APPENDIX A

MATERIALS

Chemical

Keyhole limpet hemocyanin (KLH), H-7017, Sigma Chemical Co., USA

Bovine serum albumin (BSA), A-4503, Sigma Chemical Co., USA

Oval albumin (OVA), A-388, Fisher Scientific.

Dimethylformamide (DMF) Merck

4- dimethylaminopyridine, 39405, Fluka, Sigma-Aldrich Co. USA.

N, N – dicyclohexylcarbodiimide, 36650, Fluka, Sigma-Aldrich Co. USA.

N- hydroxysuccinimide, 56480, Fluka, Sigma-Aldrich Co. USA.

Succinic anhydride, 14089, Fluka, Sigma-Aldrich Co. USA.

Glutaric anhydride, 49670, Fluka, Sigma-Aldrich Co. USA.

Pyridine, A.R. BDH, BDH Laboratory Supplies, England.

Benzene .T. Baker, Mallinckrodt Kaker, Inc., USA

Acetone .T. Baker, Mallinckrodt Kaker, Inc., USA

Ethyl acetate 9260-03, J.T. Baker, Mallinckrodt Kaker, Inc., USA

Hexane, 9309-03, J.T. Baker, Mallinckrodt Kaker, Inc., USA

Dimethyl sulfoxide, D-2650, Sigma Chemical Co., USA.

Bio-Rad protein assay, 500-0006, Bio-Rad laboratories,Inc.

Thin-layer chromatography (TLC) silica gel 60F254 20 x 20 cm aluminium sheet, Merck, Germany.

Silica gel 60F254, Merck, Germany.

Spectra/Por® membrane tubing, Spectrum, Spectrum Laboratory, Inc.

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Standard pesticides

DCBH, TCI, Japan (kindly given by Prof. Dr. Takahiko Takatori, School of Medicine, University of Tokyo)

o,p'-DDE, ch-10311, 99.8%, Laboratory of Dr. Ehrenstorfer, Augburg, Gemany.

o,p'-DDT, c-12081000, 98.0%, Laboratory of Dr. Ehrenstorfer, Augburg, Gemany.

o,p'-DDT, c-120820, 99.3%, Laboratory of Dr. Ehrenstorfer, Augburg, Gemany.

p,p'-DDA, 35484, 99.9%, Riedel-de Haen, Sigma-Aldrich Co. USA.

p,p'-DDD, 49009, 98.5%, Supelco, Bellelofente, PA, USA.

p,p'-DDE, PS-696, 99.5%, Chem Service Inc., West Chester, PA, USA.

Dicofol, 36677, 96.9%, Riedel-de Hean, Sigma-Aldrich Co. USA.

Complete Freund's adjuvant (CFA), F-5881, Sigma Chemical Co., USA

Incomplete Freund's adjuvant (IFA), F-5506, Sigma Chemical Co., USA

Immunological reagents

Dynabeads Pan Mouse IgG, Dynal A.S. N-0217 Osio, Norway.

HRP- Conjugate anti - mouse Ig G antibody, 81-6520, Zymed Laboratories Invitrogen immuno detection, USA.

Iscove's Modified Eagle Medium, 12200-036, Invitrogen Cor, USA.

Fetal Bovine Serum, Gibco, Invitrogen Cor, USA.

HAT Supplement, 21060-017, Gibco, Invitrogen Cor, USA.

HT Supplement, 1548, Invitrogen Cor, USA.

Ortho-phenyleneline diamine, P-1526, Sigma Chemical Co., USA

Polyethylene glycol (PEG) solution 50% (w/v), P-7181, Sigma Chemical Co., USA

Mouse MonoAb ID/SP Kit, 93-6556, Zymed Labolatories Inc. USA.

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Disposable products

Immuno plate Maxisorp 96F, 442404, NUNC, Denmark.

24 well culture plates, 143982, NUNC, Denmark.

96 well cell culture cluster, 3599, NUNC, Denmark.

Cell culture flasks 10 ml., 156367, NUNC, Denmark.

