



SYNTHESIS AND BIOLOGICAL ACTIVITIES OF IHEYAMINE A AND GELDANAMYCIN
DERIVATIVES

By

MISS Chanjira JARAMORNBURAPONG

A Thesis Submitted in Partial Fulfillment of the Requirements

for Doctor of Philosophy ORGANIC CHEMISTRY

Department of CHEMISTRY

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การสังเคราะห์ และฤทธิ์ทางชีวภาพของอนุพันธ์ของ Iheyamine A และ
Geldanamycin



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปรัชญาดุษฎีบัณฑิต
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Title Synthesis and biological activities of lheyamine A and Geldanamycin derivatives

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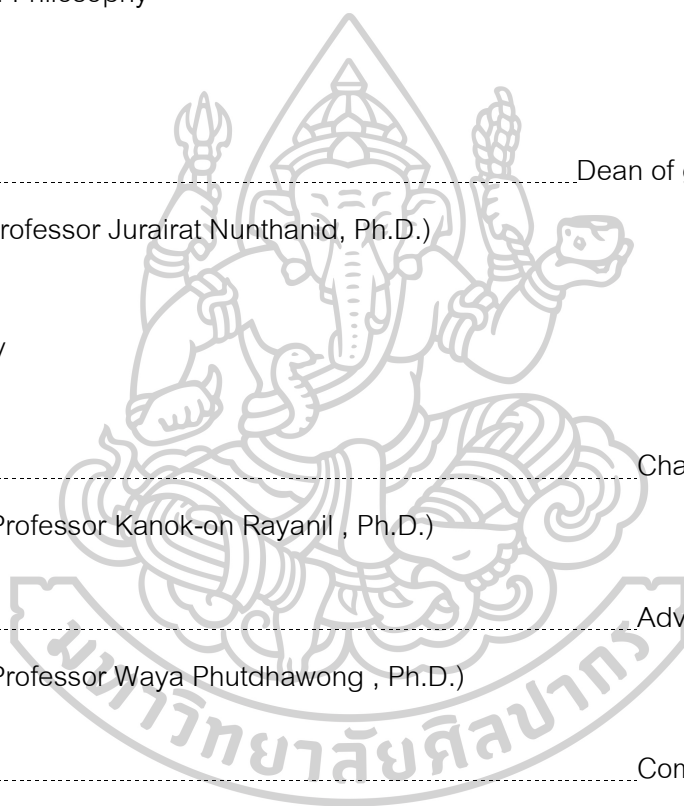
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MISS CHANJIRA JARAMORNBURAPONG : SYNTHESIS AND BIOLOGICAL ACTIVITIES OF IHEYAMINE A AND GELDANAMYCIN DERIVATIVES THESIS ADVISOR : ASSISTANT PROFESSOR WAYA PHUTDHAWONG, Ph.D.

lheyamine A is a bis-indole alkaloid isolated from ascidians *Polycitorella* sp.. lheyamine A is a heteroaromatic compound consisted of bis-indole fused with an azepine ring and showed moderately anticancer activity against lung cancer cell (A549), leukemia cell (P388), and human colon adenocarcinoma cell (HT29). Herein, we synthesized lheyamine A derivative with three synthetic routes from the different substances. The coupling of two indole rings and the formation of an azepine ring were synthesized through condensation, reduction, Pd-coupling, oxidation, nitration, substitution, hydrogenation and amide formation. However, all synthetic routes of lheyamine A derivative were not successful to fuse with an azepine ring.

Geldanamycin (GDM, 15) is an antibiotic that was isolated from *Streptomyces zerumbet* W14, which inhibited the operation of heat shock protein 90 (Hsp90). In this study, we used GDM as a core structure to generate eight Geldanamycin derivatives which were synthesized by nucleophilic substitution at C-17 of GDM with amino alcohol 36-38 and 3,4-O-disubstituted dopamine 39-43. Then, the cytotoxicity, antibacterial, and molecular docking analyses were determined. The results showed that cytotoxicity was moderately active in Hela, HepG2 and MDA cell lines with IC_{50} 19-71 $\mu\text{g/mL}$ and low cytotoxicity in normal cells. Antibacterial activity of 44, 45, and 47-51 exhibited against *B. subtilis*, *B. cereus*, and *S. aureus* (MIC>512 $\mu\text{g/mL}$). Compound 46 activated against *B. subtilis* (MIC>128 $\mu\text{g/mL}$), *B. cereus* and *S. aureus* (MIC>512 $\mu\text{g/mL}$). Their interaction with Hsp90 showed the binding energy of -97.03 to -122.41 kcal/mol which compared with GDM and DMAG in 1YET and 1OSF.

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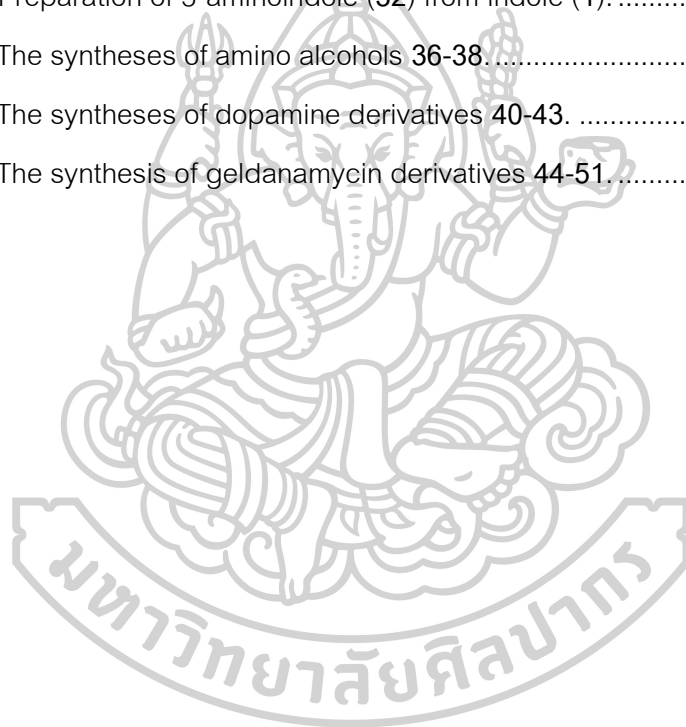
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ABBREVIATIONS

Abs	=	Absorbance
Ac ₂ O	=	Acetic anhydride
AcOH	=	Acetic acid
anh. Na ₂ SO ₄	=	Sodium sulphate anhydrous
Ar	=	Argon
Boc ₂ O	=	Di- <i>tert</i> -butyl dicarbonate
br	=	Broad (NMR spectrum)
CDCl ₃	=	Deuterated chloroform (NMR solvent)
CH ₂ Cl ₂	=	Dichloromethane
CH ₃ CN	=	Acetonitrile
CsCO ₃	=	Cesium carbonate
d	=	Doublet (NMR spectrum)
dd	=	Doublet of doublet (NMR spectrum)
DMAP	=	4-Dimethylaminopyridine
DMEM	=	Dulbecco's Modified Eagle's medium
DMF	=	Dimethylformamide
DMSO	=	Dimethyl sulfoxide
DMSO- <i>d</i> 6	=	Deuterated dimethyl sulfoxide (NMR solvent)
Et ₃ N	=	Triethylamine
EtOAc	=	Ethyl acetate
EtOH	=	Ethanol
HCl	=	Hydrochloric acid
HNO ₃	=	Nitric acid
Hz	=	Hertz
I ₂	=	Iodine
<i>J</i>	=	Coupling constant
KOH	=	Potassium hydroxide

m	=	Multiplet (NMR spectrum)
MeOD- <i>d</i> 4	=	Deuterated methanol (NMR solvent)
MeOH	=	Methanol
mmol	=	Millimole
Na ₂ S ₂ O ₅	=	Sodium metabisulfite
NaBH ₄	=	Sodium borohydride
NaN ₃	=	Sodium azide
NaOH	=	Sodium hydroxide
<i>n</i> -BuLi	=	<i>n</i> -butyl lithium
NMR	=	Nuclear Magnetic Resonance Spectroscopy
Pd(PPh ₃) ₄	=	Tetrakis (triphenylphosphine) palladium
q	=	Quartet (NMR spectrum)
s	=	Singlet (NMR spectrum)
SOCl ₂	=	Thionyl chloride
t	=	Triplet (NMR spectrum)
td	=	Triplet of doublet (NMR spectrum)
TFA	=	Trifluoroacetic acid
THF	=	Tetrahydrofuran
TLC	=	Thin-Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 Indole natural products

Natural products have been of numerous interest in the study and development of new medicines. Alkaloids are one of the natural compounds found in plants, marines, micro-organisms, and animals. Most of alkaloids are determined to possess pharmaceutical activities. An estimated quarter of total alkaloids were produced from marine organisms. The molecular structure was composed of the least one nitrogen atom (Verpoorte, 2005).

Indole (1) is a heterocyclic compound containing a nitrogen atom and bicyclic structure, comprising of a benzene ring fused to a pyrrole ring (Figure 1). Some of the indole alkaloids have interesting biological activities, such as antiviral, antibacterial, anti-inflammatory, antimalarial, antioxidant, and anticancer activities. For example, 3,6-dibromoindole (2) was isolated from the ascidian *Distaplia regina* which possessed potent antibacterial activity (Qureshi & Faulkner, 1999). Hinckdentine A (3) was isolated from the marine bryozoan *Hincksinoflustra denticulate* (Blackman, Hambley, Picker, Taylor, & Thirasasana, 1987). Bis-Indolo[1,2-c]quinazolines exhibited potent antibacterial and antifungal activities (Rohini, Reddy, Shanker, Hu, & Ravinder, 2010). Eudistomin K (4) and L (5) were isolated from the Caribbean tunicate *Eudistoma olivaceum*. They showed antiviral activity against HSV-1 (Rinehart et al., 1984) and antitumor against A-549, HCT-8, L-1210, and P-388 cell lines (Lake, Blunt, & Munro, 1989). The structures were shown in Figure 2.

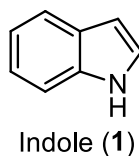


Figure 1 Structure of indole (1).

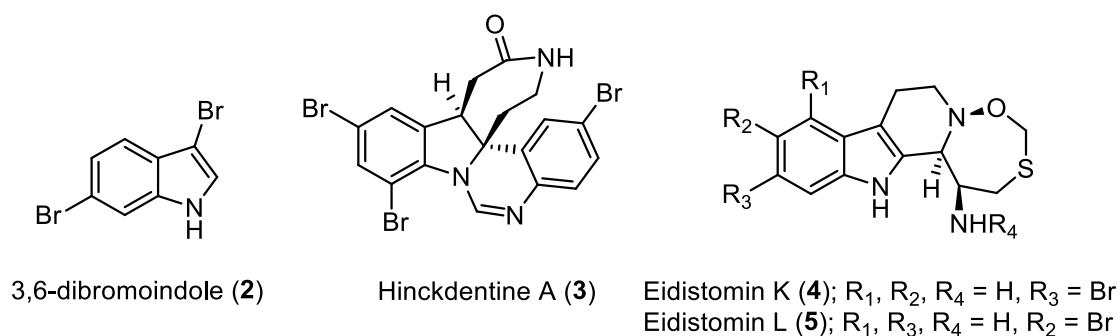


Figure 2 The structure of some bioactive indole alkaloids were bioactive compounds.

The structures shown in Figure 3 were bis-indole molecules that consist of dimeric of indole unit. The diversity of structure has been shown to exhibit more potent biological such as Dragmacidin (**6**) was isolated from the marine sponges *Dragmacidin* sp. This bis-indole alkaloid was reported to possess anticancer activity against P388 leukemia cell lines (Kohmoto et al., 1988) and exhibited anti-inflammatory activity (Capon et al., 1998). Nortopsentin A-C (**7-9**) were isolated from *Spongisorites ruetzleri*. This bis-indoles link with the five-membered ring of imidazole. They showed the cytotoxicity against P388 cancer cell lines (Sakemi & Sun, 1991). Trigonoliminiines A–C (**10-12**) were isolated from the leaves of *Trigonostemon lili*. The structures are containing bis-indole fused azepine ring. Their cytotoxicities showed modest potent antiviral activity against HIV-1 but exhibited weak potent against HeLa and U-937 cancer cell lines (Tan et al., 2010).

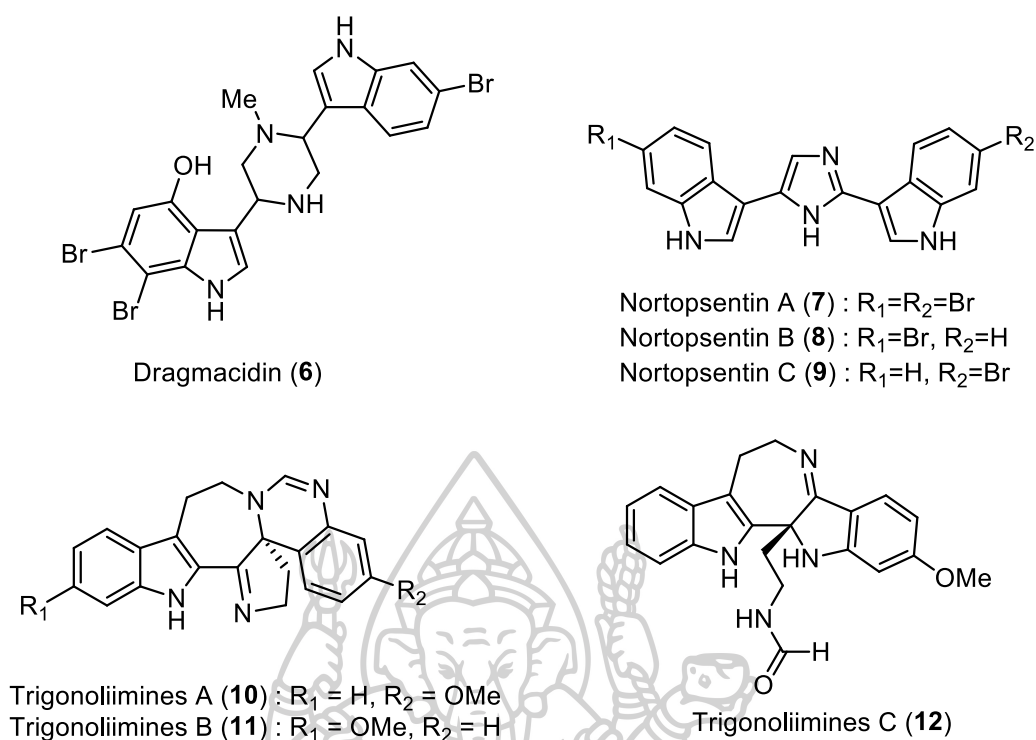


Figure 3 The structure of bioactive bis-indoles compounds.

Iheyamine A (**13**) and B (**14**) are bisindole alkaloids that were extracted and isolated from a colonial ascidian, *Polycitorella* sp. (purple) species found on Iheya Island, Okinawa, Japan. The structure of iheyamine A and B were contained polycyclic heteroaromatic system of bis-indole fused azepine which were reported and interpreted in 1999. Both iheyamine A and B showed cytotoxicity with moderate activity against P388, A549, and HT29 cell lines (Sasaki, Ohtani, Tanaka, & Higa, 1999) (Figure 4).

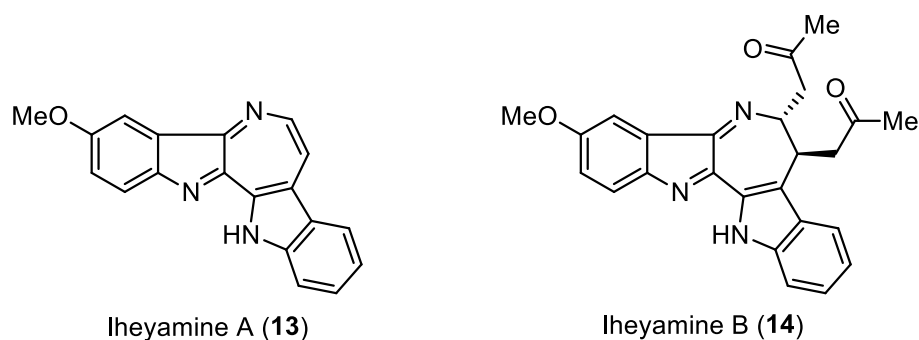


Figure 4 Structure of iheyamine A (**13**) and B (**14**).

1.2 Introduction of geldanamycin

Geldanamycin (GDM, **15**) was first isolated from *Streptomyces hygroscopicus* var. *geldanus* var. *nova* in 1970 (DeBoer, Meulman, Wnuk, & Peterson, 1970) (Figure 5). In 2019, geldanamycin was isolated from the culture medium of *Streptomyces zerumbet* W14 (Taechowisan, Puckdee, & Phutdhawong, 2019). Geldanamycin is a benzoquinone ansamycin antibiotic that was a macrocyclic lactam binding to the heat shock protein 90 (Hsp90) at the N-terminal ATP binding region (Whitesell, Mimnaugh, De Costa, Myers, & Neckers, 1994).

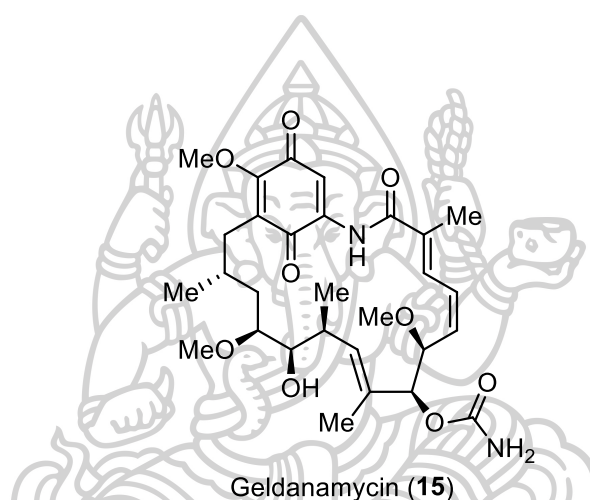


Figure 5 The structure of geldanamycin (15).

Heat shock protein 90 (Hsp90) is a chaperone protein that is highly found and expressed in the eukaryotic cytosol. The Hsp90 plays an important role in the regulation of stability, folding, and the function of oncoprotein in the cells of living organisms. It also acts as a signal linker involved in normal cell division and survival including cancer cells. Many research has reported that HSP90 shows a role in the progression of the fierce disease and exhibited is 10-fold higher in tumor cells than normal cells. Due to cancer diseases are still a main cause of death worldwide. Therefore, Hsp90 is one of the most popular chaperone proteins in the research, and inhibition of Hsp90 is an interesting new treatment to research and development of drugs for cancer disease.

Although, geldanamycin (**15**) has been recognized as the antitumor potential. The clinical evaluation of geldanamycin has not been derived due to its strongly hepatotoxic and poorly soluble in water. For this problem, many efforts have been attempted to modify

the structure of geldanamycin, generating various derivatives to increase the clinical effect and water solubility.

Among the successful analogs, 17-allylamino-17-demethoxy-geldanamycin or Tanespimycin (17-AAG, **16**) is a derivative resulting from the substitution of the methoxy (-OMe) group at C-17 of geldanamycin with allyl amino group (-NHCH₂CH=CH₂). This compound gave a lower cytotoxicity test than the geldanamycin and less able to dissolve in water. Later, another derivative of the molecule, 17-demethoxy-17-*N,N*-dimethylaminoethyl-aminogeldanamycin or Alvespimycin (17-DMAG, **17**) was developed. This molecule has water soluble and better biological activity than 17-AAG.

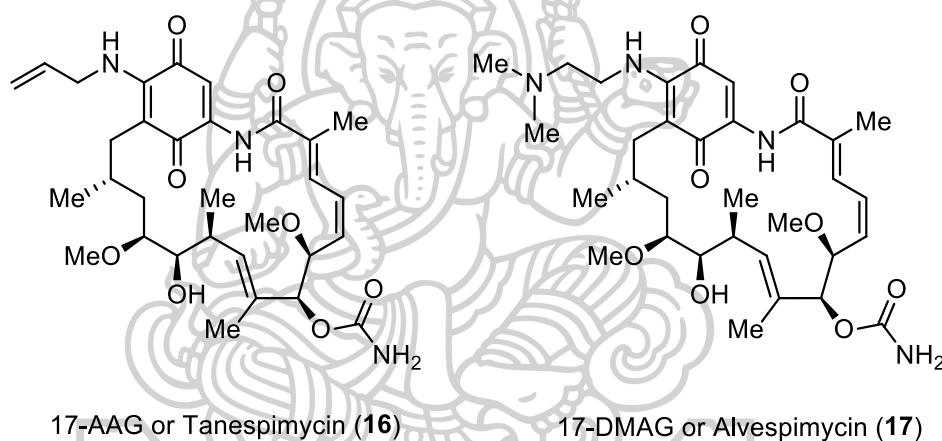


Figure 6 Structure of geldanamycin derivatives, 17-AAG (**16**) and 17-DMAG (**17**).

In this study, we are interested in the synthesis of iheyamine A and geldanamycin derivative, due to the unique structural visages and the dramatic biological activities. These two molecular moieties could develop new pharmacologically active compounds in the future.

1.3 Objective of this study

1. To develop the synthesis of iheyamine A and geldanamycin derivatives.
2. To evaluate the biological activities of the synthetic iheyamine A and geldanamycin derivatives.

1.4 Scope of this study

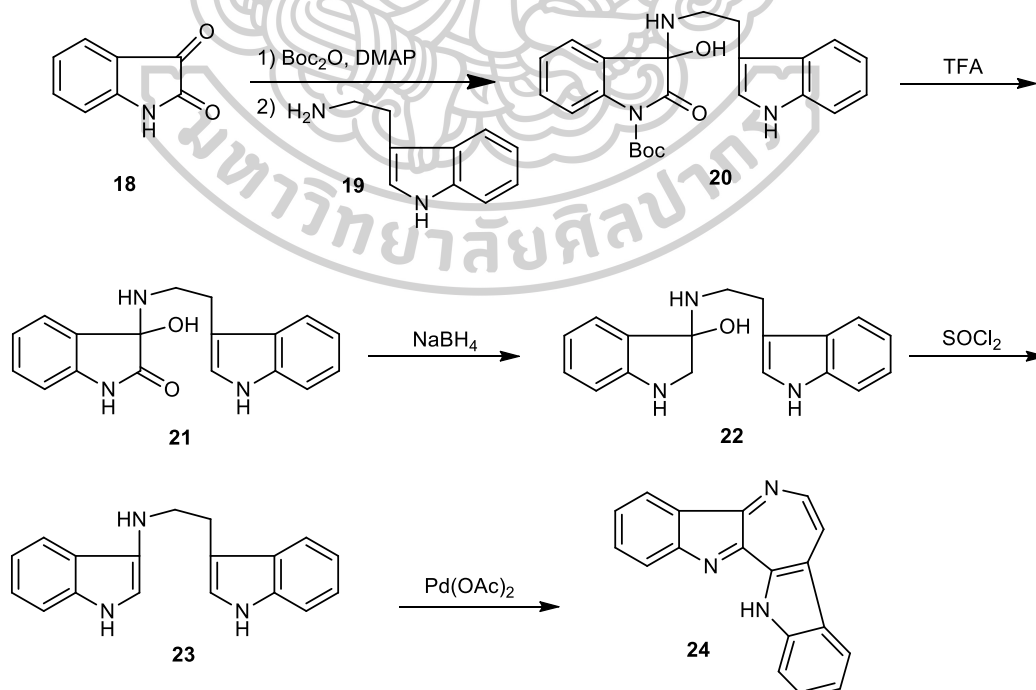
Synthesized, confirmed the structure of iheyamine A and geldanamycin derivatives and tested the biological activity.

1.5 Synthetic plan of this study

1.5.1 Synthetic plan of iheyamine A derivative

1.5.1.1 Synthetic route 1

The first synthetic route of iheyamine A derivative would be synthesized from isatin (18). The NH group of isatin would be protected with Boc group and then condensed with tryptamine (19) under the acidic condition provided the 3-hydroxyoxindole (20). Next step, deprotection of Boc group would be obtained oxindole (21). Then reduction of the oxindole (20) to bis-indole (23) using LiAlH_4 in a single step was reported in the previous our paper (Phutdhawong, Jaramornburapong, Taechowisan, & Phutdhawong, 2016). In this study, we would be changed the methodology through the reduction of oxindole and then elimination in two steps. Next, C-C coupling via palladium catalyst and oxidation would be formed the iheyamine A derivative (24) (Scheme 1).

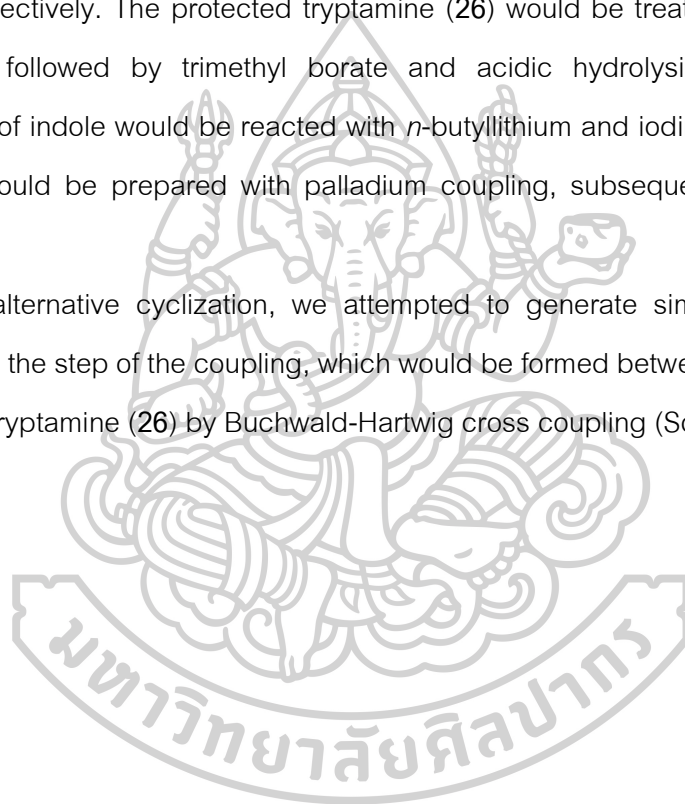


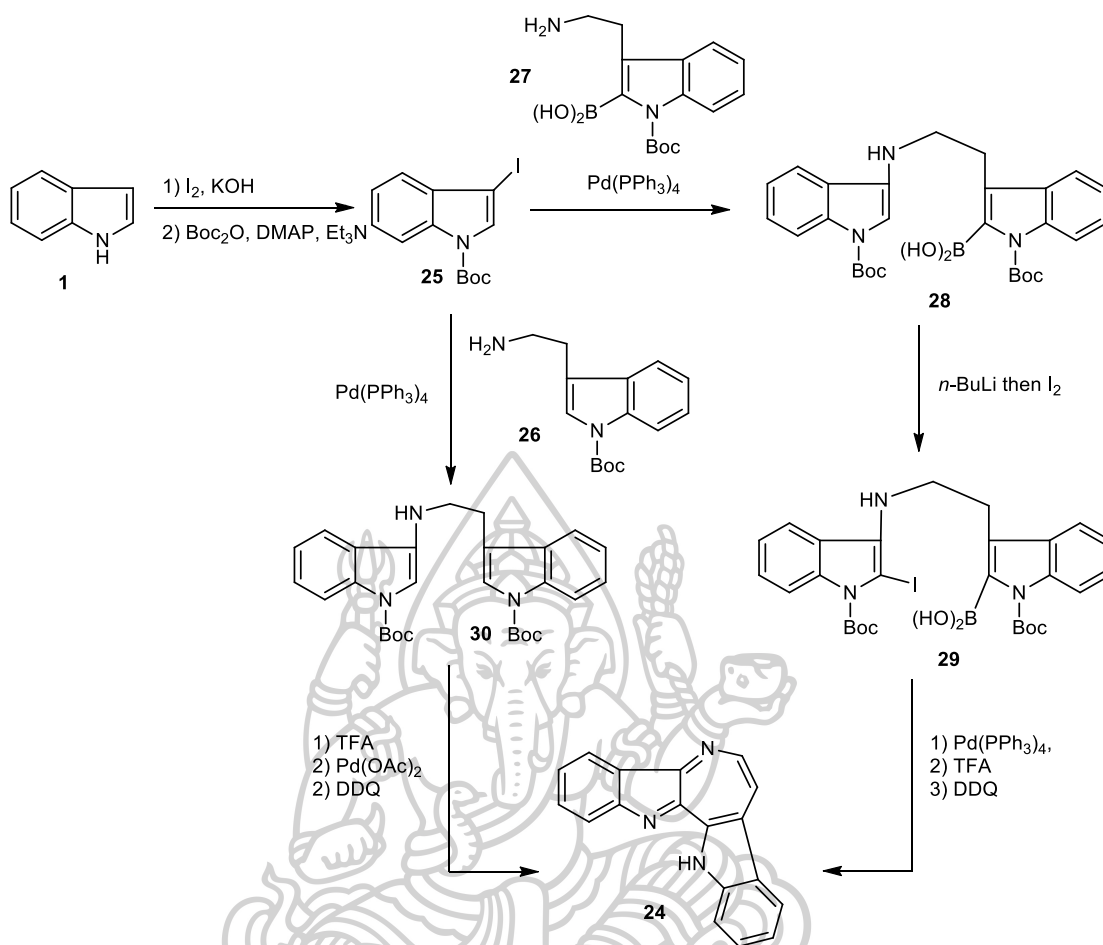
Scheme 1 The first synthetic plan of iheyamine A derivative (24).

1.5.1.2 Synthetic route 2

The second synthetic approach, we also attempted to generate the bis-indole skeleton by forming the seven membered ring system. This route was carried on the palladium catalyst of Buchwald-Hartwig and Suzuki-Miyaura cross-coupling as the key reactions. The indole (1) would be directed iodination followed by protecting of NH of indole ring. The *N*-Boc-tryptamine-2-boronic acid (27) would be synthesized from tryptamine *via* protection of primary amine and NH group with phthalic anhydride and Boc_2O , respectively. The protected tryptamine (26) would be treated *via* lithiation of *n*-butyllithium followed by trimethyl borate and acidic hydrolysis. And then 2-iodo substitution of indole would be reacted with *n*-butyllithium and iodine. The cyclization of bis-indole could be prepared with palladium coupling, subsequent deprotection and oxidation.

An alternative cyclization, we attempted to generate similar to the previous synthesis. In the step of the coupling, which would be formed between 2-iodo indole (25) and *N*-Boc tryptamine (26) by Buchwald-Hartwig cross coupling (Scheme 2).

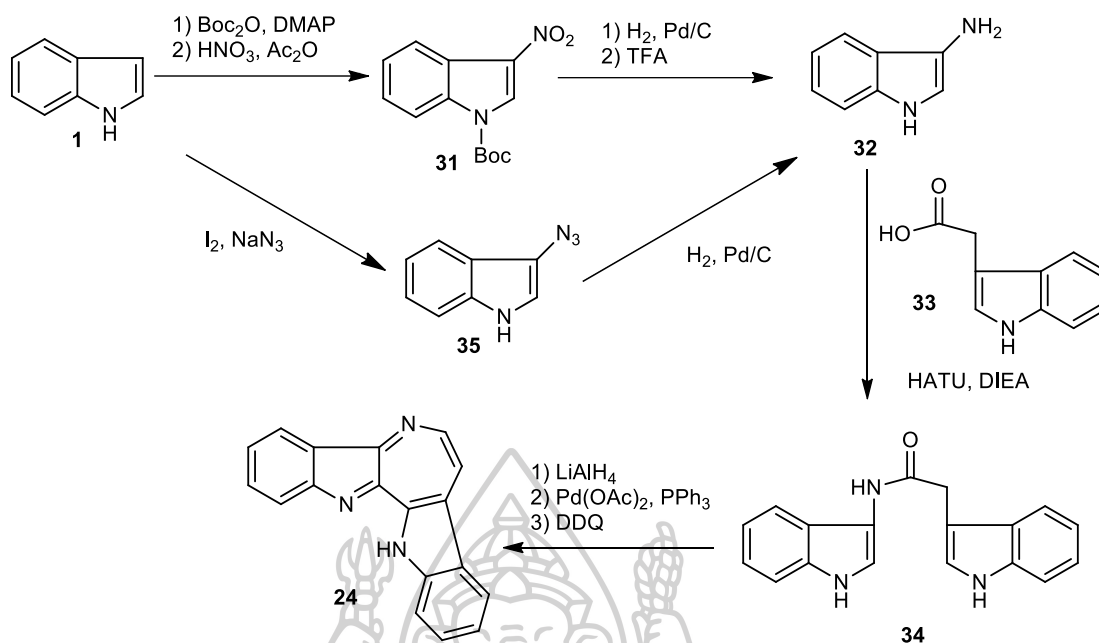




Scheme 2 The second synthetic plan of iheyamine A derivative (24).

1.5.1.3 Synthetic route 3

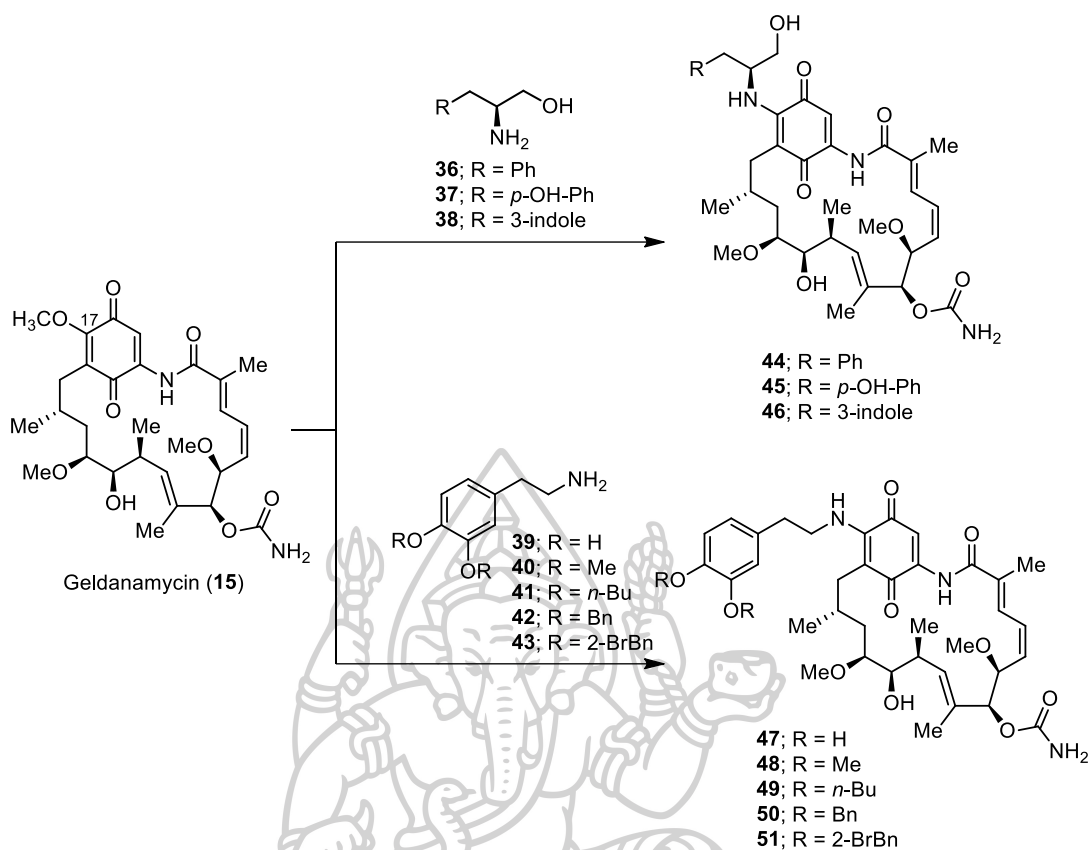
The last synthetic plan of iheyamine A, indole (1) could be converted to 3-aminoindole (32) by two methods. In the first method, indole (1) would be protected nitrogen atom with the Boc group then treated with HNO_3 , Ac_2O to undergo C-3 nitration followed by reduction with H_2 , Pd/C . The second method, iodination and substitution of indole would be prepared with iodine and sodium azide by one-pot reaction followed by hydrogenation to provide 3-aminoindole (32). The amide formation of bis-indole (34) would be generated with 3-aminoindole (32) and indole-3-acetic acid (33). The bis-indole (34) would be reduced and fused the azepine ring. The oxidation would be reacted to give the iheyamine A derivative (24) (Scheme 3).



Scheme 3 The third synthetic plan of iheyamine A derivative (24).

1.5.2 Synthetic plan of geldanamycin derivatives

The synthesis of 17-amino-17-demethoxygeldanamycin derivatives (44-51) would be generated by nucleophilic substitution with two classes of amine derivatives naming amino alcohol (36-38) and O-substituted dopamine derivatives (39-43) (Scheme 4).



Scheme 4 Synthetic plan of 17-amino-17-demethoxy geldanamycin derivatives 44-51.

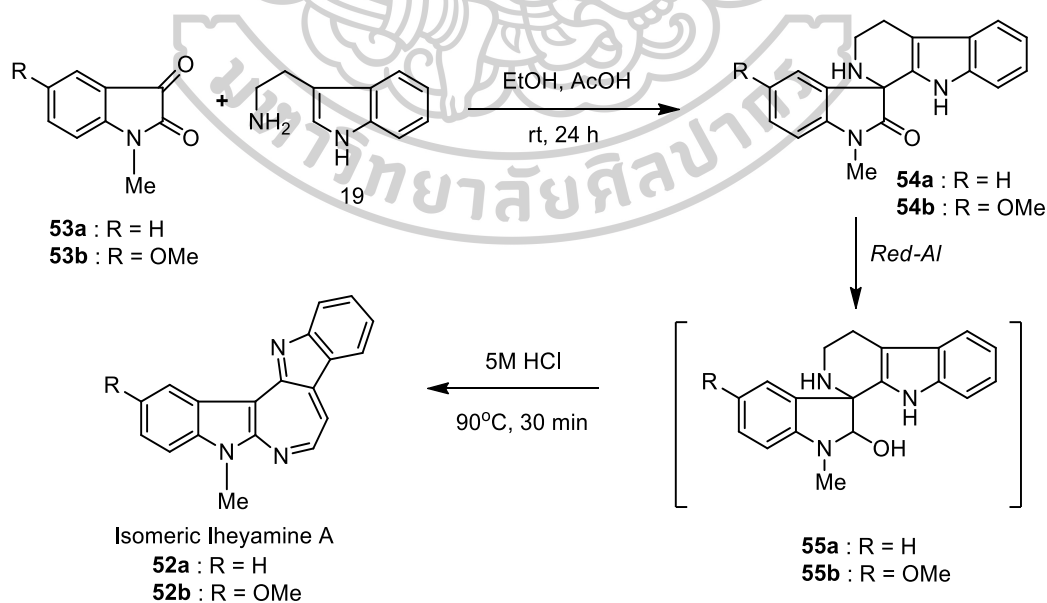
CHAPTER 2

LITERATURE REVIEW

2.1 Previous synthesis of iheyamine A

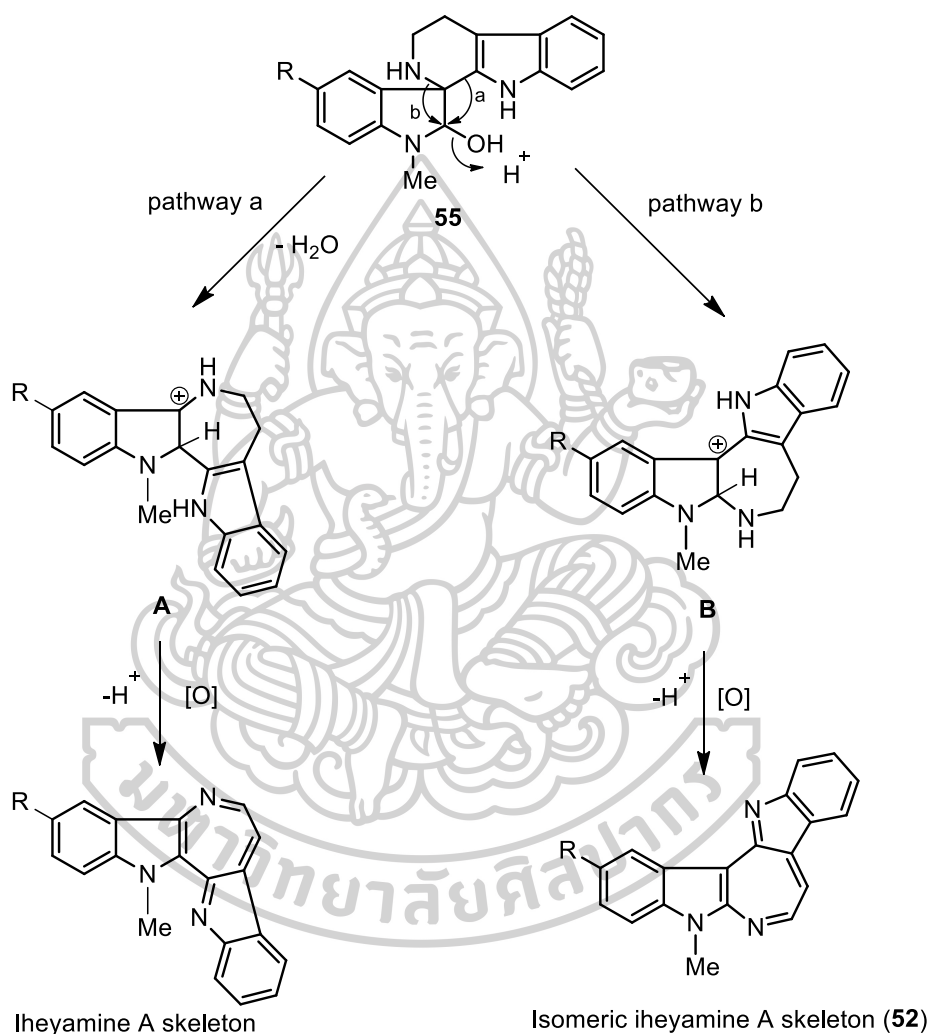
At present, indole alkaloids are one of the great interested topics to many researchers due to the unique structural features and wide range of interesting biological activities. Many indole alkaloid have been developed and used as medicines. In this research, we are interested in the synthesis of iheyamine A, which are reported in previous as follows.

In 2008, the synthesis of isomeric iheyamine A (**52a**, **52b**) have reported by Bremner and co-worker (Bremner, Sengpracha, & Skelton, 2008). They synthesized by condensation reaction between isatin derivatives (**53a**, **53b**) and tryptamine (**19**) with glacial acetic acid as a catalyst to form a spiro compound **54a** and **54b**. A partial reduction was performed using *Red-Al* as the reducing agent to give carbinolamines (**55a**, **55b**) then rearrangement under acidic conditions to form isomeric iheyamine A (**52a**, **52b**) as shown in Scheme 5. The structures were confirmed from X-ray crystal analysis.



Scheme 5 Synthesis of the isomeric iheyamine A (**52a**, **52b**).

In addition, they studied the reaction mechanism to explain the azepine ring closure. If the C-C bond migrated *via* rearrangement through intermediate A (pathway a), iheyamine A could be obtained as a product but the C-N bond migration occurred through intermediate B (pathway b) which produced the isomeric iheyamine A (Scheme 6).

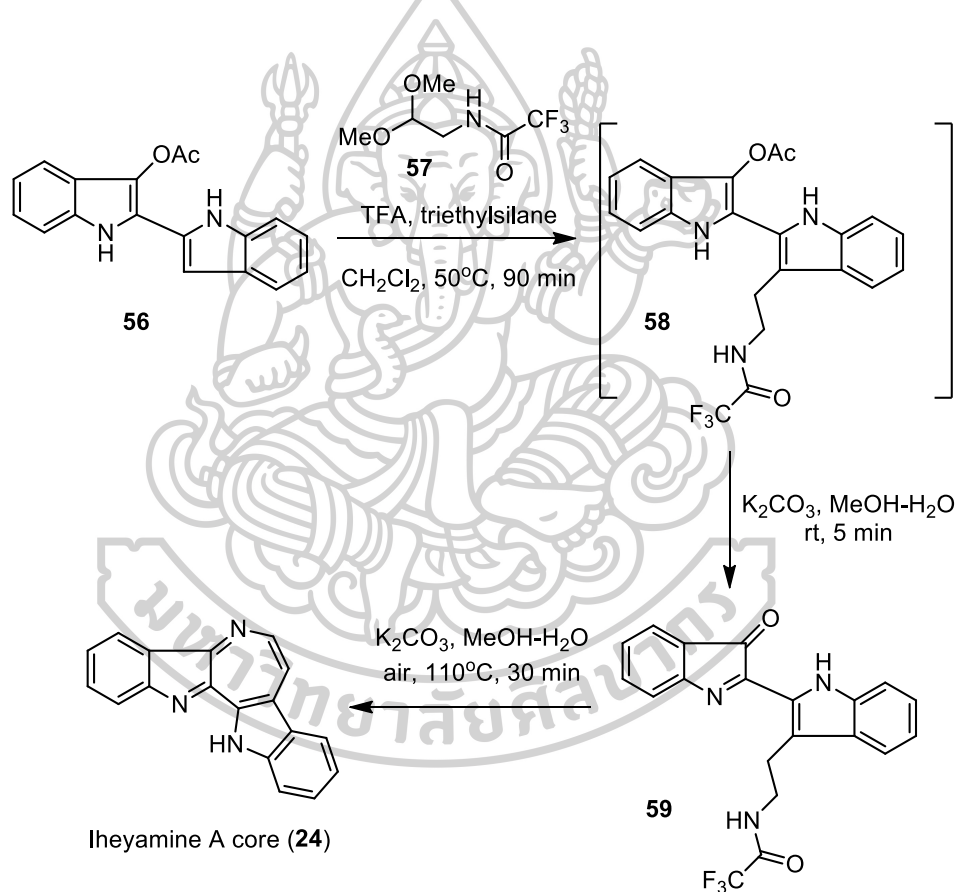


Scheme 6 Proposed mechanism for the rearrangement of carbinolamine **55**.

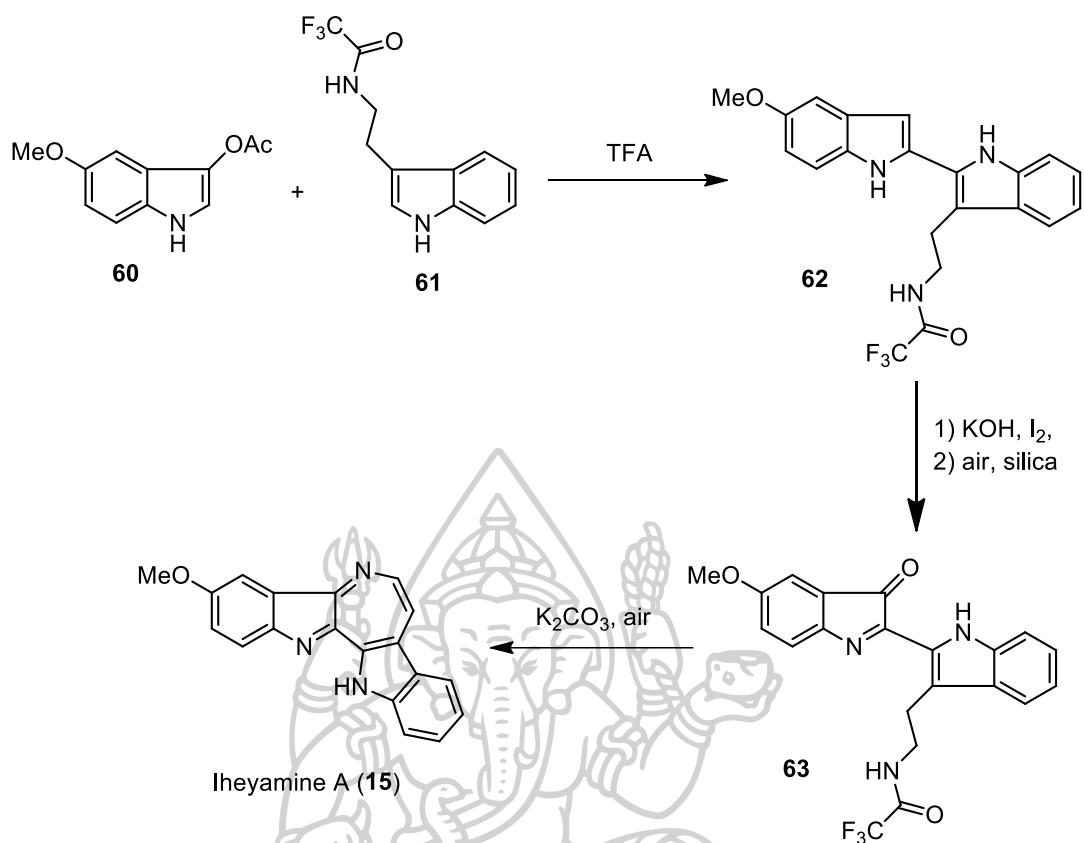
In 2016, Lindsay and co-workers reported the first total synthesis of iheyamine A (A. C. Lindsay, Leung, & Sperry, 2016). They decided to synthesize the iheyamine A core structure (**24**) which could be applied to synthesize natural product iheyamine A (**13**) as shown in Scheme 7. The side chain **57** was installed to indigo-derived acetoxybisindole **56** with reductive alkylation to obtain bisindole **58** followed by hydrolysis and oxidation.

The resulting compound, indolone **59** after reacting with potassium carbonate in methanol aqueous were cyclized to provide iheyamine A core (**24**) with this model study, iheyamine A (**13**) was synthesized through the cyclization of the azepinobisindole ring.

The unsymmetrical 2,2'-bisindole (**62**) has generated from 3-acetoxy-5-methoxyindole (**60**) coupling with tryptamine (**61**) *via* acid-mediated cross-Mannich reaction. Then, the bis-indole **62** was transformed to oxindole **63** by iodination under basic conditions and oxidation in air. Finally, the one-pot reaction of oxindole **63** gave iheyamine A with cyclization and aromatization (Scheme 8).

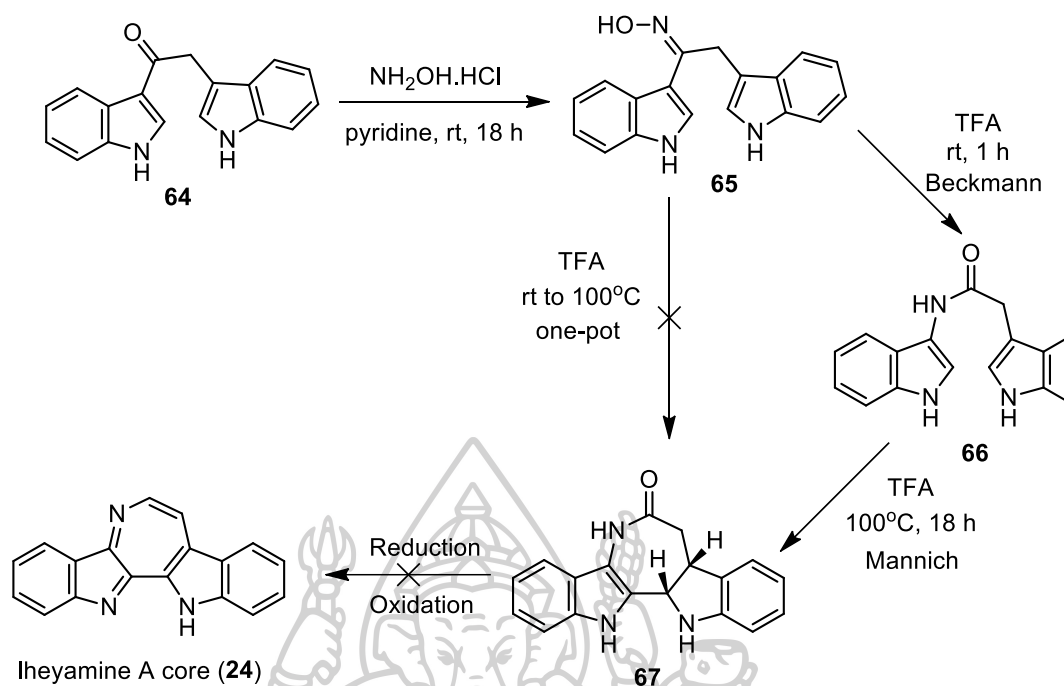


Scheme 7 Synthesis of iheyamine A core (**24**).



Scheme 8 Synthesis of the iheyamine A (15) by Lindsay and co-workers in 2016.

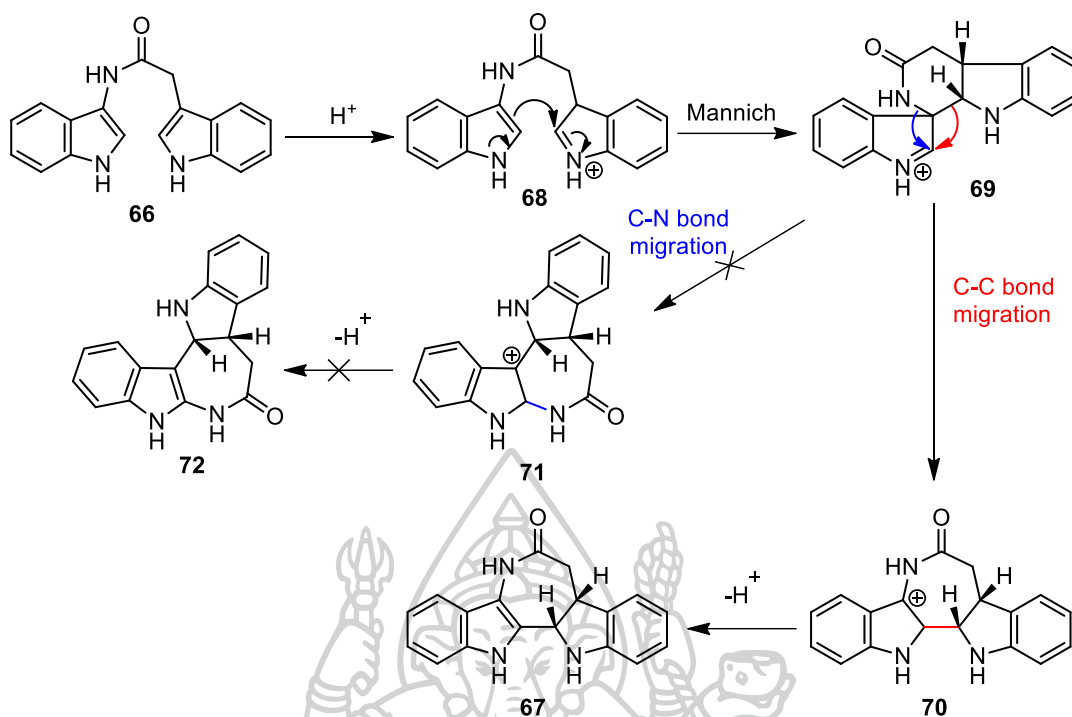
In 2017, Lindsay and Sperry were attempted to develop the synthesis of iheyamine A. They observed the cyclization through a Beckmann rearrangement-Mannich of azepinobisindole which is a core structure of iheyamine A (Ashley C. Lindsay & Sperry, 2017). They started from the condensation of bis(indolyl)ketone (64) with hydroxylamine to provide (*E*)-oxime 65, then reacted with TFA at room temperature through Beckmann rearrangement reaction to form bisindolylacetamide (66). The ring-closing of bisindolylacetamide (66) occurred with TFA at high temperature by Mannich cyclization to give 2-(indolin-2-yl)indole (67). But they cannot be synthesized to effect both of these reactions with one-pot reaction. Unfortunately, the completed azepinobisindole framework with reduction and oxidation was failed. Although they attempted using a variety of reducing agents consisted of lithium aluminium hydride, diisobutylaluminium hydride, and borane (Scheme 9).



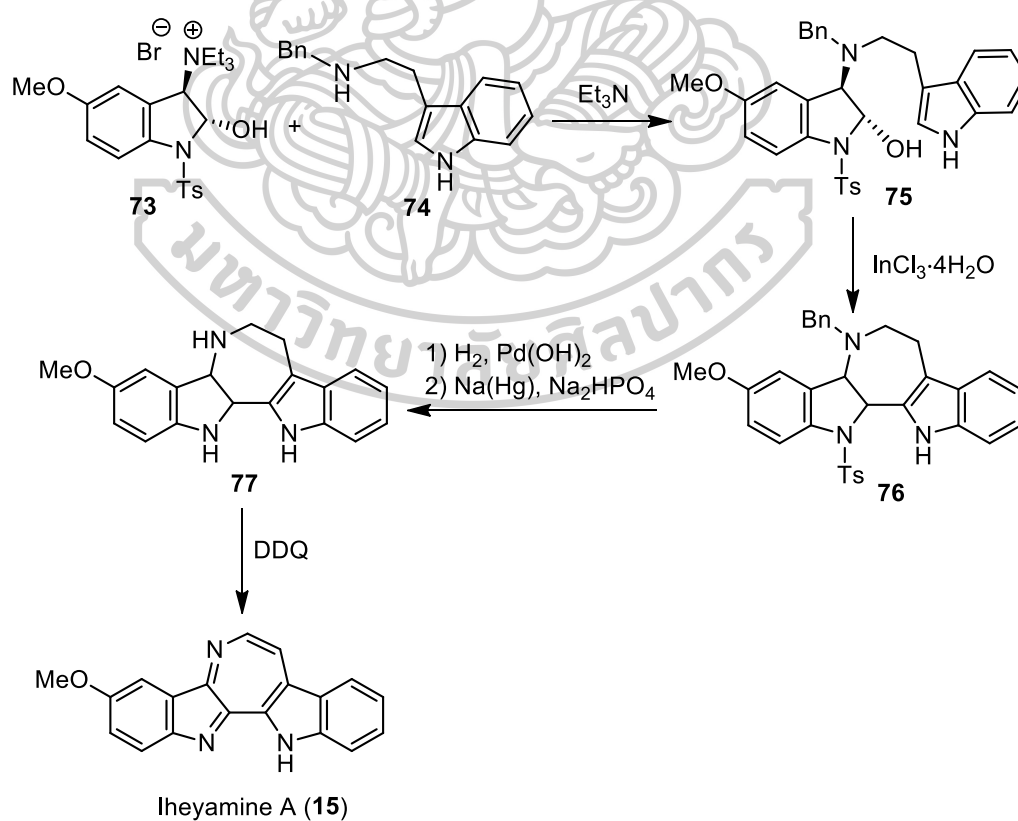
Scheme 9 Attempted to conversion of bis(indolyl)ketone (**64**) to iheyamine A core (**24**).

They described the mechanism reaction of intramolecular Mannich cyclization of bisindolylacetamide (**66**) which referred from Bremner's report, as shown in Scheme 10. The protonation of bisindolylacetamide (**66**) produced spirocyclic **69** by intramolecular Mannich cyclization. Then, C-C bond migration was occurs to provide 2-(indolin-2-yl)indole **67** through carbocation **70**, which would be stabilized from the adjacent amide. However, the C-N bond migration of spirocyclic **69** was less favoured as carbocation **71** and was not observed the bisindole **72**.

In 2018, Abe and Yamada purposed to synthesize the iheyamine A (**15**) (Abe & Yamada, 2018) which the C3-electrophilic substitution of indolinol **73** reacted with tryptamine **74** to give indolinol **75**. Then, Intramolecular Dehydrative Mannich-type reaction using Lewis acid as a catalyst occurred azepinobisindole core **76**. Finally, iheyamine A (**15**) was obtained with deprotection and oxidation of bis-indole **77** with DDQ as an oxidizing agent as shown in Scheme 11.

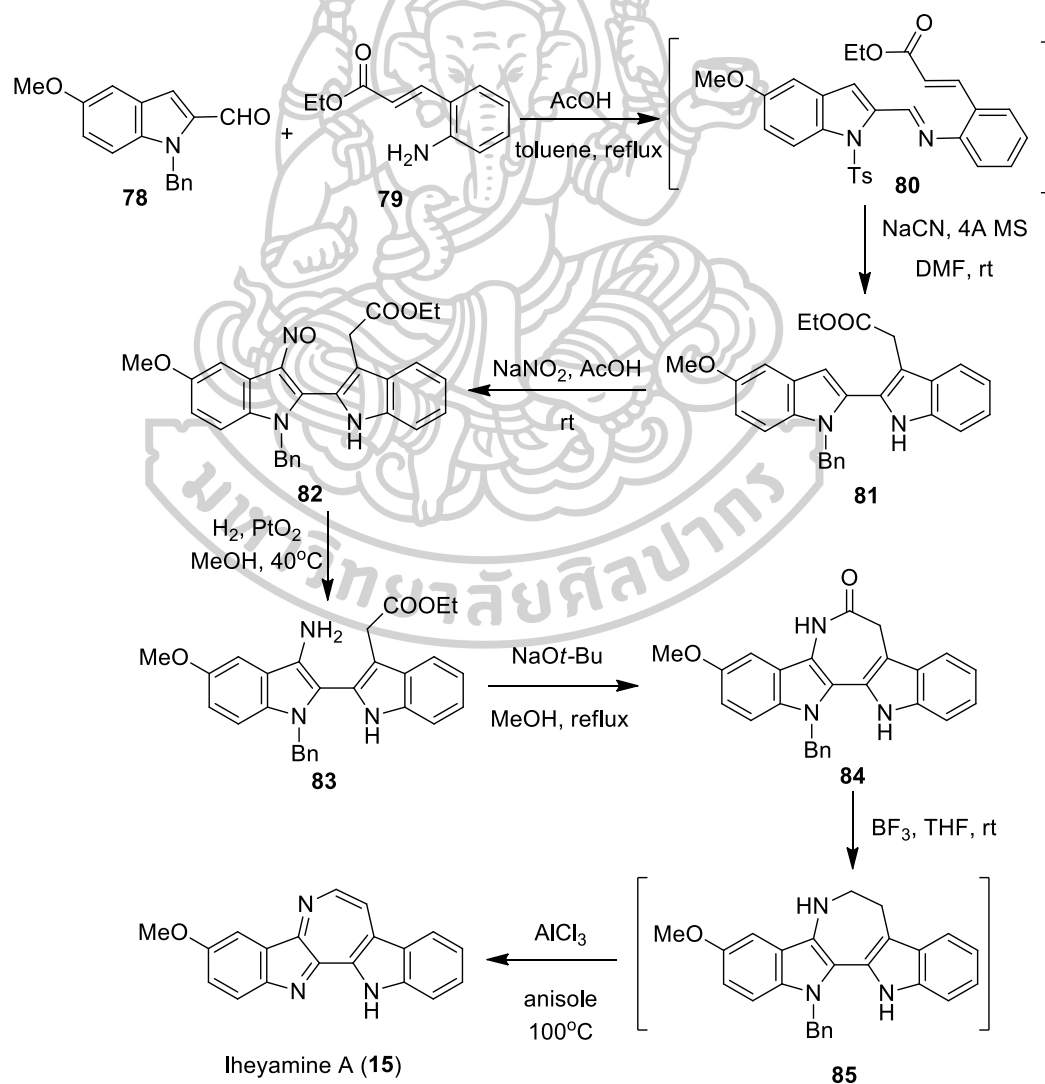


Scheme 10 Mechanism reaction of C-C bond migration of spiroindole 69.



Scheme 11 Synthesis of iheyamine A (15) by Abe and Yamada in 2018.

In 2020, Jeon and co-workers achieved to synthesize iheyamine A through the cyanide-catalyzed imino-Stetter reaction (Jeon, Kim, & Cheon, 2020). They started from the condensation of *N*-benzyl-5-methoxyindole-2-carboxaldehyde (**78**) and ethyl 2-aminocinnamate (**79**) to give aldimine (**80**). Treatment of aldimine (**80**) with sodium cyanide and addition with nitroso group at C'-3 followed by hydrogenation provided the amino ester **83**. Then, the lactam formation was occurred with NaOt-Bu to afford the desired seven-membered ring. Further reduction with BH₃ and debenzylation, followed by aerobic oxidation with AlCl₃ provided iheyamine A (**15**). This total synthesis was shown in Scheme 12.



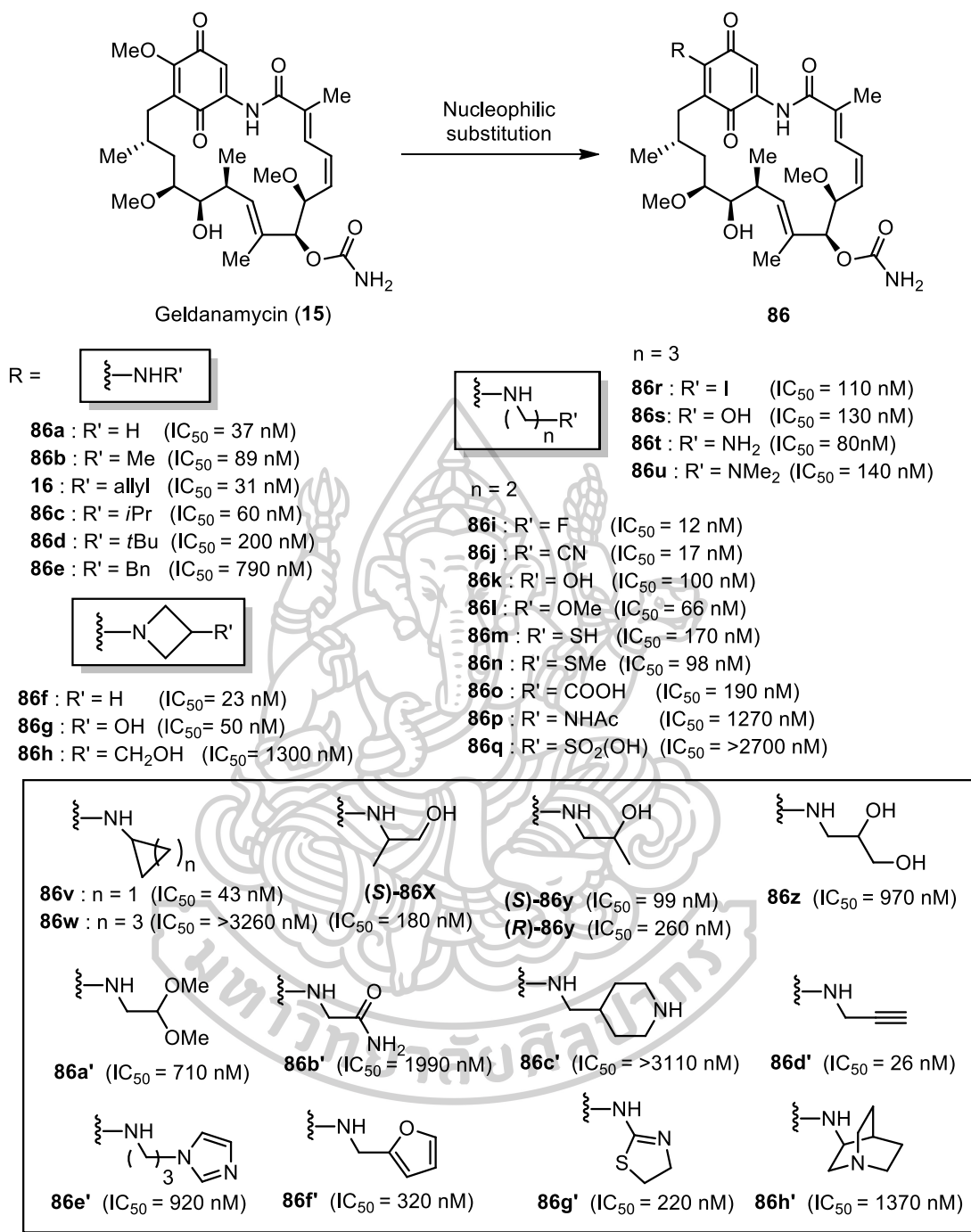
Scheme 12 Total synthesis of iheyamine A (**15**) by Jeon and co-workers in 2020.

2.2 Previous synthesis of geldanamycin derivatives

Previous reports of the development of geldanamycin derivatives by nucleophilic substitution at C-17 has attracted the attention from many researchers to study the structure- activity relationship. Geldanamycin substituted with the diversity of amine derivatives (Franke, Eichner, Zeilinger, & Kirschning, 2013) was reported below.

In 1995, Schnur and co-workers studied the semisynthesis of geldanamycin derivatives by substitution at C-17 with diverse alkylamines as shown in Scheme 13 (Schnur et al., 1995). The derivatives were evaluated the biological activity against *erbB*-2 in human breast cancer cells (SKBR-3). They found that the amino substituents at C-17 with small, sterically unstrained, and nonpolar alkyl amino groups (e.g., **16**, **86a**, **86c**, **86g**, **86l**, **86v**, **86d'**) are showed more potent than geldanamycin (**15**) ($IC_{50} \sim 70$ nM). The substituent **86i** ($IC_{50} = 12$ nM), **86j** ($IC_{50} = 17$ nM), and **86f** ($IC_{50} = 37$ nM) displayed the most potent derivatives in this series. The small amino substituents bearing the acidic functional group (**86o** and **86q**) are less potent. On the other hand, hydroxyl groups and basic functions in alkylamino (**86g**, **86s**, **86t**, and (*S*)-**86y**) were tolerated. The large and arylalkylamine substituents derivatives (**86c'**, **86w**, **86f'**, and **86e**) have reduced the activity.





