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SYNTHESIS OF FUROFURAN LIGNANS FROM SAMIN THROUGH CARBON–CARBON BOND

FORMATION WITH PHENOLICS

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                      WITH PHENOLICS
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ฟิวโรฟิวแรนลิกแนนซึ่งมีโครงสร้างแบบ 2,6-diaryl-3,7-bicyclo [3.3.0] octane ถูกสังเคราะห์ผ่าน 2 ขั้นตอน โดยขั้นตอนแรกเกี่ยวข้องกับการเปลี่ยนเซซาโมลินที่ได้จากปฏิกิริยาสะปอนนิฟิเคชันของน้ำมันงาให้อยู่ในรูปสารที่มีความว่องไวสูง ชื่อ เซซามิน ภายใต้สภาวะที่มีกรดเร่งปฏิกิริยา จากนั้นกลุ่มสารฟิวโรฟิวแรนถูกสังเคราะห์ขึ้นผ่านปฏิกิริยา Friedel-Crafts type จากการทำปฏิกิริยาระหว่างซามินกับฟิลอนิกที่หลากชนิด (a-k) ซึ่งปฏิกิริยาดังกล่าวสามารถให้ผลิตภัณฑ์ตามที่ต้องการ (3a-3k) และอิพิเมอร์ (*epi*-3a-*epi*-3k) ด้วยร้อยละที่ดี เมื่อนำฟิวโรฟิวแรนที่สังเคราะห์ได้ไปทดสอบฤทธิ์ต้านอนุมูลอิสระและยับยั้งการทำงานของแอลฟา-กลูโคซิเดส ปรากฏว่าในบรรดาสารที่ถูกสังเคราะห์ขึ้นผลิตภัณฑ์ที่มีหมู่ไฮดรอกซิลอิสระบนวงฟีนอลิก ซึ่งได้แก่ 3a, 3e, 3g, 3i, 3k, 1-3 และ 1-22 พร้อมกับอิพิเมอร์ จะมีฤทธิ์ต้านอนุมูลอิสระ มีค่า SC_{50} ในช่วง 0.22-1.45 mM และ 0.15-0.41 mM ด้วยวิธี DPPH และ ABTS ตามลำดับ อีกทั้งมีฤทธิ์ยับยั้งการทำงานของเอนไซม์แอลฟา-กลูโคซิเดสต่อมอลเทส ด้วยค่า IC_{50} ในช่วง 1.14-8.23 mM จากการศึกษากลไกการยับยั้งเอนไซม์ของสารประกอบ 1-22 แสดงให้เห็นว่าโหมดการยับยั้งของสารในกลุ่มฟิวโรฟิวแรนลิกแนน คือ mixed-competitive กับมอลเทส ด้วยค่า K_i และ $K_i{}'$ เท่ากับ 0.29 และ 0.48 mM ตามลำดับ จากข้อมูลดังกล่าวเป็นการแสดงเห็นว่าหมู่ไฮดรอกซิลอิสระบนวงฟีนอลิกมีบทบาทสำคัญในการเพิ่มฤทธิ์ต้านอนุมูลอิสระและยังยั้งการทำงานของเอนไซม์

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PHONPIMON KHONGCHAI: SYNTHESIS OF FUROFURAN LIGNANS FROM SAMIN THROUGH CARBON–CARBON BOND FORMATION WITH PHENOLICS. ADVISOR: ASSOC. PROF. PREECHA PHUWAPRAISIRISAN, Ph.D., 86 pp.

Furofuran lignans contaning 2,6-diaryl-3,7-bicyclo [3.3.0] octane skeleton were synthesized by two-step sequential process. The synthesis was first performed by converting sesamolin, obtained from saponification of sesame oil, to the more reactive compound named samin, under acid-catalyzed condition. Subsequently, a series of furofuran lignans were synthesized through Friedel-Crafts type between samin and various phenolics (namely a-k). The reaction produced desired products (3a-3k) along with their epimers (*epi*-3a-*epi*-3k) with good yields. The synthesized furofuran lignans were further evaluated for antioxidant and α-glucosidase inhibitory activity. Of synthesized compounds, the products having free hydroxyl group on phenolic ring (3a, 3e, 3g, 3i, 3k, 1-3, 1-22 and their epimers) showed remarkable both antioxidant (SC_{50} 0.22-1.45 mM and 0.15-0.41 mM toward DPPH and ABTS, respectively) and α-glucosidase inhibitory activity (IC_{50} 1.14-8.23 mM against maltase). An enzyme kinetic study represented by 1-22 revealed that mode of inhibition was mixed-competitive against maltase with K_i and $K_i{}'$ value of 0.29 and 0.48 mM, respectively. These revealed that the free hydroxyl group on phenolic ring played an important role in enhancing antioxidant activity and enzyme inhibitory potency.

Department:	Chemistry	Student's Signature	
Field of Study:	Chemistry	Advisor's Signature	
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จุฬาลงกรณ์มหาวิทยาลัย
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LIST OF SCHEMES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
brs	broad singlet (NMR)
calcd	calulated
CDCl$_3$	deuterated chloroform
d	doublet (NMR)
dd	doublet of doublet (NMR)
DPPH	2,2-diphenyl-1-picrylhydrazyl
equiv	equivalent
g	gram
HMBC	heteronuclear multiple bond correlation
HRMS	high resolution mass spectroscopy
J	coupling constant (NMR)
LDA	lithium diisopropylamide
m	multiplet (NMR)
mmol	milimole
mL	milliliter
mM	milimolar
MsCl	methanesulfonyl chloride
m/z	mass per charge
NADH	nicotinamide adenine dinucleotide
s	singlet (NMR)
SAM	*S*-adenosyl methionine

Tf$_2$O   trifluoromethanesulfonic anhydride

THF   tetrahydrofuran

TMSBR   trimethylsilyl bromide

μL   microliter

δ   chemical shift (NMR)

CHAPTER I

INTRODUCTION

1.1. Sesame seed oil

Sesame (*Sesamum indicum* L.) seed is an important oilseed crop containing various nutrient, especially high oil-soluble lignans [1]. In fact, about 70% of the world's sesame oil is used for different utilizations such as food products, formulation cosmetic and pharmaceutical products. The sesame oil is produced from roasted white or black sesame seeds at 180-200 °C prior to the extraction of an oil with a typical flavor [2] (Figure 1.1). Additionally, sesame oil is also obtained by cold-pressing extraction of unroasted; nevertheless the unroasted oil is dramatically different in flavor and noticeably lower antioxidant activity than the roasted sesame oil. During high temperature roasting process, sesamolin in oil is first decomposed into sesamol oxonium ion or sesamol dimmers by pyrolysis and then a new carbon bond was formed to produce sesaminol [3] (Scheme 1.1). These compounds, sesamol and sessaminol, play an important role in antioxidant of sesame oil.

Figure 1.1 (a) Black and white sesame seed from *Sesamum indicum* (b) Saseme oil is extracted by two processes; roasting process is that sesame was heated at high temperature prior to the extraction of oil (left) and cold-pressing of unroasted process (right).
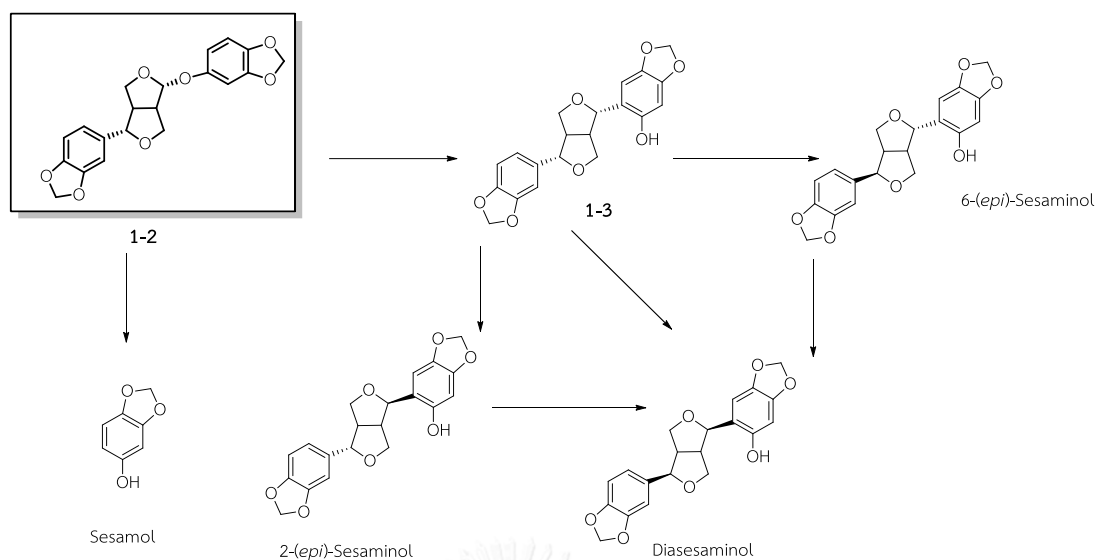
Scheme 1.1   Transformation of sesamolin in sesame oil to antioxidant sesame lignan such as sesamol and sesaminol during roasted sesame seed.

Dietary sesame lignans such as sesamin (**1-1**), sesamolin (**1-2**) and sesaminol (**1-3**) (Figure 1.2) not only contribute to antioxidant and free radical scavenging activity that may suppress oxidative stress *in vivo* [4], but are also responsible for a number of beneficial health effect in human such as controlling fatty acid metabolism in the liver, lowering of plasma cholesterol level and acceleration of alcohol and xenobiotic metabolism as well as enhancement vitamin E activities and the bioavailability of ɣ-tocopherol (**1-4)** *in vivo* [2, 5, 6]. Recently, sesame lignans were used synergistically with tocopherols for the anti-aging effect [7] and daily supplement for providing essential fatty acid.

1-1

1-2

1-3

1-4
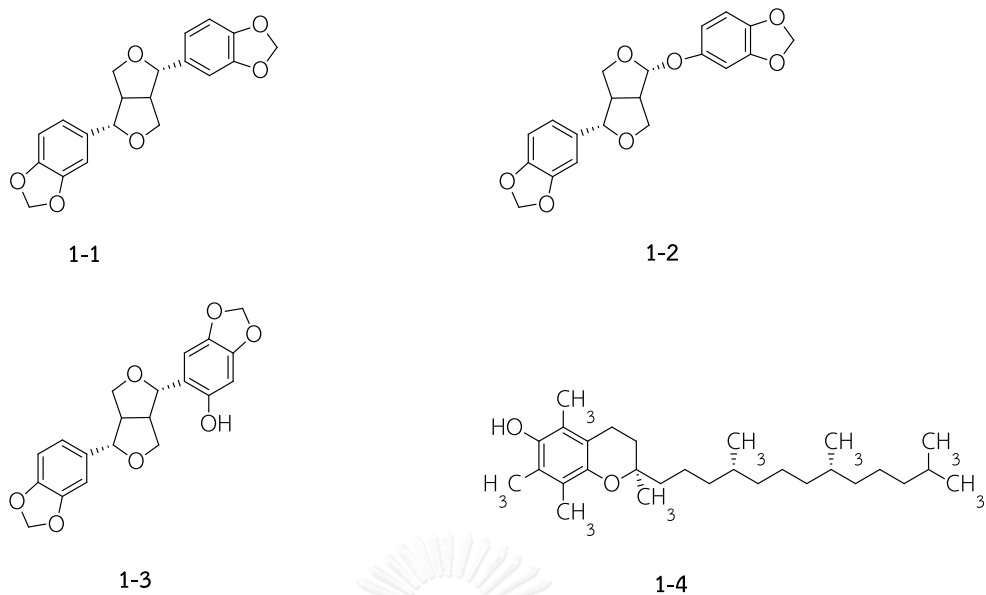
Figure 1.2 Sesame lignans found in dietary sesame oil.

1.2. Furofuran lignans
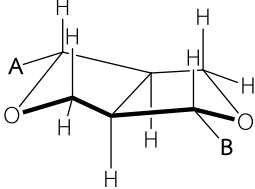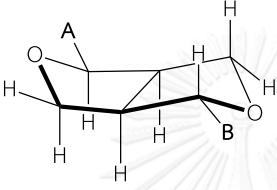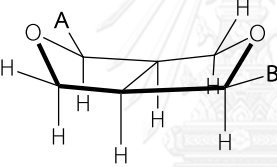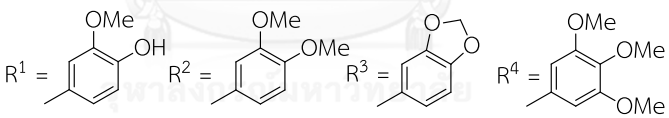
Sesame lignans are classified into furofuran lignans subclass of natural lignan. Furofuran lignans are compounds containing the 2,6-diaryl-3,7-dioxabicyclo [3.3.0] octane skeleton, which is biosynthesized from two phenylpropanoids (C_6-C_3) via oxidative radical polymerization [8] (Scheme 1.2).
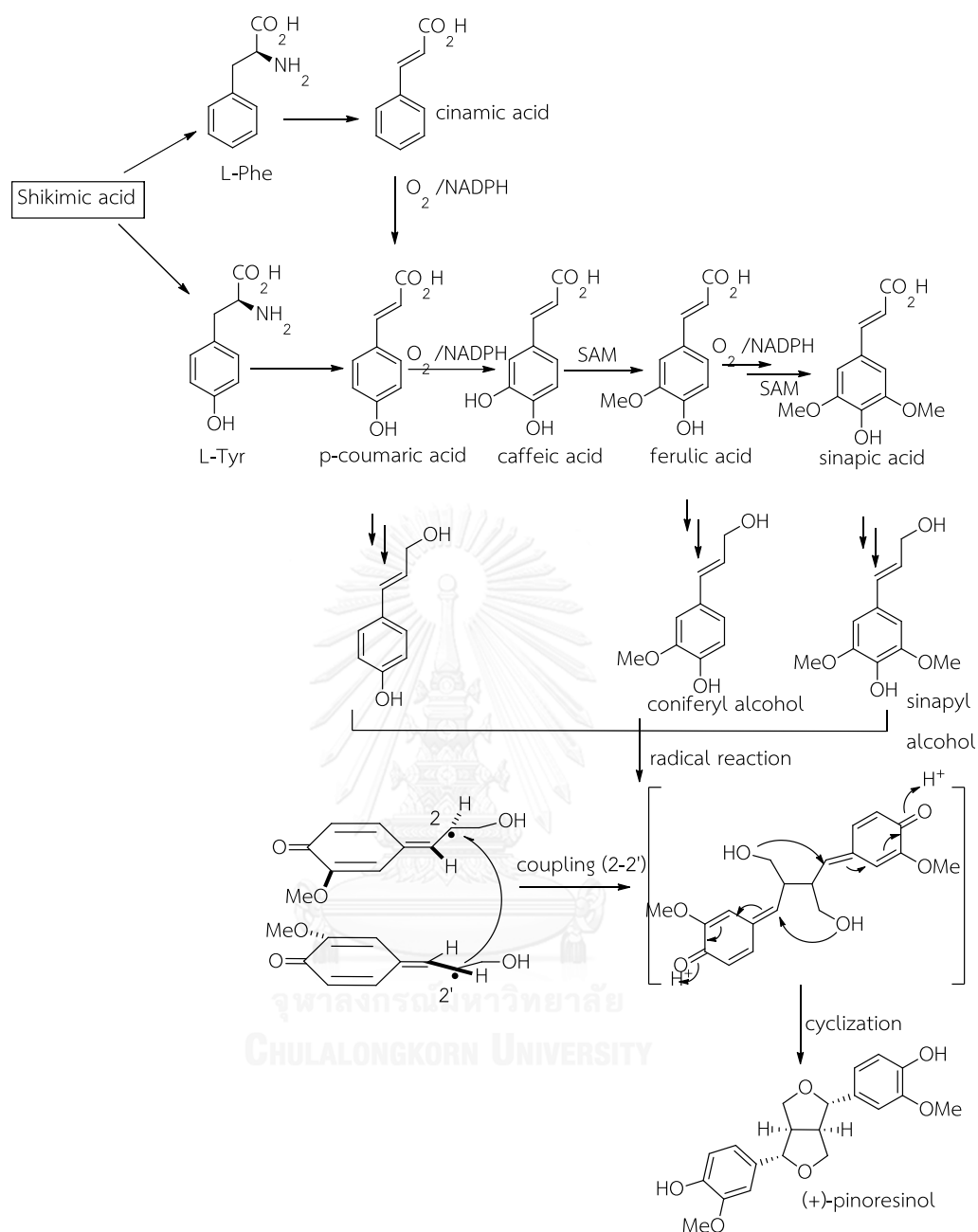
Scheme 1.2 Biosynthesis pathway of furofuran lignan via radical polymerization of phenylpropanoids (C_6-C_3).

Generally, furofuran lignans could be further divided into three different types (*exo-exo, endo-exo* and *endo-endo*), depended on arrangement of 2,6-diaryl substituent in relation to bridgehead hydrogen. The selected furofuran lignans in each class are shown in Table 1.1.

Table 1.1 The classification of furofuran lignans based on stereomer of arrangement of 2,6-diaryl sudstitutent on furofuran core structure relation to bridgehead hydrogen

Type	Structure	Compound
exo-exo		A = B = R^1 pinoresinol (**1-5**) A = B = R^2 eudesmin (**1-6**) A = R^3, B = R^2 kobusin (**1-7**)
endo-exo		A= B = R^2 : *epi*-eudesmin (**1-8**) A = R^3, B = R^1 : *epi*-piperittolin (**1-9**) A = R^2, B = R^3 : fargesin (**1-10**) A = R^4, B = R^2 : *epi*-magolin (**1-11**)
endo-endo		A = B = R^3 : *epi*-asarinin (**1-12**) A = B = R^4 : diayangambin (**1-13**)

Furofuran lignans are diverse not only in nature and stereochemistry of 2,6-diaryl substituents, but are also biological activities. The bioactivities of selected furofuran lignans are summarized in the Table 1.2

Table 1.2.   The biological activity of selected furofuran lignans

Furofuran lignan	Biological activity	Reference
sesamin (**1-1**)	controlling metabolism of lipid and glucose, antihypertensive, anti-inflammation and free radical scavenging.	[9]
sesamolin (**1-2**)	increasing both the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate and act as antioxidant compound.	[10],[11]
sesaminol (**1-3**)	antioxidant compound that inhibit the membrane lipid peroxidation as well as synergistic compound raising liver and plasma concentrations of vitamin E.	[12],[13]
pinoresinol (**1-5**)	inhibition membrane lipid peroxidation and the LDL oxidation.	[14]
epi-asarinin (**1-12**)	anti-tumor promotion, antiallergic activity and enhancement of the toxicity of certain insecticides.	[15]

1.3   Synthesis of furofuran lignans

Based on interesting core structure of furofuran lignans and a variety of their biological activity, the total synthesis and semisynthesis of furofuran lignans have been reported.

1.3.1.   Total synthesis of furofuran lignans using five-membered ring lactone as intermediate

The general method for the synthesis of furofuran lignan is the construction of five-membered lactone in the first step followed by cyclization reaction, which can be divided into three pathways, to obtain bicyclic-fused ring of tetrahydrofuran (Scheme 1.3).
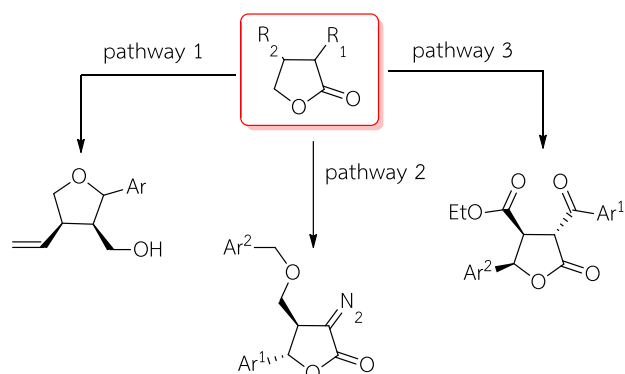
Scheme 1.3 Key intermediate lactones for synthesis of furofuran lignans.

In 1997, Marchand and coworkers [5] synthesized samin as a versatile core structure for functionalization to obtain sesamolin derivatives. Samin analogues **1-14** were synthesized from 4-vinybutylrolactone (pathway 1, Scheme 1.3) by aldol condensation followed by oxidative cleavage and intramolecular cyclisation. After treatment of **1-14** with phenols as nucleophiles under suitable condition, the desired products (e.g. *epi*-piperitolin (**1-9**) and *epi*-pinoresinolin (**1-15**)) were obtained with inversion of configuration at C-2 (Scheme 1.4.).
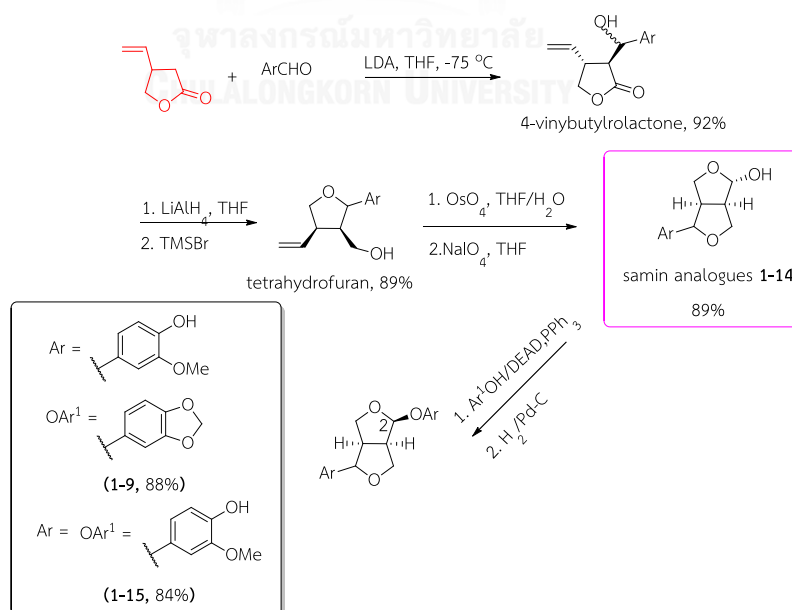
Scheme 1.4 Synthesis of *epi*-piperittolin (**1-9**) and *epi*-pinoresinolin (**1-15**).

In 2001, Brown and coworkers [15] prepared cyclobutanone from reaction of amide **1-16** and Tf$_2$O to generate keteniuminuim intermediate. Then this intermediate was reacted with allyl benzyl ether to give the targeted cyclobutanone in a good yield. Next, cyclobutanone was converted to five-membered ring lactone as key intermediate (pathway 2, Scheme 1.3) by Baeyer-Villiger oxidation in three steps. The key step was CH- insertion reaction with rhodium-catalyzed stereoselective cyclization to give endo-exo-2,6-diarylfurofurans (e.g. *epi*-magolin (**1-11**)) (Scheme 1.5).
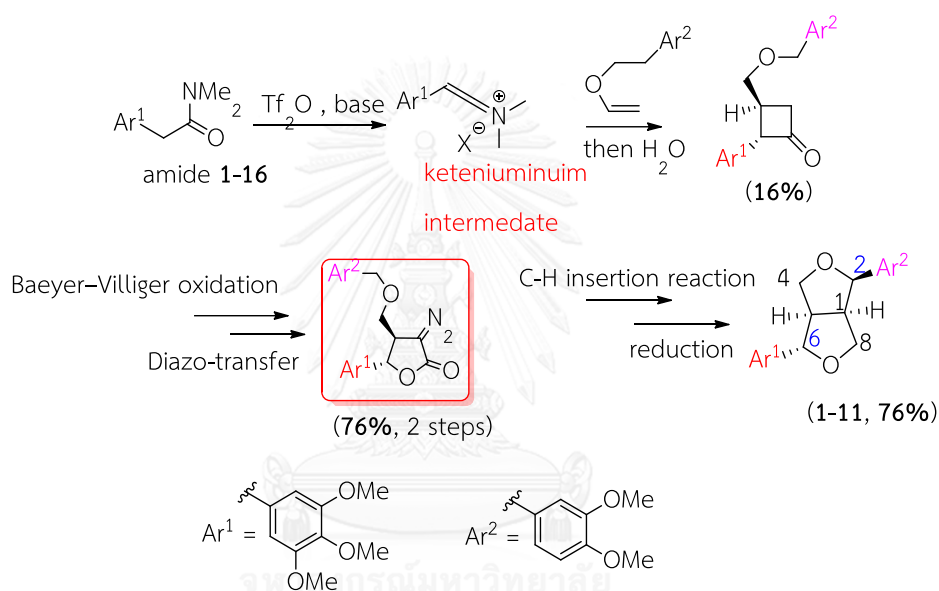
Scheme 1.5   Synthesis of *epi*-magolin (**1-11**).

In 2005, Pohmakotr and coworkers [16] prepared **α**-aroylparaconic as intermediate (pathway 3, Scheme 1.3) for the synthesis of 1-substituent *exo-endo* furofuran lignans. The **α**-aroylparaconic was obtained from the reaction of vicinal diaions derived from **α**-aroylsuccinic esters with aromatic. The synthetic sequence involved **α**-methylation or hydroxylation, reduction, bislactonization, reduction followed by furofuran formation to give (+)-gmelinol (**1-17**) (Scheme 1.6).
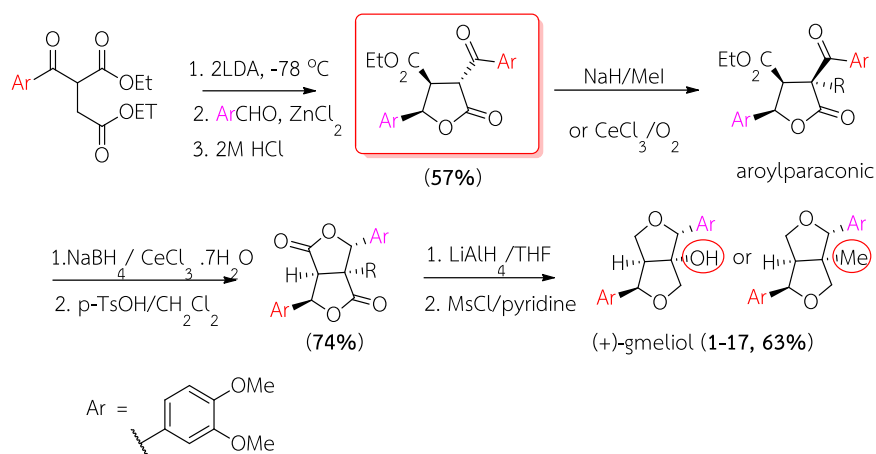
Scheme 1.6 Synthesis of (+)-gmelinol (**1-17**)

1.3.2. Semisynthesis of furofuran lignans using naturally available precursors

Sesamin and sesamolin in sesame oil are always substrates for functionalization to obtain various furofuran lignans.

In 2008, Urata and coworkers [17] found that sesamin (**1-1**) could be oxidized by lead (IV) tetraacetate [Pb(OAc)$_4$] to obtain corresponding sesamin derivatives having catechol unit (Scheme 1.7). Pb(OAc)$_4$ was used as acetoxylating agent at carbon atom of dioxymethylene group in first step. Then, acetoxylated sesamin derivatives were hydrolyzed with acetic acid (AcOH) to yield mono-catechol (**1-18**) and bis-catechol (**1-19**) sesamin derivatives. These compounds have more potent antioxidant activity than sesamin.
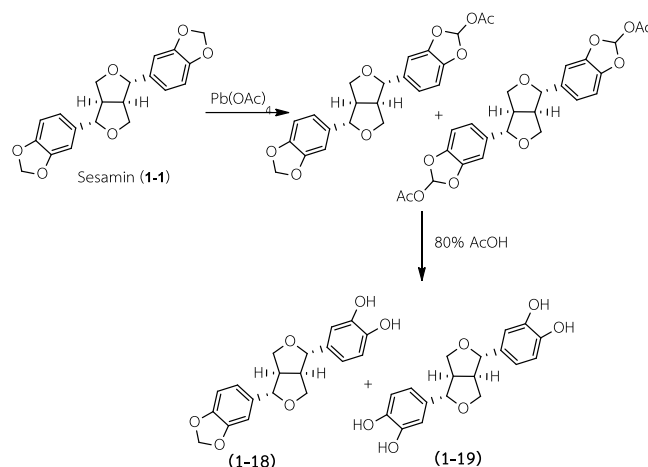
Scheme 1.7 Synthesis of mono-catechol **(1-18)** and bis-catechol **(1-19)** sesamin derivatives

In 2012, Huang and coworkers [18] demonstrated that sesamolin (**1-2**) could be used as a versatile precursor to synthesize sesaminol (**1-3**) and related isomers. Sesamolin in acid condition could be rearranged to sesamol (**1-20**) and oxocarbenium ion intermediate (**1-21**). Subsequently, two possible reaction pathways, (2)a and (2)b, could be taken place. The reaction of path (2)a underwent electrophilic aromatic substitution, Friedel-Crafts, resulting in carbon-carbon formation of sesaminol (**1-3**) and 2-*epi*-sesaminol (**1-22**).On the other hand, path (2)b was the reverse of path (1) that lead to the concurrent production of sesamolin isomer (**1-23**) (Scheme 1.8).
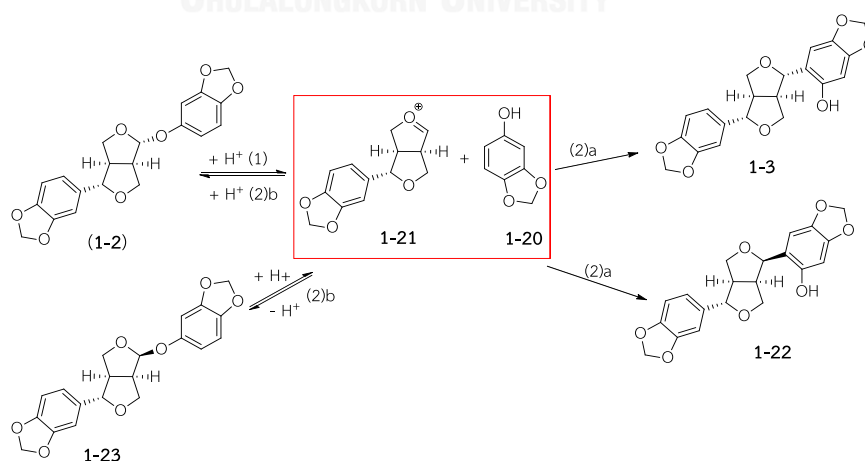
Scheme 1.8 Synthesis of sesaminol (**1-20**), 2-*epi*-sesaminol (**1-21**) and sesamolin isomer (**1-22**)

To date, there have been many reports of biological activities displayed by furofuran lignans but this is no investigation on the relation between furofuran lignan structure and their biological activity. The main problem attribute to the above synthesis approach that are not practical to produce a variety of furofuran lignans in a few steps. Therefore, the semisynthesis could be applied to solve the aforementioned problems by using naturally abundant lignans, especially sesamolin (**1-2**) as starting material. In this research, we have an idea to convert sesamolin (**1-2**) to a more reactive hemiacetal named samin (**1-14**) which can be reacted with various types of phenolics. Under acid condition, **1-14** can be protonated to produce oxocarbenium ion (**1-21**) together with the release of water. Finally, nucleophilic addition to oxocarbenium ion by phenolics (Friedel-Crafts) can produce a series of desired furofuran lignans (Scheme 1.9).
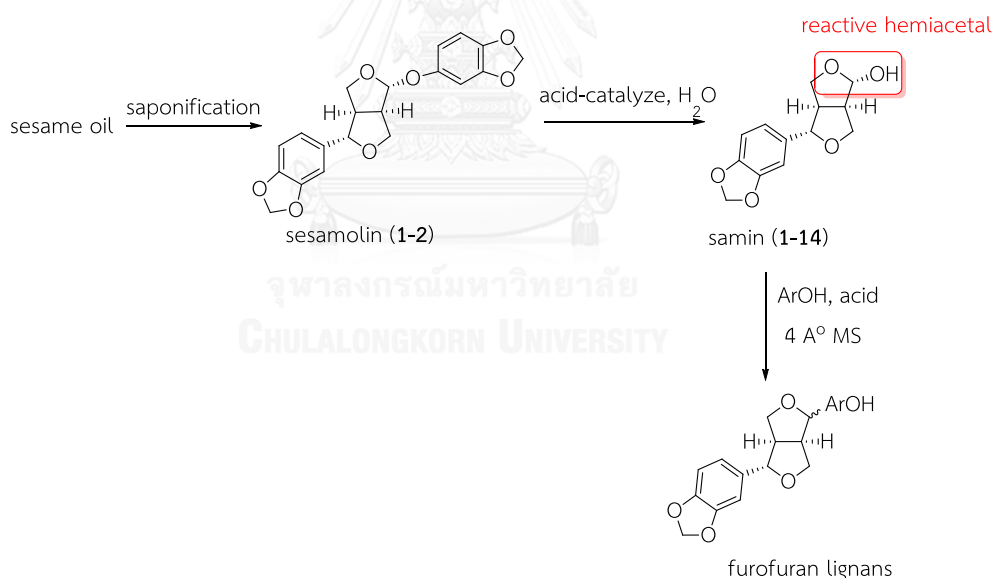
Scheme 1.9   Synthetic plan of fufofuran lignans in this study.

