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TITLE: Formal Synthesis of (±)Cladoniamide G and Synthesis of Biologically Active Compounds against the H5N1 Bird Flu Virus

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THESIS

FORMAL SYNTHESIS OF (±)CLADONIAMIDE G AND SYNTHESIS OF BIOLOGICALLY ACTIVE COMPOUNDS AGAINST THE H5N1 BIRD FLU VIRUS

ANOTHAI KONKHUM

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Anothai Konkhum 2014: Formal Synthesis of (±)Cladoniamide G and Synthesis of Biologically Active Compounds against the H5N1 Bird Flu Virus. Master of Science (Chemistry), Major Field: Chemistry, Department of Chemistry.
Thesis Advisor: Mr. Paiboon Ngernmeesri, Ph.D. 111 pages.

Andersen group reported the isolation of cladoniamides A-G from cultures of the novel actinomycete *Streptomyces uncialis* harbored within the lichen *Cladonia uncialis* collected near Pitt River, British Columbia. Cladoniamide G is an indolocarbazole alkaloid, which is structurally similar to staurosporine and rebeccamycin. The biological activities of staurosporine and rebeccamycin have made them high-profile lead compounds for the development of anticancer drugs, and several analogues have entered clinical trials. Of the cladoniamide family, cladoniamide G showed the best *in vitro* cytotoxicity against human breast cancer MCF-7 cells at 10 µg/mL (23 µM).

3-Methoxy-1*H*,1'*H*-2,2'-bisindole (**26**) was the key intermediate for the synthesis of (±)-cladoniamide G'. This key intermediate was prepared in good yield by a Suzuki cross-coupling reaction of bromoindole **187** and indolyl boronic acid **189** in the presence of catalytic amount of $PdCl_2(PPh_3)_2$.

H5N1 is a highly pathogenic causative agent of H5N1 flu, commonly known as avian influenza ("bird flu"). Generally, oseltamivir drug has been use for the treatment of this fluBased on computational data, we have found some new compounds that can bind to the influenza neuraminidase better than oseltamivir. Therefore, we are attempting to synthesize these compounds for biological testing. A precursor for the synthesis of these compounds, 1(2*H*)-phenanthrenone 3,4-dihydro-6,10-dimethoxy,-amine (**201**) has been synthesized in 6 steps with 55% overall yield from 4-methoxyphenylacetic acid and 1,3-cyclohexanedione.

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Student's signature Thesis Advisor's signature

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Anothai Konkhum
November 2014

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LIST OF ABBREVIATIONS

Ac = Acetyl
DMAP = 4-(*N*,*N*-Dimethylamino)pyridine
DMF = *N*,*N*-Dimethylformamide
DMSO = Dimethylsulfoxide
LDA = Lithium diisopropylamide
Me = Methyl
NBS = *N*-bromosuccinimide
TFA = Trifluoroacetic acid
THF = Tetrahydrofuran
δ = Chemical shift (ppm)
J = Coupling constant
cm^{-1} = Reciprocal centimeter (wave number)
m = Multiplet
s = Singlet
t = Triplet
q = Quartet
d = Doublet
dd = Doublet of doublets
ddd = Doublet of doublet of doublets
Hz = Hertz
FTIR = Fourier transform infrared spectrometry
MS = Mass spectroscopy
CI = Chemical ionization
EI = Electron impact
HRMS = High resolution mass spectroscopy
m.p. = Melting point
m/z = A value of mass divided by charge

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FORMAL SYNTHESIS OF (±)CLADONIAMIDE G AND SYNTHESIS OF BIOLOGICALLY ACTIVE COMPOUNDS AGAINST THE H5N1 BIRD FLU VIRUS

INTRODUCTION

1. Synthesis of (±)-Cladoniamide G

The indolocarbazole alkaloids, which are obtained from both soil organisms and marine sources, contain an indolo[2,3-*a*]carbazole core (Rajeshwaran and Mohanakrishnan, 2011). These alkaloids possess a variety of biological activities such as antifungal, antimicrobial, antitumor, and antihypertensive activity (Knölker and Reddy, 2002). Examples of indolocarbazole compounds are staurosporine (**1**) and rebeccamycin (**2**) (Figure 1). Compound **1**, which is a metabolite from *Streptomyces*, inhibits a variety of protein kinases C (PKC) (IC_{50} = 1 nM) by acting as the inhibitor of cyclic B/CDK1 and topoisomerase activity by ATP competitive. Compound **2**, which was isolated from an actinomycete, is a well-known natural product from a group of indolocarbazale alkaloids containing a single *N*-glycosidic bond. The structure and absolute configuration of rebeccamycin have been determined by X-ray crystallography. Rebeccamycinis a high-profile lead compound for the development of anticancer drugs (Faul *et al.*, 1999). Another group of alkaloids whose structures are very similar to indolocarbazoles is a family of indolotryptolines. This family of natural products exhibits biological activities against tumor cell lines. Examples of natural products containing an indolotryptodine core are cladoniamide A (**3**) and cladoniamide G (**4**), which are formed by successive oxidations of the indolocarbazole precursor (Chang and Brady, 2011; Du *et al.*, 2014).

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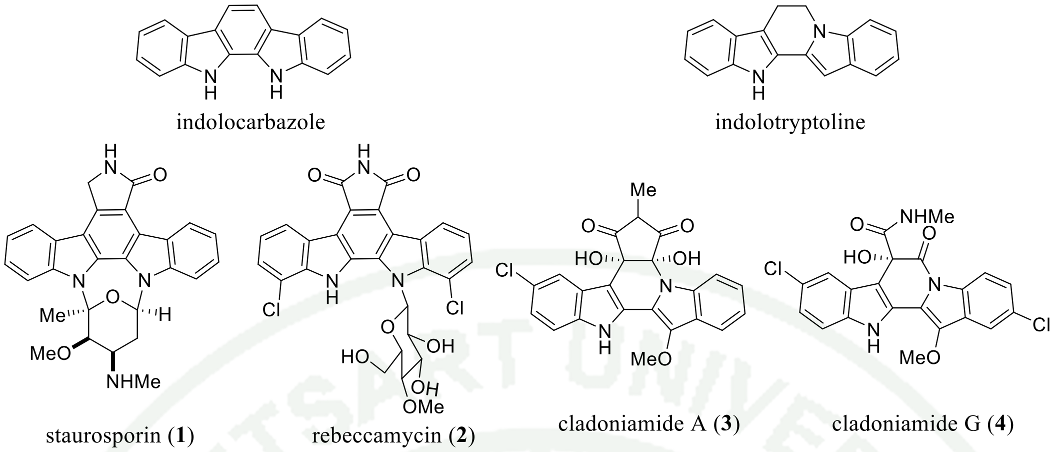

Figure 1 Cladoniamides A and G and related bisindole alkaloids.

In 2008, Anderson *et al*. reported the isolation of the new enediyne antibiotic uncialamycin from cultures of *Streptomyces uncialis* (Williams *et al.*, 2008). The *Streptomyces uncialis* is an actinomycete bacterial strain isolated from the lichen *Cladonia uncialis* near the Pitt River in British Columbia. This bacterial strain is the source of the enediyne uncialamycin and the cladoniamides A-G (**5**-**11**) as shown in Table 1. The absolute configuration of cladoniamide A has been confirmed by X-ray diffraction analysis. Therefore, the absolute configuration of cladoniamide G can be inferred because these compounds appear to be biosynthetically related. The cladoniamides are unusual among bis-indole natural products, most bis-indoles have an indolocarbazole structure such as staurosporine (**1**) and rebeccamycin (**2**), but the cladoniamides have a rarely observed indolotryptoline structure.

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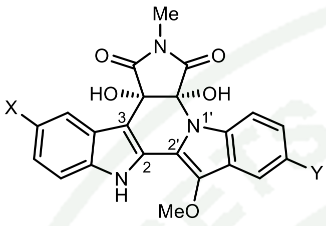
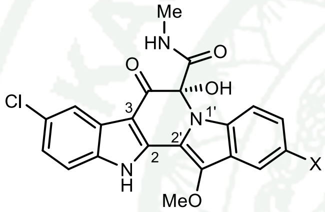
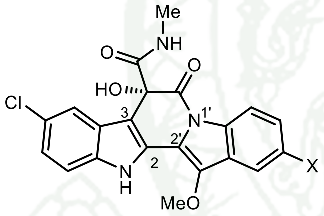
Table 1 Chemical structure of cladoniamides.

Structure	Name	X	Y
	Cladoniamide A (**5**)	Cl	H
	Cladoniamide B (**6**)	Cl	Cl
	Cladoniamide C (**7**)	H	H
	Cladoniamide D (**8**)	H	-
	Cladoniamide E (**9**)	Cl	-
	Cladoniamide F (**10**)	H	-
	Cladoniamide G (**11**)	Cl	-

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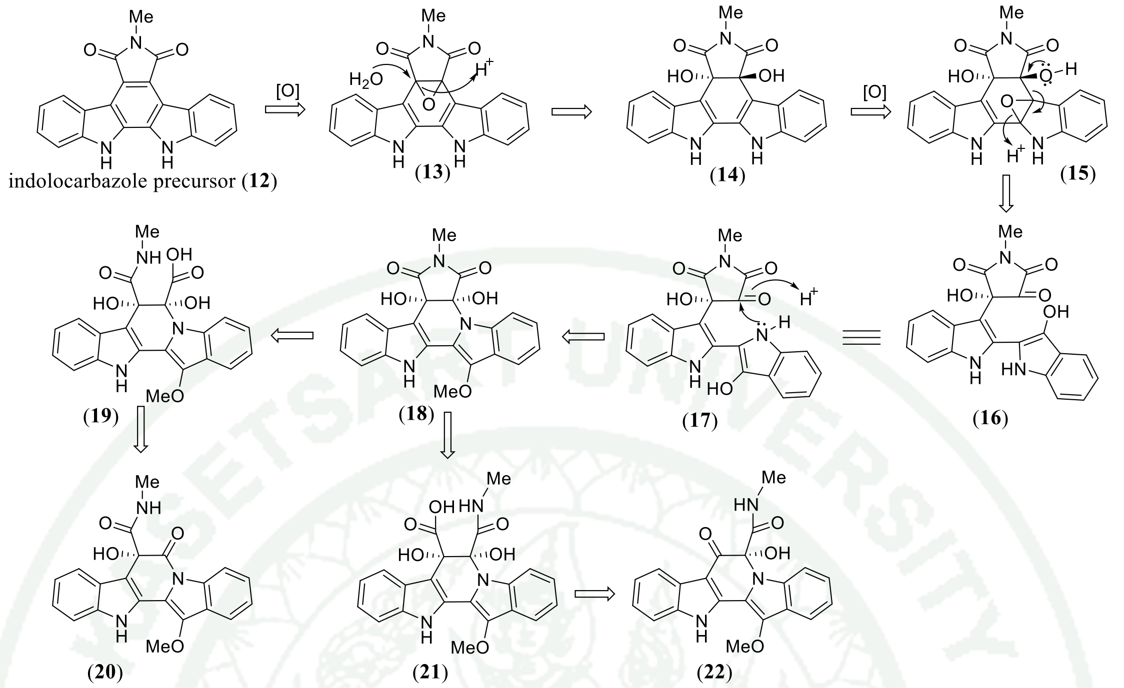

Scheme 1 Proposed biosynthesis for the cladoniamides.

Anderson *et al.* proposed the biosynthesis of cladoniamides A-G as show in scheme 1. They suggested that the cladoniamides are derived from tryptophan-derived indolocarbazole precursors **12** by rearrangement and degradation.

Among members in the family of cladoniamides, cladoniamide G (**11**) is cytotoxic against MCF-7 breast cancer cells (10 μg/mL in vitro) more than other cladoniamides which are active at concentrations < 50 μg/mL (Williams *et al.*, 2008). Because of its novel structure and significant biologically activities, Dake and coworkers reported the synthesis of cladoniamide G in 2013 (Loosley *et al.*, 2013). They synthesized cladoniamide G successfully in 9 steps with 15% overall yield from 5-chloroindole (**23**) as a precursor. The synthesis's key tremsformations consist of oxidative dimerization of 3-acetoxy-5-chloroindole (**24**) followed bimolecular carbonyl addition, lactam formation, and carbarmate removal as show in Scheme 2.

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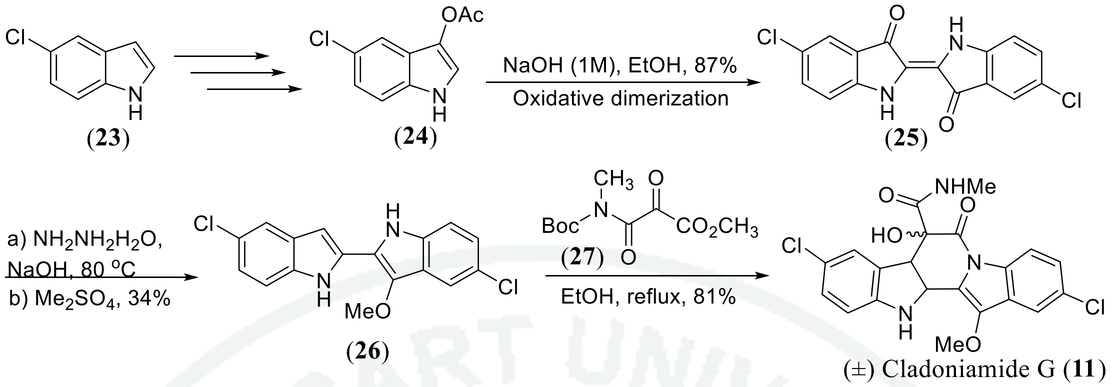
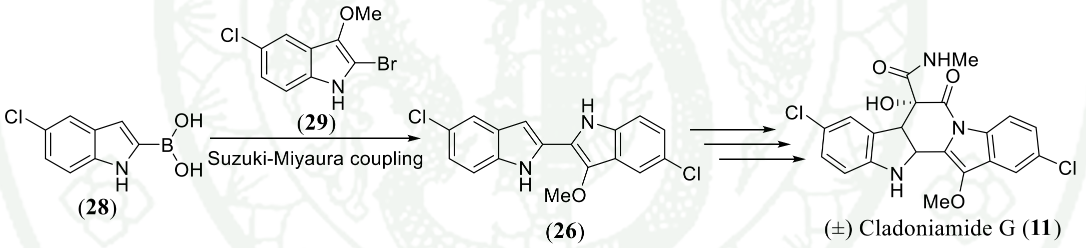


Scheme 2 Total synthesis of (±)cladoniamide G.

Our research concentrated on a method of connecting two indole units together. Our tactics was to use a Suzuki cross coupling reaction to form a functionalized bisindole compound from indole precursors **28** and **29**, which could be prepared from commercially available starting materials.



Scheme 3 Our proposed synthesis of (±)-cladoniamide G.

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2. Synthesis of biologically active compounds agent the H5N1 bird flu virus

Influenza is generally known as “the flu”, which is the infectious disease of birds and mammols caused by the RNA viruses of orthomyxoviridae family. Normally, influenza will be transmitted via the air by creating an aerosol. Influenza can also be transmitted through direct contact with bird droppings, nasal secretions and contaminated surfaces (Brankston *et al.*, 2007). Recent report from the World Health Organization (WHO) of the influenza infectiousness concluded that influenza viral shedding it can be detected within 24–48 hours before clinical onset. Shedding usually takes about less than 5 days, but in children it may be faster. There was a report that the incubation period takes 2 days on average (range: 1–4 days) (Carrat *et al.*, 2008).

Table 2 Most sensitive symptoms for diagnosing influenza.

Symptom	Sensitivity	specificity
Fever	68-86%	25-73%
Cough	84-94%	7-29%
Nasal congestion	68-91%	19-42%

Usually first symptoms of influenza are chills or a chilly feeling, but fever is also common in the early infection, Body temperatures can be in the range of 38 to 39 °C or approximately 100 to 103 °F (Suzuki *et al.*, 2007). To distinguish between the common cold and influenza can be difficult in the early stages, but it can be identified by a sudden high fever and extreme exhaustion. The symptoms of influenza is a mixture of common cold, body ache, fatigue, headache, and pneumonia. The influenza in adults have unusual symptoms with diarrhea. The symptoms of influenza are shown in Figure 2 (Hui, 2008).

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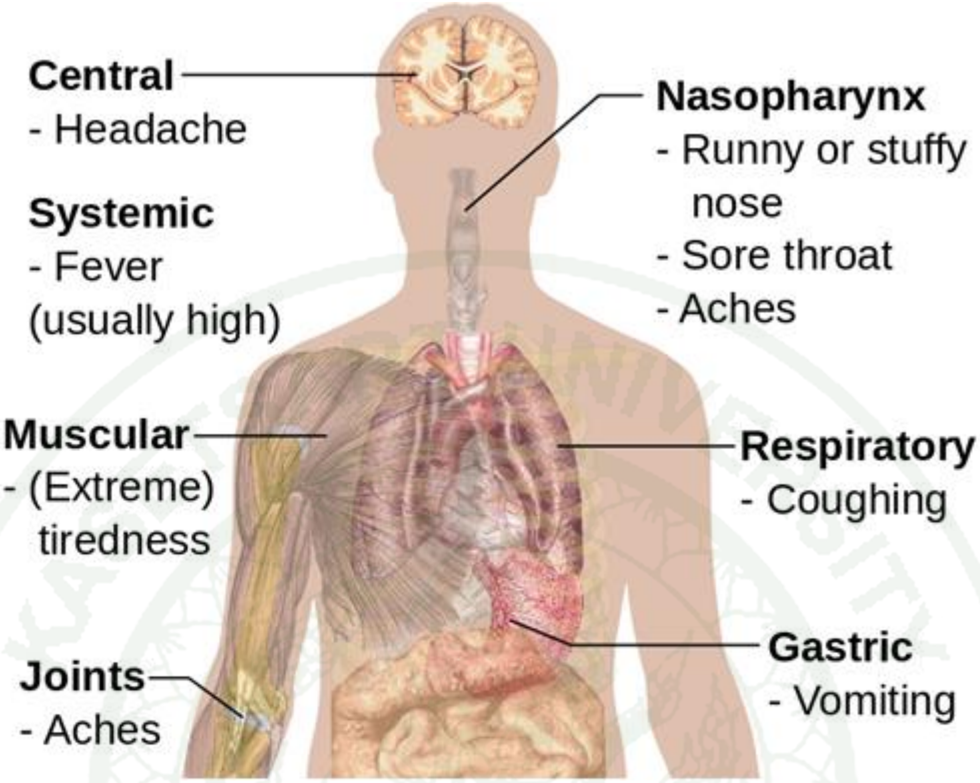

Figure 2 Symptoms of influenza[a]

In virus classification of influenza, there are three genera of the family orthomyxoviridae. Among them are RNA viruses that are influenza viruses A, B and C (Muradrasoli *et al.*, 2010). They have been reported that type A effects the human body the most and causes the severe disease. Influenza A viruses are classified into subcategories by the antigenic differences of the surface a main of antigenic determinants proteins the hemagglutinin (HA) and neuraminidase (NA) genes. They contains distinct sixteen HA (H1–H17) and nine NA (N1–N9) subtypes (Figure 3) which are membrane-associated glycoproteins (Blazejewska *et al.*, 2011).

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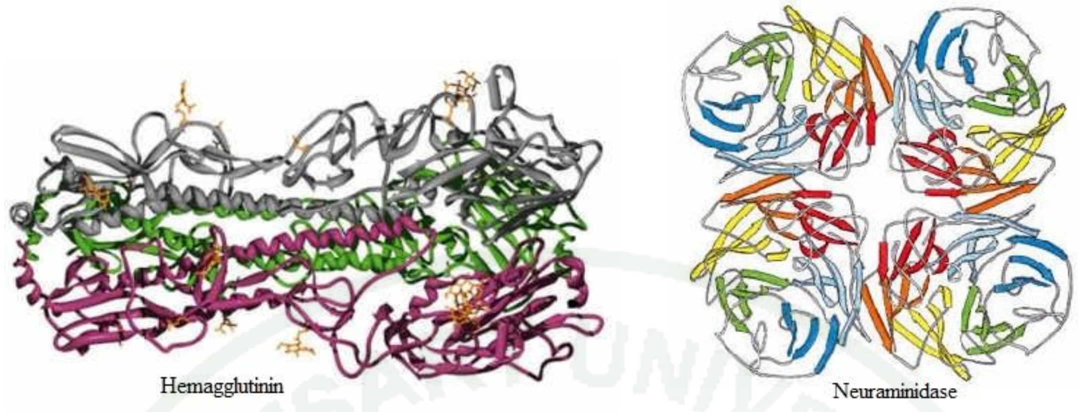

Figure 3 Hemagglutinin and Neuraminidase^{b}

Influenza viruses are spherical or ovoid in shape and range from 80 to 120 nm in diameter as show in Figure 4. The influenza is an enveloped virus that derives its lipid bilayer from the plasma membrane of a host cell. The characteristic feature of viral particles is their external layer of about 500 spike-like projections. The particles consist of glycoproteins HA (which has a rod-like shape), NA (which is mushroom-shaped), lipid envelope, capsid and nucleoprotein (RNA) (Figure 3).

The ribonucleoprotein complexes (RNPs) of the influenza A virus play an important role on the virus infection cycle. After receptor-mediated endocytosis, the viral ribonucleoprotein (vRNP) complexes are released into the cytoplasm and then move to the nucleus, where replication and transcription will take place. The messenger of RNAs to export the cytoplasm for translation. The new viral poteins and vRNA export to cytoplasm in order to assemble as virion cells. The new cells move to the host cell surface where the virus cell will be released by neuraminidase enzyme as show in Figure 5 (Zheng and Tao, 2013).

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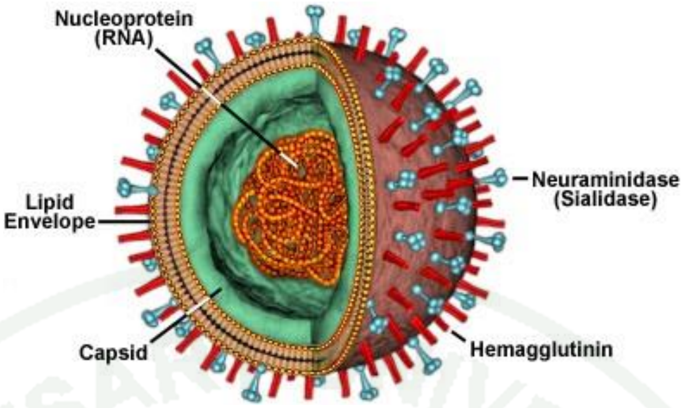

Figure 4 Model of influenza virus.[c]

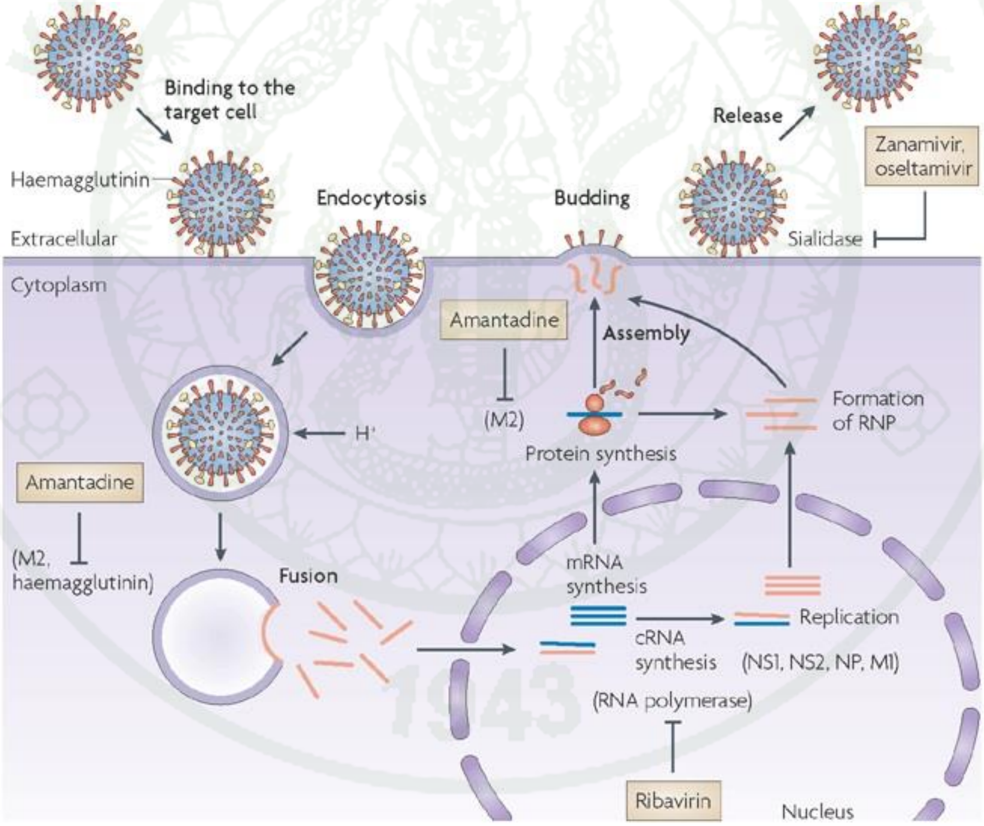

Figure 5 The influenza A virus life cycle.[d]

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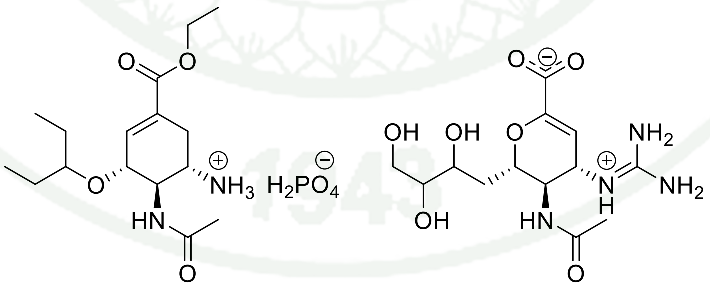
The influenza infects many animal species and transfer of viral strains between species can occur. Birds are the main animal reservoirs of great influenza viruses. The subtypes (HxNy) of avian influenza identified by sixteen forms of hemagglutinin and nine forms of neuraminidase (Webster *et al.*, 1992).

Recently, the emergence and worldwide distribution of the avian influenza A virus subtype H5N1 occur. There are concerns that the spread in human-to-human transmission becomes more easily. Therefore, the development of drugs is used for the treatment and prophylaxis of influenza infections. Neuraminidase causes influenza virus that is released from infected cells and facilitates the spread of virus within respiratory. Several potent and specific inhibitors of the neuraminidase were developed via structure-based rational design. Based on this method, only two species of oseltamivir (Tamiflu) and zanamivir (Relenza), have been approved for human use. They were discovered by Kim *et al.* and Gubareva *et al.* (C. U. Kim *et al.*, 1997) (Gubareva *et al.*, 2000). Zanamivir is low oral bioavailability, it is delivered by inhalation whereas oseltamivir is administered by mouth. At present, there are reports about oseltamivir drug-resistant is some patients (Rungrotmongkol *et al.*, 2009). Therefore, we want to search for a new compound that is a potential inhibitor of neuraminidase.



Oseltamivir
(Tamiflu)

Zanamivir
(Relenza)

Figure 6 Chemical structures of antiviral drugs inhibiting the neuraminidase of influenza A virus: oseltamivir, zanamivir

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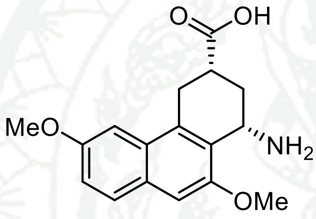
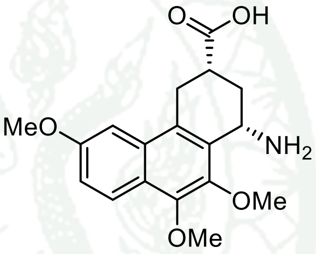
Dr. Chak Sangma and his research group have been searching for compounds that can bind to the influenza neuraminidase better than oseltamivir using computational data. They found that compounds **30** and **31** might be new good drug candidates. According to the calculation by AltoDock 4, the binding energies of the influenza neuraminidase of compound **30** and **31** are -8.90 and -9.80 kcal/mol, respectively, and these values are very close to (with the second value slightly better) that of oseltamivir (-9.72 kcal/mol). These findings have stimulated our interest to determine whether both compounds actually possess some biological activity against the influenza virus.Therefore, our research will deal with the synthesis of these two molecules to provide samples for biological testing.



(**30**)



(**31**)

Figure 7 Structures of aminoesters **30** and **31**

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OBJECTIVES

To synthesize anti-cancer cladoniamide G and potential biologically active compounds against the H5N1 Bird flu virus

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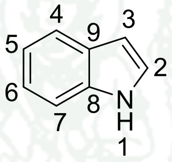
LITERATURE REVIEW

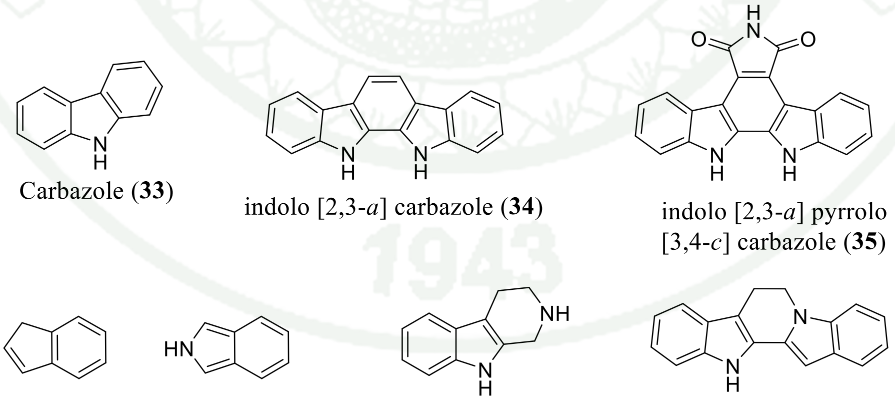
1. Synthesis of Indole Alkaloids

Indole is an aromatic heterocyclic organic compound. It is widely distributed in the natural environment and can be produced by a variety of bacteria. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring. The indole ring system is a key component in the structure of many drugs (Takayama et al., 2003). The numbering system of the indole nucleus is shown in Figure 8.



indole (**32**)

Figure 8 General structure and numbering system of indole nucleus.



Carbazole (**33**)

indolo [2,3-*a*] carbazole (**34**)

indolo [2,3-*a*] pyrrolo [3,4-*c*] carbazole (**35**)

Indene (**36**)

Isoindene (**37**)

Tryptoline (**38**)

Indeno [2,1-*a*] tryptoline (**39**)

Figure 9 General structure of indole alkaloid derivatives.

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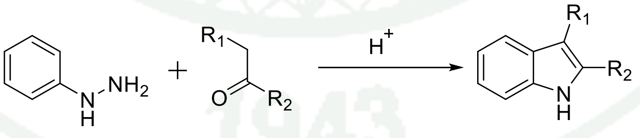
A variety of methods have been developed to synthesize the indole ring since its skeleton represents a key intermediate in the synthesis of many indole alkaloid derivatives (Figure 9). Indole compounds can be synthesized in several ways such as Castro indole synthesis, Leimgruber-Batcho indole synthesis, intramolecular Michael addition/elimination, Dieckmann condensation, and the Fischer indole synthesis. The first three methods are related to ring closure between the *N-1* and *C-2* to construct the indole ring. The Dieckmann condensation is related to ring closure between the *C-2* and *C-3*, while the Fischer indole synthesis is the bond formation the ring closing between *C-3* and *C-9*, and *N-1* and *C-2* of the indole nucleus.

1.1 Fischer indole synthesis

The Fischer indole synthesis is a chemical reaction to generate the aromatic heterocyclic indole from a reaction of phenylhydrazine (**40**) and an aldehyde or ketone (**41**) under acidic conditions. Hermann Emil Fischer at al. have discovered this reaction in 1883 (Fischer and Jourdan, 1883). In this reaction, phenylhydrazine reacts with a carbonyl compound (aldehyde or ketone) to generate phenylhydrazone and isomerizes to provide the enamine. After protonation, a cyclic [3,3]-sigmatropic rearrangement occurs to produce an imine. The resulting imine forms a cyclic aminoacetal followed by the elimination NH_3 to give the aromatic indole (**42**).



Scheme 3

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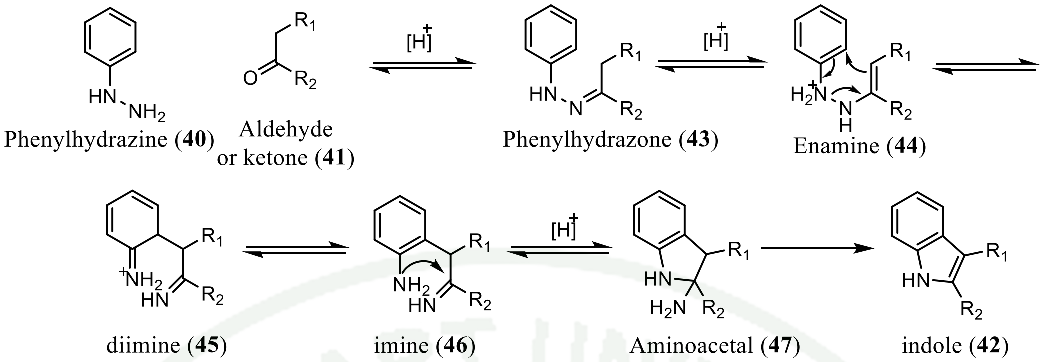

Scheme 5 Mechanism of the Fischer indole reaction.

In 1997 Liu et al. reported the preparation of a series of 5- and/or 6-substituted-3-methylindoles **52 a-d** as show in Scheme 5 (Liu *et al.*, 1997). For example, ethyl 3-methylindole-2-carboxylates **51a** and **51b** were synthesized from the proper aniline **48** and ethyl α-ethylacetoacetate **49**. This reaction was performed in HCl in ethanol or PPA, and regioisomers were also obtained as minor products. For example, indole **51a** was obtained along with its regioisomer, 4-chloro-5-methoxy, in the ratio of 14:1. Then compound **51a** was purified by simple crystallization from the crude reaction mixture. Hydrolysis of compound **51a** by using potassium hydroxide and decarboxylation under standard conditions gave indole compound **52a**. Indoles compounds **52a-d** can be used as precursors in the synthesis of a wide range of interesting natural products.