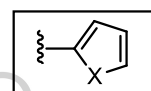
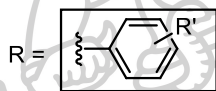
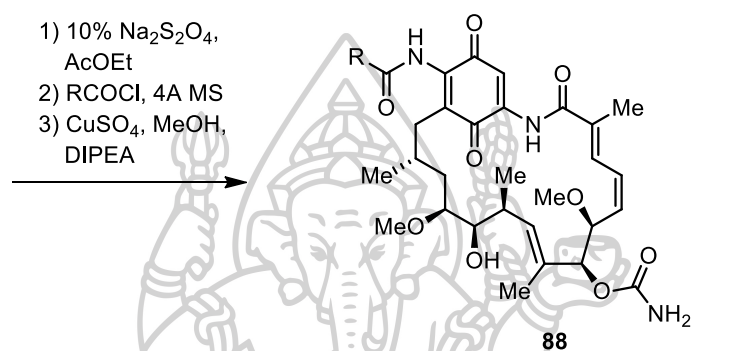
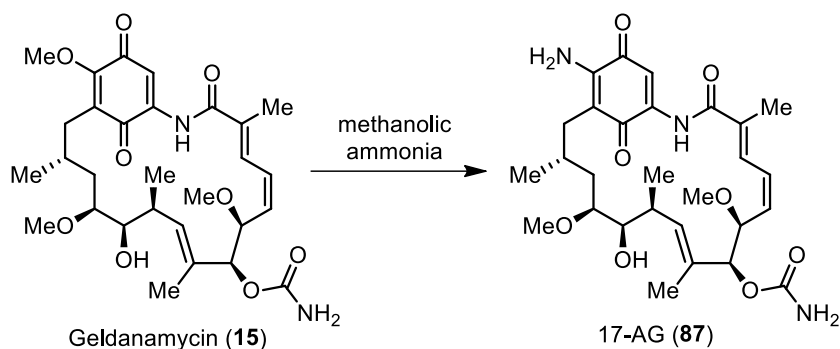
Scheme 13 Synthesis of geldanamycin derivative **86** by Schnur and co-workers.

In 2004, Brazidec and co-workers modified the synthesis of geldanamycin derivatives **88** by replacing the C-17 methoxy with amide, carbamates, and urea groups (Le Brazidec et al., 2004). The synthesis was initiated by using geldanamycin (**15**) to react

with methanolic ammonia. The resulting 17-AG (**87**) was used as a substrate to synthesize geldanamycin derivatives **88** (Scheme 14). The aromatic amides **88a-c** and **88g-h** are more active than the aliphatic derivative **88k**. When the size and polarity of the substituent groups (**88i-j**) were studied, the activity was reduced when the aromatic ring from phenyl (**88a**) ($IC_{50} = 200$ nM) was changed to 2-furyl (**88i**) and 2-thionyl (**88j**) ($IC_{50} = 500$ and 400 nM, respectively). Increasing the electron density at substitution position was also affected the activity. For example, **88d-e** ($IC_{50} = 180$ nM) appeared stronger activity in the MCF-7 cell lines than **88f** ($IC_{50} = 400$ nM). The electron withdrawing substituted compounds **88g-h** showed inactive against tested cell lines. Therefore, they used **88b** to study for structural relationships by reacting with secondary amine groups.

The *p*-chloromethylphenyl geldanamycin derivative **88b** was treated with the various secondary amines to provide amides **89**. The analogues of tertiary amine derivatives, benzylalkylamines, such as **89c**, were 3-folds more potent than dialkylamines, such as **89b**. Cyclic amine analogues **89d-f** showed more potent than **89g** which showed in Scheme 15.





88a : R' = H (IC₅₀ = 200 nM)

88b : R' = *p*-Cl-CH₂ (IC₅₀ = 550 nM)

88c : R' = *m*-Cl-CH₂ (IC₅₀ = 1000 nM)

88d : R' = *o*-OMe (IC₅₀ = 180 nM)

88e : R' = *m*-OMe (IC₅₀ = 180 nM)

88f : R' = *p*-OMe (IC₅₀ = 400 nM)

88g : R' = *p*-NO₂ (IC₅₀ = 300 nM)

88h : R' = *p*-F (IC₅₀ = 250 nM)

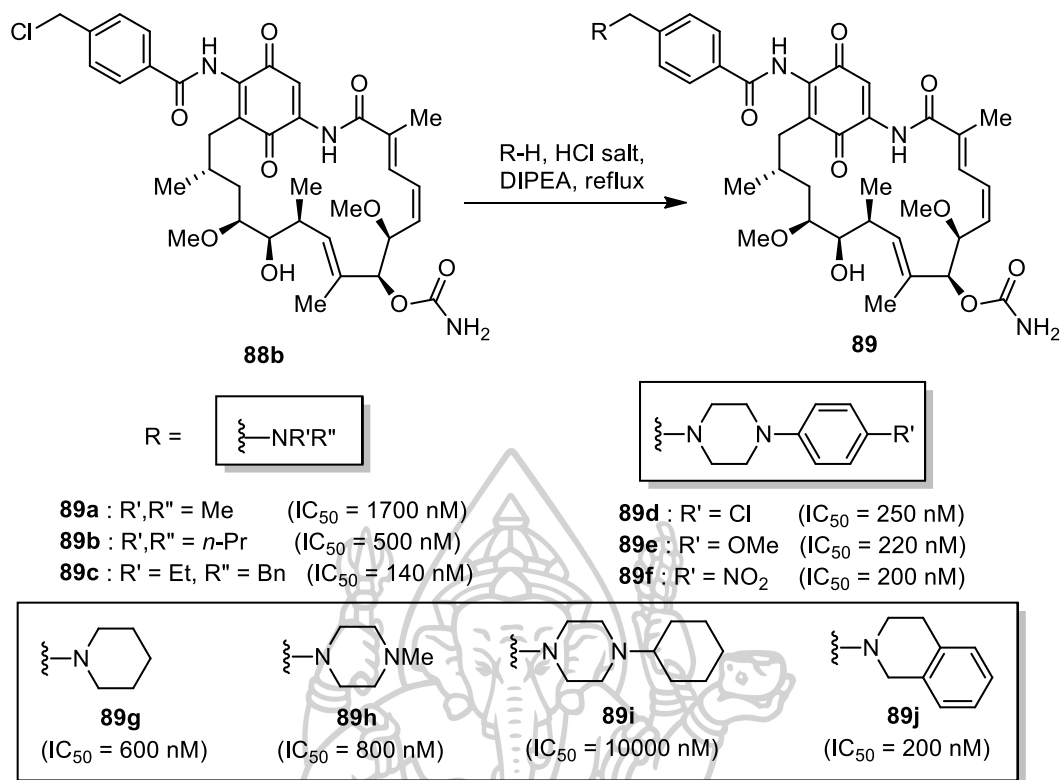
88i : X = O (IC₅₀ = 500 nM)

88j : X = S (IC₅₀ = 400 nM)

88k ; R = Me

(IC₅₀ = 1700 nM)

Scheme 14 Synthesis of geldanamycin derivatives **88a-k** and their Her-2 degradation activity in MCF-7 cell lines.

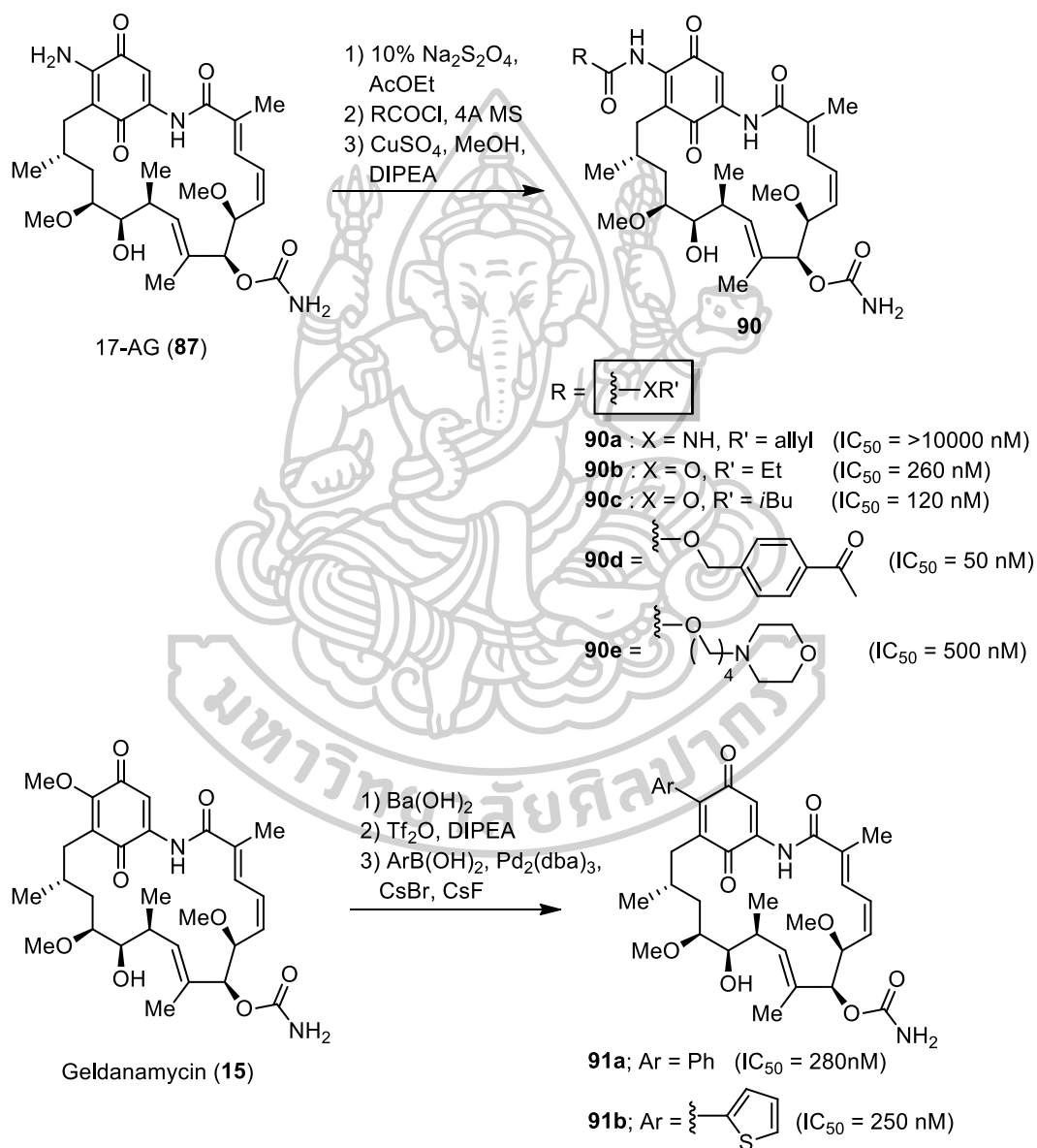


Scheme 15 Synthesis of amide geldanamycin derivative **89** by Brazidec and co-workers.

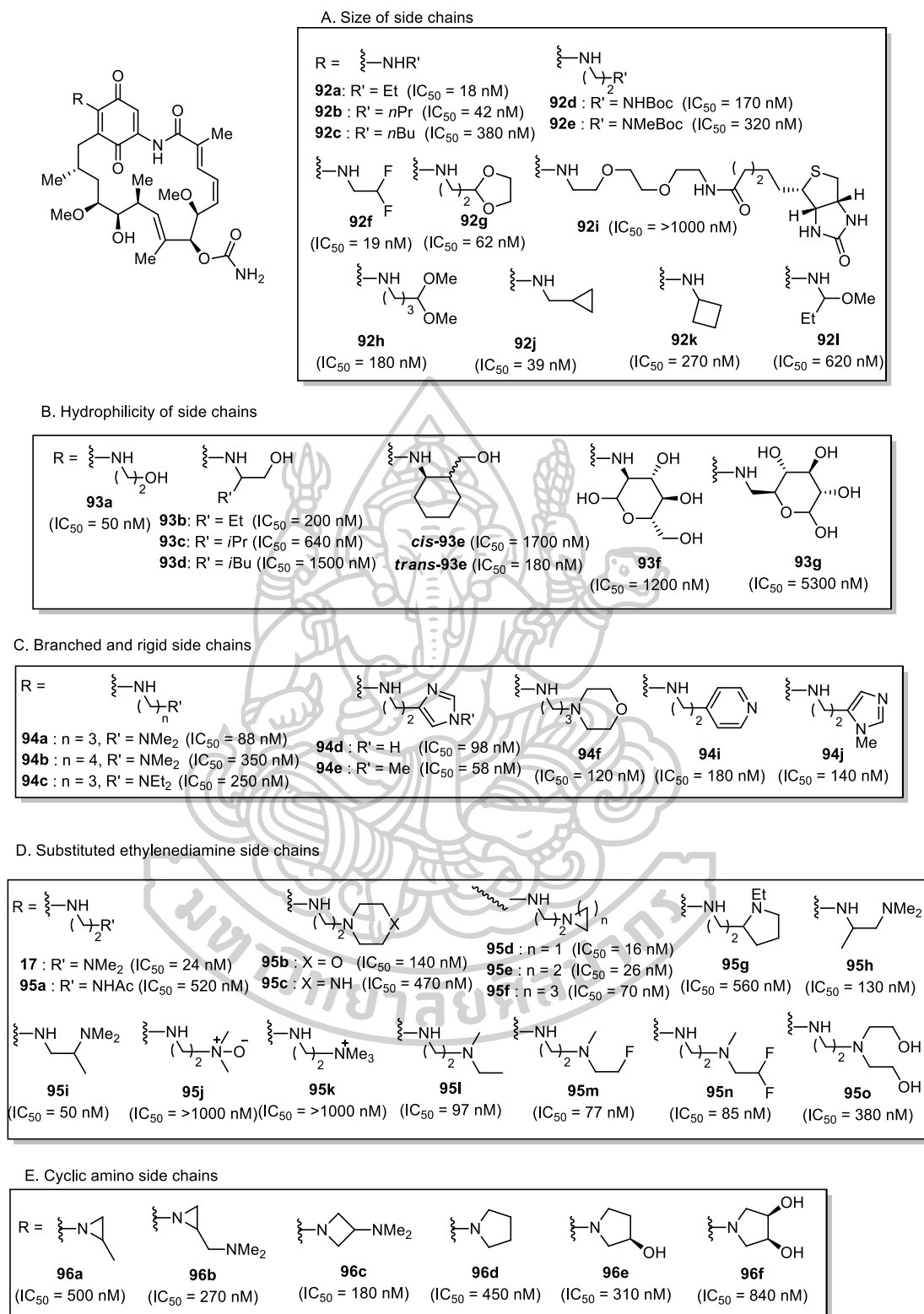
Urea **90a** and series of carbamate **90b-e** were generated from 17-AG (**87**) which *p*-acetoxybenzyl substituent **90d** was the most active compound against Her-2 in MCF-7 cell lines (IC₅₀ = 50 nM) while urea **90a** was inactive. The 17-aryl geldanamycin derivatives **91a** and **91b** were synthesized by Suzuki-cross coupling through the corresponding triflate in which both derivatives showed moderate activity (Scheme 16).

Although most of geldanamycin derivatives displayed good biological, they were not dissolve in water. Therefore, Tian and co-workers were focused on the nucleophilic substitution of geldanamycin **92-96** in order to obtain a better bioactive derivative and good water solubility. More than sixty of 17-alkylamino-17-demethoxygeldanamycin derivatives (Tian et al., 2004) were prepared and shown in Scheme 17. The studies of bioactivities to inhibit the growth of SKBr3 cells were found that the large size and steric compound **92i** reduced the potent effectiveness inhibition. The optimal length of the amine chain should be composed of two carbon atoms to provide good biological activity.

They found that there were several synthetic derivatives with good bioactivity (<100 nM) including 17, 92a-b, 92f-g, 92j, 93a-b, 95d-f. Therefore, these compounds were studied for water solubility and found to have better solubility compared to 17-AAG (16) (0.1 mg/mL). They also recognized that 17-DMAG (17) (IC_{50} = 24 nM, solubility = 1.4 mg/ mL) was the best current geldanamycin derivative.

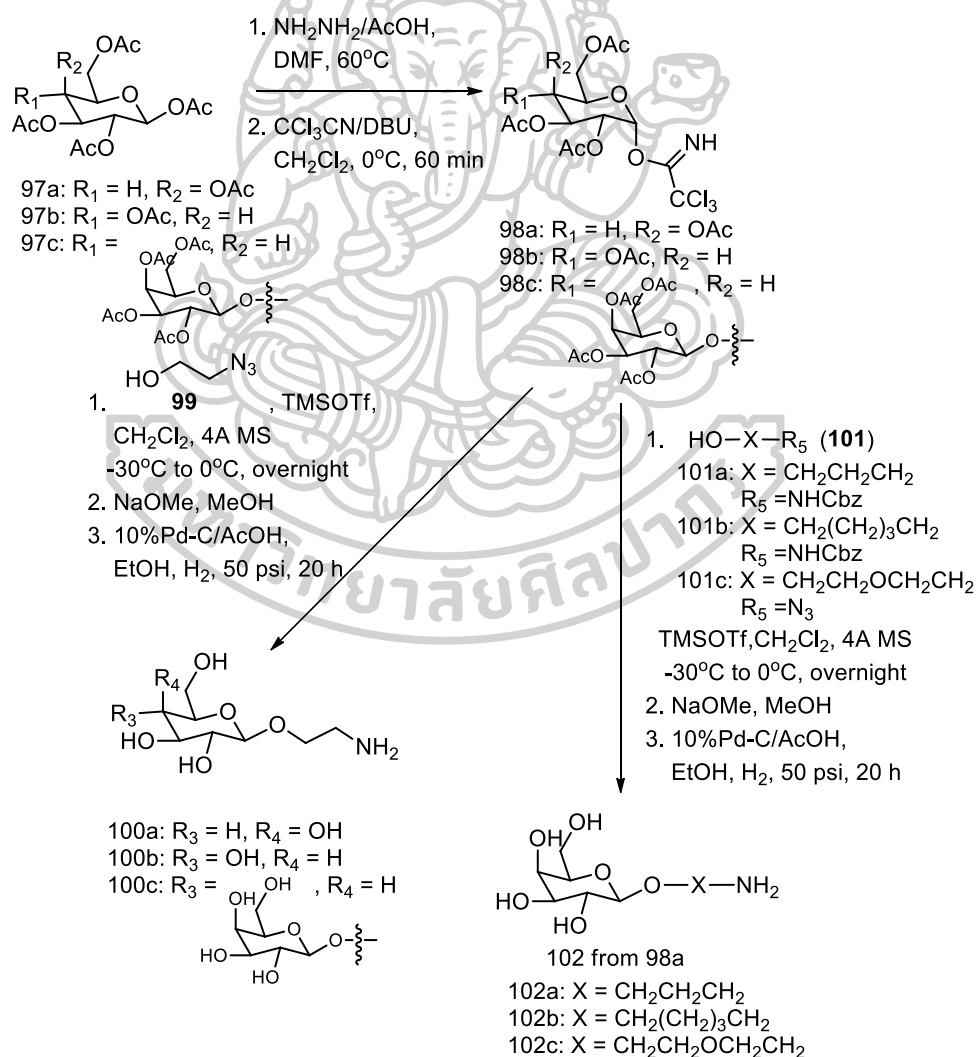


Scheme 16 Synthesis of geldanamycin derivatives 90 and 91 by Brazidec and co-workers.



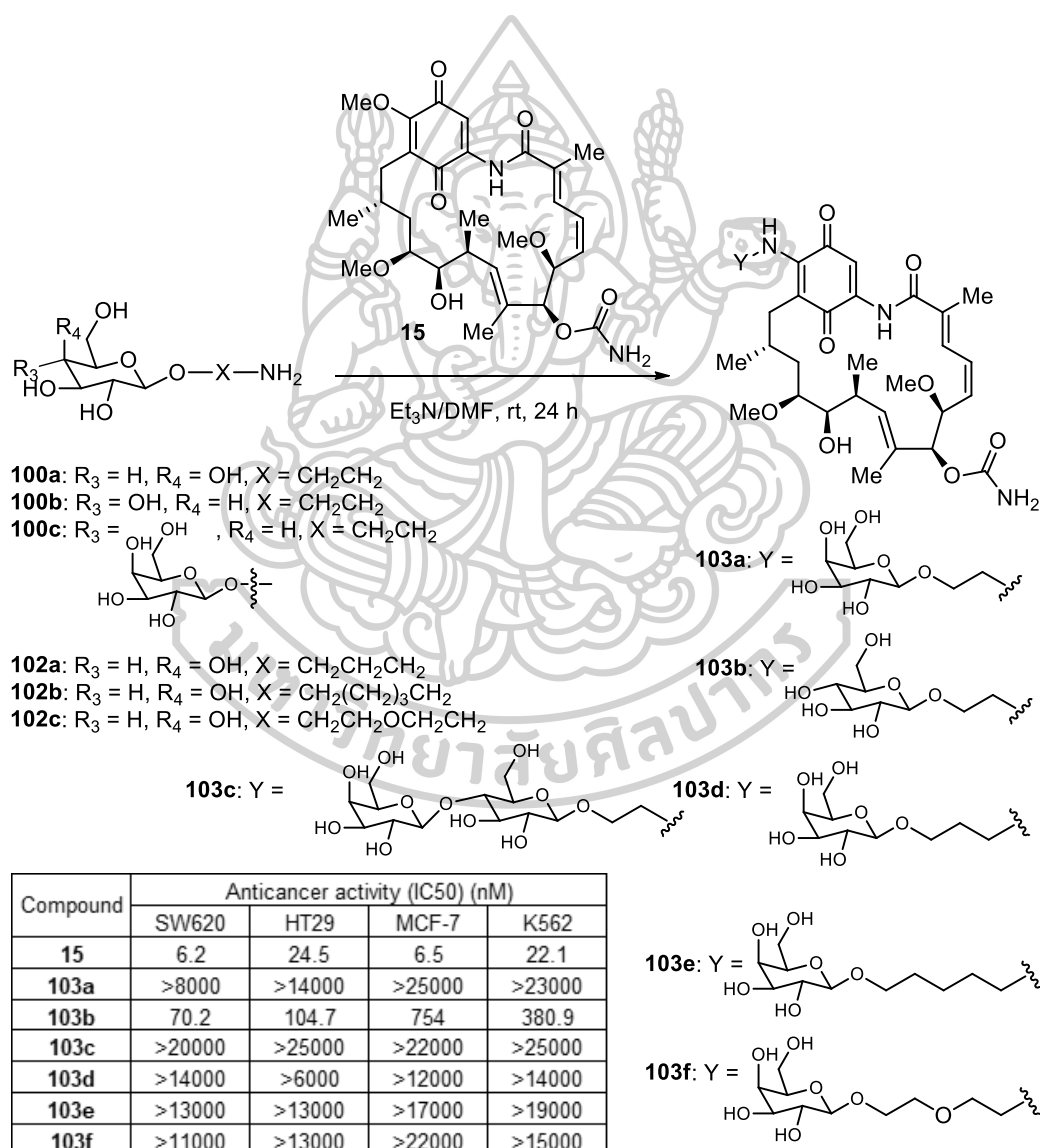
Scheme 17 Synthesis of geldanamycin derivatives 99-103 by Tian and co-workers.

In 2005, Cheng and co-workers synthesized carbohydrate-geldanamycin conjugates as a prodrug for delivery with an enzyme that was pervasively expressed in various cell types (Cheng et al., 2005). They designed the amino sugar **100** and **102** by glycosylation with varied lengths of amine linker which was shown in Scheme 18. Peracetylated sugars (galactose, glucose, and lactose) **97** were reacted with hydrazine in acid condition then treated with trichloroacetonitrile in the presence of DBU to generate trichloroacetimidates **98**. The different alcohol-linkers **99** and **101** were added to trichloroacetimidates **98** in the presence of TMSOTf followed by deprotection and hydrogenation to afford sugars **100** and **102**.



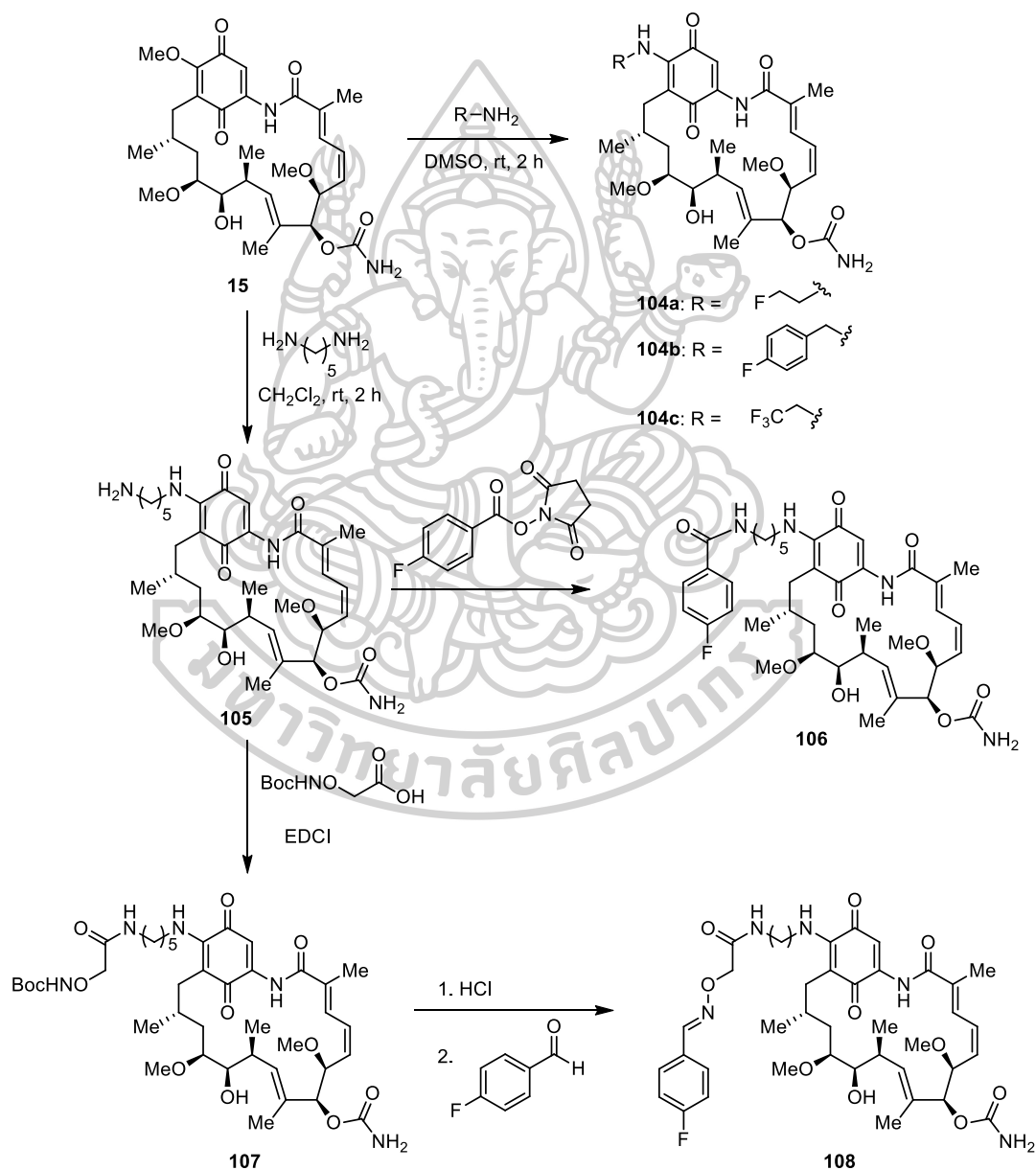
Scheme 18 Synthesis of amino linker **100** and **102** by Cheng and co-workers.

The C-17 of geldanamycin (**15**) was substituted with the prepared amino linkers **100** and **102** under a mild condition to provide the difference geldanamycin derivatives **103** which was shown in Scheme 19. These compounds were tested with cancer cell lines; leukemia cells (K562), breast cancer cells (MCF-7), and colorectal carcinoma cells (SW620, HT 29). The results showed that glucose-geldanamycin (**103b**) displayed the highest activity (IC_{50} of 70.2-380.9 nM) in various cancer cells by β -glucosidase activation.



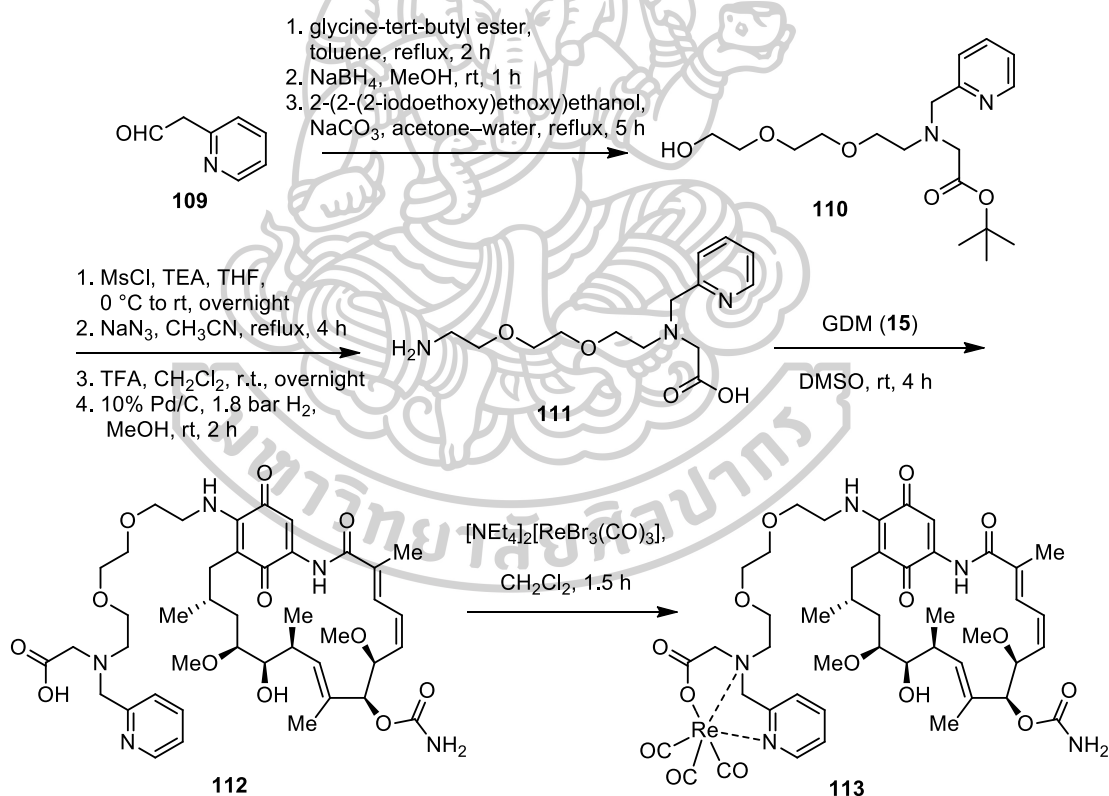
Scheme 19 Synthesis of carbohydrate-geldanamycin derivatives **103** by Cheng and co-workers.

Scheme 20 Synthesis of ^{18}F -labeled geldanamycin derivatives **104**, **106**, and **108** by Wuest and co-workers.



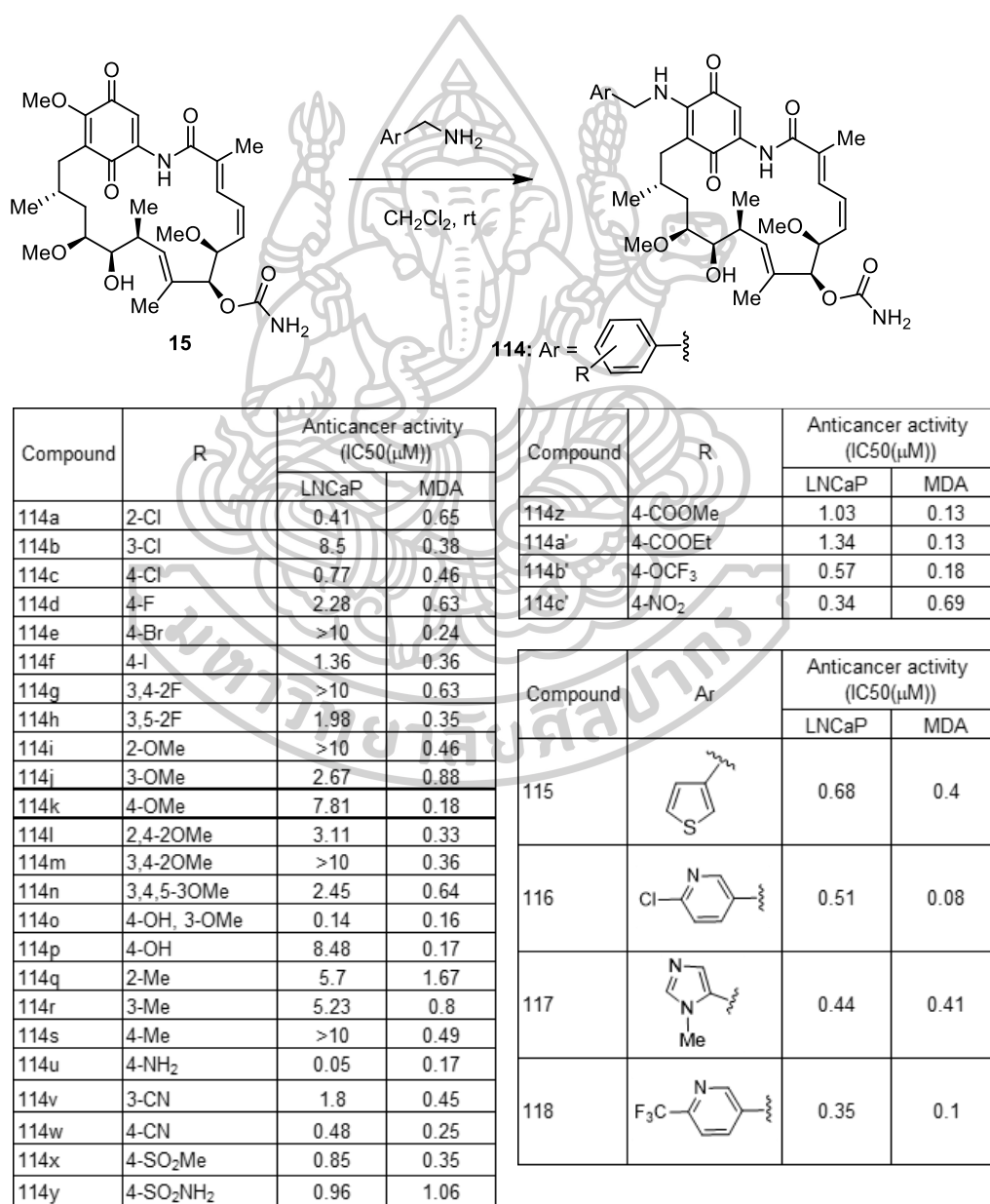
The last route involved the preparation of a rhenium-tricarbonyl complex with geldanamycin (**15**) which was interested in the design of radiopharmaceuticals. Wuest and co-workers synthesized **112** containing a donor group for chelation of a $[\text{Re}(\text{CO})_3]^+$ core via Michael reaction with a primary amine to geldanamycin. The primary amine **111** was obtained in multiple steps reaction from pyridine-2-carbaldehyde (**109**) and glycine-*tert*-butyl ester which was shown in Scheme 21.

This was showed that the fluorobenzoyl derivative **106** exhibited highest Hsp90 ATPase inhibitory potency. The fluorobenzyl geldanamycin derivative **104b** and the tricarbonyl-rhenium complex **113** were the lowest active compounds while compounds **104a** and **108** reduced ATPase activity.



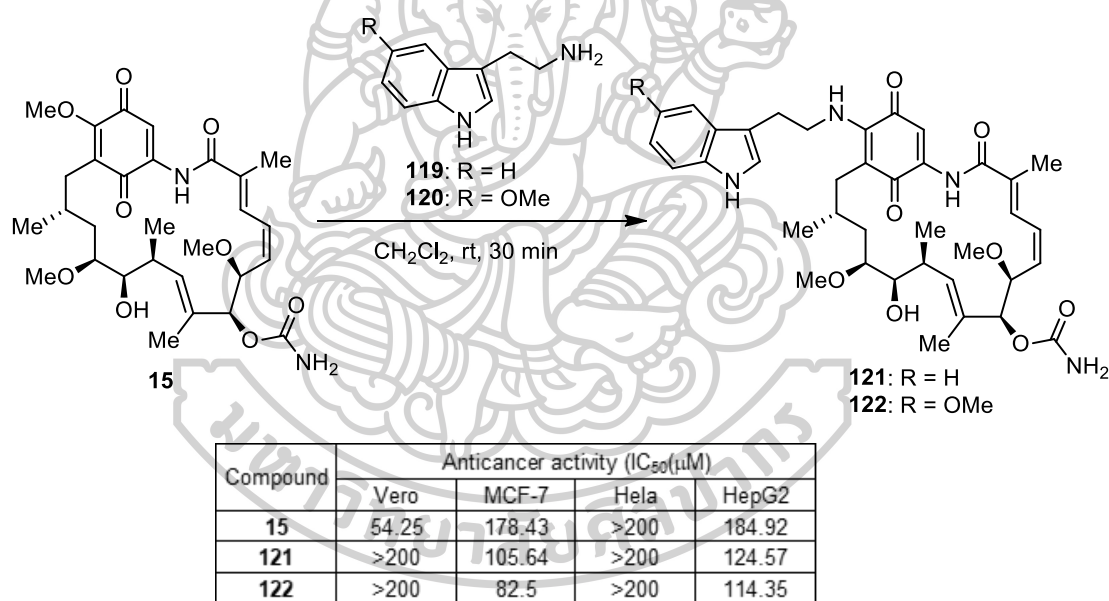
Scheme 21 Synthesis of tricarbonyl-rhenium complex geldanamycin **113**.

In 2014, Li and co-workers reported the synthesis of geldanamycin (**15**) by replacing C-17 with various arylmethylamine to obtain 17-arylmethylamine-17-demethoxygeldanamycin **114-118** which was shown in Scheme 22. (Li et al., 2014). Thirty-four compounds were evaluated for the human cancer cell lines (LNCaP and MDA-MB-231). The compounds **114u**, **117**, and **118** showed potent cytotoxicity against both cancer cell lines with IC_{50} 0.05-0.51 mM. In addition, **118** exhibited excellent *in vivo* antitumor activity in the MDA-MB-231 xenograft nude mice.



Scheme 22 Synthesis of arylmethylamine geldanamycin derivatives **114-118**.

In 2019, Taechowisan and co-workers isolated geldanamycin (**15**) from *Streptomyces zerumbet* W14 (Taechowisan et al., 2019) which was used as a substrate to modify the substituent of geldanamycin derivatives **121** and **122** with tryptamine (**119**) and 5-methoxytryptamine (**120**) as shown in Scheme 23. The derived were active to exhibit anti-inflammatory efficacy of RAW 264.7 cells. Moreover, the derivatives were evaluated cytotoxicity activity against a normal cell line, and cancer cell lines (Taechowisan, Samsawat, Puckdee, & Phutdhawong, 2020). The results exhibited moderate cytotoxic activity on Vero and human cervical carcinoma cells ($IC_{50} > 200.00 \mu\text{g/mL}$), and the strongest cytotoxic against MCF-7 and HepG2 in IC_{50} range of 82.50 to 124.57 $\mu\text{g/mL}$.



Scheme 23 Synthesis of tryptamine geldanamycin derivatives **121** and **122**.

CHAPTER 3

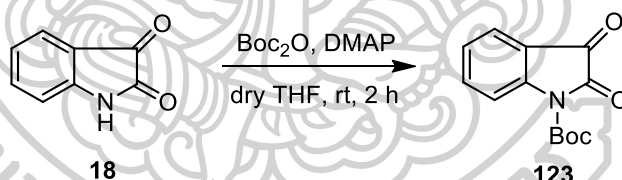
EXPERIMENTAL

3.1 General methods for the synthesis

All chemicals were purchased from Tokyo Chemical Industry (TCI), Sigma-Aldrich and Fluka Chemical Companies. All solvents were dried by standard methods. The ^1H and ^{13}C NMR spectra were recorded with a Bruker Avance 300 spectrometer (300 MHz for ^1H , 75 MHz for ^{13}C). Mass spectra were determined with a microTOF (Bruker Daltonics). Melting points were measured by a Stuart Scientific SMP 2 melting point apparatus and are uncorrected. Reaction was monitored by TLC which was pre-coated with silica gel 60 F₂₅₄ on aluminium sheets. Column chromatography was performed using Merck Kieselgel 60.

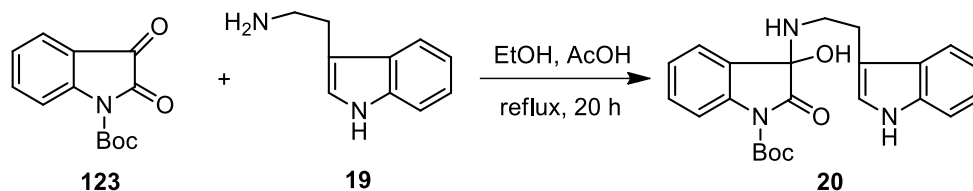
3.2 Synthesis of iheyamine A derivative

Synthesis of *N*-*tert*-butyl 2,3-dioxoindoline-1-carboxylate (123)



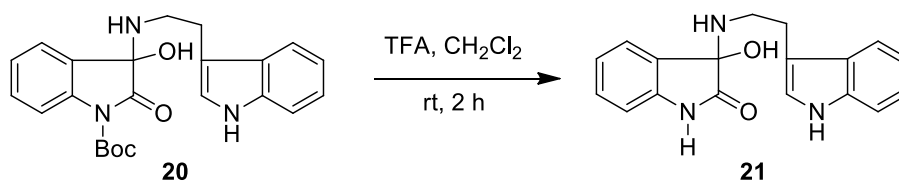
A catalyst amount of DMAP (0.08 g, 0.69 mmol) and isatin (**18**) (1.02 g, 6.94 mmol) were dissolved in dry THF (25 mL) under argon atmosphere. The mixture was added dropwise a solution of Boc₂O (1.92 mL, 8.36 mmol) in dry THF (15 mL). The reaction mixture was stirred for 2 h at room temperature. The completed reaction was quenched with water (100 mL) and extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried over with anh. Na₂SO₄ and concentrated under reduced pressure to give yellow solid (**123**) which used in the following step without further purification. ¹H NMR (300 MHz, CDCl₃) δ 1.57 (s, 9H, CH₃), 7.20 (t, *J* = 7.5, 8.1 Hz, 1H, ArH), 7.60-7.67 (m, 2H, ArH), 7.99 (d, *J* = 8.1 Hz, 1H, ArH); ¹³C NMR (75 MHz, CDCl₃) δ 28.0, 85.6, 116.8, 118.7, 125.3, 125.4, 138.8, 148.3, 152.9, 155.7, 180.2.

Synthesis of *tert*-butyl 3-((2-(1*H*-indol-3-yl)ethyl)amino)-3-hydroxy-2-oxoindoline-1-carboxylate (**20**)



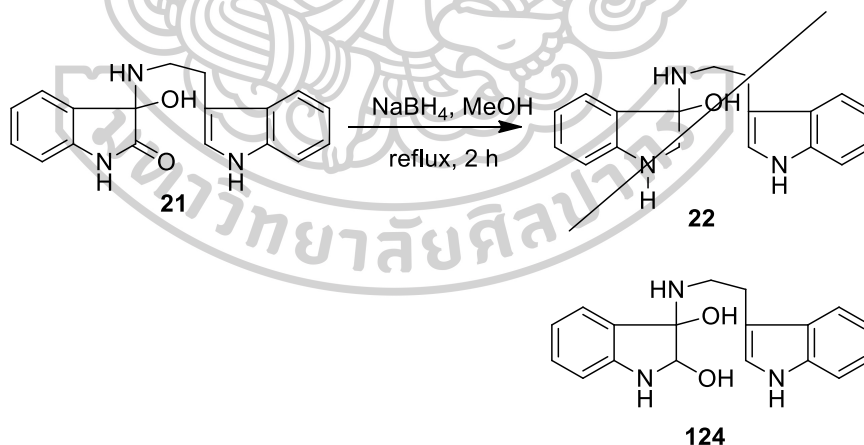
To a glacial acetic acid (0.10 mL) was added to a solution of *N*-Boc isatin (**123**) (1.36 g, 5.51 mmol) in EtOH (20 mL) which was stirred at room temperature for 5 min. Tryptamine (**19**) (1.32 g, 8.27 mmol) was then added to the mixture and refluxed at 80 °C for 20 h. The resulting mixture was cooled down to room temperature and quenched with water (100 mL) followed by extraction with EtOAc (4 x 50 mL). The combined organic phase was dried over with anh. Na₂SO₄ and then concentrated under reduced pressure to provide yellow oil crude. The crude was purified on silica gel by preparative chromatography using 4:1 hexane:EtOAc as mobile phase. Oxindole **20** (1.35 g, 73.1%) was obtained as yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 1.51 (s, 9H, CH₃), 3.05 (t, *J* = 6.6 Hz, 2H, CH₂), 3.72 (q, *J* = 6.3 Hz, 2H, CH₂NH), 6.89 (br, 1H, NHCH₂), 6.99 (t, *J* = 7.8 Hz, 1H, ArH), 7.02 (s, 1H, CHNH), 7.09-7.24 (m, 2H, ArH), 7.34 (d, *J* = 7.8 Hz, 1H, ArH), 7.53 (t, *J* = 7.8 Hz, 1H, ArH), 7.60 (d, *J* = 7.8 Hz, 1H, ArH), 8.22 (br, 1H, ArNH), 8.26 (d, *J* = 8.1 Hz, 1H, ArH), 8.41 (d, *J* = 8.4 Hz, 1H, ArH), 10.22 (s, 1H, OH); ¹³C NMR (75 MHz, CDCl₃) δ 25.0, 28.2, 39.8, 81.0, 111.4, 111.9, 118.0, 118.5, 119.0, 119.3, 121.2, 121.9, 122.4, 127.1, 134.2, 136.2, 136.5, 142.9, 152.7, 163.7, 191.9; HR-ESI MS calculated for C₂₃H₂₅N₃O₄ (M+Na)⁺ 430.1743, found 430.1743.

Synthesis of 3-((2-(1*H*-indol-3-yl)ethyl)amino)-3-hydroxyindolin-2-one (**21**)



The trifluoroacetic acid (3.24 mL, 42.40 mmol) was added dropwise to a solution of 3-hydroxyoxindole **20** (0.22 g, 0.53 mmol) in CH_2Cl_2 (18 mL) at 0°C which was stirred at room temperature for 6 h. The completed reaction was added sat. NaHCO_3 (20 mL) and extracted with CH_2Cl_2 (20 mL). The organic layer was washed with water, brine and dried over with anhydrous Na_2SO_4 . Then solvent was removed under reduced pressure and purified by preparative chromatography using hexane:EtOAc (1:1) as mobile phase to provide 3-hydroxyoxindole **21** (0.025 g, 15.2%) as yellow solid.; m.p. $168.8\text{--}170.9^\circ\text{C}$. ^1H NMR (300 MHz, CDCl_3) δ 2.99 (t, $J = 6.9$ Hz, 2H, CH_2), 3.63 (q, $J = 6.6$ Hz, 2H, CH_2NH), 6.26 (br, 1H, NHAr), 6.54 (t, $J = 7.5$ Hz, 1H, ArH), 6.57 (d, $J = 8.4$ Hz, 1H, ArH), 7.00 (s, 1H, CHNH), 7.03 (t, $J = 7.8$ Hz, 1H, ArH), 7.11 (t, $J = 7.5, 7.2$ Hz, 1H, ArH), 7.21 (td, $J = 9.6, 1.2$ Hz, 1H, ArH), 7.30 (d, $J = 8.1$ Hz, 1H, ArH), 7.55 (d, $J = 7.8$ Hz, 1H, ArH), 8.10 (d, $J = 8.1$ Hz, 1H, ArH), 8.65 (br, 1H, OH); ^{13}C NMR (75 MHz, CDCl_3) δ 25.2, 39.5, 111.3, 112.6, 114.5, 116.2, 116.8, 118.7, 119.6, 122.2, 122.3, 127.2, 134.3, 135.9, 136.4, 152.3, 164.0, 189.67; HR-ESI MS calculated for $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_2$ ($\text{M}+\text{Na}$) $^+$ 330.1218, found 330.1202.

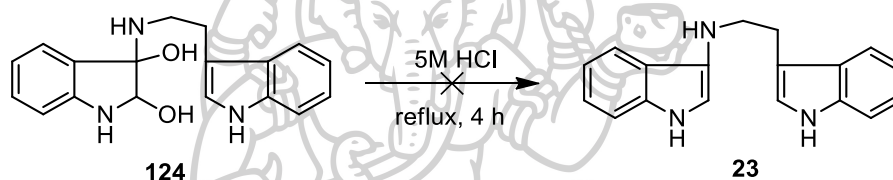
Synthesis of 3-((2-(1*H*-indol-3-yl)ethyl)amino)indolin-3-ol (**22**)



To a solution of 3-hydroxyoxindole (**21**) (0.049 g, 0.16 mmol) in MeOH (5 mL) was added with one portion of NaBH_4 (0.065 g, 1.60 mmol) at 0°C . The mixture was refluxed for 2 h. Then the completed reaction was cooled down to room temperature, the solvent was removed under reduce pressure and quenched with water at 0°C . The aqueous solution was extracted with CH_2Cl_2 , dried over with anhydrous Na_2SO_4 and concentrated. The crude

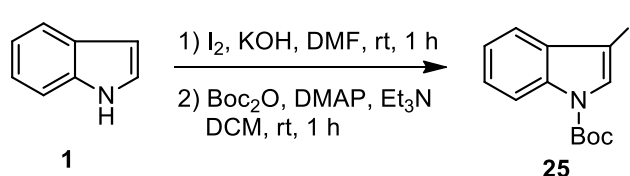
product was purified on silica gel by flash column chromatography using 1:1 hexane:EtOAc as an eluent to give only the undesired indolinol (**124**) (0.07 g, 28 % yield) as yellow solid; m.p. 179.1-182.3 °C. ^1H NMR (300 MHz, CDCl_3) δ 2.96-2.86 (2H, m, CH_2), 3.64-3.44 (2H, m, NHCH_2), 5.15 (1H, s, CHOH), 6.71 (1H, d, $J = 7.8$ Hz, ArH), 6.79 (1H, t, $J = 7.5$ Hz, ArH), 6.87 (1H, s, CHNH), 7.21-7.07 (2H, m, ArH), 7.36 (1H, d, $J = 8.1$ Hz, ArH), 7.56, (1H, d, $J = 7.8$ Hz, ArH); ^{13}C NMR (75 MHz, CDCl_3) δ 25.11, 39.63, 71.30, 111.31, 112.39, 117.44, 118.58, 119.33, 122.04, 122.28, 122.43, 127.51, 129.13, 136.33, 144.35, 173.07; HR-ESI MS calculated for $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_2$ ($\text{M}+\text{H}$) $^+$ 310.1556, found 310.1558.

Synthesis of *N*-(2-(1*H*-indol-3-yl)ethyl)-1*H*-indol-3-amine (**23**)



To a solution of indolinol **124** in 5M HCl (10 mL) was refluxed at 90 °C for 4 h. Then, the reaction mixture was cooled down to room temperature followed by adjusted pH to basic condition by using 25% NaOH. The aqueous solution was extracted with EtOAc, dried over with anhyd. Na_2SO_4 and concentrated. The reaction was not occurred the desired product **23**.

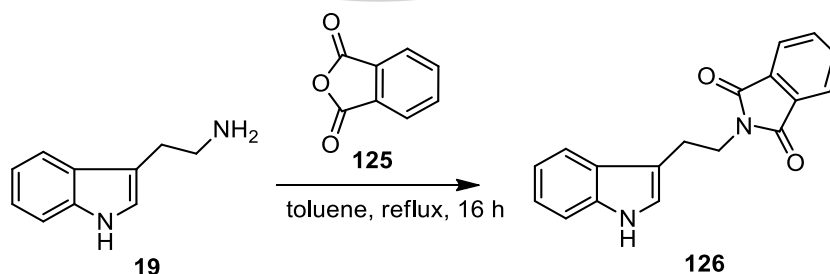
Synthesis of *tert*-butyl 3-iodo-1*H*-indole-1-carboxylate (**25**)



The solid of I_2 (0.22 g, 8.66 mmol) was dissolved in DMF (2 mL). The homogeneous solution was added dropwise to a mixture of indole (**1**) (0.10 g, 0.85 mmol) and KOH (0.12 g, 2.14 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 1 h. The resulting was poured into water with ice (200 mL) containing $Na_2S_2O_5$ (0.10% of solution) and 0.5% NH_3 (4mL). The precipitate was filtered, washed with a lot of cold water and dried over under vacuum. The crude was provided as beige solid (0.17 g) which was used in the next step without further purification and unstable. ^1H NMR (300 MHz, $CDCl_3$) δ 7.18-7.28 (m, 3H, ArH), 7.37 (d, J = 6.6 Hz, 1H, ArH), 7.47 (d, J = 7.7 Hz, 1H, ArH), 8.31 (br, 1H, NH). ^{13}C NMR was not tested.

The Boc_2O (0.17 mL, 0.74 mmol) and Et_3N (0.27 mL, 1.94 mmol) were added to a mixture of 3-iodo-1*H*-indole (0.16 g, 0.46 mmol) in CH_2Cl_2 (10 mL) and DMAP (0.008 g, 1.94 mmol) at 0 °C. The mixture was stirred for 1 h at room temperature. The mixture was then extracted with 5% $Na_2S_2O_5$ (2x20 mL). The organic layer was dried over with anh. Na_2SO_4 and concentrated under reduce pressure. The crude was purified on silica gel by flash column chromatography using 10:3 Hexane: EtOAc as an eluent to provide *tert*-butyl 3-iodo-1*H*-indole-1-carboxylate (**25**) (0.19 g, 63% yield over two steps) as brown oil which was unstable. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.66 (s, 9H, CH_3), 7.30-7.42 (m, 3H, ArH), 7.73 (s, 1H, ArH), 8.12 (d, J = 7.9 Hz, 1H, ArH); ^{13}C NMR was not tested.

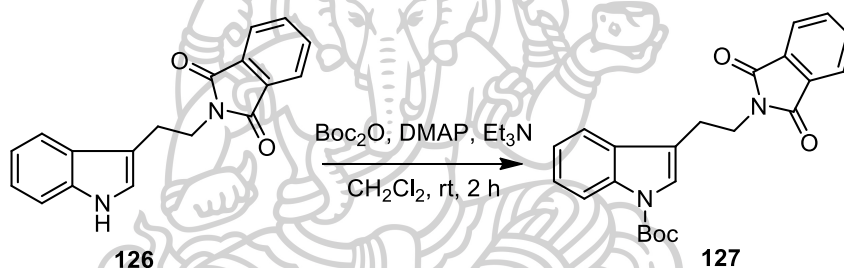
Synthesis of 2-(2-(1*H*-indol-3-yl)ethyl)isoindoline-1,3-dione (**126**)



A suspension of phthalic anhydride (**125**) (2.00 g, 13.50 mmol) and tryptamine (**19**) (2.00 g, 12.23 mmol) in toluene (60mL) was refluxed using a Dean-Stark trap water for 16 h under Ar atmosphere. The resulting mixture was concentrated under reduce

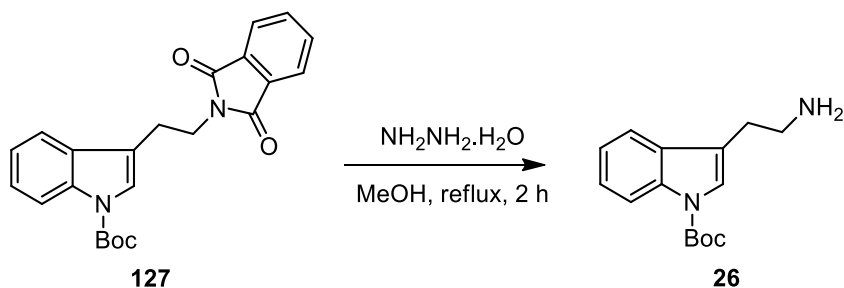
pressure to provide crude product which was purified on silica gel by flash column chromatography using 1:2 hexane:EtOAc as an eluent to give 2-(2-(1*H*-indol-3-yl)ethyl)isoindoline-1,3-dione (**126**) (2.72 g, 77% yield) as pale brown solid; m.p. 165-167 °C (lit. 165 °C); ¹H NMR (CDCl₃, 300 MHz) δ 3.15 (dd, *J* = 7.3, 8.1 Hz, 2H, CH₂), 4.00 (dd, *J* = 7.7, 7.9 Hz, 2H, CH₂), 7.19 (m, 3H, ArH), 7.34 (d, *J* = 7.7 Hz, 1H, ArH), 7.69 (dd, *J* = 3.0, 5.4 Hz, 2H, ArH), 7.73 (d, *J* = 8.0 Hz, 1H, ArH), 7.82, (dd, *J* = 3.0, 5.4 Hz, 2H, ArH), 8.04 (br, 1H, NH); ¹³C NMR (CDCl₃, 75 MHz) δ 24.5, 38.5, 111.2, 112.3, 118.8, 119.4, 122.1, 122.1, 123.2, 127.4, 132.1, 133.9, 136.2, 168.4

Synthesis of *tert*-butyl 3-(2-(1,3-dioxoisindolin-2-yl)ethyl)-1*H*-indole-1-carboxylate (**127**)



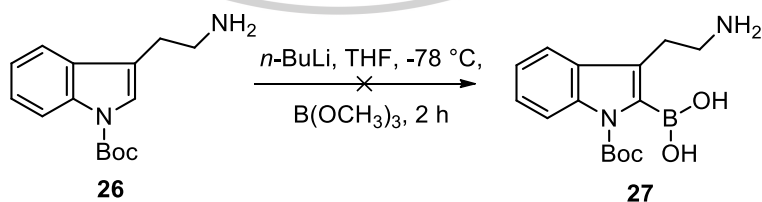
To a solution of 2-(2-(1*H*-indol-3-yl)ethyl)isoindoline-1,3-dione (**126**) (1.14 g, 3.94 mmol) in CH₂Cl₂ (50 mL) was added DMAP (0.05 g, 0.39 mmol) for 10 min at room temperature. Then followed by Boc₂O (1.36 mL, 5.91 mmol) and Et₃N (2.20 mL, 15.77 mmol) which was further stirred at room temperature for 2 h. The completed reaction was concentrated under vacuum and purified by column chromatography using 6:1 hexane:EtOAc as an eluent to provide *tert*-butyl 3-(2-(1,3-dioxoisindolin-2-yl)ethyl)-1*H*-indole-1-carboxylate (**127**) (1.26 g, 82% yield) as light brown solid; m.p. 154-155 °C (lit. 149-150 °C); ¹H NMR (CDCl₃, 300 MHz) δ 1.65 (s, 9H, CH₃), 3.08 (dd, *J* = 7.2, 8.3 Hz, 2H, CH₂), 4.00 (dd, *J* = 7.8, 8.0 Hz, 2H, CH₂), 7.28 (m, 2H, ArH), 7.48 (s, 1H, ArH), 7.68 (d, *J* = 8.2 Hz, 1H, ArH), 7.72 (dd, *J* = 3.0, 5.4 Hz, 2H, ArH), 7.86 (dd, *J* = 3.0, 5.4 Hz, 2H, ArH), 8.13 (d, *J* = 8.0 Hz, 1H, ArH); ¹³C NMR (CDCl₃, 75 MHz) δ 24.3, 28.2, 37.7, 83.5, 115.3, 116.9, 119.0, 122.6, 123.2, 123.3, 124.5, 130.3, 132.1, 133.9, 135.5, 149.6, 168.2.

Synthesis of *tert*-butyl 3-(2-aminoethyl)-1*H*-indole-1-carboxylate (**26**)



A *tert*-butyl 3-(2-aminoethyl)-1*H*-indole-1-carboxylate (**127**) (0.68 g, 1.74 mmol) was dissolved in MeOH (60 mL). Hydrazine hydrate (1.08 mL, 34.80 mmol) was added to a solution and stirred at 25 °C for 10 min, and then was further refluxed for 2 h. The reaction mixture was cooled down to room temperature and evaporated to provide crude product. The residue was purified by column chromatography using 10% MeOH in CH₂Cl₂ as eluent to provide product *tert*-butyl 3-(2-aminoethyl)-1*H*-indole-1-carboxylate (**26**) (0.45 g, 99% yield) as white solid; m.p. 191-192 °C; ¹H NMR (CDCl₃, 300 MHz) δ 1.67 (s, 9H, CH₃), 2.84 (t, *J* = 6.7 Hz, 2H, CH₂), 3.04 (dd, *J* = 6.5, 6.9 Hz, 2H, CH₂), 7.28 (m, 2H, ArH), 7.32 (s, 1H, ArH), 7.53 (d, *J* = 7.7 Hz, 1H, ArH), 8.13 (d, *J* = 7.7 Hz, 1H, ArH); ¹³C NMR (DMSO-*d*₆, 75 MHz) δ 27.7, 83.4, 114.7, 117.7, 119.2, 123.3, 124.3, 125.3, 130.2, 134.8, 149.1.

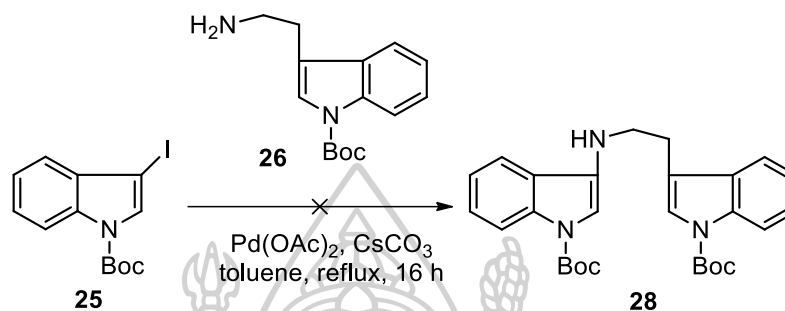
Synthesis of (3-(2-aminoethyl)-1-(*tert*-butoxycarbonyl)-1*H*-indol-2-yl)boronic acid (**27**)



To a solution of *N*-Boc tryptamine (**26**) (0.26 g, 1.00 mmol) in dry THF (10 mL) was added *n*-BuLi (0.13 mL, 1.30 mmol) at -78 °C under Ar atmosphere. The solution was cooled to 0-5 °C in an ice bath, and trimethyl borate (0.17 mL, 1.50 mmol) was added. After 2 h, the reaction mixture was quenched with 2N HCl and extracted with EtOAc. The

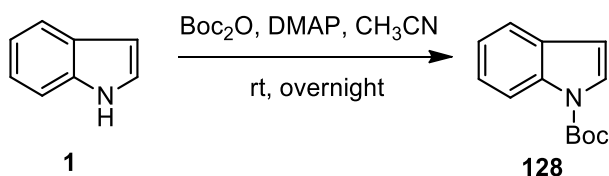
organic layer was separated, dried over with anhydrous Na_2SO_4 , and concentrated which was not achieved the desired product **27**.

Synthesis of *tert*-butyl 3-((2-(1*H*-indol-3-yl)ethyl)amino)-1*H*-indole-1-carboxylate (**28**)



A mixture of *N*-Boc-3-iodo-indole (**25**) (0.59 g, 1.70 mmol), CsCO_3 (0.55 g, 1.70 mmol) and a catalyst amount of $\text{Pd}(\text{PPh}_3)_4$ (0.10 g, 0.085 mmol) were dissolved in toluene (3 mL) under Ar atmosphere. The mixture was stirred for 30 min followed by addition of *N*-Boc tryptamine (**26**) (0.50 g, 2.04 mmol). The reaction was further refluxed for 16 h. The resulting mixture was cooled to room temperature and diluted with EtOAc which was washed with sat. NaHCO_3 and brine. The organic layer was dried over with anhydrous Na_2SO_4 and concentrated. The crude product was purified on silica gel by using preparative chromatography which was not obtained the desired product **28**.

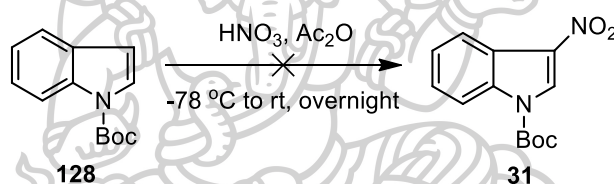
Synthesis of *tert*-butyl 1*H*-indole-1-carboxylate (**128**)



A catalyst amount of DMAP (0.008 g, 0.07 mmol) and Boc_2O (0.37 mL, 1.60 mmol) was added to a solution of indole (**1**) (0.16 g, 1.33 mmol) in CH_3CN (3 mL) at 0°C . The mixture was warmed up and stirred for overnight at room temperature. The solvent was

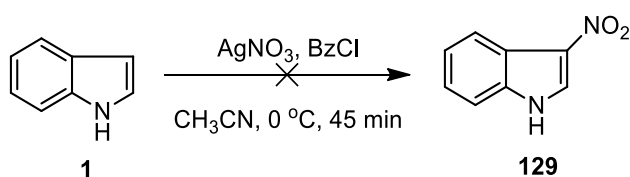
removed under reduce pressure then diluted with EtOAc and extracted with 1N HCl and brine. The organic phase was dried over with anh. Na_2SO_4 and followed by concentration to give yellow oil as crude which was purified on silica gel by column chromatography using 10:1 hexane:EtOAc as an eluent to afford *tert*-butyl 1*H*-indole-1-carboxylate (**128**) as colorless oil (0.26 g, 88%); ^1H NMR (300 MHz, CDCl_3) δ 1.67 (s, 9H, CH_3), 6.56 (dd, J = 3.0, 6.0 Hz, 1H, ArH), 7.22 (td, J = 3.0, 9.0 Hz, 1H, ArH), 7.31 (td, J = 3.0, 9.0 Hz, 1H, ArH), 7.55 (d, J = 6.0 Hz, 1H, ArH), 7.59 (d, J = 6.0 Hz, 1H, ArH), 8.15 (d, J = 6.0 Hz, 1H, ArH); ^{13}C NMR (75 MHz, CDCl_3) δ 28.1, 83.4, 107.2, 115.1, 120.9, 122.6, 124.1, 125.8, 130.5, 135.2, 149.7.

Synthesis of *tert*-butyl 3-nitro-1*H*-indole-1-carboxylate (**31**)



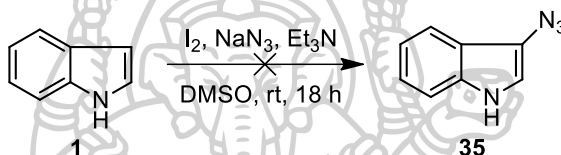
Pre-formation of acetyl nitrate, the 90% HNO_3 (0.27 mL, 6.34 mmol) was added to Ac_2O (4 mL, 42.23 mmol) at $0\text{ }^\circ\text{C}$ for 10 min. The mixture was transferred via dropping funnel to a solution of *N*-Boc indole (**128**) (0.43 g, 1.98 mmol) in Ac_2O (10 mL) at $-78\text{ }^\circ\text{C}$ for 10 min. The mixture was warmed up to room temperature and stirred further for overnight. The resulting mixture was poured onto crushed ice and extracted with EtOAc. The combined organic layers were washed with water, dried over with anh. Na_2SO_4 and then the solvent was concentrated. The desired product **31** was not occurred.

Synthesis of 3-nitro-1*H*-indole (**129**)



The mixture of silver nitrate (0.17 g, 1.00 mmol) in CH_3CN (1 mL) was added dropwise benzoyl chloride (0.12 mL, 1.03 mmol) and stirred for 30 min at 0°C . Follow by addition of a solution of indole (**1**) (0.10 g, 0.85 mmol) in CH_3CN (5 mL) which was further stirred for 2 h at room temperature. The reaction mixture was quenched with water and extracted with EtOAc. The combined organic layer was washed with solution of 10% Na_2CO_3 and dried over with anhydrous Na_2SO_4 . The solvent was removed under reduced pressure. The reaction did not occur to give the desired product **129**.