CHAPTER II

SYNTHESIS OF SAMIN

2.1   Saponification of sesame oil

Sesame oil is rich not only in edible oil and protein, but also in source of furofuran lignan such as sesamin and sesamolin. Therefore, sesame oil is interesting source to isolate furofuran lignans for starting material. However, these compounds are dissolved in triglyceride matrix of sesame oil. Isolation of furofuran lignans with direct column chromatography technique is not suitable method because sesame oil comprises of large amount of triglyceride matrix that makes separation more tedious. To solve this problem, the sesame oil should be primarily pretreated by converting to salt of fatty acid by base-catalyzed saponification reaction in first step followed by liquid-liquid extraction.

For base-catalyzed saponification reaction, sesame oil was dissolved in methanol containing potassium hydroxide in order to obtain potassium salt of fatty acid, which was easily dissolved in water. The minimum amount of potassium hydroxide (KOH) used to complete saponification could be calculated from saponification number (SN). However, the slight excess of KOH (about 1.5-2.0 times) from SN was used to ensure converting triglyceride to soap. After saponification completed, the reaction mixture was dissolved in water and extracted with ethyl acetate (EtOAc). The organic phase or unsponifiable matters was further to isolate because it mainly contains desired sesamolin (**1-2**)

Then, the unsponifiable matters after solvent removal under reduced pressure were purified by silica gel column to obtain sesamolin (**1-2**) and sesamin (**1-1**). The isolation procedure is summarized in Scheme 2.1.

Sesamolin (**1-2**) and sesamin (**1-1**) were obtained as white crystals. The structure of sesamolin (**1-2**) was deduced by ^{1}H compared with previous report [19].

Sesame seed oil (150 g)

      1) 35 g KOH in methanol 150 mL

      2) reflux at 70 °C, 3-4 h

      3) MeOH removal

Crude reaction

      - dissolved in H_2O

Solution mixture

      - partition with EtOAC (1:1,v/v)

Unsaponifiable matters   Saponifiable matters

      - silica gel column

Sesamolin (**1-2**)   Sesamin (**1-1**)
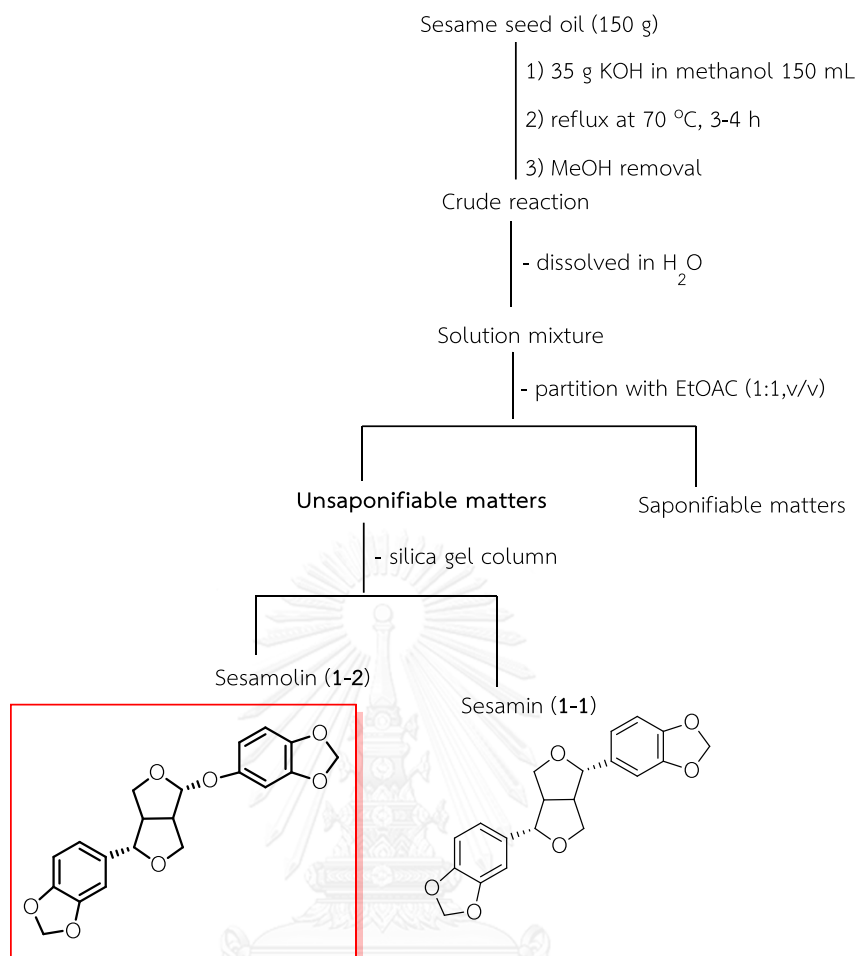
Scheme 2.1   The isolation procedure of **1-2** and **1-1** from sesame seed oil

2.2   Hydrolysis of sesamolin

Sesamolin (**1-2**) and sesamin (**1-1**) isolated from sesame oil seed are slightly different in structure in which sesamolin possess oxygen insertion between C-2 of furofuran core structure and carbon of aryl substitutent. In fact, the presence of acetal in sesamolin (**1-2**) makes it more reactive toward nucleophile substitution than sesamin (**1-1**). Therefore, reaction between **1-2** and any good nuclophile would produce a variety furofuran lignans.

However, sesamolin under acid-catalyzed condition could generate oxocabenium ion intermediate and sesamol (**1-20**) as by product, which in turn reacts with **1-21** to produce sesaminol product (**1-3**) and its epimer (Scheme 2.2). To solve

this problem, water (H_2O) as good nucleophile must be added to compete against sesamol (**1-20**), yielding samin (**1-14**) which is more reactive than sesamolin (**1-2**) (Scheme 2.2).
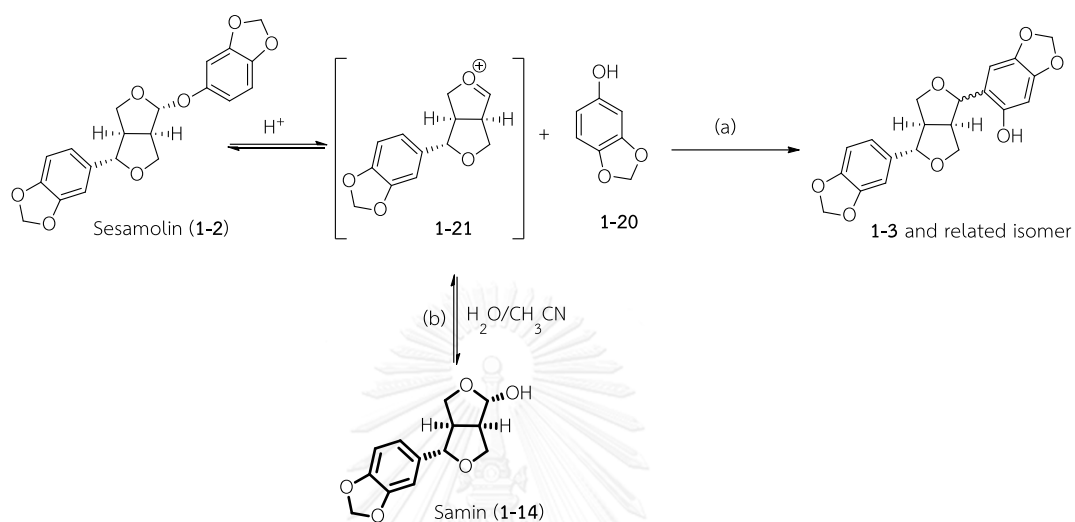
Scheme 2.2 (a) Sesamolin under acid catalyzed condition could be transformed to sesaminol and related isomer product (b) H_2O as nucleophile could compete against sesamol to afford sesamin

With the starting material in hand, sesamolin (**1-2**) was further hydrolyzed in the presence of amberlyst®-15, a strong acid resin, to afford samin (**1-14**, 90%). This conversion would make **1-14** more reactive than **1-2** toward nucleophilic substitution. **1-14** was obtained as brown crystals, and its structure was deduced by ^{1}H and ^{13}C data compared with previous report [20]

2.3 Experimental sections

2.3.1 General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded ($CDCl_3$ as solvent) at 400 and 100 MHz, respectively on a Varian Mercury 400 NMR spectrometer. The chemical shifts were reported in ppm downfield from TMS. Thin layer chromatography (TLC) was performed

on pre-coated Merk silica gel 60 F_{254} plated (0.25 mm thick layer) and visualized under 254 nm UV. Silica gel 60 Merck cat. No. 7729 was used for column chromatography.

2.3.2   Chemical

Potassium hydroxide (KOH) and amberlyst®-15 were purchased from Sigma-Aldrich. Sesame oil was purchased from Suan-pana (Samut Songkhram, Thailand).

2.3.3   Isolation of sesamolin from sesame seed oil

General procedure for isolation and extraction of sesamolin from sesame seed oil are as follows. Sesame seed oil (150 g) dissolved in MeOH (150 mL) was saponified with KOH (35 g) for 4 h. After solvent removal, the resulting mixture was dissolved in water and then extracted with EtOAc. The organic extract mainly containing unsaponifiable lignans was separated on silica gel column using 20:80 EtOAc-Hexane to obtain sesamolin (**1-2**, 1%).

Sesamolin (**1-2**): white crystals; ^{1}H NMR (400 MHz, CDCl$_3$) **δ** 6.88 (s, 1H), 6.83 – 6.76 (m, 2H), 6.71 (d, J = 8.5 Hz, 1H), 6.62 (d, J = 2.3 Hz, 1H), 6.50 (dd, J = 8.4, 2.3 Hz, 1H), 5.96 (s, 2H), 5.92 (s, 2H), 5.50 (s, 2H), 4.45 (t, J = 9.0 Hz, 1H), 4.39 (d, J = 7.3 Hz, 1H), 4.12 (dd, J = 9.2, 6.0 Hz, 1H), 3.96 (d, J = 9.2 Hz, 1H), 3.63 (m, 1H), 3.31 (q, J = 8.7 Hz, 1H), 2.94 (dd, J = 6.9 Hz, 1H).

2.3.4   Hydrolysis of sesamolin

The solution of sesamolin (**1-2**, 0.27 mmol) in a mixture of acetonitrile/H$_2$O (9:1, 10 mL) was treated with amberlyst®-15. After stirring at 70 °C for 8 h, the reaction mixture was evaporated to dryness and purified on column chromatography using 30:70 EtOAc-Hexane to give **1-14** (90%)

Samin (**1-14**): as brown crystal solid ; ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.85 (s, 1H, H-2'), 6.80-6.75 (m, 2H, H-5' and H-6'), 5.94 (s, 2H, H-7'), 5.36 (s, 1H, H-2), 4.35 (d, J = 8.4 Hz, 2H, H-6 and H-8), 4.16 (dd, J = 9.2, 6.0 Hz, 1H, H-4), 3.89 (d, J = 9.2 Hz, 1H, H-4), 3.56 (dd, J = 8.8, 7.2 Hz, 1H, H-8), 3.25 (brs, 1H, -OH), 3.05 (m, 1H, H-1), 2.86 (m, 1H, H-5); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 148.1, 147.4, 134.7, 119.7, 108.3, 106.7, 102.4, 101.2, 87.0, 71.4, 69.5, 53.7, 52.9.

CHAPTER III

CONCISE SYNTHESIS OF FUROFURAN LIGNANS FROM SAMIN

3.1   Synthesis of furofuran lignans

A series of furofuran lignans could be synthesized from reaction between samin (**1-14**) and phenolics (**a-k**) under acid catalyzed condition via Friedel – Crafts reaction (Table 3.1). The phenolics reacted with samin are shown in Figure 3.1 which can be classified into six groups based on number of hydroxyl or methoxy groups on benzene ring. To test synthetic plan in hand, we first applied it to the reaction between samin (**1-14**) and monooxygenated benzene, *m*-cresol (**a**). The reaction produced fufofuran lignans **3a** (30%) and the corresponding epimer (*epi*-**3a**, 15%). The generation of **3a** and *epi*-**3a** could be explained in Scheme 3.1. Under the acid condition, samin (**1-14**) could be protonated to produce oxocarbenium ion together with the release of water, which could be trapped by 4 °A MS. Then, oxocarbenium ion was attacked by *m*-cresol (**a**) via either pathway (a) yielded *epi*-**3a** (*exo,exo*-furofuran) or pathway (b) afforded **3a** (*endo,exo*-furofuran).
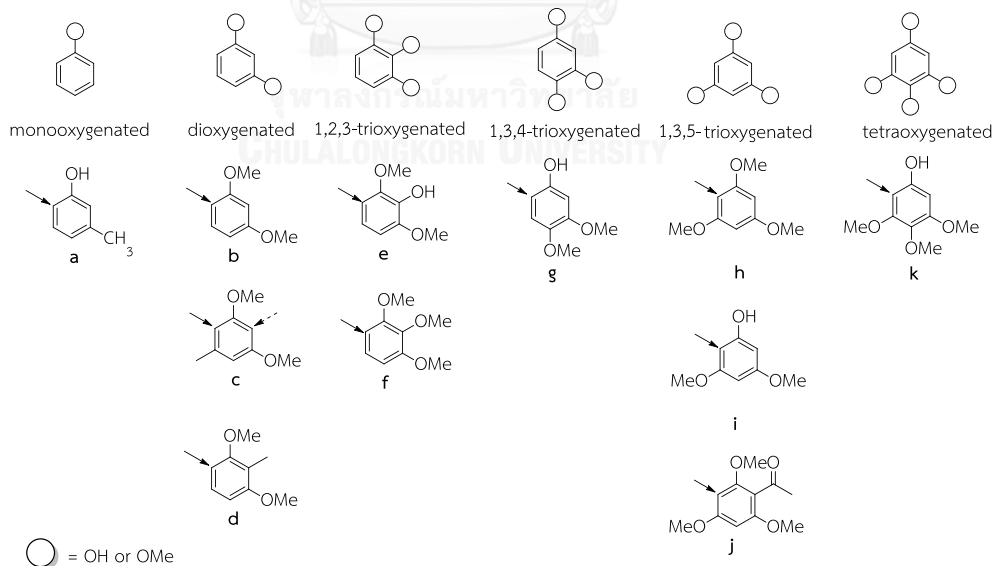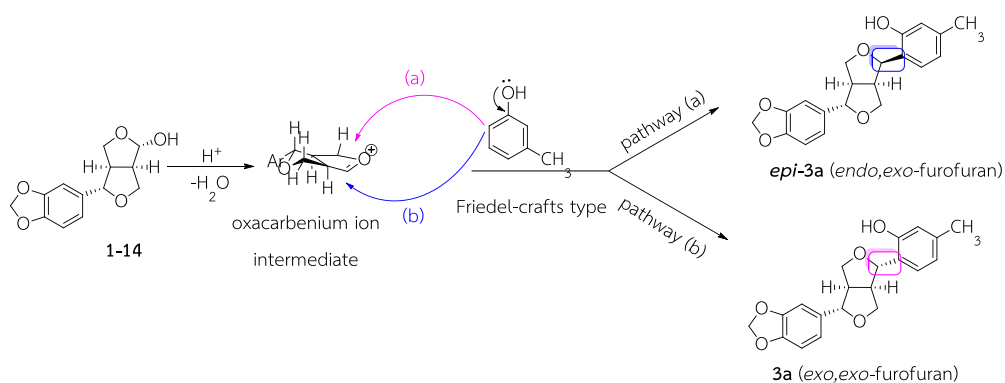

Figure 3.1   Phenolic (**a**-**e**) used to synthesize a series of furofuran lignans. The bold and dash arrows indicated the position in which bonding between C-2 of furofuran moiety and phenolics was formed.

Scheme 3.1 Propose formation of diastereomeric products from reaction between samin (**1-14**) and *m*-cresol (**a**) under acid condition

To produce diverse furofuran lignans, we further synthesized furofuran lignans using other phenolics, namely dioxygenated (**b**-**d**), trioxygenated (**e**-**j**) and tetraoxygenated (**k**) benzenes (Figure 3.1). All reactions, excepted for the reaction between samin (**1-14**) and **c**, produced a pair of diastereomeric products (Table 3.1).

Table 3.1 Synthesis of furofuran lignans
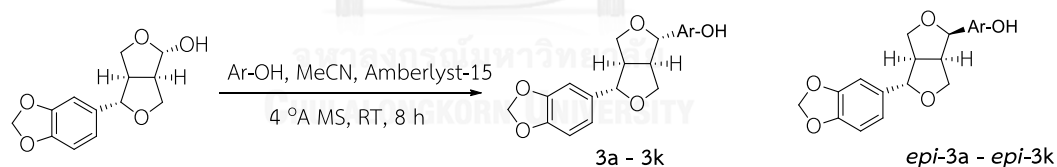
Entry	Isolated product (% Yield)	
	3	*epi*-3
1	**3a** (30%)	*epi*-**3a** (15%)
2	**3b** (50%)	*epi*-**3b** (45%)
3	**3c** (19%)	*epi*-**3c** (21%)
	3c' (trace)	*epi*-**3c'** (24%)
4	**3d** (31%)	*epi*-**3d** (42%)
5	**3e** (27%)	*epi*-**3e** (37%)
6	**3f** (31%)	*epi*-**3f** (22%)

Table 3.1 (Cont.) Synthesis of furofuran lignans

| | Isolated product (% Yield) | |
Entry	3	*epi*-3
7	**3g** (68%)	***epi*-3g** (7%)
8	**3h** (50%)	***epi*-3h** (trace)
9	**3i** (51%)	***epi*-3i** (47%)
10	**3j** (14%)	***epi*-3j** (trace)
11	**3k** (30%)	***epi*-3k** (40%)

Additionally, **1-3** and its epimer (**1-22**) could be synthesized by acid-catalyzed reaction of sesamolin (**1-2**) with good yield shown in Scheme 3.2. Under acid condition, **1-2** generate oxocarbenium ion and sesamol as nucleophile, which in turn reacted with oxocarbenium ion to produce the desired products.
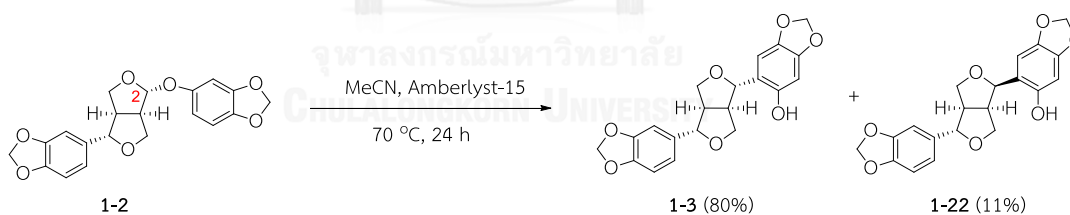
Scheme 3.2 Synthesis of **1-3** and its epimer (**1-22**)

From the results, we found that number and type of electron donating group on a phenolic aromatic ring influence the yield of products. On reaction with samin (**1-14**), phenolic **b** having two methoxy groups (2XOCH_3) as strong activating groups afforded higher yield of **3b** (50%) and ***epi*-3b** (45%); whereas phenolic **a** having a strong activating group (-OH) and a weak activating group (-CH_3) afforded **3a** and ***epi*-3a** with

lower yield of 30 and 15%, respectively. Therefore, higher electron density on the phenolic aromatic moiety was critical to force reaction with higher yield of products.

Additionally, the orientation of substituents on phenolic ring was another key factor to enhance reactivity of reaction. For example, phenolic **g** and **i** had the same a number of strong activating group (1xOH, 2XOCH$_3$) but they were different reactivity; namely phenolic **i** was more reactive toward oxocarbenium ion than phenolic **g**. This observation could be explained by constructive density of electron. In case of phenolic **i**, all substituents reinforced each other whereas phenolic **g** did not (Figure 3.2).
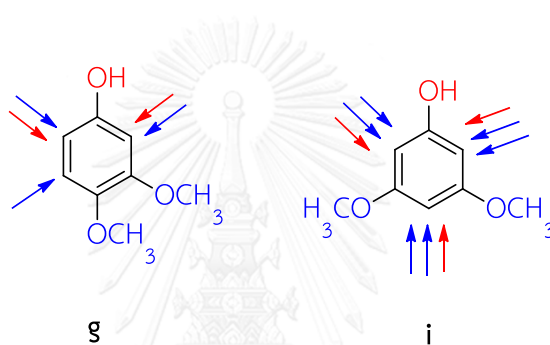

Figure 3.2 Electron density on phenolics ring from resonance effect of activating group presented by the arrows

Furthermore, when the multiple substituted phenolics underwent an electrophilic aromatic substitution reaction, the directing effects of all substituents had to be considered: phenolic **a**, for example, possessing the hydroxyl group directing to the *ortho*- or *para*- position, and the methyl group directed to *ortho*- or *para*- position. Notice that three positions were activated, but the new substituent ended up on only one of three because steric hindrance effect made the position between the substituents less accessible and the more powerful activating group , the hydroxyl group (-OH) was the dominant influence over a weakly activating substituent (-CH$_3$). Therefore, the incoming oxocarbenium ion of furofuran moiety was installed at a position that was *ortho*- or *para*- position to hydroxy or methyl groups, repectively as

indicated by bold arrow in Figure 3.1. The other phenolics could be explained in the same way.

To confirm correct structure, particular synthesized furofuran lignans, such as **3a** and *epi*-**3a**, were elucidated by HMBC. The HMBC correlations (Figure 3.3) from H-2 (δ_H = 4.85, d, J = 5.6 Hz) to C-1$^{'}$ (δ_C = 121.2), C-2$^{'}$ (δ_C = 155.5) and C-6$^{'}$ (δ_C =126.9), suggested that the favor reactivity on phenolic to furan moiety was *ortho*- or *para*-position to hydroxy or methyl groups corresponding with theoretical assumption.
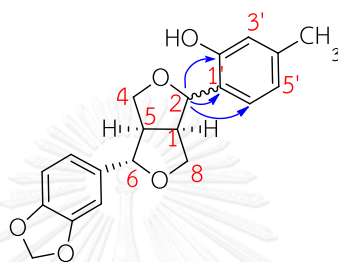

Figure 3.3 Key HMBC (H ⟶ C) for **3a** and *epi*-**3a** compound

However, multiple products were also obtained if there are more than one favored sites. This observation was demonstrated by the reaction between samin (**1-14**) and phenolic **c**. Once the oxocarbenium ion was produced, electrophilic aromatic substitution at C-2$^{'}$ of phenolic (pathway A) yielded lignan **3c** and its epimer (*epi*-**3c**) while the substitution at C-4$^{'}$ or C-6$^{'}$ (pathway B) afforded lignan **3c$^{'}$** and its epimer (*epi*-**3c$^{'}$**) (Scheme 3.3).
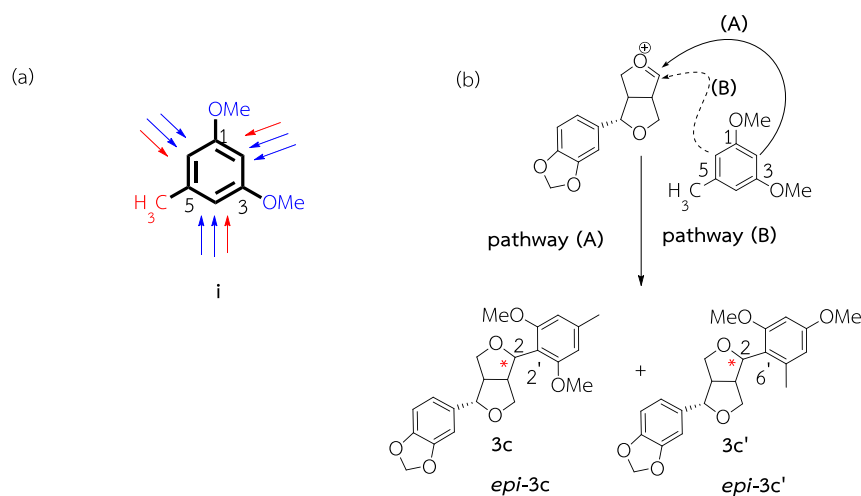
Scheme 3.3 (a) Electron density on phenolics ring from resonance effect of activating group presented by the arrows (b) pathway for generate multiple products from reaction **i** phenolics with oxocarbenium ion intermediate

3.2 Stereochemistry determination of synthesized furofuran lignans

Reactions between samin (**1-14**) and phenolics produced a pair of diastereomeric. To easily elucidate each isomer, we first drawn boat-boat and chair-boat conformation of each isomer, which was verified by X-ray analysis of samin and *epi*-samin, respectively [21].

3a and ***epi*-3a** were represented for construction this model. The relative configurations of **3a** and ***epi*-3a** were inspected by NOESY data as well as the observed ^{1}H NMR pattern. **3a** revealed key NOESY correlations from H-2 to H-8$_{ax}$ and H-6 to H-4$_{ax}$, which were indicated of *exo-exo* orientation (Figure 3.4a). In contrast, ***epi*-3a** showed key NOESY correlations from H-2 to H-4$_{ax}$ and H-6 to H-8$_{ax}$. Therefore, the structure of ***epi*-3a** was elucidated as *endo-exo* orientation (Figure 3.4b).

The difference in stereochemistry at C-2 of **3a** and ***epi*-3a** could also be easily observed in ^{1}H NMR spectra (Figure 3.5). H-2 and H-6 in **3a** revealed doublet (d) signal with nearly equal coupling constant (J = 4.0 Hz), whereas those of ***epi*-3a** demonstrated significantly different values (J = 5.6 Hz and J = 8.0 Hz for H-2 and H-6, respectively). Furthermore, the difference in ^{1}H NMR splitting patterns of **3a** and ***epi*-**

3a was also striking observed for H-4 (Figure 3.5). H-4$_{ax}$ and H-4$_{eq}$ of **3a** each showed the expected doublet of doublet (dd) signal whereas those of **epi-3a** demonstrated different splitting patterns. The H-4$_{eq}$ of **epi-3a** revealed unexpected doublet (d) signal caused solely by germinal coupling with H-4$_{ax}$. This observation could be accounted for a nearly 90° dihedral angle between H-4$_{eq}$ and H-5 that give rise $^{2}J_{4eq, 5} \approx 0$ Hz [22]. Noticeably, the differences in ^{1}H NMR splitting patterns of **3a** and its epimer were also observed in other synthesized furofuran lignans. Therefore, applying the above mentioned observation would be useful to readily discriminate the stereochemistry at C-2 of synthesized furofuran lignans.
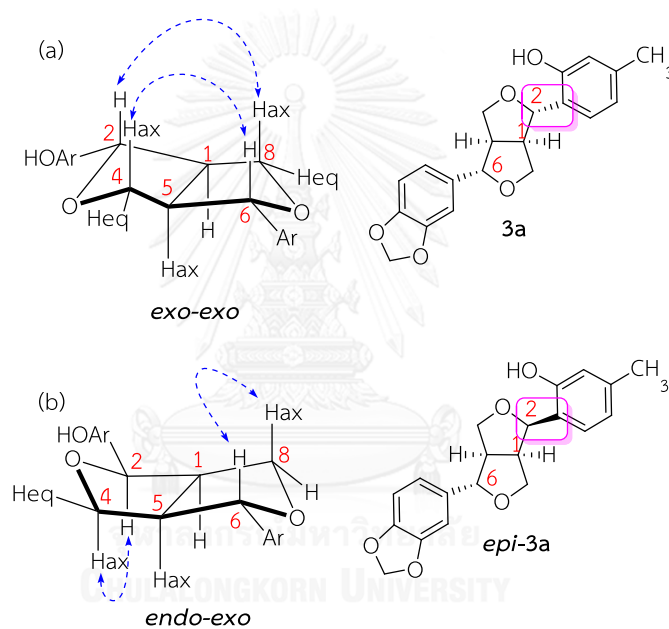

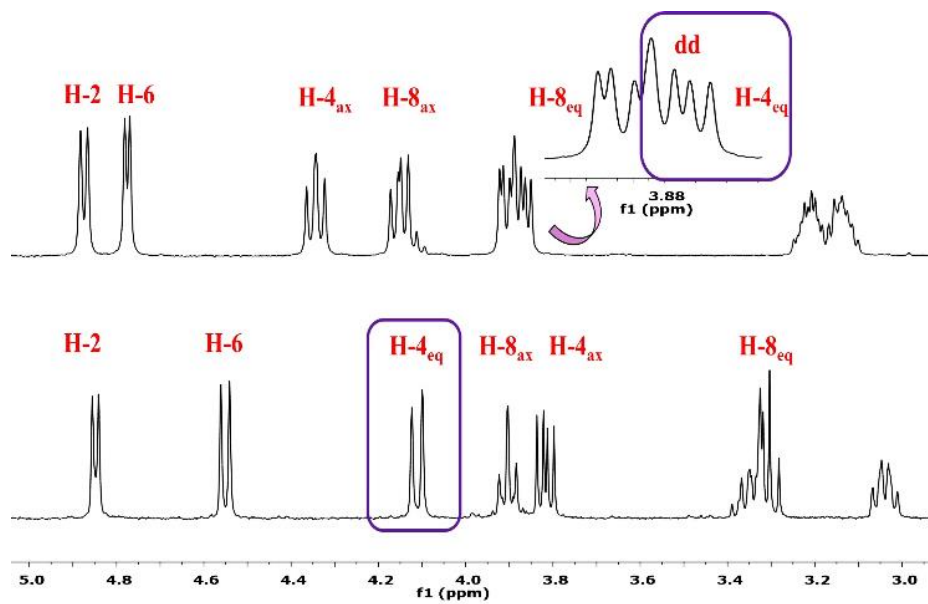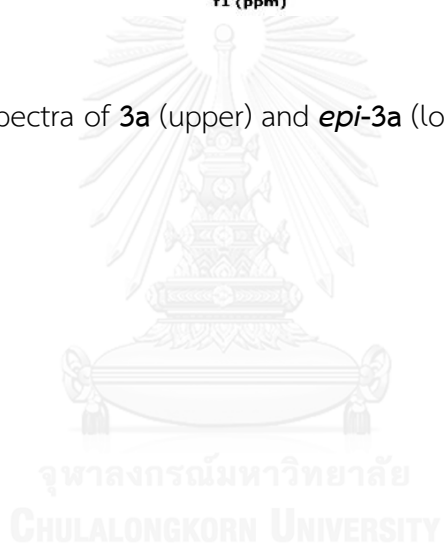**Figure 3.4** Diagnostic NOESY (H◄──►H) correlations for **3a** (a) and **epi-3a** (b)

**Figure 3.5** ^{1}H NMR spectra of **3a** (upper) and *epi*-**3a** (lower)

3.3 Experimental section

3.3.1 Synthesis of furofuran lignans 3a – 3k and their epimers

Gerneral procedure for synthesis

To a solution of samin **1-14** (1 equiv) in acetonitrile (1.0 mL/0.1 mmol of samin) was treated with phenolics **a – e** (1.5 - 2 equiv), acidic resin amberlyst-15 (1 mg/0.005 mmol of samin) and 4 Å MS. After stirring at room temperature for 8 h, the reaction mixture was evaporated to dryness and purified by column chromatography.
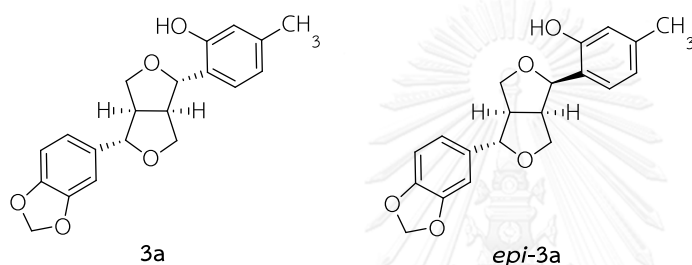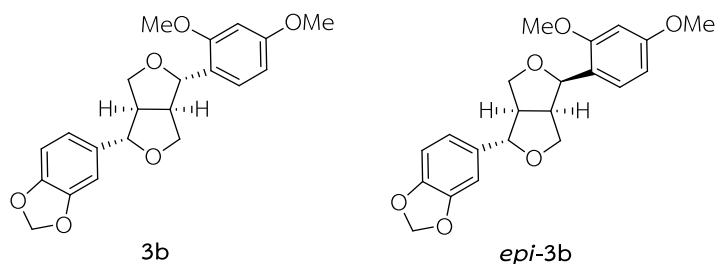

Following the above general precedure, reaction of **1-14** (64.5 mg, 0.26 mmol) and *m*-cresol (**a**, 40 µL, 0.39 mmol) in acetonitrile (2 mL) after 8 h yielded compounds **3a** (27 mg, 30%) and **_epi_-3a** (13 mg, 15%) as white solid. Note that the IUPAC nomenclutures of synthesized products were made based on "classification and nomenclature of lignans", published in 1995 [23]

3a (*1R,2S,5R,6S*)-2-(2´-hydroxy-4´-methylphenyl)-6-(3´´,4´´-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 7.89 (brs, 1H, -OH), 6.92 (d, J = 7.6 Hz, 1H, H-6''), 6.83-6.78 (m, 3H, H-2', H-5', and H-6'), 6.71 (s, 1H, H-3''), 6.67 (d, J = 7.6 Hz, 1H, H-5''), 5.95 (s, 2H, H-7'), 4.87 (d, J = 4.0 Hz, 1H, H-2), 4.78 (d, J = 4.0 Hz, 1H, H-6), 4.34 (dd, J = 9.2, 7.6 Hz, 1H, H-4), 4.15 (dd, J = 9.2, 6.8 Hz, 1H, H-8), 3.92-3.85 (m, 2H, H-4 and H-8), 3.21 (m, 1H, H-1), 3.14 (m, 1H, H-5), 2.29 (s, 3H, -CH$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 155.5, 148.2, 147.4, 139.8, 134.8, 126.8, 120.9, 120.9, 119.5, 117.9, 108.4, 106.7, 101.3, 86.7, 85.6, 72.5, 70.9, 53.6, 53.1, 21.2; HRMS m/z 363.1212 [M+Na]$^+$ (calcd for C$_{20}$H$_{20}$NaO$_5$, 363.1208).

