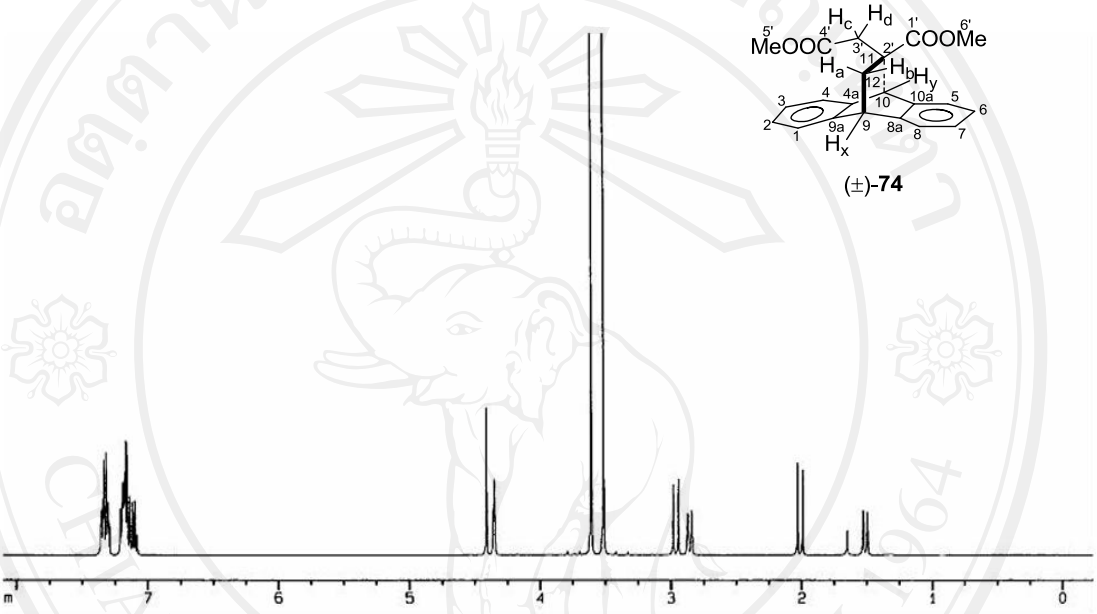
APPENDIX

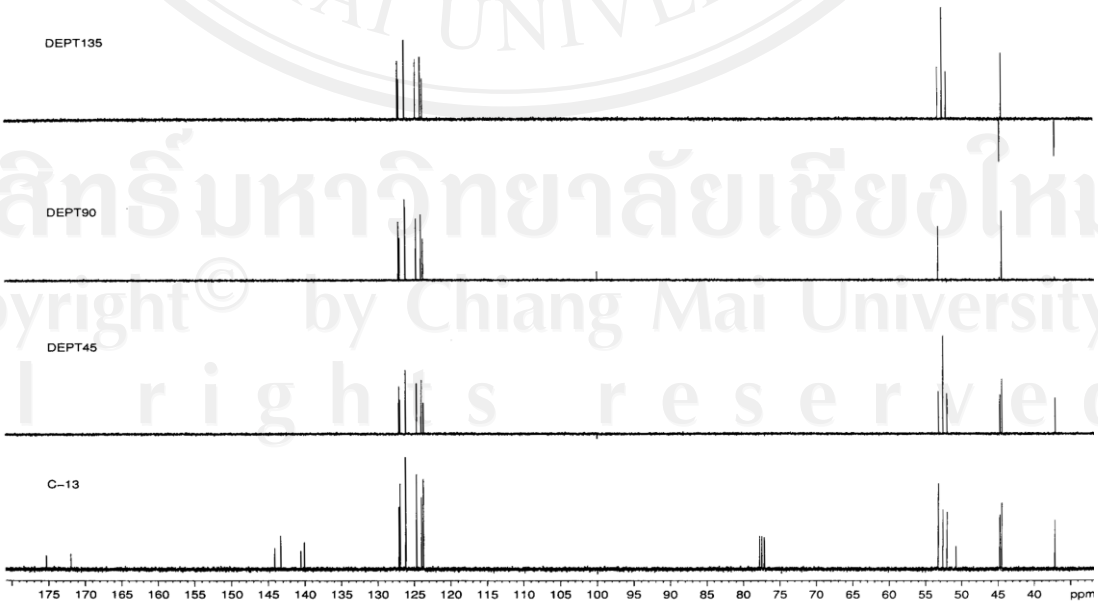
^{1}H-NMR (500 and 400 MHz) and ^{13}C-NMR (125 and 100 MHz)

^{1}H-NMR in $CDCl_3$

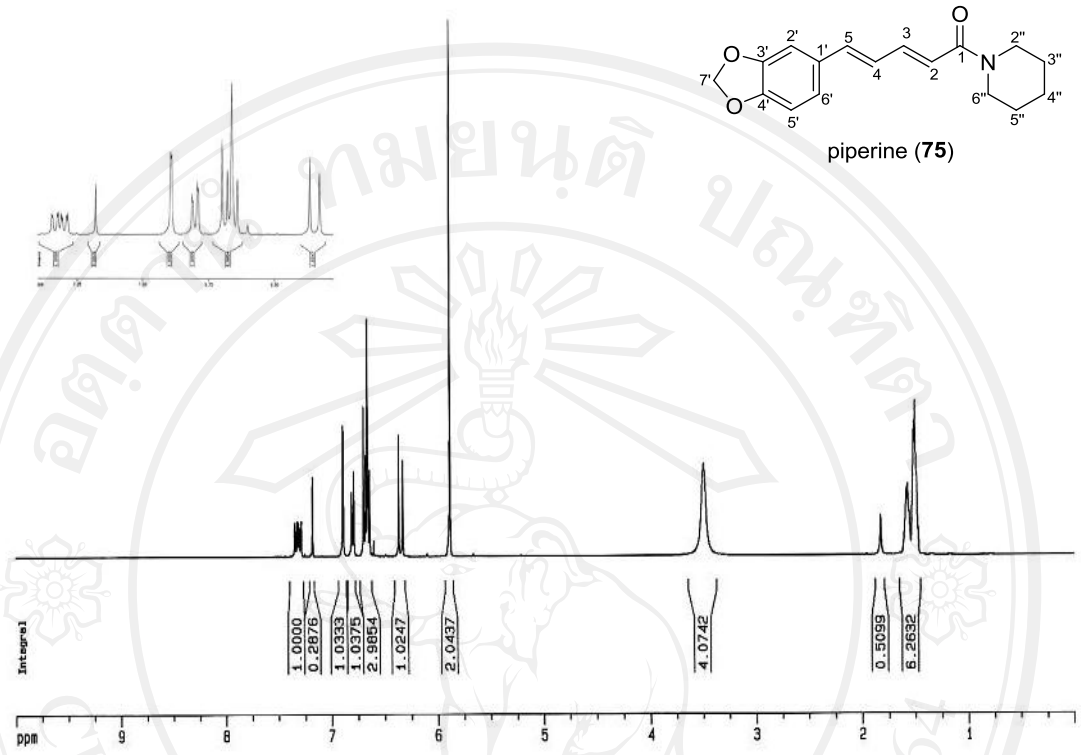


(±)-**74**

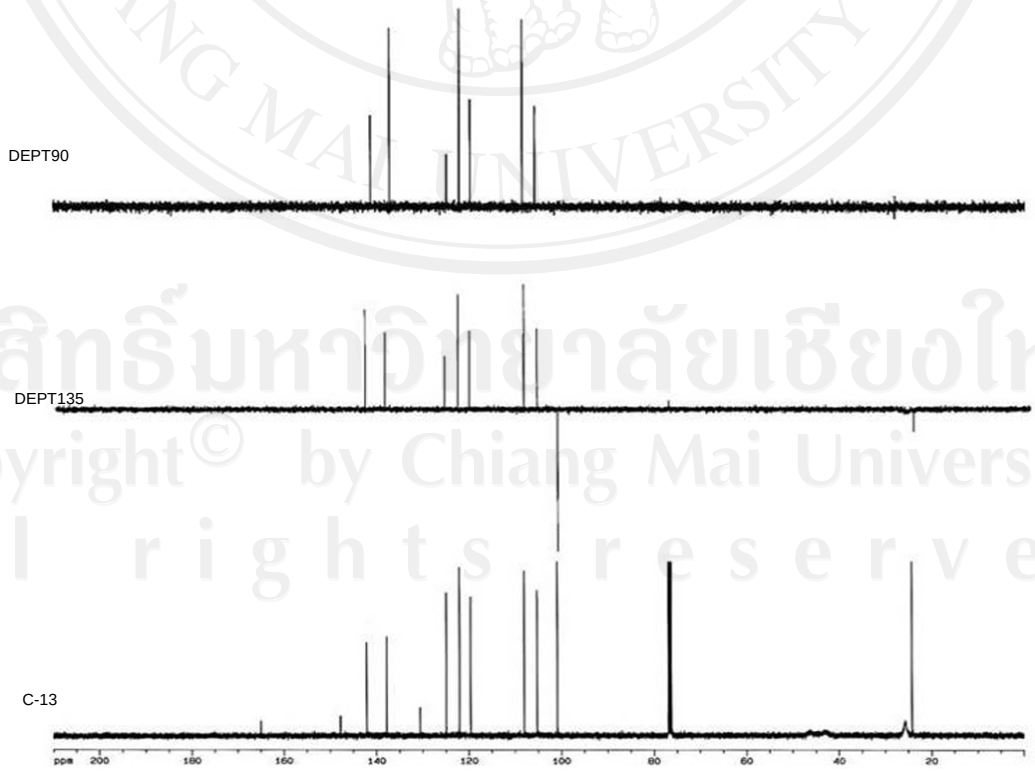
^{13}C-NMR in $CDCl_3$



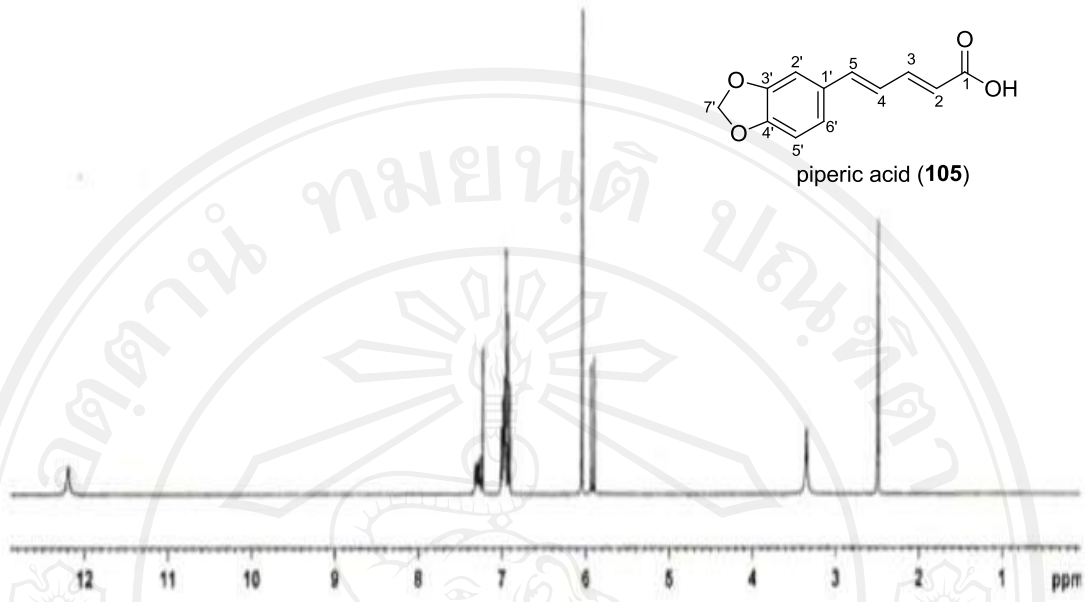
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$

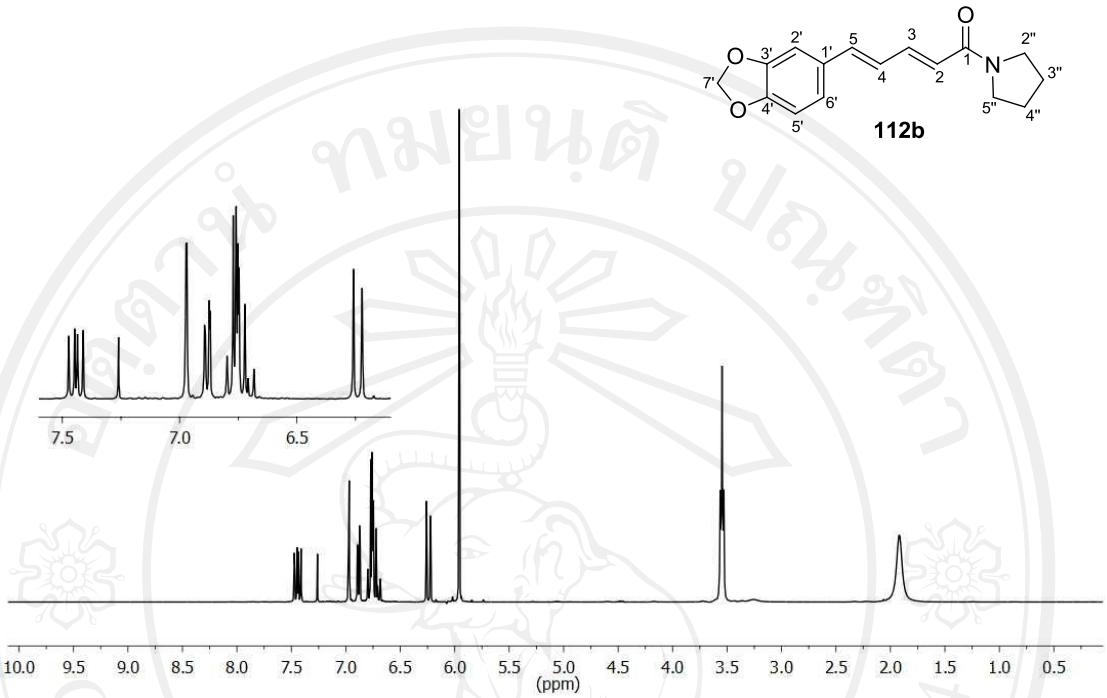


^{1}H-NMR in $CDCl_3$

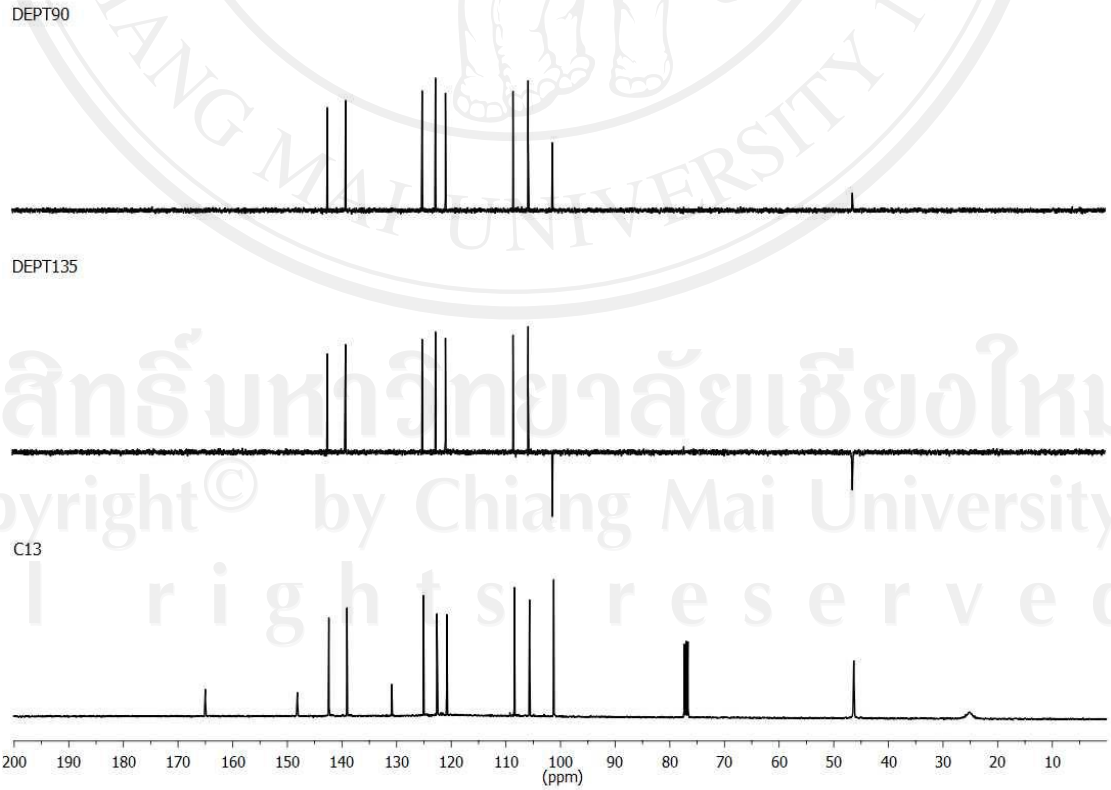


piperic acid (**105**)

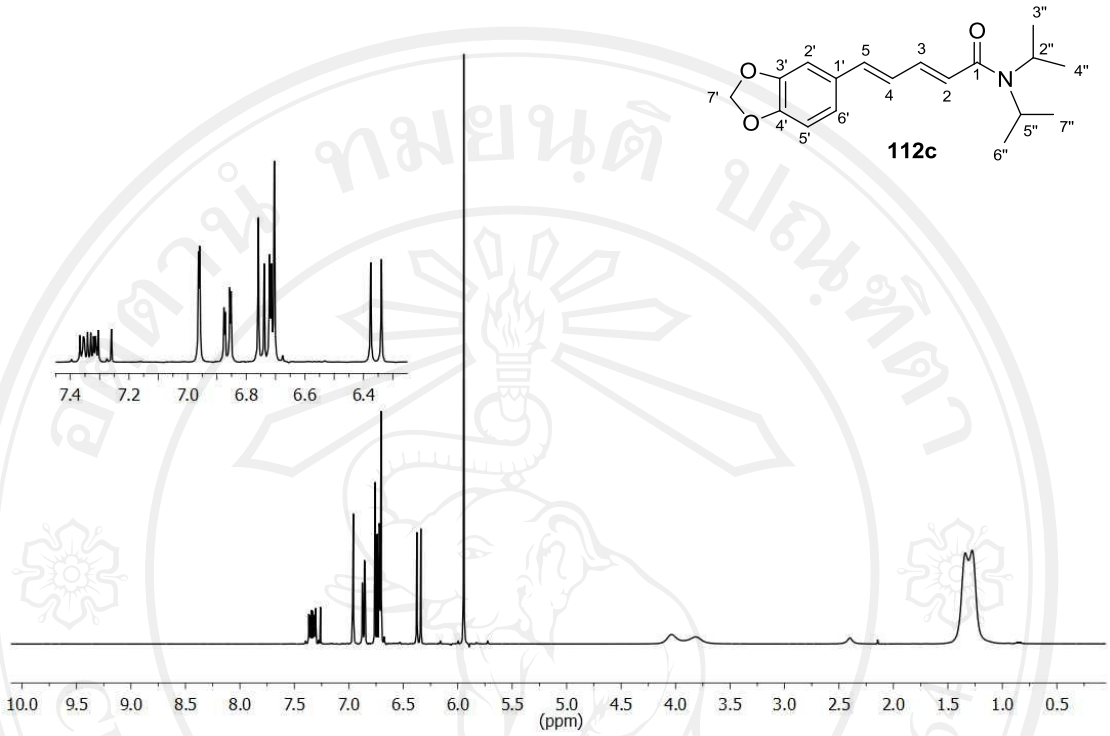
^{1}H-NMR in $CDCl_3$



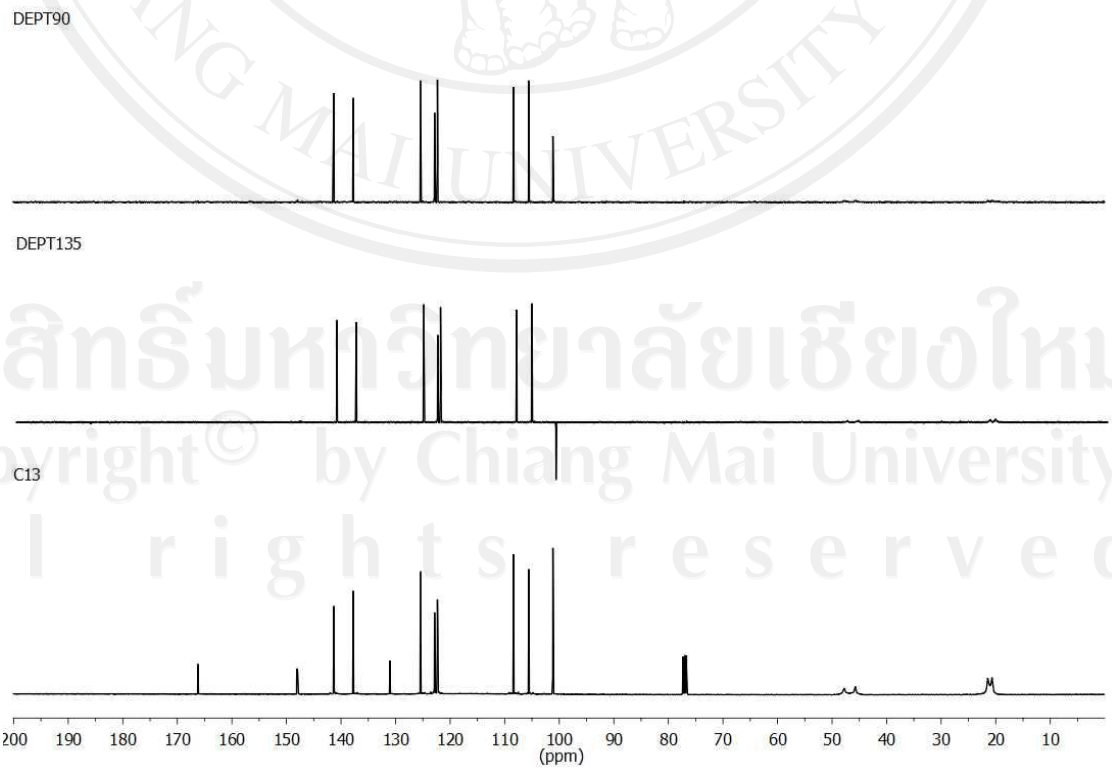
^{13}C-NMR in $CDCl_3$



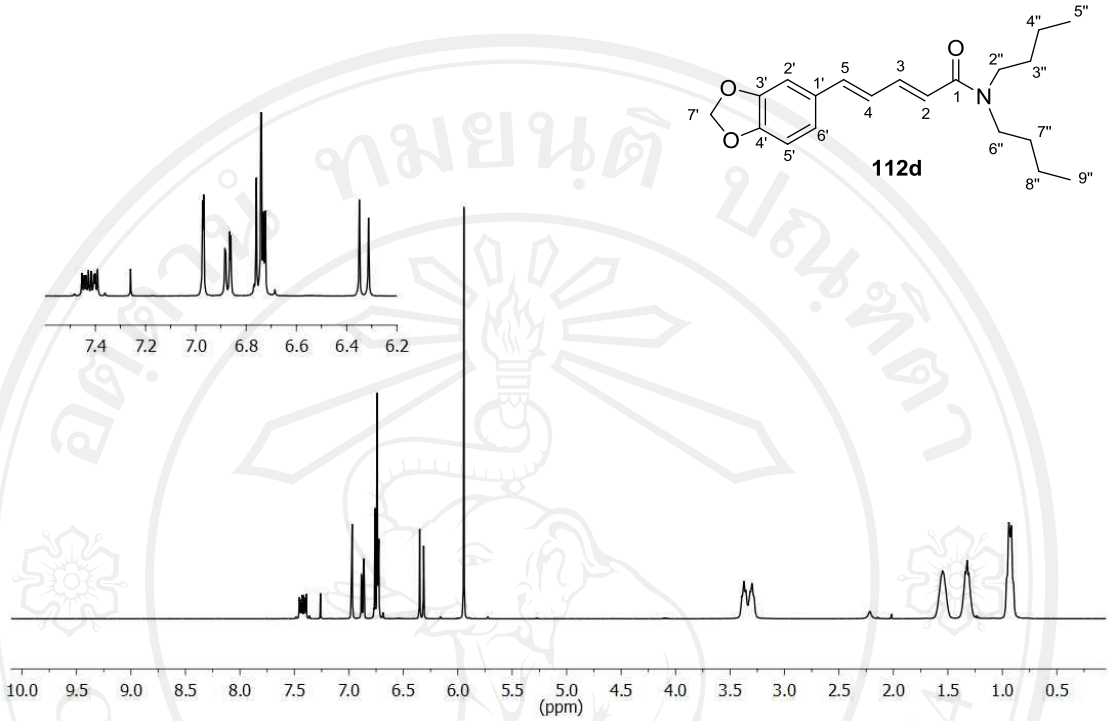
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



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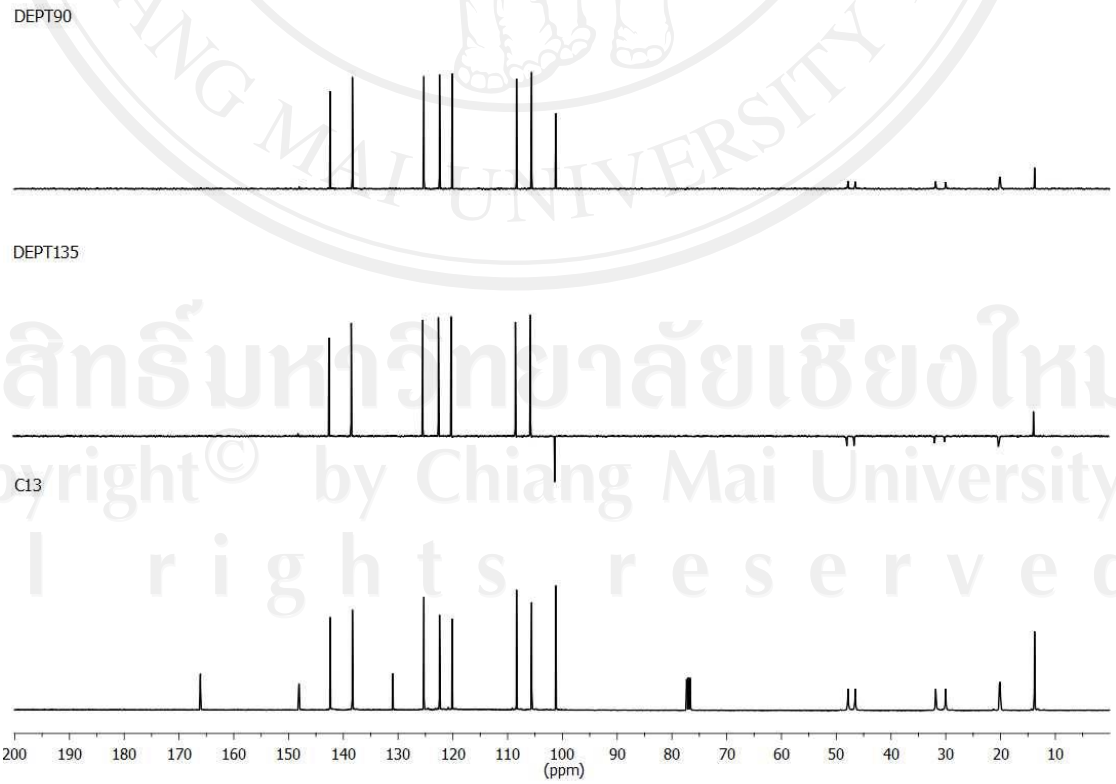
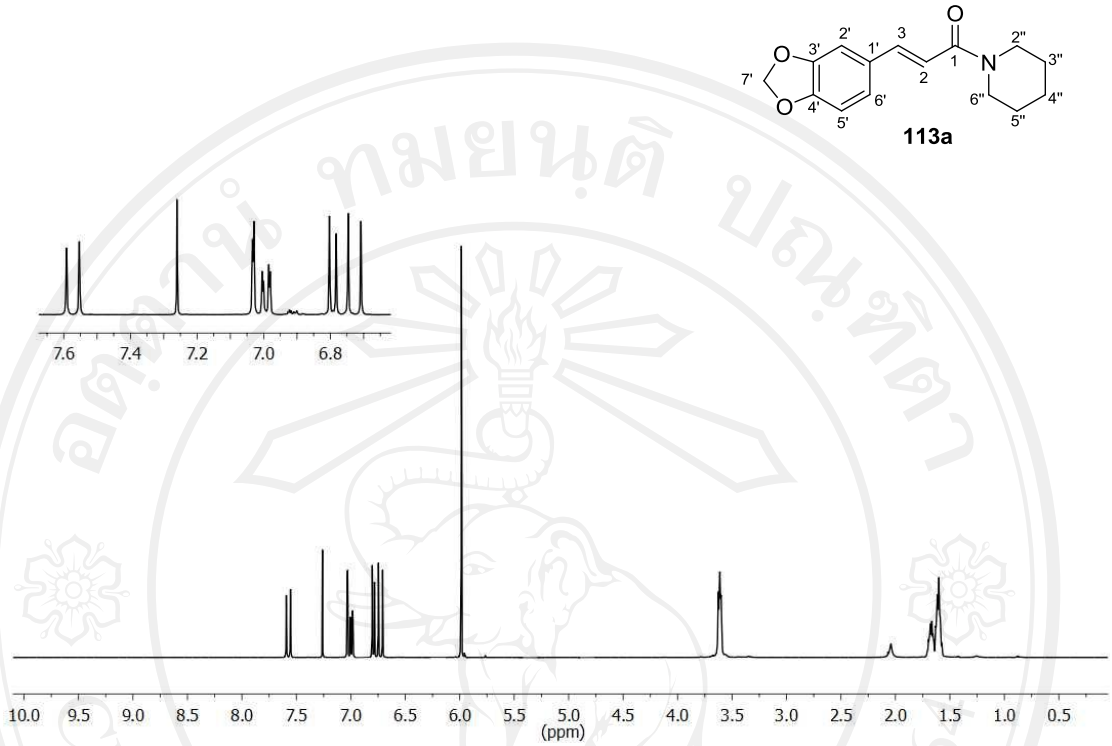
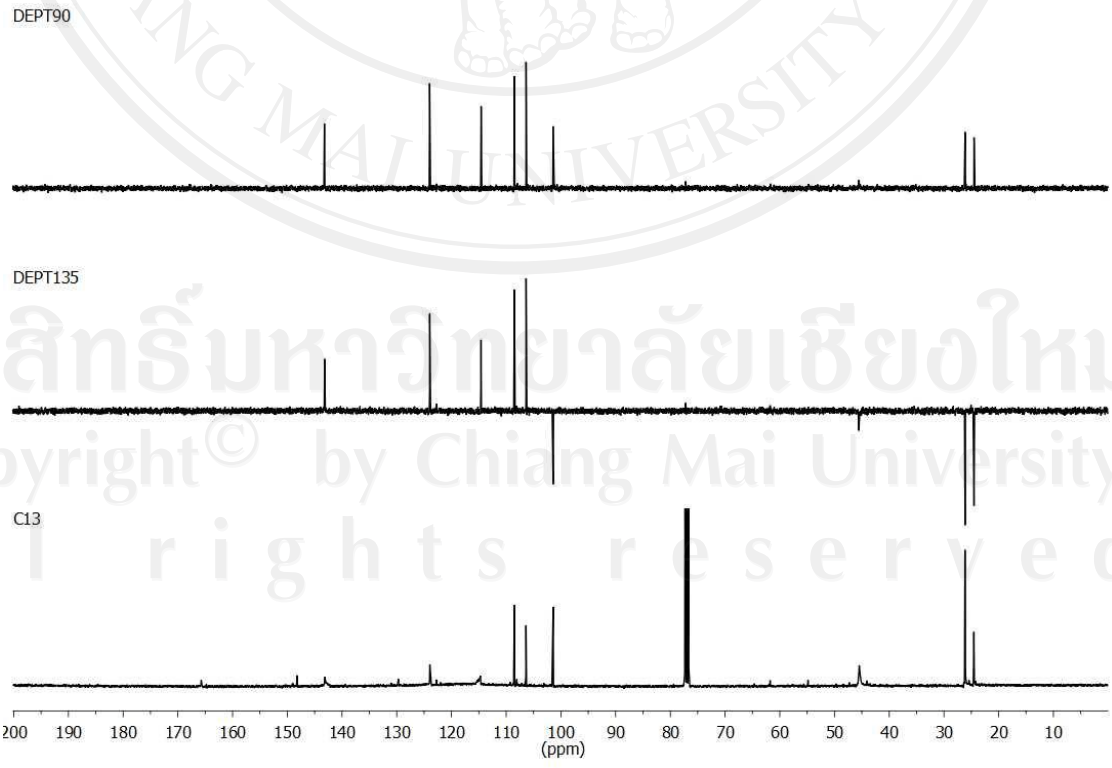


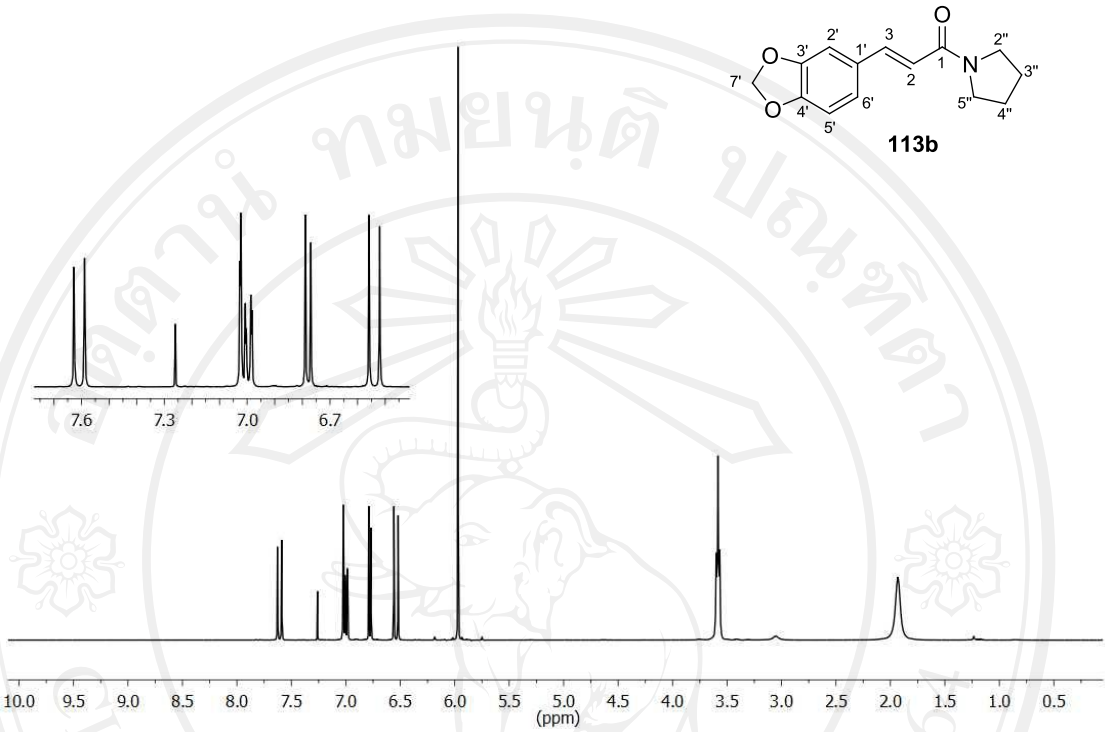
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



