SYNTHESIS OF OSELTAMIVIR DERIVATIVES WITH ANTI-TYROSINASE ACTIVITY

By
Mr.Kittiwat Srikittiwanna

A Thesis Submitted in Partial Fulfillment of the requirements for the Degree
Master of Science Program in Organic Chemistry
Department of Chemistry
Graduate School, Silpakorn University
Academic Year 2015

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การสังเคราะห์อนุพันธ์โอเซลทามิเวียร์เพื่อใช้ยับยั้งการทำงานของเอนไซม์ไทโรซิเนส

โดย

นายกิตติวัฏ ศรีกิตติวรรณา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเคมีอินทรีย์

ภาควิชาเคมี

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2558

The Graduate School, Silpakorn University has approved and accredited the Thesis title of "Synthesis of Oseltamivir derivatives with anti-tyrosinase activity" submitted by MR.Kittiwat Srikittiwanna as a partial fulfillment of the requirements for the degree of Master of Science in organic chemistry

.....
(Associate Professor Panjai Tantatsanawong,Ph.D.)
Dean of Graduate School
...../...../.....

The Thesis Advisor

Muhammad Niyomdecha, Ph.D.

The Thesis Examination Committee

..... Chairman
(Punlop Kuntiyong, Asst. Prof.)
...../...../.....

..... Member
(Anan Athipornchai, Ph.D.)
...../...../.....

..... Member
(Muhammad Niyomdecha, Ph.D.)
...../...../.....

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Oseltamivir phosphate or Tamiflu is an antiviral licensed to prevent or slow the spread of influenza A and influenza B (Bird flu). To the present date many synthesis of Oseltamivir phosphate have been documented. There were Oseltamivir derivatives (**3b-3d** and four new compounds (**2**), (**4b**), (**4c**) and (**4d**) were synthesized starting from epoxide (**1**) which can be synthesis 2 route. Fisrt, Ring opening of epoxide (**1**) via S_N^2 substitution by H_2SO_4 and NaN_3 gave Oseltamivir derivatives (**2**), (**3**) and (**4**) respectively. Oseltamivir derivatives (**3**) was converted to Oseltamivir derivatives (**3b**) by azide S_N^2 substitution, acetylation and reduction respectively. Reduction of Oseltamivir derivatives (**3**) generated Oseltamivir derivatives (**3c**) after that acetylation of oseltamivir derivative (**3c**) furnished Oseltamivir derivatives (**3d**). Oseltamivir derivatives (**4b**), (**4c**) and (**4d**) synthesis is same of Oseltamivir derivatives (**3b**), (**3c**), (**3d**). Oseltamivir derivatives were evaluated for anti-tyrosinase activity. Oseltamivir derivatives (**2**) and (**3c**) exhibited inhibitory activity of tyrosinase in 13.97 and 12.06 %.

Program of Chemistry Graduate School, Silpakorn University
Student's signature ..... Academic Year 2015
Thesis Advisor's signature .....

56302201 : สาขาวิชาเคมีอินทรีย์

คำสำคัญ : โอเซลทามิเวียร์/ไทโรซิเนส

กิตติวัฏ ศรีกิตติวรรณา : การสังเคราะห์อนุพันธ์โอเซลทามิเวียร์เพื่อใช้ยับยั้งการทำงานของเอนไซม์ไทโรซิเนส. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : อ.ดร.มูฮำหมัด นิยมเดชา. 81 หน้า.

โอเซลทามิเวียร์ฟอสเฟตหรือทามิฟลูเป็นยาที่ใช้ป้องกันหรือชะลอการแพร่กระจายของโรคไข้หวัดใหญ่สายพันธุ์ A และ B (ไข้หวัดนก) ในปัจจุบันได้มีงานวิจัยเป็นจำนวนมากที่เสนอทำการสังเคราะห์โอเซลทามิเวียร์ฟอสเฟต ในงานวิจัยนี้ได้นำสังเคราะห์อนุพันธ์ของโอเซลทามิเวียร์ได้แก่ อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3b**), (**3c**) และ (**3d**) ชุดใหม่ คือ (**2**), (**4b**), (**4c**) และ (**4d**) โดยจะใช้สารตั้งต้น คือ epoxide (**1**) ซึ่งสามารถแบ่งการสังเคราะห์ได้ 2 แนวทาง คือ 1. ปฏิกิริยาเปิดวง epoxide (**1**) ด้วย S_N2 substitution โดยใช้ H_2SO_4 จะได้ อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**2**) แนวทางที่ 2 คือ ปฏิกิริยา S_N2 substitution ด้วย NaN_3ของ epoxide (**1**) จะได้อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3**) และ (**4**) จากนั้นนำอนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3**) เปลี่ยนไปเป็น อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3b**) โดยปฏิกิริยา acetylation และ reduction ต่อไปเป็นปฏิกิริยา Reduction ของอนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3**) จะได้อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3c**) หลังจากนั้นทำปฏิกิริยา acetylation จะได้อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3d**) ส่วน อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**4b**), (**4c**) และ (**4d**) สังเคราะห์คล้ายๆ กับ อนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**3b**), (**3c**) และ (**3d**)

อนุพันธ์ของโอเซลทามิเวียร์มีผลสำหรับ anti-tyrosinase โดยอนุพันธ์ของโอเซลทามิเวียร์หมายเลข (**2**) และ (**3c**) มีความสามารถยับยั้ง tyrosinase คือ 13.97 และ 12.06 % ตามลำดับ

ภาควิชาเคมี บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ลายมือชื่อนักศึกษา..... ปีการศึกษา 2558

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ .....

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Finally, none of this would have been possible without love and encouragement of my family and friends.

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ABBREVIATIONS

OS	Oseltamivir
$RuCl_3$	Ruthenium(III) Chloride
$NaIO_4$	Sodium Periodate
EtOAc	Ethyl Acetate
CH_3CN	Acetonitrile
Ac_2O	Acetic Anhydride
Py	Pyridine
$POCl_3$	Phosphoryl Chloride
$SOCl_2$	Thionyl Chloride
SO_2Cl_2	Sulfuryl Chloride
CH_2Cl_2	Dichloromethane
PPh_3	Triphenylphosphine
THF	Tetrahydrofuran
Boc	Tert-Butyloxycarbonyl
$TMSN_3$	Trimethylsilyl Azide
t- BuOH	Tert-Butyl Alcohol
KOH	Potassium Hydroxide
TFA	Trifluoroacetic Acid
$HgCl_2$	Mercury(II) Chloride
$NaBH_3CN$	Sodium Cyanoborohydride
PCC	Pyridinium Chlorochromate

CHAPTER 1
INTRODUCTION

Skin whitening, skin lightening, and skin bleaching refer to the practice of using chemical substances in an attempt to lighten skin tone or provide an even skin complexion by reducing the melanin concentration in the skin. Several chemicals have been present to be effective in skin whitening, while some have proven to be toxic or have questionable safety profiles, adding to the controversy surrounding their use and impacts on certain ethnic groups [1-3].

Melanin is a broad term for a group of natural pigments found in most organisms. It pigmentation is able to shield from UV radiation, inhibit photocarcinogenesis and affect the synthesis of vitamin D3. In contrast, the abnormal pigmentation, such as senile lentigines, freckles, melasma, and other forms of melanin hyperpigmentation, causes serious esthetic problem. The oxidative reactions of the tyrosine catalyzed by tyrosinase mainly contributes to the melanin biosynthesis. Tyrosinase inhibitors such as arbutin, kojic acid, aloesin, glabridin and hydroquinones **(Figure 1)** have been used as whitening or antihyperpigment agents because of their ability to suppress dermal-melanin production. However, arbutin and kojic acid hardly showed inhibitory activity against pigmentation in intact melanocytes or in a clinical trial, and hydroquinones are considered to be cytotoxic to melanocytes and potentially mutagenic to mammalian cells. Therefore, it remains necessary to search for new tyrosinase inhibitors without side effects [4-10].

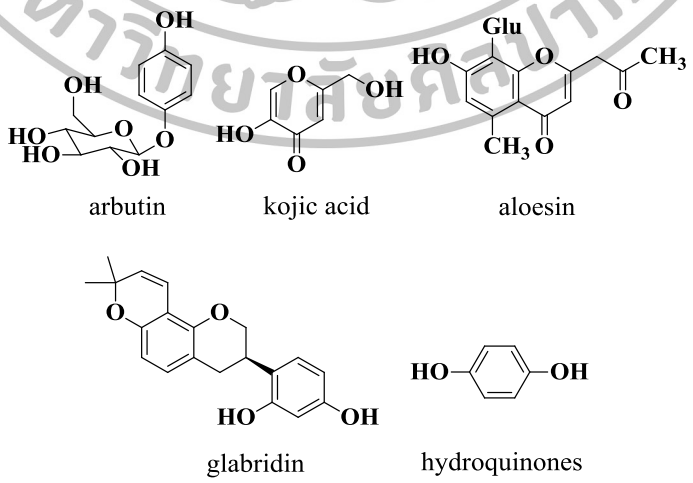


Figure 1: Compounds of Tyrosinase inhibitor.

Oseltamivir phosphate which marketed under the trade name Tamiflu® is antiviral medication used to treat influenza A and B (Bird flu). It is a neuraminidase inhibitor [11]. To the present date many syntheses of oseltamivir derivatives have been documented. Also interested in evaluation of other biological activity of their derivatives. Oseltamivir derivatives were synthesized by precursor epoxide (**1**) [12] obtained from (-)-shikimic acid **(Figure 2)**.

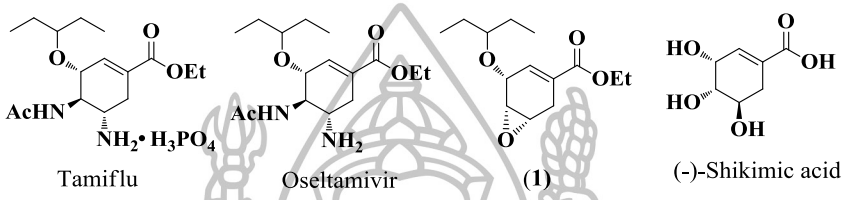
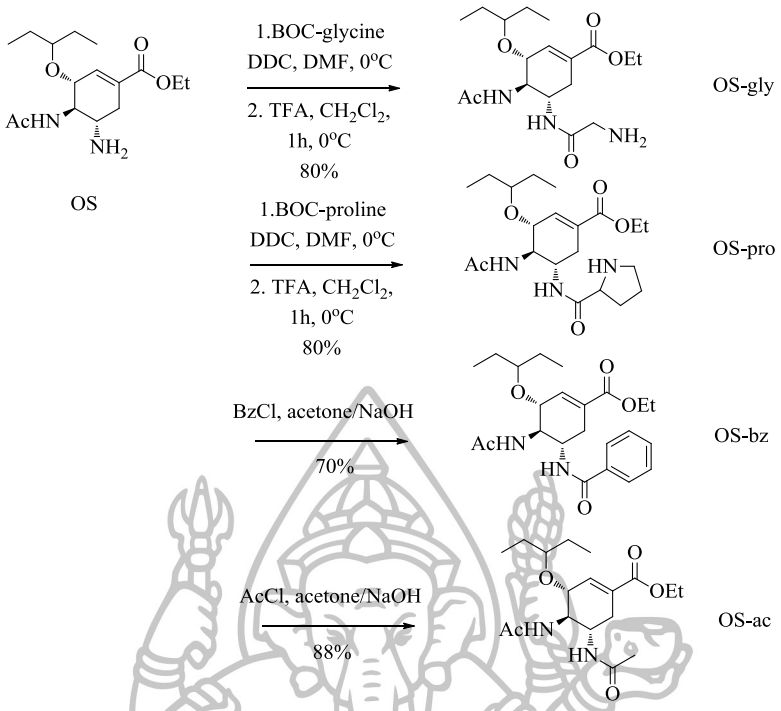


Figure 2: Structure of Tamiflu, Oseltamivir, epoxide(**1**) and shikimic acid.

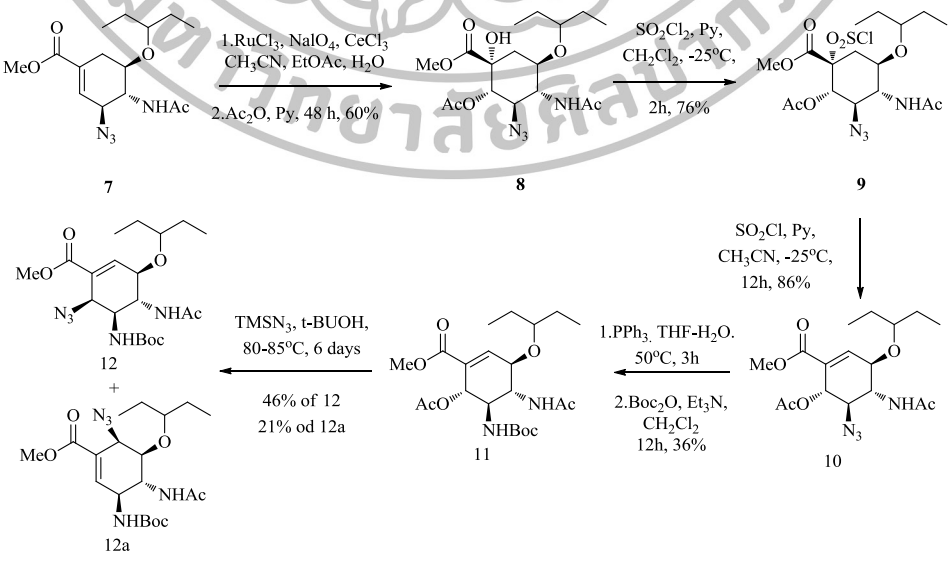
In 2012, publication of Charlotte D'Souza [13] and co-workers reported oseltamivir derivatives. New derivatives of oseltamivir were prepared by modifying the amino group with glycyl, acetyl, benzyl and prolyl moieties. Based on this suggestion, they have prepared derivatives of oseltamivir (GS-4104) (OS) with varying charge and lipophilicity, by substituting glycyl (OS-gly), acetyl (OS-ac), benzyl (OS-bz) and prolyl (OS-pro) groups at the amino group of oseltamivir.

In the synthetic studies, preparation of OS-gly and OS-pro (**Scheme 1**) of nucleophilic substitution between oseltamivir and BOC-glycine or BOC-proline and deprodection of BOC provided OS-gly or OS-pro. Oseltamivir was convented to OS-ac or OS-bz by nucleophilic substitution with acetyl chloride or benzoyl chloride.