***epi*-3a** (*1R,2R,5R,6S*)-2-(2´-hydroxy-4´-methylphenyl)-6-(3´,4´-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 7.85 (brs, 1H, -OH), 6.92 (d, *J* = 7.6 Hz, 1H, H-6''), 6.86-6.80 (m, 3H, H-2', H-5', and H-6'), 6.71 (s, 1H, H-3''), 6.67 (d, *J* = 7.6 Hz, 1H, H-5''), 5.97 (s, 2H, H-7'), 4.85 (d, *J* = 5.6 Hz, 1H, H-2), 4.55 (d, *J* = 8.0 Hz, 1H, H-6), 4.11 (d, *J* = 9.6 Hz, 1H, H-4), 3.90 (dd, *J* = 8.4, 7.6 Hz, 1H, H-8), 3.82 (dd, *J* = 9.6, 6.0 Hz, 1H, H-4), 3.38-3.28 (m, 2H, H-1 and H-8), 3.04 (m, 1H, H-5), 2.29 (s, 3H, -CH$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 155.5, 147.9, 146.9, 139.8, 132.0, 126.9, 121.2, 120.8, 118.8, 118.0, 108.4, 106.5, 101.2, 88.6, 82.0, 70.7, 70.2, 53.4, 49.9, 21.3; HRMS *m/z* 363.1213 [M+Na]$^+$ (calcd for C$_{20}$H$_{20}$NaO$_5$, 363.1208).
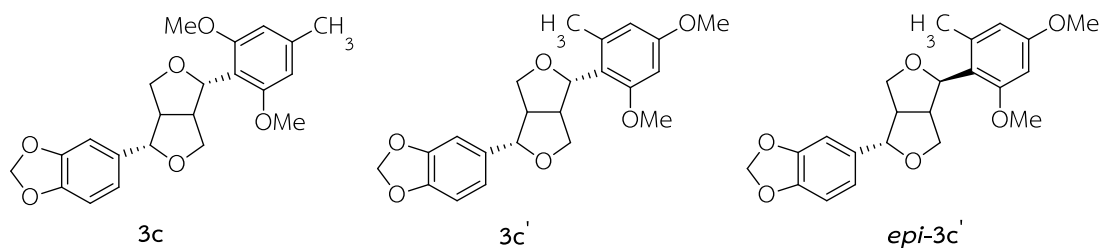
3b   epi-3b

Following the above general procedure, reaction of **1-14** (12.5 mg, 0.05 mmol) and 1,3-dimethoxybenzene (**b**, 12 mg, 0.075 mmol) in acetonitrile (0.5 mL) after 8 h yielded compounds **3b** (10 mg, 55%) and **epi-3b** (8.2 mg, 45%) as colorless oil.

3b (*1R,2S,5R,6S*)-2-(2′,4′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.25 (dd, *J* = 8.0, 2.8 Hz, 1H,), 6.84 (s, 1H), 6.81-6.74 (m, 2H), 6.45 (d, *J* = 7.2 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 5.92 (s, 2H), 5.03 (d, *J* = 4.8 Hz, 1H), 4.64 (d, *J* = 5.6 Hz, 1H), 4.30 (dd, *J* = 9.2, 7.6 Hz, 1H,), 4.19 (dd, *J* = 8.8, 6.4 Hz, 1H), 3.98 (dd, *J* = 9.2, 5.2 Hz, 1H), 3.90 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.01 (m, 1H), 2.91 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 160.2, 157.4, 147.9, 147.1, 135.4, 126.1, 122.8, 119.5, 108.1, 106.6, 103.8, 101.0, 98.6, 85.5, 82.0, 73.3, 71.2, 55.4, 55.3, 54.7, 53.7; HRMS *m/z* 393.1310 [M+Na]$^+$ (calcd for C$_{21}$H$_{22}$NaO$_6$, 393.1314).

epi-3b (*1R,2R,5R,6S*)-2-(2′,4′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.44 (d, *J* = 8.4 Hz, 1H), 6.87 (s, 1H), 6.81-6.76 (m, 2H), 6.50 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.44 (s, 1H), 5.94 (s, 2H), 4.91 (d, *J* = 6.0 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.09 (d, *J* = 9.2 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H$_3$), 3.78-3.74 (m, 2H), 3.47 (m, 1H), 3.22 (dd, *J* = 8.8, 8.8 Hz, 1H), 2.84 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 160.2, 156.6, 148.1, 147.3, 135.6, 127.3, 127.3, 119.7, 108.3, 106.8, 103.9, 101.1, 98.3, 87.6, 78.6, 70.5, 69.9, 55.5, 55.4, 54.9, 48.7; HRMS *m/z* 393.1310 [M+Na]$^+$ (calcd for C$_{21}$H$_{22}$NaO$_6$, 393.1314)
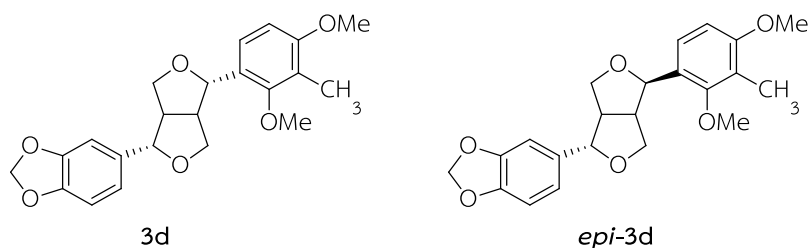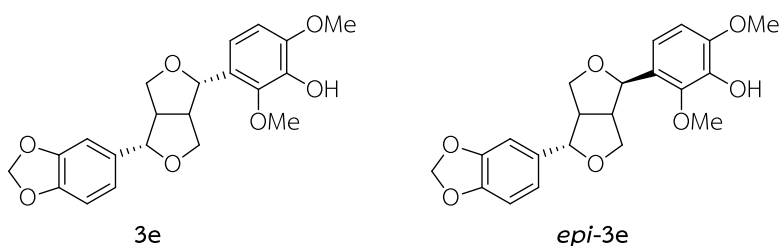
Following the above general procedure, reaction of **1-14** (68.3 mg, 0.27 mmol) and 3,5-dimethoxytoluene (**c**, 79 μL, 0.54 mmol) in acetonitrile (3 mL) after 8 h yielded compounds **3c** (21 mg, 20%), **3c'** (22 mg, 21%) and *epi*-**3c'** (24 mg,23%) as white solid.

3c *(1R,2S,5R,6S)*-2-(2′,6′-dimethoxy-6′-methylphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.90 – 6.78 (m, 3H), 6.38 (s, 2H), 5.95 (s, 2H), 5.47 (d, *J* = 5.9 Hz, 1H), 4.70 (d, *J* = 6.2 Hz, 1H), 4.27 (dd, *J* = 8.5, 6.6 Hz, 1H), 4.16 (dd, *J* = 8.9, 7.2 Hz, 1H), 3.87 – 3.83 (m, 2H), 3.80 (s, 6H), 3.50 (m, 1H), 3.07 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 158.9, 148.0, 147.1, 139.9, 136.0, 119.6, 114.1, 108.3, 106.7, 105.4, 101.1, 85.6, 78.3, 73.2, 72.3, 56.4, 55.9, 51.2, 22.2; HRMS *m/z* 407.1462 [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_6$$^+$,407.1465).

3c' *(1R,2S,5R,6S)*-2-(2′,4′-dimethoxy-6′-methylphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.87 – 6.71 (m, 3H), 6.33 (d, *J* = 6.3 Hz, 2H), 5.96 (s, 2H), 5.22 (d, *J* = 7.2 Hz, 1H), 4.81 (d, *J* = 4.6 Hz, 1H), 4.38 (t, *J* = 8.0 Hz, 1H), 4.03 (m, 1H), 3.89 (m, 1H), 3.79 (m, 7H), 3.32 (m, 1H), 3.17 (m, 1H), 2.41 (s, 3H). ; ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 160.0, 159.3, 148.1, 147.1, 139.5, 135.8, 135.4, 125.2, 119.5, 108.3, 108.0, 106.7, 101.2, 97.0, 85.1, 81.5, 72.7, 71.9, 55.8, 55.4, 55.4, 52.1, 21.0. HRMS *m/z* (calcd for C$_{22}$H$_{24}$NaO$_6$$^+$,407.1465)

***epi*-3c'** *(1R,2R,5R,6S)*-2-(2′,4′-dimethoxy-6′-methylphenyl)-6-(3′′,4′′-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.90 – 6.78 (m, 3H), 6.33 (brs, 1H), 6.32(brs, 1H), 5.96 (s, 2H), 5.02 (d, *J* = 7.7 Hz, 1H), 4.82 (d, *J* = 5.6 Hz, 1H), 4.07 (d, *J* = 9.3 Hz, 1H), 3.84 – 3.72 (m, 8H), 3.37 (m,1H), 3.26 – 3.16 (m, 2H), 2.41 (s, 3H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 159.9, 159.3, 147.7, 146.7, 146.6, 139.7, 132.7, 118.8, 108.2, 108.1, 106.6, 101.1, 96.8, 82.0, 81.9, 71.7, 69.6, 55.9, 55.4, 51.8, 51.1, 20.9; HRMS *m/z* [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_6$$^+$,407.1465)

3d   *epi*-3d

Following the above general procedure, reaction of **1-14** (63mg, 0.25 mmol) and 2,6-dimethoxytoluene (**d**, 74 µL, 0.5 mmol) in acetonitrile (2.5 mL) after 8 h yielded compounds **3d** (40 mg, 42%) and ***epi*-3d** (30 mg, 31%) as yellow oil.

3d *(1R,2S,5R,6S)*-2-(2′,4′-dimethoxy-3′-methylphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.16 (d, J = 8.5 Hz, 1H), 6.90 – 6.72 (m, 3H), 6.63 (d, J = 8.5 Hz, 1H), 5.95 (s, 2H), 5.06 (d, J = 4.6 Hz, 1H), 4.68 (d, J = 5.5 Hz, 1H), 4.30 (t, J = 8.2 Hz, 1H), 4.21 (m, 1H), 3.98 (dd, J = 9.0, 4.8 Hz, 1H), 3.90 (dd, J = 9.1, 3.9 Hz, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 3.09 (m, 1H), 3.00 (m, 1H), 2.16 (s, 3H).; ^{13}C NMR (CDCl$_3$, 100 MHz) δ 158.6, 157.0, 148.1, 147.2, 135.5, 127.0, 123.8, 120.1, 119.6, 108.3, 106.7, 106.0, 101.2, 85.7, 82.3, 73.1, 71.5, 60.9, 55.8, 54.8, 54.1, 9.2.; HRMS *m/z* 407.1470 [M + Na$^+$] (calcd for C$_{22}$H$_{24}$NaO$_6^+$, 407.1465)

***epi*-3d** *(1R,2R,5R,6S)*-2-(2′,4′-dimethoxy-3′-methylphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.36 (d, J = 8.6 Hz, 1H), 6.90 – 6.74 (m, 3H), 6.66 (d, J = 8.5 Hz, 1H), 5.94 (s, 2H), 4.96 (d, J = 6.2 Hz, 1H), 4.38 (d, J = 7.4 Hz, 1H), 4.10 (d, J = 9.3 Hz, 1H), 3.83 - 3.80 (m, 4H), 3.76 – 3.73 (m, 4H), 3.46 (m, 1H), 3.24 (t, J = 8.6 Hz, 1H), 2.87 (m, 1H), 2.16 (s, 3H) ; ^{13}C NMR (CDCl$_3$, 100 MHz) δ158.3, 155.7, 148.1, 147.3, 135.6, 124.5, 124.2, 123.6, 119.8, 108.3, 106.78, 105.9, 101.2, 87.7, 78.8, 70.6, 69.9, 60.6, 55.8, 55.0, 49.3, 9.3; HRMS *m/z* [M + Na$^+$] (calcd for C$_{22}$H$_{24}$NaO$_6^+$, 407.1465)

3e   epi-3e

Following the above general procedure, reaction of **1-14** (36 mg, 0.14 mmol) and 2,6-dimethoxyphenol (**e**, 43 mg, 0.28 mmol) in acetonitrile (1.5 mL) after 8 h yielded compounds **3e** (15.2 mg, 27%) and ***epi*-3e** (20 mg, 37%) as yellow oil.
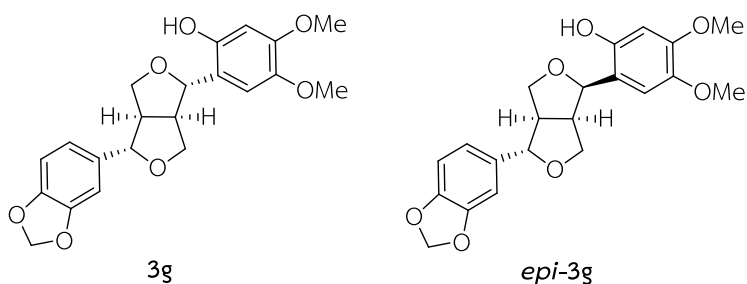
3e *(1R,2S,5R,6S)*-2-(3′-hydroxyl-2′,4′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 6.89 – 6.73 (m, 4H), 6.62 (d, J = 8.6 Hz, 1H), 5.94 (s, 2H), 5.05 (d, J = 4.8 Hz, 1H), 4.68 (d, J = 5.7 Hz, 1H), 4.31 (dd, J = 9.1, 7.3 Hz, 1H), 4.22 (dd, J = 9.1, 6.6 Hz, 1H), 4.01 (dd, J = 9.2, 4.7 Hz, 1H), 3.92 (d, J = 4.3 Hz, 4H), 3.89 (d, J = 7.1 Hz, 4H), 3.05 (m, 1H), 2.98 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 148.1, 147.4, 144.6, 138.7, 135.4, 128.3, 119.6, 115.9, 108.3, 106.7, 105.9, 101.2, 85.6, 82.4, 73.1, 71.6, 60.6, 56.4, 54.8, 54.2; HRMS *m/z* 409.1263 [M + Na$^+$] (calcd for C$_{21}$H$_{22}$NaO$_7^+$, 409.1258).

***epi*-3e** *(1R,2R,5R,6S)*-2-(3′-hydroxyl-2′,4′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.02 (d, J = 8.5 Hz, 1H), 6.87 – 6.76 (m, 3H), 6.65 (d, J = 8.4 Hz, 1H), 5.95 (s, 2H), 4.95 (d, J = 5.9 Hz, 1H), 4.36 (d, J = 8.0 Hz, 1H), 4.09 (d, J = 9.4 Hz, 1H), 3.92 – 3.86 (m, 7H), 3.83 – 3.78 (m, 2H), 3.45 (m, 1H), 3.23 (m, 1H), 2.86 (dd, J = 15.4, 7.2 Hz, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 148.1, 147.3, 147.2, 138.2, 135.5, 129.9, 124.6, 119.7, 116.8, 108.3, 106.8, 105.8, 101.2, 87.7, 78.7, 70.6, 69.9, 60.3, 56.4, 54.9, 49.2 ; HRMS *m/z* 409.1263 [M + Na$^+$] (calcd for C$_{21}$H$_{22}$NaO$_7^+$, 409.1258)

3f   *epi*-3f
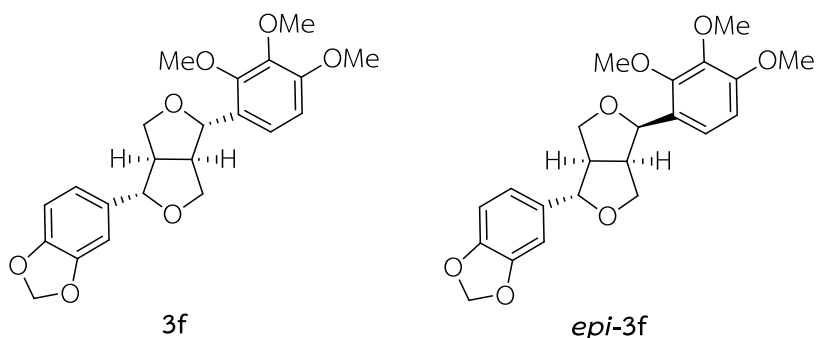
Following the above general procedure, reaction of **1-14** (81.6 mg, 0.33 mmol) and 1,2,3-trimethoxybenzene (**f**, 54.8 mg, 0.66 mmol) in acetonitrile (3 mL) after 8 h yielded compounds **3f** (41 mg, 31%) and ***epi*-3f** (29 mg, 22%) as yellow oil.

3f   *(1R,2S,5R,6S)*-2-(2′,3′,4′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (400 MHz, CDCl$_3$) **δ** 7.03 (d, *J* = 8.6 Hz, 1H), 6.89 − 6.75 (m, 3H), 6.64 (d, *J* = 8.6 Hz, 1H), 5.95 (s, 2H), 5.03 (d, *J* = 4.6 Hz, 1H), 4.67 (d, *J* = 5.7 Hz, 1H), 4.33 (dd, *J* = 8.9, 7.6 Hz, 1H), 4.21 (dd, *J* = 9.0, 6.6 Hz, 1H), 4.00 (dd, *J* = 9.1, 4.8 Hz, 1H), 3.94 − 3.88 (m, 4H), 3.87 (s, 3H), 3.85 (s, 3H), 3.05 (m, 1H), 2.97 (m, 1H). ^{13}C NMR (101 MHz, CDCl$_3$) **δ** 153.4, 151.2, 148.1, 147.3, 142.4, 135.4, 128.0, 125.2, 120.3, 119.6, 108.3, 107.1, 106.7, 101.2, 85.6, 82.4, 73.2, 71.5, 60.9, 56.2, 54.8, 54.2, HRMS *m/z* 423.1431 [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_7$, 423.1420).

***epi*-3f**   *(1R,2R,5R,6S)*-2-(2′,3′,4′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 7.21 (d, *J* = 8.6 Hz, 1H), 6.87-6.76 (m, 3H, H-2', H-5', and H-6'), 6.67 (d, *J* = 8.6 Hz, 1H), 5.94 (s, 2H), 4.93 (d, *J* = 5.6 Hz, 1H), 4.37 (d, *J* = 8.0 Hz, 1H), 4.09 (d, *J* = 9.2 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 6H), 3.83-3.77 (m, 2H), 3.43 (m, 1H), 3.24 (m, 1H), 2.86 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 153.2, 150.0, 148.1, 147.3, 141.8, 135.5, 124.4, 121.2, 119.7, 108.3, 107.0, 106.8, 101.2, 87.7, 78.6, 70.6, 69.9, 60.9, 60.8, 56.1, 55.0, 49.2; HRMS *m/z* 423.1431 [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_7$, 423.1420).

3g   *epi*-3g

Following the above general procedure, reaction of **1-14** (46.8 mg, 0.19 mmol) and 3,4-dimethoxyphenol (**g**, 58 mg, 0.37 mmol) in acetonitrile (2 mL) after 8 h yielded compounds **3g** (49 mg, 68%) and ***epi*-3g** (6 mg, 7%) as white powder.
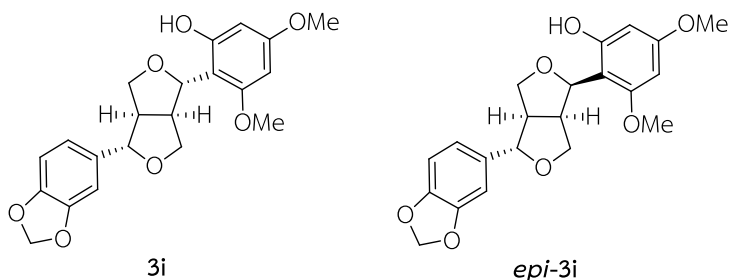
3g *(1R,2S,5R,6S)*-2-(2′-hydroxyl-4′,5′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 8.05 (brs, 1H, -OH), 6.87-6.77 (m, 3H), 6.46 (s, 1H), 6.42 (s, 1H), 5.95 (s, 2H), 5.01 (d, J = 6.0 Hz, 1H), 4.44 (d, J = 6.8 Hz, 1H), 4.19 (d, J = 9.6 Hz, 1H), 3.98 (t, J = 8.8 Hz, 1H), 3.88 (m, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.49 (dd, J = 8.4, 9.2 Hz), 3.40 (m, 1H), 2.91 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 150.1, 149.8, 148.2, 147.5, 142.7, 134.8, 125.2, 119.8, 110.5, 108.4, 106.7, 101.9, 101.2, 87.7, 84.6, 71.9, 70.1, 57.0, 56.0, 53.7, 50.8; HRMS *m/z* 409.1275 [M+Na]$^+$ (calcd for C$_{21}$H$_{22}$NaO$_7$, 409.1263).

***epi*-3g** *(1R,2R,5R,6S)*-2-(2′-hydroxyl-4′,5′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 7.71 (brs, 1H), 6.84-6.79 (m, 3H), 6.54 (s, 1H), 6.49 (s, 1H), 5.96 (s, 2H), 4.82 (d, J = 6.0 Hz,), 4.78 (d, J = 4.0 Hz), 4.36 (dd, J = 8.8, 7.2 Hz, 1H), 4.16 (dd, J = 9.6, 6.4 Hz, 1H), 3.92-3.86 (m, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.21-3.14 (m, 2H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 150.3, 150.1, 148.2, 147.4, 142.6, 134.8, 125.2, 119.5, 111.2, 108.4, 106.7, 102.1, 101.3, 86.7, 85.6, 72.6, 70.8, 57.2, 56.1, 53.6, 53.2; HRMS *m/z* 409.1275 [M+Na]$^+$ (calcd for C$_{21}$H$_{22}$NaO$_7$, 409.1263).

3h
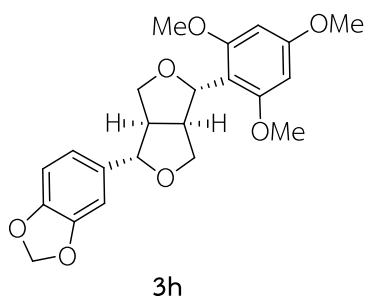
Following the above general procedure, reaction of **1-14** (25.5 mg, 0.10 mmol) and 1,3,5-trimethoxybenzene (**h**, 26 mg, 0.15 mmol) in acetonitrile (1 mL) after 8 h yielded compound **3h** (20 mg, 50%) as a colorless oil;
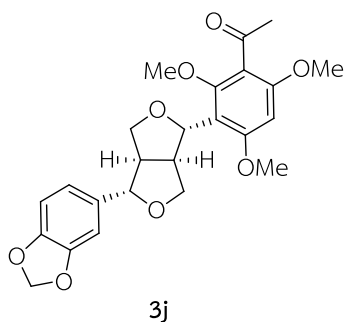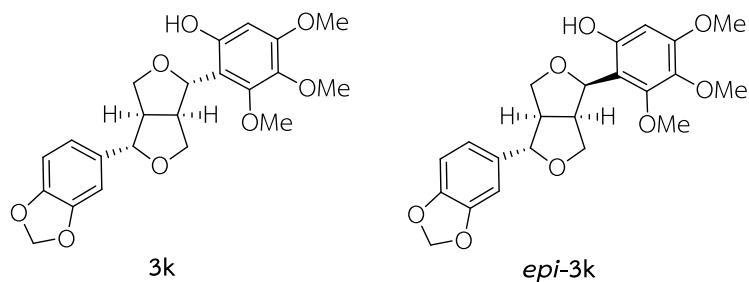
3h *(1R,2S,5R,6S)*-2-(2′,4′,6′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.89 (s, 1H, H-2'), 6.84 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.13 (s, 2H), 5.95 (s, 2H), 5.42 (d, *J* = 6.0 Hz, 1H), 4.70 (d, *J* = 6.0 Hz, 1H), 4.25 (dd, *J* = 8.8, 6.8 Hz, 1H4), 4.16 (dd, *J* = 8.8, 7.2 Hz,), 3.85-3.82 (m, 2H,), 3.81 (s, 3H), 3.80 (s, 6H), 3.49 (m, 1H), 3.07 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 161.3, 159.9, 159.9, 148.0, 147.1, 136.0, 119.6, 109.6, 108.3, 106.7, 101.1, 91.2, 91.2, 85.6, 78.3, 73.2, 72.2, 56.3, 56.0, 56.0, 55.5, 51.1; HRMS m/z 423.1417 [M+Na]$^{+}$ (calcd for C$_{22}$H$_{24}$NaO$_7$, 423.1420).

Following the above general procedure, reaction of **1-14** (56.5 mg, 0.22 mmol) and 3,5-dimethoxyphenol (**i**, 52 mg, 0.34 mmol) in acetonitrile (2 mL) after 8 h yielded compounds **3i** (41 mg, 47%) and ***epi*-3i** (44 mg, 51%) as white powder.

3i *(1R,2S,5R,6S)*-2-(2′-hydroxyl-4′,6′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 8.96 (brs, 1H), 6.82-6.77 (m, 3H), 6.06 (d, *J* = 2.4 Hz, 1H), 6.01 (d, *J* = 2.4 Hz, 1H), 5.95 (s, 2H), 5.21 (d, *J* = 7.6 Hz, 1H), 4.81 (d, *J* = 4.0 Hz, 1H), 4.47 (dd, *J* = 9.2, 8.4 Hz), 4.13 (dd, *J* = 9.2, 2.8 Hz), 4.03 (dd, *J* = 9.2, 6.8 Hz), 3.79 (m, 1H), 3.76 (s, 6H), 3.19 (m, 1H), 3.01 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 161.0, 158.0, 157.6, 148.2, 147.3, 134.9, 119.5, 108.3, 106.7, 105.0, 101.2, 94.6, 91.0, 84.2, 84.2, 72.7, 71.0, 55.5, 55.5, 54.8, 53.7; HRMS *m/z* 387.1449 [M+H]$^+$ (calcd for C$_{21}$H$_{23}$O$_7$, 387.1444).

***epi*-3i** *(1R,2R,5R,6S)*-2-(2′-hydroxyl-4′,6′-dimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 9.15 (brs, 1H), 6.87-6.77 (m, 3H), 6.07 (d, *J* = 2.0 Hz, 1H), 6.00 (d, *J* = 2.4 Hz, 1H), 5.95 (s, 2H), 5.17 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 7.2 Hz, 1H), 4.17 (d, *J* = 10.0 Hz, 1H), 3.91 (dd, *J* = 8.0, 8.0 Hz, 1H), 3.81 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.51-3.42 (m, 2H), 2.87 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 160.9, 158.1, 157.4, 148.2, 147.5, 134.9, 119.8, 108.3, 106.8, 101.7, 101.2, 94.3, 90.8, 87.5, 81.9, 71.4, 70.3, 55.7, 55.4, 53.7, 49.6; HRMS *m/z* [M+Na]$^+$ (calcd for C$_{21}$H$_{23}$O$_7$, 387.1444).

3j

Following the above general procedure, reaction of **1-14** (116.6 mg, 0.46 mmol) and 2,4,6-trimethoxyacetophenone (**j**, 196 mg, 0.92 mmol) in acetonitrile (5 mL) after 8 h yielded compound **3j** (13 mg, 15%) as white solid.

3j *(1R,2S,5R,6S)*-2-(3′-acety-2′,4′,6′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 6.88 (m, 3H), 6.27 (s, 1H), 5.95 (s, 2H), 5.23 (d, *J* = 6.0 Hz, 1H), 4.74 (d, *J* = 5.6 Hz, 1H), 4.31 (dd, *J* = 9.2, 7.2 Hz, 1H), 4.13 (dd, *J* = 8.8, 7.6 Hz, 1H), 3.92-3.90 (m, 2H), 3.85 (s, 3H$_3$), 3.83 (s, 3H), 3.77 (s, 3H), 3.44 (m, 1H) 3.12 (m, 1H), 2.49 (s, 3H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 202.0, 160.6, 158.1, 157.8, 148.1, 147.2, 135.7, 119.7, 119.5, 108.3, 106.7, 101.2, 101.2, 92.0, 85.3, 79.1, 72.8, 72.6, 64.4, 56.0, 56.0, 55.9, 51.5, 29.8; HRMS *m/z* 465.1538 [M+Na]$^+$ (calcd for C$_{24}$H$_{26}$NaO$_8$, 465.1525).

3k   epi-3k

Following the above general procedure, reaction of **1-14** (39 mg, 0.16 mmol) and 3,4,5-trimethoxyphenol (**k**, 44 mg, 0.24 mmol) in acetonitrile (1.6 mL) after 8 h yielded compounds **3k** (25.7 mg, 40%) and **epi-3k** (18.8 mg, 30%) as colorless oil.