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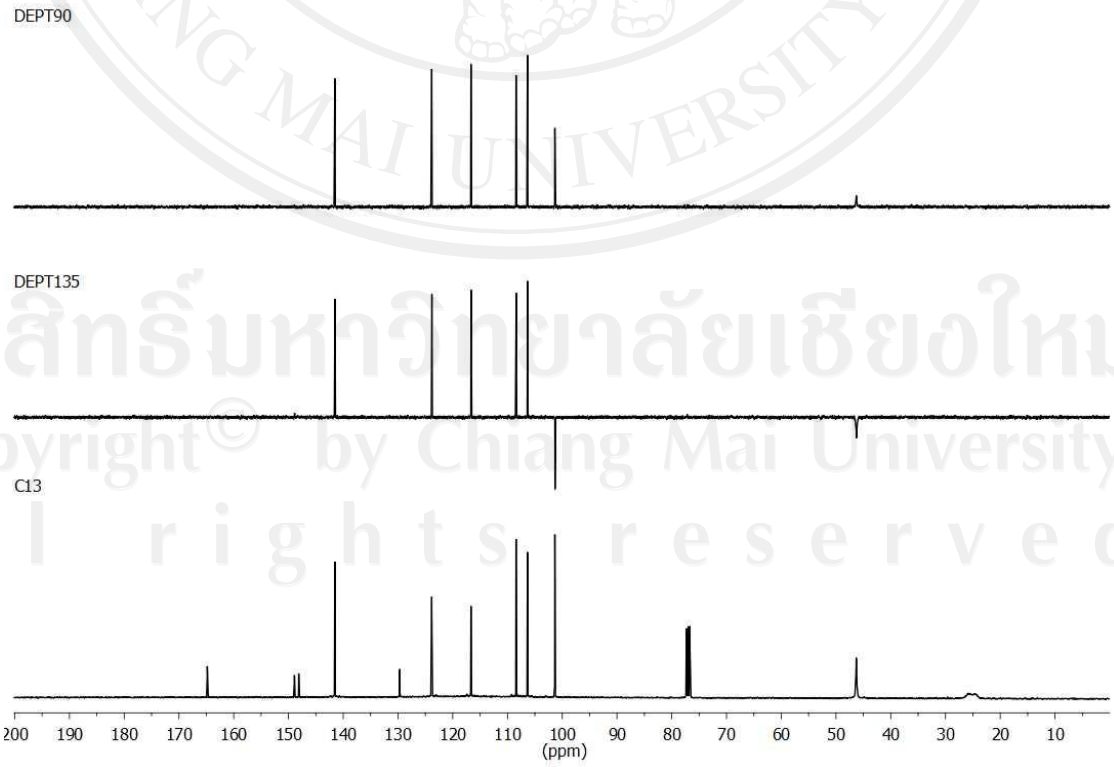
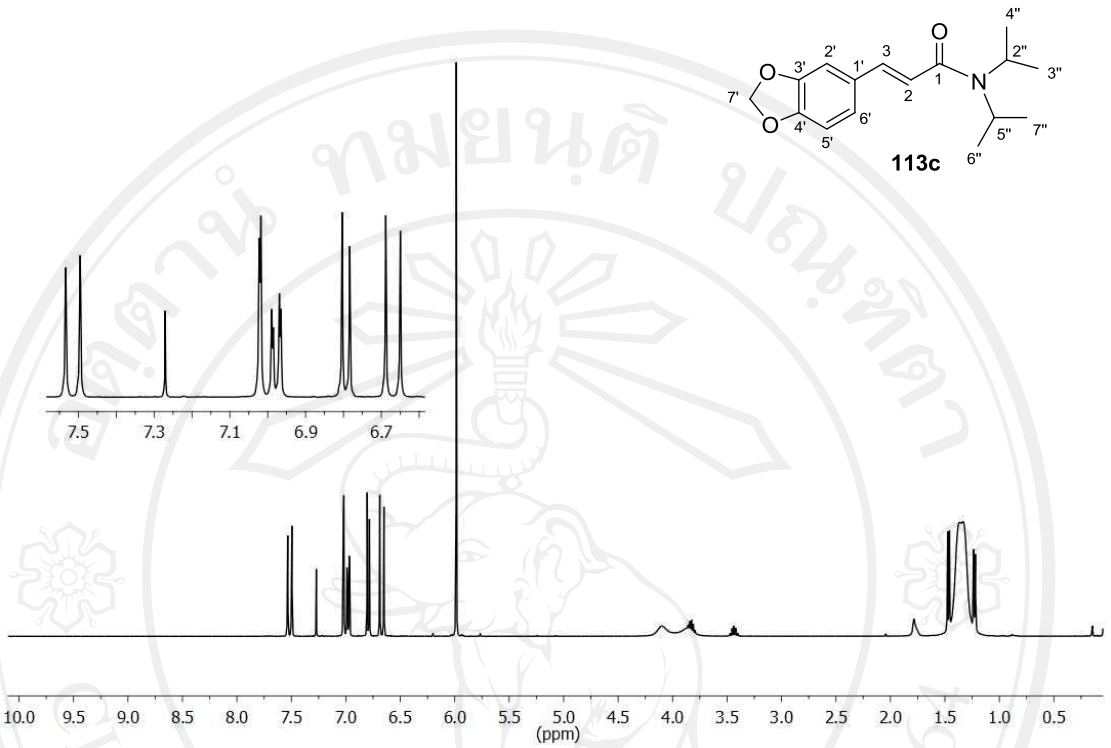
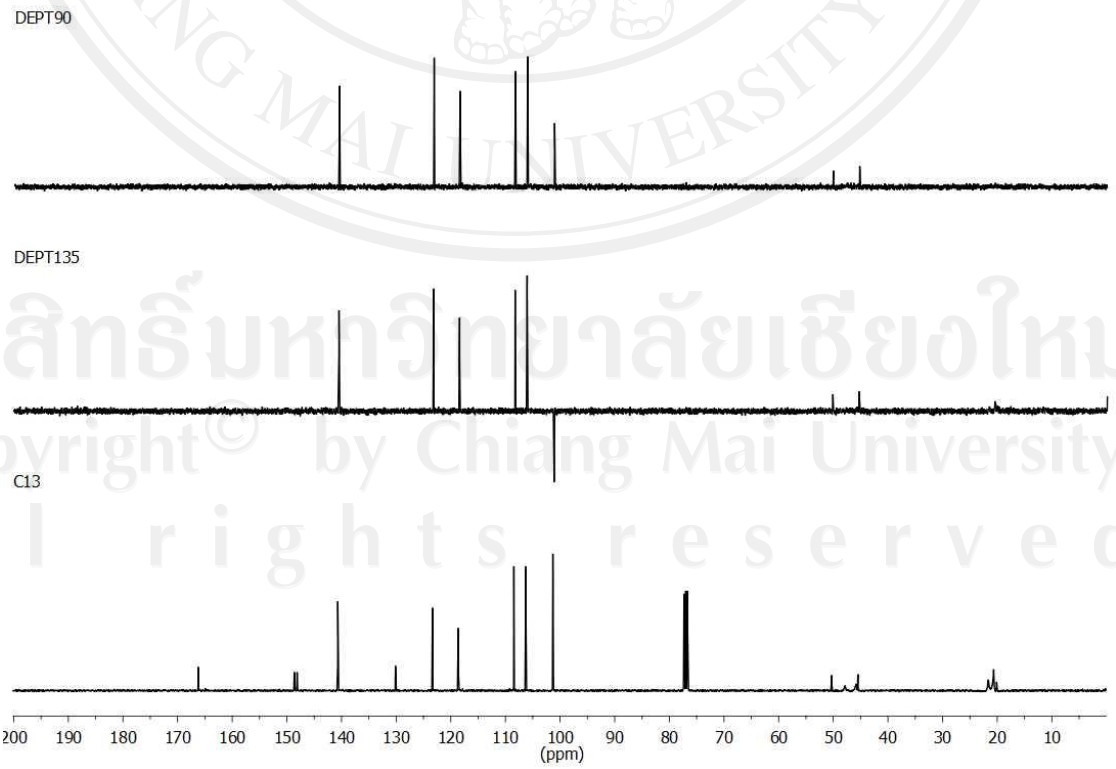


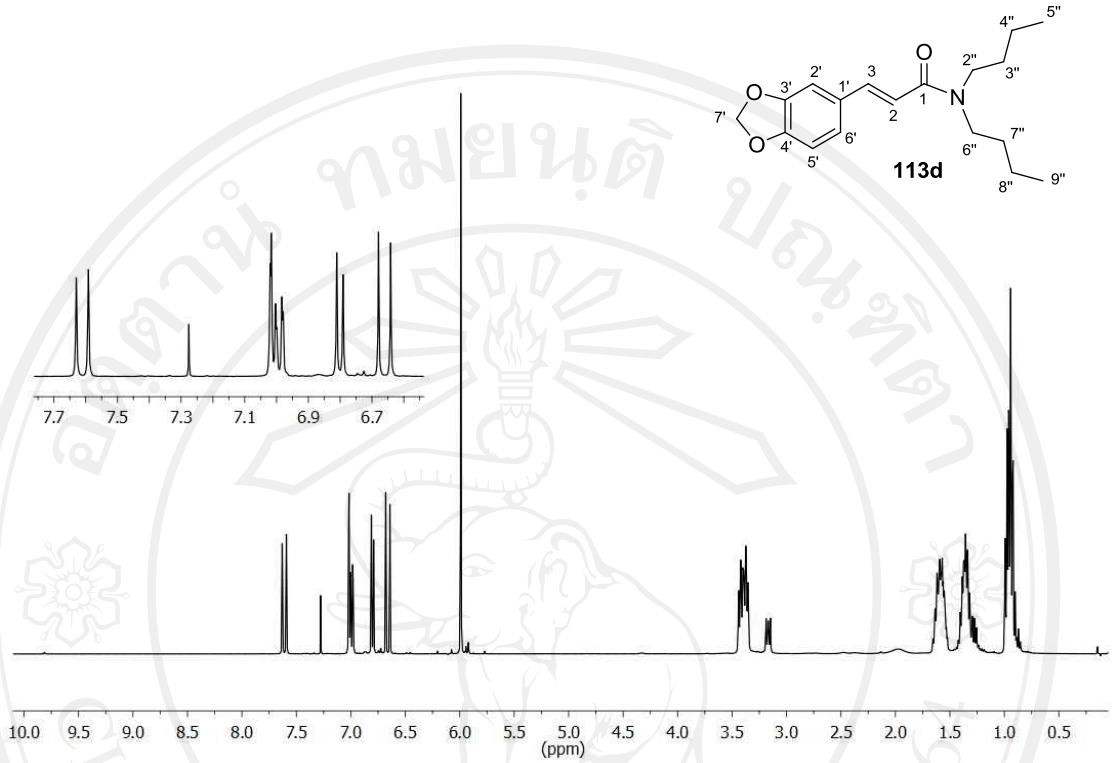
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



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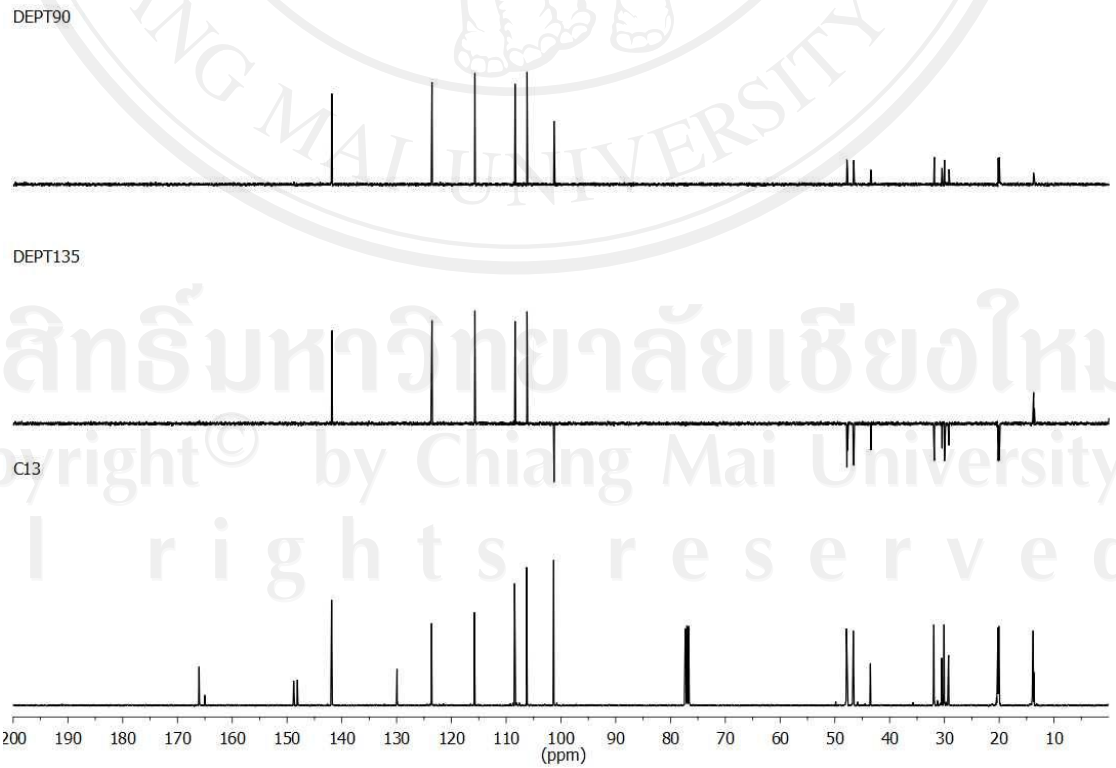
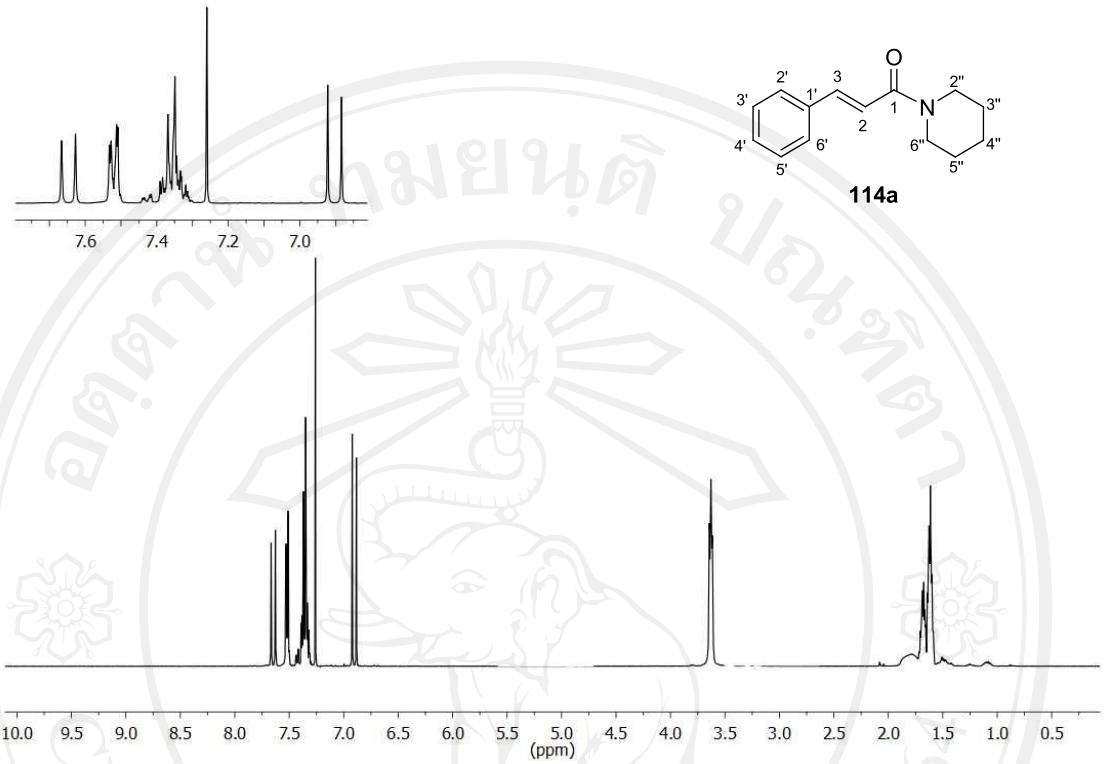
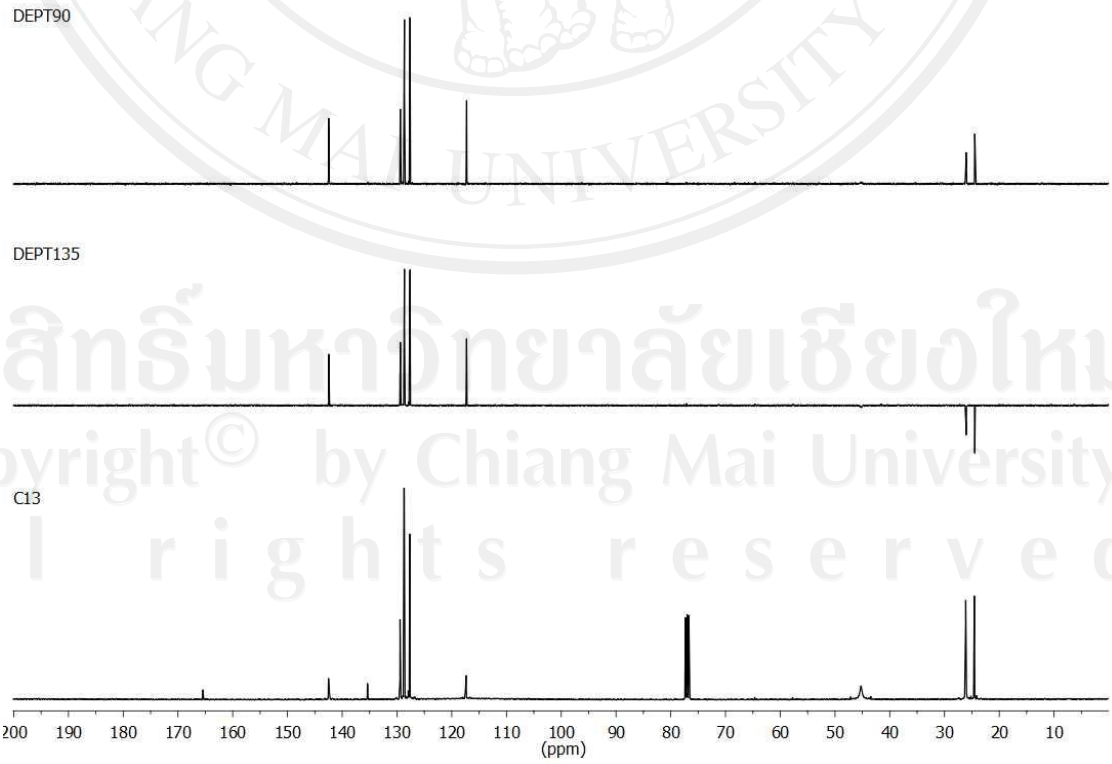


^{1}H-NMR in $CDCl_3$

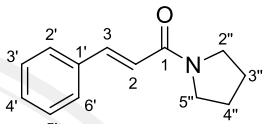


114a

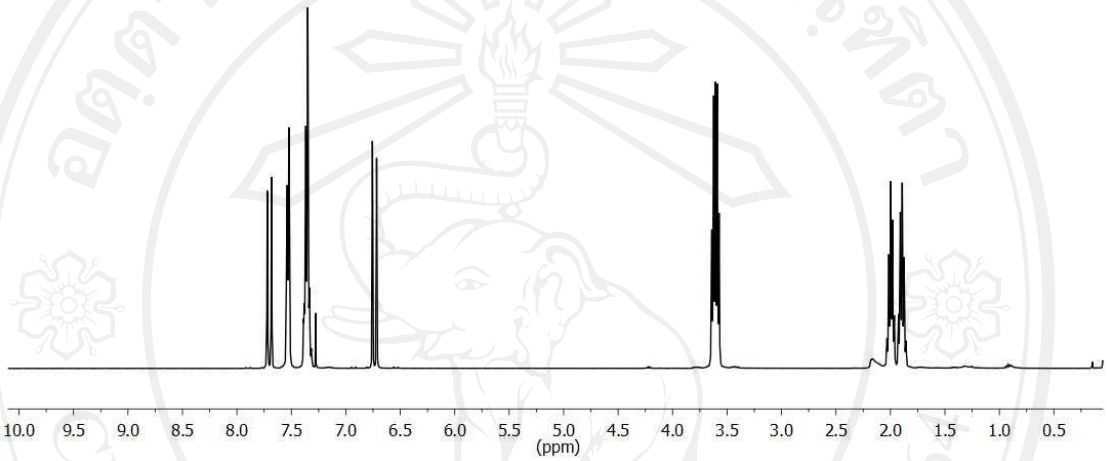
^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



114b



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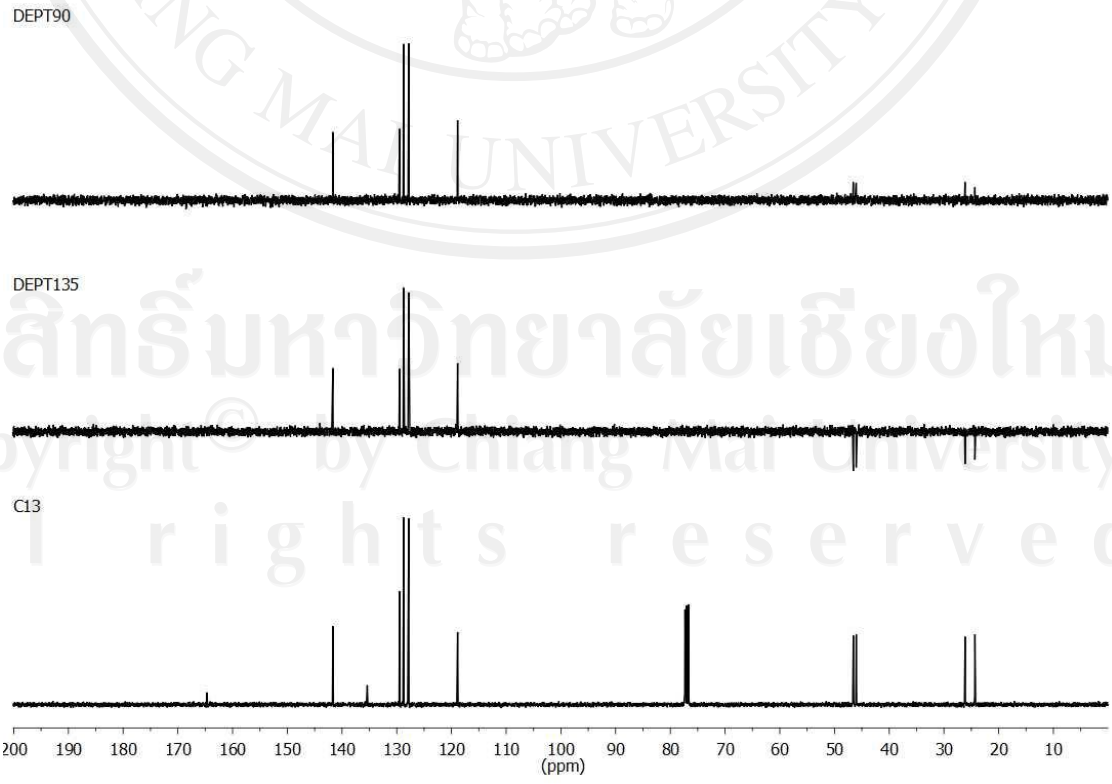

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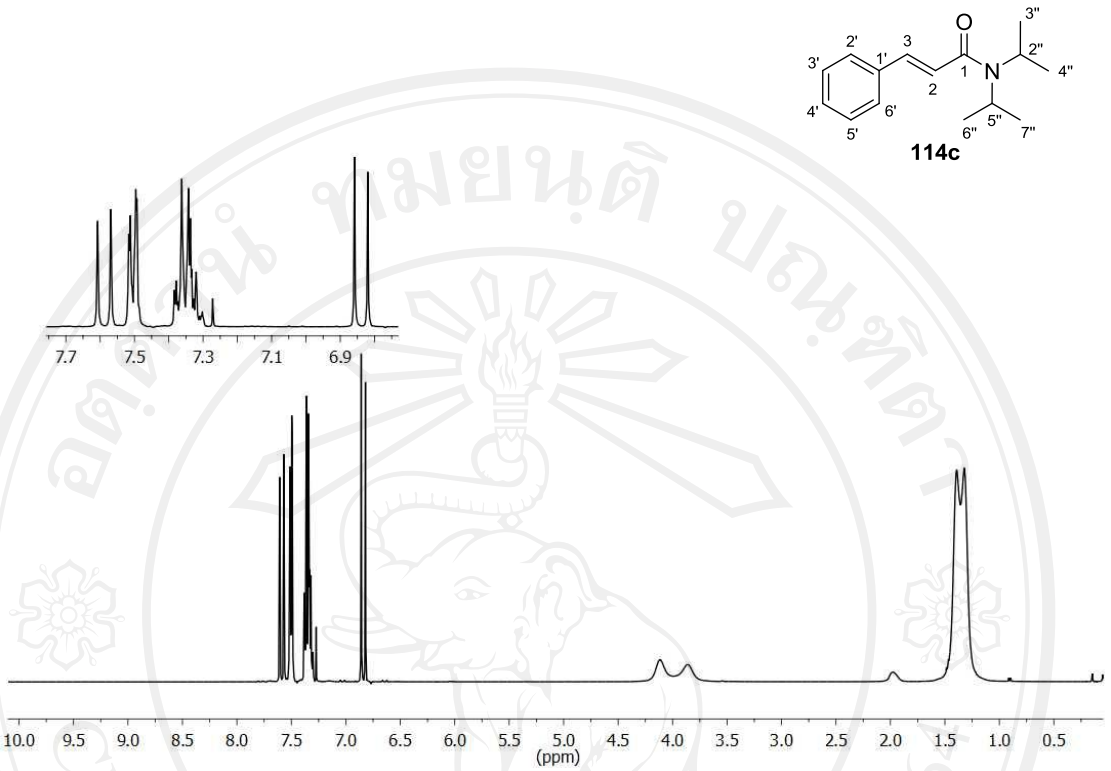

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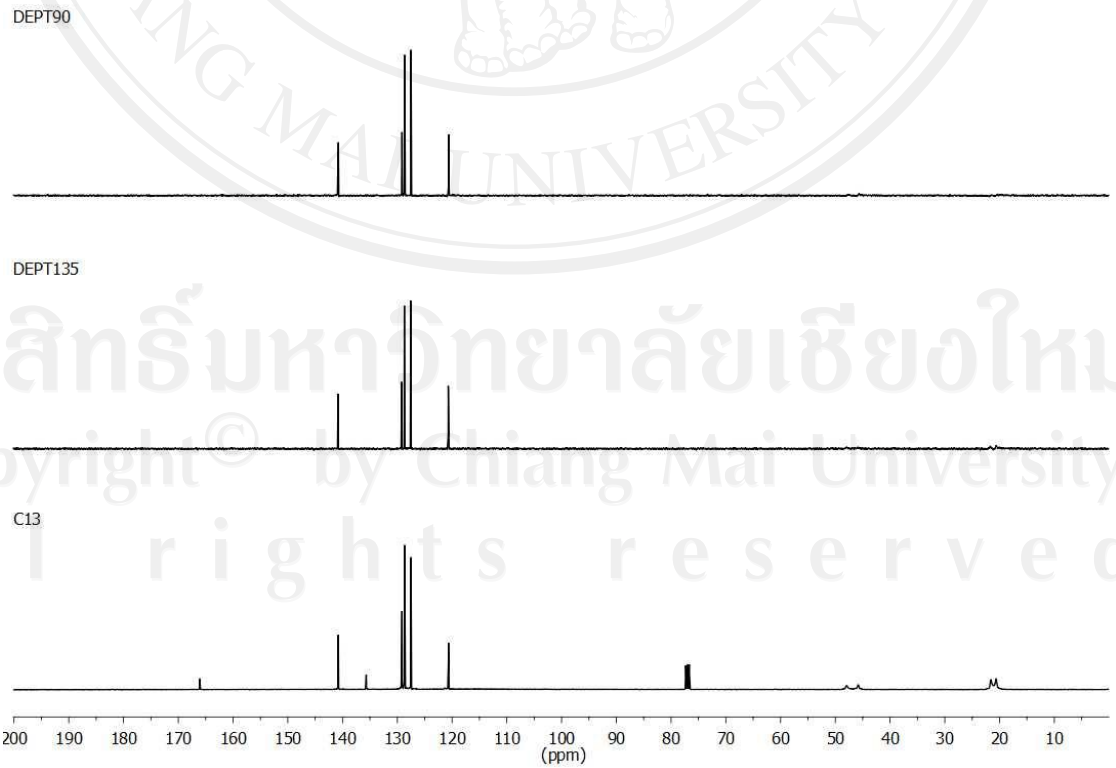
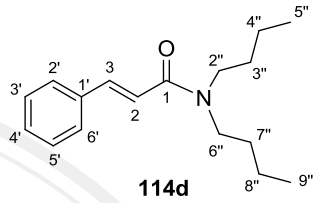
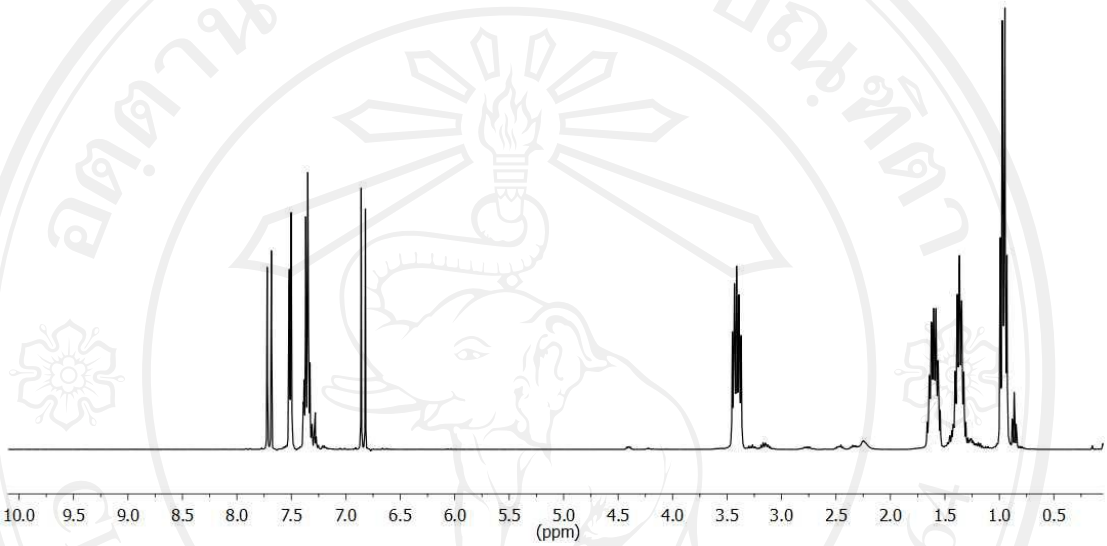


