การสังเคราะห์โรทีนอยด์ 6-ดิออกซิคลิทอริแอซิทัลที่แยกจากรากหนอนตายอยาก *Stemona collinsae* Craib. และการเป็นพิษต่อเซลล์มะเร็ง

นายประภาส ขอพึ่ง

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สาขาวิชาเคมี ภาควิชาเคมี

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SYNTHESIS OF THE ROTENOIDS 6-DEOXYCLITORIACETAL ISOLATED FROM ROOTS OF *Stemona collinsae* CRAIB. AND CYTOTOXIC ACTIVITY AGAINST CANCER CELL LINES

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By	Mr. Prapas Khorphueng
Field of Study	Chemistry
Thesis Advisor	Professor Sophon Roengsumran, Ph.D.
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ประภาส ขอพึ่ง: การสังเคราะห์โรทีนอยด์ 6-ดิออกซิคลิทอริแอซิทัลที่แยกจากรากหนอนตายอยาก *Stemona collinsae* Craib. และการเป็นพิษต่อเซลล์มะเร็ง (SYNTHESIS OF THE ROTENOID 6-DEOXYCLITORIACETAL ISOLATED FROM ROOTS OF *Stemona collinsae* CRAIB. AND CYTOTOXIC ACTIVITY AGAINST CANCER CELL LINES) อ. ที่ปรึกษา: ศ. ดร. โสภณ เริงสำราญ, อ. ที่ปรึกษาร่วม: รศ. ดร. อมร เพชรสม, 118 หน้า ISBN 974-14-1784-5.

ได้ประสบความสำเร็จในการสังเคราะห์สารประกอบ 6-deoxyclitoriacetal (**39**) ที่มีฤทธิ์เป็นพิษต่อเซลล์มะเร็ง โดยผ่านปฏิกิริยาที่สำคัญๆ คือ $PtCl_2$-catalyzed hydroarylation, Sharpless asymmetric dihydroxylation, regioselective IBX diol oxidation และ stereoselective intramolecular keto-aldehyde pinacol coupling

กระบวนการสังเคราะห์ที่สำคัญเริ่มจากการเปลี่ยนสารประกอบ 1,2-dimethoxy-4-prop-2-ynyloxybenzene (**75**) ไปเป็นสารประกอบ 6,7-dimethoxy-2*H*-chromene (**83**) โดยใช้ปฏิกิริยา $PtCl_2$-catalyzed hydroarylation reaction จากนั้นเปลี่ยนสารประกอบ **83** ให้เป็นสารประกอบ 6,7-dimethoxy-chroman-3,4-diol (**84**) โดยปฏิกิริยา Sharpless asymmetric dihydroxylation สารประกอบ **84** ที่ได้นำไปออกซิไดซ์ด้วย IBX เฉพาะที่ตำแหน่ง benzyl ในตัวทำลายลาย EtOAc ได้สารประกอบ 3-hydroxy-6,7-dimethoxy-chroman-4-one (**82**) การเชื่อมต่อวง D กับ วง A-B เข้าด้วยกันโดยปฏิกิริยา S_N2 กระทำโดยการเปลี่ยนหมู่ hydroxyl ของสารประกอบ **82** ให้เป็นหมู่ที่ถูกแทนที่ได้ดีคือ 3-trifluoromethansulfonyl-6,7-dimethoxy-4-oxo-chromanyl ester (**86**) ได้ผลิตภัณฑ์เป็นสารประกอบ 2-(6,7-Dimethoxy-4-oxo-chroman-3-yloxy)-6-hydroxy-4-methoxybenzaldehyde (**87**) 80% ขั้นตอนการสร้างวง C ทำได้โดยการใช้ปฏิกิริยา Pinacol โดยใช้ SmI_2 ได้สารประกอบ 2,3,9-trimethoxy-6,6a-dihydro-12*H*-chromeno[3,4-*b*]chromene-11,12,12a-triol (**88**) ที่มีหมู่ OH ตรงตำแหน่ง C-12a ตรงกับสารที่แยกได้จากธรรมชาติและในขั้นสุดท้ายเมื่อออกซิไดซ์สารประกอบ **88** ด้วย MnO_2 จะได้สารประกอบ **39** ตามต้องการ

สารประกอบ **39** เมื่อทดสอบฤทธิ์ความเป็นพิษต่อเซลล์มะเร็งเต้านม (BT474) เซลล์มะเร็งปอด (CHAGO) เซลล์มะเร็งตับ (HEP-G2) เซลล์มะเร็งกระเพาะอาหาร (KATO3) และเซลล์มะเร็งลำไส้ (SW620) พบว่า IC50=0.2, 0.9, 0.1, 0.3 และ 0.1 μg/mL ตามลำดับ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....

สาขาวิชา...เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา..2548.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4172348523: MAJOR CHEMISTRY
KEY WORD: ROTENOIDS/*Stemona collinsae*/CYTOTOXICITY/ TOTAL SYNTHESIS

PRAPAS KHORPHUENG: SYNTHESIS OF THE ROTENOID 6-DEOXYCLITORIACETAL ISOLATED FROM ROOTS OF *Stemona collinsae* CRAIB. AND CYTOTOXIC ACTIVITY AGAINST CANCER CELL LINES. THESIS ADVISOR: PROF. SOPHON ROENGSUMRAN, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. AMORN PETSOM, Ph.D., 118 pp. ISBN 974-14-1784-5.

The total synthesis of 6-deoxyclitoriacetal (**39**), a cytotoxic natural product, has been achieved by using $PtCl_2$-catalyzed hydroarylation, Sharpless asymmetric dihydroxylation, IBX regioselective diol oxidation and stereoselective intramolecular keto-aldehyde pinacol coupling as the key steps.

The important steps of this synthesis began with the conversion of compound 1,2-dimethoxy-4-prop-2-ynyloxybenzene (**75**) into 6,7-dimethoxy-2*H*-chromene (**83**) using $PtCl_2$-catalyzed hydroarylation reaction successfully in good yield. The Sharpless asymmetric dihydroxylation of compound **83** provided a diol **84** which was converted into 3-hydroxy-6,7-dimethoxy-chroman-4-one (**82**) by selective oxidation with IBX in EtOAc. Conversion of α-hydroxy ketone **82** to a good leaving group, Tf (**86**), leaded to connection of D ring and A-B ring which was convinced by S_N2 reaction in very good yield (80%). The C ring was formed by SmI_2-promoted intramolecular Pinacol-type cyclization of 2-(6,7-dimethoxy-4-oxo-chroman-3-yloxy)-6-hydroxy-4-methoxybenzaldehyde (**87**) which was delivered the right stereogenic center at C 12a. Finally, completed synthesis of 6-deoxyclitoriacetal (**39**) was achieved by oxidation of 2,3,9-trimethoxy-6,6a-dihydro-12*H*-chromeno[3,4-*b*]chromene-11,12,12a-triol (**88**) with MnO_2.

The compound **39** exhibited cytotoxic activity against human breast carcinoma (BT474), lung carcinoma (CHAGO), hepato carcinoma (HEP-G2), gastric carcinoma (KATO3) and colon carcinoma (SW620) at IC50 0.2, 0.9, 0.1, 0.3 and 0.1 μg/mL, respectively.

Department……Chemistry…….Student's signature……P. Khorphueng……
Field of study……..Chemistry……Advisor's signature……
Academic year…….2005……Co-advisor's signature……

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List of Abbreviations

Å	angstrom
Ac	acetyl
ADH	asymmetric dihydroxylation
aq	aqueous
bp	boiling point
Bn	benzyl
Bu	butyl
br	broad
calcd	calculated
C	carbon
ºC	celsius degree
$CDCl_3$	chloroform-*d*
CH_3CN	acetonitrile
d	doublet
dd	doublet of doublets
DCM	dichloromethane
DEA	N,N-diethylaniline
DEAD	diethyl azodicarboxylate
δ	chemical shift in ppm downfield from Me_4Si
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
ee	enantiomeric excess
equiv.	equivalent
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
FAB	fast atom bombardment
FT-IR	Fourier transform infrared
g	gram(s)
h	hour(s)

Hz	hertz
HRMS	high-resolution mass spectrum
IBX	*o*-iodoxybenzoic acid
imid.	imidazole
J	coupling constant
LAH	lithium aluminium hydride
lit.	literature
µ	micro
m	multiplet (NMR), medium (FTIR)
M	moles per liter
m-CPBA	3-chloroperbenzoic acid
MeOH	methanol
Me	methyl
mg	milligrams
MHz	megahertz
mm	millimeters
mmol	millimole
mol	mole(s)
mp	melting point
Ms	methanesulfonyl
MS	molecular sieves
m/z	mass to charge ratio
NMR	nuclear magnetic resonance
NH_4Cl	ammonium chloride
p	para
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Ph	phenyl
PPh_3	triphenylphosphine
ppm	parts per million
pyr.	pyridine
rt	room temperature
s	singlet

t	triplet
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
Tf	trifluoromethanesulfonyl
Ts	toluenesulfonyl

CHAPTER I

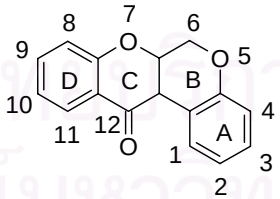
Introduction

1.1 Background

Natural products have provided various biological activities and known of chemically diverse pharmaceutics and agricultures. Rotenones and rotenoids are a natural toxin produced by several tropical plants and has been used for centuries as a selective fish poison and more recently as a commercial insecticide. They are known not only as toxicants but also as candidate for anticancer agents.

1.2 Structure

The rotenoids are known as advanced isoflavonoids, and construction of their angular A/B/C/D-ring systems. To be classified structurally as a rotenoids, a natural product must contain the heterocyclic core structure as **1**, *cis*-6a, 12a-dihydro-*6H, 12H*-[1]benzopyrano[3,4-*b*][1]benzopyran -12-one, or be a structure clearly related to it.[1, 2]



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1.3 General Bioactivities

The rotenoids possess a wealth of pharmacological properties including insecticidal,[1, 5-6, 8-10, 15] antifeedant,[1, 9, 15] piscicidal,[1, 8-10, 14-16] antiviral activity[1] and ichthyotoxic.[19] Although insecticidal and antifeedant properties are the best known, other useful biological activities such as antimicrobial,[20] and the ability to inhibit microtubule formation from tubulin, [37] are also recognized.

Rotenoids are known not only as toxicants but also as candidate anticancer agents based on three observations: dietary rotenone reduces the background incidence of liver tumors in mice and mammary tumors in rats, prevents cell proliferation induced by a peroxisome proliferator in mouse liver.[2]

1.4 Source of Rotenoids

The STEMONA genus, for example, is rich in rotenoids. *Stemona* is a type of perennial climbing plant native to continental Asia and Japan through Southeast Asia to tropical Australia. It usually grows in areas of dry vegetation, and usually consists of a single thing with alternating, spade-shaped leaves and thick, white tuberous roots. *Stemona* is the largest genus with about 25 species occurring as subshrubs or twining herbs mostly with perennial tuberous roots.

1.5 Traditional usages

S. collinsae Craib., *S. tuberosa* Lour., *S. japonica* Miq., and *S. sessilifolia* Miq. have long been used in Thailand, China and Japan for various medicinal and biological properties. Especially extracts from the fleshy tuberous roots are still used to treat cancer, respiratory disorders, including pulmonary tuberculosis and bronchitis and are also recommended to use against different insect pests.

Stemona root can be used both internally and externally. Externally, it can be applied to the skin as a poultice to rid the body of lice and fungi. Internally, it is used

to reduce the incidence of both acute and chronic coughs by relaxing the respiratory system and lowering blood pressure. One component of *Stemona*, tuberostemonine, also has been shown to have some pain-relieving properties. There is also evidence that *Stemona* root, when taken in a decoction with alcohol, can prohibit growth of the bacteria that causes tuberculosis.

1.6 The objective of this research

Knowing that rotenoids posses a variety of activities while the total synthesis of rotenoids have not much been reported. According to the report,[19, 64] the structure of 6-deoxyclitoriacetal (**39**) is determined by X-ray crystal structure while the potent cytotoxic activity against human breast carcinoma (BT479), lung carcinoma (CHAGO), hepato carcinoma (HEP-G2), gastric carcinoma (KATO3) and colon carcinoma (SW620) exhibited at IC50 0.2, 0.9, 0.1, 0.3 and 0.1 μg/mL, respectively. Moreover, this compound has also been known to show strong cytotoxic against cultured P-388 lymphocytic leukemia cell line. However, it is quite limit to modify or change functional groups on the structure as well as low abundance of this rotenoids. To obtain appreciable quantities for pharmacological studies, the compound 39 which contains hydroxyl group at C-12a position is decided to synthesize for the first time.

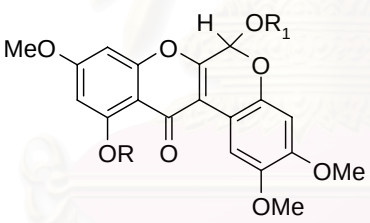
CHAPTER II

Literature Review

2.1 Isolation

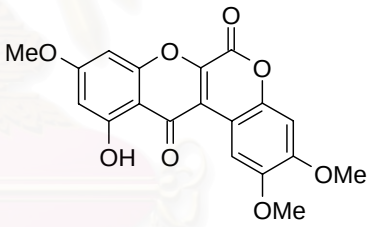
The isolation of rotenoids has been reported from variety of plants particularly tropical plants are abundant source of them. The following literatures are selected to review.

In 1974, Shiengthong[4] and co-workers reported the isolation of four new rotenoid compounds from *Stemona collinsae* Craib. namely stemonacetal (**2**), stemonal (**3**) and stemonone (**4**) respectively.



2 R=H, R_1=OCH_2CH_3

3 R=H, R_1=H



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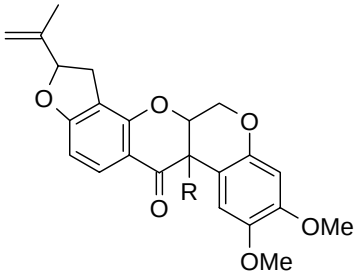
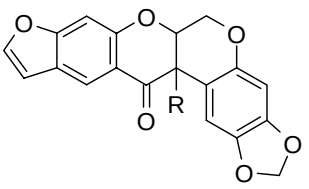
Roux and colleagues[5, 6] reported the isolation of rotenoid compounds from the roots of *Neorautanenia amboensis*. Preliminary tests showed (**9**) to be toxic to insects.



5 R=H

6 R=OH

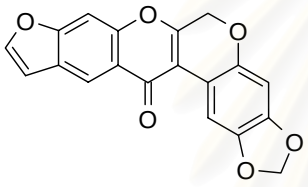
7 R=OMe



8 R=H

9 R=OH

10 R=OMe



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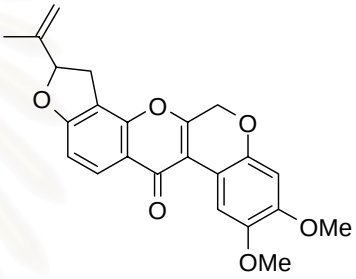

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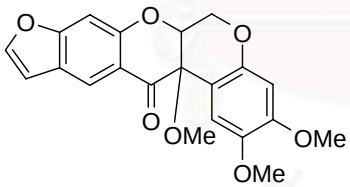

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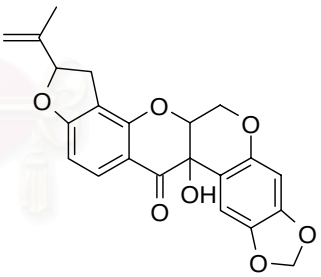

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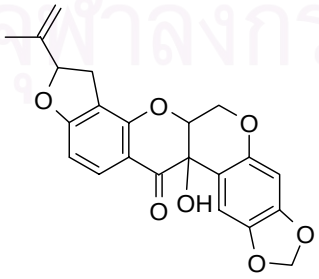

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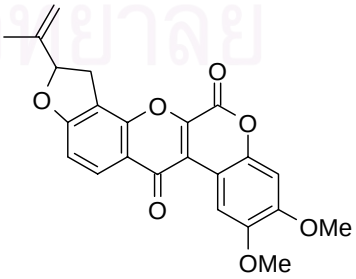
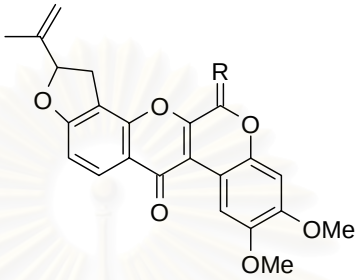


16

A new rotenoid isolated from a hexane extract of *Amorpha canescens*[7] roots and identified as 6-hydroxydehydrorotenone (**17**). The known two compounds, (**6**) and (**7**) were resulted by chemical conversion to compare their core structure to compound (**17**).

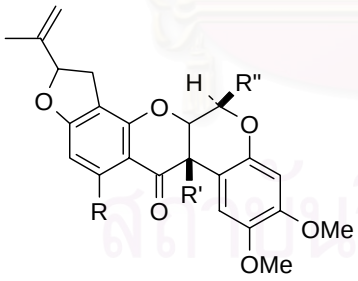


17 R=H, OH

12 R=H_2

16 R=O

Many *Tephrosia* species show piscicidal and insecticidal properties due to the presence of rotenoids. In India, the juice of leaves of *T. villosa*[8-10] is used to treat dropsy and diabetes. The isolation from whole plants of this species found 13 rotenoid compounds and three of them were known.



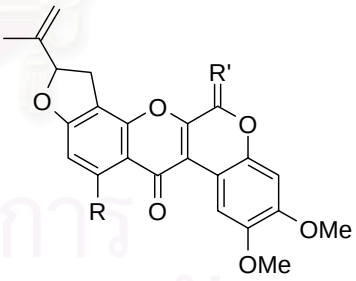
5 R, R′, R″=H

18 R=OH; R′, R″=H

19 R, R′=OH; R″=H

20 R, R″=OH; R′=H

21 R, R′, R″=OH



12 R=H; R′=H_2

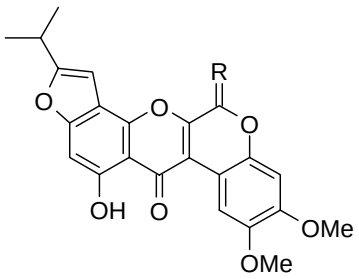
22 R=OH; R′=H_2

23 R=OH; R′=H, OH

24 R=OH; R′=H, OMe

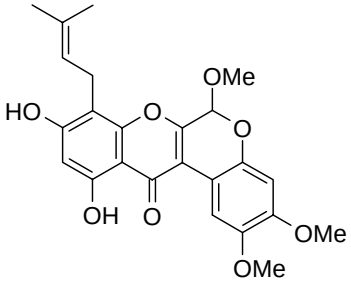
16 R=H; R′=O

25 R=OH; R′=O



26 R=O

27 R=H, OH



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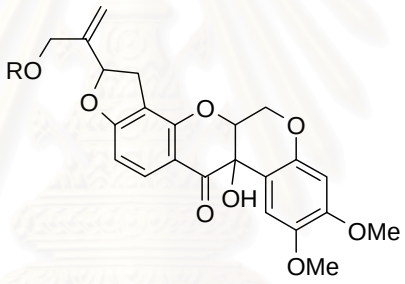
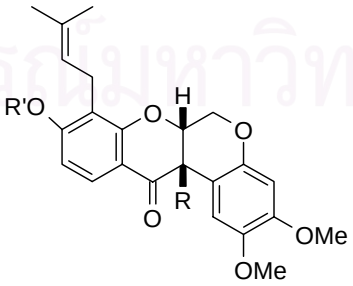
Dalbergia latifolia,[11-13] Indian Rosewood, is valued for resistant to attack by insects and microorganisms. Its seed has been examined and found a new rotenoid.



29a R=H

29b R=β-glycosyl

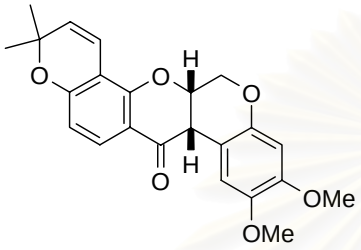
The roots of *Millettia pachycarpa* are occasionally used as a fish poison and are reputed to be insecticidal. Singhal[14] and colleagues reported the isolation of retenoids from the roots of this species **5**, **14**, **30a** and **30b**.



30a R=R′=H

30b R=OH, R′=H

Crombie[15] and co-workers noticed that the seeds of the legume *Lonchocarpus salvadorensis*, having unusually low destroyed by bruchid beetles (seed predators). To explore the composition of the seeds found deguelin (**31**),[16] rotenone (**5**), elliptone (**32**) and α-toxicarol (**33**) and also examined the insecticidal effectiveness of those rotenoids.



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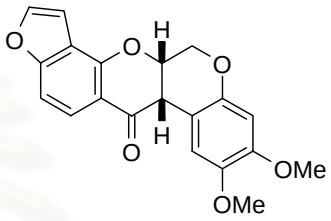

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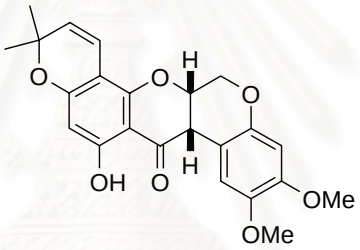
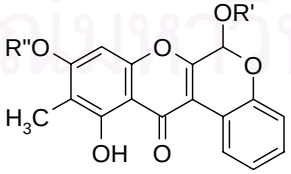


33

Boerhaavia diffusa L.[17] is widely used in traditional medicine: in Nepal as a remedy for back bone pain and as a tonic (whole plant) in association with other plant; in Sri Lanka to treat rheumatism and snake bite; in India as a medicine with multiple actions (stomachic, diuretic, antiasthmatic, diaphoretic, anthelminthic, febrifuge, antileprosy, antiscabies, anti-urethritis, etc.). Investigations on its chemical constituents found two new rotenoid analogues.

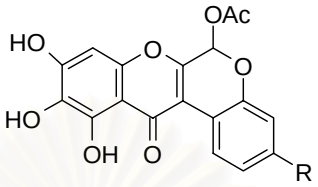


34 R′=CH_3, R″=H

35 R′=R″=H

36 R′=R″=CH_3

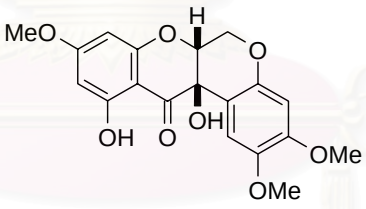
Ahmed et al.[18] reported two rotenoid derivatives, repenone (**37**) and repenol (**38**), from *Boerhaauia repens* usually grows in tropical and subtropical regions. This plant is common in Bangladesh and has a reputation for versatility.



37 R=H

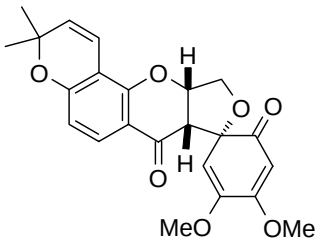
38 R=OH

Clitoria macrophylla[19] is traditionally used in Thailand for skin diseases and for pest control in horticulture and animal husbandry. It was also reported as anti-inflammatory and antipyretic activities. Phytochemical investigation resulted in the isolation of a new rotenoids, 6-deoxyclitoriacetal (**39**), from its roots. *In vitro* tests showed that this compound possessed strong cytotoxic activity against cultured P-388 lymphocytic leukemia cells, but was not active with cultured KB cells.



39

Amorpha fruticosa[20] has also been shown to exhibit feeding deterrence along with insecticidal, antiparasitic, antimicrobial and hypotensive activities. To investigate chemical constituent of the leaves found 5 rotenoid compounds.



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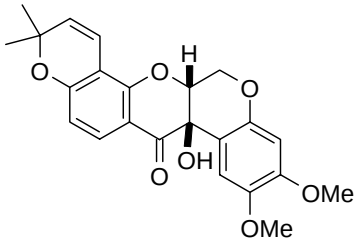

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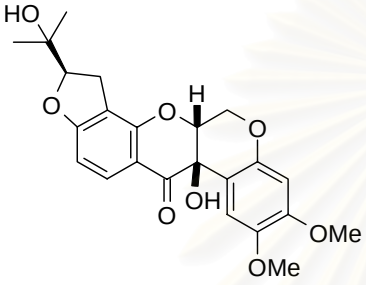

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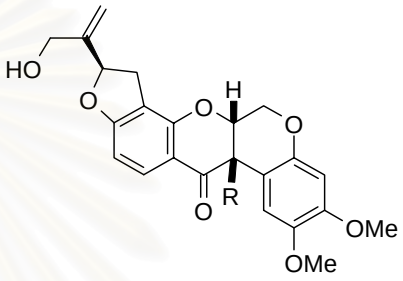
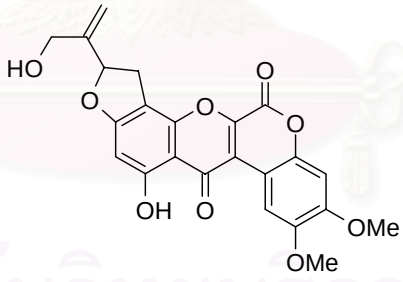


43 R=H

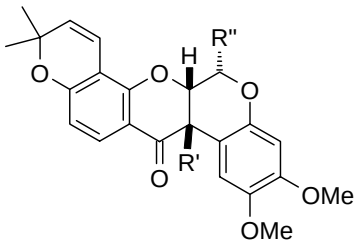
29a R=OH

Investigation of *Dalbergia sissoides*[21] stem-bark has resulted in the isolation of a new 6-ketodehydrorotenoid, characterized as 6-ketodehydroamorphigenin (**40**).



44

The bark, leaves, seeds, and roots of *Mundulea sericea* (Wild.)[22] are used as a fish poison, insecticide, and an aphrodisiac. As part of searching for naturally occurring cancer chemopreventive agents, Kinghorn et al. have reinvestigated the chemical constituents of this plant found four rotenoid derivatives, two were known.



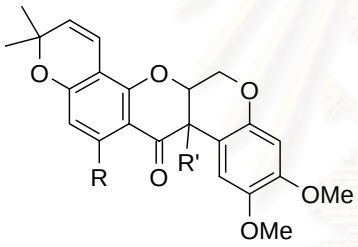
45 R′=H, R″=OH

46 R′=R″=OH

31 R′=R″=H

41 R′=OH, R″=H

The hexane and ethyl acetate extracts of roots of *Tephrosia canida*[23] afforded eight rotenoids.

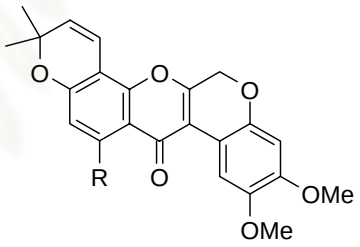


31 R=R′=H

33 R=OH; R′=H

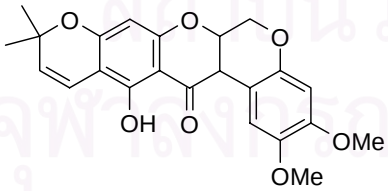
41 R=H; R′=OH

42 R= R′=OH



43 R=H

44 R=OH



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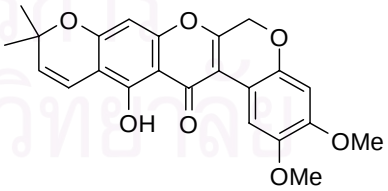
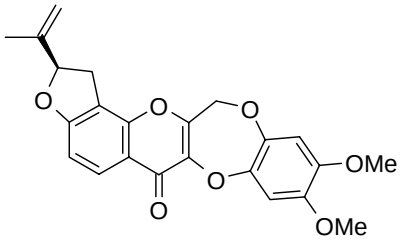


46

Two novel rotenoid-like compounds isolated from cube resin (*Lonchocarpus utilis* and *urucu* or *Derris elliptica* or *Leguminosae*),[24] used as an insecticide and piscicide, have been discovered during the study of its composition and toxicology.



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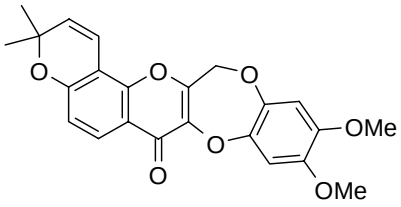
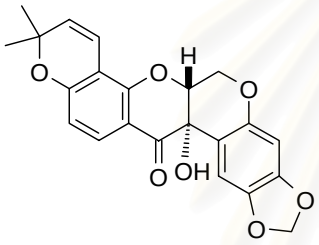


48

From the stem bark of *Millettia usaramensis* subsp., *usaramensis*[25] four new 12a-hydroxyrotenoids with the unusual *trans* B/C ring junction have been isolated and characterized.



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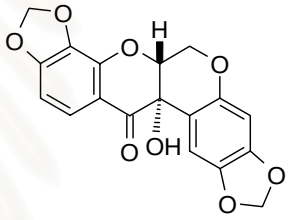

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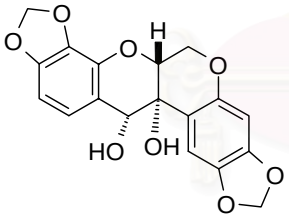

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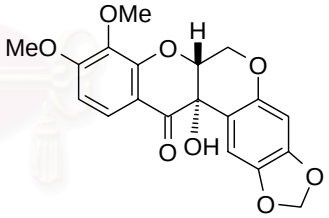
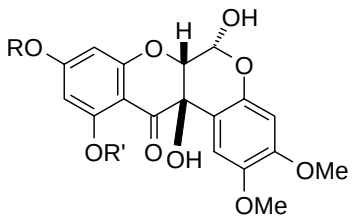


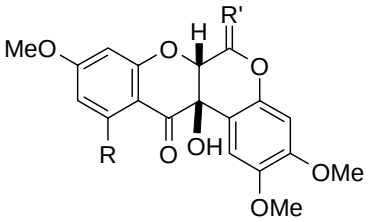
52

The genus *Clitoria* has 60 reported species, some of which possess remarkably anti-inflammatory and antipyretic activities. Moreover, from the roots of *C. macrophylla* has been used for the treatment of skin diseases in Thailand. Preliminary test showed strong cytotoxic activity against cultured P-388 lymphocytic leukemia cells. The isolation of the roots[26, 27] and seeds[28] of *Clitoria fairchildiana* afforded rotenoid derivatives.



53 R=Me; R′=β-glycosyl

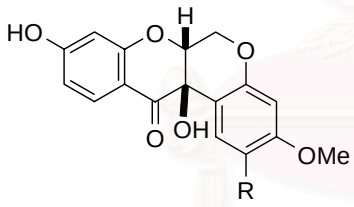
54 R= R′=H



55 R=H; R′=H, α-OH; H, β-OH

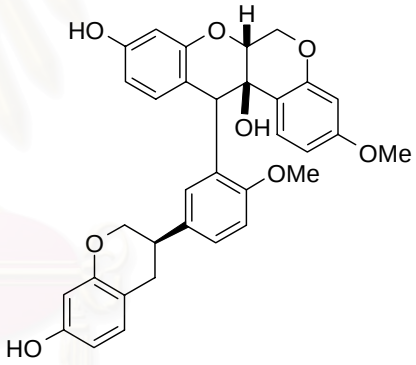
56 R=OH; R′=H, α-OH; H, β-OH

Gliricidia sepium (Jacq.)[29] is a tree native to both coasts of Mexico form above the middle of the country southward and through Central America to Columbia and Venezuela. The bark decoction is used against protozoal diseases and for the treatment of impetigo and other skin diseases. The investigation of a methanolic extract of *Gliricidia sepium* bark afforded three new rotenoids which exhibited activity against *Artemia salina* larvae.



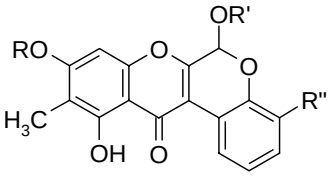
57 R=H

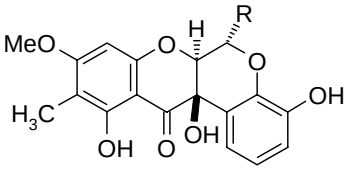
58 R=OMe



59

As the problem of low abundance active metabolites, time-consuming and high cost of collection and re-collection, and variable recovery of active compounds lead to plant cell culture which offers a good alternative to whole plant collection and allows for the production of bioactive secondary metabolites. From a manipulated plant cell culture of *Mirabilis jalapa*[30] led to the isolation and subsequent identification of phenolic compounds including rotenoids.





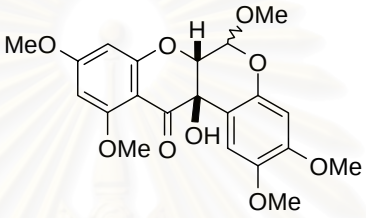
60 R=CH_3; R′=H; R″=OH

34 R= R″=H; R′=CH_3

35 R= R′=R″=H

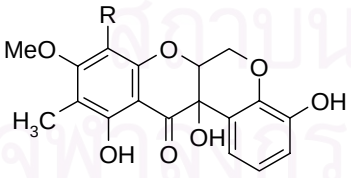
61 R=OMe

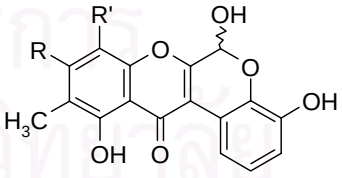
62 R=H



63

M. jalapa[31] is a plant belonging to the family *Nyctaginaceae*, widely used as a traditional folk herb to treat acute arthritis, anesthesia, inflammation, and so on. In screening of this Chinese folk herbs for anti-HIV agents, it was found that the ethyl acetate fraction found that the ethyl acetate fraction of the roots of *Mirabilis jalapa* L. showed potent inhibitory activity against HIV *in vitro*. To isolate an effective compound against HIV, *M. jalapa* collected at Kunming in Yunnan Province was chemically investigated. The isolation and structure identification of the roots of *M. jalapa* resulted four new rotenoids.



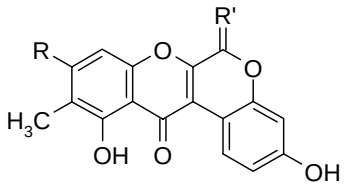


64 R=Me

62 R=H

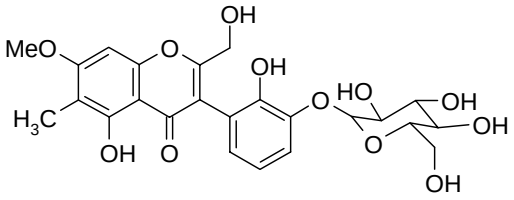
65 R=OH; R′=Me

60 R=OMe; R′=H



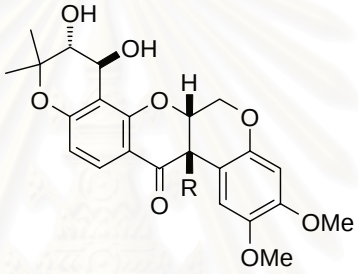
66 R=OMe; R′=H, OH

67 R=OH; R′=O



68

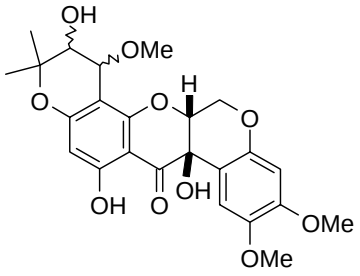
A screening of Brazilian medicinal plants for anti-*Helicobacter pylori* actives resulted in the isolation of rotenoid from the roots of *Derris malaccensis*.[32]



69 R=H

70 R=OH

Tephrosia toxicaria (Sw.) Pers.[33] is a tropical fish-poisoning plant growing in Sri Lanka and South America and is well-known as a source of rotenoids including deguelin, sumatrol, and toxicarol. As part of the discovery of novel naturally occurring cancer chemopreventive agents from plants, the stems of *T. toxicaria* were chosen for more detailed investigation. Bioassay-guided fractionation of the ethyl acetate-soluble residue of the stems of *T. toxicaria*, using quinone reductase induction assay, led to the isolation and characterization of a new rotenoid and the identification of 12 known compounds.