3k *(1R,2S,5R,6S)*-2-(2′-hydroxyl-4′,5′,6′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 8.58 (brs, 1H), 6.82-6.77 (m, 3H), 6.22 (s, 1H, H-3"), 5.95 (s, 2H), 5.12 (d, *J* = 7.6 Hz, 1H), 4.83 (d, *J* = 3.6 Hz, 1H), 4.49 (dd, *J* = 8.4, 8.4 Hz, 1H), 4.13 (dd, *J* = 9.6, 2.8 Hz, 1H), 4.04 (dd, *J* = 9.2, 6.8 Hz, 1H), 3.90 (s, 3H), 3.81 (s, 3H), 3.80 (m, 1H), 3.79 (s, 3H), 3.22 (m, 1H), 3.03 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 153.9, 152.1, 150.9, 148.2, 147.3, 135.2, 134.7, 119.5, 109.1, 108.4, 106.8, 101.2, 97.0, 84.4, 84.2, 72.9, 70.8, 61.1, 60.9, 56.0, 54.7, 53.7; HRMS *m/z* 439.1366 [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_8$, 439.1369).

epi-3k *(1R,2R,5R,6S)*-2-(2′-hydroxyl-4′,5′,6′-trimethoxyphenyl)-6-(3″,4″-metylenedioxyphenyl)-3,8-dioxabicyclo [3.3.0] octane: ^{1}H NMR (CDCl$_3$, 400 MHz) **δ** 8.87 (brs, 1H), 6.87 (s, 1H), 6.83-6.77 (m, 2H), 6.21 (s, 1H), 5.95 (s, 2H), 5.15 (d, *J* = 4.0 Hz, 1H), 4.40 (d, *J* = 4.0 Hz, 1H), 4.18 (d, *J* = 10.0 Hz, 1H), 3.92 (m, 1H), 3.90 (s, 3H), 3.82 (s, 3H), 3.79 (m, 1H), 3.78 (s, 3H), 3.48-3.43 (m, 2H), 2.90 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) **δ** 153.8, 152.6, 150.2, 148.2, 147.5, 135.0, 134.8, 119.8, 108.3, 106.7, 105.7, 101.2, 96.8, 87.5, 82.0, 71.4, 70.3, 61.1, 60.9, 55.9, 53.8, 50.2; HRMS *m/z* [M+Na]$^+$ (calcd for C$_{22}$H$_{24}$NaO$_8$, 439.1369).

3.3.2 Synthesis of sesaminol and *epi*-sesaminol

Sesaminol (**1-3**) and *epi*-sesaminol (**1-22**) were obtained from acid catalysis of sesamolin (**1-2**) using the following procedure: To a solution of sasemolin (**1-2**, 82 mg, 0.221 mmol) in acetonitrile (2.5 mL) was treated with amberlyst®-15 (40 mg) and 4 Å MS. After stirring at 70°C for 5 h, the reaction mixture was evaporated to dryness and purified by column chromatography (SiO$_2$, 30:70 EtOAc-Hexane) yielded sesaminol (**1-3**, 66 mg, 80%) and *epi*-sesaminol (**1-22**, 11 mg, 13%) as white powder.
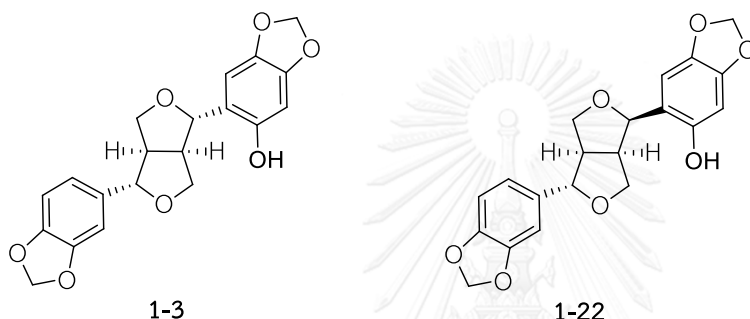
Saseminol (1-2): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.76 (brs, 1H), 6.83-6.78 (m, 3H), 6.51 (s, 1H), 6.45 (s, 1H), 5.96 (s, 2H), 5.89 (s, 2H), 4.77 (d, *J* = 4.0 Hz, 2H), 4.35 (dd, *J* = 8.8, 7.6 Hz, 1H), 4.14 (dd, *J* = 9.2, 6.0 Hz, 1H), 3.89-3.83 (m, 2H), 3.18-3.11 (m, 2H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 150.9, 148.3, 148.2, 147.4, 141.1, 134.7, 125.2, 119.5, 115.2, 108.4, 106.7, 106.3, 101.3, 99.6, 86.7, 85.5, 72.6, 70.7, 53.5, 53.1; HRMS *m/z* 393.0962 [M+Na]$^+$ (calcd for C$_{20}$H$_{18}$NaO$_7$, 393.0950).

epi-sesaminol (1-22): ^{1}H NMR (CDCl$_3$, 400 MHz) δ 8.17 (brs, 1H), 6.87-6.77 (m, 3H), 6.42 (s, 1H), 6.40 (s, 1H), 5.96 (s, 2H), 5.90 (s, 2H), 4.97 (d, *J* = 5.9 Hz, 1H), 4.41 (d, *J* = 7.0 Hz, 1H), 4.17 (d, *J* = 10.0 Hz, 1H), 4.00 (dd, *J* = 9.2, 8.8 Hz, 1H), 3.84 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.49 (dd, *J* = 9.2, 8.4 Hz, 1H), 3.37 (m, 1H), 2.89 (m, 1H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 150.8, 148.2, 147.9, 147.5, 141.2, 134.7, 119.8, 112.1, 108.3, 106.7, 105.8, 101.3, 101.2, 99.4, 87.7, 84.5, 71.9, 70.1, 53.6, 50.7; HRMS *m/z* 393.0946 [M+Na]$^+$ (calcd for C$_{20}$H$_{18}$NaO$_7$, 393.0950)

CHAPTER IV

BIOLOGICAL ACTIVITY EVALUATION

4.1   Investigation of antioxidant activity

The antioxidant activity of synthesized furofuran lignans was evaluated in order to get insight into the structure-antioxidant activity relationship. The antioxidant activity was evaluated based on free radical scavenging againt DPPH and ABTS. In DPPH bioassay, the antioxidant activity was measured in term of hydrogen atom transfer (HAT) capability to DPPH radical. On the other hand, ABTS analysis is based on a single electron transfer (SET) capability.

The synthesized compounds showed variation in antioxidant activity (Table 4.1). Generally, furofuran lignans having one hydroxyl group (namely **3a**, **3e**, **3g**, **3i**, **3k, 1-3** and **1-22** along with their epimers) showed highly potent activity with SC_{50} values in range 0.22 – 1.45 mM and 0.15 – 0.41mM toward DPPH and ABTS, repectively. On the other hand, furofuran lignans contaning no free hydroxy group reveled low or no scavenging activity. These results indicated that the presence of a free hydroxyl group on the phenolic moiety was particularly important for scavenging potency compared to methoxy ($-OCH_3$) and methyl ($-CH_3$) groups. These observations were examplified by **3i** and **3h** along with their epimers, whose structures process one and no hydroxy group, respectively.

Of synthesized compounds, the most potent antioxidant compound toward $DPPH^{\bullet}$ and $ABTS^{\bullet+}$radicals was **3e** along with epimer (Figure 4.1). The antioxidant potency of **3e** and epimer over the other products was mainly illustrated by low O-H bond dissociation enthalpies (BDE) that facillate direct hydrogen tranfer to radical as well as intramolecullar hydrgen bonding between hydroxyl and methoxy groups at *ortho*-postition on phenolic moiety.[24].

Table 4.1 Radical scavenging capability of furofuran lignans

Compound	Radical scavenging (SC_{50}, mM)	
	DPPH	ABTS
1-2	NA[a]	NA
1-14	NA	NA
3a	NA	0.34
epi-3a	NA	0.20
3b	NA	NA
epi-3b	NA	NA
3c	NA	NA
3c'	NA	NA
epi-3c'	NA	NA
3d	NA	NA
epi-3d	NA	NA
3e	0.34	0.22
epi-3e	0.17	0.15
3f	NA	NA
epi-3f	NA	NA
3g	0.41	0.34
epi-3g	0.22	0.37
3h	NA	NA

Table 4.1 (Cont.)   Radical scavenging capability of furofuran lignans

Compound	Radical scavenging (SC$_{50}$, mM)	
	DPPH	ABTS
3i	NA	0.31
*epi-*3i	NA	0.25
3j	NA	NA
3k	2.3	0.41
*epi-*3k	1.45	0.40
1-3	0.65	0.39
1-22	0.42	0.35
BHT	1.56	0.14

[a] Not active; %inhibition less than 30% at highest concentration (1 mg/mL) examined
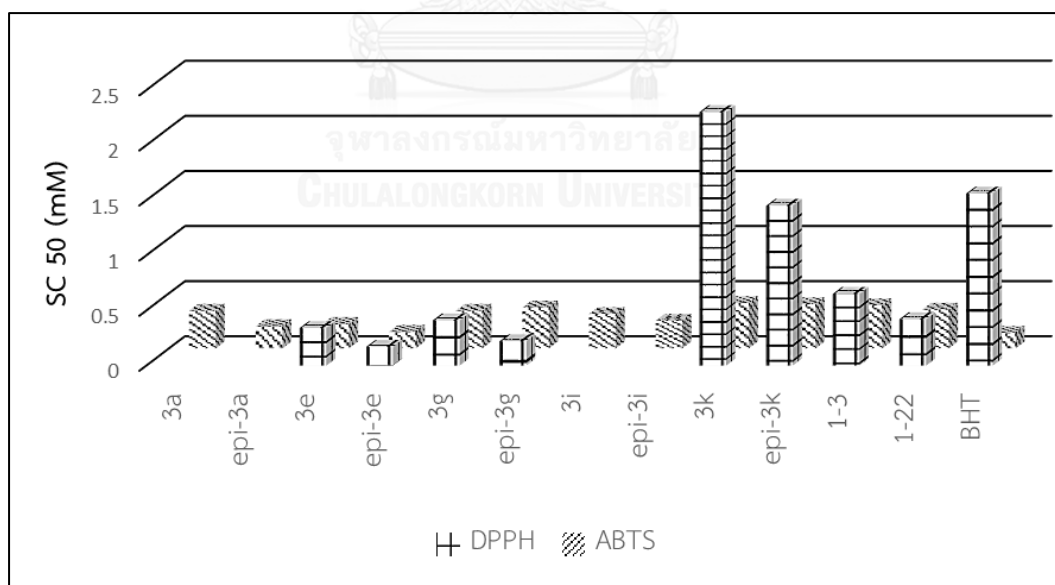
Figure 4.1   Antioxidant activity of particular furofuran lignans

4.2　α-Glucosidase inhibitory activity and kinetic analysis

Glucosidase inhibitory activity of all synthesized furofuran lignans was also evaluated againt rat intesinal maltase and and, the results expressed as IC_{50}, are summarized in Table 4.2 . Among synthesized products, the componds having free hydroxy group on phenolic moiety (namely **3a**, **3e**, **3g**, **3i**, **3k**, **1-3** and **1-22** along with their epimer) exhibited potent inhibition against maltase with IC_{5o} values in the milimolar concentration rang of 1.14 – 8.23 mM whereas the weaker inhibitory effects were observed in sucrase (3.13 – 18.89 mM) (Figure 4.2). The results suggested that hydroxy group was critical for enzyme inhibition, possibly through hydrogen bonding between hydroxy group and amino group residue of enzyme [25]. Noticeably, epimeric analogs revealed slightly enhanced inhibition than their congener; for example the inhibition against maltase of **1-22** (IC_{50} 1.14 mM) vs **1-3** (IC_{50} 3.42 mM).

Table 4.2　α-Glucosidase inhibitory effect of synthesized furofuran lignans

Compound	α-Glucosidase inhibitory effect (IC_{50}, mM)	
	Maltase	Sucrase
1-2	NA[a]	NA
1-14	NA	NA
3a	8.23	14.67
epi-**3a**	7.01	8.52
3b	NA	NA
epi-**3b**	NA	NA
3c	NA	NA
3c'	NA	NA
epi-**3c'**	NA	NA
3d	NA	NA
epi-**3d**	NA	NA

Table 4.2 (Cont.)   α-Glucosidase inhibitory effect of synthesized furofuran lignans

Compound	α-Glucosidase inhibitory effect (IC_{50}, mM)	
	Maltase	Sucrase
3e	2.15	3.83
epi-3e	1.52	3.13
3f	NA	NA
epi-3f	NA	NA
3g	5.59	8.21
epi-3g	2.44	3.30
3h	NA	NA
3i	4.67	18.89
epi-3i	2.98	11.1
3j	NA	NA
3k	3.36	3.59
epi-3k	1.31	3.84
1-3	3.42	6.90
1-22	1.14	4.01
Acarbose®	0.0015	0.0023

[a] Not active; % inhibition less than 30% at highest concentration (0.0625 mg/mL) examined
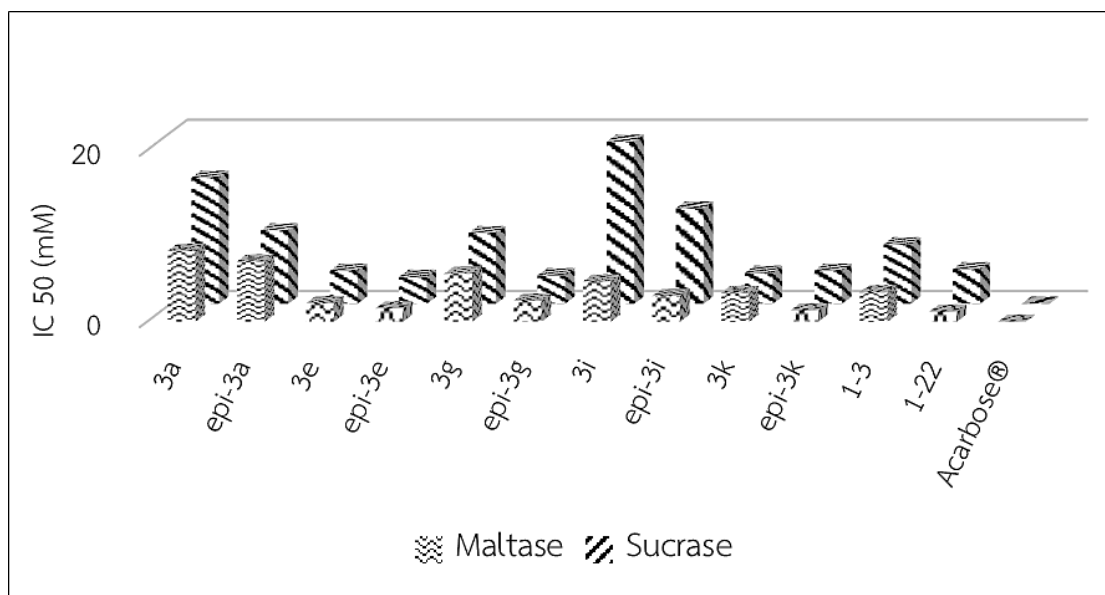
Figure 4.2 α-glucosidase inhibitory activity of active furofuran lignans

epi-Sesaminol (**1-22**), the most potent α-glucosidase inhibitor was further studied for enzyme kinetic by Lineweaver-Burk plot to gain insight into mode of inhibition. The Lineweaver - Burk plot was generated from initial velocity values (1/V) displayed on the Y-axis, and maltose concentration (1/ [maltose]) on the X-axis in the presence and absence of various concentrations of **1-22**. This plot gave a series of straight lines; all of which intersected in the second quadrant, as shown in Figure 4.3. The analysis showed that V_{max} decreased with increasing K_m in the presence of increasing concentrations of **1-22**. This behavior indicated that **1-22** inhibited maltase in a mix-type manner (Table 4.3) through two different pathways; competitively forming enzyme-inhibitor (EI) complex and interrupting enzyme-substrate (ES) intermediate by forming enzyme-substrate-inhibitor (ESI) complex in noncompetitive manner (Scheme 4.1).

Table 4.3 The K_m and V_{max} profile of enzyme inhibition

Mode of inhibition	Lineweaver-Burk plot	
	K_m	V_{max}
competitive	increased	unaffected
noncompetive	unaffected	reduced
uncompetitive	reduced	reduced
mix	increased or reduced	reduced
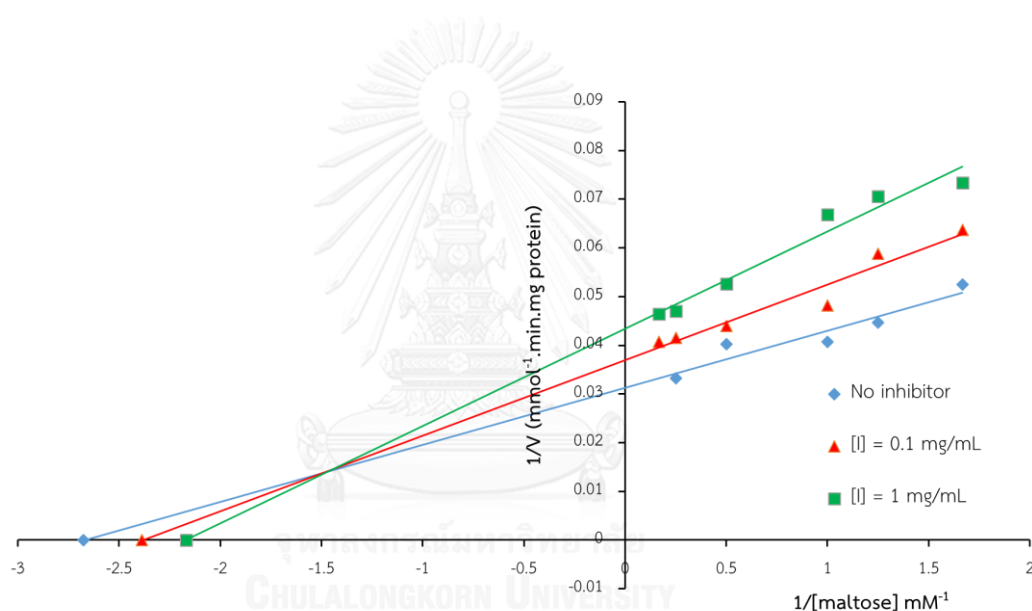
Figure 4.3 Lineweaver-Burk plot for inhibitory activity of *epi*-sesaminol (**1-22**) against rat intestinal maltase
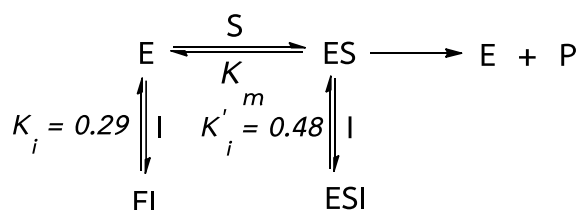
Scheme 4.1 Putative mechanism pathway for mixed type reversible inhibition of **1-22**. E, S, I and P are maltase, maltose, **1-22** and glucose, respectively.

To gain insight into preferential pathway of **1-22** to proceed, binding affinities of EI and ESI complex were investigated through dissociation constants K_i and $K_i^{'}$, respectively. The secondary replot of slope versus concentration of **1-22** revealed the K_i, value of 0.29 mM (Figure 4.4) while that of intercept versus concentration of **1-22** yielded the $K_i^{'}$ value of 0.48 mM (Figure 4.5). From this result, K_i value was 0.6 times smaller than $K_i^{'}$, suggesting that **1-22** predominantly inhibited maltase by competitive forming of EI complex over noncompetitive manner.
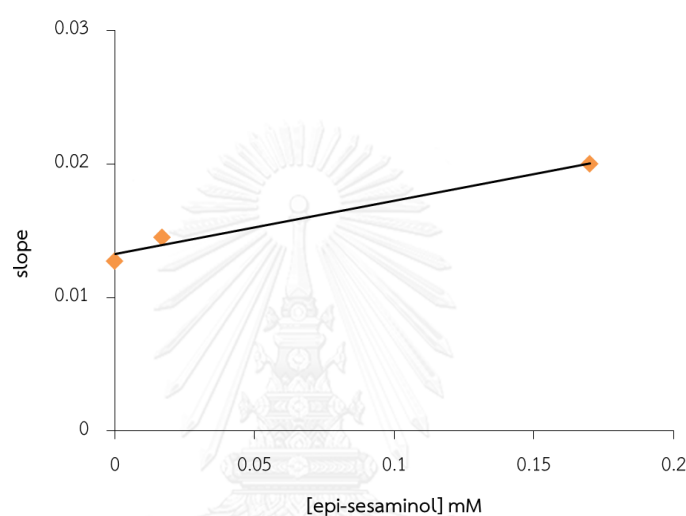
Figure 4.4 The secondary replot of slope (V_{max}/K_m) versus [*epi*-sesaminol] for determine the K_i. value
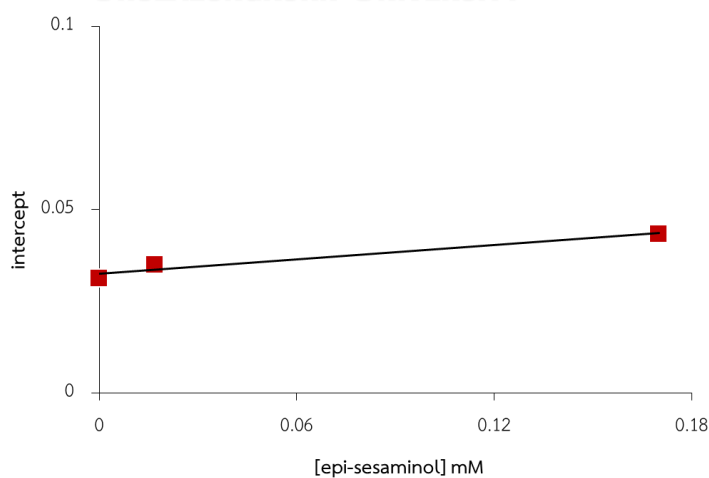
Figure 4.5 The secondary replot of intercept versus [*epi*-sesaminol] for determine the $K_i^{'}$. value

From biological acitivity results, the hydroxy group on phenolic moiety played an important role in enhancing both antioxidant acitivity and **α**-glucosidase inhibition activity (Figure 4.6). Additonally, the relation between structure of furofuran lignan and above biological activity SAR could be sumarized in Figure 4.7: (a) stereochemistry at C-2, almost epimeric products (*endo-exo*) reveal more slightly potent inhibition than their isomeric products (*exo-exo*) and (b) type of substitutent on phenolic moiety, hydroxyl groups contribute to more potent inhibition than other substituent groups (e.g. -OMe and -Me), possibly attribute to hydrogen bonding ability.
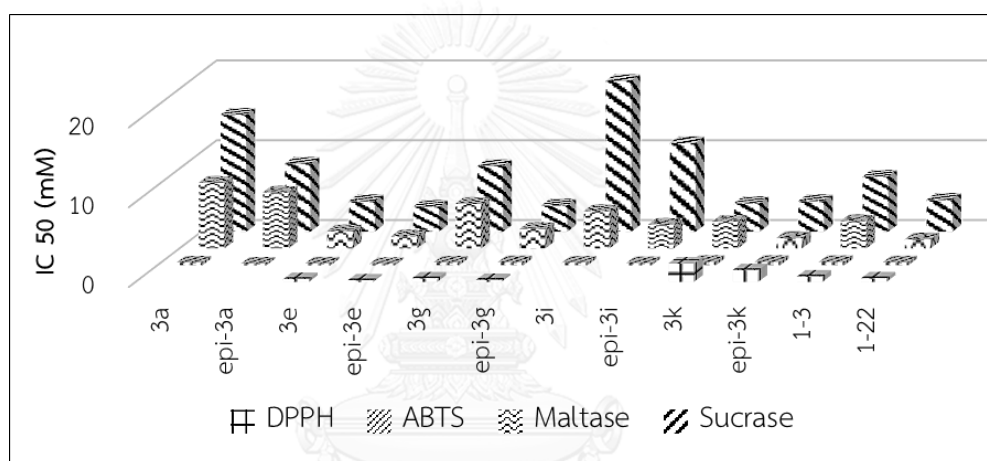
Figure 4.6 Antioxidant activity and **α**-glucosidase inhibitory activity of particular active furofuran lignans
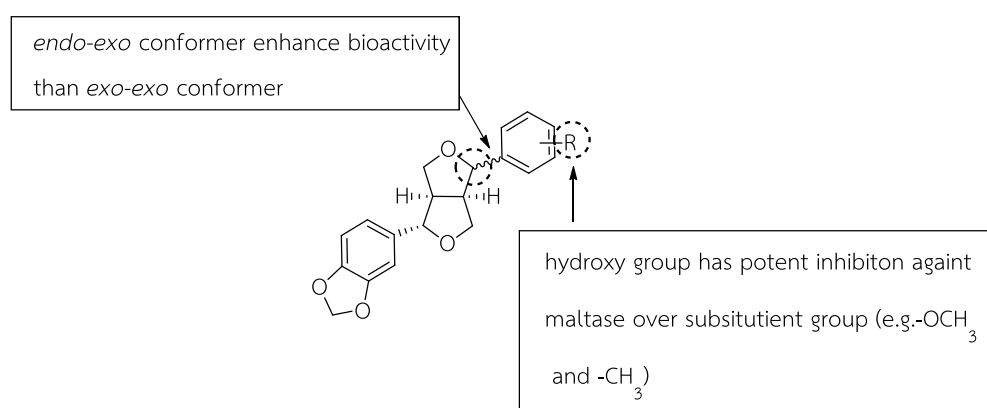
Figure 4.7 General SAR of furofuran lignans

4.3 Experimental section

4.3.1 Free radical scavenging activity

4.3.1.1 DPPH assay

The sample solution (20 µL at concentrations of 0.008, 0.04, 0.2 and 1 mg/mL in DMSO) was added to 0.1 mM methanolic solution of DPPH (180 µL). Then, the mixture was kept dark at room temperature for 15 min. The absorbance of the resulting solution was measured at 517 nm with 96-well microplate reader (Bio-Red microplate reader model 3550 UV). The percentage scavenging (%SC) was calculated by $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance without the sample, and A_1 is the absorbance with the sample. The SC_{50} value was determined from a plot of percentage inhibition versus sample concentration by sigmaplot programme. Butylated hydroxytoluene (BHT) was used as standard control and the experiment was performed in triplicate.

2,2-diphenyl-1-picrylthydrazyl
free radical (DPPH•)

Purple, 517 nm

2,2-diphenyl-1-picrylthydrazyl
(DPPH)

Colorless
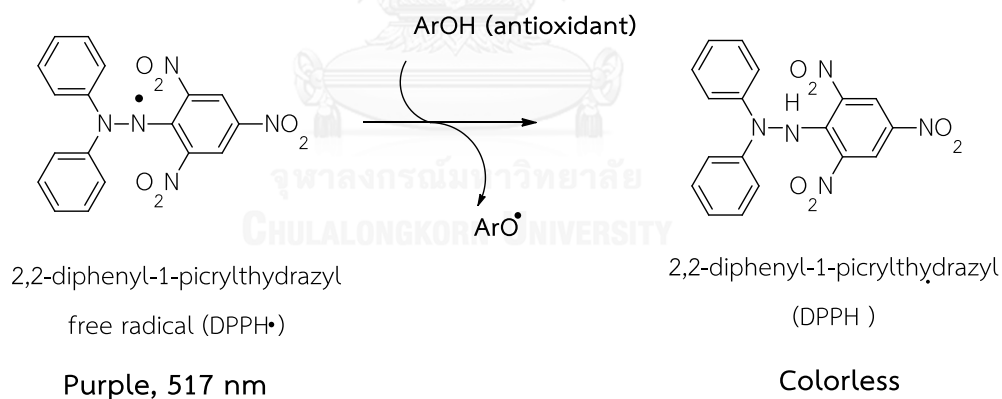
4.3.1.2 ABTS assay

$ABTS^{\bullet+}$ radical cation was produced by mixing 10 mL of 7.4 mM ABTS with 0.5 mL of 2.6 mM potassium persulphate ($K_2S_2O_8$) for 16 h in the dark at room temperature. The $ABTS\bullet^+$ stock solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 750 nm. The synthesized compound (20 µL) was mixed with 80 µL of diluted $ABTS^{\bullet+}$ solution. After 2 h of incubation, the absorbance was determined at 750

nm. The percentage inhibition could be calculated using in the above expression. Butylated hydroxytoluene (BHT) was used as standard control and the experiment was performed in triplicate.
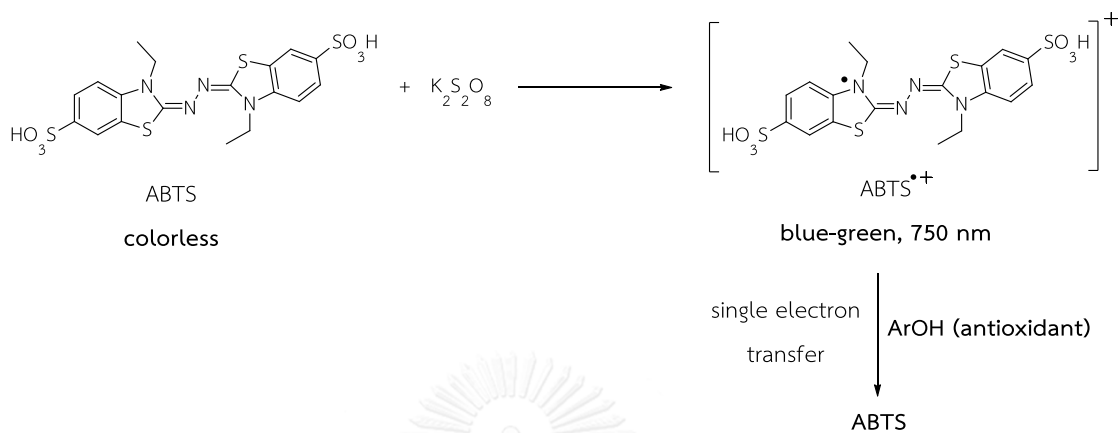
4.3.2 α -Glucosidase inhibitory activity and enzyme kinetic

4.3.2.1 α-Glucosidase inhibitory activity

The inhibitory activity of the test compounds against α-glucosidases from rat intestine (as maltase and sucrase) was based on glucose oxidase colorimetric method. Maltase and sucrase was obtained from rat intestinal acetone powder (Sigma, St.Louis). The rat intestinal acetone powder (1 g) was homogenized with 30 mL of 0.9% NaCl solution. The aliquot containing both maltase and sucrase was obtained upon centrifugation (12,000 rpm) for 30 min. The suppression aqueous layer (maltase and sucrose) was collected to test inhibitory activity of compound. To evaluate inhibition potential, a 10 μL of synthesized compounds (1 mg/mL in DMSO) was added with 30 μL of the 0.1 M phosphate buffer (pH 6.9), 20 μL of the substrate solution (maltose: 10 mM; sucrose: 100 mM) in 0.1 M phosphate buffer, 80 μL of glucose assay kit, and 20 μL of the crude enzyme solution. The reaction mixture was then incubated at 37 °C for 10 min (for maltose) and 40 min (for sucrose). Enzymatic activity was quantified by measuring the absorbance at 500 nm. The percentage inhibition was calculated by following equation: Inhibition (%) = [(Abs$_{control}$ − Abs$_{control\ blank}$)–(Abs$_{sample}$ − Abs$_{sample\ blank}$) / [(Abs$_{control}$ − Abs$_{control\ blank}$)] x 100. Acarbose$^®$ was used as standard control and the experiment was performed in triplicate.
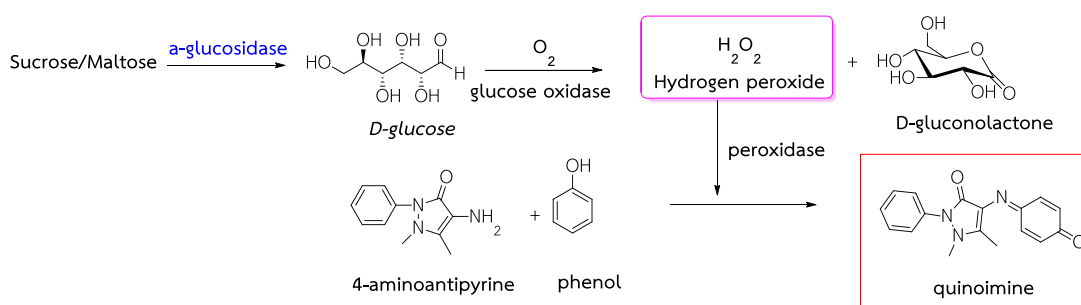
Figure 4.8 Principle of α-glucosidase inhibitory activity based on glucose oxidase colorimetric method

4.3.2.2 Kinetic study of α-glucosidase inhibition

The mode of inhibition for α-glucosidase was investigated with increasing concentrations of maltose in the presence or absence of *epi*-sesaminol. The inhibition type was determined by pattern a series straight of Lineweaver–Burk plots (1/V versus 1/[maltose]). For two secondary replots, they were constructed from slop of Lineweaver–Burk plots versus [*epi*-sesaminol] and intercept vesus [*epi*-sesaminol], repesctively by following express equation [26].