Bottle top filter, 431161, Corning Incorporated, USA

Reagents

1. Reagent for production of monoclonal antibodies

1.1 Phosphate buffer saline (PBS) pH7.2

NaCl	8.00 gm
KCl	0.20 gm
Na_2HPO_4	1.15 gm
KH_2PO_4	0.20 gm

- Added distilled water to about 900 ml and stirred on magnetic stirrer until well dissolved.
- Adjusted the pH 7.2 with HCl and NaOH.
- Filled up to 1,000 ml with water and stored at room temperature.

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1.2 Iscove's Modified Dulbecco's Medium (IMDM)

- Added IMDM powder to distilled water with gentle stirring.
- Rinsed out inside of package to remove all traces of powder.

- Added 3.024 g of $NaHCO_3$.
- Added penicillin and streptomycin to final concentration of 100 unit/ml and 100 µg/ml, respectively.
- Added distilled water to about 900 ml, mixed well.
- Filled up with distilled water to 1,000 ml.
- Sterilized by filtration and stored 4 ^{O}C.

1.3 10 % FBS-IMDM

- Mixed 10 ml of heat inactivated fetal bovine serum and 90 ml IMDM together by sterile technique in a laminar air flow and kept at 4 ^{O}C.

1.4 0.2 % trypan blue in normal saline

Trypan blue powder	0.20 g
Normal saline solution	100 ml

- Dissolved trypan blue saline solution and stored at room temperature.

1.5 ACK Lysing Buffer

NH_4Cl	8,024 mg/l
$KHCO_3$	1,001 mg/l
$EDTA.Na_2 \cdot 2H_2O$	3.722 mg/l

- Added distilled water to about 900 ml and mixed until well dissolved.
- Adjusted pH to 7.4 with HCl.
- Filled up to 1,000 ml with distilled water and stored at 4 ^{O}C.

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1.6 HAT medium

10%FBS-IMDM	98 ml
10 mM Hypoxantine, 50 µM Aminopterine, 1.6 mM Thymidine (HAT) 50X stock	2 ml

- Mixed all ingradients together until well dissolved.
- Sterilized by filtration and stored at 4 ^{O}C.

1.7 HT medium

10%FBS-IMDM	99 ml
10 mM Hypoxantine, 1.6 mM Thymidine (HT) 100X stock	1 ml

- Mixed all ingradients together until well dissolved.
- Sterilized by filtration and stored at 4 ^{O}C.

2. Reagent for ELISA

2.1 Carbonate/bicarbonate coating buffer pH 9.6, 0.1 M

Na_2CO_3	1.59 g
$NaHCO_3$	2.93 g

- Dissolved Na_2CO_3 and $NaHCO_3$ in 1,000 ml distilled water.
- Adjusted pH to 9.6 with NaOH.
- Store at 4 ^{O}C.

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2.2 Phosphate buffered saline with 0.05% Tween

Phosphate buffer saline	1,000	ml
Tween – 20	0.5	ml

- Added tween-20 into phosphate buffered saline pH 7.2 and mix well.
- Used with in day after preparation.

2.3 Blocking reagent 1% gelatin/PBS.

Gelatin	1	g
PBS, pH 7.2	100	ml

- Boiled all together.

2.4 Citrate – Phosphate buffer, pH 4.0

Citric acid	21.0	g
Na_2HPO_4	14.2	g

- Dissolved Citric acid and Na_2HPO_4 in 1,000 ml distilled water.
- Adjusted pH to 4.0 with HCL
- Store at 4 ^{O}C.

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2.5 OPD substrate 10 mg/ml

OPD	1	g
Citrate – phosphate buffer, pH 4.0	100	ml

- Mixed all together until well dissolved.
- Store at -20 ^{O}C

2.**6 OPD substrate working solution**

Citrate – phosphate buffer, pH 4.0	10	ml
OPD (10 mg/ml)	500	µl
30 % H_2O_2	5	µl

- Mixed all together until well dissolved.

2.7 2 N H_2SO_4 (stopping reagent)

Conc. H_2SO_4	56	ml
Distilled water	444	ml

- Mixed all together until well dissolved and store at room temperature.

Equipments

UV-2101 PC UV/vis spectrophotometer, Shimadzu Scientific Instrument, Inc. Japan

Micro plate spectrophotometer, Spectra MR, DYNEX Technologies, Inc.

Gas chromatograph 6890, Agilent Technologies, USA.