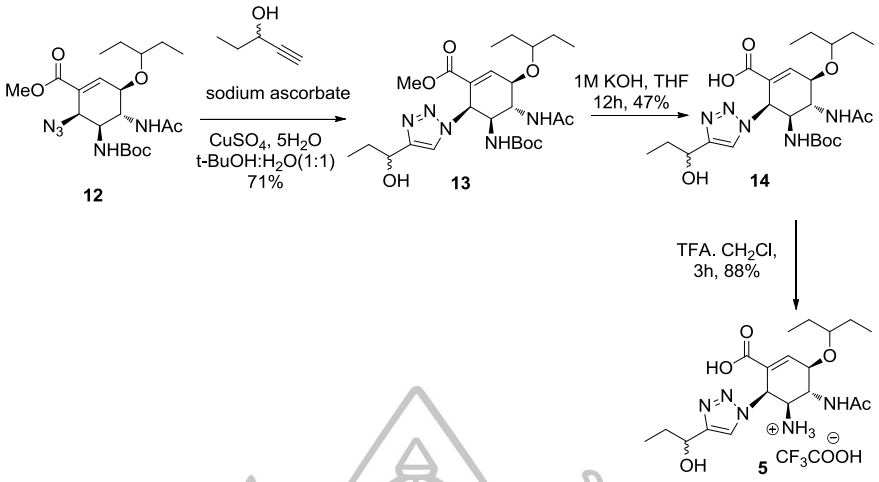
Scheme 1: Synthesis of OS-gly, OS-ac, OS-bz and OS-pro

In 2013, publication of Pal John Pal Adabala [14] and co-workers reported oseltamivir derivatives. They have successfully synthesized C-6 triazolefunctionalized Tamiflu derivatives as second-generation candidates using an azidation reaction on the cyclic Baylis−Hillman derivative **11** via allylic azide [3,3]-sigmatropic rearrangement and copper-catalyzed azide−alkyne cycloaddition as key reactions.



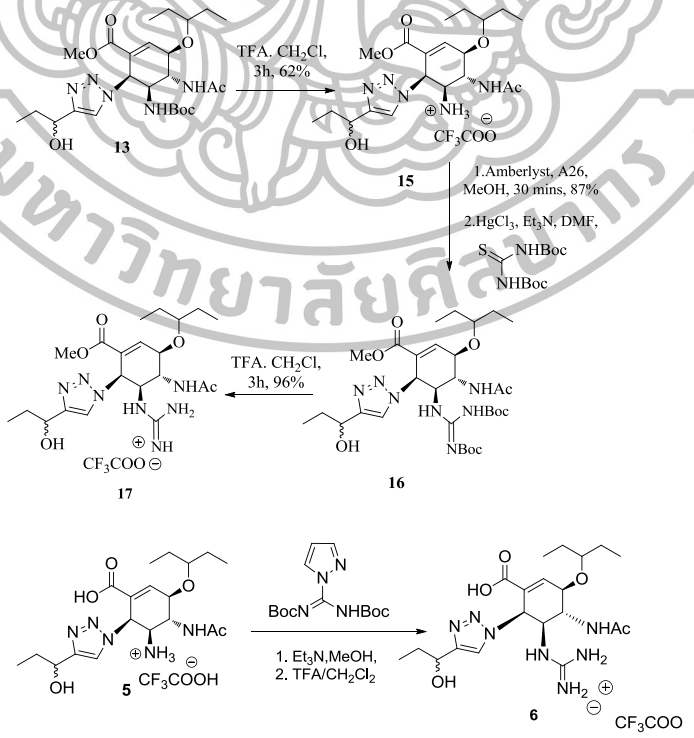
Scheme 2: Synthesis of compounds **12**.

In **Scheme 2**, treatment of unsaturated ester **7** with a catalytic amount of $RuCl_3$ in the presence of $CeCl_3/NaIO_4$ in a mixed solvent system ($EtOAc/CH_3CN/H_2O$, 3/3/1) at 0 °C provided an α-diol and acetylation of the resulting diol in the presence of Ac_2O/Py gave the monoacetate **8** in 60% overall yield. Eliminations of the alcohol function in 8 with various reagents such as Martin sulfurane, Burgess reagent, Vilsmeier reagent and other classical methods ($POCl_3$, $SOCl_2$, OTf elimination) were unsuccessful or were very low yielding. The elimination reaction was finally achieved with sulfuryl chloride. Initial experiments with SO_2Cl_2 in the presence of pyridine in CH_2Cl_2 furnished the chlorosulfate **9**, which was used for the preparation of olefin **10** using basic conditions or by heating. Subsequently, the elimination reaction was accomplished using CH_3CN as solvent with SO_2Cl_2 in the presence of pyridine. Owing to the residual sulfur products in olefin **10**. Reduction of the azide **10** with PPh_3 in THF−H_2O at 50 °C provided the corresponding free amine, which on treatment with tertbutylpyrocarbonatetert-butylpyrocarbonate afforded the Boc-protected amine **11** (**Scheme 2**). Therefore, the crucial nucleophilic substitution was examined using the allylic acetate **11** (**Scheme 2**), a cyclic Baylis−Hillman derivative. Initial attempts at substitution reactions with sodium azide in different polar solvents led to a complex mixture of products. Finally, the desired azide **12** wasobtained in 46% yield by treatment of **11** with $TMSN_3$ in *t*-BuOH, a side product **12a** also being formed. Controlling the regioselectivity of this reaction under various reaction conditions proved to be difficult and depended on the neighboring substituents and nature of the leaving groups.



Scheme 3: Synthesis of compound **5**.

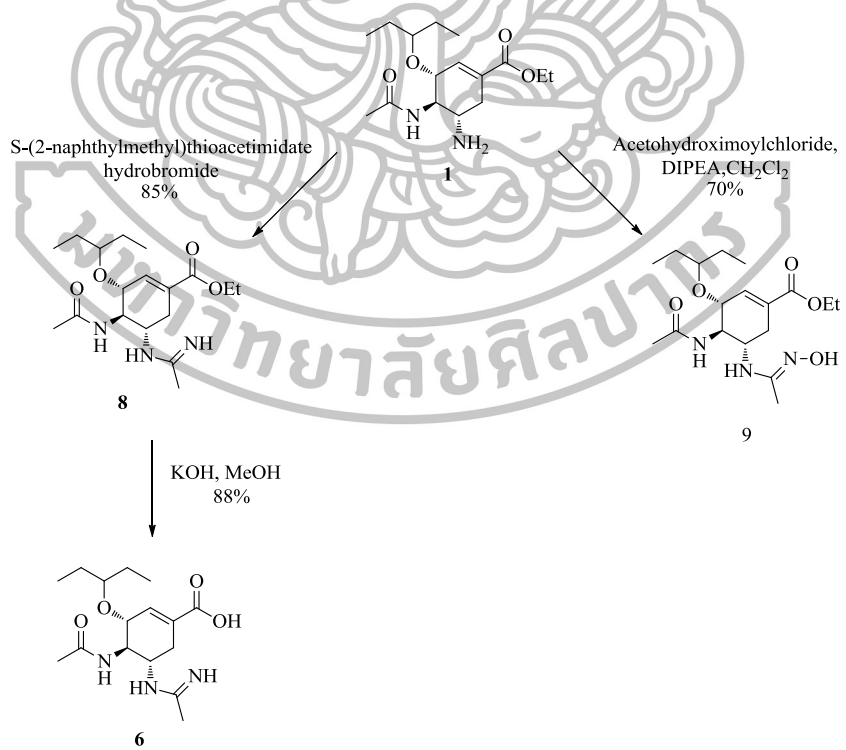
In **Scheme 3**, the copper-catalyzed azide−alkyne cycloaddition reaction with azide **12** and 1-pentyn-3-ol using the standard protocol provided the triazole **13** in 71% yield. Hydrolysis of the methyl ester **13** was performed using 1 M KOH in THF. The desired carboxylic acid **14** was precipitated by the addition of EtOAc to the column-purified acid. The NHBoc deprotection of acid **14** with TFA furnished the target compound **5** in 88% yield.



Scheme 4: Synthesis of compounds **6** and **14**.

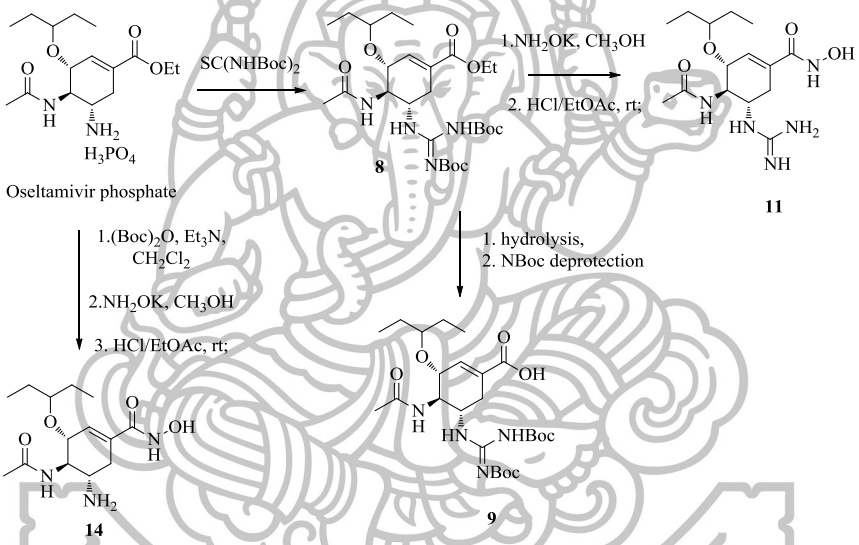
In **Scheme 4**, Boc deprotection of carbamate **13** under acidic conditions followed by treatment with basic Amberlyst A26 resin gave the free amine, which was then converted into the Boc-protected guanidine derivative **16** using Boc-protected thiourea and $HgCl_2$, and the Boc groups were removed to give **17** with TFA , as shown in **Scheme 4**. The target guanidinium salt **6** was obtained finally in modest yield from **5** by introduction of the Boc-protected guanidine group using Boc-protected 1H-pyrazole-1-carboxamidine and subsequent Boc deprotection with TFA.

In 2013, publication of Dennis Schade [15] and co-workers reported oseltamivir derivatives. Starting from **1**, the acetamidine group was installed using *S*-(2-naphtylmethyl thioacetimidate and afforded the amidine ester prodrug **8** in 85% yield. Alkaline hydrolysis of **8** furnished the amidine carboxylate drug (**6**, 88%). For the synthesis of amidoxime-type of prodrugs, they used freshly prepared acetohydroximoyl chloride as a reagent, and amidoxime **9** was readily accessible in good yields (70%) (**Scheme 5**).



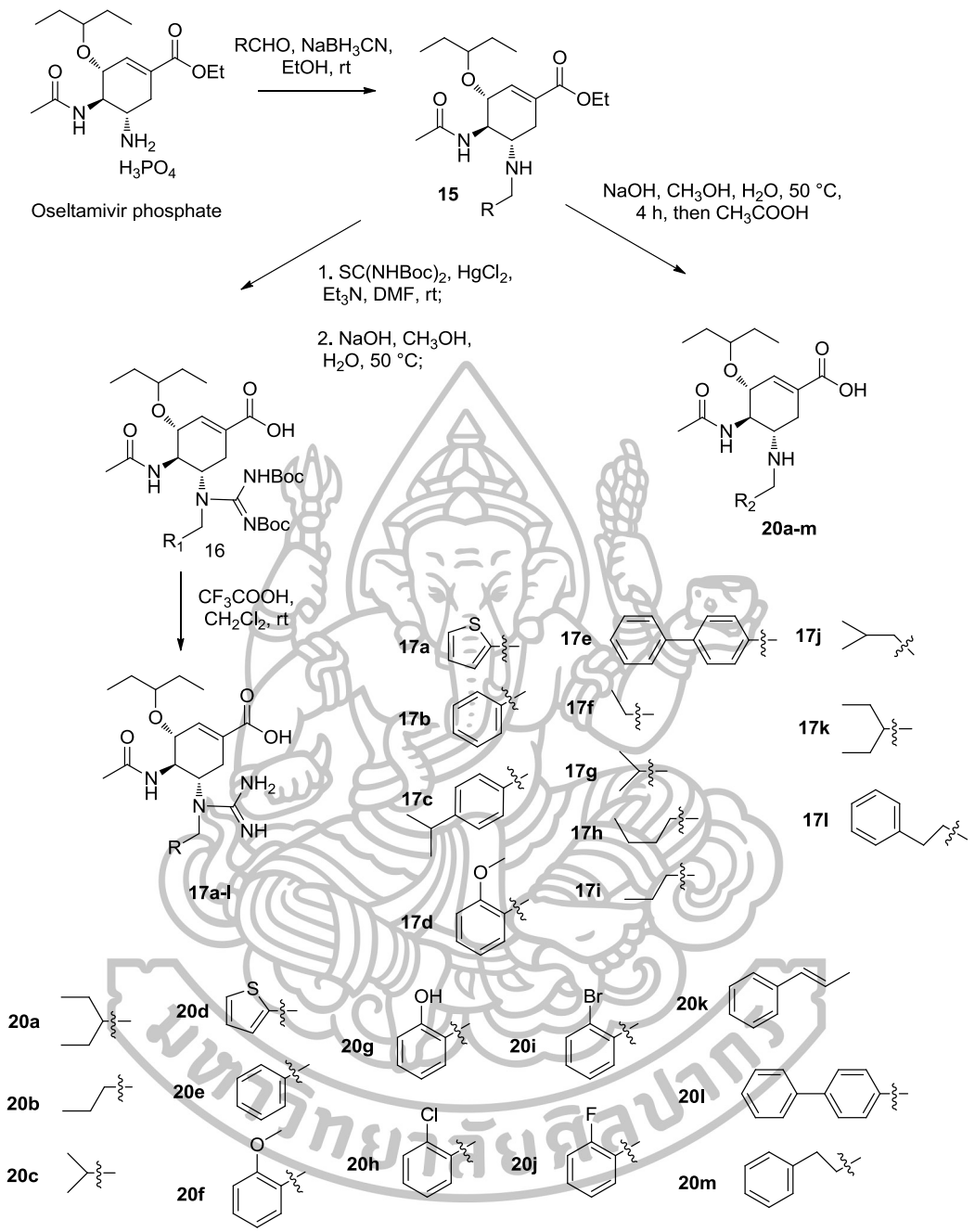
Scheme 5: Synthesis of compounds **6** and **9**.

In 2014, publication of YuanchaoXie [16] and co-workers reported *N*-Substituted oseltamivir derivatives. Commercial oseltamivir phosphate was the primary starting material. In **Scheme 6**, compound **9** was synthesized using a three-step route that included guanylation, hydrolysis, and NBoc deprotection and was similar to the reported method. The guanylation reaction was performed with N,N′-bis(tertbutoxycarbonyl) thiourea ($SC(NHBoc)_2$)/$HgCl_2$. Treatment of compound **8** with a solution of NH_2OK in CH_3OH gave intermediate **10**. Compound **13** was synthesized by the same method from Boc-protected oseltamivir **12**. The target compounds **11** and **14** were all prepared as hydrochlorides with 3 M HCl/EtOAc.

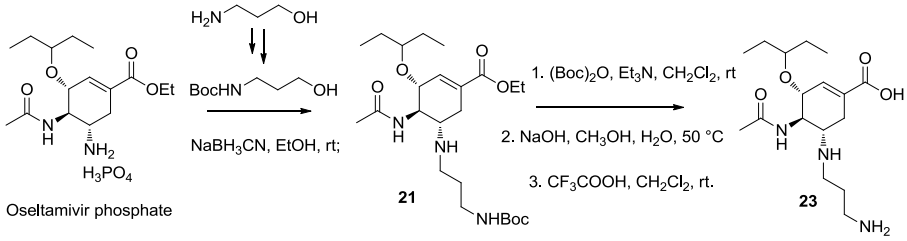


Scheme 6: Synthesis of compounds **9**, **11** and **14**.