Lineweaver-Burk plots equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}} \qquad (1)$$

Secondary replots can be constructed as

$$slope = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max}K_i} \qquad (2)$$

and

$$Y\text{-intercept} = \frac{1}{V_{max_{app}}} + \frac{1}{\propto K_i' V_{max}} [I] \qquad (3)$$

CHAPTER V

CONCLUSION

A series of furofuran lignans having different substituents at C-2 of furofuran core structure were synthesized by coupling of samin and phenolic compounds via Fridel-Crafts type reaction under acid-catalyzed condition. Samin, as versatile starting material, was obtained from naturally available sesamolin derived by saponification of sesame oil followed acid hydrolysis reaction. This present methodology allowed to synthesize various furofuran lignans with good yields compared with total synthesis. Moreover, the substituent position on phenolics were contributed to the yield of product; the strong electron donating group (-OH or –OCH$_3$) arranged meta-position enhance reaction proceeded with in high yield of products. For biological activity, the free hydroxyl on phenolic moiety is critical to enhance biological activity both antioxidant activity and α-glucosidase inhibition activity, especially **3a**, **3e**, **3g**, **3i**, **3k, 1-3** and **1-22** along with their epimers. From enzyme kinetics study, the mode of inhibition of the furofuran lignans was predominantly competitive against maltase. The present results indicated that this methodology is alternatively efficient method for synthesis of active furofuran lignans, which played the important role in prevention or treatment of diabetic mellitus type II.

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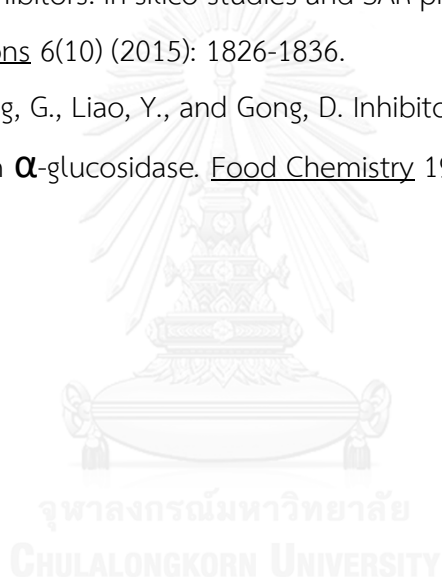
Appendix
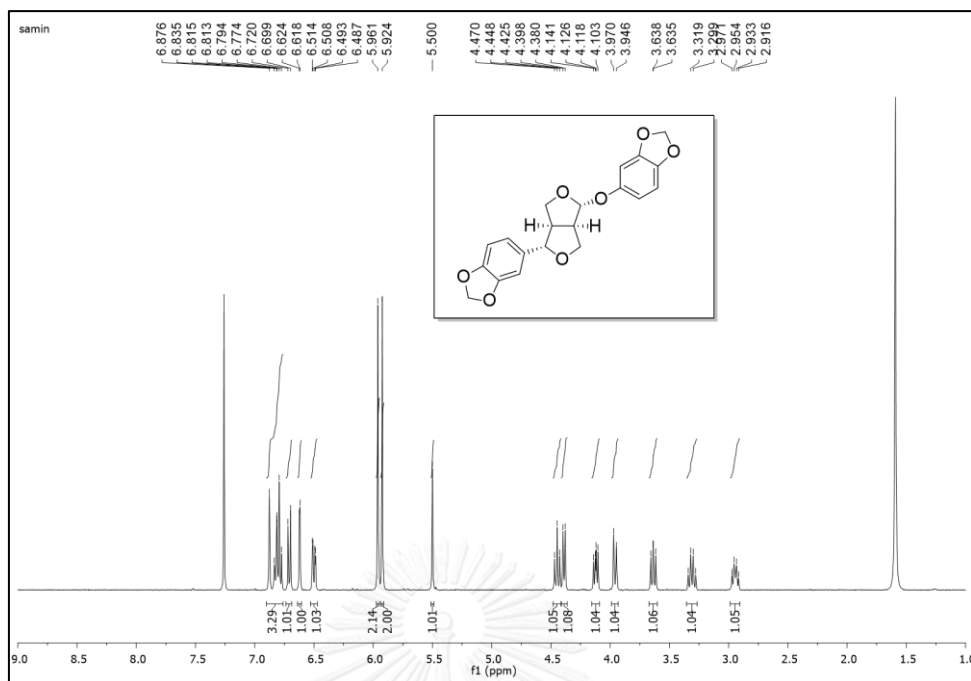
Figure 1 ^{1}H spectrum of **1-2** (CDCl$_3$)
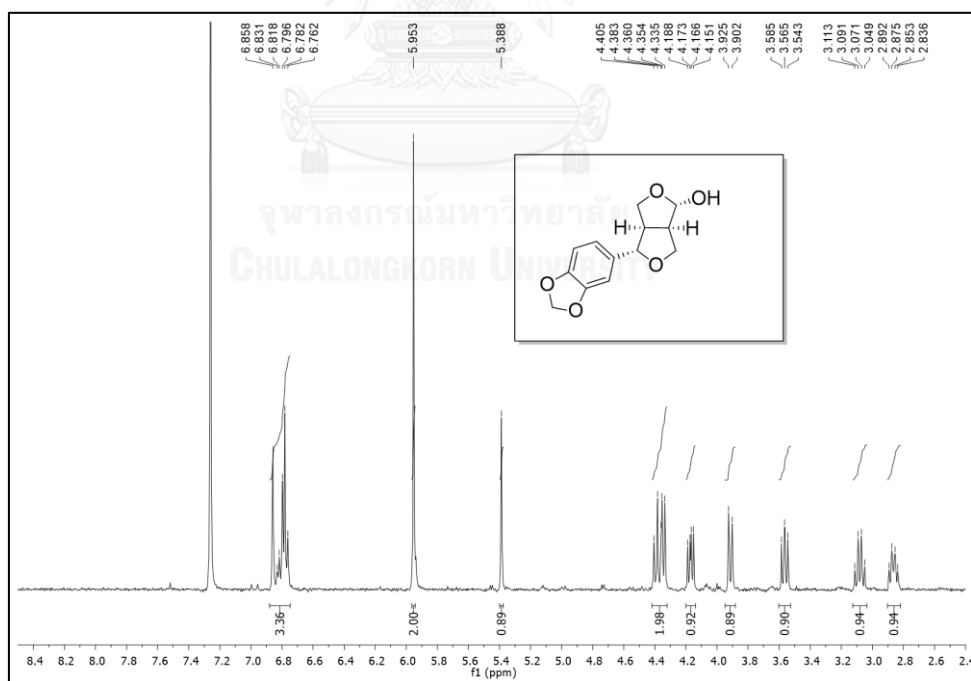
Figure 2 ^{1}H spectrum of **1-14** (CDCl$_3$)
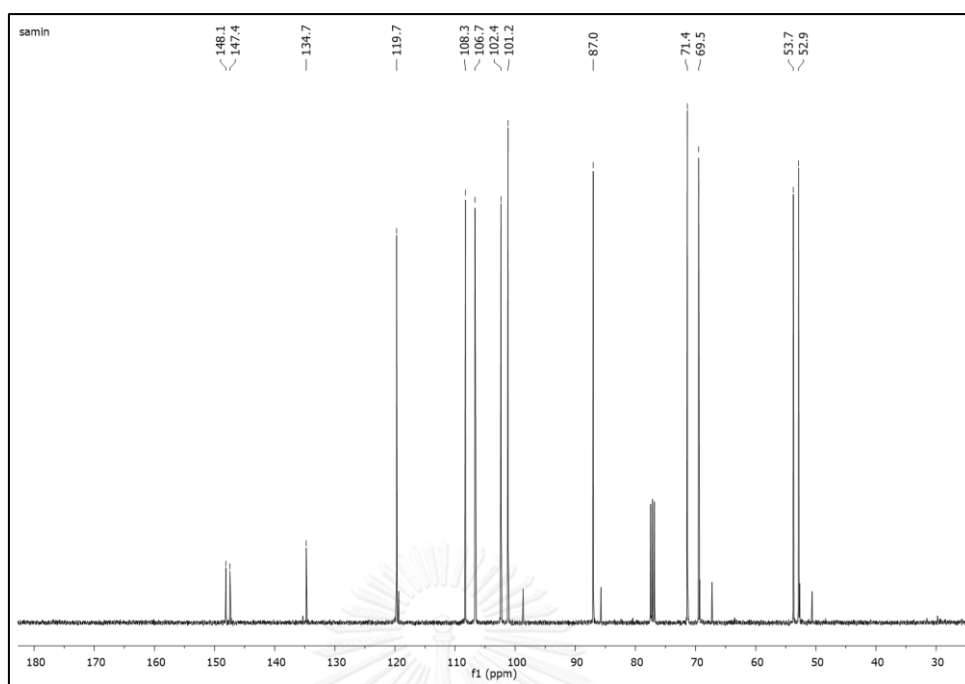
Figure 3 ^{13}C spectrum of **2-1** (CDCl$_3$)

Figure 4 ^{1}H spectrum of **3a** (CDCl$_3$)