Mass-spectro detector (MSD), Hewlett Packard, USA.

^{1}H and ^{13}C NMR, Bruker, Bruker analytic GmbH, Germany.

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APPENDIX B

CHROMATOGRAMS OF HAPTENS

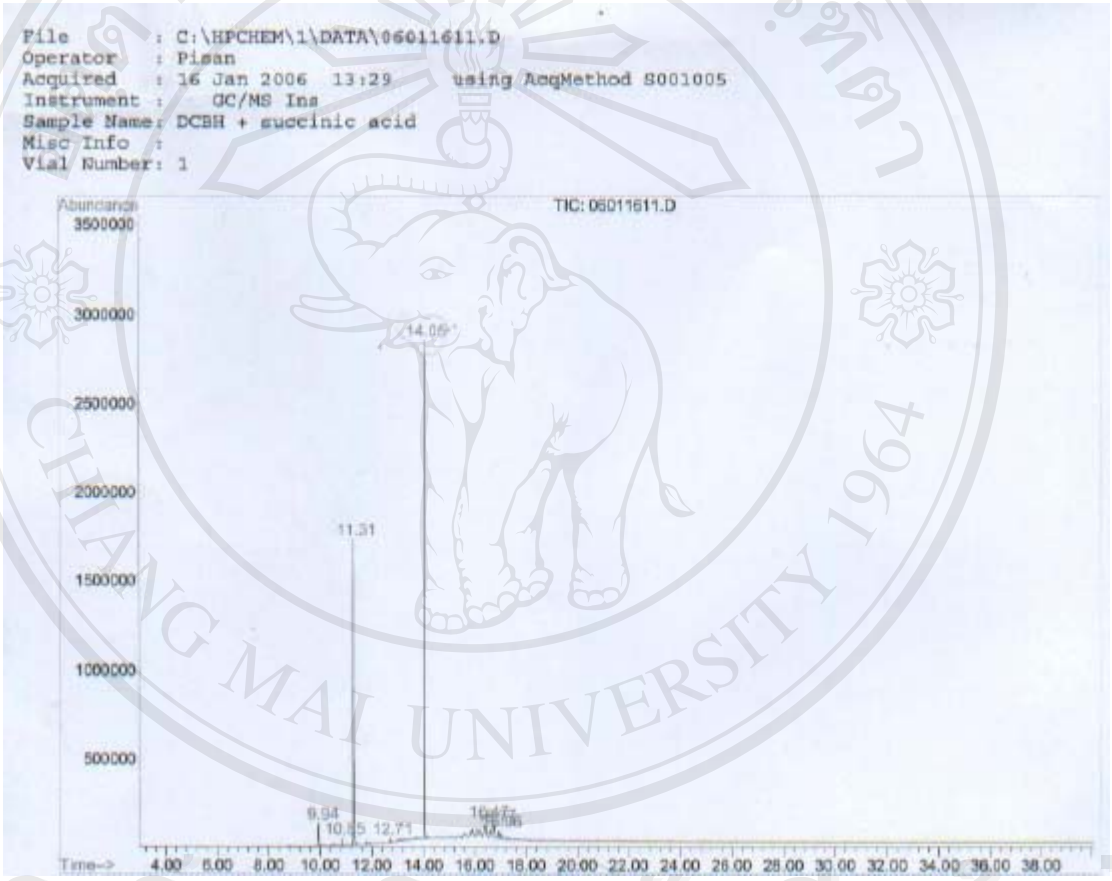

Figure B.1 The chromatogram of GC/MS used for confirmation molecular weight of haptens 1.