71

2.2 Biological activities

Rotenoids are known as insecticide for at least 150 years. They have been used even longer as fish poisons by native tribes to obtain food [34] and more recently in fish management to achieve the desired balance of species. The acute toxicity of rotenoids to insects, fish, and mammals is attributable to inhibition of NADH; ubiquinone oxidoreductase (complex I) activity as the primary target.[35-36] Rotenoids are known not only as toxicants but also as candidate anticancer agents based on three observations; exhibition of the formation of microtubules from tubulin and anti-cancer activities,[37-40] prevents cell proliferation induced by a peroxisome proliferators in mouse liver,[2] and inhibit phorbol ester-induced ornithine decarboxylase (ODC) activity as a measure of cancer chemopreventive potency.[22, 41-44]

Other interesting biological activities are anti viral and anti-malarial activities.[45-46]

2.3 Synthesis

Some of the existing rotenoid syntheses have limitations in terms of their ability to accommodate different substitution patterns, and in terms of yield. However, a number of synthetic strategies have been used to construct the retenoid system including the use of Hoesch condensation, thermal condensation of 4-ethoxycarbonylchroman-3-ones with activated phenols, reaction of isoflavones with dimethylsulfoxonium methylide, Claisen rearrangement of pro-2-ynyl ethers, aroylation of 4-lithiochromenes, enamines and 4-phenylsulfonylchromans,

intramolecular radical cyclization, and combined Wadsworth-Emmons-Mukaiyama aldol methodologies.[1]

In 1993, Crombie et al. [66-67] have developed the synthesis of rotenoids using thermal cyclization, Claisen rearrangement, and Wadsworth-Emmons coupling.

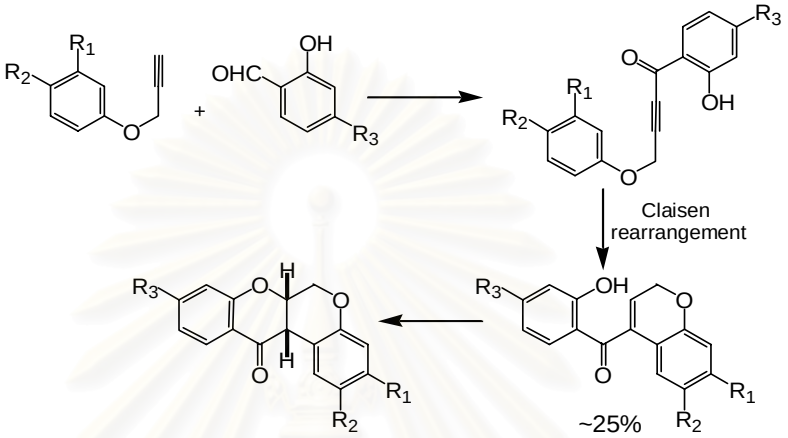


Figure 1 Claisen rearrangement in rotenoid synthesis.

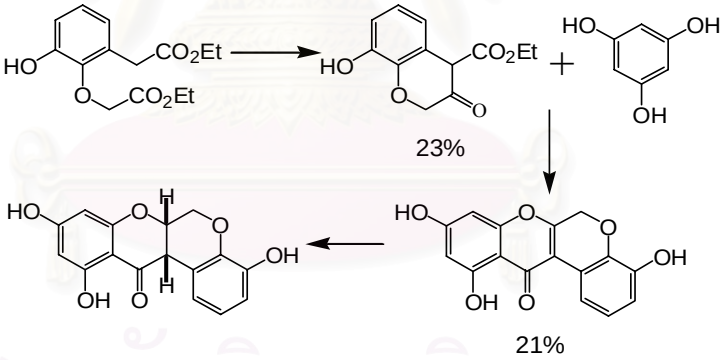


Figure 2 Wadsworth-Emmons coupling in rotenoid synthesis.

In 1998, Gabbutt et al.[1] reported the synthesis of rotenoid core structure using the hypervalent iodine-promoted oxidative ring expansion of the spirocycles as a key step.

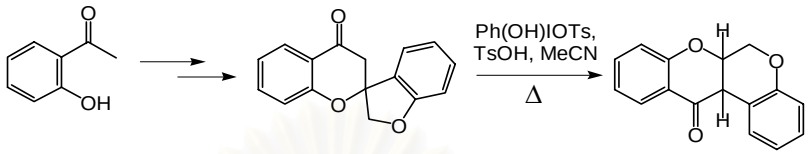


Figure 3 Hypervalent iodine in rotenoid synthesis.

Lately, Sames *et al.*[47-49] have developed a new platinum-catalyzed hydroarylation method which would be applicable to synthesize rotenoid class of natural products.

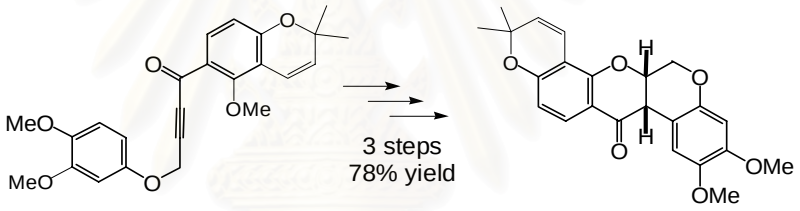


Figure 4 $PtCl_2$-catalyzed hydroarylation in rotenoid synthesis.

CHAPTER III

Experimental

3.1 Materials and Methods

3.1.1 General experimental procedures

All reactions were performed in oven- or flame-dried glassware fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation at 30°C, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} precoated plates (0.25 mm). TLC plates were visualized by exposure to ultraviolet light (UV), then were stained by submersion in aqueous ceric ammonium molybdate solution (CAM), or a basidic $KMnO_4$ solution, or acidic ethanolic vanillin solution, followed by brief heating on a hot gun (~200°C, 10-15s). Flash chromatography was performed by employing silica gel (60 Å pore size, 230-400 mesh, Merck KGA).

3.1.2 Materials

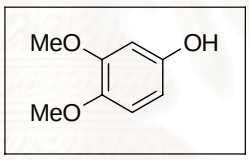
Commercial reagents and solvents were used as received with the following exceptions. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium/benzophenone ketyl. Methylene chloride (CH_2Cl_2) and triethylamine (Et_3N) were distilled from calcium hydride. All other commercially obtained reagents were distilled before.

3.1.3 Instrumentation

All melting points were obtained on a Gallenkamp capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin Elmer 1760X FT-IR Spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer operated at 400 MHz for proton nuclei and 100 MHz for carbon nuclei. Chemical shifts are reported relative to internal chloroform (^{1}H, δ 7.26 ppm; ^{13}C, δ 77.0 ppm), unless otherwise noted. High resolution mass spectra were determined on Bruker Daltonics micrOTOF-Q mass spectrometer. Specific rotations were measured on Jasco P1010 and are reported as follows: $[\alpha]_D$, concentration (c = g/100 mL).

3.2 Experimental Procedures and Characterization data

3.2.1 Synthesis of compound 78



3,4-Dimethoxyphenol (78)

To a cooled solution of 3, 4-Dimethoxybenzaldehyde (10.0 g, 60.2 mmol) in dichloromethane at 0°C (200 mL) was added *m*-chloroperbenzoic acid (16.3 g with ~30% of H_2O, ca. 66.2 mmol), and the mixture was then allowed to stir at room temperature for 15 h. The reaction was quenched with Na_2SO_3, and the mixture was diluted with dichloromethane (500 mL) and then successively washed with saturated aqueous Na_2CO_3 solution (3 × 100 mL) and brine (2 × 50 mL), and finally dried over Na_2SO_4. After removal of organic solvent, the residue was dissolved in MeOH (200 mL), then treated with K_2CO_3 (8.4 g, 61.0 mmol), and the mixture was stirred at room temperature for 30 min. After removal of MeOH, the residue was dissolved in dichloromethane (200 mL) was with H_2O (2 × 20 mL) and brine (2 × 20 mL), and dried over Na_2SO_4. The organic phase was concentrated, and residue was purified by

flash chromatography on silica gel (elution with 9:1 DCM/EtOAc) to give compound **78** (9.1 g, 98 %) as a brown solid.

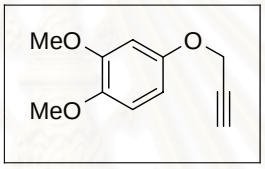
m.p. 43-45°C.

$\mathbf{R}_f$ = 0.54 (CH_2Cl_2/EtOAc = 9:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (s, 3H), 3.82 (s, 3H), 6.39 (dd, J_1 = 8.6 Hz, J_2 = 2.8 Hz, 1H), 6.50 (d, J = 2.8 Hz, 1H), 6.74 (d, J = 8.6 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 56.6, 100.7, 106.0, 112.6, 142.8, 149.8, 150.4.

3.2.2 Synthesis of compound 75[47]



1,2-Dimethoxy-4-prop-2-ynyloxybenzene (75)

To 3, 4-dimethoxyphenol **78** in acetone (0.2M) was added propargyl bromide (1.2 equiv.) and K_2CO_3 (1.2 equiv.). The reaction was stirred vigorously at room temperature. After 18 hours, saturated NH_4Cl and DCM were added. The organic layer was washed with water (× 2), brine, and dried over Na_2SO_4. Removal of volatiles and purification of the crude residue by filtration through a pad of silica (Hexane/CH_2Cl_2 = 1:1) gave **75** as light brown oil in quantitative yield.

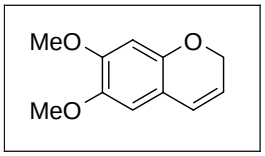
$\mathbf{R}_f$ = 0.43 (hexanes/CH_2Cl_2 = 1:1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (t, J = 2.4, 1H), 3.84 (s, 3H), 3.85 (s, 3H), 4.65 (d, J = 2.4 Hz, 2H), 6.49 (dd, J_1 = 8.7, 2.8 Hz, 1H), 6.60 (d, J_2 = 2.8 Hz, 1H), 6.79 (d, J = 8.7 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 56.3, 56.4, 75.4, 78.8, 101.3, 104.3, 111.5, 144.1, 149.8, 152.1.

IR (Chloroform) ν_{max}: 2969, 2930, 2354, 2330, 1607, 1570, 1516, 1449, 1207, 1128, 1028 cm^{-1}.

3.2.3 Synthesis of compound 83[47-50]



6,7-Dimethoxy-2H-chromene (83)

In a flame dried 50 mL round bottom flask was added **78** (1.00 g, 5.20 mmol) and $PtCl_2$ (28 mg, 2 mol %). The flask was evacuated and flushed with argon three times, followed by the addition of toluene (25 mL, 0.21 M). The reaction was allowed to stir at 55-60°C for 18 h and the volatiles were removed when reaction was completed (TLC). Purification of the crude residue by flash chromatography on silica gel (Hexane/EtOAc = 4:1) afforded **83** as a pale yellow viscous oil (750 mg, 75 %).

$\mathbf{R_f}$ = 0.41 (Hexane/EtOAc = 4:1)

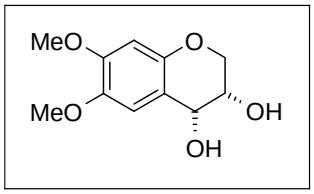
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H), 3.87 (s, 3H), 4.77 (dd, J_1=1.5 Hz, J_2=3.1 Hz, 2H), 5.69 (ddd, J_1 = 3.1 Hz, J_2=7.0 Hz, J_3=9.4 Hz 1H), 6.38 (d, J = 9.4 Hz, 1H), 6.45 (s, 1H), 6.56 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.5, 65.4, 100.5, 109.9, 114.4, 119.3, 124.4, 143.5, 148.5, 149.5.

IR (Chloroform) ν_{max}: 2941, 2833, 1614, 1509, 1458, 1279, 1226, 1193, 1135, 1035, 980, 589 cm^{-1}.

HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{11}H_{13}O_3$ 193.0859 found 193.0856.

3.2.4 Synthesis of compound 84[51]



6,7-Dimethoxy-chroman-3,4-diol (84)

The mixture of AD-mix-α (4.37 g) and $MeSO_2NH_2$ (296 mg, 3.12 mmol) in t-BuOH/H_2O (20 mL/20 mL) was stirred at room temperature for 15 min and then cooled to 0°C. To this solution was added the compound **83** (600 mg, 3.12mmol). The reaction was stirred at room temperature for 2 days and then quenched with Na_2SO_3 at room temperature for an additional 10 min. EtOAc was added to the reaction mixture, and after separation of the layers, the aqueous layer was further extracted with EtOAc twice. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by flash column using pure EtOAc afforded the corresponding diol product (650 mg, 92%) as a pale yellow solid.

m.p. 98-99°C.

R_f = 0.43 (EtOAc).

Optical Rotation: $[\alpha]_D^{25}$ = -12.4 (*c* 0.95, $CHCl_3$).

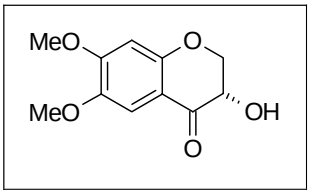
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H), 3.86 (s, 3H), 4.09 (m, 3H), 4.71 (s, 1H), 6.41 (s, 1H), 6.88 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.4, 65.8, 65.9, 66.1, 100.2, 111.8, 113.1, 144.0, 147.9, 150.3.

IR (Chloroform) ν_{max}: 3378, 2938, 1662, 1621, 1541, 1512, 1449, 1403, 1265, 1224, 1200, 1162, 1127, 1057, 938 cm^{-1}.

HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{11}H_{14}O_5Na$ 249.0739 found 249.0740.

3.2.5 Preparation of compound 82[55]



3-Hydroxy-6,7-dimethoxy-chroman-4-one (82)

The compound **84** (400 mg, 1.77 mmol) was dissolved in ethyl acetate (10 mL, 0.18 M) and IBX (846 mg, 3.0 mmol) was added. The resulting suspension was immersed in an oil bath set to 70°C and stirred vigorously open to the atmosphere. After 5 h (TLC monitoring), the reaction was cooled to room temperature and filtered. The filter was washed with 3×10 mL of ethyl acetate, and the combined filtrates were concentrated to yield a yellow crude product which was further purified by column chromatography using EtOAc/Hexane (3:2) to yield 350 mg (88%, pale yellow powder).

m.p. 160-161°C.

$\mathbf{R_f}$ = 0.52 (EtOAc/Hexane = 3:2).

Optical Rotation: $[\alpha]_D^{25}$ = -52.8 (*c* 0.65, $CHCl_3$).

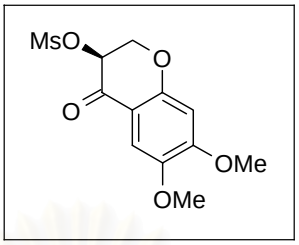
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (s, 3H) 3.86 (s, 3H), 4.13 (dd, J_1 = 11.0 Hz, J_2 = 13.4 Hz, 1H), 4.57 (dd, J_1 = 6.2 Hz, J_2 = 13.1 Hz, 1H), 4.66 (dd, J_1 = 6.2 Hz, J_2 = 10.1 Hz, 1H), 6.41 (s, 1H), 6.88 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 56.3, 56.4, 68.6, 71.1, 100.2, 106.5, 110.6, 145.0, 156.7, 159.0, 192.8.

IR (Chloroform) ν_{max}: 3379, 2925, 1674, 1612, 1509, 1454, 1265, 1211, 1175, 1114, 1032, 995, 937 cm^{-1}.

HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{11}H_{12}O_5Na$ 247.0582 found 247.0588.

3.2.6 Synthesis of compound 85



3-Methansulfonyl-6,7-dimethoxy-4-oxo-chromanyl ester (85)

To a cooled (0°C) stirred solution of the compound **82** (100 mg, 0.446 mmol) in dichloromethane (4 mL) and pyridine (36 μL) under argon, methanesulfonyl chloride (38 μL (56.2 mg), 1.1 equiv.) was dropped. The mixture was stirred at room temperature for 6 h, the solution was poured into ice-10% HCl and extracted with CH_2Cl_2, the organic layer was combined, washed with water, dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by column chromatography using Hexane/EtOAc (3:1) to give **85** as a pale yellow solid (110 mg, 82%).

m.p. 109-110°C.

$\mathbf{R_f}$ = 0.56 (Hexane/EtOAc = 3:1).

Optical Rotation: $[\alpha]_D^{25}$ = -12.4 (*c* 0.95, $CHCl_3$).

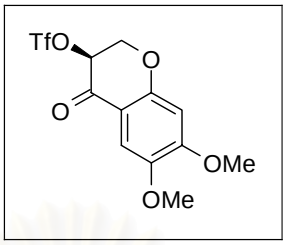
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.34 (s, 3H), 3.92 (s, 3H) 3.97 (s, 3H), 4.55 (dd, J_1 = 9.8 Hz, J_2 = 11.6 Hz, 1H), 4.67 (dd, J_1 = 5.1 Hz, J_2 = 11.5 Hz, 1H), 5.34 (dd, J_1 = 4.8 Hz, J_2 = 9.7 Hz, 1H), 6.50 (s, 1H), 7.30 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 39.2, 56.2, 56.5, 69.5, 74.8, 100.1, 106.5, 111.5, 145.4, 157.2, 158.3, 184.9.

IR (Chloroform) ν_{max}: 2925, 2853, 1685, 1613, 1508, 1470, 1429, 1360, 1271, 1214, 1174, 1072, 1032, 939, 807 cm^{-1}.

HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{12}H_{14}O_7SNa$ 325.0358 found 325.0365.

3.2.7 Synthesis of compound 86



3-Trifluoromethansulfonyl-6,7-dimethoxy-4-oxo-chromanyl ester (86)

Pyridine (54 μL, 0.669 mmol, 1.5 equiv) was added to a solution of compound **82** (100 mg, 0.446 mmol) in dichloromethane (1 mL) at -10°C. Tf_2O (112 μL, 0.669 mmol, 1.5 equiv) was then added dropwise, and the mixture solution was then stirred at that temperature for 2 h. The reaction mixture was poured into ice/10%HCl and was extracted with DCM (3×10 mL). The organic layer was dried over Na_2SO_4 and concentrated to obtain crude product. The crude was purified by column chromatography using EtOAc/Hexane (3:2) as eluent; the compound **7** was obtained as a light brown solid (140 mg, 88%).

m.p. 89-90°C.

R_f = 0.51 (EtOAc/Hexane = 3:2).

Optical Rotation: $[\alpha]_D^{25}$ = -16.2 (*c* 1.0, $CHCl_3$).

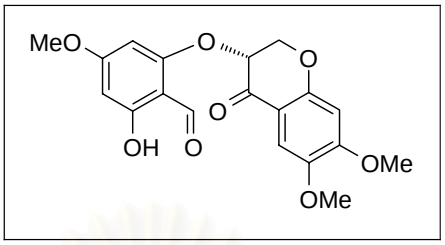
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.86 (s, 3H), 4.50 (dd, J_1 = 2.1 Hz, J_2 = 11.9 Hz, 1H), 4.58 (dd, J_1 = 5.4 Hz, J_2 = 11.7 Hz, 1H), 5.34 (dd, J_1 = 4.8 Hz, J_2 = 9.3 Hz, 1H), 6.40 (s, 1H), 7.18 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 56.2, 56.5, 68.7, 79.0, 100.0, 106.7, 111.2, 120.1, 145.7, 157.4, 158.0, 181.5.

IR (Chloroform) ν_{max}: 2927, 2847, 1677, 1614, 1508, 1471, 1433, 1271, 1217, 1033, 827, 831 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{12}H_{12}F_3O_7S$ 357.0256 found 357.0250.

3.2.8 Preparation of compound 87[57-58]



2-(6,7-Dimethoxy-4-oxo-chroman-3-yloxy)-6-hydroxy-4-methoxybenzaldehyde (87)

To a solution of compound **81** (89 mg, 0.529 mmol), K_2CO_3 (73 mg, 0.529 mmol), and 18-crown-6 (140 mg, 0.529 mmol) in MeCN (1 mL) was added a solution of **85** or **86** (80 mg (0.265 mmol) for **85** or 90 mg for **86**, 0.253 mmol) in MeCN (1 mL). The reaction mixture was stirred at room temperature for 24 h, filtered through Celite and the solvent evaporated at reduced pressure to provide a crude product. The crude product was purified by silica gel chromatography (Hexane/EtOAc; 1:4) to provide **87** (67 mg**,** 68% for **85** or 76 mg, 80% for **86**) as a white solid.

m.p. 202-203°C.

R_f = 0.43 (Hexane/EtOAc = 1:4).

Optical Rotation: $[\alpha]_D^{25}$ = +168.3 (*c* 0.55, $CHCl_3$).

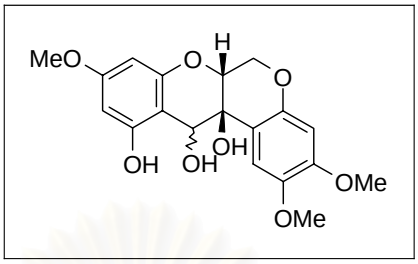
^{1}H NMR (400 MHz, acetone-d-6) δ 3.82 (s, 3H), 3.89 (s, 3H) 3.92 (s, 3H), 4.72 (dd, J_1 = 8.1 Hz, J_2 = 11.8 Hz, 1H), 4.79 (dd, J_1 = 4.7 Hz, J_2 = 12.3 Hz, 1H), 5.32 (dd, J_1 = 3.9 Hz, J_2 = 8.0 Hz, 1H), 6.11 (s, 1H), 6.48 (s, 1H), 6.62 (s, 1H), 7.19 (s, 1H), 10.09 (s, 1H), 12.37 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.2, 56.5, 69.5, 74.0, 93.2, 94.3, 99.9, 106.5, 106.8, 111.7, 145.2, 156.9, 157.9, 161.5, 166.2, 167.9, 186.2, 191.8.

IR (Chloroform) ν_{max}: 2927, 1680, 1638, 1509, 1433, 1272, 1213, 1163, 1124, 1037, 930 cm^{-1}.

HRMS (ESI-TOF) (M^++Na) *m/z* calcd for $C_{19}H_{18}O_8Na$ 397.0899 found 397.0897.

3.2.9 Synthesis of compound 88[59-63]



2,3,9-Trimethoxy-6,6a-dihydro-12*H*-chromeno[3,4-*b*]chromene-11,12,12a-triol (88)

To a 0.1 M solution of SmI_2 in THF (5.3 mL, 0.53 mmol) and t-BuOH (81, 0.84 mmol) at -78°C was added dropwise a solution of **87** (80 mg, 0.21 mmol) in THF (3 mL). After the mixture was stirred at -30°C for 3 h, the flask was opened to air to oxidize excess SmI_2, and the crude reaction mixture was filtered. The filtrate was evaporated at reduced pressure, and the residue was purified by flash chromatography (Hexane/EtOAc 4:1) to afford **88** (52 mg, 65%) as a pale yellow solid.

m.p. 149-150°C.

$\mathbf{R_f}$ = 0.47 (Hexane/EtOAc = 4:1).

Optical Rotation: $[\alpha]_D^{25}$ = -122.8 (*c* 0.50, $CHCl_3$).

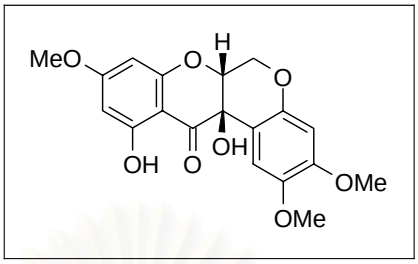
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (s, 3H), 3.76 (s, 3H) 3.79 (s, 3H), 4.30 (dd, J_1 = 3.8 Hz, J_2 = 10.5 Hz, 1H), 4.56 (dd, J_1 = 3.8 Hz, J_2 = 9.1 Hz, 1H), 4.69 (dd, J_1 = 9.1 Hz, J_2 = 10.5, 1H), 5.17 (s, 1H), 5.91 (d, J = 2.2 Hz, 1H), 6.02 (d, J = 2.2 Hz, 1H), 6.39 (s, 1H), 7.46 (s, 1H).

^{13}C NMR (100 MHz, acetone-d-6) δ 54.5, 55.0, 55.9, 64.2, 67.4, 68.9, 74.8, 92.5, 94.7, 100.0, 102.1, 111.3, 114.9, 143.6, 149.3, 150.3, 154.0, 157.9, 160.8.

IR (Chloroform): ν_{max} 3370, 2936, 2840, 1662, 1621, 1541, 1513, 1449, 1403, 1264, 1224, 1201, 1161, 1126, 1056, 938, 829 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{19}H_{21}O_8$ 377.1236 found 377.1231.

3.2.10 Synthesis of compound 39



11,12a-Dihydroxy-2,3,9-trimethoxy-6a,12a-dihydro-6H-chromeno[3,4-b]chromen-12-one (39)

The compound **88** (40 mg, 0.106 mmol) was dissolved in dichloromethane (1 mL), and MnO_2 (64.5 mg, 0.742 mmol, 7.0 equiv.) was added. After the reaction was stirred overnight at room temperature, CH_2Cl_2 was added to dilute the reaction and was filtered through a pad of celite and silica gel. Removal of volatiles revealed a brown solid that was purified by silica gel chromatography (Hexane/EtOAc; 2:3) to provide **39** (32 mg, 80%) as a pale yellow solid.

m.p. 131-132°C.

R_f = 0.52 (Hexane/EtOAc = 2:3).

Optical Rotation: $[\alpha]_D^{25}$ = +219.9 (*c* 1.0, $CHCl_3$) (lit.[19] +233, $CHCl_3$; *c* 0.1).

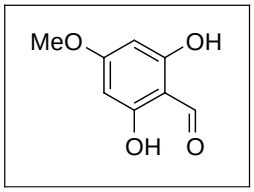
^{1}H NMR (400 MHz, DMSO-*d*-6) δ 3.57 (s, 3H), 3.70 (s, 3H) 3.74 (s, 3H), 4.33 (dd, J_1 = 12.3 Hz, J_2 = 1.6 Hz, 1H), 4.47, (dd, J_1 = 12.3 Hz, J_2 = 2.3 Hz, 1H), 4.67 (d, J = 2.3 Hz, 1H) 6.01 (d, J = 2.3 Hz, 1H), 6.06 (d, J = 2.3 Hz, 1H), 6.68 (s, 1H), 6.71 (s, 1H), 11.95 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 55.9, 56.3, 63.6, 66.9, 75.5, 94.5, 95.6, 100.1, 101.1, 108.2, 109.2, 143.9, 148.3, 151.3, 161.6, 164.3, 169.0, 195.0.

IR (Chloroform) ν_{max}: 3444, 2939, 2842, 1670, 1610, 1574, 1509, 1461, 1336, 1263, 1206, 1160, 1120, 1044, 1025, 822, 735 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{19}H_{19}O_8$ 375.1080 found 375.1074.

3.2.11 Synthesis of compound 89



2,6-Dihydroxy-4-methoxybenzaldehyde (89)

A mixture of 2,4,6-trihydroxybenzaldehyde (200 mg, 1.29 mmol), potassium carbonate (179.35 mg, 1.29 mmol), and methyl iodide (80.8 μL, 1.29 mmol) in acetone (100 mL) was stirred overnight. The mixture was filtered and the filtrate was concentrated in vacuo to a residue that was chromatographed on silica gel, eluting with ethyl acetate in hexane (2:3) to yield **89** (112.0 mg, 52%):

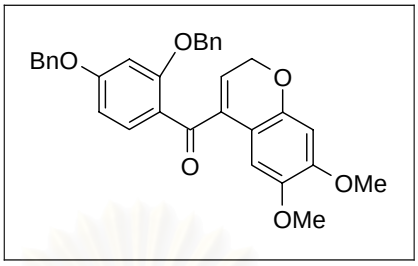
m.p. 171-172ºC.

$\mathbf{R}_f$ = 0.40 (Hexane/EtOAc = 3:2).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H), 5.90 (d, *J* = 2.3 Hz, 1H), 6.00 (d, *J* = 2.2 Hz, 1H), 10.08 (s, 1H), 12.51 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.8, 94.3, 106.7, 111.6, 161.4, 166.1, 167.8, 191.8.

3.2.12 Preparation of compound **80**[47]



(2,4-Bis-benzyloxy-phenyl)-(6,7-dimethoxy-2*H*-chromen-4-yl)-methanone (80)

In a flame dried 50 mL round bottom flask was added **79** (2.00 g, 3.93 mmol) and $PtCl_2$ (21 mg, 2 mol %). The flask was evacuated and flushed with argon three times, followed by the addition of toluene (20 mL, ~0.20 M). The reaction was allowed to stir at 55-60°C for 12h and the volatiles were removed when reaction was completed (TLC). Purification of the crude residue by flash chromatography on silica gel (Hexane/EtOAc, 3:1) afforded **80** as a brown solid (1.56 g, 78 %).

m.p. 120-121°C.

$\mathbf{R_f}$ = 0.44 (Hexane/EtOAc = 3:1).

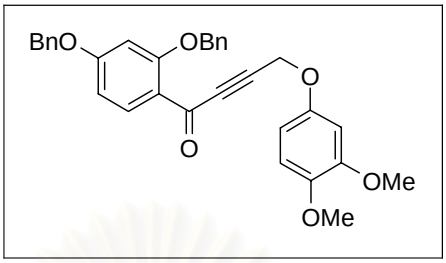
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74 (s, 3H), 3.89 (s, 3H) 4.70 (d, *J* = 3.7 Hz, 2H), 5.00 (s, 2H), 5.12 (s, 2H), 6.05 (t, *J* = 3.9 Hz, 1H), 6.47 (s, 1H), 6.62 (s, 1H), 6.65 (d, *J* = 8.6 Hz, 1H), 7.11 (s, 1H), 7.26 (m, 4H), 7.42 (m, 6H), 7.60 (d, *J* = 8.5 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.2, 64.8, 70.3, 70.4, 100.5, 100.8, 106.2, 108.7, 112.2, 122.3, 125.7, 126.9 (× 2), 127.6 (× 2), 127.8, 128.3, 128.5 (× 2), 128.7, 128.8 (× 2), 132.7, 136.1, 143.4, 148.6, 149.9, 159.3, 194.1.

IR (Chloroform) ν_{max}: 2938, 1601, 1507, 1456, 1379, 1267, 1178, 1124, 1022, 833, 742 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{32}H_{29}O_6$ 509.1964 found 509.1960.

3.2.13 Preparation of compound 79



1-(2,4-Bis-benzyloxy-phenyl)-4-(3,4-dimethoxy-phenoxy)-but-2-yn-1-one (79)

To a solution of **75** (1.50g, 7.80 mmol, 1.00 equiv.) in 80 mL of THF cooled to -78°C was added *n*-BuLi (5.46 mL, 8.19 mmol, 1.05 equiv.) under argon. After thirty minutes, **77** (2.48g, 7.8 mmol, 1.00 equiv.) in 50 mL of THF was added via cannula. The reaction was stirred for 60 min and then quenched with 30 mL of saturated NH_4Cl and extracted with EtOAc (× 3). The combined organic layer was washed with brine and dried over Na_2SO_4 and the volatiles removed under reduced pressure. The crude oil was then dissolved in dichloromethane (30 mL), and MnO_2 (4.75g, 54.60 mmol, 7.0 equiv.) was added. After the reaction was stirred overnight at room temperature, CH_2Cl_2 was added to dilute the reaction and was filtered through a pad of celite and silica gel. Removal of volatiles revealed a brown solid that was purified by silica gel chromatography (CH_2Cl_2/Hexane; 2:3) to provide **79** (3.85g, 97%) as a brown solid.

m.p. 85-86°C.

R_f = 0.50 (CH_2Cl_2/Hexane = 2:3).

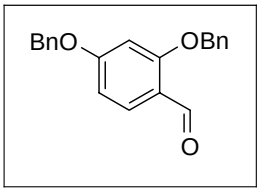
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (s, 3H), 3.81 (s, 3H), 4.62 (s, 2H), 5.05 (s, 2H), 5.06 (s, 2H), 6.43 (dd, J_1 = 2.2 Hz, J_2 = 8.7, 1H), 6.52-6.57 (m, 3H), 6.74 (d, J = 8.7 Hz), 7.32-7.47 (m, 10H), 7.94 (d, J = 8.6 Hz, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 55.9, 56.3, 56.6, 70.4, 70.5, 87.0, 87.1, 100.6, 101.5, 104.4, 106.5, 111.4, 119.9, 127.3 (× 2), 127.6, (× 2), 128.0, 128.4, 128.6 (× 2), 128.7, 128.8 (× 2), 134.9, 135.8, 136.1, 144.2, 149.8, 151.9, 161.1, 164.7, 174.0.

IR (Chloroform) ν_{max}: 2939, 1596, 1509, 1450, 1377, 1258, 1233, 1188, 1147, 1025, 833, 740 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{32}H_{29}O_6$ 509.1964 found 509.1959.

3.2.14 Synthesis of compound 77



2,4-Bis-benzyloxy-benzaldehyde (77)

The 2,4-dihydroxybenzaldehyde (4.0 g, 28.96 mmol), K_2CO_3 (8.0 g, 57.92 mmol) and $^{n}Bu_4NI$ (0.31 g, 0.87 mmol) were dissolved in acetone (80 mL), the solution was treated with benzyl bromide (8.7 mL (12.38 g), 72.40 mmol), and the reaction mixture was stirred at room temperature for 24 h. After removal of acetone, the aqueous phase was extracted with DCM (× 3), and the combined extracts were washed with H_2O, brine and dried over Na_2SO_4. The extracts were filtered and concentrated, and the residue was purified by flash column chromatography on silica gel (elution with 9:1 Hexane/EtOAc) to give compound **77** (8.5 g, 92%) as a white solid.

m.p. 155-156°C °C.

R_f = 0.60 (Hexane/EtOAc = 9:1).

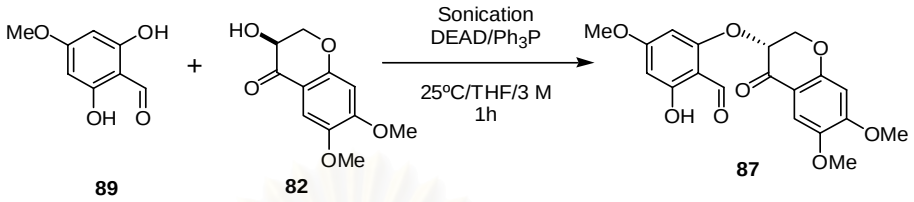
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (s, 2H), 5.17 (s, 2H), 6.67 (dd, J_1 = 1.6 Hz, J_2 = 8.7, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.74-7.75 (m, 10H), 7.88 (d, J = 8.7 Hz, 1H), 10.42 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 70.3(× 2), 100.1, 106.9, 119.4, 127.3 (× 2), 127.6 (× 2), 128.3, 128.4, 128.7 (× 4), 130.5, 135.8, 135.9, 162.7, 165.2, 188.3.

IR (Chloroform) ν_{max}: 2953, 1677, 1600, 1500, 1456, 1259, 1174, 1109, 1020, 820, 735 cm^{-1}.

HRMS (ESI-TOF) (MH^+) *m/z* calcd for $C_{21}H_{19}O_3$ 319.1334 found 319.129.

3.2.15 Attempted on Mitsunobu coupling between compound 82 and compound **89**[65]



To a round bottomed flask was added 2,6-dimehydroxy-4-mothoxybenzaldehyde **89** (50 mg, 0.297 mmol), α-hydroxyketone **82** (70 mg, 0.312 mmol), triphenylphosphine (81.83 mg, 0.312 mmol), and THF (0.1 mL). The reaction vessel was then lowered into a sonication bath and sonicated for several minutes (to allow for mixing) giving a clear and highly viscous solution. While sonicating, diethylazodicarboxylate (DEAD) (0.854 mL, 4.30 mmol) was added dropwise to the reaction mixture over the course of 2 min. Overall, the reaction mixture (amber color) was sonicated for an hour. The reaction mixture was checked by TLC, there was no coupling product (**87**) was detected.