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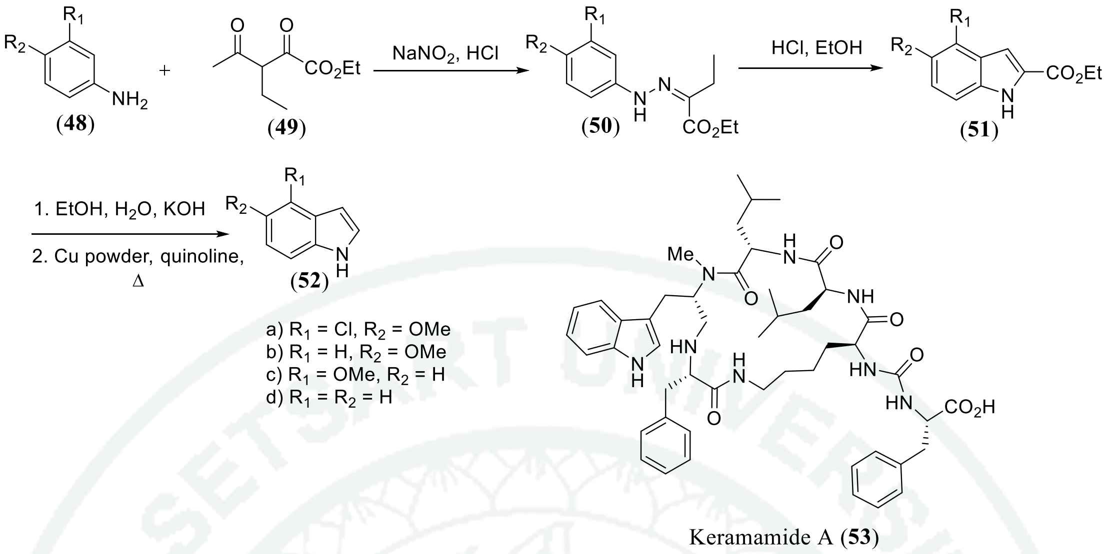
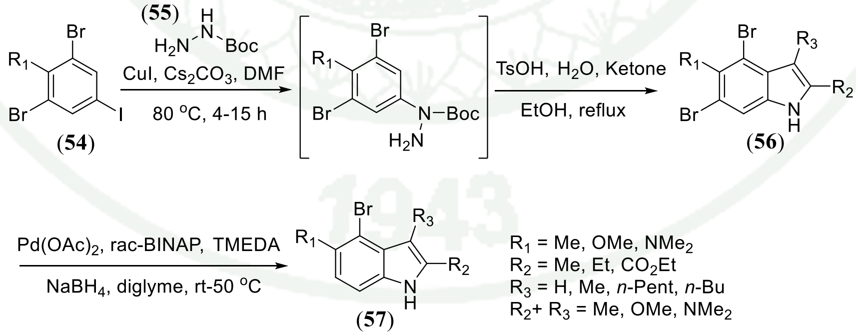


Scheme 6

Chae and Buchwald reported the methodology for the synthesis of 4-bromoindoles. They first synthesized 4,6-dibromoindoles chemoselectively using Cu-catalyzed coupling of symmetric dibromoaryliodides and *tert*-butylcarbazate via a one-pot process and followed by Fischer indolization as shown in Scheme 7. In the next step hydrodebromination by $Pd(OAc)_2$/rac-BINAP/$NaBH_4$ occurred at the less hindered 6-position to give 4-bromoindoles **57**. (Chae and Buchwald, 2004)



Scheme 7

An interesting extension of this methodology for the selective synthesis of indole **59** was reported by Lim and co-workers (Lim *et al.,* 2014). They synthesized indole compounds **59** in high yields and high selectivity from ene-hydrazides **58** using

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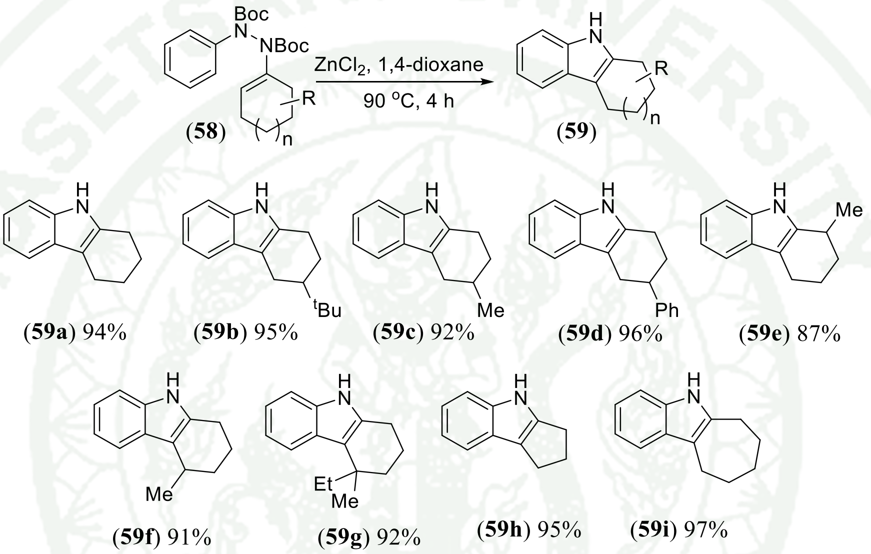
$ZnCl_2$ as a catalyst. Under these condition, the Boc group was removed from the reaction to reveal an enamine. After that, hydrogen atom was transferred through the isomerization via the hydrazine intermediate. In order to suppress *N*-Boc group deprotection and subsequent isomerization. The indolization was used in this process under Lewis acid conditions. They used the Fisher indole synthesis for reducing the regiochemistry issue.



Scheme 8

As shown in Scheme 9, interrupted Fischer indolization process has the advantage of producing three new bonds, two heterocyclic rings, and two stereogenic centers by one pot. The arylhydrazine **41** reacted with a disubstituted aldehyde **60** to enamine intermediate **61**, then [3,3]-sigmatropic rearrangement and rearomatization occurred to provide aniline **62**. Finally, intramolecular attack by a proximal heteroatom substituent (X=NR or O) and loss of NH_3 gave the desired indoline rings system. Applications of interrupted Fischer indolization to prepare furoindoline or pyrroloindoline bioactive analogs have been reported (Boal *et al.*, 2009) (Mo *et al.*, 2014).

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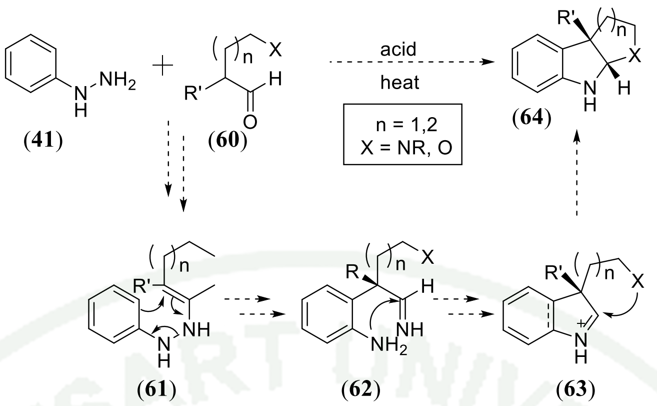
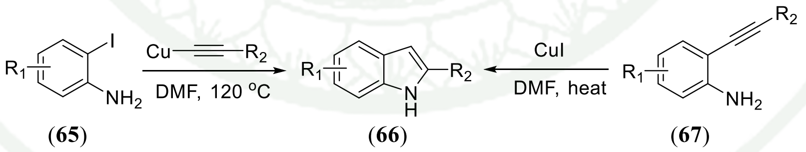


Scheme 9

1.2 Castro Indole Synthesis

The Castro indole synthesis involves cyclization of either *o*-iodo aniline derivatives with cuprous acetylides or 2-alkynylanilines with copper(I) salts, usually copper (I) iodide, as shown in Scheme 10 (Castro *et al.*, 1966). The cyclizations of 2-alkynyl anilines **67** using copper (I) have received considerable attention for the construction of indoles. The Castro indole synthesis is particularly attractive since no protecting groups are needed in this reaction (Ezquerra *et al.*, 1996).



Scheme 10 Castro indole synthesis

Farr *at al.* reported the preparation of indole **51** by modification of the procedure originally shown in Scheme 11 (Farr *et al.*, 2003). This indole is an intermediate in the synthesis of gonanotropin-releasing hormone antagonist **52**. Precursor **50** was prepared in quantitative yield by the coupling reaction between iodoaniline hydrochloride **68** and acetylene **52**.When this precursor was treated with 0.5 equivalent of copper (I) iodide in DMF/toluene at 104 ^{o}C for 3 h, compound **51**

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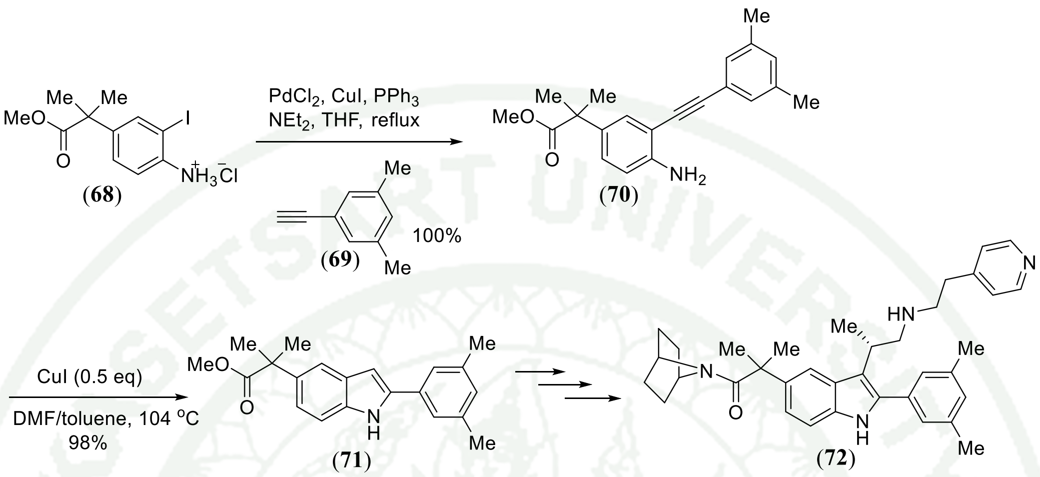
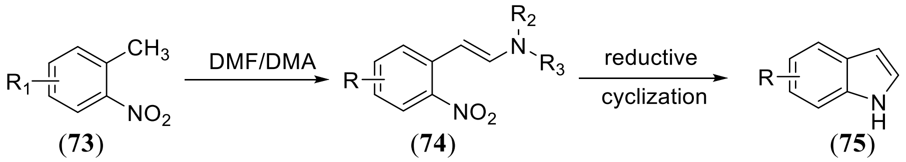
was obtained in 98% yield after crystallization. Then indole **52** was generated in four to five additional steps via two separate reaction pathways.



Scheme 11

1.3 Leimgruber-Batcho indole synthesis

The Leimgruber–Batcho indole synthesis is a series of organic reactions that produces indoles from *o*-nitrotoluenes **73** for the preparation of 2,3-unsubstituted indoles with dimethyl formamide (DMF) or dimethyl acetal (DMA) via *o*-(dimethylamino)-2-nitrostyrene intermediate **74** (Batcho and Leimgruber, 2003). In Scheme 12, the reductive cyclisation leads to substituted indoles **75**. This indole synthesis has been used extensively for the construction of both natural products and pharmaceutically important compounds.



Scheme 12 Leimgruber-Batcho indole synthesis

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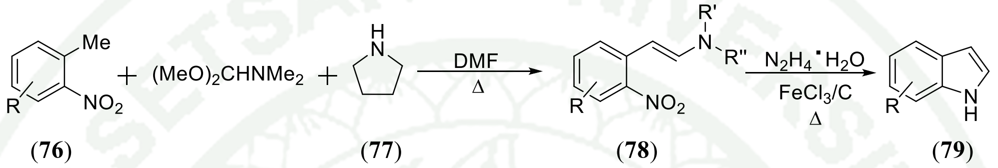
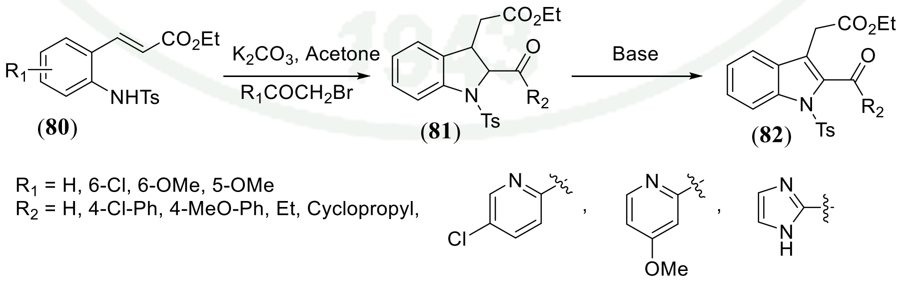
In 2011, Taydakov and coworkers reported the synthesis of indoles by the Leimgruber-Batcho synthesis (Taydakov *et al.*, 2011). The starting 2-methyl-1-nitrobenzenes **76** were converted to enamines **78** when they were treated with dimethyl formamide (DMF) dimethyl acetal (DMA) and pyrrolidine **77** in dry DMF. Reduction and cyclization of compounds **78** occurred by the addition of $FeCl_3$/C and $N_2H_4 \cdot H_2O$ at reflux to give indoles **79**.



Scheme 13

1.4 Intramolecular Michael Addition/Elimination

Nakao et al. have reported the new discovery that offers the advantage of a facile one-pot 2-acyl indole-3-acetic acid synthesis via an intramolecular Michael addition reaction at room-temperature. They treated *trans*-2-aminocinnamate **80** with K_2CO_3 to generate indoline **81**. Subsequent treatment with a base, such as DBU or Cs_2CO_3 promoted elimination and rearomatization to give indoles **82** (Scheme 14) in excellent yields (Nakao *et al.*, 2003).



Scheme 14

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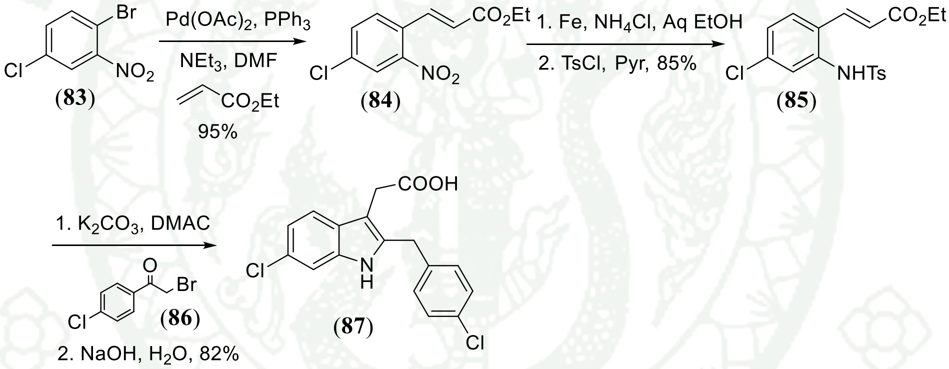
In 2003, Caron and co-workers reported the synthesis of 6-chloro-2-(4-chlorobenzoyl)-1*H*-indol-3-ylacetic acid (**87**) by intramolecular Michael addition/elimination. This method was very simple and highly efficient. In this synthesis, the Michael addition reaction of 2-bromo-5-chloronitrobenzene (**83**) with ethyl acrylate took place to generate cinnamate **84** in 95% yield. Then reduction of the nitro group of compound **84** and protection of the resulting aniline gave sulfonamide **85**. The alkylation of sulfonamide **85** by K_2CO_3 in dimethyl acetamide (DMAC) proved to be the optimal combination to afford the dihydroindole intermediate. However, addition of sodium hydroxide in the single pot promoted both the elimination of the toluenesulfonic acid and the hydrolysis of the ester to provide acid **87** in 82% overall yield.



Scheme 15

1.5 Dieckmann condensation

In 1997, Bös and co-workers reported the synthetic procedures of 10-methoxy-pyrazinol [1,2-*a*] indoles **91** and their applications in the synthesis of a partial agonist at the $5HT_{2c}$ receptor (Bös *et al.*, 1997). The indole ring was prepared from the appropriate antranilates **88** in two steps In the first step, antranilates **88** were alkylated at the N atom with bromoacetate in the presence of K_2CO_3 to provide phenylglycines **89**. In the second step, intramolecular ester condensation of compounds **89** occurred to provide 3- hydroxyindole-2-carboxylates **90** in good yields.

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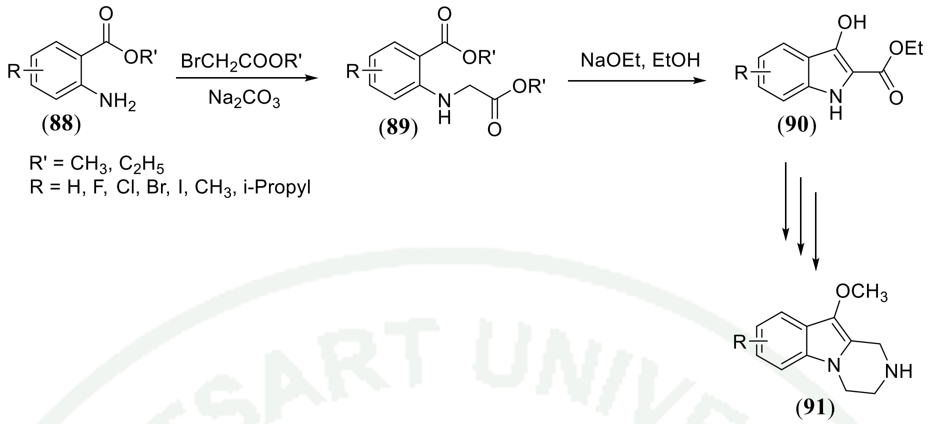
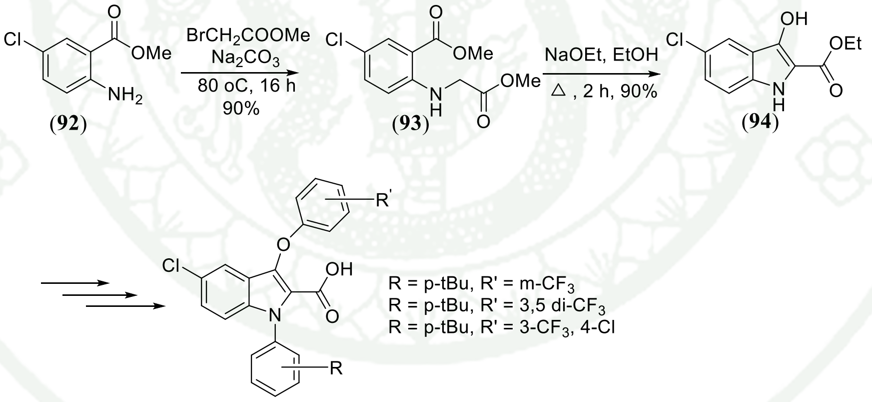


Scheme 16

Dropinski *et al*. reported a series of novel aryl indole-2-carboxylic acids, which have potent selective PPARγ modulators. They synthesized indole **94** via the Dieckmann condensation (Scheme 17) (Dropinski *et al*., 2005).



Scheme 17

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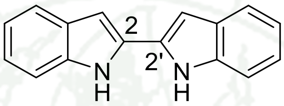
2. Synthesis of indolocarbazole alkaloid

2.1 Synthesis of bisindole alkaloid

The dimeric indole (bisindole) alkaloids have been known for over 200 years. They were discovered in living organisms through dimerization of monomeric indole bases. Of special interest is a group of natural products that are essential ingredients of drugs represented by the aglycon of antibiotic rebeccamycin **2** and staurosporine **1**.

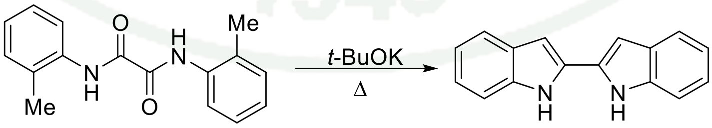


2,2' bisindole (**95**)

Figure 10 General structure of bisindole alkaloid

2.1.1 Double Madelung cyclization

2,2'-Bisindolyl was first synthesized by double Madelung involve of *N,N'*-bis(o-tolyl)oxamide, which now he named that the double Madelung cyclization reaction. The Madelung cyclization was successful by potassium tert-butoxide at high temperature gave the desire product in modest yields (Madelung, 1912) (Madelung, 1914).



(**96**) (**95**)

Scheme 18

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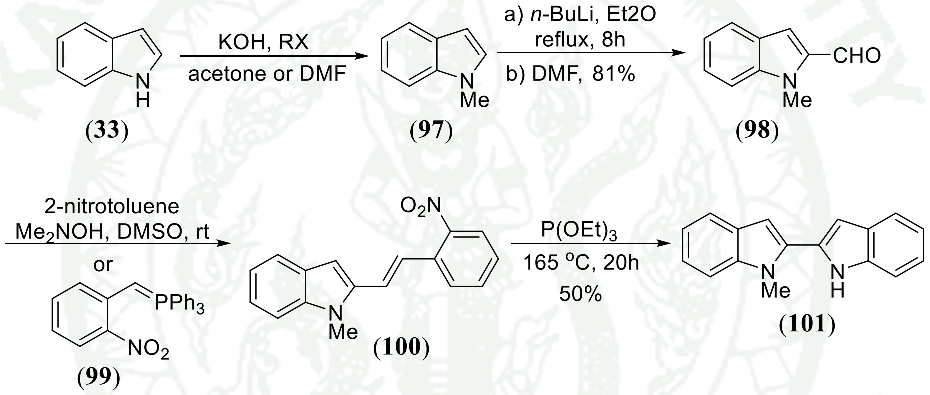
2.1.2 Nitrene cyclization reaction

In 2001, Merlic *et al.* studied the strategy to employ the nitrene cyclization reaction of Cadogan to build one indole ring (Merlic *et al.*, 2001). The *N*-methylindole-2-carboxaldehyde **98** was synthesized in two steps with *N*-alkylation or sulfonylation of indole and formylation at the 2 position of *N*-methylindole. Subsequent condensation of *N*-methylindole-2-carboxaldehyde **98** with 2-nitrotoluene **99** gave indole substituted alkene **100** in modest yield. In the last step, cyclization of *trans*-alkene **100** with triethylphosphite provided biindole **101** in 50% yield.



Scheme 19

In 2003, Kuethe *et al.* used nitrene cyclization reaction for preparation of the biindoles **106** (Kuethe *et al.*, 2003). Alcohol **104** was prepared from TMS-nitro compound **102** and indole carboxaldehyde **103** with a catalytic amount of tetrabutylammonium fluoride (TBAF). This alcohol was then treated with TFAA to generate a corresponding trifluoroacetate, which underwent an elimination reaction when treated with DBU at 60 ^{o}C to give *trans*-nitrostyrene **105** in 85% overall yield. Reductive cyclization of *trans*-nitrostyrene **105** by Cadogan/Sundberg conditions [$P(OEt)_3$] gave biindole **106** in 73% yield. Alternatively, palladium-catalyzed reductive cyclization of *trans*-nitrostyrene under Söderberg conditions provided biindole **106** in 96% yield.

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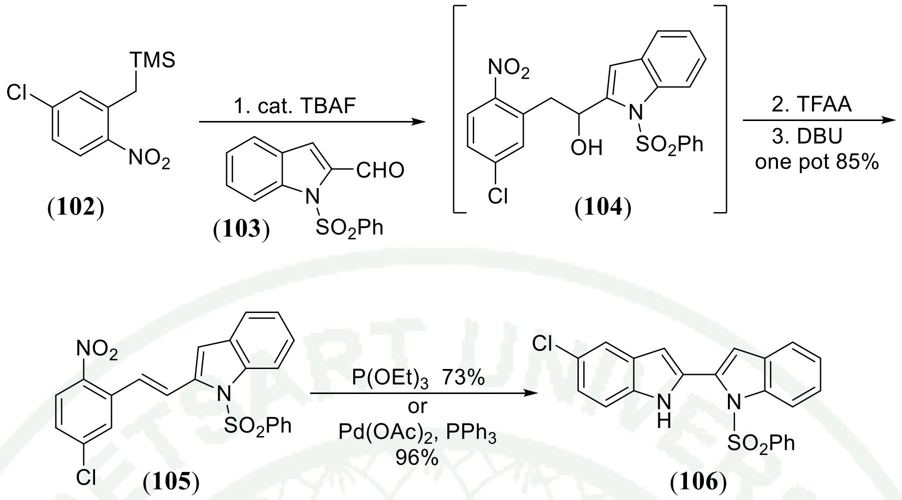
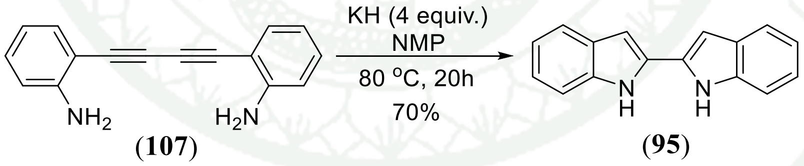


Scheme 20

2.1.3 Coupling reaction

In 2003, Koradin and co-workers (Koradin *et al.*, 2003) synthesized a bis-indole by the cyclization of symmetrical diaminodiyne derivative under basic conditions (KH (4 equiv.), 80 °C, 20 h) (Scheme 21).



Scheme 23

Bergman *at al.* reported the synthesis of 2,2-biindolyl **116** in modest yield from dimerizations between 2-chloroindoles and 3-methylindole (Bergman *et al.*, 1995).

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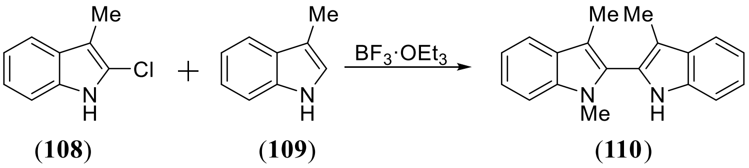
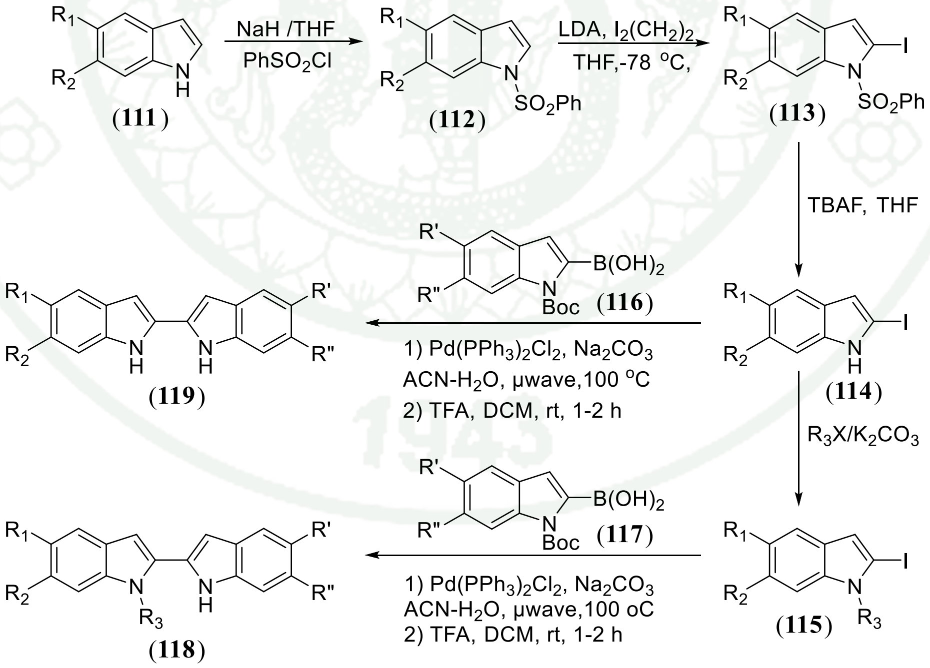


Scheme 22

2.1.4 Palladium catalyzed cross-coupling reaction

Kumar and co-workers presented a detailed account of bisindole synthesis (Kumar *et al.*, 2014). The unprotected indoles **114** were synthesized in three steps as follows: protection of indoles **111**, iodination of the resulting protected indoles, and subsequent deprotection of the iodinated products **113**. Then they treated 2-iodoindole **113** with boronic acids **116** under standard Suzuki–Miyaura conditions and finally the Boc protecting group was removed with TFA (Scheme 23).



Scheme 23

In 1993, Palmisano *et al*. published the use of Stille reaction in the synthesis of bisindoles (Palmisano and Santagostino, 1993). The Stille reaction is a means to

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couple stannane **119** with suitable halogenated partner **120** in the presence of a palladium catalyst. In this research, the desired bisindole **121** was obtained in an excellent yield (97%).

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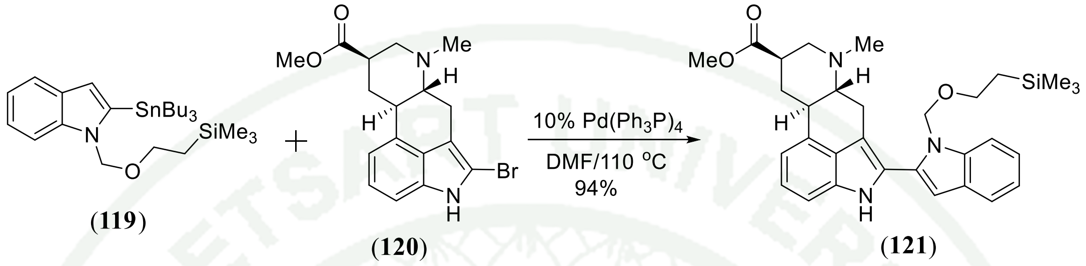

Scheme 24

2.2 Synthesis of indenotryptoline alkaloid

N-Boc-3-ethynyl-indole-2-carbaldehyde **122** was employed as a common precursor for the synthesis of indenotryptoline alkaloids. In 2010 Waldmann and co-workers synthesized homofascaplysin C **127** and fascaplysin **128** from compound **122** via two routes. In the first route, they used silver-catalyzed cascade for the synthesis of pentacyclic core **126** and *tert*-butyl ester **125**. Subsequent formylation of **126** with $POCl_3$ provided homofascaplysin C **127** with an overall yield of 53%. Also, the pentacyclic core **126** was oxidized with peracetic acid followed by salt formation to give fascaplysin **128** in 52% overall yield. Partial reduction of the *tert*-butyl ester of compound **125** by means of *in situ* generated lithium diisobutylpiperidinohydroaluminate gave the natural product homofascaplysin C **127** in 48% overall yield (Waldmann *et al.*, 2010).

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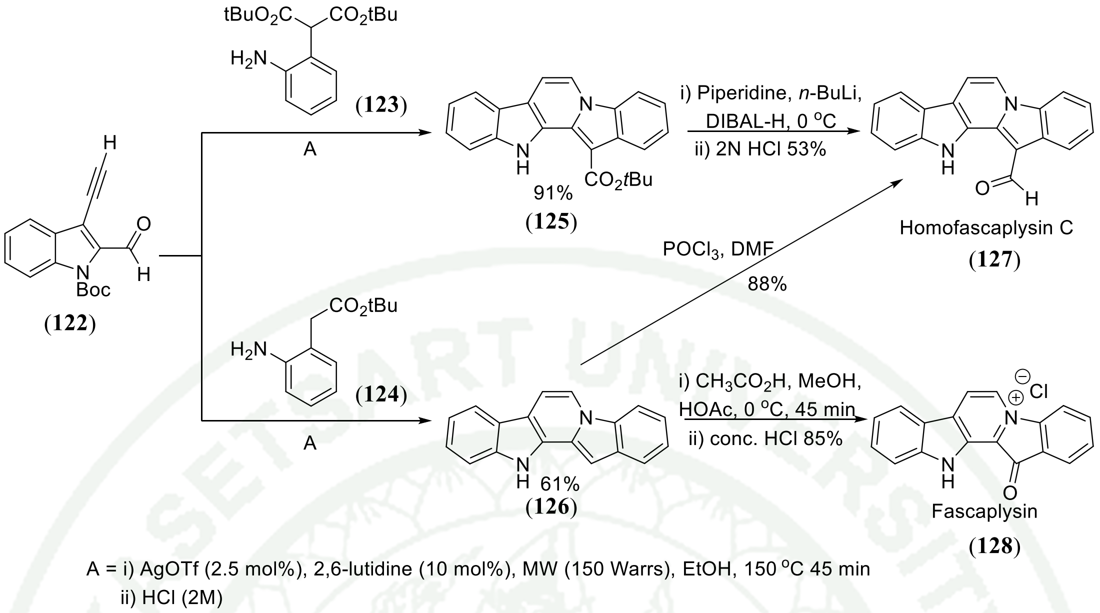

Scheme 25

In 2012, Kimura *et al*. reported the synthesis of BE-54017 whose structure is very similar to cladoniamide G, our target.. Their synthesis commenced with the Stille coupling reaction between stannane **129** and 1.2 equiv of methoxyacetyl chloride to give compound **130**. In the next step, Fischer indole synthesis protocol of the 2-acylindole derivative **130** with phenylhydrazine provided unstable bisindole intermediate **131**, which was immediately used without purification for the succeeding Michael addition to maleimide to afford **132** in 31% over two steps. The cyclization of **132** was treated by Pd black in nitrobenzene under heating at 200 °C to generate **133** in moderate yield (48%). Methylation of the resulting indenotryptoline intermediate **133** with excess MeI in the presence of KOH as a base afforded **134** in 70% yield. Finally, dihydroxylation of **134** with OsO_4 completed the synthesis of BE-54017 **135** in 37% yield as shown in Scheme 26 (Kimura *et al*., 2012).

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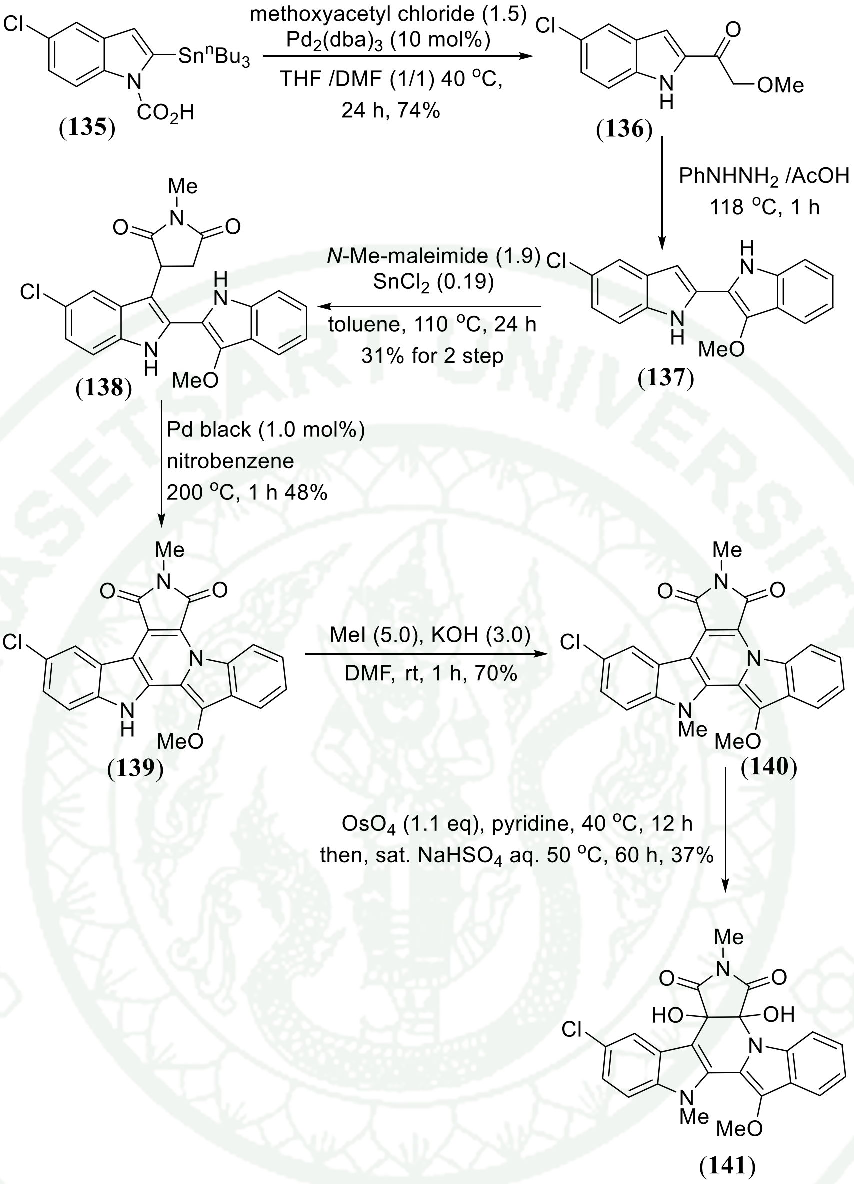

Scheme 26

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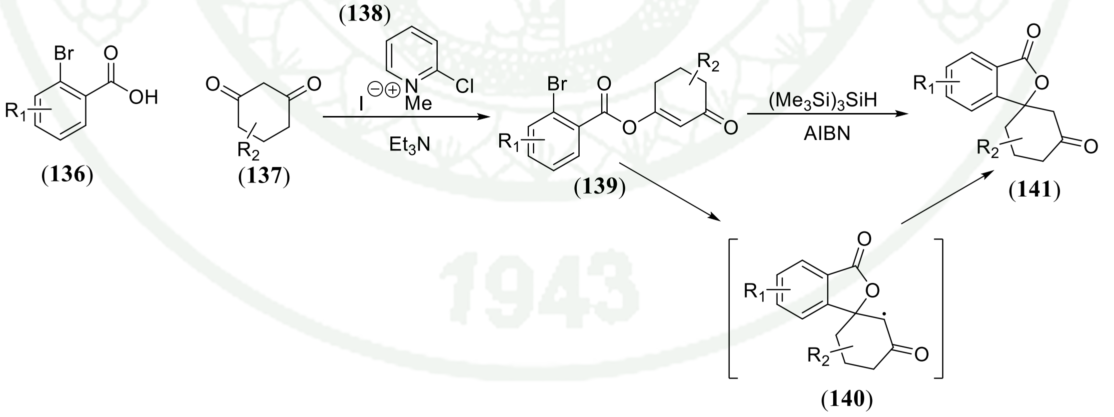
3. Synthesis of potential biologically active compounds against the H5N1 bird flu virus

3.1 Friedel–Crafts reaction

The Friedel–Crafts reactions are a set of reactions developed by Charles Friedel and James Crafts in 1877 in order to attach substituents to an aromatic ring. There are two main types of Friedel–Crafts reactions: alkylation reactions and acylation reactions. In 1999, Zhang and co-workers discovered a simple method for the synthesis of keto spiro-γ-lactones and keto spiro-γ-lactams by free radical cyclization. The synthesis of spirobenzolactones **141** proceeded via a two-step process. In the first step, 2-bromobenzoic acids **136** reacted with cyclic 1,3-diones **137** in the presence of Mukaiyama coupling agent **138** to give enol ester **139**. The enol esters were used as aryl radical precursors for spirocyclizations. The enol esters reacted with tris(trimethylsilyl)silane (1.5 equiv.) and catalytic amount of 2,2′-azobis(2-methylpropisonitrile) (AIBN) to provide keto spiro-γ-lactones **141** through an α-acyl stabilized radical **140** as shown in Scheme 27 (Zhang and Pugh, 1999).