^{1}H-NMR in $CDCl_3$



114d



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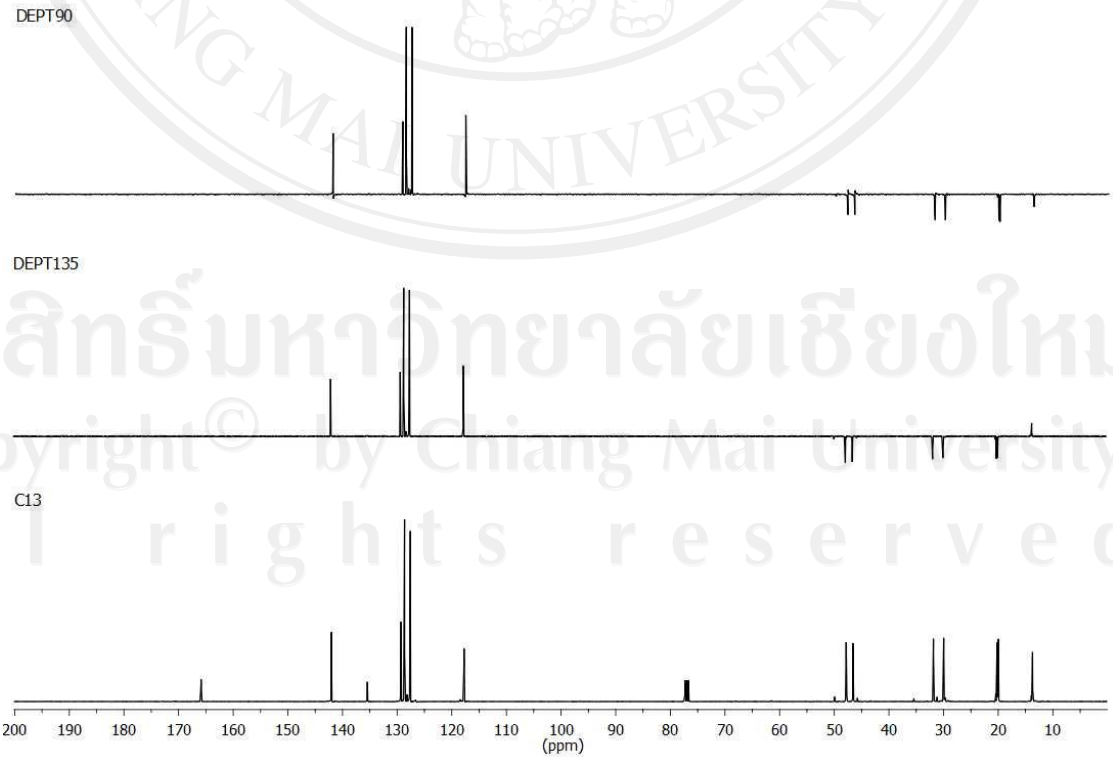
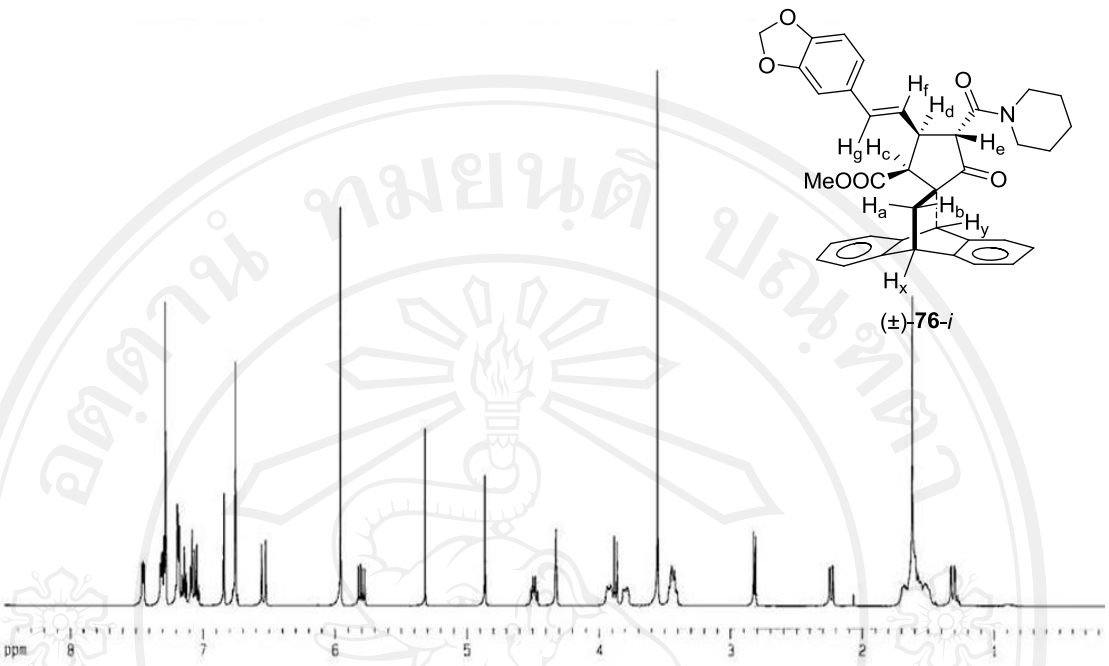
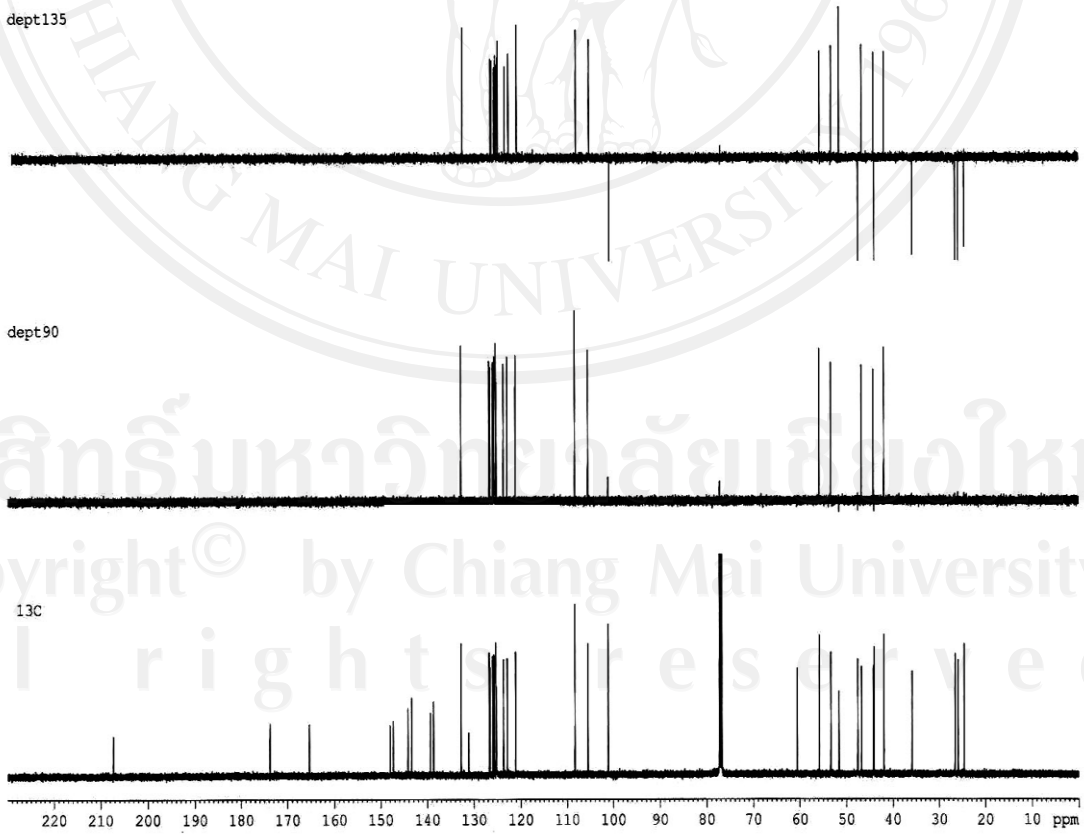


^{1}H-NMR in $CDCl_3$

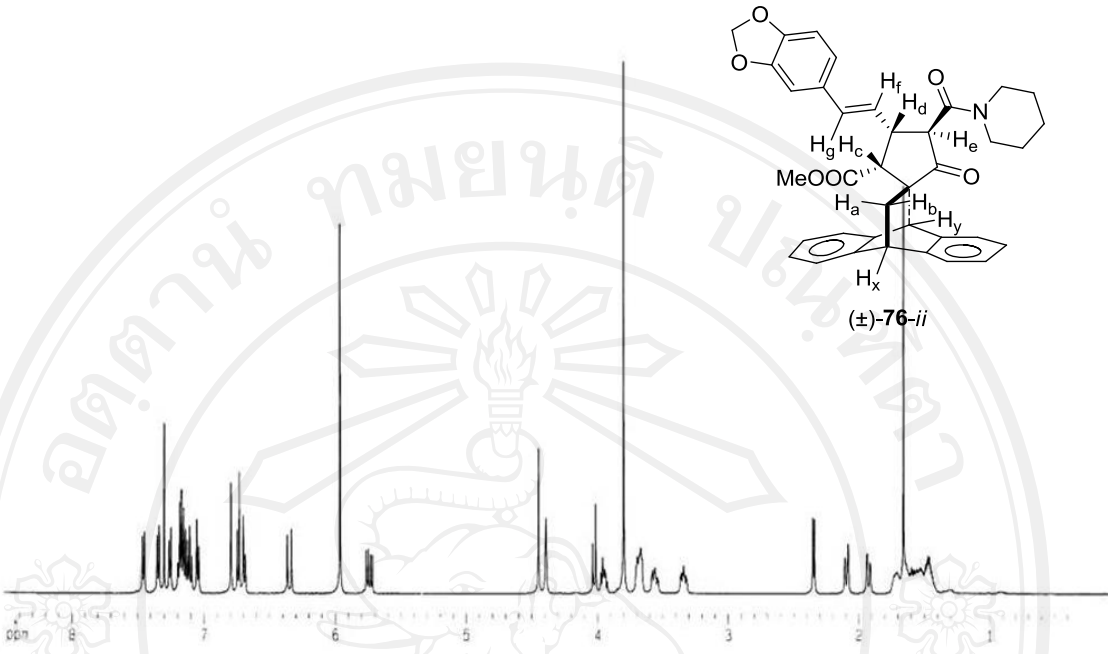


(±)-**76**-*i*

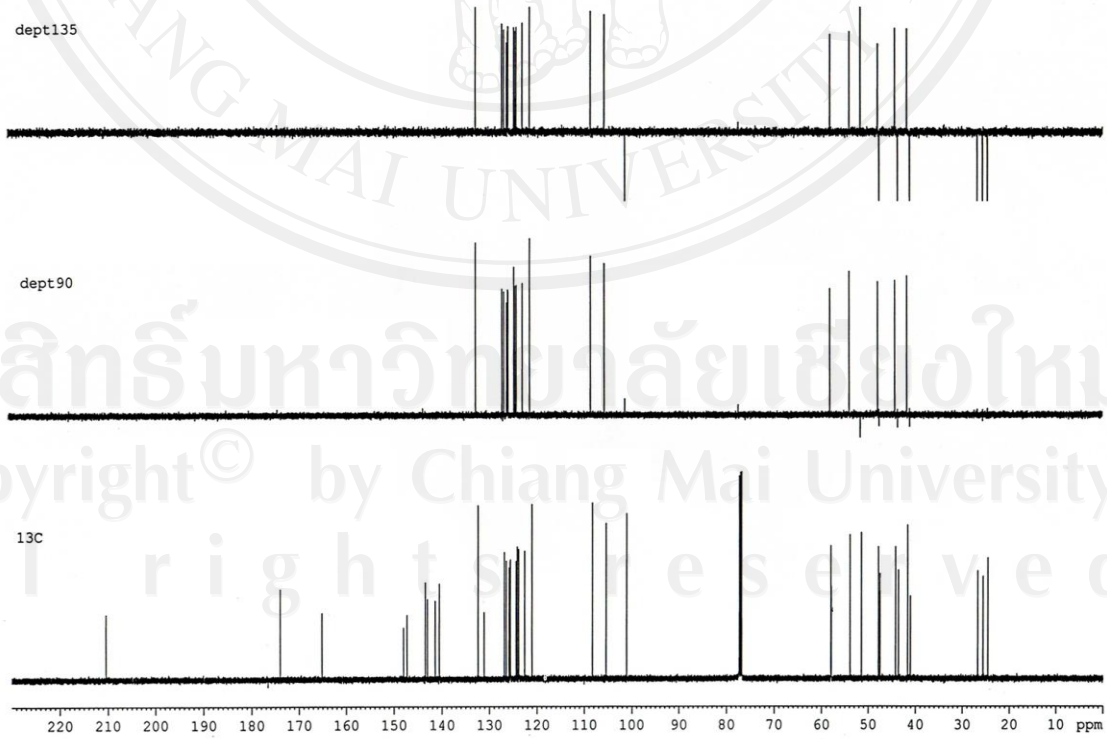
^{13}C-NMR in $CDCl_3$



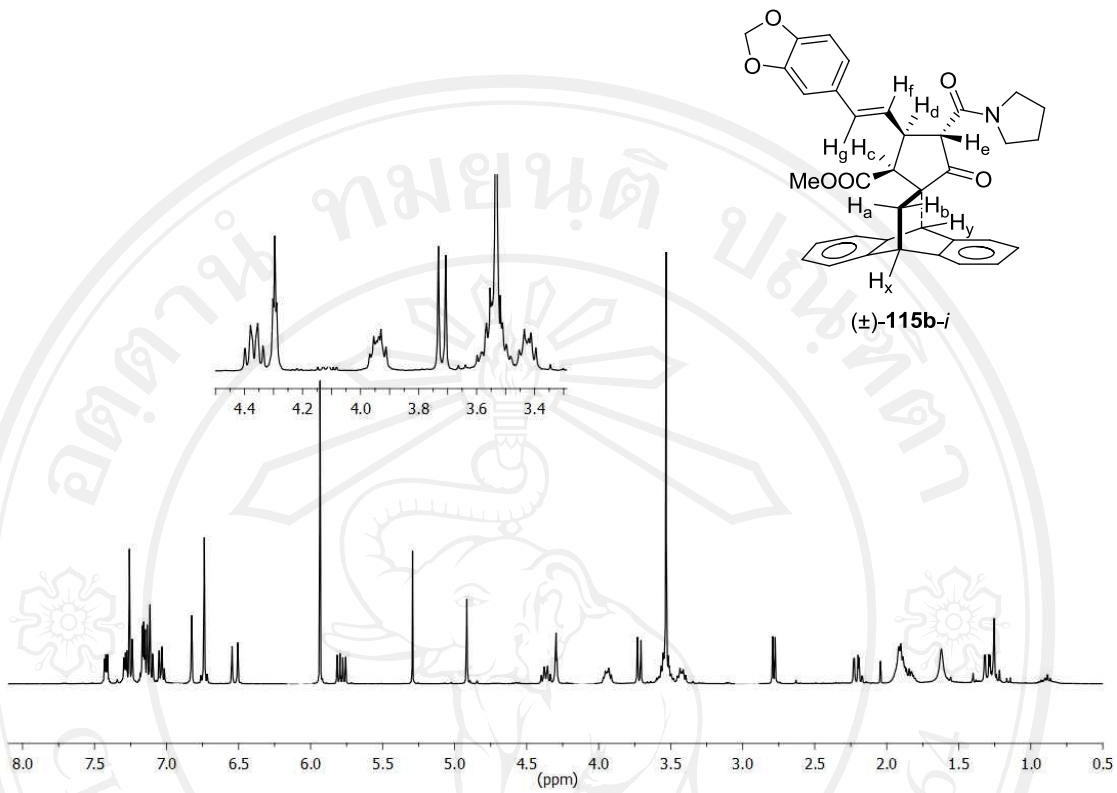
^{1}H-NMR in $CDCl_3$



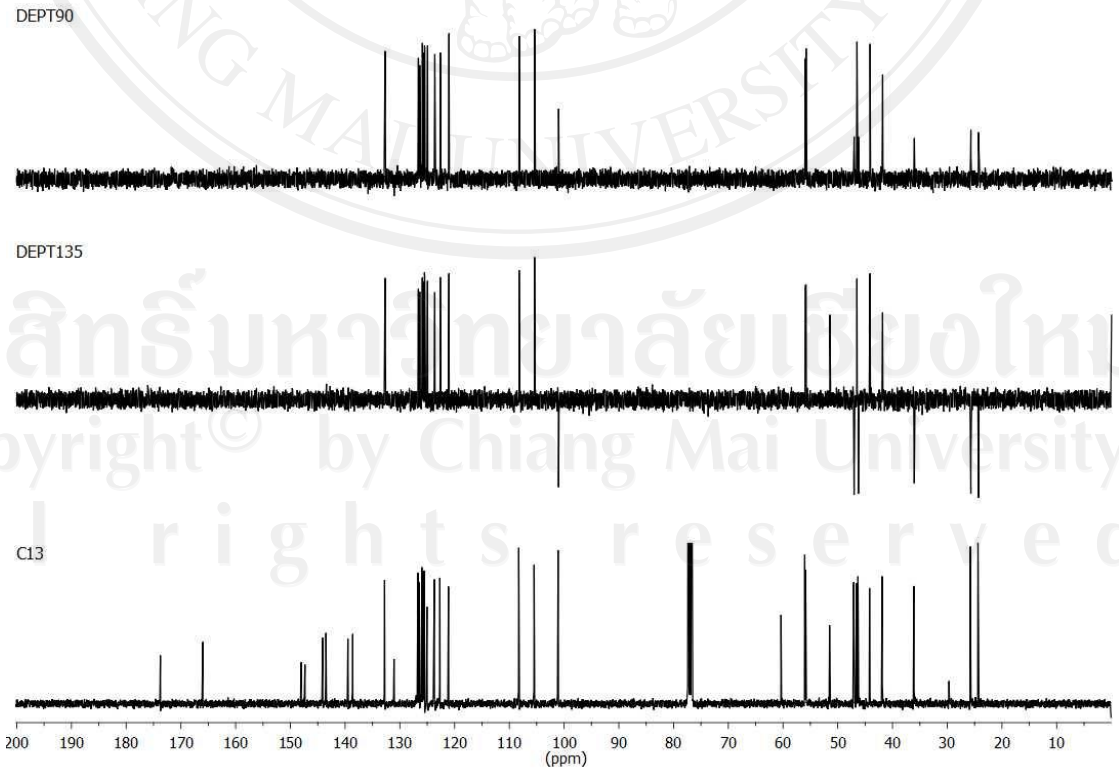
^{13}C-NMR in $CDCl_3$



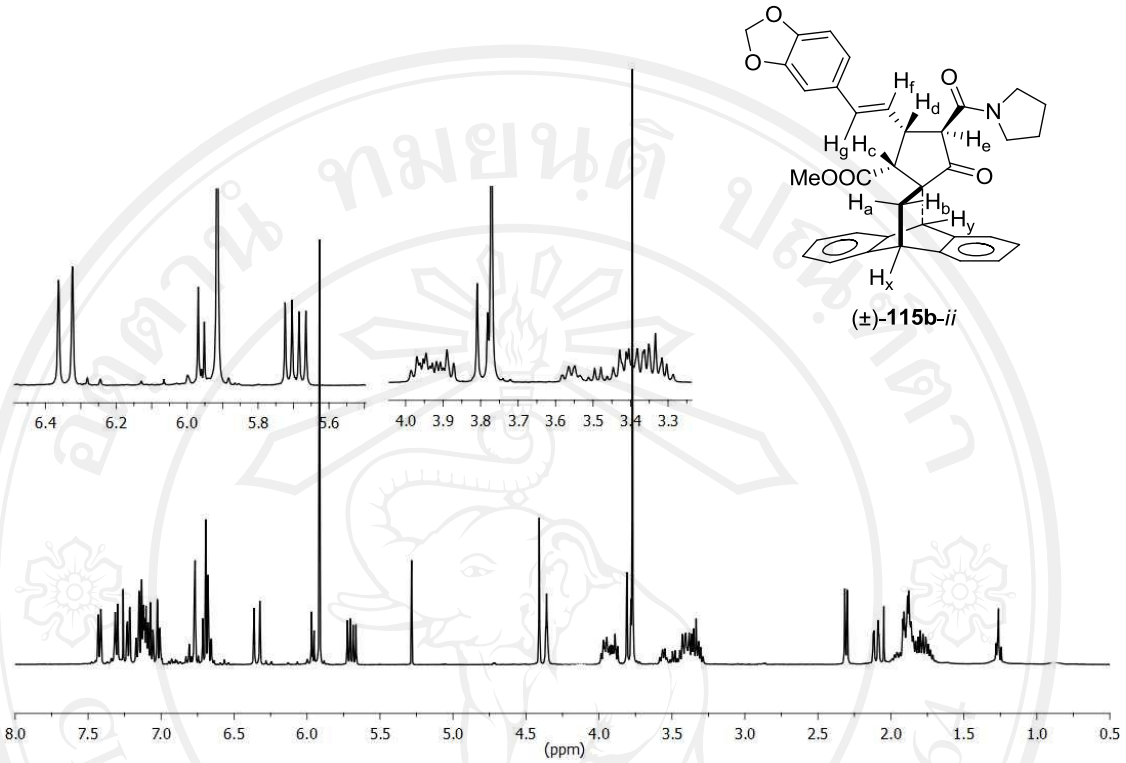
^{1}H-NMR in $CDCl_3$



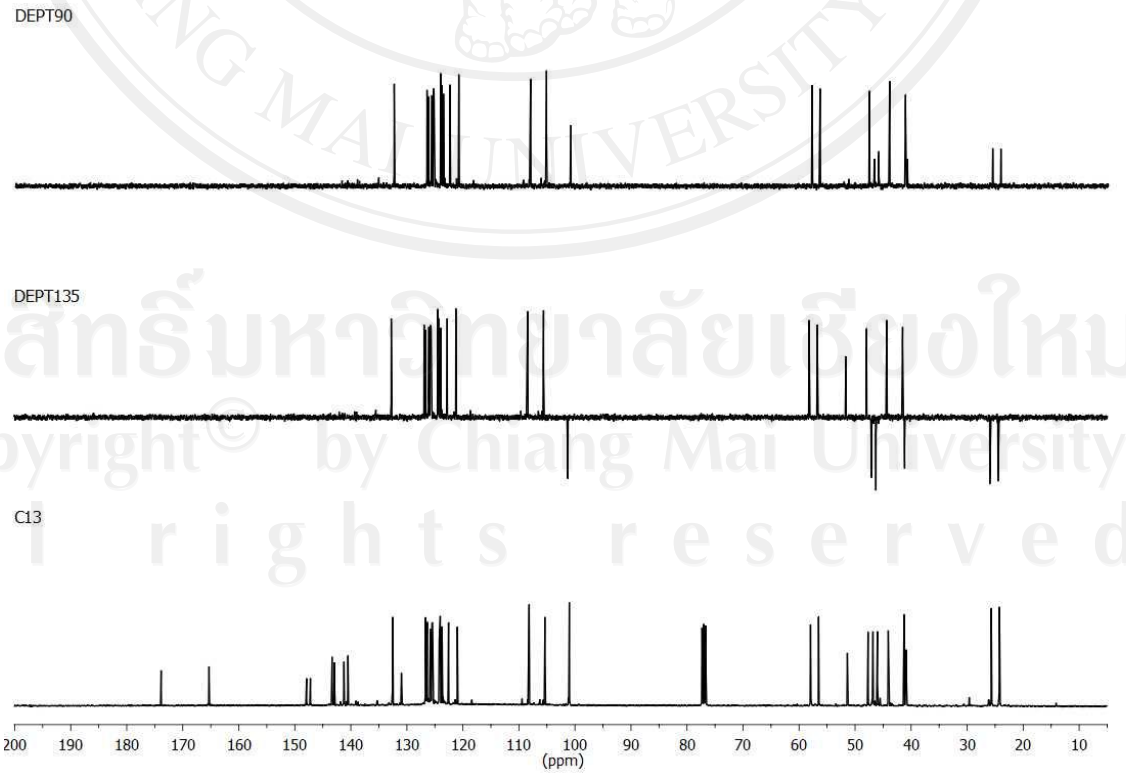
^{13}C-NMR in $CDCl_3$



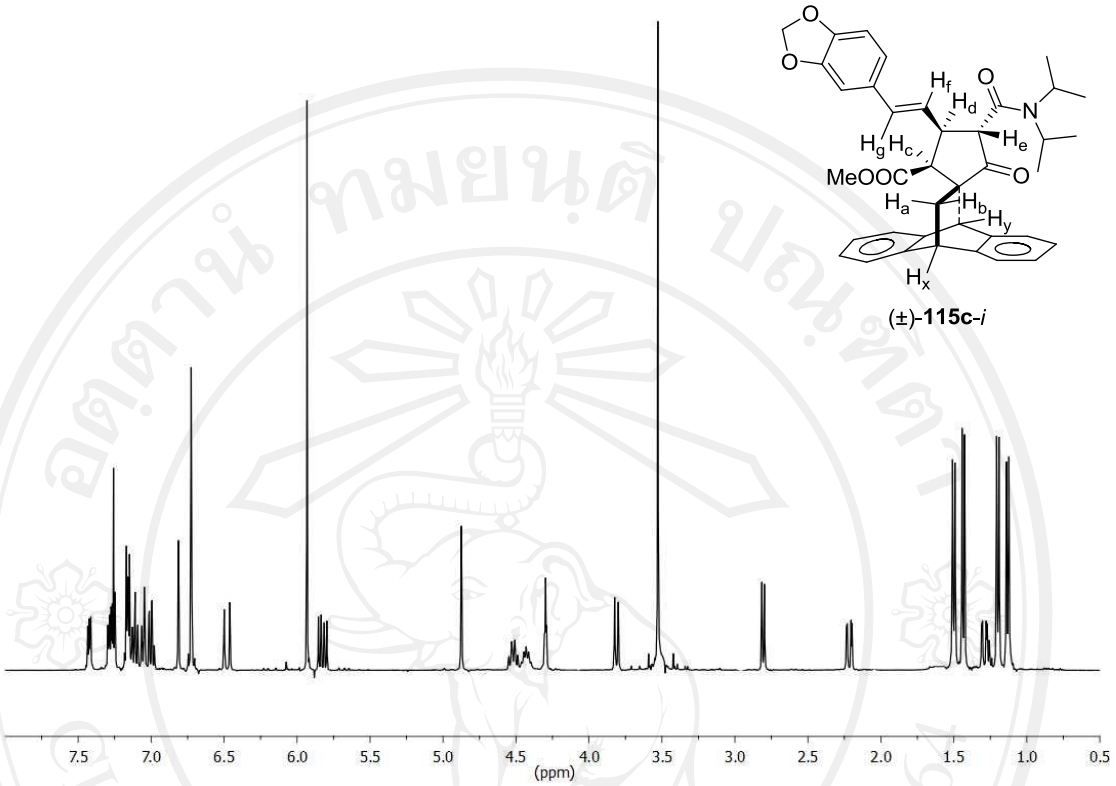
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$