In **Scheme 7** and **8**, oseltamivir phosphate was reacted with a range of different aldehydes in the presence of $NaBH_3CN$ to afford the key intermediate **15**. The synthesis of compound **17** was achieved in three steps induding guanylation, hydrolysis, and Boc deprotection. Compound **20** was also synthesized direct hydrolysis of intermediate **15** with NaOH. Because the R_2 groups of compounds **20 a-m** were all hydrophobic, they further designed compound **23**, which contained one NH_2 at the end of the substituent. The structure and its synthetic route are shown in **Scheme 8**.

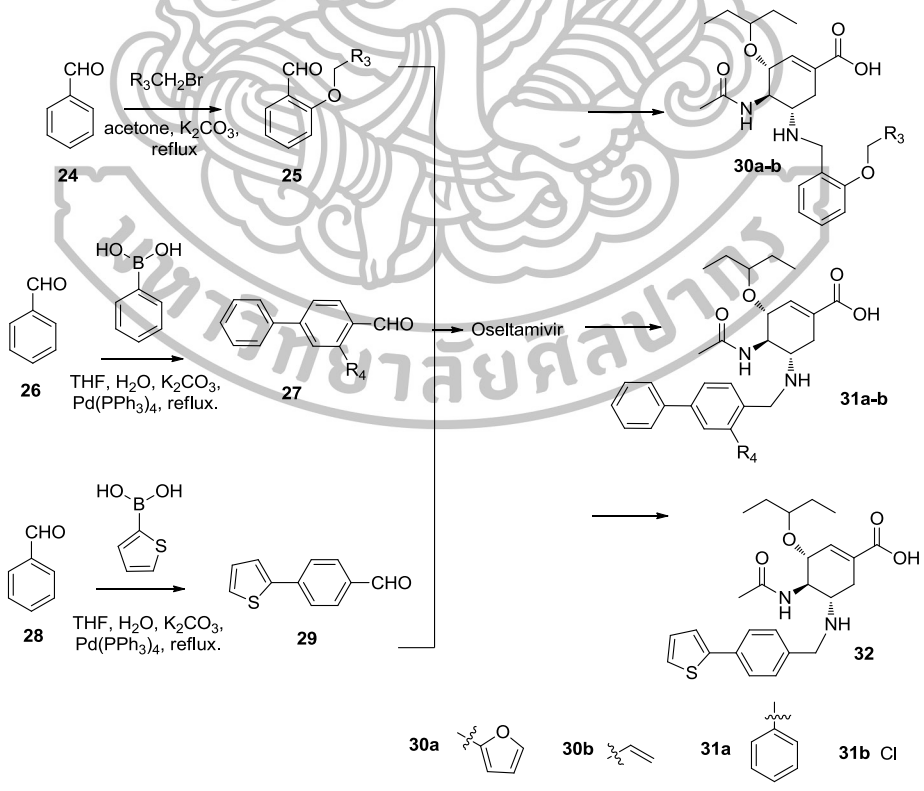


Scheme 7: Synthesis of compounds **17a-l** and **20a-m**.



Scheme 8: Synthesis of compounds **23**.

In **Scheme 9**, They continued to synthesize another five derivatives, compounds **30**−**32**. The two 2-alkoxybenzaldehydes were prepared by reaction of 2-hydroxylbenzaldehyde with 2- (bromomethyl) furan and 3-bromoprop-1-ene and were used for the synthesis of compounds **30a** and **30b**. A Suzuki reaction of the aryl bromides with boronic acid gave another three aldehydes that were used in synthesizing compounds **31** and **32**.



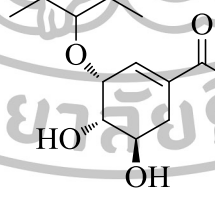
Scheme 9: Synthesis of compounds **30a-b**, **31a-b** and **32**.

CHAPTER 2
EXPERIMENTAL

General methods

Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ on a 300 MHz Bruker spectrometer. Chemical shifts are δ (ppm) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Bruker Daltonics-micrOTOF - benchtop ESI-TOF MS. Infared spectra were recorded as NaCl cell with Perkin-Elmer GX FT-IR spectrophotometer. Reagents were purchased from Sigma-Aldrich and Fluka. Ultraviolet (UV) active compounds were visualized with UV a light at 254 nm and vanillin stain. Column chromatography was performed using silica gel 60, 230-400 mesh. Mushroom tyrosinase (EC.1.14.18.1) and 3-(3,4-dihydroxyphenyl)-L-alanine (LDOPA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Kojic acid was purchased from Tokyo Chemical Industry Co., LTD. (Tokyo, Japan). All chemical and solvents used were purchased from E. Merk, Fluka, and Sigma & Alfrich Co., unless stated otherwise.

Synthesis of Oseltamivir Derivatives (**2**), (**3b**), (**3c**), (**3d**), (**4b**), (**4c**) and (**4d**)



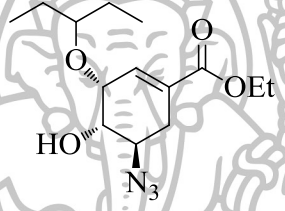
2

(3*R*,4*S*,5*R*)-ethyl 4,5-dihydroxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**2**)

Sulfuric acid (0.5 mL) was added dropwise over 5 min to a solution of epoxide (**1**) (1.0198 g, 0.21 mmol) in ethanol (5 mL). The mixture was stirred at room temperature for 24 h. The solution extracted with dichloromethane (3x10 mL). The combined dichloromethane extracts were dried anhydrous sodium sulfate, and the solvent was evaporated to give crude oil which was purified by Column chromatography (2:1

hexane/EtOAc) to afford diol (**2**) as a colorless oil (0.5802 g, 53%) R_f 0.34 (2:1 hexane/EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$) 6.86 (m, 1H); 4.20 (m, 3H); 3.96 (ddd, *J*1=*J*2=8.3 Hz, *J*3 = 5.4 Hz, 1H); 3.62 (dd, *J*1= 8.8 Hz, *J*2 = 4.6 Hz, 1H); 3.43 (quin, *J* = 5.7 Hz, 1H); 2.90 (dd, *J*1= 18.0 Hz, *J*2 = 5.3 Hz, 1H); 2.61 (s, 2H); 2.20 (dd, *J*1 = 18.1 Hz, *J*2 = 7.8 Hz, 1H); 1.42-1.69 (m, 4H); 1.30 (t, *J* = 7.1 Hz, 3H); 0.83-0.98 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 134.7, 130.9, 81.8, 72.2, 71.3, 67.7, 30.9, 31.2, 26.6, 26.0, 14.2, 9.6, 9.5 ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1064, 1244, 1383, 1463,1651, 1715, 2968, 3444 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{14}H_{24}O_5Na$= 295.1521 found 295.1525.



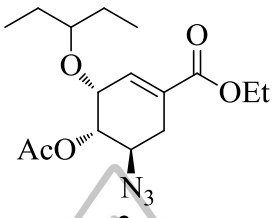
3

(3R,4S,5R)-ethyl 5-azido-4-hydroxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**3**)

Epoxide (**1**) (1.0046 g, 3.95 mmol) was dissolved in EtOH (10 mL). To this solution was added sodium azide (0.3848 g, 5.92 mmol) and ammonium chloride (0.3137 g, 5.92 mmol). The reaction mixture was heated to reflux and stirred for 24 h. The reaction mixture was then filtrated and was evaporated to gave brown oil. Water was added to aqueous residue and extracted with ethyl acetate (3 x 10 ml). The combine extracted was dried over anhydrous sodium sulfate. Filtration and evaporation afforded alcohol azido (**3**) and azido alcohol (**4**) as a brown oil. An analytical sample of azido alcohol (**3**) was prepared by column chromatography (5:1 hexane/EtOAc) as a pale yellow oil (0.8890g, 75%) R_f 0.64 (4:1 hexane/EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (m, 1H); 4.22 (q, *J* = 7.1 Hz, 2H)); 3.86 (ddd, *J*1=*J*2 = 7.9 Hz, *J*3 = 5.4 Hz, 1H); 3.77 (dd, *J*1 = 8.2 Hz, *J*2 = 4.2 Hz, 1H); 3.44 (quin, *J* = 5.8 Hz, 1H); 2.84 (dd, *J*1 = 18.3 Hz, *J2* = 5.1 Hz, 1H); 2.26 (dd, *J*1 = 18.3 Hz, *J*2 = 6.9 Hz, 1H); 1.44-1.67 (m, 4H); 1.30 (t, *J* = 7.1 Hz, 3H); 0.93 (t, *J* = 7.3 Hz, 3H); 0.91 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 135.1, 130.0, 81.9, 71.1, 70.2, 61.0, 58.9, 28.2, 26.5, 26.1, 14.2, 9.6, 9.5 ppm; FTIR (NaCl - film), υ (cm^{-1}): 1009, 1053, 1099,

1182, 1247, 1367, 1463, 1655, 1717, 2108, 2504, 2878, 2935, 2968, 3530 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{14}H_{23}N_3O_4Na$= 320.1586 found 320.1579.

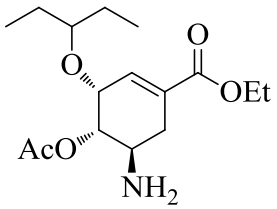


3a

(3*R*,4*S*,5*R*)-ethyl 4-acetoxy-5-azido-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**3a**)

To solution of azido alcohol (**3**) (0.3746 g, 1.26 mmol) in dichloromethane (5 mL) was added acetic anhydride (0.17 mL, 1.89 mmol) and DMAP (0.0146 g, 0.12 mmol). Triethylamine (0.26 mL, 1.89 mmol) was then dropwise added over 5 min. The reaction mixture was further stirred at room temperature for 1h. The mixture was then partitioned between dichloromethane (5 mL) and water (10 mL). The organic phase was separated and dried over anhydrous sodium sulfate. The organic solution was filtrated and concentrated under vacuum to produce a crude oil which was purified by chromatography (5:1 hexane/EtOAc,) to give acetoxy (**3a**) as a pale yellow oil (0.3866 g, 90%) R_f 0.86 (4:1 hexane/EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (m, 1H); 4.91 (dd, *J*1 = 9.7 Hz, *J*2 = 3.9 Hz, 1H); 4.28 (t, *J* = 4.2 Hz, 2H); 4.22 (q, *J* = 7.1 Hz, 2H); 4.12 (ddd, *J*1 = 14.6 Hz, *J*2 = 8.7 Hz, *J*3 = 6.0 Hz, 1H); 3.44 (quin, *J* = 5.7 Hz, 1H); 2.89 (dd, *J* 1= 18.4 Hz, *J*2 = 5.8 Hz, 1H); 2.25 (ddt, *J*1 = 18.3 Hz, *J*2 = 8.4 Hz, *J*3 = 1.0 Hz, 1H); 2.15 (s, 3H); 1.45-1.58 (m, 4H); 1.30 (t, *J* = 7.1 Hz, 3H); 0.94 (t, *J* = 7.4 Hz, 3H); 0.88 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 165.2, 135.2, 129.6, 82.9, 73.2, 69.4, 61.0, 55.6, 29.5, 26.3, 26.2, 20.9, 14.1, 9.8, 9.1 ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1014, 1076, 1103, 1170, 1239, 1368, 1463, 1655, 1717, 1743, 2108, 2878, 2936, 2969, 3055 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{16}H_{25}N_3O_5Na$ = 362.1692 found 362.1691.

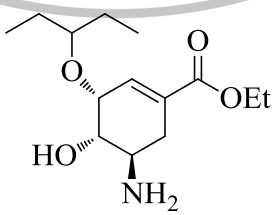


3b

(3*R*,4*S*,5*R*)-ethyl 4-acetoxy-5-amino-*3-(pentan*-3-yloxy)cyclohex-1-enecarboxylate (**3b**)

Acetoxy (**3a**) (0.3305 g, 0.97 mmol) was dissolved in EtOH:H_2O (4:1 mL). Ammonium chloride (0.1028 g, 1.94 mmol) and Zinc dust (0.1274 g, 1.94 mmol) were added in to a solution. The mixture was stirred at room temperature for 6 h. The reaction was filtrated and concentrated under vacuum to remove ethanol. The residue was extracted with ethyl acetate (3x8 mL). The combined organic layers were dried with anhydrous sodium sulfate, concentrated and the residue was purified by column chromatography (EtOAc) to give amine (**3b**) as a pale yellow oil (0.1991 g, 65%) mp 97-99°C R_f 0.27 (2:1 EtOAc/hexane).

^{1}H NMR (300 MHz, $CDCl_3$) 6.83 (m, 1H); 5.83 (d, *J* = 6.7 Hz, 1H); 4.21 (m, 3H); 4.01 (t, *J* = 4.2 Hz, 1H); 3.65 (dd, *J* 1= 9.9 Hz, *J*2 = 4.2 Hz, 1H); 3.46 (quin, *J* = 5.7 Hz, 1H); 3.05 (dd, *J* 1= 18.0 Hz, *J*2 = 5.3 Hz, 1H); 2.29 (br, 1H); 2.15 (m, 1H); 2.05-2.25 (m, 1H); 2.03 (s, 3H); 1.42-1.63 (m, 4H); 1.30 (t, *J* = 7.4 Hz, 3H); 0.95 (t, *J* = 7.4 Hz, 3H); 0.84 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 166.1, 135.2, 130.9, 82.0, 71.3, 70.9, 60.8, 47.2, 30.0, 26.4, 26.0, 23.0, 14.0, 9.6, 9.3, ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1100, 1244, 1372, 1463, 1549, 1625, 1714, 2849, 2923, 2961, 3294 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{16}H_{27}NO_5Na$ = 336.1787 found 336.1781.



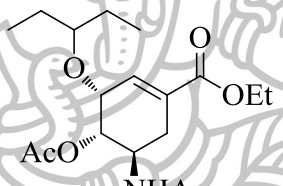
3c

(3*R*,4*S*,5*R*)-ethyl 5-amino-4-hydroxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**3c**)

To a solution of azido alcohol (**3**) (0.3403 g, 1.15 mmol) in tetrahydrofuran (5 mL) was added triphenylphosphine (0.3602 g, 1.37 mmol). The mixture was stirred for 3 h. Tetrahydrofuran was removed by vacuum distillation. Water (10 mL) was added and

extracted with ethyl acetate (3x7 mL). The organic layer was combined, dried anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by chromatography (10:1 EtOAc/MeOH) to afford amino (**3c**) as a pale yellow oil (0.2997 g, 96%) R_f 0.31 (4:1 EtOAc/MeOH).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (m, 1H); 4.21 (q, *J* = 7.1 Hz, 2H); 4.13 (t, *J* = 4.4 Hz, 1H); 3.51 (dd, *J*1 = 9.4 Hz, *J*2 = 4.4 Hz, 1H); 3.44 (quin, *J* = 5.7 Hz, 1H); 3.19 (ddd, *J*1=*J*2 = 9.1 Hz, *J*3 = 5.5 Hz, 1H), 2.86 (dd, *J*1 = 18.1 Hz, *J2* = 5.3 Hz, 1H); 2.50 (br, 1H); 2.08 (dd, *J*1 = 17.9 Hz, *J*2 = 8.9 Hz, 1H); 1.43-1.67 (m, 4H); 1.29 (t, *J* = 7.3 Hz, 3H); 0.93 (t, *J* = 7.4 Hz, 3H); 0.88 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.5, 134.9, 131.6, 81.8, 73.3, 71.2, 60.8, 48.4, 32.4, 26.6, 26.1, 14.2, 9.7, 9.5, ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1056, 1083, 1183, 1254, 1305, 1383, 1438, 1660, 1714, 2876, 2926, 2966, 3267 cm^{-1}; LC-HRMS m/z calculated for $[M+H]^+$ $C_{14}H_{26}NO_4$ = 272.1862 found 272.1861.