Scheme 27

In 2003, Nicolaou *et al*. have applied a *C*-alkylation to synthesize enol esters (Nicolaou *et al.*, 2005). Initially, ester **142** was first brominated at its free benzylic position by NBS, benzoyl peroxide, CCl_4 in high yield. The resulting bromo

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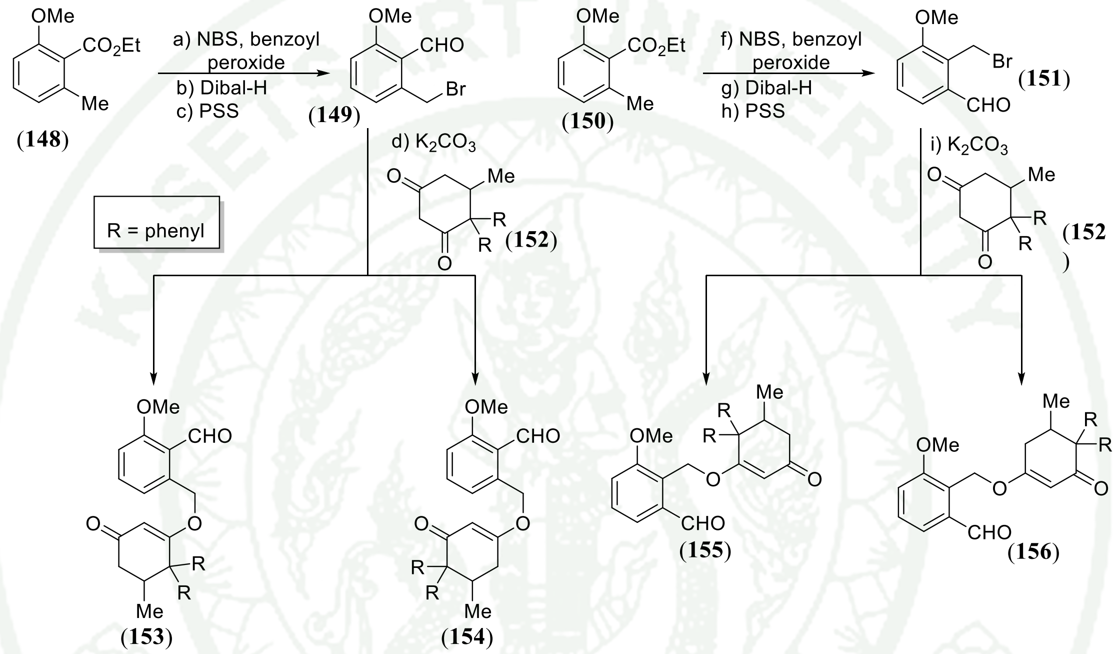
compound was then reduced to aldehydes **143** by using a two-step reduction-partial reoxidation sequence with DIBAL-H, followed by PCC. After that alkylation 1,3-cyclohexadiones **146** with benzylic bromide **143** using K_2CO_3 as a base to afforded products **147** and **148** as shown in Scheme 28. In addition, attempts to use borane dimethylsulfide instead of DIBAL-H still afforded enol ester products.



Scheme 28

In 1978, Akhrem *et al*. used this *C*-acylation to prepare 2-acylcychlohexane-1,3-dione. They synthesized 1,3-diketone in excellent yields by *C*-acylation with pyridine in chloroform or dichloromethane as shown in Scheme 29 (Akhrem *et al.*, 1978).

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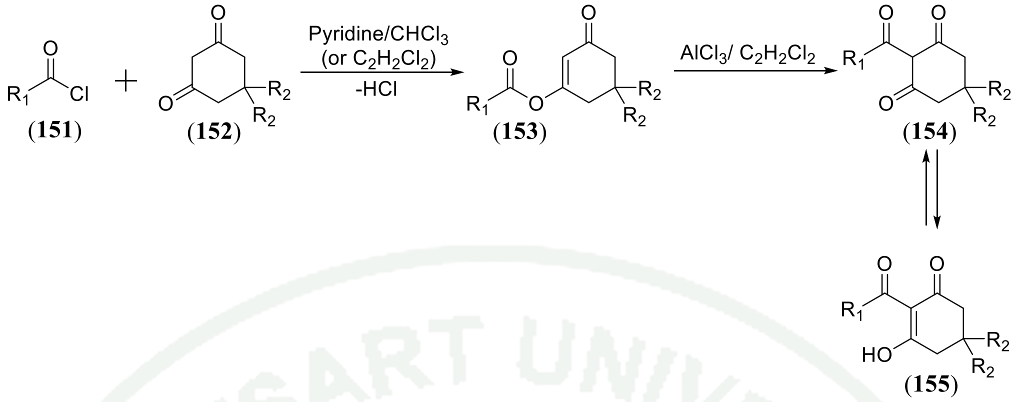
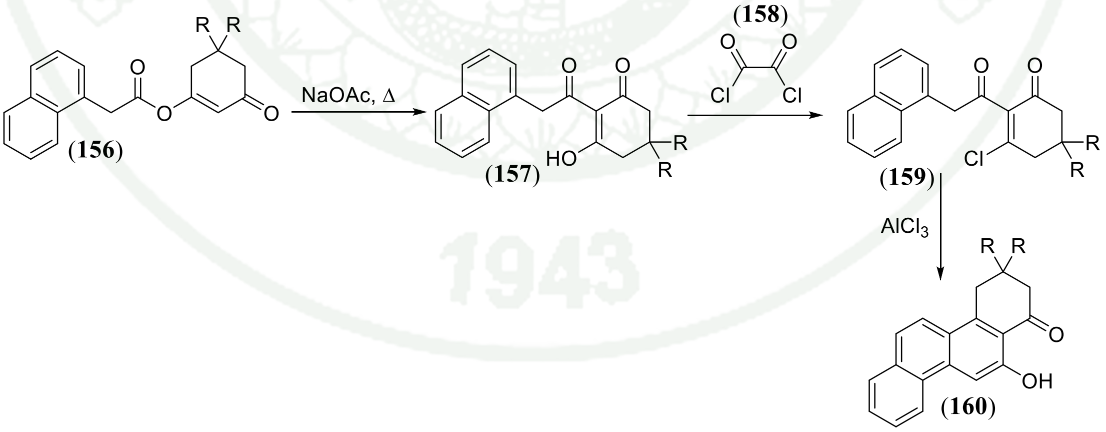


Scheme 29

In 1985, Lakhvich *et al*. used the intramolecular Friedel-Crafts reaction in the synthesis of tetrahydrochrysenone **160**. The isomerization of triketone occurred by heating 1-naphthylacetates **156** with anhydrous sodium acetate. This triketone underwent fragmentation to generate acid chloride when it reacted with oxalyl chloride **159**. Finally, cyclization of acid chlorides **159** by a Friedel-Crafts reaction provided a tetrahydrochrysenones **160** in good yield as shown in Scheme 30 (Lakhvich *et al.*, 1985).



Scheme 30

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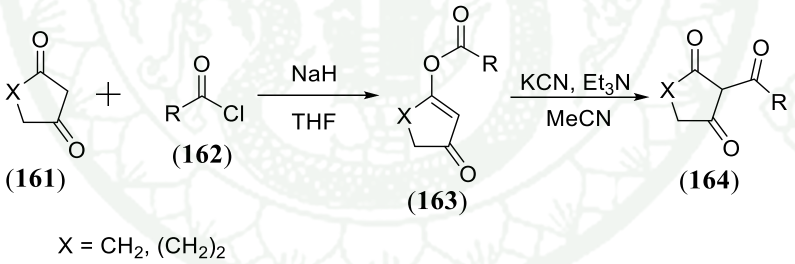
3.2 Rearrangement reaction

A rearrangement reaction is a broad class of organic reactions where the carbon skeleton of a molecule is rearranged to give a structural isomer of the original molecule. Often a substituent moves from one atom to another atom in the same molecule. An example of the rearrangement reactions was already mentioned earlier in Scheme 29. In this rearrangement, enol esters **153** were transformed to diketenes **154** by aluminium chloride in dicloromethane.

In 2003, Kim and coworkers reported the procedure to synthesize 2-alkanoyl or 2-benzoylcycloalkane-1,3-diketones (T. Kim *et al.*, 2003). In their procedure, 1,3-cyclopentanedione or 1,3-cyclohexanedione **161** and acyl chlorides **162** were used as the starting materials in the presence of sodium hydride in tetrahydrofuran to give enol esters **163**. After that rearrangement of enol ester with KCN and Et_3N in acetonitrile provided triketones **164** in good yield (Scheme 31).



Scheme 31

In 2007, Khlebnikova *et al.* reported the preparation of triketones **168** (Khlebnikova *et al.*, 2007). In this synthesis, acylation of 5,5-dimethylcyclohexane-1,3-dione **165** with fluorobenzoic acids **166** took place in the presence of pyridine in absolute chloroform to give compounds **167a-e** in 73-95% yields. The *O-C* isomerization of fluorinated enol acylates **167a-e** generated 2-benzoylcyclohexane-1,3-diones **168a-e** in excellent yields under the action of catalytic amounts of acetone cyanohydrin and triethylamine in absolute acetonitrile (Scheme 32).

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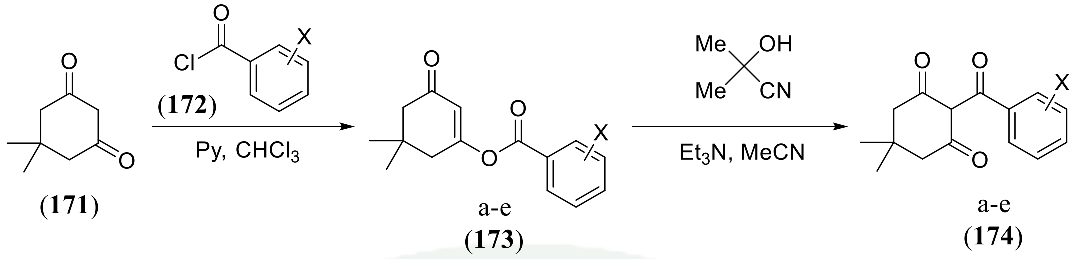
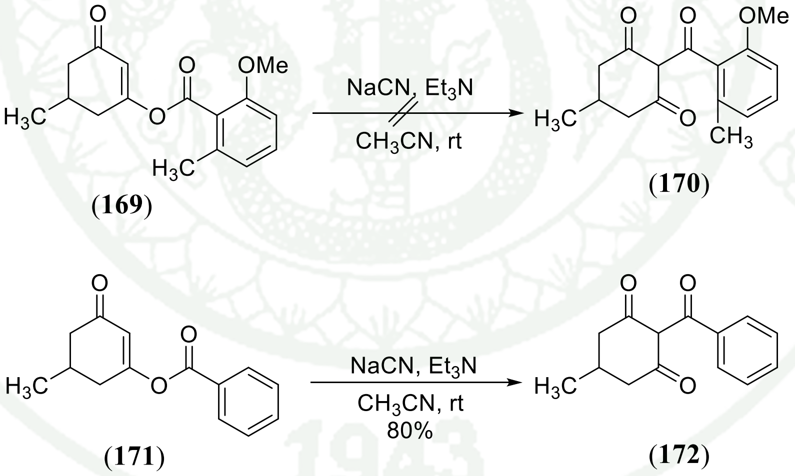


X = 2-F (a), 3-F (b), 4-F (c), 4-CF_3 (d), 2,3,4,5,6-F (e).

Scheme 32

In 2004, Patil *et al.* reported the rearrangement reaction in the presence of enol ester (Patil *et al.*, 2004). In this reaction, a cyanide-catalyzed rearrangement of enol ester **169** in triethylamine and acetonitrile at room temperature failed to give triketone **170**. However, when unsubstituted benzoate ester **171** was employed in cyanide-catalyzed rearrangement, it provided triketone **172** in good yield (Scheme 33).



Scheme 33

3.3 Methylation reaction

In the chemical sciences, methylation denotes the addition of a methyl group to a substrate or the substitution of an atom or group by a methyl group. Methylation is a form of alkylation with a methyl group, replacing a hydrogen atom. In 2007

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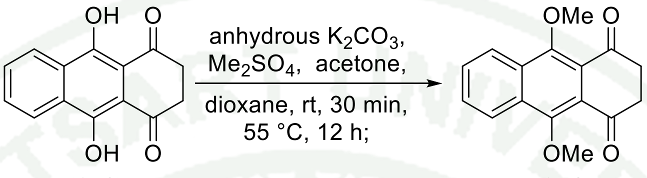
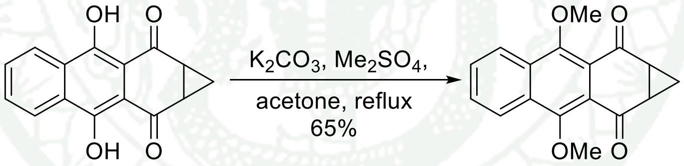
Palayangoda *et al.* reported the methylation of 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione **173** to give 2,3-dihydro-9,10-dimethoxy-1,4-anthracenedione **174** by using dimethyl sulfate in the presence of anhydrous potassium carbonate as shown in Scheme 34 (Palayangoda *et al.*, 2007).



(**173**) (**174**)

Scheme 32

In 2008, Mal and Ray applied a methylation reaction to synthesize quinone **176** (Scheme 32). Methylation of the two hydroxyl groups of compound **175** with Me_2SO_4–K_2CO_3 provided **176** in 65 % yield (Mal and Ray, 2008).



(**175**) (**176**)

Scheme 32

3.4 Reductive amination of ketones

Reductive amination also known as reductive alkylation is a form of amination that involves the conversion of a carbonyl group to an amine via an intermediate imine. The carbonyl group is most commonly a ketone or an aldehyde. In 2011, Falus and co-workers investigated a one-pot reductive amination of aliphatic, cycloaliphatic, and aromatic ketones (Scheme 33). In their method, ketones **177** were treated with

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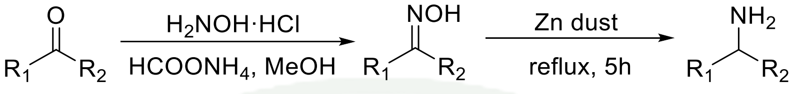
$H_2NOH{\cdot}HCl$ and $HCOONH_4$ in methanol to form oximes **178**. Then, oxime **178** were treated with Zn dust in methanol to give amides **179** (Falus *et al.*, 2011).



(**177**) (**178**) (**179**)

Scheme 33

In 2003, Kadyrov and Riermeier developed a highly active and enantioselective catalytic system for the reductive amination of ketone. The best enantioselectivities were observed Ru catalysts with a binap or a tol-binap ligand. In this reaction, they studied the conversion of phenylpyruvic acid **180** in methanolic ammonia at 60 °C in the presence of a Rh catalyst to amine **181** (Kadyrov and Riermeier, 2003).

(**180**) (**181**)

Scheme 34

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MATERIALS AND METHODS

Materials

Instrument

The following analytical procedures were used throughout this research unless indicated otherwise.

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a VARIAN$^{\text{UNITY}}$ INOVA 400 (400 MHz for ^{1}H and 300, 400, 500 MHz for ^{13}C). Chemical shifts of ^{1}H NMR spectra are reported in parts per million (ppm) relative to $CDCl_3$ (δ 7.26), CD_3OD (δ 3.31), DMSO-d_6 (δ 2.49) or acetone-d_6 (δ 2.04) if the former was absent. Data are presented as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = mutiplet, br = broad, dd = doublet of doublet), coupling constants (J) are given in Hertz. ^{13}C NMR spectra are reported in ppm relative to $CDCl_3$ (δ 77.0), CD_3OD (δ 49.0), DMSO-d_6 (δ 39.5) or acetone-d_6 (δ 29.8) if the former was absent.

Infrared (IR) spectra were recorded in cm^{-1} on a Perkin-Elmer 2000 Fourier transform infrared spectrophotometer at the Chemistry Department, Faculty of Science, Kasetsart University.

Low resolution mass spectra received on an Agilent Technology 1100 series LL/MSD Trap and on a GCMS-QP-5050QA spectrometer in electrospray ionization mode (ESI+) and high-resolution mass spectra (HRMS) were collected on Bruker Daltonics in ESI positive mode at the Scientific Equipment Center, Faculty of Science, Kasetsart University. The first number denoted m/z value, and the abundance and ion assignment are given in parentheses.

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Melting points (m.p.) were recorded on a Mel-Temp electrothermal apparatus at the Chemistry Department, Kasetsart University and are uncorrected.

Chromatographic system

Analytical thin-layer chromatography (TLC) was conducted on aluminum-backed 0.2 mm thick silica gel 60 F254 plates (Merck) and the chromatograms were visualized under a 254 nm UV and 356 mm Vis lamp and/or by spraying with a solution of vanillin (3% in ethanol with 3% sulfuric acid) followed by heating.

Flash column chromatography was performed on silica gel 60 (mesh size 0.040-0.063 mm).

Chemical reagents

Tetrahydrofuran (THF), diethyl ether and toluene were distilled from sodium and benzophenone under a nitrogen atmosphere. Dichloromethane and acetonitrile were distilled from calcium hydride under a nitrogen atmosphere. Methanol and ethanol were distilled from magnesium under a nitrogen atmosphere. All other commercially obtained chemicals were used as received.

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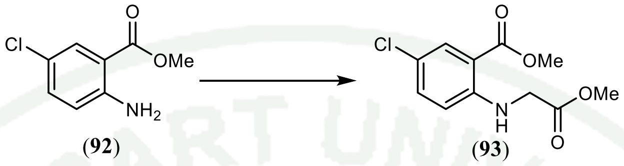
Methods

Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (93)



To a suspension of Na_2CO_3 (3.43 g, 32.4 mmol) in DMF (15 mL) was added Methyl 2-amino-5-chlorobenzoate (4 g, 21.6 mmol) followed by methylbromoacetate (3.4 mL, 25.9 mmol). The reaction mixture was heated at 80 ^{o}C for 15 h. It was allowed to cool to room temperature, then filtered through a fritted funnel and wash with EtOAc to remove Na_2CO_3. The filtrate was concentrated to dryness under reduced pressure. Water (30 mL) was added to the solid residue. The resulting suspension was then filtered through a fritted funnel and washed several times with water to collect a white solid. This solid was then dissolved in EtOAc (150 mL), dried over Na_2SO_4 and concentrated under reduced pressure to remove most of the solvent. The crude residue was allowed to sit at room temperature and ether (10 mL) was then added. A white precipitate formed immediately. The liquid phase was carefully removed with a pipette. Additional ether (5 mL) was added to the solid and the liquid phase was again carefully removed with a pipette to leave the desired product **93** as a white solid (5.20 g, 93%); m.p. 97-99 °C.

FTIR (film) 3446 (N-H), 1748 (C=O)

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.87 (d, *J* = 2.6 Hz, 1H), 7.28 (d, *J* = 8.9 Hz, 1H), 7.27 (d, *J* = 8.9 Hz, 1H), 6.45 (d, *J* = 9.0 Hz, 1H), 3.98 (s, 1H), 3.86 (s, 3H), 3.77 (s, 3H)

^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5, 167.7, 148.4, 134.4, 131.0, 120.3, 112.5, 111.8, 52.3, 51.8, 44.8

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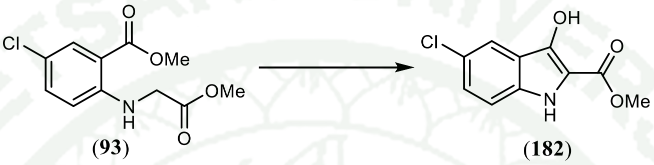
LRMS (EI), *m/z* (relative intensity): 270.4 (100%, $M+Na^+$)

HRMS (ESI) [$M+ Na^+$] Calcd. For $C_{11}H_{12}ClNNaO_4$ *m/z* 280.0347; found 280.0364

Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (182)



Anhydrous methanol (20 mL) was added slowly to a flask containing sodium (1.17 g, 38.9 mmol) at 0 °C and the suspension was stirred until sodium completely dissolved. This solution was then cannulated to a solution of α-amino ester **93** (5 g, 19.5 mmol) in THF (50 mL). The resulting solution was heated at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and water (30 mL) was added. This mixture was extracted with ether (2 × 30 mL) to remove impurities. To the remaining aqueous phase was added dry ice until a precipitate formed (pH~8). The precipitate was collected by filtration through a fritted funnel and washed several times with water. This off-white solid was dissolved in EtOAc (150 mL), dried over Na_2SO_4 and concentrated to dryness under reduced pressure to give the desired product **182** as a pale purple/brown solid (3.29 g, 66% yield); m.p. 208-210 °C.

FTIR (film) 3368 (O-H), 1647 (C=O)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.08 (s, 1H), 9.52 (s, 1H), 7.76 (d, *J* = 1.8 Hz, 1H), 7.29 (d, *J* = 8.9 Hz, 1H), 7.20 (dd, *J* = 2.2, 8.8 Hz, 1H), 3.82 (s, 3H);

^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.7, 141.9, 133.1, 125.7, 122.8, 119.1, 118.9, 114.0, 110.6, 51.1

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LRMS (ESI) *m/z* (relative intensity) 248.5 (40%, M+Na$^+$)

HRMS (ESI) [M+Na]$^+$ calcd for $C_{10}H_8ClNNaO_3$ 248.0085, found 248.0087

Methyl 5-Chloro-3-methoxy-1*H*-indole-2-carboxylate (182)

(**182**) (**183**)

To a mixture of hydroxyindole **182** (1 g, 4.44 mmol) and K_2CO_3 (0.68 g, 4.89 mmol) was added acetone (15 mL) followed by dimethyl sulfate (0.5 mL, 5.33 mmol). The reaction mixture was stirred at room temperature for 18 h, and then concentrated to dryness under reduced pressure. The crude residue was dissolved in EtOAc (30 mL) and poured into water (30 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 30 mL) The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography on SiO_2 (1:4 and 1:2 ether/hexanes) to give the desired product 186 as a white solid (1.02 g, 96% yield); m.p. 144-146 °C.

FTIR (film) 3325 (N-H), 1692 (C=O)

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.76 (s, 1H), 7.73 (s, 1H), 7.27-7.23 (m, 2H), 4.10 (s, 3H), 3.97 (s, 3H),

^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.6, 144.2, 132.5, 126.6, 125.7, 121.4, 119.4, 115.5, 113.3, 62.4, 52.0

LRMS (ESI) *m/z* (relative intensity) 262.1 (100%, M+Na$^+$)

HRMS (ESI) [M+Na]$^+$ calcd for $C_{11}H_{10}ClNNaO_3$ 262.0241, found 262.0233

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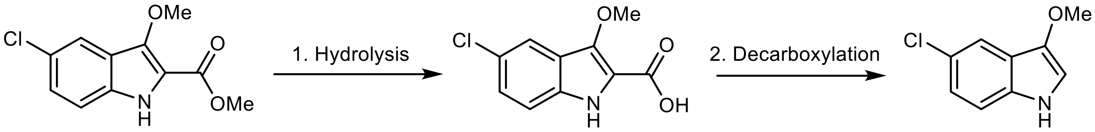
5-Chloro-3-methoxy-1*H*-indole (185)



(**183**) (**184**) (**185**)

To a solution of methoxy-indole **183** (1 mg, 4.17 mmol) in MeOH was added 1 M aq. NaOH solution (7 mL, 8.34 mmol). The reaction mixture was heated at reflux for 1 h. It was then allowed to cool to room temperature, dilute with EtOAc (20 mL) and poured into ice cooled 1M HCl (10 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (20 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure to give product **184** as a white solid; m.p. 182-184 °C (recrystallized from hexane).

FTIR (film) 3250 (N-H), 1722 (C=O), 1469 (O-H)

^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 11.29 (s, 1H), 7.64 (d, *J* = 2.0, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 7.20 (dd, *J* = 2.1, 8.8, 1H), 3.96 (s, 3H), 2.08 (s, 1H)

^{13}C NMR (DMSO-d$_6$, 125 MHz) δ 162.2, 142.1, 131.8, 124.7, 123.7, 120.8, 118.2, 118.1, 114.3, 61.8

LRMS (ESI) *m/z* (relative intensity) 248.3 (100%, M+Na$^+$)

HRMS (ESI) [M+Na]$^+$ calcd for $C_{10}H_8ClNNaO_3$ 248.0085, found 248.0087

To a mixture of carboxylic acid **184** and Cu powder (1 g, 4.43 mmol) was added distilled quinoline (5 mL). The reaction mixture was heated to 200 ^{o}C for 1 h. It was then allowed to cool to room temperature, diluted with EtOAc (100 mL) and poured into ice-cooled 1 M HCl (100 mL) The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude residue

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was purified by flash chromatography on SiO_2 (1:4, 1:3 and 1:2 ether/ hexanes) to give the desired product **185** as a brown liquid (712 mg, 94% yield over 2 steps).

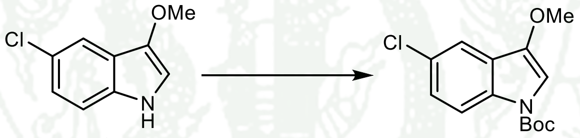
FTIR (film) 3341 (N-H)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (s, 1H), 7.53 (br s, 1H), 7.14 (m, 2H), 6.68 (d, *J* = 2.5 Hz, 1H), 3.88 (s, 3H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.4, 134.5, 124.4, 123.0, 120.5, 117.3, 112.2, 105.5, 58.1

HRMS (ESI) $[M+H]^+$ calcd for C_9H_9ClNO 182.0367, found 182.0362

***N*-Boc 5-chloro-3-methoxy-1*H*-indole (186)**



(**185**) (**186**)

To a solution of 3-methoxyindole **185** (300 mg, 1.65 mmol) in CH_3CN (10 mL) was added DMAP (40 mg, 0.17 mmol) followed by Boc_2O (432 mg, 1.98 mmol). The reaction mixture was stirred at room temperature for 1 h. It was concentrated to dryness and the crude residue was purified by flash chromatography on SiO_2 (1-3% ether in hexanes) to give the desired product **186** as a clear and coloress oil (449 mg, 97% yield).

FTIR (film) 1727 (C=O)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (br s, 1H), 7.53 (d, *J* = 2.2 Hz, 1H), 7.26 (dd, *J* = 2.2, 8.8 Hz, 1H), 6.97 (br s, 1H), 3.88 (s, 3H), 1.66 (s, 9H)

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^{13}C NMR (CDCl$_3$, 75 MHz) δ 149.7, 143.7, 127.9, 125.3, 124.8, 117.7, 116.2, 104.5, 83.5, 57.6, 28.2, 27.8

LRMS (ESI) *m/z* (relative intensity) 270.1 (100%, M+Na$^+$)

HRMS (ESI) [M+Na]$^+$ calcd for $C_{14}H_{16}ClNNaO_3$ 304.0711, found 304.0715

N-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (187)

(**186**) (**187**)

To a solution of *N*-Boc-3-methoxyindol **186** (570 mg, 2.02 mmol) in CCl$_4$ (7 mL) was added NBS (1 g, 6.07 mmol). The resulting suspension was stirred at room temperature under N$_2$ for 15 min. The reaction mixture was filtered through a pad of celite and washed with CCl$_4$ (5 mL) The filtrate was concentrated to ~3 mL under reduced pressure. This solution was purified by flash chromatography on basic Al$_2$O$_3$ (5% ether in hexanes) to give the desired product **187** as a clear and colorless oil (550 g, 76% yield).

FTIR (film) 1737 (C=O)

^{1}H NMR (Acetone d$_6$, 400 MHz) δ 8.12 (d, *J* = 8.8 Hz, 1H), 7.60 (d, *J* = 2.0 Hz, 1H), 7.31 (dd, *J* = 2.15, 8.9 Hz, 1H), 3.98 (s, 3H), 1.68 (s, 9H)

^{13}C NMR (Acetone d$_6$, 75 MHz) δ 206.1, 149.4, 143.0, 134.1, 129.0, 125.7, 124.9, 117.8, 117.2, 101.4, 86.1, 61.6, 28.2

LRMS (ESI) *m/z* (relative intensity) 259.2 (13%, M-Boc+H$^+$)

HRMS (ESI) [M+Na]$^+$ calcd for $C_{14}H_{15}ClBrNNaO_3$ 381.9816, found 381.9806

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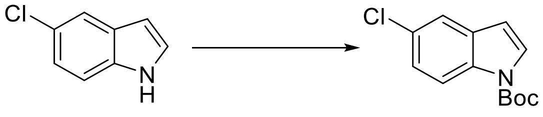
N-Boc-5-chloro indole (188)


(**23**) (**188**)

To a solution of 3-methoxyindole **23** (4 g, 26.39 mmol) in CH_3CN (25 mL) was added DMAP (40 mg, 0.17 mmol) followed by Boc_2O (349 mg, 2.64 mmol). The reaction mixture was stirred at room temperature for 1 h. It was concentrated to dryness and the crude residue was purified by flash chromatography on SiO_2 (3-5% ether in hexanes) to give the desired product **188** as a clear and coloress oil (6.56 g, 99% yield).

FTIR (film) 1737 (C=O)

^{1}H NMR (Acetone d_6, 400 MHz) δ 8.08 (d, *J* = 8.7 Hz, 1H), 7.61 (d, *J* = 3.7 Hz, 1H), 7.51 (d, *J* = 2.1 Hz, 1H), 7.26 (dd, J = 2.1, 8.8 Hz, 1H), 6.49 (d, J = 3.7 Hz, 1H), 1.68 (s, 9H)

^{13}C NMR (Acetone d_6, 75 MHz) δ 149.3, 133.5, 131.6, 128.1, 127.0, 124.2, 120.3, 116.0, 106.5, 83.9, 28.0

LRMS (ESI) *m/z* (relative intensity) 259.2 (13%, M-Boc+H^+)

HRMS (ESI) $[M+Na]^+$ calcd for $C_{13}H_{14}ClNNaO_2$ 381.9816, found 381.9806

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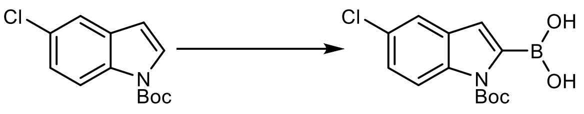
N-Boc-5-chloro-1H-indol-2-yl boronic acid (189)



(**188**) (**189**)

Preparation of LDA: A solution of diisopropylamine (12 mL, 85.82 mmol) in anhydrous THF (30 mL) was cooled to 0 °C and 1.6 M *n*-BuLi in THF (43 mL, 64.37 mmol) was added. The reaction mixture turned slightly yellow (almost colorless). It was stirred at 0 °C for 30 min. A solution of *N*-Boc indole **188** in anhydrous THF (40 mL) was cooled to 0 °C and triisopropyl borate (24.7 mL, 107.28 mmol) was added followed by the solution of LDA (prepared above). The reaction mixture turned yellow and turbid, and it was stirred in an ice bath for 1 h. This reaction mixture was then diluted with 1 M HCl (~100 mL) and extracted with ether (2 × 100 mL). The yellow organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness to give a yellow solid. This crude residue was purified by flash chromatography on SiO_2 (1:2 ether/hexanes) to give the desire product **189** as a white solid (12.73 g, 80% yield); m.p. 153-155 °C.

FTIR (film) 3353 (O-H) 1700 (C=O)

^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.24 (s, 2H), 8.04 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 1.9 Hz, 1H), 7.29 (dd, J = 2.2, 8.8 Hz, 1H), 6.59 (s, 1H), 1.58 (s, 9H)

^{13}C NMR (DMSO-d_6, 75 MHz) δ 149.5, 140.3, 134.6, 132.1, 126.9, 123.7, 119.9, 115.8, 111.2, 84.5, 27.5

LRMS (ESI) *m/z* (relative intensity) 346.4 (100%, M+Na$^+$)

HRMS (ESI) [M-H_2O+2MeOH+Na] calcd for $C_{15}H_{19}BClNNaO_4$ 346.0993, found 346.0998

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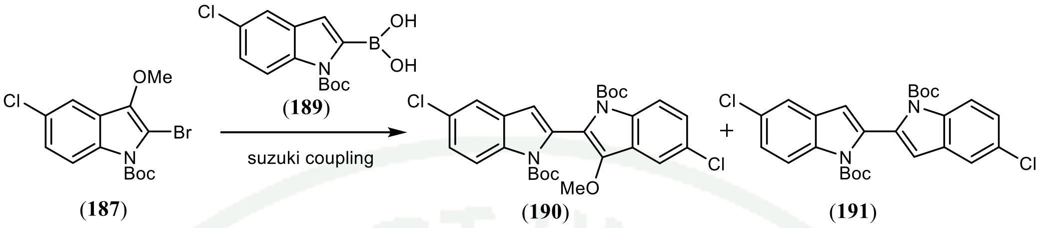
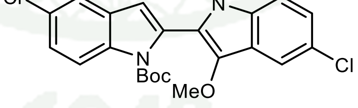
N,N'-diBoc-5,5’-dichloro-3-methoxy- 1*H*,1’*H* -2,2’-bisindole (190)



A Schlenk flask containing indolyl boronic acid **190** (898 mg, 3.04 mmol), $PdCl_2(PPh_3)_2$ (106 mg, 0.15 mmol) and Na_2CO_3 (322 mg, 3.04 mmol) was evacuated under high vacuum and then filled with N_2. A solution of bromoindole **188** (550 mg, 1.52 mmol) in 1,4-dioxane (16 mL) was added to the Schlenk flask followed by deoxygenated water (4 mL) The reaction mixture was heated at reflux for 2 h. It was then allowed to cool to room temperature and poured into ice water (100 mL) This mixture was extracted with ether (3 × 100 mL). The organic extract was dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography on SiO_2 (1-5% ether in hexanes) to give the desired product **190** as a colorless oil (559 mg, 69% yield); m.p. 99-101 °C

N,N'-diBoc-5,5’-dichloro-3-methoxy-1*H*,1’*H* -2,2’-bisindole (190)



(190)

FTIR (film) 1730 (C=O)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.23 (d, *J* = 9.0 Hz, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 2.5 Hz, 1H), 7.75 (d, *J* = 2.3 Hz, 1H), 7.45 (dd, *J* = 2.1, 9.1 Hz, 1H), 7.42 (d, *J* = 2.3, 9.0 Hz, 1H), 6.91 (s, 1H) 3.74 (s, 3H), 1.19 (s, 9H), 1.16 (s, 9H)

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^{13}C NMR (DMSO-d_6, 75 MHz) δ 148.9, 148.8, 141.4, 134.9, 132.1, 129.7, 129.2, 127.4, 127.3, 125.8, 124.9, 124.0, 120.3, 118.3, 117.5, 116.9, 116.5, 111.9, 83.7, 83.5, 61.1, 27.1

LRMS (ESI) *m/z* (relative intensity) 553.4 (100%, $M+Na^+$)

HRMS (ESI) $[M+Na]^+$ calcd for $C_{27}H_{28}Cl_2N_2NaO_5$ 553.1267, found 553.1270

3-Methoxy-1*H*,1'*H*-2,2'-bisindole (26)

(**190**) (**26**)

To an ice-cooled solution of bisindole **190** (149 mg, 0.32 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL). The reaction mixture was stirred for 2 h (0 °C to rt). The resulting yellow solution was cooled to 0 °C and sat. $NaHCO_3$ (20 mL) was added. This mixture was then extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude residue was purified by flash chromatography on SiO_2 (1:1 ether in hexanes) to give the desired product **26** as a green solid (78 mg, 90% yield); m.p. 236-234 °C.