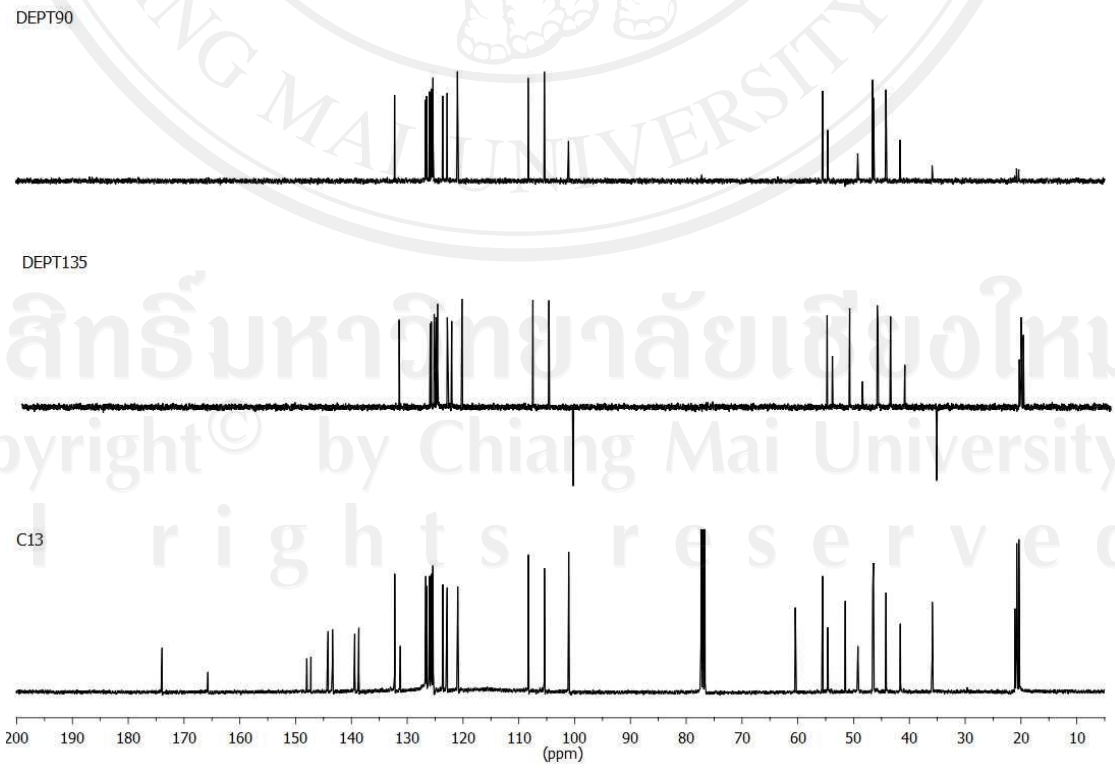


^{1}H-NMR in $CDCl_3$

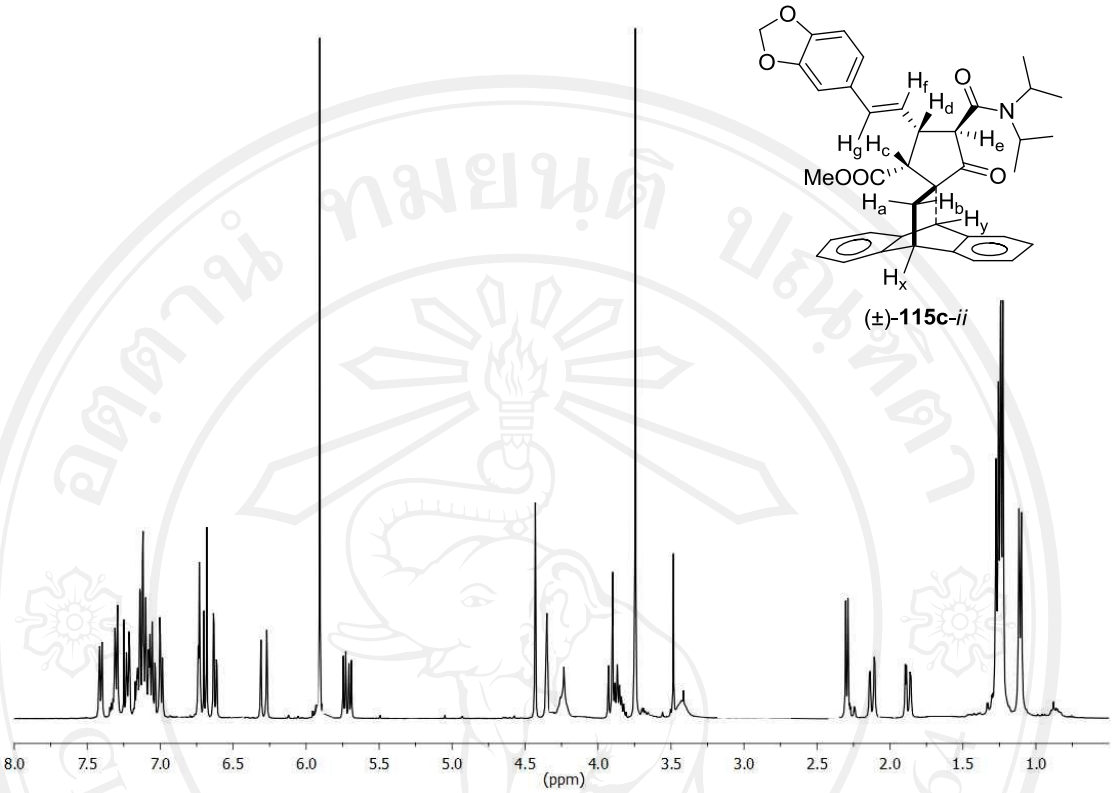


(±)-**115c**-*i*

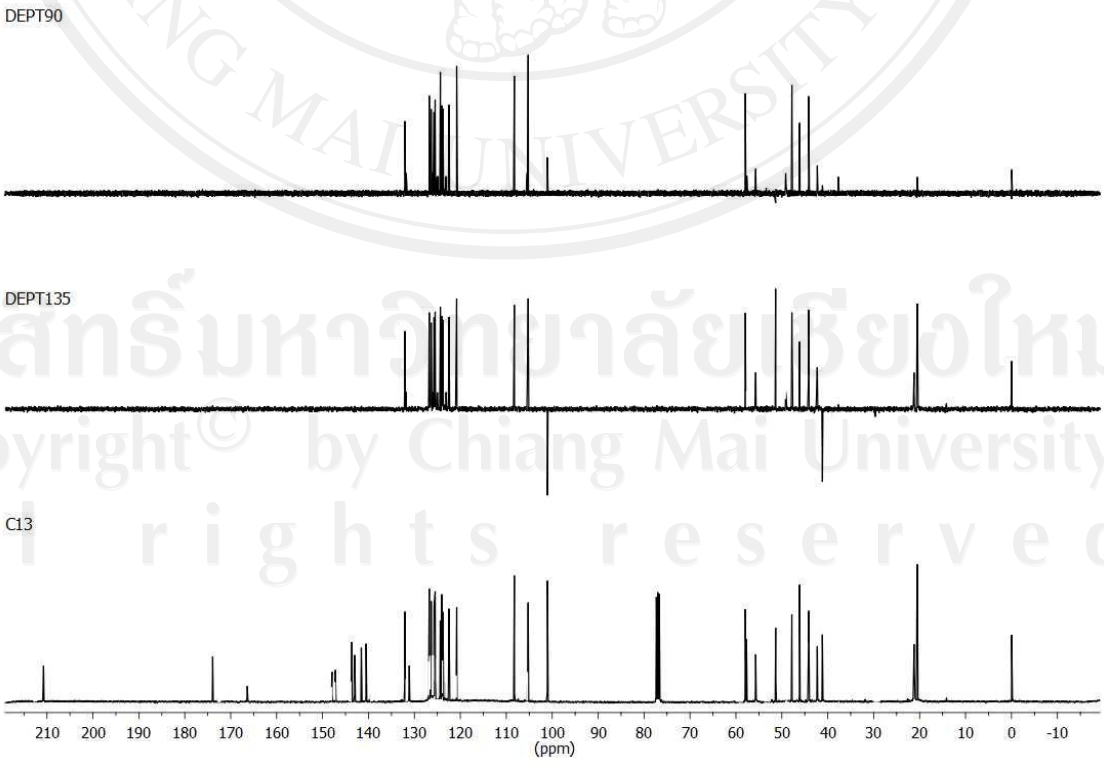
^{13}C-NMR in $CDCl_3$



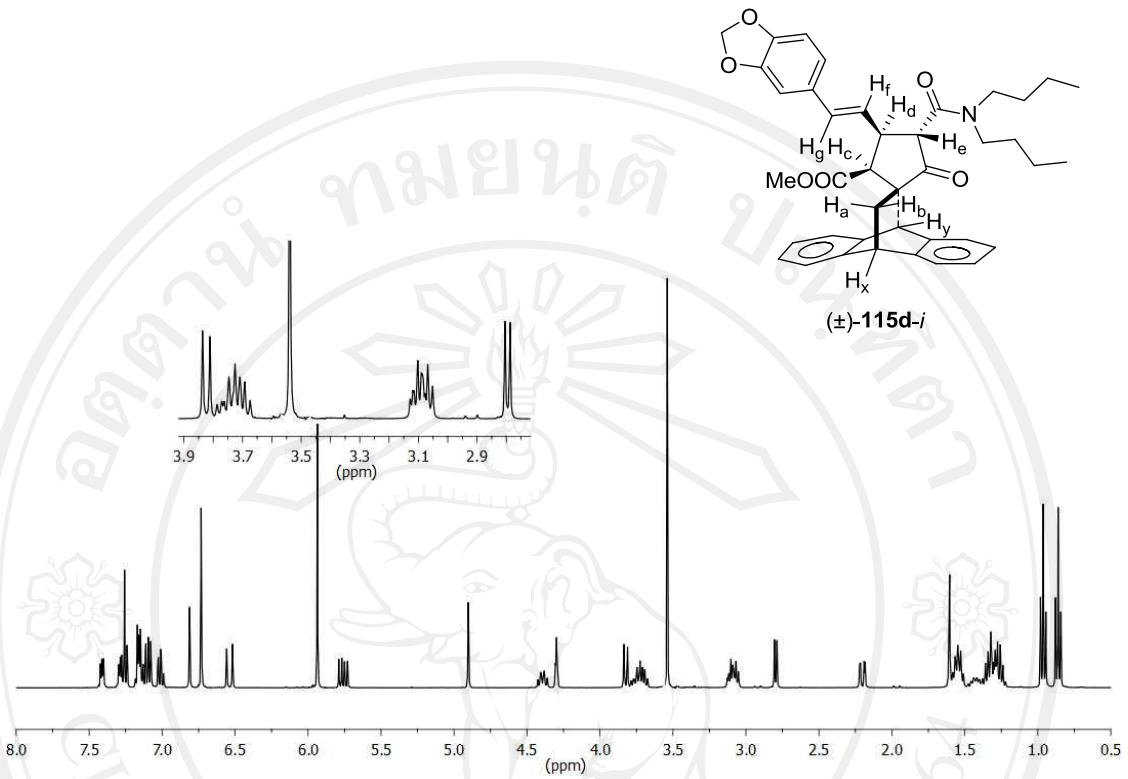
^{1}H-NMR in $CDCl_3$



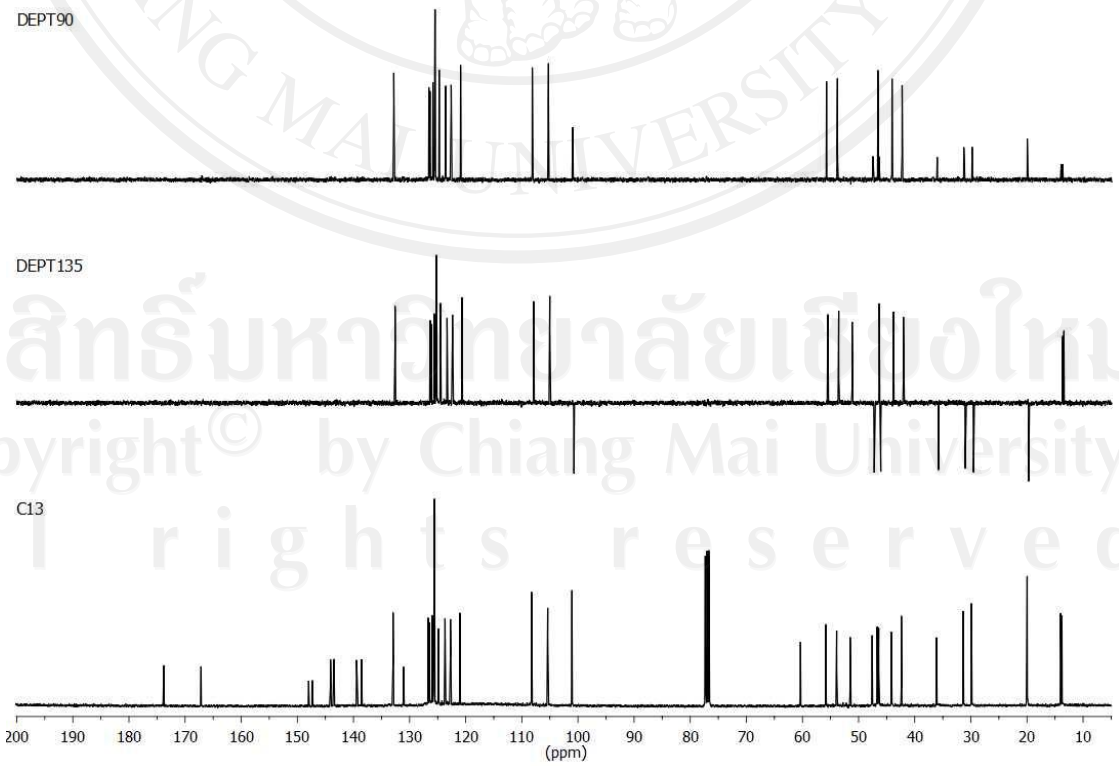
^{13}C-NMR in $CDCl_3$



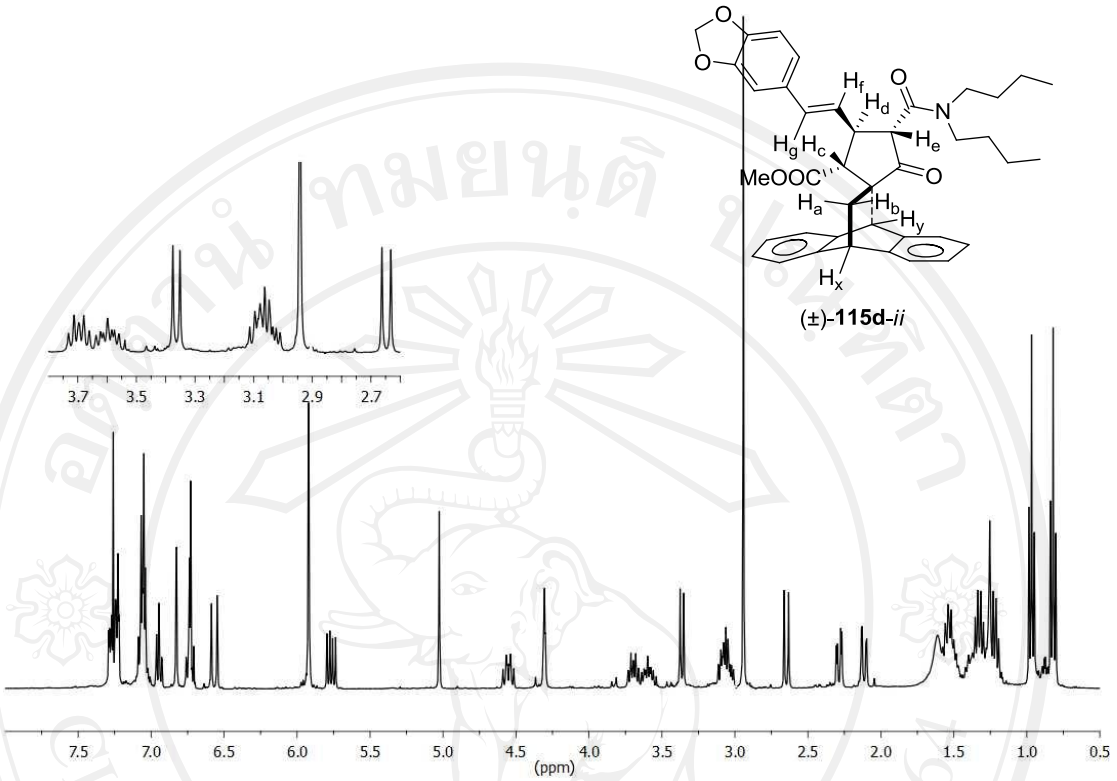
^{1}H-NMR in $CDCl_3$



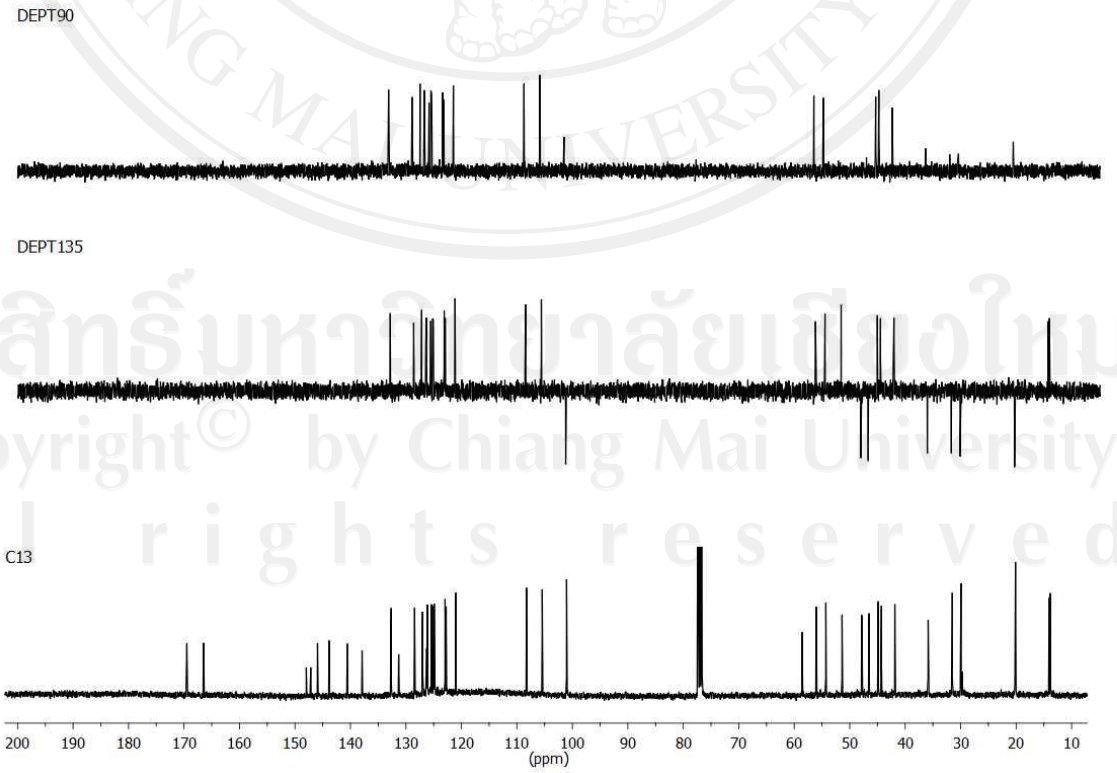
^{13}C-NMR in $CDCl_3$



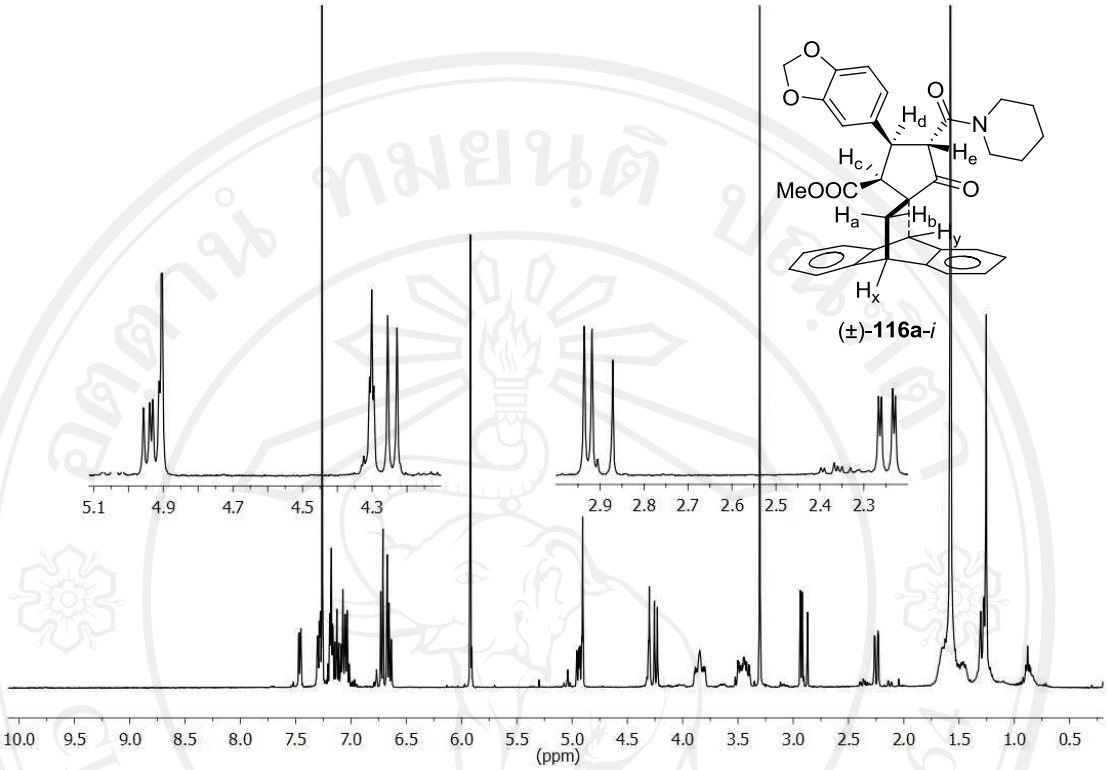
^{1}H-NMR in $CDCl_3$



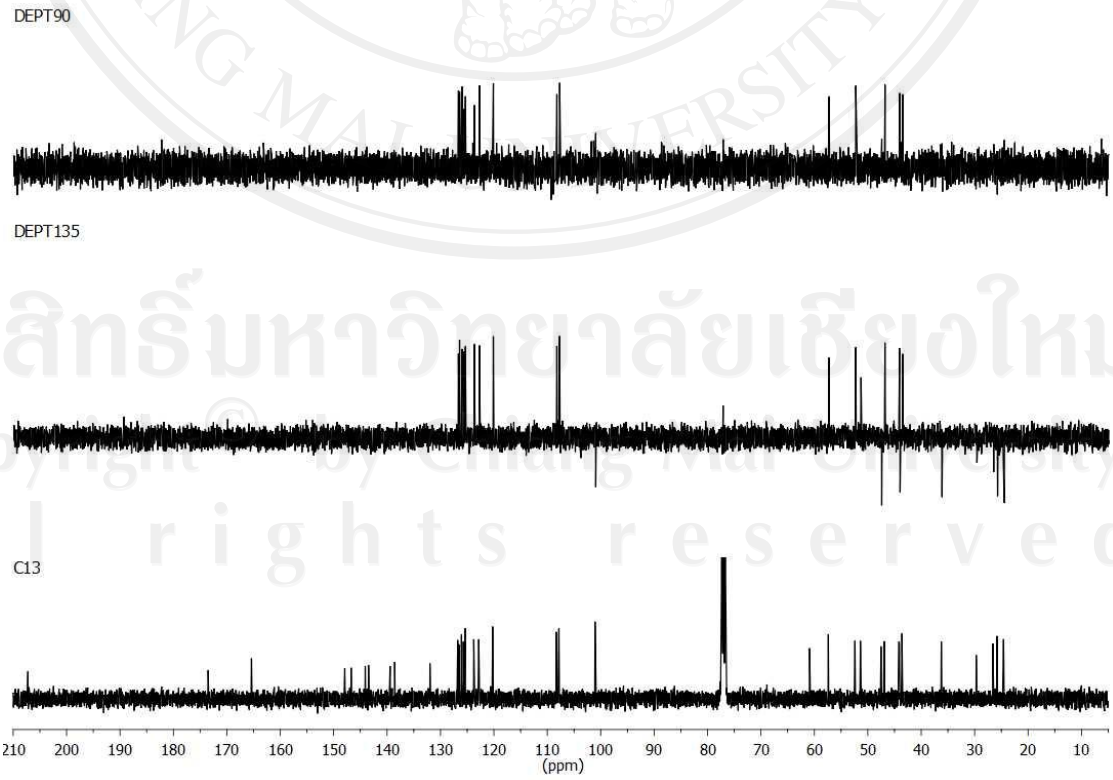
^{13}C-NMR in $CDCl_3$



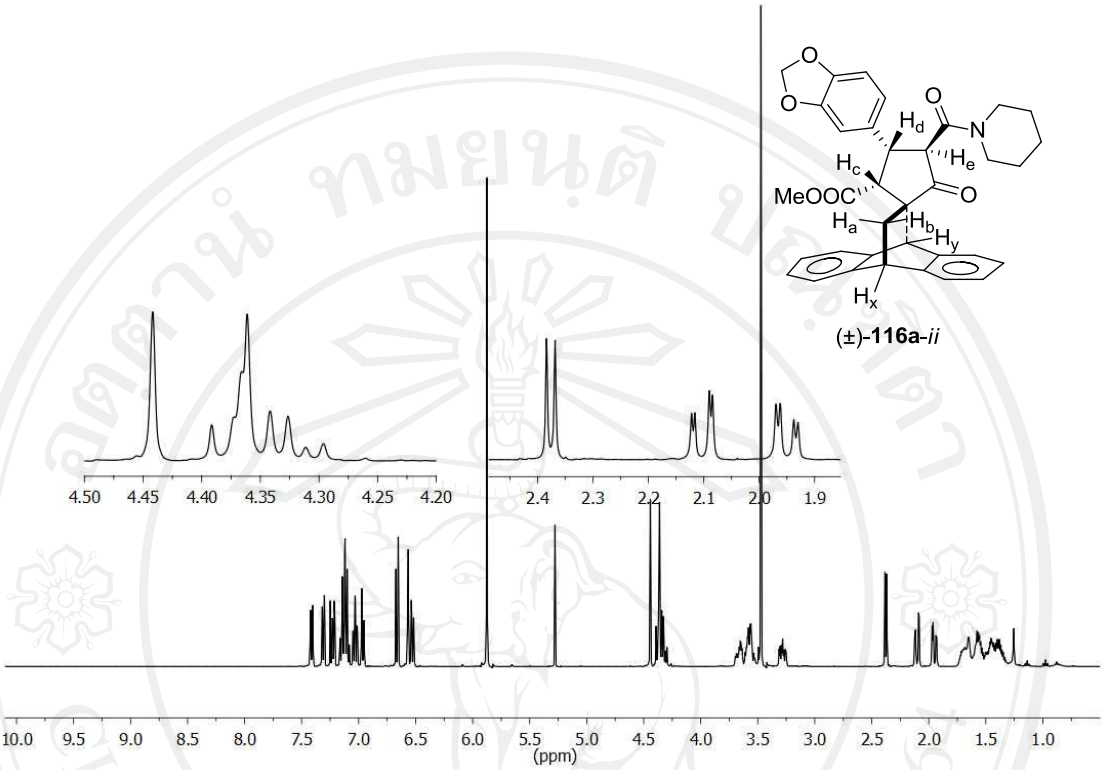
^{1}H-NMR in $CDCl_3$



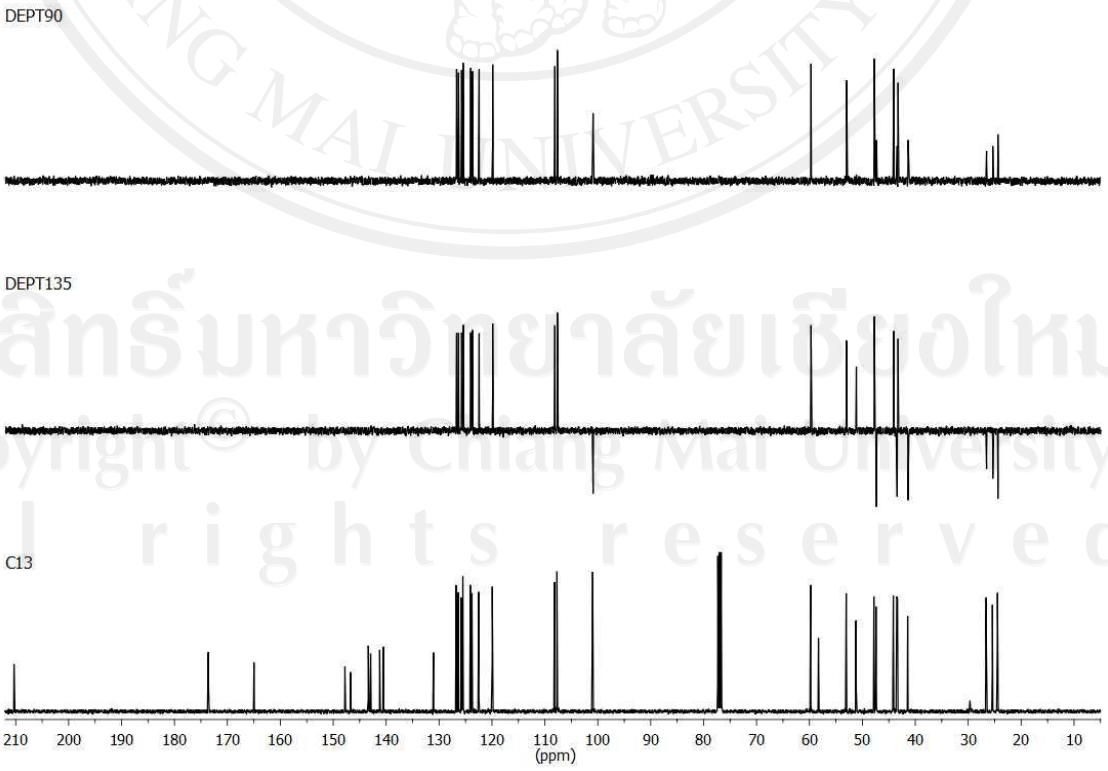
^{13}C-NMR in $CDCl_3$



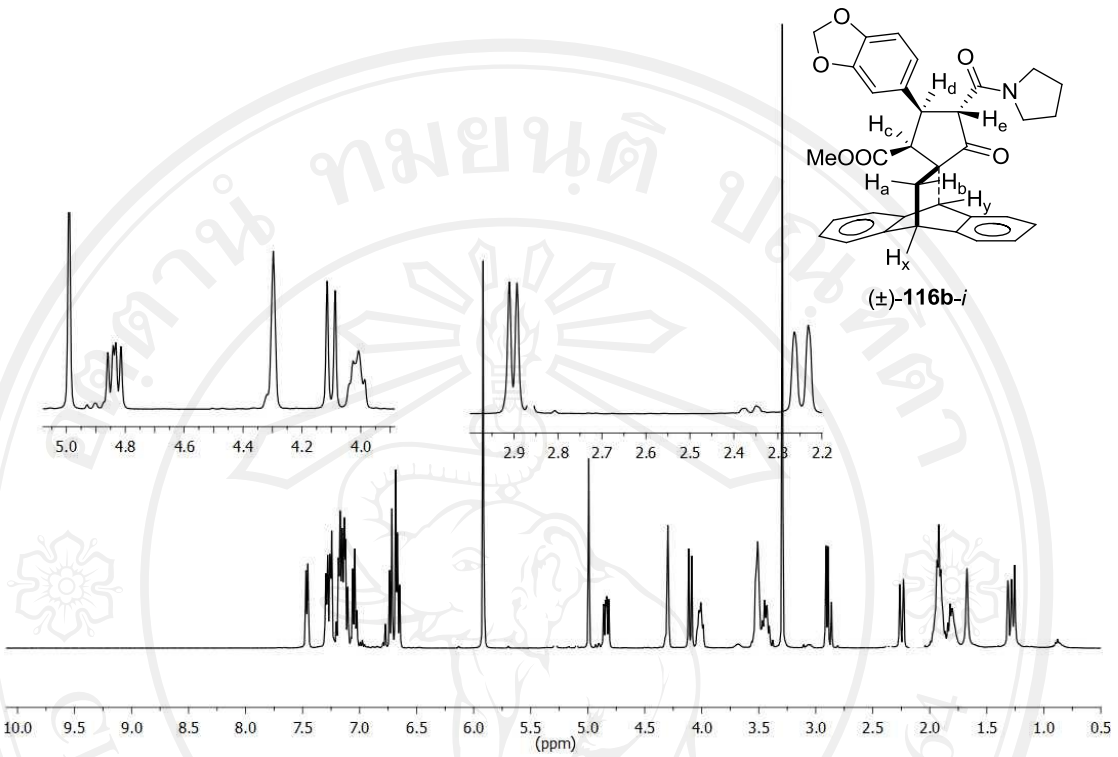
^{1}H-NMR in $CDCl_3$



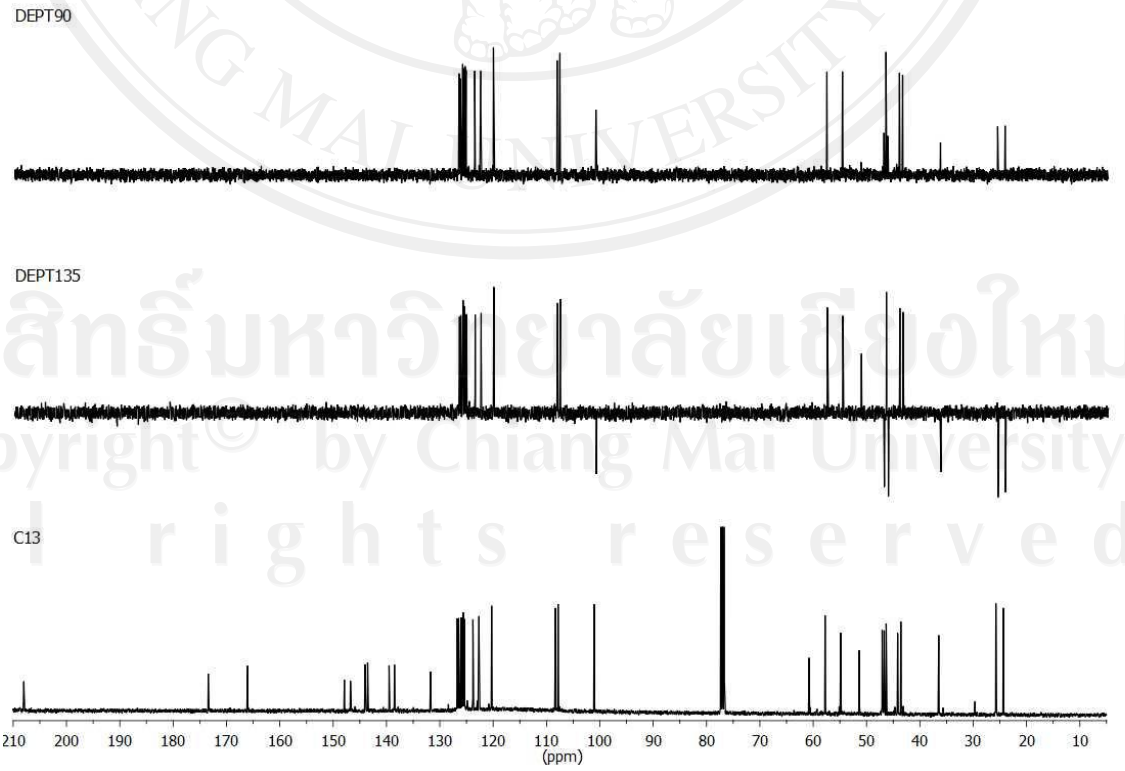
^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



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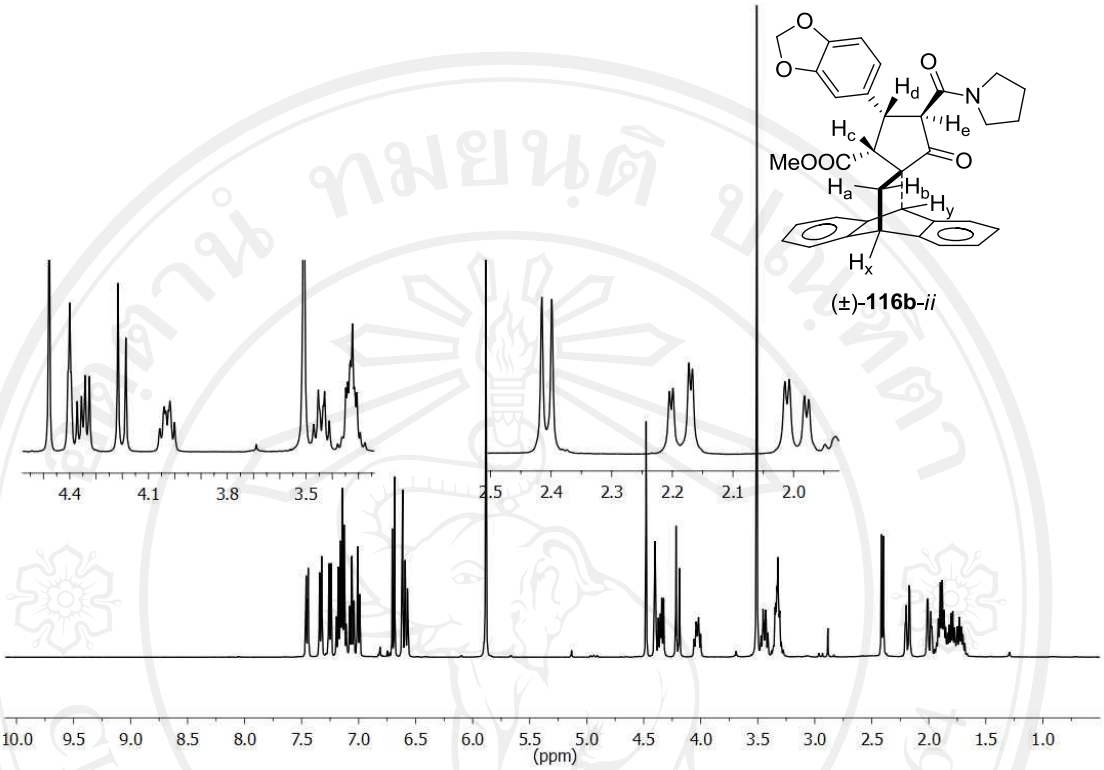
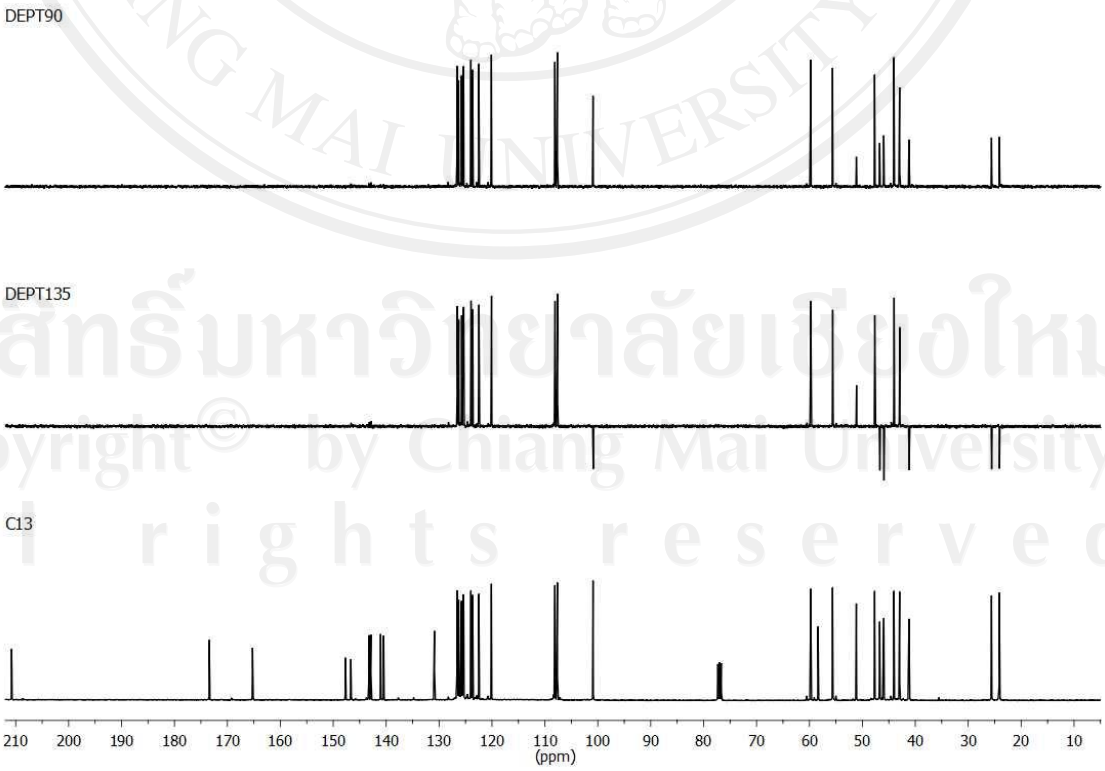
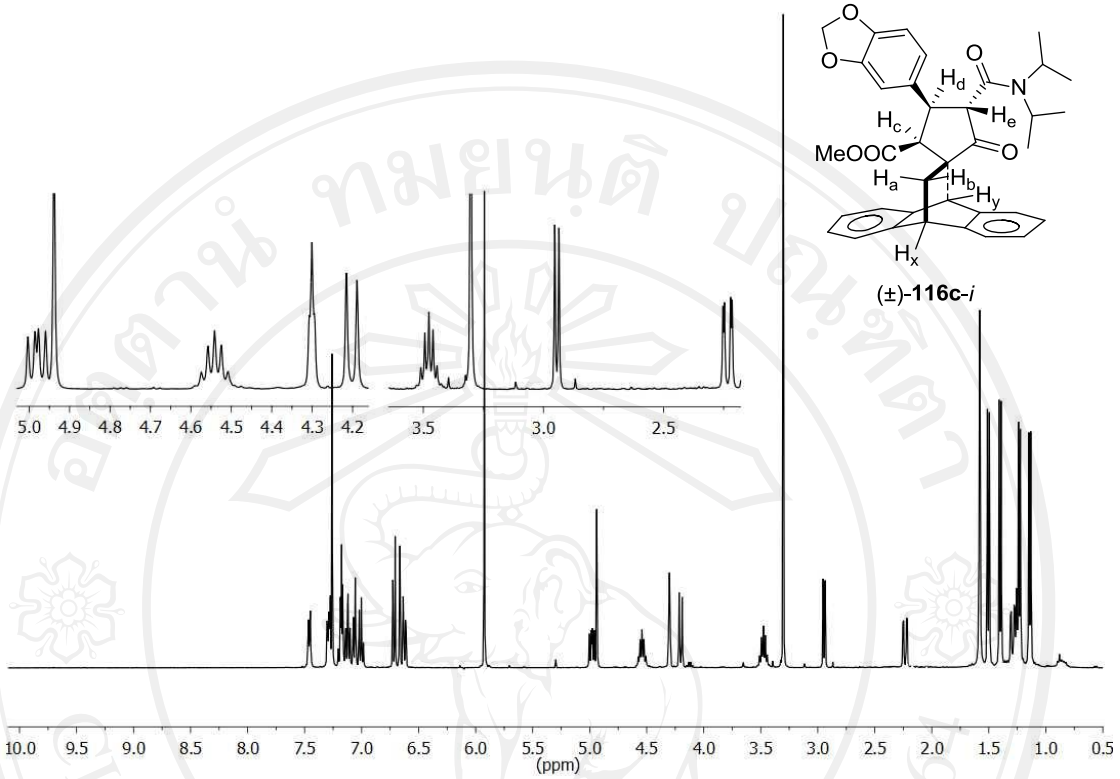