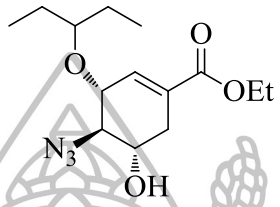
3d

(3*R*,4*S*,5*R*)-ethyl 5-acetamido-4-acetoxy-3-(pentan-3-yloxy)cyclohex-1 enecarboxylate (**3d**)

Triethylamine (0.46 mL, 3.33 mmol) was added dropwise, over 5 min, to a solution of amino (**3c**) (0.2997 g, 1.11 mmol) in dichloromethane (5 mL), acetic anhydride (0.21 ml, 2.22 mmol), and DMAP (0.0135 g, 0.11 mmol), at room temperature for 1h. Water (10 mL) was added and the aqueous solution extracted with dichloromethane (3x5 mL). The combined dichloromethane extracts were dried anhydrous sodium sulfate, and the solvent was evaporated to give crude oil which was purified by chromatography (3:1 EtOAc/hexane) to afford acetamido (**3d**) as a white crystal solid (0.3168 g, 80%) R_f 0.45 (3:1 EtOAc/hexane) mp 77-79°C.

^{1}H NMR (300 MHz, $CDCl_3$) 6.84 (m, 1H); 6.04 (d, *J* = 8.3 Hz, 1H); 4.97 (dd, *J* 1= 10.5 Hz, *J*2 = 3.8 Hz, 1H); 4.57 (ddd, *J*1 = 18.5 Hz, *J*2 = 8.8 Hz, *J*3 = 5.8 Hz, 1H); 4.2 (q, *J* = 7.0 Hz, 1H), 3.34 (quin, *J* = 5.7 Hz, 1H); 2.98 (dd, *J* 1= 18.1 Hz, *J*2 = 5.8 Hz, 1H); 2.14 (ddd, *J*1 = 18.3 Hz, *J*2 = 8.9 Hz, *J*3 = 1.4 Hz, 1H); 2.11 (s, 3H); 1.95 (s, 3H); 1.45-

1.59 (m, 4H); 1.29 (t, *J* = 7.1 Hz, 3H); 0.94 (t, *J* = 7.3 Hz, 3H); 0.88 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.0, 165.9, 135.0, 130.6, 82.8, 72.3, 69.8, 60.8, 44.6, 30.8, 26.3, 26.2, 23.1, 20.9, 14.0, 9.7, 9.1 ppm; FTIR (NaCl - film), υ (cm^{-1}) :1063, 1242, 1370,1463, 1553, 1652, 1716, 2243, 2920, 3074, 3281 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{18}H_{29}NO_6Na$ = 378.1893 found 378.1894.

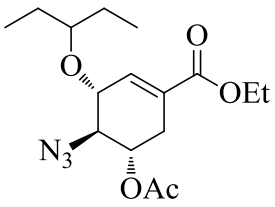


4

(3*R*,4*R*,5*S*)-ethyl 4-azido-5-hydroxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**4**)

Epoxide (**1**) (1.0046 g, 3.95 mmol) was dissolved in ethanol (10 mL). To solution was added sodium azide (0.3848 g, 5.92 mmol) and ammonium chloride (0.3137 g, 5.92 mmol). The reaction mixture was heated to reflux and stirred for 24 h. The reaction mixture was filtrated. Ethanol was removed by vacuum distillation to gave brown oil. Water was added to aqueous residue and extracted with ethyl acetate (3 x 10 ml). The organic phase was combine to extracted and was dried over anhydrous sodium sulfate, and then concentrated under vacuum to afford alcohol azido (**3**) and azido alcohol (**4**) as a brown oil. An analytical sample of azido alcohol (**4**) was prepared by column chromatography (5:1 hexane/EtOAc) as a pale yellow oil (0.0811g, 7%) R_f 0.42 (4:1 hexane/EtOAc) mp 67-69°C.

^{1}H NMR (300 MHz, $CDCl_3$) δ); 6.75 (t, *J* = 2.39 Hz, 1H); 4.21 (q, *J* = 7.0 Hz, 2H); 3.98-4.06 (m, 1H); 3.69 (ddd, *J*1=*J*2 = 10.2 Hz, *J*3 = 5.9 Hz, 1H); 3.39-.351 (m, 2H); 2.86 (dd, *J*1 = 17.7 Hz, *J2* = 5.8 Hz, 1H); 2.30 (ddt, *J*1 = 17.7 Hz, *J*2 = 9.5 Hz, *J*3 = 3.4 Hz, 1H); 1.43-1.73 (m, 4H); 1.29 (t, *J* = 7.1 Hz, 3H); 0.89-1.00 (m, 6H); ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 136.2, 128.6, 81.9, 76.2, 69.0, 68.2, 61.0, 31.9, 26.2, 25.4, 14.1, 9.4, 9.3 ppm; FTIR (NaCl - film), υ (cm^{-1}) :1016, 1096, 1248, 1370, 1463, 1654, 1716, 2108, 2878, 2919, 2967, 3445 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{14}H_{23}N_3O_4Na$ = 320.1586 found 320.1589.

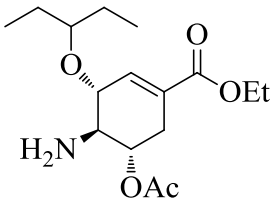


4a

(3*R*,4*R*,5*S*)-ethyl 5-acetoxy-4-azido-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**4a**)

To a solution of azido alcohol (**4**) (0.0699 g, 0.23 mmol) in dichloromethane (5 mL) was added acetic anhydride (0.03 mL, 0.35 mmol) and DMAP (0.00028 g, 0.02 mmol). Triethylamine (0.05 mL, 0.35 mmol) was dropwise added over 5 min. The reaction mixture was stirred at room temperature for 1h. The mixture was diluted with dichloromethane (5 mL) and the aqueous layer was extracted with dichloromethane (3 x 5 mL). The combined organics were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to produce a crude oil which was purified by chromatography (4:1 hexane/EtOAc) to give acetoxy (**4a**) as a pale yellow oil (0.0696 g, 91%). R_f 0.87 (4:1 hexane/EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.73 (t, *J*1 = 2.1 Hz, 1H); 4.95 (ddd, *J*1 = 10.9 Hz, *J*2 = 9.8 Hz, *J*3 = 6.0 Hz 1H); 4.21 (q, *J* = 7.1 Hz, 2H); 4.22 (q, *J* = 7.1 Hz, 2H); 4.12 (ddd, *J*1 = 14.6 Hz, *J*2 = 8.7 Hz, *J*3 = 6.0 Hz, 1H); 3.94-4.10 (m, 1H); 3.62 (dd, *J* 1= 10.9Hz, *J*2 = 8.3 Hz, 1H); 3.46 (quin, *J* = 5.7 Hz, 1H); 2.95 (dd, *J* 1= 17.3 Hz, *J*2 = 6.0 Hz, 1H); 2.30 (ddt, *J*1 = 17.4 Hz, *J*2 = 9.8 Hz, *J*3 = 3.0 Hz, 1H); 2.15 (s, 3H); 1.47-1.66 (m, 4H); 1.29 (t, *J* = 7.1 Hz, 3H); 0.94 (q, *J* = 7.3 Hz, 6H); ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 165.2, 136.8, 128.0, 82.6, 75.9, 70.2, 66.1, 61.2, 29.9, 26.3, 25.7, 20.9, 14.1, 9.4 ppm; FTIR (NaCl - film), υ (cm^{-1}) :1038, 1233, 1369, 1463, 1659, 1715, 1748, 2109, 2969 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{16}H_{25}N_3O_5Na$ = 362.1692 found 362.1695.

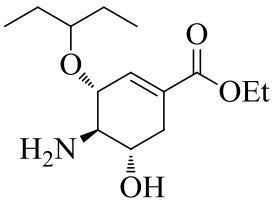


4b

(3*R*,4*R*,5*S*)-ethyl 5-acetoxy-4-amino-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**4b**)

Acetoxy (**4a**) (0.0348 g, 0.10 mmol) was dissolved in EtOH:H_2O (4:1 mL). Ammonium chloride (0.0106 g, 0.2 mmol) and Zinc dust (0.0130 g, 0.2 mmol) were added in to solution. The mixture was stirred at room temperature for 6 h. The reaction solution was filtrated and concentrated under vacuum to remove ethanol. The residue was diluted with ethyl acetate and water. The aqueous layer was extracted with ethyl acetate (3x8 mL). The combined organic layers were dried over anhydrous sodium sulfate. Filtration and evaporation afforded crude oil which was purified by column chromatography (EtOAc) to give amine (**4b)** as a pale yellow oil (0.0118 g, 35%) mp 97-99°C R_f 0.30 (EtOAc).

^{1}H NMR (300 MHz, $CDCl_3$) 6.79 (s 1H); 5.98 (d, *J* = 6.8 Hz, 1H); 4.22 (q, *J* = 7.1 Hz, 1H); 4.13 (m, 1H); 4.00 (dd, *J* 1= 9.1 Hz, *J*2 = 5.5 Hz, 1H); 3.82 (q, *J* = 7.4 Hz, 1H); 3.39 (quin, *J* = 4.5 Hz, 1H); 3.00 (br, 2H); 2.80 (dd, *J* 1= 18.1 Hz, *J*2 = 5.4 Hz, 1H); 2.39 (ddt, *J*1 = 17.9 Hz, *J*2 = 8.1 Hz, *J*3 = 2.6 Hz, 1H); 2.06 (s, 3H); 1.41-1.62 (m, 4H); 1.30 (t, *J* = 7.0 Hz, 3H); 0.95 (t, *J* = 7.3 Hz, 6H); ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 166.3, 136.7, 129.2, 81.9, 74.9, 67.9, 60.9, 57.3, 32.9, 26.2, 25.7, 23.5, 14.1, 9.5, 9.2 ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1012, 1060, 1130, 1249, 1372, 1463, 1554, 1647, 1714, 2849, 2879, 2918, 3296 cm^{-1}; LC-HRMS m/z calculated for $[M+Na]^+$ $C_{16}H_{27}NO_5Na$ = 336.1787 found 336.1782.

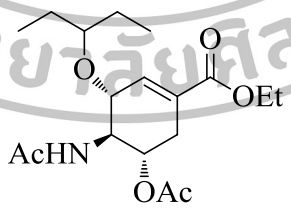


4c

(3*R*,4*R*,5*S*)-ethyl 4-amino-5-hydroxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**4c**)

To a solution of alcohol azido (**3**) (0.0762 g, 0.25 mmol) in tetrahydrofuran (5 mL). Triphenylphosphine (0.0806 g, 0.30 mmol) was added to solution. The mixture was stirred at rt for 3 h. Tetrahydrofuran was removed by vacuum distillation. Water (10 mL) was added and extracted with ethyl acetate (3x7 mL). The organic layer was combined, dried anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by chromatography (8:1 EtOAc/MeOH) to afford amino (**4c**) as a pale yellow oil (0.0568 g, 81%) R_f 0.46 (4:1 EtOAc/MeOH): mp 99-101°C.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1H); 4.21 (q, *J* = 7.1 Hz, 2H); 3.90-3.99 (m, 1H); 3.75 (ddd, *J*1= *J*2 10.0 Hz, *J*3= 5.8 Hz, 1H); 3.59 (br, 3H); 3.39 (quin, *J* = 6.4 Hz, 1H); 2.78-2.94 (m, 2H); 2.27 (ddt, *J*1= 17.1 Hz, *J*2 = 9.7 Hz, *J*3 = 3.0 Hz, 1H), 1.42-1.70 (m, 4H); 1.29 (t, *J* = 7.1 Hz, 3H); 0.88-0.99 (m, 6H); ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4, 137.0, 129.1, 81.0, 78.2, 69.0, 60.8, 58.1, 32.8, 26.4, 25.7, 14.2, 9.7, 9.4 ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1059, 1245, 1368, 1463, 1569, 1651, 1716, 2887, 2938, 2967, 3364 cm^{-1}; LC-HRMS m/z calculated for $[M+H]^+$ $C_{14}H_{27}NO_4$ = 272.1862 found 272.1865.



4d

(3*R*,4*R*,5*S*)-ethyl 4-acetamido-5-acetoxy-3-(pentan-3-yloxy)cyclohex-1-enecarboxylate (**4d**)

Triethylamine (0.08 mL, 0.63 mmol) was added dropwise, over 5 min, to a solution of amino (**4c**) (0.0568 g, 0.21 mmol) in dichloromethane (5 mL), acetic anhydride (0.04 ml, 0.42 mmol), and DMAP (0.0025 g, 0.02 mmol). The mixture was stirred at room

temperature for 1h. The mixture was diluted with water. The aqueous solution extracted with dichloromethane (3x5 mL). The combined dichloromethane extracts were dried anhydrous sodium sulfate, and the solvent was evaporated to give crude oil which was purified by chromatography (3:1 EtOAc/hexane) to afford acetamido (**4d**) as a white crystal solid (0.3168 g, 80%) R_f 0.47 (3:1 EtOAc/hexane) mp 95-97°C.

^{1}H NMR (300 MHz, $CDCl_3$) 6.82 (s, 1H); 5.72 (d, *J* = 8.6 Hz, 1H); 5.09 (ddd, *J*1=*J*2=9.7 Hz, *J*3 = 5.8 Hz, 1H); 4.05-4.29 (m, 4H); 3.34 (quin, *J* = 5.6 Hz, 1H); 2.82 (dd, *J*1= 17.4 Hz, *J*2 = 5.7 Hz, 1H); 2.14 (ddt, *J*1 = 17.2 Hz, *J*2 = 9.6 Hz, *J*3 = 2.8 Hz, 1H); 2.06 (s, 3H); 1.97 (s, 3H); 1.51 (sex, , *J* = 7.5 Hz, 4H); 1.29 (t, *J* = 7.1 Hz, 3H); 0.82-0.96 (m, 6H); ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.1, 165.7, 137.8, 128.0, 82.2, 75.6, 69.1, 61.0, 54.4, 30.0, 26.1, 25.6, 23.3, 20.9, 14.1, 9.4, 9.1 ppm; FTIR (NaCl - film), υ (cm^{-1}) : 1064, 1244, 1375,1468, 1551, 1656, 1712, 2247, 2921, 3078, 3286 cm^{-1}; LC-HRMS m/z calculated for $[M+H]^+$ $C_{18}H_{30}NO_6$ = 356.2073 found 356.2071.