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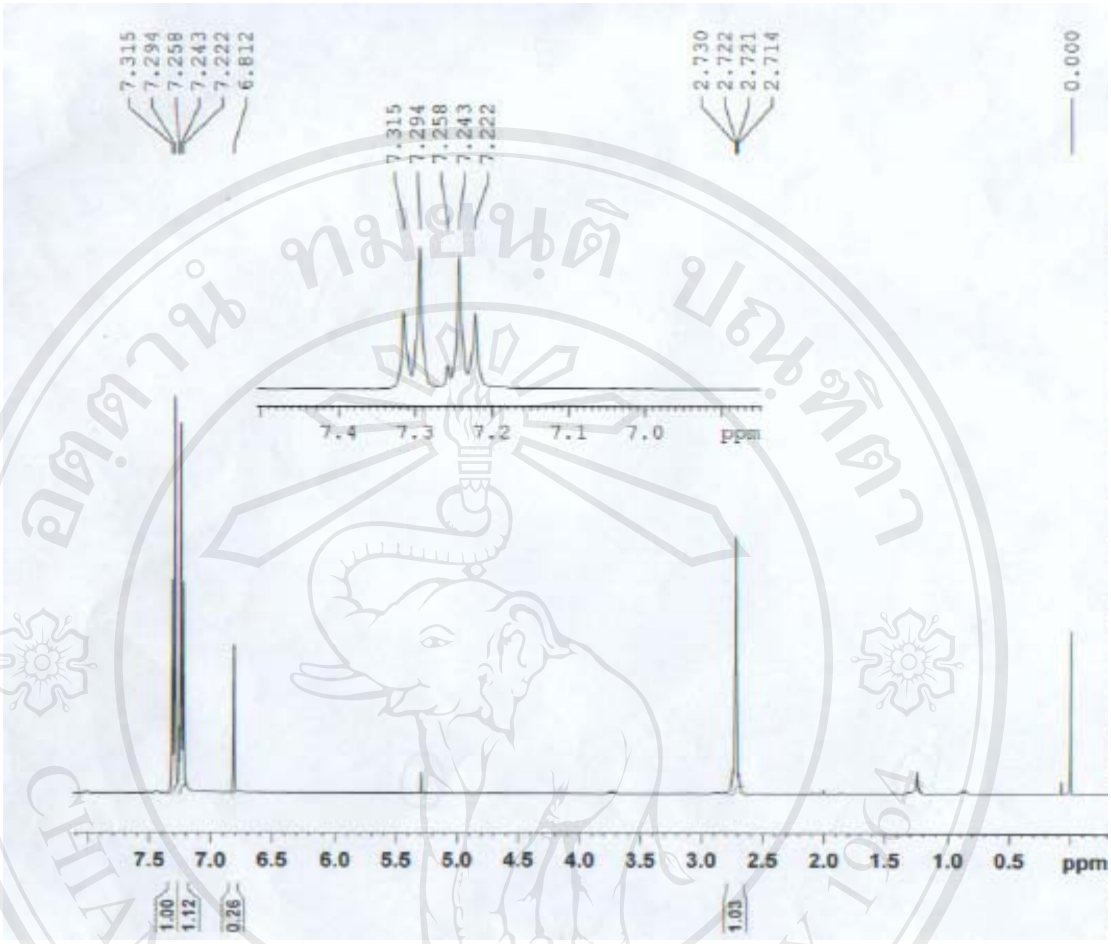

Figure B.2 ^{1}H NMR spectrum of used for confirmation number of hydrogen in haptens 1.

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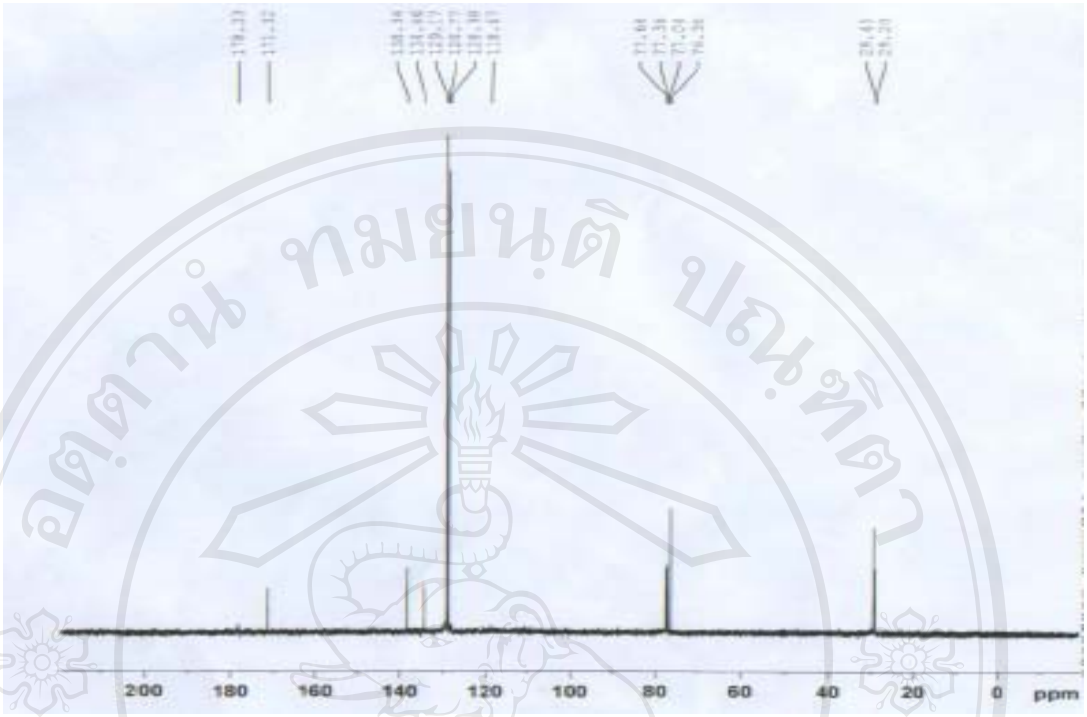