FTIR (film) 3416 (N-H)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.34 (s, 1H), 11.18 (s, 1H), 7.70 (d, *J* = 1.9 Hz, 1H), 7.63 (d, *J* = 1.9 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.39 (d, *J* = 8.6 Hz, 1H), 7.13 (dd, *J* = 2.1, 8.7 Hz 1H), 7.10 (dd, *J* = 2.1, 8.7 Hz 1H), 6.96 (d, *J* = 1.6 Hz 1H), 4.04 (s, 3H)

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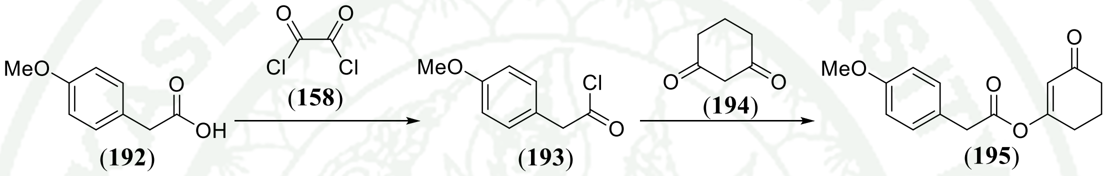
^{13}C NMR (DMSO-d_6, 75 MHz) δ 135.8, 135.3, 132.4, 130.9, 129.2, 124.1, 123.7, 122.3, 121.4, 121.3, 119.3, 118.8, 116.8, 113.2, 113.2, 98.6, 61.3

LRMS (ESI) *m/z* (relative intensity) 331.3 (69%, M+H$^+$)

HRMS (ESI) $[M+H]^+$ calcd for $C_{17}H_{13}Cl_2N_2O$ 331.0399 , found 331.0381

3-Oxocyclohex-1-en-1-yl 2-(4-methyoxyphenyl)acetate (195)



To a solution of 4-methoxyphenylacetic acid (**192**) (500 mg, 3 mmol) in dichloromethane (5 ml) was added oxalyl chloride (0.52 ml, 6.2 mmol) followed by, two drops of DMF. The reaction mixture was stirred at room temperature for 1h, Then, excess oxalyl chloride was evaporated in vacuo to give acyl chloride as a yellow solid. This solid was dissolved in dry dichlorometane (5 ml) and 1,3-cyclohexanedione (371 mg, 3.31 mmol) was added followed by pyridine (261 mg, 3.31 mmol). The reaction mixture was stirred at room temperature for 16 h, then diluted with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The organic phase was dried over sodium sulfate and evaporated to dryness in vacuo. The crude residue was purified by flash chromatography on SiO_2 (1:3 ethylacetate/hexane) to give enol ester **195** as a primrose oil (765 mg, 98% yield)

FTIR (film) 1719 (C=O), 1611 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.17 (d, *J* = 8.3 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 3.75 (s, 3H), 3.65 (s, 2H), 2.45 (t, *J* = 6.2 Hz, 2H), 2.33 (t, *J* = 6.71 Hz, 2H), 1.96 (quint, *J* = 6.4, 13.1 Hz, 2H)

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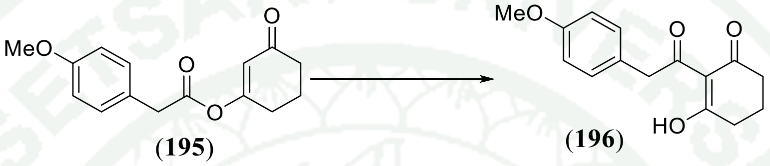
^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.5, 169.8, 168.2, 158.8, 130.1, 124.4, 117.3, 114.0, 55.0, 40.3, 36.4, 28.0, 21.0

HRMS (ESI) $[M+Na]^+$ calcd for $C_{15}H_{16}O_4$ 283.0941 , found 283.0944

3-Hydroxy-2-(2-(4-methoxyphenyl)acetyl)cyclohex-2-enone (196)



Enol ester **195** (400 mg, 1.54 mmol) was added to a stirred suspension of 4-dimethylaminopyridine (38 mg, 0.31 mmol) in dry toluene (4 mL). The reaction mixture was stirred for 2h at 110 ^{o}C. It was allowed to cool to room temperature, diluted with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic phase was dried over sodium sulfate, and concentrated in vacuo. The crude residue was purified by flash chromatography on SiO_2 (50% ethylacetate in hexanes) to provide diketone **196** as a yellow oil (330 mg, 83% yield).

FTIR (film) 1664 (C=O), 1509 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.19 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.2 Hz, 2H), 4.30 (s, 2H), 3.77 (s, 3H), 2.65 (t, *J* = 6.3 Hz, 2H), 2.49 (t, *J* = 6.6 Hz, 2H), 1.97 (m, 2H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.5, 198.4, 195.2, 158.4, 130.7, 126.3, 113.7, 112.6, 55.0, 45.4, 38.6, 32.9, 18.8

LRMS (ESI) *m/z* (relative intensity) 283.4 (100%, $M+Na^+$)

HRMS (ESI) $[M+Na]^+$ calcd for $C_{15}H_{16}O_4$ 283.0941, found 283.0949

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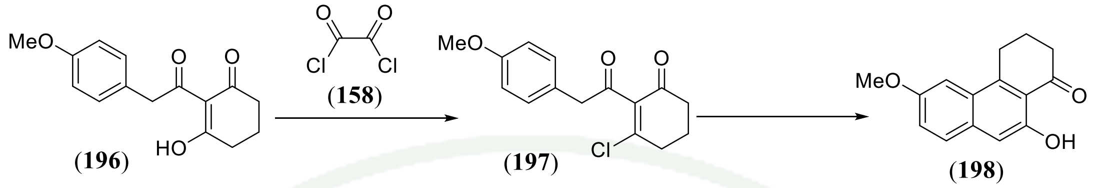
10-Hydroxy-6-methoxy-3,4-dihydrophenanthren-1(2*H*)-one (198)



To a solution of compound **196** (1.1 g, 4.2 mmol) in dichloromethane (10 mL) was added oxalyl chloride (1.1 mL, 12.7 mmol) followed by two drops of DMF. The reaction mixture was stirred at room temperature for 1h, Then, excess oxalyl chloride was evaporated in vacuo to give acid chloride **297** as a yellow oil. This oil was dissolved in dry 1,2-dichloroethane (10 mL) and anhydrous aluminium chloride (564 mg, 4.2 mmol) was added. The reaction mixture was stirred for 2 h at room temperature, then ice water (50 mL) was added. This mixture was extracted with dichlorometane (3 × 30) The extract was dried over magnesium sulfate, and evaporated in vacuo. The crude residue was purified by flash chromatography on SiO_2 (50% ethylacetate in hexanes) to provide product compound **298** as a yellow solid (1 g, 98% yield); m.p. 136-138 °C.

FTIR (film) 3443 (O-H), 1647 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.82 (s, 1H) 7.57 (d, *J* = 8.9 Hz, 1H), 7.22 (dd, *J* = 2.5 Hz, 1H), 7.19 (d, *J* = 2.5 Hz, 1H) 7.10 (s, 1H), 3.91 (s, 3H), 3.32 (d, *J* = 6.2 Hz, 2H), 2.79 (d, *J* = 6.7 Hz, 2H), 2.27 (quint, *J* = 6.4, 12.9 Hz, 2H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.7, 156.1, 155.6, 143.3, 133.6, 128.5, 126.0, 122.4, 118.2, 110.3, 102.9, 55.2, 38.7, 26.1, 22.2

LRMS (ESI) *m/z* (relative intensity) 247.7 (100%, $M+H^+$)

HRMS (ESI) $[M+Na]^+$ calcd for $C_{15}H_{14}O_3$ 265.0859, found 265.0850

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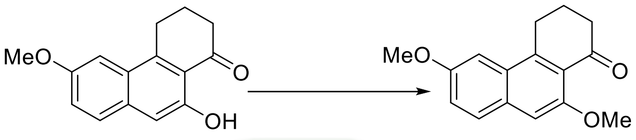
6,10-Dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one (199)



(**198**) (**199**)

To a mixture of compound **198** (50 g, 0.21 mmol) and K_2CO_3 (57 g, 0.41 mmol) was added acetone (2 mL) followed by dimethyl sulfate (52 mg, 39 mL, 0.41 mmol). The reaction mixture was stirred at room temperature for 18 h, and then concentrated to dryness under reduced pressure. The crude residue was dissolved in EtOAc (30 mL) and poured into water (30 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 30 mL) The organic extracts were combined, dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude product was purified by flash chromatography on SiO_2 (and /hexanes) to give the desired product **199** as a white solid (48 mg, 98 % yield); m.p. 110-112 °C.

FTIR (film) 2936 (C-H), 1678 (C=O)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (d, *J* = 8.6 Hz, 1H), 7.20 (m, 2H), 7.02 (s, 1H) 3.93 (s, 3H), 3.81 (s, 3H), 3.24 (t, *J* = 6.2 Hz, 2H), 2.69 (t, *J* = 6.7 Hz, 2H), 2.20 (quint, *J* = 6.5, 13.0 Hz, 2H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 198.2, 156.5, 154.9, 143.5, 131.4, 128.6, 127.2, 123.7, 120.9, 105.8, 103.4, 55.8, 55.2, 40.3, 26.4, 22.3

LRMS (ESI) *m/z* (relative intensity) 279.6 (100%, M+Na$^+$)

HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{16}O_3$ 279.0992, found 279.0993

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6,10-dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one oxime (200)

(**199**) (**200**)

To a solution of ketone **199** (50 mg, 0.2 mmol) in EtOAc (1 mL) was added hydroxylamine hydrochloride (27 mg, 0.39 mmol) followed by pyridine (1 mL). The reaction mixture was stirred at room temperature for 2 h. The solution was poured into water and extracted with EtOAc (3 × 25 mL). The combine organic extracts were dried over anh. Na_2SO_4, filtered and concentrated under reduced pressure. The crude oxime was purified by (1:1, EtOAc:Hexane) to give compound **200** as a yellow solid (45 mg, 85% yield); m.p. 202-204 °C.

FTIR (film) 3649 (O-H), 1506 (C=C)

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63 (d, *J* = 8.9 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 7.16 (dd, *J* = 2.4, 8.8 Hz, 1H) 7.09 (s, 1H), 4.00 (s, 3H), 3.93 (s, 3H), 3.11 (t, *J* = 6.1 Hz, 2H), 3.02 (t, *J* = 6.7 Hz, 2H) 1.97 (quint, *J* = 6.5, 12.8 Hz, 2H)

^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.4, 153.9, 153.8, 138.3, 129.3, 128.5, 127.7, 120.7, 119.2, 105.3, 103.1, 55.4, 55.3, 26.1, 24.3, 20.8

HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{17}NO_3$ 272.1281, found 272.1283

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6,10-Dimethoxy-1,2,3,4-tetrahydrophenanthren-1-amine (201)

(**200**) (**201**)

To a mixture of oxime **200** (65 mg, 0.24 mmol) and MoO_3 (48 mg, 0.34 mmol) in MeOH (3 mL) was added $NaBH_4$ (181 mg, 4.79 mmol) portionwise. An exothermic reaction occurred with evolution of gas. The reaction mixture was stirred at room temperature for 2 h or until all of the starting material was consumed as indicated by TLC analysis. Sat. NaCl was added and the precipitate was filtered off. The filtrate was extracted with EtOAc (3 x 10 mL). The combine organic extracts were dried over anh.Na_2SO_4 and concentrated under reduced pressure to provide amine **201** as a brownish yellow solid (90% yield).

FTIR (film) 3431 (N-H), 1634 (C=C)

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.52 (s, 2H), 7.62(d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 2.3 Hz, 1H), 7.15 (dd, *J* = 2.4, 8.8 Hz, 1H) 4.69 (s, 1H), 3.90 (s, 3H), 3.74 (s, 3H), 3.23 (d, *J* = 16.8 Hz, 1H), 2.85 (m, 2H) 2.55 (d, *J* = 13.9 Hz, 1H), 2.35 (d, *J* = 13.9 Hz, 1H), 1.95 (m, 2H)

HRMS (ESI) $[M+H]^+$ calcd for $C_{16}H_{19}NO_2$ 258.1489, found 254.1493

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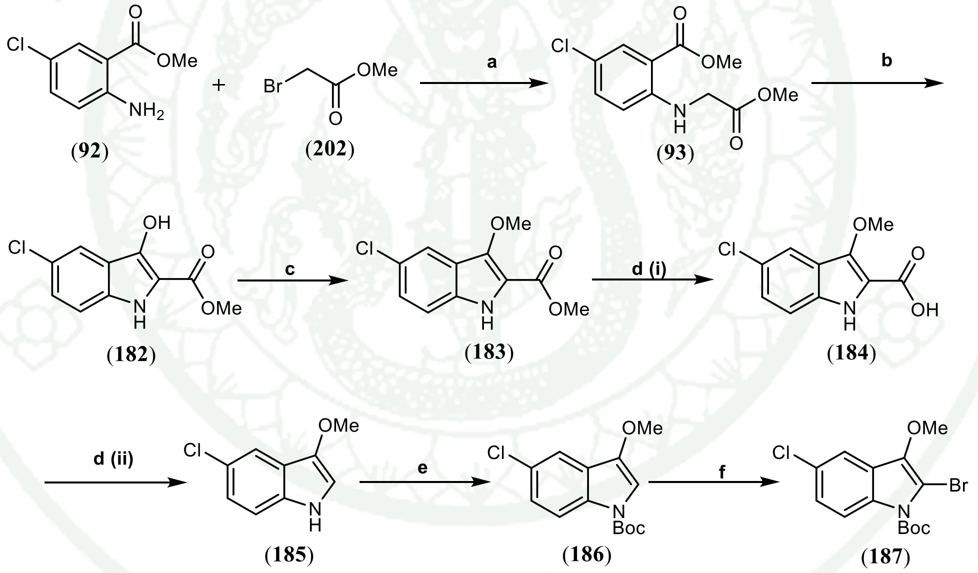
RESULTS AND DISCUSSION

Results

Synthesis of bisindole (26)

Bisindole **26**, a key intermediate for the synthesis of (±)-clado niamide G has been synthesized successfully in 11 steps with 17% overall yield from commercially available methyl-2-amino-5-chlorobenzoate and methyl bromoacetate as shown below

1. Preparation of *N*-Boc-5-chloro-2-bromo-3-methoxy-1*H*-indole (**187**)



Scheme 35

Reagents and conditions:

a) Na_2CO_3, DMF, 80 ^oC, 16 h, 83%

b) NaOMe, MeOH, THF, reflux, 2 h, 79%

c) Me_2SO_4, K_2CO_3, acetone, rt, 18 h, 91%

d) i) aq. NaOH, MeOH, reflux, 1 h

ii) Cu, quinoline, 200 ^oC, 2 h, 88% (over two steps)

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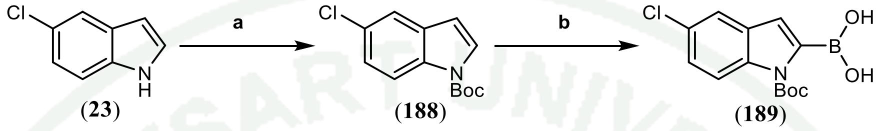
e) Boc_2O, DMAP, acetonitrile, rt, 30 min, 95%

f) NBS, CCl_4, rt, 15 min, 79%

2. Preparation of *N*-Boc-1*H*-indol-2-yl Boronic acid **189**.



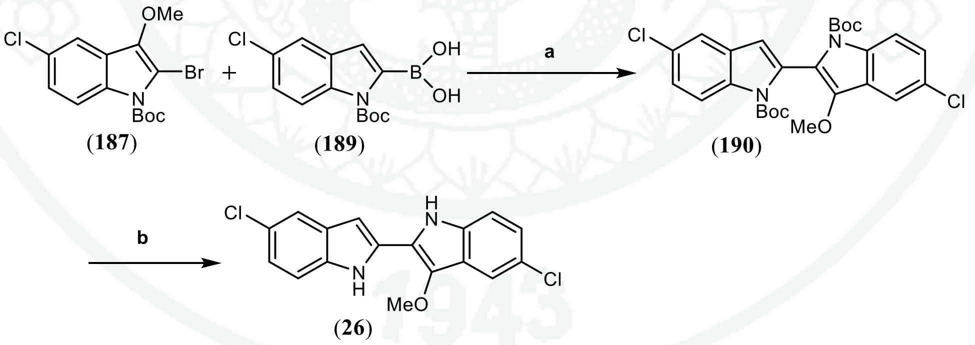
Scheme 36

Reagents and conditions:

a) Boc_2O, DMAP, acetonitrile, rt, 30 min, 95%

b) LDA, $B(^iPrO)_3$, THF, 0 ^oC, 2 h

3. Synthesis of *N*-Boc Dioxo-bisindole **32**.



Scheme 37

Reagents and conditions:

a) $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, H_2O, 80 ^oC, 2 h, 73%

b) TFA, CH_2Cl_2, 0 ^oC - rt, 18 h, 79%

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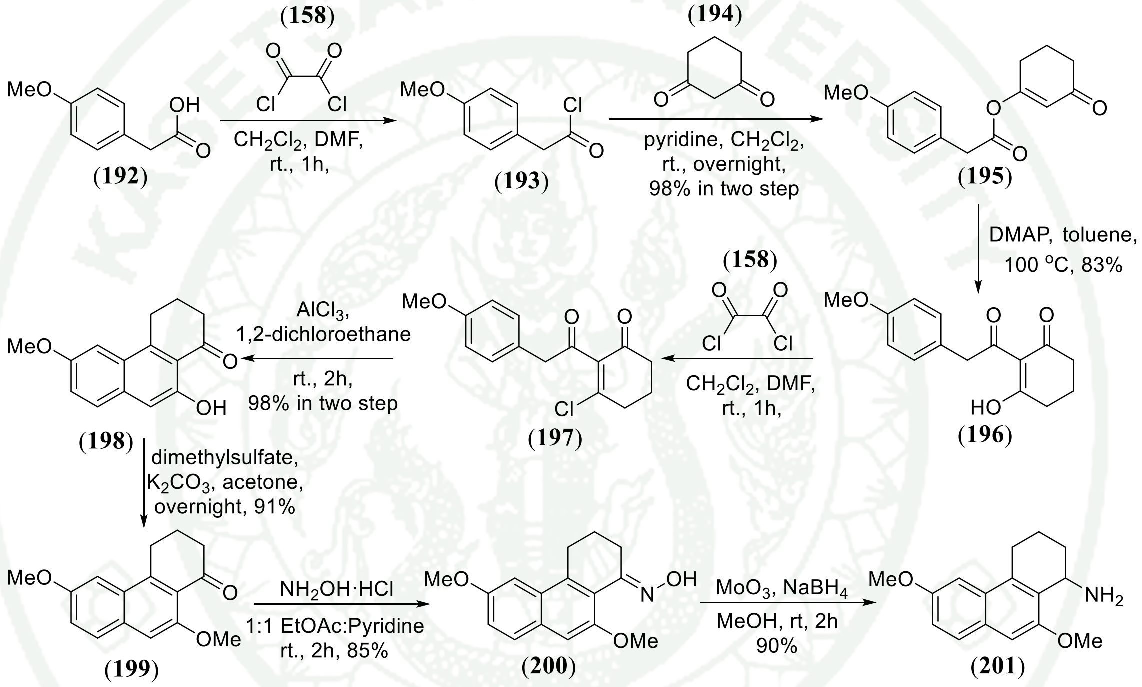
Synthesis of phenanthrenone 204

Phenanthrenone **204**, a key intermediate for the synthesis of potential biologically active compounds against the H5N1 bird flu virus has been synthesized successfully in 6 steps with 55% overall yield from commercially available 4-methoxyphenylacetic acid as shown in Scheme 35.



Scheme 38

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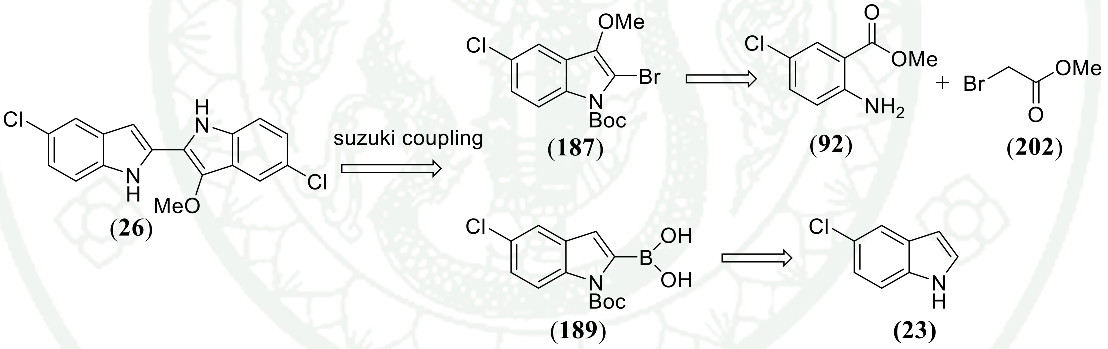
Discussion

Synthesis of bisindole (32)

In this research, we reported the synthetic studies of (±)-cladoniamide G based on our retrosynthetic analysis as shown below (Scheme 39). Miss Sarochar Soonkit had previously used this strategy to prepare a precursor for the synthesis of cladoniamide G's analog, so we chose this route as our main method. It was envisioned that two indole units of **26** could be prepared by a Suzuki cross-coupling reaction of bromoindole **187** with indolyl boronic acid **189**. Bromoindole **187** could then be prepared from commercially available methyl 2-amino-5-chlorobenzoate **92** and methyl bromoacetate **202**. In addition, boronic acid **189** could be derived from 5-cloroindole **23**.



Scheme 39

Synthesis of the *N*-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (185)

We began our research with the synthesis of methyl indole-2-carboxylate from methyl 2-amino-5-chlorobenzoate. In the first step, a bimolecular nucleophilic substitution between methyl 2-amino-5-chlorobenzoate and methyl bromoacetate took place to generate compound **93** in 93% yield. This compound then underwent the Dieckmann condensation reaction when it was treated with a base to provide **182** in

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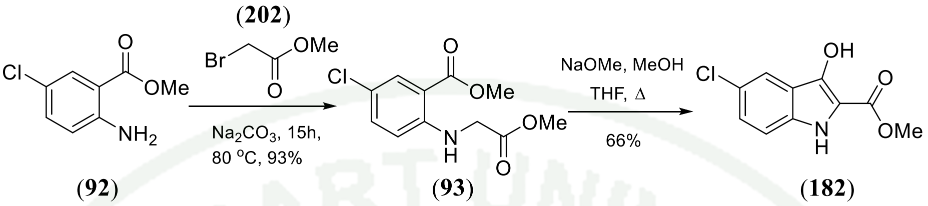
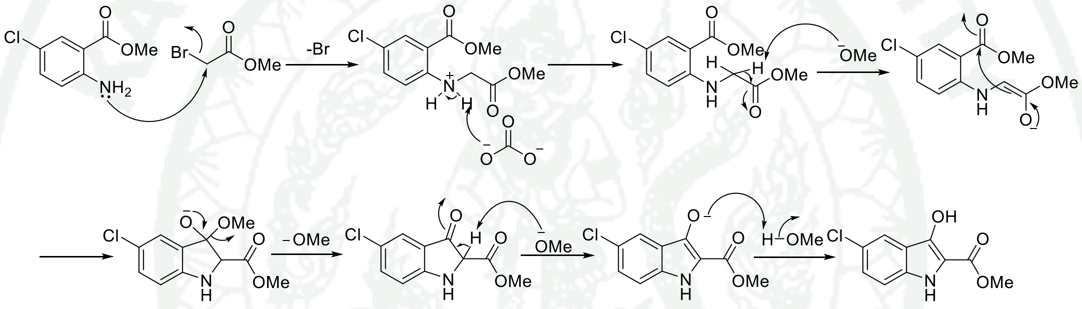
86% yield. The possible mechanism of these two steps is shown in Scheme 40 (Dropinski *et al.*, 2005).



Proposed the mechanism



Scheme 40

Treatment of methyl 5-chloro-2-(2-methoxy-2-oxoethylamino)benzoate **182** with excess dimethyl sulfate gave methyl 5-chloro-3-methoxy-1*H*-indole-2-carboxylate **183** in 96% yield (Unangst *et al.*, 1989). Methylation exclusively occurred at the hydroxyl group as *N*-methylated indole was not observed although dimethyl sulfate was used in excess. Then we prepared 5-chloro-3-methoxy-1*H*-indole **185** according to the method described by Liu et al., the ester group of **183** was hydrolyzed by aq. NaOH in MeOH at reflux for 1 h, and the resulting carboxylic acid **184** was decarboxylated with Cu powder in quinoline at 200 ^{o}C. This desired product was obtained in 94% yield over two steps (Cohen and Schambach, 1970). The possible mechanism of these two steps is shown in Scheme 41.

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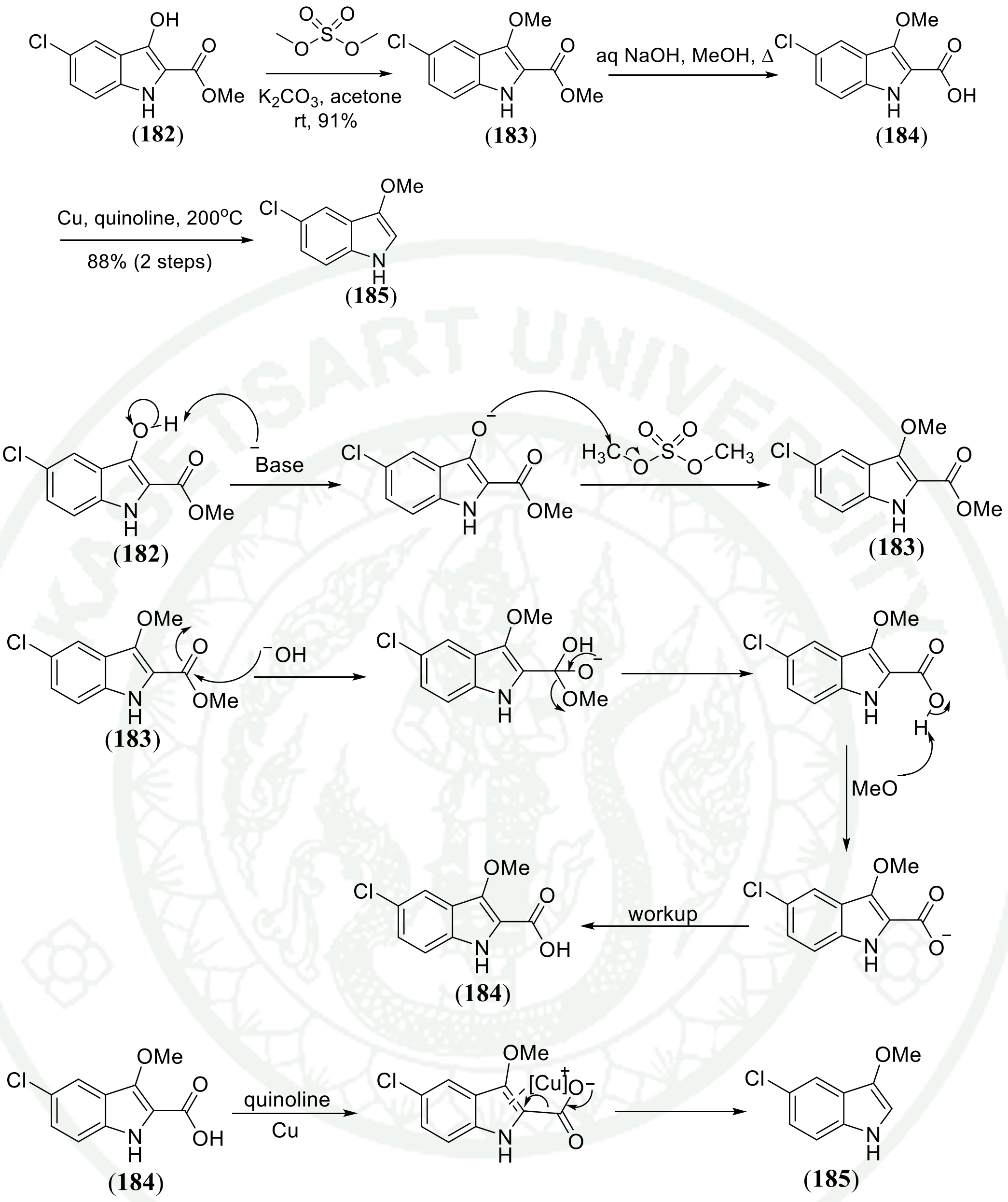

Scheme 41

Bromination of 3-methoxy indole **186** was successful when it was treated with NBS in CCl_4 at room temperature (Liu *et al.*, 1997). Bromination of this compound went to completion in 15 minutes at room temperature to give bromoindole **187** (Scheme 42). This desired product was not very stable in acidic conditions, and purification by chromatography on SiO_2 became problematic as some of this

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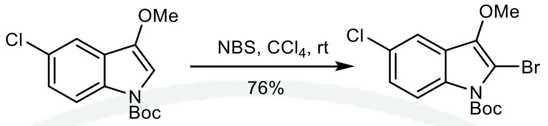
compound decomposed. Fortunately, basic Al_2O_3 could be used to replace SiO_2 so that this desired product was obtained in good yield.

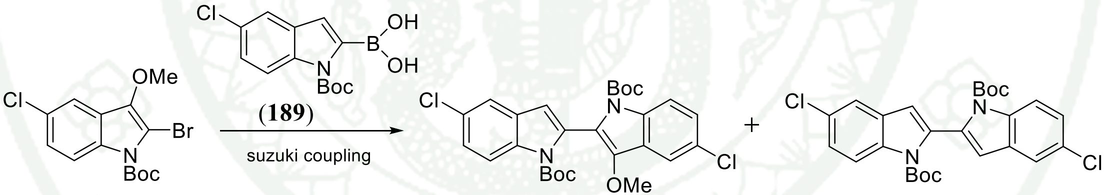


(**186**) (**187**)

Scheme 42

Synthesis of the *N,N′*-diBoc-5,5’-dichloro-3-methoxy-1*H*,1’*H* -2,2’-bisindole (190)

The Suzuki-coupling reaction of bromo indole **187** and boronic acid **189** (Vazquez *et al.*, 2002) generated bisindole **190** in 69% yield along with presumably minor product **191** (Scheme 43). The identity of this minor product was based on TLC analysis and our previous study on the synthesis of cladoniamide G’s analog.



(**187**) (**190**) (**191**)

Scheme 43

The possible mechanism of the Suzuki coupling reaction is shown in Scheme 44. It was found that the by-product **191** became dominant over the desired product **190** in some runs. We believed that the role of O_2 possibly has an effect to the reaction mechanism (Nising *et al.*, 2004) as it can oxidize the active Pd(0) catalyst to form Pd(II) (Scheme 45). This Pd(II) form does not allow an oxidative addition step to take place; only transmetallation is possible. Therefore, a homocoupling reaction occurred to give the minor product. The ratio of **190**:**191** was different in each run and it seemed that the amount of **191** increased if the evacuation of O_2 was not done properly.

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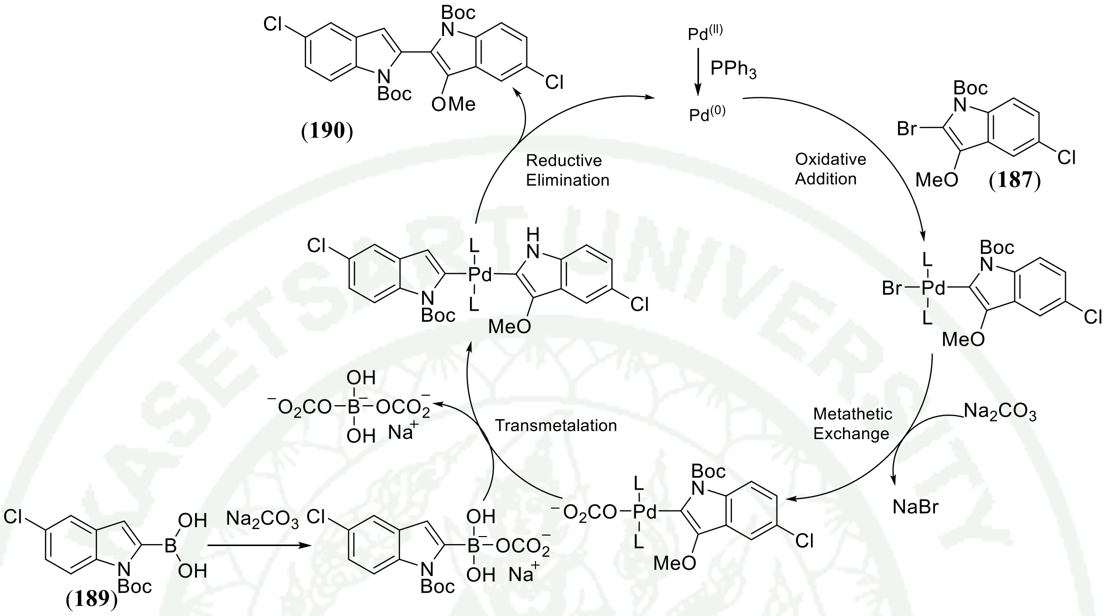
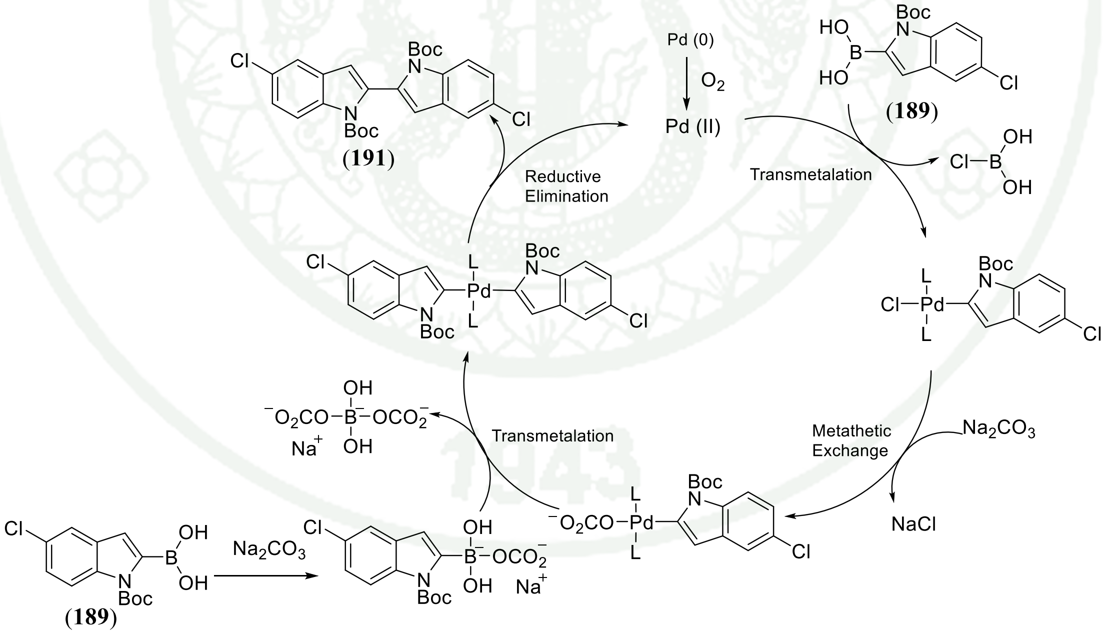
Mechanism for the Formation of **190**



Possible Mechanism for the Formation of **191**



Scheme 44

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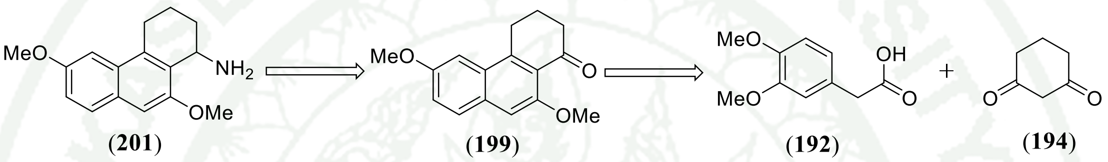
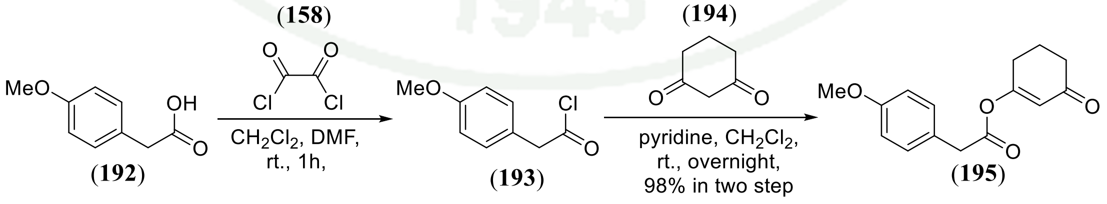
Synthesis of potential biologically active compounds against the H5N1 bird flu virus

Herein, we reported the progress on the synthesis of 1-amine-phenanthrenone. Our retrosynthetic analysis is shown in Scheme 45. We envisioned that 1-amine-phenanthrenone could be formed from compound **201** via reductive amination. The α,β-unsaturated ketone **199** could be formed via Friedel-Crafts reaction of acid chloride **192** and diketone **194**.



