) 2-hydrofissinolide (**13**) และ hispidone (**15**) การพิสูจน์ทราบโครงสร้างทางเคมีของสารที่แยกได้ทำโดยอาศัยการวิเคราะห์ข้อมูลทางสเปกโทรสโกปีและในกรณีของสารที่มีการรายงานมาก่อนจะทำการการเปรียบเทียบข้อมูลทางสเปกโทรสโกปีกับข้อมูลในวารสารตีพิมพ์ต่างๆ นอกจากนี้ ในงานวิจัยนี้ยังได้รายงานข้อมูล NMR ที่สมบูรณ์และโครงสร้างผลึกของ andirobin (**3**) ที่ได้จากพืชทั้งสองชนิดเป็นครั้งแรก จากนั้นนำสารที่แยกได้มาทดสอบฤทธิ์ต้านการอักเสบด้วยวิธีการตรวจวัดการยับยั้งการผลิตไนตริกออกไซด์ในmacrophage RAW 264.7 ที่ถูกกระตุ้น พบว่ามีพเพียง 7-deacetylgedunin (**7**) ซึ่งเป็นลิโมนอยด์ประเภท gedunin ให้ฤทธิ์การยับยั้งที่ดีที่สุดด้วยค่า IC_{50} 4.75 μM

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....
สาขาวิชา.....เคมี…….....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
ปีการศึกษา.....2554.....

5272677323 : MAJOR CHEMISTRY
KEYWORDS : limonoids / *Xylocarpus granatum / Xylocarpus moluccensis* / anti-inflammatory

CHUTIMA JITTANIYOM: LIMONOIDS FROM THE SEEDS OF *Xylocarpus granatum* and *Xylocarpus moluccensis*, SURATTHANI PROVINCE. ADVISOR : ASST. PROF. KHANITHA PUDHOM, Ph.D., 135 pp.

The present study is to isolate and characterize the bioactive limonoids from the seeds of *Xylocarpus granatum* Koeing. and *Xylocarpus moluccensis* Roem. collected in Suratthani province. Chromatographic fractionation of EtOAc crude extract of *X. granatum* led to the isolation of ten known compounds, namely xyloccensin K (**1**), 6-acetoxycedrodorin (**2**), andirobin (**3**), methylangolensate (**4**), methyl-6-*β*-hydroxy angolensate (**5**), gedunin (**6**), 7-deacetylgedunin (**7**), kihadalactone A (**8**), toonaciliatavarin E (**9**) and protoxylocarpin G (**10**). Likewise, the isolation of EtOAc crude extract of *X. moluccensis* provided a new mexicanolide, named as thaimoluccensin D (**14**), and additional three known compounds including protoxylocarpin H (**11**), moluccensin I (**12**), 2-hydrofissinolide (**13**) and hispidone (**15**), other than compounds **3**, **4**, **6** and **7** also found in *X. granatum*. The structures of isolated compounds were established mainly by analysis of spectroscopic data and by comparison with data in the literature for known compounds. Furthermore, the complete assignments of NMR and the crystal structure of andirobin (**3**) obtained from both species were herein reported for the first time. Isolated compounds were further tested for their anti-inflammatory effects by monitoring the inhibition of nitric oxide (NO) production in activated macrophage RAW 264.7 cells. Only a gedunin type limonoid, 7-deacetylgedunin (**7**), showed the most potent activity with an IC_{50} value of 4.75 μM.

Department : Chemistry Student's Signature .....
Field of Study : Chemistry Advisor's Signature .....
Academic Year : 2011

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my advisor, Assistant Professor Dr. Khanitha Pudhom, for their generous assistance, benevolent guidance and encouragement throughout the course of this research.

Many thanks to the thesis committees, Assistant Professor Dr. Warinthorn Chavasiri, Assistant Professor Dr. Pattara Sawasdee Department of chemistry, Faculty of science, Chulalongkorn University and Dr. Damrong sommit, Mahanakorn University of Technology, For their invaluable discussion and suggestion.

I am grateful to Associate Professor Dr. Nongnuj Muangsin, Department of Chemistry, Faculty of Science, Chulalongkorn University who kindly preformed the X-ray Crystallographic analysis.

I would like to acknowledge the grant and funding supports provided by Center for Petroleum, Petrochemicals, and Advanced Materials. I wish to thank The 100th Chemistry of Chulalongkorn University for supporting scholarship.

I wish to thank all member of laboratory room 1528/1 and 1532 for their help and kind friendship, especially Mr. Thanesuan Nuanyai, for his valuable suggestions.

Finally, I am deeply grateful to my mother for their love and encouragement throughout my M.Sc. Study.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

Figure Page

Figure Page

Figure Page

LIST OF SCHEMES

LIST OF ABBREVIATIONS

J	Coupling constant
δ	Chemical shift
δ_H	Chemical shift of proton
δ_C	Chemical shift of carbon
s	Singlet (for NMR spectra)
d	Doublet (for NMR spectra)
dd	Doublet of doublet (for NMR spectra)
t	Triplet (for NMR spectra)
m	Multiplet (for NMR spectra)
q	Quartet (for NMR spectra)
brs	Broad singlet (for NMR spectra)
qC	Quaternary carbon
calcd.	Calculated
^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
2D NMR	Two dimensional nuclear magnetic resonance
^{1}H-^{1}H COSY	Homonuclear (proton-proton) correlation spectroscopy
NOESY	Nuclear overhauser effect spectroscopy
HSQC	Heteronuclear single quantum coherence
HMBC	Heteronuclear multiple bond correlation
ORTEP	Oak ridge thermal ellipsoid plot
HRESIMS	Hight resolution electrospray ionization mass spectrometry
CC	Column chromatography
IC_{50}	Half maximal inhibitory concentration

$CDCl_3$	Deuterated chloroform
MeOH	Methanol
EtOH	Ethanol
CH_2Cl_2	Dichloromethane
EtOAc	Ethyl acetate
KBr	Potassium bromide
SiO_2	Silicon dioxide
g	Gram (s)
mg	Milligram (s)
mL	Milliliter (s)
μg	Microgram (s)
μL	Microliter (s)
L	Liter (s)
h	Hour
nm	Nanometer
Hz	Hertz
MHz	Megahertz
cm^{-1}	Reciprocal centimeter (unit of wave number)
ppm	part per million
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
IR	Infared
UV	Ultraviolet
m.p.	Melting point
α	Alpha
β	Beta

Δ	Delta
m/z	Mass to charge ratio
$[M+H]^+$	Protonated molecule
$[M+Na]^+$	Pseudomolecular ion
$[\alpha]_D^{20}$	Specific rotation at 20 °C and sodium D line (589 nm)
λ_{max}	Wavelength of maximum absorption
c	Concentration
ε	Molar extinction coefficient
Å	Angstrom
°C	Degree celcius
deg.	Degree
sp.	Species

CHAPTER I

LITERATURE REVIEWS

1.1 The influence of natural products

The basic needs of life have relied on nature including foodstuffs, shelters, clothing, and medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. The first record, from Mesopotamia in 2600 BC, the approximately 1000 plant-derived substances used were oils of *Cedrus* species (cedar), *Cupressus sempevirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh), and *Papaver somniferum* (poppy juice), all of which are still used for the treatment of various diseases from coughs and colds to parasitic infections and inflammation (Newman, 2002). In traditional Chinese medicine (TCM), the starting compound, artemisinin is isolated from the plant *Artemisia annua* for anti-malaria. The production of opium and poppy straw can be used for the therapeutic properties such as morphine (and its derivative heroin) thebaine, codeine, papaverine, and noscapine (Newman *et al*., 1997).

As mentioned above, plant products play an important role in the health care systems of about 20% of the population, mainly residing in developed countries such as China and India. The use of plants in the traditional medicine systems of many other cultures has been extensively documented. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization (WHO) that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Newman *et al*., 1997).

1.2 Natural products in drug discovery

Natural products have been the source of most active ingredients of medicines. They can be found in nature including plant, microorganism, marine source and animal source. They have played an important role in pharmacological or biological activity for use in pharmaceutical drug discovery and drug design (Newman and Cragg, 2007). The value of natural products in this regard can be assessed using three criteria: (1) the rate of introduction of new chemical entities of wide structural

diversity, including serving as templates for semisynthetic and total synthetic modification, (2) the number of diseases treated or prevented by these substances, and (3) their frequency of use in the treatment of disease. Between 1981 and 2007, comparisons of the information indicated that more than 80 percent of the new drugs were natural products or inspired by a natural compound (Newman, 2007; Butler, 2008). During 2000 to 2005, the twenty-three drugs derived from natural sources launched on the market such as Galantamine hydrobomide, Micafungin sodium, Daptomycin, Ziconotide and Bivalirudin, all of which have been established to treat for cancer, neurological diseases, infectious disease, cardiovascular and metabolic diseases, immunological, inflammatory and related diseases, and the common human diseases (Kinghorn *et al*., 2006).

1.3 Mangrove plants

Mangrove plants are one of important sources of people which found mainly in the tropical and subtropical intertidal regions of the world, especially in Southeast Asia and South Africa (Thatoi and Patra, 2011). There are a number of tradition uses of mangroves for medicines, for example, the leaf extracts of *B. cylindrical* and bark of *R. mucronata* revealed to possess antiviral activity against all the viruses tested (Premnathan *et al*, 1992), the leaves and roots of *Pluchea indica* for diaphoretic in fever in Thailand and Java (Bandaranayake, 1998), the bark pressing of *X. granatum* and *X. moluccensis* for treatment of fever caused malaria (Alvi *et al*., 1994). Mangrove plants are the rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins (Bandaranayake, 1998). Extracts and chemicals from mangrove plants showed a wide range of pharmacological activities, such as cancer, acquired immouno-deficiency syndrome (AIDS), arthritis, etc,. Some compounds have been tested for drug discovery with clinical trials as *Rhizophora mangla* used in clinical for control of diabetes (Bandaranayake, 2002), as well as *B. sexangula* and *A. Africana* tested for treating cancer and tumors (Kinghorn *et al*., 2006).

Thailand is located in tropical areas which have a great biodiversity of plant species. Thai medicinal plants have many sources for bioactive compounds that are applicable in various fields. Furthermore, the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs, because they must accumulate

within living cells (El-Shemy, 2007). In addition, the uses of traditional medicine based on plants have received considerable interest (Han *et al*., 2002).

The biological activities and chemical constituents of mangrove plants are attractive because the discovery of new therapeutic agents and the information of value in disclosing new sources of already known biologically active compounds. They may be valuable for pharmaceutical industry and for the discovery of new therapeutic agents (Thatoi and Patra, 2011; Bandaranayake, 2002).

1.4 Plants in the genus *Xylocarpus*

The genus *Xylocarpus* belongs to the order Geraniales of the family meliaceae (Sastri, 1950). The family Meliaceae comprises of the 50 genera including *Xylocarpus* and 1400 other species distributed all over the world (Banerji and Nigam, 1984). They are distributed in the tropical, tidal forests of old world and are spread in southeastern Asia, Australia, East Africa and Polynesia (Wu *et al*., 2006). The *Xylocarpus* genus is a small genus comprising only three species, *X. granatum* Koenig, *X. moluccensis* (Lam.) M. Roem., and empirical *X. runphii* (Kostel.) Mabb. All three species can be found in Thailand (Ximu and Pongumphai, 1994). Especially, *X. granatum* and *X. moluccensis* are the most popular plants in practical study (Huo *et al*., 2009). In addition, the plants in the genus *Xylocarpus* are reported to contain a special class of bitter substances termed as limonoids (Taylor *et al*., 1984).

1.4.1 Tradition and Medicinal use of the genus *Xylocarpus*

All parts of the species of *Xylocarpus* are used as the folk medicine. The bark of this plant is also used in dysentery, diarrhea and other abdominal problems and febrifuge (Sastri, 1950; Chopra *et al*., 1956). The root is used to treat cholera from Burma to Philippines. Traditionally, the mangrove plant *X. granatum* is used in Southeast Asia and Indian for the treatment of diarrhea, cholera and fever disease (Bandarnayake, 1998). The *X. moulccensis* is used in cholrea and fever and the fruits of this species are used as a cure for elephantiasis and an anaphrodisiac (Chopra *et al*., 1956). The kernels are used in tonics and in relieving colic. The seeds or peels of the fruits are utilized to poultice swellings and ash of the seeds is applied to itch. The bark pressings are used to treat fevers including those caused by malaria (Bandarnayake, 1998).

Various biological activities have been reported for the extracts and compounds from the genus *Xylocarpus*. such as antidiarrhoeal activity (Uddin *et al*., 2005). In 2005, the methanol extract of the barks of *X. moluccensis* in castor oil and magnesium sulphate induced diarrhea models in mice have been studied. Antibacterial activity, the extract of *X. granatum* showed inhibition of the growth of six virulent strains of bacteria pathogenic to fish viz. *Edwradsiella tarda, Vibrio alginolyticus*, *Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Acromonas hydrophila*, reported in 2005 by Choudhary and coworkers (Choudhary *et al*., 2005). The gedunin compound from *X. granutum* showed significant in vitro antimalarial activity (Omar *et al*., 2003). *N*-methylflindersine from *X. granatum* was found to have antifeedant, insect repellant, antimicrobial, antiyeast and antifungal activities (Chou *et al*., 1977; Bandarnayake, 2002). Xyloccensins Q-V from *X. granatum* have been reported to have antifeedant activity (Wu *et al*., 2005).

1.4.2 Morphology of the *Xylocarpus* genus.

The genus *Xylocarpus* has three distinct spices. They grow in the mangrove habitat or in sandy or coastal habitat. They are difficult to distinguish these three spices based on herbarium specimens without observing the plant in *situ*. They are easily recognized from each other in the field based on the characters of root, trunk, bark, leaves and fruit (Tomlinson, 1986). *X. granatum* is a large spreading mangrove, with rounded coriaceous leaves, smooth thin bark, and abundant red heartwood, which furnishes a useful, if rather hard, timber of the characteristic mahogany type. The fruit is grape fruit sized, hard and heavy. *X. moluccensis* is a smaller, less branched mangrove, with pointed leaves, deeply serrated bark and an undistinguished timber. The fruit is the size of a mandarin orange. In the case of *X. rumphii*, it is a rare plant on the East African coast, similar to *X. granatum*, but having the small fruit typical of *X. moluccensis*, which has been considered as a possible hybrid of these two species (Mullholand and Taylor, 1992).

1.5 Taxonomical *Xylocarpus granatum* Koenig, *Xylocarpus moluccesis* (Lam) Roem *and Xylocarpus rumphii* (Kostel) Mabb.

Taxonomy of *Xylocarpus* is categorized as

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Rutales

Family : Meliaceae

Genus : *Xylocarpus*

1.5.1. Botanical characteristics of *Xylocarpus moluccesis* (Lam) Roem.

Medium-sized crooked much-branched evergreen tree up to 10 m. tall (taller elsewhere); bark smooth and yellowish, or brown and green and flaking surface roots laterally compressed and forming and spreading network of ribbon-like pneumatophores with the upper edges protruding above the mud and suggesting and mass of snakes. Leaved paripinnate, drying orange-brown; petiole and rhachis up to 8·5 cm. long, glabrous; leaflets up to 12 × 5 cm., usually much smaller, opposite, 1-2 (3-jugate), elliptic, oblong-elliptic or obovate-elliptic, apex usually rounded, rarely obtuse or emarginated, base narrowly or broadly cuneate, glabrous, coriaceous venation prominent on both sides; petiolules 2-5 mm. long. Flowers whitish or pale pink, in lax racemes of (2) 3-flowered cymes; peduncle plus rhachis 4-7 cm. long; bracts minute, usually caduceus. Calyx about 3 mm. long, glabrous, lobed to the middle, lobes rounded. Petals 5-6.5 × 2·5 mm., glabrous. Staminal tube 4-5 mm. long, glabrous. Ovary less than 1 mm. in diam.; style 1·5 mm. long; disk fused to the lower half of the ovary. Fruit large, up to 20 cm. in diam., obscurely 4-sulcate. Seeds 4-8 cm. long.

Figure 1.1 Botanical characteristics of *Xylocarpus moluccensis* (Lam)

Figure 1.2 Botanical characteristics of *Xylocarpus rumphii* (Kostel.) Mabb.

1.5.2. Botanical characteristics of *Xylocarpus rumphii* (Kostel.) Mabb.

X. rumphii is a tree up to 4-12 m with neither conspicuous buttresses nor pneumatophores; bole usually solitary, to 50 cm diameters, frequently of poor form. Bark lenticellate to finely fissured, grayish; inner bark bright pink to red. Leaf rachis and petiole to 22 cm with terminal spike to 1 mm. Leaflets in 2-4 pairs, 5-10 by 3-5 cm, ovate to cordate, sometimes falcate, base broadly cuneate or rounded to truncate or cordate, asymmetric, apex acute to acuminate; venation prominent on both surfaces in sicco, conspicuous in vivo; petiolule 1-3 mm. Thyrses 10-18 cm long, lax, pendent, main axis distinct; lateral branches to 8 cm; bracts and bracteoles 0.5 mm, narrowly triangular, persistent; pedicles 3-8 mm, not conspicuously swollen near calyx. Calyx lobes 1-1.1.5 mm long. Petals 3.5-6 by 2-2.5 mm, elliptic-oblong, creamy white. Staminal tube 2-2.5 mm diameters, lobes apiculate or bifid to retuse. Fruit 6-8 cm diameters, globose. Seeds 8-16, 3.6-7 cm long (Mabberley *et al*., 1995) In addition, the vernacular names of this plant are “Niri” or “Nyireh” and local name in Thailand is “Ta Ban” (Mabberley *et al*., 1995).

1.5.3. Botanical characteristics of *Xylocarpus granatum* Koenig.

In *X. granatum*, erect, conical knee roots are absent but the horizontal cable roots develop into ribbon-like plank roots, which is a tree, 3-8 m. tall; buttresses are long and snaking laterally; the bark is smooth, unfissured and thin, displaying a pattern of light-brown to yellowish or greenish patches, acused by peeling of the bark. Leaves pinnate, the leaflets are bright light green and the leaf are dark green when old, narrowly drop-shaped, with rounded tips and on average 10 cm. long and 4 cm. wide. Petiole of the leaf is short and corky. The flowers are white in color and very small about 8 mm. across; capsule woody. The fruit is large, globose up to 20-30 cm. across, in which several seeds are enclosed leading to the common name of the “cannon ball tree”(Tomlinson, 1986). Furthermore, *X.mangrove* have the local names in thai e.g. Tabun khao, Tabun and Krabunkhao. The picture of *X.granagum* is shown in Figure 1.3

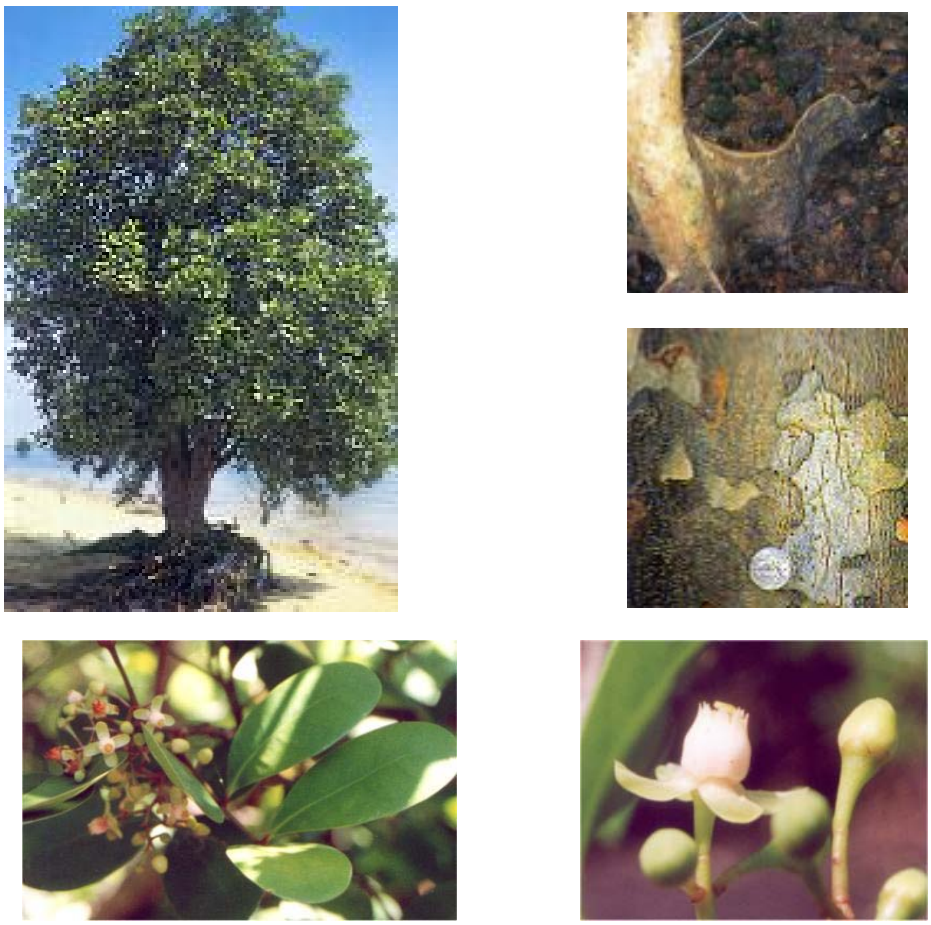
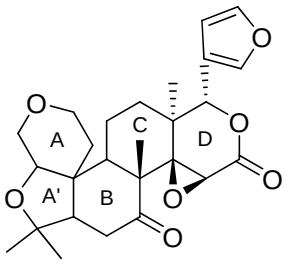


Figure 1.3 Botanical characteristics of *Xylocarpus granatum* Koenig.

1.6 Limonoid

The term limonoid was derived from the limonin compound which was first obtained from the bitter principles (Devakumar, 1996) of citrus fruits. Limonoid is usually defined as a triterpene derivative in which the side chain has become a furan ring by the loss of four carbons; it was thus termed as tetranortriterpenoids. (Lakshmi, 2008)

The scientist studies show that limonoids are highly oxygenated, modified terpenoids and have recently attracted attention because compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans (Koul, 2004; Nakagawa, 2001).



Limonin

Figure 1.4 Citrus limonoid (limonin)

Although hundreds of limonoids have been isolated from various plants, their occurrence in the plant kingdom is confined to only plant families of order Rutales and that too more abundantly in Meliaceae and Rutaceae, and less frequently in Cneoraceae and *Harrisonia* sp. of Simaroubaceae (Roy and Saraf, 2006). *Azadirachta indica* (Neem tree), a species of meliaceae family is a storehouse of limonoids containing more than hundred different limonoids and their derivative in its different plant parts (Devakumar, 1996; Charleston, 2002; Kraus, 1995).

1.6.1 Chemistry and biosynthesis of limonoids

Limonoids are of moderate polarity, insoluble in water and hexane but soluble in hydrocarbons, alcohol and ketone (Aliero, 2003). They are present in neutral (noncarboxylated/aglycon) as well as acidic (carboxylated/glucoside) forms, the former are insoluble and bitter while latter are soluble and tasteless (Roy and Saraf, 2006)

These compounds are stereochemically homogenous compounds, which are triterpene derivatives from a 4,4,8-trimethyl-17-furanylsteroid skeleton (Zhou *et al*., 2006). All naturally occurring limonoids contain a furan ring attached to the D-ring, at C-17, as well as oxygen containing functional groups at C-3, C-4, C-7, C-16 and C-17 (Somrutai *et al*., 2005).

The biosynthesis of limonoids shows that limonoids are synthesized via terpenoid biosynthetic pathway, starting with cyclization of squalene, which results into a tetracyclic ion (Figure 1.5). Euphane and tirucallane, two chemically similar compounds (Figure 1.6), may be the ultimate biogenetic precursors. Oxidative degradation at the C-17 side chain of either of these nucleus results in loss of four

carbon atoms and formation of β-substituted furan, further oxidations and skeletal rearrangements in one or more of the four rings, which are designated as A, B, C and D (Scheme 1.1), give rise to different groups of limonoids as shown in Figure 1.7 (Endo *et al*., 2002; Suarez *et al*., 2002). However, the oxidations are either epoxidations of double bonds or Baeyer Villiger attacks on ketones and are all of the types to be expected from a biological peracid equivalent, presumably a peroxidase as described in Scheme 1.2 and 1.3 (Lakshmi and Gupta, 2008).

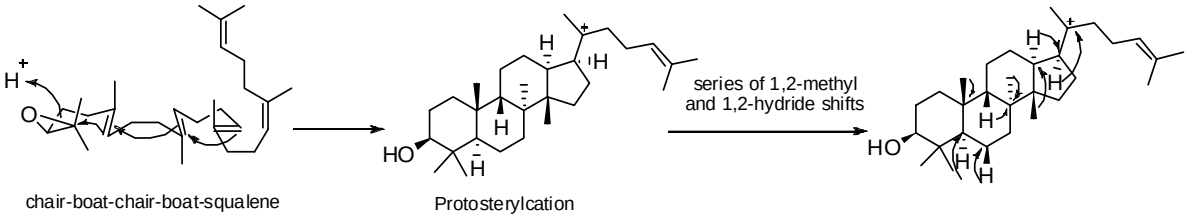
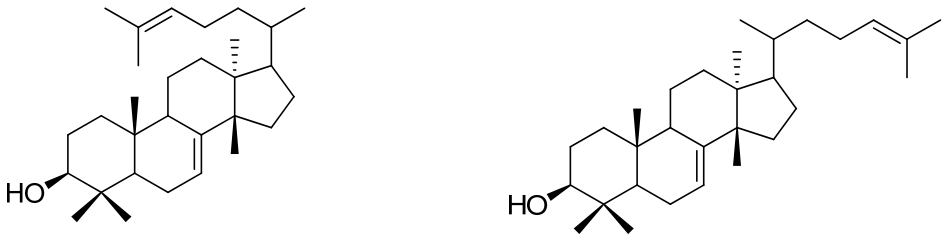
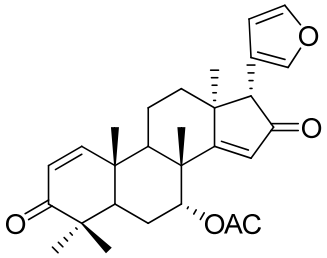


Figure 1.5 Squalene epoxide leading to different intermediate triterpene cations.

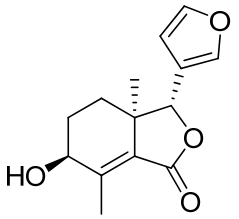


Euphane Tirucallane

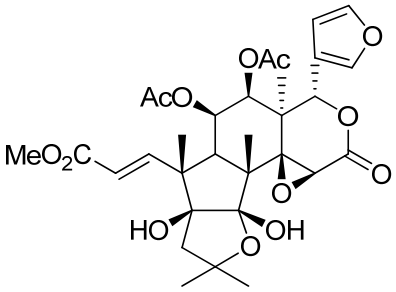
Figure 1.6 Precursors of limonoids



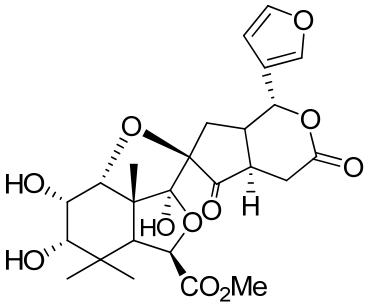
Intact limonoid



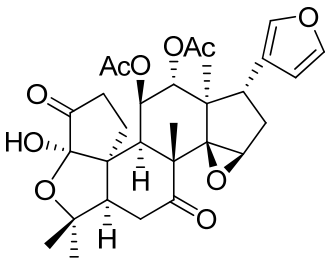
Degraded limonoid



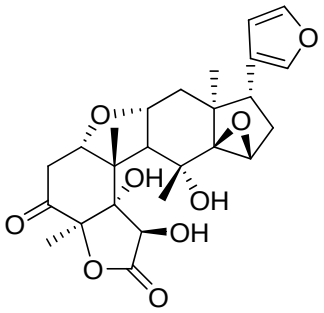
Highly modified limonoid



Highly cleaved limonoid

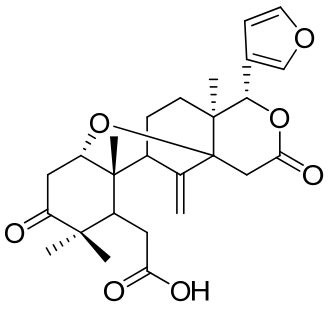


Ring A-*seco* limonoid

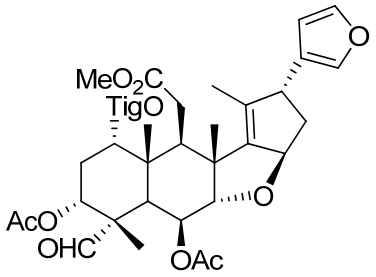


Ring-A,C,D-intact-ring-B-*seco* limonoid

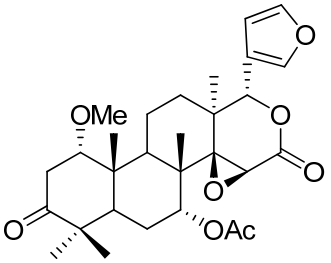
Figure 1.7 Example of limonoids with different degree of oxidation and skeleton arrangement.



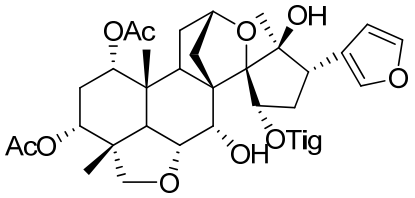
Ring-B,D-*seco* limonoid



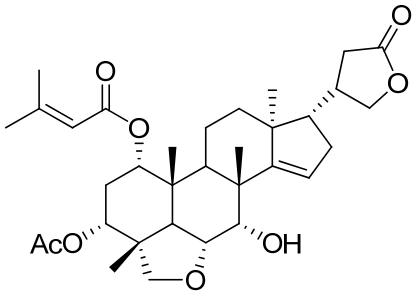
Ring-C cleaved limonoid



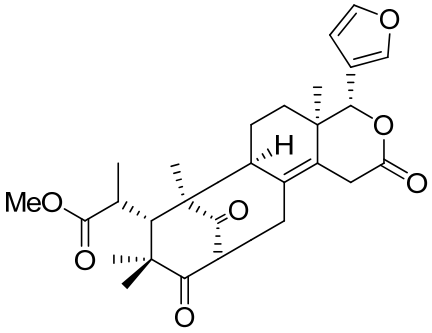
Ring-D-lactone-limonoid



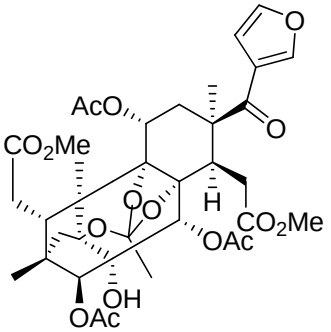
Ring-C-*seco* limonoid



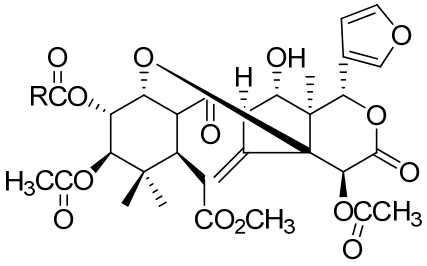
Gamma-lactone side chain limonoid



Mexicanolide

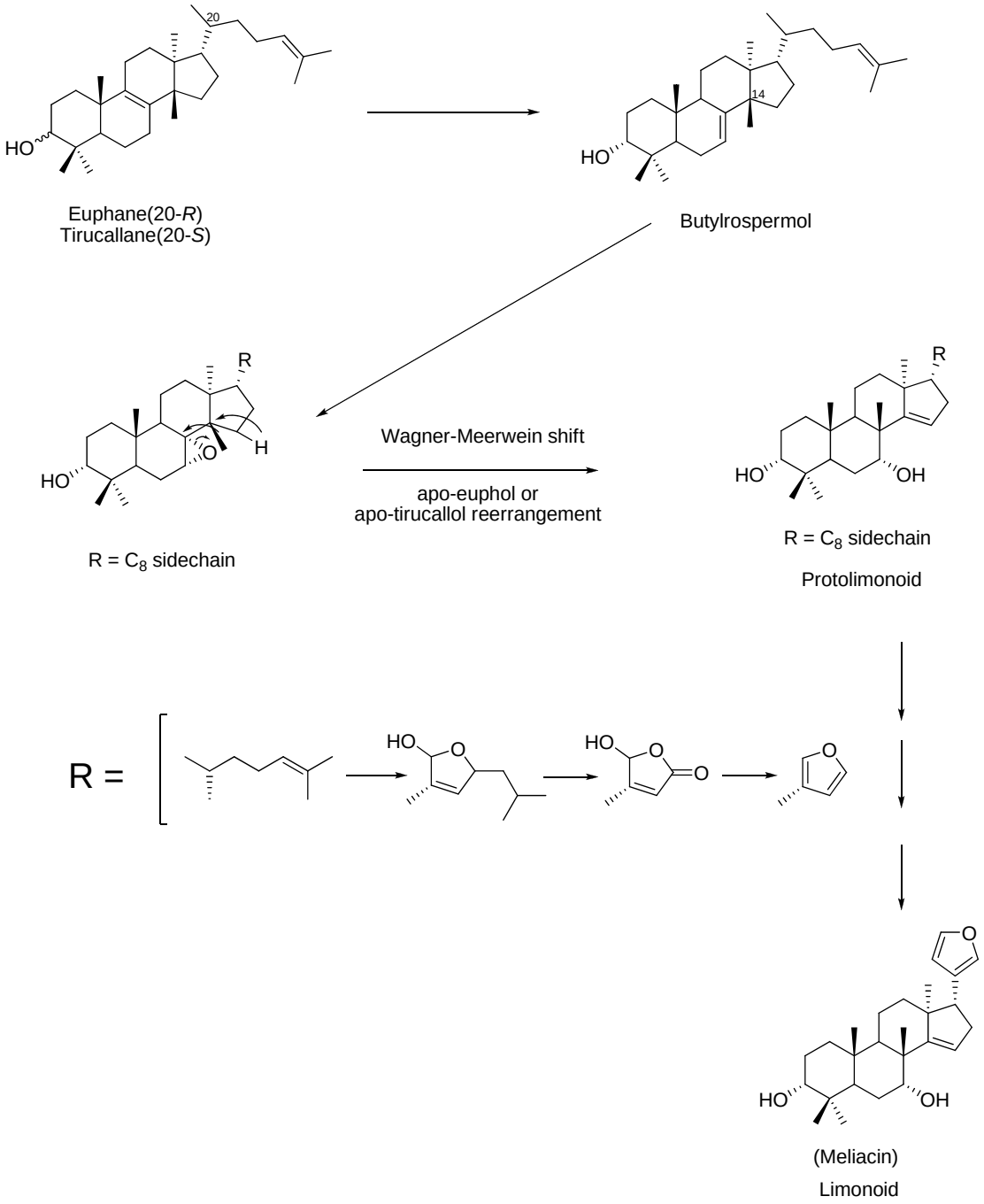


Phragmalin

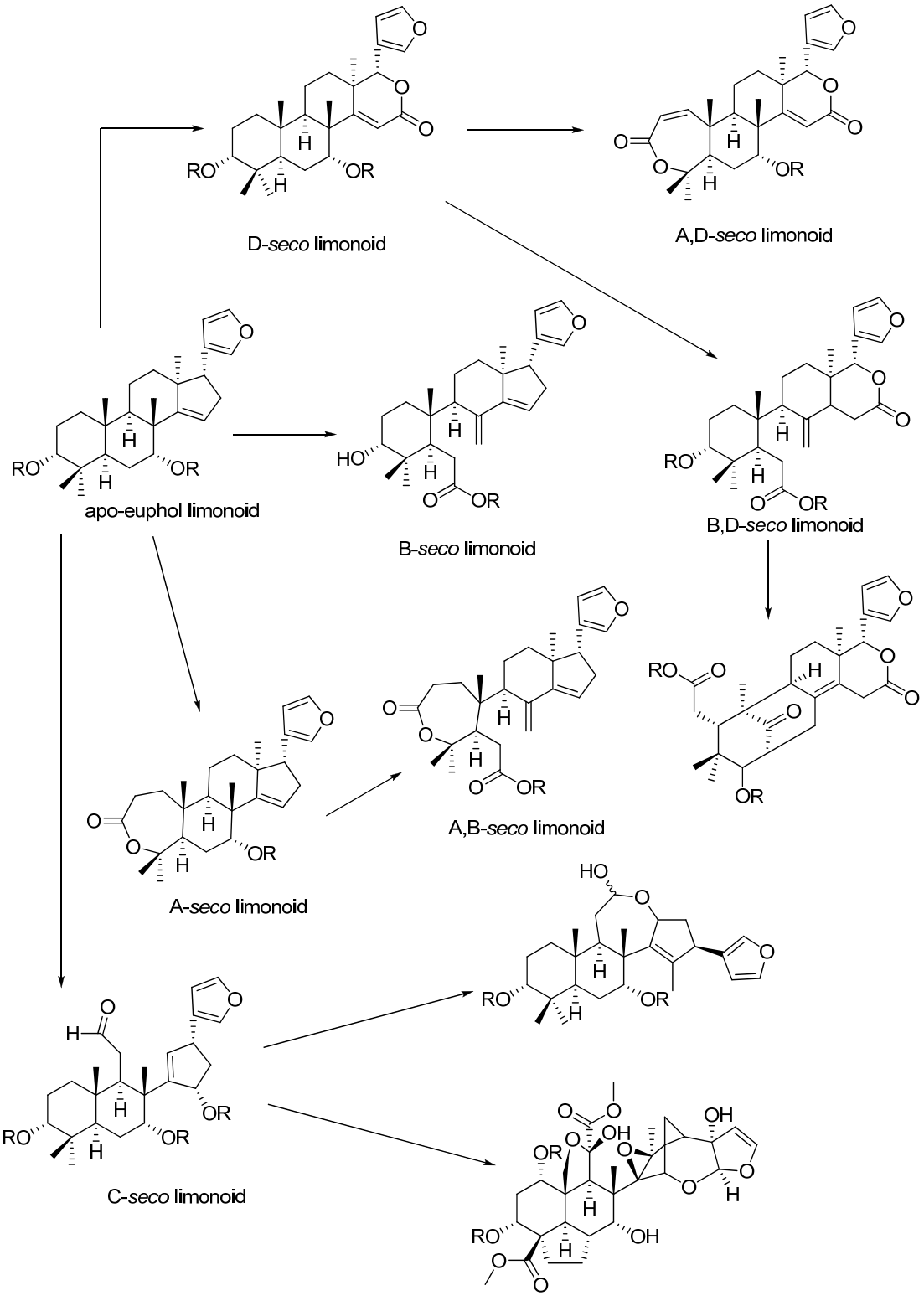


Trijugin-type-limonoid

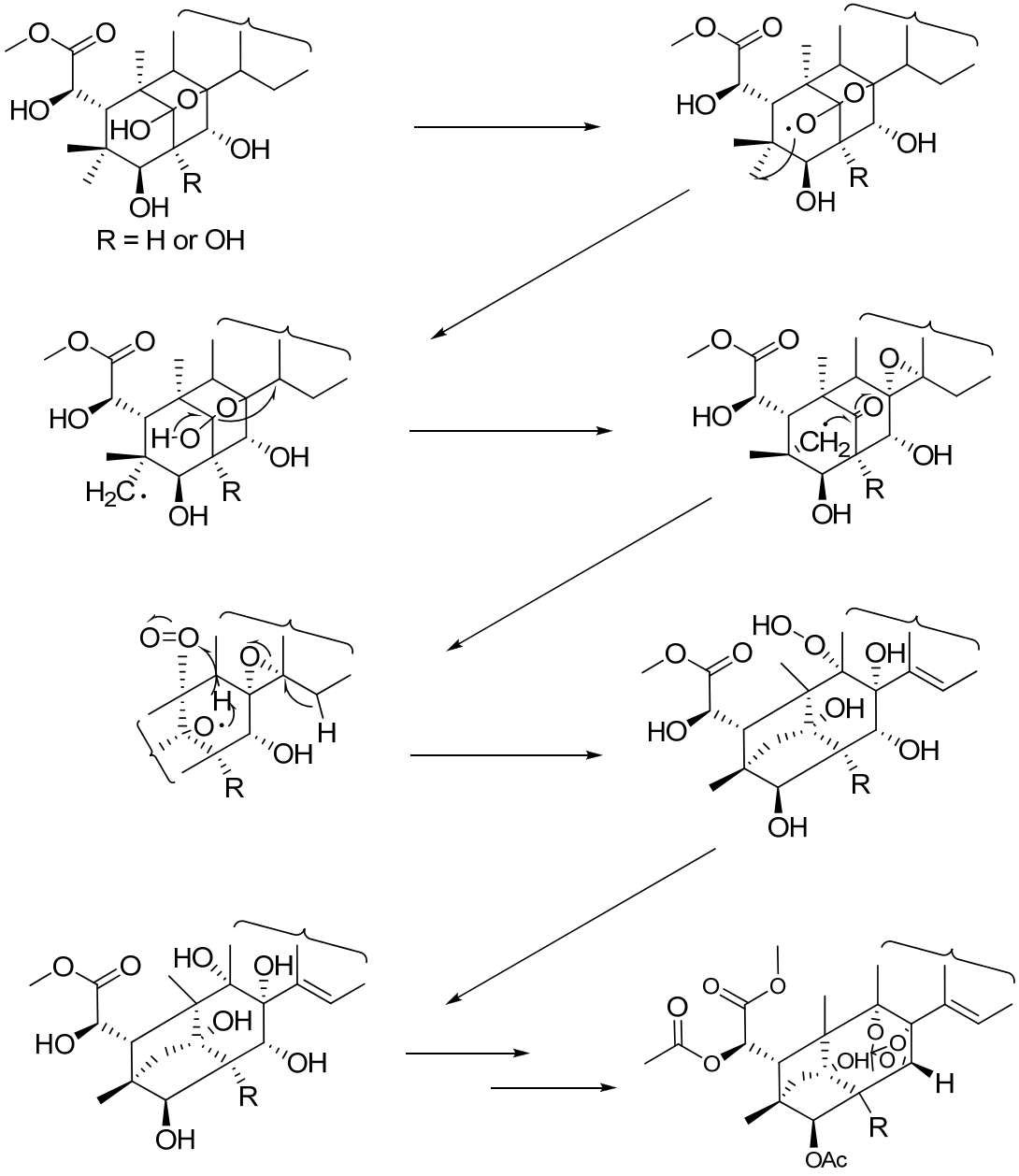
Figure 1.7 Example of limonoids with different degree of oxidation and skeleton arrangement (continued)



Scheme 1.1 Biosynthetic pathway leading to the formation of a simple limonoid (Champagne *et al*., 1992)



Scheme 1.2 Major biosynthetic routes of limonoids (Champagne *et al*., 1992)

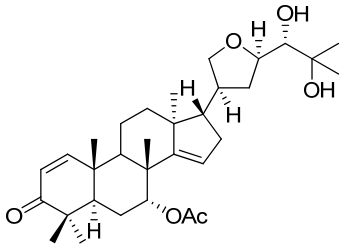


Scheme 1.3 Proposed biosynthetic pathway to 8,9,30-phragmalin *ortho* esters from a mexicanolide (Wu *et al*., 2004)

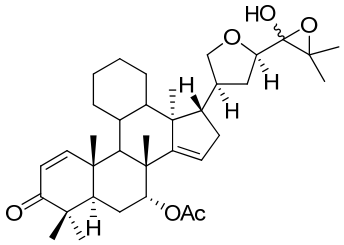
1.7 Classification of limonoids

The chemical constituents of genus *Xylocarpus* include triterpenoids, alkaloids, phenolic acids, flavanol, steroids, monoterpenes and some others (Mulholland, 1992). Of these reported compounds, limonoids are the most component within the genus *Xylocarpus*. The genus *Xylocarpus* have many various structural patterns, which could be classified into protolimonoids, gedunin, obacunol, andirobin, mexicanolide, phragmalin and miscellaneous type limonoid (Chang-Hong, 2009).

1.7.1 Protolimonoid. This group is limonoid with intact side chain and it is a precursor of other limonoids. Its structure still contains 30 carbon atoms as normal triterpenes. An example of this class is protoxylocarpins G and H isolated from sed kernels of *X. granatum* by our group (Pudhom *et al*., 2009)

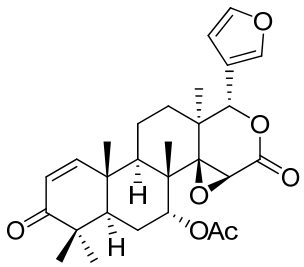


Protoxylocarpin G

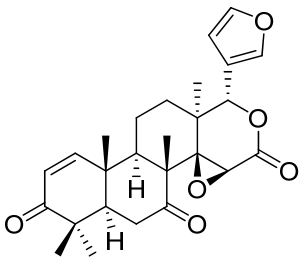


Protoxylocaprin H

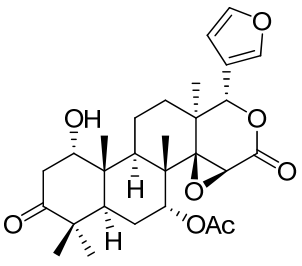
1.7.2 Gedunin. The characteristic of this group is D-ring opened. Only three compounds in gedunin series, gedunin (Taylor *et al*, 1995; Mulholland *et al*, 1992;), 7-oxogedunin (Ng *et al*., 1979), and 1α-hydroxy-1,2-dihydrogedunin (Uddin *et al*., 2007), were isolated from the genus *Xylocarpus*. Gedunin, the first limonoid from this genus, was identified by Taylor and his coworkers from the timber of the east African *X. granatum*, while 7-oxogedunin was isolated from the Kenya *X. moluccensis* and has been recently obtained again from the seeds of the Thai *X. granatum.*



Gedunin

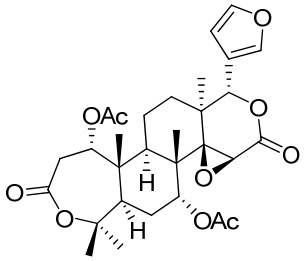


7-Oxogedunin



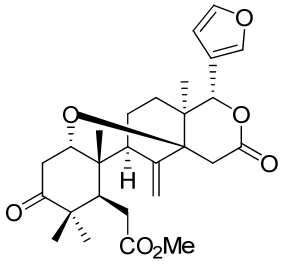
1α-Hydroxy-1,2-dihydrogedunin

1.7.3 Obacunol. This type limonoid is characterized by the presence of ring A and D opened. It was found to be rare in Meliaceae and only one compound, 7α-acetoxydihydronomilin, was reported from the seeds of *X. granatum* (Ng *et al*., 1979 Ahmed *et al*., 1978).

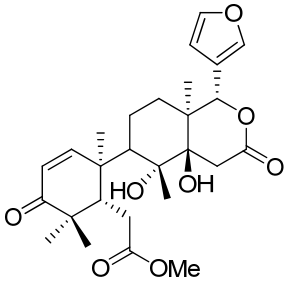


7α-acetoxydihydronomilin

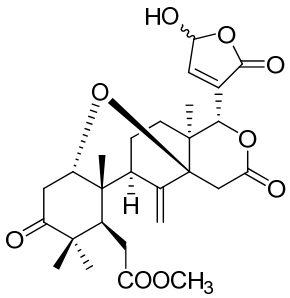
1.7.4 Andirobin. This type limonoid is identified by the B-ring opened, other than the D-ring opened as seen in Gedunin series. Methyl angolensate was found in all Xylocarpus genus (Molholland *et al*., 1992). Recently, two new andirobins, moluccensins N and O, have been reported from *X. moluccensis* by Wu and his coworkers (Wu *et al*., 2010). In addition, our group also reported the isolation and characterization of an additional new andirobin, thaimoluccensin A from the same species collected from southern part of Thailand (Pudhom *et al*., 2011).