Figure B.3 ^{13}C NMR spectrum of used for confirmation number of carbon in haptens 1.

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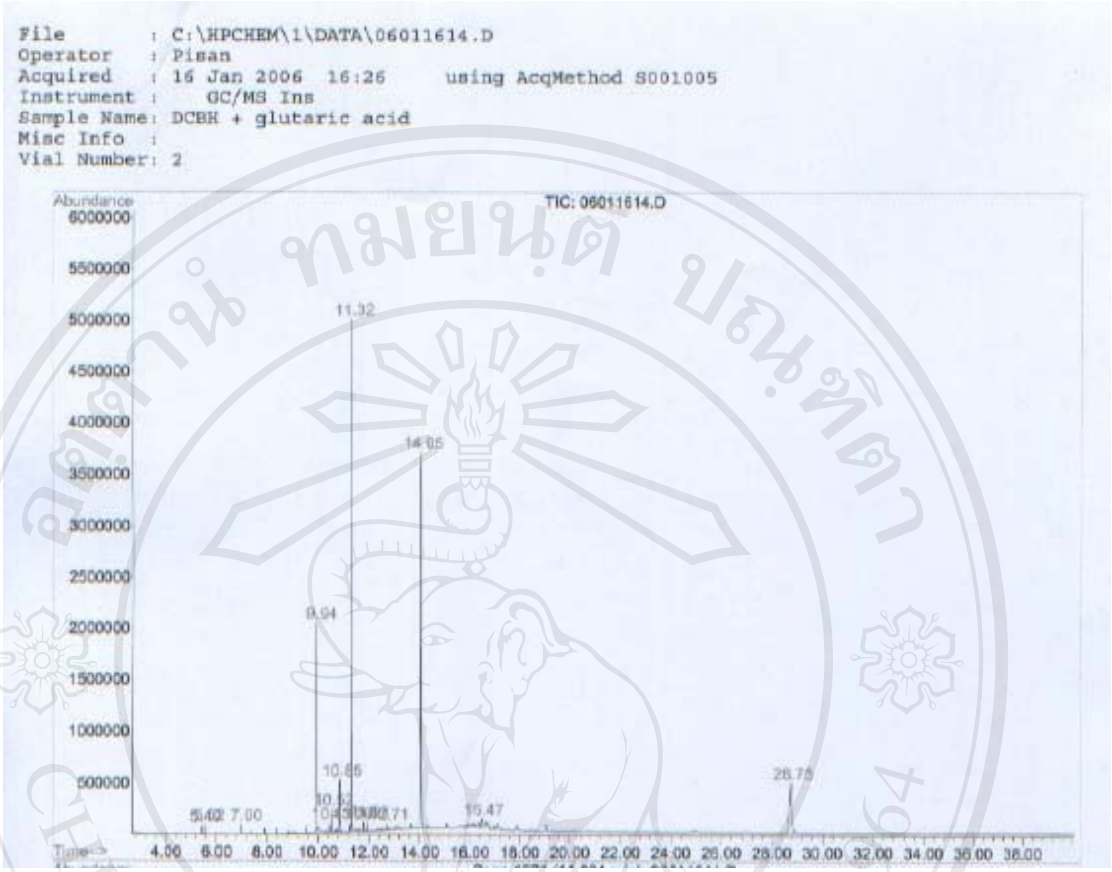

Figure B.4 The chromatogram of GC/MS used for confirmation molecular weight of haptens 2.