Synthesis of 3-azido-1*H*-indole (**35**)



To a mixture of indole (**1**) (0.11 g, 1.00 mmol) and I_2 (0.26 g, 1.00 mmol) in dry DMSO (8 mL) was added NaN_3 (0.11 g, 1.50 mmol) at 0°C and then added dropwise Et_3N (0.14 mL, 1.00 mmol). The reaction was stirred at room temperature for 18 h and kept away from light. After completion of the reaction, the mixture was quenched with water at 0°C followed by extraction with EtOAc. The combined organic layers were washed with brine and concentrated under reduced pressure. The crude product was purified on silica gel by preparative chromatography using 2:1 hexane:EtOAc as mobile phase to provide the desired product.

3.3 Synthesis of geldanamycin derivatives

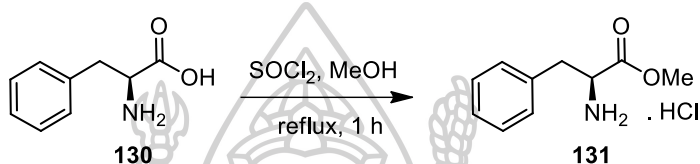
3.3.1 Extraction, purification and structure elucidation of geldanamycin

The spores of *Streptomyces zerumbet* W14 were streaked on 220 petri dishes of ISP-2 agar medium and incubated at 30°C for 14 days. Next, the culture medium was cut into small pieces which were extracted with EtOAc about 3 times or until the solvents were colorless. The organic extracts were filtered through cotton and evaporated to provide a crude EtOAc extract.

The crude EtOAc extract was isolated by column chromatography by using 1:3 hexane:EtOAc as an eluent. The fractions were monitored by TLC which were collected until yellow band was cleared. The yellow fractions were evaporated and elucidated by NMR spectroscopy.

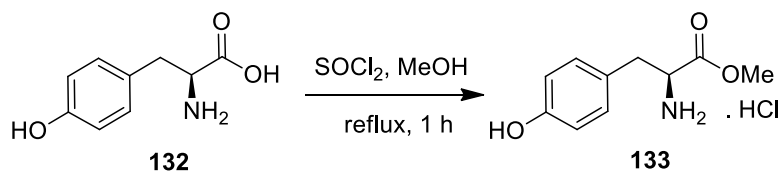
3.3.2 Synthesis of amine derivatives

Synthesis of (S)-methyl 2-amino-3-phenylpropanoate hydrochloride (131)



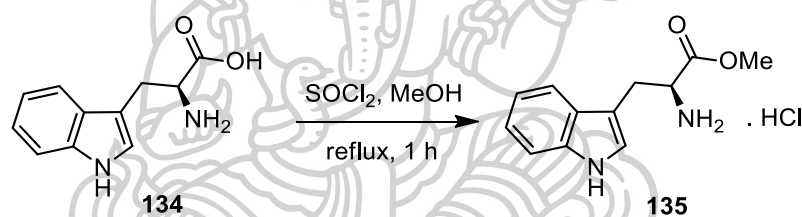
To a solution of (L)-phenylalanine (130) (0.83 g, 5.00 mmol) in dry methanol (10 mL), the freshly distilled thionyl chloride (0.40 mL, 5.50 mmol) was slowly added at 0 °C and refluxed for 1 h. The mixture was cooled down to room temperature and the solvent was concentrated under vacuum and recrystallized from methanol to provide (L)-phenylalanine methyl ester hydrochloride **131** (1.08 g, 100%) as white crystals; m.p. 165-166 °C (lit. (Bushkova, Parchinsky, & Krasavin, 2010) 159-161 °C); ^1H NMR (D_2O , 300 MHz) δ 3.23 (dd, $J = 7.4, 14.5$ Hz, 1H, CH), 3.35 (dd, $J = 5.6, 14.5$ Hz, 1H, CH), 3.84 (s, 3H, CH_3), 4.43 (dd, $J = 5.9, 7.1$ Hz, 1H, CH), 7.28-7.45 (m, 5H, ArH); ^{13}C NMR (D_2O , 75 MHz) δ 35.5, 53.5, 54.0, 128.0, 129.2, 129.3, 133.6, 170.0.

Synthesis of (S)-methyl 2-amino-3-(4-hydroxyphenyl)propanoate hydrochloride (133)



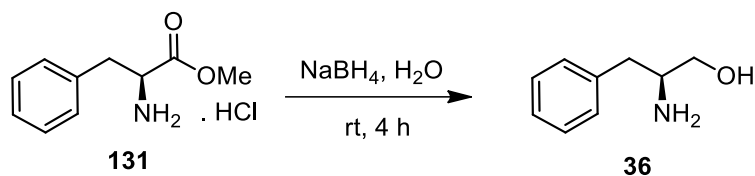
To a solution of (*L*)-tyrosine (**132**) (0.91 g, 5.00 mmol) in dry methanol (10 mL), the freshly distilled thionyl chloride (0.40 mL, 5.50 mmol) was slowly added at 0 °C and refluxed for 1 h. The mixture was cooled down to room temperature and the solvent was concentrated under vacuum and recrystallized from methanol to provide (*L*)-tyrosine methyl ester hydrochloride **133** (1.15 g, 99%) as white crystals; m.p. 195-197 °C (lit. (Jung & Rohloff, 1985) 188-189 °C); ¹H NMR (D₂O, 300 MHz) δ 3.16 (dd, *J* = 7.5, 14.7 Hz, 1H, CH), 3.26 (dd, *J* = 5.8, 14.7 Hz, 1H, CH), 3.83 (s, 3H, CH₃), 4.25 (dd, *J* = 5.9, 7.3 Hz, 1H, CH), 6.89 (d, *J* = 8.6 Hz, 2H, CH), 7.16 (d, *J* = 8.6 Hz, 2H, CH); ¹³C NMR (D₂O, 75 MHz) δ 34.7, 53.5, 54.1, 115.9, 125.3, 130.8, 155.1, 170.0.

Synthesis of (*S*)-methyl 2-amino-3-(1*H*-indol-3-yl)propanoate hydrochloride (**135**)



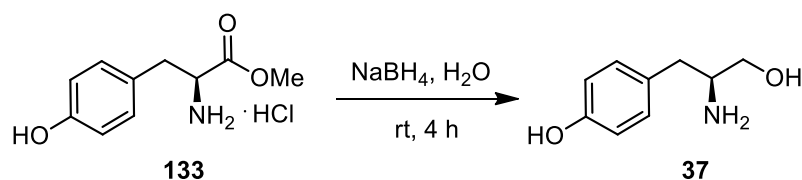
To a solution of (*L*)-tryptophan (**134**) (1.00 g, 5.00 mmol) in dry methanol (10 mL), the freshly distilled thionyl chloride (0.40 mL, 5.50 mmol) was slowly added at 0 °C and refluxed for 1 h. The mixture was cooled down to room temperature and the solvent was concentrated under vacuum and recrystallized from methanol to provide (*L*)-tryptophan methyl ester hydrochloride **135** (1.27 g, 99%) as white crystals; m.p. 218-219 °C (lit. (Kachhwaha, Varghese, Gupta, Mhaske, & Dhaneshwar, 2002) 220-223 °C); ¹H NMR (D₂O, 300 MHz) δ 3.34 (dd, *J* = 4.4, 6.5 Hz, 2H, CH₂), 3.68 (s, 3H, CH₃), 4.32 (dd, *J* = 5.9, 6.6 Hz, 1H, CH), 7.07 (td, *J* = 0.9, 6.9 Hz, 1H, CH), 7.16 (m, 1H, CH), 7.18 (s, 1H, CH), 7.41 (d, *J* = 8.19 Hz, 1H, CH), 7.48 (d, *J* = 7.9 Hz, 1H, CH); ¹³C NMR (D₂O, 75 MHz) δ 25.6, 53.2, 53.5, 105.9, 112.0, 118.0, 119.5, 122.2, 125.3, 126.3, 136.2, 170.4.

Synthesis of (S)-2-amino-3-phenylpropan-1-ol (36)



A solution of (*L*)-phenylalanine methyl ester hydrochloride (**131**) (0.22 g, 1.00 mmol) in THF:H₂O (10:1) (3 mL) was added to a solution of NaBH₄ (0.15 g, 4.00 mmol) in H₂O (5 mL) under 0 °C. The reaction mixture was stirred at room temperature for 4 h upon monitoring the process by TLC. The resulting mixture was acidified with 2N HCl and extracted with ether. The aqueous phase was adjusted to base with 2N NaOH and extracted with EtOAc. The organic phases were combined and washed with H₂O. Then dried over with anh. Na₂SO₄. The organic phase was concentrated under reduced pressure to provide crude product of (*L*)-phenylalaninol (**36**) (0.11 g) as pale yellow oil which was used in the following step without further purification.; ¹H NMR (CD₃OD, 300 MHz) δ 2.59 (dd, *J* = 7.7, 13.4 Hz, 1H, CH), 2.79 (dd, *J* = 6.2, 13.4 Hz, 1H, CH), 3.04-3.13 (m, 1H, CH), 3.41 (dd, *J* = 6.8, 10.9 Hz, 1H, CH), 3.57 (dd, *J* = 4.3, 10.9 Hz, 1H, CH), 7.18-7.32 (m, 5H, ArH); ¹³C NMR (CD₃OD, 75 MHz) δ 40.5, 55.4, 66.2, 127.5, 129.6, 130.3, 139.8.

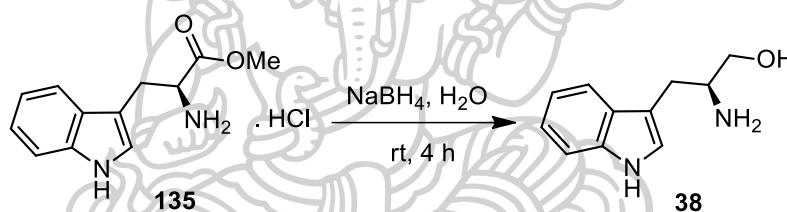
Synthesis of (S)-4-(2-amino-3-hydroxypropyl)phenol (37)



A solution of (*L*)-tyrosine methyl ester hydrochloride (**133**) (0.23 g, 1.00 mmol) in THF:H₂O (10:1) (3 mL) was added to a solution of NaBH₄ (0.15 g, 4.00 mmol) in H₂O (5 mL) under 0 °C. The reaction mixture was stirred at room temperature for 4 h upon monitoring the process by TLC. The resulting mixture was acidified with 2N HCl and

extracted with ether. The aqueous phase was adjusted to base with 2N NaOH and extracted with EtOAc. The organic phases were combined and washed with H₂O. Then dried over with anh. Na₂SO₄. The organic phase was concentrated under reduced pressure to provide crude product of (*L*)-tyrosinol (**37**) (0.14 g) as pale orange color oil which was used in the following step without further purification.; ¹H NMR (CD₃OD, 300 MHz) δ 2.50 (dd, *J* = 7.7, 13.6 Hz, 1H, CH), 2.70 (dd, *J* = 6.3, 13.6 Hz, 1H, CH), 2.99-3.04 (m, 1H, CH), 3.38 (dd, *J* = 6.9, 10.8 Hz, 1H, CH), 3.55 (dd, *J* = 4.4, 10.9 Hz, 1H, CH), 6.76 (d, *J* = 8.5 Hz, 2H, CH), 7.05 (d, *J* = 8.5 Hz, 2H, CH); ¹³C NMR (CD₃OD, 75 MHz) δ 39.5, 55.6, 66.3, 116.5, 130.4, 131.3, 157.2.

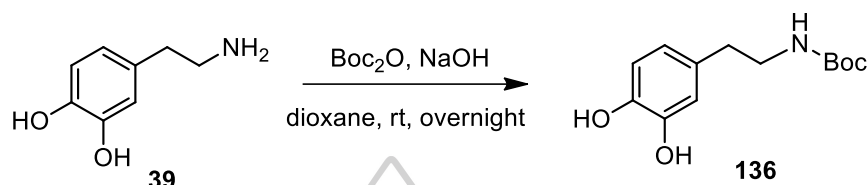
Synthesis of (*S*)-2-amino-3-(1*H*-indol-3-yl)propan-1-ol (**38**)



A solution of (*L*)-tryptophan methyl ester hydrochloride (**135**) (0.25 g, 1.00 mmol) in THF:H₂O (10:1) (3 mL) was added to a solution of NaBH₄ (0.15 g, 4.00 mmol) in H₂O (5 mL) under 0 °C. The reaction mixture was stirred at room temperature for 4 h upon monitoring the process by TLC. The resulting mixture was acidified with 2N HCl and extracted with ether. The aqueous phase was adjusted to base with 2N NaOH and extracted with EtOAc. The organic phases were combined and washed with H₂O. Then dried over with anh. Na₂SO₄. The organic phase was concentrated under reduced pressure to provide crude product of (*L*)-tryptophanol (**38**) (0.12 g) as light brown oil which was used in the following step without further purification.; ¹H-NMR (CD₃OD, 300 MHz) δ 2.71 (dd, *J* = 7.5, 14.3 Hz, 1H, CH), 2.92 (dd, *J* = 5.9, 14.3 Hz, 1H, CH), 3.11-3.20 (m, 1H, CH), 3.42 (dd, *J* = 7.0, 10.8 Hz, 1H, CH), 3.60 (dd, *J* = 4.8, 10.8 Hz, 1H, CH), 7.02 (td, *J* = 1.1, 7.0 Hz, 1H, CH), 7.07 (s, 1H, CH), 7.11 (td, *J* = 1.1, 7.0 Hz, 1H, CH), 7.37 (d, *J* =

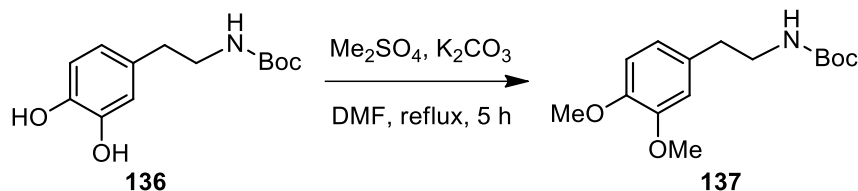
8.0 Hz, 1H, CH), 7.58 (d, $J = 7.7$ Hz, 1H); ^{13}C -NMR (CD_3OD , 75 MHz) δ 30.0, 54.4, 67.0, 112.3, 112.4, 119.52, 119.8, 122.5, 124.4, 128.9, 138.2.

Synthesis of *N*-tert-butyl 3,4-dihydroxyphenethylcarbamate (**136**)



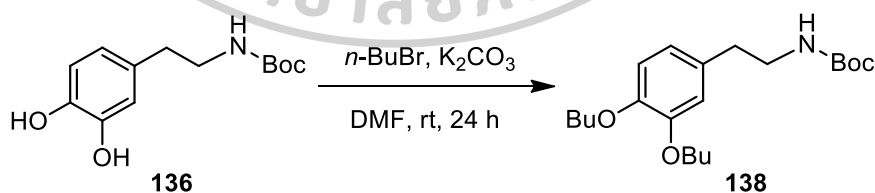
To a solution of dopamine hydrochloride (**39**) (0.48 g, 2.54 mmol) in the mixture of dioxane (5 mL) and H_2O (2.5 mL) were added 2N NaOH (1.27 mL, 2.54 mmol) and stirred for 10 minutes at room temperature. Then the solution of Boc_2O (0.6 mL, 2.54 mmol) in dioxane (4 mL) was added to the mixture. The reaction was stirred at room temperature under argon atmosphere for overnight. The resulting mixture was cooled and added EtOAc then the mixture was acidified with 1N HCl to adjust pH about 3. The aqueous layer was washed with EtOAc. The combined organic layers were washed with water, brine and dried over with anhydrous Na_2SO_4 , then the organic layer was concentrated under reduced pressure. The crude product was purified on silica gel by column chromatography using hexane:EtOAc 3:1 as eluent to provide product **136** (0.47 g, 73% yield) as a brown solid; m.p. 134-135 °C (lit. (Fletcher, Ganellin, Piergentili, Dunn, & Jenkinson, 2007) 132-134 °C); ^1H NMR ($\text{DMSO}-d_6$, 300 MHz) δ 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.47-2.52 (m, 2H, CH_2), 3.04 (m, 2H, CH_2), 6.42 (dd, $J = 2.0, 8.0$ Hz, 1H, ArH), 6.56 (d, $J = 2.0$ Hz, 1H, ArH), 6.63 (d, $J = 8.0$, 1H, ArH), 6.78 (br, 1H, NH), 8.64 (br, 1H, OH), 8.72 (br, 1H, OH); ^{13}C NMR ($\text{DMSO}-d_6$, 75 MHz) δ 28.3, 35.0, 41.9, 77.4, 115.4, 115.9, 119.1, 130.2, 143.4, 145.0, 155.5.

Synthesis of *N*-tert-butyl 3,4-dimethoxyphenethylcarbamate (**137**)



N-Boc Dopamine (**136**) (0.39 g, 1.56 mmol) was dissolved in anh. MeOH (10 mL). K_2CO_3 (0.95 g, 6.87 mmol) and dimethyl sulfate (0.33 mL, 3.44 mmol) were added. The mixture was refluxed for 5 h under Ar atmosphere. The resulting mixture was filtered through celite using sintered glass and washed with MeOH. The filtrate was concentrated to provide a residue which was purified on silica gel by flash column chromatography using hexane:EtOAc 2:1 as eluent to provide product **137** (0.31 g, 76% yield) as pale yellow solid; m.p. 52-54 °C (lit. (Khaligh, 2012) 55-57 °C); ^1H NMR (CDCl_3 , 300 MHz) δ 1.41 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.71 (t, $J = 7.1$ Hz, 2H, CH_2), 3.32 (t, $J = 7.1$ Hz, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.56 (br, 1H, NH), 6.69-6.72 (m, 2H, 2ArH), 6.78 (d, $J = 8.5$ Hz, 1H, ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 28.5, 35.9, 42.1, 55.9, 56.0, 79.3, 111.4, 112.1, 120.8, 131.6, 147.7, 149.0, 156.0.

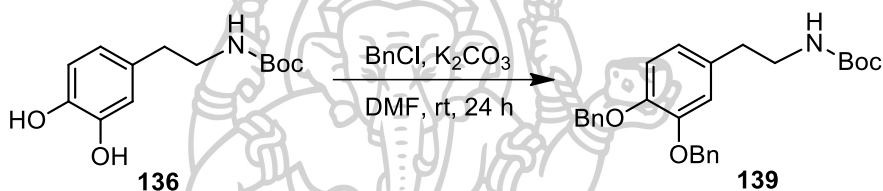
Synthesis of *N*-tert-butyl 3,4-dibutoxyphenethylcarbamate (**138**)



N-Boc Dopamine (**136**) (0.12 g, 0.47 mmol) was dissolved in anh. DMF (4 mL) then added K_2CO_3 (0.39 g, 2.84 mmol) and *n*-butyl bromide (0.10 mL, 0.95 mmol). The mixture was stirred under Ar atmosphere at room temperature for 24 h then was filtered through celite and washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was

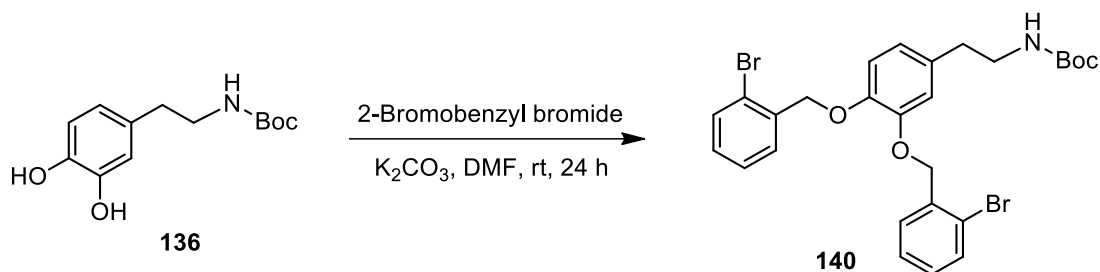
purified by preparative chromatography using hexane:EtOAc (4:1) as a mobile phase to provide **138** (0.02 g, 12% yield) as colorless oil; ^1H NMR (CDCl_3 , 300 MHz) δ 0.95-1.00 (dt, $J = 1.9, 7.4$ Hz, 6H, 2CH_3), 1.44 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.46-1.54 (m, 4H, 2CH_2), 1.74-1.82 (m, 4H, 2CH_2), 2.71 (t, $J = 7.0$ Hz, 2H, CH_2), 3.33 (q, $J = 6.2$ Hz, 2H, CH_2), 3.98 (dt, $J = 2.4, 6.6$ Hz, 4H, 2CH_2), 4.53 (br, 1H, NH), 6.68-6.71 (m, 2H, 2ArH), 6.81 (d, $J = 8.0$ Hz, 1H, ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 13.8, 19.2, 28.4, 31.4, 35.7, 41.9, 69.0, 69.1, 79.2, 114.2, 114.7, 120.9, 131.7, 147.8, 149.2, 155.9.

Synthesis of *N*-tert-butyl 3,4-bis(benzyloxy)phenethylcarbamate (**139**)



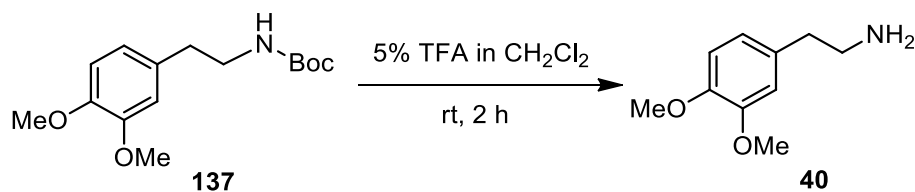
N-Boc Dopamine (**136**) (0.04 g, 0.17 mmol) was dissolved in anh. DMF (2 mL) then added K_2CO_3 (0.14 g, 1.02 mmol) and benzyl chloride (0.04 mL, 0.37 mmol). The mixture was stirred for 24 h under Ar atmosphere at room temperature then was filtered through celite and washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was purified on silica gel by column chromatography using hexane:EtOAc (2:1) as eluent to provide product **139** (0.065 g, 88% yield) as white solid; m.p. 111-113 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 1.45 (s, 9H, $\text{C}(\text{CH}_3)_3$), 2.69 (t, $J = 6.9$ Hz, 2H, CH_2), 3.32 (q, $J = 5.9$ Hz, 2H, CH_2), 4.52 (br, 1H, NH), 5.14 (s, 2H, OCH_2), 5.15 (s, 2H, OCH_2), 6.71 (dd, $J = 1.8, 8.1$ Hz, 1H, ArH), 6.80 (s, 1H, ArH), 6.88 (d, $J = 8.1$, 1H, ArH), 7.30-7.47 (m, 10H, 10ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 28.6, 35.8, 41.9, 71.5, 71.6, 79.4, 115.6, 116.0, 121.8, 127.4, 127.6, 127.9, 127.9, 128.6, 132.6, 137.4, 137.6, 147.8, 149.2, 156.0.

Synthesis of *N*-tert-butyl 3,4-bis((2-bromobenzyl)oxy)phenethylcarbamate (**140**)



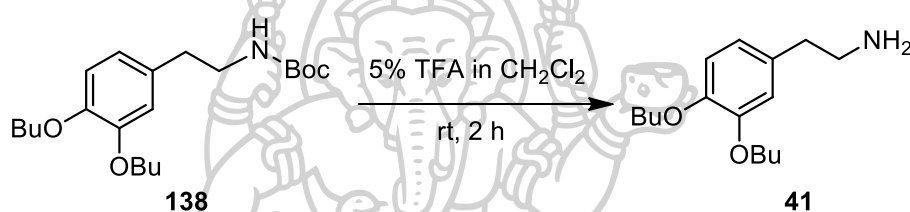
N-Boc Dopamine (**136**) (0.06 g, 0.24 mmol) was dissolved in anh. DMF (2 mL) then added K_2CO_3 (0.27 g, 1.42 mmol) and 2-bromobenzyl bromide (0.06 mL, 0.46 mmol). The mixture was stirred under Ar atmosphere at room temperature for 24 h. then was filtered through celite and washed with EtOAc. The filtrate was washed with water and brine. The organic layer was dried over with anh. Na_2SO_4 and concentrated. The crude product was purified on silica gel by column chromatography using hexane:EtOAc (4:1) as eluent to provide **140** (0.10 g, 72% yield) as white solid; m.p. 82-84 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.43 (s, 9H, $C(CH_3)_3$), 2.71 (t, J = 6.9 Hz, 2H, CH_2), 3.31 (br, 2H, CH_2), 4.55 (br, 1H, NH), 5.18 (s, 2H, OCH_2), 5.19 (s, 2H, OCH_2), 6.72 (dd, J = 1.8, 8.1 Hz, 1H, ArH), 6.81 (d, J = 1.6 Hz, 1H, ArH), 6.89 (d, J = 8.2, 1H, ArH), 7.12-7.18 (tt, J = 2.0, 7.6 Hz, 2H, 2ArH), 7.26-7.32 (td, J = 2.6, 7.5 Hz, 2H, 2ArH), 7.53-7.57 (dd, J = 2.3, 7.9 Hz, 2H, 2ArH), 7.61-7.65 (td, J = 1.3, 7.2 Hz, 2H, 2ArH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.5, 35.7, 41.9, 70.7, 70.8, 79.3, 115.2, 115.7, 122.0, 122.2, 127.5, 128.8, 129.0, 129.1, 129.2, 132.5, 132.6, 132.8, 136.6, 136.7, 147.3, 148.7, 155.9.

Synthesis of 2-(3,4-dimethoxyphenyl)ethanamine (**40**)



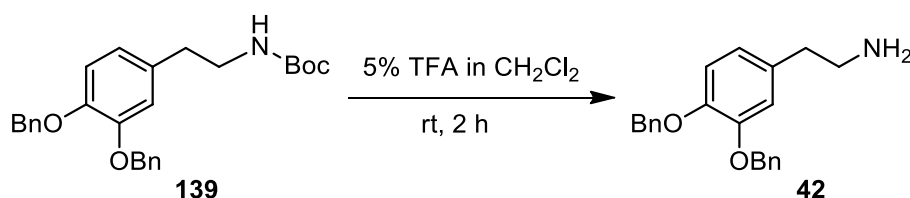
A solution of 5% TFA in dry CH_2Cl_2 (30.7 mL, 20.60 mmol) was added to amine **137** (0.30 g, 1.07 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum and purified by preparative chromatography using 15% MeOH in CH_2Cl_2 as a mobile phase to provide **40** (0.15 g, 47% yield) as yellow oil; ^1H NMR (CDCl_3 , 300 MHz) δ 2.75 (t, $J = 7.1$ Hz, 2H, CH_2), 2.99 (t, $J = 7.1$ Hz, 2H, CH_2), 3.81 (s, 3H, OCH_3) 3.82 (s, 3H, OCH_3), 4.47 (br, 2H, NH_2), 6.65-6.84 (m, 3H, 3ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 36.7, 42.5, 55.9, 56.0, 111.5, 112.1, 120.8, 130.7, 147.9, 149.1.

Synthesis of 2-(3,4-dibutoxyphenyl)ethanamine (**41**)



A solution of 5% TFA in dry CH_2Cl_2 (1.7 mL, 1.12 mmol) was added to amine **138** (0.02 g, 0.06 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum to provide crude product of **41** which was used in the following step without further purification.

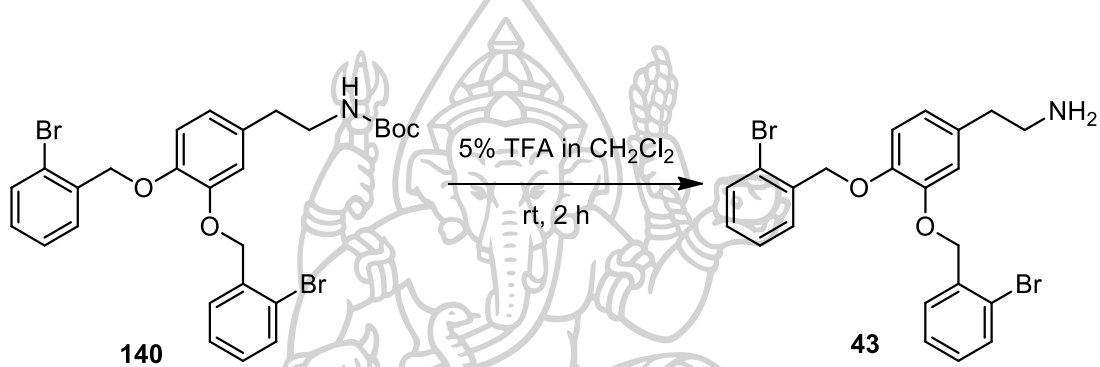
Synthesis of 2-(3,4-bis(benzyloxy)phenyl)ethanamine (**42**)



A solution of 5% TFA in dry CH_2Cl_2 (28.5 mL, 18.60 mmol) was added to amine **139** (0.40 g, 0.93 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum and purified by preparative chromatography using 15% MeOH in CH_2Cl_2

as a mobile phase to provide **42** (0.30 g, 72% yield) as yellow oil; ^1H NMR (CDCl_3 , 300 MHz) δ 2.64 (t, $J = 7.0$ Hz, 2H, CH_2), 2.86 (t, $J = 6.8$ Hz, 2H, CH_2), 3.94 (br, 2H, NH_2), 5.07 (s, 2H, OCH_2), 5.10 (s, 2H, OCH_2), 6.65 (dd, $J = 1.8, 8.1$ Hz, 1H, ArH), 6.76 (d, $J = 1.8$ Hz, 1H, ArH), 6.83 (d, $J = 8.1$, 1H, ArH), 7.27-7.38 (m, 10H, 10ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 37.1, 42.5, 71.4, 71.5, 115.5, 116.0, 121.8, 127.4, 127.6, 127.9, 128.0, 128.6, 131.9, 137.3, 137.4, 147.8, 149.0.

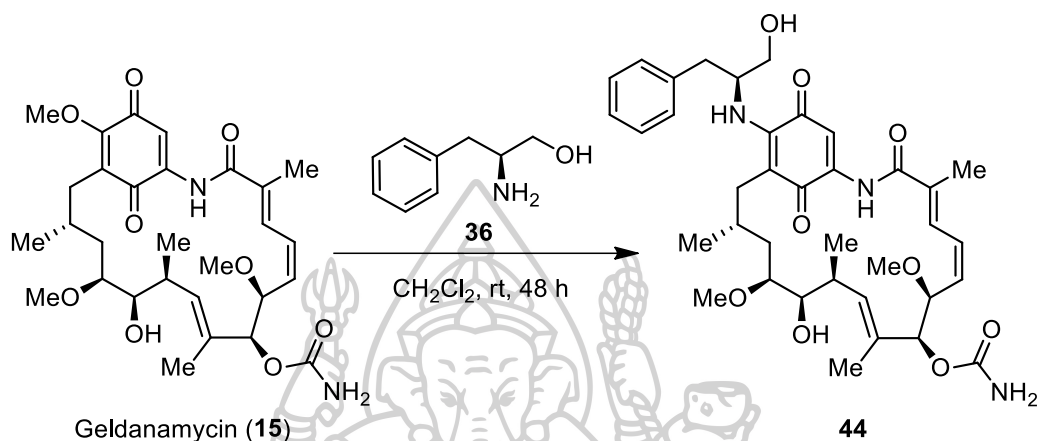
Synthesis of 2-(3,4-bis((2-bromobenzyl)oxy)phenyl)ethanamine (**43**)



A solution of 5% TFA in dry CH_2Cl_2 (9.2 mL, 6.05 mmol) was added to amine **140** (0.25 g, 0.42 mmol) and stirred at room temperature for 2 h. The solvent was removed under vacuum and purified by preparative chromatography using 15% MeOH in CH_2Cl_2 as a mobile phase to provide **43** (0.19 g, 76% yield) as pale yellow solid; m.p. 68-69 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 2.69 (t, $J = 6.8$ Hz, 2H, CH_2), 2.93 (t, $J = 6.6$ Hz, 2H, CH_2), 3.02 (br, 2H, NH_2), 5.17 (s, 2H, OCH_2), 5.19 (s, 2H, OCH_2), 6.73 (dd, $J = 1.6, 8.2$ Hz, 1H, ArH), 6.80 (d, $J = 1.6$ Hz, 1H, ArH), 6.87 (d, $J = 8.2$, 1H, ArH), 7.27-7.38 (td, $J = 1.4, 7.7$ Hz, 2H, 2ArH), 7.25-7.31 (m, 2H, 2ArH), 7.55 (dd, $J = 0.7, 7.9$ Hz, 2H, 2ArH), 7.60-7.62 (d, $J = 7.7$ Hz, 2H, 2ArH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 38.1, 43.0, 70.8, 70.9, 115.2, 115.9, 122.2, 122.3, 127.7, 129.0, 129.1, 129.2, 129.3, 132.6, 132.7, 132.8, 136.6, 136.7, 147.4, 148.7.

3.3.3 Synthesis of 17-amino-17-demethoxygeldanamycin derivatives

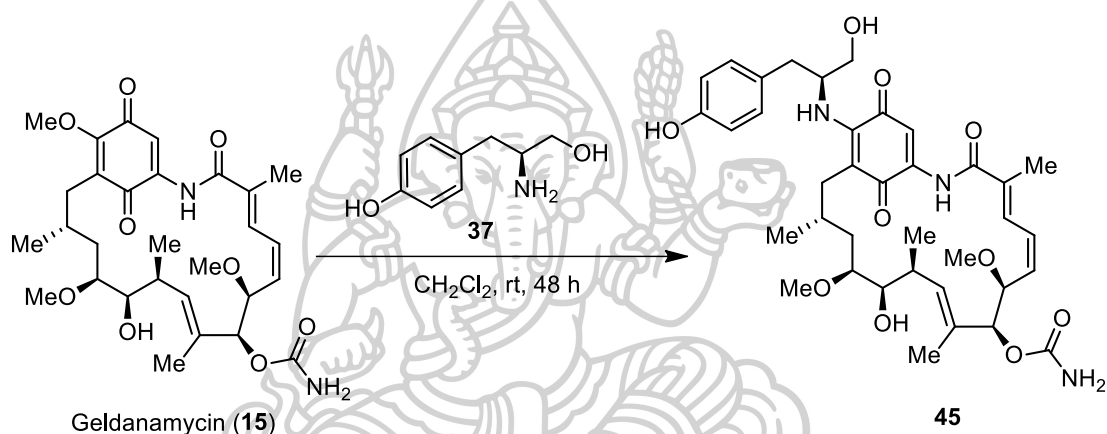
Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-13-hydroxy-19-(((*S*)-1-hydroxy-3-phenylpropan-2-yl)amino)-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1] docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (44)



A solution of (*L*)-phenylalaninol (**36**) (0.05 g, 0.30 mmol) in MeOH (2 mL) was added to a solution of geldanamycin (**15**) (0.08 g, 0.14 mmol) in CH₂Cl₂ (10 mL). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc and then washed with 1N HCl, H₂O and brine. The organic phases were combined and dried over with anh. Na₂SO₄. The organic phase was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with 40% CH₂Cl₂ in EtOAc to afford the product **44** (0.016 g, 40%) as purple solid; m.p. 129-131 °C; ¹H NMR (CDCl₃, 300 MHz) δ 0.84 (d, *J* = 6.4 Hz, 3H, CH₃), 0.98 (d, *J* = 6.4 Hz, 3H, CH₃), 1.75 (m, 4H, hydrocarbon), 1.78 (s, 3H, CH₃), 2.02 (s, 3H, CH₃), 2.21 (m, 1H, CH), 2.63 (d, *J* = 13.1 Hz, 1H, CH), 2.73 (m, 1H, CH), 2.93 (dd, *J* = 6.8, 13.6 Hz, 1H, CH), 3.08 (dd, *J* = 6.6, 13.6 Hz, 1H, CH), 3.26 (s, 3H, CH₃), 3.35 (s, 3H, CH₃), 3.42 (m, 1H, CH), 3.55 (dd, *J* = 1.9, 8.8 Hz, 1H, CH), 3.64 (d, *J* = 3.8 Hz, 2H, CH₂), 4.29 (d, *J* = 9.8 Hz, 1H, CH), 4.40 (m, 1H, CH), 4.95 (br, 2H, NH₂), 5.16 (s, 1H, CH), 5.86 (m, 2H, CH), 6.37 (d, *J* = 9.1 Hz, 1H, CH), 6.57 (t, *J* = 11.6 Hz, 1H, CH), 6.92 (d, *J* = 11.5 Hz, 1H, CH), 7.22 (s, 1H, CH), 7.28 (m, 5H, ArH), 9.10 (br, 1H, NH); ¹³C NMR (CDCl₃, 75 MHz) δ 12.4, 12.5, 12.8, 23.0, 28.7, 32.2, 34.5, 35.0, 38.2, 56.5, 56.7,

57.1, 63.0, 72.6, 81.1, 81.3, 81.7, 109.0, 109.3, 126.5, 126.9, 128.7, 129.4, 132.8, 133.6, 134.9, 135.8, 136.7, 140.9, 144.9, 156.2, 168.3, 180.4, 184.1; HRMS calculated for $C_{37}H_{49}N_3O_9$ ($M+Na$)⁺ 702.3361, found 702.3360.

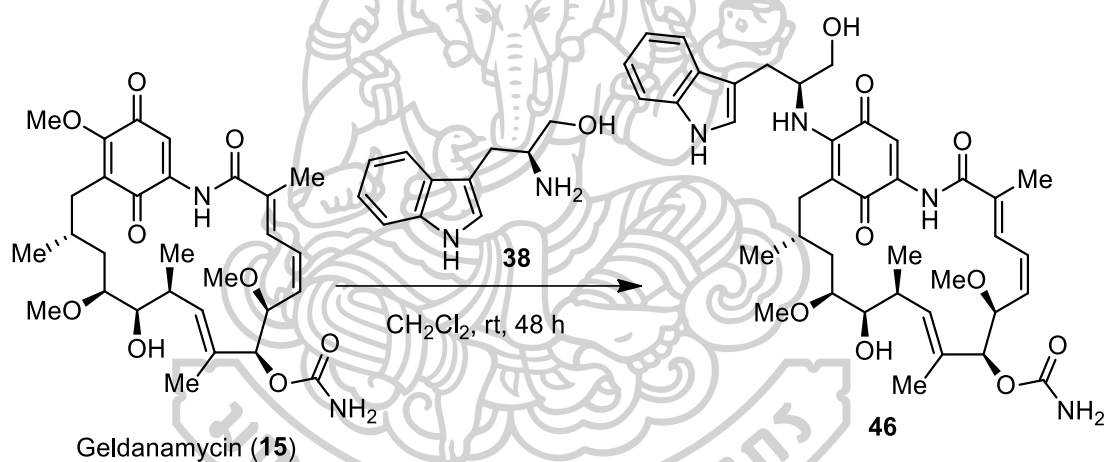
Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-13-hydroxy-19-(((*S*)-1-hydroxy-3-(4-hydroxyphenyl)propan-2-yl)amino)-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo [16.3.1]docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (45)



A solution of (*L*)-tyrosinol (**37**) (0.05 g, 0.30 mmol) in MeOH (2 mL) was added to a solution of geldanamycin (**15**) (0.08 g, 0.14 mmol) in CH_2Cl_2 (10 mL). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc and then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anh. Na_2SO_4 . The organic phase was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with 40% CH_2Cl_2 in EtOAc to afford the product **45** (0.028 g, 29%) as purple solid; m.p. 125-127 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.89 (d, J = 6.2 Hz, 3H, CH_3), 1.01 (d, J = 6.2 Hz, 3H, CH_3), 1.78 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.14 (m, 2H, CH_2), 2.65 (d, J = 13.4 Hz, 1H, CH), 2.75 (m, 1H, CH), 2.94 (d, J = 5.0 Hz, 2H, CH_2), 3.27 (s, 3H, CH_3), 3.36 (s, 3H, CH_3), 3.45 (m, 1H, CH), 3.59 (m, 3H, CH, CH_2), 4.30 (d, J = 9.8 Hz, 1H, CH), 4.38 (m, 2H, CH_2), 4.79 (br, 2H, NH_2), 5.17 (s, 1H, CH), 5.54, (br, 1H, NH), 5.86 (dd, J = 10.6, 10.8 Hz, 2H, CH), 6.37 (d, J = 8.8 Hz, 1H,

CH), 6.58 (t, $J = 11.3$ Hz, 1H, CH), 6.81 (d, $J = 8.5$ Hz, 2H, CH), 6.93 (d, $J = 11.6$ Hz, 1H, CH), 7.11 (d, $J = 8.5$ Hz, 2H, CH), 7.24 (s, 1H, CH), 9.11 (br, 1H, NH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 12.5, 12.7, 12.9, 23.1, 28.8, 32.0, 32.4, 34.5, 35.1, 37.1, 56.4, 56.8, 57.3, 62.7, 72.8, 81.3, 81.4, 82.0, 109.1, 109.3, 115.8, 126.8, 127.0, 128.0, 130.8, 133.0, 133.6, 135.1, 135.9, 141.0, 155.2, 156.4, 168.5, 180.5, 184.2; HRMS calculated for $\text{C}_{37}\text{H}_{49}\text{N}_3\text{O}_9$ ($\text{M}+\text{Na}$) $^+$ 718.3310, found 718.3309.

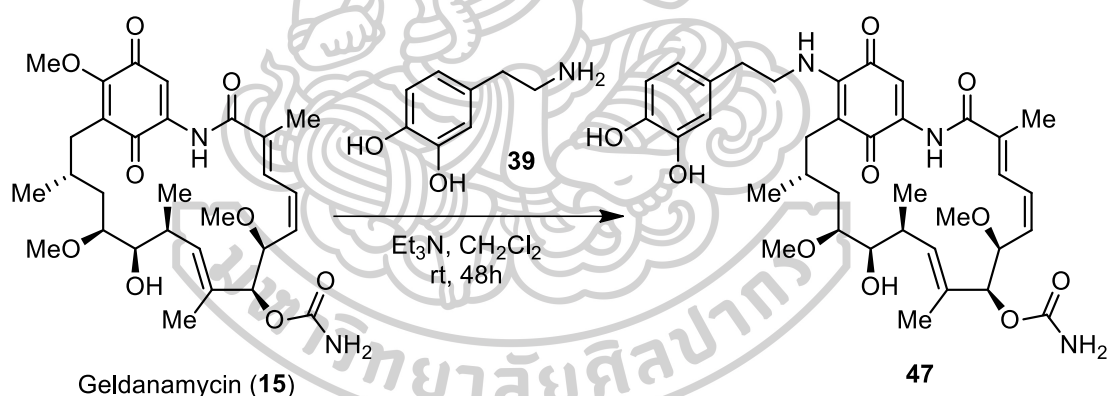
Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-13-hydroxy-19-(((*S*)-1-hydroxy-3-(1*H*-indol-3-yl)propan-2-yl)amino)-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1] docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (46)



A solution of (*L*)-tryptanol (38) (0.06 g, 0.30 mmol) in MeOH (2 mL) was added to a solution of geldanamycin (15) (0.08 g, 0.14 mmol) in CH_2Cl_2 (10 mL). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc and then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anhydrous Na_2SO_4 . The organic phase was concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel eluting with 40% CH_2Cl_2 in EtOAc to afford the product 46 (0.032 g, 32%) as purple solid; m.p. 144-146 $^\circ\text{C}$; ^1H NMR (CDCl_3 , 300 MHz) δ 0.86 (d, $J = 6.3$ Hz, 3H, CH_3), 0.97 (d, $J = 6.9$ Hz, 3H, CH_3), 1.74 (m, 4H, hydrocarbon), 1.79 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 2.24 (m, 1H, CH), 2.60 (d, $J = 13.9$ Hz, 1H, CH), 2.73 (m, 1H, CH),

3.16, (d, $J = 6.0$ Hz, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.33 (m, 4H, CH, CH_3), 3.42 (m, 1H, CH), 3.56 (d, $J = 7.5$ Hz, 1H, CH), 3.64 (d, $J = 4.4$ Hz, 2H, CH_2), 4.28 (d, $J = 9.8$ Hz, 1H, CH), 4.52 (m, 1H, CH), 4.93 (br, 2H, NH_2), 5.15 (s, 1H, CH), 5.85 (m, 2H, CH), 6.49 (m, 2H, CH), 6.88 (d, $J = 11.5$ Hz, 1H, CH), 7.17 (m, 4H, CH), 7.37 (d, $J = 7.9$ Hz, 1H, CH), 7.66 (d, $J = 7.7$ Hz, 1H, CH), 8.52 (br, 1H, NH), 9.08 (br, 1H, NH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 12.3, 12.5, 12.7, 22.8, 27.3, 28.6, 32.2, 34.5, 34.9, 55.4, 56.7, 57.1, 63.1, 72.6, 81.2, 81.3, 81.8, 109.0, 110.2, 111.3, 118.6, 119.7, 119.9, 122.3, 123.5, 126.6, 126.8, 127.6, 132.8, 133.7, 135.0, 135.7, 136.3, 140.8, 145.3, 156.2, 168.3, 180.2, 184.2; HRMS calculated for $\text{C}_{39}\text{H}_{50}\text{N}_4\text{O}_9$ ($\text{M}+\text{Na}$) $^+$ 741.3470, found 741.3473.

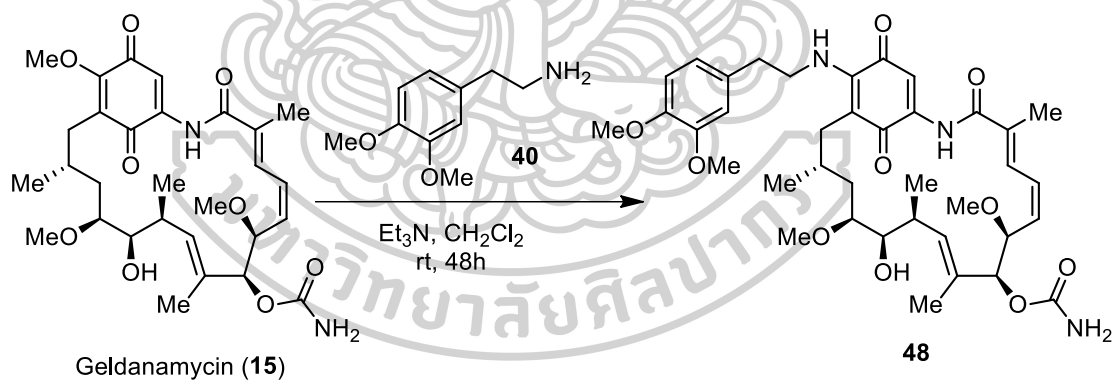
Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-19-((3,4-dihydroxyphenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21), 4,6,10,18-pentaen-9-yl carbamate (47)



To a solution of geldanamycin (15) (0.06 g, 0.10 mmol) in CH_2Cl_2 was added with dopamine hydrochloride (39) (0.04 g, 0.20 mmol) in MeOH and Et_3N (0.03 mL, 0.24 mmol). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anhydrous Na_2SO_4 . The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography using 1:2 Hexane:EtOAc as a mobile phase to afford the product 47 (0.0167 g, 21% yield) as a purple solid; m.p. 114-116 $^\circ\text{C}$; ^1H NMR (CDCl_3 , 300

MHz) δ 0.93 (d, J = 6.2 Hz, 3H, CH₃), 1.00 (d, J = 6.9 Hz, 3H, CH₃), 1.60-1.78 (m, 3H, hydrocarbon), 1.80 (s, 3H, CH₃), 2.01 (s, 3H, CH₃), 2.35-2.43 (m, 1H, CH), 2.62-2.83 (m, 4H, hydrocarbon), 3.27 (s, 3H, OCH₃), 3.36 (s, 3H, OCH₃), 3.46 (m, 1H, CH), 3.59-3.73 (m, 3H, hydrocarbon), 4.33 (d, J = 9.7 Hz, 1H, CH), 4.97 (br, 2H, NH₂), 5.18 (s, 1H, CH), 5.83-5.90 (m, 2H, 2CH), 6.41 (br, 1H, NH), 6.54-6.61 (m, 2H, 2CH), 6.73 (d, J = 1.8 Hz, 1H, ArH), 6.81 (d, J = 8.0 Hz, 1H, ArH), 6.94 (d, J = 11.5 Hz, 1H, CH), 7.21 (s, 1H, CH), 9.18 (br, 1H, NH); ¹³C NMR (CDCl₃, 75 MHz) δ 12.5, 12.6, 12.8, 22.8, 28.6, 29.7, 32.3, 34.3, 35.0, 35.2, 47.0, 56.7, 57.1, 72.8, 81.1, 81.4, 81.9, 108.5, 108.7, 115.6, 115.7, 120.8, 126.6, 127.0, 129.6, 132.8, 133.6, 134.9, 141.3, 143.1, 144.3, 144.9, 156.3, 168.5, 180.4, 183.9; HRMS calculated for C₃₆H₄₇N₃O₁₀ (M+Na)⁺ 704.3154, found 704.3157.

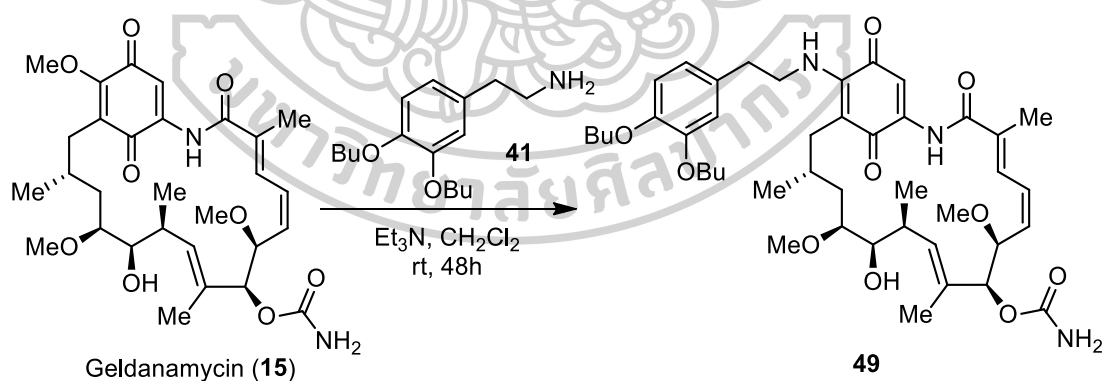
Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-19-((3,4-dimethoxyphenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21), 4,6,10,18-pentaen-9-yl carbamate (48)



To a solution of geldanamycin (15) (0.08 g, 0.14 mmol) in CH₂Cl₂ was added with 2-(3,4-dimethoxyphenyl)ethanamine (40) (0.05 g, 0.30 mmol) in MeOH and Et₃N (0.05 mL, 0.33 mmol). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc then washed with 1N HCl, H₂O and brine. The organic phases were combined and dried over with anh. Na₂SO₄. The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography using 1:1 Hexane:EtOAc as a mobile phase to afford the

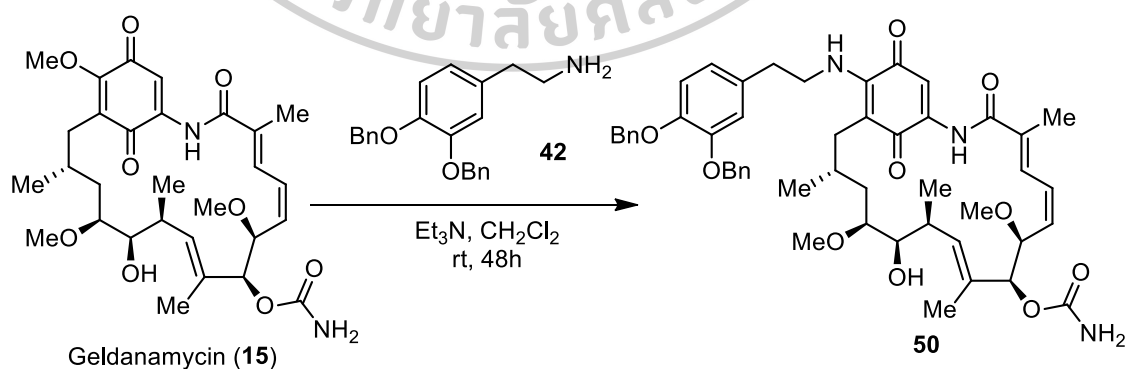
product **48** (0.0508 g, 50% yield) as a purple solid; m.p. 127-129 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 0.95 (d, J = 6.4 Hz, 3H, CH_3), 1.00 (d, J = 6.9 Hz, 3H, CH_3), 1.62-1.78 (m, 3H, hydrocarbon), 1.80 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.39-2.46 (m, 1H, CH), 2.67-2.93 (m, 4H, hydrocarbon), 3.27 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.45 (m, 1H, CH), 3.57 (dd, J = 1.8, 9.0 Hz, 1H, CH), 3.62-3.85 (m, 1H, CH), 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.31 (d, J = 9.8 Hz, 1H, CH), 4.86 (br, 2H, NH_2), 5.19 (s, 1H, CH), 5.83-5.92 (m, 2H, 2CH), 6.38 (br, 1H, NH), 6.58 (t, J = 11.5 Hz, 1H, CH), 6.71-6.91 (m, 3H, 3ArH), 6.95 (d, J = 11.4 Hz, 1H, CH), 7.26 (s, 1H, CH), 9.17 (br, 1H, NH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 12.3, 12.6, 12.8, 22.9, 28.5, 32.3, 34.4, 35.1, 35.5, 47.1, 55.9, 56.7, 57.1, 72.65, 81.2, 81.4, 81.6, 108.6, 108.7, 111.6, 111.7, 120.7, 126.5, 126.9, 129.6, 132.7, 133.7, 134.9, 135.8, 141.3, 144.7, 148.2, 149.3, 156.0, 168.4, 180.7, 183.8; HRMS calculated for $\text{C}_{38}\text{H}_{51}\text{N}_3\text{O}_{10}$ ($\text{M}+\text{Na}$) $^+$ 732.3467, found 732.3469.

Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-19-((3,4-dibutoxyphenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21), 4,6,10,18-pentaen-9-yl carbamate (**49**)



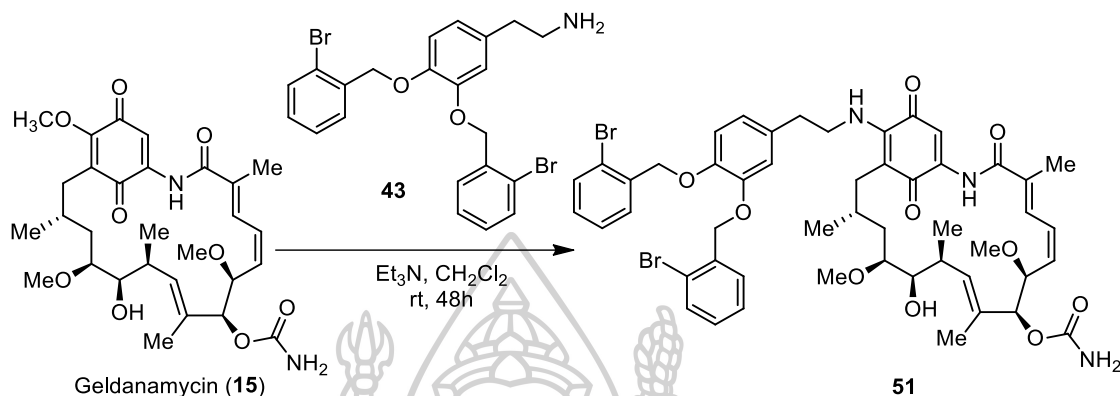
To a solution of geldanamycin (**15**) (0.03 g, 0.05 mmol) in CH_2Cl_2 was added with crude product of 2-(3,4-dibutoxyphenyl)ethanamine (**41**) (0.02 g, 0.09 mmol) in MeOH and Et_3N (0.02 mL, 0.11 mmol). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anh.

Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-19-((3,4-bis(benzyloxy)phenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1]docosa-1(21), 4,6,10,18-pentaen-9-yl carbamate (50)



To a solution of geldanamycin (**15**) (0.11 g, 0.20 mmol) in CH_2Cl_2 was added with 2-(3,4-bis(benzyloxy)phenyl)ethanamine (**42**) (0.13 g, 0.40 mmol) in MeOH and Et_3N (0.07 mL, 0.46 mmol). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anhydrous Na_2SO_4 . The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography using 5:2 CH_2Cl_2 :EtOAc as a mobile phase to afford the product **50** (0.0501 g, 14% yield) as purple solid; m.p. 92-94 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 0.93 (d, J = 6.4 Hz, 3H, CH_3), 1.00 (d, J = 6.9 Hz, 3H, CH_3), 1.62-1.78 (m, 3H, hydrocarbon), 1.79 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.34-2.42 (m, 1H, CH), 2.64-2.77 (m, 1H, CH), 2.84 (t, J = 6.9 Hz, 2H, CH_2), 3.26 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.43-3.46 (m, 1H, CH), 3.58 (dd, J = 1.7, 8.9 Hz, 1H, CH), 3.62-3.82 (m, 2H, CH_2), 4.31 (d, J = 9.9 Hz, 1H, CH), 4.96 (br, 2H, NH_2), 5.14 (s, 2H, OCH_2), 5.16 (s, 2H, OCH_2), 5.18 (s, 1H, CH), 5.82-5.91 (m, 2H, 2CH), 6.32 (br, 1H, NH), 6.58 (t, J = 11.5 Hz, 1H, CH), 6.73 (dd, J = 2.0, 8.1 Hz, 1H, ArH), 6.79 (d, J = 2.0 Hz, 1H, ArH), 6.91 (d, J = 8.2 Hz, 1H, ArH), 6.95 (d, J = 10.8 Hz, 1H, CH), 7.26 (s, 1H, CH), 7.29-7.40 (m, 6H, 6ArH), 7.40-7.55 (m, 4H, 4ArH), 9.17 (br, 1H, NH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 12.3, 12.6, 12.7, 22.9, 28.5, 32.3, 34.4, 35.0, 35.4, 46.9, 56.7, 57.1, 71.4, 71.5, 72.6, 81.2, 81.4, 81.6, 108.6, 108.7, 115.4, 115.6, 121.6, 126.5, 126.9, 127.3, 127.8, 128.5, 130.4, 132.7, 133.7, 134.9, 135.8, 137.1, 137.2, 141.3, 144.7, 148.2, 149.3, 156.1, 168.3, 180.6, 183.7; HRMS calculated for $\text{C}_{50}\text{H}_{59}\text{N}_3\text{O}_{10}$ ($\text{M}+\text{Na}$) $^+$ 884.4093, found 884.4094.

Synthesis of (4*E*,6*Z*,8*S*,9*S*,10*E*,12*S*,13*R*,14*S*,16*R*)-19-((3,4-bis((2-bromobenzyl)oxy)phenethyl)amino)-13-hydroxy-8,14-dimethoxy-4,10,12,16-tetramethyl-3,20,22-trioxo-2-azabicyclo[16.3.1] docosa-1(21),4,6,10,18-pentaen-9-yl carbamate (51)



To a solution of geldanamycin (**15**) (0.08 g, 0.14 mmol) in CH_2Cl_2 was added with 2-(3,4-bis((2-bromobenzyl)oxy)phenethyl)ethanamine (**43**) (0.15 g, 0.30 mmol) in MeOH and Et_3N (0.05 mL, 0.33 mmol). The reaction was kept away from light and stirred at room temperature for 48 h. The solvent was removed and diluted with EtOAc then washed with 1N HCl, H_2O and brine. The organic phases were combined and dried over with anhydrous Na_2SO_4 . The organic phase was concentrated under reduced pressure. The crude product was purified by preparative chromatography using 2:1 CH_2Cl_2 :EtOAc as a mobile phase to afford the product **51** (0.0757 g, 53% yield) as purple solid; m.p. 174-176 °C; ^1H NMR (CDCl_3 , 300 MHz) δ 0.94 (d, $J = 6.4$ Hz, 3H, CH_3), 1.00 (d, $J = 6.9$ Hz, 3H, CH_3), 1.62-1.79 (m, 3H, hydrocarbon), 1.80 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.36-2.44 (m, 1H, CH), 2.65-2.80 (m, 1H, CH), 2.88 (t, $J = 6.9$ Hz, 2H, CH_2), 3.26 (s, 3H, OCH_3), 3.36 (s, 3H, OCH_3), 3.43-3.46 (m, 1H, CH), 3.57 (dd, $J = 1.4, 8.6$ Hz, 1H, CH), 3.60-3.85 (m, 2H, CH_2), 4.31 (d, $J = 9.9$ Hz, 1H, CH), 5.06 (br, 2H, NH_2), 5.18 (s, 1H, CH), 5.20 (s, 2H, OCH_2), 5.22 (s, 2H, OCH_2), 5.82-5.91 (m, 2H, 2CH), 6.33 (br, 1H, NH), 6.58 (t, $J = 11.6$ Hz, 1H, CH), 6.77-6.86 (m, 2H, 2ArH), 6.93 (d, $J = 8.1$ Hz, 1H, ArH), 6.95 (d, $J = 11.6$ Hz, 1H, CH), 7.17 (td, $J = 0.5, 7.4$ Hz, 2H, 2ArH), 7.26 (s, 1H, CH), 7.31 (td, $J = 0.6, 7.5$ Hz, 2H, 2ArH), 7.56 (d, $J = 7.9$ Hz, 2H, ArH), 7.61 (ddd, $J = 1.8, 2.3, 7.7$ Hz, 2H, 2ArH), 9.17 (br, 1H, NH); ^{13}C NMR (CDCl_3 , 75 MHz) δ 12.3, 12.5, 12.7, 22.9, 28.4, 32.3, 34.4, 35.0, 35.3, 46.8, 56.7,

57.0, 70.6, 72.5, 81.2, 81.4, 81.5, 108.6, 108.7, 115.1, 115.2, 121.9, 122.0, 122.1, 126.5, 126.9, 127.5, 127.6, 128.8, 128.9, 129.1, 129.2, 130.7, 130.8, 132.4, 132.8, 133.6, 134.9, 135.8, 136.3, 136.4, 141.2, 144.7, 147.7, 147.8, 148.8, 148.9, 156.2, 168.3, 180.6, 183.6; HRMS calculated for $C_{50}H_{57}Br_2N_3O_{10}$ (M+Na)⁺ 1040.2303, found 1040.2323.

3.4 Biological activities

3.4.1 Antibacterial activity

The minimal inhibitory concentration (MIC) of antibacterial activity was studied with microbroth dilution method. The geldanamycin (**15**) and its derivatives (**44-51**) were determined against three gram-positive bacterial pathogens (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*). Chloramphenicol was used as the standard antibiotic to compare the bacterial cultures.

These bacterial were cultured in Nutrient broth (NB) with shaking incubation at 180 cycles/min for 24 h at 37 °C which was adjusted to the 0.5 McFarland standard (1×10^5 CFU / mL). The test compounds were dissolved in DMSO to prepare 1,024 µg/mL stock solution and chloramphenicol were dissolved in EtOH to 50 mg/mL. These stock solutions were transferred 10 µL of stock solution into the first well containing 190 µL of NB. The 2-fold dilutions were taken by transferring the solution from the first well (100 µL) into the next well and continued until the 11th well to obtain the various concentrations (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 µg/mL). The NB containing 20% DMSO was used as a positive control. The 50 µL of bacterium in NB was added into each well and incubated for 48 h at 37 °C. The results were determined by comparison of the turbidity of NB.

3.4.2 Cytotoxicity activity

The cytotoxicity activity studies were evaluated using MTT assay. Three cancer cell lines including human cervical cancer cells (HeLa) were obtained from the Department of Microbiology, Faculty of Science (Silpakorn University, Nakhon pathom); human hepatocellular carcinoma cells (HepG2) were obtained from the Department of

Biology, Faculty of Science (Silpakorn University, Nakhon pathom); Human breast carcinoma cells (MDA-MB-231) were obtained from the Faculty of Pharmacy (Silpakorn University, Nakhon pathom). Two normal cell lines including African green monkey kidney cells (Vero) were obtained from Siriraj Hospital (Bangkok) and Rhesus monkey kidney cells (LLC-MK2) were derived from the Department of Biology, Faculty of Science (Silpakorn University, Nakhon pathom).

Preparation of Dulbecco's Modified Eagle Medium (DMEM) for cell cultures

The 1,000 mL of sterile deionized water in 1,000 mL Duran bottle was autoclaved and cooled down to room temperature then added DMEM powder 10 g. The solution was stirred which was prepared under the vertical laminar air flow. The stock solution of DMEM was added with an antibiotic; penicillin (100 $\mu\text{g/mL}$) and streptomycin sulfate (100 $\mu\text{g/mL}$) and 10% Fetal Bovine Serum (FBS) then was adjusted pH to 7.1-7.2 with sterile solution of NaHCO_3 . The completed DMEM was filtered through 0.22 μm microdisc filter.

Cell cultures

These cell lines were cultured as monolayer in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and antibiotic then incubated at 37 °C with 5% CO_2 atmosphere. The medium was changed every 2 days until cells increased to 80-90% confluent.

MTT assay for anticancer and cytotoxicity activity

Cells in cell culture flask were detached with enzyme trypsin then transferred to 96 well plates to a density of 2×10^5 cells per well containing 100 μL of DMEM medium with 10% FBS and incubated at 37 °C with 5% CO_2 for overnight. Then the crude extract, geldanamycin (15) and its derivatives (44-51) were dissolved in DMSO for 1,024 $\mu\text{g/mL}$ as a stock solution. The various concentrations were diluted 2-fold from stock solution. Cells were treated with the reducing concentration of test compounds (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 $\mu\text{g/mL}$) in medium without FBS for 24 h. The 5% and 20% of

DMSO in medium without FBS were used as a control. Cells were once rinsed with phosphate buffer saline (PBS). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) solution (20 μ L of 5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The MTT solution was removed. The resulting formazan crystal was dissolved with 100 μ L of DMSO to each well. The absorbance was measured at 540 nm with microplate spectrometer. Percentage of cell viability was determined and calculated using the following formula.

$$\% \text{ cell viability} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{reference}} - \text{Abs}_{\text{blank}}} \times 100$$

The IC_{50} of each cell was calculated from the calibration curve with X-axis as a concentration and Y-axis as the percentage of cell viability.

3.5 Molecular docking studies

The structure of geldanamycin and its derivatives were designed with GaussView 6.0. These structures were optimized and converted to three dimensional structure. The human Hsp90 with difference co-crystallized geldanamycin (GDM; 15) (PDB ID: 1OSF) and dimethylamino ethylaminogeldanamycin (DMAG; 17) (PDB ID: 1YET) were chosen for the studied of binding energy and hydrogen bond. Geldanamycin (15) and DMAG (17) were redocked into 1YET and 1OSF. The molecular docking were docked by using the iGEMDOCK v2.1 software. The resulted interactions of protein-ligands were analyzed with UCSF Chimera 1.13.1 and Discovery Studio 2020.

CHAPTER 4

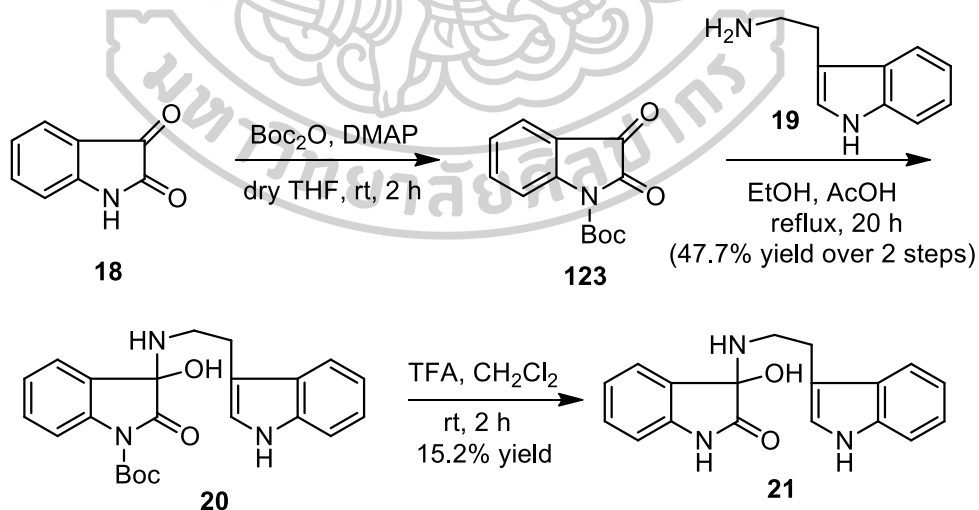
RESULTS AND DISCUSSION

4.1 Results of the synthesis of iheyamine A derivative

The synthetic studies of iheyamine A derivatives were designed with three routes by the different starting materials.