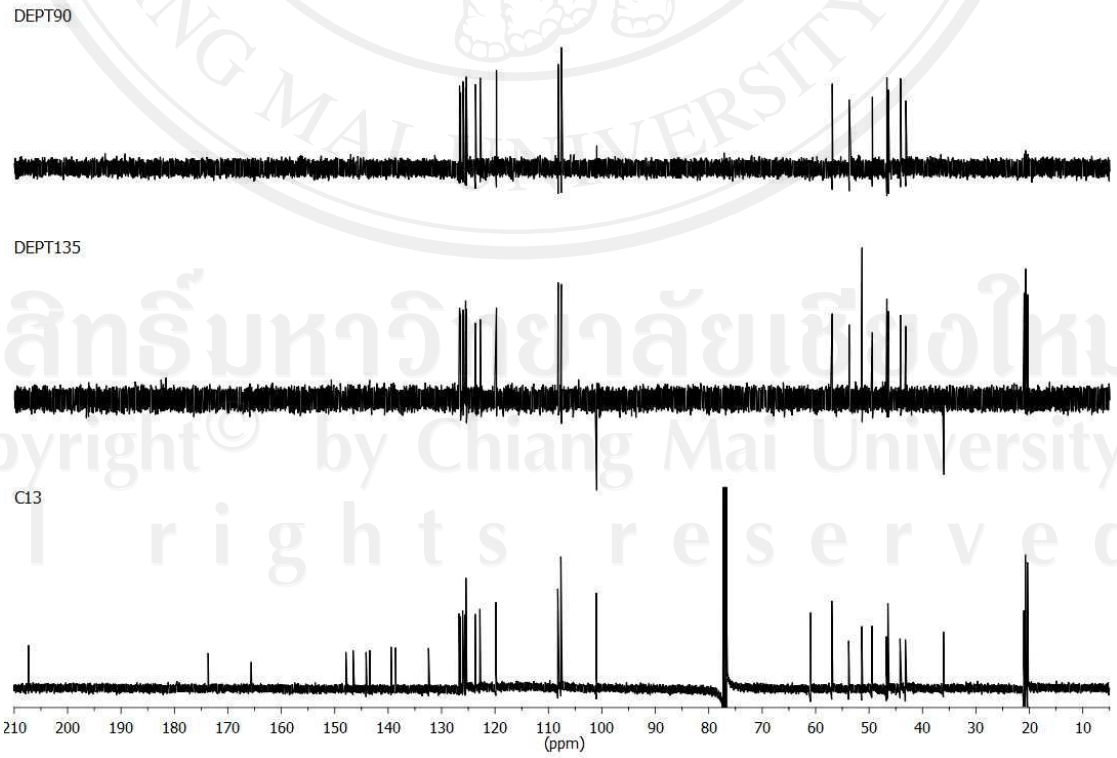
^{13}C-NMR in $CDCl_3$



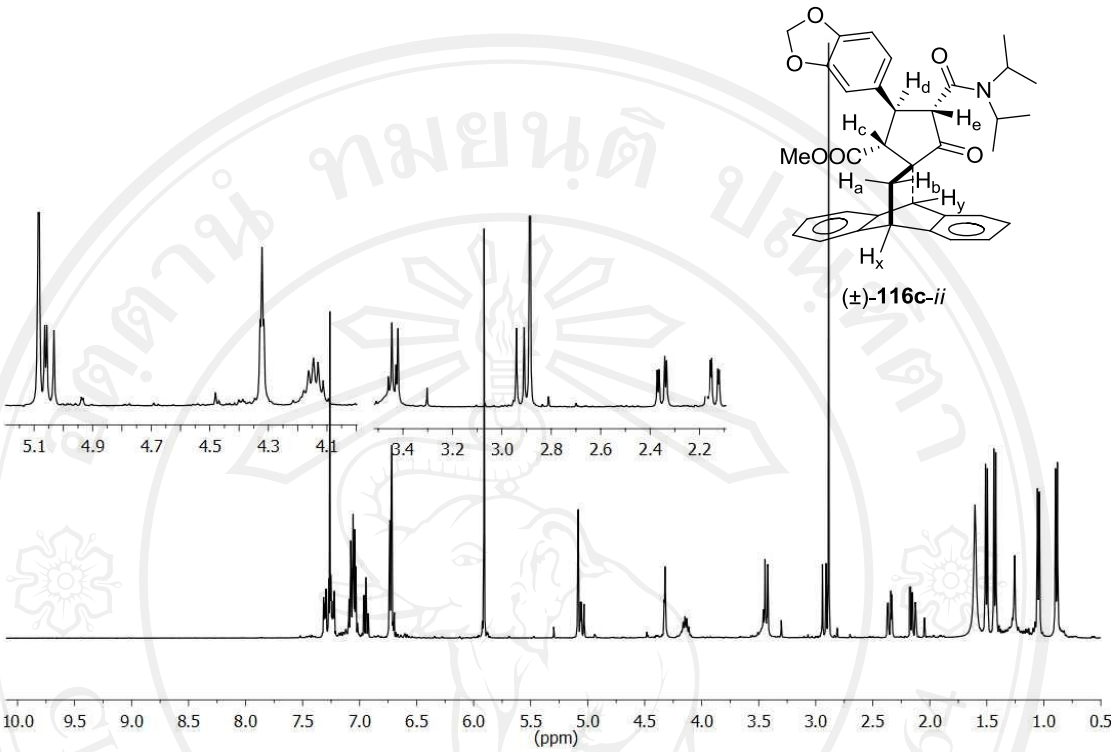
^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



^{1}H-NMR in $CDCl_3$



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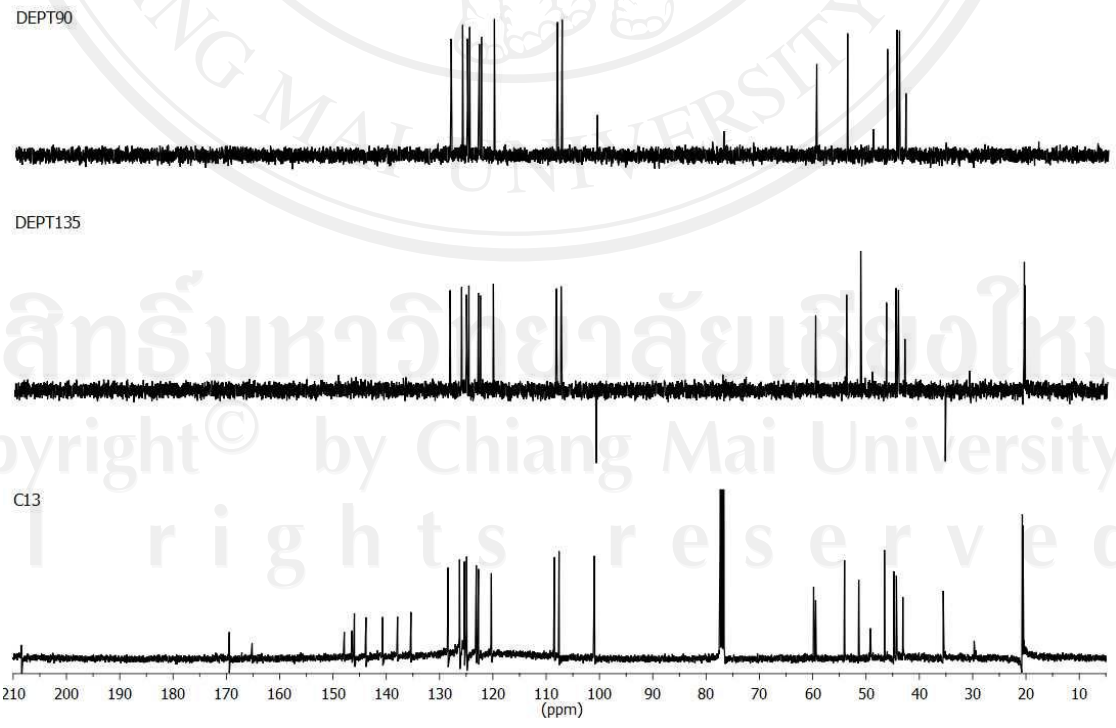

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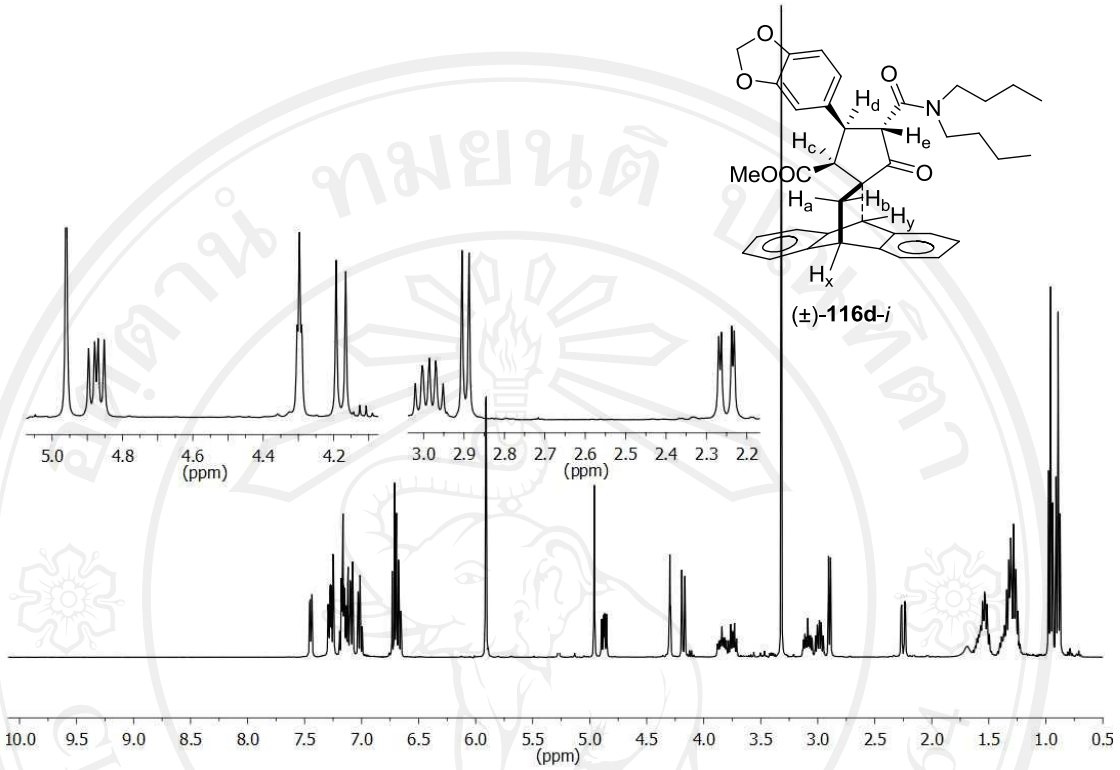

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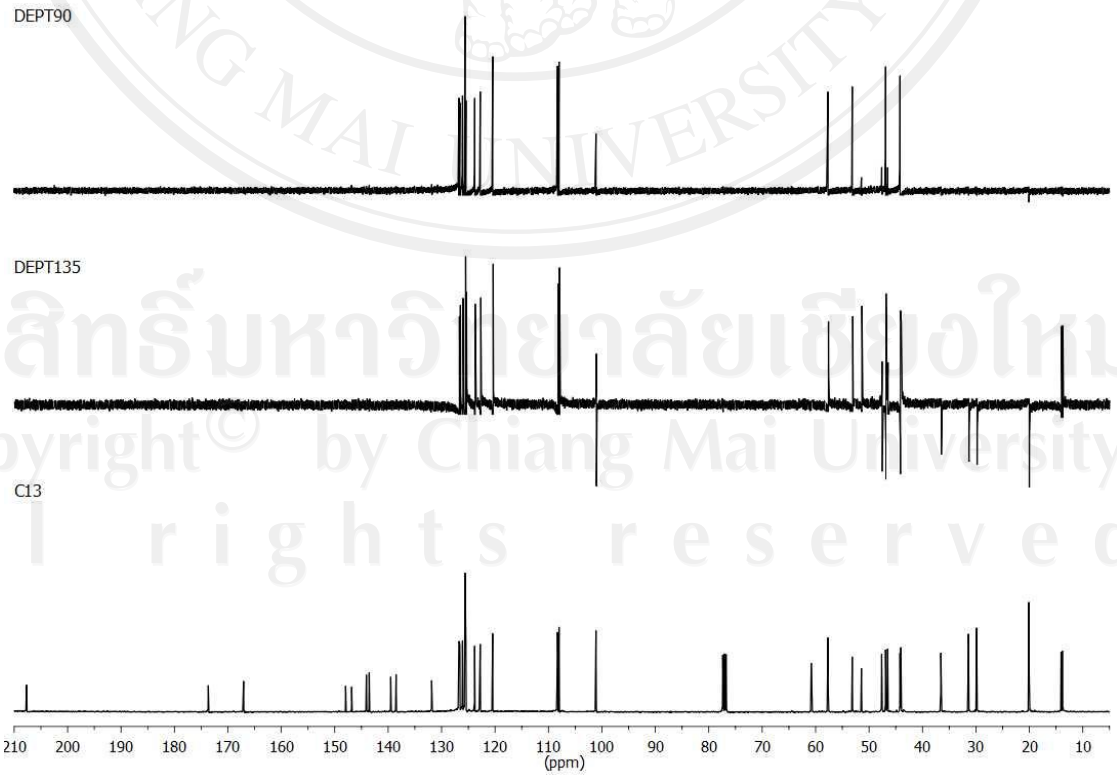

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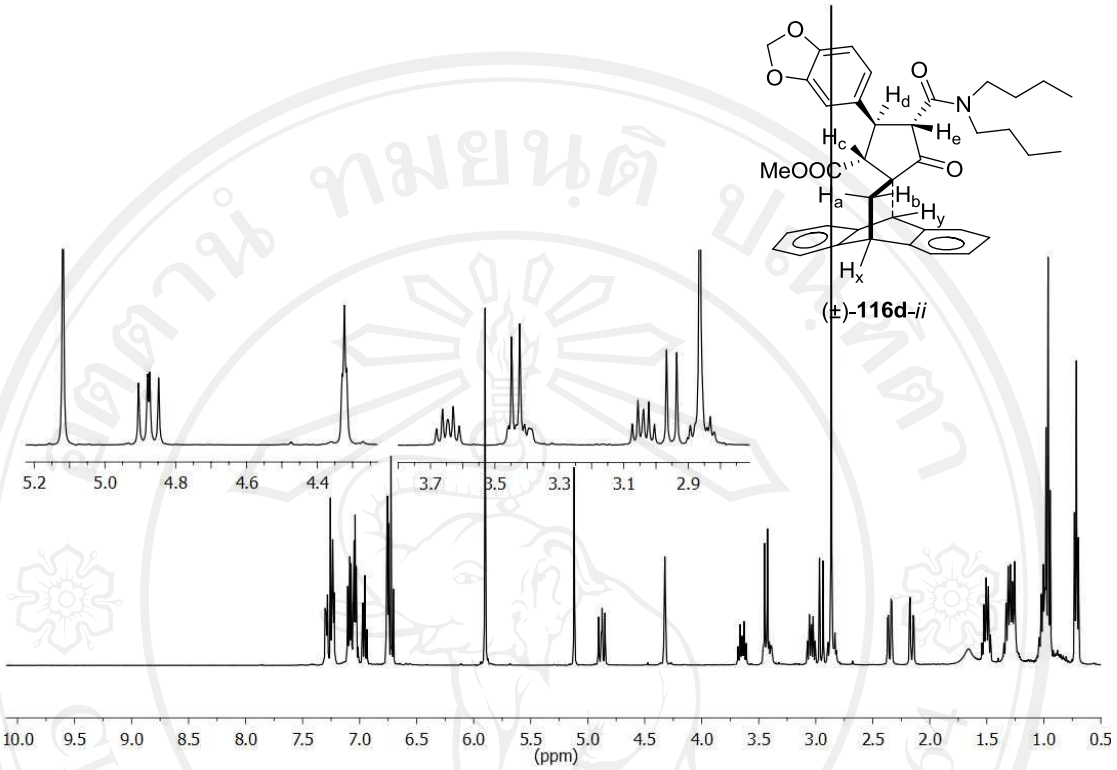

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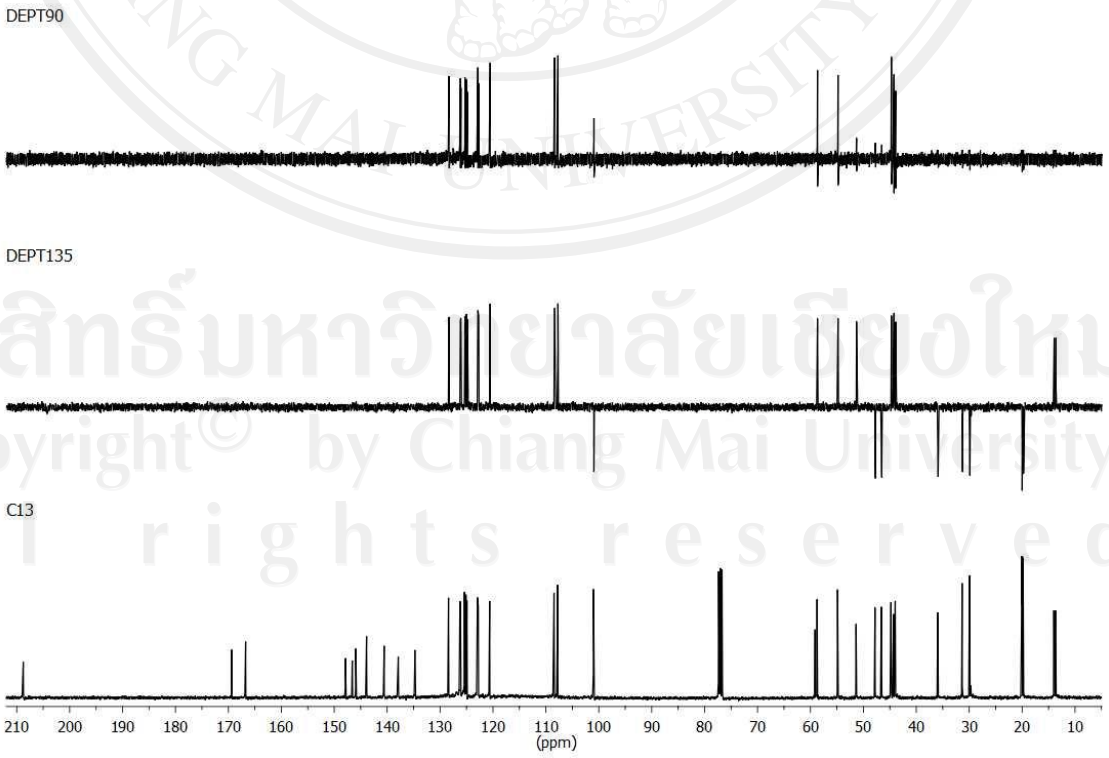

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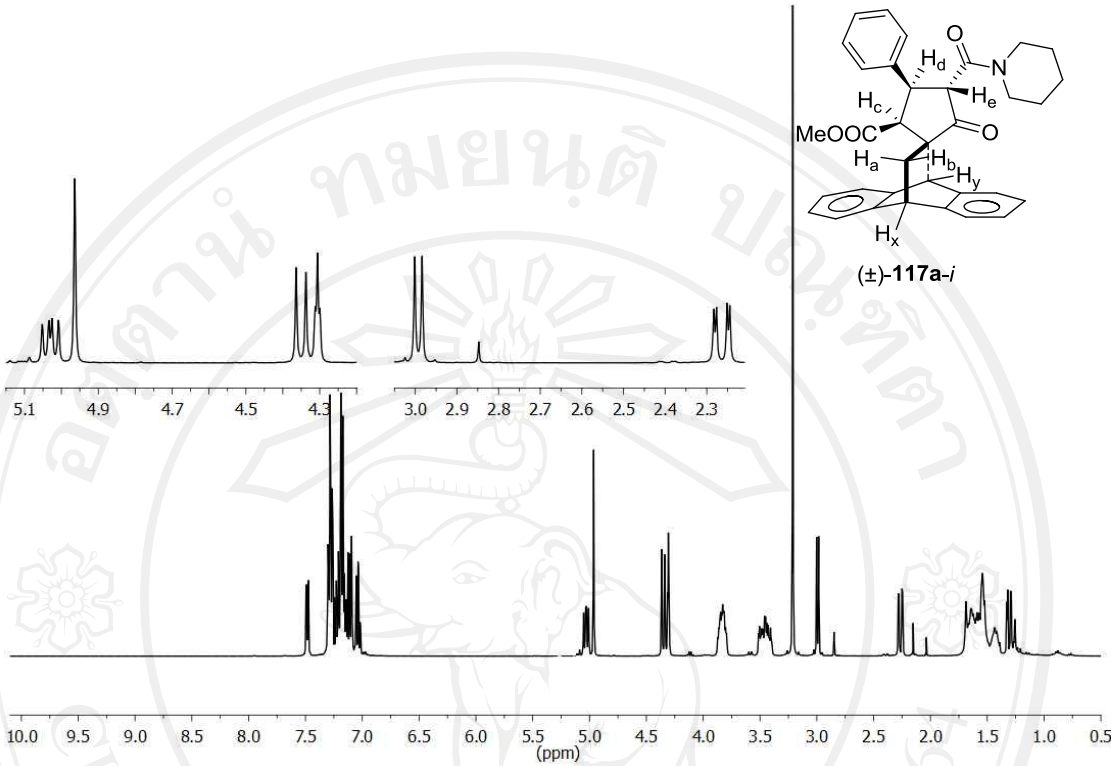

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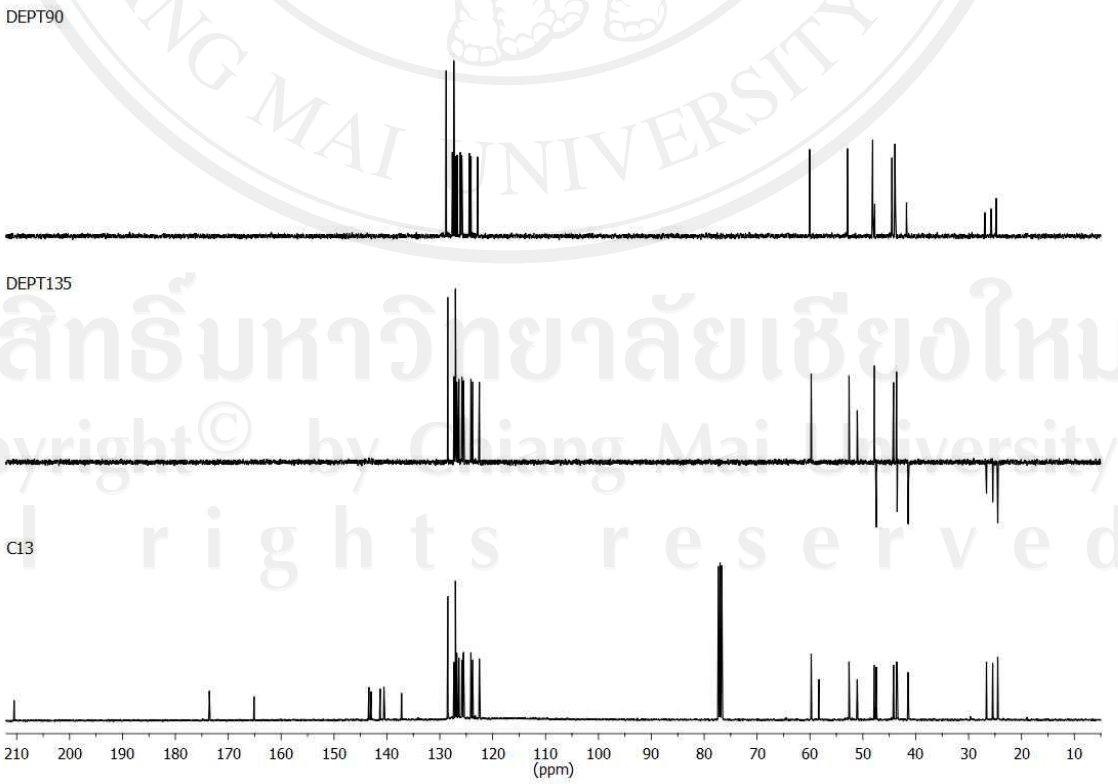

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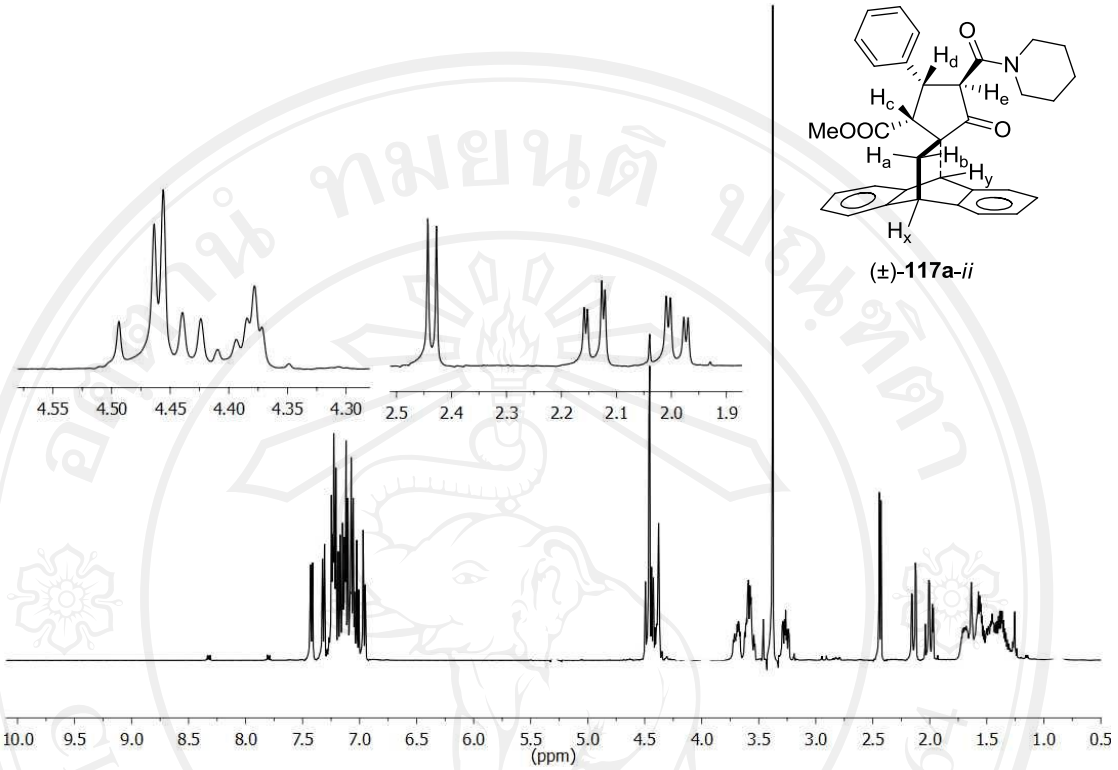

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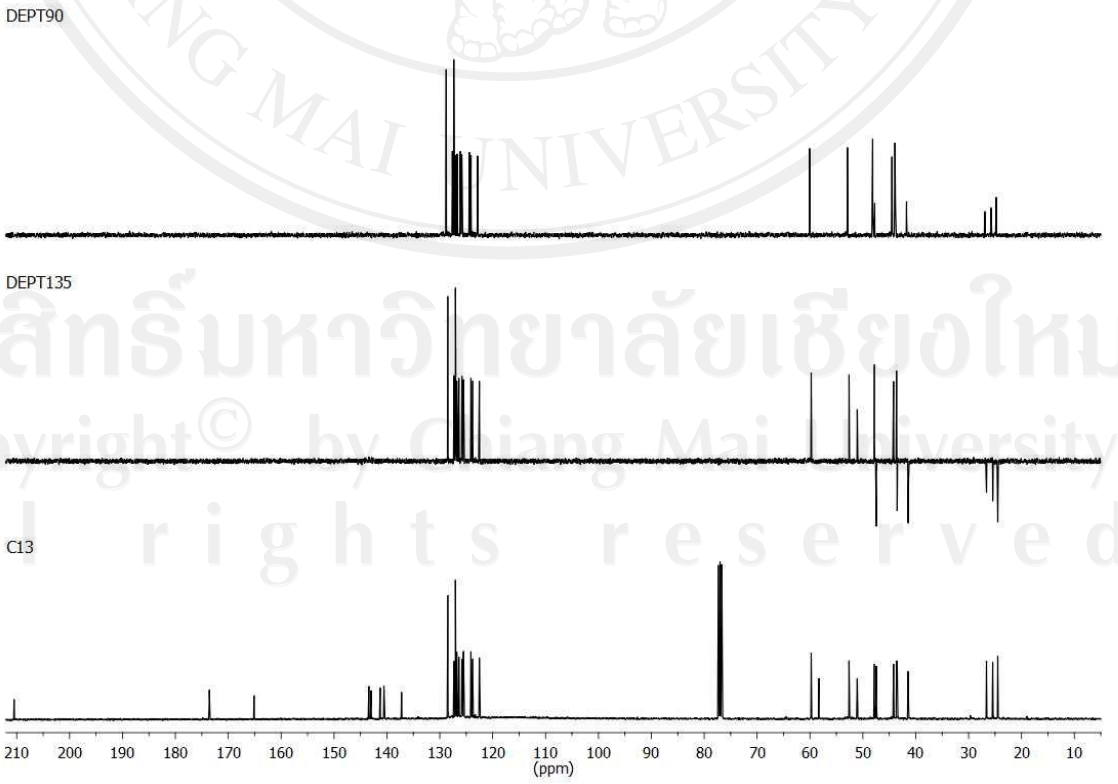

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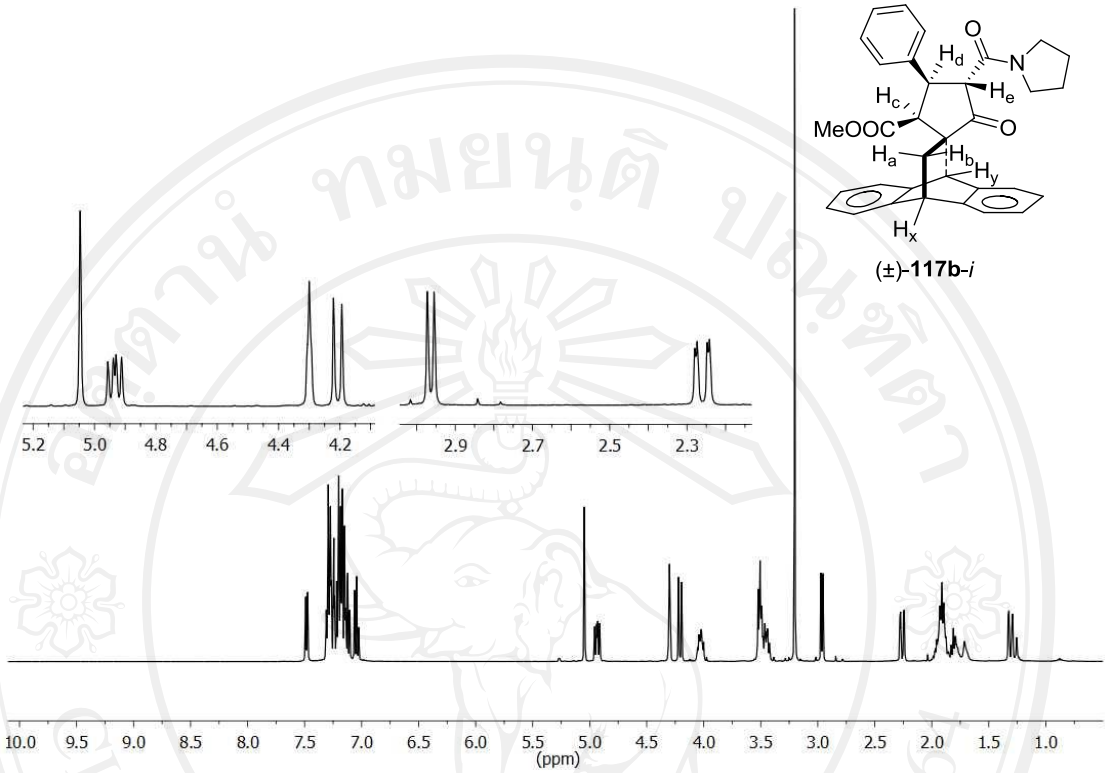

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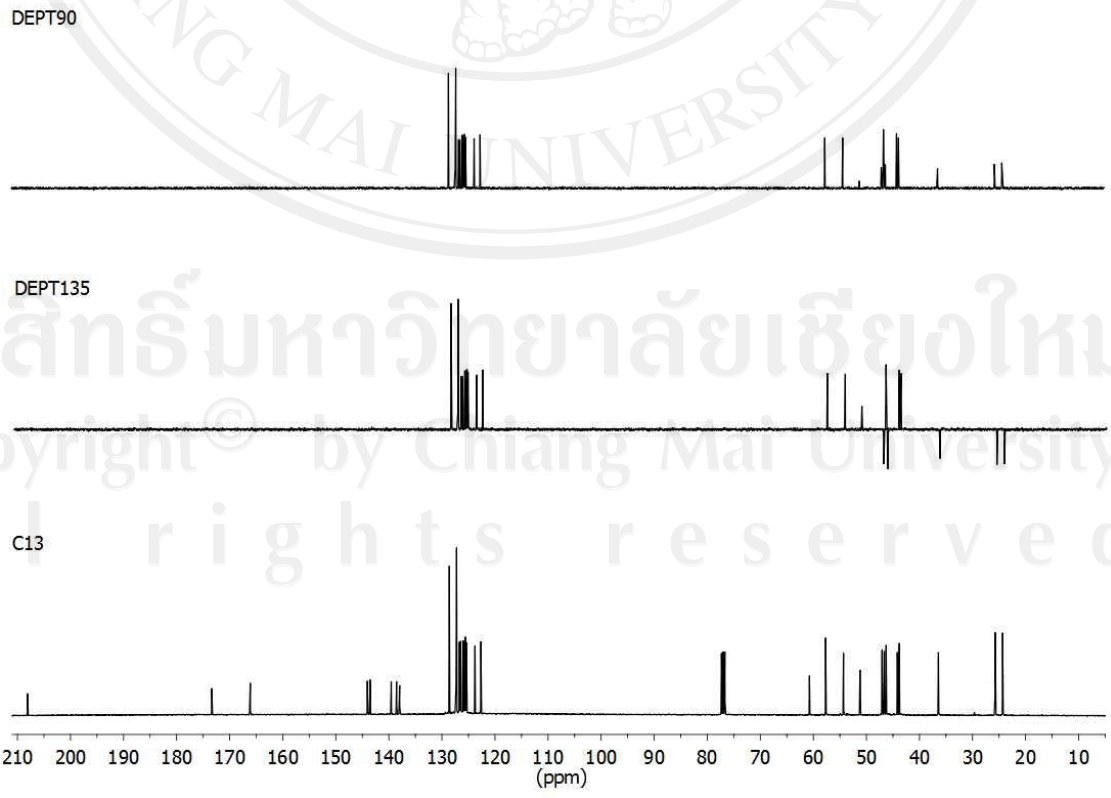

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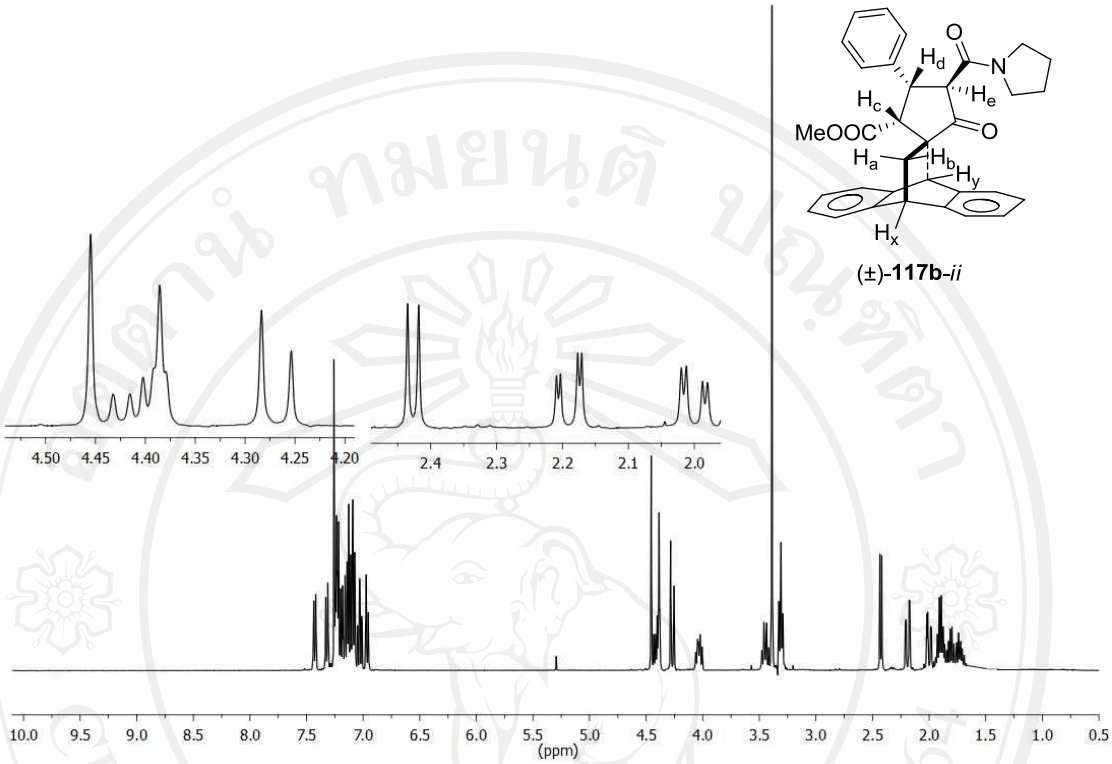