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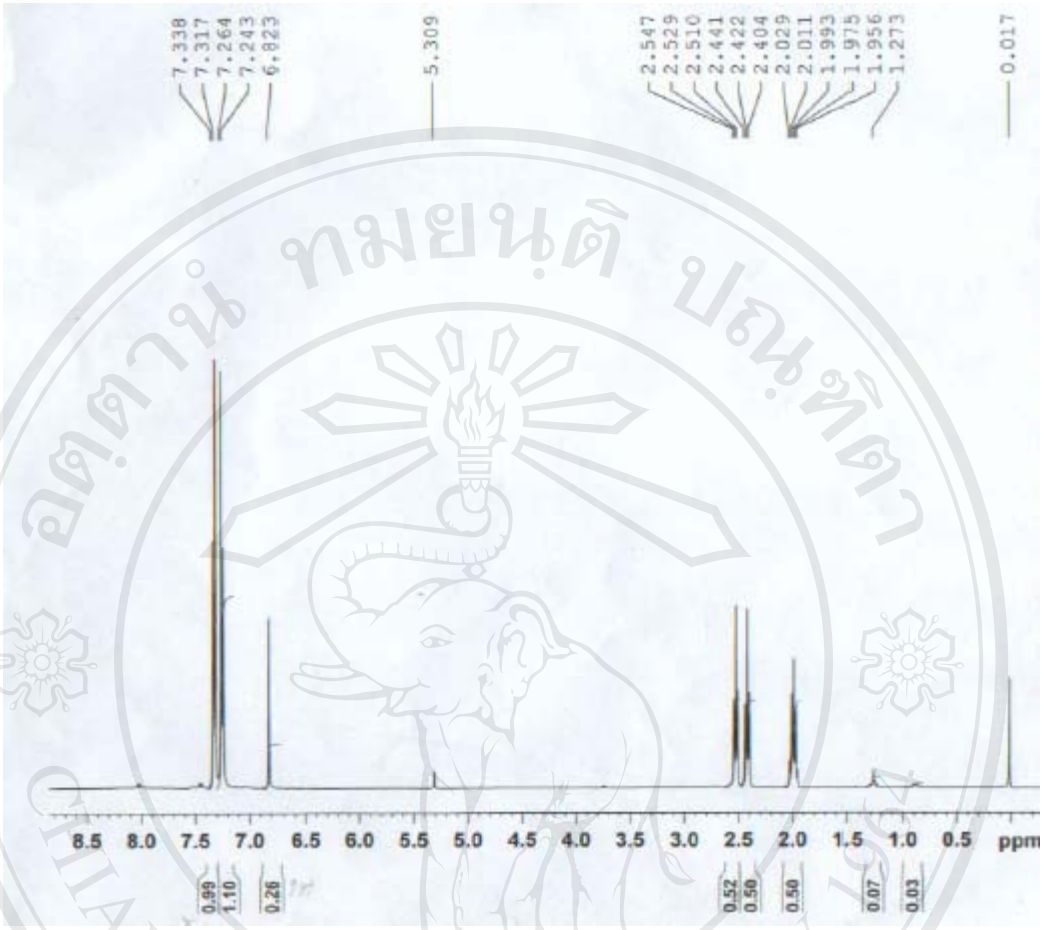

Figure B.5 ^{1}H NMR spectrum of used for confirmation number of hydrogen in haptens 2.