Scheme 45

We prepared compound **198** according to the method described by Lakhvich *et al.* (Lakhvich *et al.*, 1985). Carboxylic acid **192** was converted to acid chloride **193** by oxalyl chloride (Scheme 46). This reactive acid chloride was carried on to the next step immediately after the solvent and unreacted (excess) oxalyl chloride were removed under reduced pressure. When this acid chloride reacted with the enol form of 1,3-cyclohexanedione **194** in the presence of pyridine, enol ester **195** was obtained in 98% over 2 steps.



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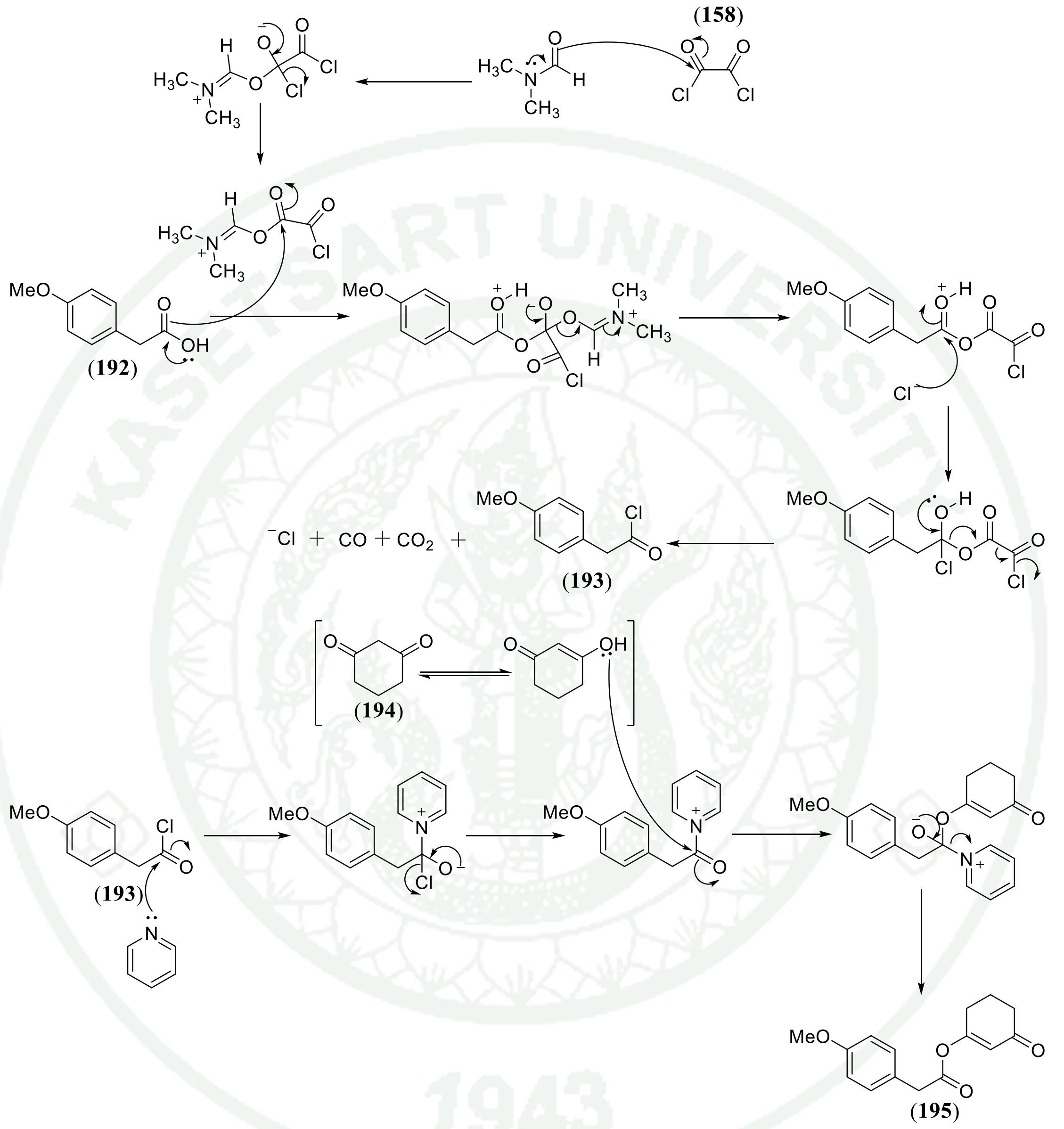
Possible Mechanism



Scheme 46

In the next step, we wanted to convert enol ester **198** to vinylogous carboxylic acid **196** via a rearrangement reaction. We first attempted to use aluminium trichloride for this rearrangement as the use of this Lewis acid had previously to produce this type of rearrangement (Akhrem *et al.*, 1978). Unfortunately, our first attempt following the described method did not yield the desired product. We tried to vary the reaction

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conditions by changing the amount of Lewis acid, solvent, and temperature (Table 3). However, We were still unable to obtain the desired product. When ester **195** was treated with DMAP in toluene at reflux rearrangement occurred to provide the desired product. The best yield (83%) was obtained when 0.2 equiv. DMAP was use and the reaction mixture was heated at reflux for 2 h.

Table 3 Condition of rearrangement reaction

Catalyst	**Equiv.**	**solvent**	**Temp (^{o}C)**	**Reaction time (h)**	**Product**
$AlCl_3$	1	Toluene	rt.	2	No desired product
$AlCl_3$	2	1,2-dichloroethane	rt.	2	No desired product
$AlCl_3$	2	1,2-dichloroethane	0	4	No desired product
$AlCl_3$	2	Toluene	rt.	1	No desired product
$AlCl_3$	4	1,2-dichloroethane	0	4	No desired product
$AlCl_3$	4	1,2-dichloroethane	0, rt.	1	No desired product
$AlCl_3$	4	Toluene	110	1	No desired product
$AlCl_3$	6	Toluene	rt.	1	No desired product
NaOAc	1	-	160	15	No desired product
DMAP	2	Toluene	rt-110	Overnight	trace amount[a]
DMAP	4	Toluene	rt-110	3	trace amount[a]
DMAP	4	Toluene	110	3	20%[b]

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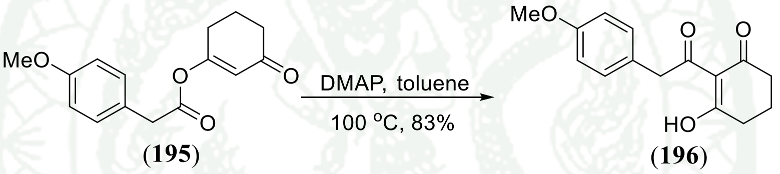
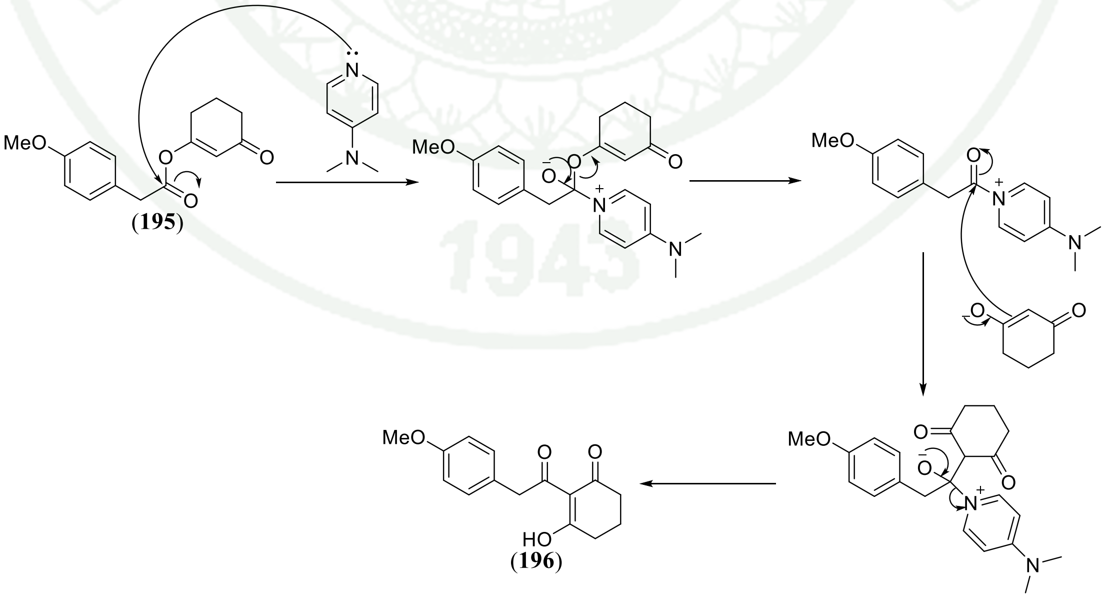
Catalyst	Equiv.	solvent	Temp (^{o}C)	Reaction time (h)	Product
DMAP	2.1	Toluene	110	3	34% ^{b}
DMAP	1.2	Toluene	110	1	73% ^{b}
DMAP	0.2	Toluene	110	2	83% ^{b}

[a] The starting material was dissolved in toluene then DMAP was added. The mixture stirred roomtemperature and warm up to 110 ^{o}C.

[b] The starting material and DMAP were dissolved in toluene separately and the resulting solutions were mixed together. The reaction mixture was submerged in an oil bath, which was preheated to 110 ^{o}C.



Possible Mechanism



Scheme 47

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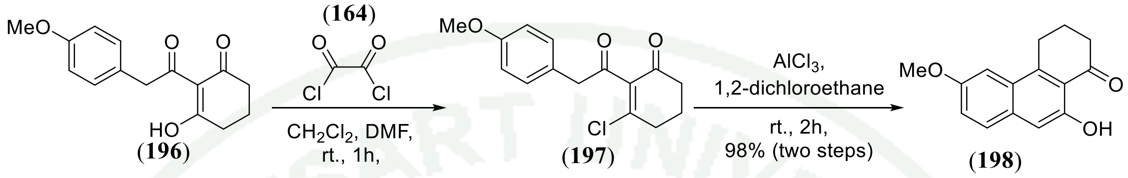
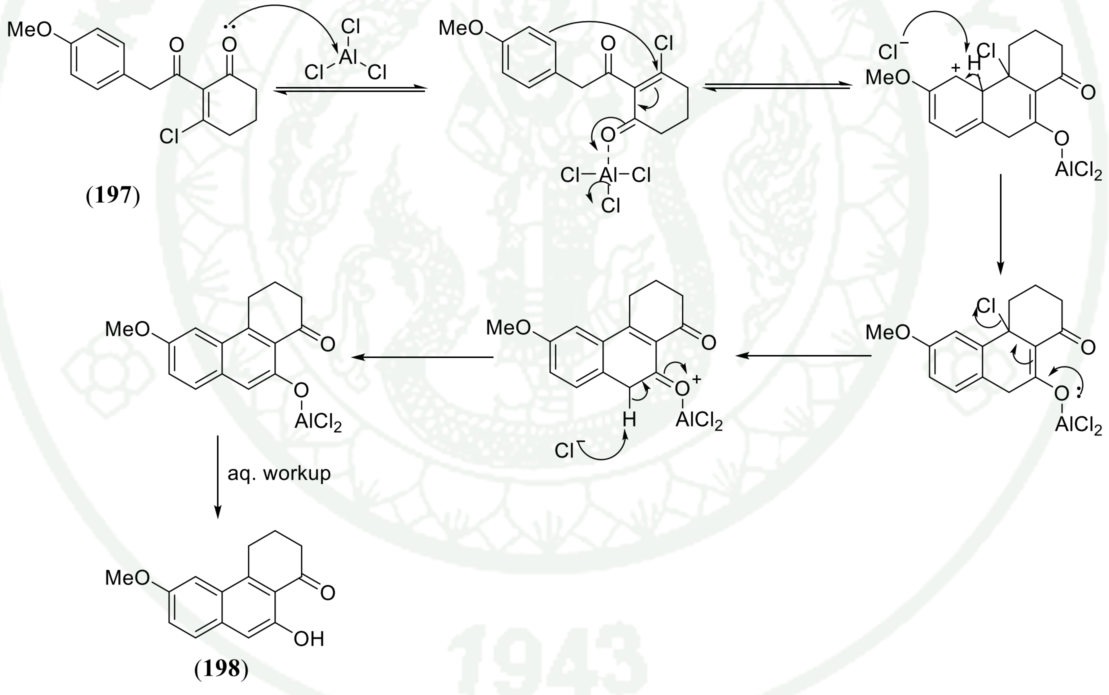
Compound **196** was then convweted to vinylogous acid chloride **197** Friedel–Crafts reaction of this product allowed the cyclization of the middle ring to furnish the desired dihydrophenanthrenone **198** in 98% yield over two steps.



Possible Mechanism



Scheme 48

Reductive amination of ketone **199** began with the formation oxime. When compound **199** was treated with hydroxylamine hydrochloride in EtOH and pyridine, oxime **200** was obtained in 85% yield. Treatment of this oxime with molybdenum oxide and sodium borohydride in methanol gave amine **201** in 90% yield. However, the identity of the final product could only be tentatively confirmed by mass

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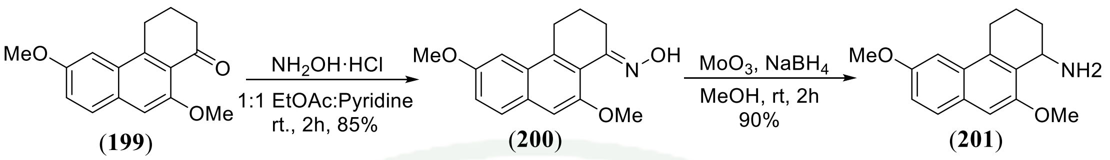
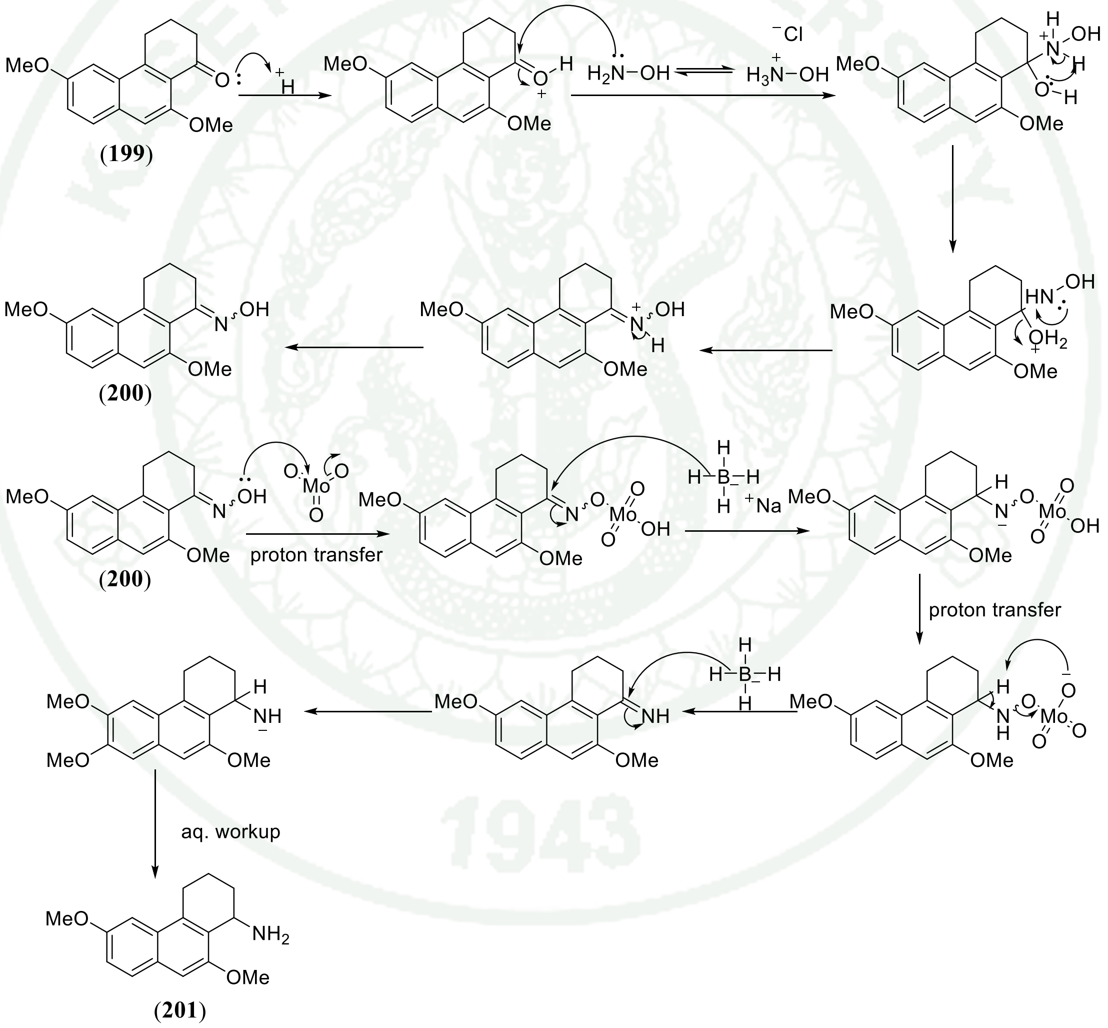
spectroscopy and ^{1}HNMR spectrum as this compound slowly decomposed overnight in $CDCl_3$. Therefore, ^{13}C NMR spectrum could not yet be obtained.



Possible Mechanism



Scheme 49

Owe also try to oxidize compound **198** by using 2-iodoxybenzoic acid or IBX in toluene 1:1 dimethyl sulfoxide in order to produce enone **203**. Unfortunately,

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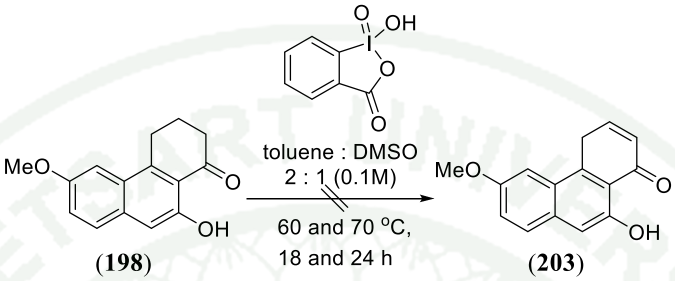
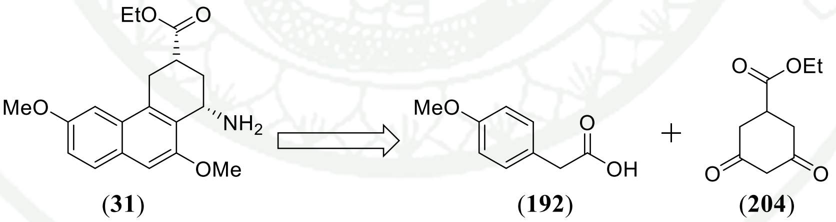
oxidation did not take place and no desired product was obtained. We soon realized that if the oxidation occurred, the enone product would tautomerize to give a phenol. Therefore, this route was abandoned.



Scheme 50

Since we could not synthesize enone **203**, functionalization at the β position of ketone **198** became a problem. Therefore, it would be necessary to install a functional group at this position at the beginning of the synthesis. Our idea was to change 1,3-cyclohexanedione to dione **204** as this compound could be prepared from commercially available starting materials.



Scheme 51

Dione **204** could be prepared from 3,5-dihydroxybenzoic acid by esterification and hydrogenation. However, reaction of the aromatic ring took place very slowly even at high pressure (59.9 bar) and high temperature (200 °C)

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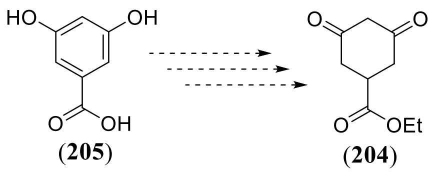
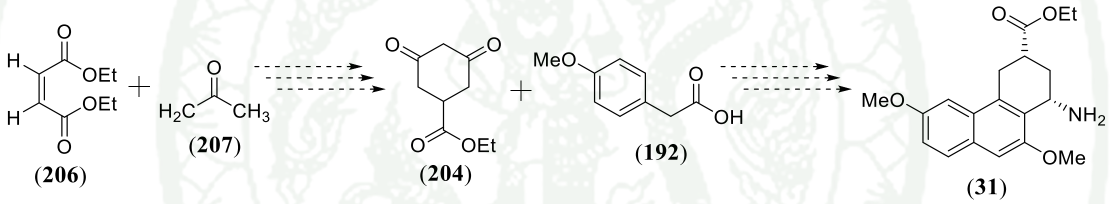


Scheme 52

We later found a synthetic method to prepare compound **204** without the use of high pressure gas and a complicated parr reactor. In this new route, dione **204** would be prepared via enolate chemistry using diethyl maleate (**206**) and acetone (**207**) as precursers. Although this route seemed to be promising based on reported methods, our attempts thus for produced no desired product.



Scheme 53

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CONCLUSION

The synthesis of *N*,*N*'-diBoc-5,5’-dichloro-3-methoxy-1*H*,1’*H*-2,2’-bisindole **190**, a key intermediate for the synthesis of (±)-cladoniamide G, has been synthesized successfully in 11 steps with 15% overall yield. This synthesis started with a nucleophilic substitution of methyl bromoacetate with methyl 2-amino-5-chlorobenzoate **92** to give α-amino ester **93** in 93% yield. Dieckmann condensation of α-amino ester **93** gave methyl 5-chloro-2-(2-methoxy-2-oxoethyl-amino)benzoate **182** in 66% yield. Methylation of this benzoate afforded methyl 5-chloro-3-methoxy-1*H*-indole-2-carboxylate **183** in 96% yield. Hydrolysis of the ester group of **183** followed by decarboxylation of the resulting carboxylic acid gave 3-methoxy indole **184** in 94% over two steps. Subsequent protection of the indole nitrogen of **185** with Boc_2O provided *N*-Boc-3-methoxy indole **186** in 97% yield. Bromination at the C-2 position of **187** followed by chromatography on Al_2O_3 gave bromo indole **187** in 76% yield. The key step of our synthesis was the Suzuki-coupling reaction to join indole **187** and boronic acid **189** to form bisindole **190** in 69% yield. Finally, the Boc protecting groups were removed to provide unprotected bisindole **26** in 90% yield. Compound **26** was successfully converted to cladoniamide G as reported by Dake and coworkers (Loosley *et al.*, 2013).

The synthesis of 1-amine-phenanthrenone has been achieved in 6 steps with 55% overall yield. This synthesis started with the alkylation of 4-methoxyphenylacetic acid **192** with 1,3-cyclohexanedione **194** to give 3-oxocyclohex-1-en-1-yl 2-(4-methyoxyphenyl)acetate **195** in 98% yield. Rearrangement of **195** with DMAP in toluene to provided 3-hydroxy-2-(2-(4-methoxyphenyl)acetyl)cyclohex-2-enone **196** in 83% yield. Vinylogous carboxylic acid **196** was then converted to vinylogous acid chloride **197**, and Friedel–Crafts acylation of the resulting acid chloride with $AlCl_3$ generated 10-hydroxy-6-methoxy-3,4-dihydrophenanthren-1(2*H*)-one **198** in 98% over two steps. Methylation of the hydroxyl group of **198** gave 6,10-dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one **199** in 91% yield. Reductive amination of ketone **199** provided 6,10-dimethoxy-1,2,3,4-tetrahydrophenanthren-1-amine **201** in 77% yield over 2 steps. Based on this method, we are currently attempting to synthesize

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compounds **31** and **32** for biological-activity testing by replacing 1,3-cyclohexanedione with 3,5-dioxocyclohexanecarboxylic acid ethyl ester.

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APPENDIX

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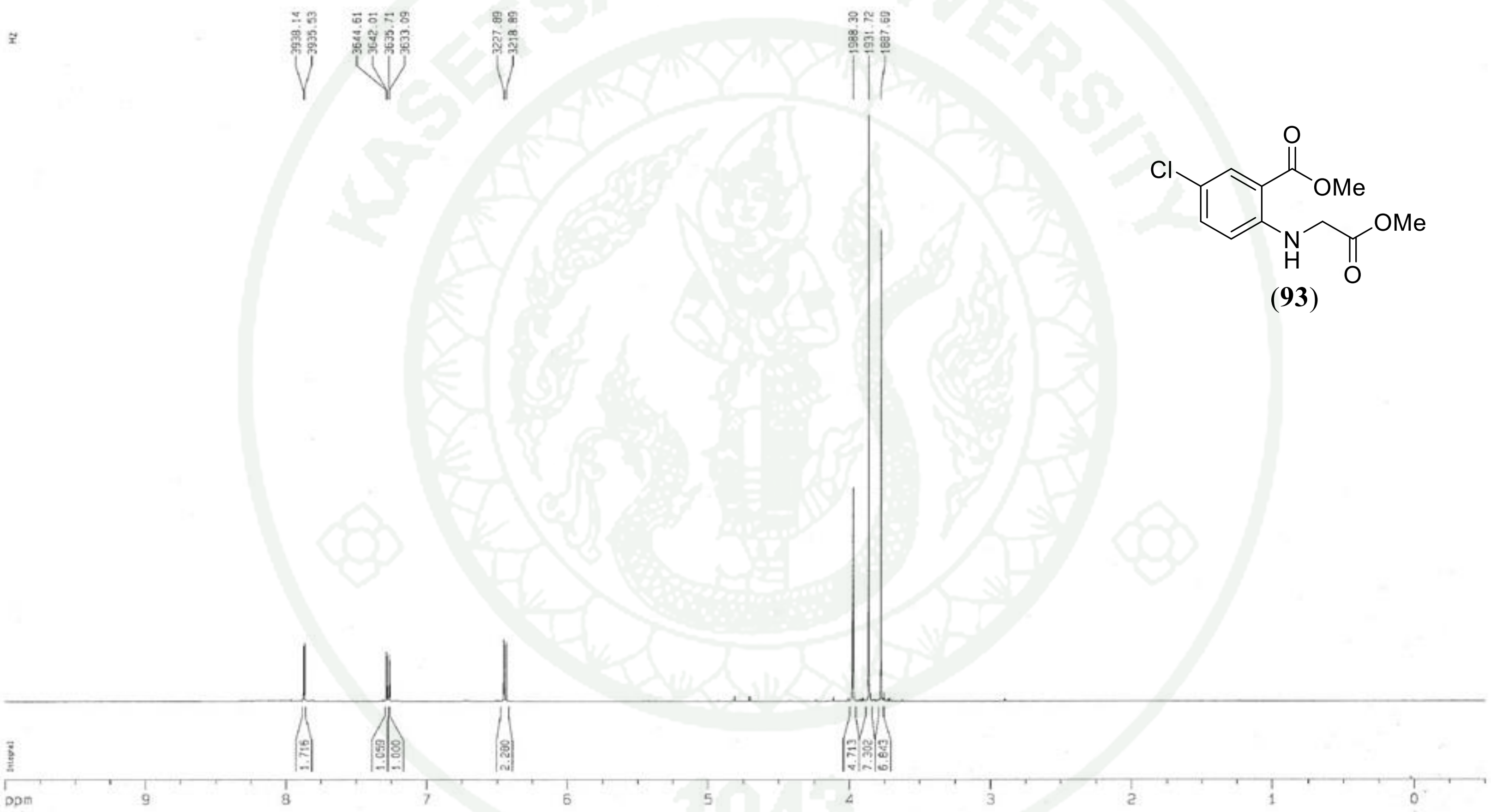

Appendix Figure 1 500 MHz ^{1}H NMR spectrum of Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (**93**)

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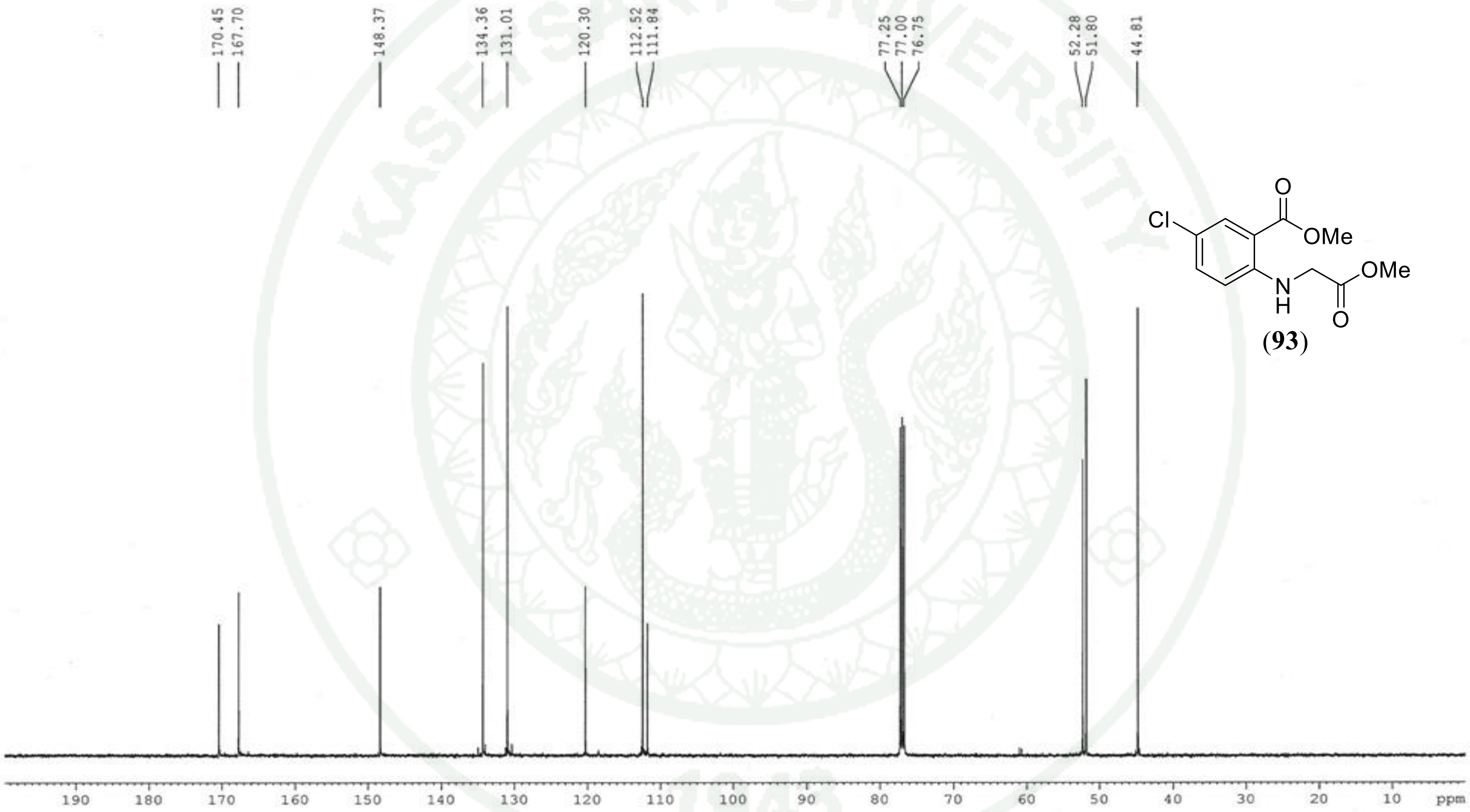

Appendix Figure 2 125 MHz ^{13}C NMR spectrum of Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (**93**)

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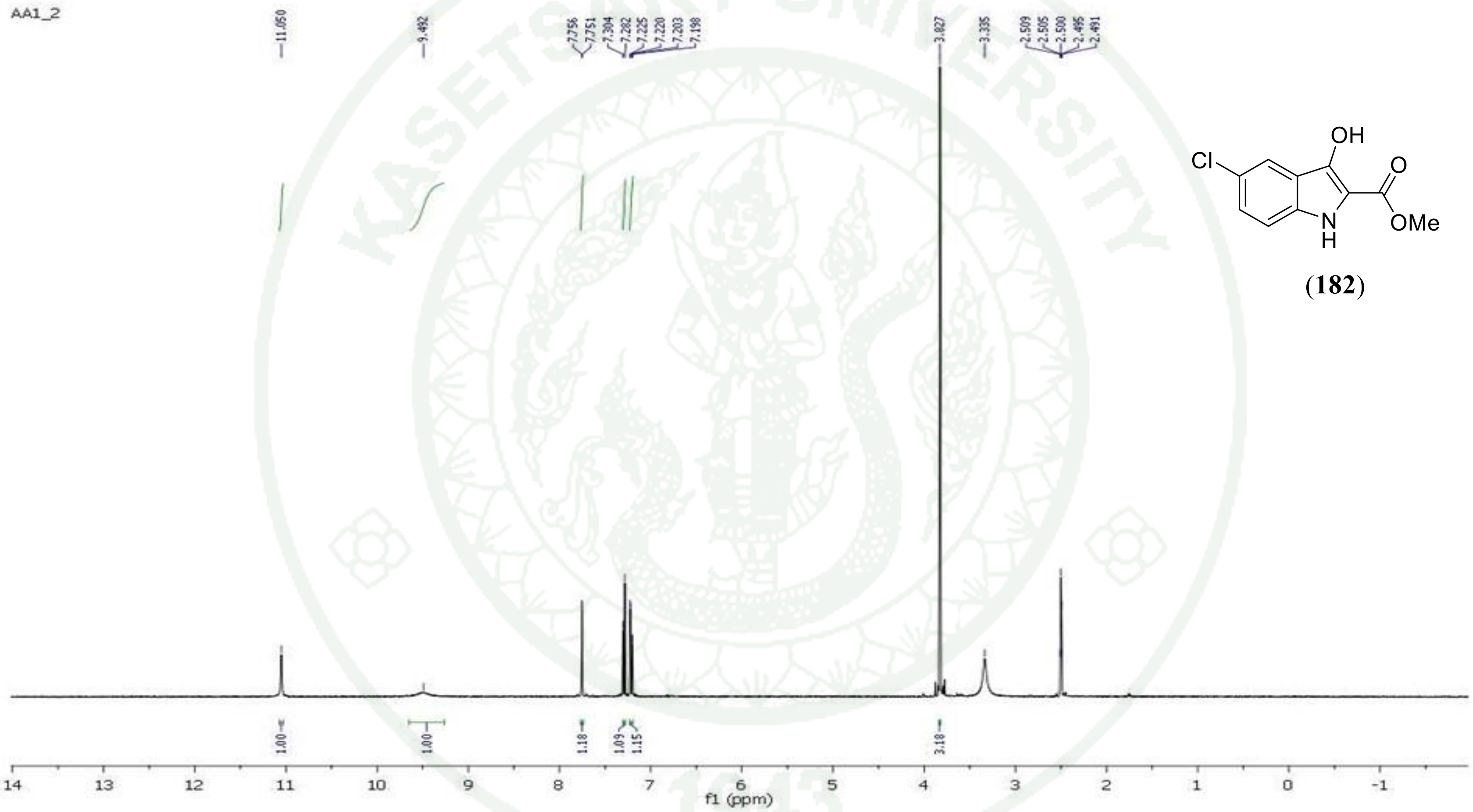

Appendix Figure 3 400 MHz ^{1}H NMR spectrum of Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (**182**)

