SYNTHESIS AND BIOACTIVITY ASSAY OF TETRAHYDRO-BETA-CARBOLINE AND BETA-CARBOLINE DERIVATIVES AND EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANT FOR MEDICAL PURPOSES

By
MISS Koonchira BUABAN

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy (CHEMISTRY)
Department of CHEMISTRY
Graduate School, Silpakorn University
Academic Year 2021

การสังเคราะห์และทดสอบฤทธิ์ทางชีวภาพของอนุพันธ์เตตระไฮโดร-เบต้า-คาร์โบลีน และเบต้า-คาร์โบลีน และการสกัดสารออกฤทธิ์จากพืชเพื่อนำไปใช้ประโยชน์ทางการแพทย์

โดย
นางสาวกุลจิรา บัวบาน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปรัชญาดุษฎีบัณฑิต
สาขาวิชาเคมี แบบ 1.1 ปรัชญาดุษฎีบัณฑิต
ภาควิชาเคมี
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2564

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Title	Synthesis and bioactivity assay of Tetrahydro-beta-Carboline and Beta-Carboline derivatives and Extraction of bioactive compounds from plant for medical purposes
By	Koonchira BUABAN
Field of Study	(CHEMISTRY)
Advisor	Assistant Professor Waya Phutdhawong , Ph.D.

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Keyword : Tetrahydro-beta-carboline, beta-carboline, biological activity, Pictet-Spengler reaction, cytotoxicity

MISS KOONCHIRA BUABAN : SYNTHESIS AND BIOACTIVITY ASSAY OF TETRAHYDRO-BETA-CARBOLINE AND BETA-CARBOLINE DERIVATIVES AND EXTRACTION OF BIOACTIVE COMPOUNDS FROM PLANT FOR MEDICAL PURPOSES THESIS ADVISOR : ASSISTANT PROFESSOR WAYA PHUTDHAWONG, Ph.D.

Tetrahydro-beta-carboline (THβC) and beta-carboline (βC) derivatives are one of indole scaffolds and are widely found in natural products such as plants, fungi and marines. Many of these compounds display various biological and pharmacological activities such as inhibit the monoamine oxidase (MAO-A), antioxidant, anticancer, and antifungal activities. Moreover, the structure-activity relationship (SAR) studies indicated that THßC derivatives with substitutions with aromatic ring at 1-position and also ester at 3-position would make great influence on their biological activities. Thus, the synthesis of THßCs derivatives bearing the substituted at C-1 and C-3 was of interest and Pictet-Spengler reaction was used as a key step to evaluate for their biological activities. In first plan, the synthesis was started from tryptamine or *L*-tryptophan methyl ester with various aldehydes *via* Pictet-Spengler to provide 1-substituted THßCs and diastereomeric mixture of 1,3-disubstituted THßCs, respectively. The obtained THβCs were oxidized with sulfur powder under ambient oxygen to afford the C1 and/or C3 substituted ßCs. All the synthesized compounds were confirmed by NMR analysis. Moreover, all compounds were investigated for *in vitro* cytotoxicity against human cancer cell lines by MTT assay and antioxidant activity by DPPH radical-scavenging assay. Doxorubicin, acridine orange and trolox were used as a standard drug for cytotoxicity and antioxidant activity, respectively. However, all synthesized compounds showed potency less than that of the standard drug both in cytotoxicity and antioxidant activity.

And in second plan, in continuous study for investigation of the influence of configuration at C-1 position on their biological activities, 1-substituted THβC derivatives were reported in enantioselective synthesis. At first, we attempted to construct of piperidine ring *via* asymmetric Pictet-Spengler by using Boc-protected proline and *N*,*N*-phthaloyl-protected amino acid chloride as chiral auxiliary and Titanium isopropoxide as catalyst but this synthetic strategy was not successful regarding to the steric repulsion between chiral auxiliary and substituent of the parent compound. Then, the synthetic strategy of enantioselective synthesis of 1-substituted THßCs was designed *via* asymmetric Pictet-Spengler with cocatalyzed by a chiral thiourea and benzoic acid. Moreover, we attempted to SAR study of substituted methyl ester at C-3 compared the parents by chromatographic separation of *cis*/*trans* 1,3-disubstituted THßCs to pure *trans* 1,3-disubstituted THßCs. Their structures were elucidated by ^{1}H, ^{13}C NMR and ^{1}H-^{1}H NOESY spectroscopic analyses. Later on, all of enantioselective 1-substituted THßCs and *trans* 1,3-disubstituted THßCs have been investigated their biological activities compared to the mixture compounds in first plan.

Moreover, the isolation of Cannabinoids led to study the bioassay of *Cannabis indica*. Their isolated structures were elucidated by spectroscopic analysis and compared with previous literatures. All compounds were conducted to explore cytotoxic activities using MTT assay. In particular, CBD was found to exhibit the most promising cytotoxic effect against MDA-MB-231 cell with the lowest IC_{50} value of 4.14 µg/mL.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my thesis adviser, Asst. Prof. Dr. Waya Phutdhawong, for enthusiastic encouragement and invaluable advice throughout of this research.

Moreover, I would like to express my special thanks to Asst. Prof. Dr. Anan Athipornchai, Prof. Nantanit Wanichacheva, Dr. Sirirat Choosakoonkriang and Dr. Muhammad Niyomdecha, my thesis committees, for spent their valuable time, advice and support.

Next, I would like to thank to Assoc. Prof. Dr. Weerachai Phutdhawong, Department of Science, Faculty of Liberal Arts and Science, Kasetsart University for their support in collecting and enabling me to carry out the isolation of the plant material.

Next, I would like to express my very great appreciation to Assoc. Prof. Dr. Thongchai Taechowisan for assistance, advice and knowledge in bioactivity assay. And, I would also like to thank Department of Chemistry and Department of Microbiology, Faculty of Science, Silpakorn University for helpful suggestion and facilities in cytotoxicity assay.

Besides, I would like to thank all the teachers and staffs of Department of Chemistry, Faculty of Science, Silpakorn University who helped us by giving me advice and providing the research facilities.

Also, special thanks should be given to the Chulabhorn Research Institute for HRMS measurement.

Next, I would like to thank the Science Achievement Scholarship of Thailand (SAST) for providing me ascholarships.

Finally, I would like to thank my family and friends for their support and encouragement throughout my study.

MISS Koonchira BUABAN

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CHAPTER 1
INTRODUCTION

1.1 Background and Signification of the Research Problem

Tetrahydro-beta-carboline (THβC) and beta-carboline (βC) derivatives are one of the important classes of indole alkaloids which display various biological activities such as anti-inflammatory, antileishmanial, antioxidant, anticancer, antimalarial, antiplasmodial, antiviral, antifungal and antibacterial activities. The structure of THβC consists of piperidine ring fused to indole ring while βC shows unique fused between pyridine ring and indole ring. Moreover, this group was found in many plants, fungi and marine organisms (Figure 1). For example, Tetrahydrohymine (**1**) was isolated from *Banisteriopsis caapi* which found in South America [1]. (+)-Harmicine (**2**) was extracted from *Kopsia griffithii* in Malaysia [2]. Furthermore, Harmane (**3**) was found in bark of *Hippophaë rhamnoides L.*, *Elaeagnus angustifolia L.*, and *E. orientalis L* [3]. Siliendines A (**4**) was extracted from the aerial parts of *Silene seoulensis* with EtOH [4]. Additionally, Eudistomin V (**5**), H (**6**) and I (**7**) were isolated from the Australian Ascidian *Pseudodistoma aureum* [5].

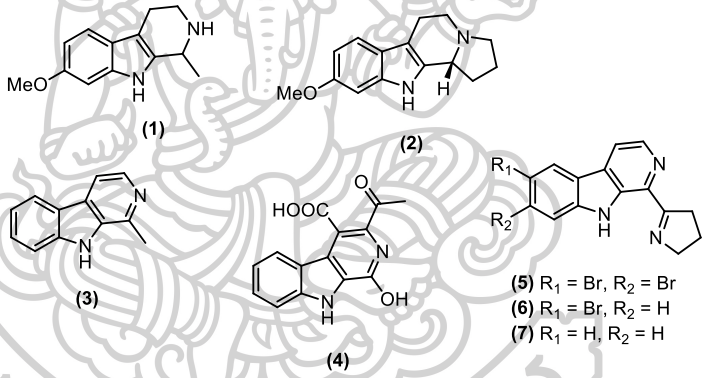


Figure 1. Natural THβCs and βCs

Furthermore, THβCs and βCs display significant range of biological activities. For instance, in Figure 2, Tryptoline (**8**) and Pinoline (**9**) were used as antidepressant including monoamine oxidase type A (MAO-A) inhibitor [6-8]. 2-Acetyl-1,2,3,4-tetrahydro-ß-carboline (**10**) displays antifungal activity against *Fusarium incarnatum* (HKI0504) [9]. Sacleuximine A (**11**) has been isolated from aerial parts of *Triclisia sacleuxii* and exhibited antibacterial and cytotoxic activities against human adenocarcinoma, hepatocarcinoma and breast carcinoma cell lines [10]. In addition, β-carboline-1-propionic acid (**12**) displays production of nitric oxide (NO) by infected leishmanicidal activity with inhibition effect on the macrophages [11]. The Papua New Guinea marine sponge *Hyrtios reticulatus* was extracted to furnish hyrtiocarboline (**13**) which showed selective antiproliferative activity against H522-T1 non-small cell lung, MDA-MB-435 melanoma and U937 lymphoma cancer cell lines [12]. Moreover, Xestomanzamine A (**14**) was isolated from an Indonesian sponge *Acanthostrongylophora spp.* and displayed antifungal activities against *Cryptococcus neoformans* [13].

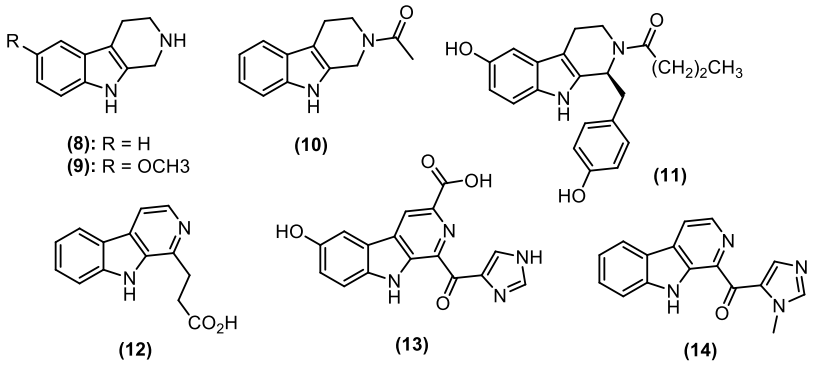


Figure 2. THβC and βC derivatives

According to a broad range of biological activities of THβCs and βCs, synthetic chemists interested in the development of the synthetic route for improvement their pharmaceutical activities. In previous work, we synthesized 1, 2-substituted THβCs (**19**) and 1, 2, 3-substituted THβCs (**20**) through the key steps of Pictet-Spengler reaction and *N*-Alkylation or *N*-Acylation (Figure 3). 1-Substituted THßCs (**17-18**) were synthesized from tryptamine (**15**) and L-tryptophan methyl ester (**16a**) with vary appropriate aldehydes *via* Pictet-Spengler reaction followed by *N*-Alkylation or *N*-Acylation with alkyl halides or acyl halide to provide 1,2-substituted and 1,2,3-substituted THßCs (**19-20**). Interestingly, most of them display significant range of biological activities such as anticancer activity against two cancer cell lines (HepG2 and HeLa) with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay as well as inhibition of five plant pathogenic fungi (*Rhizopus sp*., *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium semitectum* and *Fusarium fujikuroi*). Especially, substituent at position 1 enhanced anticancer activity such as ethylbenzene (**17b**) displayed the highest cytotoxicity against Hep G2 and HeLa with IC_{50} values of 23.67-33.33 µg/mL. However, these synthetic routes provided a racemic mixture of THβC (**17**) at C-1. Then, the synthetic routes of enantioselective 1-substituted THβCs have been studied and evaluated their biological activities in this work.

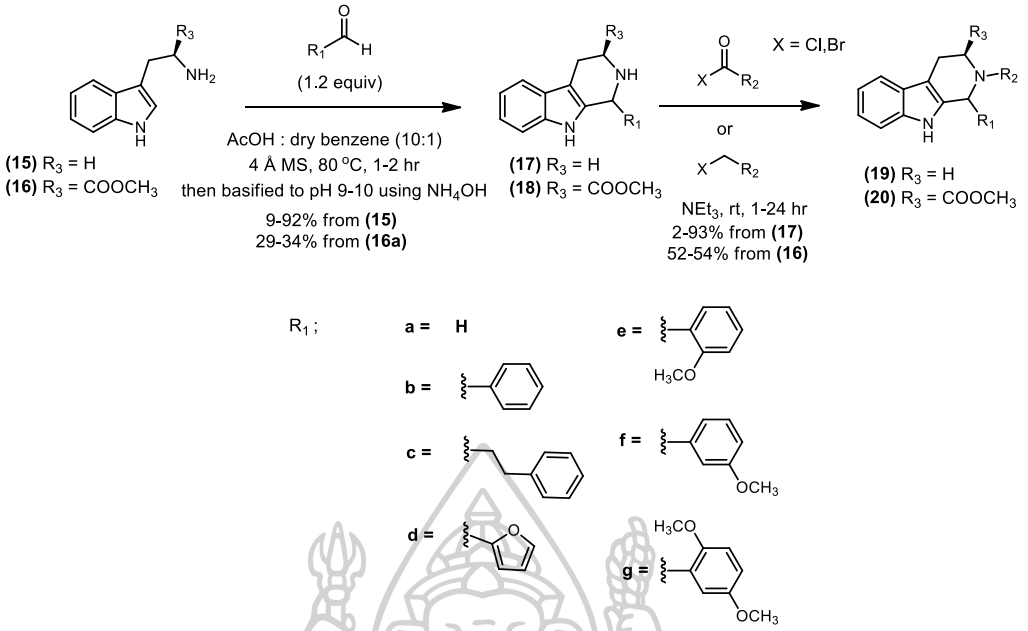


Figure 3. The synthetic route of THβCs in previous work

Part A: Synthesis of THβC and βC derivatives

Thus, the development of a 1,3-disubstituted ßCs (**21-22**) is of interest. The synthesized ßCs (**21-22**) have been prepared from tryptamine (**15**) or *L*-tryptophan methyl ester (**16**) *via* key steps as aromatization and Pictet-Spengler reaction which is depicted in Fig. 4. After that, all of them will be evaluated *in vitro* biological activities such as anticancer, antifungal and antibacterial activity.

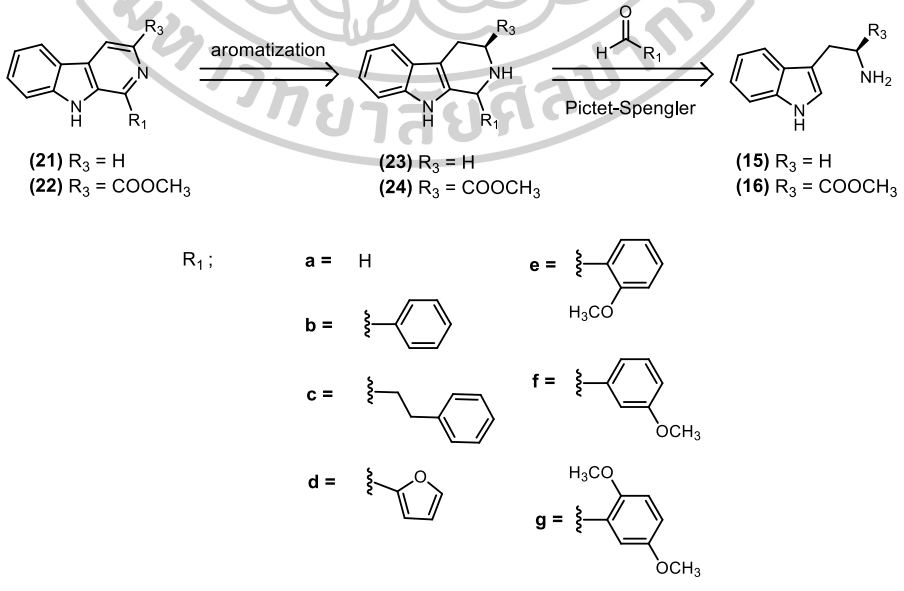


Figure 4. The synthetic route of βCs in this work

Part B: Enantioselective synthesis of C-1 substituted THβC derivatives (17) and stereoselective synthesis of *trans*-1,3-disubstituted THβCs (*trans*-24)

Based on anticancer screening of racemic synthesis of C-1 substituted THβC derivatives (**17**) in part A, and in continuation to our SAR studies, the enantioselective synthesis of 1-substituted THβC derivatives (**17**) have been studied in this part. Thus, 1-substituted THßCs (**17**) have been synthesized enantioselectively *via* asymmetric Pictet-Spengler using Boc-protected proline (**27**) and *N*,*N*-phthaloyl-protected amino acid chloride (**28**) as chiral auxiliary using Titanium isopropoxide as catalyst (Figure 5). The key steps involved imine formation from tryptamine (**15**), construction of piperidine ring *via* asymmetric Pictet-Spengler as well as reduction of chiral auxiliary at last stage. However, this plan was not successful due to the steric repulsion between chiral auxiliary and substituent of the parent compound including decomposition after purification.

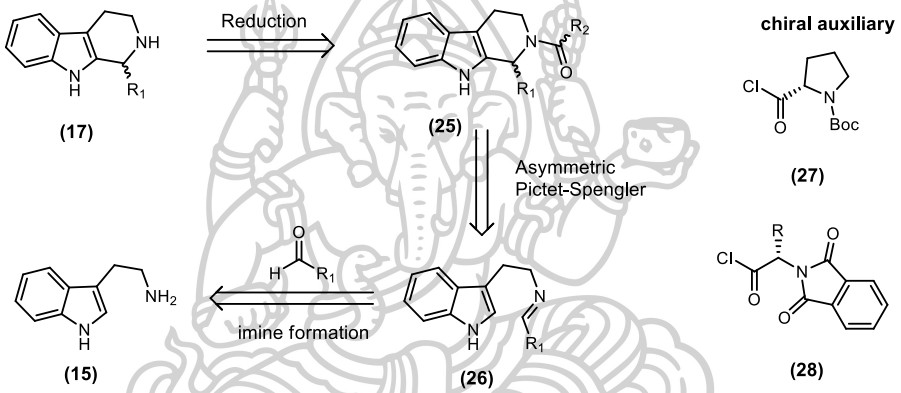


Figure 5. The enantioselective synthesis of THβCs (**17**) in this work

According to the failure of enantioselective synthesis by using chiral auxiliary, we attempted to synthesize 1-substituted THßCs (**17**) from tryptamine (**15**) and aldehyde precursors under simple and mild condition with cocatalyzed by a chiral thiourea and Brønsted acid (benzoic acid) (Figure 6).

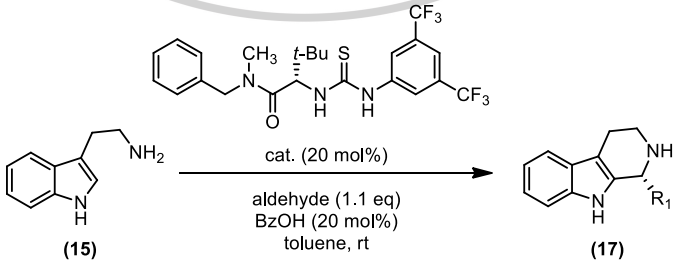


Figure 6. The enantioselective synthesis of THβCs (**17**) by a chiral thiourea catalyst

To evaluate the SAR of substituted methyl ester at C-3 compared the parents (**17**) (Figure 7), the synthetic strategy of *cis*/*trans* isomers of 1,3-disubstituted THβCs (**24b-k**) were synthesized from *L*-tryptophan methyl ester (**16**) *via* Pictet-Spengler reaction depicted in part A. In this regard, chromatographic separation of *cis*/*trans* isomers (**24b-k**) have been studied

for investigation of the pure *trans* diastereomer (***trans*-24b-k**) which were investigated by dynamic ^{1}H, ^{13}C NMR and ^{1}H-^{1}H NOESY spectroscopic analyses.

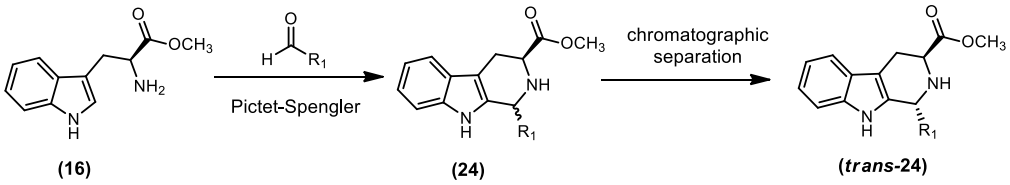


Figure 7. Stereoselective synthesis of *trans*-1,3-disubstituted THβCs (***trans*-24**)

Part C: Extraction of bioactive compounds from plant for medical purposes

Moreover, natural products represent an important source of lead compounds in drug discovery research [14-19]. Generally, the plant kingdom was increasingly interested as a potential source of therapeutic agents [20-22]. The chromatographic separation techniques have been popularly applied for isolation bioactive compounds [23-26]. The biological activities of plant-derived natural products have been diversely displayed including anticancer agents [27], antioxidant [25, 28], antifungal [29], anti-inflammatory [30], antimicrobial [31] properties. Additionally, the family Cannabaceae, *Cannabis indica*, was used for medical purposes and found bioactive compounds as the cannabinoids [32-35] (Figure 8). Thus, in this work, *Cannabis indica* were isolated of bioactive compounds for medical purposes using supercritical CO_2 extraction and preliminary examined *in vitro* anticancer activity.

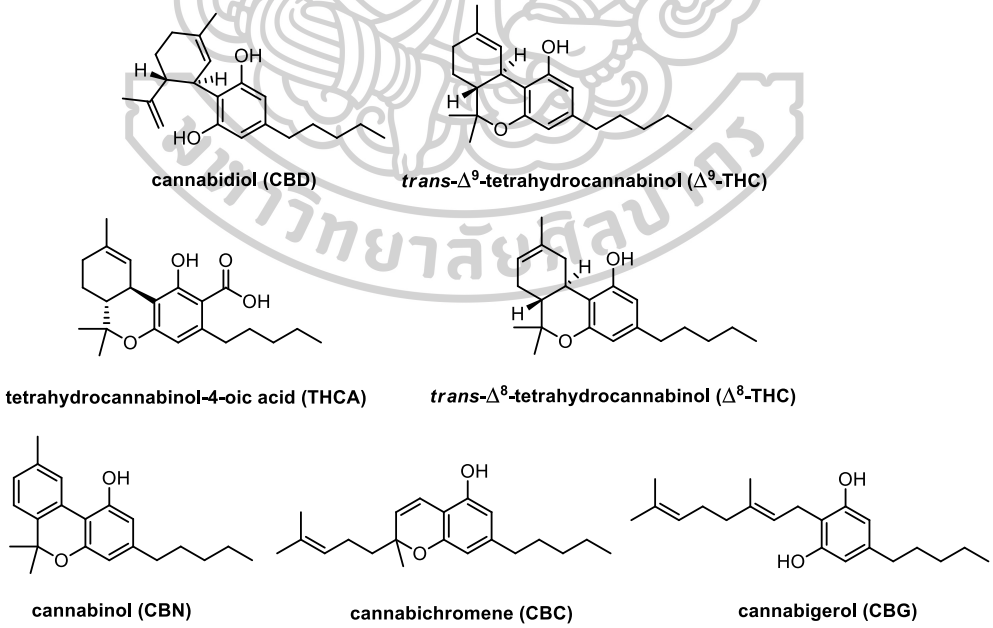


Figure 8. Some natural cannabinoids and their structures

1.2 Objectives

1. To design and study the synthetic routes of tetrahydro-beta-carboline and beta-carboline derivatives.

2. To study the biological activities of tetrahydro-beta-carboline and beta-carboline derivatives.

3. To investigate the structure activity relationship (SAR) of the synthesized compounds.

4. To isolate the bioactive compounds from plant for medical purposes.

1.3 Research hypothesis

The obtained tetrahydro-beta-carboline and beta-carboline derivatives will synthesize in short period of time, efficient procedure as well as high yields. Moreover, all of synthesized compounds could display *in vitro* biological activities. Additionally, the bioactive compounds from plant will obtain in high yield and display in vitro biological activity.

1.4 Scope of study

In this research, tetrahydro-beta-carboline and beta-carboline derivatives have been designed and synthesized for investigation the structure by spectroscopy method including evaluation their biological activities. Moreover, the plant has been investigated for their bioactive compounds and biological activity.

1.5 Expected Benefits

This study will find the ways to develop the suitable, short and effective synthetic route of tetrahydro-ß-carboline and ß-carboline derivatives. Especially, the synthesized compounds will have highly biological activities such as anticancer and antioxidant activities. Furthermore, the bioactive compounds from plant will display in vitro preliminarily biological activity for further medical purposes.

1.6 List of Abbreviations

anh. Na_2SO_4	=	sodium sulphate anhydrous
aq	=	aqueous
Abs	=	absorbance
AcOH	=	acetic acid
Ar	=	argon
br s	=	broad singlet (NMR spectrum)
cat.	=	catalyst
$CDCl_3$	=	deuterated chloroform
CH_2Cl_2	=	dichloromethane
^oC	=	degree Celsius
d	=	doublet (NMR spectrum)
dd	=	doublet of doublet (NMR spectrum)
ddd	=	doublet of doublet of doublet (NMR spectrum)
ddt	=	doublet of doublet of triplet (NMR spectrum)
dt	=	doublet of triplet (NMR spectrum)
DMSO	=	dimethyl sulfoxide

ee	=	enantiomeric excess
equiv	=	equivalent
Et_3N	=	triethylamine
EtOAc	=	ethyl acetate
g	=	gram
hr	=	hour
H_2SO_4	=	sulfuric acid
IC_{50}	=	half maximal inhibitory concentration
J	=	coupling constant
LD_{50}	=	50% lethal dose
m	=	multiplet (NMR spectrum)
mg/mL	=	milligram per milliliter
min	=	minute
m.p.	=	melting point
mL	=	milliliter
mmol	=	millimole
MeOH	=	methanol
MHz	=	megahertz
nm	=	nanometer
N	=	Normality
rt	=	room temperature
s	=	singlet (NMR spectrum)
SD	=	standard deviation
t	=	triplet (NMR spectrum)
td	=	triplet of doublet (NMR spectrum)
tdd	=	triplet of doublet of doublet (NMR spectrum)
TFA	=	trifluoroacetic acid
TLC	=	thin-layer chromatography
µg/mL	=	microgram per milliliter

CHAPTER 2
REVIEW OF LITERATURE

2.1 Previous studies of THβC and βC derivatives

Indole alkaloids were widely found in nature including plants, fungi and marine organisms. Therefore, the novel indole alkaloids were synthesized and developed for improvement their pharmaceutical activities including tetrahydro-beta-Carbolines (THβCs) and beta-Carbolines (βCs). βCs possess a fully aromatic tricyclic pyrido[3,4-*b*]indole ring structure, whereas the saturated ones are known as THβCs. Due to a broad range of biological activities, these compounds are of great interest. Callophycin A (**29**) was isolated from the red alga *Callophycus oppositifolius* collected from Australia which shown as anticancer activity against Cellosaurus cell line (SF-268) and Human colon adenocarcinoma (HT-29) [36] (Figure 9). Hydroxytrypargine (**30**) was isolated from the crude venom of the web spider *Parawixia bistriata*, which represented *in vivo* insecticidal activity against honeybee *Apis mellifera* with LD_{50} of 8 ng/g [37]. Reserpine (**31**), an antihypertensive and antipsychotic drug in Africa, was isolated from the root of *Rauwolfia serpentine* and *Rauwolfia vomitoria* [38]. 14-Methyleudistomidin C (**32**) was extracted from the marine ascidian *Eudistoma gilboverde* and was reported to exhibit the potent cytotoxic activity against four different human tumor cell lines with IC_{50} values of < 1.0 μg/mL [39]. The novel ß-carboline, Eudistomin Y6 (**33**), was isolated from the genus Eudistoma collected near Tong-Yeong City, South Sea, Korea and displayed moderate antibacterial activity against Gram-positive bacteria *Staphylococcus epidermis* and *Bacillus subtilis* [40]. Moreover, Indolactam alkaloids named as marinacarbolines C (**34**) was obtained from the fermentation broth of *Marinactinospora thermotolerans* SCSIO 00652 [41] which exhibited antiplasmodial activities against Plasmodium falciparum lines 3D7 and Dd2. Dichotomide X (**35**) was characterized from the roots of *Stellaria dichotoma* var. *lanceolate* and displayed significantly anti-inflammatorial activity for the inhibition of NO production in LPS-treated RAW 264.7 with IC_{50} values of 11.3 μM [42].

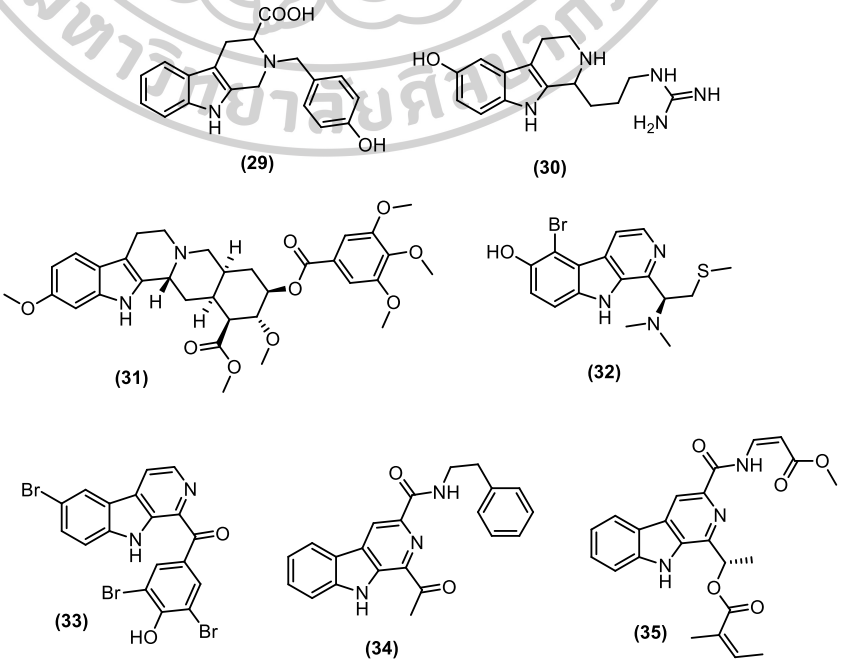


Figure 9. Some structures of bioactive tetrahydro-beta-Carbolines (THβCs) and beta-Carbolines (βCs)

Previous synthesis of THβC and βC derivatives *via* Pictet-Spengler reaction

Previous studies have demonstrated that substitution of THβCs and βCs at the position-1, 2 and 3 display wide range of biological activities including pharmacological activities (Figure 10). The structure-activity relationships (SAR) analysis previously indicated that various substituents at different positions might play a crucial role in determining their multiply pharmacological functions such as antitumor, antibacterial, anti-inflammatorial, antimalarial, antiviral, PDE5-inhibitory, antiparasitic and antithrombotic activities [43].

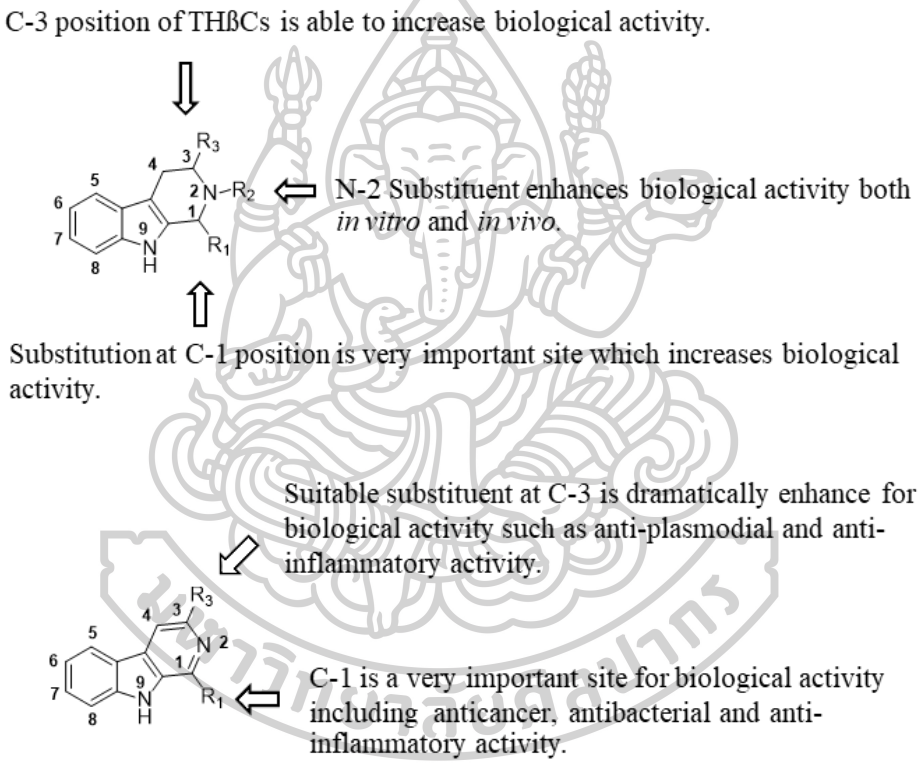


Figure 10. The structure-affinity relationships (SAR) of Tetrahydro-beta-Carboline and Beta-Carboline derivatives

Furthermore, it is well-known that the Pictet-Splengler reaction is one of the most potent methods for the formation of ring system form tryptophan or tryptamine and aldehyde as substrates (Figure 11). This reaction commonly represents the formation of an iminium intermediate under an acid-catalyzed condensation reaction, followed by intramolecular cyclization to form THβC by Brønsted acids or Lewis acids of the iminium ion to obtain pyrrole ring by direct attack from the indole at position-2. After that, oxidation of THβC furnish βC.

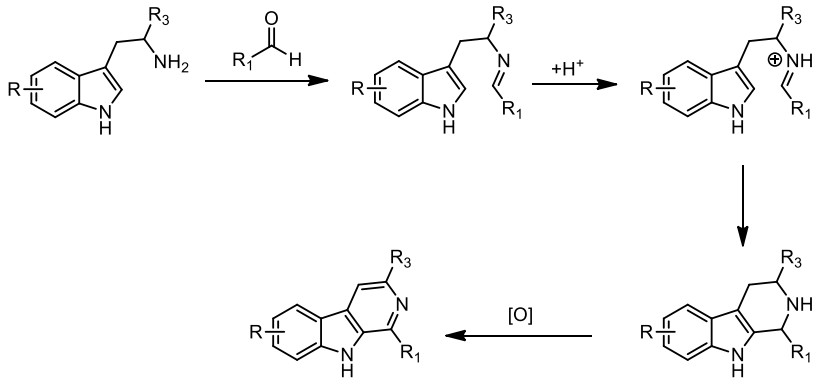


Figure 11. Mechanism of Pictet-Spengler condensation

For example, Narayanan et al. reported synthesis of THβC derivatives (**41a-d**) in 1990 through Pictet-Spengler condensation from tryptamine-2-carboxylic acid (**40a-d**) [44] (Figure 12). Tryptamine-2-carboxylic acid (**40a-d**) was synthesized from aryl diazonium salts (**36a-d**) and ß-keto-acids (**37**) *via* Japp-Klingemann, followed by closing indole ring to THβCs (**39a-d**) and hydrolysis of the resulting THβCs (**39a-d**) to provide tryptamine-2-carboxylic acids (**40a-d**). Then, Pictet-Spengler reaction of tryptamine-2-carboxylic acid (**40a-d**) in benzene/dioxane/trifluoroacetic acid provided THβC derivatives (**41a-d**).

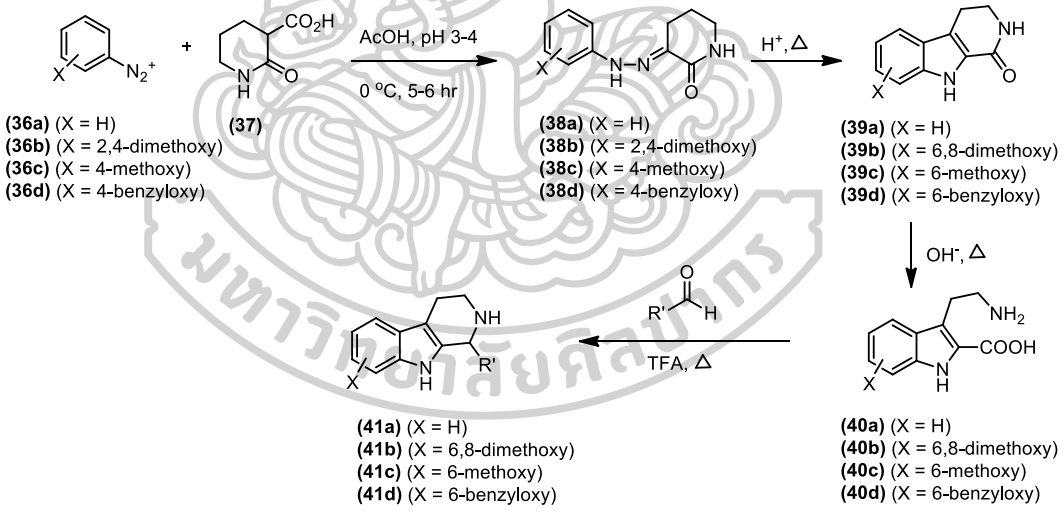


Figure 12. The synthetic route of THβCs (**41a-d**) by Narayanan et al.

Later in 2012, Ascic et al. reported the metal-catalyzed tandem isomerization and *N*-alkyliminium cyclization of *N*-allyltryptamines constitutes (**42**) as an efficient alternative of the Pictet-Spengler reaction for the synthesis of THBCs [45] (Figure 13). Especially, Tsuji–Trost reaction with Pd catalyst was able to synthesize novel tetrahydro-ß-carboline (**43**).

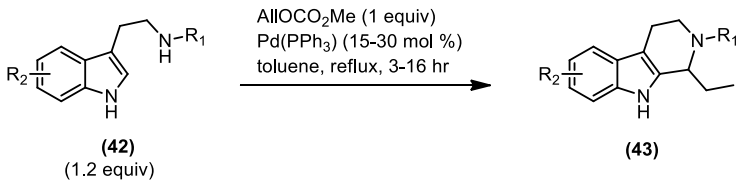


Figure 13. The metal-catalyzed cyclization of THβCs (**43**) by Ascic et al.

In 2016, Spindler et al. synthesized novel THβCs with different modifications at position 1, 2 and 6 [46] (Figure 14). The synthesis began with Pictet-Spengler reaction of tryptamine or 5-methoxytrypamine (**44a**) and various aldehydes in presence of trifluoroacetic acid (TFA) in dichloromethane (DCM) to form the 1-substituted-THβCs or the 1,3-disubtituted-THβCs with a chiral centre at C1. The resulting racemic mixtures of THβCs (**45**) were reacted with differently substituted acid chlorides by *N*-Acylation or *N*-Alkylation to obtain 1,2-substituted-THβCs or the 1,2,3-disubtituted-THβCs. Moreover, all synthesized THBCs (**46a-q**) were evaluated for inhibition on Breast Cancer Resistance Protein ($ABCG_2$) using the MDCK II BCRP Cell Line (Table 1). The results showed that the effect of phenyl group substituent at C-1 with Cl and Br at *meta* or *para* position showed good activity toward Breast Cancer Resistance Protein ($ABCG_2$), especially 3,4-dichlorophenyl (**46n-o**). Both 4-OCH_3-Phenyl (**46n**) and 3-OCH_3-Phenyl (**46o**) showed the highest cytotoxicity with IC_{50} values of 0.233 and 0.237 μM, respectively.

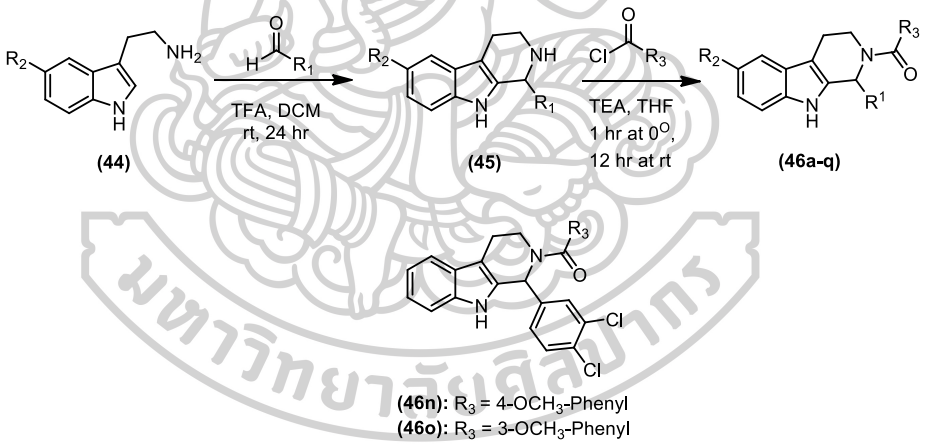


Figure 14. The synthetic route of THβCs (**46a-q)** by Spindler et al.

Table 1. Inhibitory effect on breast cancer resistance protein (ABCG2) of THβCs (**46a-q**) in Hoechst 33342 assays using the MDCK II BCRP Cell Line

cpds	R_1	R_2	R_3	R_4	IC_{50} ± SD [μM]
(46a)	phenyl	H	phenyl	H	2.78 ± 0.15
(46b)	3,4-OCH_3-phenyl	H	phenyl	H	3.62 ± 0.41
(46c)	3-CN-phenyl	H	phenyl	H	2.00 ± 0.18
(46d)	3,4-F-phenyl	H	phenyl	H	1.10 ± 0.10

cpds	R_1	R_2	R_3	R_4	IC_{50} ± SD [μM]
(46e)	3-Cl-phenyl	H	phenyl	H	0.869 ± 0.068
(46f)	4-Cl-phenyl	H	phenyl	H	0.741 ± 0.123
(46g)	3,4-Cl-phenyl	H	phenyl	H	0.328 ± 0.055
(46h)	3,4-F-phenyl	H	4-Cl-phenyl	H	1.26 ± 0.30
(46i)	4-Br-phenyl	H	4-Cl-phenyl	H	1.78 ± 0.19
(46j)	3,4-Cl-phenyl	H	4-Cl-phenyl	H	1.03 ± 0.10
(46k)	3,4-Cl-phenyl	H	3,4-OCH_3-phenyl	H	0.698 ± 0.116
(46l)	3,4-F-phenyl	H	3,4-OCH_3-phenyl	H	1.10 ± 0.20
(46m)	3,4-Cl-phenyl	H	1-naphthyl	H	1.32 ± 0.23
(46n)	3,4-Cl-phenyl	H	4-OCH_3-phenyl	H	0.233 ± 0.044
(46o)	3,4-Cl-phenyl	H	3-OCH_3-phenyl	H	0.238 ± 0.044
(46p)	3,4-Cl-phenyl	OCH_3	phenyl	H	0.382 ± 0.077
(46q)	3,4-Cl-phenyl	H	3-OCH_3-phenyl	ethyl	>> 10
Ko143					0.221 ± 0.024

THβC derivatives (**48a-r**) were also synthesized under ultrasonic irradiation by Reddy et al. [47] (Figure 15). The efficient synthesis of novel THBCs were obtained *via* Pictet-Spengler condensation from tryptamine (**15**) and aryl or heteroaryl aldehydes (**47**) in the presence of a non-ionic surfactant, Triton X-100 catalyst, at 70 ºC in aqueous media. This developed procedure provided clean reaction, simple methodology, short reaction time as well as the excellent yields depicted in Table 2.

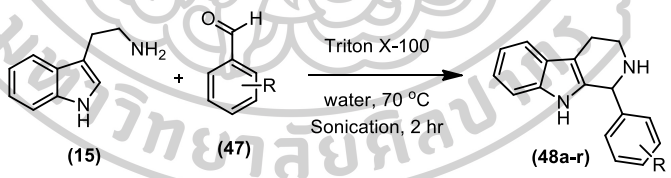


Figure 15. The synthetic route of THβCs (**48a-r**) by Spindler et al.

Table 2. Comparative study on ultrasound-promoted synthesis of THβCs (**48a-r**)

Entry	Substrate	Time (h)	Yield (%)	Entry	Substrate	Time (h)	Yield (%)
(48a)	(4-Cl)Ph	2	92	**(48j)**	(4-OH)(3-OMe)Ph	3	85
(48b)	(4-Me)Ph	2	86	**(48k)**	(4-Piperidinyl)Ph	2	92
(48c)	(4-NO_2)Ph	4	94	**(48l)**	2-Furyl	2	95

Entry	Substrate	Time (h)	Yield (%)	Entry	Substrate	Time (h)	Yield (%)
(48d)	(4-OMe)Ph	2	89	**(48m)**	2-Thiophenyl	2.5	85
(48e)	(4-OH)Ph	2	88	**(48n)**	2-Pyrrolyl	2	90
(48f)	(4-$N(CH_3)_2$)Ph	3	84	**(48o)**	4-Pyridinyl	2	93
(48g)	(4-F)Ph	2	89	**(48p)**	(2-Methyl)-4-Pyridinal	3	87
(48h)	(2-Br)Ph	3	86	**(48q)**	5-Indolyl	2	92
(48i)	(2-OH)Ph	3	82	**(48r)**	1,3-benzo[*d*]dioxole-5-yl	2	88

Previous enantioselective synthesis of 1-substituted-THβC derivatives

Many studies have been developed for the enantioselective synthesis of THβCs regarding to improve their biological activities and the most of the syntheses rely on the Pictet Spengler reaction. Introduction of chiral auxiliaries on the nitrogen atom of side chain of tryptamine (**15**) was played an important role in asymmetric Pictet Spengler reaction to construct of chiral THβCs. For example, Lamas et al. reported the synthesis of 1,3,4-substituted THβCs (**51**) from indole (**49**) and azalactones (**50**) through Pictet-Spengler reaction under acidic condition [48] (Figure 16). The obtained single diastereomer (**51**) was generated by the conformational restrictions of tryptamine substituents as stereocontrol generation.

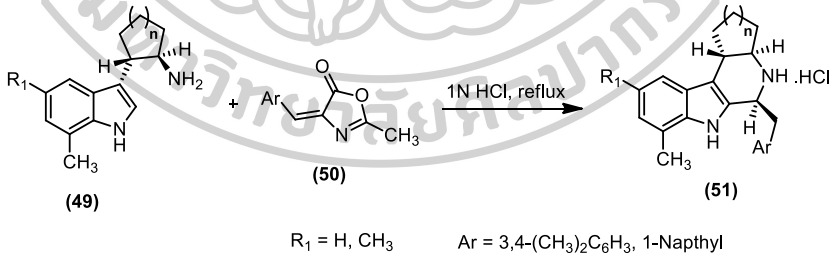


Figure 16. The synthetic route of THβCs (**51**) by Lamas et al.

Besides chiral tryptamine, tryptophan also used as the starting material for the preparation of chiral THβCs. For example, Horiguchi et al. reported the formation of diastereomeric mixtures of THβC (**55a**) and (**55b**) *via* Pictet-Spengler cyclization of the imines (**54**), which was prepared by the condensation of L-tryptophan methyl ester (**52**) and aryl methyl ketones (**53**) in the presence of titanium (IV) isopropoxide as an imitating reagent [49] (Figure 17).

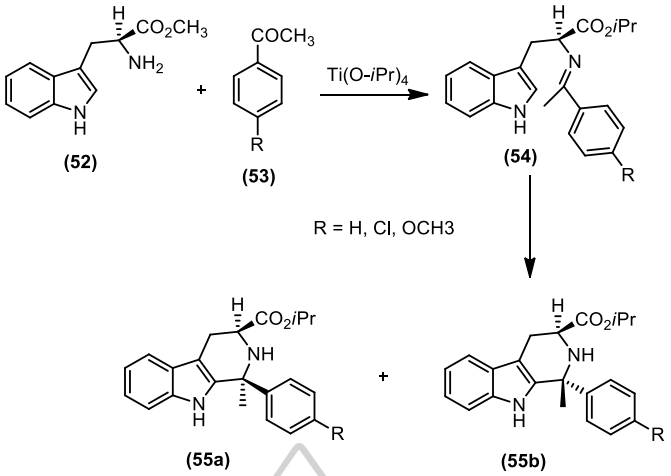


Figure 17. The synthetic route of chiral THβCs (**55a-b**) by Horiguchi et al.

Additionally, many enantiospecific synthesis of 1,3-disubstituted THβCs produced by tryptophan derivatives with aldehydes have long been investigated [50-54]. For example, Cook et al. prepared *trans* 1,3-disubstituted THβCs (**59**) in a stereospecific fashion with *N*-benzyl tryptophan benzylester (**57**) *via* asymmetric Pictet-Spengler reaction [55] (Figure 18). The asymmetric total synthesis of THβC began with monoalkylation of benzyl ester (**57**) with the allylic bromide to provide secondary amine (**56**) using Cs_2CO_3 as base in a mixture of DMF and THF. Next, asymmetric Pictet-Spengler reaction between 4-methoxy-D-tryptophan benzyl ester (**57**) and aldehyde (**58**) provided *trans*-THβCs (**59**) in 90%.

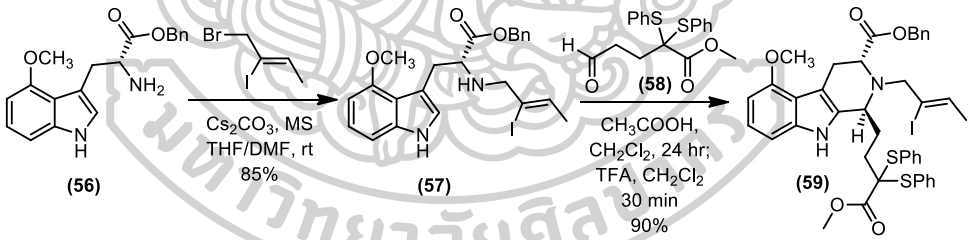


Figure 18. The synthetic route of *trans* 1,3-disubstituted THβCs (**59**) by Cook et al.

Moreover, Cox et al. studied the epimerization of *cis* to *trans* isomer by equilibration experiments in TFA (Figure 19). *Trans*-1-alkyl-2-benzyl-3-(alkoxycarbonyl)-THβCs (***trans*-62**) was synthesized *via* Pictet-Spengler cyclization by heating tryptophan methyl ester (**60**) with steric bulky aldehyde (**61**) under aprotic and acidic conditions to form *cis* to *trans* diastereomers (**62**) [51]. Upon exposure to acid, the diastereomeric ratios shift to provide the stable *trans* N-substituted diastereomers (***trans*-62**) by cleavage of the carbon (C-1)-nitrogen (N-2) bond in the *cis*- to *trans*-isomerization.

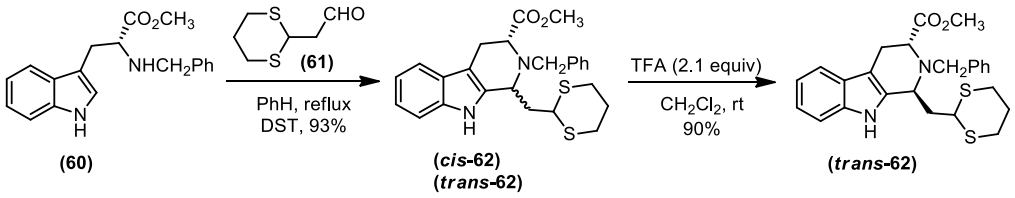


Figure 19. The enantiospecific synthesis of *trans*-1-alkyl-2-benzyl-3-(alkoxycarbonyl)-THβCs (***trans*-62**) by Cox et al.

Furthermore, the asymmetric THβC synthesis have also been developed a new and highly enantioselective Pictet-Spengler reaction by chiral catalysts. Some Brønsted or Lewis acid or thiourea catalysts are frequently used as chiral catalysts. For example, diisopinocampheylchloroborane (Ipc_2BX) is used as a chiral Lewis acid catalyst for enantioselective asymmetric synthesis of THβCs by Nakagawa et al. in 1996 [56] (Figure 20). The enantioselective asymmetric Pictet-Spengler reaction of nitrone (**63**) in the presence of Ipc_2BX provided 2-hydroxy THβC (**64**) up to 90% ee. Hydrogenolysis of 2-hydroxy THβC (**64**) with 10% Pd/C at last stage obtained THβCs (**65**).

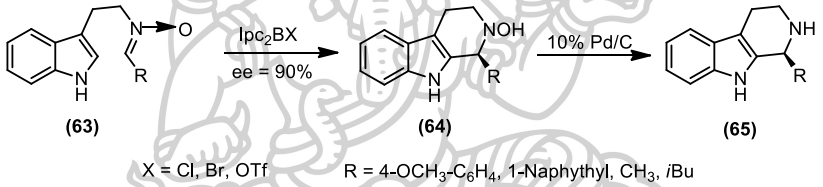


Figure 20. The asymmetric synthesis of chiral THβCs (**65**) by Nakagawa et al.

Another chiral catalyst, pyrrolidine (**68**), was found to be very effective towards an enantioselective one-pot synthesis of THβC (**69**) *via* organocatalytic Michael addition-Pictet-Spengler reaction [57]. Zhao et al. reported the enantioselective synthesis of THβC (**69**) from tryptamine (**15**) and cinnamic aldehyde (**67**) in the presence of pyrrolidine catalyst and benzoic acid in toluene through three-component Michael addition and Pictet-Spengler sequence of beta-ketoesters (**69**) in one-pot synthesis with good to excellent enantioselectivities (Figure 21).

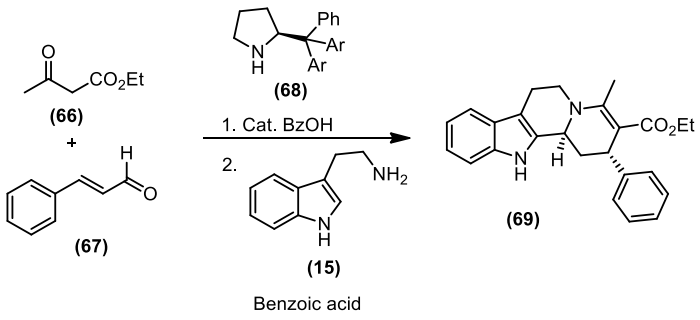


Figure 21. The enantioselective one-pot synthesis of THβC (**69**) by Zhao et al.

Moreover, Jacobsen et al. developed enantioselective Pictet-Spengler reactions by cocatalyzed with a chiral thiourea catalyst and benzoic acid [58]. THβCs (**71**) were obtained in high ee and yield from tryptamine or 6-methoxytryptamine (**70**) and aldehyde precursors (Figure 22).

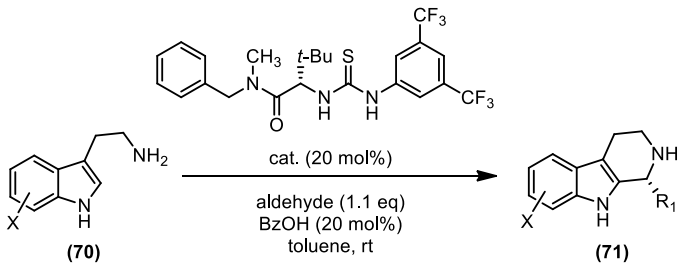


Figure 22. The enantioselective synthesis of THβC (**71**) by Jacobsen et al.

Besides the chiral catalyst, the chiral auxiliaries were also important role to construction of asymmetric THβC through Pictet-Spengler reaction. For instance *p*-tolylsulfinyl chiral auxiliary (**72**), the enantioselective of THβCs (**75**) *via* Pictet-Spengler reaction was reported in 2000 [59] (Figure 23). Tryptamine (**15**) was treated with Andersen reagent [(1*R*,2*S*,5*R*)-(*S*)-menthyl *p*-toluene sulfinate] to form racemic *R*-sulfinamine (**73**) which was cyclized to THβCs (**74**) when treated with various aldehydes in the presence of 10-camphorsulfonic acid (CSA) catalyst in a 1:1 mixture of dry methylene chloride and chloroform at -78 °C. Major diastereomers were obtained after crystallization. Removing of *p*-tolylsulfinyl chiral auxiliary with hydrochloric acid in methanol furnished THβCs (**75**) in high yield.

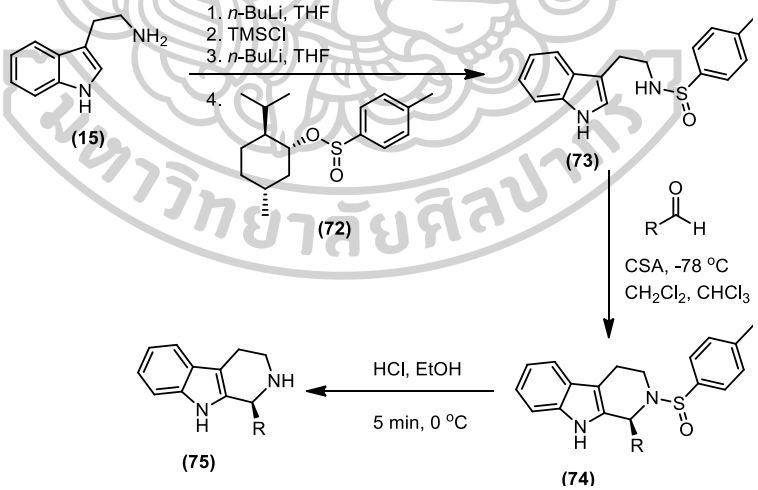


Figure 23. The asymmetric synthesis of chiral THβC (**75**) by Gremmen et al.

An alternative of chiral auxiliary was reported by Zhang et al. [60] (Figure 24). The enantioselective total synthesis of THβC (**79a**) and its C-1 diastereomer (**79b**) was achieved through the asymmetric Pictet-Spengler cyclization. The reaction of tryptamine (**15**) and isobutyraldehyde (**76**) was generate acyl iminium (**77**) which was treated with *L*-*N*,*N*-phthaloyl-

protected amino acid chloride (**78**) as chiral auxiliary to provide diastereomeric mixture of THβCs (**79a-b**) in 1:7 ratio.

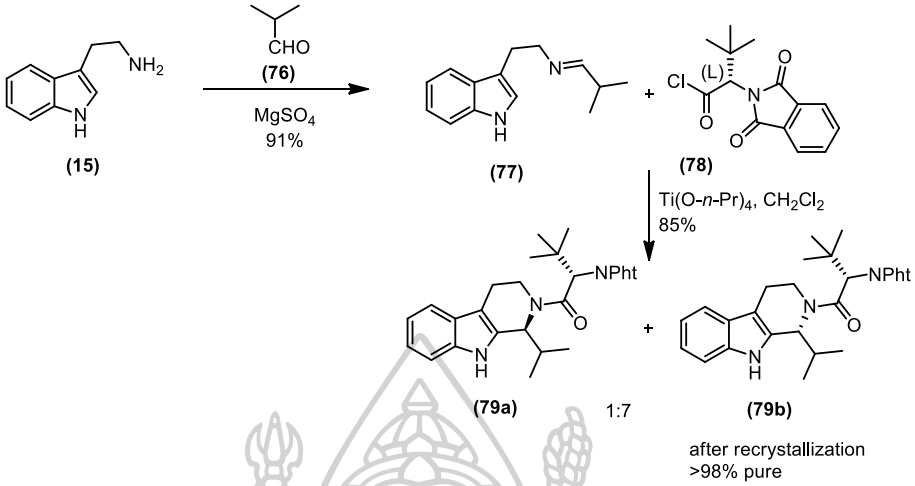


Figure 24. The enantioselective synthesis of THβCs (**79a-b**) by Zhang et al.

The chiral aldehydes are also an essential influence for asymmetric Pictet-Spengler reaction. For example, Thal et al. developed a stereoselective route of THβCs (**81**) using chiral aminoaldehydes (**80**) [61] (Figure 25). The asymmetric Pictet-Spengler reaction were obtained from tryptamine (**15**) and various aminoaldehydes (**80**) to provide highly stereoselective THβCs (**81**) in the presence of trifluoroacetic acid (TFA). A result shown that the use of bulky amino-protecting groups led to the *trans* system under thermodynamic conditions, whereas smaller protecting groups led to the *cis* product under kinetic conditions.

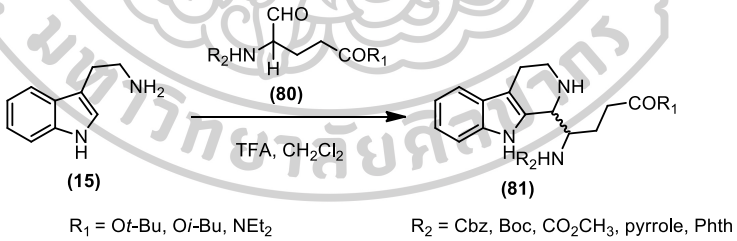


Figure 25. The asymmetric synthesis of THβCs (**81**) by Thal et al.

Previous synthesis of βC derivatives

Moving to the synthesis of βC derivatives, the most of them start from tryptamine or its derivatives to construct THβCs *via* the Pictet-Spengler reaction, followed by aromatization for construction of pyridine ring with an oxidizing agent or dehydrogenation catalyst. For example, βC derivatives (**88**) were synthesized and evaluated their inhibitory activity against both HIV-1 and HIV-2 strains by Murugesan et al. in 2015 [62] (Figure 26). Esterification of DL-Tryptophan (**82**) with thionyl chloride in ethanol was furnished tryptophan ethyl ester (**83**). Next, Pictet-Spengler reaction of the resulting ester (**83**) in the presence of trifluoroacetic acid (TFA) provided tricyclic ethyl 2,3,4,9-tetrahydro-1-phenyl-1*H*-pyrido[3,4-*b*]indole-3-

carboxylate (**84**) which was followed by oxidation with potassium permanganate to give ethyl 1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**85**). Next, 9-*N* methylation with methyl iodide in the presence of potassium hydroxide obtained ethyl 9-methyl-1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**86**) which was converted to 9-methyl-1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**87**) by alkaline ester hydrolysis. Finally, carboxylic acid (**87**) was treated with appropriate amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and hydroxybenzotriazole (HOBt) to give the desired amides (**88**) in excellent yields. Moreover, the most of synthesized βCs showed *in-vitro* anti-HIV activity against both HIV-1 (IIIB) and HIV-2 (ROD) in moderate concentration.

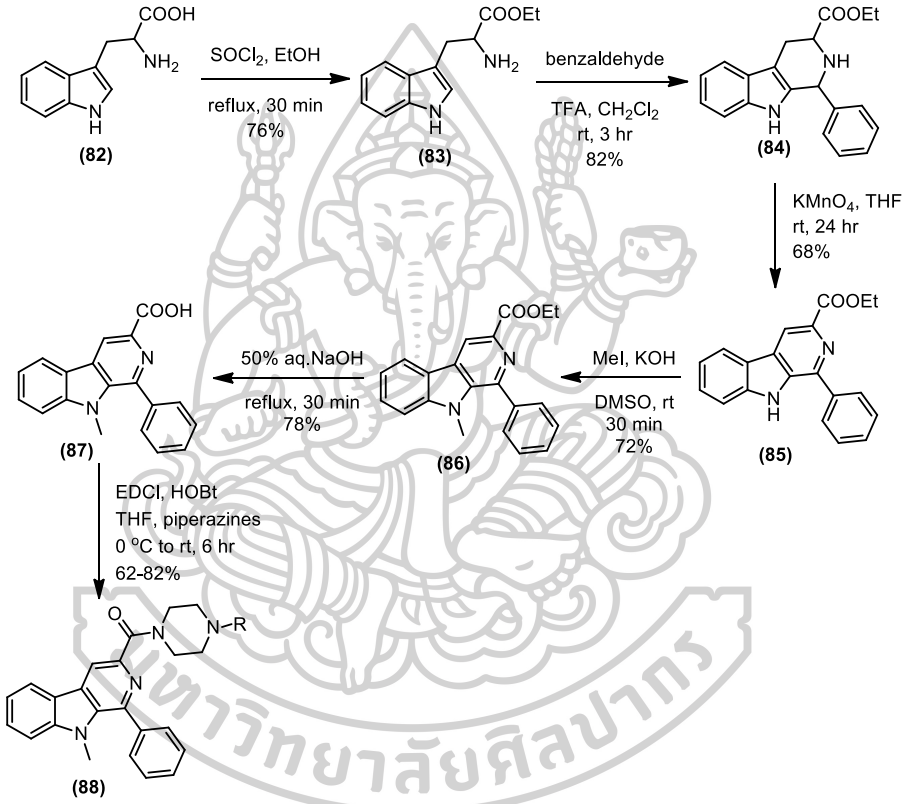


Figure 26. The synthesis route of βCs (**88**) by Murugesan et al.

In 2018, Lokhande et al. developed the temperature controlled chemoselective dehydrogenation and aromatization of βCs in the presence of I_2 and H_2O_2 in DMSO [63] (Figure 27). Consequently, THβC (**89**) was treated with 10 mol% molecular iodine at room temperature in DMSO to provide undesired 3,4-dihydro-β-carboline (**90a**) with 50% yield (entry 1). Increase of molecular iodine to 25 mol% slightly improved the yield (entry 2). Addition of 100 mol% of H_2O_2 at room temperature resulted in 60% yield of 3,4-dihydro-b-carbolines (**90a**) (entry 3-4). Interestingly, the increase of temperature to 80 °C resulted yields of the dehydrogenated product (**90b**) (entries 6-9). This study showed that the controlled of oxidative dehydrogenation and aromatization reaction is temperature dependent (Table 3).

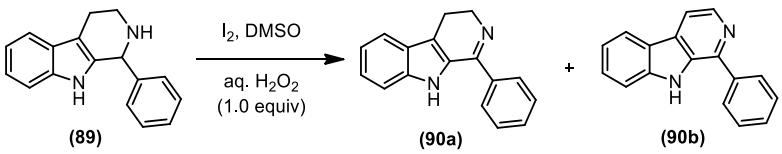


Figure 27. The synthesis route of βCs (**90b**) by Lokhande et al.

Table 3. Optimization of oxidative dehydrogenation and aromatization reaction

Entry	I_2 (mol%)	Oxidant (100 mol%)	Temp (°C)	Time (hr)	Yield % (90a)	Yield % (90b)
1	10	-	RT	4	50	-
2	25	-	RT	4	55	-
3	10	H_2O_2	RT	3	60	-
4	25	H_2O_2	RT	3	60	-
5	25	H_2O_2	80	2	87	Trace
6	25	H_2O_2	100	10	-	88
7	50	H_2O_2	100	8	-	89
8	75	H_2O_2	100	8	-	90
9	100	H_2O_2	100	8	-	90

Recently, the 1-substituted βCs (**94a-c**) which were prepared from tryptamine derivatives (**91a-c**) and glyoxylic acid monohydrate *via* Pictet-Spengler reaction in excellent yields under mild conditions [64] (Figure 28). Then, esterification of the resulting THβCs (**92a-c**) with thionyl chloride in methanol at low temperature (-25°C to rt) for 24 hr gave methyl esters (**93a-c**). After that, the resulted compounds (**93a-c**) were reacted with sulfur in xylene at reflux for 5 hr for aromatization of the C-ring to furnish the desired 1-substituted βCs (**94a-c**) in excellent yields (71–100%).

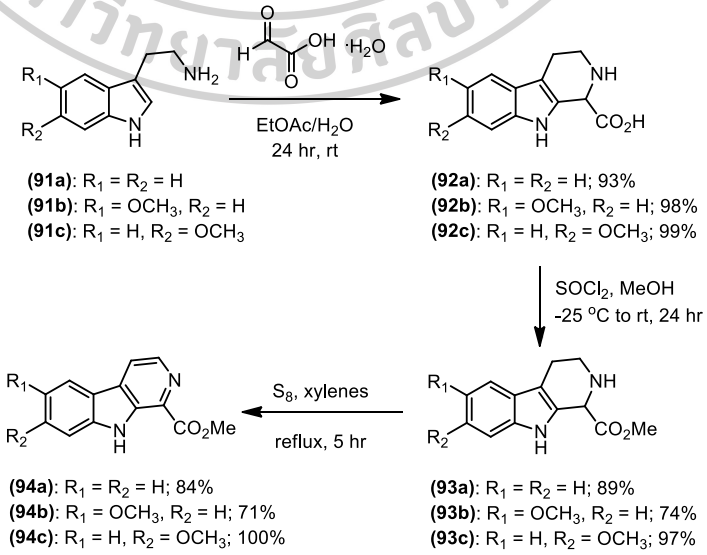


Figure 28. The synthesis route of βCs (**94a-c**) by Milen et al.

Moreover, 1-substituted βCs (**98**) were synthesized through *aza*-alkylation/Michael addition cascade reaction and aromatization as key steps by Bracher et al. in 2020 [65] (Figure 29). In first step, diketoindoles (**97**) were synthesized from enones (**95a-c**) and aliphatic or aromatic α-bromoketones (**96**) in the presence of trimethylamine (NEt_3) *via N*-alkylation subsequented by treatment with DBU under intramolecular Michael addition. Diketoindoles (**97**) were converted into the hydroxycarbazoles (**98**) in 41-69% yield at the last stage by treatment with ammonium acetate in glacial acetic acid for 12 h *via* aromatization.

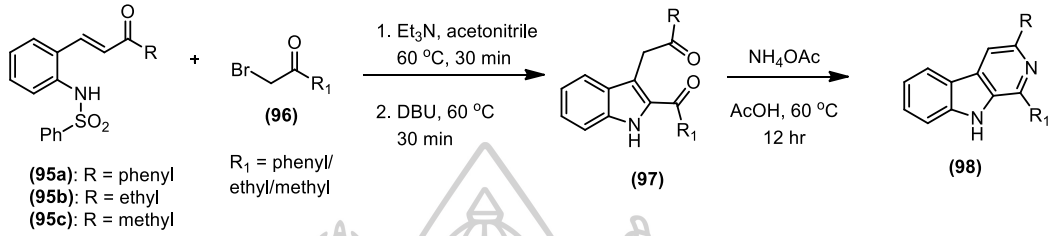


Figure 29. The synthesis route of βCs (**98**) by Bracher et al.

Over the same year, Melo et al. synthesized the novel βCs (**105a-c**) and evaluated *in vitro* anticancer activity [66] (Figure 30). Tryptamine (**102**) was prepared from nitrosoalkenes (**99**) and indole (**100**) *via* hetero-Diels-Alder reaction, followed by 1,2-oxazine ring opening of the resulting cycloadduct (**101**) using Zn/AcOH to furnish oxime (**100**). Then, Tryptamine (**102**) was converted to THβC derivatives (**104a-c**) *via* the Pictet-Spengler reaction. These derivatives were oxidized with sulfur in xylene at reflux for 24 h to furnish βC derivatives (**105a-c**) with good yields. Among these, βC (**105b**), bearing 4-methoxy at the C-1, displayed good anticancer activity against breast adenocarcinoma (MCF-7), lung carcinoma (NCIeH460) and ovarian carcinoma (OVCAR-3) with GI_{50} between 1.32 and 1.62 mM.