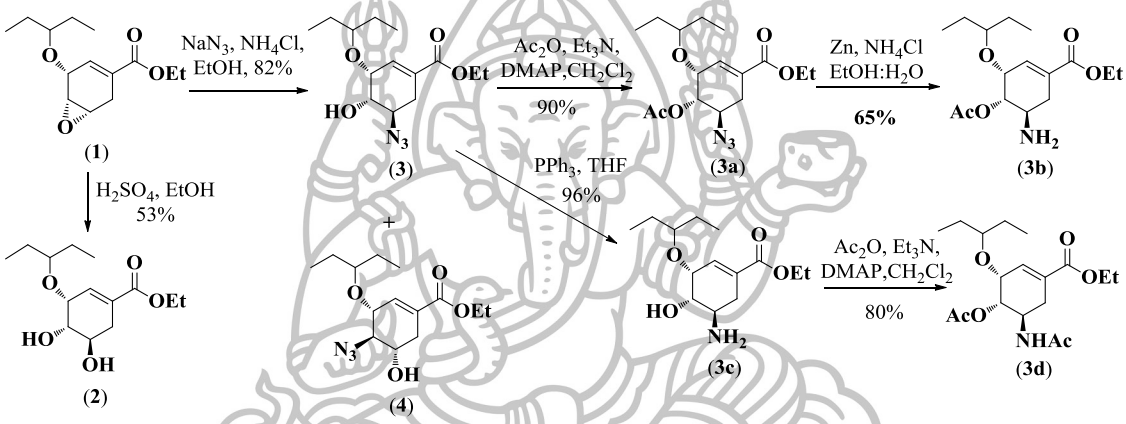
CHAPTER 3

RESULT AND DISCUSSION

Synthesis of Oseltamivir derivative

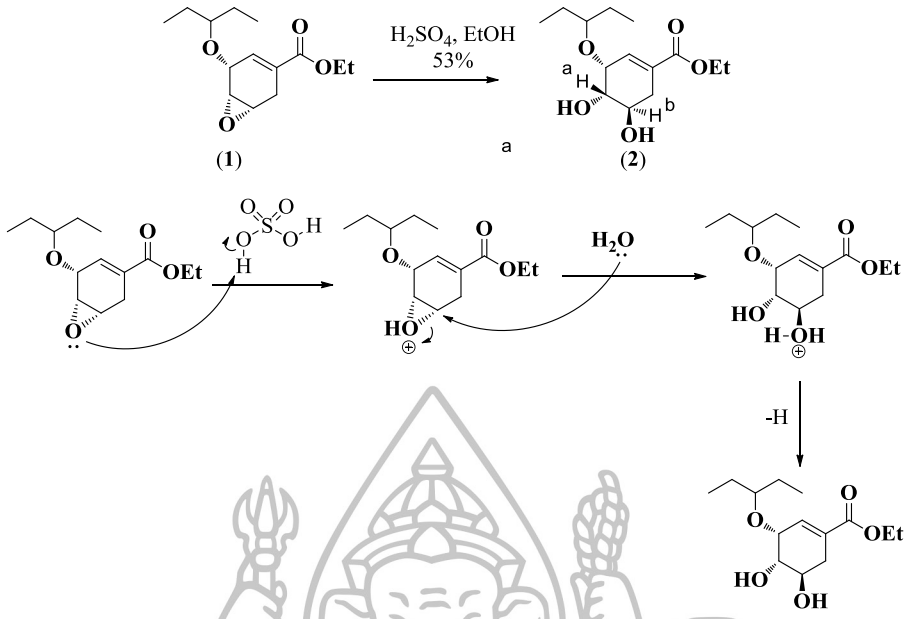
In this chapter we describe synthetic studies of Oseltamivir derivative. We designed key reactions of ring opening for synthesis Oseltamivir derivative. Ring opening give 2 line synthesis with H_2SO_4 and NaN_3.

Synthesis of Oseltamivir derivatives (2), (3b), (3c) and (3d) (Scheme 10).



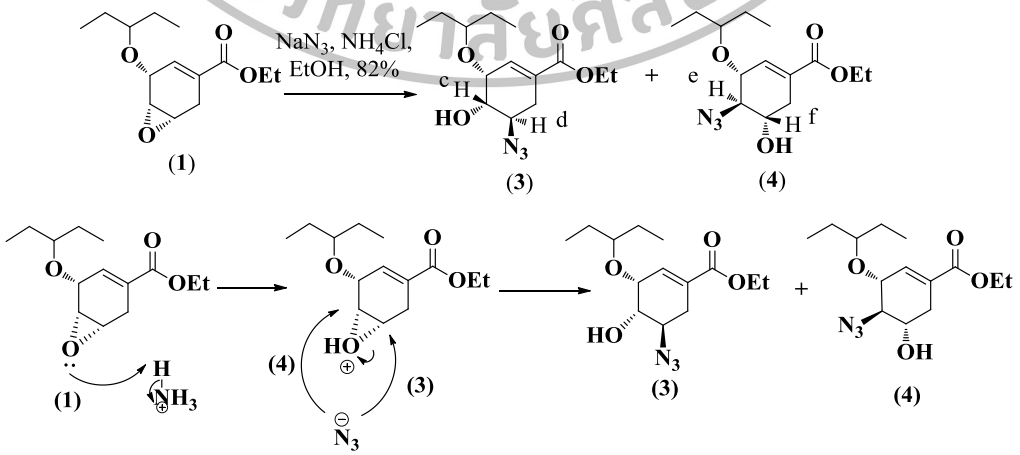
Scheme 10: Synthesis of Oseltamivir derivatives (**2**), (**3b**), (**3c**) and (**3d**).

First, Oseltamivir derivative (**2**) was synthesized by ring opening of epoxide (**1**) with H_2SO_4 in ethanol to moderate yield 53%. The mechanism is shown in **scheme 11**. FTIR spectrum of Oseltamivir derivative (**2**) showed a broad of hydroxyl group (-OH) at 3444.64 cm^{-1}(page 39 in Appendix) and ^1H NMR showed in downfield of H^a from δ 3.50 to 3.62 ppm and H^b from δ 3.50 to 3.96 ppm). (page 37 in Appendix).



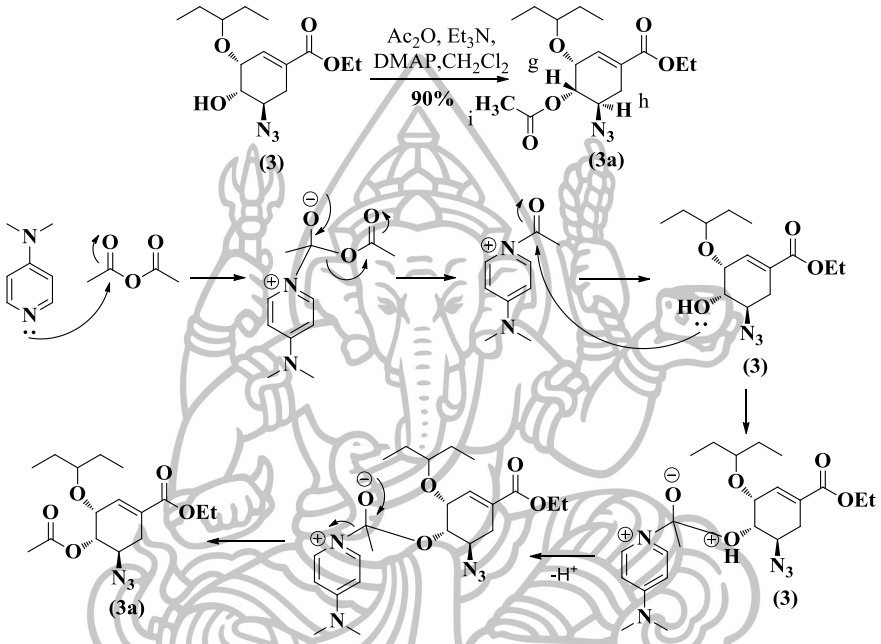
Scheme 11: Reaction of ring opening of epoxide (**1**) with H_2SO_4 and mechanism.

In this thesis, the azido group was cleated to the know intermediate (**3**) and (**4**). sodium azide and ammonium chloride in ethanol were used in this reaction to give 10:1 mixture of isomeric azido hydroxyl (**3:4**) in 82% yield, compound (**3**) is major product in **Scheme 12**. Analysis of ^{1}H NMR showed that the ring opening of epoxide (**1**) showed in downfield shift of H^c from δ 3.50 to 3.77, H^d from δ 3.50 to 3.86 and H^f from δ 3.50 to 3.69 ppm (page 41 and 61 in Appendix). FTIR spectrum of compounds (**3**) and (**4**) showed a of azido group (-N≡N) at 2108 cm^{-1} and hydroxyl group (-OH) at 3530 cm^{-1} (page 43 and 63 in Appendix).



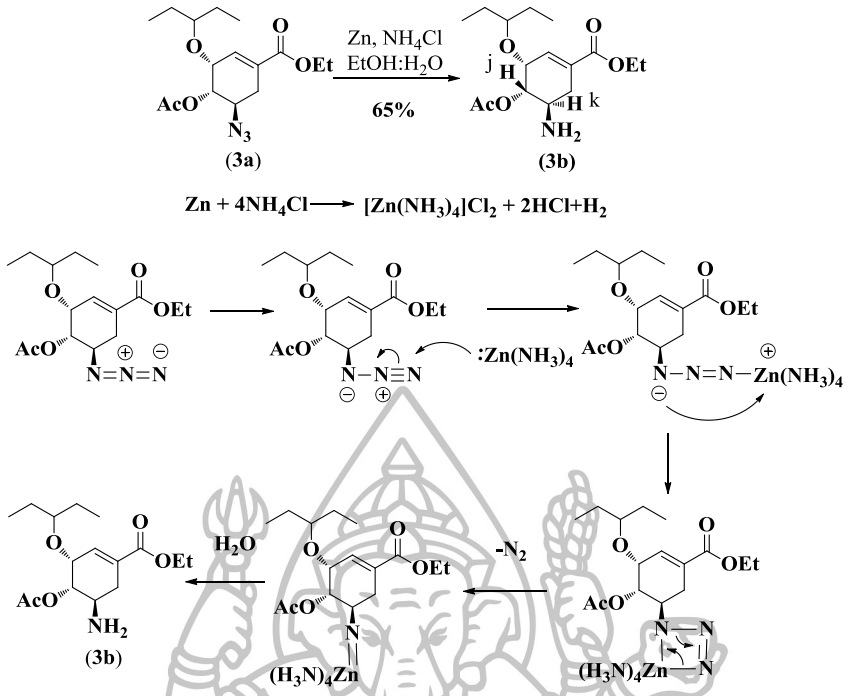
Scheme 12: Reaction of ring opening of epoxide (**1**) with NaN_3 and mechanism.

Acetylation of hydroxyl group of compound (**3**) with acetic anhydride generated compound (**3a**) in 90% yield as show the mechanism in **Scheme 13**. Analysis of ^{1}H NMR of compound (**3a**) showed in downfield shift of H^{g} from δ 3.77 to 4.91, H^{h} from 3.86 to 4.12 and new (CH_3^{i}) Singlet at 2.15 ppm (page 45 in Appendix). ^{13}C NMR spectrum had a new carbonyl group at δ 170.4 ppm (page 46 in Appendix). FTIR spectrum of compound (**3a**) showed a lost of hydroxyl group (-OH) (page 47 in Appendix).



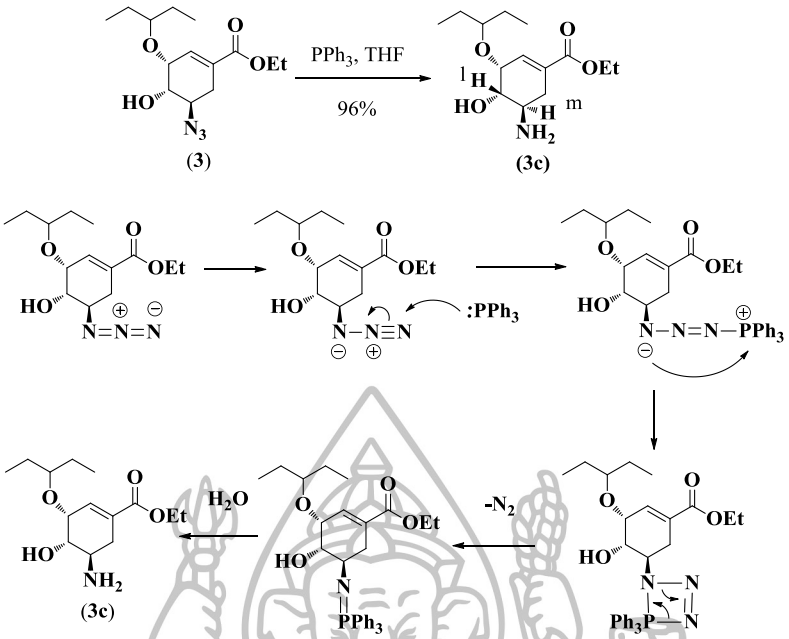
Scheme 13: Acetylation of Oseltamivir derivative (**3**) and mechanism.

New oseltamivir derivative (**3b**) was synthesized by reduction azido group of compound (**3a**) with zinc dust to give 65% yield. Analysis of ^{1}H NMR showed that the reduction of Oseltamivir derivative (**3a**) showed in upfield shift of H^{j} from δ 4.91 to 3.65 and downfield shift of H^{k} from 4.12 to 4.21 ppm (page 49 in Appendix). FTIR spectrum of Oseltamivir derivative (**3b**) showed a lost of azido group (-N≡N) and new peak amino group (-NH_2) at 3294 cm^{-1} (page 51 in Appendix).



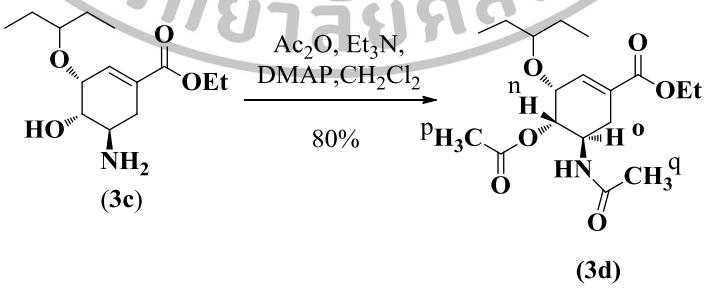
Scheme 14: Reduction of compound (**3a**) with zinc dust and mechanism.

Reduction of azido group of compound (**3**) with triphenylphosphine gave Oseltamivir derivative (**3c**) in 96% yield. Analysis of ^{1}H NMR showed that the reduction of Oseltamivir derivative (**3**) showed in upfield shift of H^{l} from δ 3.77 to 3.51 and H^{m} from 3.86 to 3.19 ppm (page 53 in Appendix). FTIR spectrum of Oseltamivir derivative (**3c**) showed a lost of azido group (-N≡N) and new peak amino group ($-NH_2$) at 3267 cm^{-1} (page 55 in Appendix).