Figure 5 ^{13}C spectrum of **3a** (CDCl$_3$)
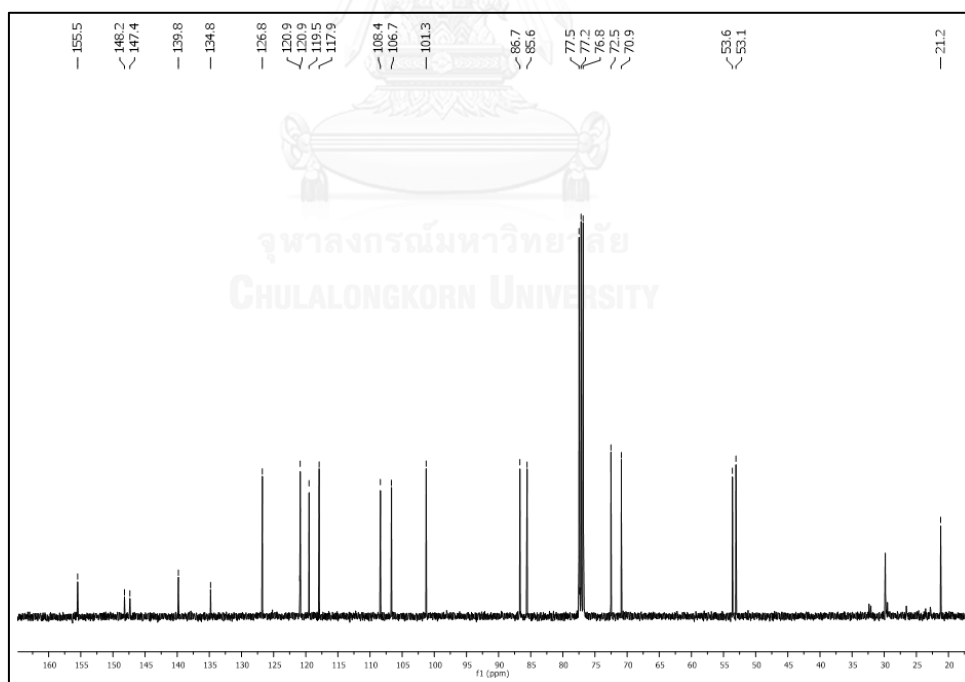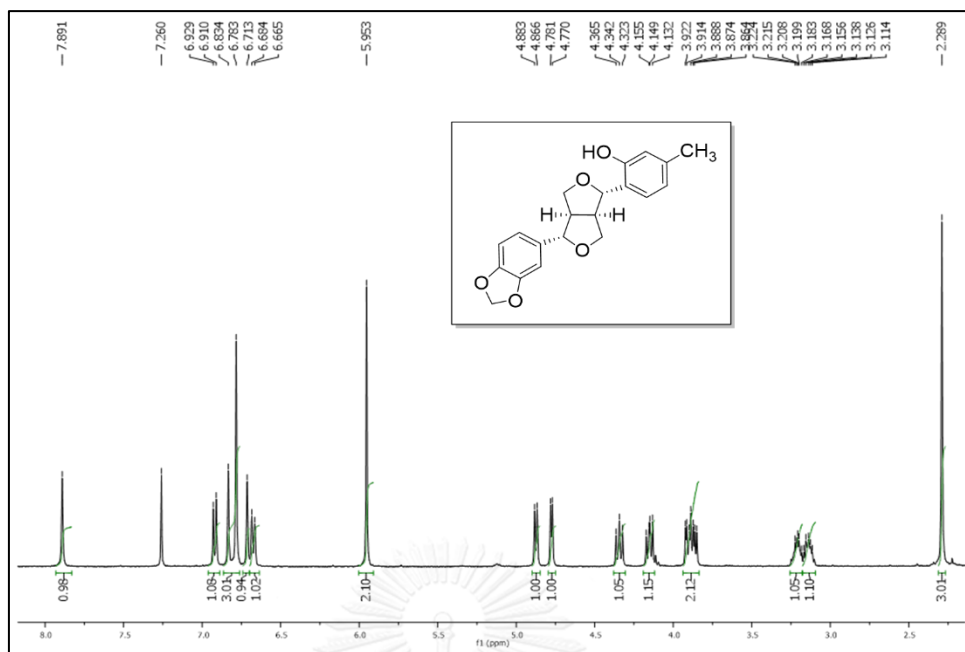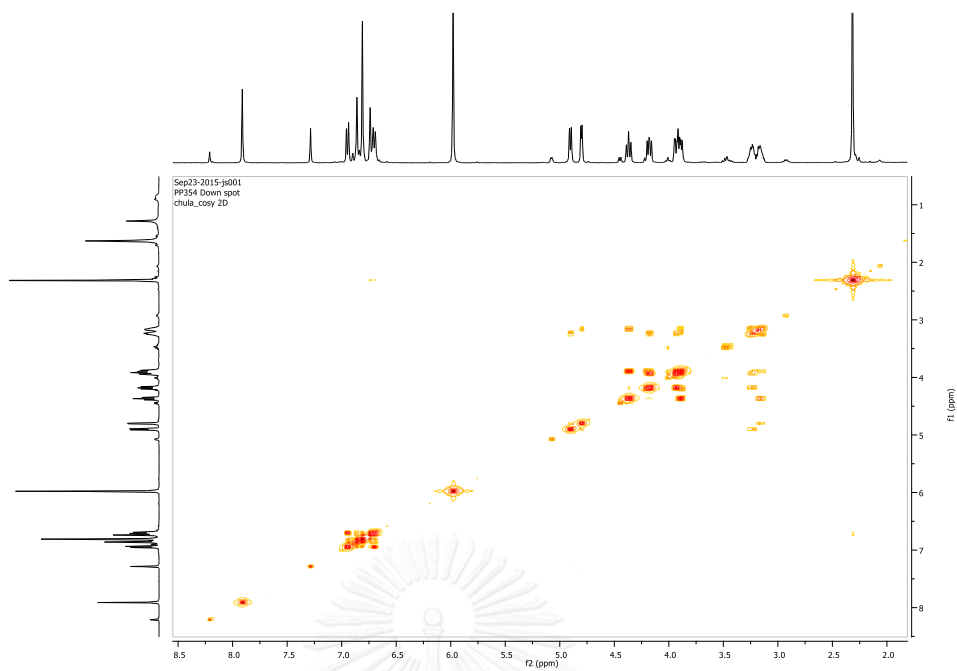
Figure 6   COSY experiment of **3a** (CDCl$_3$)
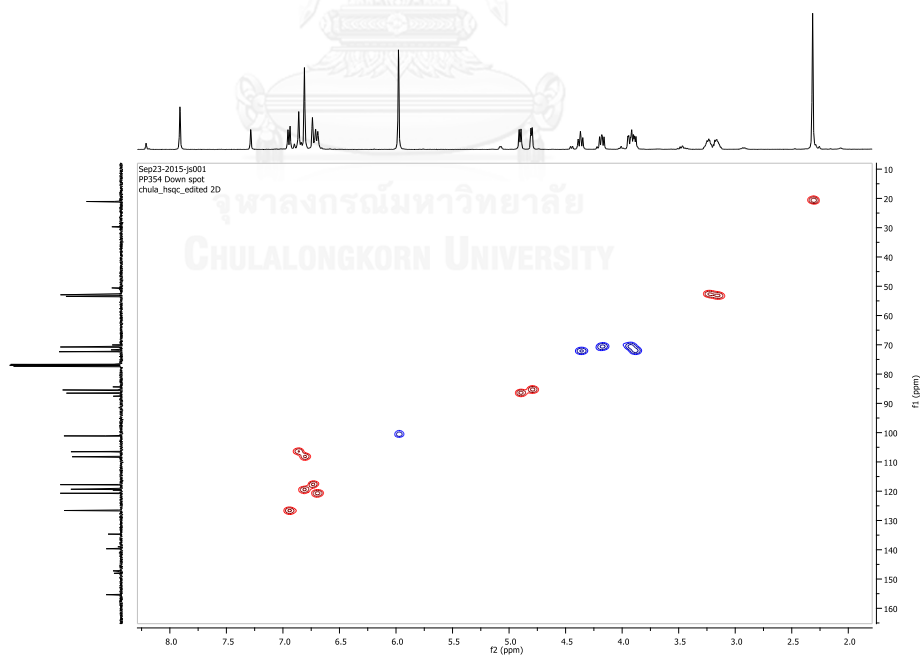
Figure 7   HSQC experiment of **3a** (CDCl$_3$)
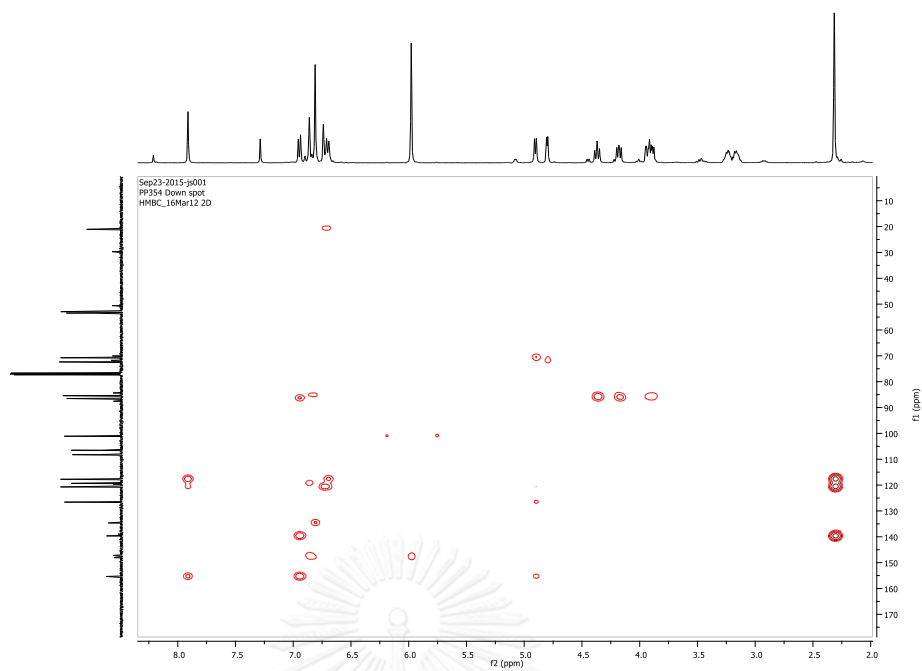
Figure 8   HMBC experiment of **3a** (CDCl$_3$)
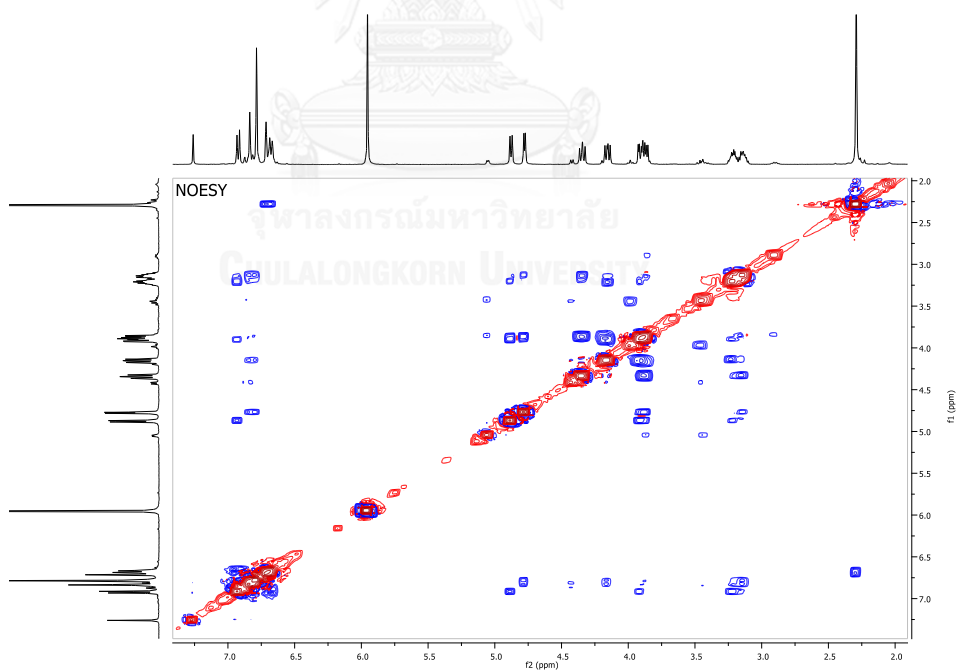
Figure 9   NOESY experiment of **3a** (CDCl$_3$)
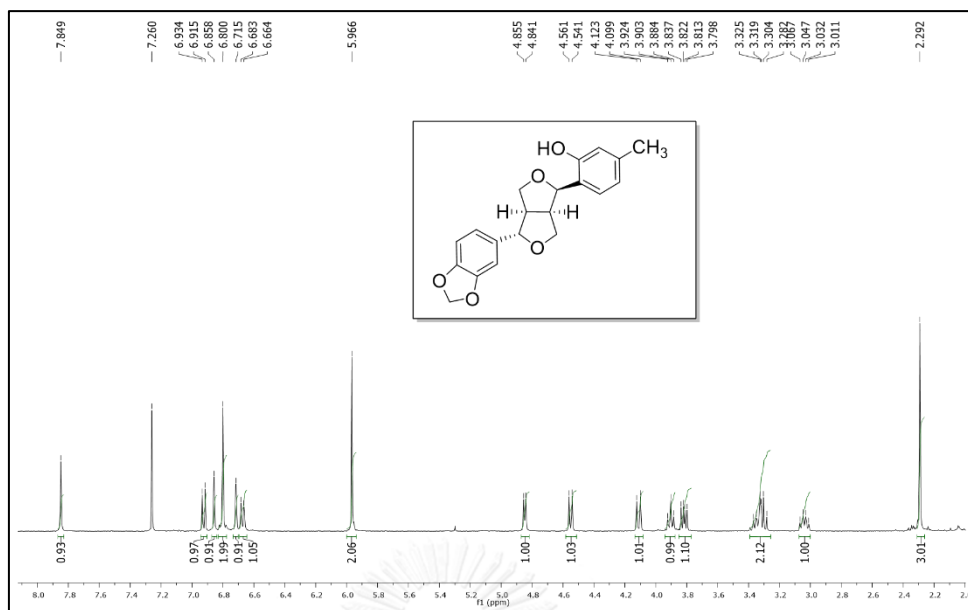
Figure 10 ^{1}H spectrum of *epi*-**3a** (CDCl$_3$)
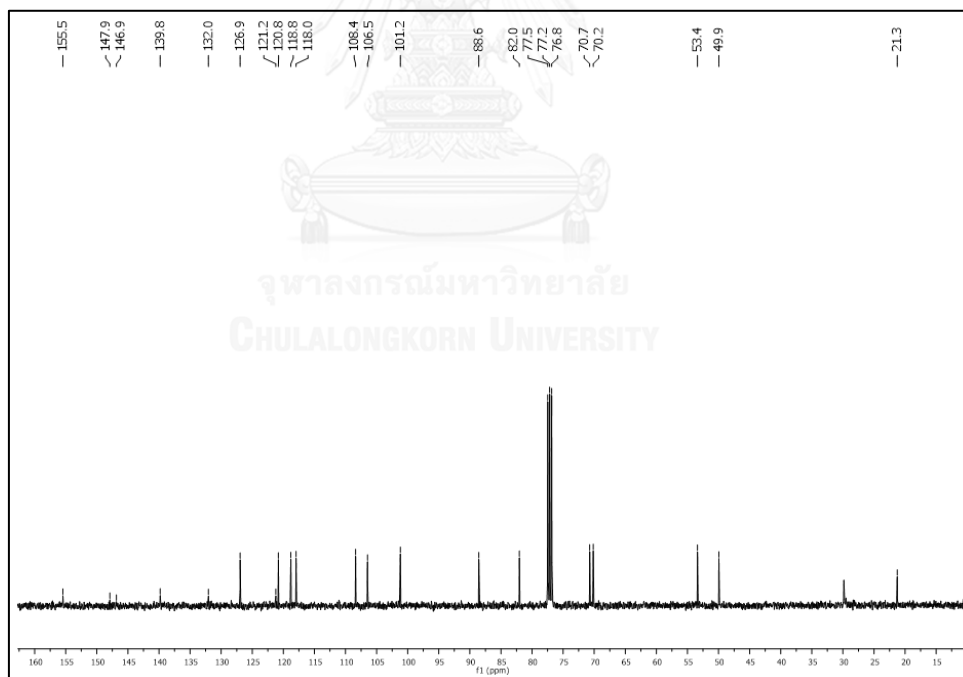
Figure 11 ^{13}C spectrum of *epi*-**3a** (CDCl$_3$)
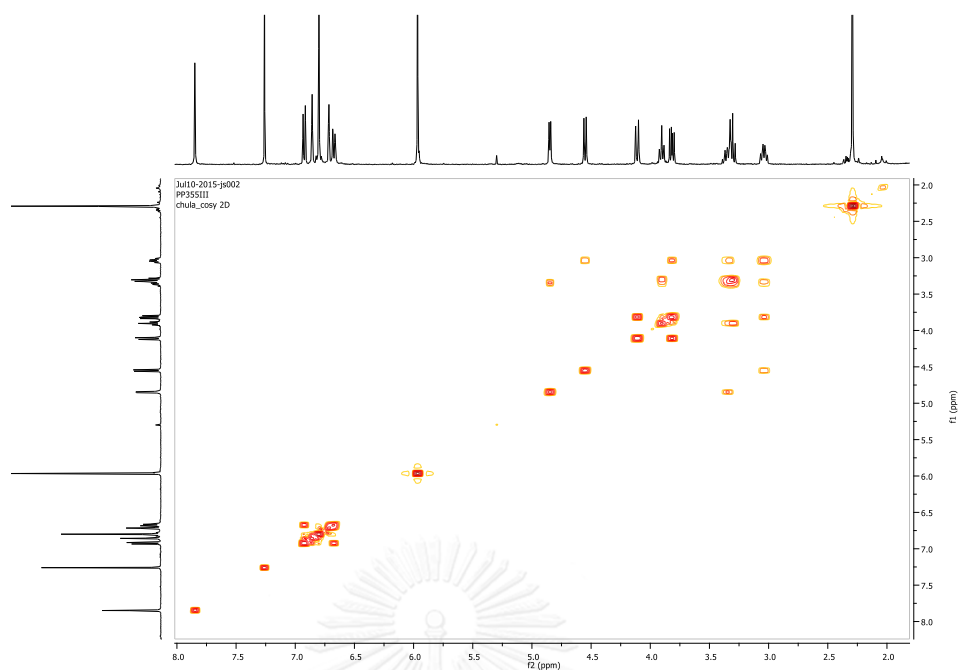
Figure 12   COSY experiment of *epi*-**3a** (CDCl₃)
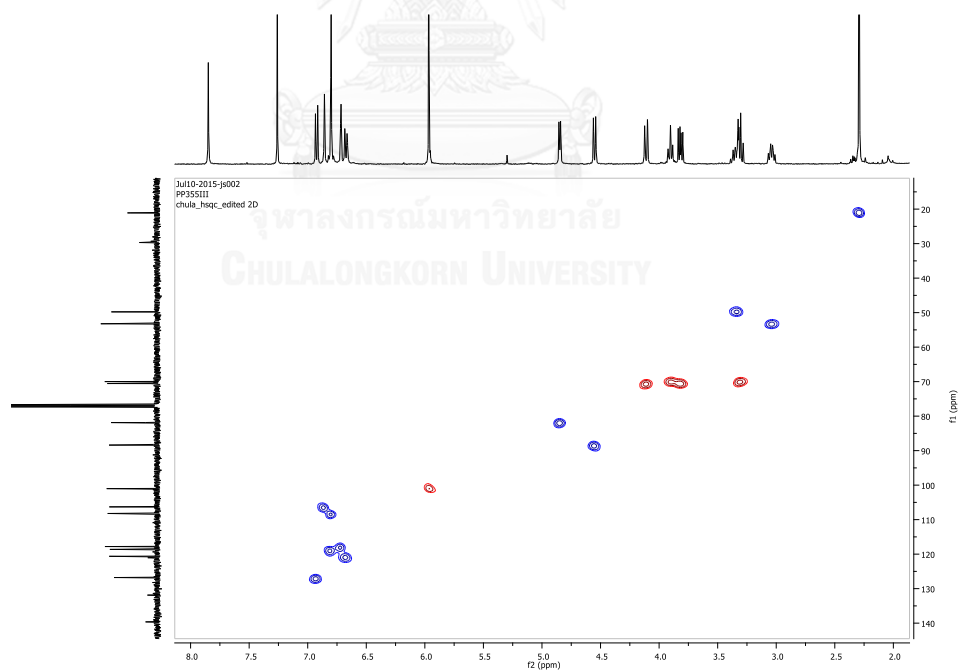
Figure 13   HSQC experiment of *epi*-**3a** (CDCl₃)
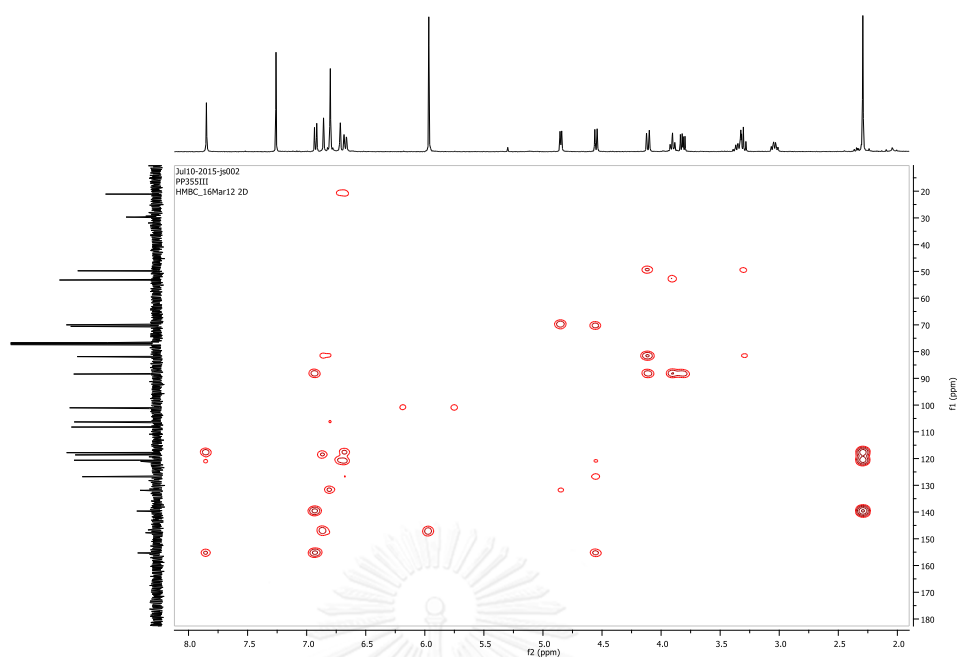
Figure 14   HMBC experiment of *epi*-3a (CDCl₃)
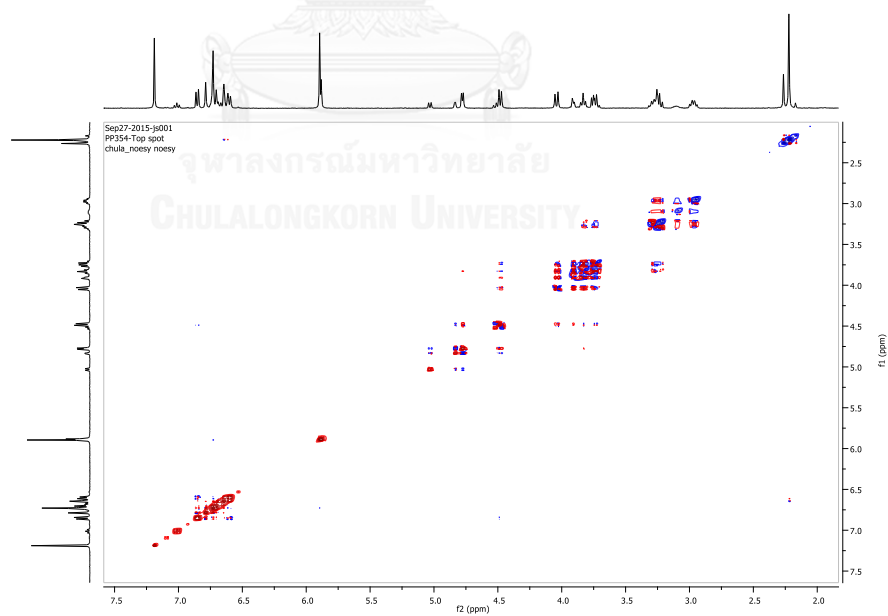
Figure 15   NOESY experiment of *epi*-3a (CDCl₃)
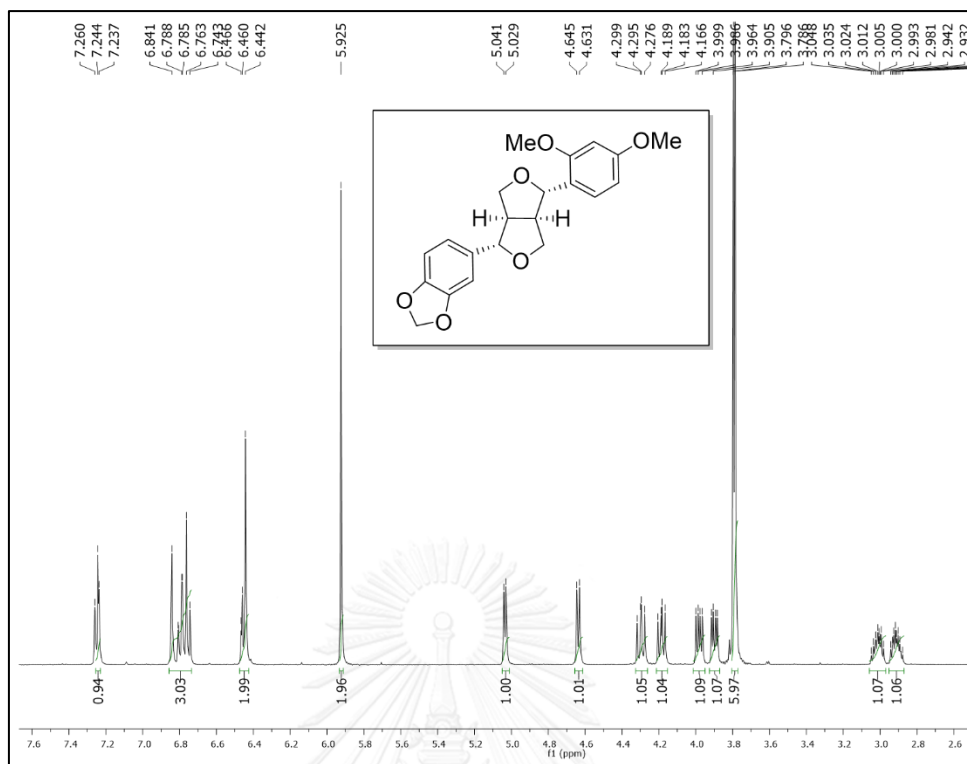
Figure 16 ^{1}H spectrum of **3b** (CDCl$_3$)
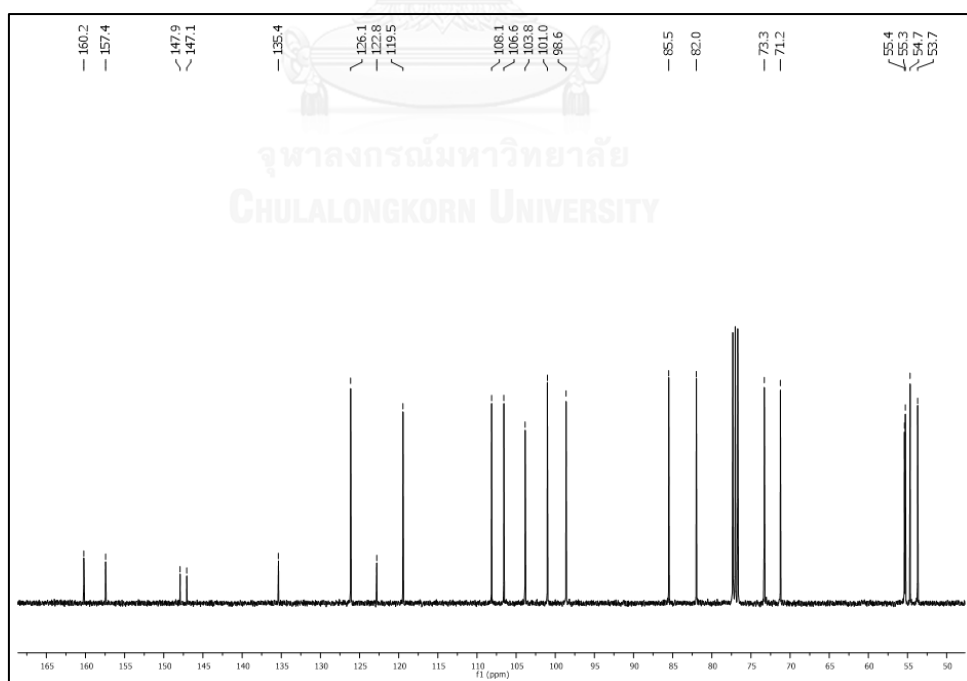
Figure 17 ^{13}C spectrum of **3b** (CDCl$_3$)
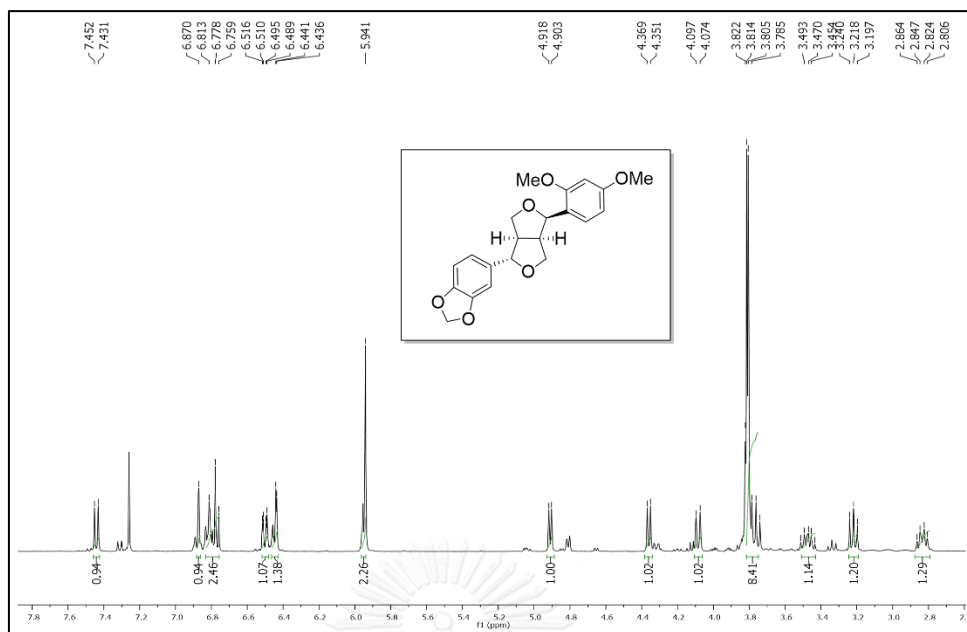
Figure 18 ¹H spectrum of *epi*-3b (CDCl₃)
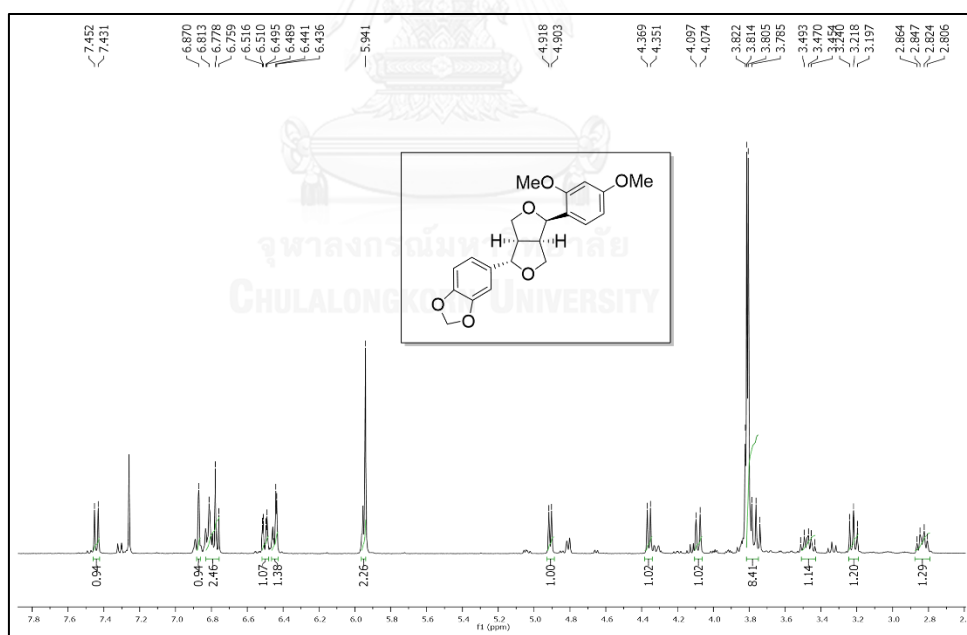
Figure 19 ¹³C spectrum of *epi*-3b (CDCl₃)
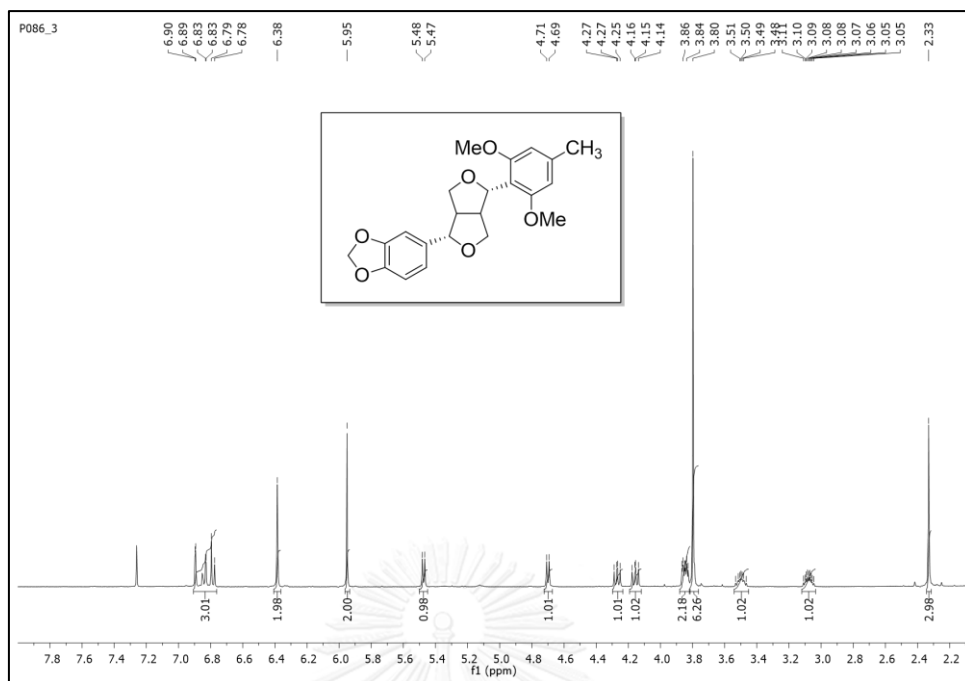
Figure 20 ^{1}H spectrum of **3c** (CDCl$_3$)
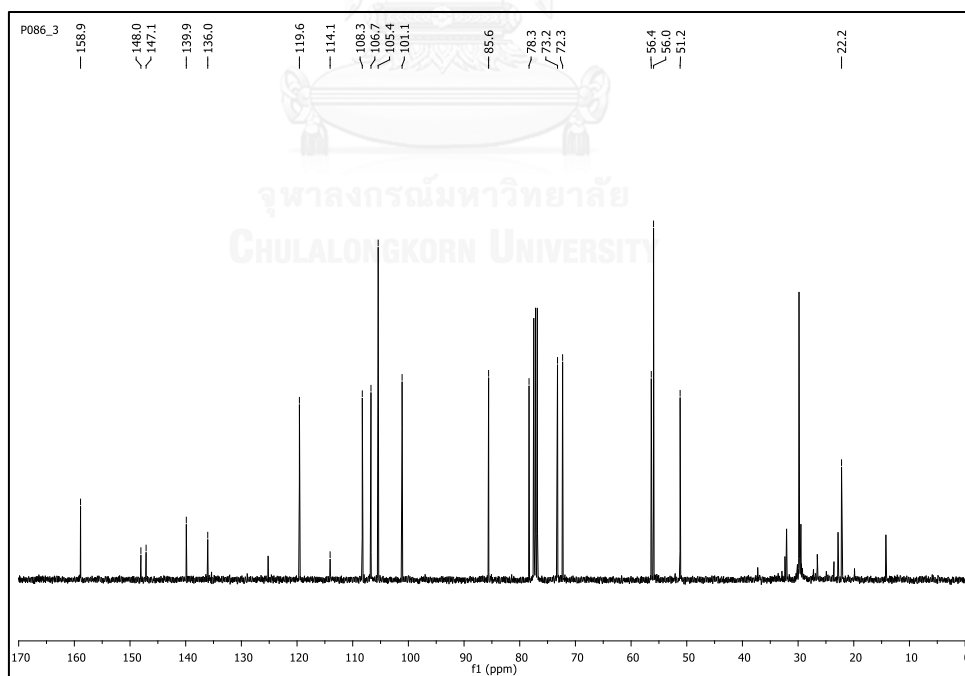
Figure 21 ^{13}C spectrum of **3c** (CDCl$_3$)
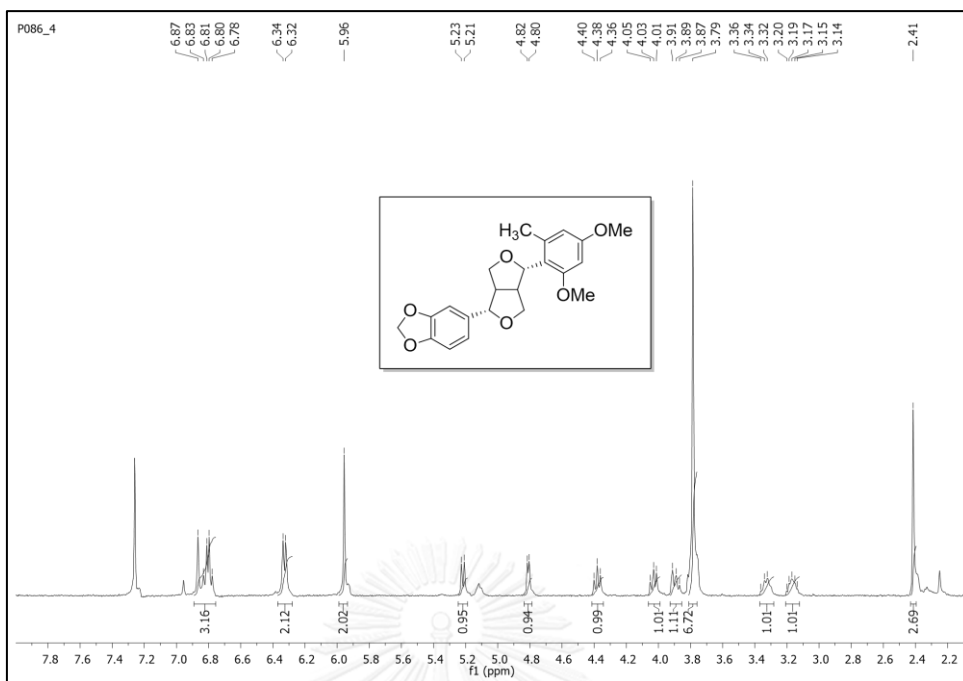
Figure 22 ^{1}H spectrum of **3c'** (CDCl$_3$)
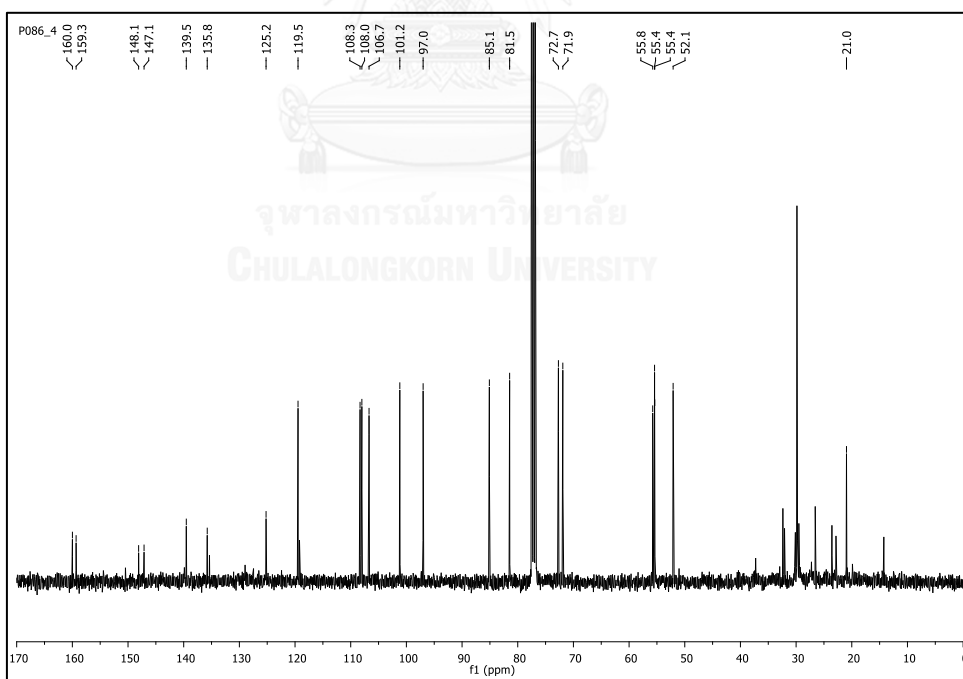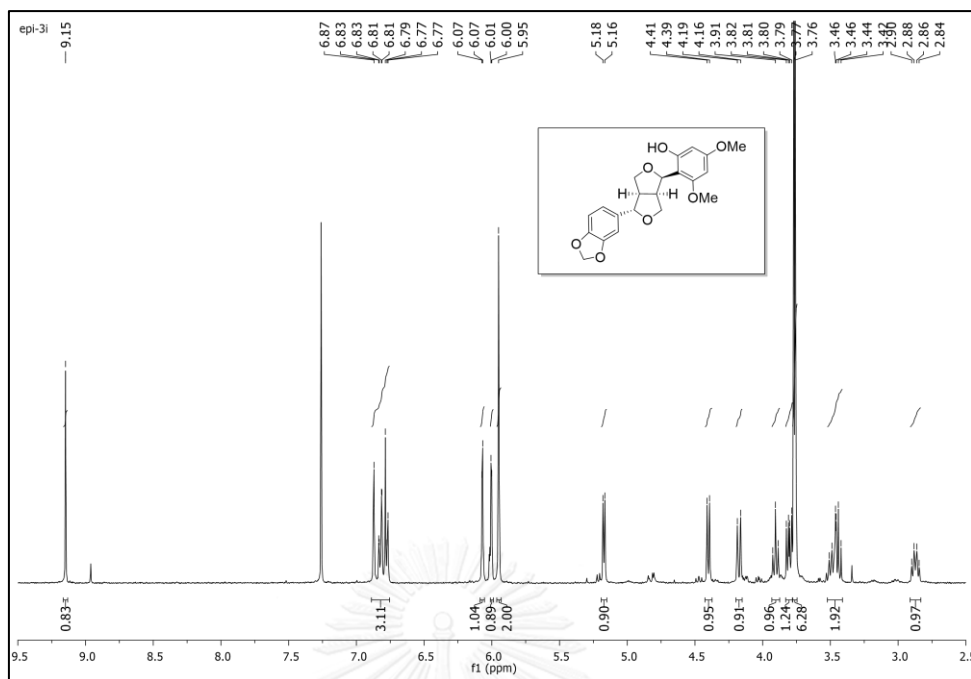