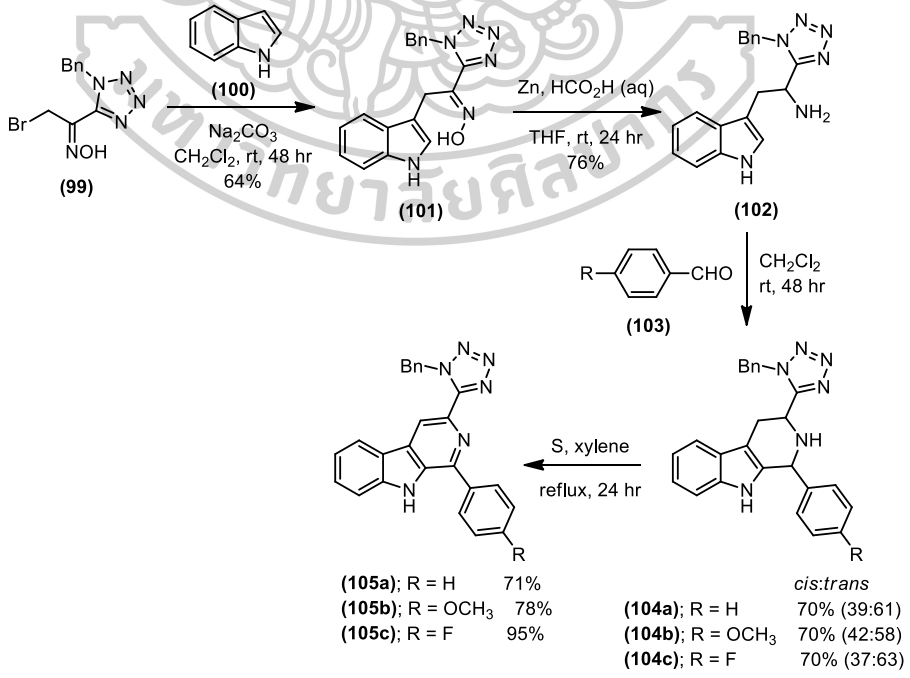


Figure 30. The synthesis route of βCs (**105a-c**) by Melo et al.

Interestingly, gram-scale of βC (**108**) was prepared from tryptophan methyl ester hydrochloride (**106**) and benzaldehyde (**107**) under a mild and efficient *n*Bu_4NBr-mediated oxidative cycloaromatization [67] (Figure 31). Wang et al. developed the synthesis of βC using various catalysts and hydroperoxides as oxidants (Table 4). According to the result of optimization of oxidative dehydrogenation and aromatization reaction conditions, it was noticeable that the combination of *n*Bu_4NBr with cumene hydroperoxide (CHP) promoted the cycloaromatization to provide βC (**108**) in 91% yield (entry 4).

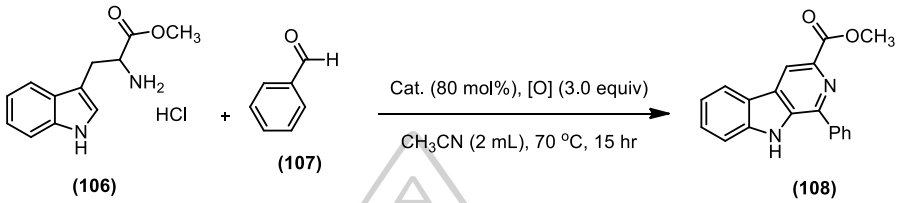


Figure 31. The synthesis route of βCs (**108**) by Wang et al.

Table 4. Screening of the reaction conditions[a].

Entry	Cat.	[O]	Yield (%) [b]
1	I_2	THBP	trace
2	*n*Bu_4NI	THBP	52
3	*n*Bu_4NBr	THBP	60
4	*n*Bu_4NBr	CHP	91
6	Et_4NBr	CHP	42
7	NBS	CHP	35
10	$ZnCl_2$	CHP	17
12	PhCOOH	CHP	25
13	TFA	CHP	46

[a] Reaction conditions: **106** (0.25 mmol), **107** (1.5 eq., 0.38 mmol), catalyst (80 mol%), oxidant (3.0 eq.), under air.

[b] isolated yields.

2.2 Previous studies of the cannabinoids for medical purposes

Natural products and traditional medicines played an important role of a source of therapeutic agents and diversity of their structures [18, 21, 22, 25, 27, 33]. Extensive studies have indicated that the plants contain plentiful of active compounds to possess strong antitumor [14, 19, 27], antioxidant [25], antimicrobial [31], and herbicidal properties [22]. Therefore, the exploration of their natural bioactive molecules has become essential for additional values of natural sources.

The *Cannabis indica* (marijuana) plant was placed in the Middle East, Asia and South East Asia [68] which belongs to the Cannabaceae family. The plant has been a source of fiber,

oil, food and medical applications [69, 70] with a long history. The type of Cannabis and quantity of the constituents is associated with their geographic origins, cultivation, storage and climatic conditions [71]. Moreover, exclusively bioactive compounds in this plant are cannabinoids [32, 72]. Cannabinoids are terpenophenolic compounds which are produced by glandular trichomes on aerial surfaces of the plant [73]. The most important cannabinoids are cannabidiol (CBD), cannabidiol acid (CBDA), cannabinol (CBN), and Δ^9-tetrahydrocannabinol (Δ^9-THC) but Δ^9-THC, a major constituent of marijuana, shows hallucinogenic property [74] (Figure 32). However, the *Cannabis indica* extracts were commonly used in Western medicine, while Europe usually considered that the common hemp was unsuitable for medicinal use [75].

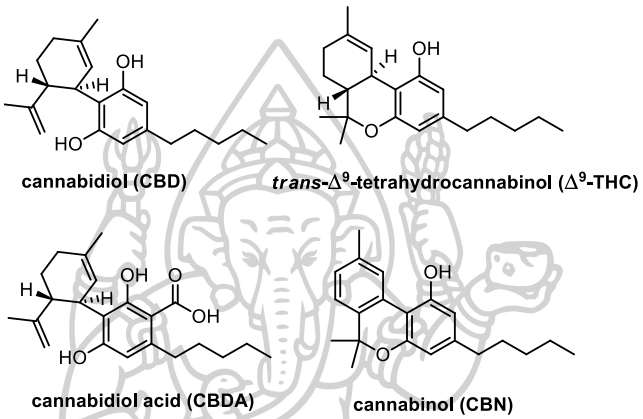


Figure 32. The most important cannabinoids.

Among these cannabinoids, numerous researchers have been attempted to investigation of the potential use in medical applications and natural constituent in marijuana. For example, CBD, a nonpsychoactive cannabinoid, was one of the most important cannabis compounds and was introduced as a potent inhibitor of cytochrome P450 3A11 metabolism [76], anticonvulsant [77] including antibacterial activity [78]. Among other pharmacological effects of cannabinoids have been shown an effective option for pain relief in patients of AIDS [79], antinociceptive [80], anticancer [81], anti-inflammatory [82], treatment of Chemotherapy-induced nausea and vomiting (CINV) [83] as well as neuroprotective antioxidants [84].

In addition, several analytical methods have been applied for the characterization and determination of cannabinoids with various detection techniques. Chromatography is also suitable for both qualitative and quantitative determination of cannabinoids within raw plants. For example, CBD, THC, and CBN were isolated by thin-layer chromatography with solvent system as benzene-*n*-hexane-diethylamine (25:10:1) in which *Rf*-values of 0.45, 0.35, and 0.25, respectively [85]. Moreover, Tewari et al. presented the separation and identification of *Cannabis Indica Linn*. components by thin-layer chromatography (TLC) and its application in forensic Analysis [86]. The main constituents, cannabinol (CBN), cannabidiol (CBD), tetrahydrocannabinol (THC-three isomers) and cannabidiolic acid (CBDA), were confirmed by R_f values and the colours under ultraviolet light (254 nm) and Fast-Blue-B chromogenic reagent for the location of the separated spots on alumina plates developed by benzene-chloroform (1:1 v/v). By the way, Fischedick et al. developed a rapid high performance thin-layer chromatography (HPTLC) method for the qualitative screening of the major neutral cannabinoids in cannabis [87]. Next example, Gambelunghe et al. presented to test CBN, Δ^9-

THC, and CBD in a sweat analysis which emerged as suitable for monitoring chronic cannabis intake using gas chromatography-mass (GC/MS) spectrometry [88].

Moreover, cultivated cannabis plants were studies to determine major and minor cannabinoids by an ultrahigh-performance liquid chromatographic method coupled with photodiode array and single quadruple mass spectrometry detectors (UHPLC-UV-SQD) [89]. In this technique, the standard cannabinoids (CBDA, CBGA, CBG, CBD, THCV, CBN, Δ^9-THC, Δ8-THC, CBL, CBC, and THCAA) were separated with gradient formed from acetonitrile (with 0.05% formic acid) and water (with 0.05% formic acid) and the recovery was in the range of 97–105%. Another quantitative determination of cannabinoids, Weinmann et al. reported the characterization of 110 cannabis samples seized by the Swiss police by HPLC-DAD method [90]. This method presented to be sensitive, accurate, and selective to determine of THC, THCA, CBD, CBDA, and CBN from forensic chemistry laboratories.

Furthermore, McRae et al. developed a quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method which was sensitive, simple, reliable, selective, and accurate for the analysis of both major and minor cannabinoids in cannabis and hemp over a range of 0.002 to 200 mg/g (0.0002 to 20%) [91].

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Laboratory equipments

3.1.1 Nuclear Magnetic Resonance 300 MHz: Bruker 300
3.1.2 Stuart SMP2 melting point apparatus
3.1.3 UV-visible spectrometer: ELISA Microplate Reader
3.1.4 Mass spectrometer
3.1.5 Rotary evaporator: Buchi Rotavapor R-114
3.1.6 Vacuum pump: Tokyo Rikakikai Co., Ltd. model A-3S
3.1.7 Analytical balance: Mettler Toledo model AB204
3.1.8 Hotplate and stirrer: Heidolph MR 3001
3.1.9 Ultrasonic Bath: Elmasonic S 30 H
3.1.10 Micropipette: Finnpipette, HH10711 ขนาด 1-10 µL, 20-200 µL และ 100-1000 µL
3.1.11 Preparative TLC: Desaga Brinkmann
3.1.12 TLC Silica gel 60 F_{254} aluminium sheet, Merck
3.1.13 Column
3.1.14 Filter paper: Advantec dia. 110 mm and 70 mm
3.1.15 Laboratory glassware
3.1.16 Vacuum filtration buchner set
3.1.17 Parafilm
3.1.18 Syringes
3.1.19 Magnetic bars
3.1.20 Clamp and Clamp Holder
3.1.21 CO_2 Incubator
3.1.22 Laminar Flow Cabinet
3.1.23 Automatic Autoclave
3.1.24 96-well microplate
3.1.25 Petri dish
3.1.26 Sealed tube
3.1.27 Digital polarimeters: Kruss P3000 series

3.2 Chemicals

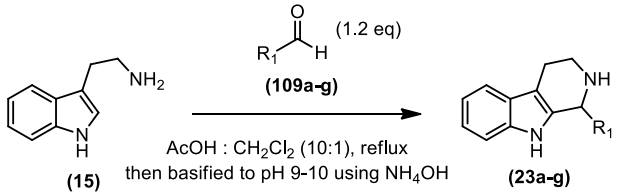
3.2.1 Acetone (AR) (BDH Analar)
3.2.2 Ammonium solution (Carlo erba)
3.2.3 Argon gas (Masser Specialty Gas Co., Ltd.)
3.2.4 Benzaldehyde (Sigma-aldrich)
3.2.5 Benzene (AR) (Merck)
3.2.6 (*S*)-2-[[3,5-Bis(trifluoromethyl)phenyl]thioureido]-*N*-benzyl-*N*,3,3-trimethylbutanamide (Sigma-Aldrich)
3.2.7 Benzoic acid
3.2.8 3-bromobenzaldehyde (Acros Organics)
3.2.9 Di-*tert*-butyl decarbonate (Sigma-aldrich)
3.2.10 Celite (Fluka)
3.2.11 Chloroform-d contains 1% v/v of TMS (Sigma-Aldrich)
3.2.12 Cinnamaldehyde (Fluka)
3.2.13 3-(3,5-Dimethylthiazol-2,5-diphenyltetra-zolium bromide) (MTT)
3.2.14 2,5-Dimethoxybenzaldehyde (Fluka)
3.2.15 Dichloromethane (distillation)

3.2.16 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (TCI)
3.2.17 Dimethyl sulfoxide (BDH VWR Analytical)
3.2.18 Dulbecco's modified eagle medium (DMEM)
3.2.19 *N*,*N*-Dimethylformamide (Merck)
3.2.20 di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) (Sigma-aldrich)
3.2.21 Ethanol (Carlo Erba)
3.2.22 Ethylacetate (distillation)
3.2.23 10% Fetal bovine serum
3.2.24 2-Furaldehyde (Fluka)
3.2.25 *para*-formaldehyde
3.2.26 Furfuraldehyde
3.2.27 Glacial acetic acid (Lab scan)
3.2.28 *D*-(+)-glucose (Riedel-de Haen)
3.2.29 Hexane (distillation)
3.2.30 Hydrochloric acid (Lab-scan)
3.2.31 Hydrogen gas (Masser Specialty Gas Co., Ltd.)
3.2.32 3-Hydroxybenzaldehyde (TCI)
3.2.33 *L*-Leucine (TCI)
3.2.34 3-Methoxybenzaldehyde (Fluka)
3.2.35 2-Methoxybenzaldehyde (Fluka)
3.2.36 4-Methoxybenzaldehyde (Merck)
3.2.37 Methanol (distillation)
3.2.38 Methanol-d (Sigma-Aldrich)
3.2.39 *N*-Methyl-2-pyrrolidone (Fluka)
3.2.40 Molecular sieve 3 Å
3.2.41 Molecular sieve 4 Å
3.2.42 3-Nitrobenzaldehyde (TCI)
3.2.43 Oxalyl chloride (Fluka)
3.2.44 10% Palladium on carbon (BDH Analar)
3.2.45 *L*-Phenylalanine
3.2.46 Phthalic anhydride
3.2.47 Potassium carbonate (Riedel-de Haen)
3.2.48 *L*-Proline
3.2.49 Pyridine (Lab scan)
3.2.50 Silica gel 60 (0.063-0.200 mm) for column chromatography (Merck)
3.2.51 Silica gel 60 F254 containing gypsum for preparative thin layer chromatography (Merck)
3.2.52 Sodium bicarbonate (Riedel-de Haen)
3.2.53 Sodium sulfate anhydrous (Sigma-Aldrich)
3.2.54 Sulfuric acid (Carlo-erba)
3.2.55 Sulfur powder
3.2.56 Tetrahydrofuran (A.R.) (Lab-scan)
3.2.57 Thionyl chloride
3.2.58 Titanium isopropoxide (Fluka)
3.2.59 Toluene
3.2.60 Triethylamine (Fluka)
3.2.61 Trolox (Sigma-aldrich)
3.2.62 Tryptamine (Aldrich)
3.2.63 Tryptophane (Sigma-aldrich)
3.2.64 Xylene (Carlo Erba)
3.2.65 *p*-Xylene (BDH Analar)

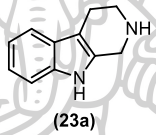
3.3 Research methodology

3.3.1. Synthesis of THβC derivatives

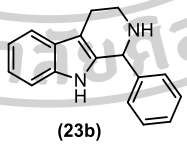
Procedure for the synthesis of 1-substituted THßCs (23a-g)



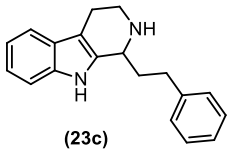
Tryptamine (**15**) was dissolved in AcOH: dry CH_2Cl_2 (5:10 mL) in a round-bottom flask. Then, aldehyde (1.2 eq) was slowly added to the solution, and the solution was refluxed for 1-2 h. After completion, the reaction mixture was cooled to room temperature and basified to pH 9-10 using NH_4OH. Afterwards, the solution was extracted with CH_2Cl_2, and the organic layer was dried over anh. Na_2SO_4 and evaporated under reduced pressure. The crude product was purified using column chromatography (silica gel, 15% MeOH:CH_2Cl_2).


(23a)

2,3,4,9-Tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23a**). The title compound was synthesized from (**15**) (0.2681 g, 1.7 mmol) and *p*-formaldehyde (0.060 g, 2.0 mmol) to afford (**23a**) (0.1527 g, 53%) as a yellow solid. m.p. 185.9-187.5°C (lit. 109-221°C) [92, 93]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.86 (br s, 1H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.14 (dt, *J* = 7.1, 1.3 Hz, 1H), 7.09 (dt, *J* = 7.1, 1.1 Hz, 1H), 4.01 (s, 2H), 3.18 (t, *J* = 5.7 Hz, 2H), 2.75 (t, *J* = 5.7 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) [94] δ 135.6 (C), 132.7 (C), 127.6 (C), 121.5 (CH), 119.4 (CH), 117.9 (CH), 110.7 (CH), 108.7 (C), 43.9 (CH_2), 43.2 (CH_2), 22.5 (CH_2).

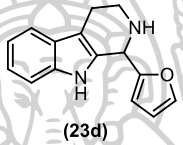

(23b)

1-Phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23b**). The title compound was synthesized from (**15**) (0.3210 g, 2.003 mmol) and benzaldehyde (0.24 mL, 2.4 mmol) to afford (**23b**) (0.1244 g, 25%) as a yellow solid. m.p. 162.9-164.7°C (lit. 160-161°C) [95]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.56-7.52 (m, 1H), 7.37-7.27 (m, 5H), 7.23-7.17 (m, 1H), 7.14 (dt, *J* = 6.9, 1.9 Hz, 1H), 7.10 (dt, *J* = 6.9, 1.9 Hz, 1H), 5.17 (s, 1H), 3.37 (dt, *J* = 12.5, 3.9 Hz, 1H), 3.18-3.07 (m, 1H), 3.00-2.78 (m, 1H), 1.97 (br s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 141.7 (C), 135.9 (C), 134.4 (C), 128.8 (2CH), 128.5 (2CH), 128.2 (CH), 127.4 (C), 121.8(CH), 119.4 (CH), 118.3 (CH), 110.8 (CH), 110.2 (C), 58.1 (CH), 42.9 (CH_2), 22.5 (CH_2).



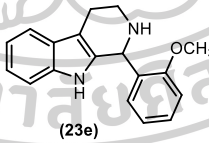
(23c)

1-Phenethyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23c**). The title compound was synthesized from (**15**) (0.4889 g, 3.052 mmol) and 3-phenylpropanal (0.4913 g, 3.662 mmol) to afford (**23c**) (0.3964 g, 47% yield) as an orange oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.74 (br s, NH), 7.47 (d, *J* = 7.0 Hz, 1H), 7.33-7.16 (m, 6H), 7.12 (dt, *J* = 7.0, 1.3 Hz, 1H), 7.07 (dt, *J* = 7.1, 1.3 Hz, 1H), 4.06 (d, *J* = 5.2 Hz, 1H), 3.33 (td, *J* = 12.8, 4.7 Hz, 1H), 3.08-2.96 (m, 1H), 2.92-2.76 (m, 2H), 2.76-2.68 (m, 2H), 2.20-2.05 (m, 1H), 2.04-1.89 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 141.9 (C), 135.9 (C), 135.6 (C), 128.5 (2CH), 128.4 (2CH), 127.5 (C), 126.0 (CH), 121.5 (CH), 119.0 (CH), 118.0 (CH), 110.7 (CH), 109.1 (C), 52.2 (CH), 42.4 (CH_2), 36.7 (CH_2), 29.7 (CH_2), 22.7 (CH_2); HRESI-MS: calcd for $C_{19}H_{20}N_2$ $[M+H]^+$: 277.1700, found: 277.1706.



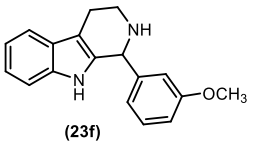
(23d)

1-(furan-2-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23d**). The title compound was synthesized from (**15**) (0.2560 g, 1.598 mmol) and furfuraldehyde (0.16 mL, 1.158 mmol) to afford (**23d**) (0.3209 g, 84% yield) as an orange oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.53 (d, *J* = 7.5 Hz, 1H), 7.42 (dd, *J* = 1.8, 0.8 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.20-7.07 (m, 2H), 6.34 (dd, *J* = 3.1, 1.9 Hz, 1H), 6.21 (d, *J* = 3.1 Hz, 1H), 5.31 (s, 1H), 3.40-3.27 (m,1H), 3.20-3.10 (m, 1H), 2.87-2.79 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 154.5 (C), 142.5 (C), 135.8 (C), 131.9 (C), 127.3 (C), 121.9 (CH), 119.5 (CH), 118.3 (C), 110.9 (CH), 110.3 (CH), 110.1 (C), 107.4 (CH), 50.9 (CH), 41.8 (CH_2), 22.7 (CH_2).



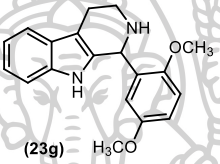
(23e)

1-(2-Methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23e**). The title compound was synthesized from (**15**) (0.1984 g, 1.238 mmol) and 2-methoxybenzaldehyde (0.2023 g, 1.486 mmol) to afford (**23e**) (0.1863 g, 55 % yield) as an orange solid. m.p. 97.8-99.3°C (lit. 95-96°C) [96]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.94 (s, NH), 7.50 (d, *J* = 3.7 Hz, 1H), 7.26 (dd, *J* = 15.6, 1.5 Hz, 1H), 7.18-7.06 (m, 3H), 6.98 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 6.83 (t, *J* = 7.4 Hz, 1H), 5.57 (s, 1H), 3.85 (s, 3H), 3.28-3.16 (m, 1H), 3.11-3.00 (m, 1H), 2.81 (d, *J* = 4.9 Hz, 2H), 2.28 (br s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 157.2 (C), 135.8 (C), 134.1 (C), 129.7 (C), 129.2 (CH), 129.0 (CH), 127.3 (C), 121.4 (CH), 120.6 (CH), 119.2 (CH), 118.0 (CH), 110.8 (CH), 110.7 (CH), 110.2 (C), 55.5 (CH), 50.9 (CH_3), 41.7 (CH_2), 22.4 (CH_2).



(23f)

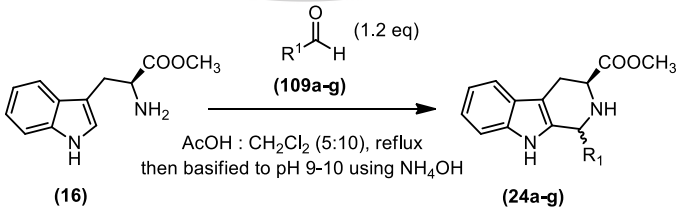
1-(3-Methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23f**). The title compound was synthesized from (**15**) (0.3034 g, 1.894 mmol) and 3-methoxybenzaldehyde (0.20 mL, 2.3 mmol) to afford (**23f**) (0.3848 g, 73% yield) as an orange solid. m.p. 150.8-153.8°C (lit. 154-156°C) [97]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.80 (br s, NH), 7.53 (dd, *J* = 7.0, 2.0 Hz, 1H), 7.29-7.23 (m, 1H), 7.22 (d, *J* = 2.0 Hz, 1H), 7.16 (dt, *J* = 7.0, 1.5 Hz, 1H), 7.11 (dt, *J* = 7.0, 1.5 Hz, 1H), 6.91-6.84 (d, *J* = 3.9 Hz, 3H), 5.32 (s, 1H), 3.75 (s, 3H), 3.43-3.32 (m, 1H), 3.22-3.10 (m, 1H), 3.04-2.82 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 160.1 (C), 140.9 (C), 136.1 (C), 132.3 (C), 129.9 (CH), 127.0 (C), 122.1 (CH), 121.0 (CH), 119.6 (CH), 118.3 (CH), 114.2 (CH), 111.5 (CH), 111.0 (CH), 109.8 (C), 57.2 (CH), 55.3 (CH_3), 41.7 (CH_2), 21.3 (CH_2); HRESI-MS: calcd for $C_{18}H_{18}N_{2O}$ $[M+H]^+$: 279.1492, found: 279.1486.



(23g)

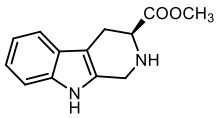
1-(2,5-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23g**). The title compound was synthesized from (**15**) (0.4942 g, 3.085 mmol) and 2,5-dimethoxybenzaldehyde (0.6150 g, 3.701 mmol) to afford (**23g**) (0.08561 g, 9% yield) as a yellow solid. m.p. 132.2-133.6°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83 (br s, NH), 7.52 (d, *J* = 7.0 Hz, 1H), 7.24-7.22 (m, 1H), 7.14 (dt, *J* = 7.0, 1.4 Hz, 1H), 7.10 (dt, *J* = 7.1, 1.4 Hz, 1H), 6.93-6.80 (m, 2H), 6.68 (d, *J* = 2.9 Hz, 1H), 5.65 (s, 1H), 3.84 (s, 3H), 3.67 (s, 3H), 3.39-3.29 (m, 1H), 3.25-3.15 (m, 1H), 2.92-2.87 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 175.9 (C), 153.7(C), 151.5 (C), 136.0 (C), 128.5 (C), 127.0 (C), 121.9 (CH), 119.5 (CH), 118.3 (CH), 116.1 (CH), 113.9 (CH), 111.9 (CH), 110.9 (CH), 109.6 (C), 56.1 (CH_3), 55.8 (CH_3), 51.3 (CH), 41.0 (CH_2), 21.1 (CH_2); HREI-MS: calcd for $C_{19}H_{20}N_2O_2$ $[M]^+$: 308.1525, found: 308.1527.

Procedure for the synthesis of 1,3-disubstituted THßCs (24a-g)



(16) (24a-g)

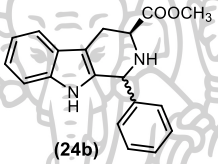
L-tryptophan methyl ester (**16**) was dissolved in AcOH: dry CH_2Cl_2 (5:10 mL) in a round-bottom flask. Then, aldehyde (1.2 eq) was slowly added to the solution, and the solution was refluxed for 1-2 h. After completion, the reaction mixture was cooled to room temperature and basified to pH 9-10 using NH_4OH. Afterwards, the solution was extracted with CH_2Cl_2, and the organic layer was dried over anh. Na_2SO_4 and evaporated under reduced pressure. The crude product was purified using column chromatography (silica gel, 15% MeOH:CH_2Cl_2) to afford *cis*/*trans* mixture of diastereoisomers. Finally, the diastereomeric mixture was again subjected to thin layer chromatography (1% MeOH:CH_2Cl_2) to separate both the isomers.



(24a)

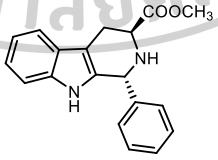
Methyl 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24a**). The title compound was synthesized from (**16**) (0.2710 g, 1.242 mmol) and *para*-formaldehyde (0.0447 g, 1.490 mmol) to afford (**24a**) (0.1973 g, 69% yield) as a yellow solid. m.p. 171.8-173.6°C (lit. 187.2-188.8°C) [98]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.87 (br s, NH), 7.48 (d, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.15 (dt, *J* = 7.0, 1.3 Hz, 1H), 7.10 (dt, *J* = 7.0, 1.3 Hz, 1H), 4.30-4.08 (m, 2H), 3.85-3.81 (m, 1H), 3.79 (s, 3H), 3.14 (tdd, *J* = 15.2, 4.2, 1.5 Hz, 1H), 2.89 (tdd, *J* = 15.2, 4.2, 1.5 Hz, 1H), 2.05 (br s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.7 (C), 135.0 (C), 130.6 (C), 126.0 (C), 120.5 (CH), 118.2 (CH), 116.6 (CH), 109.8 (CH), 105.5 (C), 54.7 (CH), 51.1 (CH_3), 40.7 (CH_2), 24.2 (CH_2).

Methyl 1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24b**)



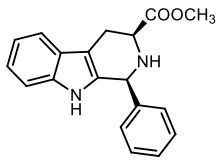
(24b)

Diastereomeric mixture (**24b**). The title compound was synthesized from (**16**) (0.1993 g, 9.133 mmol) and benzaldehyde (0.11 mL, 1.096 mmol) to afford (**24b**) (0.2238 g, 80% yield) as a yellow solid. m.p. 156.0-158.0 °C (lit. 152-153°C) [95]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.80 (br s, NH), 7.60 (br s, NH), 7.52-7.47 (m, 3H), 7.27-7.20 (m, 3H), 7.19-7.11 (m, 3H), 7.10-7.04 (m, 9H), 5.29 (s, 1H), 5.16 (s, 1H), 3.94-3.85 (m, 2H), 3.75 (s, 3H), 3.64 (s, 3H), 3.30-3.15 (m, 2H), 3.12-2.96 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.0 (C), 173.2 (C), 141.9 (C), 140.7 (C), 136.1 (2C), 134.6 (C), 133.1 (C), 128.7 (2CH), 128.6 (4CH), 128.4 (2CH), 128.0 (2CH), 127.0 (C), 126.9 (C), 121.8 (2CH), 119.5 (CH), 119.4 (CH), 118.1 (2CH), 110.9 (2CH), 108.7 (C), 108.3 (C), 58.6 (CH), 56.8 (CH), 54.8 (CH), 52.3 (CH), 52.1 ($2CH_3$), 25.7 (CH_2), 24.7 (CH_2).



(***trans*-24b**)

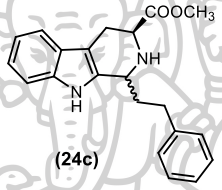
Trans* isomer** (trans*-24b**). Yellow solid (0.0362 g, 17% yield). m.p. 167.8-168.2 °C. $[\alpha]_D^{25}$ = -5.7° (c = 0.0035, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83 (br s, NH), 7.56-7.51 (m, 1H), 7.29 (d, *J* = 1.1 Hz, 1H), 7.27 (d, *J* = 2.3 Hz, 1H), 7.21-7.16 (m, 1H), 7.16-7.08 (m, 2H), 5.31 (s, 1H), 3.90 (dd, *J* = 7.1, 5.4 Hz, 1H), 3.67 (s, 3H), 3.24 (ddd, *J* = 15.5, 5.4, 0.9 Hz, 1H), 3.07 (ddd, *J* = 15.4, 7.2, 1.4 Hz, 1H), 2.37 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1 (C), 141.9 (C), 136.2 (C), 133.1 (C), 128.7 (2CH), 128.4 (2CH), 128.1 (CH), 126.9 (C), 121.9 (CH), 119.5 (CH), 118.2 (CH), 110.9 (CH), 108.4 (C), 54.9 (CH), 52.5 (CH), 52.1 (CH_3), 24.6 (CH_2).



(*cis*-24b)

Cis* isomer** (cis*-24b**). Yellow solid (0.0351 g, 17% yield). m.p. 186.3-188.2 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.54 (br s, NH), 7.53-7.47 (m, 1H), 7.39-7.32 (m, 5H), 7.20-7.06 (m, 3H), 5.22 (s, 1H), 3.96 (dd, *J* = 11.1, 4.3 Hz, 1H), 3.79 (s, 3H), 3.22 (ddd, *J* = 15.1, 4.3, 1.9 Hz, 1H), 3.00 (ddd, *J* = 15.0, 10.9, 2.6 Hz, 1H), 2.29 (br, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) [99] δ 173.2 (C), 140.7 (C), 136.2 (CH), 134.7 (CH), 128.9 (C), 128.8 (C), 127.1 (CH), 127.1 (C), 121.9 (CH), 119.8 (CH), 118.2 (CH), 110.9 (CH), 106.9 (CH), 58.7 (CH), 56.9 (CH), 52.2 (CH_3), 25.7 (CH_2).

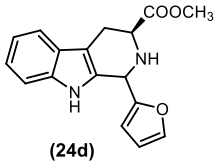
Methyl 1-phenethyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (**24c**).



(24c)

Diastereomeric mixture (**24c**). The title compound was synthesized from (**16**) (0.1412 g, 0.6470 mmol) and 3-phenylpropanal (0.1042 g, 0.7770 mmol) to afford (**24c**) (0.1148 g, 53% yield)as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.50-7.44 (m, 2H), 7.33-7.25 (m, 8H), 7.25-7.20 (m, 4H), 7.15-7.11 (m, 2H), 7.11-7.05 (m, 2H), 4.34 (t, *J* = 6.2 Hz, 1H), 4.30-4.24 (m, 1H), 4.21 (dd, *J* = 6.0, 4.0 Hz, 1H), 4.08-4.02 (m, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.10-3.00 (m, 4H), 2.90-2.70 (m, 4H), 2.40-2.00 (m, 4H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.8 (2C), 167.0 (2C), 144.9 (2C), 141.4 (2C), 141.1 (2C), 140.5 (2C), 128.6 (4CH), 128.5 (2CH), 128.4 (2CH), 126.2 (2CH), 121.9 (2CH), 119.7 (2CH), 118.1 (CH), 118.0 (CH), 111.1 (CH), 111.0 (CH), 56.4 (CH), 52.9 (CH), 52.6 (CH), 52.5 (CH_3), 52.4 (CH_3), 50.7 (CH), 36.5 (CH_2), 36.3 (CH_2), 32.2 (CH_2), 31.6 (CH_2), 25.5 (CH_2), 24.1 (CH_2). HRESI-MS: calcd for $C_{21}H_{22}N_2O_2$ $[M+H]^+$: 335.1754, found: 335.1757.

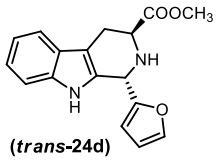
Methyl 1-(furan-2-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24d**).



(24d)

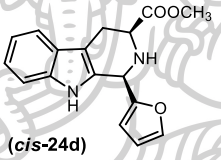
Diastereomeric mixture (**24d**). The title compound was synthesized from (**16**) (0.0817 g, 0.0374 mmol) and furfuraldehyde (0.04 mL, 0.0449 mmol) to afford (**24d**) (0.0650 g, 59% yield) as a black oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.09 (br s, NH), 7.50 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 1.1 Hz, 1H), 7.25 (d, *J* = 7.3 Hz, 1H), 7.16 (td, *J* = 7.1, 1.3 Hz, 1H), 7.10 (td, *J* = 7.1, 1.2 Hz, 1H), 6.27 (dd, *J* = 3.2, 1.9 Hz, 1H), 6.04 (d, *J* = 3.2 Hz, 1H), 5.41 (s, 1H), 3.95

(dd, *J* = 9.2, 1.7 Hz, 1H), 3.74 (s, 3H), 3.18 (dd, *J* = 15.4, 4.8 Hz, 1H), 2.95 (ddd, *J* = 15.4, 9.3, 1.4 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.6 (C), 154.2 (C), 142.7 (CH), 136.2 (C), 130.8 (C), 126.8 (C), 122.2 (CH), 119.5 (CH), 118.3 (CH), 111.1 (CH), 110.2 (CH), 108.2 (C), 107.9 (CH), 52.3 (CH), 52.2 (CH_3), 49.1 (CH), 25.0 (CH_2). HRESI-MS: calcd for $C_{17}H_{16}N_2O_3$ $[M+H]^+$: 297.1234, found: 297.1235.



(*trans*-24d)

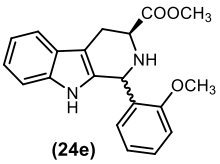
Trans* isomer** (trans*-24d**). Brown oil (0.0457 g, 26% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.23 (br s, NH), 7.49 (d, *J* = 7.2 Hz, 1H), 7.40-7.34 (m, 1H), 7.29-7.19 (m, 1H), 7.18-7.04 (m, 2H), 6.34-6.22 (m, 1H), 5.99 (d, *J* = 3.2 Hz, 1H), 5.34 (s, 1H), 3.91 (dd, *J* = 9.3, 4.7 Hz, 1H), 3.72 (s, 3H), 3.17 (dd, *J* = 15.4, 4.7 Hz, 1H), 2.92 (ddd, *J* = 15.3, 9.4, 0.8 Hz, 1H), 2.57 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.3 (C), 151.9 (C), 140.2 (CH), 133.8 (C), 128.5 (C), 124.3 (C), 119.7 (CH), 117.1 (CH), 115.8 (CH), 108.7 (CH), 107.8 (CH), 106.2 (C), 105.6 (CH), 49.8 (2CH), 46.6 (CH_3), 22.7 (CH_2).



(*cis*-24d)

Cis* isomer** (cis*-24d**). Brown oil (0.0190 g, 11% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.91 (br s, NH), 7.68-7.60 (m, 1H), 7.54-7.48 (m, 1H), 7.46-7.33 (m, 2H), 7.31-7.25 (m, 1H), 7.21-7.08 (m, 2H), 5.41 (s, 1H), 3.93 (dd, *J* = 11.0, 4.4 Hz, 1H), 3.81 (s, 3H), 3.19 (ddd, *J* = 15.3, 4.4, 1.9 Hz, 1H), 2.95 (ddd, *J* = 15.2, 11.0, 2.5 Hz, 1H), 2.32 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 170.6 (C), 150.6 (C), 140.4 (CH), 133.6 (C), 129.4 (C), 124.6 (C), 119.7 (CH), 117.2 (CH), 115.8 (CH), 108.6 (CH), 108.0 (CH), 106.2 (C), 105.2 (CH), 54.1 (CH), 49.9 (CH), 49.2 (CH_3), 23.0 (CH_2).

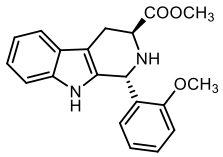
Methyl 1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24e**).



(24e)

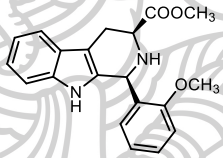
Diastereomeric mixture (**24e**). The title compound was synthesized from (**16**) (0.2652 g, 1.215 mmol) and 2-methoxybenzaldehyde (0.1654 g, 1.458 mmol) to afford (**24e**) (0.3594 g, 88% yield) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.11 (br s, NH), 7.75 (br s, NH), 7.55-7.47 (m, 2H), 7.34-7.27 (m, 2H), 7.26-7.20 (m, 2H), 7.19-7.15 (m, 1H), 7.14-7.06 (m, 4H), 6.94 (d, *J* = 6.9 Hz, 1H), 6.92-6.86 (m, 2H), 6.82-6.74 (m, 2H), 5.78 (s, 1H), 5.68 (s, 1H), 3.94 (dd, *J* = 10.9, 4.2 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.81 (t, *J* = 4.4 Hz, 1H), 3.77 (s, 3H), 3.69 (s, 3H), 3.26-3.15 (m, 2H), 3.07-2.20 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.8 (C),

173.4 (C), 157.3 (C), 156.9 (C), 136.2 (C), 135.9 (C), 134.9 (C), 132.7 (C), 129.5 (C), 129.3 (CH), 129.2 (CH), 129.1 (2CH), 128.9 (C), 127.1 (C), 126.9 (C), 121.8 (CH), 121.6 (CH), 121.3 (CH), 120.3 (CH), 119.4 (CH), 119.3 (CH), 118.1 (CH), 117.9 (CH), 111.0 (CH), 110.9 (CH), 110.8 (CH), 110.6 (CH), 108.9 (C), 108.4 (C), 56.9 (CH), 55.7 (CH_3), 55.5 (CH_3), 52.1 ($2CH_3$), 52.0 (CH), 51.8 (CH), 49.3 (CH), 25.7 (CH_2), 24.9 (CH_2).



(***trans*-24e**)

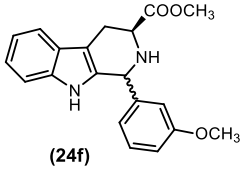
Trans* isomer** (trans*-24e**). Orange solid (0.0243 g, 9% yield). m.p. 153.6-155.3 °C. $[\alpha]_D^{25}$ = -34.6° (c = 0.0035, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.91 (br s, NH), 7.54 (dd, *J* = 6.7, 1.6 Hz, 1H), 7.29-7.22 (m, 2H), 7.19-7.08 (m, 2H), 6.95 (d, *J* = 8.3 Hz, 1H), 6.86-6.77 (m, 2H), 5.80 (s, 1H), 3.94 (s, 3H), 3.84 (dd, *J* = 9.0, 4.7 Hz, 1H), 3.72 (s, 3H), 3.23 (dd, *J* = 15.1, 4.7 Hz, 1H), 3.01 (ddd, *J* = 15.1, 8.9, 1.3 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.9 (C), 156.9 (C), 136.3 (C), 136.2 (C), 132.9 (C), 129.9 (CH), 128.9 (CH), 126.9 (C), 121.7 (CH), 120.3 (CH), 119.3 (CH), 118.1 (CH), 110.9 (CH), 110.6 (CH), 109.1 (C), 55.5 (CH), 52.1 (CH_3), 52.0 (CH_3), 49.2 (CH), 25.2 (CH_2). HRESI-MS: calcd for $C_{20}H_{20}N_2O_3$ $[M+H]^+$: 337.1547, found: 337.1547.



(***cis*-24e**)

Cis* isomer** (cis*-24e**). Yellow solid (0.0351 g, 14% yield). m.p. 83.5-84.9 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.73 (br s, NH), 7.54-7.47 (m, 1H), 7.36-7.26 (m, 2H), 7.22-7.17 (m, 1H), 7.16-7.05 (m, 2H), 6.96 (d, *J* = 7.8 Hz, 1H), 6.91 (dd, *J* = 7.2, 0.9 Hz, 1H), 5.70 (s, 1H), 3.97 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.88 (s, 3H), 3.79 (s, 3H), 3.21 (dd, *J* = 15.0, 4.2, 1.8 Hz, 1H), 2.99 (ddd, *J* = 15.0, 11.1, 2.4 Hz, 1H), 2.43 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.1 (C), 156.9 (C), 135.6 (C), 134.6 (C), 128.9 (CH), 128.8 (CH), 128.6 (C), 126.8 (C), 121.3 (CH), 120.9 (CH), 119.1 (CH), 117.7 (CH), 110.6 (CH), 110.5 (CH), 108.1 (C), 56.6 (CH), 55.4 (CH_3), 51.9 (CH_3), 51.4 (CH), 25.4 (CH_2).

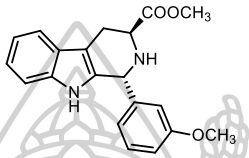
Methyl 1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24f**).



(**24f**)

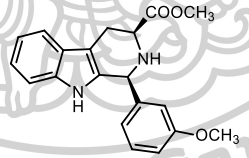
Diastereomeric mixture (**24f**). The title compound was synthesized from (**16**) (0.1158 g, 0.5306 mmol) and 3-methoxybenzaldehyde (0.08 mL, 0.6368 mmol) to afford (**24f**) (0.1417

g, 79% yield) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.64 (br s, NH), 7.49 (br s, NH), 7.54 (dd, *J* = 6.2, 1.4 Hz, 2H), 7.32-7.19 (m, 4H), 7.18-7.08 (m, 4H), 6.99-6.80 (m, 6H), 5.43 (s, 1H), 5.22 (s, 1H), 4.03 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.97 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.72 (s, 3H), 3.31 (ddd, *J* = 15.5, 5.5, 0.9 Hz, 1H), 3.22 (ddd, *J* = 15.3, 4.4, 1.9 Hz, 1H), 3.15 (ddd, *J* = 15.5, 6.5, 1.4 Hz, 1H), 3.01 (ddd, *J* = 15.1, 11.1, 2.6 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.7 (C), 173.1 (C), 160.1 (C), 160.0 (C), 142.8 (C), 142.1 (C), 136.2 (2C), 134.5 (C), 132.6 (C), 129.9 (CH), 129.8 (CH), 127.1 (C), 126.9 (C), 122.1 (CH), 121.9 (CH), 120.8 (2CH), 119.7 (CH), 119.6 (CH),118.3 (CH), 118.2 (CH), 114.3 (CH),114.1 (CH), 113.9 (CH), 113.8 (CH), 110.9 (2CH), 108.8 (C), 108.2 (C), 58.7 (CH), 56.9 (CH), 55.3 ($2CH_3$), 54.9 (CH), 52.7 (CH), 52.3 (CH_3), 52.2 (CH_3), 25.6 (CH_2), 24.3 (CH_2). HRESI-MS: calcd for $C_{20}H_{20}N_2O_3$ $[M+H]^+$: 337.1547, found: 337.1544.



(*trans*-**24f**)

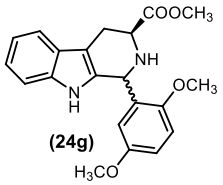
Trans* isomer** (trans*-24f**). Yellow solid (0.0581 g, 28% yield). m.p. 87.0-88.0 °C. $[\alpha]_D^{25}$ = -37.1° (c = 0.0038, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.73 (br, NH), 7.56-7.51 (m, 1H), 7.26-7.17 (m, 2H), 7.17-7.07 (m, 2H), 6.84 (d, *J* = 2.1 Hz, 2H), 6.82 (s, 1H), 5.34 (s, 1H), 3.97 (t, *J* = 6.4 Hz, 1H), 3.73 (s, 3H), 3.69 (s, 3H), 3.25 (ddd, *J* = 15.4, 5.4, 1.1 Hz, 1H), 3.11 (ddd, *J* = 15.4, 6.8, 1.3 Hz, 1H), 2.39 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1 (C), 159.9 (C), 143.6 (C), 136.2 (C), 133.1 (C), 129.7 (CH), 126.9 (C), 121.9 (CH), 120.7 (CH), 119.5 (CH), 118.2 (CH), 113.9 (CH), 113.5 (CH), 110.9 (CH), 108.3 (C), 55.3 (CH), 54.9 (CH_3), 52.6 (CH), 52.1 (CH_3), 24.6 (CH_2). HRESI-MS: calcd for $C_{20}H_{20}N_2O_3$ $[M+H]^+$: 337.1547, found: 337.1547.



(*cis*-**24f**)

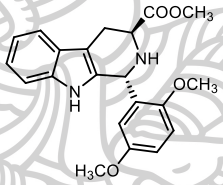
Cis* isomer** (cis*-24f**). Yellow solid (0.0488 g, 23% yield). m.p. 74.8-75.9 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.55-7.50 (m, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.21-7.14 (m, 1H), 7.14-7.06 (m, 2H), 6.98-6.85 (m, 3H), 5.19 (s, 1H), 3.95 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 3.21 (ddd, *J* = 15.3, 4.5, 1.8 Hz, 1H), 2.99 (ddd, *J* = 15.0, 11.1, 2.7 Hz, 1H), 2.12 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.7 (C), 158.5 (C), 140.7 (C), 134.6 (C), 133.0 (C), 128.4 (CH), 125.6 (C), 120.4 (CH), 119.3 (CH), 118.1 (CH), 116.7 (CH), 112.7 (CH), 112.4 (CH), 109.5 (CH), 107.2 (C), 57.2 (CH), 55.4 (CH_3), 53.8 (CH), 50.7 (CH_3), 24.1 (CH_2)

Methyl 1-(2,5-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24g**).



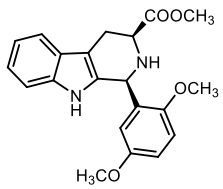
(**24g**)

Diastereomeric mixture (**24g**). The title compound was synthesized from (**16**) (0.2478 g, 1.135 mmol) and 2,5-dimethoxybenzaldehyde (0.2260 g, 1.368 mmol) to afford (**24g**) (0.2065 g, 50% yield) as a yellow solid. m.p. 107.2-109.0 ºC (lit. 100-102°C) [100]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.92 (br s, NH), 7.79 (br s, NH), 7.55-7.49 (m, 2H), 7.24-7.20 (m, 2H), 7.17-7.06 (m, 4H), 6.99 (d, *J* = 3.1 Hz, 1H), 6.90 (t, *J* = 8.9 Hz, 1H), 6.82 (dd, *J* = 8.9, 3.1 Hz, 1H), 6.76 (dd, *J* = 8.9, 3.1 Hz, 1H), 6.58 (d, *J* = 3.1 Hz, 1H), 5.79 (s, 1H), 5.67 (s, 1H), 3.97 (dd, *J* = 11.0, 4.2 Hz, 2H), 3.92 (s, 3H), 3.86 (s, 3H), 3.81 (s, 3H), 3.73 (s, 3H), 3.69 (s, 3H), 3.64 (s, 3H), 3.27-3.16 (m, 2H), 3.09-2.93 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1 (C), 173.4 (C), 154.1 (C), 153.5 (C), 151.3 (C), 151.2 (C), 136.1 (C), 135.9 (C), 134.8 (C), 132.8 (C), 131.5 (C), 130.2 (C), 127.1 (C), 126.9 (C), 121.7 (CH), 121.6 (CH), 119.4 (CH), 119.3 (CH), 118.1 (CH), 118.0 (CH), 115.6 (2CH), 114.6 (CH), 113.9 (CH), 112.4 (CH), 112.2 (CH), 111.5 (CH), 110.9 (CH), 108.8 (C), 108.3 (C), 56.9 (2CH), 56.4 (CH_3), 56.1 (CH_3), 55.8 (CH_3), 55.7 (CH_3), 52.4 (2CH), 52.2 (CH_3), 52.1 (CH_3), 25.6 (CH_2), 24.9 (CH_2).



(***trans*-24g**)

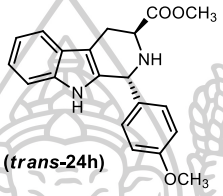
Trans* isomer** (trans*-24g**). Yellow solid (0.0258 g, 12% yield). m.p. 92.5-94.3 ºC. $[\alpha]_D^{25}$ = -12.6º (c = 0.0034, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.07 (br s, NH), 7.52 (d, *J* = 6.6, 1.7 Hz, 1H), 7.26-7.21 (m, 1H), 7.13 (td, *J* = 5.5, 1.5 Hz, 1H), 7.09 (td, *J* = 5.5, 1.5 Hz, 1H), 6.86 (d, *J* = 8.9 Hz, 1H), 6.75 (dd, *J* = 8.9, 3.1 Hz, 1H), 6.53 (d, *J* = 3.1 Hz, 1H), 5.76 (s, 1H), 3.93-3.86 (m, 1H), 3.88 (s, 3H), 3.71 (s, 3H), 3.61 (s, 3H), 3.22 (dd, *J* = 15.2, 4.3 Hz, 1H), 3.04 (ddd, *J* = 14.0, 8.6, 1.2 Hz, 1H), 2.53 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.0 (C), 153.5 (C), 151.1 (C), 136.2 (C), 132.7 (C), 131.3 (C), 126.9 (C), 121.7 (CH), 119.3 (CH), 118.1 (CH), 115.7 (CH), 112.4 (CH), 111.5 (CH), 110.9 (CH), 108.8 (C), 56.1 (CH), 55.7 (CH_3), 52.3 (CH_3), 52.1 (CH_3), 49.1 (CH), 24.9 (CH_2).



(***cis*-24g**)

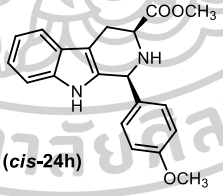
Cis* isomer** (cis*-24g**). Yellow solid (0.0314 g, 14% yield). m.p. 148.3-149.2 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83 (br s, NH), 7.53-7.47 (m, 1H), 7.22-7.18 (m, 1H), 7.14-7.05 (m, 2H), 6.98 (d, *J* = 3.0 Hz, 1H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.81 (dd, *J* = 9.0, 3.0 Hz, 1H), 5.66 (s, 1H), 3.96 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.68 (s, 3H), 3.20 (dd, *J* = 15.1, 4.2, 1.7 Hz, 1H), 2.98 (ddd, *J* = 14.9, 11.1, 2.5 Hz, 1H), 2.35 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) [101] δ 173.1 (C), 154.2 (C), 151.3 (C), 135.9 (C), 134.4 (C), 127.0 (C), 121.6 (CH), 119.4 (CH), 117.9 (CH), 114.7 (CH), 114.1 (CH), 112.3 (CH), 110.8 (CH), 108.1 (C), 56.9 (CH_3), 56.4 (CH_3), 55.8 (CH), 52.2 (CH), 52.0 (CH_3), 25.5 (CH_2).

methyl 1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24h**).



(***trans*-24h**)

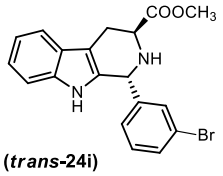
Trans* isomer** (trans*-24h**). Yellow solid (0.0284 g, 10% yield). m.p. 172.7-173.5 °C (lit. 205 °C) [102]. $[\alpha]_D^{25}$ = -10.6° (c = 0.0038, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.62 (br s, NH), 7.55 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.26-7.21 (m, 2H), 7.19 (t, *J* = 1.9 Hz, 1H), 7.17-7.09 (m, 2H), 6.89-6.82 (m, 2H), 5.42 (s, 1H), 3.99 (t, *J* = 6.3 Hz, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.29 (ddd, *J* = 15.5, 5.4, 1.1 Hz, 1H), 3.14 (ddd, *J* = 15.5, 6.7, 1.4 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.8 (C), 159.6 (C), 136.2 (C), 134.1 (C), 133.2 (C), 129.7 (2CH), 126.9 (C), 122.0 (CH), 119.5 (CH), 118.3 (CH), 114.1 (2CH), 110.9 (CH), 108.3 (C), 55.3 (CH), 54.3 (CH), 52.5 (CH_3), 52.2 (CH_3), 24.4 (CH_2).



(***cis*-24h**)

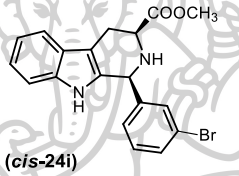
Cis* isomer** (cis*-24h**). Yellow solid (0.0075 g, 3% yield). m.p. 69.7-71.1 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.55-7.50 (m, 1H), 7.48 (br, 1H), 7.32-7.26 (m, 1H), 7.23-7.19 (m, 1H), 7.17-7.07 (m, 2H), 6.92-8.86 (m, 2H), 5.19 (s, 1H), 3.96 (dd, *J* = 11.4, 4.5 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.22 (ddd, *J* = 15.3, 4.2, 2.1 Hz, 1H), 2.99 (ddd, *J* = 15.0, 11.1, 2.4 Hz, 1H), 2.09 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) [102] δ 173.2 (C), 159.7 (C), 135.9 (C), 134.8 (C), 132.6 (C), 129.7 (2CH), 121.8 (CH), 119.5 (CH), 118.1 (CH), 114.2 (2CH), 110.8 (CH), 108.6 (C), 57.8 (CH), 56.8 (CH), 55.3 (CH_3), 52.1 (CH_3), 25.6 (CH_2).

methyl 1-(3-bromophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24i**).



(***trans*-24i**)

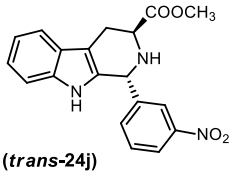
Trans* isomer** (trans*-24i**). Pale Yellow solid (0.0276 g, 22% yield). m.p. 104.3-105.5 ^{o}C. $[\alpha]_D^{25}$ = -5.2^{o} (c = 0.0038, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.69 (s, NH), 7.57-7.52 (m, 1H), 7.46-7.40 (m, 2H), 7.24-7.09 (m, 4H), 5.35 (s, 1H), 3.94 (t, *J* = 6.4 Hz, 1H), 3.70 (s, 3H), 3.26 (ddd, *J* = 15.5, 5.4, 1.2 Hz, 1H), 3.11 (ddd, *J* = 15.5, 6.6, 1.5 Hz, 1H), 2.23 (br s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.0 (C), 144.3 (C), 136.2 (C), 132.4 (C), 131.4 (CH), 131.3 (CH), 130.3 (CH), 127.1 (CH), 126.9 (C), 122.9 (C), 122.2 (CH), 119.6 (CH), 118.3 (CH), 111.0 (CH), 108.6 (C), 55.4 (CH), 52.6 (CH), 52.2 (CH_3), 24.5 (CH_2). HRESI-MS: calcd for $C_{19}H_{17}BrN_2O_2$ $[M+H]^+$: 385.0546, found: 385.0544.



(***cis*-24i**)

Cis* isomer** (cis*-24i**). Yellow oil (0.0318 g, 25% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.56-7.49 (m, 3H), 7.49-7.44 (m, 1H), 7.32-7.27 (m, 1H), 7.25-7.17 (m, 2H), 7.16-7.07 (m, 2H), 5.17 (s, 1H), 3.93 (dd, *J* = 11.1, 5.7 Hz, 1H), 3.80 (s, 3H), 3.21 (ddd, *J* = 15.1, 4.2, 1.8 Hz, 1H), 2.99 (ddd, *J* = 15.1, 11.1, 2.5 Hz, 1H), 2.36 (br s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.4 (C), 142.4 (C), 135.6 (C), 133.1 (C), 131.2 (CH), 130.9 (CH), 129.9 (CH), 126.7 (CH), 126.3 (C), 122.4 (C), 121.6 (CH), 119.2 (CH), 117.7 (CH), 110.4 (CH), 108.5 (C), 57.6 (CH), 56.1 (CH), 51.7 (CH_3), 24.9 (CH_2).

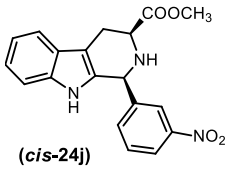
methyl 1-(3-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (**24j**).



(***trans*-24j**)

Trans* isomer** (trans*-24j**). Yellow oil (0.0089 g, 4% yield). $[\alpha]_D^{25}$ = -8.8^{o} (c = 0.0021, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 7.65 (d, *J* = 7.7 Hz, 1H), 7.60 (br s, NH), 7.57 (d, *J* = 7.1 Hz, 1H), 7.50 (t, *J* = 7.9 Hz, 1H), 7.29-7.24 (m, 1H), 7.19 (td, *J* = 7.1, 1.3 Hz, 1H), 7.14 (td, *J* = 7.0, 1.3 Hz, 1H), 5.54 (s, 1H), 3.59 (t, *J* = 5.8 Hz, 1H), 3.72 (s, 3H), 3.30 (ddd, *J* = 11.0, 4.5, 1.2 Hz, 1H), 3.18 (ddd, *J* = 15.5, 6.1, 1.2 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 174.1 (C), 148.6 (C), 144.4 (C), 136.3 (C), 134.5 (CH), 131.8 (C), 129.7 (CH), 126.9 (C), 123.4 (CH), 123.2 (CH), 122.5 (CH), 119.8 (CH), 118.5 (CH),

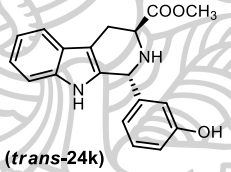
111.0 (CH), 108.6 (C), 54.2 (CH), 52.8 (CH), 52.3 (CH_3), 24.5 (CH_2). HRESI-MS: calcd for $C_{19}H_{17}N_3O_4$ $[M+H]^+$: 352.1292, found: 352.1290.



(*cis*-24j)

Cis* isomer** (cis*-24j**). Yellow oil (0.0085 g, 4% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.29 (t, *J* = 1.9 Hz, 1H), 8.21 (ddd, *J* = 8.1, 2.1, 0.9 Hz, 1H), 7.76 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.45 (br s, NH), 7.25-7.19 (m, 1H), 7.18-7.11 (m, 2H), 5.39 (s, 1H), 3.99 (dd, *J* = 11.1, 4.2 Hz, 1H), 3.83 (s, 3H), 3.25 (ddd, *J* = 15.2, 4.2, 1.8 Hz, 1H), 3.04 (ddd, *J* = 15.1, 11.1, 0.5 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.5 (C), 147.1 (C), 141.8 (C), 134.9 (C), 133.5 (CH), 131.6 (C), 128.6 (CH), 125.5 (C), 122.3 (CH), 122.2 (CH), 121.0 (CH), 118.6 (CH), 117.0 (CH), 109.6 (CH), 108.2 (C), 56.7 (CH), 55.2 (CH), 51.0 (CH_3), 24.1 (CH_2).

(1*R*,3*S*)-methyl 1-(3-hydroxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylate (***trans*-24k**)



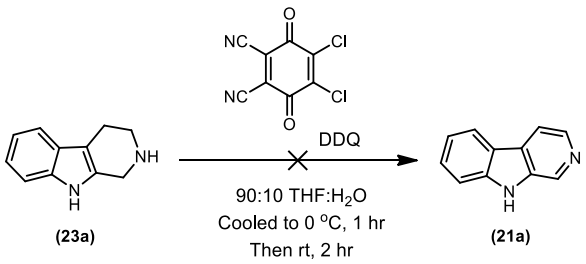
(*trans*-24k)

Trans* isomer** (trans*-24k**). Pale yellow solid (0.0195 g, 11% yield). m.p. 191.4-192.5 ^{o}C. $[\alpha]_D^{25}$ = -8.8^o (c = 0.0045, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.29 (br s, NH), 7.53 (d, *J* = 7.2 Hz, 1H), 7.24 (d, *J* = 7.3 Hz, 1H), 7.20-7.09 (m, 3H), 6.80-6.66 (m, 2H), 5.29 (s, 1H), 3.95 (t, *J* = 6.6 Hz, 1H), 3.72 (s, 3H), 3.26 (dd, *J* = 15.4, 4.9 Hz, 1H), 3.08 (dd, *J* = 15.2, 7.6 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.9 (C), 157.1 (C), 142.8 (C), 132.7 (C), 129.8 (CH), 126.5 (2C), 121.9 (CH), 119.8 (CH), 119.4 (CH), 118.2 (CH), 115.6 (CH), 115.3 (CH), 111.0 (CH), 108.1 (C), 54.8 (CH), 52.3 (CH), 52.2 (CH_3), 24.6 (CH_2). HRESI-MS: calcd for $C_{19}H_{18}N_2O_3$ $[M+H]^+$: 323.1390, found: 323.1391.

3.3.2. Synthesis of βC derivatives

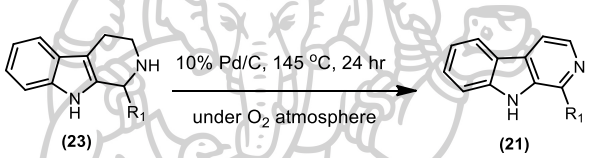
Procedure for the synthesis of 1-substituted βCs (21a-g)

method 1;
Attempted synthesis of 9*H*-pyrido[3,4-*b*]indole (21a)

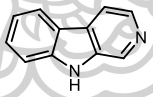


2,3,4,9-Tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23a**) (0.0739 g, 0.270 mmol) were dissolved in 90:10 THF:H_2O (5 mL) at 0 °C. Next, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.105 g, 0.270 mmol) in THF (100 mL) were added dropwise over 1 hr. The mixture was continually stirred for 2 hr at room temperature. Then, the solution was evaporated to dryness. As the ^{1}H-NMR result shown this synthetic route could not give the desired product (**21a**).

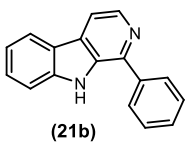
method 2;



1-substituted βCs (**23a**) and 10% Pd/C were added in *p*-xylene (3.5 mL) and refluxed at 145 °C for 24 hr under O_2 atmosphere. After cooled to room temperature, the solution mixture was concentrated under reduced pressure and was purified by column chromatography (silica gel, 1% DCM:EtOAc).

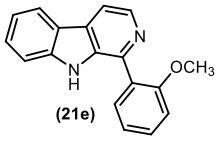


2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**21a**). The title compound was synthesized from (**23a**) (0.12 g, 0.69 mmol) to yield (**21a**) (0.019 g, 17%) as a yellow solid. m.p. 187.3-188.6 °C (lit. 196 °C) [103]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.03 (s, 1H), 9.36 (br, NH), 8.44 (d, *J* = 5.1 Hz, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 8.01 (d, *J* = 5.2 Hz, 1H), 7.58 (d, *J* = 3.8 Hz, 1H), 7.57 (s, 1H), 7.35-7.27 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9 (C), 137.6 (CH), 136.0 (CH), 132.8 (C), 129.6 (C), 128.9 (C), 121.9 (CH), 121.2 (CH), 120.4 (CH), 114.9 (CH), 111.9 (CH); ESI-MS [103] $[M+H]^+$: 169.



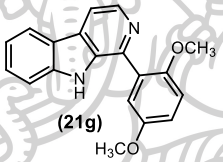
1-phenyl-9*H*-pyrido[3,4-*b*]indole (**21b**). The title compound was synthesized from (**23b**) (0.051 g, 0.246 mmol) to yield (**21b**) (0.0098 g, 16%) as a yellow solid. m.p. 227.6-228.7 °C (lit. 239-241 °C) [104]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.74 (br s, NH), 8.57 (d, *J* = 5.3 Hz, 1H), 8.17 (d, *J* = 7.9 Hz, 1H), 7.96 (d, *J* = 5.6 Hz, 3H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.54 (d, *J* = 3.2

Hz, 1H), 7.57-7.44 (m, 1H), 7.32 (td, *J* = 7.9, 1.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.5 (C), 139.1 (C), 139.0 (CH), 133.42 (C), 130.1 (C), 129.3 (2CH), 129.2 (C), 129.0 (CH), 128.7 (CH), 128.2 (2CH), 121.9 (CH), 121.8 (C), 120.4 (CH), 113.9 (CH), 111.6 (CH).



(21e)

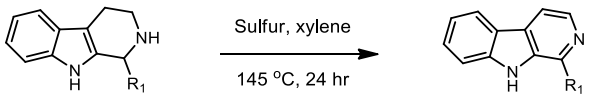
1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**21e**). The title compound was synthesized from (**23e**) (0.1618 g, 0.592 mmol) to yield (**21e**) (0.0217 g, 14%) as a brown solid m.p. 163.6-64.5 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.78 (br s, NH), 8.53 (d, *J* = 5.3 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.94 (d, *J* = 5.3 Hz, 1H), 7.70 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.50 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 7.46-7.38 (m, 2H), 7.26 (ddd, *J* = 7.9, 6.9, 1.1 Hz, 1H), 7.12 (td, *J* = 7.5, 0.9 Hz, 1H), 7.06 (dd, *J* = 8.3, 0.9 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.6 (C), 140.9 (C), 140.5 (C), 138.9 (CH), 134.8 (C), 132.3 (CH), 130.3 (CH), 129.4 (C), 128.3 (CH), 127.4 (C), 121.8 (CH), 121.7 (C), 121.6 (CH), 119.9 (CH), 113.7 (CH), 112.2 (CH), 111.6 (CH), 56.3 (CH_3) HRESI-MS: calcd for $C_{18}H_{14}N_2O$ $[M+H]^+$: 275.1179, found: 275.1181.



(21g)

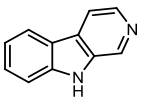
1-(2,5-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**21g**). The title compound was synthesized from (**23**g) (0.961 g, 3.119 mmol) to (**21g**) (0.0153 g, 2%) as an orange oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.74 (br s, NH), 8.58 (d, *J* = 5.3 Hz, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 5.3 Hz, 1H), 7.55 (td, *J* = 6.7, 1.0 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 2.9 Hz, 1H), 7.30 (ddd, *J* = 14.5, 6.5, 1.4 Hz, 1H), 7.07 (t, *J* = 8.9 Hz, 1H), 7.02 (dd, *J* = 8.9, 3.0 Hz, 1H), 3.85 (s, 1H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.8 (C), 154.7 (C), 150.8 (C), 140.7 (C), 140.6 (C),139.1 (CH), 134.7 (C), 129.6 (C), 128.4 (CH), 121.9 (C), 121.7 (CH), 120.0 (CH), 116.7 (CH), 116.6 (CH), 114.9 (CH), 113.9 (CH), 111.7 (CH), 57.8 (CH_3), 55.9 (CH_3).

method 3;



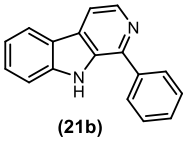
(23a-g) (21a-g)

1-substituted THβCs (**23a-g**) and Sulfur powder (3.0 eq) were added in xylene (5 mL) and refluxed at 145 °C for 24 hr. After cooled to room temperature, the solution mixture was extracted with CH_2Cl_2 and concentrated under reduced pressure and was purified by column chromatography (silica gel, 1% CH_2Cl_2:EtOAc).



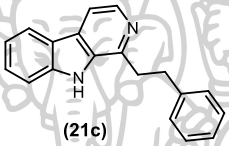
(21a)

9*H*-pyrido[3,4-*b*]indole (**21a**). The title compound was synthesized from (**23a**) (0.1859 g, 1.079 mmol) and Sulfur powder (0.1051 g, 3.278 mmol) to afford (**21a**) (0.0129 g, 40%) as a yellow solid.



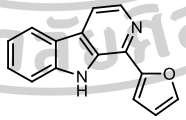
(21b)

1-phenyl-9*H*-pyrido[3,4-*b*]indole (**21b**). The title compound was synthesized from (**23b**) (0.0533 g, 0.0215 mmol) and Sulfur powder (0.0210 g, 0.0644 mmol) to afford (**21b**) (0.0238 g, 45%) as a yellow solid.



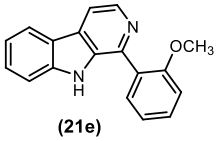
(21c)

1-phenethyl-9*H*-pyrido[3,4-*b*]indole (**21c**). The title compound was synthesized from (**23c**) (0.0519 g, 0.1878 mmol) and Sulfur powder (0.0181 g, 0.5634 mmol) to afford (**21c**) (0.0240 g, 47%) as a brown oil.^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.92 (br s, NH), 8.33 (d, *J* = 5.5 Hz, 1H), 8.07 (d, *J* = 7.9 Hz, 1H), 7.83 (d, *J* = 5.4 Hz, 1H), 7.51 (t, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 8.2 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.17-7.10 (m, 5H), 3.45 (t, *J* = 7.2 Hz, 2H), 3.18 (t, *J* = 8.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.1 (C), 141.4 (C), 140.9 (C), 136.9 (CH), 134.4 (C), 129.4 (C), 128.8 (CH), 128.5 (4CH), 126.2 (CH), 121.8 (CH), 121.5 (C), 120.3 (CH), 113.3 (CH), 111.9 (CH), 35.9 (CH_2), 34.9 (CH_2). HRESI-MS: calcd for $C_{19}H_{16}N_2$ $[M+H]^+$: 273.1386, found: 273.1382.



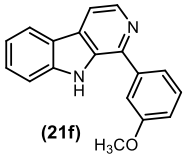
(21d)

1-(furan-2-yl)-9*H*-pyrido[3,4-*b*]indole (**21d**). The title compound was synthesized from (**23c**) (0.0555 g, 0.233 mmol) and Sulfur powder (0.0224 g, 0.6987 mmol) to afford (**21d**) (0.0258 g, 47%) as a black oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.39 (br, NH), 8.46 (d, *J* = 5.2 Hz, 1H), 8.14 (d, *J* = 7.9 Hz, 1H), 7.88 (d, *J* = 5.2 Hz, 1H), 7.73 (dd, *J* = 1.7, 0.7 Hz, 1H), 7.60-7.57 (m, 2H), 7.34-7.28 (m, 2H), 6.67 (dd, *J* = 3.5, 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9 (C), 141.1 (CH), 138.8 (C), 137.3 (CH), 131.8 (C), 129.7 (C), 128.6 (C), 127.0 (CH), 120.1 (CH), 119.7 (C), 118.5 (CH), 112.0 (CH), 110.7 (CH), 110.0 (CH), 107.1 (CH); MS [105]: 234.



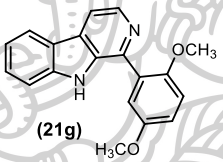
(21e)

1-(2-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (**21e**). The title compound was synthesized from (**23e**) (0.3017 g, 1.085 mmol) and Sulfur powder (0.1040 g, 3.254 mmol) to afford (**21e**) (0.1294 g, 43%) as a brown solid.



(21f)

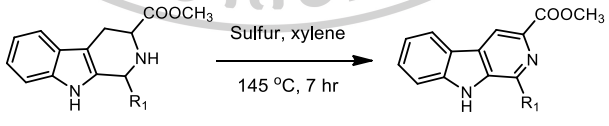
1-(3-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (**21f**). The title compound was synthesized from (**23f**) (0.3340 g, 1.201 mmol) and Sulfur powder (0.1160 g, 3.603 mmol) to afford (**21f**) (0.2581 g, 78%) as a brown oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.02 (br s, NH), 8.92 (d, *J* = 5.3 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.91 (d, *J* = 5.3 Hz, 1H), 7.55-7.43 (m, 4H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.28 (ddd, *J* = 7.8, 6.9, 1.3 Hz, 1H), 7.02 (ddd, *J* = 7.9, 2.6, 1.2 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4 (C), 142.8 (C), 140.4 (C), 139.8 (C), 139.4 (CH), 133.5 (C), 130.2 (CH), 129.9 (C), 128.6 (CH), 121.9 (C), 121.8 (CH), 120.3 (2CH), 114.9(CH), 113.9 (CH), 113.5 (CH), 111.6 (CH), 55.5 (CH_3).



(21g)

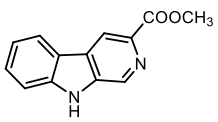
1-(2,5-dimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole (**21g**). The title compound was synthesized from (**23g**) (0.0893 g, 0.291 mmol) and Sulfur powder (0.0280 g, 0.872 mmol) to afford (**21g**) (0.0386 g, 44%) as an orange oil.