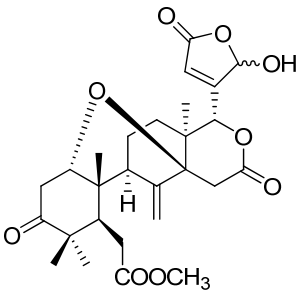
Methyl angolensate



Thaimoluccensin A

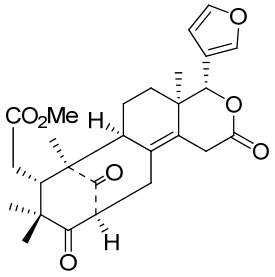


Moluccensin N

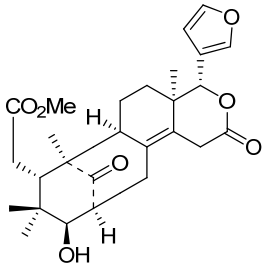


Moluccensin O

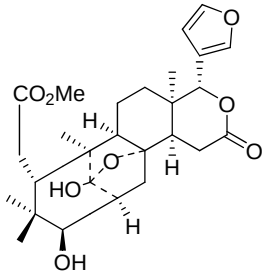
1.7.5 Mexicanolide. This limonoid series is derived from 1,3-diketodiene lactone of the andirobin group by spontaneous Michael cyclization, forming mexicanolide. This group limonoid is one of the major limonoids found in the genus *Xylocarpus*. The simplest member of this group namely mexicanolide was reported from *X. moluccensis* (Ng *et al*., 1979; Connolly *et al*., 1968). Until the present, a number of new compounds from the plants belonging to *Xylocarpus* genus have still been added to this group, for example, xyloccensin X_1 (Cheng *et al*., 2006) and proceranolide (Cui *et al*., 2007) from *X. moluccensis* and *X. granatum*. In this group, there are also the 8α-hydroxyl compounds which spontaneously form the 1,8-ketals such as xylogranatins B-D from *X.granatum* (Wu *et al*., 2006). Another example of limonoid in this series is 3,8-oxide bridge, for example, xyloccensin W and xylorumphiin D from *X.granatum* (Wu *et al*., 2006; Pudhom *et al*., 2010).



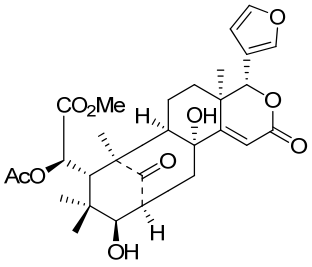
Mexicanolide



Proceranolide



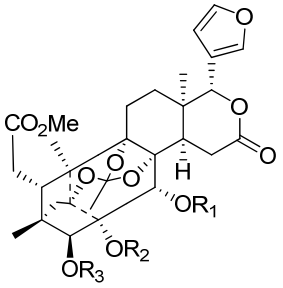
3-Deacetylxyloccensin M



Xyloccensin X_1

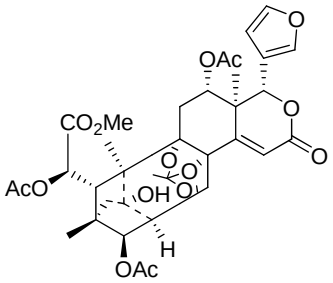
1.7.6 Phragmalin. Limonoid in this series is derived from the addition of C-28 to the keto carbonyl (C-1) of mexicanolide, producing phragmalin type limonoid. Similar to mexicanolide type, it is another type of the major limonoids found in the genus *Xylocarpus*. In 2004, two unique 8,9,30-phragmalin *ortho*-esters, xyloccensins O and P, were isolated from the stem barks of *X. granatum* (Wu *et al*., 2004). Furthermore, the reinvestigation of the same plant resulted in the isolation of the other

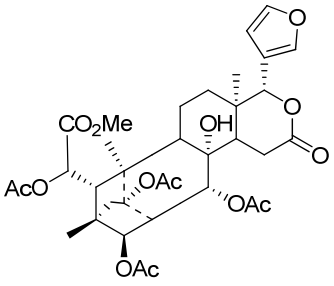
six new 8,9,30-phragmalin *ortho*-esters, namely xyloccensins Q-V (Wu *et al*., 2005; Cui *et al*., 2005; Wu *et al*., 2006). Recently, a new phragmalin xylogranatin E_2, which contains a 3-*O*-*β*-tigloyl group and a C(14)=C(15) bond, was obtained from the seeds of *X. granatum* (Wu *et al*., 2007).



	R_1	R_2	R_3
Phragmalin	H	OH	H
3*β*,30*α*-diacetylphragmalin	Ac	OH	Ac
Xyloccensin E	Ac	OAc	Ac

	R_1	R_2
Xyloccensin O	OAc	H
Xyloccensin P	OAc	OAc
Xyloccensin Q	OAc	OH
Xyloccensin R	OH	OH
Xyloccensin S	OH	OAc
Xyloccensin T	OH	H
Xyloccensin U	H	OH
Xyloccensin V	H	OAc

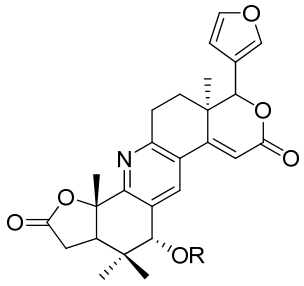




Xyloccensin E_2

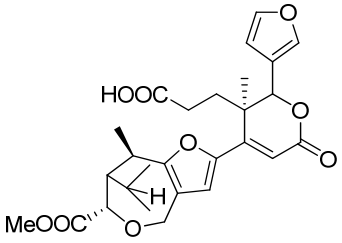
1**.7.7 Miscellaneous limonoid.** Apart from limonoids mentioned above, some limoniods with the unique structures have been recently reported from *Xylocarpus* spp. In 2007, the first aromatic B-ring limonoids were isolated from the seeds of Chinese *X. granatum*. They belong to two substructural classes, of which one contains

a pyridine ring including xylogranatins F and G, while xylogranatins I-M and Q contains a central furan core (Wu *et al*., 2008).

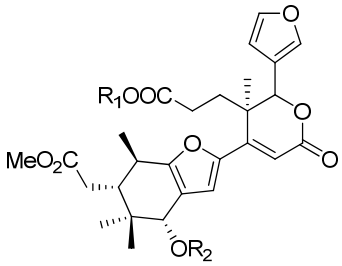


Xylogranatin F: R = H

Xylogranatin G: R = Ac



Xylogranatin Q



	R_1	R_2
Xylogranatin I	H	H
Xylogranatin J	Me	Me
Xylogranatin K	H	Me
Xylogranatin L	H	Et
Xylogranatin M	Me	Ac

1.8 Biological activities of limonoids from *Xylocarpus* species

1.8.1 Cytotoxic Activities

In 2006, xylograntins A-D isolated from *X. granatum* were evaluated for their *in vitro* cytotoxicitys against two tumor cell lines, P-388 murine leukemia and A-549 human lung carcinoma. Results showed xylograntins B-D showed moderate cytotoxicity against the P-388 cells with the corresponding IC_{50} values of 8.9, 6.3 and 14.6 μM, respectively. Moreover, xylograntins A and B displayed cytotoxicity against A-549 cell lines with IC_{50} values of 15.7 and 11.3 μM, respectively, (Yin *et al*., 2006).

In 2007, the cytotoxic activity of granaxylocarpins A and B from *X.granatum* against the P-388 murine leukemia and A-549 human lung carcinoma cell lines were tested. They showed weak cytotoxicity toward P-388 cells with IC_{50} values of 9.3 and 4.9 μM, respectively, but both were inactive on A-549 cell lines (Yin *et al*., 2007).

In 2009, five new protolimonoids, protoxylocarpins A-E, and two new limonoids, xylocarpins J and K, together with xyloccensins M and Y were obtained from the fruits of a Chinese mangrove plant *X. granatum*. These compounds exhibited moderate to weak activity against HCT-8, Bel-7402, BGC-823 and A2780 cell lines (Cui *et al*., 2009).

1.8.2 Antifeedant Activities

In 2005, the xyloccensins O-V from *X. granatum* were tested on the antifeedant activity with a conventional leaf disk method against the third instar larvae of *Mythimna separate*. Only xyloccensins P and Q were strongly active at the concentration of 500 ppm, with 50 ppm corresponding to the concentration of *ca*. 1 μg/leaf·cm^2 (Wu *et al*., 2005).

In 2007, xylogranatins C-D, F-G, I, K, P and R obtained from *X.granatum*, were also tested for their antifeedant activity by using the same method against Xylogranatins F, G and R were found to be active at a concentration of 1 mg/mL, whereas xylogranatin G exhibited the most potent antifeedant activity by AFC_{50} values of 0.31 and 0.30 mg/mL at 24 and 48 h, respectively (Wu *et al*., 2008).

In 2010, moluccensins H and I from the seeds of an Indian mangrove, *X. moluccensis* displayed moderate insecticidal activity against the fifth instar larvae of *Brontispa longissima* (Gestro) at a concentration of 100 mg/L. The lethal rates of moluccensin H at exposure times of 72 and 96 h were 20.7% and 27.6%, respectively, while those of moluccensin I were 10.7% and 28.7%, respectively (Wu *et al*., 2010).

1.8.3 Antimicrobial Activities

In 2010, three new phragmalin limonoids, moluccensins H-J, isolated from seed kernels of the cedar mangrove, *X. moluccensis* were tested for their antibacterial activity. Only moluccensin I displayed weak antibacterial activity against *Staphylococcus hominis* ATCC 27844 and *Enterococcus faecalis* ATCC 29212, with a MIC at 256 μg/mL (Pudhom *et al*., 2010)

1.8.4 Antimalaria Activities

Gedunin and methyl angolensate were found to be active *in vitro* against *Plasmodium falciparum* with IC_{50} values of 12.5 and 9.63 µg/mL. Furthermore, it was found that gedunin could give additive effect when combined with chloroquine,, a standard drug (Bickii *et al*., 2000).

Limonoid derivatives have been found in all *Xylocarpus* plants, including *X. moluccensis*, *X. granatum* and *X. rumphii*. The *Xylocarpus* plants could be found in Thai mangrove areas both eastern and southern. In addition, it was found that the ecological system in each area has much effect on limonoid structures. This prompted us to investigate limonoids of the seed kernels of *X. moluccensis*. and *X. granatum*. collected from a mangrove area in Surat-thani province, the southern part of Thailand. This is because there still are very few reports of limonoids from Thai *Xylocarpus* sp. collected from southern areas, including *X. moluccensis*. and *X. granatum*. Moreover, biological activity, particularly anti-inflammatory effect of isolated limonoids would be evaluated.

Therefore, the objectives of this research are summarized as follow;

1. To extract, isolate and purify limonoids from the seed kernels of *X. granatum* Koenig. and *X.moluccensis* Lam. collected from Surat-thani province.
2. To elucidate structures of the isolated limonoids by spectroscopic technique.
3. To evaluate biological activity of pure limonoids, particularly anti-inflammatory activity.

CHAPTER II

EXPERIMENTS

2.1 Plant material

Fruits of *X. granatum* Koenig. and *X. moluccesis* (Lam) Roem. were collected from Surat-thani Province, Thailand, in February 2010 and December 2009, respectively. Plant materials were identified by Royal Forest Department, Bangkok, Thailand.

2.2 General Experimental Procedures

2.2.1 Nuclear magnetic resonance spectrometer (NMR)

The NMR spectra were recorded in $CDCl_3$ using a Bruker AV400 and Varian Mecury 400 plus spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR using TMS (tetramethylsilane) as internal standard.

2.2.2 Mass spectrometer (MS)

HRESIMS spectra were obtained with a Bruker micrOTOF.

2.2.3 Ultraviolet-visible spectrophotometer (UV-vis)

UV data were recorded on a CARY 50 Probe UV-visible spectrophotometer.

2.2.4 Fourier transforms infrared spectrophotometer (FT-IR)

FT-IR spectra were recorded on a Perkin-Elmer Model 1760X Fourier Transform Infrared Spectrophotometer. Solid samples were formally examined by incorporating the sample with potassium bromide (KBr) to form a pellet.

2.2.5 Optical rotation

Optical rotations were measured on a Perkin-Elmer 341 polarimeter at 589 nm.

2.2.6 Melting point

Melting points were recorded on a Fisher-Johns melting point apparatus.

2.2.7 X-ray crystallography

Crystal structures were solved by direct methods and using the SHELXS97 program. Crystallographic data, excluding structure factors, have been deposited at the Cambridge Crystallographic Data Centre.

2.3 Chemicals

2.3.1 Solvent

All commercial grade solvents, used in the present study such as hexane, chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), acetone and methanol (MeOH), were purified by distillation prior to use.

2.3.2 Other chemicals

- Merck's silica gel 60 No. 7734 and No. 9385 were used as adsorbents for open column chromatography.

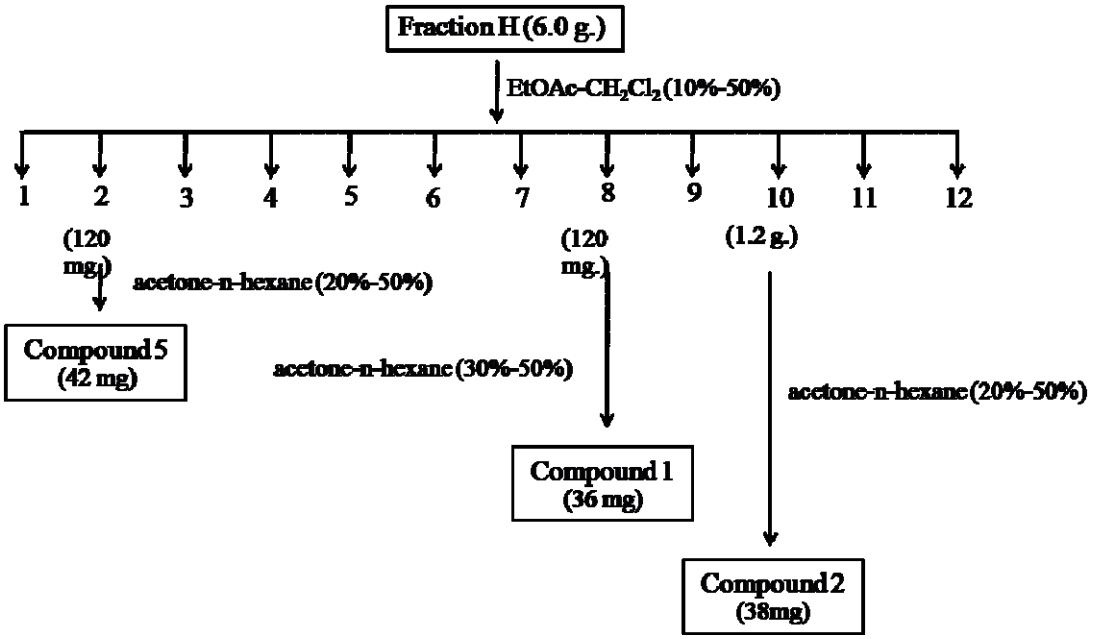
- Merck's Thin layer chromatography (TLC) aluminum sheets, silica gel 60 F_{254} precoated 25 sheets, 20x20 cm, layer thickness 0.2 mm were used for TLC analysis. Detection was visualized under ultraviolet light at wavelengths of 254 nm and dipped with $(NH_4)_6Mo_7O_{24}$ solution in 5% H_2SO_4 /EtOH.

2.4 Extraction and Isolation

2.4.1 *Xylocarpus granatum* seed kernels

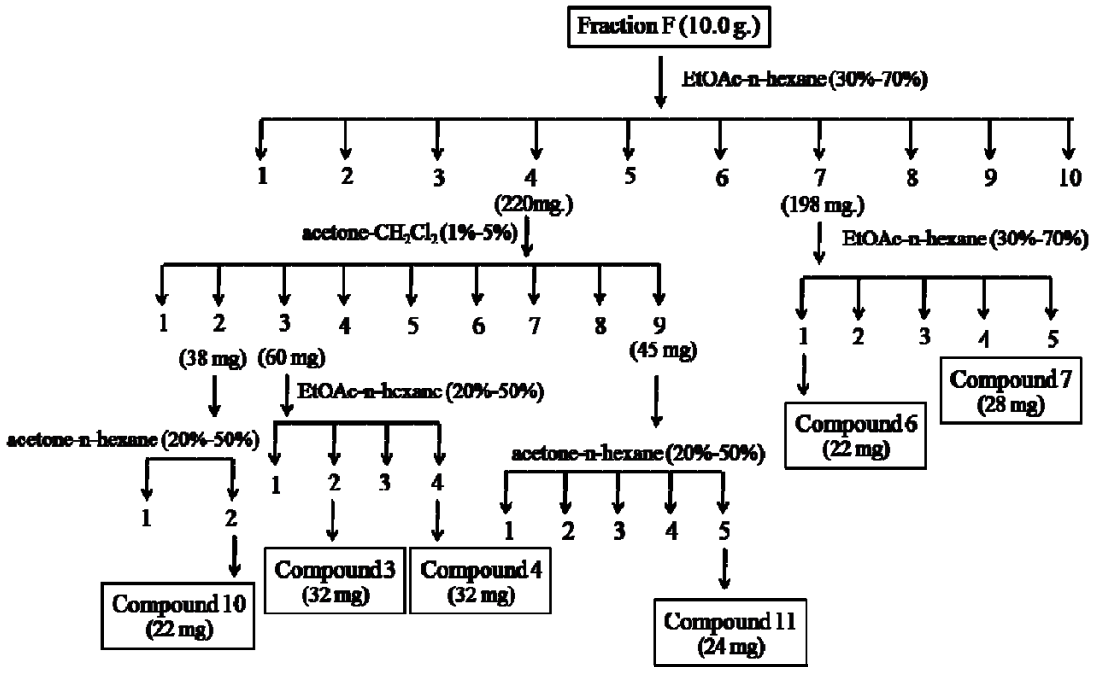
Air-dried powder seed kernels of *X. grantum* (15 kg) were extracted with MeOH (30 L x 3, each 3 days) at room temperature. After removing the solvent in vaccuo, the combined MeOH crude extract was suspended in H_2O (300 mL), then partitioned with EtOAc (300 mL x 3) to afford the EtOAc crude extract (96.0 g). The extract was fractionated by silica gel column chromatography (CC) with a gradient of EtOAc-hexane (10%-50%) to yield 13 pooled fractions (A-M).

Fraction H (6.0 g) was chromatographed on a silica gel column eluted with a gradient of acetone-CH_2Cl_2 (10%-50%) to obtain 12 subfractions (H.1-H.12). Subfraction H.8 (160 mg) was further subjected to a silica gel column eluting with the mixture of acetone-hexane (20%-50%) to yield compound **1** (36 mg), while subfraction H.10 (87 mg) gave compound **2** (52 mg) by using the same condition. Subfraction H.2 was rechromatographed on a silica gel column (30%-50% acetone-hexane) to obtain compound **5** (42 mg). The isolation procedure of this fraction is summarized in Scheme 2.1.

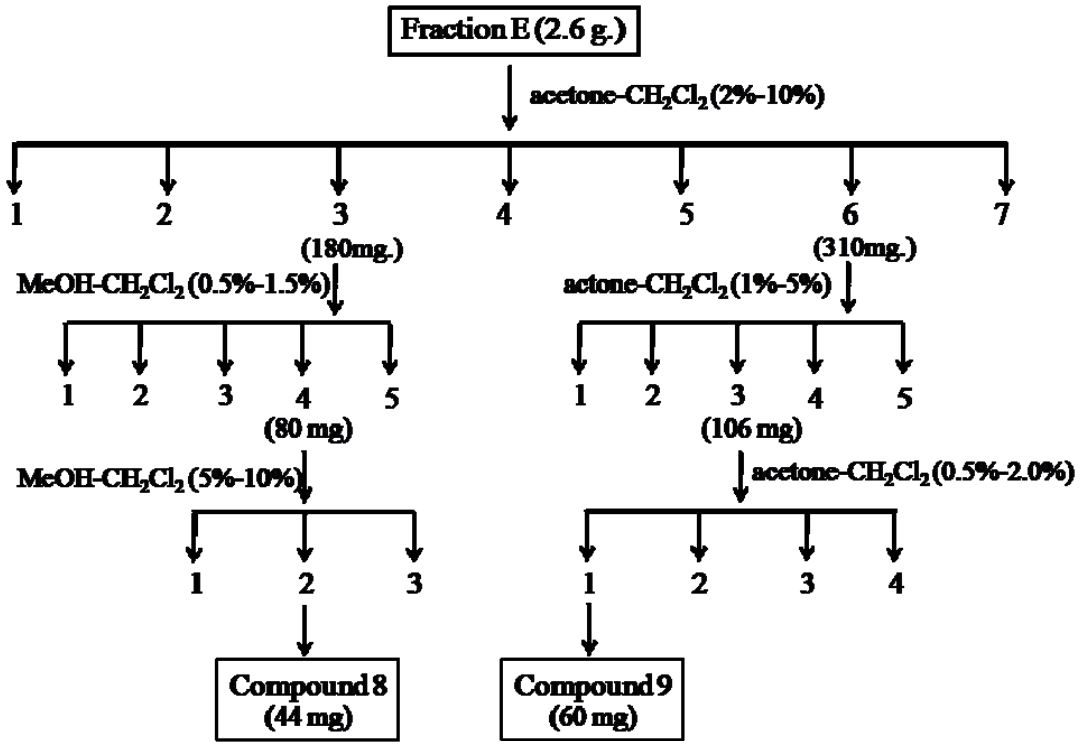


Scheme 2.1 Isolation of fraction H of *X. granatum* crude extract

Fraction F (36 g) was chromatographed on a silica gel column eluted with EtOAc-hexane (30%-70%) mixture to give ten subfractions (F.1-F.10). Subfraction F.4 (220 mg) was further separated on a silica gel column with acetone-CH_2Cl_2 (1%-5%) to give ten fractions (F.4.1-F.4.10). Subsequently, fraction F.4.2 (38 mg) was subjected to silica gel CC eluting with acetone-hexane (20%-50%) to afford compound **10** (22 mg), while separation of fraction F.4.3 (60 mg) with EtOAc-hexane (20%-50%) yielded compound **3** (32 mg) and compound **4** (32 mg). Fraction F.4.9 (45 mg) was applied to a silica gel column and eluted with EtOAc-hexane (20%-50%) to give compound **11** (24 mg). Fraction F.7 (198 mg) was rechromatographed on a silica gel column (1%-5% acetone-CH_2Cl_2) to give five fractions (F.7.1-F.7.5). Subsequent isolation of fraction F.7.1 by silica gel CC with EtOAc-hexane (30%-70%) provided compound **6** (22 mg) and compound **7** (28 mg). The isolation procedure is summarized in Scheme 2.2.



Scheme 2.2 Isolation of fraction F of *X. granatum* crude extract



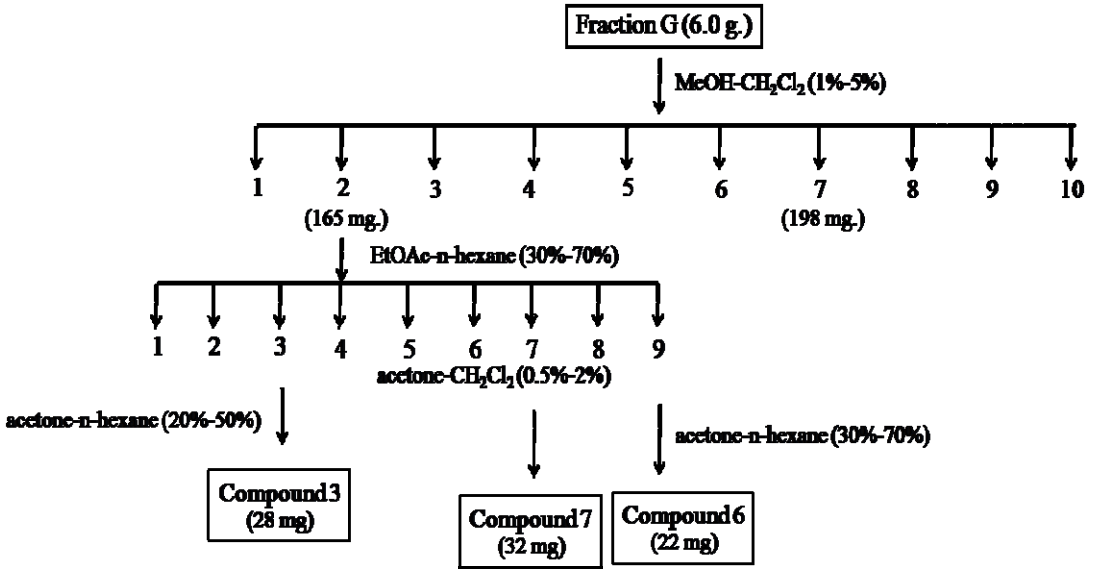
Scheme 2.3 Isolation of fraction E of *X. granatum* crude extract

As shown in Scheme 2.3, Fraction E (2.6 g) was chromatographed on a silica gel column eluted with a gradient of acetone-CH_2Cl_2 (2%-10%) to obtain seven subfractions (E.1-E.7). Subfraction E.3 (180 mg) was subjected to silica gel CC eluting with MeOH-CH_2Cl_2 (0.5%-1.5%) to afford five fractions (E.3.1-E.3.5). Fraction E.3.4 (80 mg) was further purified by silica gel CC with MeOH-CH_2Cl_2 (5%-10%) to give compound **8** (44 mg). Subfraction E.6 (310 mg) was rechromatographed on a silica gel column eluted with actone-CH_2Cl_2 (1%-5%) to yield five fractions (E.6.1-E.6.5), then fraction E.6.3 (106 mg) was subjected to another silica gel column eluting with acetone-CH_2Cl_2 (0.5%-2.0%) to give compound **9** (60 mg).

2.4.2 *X. moluccesis* seed kernels

The EtOAc crude extract (98.0 g) of *X. moluccensis* seed kernels (15 kg) was obtained by the same manner with that of *X. granatum* in section 2.4.1 The extract was chromatographed on a silica gel column eluted with a gradient of acetone-hexane (10%-50%) to yield 12 pooled fractions (A-L). Each fraction was analyzed by TLC and ^{1}H NMR.

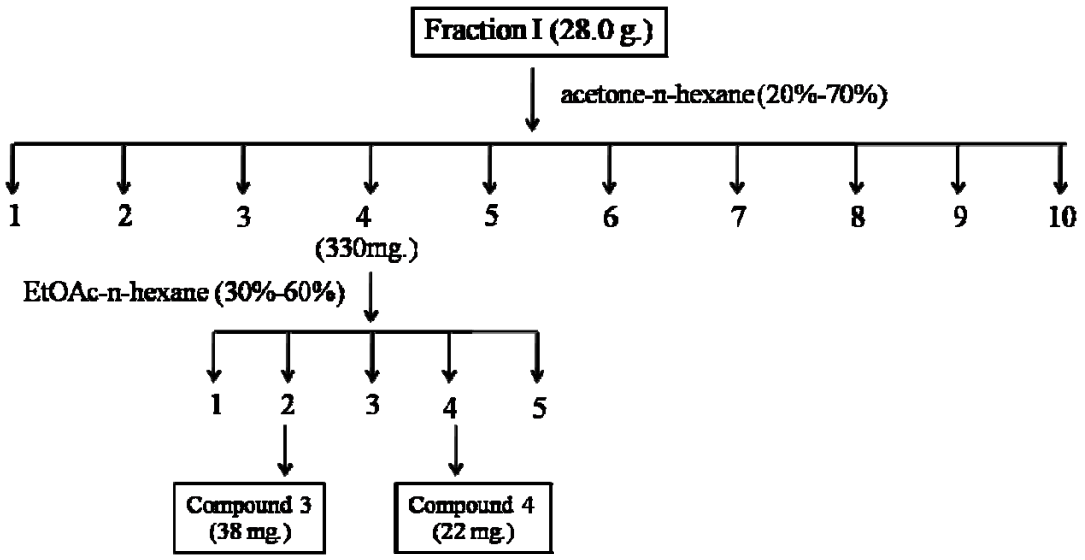
Fraction G (6.0 g) was subjected to CC over silica gel eluting with MeOH-CH_2Cl_2 (1%-5%) to give 12 subfractions (G.1-G.12). Subfraction G.2 (165 mg) was rechromatograhped on a silica gel column eluted with EtOAc-hexane (20%-60%) to obtain compound **3** (28 mg) and additional nine fractions (G.2.1-G.2.9). Fraction G.2.7 was further separated on a silica gel column with acetone-CH_2Cl_2 (0.5%-2.0%) to afford compound **7** (13 mg), whereas isolation of fraction G.2.9 with MeOH-CH_2Cl_2 (5%-10%) gave compound **6** (22 mg). The isolation procedure is summarized in Scheme 2.4.



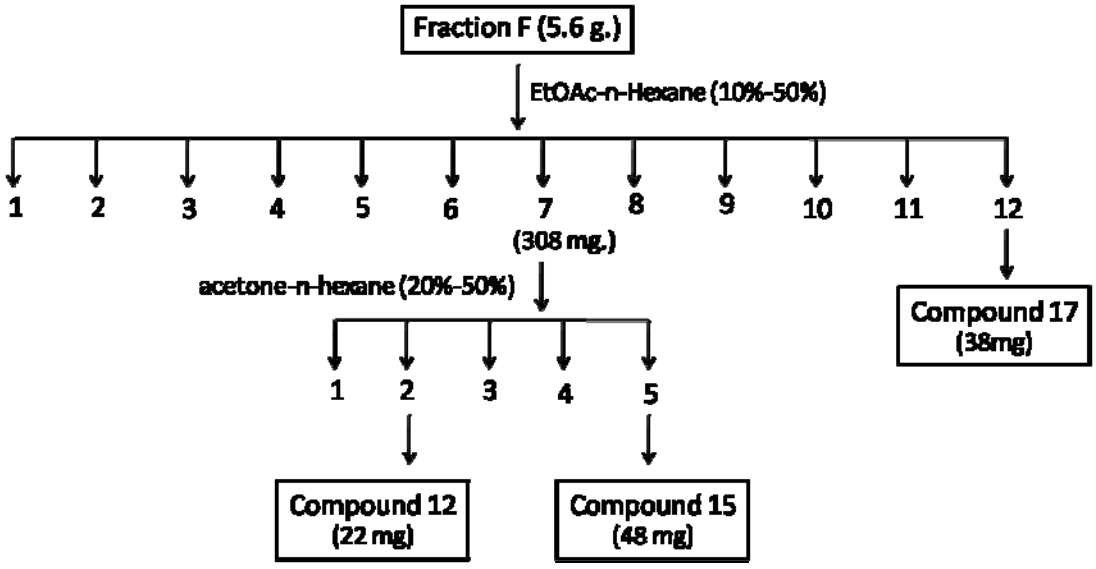
Scheme 2.4 Isolation of fraction G of *X. moluccensis* crude extract

Fraction I (8 g) was chromatographed on a silica gel column using acetone-hexane (30%-70%) to give 11 fractions (I.1-I.11). Fraction I.4 (168 mg) was separated by silica gel CC with EtOAc-hexane (30%-60%) to yield compound **12** (68 mg) and **15** (33 mg). Fraction I.7 (308 mg) was subjected to silica gel CC eluting with EtOAc-hexane (40%-60%) to afford compound **13**. Furthermore, fractionation of fraction F

(5.6 g) over a silica gel column with EtOAc-hexane (10%-50%) led to the isolation of four fractions (F.1-F.14). Fraction F.7 (108 mg) was further purified by silica gel CC with acetone-hexane (20%-50%) to afford compound **14** (22 mg). The isolation procedures are summarized in Scheme 2.5 and 2.6, respectively.



Scheme 2.6 Isolation of fraction I of *X. moluccensis* crude extract



Scheme 2.7 Isolation of fraction F of *X. moluccensis* crude extract

2.5 Evaluation of biological activity

Isolated compounds were assessed for their anti-inflammatory effects and acute toxicity on macrophage cell lines RAW264.7. Their anti-inflammatory activity were evaluated by monitoring the inhibition of nitric oxide (NO) production in activated macrophages, and the toxicity test was performed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphynyltrazolium bromide) colorimetric method as follows.

2.5.1 Nitric oxide inhibitory assay

The macrophage cell lines RAW 264.7 (ATCC TIB-71) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, penicillin G (100 units/mL) and streptomycin (100 µg/mL). The cells were seeded in 96-well plates with 1×10^4 cells/well and allowed to adhere for 24 h at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Further, the cells was pretreated with various doses of compounds or vehicle control (DMSO) for 1 h, then lipopolysaccharide from *Escherichia coli* (100 ng/mL) and recombinant interferon γ (10 ng/mL) were added to stimulate macrophages. Cells were incubated for additional 24 h under the same condition, and the culture supernatant (50 µL) of each well was collected. The amount of nitric oxide (NO) in the form of nitrite was measured by the Griess reaction. Those collected supernatants were added 50 µL of 1% sulfanilamide (per well), incubated under the dark condition at room temperature for 10 min. After that 50 µL of 0.1% N-1-napthylethylenediamine dihydrochloride (NED) were added and incubated under the dark condition for further 10 min, and the absorbance was measured with a microplate reader at 540 nm. Nitrite levels in the samples were calculated from the standard curve made from known concentrations of sodium nitrite.

2.5.2 Toxicity assay

To determine the cell viability of the active compounds, an MTT assay was carried out. After 50 µL of culture supernatant of each well was taken out for NO inhibition assay, MTT solution (10 µL, 5 mg/mL) were added into each well and the cells were further incubated for another 4 h under the same condition. The medium was further removed and isopropanol in 0.4 N HCl (100 µL/well) was added to

dissolve formazan crystals, and then the absorbance was measured with a microplate reader at 540 nm. Cells treated with only DMSO were used as a positive control.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Isolated limonoids from *Xylocarpus grantum* Koenig. and *Xylocarpus moluccensis* Roem.

The EtOAc crude extract of the seed kernels of *X. granatum* collected in Surat-thani province was separated by chromatographic techniques to obtain ten limonoids including xyloccensin K (**1**), 6-acetoxycedrodorin (**2**), andirobin (**3**), methylangolensate (**4**), methyl-6-*β*-hydroxy angolensate (**5**), gedunin (**6**), 7-deacetylgedunin (**7**), kihadalactone A (**8**), toonaciliatavarin E (**9**), and protoxylocarpin G (**10**). In addition, the chromatographic fractionation of the EtOAc crude extract of *X. moluccensis* seed kernels collected in the same province led to the isolation of a new mexicanolide, namely thaimoluccensin D (**13**). Limonoids **3**-**4** and **6**-**7** were also obtained from *X. moluccensis*, together with three additional known limonoids, protoxylocarpin H (**11**), moluccensin I (**12**), 2-hydrofissinolide (**14**) and hispidone (**15**). The structures of all isolated limonoids are presented in Figure 3.1.

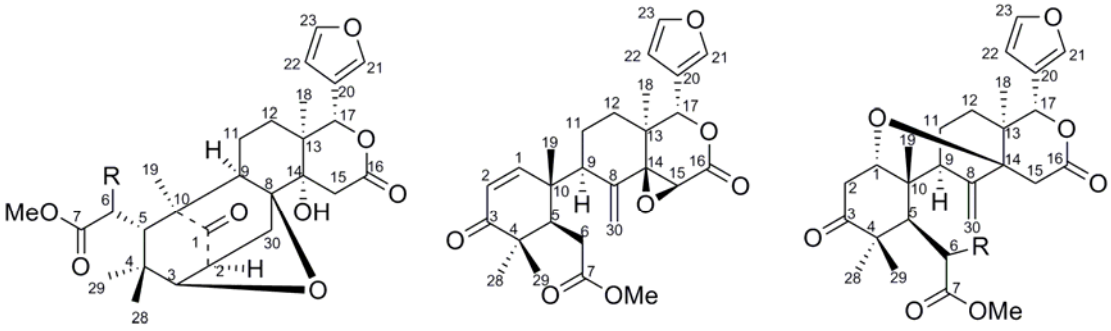
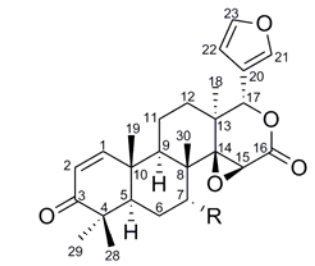
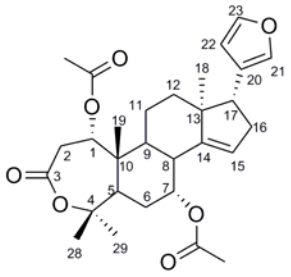


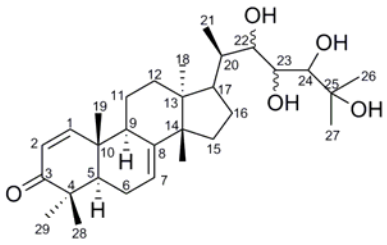
Figure 3.1 Structures of isolated limonoids from *X. granagtum* and *X. moluccensis*.



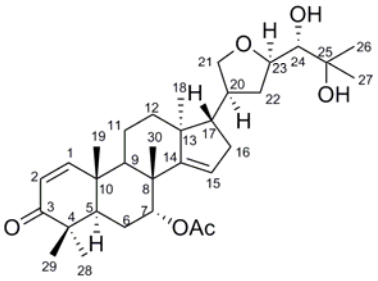
6: R = OAc
7: R = OH



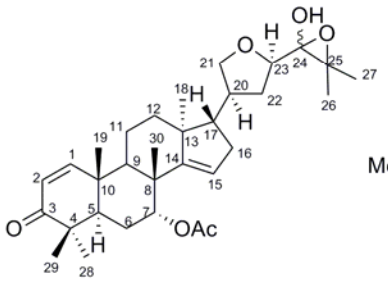
8



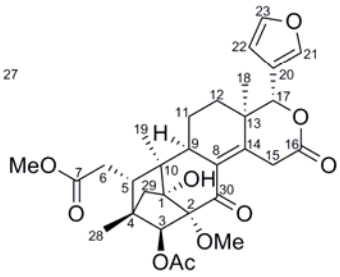
9



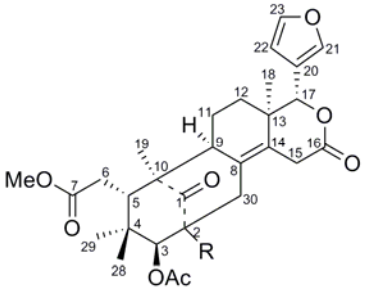
10



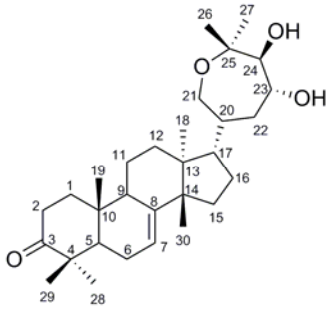
11



12



13: R = H
14: R = OH



15

3.1.1 Structure elucidation of compound 1

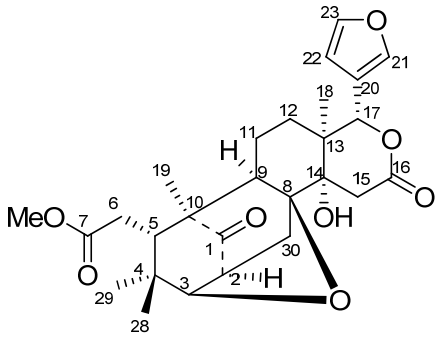


Figure 3.2 Compound **1**

Molecular formula	$C_{27}H_{34}O_8$
Appearance	White amorphous solid
IR (KBr)	3531, 3445, 3469, 2968, 2959, 2358, 2329, 1747, 1733, 1466, 1375, 1366, 1237, 1170, 1094, 1056, 1027 and 1022 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.1

Compound **1** was isolated as a white amorphous solid and its molecular formula $C_{27}H_{34}O_8$ was assigned by 1D NMR and HSQC data. The ^1H NMR spectrum of **1** (Table 3.1) displayed typical signals for a *β*-substituted furanyl ring (δ_H 6.49, 7.45 and 7.57), four tertiary methyls (δ_H 0.66, 0.94, 1.09 and 0.99), one methoxy group (δ_H 3.69) and two oxymethines (δ_H 6.28 and 4.22). The ^{13}C NMR spectrum revealed the presence of three carbonyl carbons (δ_C 170.3. 175.0 and 215.1), four olefinic carbons (δ_C 110.3, 121.0, 141.3 and 143.3) and five methyl carbons (δ_C 16.4, 17.2, 20.4, 28.4 and 52.2). The remaining carbons were assigned to five methylenes, five methines, and five quaternary carbons, based on the results of HSQC experiment. The data from decouplings and the subsequent 2D NMR studies (HMBC and HSQC) suggested that **1** was a mexicanolide-type limonoid. Proton of a methine group at δ_H 4.22 exhibiting HMBC correlations to C-8, C-5 and C-29, was identified as H-3 (Figure 3.3). In addition, HMBC cross peaks from H-3 to a keto carbonyl at δ_C 215.1 and C-8 clarified the existence of a keto group at C-1 and an *O*-bridge between C-3 and C-8. An oxygenated quaternary carbon at δ_C 74.8 was assigned as C-14 due to its HMBC

correlations with H_2-15 and H-17. Based on the information of the above NMR data, compound **1** was identified as xyloccensin K. Finally, comparison of ^{1}H and ^{13}C NMR data with those reported in the literature confirmed **1** was xyloccensin K as shown in Table 4.1 (Kokpol *et al*. 1996).

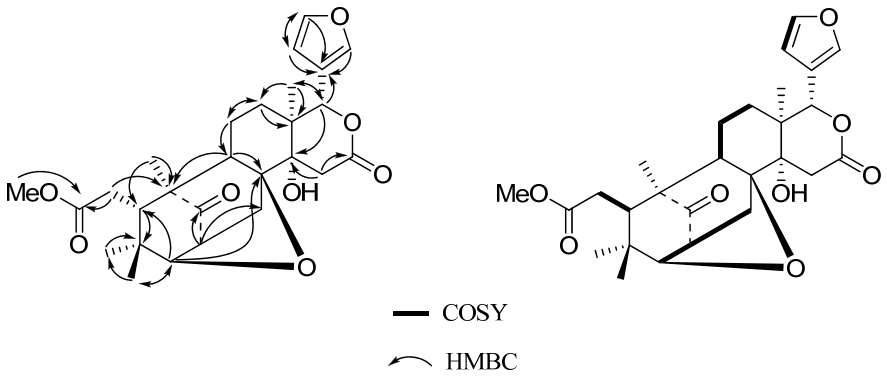


Figure 3.3 Key HMBC and COSY correlations of compound **1**

Table 3.1. NMR data ($CDCl_3$) of xyloccensin K and compound **1**

Position	Xyloccensin K		Compound **1**	
	^1H	^{13}C	^1H	^{13}C
1		215.2		215.1
2	2.96 (dd, *J* = 6.0 Hz, 1H)	48.9	2.97 (t, *J* = 6.0 Hz, 1H)	49.3
3	4.22 (d, *J* = 6.0 Hz, 1H)	91.3	4.22 (d, *J* = 5.6 Hz, 1H)	91.7
4		37.5		37.3
5	3.08 (dd, *J* = 2.0, 11.0, 1H)	42.9	3.07 (m, 1H)	43.3
6	2.14 (dd, *J* = 2.0, 17.0 Hz, 1H) 2.24 (dd, *J* = 11.0, 17.0 Hz, 1H)	32.6	2.11 (m, 1H) 2.23 (m, 1H)	32.9
7		174.2		175.0
8		85.4		85.8
9	1.97 (dd, *J* = 5.0, 12.5 Hz, 1H)	52.0	1.95 (dd, *J* = 12.6, 4.0 Hz, 1H)	52.4
10		51.0		51.5
11	1.46 (m, 1H) 2.10 (m, 1H)	17.8	1.47 (m, 1H) 2.11 (m, 1H)	18.0
12	1.50 (ddd, *J* = 1.5, 14.0 Hz, 1H) 1.70 (ddd, *J* = 1.5, 14.0 Hz, 1H)	28.6	1.53 (m, 1H) 1.69 (m, 1H)	29.1
13		40.0		40.4
14		74.1		74.8
15	2.54 (d, *J* = 17.0 Hz. 1H) 3.13 (d, *J* = 17.0 Hz, 1H)	36.8	2.52 (m, 1H) 3.15 (d, *J* = 17.7 Hz, 1H)	37.4
16		170.7		170.3
17	6.28 (br s, 1H)	76.7	6.28 (s, 1H)	76.8
18	0.67 (s, 3H)	16.0	0.66 (s, 3H)	16.4
19	0.94 (s, 3H)	16.8	0.94 (s, 3H)	17.2
20		120.6		121.0
21	7.45 (dd, *J* = 2.0 Hz, 1H)	142.9	7.45 (br s, 1H)	141.3
22	6.49 (br d, *J* =2.0 Hz, 1H)	109.9	6.49 (s, 1H)	110.3
23	7.56 (br s, 1H)	140.7	7.55 (d, *J* = 0.5 Hz, 1H)	143.3
28	1.03 (s, 3H)	20.0	1.09 (s, 3H)	20.4
29	0.98 (s, 3H)	27.9	0.99 (s, 3H)	28.4
30	2.04 (d, *J* = 12 Hz, 1H) 2.52 (dd, *J* = 7.0,12.0 Hz, 1H)	42.4	2.05 (m, 1H)	42.8
7-*OMe*	3.70 (s, 3H)	51.8	3.69 (s, 3H)	52.2

3.1.2 Structure elucidation of compound 2

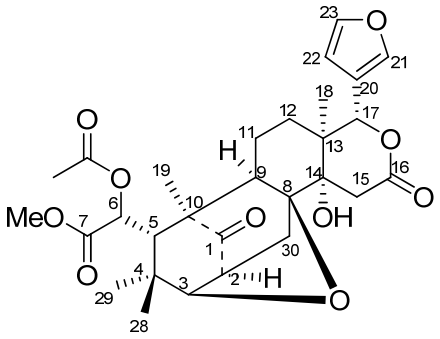


Figure 3.4 Compound **2**

Molecular formula	$C_{29}H_{36}O_9$
Appearance	White amorphous solid
IR (KBr)	3427, 2939, 2360, 2320, 1722, 1627, 1476, 1367, 1238, 1161, 1055, 1026, 873, 797, 600
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.2

Compound **2**, obtained as a white amorphous solid, had the molecular formula of $C_{29}H_{36}O_9$, as established by 1D NMR and HSQC data. The NMR data of **2** (Table 3.2) also displayed characteristic signals associated with a mexicanolide limonoid, including a *β*-furanyl ring [δ_H 6.45 s, 7.47 s, 7.49 s; δ_C 109.9 CH, 120.7 qC, 140.7 CH, 143.2 CH], four tertiary methyls [δ_H 0.99 s, 1.00, s, 1.04 s, 1.05 s; δ_C 16.2, 18.1, 21.3, 29.7] and an acetoxy group [δ_H 2.12 s; δ_C 20.6, 169.5]. Moreover, the NMR data of **2** were similar to those of **1**, with the only difference being the presence of an acetoxy group at C-6 in **2** instead of a hydrogen in **1**. This was confirmed by HMBC correlation between H-6 at δ_H 5.09 and carbonyl carbon of acetyl group at 169.5 as shown in Figure 4.5. Therefore, the structure of **2** was elucidated as 6-acetoxycedrodorin which has been reported in the literature (Veitch *et al*. 1999). Comparison of ^{13}C NMR data between both compounds (Table 4.2) also clarified that compound **2** was 6-acetoxycedrodorin.