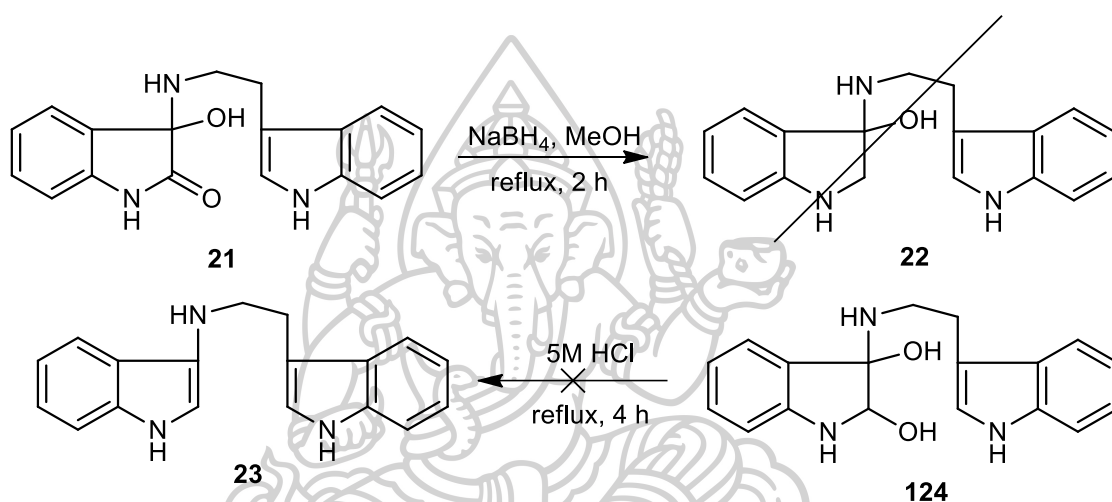
4.1.1 The first synthetic route of iheyamine A derivative

The first synthetic route was started from isatin (**18**) which was *N*-protected with Boc group to produce the compound **123**. The chemical shift of 3CH₃ or Boc group was added 1.57 and 28.0 ppm of ¹H and ¹³C NMR, respectively. The condensation of *N*-Boc isatin (**123**) was refluxed with tryptamine (**19**) under acidic conditions. This formation was provided the 3-hydroxy oxindole **20**. This was also confirmed by the previous reports of Phutdhawong and co-workers (Phutdhawong et al., 2016) that the hydroxyl group was presented when the isatin (**18**) was protected by electron withdrawing group. Removal of Boc group was performed with trifluoroacetic acid which was provided 3-hydroxy oxindole **21** in low yield and ¹H NMR of this compound showed no signal of 3CH₃ at 1.5 ppm.



Scheme 24 Preparation of 3-hydroxy oxindole (**21**).

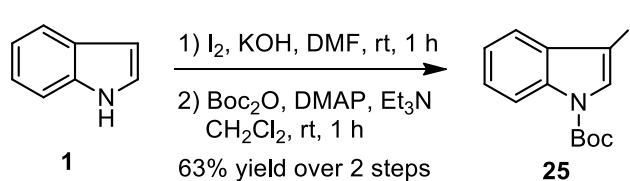
Then, the oxindole **21** was reduced with sodium borohydride in methanol. However, the indoline **22** was not formed. But the resulting the indolinol **124** was obtained instead. The consequence was confirmed by ^1H NMR at 5.05 ppm signal of H-2 singlet of indolinol. Attempt to dehydration by heating under acidic condition failed to give bis-indole **23**. Therefore, this method of synthesis was unable to form the azepine fusing indole. So, we were attempted to synthesize by changing the starting material to indole.



Scheme 25 Reduction of 3-hydroxy oxindole **21**.

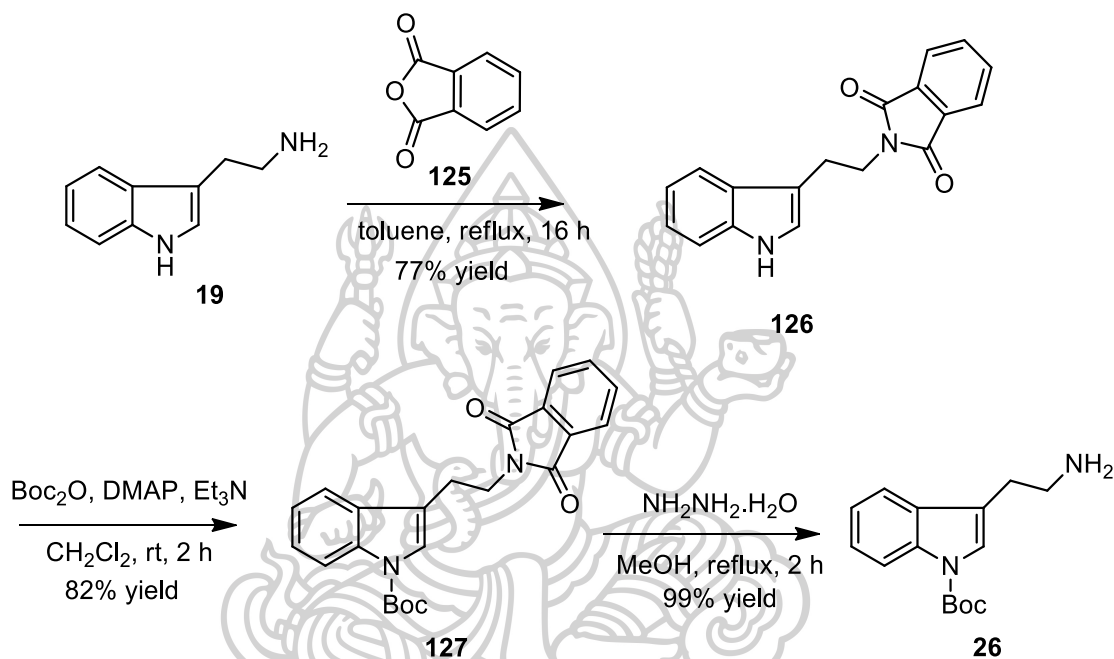
4.1.2 The second synthetic route of iheyamine A derivative

Indole (**1**) was reacted with iodine in the presence of potassium hydroxide followed by protection with the electron withdrawing group as a Boc group to provide *N*-Boc-3-iodoindole (**25**). The ^1H NMR spectrum was shown the singlet of H-2 (indole) at 7.73 ppm.



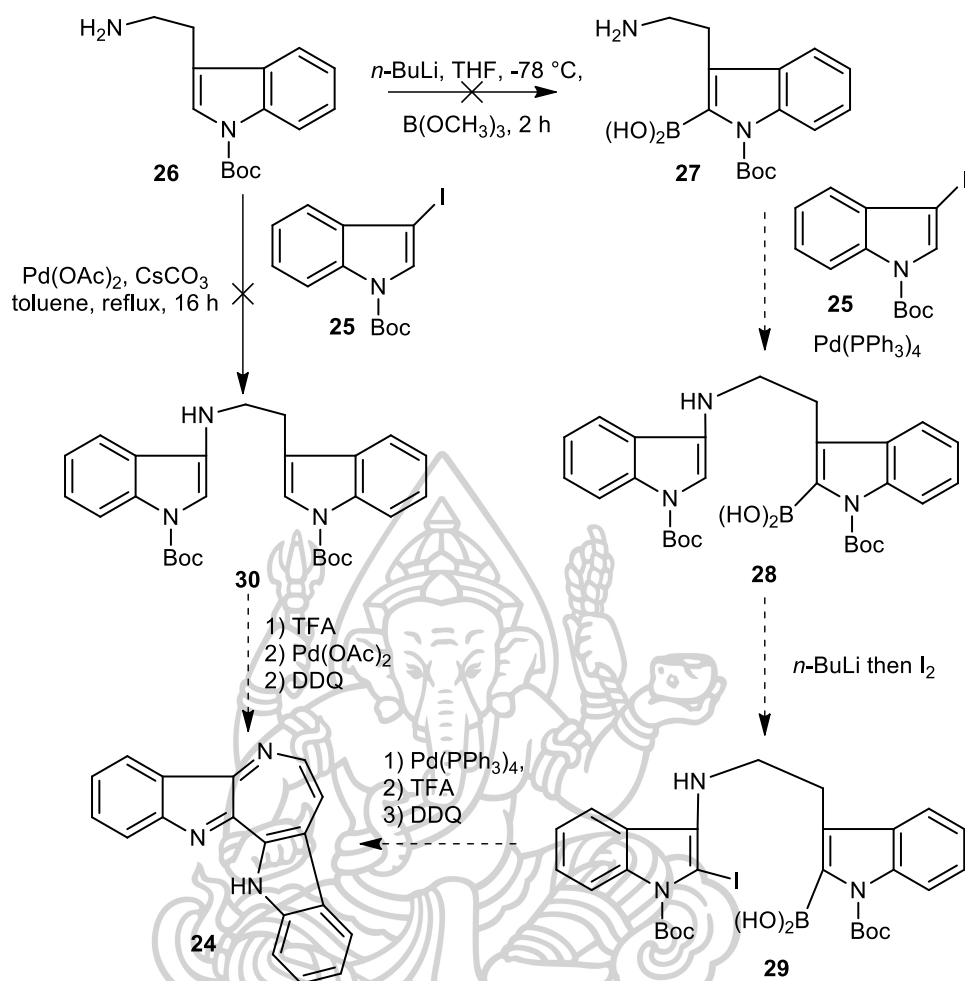
Scheme 26 Iodination of indole (**1**).

Next step, we attempted to synthesize the *N*-Boc tryptamine (26) which was generated from tryptamine (19). The primary amine was protected with phthalic anhydride which was obtained in high yield. And then NH of indole was prevented with Boc group. After that, the removal of primary amine was achieved by hydrazine and ^1H spectrum of CH_2 adjacent to the free amine was shifted to upfield at 2.84 ppm.



Scheme 27 Preparation of *N*-Boc tryptamine (26).

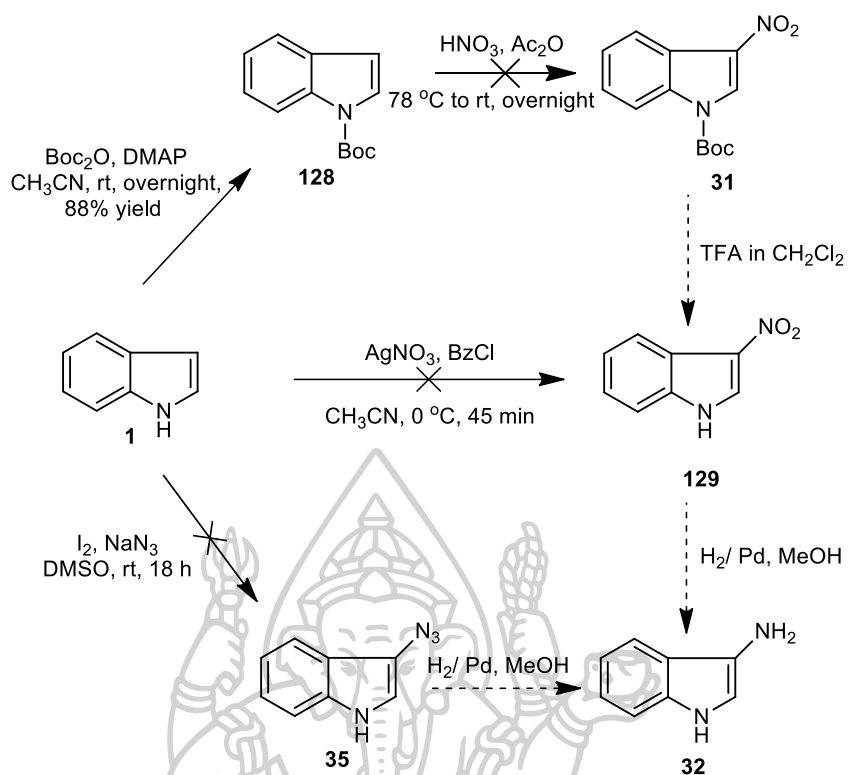
For the synthesis of the C-N bond of bis-indole (28), we attempted to generate through Buchwald-Hartwig cross coupling of *N*-Boc tryptamine boronic acid (27) with *N*-Boc-3-iodoindole (25). The lithiation and substitution of boronate to generate *N*-Boc tryptamine boronic acid (27) from *N*-Boc tryptamine (26) was failed due to the steric effect of the side chain at C-3 of tryptamine. Therefore, we changed a coupling precursor from the *N*-Boc tryptamine boronic acid (27) to *N*-Boc tryptamine (26). *N*-Boc-3-iodoindole (25) was reacted with *N*-Boc tryptamine (26) by Buchwald-Hartwig amination. In this step was not obtained the desired bis-indole (30). Thus, our efforts have resulted in the third synthetic route.



Scheme 28 Preparation of iheyamine A derivative (24).

4.1.3 The third synthetic route of iheyamine A derivative

The final attempted synthesis of iheyamine A derivative was started from 3-aminoindole (32) as a key precursor. Protection of indole (1) with Boc group followed by nitration with HNO_3 and Ac_2O failed to achieve compound 31. Moreover, reaction of indole (1) which was unsuccessful. Moreover, we planned the substitution of indole (1) with iodine and sodium azide was also not given the desired compound. Therefore, these synthetic plans could not be successful to synthesize the iheyamine A derivative.



Scheme 29 Preparation of 3-aminoindole (32) from indole (1).

4.2 Results of the synthesis of geldanamycin derivatives

4.2.1 Extraction, purification and structure elucidation of geldanamycin

The geldanamycin (GDM, 15) was obtained as a yellow solid in 1.15 g from 4.20 g of crude EtOAc extract. The structure of the geldanamycin (15) was compared with previous report (Qin & Panek, 2008) (Table 1) and used as a precursor to the synthesis of geldanamycin derivatives.

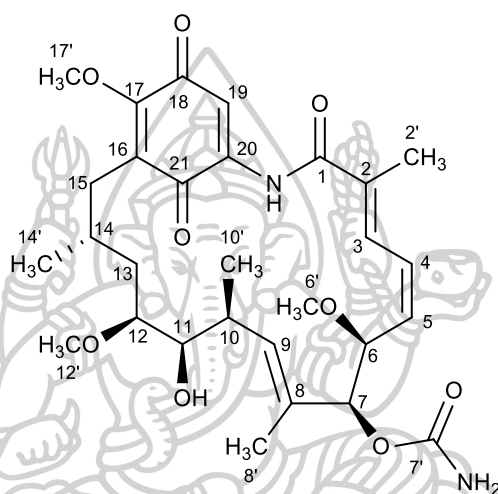


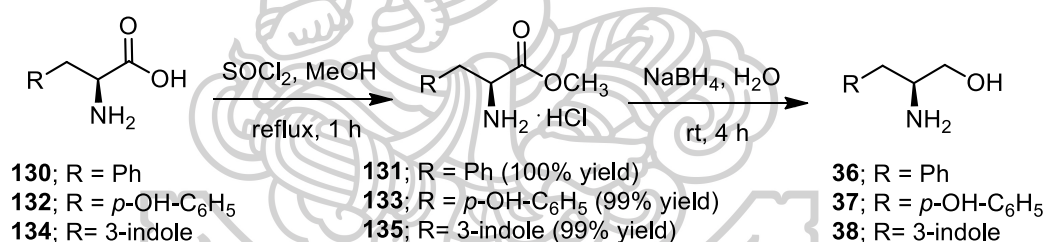
Figure 7 Structural elucidation of geldanamycin.

Table 1 The comparison of ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) of geldanamycin to authentic data of ^1H NMR (400 MHz) and ^{13}C NMR (75 MHz) in CDCl_3 .

No.	position	δ_{C} GDM	δ_{C} 15	δ_{H} GDM	δ_{H} 15
1	CONH	168.2	168.3	8.68 (s, 1H)	8.71 (br, 1H)
2	C	134.8	134.8	-	-
2'	CH_3	12.9	12.9	2.01 (s, 3H)	2.02 (s, 3H)
3	CH	127.2	127.4	6.93 (d, 1H, $J = 11.6$ Hz)	6.94 (d, 1H, $J = 11.1$ Hz)
4	CH	126.3	126.3	6.55 (t, 1H, $J = 11.6$ Hz)	6.56 (t, 1H, $J = 11.6$ Hz)
5	CH	136.4	136.4	5.86 (m, 1H)	5.88 (m, 1H)
6	CH	81.2	81.3	4.30 (d, 1H, $J = 9.2$ Hz)	4.31 (d, 1H, $J = 9.3$ Hz)
6'	OCH_3	57.3	57.3	3.28 (s, 3H)	3.29 (s, 3H)
7	CH	81.7	81.7	5.17 (s, 1H)	5.19 (s, 1H)
7'	OCONH_2	155.9	156.2	4.73 (br, 2H)	4.75 (br, 2H)
8	C	133.3	133.3	-	-
8'	CH_3	12.5	12.5	1.78 (d, 3H, $J = 1.2$ Hz)	1.79 (s, 3H)
9	CH	133.1	133.1	5.81(d, 1H, $J = 9.6$ Hz)	5.81 (d, 1H, $J = 9.9$ Hz)
10	CH	32.1	32.2	2.76 (m, 1H)	2.78 (m, 1H)
10'	CH_3	34.7	12.4	0.95 (overlap, 3H)	0.97 (overlap, 3H)
11	CHOH	72.6	72.7	3.53 (m, 1H)	3.54 (m, 1H)
12	CH	80.9	80.9	3.38 (m, 1H)	3.39 (m, 1H)
12'	OCH_3	56.7	56.7	3.34 (s, 3H)	3.36 (s, 3H)
13	CH_2	34.7	34.6	1.80, 1.74 (m, 2H)	1.77, 1.72 (m, 2H)
14	CH	27.9	27.9	1.65 (m, 1H)	1.69 (m, 1H)
14'	CH_3	27.9	22.9	0.95 (overlap, 3H)	0.97 (overlap, 3H)
15	CH_2	32.8	32.8	2.46 (m, 2H)	2.46 (m, 2H)
16	C	127.5	127.6	-	-
17	C	157.0	157.0	-	-
17'	OCH_3	61.7	61.7	4.11 (s, 3H)	4.12 (s, 3H)
18	CO	184.1	184.2	-	-
19	CH	111.7	111.8	7.27 (s, 1H)	7.28 (s, 1H)
20	C	138.0	138.0	-	-
21	CO	185.0	185.0	-	-

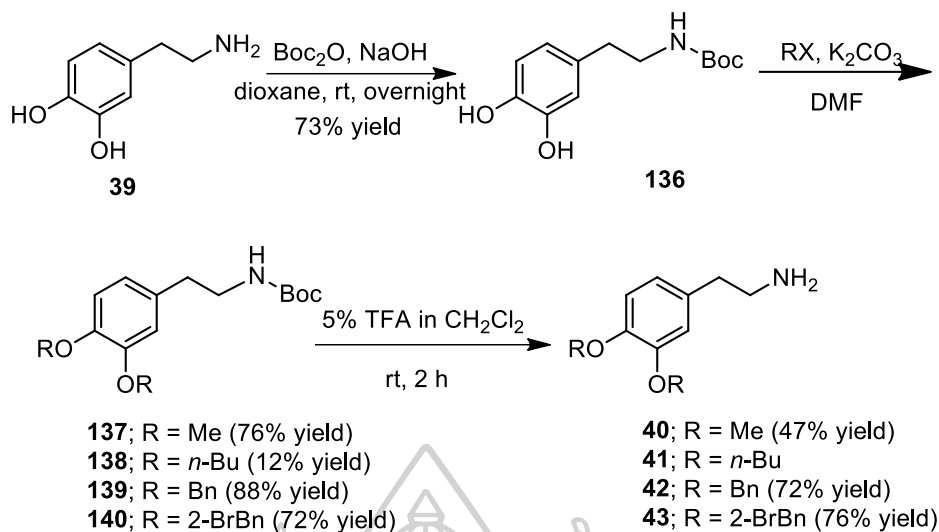
4.2.2 The Synthesis of geldamanycin derivatives

The synthesis of geldamanycin derivatives were divided into two parts. The first part involved the syntheses of amino alcohols and dopamine derivatives, and the second part concerned about the nucleophilic substitution at the C-17 of geldamanycin. In the initial step, the amino alcohols **36-38** were prepared from amino acids **130**, **132**, and **134**. Phenylalanine (**130**), tyrosine (**132**), and tryptophan (**134**) were methylated with thionyl chloride in methanol by esterification to afford in high yield. The ^1H NMR spectra were shown the 3H signal of OCH_3 about 3.6-3.8 ppm. Then, the reduction of amino esters **131**, **133**, and **135** under basic condition gave the crude oil of amino alcohols **36-38**. The ^1H NMR spectra appeared the splitting 2H of CH_2 at 3.4-3.6 ppm instead of methyl ester and the CH_2 at α -C of amino alcohol was shifted to upfield estimate 2.5-2.9 from 3.3-3.6 ppm. The amino alcohol **36-38** as crude products which were used in the next step without further purification.



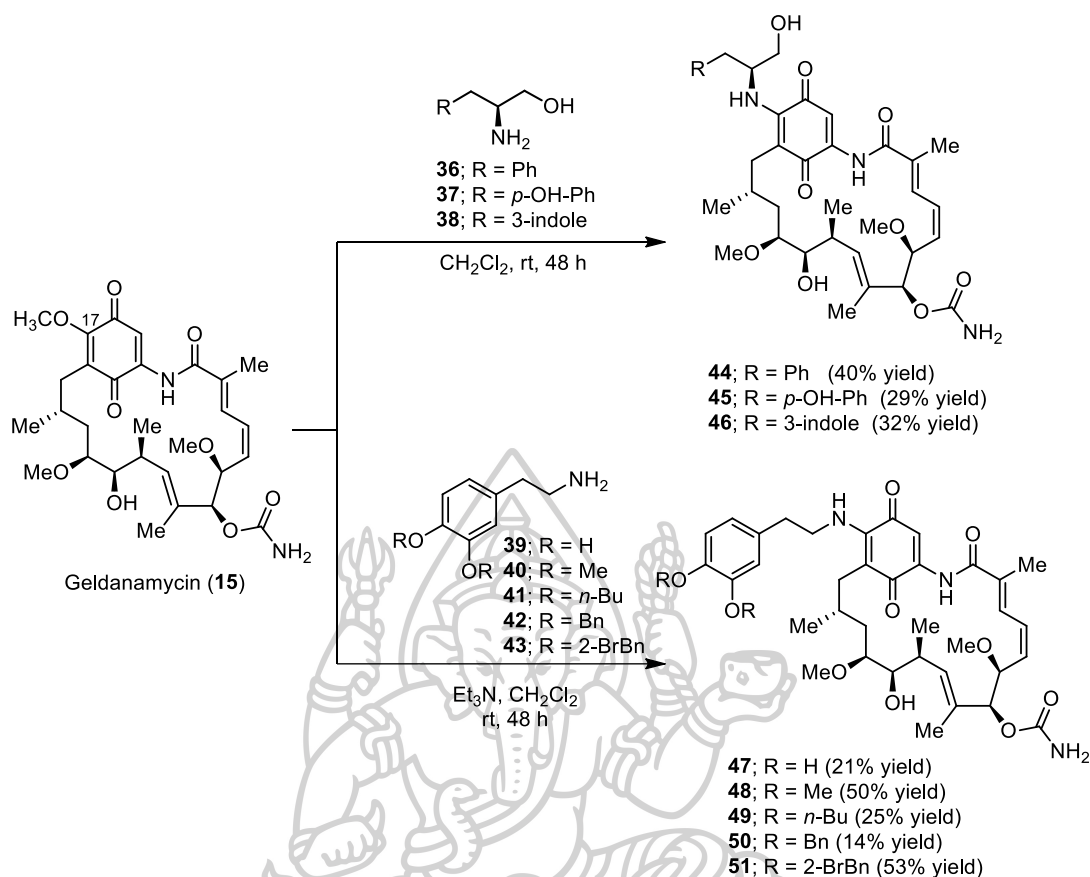
Scheme 30 The syntheses of amino alcohols **36-38**.

In the part of the synthesis of dopamine derivatives, the dopamine hydrochloride (**39**) was NH-protected with Boc group. Then, protection of dihydroxy group of dopamine with the electron donating groups was carried on by methyl, butyl, benzyl, and 2-bromo benzyl groups. The ^1H NMR spectrum was shown the singlet at 3.8 ppm of dimethylation, and 0.95, 1.46, 1.74, 3.98 ppm of the butylation. While the benzylation of **139** and **140** were displayed the chemical shift at 5.1 ppm of OCH_2 of benzyl group. The Boc group were removed with trifluoroacetic acid to give the final products of dopamine derivatives **40-43**.



Scheme 31 The syntheses of dopamine derivatives 40-43.

All of the prepared amino derivatives 36-43 were reacted with geldanamycin (15) by substitution at C-17. Geldanamycin derivatives 44-51 were provided as a purple solid. The chemical shift at 4.12 ppm of OCH₃ at C-17 of geldanamycin was disappeared. The structure of geldanamycin derivatives 44-51 were confirmed by NMR and Mass spectra analysis. Finally, we were successful to synthesized eight geldanamycin derivatives.



Scheme 32 The synthesis of geldanamycin derivatives 44-51.

4.3 Results of the Biological activities

4.3.1 Antibacterial activities

The results of antibacterial activity of the geldanamycin (15) and its derivatives 44-51 were determined against three bacterial strains that was shown in Table 2. For the substitution at C-17 of geldanamycin with amino alcohol 36-38 and dopamine derivatives 39-43, Compound 46 had higher activity to against *B. subtilis* than other derivatives with 128 μ g/mL. Compound 45 was showed activity against *B. subtilis* and *S. aureus* with 512 μ g/mL and displayed more than 512 μ g/mL to active against *B. cereus*. Compound 44, 44-51 exhibited activity against three bacterial strains more than 512 μ g/mL. While geldanamycin (15) presented activity against *B. subtilis* and *B. cereus* with 64 and 128 μ g/mL, respectively. Geldanamycin also displayed activity against *S. aureus* at 512 μ g/mL.

Table 2 The minimum inhibitory concentration (MIC) of antibacterial activity of geldanamycin (15) and its derivatives 44-51

Compound	MIC ($\mu\text{g/mL}$)		
	<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>
44	> 512	> 512	> 512
45	512	> 512	512
46	128	> 512	> 512
47	> 512	> 512	> 512
48	> 512	> 512	> 512
49	> 512	> 512	> 512
50	> 512	> 512	> 512
51	> 512	> 512	> 512
Geldanamycin (15)	64	128	512
Chloramphenicol	1	2	2

4.3.2 Cytotoxicity activity

Geldanamycin (15), and its derivatives 44-51 were studied *in vitro* for their cytotoxic activities by MTT assay on three cancer cell lines (HeLa, HepG2, MDA) and two normal cell lines (Vero, LLC-MK2). All of IC_{50} were shown in Table 3. From all of the tested compounds, the geldanamycin derivatives 44-49 were more active against Hela cell lines than geldanamycin (15) and its derivatives 50 and 51. In addition, compounds 44-47, 49, and 51 were moderately active against MDA cancer cell lines. Geldanamycin (15) and its derivatives 45-48, 50-51 have more toxic against HepG2 and LLC-MK2 than compound 44. Geldanamycin (15) and its derivative 44 were active cytotoxic against LLC-MK2. Similarly, the derivatives 44, 45, 47, and 48 were cytotoxic against Vero cell.

Table 3 The cytotoxicity of test compounds against cancer cell lines (HeLa, HepG2 and MDA) and normal cell (Vero and LLC-MK2)

Compounds	IC ₅₀ (μg/mL)				
	Hela	HepG2	MDA	LLC-MK2	Vero
44	22.02	24.62	95.87	97.30	73.49
45	45.66	616.77	96.54	339.98	94.21
46	19.36	250.81	112.96	330.58	225.46
47	62.17	238.64	41.88	341.19	104.52
48	45.05	932.88	263.07	432.42	181.00
49	53.44	175.66	70.93	408.50	369.44
50	150.29	637.76	271.84	648.70	376.70
51	172.03	649.40	52.12	860.11	364.31
Geldanamycin (15)	111.59	677.49	68.98	45.61	263.42
crude extracts	15.73	20.61	ND ^a	19.46	12.63
Doxorubicin	1.95	92.16	3.16 ^b	98.92	99.48

^a not determined.

^b IC₅₀ values of cytotoxicity against MDA-231 of Doxorubicin. (Wen, Su, Liou, Lin, & Lee, 2018)

4.4 Results of the Molecular docking

4.4.1 Binding energy

The molecular docking results were compared with two different binding sites of 1YET and 1OSF that were shown in Table 4. The binding energy into 1OSF of these compounds have lower than 1YET which was excepted compound **46**. The synthesized compounds **44-51** had the energy -97.03 to -122.41 kcal/mol which were higher than Geldanamycin (**15**) and DMAG (**17**). The core structure of amino alcohol substituent **44-46** well bound at the active site of both proteins that have similar to the geldanamycin and DMAG (Figure 10). In addition, the dopamine derivatives substituent **47-49** were partially bound to the active site. While the dopamine derivatives substituent **50-51** containing the aromatic on the dopamine were outside bound to the active site (Figure 11). The

orientation of each compound was compared binding to the active site with the co-crystallized which were shown in an appendix. However, all synthesized compounds 44-51 were rather different from the original geldanamycin (15) and DMAG (17).

Table 4 The comparison of the binding energy in the binding site of Hsp90 (1YET and 1OSF)

Compound	binding energy (kcal/mol)	
	1YET	1OSF
Geldanamycin (15)	-133.06	-141.296
DMAG (17)	-136.55	-145.307
44	-116.34	-122.41
45	-113.65	-115.29
46	-112.00	-98.33
47	-101.06	-101.67
48	-98.54	-102.36
49	-97.03	-100.50
50	-108.12	-114.28
51	-105.20	-106.63

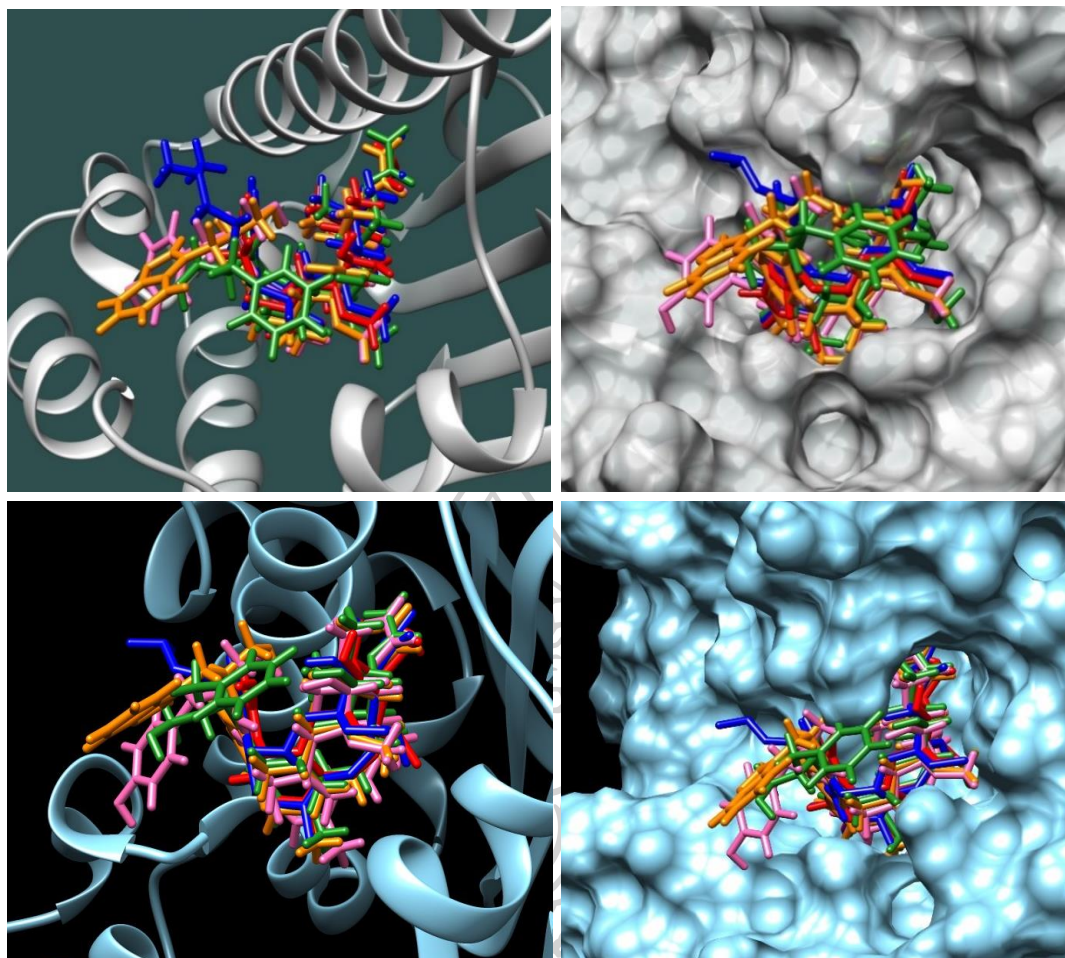


Figure 8 Comparison of the binding of 44 (green), 45 (pink), 46 (orange), redocked geldanamycin (15) (red) and DMAG (17) (blue) in the cavity of Hsp90 (1YET (up) and 1OSF (down)).

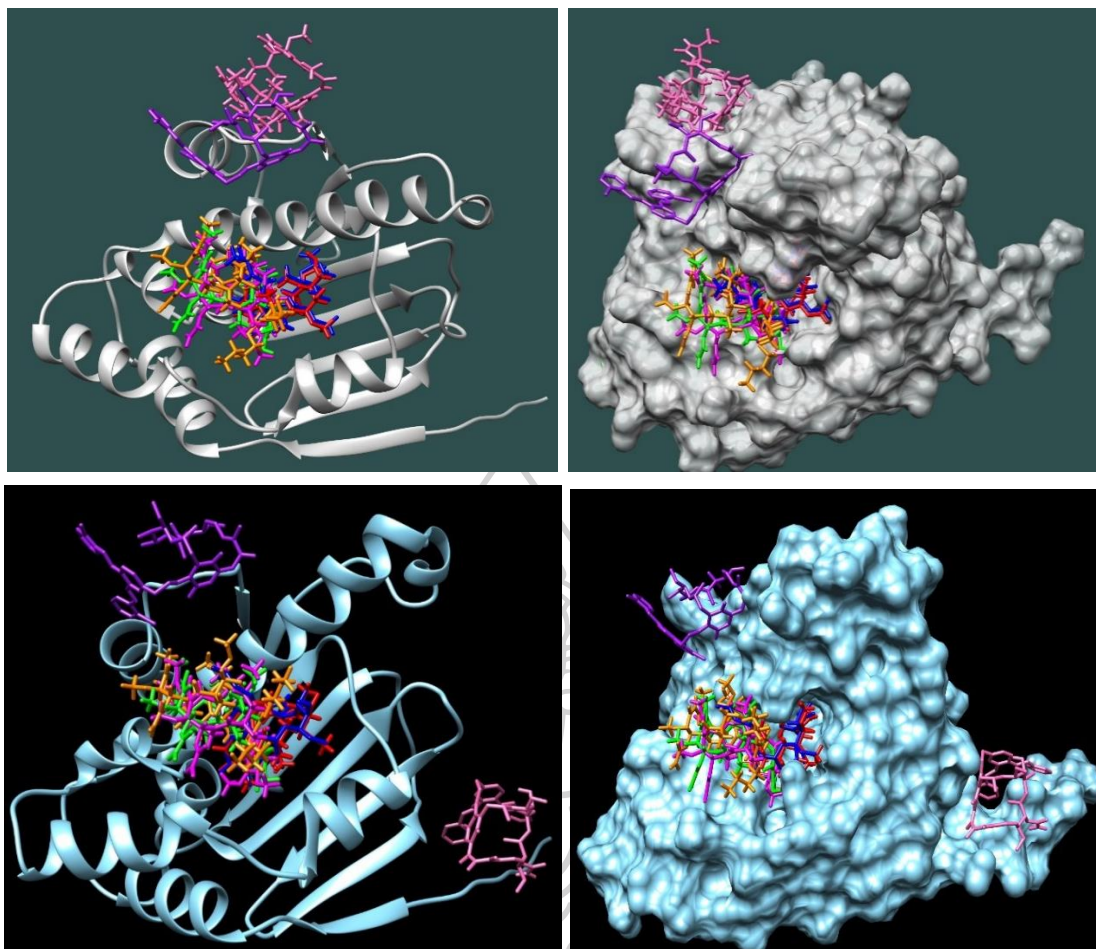


Figure 9 Comparison of the binding of 47 (green), 48 (magenta), 49 (orange), 50 (pink), 51 (purple), redocked geldanamycin (15) (red) and DMAG (17) (blue) in the cavity of Hsp90 (1YET (up) and 1OSF (down)).

4.4.2 Interaction of amino acid residues

The hydrogen bonds showed the interaction between amino acid residues and ligand. These compounds had hydrogen bonding various interactions with Hsp90 (1YET and 1OSF) which were shown the bond length in Table 5 and 6 for 1YET and 1OSF, respectively. Compound 50 and 51 had the aromatic ring substituent of benzyl and 2-bromobenzyl group, which were interacted the hydrogen bonding at the N-terminal amino acid residue. In contrast, geldanamycin (15), DMAG (17), and the derivatives 44-49 had hydrogen bonding interaction at the C-terminal amino acid residue. The hydrogen bond of amino alcohol substituents 44-46 had LYS112, VAL136, PHE 138, and THR184 similar

to geldanamycin (15) and DMAG (17) for 1YET, and ASN106, LYS112, VAL136, PHE 138 for 1OSF. In addition, ASP93 for 1YET and ASP93, ASP102, THR184 for 1OSF shown the interaction of hydrogen bonding similar to the DMAG (17), respectively. In contrast, the substituted dopamines 47-51 had the different alkyl group which compounds 47-49 were bound to the active site with the opposite core structure of geldanamycin. Thus, these compounds had the different amino residues from the geldanamycin (15).



Table 5 Summary of amino acid interactions and H-Bond length of GDM (15), DMAG (17) and geldanamycin derivatives 51-58 in binding site of Hsp90 (1YET)

Compound	Amino acid residue	Hydrogen bond length (Å)
GDM (15)	LYS58, LYS112 (2 bonds), VAL136, PHE138, THR184	2.21, 2.88 and 2.93, 2.70, 1.71, 2.88
DMAG (17)	ASP54 , ASP93, LYS112, VAL136, PHE138, THR184	2.79, 1.71, 3.04, 2.78, 1.67, 2.74
44	ASP93, ASP102 (2 bonds), LYS112 (2 bonds), VAL136, PHE138, THR184	1.51, 2.69 and 2.99, 2.81 and 3.01, 2.90, 1.68, 2.43
45	ASP54, ASP93, LYS112 (2 bonds), TYR139, THR184	2.65, 1.99, 2.40 and 2.49, 2.91, 3.04
46	ASP54, ASP93, ASP102, LYS112 (2 bonds), VAL136, PHE138	2.61, 2.10, 3.03, 2.25 and 2.49, 2.48, 1.95
47	GLU47, SER50, LYS58, ASN106, GLY132 (2 bonds), GLY135, GLY137, PHE138	2.04, 2.69, 3.02, 1.95, 2.47 and 2.64, 2.40, 2.32, 2.44
48	GLU47, LYS58, ASN106 (2 bonds), LYS112, GLY132, GLN133 (2 bonds), PHE134, GLY135, GLY137, PHE138	2.66, 2.93, 2.39 and 2.52, 2.90, 2.83, 2.09 and 2.29, 2.17, 1.75, 2.64, 2.47
49	GLU47, SER50, ASP54, ASN106, LYS112, GLY132, GLN133, GLY135, GLY137	1.90, 2.23, 2.99, 2.70, 2.36, 2.47, 2.21, 2.89, 2.23
50	GLN212 (2 bonds), ILE214, ILE218 (2 bonds)	1.94 and 2.73, 2.86, 2.12 and 3.04
51	ARG60 (2 bonds), GLN212, PHE213, TYR216	2.36 and 2.52, 3.11, 3.13, 3.61

Table 6 Summary of amino acid interactions and H-Bond length of GDM (15), DMAG (17) and geldanamycin derivatives **44-51** in binding site of Hsp90 (1OSF)

Compound	Amino acid residue	Hydrogen bond length (Å)
GDM (15)	LYS58, ASN106, LYS112 (2 bonds), VAL136, PHE138	2.46, 2.19, 2.58 and 2.74, 2.55, 1.59
DMAG (17)	ASP93, ASP102, ASN106, LYS112 (2 bonds), VAL136, PHE138, THR184	1.80, 2.52, 2.08, 2.68 and 2.69, 2.56, 1.77, 2.93
44	ASP93, ASP102 (2 bonds), ASN106, LYS112 (2 bonds), VAL136, PHE138, THR184	1.65, 2.99 and 3.01, 1.79, 2.47 and 2.86, 2.48, 1.84, 2.74
45	ASP54, ASP93, ASP102 (2 bonds), ASN106, LYS112 (2 bonds), PHE134, VAL136, TYR139, THR184	2.69, 1.99, 2.80 and 2.89, 1.58, 2.45 and 2.60, 2.60, 2.94, 2.71, 3.07
46	ASP93, ASP102 (2 bonds), ASN106, LYS112 (2 bonds), VAL136, PHE138, THR184	1.65, 2.99 and 3.01, 1.79, 2.48 and 2.86, 2.48, 1.84, 2.74
47	GLU47, SER50, ASP54, ASN106, GLN133, GLY135, GLY137, PHE138	2.61, 2.68, 2.93, 2.95, 2.04, 2.62, 2.05, 2.65
48	LYS58 (2 bonds), ASN106, LYS112, GLN133, PHE134, GLY135 (2 bonds), GLY137	2.02 and 2.39, 2.29, 2.90, 1.93, 2.35, 2.19 and 3.08, 2.46
49	GLU47, SER50, ASP54, LYS112, GLY132, GLN133, GLY135, GLY137	2.06, 1.60, 3.07, 2.41, 2.52, 1.85, 2.69, 2.10
50	MET12, GLU14 (2 bonds)	2.23, 2.18 and 2.29
51	ARG60 (2 bonds), GLN212, PHE213, GLY215	2.26 and 1.98, 3.37, 2.85, 3.40

CHAPTER 5

CONCLUSIONS

In conclusion of this studied, we attempted to synthesis iheyamine A derivative with several plans. The first route was started from *N*-Boc isatin (**123**) which was used as substrate to react through condensation with tryptamine (**19**), deprotection of *N*-Boc, and reduction. The oxindole (**21**) was reduced with NaBH₄ in MeOH to give the undesired indolinol (**124**) which was not hydrolyzed to generate bis-indole (**23**). The construction of bis-indole was failed in this step. Thus, we altered the starting material as indole (**1**). The second route was synthesized through the palladium cross-coupling *via* Buchwald-Hartwig and Suzuki reaction. Unfortunately, the synthesis of tryptamine boronic acid (**27**) was not successful. And the coupling of *N*-Boc-3-iodoindole (**25**) with *N*-Boc tryptamine (**26**) was failed too. Moreover, we tried to synthesize by the nitration of indole (**1**) and followed by amide formation, reduction, cyclization and oxidation. Despite, the nitration with the difference method was still unable to provide the 3-nitroindole (**129**). So, we were not successful to synthesize the iheyamine A derivative.

The part of the synthesis of geldanamycin derivatives **44-51** that consisted of the different side chain substituents at C-17. In the initial step, the spores of *Streptomyces zerumbet* W14 were restreaked on ISP-2 agar medium and treated for 14 days. Then, the cut culture medium into small were extracted with EtOAc and purified by column chromatography to provide yellow solid in 27% yield from crude EtOAc extract. The yellow solid was determined and elucidated by NMR spectroscopy which was indicated as geldanamycin (**15**). The geldanamycin (**15**) was substituted with two groups of amine including amino alcohol **36-38** and dopamine (**39**) and its derivatives **40-43**. The phenylalaninol (**36**), tyrosinol (**37**), and tryptophanol (**38**) were synthesized from phenylalanine (**130**), tyrosine (**132**) and tryptophan (**134**), respectively. These amino acids were converted to methyl ester followed by reduction to provide amino alcohol **36-38** in good yield. And the synthesis part of dopamine derivatives **40-43** were synthesized through *N*-Boc protection of free-NH₂ followed by *O*-alkylation with methyl, buthyl, benzyl, and 2-bromo benzyl groups. The Boc protecting group was removed to provide dopamine

derivatives **40-43**. Then, geldanamycin (**15**) were substituted at C-17 with amines **39-43** by demethylation. We achieved to synthesize geldanamycin derivative **44-51** which was purple solid. These derived were evaluated the biological activity and molecular docking. We found that the synthesized compound **44-45** and **47-51** were active against *B. subtilis*, *B. cereus*, and *S. aureus* with 512 $\mu\text{g/mL}$ or more than. Moreover, the derivative **46** was active against *B. subtilis* with 128 $\mu\text{g/mL}$ and more than 512 $\mu\text{g/mL}$ in *B. cereus* and *S. aureus*. The determination cytotoxicity of geldanamycin derivatives **44-51** were assessed, compounds **44-49** were more potentially active than geldanamycin with range 19-62 $\mu\text{g/mL}$ and compounds **50-51** were moderately active with 150.29-172.03 $\mu\text{g/mL}$ against Hela cancer cell lines. Compound **44** was high activity in 24.62 $\mu\text{g/mL}$ and compound **49** showed moderate cytotoxicity against HepG2 cancer cell lines. In addition, compound **47**, **49** and **51** were showed high activity against MDA cancer cell lines with 41.88, 70.93 and 52.12 $\mu\text{g/mL}$, respectively. While compound **46** and **50** were showed moderate potential against MDA cancer cell lines. According to the results of this study showed that the compounds were effective three cancer cell lines with low cytotoxicity against normal cell lines. Although these compound had the binding energy and hydrogen bonding different geldanamycin (**15**). However, these results could be gainful for drug development in the future.

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APPENDIXES

APPENDIX A NMR spectrum

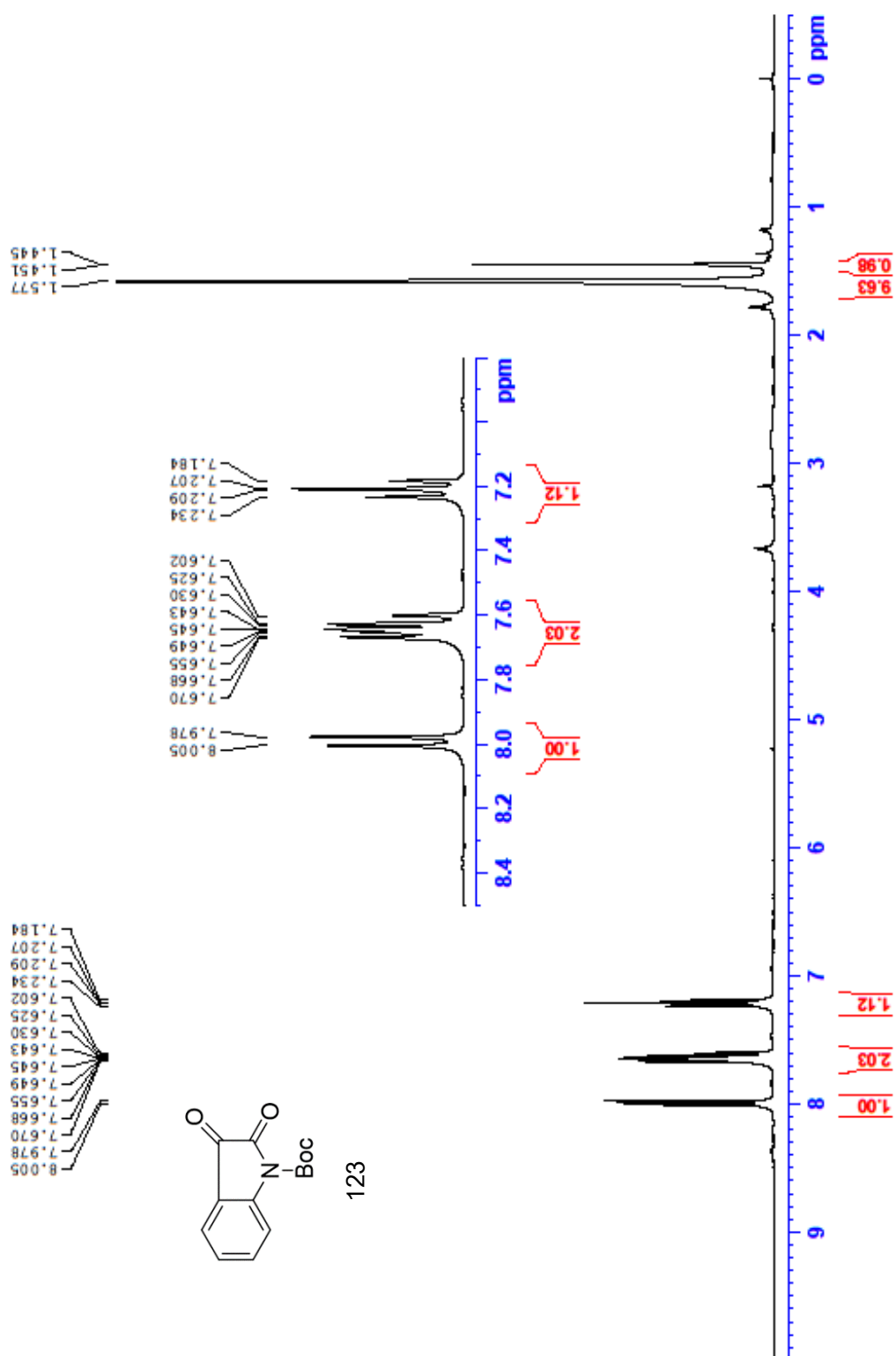
APPENDIX B MTT assay of cytotoxicity activities

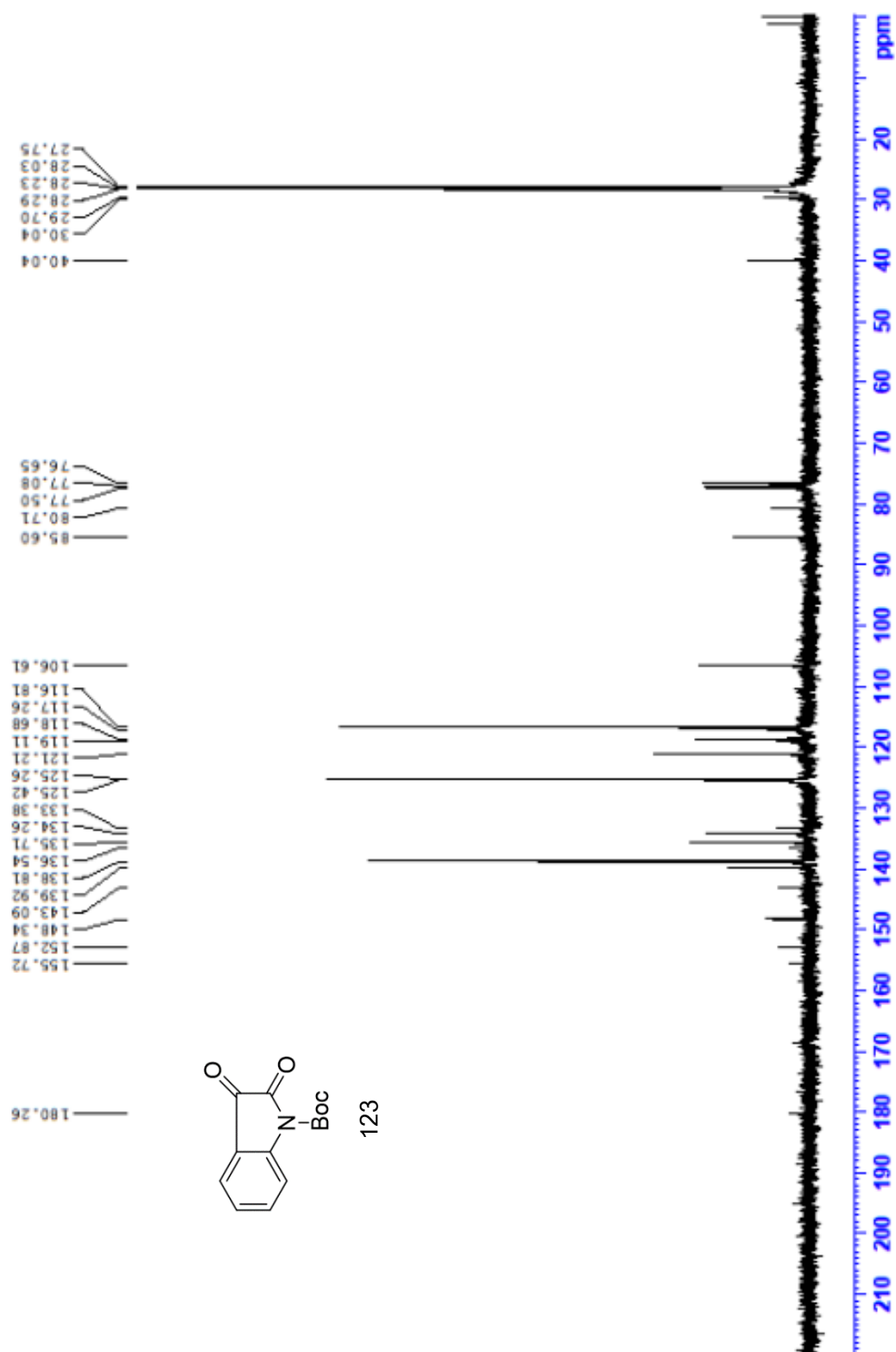
APPENDIX C Binding site of Hsp90 (1YET and 1OSF)

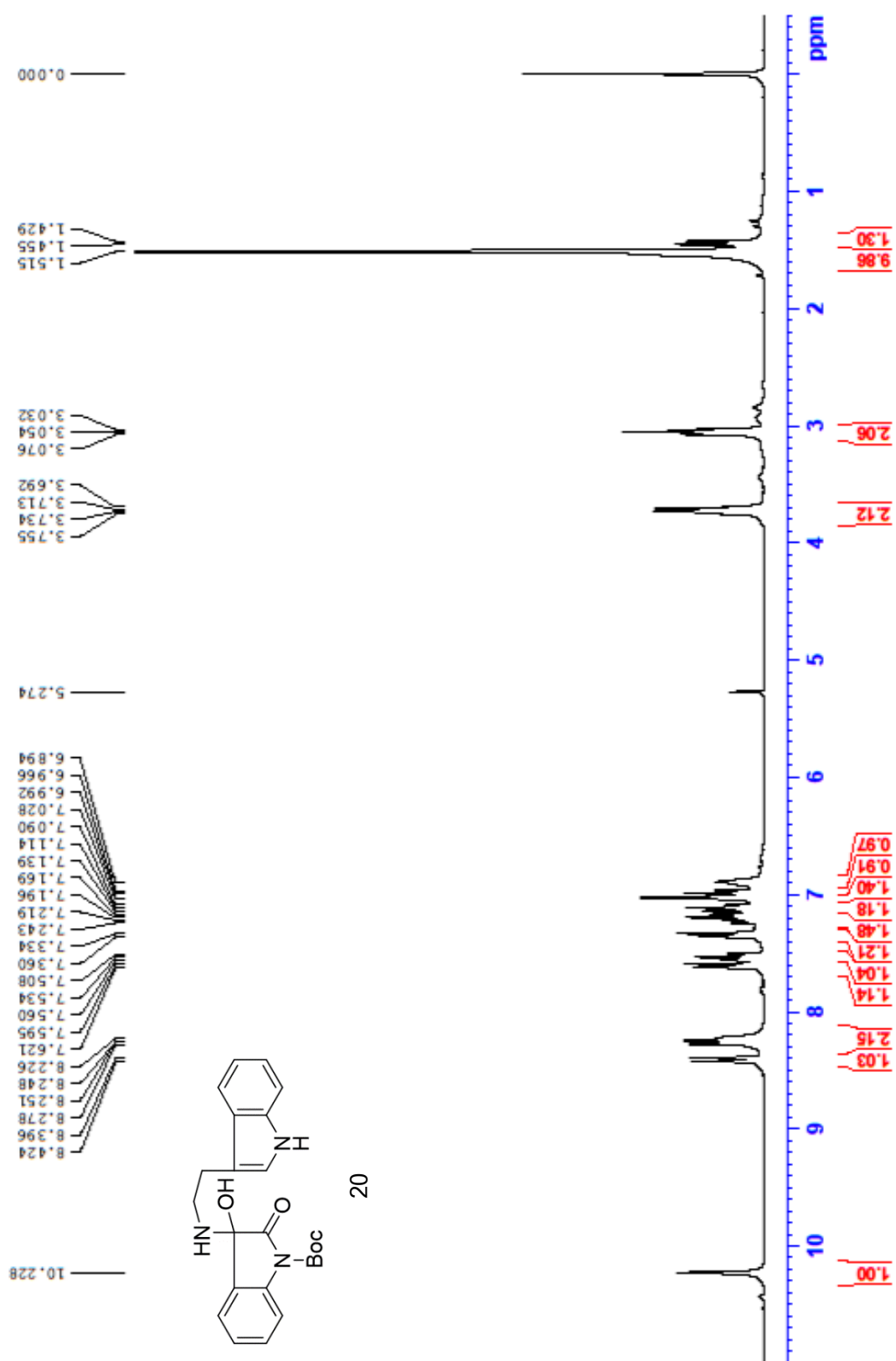
APPENDIX D Amino acid interactions and H-Bond at binding site of Hsp90 (1YET and 1OSF)



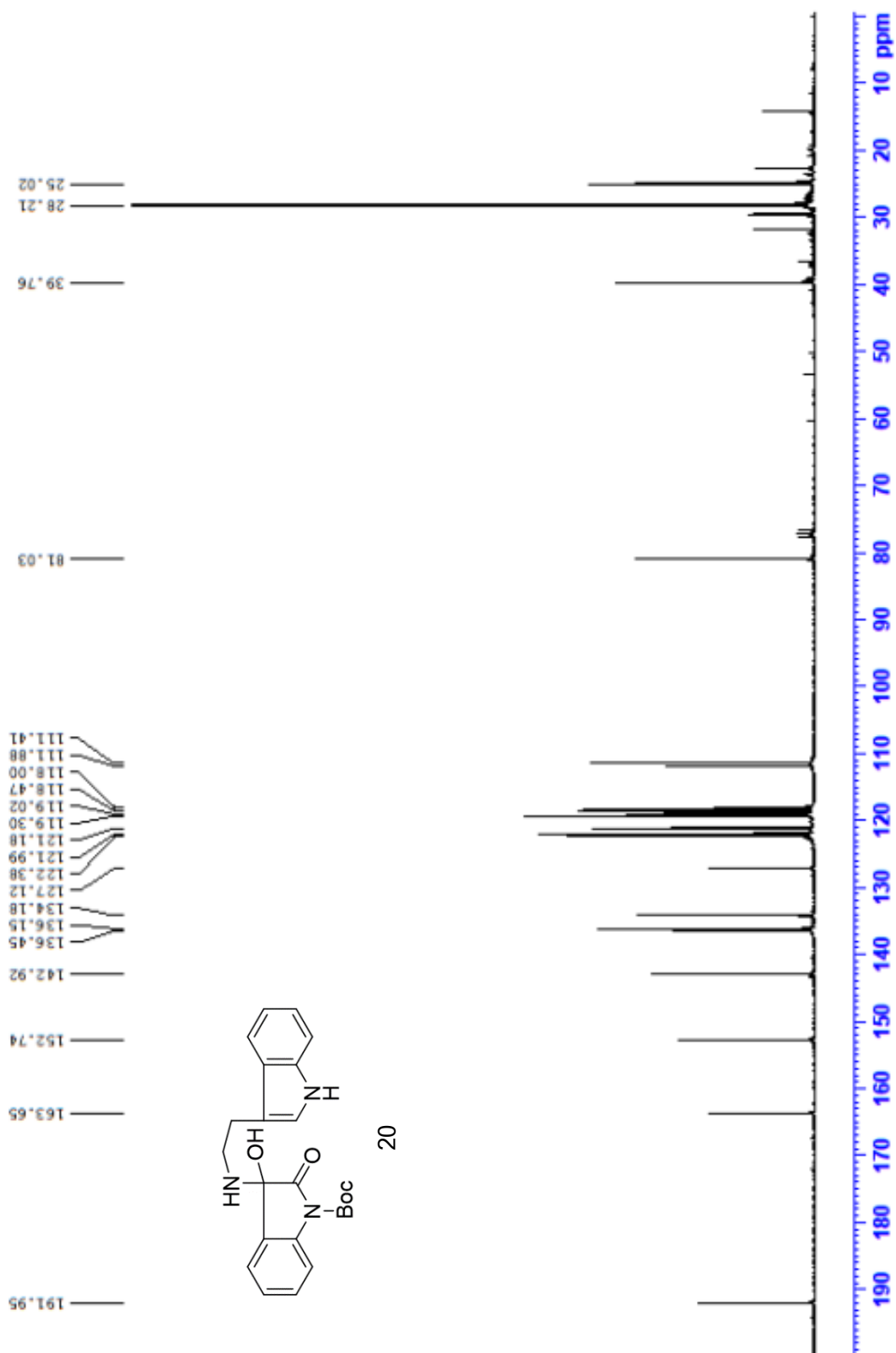
APPENDIX A
NMR spectrum

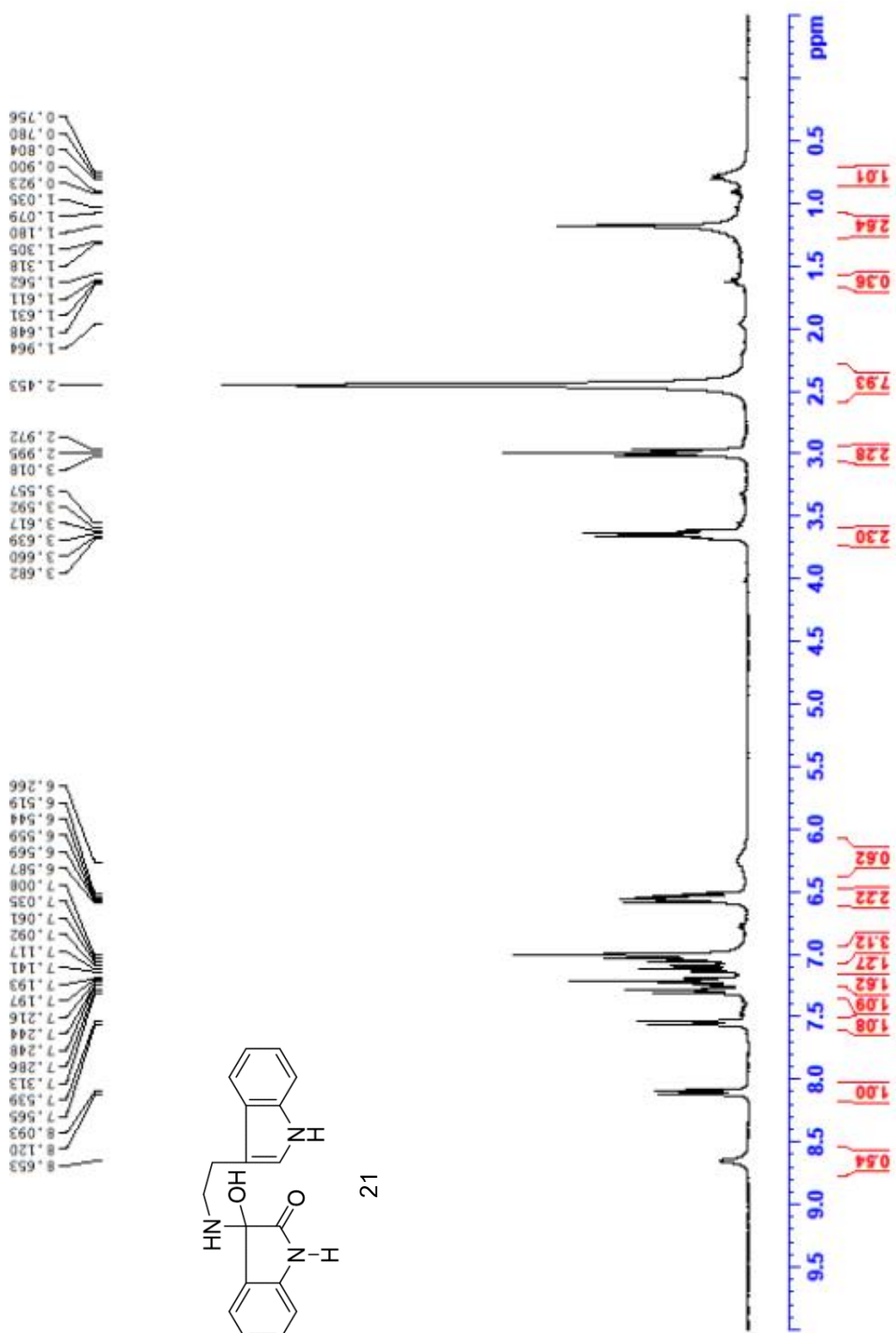




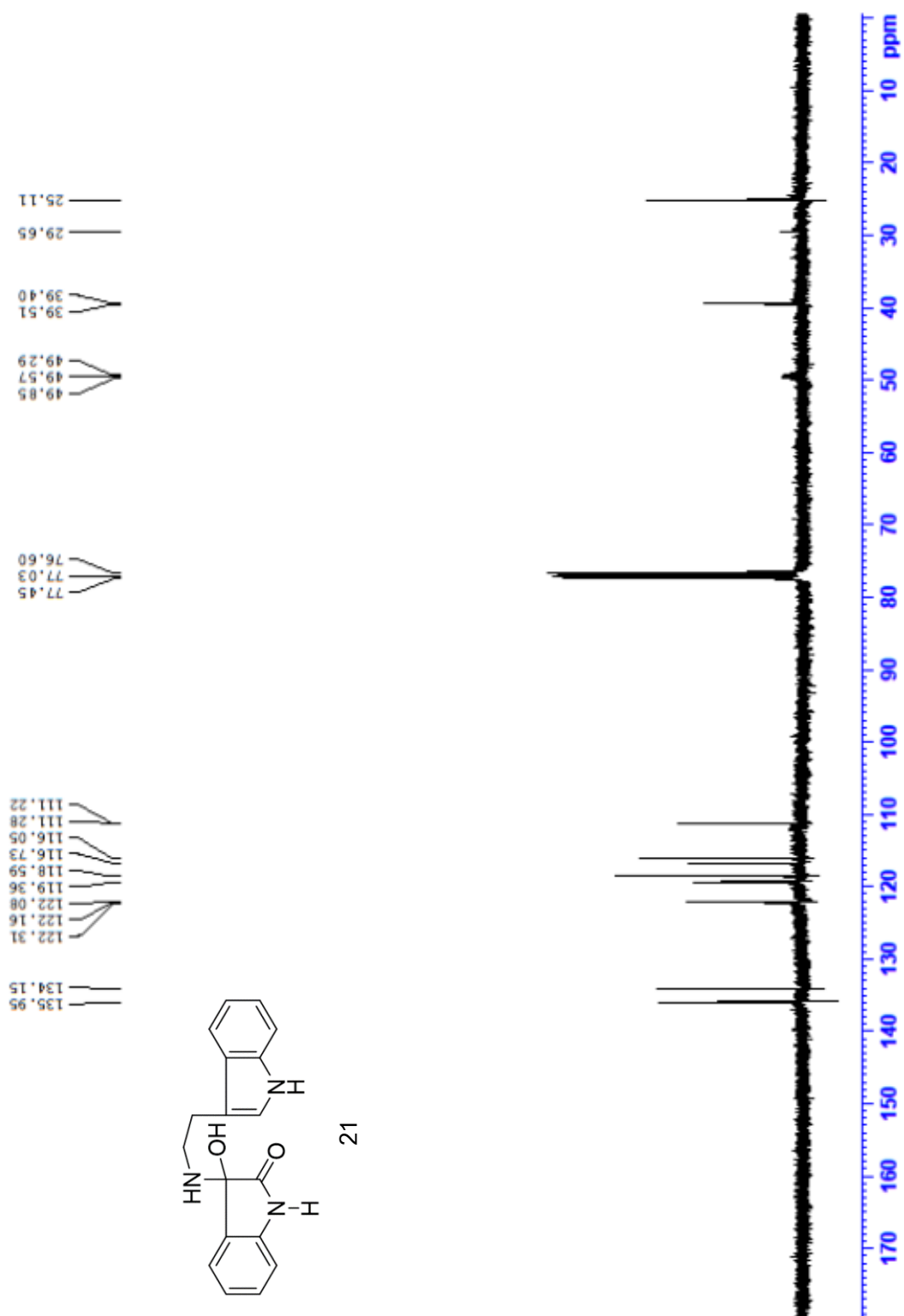


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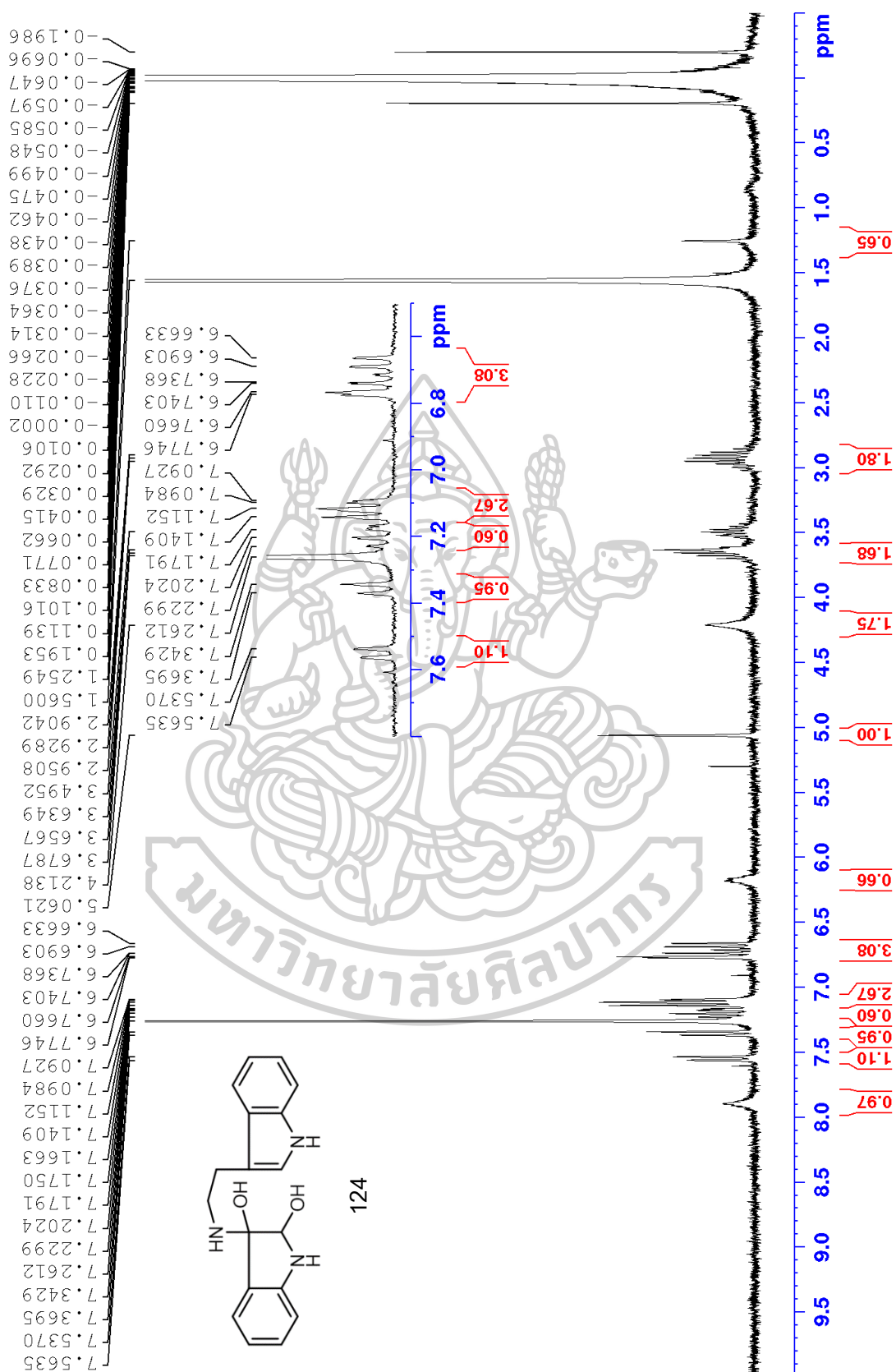