Procedure for the synthesis of 1,3-disubstituted βCs (22a-g)



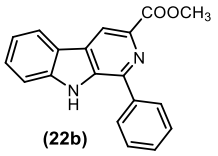
(24a-g) (22a-g)

1,3-disubstituted-tetrahydro-β-carbolines (**24a-g**) and Sulfur powder (3.0 eq) were added in xylene (5 mL) and refluxed at 145 °C for 7 hr. After cooled to room temperature, the solution mixture was extracted with CH_2Cl_2 and concentrated under reduced pressure and was purified by column chromatography (silica gel, 1% MeOH: CH_2Cl_2).



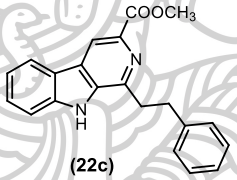
(22a)

methyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22a**). The title compound was synthesized from (**24a**) (0.1985 g, 0.862 mmol) and Sulfur powder (0.083 g, 2.587 mmol) to afford (**22a**) (0.1047 g, 53%) as a brown solid. m.p. 242.9-243.5 ºC (lit. 310 ºC) [106]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 10.87 (br s, NH), 9.27 (s, 1H), 8.87 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 1H), 7.63 (td, *J* = 7.0, 1.1 Hz, 1H), 7.38 (td, *J* = 8.0, 0.9 Hz, 1H), 4.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9 (C), 141.7 (C), 137.6 (C), 135.5 (C), 132.8 (CH), 129.4 (CH), 129.3 (C), 121.8 (CH), 121.3 (C), 121.0 (CH), 118.1 (CH), 112.8 (CH), 52.8 (CH_3).



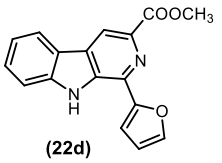
(22b)

methyl 1-phenyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22b**). The title compound was synthesized from (**24b**) (0.5779 g, 1.886 mmol) and Sulfur powder (0.181 g, 5.659 mmol) to afford (**22b**) (0.4442 g, 78%) as a brown solid. m.p. 206.4-208.5 ºC (lit. 233-238 ºC) [107]. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.26 (s, NH), 8.84 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 7.85 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 3.8 Hz, 2H), 7.45-7.32 (m, 4H), 4.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7 (C), 142.8 (C) 140.8 (C), 137.6 (C), 137.3 (C), 135.1 (C), 129.8 (C), 129.2 (CH), 129.1 (CH), 129.0 (2CH), 128.4 (2CH), 122.0 (C), 121.9 (CH), 121.1 (CH), 116.9 (CH), 112.2 (CH), 52.7 (CH_3).



(22c)

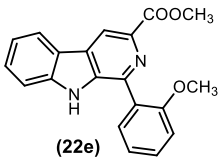
methyl 1-phenethyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22c**). The title compound was synthesized from (**24c**) (0.0281 g, 0.0848 mmol) and Sulfur powder (0.081 g, 0.2521 mmol) to afford (**22c**) (0.0169 g, 61%) as a brown oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.45 (br s, NH), 8.75 (s, 1H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.50 (td, *J* = 8.2, 0.9 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 7.29 (td, *J* = 6.8, 1.1 Hz, 1H), 7.09-7.03 (m, 3H), 6.97 (d, *J* = 3.4 Hz, 1H), 6.95 (d, *J* = 2.1 Hz, 1H), 3.98 (s, 3H), 3.43 (t, *J* = 8.2 Hz, 2H), 3.07 (t, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.7 (C), 145.2 (C), 141.4 (C), 140.9 (C), 136.7 (C), 136.3 (C), 128.8 (CH), 128.6 (C), 128.4 (2CH), 128.3 (2CH), 126.1 (CH), 121.8 (C), 121.8 (CH), 120.8 (CH), 116.6 (CH), 112.2 (CH), 52.6 (CH_3), 36.4 (CH_2), 34.9 (CH_2). HRESI-MS: calcd for $C_{21}H_{18}N_2O_2$ $[M+Na]^+$: 353.1266, found: 353.1266.



(22d)

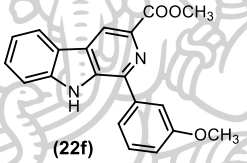
methyl 1-(furan-2-yl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22d**). The title compound was synthesized from (**24d**) (0.0650 g, 0.219 mmol) and Sulfur powder (0.0211 g, 0.6581 mmol) to afford (**22d**) (0.0402 g, 62%) as a black oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.76 (br s, NH), 8.75 (s, 1H), 8.15 (d, *J* = 7.9 Hz, 1H), 8.69 (s, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 7.58 (td, *J* = 8.2, 0.8 Hz, 1H), 7.47 (d, *J* = 3.4 Hz, 1H), 6.65 (dd, *J* = 3.5, 1.7 Hz, 1H), 4.05 (s,

3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.4 (C), 153.3 (C), 143.3 (CH), 140.8 (C), 137.2 (C), 133.1 (C), 132.7 (C), 130.3 (C), 129.1 (CH), 121.8 (CH), 121.5 (C), 121.1 (CH), 116.5 (CH), 112.5 (CH), 112.1 (CH), 110.5 (CH), 52.7 (CH_3).



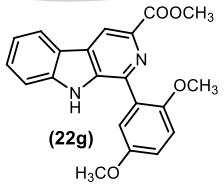
(22e)

methyl 1-(2-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22e**). The title compound was synthesized from (**24e**) (0.3594 g, 1.068 mmol) and Sulfur powder (0.103 g, 3.205 mmol) to afford (**22e**) (0.3865 g, 98%) as a brown solid. m.p. 167.0-169.7 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) 8.95 (br s, NH), 8.90 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.76 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.61-7.53 (m, 2H), 7.43 (ddd, *J* = 15.6, 8.1, 1.6 Hz, 1H), 7.12 (ddd, *J* = 7.9, 5.6, 2.4 Hz, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 4.04 (s, 3H), 3.81 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.9 (C),156.7 (C), 141.4 (C), 140.7 (C), 137.6 (C), 136.3 (C), 132.8 (CH), 130.7 (CH), 129.2 (C), 128.8 (CH), 122.1 (CH), 121.9 (C), 121.8 (CH), 120.8 (CH), 116.9 (CH), 112.3 (CH), 112.0 (CH), 99.9 (C), 56.5 (CH_3), 52.6.



(22f)

methyl 1-(3-methoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22f**). The title compound was synthesized from (**24f**) (0.1417 g, 0.4212 mmol) and Sulfur powder (0.0405 g, 1.264 mmol) to afford (**22f**) (0.1947 g, 98%) as a brown solid. m.p. 176.0-177.8 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.55 (br s, NH), 8.82 (s, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 7.60 (t, *J* = 14.8 Hz, 1H), 7.60 (d, *J* = 13.8 Hz, 1H), 7.43-7.33 (m, 3H), 7.31-7.23 (m, 1H), 6.82 (dd, *J* = 8.0, 1.9 Hz, 1H), 4.04 (s, 3H), 3.76 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.4 (C), 160.1 (C), 142.4 (C), 141.1 (C),138.1 (C), 136.9 (C), 134.9 (C), 130.0 (CH),129.9 (C), 129.2 (CH), 121.9 (CH), 121.2 (CH), 120.6 (CH), 116.9 (CH), 115.0 (CH), 113.9 (CH), 112.4 (CH), 99.9 (C), 55.4 (CH_3), 52.7 (CH_3).

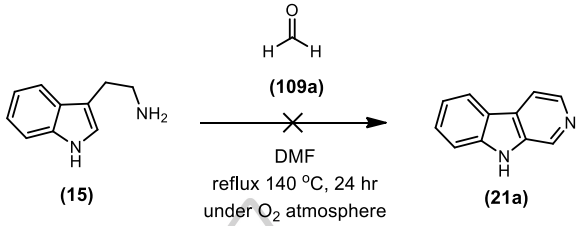


(22g)

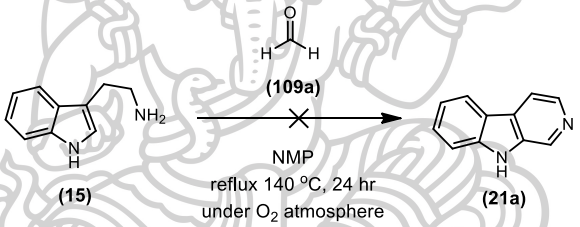
methyl 1-(2,5-dimethoxyphenyl)-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (**22g**). The title compound was synthesized from (**24g**) (0.2510 g, 0.685 mmol) and Sulfur powder (0.066 g, 2.055 mmol) to afford (**22g**) (0.2065 g, 83%) as a yellow solid. m.p. 190.5-192.5 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.33 (br s, NH), 8.89 (s, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.64-7.53 (m, 2H), 7.39-7.31 (m, 2H), 7.03-6.92 (m, 2H), 4.05 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 166.9 (C), 154.8 (C), 150.9 (C),140.8 (C), 140.7 (C), 137.6 (C), 136.3 (C), 129.4 (C), 128.9 (CH), 128.1 (C), 121.9 (C), 121.9 (CH), 120.8 (CH), 117.2 (CH), 116.9

(CH), 116.7 (CH), 114.8 (CH), 112.1 (CH), 57.8 (CH_3), 55.9 (CH_3), 52.6 (CH_3). HRESI-MS: calcd for $C_{21}H_{18}N_2O_4$ $[M+Na]^+$: 385.1164, found: 385.1156.

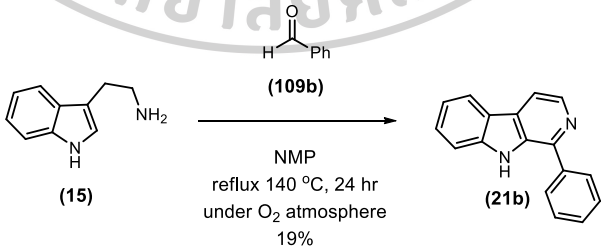
method 4 (One pot synthesis);
Attempted synthesis of 9*H*-pyrido[3,4-*b*]indole (21a)



Tryptamine (**15**) (0.2583 g, 1.612 mmol) was dissolved in DMF (8 mL). Then, *p*-formaldehyde (0.0598 g, 1.935 mmol) was added into solution and reflux at 140 ºC for 24 hr under O_2 atmosphere. After cooled to room temperature, the solution mixture was concentrated under reduced pressure. As the ^{1}H-NMR result shown this synthetic route could not give the desired product (**21a**).



Tryptamine (**15**) (0.3784 g, 2.362 mmol) was dissolved in NMP (9 mL). Then, *p*-formaldehyde (0.0780 g, 2.598 mmol) was added into solution and reflux at 140 ºC for 24 hr under O_2 atmosphere. After cooled to room temperature, the solution mixture was concentrated under reduced pressure. As the ^{1}H-NMR result shown this synthetic route could not give the desired product (**21a**).



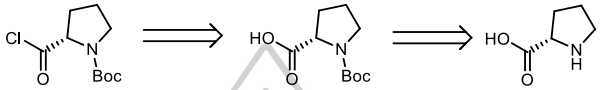
Tryptamine (**15**) (0.2226 g, 1.389 mmol) was dissolved in NMP (10 mL). Then, benzaldehyde (0.16 mL, 1.528 mmol) was added into solution and reflux at 140 ºC for 24 hr under O_2 atmosphere. After cooled to room temperature, the solution mixture was concentrated under reduced pressure to furnish (**21b**) (0.0645 g, 19%) as a yellow solid.

3.3.3. Enantioselective synthesis of C-1 substituted THβC derivatives

3.3.3.1: Enantioselective synthesis of 1-substituted THßCs using chiral auxiliary

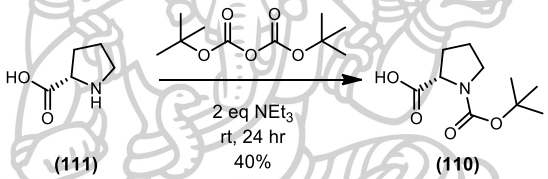
Procedure for the synthesis of chiral auxiliary (27-28)

Preparation of chiral auxiliary (27)



(27) (110) (111)

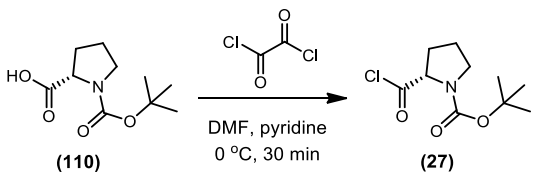
Synthesis of (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (110)



(111) (110)

L-proline (**115**) (0.3957 g, 3.437 mmol) and NEt_3 (0.55 mL, 6.874 mmol) were added into dry THF (10 mL). Next, Di-*tert*-butyl decarbonate (1.11 mL, 3.612 mmol) were slowly dropped into the solution mixture for 24 hr. After the completion of reaction, the solution was extracted with CH_2Cl_2 and the organic layer was dried over anh. Na_2SO_4. The solution was concentrated under reduced pressure the crude product was purified by column chromatography (silica gel, 4:1 Hexane:EtOAc) to yield the desired (*S*)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxylic acid (**110**) (0.2928 g, 40%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.09 (dd, *J* = 7.4, 4.9 Hz, 1H), 3.50-3.25 (m, 2H), 2.15-1.80 (m, 3H), 1.80-1.67 (m, 1H), 1.42 (s, 9H).

Synthesis of (*S*)-*tert*-butyl 2-(chlorocarbonyl)pyrrolidine-1-carboxylate (27)

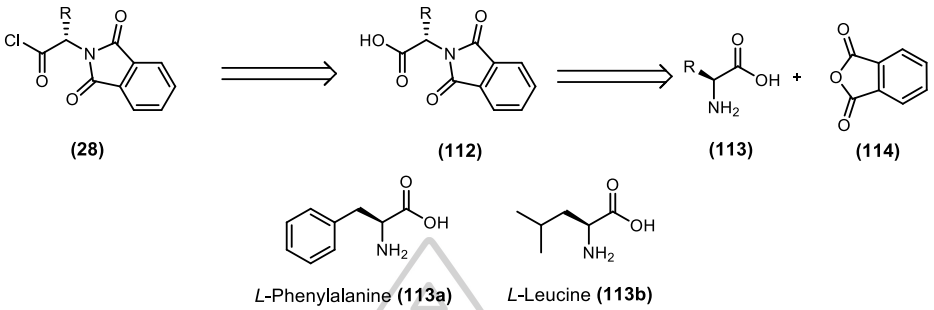


(110) (27)

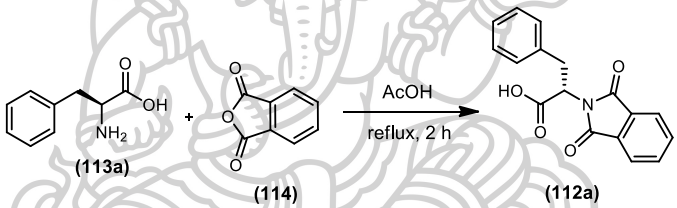
N,*N*-dimethylformide (0.09 mL, 1.155 mmol) was dissolved in CH_2Cl_2 (10 mL). Then, oxalyl chloride (0.1 mL, 1.155 mmol) was added at 0 °C and pyridine (0.09 mL, 1.155 mL) was dropped. (*S*)-1-(*tert*-butoxycarbonyl) pyrrolidine-2-carboxylic acid (**110**) (0.2485 g, 1.155 mmol) was added at 0°C and the solution was stirred for 30 min. After the completion of

reaction, the solution was concentrated by rotary evaporator to give crude product with used to the next step without purification.

Preparation of chiral auxiiary (28)

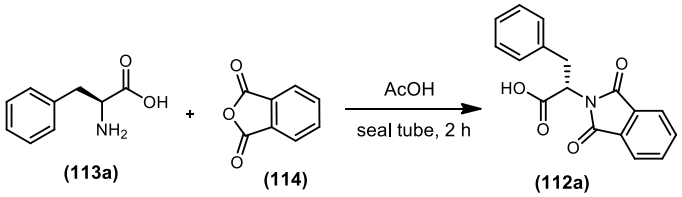


Synthesis of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (112a)
Method 1:



Phthalic anhydride (**114**) (1.900 g, 12.9 mmol) and *L*-phenylalanine (**113a**) (0.1425 g, 0.863 mmol) were refluxed in (15 ml) acetic acid for 2 hr. After the completion of reaction, the solution was cooled to room temperature and diluted the solution with CH_2Cl_2. Then, the organic layer was extracted with water and dried over anh. Na_2SO_4. The solvent was evaporated and recrystallized from ethyl acetate to give (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**112a**) (0.0167 g, 7% yield) as a white solid. m.p. 146.2-1467.5 ^oC (lit. 167-170 ^oC) [108]. ^{1}H-NMR (300 MHz, CDCl3) δ 7.80-7.74 (m, 2H), 7.12-7.65 (m, 2H), 7.23-7.10 (m, 5H), 5.22 (t, *J* = 9.1 Hz, 1H), 3.59 (d, *J* = 8.9 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 34.0 (CH_2), 52.9 (CH), 123.4 (2C), 126.6 (2C) 128.3 (CH) 128.7 (CH), 130.7 (CH), 135.0 (2C), 137.3 (C), 167.2 (2CO), 170.2 (CO_2H).

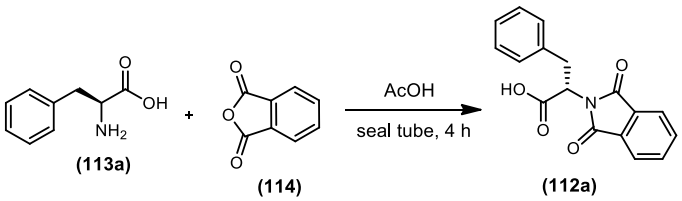
Method 2:



Phthalic anhydride (**114**) (0.3642 g, 2.475 mmol) and *L*-phenylalanine (**113a**) (0.2063 g, 1.249 mmol) were added into seal tube and refluxed in (15 ml) acetic acid for 2 hr. After the completion of reaction, the solution was cooled to room temperature and diluted the solution

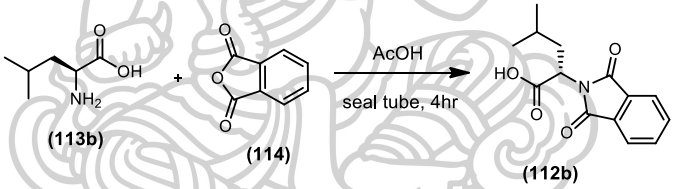
with CH_2Cl_2. Then, the organic layer was extracted with water and dried over anh. Na_2SO_4. The solvent was evaporated and recrystallized from ethyl acetate to give (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**112a**) (0.1320 g, 36% yield).

Method 3:



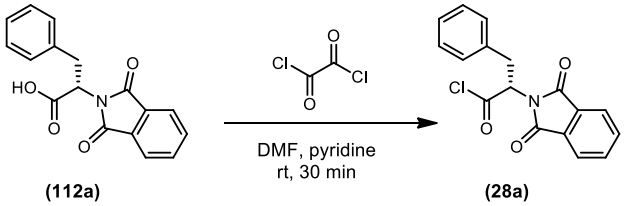
Phthalic anhydride (**114**) (0.2390 g, 1.624 mmol) and *L*-phenylalanine (**113a**) (0.2680 g, 1.622 mmol) were added into seal tube and refluxed in (15 ml) acetic acid for 4 hr. After the completion of reaction, the solution was cooled to room temperature and diluted the solution with CH_2Cl_2. Then, the organic layer was extracted with water and dried over anh. Na_2SO_4. The solvent was evaporated and recrystallized from ethyl acetate to give (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**112a**) (0.267 g, 56% yield).

Synthesis of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoic acid (112b)



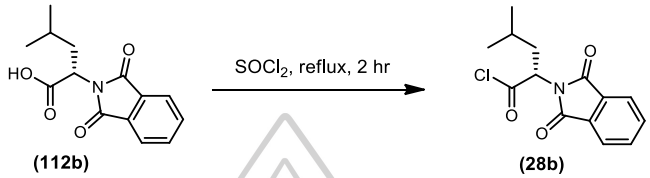
Phthalic anhydride (**114**) (0.2367 g, 1.609 mmol) and *L*-Leucine (**113b**) (0.2639 g, 1.609 mmol) were added into seal tube and refluxed in (15 ml) acetic acid for 4 hr. After the completion of reaction, the solution was cooled to room temperature and diluted the solution with CH_2Cl_2. Then, the organic layer was extracted with water and dried over anh. Na_2SO_4. The solvent was evaporated and recrystallized from ethyl acetate to give (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoic acid (**112b**) (0.3236 g, 77% yield) as a white solid. m.p. 125.0-126.1 ^oC (lit. 115-117 ^oC) [109]; ^{1}H-NMR (300 MHz, CDCl3) δ 7.90-7.84 (m, 2H), 7.76-7.71 (m, 2H), 5.00 (dd, *J* = 11.5, 4.4 Hz, 1H) 2.37 (ddd, *J* = 14.2, 11.5, 4.0 Hz, 1H), 1.95 (ddd, *J* = 14.5, 10.2, 4.4 Hz, 1H), 1.58-1.42 (m, 1H), 0.94 (t, *J* = 6.6 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 175.4 (C), 167.7 (2C), 134.2 (2CH), 131.8 (2C), 123.6 (2CH), 50.5 (CH), 37.0 (CH_2), 25.1 (CH), 23.1 (CH_3), 20.9 (CH_3).

Synthesis of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoyl chloride (28a)



N,*N*-dimethylformide (0.03 mL, 0.385 mmol) was dissolved in CH_2Cl_2 (5 mL). Then, oxalyl chloride (0.04 mL, 0.385 mmol) was added at 0°C and pyridine (0.03 mL, 0.385 mL) was dropped. (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**112a**) (0.115 g, 0.385 mmol) was added at 0 °C and the solution was stirred for 30 min. After the completion of reaction, the solution was concentrated by rotary evaporator to give crude product with used to the next step without purification.

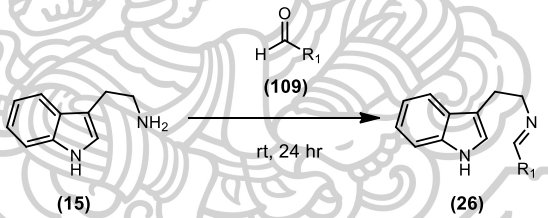
Synthesis of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoyl chloride (28b)



(112b) (28b)

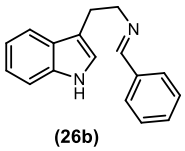
(*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoic acid (**112b**) (0.1587 g, 0.609 mmol) was dissolved in dry benzene (8 mL). Then, thionyl chloride (0.09 mL, 1.219 mmol) was slowly dropped into solution and stirred to reflux for 2 hr. After the completion of reaction, the solution was concentrated by rotary evaporator to give crude product with used to the next step without purification.

Procedure for the synthesis of Imine (26)



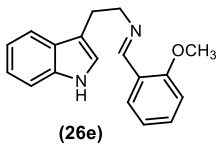
(15) (26)

Tryptamine (**15**) (1 eq) was dissolved in CH_2Cl_2. Then, aldehydes (**109**) (1 eq) were added to the solution while stirring at room temperature for 24 hr. Next, the product was extracted with CH_2Cl_2, and the organic layer was dried over anh. Na_2SO_4 and evaporated under reduced pressure. The crude product was recrystallized with 5% EtOAc:Hexane.



(26b)

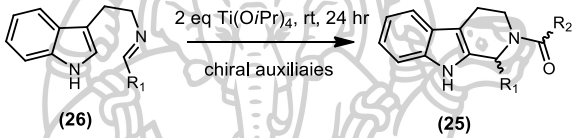
(*E*)-*N*-benzylidene-2-(1*H*-indol-3-yl)ethanamine (**26b**). The title compound was synthesized from tryptamine (**15**) (0.3394 g, 2.118 mmol) and benzaldehyde (0.22 mL, 2.118 mmol) to afford (**26b**) (0.4021 g, 76% yield) as a brown solid. m.p. 114.5-115.5 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 8.05 (br s, NH), 7.78-7.65 (m, 3H), 7.45-7.40 (m, 3H), 7.39 (d, *J* = 3.3 Hz, 1H), 7.18 (t, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 6.99 (s, 1H), 3.94 (t, *J* = 7.1 Hz, 2H), 3.17 (t, *J* = 7.2 Hz, 2H).



(26e)

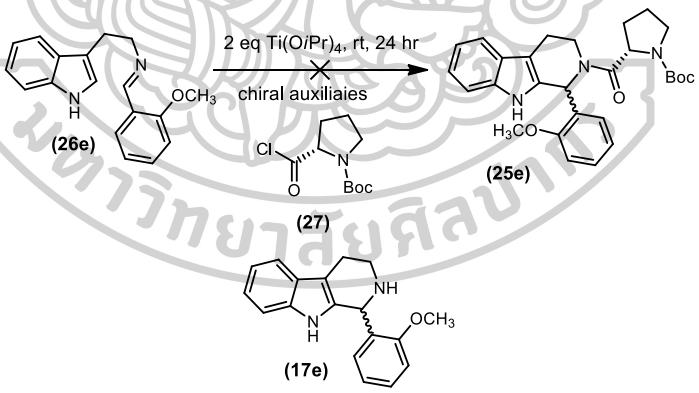
(*E*)-2-(1*H*-indol-3-yl)-*N*-(2-methoxybenzylidene)ethanamine (**26e**) The title compound was synthesized from tryptamine (**15**) (0.2197 g, 1.371 mmol) and 2-methoxybenzaldehyde (0.2198 g, 1.371 mmol) to afford (**26e**) (0.0751 g, 24% yield) as a brown oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.10 (br s, NH), 7.97 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.41-7.33 (m, 2H), 7.19 (td, *J* = 7.1, 1.3 Hz, 1H), 7.12 (d, *J* = 7.8, 1.3 Hz, 1H), 7.06-3.96 (m, 2H), 6.89 (d, *J* = 8.3 Hz, 1H), 3.94 (t, *J* = 7.7 Hz, 1H), 3.83 (s, 3H), 3.16 (t, *J* = 7.1 Hz, 1H).

Procedure for the asymmetric synthesis of 1-substituted THβCs



(26) (25)

Attempted synthesis of (2*S*)-*tert*-butyl 2-(1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-2-carbonyl)pyrrolidine-1-carboxylate (25e)

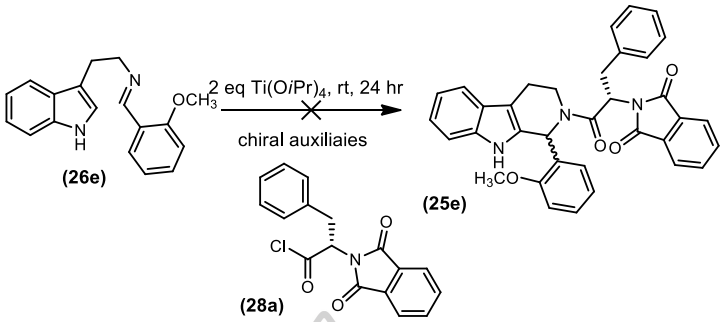


(26e) (27) (25e)

(17e)

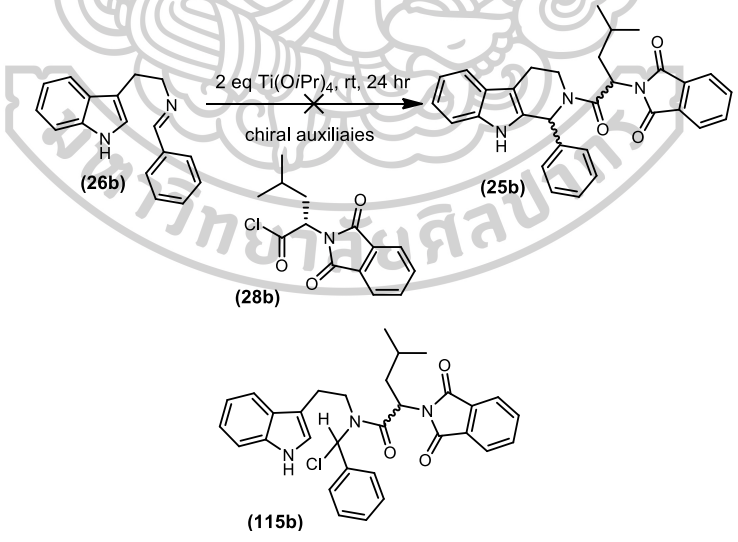
To a stirred solution of (*S*)-*tert*-butyl 2-(chlorocarbonyl)pyrrolidine-1-carboxylate (**27**) (0.0601 g, 0.0331 mmol) in CH_2Cl_2 (10 mL) was added dropwise Titanium isopropoxide (0.10 mL, 0.0662 mmol). After 5 min, a solution of the (*E*)-2-(1*H*-indol-3-yl)-*N*-(2-methoxybenzylidene)ethanamine (**26e**) (0.0053 g, 0.0331 mmol) in CH_2Cl_2 (10 mL) were added and stirred at room temperature for 24 hr. Then, the solvent was removed under reduced pressure to give crude product. The crude product was purified using column chromatography (silica gel, 1% MeOH: CH_2Cl_2) to give 1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17e**) (3 mg, 33% yield).

Attempted synthesis of (*S*)-2-(1-(1-(2-methoxyphenyl)-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)-1-oxo-3-phenylpropan-2-yl)isoindoline-1,3-dione (25e)



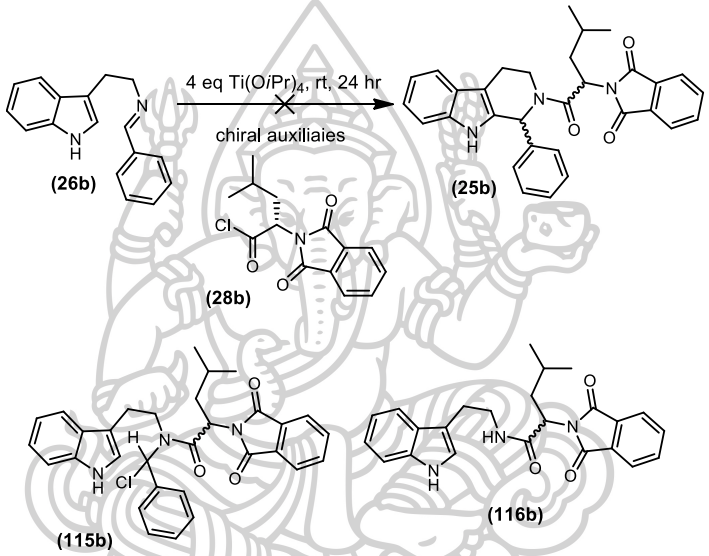
To a stirred solution of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoyl chloride (**28a**) (0.1163 g, 0.371 mmol) in CH_2Cl_2 (5 mL) was added dropwise Titanium isopropoxide (0.10 mL, 0.371 mmol). After 5 min, a solution of the (*E*)-2-(1*H*-indol-3-yl)-*N*-(2-methoxybenzylidene)ethanamine (**26e**) (0.0747 g, 0.186 mmol) in CH_2Cl_2 (10 mL) were added and stirred at room temperature for 24 hr. Then, the solvent was removed under reduced pressure. As the ^{1}H-NMR result shown this synthetic route could not give the desired product (**25e**).

Attempted synthesis of (*S*)-2-(1-(1-(2-methoxyphenyl)-3,4-dihydro-1*H*-pyrido[3,4-*b*]indol-2(9*H*)-yl)-1-oxo-3-phenylpropan-2-yl)isoindoline-1,3-dione (25b)



To a stirred solution of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoyl chloride (**28b**) (0.3101 g, 1.112 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise Titanium isopropoxide (0.33 mL, 1.112 mmol). After 5 min, a solution of the ((*E*)-*N*-benzylidene-2-(1*H*-indol-3-yl)ethanamine (**26b**) (0.1381 g, 0.556 mmol) in CH_2Cl_2 (10 mL) were added and stirred at room temperature for 8 days. Then, the solvent was removed under reduced pressure. The crude product was purified by chromatography (silica gel, 10:1 CH_2Cl_2:EtOAc) acetate to give *N*-(2-

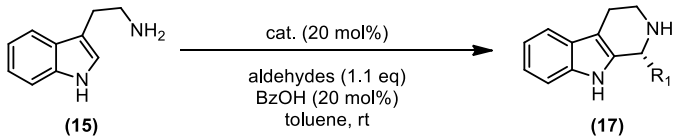
(1*H*-indol-3-yl)ethyl)-*N*-(chloro(phenyl)methyl)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanamide (**115b**) (0.0070 g, 3%) as a pale yellow oil; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.14 (s, NH), 7.82 (dd, *J* = 5.3, 3.1 Hz, 2H), 7.71 (d, *J* = 3.1 Hz, 1H), 7.69 (ddd, *J* = 3.2 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.32-7.22 (m, 4H), 7.22-7.03 (m, 4H), 6.99 (s, 1H), 5.25 (dd, *J* = 11.4, 3.5 Hz, 1H), 3.91 (dd, *J* = 14.4, 3.7 Hz, 1H), 3.44 (td, *J* = 11.4, 4.4 Hz, 1H), 2.89-2.73 (m, 1H), 2.80 (d, *J* = 3.1 Hz, 1H), 2.68 (td, *J* = 14.2, 3.0 Hz, 1H), 1.70-1.60 (m, 1H), 1.60-1.45 (m, 1H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.93 (ddd, *J* = 6.7 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 168.4 (C), 168.1 (2C), 139.7 (C), 139.5 (C), 136.3 (C), 134.2 (2CH), 131.6 (2C), 128.8 (2CH), 128.6 (2CH), 128.1 (CH), 123.5 (2CH), 122.1 (CH), 119.5 (CH), 118.1 (CH), 111.2 (2CH), 99.9 (C), 52.7 (CH), 50.2 (CH),39.9 (CH_2), 37.2 (CH_2), 25.1 (CH), 23.2 (CH_3), 22.2 (CH_2), 21.3 (CH_3).



To a stirred solution of (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanoyl chloride (**28b**) (0.2918 g, 1.047 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise Titanium isopropoxide (0.67 mL, 2.0932 mmol). After 5 min, a solution of the ((*E*)-*N*-benzylidene-2-(1*H*-indol-3-yl)ethanamine (**26b**) (0.1299 g, 0.5233 mmol) in CH_2Cl_2 (10 mL) were added and stirred at room temperature for 8 days. Then, the solvent was removed under reduced pressure. The crude product was purified by chromatography (silica gel, 10:1 CH_2Cl_2:EtOAc) acetate to give *N*-(2-(1*H*-indol-3-yl)ethyl)-*N*-(chloro(phenyl)methyl)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanamide (**115b**) (0.0025 g, 1%) as a pale yellow oil and *N*-(2-(1*H*-indol-3-yl)ethyl)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanamide (**116b**) (0.0475 g, 23%) as a pale yellow oil; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.45 (br s, NH), 7.80-7.65 (m, 4H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 8.1 Hz, 1H), 7.09 (td, *J* = 7.8, 0.9 Hz, 1H), 7.04 (td, *J* = 7.8, 0.9 Hz, 1H), 6.98 (s, 1H), 6.34 (t, *J* = 5.3 Hz, 1H), 4.85 (dd, *J* = 11.4, 4.9 Hz, 1H), 3.62-3.50 (m, 2H), 2.94 (t, *J* = 6.8 Hz, 2H), 2.32 (ddd, *J* = 15.6, 11.5, 4.2 Hz, 1H), 1.72 (ddd, *J* = 14.4, 9.8, 4.3 Hz, 1H), 1.47-1.33 (m, 1H), 0.87 (d, *J* = 6.6 Hz, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 169.4 (C), 168.2 (2C), 136.3 (C), 134.2 (2CH), 131.5 (2C), 127.2 (C), 123.5 (2CH), 122.4 (CH),121.9 (CH), 119.3 (CH), 118.4 (CH),112.4 (C), 111.3 (CH), 53.1 (CH), 40.3 (CH_2), 37.4 (CH_2), 25.2 (CH), 24.9 (CH_2), 23.1 (CH_3), 21.2 (CH_3).

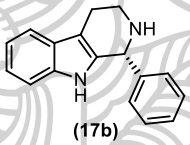
3.3.3.2: Enantioselective synthesis of 1-substituted THßCs using chiral catalyst

Procedure for the synthesis of 1-substituted βCs (17)

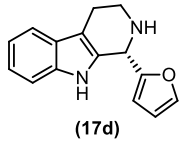


Aldehyde (7.00 mmol) was dissolved in dichloromethane (10 mL) in a separatory Funnel and was with sat. $NaHCO_3$ (3*10 mL). The organic layer was dried over Na_2SO_4, concentrated, and was used immediately.

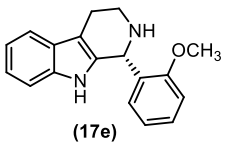
Tryptamine (**15**) (1.0 eq.) was added into a dried 25 ml round bottom flask. Toluene (2 mL), (*S*)-2-[[3,5-Bis(trifluoromethyl)phenyl]thioureido]-*N*-benzyl-*N*,3,3-trimethylbutan-amide (0.2 eq.), and benzoic acid (0.2 eq.) were added into the flask. The flask was purged with argon atmosphere for 2 minutes followed by benzoic acid (1.1 eq.) was added to stir for 10 days at room temperature. After that, the mixture was quenched by sat. $NaHCO_3$ (6 mL) and was extracted with EtOAc. the combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography using a step gradient from 1% MeOH: CH_2Cl_2 to 5% CH_2Cl_2.



(*R*)-1-phenyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17b**). The title compound was synthesized from (**15**) (0.0238 g, 0.1485 mmol), catalyst (0.0150g, 0.0297 mmol), benzoic acid (0.0036 g, 0.02971 mmol), and benzaldehyde (0.017 mL, 0.1634 mmol) to yield (**17b**) (0.0162 g, 44%) as a yellow solid. m.p. 154.2-155.6 ºC. $[\alpha]_D^{25}$ = +4.6º (c = 0.0065, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.66 (br s, NH), 7.56-7.51 (m, 1H), 7.37-7.27 (m, 5H), 7.23-7.17 (m, 1H), 7.17-7.07 (m, 2H), 5.19 (s, 1H), 3.35 (dt, *J* = 12.6, 4.9 Hz, 1H), 3.12 (ddd, *J* = 13.4, 8.5, 4.9 Hz, 1H), 3.00-2.87 (m, 1H), 2.82 (td, *J* = 4.9, 1.7 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 141.1 (C), 135.9 (C), 133.9 (C), 128.8 (2CH), 128.6 (2CH), 128.3 (CH), 127.3 (C), 121.8(CH), 119.4 (CH), 118.3 (CH), 110.9 (CH), 110.1 (C), 57.9 (CH), 42.9 (CH_2), 22.7 (CH_2).

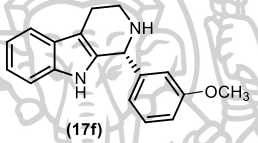


(*S*)-1-(furan-2-yl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17d**). The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and furfuraldehyde (0.05 mL, 0.5492 mmol) to yield (**17d**) and the product decomposed after purification.



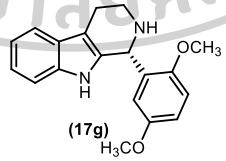
(17e)

(*R*)-1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17e**). The title compound was synthesized from (**15**) (0.0317 g, 0.1978 mmol), catalyst (0.0200g, 0.0396 mmol), benzoic acid (0.0048 g, 0.0396 mmol), and 2-methoxybenzaldehyde (0.03 mL, 0.2176 mmol) to yield (**17e**) (0.0182 g, 33%) as a yellow solid. m.p. 90.5-91.2 ºC. $[\alpha]_D^{25}$ = +67.2º (c = 0.0033, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.80 (br s, NH), 7.55-7.50 (m, 1H), 7.32-7.17 (m, 2H), 7.16-7.06 (m, 2H), 7.02 (dd, *J* = 7.5, 1.7 Hz, 1H), 6.95 (dd, *J* = 8.3, 0.7 Hz, 1H), 6.86 (td, *J* = 7.4, 4.7 Hz, 1H), 5.62 (s, 1H), 3.89 (s, 3H), 3.31-3.21 (m, 1H), 3.18-3.08 (m, 1H), 2.90-2.82 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 157.2 (C), 135.8 (C), 133.7 (C), 129.3 (C), 129.2 (CH), 129.1 (CH), 127.3 (C), 121.6 (CH), 120.7 (CH), 119.3 (CH), 118.1 (CH), 110.8 (CH), 110.7 (CH), 110.1 (C), 55.6 (CH), 50.9 (CH_3), 41.7 (CH_2), 22.2 (CH_3). HRESI-MS: calcd for $C_{18}H_{18}N_2O$ $[M+H]^+$: 279.1492, found: 279.1497.



(17f)

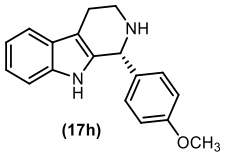
(*R*)-1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17f**). The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and 3-methoxybenzaldehyde (0.07 mL, 0.5492 mmol) to yield (**17f**) (0.0234 g, 17% yield) as an orange solid. m.p. 107.0-708.5 °C. $[\alpha]_D^{25}$ = +68.6º (c = 0.0034, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.77 (br s, NH), 7.53 (dd, *J* = 6.5, 3.5 Hz, 1H), 7.28-7.20 (m, 2H), 7.20-7.08 (m, 2H), 6.90-6.82 (m, 3H), 5.15 (s, 1H), 3.74 (s, 3H), 3.40-3.30 (m, 1H), 3.13 (ddd, *J* = 15.8, 8.5, 4.8 Hz, 1H), 3.00-2.75 (m, 2H) 2.36 (br s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 159.9 (C), 143.2 (C), 135.9 (C), 134.2 (C), 129.8 (CH), 127.3 (C), 121.7 (CH), 120.8 (CH), 119.3 (CH), 118.2 (CH), 113.9 (CH), 113.8 (CH), 110.9 (CH), 110.0 (C), 58.1 (CH), 55.3 (CH_3), 42.8 (CH_2), 22.4 (CH_2). HRESI-MS: calcd for $C_{18}H_{18}N_2O$ $[M+H]^+$: 279.1492, found: 279.1495.



(17g)
H_3CO

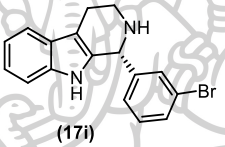
(*R*)-1-(2,5-dimethoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17g**). The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and 2,5-dimethoxybenzaldehyde (0.08 mL, 0.5492 mmol) to yield (**17g**) (0.0337 g, 22% yield) as a brown oil. $[\alpha]_D^{25}$ = +30.6º (c = 0.0036, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83 (br s, NH), 7.51 (dd, *J* = 6.8, 2.1 Hz, 1H), 7.24-7.19 (m, 1H), 7.16-7.05 (m, 2H), 6.89 (d, *J* = 8.9 Hz, 1H), 6.79 (dd, *J* = 8.9, 3.0 Hz, 1H), 6.70 (d, *J* = 3.0 Hz, 1H), 5.59 (s, 1H), 3.85 (s, 3H), 3.66 (s, 3H), 3.31 (dt, *J* = 12.4, 5.2 Hz, 1H), 3.15 (ddd, *J* = 12.3, 6.9, 5.3 Hz, 1H), 2.96-2.78 (m, 2H), 2.34 (br, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 151.6 (C), 149.2 (C), 133.7 (C), 131.5 (C), 128.4 (C), 125.2 (C), 119.4 (CH), 117.1 (CH), 115.9 (CH), 113.3 (CH), 111.1 (CH), 109.7 (CH), 108.7 (CH), 107.9 (C), 54.1 (CH_3), 53.6

(CH_3), 49.0 (CH), 39.9 (CH_2), 20.1 (CH_2). HRESI-MS: calcd for $C_{19}H_{20}N_2O_2$ $[M+H]^+$: 309.1598, found: 309.1595.



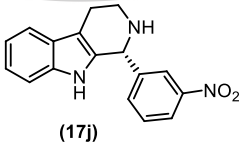
(17h)

(*R*)-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17h**). The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and 4-methoxybenzaldehyde (0.07 mL, 0.5492 mmol) to yield (**17f**) (0.0064 g, 5% yield) as a yellow oil. $[\alpha]_D^{25}$ = +36.7° (c = 0.0035, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.64 (br s, NH), 7.53 (dd, *J* = 6.1, 2.0 Hz, 1H), 7.26-7.21 (m, 3H), 7.18-7.08 (m, 2H), 6.88 (t, *J* = 1.8 Hz, 1H), 6.85 (d, *J* = 1.9 Hz, 1H), 5.21 (s, 1H), 3.79 (s, 3H), 3.35 (dt, *J* = 12.2, 4.8 Hz, 1H), 3.12 (ddd, *J* = 13.2, 8.2, 4.8 Hz, 1H), 2.96-2.78 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 159.7 (C), 135.9 (C), 133.6 (C), 132.4 (C), 129.9 (2CH), 127.2 (C), 121.9 (CH), 119.5 (CH), 119.3 (CH), 114.2 (2CH), 110.9 (CH), 109.9 (C), 57.2 (CH), 55.3 (CH_3), 42.4 (CH_2), 21.8 (CH_2).



(17i)

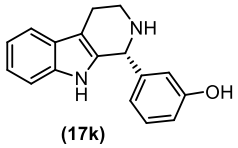
(*R*)-1-(2-bromophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17i**). The title compound was synthesized from (**15**) (0.050 g, 0.3121 mmol), catalyst (0.0320 g, 0.0624 mmol), benzoic acid (0.0076 g, 0.0624 mmol), and 3-bromobenzaldehyde (0.04 mL, 0.3433 mmol) to yield (**17i**) (0.0265 g, 26%) as a yellow oil. $[\alpha]_D^{25}$ = +27.9° (c = 0.0039, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.77 (br s, NH), 7.53 (dd, *J* = 6.3, 2.0 Hz, 1H), 7.47-7.40 (m, 2H), 7.22-7.16 (m, 3H), 7.15-7.7 (m, 2H), 5.09 (s, 1H), 3.29 (dt, *J* = 12.5, 4.7 Hz, 1H), 3.08 (ddd, *J* = 12.7, 7.9, 4.9 Hz, 1H), 2.96-2.74 (m, 2H), 2.35 (br s, NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 143.1 (C), 135.9 (C), 133.2 (C), 131.5 (CH), 131.4 (CH), 130.4 (CH), 127.3 (CH), 127.2 (C), 122.9 (C), 121.9 (CH), 119.5 (CH), 118.3 (CH), 110.9 (CH), 110.4 (C), 57.4 (CH), 42.4 (CH_2), 22.2 (CH_2). HRESI-MS: calcd for $C_{17}H_{15}BrN_2$ $[M+H]^+$: 327.0491, found: 327.0488.



(17j)

(*R*)-1-(3-nitrophenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (**17j**). The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and 3-nitrobenzaldehyde (0.08 mL, 0.5492 mmol) to yield (**17j**) (0.0514 g, 35%) as an orange oil. $[\alpha]_D^{25}$ = +9.0° (c = 0.0033, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.14 (s, 1H), 8.12-8.07 (m, 1H), 7.81 (br s, NH), 7.58 (t, *J* = 7.7 Hz, 1H), 7.53 (dd, *J* = 5.6, 2.3 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 7.24-7.07 (m, 3H), 5.19 (s, 1H), 3.24 (dt, *J* = 12.6, 5.0 Hz, 1H), 3.11 (ddd, *J* = 12.6, 7.7, 5.0 Hz, 1H), 2.97-2.75 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 147.5 (C), 143.4 (C), 135.2 (C), 133.8 (CH), 131.8 (C), 128.8 (CH), 126.3 (C), 122.5 (CH), 122.2 (CH), 121.3 (CH), 118.7 (CH), 117.5 (CH), 110.1 (CH), 109.9

(C), 53.2 (CH), 41.3 (CH_2), 21.5 (CH_2). HRESI-MS: calcd for $C_{17}H_{15}N_3O_2$ $[M+H]^+$: 294.1237, found: 294.1237.



(17k)

(*R*)-3-(2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-yl)phenol (**17k**) The title compound was synthesized from (**15**) (0.0800 g, 0.4993 mmol), catalyst (0.050 g, 0.0999 mmol), benzoic acid (0.0120 g, 0.0999 mmol), and 3-hydoxybenzaldehyde (0.0670 g, 0.5492 mmol) to yield (**17j**) (0.0039 g, 3%) as a yellow oil. $[\alpha]_D^{25}$ = +10.0° (c = 0.0030, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.54 (d, *J* = 9.0 Hz, 1H), 7.27-7.02 (m, 4H), 6.86-6.73 (m, 3H), 5.12 (s, 1H), 3.47-3.35 (m, 2H), 3.20-3.08 (m, 2H), 2.97-2.61 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 157.7 (C), 142.2 (C), 136.6 (C), 132.4 (C), 129.9 (CH), 121.9 (CH), 126.9 (C), 119.8 (CH), 118.2 (CH), 116.0 (CH), 115.5 (CH), 115.1 (CH), 111.2 (CH), 110.9 (C), 57.9 (CH), 42.8 (CH_2), 22.1 (CH_2). HRESI-MS: calcd for $C_{17}H_{16}N_2O$ $[M+H]^+$: 265.1335, found: 265.1332.

3.4 Biological assays

3.4.1 *In vitro* anti-cancer activities

The cytotoxic activities of compounds against L929, MK_2-LLC, Hep G2 and HeLa cells were assayed by the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide} colorimetric assay. The growth of the cell lines was cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 µg/mL penicillin and 100 µg/mL streptomycin and were maintained at 37 ºC with 5% CO_2. Each cell lines were seeded at $1x10^5$ cell/well (100 µL/well) in a 96-well plate and incubated overnight. Then two-fold serial dilutions of tested compounds were added into the cell culture and incubated for an additional 24 hr. After that, 100 µL MTT solution (400 µg/mL) was added into each well and incubated at 37 ºC for 4 hr. The supernatant was removed, 100 µL DMSO was added into each well to dissolve the MTT formazan, and the then immediately read on a microplate reader (ELISA) at 540 nm. The IC_{50} was defined as the concentration that induces 50% cellular death in comparison with untreated controls and calculated based on the following formular: $(Absorbance_{control} - Absorbance_{test})/ Absorbance_{control} \times 100$.

3.4.2 DPPH radical scavenging activity

DPPH scavenging activity was determined by following the modified method of Mohammad et al. (2019) [110]. All compounds were dissolved in 95% ethanol and were prepared for a series of concentrations. 50 µL of all samples and control (95 %ethanol) were mixed with the ethanolic DPPH solution (0.24 mM) 100 µL and was incubated in the dark for 30 min at room temperature. The absorbance was measured at 515 nm using microplate reader. The percentage radical-scavenging activity of the samples was calculated as DPPH inhibition (%) as displayed in Eq. (1) (A_0 is blank; A_1 is absorbance of sample) by comparing the activity of a control and were expressed in terms of the IC_{50} value which was determined as the

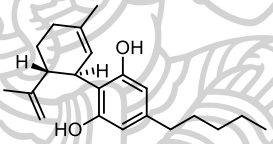
concentration of sample that inhibited 50% of the DPPH radical formation. The IC_{50} values were calculated by interpolation with linear regression, where the x-axis is the concentration of samples (μM) and the y-axis represented the scavenging effect (% inhibition).

$$\text{DPPH Inhibition} = \frac{A_0 - A_1}{A_0} \quad (1)$$

3.5 Extraction of bioactive compounds from plant for medical purposes

3.5.1 Cannabinoid extraction and isolation

Dried cannabis was obtained from the Office of the Narcotics Control Board (ONCB) of Thailand. Then, the plant extracts were subjected to a supercritical CO_2 extractor (Model HA120-50-05-C, Nantong Huaan Supercritical Extraction Co. China) with a pressure of 4.5 MPa at 37°C for 120 min. Then, the crude extract was obtained for isolation. Next, the residue was dissolved in ethanol in a freezer for 24 hr to separate fats, lipids and other oil compounds. After the cooling process, fats and lipids were removed by filtration under reduce pressure. Then, the filtrate was continuously evaporated under reduced pressure. Next, crude extracts were purified by column chromatography using a silica gel 60 from Merck (0.063–0.200 mm) (hexane: EtOAc (10:1)) to provide the cannabinoids which were CBD, CBG, CBN, and a mixture between Δ^8-THC and Δ^9-THC. The structures of bioactive compounds were confirmed by ^{1}H-NMR spectra [72].

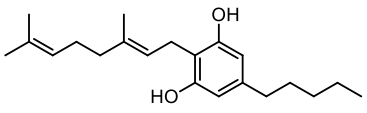


cannabidiol (CBD)

Cannabidiol (CBD). The purified compound was obtained as a yellow viscous liquid (4% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.22 (br s, 2H), 5.99 (br s, OH), 5.57 (s, 1H), 4.66 (s, 1H), 4.55 (s, 1H), 3.90-3.80 (m, 1H), 2.45-2.30 (m, 1H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.30-2.02 (m, 2H), 1.84-1.73 (m, 2H), 1.79 (s, 3H), 1.66 (s, 3H), 1.60-1.51 (m, 2H), 1.35-1.26 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H).

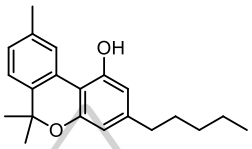
trans-Δ^8-tetrahydrocannabinol (Δ^8-THC) *trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC)

A mixture of *trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC) and *trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC). A mixture compound was obtained as a brown viscous liquid (13% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.31 (s, 1H), 6.27 (s, 2H), 6.13 (s, 2H), 5.43 (m, 1H), 3.20 (d, *J* = 9.0 Hz, 1H), 2.82-2.73 (m, 1H), 2.42 (t, *J* = 2.0 Hz, 4H), 2.20-2.10 (m, 2H), 2.10-2.00 (m, 3H), 1.96-1.87 (m, 2H), 1.80-1.71 (m, 1H), 1.70 (s, 3H), 1.68 (s, 3H), 1.60-1.50 (m, 4H), 1.41 (s, 3H), 1.40-1.30 (m, 5H), 1.29 (m, 4H), 1.20 (s, 3H), 1.10 (s, 3H), 0.89 (t, *J* = 6.9 Hz, 3H).



cannabigerol (CBG)

Cannabigerol (CBG). The purified compound was obtained as a yellow viscous liquid (3% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.32 (s, 1H), 6.28 (s, 1H), 5.40-5.30 (m, 4H), 2.30 (t, *J* = 8.0 Hz, 2H), 2.09 (s, 3H), 1.70 (s, 3H), 1.60 (s, 3H), 1.70-1.52 (m, 6H), 1.40-1.20 (m, 4H), 1.10 (s, 3H).



cannabinol (CBN)

Cannabinol (CBN). The purified compound was obtained as a brown viscous liquid (11% yield). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 6.43 (d, *J* = 1.5 Hz, 1H), 6.26 (d, *J* = 1.5 Hz, 1H), 5.48 (s, OH), 2.47 (t, *J* = 7.5 Hz, 2H), 2.37 (s, 3H), 1.62 (s, 6H), 1.32-1.24 (m, 4H), 0.89 (t, *J* = 6.8 Hz, 3H).

3.5.2 Cytotoxicity assay of cannabinoid extracts

The crude extracts and the obtained cannabinoids were evaluated *in vitro* cytotoxic activities against two cancer cell lines (HeLa and MDA-MB-231 cells) as well as two normal cell lines (LLC-MK 2 and Vero) by the MTT assay and IC_{50} determination. Cell cultures were seeded in Dulbecco's modified eagle medium containining 10% fetal bovine serum at 37°C and 5% CO_2 and diluted to $1x10^5$ cells/mL/well in a 96-well microplate. Then, two-fold serial dilutions of tested compounds were added into the cell culture and incubated for 24 hr. and then, 100 µL of MTT solution (400 µg/mL) were added into each well and incubated at 37°C and 5% CO_2 for 4 hr. After that, the supernatant was removed and DMSO was added at 100 µL/well to dissolve the formazan crystals. The MTT formazan was immediately measured at the absorbance of 540 nm on a microplate reader (ELISA). The experiment was repeated in two independent experiments and the IC_{50} were calculated. Doxorubicin was used as positive control and solvent was used as negative control.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Part A: Synthesis of THßCs (23-24) and ßCs (21-22)

The synthetic studies of 1,3-disubstituted THßCs and ßCs were discussed in this chapter. The tricyclic 1,3-disubstituted THßCs (**23-24**) would be prepared from tryptamine (**15**) or *L*-tryptophan methyl ester (**16**) *via* Pictet-Spengler reaction. Moreover, the synthesized ßC derivatives (**21-22**) were obtained from THßC derivatives (**23-24**) *via* aromatization (Figure 33). All 1,3-disubstituted THßCs (**23-24**) and ßCs (**21-22**) has been developed for investigation their biological activities such as anticancer and antioxidant activity.

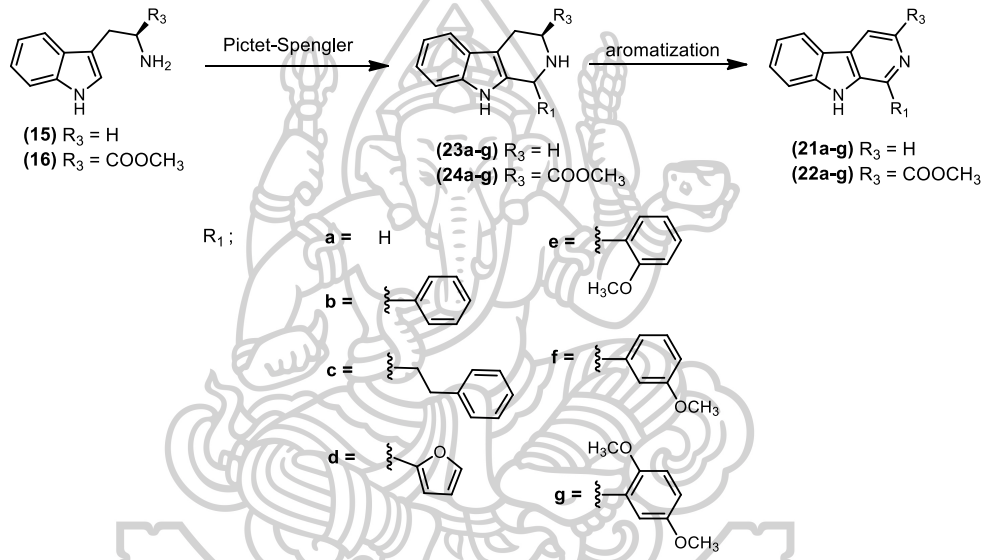
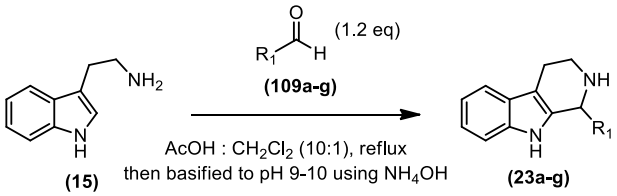


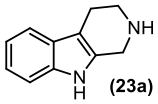
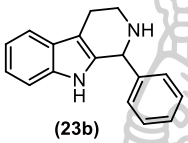
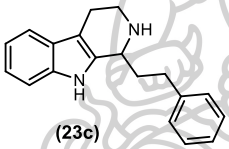
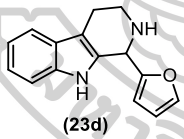
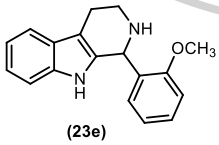
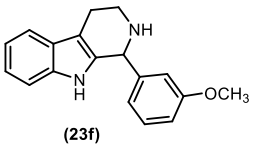
Figure 33. The synthetic procedure of THßCs (**23-24**) and ßCs (**21-22**)

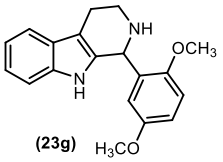
4.1.1 Synthetic study of 1-substituted THßCs (23 and 24)

First, the synthesis of tricyclic THβC derivatives (**23a-g**) started from tryptamine (**15**) and various aldehydes (**109a-g**) under acid condition *via* Pictet-spengler reaction to give 1-disubstituted THßCs (**23a-g**) in 9-84% yield (Table 5). Mechanism of Pictet-Spengler condensation is depicted in Fig. 10. Yields of the THβC derivatives (**23a-g**) are dependent on the electronic characteristics and steric hindrances of the substituent at C-1. The formation of the desired product was confirmed by NMR with the appearance of the methine singlet at C-1 position in the range of δ 5.65-4.01 ppm in the ^{1}H NMR spectrum.

Table 5. Conditions for the synthetic studies of 1-substituted THßCs (**23a-g**)

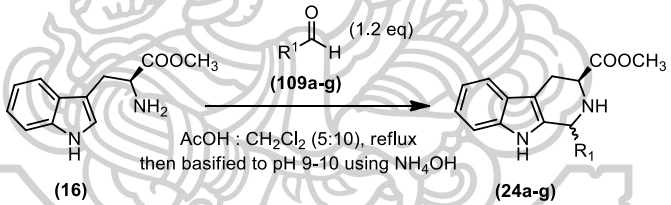


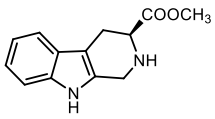
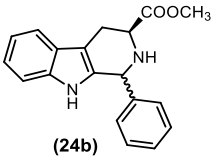
products	time (hr)	% yields
 (23a)	1	53
 (23b)	2	25
 (23c)	2	47
 (23d)	1	84
 (23e)	2	55
 (23f)	2	73

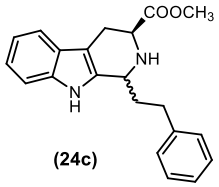
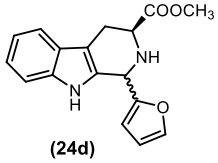
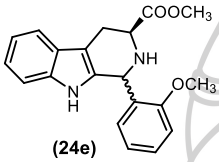
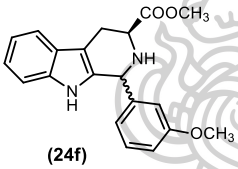
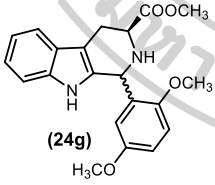
products	time (hr)	% yields
 (23g)	2	9

In the same way, 1,3-disubstituted THßCs (**24a-g**) were synthesized *via* Pictet-Spengler reaction (Table 6). *L*-tryptophan ester (**16**) reacted with aldehydes (**109a-g**) in the presence of acetic acid for 1-2 hr to give diastereomeric mixture of 1,3-disubstituted THßCs (**24a-g**) which was identified by ^{1}H and ^{13}C-NMR spectra. In this condition, diastereomeric mixture of 1,3-disubstituted THßCs (**24a-g**) were obtained in 50-88% yield. Low yield of 1,3-disubstituted THßCs (**24c** and **23g**) may occur from the steric repulsion between methyl ester group at C-3 and substituent at C-1. The desired compounds were identified by NMR data. The characteristic methine proton at 1-position was found in the range of δ 5.79-4.08 ppm in the ^{1}H-NMR spectrum.

Table 6. Conditions for the synthetic studies of 1,3-disubstituted THßCs (**24a-g**)



products	time (hr)	% yields
 (24a)	1	69
 (24b)	2	80

products	time (hr)	% yields
 (24c)	2	53
 (24d)	2	59
 (24e)	2	88
 (24f)	2	79
 (24g)	2	50

4.1.2 Synthetic study of 1-substituted-β-carbolines (21, 22)

Next, the obtained 1-substituted-β-carbolines (**23a-g**) were converted to βC derivatives (**21a-g**) *via* aromatization with oxidant promotor.

method 1;

Initially, aromatization used DDQ as friendly oxidant promotor under mild and environmentally friendly condition. Synthesis of ßC (**21a**) started from 2,3,4,9-Tetrahydro-1*H*-pyrido[3,4-*b*]indole (**23a**) which was treated with DDQ in the presence of 90:10 THF:H_2O

under cooled to 0 °C for 1 hr, followed by room temperature for 2 hr. However, aromatization in this condition was not successful (Figure 34).

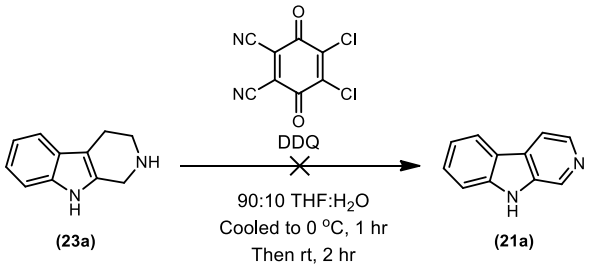
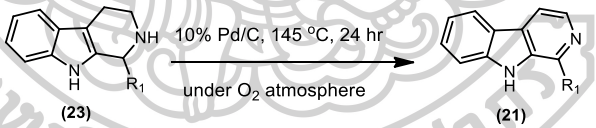


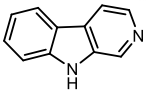
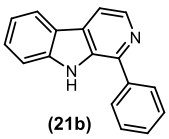
Figure 34. Aromatization of THßCs (**23a**) with DDQ

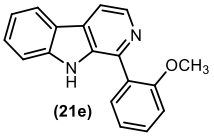
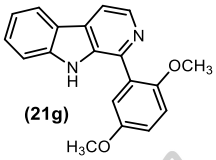
method 2;

Attempts will also be made to convert THßCs (**23a-g**) to βCs (**21a-g**). Hence, aromatization of THßCs (**23a-g**) by treat with Pd is an interesting approach (Table 7). Initially, THßC (**23a**) was treated with 10% Pd/C in *p*-xylene, and then the solution mixture was heated to 145 °C for 24 h under O_2 atmosphere to provide ßC (**21a**) with 17% yield. While, phenyl group at C-1 (**23b**) furnished ßC (**21b**) in 16% yield. In addition, 2-methoxy phenyl substituent (**23e**) gave the desired ßC (**21e**) in 14% yield. Moreover, dimethoxy phenyl ßC (**21g**) was obtained in low yield. As the result indicated that the aromatization of THßCs (**23a-g**) by heating with palladium on carbon are not suitable condition because the yields are often unsatisfactory.

Table 7. Conditions for the synthetic studies of 1-disubstituted ßCs (**21a-g**)



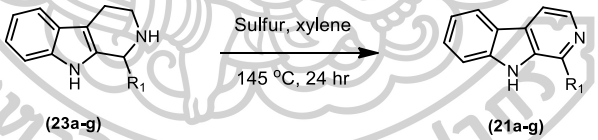
products	% yields
 (21a)	17
 (21b)	16

products	% yields
 (21e)	14
 (21g)	2

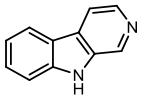
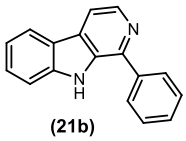
method 3;

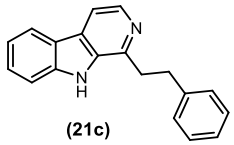
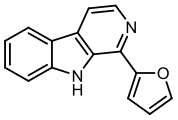
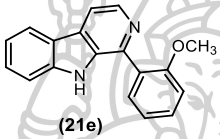
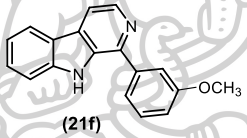
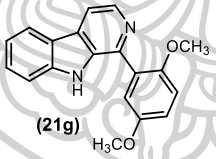
Then, other oxidizing agents are required such as sulfur powder. 1-substituted ßCs (**21a-g**) were synthesized by heating substates with sulfur in xylene under ambient oxygen (Table 8). The target ßCs (**21a-g**) were obtained in moderate to high yield (40-78%). The structure of ßCs (**21a-g**) was confirmed by NMR data by the appearance of additional peaks in the aromatic region and the absence of methine singlet (C-1) and methylene (C-3 and C-4) proton signal in ^{1}H-NMR spectrum.

Table 8. Conditions for the synthetic studies of 1-disubstituted ßCs (**21a-g**)



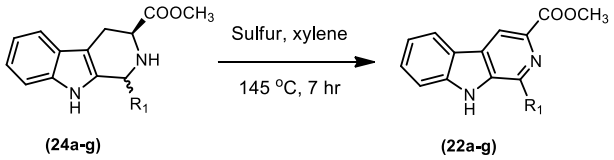
(23a-g) (21a-g)

ßCs	% yields
 (21a)	40
 (21b)	45

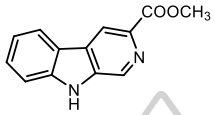
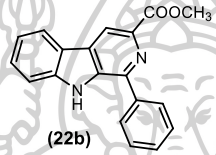
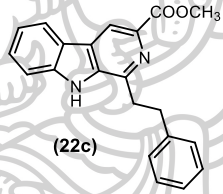
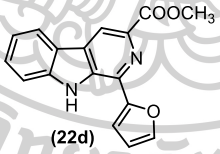
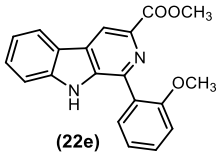
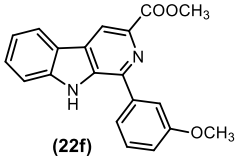
ßCs	% yields
 (21c)	47
 (21d)	47
 (21e)	43
 (21f)	78
 (21g)	44

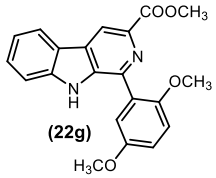
Moreover, 1,3-disubstituted βCs (**22a-g**) were synthesized from the diastereomeric mixture of 1,3-disubstituted THβCs (**24a-g**) in same condition (Table 9). The result shown that addition of methyl ester at C-3 enhance yield of the product to provide the desired 1,3-disubstituted βCs (**22a-g**) in 53-98% yield. The desired structures of 1-disubstituted ßCs (**22a-g**) were confirmed by NMR data by the appearance of additional peaks in the aromatic region and the absence of methine singlet (C-1 and C-3) and methylene (C-4) proton signal in ^{1}H-NMR spectrum.

Table 9. Conditions for the synthetic studies of 1,3-disubstituted βCs (**22a-g**)



(24a-g) (22a-g)

ßCs	% yields
 (22a)	53
 (22b)	78
 (22c)	61
 (22d)	62
 (22e)	98
 (22f)	98

ßCs	% yields
 (22g)	83

method 4 (One pot synthesis);

Aiming to improve methodologies, the development of usage of an inexpensive oxidant, environmentally safe methods as well as reduction prolonged reaction time were desired. Then, we attempted to synthesize βCs (**21a-g**) in a domino Pictet-Spengler reaction and aromatization without the use of oxidant from tryptamine (**15**) as substrate to reduce the synthetic steps. Also, domino transformation of tetrahydro-β-carbolines is carried out on Pictet-Spengler reaction through imine formation and the cyclisation requires catalysis by an acid. In this reaction, the acid should be generated in situ by the oxidation of the aldehyde in NMP at 140 °C and the generated acid in the presence of oxygen promoted by NMP should have been catalyzed the imine formation as well as the cyclisation.

Hence, aromatization of tryptamine (**15**) in one pot synthesis with solvent as DMF was not successful to give βC (**21a**) (Figure 35).

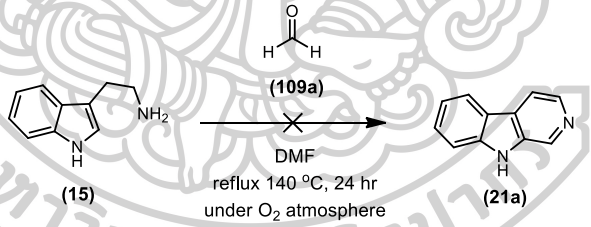


Figure 35. The synthetic route of βCs (**21a**) in one pot synthesis

Although, solvent was changed as NMP (Figure 36). The reaction was also unsuccessful too because the reaction was strictly excluded oxygen which was the crucial role for the aromatization. Moreover, paraformaldehyde undergoes decomposition at high temperature [111].

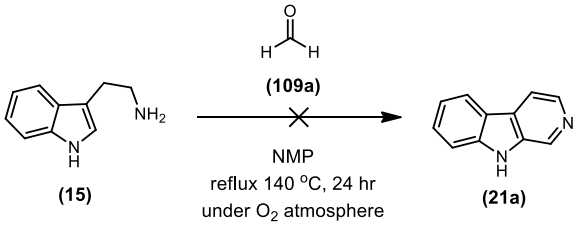


Figure 36. The one pot synthetic route of βC (**21a**)

While, tryptamine (**15**) was reacted with benzaldehyde (**109b**) in the same condition provided the product (**21b**) in 19% yield (Figure 37). The one pot synthesis of βC (**21b**) provided product in low due to difficult separation of the crude.