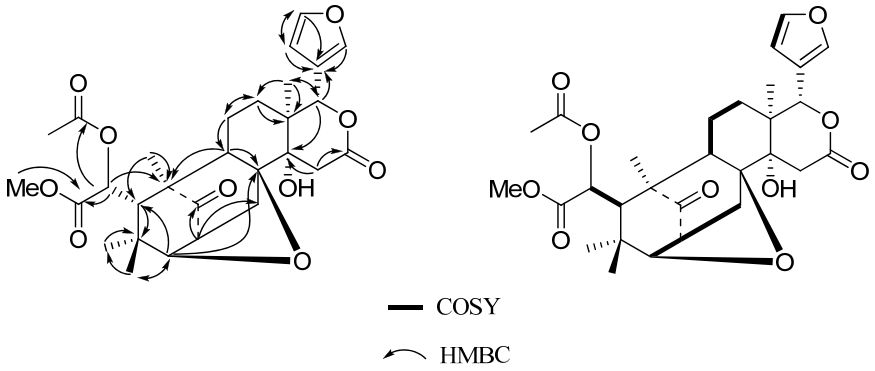


Figure 3.5 Key HMBC and COSY correlations of compound **2**

Table 3.2. NMR data ($CDCl_3$) of 6-acetoxycedrodorin and compound **2**

Position	6-acetoxycedrodorin	Compound **2**	
	^{13}C	^{1}H	^{13}C
1	212.7		212.9
2	48.3	2.97 (s, 1H)	48.9
3	92.0	4.05 (d, *J* = 5.5 Hz, 1H)	92.9
4	37.6		38.2
5	46.4	3.02 (s, 1H)	47.0
6	71.6	5.09 (s, 1H)	72.0
7	169.9		170.1
8	85.3		85.0
9	52.2	1.95 (dd, *J* = 12.8, 3.9 Hz, 1H)	53.0
10	50.1		50.7
11	17.6	1.55 (d, *J* = 11.7 Hz,1H) 1.91 (d, *J* = 12.0 Hz,1H)	18.2
12	28.5	1.54 (d, *J* = 11.7 Hz,1H) 1.73 (d, *J* = 11.7 Hz,1H)	28.9
13	39.7		40.4
14	73.0		74.8
15	36.1	2.51 (m, 1H) 3.15 (d, *J* = 17.8 Hz, 1H)	37.4
16	169.3		170.0
17	75.3	6.21 (s, 1H)	76.3
18	16.1	1.00 (s, 3H)	16.2
19	17.5	1.04 (s, 3H)	18.1
20	121.1		120.7
21	140.5	7.49 (s, 1H)	140.7
22	110.0	6.45 (s, 1H)	109.9
23	143.5	7.47 (s, 1H)	143.2
28	20.7	0.99 (s, 3H)	21.3
29	29.1	1.05 (s, 3H)	29.7
30	42.6	2.07 (m, 1H) 2.56 (m, 1H)	42.8
7-OMe	52.2	3.78 (s, 3H)	52.4
6-OAc	20.2	2.12 (s, 3H)	20.6
	169.2		169.5

3.1.3 Structure elucidation of compound 3

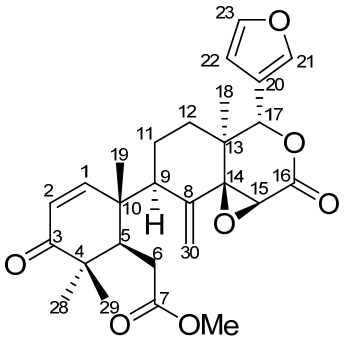


Figure 3.6 Compound **3**

Molecular formula	$C_{27}H_{32}O_7$
Appearance	Colorless prisms
IR (KBr)	3446, 2943, 2349, 2323, 1730, 1715, 1660, 1463, 1285, 1263, 1216, 1176, 1015, 848, 811, 709, 604
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.3

Compound **3** was isolated as colorless prisms. Its molecular formula of $C_{27}H_{32}O_7$ was deduced by 1D and 2D NMR data. The ^1H NMR data (Table 3.3) displayed four tertiary methyls (δ_H 0.86, 0.89, 1.00 and 1.03), two oxymethines (δ_H 3.90 and 5.40), and a β-substituted furan ring (δ_H 6.26, 7.38 and 7.40). The ^{13}C NMR and HSQC data indicated the presence of five methyls, four methylenes, nine methines, and six quaternary carbons, of which three at δ_C 167.0, 166.7 and 203.6 were assigned to two ester and a ketone carbonyl carbon, respectively. The aforementioned data indicated that **3** was an andirobin-type limonoid. The ^{13}C NMR signals of C-1 (δ_C 153.4), C-2 (δ_C 125.7), and C-3 (203.6), and the ^1H NMR signals of a pair of AB doublet at δ_H 6.00 and 7.08 suggested that the A-ring of **3** possessed a 1-en-3-one system. The location of $\Delta^{8,30}$ double bond was confirmed by HMBC correlations (Figure 3.7) from methylene protons at δ_H 5.19 and 5.30 to C-8 and C-14. The cross peaks between H-15/C-14, H-15/C-16, H-17/C-13 and H-17/C-14 in HMBC spectrum suggested the presence of a δ-lactone group with a 14,15-epoxide in the D-ring. From the above observations, compound **3** was determined as andirobin (Oills *et*

al, 1964). Also, it was confirmed by comparing its ^{1}H NMR data with those previously reported as shown in Table 3.3.

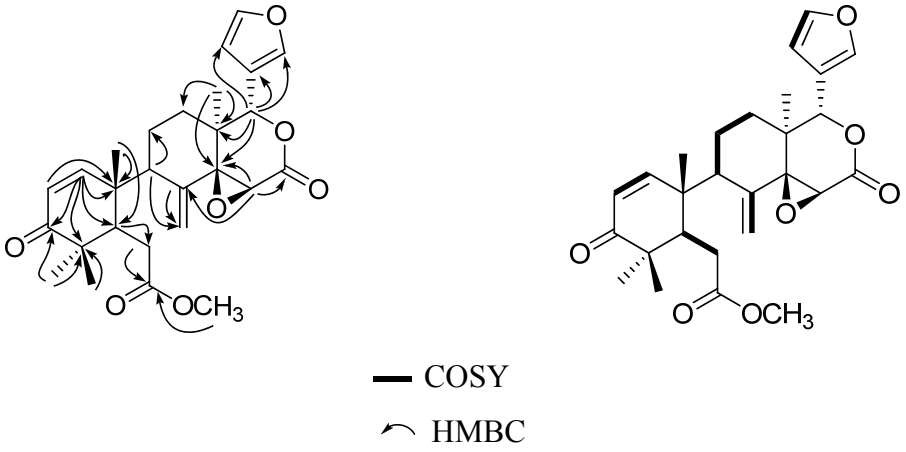


Figure 3.7 Key HMBC and COSY correlations of compound **3**

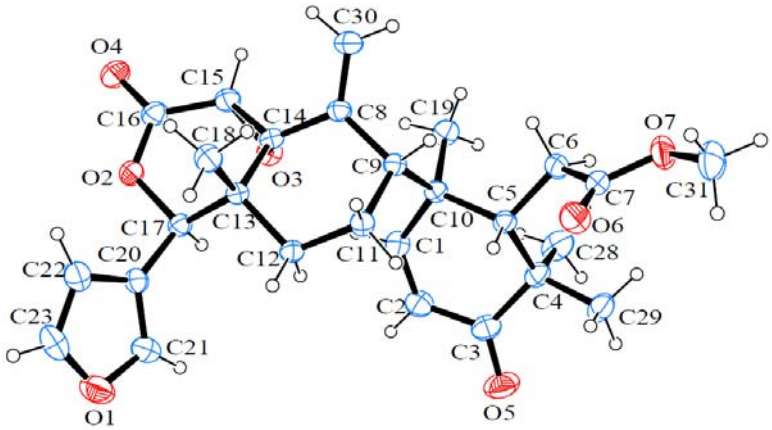


Figure 3.8 ORTEP diagram of compound **3**.

Table 3.3. NMR data ($CDCl_3$) of andirobin of compound **3**

Position	Andirobin	Compound **3**	
	^1H	^1H	^{13}C
1	7.46 (s, 1H)	7.08 (s,1H)	153.4
2	6.38 (s. 1H)	6.00 (s, 1H)	125.7
3			203.6
4			46.4
5	2.80 (m,1H)	2.62 (m, 1H)	42.8
6	2.42 (m, 1H)	2.32 (m, 1H)	33.2
	2.56 (m, 1H)	2.40 (m, 1H)	
7			170.1
8			138
9	2.25 (m, 1H)	2.18 (m, 1H)	48.7.0
10			43.7
11	1.82 (m,1H)	1.71 (m, 1H)	21.2
	2.02 (m, 1H)	1.92 (m, 1H)	
12	1.73 (m, 1H)	1.61 (m, 1H)	29.5
	1.89 (m, 1H)	1.77 (m, 1H)	
13			38.5
14			67.8
15	4.08 (m, 1H)	3.93 (m, 1H)	55.4
16			166.7
17	5.53 (s,1H)	5.40 (s, 1H)	77.4
18	0.95 (s, 3H)	0.86 (s, 3H)	14.5
19	0.99 (s, 3H)	0.89 (s, 3H)	20.2
20			119.7
21	7.52 (s, 1H)	7.47 (s, 1H)	140.9
22	6.38 (s, 1H)	6.26 (s, 1H)	109.0
23	7.56 (s, 1H)	7.38 (s, 1H)	143.0
28	1.12 (s, 1H)	1.00 (s, 3H)	22.7
29	1.12 (s, 1H)	1.03 (s, 3H)	22.5
30	5.33 (s, 1H)	5.19 (s, 1H)	122.3
	5.45 (s, 1H)	5.30 (s, 1H)	
6-OAc	3.75 (s, 3H)	3.70 (s, 3H)	52.4
			169.4

Table 3.4 Crystaldata and structure refinement for compound 3

Formula	$C_{27}H_{32}O_7$
Molecular weight	468.53
Crystal size (mm)	0.48× 0.40 × 0.36
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
***a* (Å)**	8.8125 (5) Å
***b* (Å)**	12.5907 (7) Å
***c* (Å)**	21.9393 (11) Å
***V* (Å^3)**	2434.3 (2) Å^3
Z	4
D_{calc} (g/cm^3)	1.278 Mg/m^3
μ (mm^{-1})	0.09 mm^{-1}
F(000)	1000
Independent reflections/ Observed reflection [I>4σ (I)], R_{int}	3132/13719/0.020
R_1	0.041
***w*R_2[I>2σ(I)]**	0.1423

3.1.4 Structure elucidation of compound 4

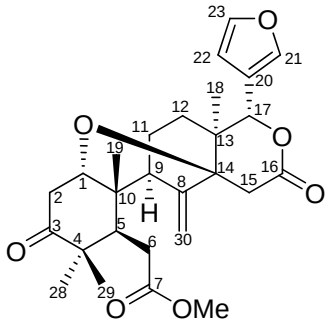


Figure 3.9 Compound **4**

Molecular formula	$C_{27}H_{34}O_7$
Appearance	White amorphous solid
IR (KBr)	3438, 3114, 2972, 1742, 1738, 1460, 1394, 1274, 1026, 902, 873, 821,731, 604
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.5

Compound **4** was isolated as white amorphous solid, with molecular formula $C_{29}H_{32}O_{10}$. Analysis of 1D and 2D NMR data of **4** (Table 3.5) revealed the presence of a *β*-substituted furanyl ring [δ_H 6.37 (d, J = 1.0 Hz), 7.36 (t, J = 1.6 Hz), 7.42 s; δ_C 120.8 qC, 140.7 CH, 109.9 CH, 142.7 CH], a methoxy [δ_H 3.70 s; δ_C 52.1 CH_3, 173.8 qC], a ketone carbonyl [δ_C 212.8 qC], an ester carbonyl [δ_C 170.1 qC], four methyls [δ_H 0.85 s, 0.93 s, 1.03 s, 1.18 s; δ_C 13.7, 21.6, 25.8, 21.6], two sp^2 methylenes [δ_H 4.88 s, 5.14 s; δ_C 111.5 CH_2, 145.6 qC], two oxygenated methines [δ_H 3.51 (dd, J = 6.1, 4.0 Hz), 5.65 s; δ_C 77.2, 79.5]. The NMR data of **4** was closely related to those of **3**, except for the absence of $\Delta^{1,2}$ double bond and the downfield shift of an oxygenated methine to δ_C 77.2. Key HMBC correlation from H-1 to C-14 allowed the assignment of the oxygen bridge between C-1 and C-14. Based on these findings and comparison of its NMR data with those reported in the literature (Table 3.2), it could be concluded that compound **4** was methyl angolensate and had the structure as shown (Connolly *et al*., 1976).

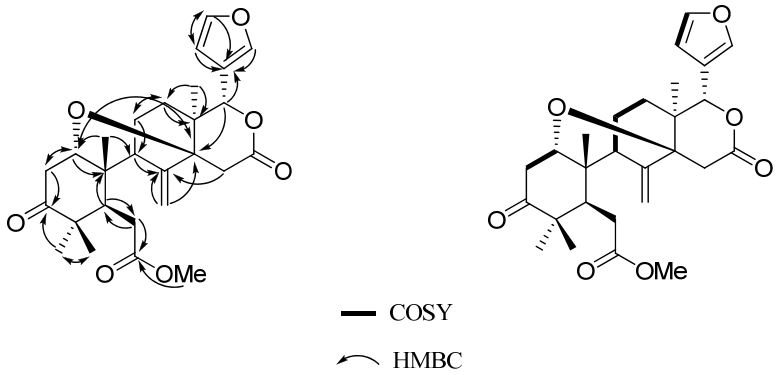


Figure 3.10 Key HMBC and COSY correlations of compound **4**

Table 3.5. NMR data ($CDCl_3$) of methyl angolensate and compound **4**

Position	Methyl angolensate		Compound **4**	
	^1H	^{13}C	^1H	^{13}C
1	3.52 (dd, *J* = 6.5,4.0 Hz, 1H)	77.2	3.51 (dd, *J* = 6.1, 4.0 Hz, 1H)	77.2
2	2.51 (dd, *J* = 14.5,4.0 Hz, 1H) 2.90 (dd, *J* = 14.5,6.0 Hz, 1H)	39.5	2.48 (dd, *J* = 14.3, 4.0 Hz, 1H) 2.91 (m, 1H)	39.3
3		212.6		212.8
4		48.0		48.0
5	2.88 (d, *J* = 10.5 Hz, 1H)	43.0	2.87 (m, 1H)	42.8
6	2.25 (d, *J* = 16.5 Hz, 1H) 2.61 (dd, *J* = 16.5,10.5 Hz, 1H)	33.6	2.26 (m, 1H) 2.60 (m, 1H)	33.7
7		173.8		173.8
8		145.9		145.6
9	2.17 (dd, *J* = 5.0,1.5 Hz, 1H)	50.0	2.15 (m, 1H)	49.8
10		44.1		43.9
11	1.57 (t, *J* = 14.5 Hz, 1H) 2.20 (m, 1H)	23.8	1.56 (m, 1H) 2.22 (m, 1H)	23.7
12	1.14 (ddd, *J* = 16.5 Hz, 1H) 2.61 (dd, *J* = 16.5,10.5 Hz, 1H)	29.3	1.12 (m, 1H) 2.61 (m, 1H)	29.3
13		41.5		41.4
14		80.2		80.2
15	2.91(d, *J* = 18.0 Hz, 1H) 2.58 (d, *J* = 18.0 Hz, 1H)	33.8	2.91 (m, 1H) 2.59 (m, 1H)	33.7
16		169.9.		170.1
17	5.67 (s, 1H)	79.6	5.65 (s, 1H)	79.5
18	0.84 (s, 3H)	13.8	0.85 (s, 3H)	13.7
19	0.95 (s, 3H)	21.7	0.93 (s, 3H)	21.6
20		120.9		120.8
21	7.44 (dd, *J* = 1.5, 0.8, 1H)	140.8	7.42 (s, 1H)	140.7
22	6.39 (dd, *J* = 1.5, 0.8 Hz, 1H)	109.9	6.37 (d, *J* = 1.0 Hz, 1H)	109.9
23	7.38 (t, *J* = 1.5 Hz, 1H)	142.7	7.36 (t, *J* = 1.6 Hz, 1H)	142.7
28	1.05 (s, 3H)	26.0	1.03 (s, 3H)	25.8
29	1.19 (s, 3H)	21.5	1.18 (s, 3H)	21.6
30	4.90 (s, 1H) 5.15 (s, 1H)	111.5	4.88 (s, 1H) 5.14 (s, 1H)	111.5
7-*OMe*	3.72 (s, 3H)	52.1	3.70 (s, 3H)	52.1

3.1.5 Structure elucidation of compound 5

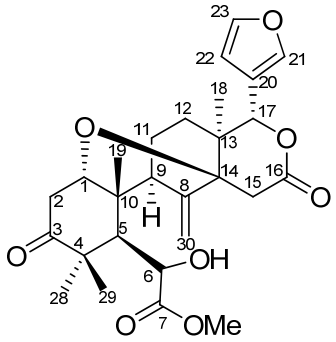


Figure 3.11 Compound **5**

Molecular formula	$C_{27}H_{34}O_8$
Appearance	Colorless gum
IR (KBr)	3446, 2940, 2848, 1735, 1631, 1463, 1369, 1238, 1023, 917, 877, 808, 760, 600
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.6

Compound **5**, colorless gum, had the molecular formula $C_{27}H_{34}O_8$. Analysis of the ^1H and ^{13}C NMR data of **5** (Table 3.6) indicated that **5** was also an andirobin type limonoid. Its NMR data were virtually identical to those of **4**, implying that both compounds had the same basic structure. The obvious difference was the appearance of an additional oxygenated methine (δ_C 72.4) in place of a methylene in **4**. This methine was assigned as C-6 due to ^1H-^1H COSY correlation of —CH(5)—CH(6)— fragment and HMBC correlation of H-5/C-6 as shown in Figure 4.11. Thus, compound **5** was determined as methyl-6-*β*-hydroxy angolensate. Also, it was further confirmed by comparing its ^{13}C NMR data with those previously reported as shown in Table 3.5(Connolly *et al*, 1967). Methyl-6-*β*-hydroxy angolensate was previously isolated from *Khaya grandifoliola* and *K. anthotheca* and this is the first report from *Xylocarpus* species.

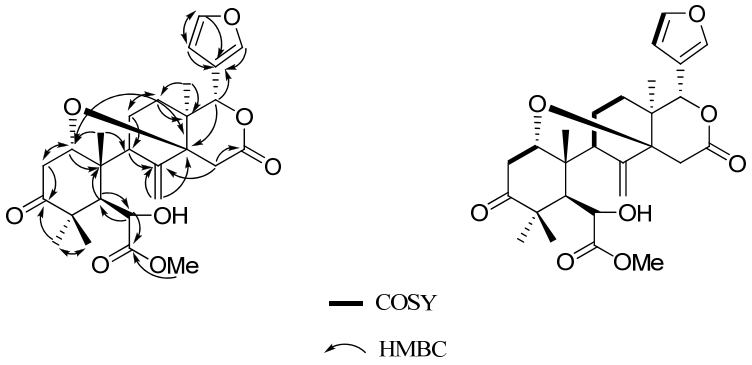


Figure 3.12 Key HMBC and COSY correlations of compound **5**

Table 3.6. NMR data ($CDCl_3$) of methyl-6-*β*-hydroxy angolensate and compound **5**

Position	methyl-6-*β*-hydroxy angolensate	Compound **5**	
	^{13}C	^{1}H	^{13}C
1	78.1	3.57 (dd, *J* = 5.56, 2.47 Hz, 1H)	78.6
2	39.2	2.33 (m, 1H)	39.4
		3.11 (m, 1H)	
3	211.2		212.3
4	48.8		49.1
5	46.5	2.73 (s, 1H)	47.9
6	72.4	4.41 (s, 1H)	72.7
7	170.9		170.9
8	146.3		145.8
9	51.2	2.32 (m, 1H)	50.8
10	44.5		45.0
11	24.0	1.56 (m, 1H)	24.4
		2.12 (m, 1H)	
12	29.3	1.61 (m, 1H)	29.0
		1.77 (m, 1H)	
13	41.4		41.7
14	80.6		80.7
15	33.7	2.57 (m, 1H)	34
		2.90 (m, 1H)	
16	169.9		170.2
17	79.5	5.63 (s, 1H)	79.7
18	13.8	0.88 (s, 3H)	14.0
19	22.7	1.39 (s, 3H)	23.7
20	120.7		121.1
21	140.8	7.43 (s, 1H)	141.1
22	109.9	6.36 (s, 1H)	110.3
23	142.6	7.38 (s, 1H)	143.1
28	24.8	1.05 (s, 3H)	24.1
29	23.8	1.46 (s, 3H)	25.1
30	111.7	4.91 (s, 1H)	111.9
		5.63 (s, 1H)	

3.1.6 Structure elucidation of compound 6

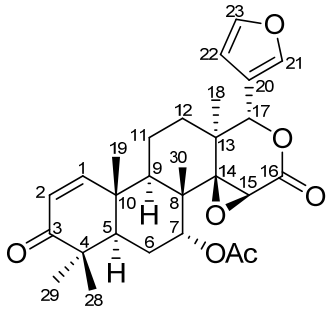


Figure 3.13 Compound **6**

Molecular formula	$C_{28}H_{34}O_7$
Appearance	White amorphous solid
IR (KBr)	3431, 2917, 2363, 1737,1688, 1436, 1387, 1230, 1026, 873, 600
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.7

Compoud **6** was isolated as colorless prisms. Its molecular formula was determined to be $C_{28}H_{34}O_7$ by 1D NMR and HSQC data. The ^1H NMR spectrum of **6** (Table 3.7) showed the presence of six tertiary methyls (δ_H 0.99, 1.00, 1.08, 1.15, 1.18 and 2.10), a β-substituted furan ring (δ_H 6.23, 7.35 and 7.40, 1H each) and three oxymethine protons (δ_H 3.46, 4.48 and 5.55). The ^{13}C NMR and HSQC data indicated the presence of six methyls, three methylenes, ten methines, and six quaternary carbons, of which three at δ_C 166.0, 169.0 and 203.6 were assigned to an acetyl, and ester, and a ketone carbonyl carbon, respectively. The ^{13}C NMR signals of C-1 (δ_C 157.8), C-2 (δ_C 125.7), and C-3 (δ_C 204.6), together with the ^1H NMR signals of a pair of AB doublet at δ_H 5.84 and 7.10, indicated the existence of a 1-en-3-one system in the A-ring of **6**. The above spectral data suggested **6** was a gedunin limonoid. The presence of an epoxide ring between C-14 and C-15 was confirmed by HMBC correlations of H-15/C-14, Me-18/C-14, and H-17/C-14. An oxygenated methine carbon at δ_C 73.0 was assigned as C-7 due to its HMBC correlations with H-6, Me-30 and H-5. Observed HMBC correlation between H-7 at δ_H 4.48 and carbonyl carbon of acetyl group at δ_C 169.0 gave evidence of an acetoxy group being attached at C-7. On

the basis of these spectral data, as well as data comparison of **6** with those in the literature (Table 3.6), compound **6** was identified as gedunin (Taylor, 1974).

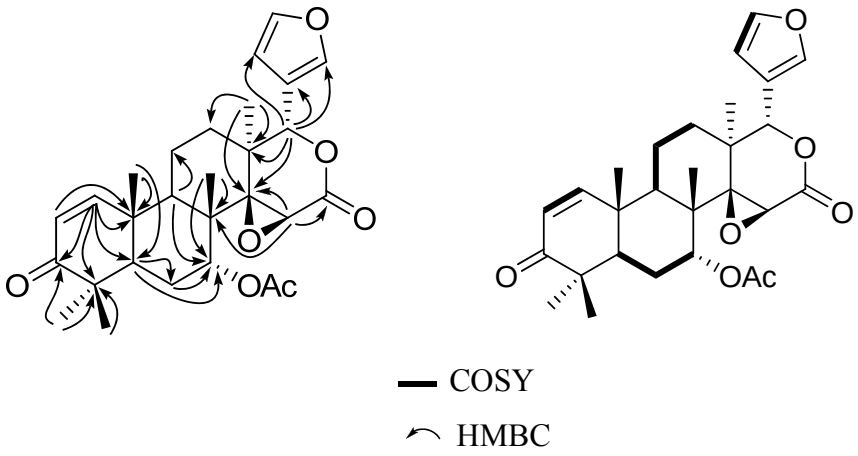


Figure 3.14 Key HMBC and COSY correlations of compound **6**

Table 3.7. NMR data ($CDCl_3$) of gedunin and compound **6**

Position	Gedunin		Compound **6**	
	^1H	^{13}C	^1H	^{13}C
1	7.07 (d, *J* = 10.0 Hz, 1H)	157.0	7.02 (d, *J* = 9.8 Hz, 1H)	156.8
2	5.81 (d, 1H)	125.9	5.78	126.2
3		203.8		203.6
4		44.0		44.0
5		39.5	2.45 (m, 1H)	39.5
6		14.9	1.80 (m, 1H)	14.9
			1.95 (m, 1H)	
7	4.05 (m, 1H)	73.2	4.48 (m,1H)	73.0
8		42.6		42.6
9		46.0	2.10 (m, 1H)	46.0
10		40.0		40.0
11		17.7	1.47 (m, 1H)	17.7
			2.11 (m, 1H)	
12		18.3	1.53 (m, 1H)	18.3
			1.69 (m, 1H)	
13		38.7		38.7
14		69.8		69.0
15	3.50 (s, 1H)	56.9	3.46 (m, 1H)	56.0
16		167.4		166.0
17	5.57 (s, 1H)	78.2	5.55 (s, 1H)	78.0
18		19.7	1.15 (s, 3H)	19.7
19		21.7	1.00 (s, 3H)	21.1
20		120.5		120.0
21		143.0	7.35 (br s, 1H)	143.3
22		109.8	6.23 (s, 1H)	109.8
23		141.4	7.55 (bs s, 1H)	141.0
28		21.0	2.04 (s, 3H)	21.0
29		27.2	0.99 (s, 3H)	27.1
30		26.0	1.49 (m, 1H)	26.0
7-*OMe*	2.07 (s, 3H)	23.3	1.85 (s, 3H)	23.2
		169.8		169.0

3.1.7 Structure elucidation of compound 7

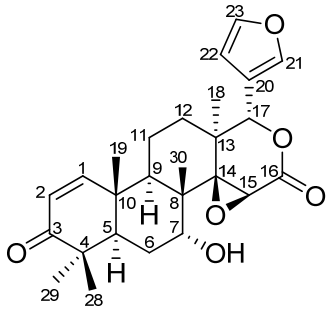


Figure 3.15 Compound **7**

Molecular formula	$C_{26}H_{32}O_6$
Appearance	White amorphous solid
IR (KBr)	3530, 3486, 3121, 2956, 2865, 1734, 1656, 1465, 1391, 1260, 1169, 1021 and 921 cm^{-1}
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.8

Compound **7** was isolated as white amorphous solid. Its molecular formula was determined to be $C_{26}H_{32}O_6$. It was revealed that the ^1H and ^{13}C NMR data (Table 3.8) also displayed characteristic signals associated with a gedunin skeleton, including a *β*-substituted furan ring, A-ring with a 1-en-3-one system, an epoxide ring, and five tertiary methyls. The NMR data of **7** was very similar to those of **6**, with the only difference being the absence of the acetyl resonances in **6**, thus indicating that **7** was a 7-deacetyl anologue of **6**. This conclusion was also confirmed by ^1H-^1H COSY correlation of –CH(5)—CH_2(6)—CH(7)— fragment and HMBC correlations of H-7/C-6, H-5/C-7 and H-7/C-30 as shown in Figure 3.15. Therefore, the structure of **7** was elucidated as 7-deacetylgedunin, previously reported by Payla *et al* in 2006. In addition, comparison of ^1H NMR data of 7-deacetylgedunin and compound **7** is shown in Table 3.7.

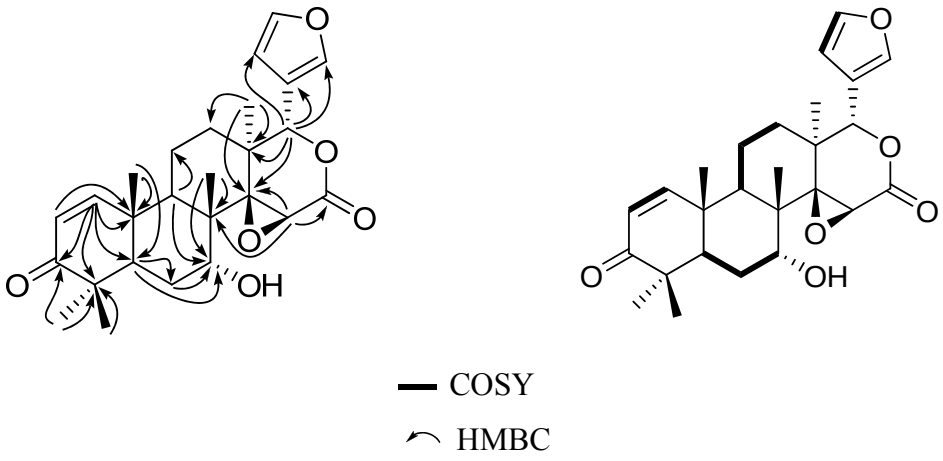


Figure 3.16 Key HMBC and COSY correlations of compound **7**

Table 3.8. NMR data ($CDCl_3$) of 7-deacetylgedunin and compound **7**

Position	7-deacetylgedunin	Compound **7**	
	^1H	^1H	^{13}C
1	7.11 (d, *J*= 10.2 Hz, 1H)	7.10 (d, *J*= 10.4 Hz, 1H)	157.8
2	5.85 (d, *J*= 10.2 Hz, 1H)	5.84 (d, *J*= 10.4 Hz, 1H)	125.7
3			204.6
4			44.2
5	2.49 (dd, *J*= 13.4, 2.4 Hz, 1H)	2.49 (m, 1H)	44.6
6	1.92 (m, 1H) 1.83 (m, 1H)	1.89	27.3
7	1.83 (m, 1H)	1.69 (m, 1H)	69.7
8	3.58 (s, 1H)	3.57 (s, 1H)	43.7
9			38.0
10	2.58 (m, 1H)	2.52 (m, 1H)	40.7
11	2.00 (m, 1H) 1.81 (m, 1H)		15.0
12	2.00 (m, 1H) 1.81 (m, 1H)	1.95 (m, 1H) 1.80(m, 1H)	26.4
13			38.3
14			70.0
15	3.91 (s, 1H)	3.90 (s, 1H)	57.8
16			168.3
17	5.60 (s, 1H)	5.60 (s, 1H)	78.5
18	1.24 (s, 3H)	1.23 (s, 3H)	17.8
19	1.20 (s, 3H)	1.19 (s, 3H)	19.9
20			120.6
21	7.41 (m, 1H)	7.41 (s, 1H)	141.2
22	6.35 (m, 1H)	6.35 (s, 1H)	110.0
23	7.41 (m, 1H)	7.40 (s, 1H)	143.0
28	1.09 (s, 3H)	1.14 (s, 3H)	27.2
29	1.10 (s, 3H)	1.09 (s, 3H)	21.5
30	1.15 (s, 3H)	1.00 (s, 3H)	18.7

3.1.8 Structure elucidation of compound 8

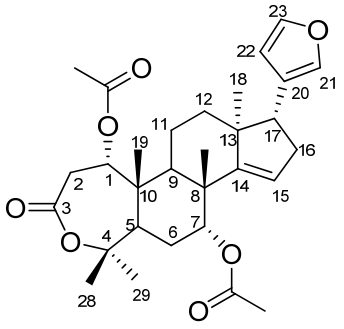


Figure 3.17 Compound **8**

Molecular formula	$C_{30}H_{40}O_7$
Appearance	Light yellow gum
IR (KBr)	3446, 2943, 2863, 2366, 2247, 1742, 1635, 1380, 1252, 1117, 1026, 880, 731, 600
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.9

Compound **8**, a light yellow gum, had the molecular formula $C_{30}H_{40}O_7$. The ^1H NMR spectrum (Table 3.9) displayed resonances of a β-substituted furan ring (δ_H 6.25, 7.22 and 7.37), two acetyls (δ_H 2.00 and 2.05), two oxymethine protons (δ_H 4.82 and 5.19) and five tertiary methyls (δ_H 0.74, 1.16, 1.20, 1.40 and 1.50). In the ^{13}C NMR spectrum 30 nonequivalent carbon resonances were observed, including three carbonyl carbons (δ_C 169.7, 170.0 and 170.3), six olefinic carbons (δ_C 110.9, 119.0, 124.4, 139.6, 142.5 and 158.6), and seven methyl carbons (δ_C 15.1, 19.7, 20.6, 21.0, 23.5, 27.1 and 34.3). The remaining carbons were assigned to five methylenes, nine methines, and six quaternary carbons, based on HSQC data. Five of the seven oxygens were accounted for the furan and two acetoxyl groups, the sixth and seventh oxygens must thus be present as a lactone ring in view of the presence of a signal at δ_C 169.7 in the ^{13}C NMR spectrum. This confirmed by HMBC correlations from H-1, H-2 and H-5 to this carbonyl carbon (C-3) as shown in Figure 4.17. Observed HMBC correlations from H-1 (δ_H 4.82) and H-7 (δ_H 5.19) to the carbonyl carbons of the acetoxy groups at δ_C 170.3 and 170.0 clarified their location at C-1 and C-7, respectively. The existence

of $\Delta^{14,15}$ double bond was confirmed by HMBC correlations of H-16/C-14, H-17/C-15 and H-15/C-13. Based on the information of NMR data and literature data comparison (Table 3.8), compound **8** was thus determined as kihadalactone A (Yoshikawa *et al*, 1992) and its structure is shown in Figure 3.16. Kihadalactone A was previously isolated from the fresh fruits of *Phellodendron amurense*. To the best of our knowledge, this is the first report of kihadalactone A from plant in *Xylocarpus* species.

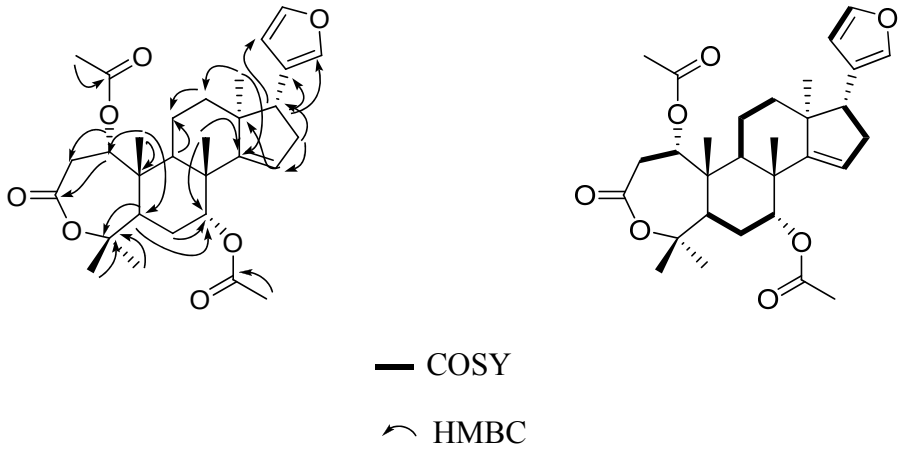


Figure 3.18 Key HMBC and COSY correlations of compound **8**

Table 3.9 NMR data ($CDCl_3$) of kihadalactone A and compound **8**

Position	Kihadalactone A		Compound **8**	
	^1H	^{13}C	^1H	^{13}C
1	4.82 (d, *J* = 7.0 Hz)	71.0	4.82 (d, *J* = 7.0 Hz)	70.9
2	3.12 (d, *J* = 15.0 Hz, 1H) 3.17 (dd, *J* = 15.0, 7.0 Hz, 1H)	34.9	1.55 (m, 1H) 1.82 (m, 1H)	34.8
3		170.2		169.7
4		85.5		85.0
5	2.52 (m)	44.1	2.50 (dd, *J* = 12.50, 2.85 Hz,1H)	44.0
6	1.95 (m)	26.4	1.94 (m, 1H)	26.3
7	5.19 (br, s)	74.3	5.19 (s, 1H)	74.2
8		41.8		41.8
9	2.56 (m)	36.0	2.54 (m, 1H)	35.9
10		44.3		44.2
11	1.42 (m) 1.57 (m)	16.3	1.44 (m, 1H) 1.55 (m, 1H)	16.2
12	1.57 (m)	32.9	1.55 (m, 1H) 1.82 (m, 1H)	32.8
13		47.1		47.0
14		158.1		158.6
15	5.36 (br, s)	119.1	5.35 (s, 1H)	119.0
16	2.35 (m) 2.45 (m)	34.4	2.34 (m, 1H) 2.42 (m, 1H)	34.2
17	2.79 (m)	51.3	2.78 (m, 1H)	51.2
18	0.75 (s)	19.8	0.74 (s, 1H)	19.7
19	1.17 (s)	15.2	1.16 (s, 1H)	15.10
20		124.5		124.4
21	7.23 (br, s)	139.6	7.22 (s, 1H)	139.6
22	6.26 (br, s)	111.0	6.25 (s, 1H)	110.9
23	7.37 (br, s)	142.6	7.37 (s, 1H)	142.5
28	1.51 (s)	23.6	1.50 (s, 3H)	23.5
29	1.40 (s)	34.4	1.40 (s, 3H)	34.3
30	1.21 (s)	27.2	1.20 (s, 3H)	27.1
1-OAc	2.01 (s)	20.7 170.0	2.05 (s, 3H)	20.6 170.3
7-OAc	2.11 (s)	21.1 169.7	2.00 (s, 3H)	21.0 170.0

3.1.9 Structure elucidation of compound 9

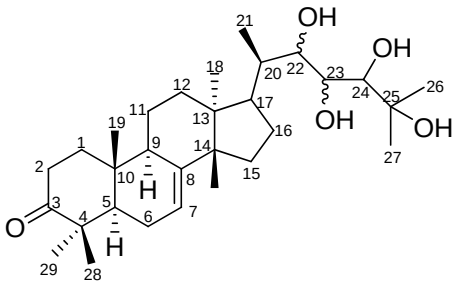


Figure 3.19 Compound **9**

Molecular formula	$C_{30}H_{48}O_4$
Appearance	White amorphous solid
IR (KBr)	3448, 2957, 1689, 1635, 1456, 1380, 1121, 966
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.10

Compound **9** was isolated as white amorphous solid. The molecular formula of **9** was established to be $C_{30}H_{48}O_4$. The ^1H NMR spectrum (Table 3.10) of **9** showed signals of eight methyls [δ_H 0.81, 0.84, 1.00, 1.04 (Me×2), 1.11 and 1.21 (Me×2)] and an olefinic proton (δ_H 5.30). A combined analysis of ^{13}C NMR (Table 3.9) and HSQC spectra revealed 30 nonequivalent carbon resonances due to one carbonyl (δ_C 217.3), two olefinics (δ_C 117.8 and 145.9), four oxymethine carbons (δ_C 72.3, 72.8, 80.9 and 83.7), eight methyls (δ_C 12.4, 12.8, 21.3, 21.7, 21.8, 24.5, 27.6 and 27.7), together with seven methylenes, eight methines, and six quaternary carbons. These data suggested that the structure of **9** was a tirucallane-type triterpene. The location of $\Delta^{7,8}$ double bond was confirmed by HMBC correlations of H-7/C-8, H-7/C-14, H-9/C-7 and H-5/C-7. The ketone carbon at δ_C 217.3 was assigned as C-3 due to its HMBC correlations with H_2-1, H_2-2, Me-28 and Me-29. Analysis of ^1H-^1H COSY data allowed the establishment of —CH_2(15)—CH_2(16)—CH(17)—MeCH(20)—CH(22)—CH(23)—CH(24)— fragment, which was connected to C-13 and C-14 by HMBC correlations of Me-18/C-17 and Me-30/C-15 (Figure 3.19). Observed HMBC

cross peaks from Me-26 and Me-27 to an oxymehtine carbon at δ_C 80.9 led to the connectivity of *gem*-dimethyl to C-25. On the basis of these spectral evidence and data comparison of **9** with those in the literature (Table 3.10), compound **9** was determined as toonaciliatavarin E previously isolated from *Toona ciliata*. To the best of our knowledge, this is the first report of compound **9** from the plant belonging to *Xylocarpus* species.

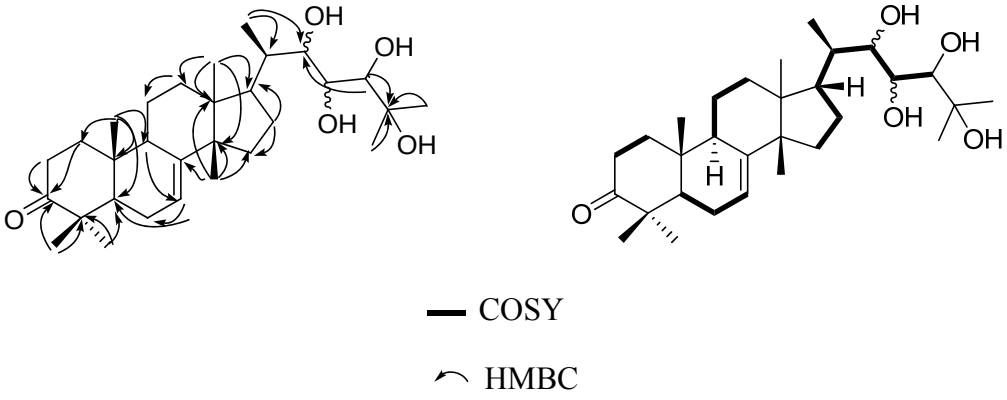


Figure 3.20 Key HMBC and COSY correlations of compound **9**

Table 3.10. NMR data ($CDCl_3$) of toonaciliatavarin E and compound **9**

Position	Toonaciliatavarin E		Compound **9**	
	^1H	^{13}C	^1H	^{13}C
1	1.47 (td, *J* = 14.0, 4.0 Hz,1H)	39.7	1.45 (m, 1H)	38.6
	2.05		1.98 (m, 1H)	
2	2.19 (td, *J* = 14.5, 2.5 Hz,1H)	35.8	2.23 (m, 1H)	34.9
	2.84 (dt, *J*= 14.5, 2.6 Hz, 1H)		2.76 (m, 1H)	
3		219.2		217.3
4		49.7		47.9
5	1.74 (dd, *J*= 11.0, 6.0 Hz, 1H)	53.8	1.71 (d, *J* = 8.64 Hz,1H)	52.4
6	2.13 (m, 1H)	25.4	2.08 (m, 2H)	24.3
7	5.34 (s, 1H)	119.0	5.30 (s, 1H)	117.8
8		147.3		145.9
9	2.35 (m, 1H)	49.9	2.28 (m, 1H)	48.5
10		36.1		35.0
11	1.62	19.2	1.53 (m, 1H)	18.3
	1.65		1.53 (m, 1H)	
12	1.68 (dd, *J*= 15.0, 9.0 Hz, 1H)	35.1	1.61 (m, 1H)	33.5
	1.87 (m, 1H)		1.86 (m, 1H)	
13		44.6		43.5
14		52.5		51.2
15	1.55 (dd, *J*= 12.0, 7.0 Hz, 1H)	35.2	1.50 (d, *J* = 7.90 Hz,2H)	34.0
	1.64			
16	1.37 (m, 1H)	28.4	1.33 (m, 1H)	27.6
	2.04		2.01 (m, 1H)	
17	1.95 (dd, *J*= 10.5, 8.2 Hz, 1H)	49.7	1.85 (m, 1H)	49.3
18	0.88 (s, 3H)	22.5	0.81 (s, 3H)	21.8
19	1.05 (s, 3H)	13.2	1.00 (s, 3H)	12.8
20	1.91 (t, *J*= 6.0 Hz 3H)	37.6	1.64 (m, 1H)	37.5
21	0.91 (s, 3H)	12.3	0.84 (s, 3H)	12.4
22	3.73 (d, *J* = 8.5 Hz, 1H)	77.0	3.82 (d, *J* = 6.17 Hz, 1H)	83.7
23	3.61 (d, *J* = 8.5, 7.5 Hz,1H)	71.9	3.97 (t, *J*= 6.02, 6.02 Hz,1H)	72.8
24	3.42 (d, *J* = 7.5 Hz, 1H)	80.9	3.65 (d, *J* = 5.98 Hz, 1H)	77.3
25		74.6		80.9
26	1.26 (s, 3H)	24.6	1.21 (s, 3H)	21.3
27	1.24 (s, 3H)	27.6	1.11 (s, 3H)	21.7
28	1.03 (s, 3H)	25.1	0.81 (s, 3H)	21.8
29	1.13 (s, 3H)	22.0	1.04 (s, 3H)	24.5
30	1.09 (s, 3H)	28.1	1.04 (s, 3H)	27.6

3.1.10 Structure elucidation of compound 10

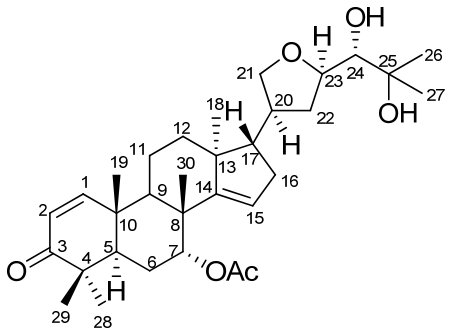


Figure 3.21 Compound **10**

Molecular formula	$C_{32}H_{48}O_6$
Appearance	colorless gum
IR (KBr)	3435, 2954, 2917, 1638, 1387, 1267, 1095, 791, 709, 593, 469
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 3.11

Compound **10** was isolated as a colorless gum, and its molecular formula was $C_{32}H_{48}O_6$. The ^{1}H NMR spectrum (Tabel 3.11) showed signal of an *α,β*-unsaturated ketone moiety indicated by a pair of dublets at δ_H 7.10 and 5.97, seven tertiary methyls [δ_H 0.90, 1.14, 1.30, 1.25 (Me×2), 1.05 (Me×2), and 1.18], an olefinic proton (δ_H 5.30), an acetoxy group (δ_H 1.92). Analysis of ^{13}C NMR and HSQC data revealed the presence of 32 noneququivalent carbon resonances indicating as one carbonyl (δ_c 204.8), four olefinic carbons (δ_c 159.0, 158.5, 125.5 and 119.6), the acetyl carbon (δ_c 170.2 and δ_C 21.2) and seven methyl carbons (δ_c 27.3, 27.0, 28.6, 23.8, 21.3, 20.4 and 19.1), together with six methylenes and seven methines. These data suggested that the structure of **10** possessed a protolimonoid skeleton. The A-ring of **10** possesses a 1-en-3-one system confirmed by HMBC correlations of H-1/C-2, H-2/C-3 and Me-28/C-3, together with by ^{1}H-^{1}H COSY correlation of –CH(1)—CH(2)— fragment shown in Figure 3.23. The presence of $\Delta^{14,15}$ double bond was indicated by the HMBC correlations from Me-18/C14, Me-30/C-14 and H-15/C-8. An oxygenated methine carbon at δ_C 86.5 was assigned as C-24 due to its ^{1}H-^{1}H COSY correlations between H-23 and H-24, whereas another at δ_C 74.3 was identified as C-25 confirmed by

HMBC correlations Me-26/ C25 and Me-27/C25. Further, the HMBC cross-peak from H-7 (δ_H 5.20) to acetyl carbon (δ_C 170.2) indicated that the above acetoxyl group was attached to C-7. This compound was determined to be protoxylocarpin G, which has been reported by our group in 2009 (Pudhom *et al*., 2009).