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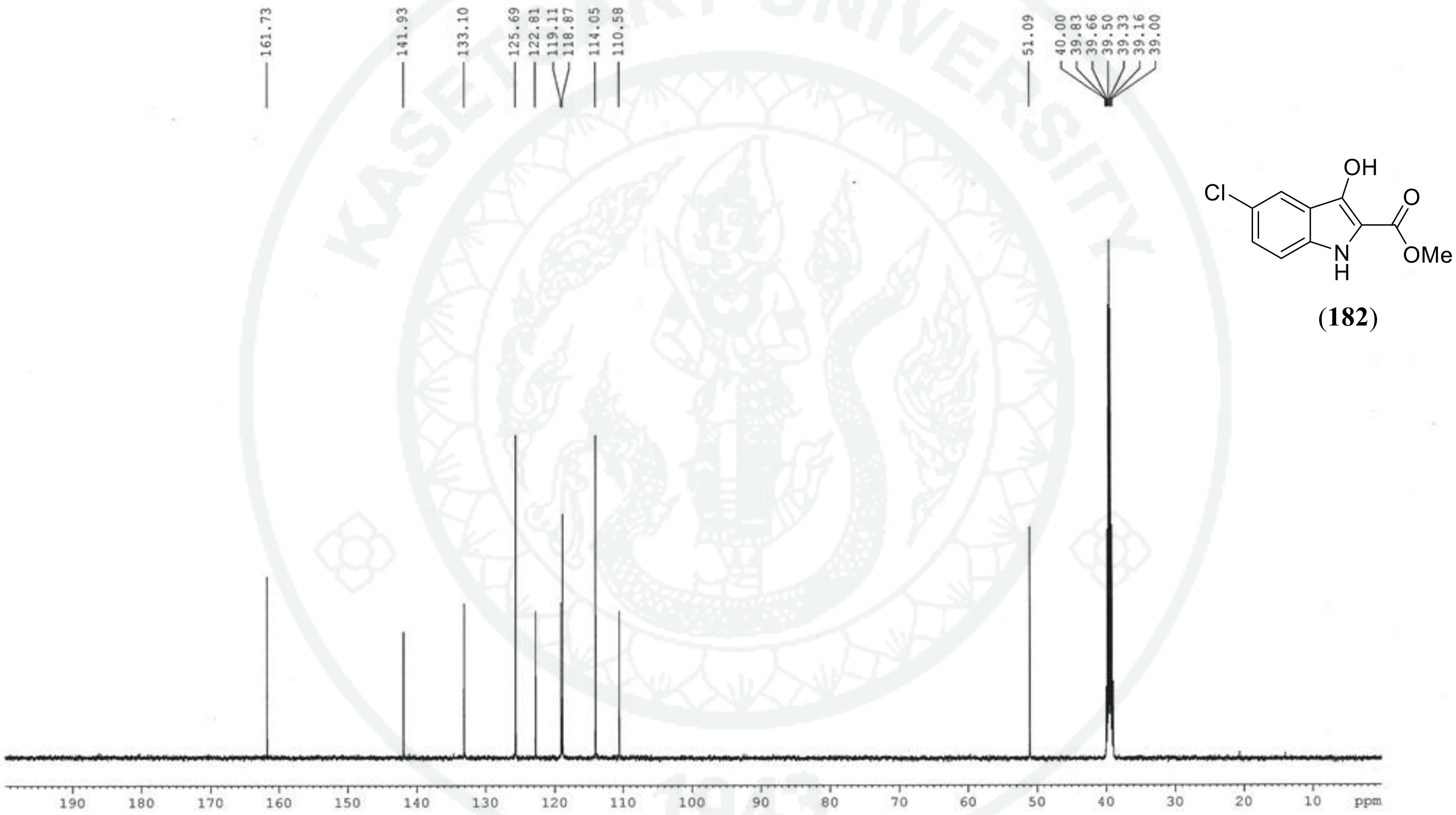

Appendix Figure 4 125 MHz ^{13}C NMR spectrum of Methyl 5-Chloro-2-(2-methoxy-2-oxoethylamino)benzoate (**182**)

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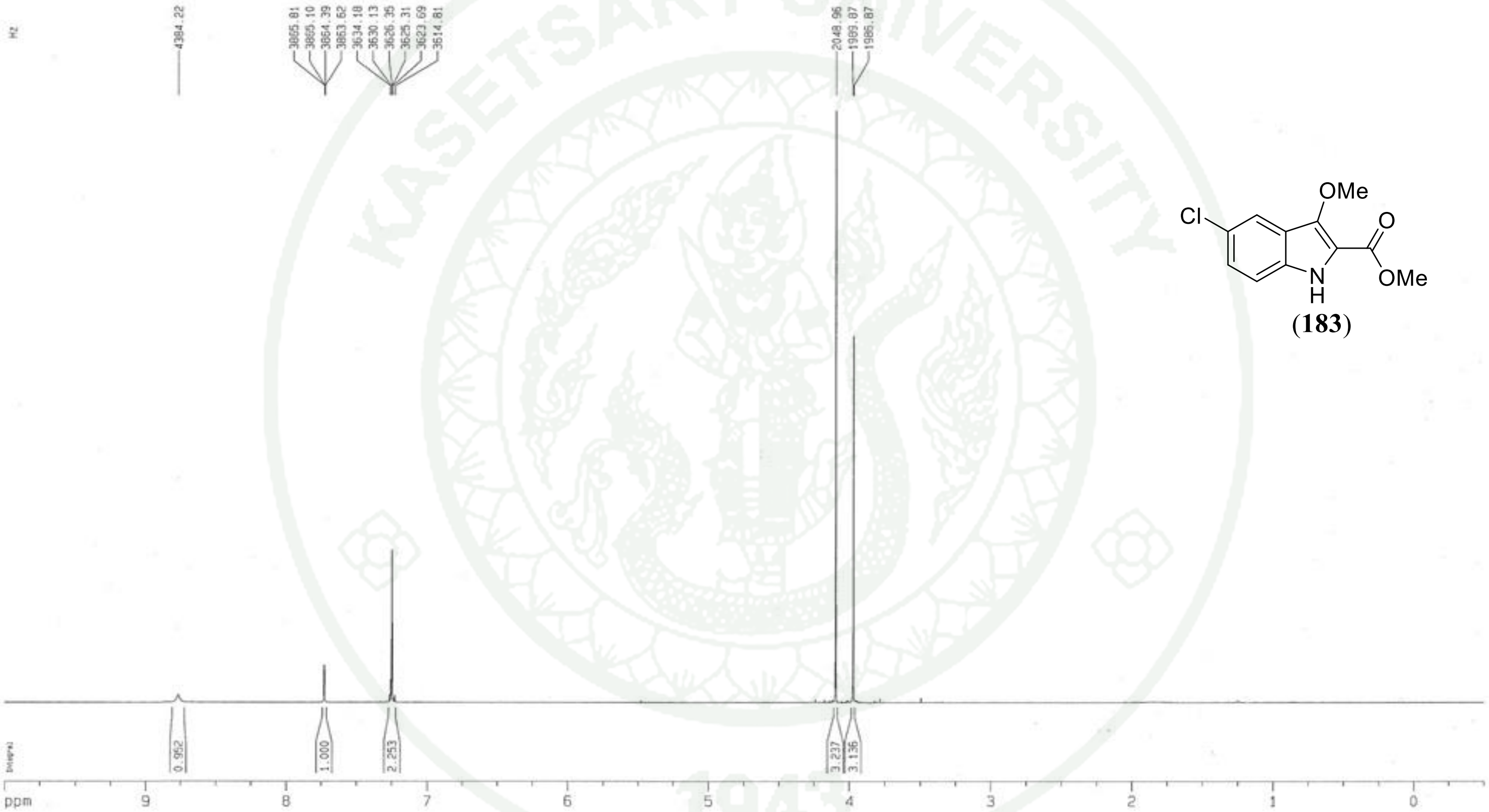

Appendix Figure 5 500 MHz ^{1}H NMR spectrum of Methyl 5-Chloro-3-methoxy-1*H*-indole-2-carboxylate (**183**)

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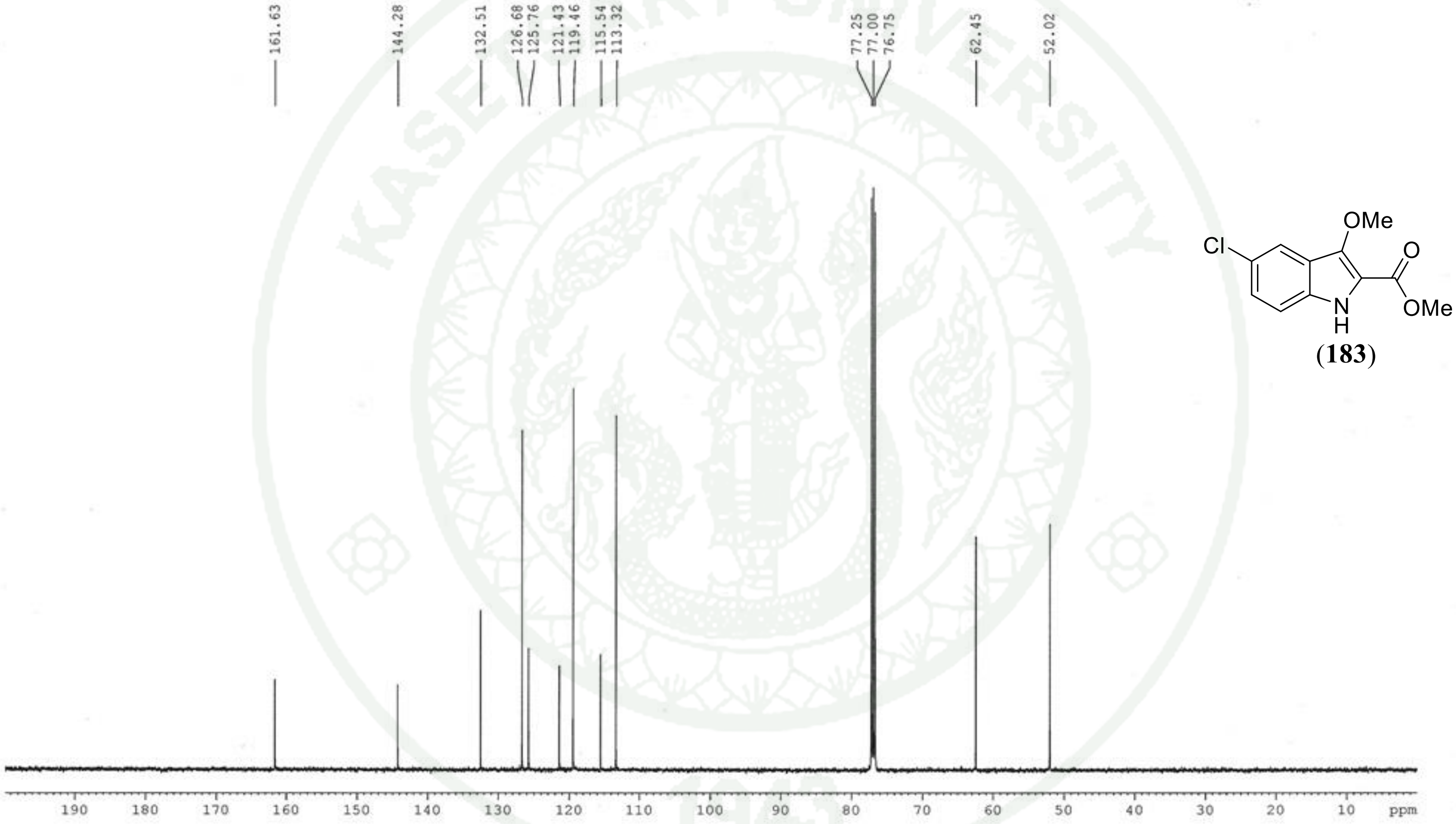

Appendix Figure 6 125 MHz ^{13}C NMR spectrum of Methyl 5-Chloro-3-methoxy-1*H*-indole-2-carboxylate (**183**)

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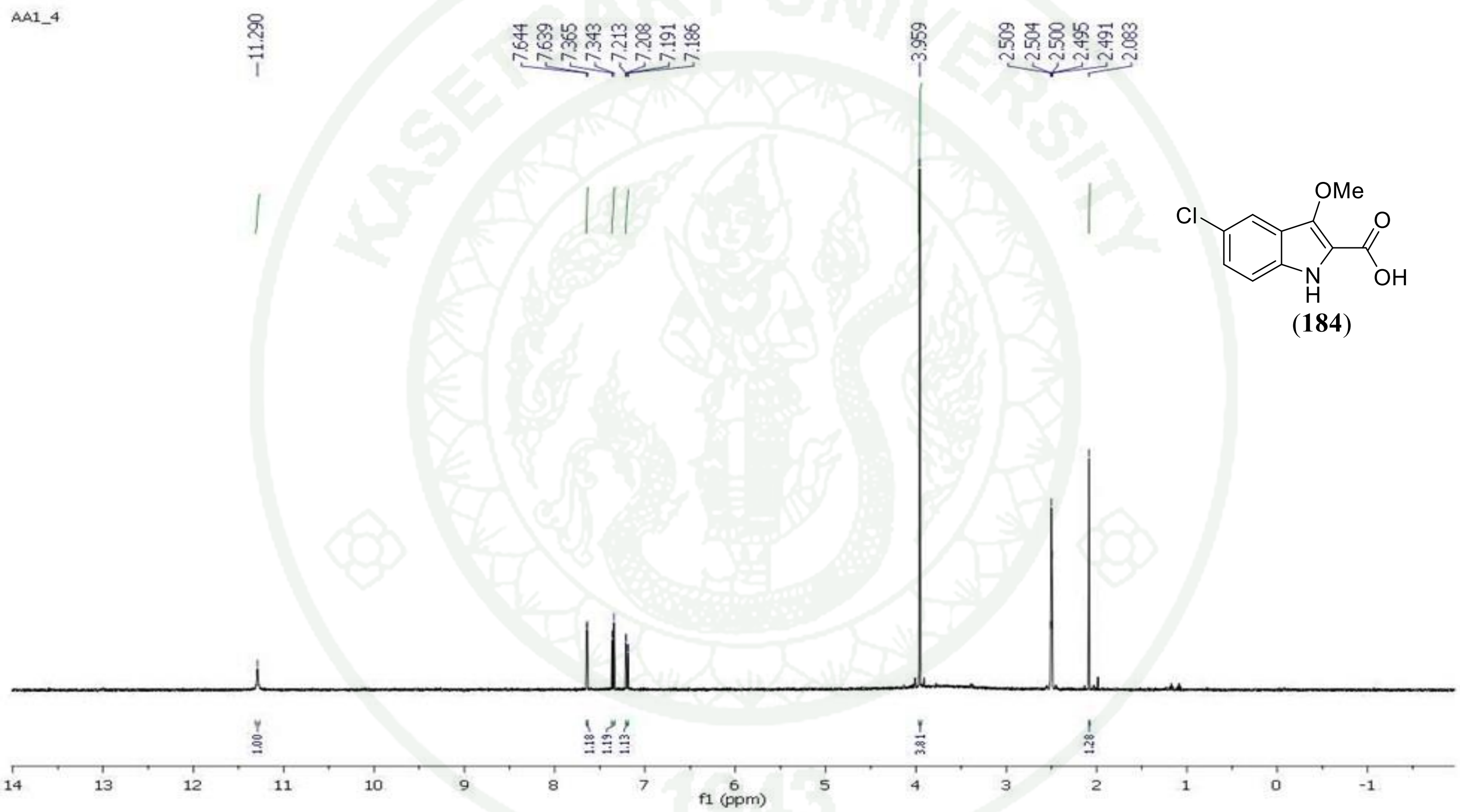

Appendix Figure 7 400 MHz ^{1}H NMR spectrum of 5-chloro-3-methoxy-1*H*-indole (**184**)

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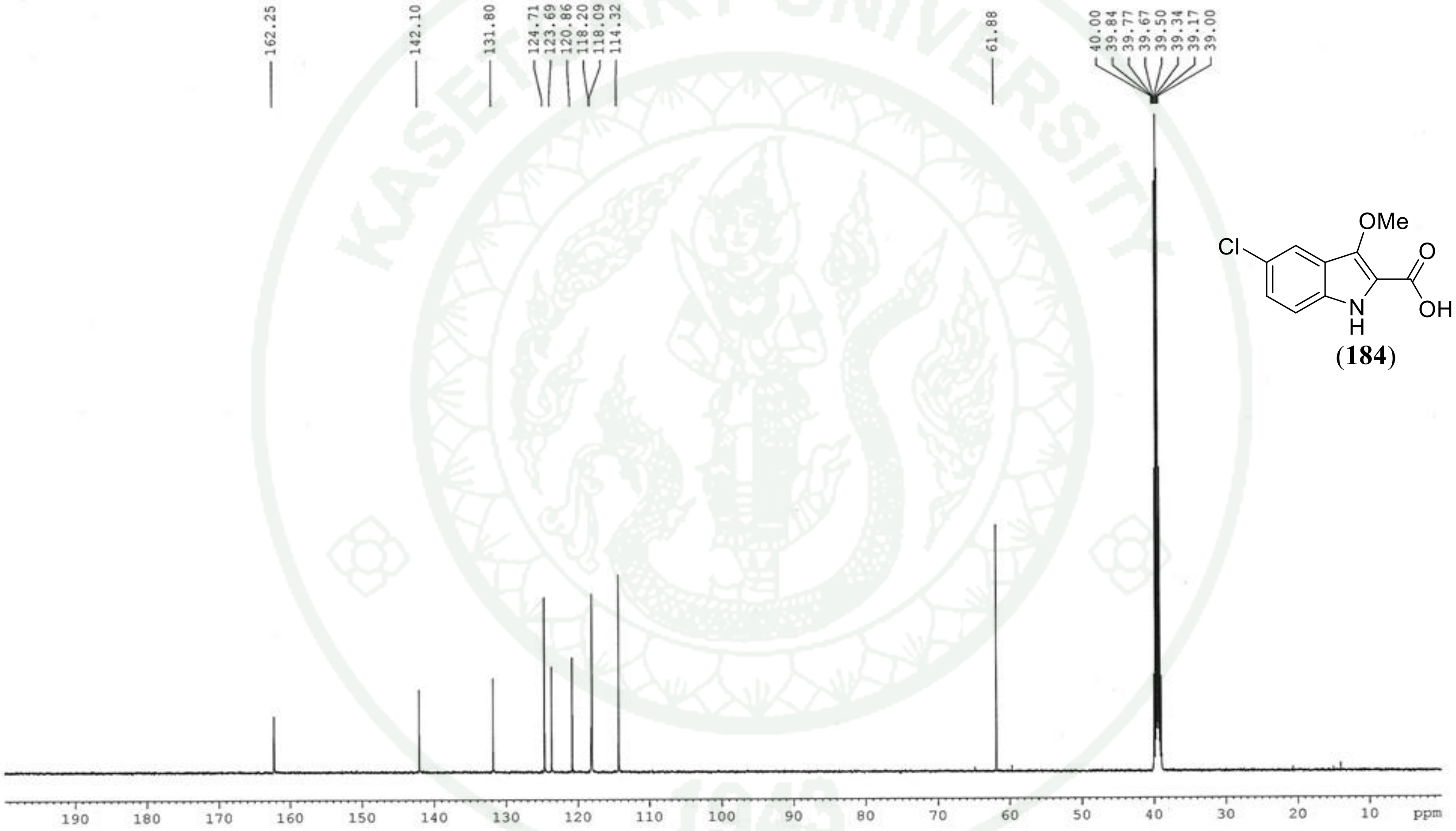

Appendix Figure 8 125 MHz ^{13}C NMR spectrum of 5-chloro-3-methoxy-1*H*-indole (**184**)

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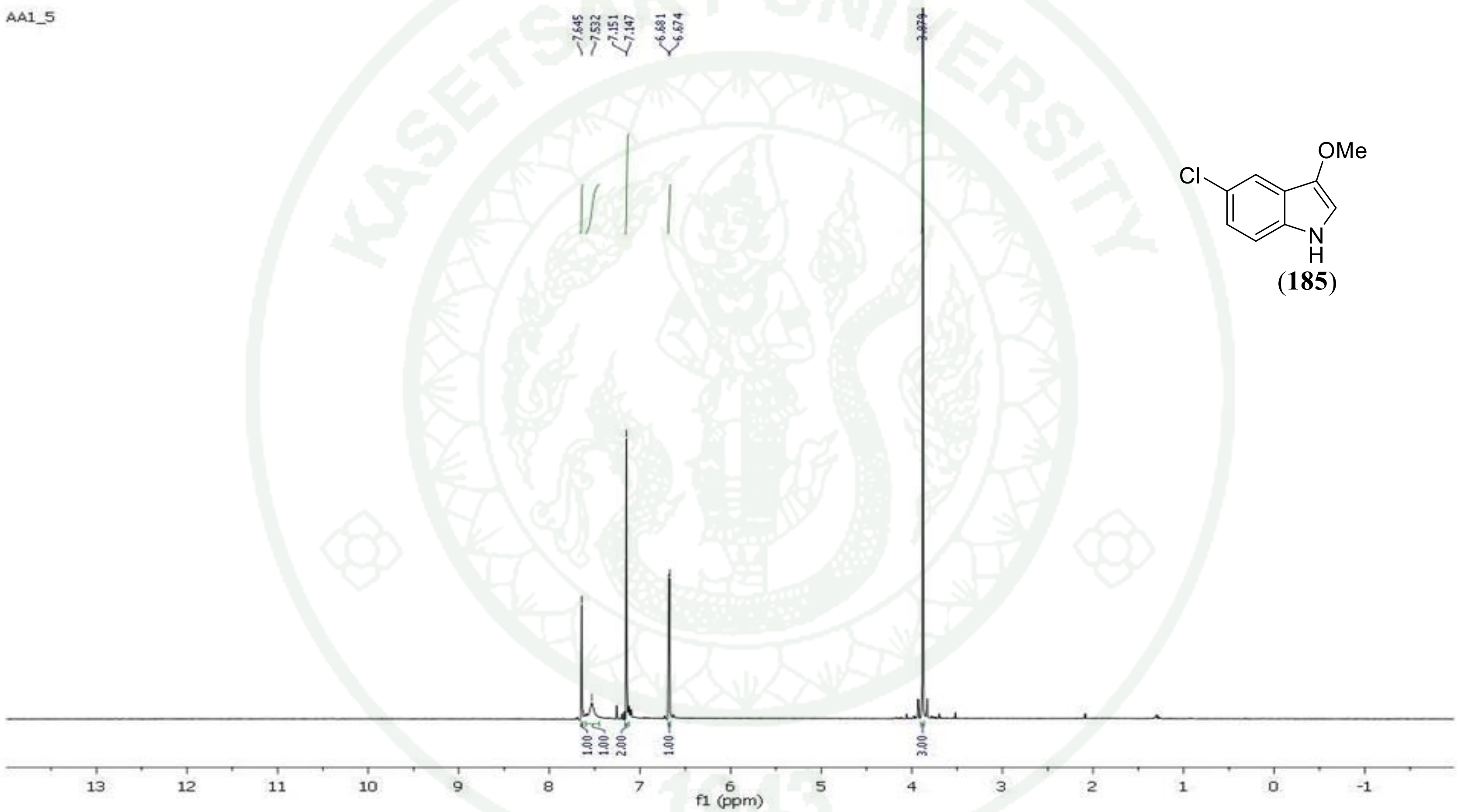

Appendix Figure 9 400 MHz ^{1}H NMR spectrum of *N*-Boc 5-chloro-3-methoxy-1*H*-indole (**185**)

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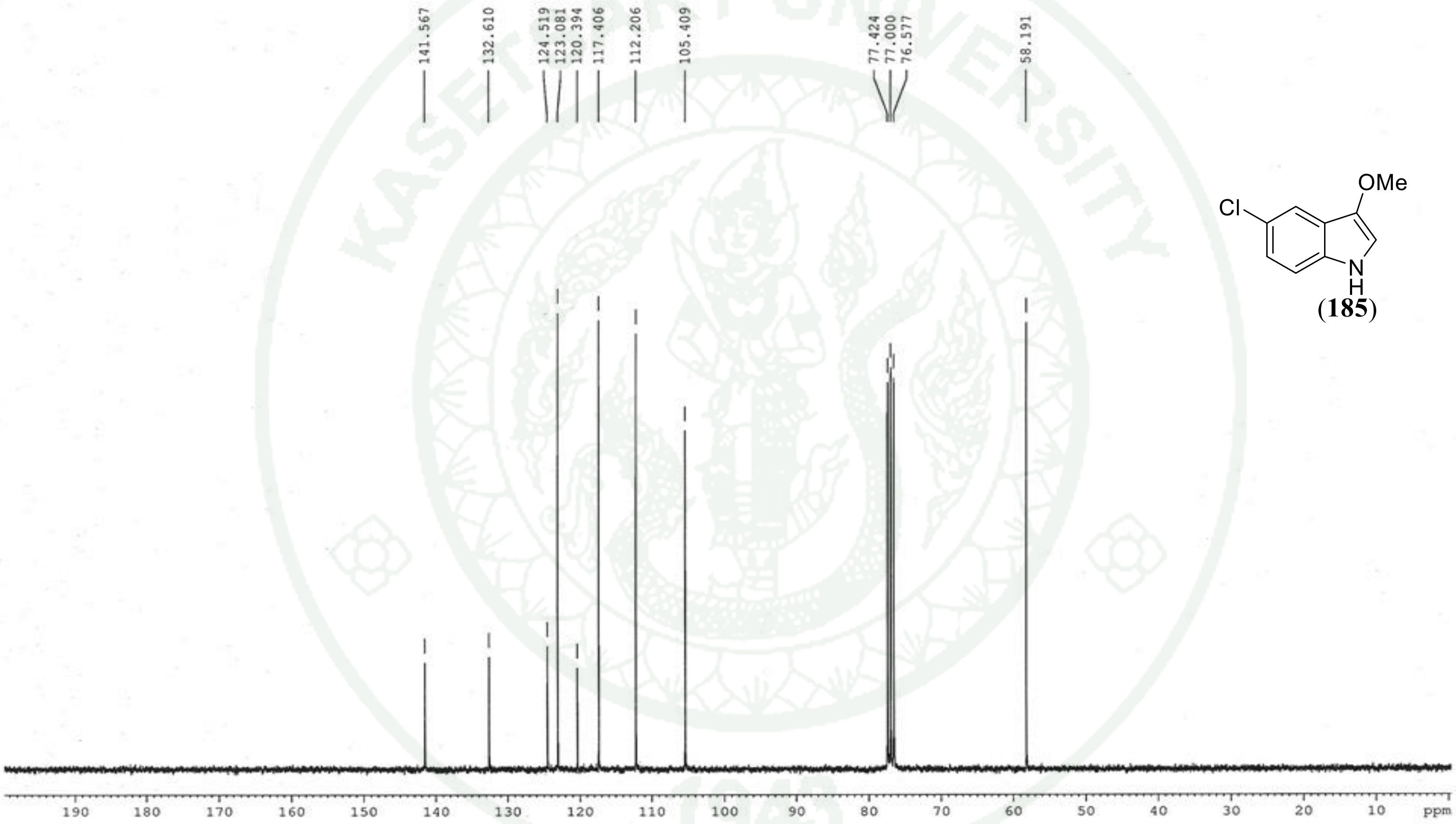

Appendix Figure 10 75 MHz ^{13}C NMR spectrum of *N*-Boc 5-chloro-3-methoxy-1*H*-indole (**185**)

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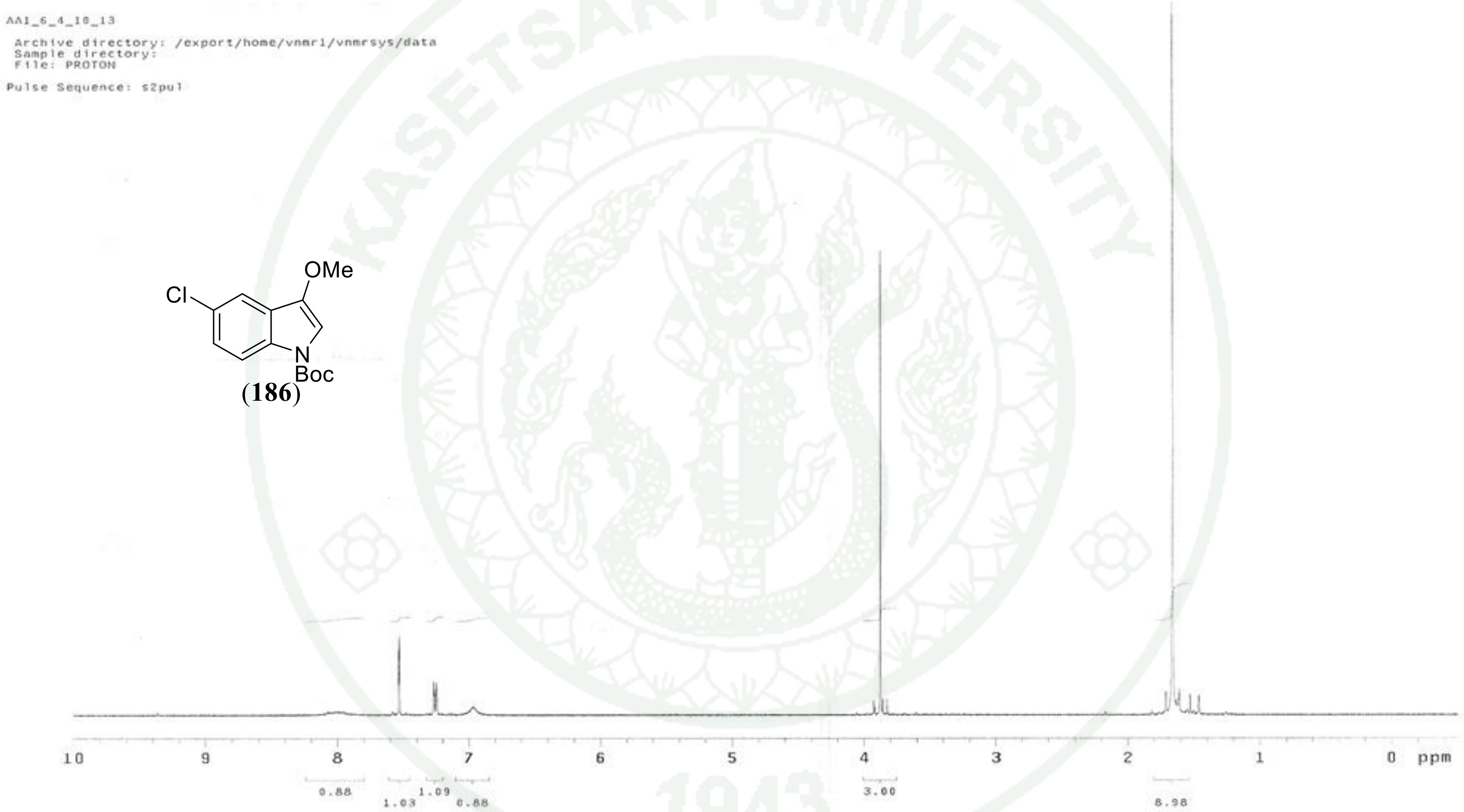

Appendix Figure 11 400 MHz ^{1}H NMR spectrum of *N*-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (**186**)

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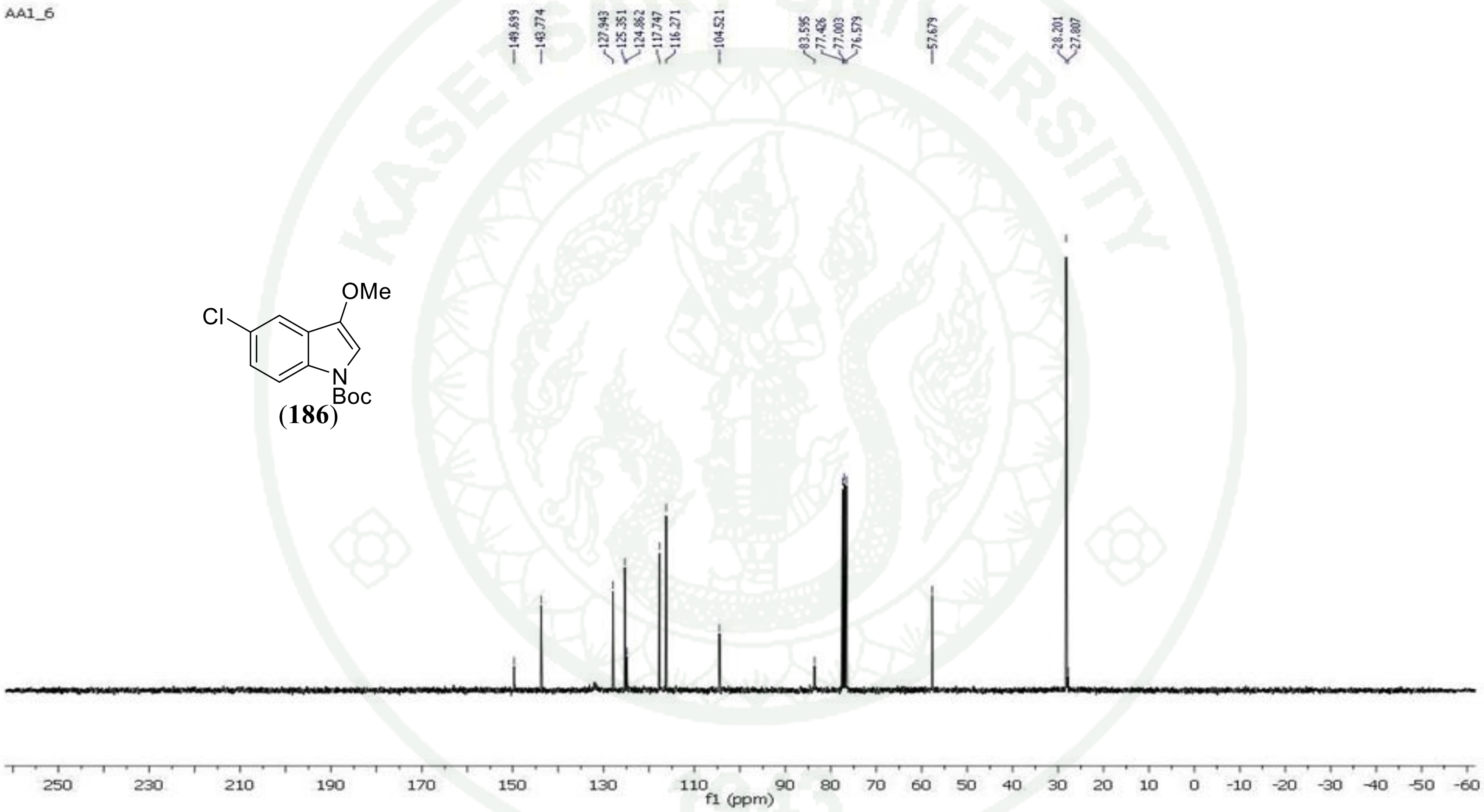

(**186**)

Appendix Figure 12 75 MHz ^{13}C NMR spectrum of *N*-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (**186**)