3.3 Isolation

The dried root of *Stemona collinsae* Craib. (4 kg) was pulverized and extracted by maceration with MeOH (2×6L) for one week. The combined MeOH extract was concentrated to dryness under reduced pressure to obtain a deep brown resinous crude extract (400 g). The crude extract was then partitioned between $CHCl_3$ and H_2O, then the $CHCl_3$ extract which is the most active part was chromatographed on silica gel column using $CHCl_3$-MeOH with increasing polarity as eluent. The compound **39** was obtained as light yellow needles. Its melting point, IR, NMR and MS are in good agreement with those reported in the literature.[19] X-ray crystal structure and cytotoxic activity against human cell lines have also been reported.[64]

CHAPTER IV

Result and Discussion

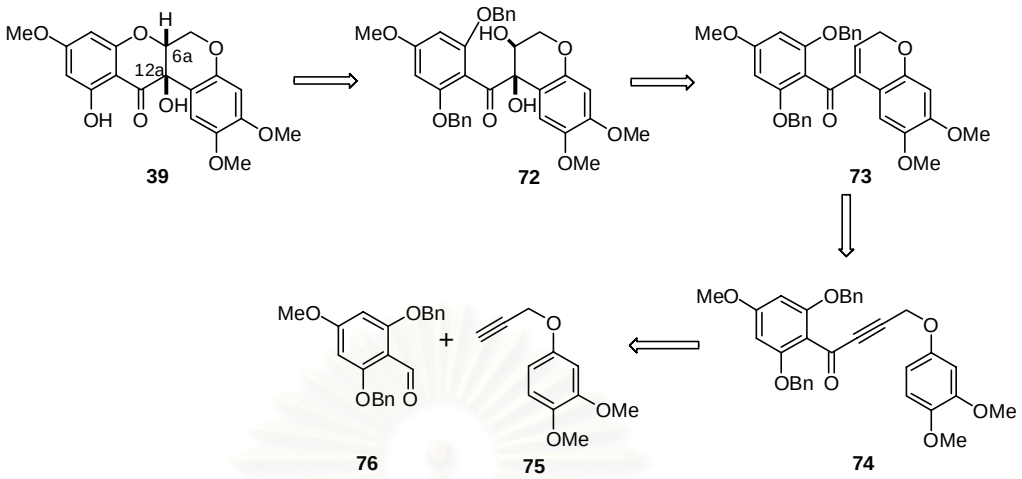
4.1 Structural Analysis

Caused by *cis* conformation at the B/C fusion, the 6-deoxyclitoriacetal **39** adopts a roof-shaped conformation that same as most naturally occurred rotenoids do and the configuration at C-6a and C-12a are *R*, *R*, respectively. The compound **39** has only two chiral centers at C-6a and C-12a and can say highly oxygenated molecule. The challenges arise from the facts that the carbons C-6a and C-12a are chiral and they have to be synthesized by asymmetric means.

4.2 The First Retrosynthetic Analysis of compound 39

4.2.1 Synthetic approach to compound 39

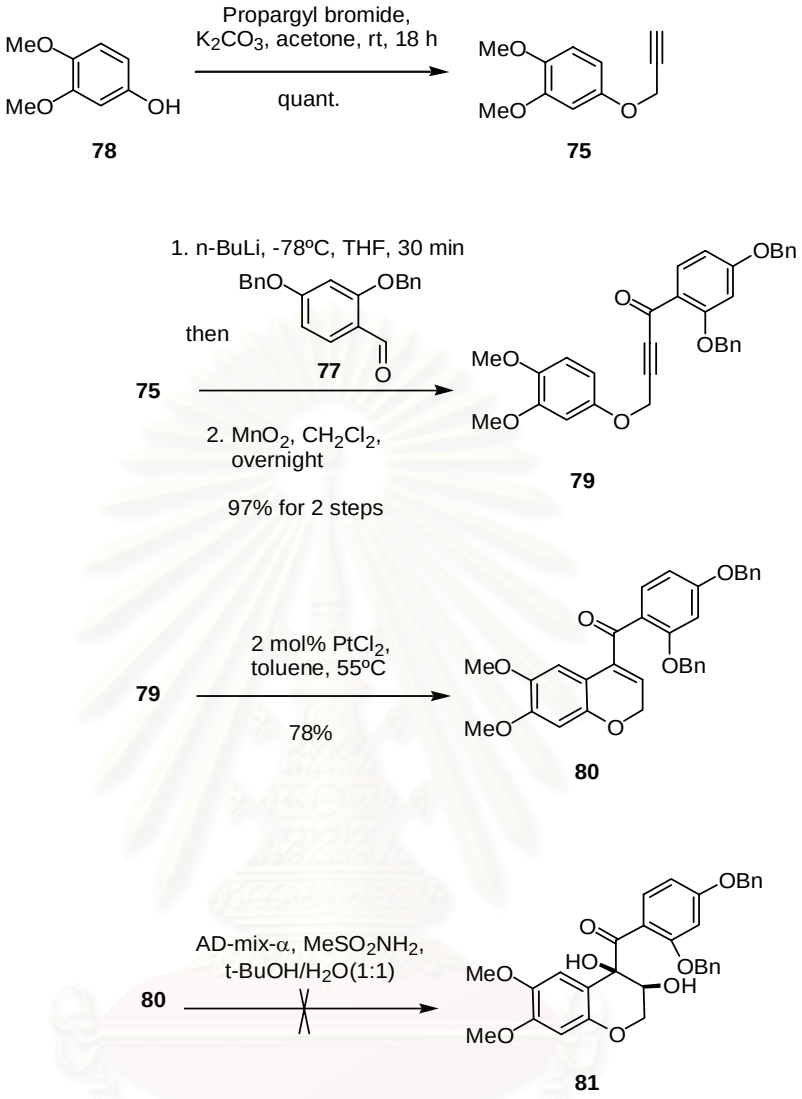
Scheme 1 was outlined the retrosynthetic strategy for compound **39** where the synthetic approach relied on two key cyclization steps, including the Mitsunobu intramolecular coupling for diol **72** to compound **39** and the platinum-cyclized 6-endo hydroarylation of alkynone **74** to alkene **73** which could be converted to the diol **72** successfully by Sharpless asymmetric dihydroxylation. The precursor **74** could then be assembled by the convergence of intermediates **75** and **76**.



Scheme 1 First disconnection of compound **39**

4.2.1.1 Investigation of the first route

To test this approach, the compound **76** had been changed to compound **77** because it is much cheaper. The synthesis commenced with conversion of phenol **78** to propagyl ether **75** in quantitative yield (Scheme 2). Treatment of compound **75** with *n*-BuLi in THF followed by reaction with aldehyde **77** produced the crude alcohol, which was oxidized without purification with manganese dioxide to obtain alkynone **79** in 97% yield for two steps.[47]



Scheme 2 Route to compound **81**

Under the action of 2 mol% $PtCl_2$ (~0.2 M in toluene, 55°C), alkynone **79** was converted to the desired cyclization product **80** in good yield (78%).[47-50] Before a last key step, Mitsunobu intermolecular coupling, alkene **80** should be converted to diol **81** under Sharpless asymmetric dihydroxylation condition.[51] Unfortunately, even after extensive try, diol product could not be obtained. The failure of dihydroxylation might be due to the steric effect of benzyl group (Figure 1).

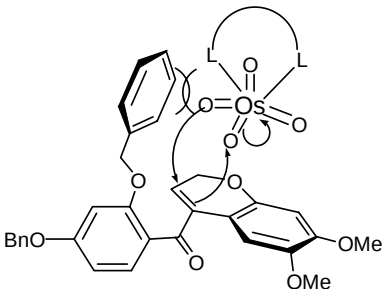
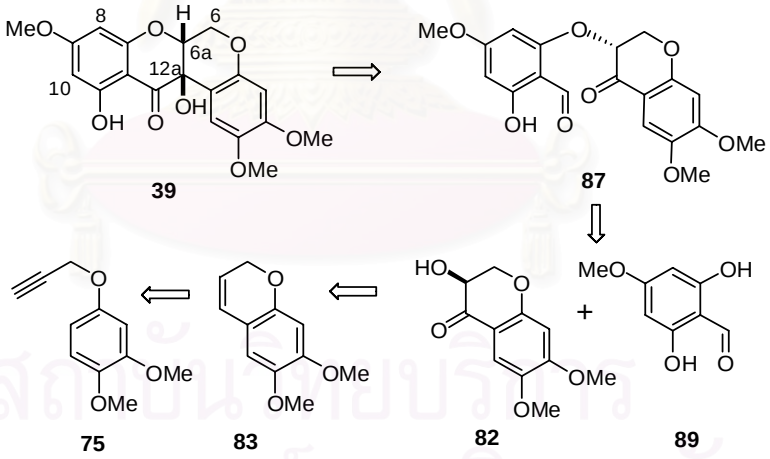


Figure 5. The possibly steric coordination of OsO_4 on compound 80

4.3 The Second Retrosynthetic Analysis of compound 39

After unsuccessful accommodation of diol on alkene **80**, I decided to change to the second synthetic approach as shown in Scheme 3. The synthetic strategy is based on the intramolecular Pinacol-type coupling between aldehyde and ketone of compound **87**. The compound **82** could be assembled from elaboration of alkene **83** which planned to obtain from propargyl aryl ether **75** using hydroarylation methodology.



Scheme 3 Second disconnection of compound **39**

4.3.1 Synthetic approach to compound 39

4.3.1.1 Synthetic route to compound **82**

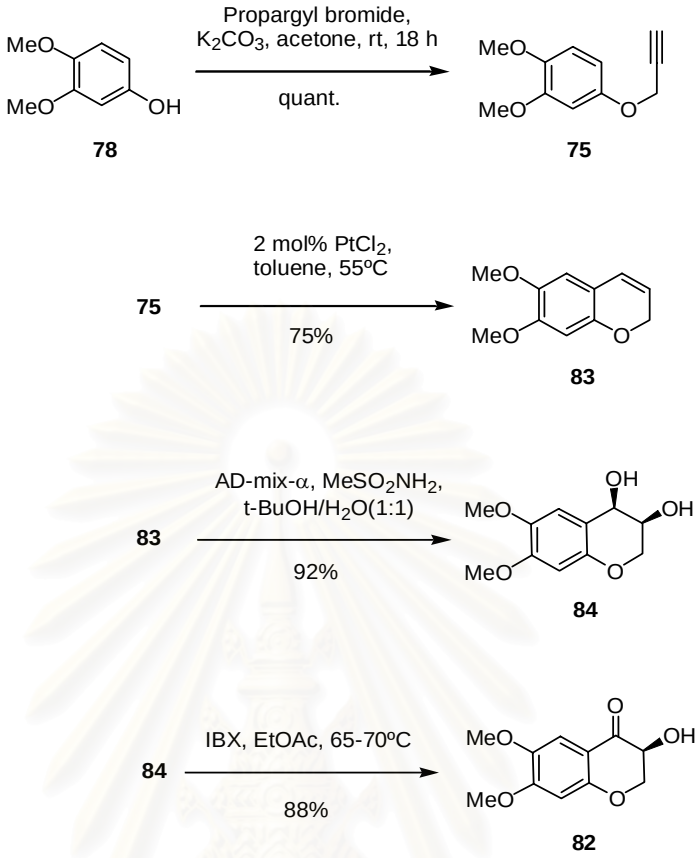
The synthesis of compound **75** was completed as presented in Scheme 4. Reaction of 3,4-dimethoxyphenol **78** with propargyl bromide and K_2CO_3 in acetone at room temperature gave compound **75** in quantitative yield. Cyclization of compound **75** could be accomplished by platinum-catalyzed hydroarylation[47-50] using $PtCl_2$ in toluene to give the required alkene **83** in reasonable yield (75%). However, Claisen rearrangement[52-53] of **75** in N,N-diethylaniline was also tried but formation of cyclic product was observed in lower yield as well as the composition of starting material.

4.3.1.2 The Sharpless asymmetric dihydroxylation.[51]

The Sharpless asymmetric dihydroxylation was then effected by treatment of alkene **83** with AD-mix-α in t-BuOH-H_2O giving diol **84** in 92% yield ($[\alpha]_D^{25}$ = -12.4 (*c* 0.95, $CHCl_3$).

4.3.1.3 Regioselective Oxidation of diol (**84**) to ketone (**82**)

Regioselective oxidation of diol **84** at the benzylic position with IBX in DMSO[54] provided the desired ketone **82** in 40% yield. However, the yield of this oxidation was significantly improved (88%) by changing the solvent to ethyl acetate.[55] Other regioselective oxidation reagents, PCC, PDC and MnO_2, were also tried, unfortunately resulted in lower yield.

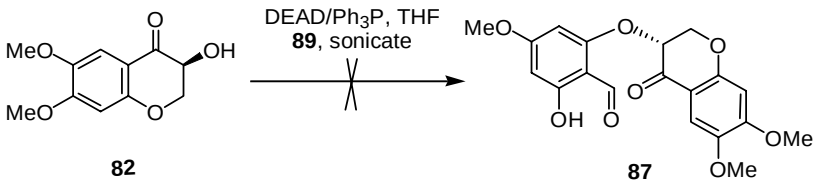


Scheme 4 Route to compound **82**

4.3.1.4 Attempted on Mitsunobu reaction.[56, 65]

The Mitsunobu reaction is extensively used in organic synthesis for the preparation of alkyl aryl ethers under mild conditions. The method has proven successful in the coupling of a wide variety of phenol and alcohol substrates.

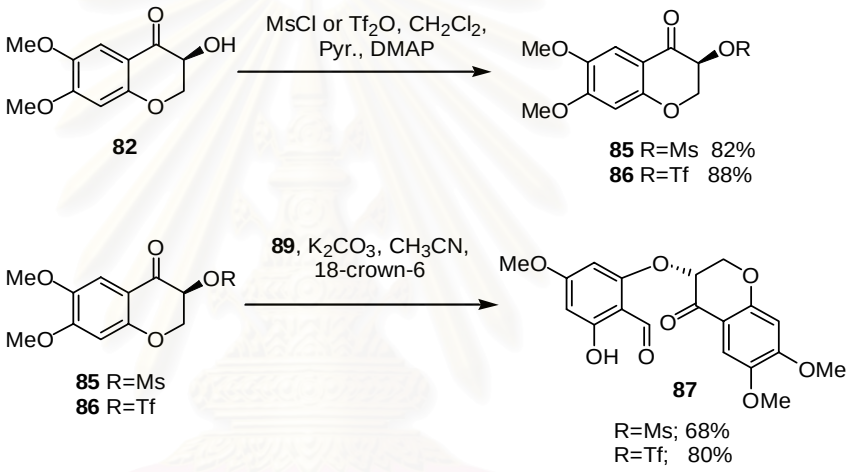
The investigation of Mitsunobu reaction (Scheme 5) between compound **82** and compound **89** was tried. Unfortunately, all tested conditions failed to give any desired product, owing to the low reactivity of phenol **89**.



Scheme 5 Attempted on Mitsunobu reaction

4.3.1.5 The S_N2 Coupling

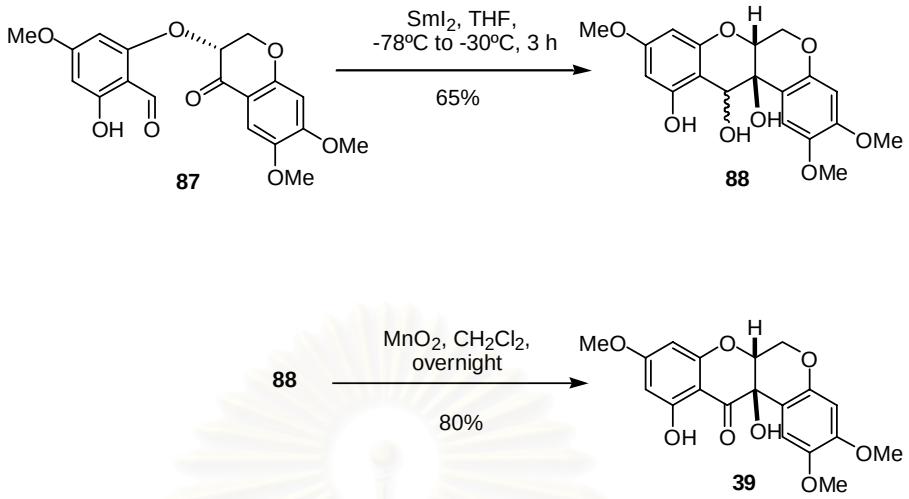
Having achieved efficient synthesis of the S_N2 coupling, the alcohol must be converted to a good leaving group. So that the α-hydroxyketone **82** was next converted into the mesylate **85** and the triflate **86** in yields of 82% and 88%, respectively under standard conditions (Scheme 6). The S_N2[57-58] coupling of mesylate **85** with the known phenol **89** using 18-crown-6 and K_2CO_3 in CH_3CN generated keto-aldehyde **87** in 67% yield. However, treatment of compound **89** with triflate **86** under the same conditions provided compound **87** in a higher yield of 80%.



Scheme 6 S_N2 coupling

4.3.1.6 Completed synthesis of 6-deoxyclitoriacetal (**39**)

To complete the synthesis of 6-deoxyclitoriacetal (**39**), two remaining steps were required (Scheme 7). Treatment of **87** with samarium diiodide in THF/t-BuOH at -78°C provided compound **88** in 65% yield through a stereoselective intra-molecular keto-aldehyde pinacol coupling (Figure 2).[59-63] Finally, oxidation of **88** with MnO_2 in dichloromethane gave 6-deoxyclitoriacetal (**39**) in 80% yield.



Scheme 7 Completed synthesis of compound **39**

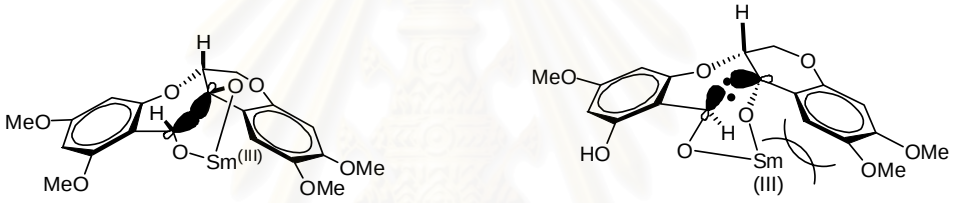


Figure 6. Stereoselectivity of Pinacol-type by SmI_2

The synthetic product **39** exhibited ^{1}H and ^{13}C NMR spectral data [a small doublet at δ 4.67 (d, J = 2.3 Hz), assigned to H-6a and an AB quartet at δ 4.33 (dd, J_1 = 12.3, J_2 = 1.6 Hz, 1 H), and δ 4.47 (dd, J_1 = 12.3 Hz, J_2 = 2.3 Hz, 1 H), attributed to H-6], m.p. (131-132 °C; lit.[19] m.p. 130-131°C) and optical rotation { $[\alpha]_D^{25}$ = +219.9 (*c* 1.0, $CHCl_3$), lit.[19] $[\alpha]_D$ = +233 (*c* 0.1, $CHCl_3$)} closely matching those published for the related natural product.[19, 25-28, 46]

Table 1 Comparison of ^{1}HNMR for synthetic and isolated compound **39**.

Position	Synthesis	Literature
1	6.68 *s*	6.66 *s*
2-OMe	3.57 *s*	3.66 *s*
3-OMe	3.75 *s*	3.75 *s*
4	6.68 *s*	6.41 *s*
6	4.33 *dd* (*J*=1.6, 12.3 Hz) 4.67 *dd* (*J*=2.3, 12.3 Hz)	4.39 *dd* (*J*=2.0, 12.0 Hz) 4.51 *dd* (*J*=2.5, 12.0 Hz)
6a	4.47 *d* (*J*=2.3 Hz)	4.49 *d* (*J*=2.0 Hz)
8	6.01 *d* (*J*=2.3 Hz)	5.88 *d* (*J*=2.3 Hz)
9-OMe	3.75 *s*	3.75 *s*
10	6.06 *d* (*J*=2.3 Hz)	6.04 *d* (*J*=2.3 Hz)
11-OH	11.95 *s*	11.50 *s*
12a-OH	7.30 *s*	7.80 *s*

4.4 Cytotoxic test[64]

Table 2 Cytotoxicity (IC50, μg/mL) of compounds **39** against human cancer cell lines.

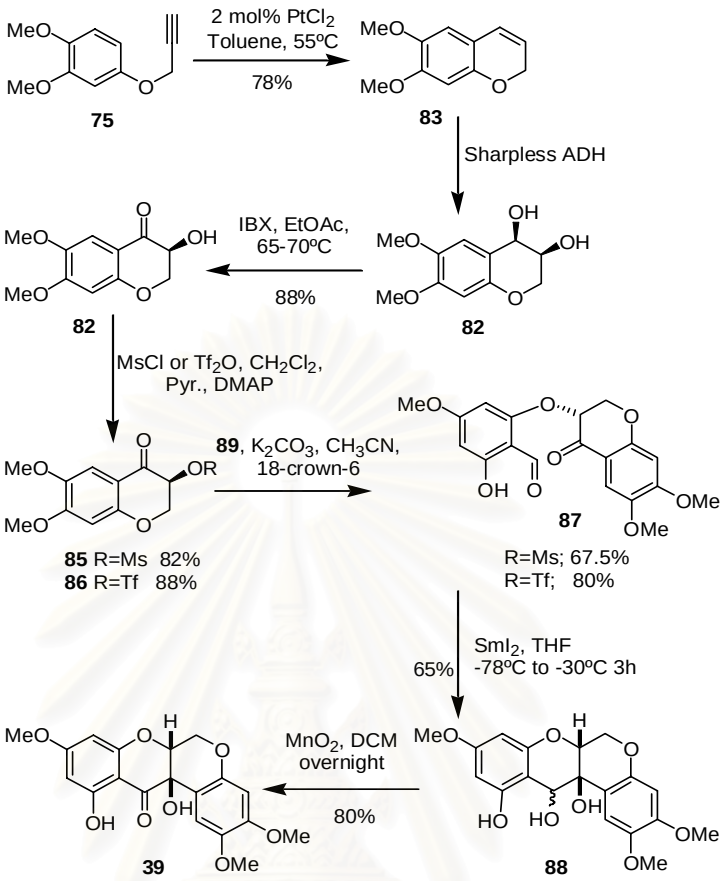
	Breast (BT474)	Lung (CHAGO)	Hepato (HEP-G2)	Gastric (KATO3)	Colon (SW620)
6-deoxyclitoriacetal	0.2	0.9	0.1	0.3	0.1
Doxorubicin HCl	0.1	2.3	0.9	1.7	1.1

CHAPTER V

Conclusions

The first total synthesis of 6-deoxyclitoriacetal (**39**) has been synthesized successfully in 8 steps starting from commercially available 3,4-dimethoxyphenol (**78**). The highlights of this synthesis are involved the S_N2 reaction, $PtCl_2$-catalyzed hydroarylation reaction and the SmI_2-promoted intramolecular Pinacol-type cyclization.

The conversion of compound **75** into compound **8**3 was achieved by $PtCl_2$-catalyzed hydroarylation reaction and easily converted into compound **82** by Sharpless asymmetric dihydroxylation followed by selective oxidation with IBX in EtOAc. Connection of D ring and A-B ring were accomplished by S_N2 reaction in high yield (80%). The C ring was constructed by SmI_2-promoted intramolecular Pinacol-type cyclization of compound **87** which was delivered the right stereogenic center at C-12a. Completion of synthetic 6-deoxyclitoriacetal (**39**) was achieved by oxidation of compound **88** with MnO_2.