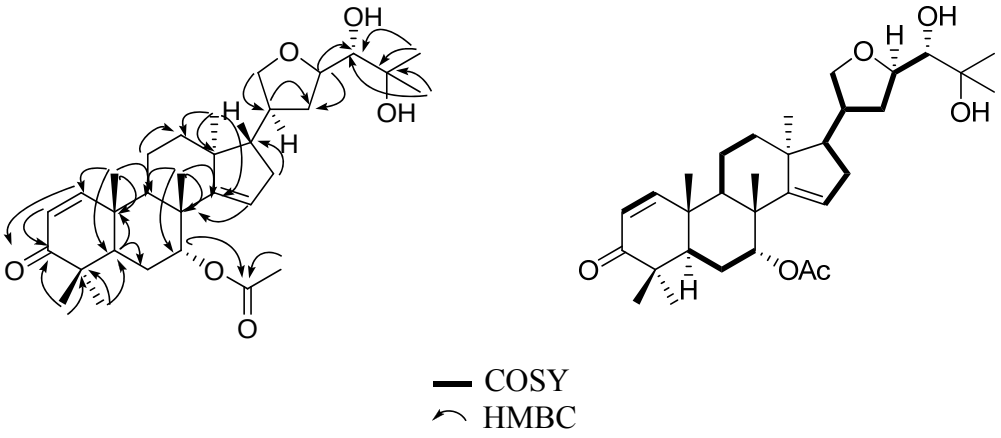


Figure 3.22 Key HMBC and COSY correlations of compound **10**

Table 3.11. NMR data ($CDCl_3$) of protoxylocarpin G and compound **10**

Position	Protoxylocarpin G		Compound **10**	
	^1H	^{13}C	^1H	^{13}C
1	7.14 (d, *J*= 10.4 Hz)	158.5	7.14 (d, *J*= 10.4 Hz)	158.5
2	5.80 (d, *J*= 10.4 Hz)	125.5	5.80 (d, *J*= 10.4 Hz)	125.5
3		204.8		204.8
4		44.2		44.2
5	2.16 (m, 1H)	46.4	2.16 (m, 1H)	46.4
6	1.80 (m, 1H)	24.0	1.80 (m, 1H)	24.0
	1.90 (m, 1H)		1.90 (m, 1H)	
7	5.20 (br, s)	74.8	5.20 (br, s)	74.8
8		42.8		42.8
9	2.2 (m, 1H)	38.7	2.2 (m, 1H)	38.7
10		39.7		39.7
11	1.55 (m, 1H)	16.8	1.55 (m, 1H)	16.8
	2.00 (m, 1H)		2.00 (m, 1H)	
12	2.26 (m, 1H)	34.9	2.26 (m, 1H)	34.9
13		46.2		46.2
14		159.0		159.0
15	5.30 (br, d *J* = 2.4 Hz, 1H)	119.6	5.30 (br, d *J* = 2.4 Hz, 1H)	119.6
16	2.26 (m, 1H)	35.0	2.26 (m, 1H)	35.0
17	2.00 (m, 1H)	52.3	2.00 (m, 1H)	52.3
18	0.90 (s, 1H)	20.4	0.90 (s, 1H)	20.4
19	1.14 (s, 1H)	19.1	1.14 (s, 1H)	19.1
20	1.88 (m, 1H)	35.8	1.88 (m, 1H)	35.8
21	3.42 (dd, *J* = 2.0, 12.0 Hz, 1H)	70.0	3.42 (dd, *J* = 2.0, 12.0 Hz, 1H)	70.0
	3.98 (br, d, *J* = 11.4 Hz, 1H)		3.98 (br, d, *J* = 11.4 Hz, 1H)	
22	1.52 (m, 1H)	36.2	1.52 (m, 1H)	36.2
	2.04 (m, 1H)		2.04 (m, 1H)	
23	3.86 (ddd, *J* = 2.8, 8.8, 13.2, 1H)	64.4	3.86 (ddd, *J* = 2.8, 8.8, 13.2, 1H)	64.4
24	2.98 (d, *J* = 8.8)	86.5	2.98 (d, *J* = 8.8)	86.5
25		74.3		74.3
26	1.30 (s, 1H)	28.6	1.30 (s, 1H)	28.6
27	1.25 (s, 1H)	23.8	1.25 (s, 1H)	23.8
28	1.05 (s, 1H)	21.3	1.05 (s, 1H)	21.3
29	1.05 (s, 1H)	27.0	1.05 (s, 1H)	27.0
30	1.18 (s, 1H)	27.3	1.18 (s, 1H)	27.3
7-OAc	1.92 (s, 1H)	21.2	1.92 (s, 1H)	21.2
		170.2		170.2

3.1.11 Structure elucidation of compound 11

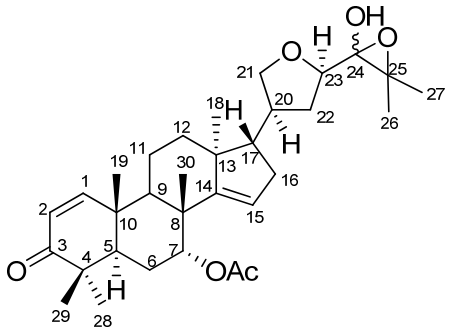


Figure 3.23 Compound **11**

Molecular formula	$C_{32}H_{46}O_6$
Appearance	colorless gum
IR (KBr)	3431, 2928, 1740, 1649, 1467, 1383, 1258, 1099, 1023, 921, 797, 727, 604
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 3.12

Compound **11** was isolated as a colorless gum and had the molecular formula $C_{32}H_{46}O_6$, which indicated an additional double-bond equivalent relative to **11**. Similar to compound **10**, the ^{1}H and ^{13}C NMR data of **11** also displayed characteristic signals of protolimonoid consisting of seven tertiary methyls [δ_H 1.04 s, 1.13 s, 1.39 s, 1.25 s, 1.04 (Me×2) s, 1.13 s; δ_C 20.4 CH_3, 19.0 CH_3, 24.3 CH_3, 23.1 CH_3, 21.2 CH_3, 27.0 CH_3, 27.3 CH_3], an A-ring 1-en-3-one moiety [δ_H 5.83, 7.14 (each, d, J = 10.4 Hz), δ_C 125.4, 158.5, 204.8], one double bond (δ_H 5.25 s; δ_C 119.2 CH, 158.7 qC), and an acetyl group (δ_H 1.92 s; δ_C 21.1 CH_3; 170.2). The NMR data of **11** was similar to those of **10**, except for the presence of the hemiacetal quaternary carbon (δ_C 95.5) instead of the oxygenated methine carbon in **11** (δ_C 86.5) in the side chain. An epoxide ring was put between C-24 (δ_C 95.5) and C-25 (δ_C 76.3) due to the HMBC correlations of H-23-C-24, Me-26/C-24 and Me-27/C-24 in Figure 4.23. This compound has also been reported by our group in 2009 (Pudhom *et al*., 2009).

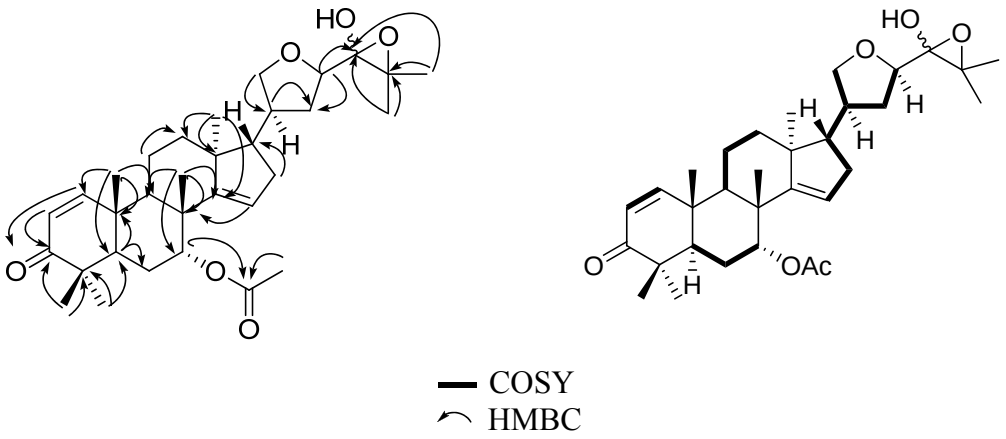


Figure 3.24 Key HMBC and COSY correlations of compound **11**

Table 3.12. NMR data ($CDCl_3$) of protoxylocarpin H and compound **11**

Position	Protoxylocarpin H		Compound **11**	
	^1H	^{13}C	^1H	^{13}C
1	7.14 (d, *J*= 10.4 Hz)	158.5	7.14 (d, *J*= 10.4 Hz)	158.5
2	5.83 (d, *J*= 10.4 Hz)	125.4	5.83 (d, *J*= 10.4 Hz)	125.4
3		204.8		204.8
4		44.1		44.1
5	2.14 (m, 1H)	46.1	2.14 (m, 1H)	46.1
6	1.76 (m, 2H)	23.8	1.76 (m, 2H)	23.8
7	5.19 (br, s)	74.6	5.19 (br, s)	74.6
8		42.6		42.6
9	2.16 (m, 1H)	38.4	2.16 (m, 1H)	38.4
10		39.8		39.8
11	1.49 (m, 1H)	16.7	1.49 (m, 1H)	16.7
	1.73 (m, 1H)		1.73 (m, 1H)	
12	1.92 (m, 1H)	33.9	1.92 (m, 1H)	33.9
	2.16 (m, 1H)		2.16 (m, 1H)	
13		46.5		46.5
14		158.7		158.7
15	5.25 (br, s)	119.2	5.25 (br, s)	119.2
16	2.14 (m, 1H)	29.8	2.14 (m, 1H)	29.8
17	1.40 (m, 1H)	57.1	1.40 (m, 1H)	57.1
18	1.04 (s, 1H)	20.4	1.04 (s, 1H)	20.4
19	1.13 (s, 1H)	19.0	1.13 (s, 1H)	19.0
20	1.76 (m, 1H)	34.5	1.76 (m, 1H)	34.5
21	3.60 (m, 1H)	65.3	3.60 (m, 1H)	65.3
	3.79 (m, 1H)		3.79 (m, 1H)	
22	1.69 (m, 1H)	32.8	1.69 (m, 1H)	32.8
23	3.85 (m, 1H)	67.5	3.85 (m, 1H)	67.5
24		95.5		95.5
25		76.3		76.3
26	1.39 (s, 1H)	24.3	1.39 (s, 1H)	24.3
27	1.25 (s, 1H)	23.1	1.25 (s, 1H)	23.1
28	1.04 (s, 1H)	21.2	1.04 (s, 1H)	21.2
29	1.04 (s, 1H)	27.0	1.04 (s, 1H)	27.0
30	1.13 (s, 1H)	27.3	1.13 (s, 1H)	27.3
7-OAc	1.92 (s, 1H)	21.1	1.92 (s, 1H)	21.1
		170.2		170.2

3.1.12 Structure elucidation of compound 12

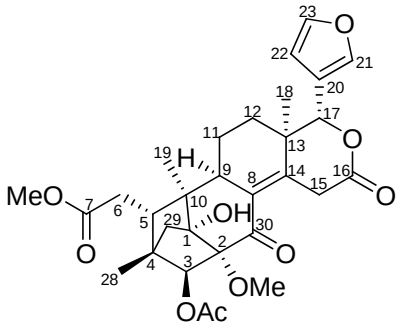


Figure 3.24 Compound **12**

Molecular formula	$C_{30}H_{36}O_{10}$
Appearance	Light yellow gum
IR (KBr)	3449, 2939, 2254, 1735, 1620, 1460, 1369, 1230, 1161, 1110, 1023, 913, 877, 804, 738, 604
^{1}H and ^{13}C NMR ($CDCl_3$)	See Table 3.13

Compound **12** was isolated as a light yellow gum, with molecular formula $C_{30}H_{36}O_{10}$. The ^{1}H NMR spectrum (Table 3.13) displayed resonances of a β-substituted furanyl ring (δ_H 6.42, 7.41, and 7.46), three tertiary methyl (δ_H 0.98, 1.02 and 1.05), an two methoxy groups (δ_H 3.43 and 3.66), and one acetoxyl group (δ_H 2.17). In the ^{13}C NMR spectrum, 30 nonequivalent carbon resonances were observed, including four carbonyl carbons (δ_C 169.8, 170.1, 172.9 and 203.4), six olefinic carbons (δ_C 110.1, 120.5, 133.9, 139.3, 141.2, and 142.9), and five methyl carbons (δ_C 15.1, 17.1, 19.7, 20.6, 51.7 and 55.2). The remaining carbons were assigned to five methylenes, four methines, and five quaternary carbons, based on the results of an HSQC experiment. The data from decouplings and the subsequent 2D NMR studies (HMBC and HSQC) suggested that **12** was a phragmalin limonoid. Two protons at δ_H 1.75 and 2.22, coupled to the carbon resonance at δ_C 43.8 in the HSQC spectrum, were indicative of the H_2-29, a characteristic 4,29,1-ring bridge of phragmalin limonoids. This was confirmed by the HMBC correlations (Figure 4.25) observed from H_2-29 to

methine carbon at δ_C 39.6 (C-5) and to quaternary carbons at δ_C 84.7 (C-1) , 40.2 (C-4) and 55.4 (C-10). The HMBC correlations between C-7 (δ_C 172.9) and H_2-6 (δ_H 2.29 and 2.45) and the methoxy proton at δ_H 3.66 also confirmed the typical C-6–C-7 appendage of phragmalin. A proton singlet at δ_H 5.19 was assignable to H-17 by correlation with the furanyl carbon at δ_C 120.5 (C-20) and the C-18 methyl carbon at δ_C 17.1. A δ-lactone ring was corroborated by the HMBC cross-peaks from H-17 to both bridgehead carbons, C-13 and C-14. Further the presence of $\Delta^{8,14}$ double bond was determined by the HMBC correlations of Me-18/C-14, H_2-15/C-8 and H_2-11/C-8. The ketone carbonyl was positioned at C-30 due to its HMBC correlation with H-3, as well as the methoxy group (δ_H 3.43 and δ_C 55.2) was attached to C-2 because it showed HMBC cross peak to C-2. Compound **12** thus determined to be moluccensin I, which has been reported by our group in 2010 (Pudhom *et al.,* 2010).

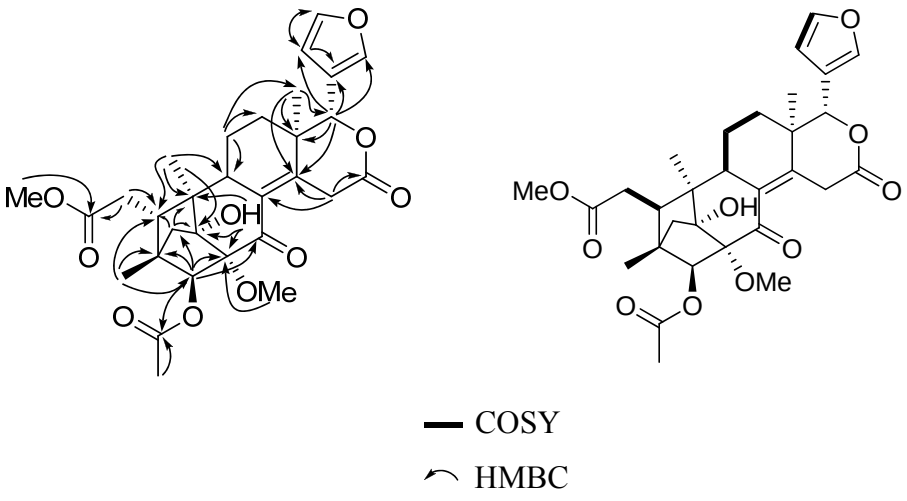


Figure 3.25 Key HMBC and COSY correlations of compound **12**

Table 3.13. NMR data ($CDCl_3$) of moluccensin I and compound **12**

Position	Moluccensin I		Compound **12**	
	^1H	^{13}C	^1H	^{13}C
1		84.7		84.7
2		92.2		92.2
3	4.92 (s, 1H)	82.8	4.94 (s, 1H)	82.8
4		40.1		40.2
5	2.37 (d, *J*= 10.0 Hz, 1H)	39.6	2.39 (d, *J*= 8.4 Hz, 1H)	39.6
6	2.25 (d, *J*= 12.0 Hz, 1H)	34.2	2.29 (d, *J*= 14.8 Hz, 1H)	34.3
	2.43 (m, 1H)		2.45 (m, 1H)	
7		172.9		172.9
8		133.9		133.9
9	2.46 (m, 1H)	46.9	2.47 (m, 1H)	46.9
10		55.4		55.4
11	1.43 (m, 1H)	18.7	1.44 (m, 2H)	18.8
	1.74 (m, 1H)			
12	1.41 (m, 1H)	31.4	1.41(m, 1H)	31.5
	1.49 (m, 1H)		1.53 (m, 1H)	
13		40.8		40.8
14		139.2		139.3
15	3.75 (m, 2H)	33.0	3.77 (m, 2H)	33.0
16		169.9		169.8
17	5.17 (s, 1H)	80.2	5.19 (s, 1H)	80.2
18	1.00 (s, 3H)	17.1	1.02 (s, 3H)	17.1
19	1.03 (s, 3H)	15.1	1.05 (s, 3H)	15.1
20		120.4		120.5
21	7.45 (s, 1H)	141.2	7.46 (s, 1H)	141.2
22	6.40 (s, 1H)	110.0	6.42 (s, 1H)	110.1
23	7.40 (s, 1H)	143.0	7.41 (s, 1H)	142.9
28	0.96 (s, 3H)	19.7	0.98 (s, 3H)	19.7
29	1.72 (m, 1H)	43.7	1.75 (m, 1H)	43.8
	2.20 (d, *J*= 13.2 Hz, 1H)		2.22 (d, *J*= 13.2 Hz, 1H)	
30		203.5		203.4
1′		170.1		170.1
2′	2.15 (s, 3H)	20.5	2.17 (s, 3H)	20.6
1-OH	2.93 (brs,1H)		2.90 (brs, 1H)	
2-OMe	3.40 (s, 3H)	55.1	3.43 (s, 3H)	55.2
7-OMe	3.65 (s, 3H)	51.8	3.66 (s, 3H)	51.7

3.1.13 Structure elucidation of compound 13

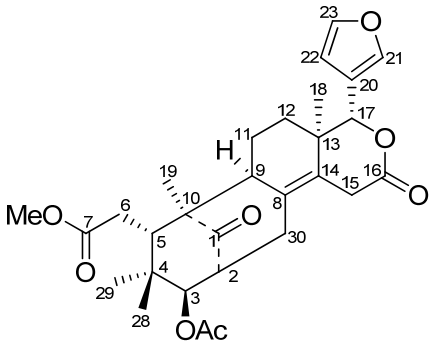


Figure 3.26 Compound **13**

Molecular formula	$C_{29}H_{36}O_8$
Appearance	Colorless prisms
IR (KBr)	3449, 2954, 2848, 1740, 1504, 1372, 1238, 1187, 1019, 877, 808, 757, 731, 600
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.14

Compound **13** was isolated as a white colorless prisms, with molecular formula $C_{29}H_{36}O_8$. The ^1H NMR spectrum (Table 3.14) displayed typical signals for a ketone carbonyl (δ_C 217.9), an ester carbonyl (δ_C 169.8), a β-substituted furanyl ring (δ_H 6.47 s, 7.40 s, 7.54 s; δ_C 109.9 CH, 120.6 qC, 141.7 CH, 142.8 CH), a methoxycarbonyl group (δ_H 3.70 s; δ_C 52.0 CH_3; 174.2 qC), an acetyl group (δ_H 2.16; δ_C 21.2 CH_3; 170.3 qC), and four tertiary methyls (δ_H 0.70 s, 0.80 s, 1.01 s, 1.14 s; δ_C 16.7 CH_3, 17.9 CH_3, 20.5 CH_3, , 23.2 CH_3). The NMR data and its 2D information (^1H-^1H COSY, HMBC and HSQC) strongly suggested that **13** was a mexicanolide-type limonoid. In the HMBC spectrum (Figure 3.29), the key correlations of OMe/C-7, H_2-6/C-7 and H-5/C-6 enabled the methoxy group to be placed at C-7 and typical C-6–C-7 appendage of a mexicanolide limonoid to be linked at C-5. The $\Delta^{8,14}$ double bond was corroborated by the HMBC correlations from H_2-30 to C-2, C-8, C-9 and C-14, as well as from H_2-15 to C-14 and C-16. The ketone group (δ_C 217.9) was located at C-1 by its HMBC correlation with Me-19 and H-2. Further, observed HMBC correlation between H-3 at δ_H 5.00 and carbonyl carbon of acetyl group at δ_C 169.5

gave evidence of acetoxy group being attached to C-3. The single-crystal X-ray diffraction analysis of **13** confirmed its planar structure and allowed the determination of its relative configuration. This compound was found to be new and thus named as Thaimoluccensin D.

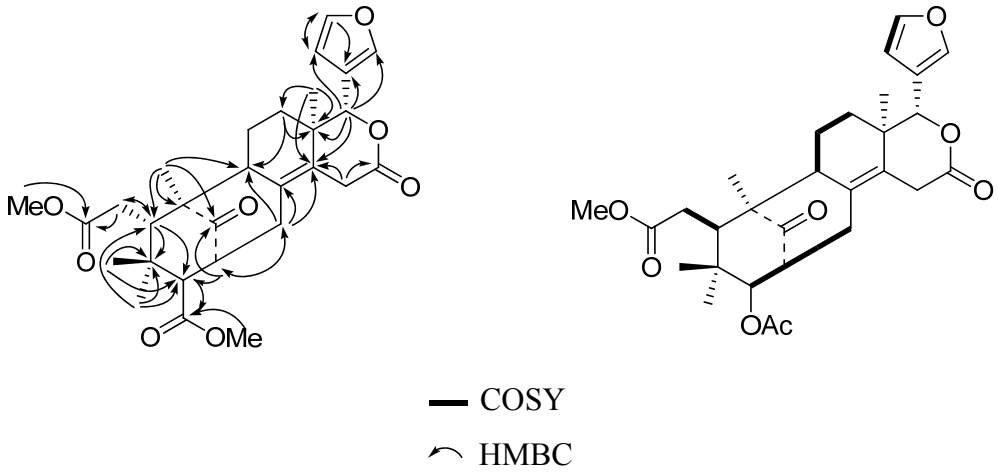


Figure 3.27 Key HMBC and COSY correlations of compound **13**

Figure 3.28 ORTEP diagram of compound **13**.

Table 3.14 NMR data ($CDCl_3$) of thaimoluccensin D and compound **13**

Position	^1H	^{13}C	COSY	HMBC
1		217.9		
2	3.13 (dd, *J*= 13.7, 8.0 Hz, 1H)	48.0	H-3, H-30	C-1, C-3, C-4, C-8, C-30
3	4.97 (s, 1H)	78.4	H-2	C-2, C-4, C-30, C-1’
4		38.2		
5	3.20 (dd, *J*= 8.5, 4.2 Hz, 1H)	40.8	H-6	C-4, C-6, C-7, C-10
6	2.35 (m, 2H)	33.48	H-5	C-5, C-7
7		174.2		
8		127.8		
9	2.04 (br, s)	52.3	H-11	C-8, C-10, C-11
10		52.9		
11	1.73 (m, 1H) 1.88 (m, 1H)	18.8	H-9, H-12	C-8, C-9, C-12, C-13
12	1.10 (m, 1H) 1.75 (m, 1H)	29.2	H-11	C-9, C-11, C-17
13		38.1		
14		131.8		
15	3.47 (td, 20.76, 20.76 Hz, 1H) 3.72 (m, 1H)	33.3		C-8, C-13, C-14, C-16
16		169.8		
17	5.69 (s, 1H)	80.7		C-12, C-13, C-20, C-21, C-22
18	1.01 (s, 1H)	17.9		C-12, C-13, C-14, C-17,
19	1.14 (s, 1H)	16.7		C-1, C-5, C-9, C-10
20		120.6		
21	7.54 (s, 1H)	141.7		C-20, C-22, C-23
22	6.47 (s, 1H)	109.9	H-23	C-20, C-21, C-23
23	7.40 (s,1H)	142.8	H-22	C-20, C-21, C-22
28	0.7 (s, 3H)	21.2		C-3, C-4, C-5, C-29
29	0.8 (s, 3H)	20.5		C-3, C-4, C-5, C-28
30	2.12 (dd, *J*= 15.17, 5.73 Hz, 1H) 2.79 (dd, *J*= 15.15, 2.13 Hz, 1H)	33.4	H-2	C-2, C-8, C-9, C-14
3-OAc		170.3		
	2.16	21.2		C-1`
7-OMe	3.70 (s, 1H)	52.0		C-7

Table 3.15. Crystaldata and structure refinement for compoung 13

Formula	$C_{29}H_{36}O_8$
Molecular weight	
Crystal size (mm)	0.48× 0.40 × 0.20
Crystal system	Orthorhombic
Space group	
***a* (Å)**	
***b* (Å)**	
***c* (Å)**	
***V* (Å^3)**	
Z	
D_{calc} (g/cm^3)	
μ (mm^{-1})	
F(000)	
Independent reflections/ Observed reflection [I>46(I)], R_{int}	
R_1	
***w*R_2[I>26(I)]**	

4.1.14 Structure elucidation of compound 14

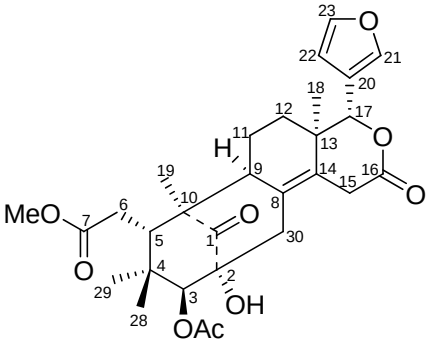


Figure 3.29 Compound **14**

Molecular formula	$C_{29}H_{36}O_9$
Appearance	White amorphous solid
IR (KBr)	3438, 2968, 2914, 2356, 2331, 1733, 1635, 1230, 1026, 873, 797, 727, 607, 465
^1H and ^{13}C NMR ($CDCl_3$)	See Table 3.16

Compound **14**, a white amorphous solid, had the molecular formula $C_{29}H_{36}O_9$. The ^1H NMR spectrum (Table 3.16) displayed typical signals for a ketone carbonyl (δ_C 217.9), an ester carbonyl (δ_C 169.8), a β-substituted furanyl ring (δ_H 6.40 s, 7.43 s, 7.49 s; δ_C 109.7CH, 120.4 qC, 141.5 CH, 142.7CH), a methoxycarbonyl group [δ_H 3.65 s; δ_C 52.0 CH_3; 173.9 qC], an acetyl group (δ_H 2.14; δ_C21.0; 169.5 qC), and four tertiary methyls (δ_H 0.63 s, 0.70 s, 1.01 s, 1.18 s; δ_C 16.6, 17.7, 196, 22.5). Analysis of the NMR data of **14** (Table 3.16) revealed it had the same mexicanolide skeleton as **13**, and their NMR data were closely related. The obvious difference was the appearance of the oxygenated quaternary carbon at δ_C 77.9, showing HMBC cross-peaks with H-3 and H-30 (Figure 3.31), in place of a C-2 methylene in **13**. This was concluded that it was a 2-hydroxy derivative of **13**, namely 2-hydroxyfissinolide which was also previously isolated from *Xylocarpus* species (Adesogan *et al*, 1970). Moreover, comparison of ^{13}C NMR data of **14** with those reported are shown in Table 3.16.

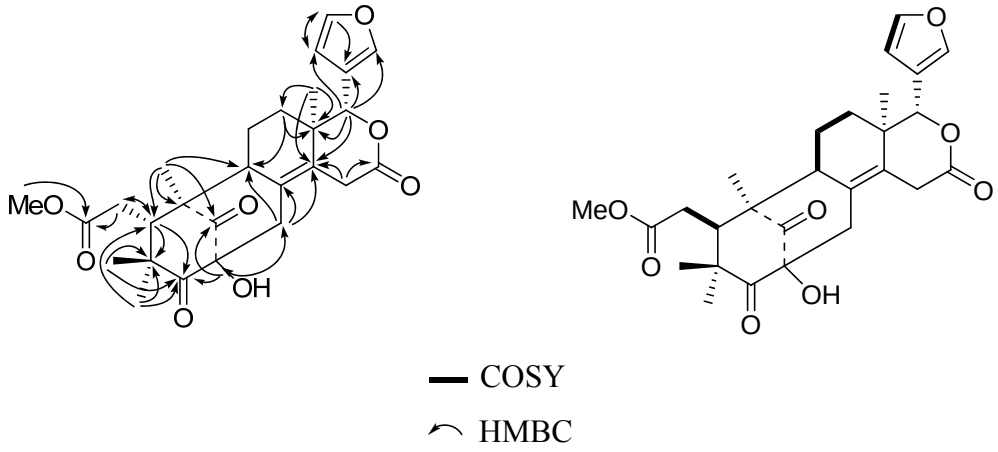


Figure 3.30 Key HMBC and COSY correlations of compound **14**

Table 3.16. NMR data ($CDCl_3$) of 2-hydroxyfissinolide and compound **14**

Position	2-hydroxyfissinolide	Compound **14**	
	^{13}C	^{1}H	^{13}C
1	217.4		217.9
2	78.0		77.9
3	85.7	5.00 (s, 1H)	85.4
4	39.0		38.7
5	52.2	3.05 (m, 1H)	40.6
6	33.3	2.31 (m, 1H)	33.0
7	174.0	2.30 (m, 1H)	
8	133.0		173.9
9	40.9	1.97 (m, 1H)	125.6
10	52.2		51.9
11	18.8	1.95 (m, 1H)	18.5
		1.73 (m, 1H)	
12	29.2	1.71 (m, 1H)	28.9
		1.07 (m, 1H)	
13	38.3		38.0
14	125.7		132.8
15		3.79 (d, *J*= 20.8 Hz, 1H)	33.2
	33.5	3.43 (d, *J*= 19.6 Hz, 1H)	
16	169.9		169.8
17	80.6	5.61 (s, 1H)	80.4
18	18.1	1.01 (s, 3H)	17.7
19	16.8	1.18 (s, 3H)	16.6
20	120.5		120.4
21	141.8	7.49 (s, 1H)	141.5
22	109.8	6.40 (s, 1H)	109.7
23	141.8	7.43 (s, 1H)	142.7
28	22.7	0.63 (s, 3H)	22.5
29	19.9	0.70 (s, 3H)	19.6
30	44.2	3.18 (d, *J*= 14.4 Hz, 1H)	43.9
		1.71 (m, 1H)	
3-OAc	169.6		169.5
	21.2	2.14 (s, 3H)	21.0
2-OH			
7-OMe	52.2	3.65 (s, 3H)	52.0

3.1.15 Structure elucidation of compound 15

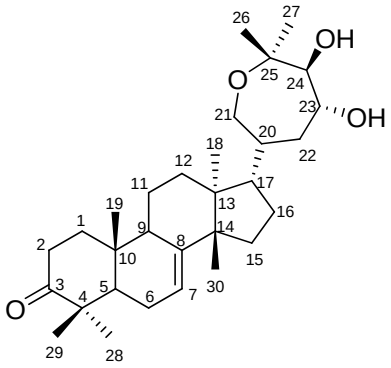


Figure 3.31 Compound **15**

Molecular formula	$C_{30}H_{48}O_4$
Appearance	White amorphous solid
IR (KBr)	3431, 2925, 1711, 1631, 1482, 1380, 1252, 1165, 1106, 1055, 880, 800, 710, 582
^1H and ^{13}C NMR (CDCl3)	See Table 3.17

Compound **15** was isolated as white amorphous solid. Its molecular formula was determined to be $C_{30}H_{48}O_4$ as established by NMR data. The ^1H NMR spectrum displayed signals for seven tertiary methyls (δ_H 0.97, 0.77, 1.14, 1.27, 1.00, 1.02 and 1.09), an olefinic proton (δ_H 5.28), four oxymethine proton (δ_H 3.59, 3.40, 3.81 and 3.39). The ^{13}C NMR spectrum showed resonances one ketone carbonyl (δ_C 216.9), four olefinic carbons (δ_C 118.0 and 145.7), and seven methyls (δ_C 12.8, 22.3, 22.2, 26.3, 27.4, 24.5 and 21.6). The remaining carbons were assigned to nine methylenes, seven methines, and six quaternary carbons based on the HSQC data as described in Table 4.16. The above analysis suggested that **15** was a tirucallane-type triterpene. The structure of **15** was further elucidated by analysis of 2D-NMR spectra, especially by ^1H-^1H COSY and HMBC correlations. The ketone carbonyl (δ_C 216.9) was placed at C-3 by its correlations with CH_2-1, CH_2-2, Me-28, and Me-29. Observed HMBC correlations of H_2-6/C-7, H_2-6/C-8, H_2-11/C8 and Me-30/C-8 helped to establish the

$\Delta^{7,8}$ double bond. A key partial structure, the C-15–C-17 fragment connecting to another C-20–C-24 fragment at C20, was obtained by analysis of ^{1}H-^{1}H COSY spectra data (Figure 3.33). Further, the HMBC correlations of two tertiary methyls at δ_H 1.14 and 1.27 with the oxymethine carbon at δ_C 80.7 led to the connectivity of gem-dimethyl at C-24. Seven-membered ring closure between C-21 and C-25 through an ether linkage was corroborated by the key HMBC correlation from H_2-21 to C-25. Based on the information of NMR spectral data above, compound **15** was identified as hispidone. It was further confirmed by comparing its ^{1}H and ^{13}C NMR data with those previously reported by Cole et al. (Cole *et al*., 1980) as shown in Table 3.17, hispidone previously isolated from *Toona ciliata*. To the best of our knowledge, this is the first report of hispidone from plant in *Xylocarpus* species

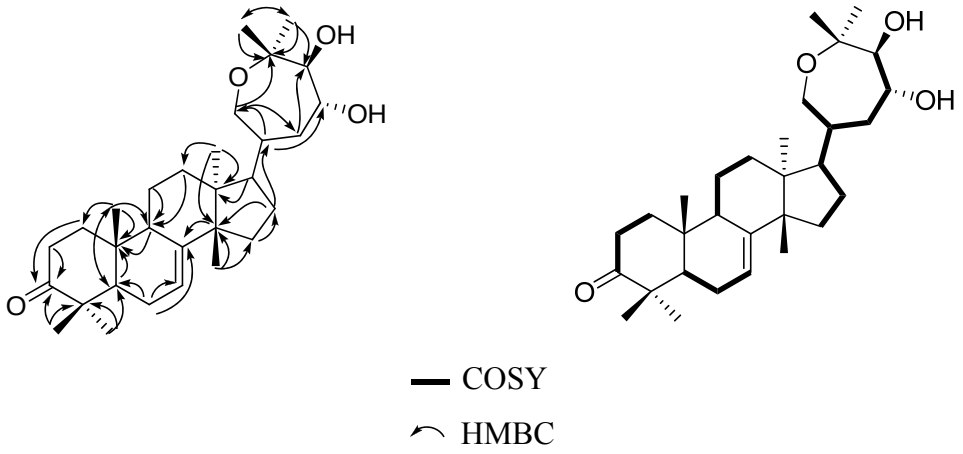


Figure 3.32 Key HMBC and COSY correlations of compound **15**

Table 3.17. NMR data ($CDCl_3$) of hispidone and compound **15**

Position	Hispidone		Compound **15**	
	^1H	^{13}C	^1H	^{13}C
1	-	38.6	1.42 (m, 1H)	38.5
			1.97 (m, 1H)	
2	-	34.9	2.21 (m, 1H)	34.9
			2.74 (m, 1H)	
3	-	217.1		216.9
4	-	47.9		47.9
5	-	52.4	1.67 (br, s)	52.3
6	-	24.4	2.06 (m, 2H)	24.4
7	5.3	118.1	5.28 (s, 1H)	118.0
8	-	145.8		145.7
9	-	48.4	2.23 (m, 1H)	48.4
10	-	34.9		35.0
11	-	18.1	1.51 (m, 1H)	18.1
			1.54 (m, 1H)	
12	-	32.6	1.46 (m, 1H)	33.6
			1.86 (m, 1H)	
13	-	43.4		43.3
14	-	51.3		51.5
15	-	33.9	1.49 (m, 2H)	33.9
16	-	28.2	1.31 (m, 1H)	28.1
			1.98 (m, 1H)	
17	-	47.5	1.87 (m, 1H)	47.5
18	0.8	12.8	0.97 (s, 3H)	12.8
19	1.1	21.6	0.77 (s, 3H)	22.3
20	-	38.6	1.75 (m, 1H)	38.6
21	-	64.4	3.40 (m, 1H)	64.4
			3.59 (d, *J*= 14.8 Hz, 1H)	
22	-	37.5	1.61 (m, 1H)	37.4
			1.92 (m, 1H)	
23	3.3 - 3.9	68.7	3.81 (t, *J*= 10.8, 10.8 Hz, 1H)	68.6
24	3.3 - 3.9	80.8	3.39 (m, 1H)	80.7
25	-	76.2		76.1
26	1.15	22.4	1.14 (s, 3H)	22.2
27	1.3	26.3	1.27 (s, 3H)	26.3
28	1.05	27.4	1.00 (s, 3H)	27.4
29	1.05	24.6	1.02 (s, 3H)	24.5
30	1.00	22.4	1.09 (s, 3H)	21.6

3.3 Anti-inflammatory activity of isolated compounds

The anti-inflammatory effects of isolated compounds 1-15 were evaluated by monitoring the inhibition of nitric oxide (NO) production in activated macrophages. Results expressed as IC_{50} are shown in Table 3.16.

Figure 3.18 Inhibitory effects of isolated compounds on nitric oxide production.

	Compound	IC_{50} (μM)	
		Anti-imflammatory	**Cytotoxicity**
1	xyloccensin K	>50	n.d.
2	6-acetoxycedrodorin	n.d.	n.d.
3	Andirobin	>50	n.d.
4	Methylangolensate	>50	n.d.
5	methyl-6-*β*-hydroxy angolensate	n.d.	n.d.
6	Gedunin	n.d.	n.d.
7	7-deacetylgedunin	4.75	n.d.
8	kihadalactone A	n.d.	n.d.
9	toonaciliavarin E	n.d.	n.d.
10	protoxylocarpin G	n.d.	n.d.
11	protoxylocarpin H	n.d.	n.d.
12	moluccensin I	>50	n.d.
13	thaimoluccensin D	n.d.	n.d.
14	2-hydrofissinolide	>50	n.d.
15	Hispidone	n.d.	n.d.

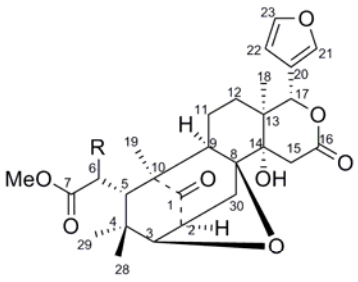
Based on the above results, only a gedunin-type limonoid, 7-deacetylgedunin (**4**), exhibited potent anti-inflammatory activity with an IC_{50} value of 4.75 μM. Generally, phragmarin and mexicanolide type limonoids did not show any significant activity even at a concentration of 50 μM.

CHAPTER IV

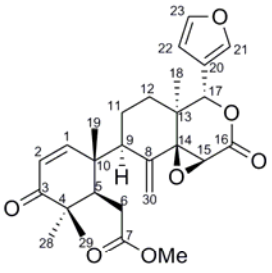
CONCLUSION

Chemical examination of the seed kernels of *Xylocarpus granatum* Koeing. collected from Surat-thani province led to the isolation of ten limonoids namely xyloccensin K (**1**), 6-acetoxycedrodorin (**2**), andirobin (**3**), methyl angolensate (**4**), methyl-6-*β*-hydroxy angolensate (**5**), gedunin (**6**), 7-deacetylgedunin (**7**), kihadalactone A (**8**) and protoxylocarpin G (**10**), and a tirucallane-type triterpene, toonaciliatavarin E (9). Apart from limonoids 3-4 and 6-7, the isolation of the *X. moluccensis* seed kernels collected from the same province gave a new mexicanolide, thaimoluccensin D (**13**), together with three known limonoids, protoxylocarpin H (**11**), moluccensin I (**12**) and 2-hydroxyfissinolide (**14**), and one tirucallane triterpene, hispidone (**15**). The structure of a novel compound (**13**) was elucidated by analysis of spectroscopic data, as well as single-crystal X-ray diffraction analysis, while those of known compounds were determined on the basis of 1D and 2D NMR spectroscopic data and by comparing their spectral data with those previously reported in literature.

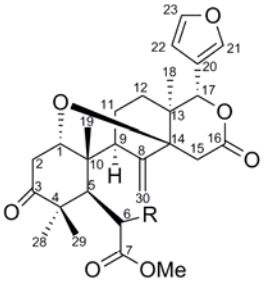
The isolated compounds were evaluated for their anti-inflammatory activity by monitoring the inhibition of nitric oxide (NO) production in activated macrophages (RAW 264.7 cells) in a concentration-dependent manner. Generally, gedunin-type limonoid showed promising acitivity, while phragmalin and mexicanolide limonid did not display any significant activity or was inactive. Based on our results, 7-deacetylgedunin exhibited potent inhibitory activity against NO production from activated macrophages with an IC_{50} value of 4.75 μM, suggesting that this compound has anti-inflammatory activity.



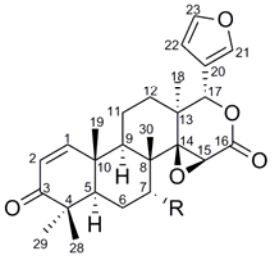
1: R = H
2: R = OAc



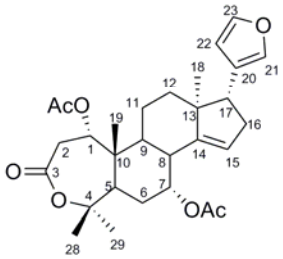
3



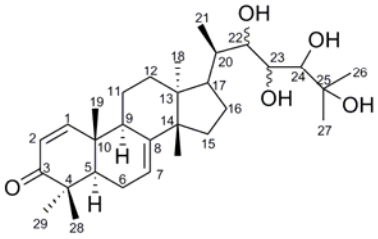
4: R = H
5: R = OH



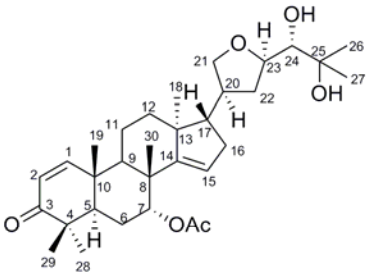
6: R = OAc
7: R = OH



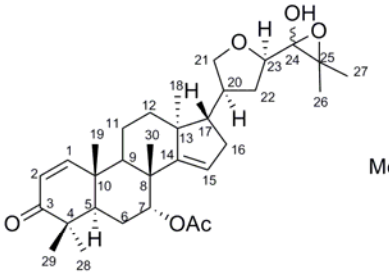
8



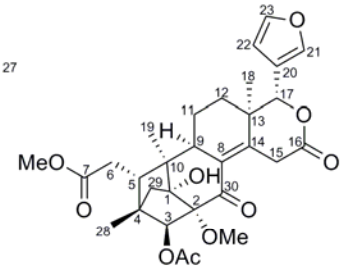
9



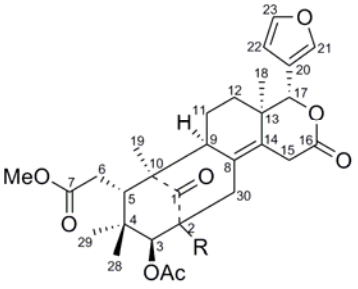
10



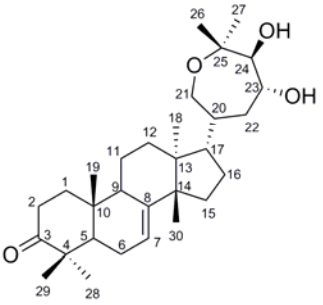
11



12



13: R = H
14: R = OH



15

REFERENCES

Abdelgaleil, S. A. M., Iwagawa, T., Doe, M. and Nakatani, M. 2004. Antifungal limonoids from the fruits of *Khaya senegalensis*. Fitoterapia. 75: 566-572.

Abdelgaleil, S. A. M., Hashinaga, F. and Nakatani, M. 2005. Antifungal activity of limonoids from *Khaya ivorensis*. Pest. Manag. Sci. 61: 186-190.

Adesogan, E. K., and Taylor, D. A. H. 1970. Limonoid Extractives from *Khaya ivorensis*. J. Chem. Soc. (C): 1710-1714.

Ahmed, F. R., Ng, A. S. and Fallis, A. G. 1978. 7α-Acetoxydihydronomilin: isolation, spectra, and crystal structure. Can. J. Chem. 56: 1020-1025.

Aliero, B. L. 2003. Larvaecidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of *Anopheles mosquito.* Afr. J. Biotechnol. 2: 325-327.

Alvi, K. A., Crews, P., Aalbersberg, B. and Prasad, R. 1991. Limonoids from the Fijian medicinal plant Dabi (*Xylocarpus*). Tetrahedron. 47: 8943-8948.

Bandaranayake, W. M. 1998. Traditional and medicinal uses of mangroves. Mangroves and Salt Marshes. 2: 133-148.

Bandarnayake, W. M. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol. Manage. 10: 421-452.

Battinelli, L., Mengoni, F., Lichtner, M., Mazzanti, G., Saija, A., Mastroianni, C. M. and Vullo, V. 2003. Effect of Limonin and Nomilin on HIV-1 Replication on Infected Human Mononuclear Cells. Planta Med. 69: 910-913.

Bickii, J., Njifutie, N., Ayafor-Foyere, J., Basco, L. K. and Ringwald, P. 2000. *In vitro* antimalarial activity of limonoids from *Khaya grandifoliola* C.D.C. (Meliaceae). J. Ethnopharmacol. 69: 27-33.

Bultler, M.S. 2008. Natural products to drugs: natural product-derived compounds in clinical trials. Nat Prod Rep. 25: 475-516

Carpinella, M. C., Defago, M. T., Valladares, G. and Palacios, S. M. 2003. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. J. Agric. Food Chem. 51: 369-374.

Carpinella, C., Ferrayoli, C., Valladares, G., Defago, M. and Palacios, S. 2002. Potent limonoid insect antifeedant from *Melia azedarach*. Biosci. Biotechnol. Biochem. 66: 1731-1736.

Cespedes, C. L., Calderon, J. S., Salazar, J. R., Lotina-Hennsen, B. and Segura, R. 2001. Plant-growth inhibitory activity of cedrelanolide from *Cedrela salvadorensis*. J. Chem. Ecol. 27: 137-149.

Cheng, F., Zhou, Y., Wu, J. and Zou, K. 2006. Xyloccensins X_1 and X_2, two new mexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum* J. Chem. Sci. 61: 626-628.

Chopra, R. N., Nayar, S. L. and Chopra, I. C. 1956. Glossary of Indian Medicinal Plants. Ed.V, New Delhi: CSIR.

Chou, F. Y., Hosttmann, K., Kubo, I., Nakanishi, K. and Taniguchi, M. 1977. Isolation of an insect antifeedant *N*-methylflindersine and several benz[c]phenanthridine alkaloids from East African plants; A comment on chelerythrine Heterocycle. 7: 969-977.

Choudhary, S., Sree, A., Mukherjee, S. C., Patnaik, P. and Bapuji, M. 2005. *In Vitro* Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens. Asian Fisheries Sci. 18: 285.

Connolly, J. D., MacLellan, M., Okorie, D. A. and Taylor, D. A. H. 1976. Limonoids from *Xylocarpus moluccensis* (Lam.) M. Roem. J. Chem. Soc. 1: 1993-1996.

Cui, J., Deng, Z., Li, J., Fu, H., Proksch, P. and Lin, W. 2005. Phragmalin-type limonoids from the mangrove plant *Xylocarpus granatum.* Phytochemistry. 66: 2334-2339.

Cui, J., Deng, Z., Xu, M., Proksch, P., Li, Q. and Lin, W. 2009. Protolimonoids and limonoids from the chinese mangrove plant *Xylocarpus granatum.* Helv. Chem. Acta. 92: 139-150.

Cui, J., Wu, J., Deng, Z., Proksch, P. and Lin, W. 2007. Xylocarpins A-I, limonoids from the Chinese mangrove plant *Xylocarpus granatum.* J. Nat. Prod. 70: 772-778.

Devakumar, C. and Sukh, D. “Chemistry”, ed by Randhawa N.S., Parmar, B.S. 1996. Neem research and development. Soc. pest. sci. New delhi India. 3: 63-99

El-Shemy, H. A., Aboul-Enein, A. M., Aboul-Enein, K. M. and Fujita, K. 2007. Willow Leaves Extracts Contain Anti-Tumor Agents Effective against Three Cell Types. PLoS One. 2: 178.

Endo, T., Kita, M., Shimada, T., Moriguchi, T., Hidaka, T., Matsumoto, R., Hasegawa, S. and Omura, M. 2002. Modification of Limonoid Metabolism in Suspension Cell Cultures of Citrus. Plant Biotechnol. 19: 397-403.

Germano, M. P., D Angelo, V., Sanogo, R., Catania, S., Alma, R., Pasquale, R. D. and Bisignano, G. 2005. Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl. (Meliaceae). J. Ethnopharmacol. 96: 227-232.

Govindachari, T. R., Suresh, G., Banumathy, B., Masilamani, S., Gopalakrishnan, G. and Krishna Kumari, G. N. 1999. Antifungal activity of some B,D-*seco* limonoids from two meliaceous plants. J. Chem. Ecol. 25: 923-933.

Govindachari, T. R., Suresh, G., Gopalakrishnan, G., Masilamani, S. and Banumathi, B. 2000. Antifungal activity of some tetranortriterpenoids. Fitoterapia. 71: 317-320.

Han, S. S., Keum, Y. S., Seo, H. J. and Surh, J. 2002. Curcumin suppresses activation of NF-*k*B and AP-1 induced by phorbol ester in cultured human promyelocytic Leukemia cells. J. Biochem. Mol. Biol. 35: 337–342.

Huo, C-H., Shen, L-R., Guo, D., Yu, Y-M., Yin, B-W., Zhao, L., Shi Q-W., Wang, Y-L. 2009. Chemical constituents of plants from the genus *xylocarpus*. Chem. Bio. 6: 1293-1308.

Jacob, R., Hasegawa, S. and Manners, G. 2000. The potential of Citrus Limonoids as anticancer agents. Perishables Handling. 102: 6-8.

Kadota, S., Marpaung, L., Kikuchi, T., and Ekimoto, H. 1990. Mahagonin, a novel dimeric tetranortriterpenoid from Swietenia mahagoni Jacq. Chem. Pharm. Bull. 38: 639

Kathiresan, K., Boopathy, NS. and Kavitha, S. 2006. Coastal vegetation: and underexplored source of anticancer drugs. Nat. Prod. Radiance. 5(2): 114-119

Kayser, O., Kiderlen, A. F. and Croft, S. L. 2003. Natural products as antiparasitic drugs. Parasitol. Res. 90: 55-62.

Kinghorn, D.A., Chin, Y.W., Balunas M.J. and Chai, H.B. 2006. Drug Discovery from natural sources. The AAPS Journal. 28: E239-E353

Kiyota, H., Shi, Q-W., Huo, C-H., Guo, D., Shen, L-R., Yin, B-W., Saruiol, F., Li, L-G., and Zhang, M-L. 2010. Xylocarpanoids A and B, unique C28 skeleton limonoids from xylocarpus granatum. Tetrahedron Lett. 51: 754-757.

Kokpol, U., Chavasiri, W., Tip-pyang, S., Veerachato, G., Zhao, F., Simpson, J. and Weavers, R. T. 1996. A limonoid from *Xylocarpus granatum.* Phytochemistry. 41: 903-905.

Koul, O., Singh, G., Singh, R., Singh, J., Daniewski, W. M. and Berlozecki, S. 2004. Bioefficacy and mode-of-action of some limonoids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J. Biosci. 29: 409-416.