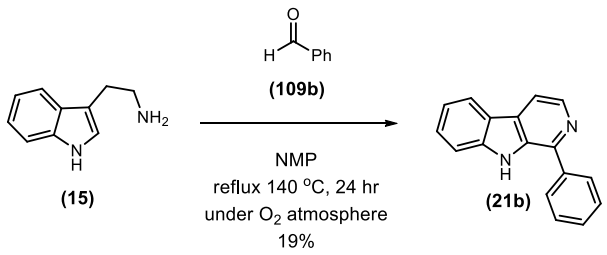


Figure 37. The one pot synthetic route of βC (**21b**)

4.2 Part B: Enantioselective synthesis of C-1 substituted THβC derivatives (17) and chromatographic separation of *trans*-1,3-disubstituted THβCs (*trans*-24)

4.2.1 Synthetic study of enantioselective synthesis of C-1 substituted THβC derivatives (17) by using chiral auxiliary

The continuous study was focused on the configuration of C-1 position prior to study on their biological activities. The stereochemistry at C1 of THβCs in the Pictet-Spengler reaction can be controlled by using chiral inductors such as chiral auxiliary [112]. Thus, in this preparation of 1-substituted THßCs (**17**) have been enantioselectively by construction of piperidine ring *via* asymmetric Pictet-Spengler using Boc-protected proline (**27**) and *N*,*N*-phthaloyl-protected amino acid chloride (**28**) as chiral auxiliary using Titanium isopropoxide as catalyst as well as reduction of chiral auxiliary at last stage (Figure 36). The stereoselectivity at C-1 is proposed in which the titanium atom coordinates both the carbonyl group of the *N*-acyliminium intermediates and the amino acid protecting group [112, 113].

Retrosynthetic analysis

The enantioselective synthesis of 1-substituted THßCs (**17**) is depicted in Figure 38. 1-Substituted THßCs (**17**) would be obtained by reduction of tricyclic amide (**25**). The tricyclic amide (**25**) would be synthesized by asymmetric Pictet-Spengler of imine (**26**) using protected amino acid chloride (**27-28**) as chiral auxiliary. Furthermore, the schiff bases (**26**) could be derived from tryptamine (**15**) and aldehyde (**109**).

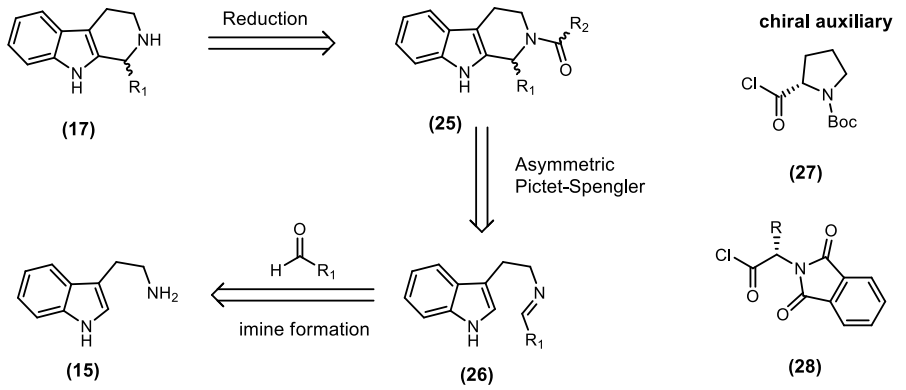


Figure 38. The retrosynthetic analysis for enantioselective synthesis of 1-substituted THßCs (**17**)

Synthetic study of chiral auxiliary (27-28)

The synthesis was started with preparation of chiral auxiliary (**27-28**). *L*-proline (**111**) were protected with Di-*tert*-butyl decarbonate at room temperature for 24 hours to give Boc-protected *L*-proline (**110**) which was converted to acid chloride (**27**) by combination of oxalyl chloride and *N*,*N*-dimethylformamide (DMF) catalyst (Figure 39). The resulted Boc-protected *L*-proline acid chloride (**27**) was used immediately in the next step without purification.

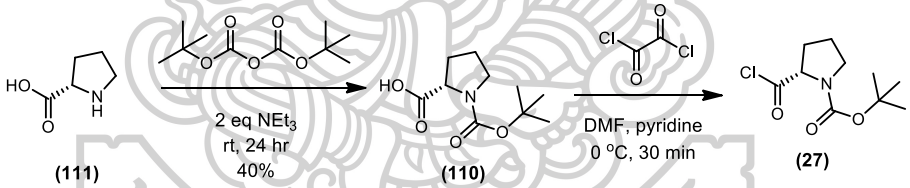
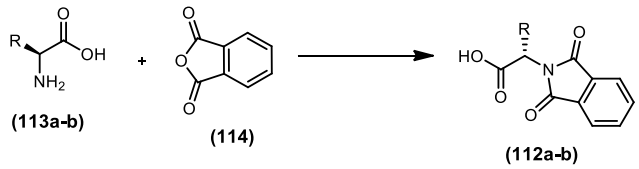
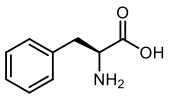
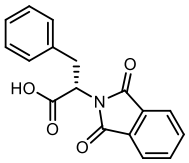
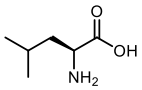
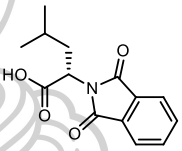


Figure 39. Synthesis of Boc-protected L-proline acid chloride (**27**)

Preparation of *N*,*N*-phthaloyl-protected amino acid (**112a-b**) was performed to use as chiral auxiliaries (**28a-b**). Initially, treatment of *L*-phenylalanine (**113a**) by heating with acetic acid for 2 hours gave (*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**112a**) in 7% yield. An attempt to increase yield by refluxing under acidic condition in seal tube for 2 hours, the targeted product (**112a**) was obtained in 36% yield. Moreover, *L*-phenylalanine (**113a**) were treated with acetic acid in seal tube for 4 hours provided (**113a**) in moderate yield (56% yield). Under the optimal condition, *L*-leucine (**113b**) were treated with acetic acid in the same reaction to give *N*,*N*-phthaloyl-protected amino acid chloride (**112b**) in high yield (77% yield) comparing to (**112a**) due to the less steric hindrance of isobutyl group at stereogenic center (Table 10).

Table 10. Conditions for the synthetic studies of carboxylic acids (**112a-b**)



amino acids	conditions	products	% yields
 (113a)	AcOH, reflux, 2 hr	 (112a)	7
	AcOH, seal tube, reflux, 2 hr		36
	AcOH, seal tube, reflux, 4 hr		56
 (113b)	AcOH, seal tube, reflux, 4 hr	 (112b)	77

Then, esterification of carboxylic acid (**112a**) in the presence of oxalyl chloride and DMF yielded *N*,*N*-phthaloyl-protected amino acid chloride (**28a**) which was used in the next step without purification (Figure 40). However, this reaction found that the remaining oxalyl chloride in this step which was disturbed the reaction in the cyclization step.

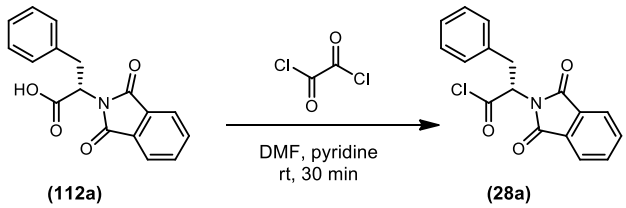


Figure 40. Esterification of (**111a**) in the presence of oxalyl chloride and DMF

Hence, in the synthesis of acid chloride (**28b**), *N*,*N*-phthaloyl-protected amino acid (**111b**) was heated with $SOCl_2$ for 2 hours (Figure 41). After the completion, the reaction was monitored by TLC and were used immediately.

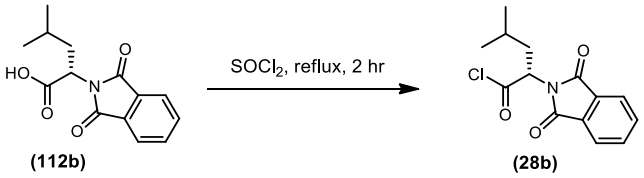
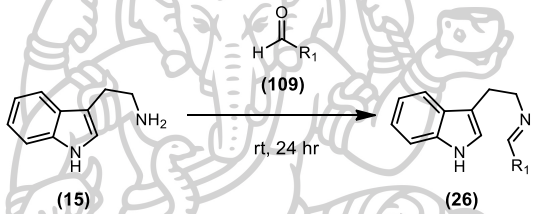


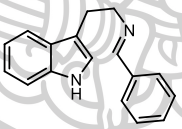
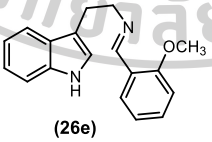
Figure 41. Synthesis of acid chloride (**28b**)

Synthetic study of Imine (26)

Move to preparation of imine (**26**), we attempted to synthesis imine (**26b**) and (**26e**) regarding to potently cytotoxicity which showed in part of biological evaluation. Imine formation of tryptamine (**15**) and aldehydes (**109**) at room temperature for 24 hours gave imine (**26b**) and (**26e**) in moderate to high yield (Table 11). Imine (**26e**) furnished moderate yield because of the steric hindrance of methoxy group at *ortho* position on phenyl ring.

Table 11. Conditions for the synthetic studies of Imine **(26)**



products	% yields
 (26b)	76
 (26e)	24

Synthetic study in asymmetric synthesis of 1-substituted THβCs (25)

After the preparation of Schiff bases (**26**) and chiral auxiliary (**27-28**), the synthesis of 1-substituted THβCs (**25**) were attempted under asymmetric Pictet-Spengler reaction. Originally, Boc-protected *L*-proline acid chloride (**27**) and Ti(O*i*Pr)$_4$ were used as chiral auxiliary and Lewis acid, respectively. Attempts to synthesis of (2*S*)-*tert*-butyl 2-(1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-2-carbonyl) pyrrolidine-1-carboxylate (**25e**) from imine (**26e**) and chiral auxiliary (**27**) under acidic condition at room temperature for 24 hours led to undesired racemic mixture of THβC (**17e**) (Figure 42). The

deprotection of a BOC-protected THβC (**17e**) is maybe resulted by hydrolysis of carbamate group in acidic conditions.

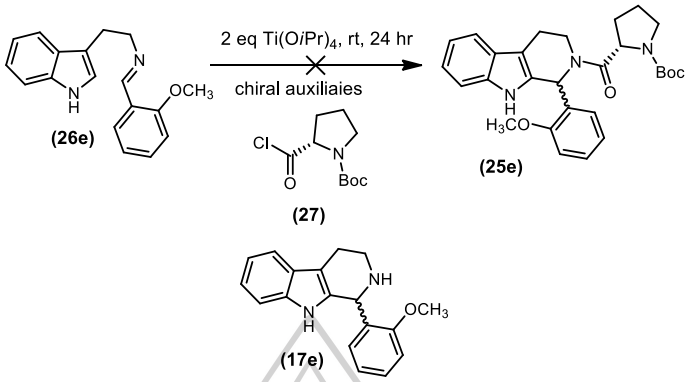


Figure 42. Attempt to synthesis of (2S)-tert-butyl 2-(1-(2-methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-2-carbonyl) pyrrolidine-1-carboxylate (**25e**)

Regarding to the unsuccessful asymmetric synthesis of 1-substituted THβCs (**25e**), we attempted to use the other chiral auxiliaries such as *N*,*N*-phthaloyl-protected amino acid chloride (**28a-b**) (Figure 43). Imine (**26e**) was reacted with chiral auxiliary (**28a**) and Ti(O*i*Pr)$_4$ at room temperature for 24 hours. However, the asymmetric Pictet-Spengler reaction using chiral auxiliary (**28a**) was unsuccessful, this might be due to the steric repulsion between benzyl group of chiral auxiliary and substituent at position 1 of the parent compound.

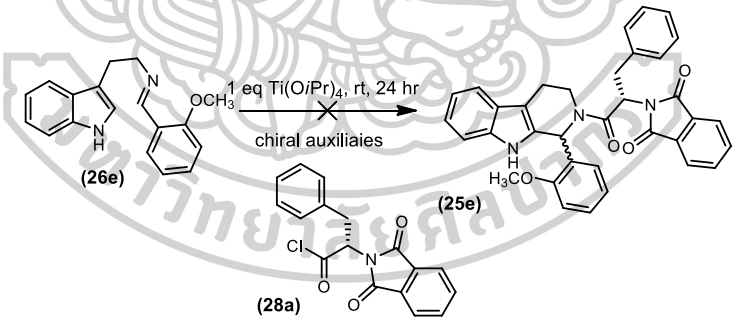


Figure 43. Attempt to synthesis of THβCs (**25e**) with Imine (**26e**) and chiral auxiliary (**28a**)

Another way of asymmetric Pictet-Spengler reaction of 1-substituted THβCs was developed with the combination of less steric Schiff bases (**26b**) and (*S*)-2-(1,3-dioxoisoindolin-2-yl)-4-methyl pentanoyl chloride (**28b**) (Table 12). Firstly, Imine (**26b**) and chiral auxiliary (**28b**) were reacted under acidic condition at room temperature for 1, 2 and 4 days using Ti(O*i*Pr)$_4$ as Lewis acids (2 equiv) (entry 1-3). However, this reaction was found that this condition was not successful. By extending the reaction time to 8 days (entry 4), the *N*-(2-(1*H*-indol-3-yl)ethyl)-*N*-(chloro(phenyl)methyl)-2-(1,3-dioxoisoindolin-2-yl)-4-methyl pentanamide (**115b**) was obtained in 3% yield without cyclized product THβC (**25b**). To improve the scope of this reaction, the amount of Ti(O*i*Pr)$_4$ was increase to 4 equiv (entry 5).

The observed result shown that the chloride (**115b**) and *N*-(2-(1*H*-indol-3-yl)ethyl)-2-(1,3-dioxoisoindolin-2-yl)-4-methylpentanamide (**116b**) were obtained in 1% and 23% yield, respectively. Moreover, increasing temperature to 45ºC in entry 6 did not improve the desired THβC (**25b**). When AcOH was used as a Lewis acid in entry 7, chloride (**115b**) only found in 8% yield. In contrast, use of $BF_3.OEt_2$ as Lewis acid (entry 8) and without Lewis acid (entry 9) did not give the desired product (**25b**). Furthermore, the result in entry 4-7 shown a complex mixture of unidentified compounds.

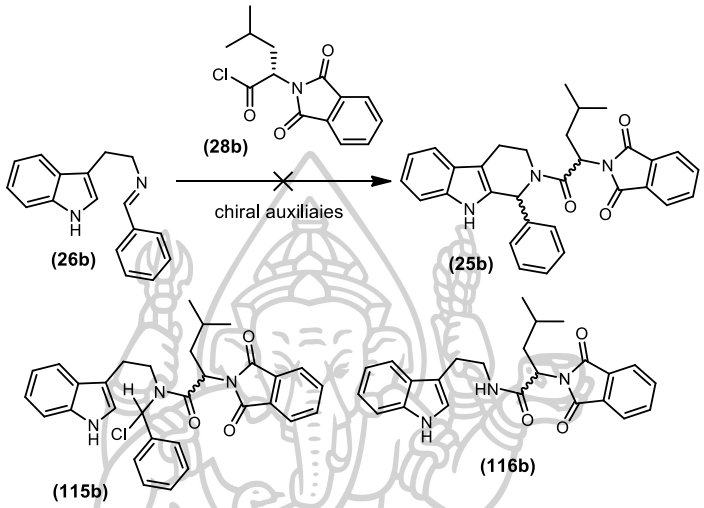


Table 12. Optimization of the Reaction Conditions for asymmetric Pictet-Spengler reaction of 1-substituted THβCs

entry	Lewis acids	temperature (ºC)	time (D)	Products (%)		
				(25b)	(115b)	(116b)
1	Ti(O*i*Pr)$_4$	rt	1	-	-	-
2	Ti(O*i*Pr)$_4$	rt	2	-	-	-
3	Ti(O*i*Pr)$_4$	rt	4	-	-	-
4	Ti(O*i*Pr)$_4$	rt	8	-	3	-
5	Ti(O*i*Pr)$_4$ [b]	rt	8	-	1	23
6	Ti(O*i*Pr)$_4$	45	1	-	1	4
7	AcOH	rt	1	-	8	-
8	$BF_3.OEt_2$	rt	1	-	-	-
9	-	rt	1	-	-	-

[a] Reaction conditions: acid chloride (**28b**) (2 equiv) in dry CH_2Cl_2 and dropwise Lewis acids (2 equiv) for 5 min, then addition of imine (**26b**) (1 equiv) and stirring during condition time.

[b] Lewis acids (4 equiv)

4.2.2 Synthetic study of Enantioselective synthesis of C-1 substituted THβC derivatives (17) by using benzoic acid and a chiral thiourea co-catalysts

Moreover, the enantioselective THßCs (**17**) were synthesized *via* asymmetric Pictet-Spengler reaction by benzoic acid and a chiral thiourea co-catalysts from tryptamine (**15**) and various aldehydes (Figure 44).

Retrosynthetic analysis

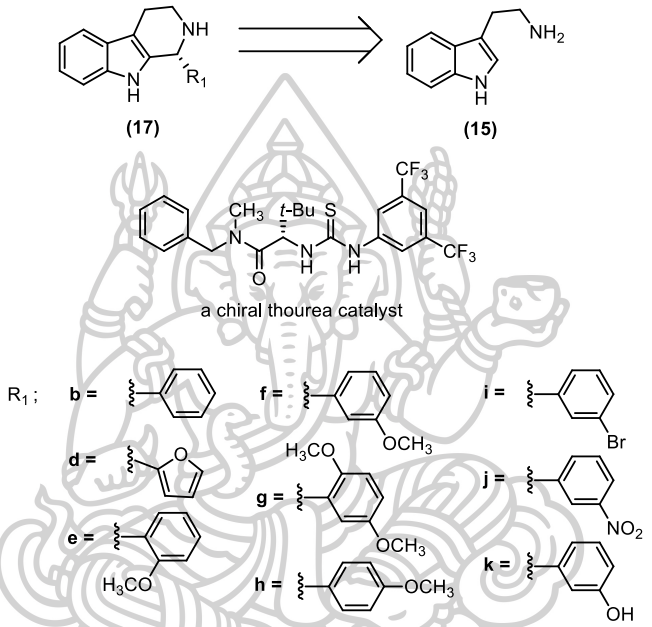


Figure 44. The retrosynthetic analysis of enantioselective synthesis of 1-substituted THßCs (**15b-k**) by benzoic acid and a chiral thiourea co-catalysts

The 1-substituted THßCs (**17b-k**) were synthesized from tryptamine (**15**) and aldehyde precursors *via* catalytic asymmetric Pictet-Spengler reactions cocatalyzed by a chiral thiourea and benzoic acid. In this case, 1-substituted THßCs (**17b-k**) are obtained in moderate yield and high ee which were determined by ^{1}H NMR (Table 13) [58]. Originally, 1-substituted THßCs (**17b**) were synthesized between tryptamine (**15**) and benzaldehyde for 2 days to provide the desired product **(17b)** in 4% yield. Moreover, THßCs (**17b**) were synthesized under extended reaction times for 10 days to furnish product in moderate yield (44%) and high ee. Under the suitable time, 1-substituted THßCs (**17e-g and 17i-j**) were obtained in high ee and moderate yield (17-35%). In contract, both *para*-substituted and 3-hydroxy benzaldehyde gave THßCs (**17h** and **17k**) in low yield due to the electronic characteristic of aldehydes. Additionally, furan (**17d**) decomposed after purification.

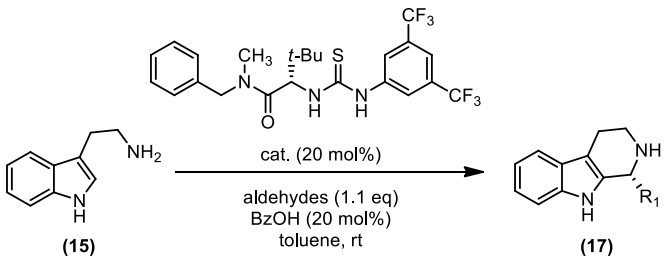
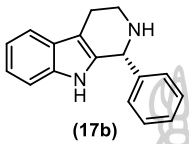
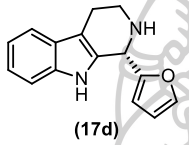
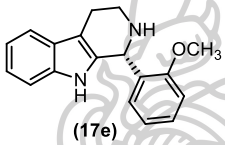
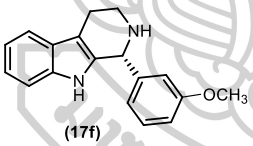
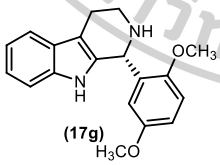
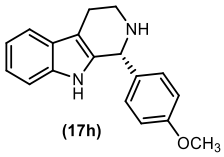
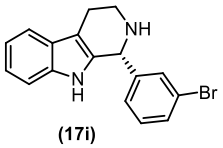
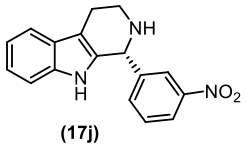
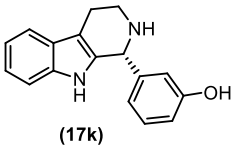


Table 13. Optimization Studies of the One-Pot Pictet-Spengler Reaction

Products	time (days)	%yield	%ee[a]
 (17b)	2	4	99
	10	44	99
 (17d)	10	-[b]	-
 (17e)	10	33	99
 (17f)	10	17	99
 (17g)	10	22	99
 (17h)	10	5	99
 (17i)	10	26	99

Products	time (days)	%yield	%ee[a]
 (17j)	10	35	99
 (17k)	10	3	99

[a] Enantioselectivity determined by ^{1}H NMR
[b] Decomposition after purification

4.2.3 Studies of chromatographic separation of *trans*-1,3-disubstituted THβCs (*trans*-24)

To investigation the influence of substituted methyl ester at C-3 compared the parents (**17**), in this study, we attempted to chromatographic separation of *cis*/*trans* isomers (**24b-k**) which were synthesized from *L*-tryptophan methyl ester (**16**) to the pure *trans* diastereomer (***trans*-24b-k**) *via* Pictet-Spengler reaction (Table 14). The *trans* diastereomer (***trans*-24b-k**) were obtained in 4-28% yield. While, the *cis* diastereomer (***cis*-24b-j**) were obtained in 3-25% yield. In addition, Pictet-Spengler reaction from *L*-tryptophan methyl ester (**16**) and 3-hydroxybenzaldehyde was only provided *trans* diastereomer (***trans*-24k**) in 11% yield.

We confirmed and assigned the stereochemistry of *trans*-stereoisomer (***trans*-24b-k**) by comparison of coupling constants of the C(3) in their 1D and 2D NMR spectra. From ^{1}H NMR data, the coupling constants (^{3}J) between H-3 and H-4 of *trans*-diastereomer observed not very large with 7.1, 5.4 Hz for (***trans*-24b**), 9.3, 4.7 Hz for (***trans*-24d**), 9.0, 4.7 Hz for (***trans*-24e**), 6.4 Hz for (***trans*-24f**), 6.3 Hz for (***trans*-24h**), 6.4 Hz for (***trans*-24i**), 5.8 Hz for (***trans*-24j**) and 6.6 Hz for (***trans*-24k**). To confirm this result, *trans* diastereomer (***trans*-24b**) was investigated in ^{1}H-^{1}H NOESY spectroscopic analyses (Figure 45). The result shown that the proton at the C-3 position does not correlate with the proton at C-1 position, but the H-3 position was correlated the neighboring protons between the methylene protons of the benzyl group [52, 54].

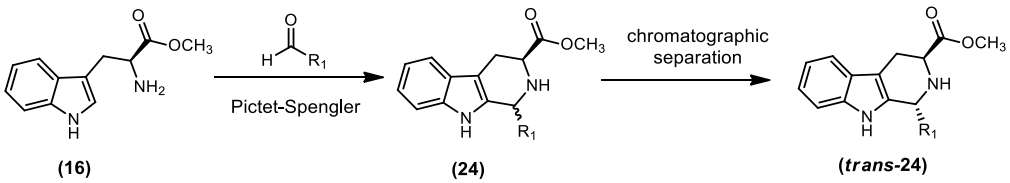
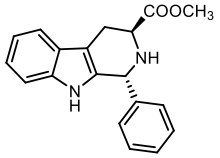
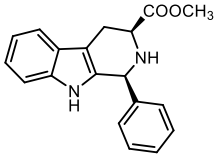
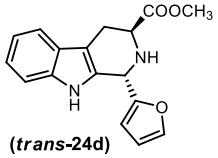
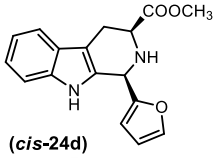
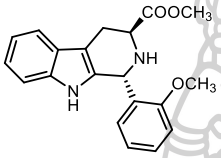
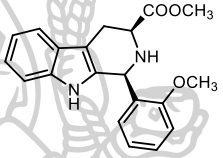
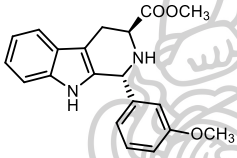
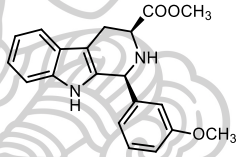
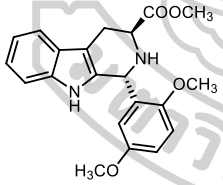
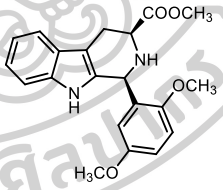
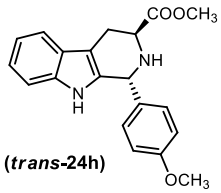
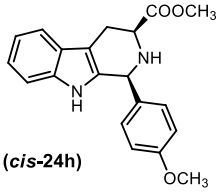
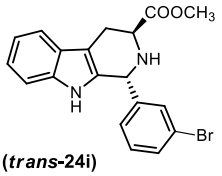
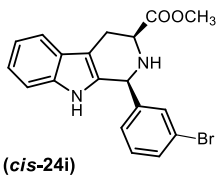
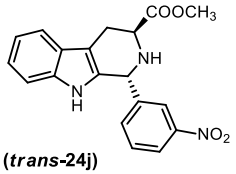
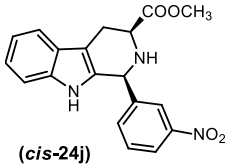
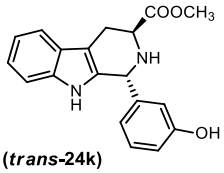
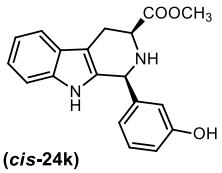


Table 14. Results of the chromatographic separation of *cis*/*trans* isomers (**24b-k**) to the pure *trans* diastereomer (***trans*-24b-k**)

Products	%yield	Products	%yield
 (*trans*-24b)	17	 (*cis*-24b)	17
 (*trans*-24d)	26	 (*cis*-24d)	11
 (*trans*-24e)	9	 (*cis*-24e)	14
 (*trans*-24f)	28	 (*cis*-24f)	23
 (*trans*-24g)	12	 (*cis*-24g)	14
 (*trans*-24h)	10	 (*cis*-24h)	3
 (*trans*-24i)	22	 (*cis*-24i)	25

Products	%yield	Products	%yield
 (*trans*-24j)	4	 (*cis*-24j)	4
 (*trans*-24k)	11	 (*cis*-24k)	-

- no reaction

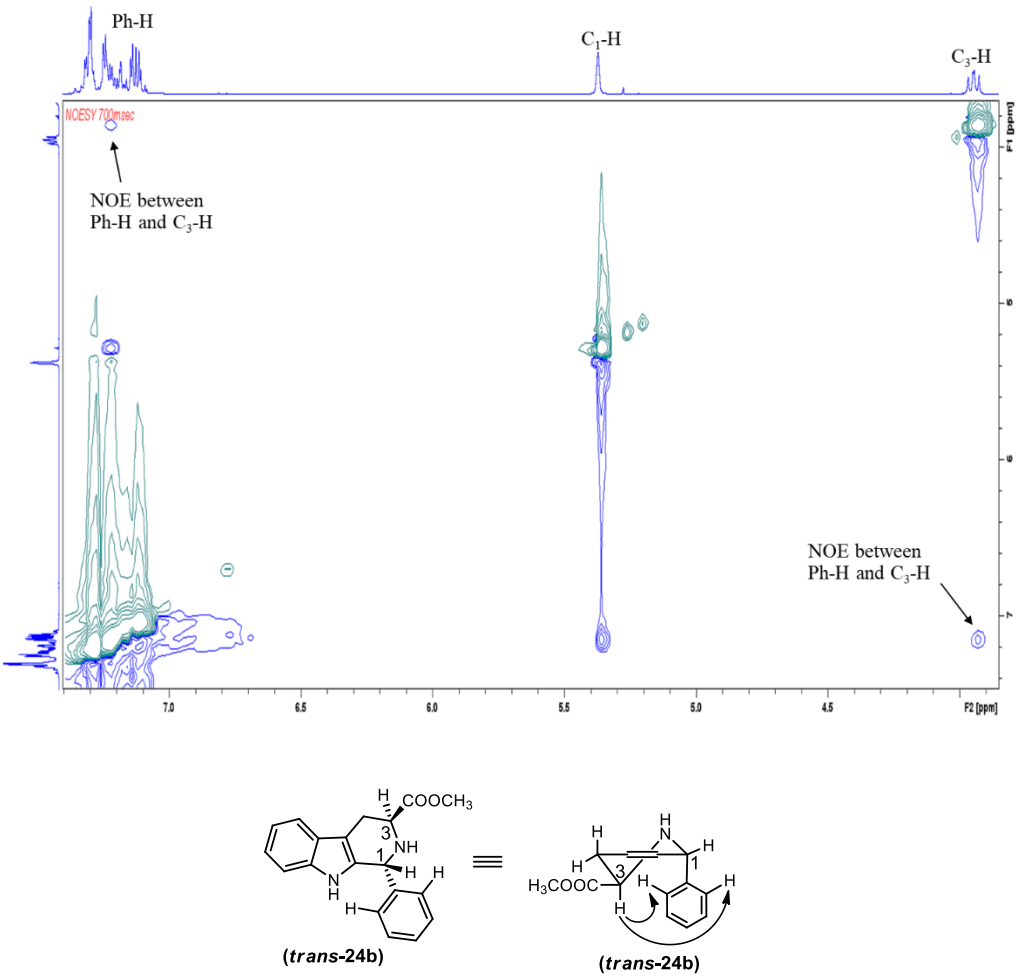


Figure 45. ^{1}H-^{1}H NOESY spectra of trans-diastereomer (***trans*-24b**)

4.3 *In vitro* biological assays

4.3.1 Cytotoxicity of synthetic compounds in Hep-G2, Hela, L929 and LLC-MK2 cells

Initially, THβC (**23a-g**, 2**4a-g**) and βC (**21a-g**, **22a-g**) derivatives were screened for their potential *in vitro* cytotoxic effects on two cell human cancer cells (Hep G2 and HeLa), mouse connective tissue fibroblast cell (L929) and rhesus monkey kidney epithelial cell (LLC-MK2) (Table 15). The normal LLC-MK2 cell line was used for study in order to evaluate the cancer selectivity of the most active compound. After exposing the cells to these compounds for 24 h, the cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay and their IC_{50} values were represented in Table 15. Two-point modification have been investigated in synthesized compounds with differentiation in C1 of THβC derivatives (**23a-g**, **24a-g**) and C1 of βC derivatives (**21a-g**, **22a-g**).

C1 of THβC derivatives: Unfortunately, analysis of SAR reveals that all of synthesized compounds shown poor activities against Hep G2. Moreover, the result indicated that the length of hybrids linkers influences the cytotoxicity activities. For instance, Compounds with ethylbenzene (**23c**) of the THßCs showed high inhibitory activity against HeLa cell line than phenyl substitutes at the 1-position with IC_{50} value of 85.64 μM but (**23c**) displayed high cytotoxic effects on normal cell (LLC-MK2) with IC_{50} value of 43.42 μM. Additionally, furan ring (**23d**) displayed high inhibitory activity among all compounds in the tested against HeLa cell line with IC_{50} value of 89.93 μM. Moreover, substitution with methoxy group (OCH_3) on phenyl group (**23e-23g**) slightly inhibited HeLa cell, especially dimethoxy (**23g**). Moreover, no one of the **THβCs** (**23a-23g**) showed high selectivity index index when compared to the normal cell, LLC-MK2.

To evaluate the effects of substituent at the C-3 position of the 1,3-disubstituted THßCs (**24a-g**) on activity, substitution with ethylbenzene (**24c**) or phenyl ring with methoxy group (**24e-f**) at position 1 exhibited strong antitumor activities against of HeLa cell line with IC_{50} ranging of 8.59-30.62 μM. However, 3-methoxy phenyl (**24f**) only displayed the highest activity against HeLa cell line with IC_{50} value of 8.59 μM and low cytotoxic effects on LLC-MK2 with high selectivity index (SI = 25.91). Furthermore, unsubstituent (**24a**), phenyl (**24b**) and furan ring (**24d**) at position 1 shown good inhibitory activities compared with unsubstitution (**23a**, **23b** and **23d**).

C1 of βC derivatives: It has been observed that most of them exhibited low cytotoxic activities compared their parent THßC analogs (**23a-g**, **24a-g**), but 2-methoxy phenyl (**21e**) showed moderate cytotoxicity *against* HeLa cell line (IC_{50} values = 44.11 μM) and good selectivity towards cancer cell lines with SI = 8.01. Hence, the fully aromatic (**21-22**) could not be enhance anticancer activity compared to the parent compounds (**23-24**) with regard to SAR investigation.

4.3.2 Antioxidant activity determinations

The antioxidant capacity of designing THßC and ßC derivatives was determined by DPPH radical-scavenging assay. The DPPH assay is simple method which is often used to determine the ability of antioxidants to scavenge free radicals. The relationship between synthesized analogues and their scavenging activity for the DPPH radical were measured in terms of IC_{50} value (mM) which were depicted in Table 15.

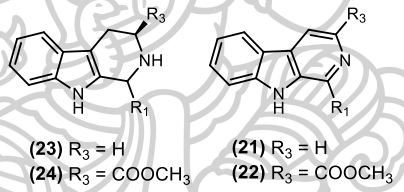
C1 of THβC derivatives: On the study of the substitution at position 1 (**23a-g**), non-substituent (**23a**), furan ring (2**3d**) and 2-methoxy phenyl group (**23e**) displayed good activities with IC_{50} ranging of 11.22-18.88 mM. While, the others exhibited moderate activities.

Compared with the substitution of methyl ester at position 3, compounds (**24a-g**) showed greater activity than the parent compound (**23a-g**). Further, replacement of the phenyl ring by ethyl benzene (**24c**) and furan (2**4d**) displayed high activity with IC_{50} value of 9.56 and 4.86 mM, respectively. Moreover, 2-methoxy phenyl group (**24e**) also showed good activity with IC_{50} value of 18.80 mM.

C1 of βC derivatives: The investigation indicated that these analogs showed worse antioxidant activities than the parent THßC. Whereas, 1-substituted ßC derivatives with substituent ethyl benzene (**21c**), furan (**21d**) and disubstituent on phenyl ring (**21g**) showed moderate activity with IC_{50} ranging of 21.01-49.83 mM. Continually, the addition of methyl ester on C-3 of ßC (**22a**) exhibited the least antioxidant activity. Moreover, aromatic ring (**22b** and **22d**) or ethyl benzene (**22c**) displayed moderate activity with IC_{50} ranging of 23.32-38.72 mM. On the other hand, the substituted ßC with electron donating group on phenyl ring (**22e-g**) show good activities with IC_{50} ranging of 9.69-13.69 mM.

In brief, these *in vitro* biological assays were evident that the aromatic substituents at position 1 improve their activities. Moreover, the presence of methyl ester at position 3 is playing an important role for enhancement the activity on THßC derivatives.

Table 15. *In Vitro* cytotoxicity (IC_{50}, µM) and antioxidant (IC_{50}, mM) activities of THßC (**23, 24**) and ßC (**21-22**) derivatives.



Cpds	IC_{50}[a] (µM)						DPPH (IC_{50} mM)
	Hep-G2[b]	SI[d]	Hela[b]	SI	L929[b]	LLC-MK2[b]	
23a	708.36	0.65	514.78	0.89	29.03	459.33	12.048 ± 0.205
23b	365.17	0.73	149.23	1.79	153.03	267.19	43.355 ± 0.421
23c	100.73	0.43	85.64	0.51	500.69	43.42	37.421 ± 4.045
23d	394.49	0.63	89.93	2.78	-[c]	250.01	11.222 ± 2.818
23e	193.07	0.57	225.44	0.49	392.64	109.57	18.876 ± 0.000
23f	322.39	0.66	362.31	0.59	865.06	212.07	74.085 ± 4.509
23g	300.26	0.58	480.95	0.36	319.58	174.76	58.300 ± 12.892
24a	556.98	1.67	638.89	1.46	790.71	932.90	27.239 ± 0.485
24b	591.62	0.64	448.46	0.85	222.42	379.13	37.335 ± 0.000
24c	211.96	0.31	23.98	2.74	-	65.79	9.560 ± 0.152
24d	506.24	0.83	268.66	1.56	-	419.58	4.861 ± 0.112

Cpds	IC_{50}[a] (μM)						DPPH (IC_{50} mM)
	Hep-G2[b]	SI[d]	Hela[b]	SI	L929[b]	LLC-MK2[b]	
24e	125.19	1.67	30.62	6.81	-	208.54	18.803 ± 0.341
24f	231.88	0.96	8.59	25.91	-	222.58	23.267 ± 0.427
24g	183.16	3.16	236.07	2.45	-	577.93	24.381 ± 0.994
21a	832.09	0.39	372.61	0.88	132.77	326.48	57.387 ± 1.488
21b	517.46	1.62	597.81	1.40	-	839.04	141.191 ± 18.638
21c	190.94	0.37	57.65	1.22	-	70.13	38.588 ± 4.149
21d	262.71	1.51	421.69	0.94	78.68	397.35	21.009 ± 0.462
21e	260.64	1.36	44.11	8.01	-	353.20	128.740 ± 9.625
21f	156.75	0.61	128.49	0.75	-	96.31	119.401 ± 0.629
21g	170.86	1.46	56.58	4.40	-	248.89	49.834 ± 1.243
22a	280.47	0.77	424.39	0.51	-	217.30	430.051 ± 20.795
22b	558.46	0.28	354.58	0.44	-	157.11	38.724 ± 0.774
22c	180.55	2.36	355.71	1.20	-	425.46	24.582 ± 3.457
22d	301.07	1.99	121.45	4.94	-	599.92	23.318 ± 0.375
22e	130.89	1.52	90.54	2.20	-	199.01	10.842 ± 0.317
22f	405.09	0.34	540.42	0.26	-	139.10	9.692 ± 0.204
22g	279.93	0.46	274.02	0.47	-	129.42	13.698 ± 0.000
trolox	-	-	-	-	-	-	0.189 ± 0.014
doxorubicin	1.91	33.23	1.18	53.79	-	63.47	-
acridine orange	5.72	14.39	6.59	12.49	27.28	82.30	-

[a] IC_{50} = 50% Inhibitory concentration after samples treatment for 24 hr. The data represented the mean values of three independent determinations.

[b] Cell lines include human hepatocyte carcinoma (Hep G2), human cervix epitheloid carcinoma (HeLa), mouse connective tissue fibroblast (L929) rhesus monkey kidney epithelial (LLC-MK2).

[c] -; not test

[d] SI = Selectivity index calculated as the ratio of IC_{50} value of the compound in normal cell (LLC-MK2)/ IC_{50} of the compound in cancer cell.

4.4 Extraction of bioactive compounds from plant for medical purposes

The dried leaves of *Cannabis indica* have been investigated for their constituents and biological activity. The plant was extracted by a supercritical CO_2 extractor at 37°C for 120 min. with a pressure of 4.5 MPa. After winterization of the residue by dissolving in ethanol, the crude extract was received without lipids and waxes. The bioactive extracts were purified using column chromatography to the isolation of CBD, CBN and CBG. The cannabinoid extract yielded CBD (4%), CBG (3%), CBN (11%), and a mixture of THC (13%). The structures of these compounds were confirmed by ^{1}H-NMR and compared with previous reports [72]. For the ^{1}H-NMR spectrum of CBD, the presence of vicinal proton at δ 5.57 (s, 1H, H-2), and the geminal proton at δ 4.66 (s, 1H, H-9), and 4.55 (s, 1H H-9) was indicated a characteristic of a cannabinoid. Three methyl groups showed signal at δ 1.79 (s, 3H, H-7 methyl), 1.66 (s, 3H, H-10 methyl), and 0.88 (t, J = 6.9 Hz, 3H, H-5″ methyl). The ^{1}H-NMR spectrum of CBN exhibited the extra aromatic protons at δ 8.18 (s, 1H, H-2), 7.14 (d, J = 7.8 Hz, 1H, H-4), and 7.06 (d, J = 7.8 Hz, 1H, H-5) including a methyl attached to a benzene ring at δ 2.37 (s, 3H, C-3 methyl). In the ^{1}H-NMR spectrum of CBG, the appearance of singlet at δ 6.32 (s, 1H) and 6.28 (s, 1H) corresponded to the two aromatic protons. The four methyl peaks showed singlets at δ 2.09 (s, 3H, H-3′), 1.70 (s, 3H, H-9′), 1.60 (s, 3H, H-8′), and 1.10 (s, 3H, H-5″) that confirmed the cannabinoid structure. In addition, a mixture of Δ^8-THC and Δ^9-THC failed to separate under this condition and the ^{1}H-NMR spectra showed peaks at a ratio of 1:1. The ^{1}H-NMR spectrum of Δ^8-THC was similar to Δ^9-THC but the vinyl proton of Δ^8-THC was moved upfield to δ 5.43 (m, 1H, H-4). While, this proton of Δ^9-THC exhibited 6.31 (s, 1H, H-4) due to deshielding effect on this proton with the OH group.

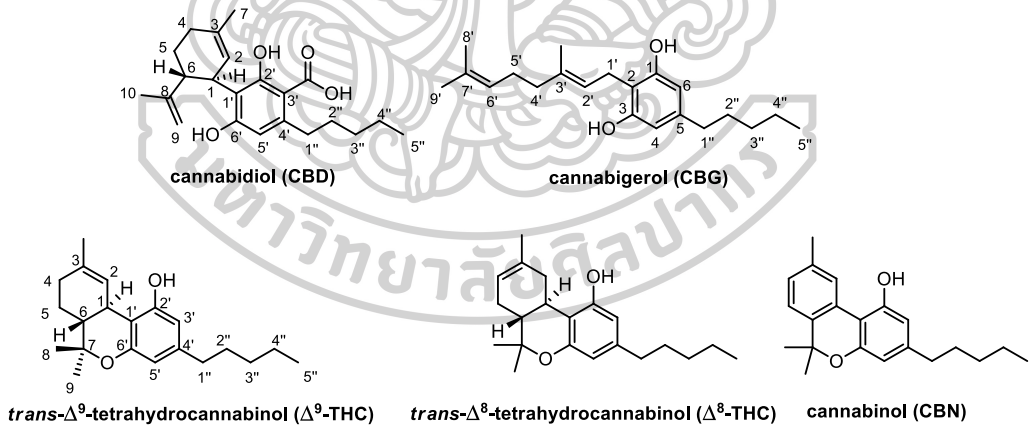


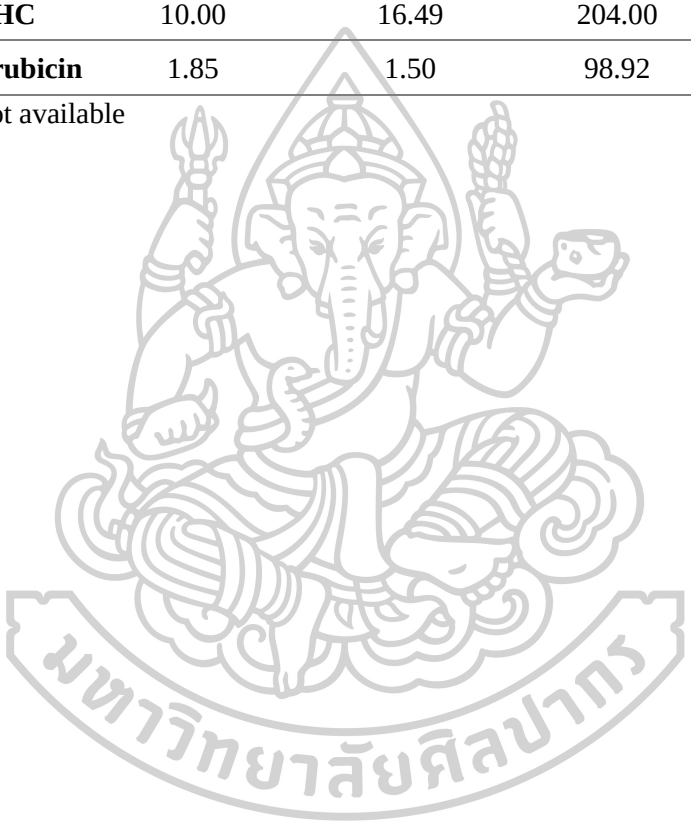
Figure 46. Structures of the cannabinoids

Next, we demonstrated *in vitro* cytotoxicity of all the isolated compound and crude extract against two tumor cell lines including two normal cell line. The results of their cytotoxic activities are depicted in Table 16. Most of the test compounds showed moderate anticancer activities except CBG. In particular, CBD exhibited significant cytotoxic activities against MDA-MB-231 cell line with IC_{50} values of 4.14 µg/mL with low cytotoxicity to LLC-MK2 normal cells.

Table 16. *In Vitro* cytotoxicity (IC_{50}, µg/mL) activities of cannabinoid extracts

Sample	IC_{50} (µg/mL)			
	HeLa	**MDA-MB-231**	**LLC-MK2**	**Vero**
Crude	9.97	na[a]	174.00	13.41
CBG	73.75	253.35	725.76	699.77
CBN	6.16	na	237.00	10.62
CBD	7.00	4.14	200.00	39.77
THC	10.00	16.49	204.00	67.23
Doxorubicin	1.85	1.50	98.92	99.48

[a] na = not available



CHAPTER 5
CONCLUSIONS

In brief, twenty-eight compounds (**21-24**) were synthesized *via* the key steps as Pictet-Springler reaction and aromatization. Pictet-spengler reaction from tryptamine (**15**) or *L*-tryptophan methyl ester (**16**) and various aldehydes gave 1-disubstituted THßCs (**23a-g**) in 9-84% yield and diastereomeric mixture of 1,3-disubstituted THßCs (**24a-g**) in 50-88% yield. Moreover, aromatization by heating substates with sulfur in xylene under ambient oxygen provided 1-disubstituted ßCs (**21a-g**) and 1,3-disubstituted βCs (**22a-g**) in moderate to high yield. The first investigation of the obtained analogs (**21-24**) derivatives was screened for their potential *in vitro* cytotoxic effects on two human cancer cells and two normal cell lines including antioxidant activity by DPPH radical-scavenging assay. Unfortunately, the synthesized compounds shown poor both activities. And the result reveals that the aromatic substituents at position 1 and the presence of methyl ester at position 3 displayed an important role for enhancement the anticancer activity on THßC derivatives.

In continuous study, we attempted to investigate the influence of configuration at C-1 position on their biological activities. Then, 1-substituted THßCs (**17b-i**) were synthesized in enantioselective synthesis. Firstly, 1-substituted THßCs (**17b-i**) have been synthesized by construction of piperidine ring *via* asymmetric Pictet-Spengler from Schiff bases (**26b** and **26e**) using Boc-protected proline (**27**) and *N,N*-phthaloyl-protected amino acid chloride (**28**) as chiral auxiliary. The result found that this condition was unsuccessful due to the steric repulsion between chiral auxiliary and substituent at position 1 of the parent compound. Furthermore, the enantioselective synthesis of THßCs (**17b-i**) were synthesized *via* asymmetric Pictet-Spengler reaction by benzoic acid and a chiral thiourea co-catalysts from tryptamine (**15**) and various aldehydes for 10 days. The 1-substituted THßCs (**17b-i**) are obtained in high ee and low to moderate yield. Additionally, comparison the influence of substituted methyl ester at C-3, the *trans* diastereomer (***trans*-24b-i**) were separated from the diastereomeric mixture and assigned the stereochemistry by their 1D and 2D NMR spectra. Afterward, all of them have been investigated their biological activities.

Furthermore, the leave extracts of *Cannabis indica* yielded the cannabinoids which were CBD (4%), CBG (3%), CBN (11%), and a mixture of THC (13%). The crude extract and the isolated cannabinoids were investigated for their *in vitro* cytotoxicity activity against two cancer cell lines. Most of them displayed low to moderate cytotoxic effect. Whereas, CBD exhibited the most significant anticancer property against MDA-MB-231 cell line along with low cytotoxic activity against normal cell LLC-MK2. The initial biological activity showed that these cannabinoids may be a potential candidate for anticancer drug development but they should be further pharmaceutical and medicinal studies in terms of the long-term adverse effects.

REFERENCES

1. Callaway, C.J., *Various Alkaloid Profiles in Decoctions of Banisteriopsis Caapi.* J. Psychoactive Drugs, 2005. **37**(2): p. 151-155.
2. Kam, S.T.S., M. K. , *Alkaloids from Kopsia griffithii.* Phytochemistry, 1998. **47**(1): p. 145-147.
3. Tolkachev, O.N., et al., *Phytochemical study of the bark of some plants of the Elaeagnaceae family as a natural source of β-carboline indole alkaloids.* Pharmaceutical Chemistry Journal, 2008. **42**(11): p. 630-632.
4. Seo, C., et al., *Isolation and structure elucidation of siliendines A–D, new β-carboline alkaloids from Silene seoulensis.* Phytochemistry Letters, 2020. **36**: p. 58-62.
5. Davis, R.A., A.R. Carroll, and R.J. Quinn, *Eudistomin V, a New β-Carboline from the Australian Ascidian Pseudodistoma aureum.* Journal of Natural Products, 1998. **61**(7): p. 959-960.
6. Rommelspacher, H., et al., *Pharmacological properties of tetrahydronorharmane (Tryptoline).* Naunyn-Schmiedeberg's Archives of Pharmacology, 1977. **298**(2): p. 83-91.
7. Airaksinen, M.M., et al., *The Uptake of 6-Methoxy-1,2,3,4-Tetrahydro-β-carboline and its Effect on 5-Hydroxytryptamine Uptake and Release in Blood Platelets.* Acta Pharmacologica et Toxicologica, 1978. **43**(5): p. 375-380.
8. Pähkla, R., et al., *Comparison of the antioxidant activity of melatonin and pinoline in vitro.* Journal of Pineal Research, 1998. **24**(2): p. 96-101.
9. Ding, L., H.-M. Dahse, and C. Hertweck, *Cytotoxic Alkaloids from Fusarium incarnatum Associated with the Mangrove Tree Aegiceras corniculatum.* Journal of Natural Products, 2012. **75**(4): p. 617-621.
10. Samita, F., et al., *Isolation of a new β-carboline alkaloid from aerial parts of Triclisia sacleuxii and its antibacterial and cytotoxicity effects.* Natural Product Research, 2017. **31**(5): p. 529-536.
11. Gabriel, R.S., et al., *β-Carboline-1-propionic acid alkaloid: antileishmanial and cytotoxic effects.* Revista Brasileira de Farmacognosia, 2019. **29**(6): p. 755-762.
12. Inman, W.D., et al., *A β-Carboline Alkaloid from the Papua New Guinea Marine Sponge Hyrtios reticulatus.* Journal of Natural Products, 2010. **73**(2): p. 255-257.
13. Peng, J., et al., *Manadomanzamines A and B: A Novel Alkaloid Ring System with Potent Activity against Mycobacteria and HIV-1.* Journal of the American Chemical Society, 2003. **125**(44): p. 13382-13386.
14. Lone, S.H., K.A. Bhat, and M.A. Khuroo, *Arglabin: From isolation to antitumor evaluation.* Chemico-Biological Interactions, 2015. **240**: p. 180-198.
15. Huestis, M.A., et al., *Cannabidiol Adverse Effects and Toxicity.* Current neuropharmacology, 2019. **17**(10): p. 974-989.
16. Steinmann, J., et al., *Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea.* British journal of pharmacology, 2013. **168**(5): p. 1059-1073.
17. Ferraz da Costa, D.C., et al., *Anticancer Potential of Resveratrol, β-Lapachone and Their Analogues.* Molecules, 2020. **25**(4).

18. Ardalani, H., A. Avan, and M. Ghayour-Mobarhan, *Podophyllotoxin: a novel potential natural anticancer agent.* Avicenna journal of phytomedicine, 2017. **7**(4): p. 285-294.
19. Maloney, K.N., et al., *Lodopyridone, a Structurally Unprecedented Alkaloid from a Marine Actinomycete.* Organic Letters, 2009. **11**(23): p. 5422-5424.
20. Abdallah, M.S., et al., *Determination of Phenolics and Flavonoids of Some Useful Medicinal Plants and Bioassay-Guided Fractionation Substances of Sclerocarya birrea (A. Rich) Hochst Stem (Bark) Extract and Their Efficacy Against Salmonella typhi.* Frontiers in Chemistry, 2021. **9**(541).
21. Heinrich, M. and S. Anagnostou, *From Pharmacognosia to DNA-Based Medicinal Plant Authentication – Pharmacognosy through the Centuries.* Planta Med, 2017. **83**(14/15): p. 1110-1116.
22. Atanasov, A.G., et al., *Discovery and resupply of pharmacologically active plant-derived natural products: A review.* Biotechnology Advances, 2015. **33**(8): p. 1582-1614.
23. Shams Eldin, S.M., et al., *Bioactivity-Guided Isolation of Potential Antidiabetic and Antihyperlipidemic Compounds from Trigonella stellata.* Journal of Natural Products, 2018. **81**(5): p. 1154-1161.
24. Krumsri, R., et al., *Assessment of Allelopathic Potential of Senna garrettiana Leaves and Identification of Potent Phytotoxic Substances.* Agronomy, 2022. **12**(1): p. 139.
25. Minh, T.N., et al., *Isolation and Purification of Bioactive Compounds from the Stem Bark of Jatropha podagrica.* Molecules (Basel, Switzerland), 2019. **24**(5): p. 889.
26. Cavaliere, C., et al., *Liquid Chromatographic Strategies for Separation of Bioactive Compounds in Food Matrices.* Molecules (Basel, Switzerland), 2018. **23**(12): p. 3091.
27. Dholwani, K.K., et al., *A review on plant-derived natural products and their analogs with anti-tumor activity.* Indian journal of pharmacology, 2008. **40**(2): p. 49-58.
28. Jebali, J., et al., *Tunisian Native Mentha pulegium L. Extracts: Phytochemical Composition and Biological Activities.* Molecules, 2022. **27**(1): p. 314.
29. Gómez, J.V., et al., *Selected plant essential oils and their main active components, a promising approach to inhibit aflatoxigenic fungi and aflatoxin production in food.* Food Additives & Contaminants: Part A, 2018. **35**(8): p. 1581-1595.
30. Salehi, B., et al., *The Therapeutic Potential of Naringenin: A Review of Clinical Trials.* Pharmaceuticals, 2019. **12**(1).
31. Cui, H., et al., *Chapter 11 - Application of antimicrobial-loaded nano/microcarriers in different food products*, in *Application of Nano/Microencapsulated Ingredients in Food Products*, S.M. Jafari, Editor. 2021, Academic Press. p. 469-517.
32. Hillig, K.W. and P.G. Mahlberg, *A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae).* American Journal of Botany, 2004. **91**(6): p. 966-975.
33. McPartland, J.M. and E.B. Russo, *Cannabis and Cannabis Extracts.* Journal of Cannabis Therapeutics, 2001. **1**(3-4): p. 103-132.

34. Hillig, K.W., *Genetic evidence for speciation in Cannabis (Cannabaceae).* Genetic Resources and Crop Evolution, 2005. **52**(2): p. 161-180.
35. Salami, S.A., et al., *It Is Our Turn to Get Cannabis High: Put Cannabinoids in Food and Health Baskets.* Molecules, 2020. **25**(18): p. 4036.
36. Ovenden, P.B.S.N., L. J.; Liptrol, H. C.; Willis, H. R.; Tapiolas, M. D.; Wright, D. A.; Motti, A. C. , *Callophycin A, a cytotoxic tetrahydro-β-carboline from the red alga Callophycus oppositifolius.* Phytochemistry Letters., 2011. **4**(2): p. 69-71.
37. Cesar, L.M.M., et al., *Structure Determination of Hydroxytrypargine: A New Tetrahydro-β-Carboline Toxin from the Venom of the Spider Parawixia bistriata.* 2005. **88**(4): p. 796-801.
38. Baumeister, A.A.H., M. F.; Uzelac, S. M. , *The myth of reserpine-induced depression: role in the historical development of the monoamine hypothesis.* J. Hist. Neurosci., 2003. **12**(2): p. 207-22.
39. Rashid, M.A., K.R. Gustafson, and M.R. Boyd, *New Cytotoxic N-Methylated β-Carboline Alkaloids from the Marine Ascidian Eudistoma gilboverde.* Journal of Natural Products, 2001. **64**(11): p. 1454-1456.
40. Wang, W., et al., *β-Carboline Alkaloids from a Korean Tunicate Eudistoma sp.* Journal of Natural Products, 2008. **71**(2): p. 163-166.
41. Huang, H., et al., *Antimalarial β-Carboline and Indolactam Alkaloids from Marinactinospora thermotolerans, a Deep Sea Isolate.* Journal of Natural Products, 2011. **74**(10): p. 2122-2127.
42. Chen, Y.-F., et al., *β-Carboline Alkaloids from Stellaria dichotoma var. lanceolata and Their Anti-inflammatory Activity.* Journal of Natural Products, 2010. **73**(12): p. 1993-1998.
43. Cao, R., et al., *β-Carboline Alkaloids: Biochemical and Pharmacological Functions.* Current Medicinal Chemistry, 2007. **14**(4): p. 479-500.
44. Narayanan, K.S., L.; Cook, M. J., *Carboxyl-Mediated Pictet-Spengler Reaction. Direct Synthesis of 1,2,3,4-Tetrahydro-/3-carbolines from Tryptamine-2-carboxylic Acids.* J. Org. Chem., 1991. **56**(1): p. 359-365.
45. Ascic, E.H., L. C.; Le Quement, T. S.; Nielsen, E. T. , *Synthesis of tetrahydro-β-carbolines via isomerization of N-allyltryptamines: a metal-catalyzed variation on the Pictet–Spengler theme.* Chem. Commun., 2012. **48**(27): p. 3345–3347.
46. Spindler, A., K. Stefan, and M. Wiese, *Synthesis and Investigation of Tetrahydro-β-carboline Derivatives as Inhibitors of the Breast Cancer Resistance Protein (ABCG2).* Journal of Medicinal Chemistry, 2016. **59**(13): p. 6121-6135.
47. Ramana, V.P.S., P.; Krishna, S. B.; Reddy, M. K. K.; Mohan, G.; Reddy, S. C., *Ultrasound-Promoted Synthesis of Tetrahydro-ß-Carbolines Using Triton-X 100 Catalyst via Pictet-Spengler Reaction.* Can. Chem. Trans., 2016. **4**(3): p. 415-424.
48. Ezquerra, J., et al., *Stereoselective synthesis of 1,3,4-substituted tetrahydro-β-carbolines from indoles based on selective transformations.* Tetrahedron, 1997. **53**(24): p. 8237-8248.
49. Horiguchi, Y., et al., *A Synthesis of Chiral 1,1,3-Trisubstituted 1,2,3,4-Tetrahydro-β-carbolines by the Pictet–Spengler Reaction of Tryptophan and Ketones: Conversion of (1R,3S)-Diastereomers into Their (1S,3S)-Counterparts*

by Scission of the C(1)–N(2) Bond. Chemical and Pharmaceutical Bulletin, 2003. **51**(12): p. 1368-1373.

50. Ungemach, F., et al., *Stereospecific synthesis of trans-1,3-disubstituted-1,2,3,4-tetrahydro-.beta.-carbolines.* The Journal of Organic Chemistry, 1981. **46**(1): p. 164-168.
51. Cox, E.D., et al., *Enantiospecific Formation of Trans 1,3-Disubstituted Tetrahydro-β-carbolines by the Pictet−Spengler Reaction and Conversion of Cis Diastereomers into Their Trans Counterparts by Scission of the C-1/N-2 Bond.* The Journal of Organic Chemistry, 1997. **62**(1): p. 44-61.
52. Han, D., et al., *Conformational Analysis of the cis- and trans-Adducts of the Pictet−Spengler Reaction. Evidence for the Structural Basis for the C(1)−N(2) Scission Process in the cis- to trans-Isomerization.* Journal of Natural Products, 2007. **70**(1): p. 75-82.
53. Kumpaty, H.J., et al., *Study of the Cis to Trans Isomerization of 1-Phenyl-2,3-disubstituted Tetrahydro-β-carbolines at C(1). Evidence for the Carbocation-Mediated Mechanism.* The Journal of Organic Chemistry, 2009. **74**(7): p. 2771-2779.
54. Xiao, S., et al., *Syntheses of chiral 1,3-disubstituted tetrahydro-β-carbolines via CIAT process: highly stereoselective Pictet–Spengler reaction of d-tryptophan ester hydrochlorides with various aldehydes.* Tetrahedron: Asymmetry, 2009. **20**(4): p. 430-439.
55. Ma, J., et al., *Total Synthesis of the Opioid Agonistic Indole Alkaloid Mitragynine and the First Total Syntheses of 9-Methoxygeissoschizol and 9-Methoxy-Nb-methylgeissoschizol.* Organic Letters, 2007. **9**(18): p. 3491-3494.
56. Kawate, T., et al., *Enantioselective asymmetric Pictet-Spengler reaction catalyzed by diisopinocampheylchloroborane.* Tetrahedron: Asymmetry, 1996. **7**(5): p. 1249-1252.
57. Wu, X., et al., *Organocatalyzed enantioselective one-pot three-component access to indoloquinolizidines by a Michael addition–Pictet–Spengler sequence.* Chemical Communications, 2010. **46**(16): p. 2733-2735.
58. Klausen, R.S. and E.N. Jacobsen, *Weak Brønsted Acid−Thiourea Co-catalysis: Enantioselective, Catalytic Protio-Pictet−Spengler Reactions.* Organic Letters, 2009. **11**(4): p. 887-890.
59. Gremmen, C., et al., *Enantiopure Tetrahydro-β-carbolines via Pictet−Spengler Reactions with N-Sulfinyl Tryptamines.* Organic Letters, 2000. **2**(13): p. 1955-1958.
60. Zhang, X., W. Jiang, and Z. Sui, *Concise Enantioselective Syntheses of Quinolactacins A and B through Alternative Winterfeldt Oxidation.* The Journal of Organic Chemistry, 2003. **68**(11): p. 4523-4526.
61. Ducrot, P., C. Rabhi, and C. Thal, *Synthesis of Tetrahydro-β-carbolines and Studies of the Pictet–Spengler Reaction.* Tetrahedron, 2000. **56**(17): p. 2683-2692.
62. Ashok, P., et al., *Design, synthesis of new β-carboline derivatives and their selective anti-HIV-2 activity.* Bioorganic & Medicinal Chemistry Letters, 2015. **25**(6): p. 1232-1235.
63. Gaikwad, S., D. Kamble, and P. Lokhande, *Iodine-catalyzed chemoselective dehydrogenation and aromatization of tetrahydro-β-carbolines: A short*

synthesis of Kumujian-C, Eudistomin-U, Norharmane, Harmane Harmalan and Isoeudistomine-M. Tetrahedron Letters, 2018. **59**(25): p. 2387-2392.
64. Szabó, T., et al., *First total synthesis of the β-carboline alkaloids trigonostemine A, trigonostemine B and a new synthesis of pityriacitrin and hyrtiosulawesine.* Tetrahedron Letters, 2019. **60**(22): p. 1471-1475.
65. Untergehrer, M. and F. Bracher, *A short divergent approach to highly substituted carbazoles and β-carbolines via in situ-generated diketoindoles.* Tetrahedron Letters, 2020. **61**(12): p. 151597.
66. Panice, M.R., et al., *New 3-tetrazolyl-β-carbolines and β-carboline-3-carboxylates with anti-cancer activity.* European Journal of Medicinal Chemistry, 2019. **179**: p. 123-132.
67. Wang, Z., et al., *A practical synthesis of β-carbolines by tetra-n-butylammonium bromide (TBAB)-mediated cycloaromatization reaction of aldehydes with tryptophan derivatives.* Chinese Chemical Letters, 2019. **30**(8): p. 1541-1544.
68. Thomas, B.F. and M.A. ElSohly, *Chapter 1 - The Botany of Cannabis sativa L*, in *The Analytical Chemistry of Cannabis*, B.F. Thomas and M.A. ElSohly, Editors. 2016, Elsevier. p. 1-26.
69. Chopra, G.S., *Man and Marijuana.* International Journal of the Addictions, 1969. **4**(2): p. 215-247.
70. Small, E. and A. Cronquist, *A PRACTICAL AND NATURAL TAXONOMY FOR CANNABIS.* TAXON, 1976. **25**(4): p. 405-435.
71. Pothier, J., *NATURAL PRODUCTS | Thin-Layer (Planar) Chromatography*, in *Encyclopedia of Separation Science*, I.D. Wilson, Editor. 2000, Academic Press: Oxford. p. 3459-3482.
72. Choi, Y.H., et al., *NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa.* Phytochemical Analysis, 2004. **15**(6): p. 345-354.
73. Dayanandan, P. and P.B. Kaufman, *Trichomes of Cannabis sativa L. (Cannabaceae).* American Journal of Botany, 1976. **63**(5): p. 578-591.
74. Barrett, F.S., et al., *"Hallucinations" Following Acute Cannabis Dosing: A Case Report and Comparison to Other Hallucinogenic Drugs.* Cannabis and cannabinoid research, 2018. **3**(1): p. 85-93.
75. Winek, C.L., *Some Historical Aspects of Marijuana.* Clinical Toxicology, 1977. **10**(2): p. 243-253.
76. Bornheim, L.M., et al., *Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain.* Drug Metabolism and Disposition, 1995. **23**(8): p. 825-831.
77. Devinsky, O., et al., *Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders.* Epilepsia, 2014. **55**(6): p. 791-802.
78. van Klingeren, B. and M. ten Ham, *Antibacterial activity of Δ9-tetrahydrocannabinol and cannabidiol.* Antonie van Leeuwenhoek, 1976. **42**(1): p. 9-12.
79. Ellis, R.J., et al., *Smoked medicinal cannabis for neuropathic pain in HIV: a randomized, crossover clinical trial.* Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 2009. **34**(3): p. 672-680.

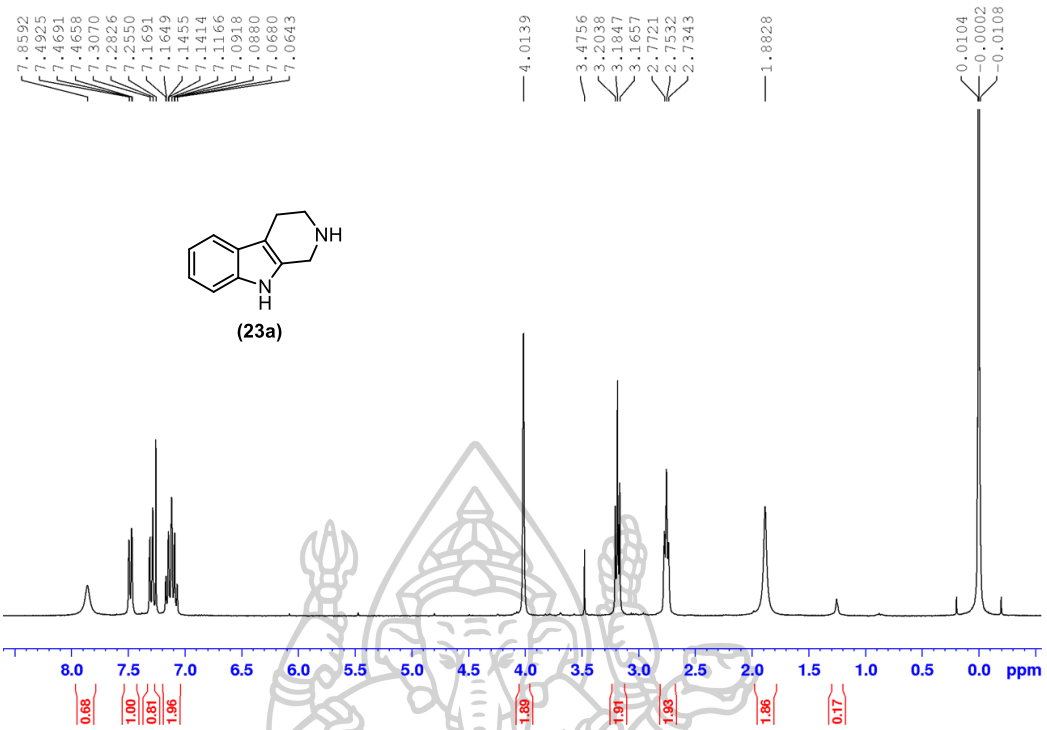
80. Ameri, A., *The effects of cannabinoids on the brain.* Progress in Neurobiology, 1999. **58**(4): p. 315-348.
81. Massi, P., et al., *Antitumor Effects of Cannabidiol, a Nonpsychoactive Cannabinoid, on Human Glioma Cell Lines.* Journal of Pharmacology and Experimental Therapeutics, 2004. **308**(3): p. 838-845.
82. Formukong, E.A., A.T. Evans, and F.J. Evans, *Analgesic and antiinflammatory activity of constituents ofCannabis sativa L.* Inflammation, 1988. **12**(4): p. 361-371.
83. Navari, R.M., *Pharmacological Management of Chemotherapy-Induced Nausea and Vomiting.* Drugs, 2009. **69**(5): p. 515-533.
84. Hampson, A.J., et al., *Cannabidiol and (−)Δ⁹-tetrahydrocannabinol are neuroprotective antioxidants.* Proceedings of the National Academy of Sciences, 1998. **95**(14): p. 8268-8273.
85. Aramaki, H.I., et al., *Forensic Chemical Study on Marihuana. I. A Detection Method of the Principal Constituents by Thin-layer and Gas Chromatographies.* CHEMICAL & PHARMACEUTICAL BULLETIN, 1968. **16**(5): p. 822-826.
86. Tewari, S.N., S.P. Harpalani, and S.C. Sharma, *Separation and identification of the constituents of hashish (cannabis indica linn.) by thin-layer chromatography and its application in forensic analysis.* Microchimica Acta, 1974. **62**(6): p. 991-995.
87. Fischedick, J.T., et al., *A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in Cannabis sativa L.* Phytochemical Analysis, 2009. **20**(5): p. 421-426.
88. Gambelunghe, C., et al., *Cannabis Use Surveillance by Sweat Analysis.* Therapeutic Drug Monitoring, 2016. **38**(5).
89. Wang, Y.-H., et al., *Quantitative Determination of Δ9-THC, CBG, CBD, Their Acid Precursors and Five Other Neutral Cannabinoids by UHPLC-UV-MS.* Planta Med, 2018. **84**(04): p. 260-266.
90. Hädener, M., S. König, and W. Weinmann, *Quantitative determination of CBD and THC and their acid precursors in confiscated cannabis samples by HPLC-DAD.* Forensic Science International, 2019. **299**: p. 142-150.
91. McRae, G. and J.E. Melanson, *Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry.* Analytical and Bioanalytical Chemistry, 2020. **412**(27): p. 7381-7393.
92. Dora, L. and O. Clauder, *[The reduction of 1,2,3,4-tetrahydro-1-oxo-beta-carboline].* Acta Pharm. Hung., 1968. **38 2**: p. 78-83.
93. Singh, U.P., et al., *Antifungal activity of venenatine, an indole alkaloid isolated fromAlstonia venenata.* Folia Microbiol. (Praha), 2000. **45**(2): p. 173.
94. Qi, S.H., et al., *Antifouling and antibacterial compounds from the gorgonians Subergorgia suberosa and Scripearia gracillis.* Nat. Prod. Res., 2008. **22**(2): p. 154-166.
95. Saha, B., et al., *Water as an efficient medium for the synthesis of tetrahydro-β-carbolines via Pictet–Spengler reactions.* Tetrahedron Letters, 2007. **48**(8): p. 1379-1383.
96. Barbero, M., et al., *o-Benzenedisulfonimide as a reusable acid catalyst for an easy, efficient, and green synthesis of tetrahydroisoquinolines and tetrahydro-β-*

carbolines through Pictet–Spengler reaction. Tetrahedron Lett., 2010. **51**(48): p. 6356-6359.
97. Cheve, G.D., P.; Fruchart, J.; Teissier, E.; Poupaert, J.; Lesieur, D., *Antioxidant activity of pinoline analogues in the LDL oxidation model.* J. Med. Chem., 2002. **11**(7): p. 361-379.
98. Ikeda, R.I., T.; Iidaa, T.; Okabayashi, T.; Nishi, E.; Kurosawa, M.; Sakai, N.; Konakahara, T., *3-Benzylamino-ß-carboline derivatives induce apoptosis through G2/M arrest in human carcinoma cells HeLa S-3.* Eur. J. Med. Chem., 2011. **46**(5): p. 636-646.
99. Rashid, N., et al., *Cis-Diastereoselectivity in Pictet–Spengler Reactions of L-Tryptophan and Electronic Circular Dichroism Studies.* Chirality, 2012. **24**(10): p. 789-795.
100. Gary A. Piazza, A.H.A., *TETRAHYDRO-beta-CARBOLINE DERIVATIVES, SYNTHESIS AND USE THEREOF*, in *United States*, A. Birmingham, Editor. 2015, Southern Research Institute: United States.
101. Abadi, A.H., et al., *Synthesis of novel tadalafil analogues and their evaluation as phosphodiesterase inhibitors and anticancer agents.* Arzneimittel-Forschung, 2009. **59**(8): p. 415-421.
102. Singh, K., P.K. Deb, and P. Venugopalan, *Modified Pictet–Spengler reaction. A highly diastereoselective approach to 1,2,3-trisubstituted-1,2,3,4-tetrahydro-β-carbolines using perhydro-1,3-heterocycles.* Tetrahedron, 2001. **57**(37): p. 7939-7949.
103. Parameswaran, P.S., C.G. Naik, and V.R. Hegde, *Secondary Metabolites from the Sponge Tedania anhelans: Isolation and Characterization of Two Novel Pyrazole Acids and Other Metabolites.* J. Nat. Prod., 1997. **60**(8): p. 802-803.
104. Li, S., et al., *Synthesis and Bioactivity of β-Carboline Derivatives.* Natural Product Communications, 2010. **5**(10): p. 1934578X1000501016.
105. Kanamori, H., et al., *The Formation of the Mutagenic Substances in the Reaction between L-Ascorbic Acid and L-Tryptophan.* Chem. Pharm. Bull., 1980. **28**(10): p. 3143-3144.
106. Lippke, K.P., et al., *beta-Carbolines as benzodiazepine receptor ligands. 1. Synthesis and benzodiazepine receptor interaction of esters of beta-carboline-3-carboxylic acid.* J Med Chem, 1983. **26**(4): p. 499-503.
107. Snyder, H.R., et al., *The Synthesis of Derivatives of β-Carboline. II. Syntheses from dl-Tryptophan and Aldehydes1.* Journal of the American Chemical Society, 1948. **70**(1): p. 219-221.
108. Leite, A.C.L., et al., *Phthaloyl amino acids as anti-inflammatory and immunomodulatory prototypes.* Medicinal Chemistry Research, 2014. **23**(4): p. 1701-1708.
109. Homsi, A. and A. Kasideh, *Synthesis of some N-phthalimide amino acids derivatives and evaluation their biological activity.* International Journal of ChemTech Research, 2015. **8**: p. 1817-1825.
110. Mohammad, N.A., et al., *Optimization of the antioxidant-rich xanthone extract from mangosteen (Garcinia mangostana L.) pericarp via microwave-assisted extraction.* Heliyon, 2019. **5**(10): p. e02571.
111. Reuss, G., et al., *Formaldehyde, Ullmann's Encyclopedia of Industrial Chemistry.* Wiley-VCH, Weinheim,, 2000: p. 1-34.