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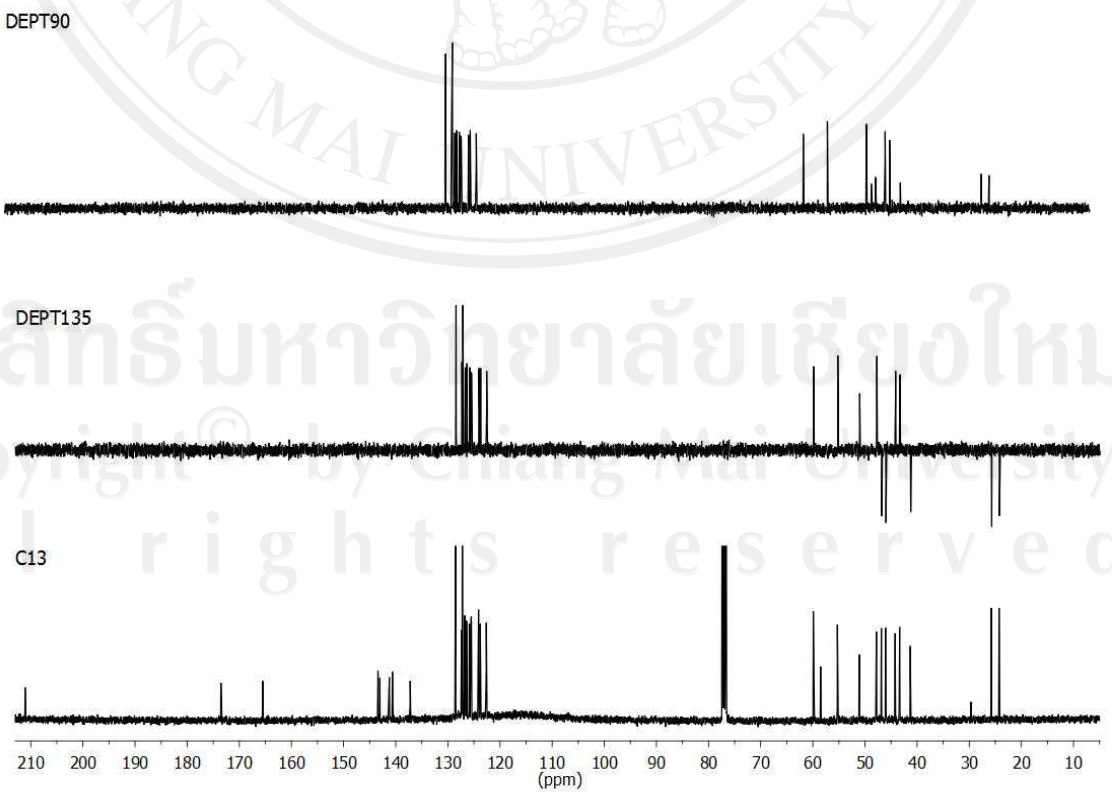

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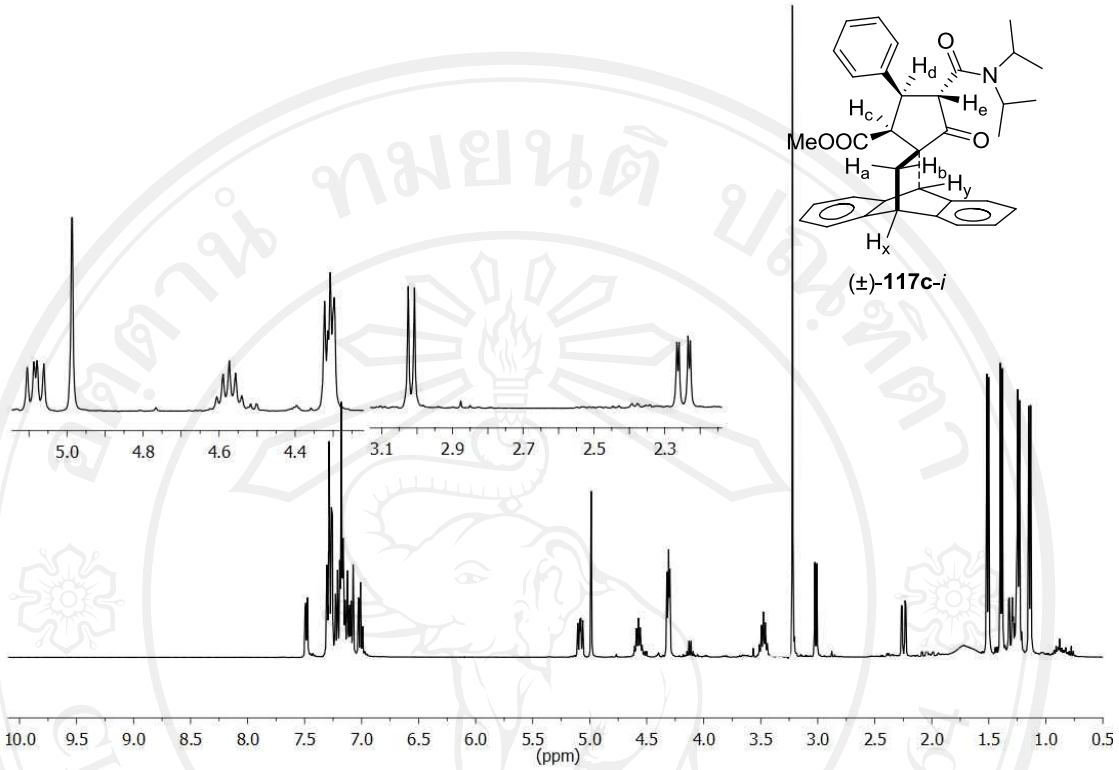

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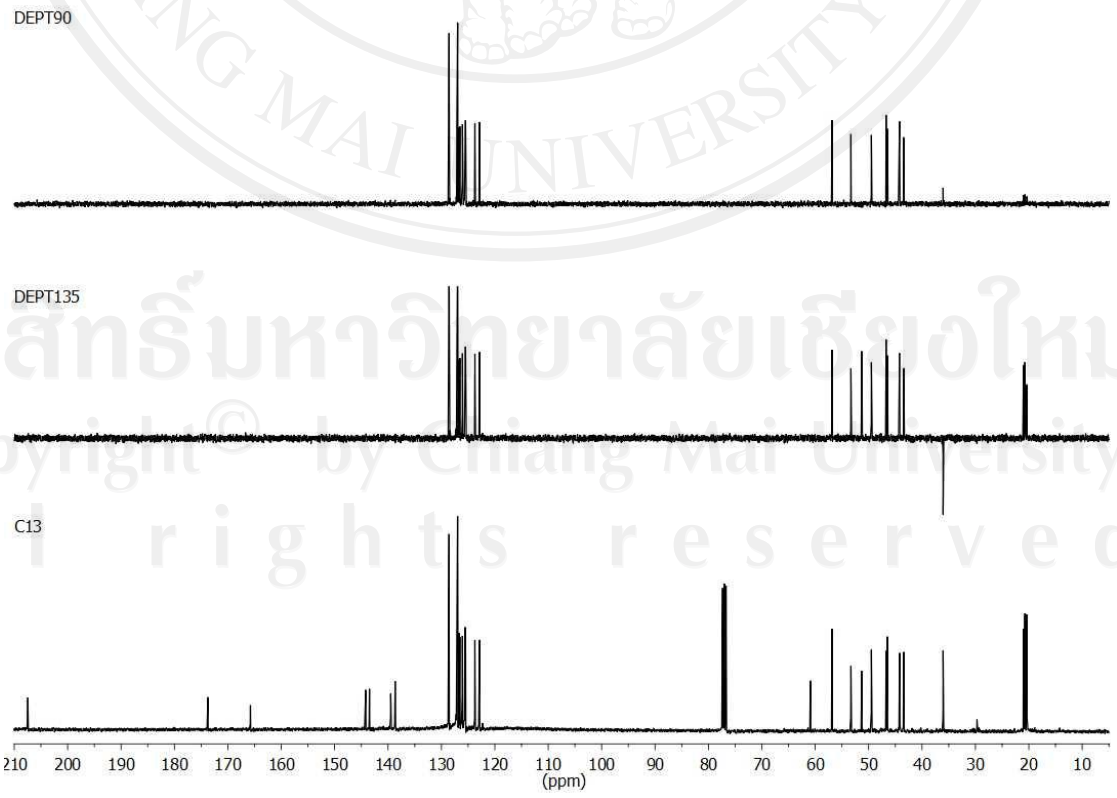

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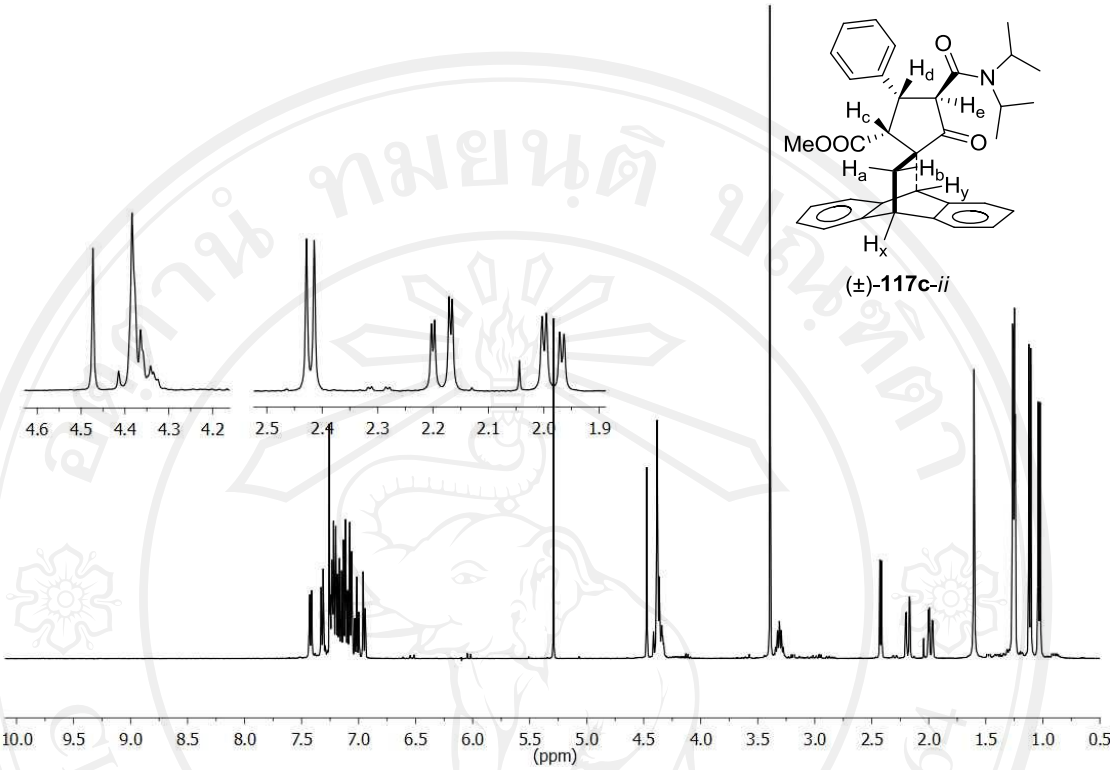

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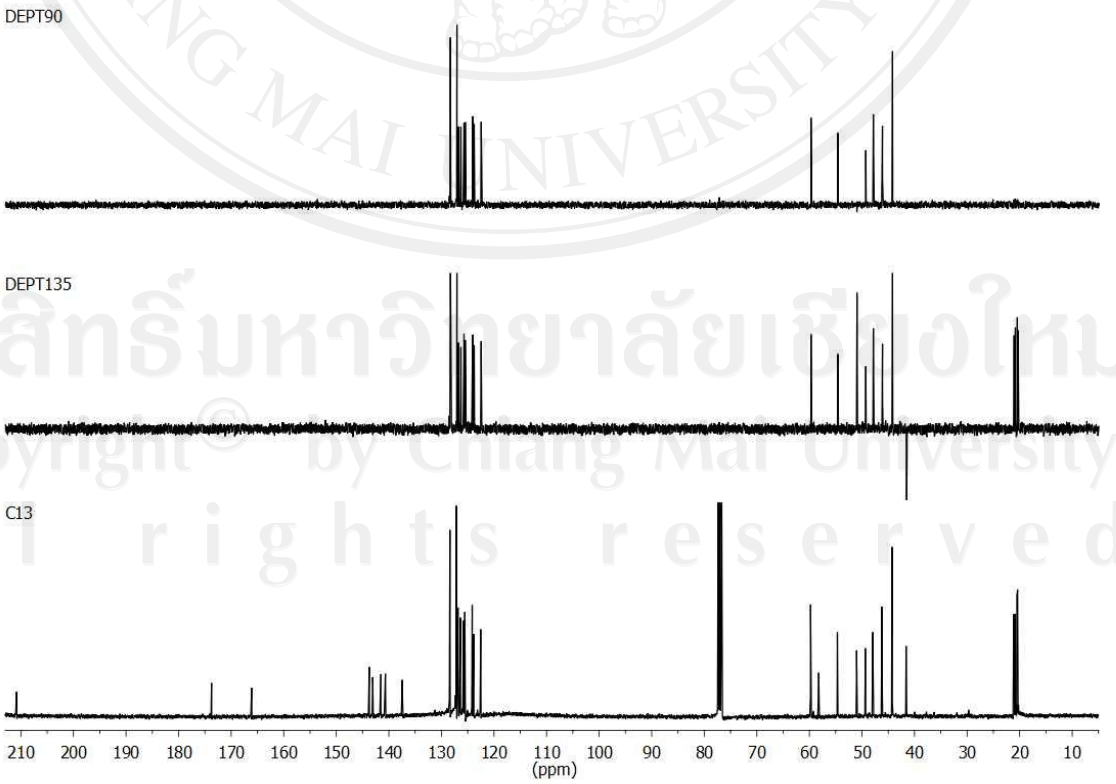

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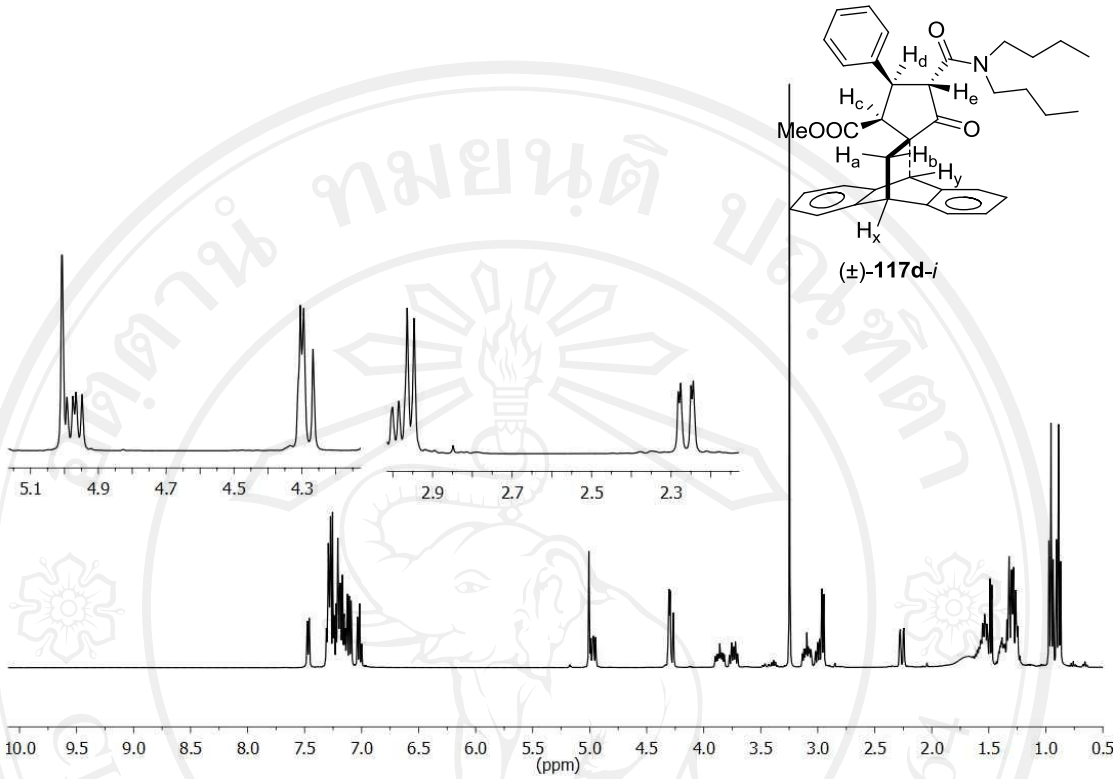

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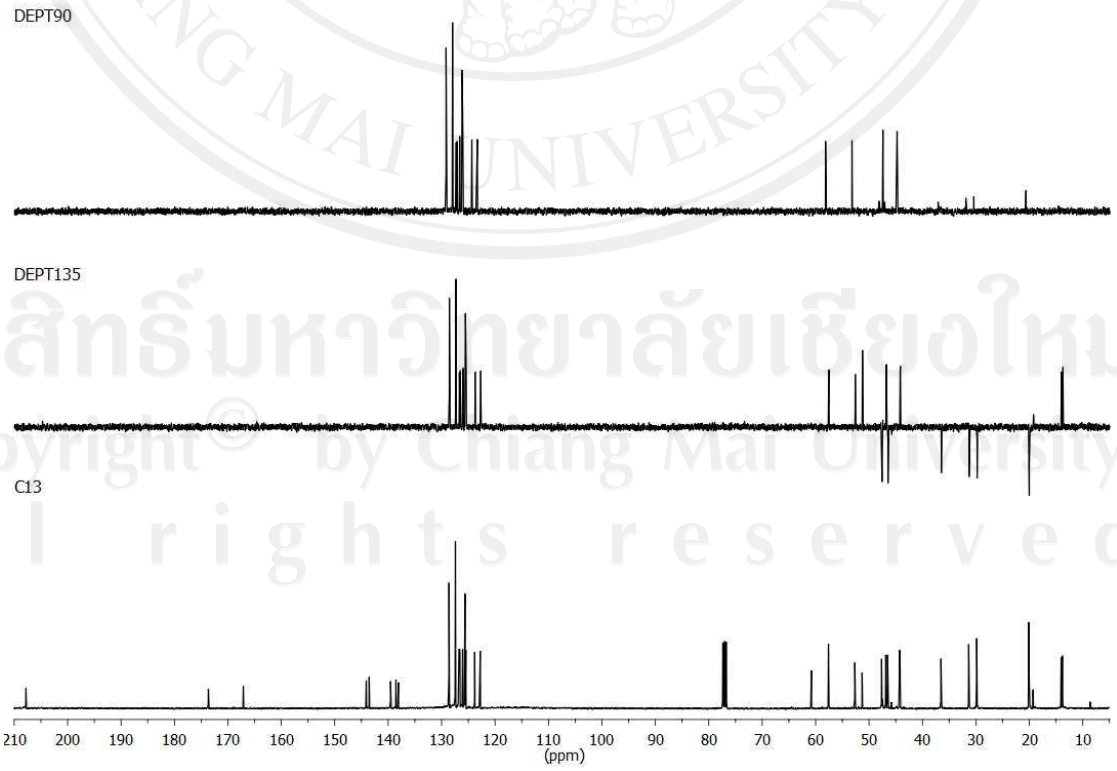

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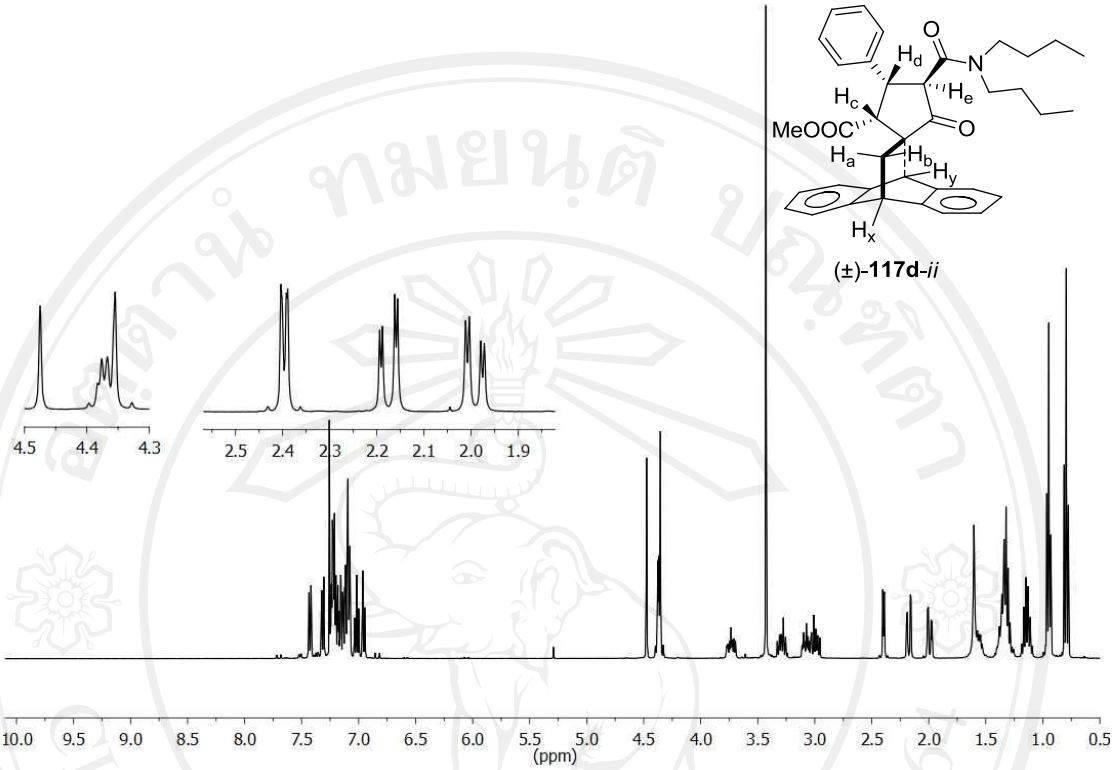

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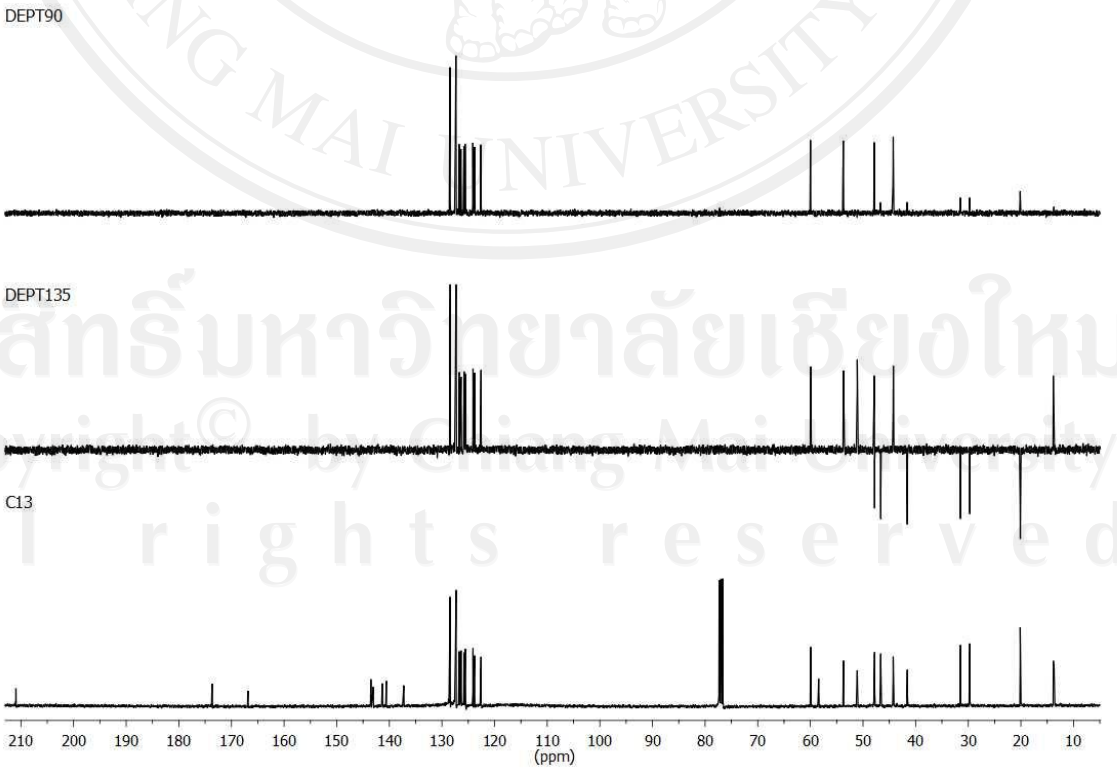

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International Conference:

Poster presentation

Ruangrat Choommongkol, Puttinan Meepowpan1, Tienthong Thongpanchang and Bongkoch Tarnchompoo, "Syntheses of spirocyclopentanone–anthracene adduct *via* tandem Michael addition–Dieckmann condensation approach", Pure and Applied Chemistry Conference 2010 (PACCON 2010) Jan 21-23, 2010, Sunee Grand Hotel and Convention Center, Ubonratchathani, Thailand. *(Proceeding)*

Laddawan Potprommanee, Puttinan Meepowpan, Ruangrat Choommongkol, Lalida Shank, "The Effects of Spirolactone-anthracene Adducts on Activity of Cytochrome P450 from Porcine Liver Microsomes", Pure and Applied Chemistry International Conference (PACCON 2011), January 5-7, 2011 at the Miracle Grand Hotel in Bangkok, Organized by Department of Chemistry, Faculty of Science, Srinakharinwirot University and Chemical Society of Thailand. *(Proceeding)*

National Conferences:

Poster presentation

Puttinan Meepowpan, **Reuangrat Choommongkol** and Pasunad Pongpijid, "Synthesis of Novel Antimalarial Agent, Cyclopentanone Anthracene Adduct, via Tandem Michael Addition–Dieckmann Condensation Reactions", The 33rd Congress

on Science and Technology of Thailand on 18-20 October 2007, Walailak University, Nakhon Si Thammarat, Thailand (2007). *(Proceeding)*

Ruangrat Choommongkol, Puttinan Meepowpan and Tientong Thongpanchang, "Design and Synthesis of Novel Antimalarial Substances: Cyclopentanone–Anthracene Adduct and Their Derivatives" Commission on Higher Education Congress I University Staff Development Consortium, 5–7 September 2008, Ambassador City Jomtien, Pattaya, Thailand.

Ruangrat Choommongkol and Puttinan Meepowpan, "Design and synthesis of spirocyclopentanone–anthracene dimer adducts as antimalarial substances", The International Congress for Innovation in Chemistry (PERCH-CIC CONGRESS VI), Pattaya, Chonburi, Thailand (2009).

Publications:

Ruangrat Choommongkol, Rattana Jongkol, Samran Prabpai, Palangpon Kongsaeree and Puttinan Meepowpan,* "Enantioselective synthesis of novel antimalarial agent, spirocyclopentanone–anthracene adduct, *via* tandem Michael addition–Dieckmann condensation reactions as the key steps", *Tetrahedron: Asymmetry*, manuscript in preparation. (Impact factor = 2.484)

Rattana Jongkol, **Ruangrat Choommongkol**, Bongkoch Tarnchompoo, Piyarat Nimmanpipug and Puttinan Meepowpan,* "Syntheses of methylenolactocin and nephrosterinic acid *via* diastereoselective acylation and chemoselective reduction–lactonization", *Tetrahedron* **2009**, *65*, 6382–6389. (Impact factor = 3.011)

Syntheses of spirocyclopentanone-anthracene adduct *via* tandem Michael addition–Dieckmann condensation approach

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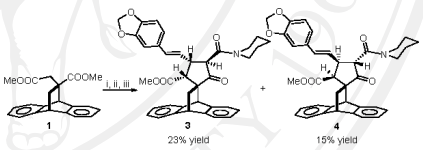
Abstract: The tandem Michael addition–Dieckmann condensation reactions has become an active topic of research. In this approach, treatment of dimethyl itaconate-anthracene adduct and an α,β-unsaturated carbonyl compound with LDA resulted in Michael addition followed by a Dieckmann condensation. It is one of the most useful carbon–carbon bond reactions in organic synthesis. It is reported here a synthesis of novel antimalarial spirocyclopentanone–anthracene adducts from the racemic and optically active dimethyl itaconate–anthracene adducts and piperine via the tandem Michael addition–Dieckmann condensation reactions, as a key step. All adducts exhibited antimalarial activity with IC_{50} values of 3.4–4.7 • g/mL, and importantly displayed no cytotoxicity to vero cells. Spirocyclopentanone–anthracene adducts derived from the racemic dimethyl itaconate–anthracene adducts and derivatives of piperine, methylene-1,3-dioxy cinnamide and cinnamide have been successfully synthesized in the moderate yields.

Introduction

Malaria is one of the world's most serious, widespread and common diseases because of its prevalence, virulence and drug resistance. It is caused by a parasite called Plasmodium, such as *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, which are transmitted *via* the bites of infected mosquitoes. In the human body, the parasites multiply in the liver, and then infect red blood cells. According to recent estimates, malaria affects more than 2,400 million people that account for approximately 40% of the world's population and 300-500 million people suffer from this disease and 2-3 million people die of malaria every year. Thus, many research groups are interesting to study and synthesize of new class of antimalarial agents.

Dimethyl itaconate-anthracene adduct (**1**) was used as starting material and chiral auxiliaries for synthesize biologically active natural product activities such as sakomycin, methyl deepoxy-4,5-didehydromethyleno mycin A and methyl methylnomycin A. Piperine (**2**) is one of a major alkaloids from Piper nigrum it display a variety of pharmacological and biological such as antifungal, antidiarrheal, insecticidal, nematocidal activity, inhibition of live metabolism and anti-inflammatory.

In previous report, adduct **1** and piperine (**2**) were reacted via tandem Michael addition–Dieckmann condensation reactions to give spirocyclopentanone-anthracene adduct **3** and **4** (Scheme 1). Adducts **3** and **4** exhibited antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} value of 4.7 and 3.4 • g/mL respectively. Interestingly, adducts **3** and **4** displayed non-cytoxicity against human epidermoid catcinima (KB), human breast cancer cells (BC-1) and *vero* cells. Therefore, aim of this work is to design and synthesis of novel antimalarial substances, spirocyclopentanone–anthracene adducts and their derivatives, using adduct **1** and amide compounds as starting materials.



Scheme 1. Synthesis of spirocyclopentanone adducts *via* tandem Michael addition–Dieckmann condensation reactions. *Reagents and Conditions:* i) 1.2 equiv LDA, THF, –78 °C to 0 °C, 2 hr; ii) 1.2 equiv piperine (**2**), THF, 0 °C to rt, 3 days; iii) 30% aq. HCl.

Materials and Methods

All reactions were carried out under nitrogen or argon. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. The ^1H and ^{13}C NMR spectra were recorded on Bruker DRX 400 MHz spectrometers and chemical shifts were given in ppm downfield from tetramethylsilane (TMS). Infrared spectra were taken with a FT-IR model TENSER 27 (Bruker) spectrometer and absorption frequencies were reported in reciprocal centimeters (cm^{-1}). Mass pectra (electrospray ionization mode, ESI-MS) were measured on a micromass Q-TOF-2^{Tm} (Waters) spectrometer. Flash column chromatography was performed employing Merck silica gel 60 and Merck silica gel 60H. Preparative thin layer chromatography (PLC) plates were carried out using Merck silica gel 60 PF_{254}. Solvents were dried over CaH2 and distilled

before used. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone ketyl under nitrogen. Diisopropylamine was distilled over CaH_2 and stored under nitrogen. *n*-Butyllithium was purchased from Fluka and Across as solution in hexane and titrated periodically according to the 2,5-dimethoxybenzyl alcohol method.

Preparation of piperic acid (5) from piperine (2)

A solution of KOH (26.28 mmol) in H_2O (50 mL) was added to a solution of piperine (**2**) (17.52 mmol), in MeOH (100 mL) and heated to reflux for 2 hr. The cooled reaction mixture was diluted with water (15 mL) and acidified to pH 2-3 by 30% HCl, then extracted with CH_2Cl_2, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was crystallized from CH_2Cl_2/hexane to give the corresponding piperic acid (**5**) (3.75 g, 98% yield) as yellow solids.

General procedure for synthesis of (2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-1-(pyrrolidin-1-yl)penta-2,4-dien-1-one, amide 7

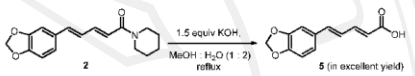
A mixture of piperic acid (**5**) (4.58 mmol) and oxalyl chloride (6.87 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 2 hr and the solvent was removed under reduced pressure. The mixture of the acid chloride obtained, CH_2Cl_2 (15 mL), triethylamine (5.50 mmol) and pyrrolidine (**6**) (5.50 mmol) was stirred at room temperature for 2 hr, filtered through Celite 545, diluted with H_2O and extracted with CH_2Cl_2. The solution was washed with H_2O, saturated NaCl solution, dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (silica gel) using EtOAc : hexane = 1 : 9 as eluent to give amide **7**.

General procedure for synthesis of spirocyclopentanone-anthracene adduct, 4'-(2'''-(benzo[c][1,3]dioxolyl)vinyl-3'-methoxy-carbonyl-5'-pyrrolidine-carbonyl-1'-cyclopentanone-2'-spiro-11-9,10-dihydro-9,10-ethanoanthracenes, 12 and 13

To a 50 mL round-bottomed flask equipped with a magnetic stirrer was fitted with a three-way stopcock with a septum cap and nitrogen inlet was added THF (8 mL) and dry diisopropylamine (4.28 mmol) *via* syringes. The mixture was cooled down to –78 °C, *n*-butyl lithium (1.4 N in hexane, 3.86 mmol) was added and the mixture left stirring at 0 °C for 1 hr. A solution of dimethyl itaconate–anthracene adduct, (**1**) (2.97 mmol) in THF (10 mL) was introduced to the LDA solution at –78 °C, then stirred at 0 °C for 2 hr. The solution of amide **7** (3.86 mmol) in THF (10 mL) was added to the anion solution at –78 °C after which the reaction mixture was left stirring at room temperature for 3 days. The reaction mixture was quenched with saturated aqueous ammonium chloride solution at 0 °C and the crude mixture was extracted several times with CH_2Cl_2. The dichloromethane solution was washed with H_2O, saturated NaCl solution, then dried over $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash column chromatography (silica gel) using EtOAc : CH_2Cl_2 : hexane = 2 : 0.5 : 7.5 as eluent to give the spirocyclopentanone-anthracene adduct, **12** and **13** respectively.