Koul, O., Multani, J. S., Singh, G. and Wahab, S. 2002. Bioefficacy of toosendanin from *Melia dubia* (syn. *M. azedarach*) against gram pod-borer, *Helicoverpa armigera* (Hubner). Curr. Sci. 83: 1387-1391.

Lakshmi, V. and Gupta, P. 2008. An overview of the genus *Xylocarpus*. Nat. Prod. Res. 22: 1197-1224.

Li, M., Wu, J., Zhang, S., Xiao, Q. and Li, Q. 2007. Xylocarpins A and B, two new mexicanolides from the seeds of a Chinese mangrove *Xylocarpus granatum*: NMR investigation in mixture. Magn. Reson. Chem. 45: 705-709.

Li, M. Y., Wu, J., Zhang, S., Xiao, Q. and Li, Q. X. 2008. The absolute stereochemistry of protoxylogranatin A - A new protolimonoid from the seeds of Chinese mangrove *Xylocarpus granatum*. J. Asian Nat. Prod. Res. 10: 503-508.

Mabberley, D. J., Pannell, C. M. and Sing, A. M. 1995. Flora Malesiana. vol. 12. pp. 407-408. Leiden, The Netherland: Rijksherbarium/Hortus Botanicus.

Miller, E. G., Porter, J. L., Binnie, W. H., Guo, I. Y. and Hasegawa, S. 2004. Further studies on the anticancer activity of citrus limonoids. J. Agric. Food Chem. 52: 4908-4912.

Mulholland, D. A. and Taylor, D. A. H. 1992. Limonoids from Australian members of the meliaceae. Phytochemistry. 31: 4163-4166.

Nakagawa, H., Duan, H. and Takaishi, Y. 2001. Limonoids from *Citrus sudachi* Chem. Pharm. Bull. 49: 649-651.

Newman, D. J. and Cragg, G. M., and Snader K.M. 2000. The influence of natural product upon drug discovery. Nat. Prod. Rep. 17: 215-234.

Newman, D. J. and Cragg, G. M., and Snader K.M. 2003. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod. 66: 1022-1037.

Newman, D. J. and Cragg, G. M. 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70: 461-477.

Ng, A. S. and Fallis, A. G. 1979. 7α-Acetoxydihydronomilin and mexicanolide: limonoids from *Xylocarpus gralzatum* (Koenig). Can. J. Chem. 57: 3088.

Okorie, D. A., Taylor, D. A. H. 1970. Limonoids from *Xylocarpus granatum* Koenig. J. Chem. Soc. C: Org. 2: 211-213.

Omar, S., Zhang, J., MacKinnon, S., Leaman, D., Durst, T., Philogene, B. J., Arnason, J. T. and Pezzuto, J. M. 2003. Traditionally-used antimalarials from the Meliaceae. Curr. Top Med. Chem. 3: 133-139.

Payla, P. A. *et, al*. 2006. Limonoids from Andiroba Oil and *Cedrela fissilis* and their Insecticidal Activity. J. Braz. Chem. Soc. 17: 542-547

Perusquia, M., Hernandez, R., Jimenez, M. A., Pereda-Miranda, R. and Mata, R. 1997. Contractile response induced by a limonoid (Humilinolide A) on spontaneous activity of isolated smooth muscle. Phytother Res. 11: 354-357.

Premnatha, M., Chandra, K., Bajpai, S.K. and kathiresan, K. 1992. A survey of some Indian marine plants for antiviral activity. Botanica Marina. 35: 321-324

Pudhom, K., Sommit, D., Nuclear, P., Ngamrojanavanich, N. and Petsom, A. 2010. Moluccensins H-J, 30-Ketophragmalin Limonoids from *Xylocarpus moluccensis*. J. Nat. Prod. 73: 263-266.

Pudhom, K., Sommit, D., Nuclear, P., Ngamrojanavanich, N. and Petsom, A. 2009. Protoxylocarpins F-H, Protolimonoids from Seed Kernels of *Xylocarpus granatum*. J. Nat. Prod. 72: 2188-2191.

Pudhom, K., Muangsin, Nongnuj., Pengpreecha, S., Teerawatananond, T., Nuanyai, T. and Sarigaputi, C. 2010. Xylorumphiins A-D, Mexicanolide limonoids from the seed kernels of *xylocarpus rumphii*. 2010. J. Nat. Prod. 73: 1456-1459.

Roy, A. and Saraf, S. 2006. Limonoids: Overview of Significant Bioactive Triterpenes Distributed in Plants Kingdom. Biol. Pharm. Bull. 29: 191-201.

Sastri, B. N. 1950. The wealth of India; Raw Materiales, Vol. 2(C), pp. 74–75, New Delhi, Publication and Information Directorate, CSIR

Saxena, S., Pant, N., Jain, D. C. and Bhakuni, R. S. 2003. Antimalarial agents from plant sources. Curr. Sci. 85: 1314-1329.

Simmonds, M. S. J., Stevenson, P. C., Porter, E. A. and Veitch, N. C. 2001. Insect antifeedant activity of three new tetranortriterpenoids from *Trichilia pallida.* J. Nat. Prod. 64: 1117-1120.

Shi, Q., Yin, B., Huo, C., Shen, L., Wang, C, Zhao, L. and Wang, Y. 2009. Protolimonoids from the seeds of *Xylocarpus granatum*. Bio. Sys. Eco. 37: 218-220

Somrutai, J., Chantachum, S., Ratanaphan, A. and Chantrapromma, K. 2005. Stability of limonin from lime seeds. Electron. J. Environ. Agric. Food Chem. 4: 938-944.

Suarez, L. E. C., Menichini, F. and Monache, F. D. 2002. Tetranortriterpenoids and Dihydrocinnamic Acid Derivatives from *Hortia colombiana.* J. Braz. Chem. Soc. 13: 339-344

Sunthitikawinsakul, A., Kongkathip, N., Kongkathip, B., Phonnakhu, S., Daly, J. W., Spande, T. F., Nimit, Y. and Yoosook, C. 2003. Anti-HIV-1 Limonoid: First Isolation from *Clausena excavate.* Phytother. Res. 17: 1101-1103.

Tada, K., Takido, M. and Kitanaka, S. 1999. Limonoids from fruit of *Melia toosendan* and their cytotoxic activity. Phytochemistry. 51: 787-791.

Taylor, D. A. H. 1965. Extractives from East African timbers. Part I. J. Chem. Soc. (Resume): 3495-3496.

Taylor, D. A. H. 1983. Limonoid extractives from *Xylocarpus moluccensis*. Phytochemistry. 22: 1297-1299.

Taylor, D. A. H. 1984. The chemistry of the limonoids from Meliaceae. *Progress in the Chemistry of Organic Natural Products*. 45: 1-102.

Thaitoi, H.N., Patra, J.K. 2011. Metabolic diversity and bioactivity screenin of mangrove plants: a review. Acta Physiol Plant. 33: 1051-1061

Tomlinson, P.B. 1986. The botany of mangroves. Cambridge university press, Cmabridge, 274-282.

Uddin, S. J., Nahar, L., Shilpi, J. A., Shoeb, M., Borkowski, T., Gibbons, S., Middleton, M. and Sarker, S. D. 2007. Gedunin, a limonoid from *Xylocarpus granatum*, inhibits the growth of CaCo-2 colon cancer cell line *in vitro*. Phytother. Res. 21: 757-761.

Uddin, S. J., Shilpi, J. A., Alam, S. M. S., Alamgir, M., Rahman, M. T. and Sarker, S.D. 2005. Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus moluccensis* in castor oil- and magnesium sulphate-induced diarrhoea models in mice. J. Ethnopharmacol. 101: 139-143.

White, NJ. 1997. Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. Antimicrob. Agents Chemother. 41(7): 1413–22

Wu, J., Ding, H., Li, M. and Zhang, S. 2007. Xylogranatin E, a new phragmalin with a rare oxygen bridge between C_1 and C_{29}, from the fruit of a Chinese mangrove *Xylocarpus granatum*. J. Chem. Sci. 62: 569-572.

Wu, J., Li, M., Zhang, S., Xiao, Q. and Li, Q. 2007. Two new limonoids with a 3-*O*-*β*-tigloyl group from the seeds of the Chinese mangrove *Xylocarpus granatum*. J. Chem. Sci. 62: 859-862.

Wu, J., Xiao, Q., Huang, J., Xiao, Z., Qi, S., Li, Q. and Zhang, S. 2004. Xyloccensins O and P, Unique 8,9,30-Phragmalin *Ortho* Esters from *Xylocarpus granatum*. Org. Lett. 6: 1841-1844.

Wu, J., Xiao, O. and Li, Q. 2006. Limonoids from the Mangrove *Xylocarpus granatum*. Biochem. Syst. Ecol. 34: 838-841.

Wu, J., Xiao, Q., Xu, J., Li, M. Y., Pan, J. Y. and Yang, M. H. 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. Nat. Prod. Rep. 25: 955-981.

Wu, J., Xiao, Q., Zhang, S., Li, X., Xiao, Z., Ding, H. and Li, Q. 2005. Xyloccensins Q-V, six new 8,9,30-phragmalin *ortho* ester antifeedants from the Chinese mangrove *Xylocarpus granatum*. Tetrahedron. 61: 8382-8389.

Wu, J., Xiao, Z., Song, Y., Zhang, S., Xiao, Q., Ma, C., Ding, H. and Li, Q. 2006. Complete assignments of ^{1}H and ^{13}C NMR data for two 3*β*,8*β*-epoxymexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum.* Magn. Reson. Chem. 44: 87-89.

Wu, J., Yang, S. X., Li, M. Y., Feng, G., Pan, J. Y., Xiao, Q., Sinkkonen, J. and Satyanandamurty, T. 2010. Limonoids and Tirucallane Derivatives from the Seeds of a Krishna Mangrove, *Xylocarpus moluccensis*. J. Nat. Prod. 73: 644-649.

Wu, J., Zhang, S., Xiao, Q., Li, Q., Huang, J., Xiao, Z. and Long, L. 2003. Xyloccensin M and N, Two New B, D-*seco* Limonoids from *Xylocarpus granatum.* J. Chem. Sci. 58: 1216-1219.

Wu, J., Zhang, S., Xiao, Q., Li, Q., Huang, J., Long, L. and Huang, L. 2004. Xyloccensin L, a novel limonoid from *Xylocarpus granatum.* Tetrahedron Lett. 45: 591-593.

Wu, J., Zhang, S., Song, Y., Xiao, Z., Xiao, Q. and Li, Q. 2005. Two new mexicanolides from the fruit of the Chinese mangrove *Xylocarpus granatum.* J. Chem. Sci. 60: 1291-1294.

Wu, J., Zhang, S., Li, M., Zhou, Y. and Xiao, Q. 2006. Xylogranatins A-D, new mexicanolides from the fruit of a Chinese mangrove *Xylocarpus granatum*. Chem. Pharm. Bull. 54: 1582-1585.

Wu, J., Li, M-Y., Yang, X-B., Pan, J-Y., Feng, G., Sinkkonen, J. and Satyanandamurty, T. 2009. Granatumins A-G, limonoids from the seeds of a Krishna mangrove, *Xylocarpus granatum*. J. Nat. Prod. 72: 2110-2114.

Wu, J., Li, J., Li, M-Y., Feng, G., Xiao, Q., Sinkkonen, J. and Satyanandamurty, T. 2010. Limonoids from the seeds of a Godavari mangrove, Xylocarpus moluccensis. Phytochemistry. 71: 1917-1924.

Wu, J., Li, J., Lin, M-Y. and Satyanandamurty, T. 2011. Godavarin K: a new limonoid with an oxygen bridge between C(1) and C(29) from the godavari mangrove *Xylocarpus moluccensis*. Hel. Chim. Acta. 94: 1651-1656

Ximu, C. and Pongumphai, S. 1994. Preliminary revision of Swietenioideae (Meliaceae) in Thailand. Thai J. For. 13: 1-9.

Yin, B., Huo, C., Shen, L., Wang, C., Zhoa, L. and Wang, Y. 2009. Protolimonoids from the seeds of *Xylocarpus granatum*. Biochem. Syst. Ecol. 37: 218-220.

Yin, S., Wang, X. N., Fan, C. Q., Lin, L. P., Ding, J. and Yue, J. M. 2007. Limonoids from the seeds of the marine mangrove *Xylocarpus granatum.* J. Nat. Prod. 70: 682-685.

Yue, J-M., Dong, S-H., He, X-F., Dong, L. and Wu, Y. 2012. Tritrepene from Melia toosendan. Helvetica Chimica Acta. 95: 286-300.

Yoshikawa, K., Arihara, S. and Kishi, K. 1992. Limonoids and protolimonoids from the fruits of *Phellodenron Amurense*. Phytochemistry. 31: 1335-1338.

Zhou, Y., Cheng, F. and Zou, K. 2006. Polyhydroxylated Phragmalins from the Fruit of a Chinese Mangrove, *Xylocarpus granatum.* J. Nat. Prod. 69: 1083-1085.

APPENDIX

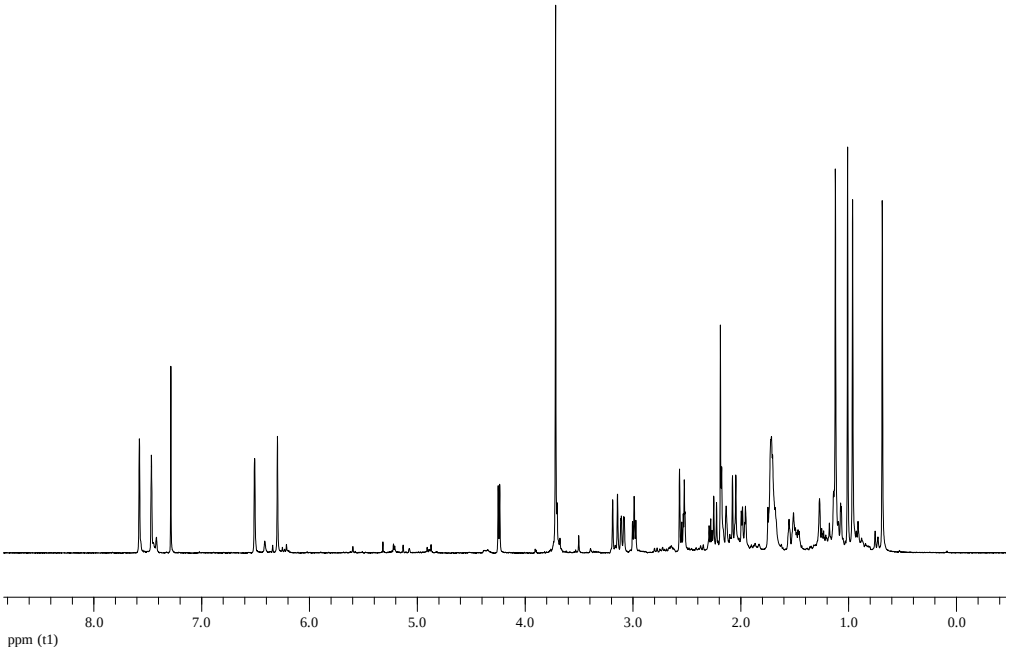


Figure S-1 ^{1}H NMR (400 MHz) spectrum of compound **1** ($CDCl_3$)

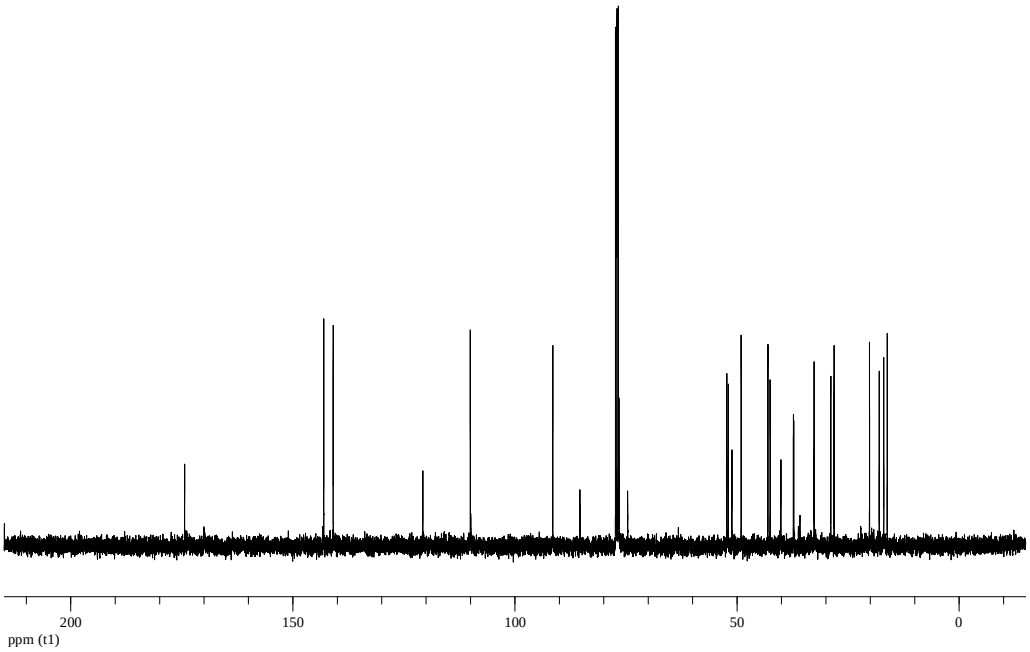


Figure S-2 ^{13}C NMR (100 MHz) spectrum of compound **1** ($CDCl_3$)

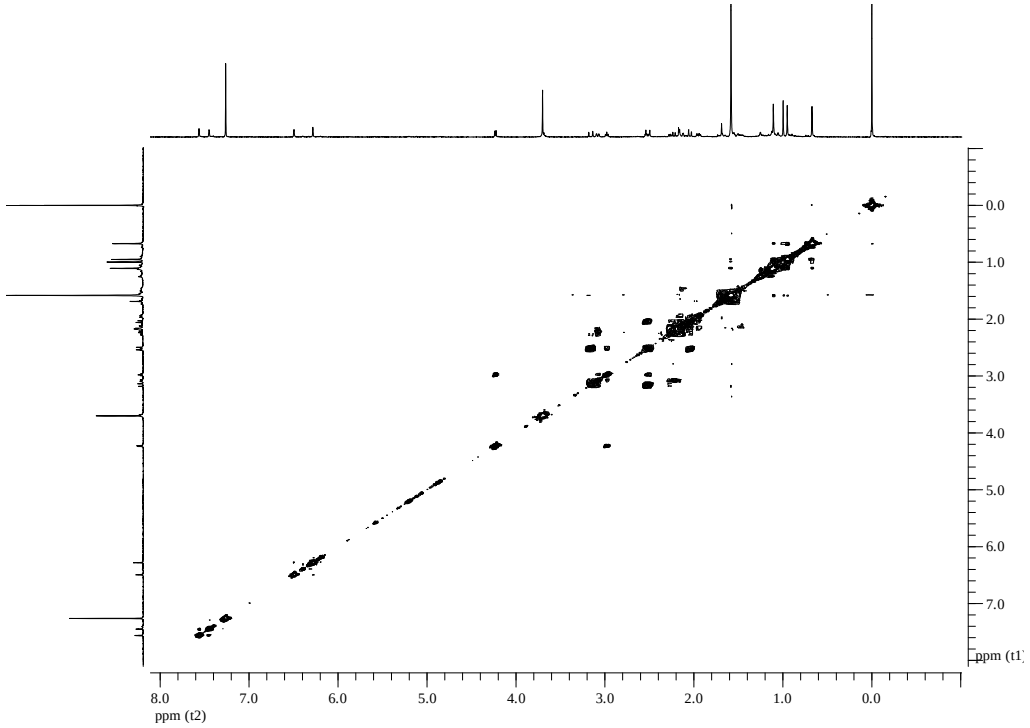


Figure S-3 ^{1}H-^{1}H COSY spectrum of compound **1** ($CDCl_3$)

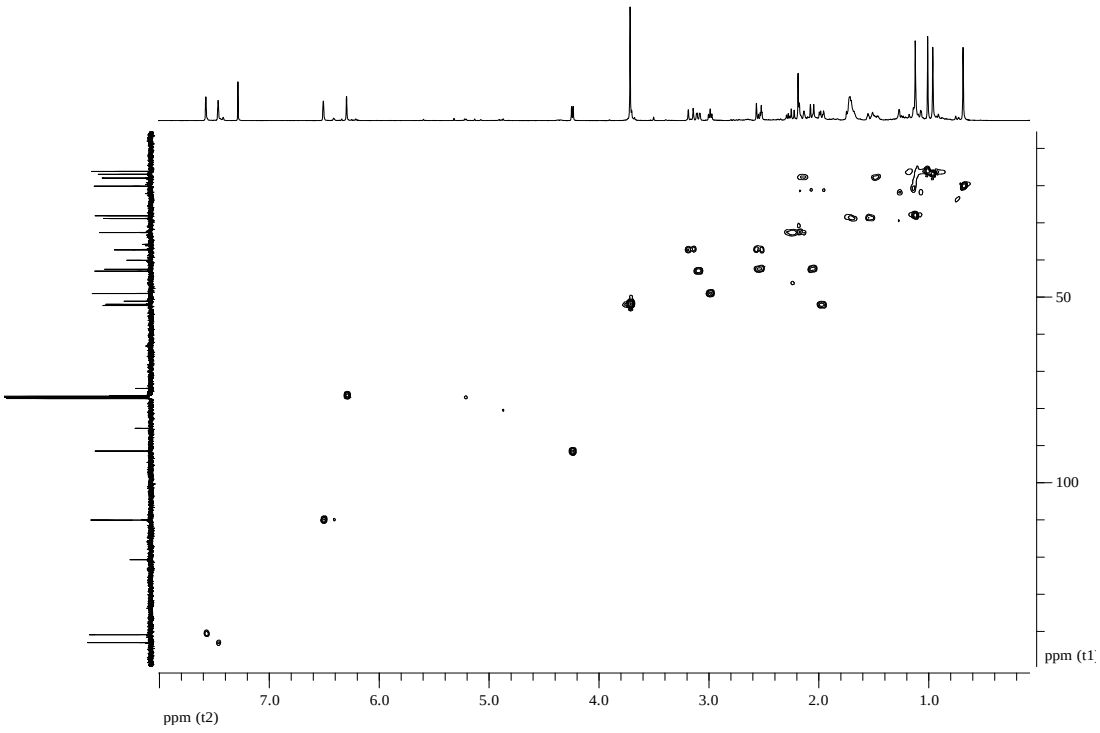


Figure S-4 HSQC spectrum of compound **1** ($CDCl_3$)

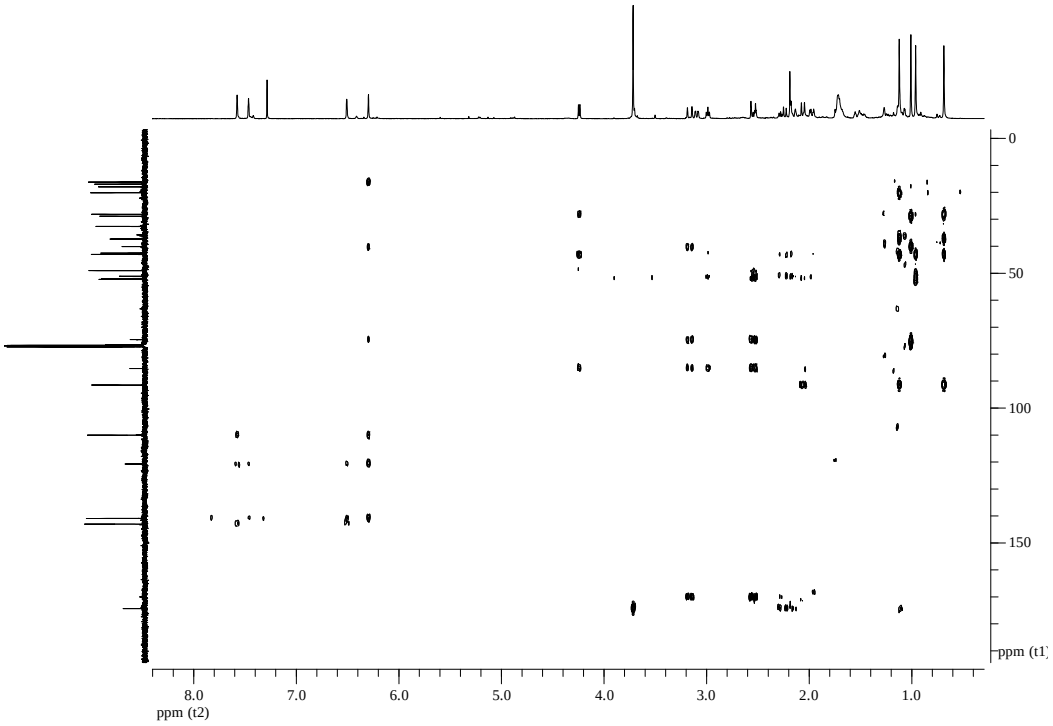


Figure S-5 HMBC spectrum of compound **1** ($CDCl_3$)

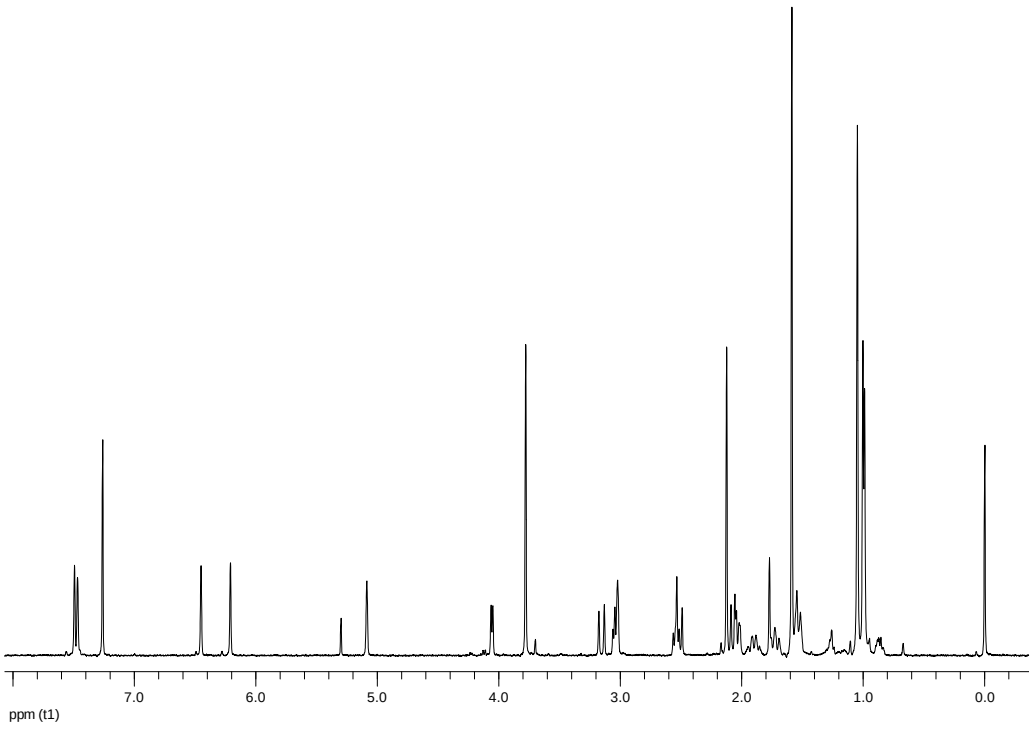


Figure S-6 ^{1}H NMR (400 MHz) spectrum of compound **2** ($CDCl_3$)

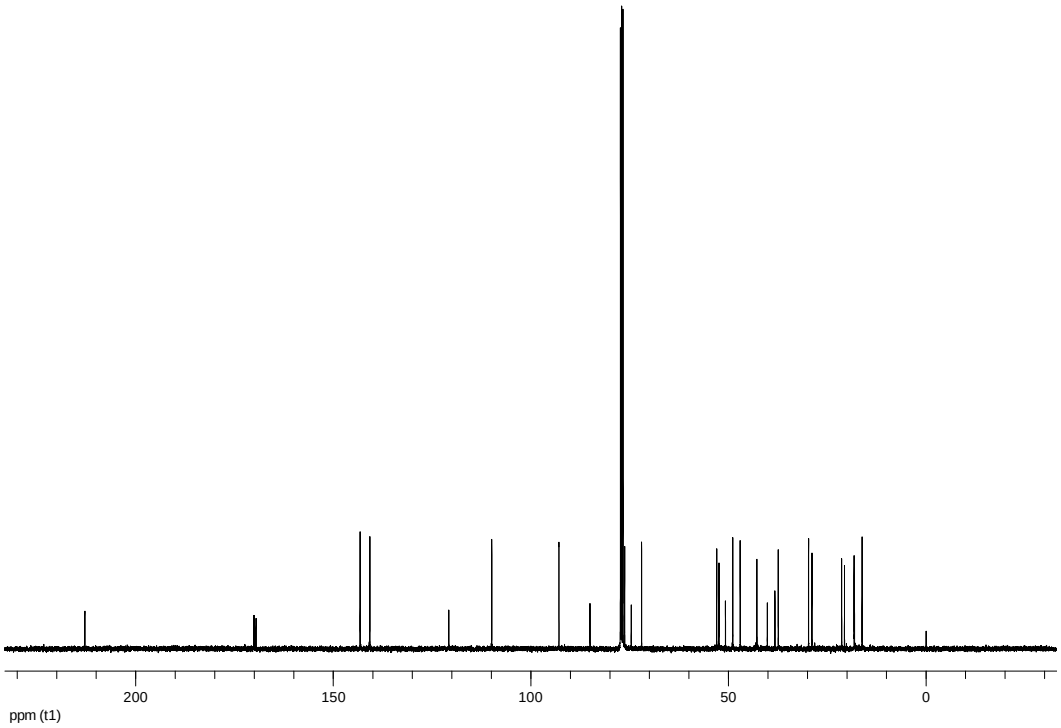


Figure S-7 ^{13}C NMR (100 MHz) spectrum of compound **2** ($CDCl_3$)

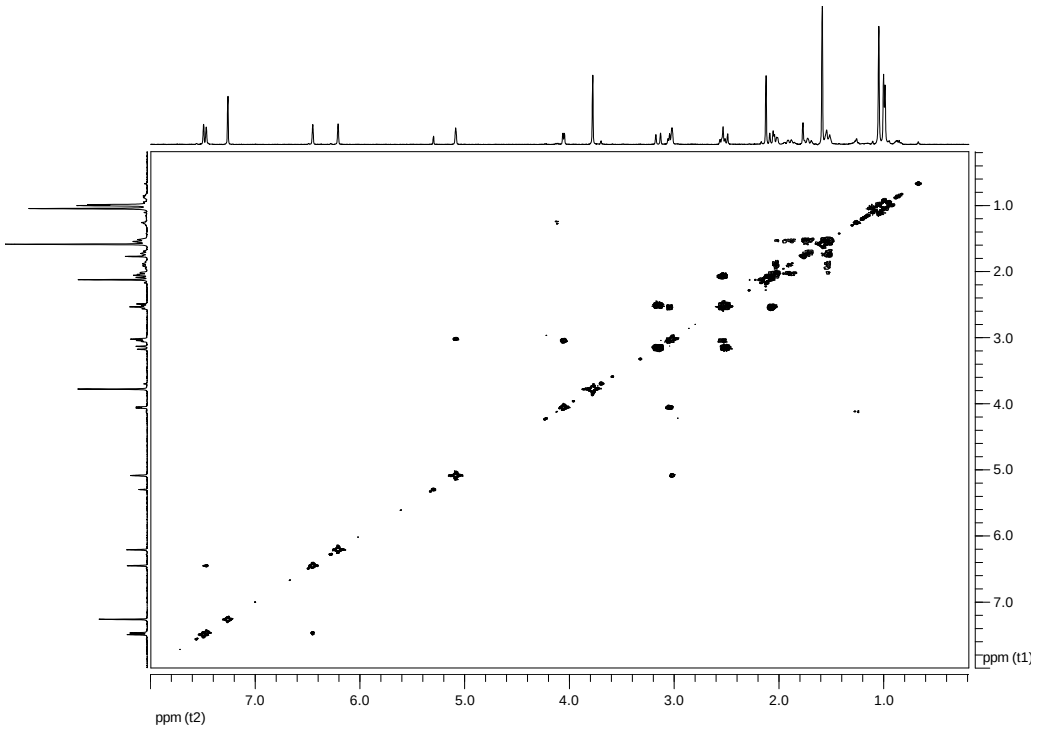


Figure S-8 ^{1}H-^{1}H COSY spectrum of compound **2** ($CDCl_3$)

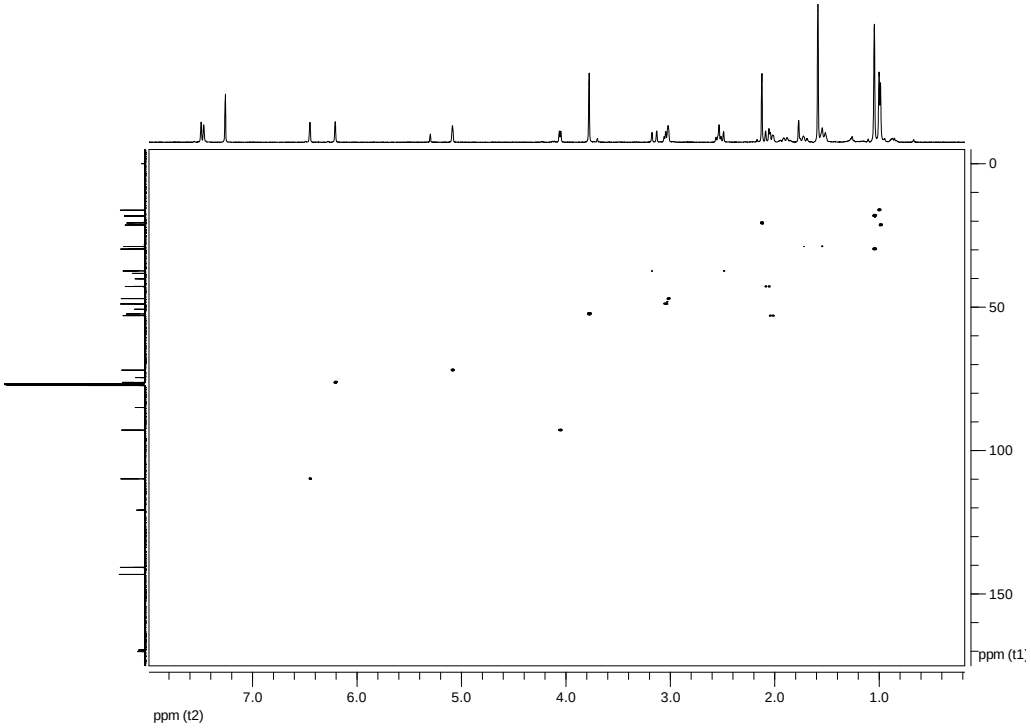


Figure S-9 HSQC spectrum of compound **2** ($CDCl_3$)

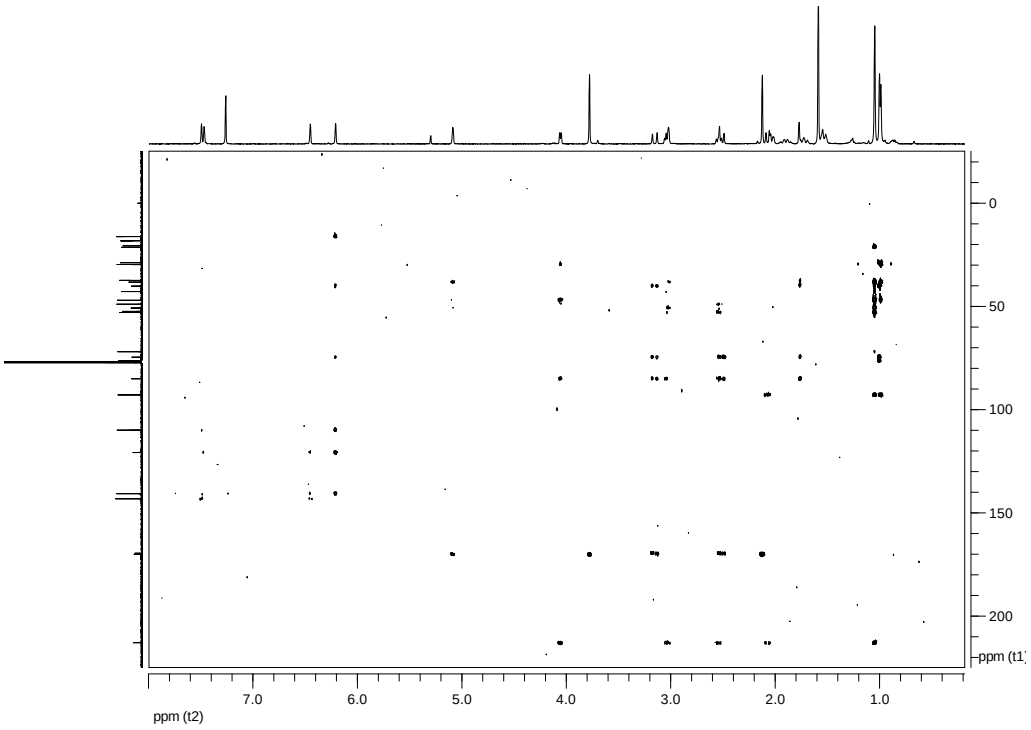


Figure S-10 HMBC spectrum of compound **2** ($CDCl_3$)

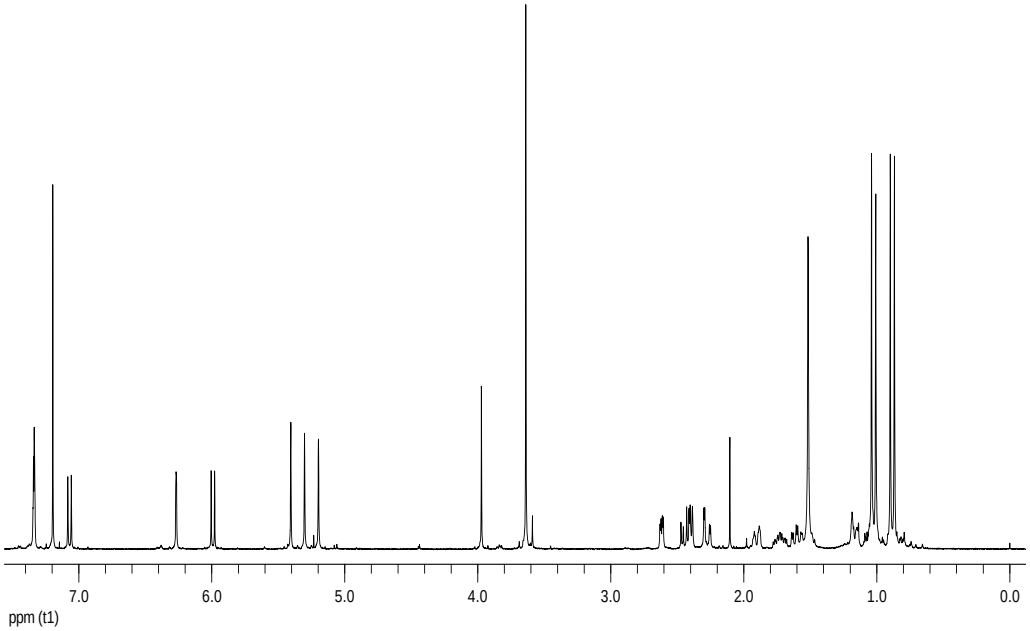


Figure S-11 ^{1}H NMR (400 MHz) spectrum of compound **3** ($CDCl_3$)

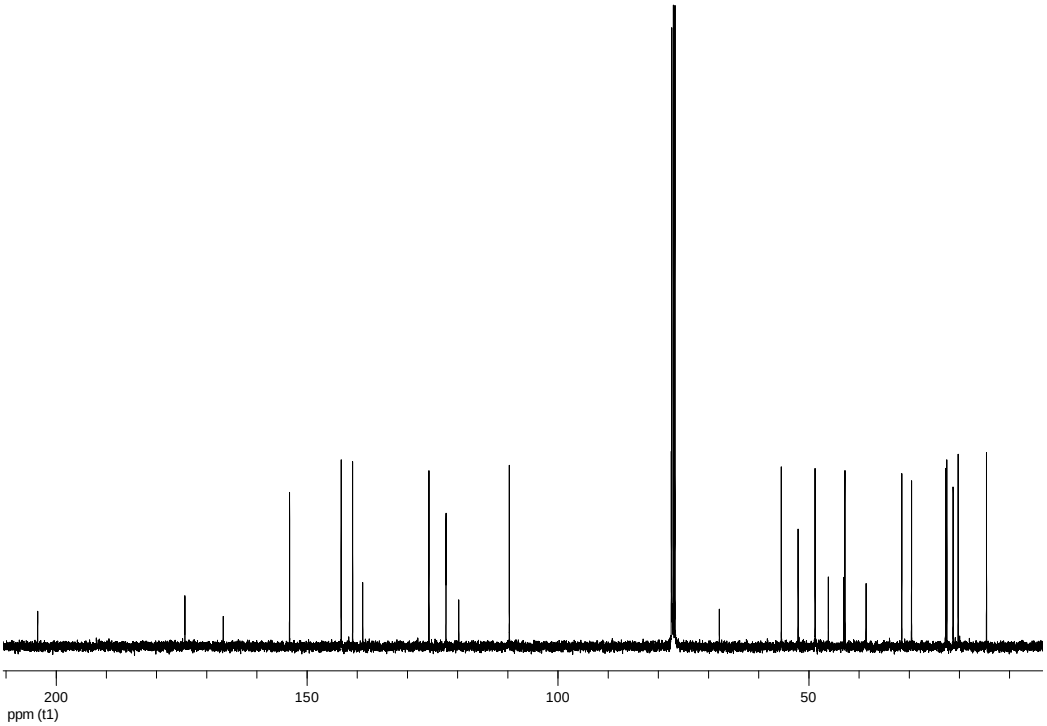


Figure S-12 ^{13}C NMR (100 MHz) spectrum of compound **3** ($CDCl_3$)

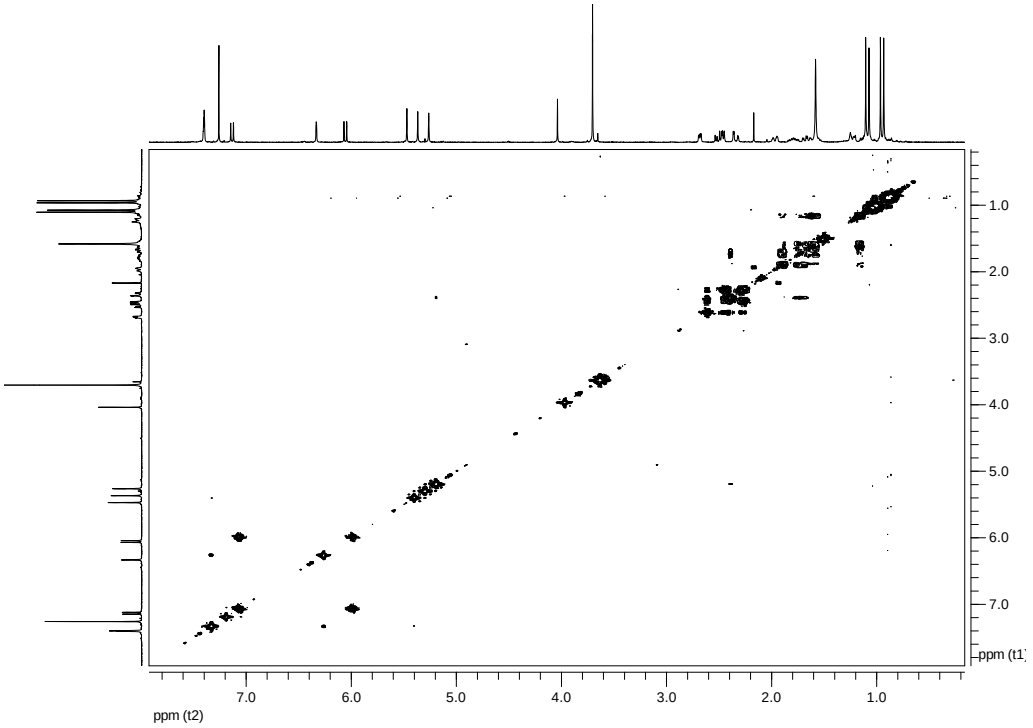


Figure S-13 ^{1}H-^{1}H COSY spectrum of compound **3** ($CDCl_3$)

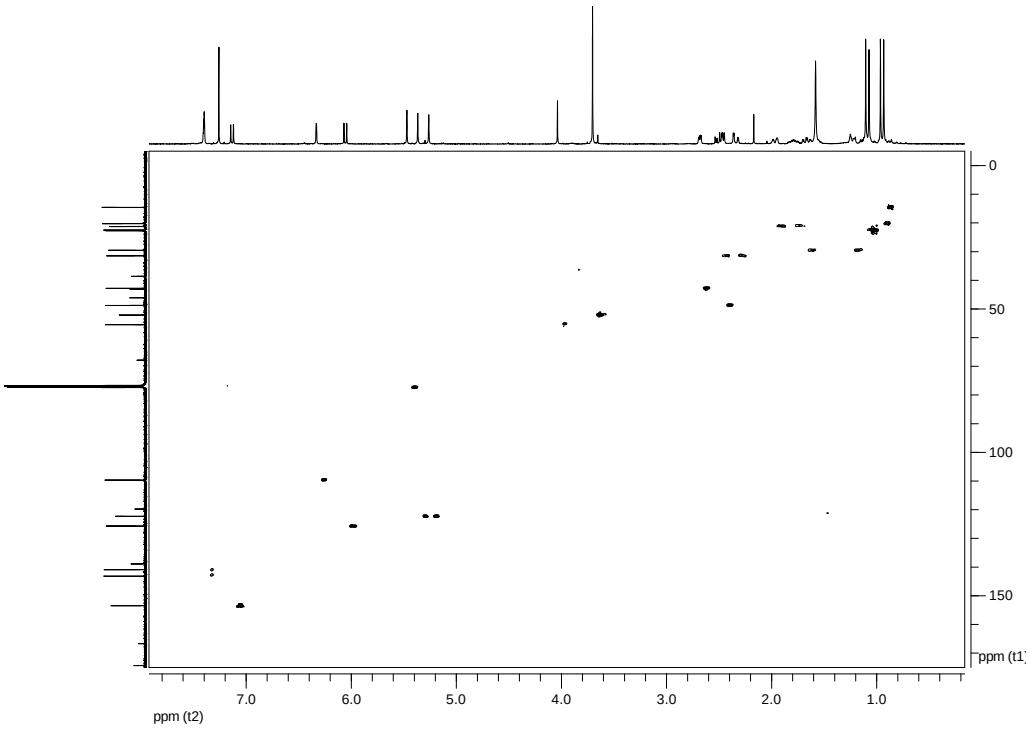


Figure S-14 HSQC spectrum of compound **3** ($CDCl_3$)

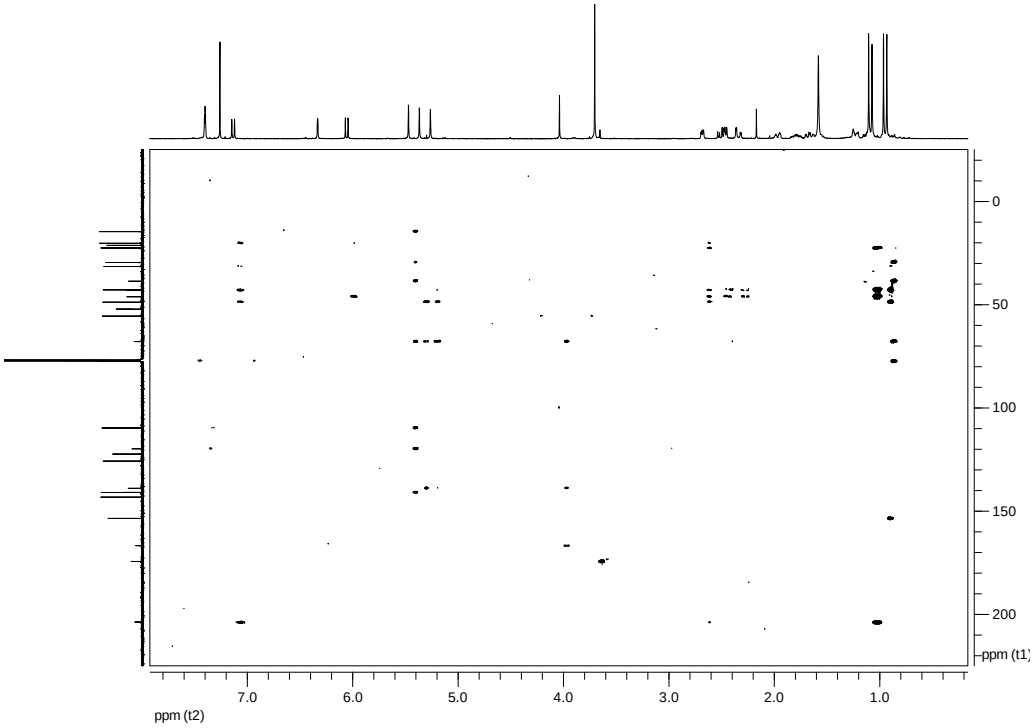


Figure S-15 HMBC spectrum of compound **3** ($CDCl_3$)