Scheme 8 Summarized in total synthesis of compound **39**

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APPENDIX

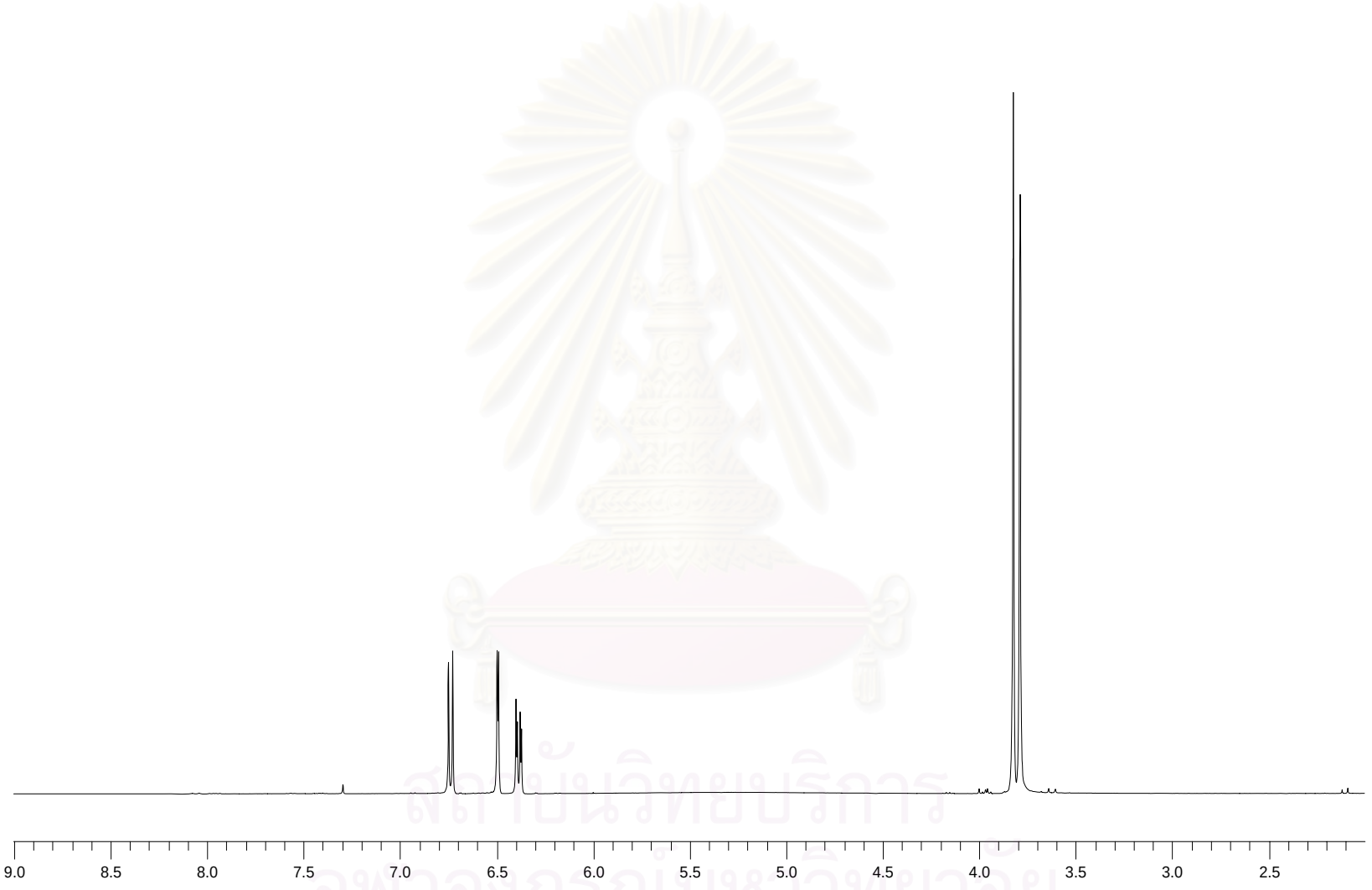

Figure 7 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 78

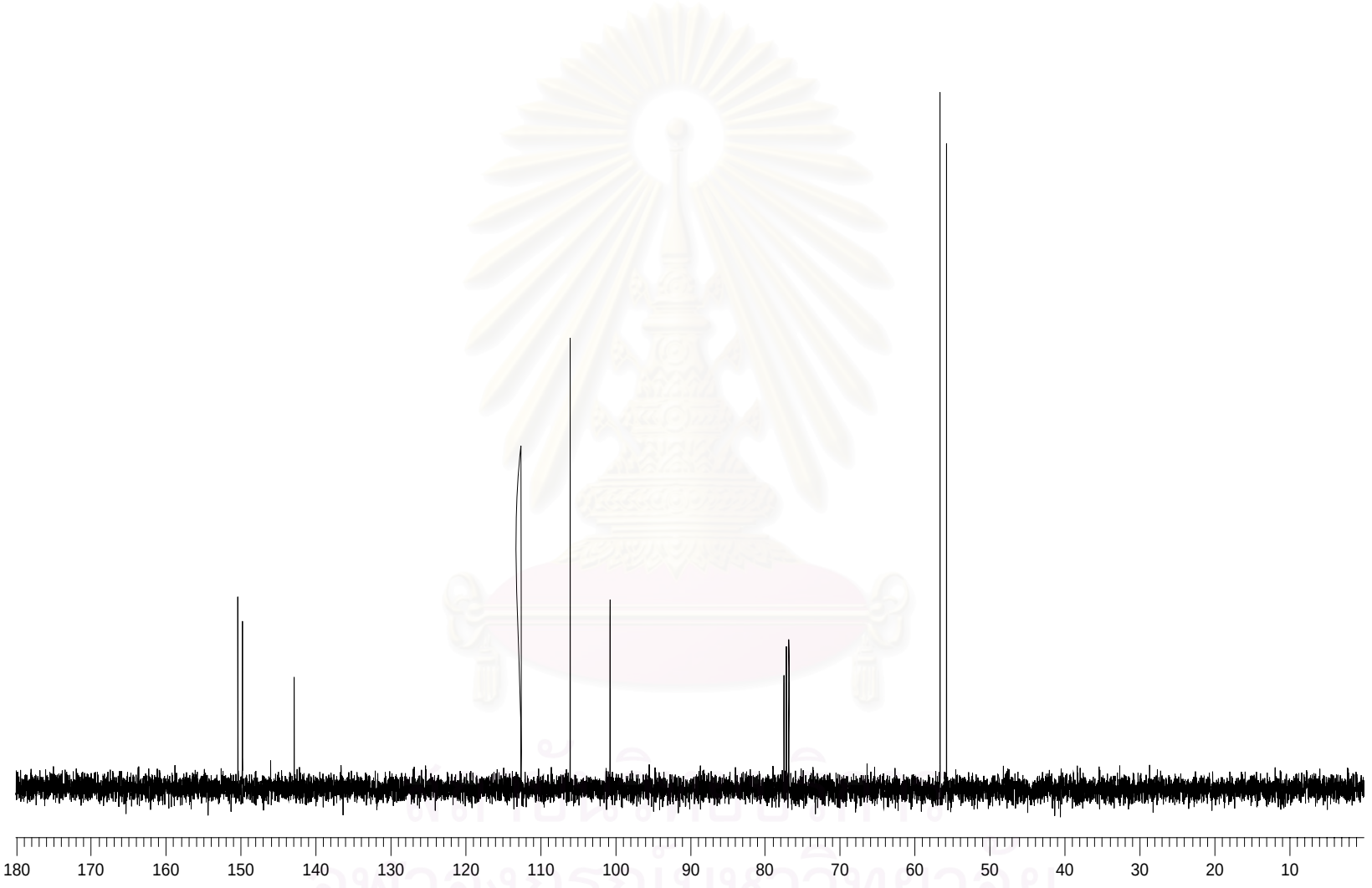

Figure 8 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 78

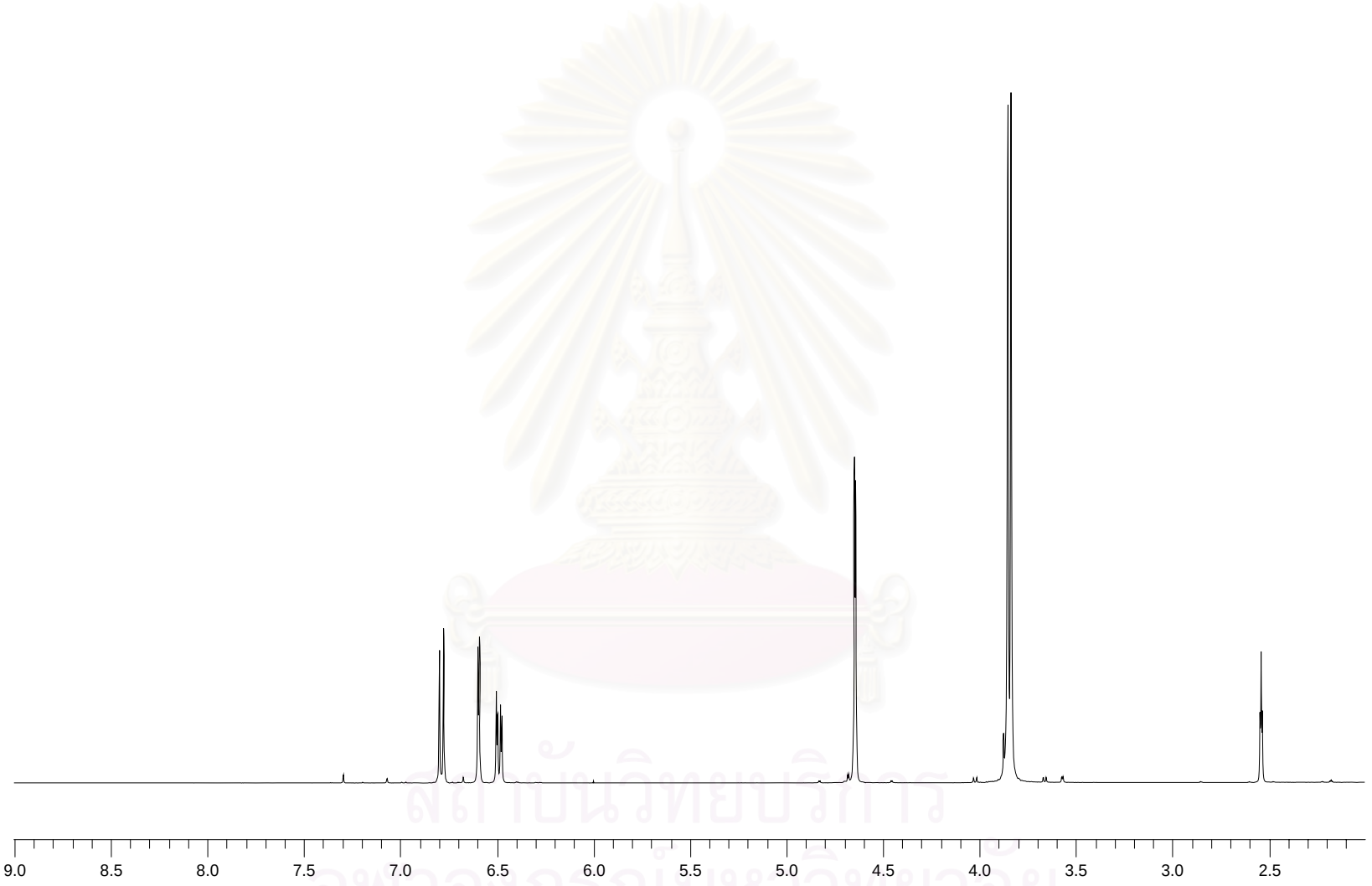

Figure 9 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 75

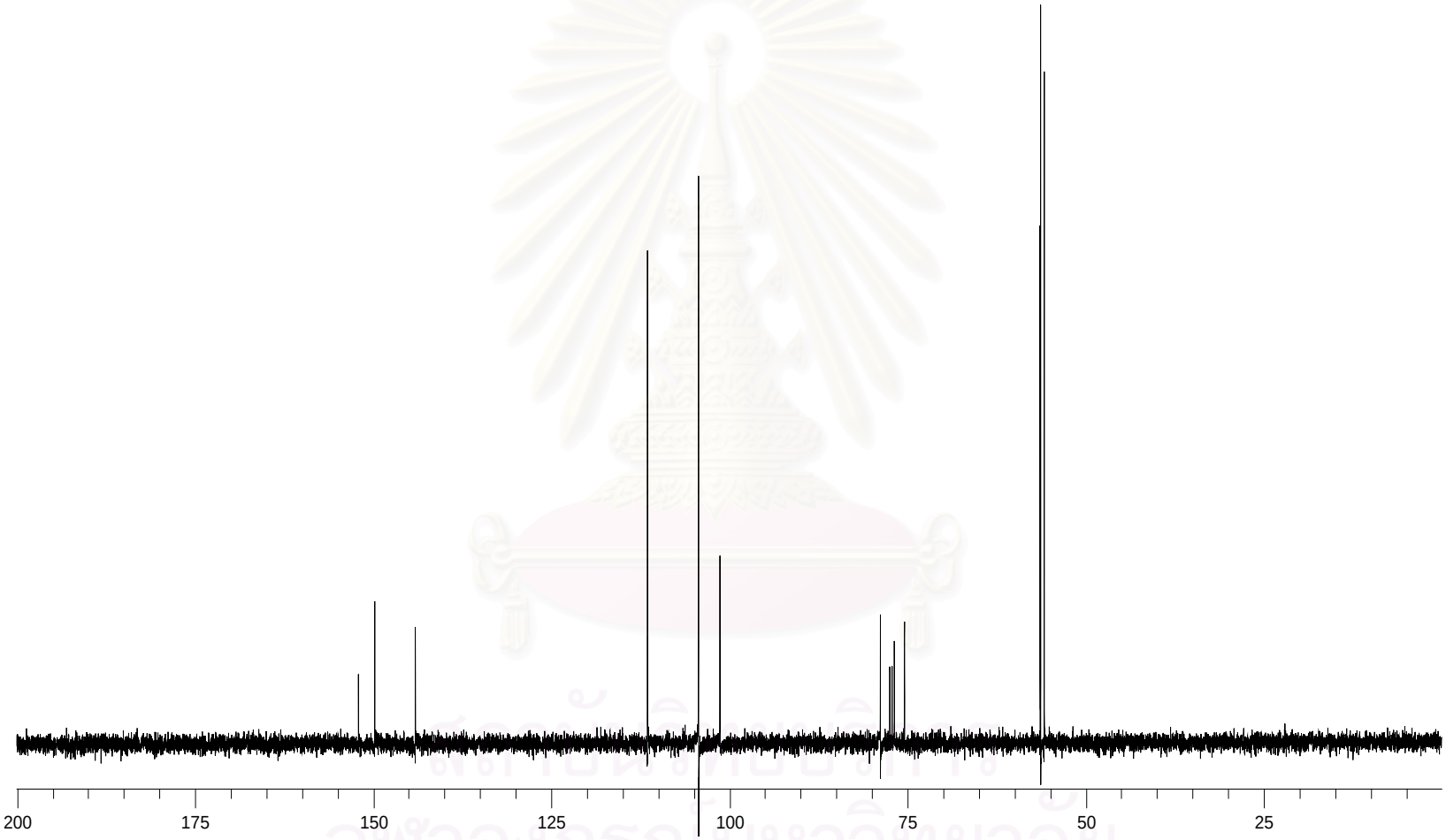

Figure 10 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 75

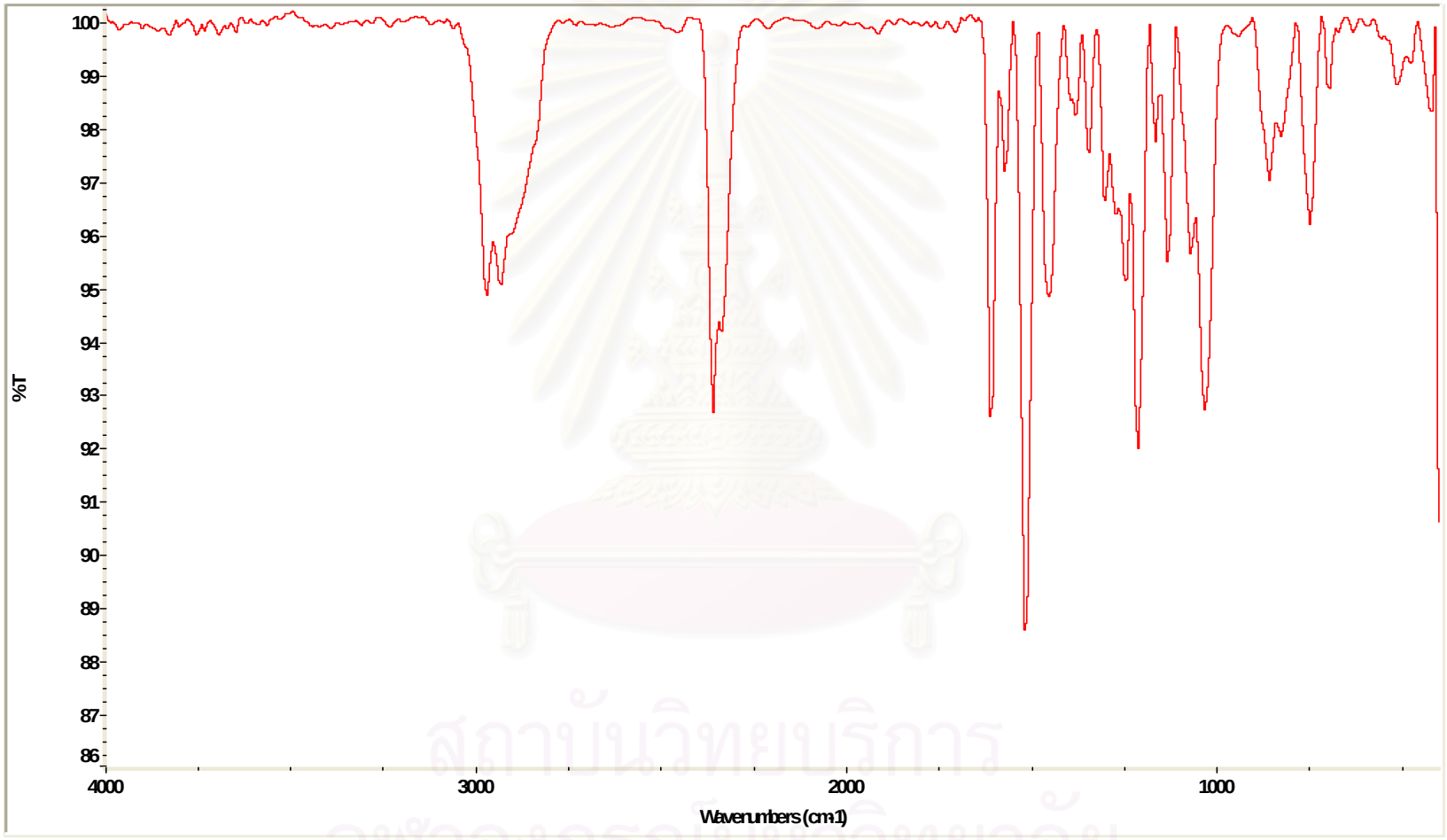

Figure 11 FT-IR of compound 75

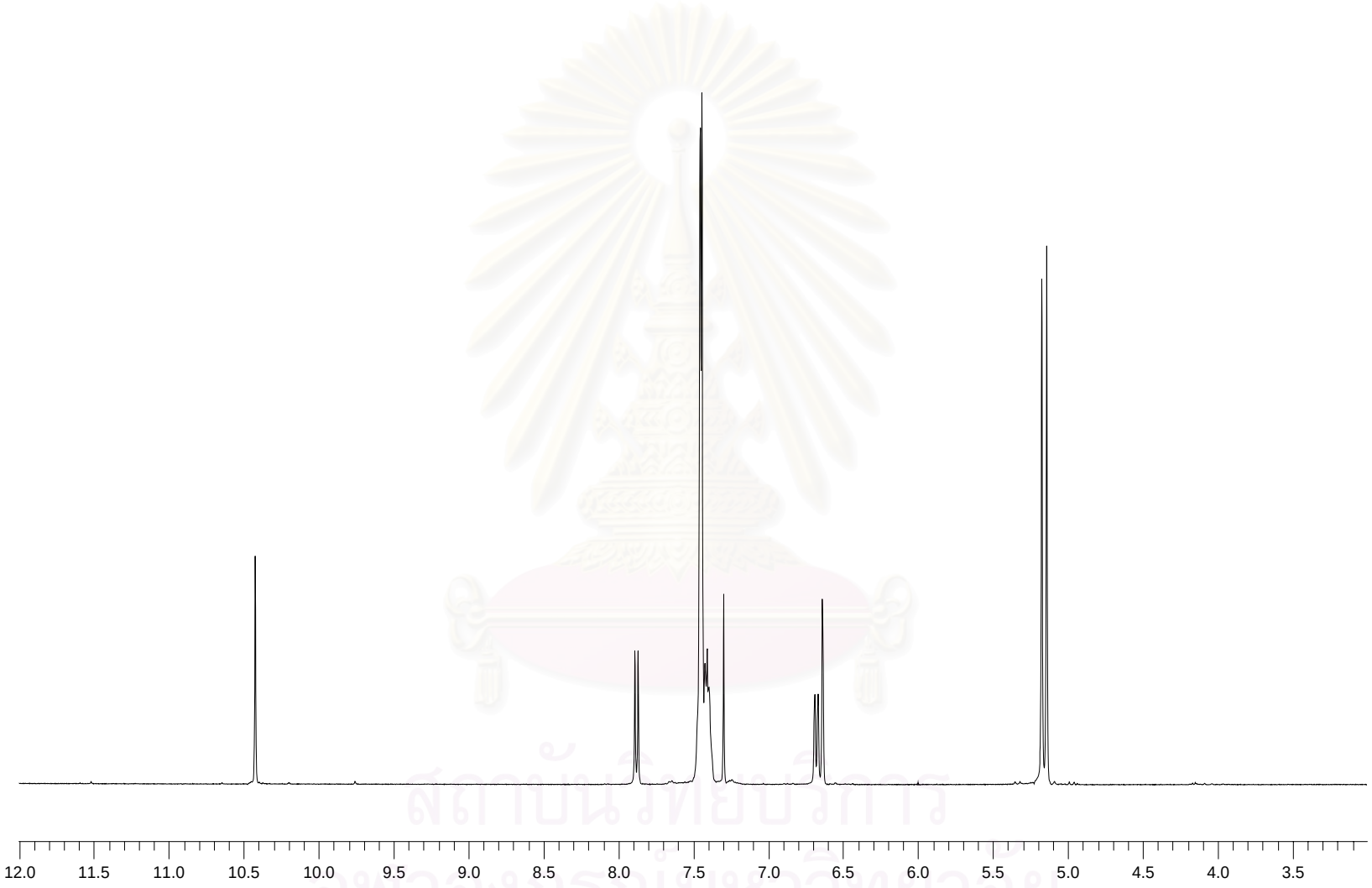

Figure 12 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 77

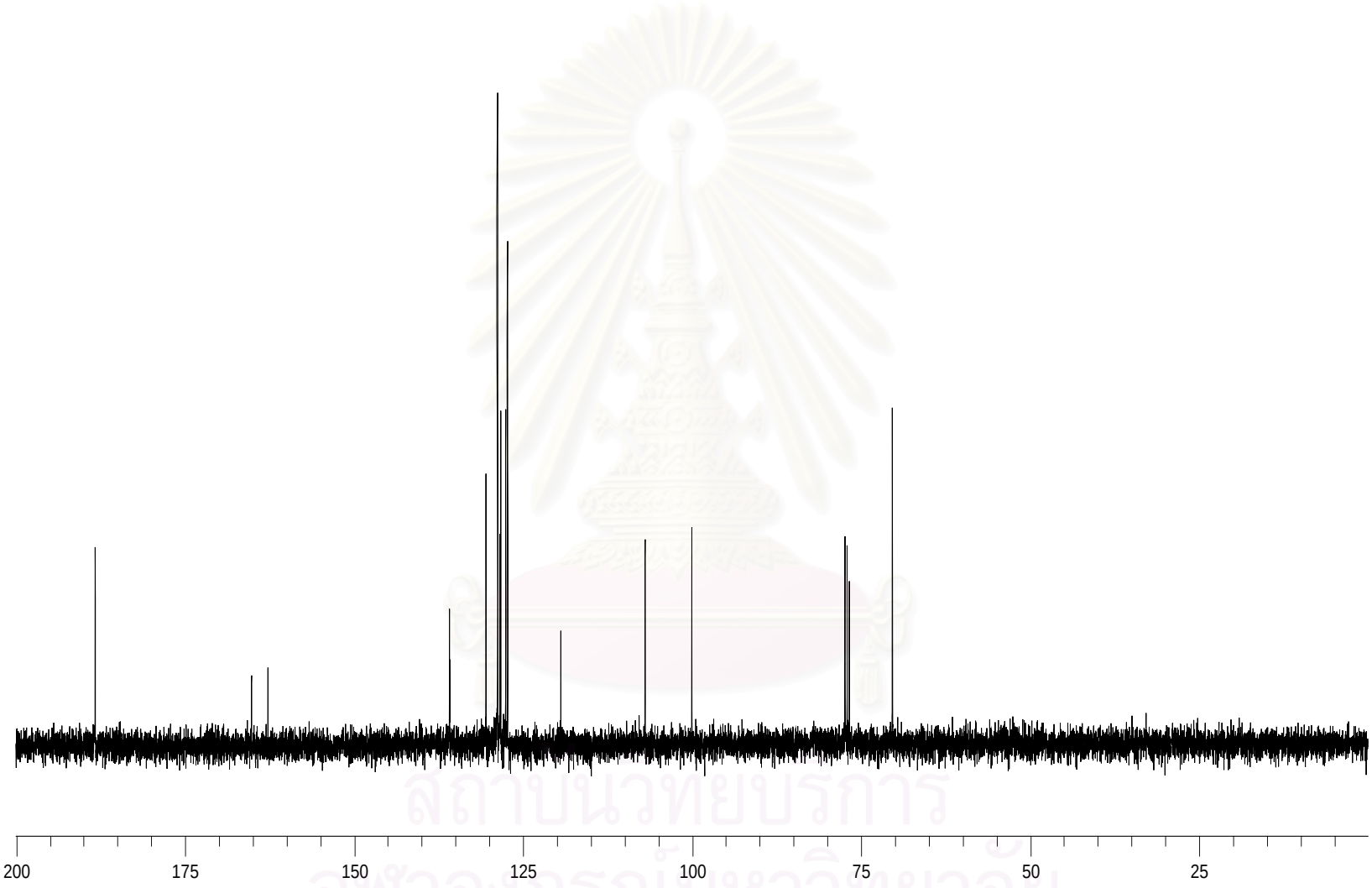

Figure 13 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 77

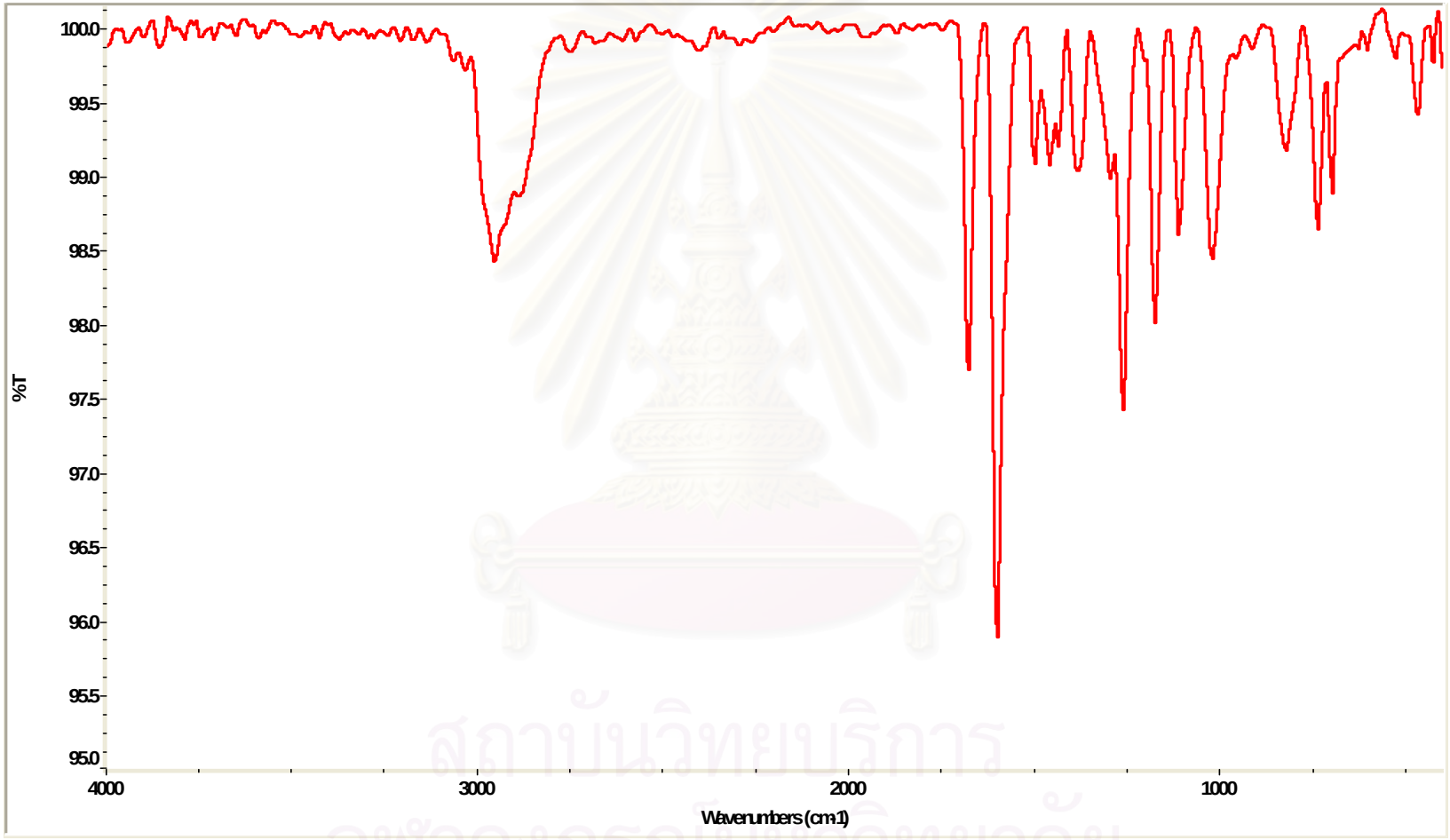

Figure 14 FT-IR of compound 77

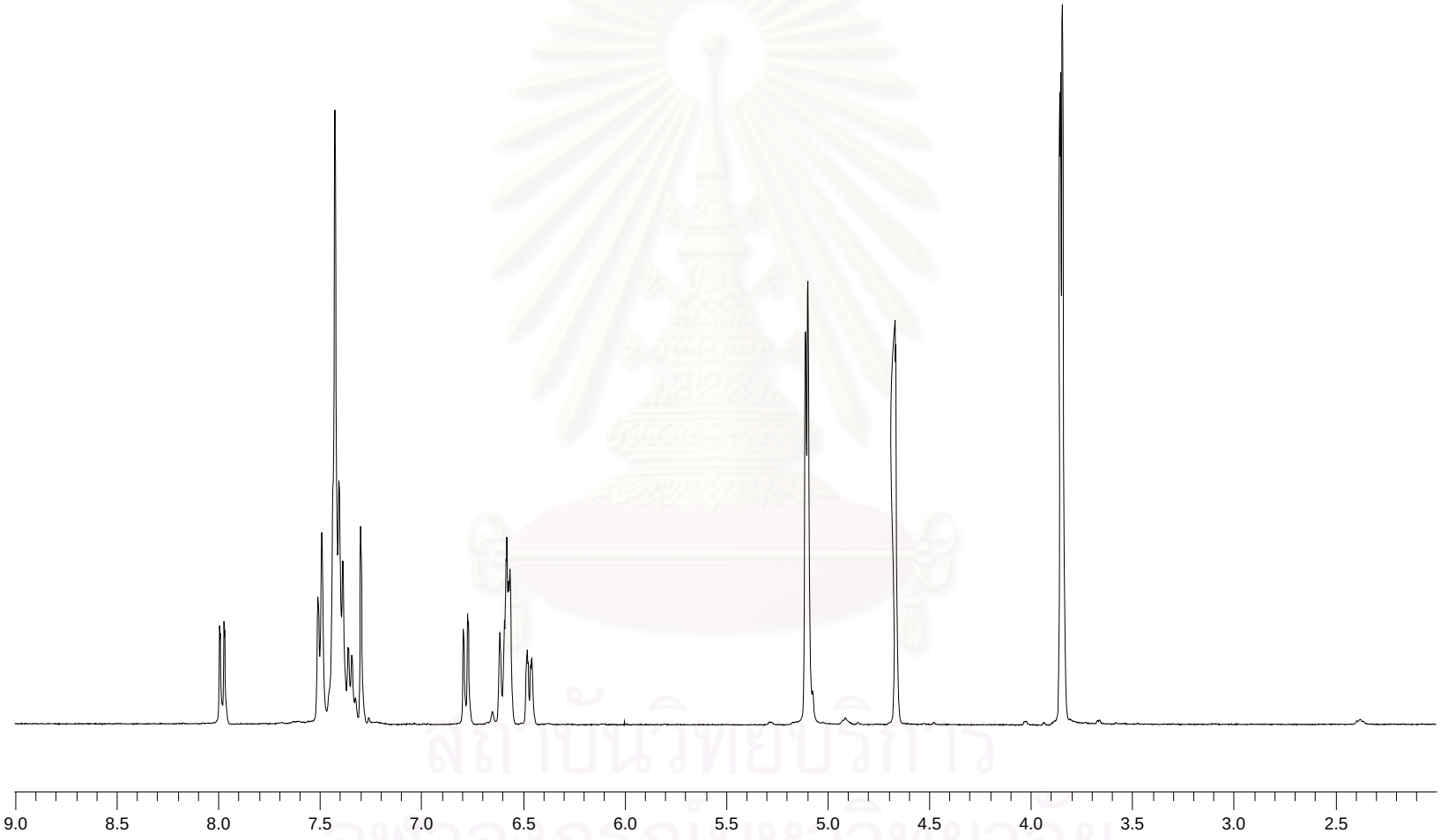

Figure 15 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 79

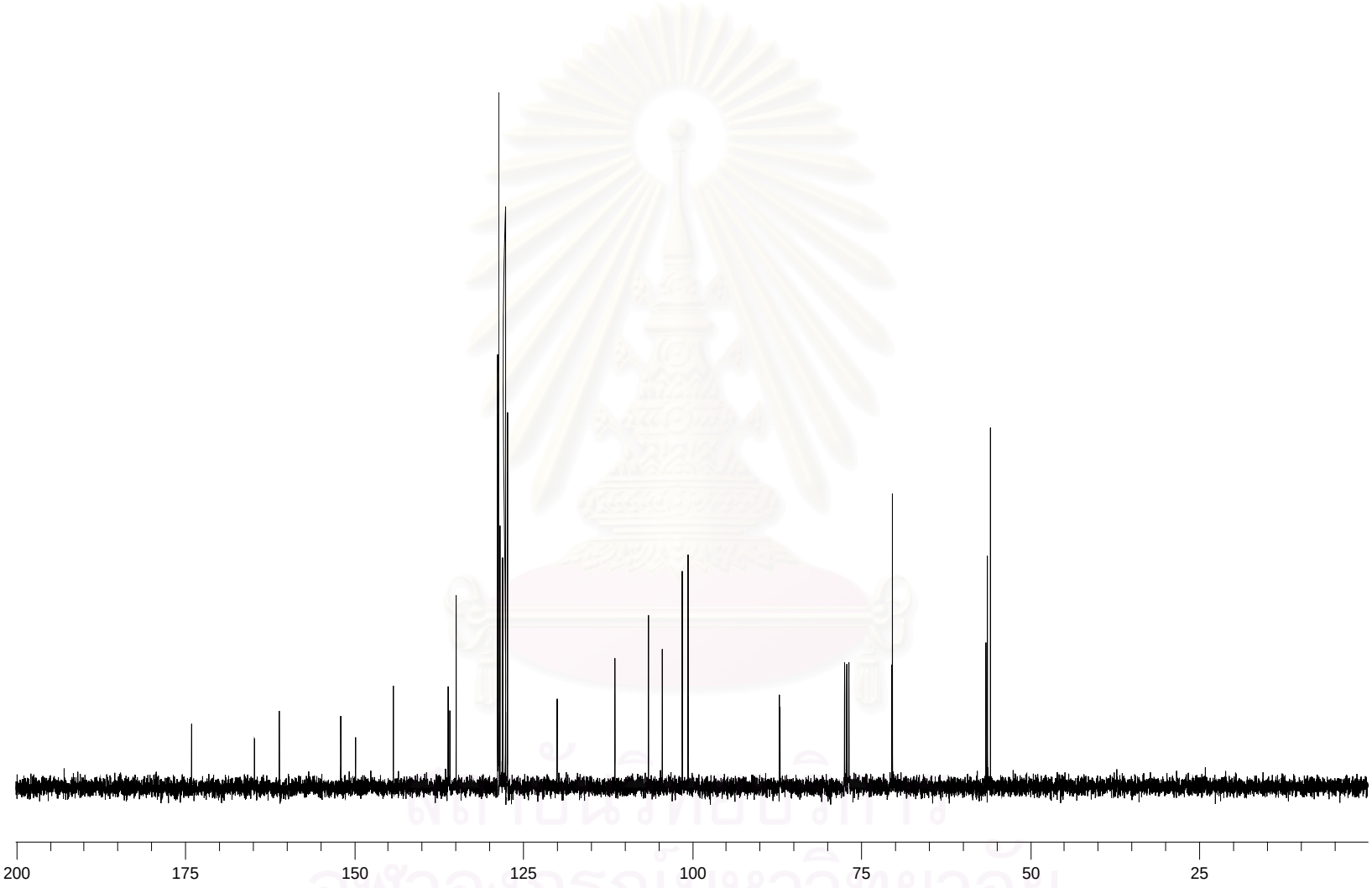

Figure 16 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 79

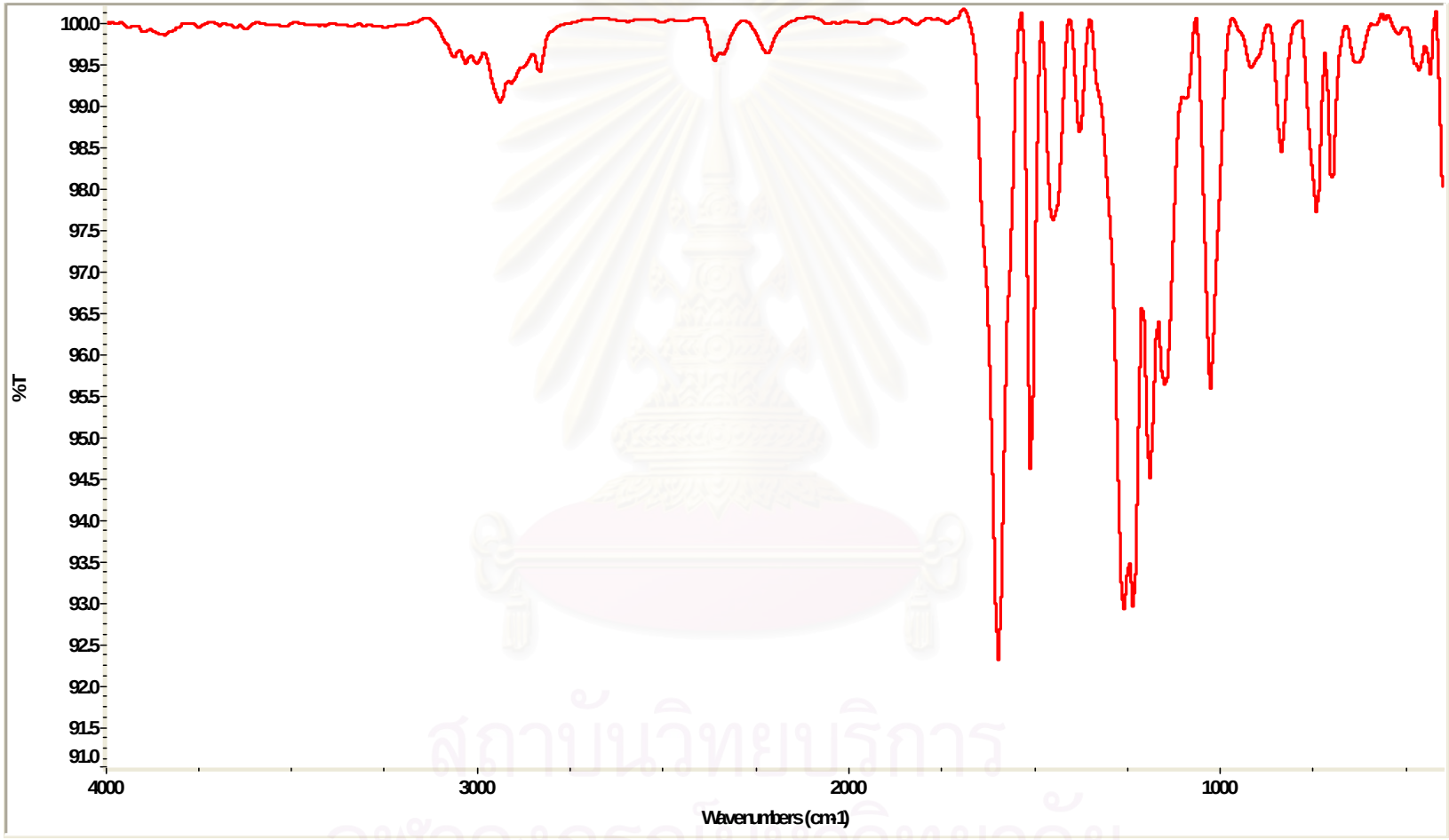

Figure 17 FT-IR of compound 79

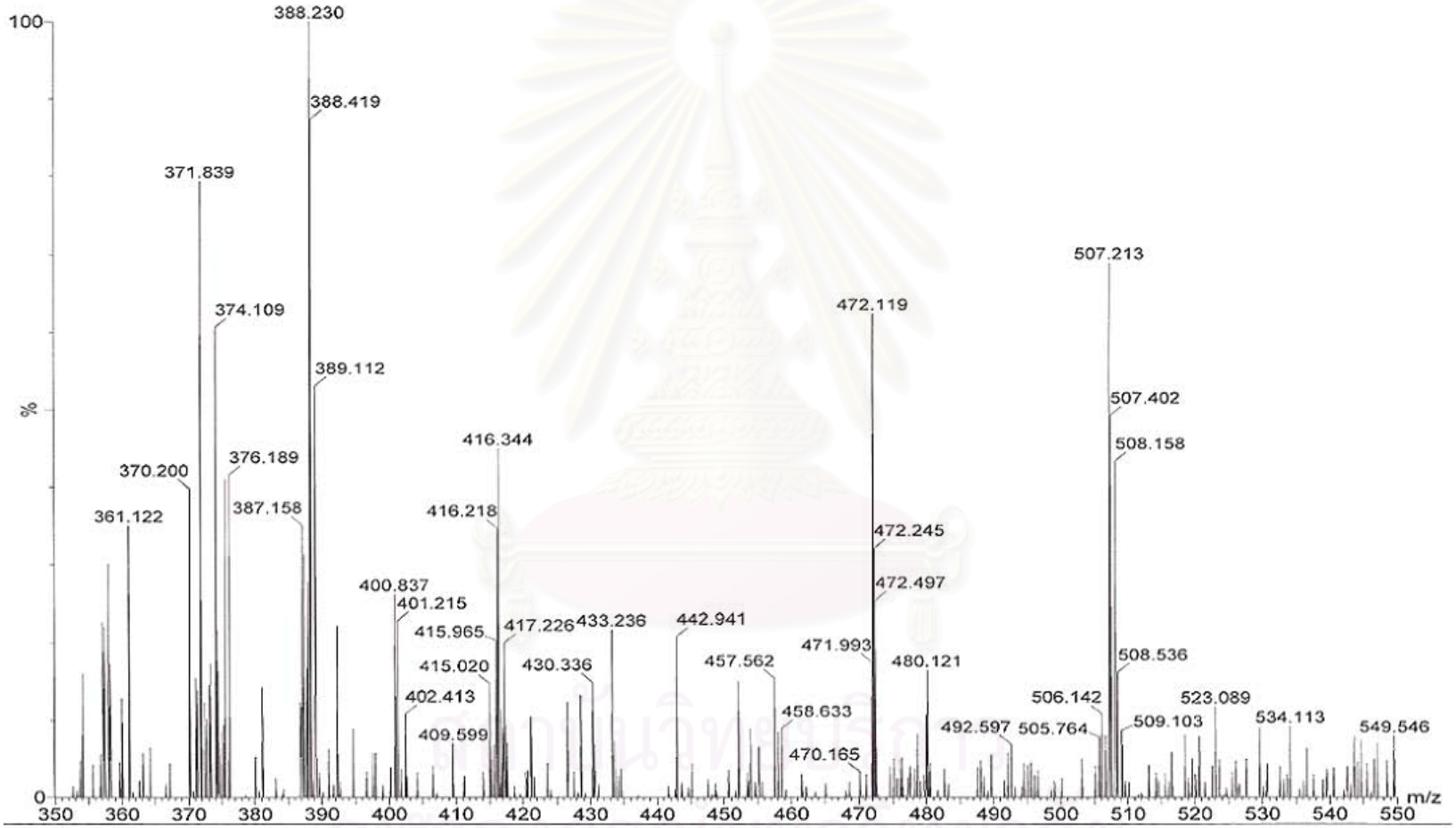

Figure 18 Low resolution MS of compound 79