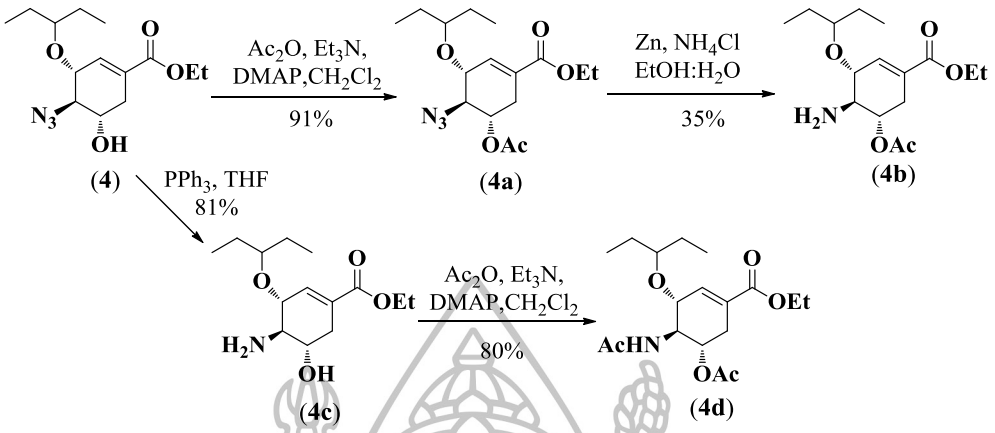
Scheme 15: Staudinger reaction of compound (**3**) with PPh_3 and mechanism.

Acetylation of hydroxyl and amino group of Oseltamivir derivative (**3**c) with acetic anhydride to afford Oseltamivir derivative (**3d**) in 80% yield. Analysis of ^{1}H NMR showed that the Acetylation of Oseltamivir derivative (**3**c) showed in downfield shift of H^{n} from δ 3.51 to 4.97, H^{o} from 3.19 to 4.57 ppm and new $CH_3^{p,q}$ Singlet at 1.99 and 2.11 ppm (page 57 in Appendix). ^{13}C NMR spectrum had a new carbonyl group at δ 171.2 and 170.0 ppm (page 58 in Appendix). FTIR spectrum of Oseltamivir derivative (**3d**) showed a lost of amino ($-NH_2$) and hydroxyl (-OH) group (page 59 in Appendix).



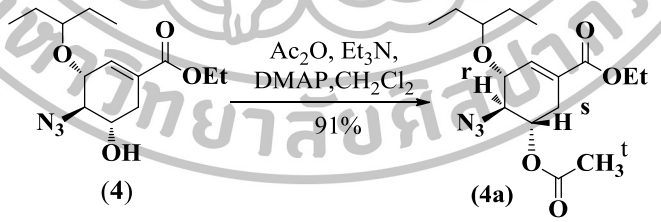
Scheme 16: Acetylation of Oseltamivir derivative (**3c**).

Synthesis of Oseltamivir derivatives (4b), (4c) and (4d) (Scheme 17)



Scheme 17: Synthesis of Oseltamivir derivatives (**4b**), (**4c**) and (**4d**).

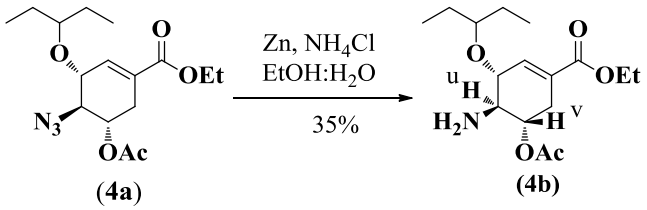
Acetylation of hydroxyl group of compound (**4**) with acetic anhydride delivered compound (**4a**) in 91% yield (**Scheme 17**). Analysis of ^{1}H NMR showed that the Acetylation of Oseltamivir derivative (**4**) showed in downfield shift of H^{r} from 3.45 to 3.62, H^{s} from 3.69 to 4.95 and new CH_3^{t} Singlet at 2.15 ppm (page 65 in Appendix). ^{13}C NMR spectrum had a new carbonyl group at δ 170.06 ppm (page 66 in Appendix). FTIR spectrum of Oseltamivir derivative (**4a**) showed a lost of hydroxyl group (-OH) (page 67 in Appendix).



Scheme 18: Acetylation of compound (**4**).

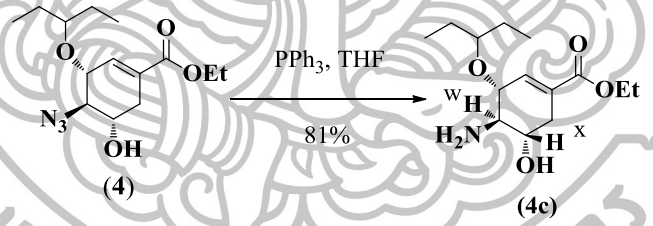
Oseltamivir derivative (**4b**) was synthesized by reduction azido group of compound (**4a**) with zinc dust to give 35% yield. Analysis of ^{1}H NMR showed that the reduction of compound (**4a**) showed in downfield shift of H^{u} δ 3.62 to 3.82 and upfield shift of H^{v} 4.95 to 4.00 ppm (page 69 in Appendix). ^{13}C NMR spectrum had a new carbonyl group at δ 170.06 ppm (page 70 in Appendix). FTIR spectrum of Oseltamivir derivative (**4b**) showed

a lost of azido group (-N≡N) and new peak amino group (-NH_2) at 3294 cm^{-1} (page 71 in Appendix).



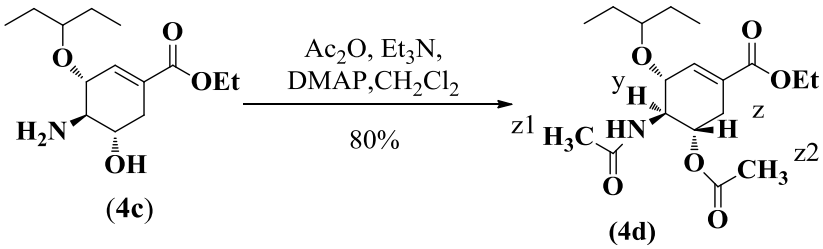
Scheme 19: Reduction of Oseltamivir derivative (**4a**) with zinc dust.

Reduction of azido group of compound (**4**) with triphenylphosphine gave Oseltamivir derivative (**4c**) in 96% yield. Analysis of ^1H NMR showed that the reduction of compound (**4**) showed in upfield shift of H^w from δ 3.45 to 2.90 and downfield shift of H^x from 3.69 to 3.75 ppm (page 73 in Appendix). FTIR spectrum of Oseltamivir derivative (**4c**) showed a lost of azido group (-N≡N) and new peak amino group (-NH_2) at 3267 cm^{-1} (page 75 in Appendix).



Scheme 20: Staudinger reaction of compound (**4**) with PPh_3.

Acetylation of hydroxyl and amino group of Oseltamivir derivative (4c) with acetic anhydride afforded Oseltamivir derivative (**4d**) in 80% yield. Analysis of ^1H NMR showed that the Acetylation of Oseltamivir derivative (**4**c) showed in downfield shift of H^y from δ 2.90 to 4.12, H^z from 3.75 to 5.09 and new $CH_3^{z1,z2}$ Singlet at 1.97 and 2.06 ppm ppm (page 77 in Appendix). ^{13}C NMR spectrum had a new carbonyl group at δ 171.2 and 170.0 ppm (page 78 in Appendix). FTIR spectrum of Oseltamivir derivative (**4d**) showed a lost of amino (-NH_2) and hydroxyl (-OH) group (page 79 in Appendix).



Scheme 21: Acetylation of Oseltamivir derivative (**4c**).

Anti-tyrosinase activity: Tyrosinase inhibition activity was determined by the modified dopachrome method using L-DOPA as a substrate. Briefly, in the 96-well plates, 120 µl of 50 mM sodium phosphate buffer (pH 6.8), 40 µl of a solution of mushroom tyrosinase (100 units/mL in 50mM sodium phosphate buffer pH 6.8) and 20 µl of test compound solution dissolved in ethanol (a final concentration of 0.1 mg/mL) were mixed and incubated at room temperature for 10 min.

The reaction was stated by adding 20 µl of 1.5 mM *L*-DOPA in 50 mM sodium phosphate buffer (pH 6.8). The assay mixture was incubated at room temperature for 20 min. The amount of dopachrome produced in the reaction mixture was measured at 492 nm on a microtiter plate reader (Sunrise, Tecan). The percentage of inhibition of tyrosinase activity was calculated as follows:

$$\text{Inhibition (\%)} = (1\text{-}B/A) \times 100$$

Where A is the enzyme activity without inhibitor and B is the activity in presence of inhibitor. Kojic acid was used as a reference standard. Every experiment was done in triplicate.

Results of tryosinase inhibitory activity: Tyrosinase-inhibition activity was dertermined by the modified dopachrome method using *L*-DOPA as a substrate. Inhibitory activity evaluation of Oseltamivir derivatives (**2**), (**3b**), (**3c**), (**3d**), (**4b**), (**4c**) and (**4d**) indicated that compound (**2**) is more potent than compound (**3c**), however the degree of inhibitor is not as good as the reference kojic acid. The results are illustrated in **Table 2**.

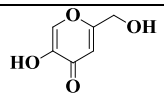
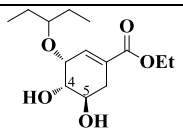
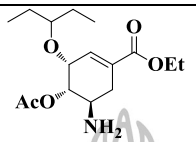
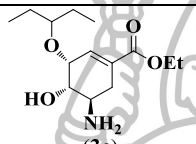
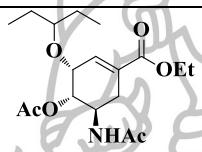
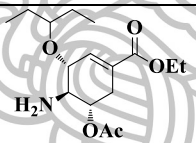
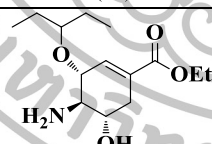
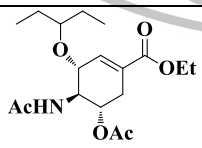
Compounds	**% Inhibition at 1.0 mM**
 Kojic acid	95.09±0.10
 (2)	**13.97±0.19**
 (3b)	8.13±0.17
 (3c)	**12.06±0.19**
 (3d)	8.97±0.10
 (4b)	1.40±0.17
 (4c)	0.45±0.10
 (4d)	2.63±0.19

Table 1: Anti-tyrosinase activity of Oseltamivir derivatives (**2**), (**3b**), (**3c**), (**3d**), (**4b**), (**4c**) and (**4d**).

CHAPTER 4
GENERAL CONCLUSION

Oseltamivir derivatives (**3b**), (**3c**), (**3d)** and four news (**2**), (**4b**), (**4c**) and (**4d**) were synthesized in this work in high yield. Oseltamivir derivatives was evaluated for anti-tyrosinase activity. If could be noted that the 4 or 5 dihydroxy group on the core structure of Oseltamivir derivatives is important for tyrosinase activity.

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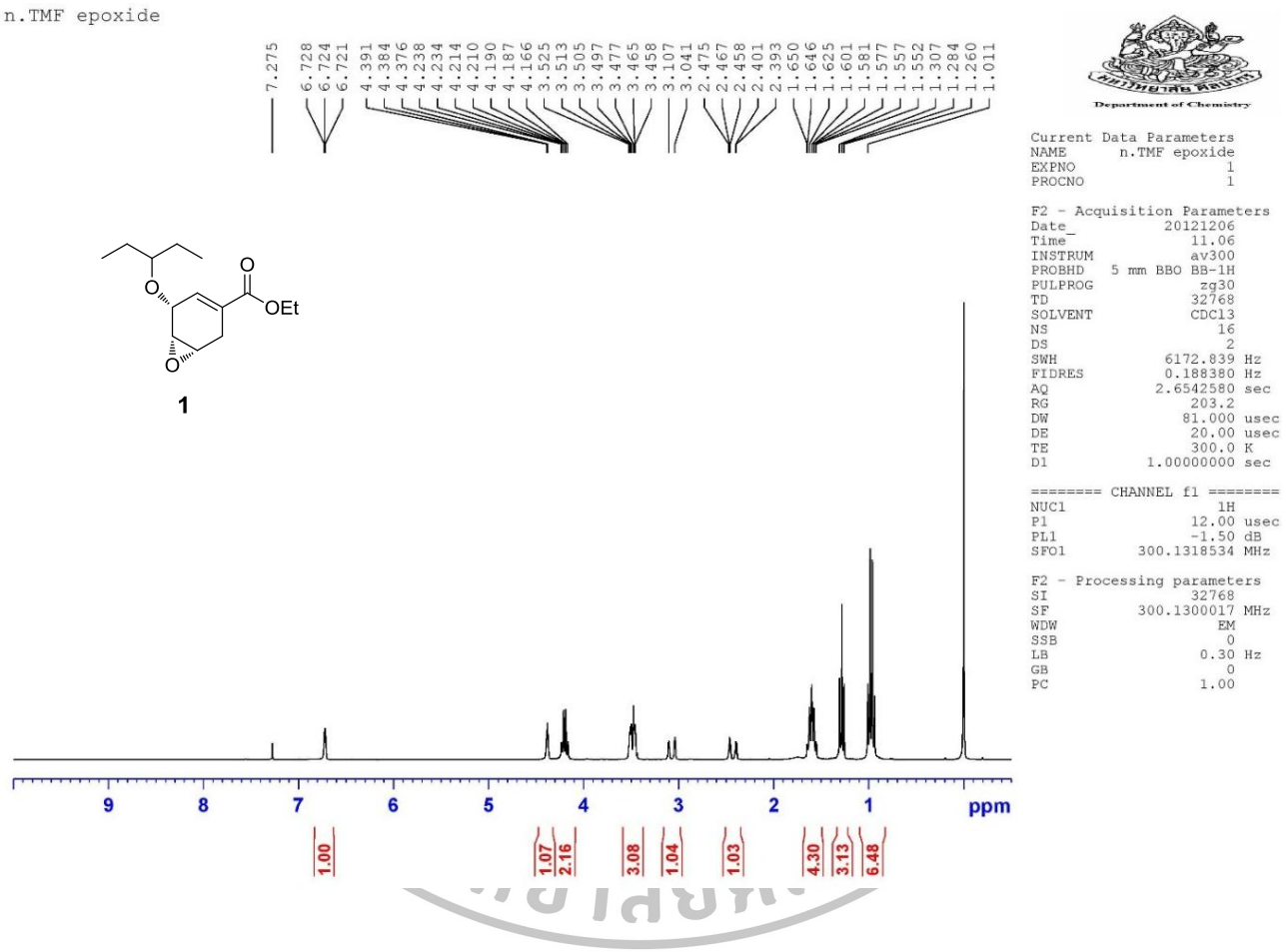
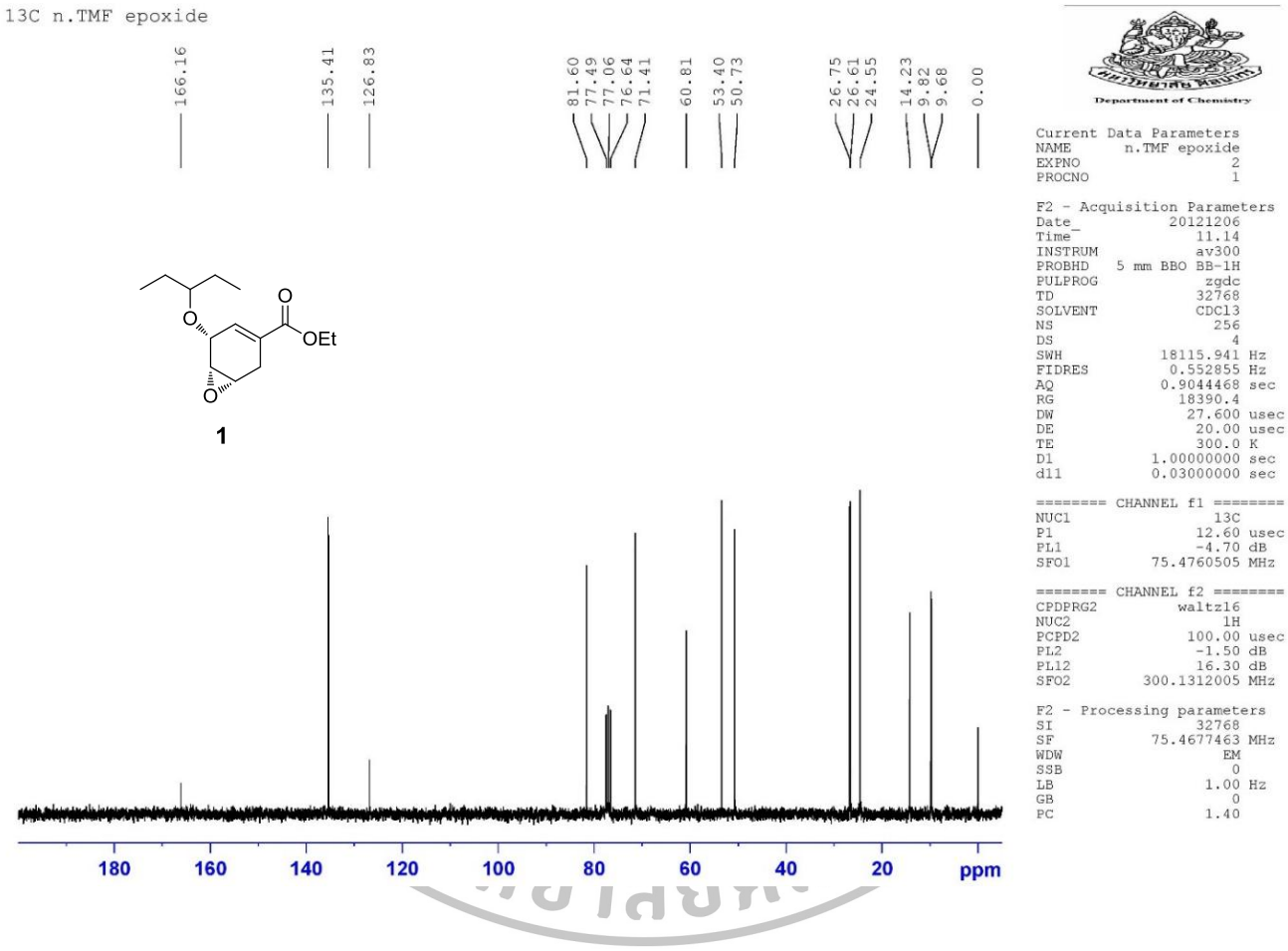
Appendix