Figure 23 ^{13}C spectrum of **3c'** (CDCl$_3$)
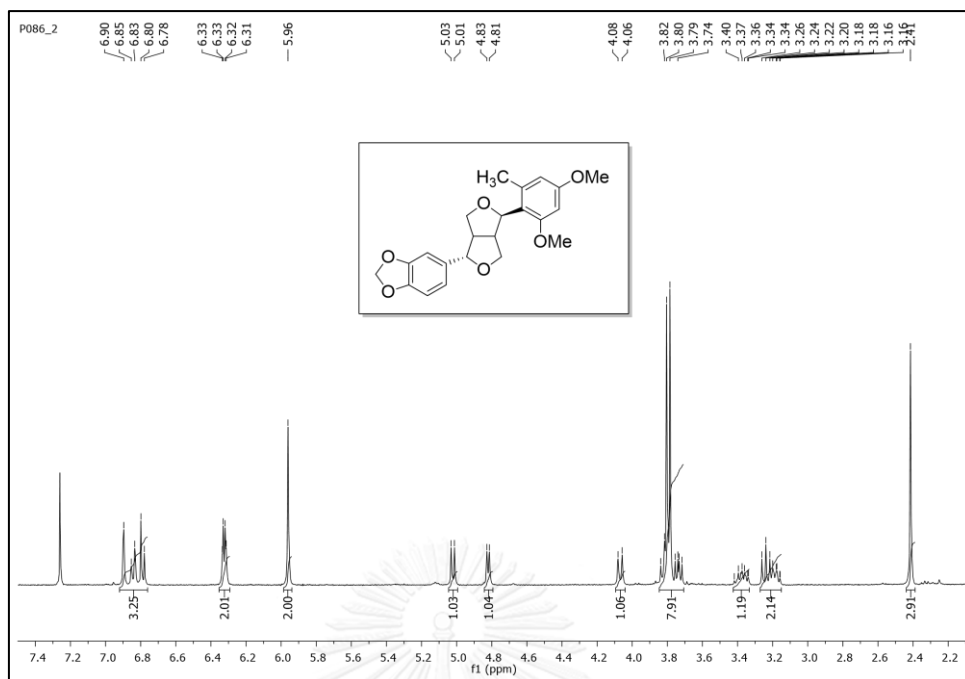
Figure 24 ¹H spectrum of *epi-3c'* (CDCl₃)
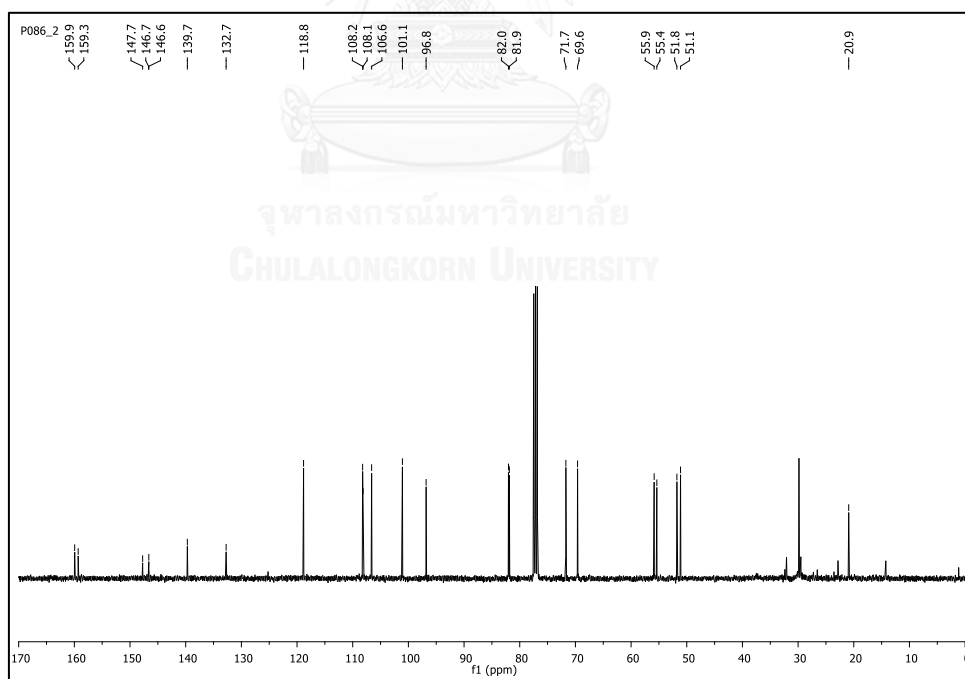
Figure 25 ¹³C spectrum of *epi-3c'* (CDCl₃)
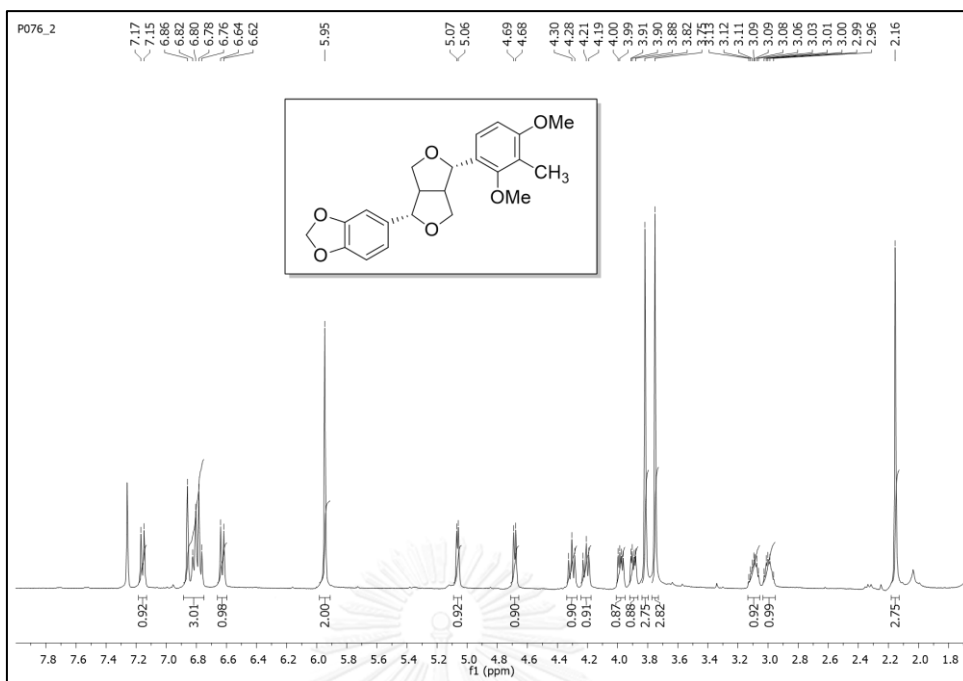
Figure 26 ^{1}H spectrum of **3d** (CDCl$_3$)
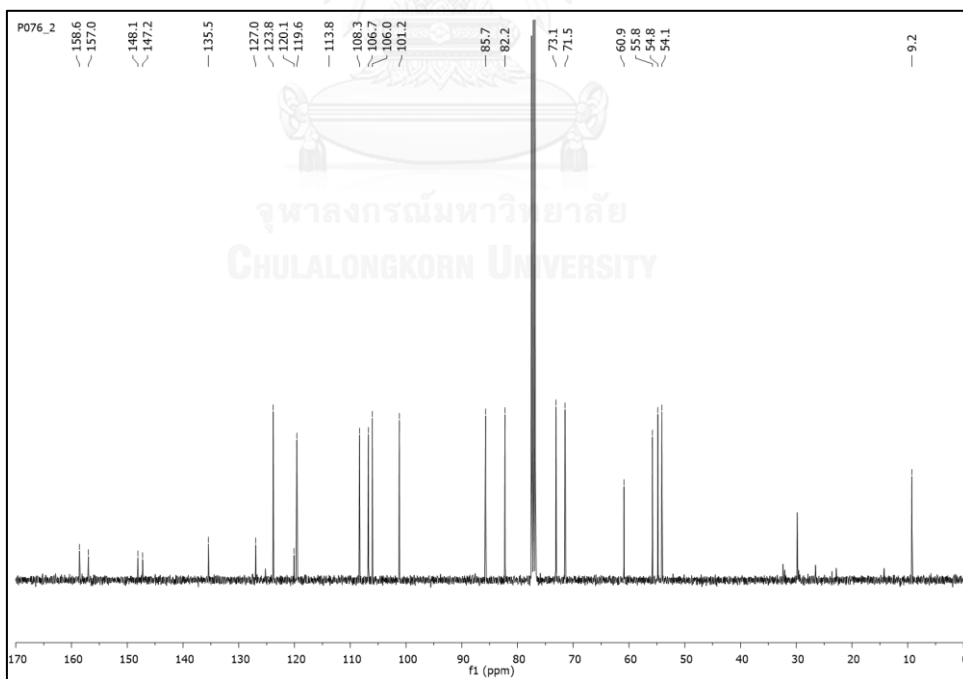
Figure 27 ^{13}C spectrum of **3d** (CDCl$_3$)
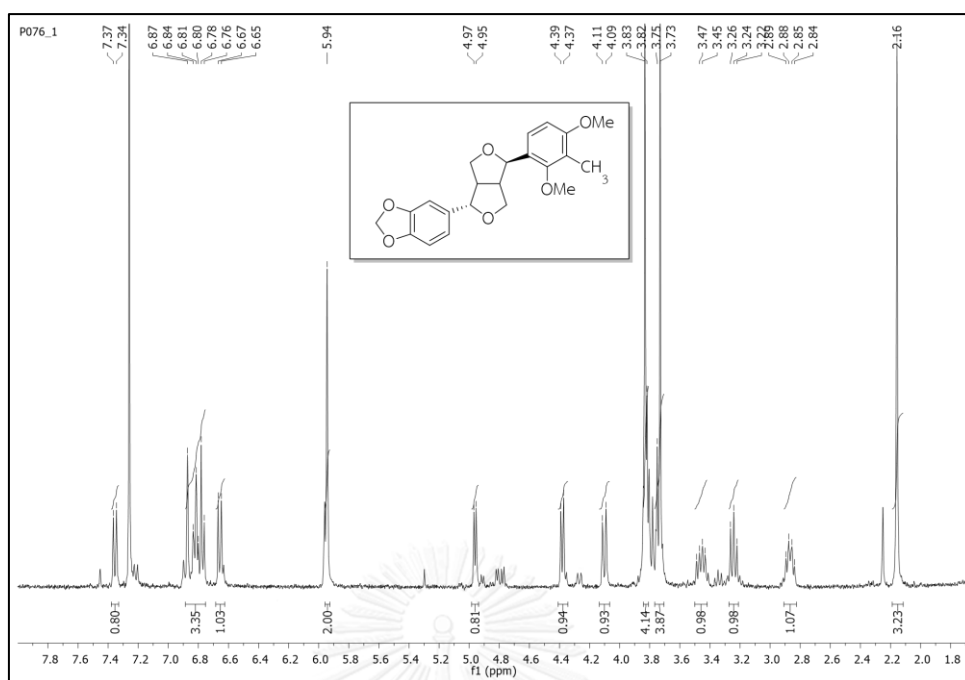
Figure 28 ^{1}H spectrum of *epi*-**3d** (CDCl$_3$)
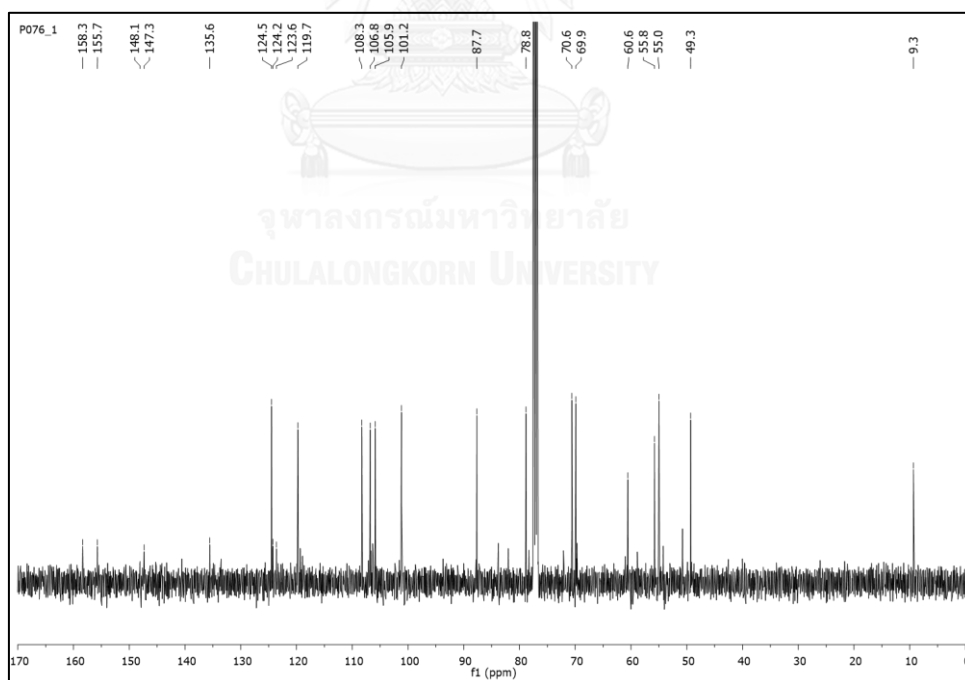
Figure 29 ^{13}C spectrum of *epi*-**3d** (CDCl$_3$)
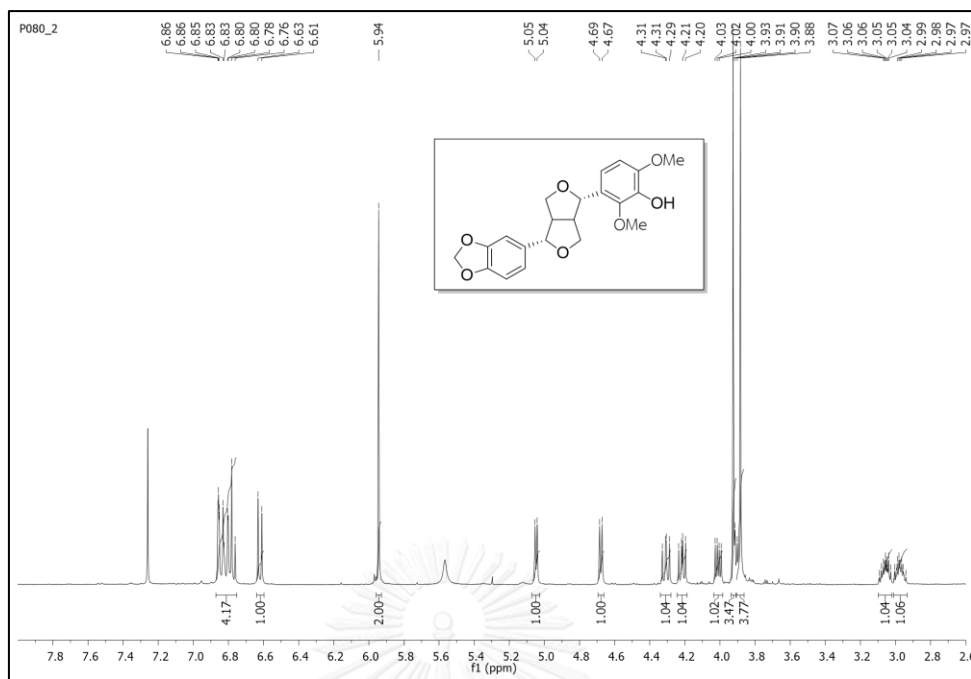
Figure 30 ^{1}H spectrum of **3e** (CDCl$_3$)
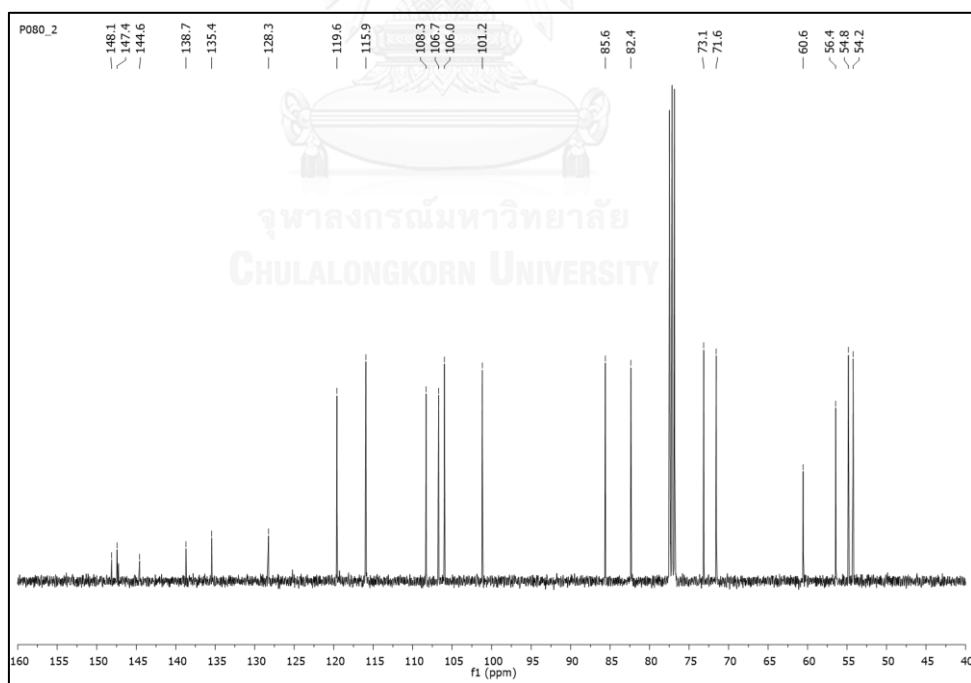
Figure 31 ^{13}C spectrum of **3e** (CDCl$_3$)
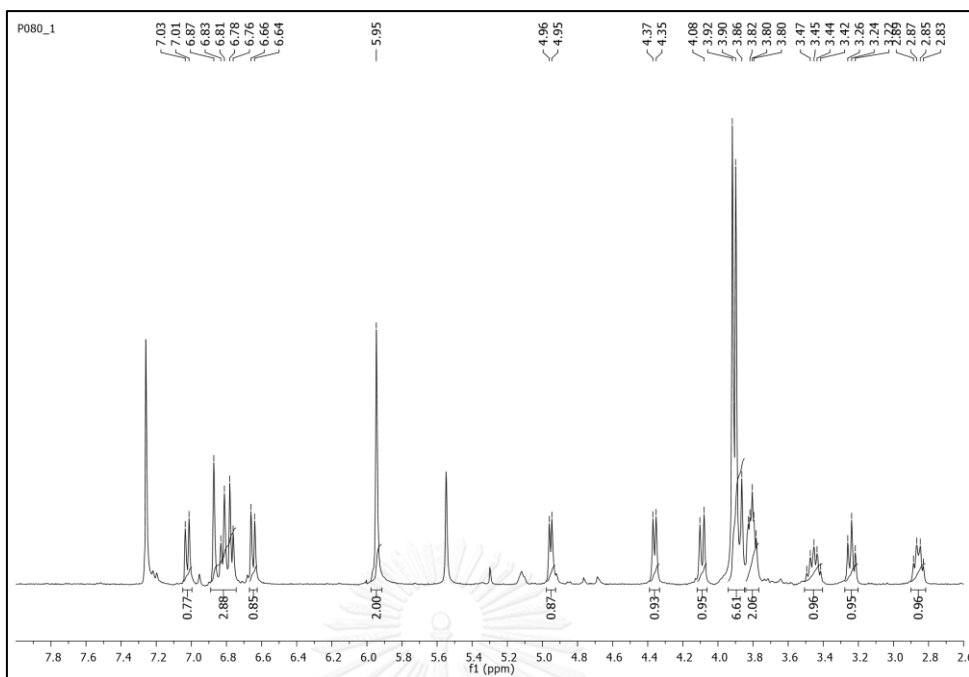
Figure 32 ^{1}H spectrum of *epi*-**3e** (CDCl$_3$)
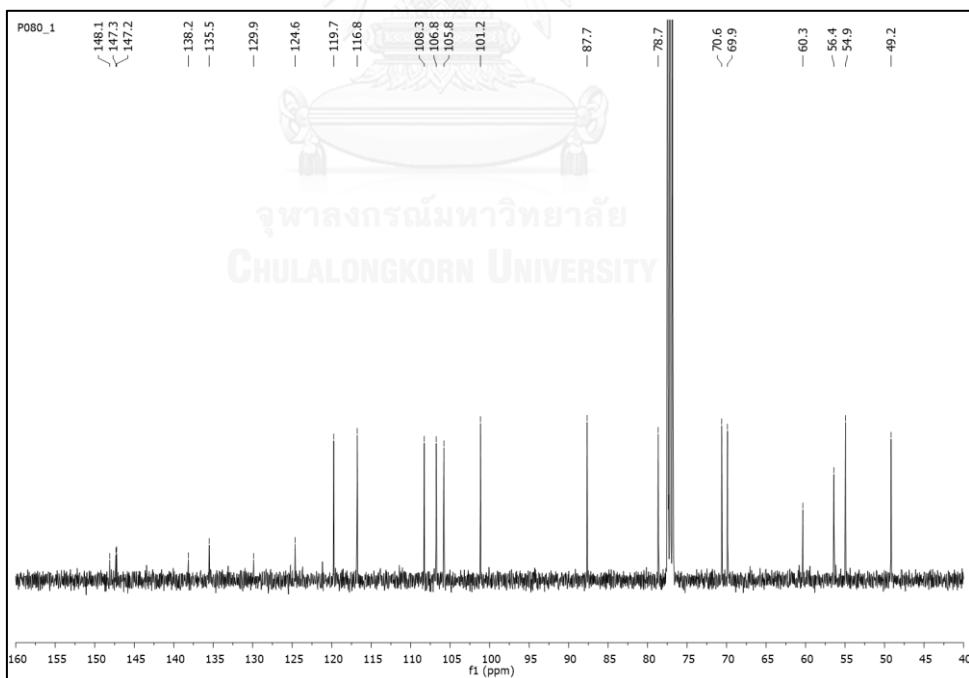
Figure 33 ^{13}C spectrum of *epi*-**3e** (CDCl$_3$)
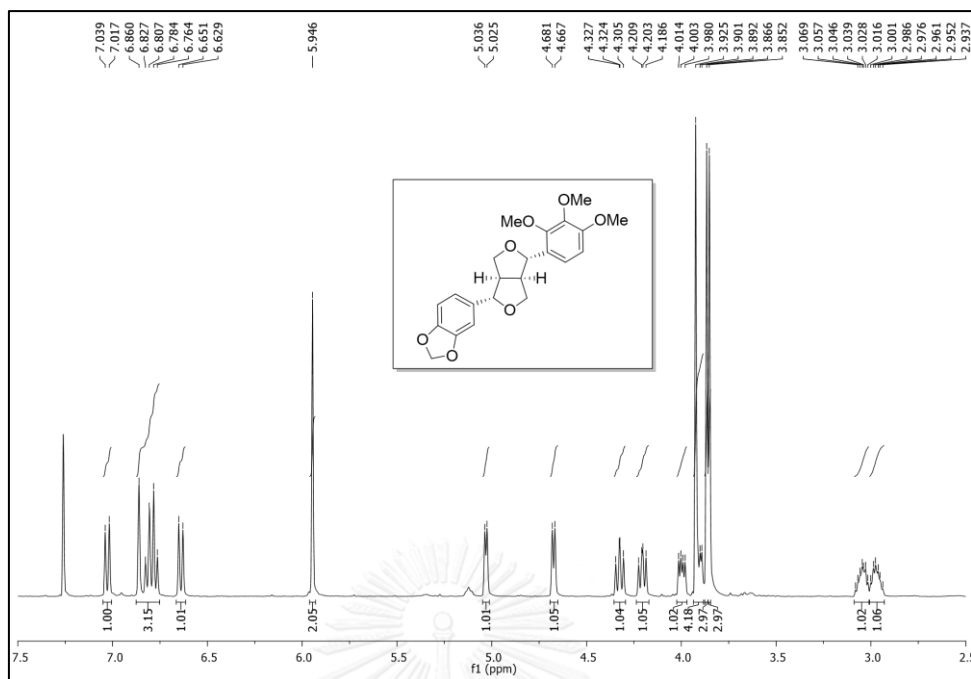
Figure 34 ¹H spectrum of **3f** (CDCl₃)
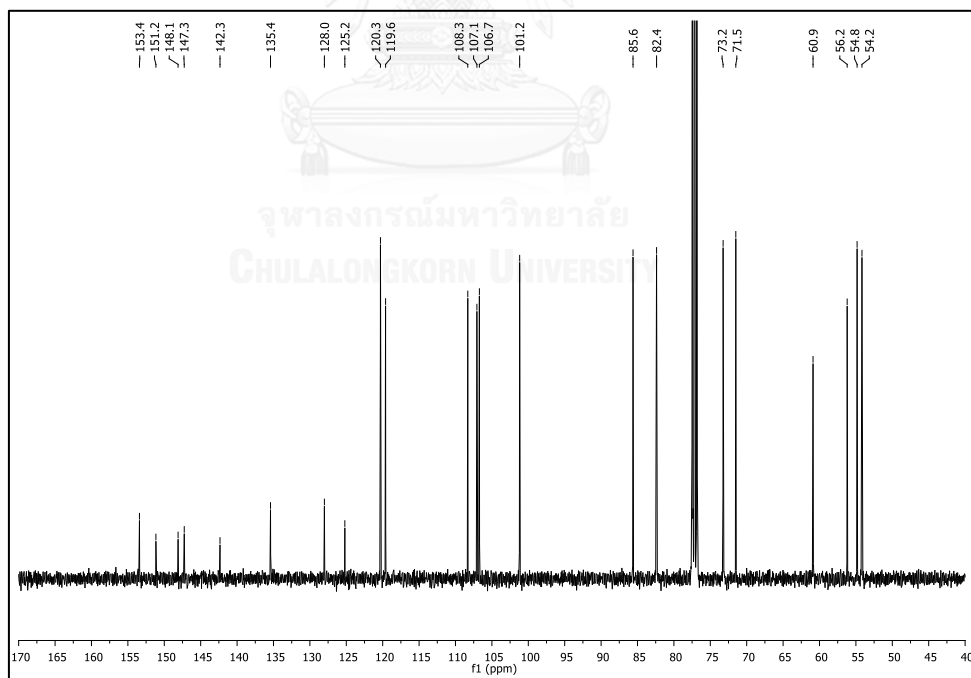
Figure 35 ¹³C spectrum of **3f** (CDCl₃)
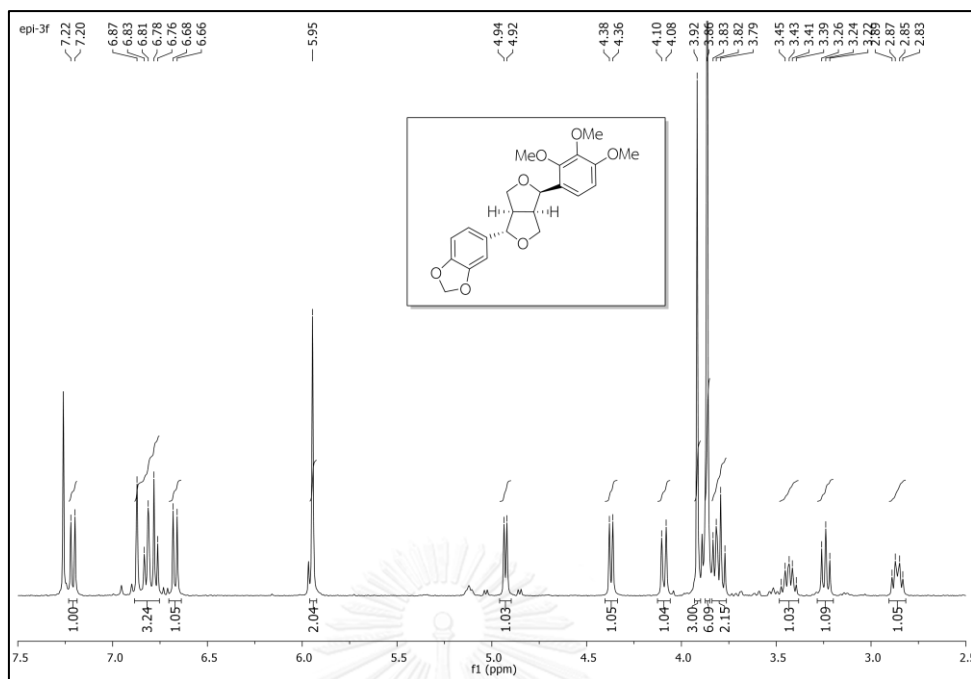
Figure 36 ^{1}H spectrum of *epi*-3f (CDCl$_3$)
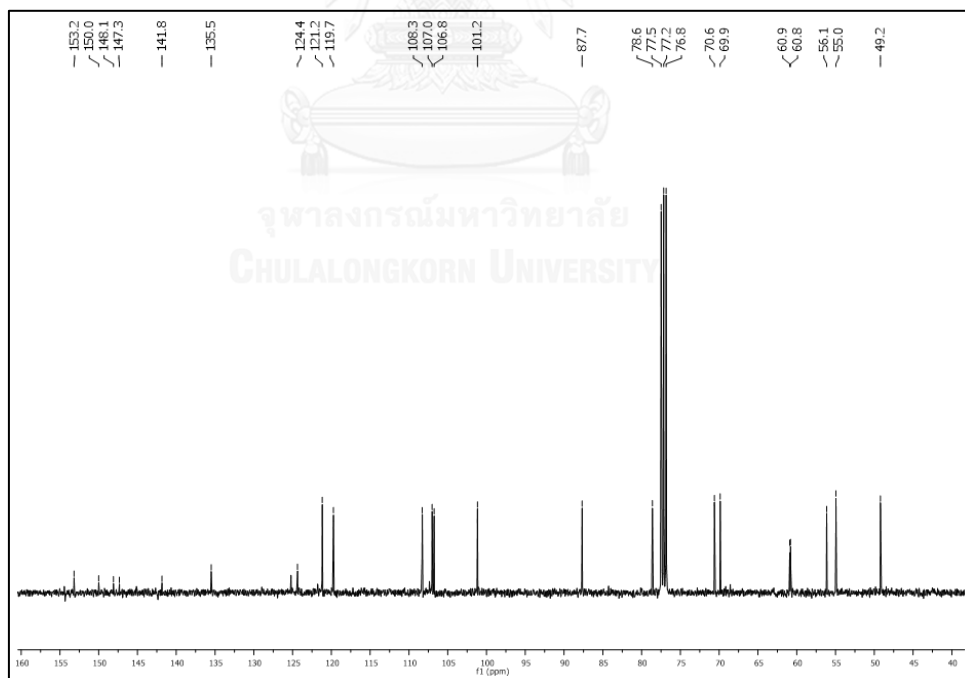
Figure 37 ^{13}C spectrum of *epi*-3f (CDCl$_3$)
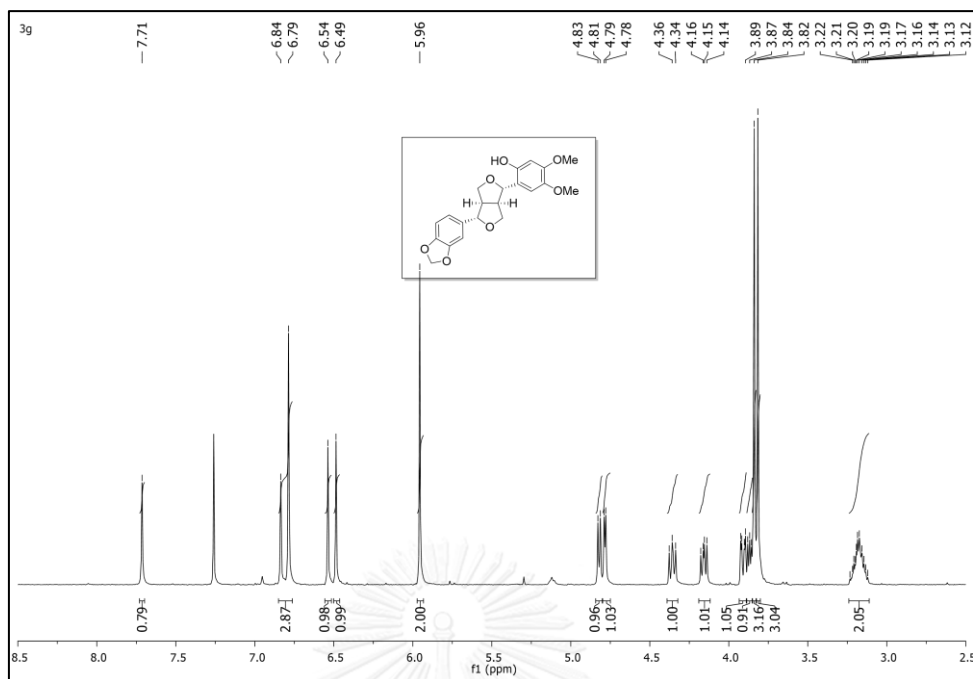
Figure 38 ^{1}H spectrum of **3g** (CDCl$_3$)
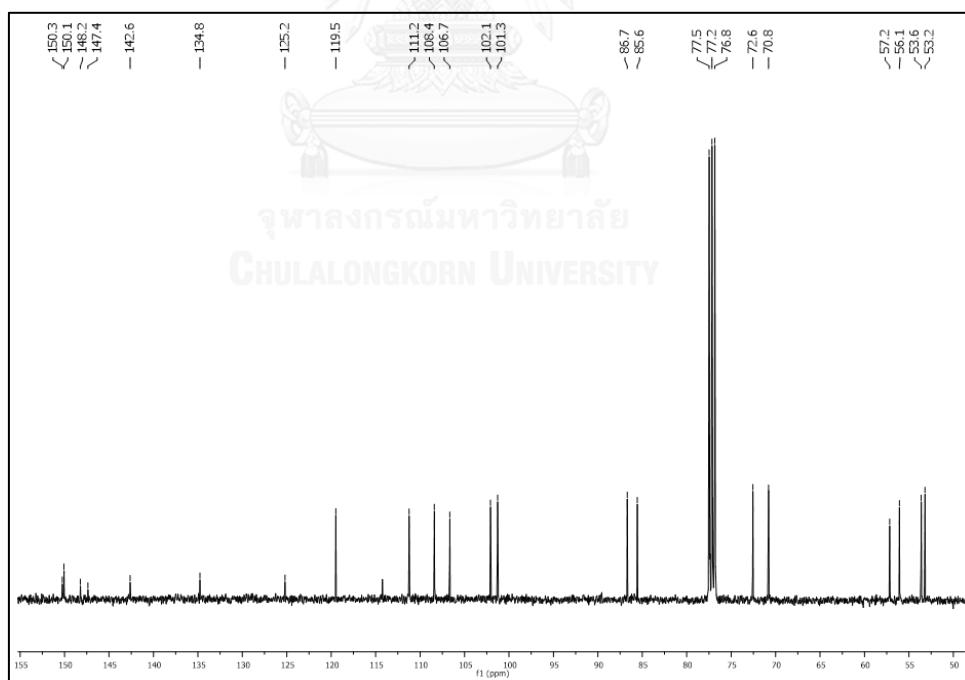
Figure 39 ^{13}C spectrum of **3g** (CDCl$_3$)
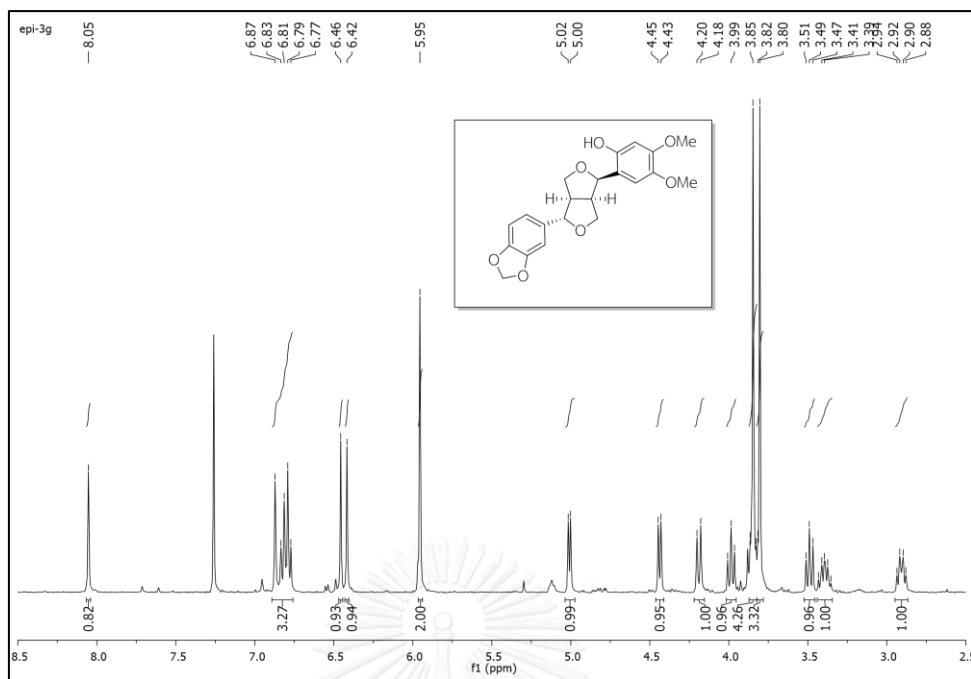
Figure 40 ^{1}H spectrum of *epi*-3g (CDCl$_3$)
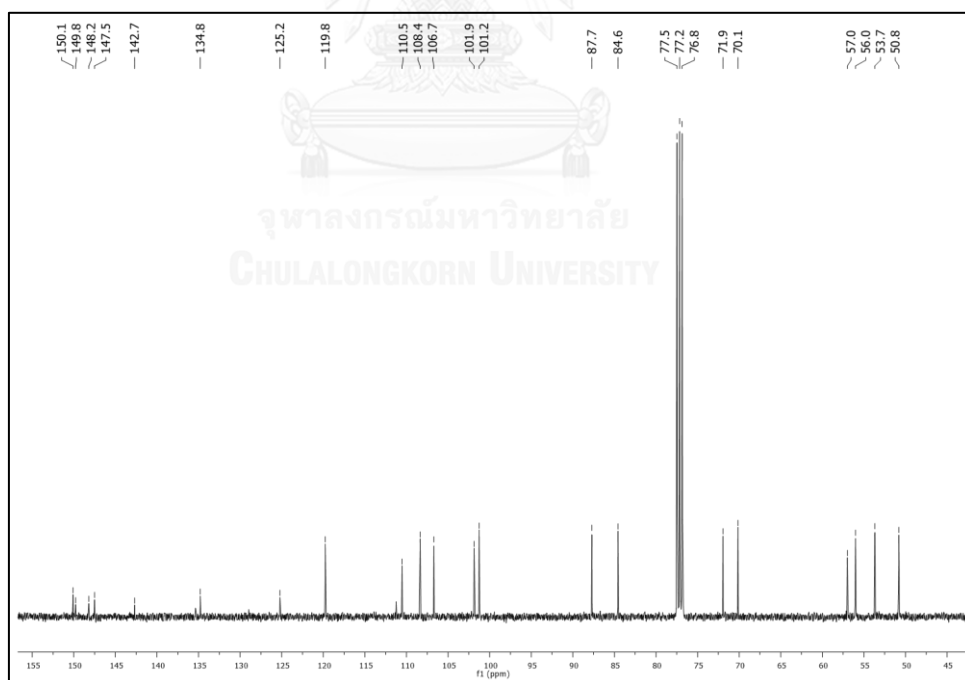
Figure 41 ^{13}C spectrum of **epi-3g** (CDCl$_3$)

Figure 42 ^{1}H spectrum of **3h** (CDCl$_{3}$)

Figure 43 ^{13}C spectrum of **3h** (CDCl$_{3}$)

Figure 44 ^{1}H spectrum of **3i** (CDCl$_3$)