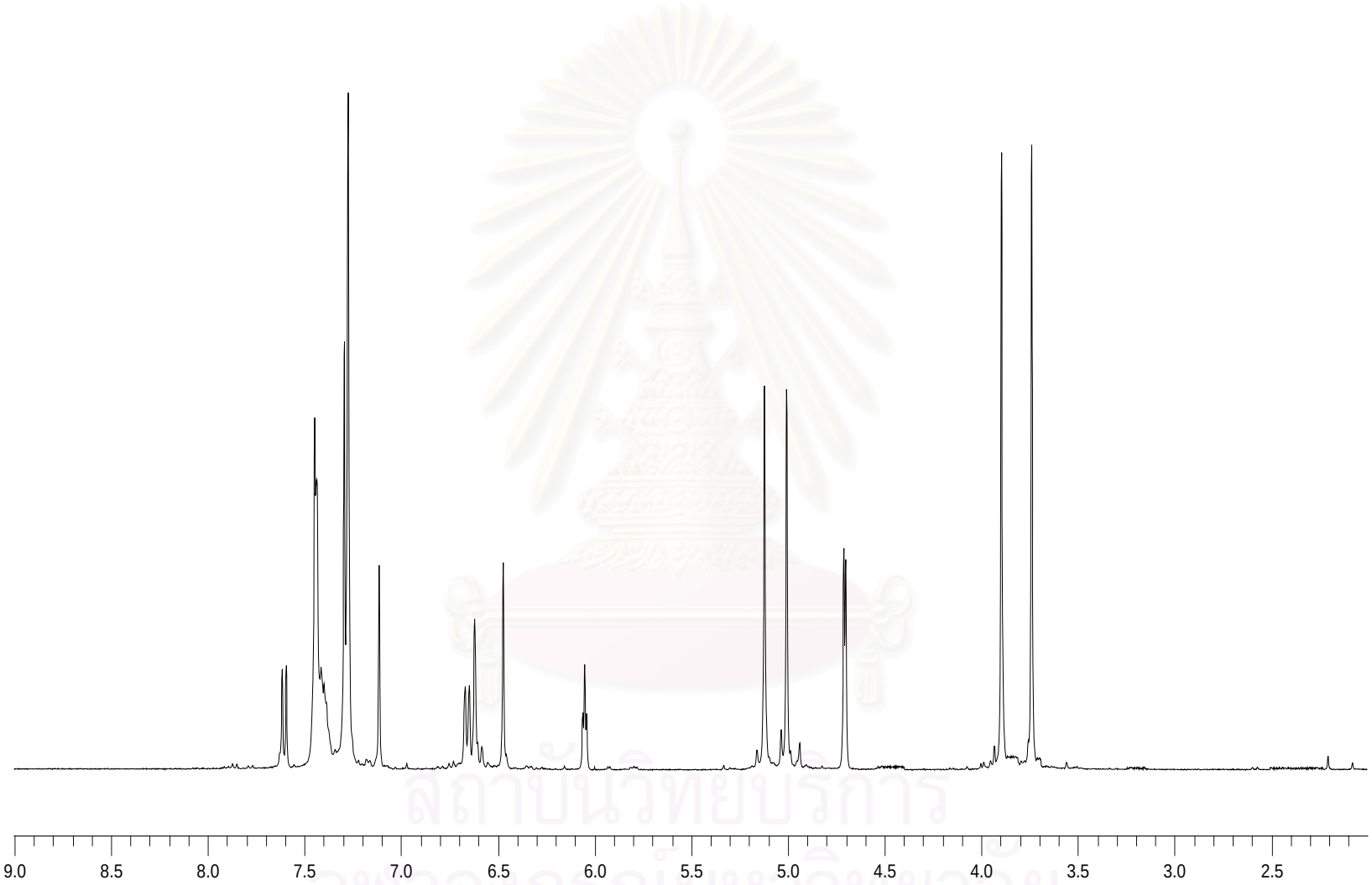

Figure 19 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 80

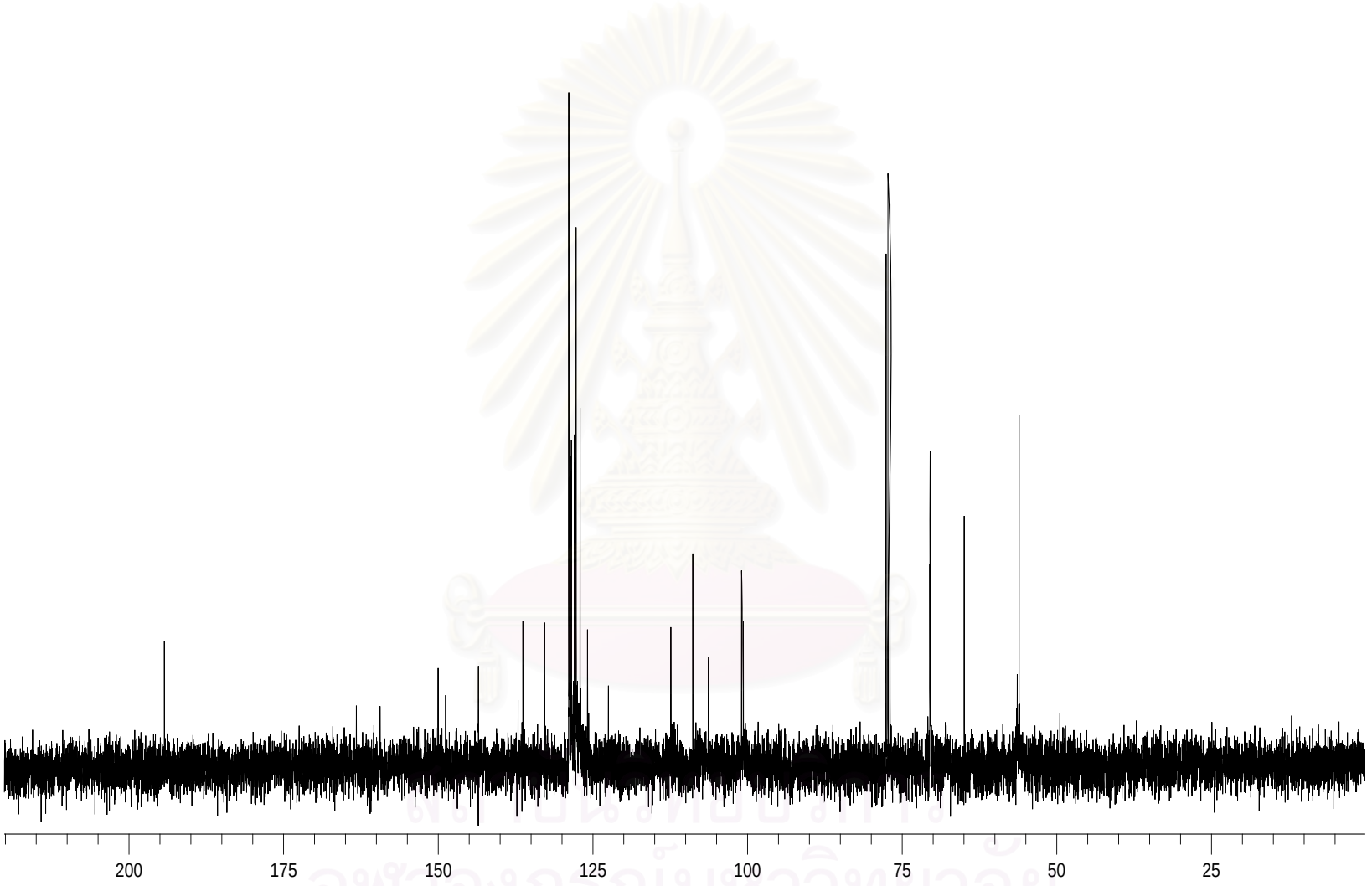

Figure 20 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 80

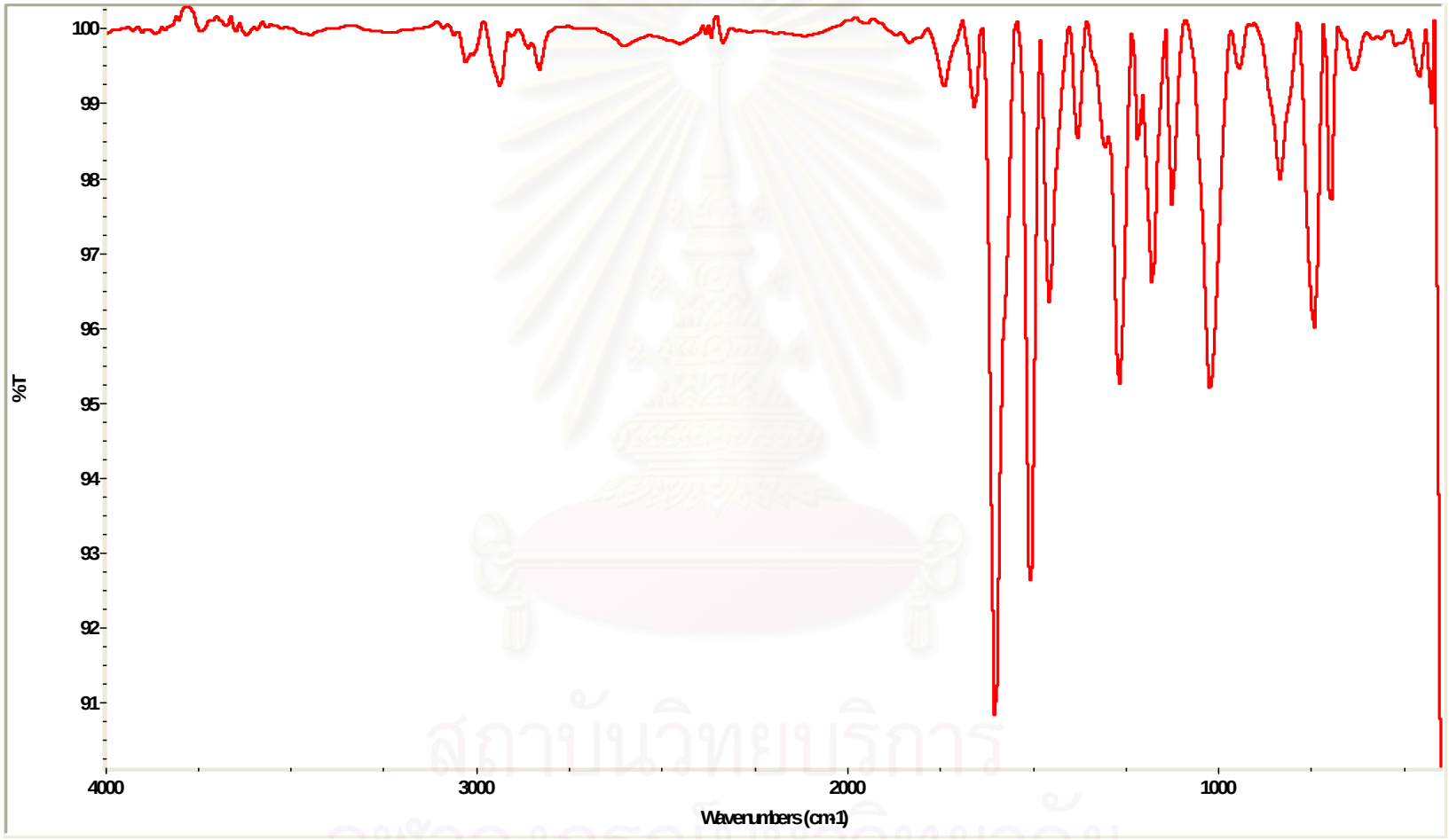

Figure 21 FT-IR of compound 80

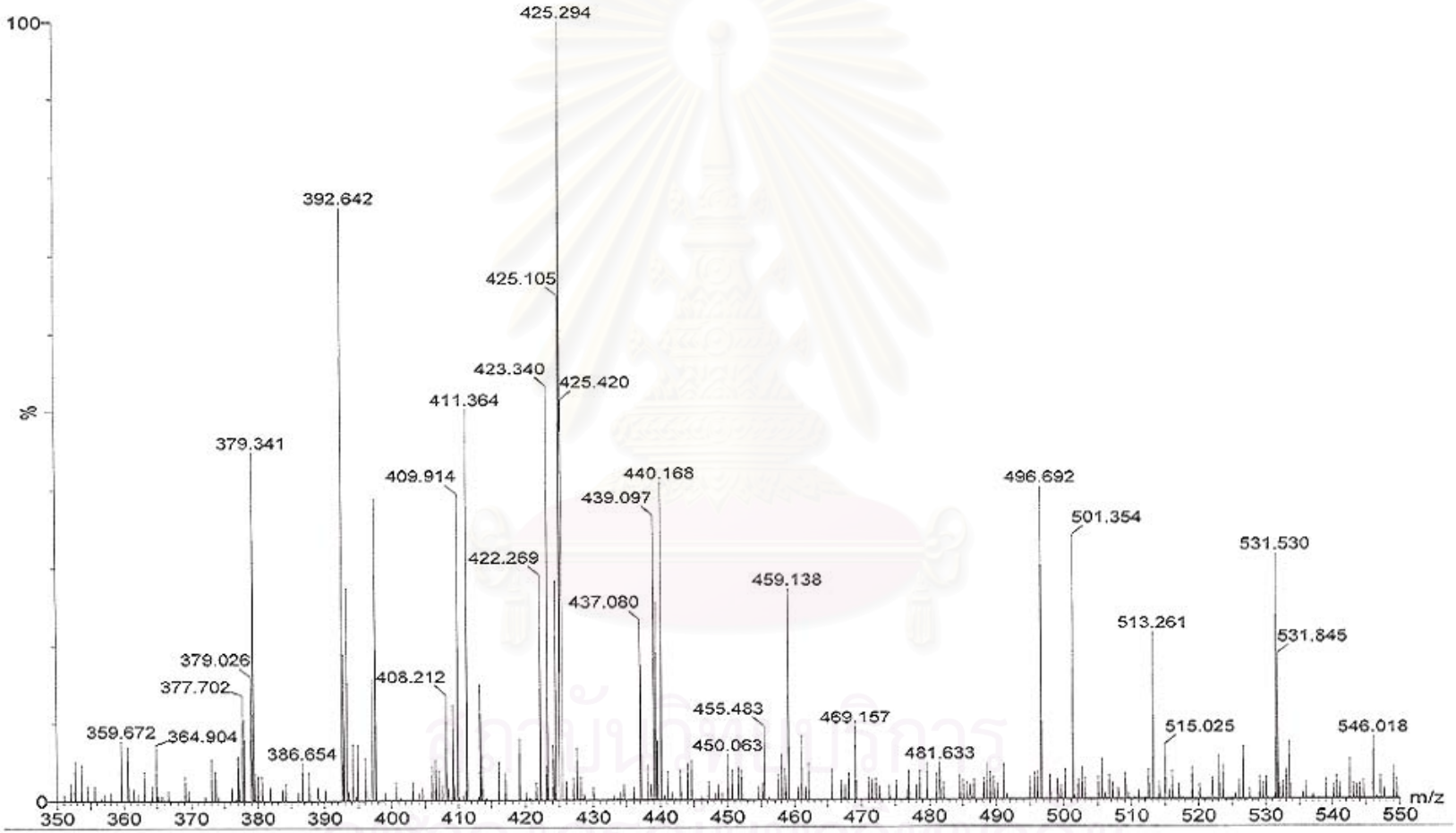

Figure 22 Low resolution MS of compound 80

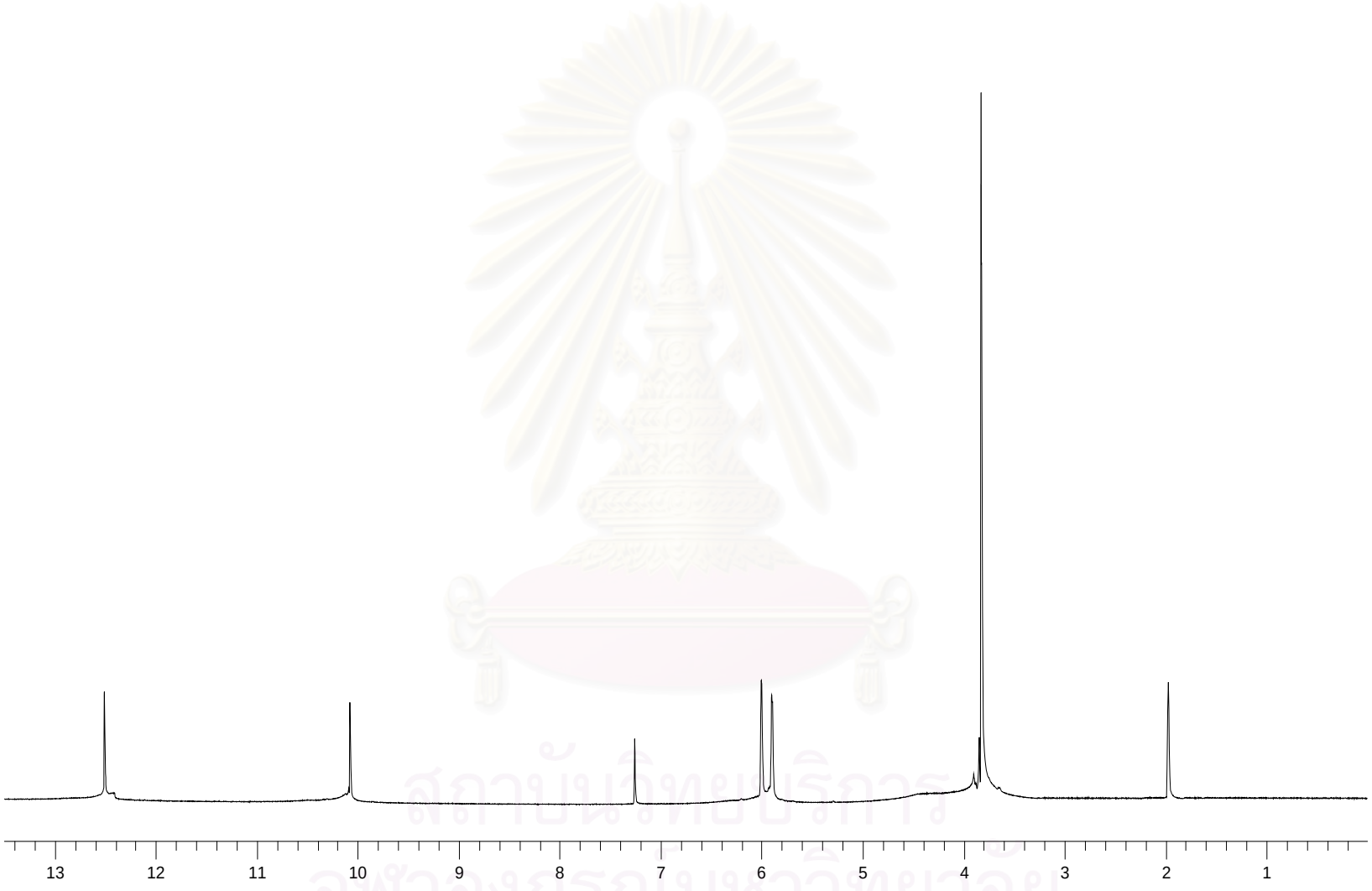

Figure 23 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 89

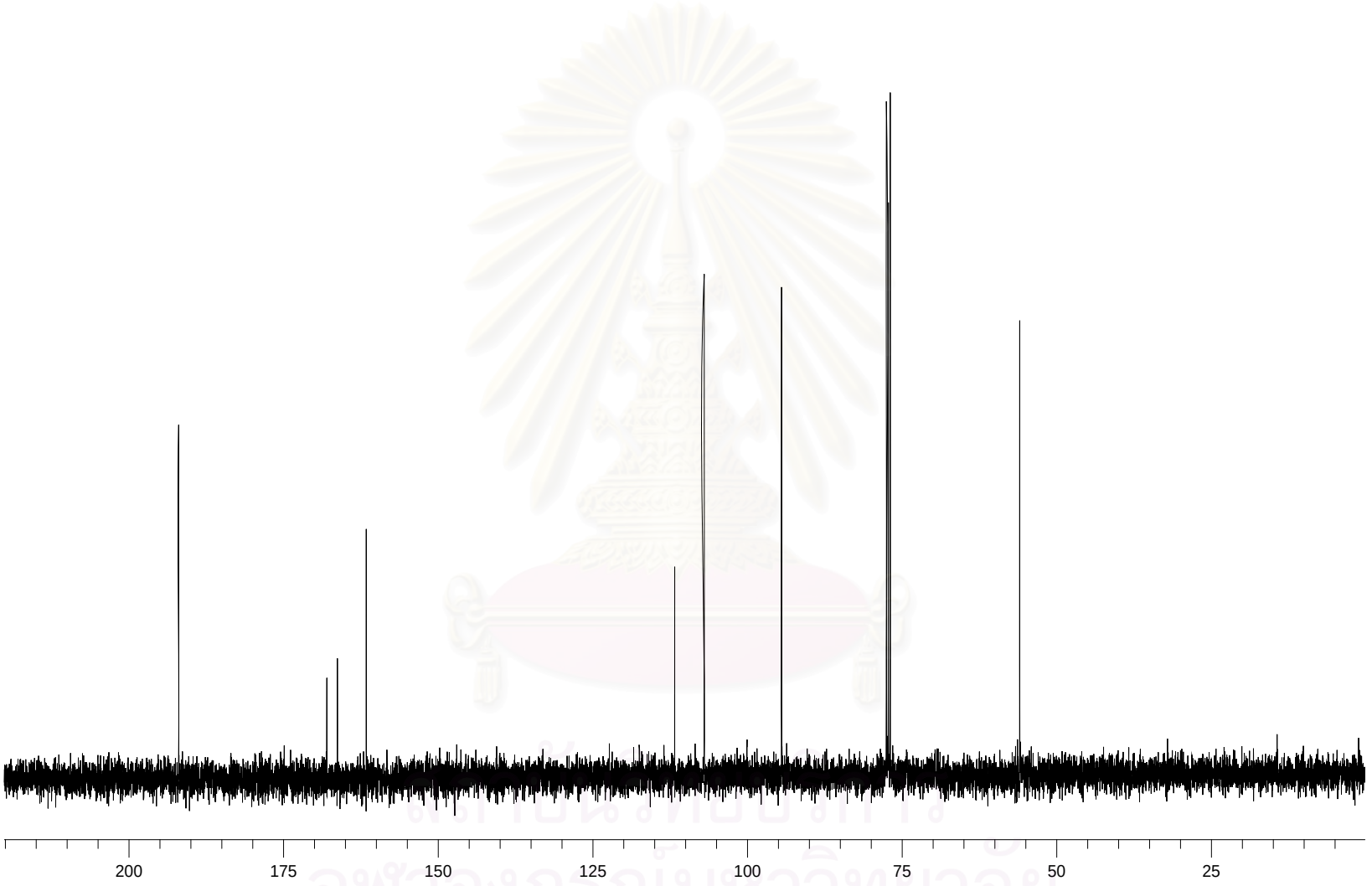

Figure 24 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 89

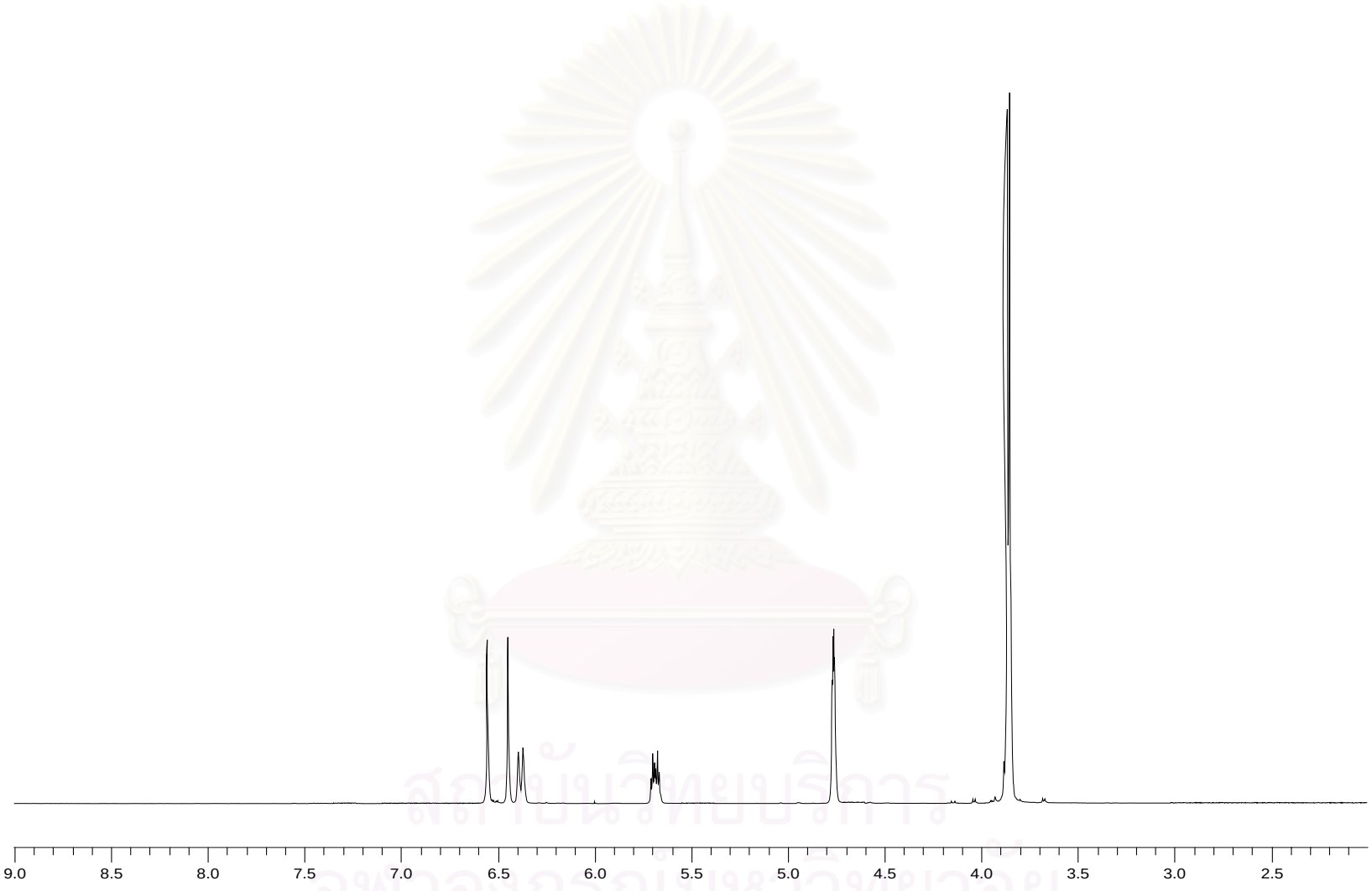

Figure 25 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 83

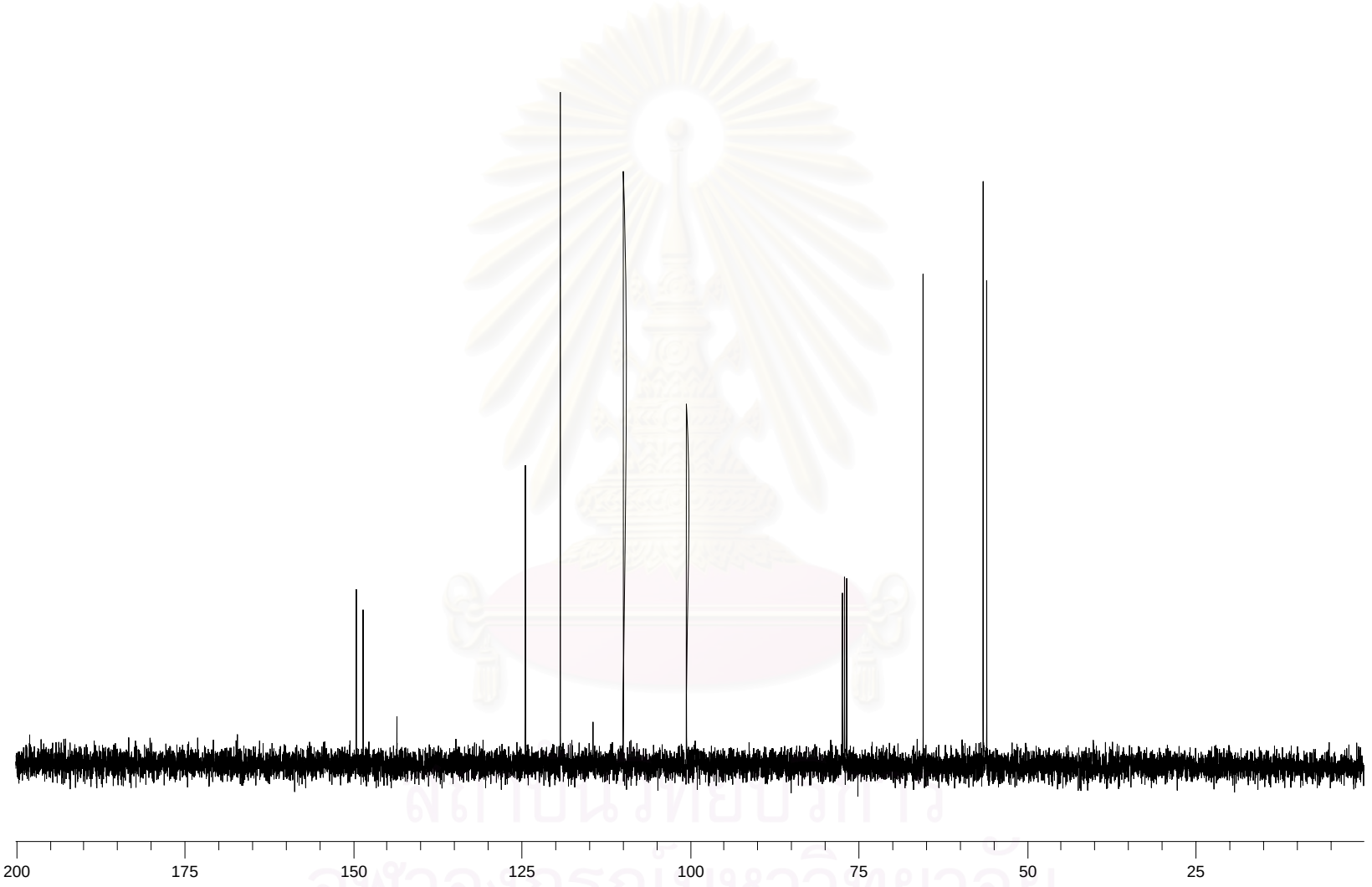

Figure 26 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 83

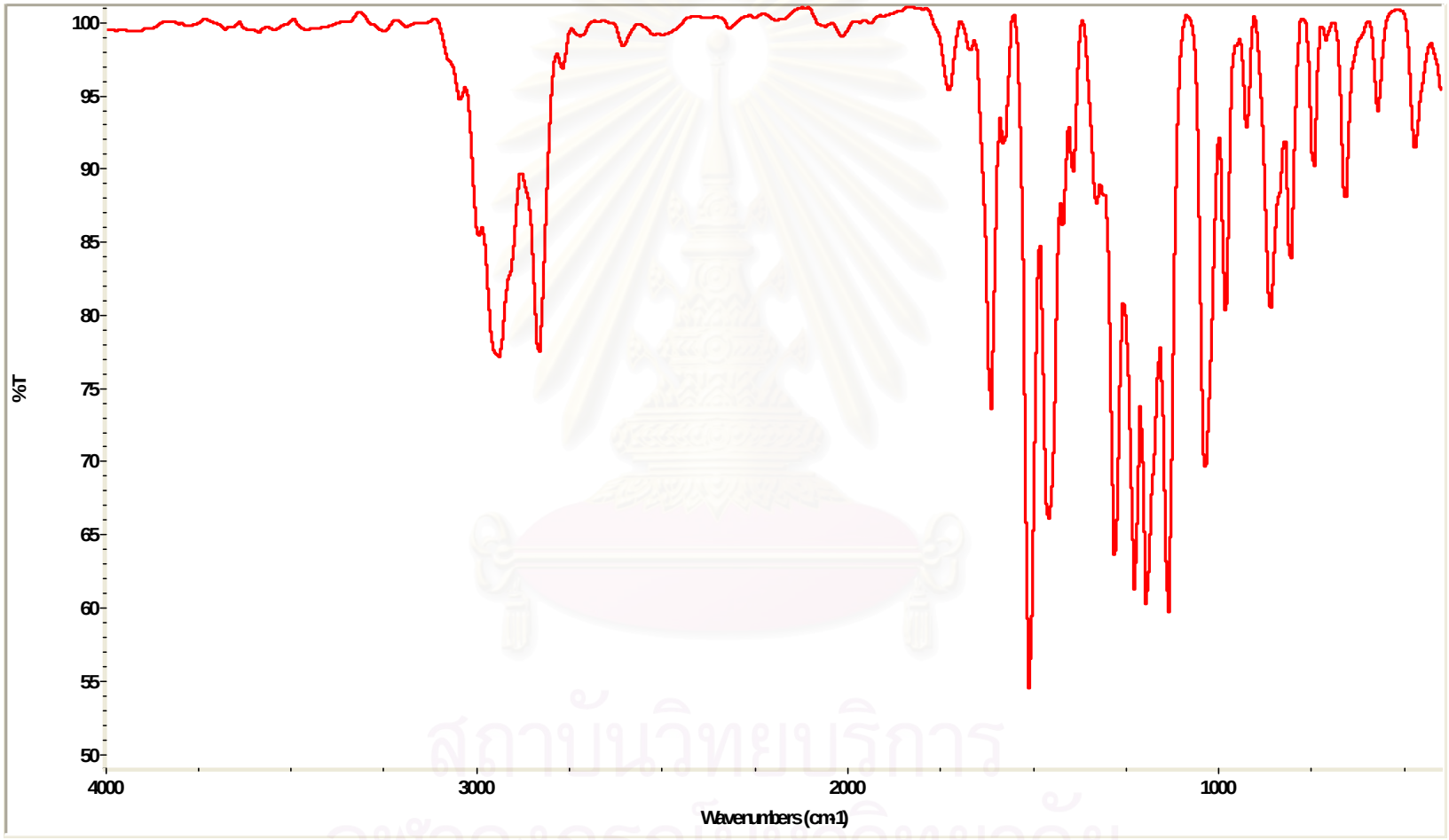

Figure 27 FT-IR of compound 83

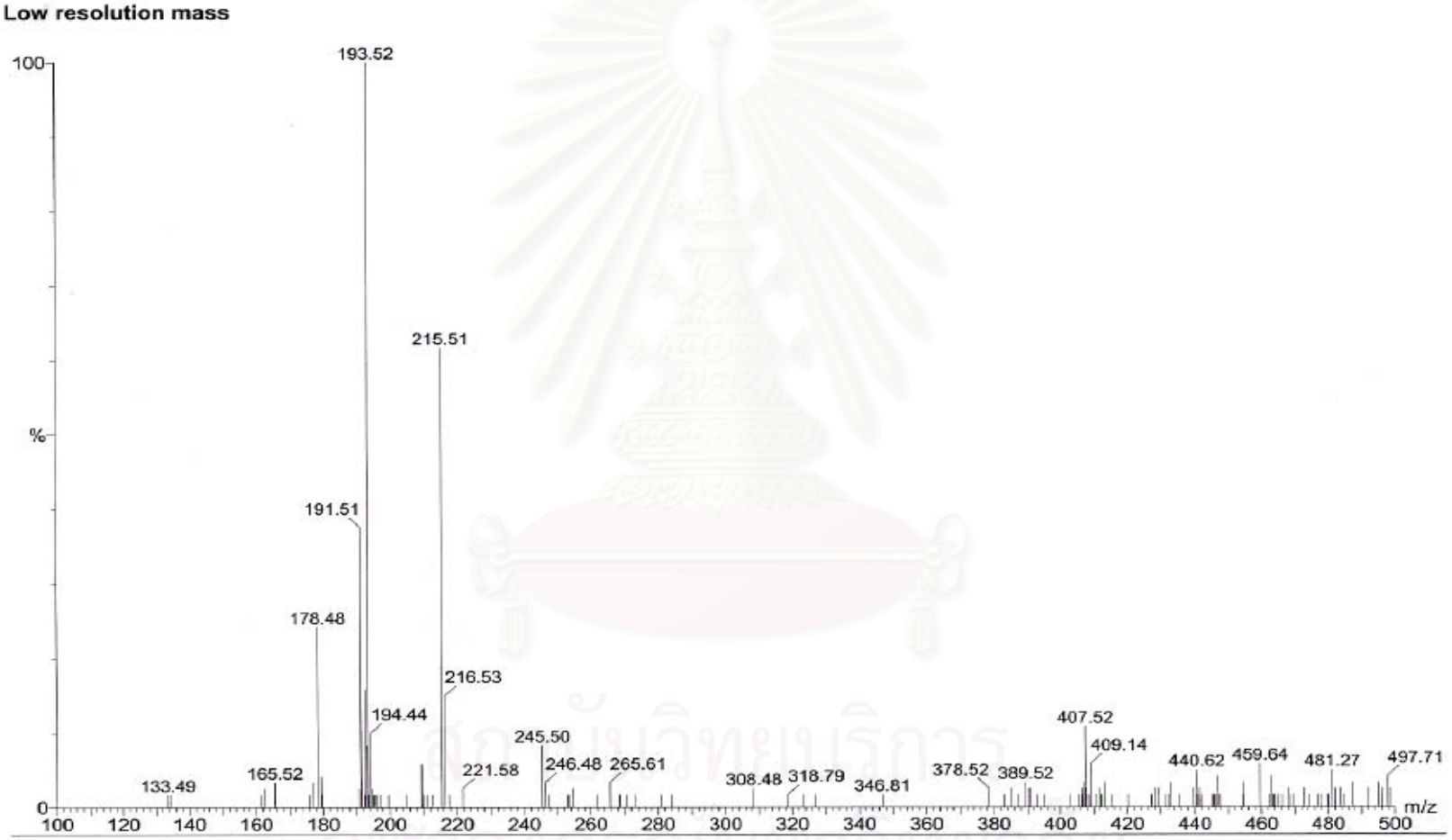

Figure 28 Low resolution MS of compound 83

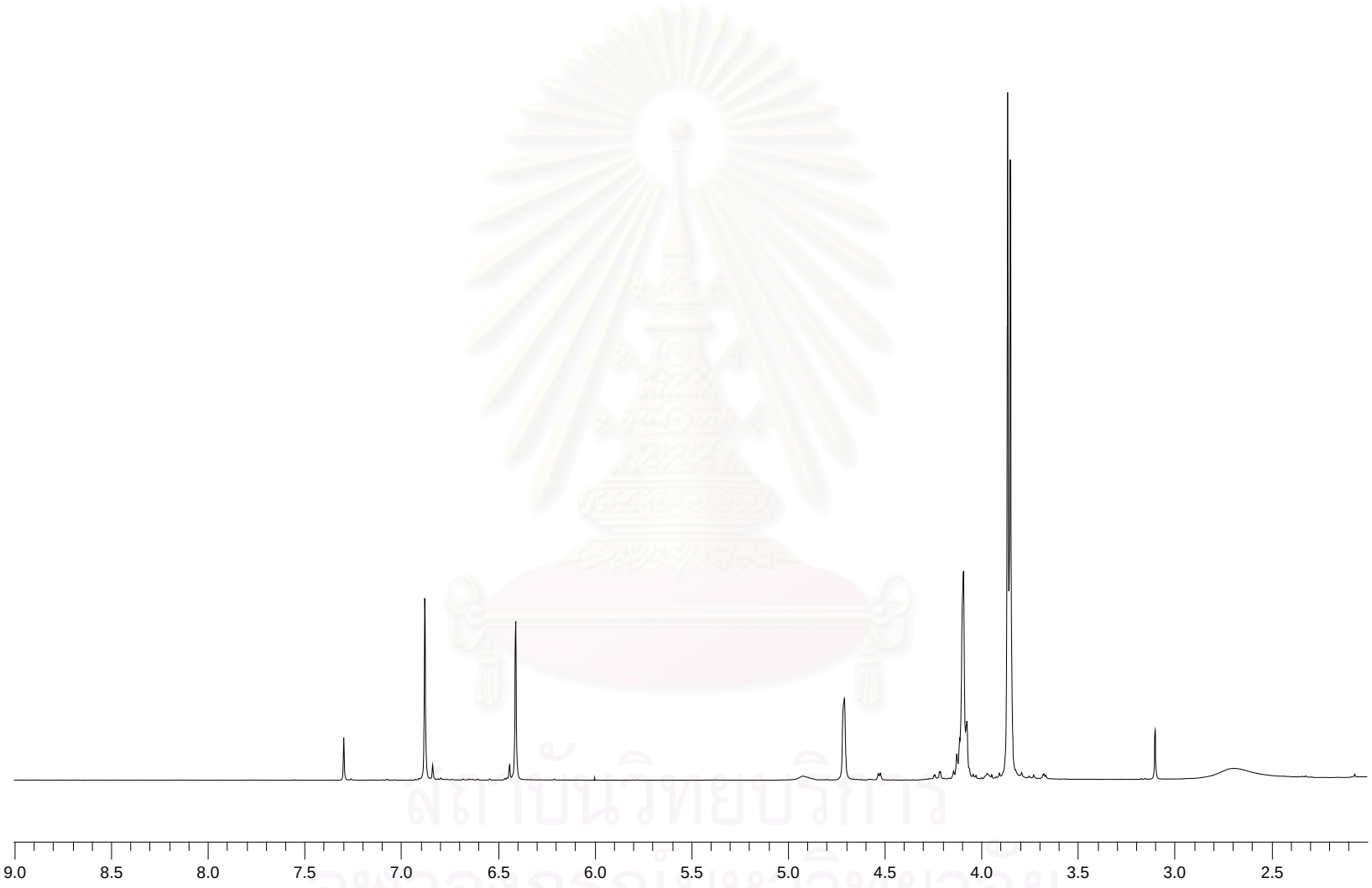

Figure 29 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 84

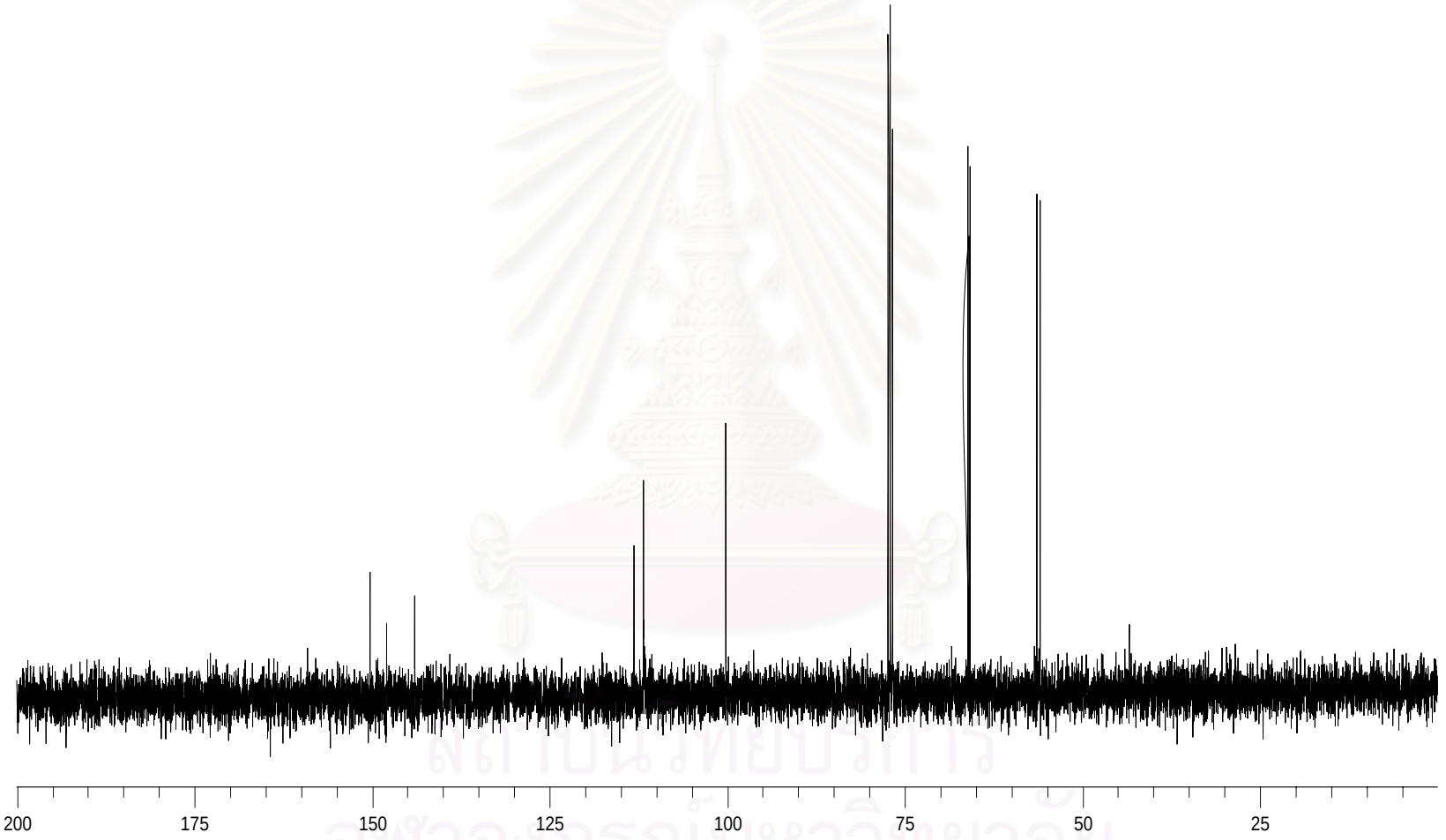

Figure 30 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 84

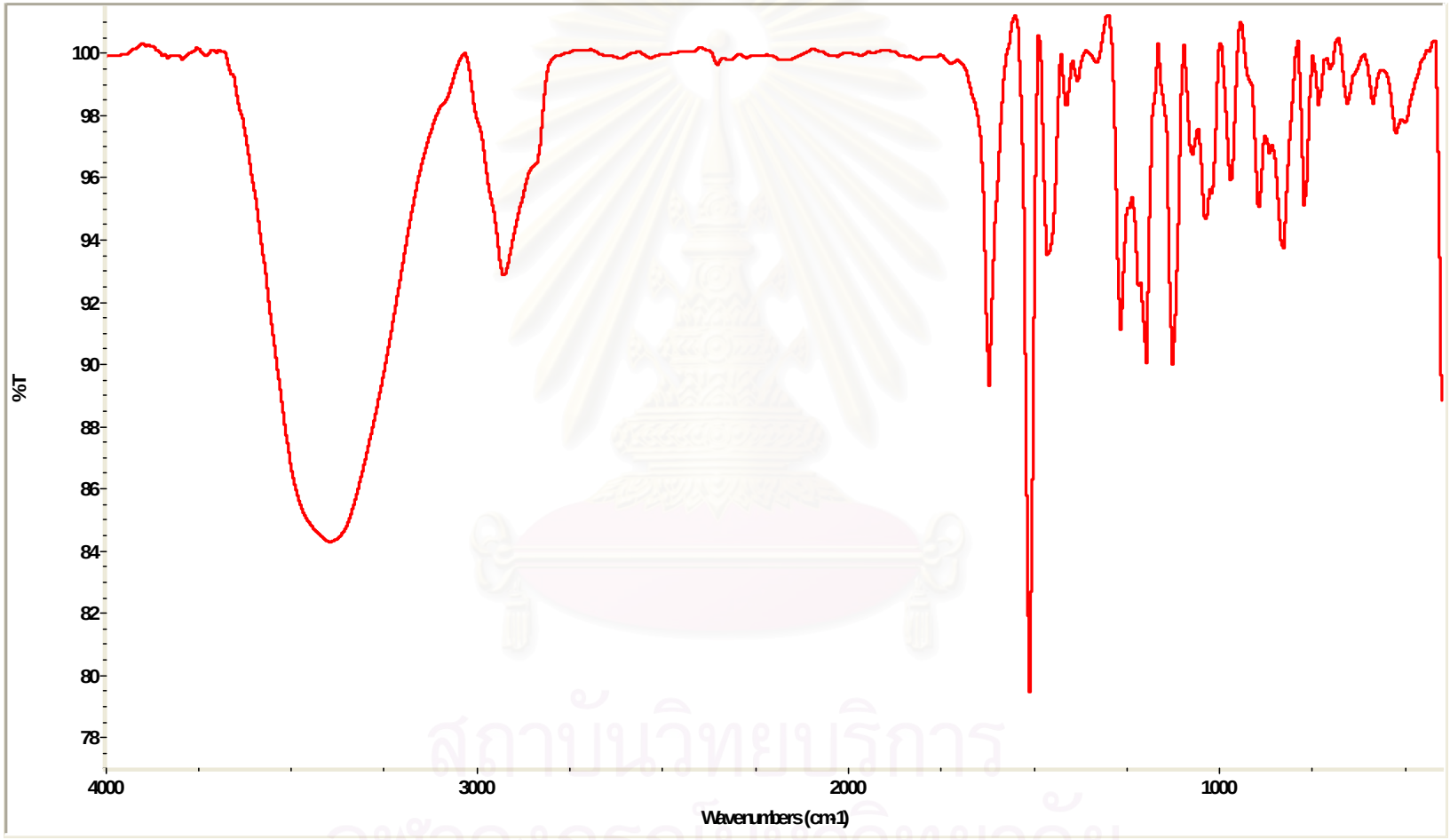

Figure 31 FT-IR of compound 84