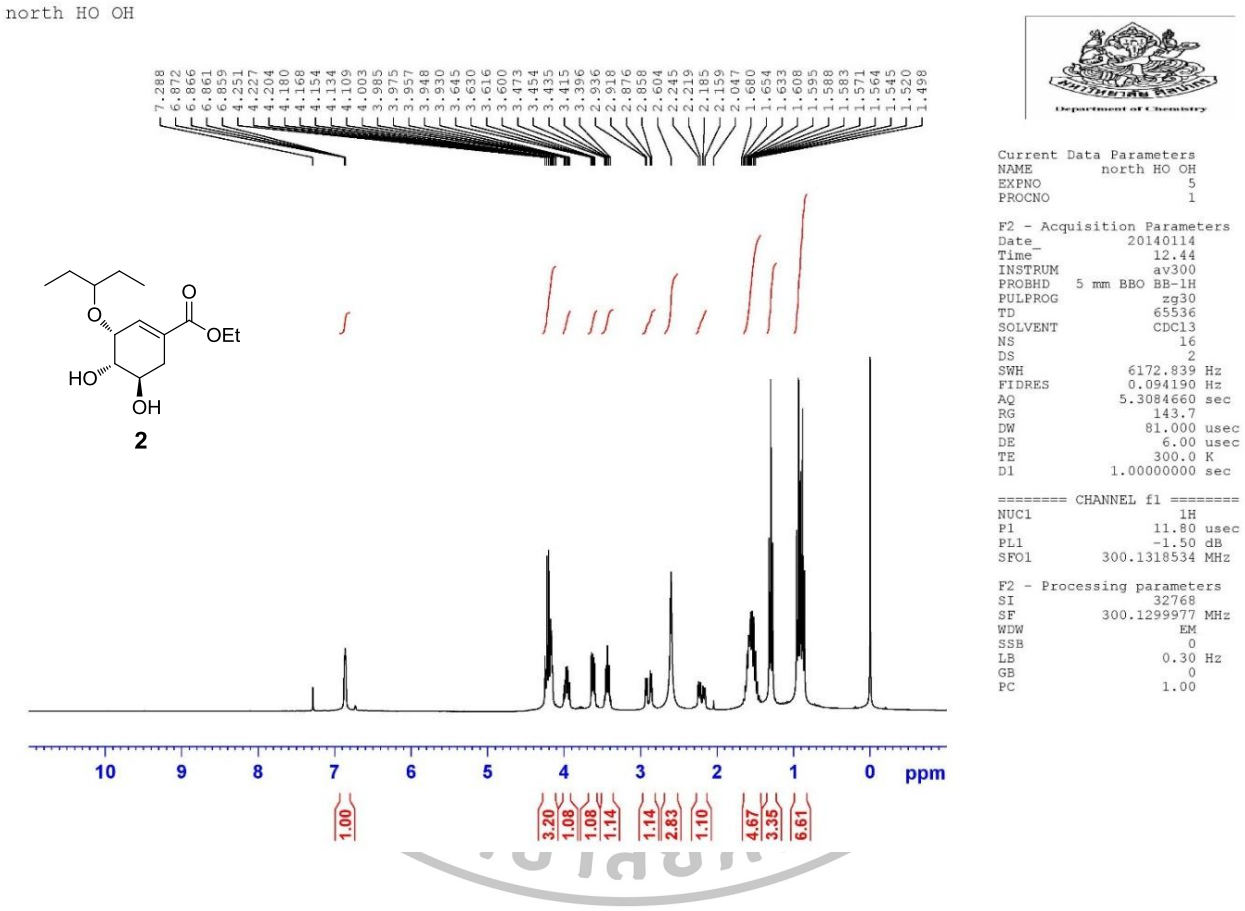
^{1}H NMR and ^{13}C NMR spectra of compounds



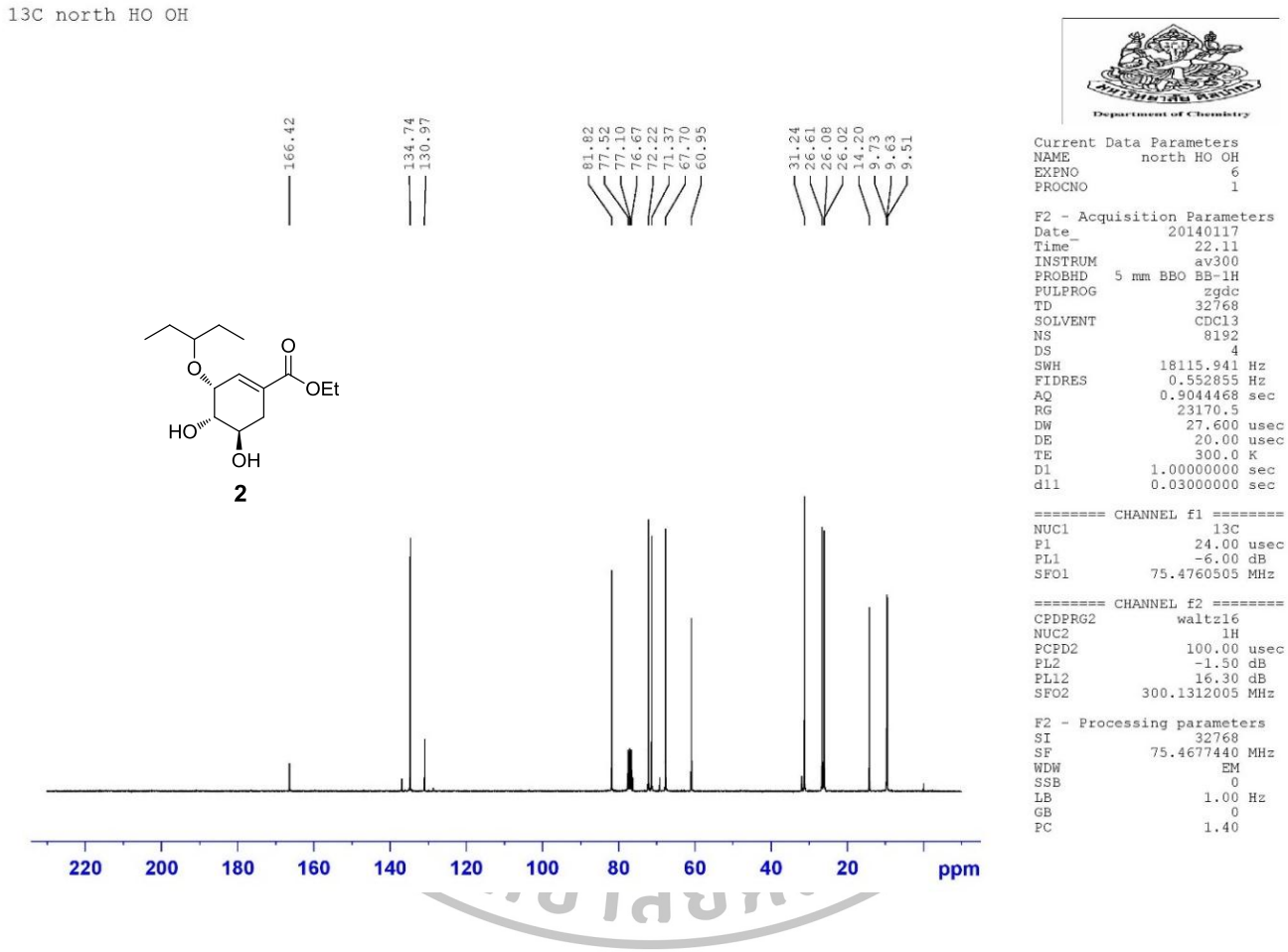
^{1}H NMR spectrum of compound **1**



^{13}C NMR spectrum of compound **1**



^{1}H NMR spectrum of compound **2**

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EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
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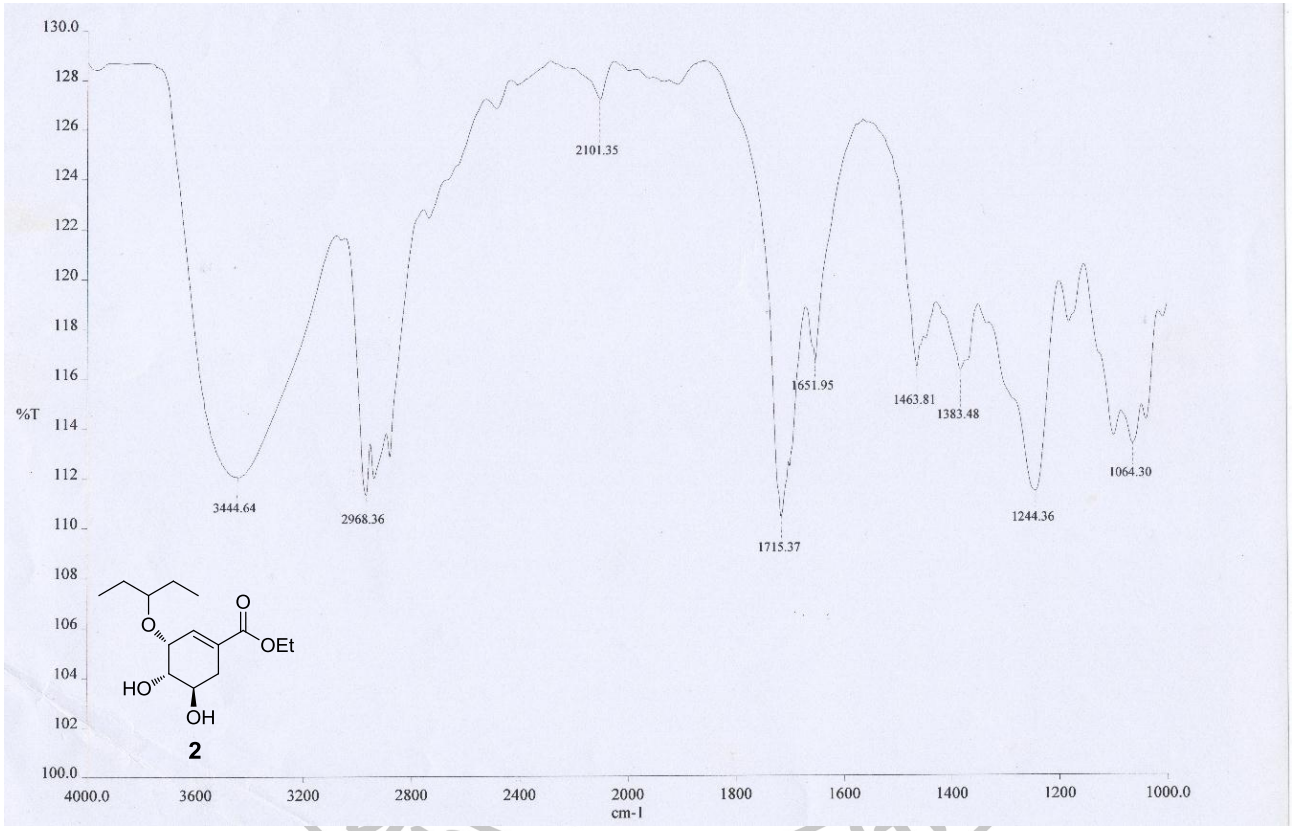
Time                22.11
INSTRUM             av300
PROBHD   5 mm BBO BB-1H
PULPROG             zgdc
TD                 32768
SOLVENT            CDCl3
NS                  8192
DS                     4
SWH           18115.941 Hz
FIDRES         0.552855 Hz
AQ            0.9044468 sec
RG              23170.5
DW               27.600 usec
DE               20.00 usec
TE               300.0 K
D1           1.00000000 sec
d11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                24.00 usec
PL1               -6.00 dB
SFO1         75.4760505 MHz