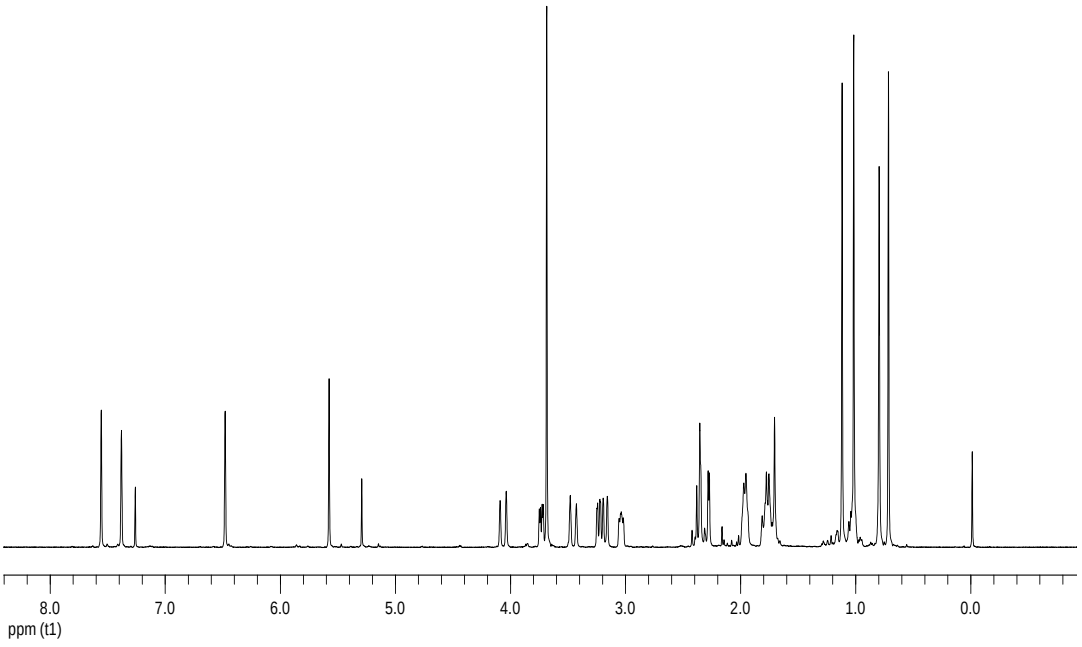


Figure S-16 ^{1}H NMR (400 MHz) spectrum of compound **4** ($CDCl_3$)

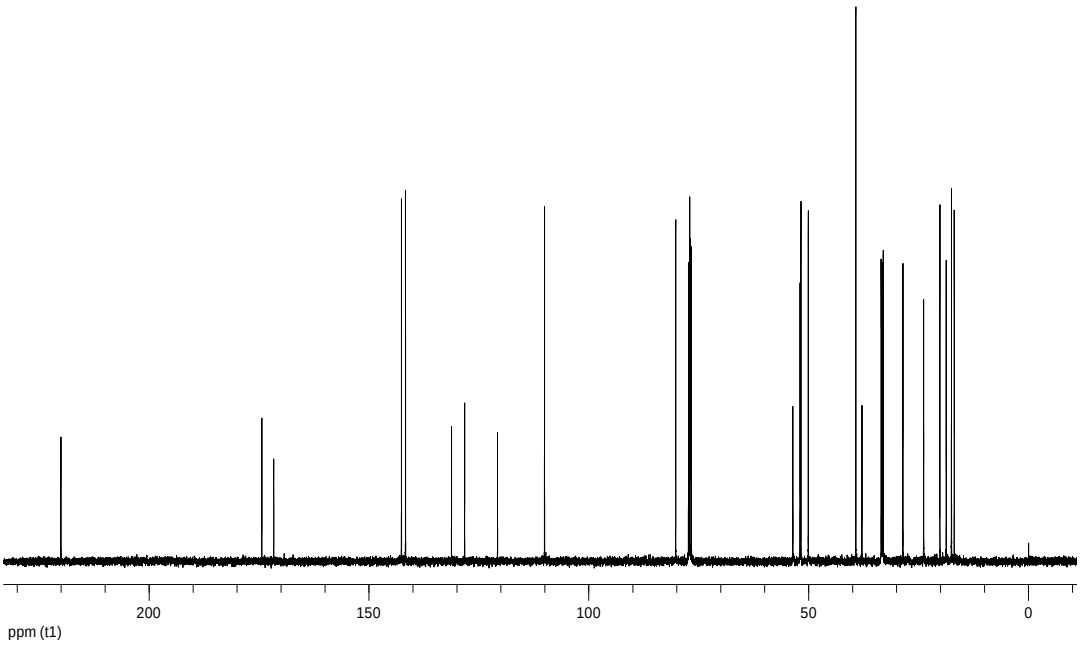


Figure S-17 ^{13}C NMR (100 MHz) spectrum of compound **4** ($CDCl_3$)

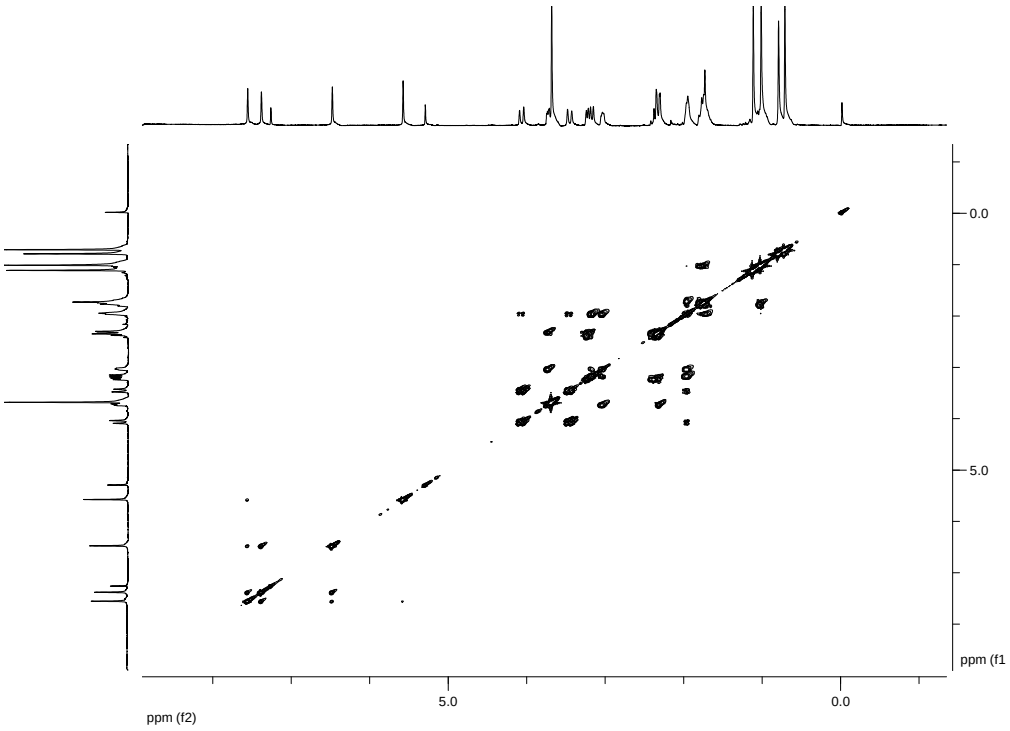


Figure S-18 ^{1}H-^{1}H COSY spectrum of compound **4** ($CDCl_3$)

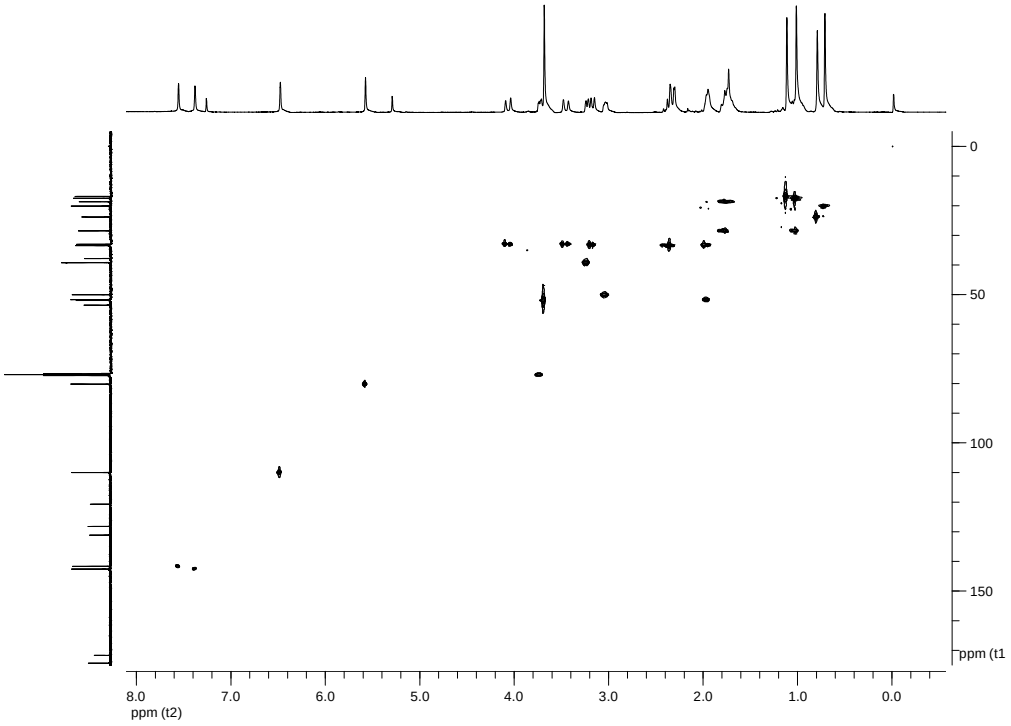


Figure S-19 HSQC spectrum of compound **4** ($CDCl_3$)

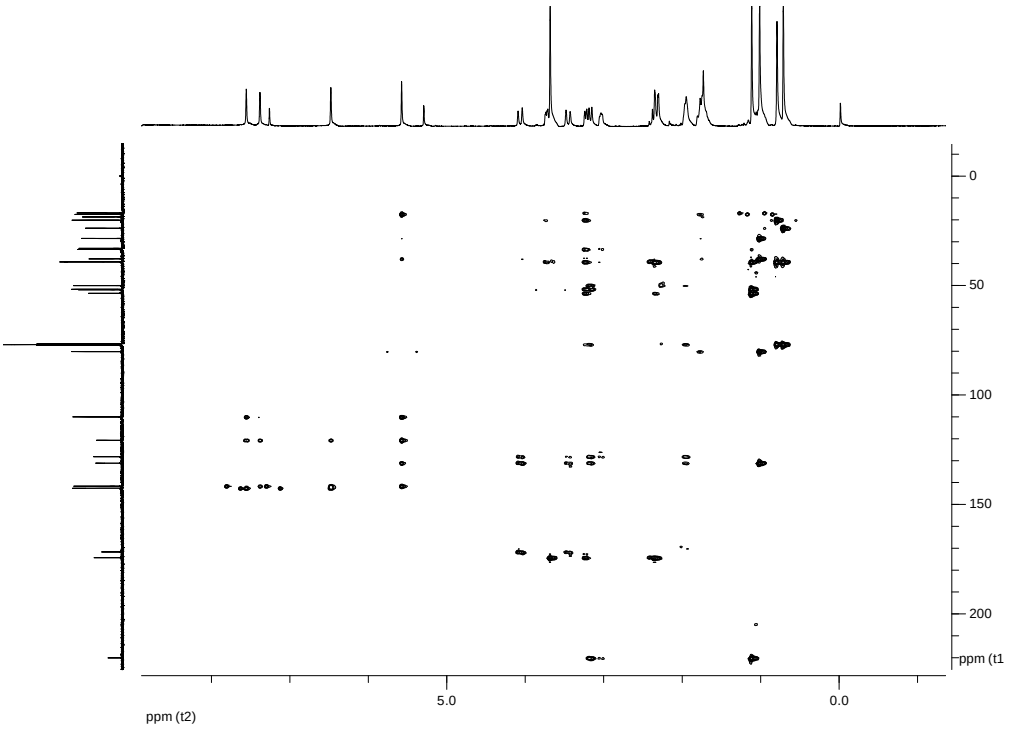


Figure S-20 HMBC spectrum of compound **4** ($CDCl_3$)

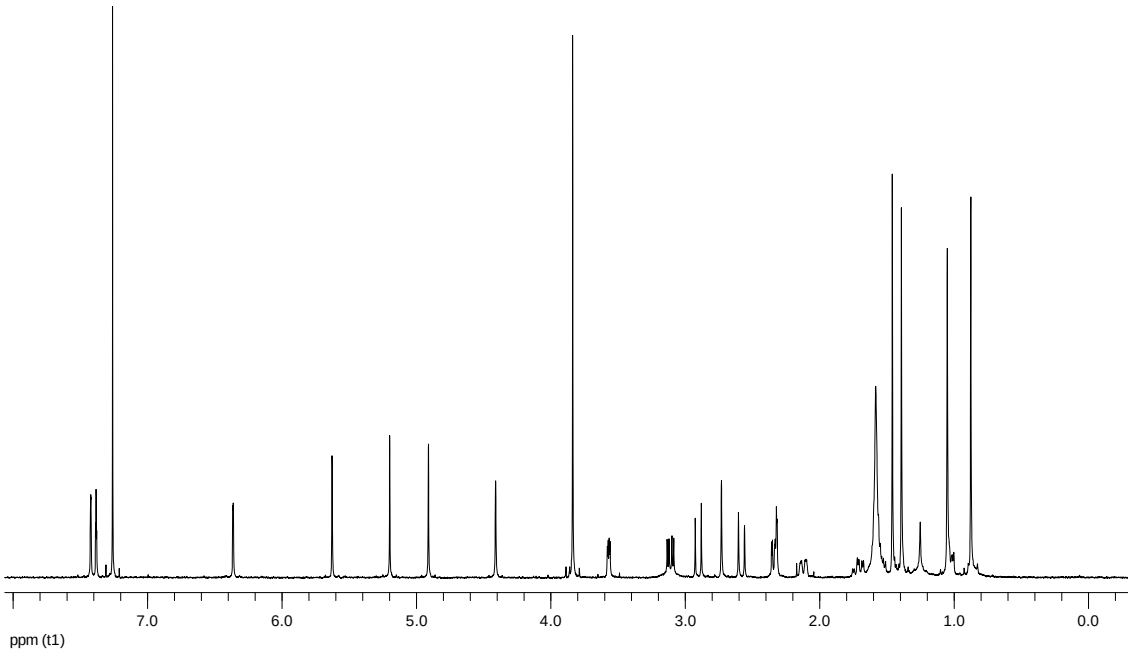


Figure S-21 ^{1}H NMR (400 MHz) spectrum of compound **5** ($CDCl_3$)

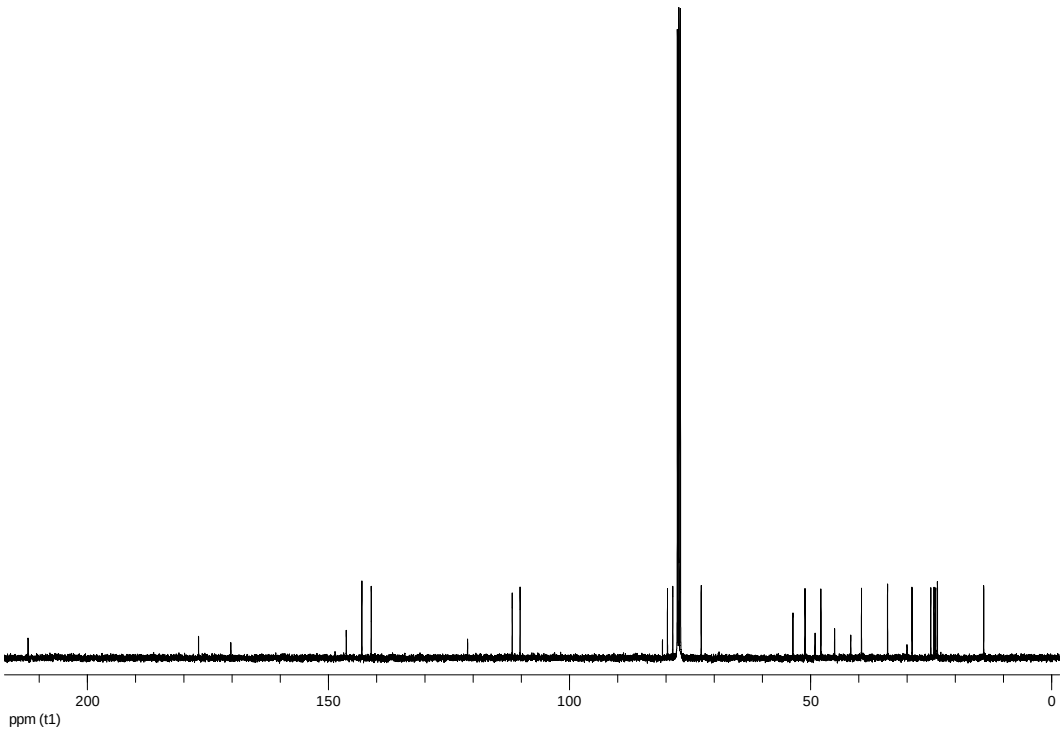


Figure S-22 ^{13}C NMR (100 MHz) spectrum of compound **5** ($CDCl_3$)

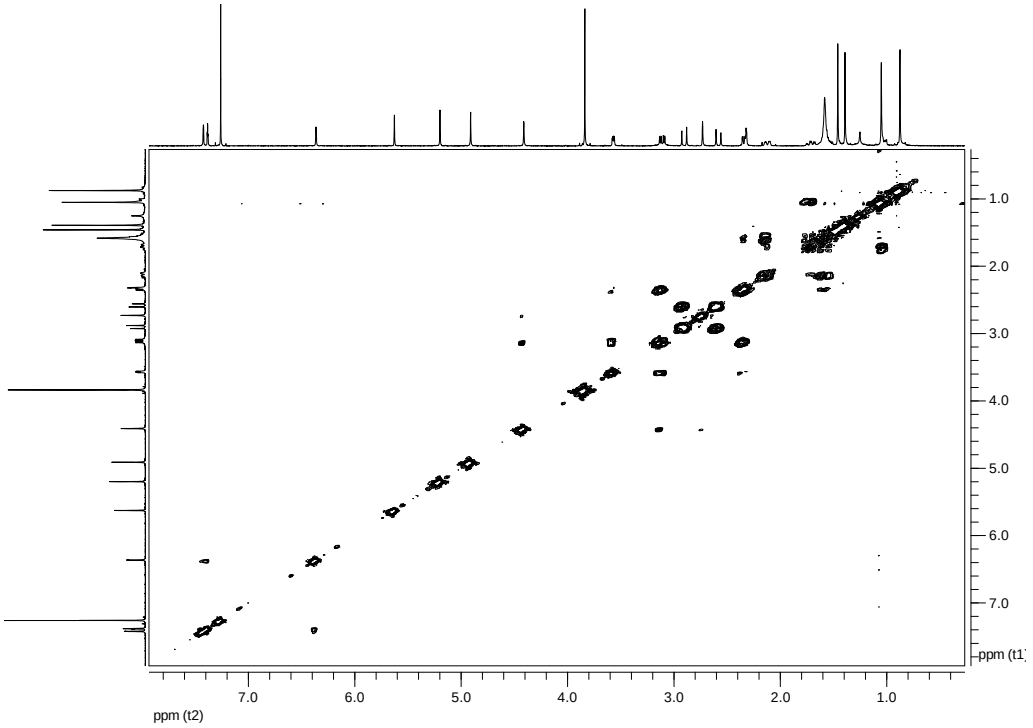


Figure S-23 ^{1}H-^{1}H COSY spectrum of compound **5** ($CDCl_3$)

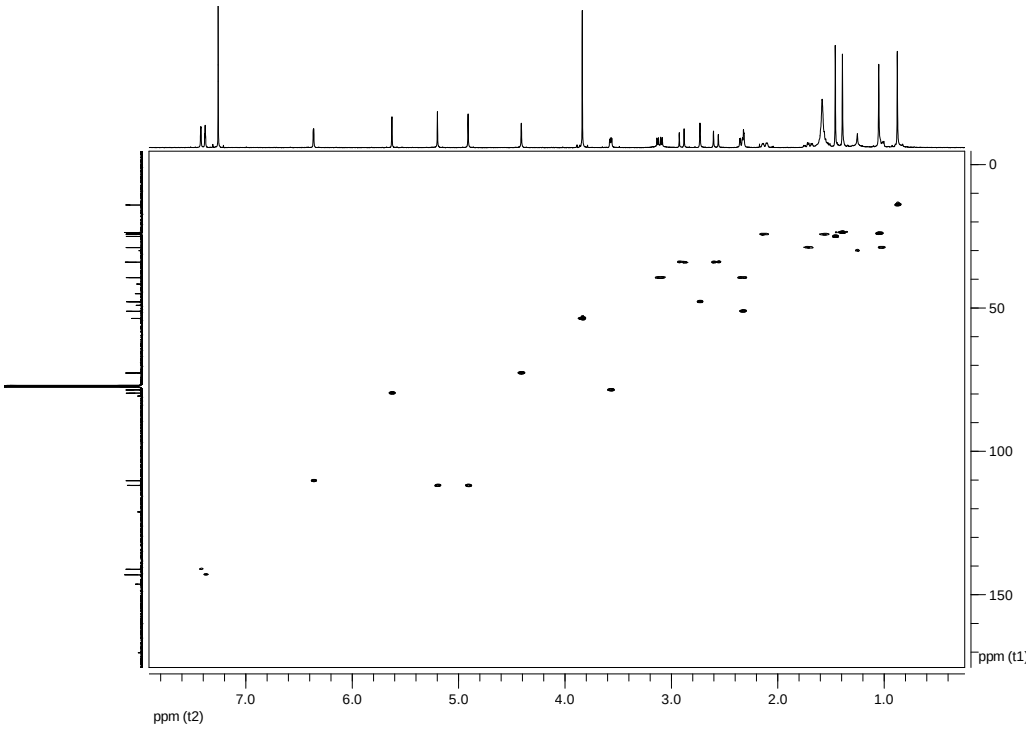


Figure S-24 HSQC spectrum of compound **5** ($CDCl_3$)

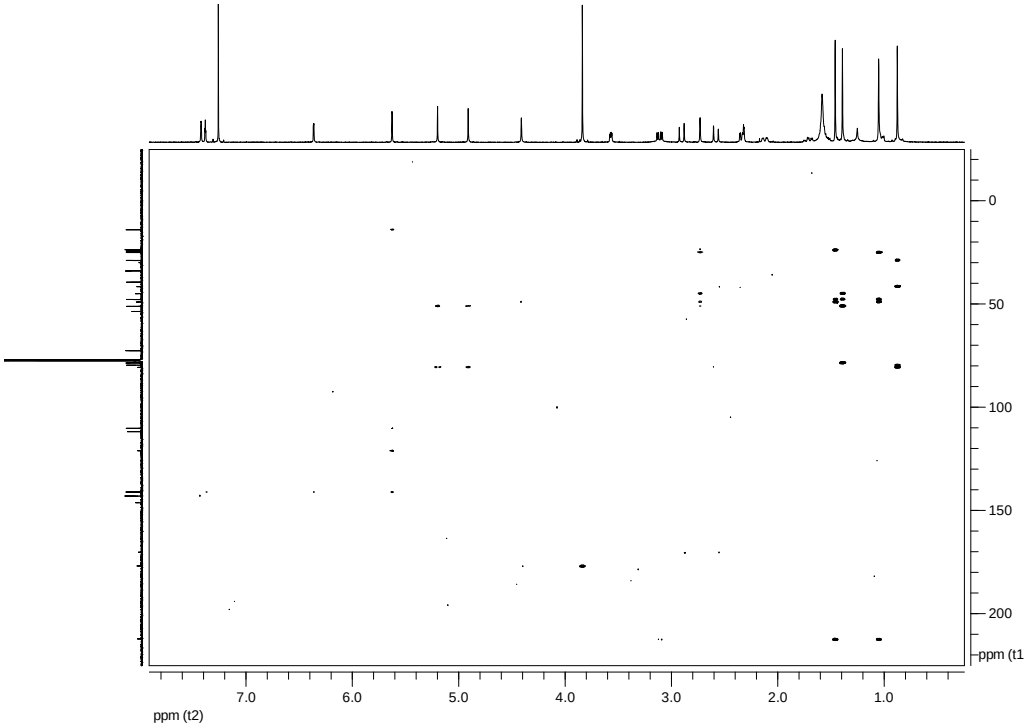


Figure S-25 HMBC spectrum of compound **5** ($CDCl_3$)

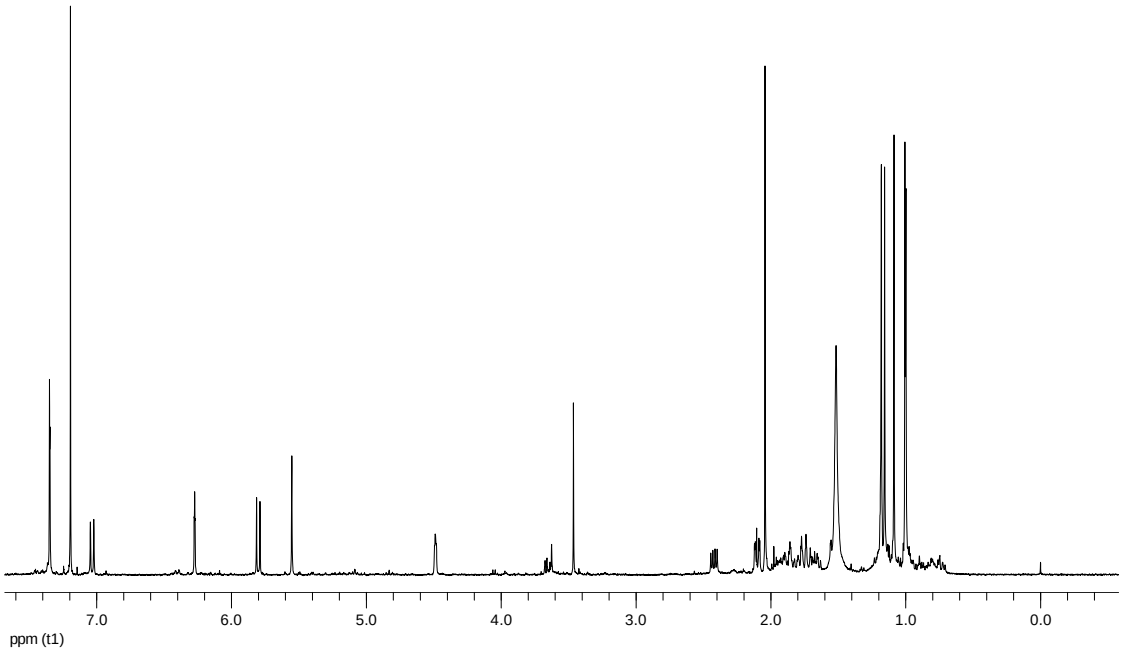


Figure S-26 ^{1}H NMR (400 MHz) spectrum of compound **6** ($CDCl_3$)

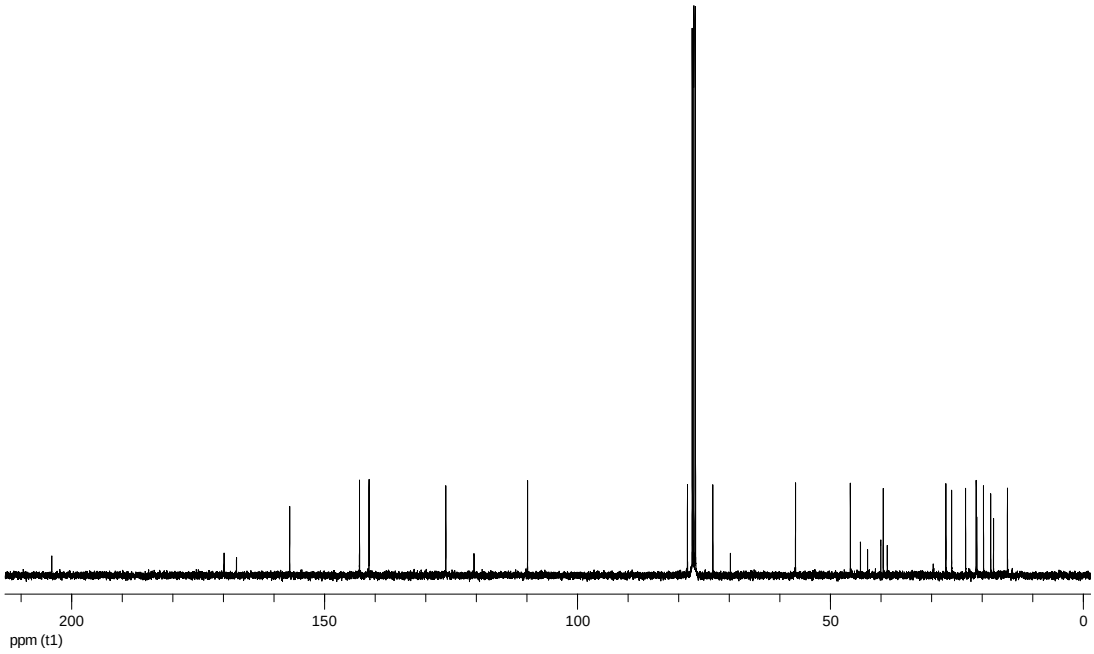


Figure S-27 ^{13}C NMR (100 MHz) spectrum of compound **6** ($CDCl_3$)

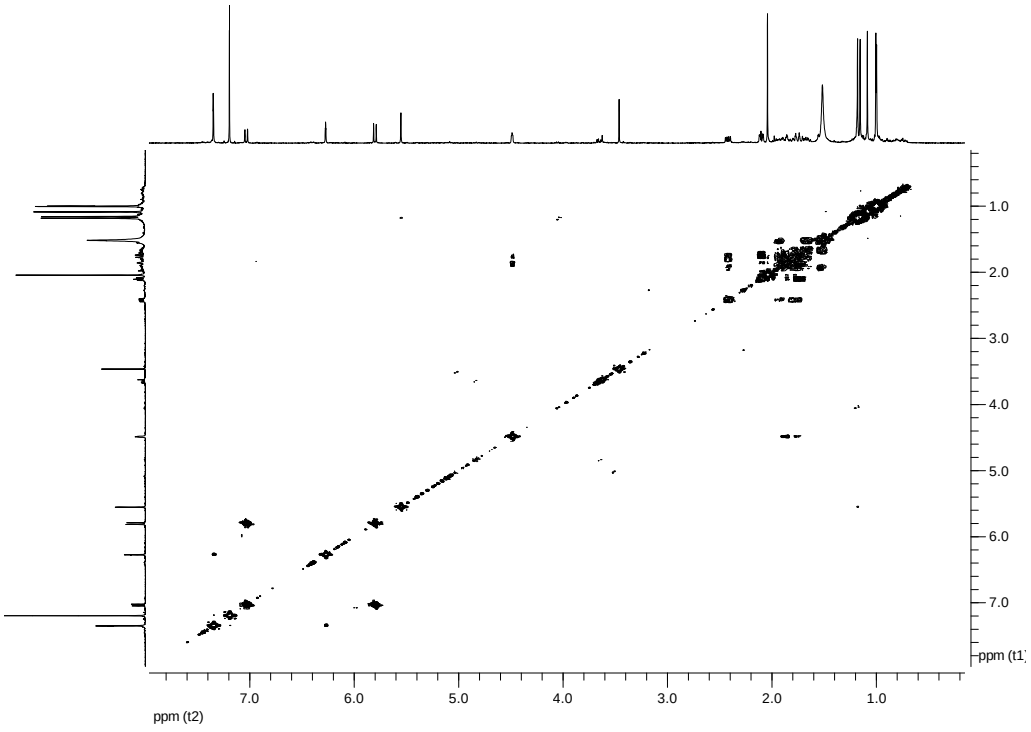


Figure S-28 ^{1}H-^{1}H COSY spectrum of compound **6** ($CDCl_3$)

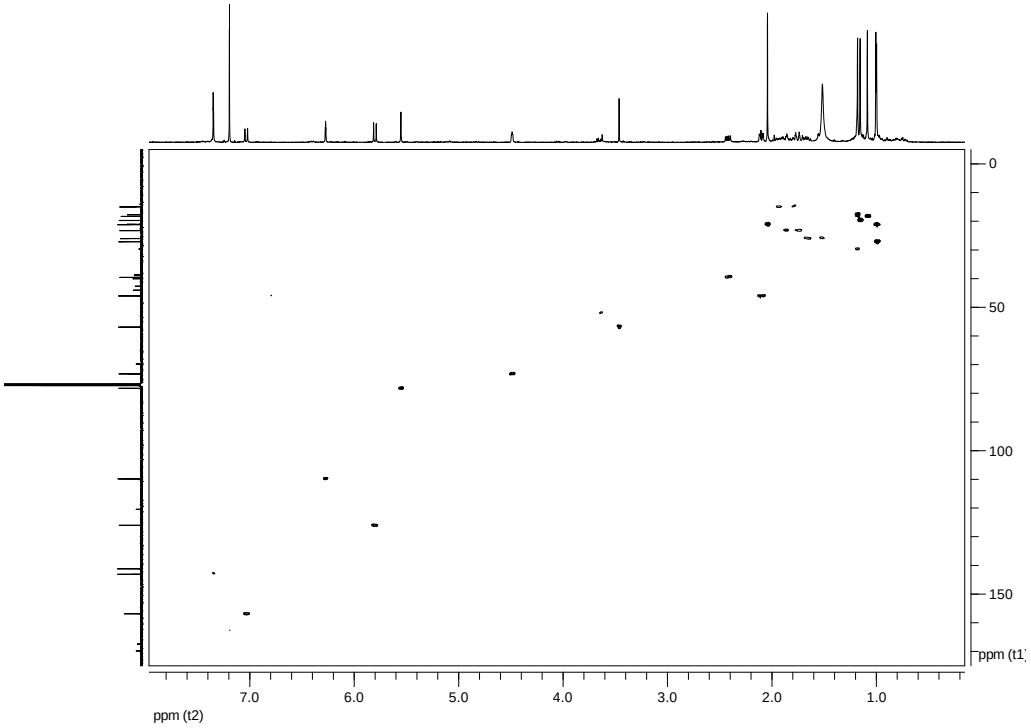


Figure S-29 HSQC spectrum of compound **6** ($CDCl_3$)

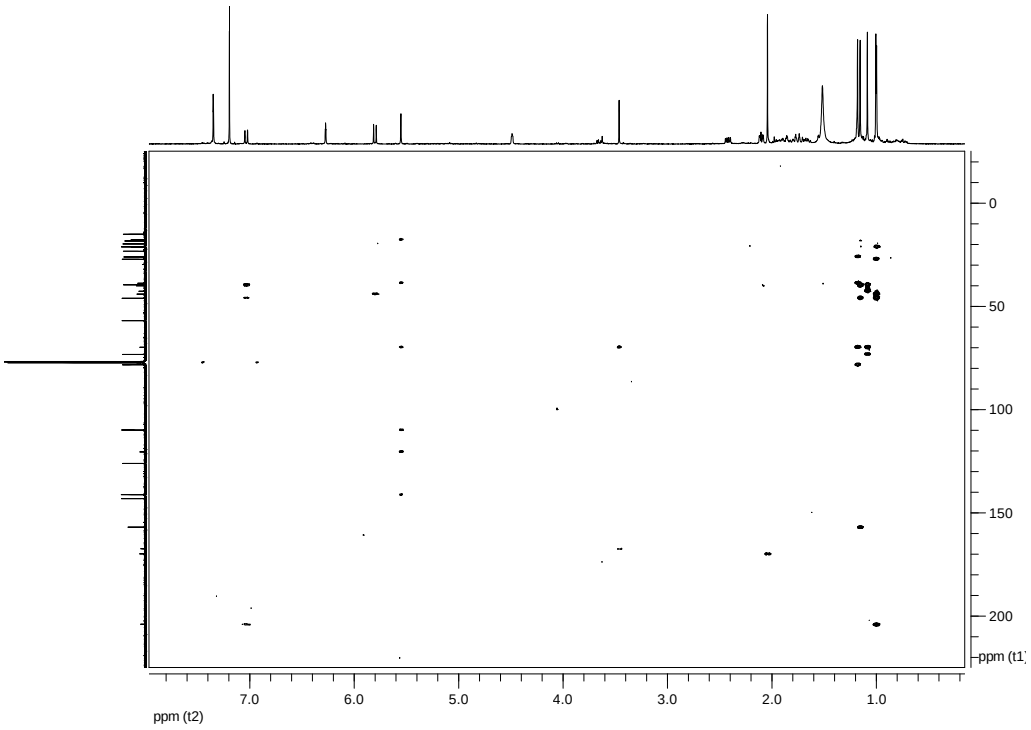


Figure S-30 HMBC spectrum of compound **6** ($CDCl_3$)

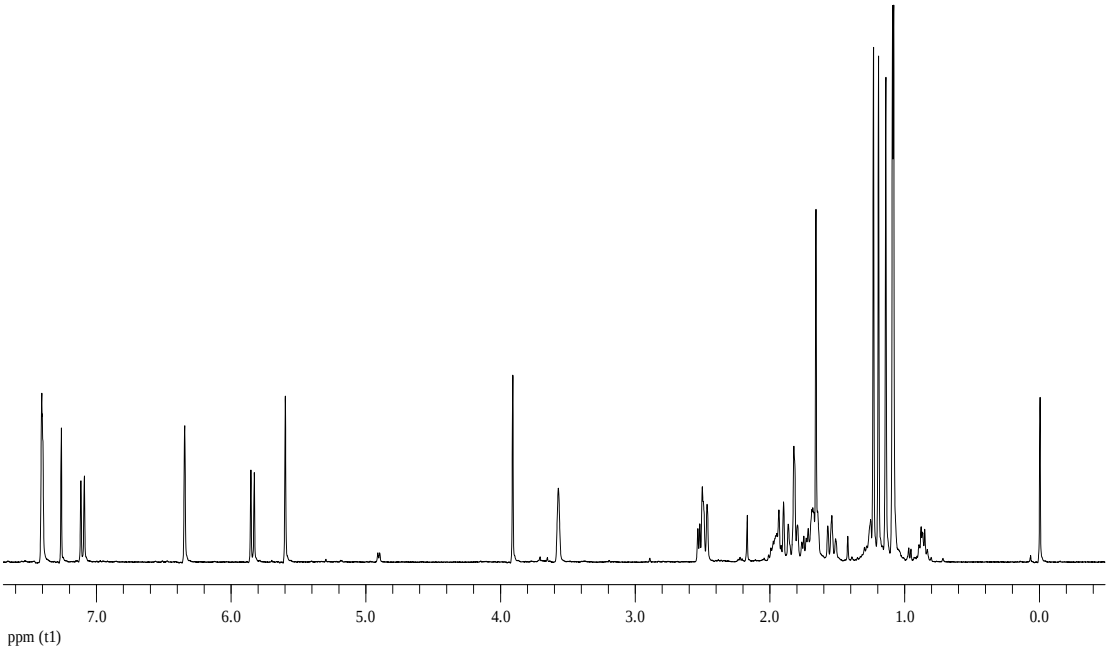


Figure S-31 ^{1}H NMR (400 MHz) spectrum of compound **7** ($CDCl_3$)

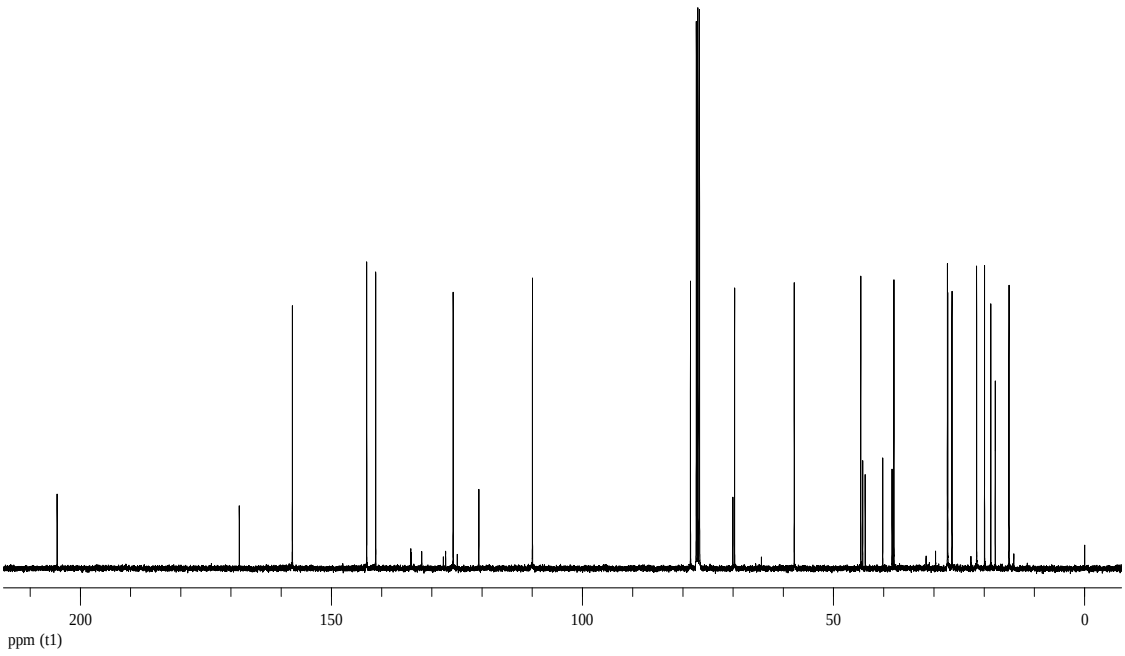


Figure S-32 ^{13}C NMR (100 MHz) spectrum of compound **7** ($CDCl_3$)

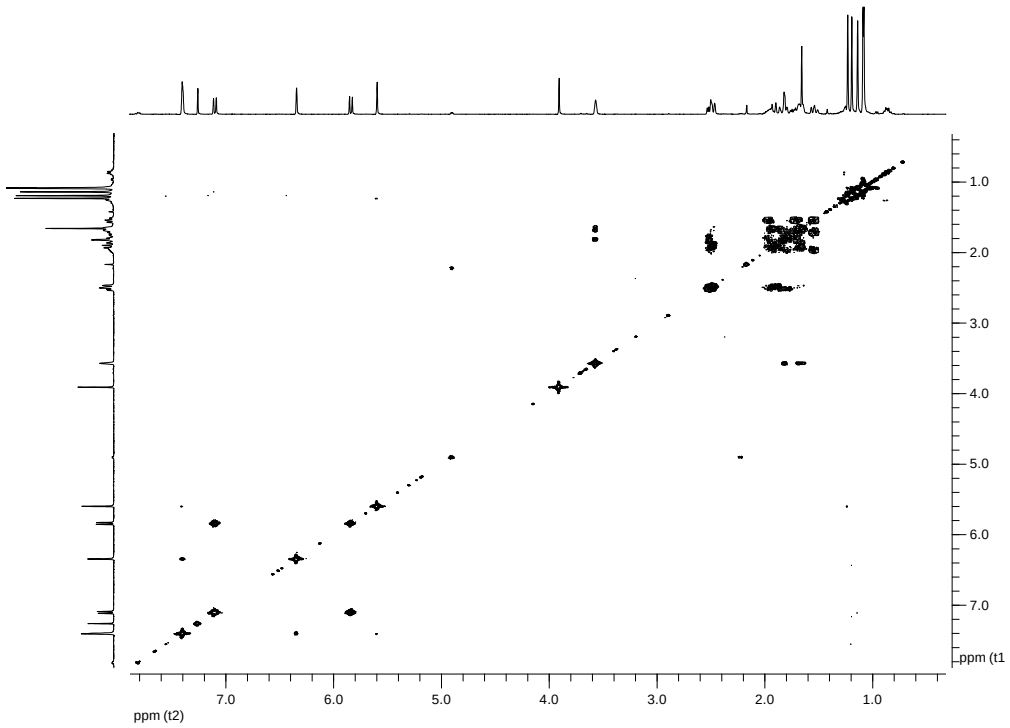


Figure S-33 ^{1}H-^{1}H COSY spectrum of compound **7** ($CDCl_3$)

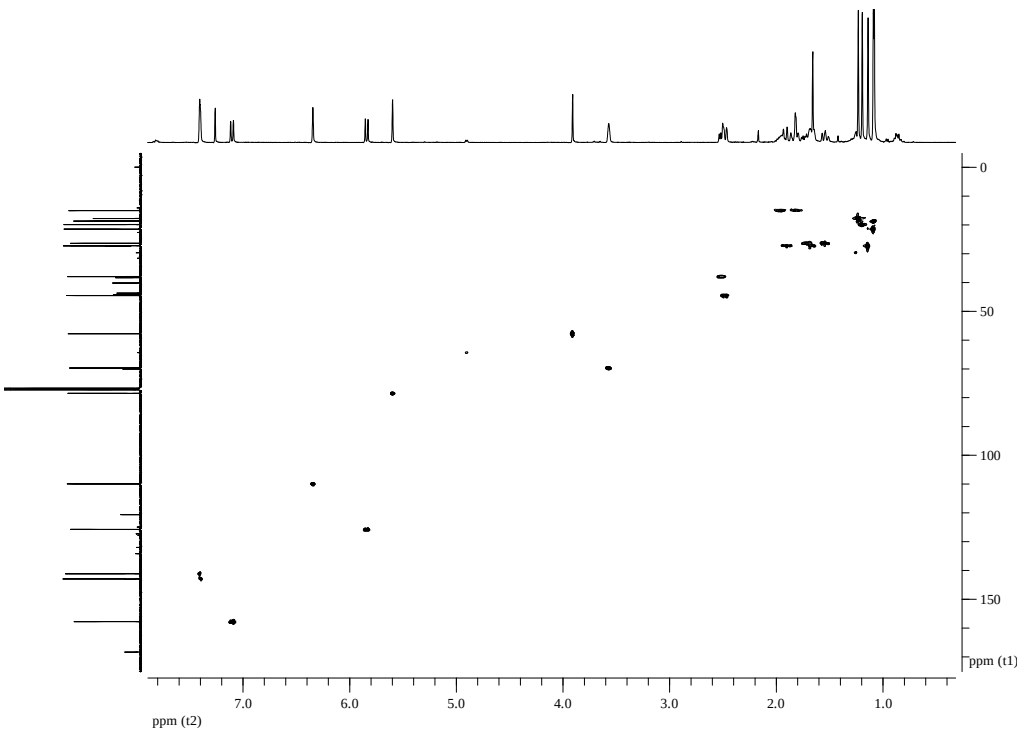


Figure S-34 HSQC spectrum of compound **7** ($CDCl_3$)

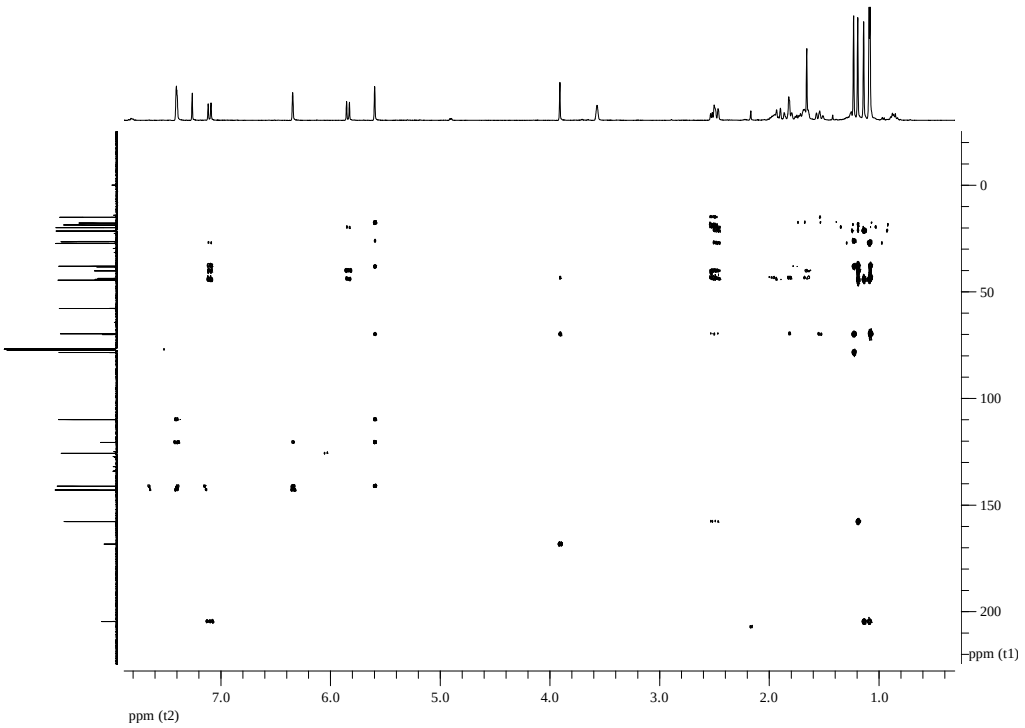


Figure S-35 HMBC spectrum of compound **7** ($CDCl_3$)