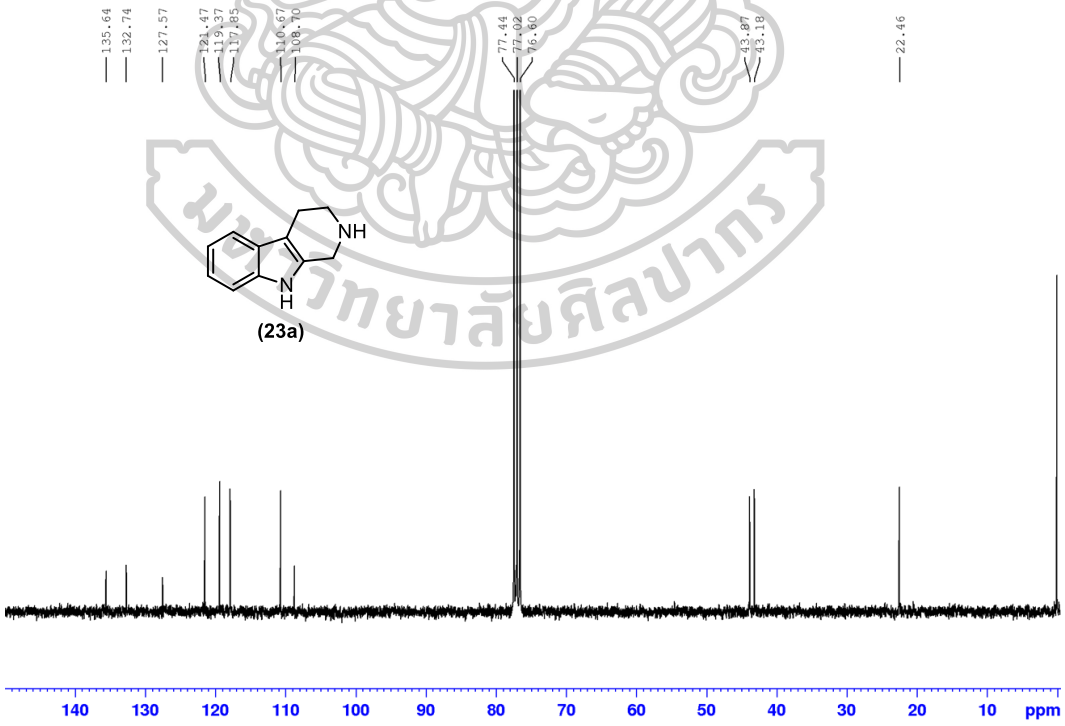
112. Waldmann, H., et al., *Asymmetric Pictet–Spengler Reactions Employing N,N-Phthaloyl Amino Acids as Chiral Auxiliary Groups.* Angewandte Chemie International Edition in English, 1995. **34**(21): p. 2402-2403.
113. Schmidt, G., et al., *Asymmetric Control in the Pictet–Spengler Reaction by Means of N-Protected Amino Acids as Chiral Auxiliary Groups.* Chemistry – A European Journal, 1996. **2**(12): p. 1566-1571.

APPENDIX

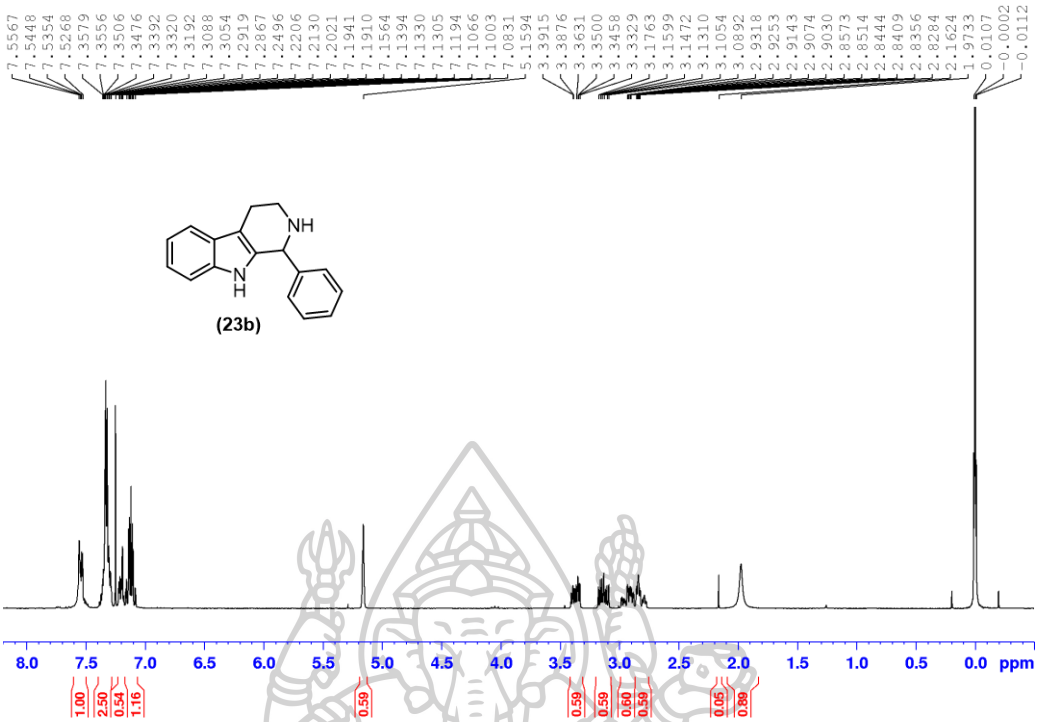
LIST OF NMR SPECTRAL DATA



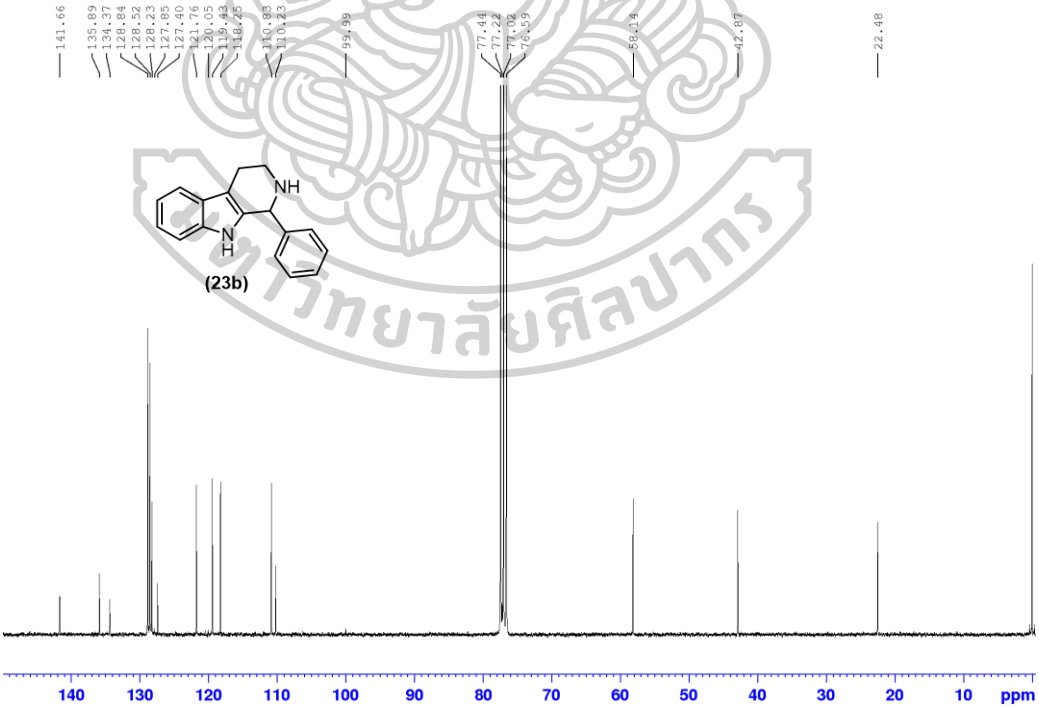
^{1}H-NMR spectrum of (**23a**) (300 MHz, $CDCl_3$)



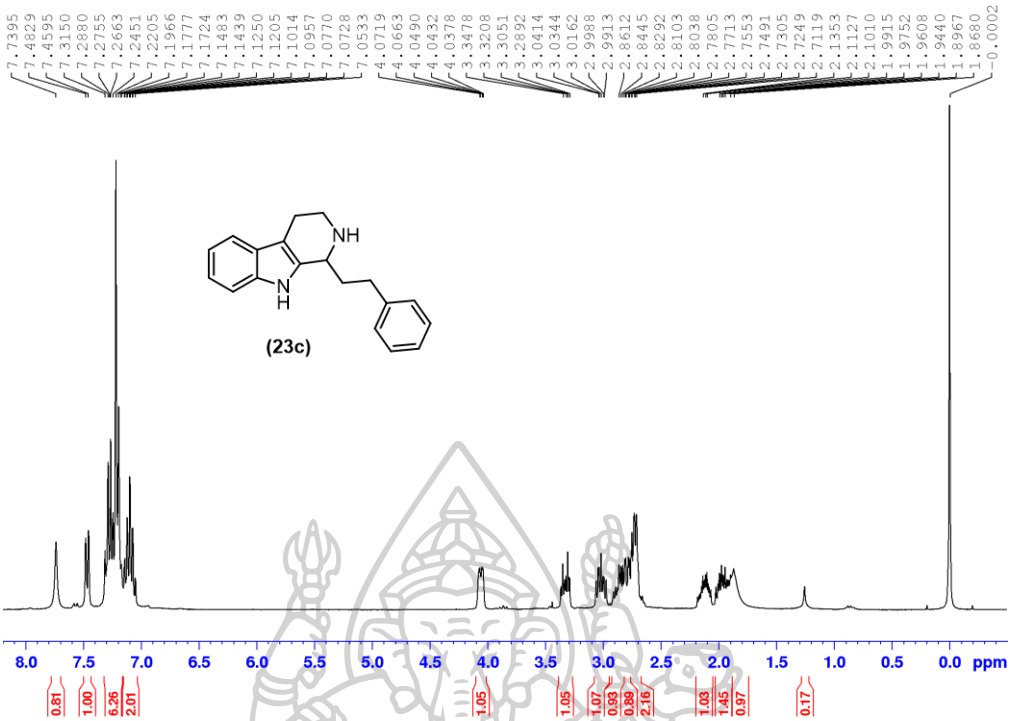
^{13}C-NMR spectrum of (**23a**) (75 MHz, $CDCl_3$)



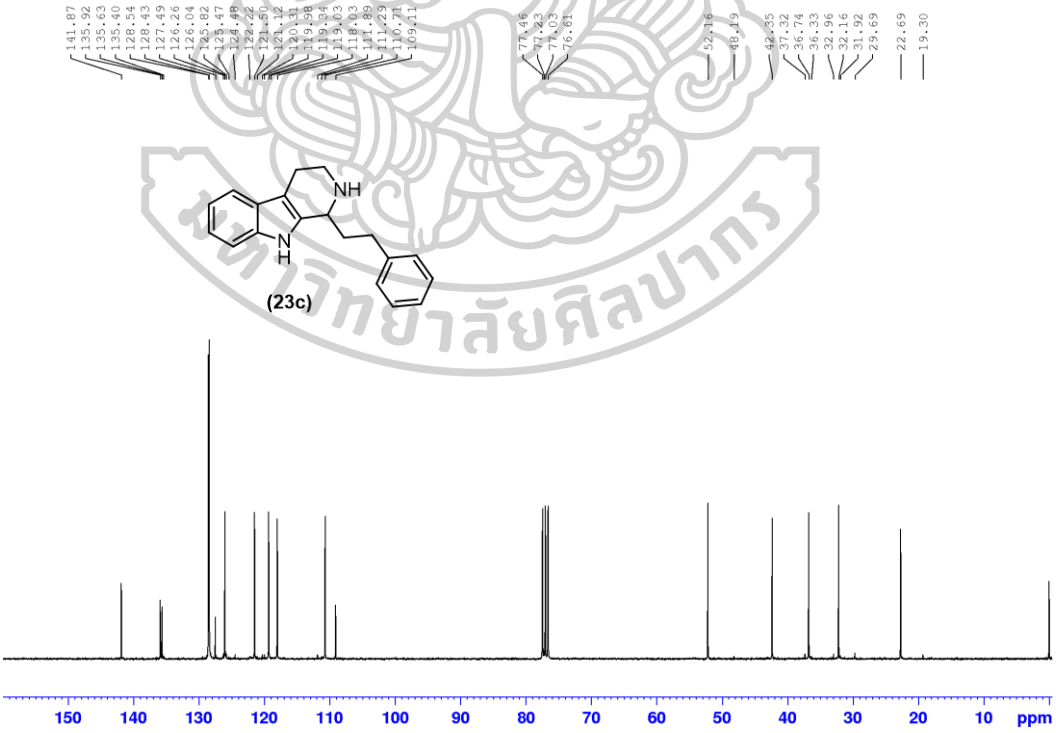
^{1}H-NMR spectrum of (**23b**) (300 MHz, $CDCl_3$)



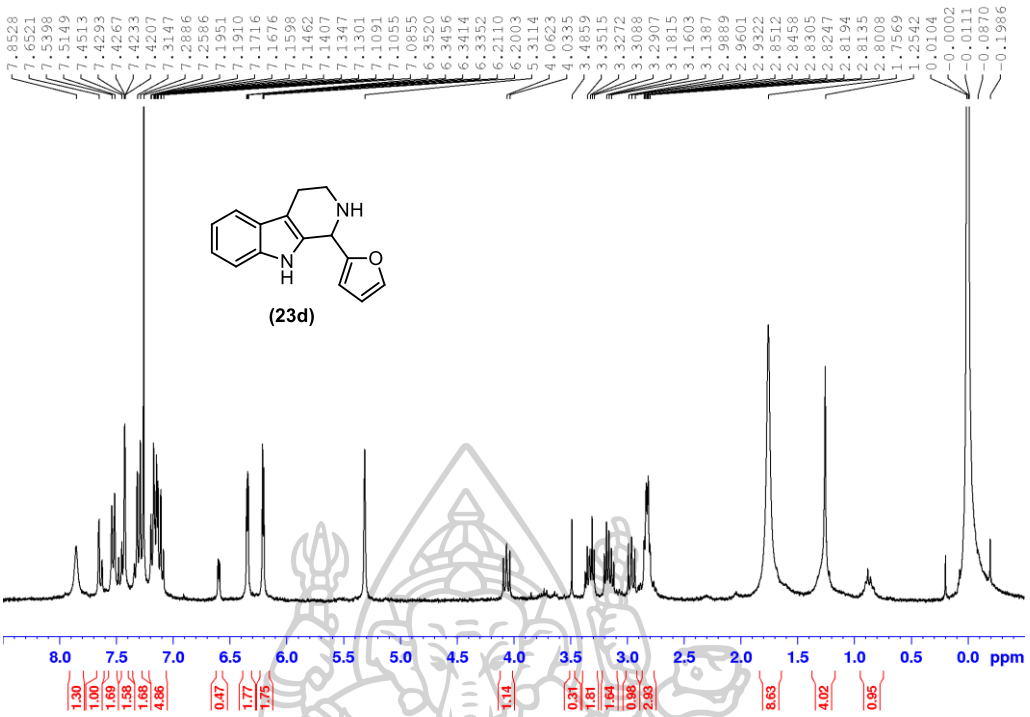
^{13}C-NMR spectrum of (**23b**) (75 MHz, $CDCl_3$)



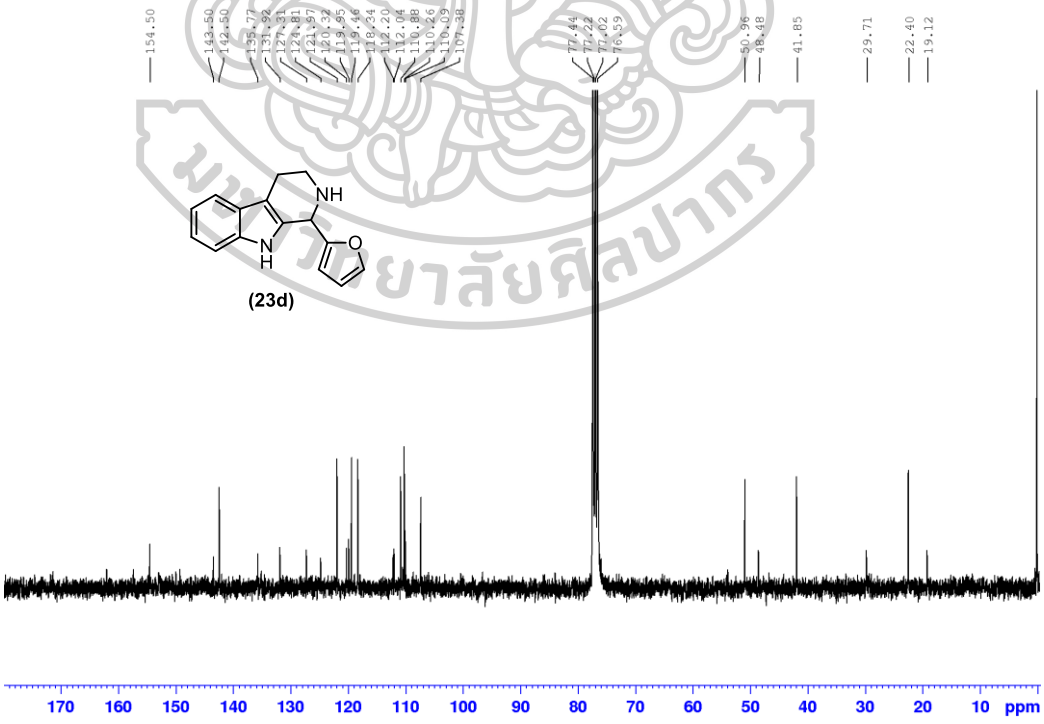
^{1}H-NMR spectrum of (**23c**) (300 MHz, $CDCl_3$)



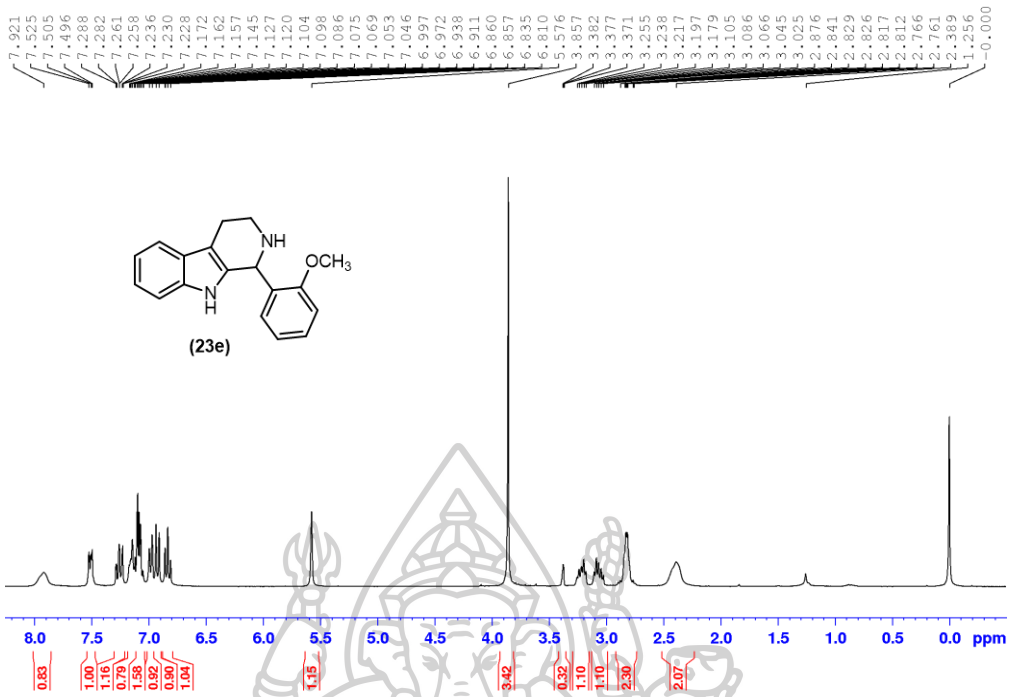
^{13}C-NMR spectrum of (**23c**) (75 MHz, $CDCl_3$)



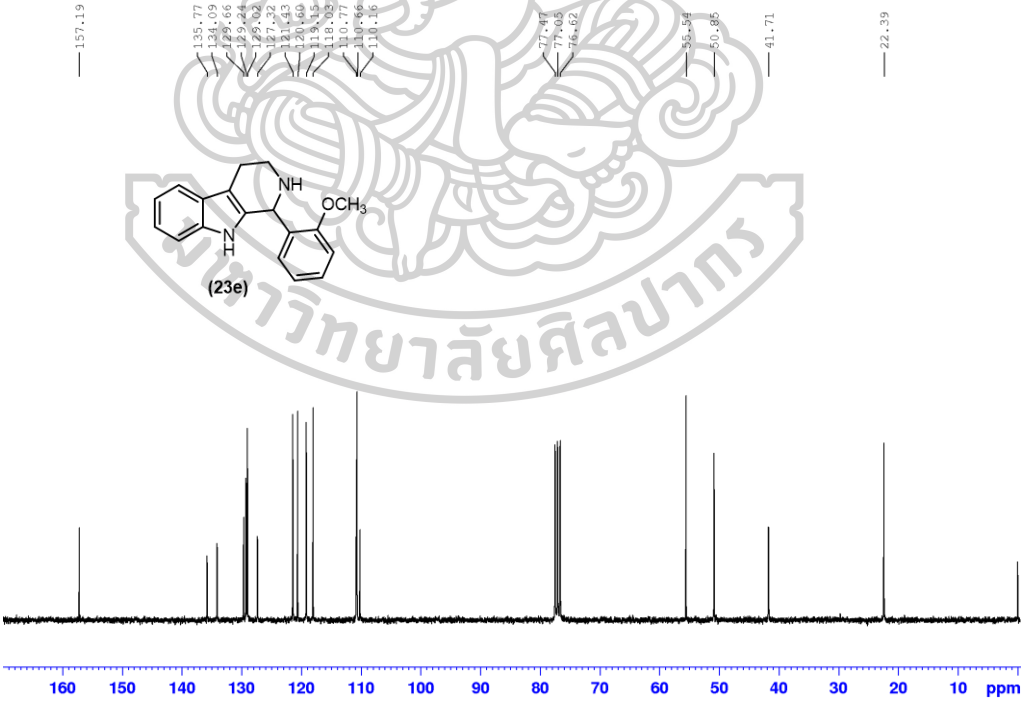
^{1}H-NMR spectrum of (**23d**) (300 MHz, $CDCl_3$)



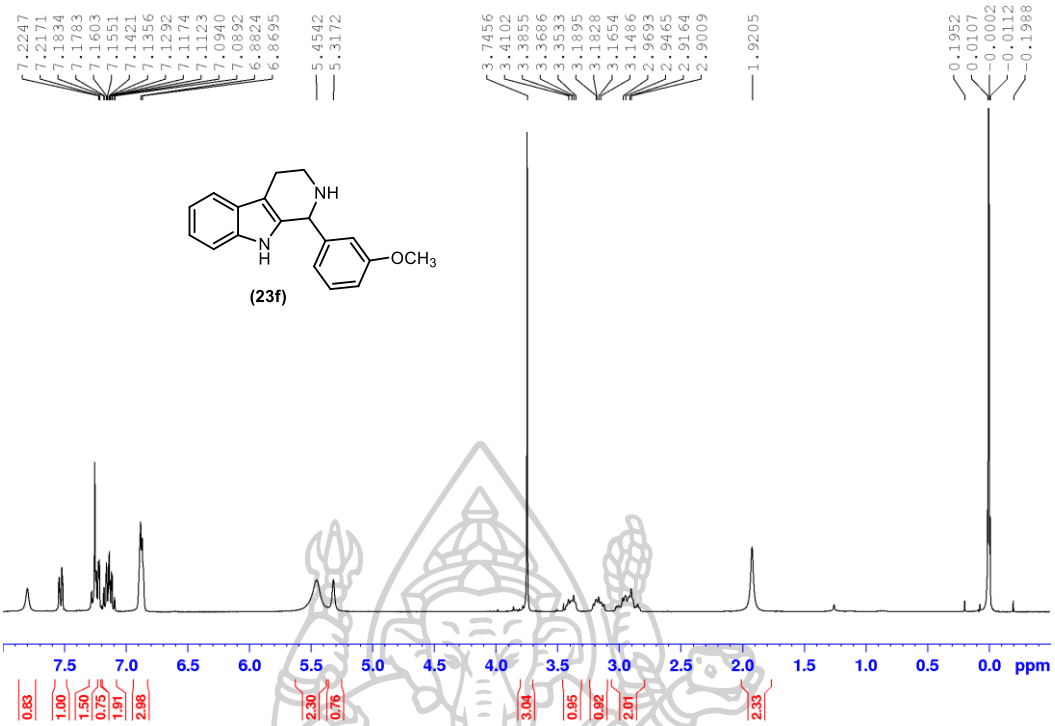
^{13}C-NMR spectrum of (**23d**) (75 MHz, $CDCl_3$)



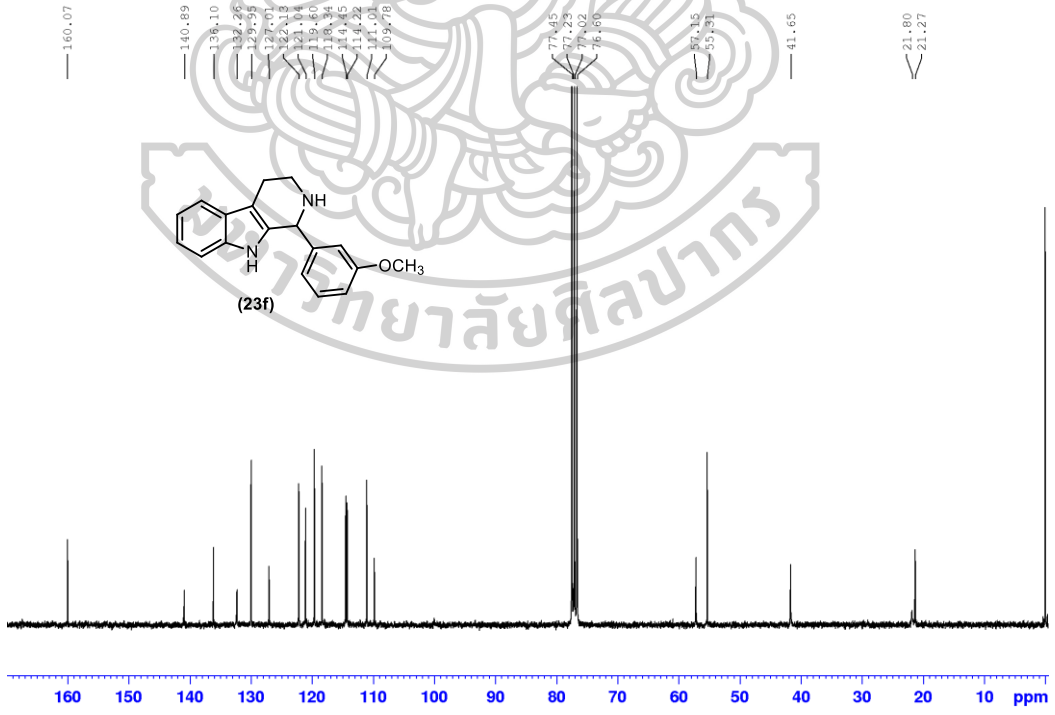
^{1}H-NMR spectrum of (**23e**) (300 MHz, $CDCl_3$)



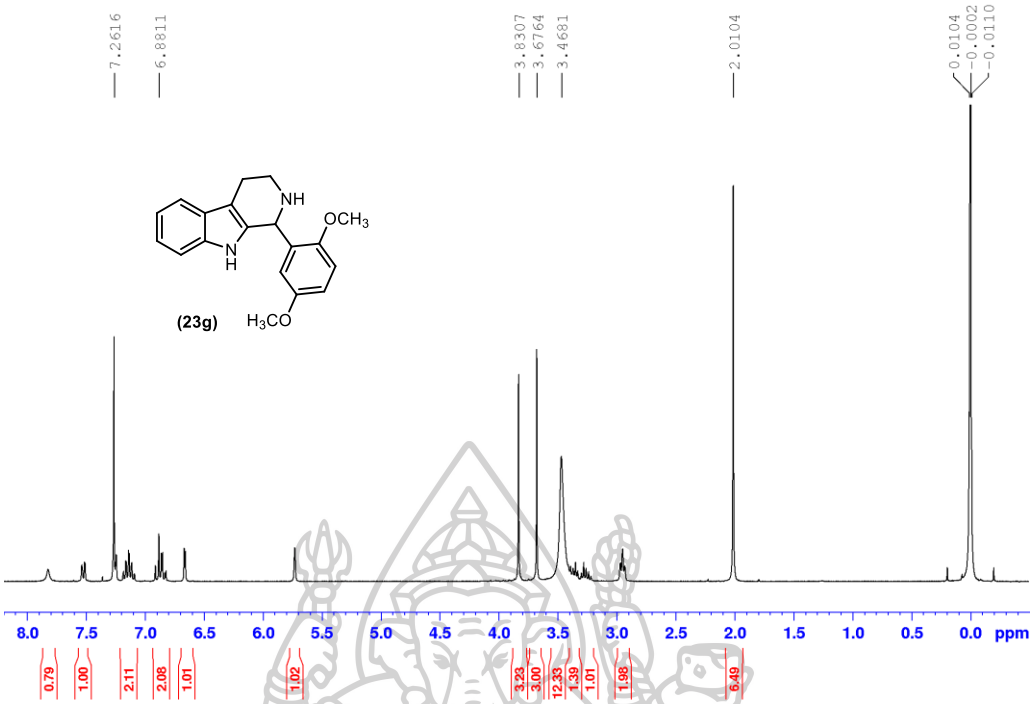
^{13}C-NMR spectrum of (**23e**) (75 MHz, $CDCl_3$)



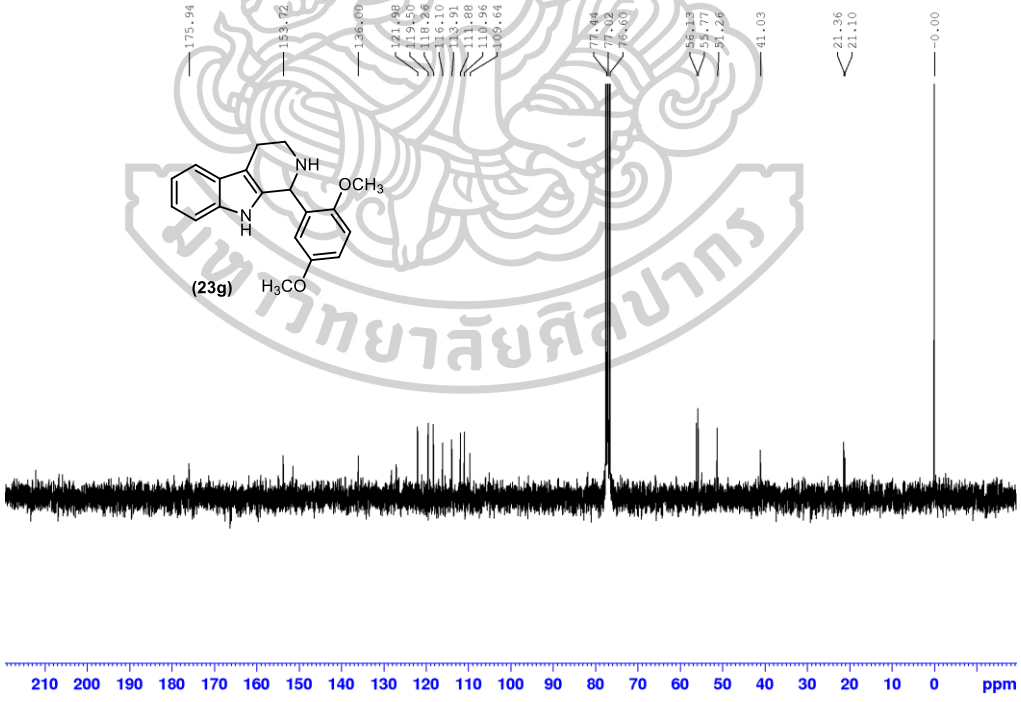
^{1}H-NMR spectrum of (**23f**) (300 MHz, $CDCl_3$)



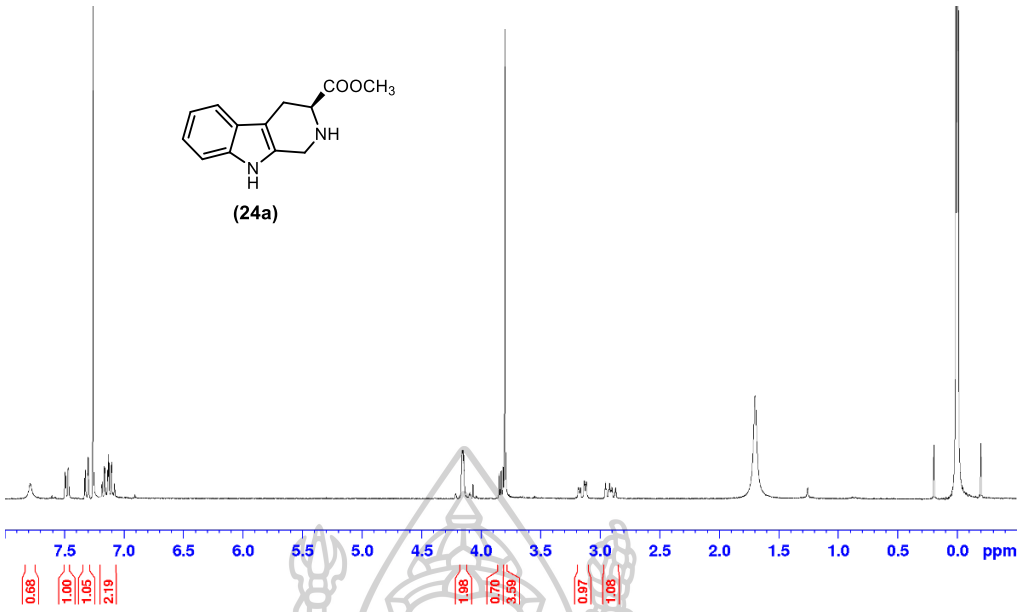
^{13}C-NMR spectrum of (**23f**) (75 MHz, $CDCl_3$)



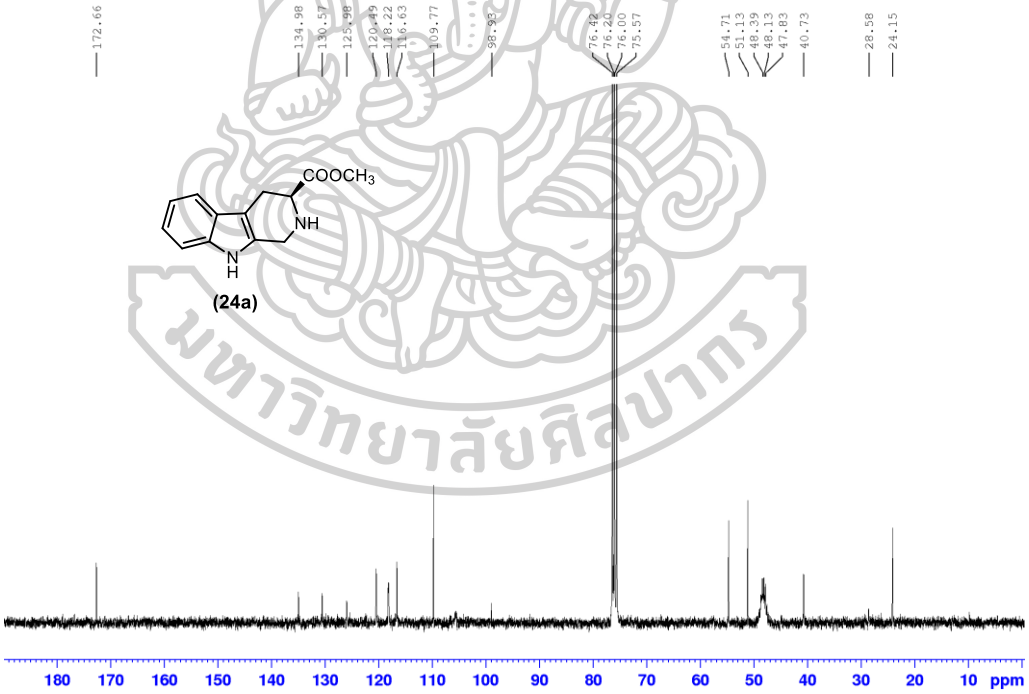
^{1}H-NMR spectrum of (**23g**) (300 MHz, $CDCl_3$)



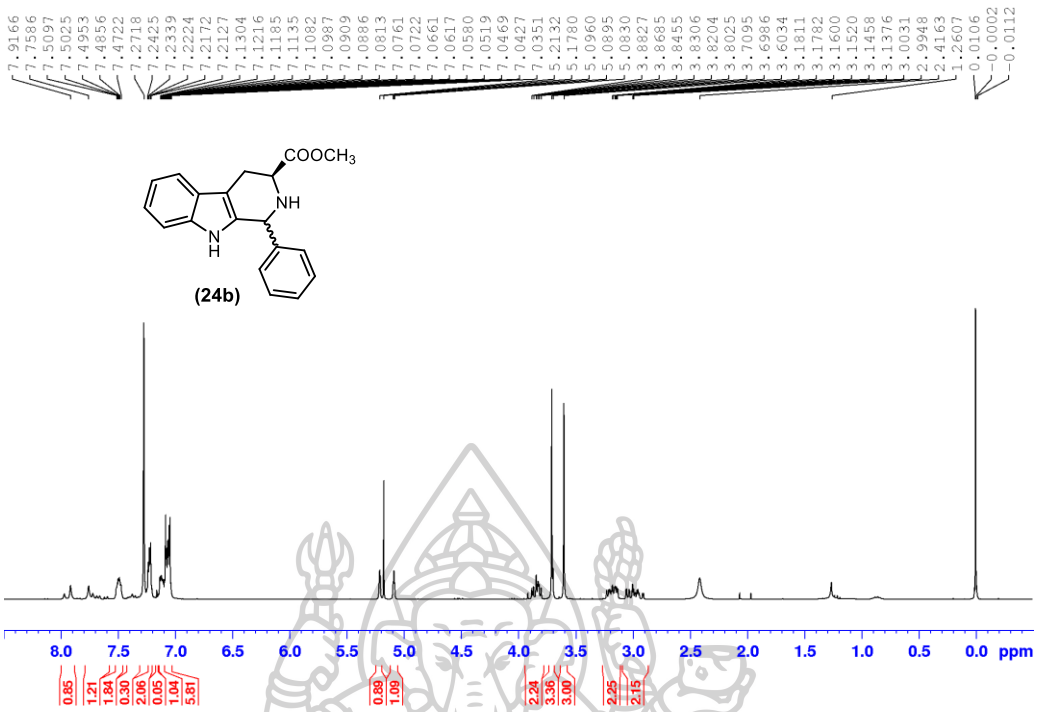
^{13}C-NMR spectrum of (**23g**) (75 MHz, $CDCl_3$)



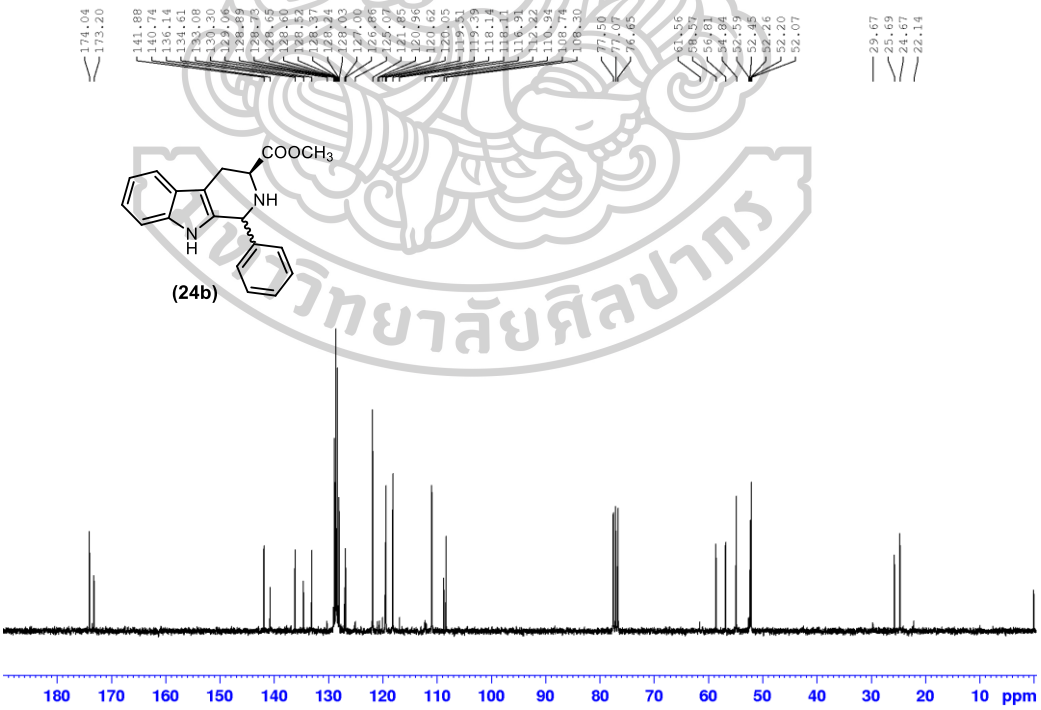
^{1}H-NMR spectrum of (**24a**) (300 MHz, $CDCl_3$)



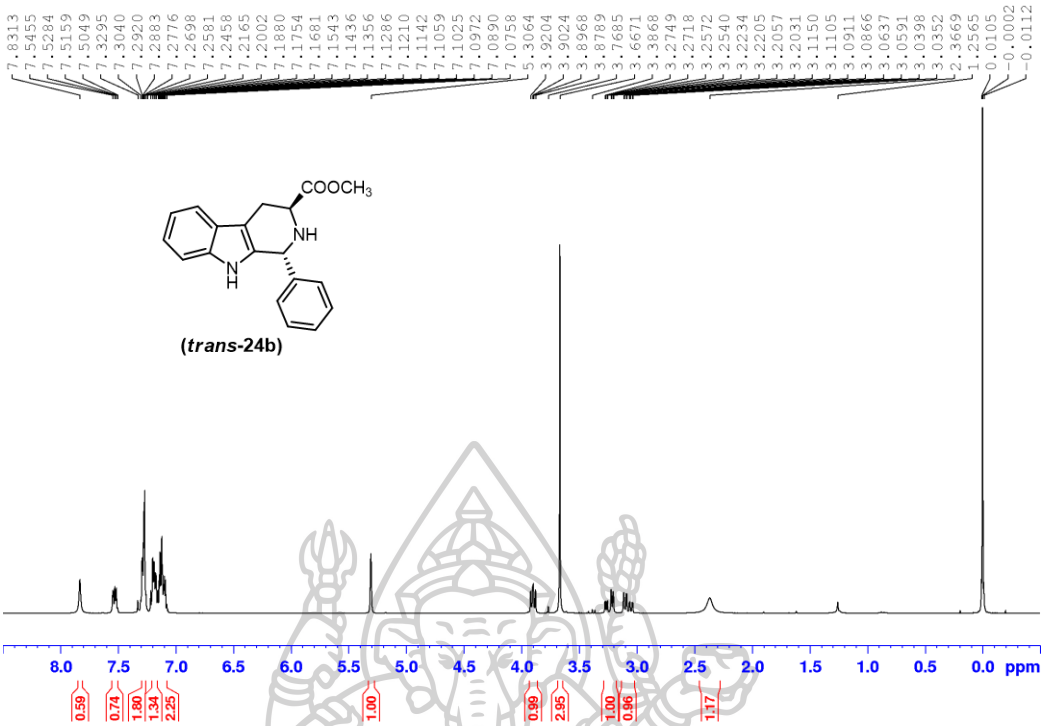
^{13}C-NMR spectrum of (**24a**) (75 MHz, $CDCl_3$)



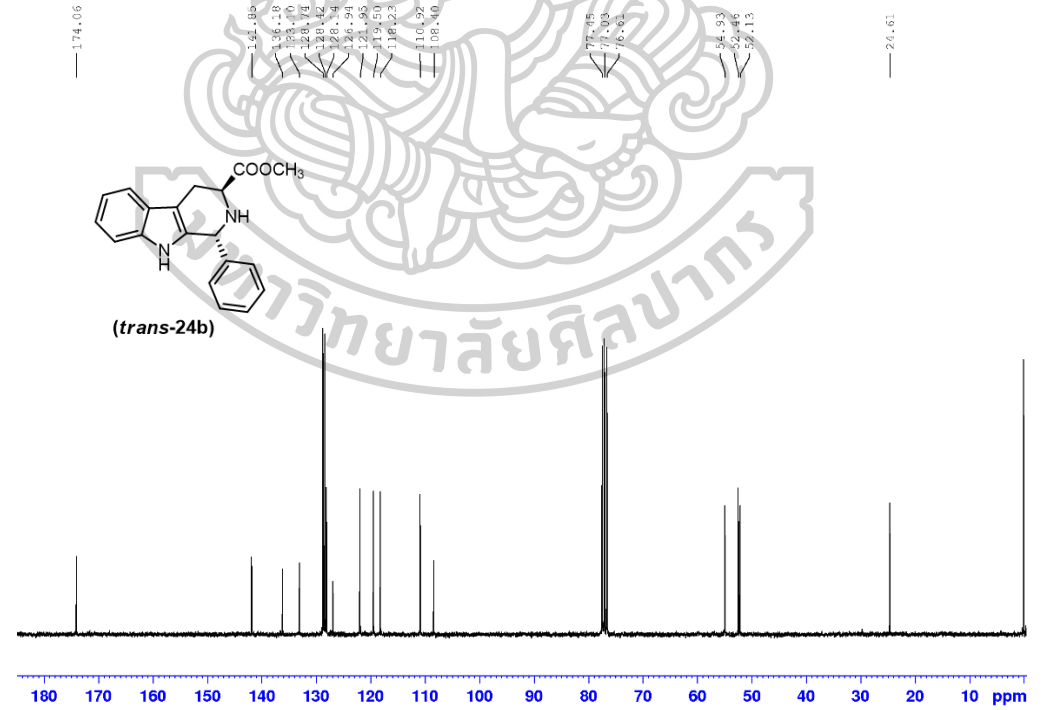
^{1}H-NMR spectrum of (**24b**) (300 MHz, $CDCl_3$)



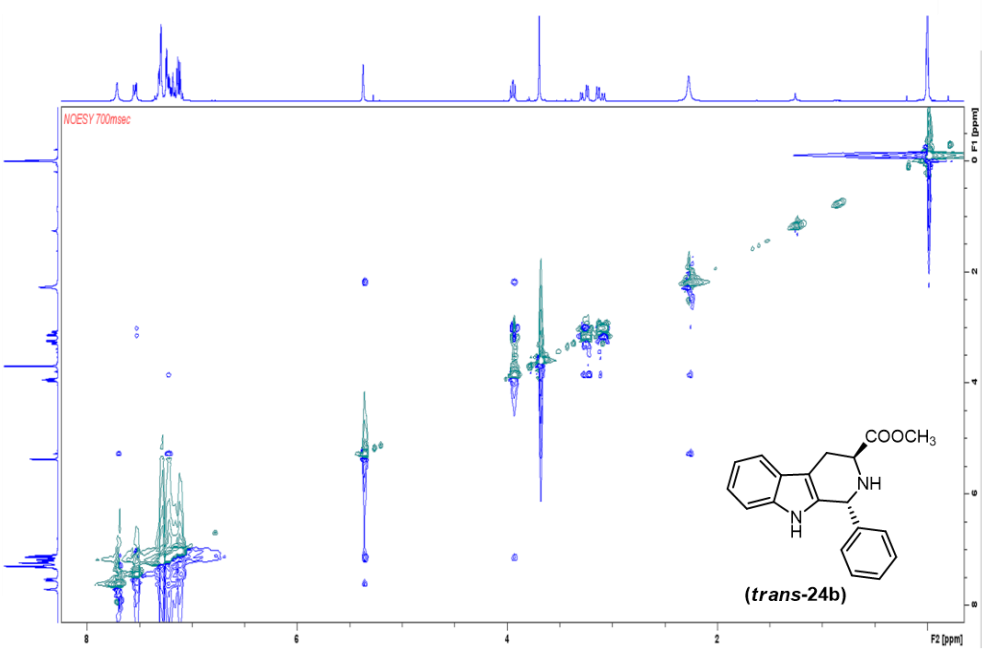
^{13}C-NMR spectrum of (**24b**) (75 MHz, $CDCl_3$)



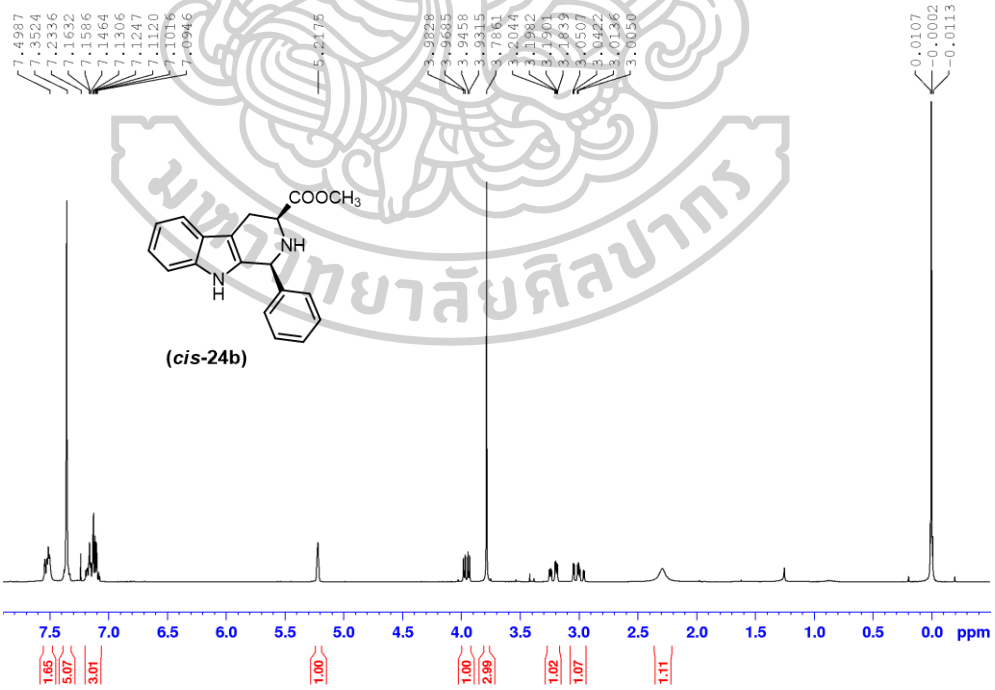
^{1}H-NMR spectrum of (***trans*-24b**) (300 MHz, $CDCl_3$)



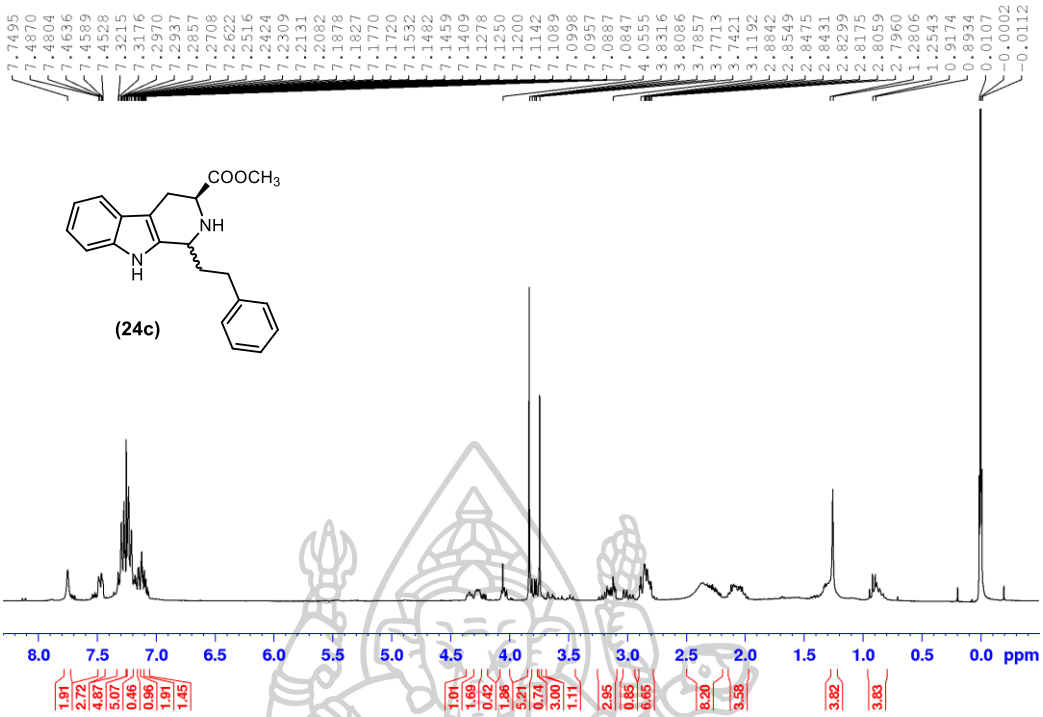
^{13}C-NMR spectrum of (***trans*-24b**) (75 MHz, $CDCl_3$)



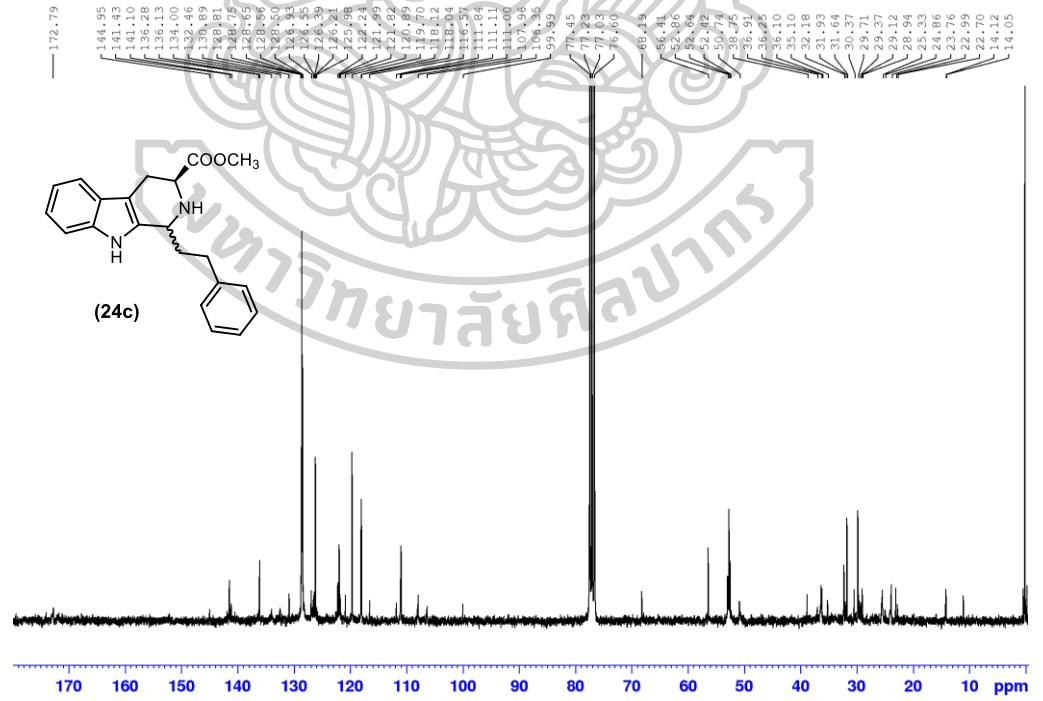
^{1}H-^{1}H NOESY spectra of *trans*-diastereomer (***trans*-24b**)



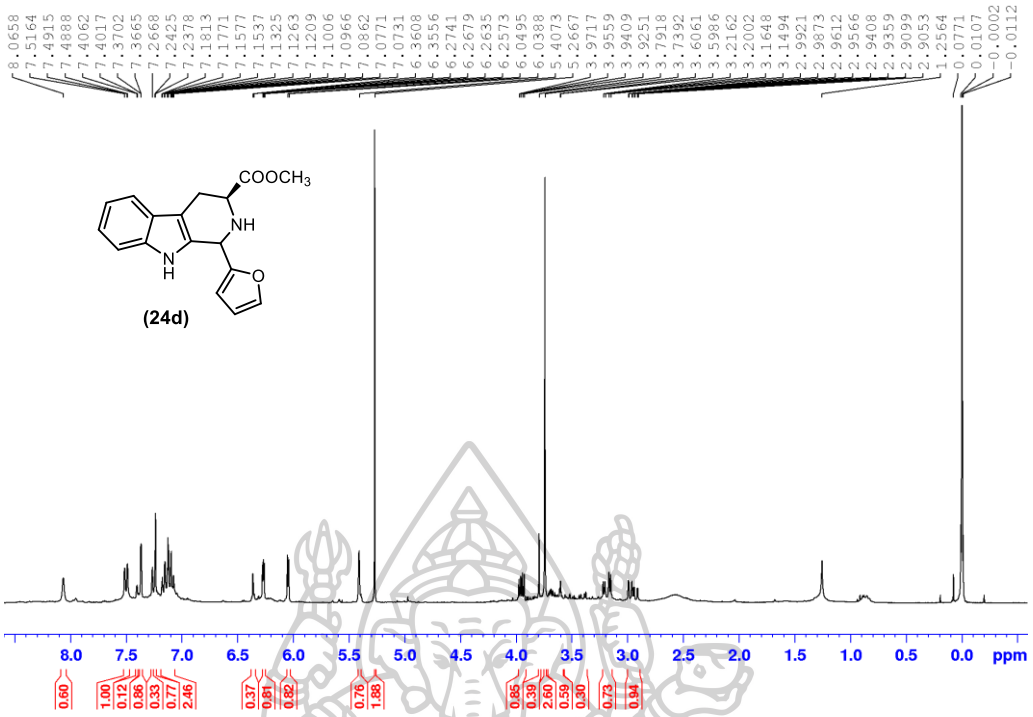
^{1}H-NMR spectrum of (***cis*-24b**) (300 MHz, $CDCl_3$)



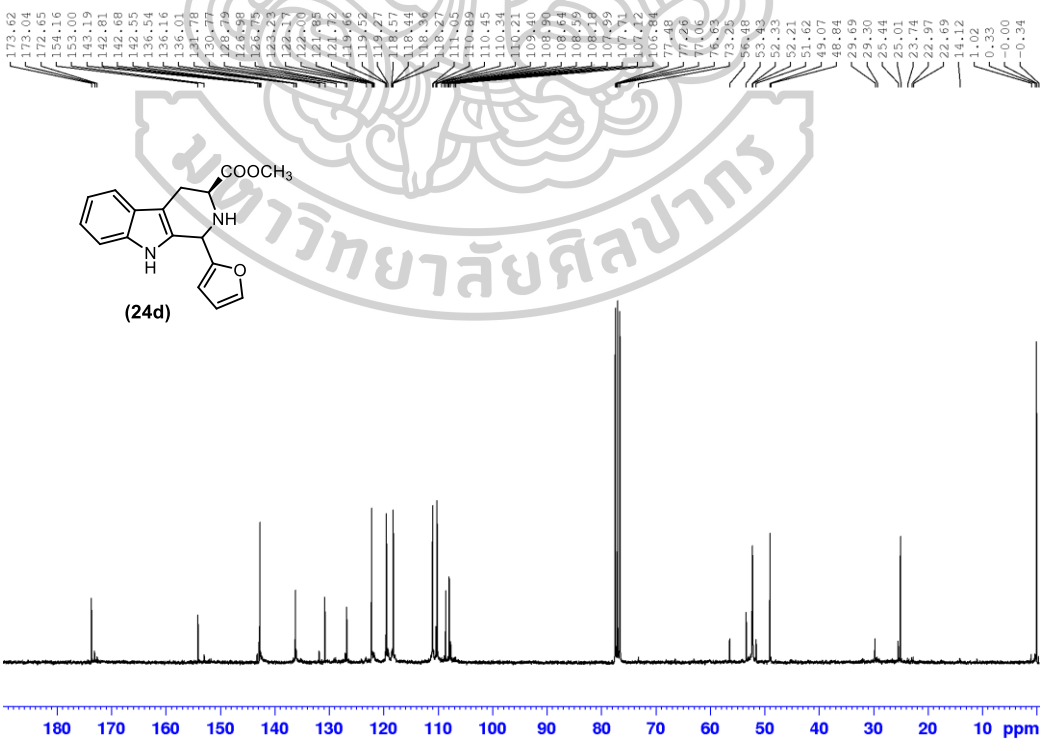
^{1}H-NMR spectrum of (**24c**) (300 MHz, $CDCl_3$)



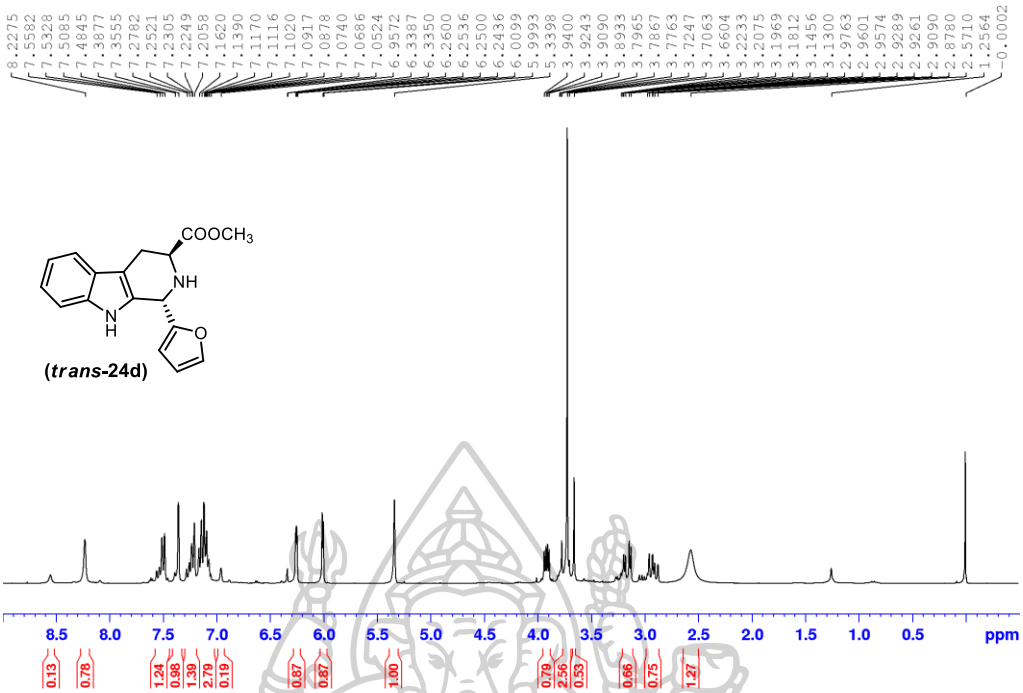
^{13}C-NMR spectrum of (**24c**) (75 MHz, $CDCl_3$)



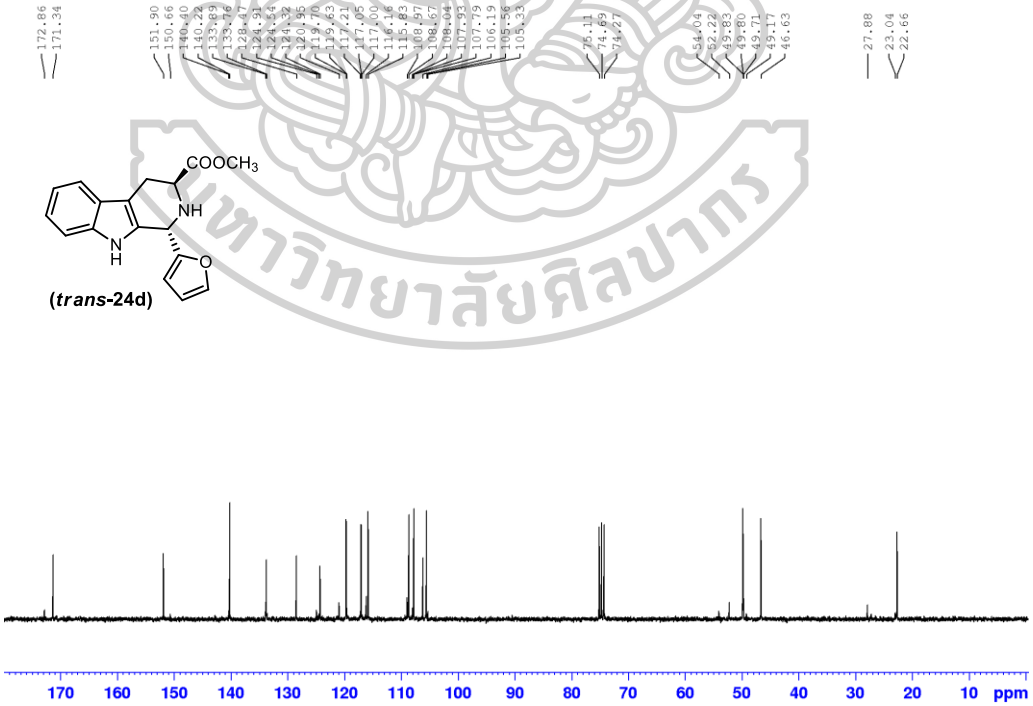
^{1}H-NMR spectrum of (**24d**) (300 MHz, $CDCl_3$)



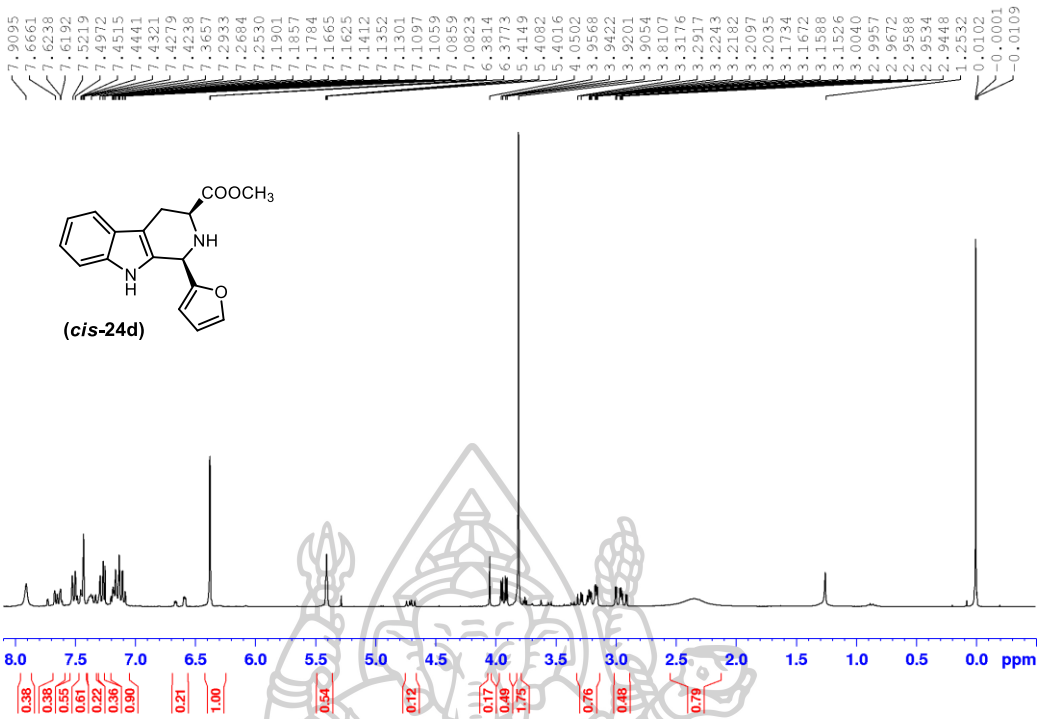
^{13}C-NMR spectrum of (**24d**) (75 MHz, $CDCl_3$)