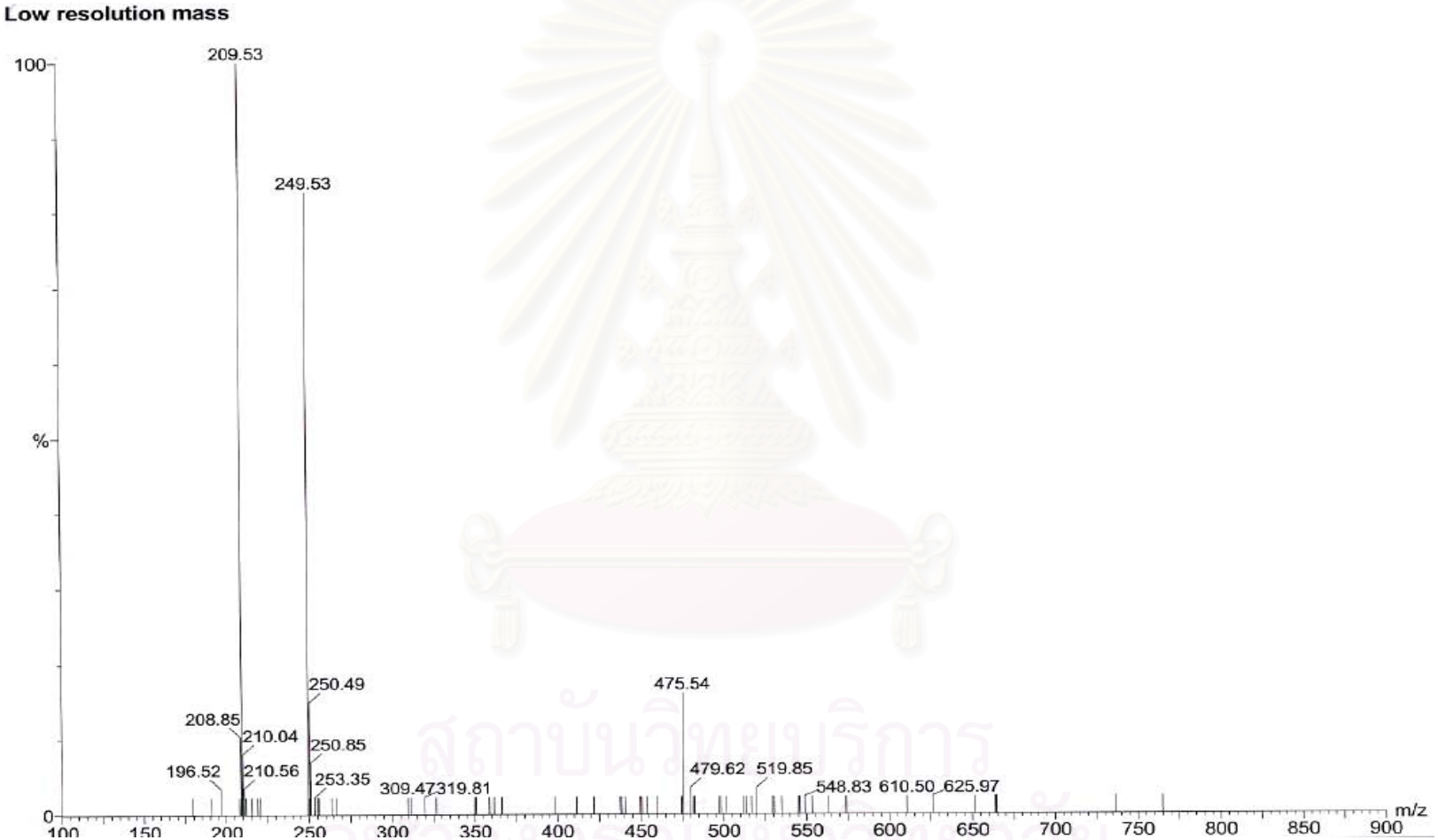

Figure 32 Low resolution MS of compound 84

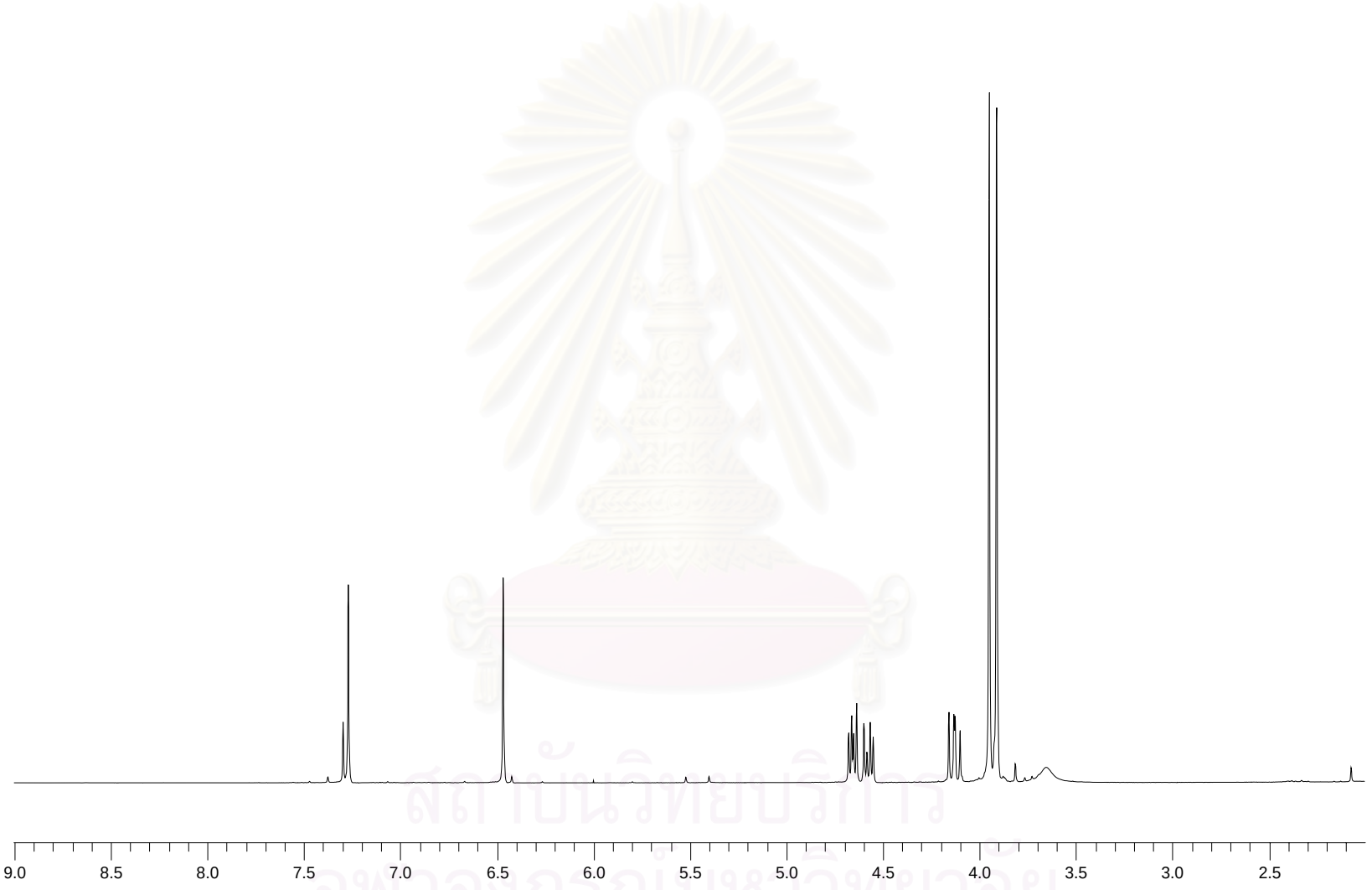

Figure 33 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 82

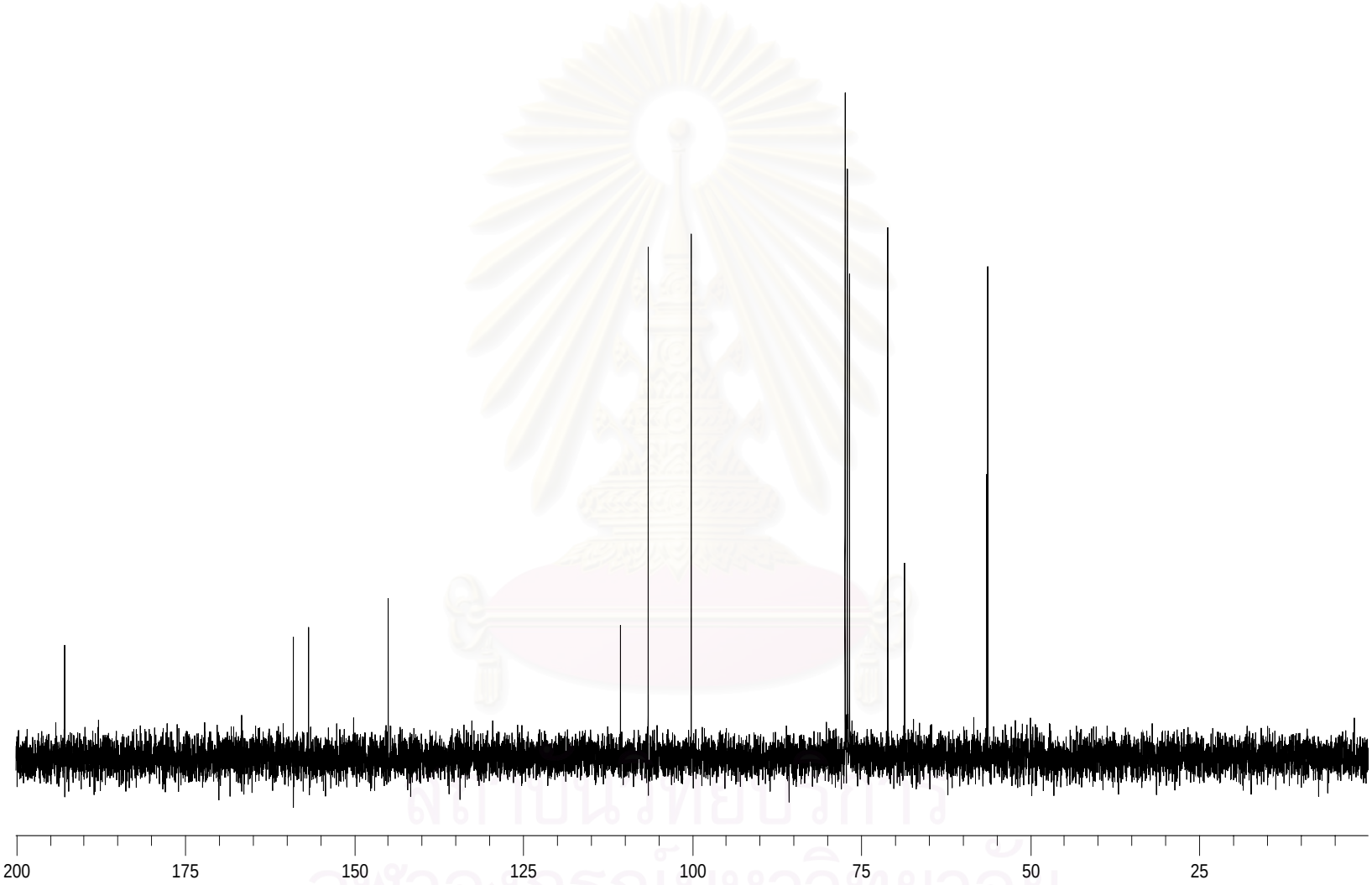

Figure 34 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 82

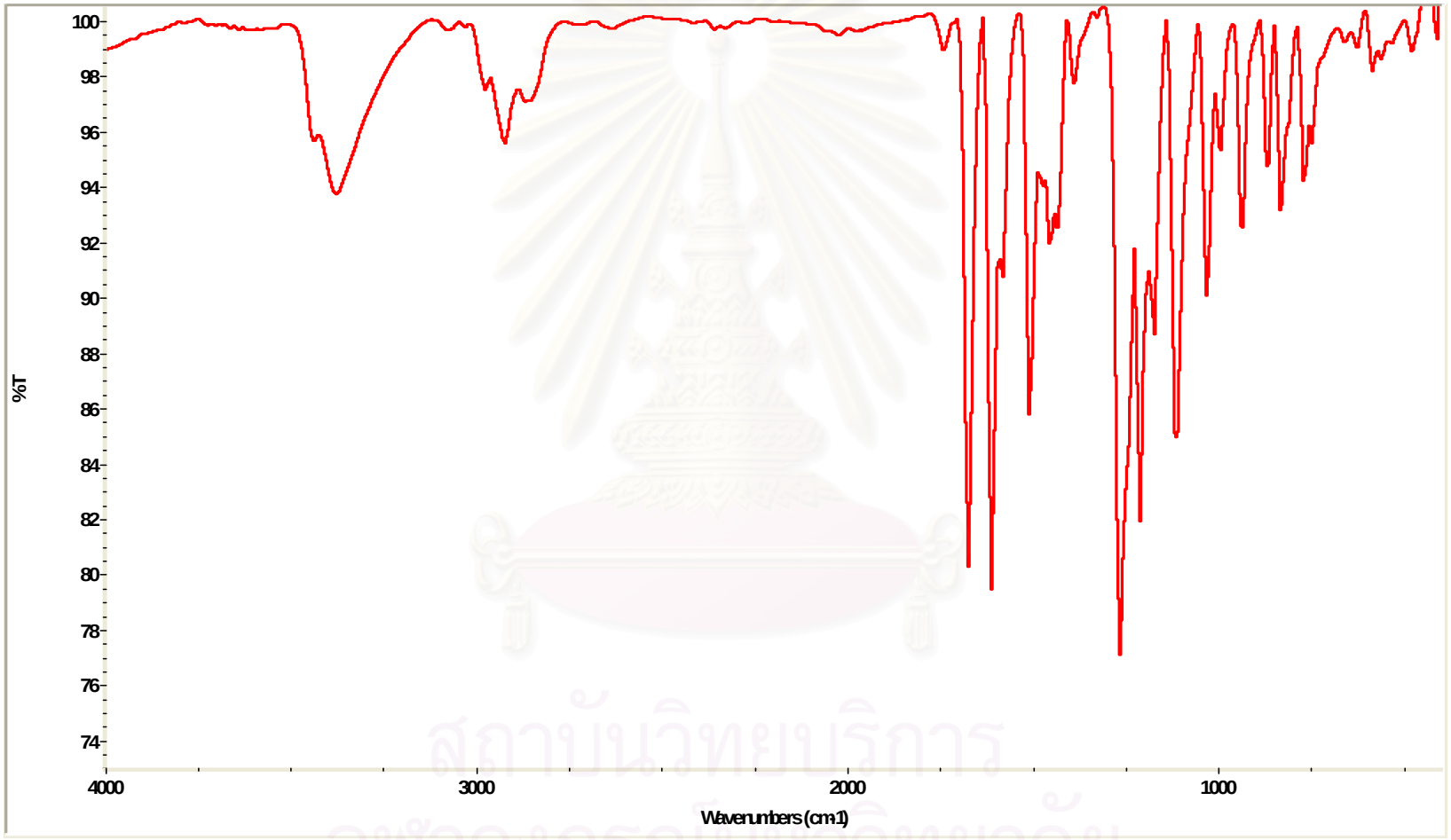

Figure 35 FT-IR of compound 82

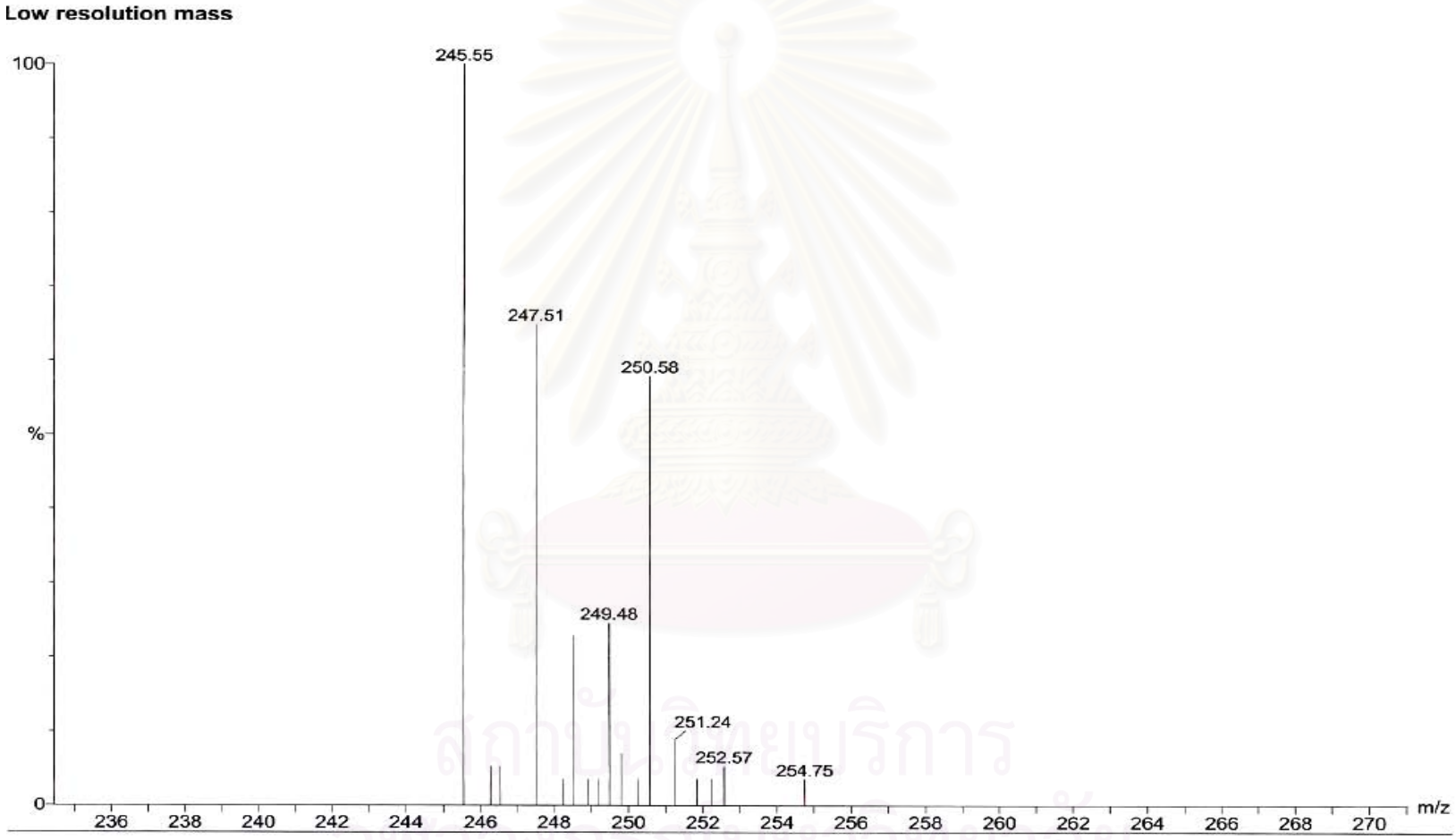

Figure 36 Low resolution MS of compound 82

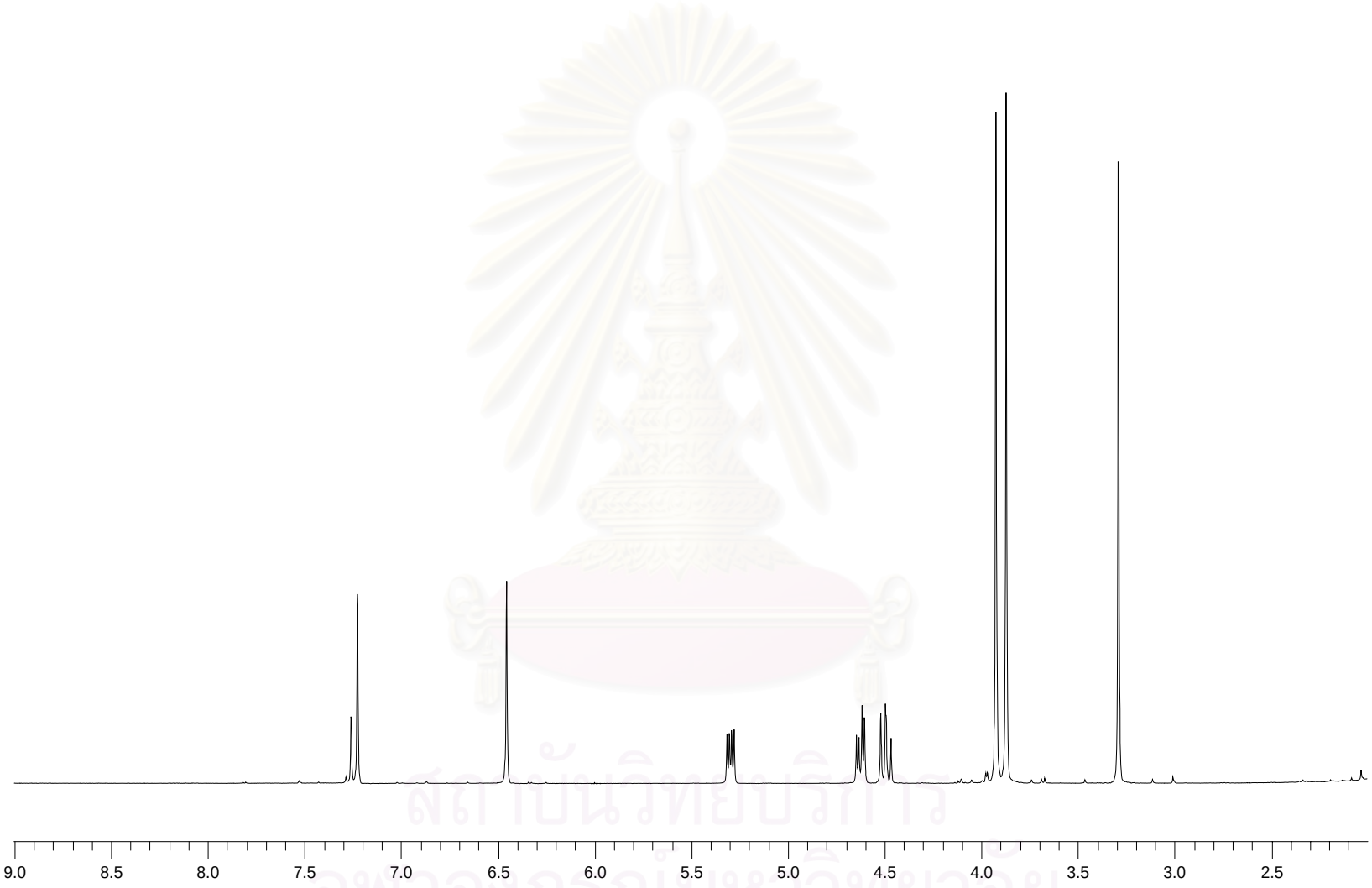

Figure 37 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 85

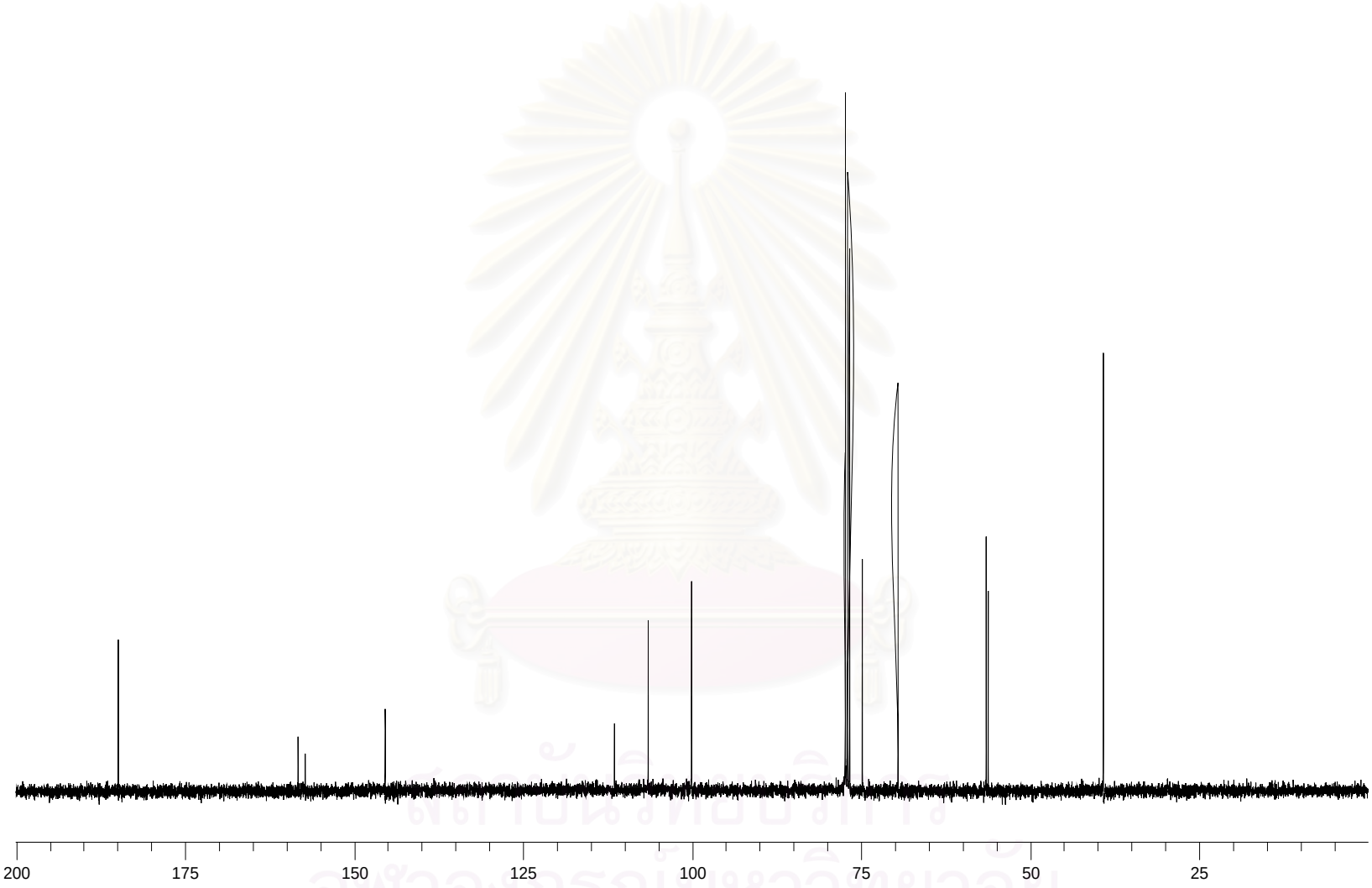

Figure 38 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 85

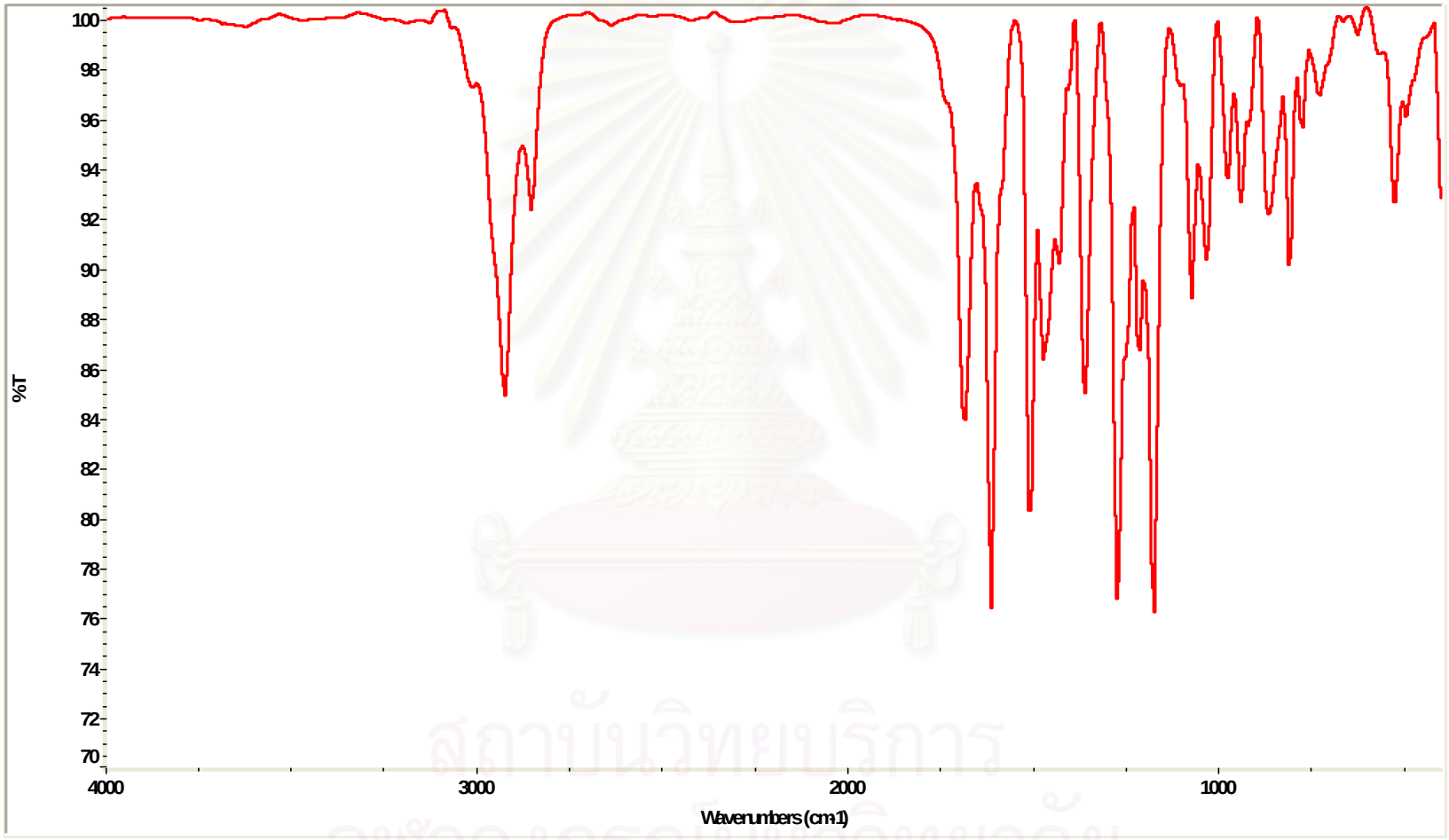

Figure 39 FT-IR of compound 85

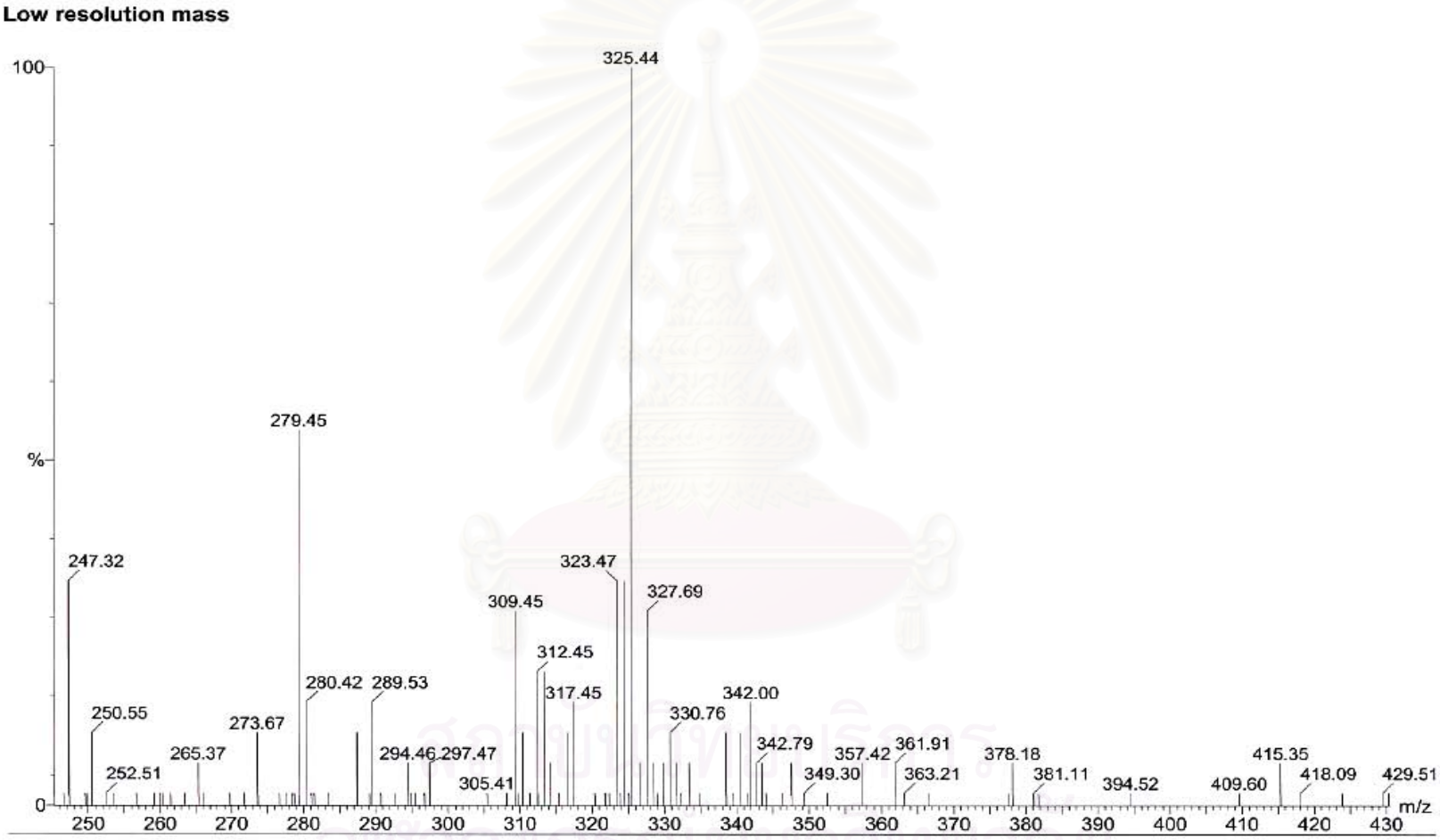

Figure 40 Low resolution MS of compound 85

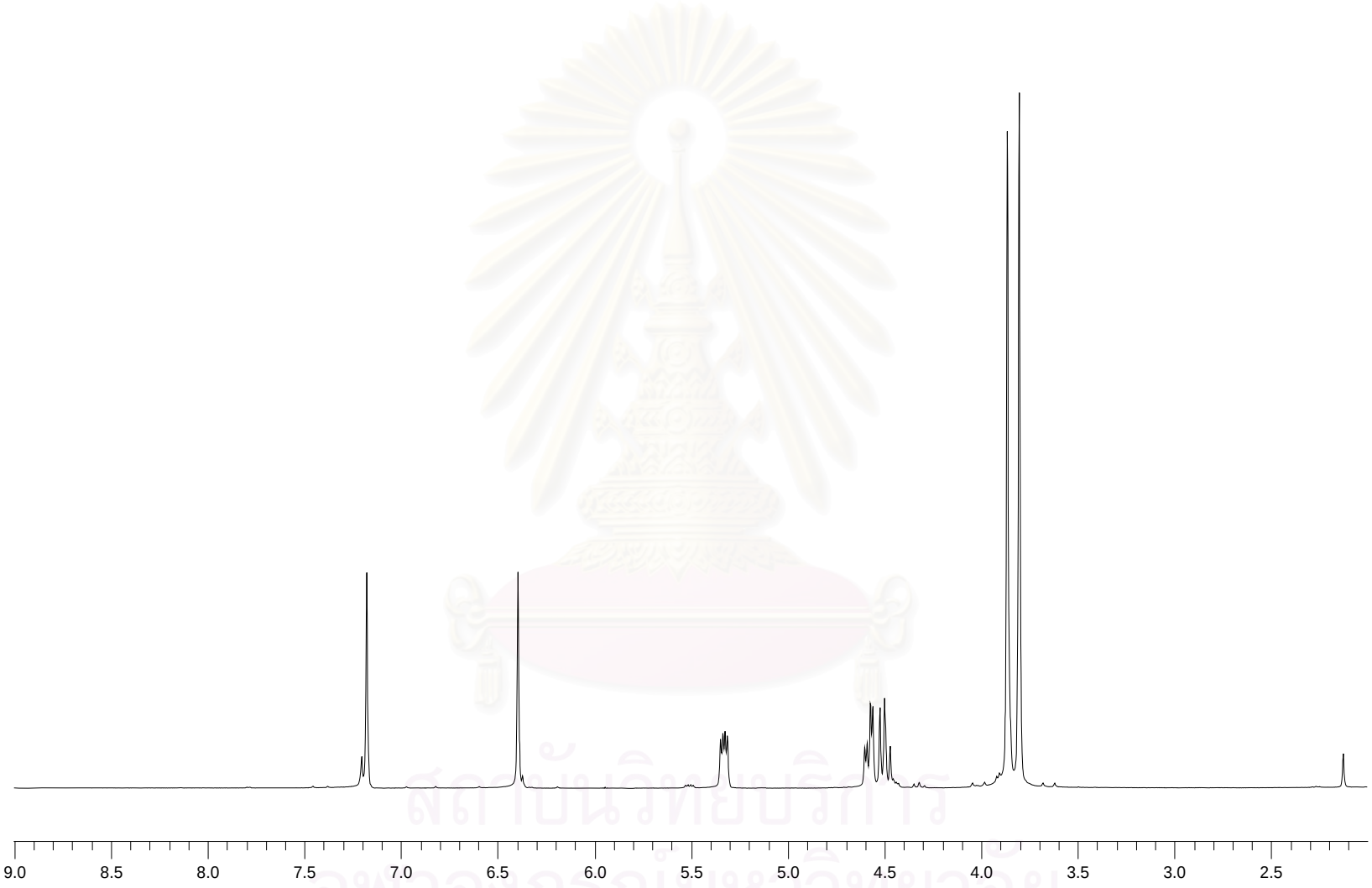

Figure 41 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 86

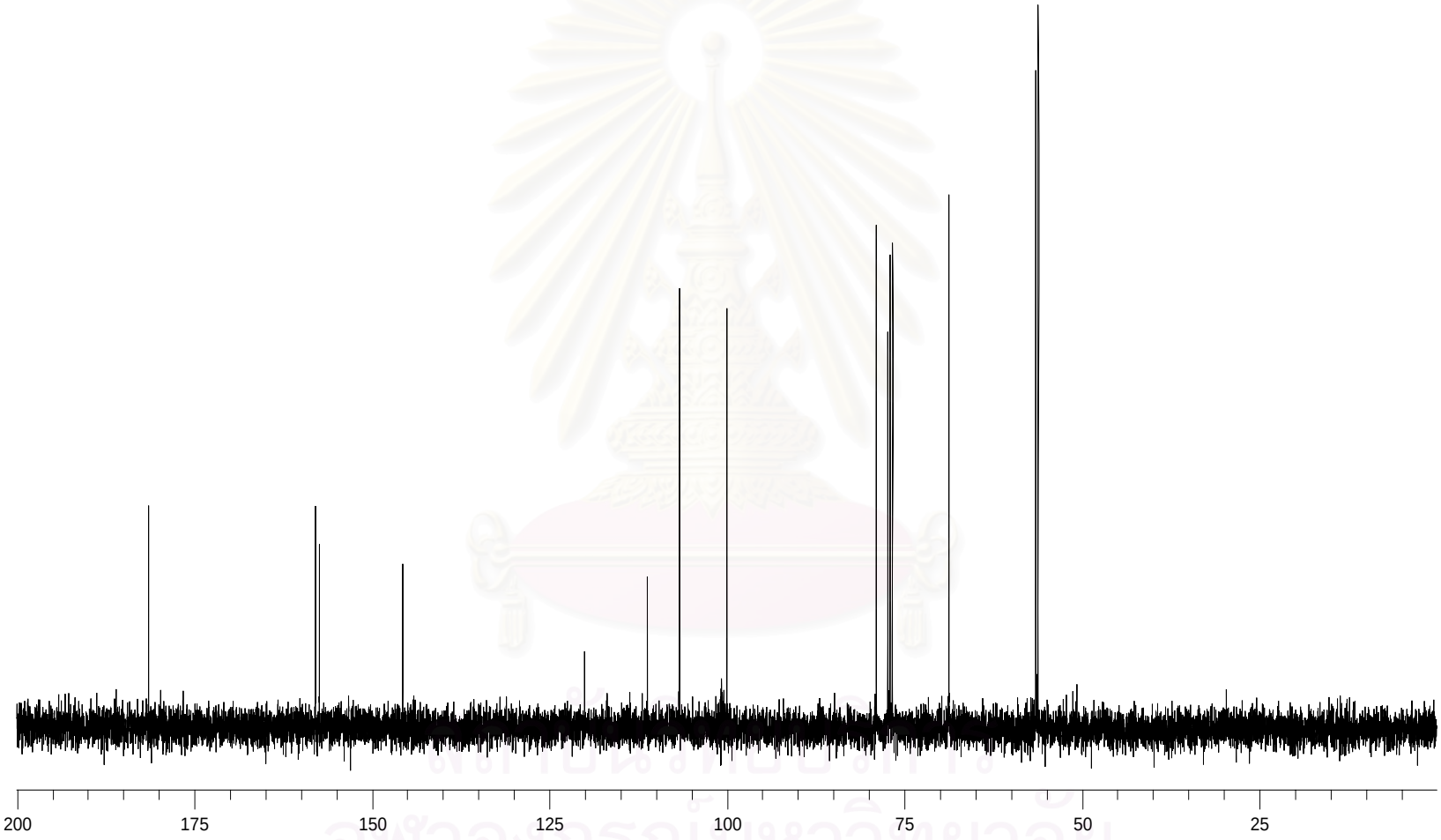

Figure 42 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 86

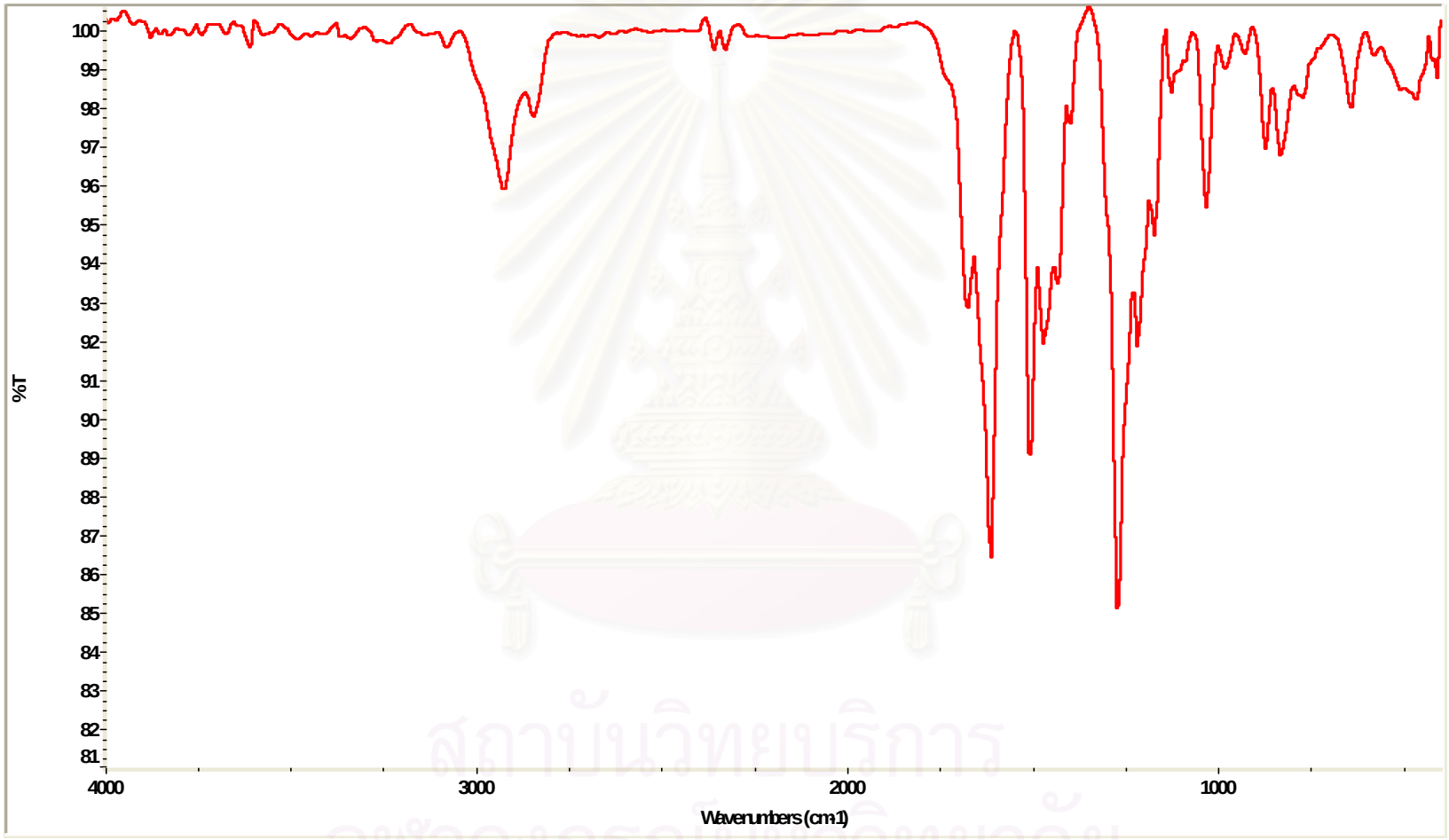

Figure 43 FT-IR of compound 86

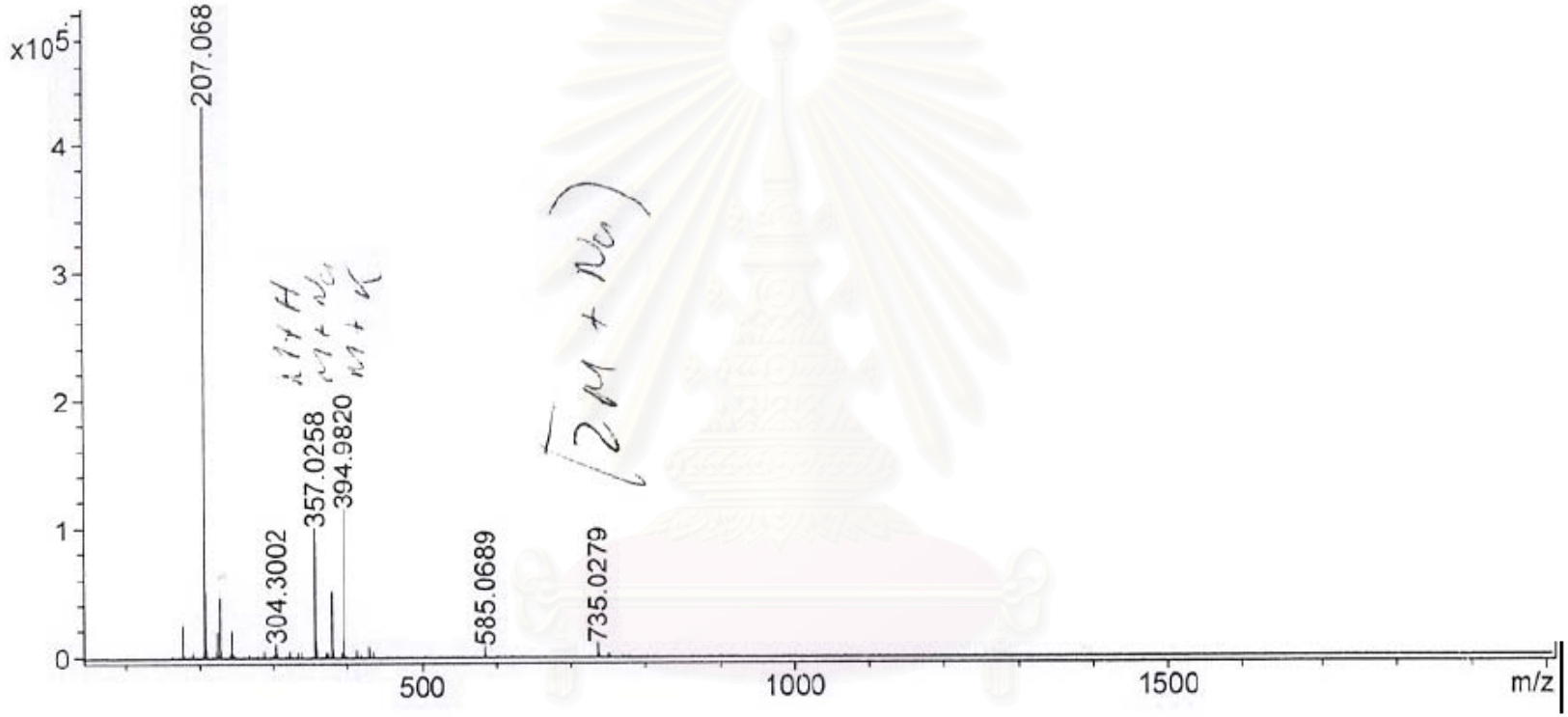

Figure 44 Low resolution MS of compound 86

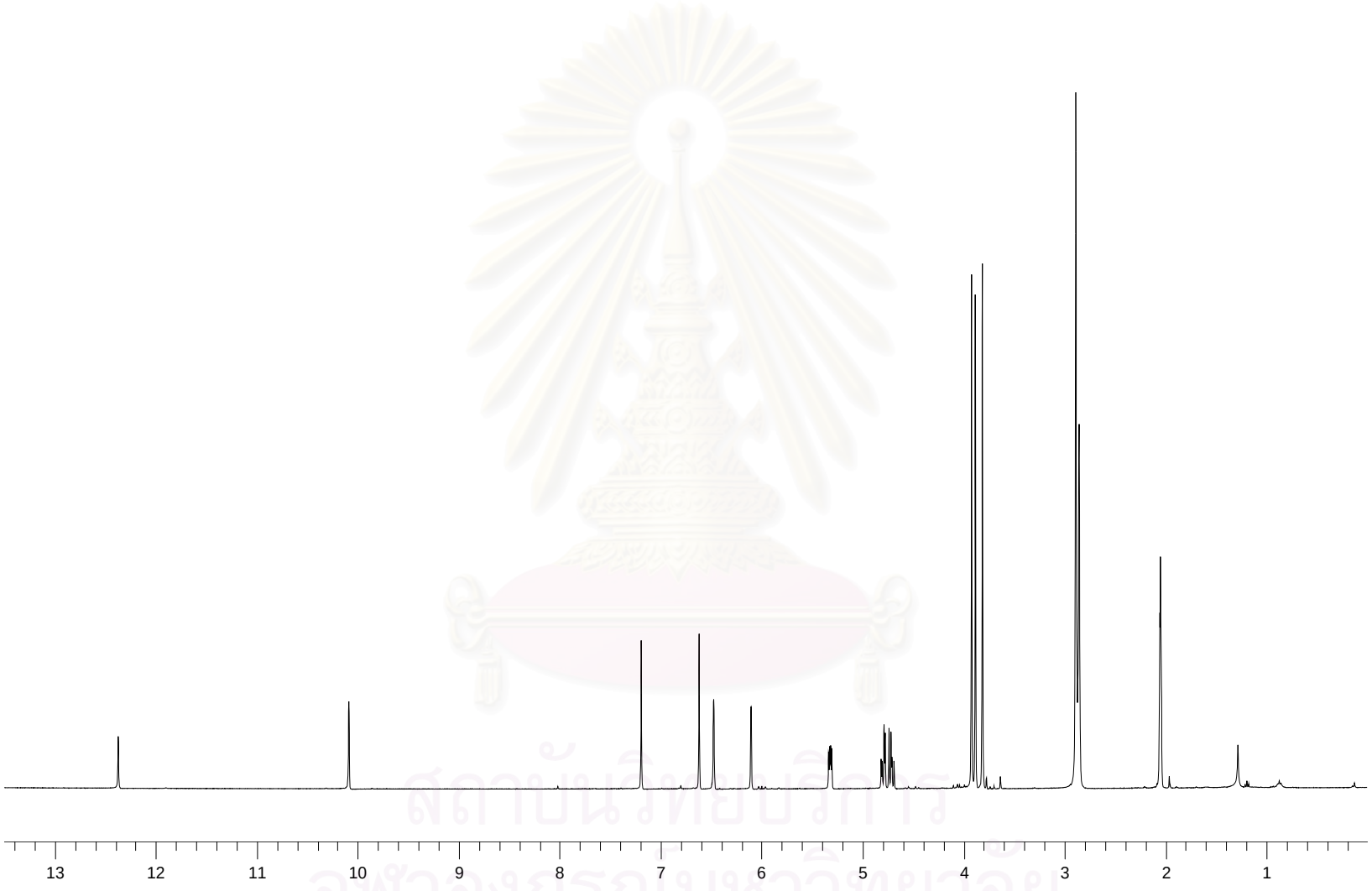