Figure 45 ^{13}C spectrum of **3i** (CDCl$_3$)
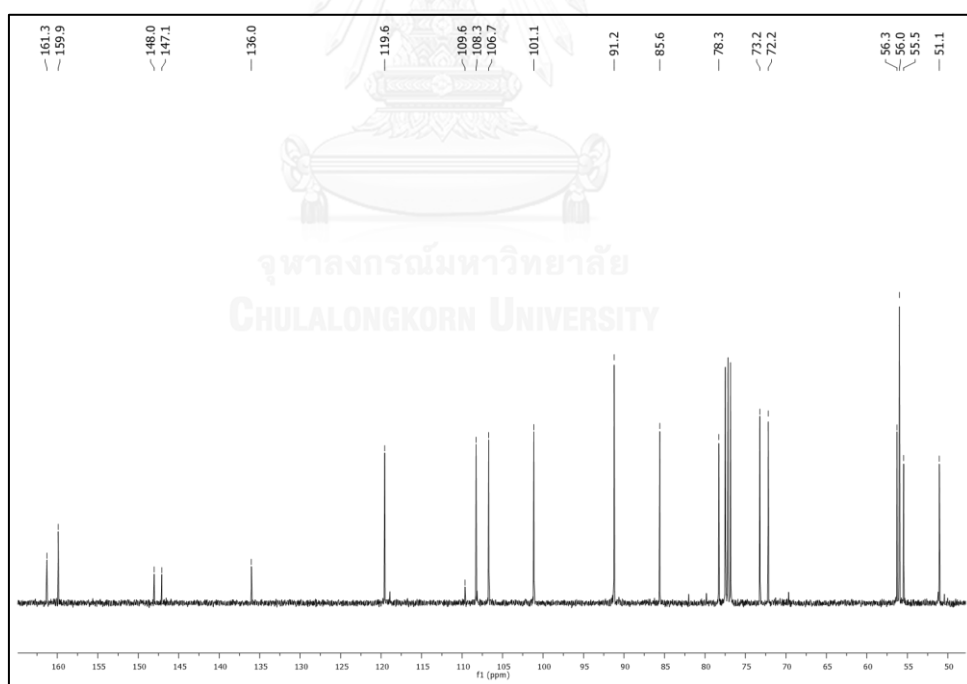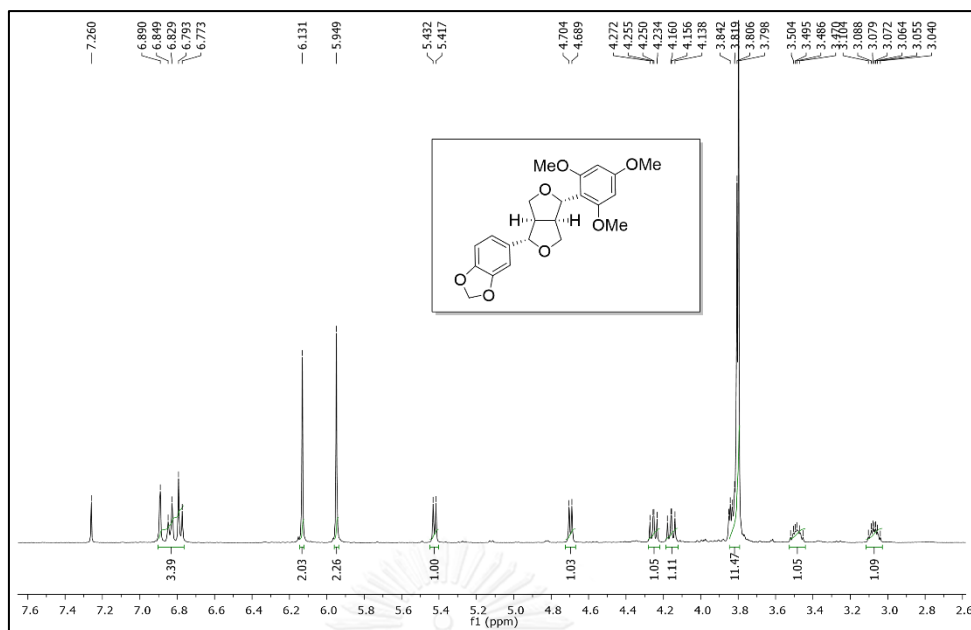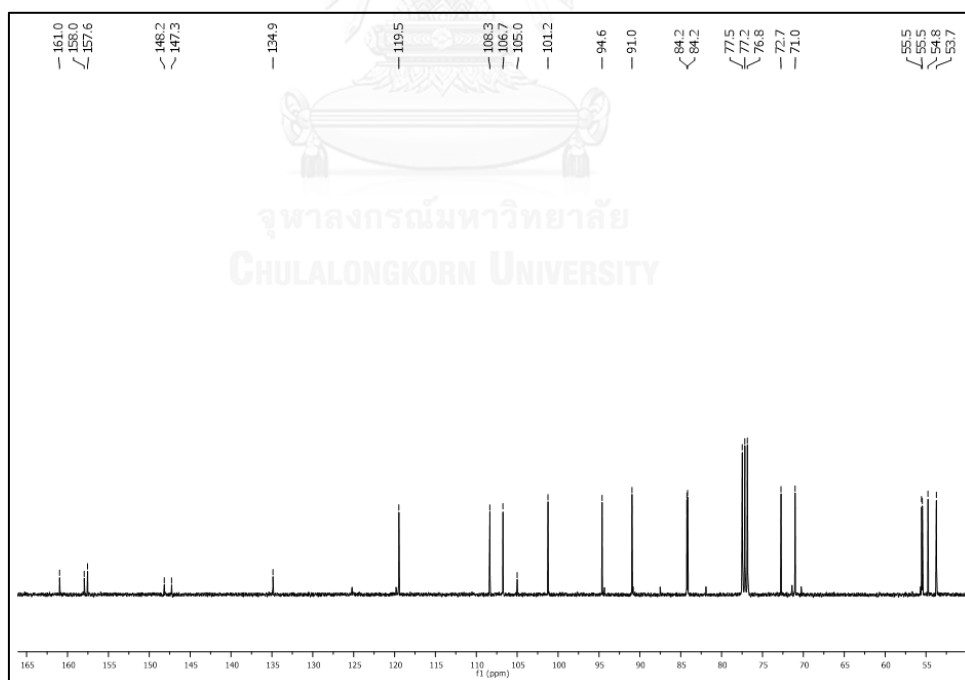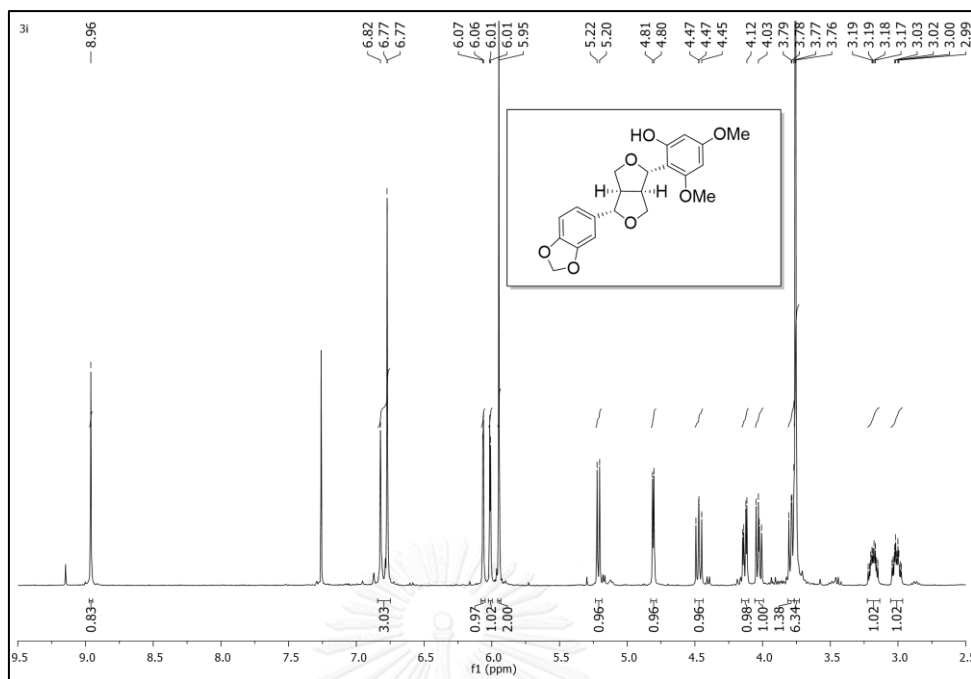
Figure 46 ^{1}H spectrum of *epi*-**3i** (CDCl$_3$)
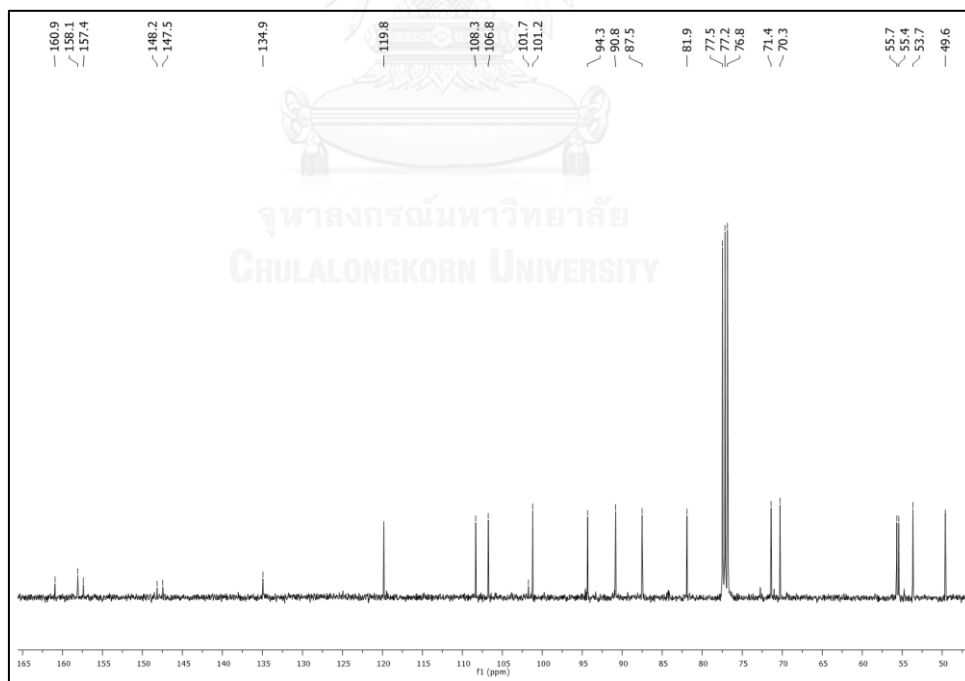
Figure 47 ^{13}C spectrum of *epi*-**3i** (CDCl$_3$)

Figure 48 ^{1}H spectrum of **3j** (CDCl$_3$)

Figure 49 ^{13}C spectrum of **3j** (CDCl$_3$)
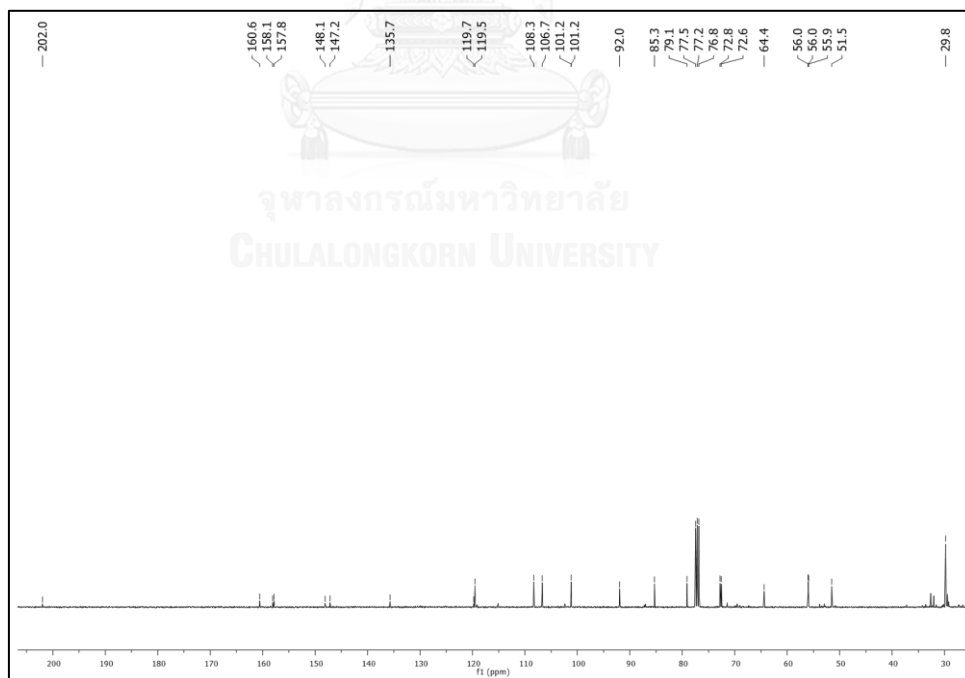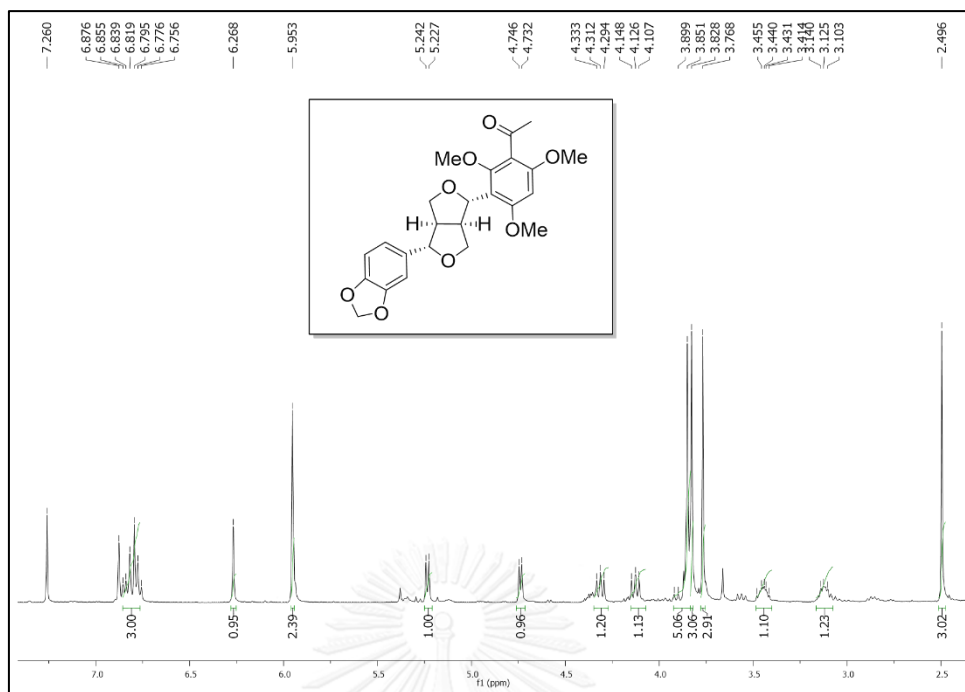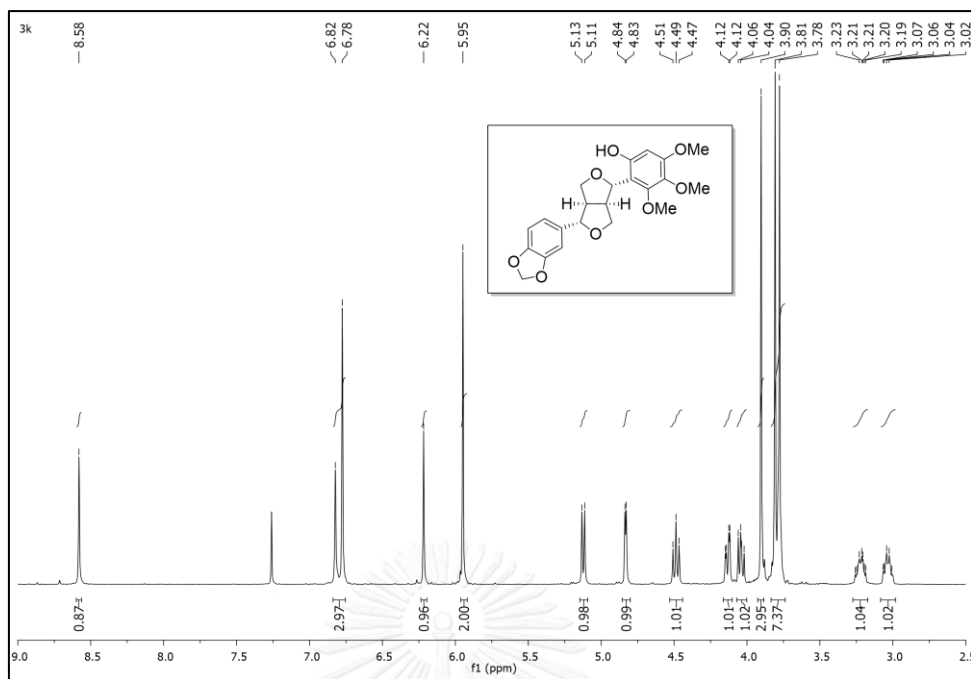
Figure 50 ^{1}H spectrum of **3k** (CDCl$_3$)
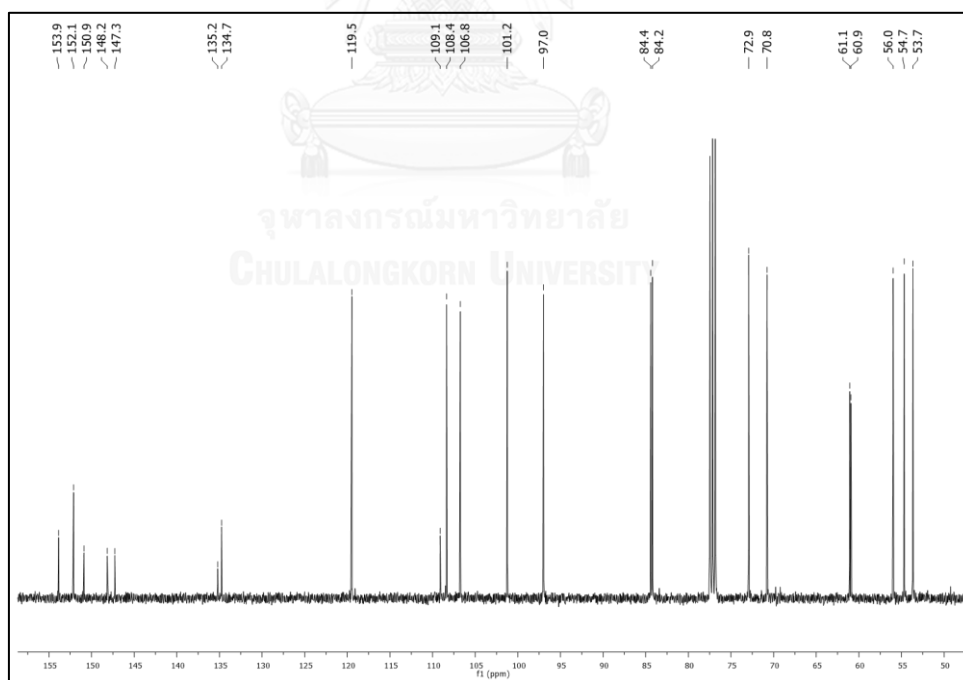
Figure 51 ^{13}C spectrum of **3k** (CDCl$_3$)
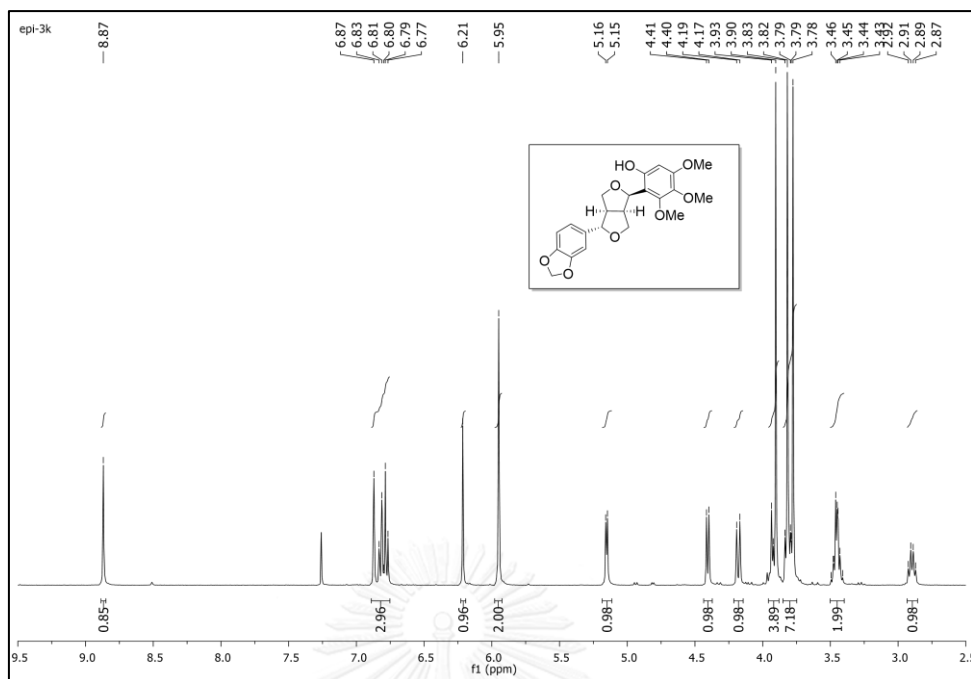
Figure 52 ^{1}H spectrum of *epi*-3k (CDCl$_3$)
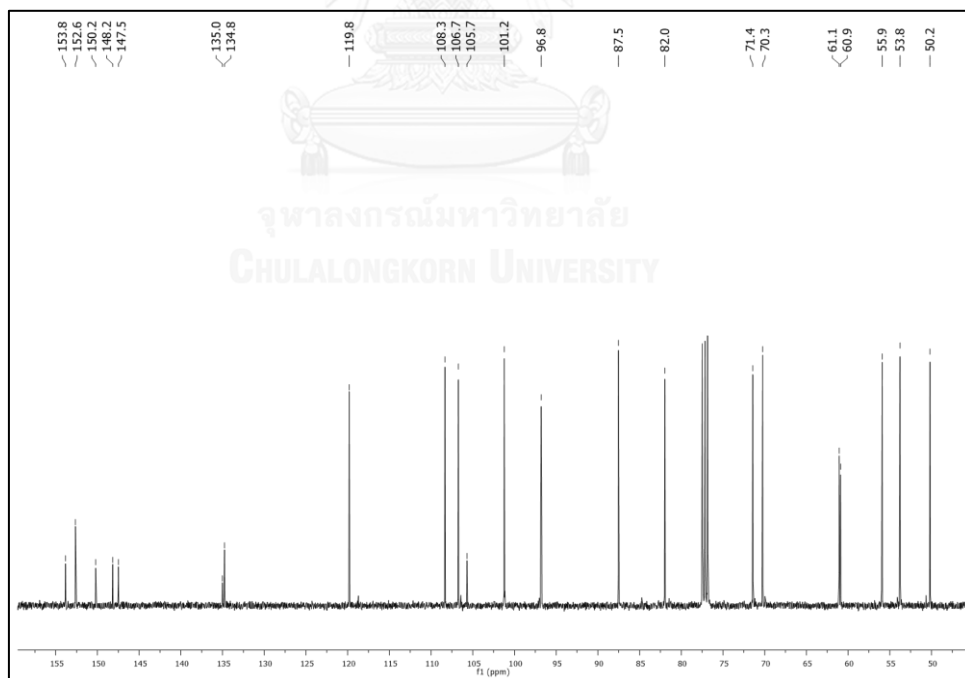
Figure 53 ^{13}C spectrum of *epi*-3k (CDCl$_3$)

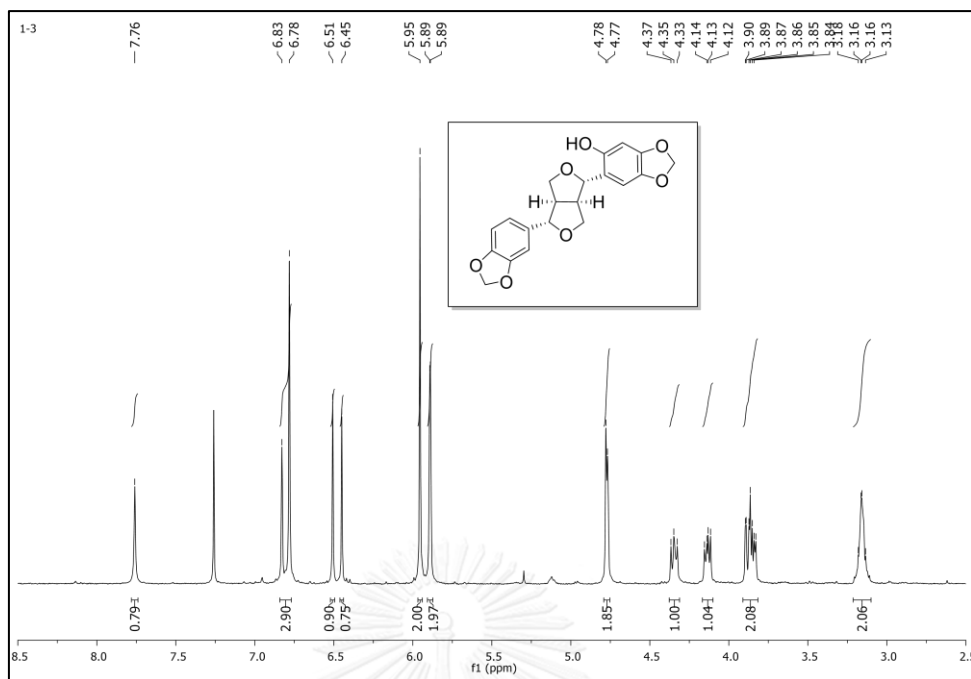**Figure 54** ^{1}H spectrum of **1-3** (CDCl$_3$)
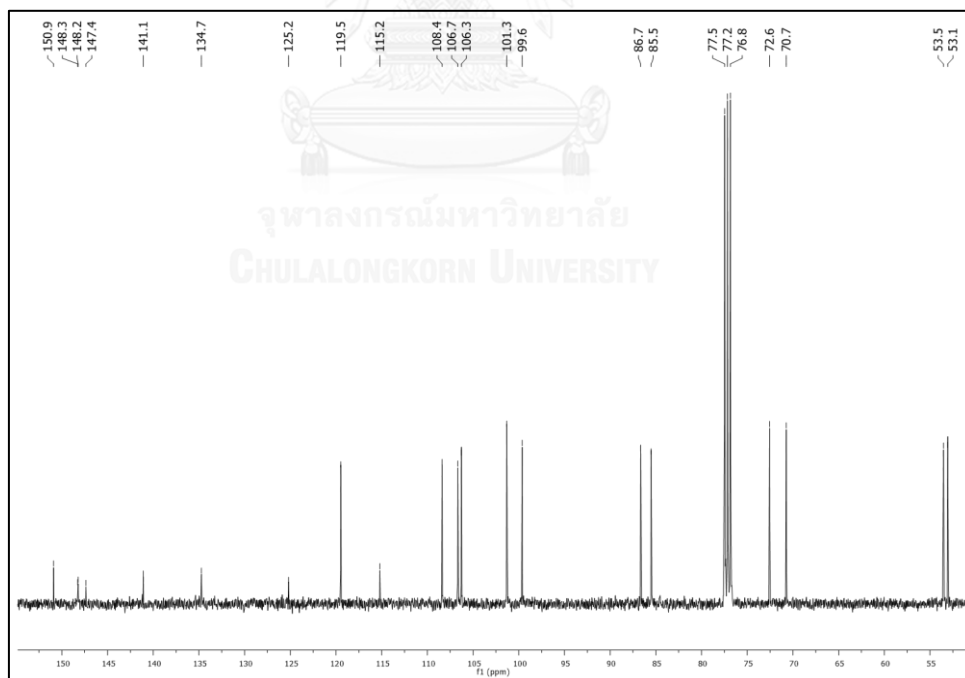
Figure 55 ^{13}C spectrum of **1-3** (CDCl$_3$)
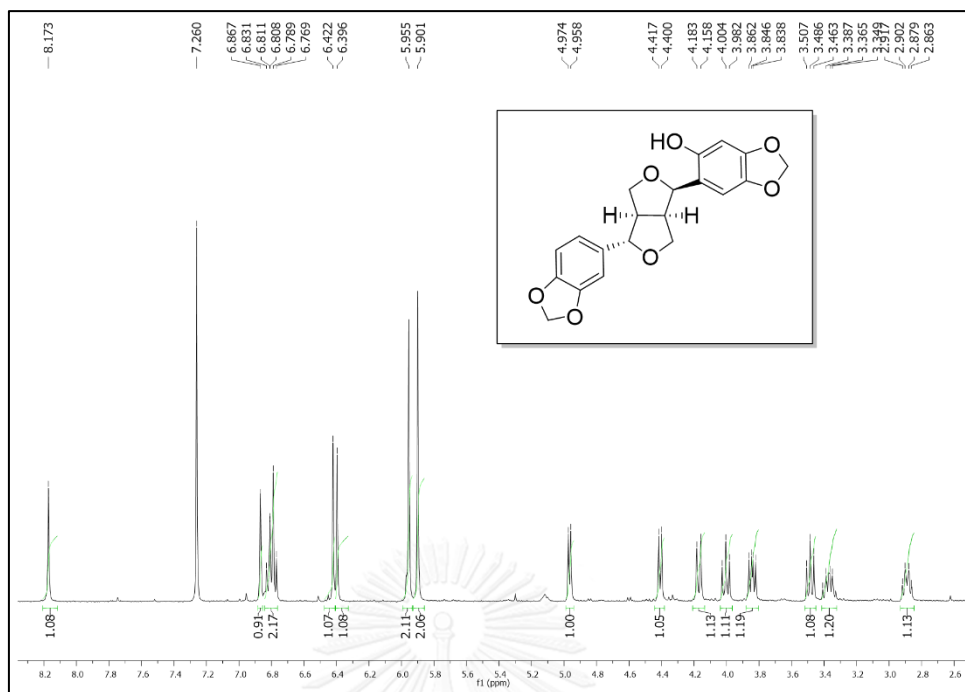
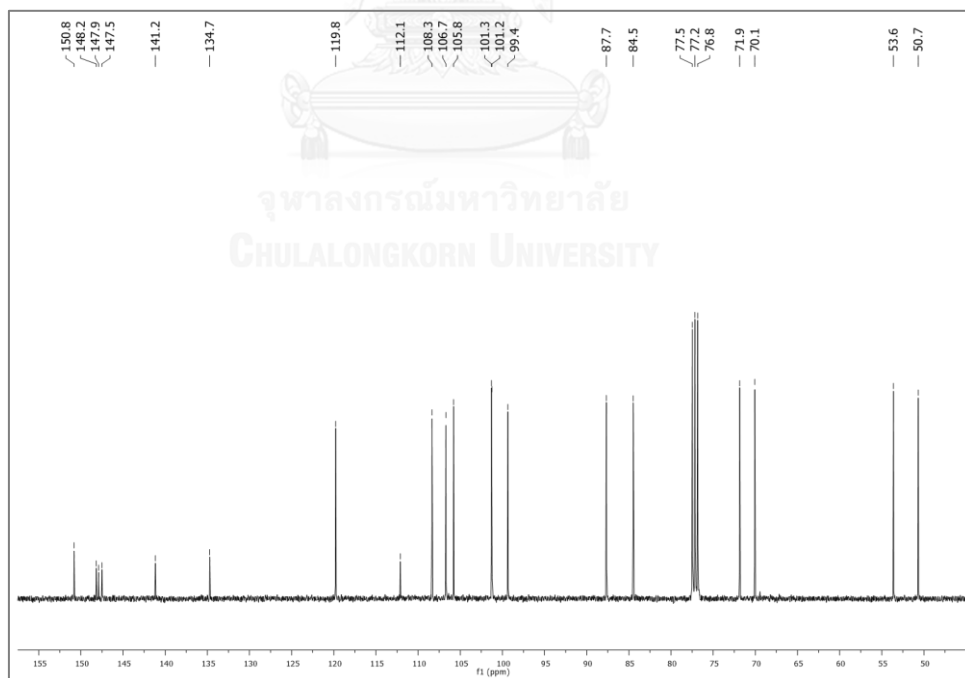Figure 56   ¹H spectrum of **1-22** (CDCl₃)

Figure 57   ¹³C spectrum of **1-22** (CDCl₃)

VITA

Mr. Phonpimon Khongchai was born on December 18, 1991 in Nakhon Si Thammarat, Thailand. He graduated with Bachelor's Degree of Chemistry from Faculty of Science, King Mongkut's University of Technology Thonburi, in 2014. He has been member of Science Achievement Scholarship of Thailand (SAST) since 2010 – present.