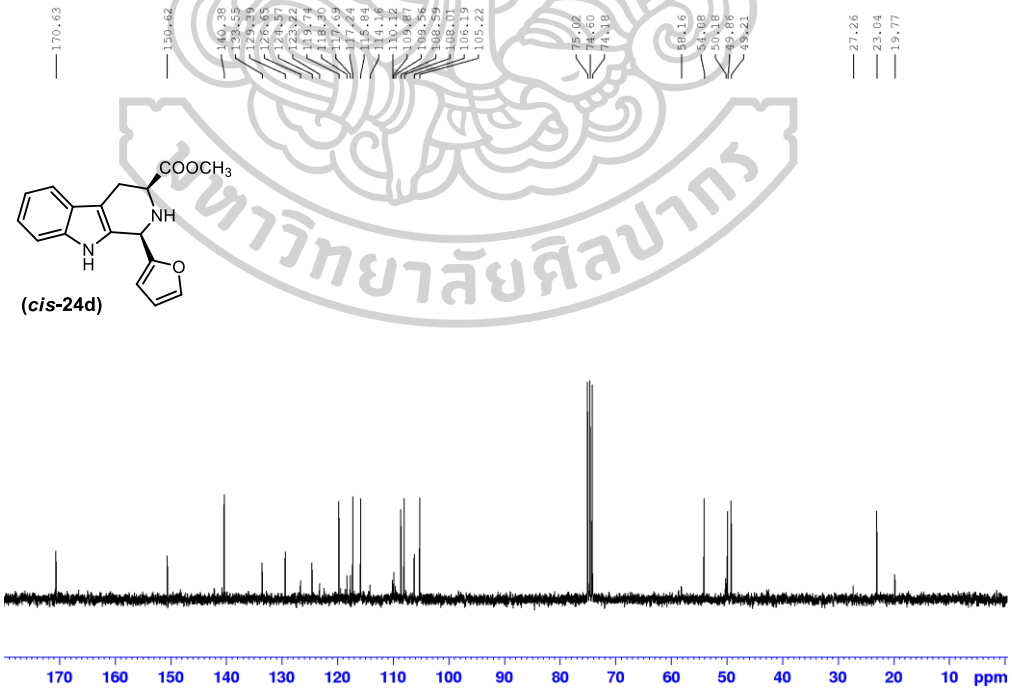
^{1}H-NMR spectrum of (***trans*-24d**) (300 MHz, $CDCl_3$)



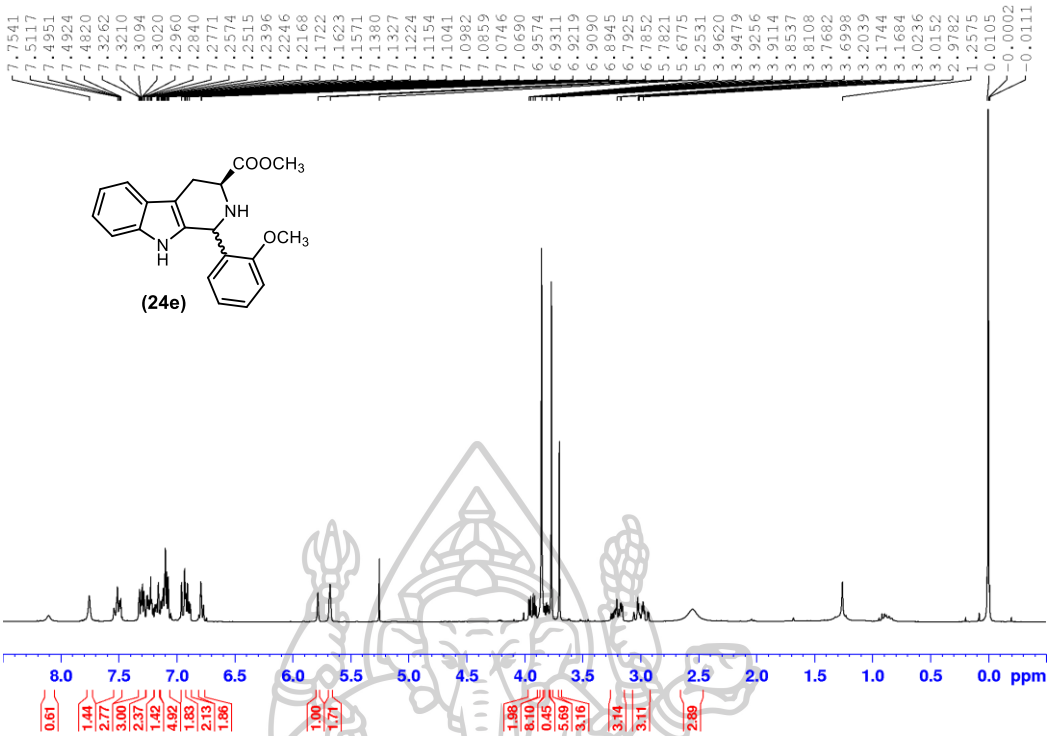
^{13}C-NMR spectrum of (***trans*-24d**) (75 MHz, $CDCl_3$)



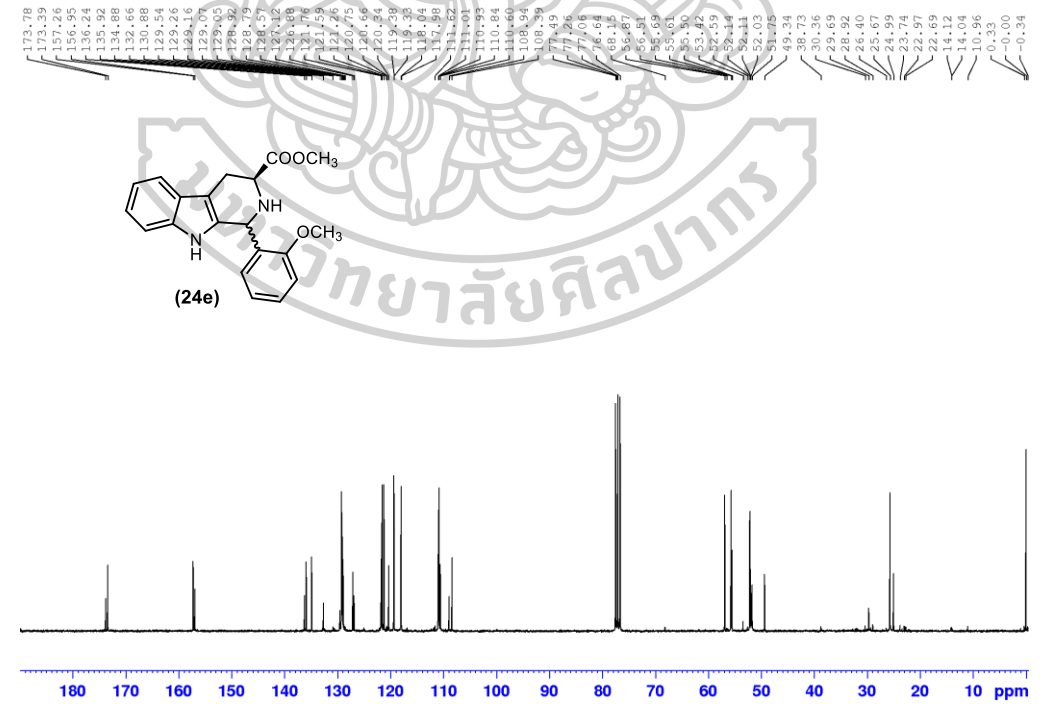
^{1}H-NMR spectrum of (***cis*-24d**) (300 MHz, $CDCl_3$)



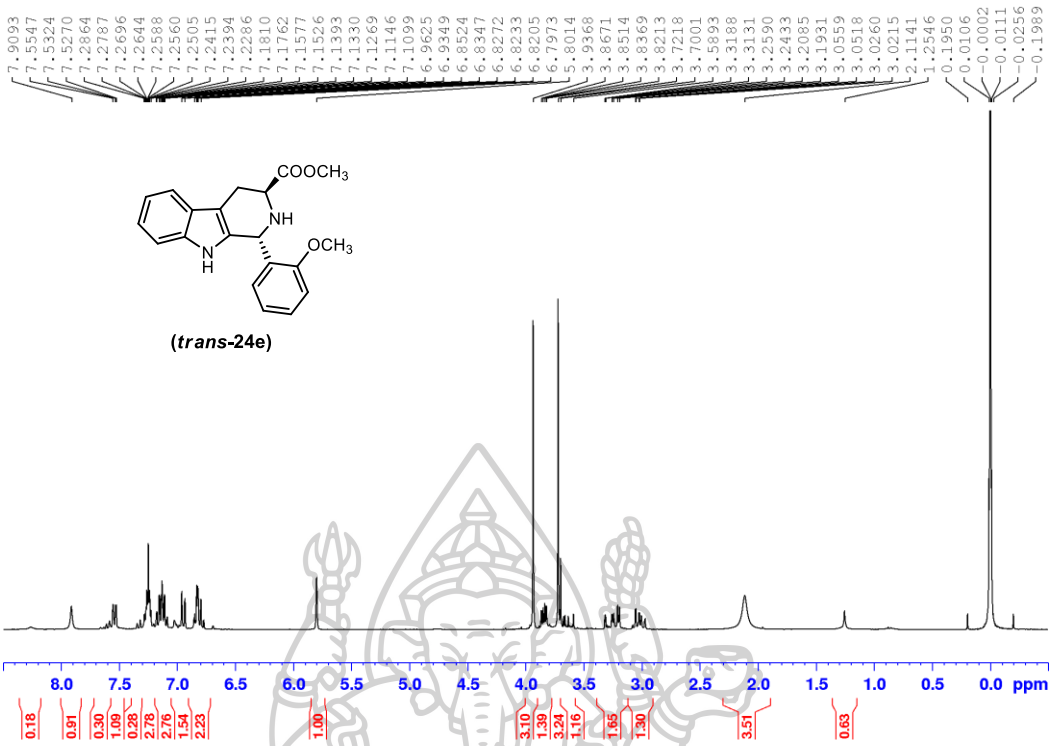
^{13}C-NMR spectrum of (***cis*-24d**) (75 MHz, $CDCl_3$)



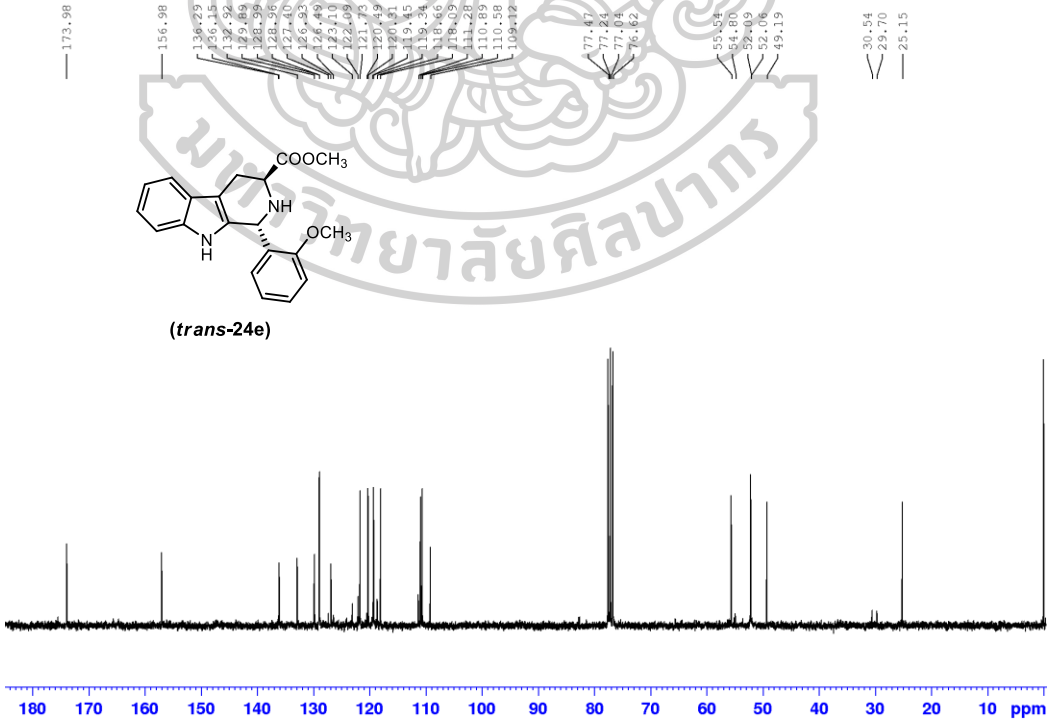
^{1}H-NMR spectrum of (**24e**) (300 MHz, $CDCl_3$)



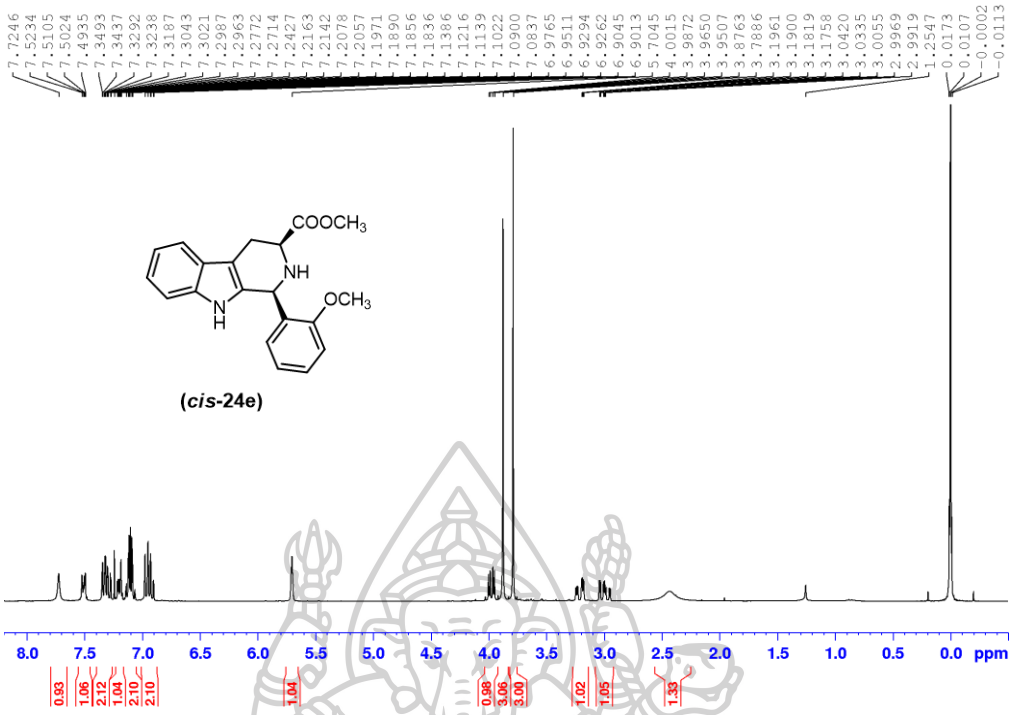
^{13}C-NMR spectrum of (**24e**) (75 MHz, $CDCl_3$)



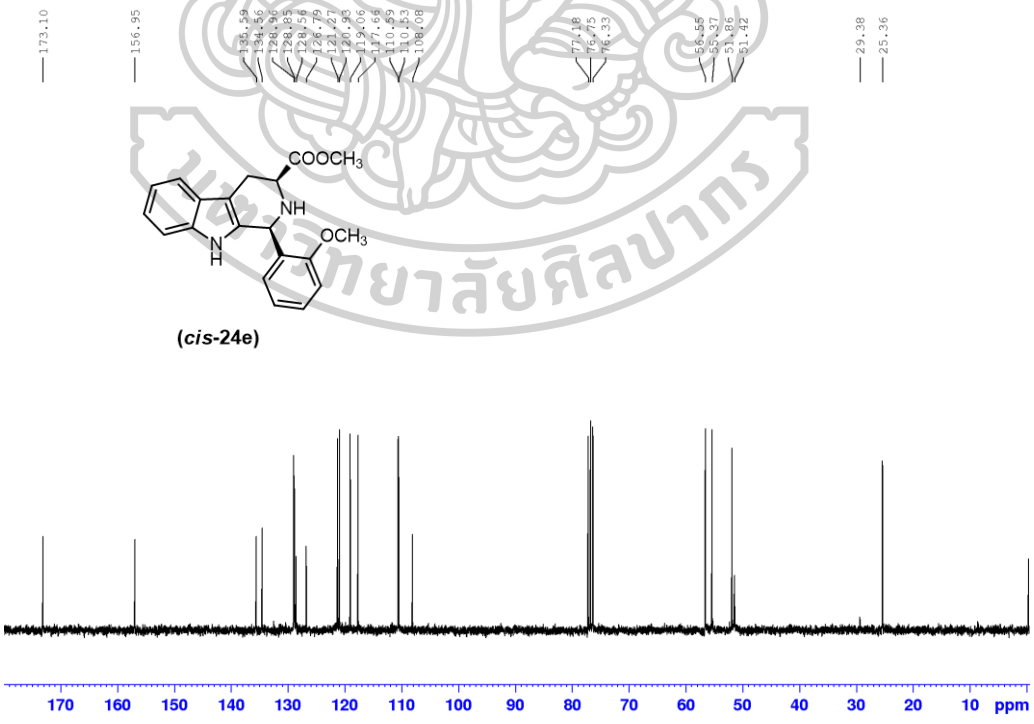
^{1}H-NMR spectrum of (***trans*-24e**) (300 MHz, $CDCl_3$)



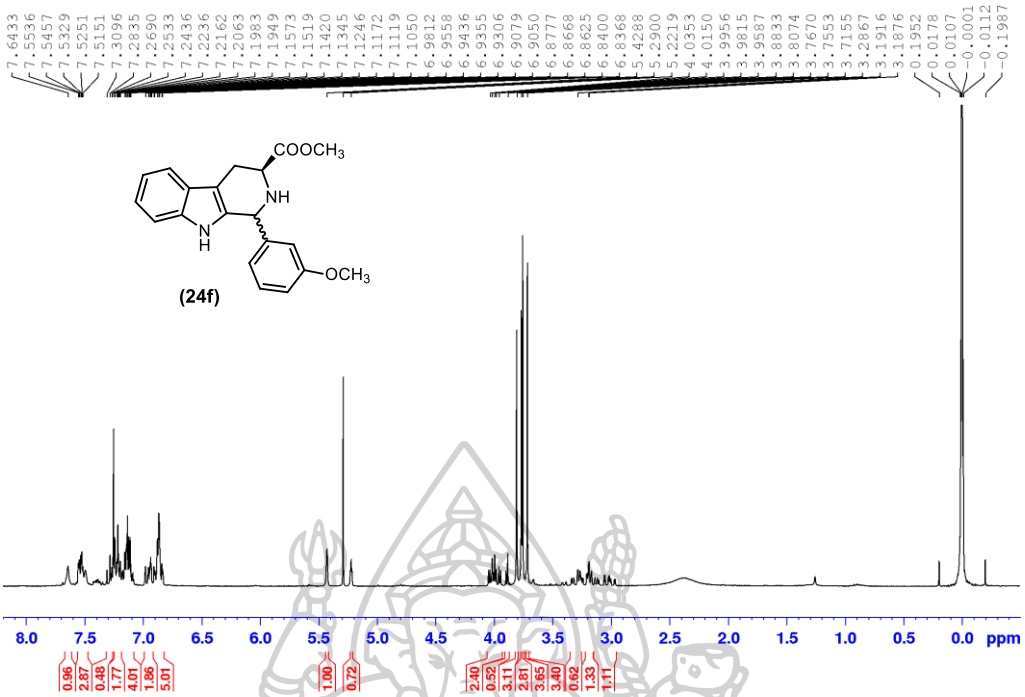
^{13}C-NMR spectrum of (***trans*-24e**) (75 MHz, $CDCl_3$)



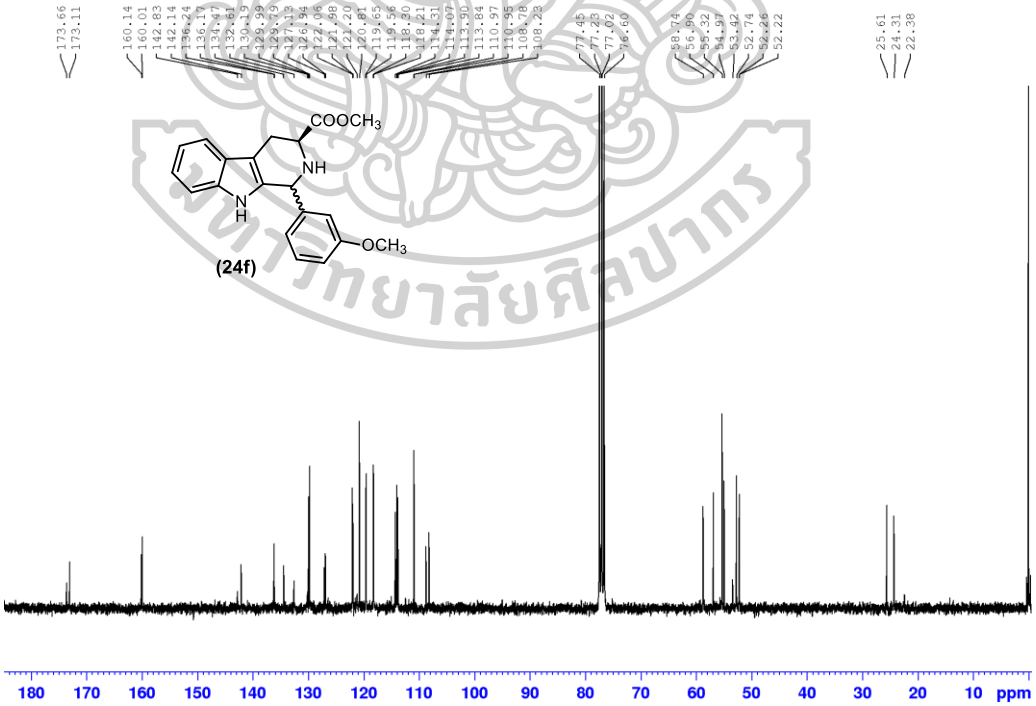
^{1}H-NMR spectrum of (***cis*-24e**) (300 MHz, $CDCl_3$)



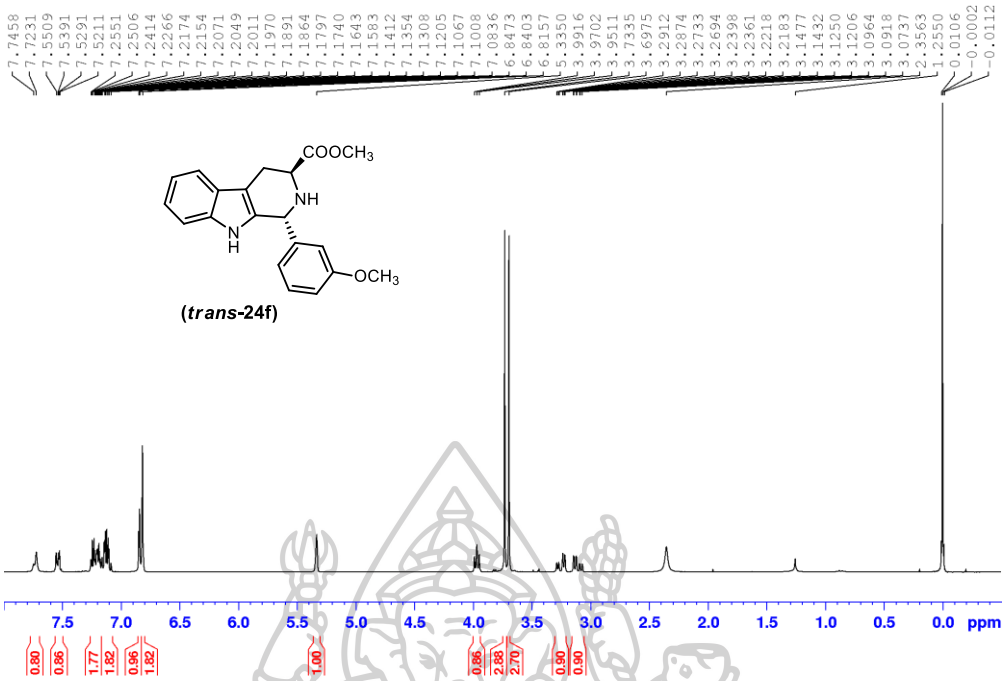
^{13}C-NMR spectrum of (***cis*-24e**) (75 MHz, $CDCl_3$)



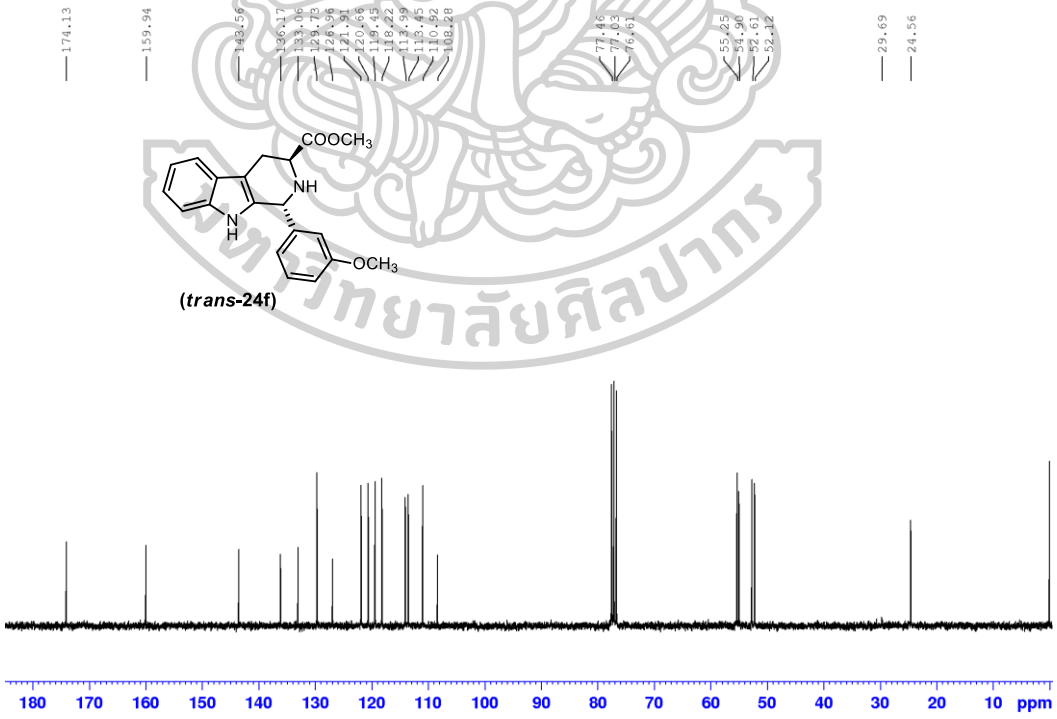
^{1}H-NMR spectrum of (**24f**) (300 MHz, $CDCl_3$)



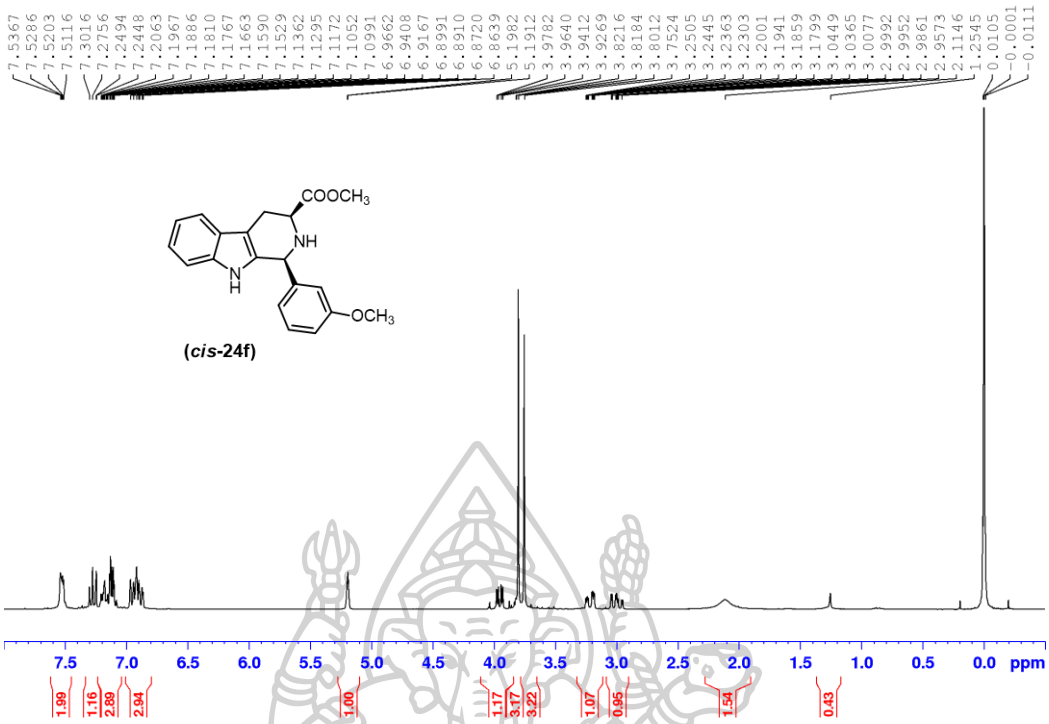
^{13}C-NMR spectrum of (**24f**) (75 MHz, $CDCl_3$)



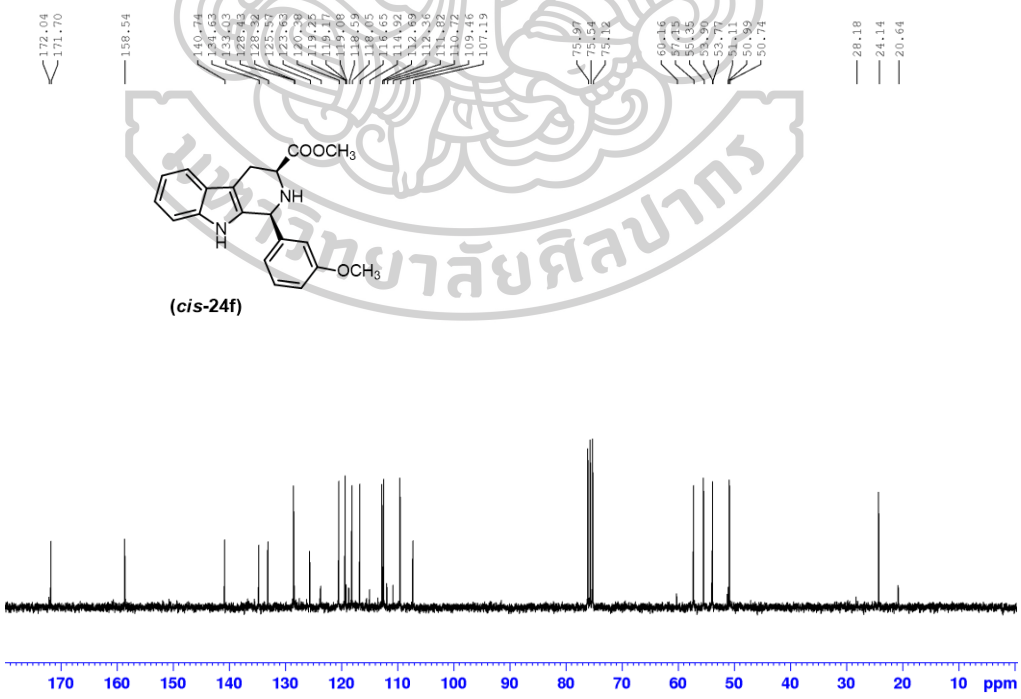
^{1}H-NMR spectrum of (***trans*-24f**) (300 MHz, $CDCl_3$)



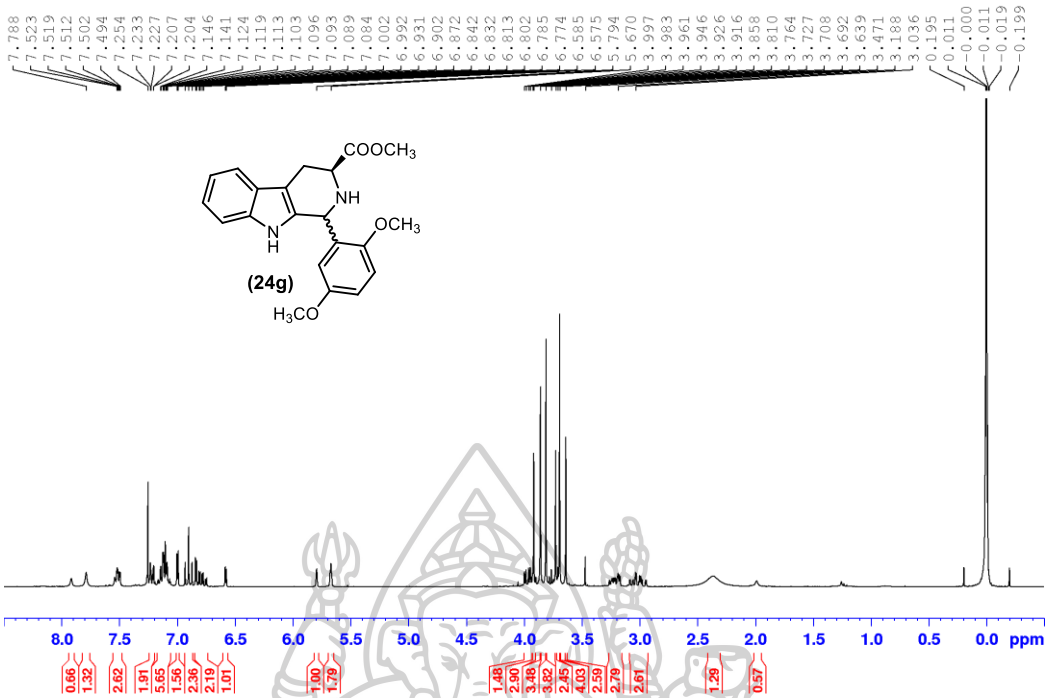
^{13}C-NMR spectrum of (***trans*-24f**) (75 MHz, $CDCl_3$)



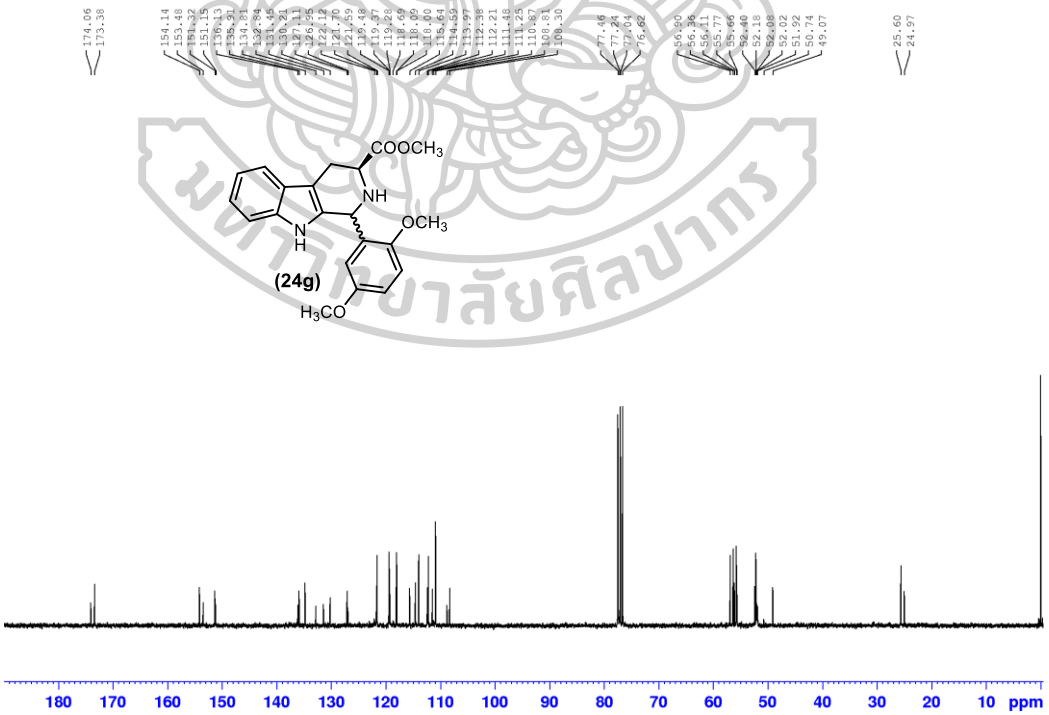
^{1}H-NMR spectrum of (***cis*-24f**) (300 MHz, $CDCl_3$)



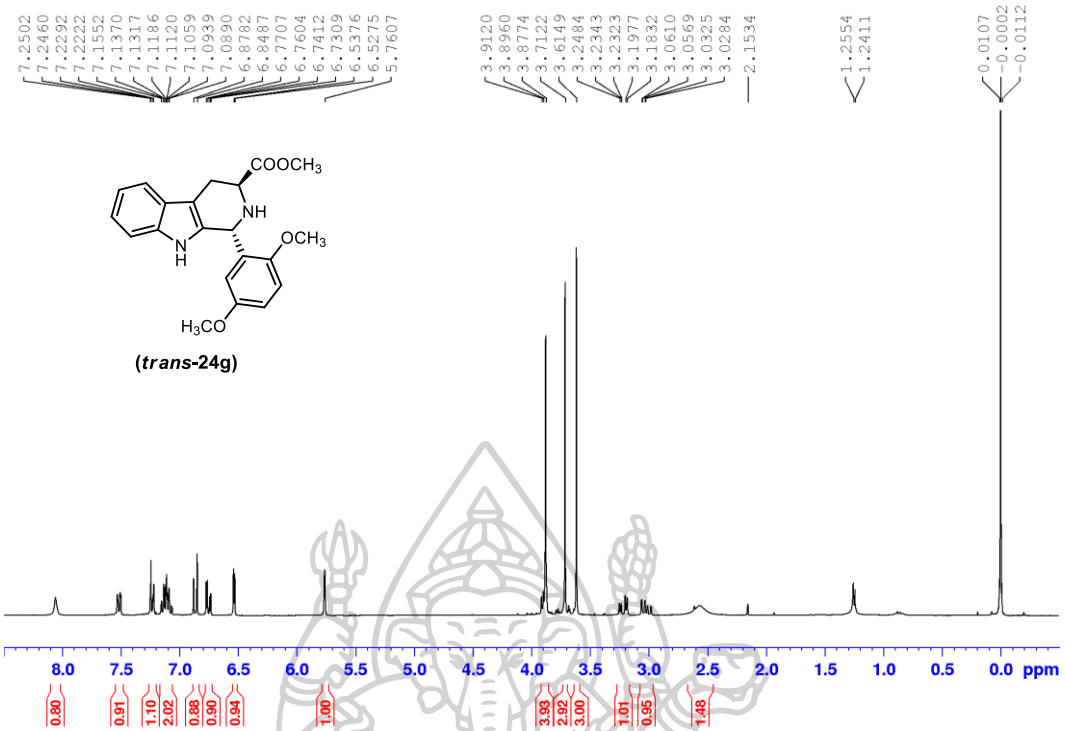
^{13}C-NMR spectrum of (***cis*-24f**) (75 MHz, $CDCl_3$)



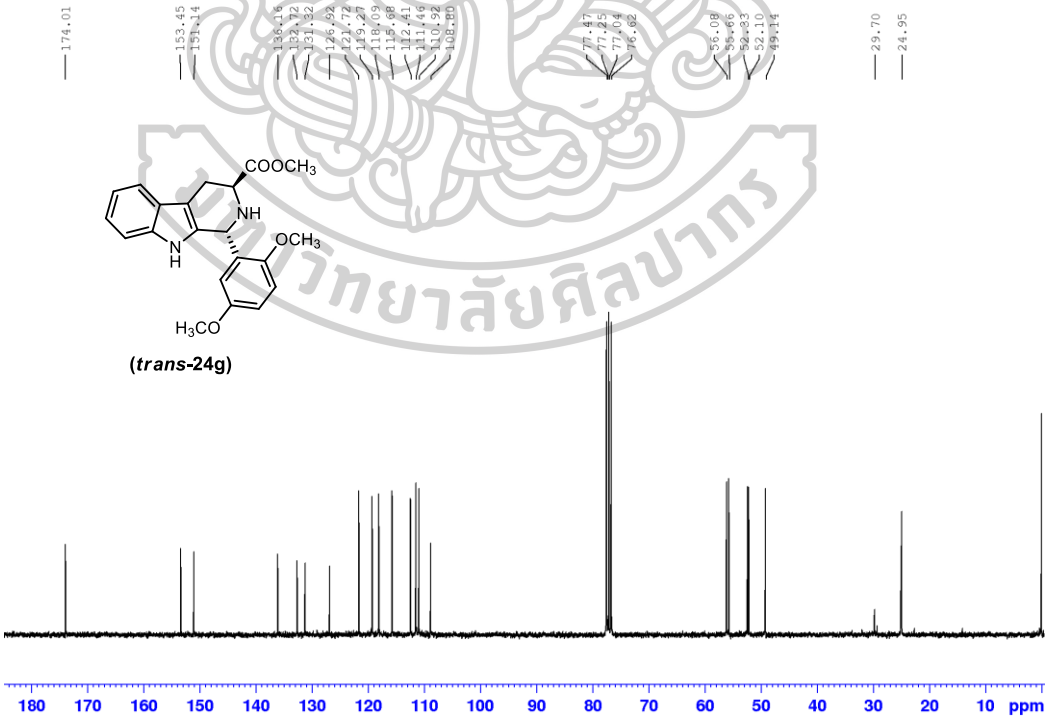
^{1}H-NMR spectrum of (**24g**) (300 MHz, $CDCl_3$)



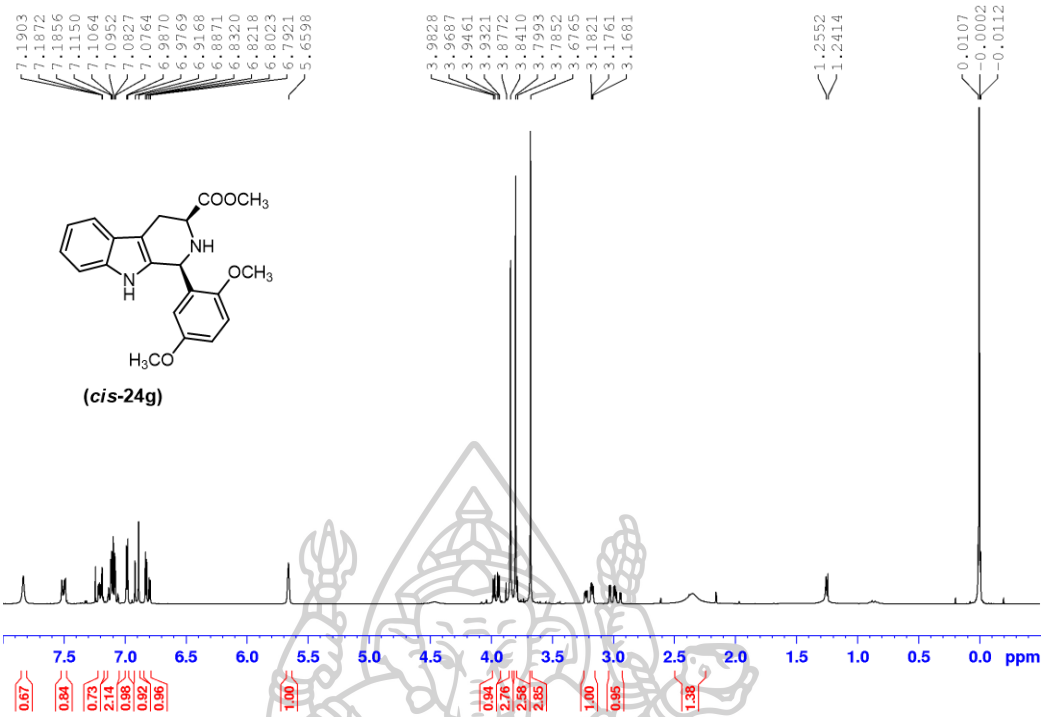
^{13}C-NMR spectrum of (**24g**) (75 MHz, $CDCl_3$)



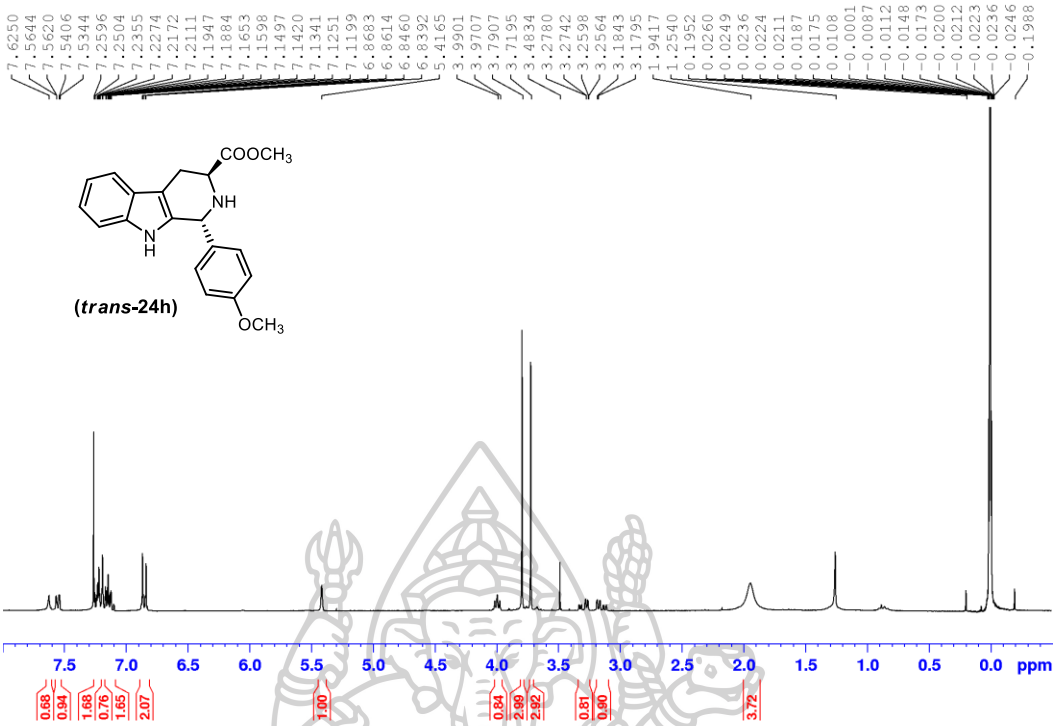
^{1}H-NMR spectrum of (***trans*-24g**) (300 MHz, $CDCl_3$)



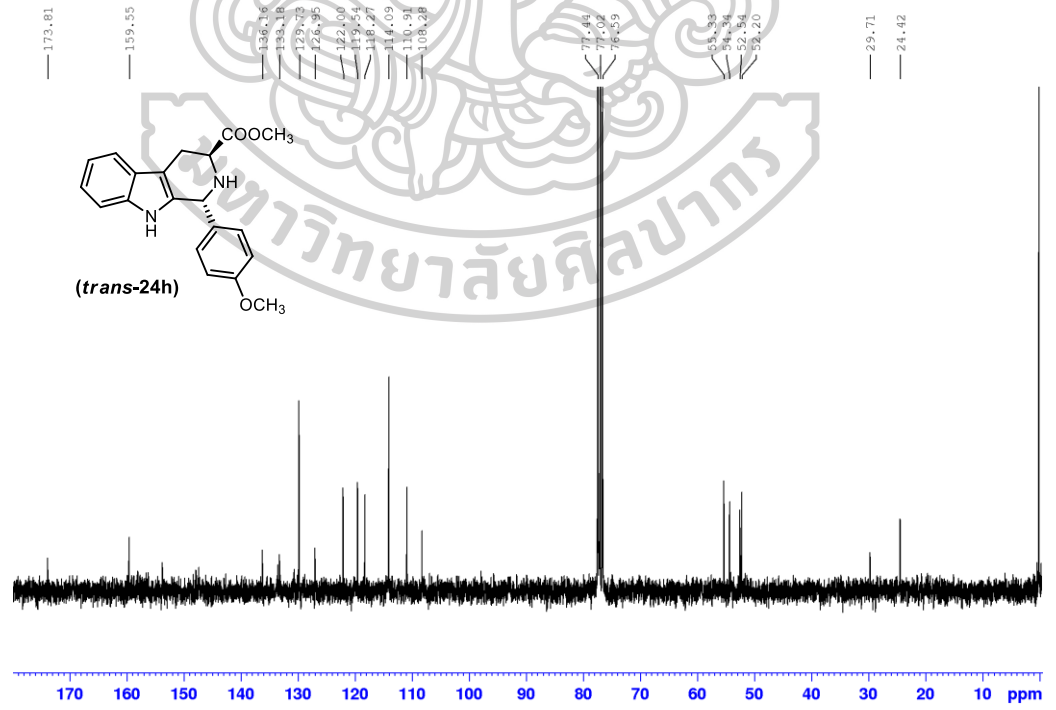
^{13}C-NMR spectrum of (***trans*-24g**) (75 MHz, $CDCl_3$)



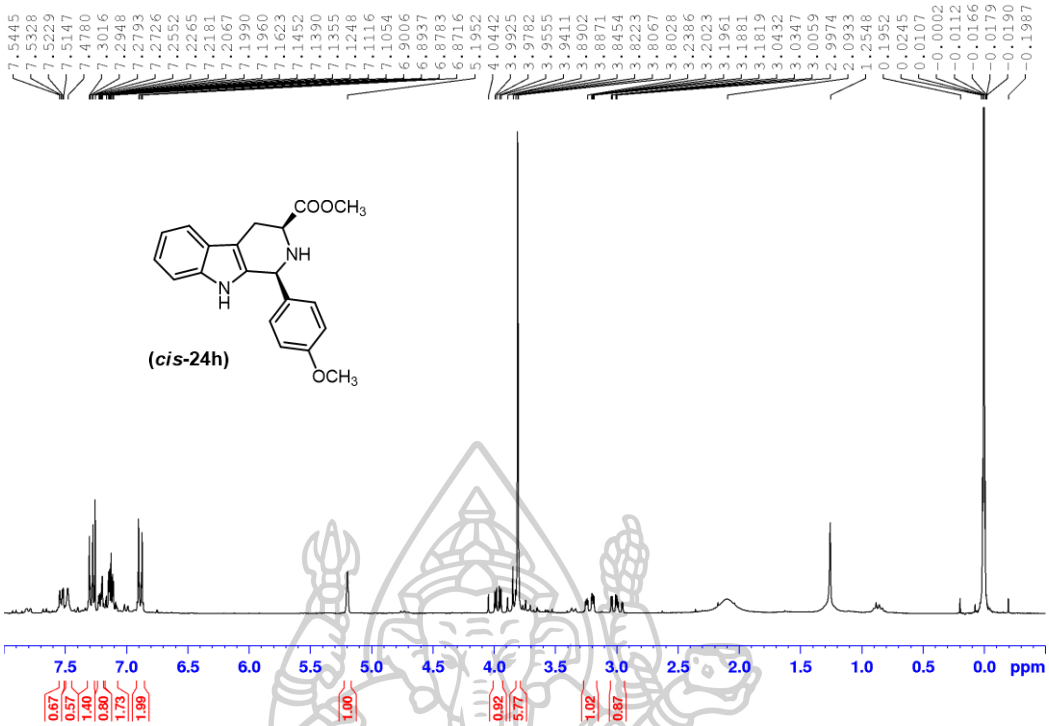
^{1}H-NMR spectrum of (***cis*-24g**) (300 MHz, $CDCl_3$)



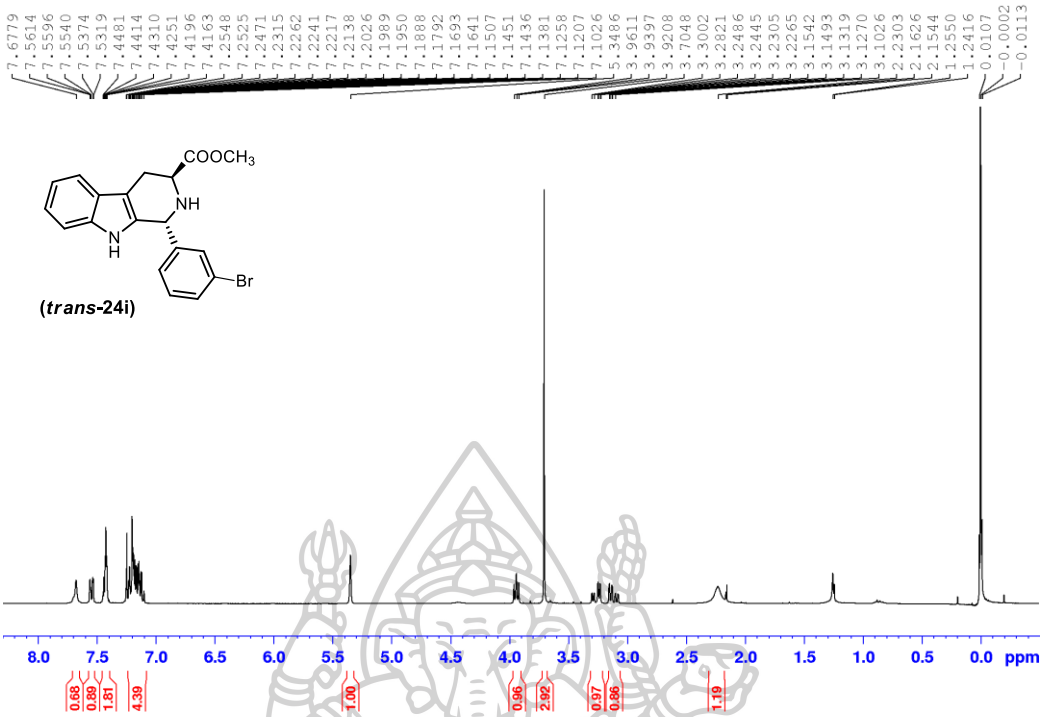
^{1}H-NMR spectrum of (***trans*-24h**) (300 MHz, $CDCl_3$)



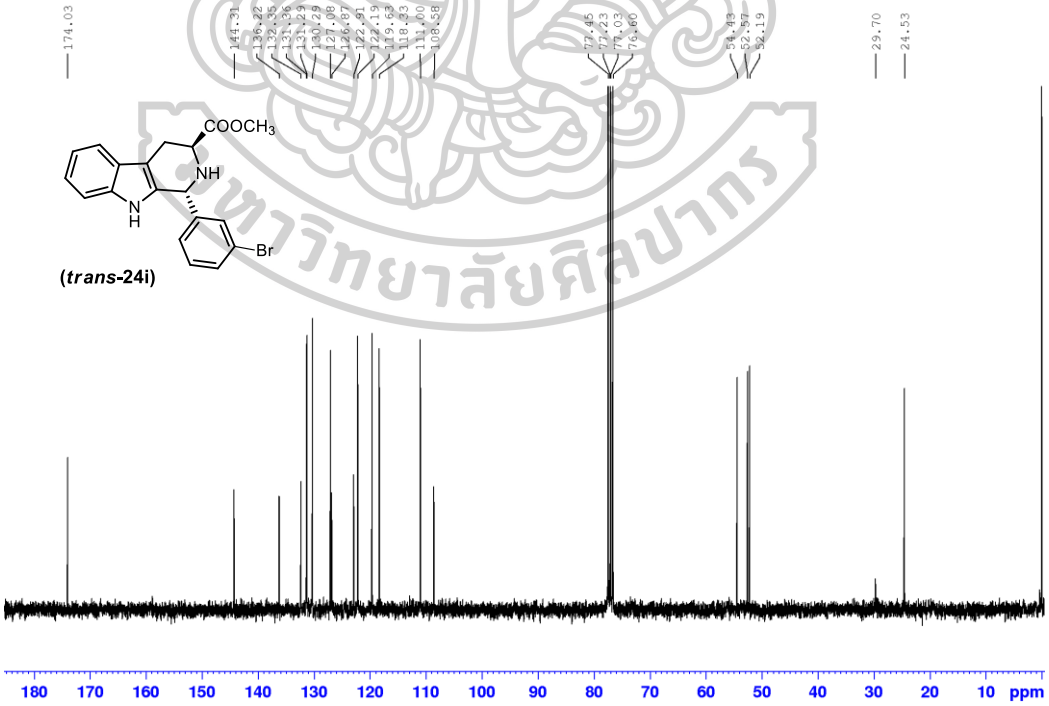
^{13}C-NMR spectrum of (***trans*-24h**) (75 MHz, $CDCl_3$)



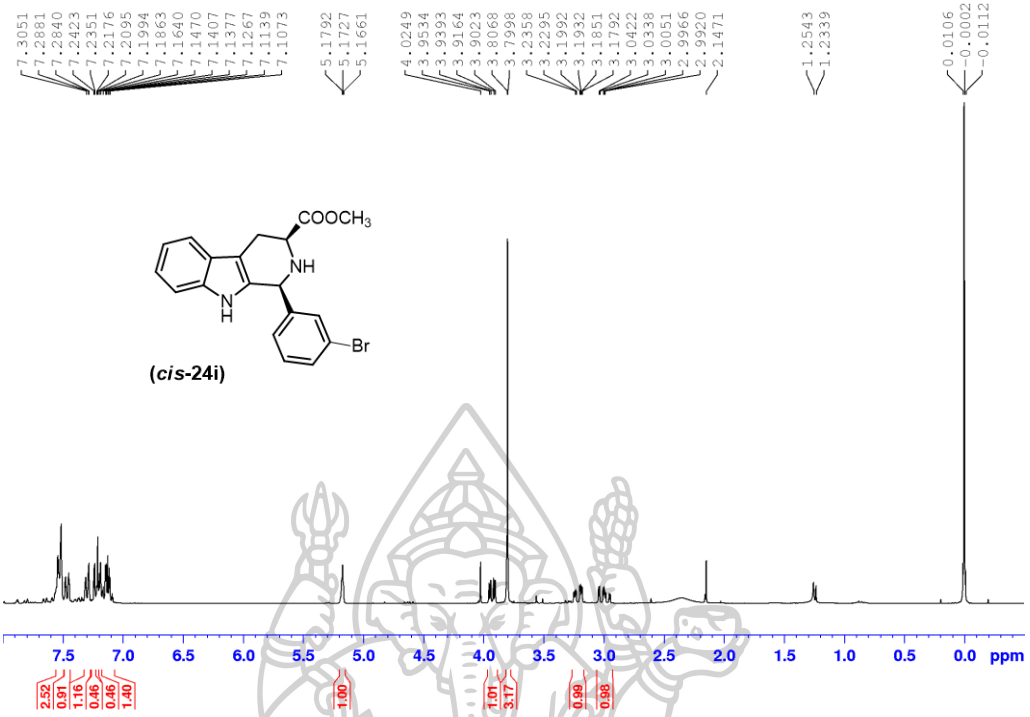
^{1}H-NMR spectrum of (***cis*-24h**) (300 MHz, $CDCl_3$)



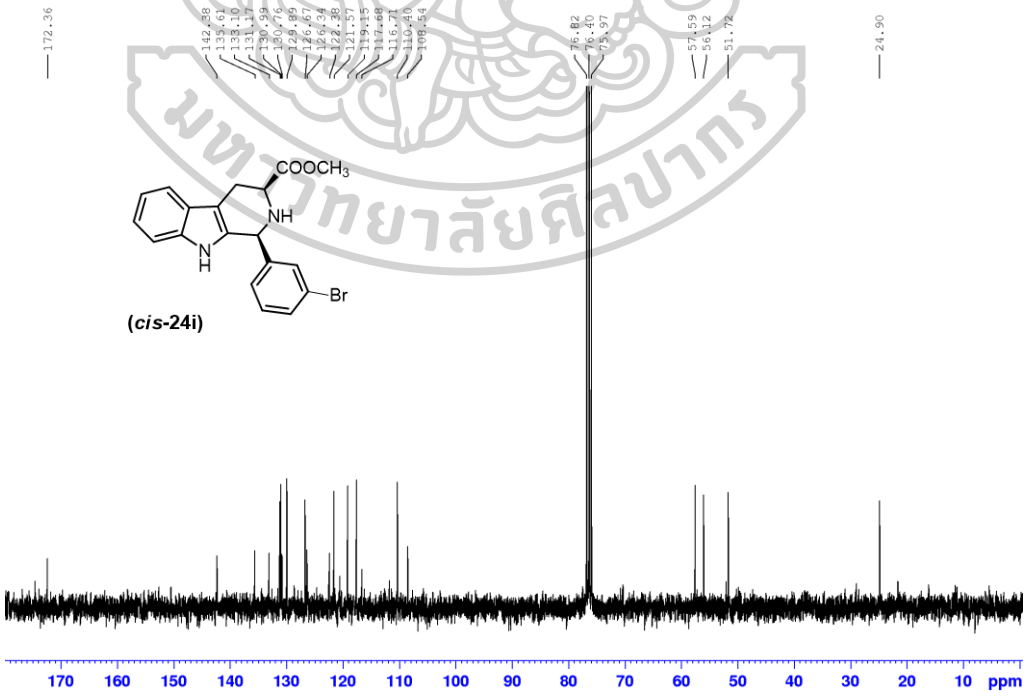
^{1}H-NMR spectrum of (***trans*-24i**) (300 MHz, $CDCl_3$)



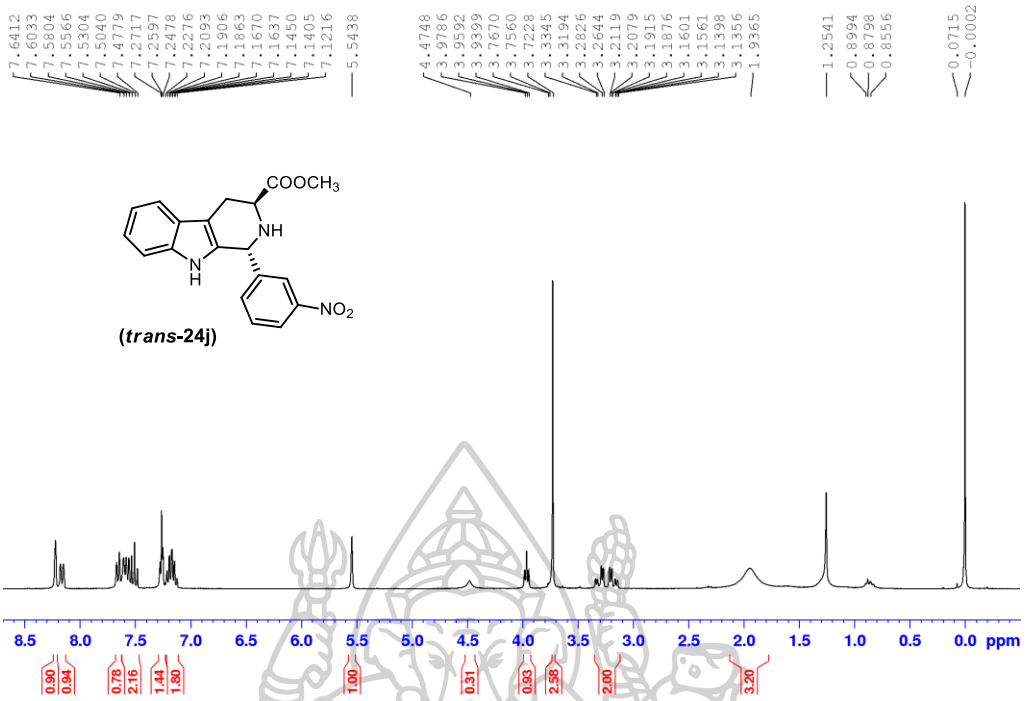
^{13}C-NMR spectrum of (***trans*-24i**) (75 MHz, $CDCl_3$)



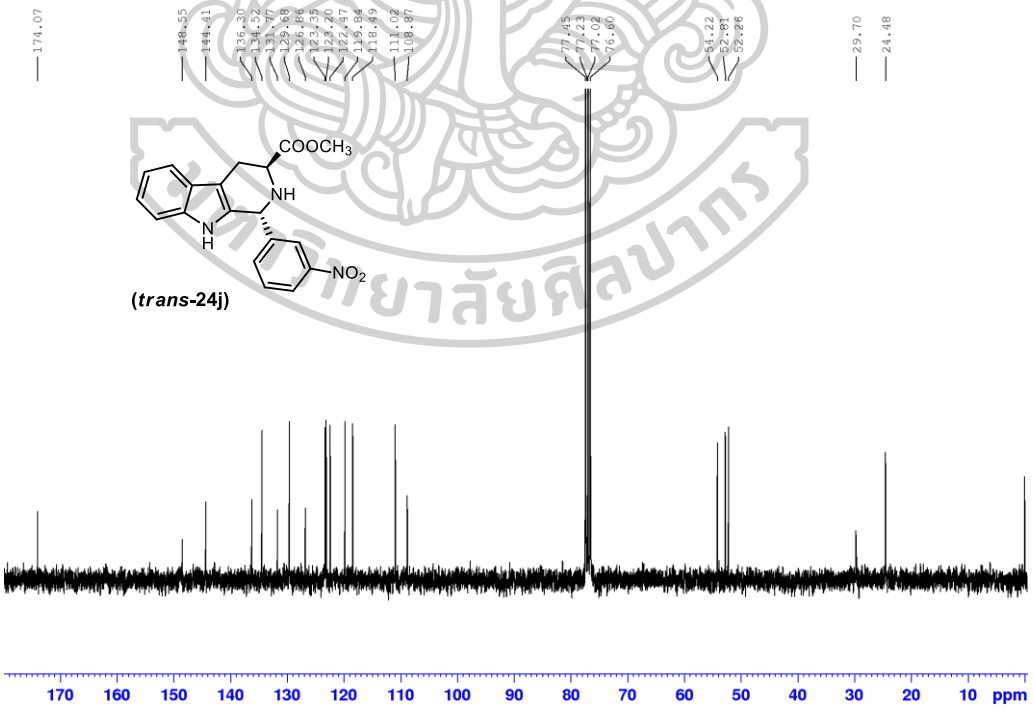
^{1}H-NMR spectrum of (***cis*-24i**) (300 MHz, $CDCl_3$)



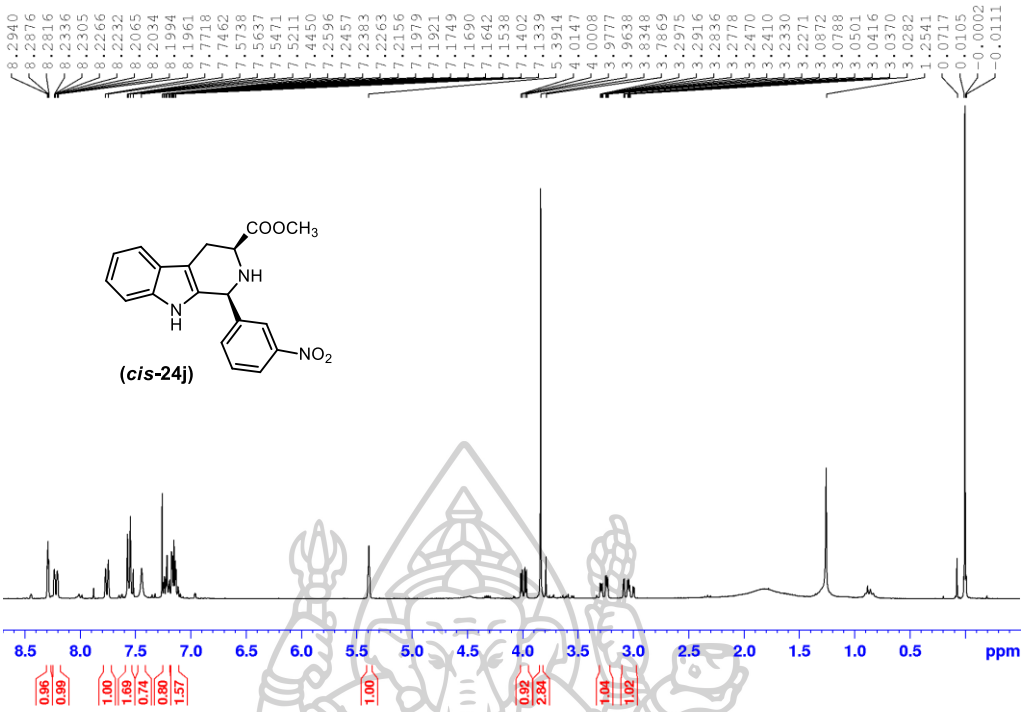
^{13}C-NMR spectrum of (***cis*-24i**) (75 MHz, $CDCl_3$)



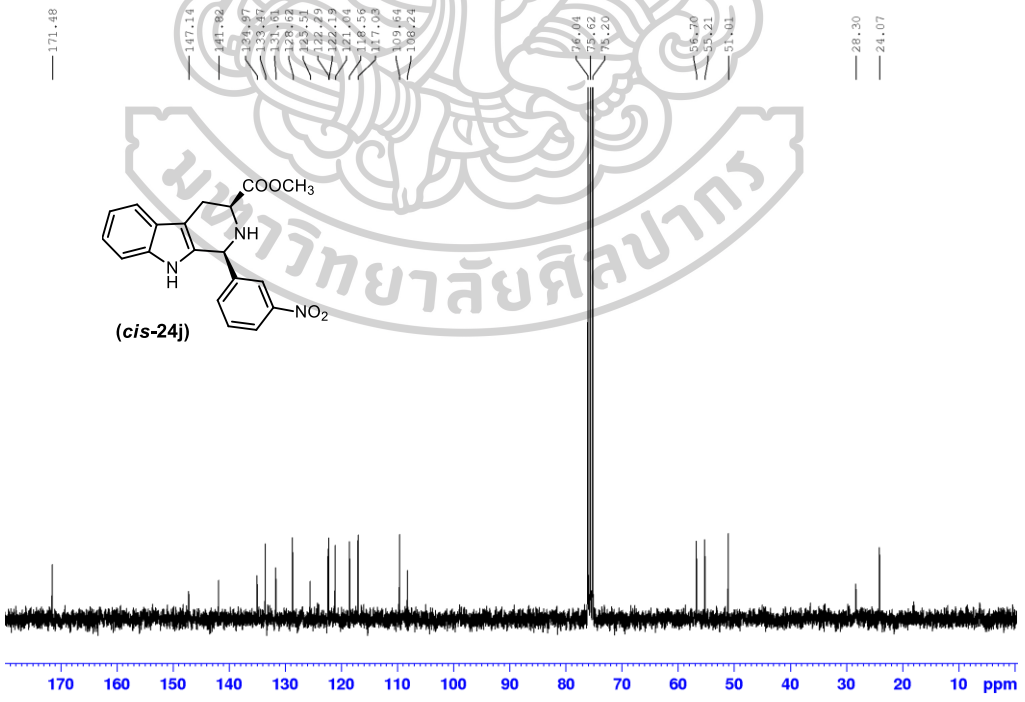
^{1}H-NMR spectrum of (***trans*-24j**) (300 MHz, $CDCl_3$)