Figure 45 ^{1}H NMR (400 MHz, Acetone-d-6) of compound 87

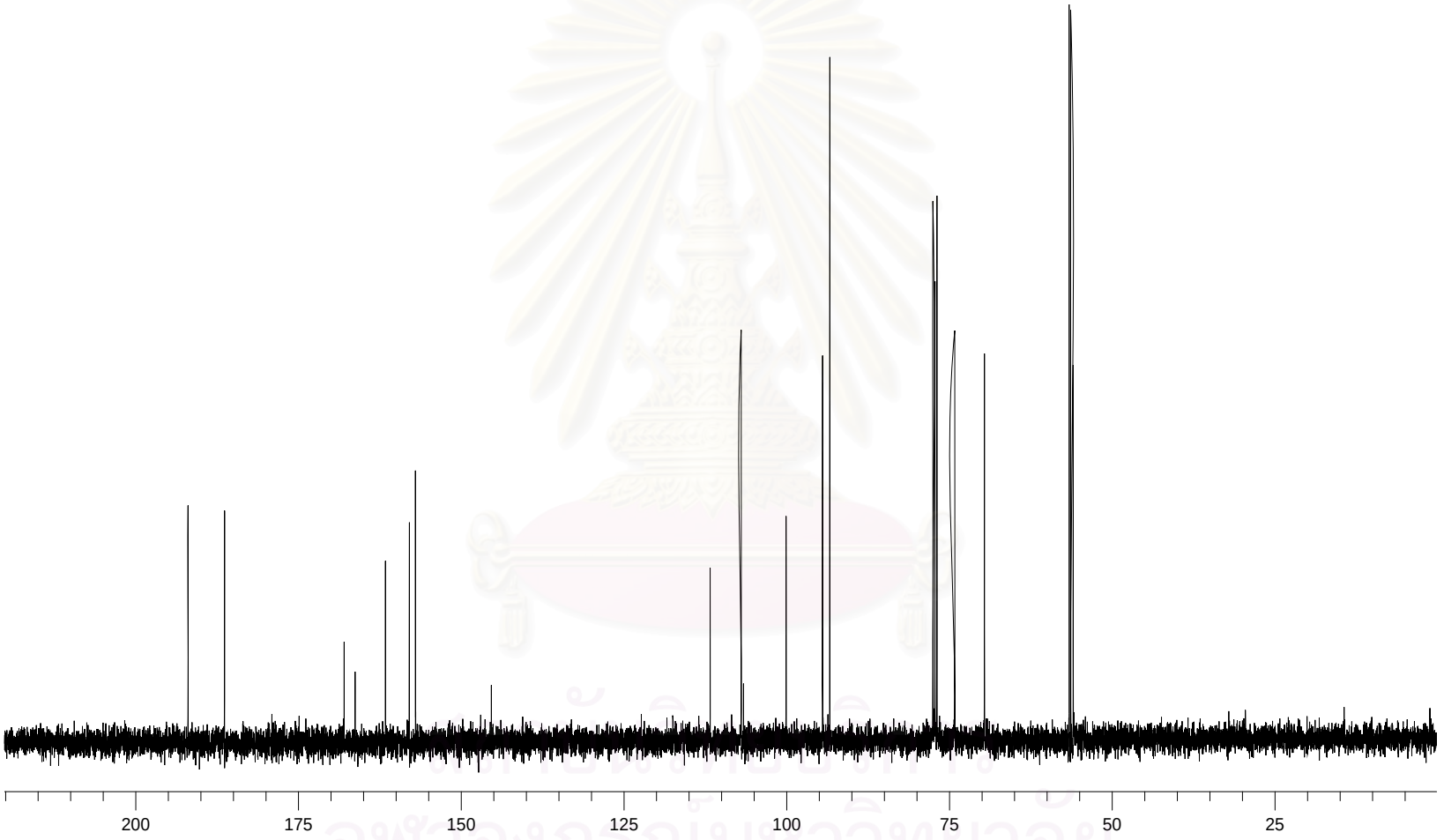

Figure 46 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 87

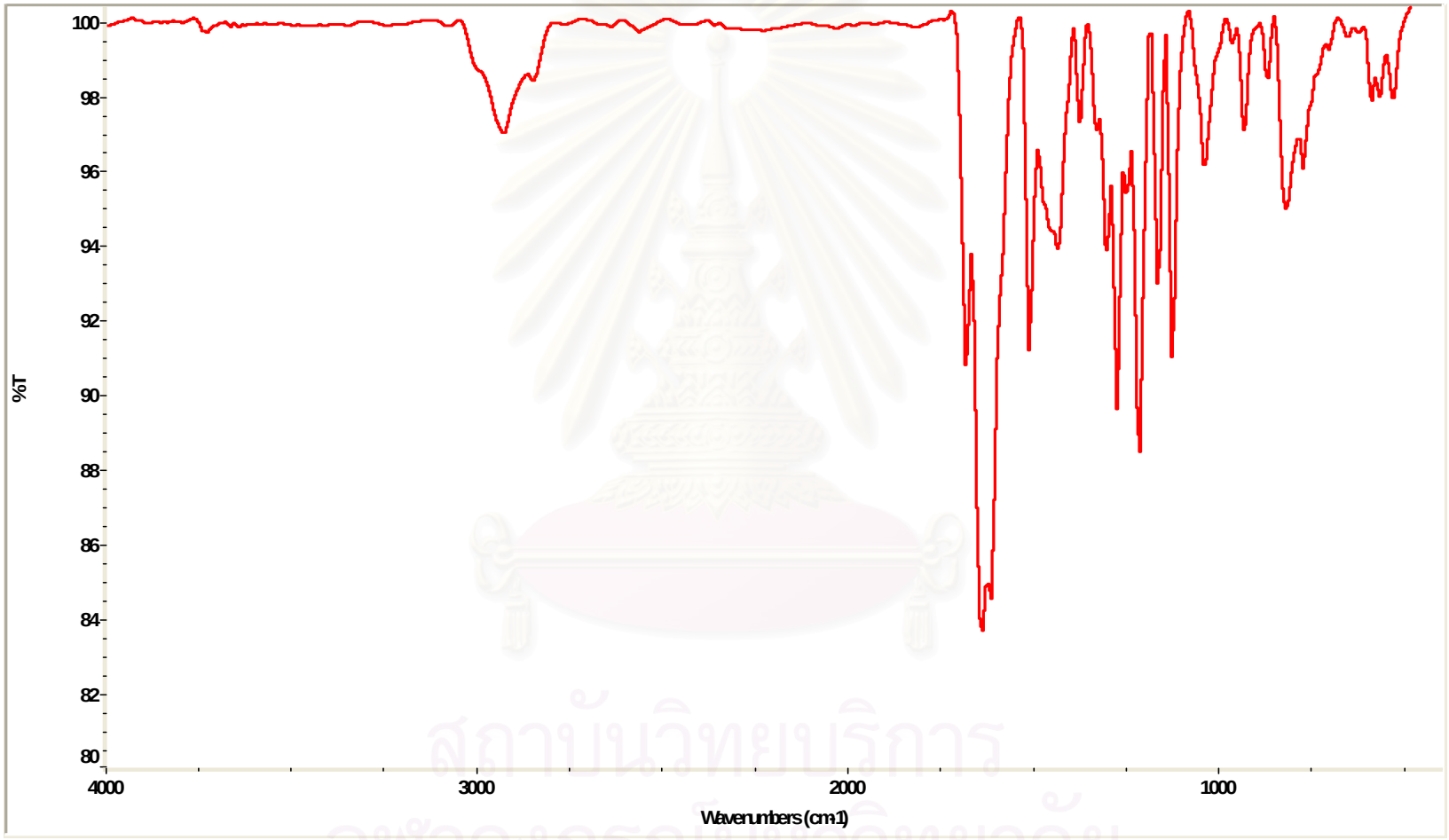

Figure 47 FT-IR of compound 87

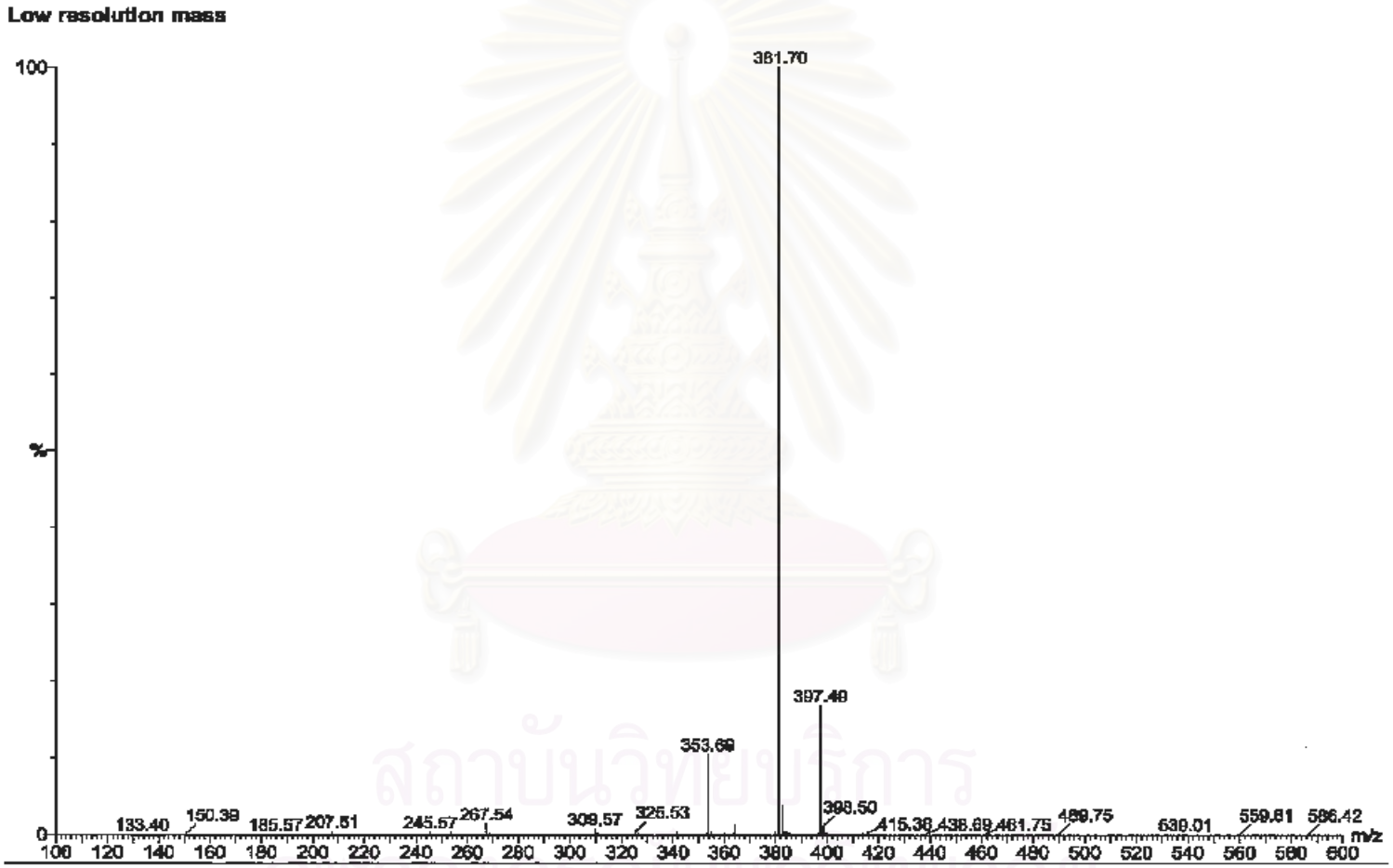

Figure 48 Low resolution MS of compound 87

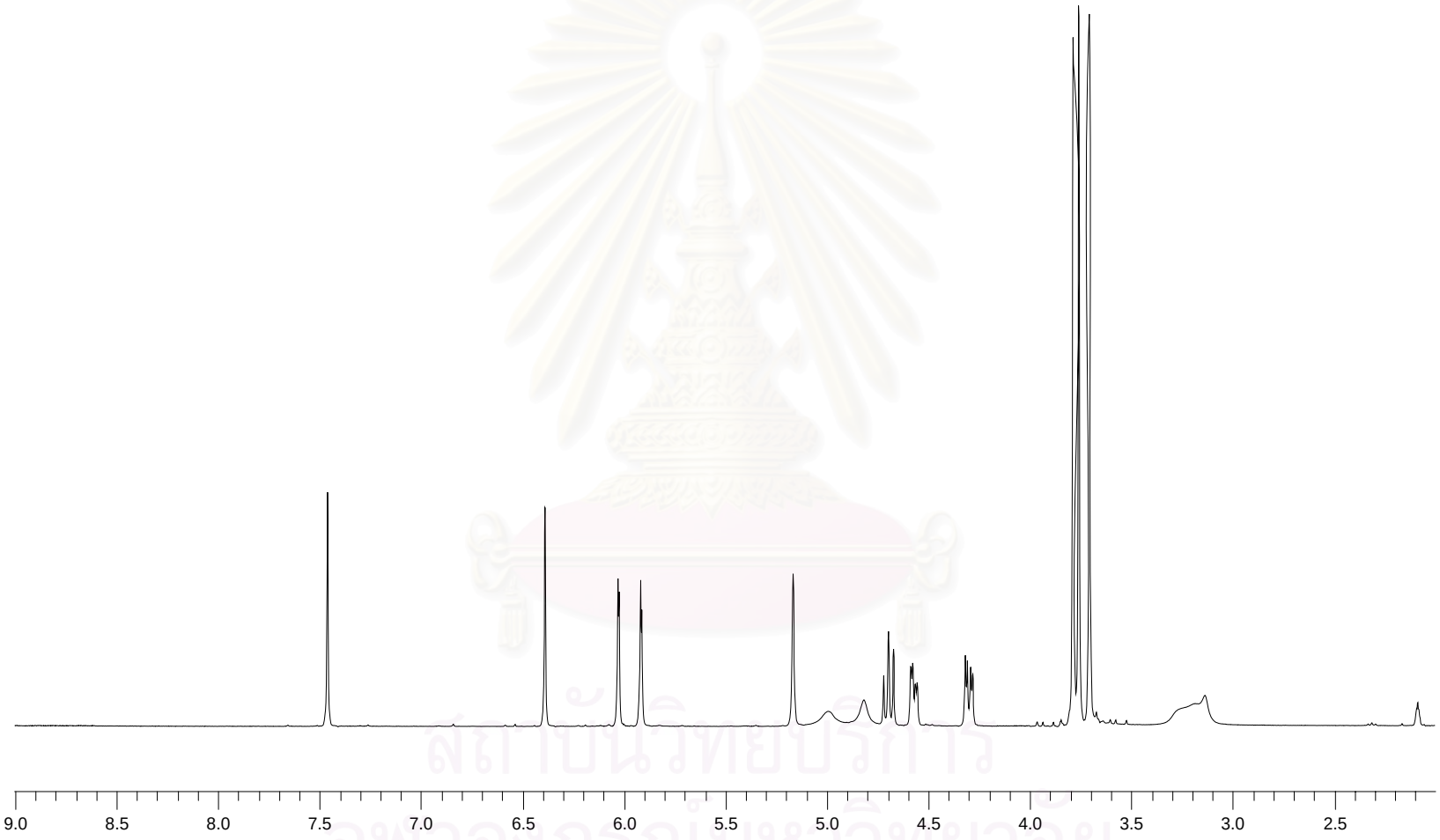

Figure 49 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 88

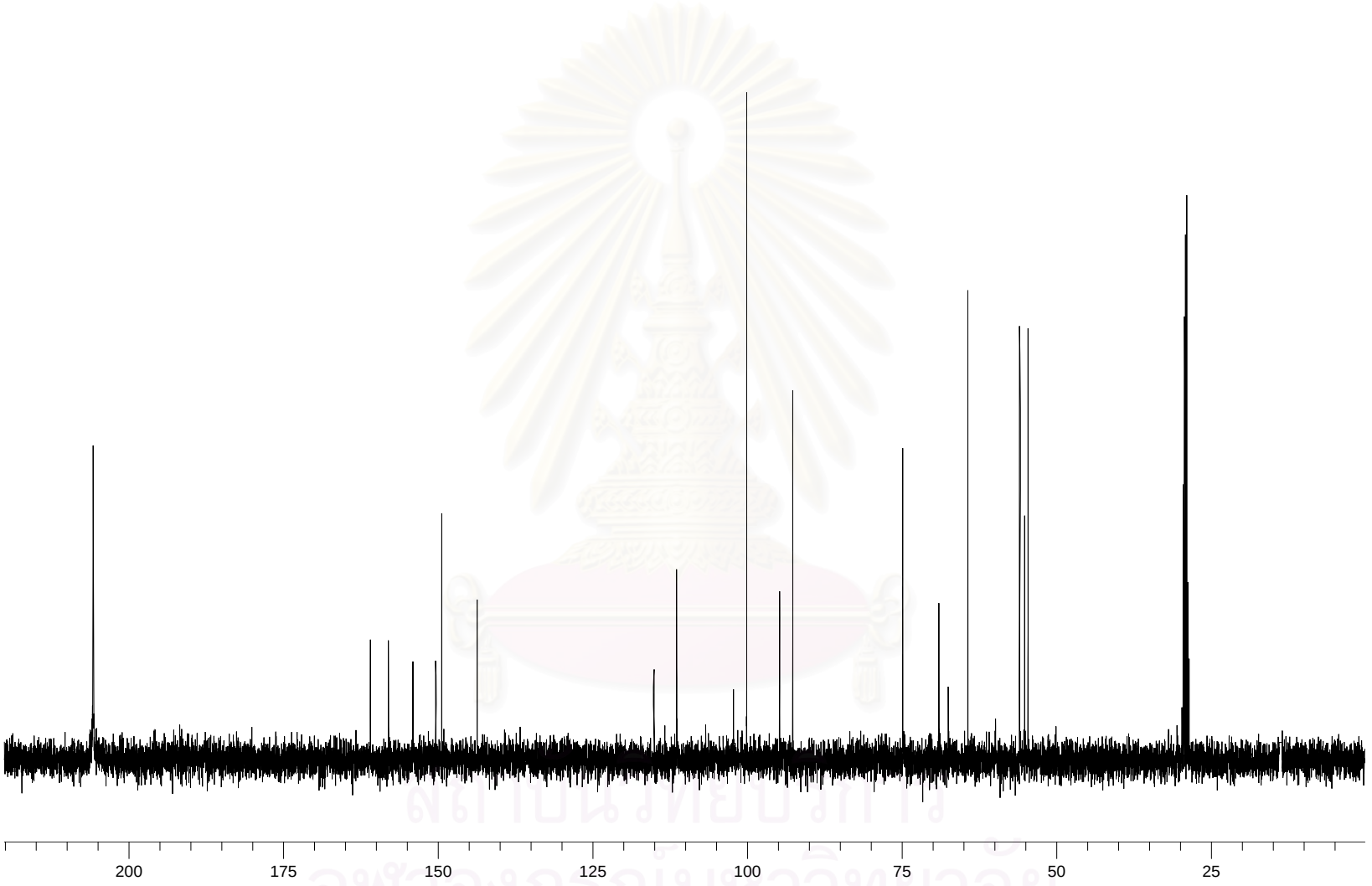

Figure 50 ^{13}C NMR (100 MHz, Acetone-d-6) of compound 88

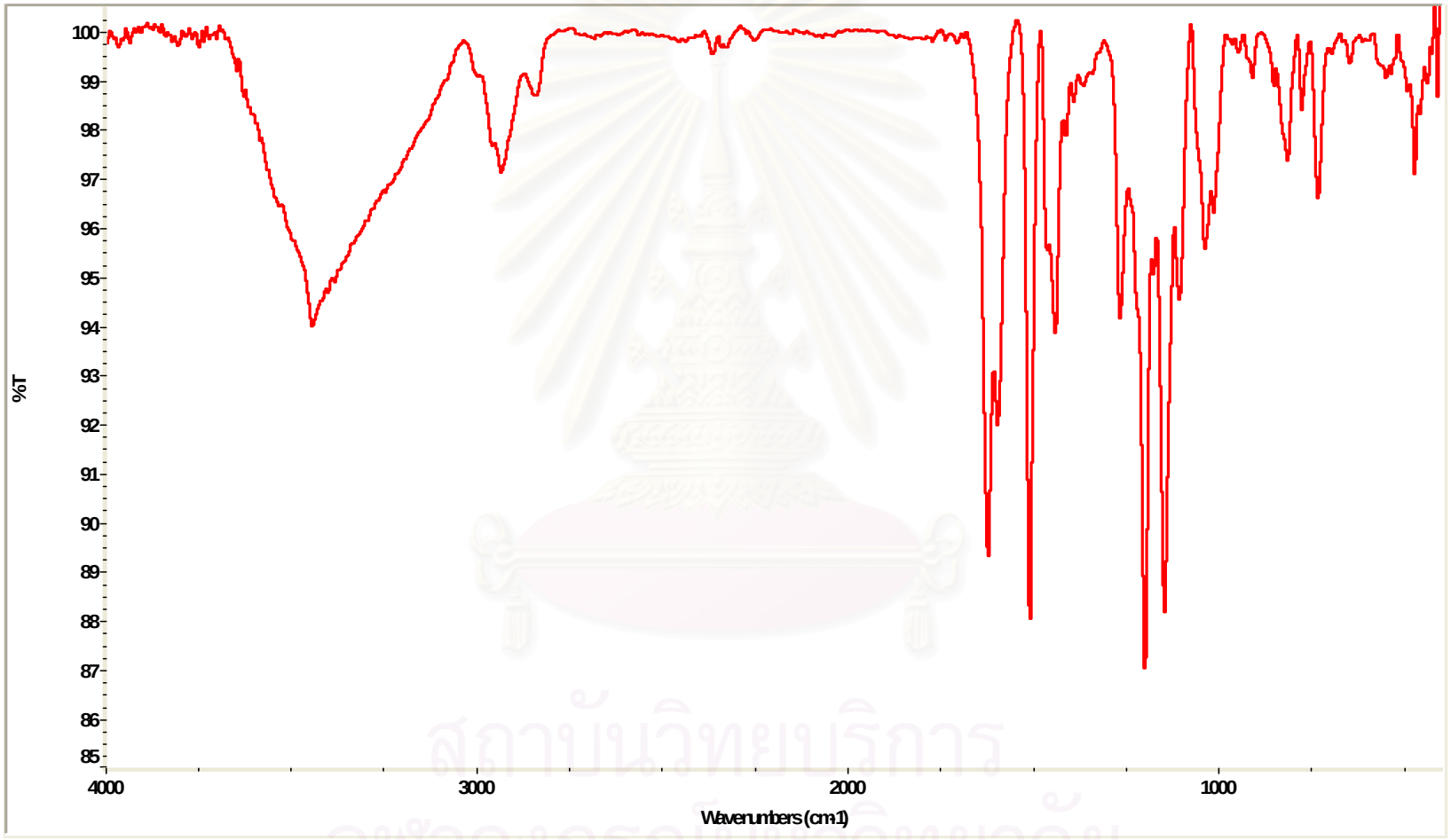

Figure 51 FT-IR of compound 88

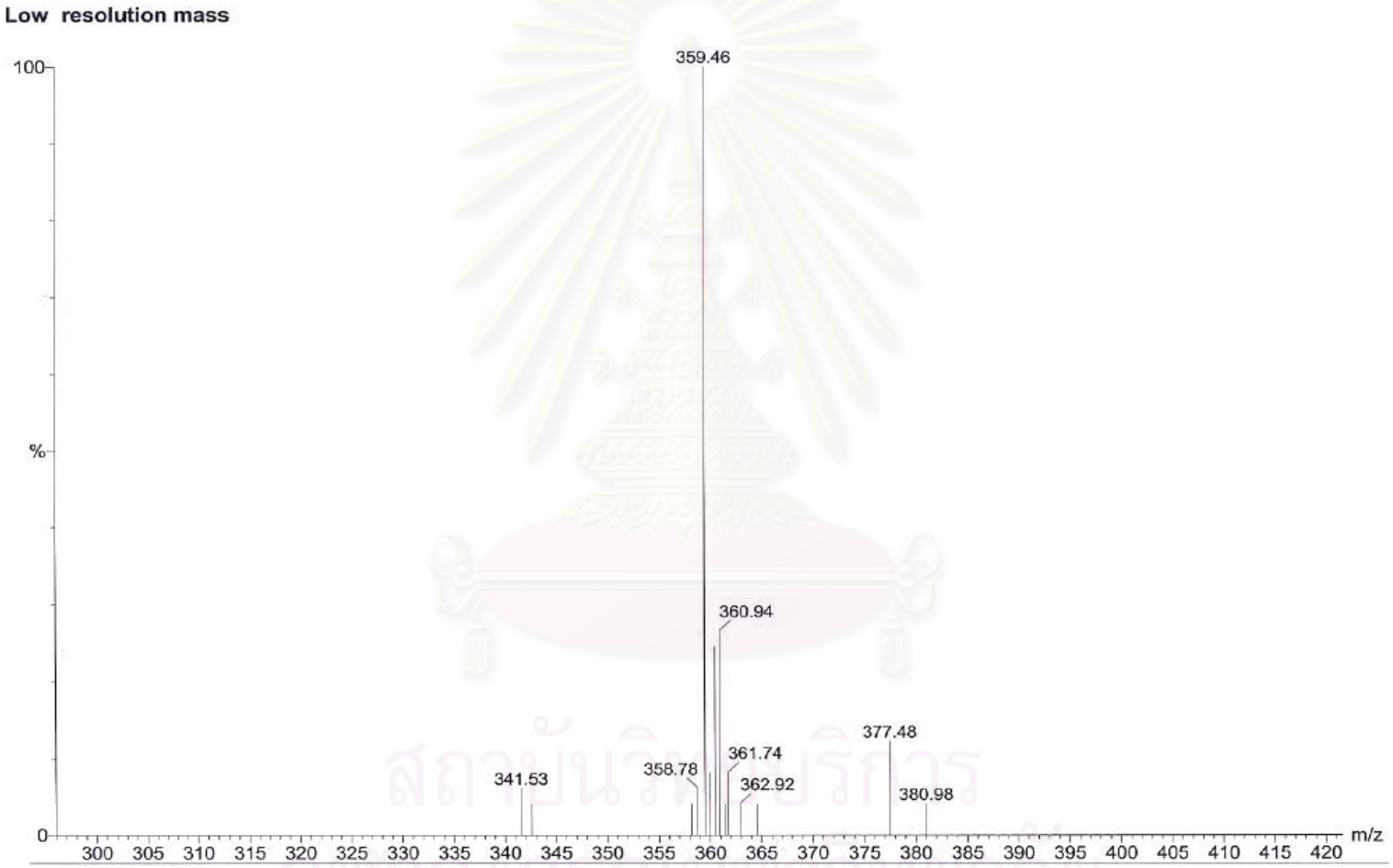

Figure 52 Low resolution MS of compound 88

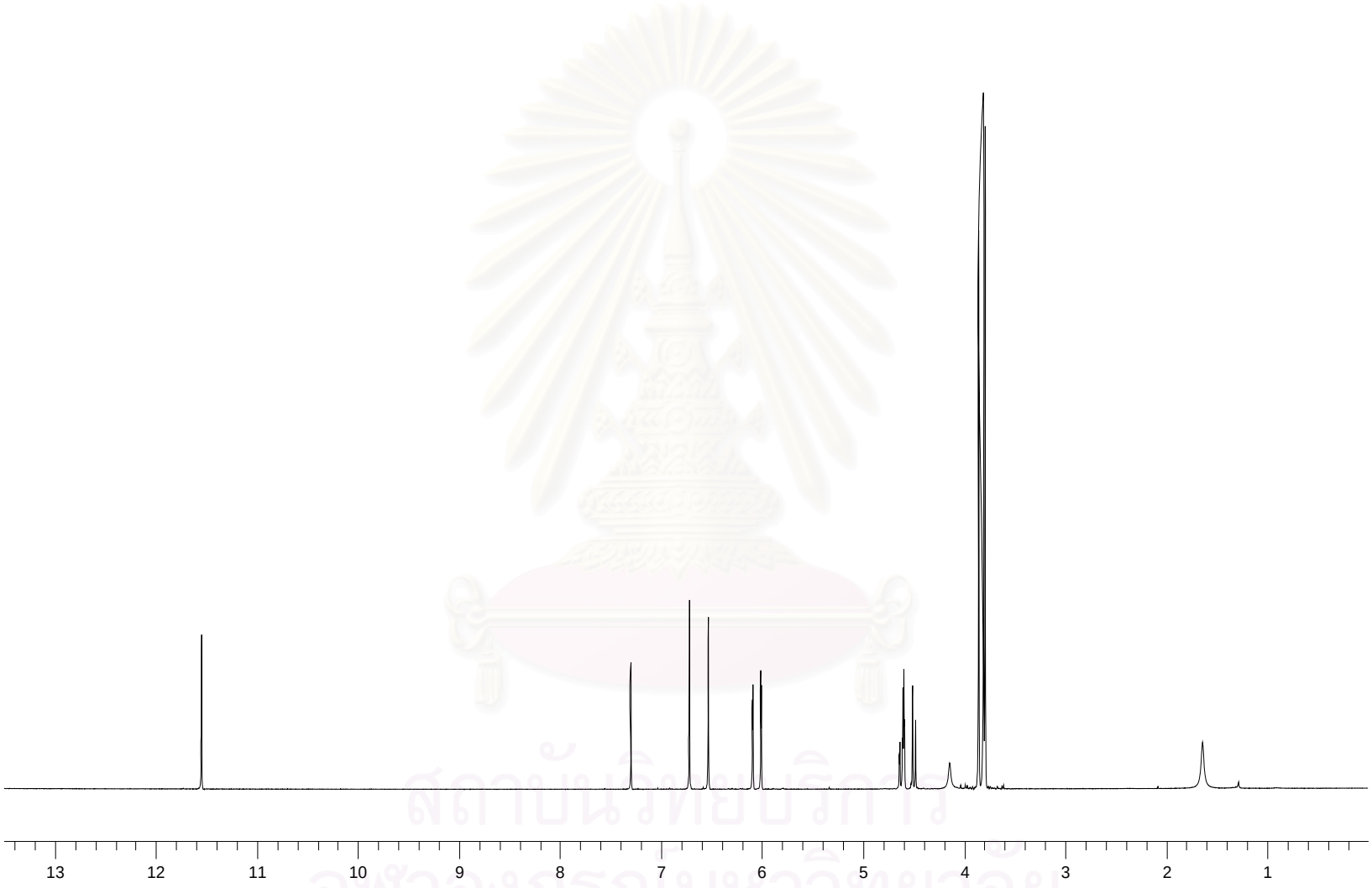

Figure 53 ^{1}H NMR (400 MHz, $CDCl_3$) of compound 39

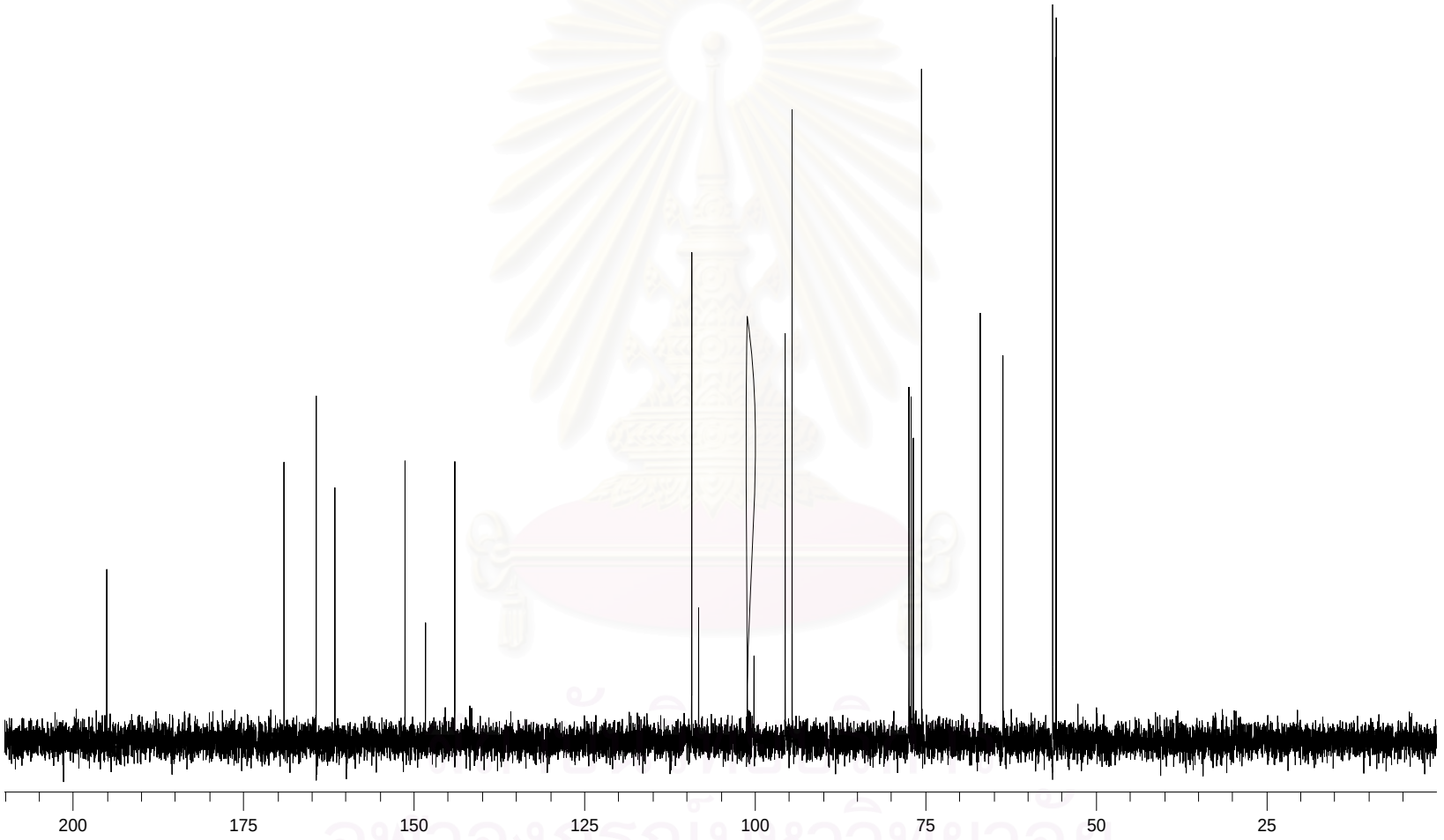

Figure 54 ^{13}C NMR (100 MHz, $CDCl_3$) of compound 39

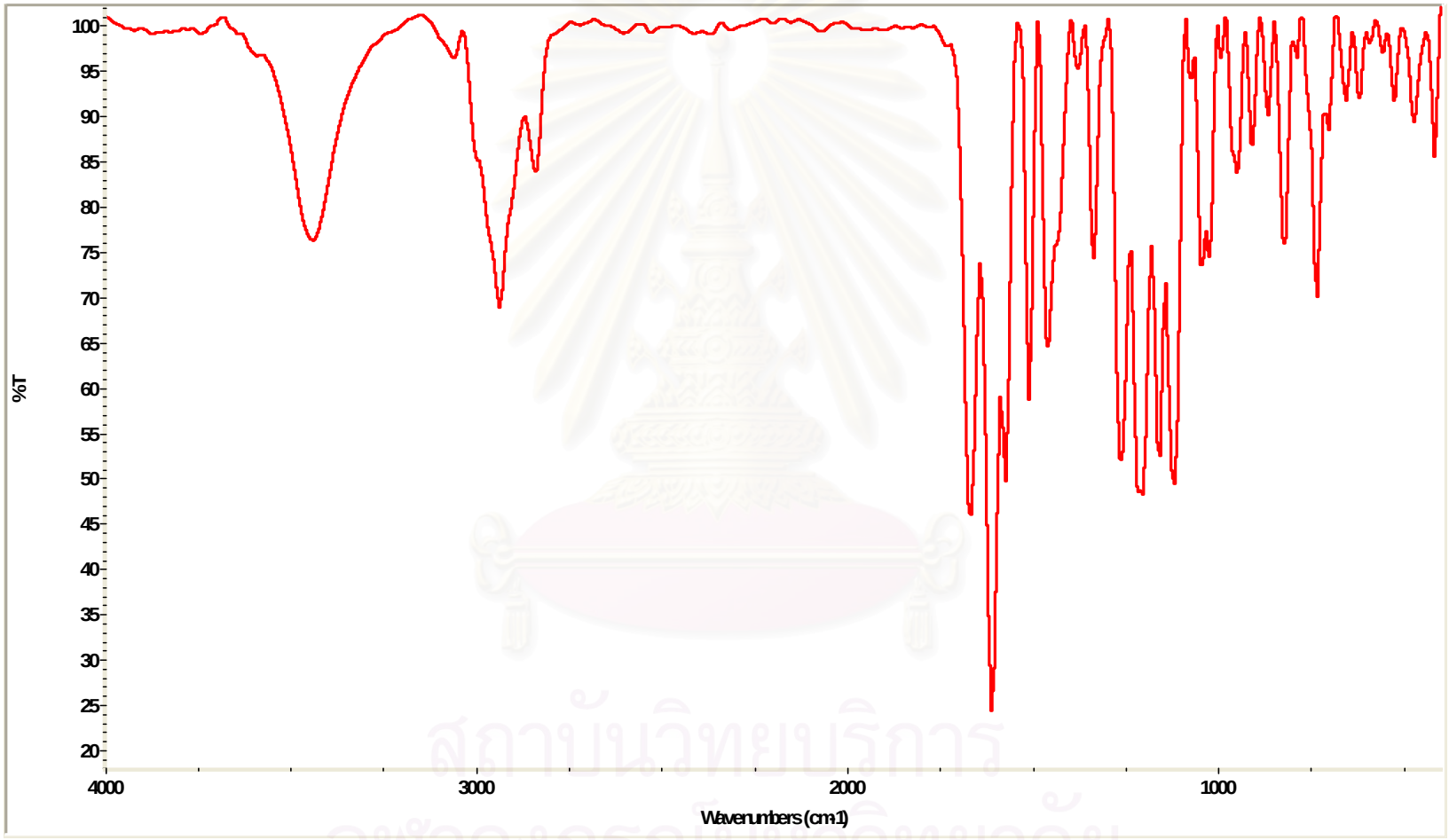

Figure 55 FT-IR of compound 39

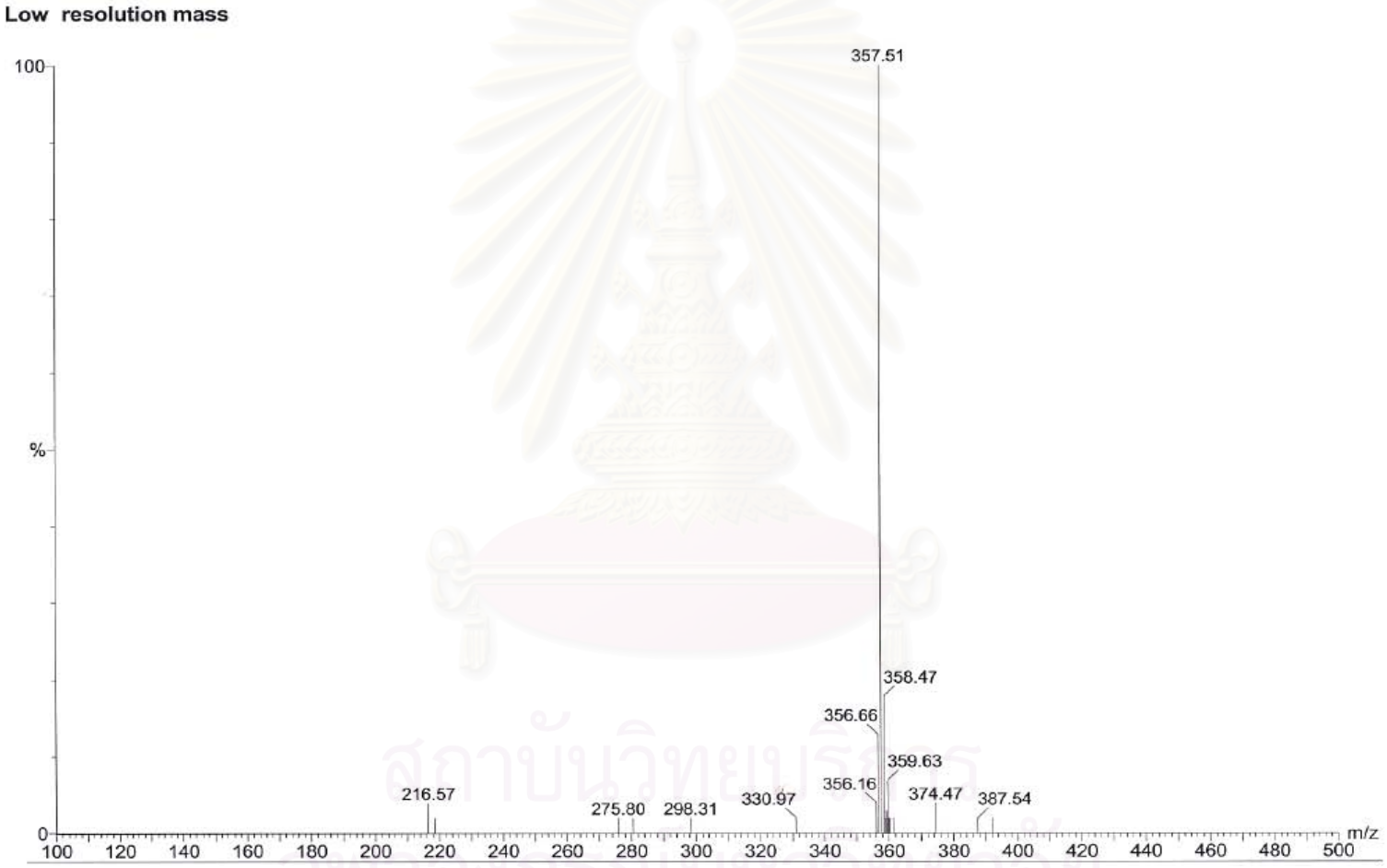

Figure 56 Low resolution MS of compound 39

VITA

The only son of poor farmer family, Prapas was born April 07, 1966 in Angthong Province. He firstly attended to elementary school when he was 6 year old. When he was 12 year old he moved to a city where he studied Intermediate and High school at Visetchaichan “Tantivitayapoom”. He lived in a temple, Nangnaitammigaram temple, during his study.

Once he finished High school, he moved to Bangkok and began undergraduate studies in Chemistry at Ramkhamhaeng University. He spent 6 and a half years for only undergraduate, but this time was so beautiful memories including happiness, broken heart, sorrow, laugh, friendships etc. Then after, he worked at Chulalongkorn University as a research assistant. He was convinced by Professor Sophon Roengsumran to continue higher study in Petrochemical and Polymer program. After spent 3 years for Master Degree, he worked as a Sale Representative for 2 years before moved to Mahanakorn Unversity of Technology as a Lecturer.

To be a lecturer, higher education is needed. He decided to study a Ph.D. at Chulalongkorn Unversity in Organic Chemistry (Synthetic Chemistry) under supervision of Professor Sophon Roengsumran. He handed a Thailand Research Fund under the Royal Golden Jubilee program scholarship for 3 years. Within this scholarship, he went to Professor Richard J.K. Taylor’s laboratory at The University of York, UK for a year as a visiting student. Eight years for Ph.D. studies with so many tries and errors, he finally graduated a Ph.D. in 2005.

He married to Jumnien with a lovely daughter.