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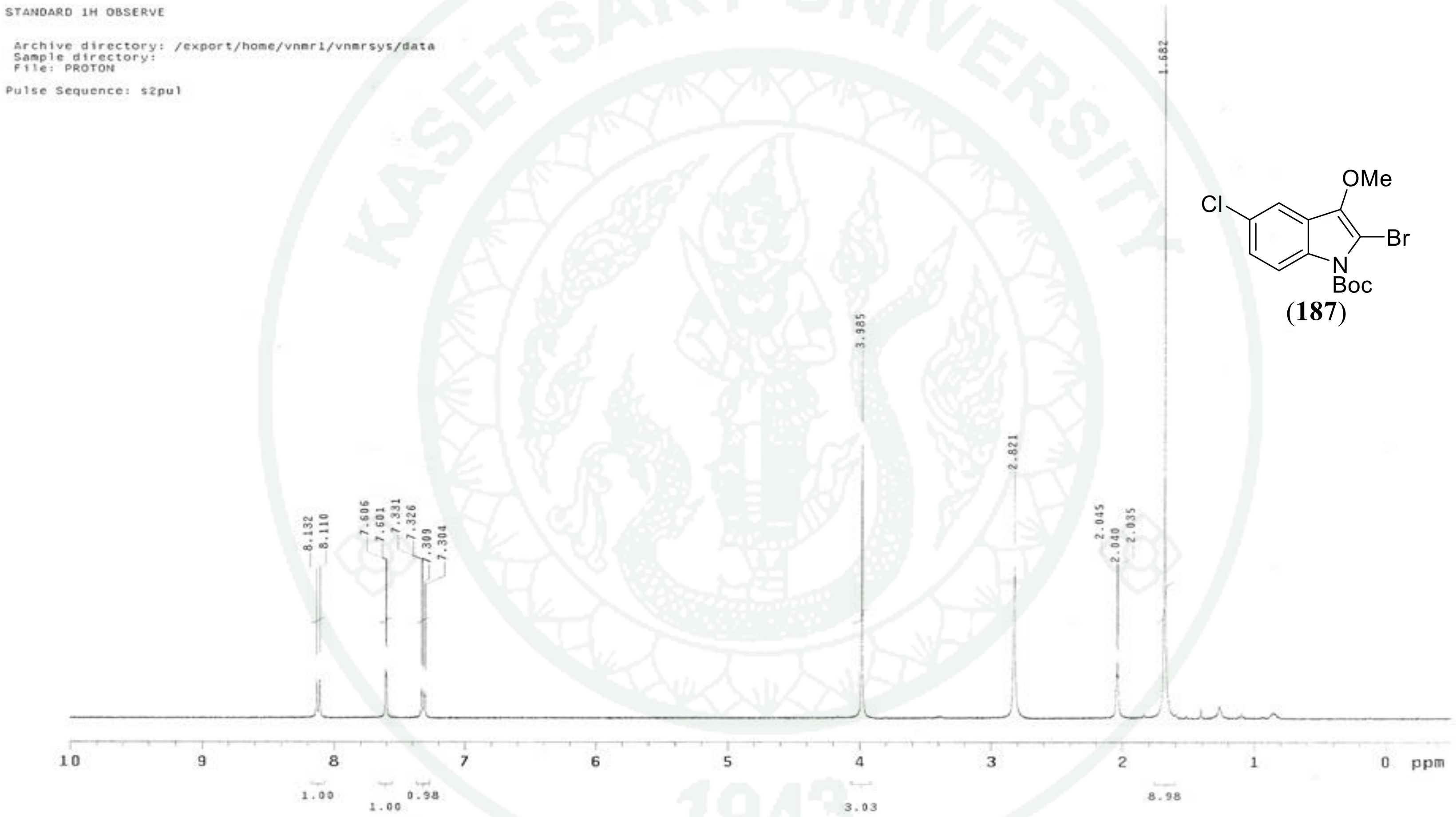

Appendix Figure 15 400 MHz ^{1}H NMR spectrum of *N*-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (**187**)

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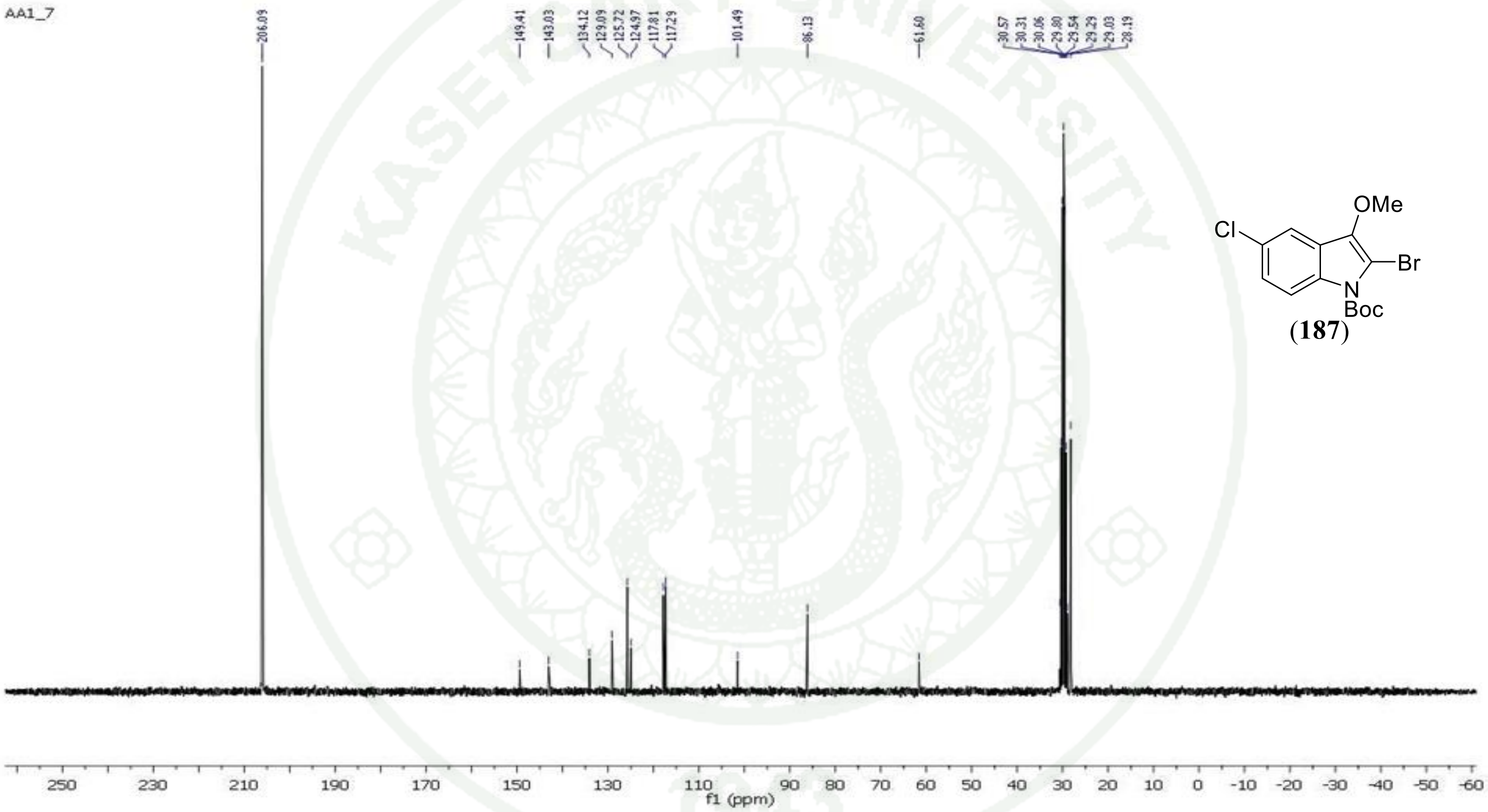

Appendix Figure 16 75 MHz ^{13}C NMR spectrum of of *N*-Boc 5-chloro-2-bromo-3-methoxy-1*H*-indole (**187**)

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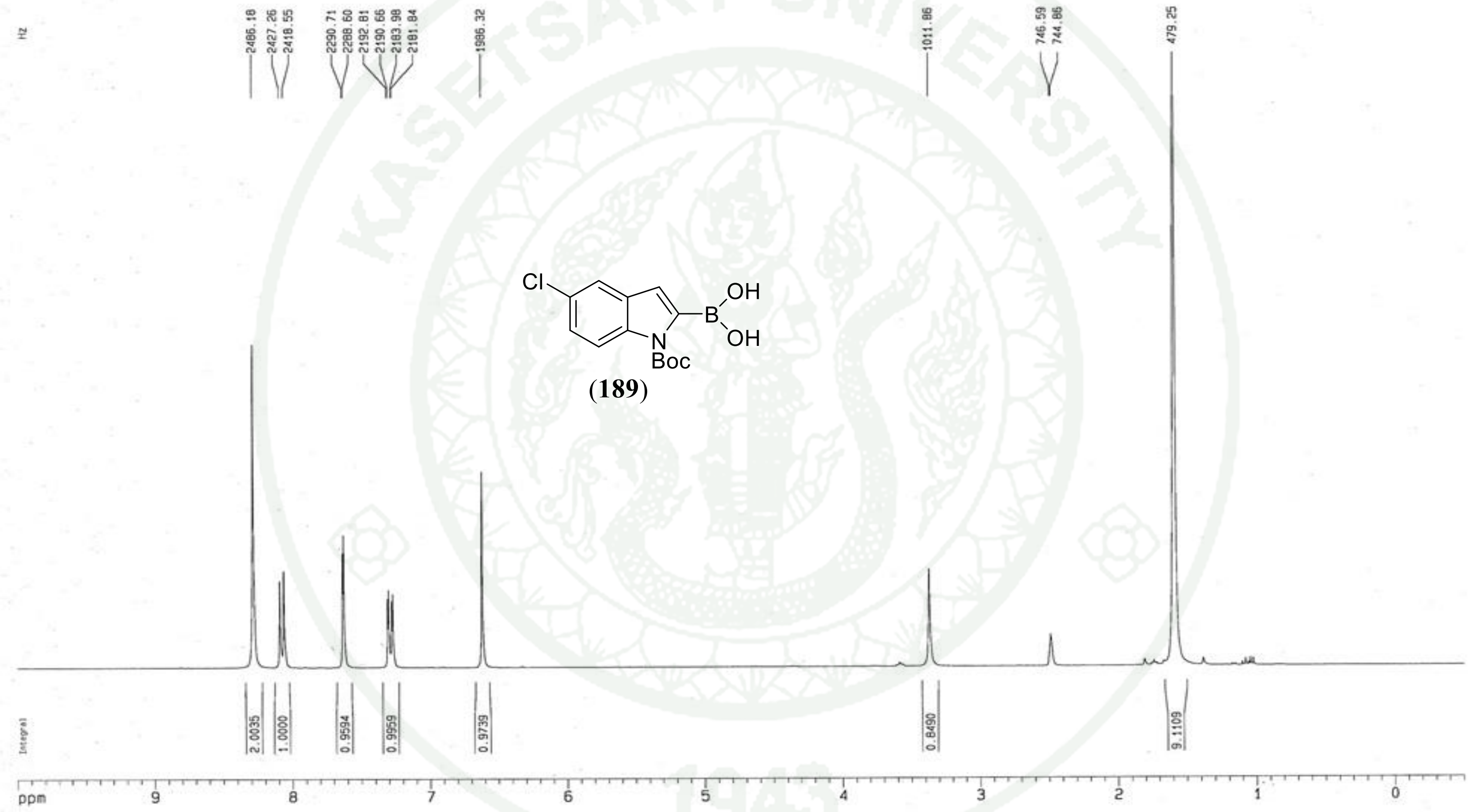

Appendix Figure 17 400 MHz ^{1}H NMR spectrum of *N*-Boc-5-chloro-1*H*-indol-2-yl boronic acid (**189**)

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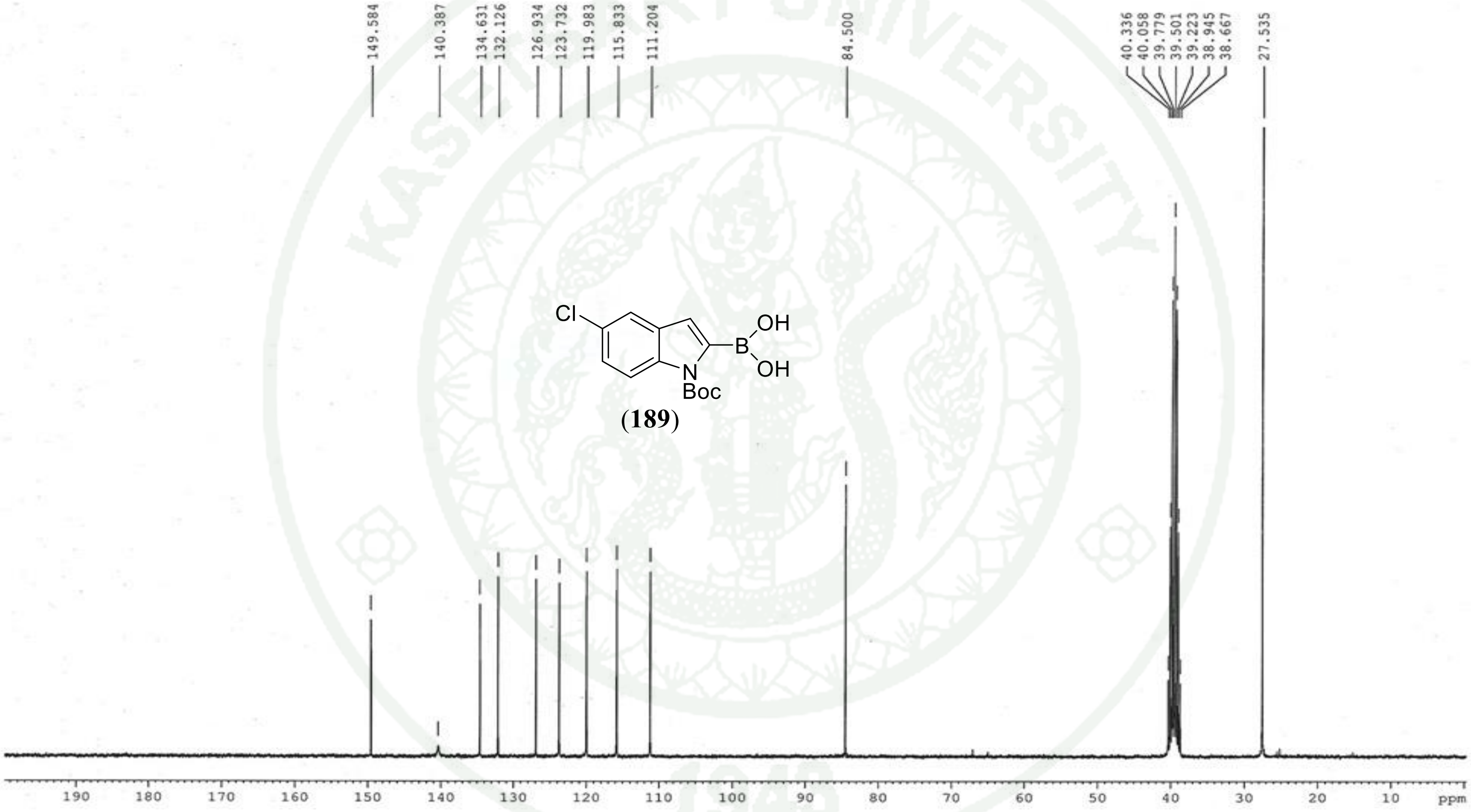

Appendix Figure 18 75 MHz ^{13}C NMR spectrum of *N*-Boc-5-chloro-1*H*-indol-2-yl boronic acid (**189**)

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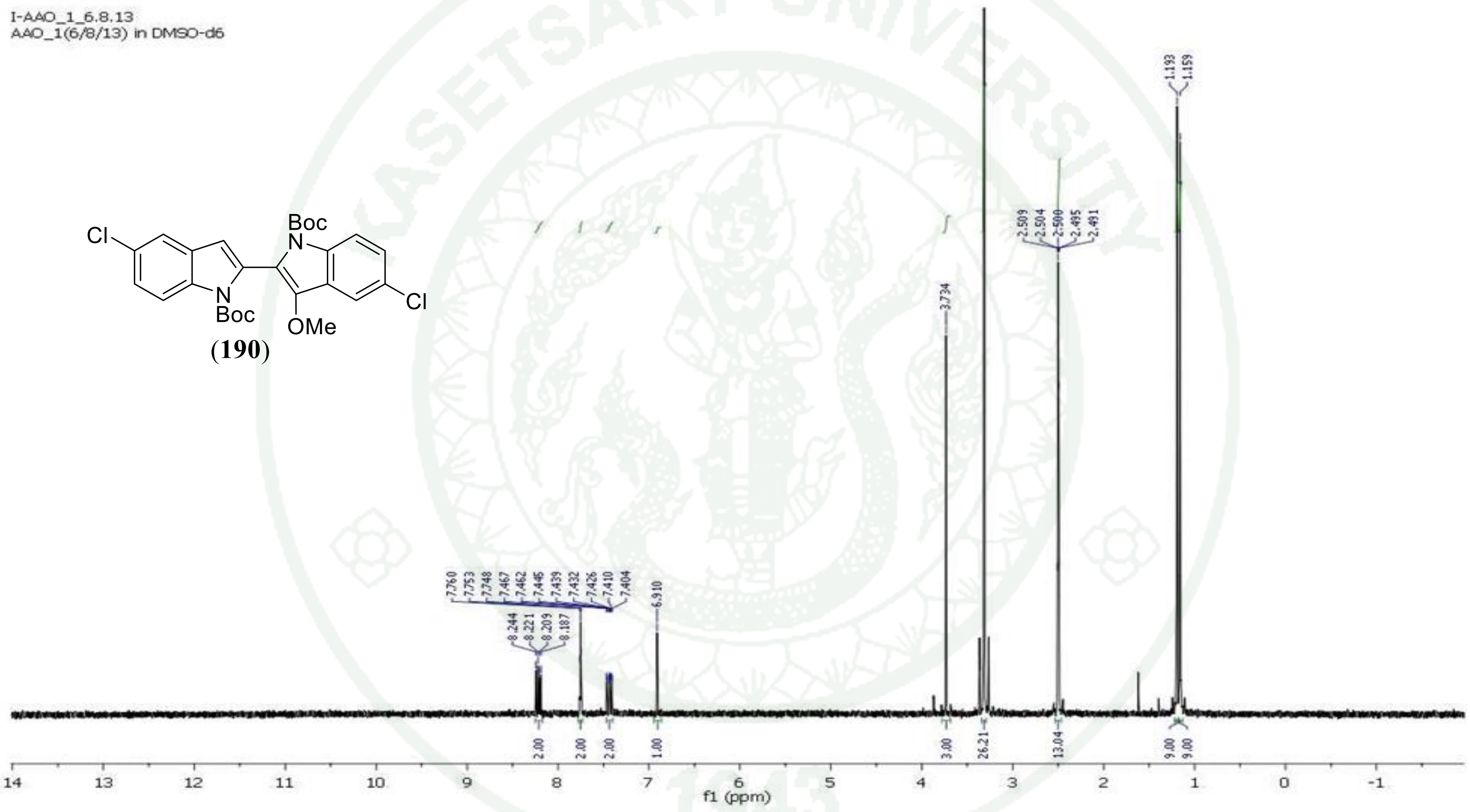

Appendix Figure 21 400 MHz ^{1}H NMR spectrum of *N*,*N'*-diBoc-5,5'-dichloro-3-methoxy- 1*H*,1'*H* -2,2'-bisindole (**190**)

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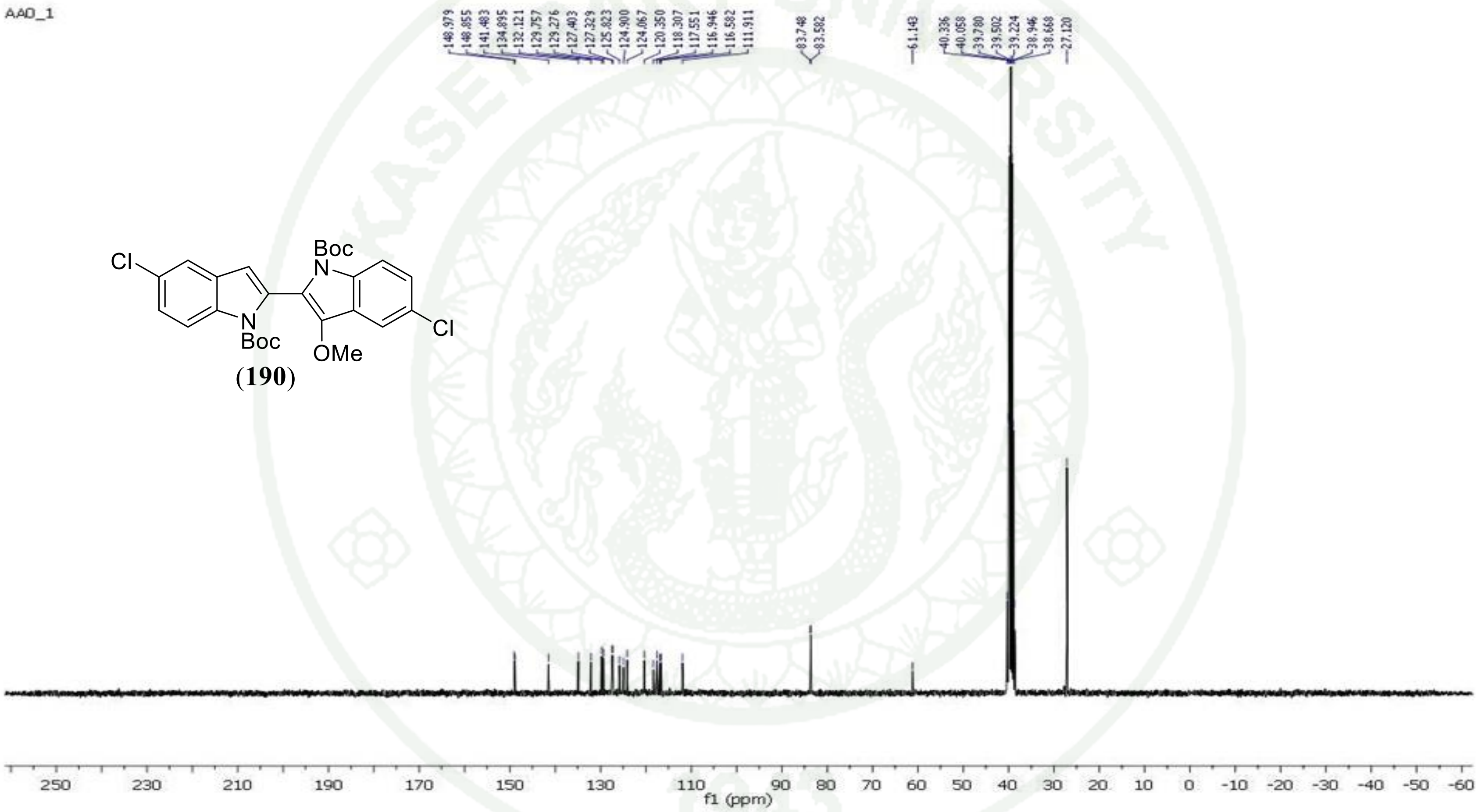

Appendix Figure 22 75 MHz ^{13}C NMR spectrum of *N*,*N*'-diBoc-5,5’-dichloro-3-methoxy- 1*H*,1’*H* -2,2’-bisindole (**190**)

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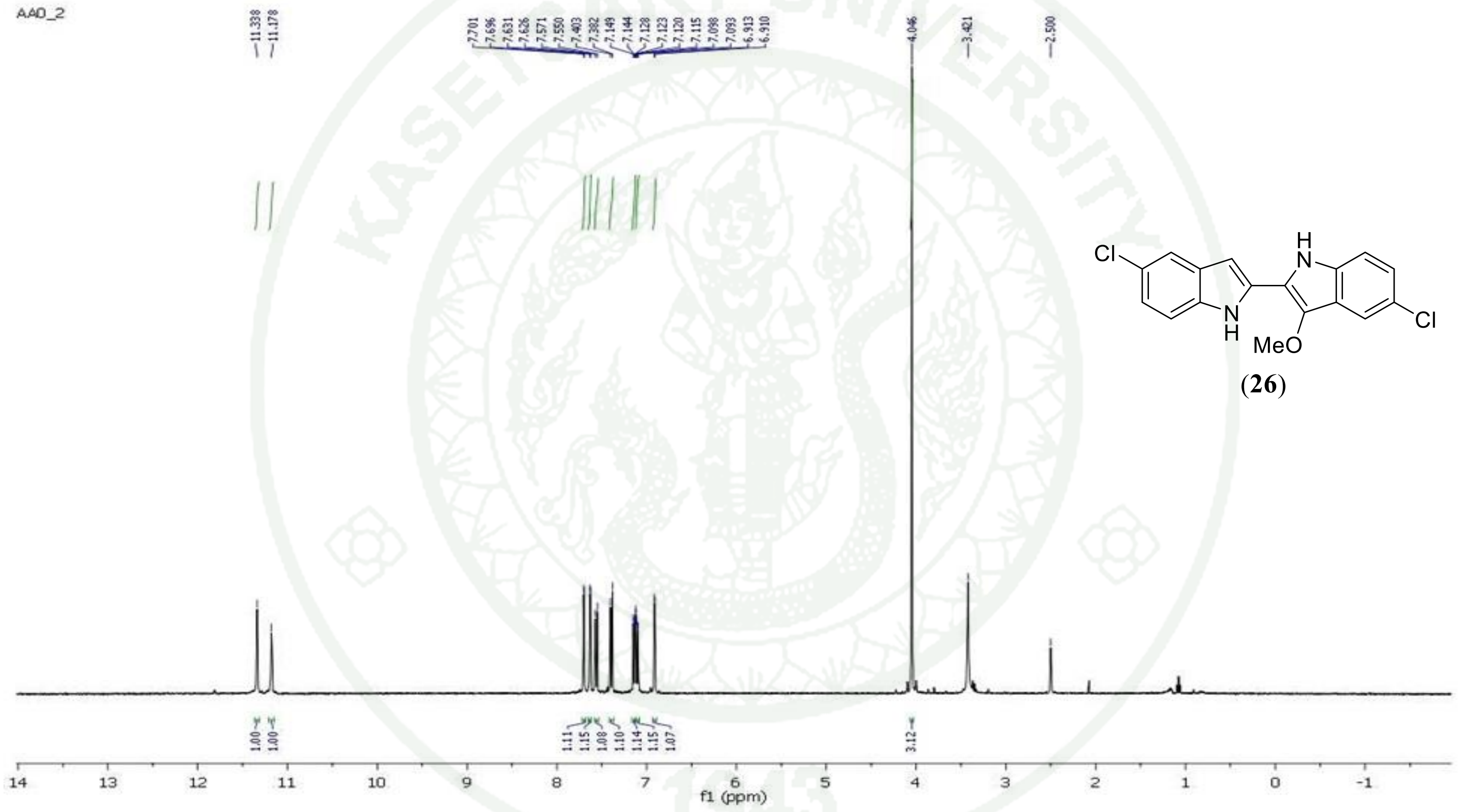

Appendix Figure 23 400 MHz ^{1}H NMR spectrum of 3-Methoxy-1*H*,1'*H*-2,2'-bisindole (**26**)

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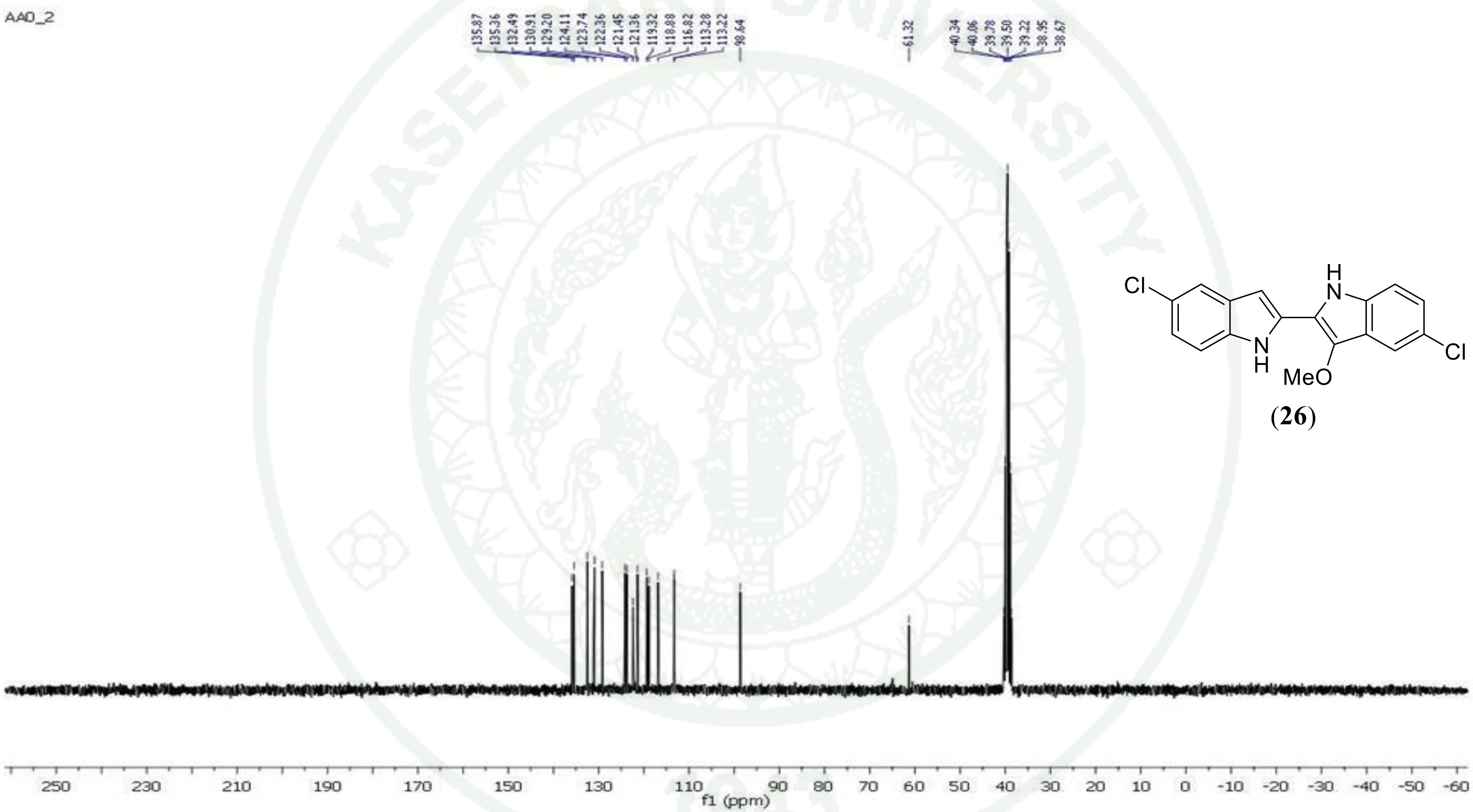

Appendix Figure 24 300 MHz ^{13}C NMR spectrum of 3-Methoxy-1*H*,1'*H*-2,2'-bisindole (**26**)

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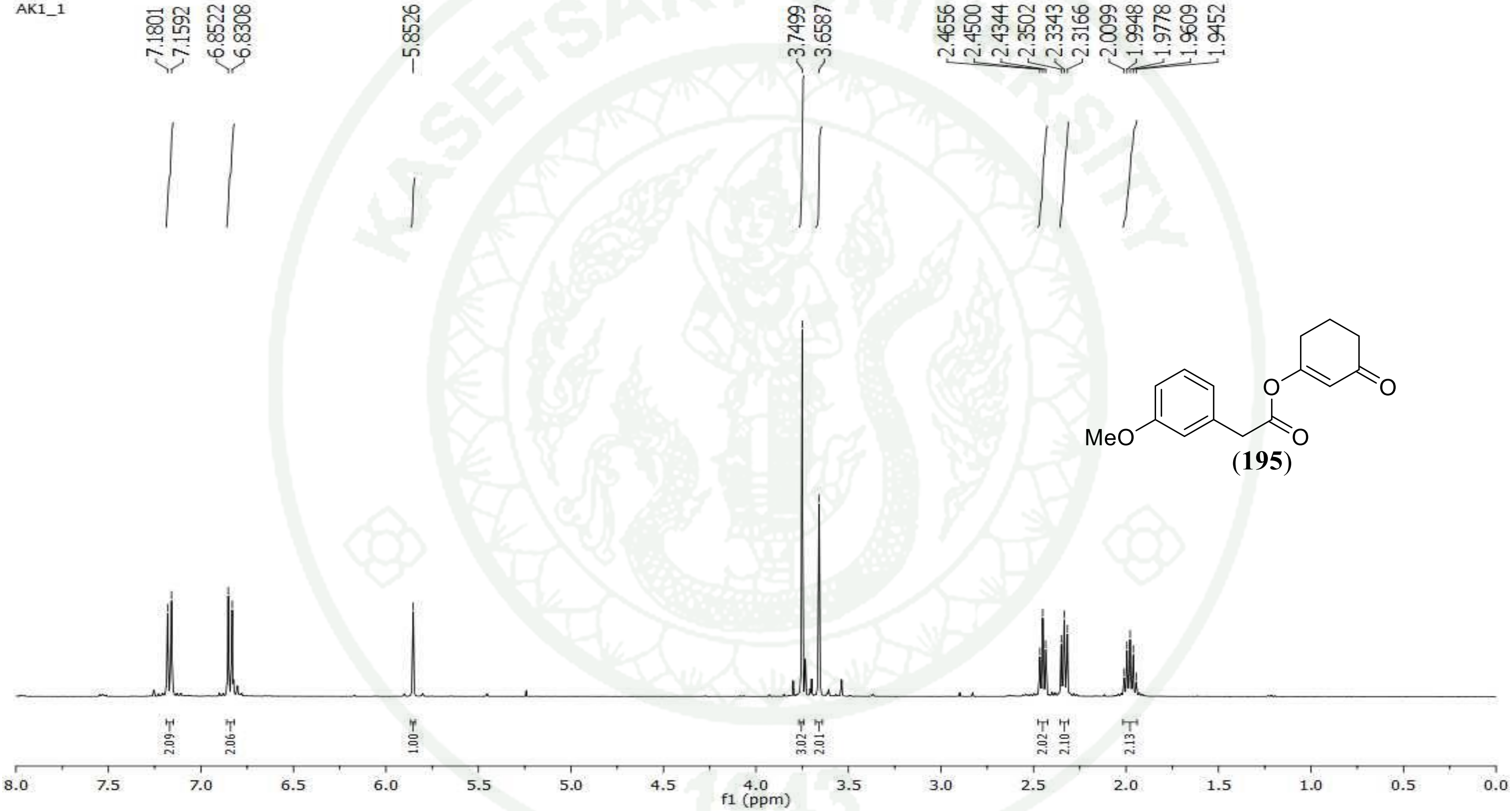

Appendix Figure 25 400 MHz ^{1}H NMR spectrum of 3-Oxocyclohex-1-en-1-yl 2-(4-methyoxyphenyl)acetate (**195**)

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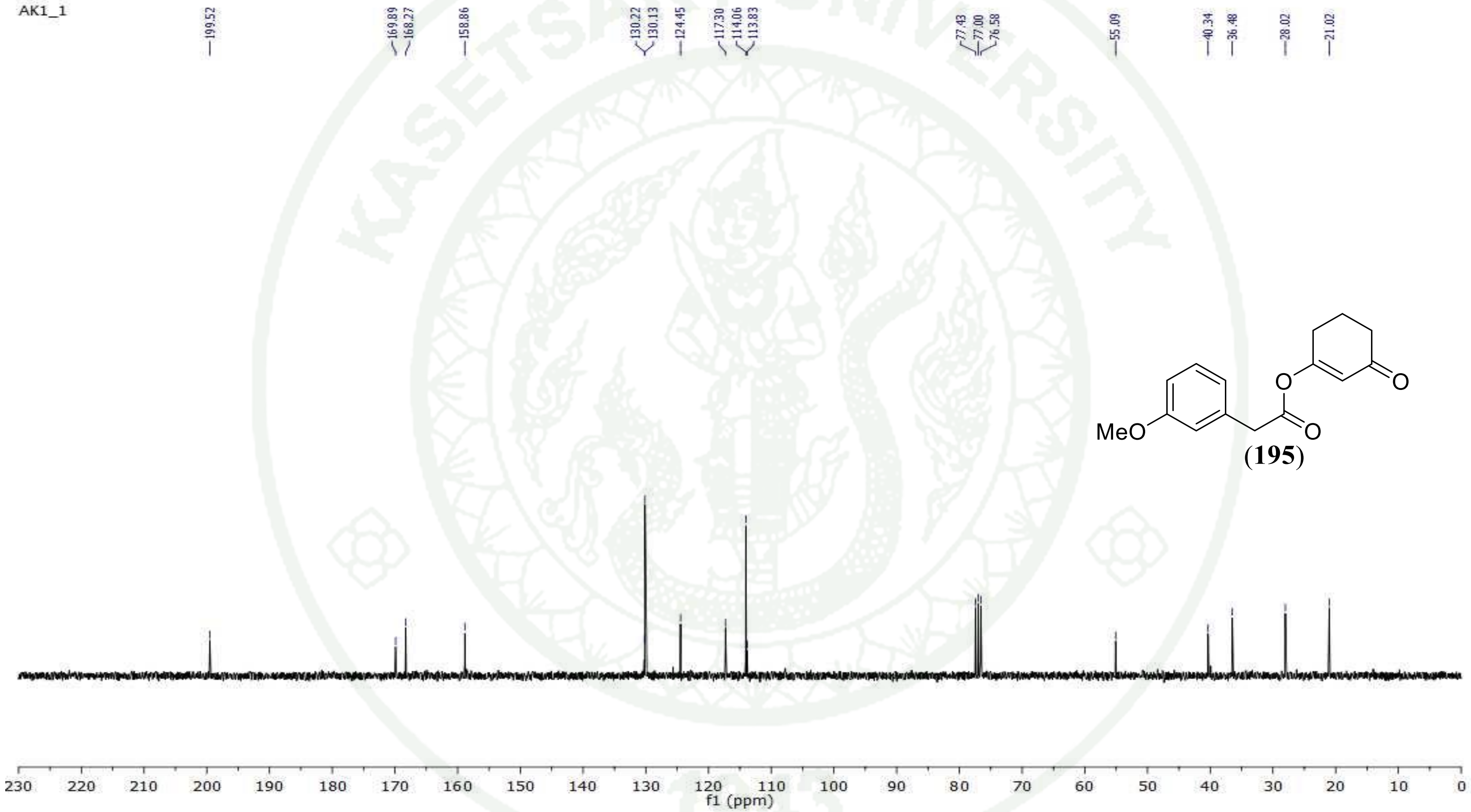