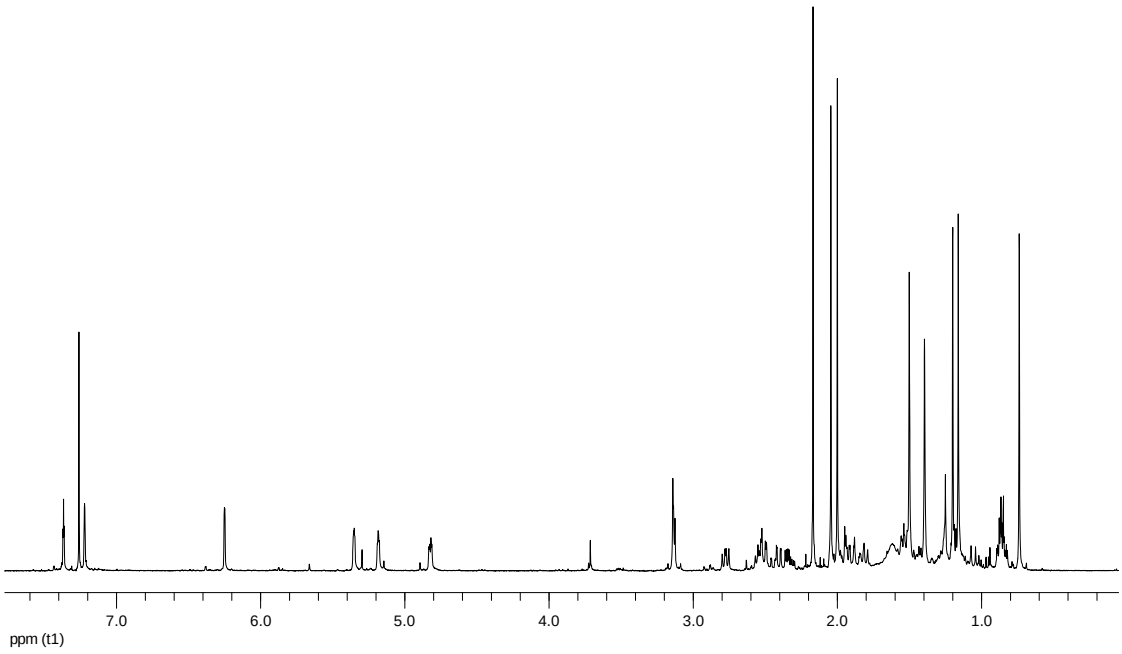


Figure S-36 ^{1}H NMR (400 MHz) spectrum of compound **8** ($CDCl_3$)

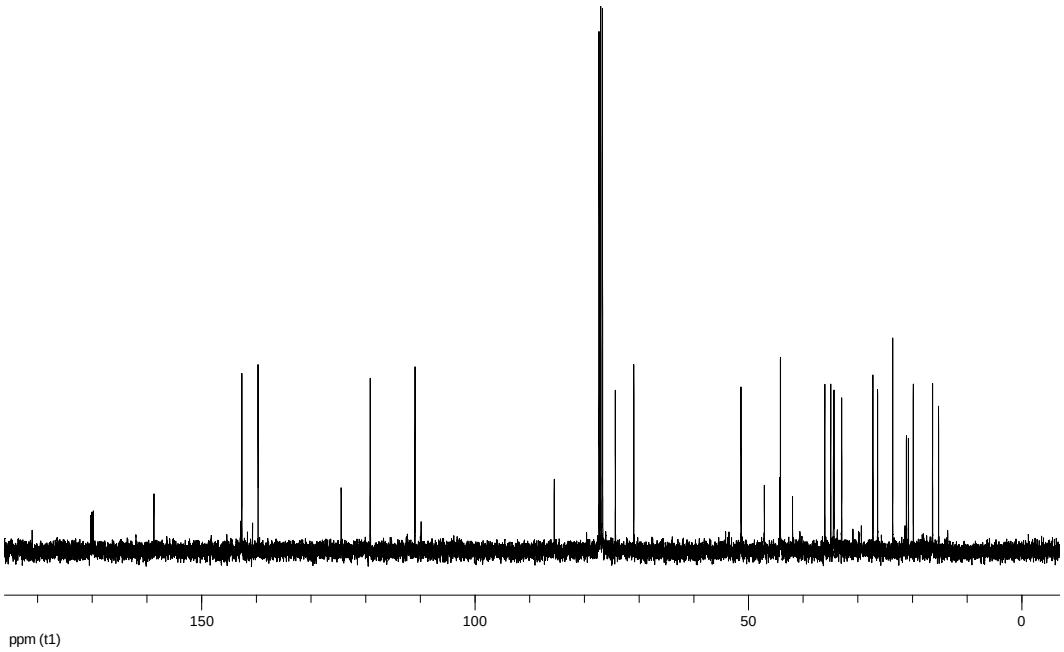


Figure S-37 ^{13}C NMR (100 MHz) spectrum of compound **8** ($CDCl_3$)

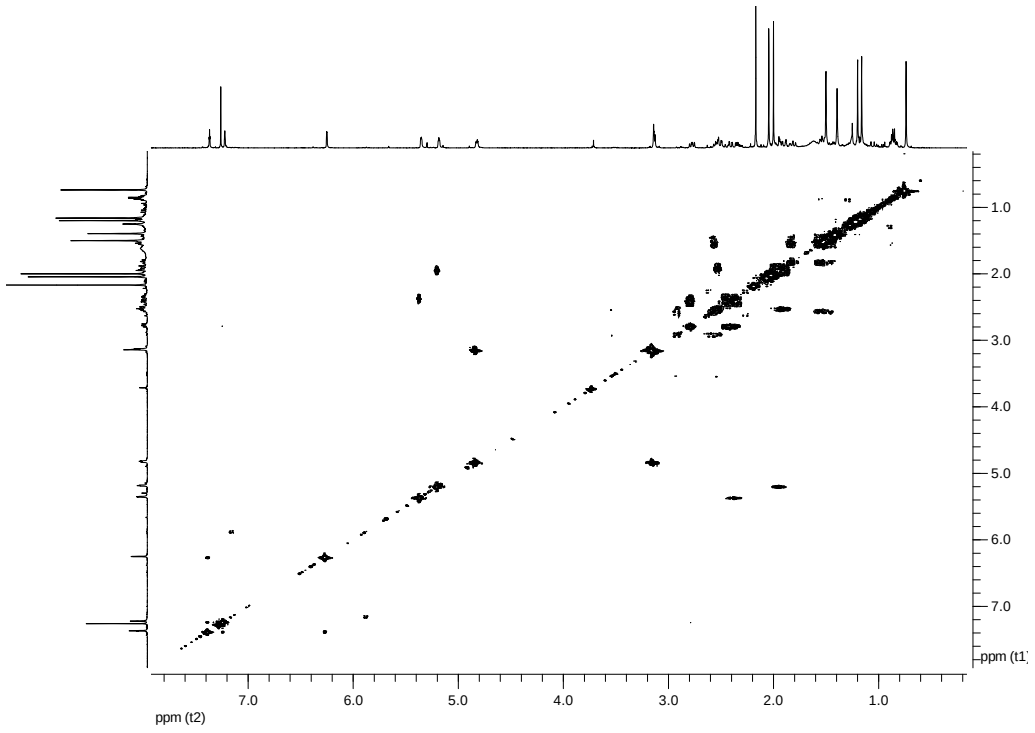


Figure S-38 ^{1}H-^{1}H COSY spectrum of compound **8** ($CDCl_3$)

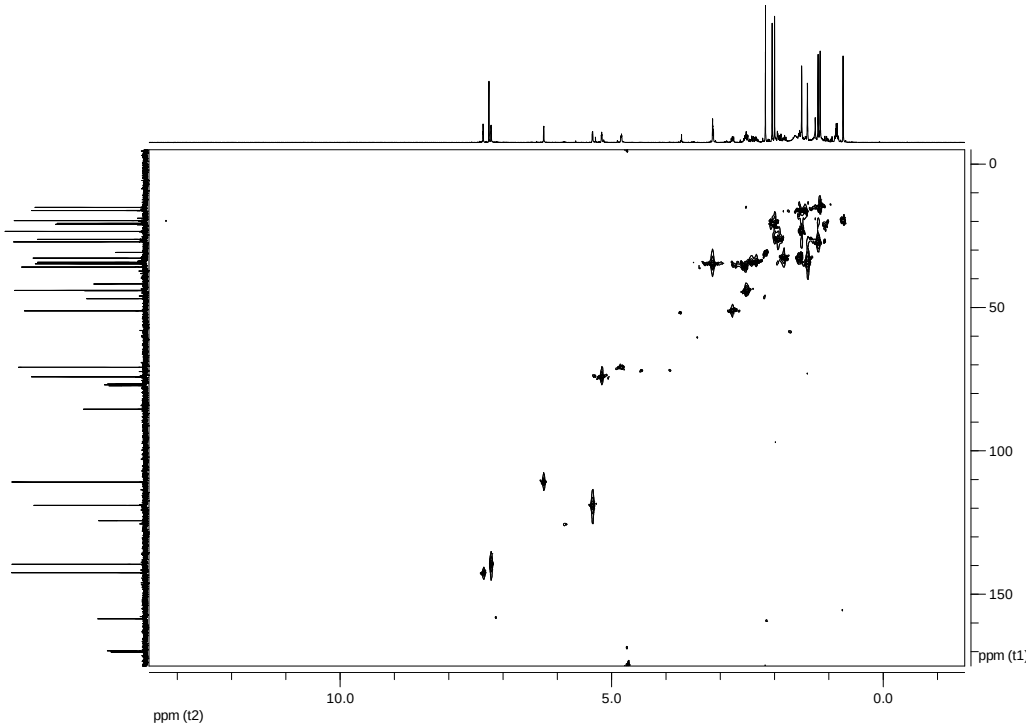


Figure S-39 HSQC spectrum of compound **8** ($CDCl_3$)

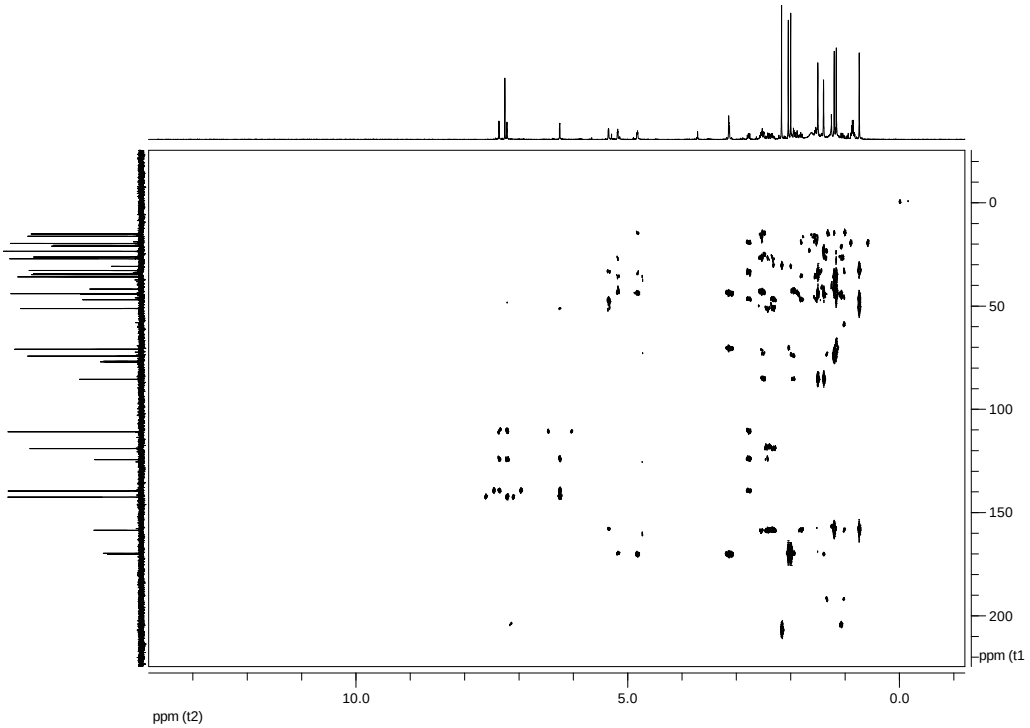


Figure S-40 HMBC spectrum of compound **8** ($CDCl_3$)

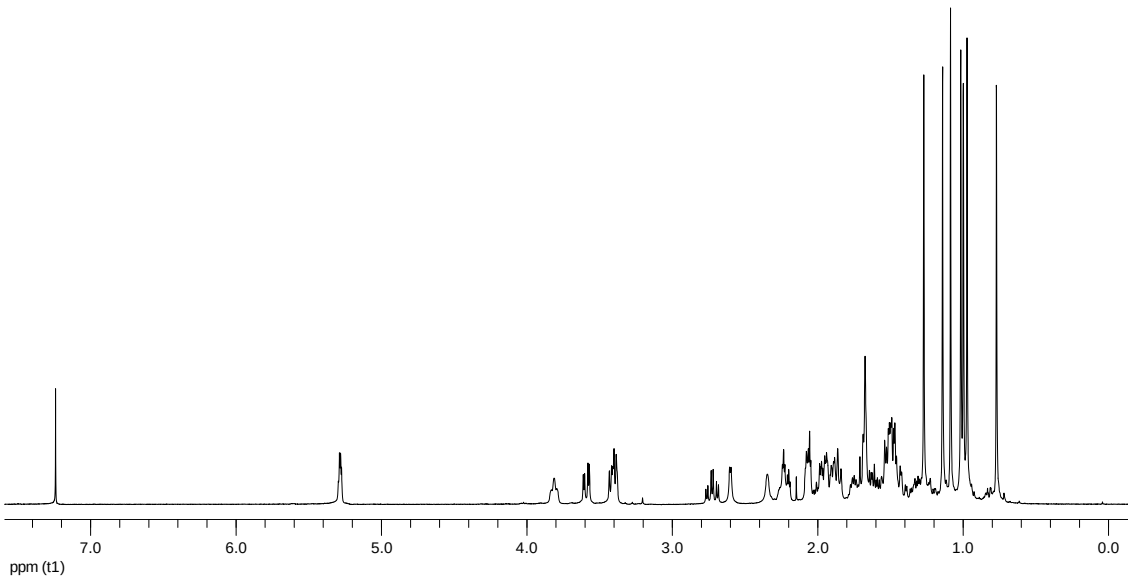


Figure S-51 ^{1}H NMR (400 MHz) spectrum of compound **9** ($CDCl_3$)

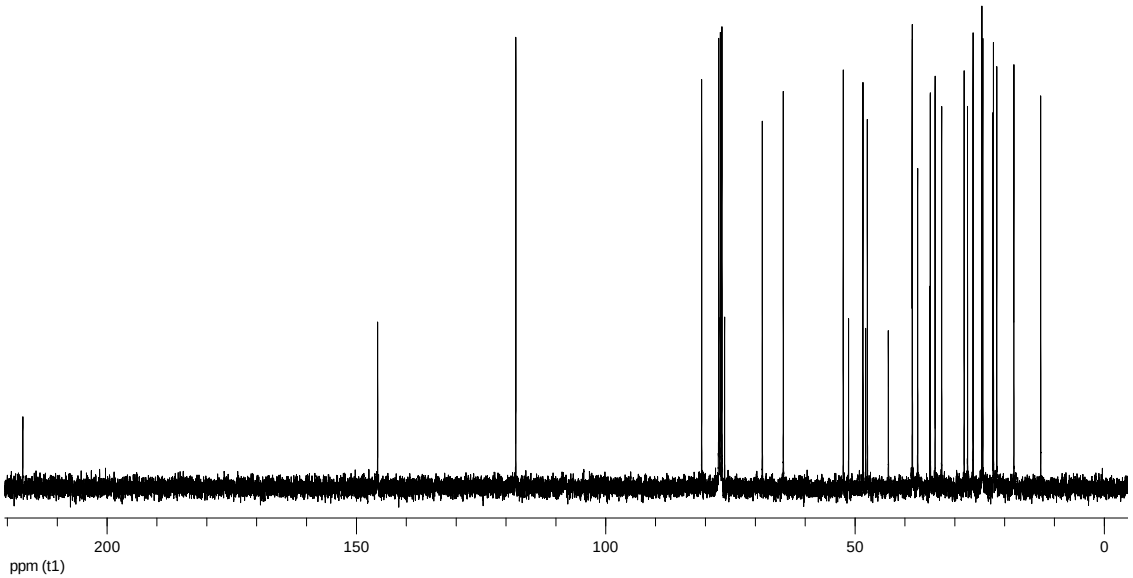


Figure S-52 ^{13}C NMR (100 MHz) spectrum of compound **9** ($CDCl_3$)

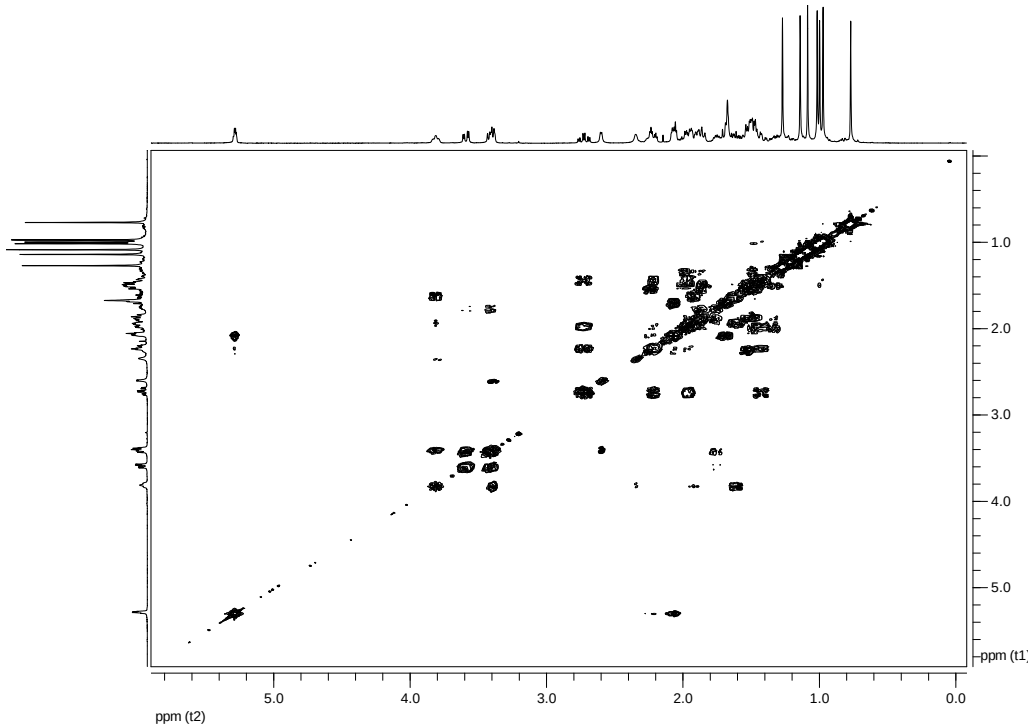


Figure S-53 ^{1}H-^{1}H COSY spectrum of compound **9** ($CDCl_3$)

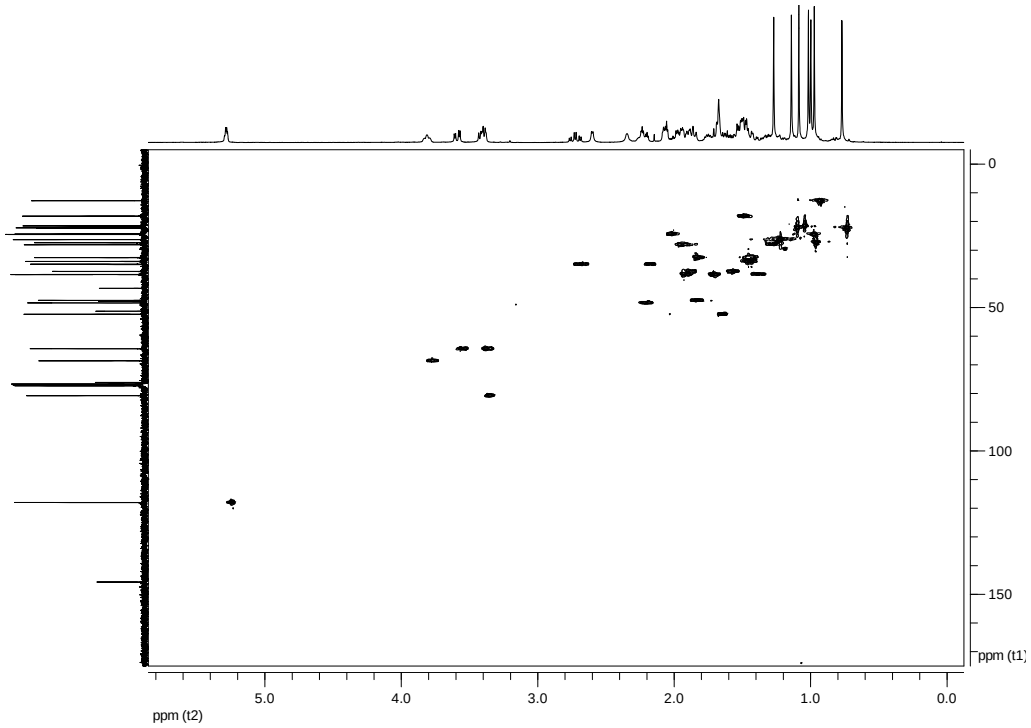


Figure S-50 HSQC spectrum of compound **9** ($CDCl_3$)

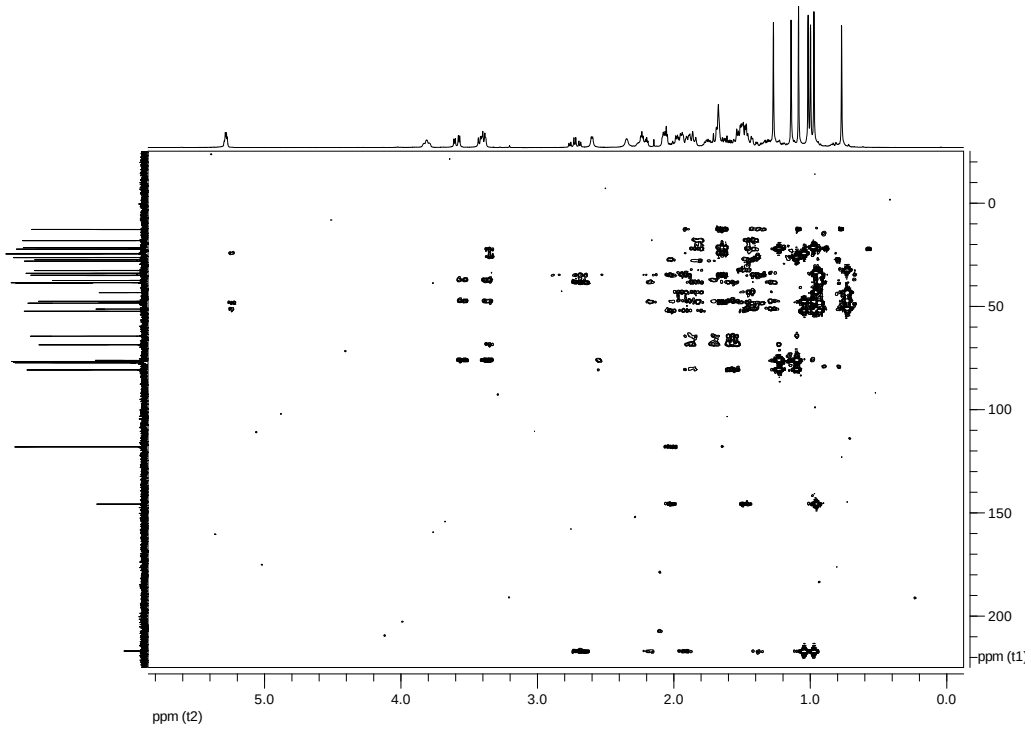


Figure S-55 HMBC spectrum of compound **9** ($CDCl_3$)

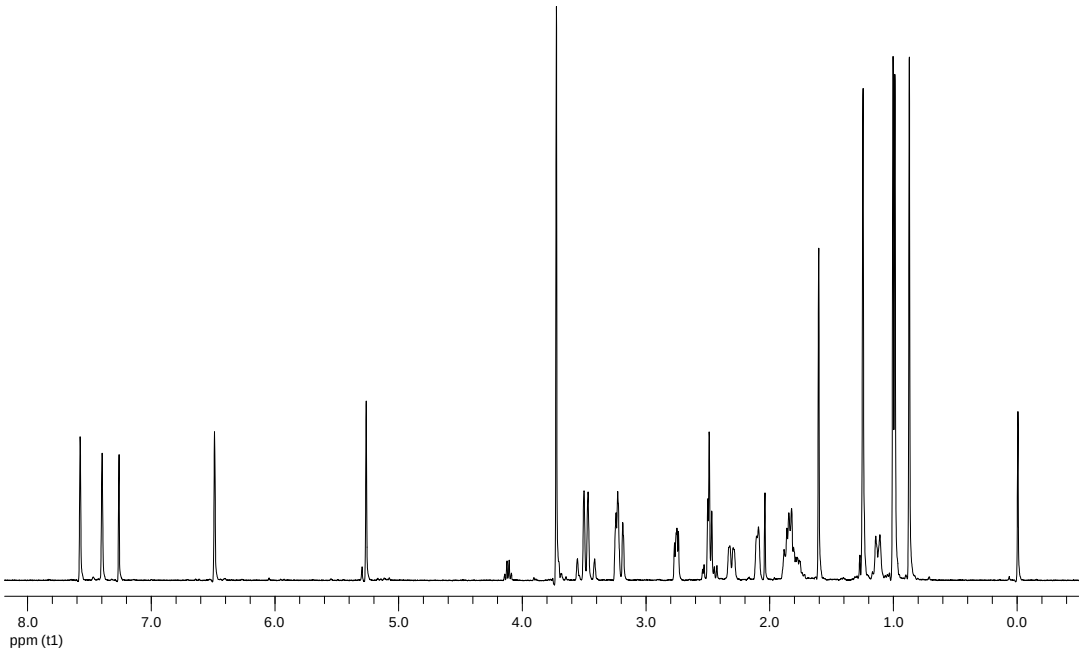


Figure S-56 ^{1}H NMR (400 MHz) spectrum of compound **10** ($CDCl_3$)

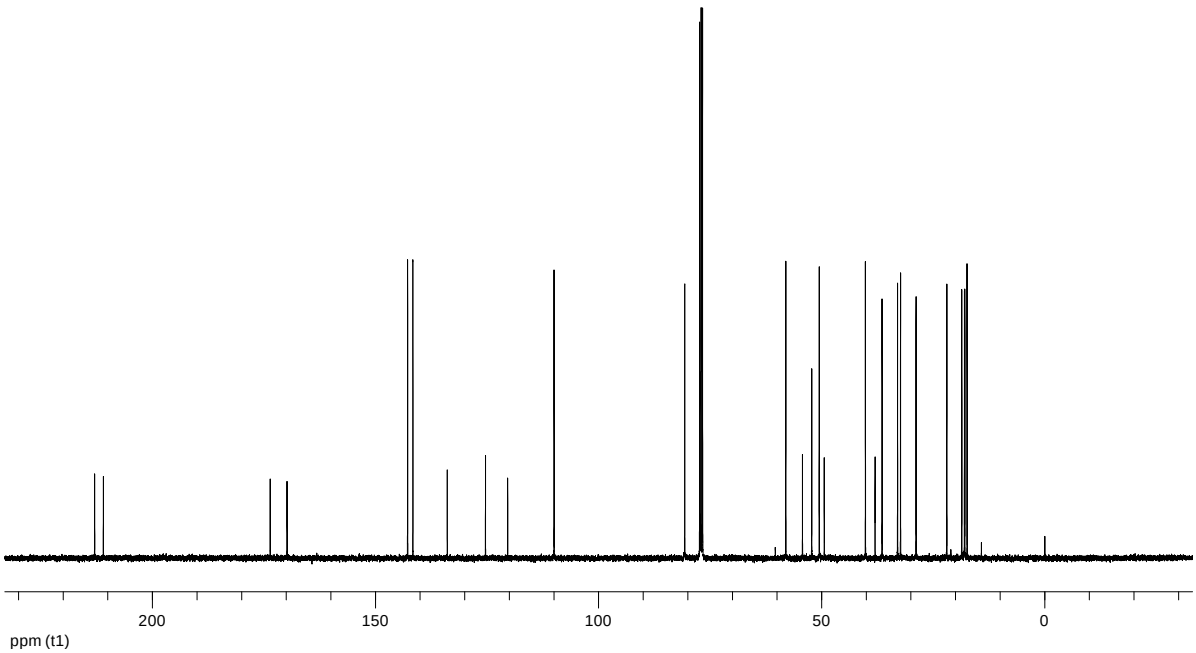


Figure S-57 ^{13}C NMR (100 MHz) spectrum of compound **10** ($CDCl_3$)

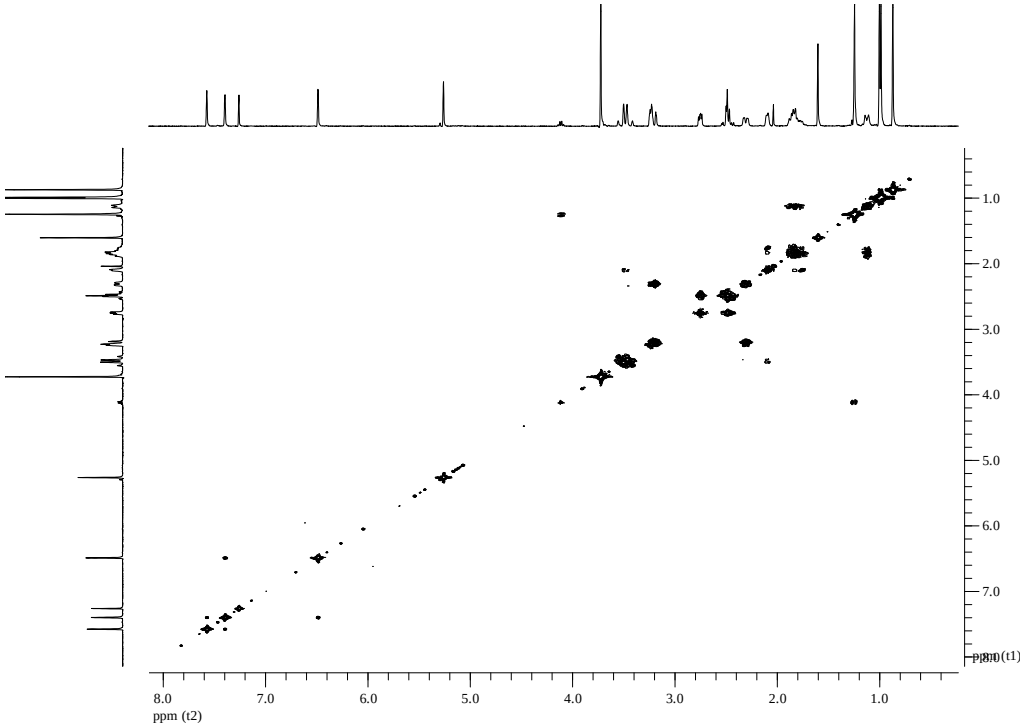


Figure S-58 ^{1}H-^{1}H COSY spectrum of compound **10** ($CDCl_3$)

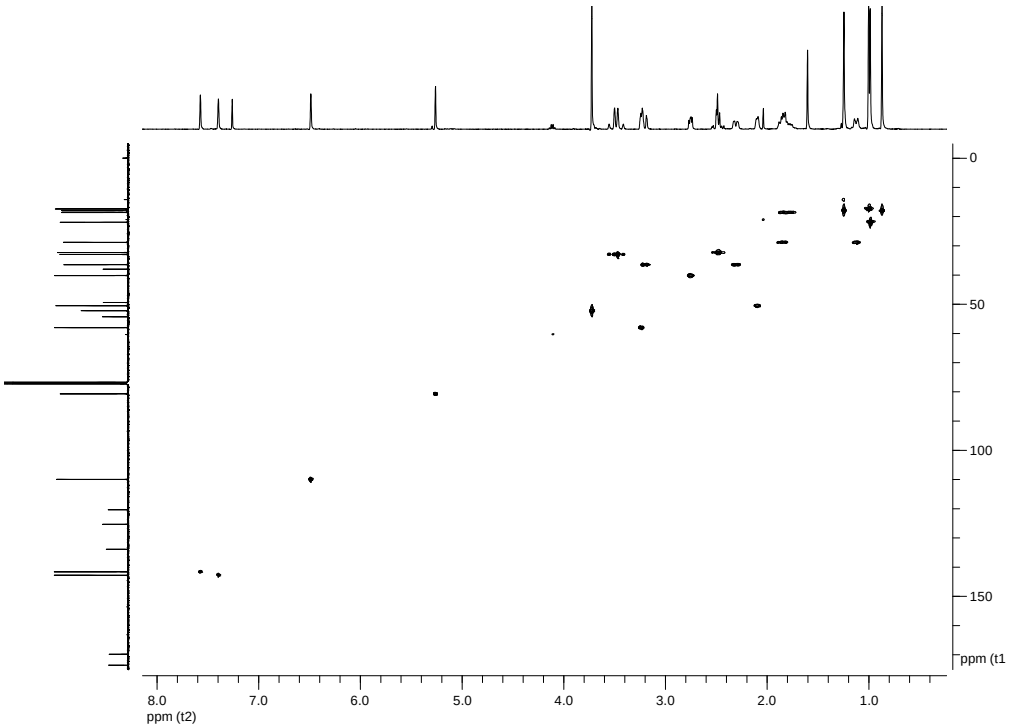


Figure S-59 HSQC spectrum of compound **10** ($CDCl_3$)

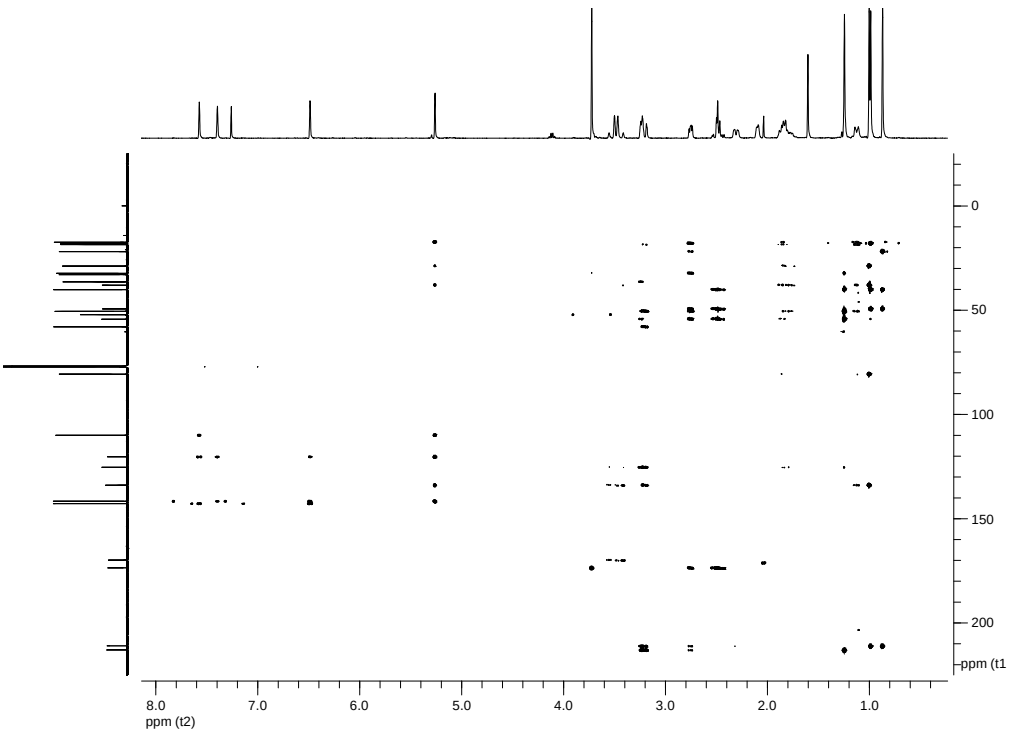


Figure S-60 HMBC spectrum of compound **10** ($CDCl_3$)

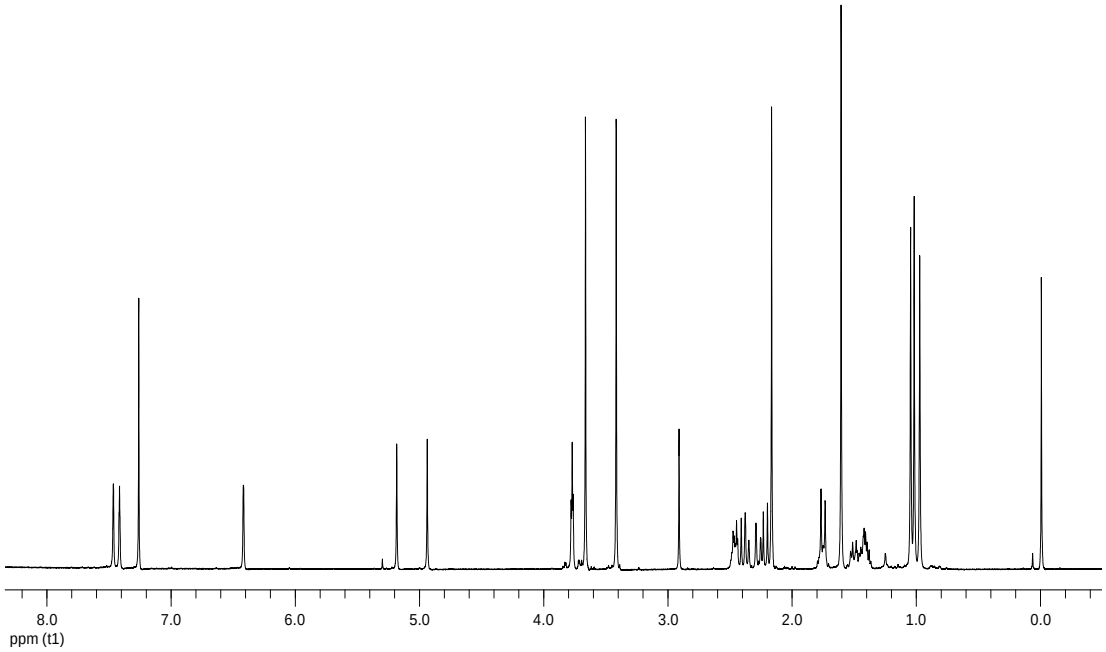


Figure S-56 ^{1}H NMR (400 MHz) spectrum of compound **11** ($CDCl_3$)

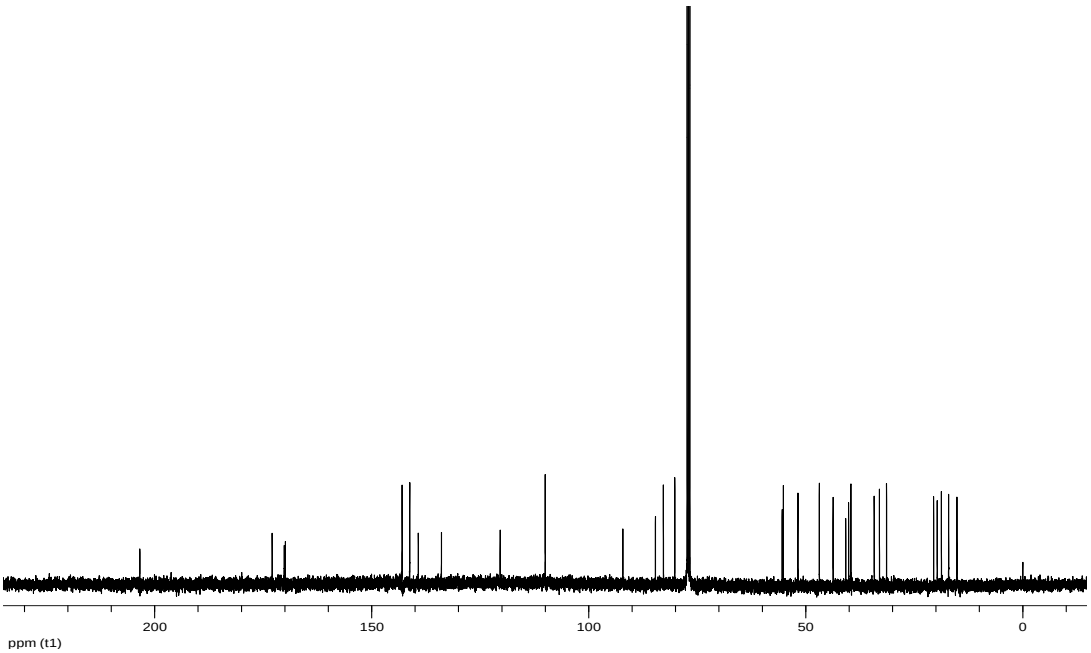


Figure S-57 ^{13}C NMR (100 MHz) spectrum of compound **11** ($CDCl_3$)

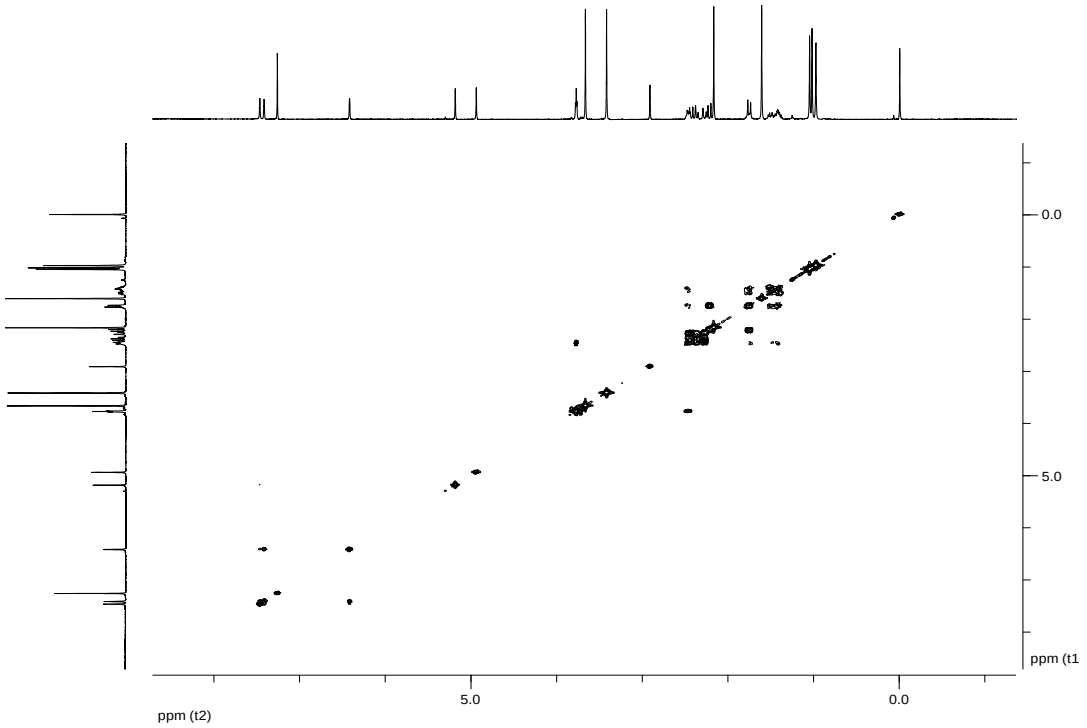


Figure S-58 ^{1}H-^{1}H COSY spectrum of compound **11** ($CDCl_3$)

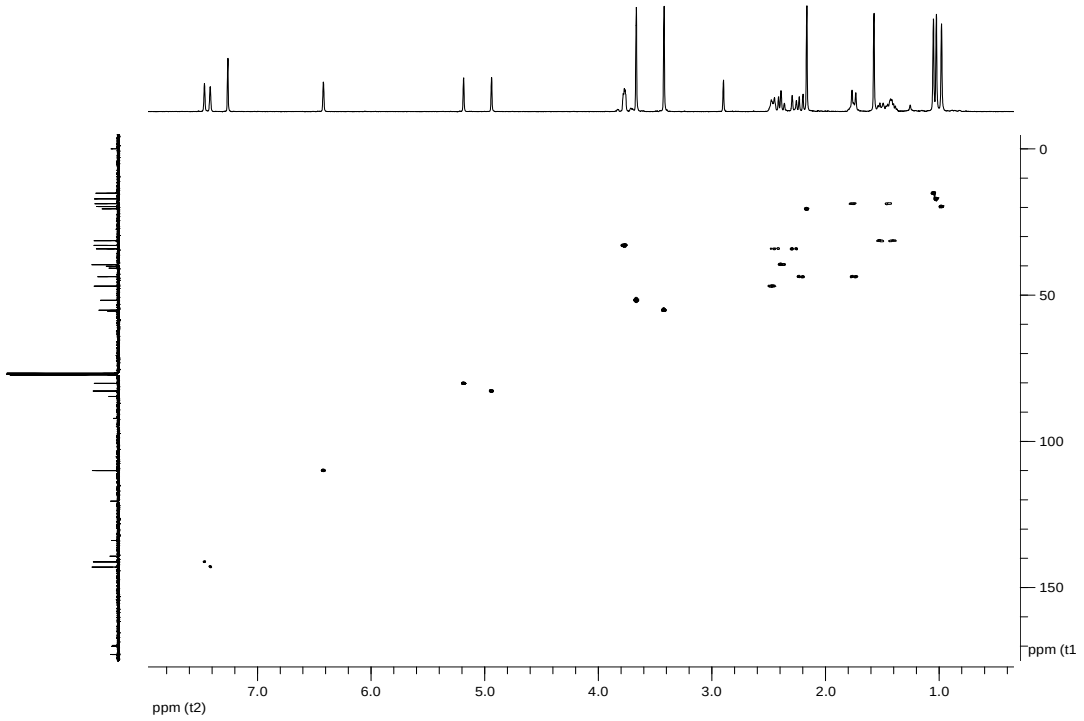


Figure S-59 HSQC spectrum of compound **11** ($CDCl_3$)

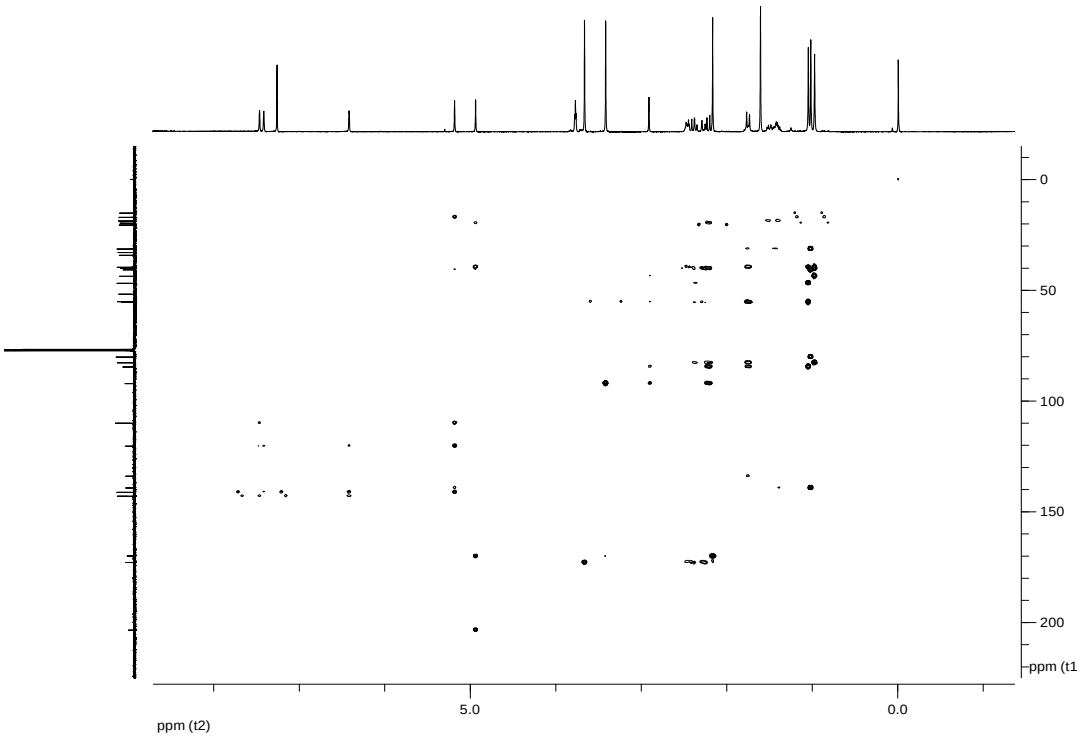


Figure S-60 HMBC spectrum of compound **11** ($CDCl_3$)