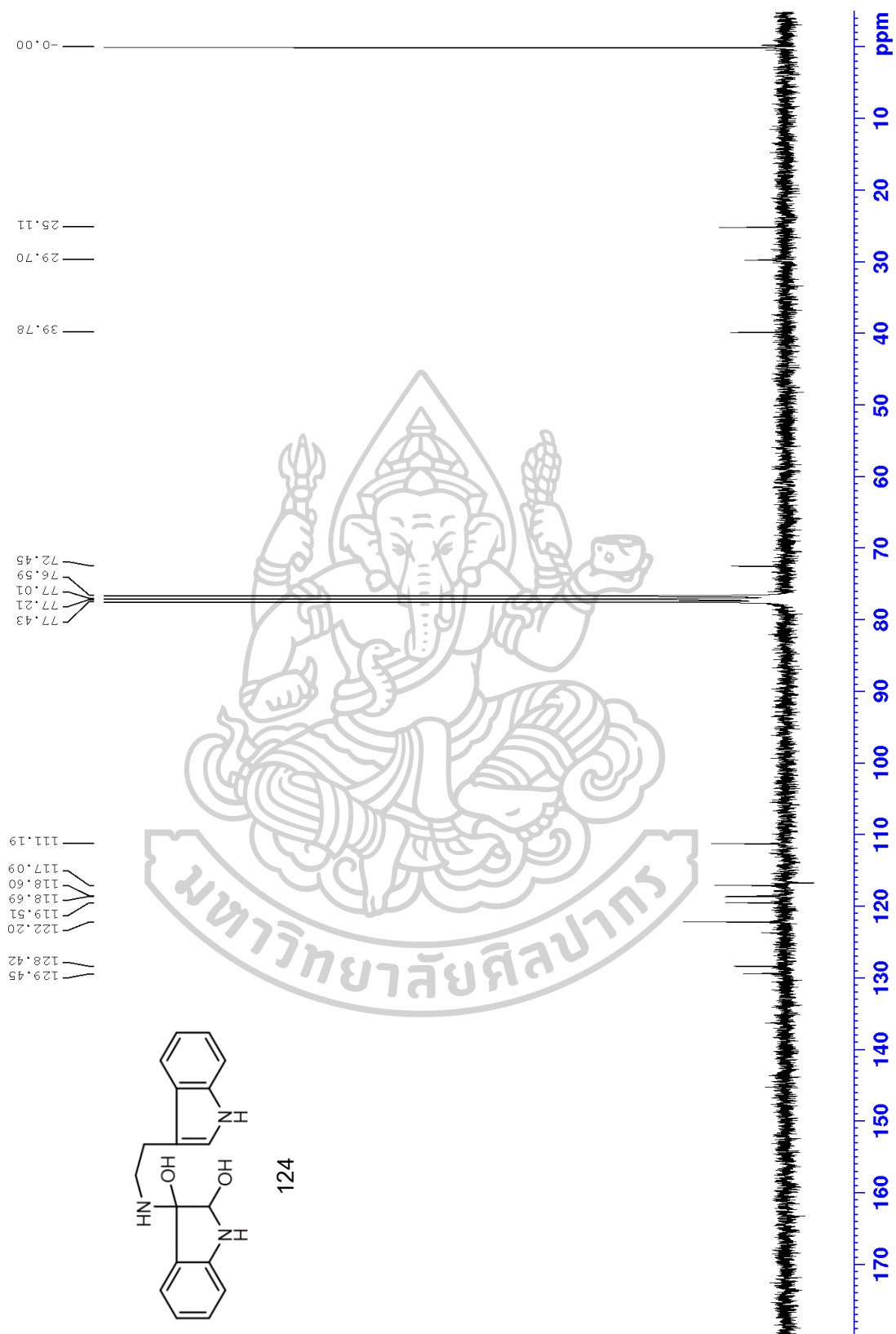


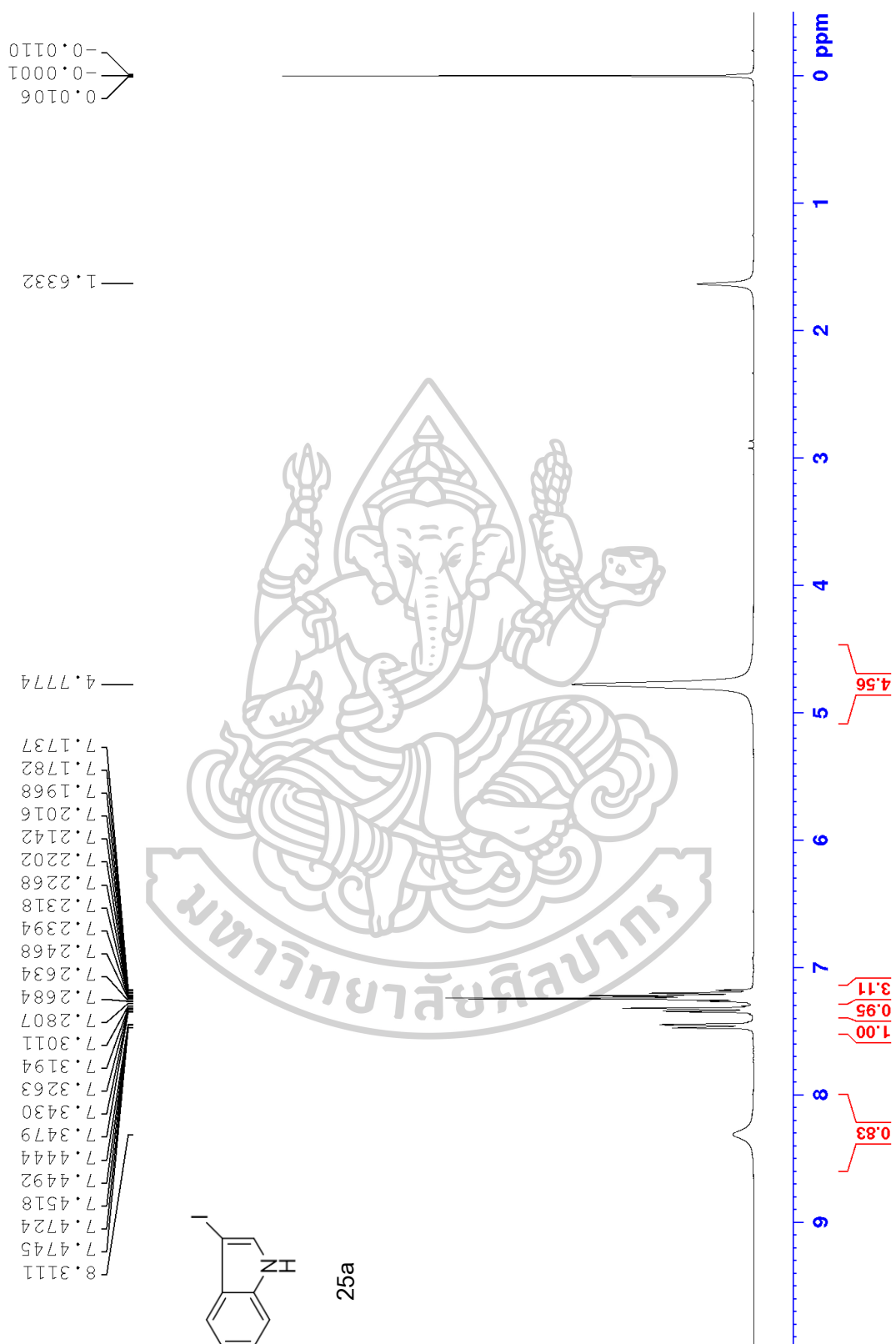
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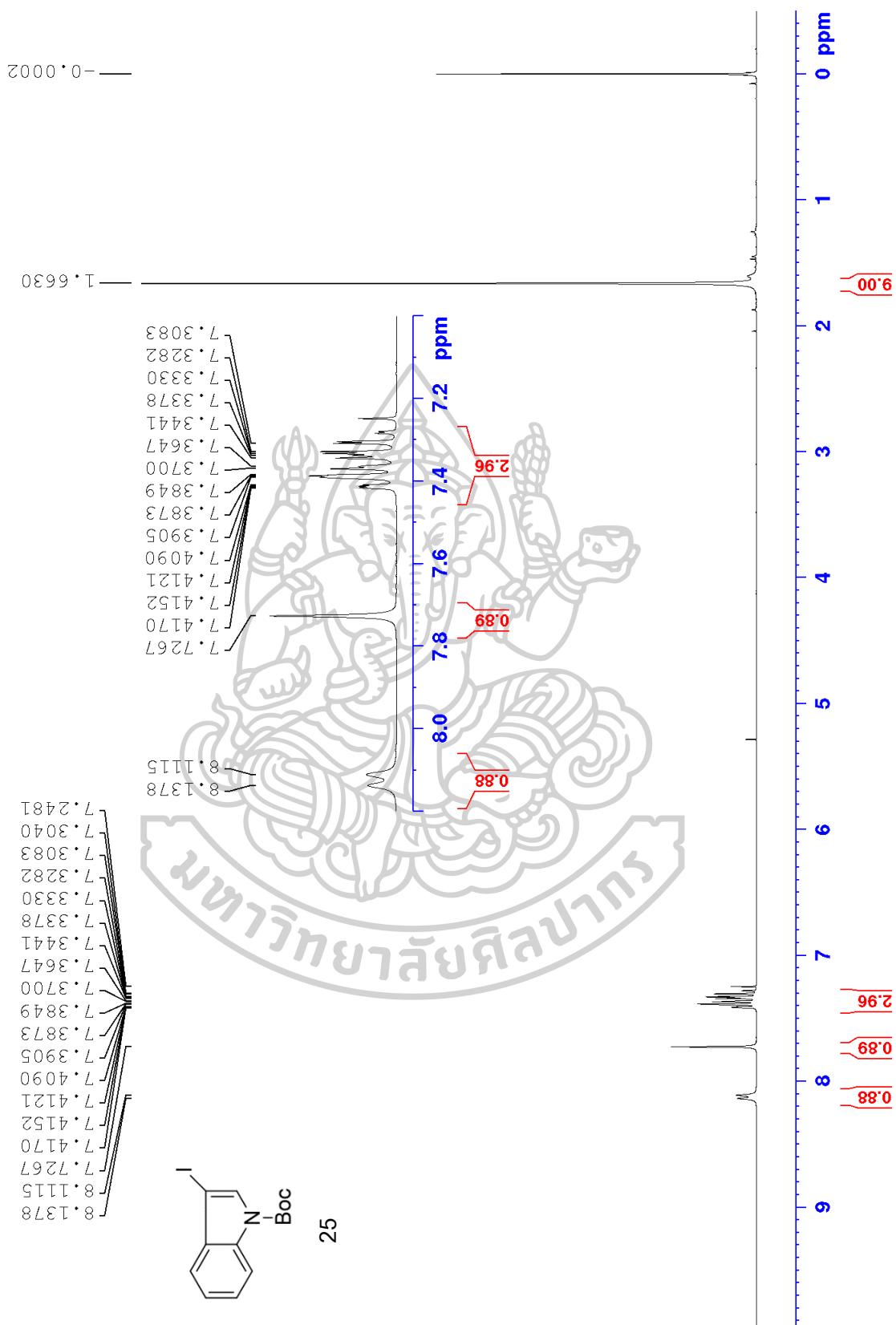


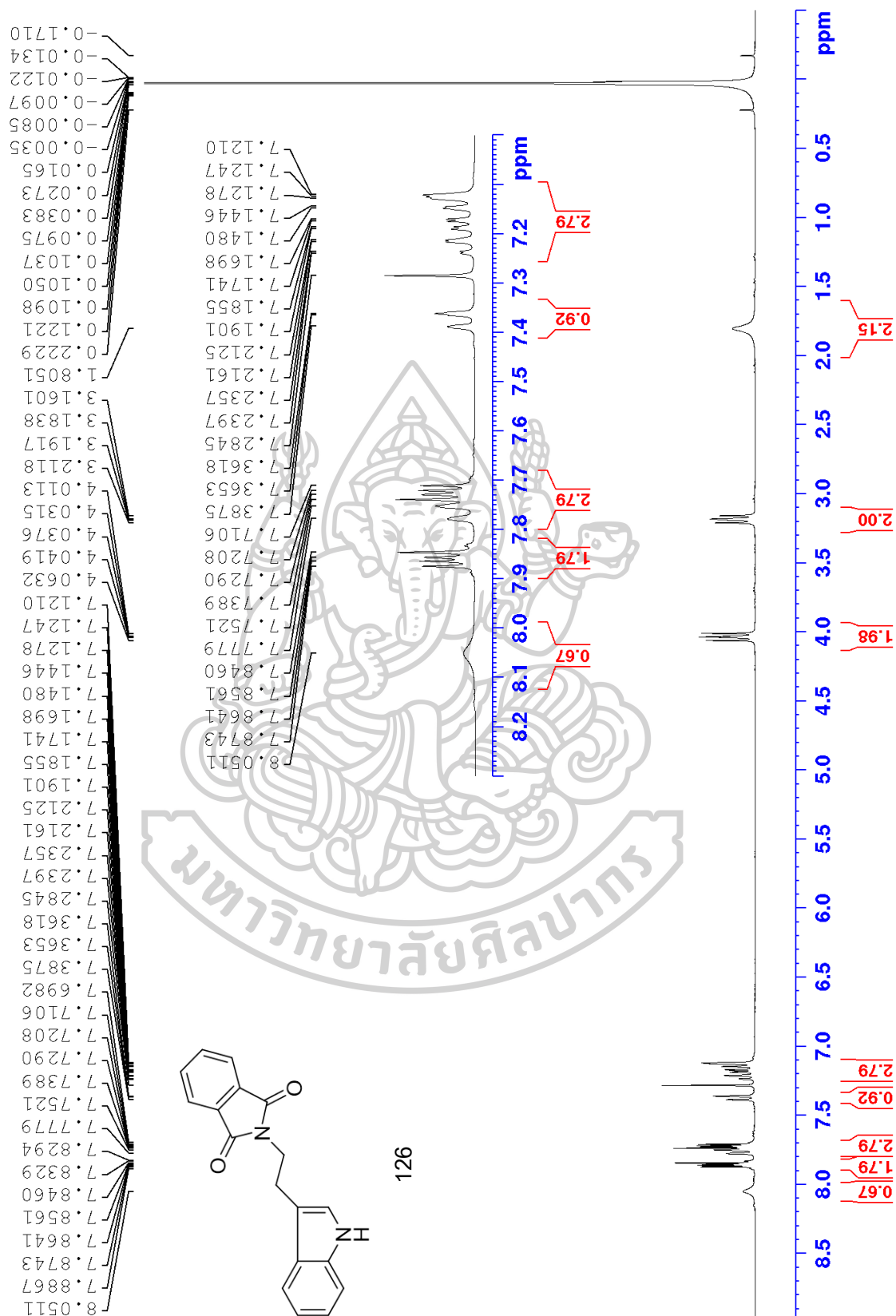
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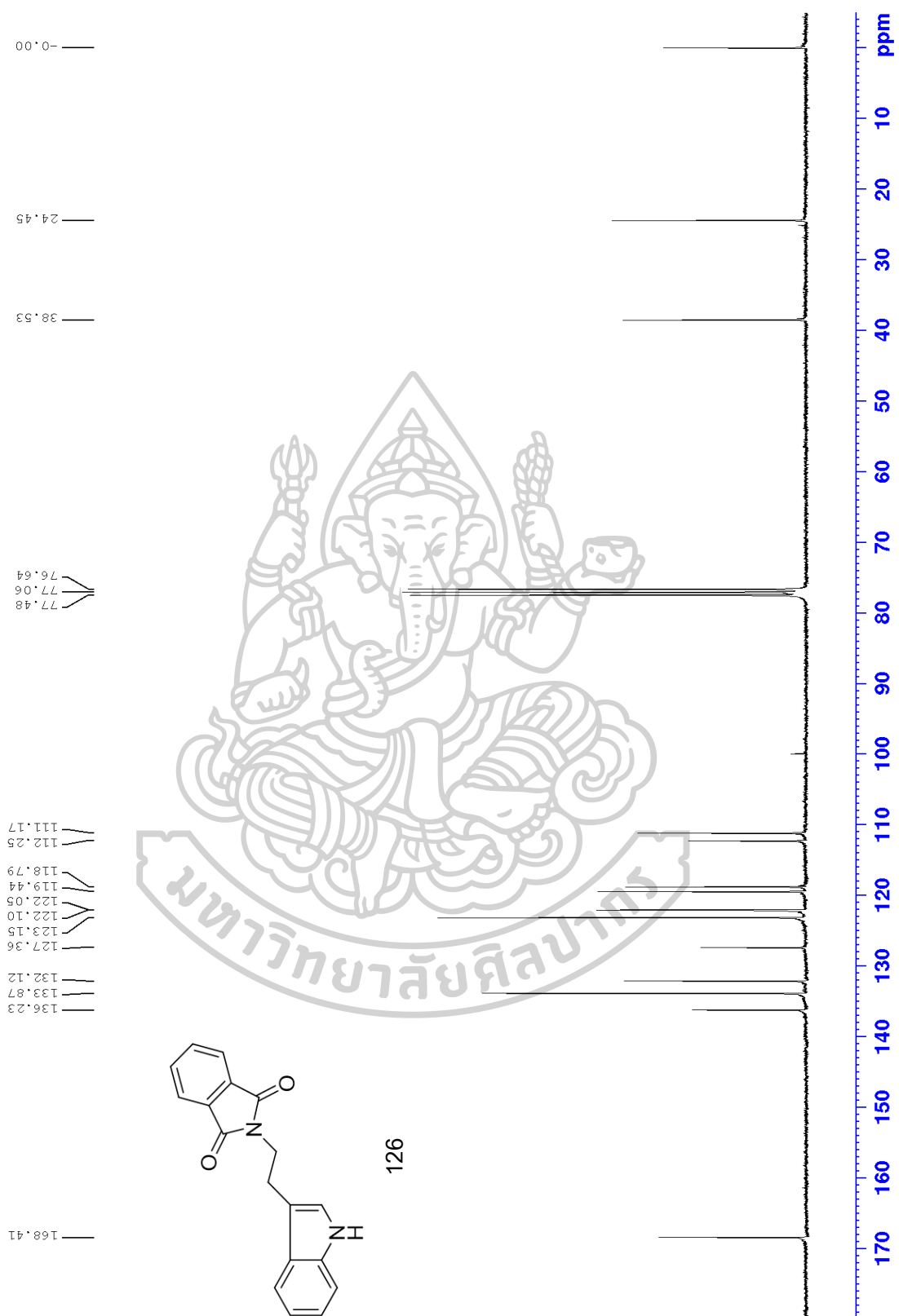


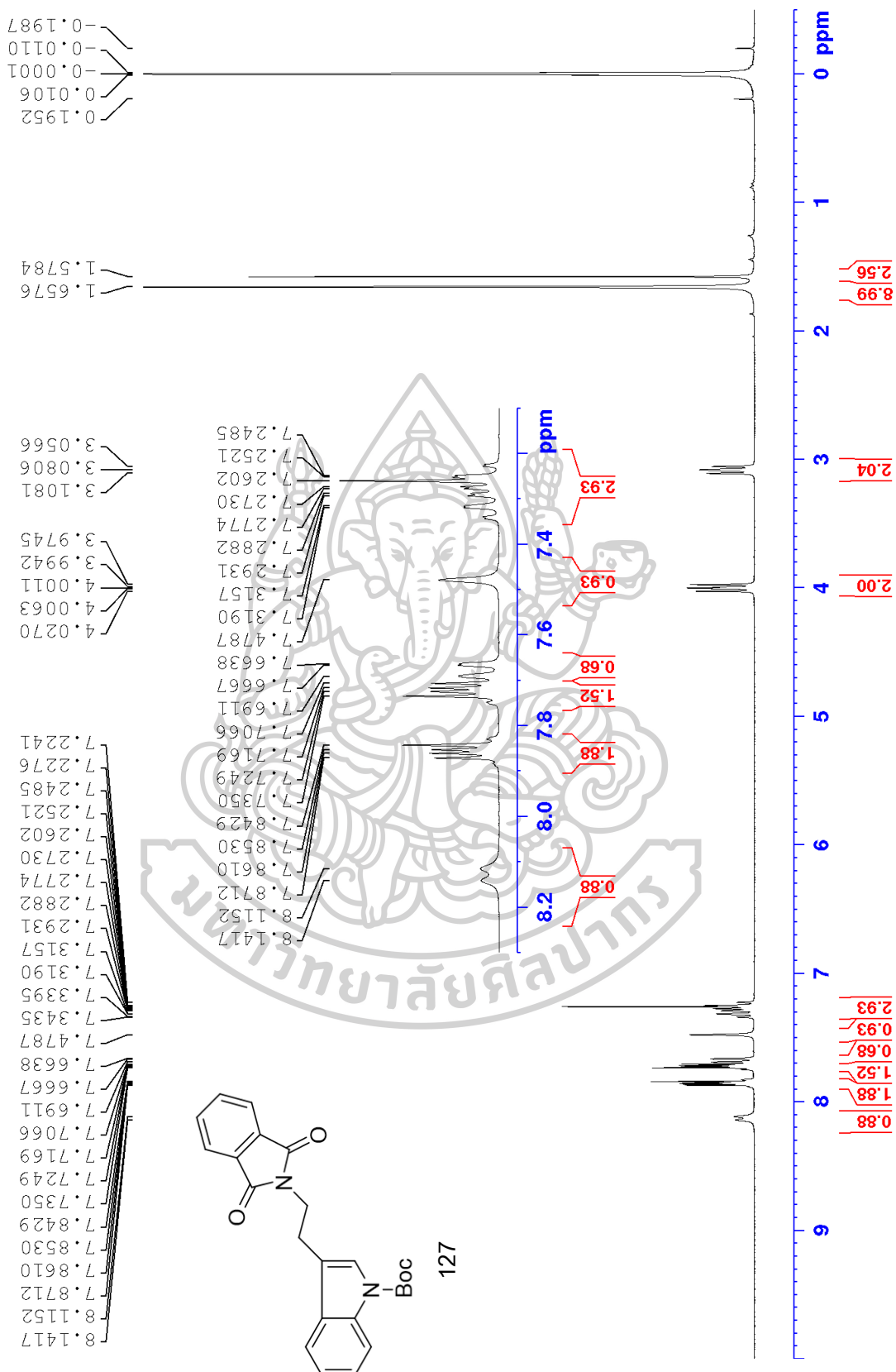


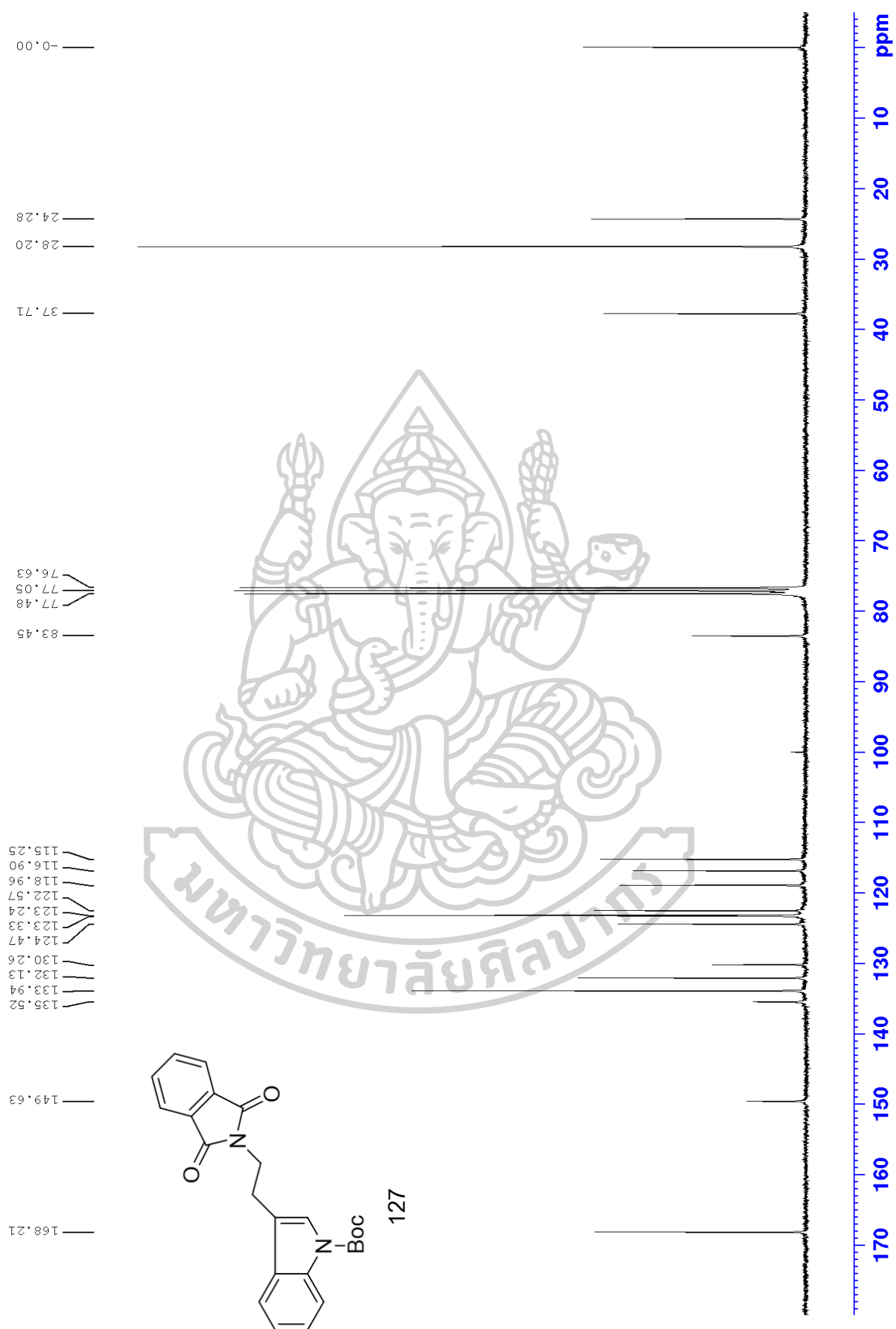


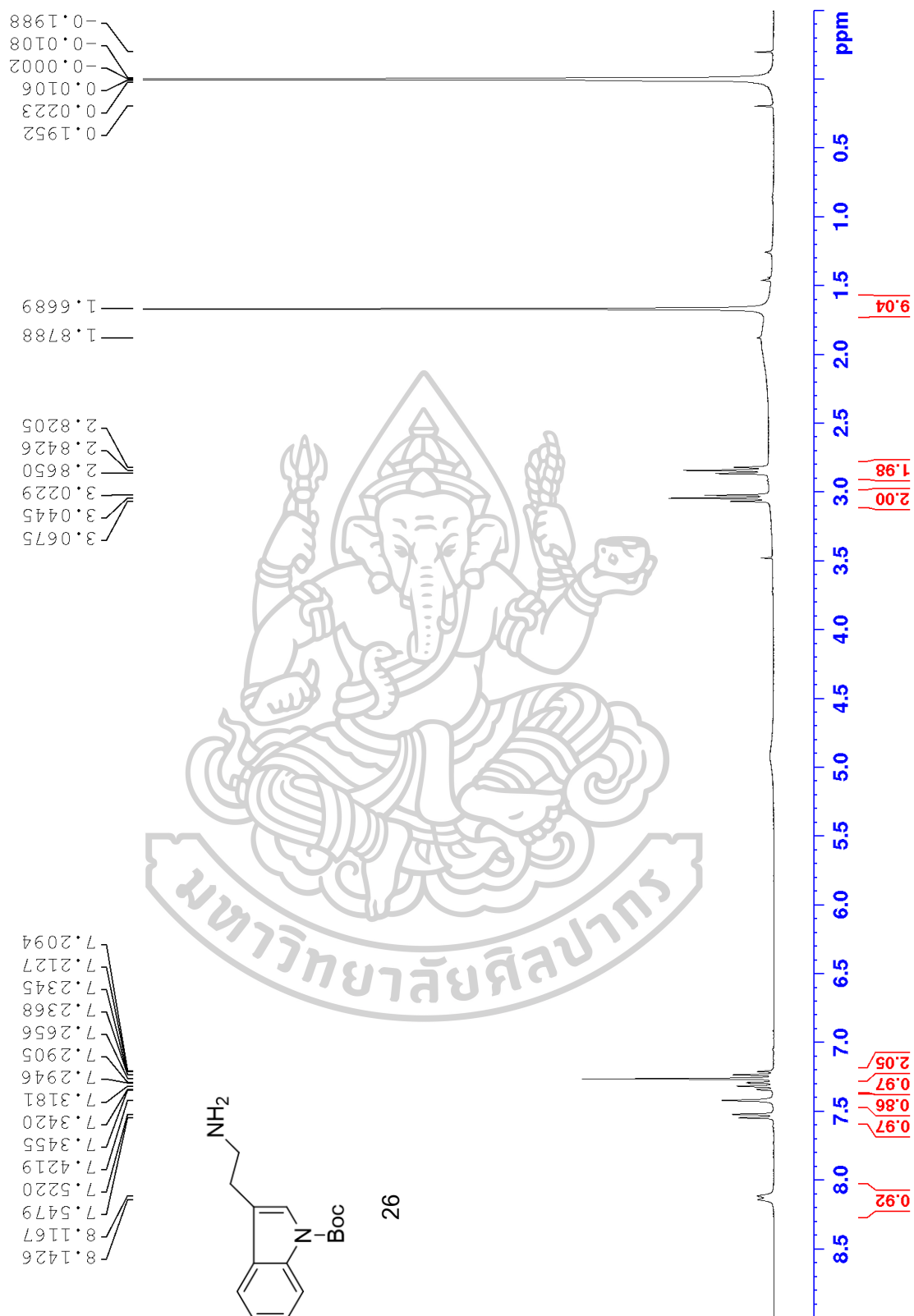


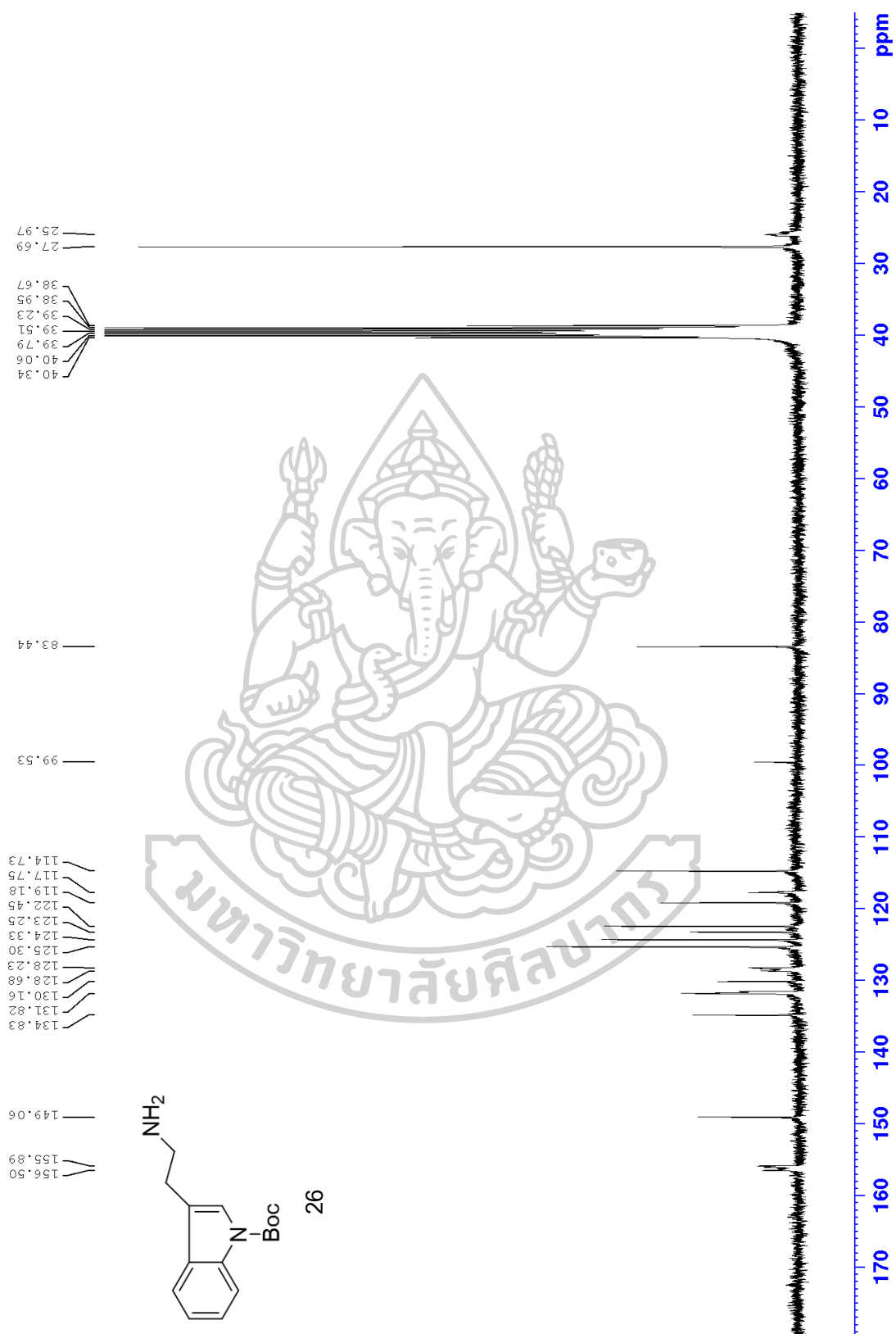


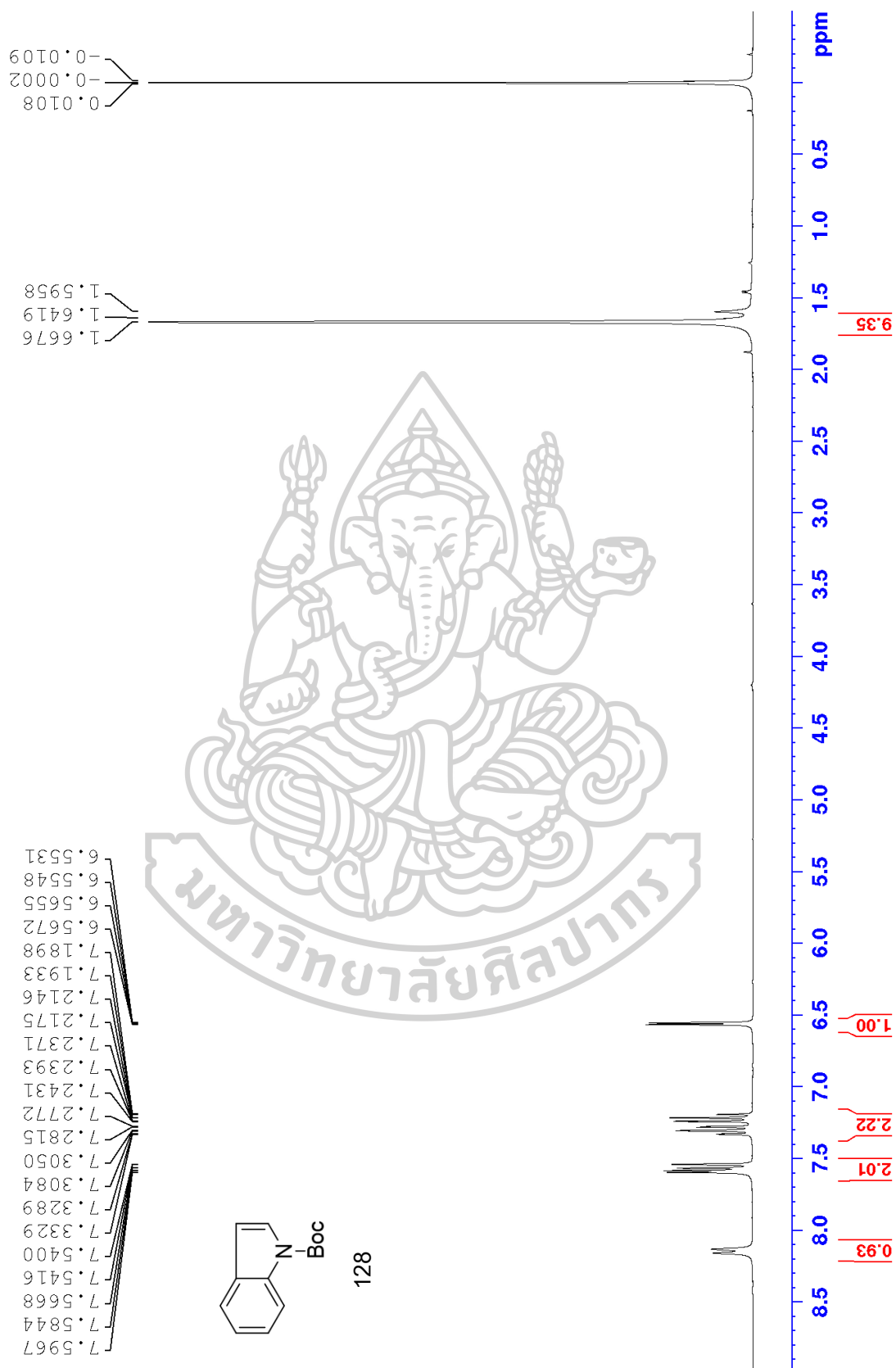


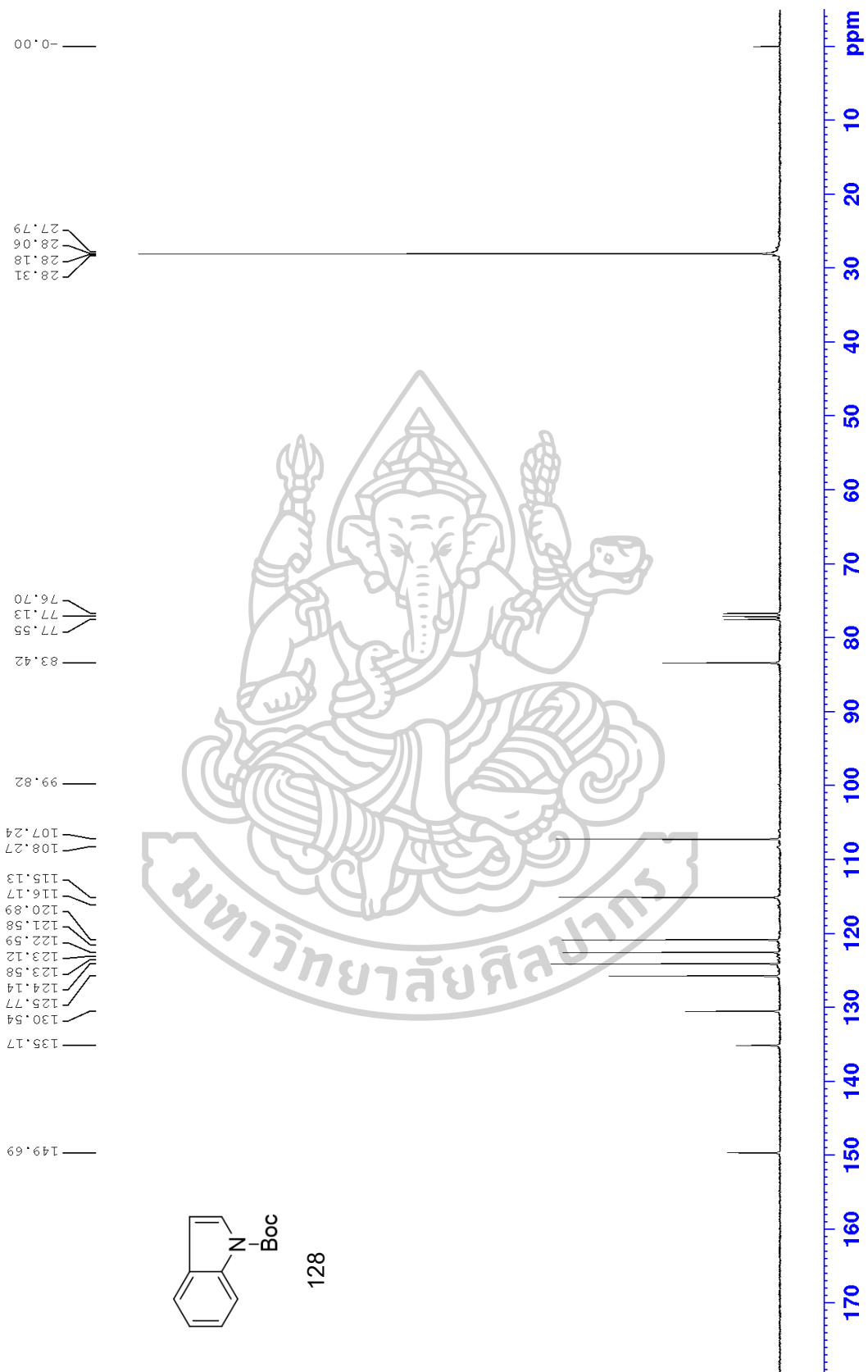




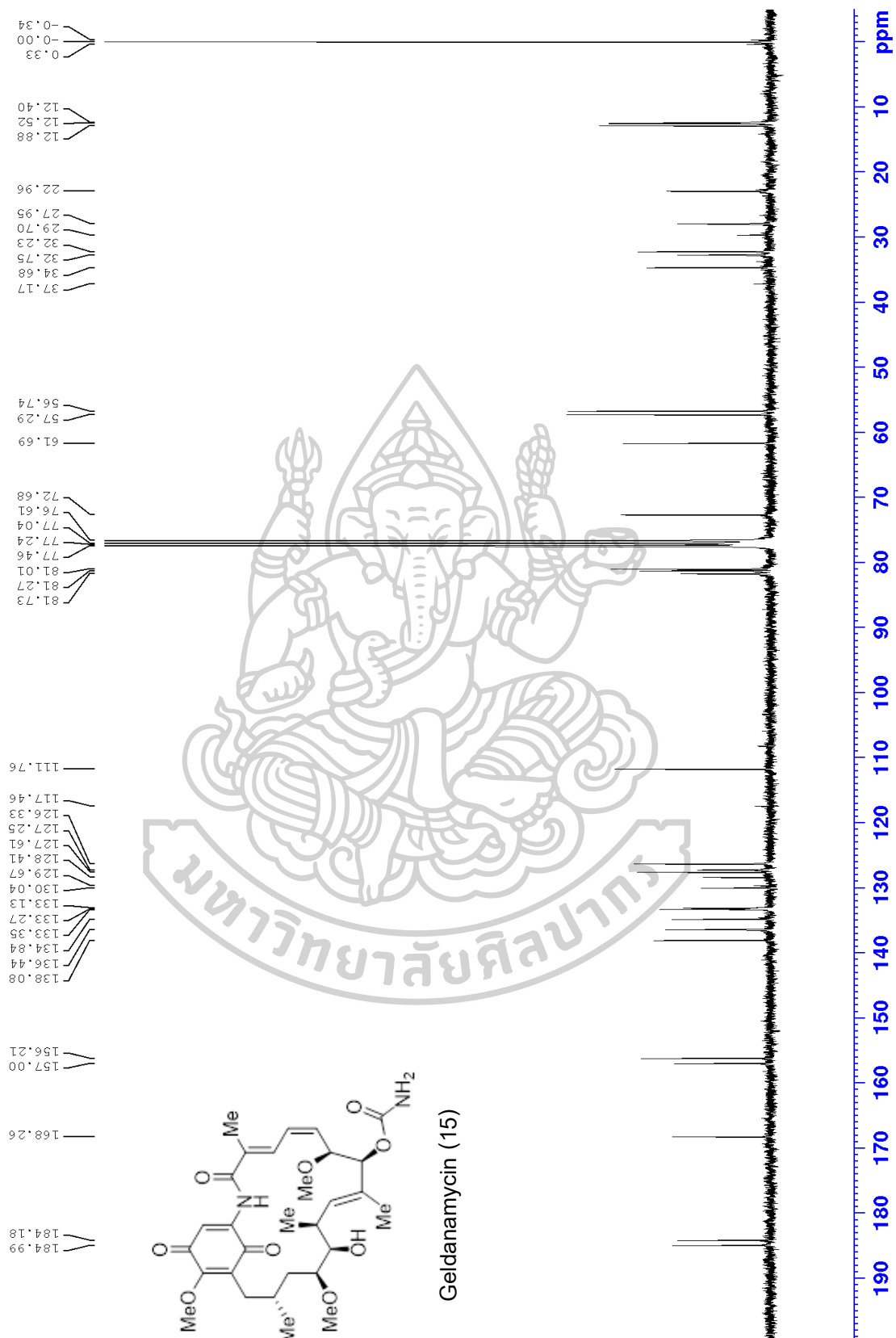


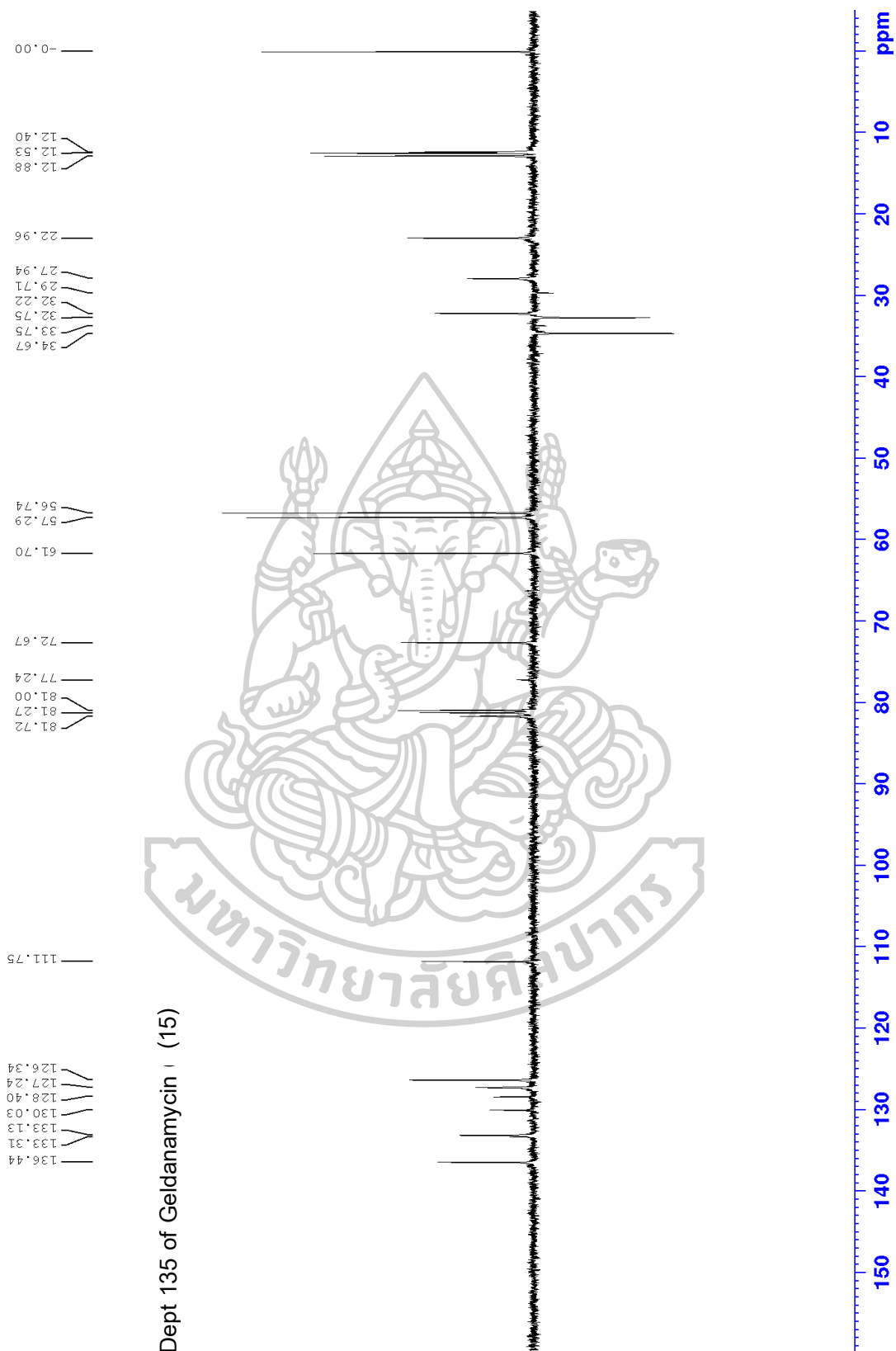




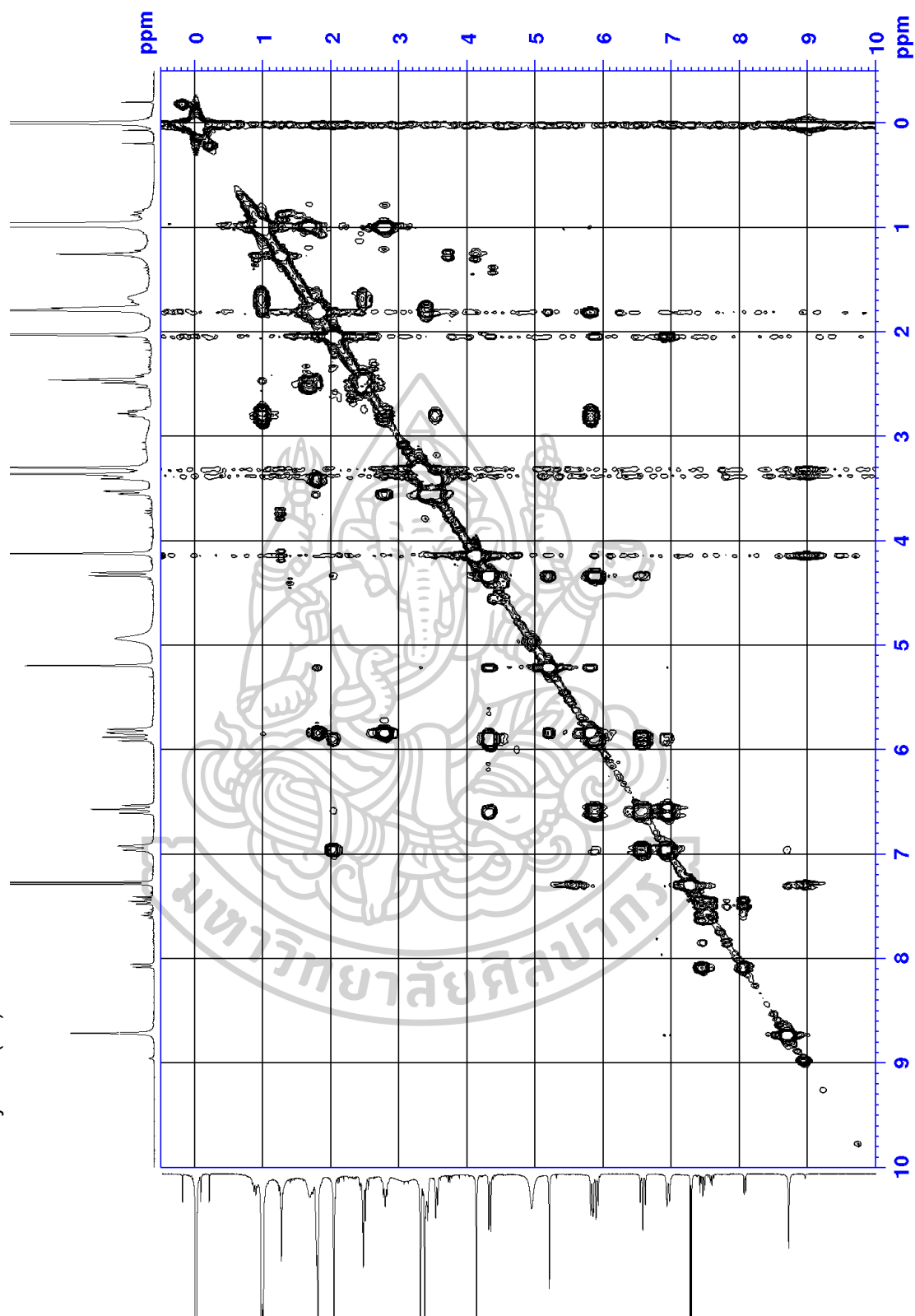


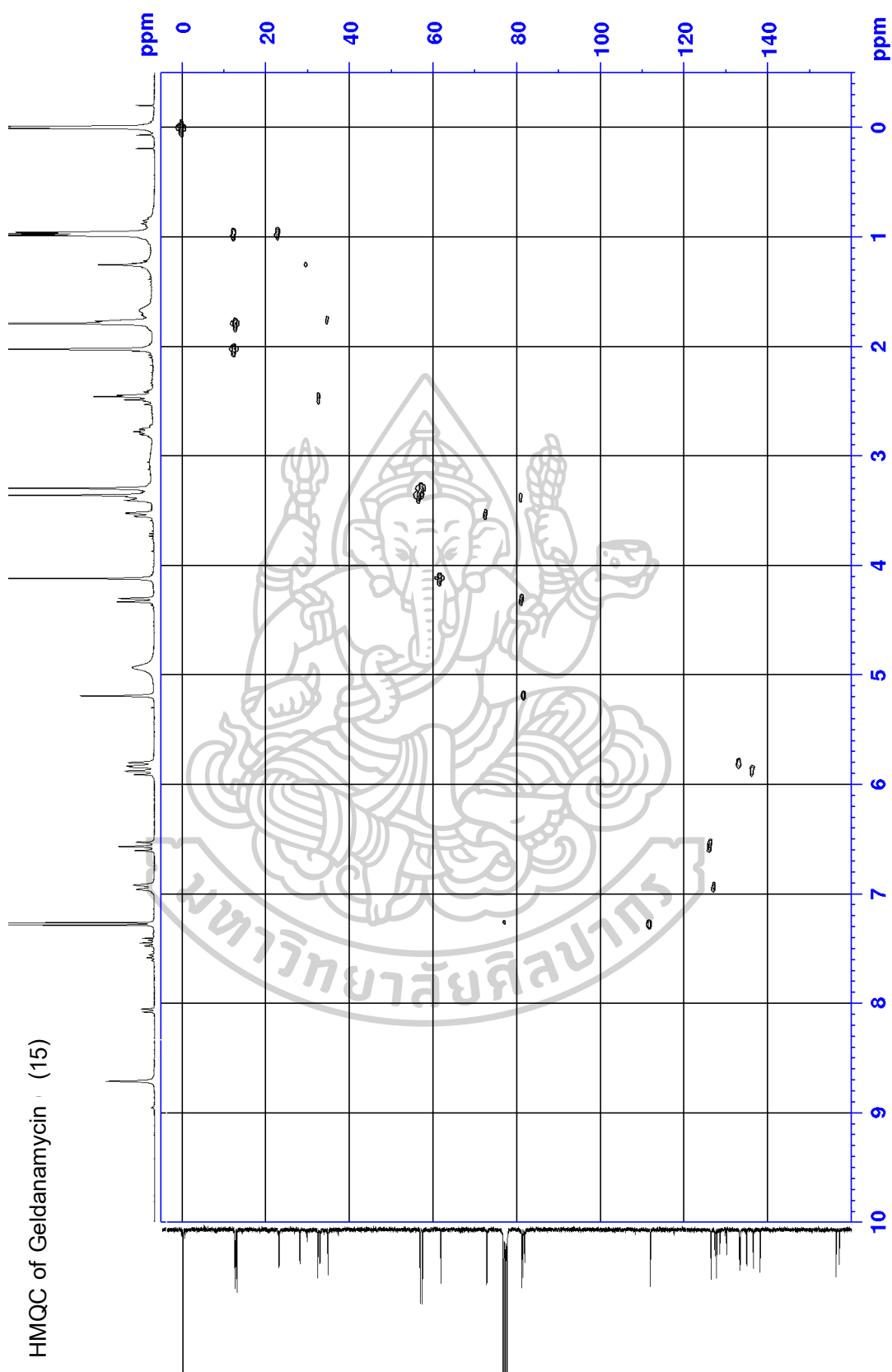
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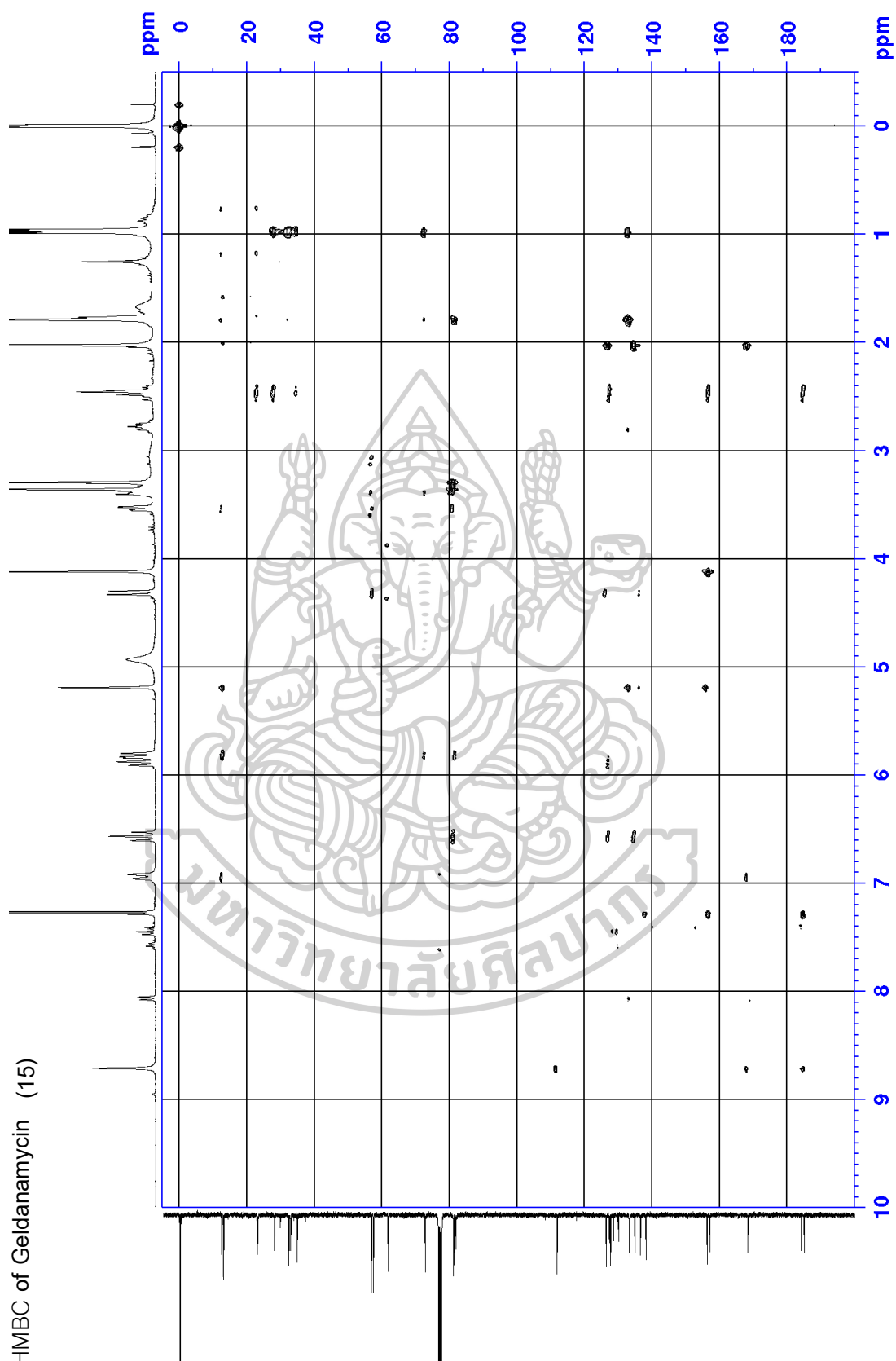


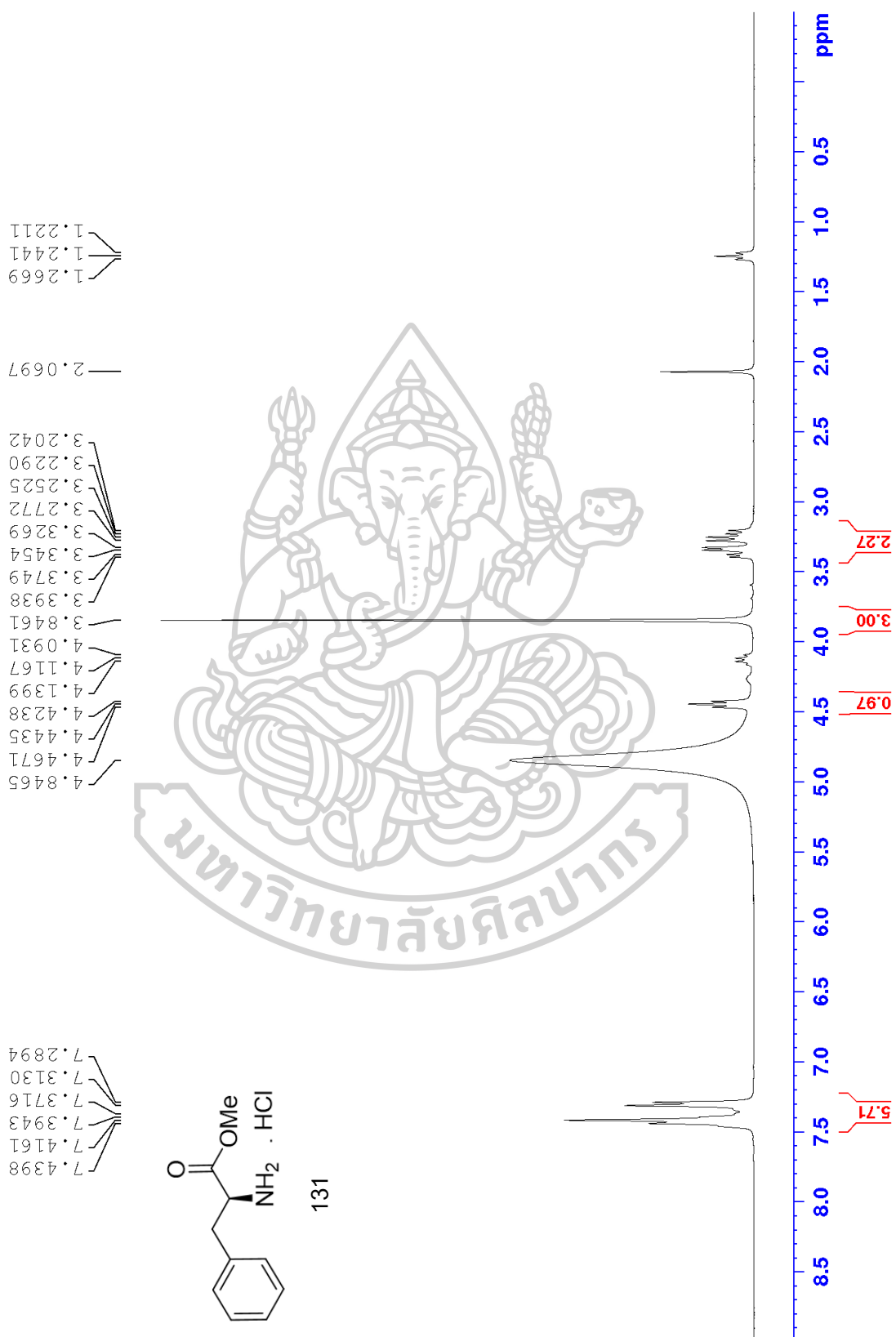


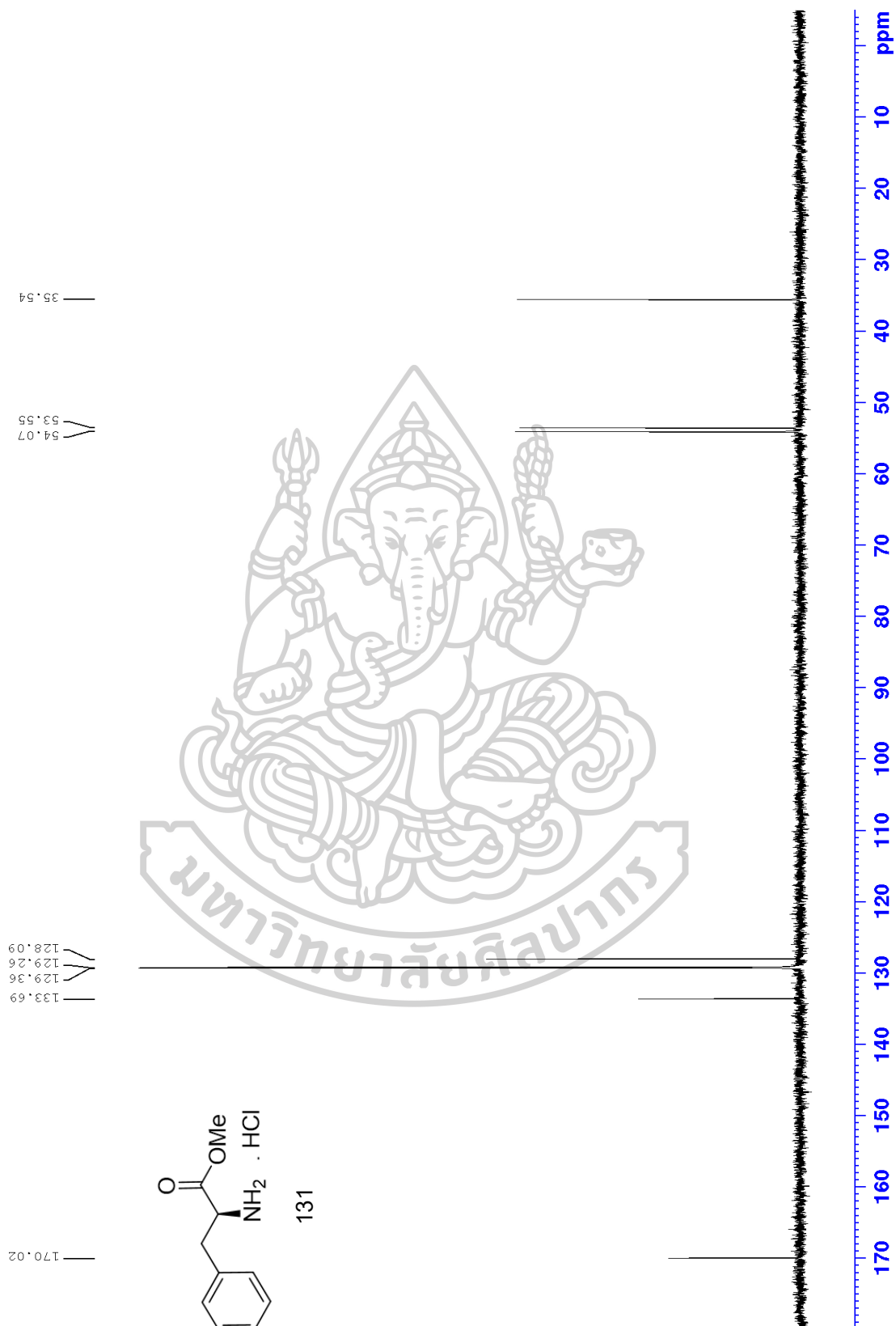
COSY of Geldanamycin (15)