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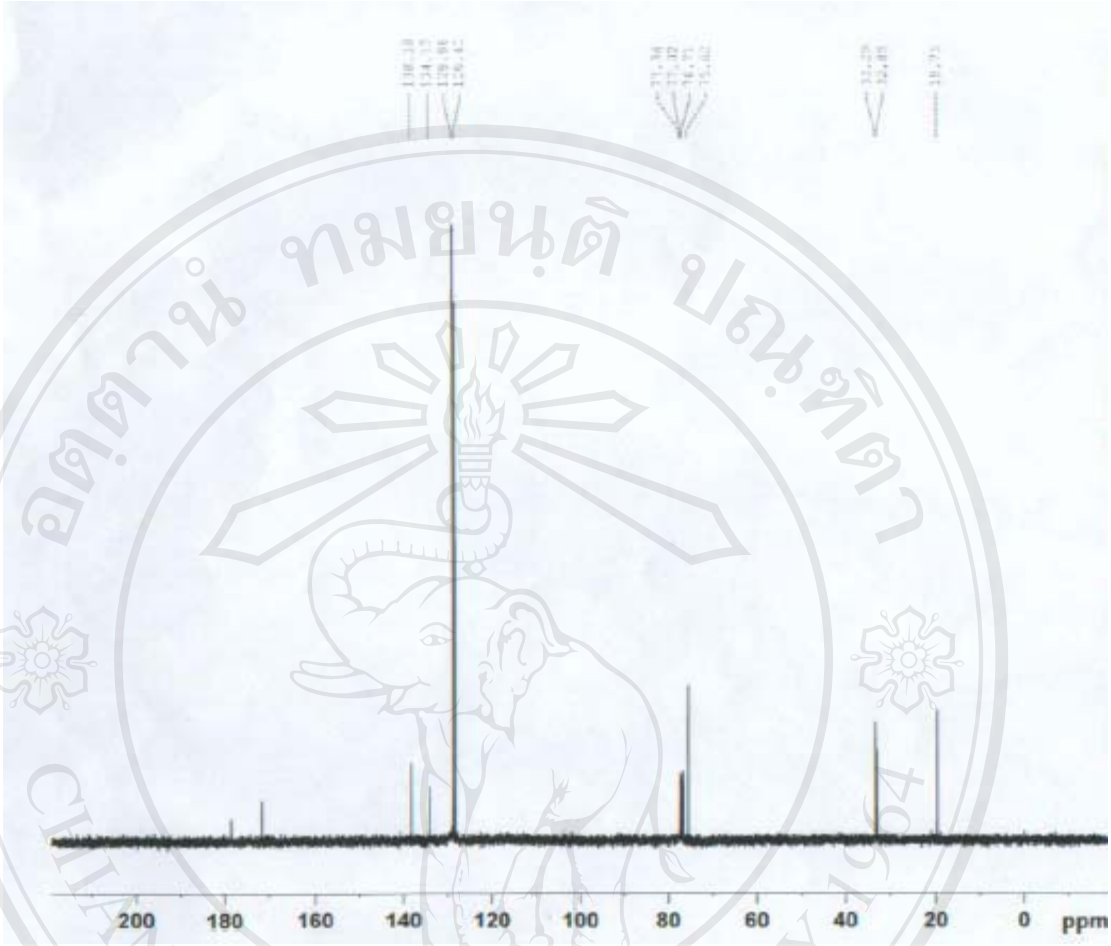

Figure B.6 ^{13}C NMR spectrum of used for confirmation number of carbon in haptens 2.

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CURRICULUM VITAE

Name: Mr. Surat Hongsibsong

Date of birth: May 6, 1978

Education background: BSc. (Medical Technology). Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand, 1997 – 2000.

Work Experience: Research assistant at Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand, 2000 – 2006.

Scholarships: Research Institute for Health Sciences, Chiang Mai University, Thailand, 2004 – 2006.
Graduated School, Chiang Mai University, Thailand, 2005-2006.

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Presentation: 1) Surat Hongsibsong, Tippawan Prapamontol, Chaisuree Suphavilai, Jiraprapa Wipasa, Mookda Pattarawarapan, Watchara Kasinrerk. Production of antibody against a metabolite of an acaricide Dicofol.

29th Annual Scientific Meeting on mahidol's Day September 23, 2005. p. 14.

2) Surat Hongsibsong, Chaisuree Suphavilai, Jiraprapa Wipasa, Mookda Pattarawarapan, Watchara Kasinrerk, Tippawan Prapamontol. Production of DDT antibodies for an ELISA test kit. The 5th National Symposium on graduate Research, October 10 -11, 2005. p. 295.

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