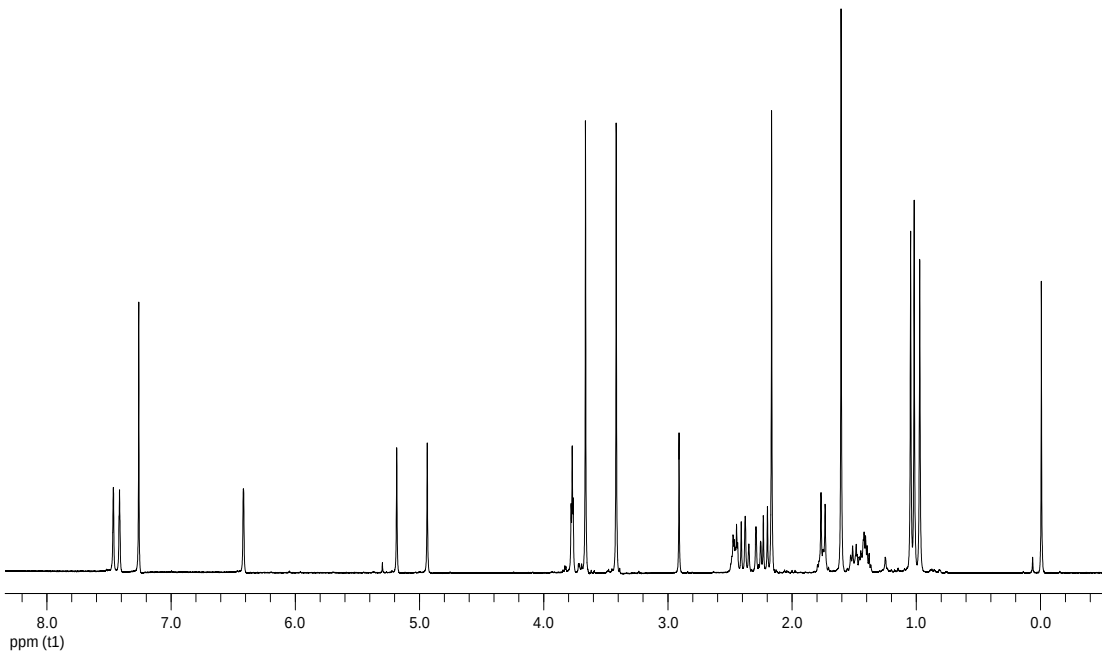


Figure S-61 ^{1}H NMR (400 MHz) spectrum of compound **12** ($CDCl_3$

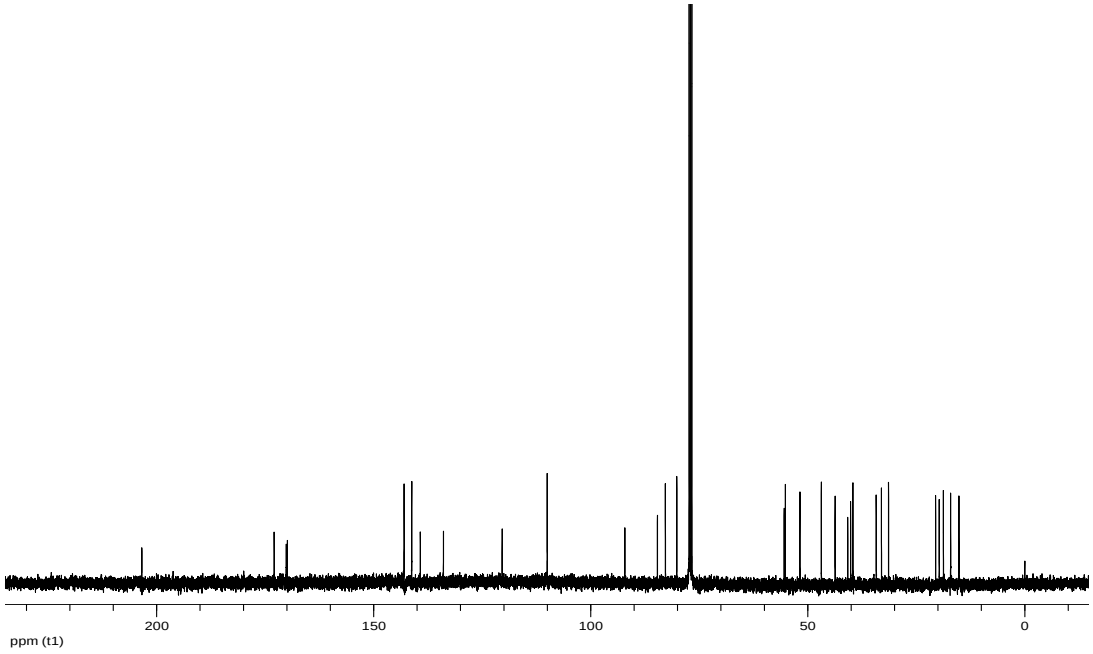


Figure S-62 ^{13}C NMR (100 MHz) spectrum of compound **12** ($CDCl_3$)

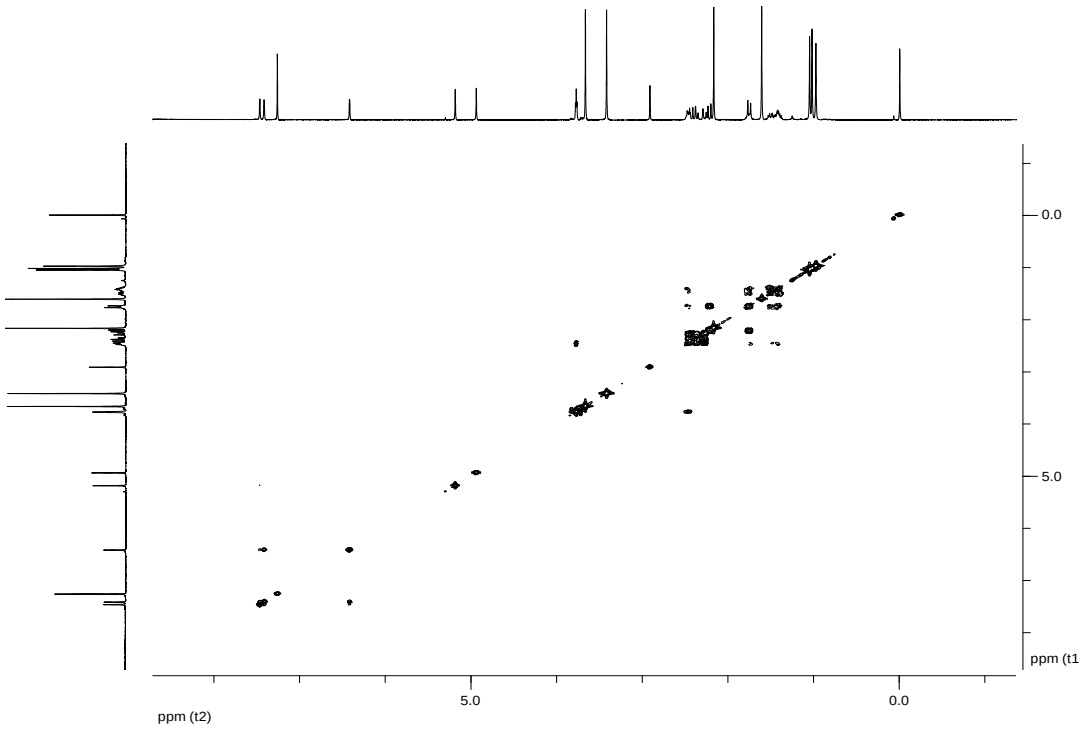


Figure S-63 ^{1}H-^{1}H COSY spectrum of compound **12** ($CDCl_3$)

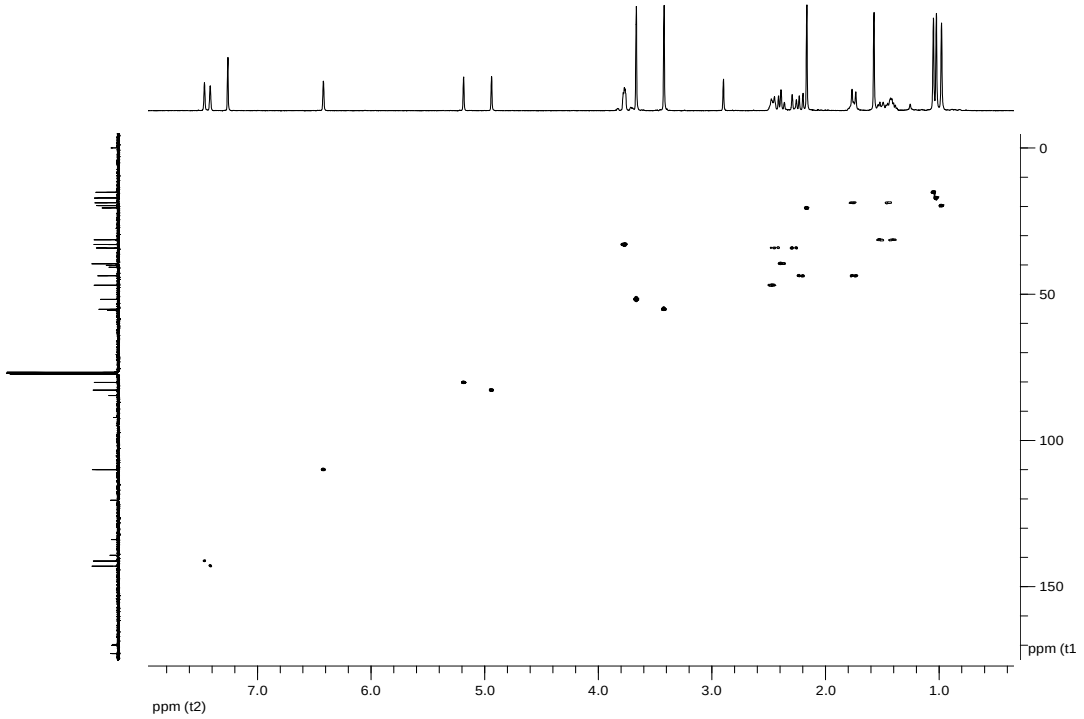


Figure S-64 HSQC spectrum of compound **12** ($CDCl_3$)

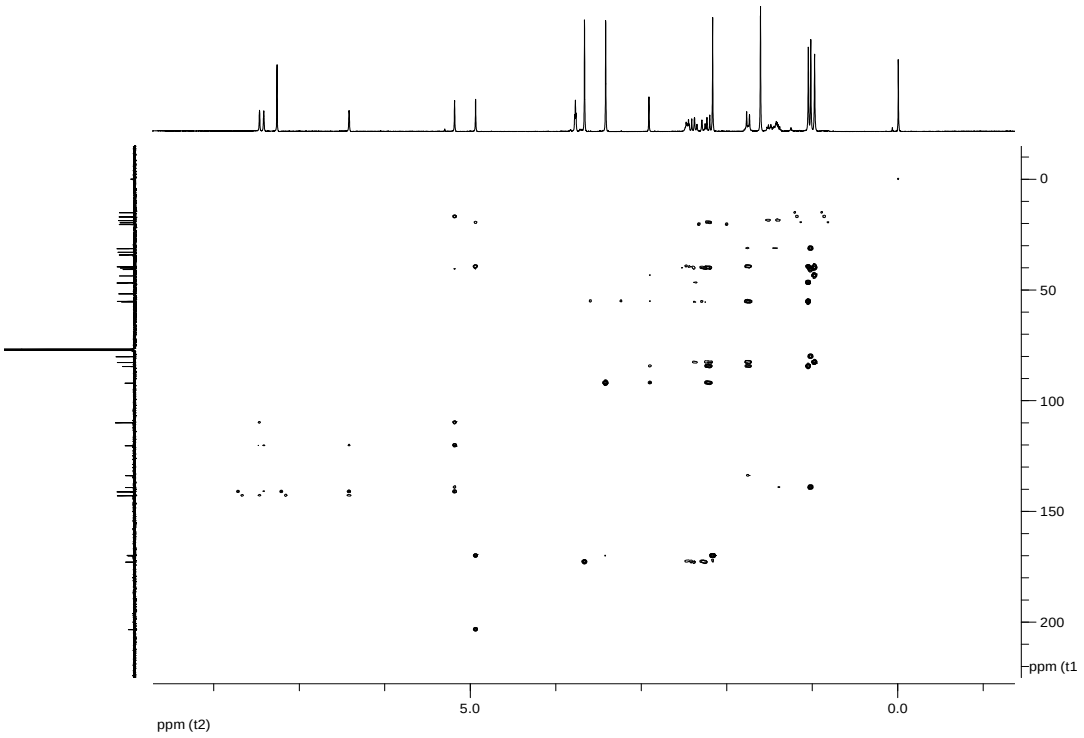


Figure S-65 HMBC spectrum of compound **12** ($CDCl_3$)

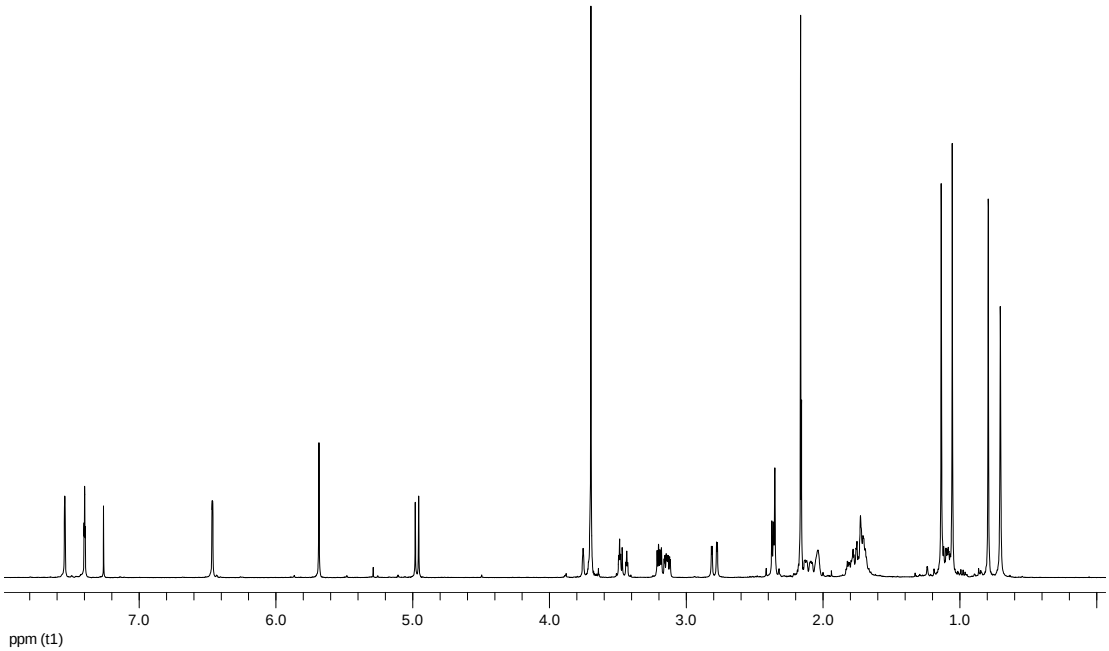


Figure S-66 ^{1}H NMR (400 MHz) spectrum of compound **13** ($CDCl_3$)

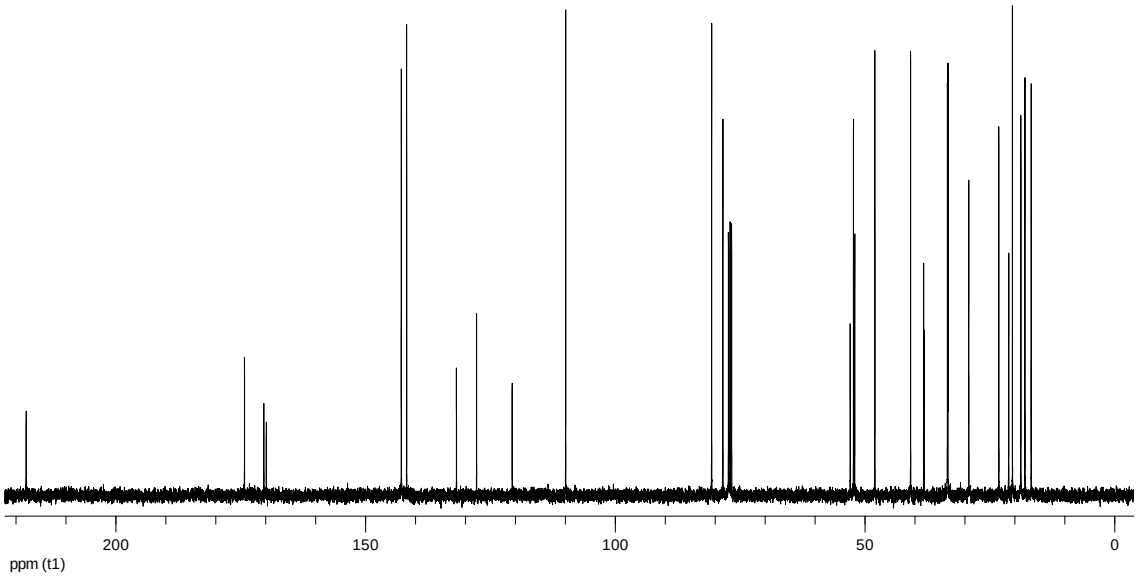


Figure S-67 ^{13}C NMR (100 MHz) spectrum of compound **13** ($CDCl_3$)

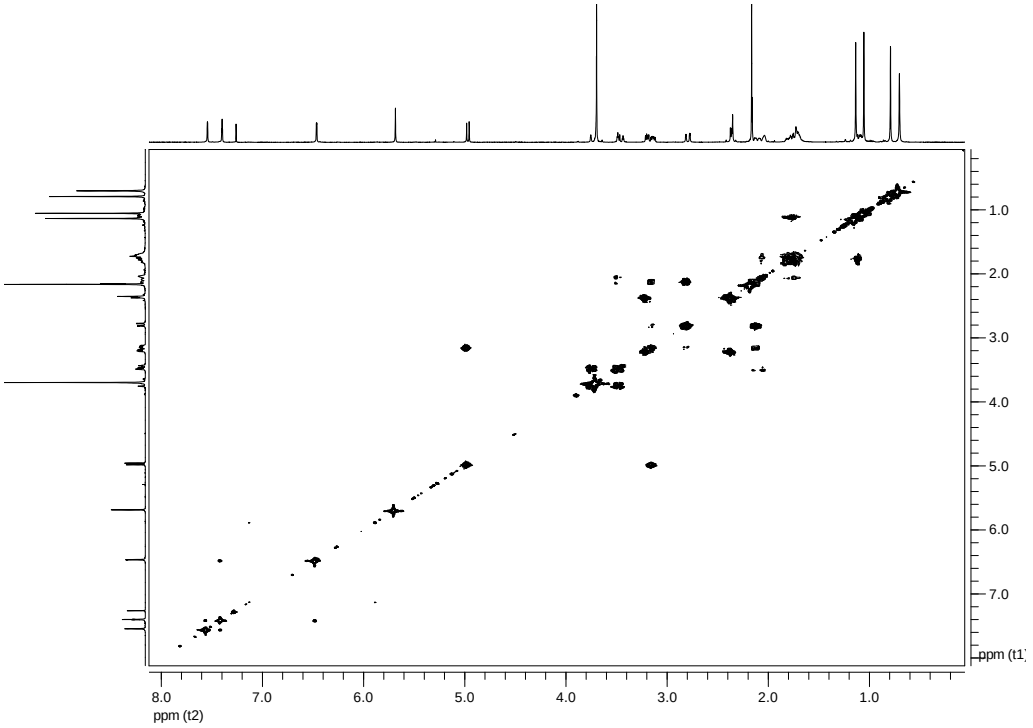


Figure S-68 ^{1}H-^{1}H COSY spectrum of compound **13** ($CDCl_3$)

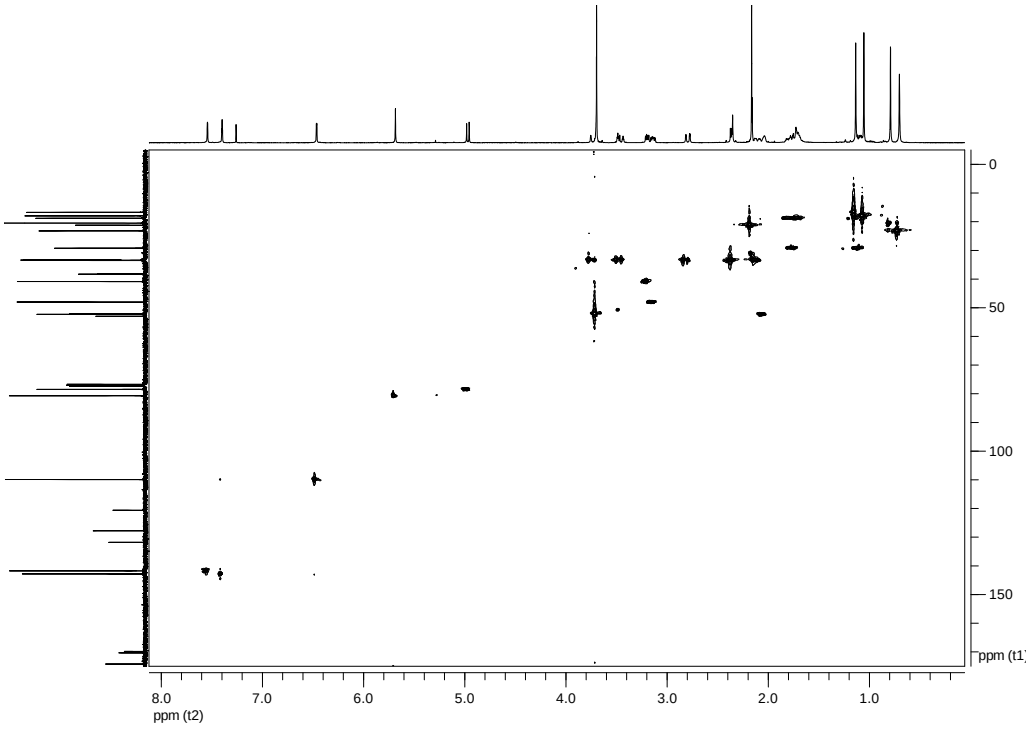


Figure S-69 HSQC spectrum of compound **13** ($CDCl_3$)

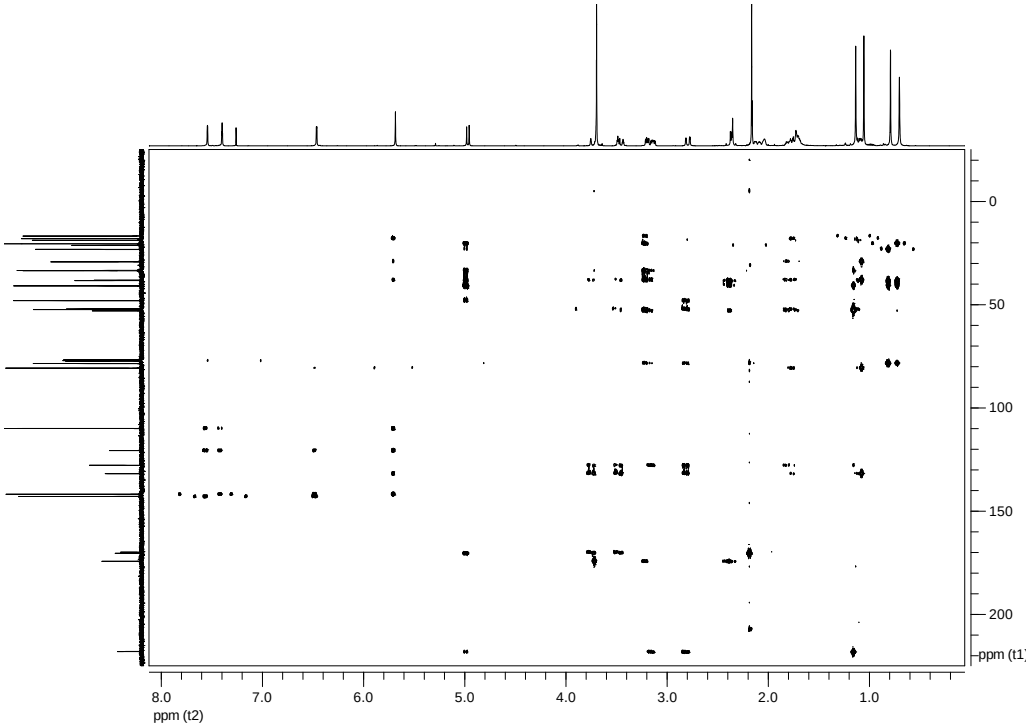


Figure S-70 HSQC spectrum of compound **13** ($CDCl_3$)

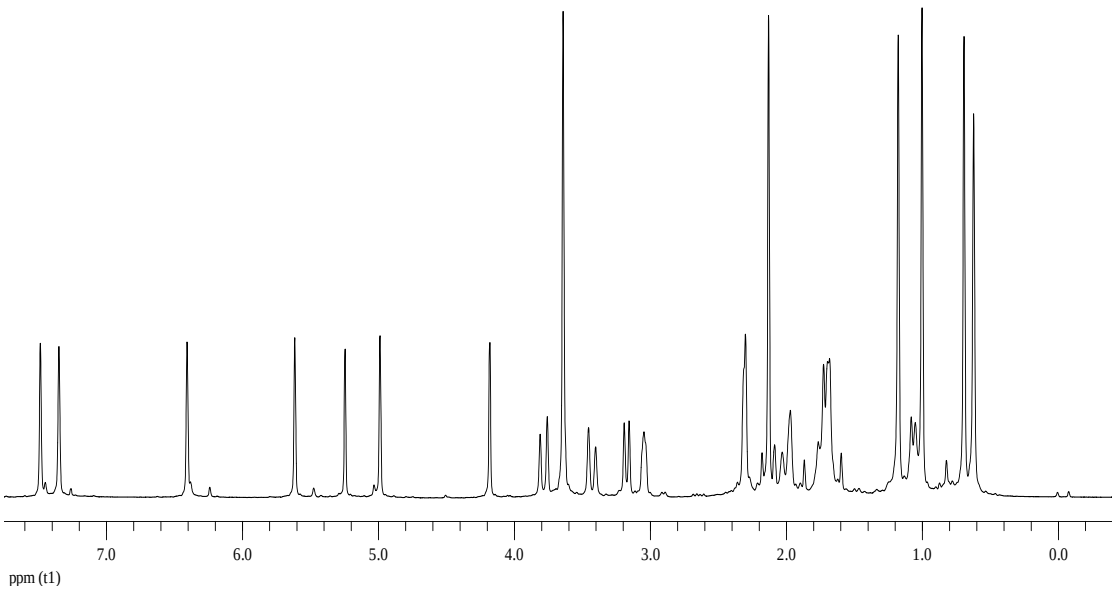


Figure S-71 ^{1}H NMR (400 MHz) spectrum of compound **14** ($CDCl_3$)

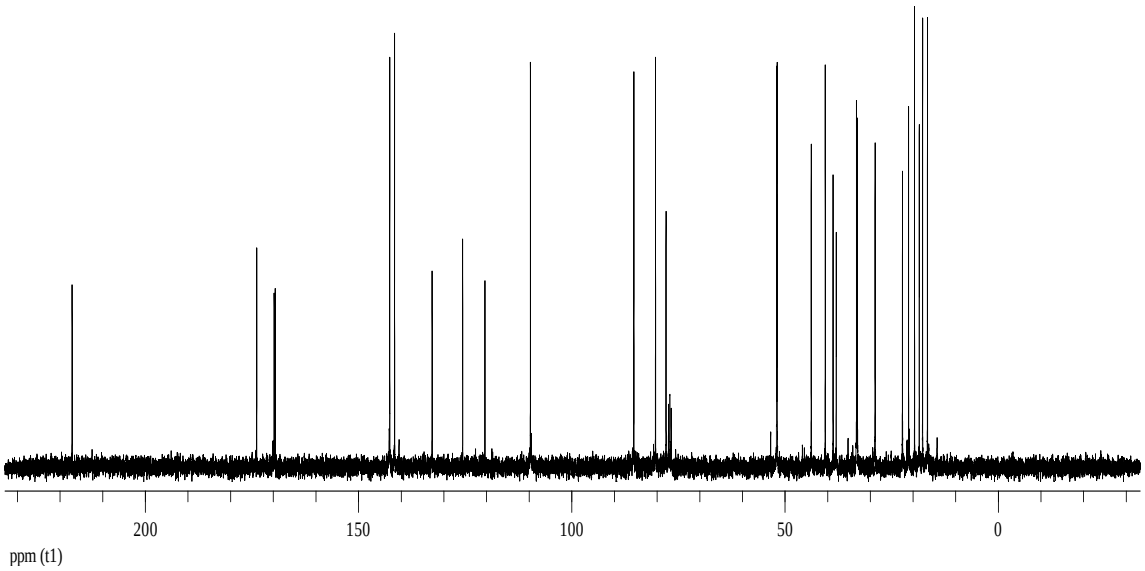


Figure S-72 ^{13}C NMR (100 MHz) spectrum of compound **14** ($CDCl_3$)

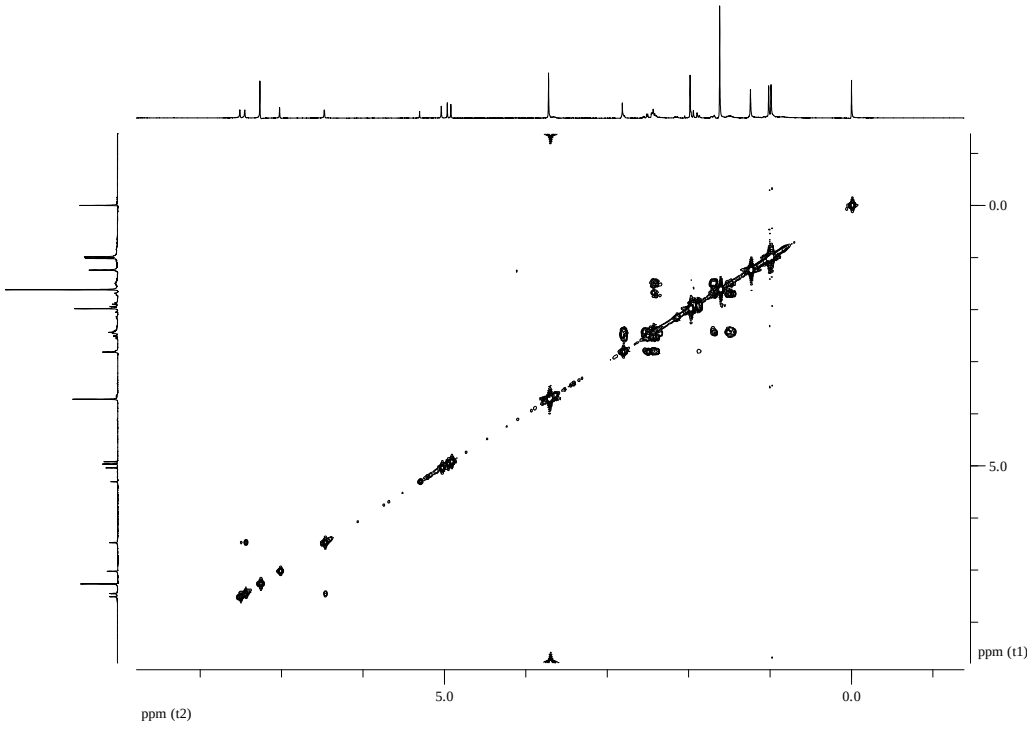


Figure S-73 ^{1}H-^{1}H COSY spectrum of compound **14** ($CDCl_3$)

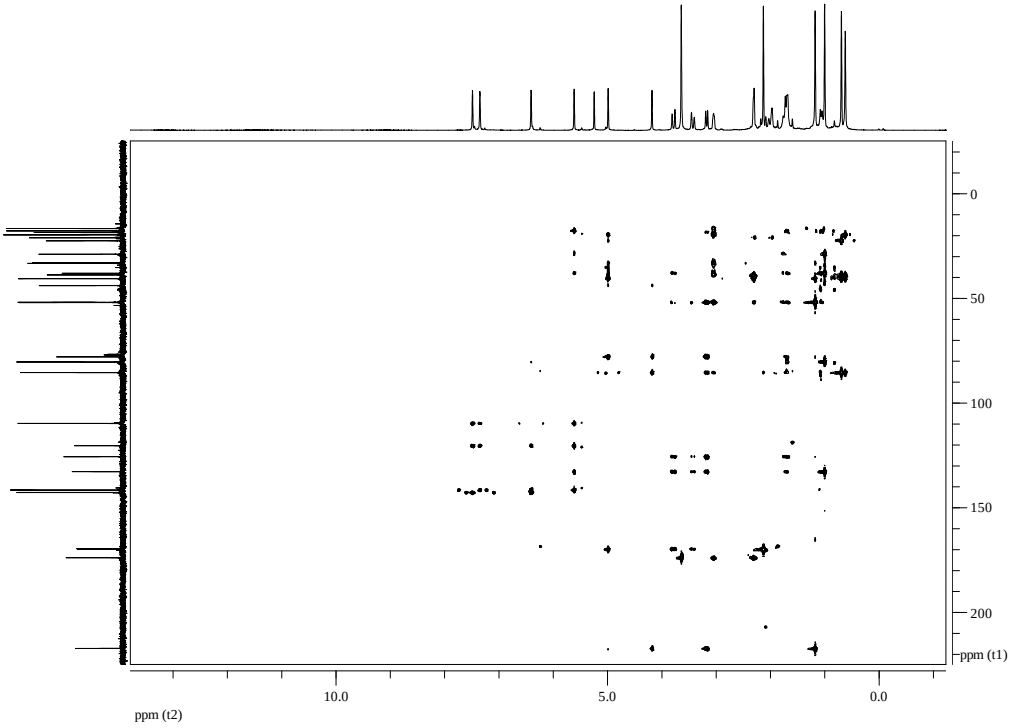


Figure S-74 HSQC spectrum of compound **14** ($CDCl_3$)

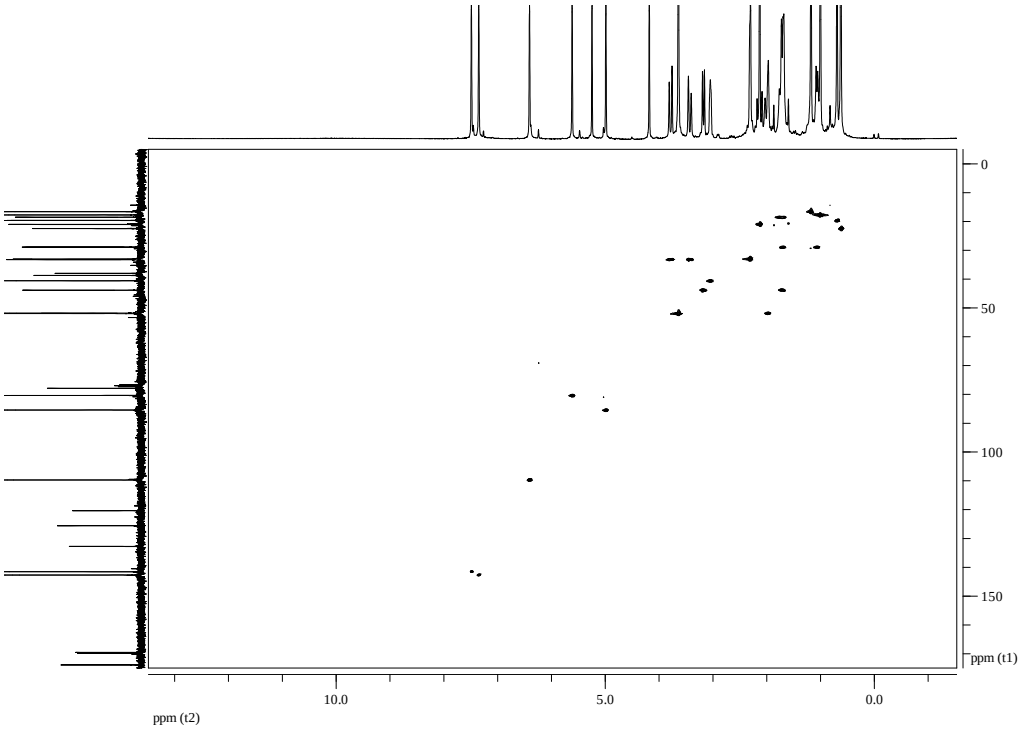


Figure S-75 HMBC spectrum of compound **14** ($CDCl_3$)

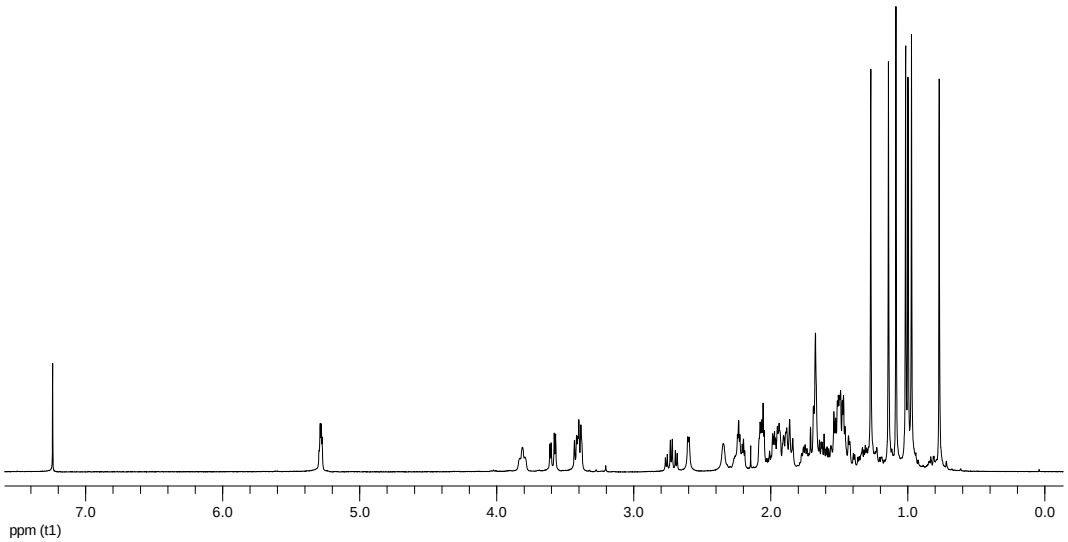


Figure S-76 ^{1}H NMR (400 MHz) spectrum of compound **15** ($CDCl_3$)

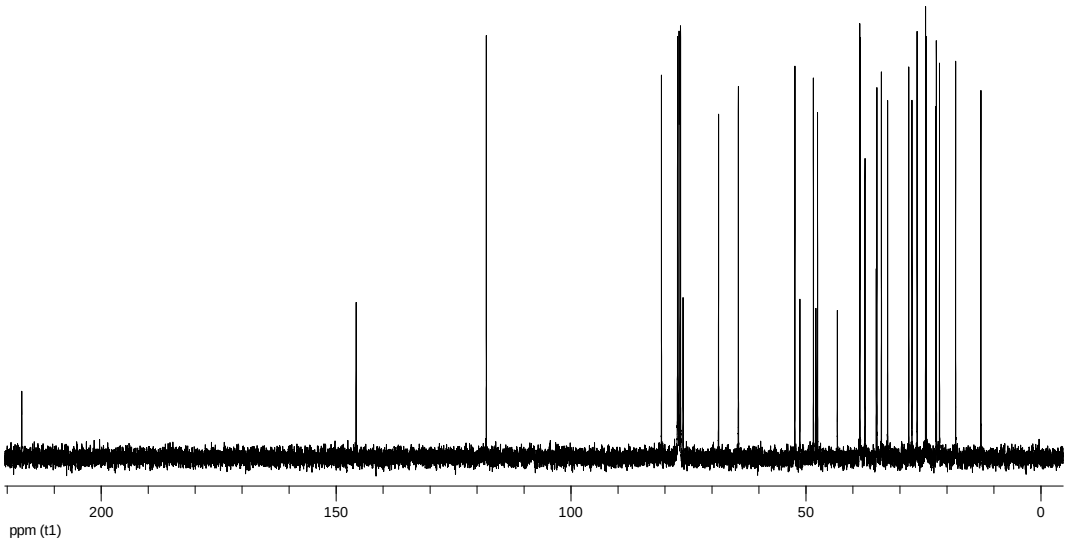


Figure S-77 ^{13}C NMR (100 MHz) spectrum of compound **15** ($CDCl_3$)

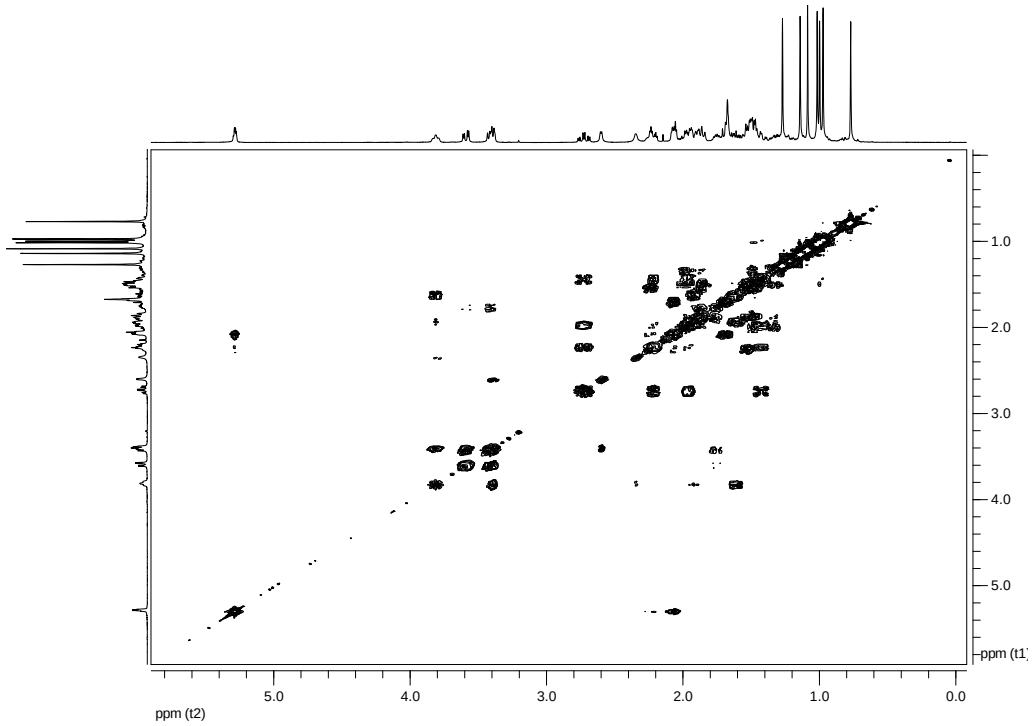


Figure S-78 ^{1}H-^{1}H COSY spectrum of compound **15** ($CDCl_3$)

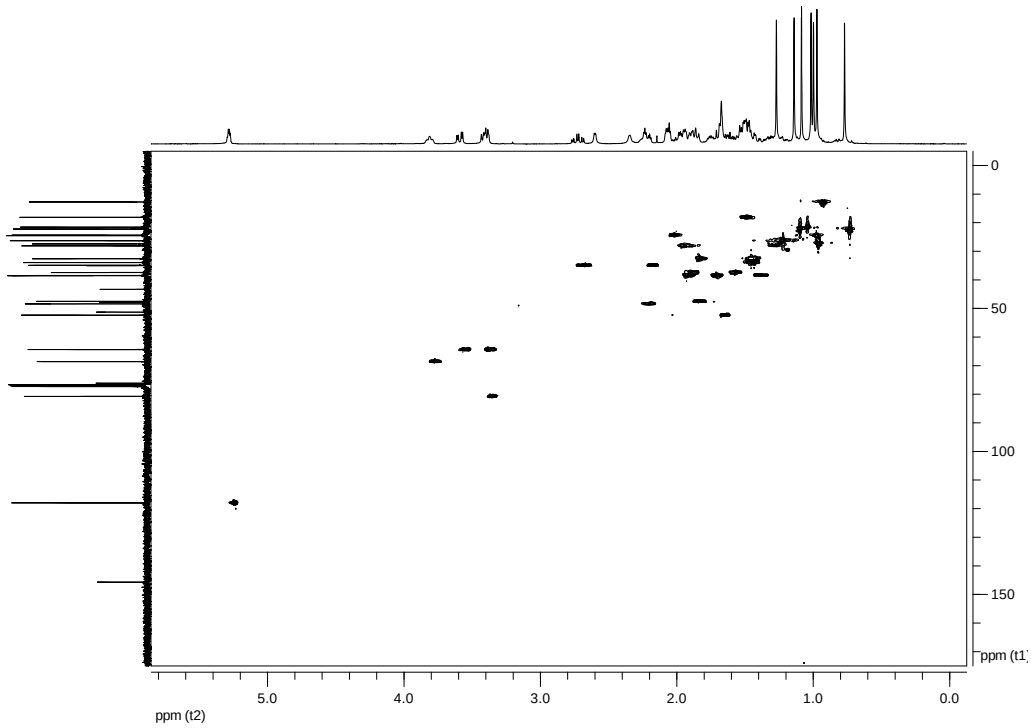


Figure S-79 HSQC spectrum of compound **15** ($CDCl_3$)

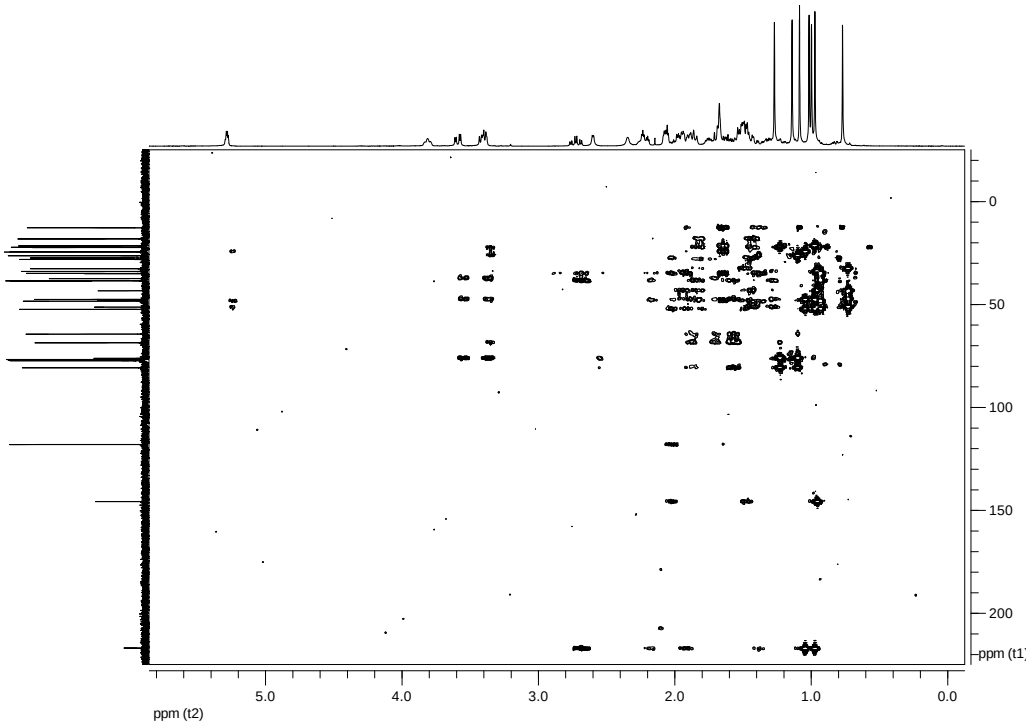


Figure S-80 HSQC spectrum of compound **15** ($CDCl_3$)

VITAE

Miss Chutima Jittaniyom was born on October 31, 1986 in Chonburi, Thailand. She was graduated with Bachelor’s Degree of science in Chemistry from Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, in 2009. During the time Hi was studying in the Master Degree in Chemistry program, Faculty of science, Chullongkorng University

Her present address is 4/84 Moo 9 Tambon Thachang, Muang Direct, Chanthaburi 22000 Tel. 0859982688.