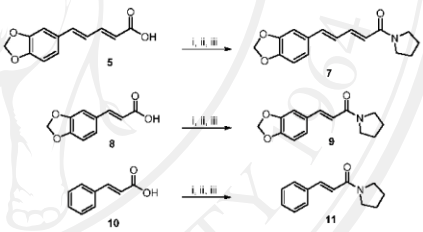
Results and Discussion

Piperine (**2**) was hydrolyses with KOH in MeOH : H_2O (1 : 2) to give piperic acid in excellent yield, (Scheme 2).



Scheme 2. Synthesis of piperic acid (**5**) from piperine (**2**)

Amide **7**, **9**, **11** were synthesized from acid **5**, **8**, and **10** respectively, in the present of oxalyl chloride and reacted with pyrrolydine (**6**) under N_2 gas (Scheme 3) and after purified by column chromatography to give amide **7**, **9** and **11** in good yields (Table 1).

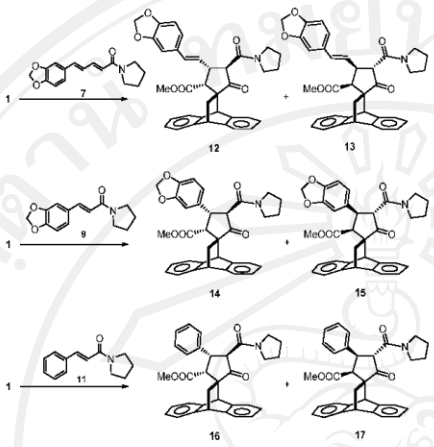


Scheme 3. Syntheses of amide **7**, **9** and **11**. *Reagents and Conditions:* i) 1.5 equiv $(COCl)_2$, 0 °C,15 min to rt 2 hr; ii) 1.2 equiv NEt_3, 0 °C 15 min; iii) 1.2 equiv pyrrolydine (**6**), 0 °C to rt, 2 hr.

Table 1: % Yields and % conversions of amide **7**, **9** and **11**

Compounds	Yield (%)	Conversion (%)
7	70	99
9	82	99
11	83	85

Then, the enolate anion of dimethyl itaconate–anthracene adduct (**1**) was generated by treated with LDA, at 0 °C for 2 hr and then reacted with amide **7** *via* tandem Michael addition–Dieckmann condensation reactions to give the spirocyclopentanone–anthracene adducts **12** and **13** in 9 % and 12 % yield, respectively (Scheme 4 and Table 2). Also amide **9** was allowed to react with the enolate anion of adduct **1** to give adducts **14** and **15** (in 23 % and 57 %), and adducts **16** and **17** (in 15 % and 15 %) were obtained from amide **11** whose results are shown in Table 2.



Scheme 4. Syntheses of spirocyclopentanone–anthracene adducts (**12**–**17**). *Reagents and Conditions:* i) 1.2 equiv LDA, THF, –78 °C to 0 °C, 2 hr; ii) 1.2 equiv amide **7** or **9** or **11**, THF, 0 °C to rt, 3 days; iii) 30% aq. HCl.

Table 2**:** % Yields of the spirocyclopentanone–anthracene adducts from tandem Michael addition–Dieckmann condensation reactions

Compounds	Yield (%)	Conversion (%)
12	9	70
13	12	
14	23	47
15	57	
16	15	80
17	15	

The relative stereochemistries of spirocyclopentanone–anthracene adducts (**12**–**17**) were determined on the basis of their analytical and spectroscopic data *e.g.* ^{1}H NMR, ^{13}C NMR and NOE experiment.

Conclusions

Amide **7**, **9** and **11** could be synthesized from piperic acid (**5**), methylene-1,3-dioxycinnamic acid (**8**), and cinnamic acid (**10**) reacted with pyrrolydine (**6**) in the present of oxalyl chloride in a good yield. Then, the spirocyclopentanone–anthracene adducts (**12**–**17**) could be synthesized using adduct **1** reacted with amides *via* tandem Michael addition–Dieckmann condensation reactions. In the future, all adducts **12**–**17** will be test antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) and cytoxicity against human epidermoid catcinima (KB), human breast cancer cells (BC-1) and *vero* cells.

Acknowledgement

I would like to thank the Office of the Higher Education Commission, Thailand for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree for this research. We would like to thank the faculty of science and the Graduate School, Chiang Mai University and we also would like to thank Center for Innovation in Chemistry Postgraduate Education and Research Program in Chemistry (PERCH-CIC).

References

[1] Lertvorachon, J.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron* **1998**, *54*, 14341–58.
[2] Kongsaeree, P.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1913–22.
[3] Tsukamoto, S.; Cha, B. C.; Ohta, T. *Tetrahedron* **2002**, *58*, 1667–71.
[4] Takahashi, M.; Ichikawa, M.; Aoyagi, S.; Kibayashi, C. *Tetrahedron Letters* **2005**, *46*, 57–9.
[5] Semler, U.; Gross, G. G. *Phytochemistry* **1988**, *27*, 1566–7.
[6] Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamrarn, A. *Planta Medica* **2002**, *68*, 853–5.

Syntheses of spirocyclopentanone-anthracene adduct *via* tandem Michael addition–Dieckmann condensation approach

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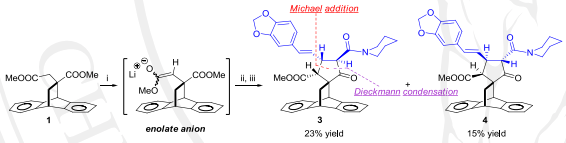
Introduction

Malaria is one of the world's most serious, widespread and common diseases because of its prevalence, virulence and drug resistance. It is caused by a parasite called *Plasmodium*, such as *P. falciparum, P. vivax, P. ovale* and *P. malariae*, which is transmitted *via* the bites of infected mosquitoes. In the human body, the parasites multiply in the liver, and then infect red blood cells. According to recent estimates, malaria affects more than 2,400 million people that account for approximately 40% of the world's population and 300-500 million people suffer from this disease and 2-3 million people die of malaria every year. Thus, many research groups are interesting to study and synthesize of new class of antimalarial agents.

Dimethyl itaconate–anthracene adduct (**1**) was used as starting material and chiral auxiliaries for synthesize biologically active natural product activities such as sakomycin, methyl deepoxy-4,5-didehydromethylemomycin A and methyl methylenomycin A. Piperine (**2**) is one of a major alkaloids from *Piper nigrum* it display a variety of pharmacological and biological such as antifungal, antidiarrheal, insecticidal, nematocidal activity, inhibition of live metabolism and anti-inflammatory.

Adduct **1** and piperine (**2**) were reacted *via* tandem Michael addition–Dieckmann condensation reactions to give spirocyclopentanone-anthracene adducts **3** and **4** (Scheme 1). Adducts **3** and **4** exhibited antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} value of 4.7 and 3.4 μg/ml respectively. Interestingly, compounds **3** and **4** displayed non-cytoxicity against human epidermoid catcinima (KB), human breast cancer cells (BC-1) and *vero* cells.

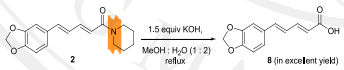
Therefore, aim of this work is design and syntheses of novel antimalarial substances, spirocyclopentanone–anthracene adducts and their derivatives using varied amides.



Scheme 1. Synthesis of spirocyclopentanone adducts *via* tandem Michael addition–Dieckmann condensation reactions. *Reagents and Conditions:* i) 1.2 equiv LDA, THF, –78°C to 0°C, 2 hr; ii) 1.2 equiv piperine (**2**), THF, 0°C to rt, 3 days; iii) 30% aq. HCl.

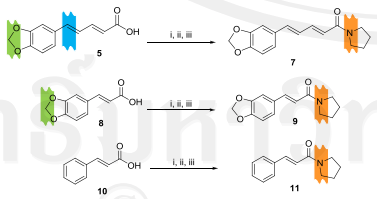
Method, Results and Discussion

Piperine (**2**) was hydrolyses with KOH in MeOH : H_2O (1 : 2) to give piperic acid in excellent yield (Scheme 2).



Scheme 2. Synthesis of piperic acid (**5**) from piperine (**2**)

Amides **11** were synthesized from acids **5**, **8**, and **10** respectively, in the present of oxalyl chloride and reacted with pyrrolidine (**6**) under N_2 gas (Scheme 3) and after purified by column chromatography to give amides **7**, **9** and **11** in good yields, respectively (Table 1).



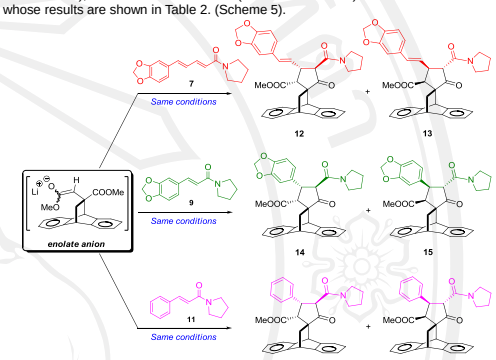
Scheme 3. Syntheses of amides **7**, **9** and **11**. *Reagents and Conditions:* i) 1.5 equiv $(COCl)_2$, 0°C,15 min to rt 2 hr; ii) 1.2 equiv NEt_3, 0°C 15 min; iii) 1.2 equiv pyrrolidine (**6**), 0°C to rt, 2 hr.

Table 1. % Yields and % conversions of amides **7**, **9** and **11**

Entries	Compounds	Yields (%)	Conversions (%)
1	**7**	70	99
2	**9**	82	99
3	**11**	83	85

Keywords: Tandem, Michael addition, Dieckmann condensation and Piperine

Then, the enolate anion of dimethyl itaconate–anthracene adduct (**1**) was generated by treated with LDA, at 0 °C for 2 hr and then reacted with amide **7** *via* tandem Michael addition–Dieckmann condensation reactions to give the spirocyclopentanone–anthracene adducts **12** and **13** in 9 % and 12 % yield, respectively (Scheme 4 and Table 2). Also amide **9** was allowed to react with the enolate anion of adduct **1** to give adducts **14** and **15** (in 23 % and 57 %), and adducts **16** and **17** (in 15 % and 15 %) were obtained from amide **11** whose results are shown in Table 2. (Scheme 5).



Scheme 4. Syntheses of spirocyclopentanone–anthracene adducts (**12**–**17**). *Reagents and Conditions:* i) 1.2 equiv LDA, THF, –78°C to 0°C, 2 hr; ii) 1.2 equiv amide **7** or **9** or **11**, THF, 0°C to rt, 3 days; iii) 30% aq. HCl.

Table 2. % Yields of the spirocyclopentanone–anthracene adducts from tandem Michael addition–Dieckmann condensation reactions

Entries	Compounds	Yields (%)	Conversions (%)
1	**12**	9	70
	13	12	
2	**14**	23	47
	15	57	
3	**16**	15	80
	17	15	

The relative stereochemistries of spirocyclopentanone–anthracene adducts (**12**–**17**) were determined on the basis of their analytical and spectroscopic data *e.g.* ^{1}H–NMR, ^{13}C–NMR and NOE experiment.

Conclusions

Amides **7**, **9** and **11** could be synthesized from piperic acid (**5**), methylene-1,3 dioxy cinnamic acid (**8**) and cinnamic acid (**10**) reacted with pyrrolidine (**6**) in the present of oxalyl chloride in a good yield. Then, the spirocyclopentanone–anthracene adducts (**12**–**17**) could be synthesized using adduct **1** reacted with amides *via* tandem Michael addition–Dieckmann condensation reactions. In the future, all adducts **12**–**17** will be test antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) and cytoxicity against human epidermoid catcinima (KB), human breast cancer cells (BC-1) and *vero* cells.

Acknowledgement

I would like to thank the Office of the Higher Education Commission, Thailand for supporting by grant fund under the program Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree for this research. We would like to thank the faculty of science and the Graduate School, Chiang Mai University and we also would like to thank Center of Innovation in Chemistry Postgraduate Education and Research Program in Chemistry (PERCH-CIC).

References

[1] Lertvorachon, J.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron* **1998**, 54, 14341–58.
[2] Kongsaeree, P.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron: Asymmetry* **2001**, 12, 1913–22.
[3] Tsukamoto, S.; Cha, B. C.; Ohta, T. *Tetrahedron* **2002**, 58, 1667–71.
[4] Takahashi, M.; Ichikawa, M.; Aoyagi, S.; Kibayashi, C. *Tetrahedron Letters* **2005**, 46, 57–9.
[5] Semler, U.; Gross, G. G. *Phytochemistry* **1988**, 27, 1566–7.
[6] Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamrarn, A. *Planta Medica* **2002**, 68,853–5.

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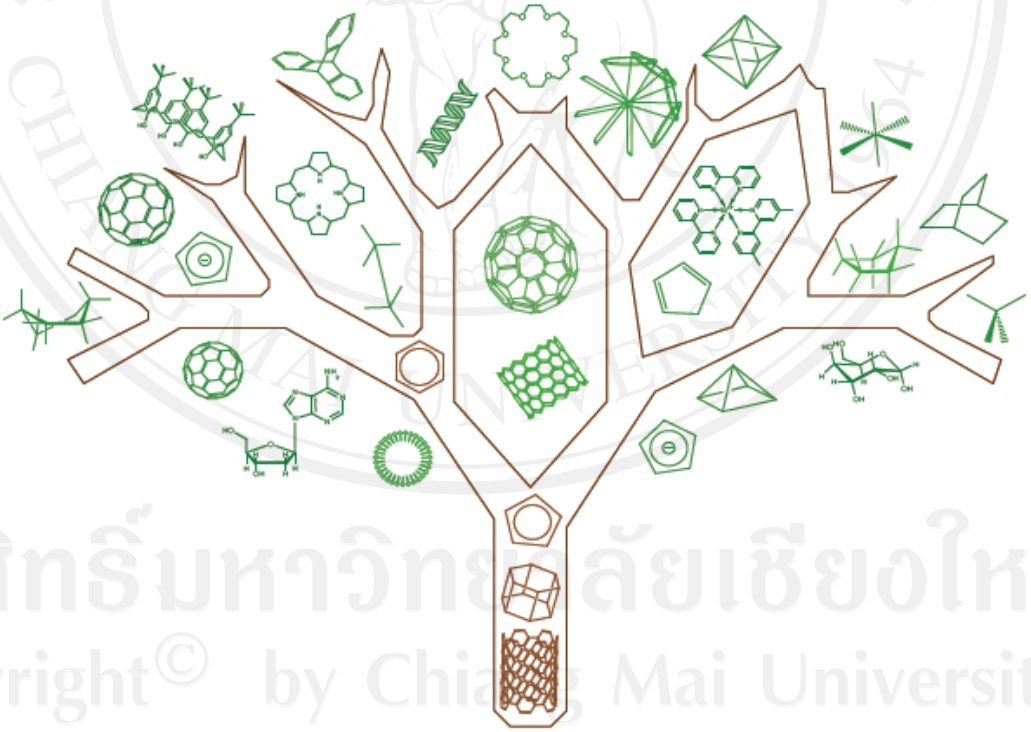

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The Effects of Spirocyclopentanone–Anthracene Adducts on Activity of Cytochrome P450 from Porcine Liver Microsomes

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Abstract: Piperine (1), an alkaloid compound, is a secondary metabolite considered as natural product found in pepper (*Piper nigrum* L.). Piperine, a main constituent of pepper, displays a variety of pharmacological activities and is used for treatment of many disorders. In this study piperine was utilized as the starting material for synthesis of the amide adducts *via* tandem Michael addition–Dieckmann condensation reactions to give spirocyclopentanone–anthracene adducts 3 and 4 for preparation of potentially bioactive compounds. The characterizations of both compounds were carried out using ^{1}H-NMR and ^{13}C-NMR spectra. Piperine, the amide adducts 3 and 4 were tested with cytochrome P450 (CYP 450) from porcine liver in the presence of β-NADPH. The absorbance of the product from the reaction of formaldehyde release from CYP 450 catalysis with Nash reagent, diacetyldihydrolutidine (DDL), was monitored at 405 nm and used to calculate the CYP 450 activity. It was found that piperine, the amide 3 and 4 were all substrates of CYP 450 judging from formaldehyde release from individual reaction (using erythromycin as a positive control) with K_m values of 29.24, 9.71 and 14.47 mM and V_{max} values of 1.63, 1.15 and 1.41 mM/min/mg, respectively. This suggests that erythromycin, piperine, spirocyclopentanone–anthracene adducts 3 and 4 share the common metabolic pathway in the catalytic process by CYP 450.

Introduction

Pepper (*piper nigrum* L.), known in Thai as Phrik-thai, is a common plant in Indian, South East Asia, Latin America and a number of African countries. The species belong to the family Piperaceae which includes a total of 700 species [1]. Pepper is a commonly used name for the spice due to the pungent and biting taste of piperine which exists and may be extracted from the dry fruits with a yield of 3-7% [2]. *P. nigrum* is extensively used in the Ayurvedic system of medicine [3]. A number of piperidine and pyrrolidine alkamides are known to occur in *P. nigrum*, the most important being piperine which is known to possess a variety of pharmacological activities such as antifungal, anti-inflammatory and biological activities such as insecticidal, nematocidal and inhibition of liver metabolism [4-8]. CYP 450 enzymes are superfamily of hemeprotein that catalyze the oxidative metabolism of drugs and other xenobiotics [9]. Drug metabolism via the CYP 450 system has emerged as an important determinant in the occurrence of several drug interactions that can result in drug toxicities, reduced pharmacological effect and adverse drug reaction [10]. At present, it appears that from about 30 isoenzymes, only six isoenzymes from the CYP1, CYP2 and CYP3 families are involved in the hepatic metabolism of various drugs. The isoenzymes include CYP1A2, 3A4, 2C9, 2C19, 2D6 and 2E1 [11]. Members of 3A subfamily are the most abundant CYP 450 and account for about 30% of CYP 450 enzymes in the liver. This study reports the synthesis of amide adducts, which were prepared from the dimethyl itaconate–anthracene adduct (**2**) and amides, for preparation of potentially bioactive compounds by CYP 450, which may be useful in pharmaceutical drug discovery and new drug design to treat human diseases. The finding of metabolism of piperine, spirocyclopentanone–anthracene adducts **3** and **4**, Figure 1, synthesized from piperine, via CYP 450 from porcine liver microsomes is also revealed here.

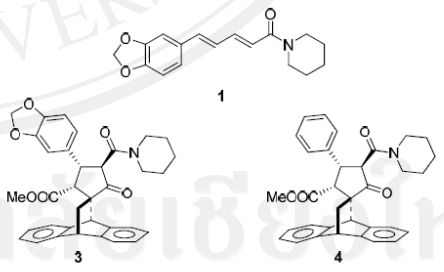
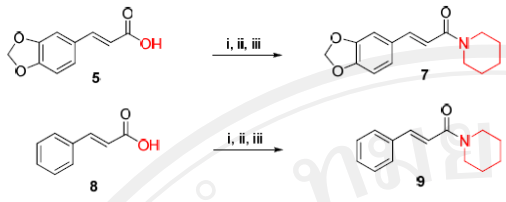


Figure 1. Chemical structures of spirocyclopentanone–anthracene adducts **3** and **4**

Materials and Methods

Synthesis Procedures

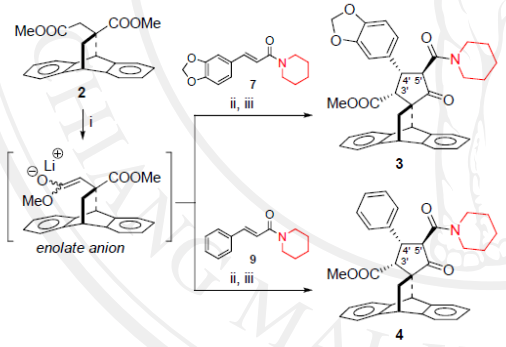
Amides **7** and **9** were synthesized from acid compounds **5** and **8** respectively, with piperidine **6** in the presence of oxalyl chloride and triethylamine under N_2 gas, Scheme 1.



Scheme 1. Synthesis of amides **7** and **9**. *Reagents and Conditions:* (i) 1.5 equiv $(COCl)_2$, 0 °C,15 min, rt 2 h; (ii) Remove DMC, then 1.2 equiv NEt_3, 0 °C, 15 min; (iii) 1.2 equiv piperidine (**6**), 0 °C → rt, 2 h.

After the reaction, the crude mixture was purified by column chromatography (silica gel) using EtOAc : hexane = 2 : 8 obtained amide **7**, *(E)-3-(benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)prop-2-en-1-one*, in 74% yield and amide **9**, *(E)-3-phenyl-1-(piperidin-1-yl)prop-2-en-1-one*, in 81% yield.

Subsequently, the enolate anion of dimethyl itaconate–anthracene adduct (**2**) was generated by treatment with LDA, at 0 °C for 2 h and reaction with amide **7** through tandem Michael addition–Dieckmann condensation reactions as shown in Scheme 2.



Scheme 2. Synthesis of spirocyclopentanone–anthracene adducts **3** and **4**. *Reagents and Conditions:* (i) 1.2 equiv LDA, THF, –78 °C to 0 °C 2 h; (ii) 1.2 equiv amides **7** (or **9**), 0 °C to rt 3 h; (iii) 30% HCl.

Spirocyclopentanone–anthracene adduct **3**, *4′-(2‴-(benzo[c][1,3]dioxolyl)vinyl-3′-methoxycarbonyl-5′-piperidinecarbonyl-1′-cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracene*, in 23% (2.3725 g) was obtained from column chromatography. Adduct **2** was allowed to react with amide **9** to give the amide adduct **4**, *4′-(2‴-(benzo[c][1,3]dioxolyl)vinyl-3′-methoxycarbonyl-5′-piperidinecarbonyl-1′-cyclopentanone-2′-spiro-11-9,10-dihydro-9,10-ethanoanthracene*, in 23% yield (2.3725 g), Scheme 2. All amide adducts were characterized by spectroscopic techniques *e.g.* ^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC and HMBC.

Amide adduct **3**: White powder, m.p. 114.7–118.3 °C (from CH_2Cl_2/hexane); ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H 1.38–1.72 (m, CH_2-4″, 5″, 6″, 6H), 1.29, 2.25, 4.30 (ABX system, J = 12.8, 2.7, 2.5 Hz, H_a, H_b, H_x, 3H), 2.93 (d, J = 7.0 Hz, Hc, 1H), 3.30 (s, COOMe, 3H), 3.37–3.51, 3.78–3.91, (m, CH_2-3″, 7″, 4H), 4.24 (d, J = 10.7 Hz, H_e, 1H), 4.90 (s, H_y, 1H), 4.93 (dd, J = 10.8, 7.1 Hz, H_d, 1H), 5.92 (s, CH_2-7‴, 2H), 7.00–7.31, 7.46 (m, ArH-aromatic, 11H); ^{13}C-NMR (100 MHz, $CDCl_3$), δ_C 24.6, 25.8, 26.6, 36.2, 43.6, 44.1, 44.2, 46.9, 47.5 51.4, 52.4, 57.4, 60.9, 101.1, 107.8, 108.3, 120.2, 122.8, 123.8, 125.4, 125.5, 125.7, 126.1, 126.6, 126.8, 132.0, 138.6, 139.4, 143.5, 144.1, 146.7, 147.9, 165.4, 173.5, 207.2.

Amide adduct **4**: White powder, m.p. 207.5–208.3 °C (from CH_2Cl_2/hexane); ^{1}H-NMR (400 MHz, $CDCl_3$), δ_H 1.76–1.48 (m, CH_2-4″, 5″, 6″, 6H), 1.31, 2.26, 4.31 (ABX system, J = 12.8, 2.7, 2.5 Hz, H_a, H_b, H_x, 3H), 2.99 (d, J = 7.1 Hz, H_c, 1H), 3.89–3.77 and 3.52–3.37 (m, CH_2-3″, 7″, 4H), 3.21 (s, COOMe, 3H), 4.35 (d, J = 10.6 Hz, H_e, 1H), 4.96 (s, Hy, 1H), 5.03 (dd, J = 10.5, 7.1 Hz, H_d, 1H), 7.00–7.32, 7.49 (m, ArH-aromtic, 13H); ^{13}C NMR (100 MHz, $CDCl_3$), δ_C 24.5, 25.8, 26.5, 36.2, 43.8, 44.0, 44.2, 46.8, 47.5, 51.2, 51.8, 57.3, 60.8, 122.8, 123.7, 125.3, 125.4, 125.7, 126.0, 126.5, 126.7, 127.2(2), 127.3, 128.6(2), 138.1, 138.6, 139.4, 143.4, 144.1, 165.4, 173.4, 207.3.

Microsomes Preparation

Porcine liver sample was collected immediately after slaughtering and weighed to approximately 20 g. The sample was washed thoroughly with distilled water at room temperature three times, cut into small pieces and then homogenized in the blender with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M KCl, 1 mM EDTA and 0.25 M sucrose for 5-10 min prior to homogenization with the homogenizer. To obtain the microsomal fraction the homogenate was centrifuged at 6,000 × g for 20 min at 4 °C and the supernatant was ultracentrifuged at 40,000 × g for 2 hr at 4 °C. The microsomal pellet was suspended in 0.05 M Tris-HCl buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA and 0.25 M sucrose and stored at –20 °C until the time of analysis. Just prior to the assay, the microsomal pellet was thawed to room temperature. Microsomal protein content was determined at 595 nm by using Bradford method and with bovine serum albumin (BSA) as a protein standard [12].

CYP 450 Activity

CYP 450 activity from porcine liver microsomes was determined using erythromycin as a substrate (positive control) at concentration of 1 mM in the presence of β-NADPH. The reaction was initiated by the addition of mixture solution containing 5.0 mM $MgCl_2$, 1 mM EDTA and 0.50 mg/ml of porcine liver microsomes. The mixture was vortexed and incubated at 37 °C for 30 min. The reaction was terminated with 0.50 ml of 25% $ZnSO_4$ and 0.50 ml of 1 M $Ba(OH)_2$. After the samples had been vortexed and centrifuged at 8,000 × g for 10 min at 4 °C and 0.50 ml of the supernatant was transferred to another tube and then mixed with 0.15 ml of Nash reagent [13]. The mixture was vortexed and incubated for 30 min at 56 °C.

Samples were analyzed by measuring the product from the reaction, formaldehyde release, of CYP 450 catalysis at 405 nm with spectrophotometer (Lamda 25, FALL Instrument, UK). The concentration of formaldehyde released was calculated from the absorbance of its product with Nash reagent, diacetyldihydrolutidine (DDL), using a standard curve constructed from various concentrations of formaldehyde undergoing the same protocol. The concentration of piperine, spirocyclopentanone–anthracene adducts **3** and **4** were 1 mM. The activity of CYP 450 was assayed as described above. All reactions were performed in triplicate.

Kinetic Parameters

The kinetic parameters of CYP 450 activity were determined for piperine, the amide adducts **3** and **4** in the presence of β-NADPH to observe their metabolisms via CYP 450. The final concentrations of piperine, the amide adducts **3** and **4** were varied to be at 0.5, 0.6, 0.7, 0.9, 1.0, 2.0, 2.5 and 3.0 mM, respectively. The CYP 450 activity was assayed as described above. Kinetic parameters of CYP 450 for each compound were calculated from the Lineweaver–Burk plots between 1/[S] and 1/initial to obtain Michaelis–Menten constant (K_m) and maximum velocity (V_{max}) for the enzyme.

Results and Discussion

CYP 450 activity in porcine liver microsomes was first detected by observing formaldehyde released as a product of erythromycin *N*-demethylation. Microsomes containing CYP 450 were able to oxidize erythromycin and release formaldehyde. Piperine, spirocyclopentanone–anthracene adducts **3** or **4** were used as substrates of CYP 450 catalysis. Formaldehyde was also liberated from each compound and detected by reaction with Nash reagent to form the colored product of diacetyldihydrolutidine as shown in Table 1. The concentration of formaldehyde release was attributed to the process of CYP 450 catalysis using the amide adducts **3** and **4** which was greater than when piperine was used as a substrate. CYP 450 activity was calculated from the formaldehyde released from each compound. The amide adduct **3** was the best compound from the reaction of CYP 450 catalysis with CYP 450 activity of 68.66 nM/min/mg, followed by the amide adduct **4** at 67.63 and piperine at 34.00 nM/min/mg, respectively.

Table 1 The CYP 450 activity and concentration of formaldehyde release form the catalytic activity of CYP 450

Compounds	[Formaldehyde] (nM)	Activity (nM/min/mg)
Piperine (1 mM)	510 ± 0.007	34.00
3 (1 mM)	1030 ± 0.005	68.66
4 (1 mM)	1010 ± 0.006	67.63

Table 2 K_m and V_{max} values of CYP 450 for piperine, spirocyclopentanone–anthracene adducts **3** and **4**

Compounds	K_m (mM)	V_{max}(mM/min/mg)
Piperine	29.24	1.63
3	9.71	1.15
4	14.47	1.41

The kinetic studies of CYP 450 were carried out using piperine, the amide adducts **3** and **4** in the presence of β-NADPH to observe their metabolisms via CYP 450. The kinetic parameters of the CYP 450 were determined from the Lineweaver–Burk plots between 1/[S] and 1/initial velocity as shown in Figure 2. The K_m values of the CYP 450 enzyme for piperine, spirocyclopentanone–anthracene adducts **3** and **4** were at 4.76, 4.90 and 15.7 mM and V_{max} values at 0.21, 0.38 and 0.73 mM/min/mg as shown in Table 2. The results suggest that piperine, spirocyclopentanone–anthracene adducts **3** and **4** are substrates for microsomal enzymes and thus are likely to be affected by the presence of one another may affect metabolism of one another.

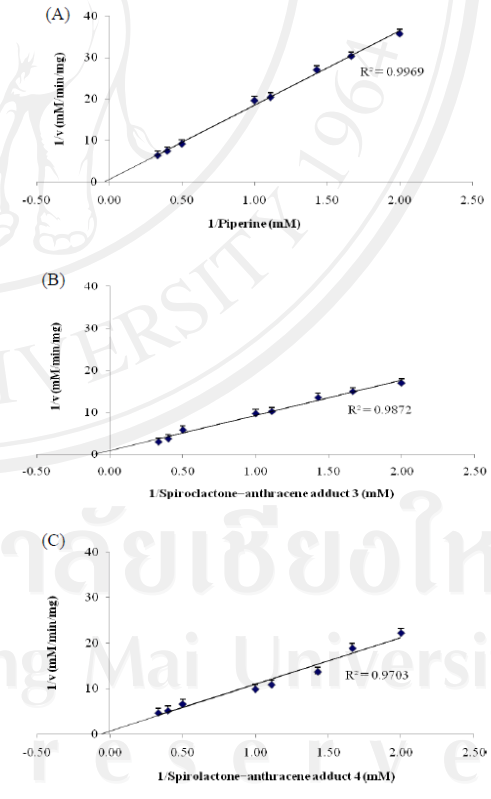


Figure 2. Lineweaver–Burk plots between 1/initial velocity of reaction versus 1/concentration of piperine (A), spirocyclopentanone–anthracene adducts **3** (B) and **4** (C) respectively

Porcine liver microsomes were selected as the source of CYP 450 in this experiment due to the high similarity of amino acid sequence of porcine CYP 450 to that of human [14]. This characteristic assists the preliminary study of drug interaction. Erythromycin which was the substrate of choice has long been used by researchers due to its availability and well known CYP 450 catalytic mechanism of *N*-demethylation [15]. Interestingly, the same mechanism is likely the case for piperine, spirocyclopentanone–anthracene adducts **3** and **4** at the piperidine ring. *N*-demethylation of the compounds catalyzed by CYP 450 results in lower hydrophobicity which in turn offering higher solubility for excretion. More compounds with related structures have been examined for their bioactivities. Our goal is to seek the candidates with potential pharmaceutical usages, high production yield and low toxicity. It is important that metabolisms of these compounds in the body are characterized first by using *in vitro* system. The common metabolic pathway demonstrated by CYP 450 from porcine microsomes suggested possible interaction and interference. The kinetic parameters obtained from this study also revealed that the maximal velocity of catalysis for piperine, the amide adducts **3** and **4** are similar. The amide adduct **3** has the highest binding affinity for the active site of CYP, followed by that of the amide adduct **4** and piperine, respectively.

Conclusion

Spirocyclopentanone–anthracene adducts **3** and **4** were successfully synthesized by tandem Michael addition–Dieckmann condensation reaction. The compounds were tested with CYP 450 from porcine liver. It was found that piperine, the amide adducts **3** and **4** are all substrates of CYP 450 as they produced formaldehyde upon the catalysis. This implies that their metabolisms may be affected by the presence of other compounds sharing the CYP 450 catalytic process. Thus, these compounds that have been prepared as potential bioactive agents may affect the metabolism of other drugs by acting as substrates and competing with one another for the binding site of CYP 450 enzymes.

Acknowledgements

The authors are grateful to the Center of Excellence for Innovation in Chemistry (PERCH-CIC), The Thailand Research Fund (TRF), Commission on Higher Education, Ministry of Education, Faculty of Graduate School, Bioorganic and Natural Product Research Unit, Phytochemica Research Unit and the Faculty of Science, Chiang Mai University for financial support.

References

[1] Parmar, V. S., Jain, S. C., Bist, K. S., Jain, R., Taneja, P., Jha, A., Tyagi, O. D., Prasad, A. K., Wengel, J., Olsen, C. E. and Boll, P. M. (1997). Phytochemistry of the genus *piper*. *Phytochem*. 46: 597-673.

[2] Ikan, R. (1991). *Natural Products: A Laboratory Guide*. 2nd ed., Academic Press. New York. pp. 233-238.

[3] Kirtikar, K. R. and Basu, B. D. (1981). *Indian Medicinal Plants*. 3rd ed., Periodical Export Book Agency, Vivek Vihar, New Delhi, India. pp. 2133.

[4] Navickiene, H. M. D., Alecio, A. C., Kato, M. J., Bolzani, V. da S., Young, M. C. M., Cabalheiro, A. J. and Furlen, M. (2000). Antifungal amides from piper hispidum and piper tuberculatum. *Phytochem*. 55: 621-626.

[5] Mujumdar, A. M., Dhuley, J. N., Deshmukh, V. K., Raman, P. H. and Naik, S. R. (1990). Anti-inflammatory activity of piperine. *Jpn. J. Med. Sci. Biol*. 43: 95-100.