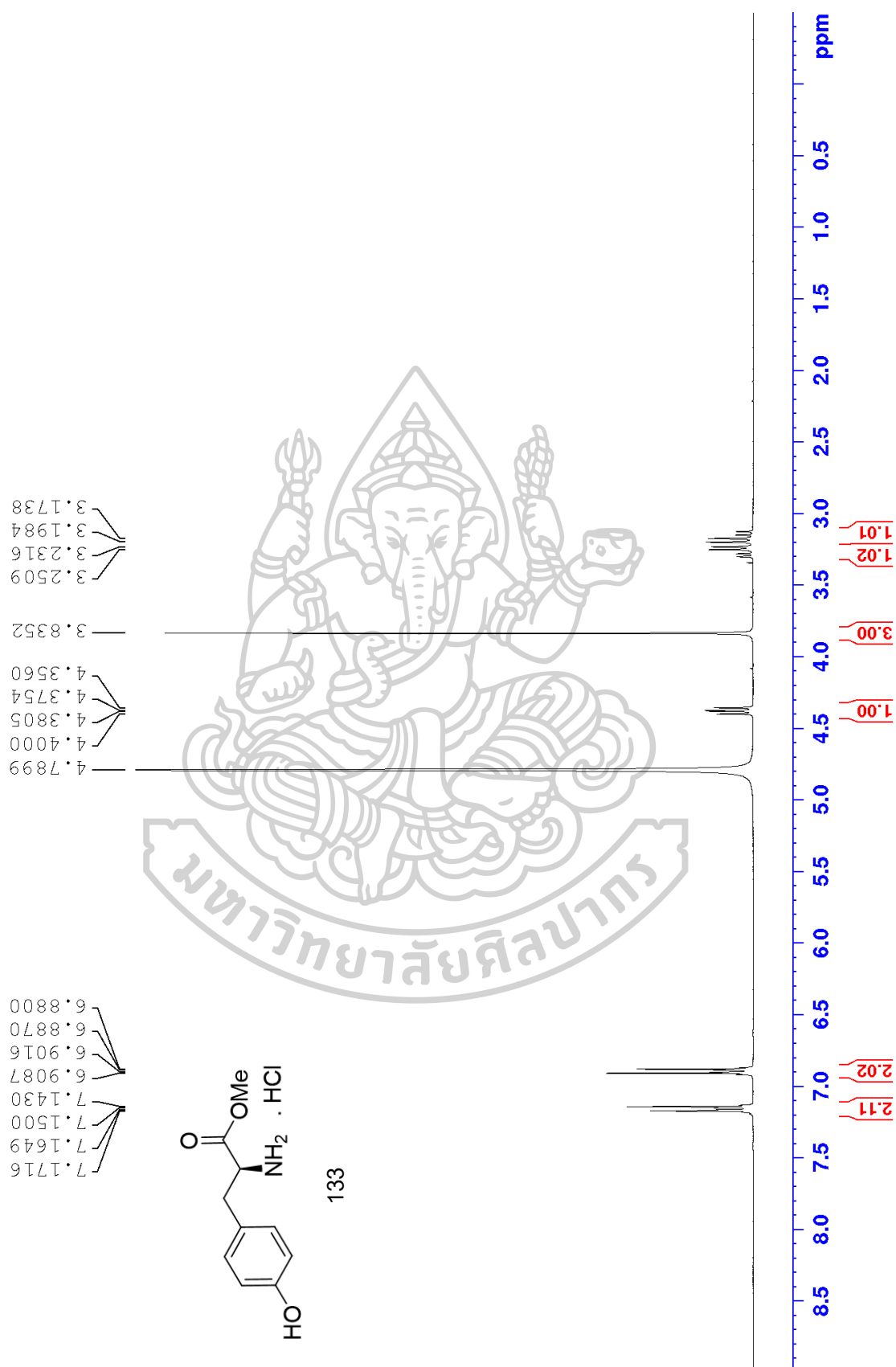


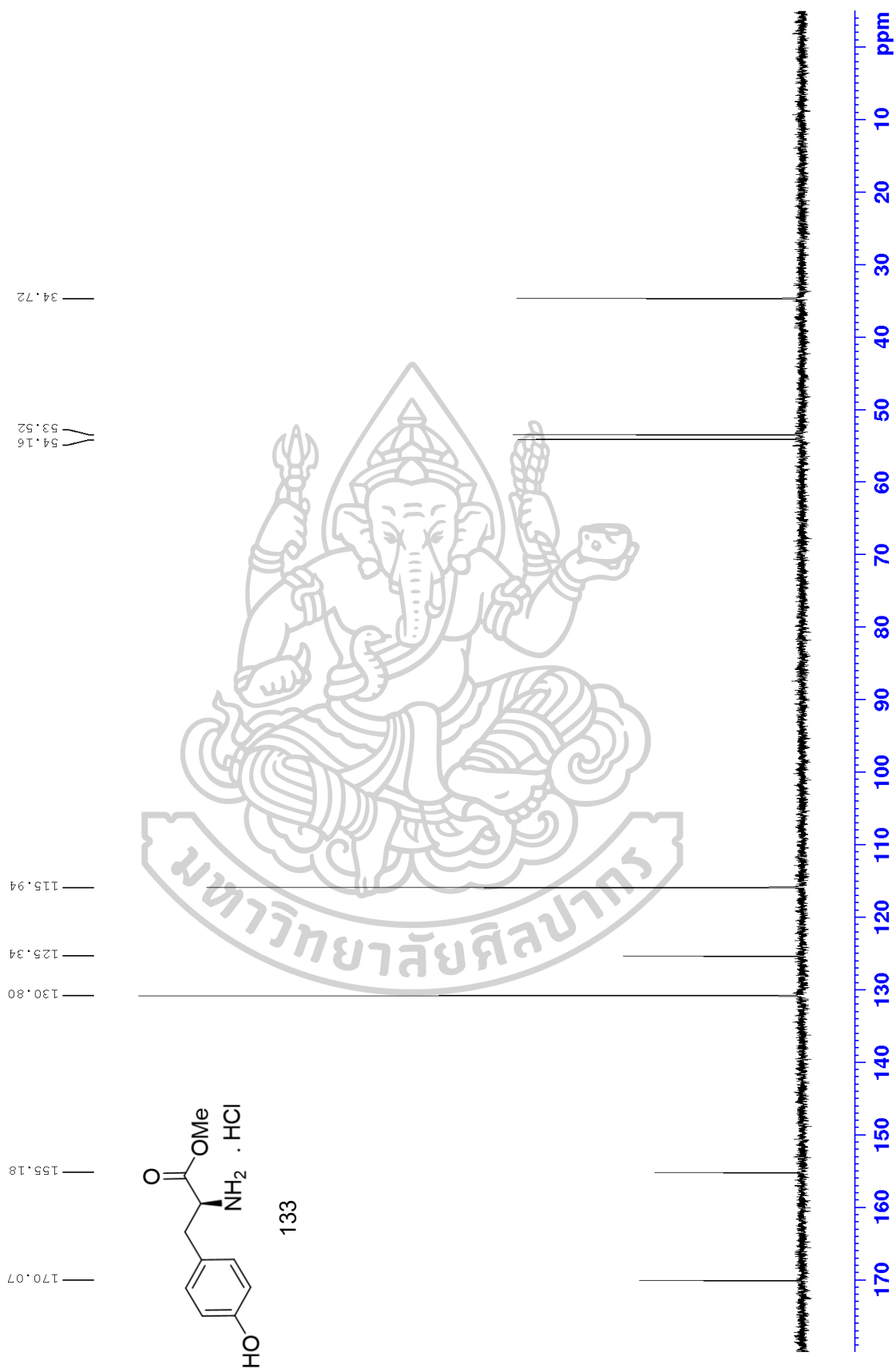


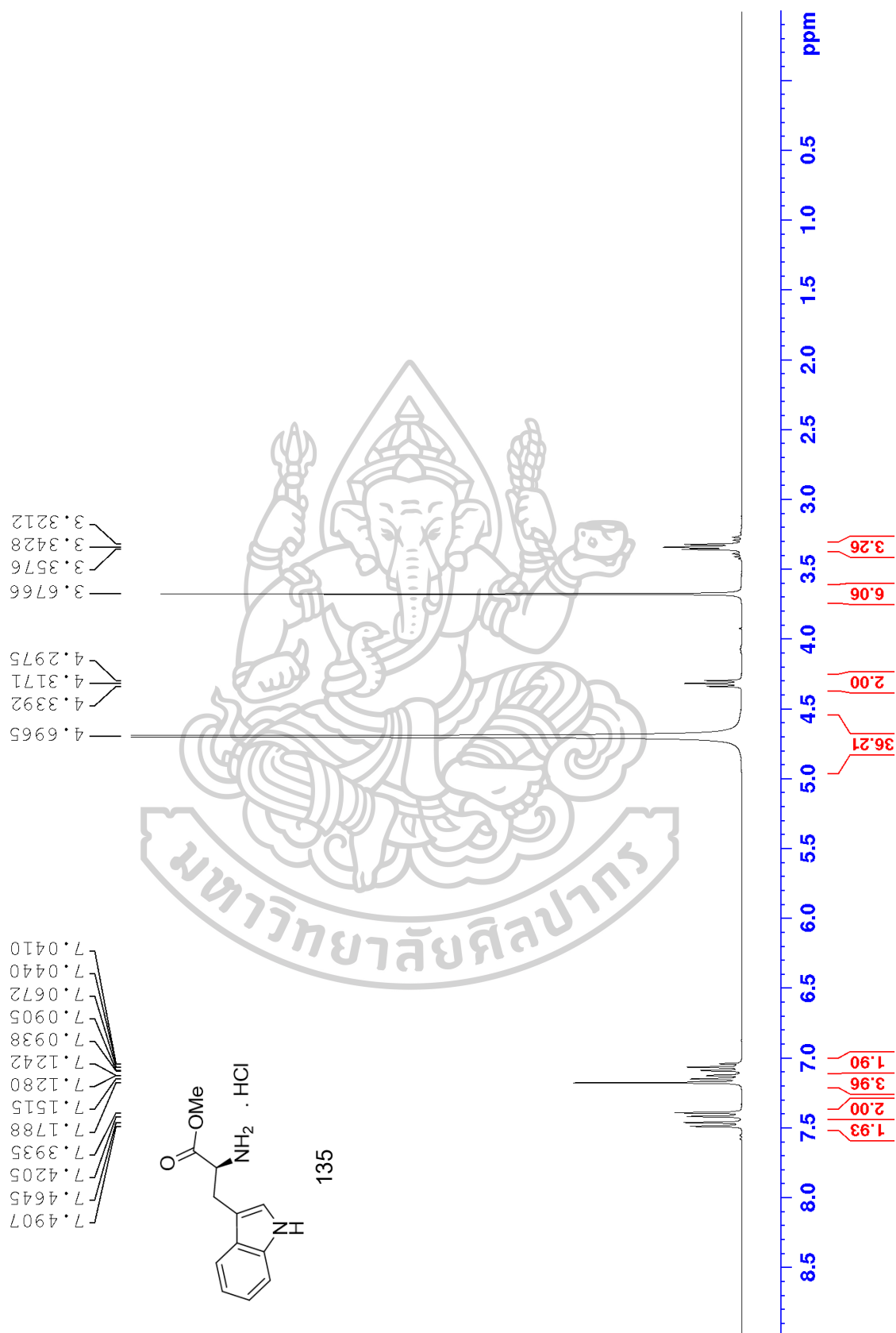


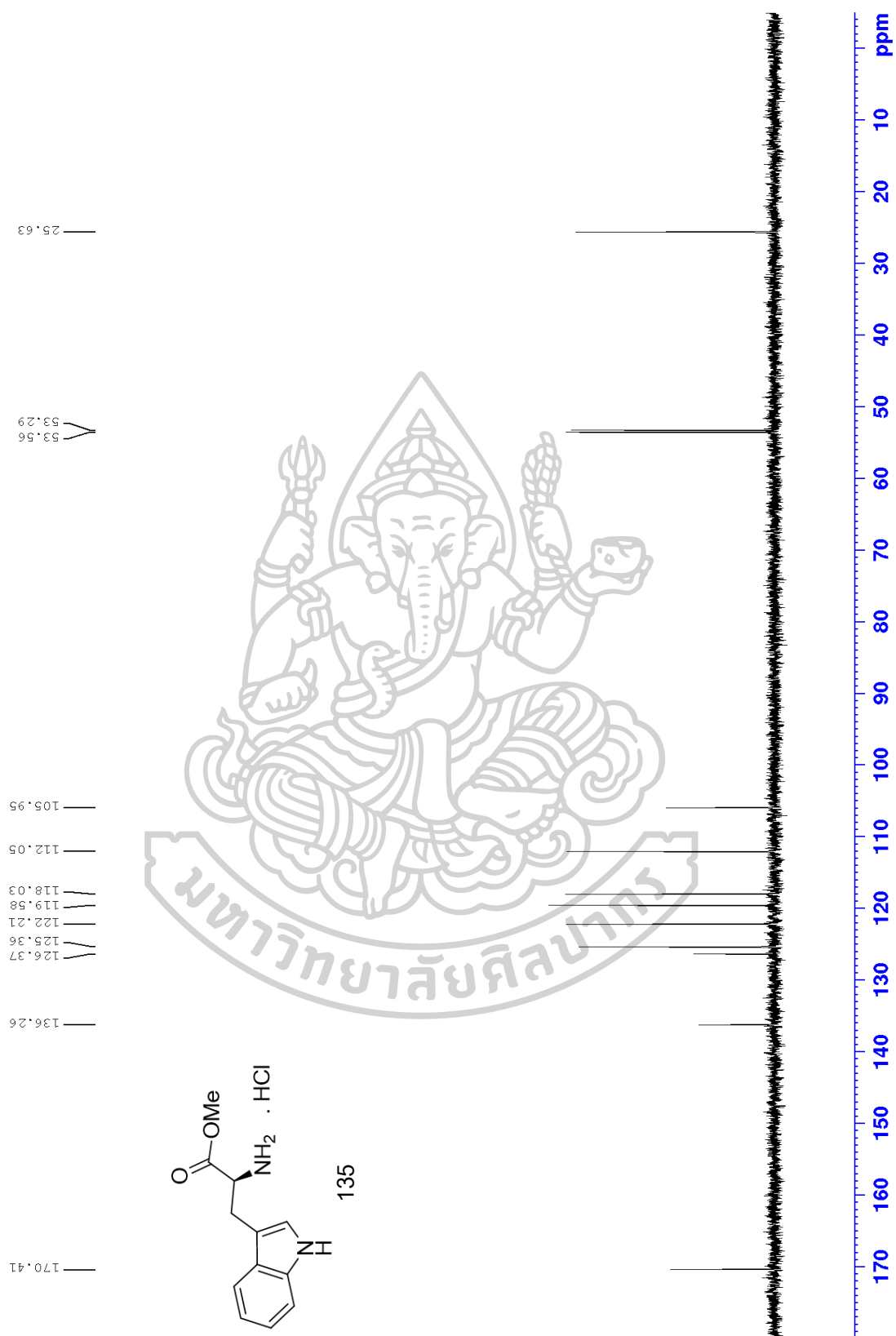


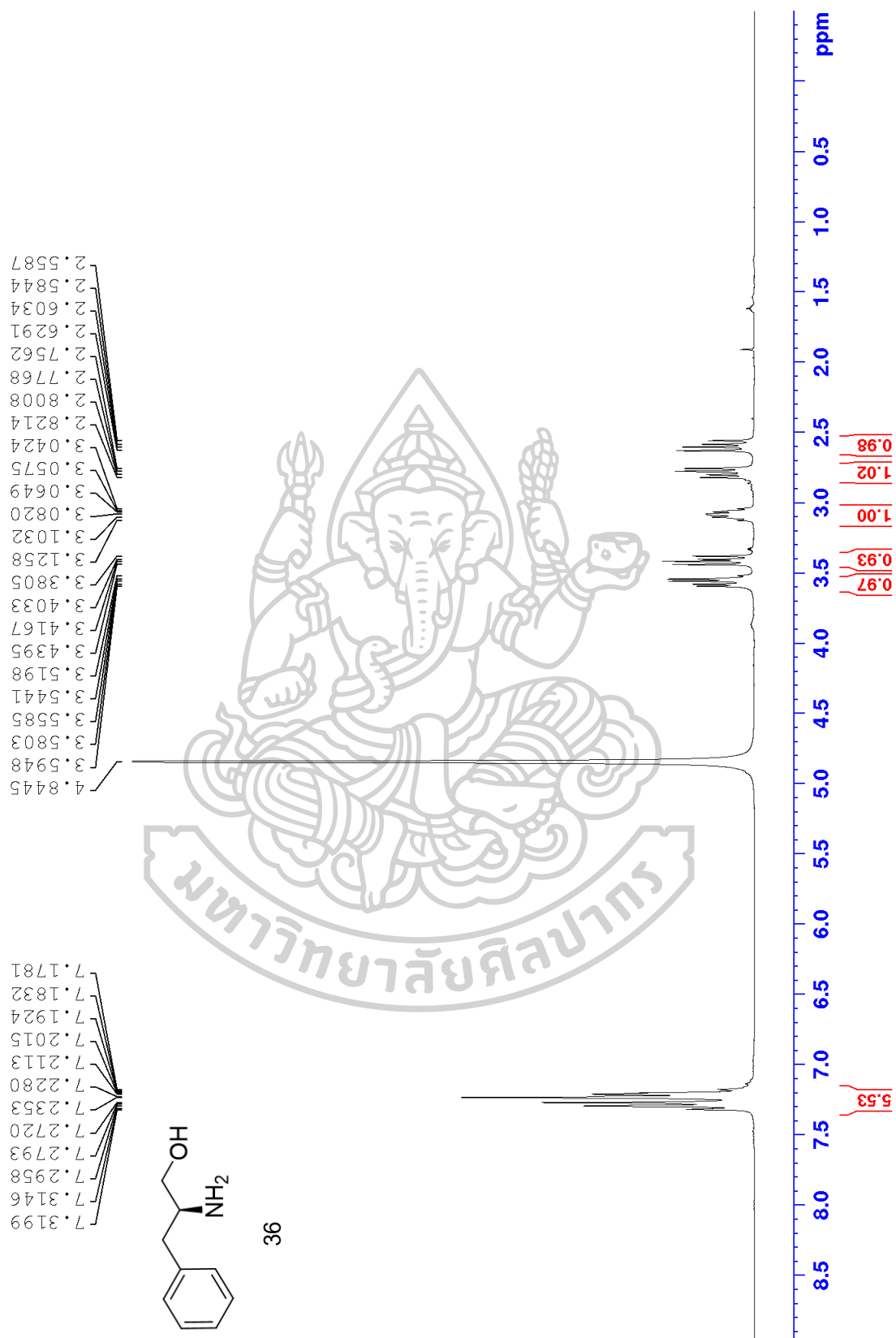


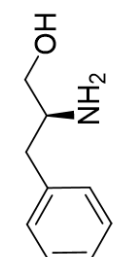




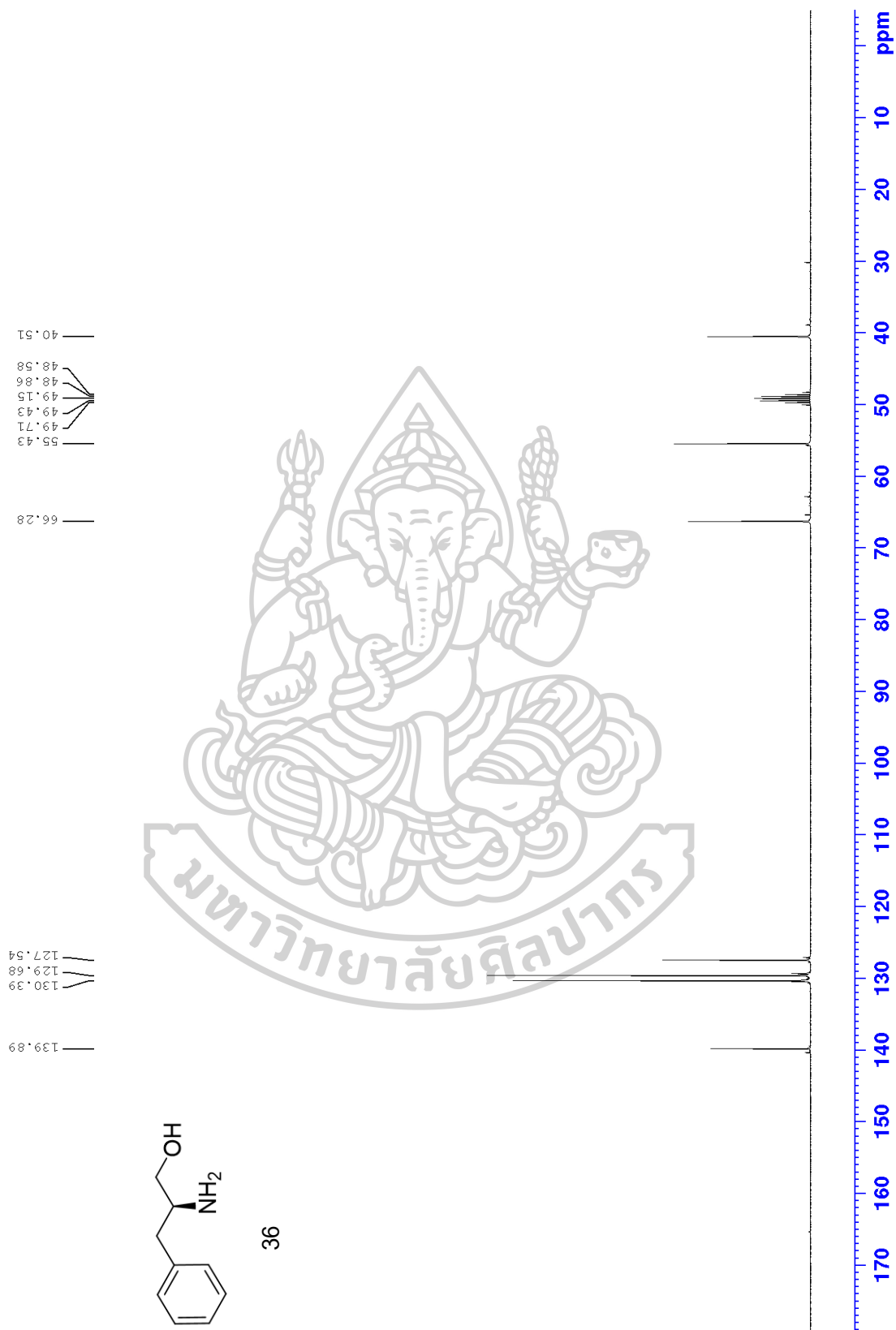


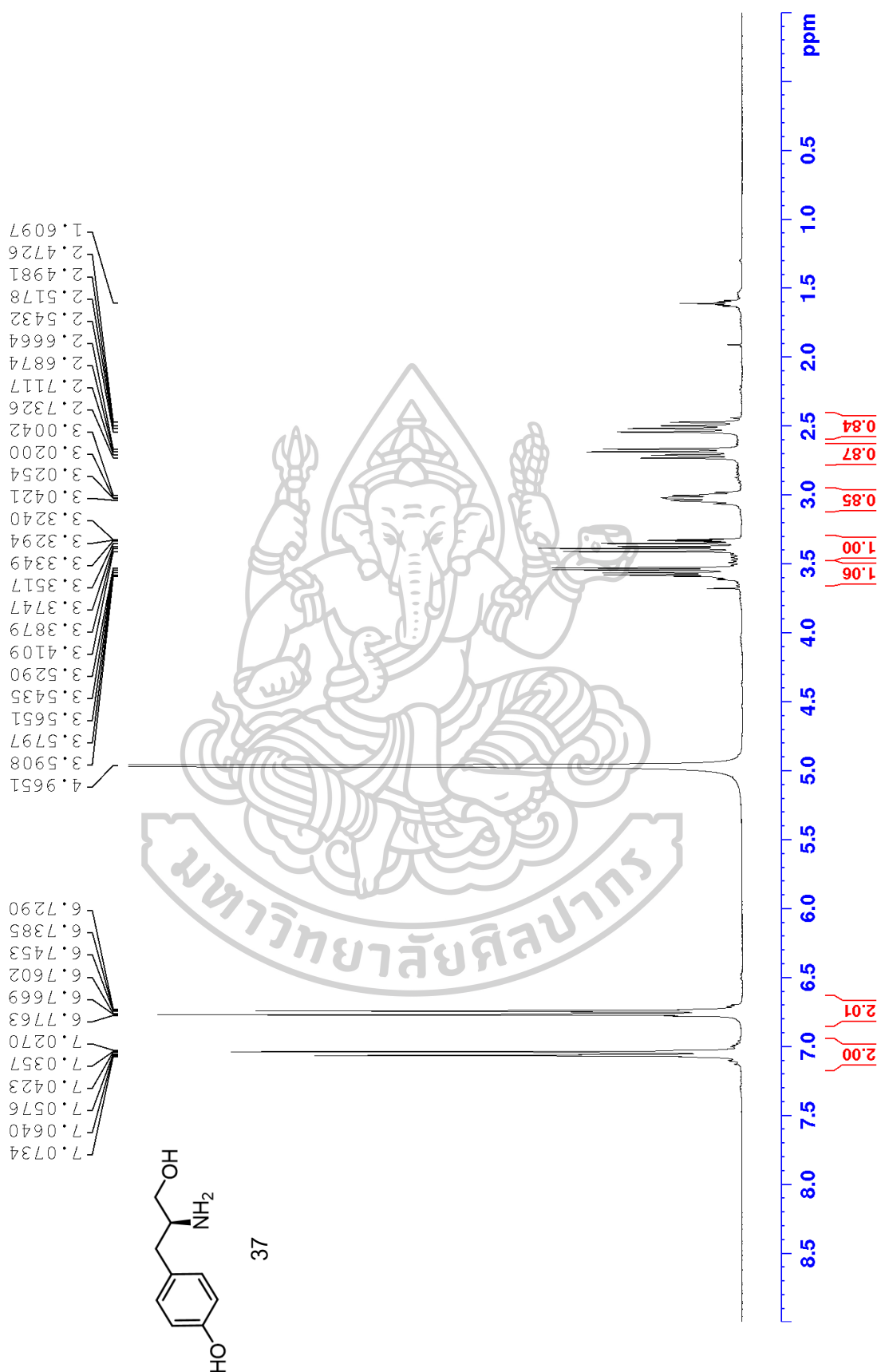


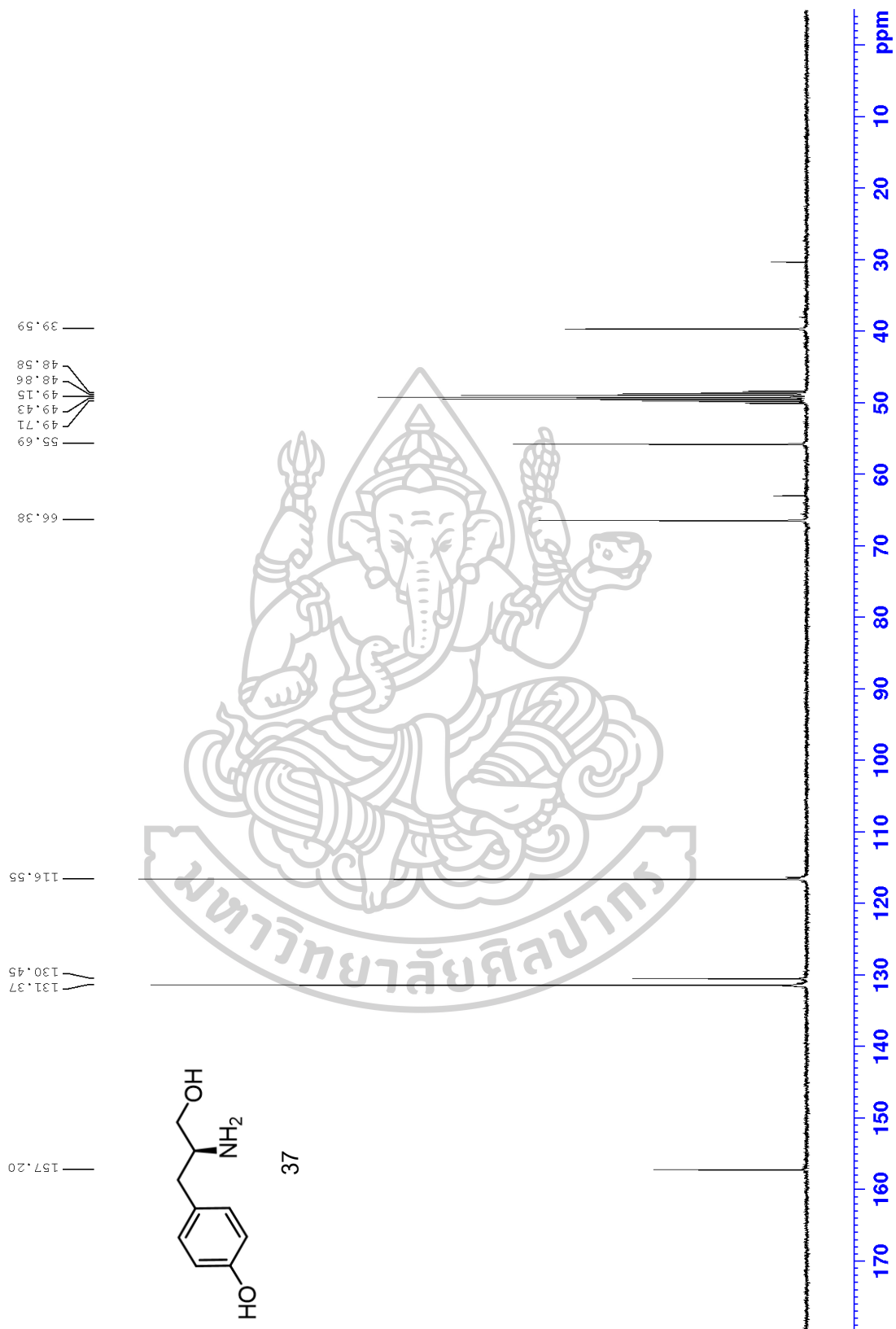


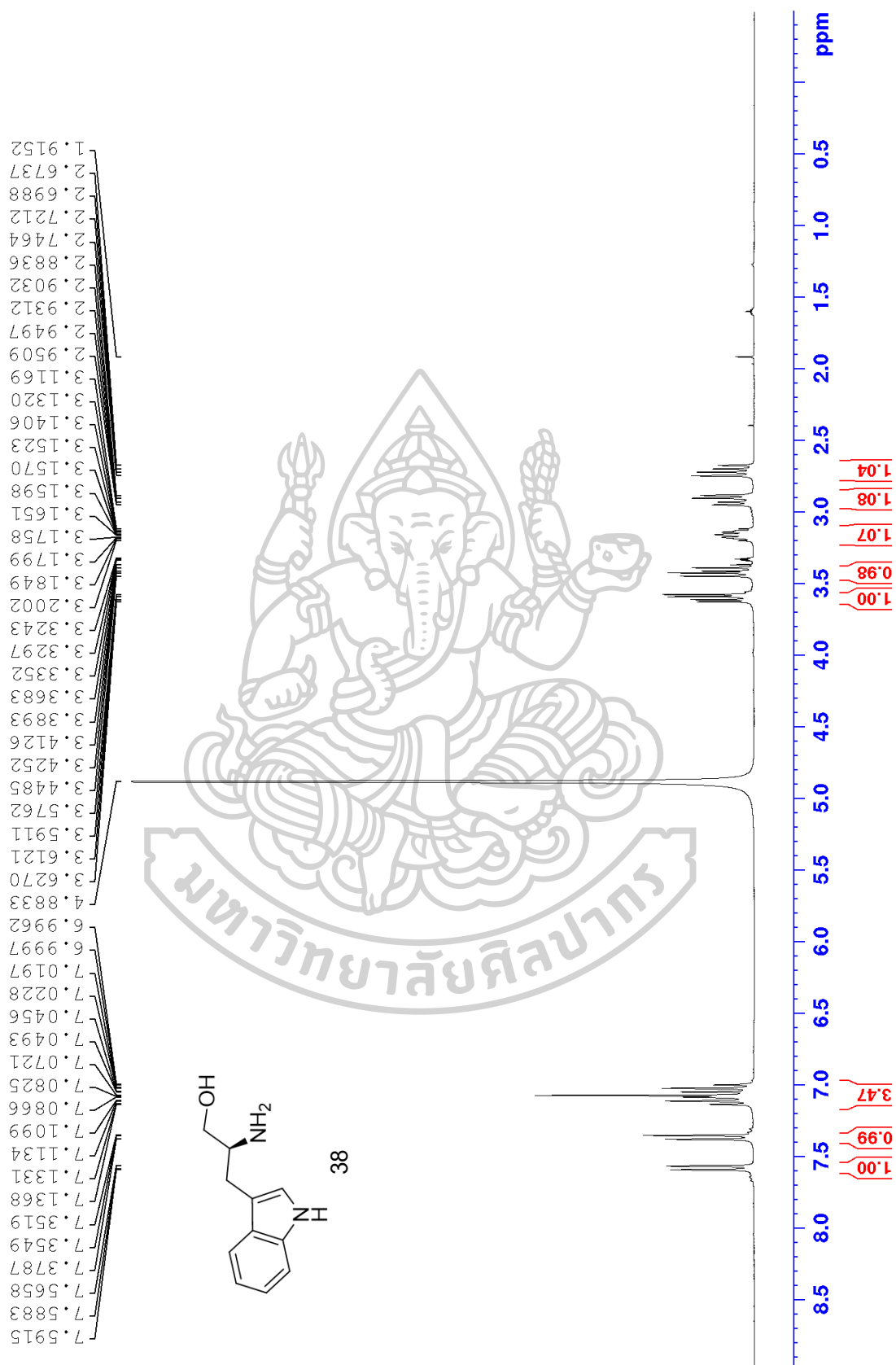


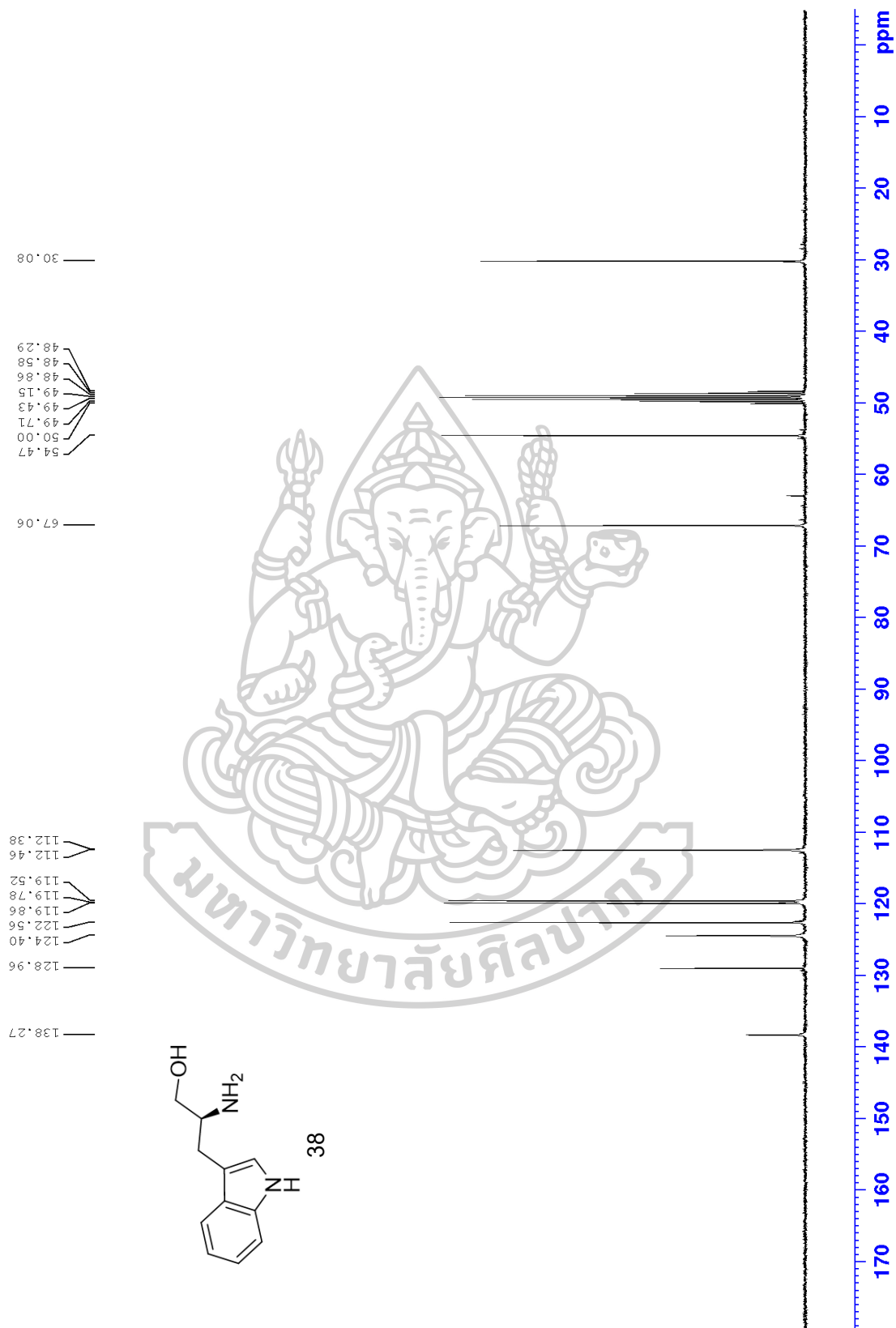
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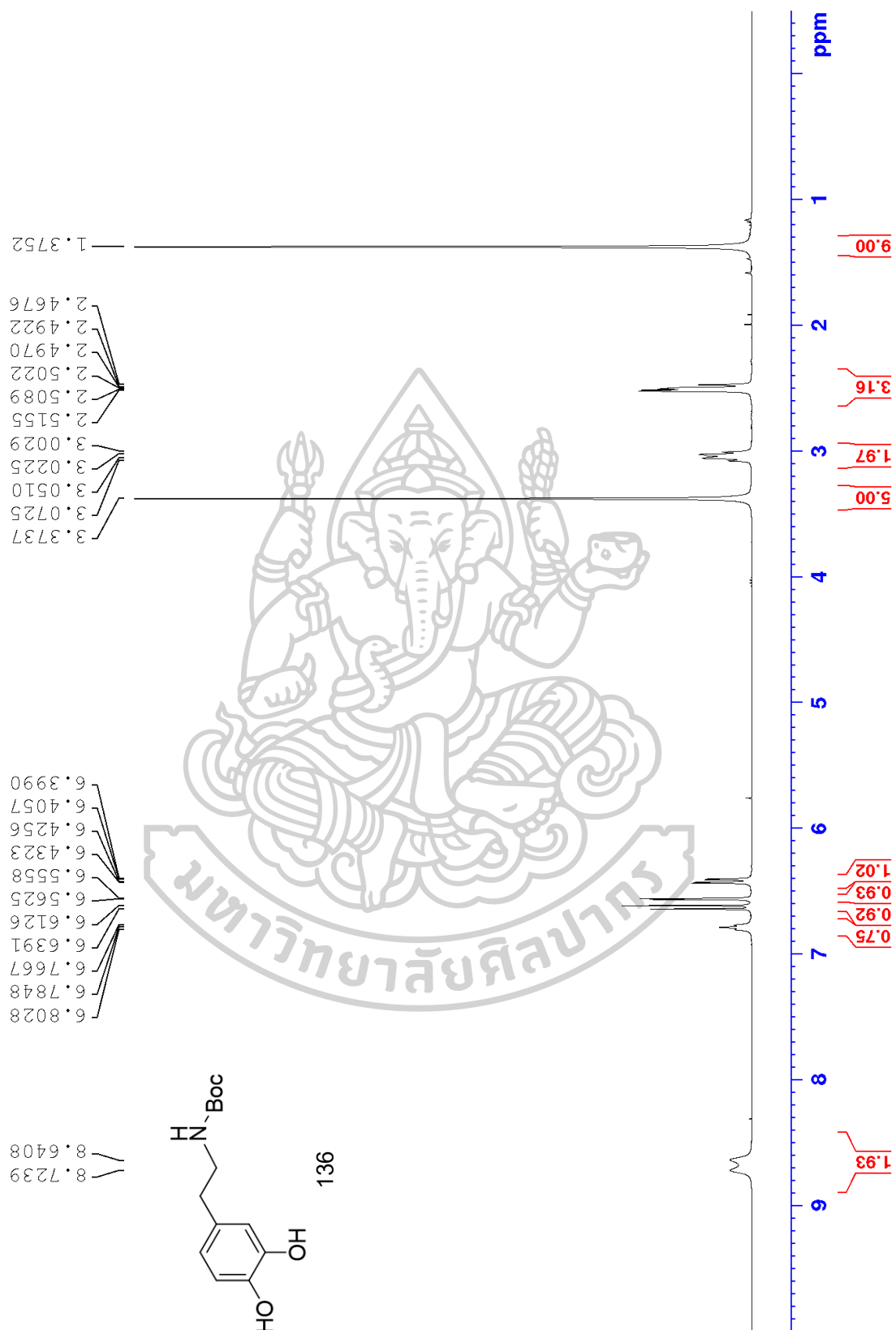