Appendix Figure 26 75 MHz ^{13}C NMR spectrum of 3-Oxocyclohex-1-en-1-yl 2-(4-methyoxyphenyl)acetate (**195**)

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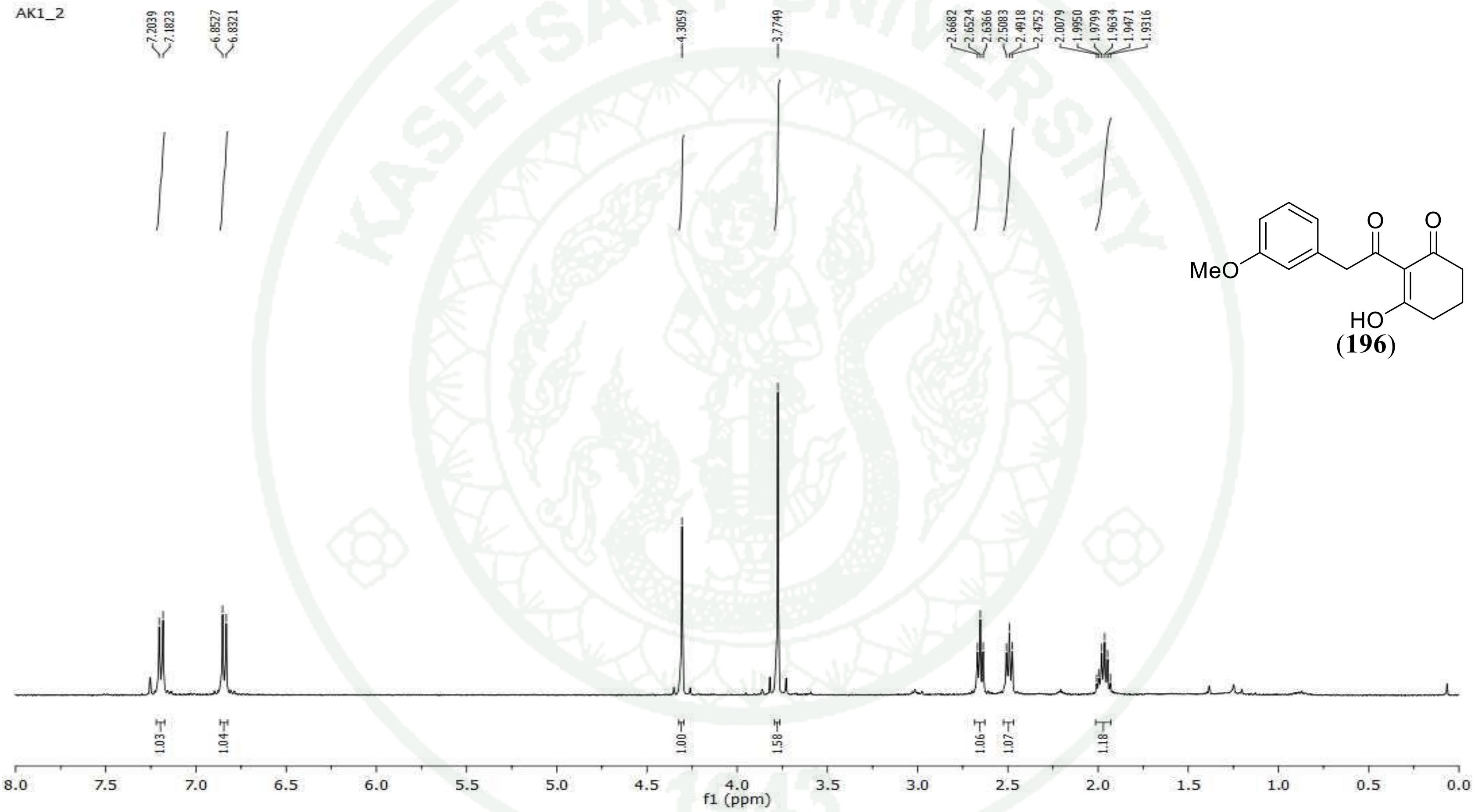

Appendix Figure 27 400 MHz ^{1}H NMR spectrum of 3-Hydroxy-2-(2-(4-methoxyphenyl)acetyl)cyclohex-2-enone (**196**)

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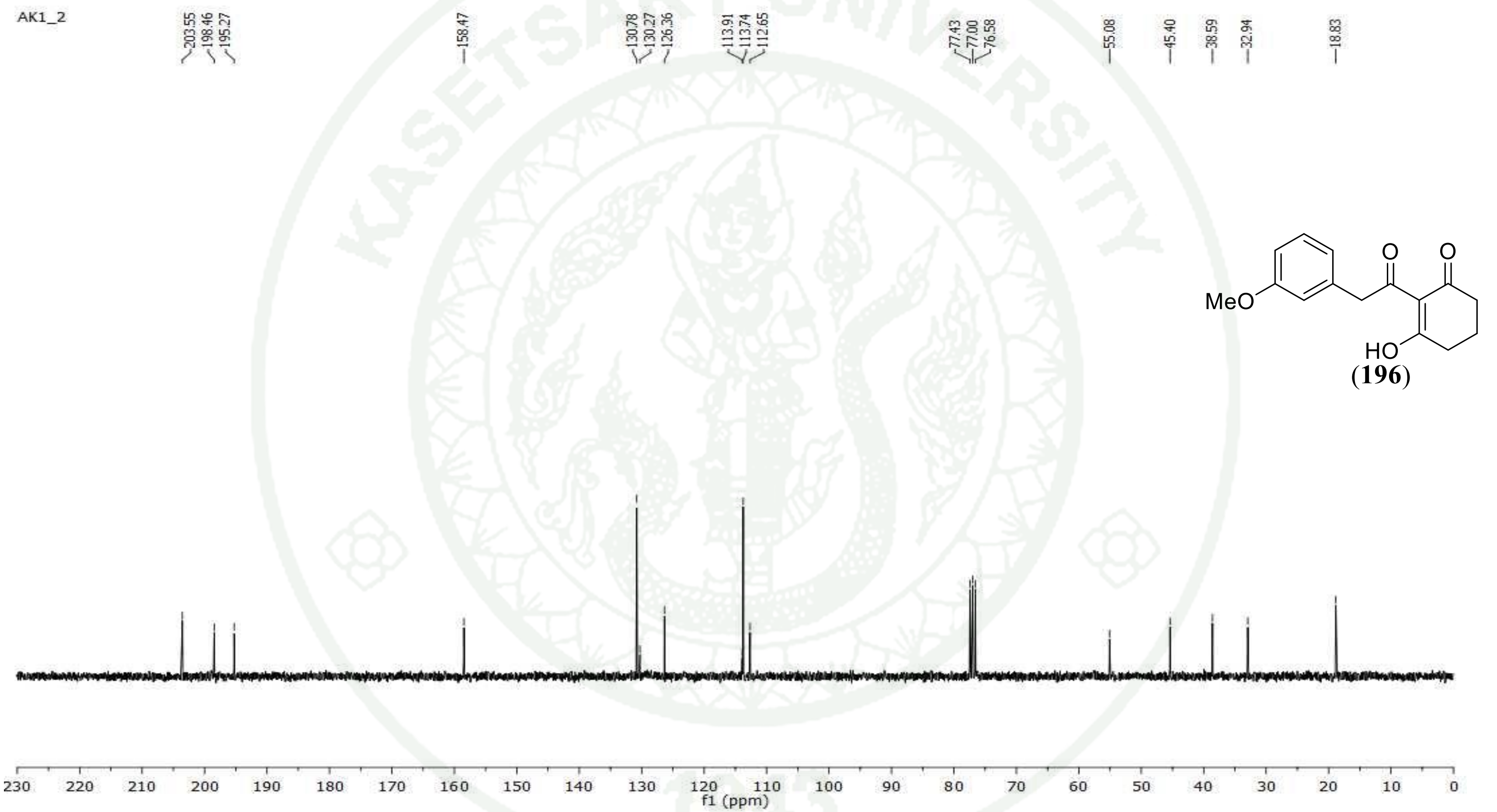

Appendix Figure 28 75 MHz ^{13}C NMR spectrum of 3-Hydroxy-2-(2-(4-methoxyphenyl)acetyl)cyclohex-2-enone (**196**)

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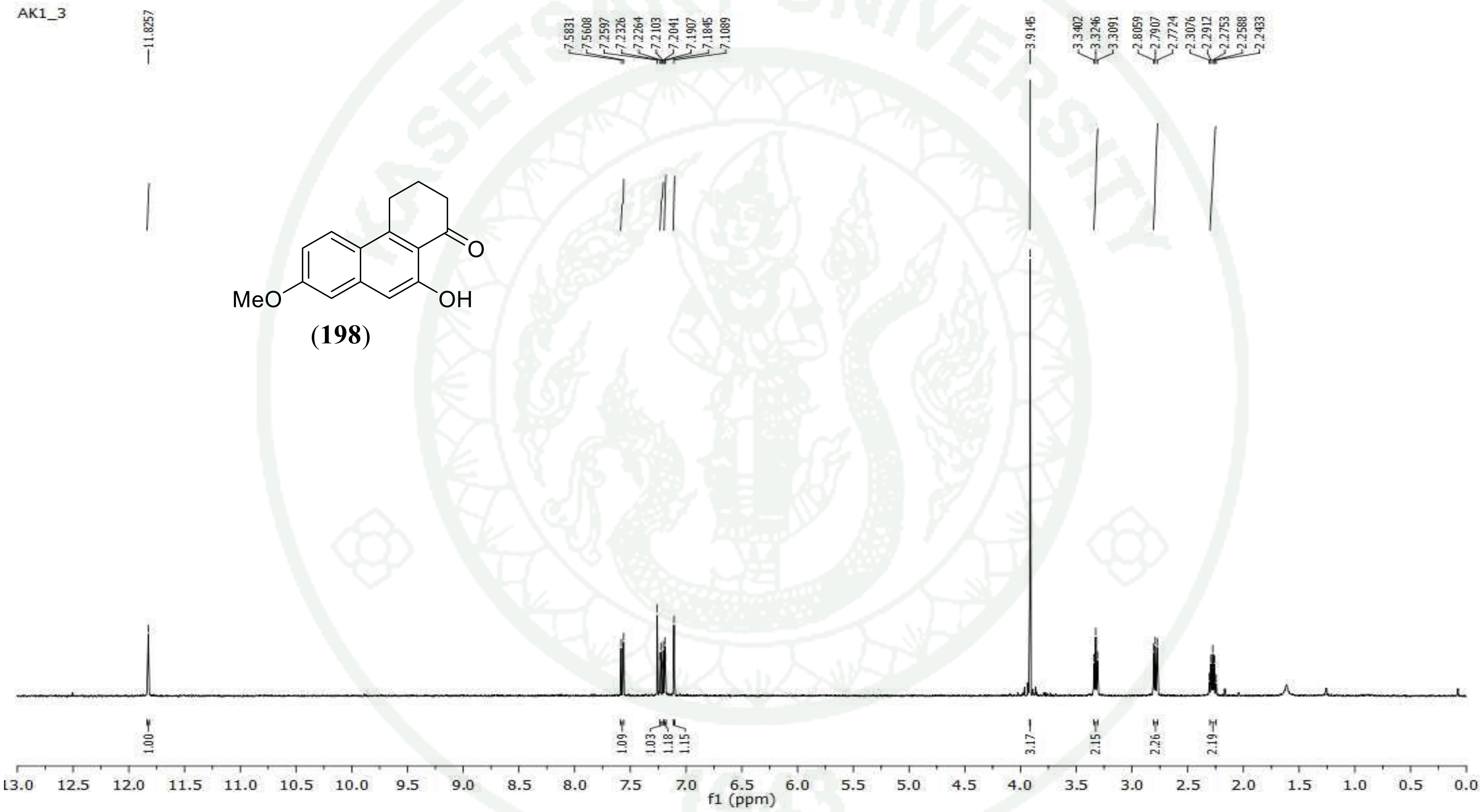

Appendix Figure 29 400 MHz ^{1}H NMR spectrum of 10-Hydroxy-6-methoxy-3,4-dihydrophenanthren-1(2*H*)-one (**198**)

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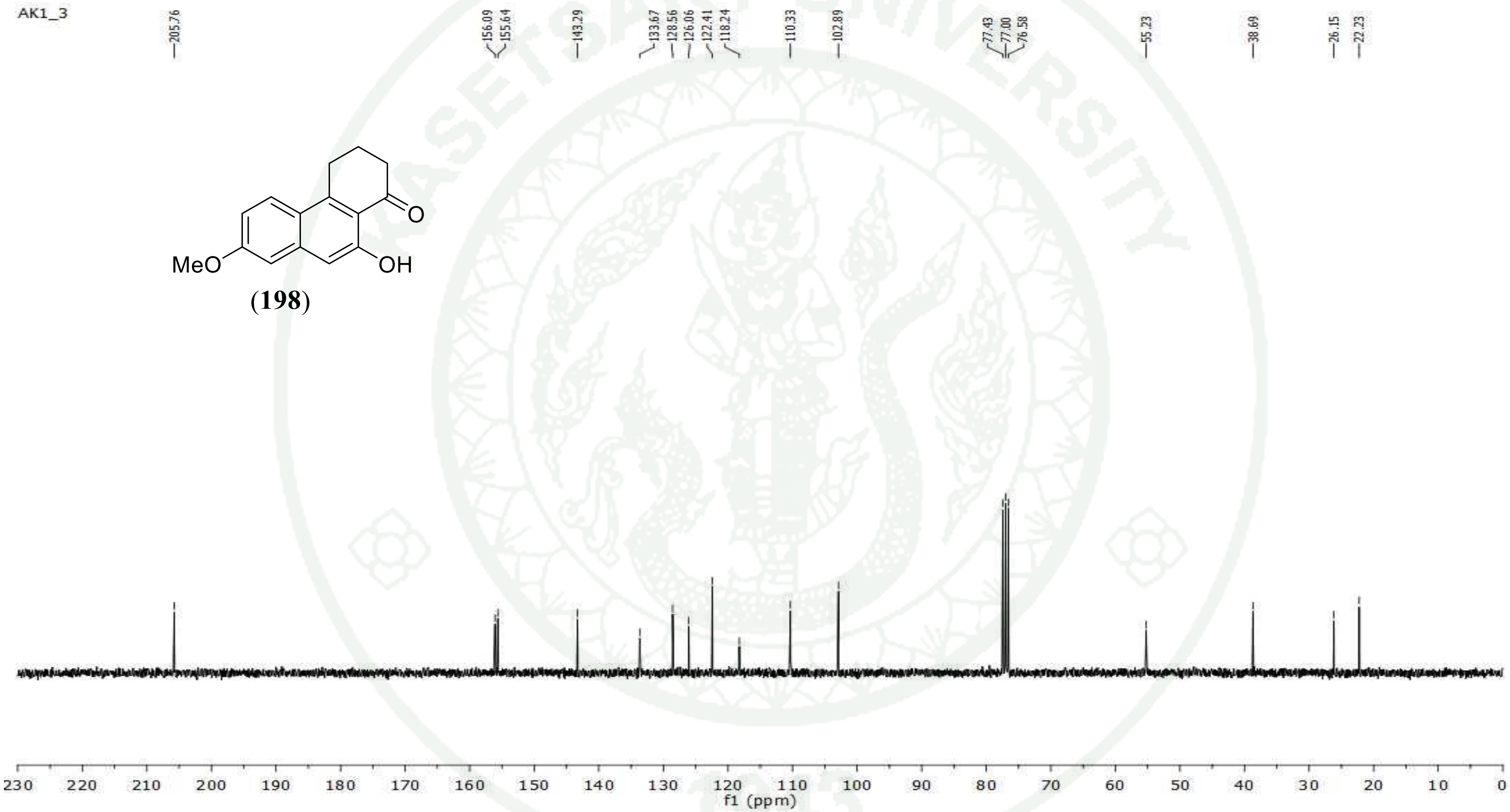

Appendix Figure 30 75 MHz ^{13}C NMR spectrum of 10-Hydroxy-6-methoxy-3,4-dihydrophenanthren-1(2*H*)-one (**198**)

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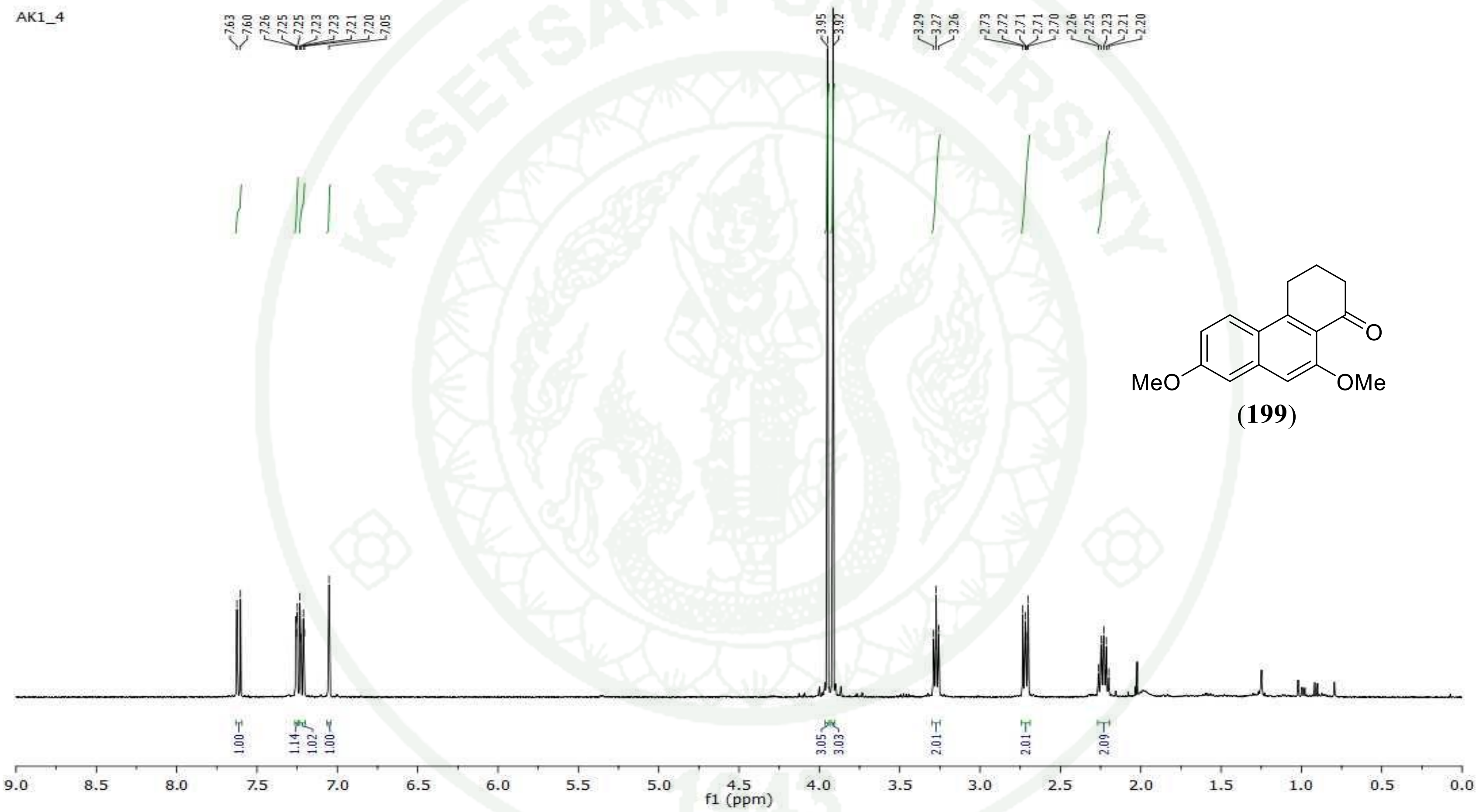

Appendix Figure 31 400 MHz ^{1}H NMR spectrum of 6,10-Dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one (**199**)

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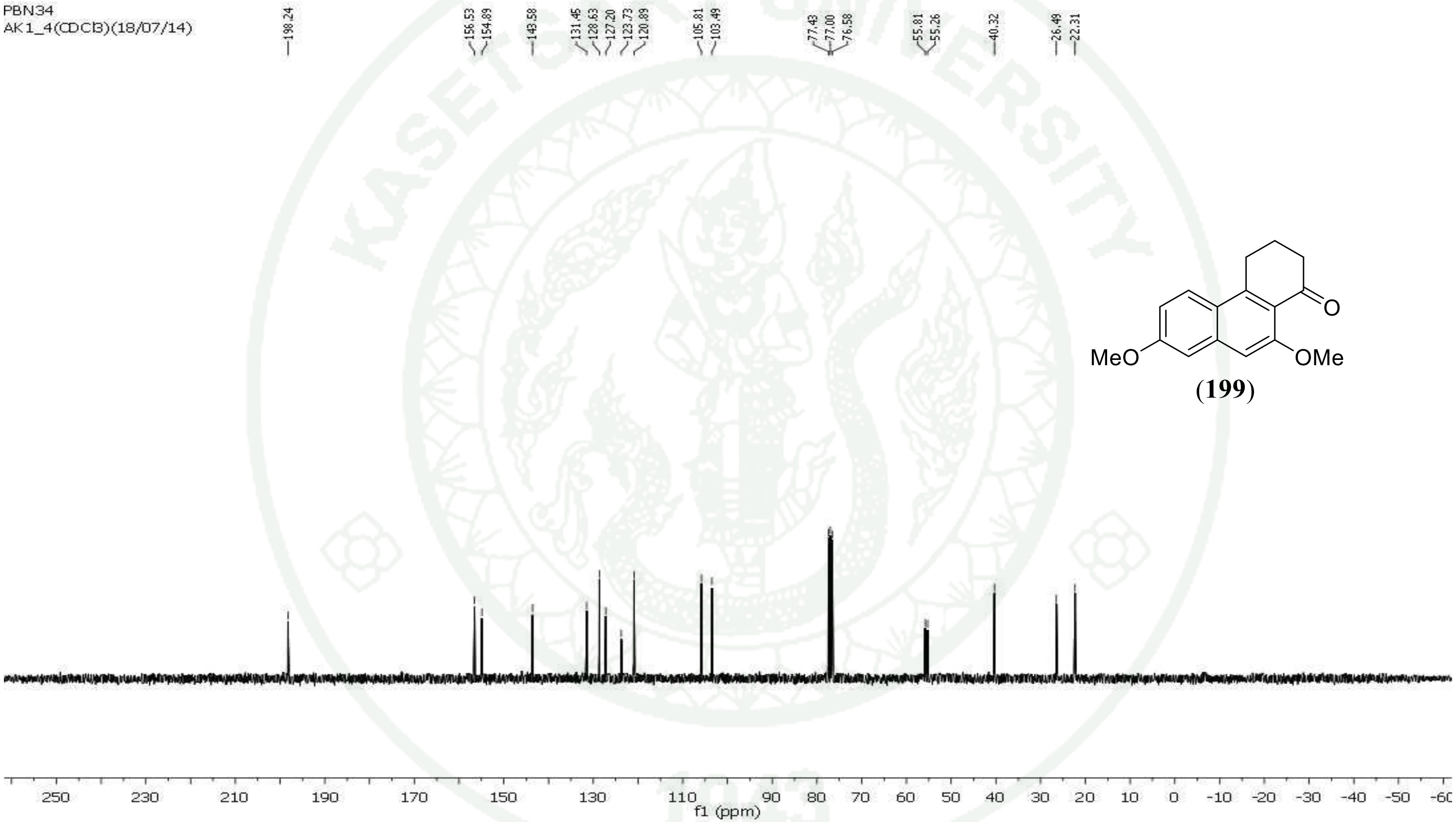

Appendix Figure 32 75 MHz ^{13}C NMR spectrum of 6,10-Dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one (**199**)

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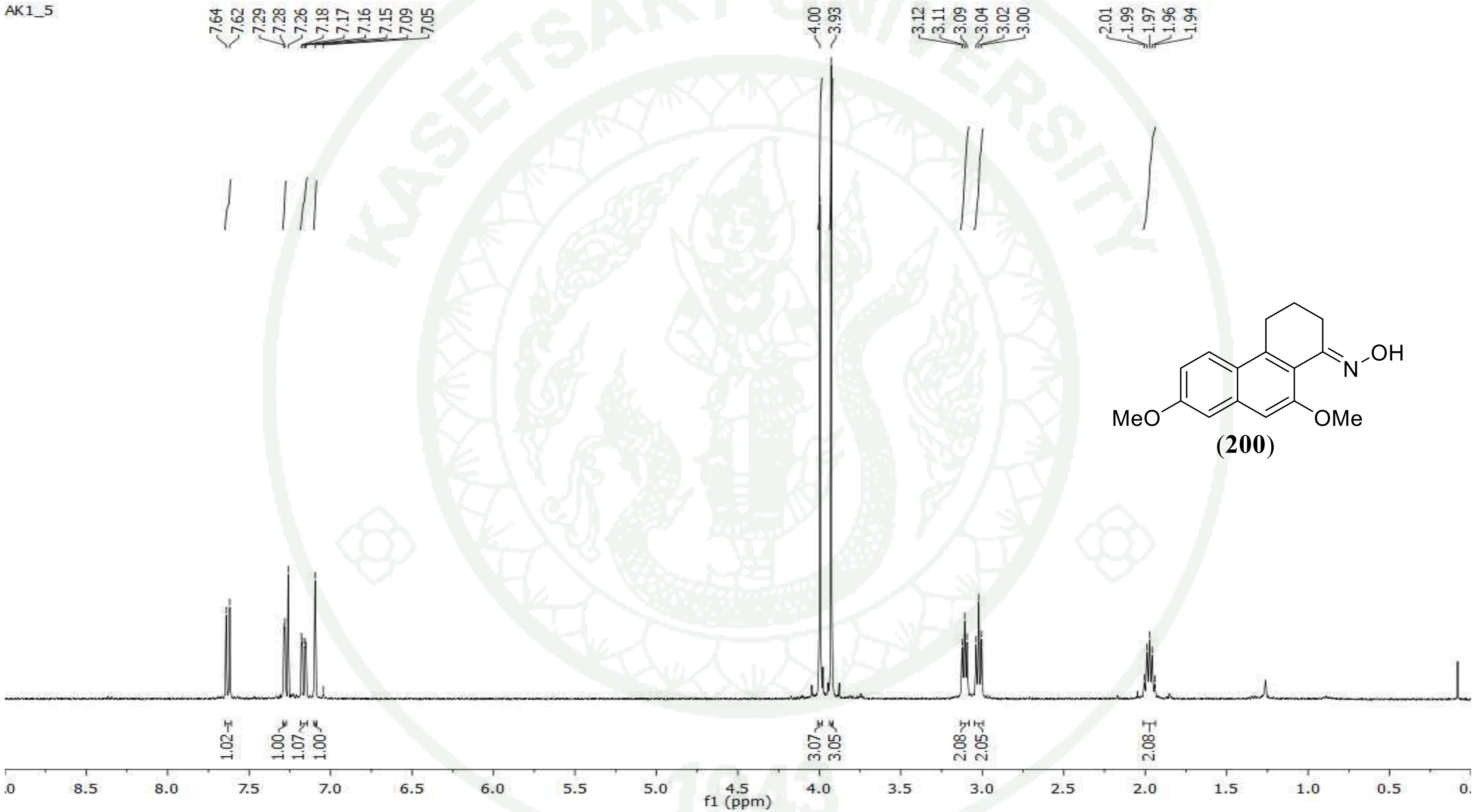

Appendix Figure 33 400 MHz ^{1}H NMR spectrum of 6,10-dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one oxime (**200**)

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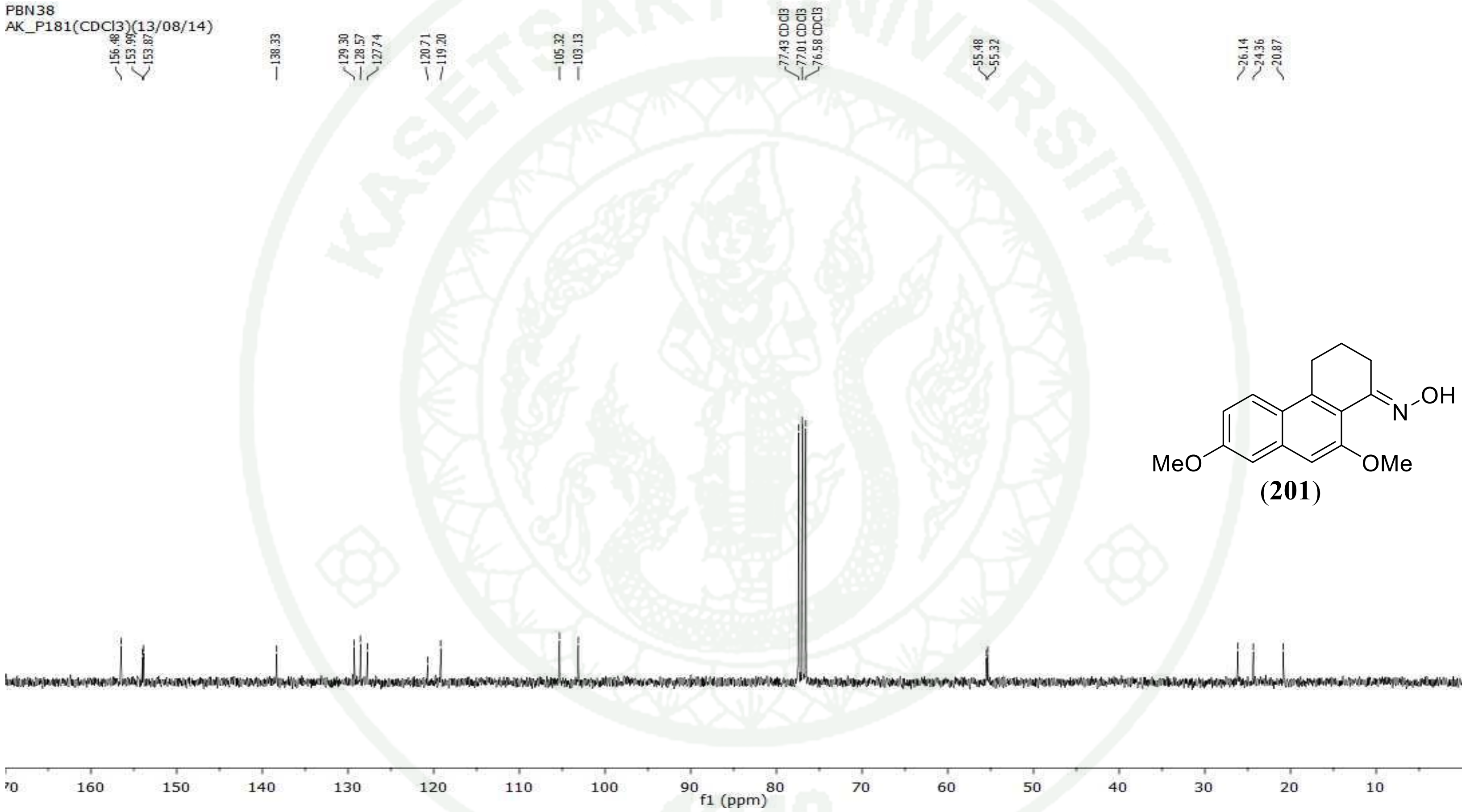

(**201**)

Appendix Figure 32 75 MHz ^{13}C NMR spectrum of6,10-dimethoxy-3,4-dihydrophenanthren-1(2*H*)-one oxime (**201**)

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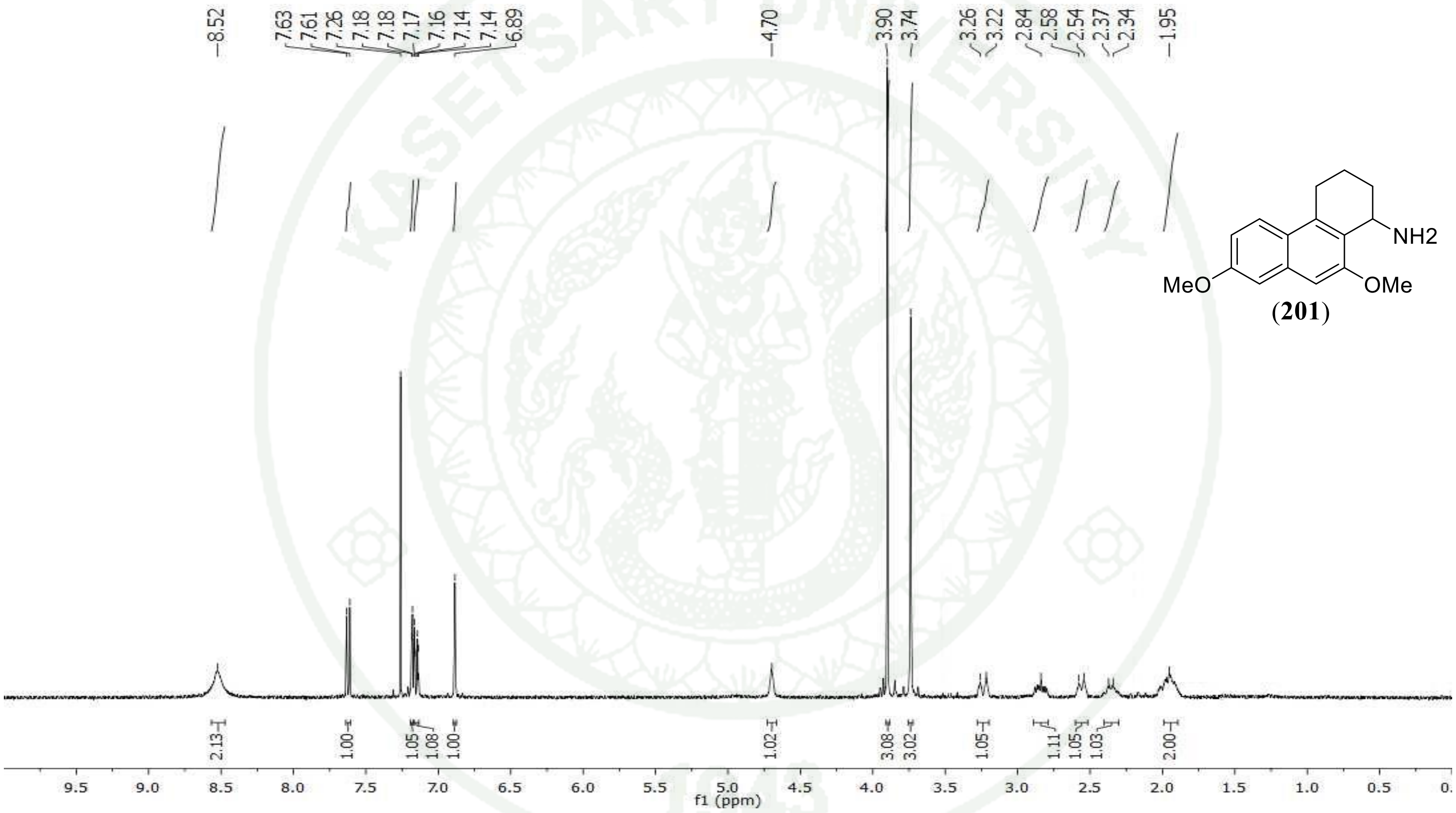

Appendix Figure 33 400 MHz ^{1}H NMR spectrum of 6,10-Dimethoxy-1,2,3,4-tetrahydrophenanthren-1-amine (**201**)

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