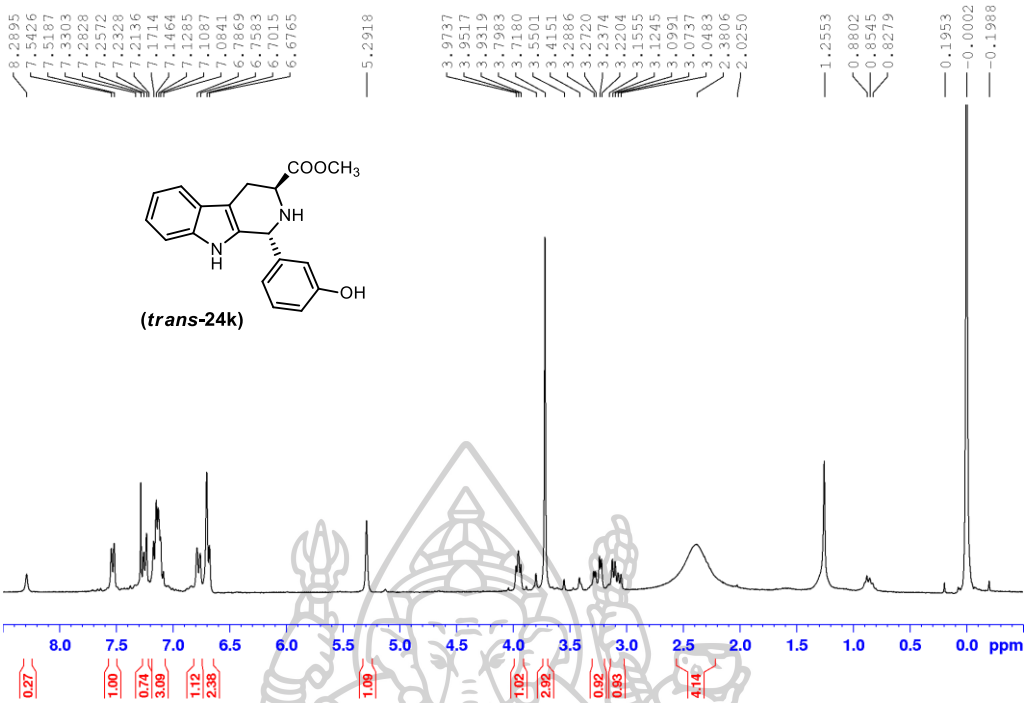
^{13}C-NMR spectrum of (***trans*-24j**) (75 MHz, $CDCl_3$)



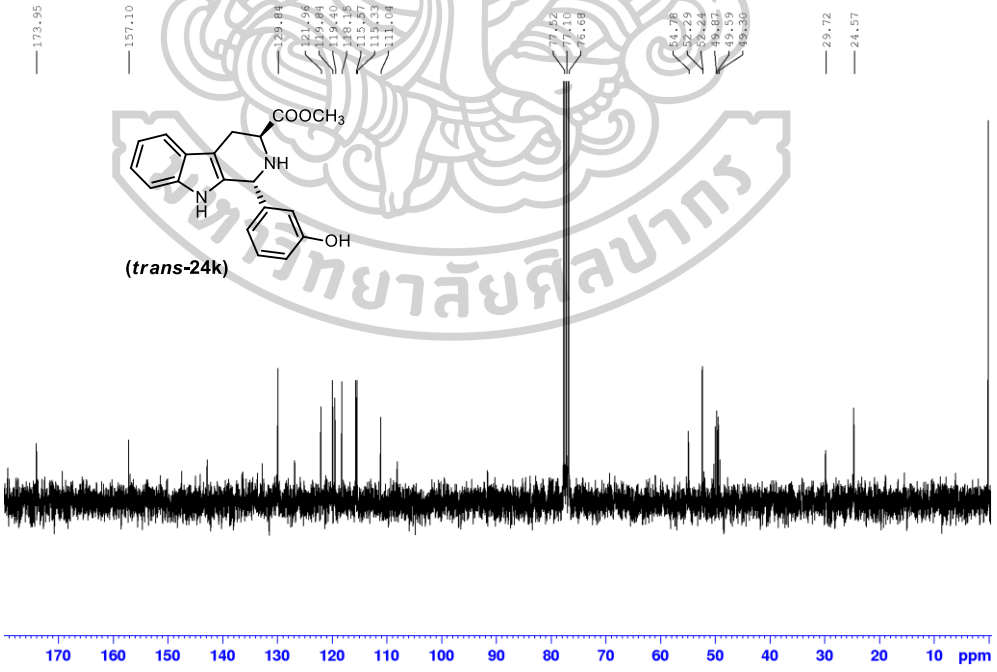
^{1}H-NMR spectrum of (***cis*-24j**) (300 MHz, $CDCl_3$)



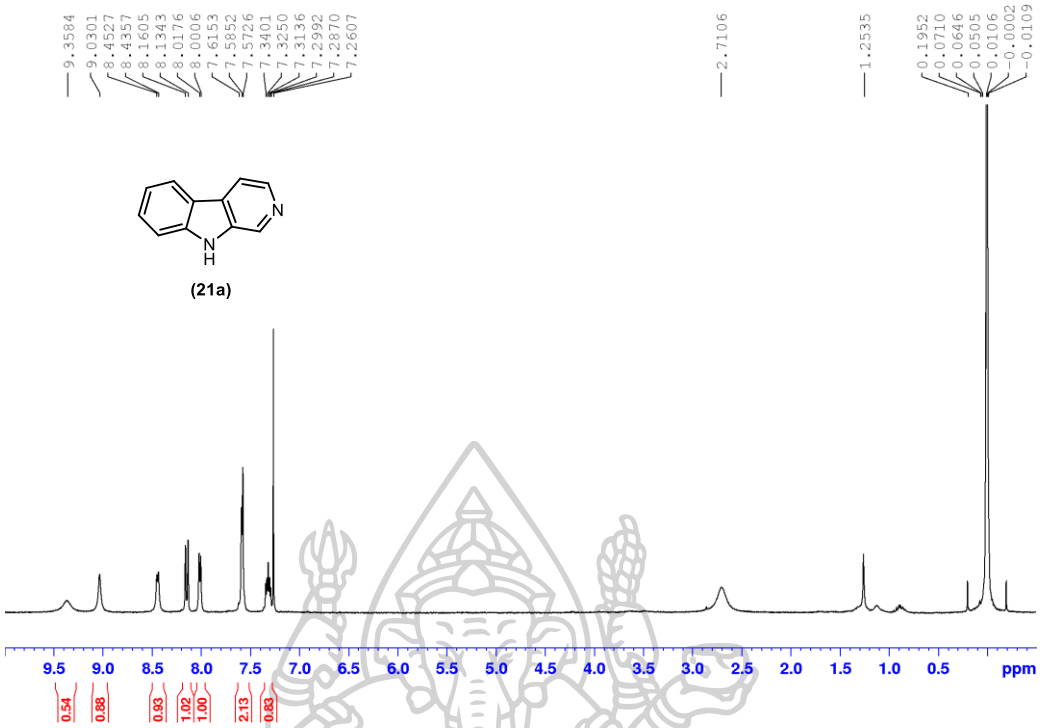
^{13}C-NMR spectrum of (***cis*-24j**) (75 MHz, $CDCl_3$)



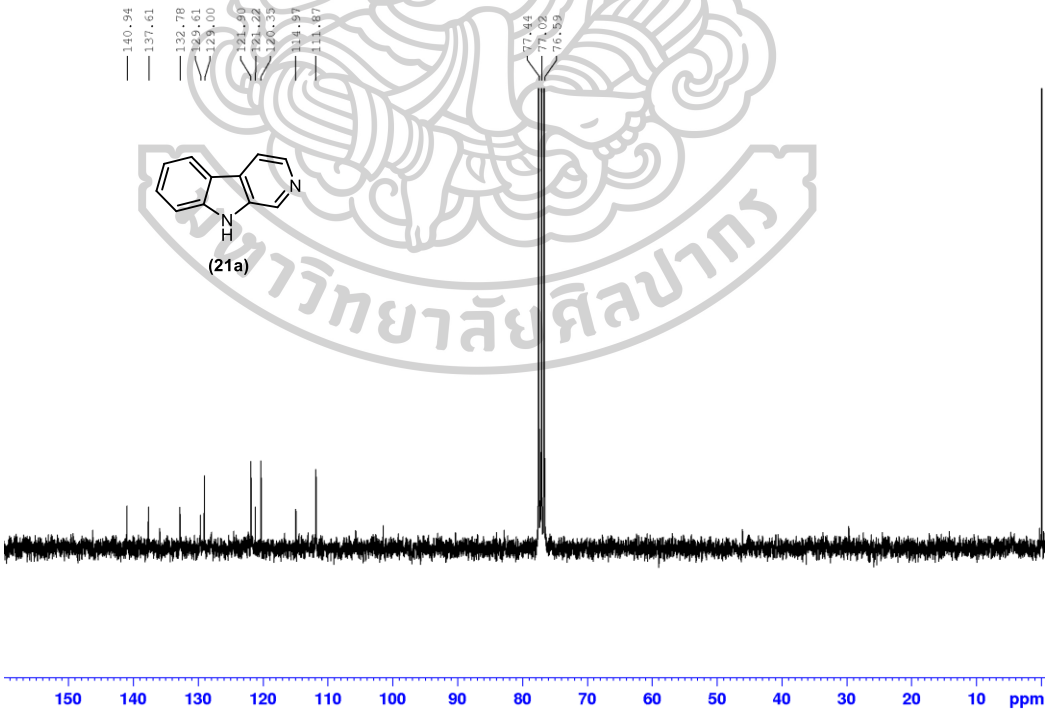
^{1}H-NMR spectrum of (***trans*-24k**) (300 MHz, $CDCl_3$)



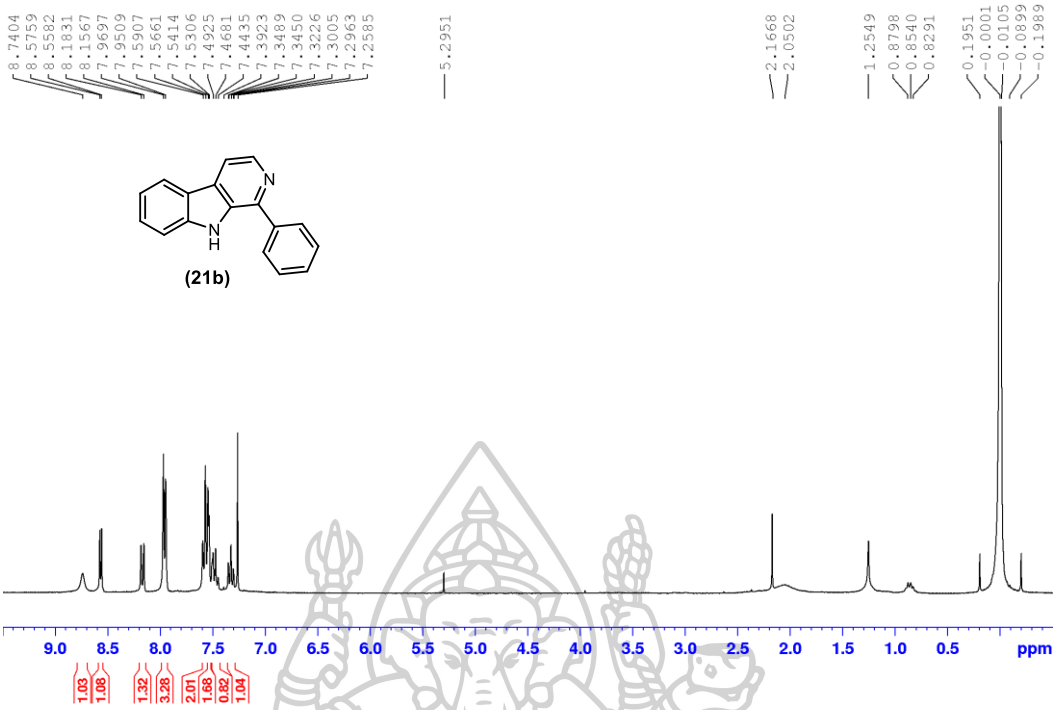
^{13}C-NMR spectrum of (***trans*-24k**) (75 MHz, $CDCl_3$)



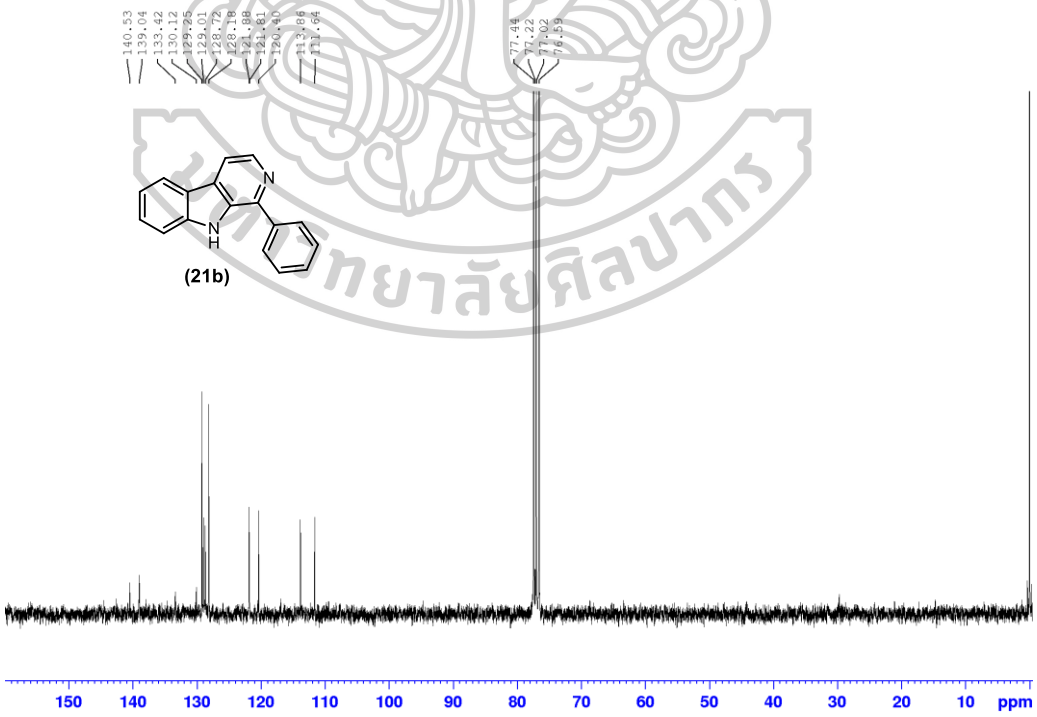
^{1}H-NMR spectrum of (**21a**) (300 MHz, $CDCl_3$)



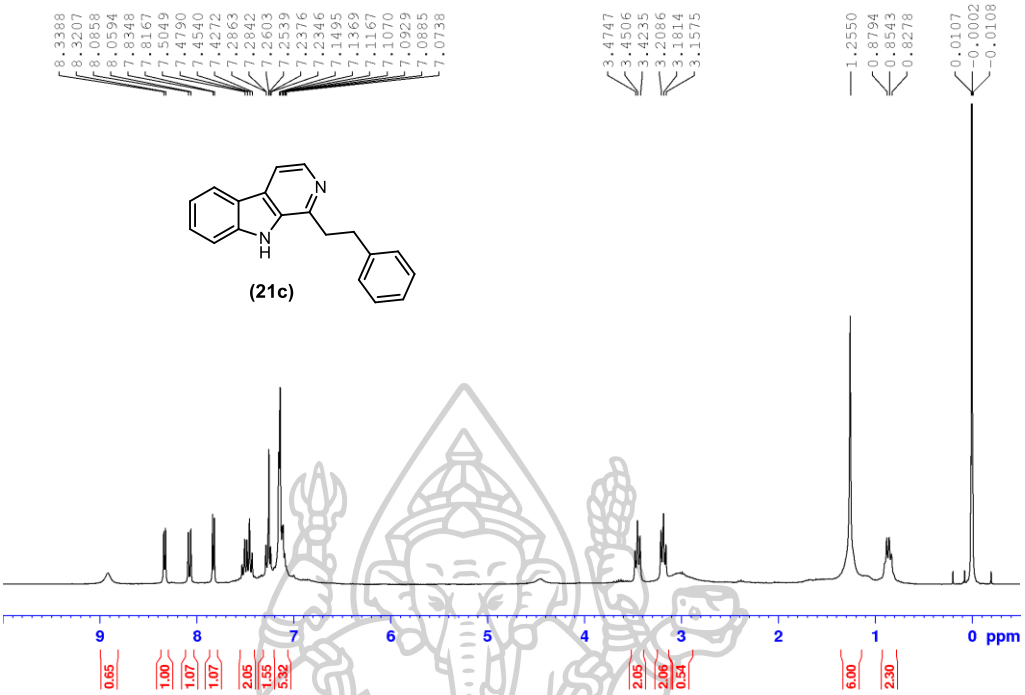
^{13}C-NMR spectrum of (**21a**) (75 MHz, $CDCl_3$)



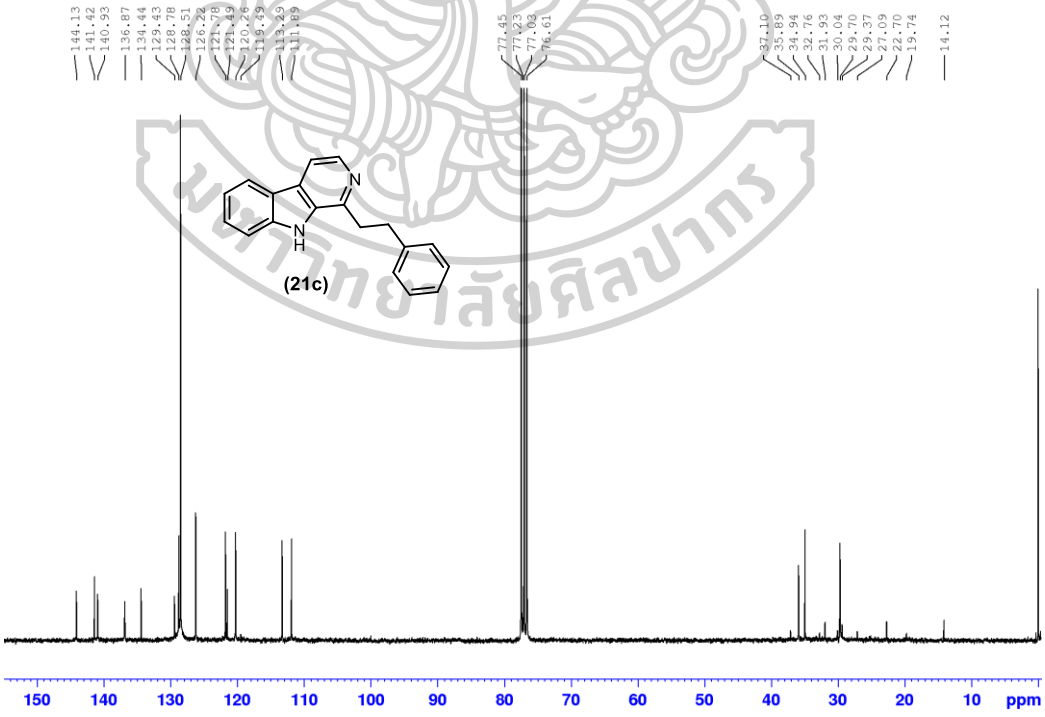
^{1}H-NMR spectrum of (**21b**) (300 MHz, $CDCl_3$)



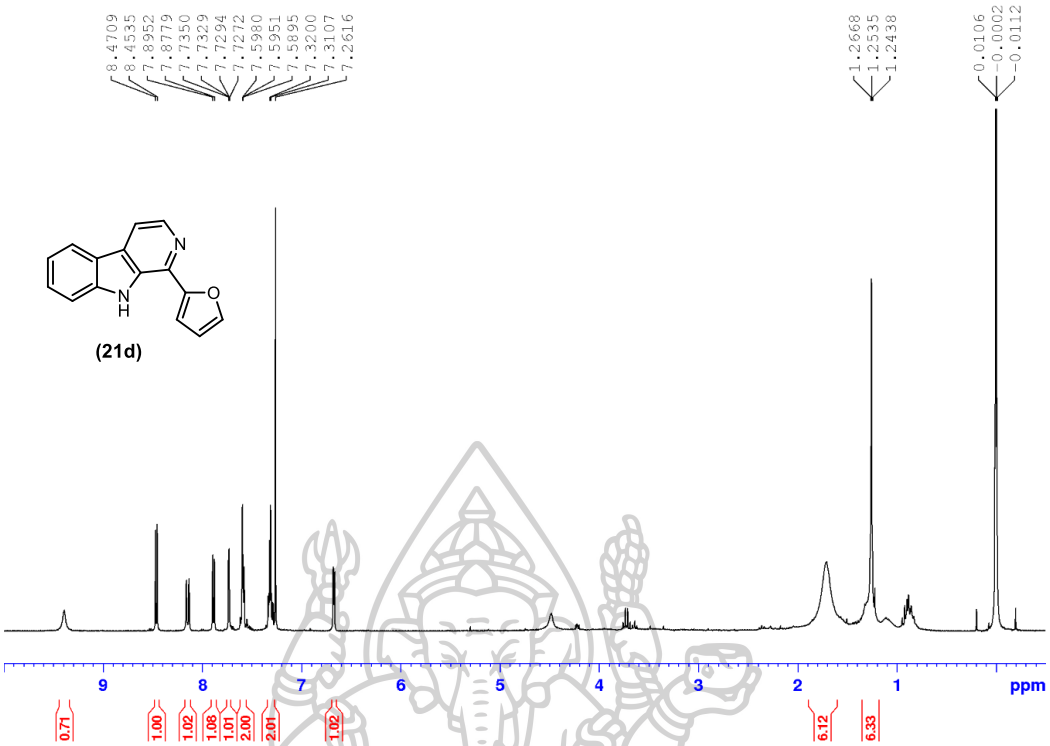
^{13}C-NMR spectrum of (**21b**) (75 MHz, $CDCl_3$)



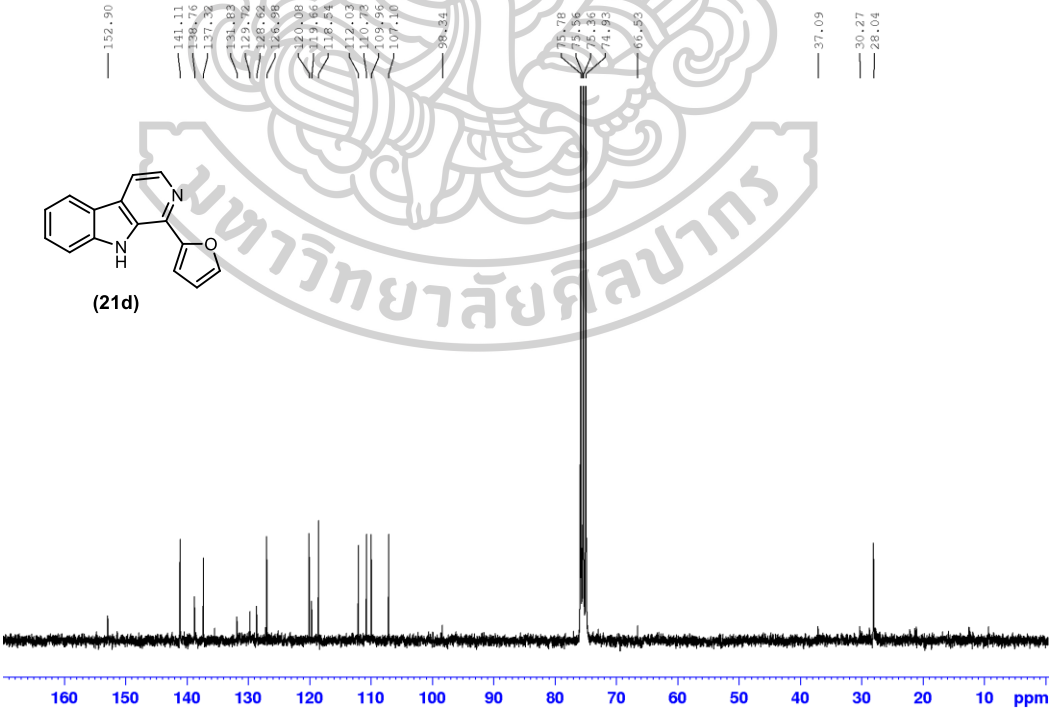
^{1}H-NMR spectrum of (**21c**) (300 MHz, $CDCl_3$)



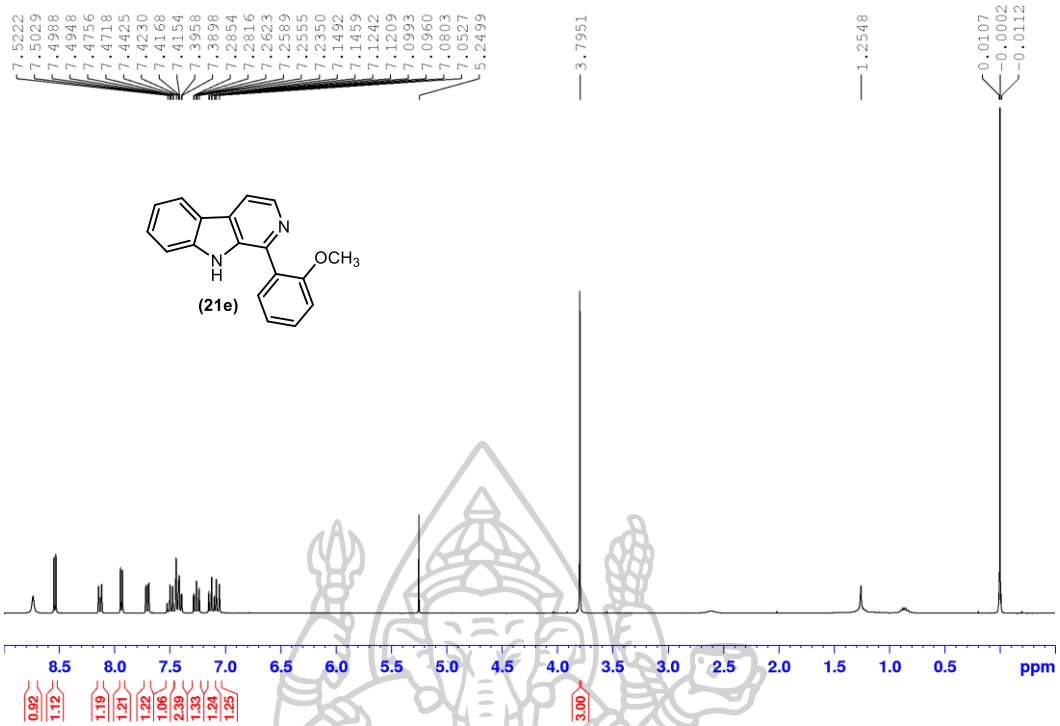
^{13}C-NMR spectrum of (**21c**) (75 MHz, $CDCl_3$)



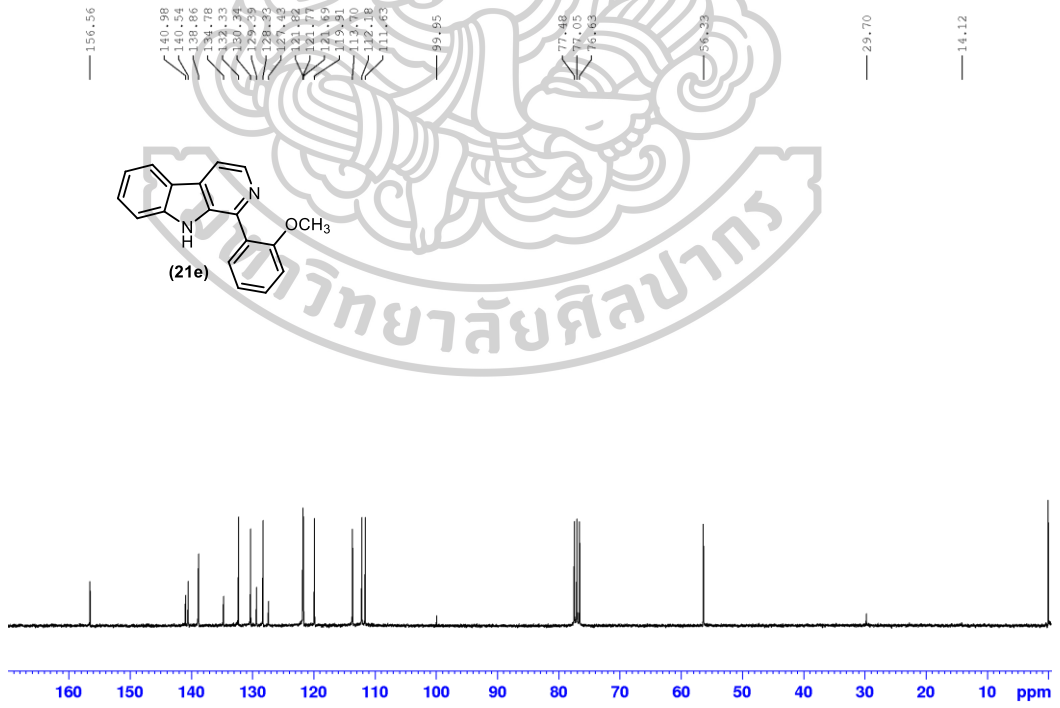
^{1}H-NMR spectrum of (**21d**) (300 MHz, $CDCl_3$)



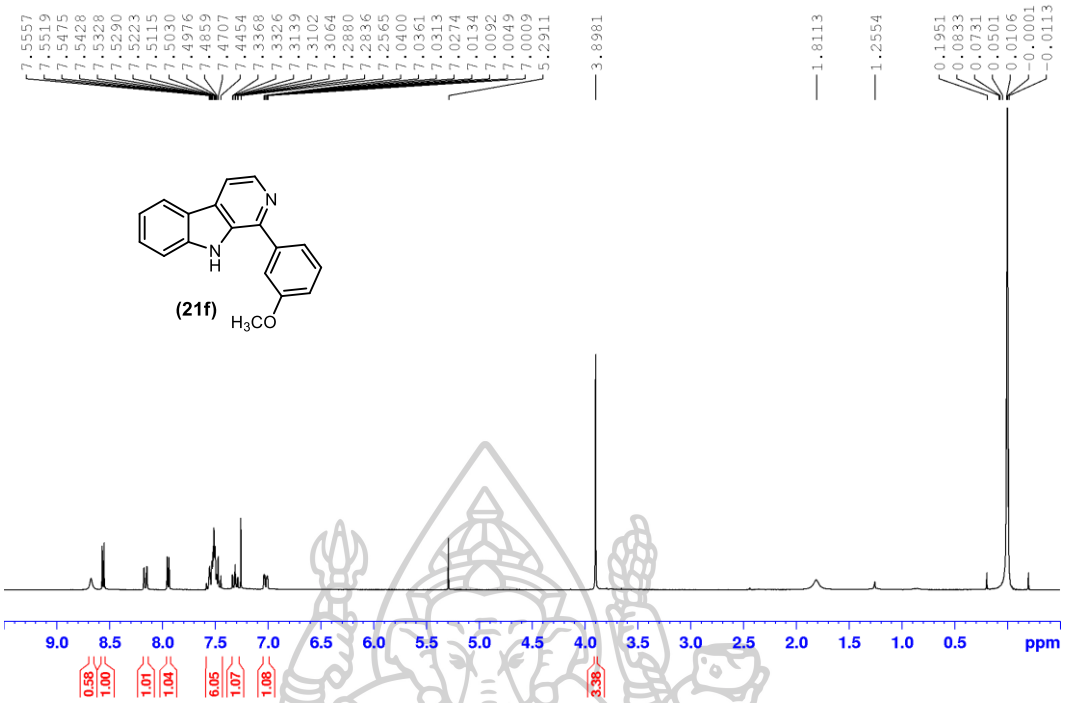
^{13}C-NMR spectrum of (**21d**) (75 MHz, $CDCl_3$)



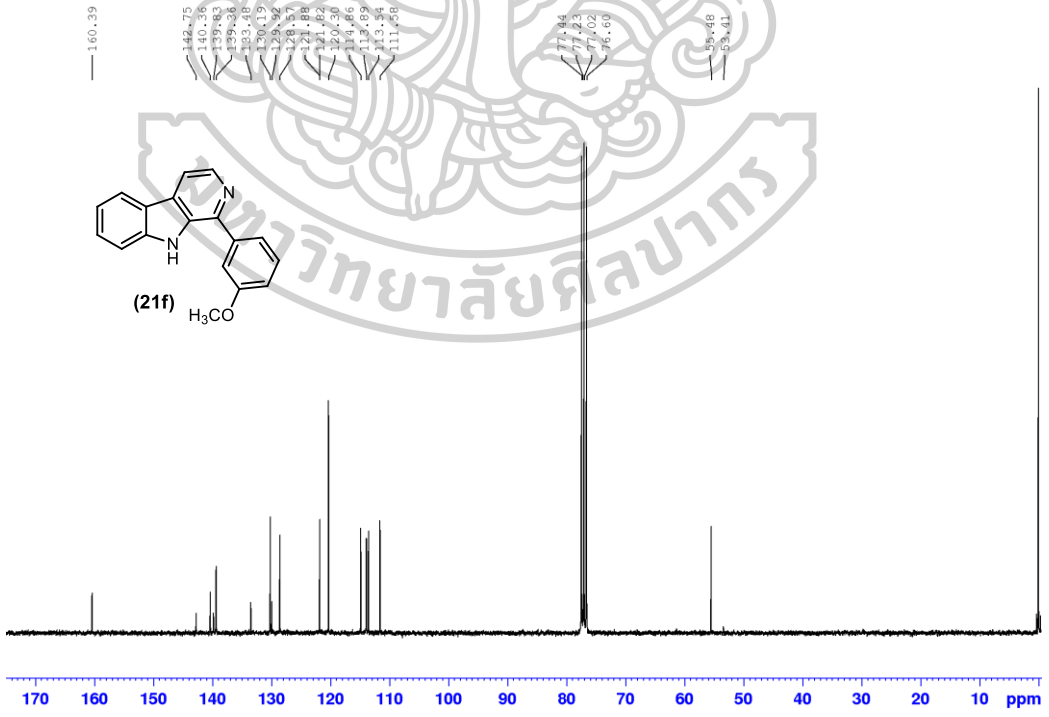
^{1}H-NMR spectrum of (**21e**) (300 MHz, $CDCl_3$)



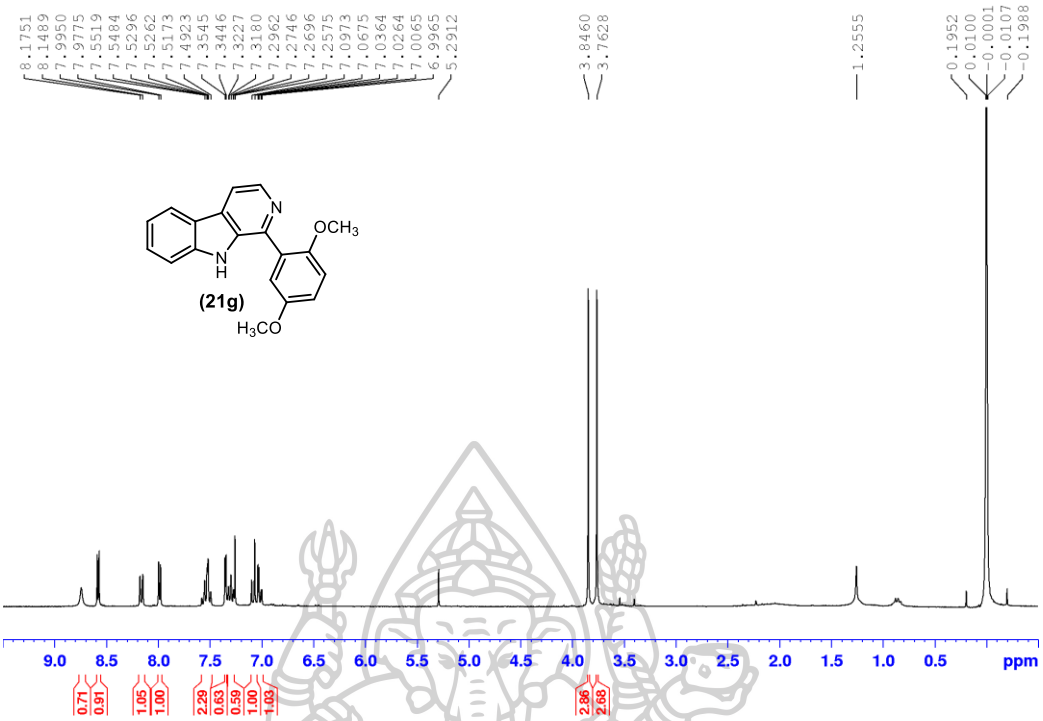
^{13}C-NMR spectrum of (**21e**) (75 MHz, $CDCl_3$)



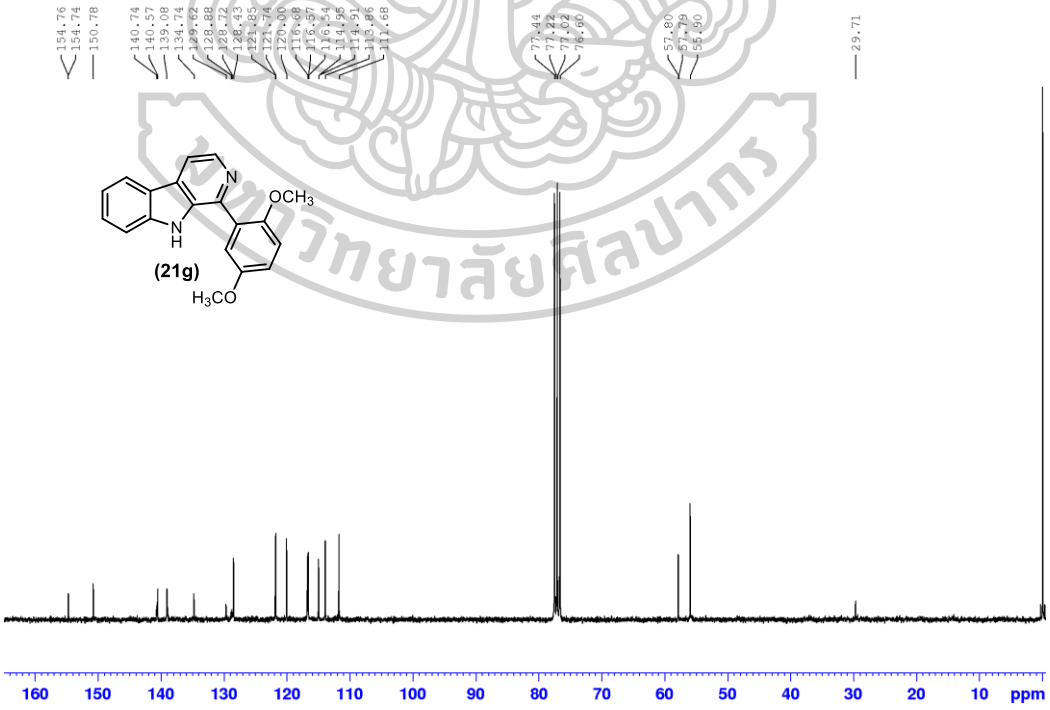
^{1}H-NMR spectrum of (**21f**) (300 MHz, $CDCl_3$)



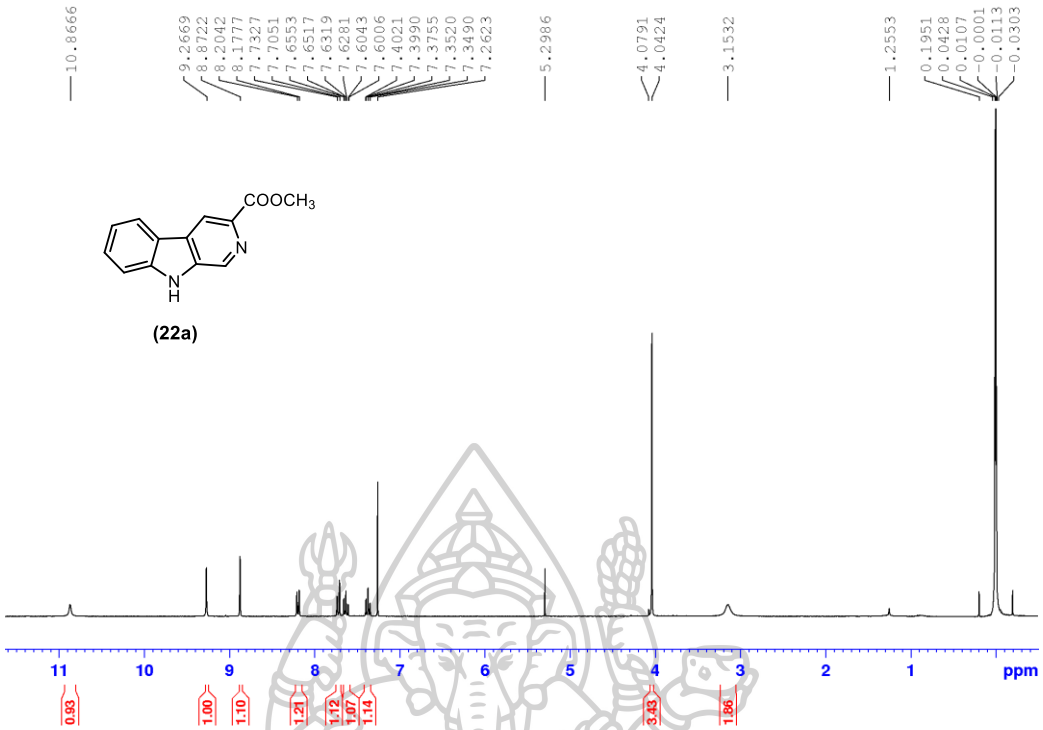
^{13}C-NMR spectrum of (**21f**) (75 MHz, $CDCl_3$)



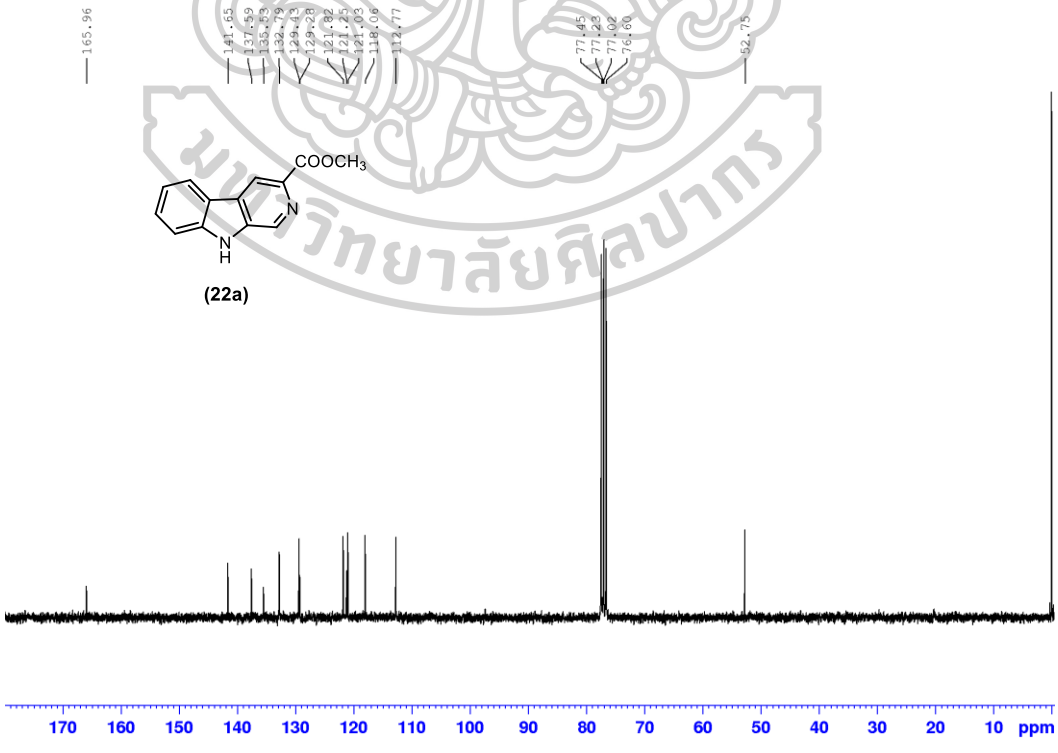
^{1}H-NMR spectrum of (**21g**) (300 MHz, $CDCl_3$)



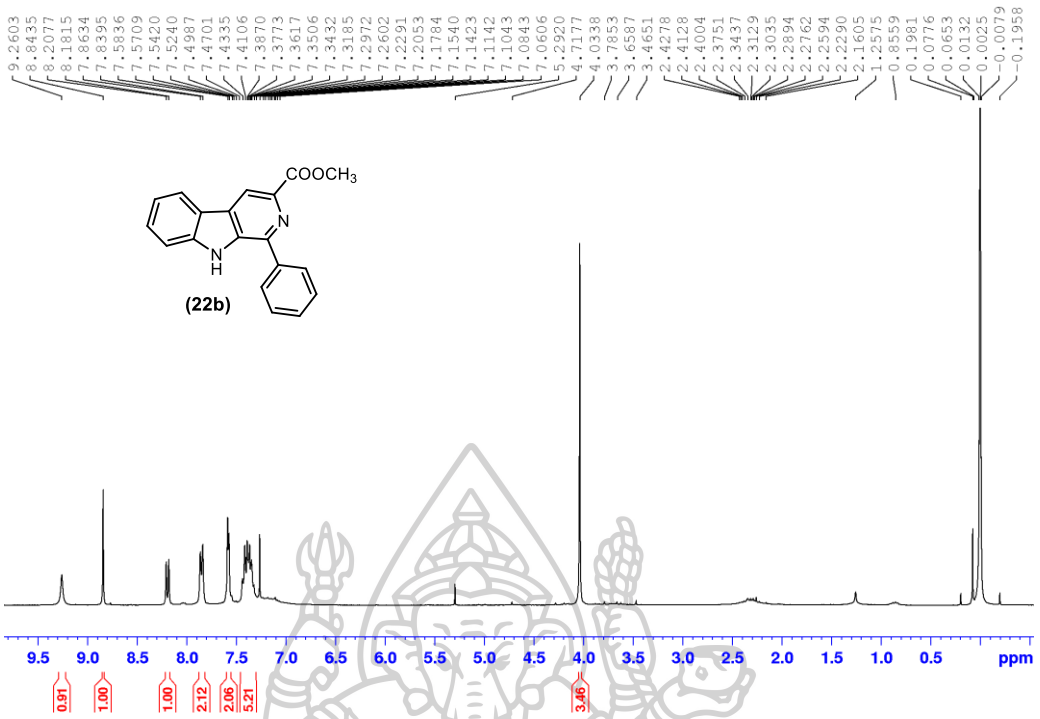
^{13}C-NMR spectrum of (**21g**) (75 MHz, $CDCl_3$)



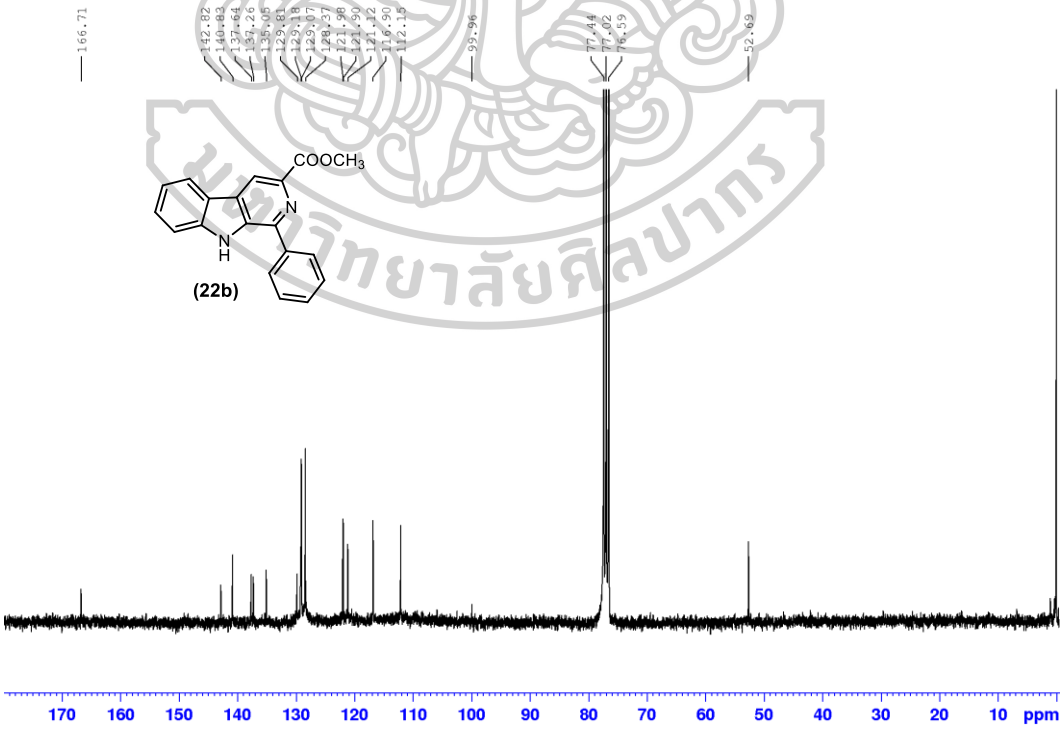
^{1}H-NMR spectrum of (**22a**) (300 MHz, $CDCl_3$)



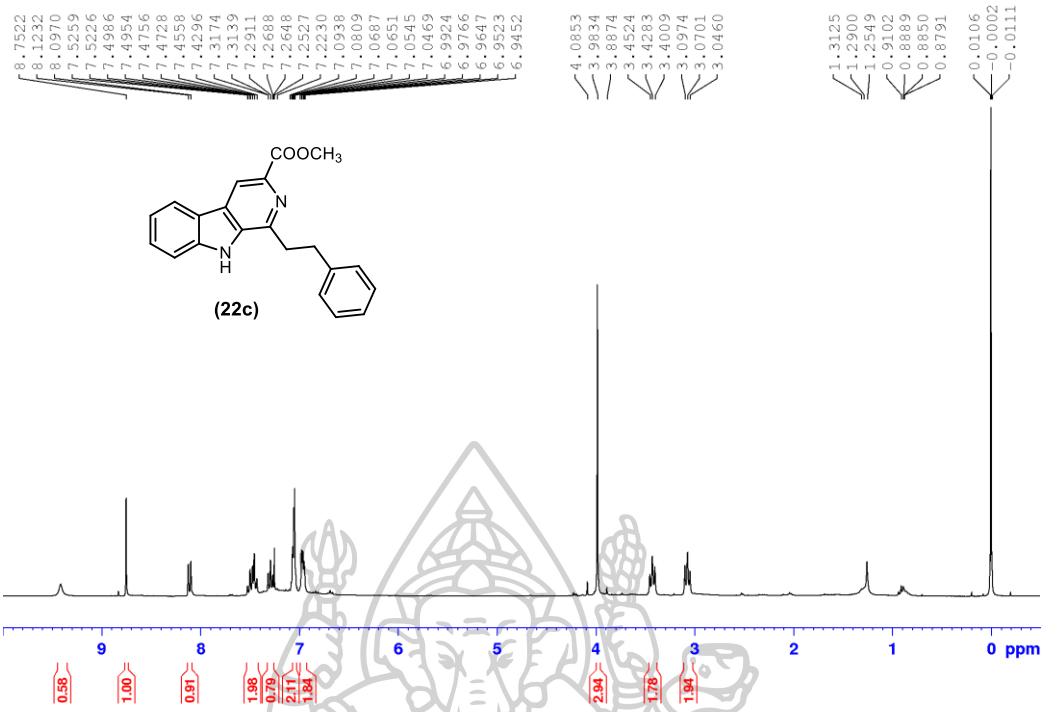
^{13}C-NMR spectrum of (**22a**) (75 MHz, $CDCl_3$)



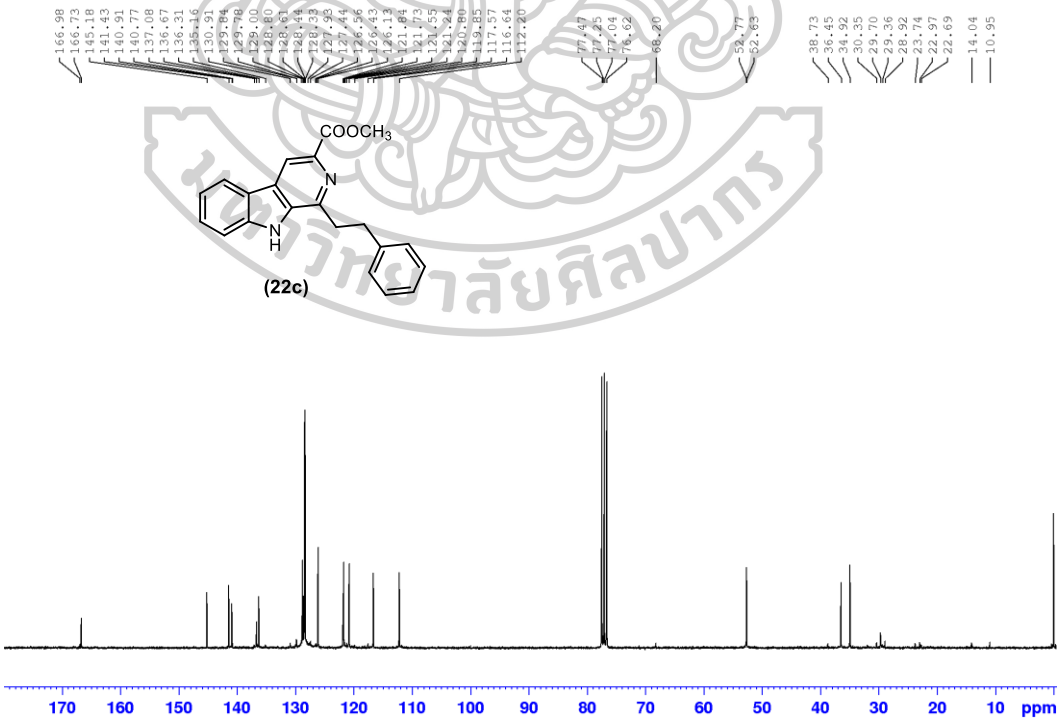
^{1}H-NMR spectrum of (**22b**) (300 MHz, $CDCl_3$)



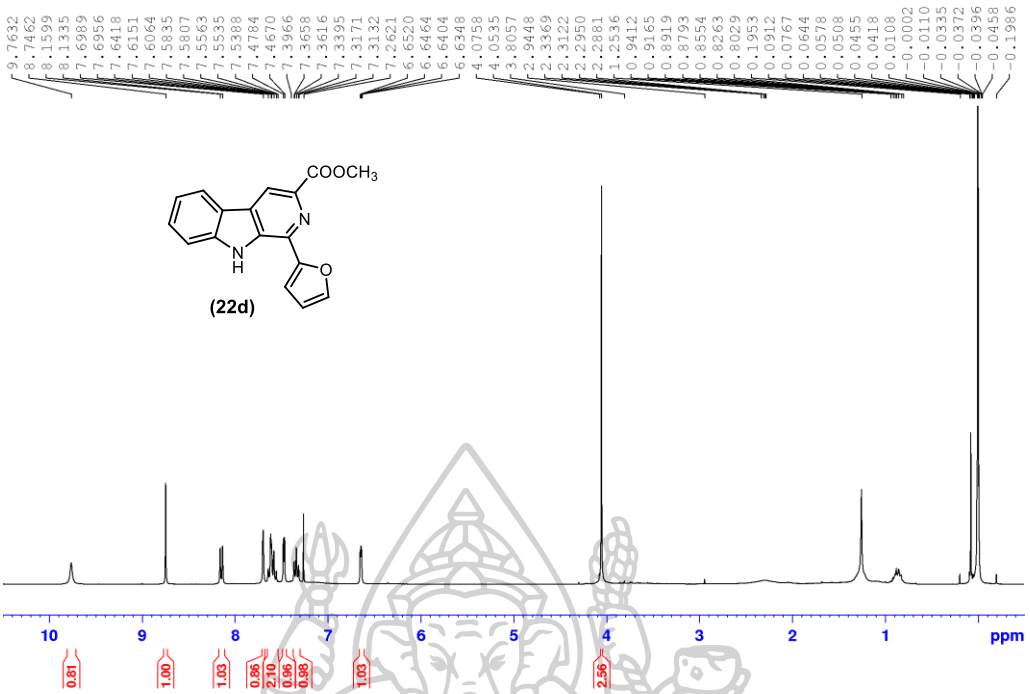
^{13}C-NMR spectrum of (**22b**) (75 MHz, $CDCl_3$)



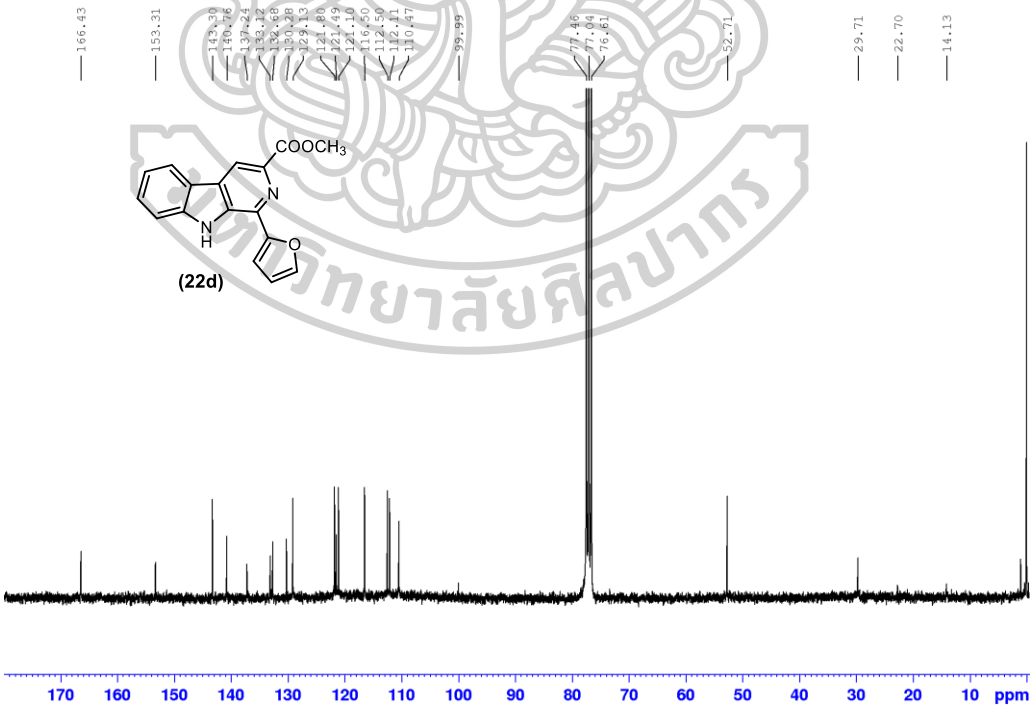
^{1}H-NMR spectrum of (**22c**) (300 MHz, $CDCl_3$)



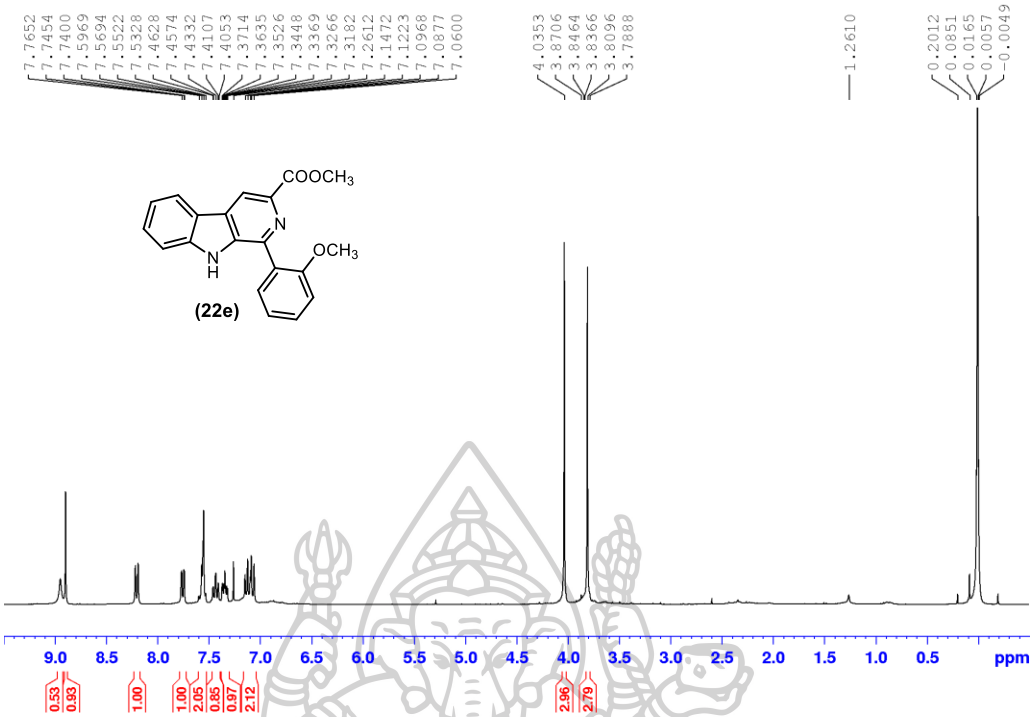
^{13}C-NMR spectrum of (**22c**) (75 MHz, $CDCl_3$)



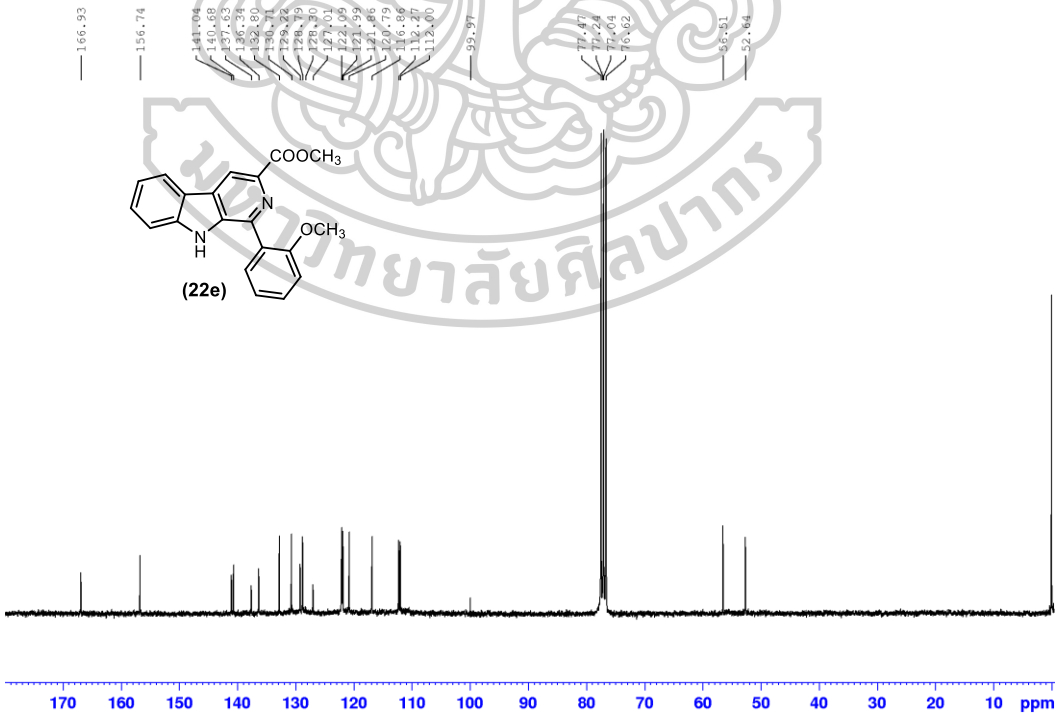
^{1}H-NMR spectrum of (**22d**) (300 MHz, $CDCl_3$)



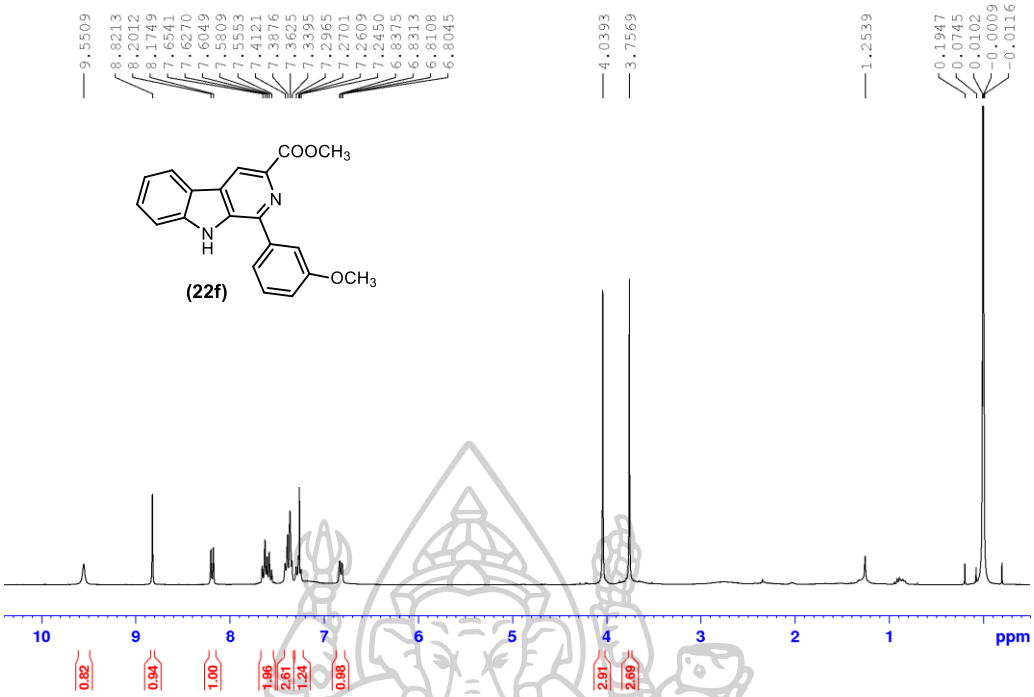
^{13}C-NMR spectrum of (**22d**) (75 MHz, $CDCl_3$)



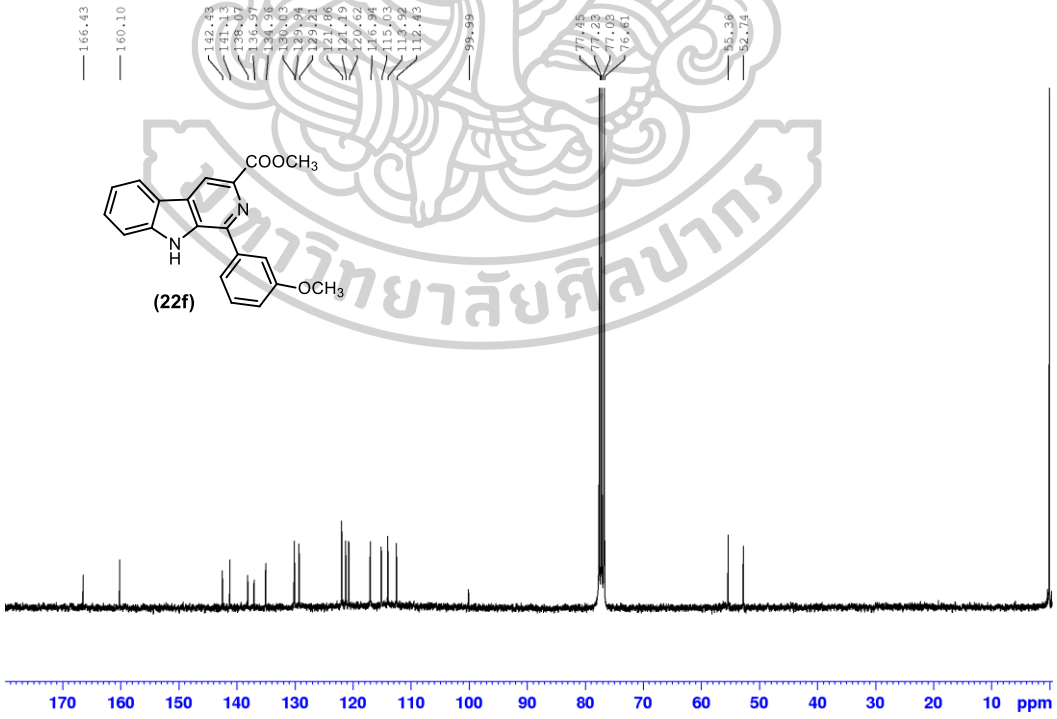
^{1}H-NMR spectrum of (**22e**) (300 MHz, $CDCl_3$)



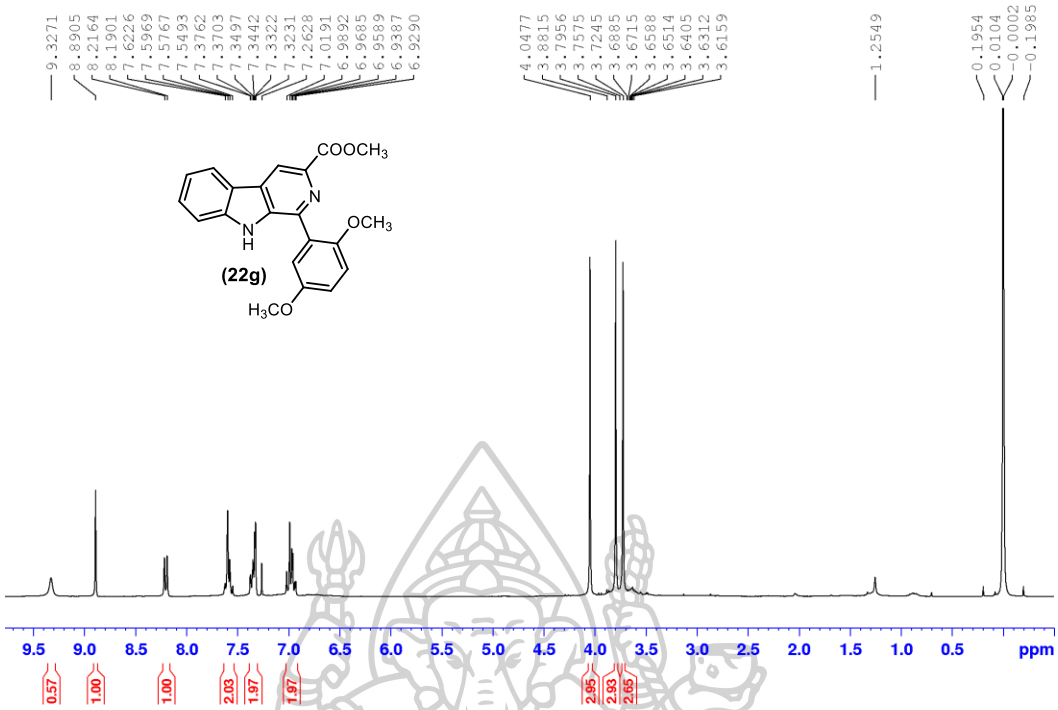
^{13}C-NMR spectrum of (**22e**) (75 MHz, $CDCl_3$)



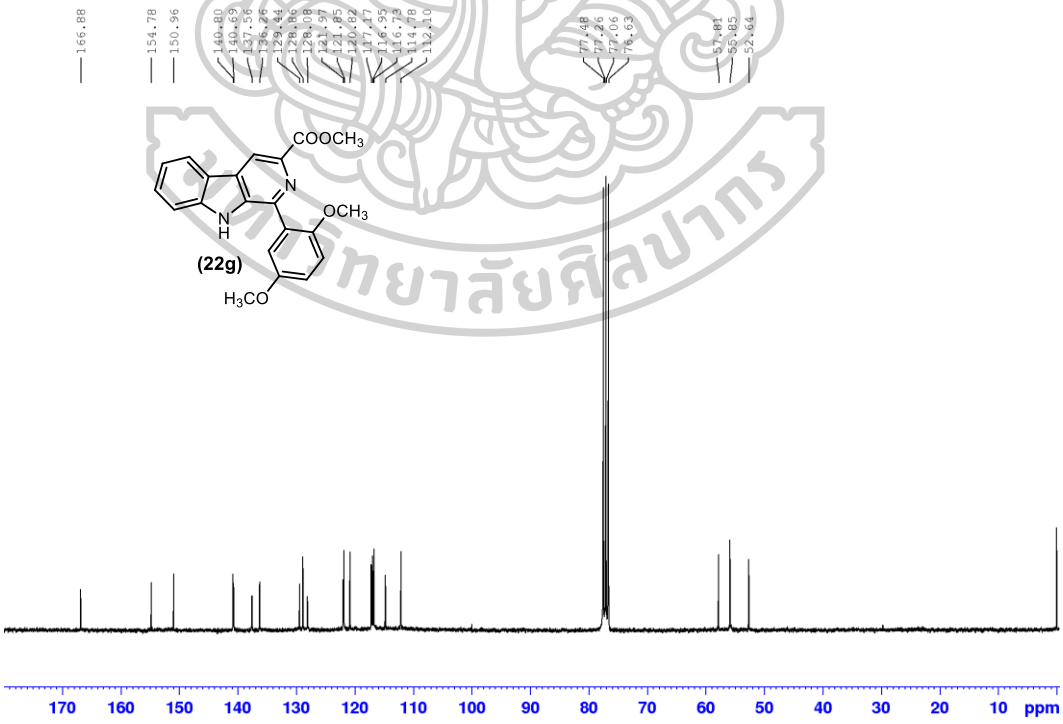
^{1}H-NMR spectrum of (**22f**) (300 MHz, $CDCl_3$)