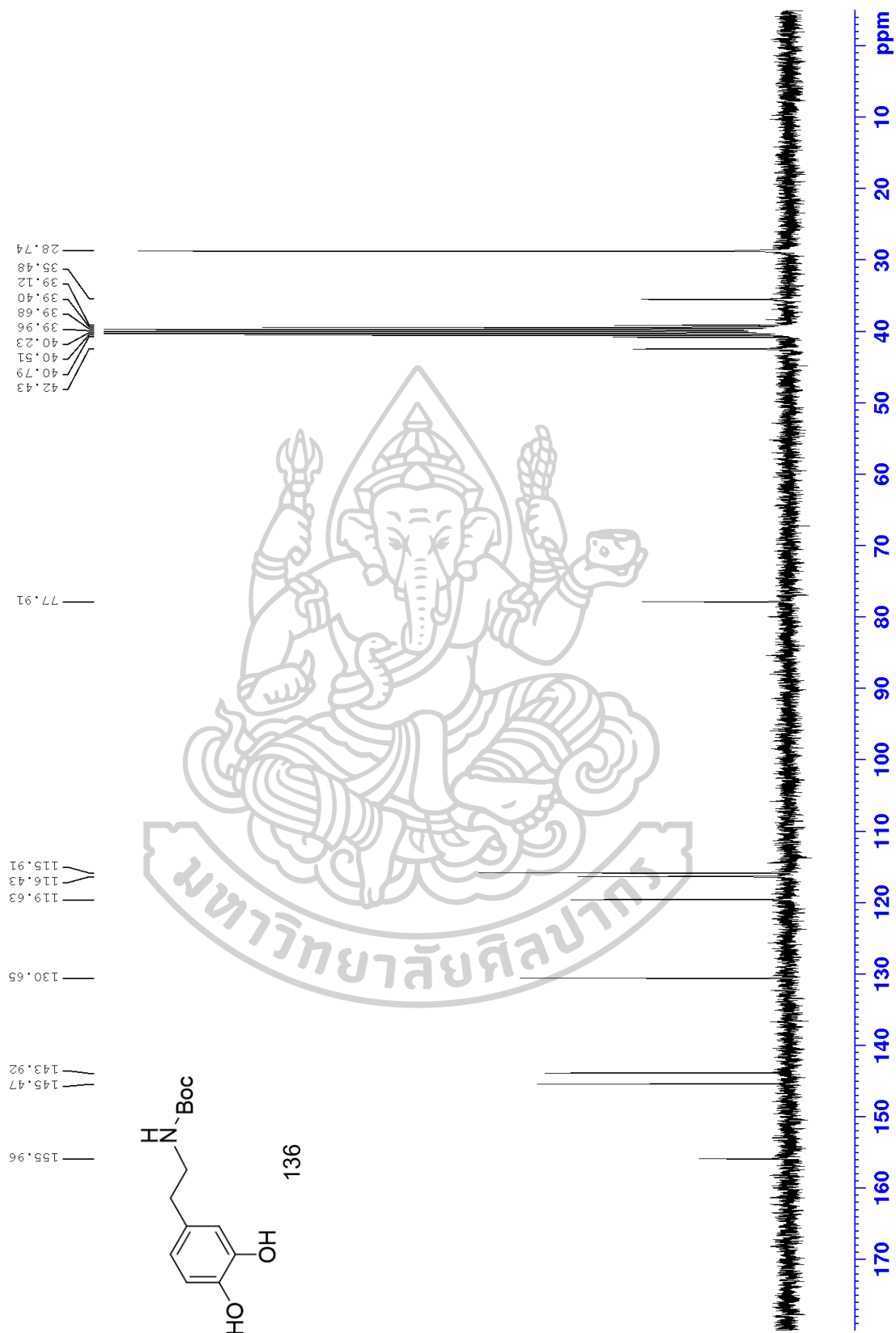


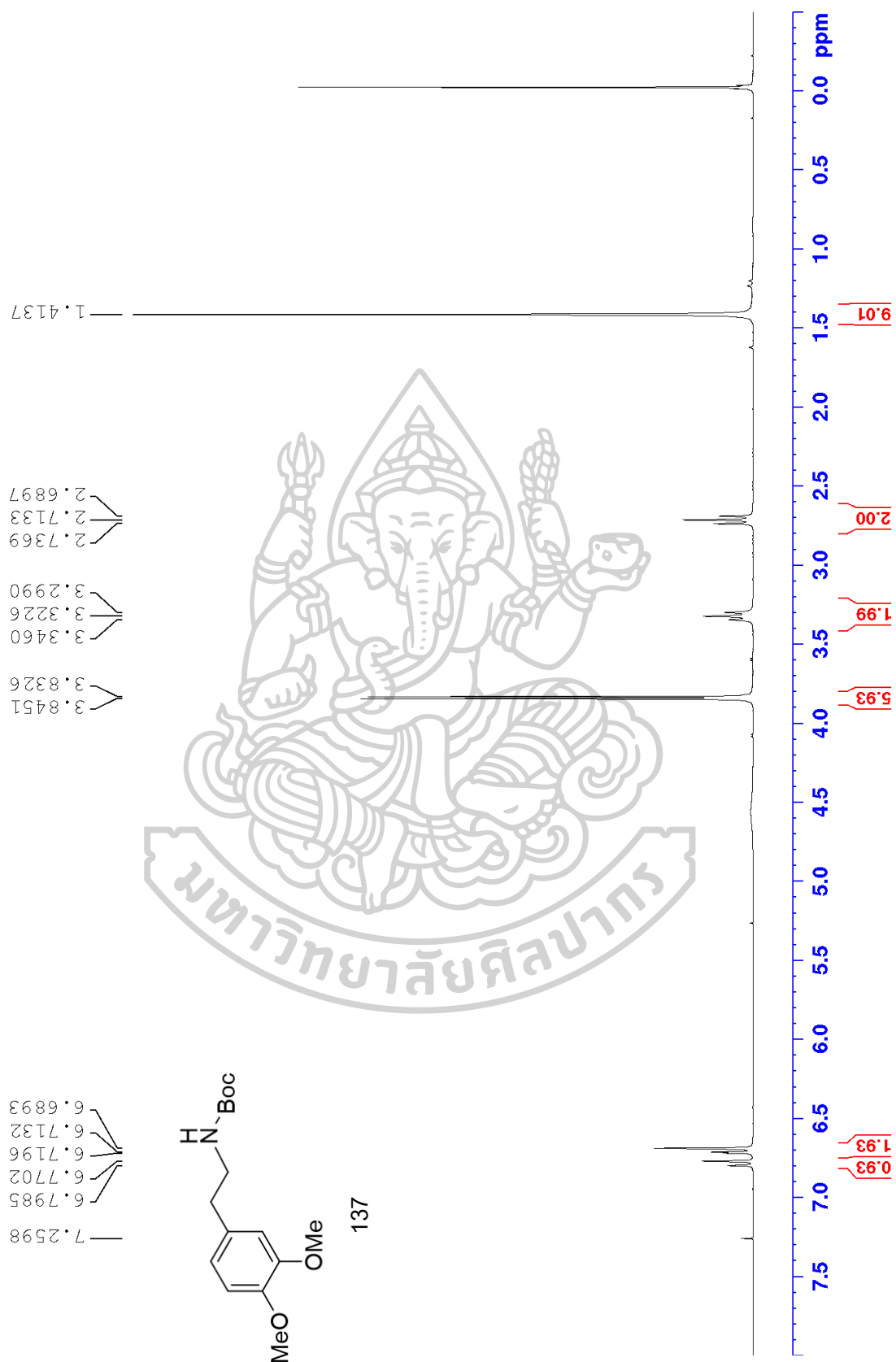


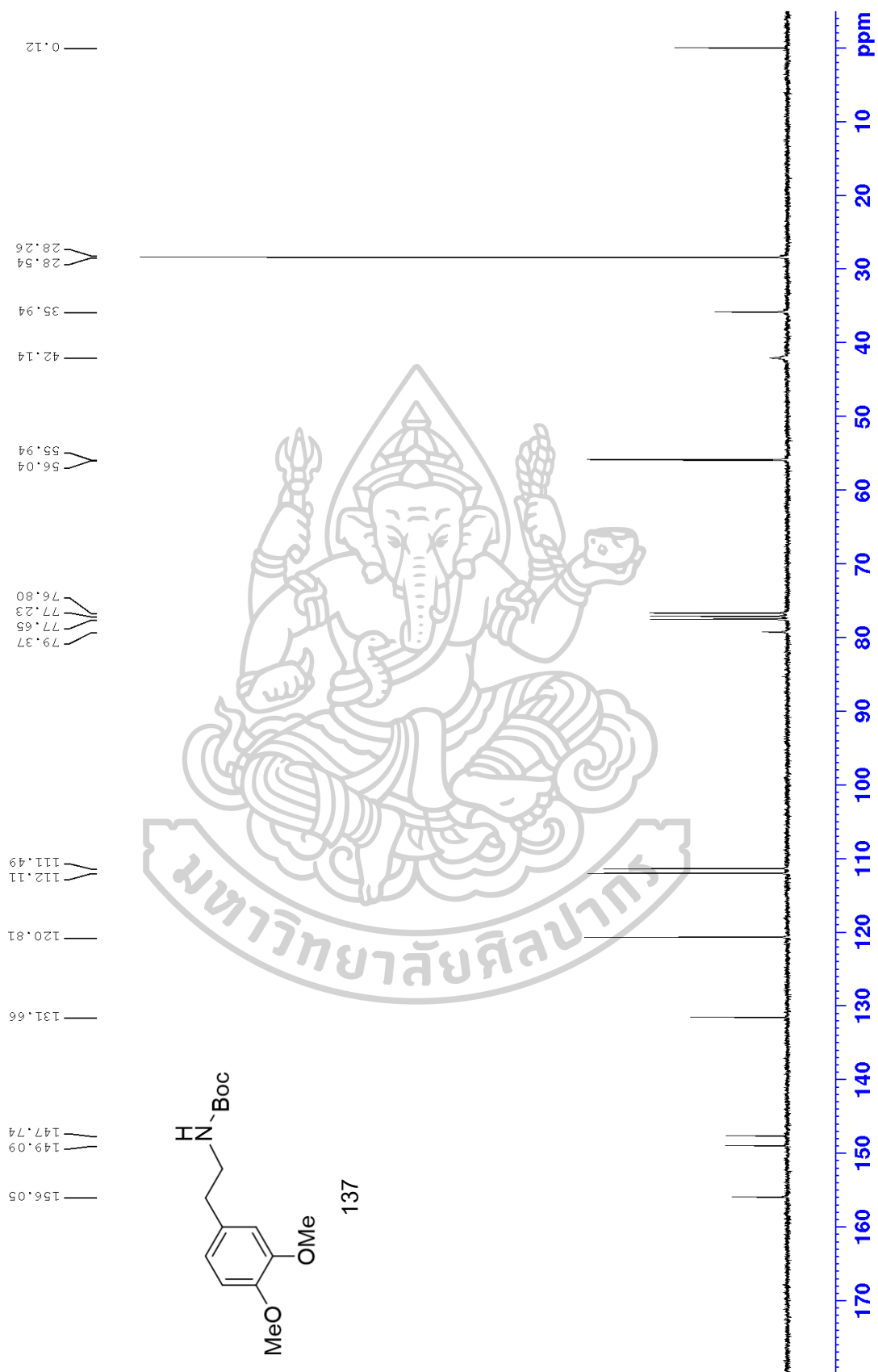


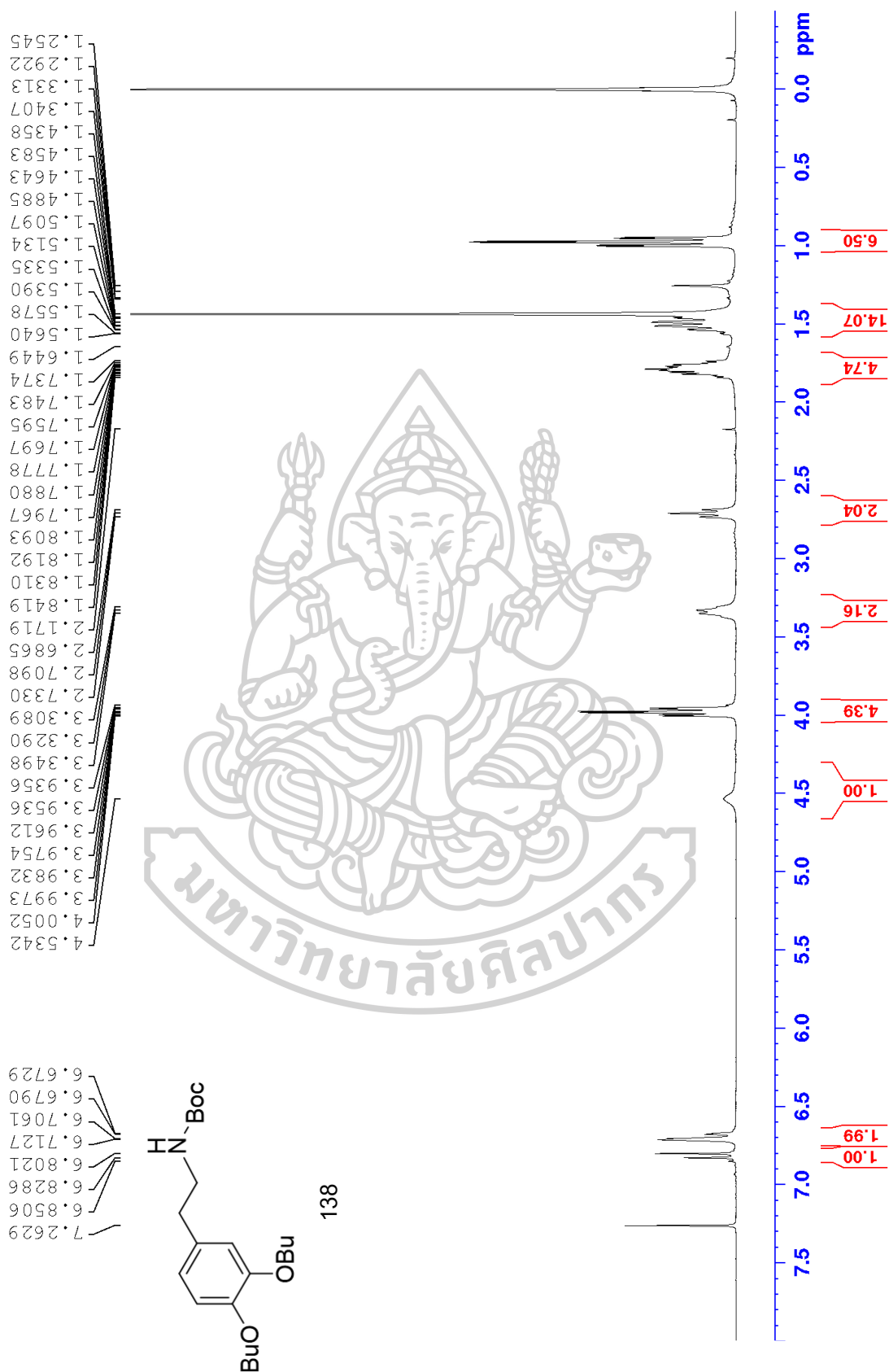


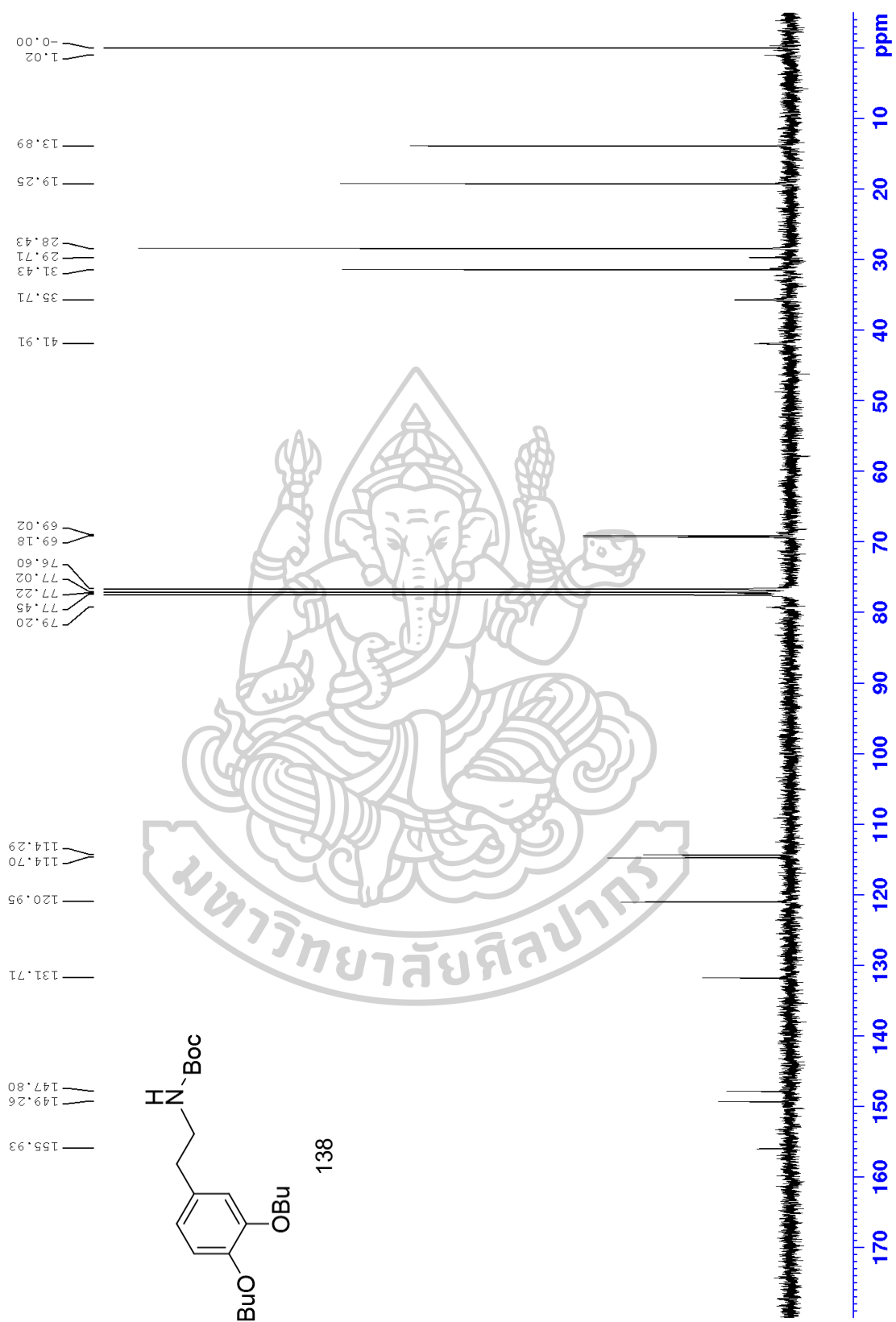


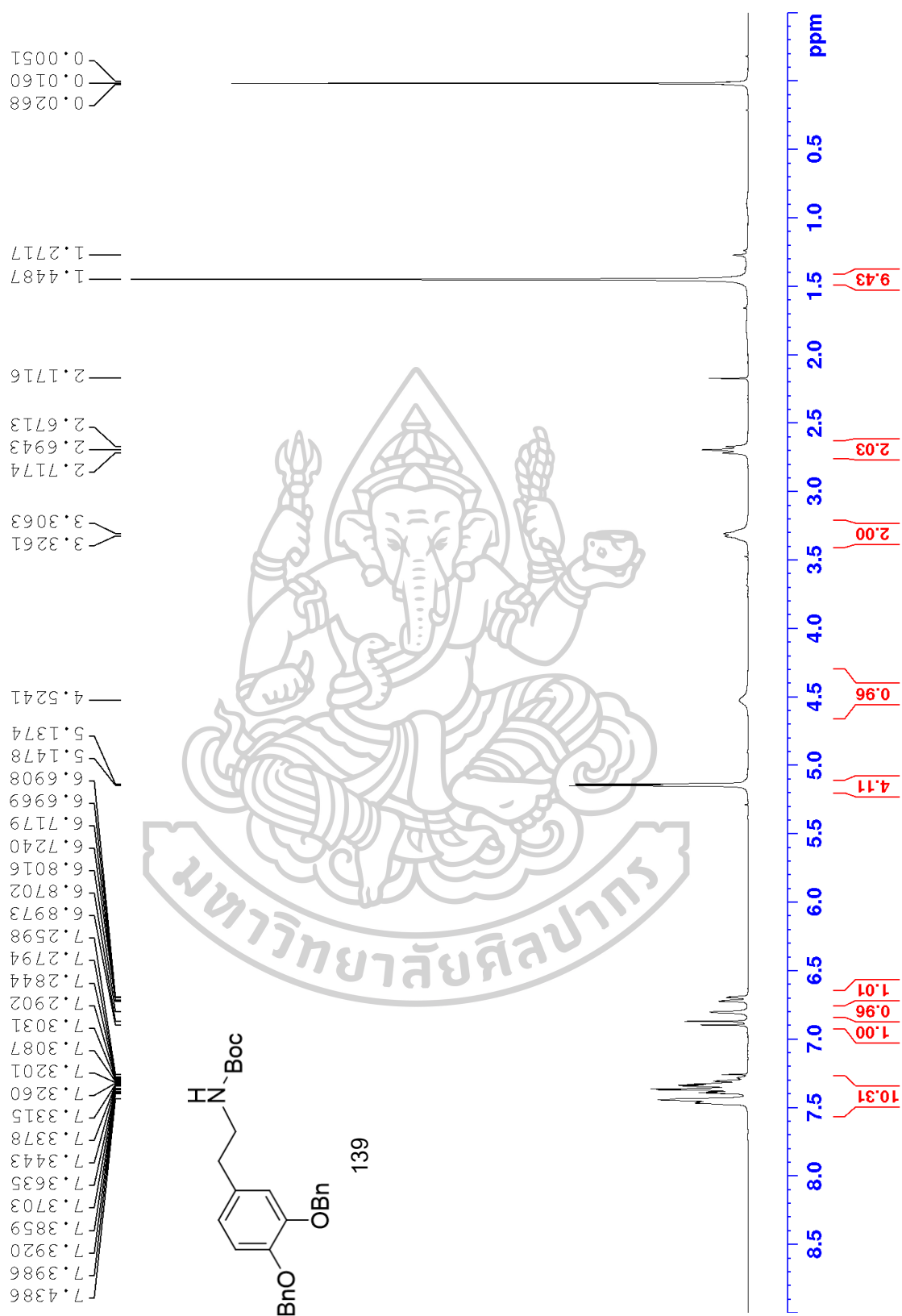


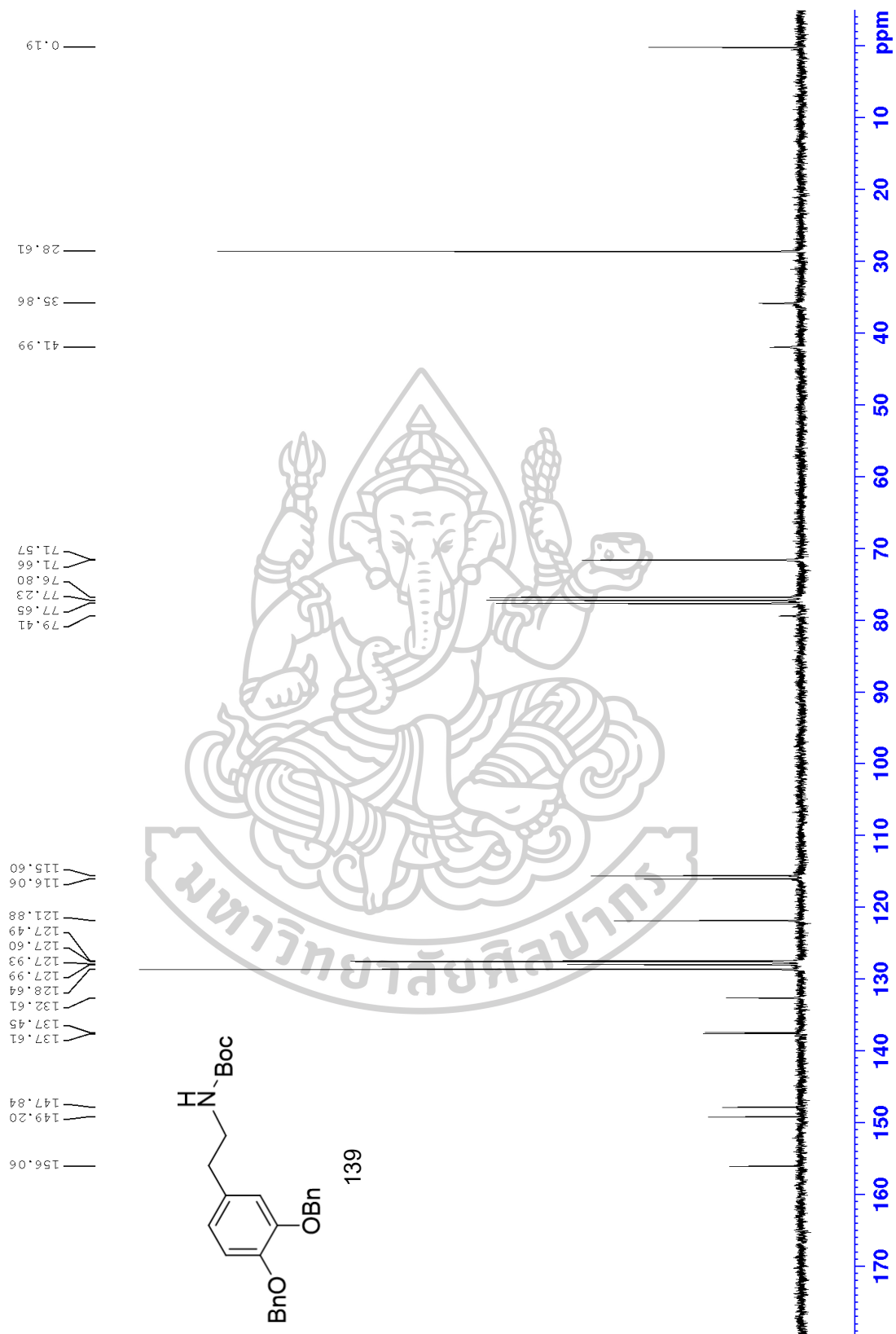


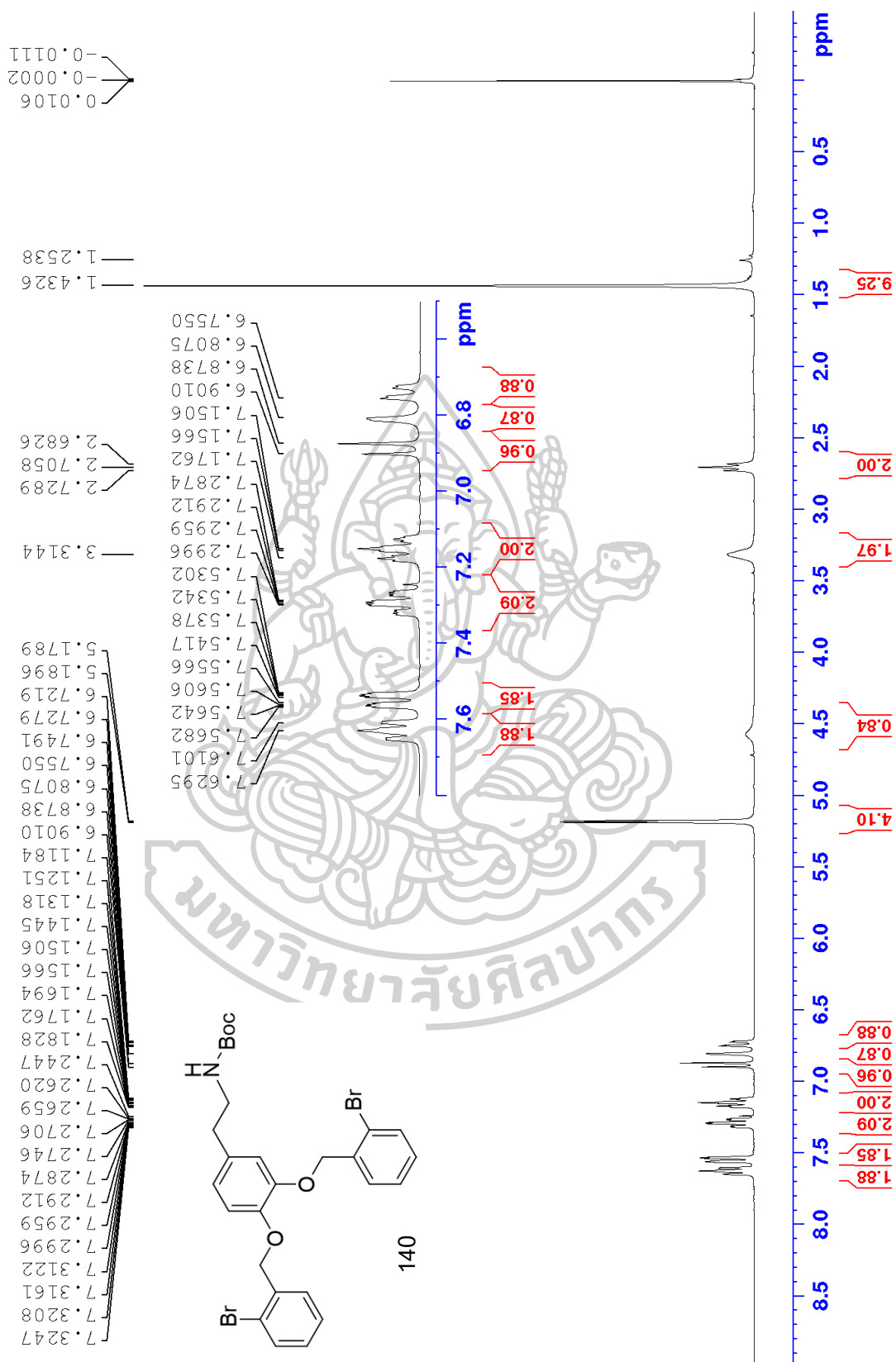


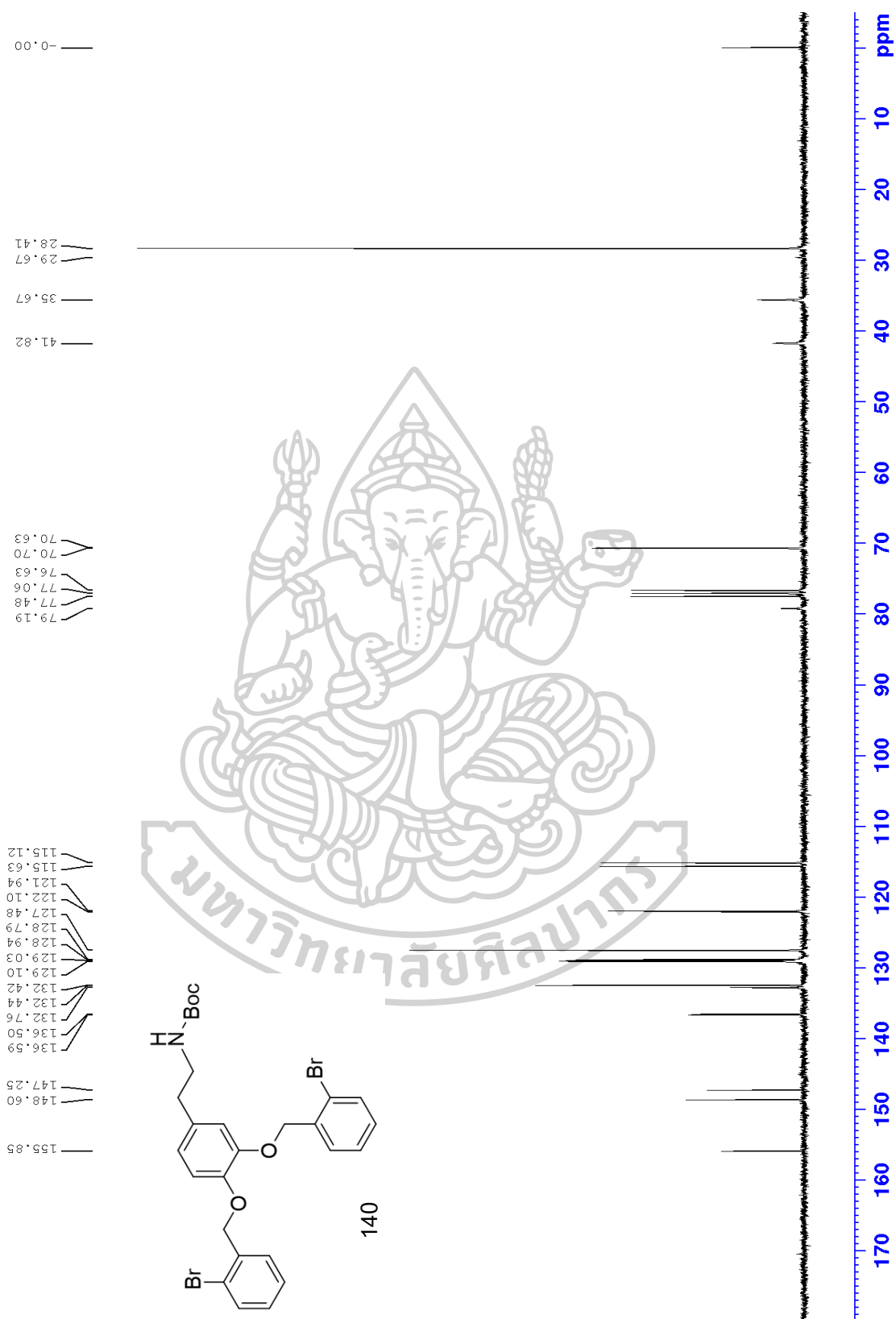


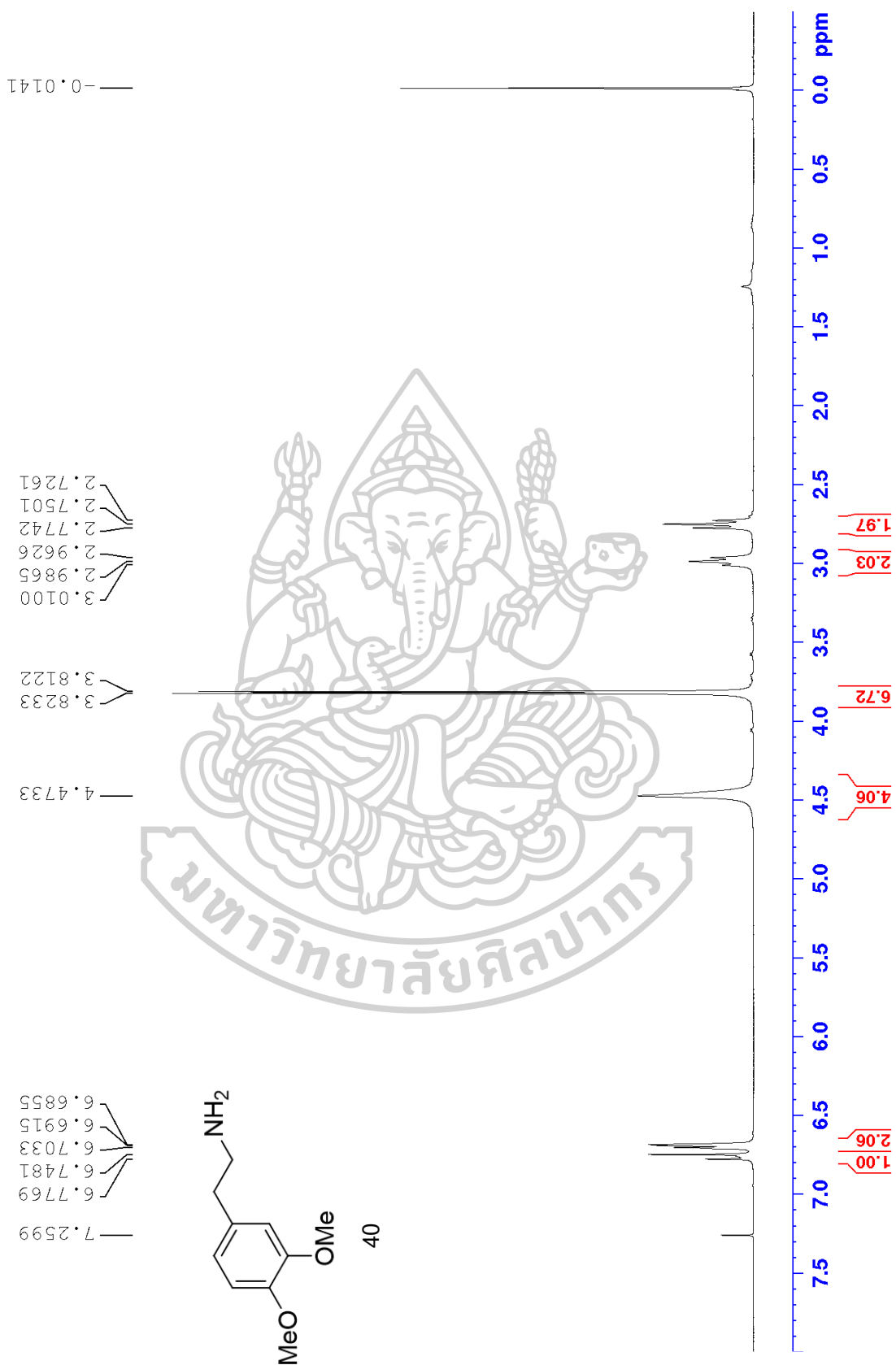


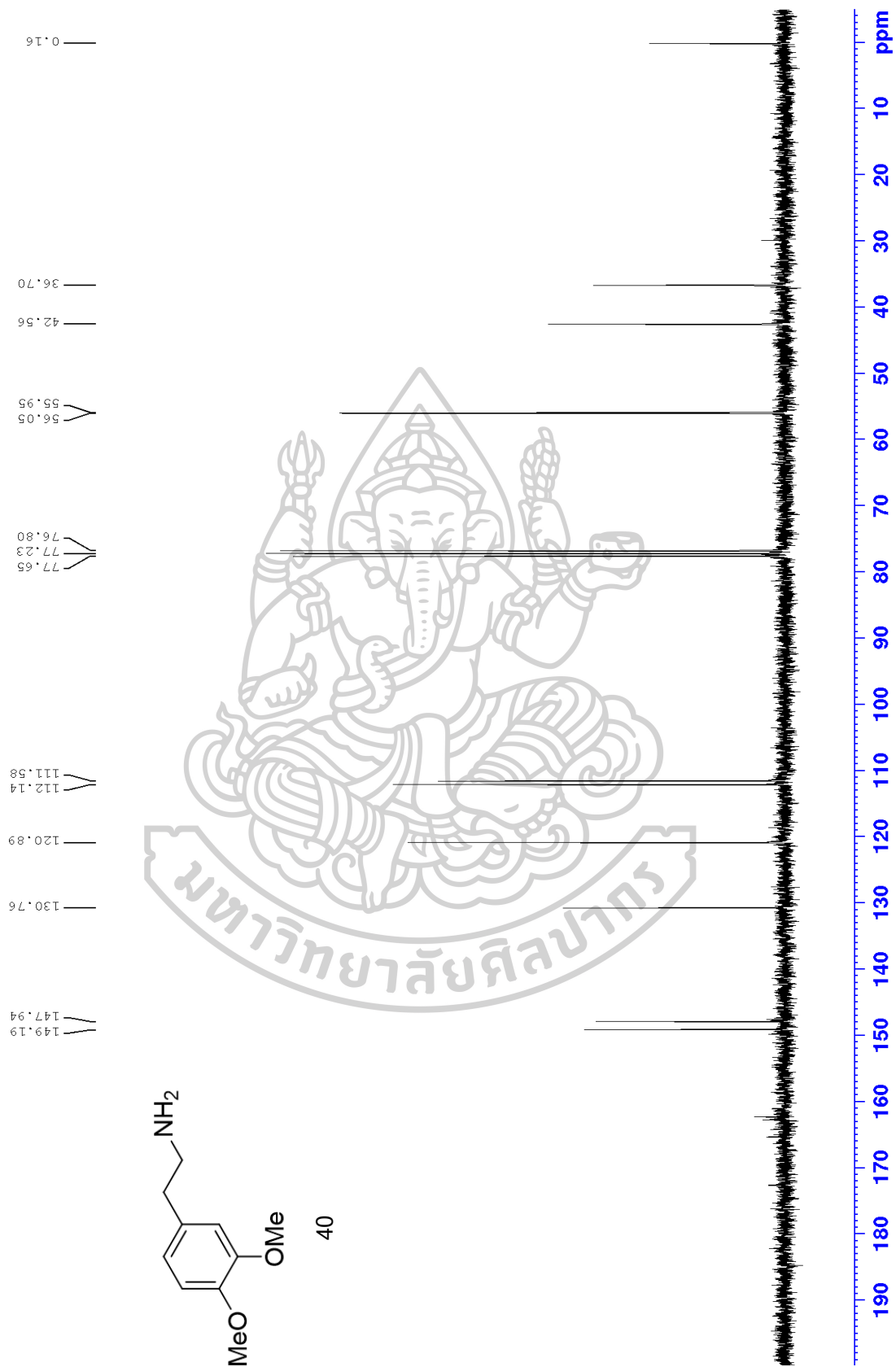


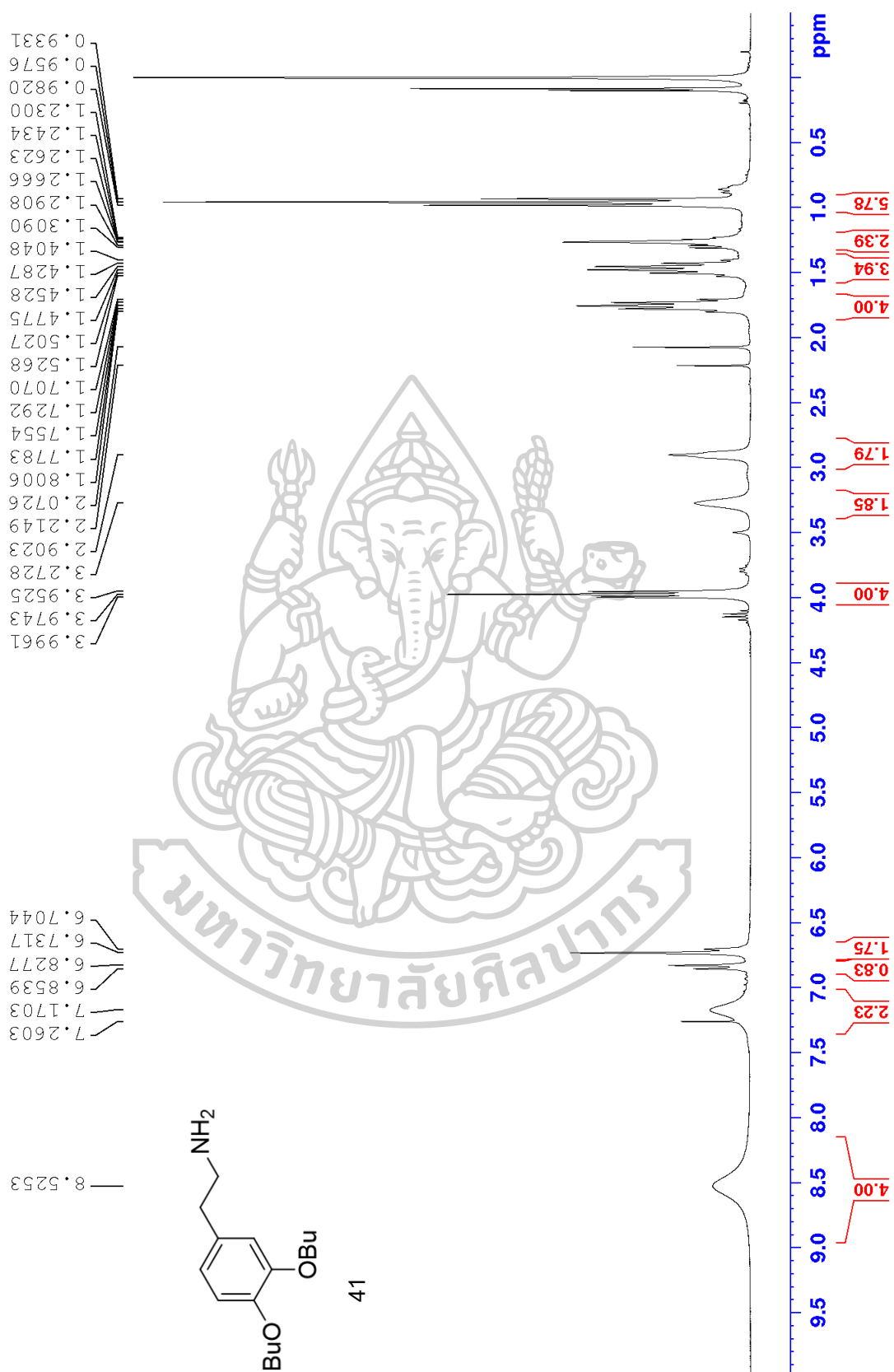




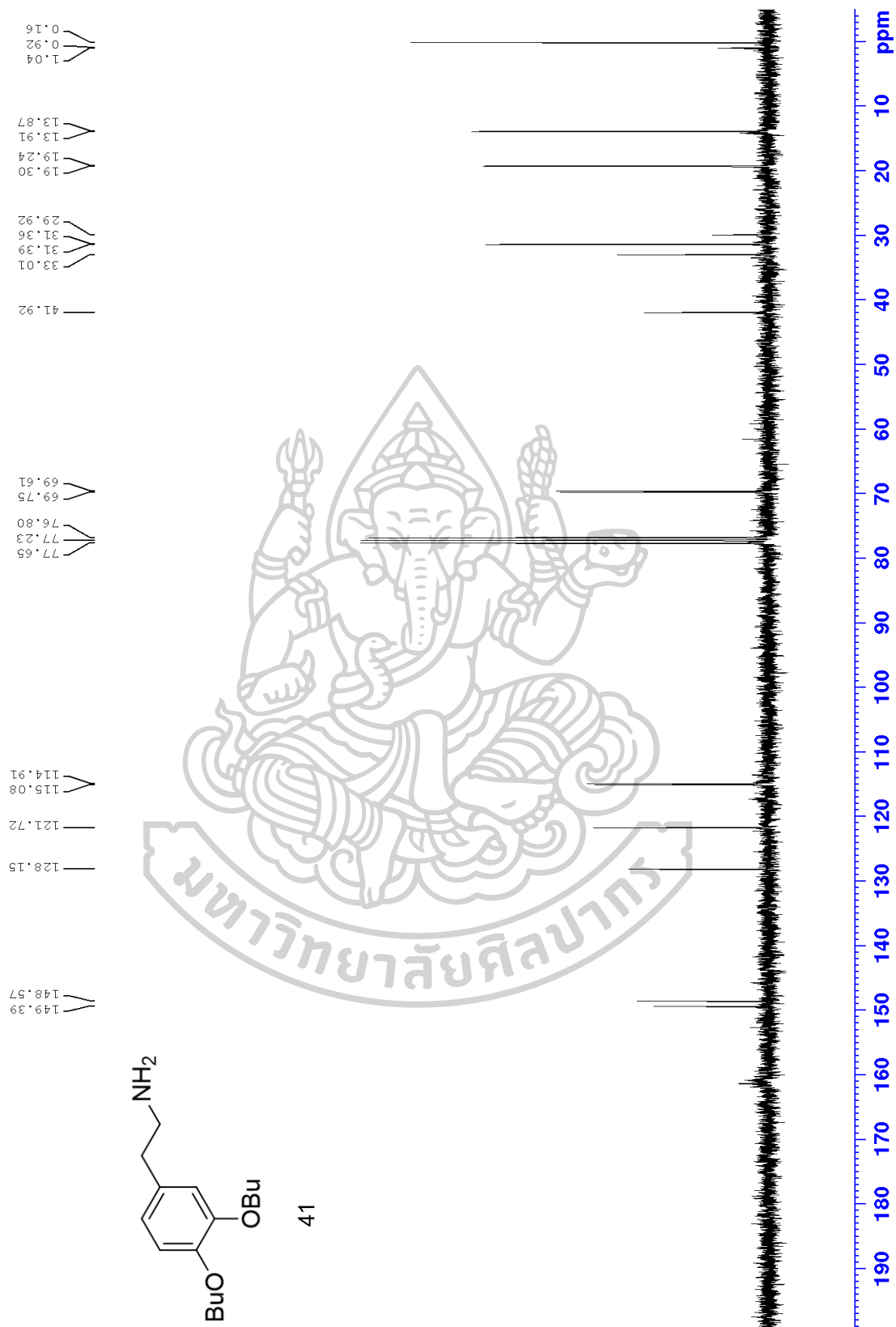




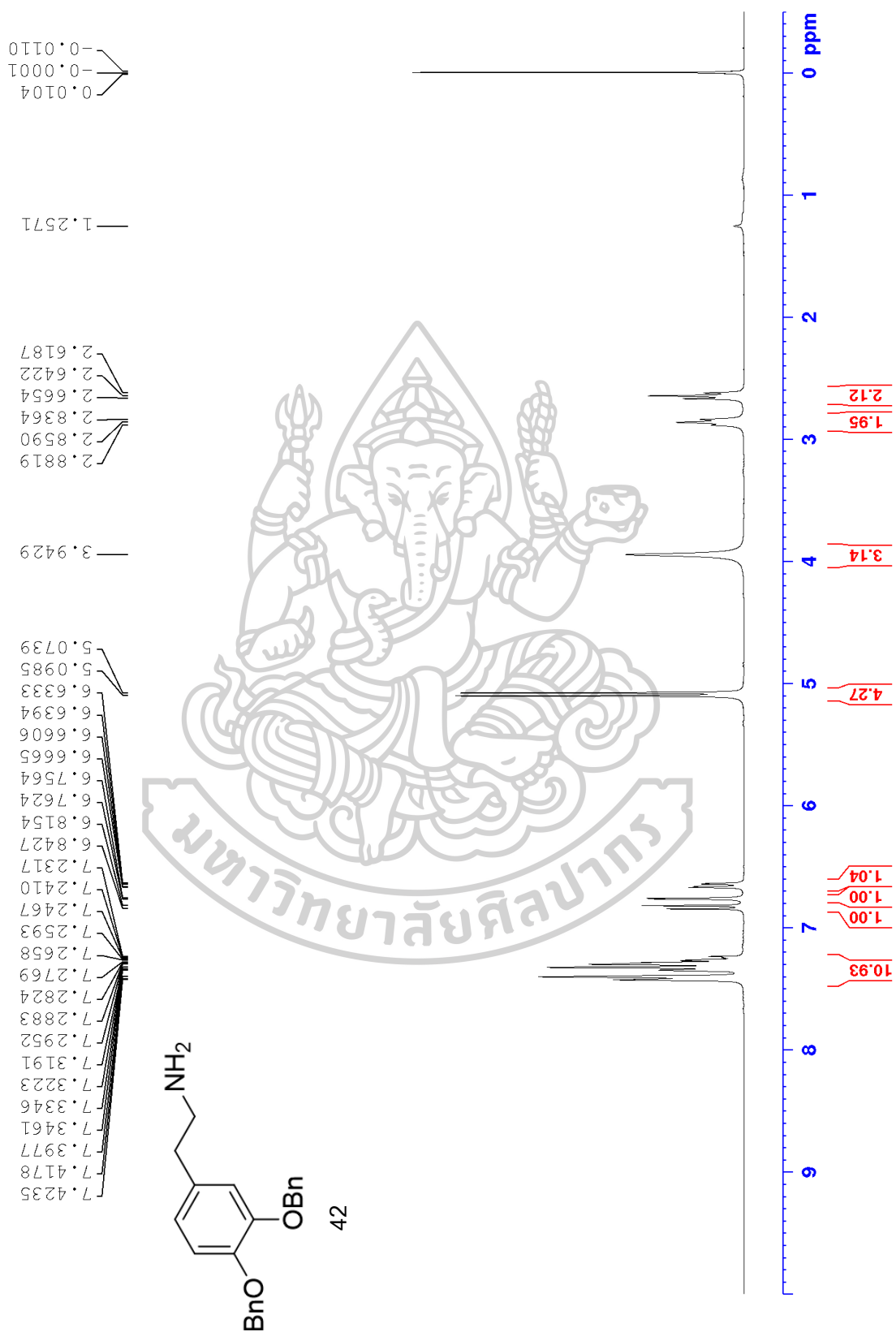


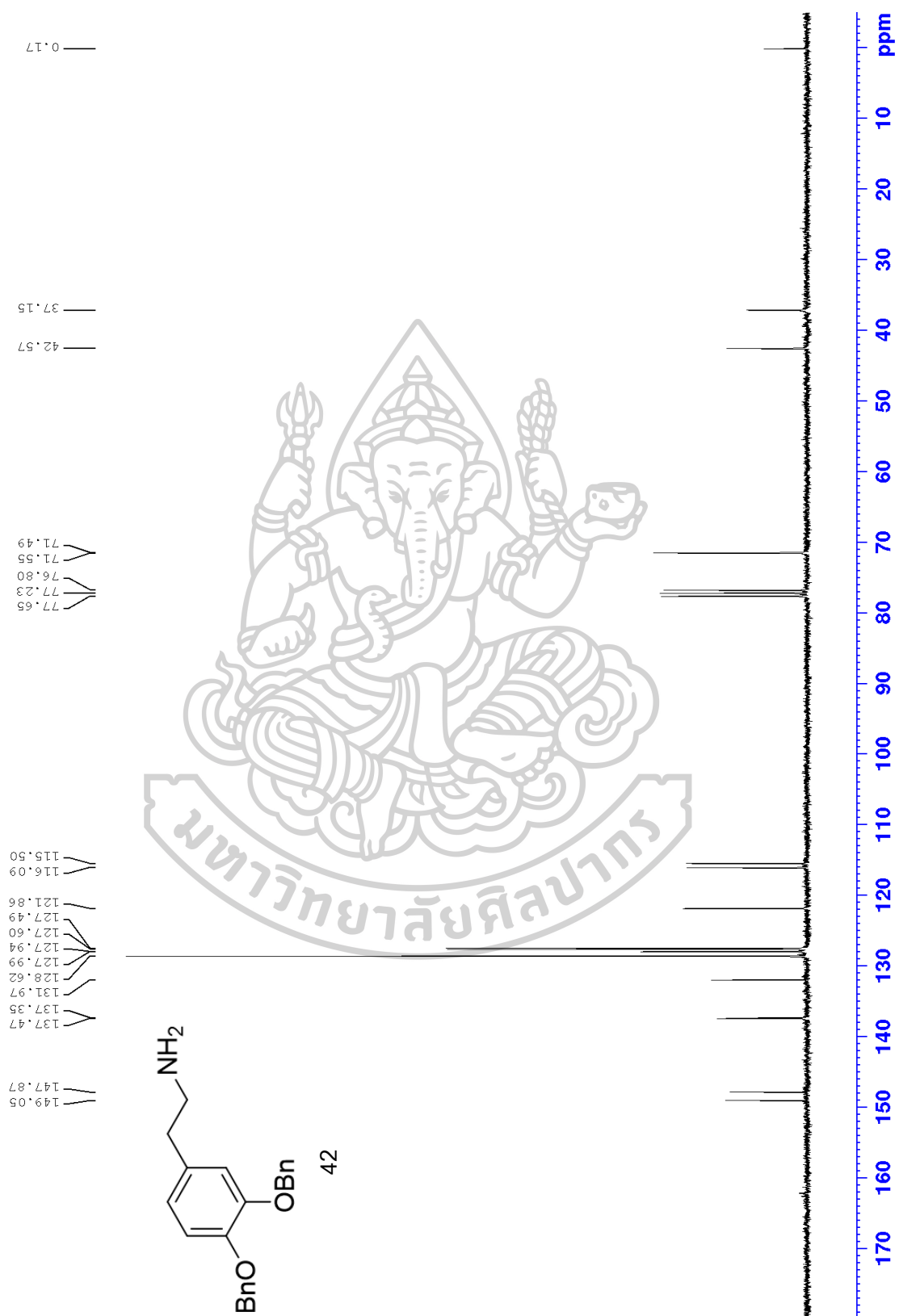


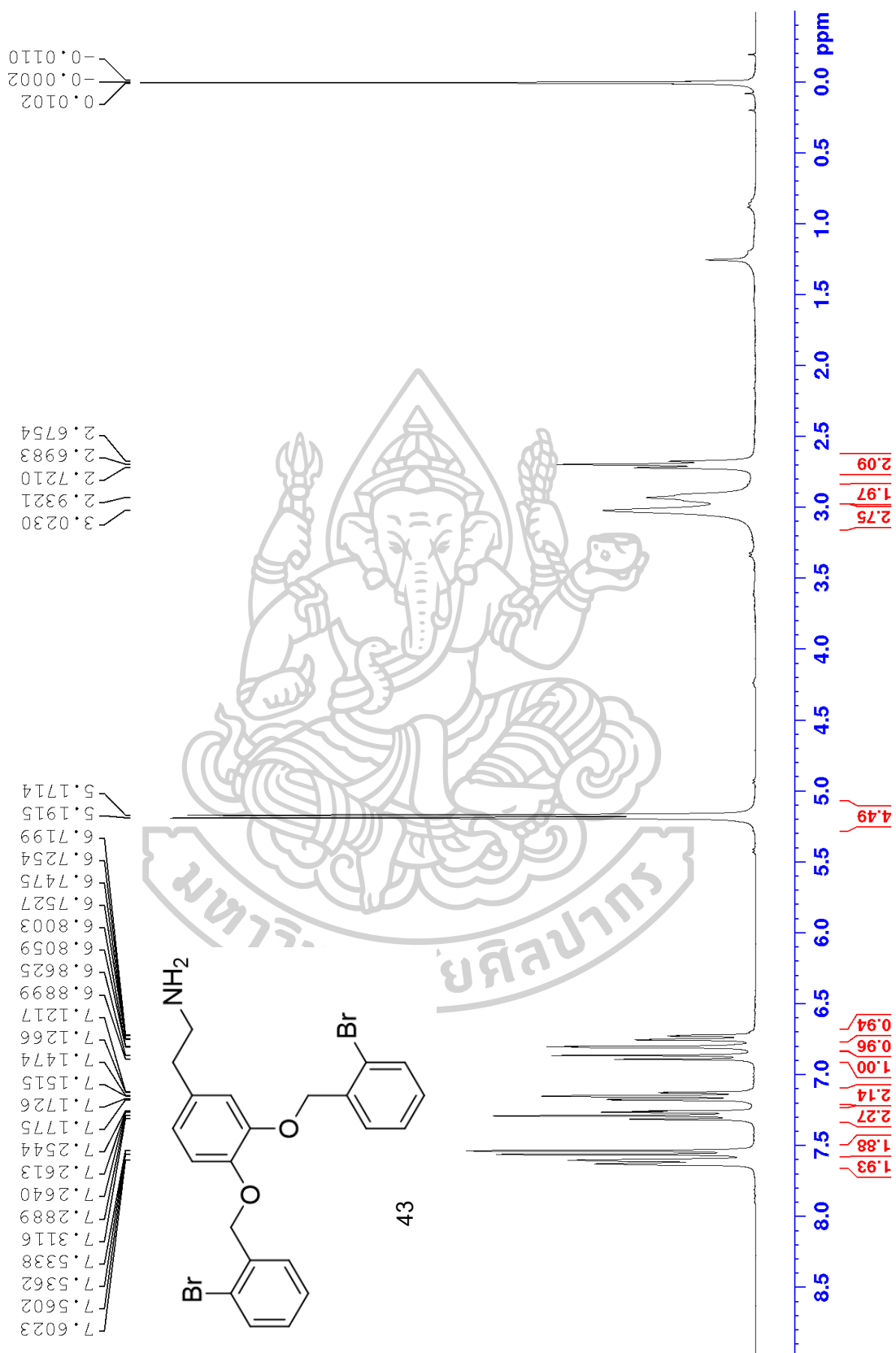
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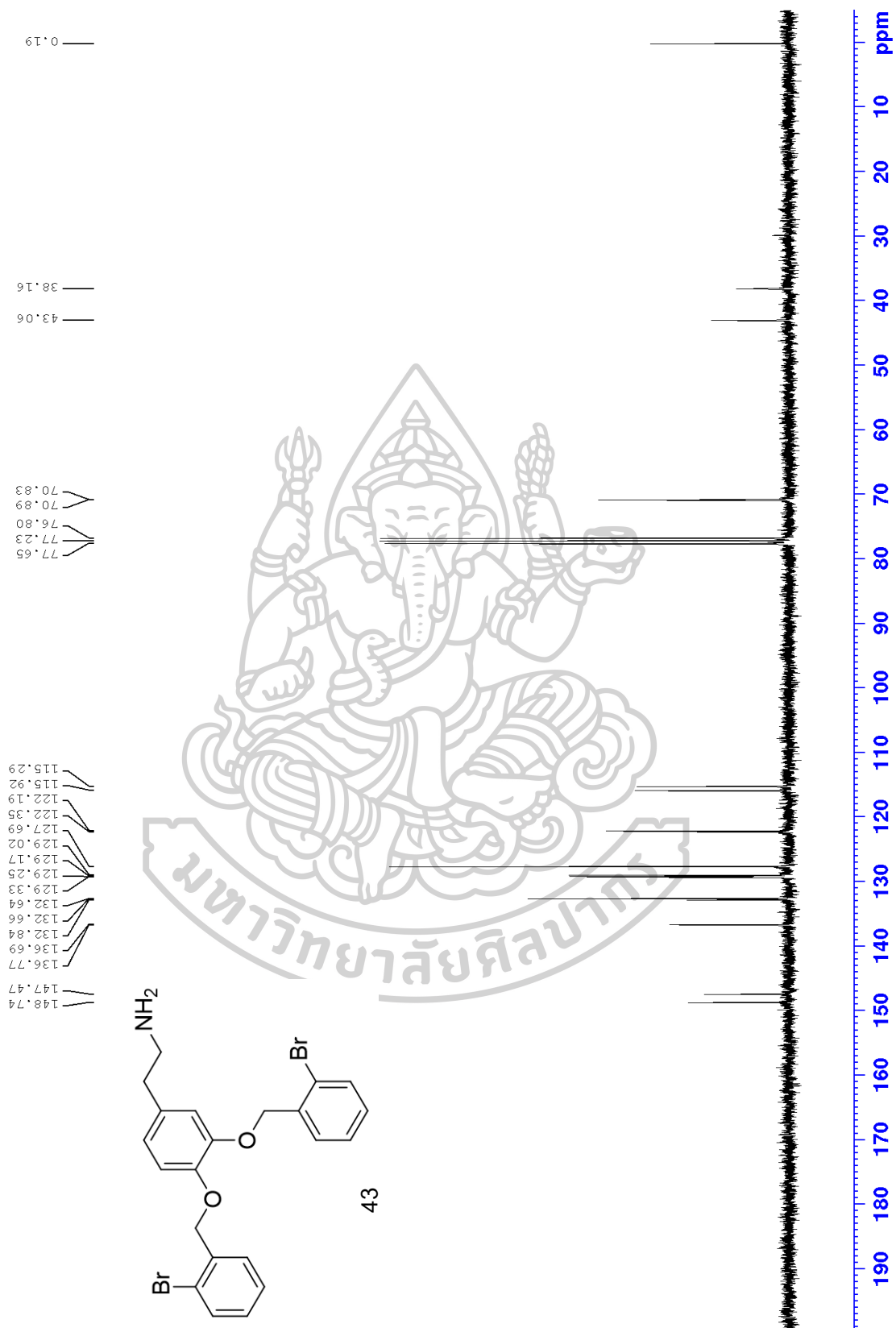


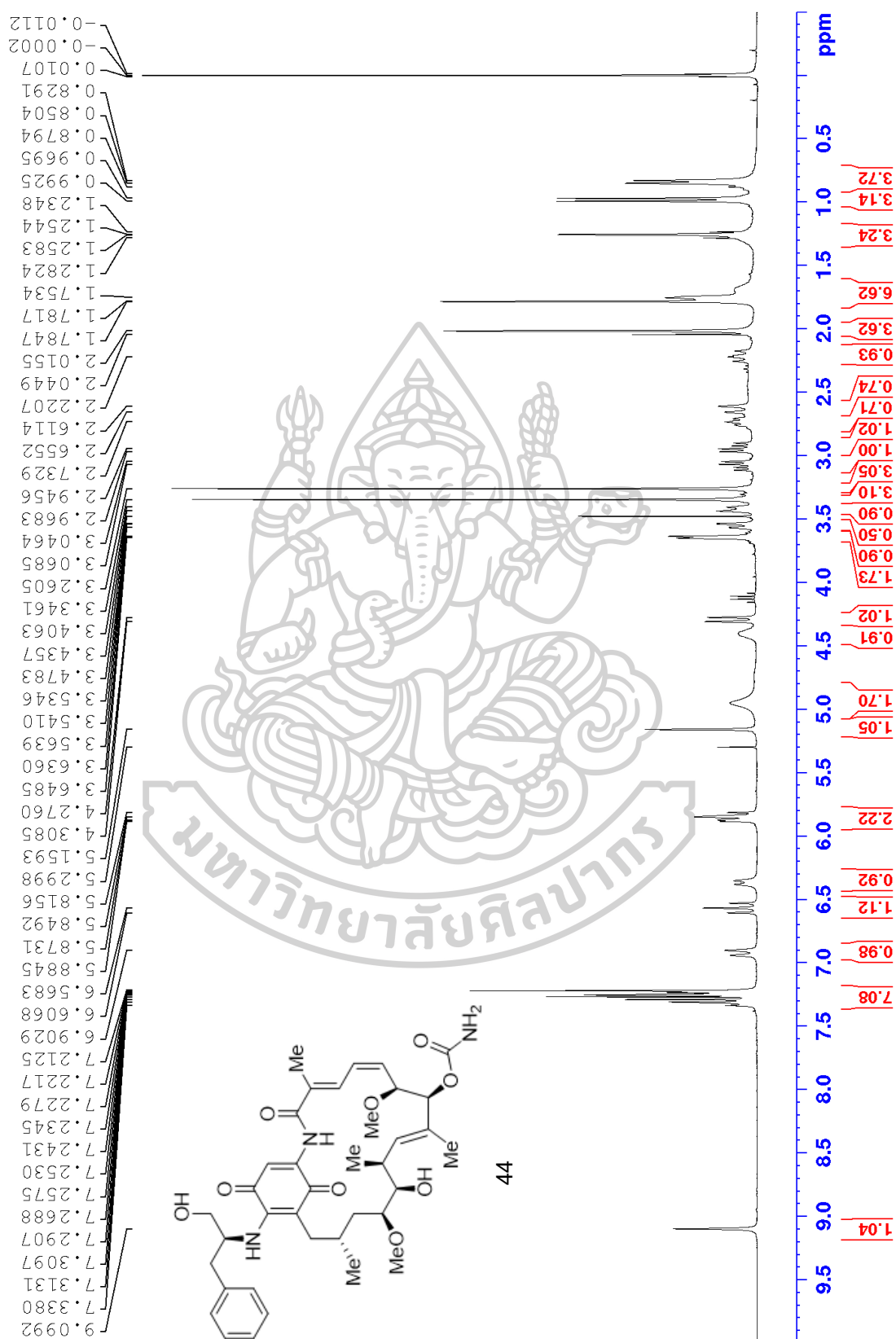
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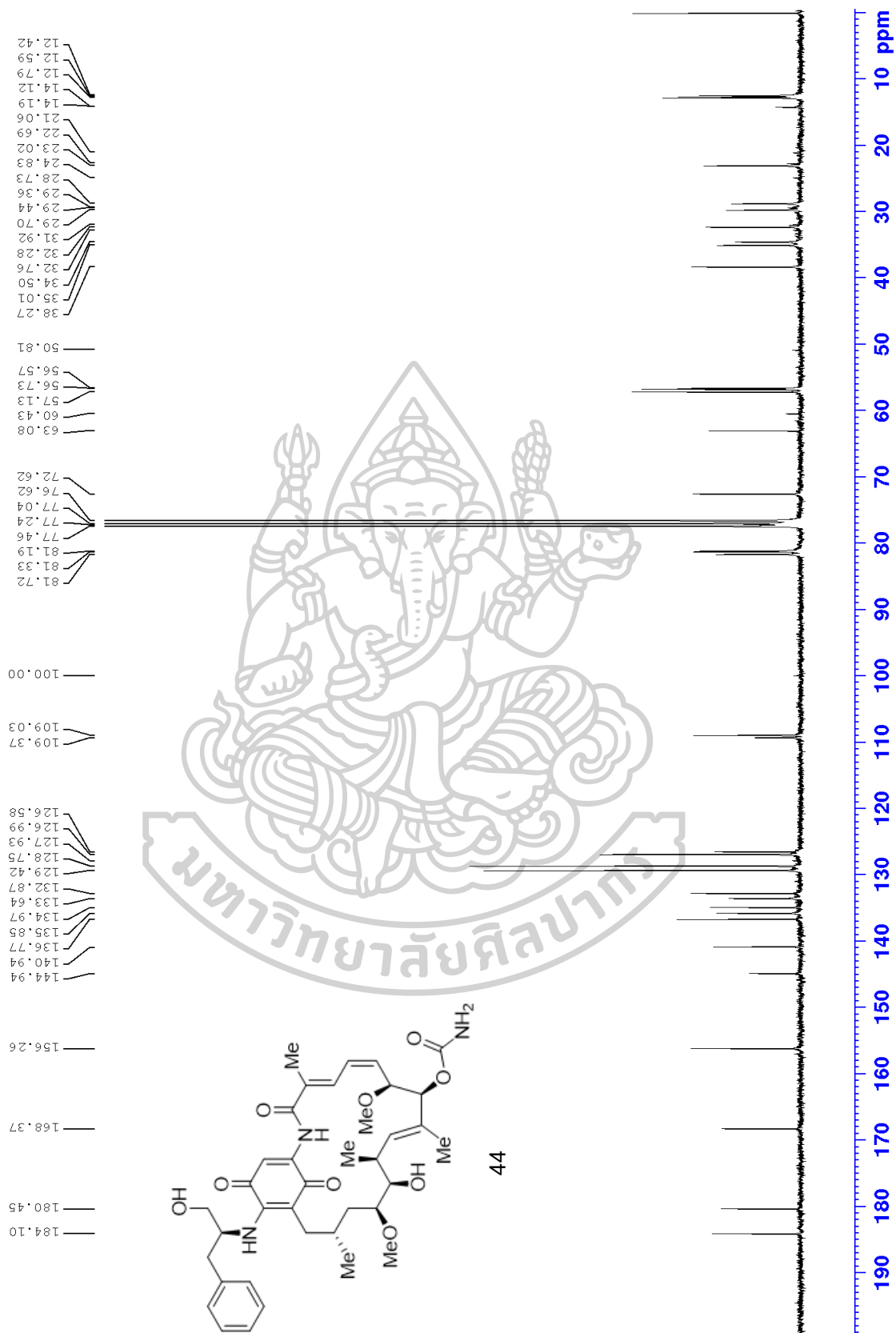