[6] de Paula, V. F., de Barbosa, L.C., Demuner., A., Pilo-Veloso, D. and Picanco, M. C. (2000). Synthesis and insecticidal activity of new amide derivative of piperine. *Pest Manag. Sci*. 56: 168-174.

[7] Kiuchi, F., Nakamura, N., Tsuda, Y., Kondo, K. and Yoshimura, H. (1988). Studies on clude drugs effective on visceral larva migrans. IV Isolation and identification of larvicidal principles in pepper. *Chem. Pharm. Bull*. 36: 2452-2465.

[8] Koul, S., Koul, I. L., Taneja, S. C. Dhar, K. L., Jamwal, D. S., Singh, K., Reen, R. K. and Sig, J. (2000). Structure-activity relationship of piperine ant its synthetic analogues for their inhibitory potentials of rat hepatic microsomal constitutive and inducible cttochrome P450 activities. *Bioorg. Med. Chem*. 8: 251-268.

[9] Goshman, L., Fish, J. and Roller, K. (1998). Clinically significant cytochrome P-450 drug interactions. *J. Pharm. Soc. Wis*. 18: 84-112.

[10] Bernhaedt, R. (2006). Cytochromes P450 as versatile biocatalysts. *Biotechmol*. 124: 128-45.

[11] Levy, R. H. (1995). Cytochrome P450 isozymes and antiepileptic drug interacttions. *Epilepsia*. 36: S8-13.

[12] Bradford, M.M. (1917). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem* 72: 248-254.

[13] Nash, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem*. 55: 416-42.

[14] Joan, K. L. (2007). Advances in swine biomedical model genomics. *Int. J. Biol. Sci*. 3: 179-184.

[15] Wang, R. W., Newton D. J., Scheri, T. D. and Lu, A. Y. H. (1997). Human cytochrome P450 3A4-catalyzed testosterone 6β-hydroxylation and erythromycin *N*-demethylation competition during catalysis. *Drug. Metabol. Dispos*. 25: 502-507.

การสังเคราะห์สารใหม่ที่ยับยั้งมาลาเรียไซโคลเพนทาโนน-แอนทราซีนแอดดัคโดยผ่านปฏิกิริยาแทนเดมไมเคิลแอดดิชัน-ดิคแมนน์ คอนเดนเซชัน

SYNTHESIS OF NOVEL ANTIMALARIAL AGENT, CYCLOPENTANONE ANTHRACENE ADDUCT, VIA TANDEM MICHAEL ADDITION – DIECKMANN CONDENSATION REACTIONS

พุฒินันท์ มีเผ่าพันธุ์ เรืองเรศ ชุมมงคล, พสุนาถ พงศ์ไพจิตร

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บทคัดย่อ:

สารพิพเพอริน (**1**) เป็นสารผลิตภัณฑ์ธรรมชาติถูกใช้เป็นสารตั้งต้นในการทำปฏิกิริยากับ ไดเมทิลอิทาโคเนท–แอนทราซีนแอดดัค (**2**) และ ไดเมทิล 2,2-ไดเมทิลซัคซิเนท (**3**) ผ่านปฏิกิริยาแทนเดมไมเคิลแอดดิชัน–ดิคแมนน์คอนเดนเซชัน ให้อนุพันธ์ของสารต้านมาลาเรีย ไซโครเพนทาโนนแอนทราซีนชนิดราซีมิก (±)-**4**-*i* (±)-**4**-*ii* และสารบริสุทธ์ออพติคัลแอคทิฟ (–)-**4**-*i* (–)-**4**-*ii* (+)-**4**-*i* และ (+)-**4**-*ii* พบว่าออกฤทธิ์ต้านมาลาเรียชนิด*พี. ฟัลซิพาลัม* (เค1 ชนิดสายพันธุ์ที่ดื้อยาหลายชนิด) โดยมีค่า IC_{50} ที่ 4.7 3.4 4.3 3.6 4.0 และ 4.5 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ และที่สำคัญสารประกอบเหล่านี้แสดงความไม่เป็นพิษต่อเซลล์ปกติ สารไดเมทิล 2,2-ไดเมทิลซัคซิเนทแอดดัค (±)-**5**-*i* และ (±)-**5**-*ii* ไม่ออกฤทธิ์ยับยั้งต่อเชื้อ *พี. ฟัลซิพาลัม* และเซลล์ปกติ เป็นที่น่าสนใจว่าหมู่แอนทราซีนมีส่วนสำคัญในการออกฤทธิ์ต้านมาลาเรียและไซโครเพนทาโนน-แอนทราซีนแอดดัคสามารถสังเคราะห์ได้ง่ายและรวดเร็วจากสารตั้งต้นที่หาง่ายและสะดวกในการซื้อหา

Abstract:
The naturally available piperine (**1**) was utilized as the starting material which was reacted with dimethyl itaconate-anthracene adduct (**2**) and dimethyl 2,2-dimethylsuccinate (**3**) *via* tandem Michael addition–Dieckmann condensation reactions provided antimalarial analogs. The racemic cyclopentanone-anthracene adducts, (±)-**4**-*i* and (±)-**4**-*ii*, and optically active pure forms, (–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4**-*i* and (+)-**4**-*ii*, displayed antimalarial activity against parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} value of 4.7, 3.4 4.3, 3.6, 4.0 and 4.5 μg/ml respectively. Interestingly, none of these compounds showed cytoxicity against vero cells. The dimethyl 2,2-dimthyl succinate adducts ((±)-**5**-*i* and (±)-**5**-*ii*) were inactive against *P. falciparum* and vero cell. Interestingly, the anthracene moiety are important for antimalarial activity and the cyclopentanone-anthracene adducts are easily and rapidly synthesized from simple, commercially available starting materials.

Introduction:

Malaria disease[1] is a serious problem spreading in tropical and subtropical areas of the world. Approximately 300-500 million people were infected and over 1 million deaths each year worldwide. Malaria is an infectious disease caused by protozoa parasite of the genus *plasmodium* and carried from person to person by anopheles mosquitoes. The parasites have many types, but only four types cause malaria in humans. The parasite that cause malaria humans are *Plasmodium falciparum* (malaria tropica), *Plasmodium vivax* (malaria tertiana),

Plasmodium ovale (malaria quartana) and *Plasmodium malariae* (malaria tertiana). The first two species cause the most infections worldwide. *P. falciparum* is the agent of severe, potentially fatal malaria because of antimalaria drugs resistant.

In this work, we report the synthesis of novel antimalarial agents, cyclopentanone-anthracene adducts **4**[2] *via* tandem Michael addition–Dieckmann condensation reactions by utilizing the naturally available piperine (**1**)[3] and the anthracene adduct **2**,[4] as starting materials.

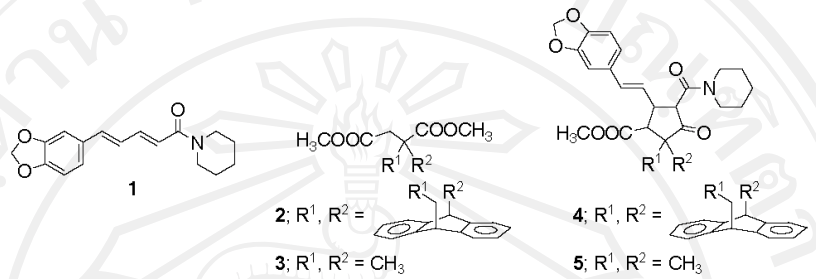
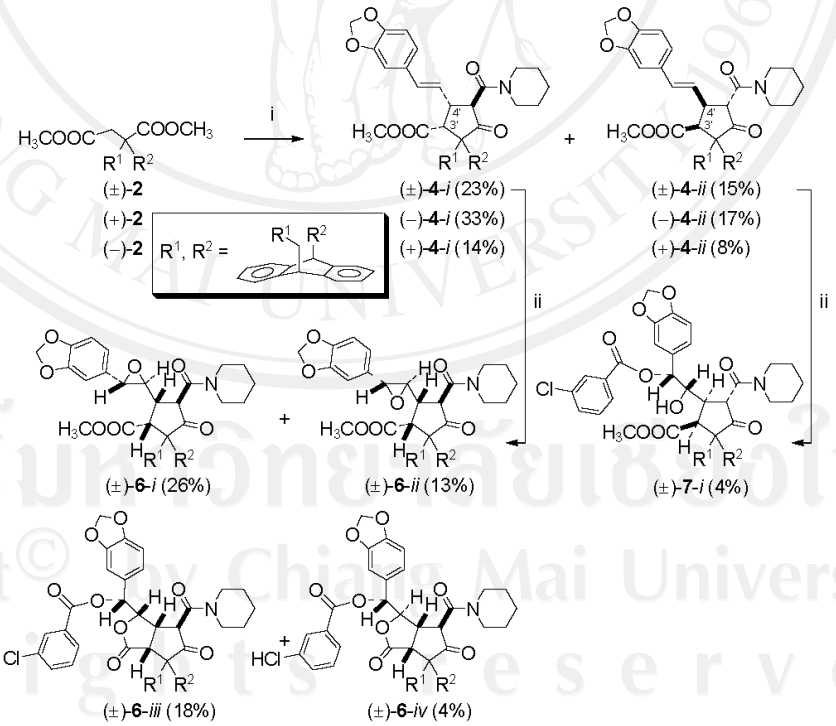
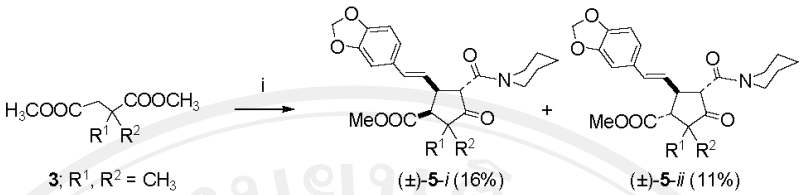


Figure 1. Structures of piperine (**1**), dimethyl itaconate-anthracene adduct (**2**), dimethyl 2,2-dimethylsuccinate (**3**) and cyclopentanone-anthracene adduct (**4**) and cyclopentanone adduct (**5**)

Methodology, Results and Discussion: The cyclopentanon-anthracene adduct (**4**) in racemic and enantiomerically pure form were synthesized using the readily available dimethyl itaconate-anthracene adducts in racemic, (±)-**2** and optically pure form, (+)-**2** and (–)-**2**,[4] as shown in Scheme.



Scheme 1. *Reagents and conditions:* (i) (a) 1.2 equiv. LDA, THF, -78 to 0°C 2 h, (b) 1.3 equiv. piperine (**1**), THF, 0°C to rt 3 days, (c) aq. NH_4Cl; (ii) (a) 1.5 eq. *m*-CPBA, $CHCl_3$, (b) aq. $NaHCO_3$



Scheme 2. *Reagents and conditions:* (i) (a) 1.2 equiv. LDA, THF, -78 to 0°C 2 h, (b) 1.3 equiv. piperine (**1**), THF, 0°C to rt 3 days, (c) aq. NH_4Cl

Tandem Michael addition – Dieckmann condensation reactions of piperine (**1**) and compound (±)-**2** by treating with LDA (1.2 equiv.) at 0°C for 2 h and then stirred at room temperature for 3 days after added compound **1** gave the cyclopenatanone-anthracene adducts **4**-*i* (23%) and **4**-*ii* (15%). Similar reaction of the enolate anion of (+)-**2** with piperine provided (–)-**4**-*i* and (–)-**4**-*ii* in 23% (>99% e.e., $[\alpha]_{589}^{24.4}$ = –18.49 (*c*, 0.87, $CHCl_3$)) and 15% (>99% e.e., $[\alpha]_{589}^{24.4}$ = –70.50 (*c*, 0.58, $CHCl_3$)) yields and, likewise, compounds (+)-**4**-*i* and (+)-**4**-*ii* were obtained in 14% (>99% e.e., $[\alpha]_{589}^{24.4}$ = +17.54 (*c*, 0.76, $CHCl_3$)) and 8% (>99% e.e., $[\alpha]_{589}^{24.4}$ = +67.57 (*c*, 0.60, $CHCl_3$)) yields when piperine was allowed to react with the enolate anion of (–)-**2**. The structure of all products were elucidated by analysis of NMR data while the relative stereochemistry at the C(3′) and C(4′) stereogenic centers of compounds **4**-*i* and **4**-*ii* were determined by X-ray crystallographic analysis. In similar reaction, the enolate anion of dimethyl 2,2-dimthylsuccinate (**3**) as no anthracene moiety reacted with piperine to obtain (±)-**5**-*i* (16%) and (±)-**5**-*ii* (11%), as shown in Scheme.

The epoxidation of major product (±)-**4**-*i* was affected by treatment with *m*-CPBA (1.5 equiv.) in $CHCl_3$ to furnish the epoxide-cyclopentanone adducts (±)-**6**-*i* - (±)-**6**-*ii* and the unexpected γ-lactone-cyclopentanone adducts (±)-**6**-*iii* - (±)-**6**-*iv* in 26, 13, 18 and 4% yields respectively, as shown in Scheme. Similarly, the minor product (±)-**4**-*i* gave only the hydroxyl-cyclopentanone adduct (±)-**6**-*i* in 4% yield.

Table Antimalarial[5] and cytotoxicity[6] of compounds **1**, (±)-**4**-*i*, (±)-**4**-*ii*, (–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4**-*i*, (+)-**4**-*ii*, mixture of (±)-**6**-*i* and (±)-**6**-*ii*, (±)-**6**-*iii*, (±)-**6**-*iv*, (±)-**5**-*i*, and (±)-**5**-*ii*.[a]

Entry	Compounds[a]	Bioactive (IC_{50} : μg/ml)		Entry	Compounds[a]	Bioactive (IC_{50} : μg/ml)	
		Anti-malarial[b]	Cytotoxicity (vero cell)[c]			Anti-malarial[b]	Cytotoxicity (vero cell)[c]
1	piperine (**1**)	inactive	non-cytotoxic	8	mixture (±)-**6**-*i* and (±)-**6**-*ii*	5.04	non-cytotoxic
2	(±)-**4**-*i*	4.7	non-cytotoxic				
3	(±)-**4**-*ii*	3.4	non-cytotoxic	9	(±)-**6**-*iii*	9.25	non-cytotoxic
4	(–)-**4**-*i*	4.3	non-cytotoxic	10	(±)-**6**-*iv*	4.45	non-cytotoxic
5	(–)-**4**-*ii*	3.6	non-cytotoxic	11	(±)-**6**-*i*	4.75	non-cytotoxic
6	(+)-**4**-*i*	4.0	non-cytotoxic	12	(±)-**5**-*i*	inactive	45.55
7	(+)-**4**-*ii*	4.5	non-cytotoxic	13	(±)-**5**-*ii*	inactive	non-cytotoxic

[a]All biological activities resulted from the average of multiple (three) determination. [b]IC_{50} values of the standard antimalarial compounds dihydroartemisinin was 0.0011 μg/mL, respectively. [c]The IC_{50} value of the standard compound ellipticine was 1.0 μg/mL for the vero cells.

The antimalarial activity of the synthetic compounds was determined by means of the microculture radioisotope technique base on the method described by Desjardins *et. al.*[5] Compounds were screened in vitro for activity against the parasite *P. falciparum* (K1, multi-drug resistance strain), and dihydroartemisinin (DHA) was used as a positive standard

control. Compounds (±)-**4**-*i*, (±)-**4**-*ii*, (–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4**-*i*, and (+)-**4**-*ii* displayed antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} value of 4.7, 3.4, 4.3, 3.6, 4.0 and 4.5 μg/mL respectively, as shown in Table. From the above results, interestingly, all compounds have anthracene moiety displayed non-cytoxicity against vero cells and all optically active products showed non-specifically antimalarial activity against the parasite *P. falciparum*. On the other hand, the cyclopentanone adducts, as no anthracene moiety are inactive against the parasite *P. falciparum*.

Conclusion: This work provides preliminary survey of the synthesis of novel antimalarial agents, cyclopentanone-anthracene adducts in racemic ((±)-**4**-*i* and (±)-**4**-*ii*) and optically pure form ((–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4'**-*i*, and (+)-**4'**-*ii*). These compounds exhibited in *vitro* antimalarial activity, and importantly exhibited non-cytotoxicity against vero cells. Obviously, the anthracene moiety are important for antimalarial activity and the cyclopentanone-anthracene adducts are easily and rapidly synthesized from simple, commercially available starting materials.

References:

1. WHO. Guideline for the treatment of malaria, World Health Organization; (2006), WHO/HTM/MAL/2006.1108.
2. R. Choommongkol, *M.S. Thesis*, 2007.
3. (a) F. Kiuchi, N. Nakamura, Y. Tsuda, K. Kondo, H. Yoshimura, *Chem. Pharm. Bull*., 1988, **36**, 2452-2465. (b) A. M. Mujumdar, J. N. Dhuley, V. K. Deshmukh, P. H. Raman, S. R. Naik, *Jpn. J. Med. Sci. Biol*., 1990, **42**, 95-100. (c) H. M. D. Navickiene, A. C. Alecio, M. J. Kato, V.da S. Bolzani, M. C. M. Young, A. J. Cabalheiro, M. Furlen, *Phytochemistry*, 2000, **55**, 621-626. (d) V. F. de Paula, L. C. de Barbosa, A. J. Demuner, D. Pilo-Veloso, M. C. Picanco, *Pest Manag. Sci*., 2000, **56**, 168-174. (e) S. Koul, J. L. Koul, S. C. Taneja, K. L. Dhar, D. S. Jamwal, K. Singh, R. K. Reen, J. Sing, *Bioorg. Med. Chem*., 2000, **8**, 251-268. (f) S. Bajad, K. L. Bedi, A. K. Singla, R. K. Johri, *Planta Med*., 2001, **67**, 284-287.
4. (a) J. Lertvorachon, P. Meepowpan and Y. Thebtaranonth, *Tetrahedron*, 1998, **54**, 14341-14358. (b) P. Kongsaeree, P. Meepowpan and Y. Thebtaranonth, *Tetrahedron: Asymmetry*, 2001, **12**, 1913-1922.
5. Re. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother*., 1979, **16**, 710-718.
6. P. Skehan, S. Ritsa, S. Dominic, *J. Natl. Cancer Inst*., 1990, **82**, 1107-1112.

Keywords: antimalarial, tandem Michael addition – Dieckmann condensation reactions, cyclopentanone-anthracene, piperine.

Acknowledgements: We are grateful to the Thailand Research Fund (TRF), National Center for Genetic Engineering and Biotechnology (BIOTEC), the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Department of Chemistry, Faculty of Science, Chiang Mai University.

Synthesis of Novel Antimalarial Agent, Cyclopentanone Anthracene Adduct, via Tandem Michael Addition-Dieckmann Condensation Reactions

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Introduction

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In this work, we report the synthesis of novel antimalarial agents, cyclopentanone-anthracene adducts **4**[2] via tandem Michael addition-Dieckmann condensation reactions by utilizing the naturally available piperine (**1**)[3] and the anthracene adduct [2,3] as starting materials.

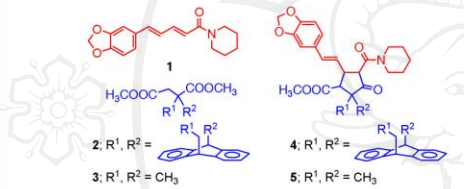
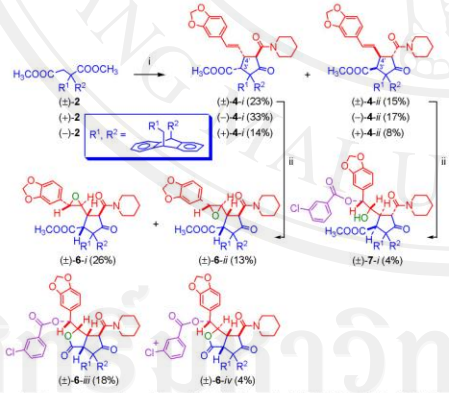


Figure 1. Structures of piperine (**1**), dimethyl itaconate-anthracene adduct (**2**), dimethyl 2,2-dimethylsuccinate (**3**) and cyclopentanone-anthracene adduct (**4**) and cyclopentanone adduct (**5**)

Methodology, Results and Discussion

The cyclopentanone-anthracene adducts (**4**) in racemic and enantiomerically pure form were synthesized using the readily available dimethyl itaconate-anthracene adducts in racemic, (±)-**2** and optically pure form, (+)-**2** and (–)-**2**,[2,3] as shown in Scheme 1.



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Tandem Michael addition - Dieckmann condensation reactions of piperine (**1**) and compound (±)-**2** by treating with LDA (1.2 equiv.) at 0°C for 2 h and then stirred at room temperature for 3 days. Compound **1** gave the cyclopenatanone-anthracene adducts **4**-*i* (23%) and **4**-*ii* (15%). Similar reaction of the enolate anion of (+)-**2** with piperine provided (–)-**4**-*i* and (–)-**4**-*ii* in 23% (>99% e.e., $[\alpha]_{589}^{24.4}$ = –18.49 (*c*, 0.87, $CHCl_3$)) and 15% (>99% e.e., $[\alpha]_{589}^{24.4}$ = –70.50 (*c*, 0.58, $CHCl_3$)) yields, respectively and, likewise, compounds (+)-**4**-*i* and (+)-**4**-*ii* were obtained in 14% (>99% e.e., $[\alpha]_{589}^{24.4}$ = +17.54 (*c*, 0.76, $CHCl_3$)) and 8% (>99% e.e., $[\alpha]_{589}^{24.4}$ = +67.57 (*c*, 0.60, $CHCl_3$)) yields, respectively when piperine was allowed to react with the enolate anion of (–)-**2**. The structures of all product were elucidated by analysis of NMR data while the relative stereochemistry at the C(3′) and C(4′) stereogenic centers of compounds **4**-*i* and **4**-*ii* were determined by X-ray crystallographic analysis. In similar reaction, the enolate anion of dimethyl 2,2-dimthylsuccinate (**3**) with no anthracene moiety reacted with piperine to obtain (±)-**5**-*i* (16%) and (±)-**5**-*ii* (11%), as shown in Scheme 2.

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Entry	Compounds[a]	Bioactive (IC_{50} : μg/mL)		Entry	Compounds[a]	Bioactivity (IC_{50} : μg/mL)	
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4	(–)-**4**-*i*	4.3	non-cytotoxic	10	(±)-**6**-*iv*	4.45	non-cytotoxic
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7	(+)-**4**-*ii*	4.5	non-cytotoxic	13	(±)-**5**-*ii*	inactive	non-cytotoxic

[a]All biological activities resulted from the average of multiple (three) determination. [b]IC_{50} values of the standard antimalarial compound dihydroartemisinin was 0.0011 μg/mL. [c]The IC_{50} value of the standard compound ellipticine was 1.0 μg/mL for the *vero* cells.

The antimalarial activity of the synthetic compounds was determined by means of the microculture radioisotope technique based on the method described by *Desjardins et. al.*[4] Compounds were screened in vitro for activity against the parasite *P. falciparum* (K1, multi-drug resistance strain), and dihydroartemisinin (DHA) was used as a positive standard control. Compounds (±)-**4**-*i*, (±)-**4**-*ii*, (–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4**-*i*, and (+)-**4**-*ii* displayed antimalarial activity against the parasite *P. falciparum* (K1, multi-drug resistance strain) with IC_{50} values of 4.7, 3.4, 4.3, 3.6, 4.0 and 4.5 μg/mL respectively, as shown in Table 1. From the above results, interestingly, all compounds having anthracene moiety displayed non-cytoxicity against *vero* cells and all optically active products showed non-specific antimalarial activity against the parasite *P. falciparum*. On the other hand, the cyclopentanone adducts, with no anthracene moiety are inactive against the parasite *P. falciparum*.

Conclusion

This work provides preliminary survey for the synthesis of novel antimalarial agents, cyclopentanone-anthracene adducts in racemic ((±)-**4**-*i* and (±)-**4**-*ii*) and optically pure forms ((–)-**4**-*i*, (–)-**4**-*ii*, (+)-**4'**-*i*, and (+)-**4'**-*ii*). These compounds exhibited in vitro antimalarial activity, and importantly exhibited non-cytotoxicity against *vero* cells. Obviously, the anthracene moiety is important for antimalarial activity and the cyclopentanone-anthracene adducts are easily and rapidly synthesized from simple, commercially available starting materials.

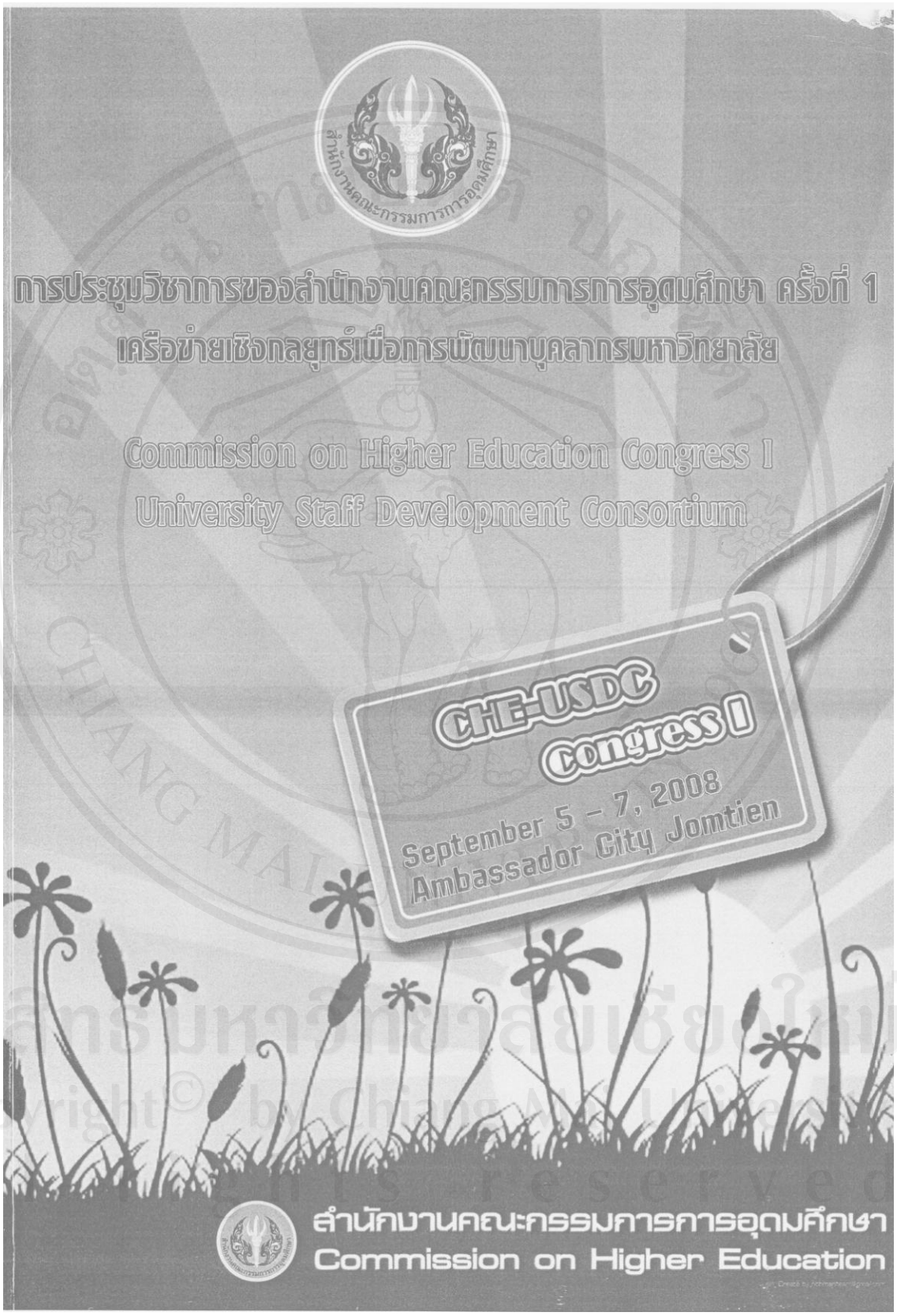
Acknowledgements

We are grateful to the Thailand Research Fund (TRF), National Center for Genetic Engineering and Biotechnology (BIOTEC), the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Department of Chemistry, Faculty of Science, Chiang Mai University.

References

(1) WHO. Guideline for the treatment of malaria, World Health Organization; **2006**, WHO/HTM/MAL/2006.1108.
(2) R. Choommongkol, M.S. Thesis **2007**.
(3) a) J. Lertvorachon, P. Meepowpan and Y. Thebtaranonth, *Tetrahedron* **1998**, *54*, 14341-58 and references therein. b) P. Kongsaeree, P. Meepowpan and Y. Thebtaranonth, *Tetrahedron: Asymmetry* **2001**, *12*, 1913-22 and references therein.
(4) Re. Desjardins, C. J. Canfield, J. D. Haynes, J. D. Chulay, *Antimicrob. Agents Chemother.* **1979**, *16*, 710-718.
(5) P. Skehan, S. Ritsa, S. Dominic, *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.

Keywords: antimalarial, tandem Michael addition - Dieckmann condensation reactions, cyclopentanone-anthracene, piperine.



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Design and Synthesis of Novel Antimalarial Substances: Cyclopentanone-Anthracene Adduct and Their Derivatives

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Objectives

To synthesize spiro-cyclopentanone-anthracene adducts (**3a** and **3b**) and th derivatives for antimalarial and CYP450 test

To synthesize spiro-cyclopentanone-anthracene adduct linked with dihydroartemisir (**5a**), spiro-cyclopentanone-anthracene adduct (**5b**) and pyrimidine compounds (**5c**) f antimalarial and CYP450 test

Methods

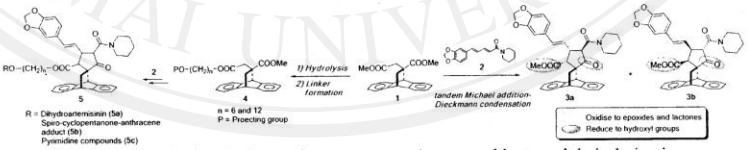
Spiro-cyclopentanone-anthracene adducts (**3**) were synthesized by utilizing dimetl itaconate-anthracene adduct (**1**) and the naturally available piperine (**2**) as starting materi (Scheme 1). Compound **1** was treated with LDA (1.2 equiv) at 0 °C for 2 h, and then trapp with piperine (**2**) *via* tandem Michael addition followed by Dieckmann condensati reactions, then stirred at room temperature for 3 days. After extraction and purification ga adducts **3a** and **3b**.

Adducts **3** were affected by treatment with *m*-CPBA (1.2 equiv) in $CHCl_3$ to furn the epoxide adducts and γ-lactone adducts. Next, adducts **3** were reduced with $NaBH_4$ LAH (2 equiv) in THF at room temperature to give complex mixture products.

Adduct **3** linked with dihydroartemisinin (**5a**), spiro-cyclopentanone-anthrace adduct (**5b**) and pyrimidine compounds (**5c**) were prepared from **1** using hydrolysis, lin formation, protection of hydroxyl group, tandem Michael addition-Dieckmann condensat reactions and linked with dihydroartemisinin, adduct **3** and pyrimidine compou respectively.

Results

Tandem Michael addition-Dieckmann condensation reactions of compound **1** a piperine (**2**) gave adduct **3a** (23 %) and **3b** (15 %) which displayed antimalarial agai parasite *P. falciparum* (K1, multi-drug resistance stain) with IC_{50} value 4.7 and respectively.



Scheme 1 Synthesis of spiro-cyclopentanone-anthracene adducts and their derivatives

Discussion

This work provides preliminary survey for the synthesis of novel antimalarial age adducts **3**. These compounds exhibited *in vitro* antimalarial activity, and importa exhibited non-cytotoxicity against *vero* cells.

PERCH-CIC CONGRESS VI

Theme: Towards a Sustainable Future

การประชุมวิชาการ
ศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี ครั้งที่ 6

3-6 May 2009
Jomtien Palm Beach Hotel & Resort Pattaya, Chonburi

PERCH-CIC Center of Excellence for Innovation in Chemistry

S2-P13

Design and Synthesis of Spirocyclopentanone-Anthracene Dimer Adducts as Antimalarial Substances

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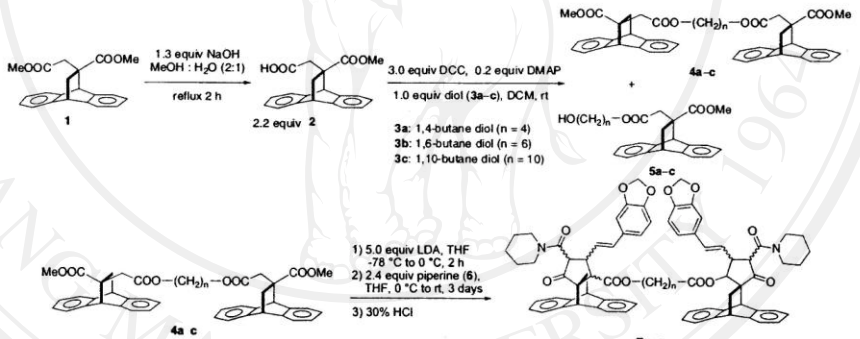
Objective

To synthesize spirocyclopentanone-anthracene dimer adducts *via* tandem Michael addition–Dieckmann condensation reactions for antimalarial activity test.

Methods

Spirocyclopentanone-anthracene dimer adducts (**7a–c**) could be synthesized *via* tandem Michael addition–Dieckmann condensation reactions of dimethyl itaconate-anthracene dimer adduct (**4a–c**) and piperine (**6**). Dimer adducts **4a–c** were prepared using base hydrolysis of **1** to give monomethyl itaconate-anthrcenene adduct (**2**), followed by reacted with DCC and diols **3a–c** to yield of dimer adducts **4a–c** as showed below.



Results

Dimer adducts **4a–c**, as major product and hydroxyalkyl adducts **5a–c**, as minor product, were obtained after purified by flash column chromatography using EtOAc : Hexane, 2 : 8 as eluent. Tandem Michael addition–Dieckmann condensation adducts (**7a** and **7b**) from dimer adducts (**4a** and **4b**) reacted with piperine could not observed. However, spirocyclopentanone-anthracene dimer adduct (**7c**) could be obtained.

Conclusion

Dimer adducts **4a–c** and hydroxyalkyl adducts **5a–c** could be achieved using practical hydrolysis and dimerization respectively. Tandem Michael addition–Dieckmann condensation reactions of **4a–c** with piperine are under investigated.

Keywords: spirocyclopentanone, antimalarial, tandem Michael addition-Dieckmann condensation reactions

Selected References:

1. Lertvorachon, J.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron* **1998**, *54*, 14341-14358.
2. Kongsaeree, P.; Meepowpan, P.; Thebtaranonth, Y. *Tetrahedron: Asymmetry* **2001**, *12*, 1319-1922.
3. Tarnchompoo, B.; Thebtaranonth, C.; Thebtaranonth, Y. *Tetrahedron Lett.* **1987**, *28*, 6671-6674.
4. Tsukamoto, S.; Cha, B.-C.; Ohta, T. *Tetrahedron* **2002**, *58*, 1667-1671.
5. Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamrarn, A. *Planta Med.* **2002**, *68*, 853-855.

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