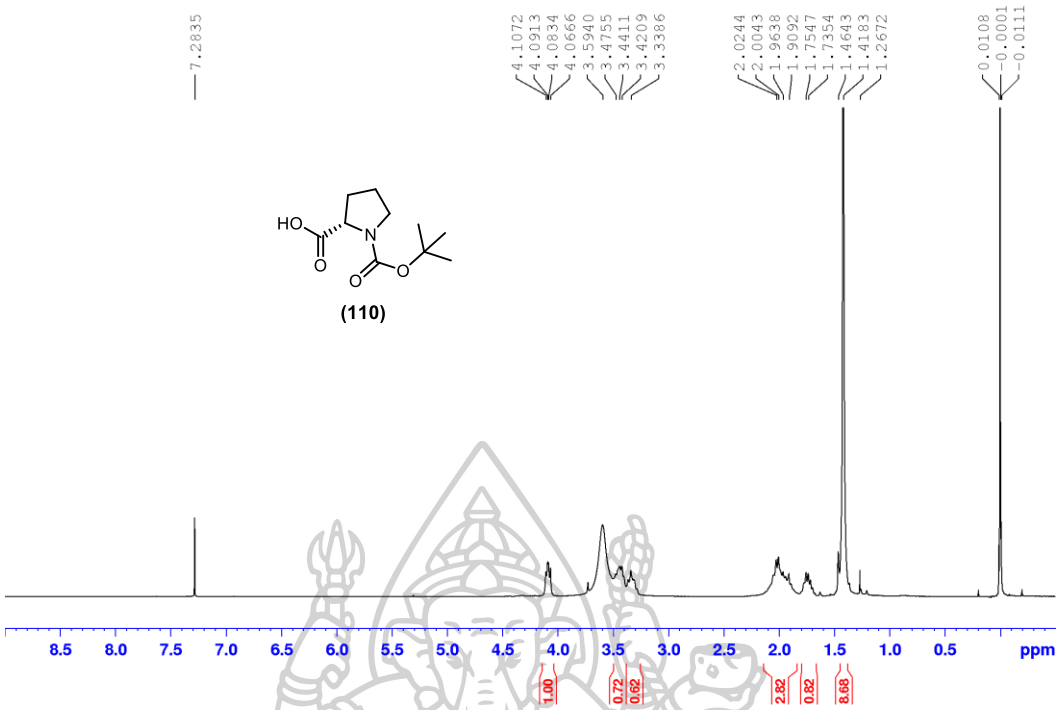
^{13}C-NMR spectrum of (**22f**) (75 MHz, $CDCl_3$)



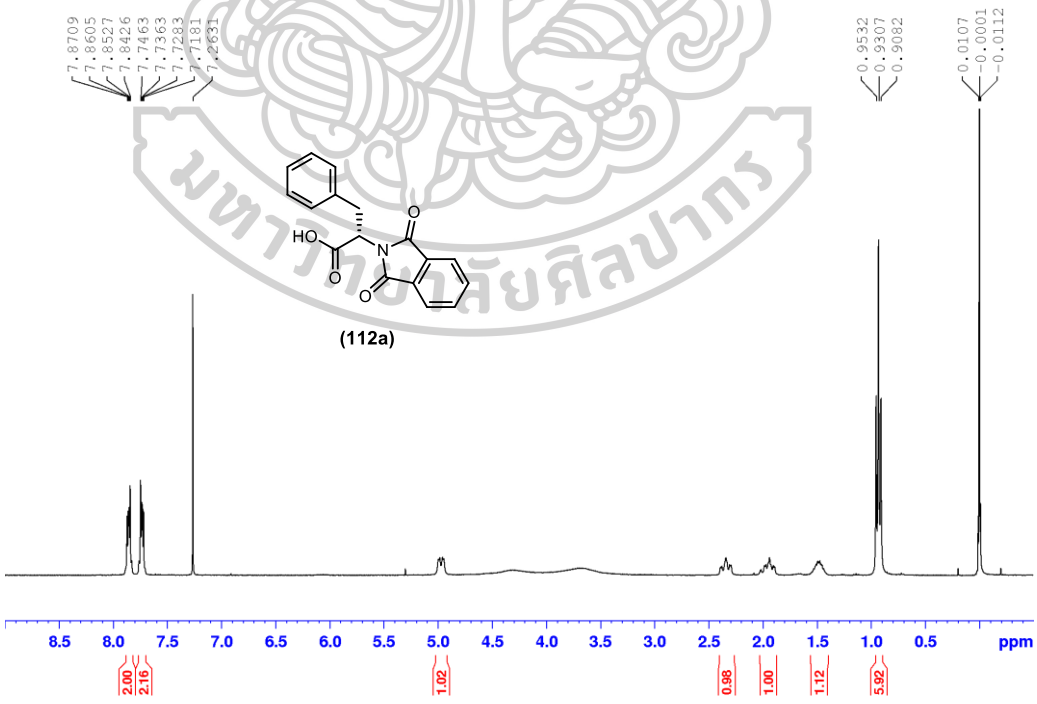
^{1}H-NMR spectrum of (**22g**) (300 MHz, $CDCl_3$)



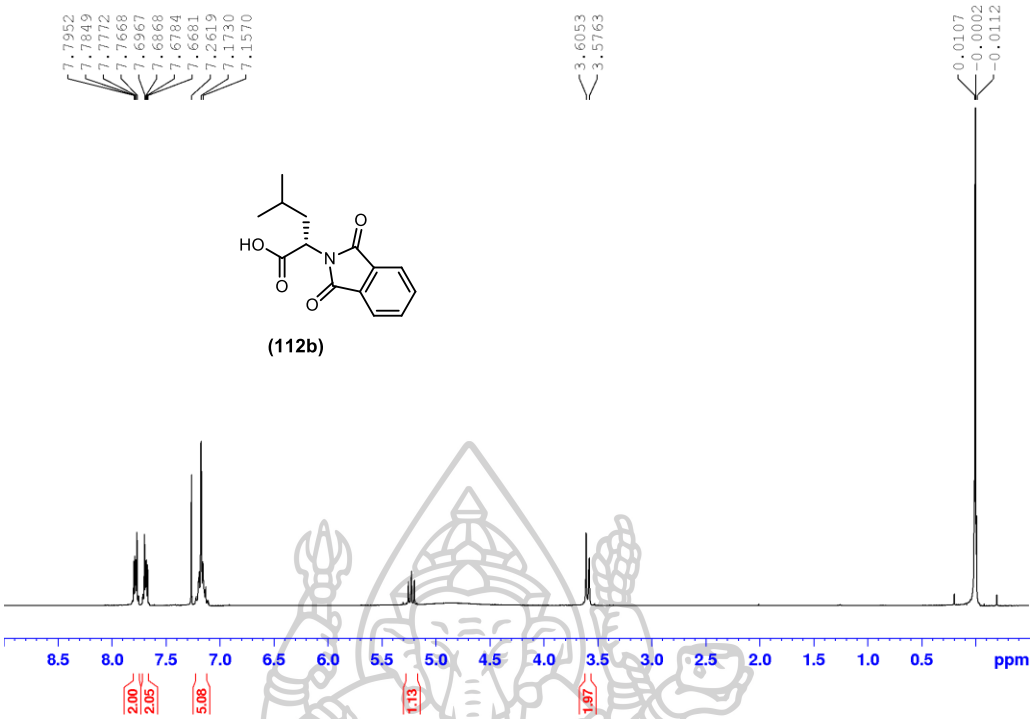
^{13}C-NMR spectrum of (**22g**) (75 MHz, $CDCl_3$)



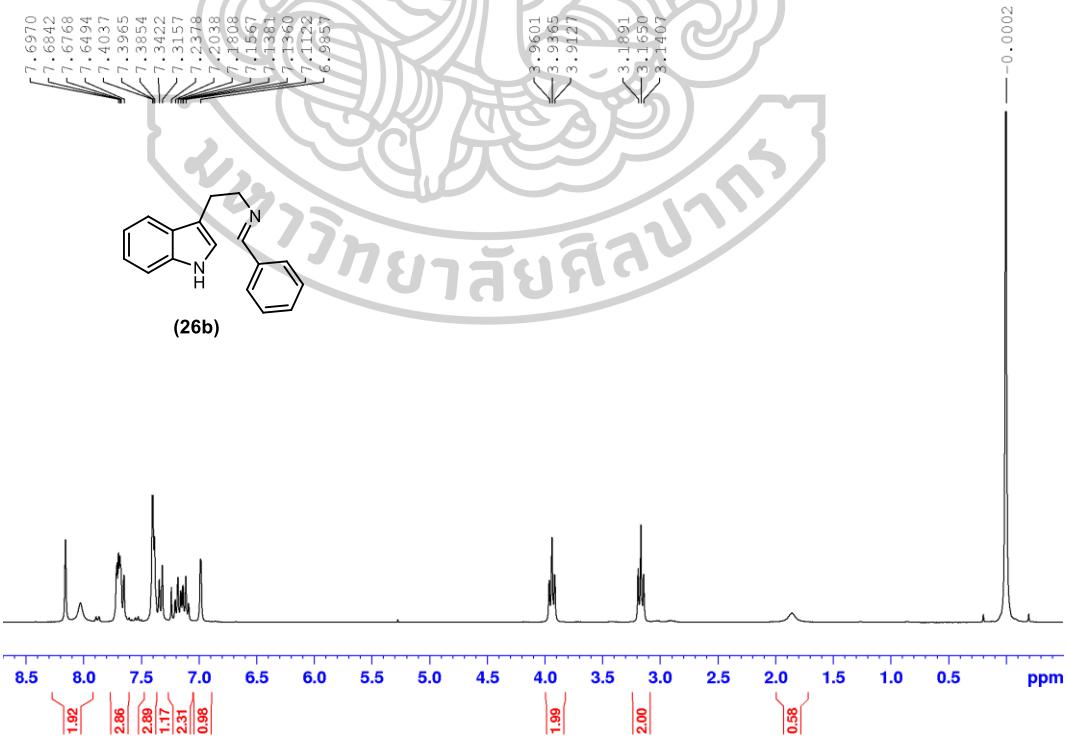
^{1}H-NMR spectrum of (**110**) (300 MHz, $CDCl_3$)



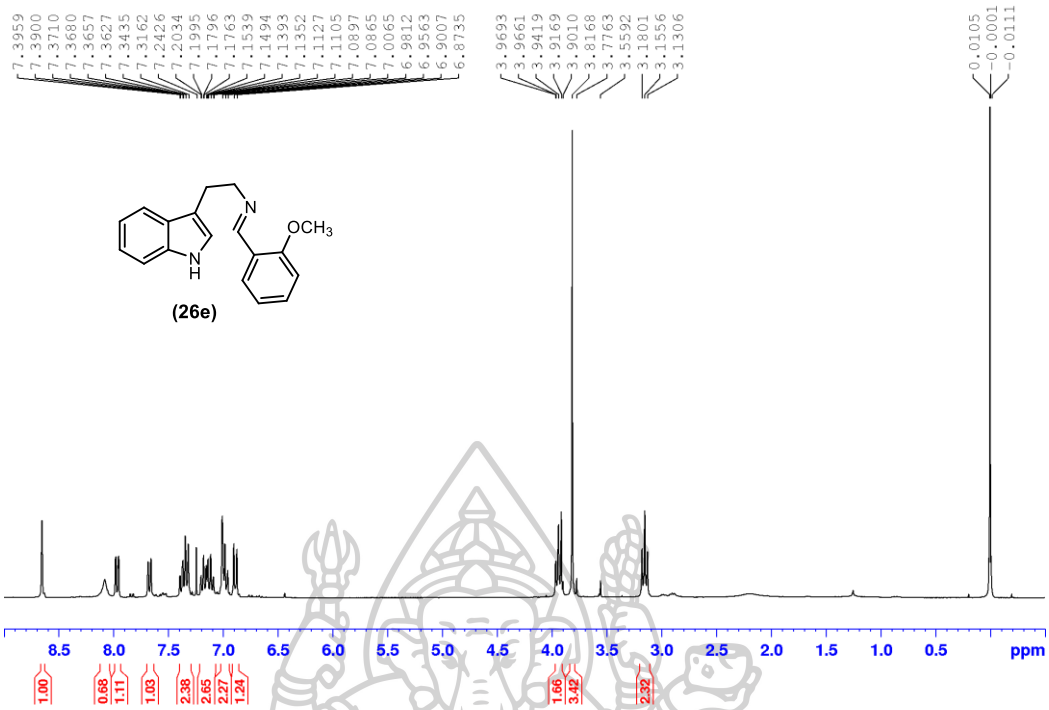
^{1}H-NMR spectrum of (**112a**) (300 MHz, $CDCl_3$)



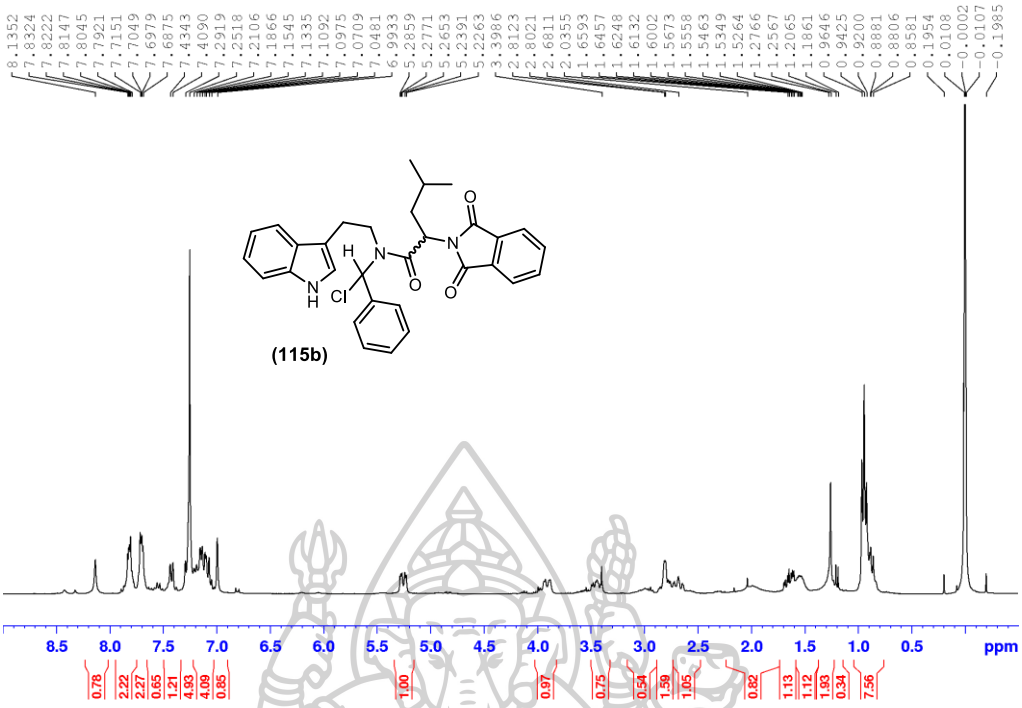
^{1}H-NMR spectrum of (**112b**) (300 MHz, $CDCl_3$)



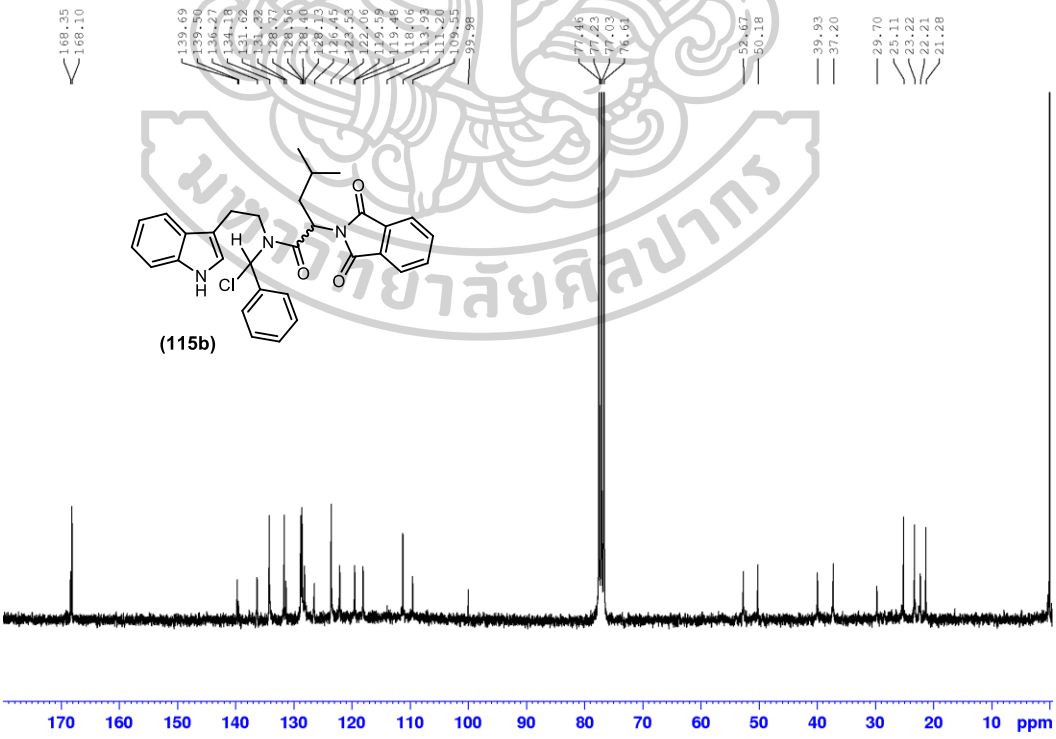
^{1}H-NMR spectrum of (**26b**) (300 MHz, $CDCl_3$)



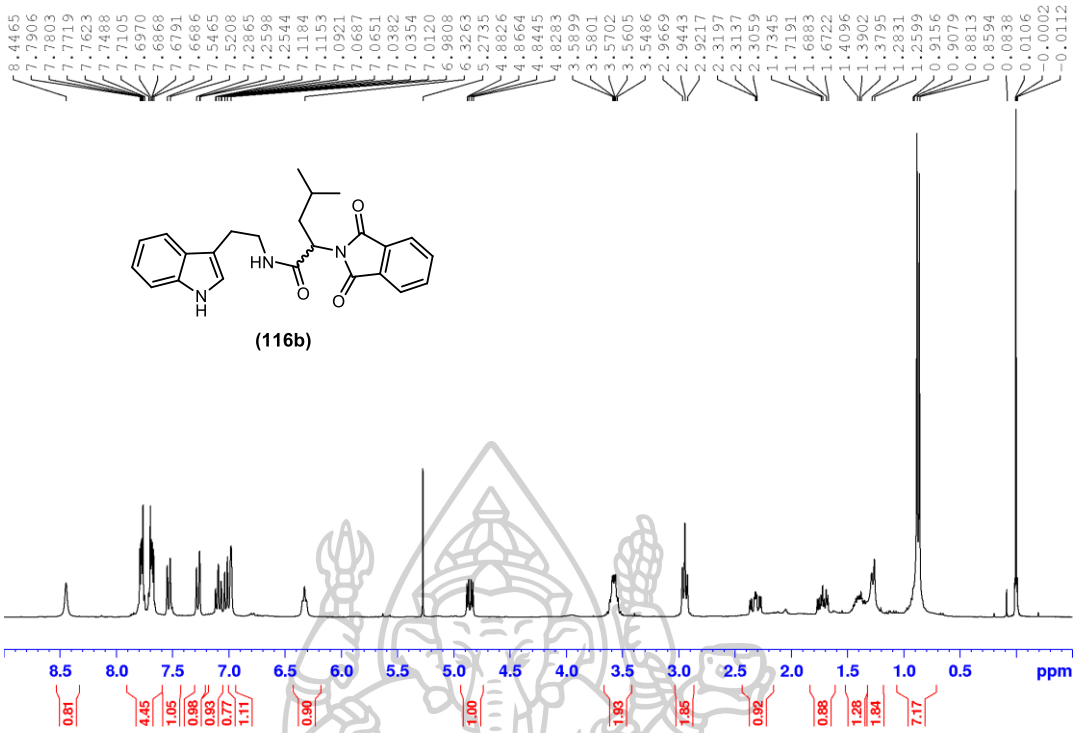
^{1}H-NMR spectrum of (**26e**) (300 MHz, $CDCl_3$)



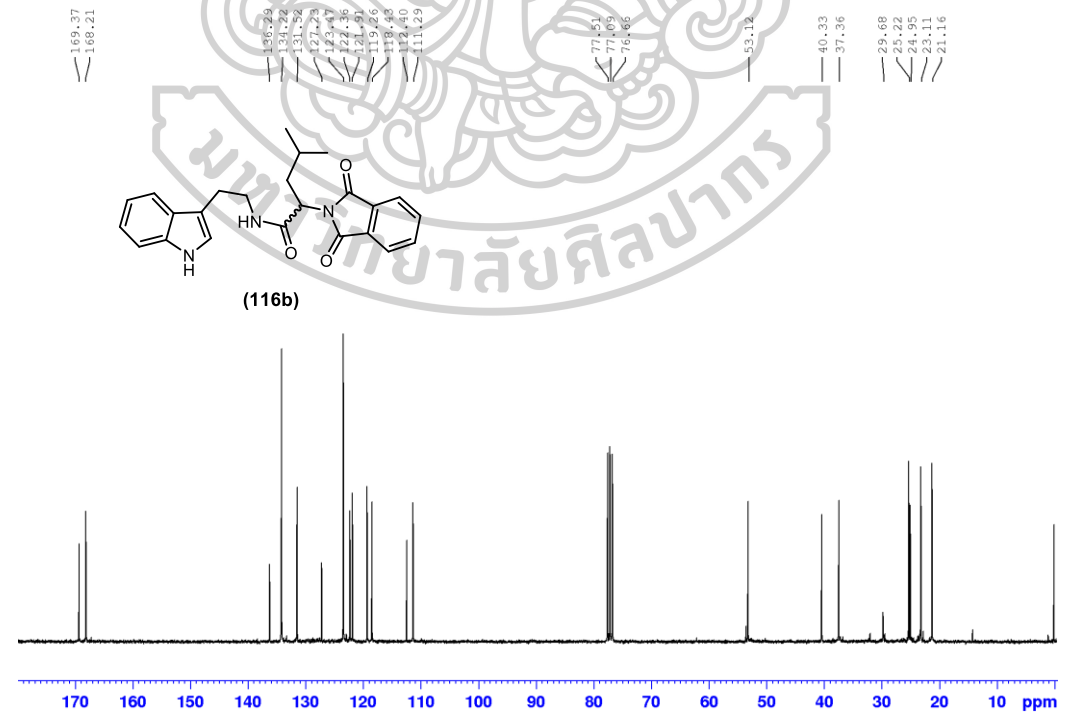
^{1}H-NMR spectrum of (**115b**) (300 MHz, $CDCl_3$)



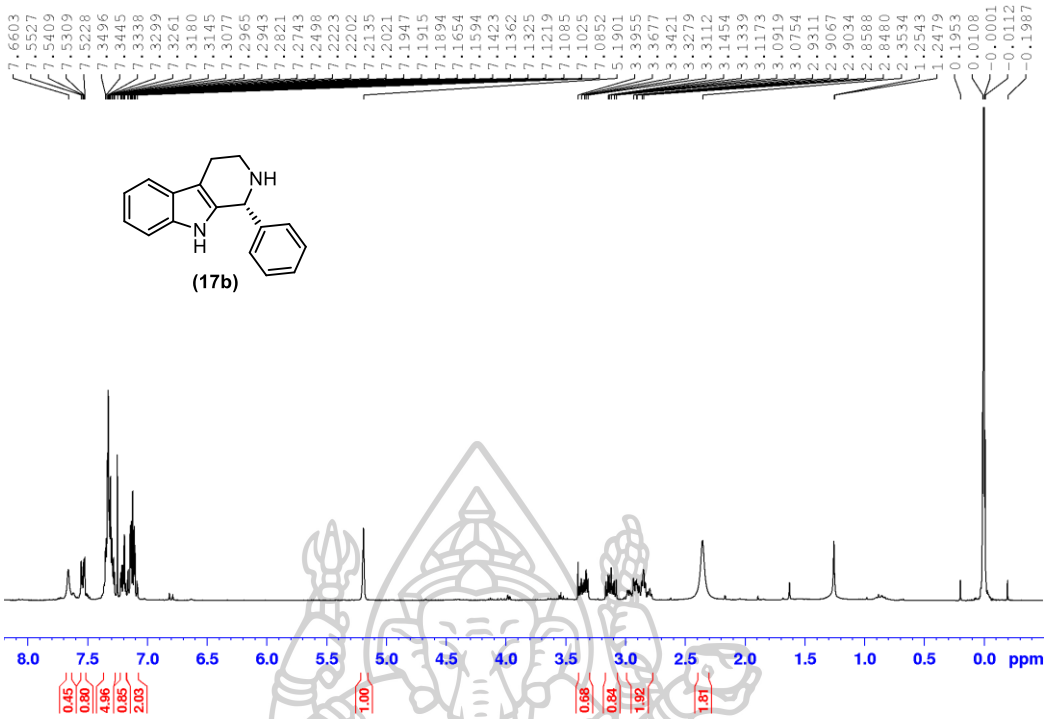
^{13}C-NMR spectrum of (**115b**) (75 MHz, $CDCl_3$)



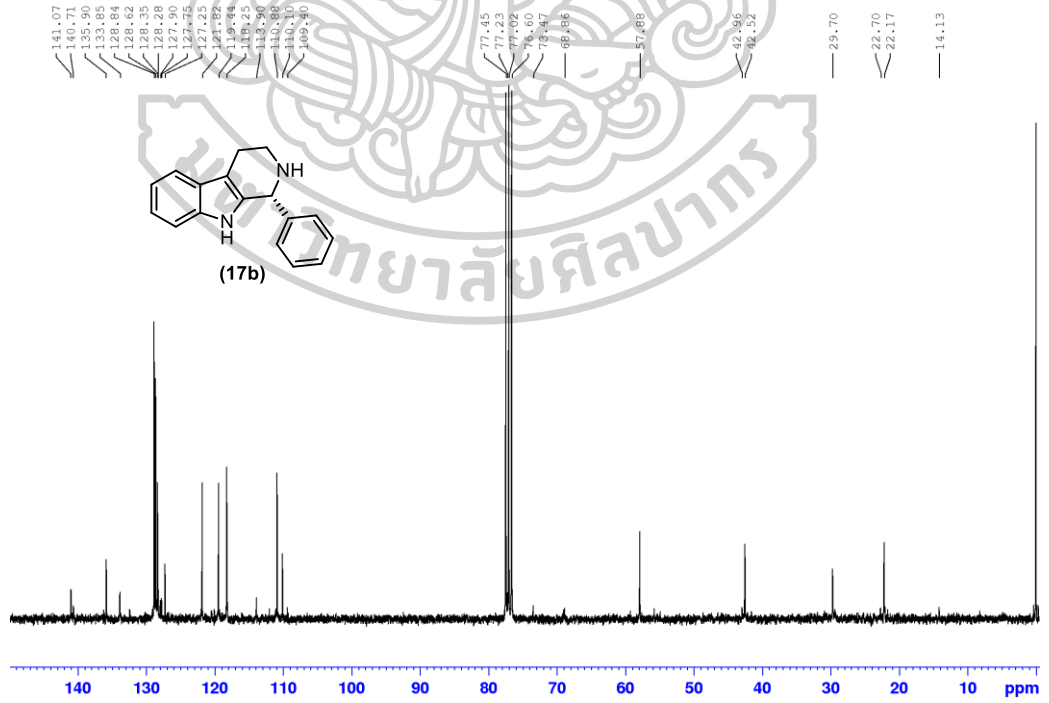
^{1}H-NMR spectrum of (**116b**) (300 MHz, $CDCl_3$)



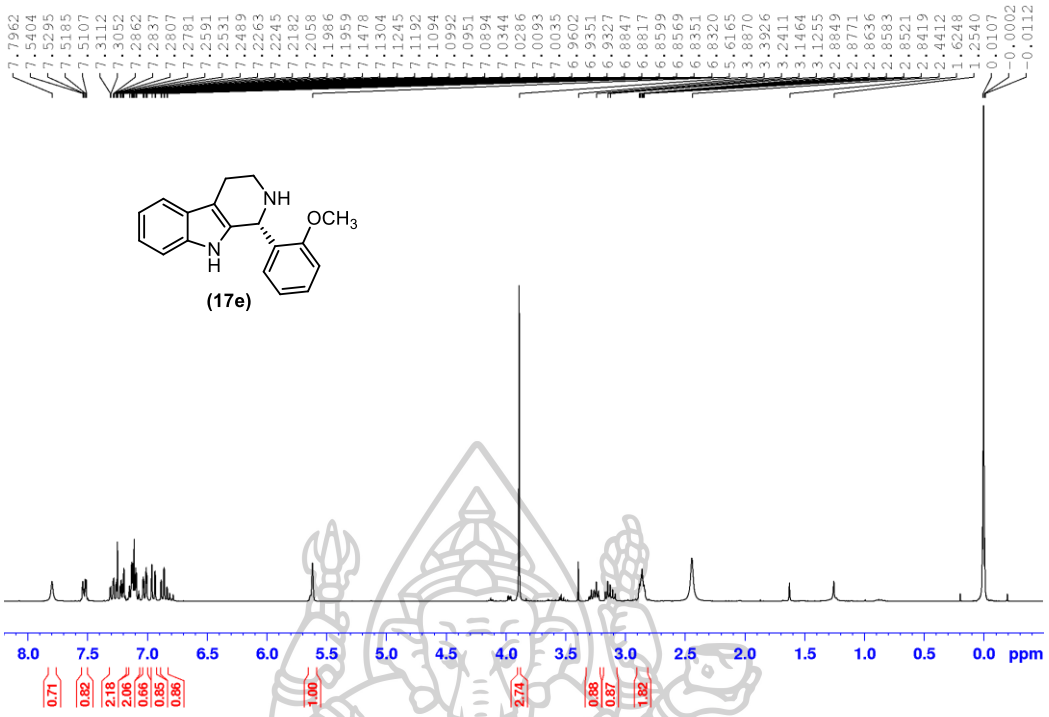
^{13}C-NMR spectrum of (**116b**) (75 MHz, $CDCl_3$)



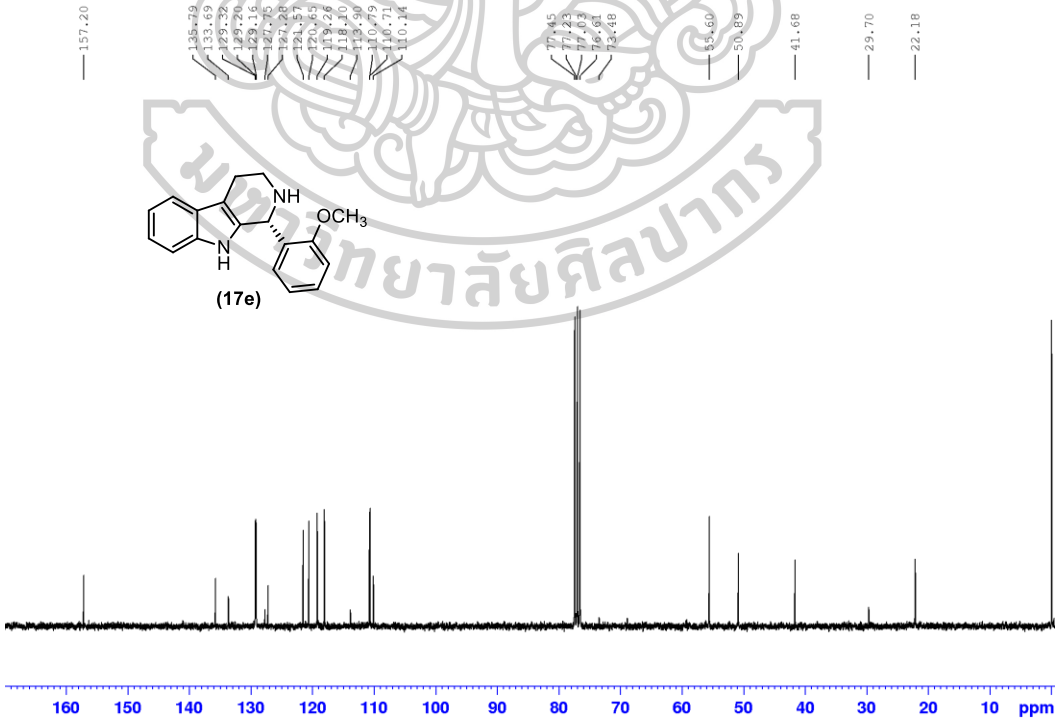
^{1}H-NMR spectrum of (**17b**) (300 MHz, $CDCl_3$)



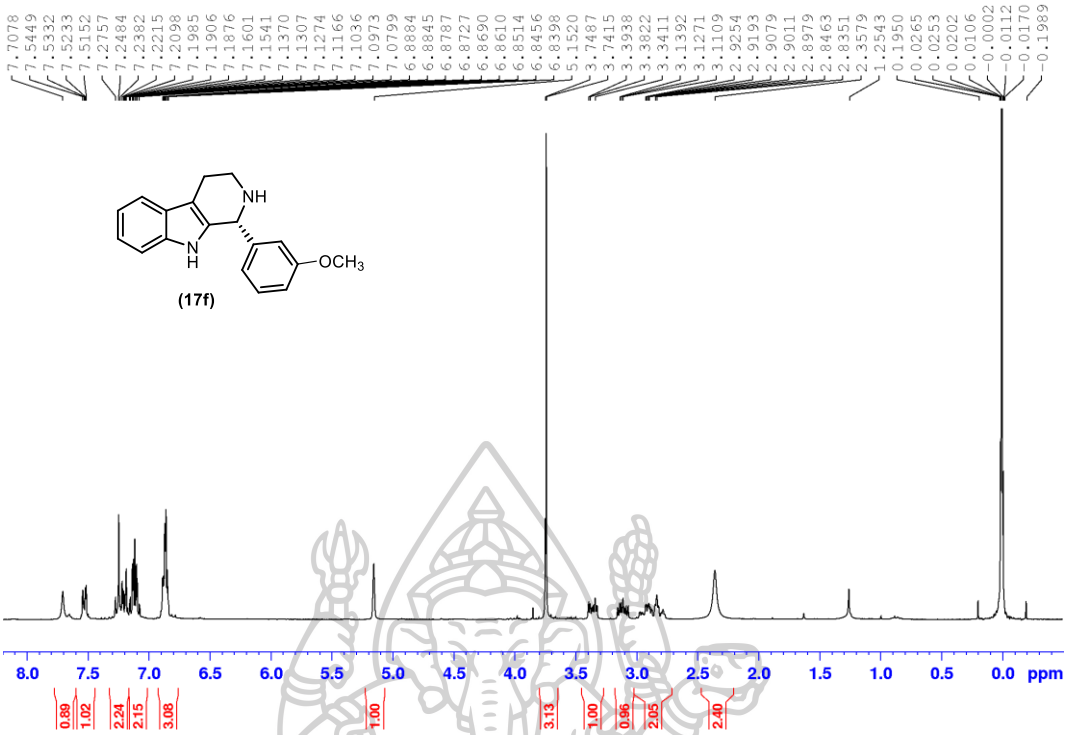
^{13}C-NMR spectrum of (**17b**) (75 MHz, $CDCl_3$)



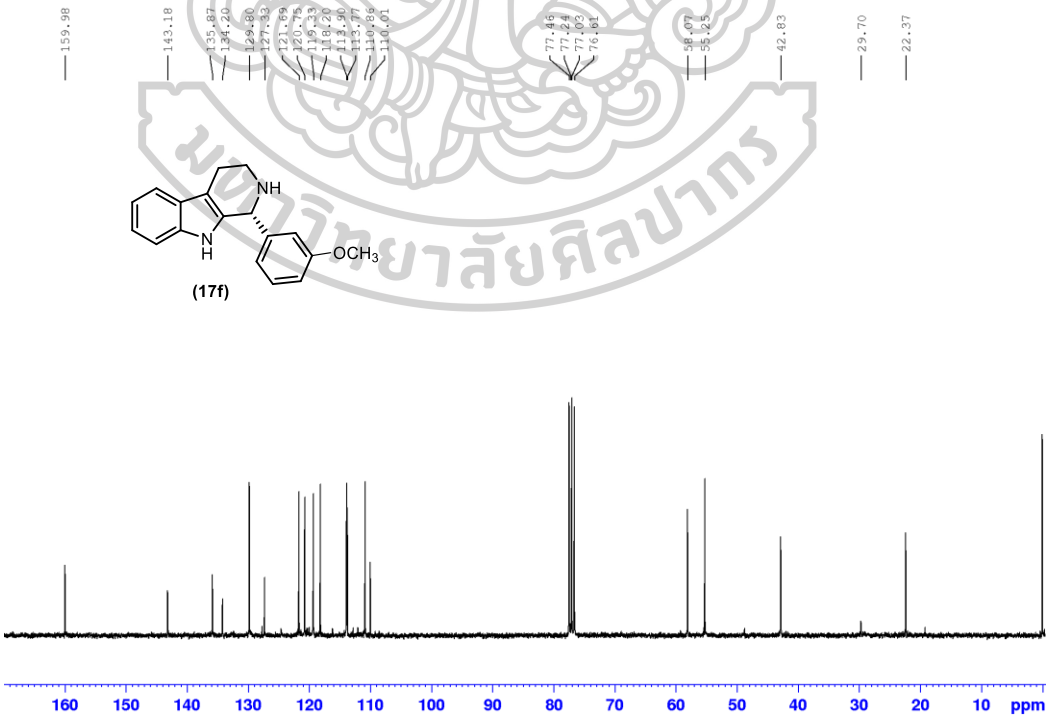
^{1}H-NMR spectrum of (**17e**) (300 MHz, $CDCl_3$)



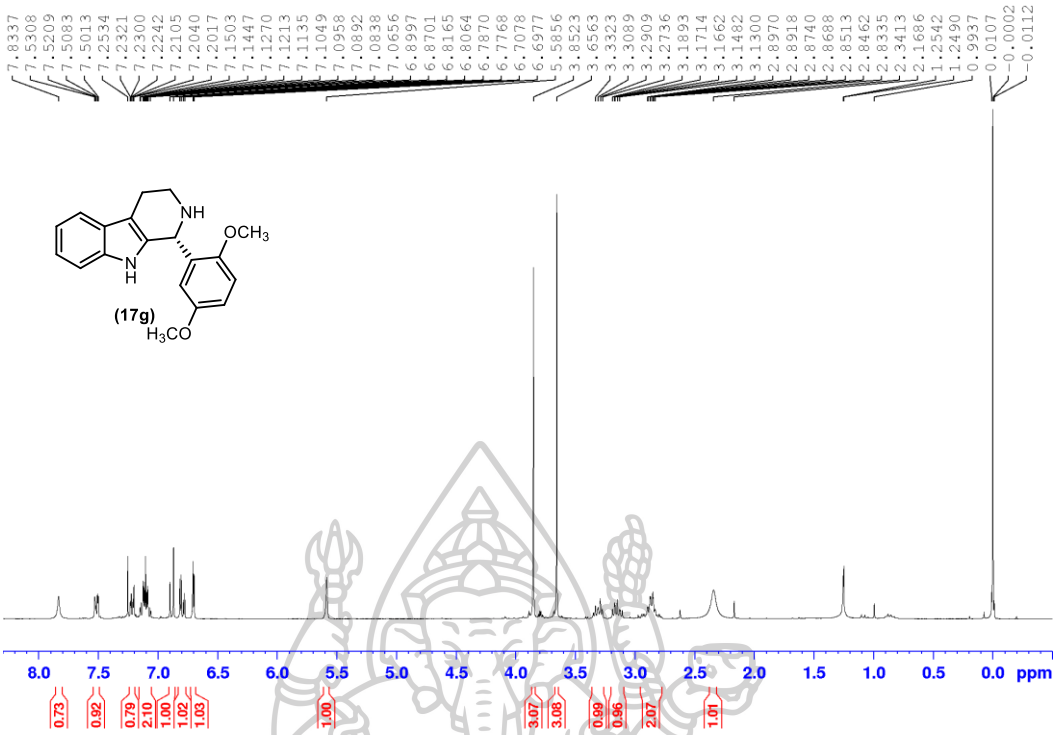
^{13}C-NMR spectrum of (**17e**) (75 MHz, $CDCl_3$)



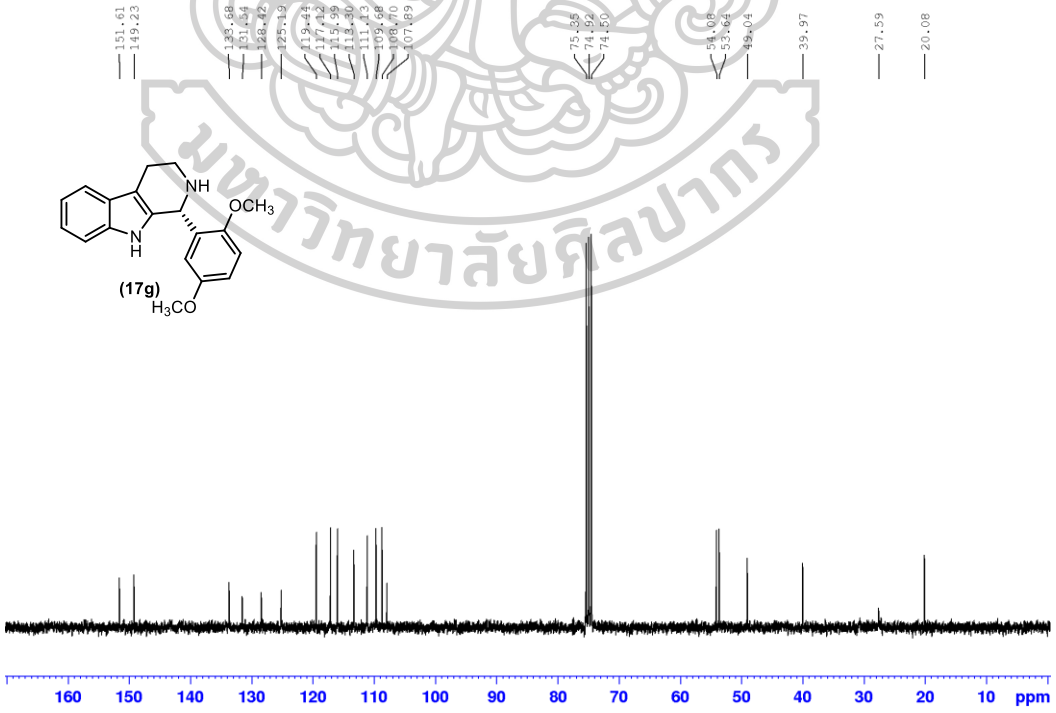
^{1}H-NMR spectrum of (**17f**) (300 MHz, $CDCl_3$)



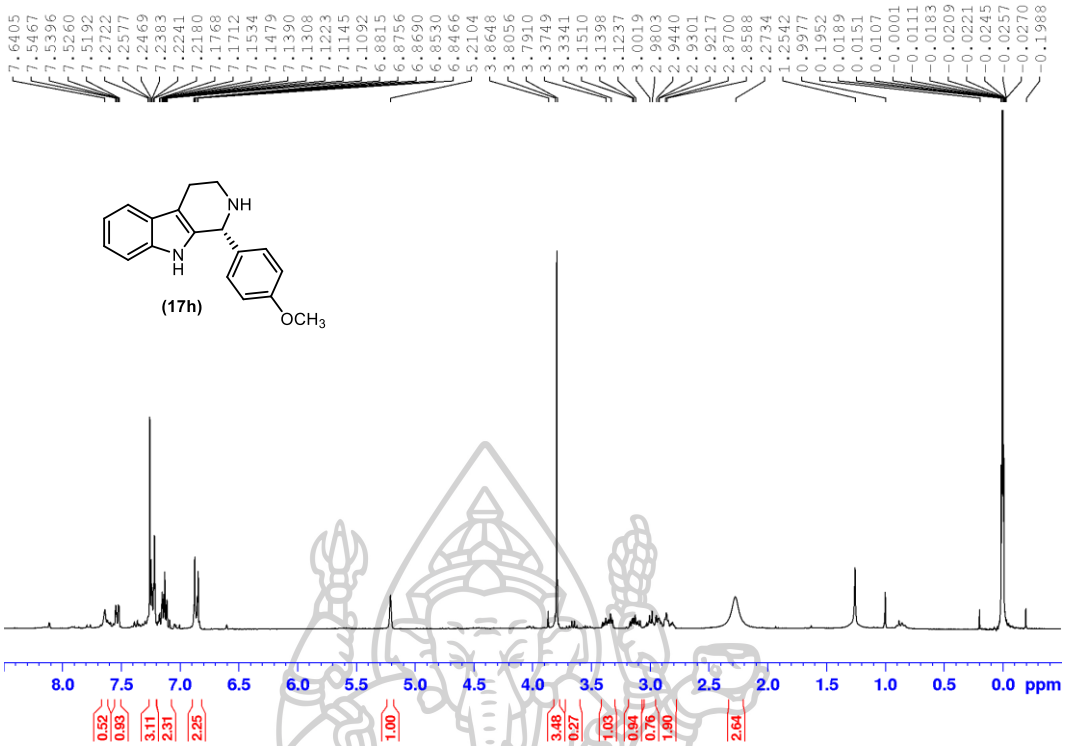
^{13}C-NMR spectrum of (**17f**) (75 MHz, $CDCl_3$)



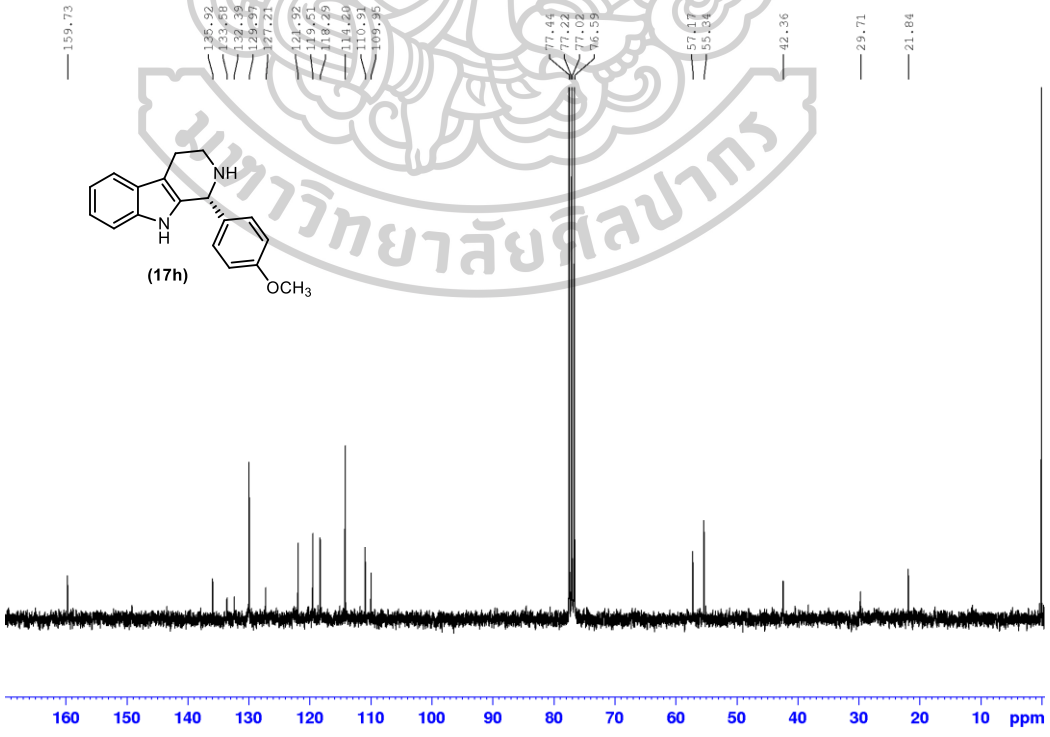
^{1}H-NMR spectrum of (**17g**) (300 MHz, $CDCl_3$)



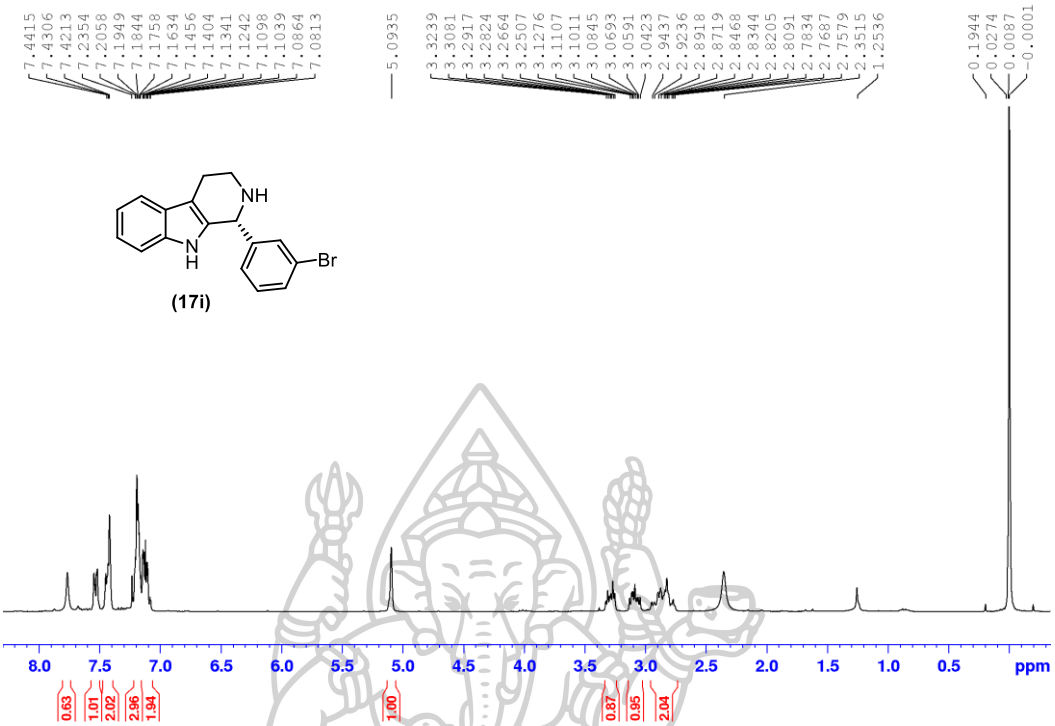
^{13}C-NMR spectrum of (**17g**) (75 MHz, $CDCl_3$)



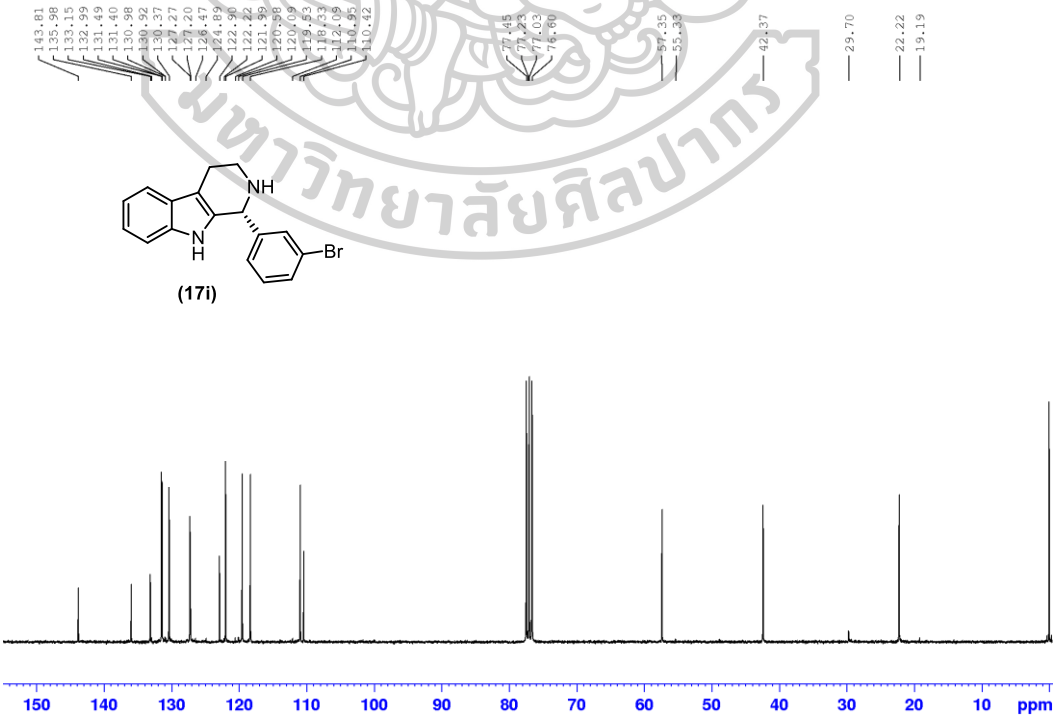
^{1}H-NMR spectrum of (**17h**) (300 MHz, $CDCl_3$)



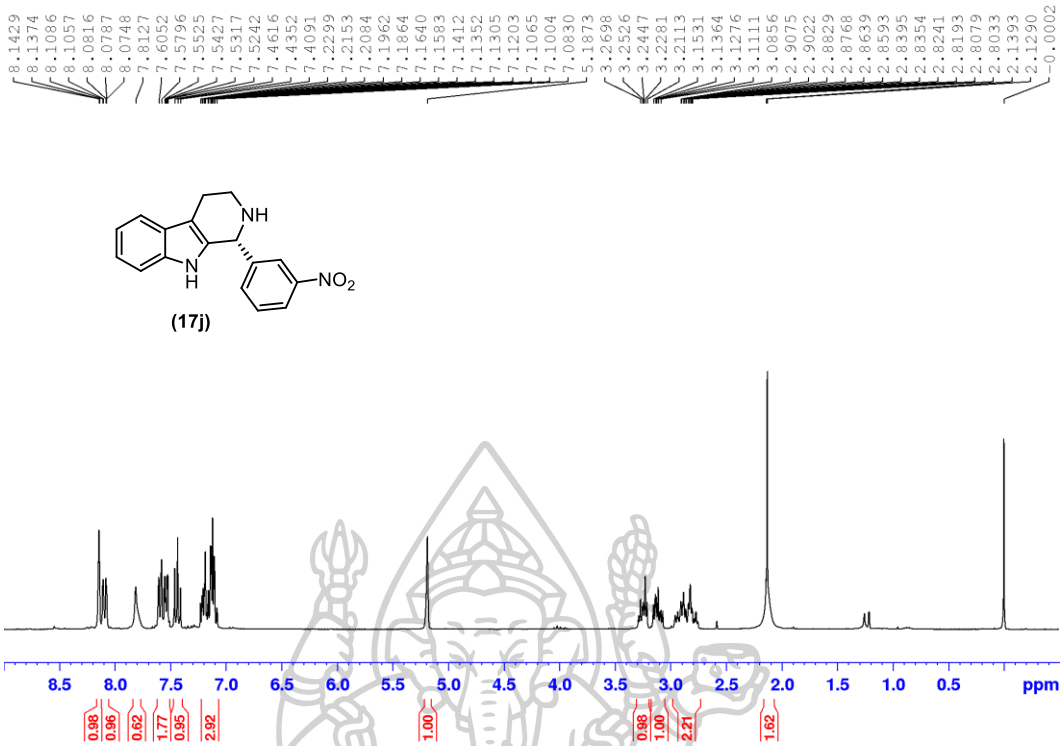
^{13}C-NMR spectrum of (**17h**) (75 MHz, $CDCl_3$)



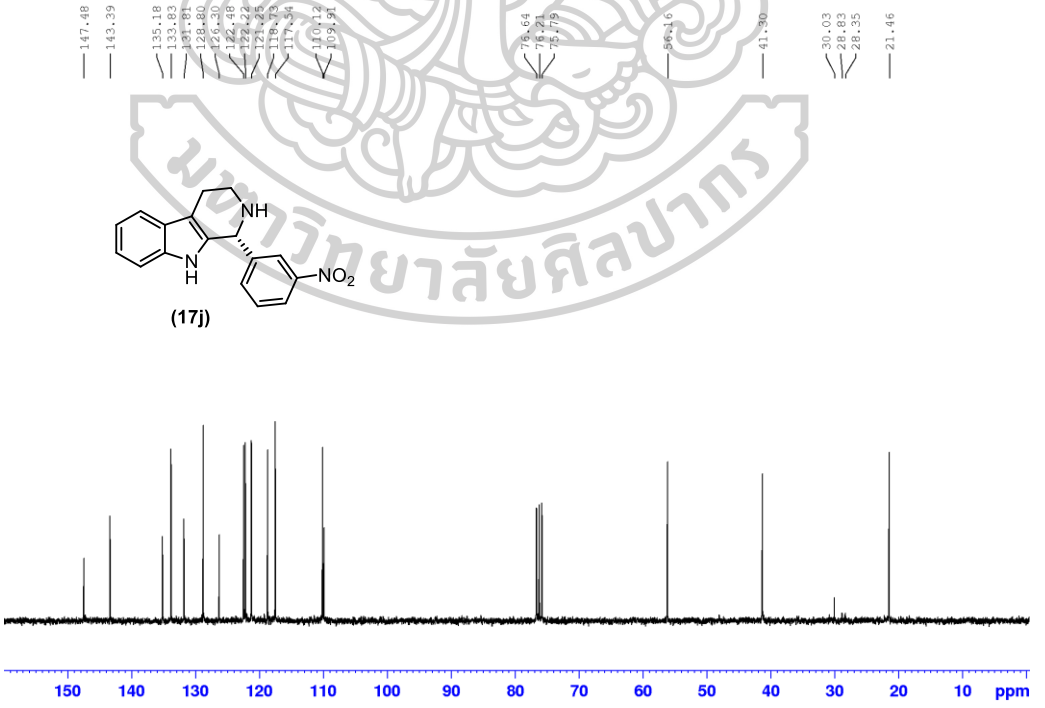
^{1}H-NMR spectrum of (**17i**) (300 MHz, $CDCl_3$)



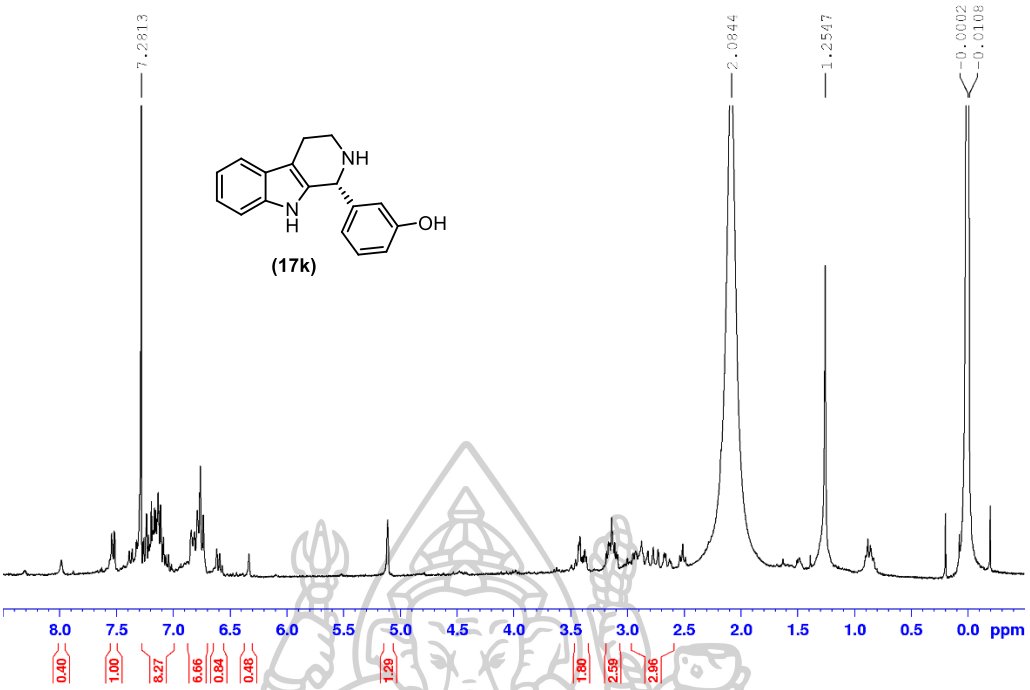
^{13}C-NMR spectrum of (**17i**) (75 MHz, $CDCl_3$)



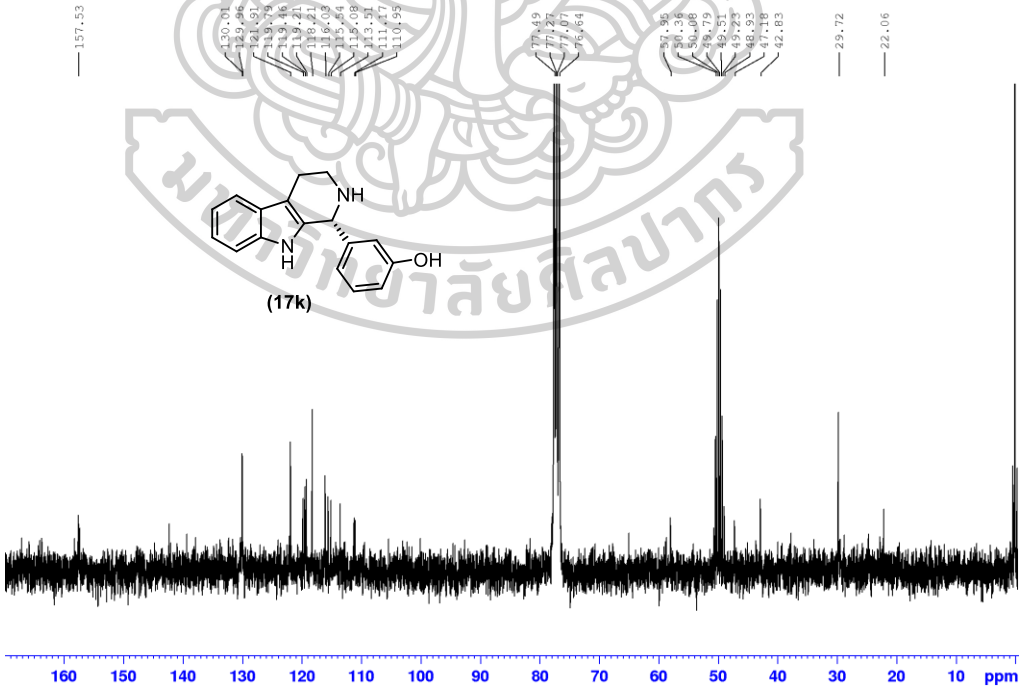
^{1}H-NMR spectrum of (**17j**) (300 MHz, $CDCl_3$)



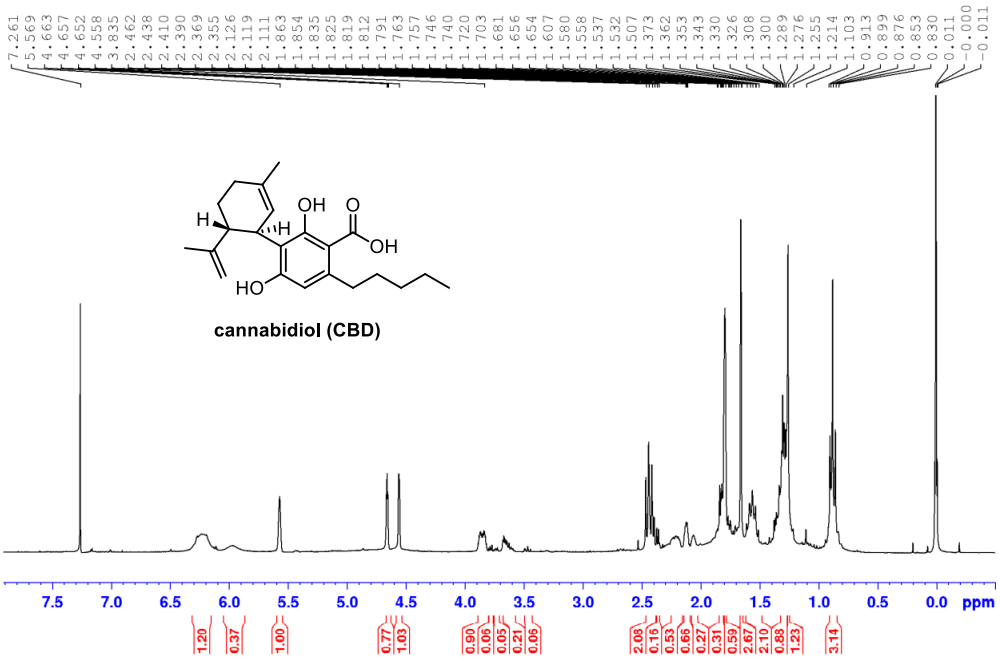
^{13}C-NMR spectrum of (**17j**) (75 MHz, $CDCl_3$)



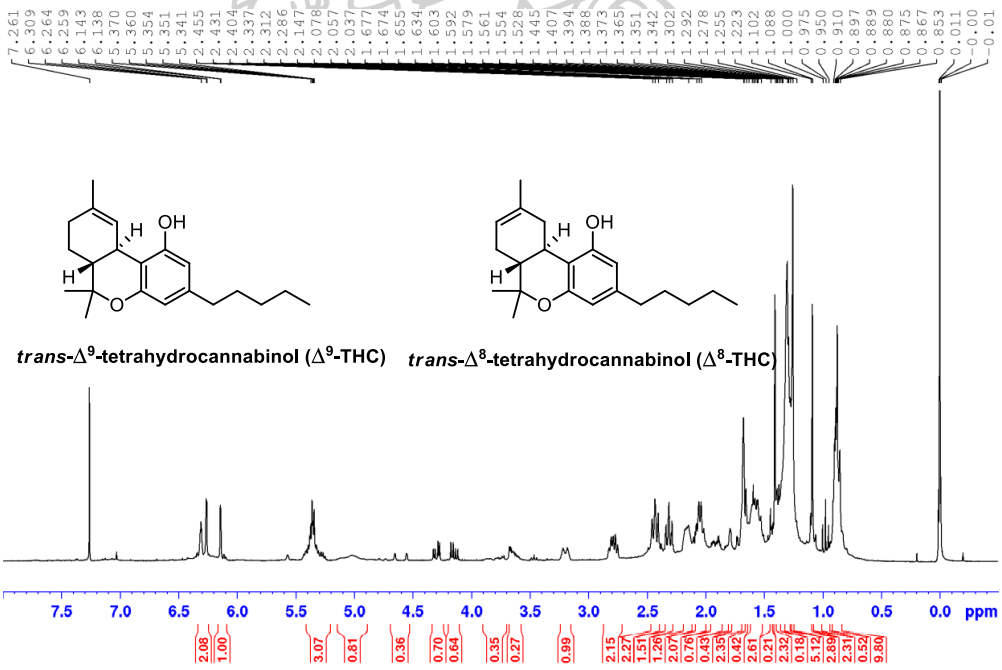
^{1}H-NMR spectrum of (**17k**) (300 MHz, $CDCl_3$)



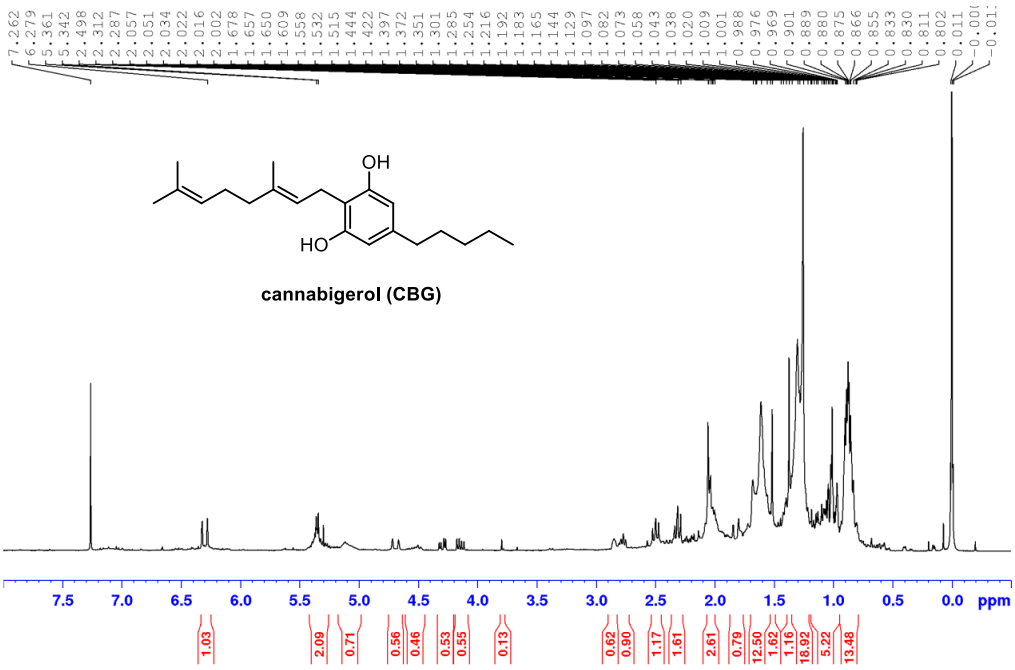
^{13}C-NMR spectrum of (**17k**) (75 MHz, $CDCl_3$)



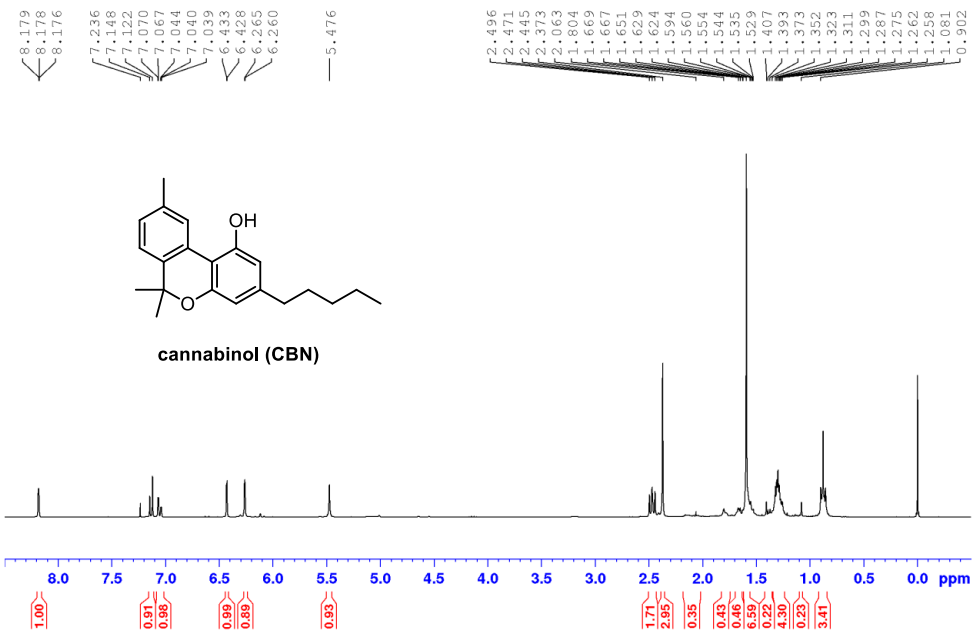
^{1}H-NMR spectrum of CBD (300 MHz, $CDCl_3$)



^{1}H-NMR spectrum of Δ^{8}-THC and Δ^{9}-THC (300 MHz, $CDCl_3$)



^{1}H-NMR spectrum of CBG (300 MHz, $CDCl_3$)



^{1}H-NMR spectrum of CBN (300 MHz, $CDCl_3$)

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