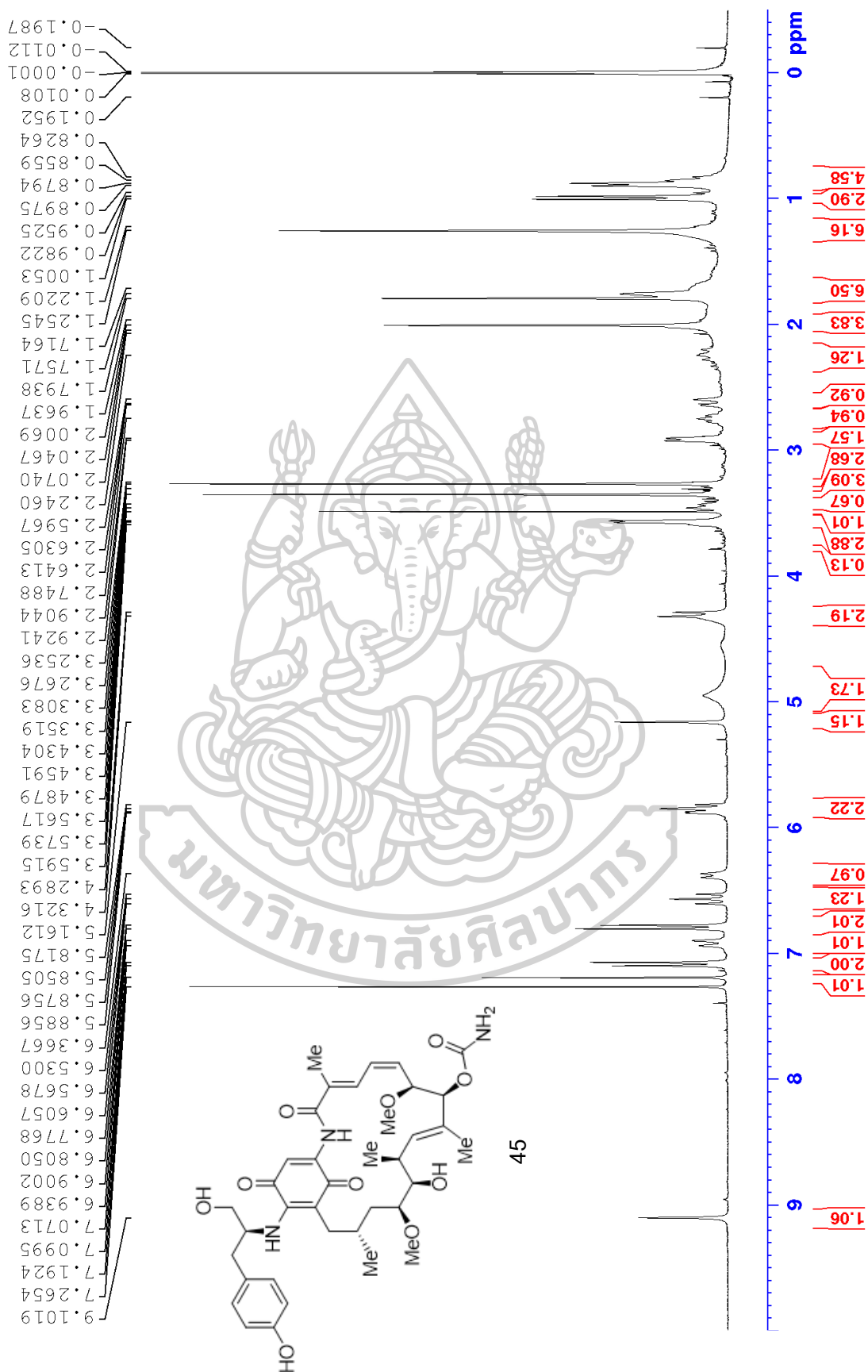


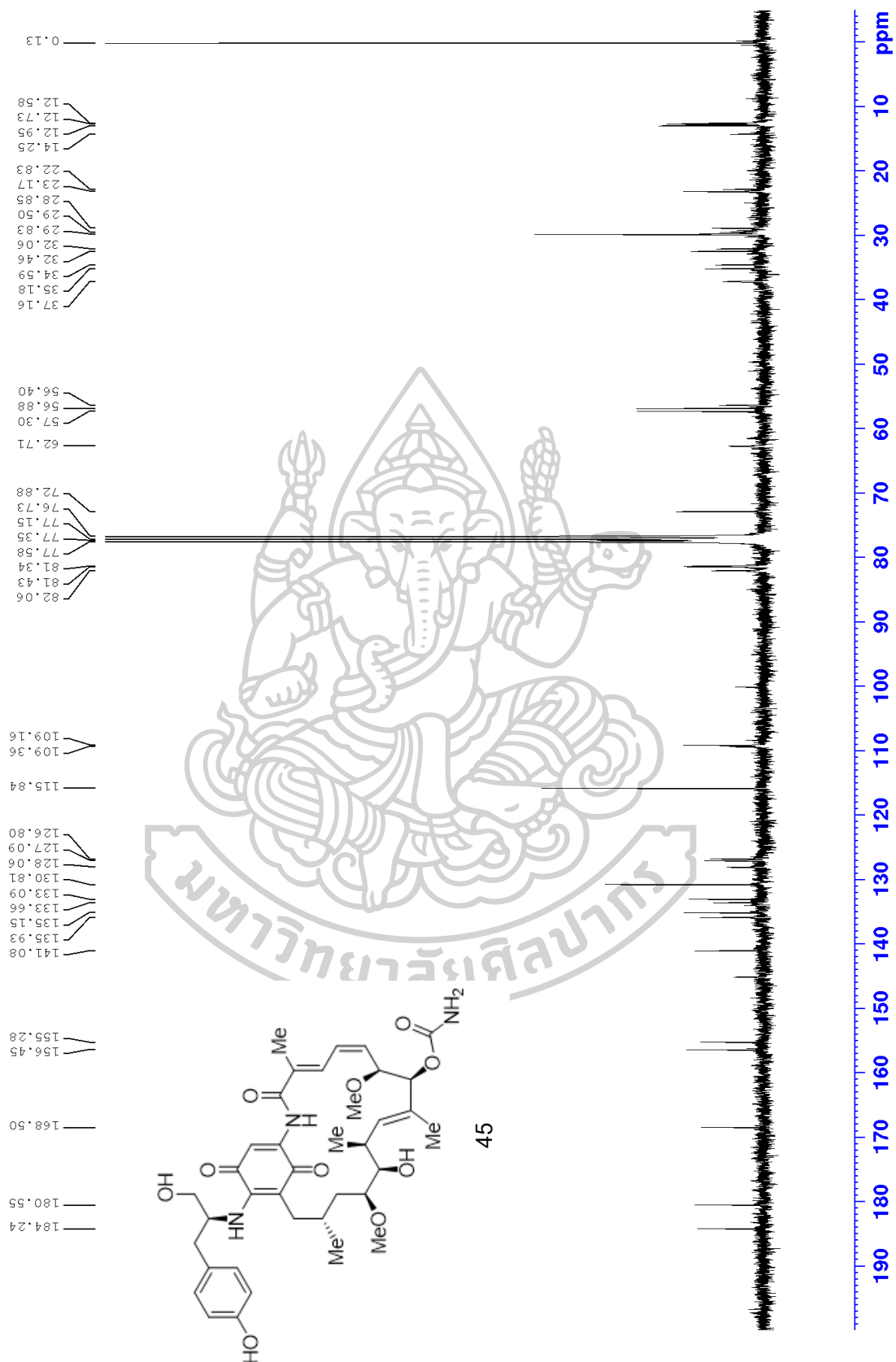


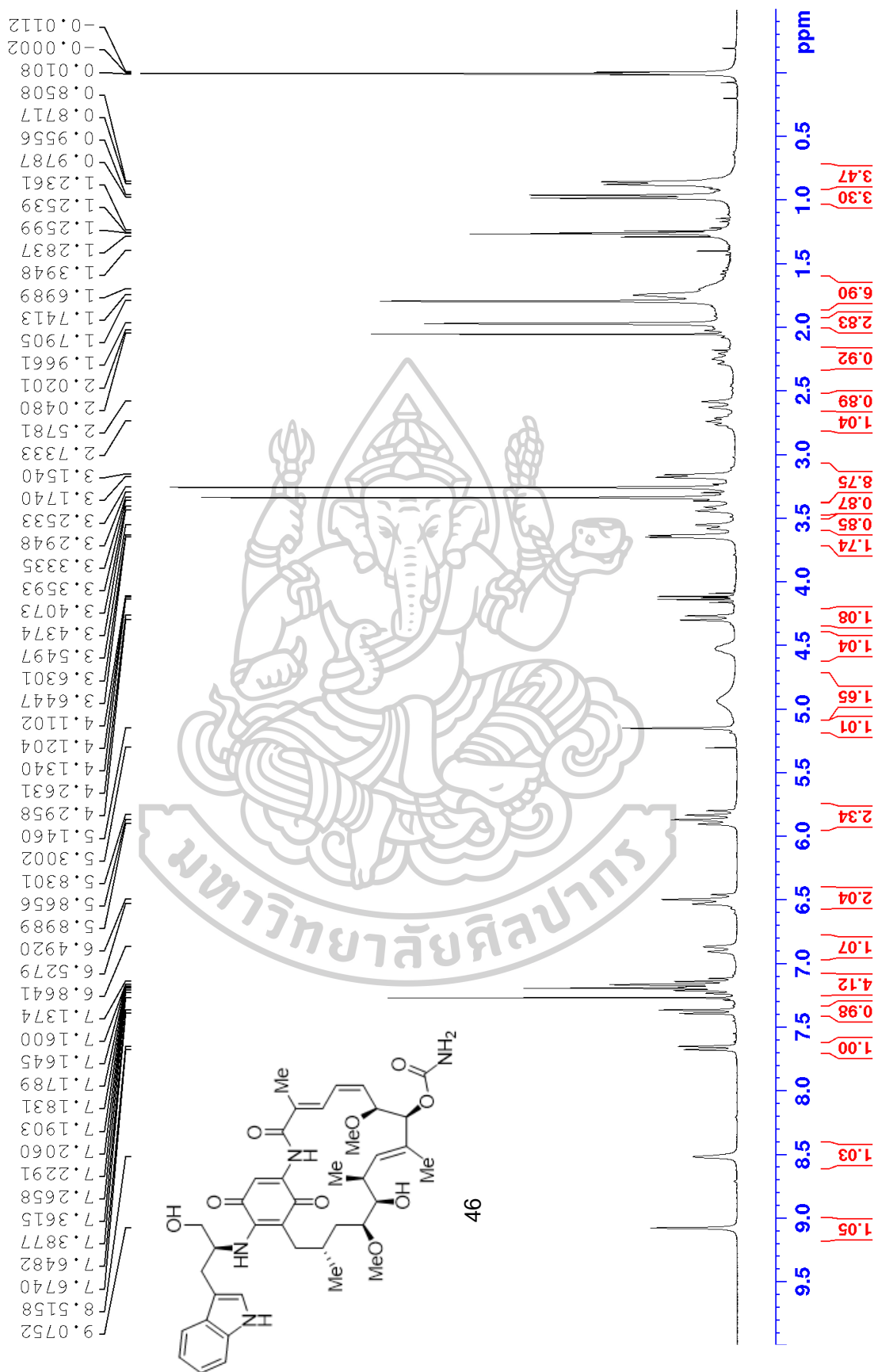


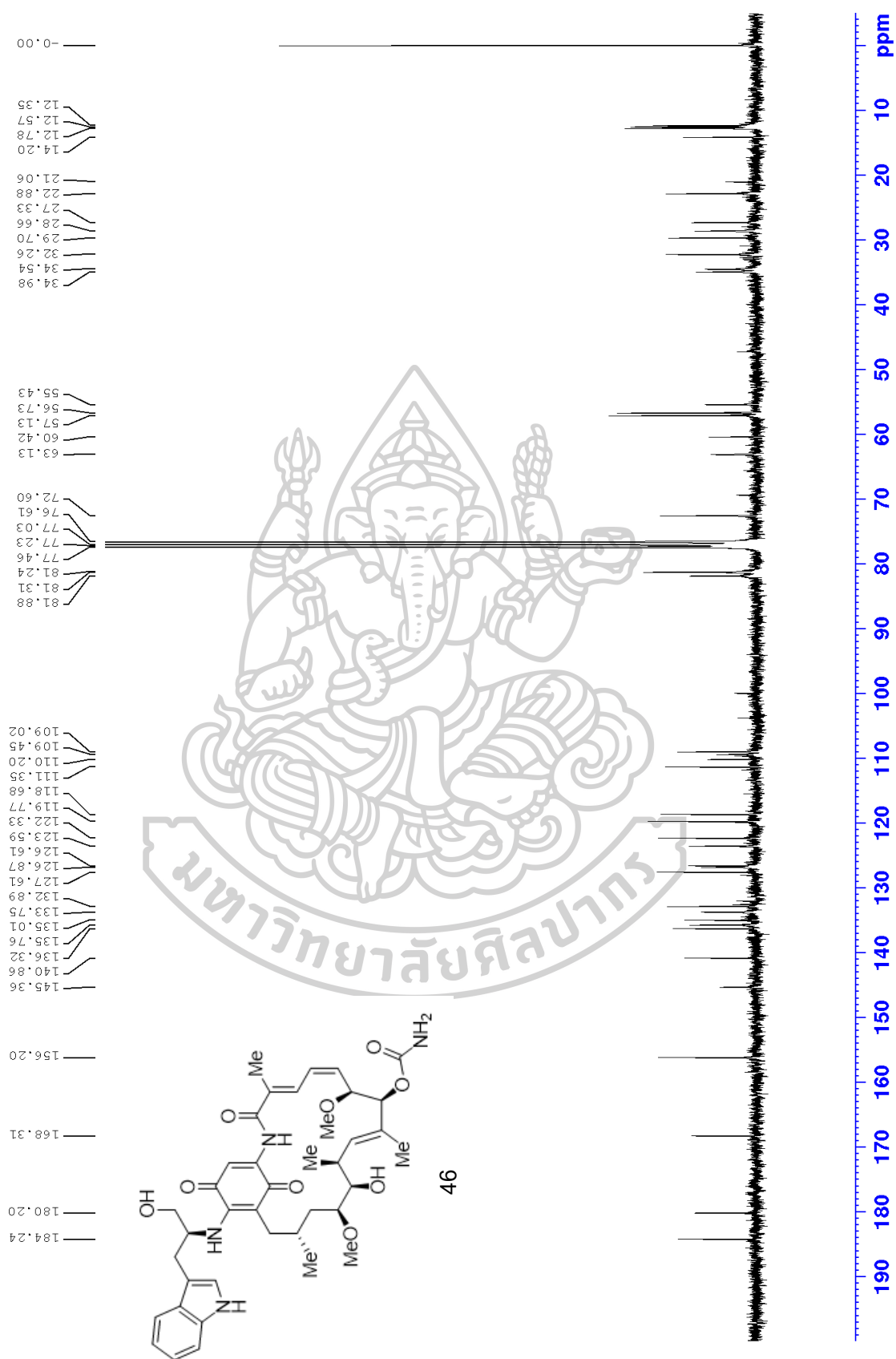


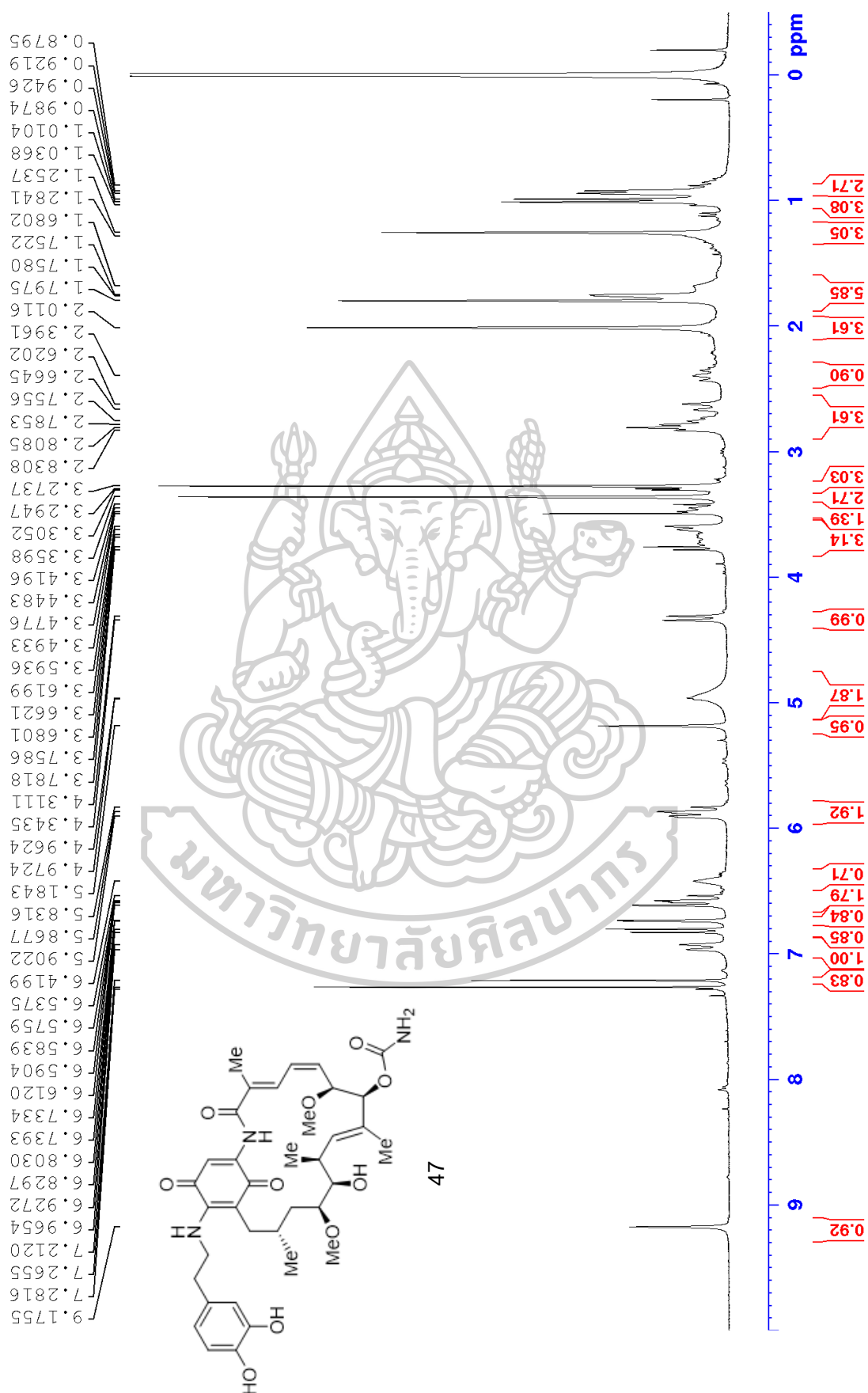


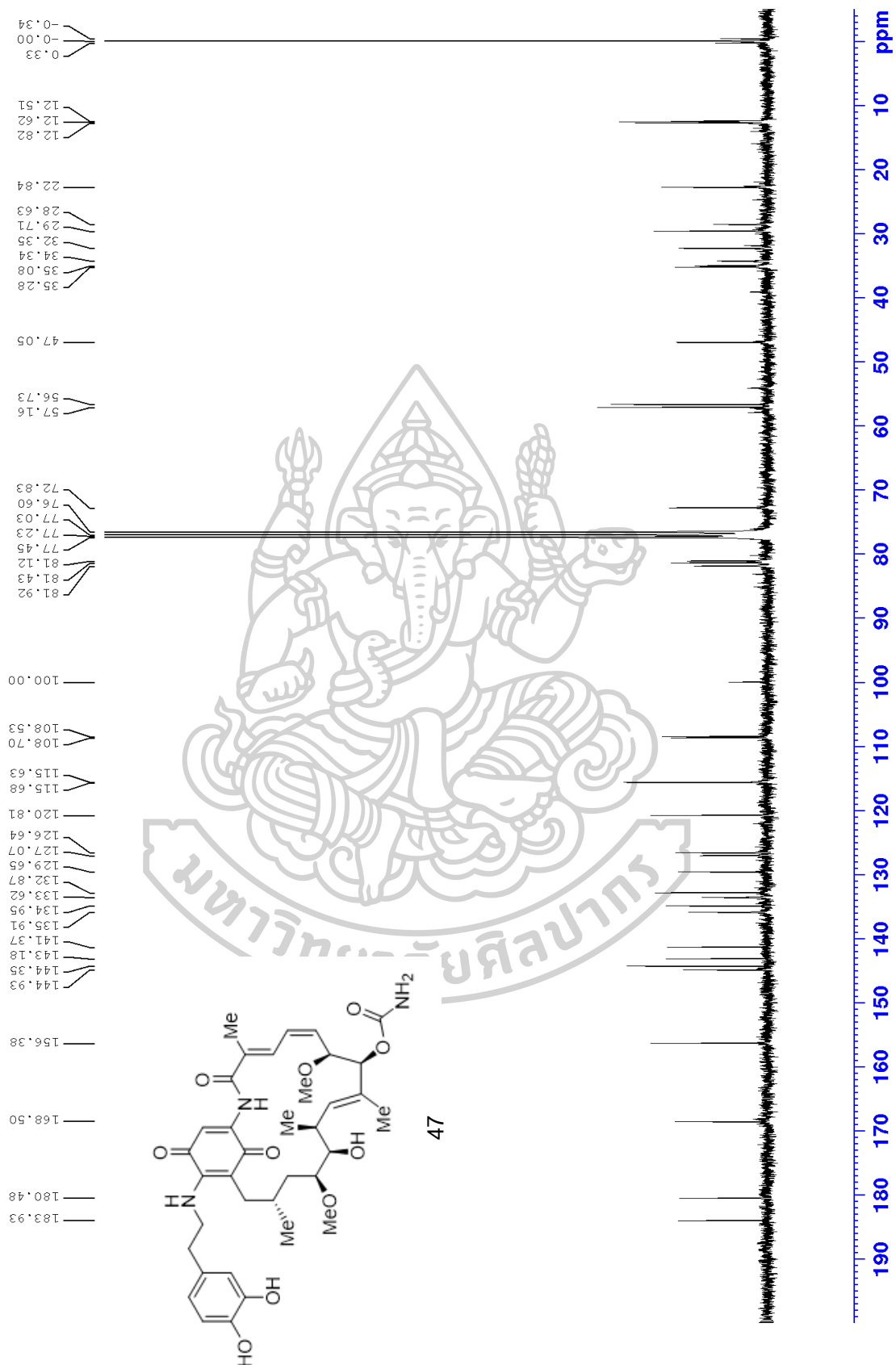


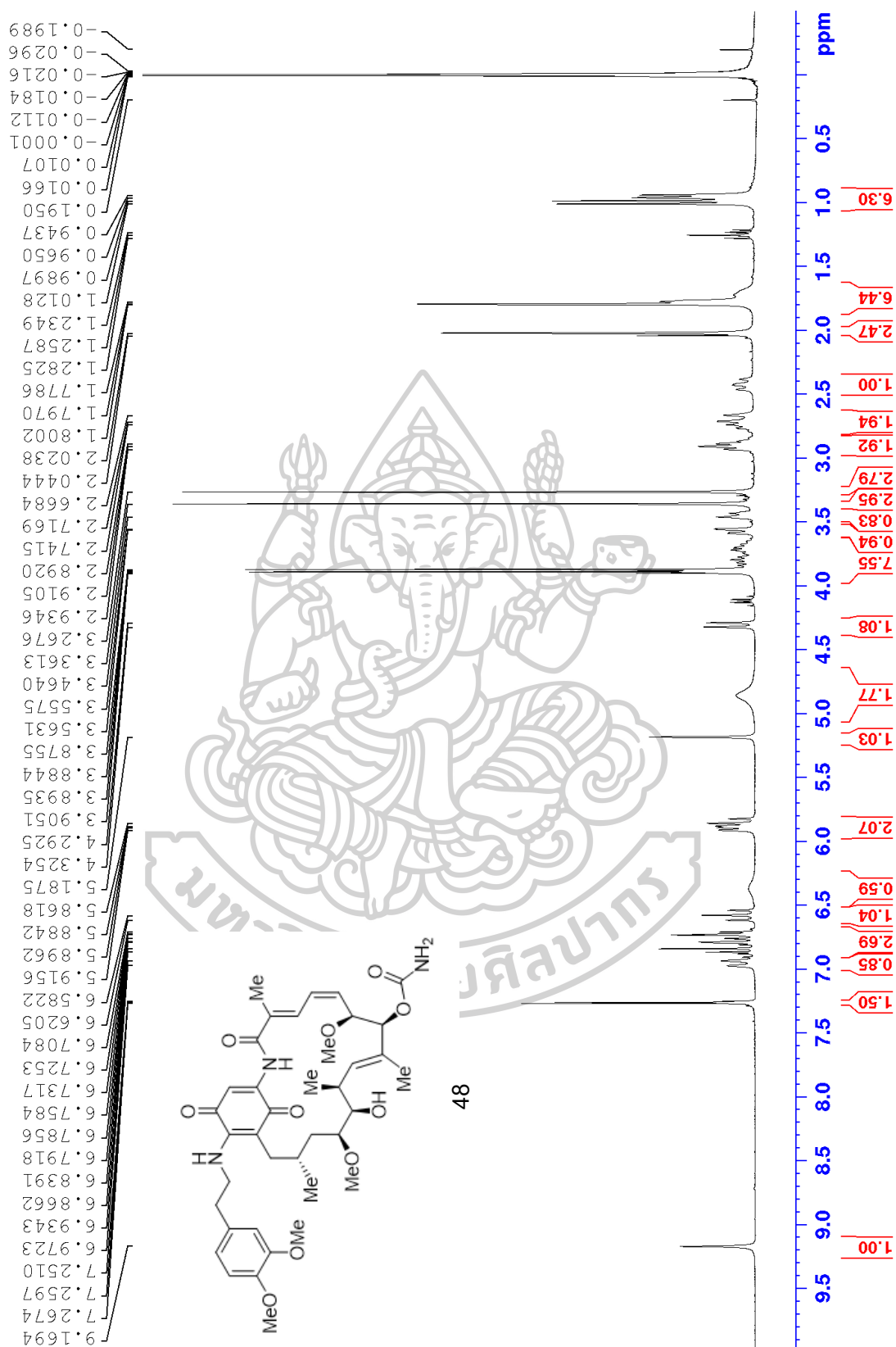


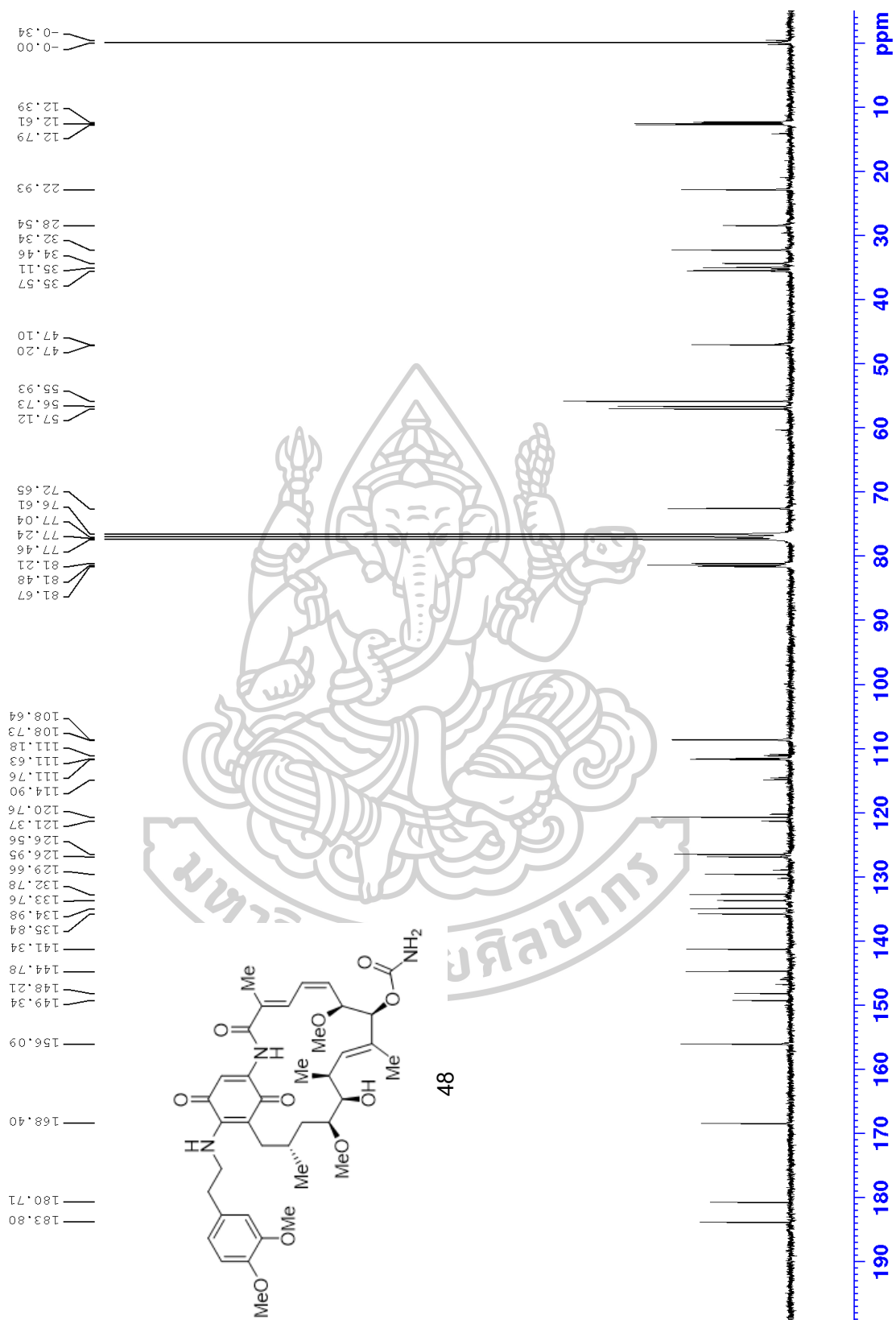


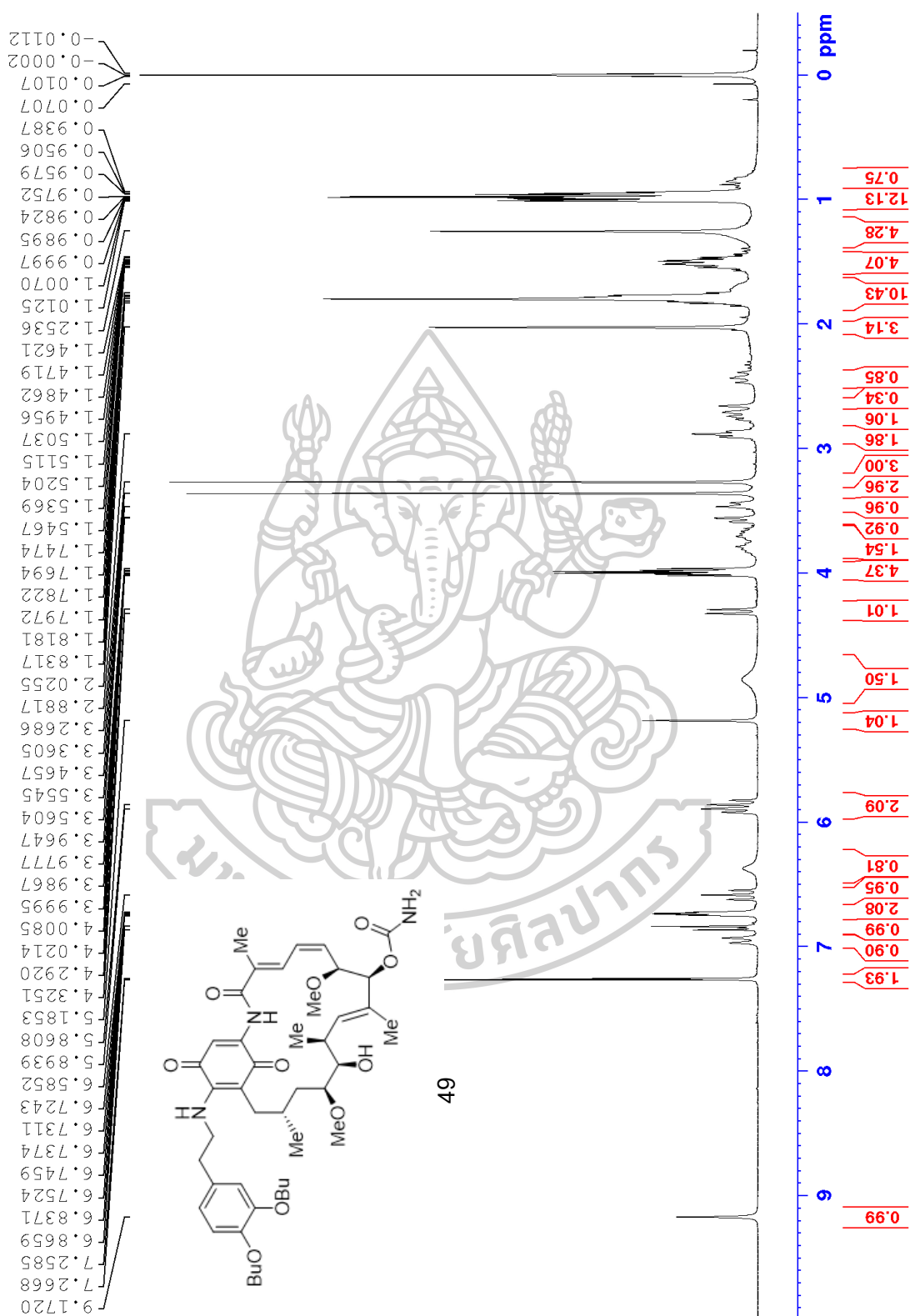


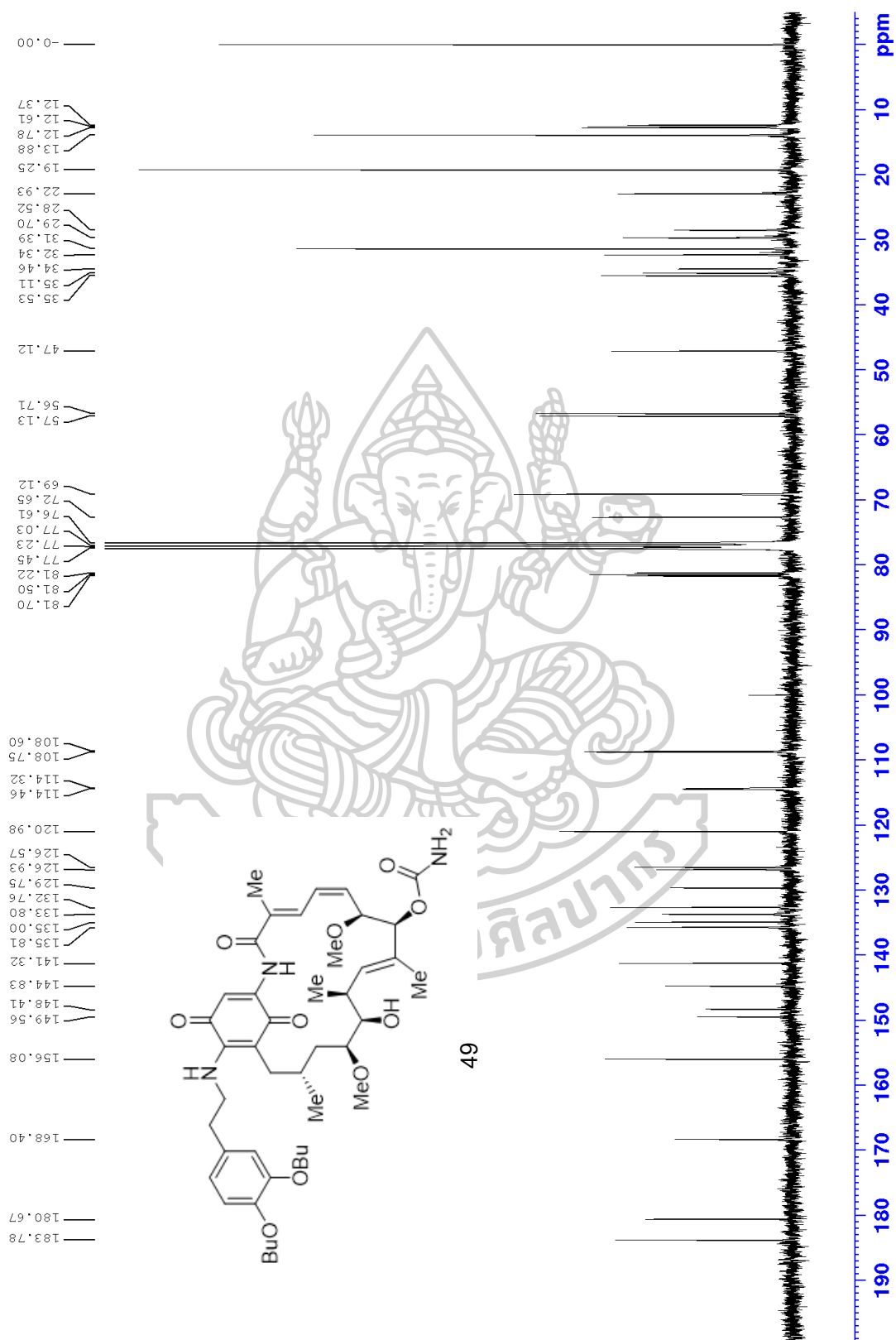


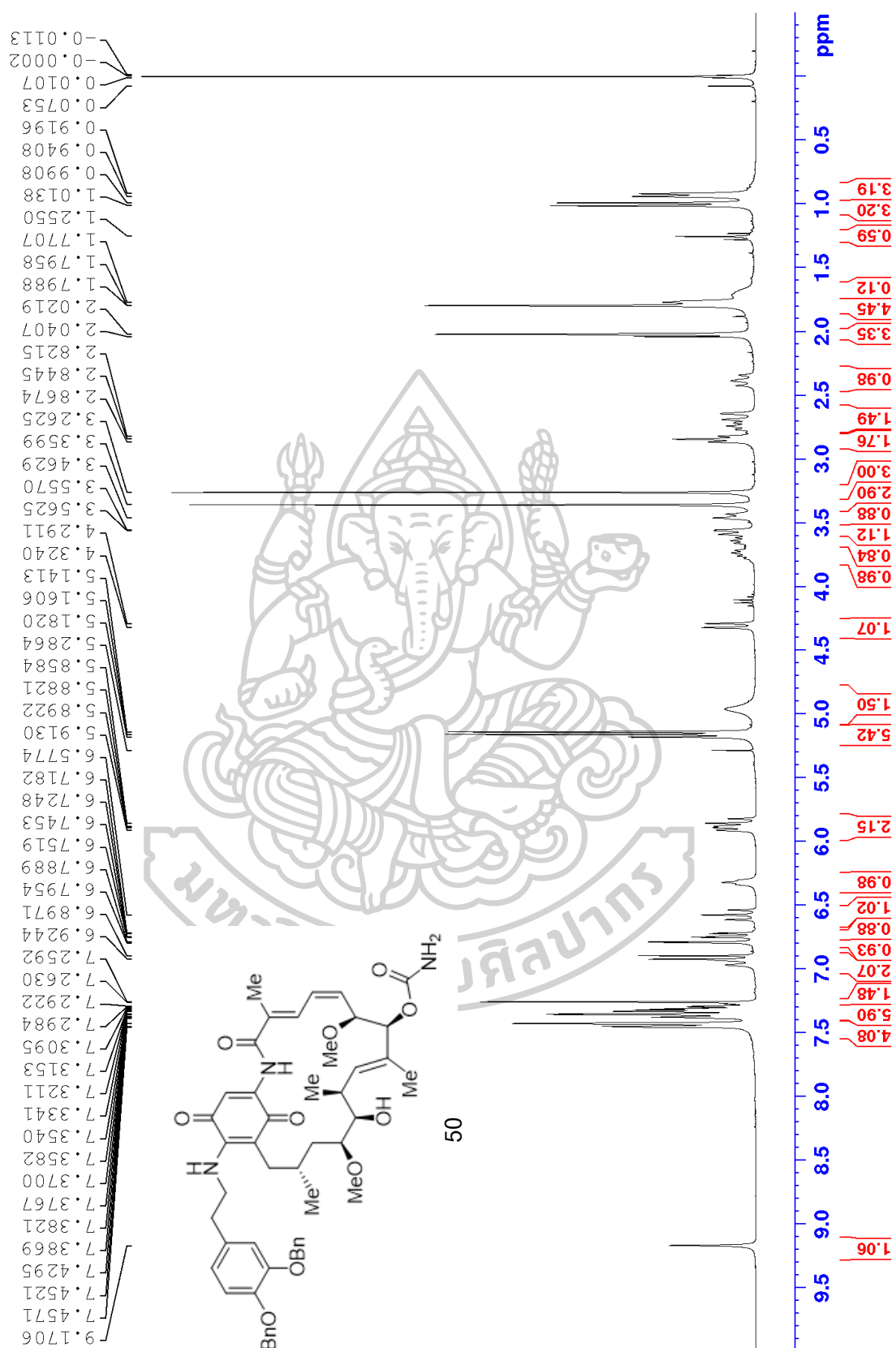


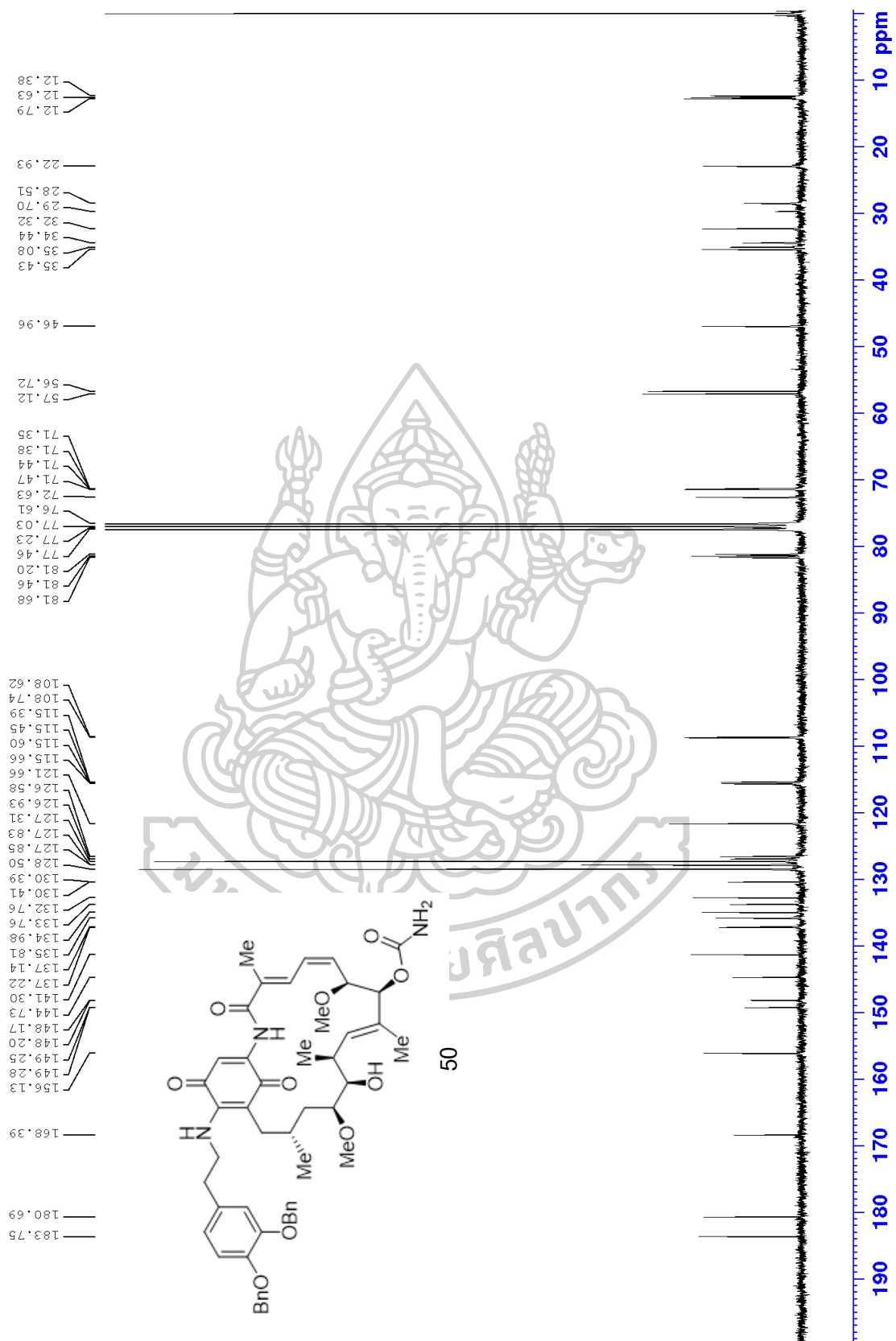


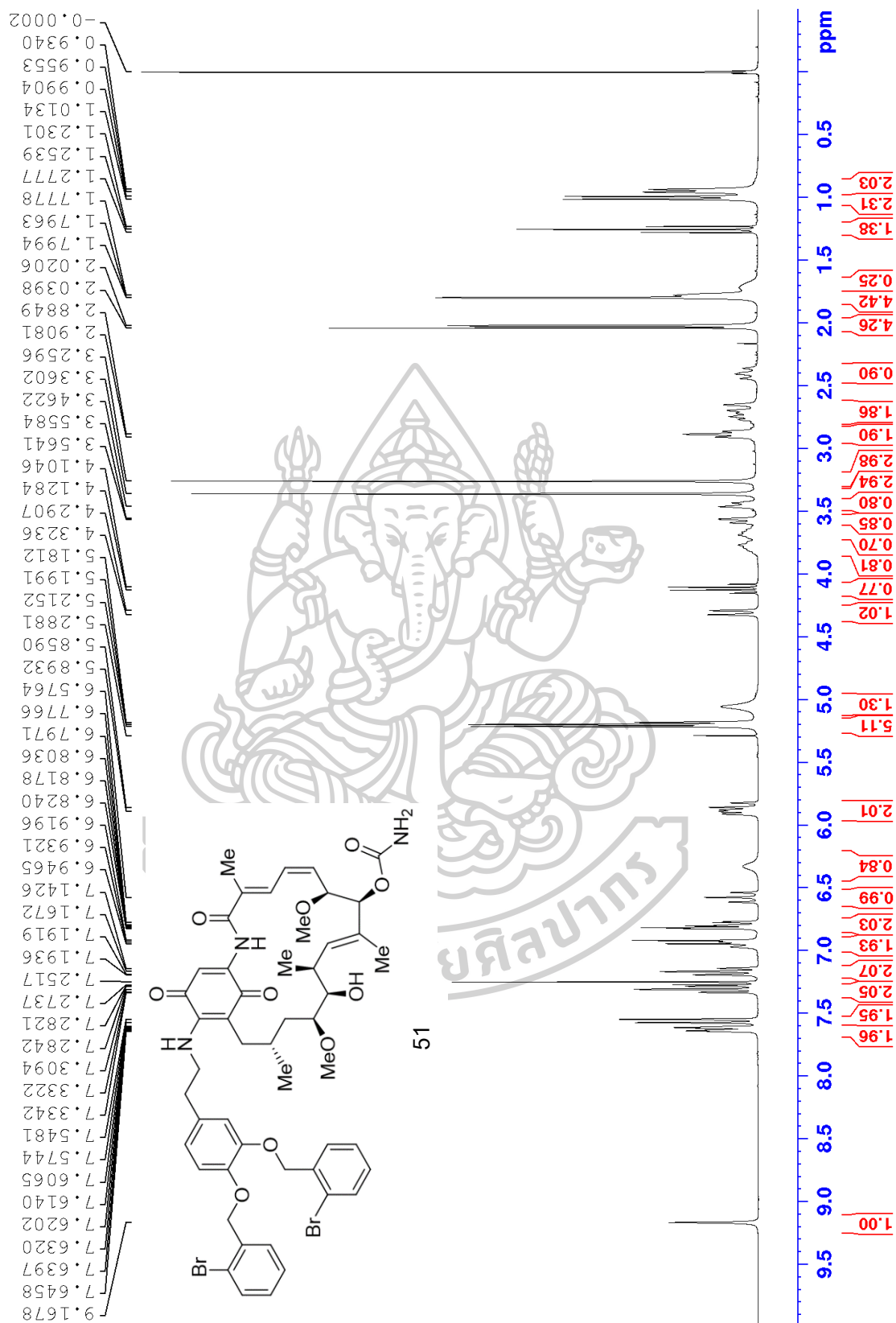


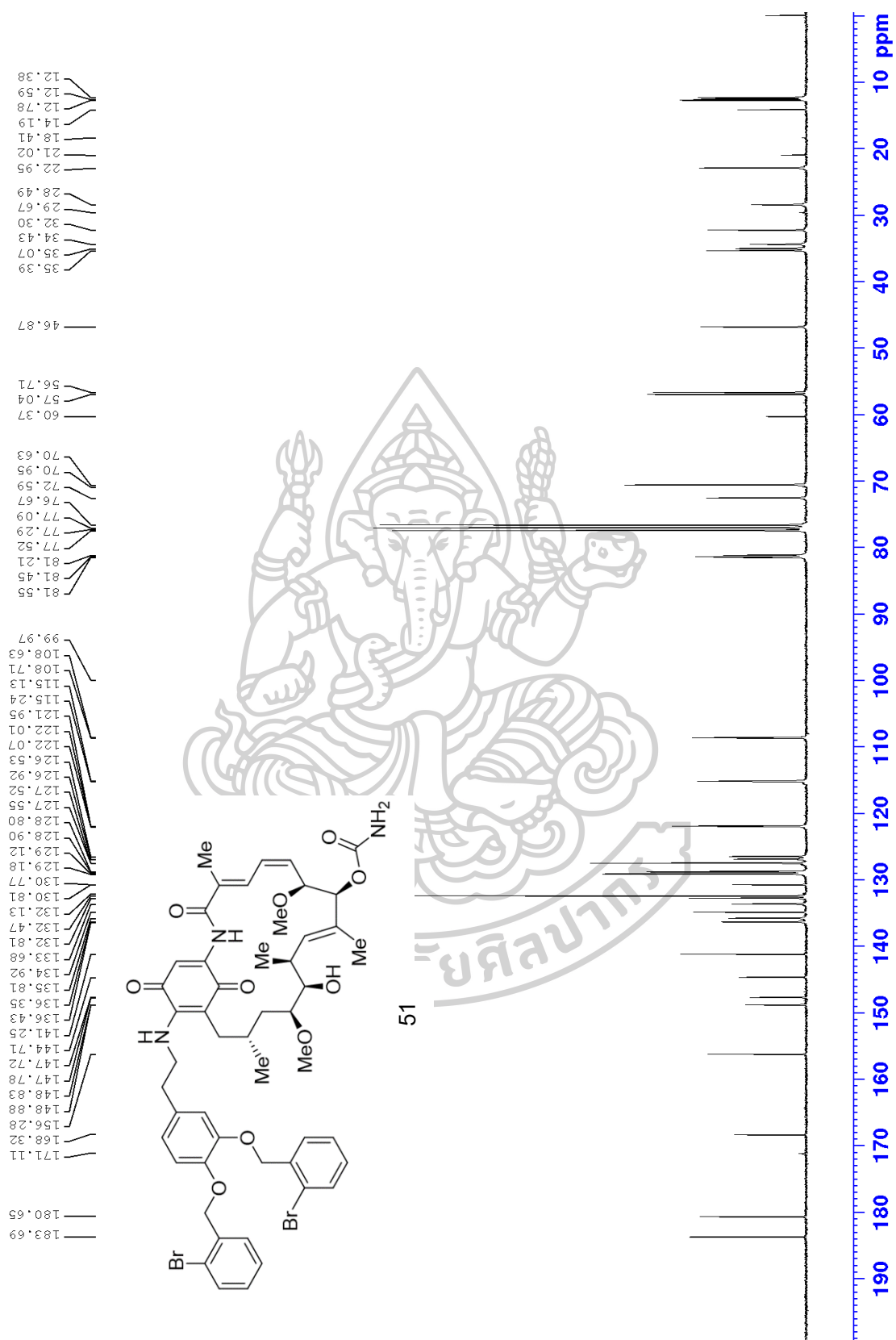












APPENDIX B

MTT assay of cytotoxicity activities

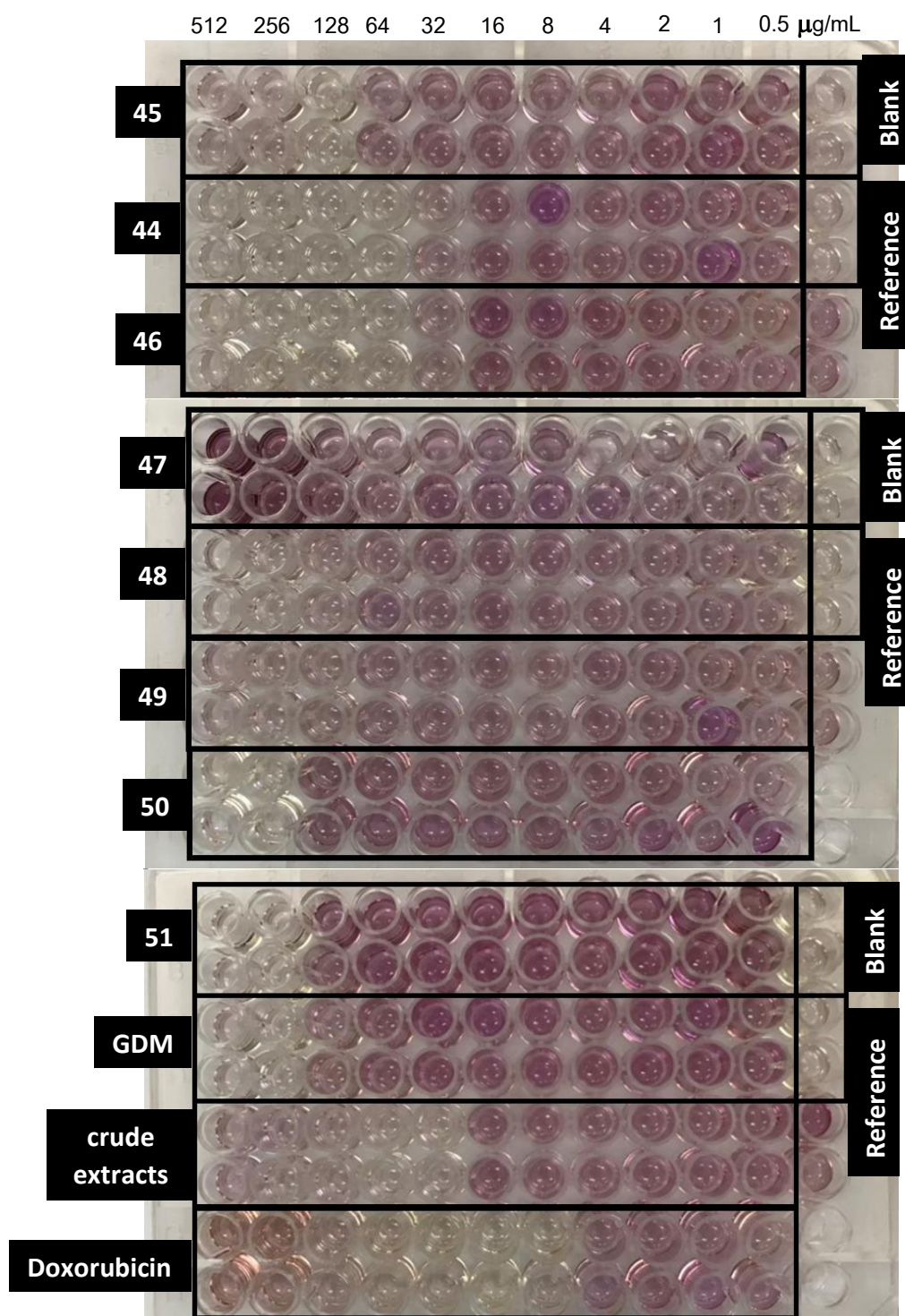


Figure 10 The results of cytotoxicity against human cervical cancer cells (HeLa).

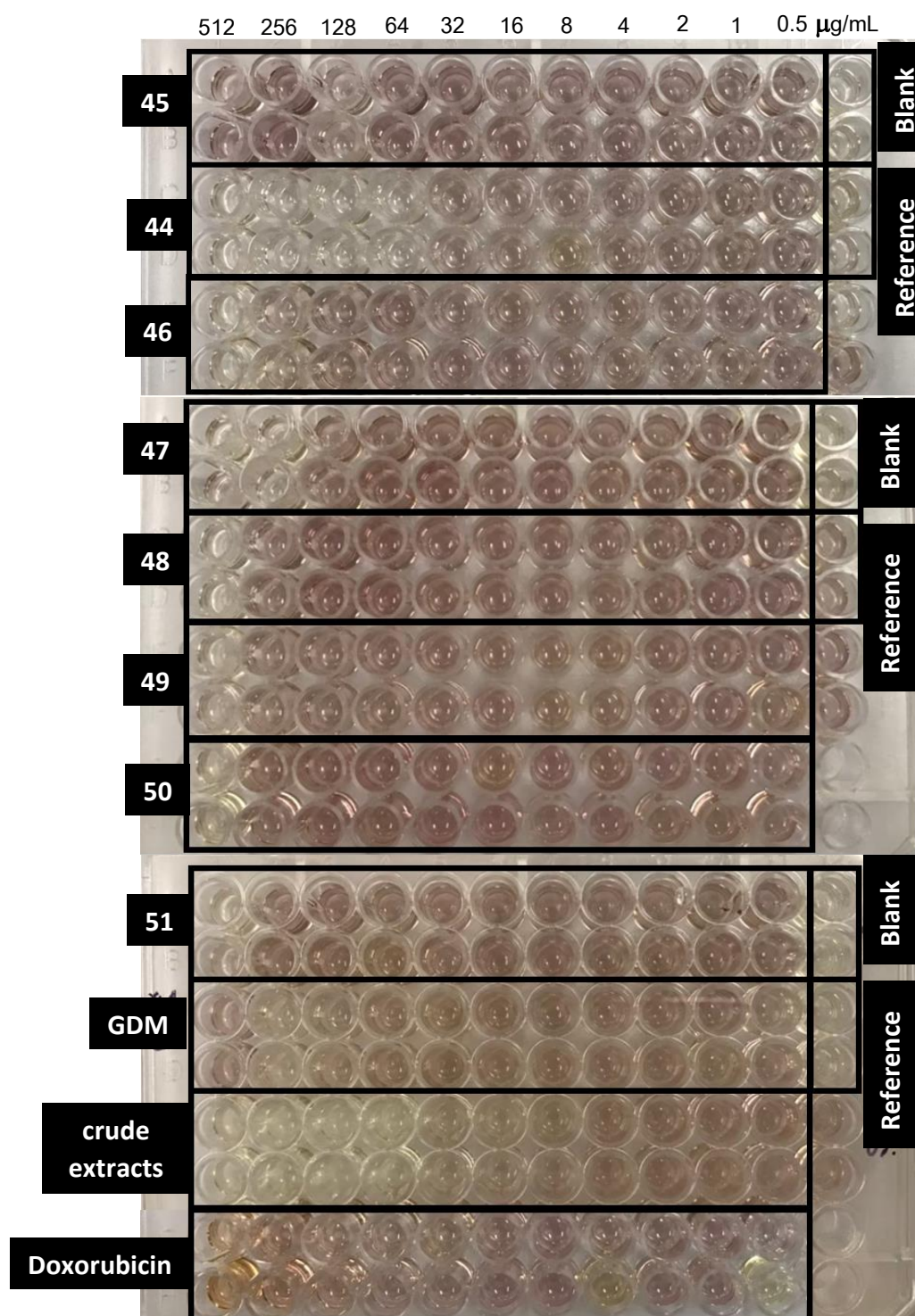


Figure 11 The results of cytotoxicity against human hepatocellular carcinoma cells (HepG2).

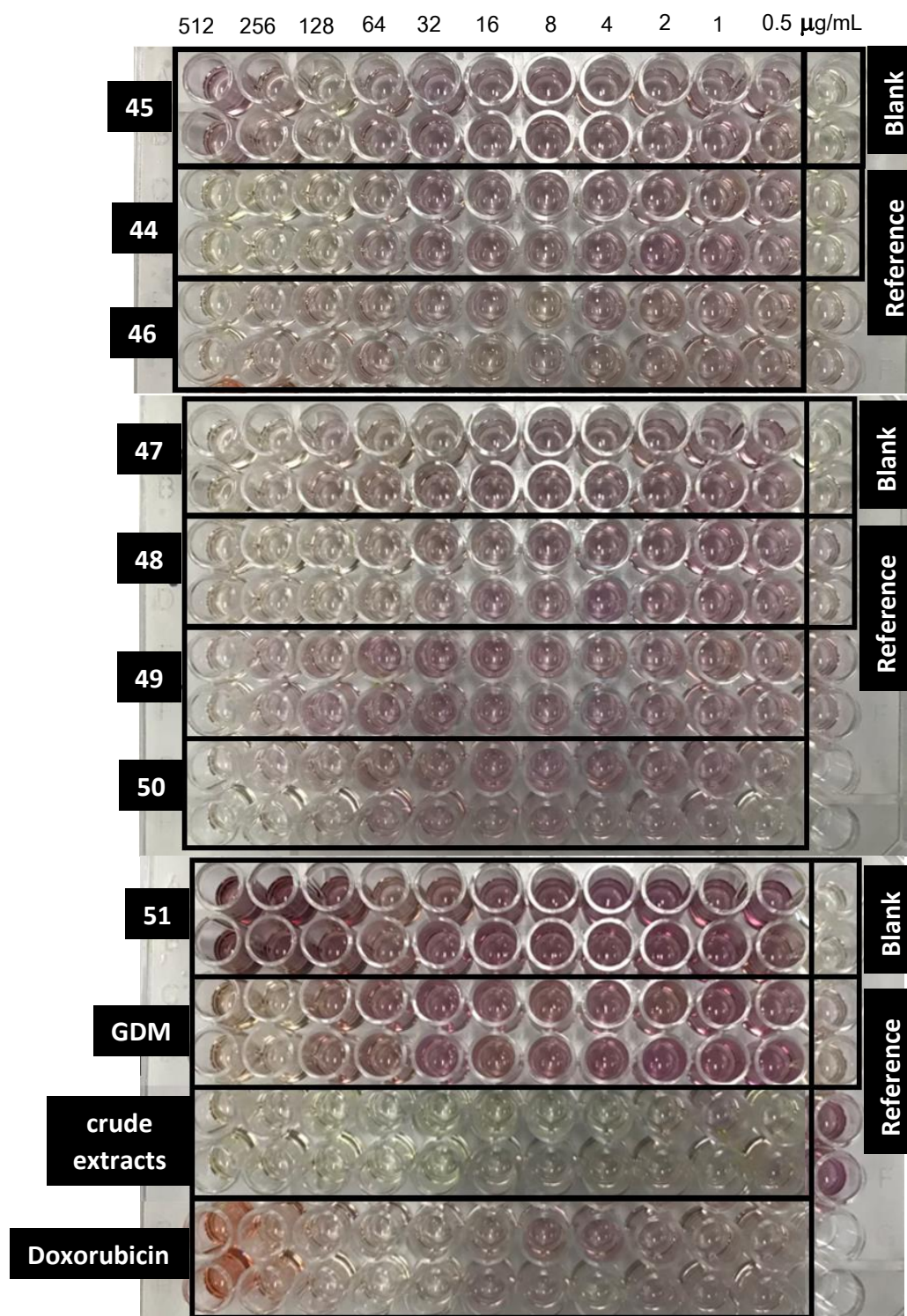


Figure 12 The results of cytotoxicity against human breast carcinoma cells (MDA-MB-231).

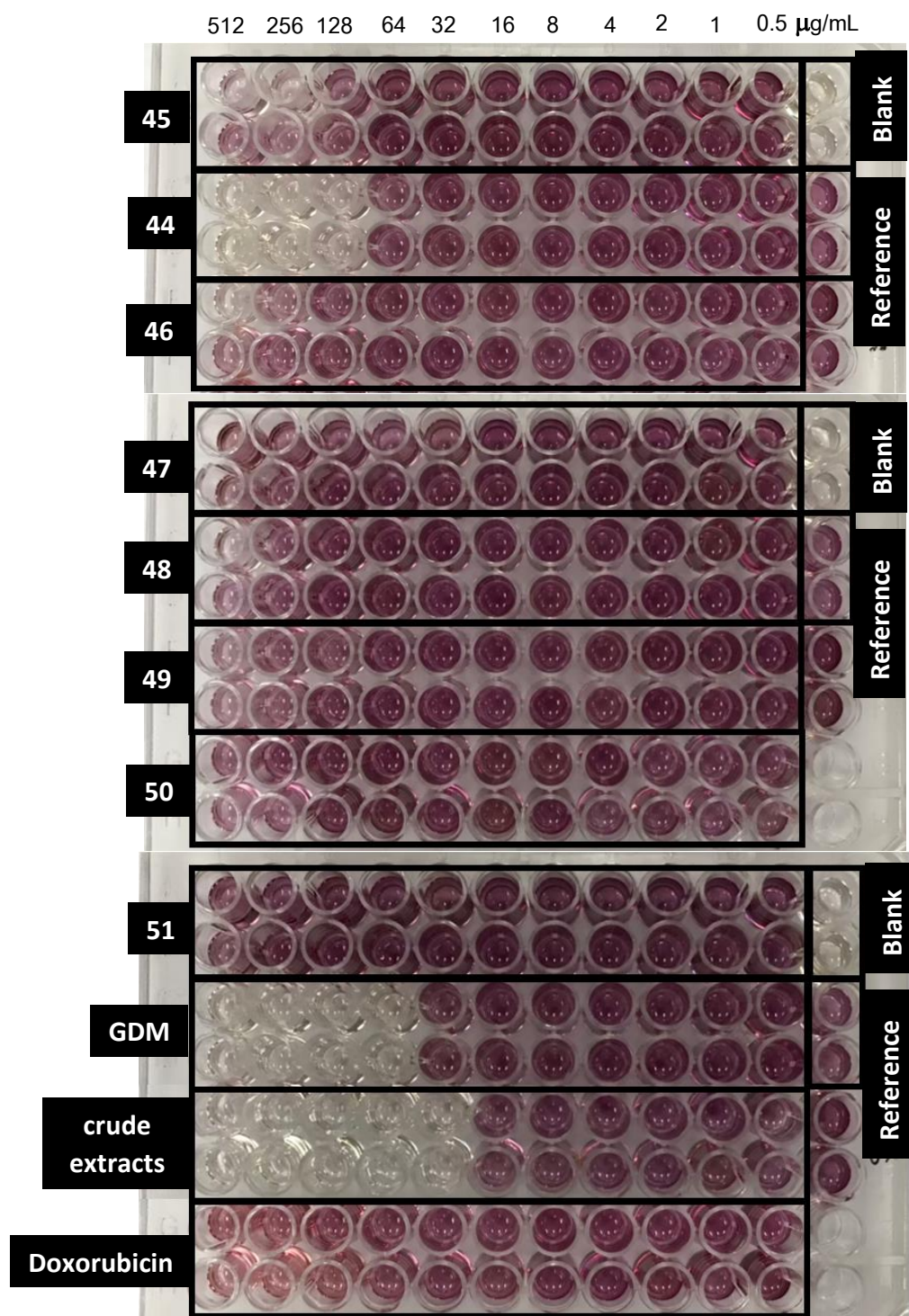


Figure 13 The results of cytotoxicity against Rhesus monkey kidney cells (LLC-MK2).

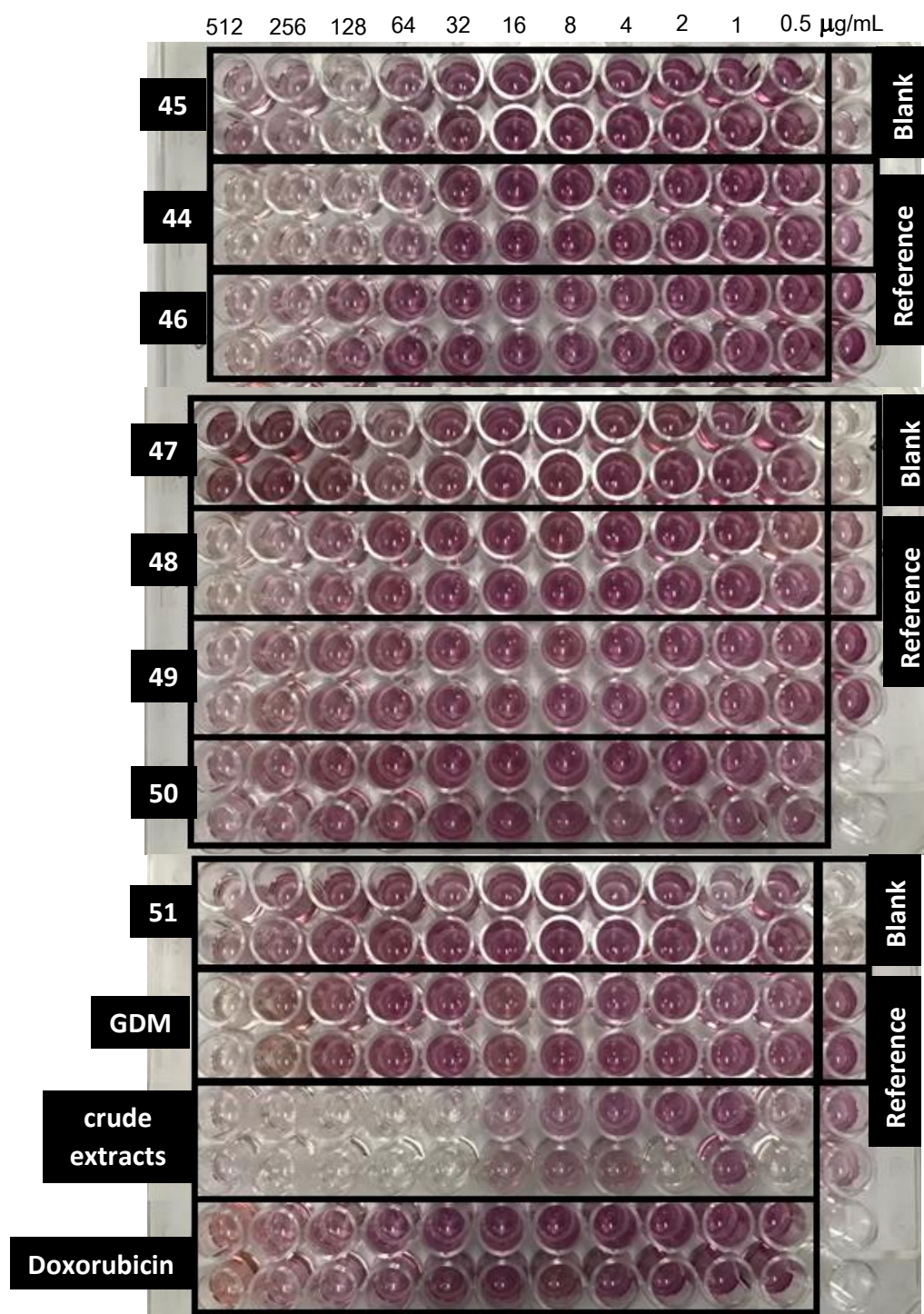


Figure 14 The results of cytotoxicity against African green monkey kidney cells (Vero).

APPENDIX C

Binding site of Hsp90 (1YET and 1OSF)

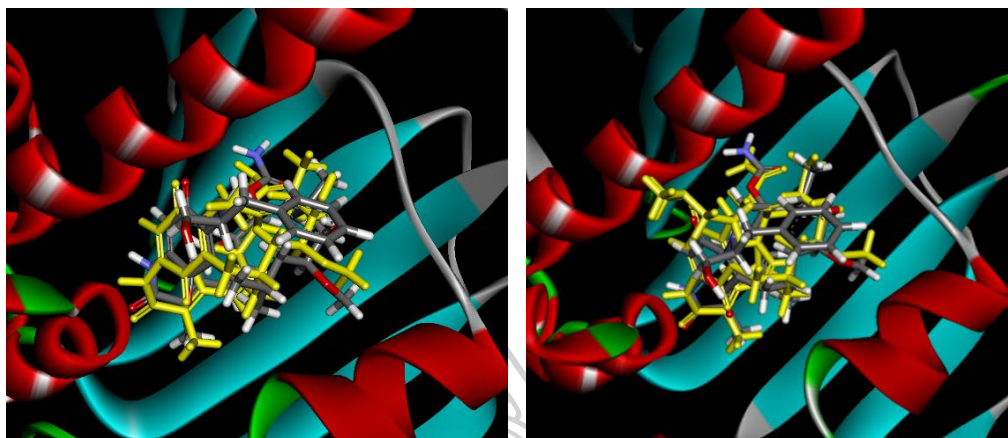


Figure 15 Comparison of the binding of 44 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

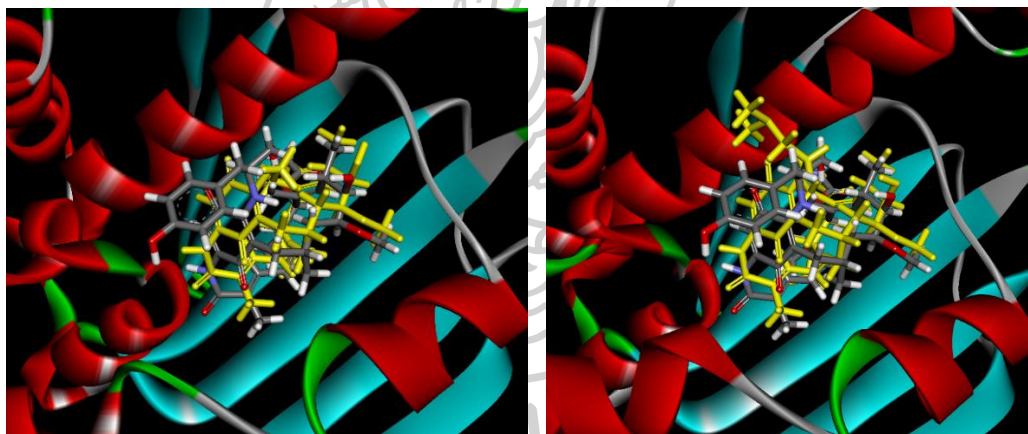


Figure 16 Comparison of the binding of 45 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

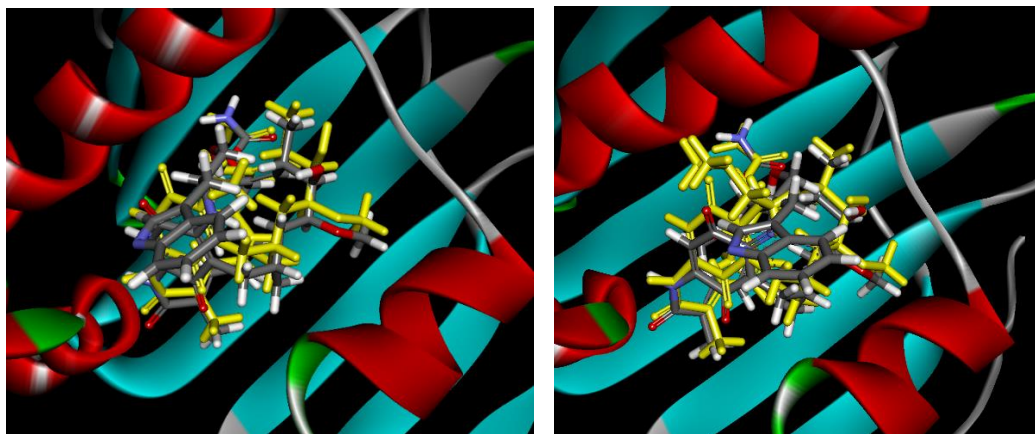


Figure 17 Comparison of the binding of 46 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

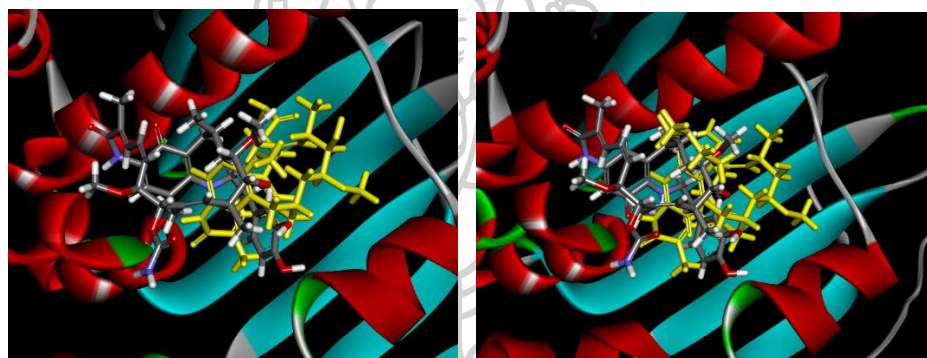


Figure 18 Comparison of the binding of 47 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

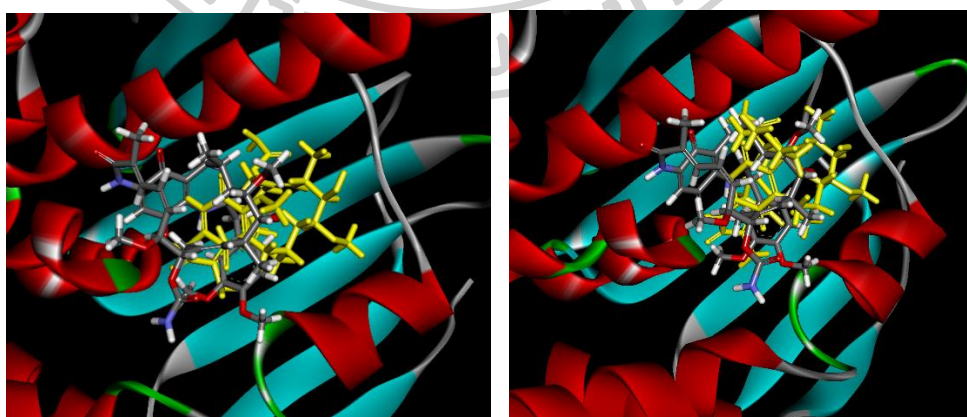


Figure 19 Comparison of the binding of 48 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

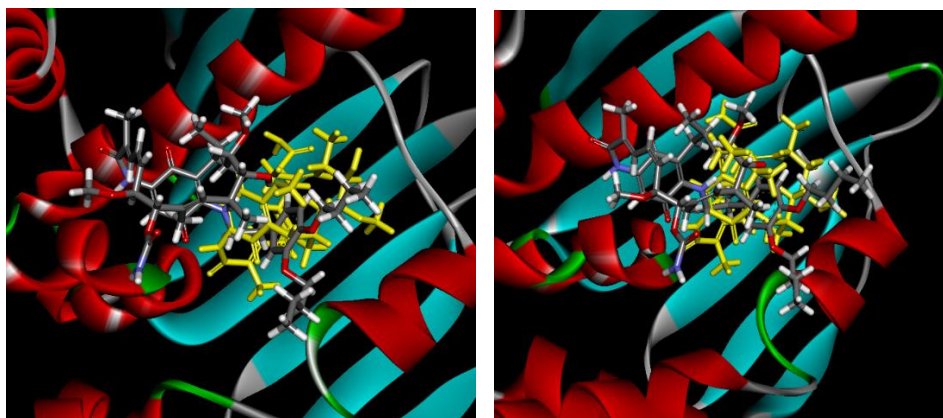


Figure 20 Comparison of the binding of 49 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

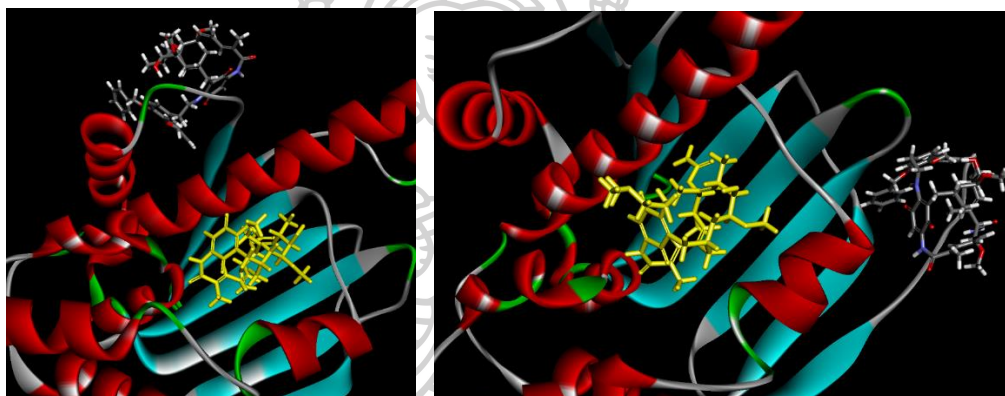


Figure 21 Comparison of the binding of 50 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

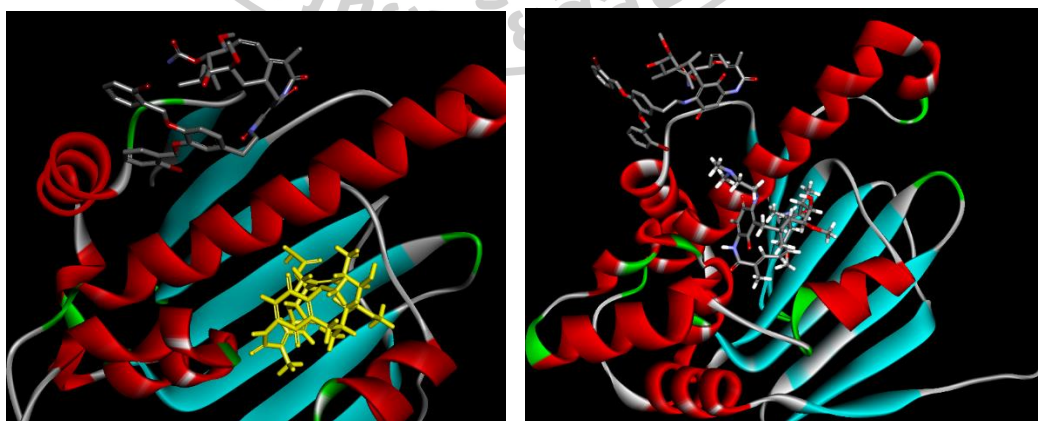


Figure 22 Comparison of the binding of 51 and the redocked co-crystallized (yellow) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

APPENDIX D

Amino acid interactions and H-Bond at binding site of Hsp90 (1YET and 1OSF)

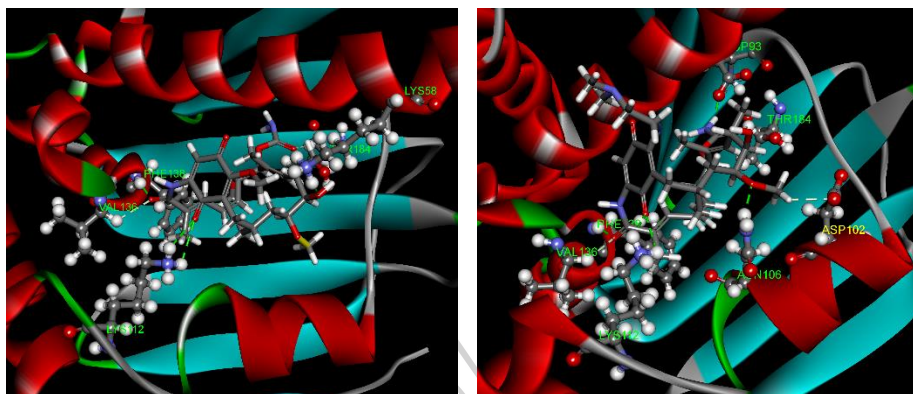


Figure 23 Comparison of amino acid interactions and H-Bond of geldanamycin (15) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

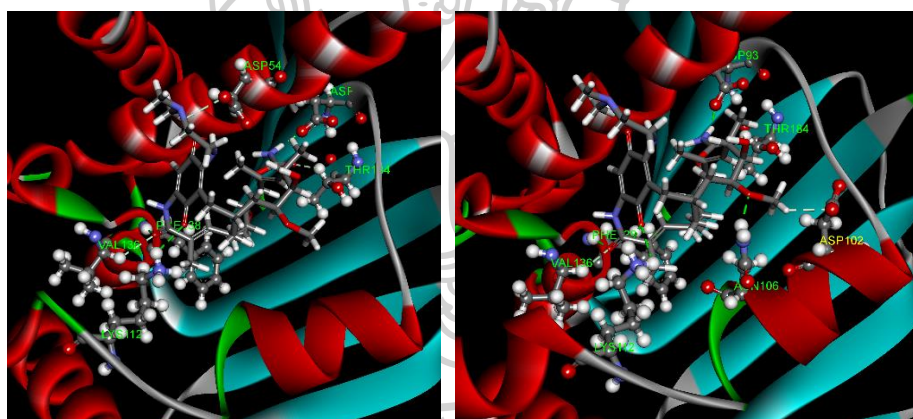


Figure 24 Comparison of amino acid interactions and H-Bond of DMAG (17) in the active site of Hsp90 (1YET (left) and 1OSF (right)).

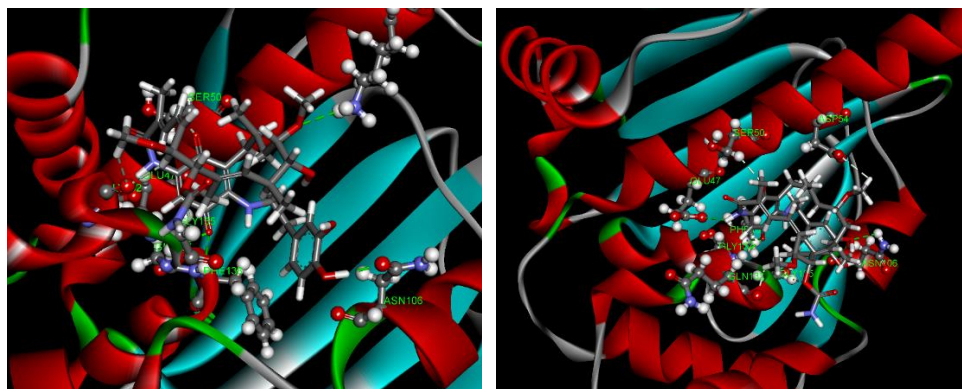


Figure 28 Comparison of amino acid interactions and H-Bond of **47** in the active site of Hsp90 (1YET (left) and 1OSF (right)).

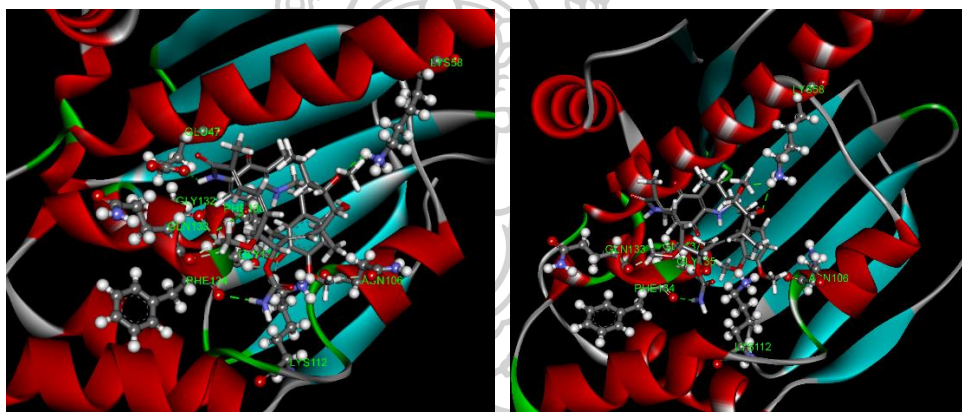


Figure 29 Comparison of amino acid interactions and H-Bond of **48** in the active site of Hsp90 (1YET (left) and 1OSF (right)).

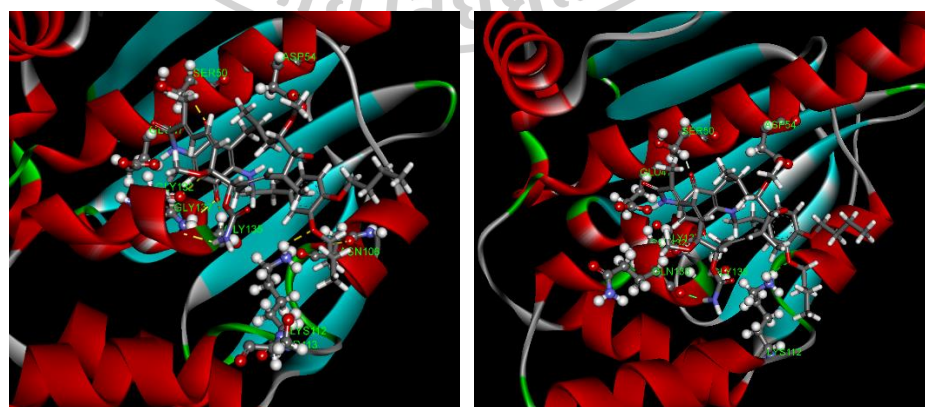


Figure 30 Comparison of amino acid interactions and H-Bond of **49** in the active site of Hsp90 (1YET (left) and 1OSF (right)).

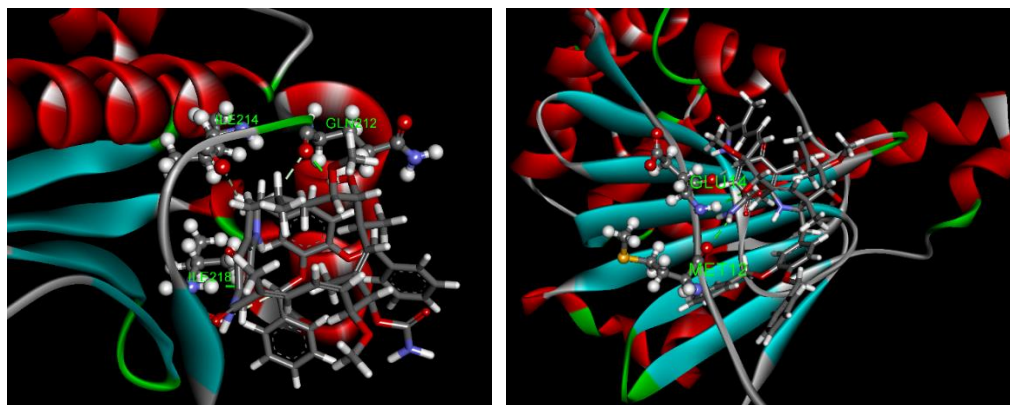


Figure 31 Comparison of amino acid interactions and H-Bond of 50 in the active site of Hsp90 (1YET (left) and 1OSF (right)).

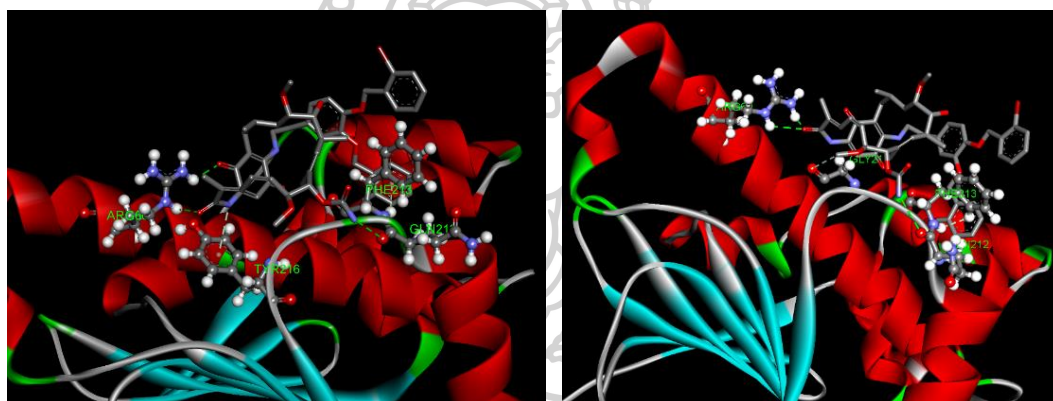


Figure 32 Comparison of amino acid interactions and H-Bond of 51 in the active site of Hsp90 (1YET (left) and 1OSF (right)).

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Phutdhawong, W.S. (2016). Synthesis and Evaluation of 3-
((2-(1H-Indol-3-Yl)Ethyl)Amino)-3-Hydroxyoxindoles and
Derivatives for Antibacterial Activity. J Pharm Chem Biol Sci,
4(4), 455-464.

- Poster Presentation
Jaramornburapong, C., Phutdhawong, W., Phutdhawong,
W.S. (2015), Synthesis of Iheyamine derivatives; the
bisindole alkaloids, Pure and Applied Chemistry
International Conference (PACCON 2015), 21-23 January
2015, Bangkok, Thailand.