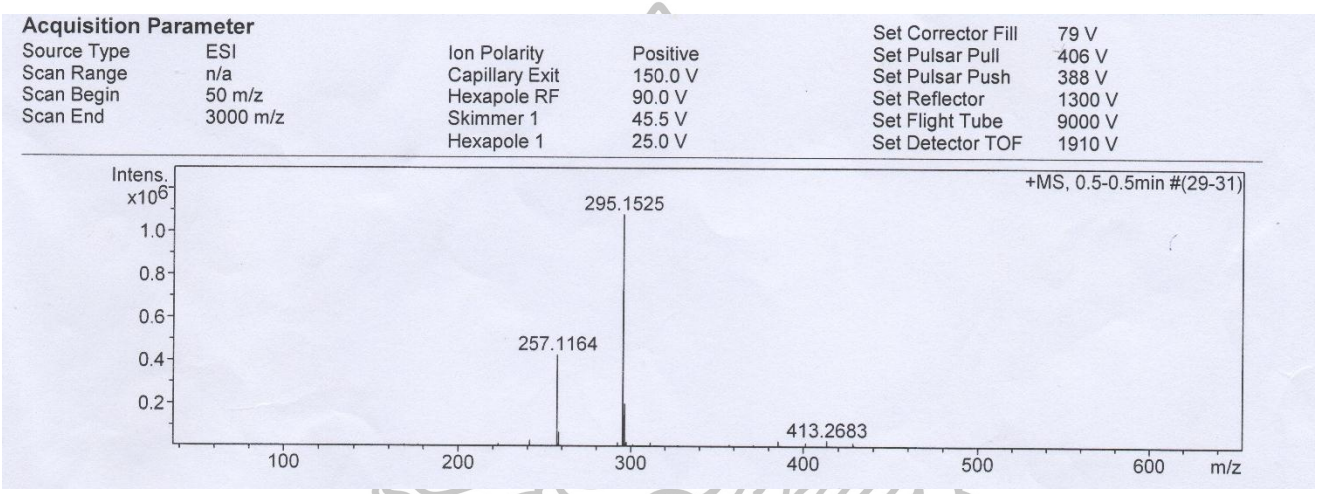
======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                1H
PCPD2           100.00 usec
PL2              -1.50 dB
PL12             16.30 dB
SFO2        300.1312005 MHz

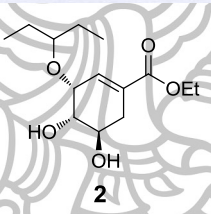
F2 - Processing parameters
SI                 32768
SF          75.4677440 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

^{13}C NMR spectrum of compound **2**



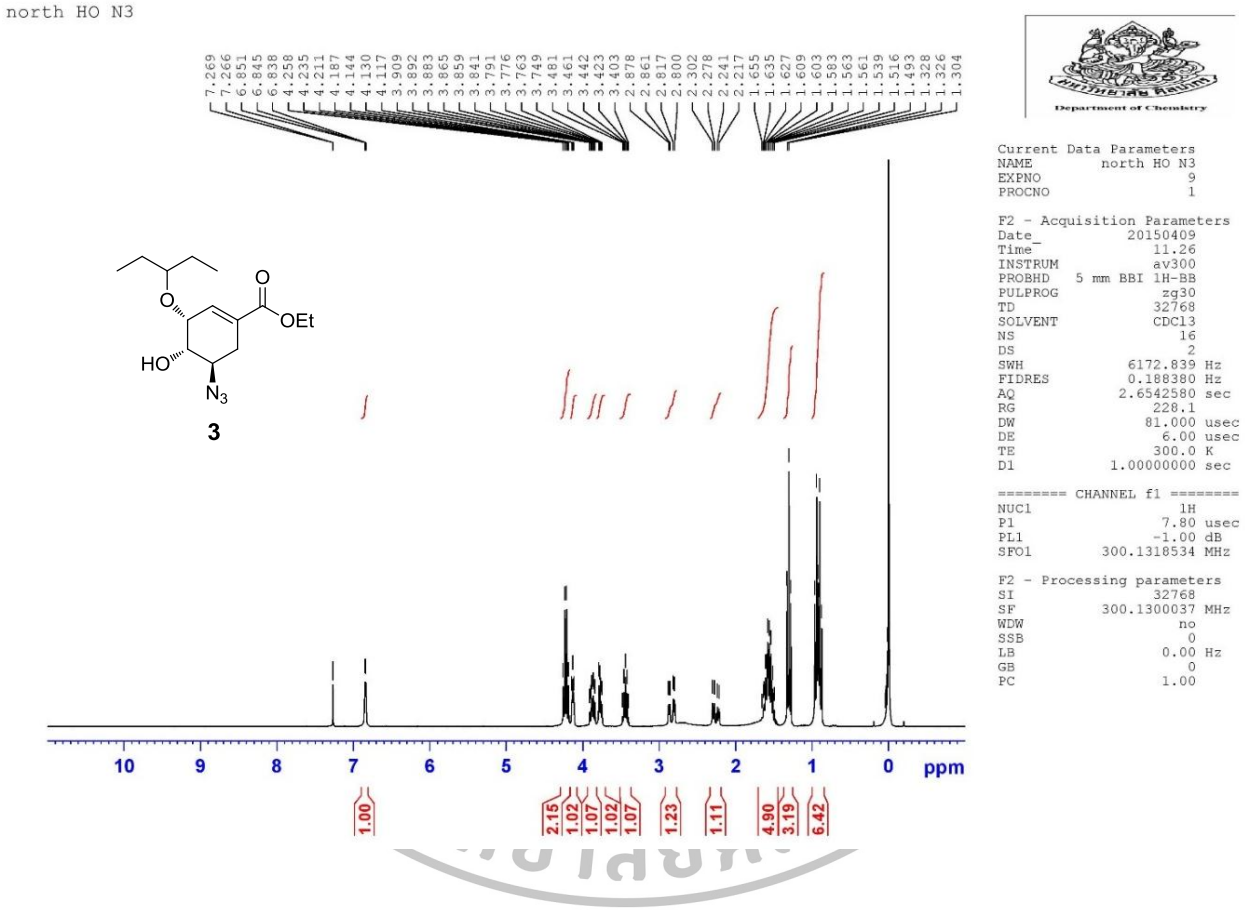
FTIR spectrum of compound **2**



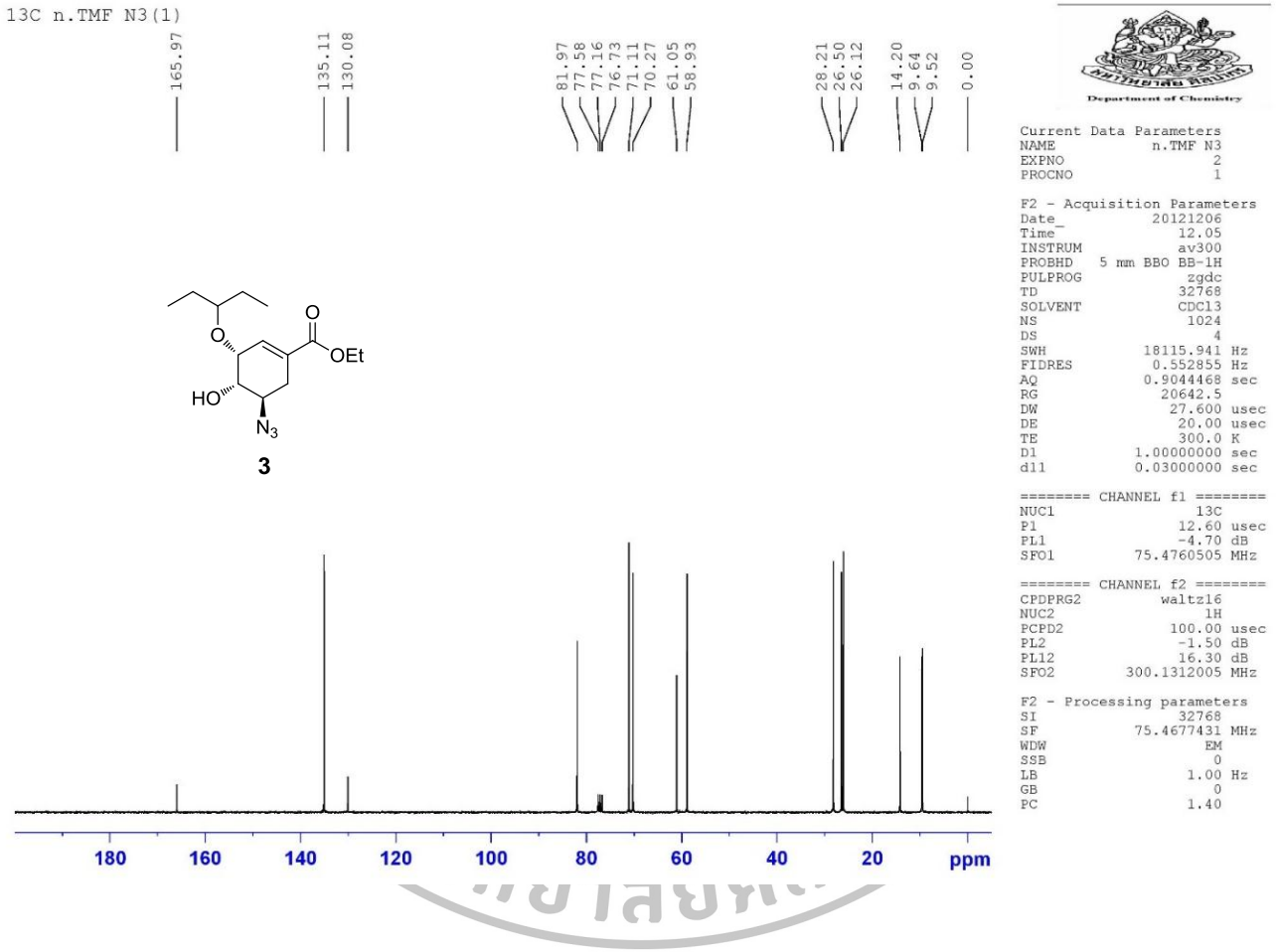


2

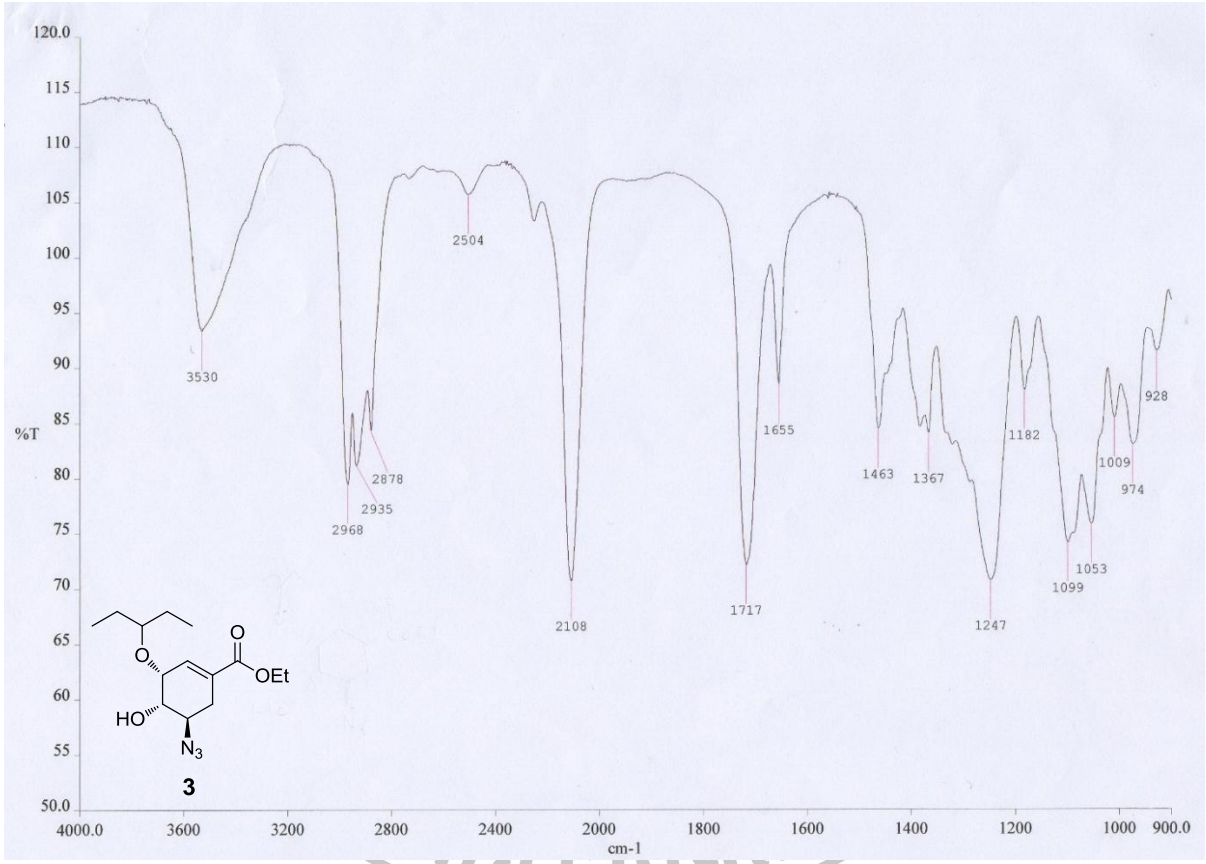
Mass spectrum of compound **3**



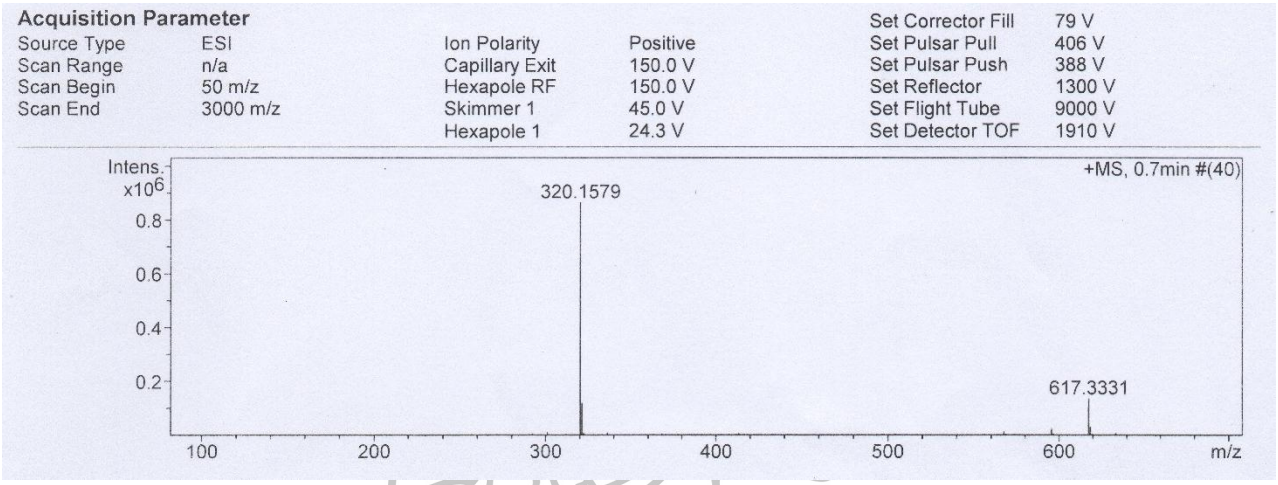
^{1}H NMR spectrum of compound **3**

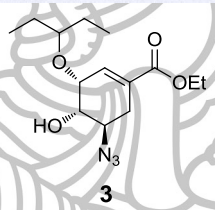


^{13}C NMR spectrum of compound **3**



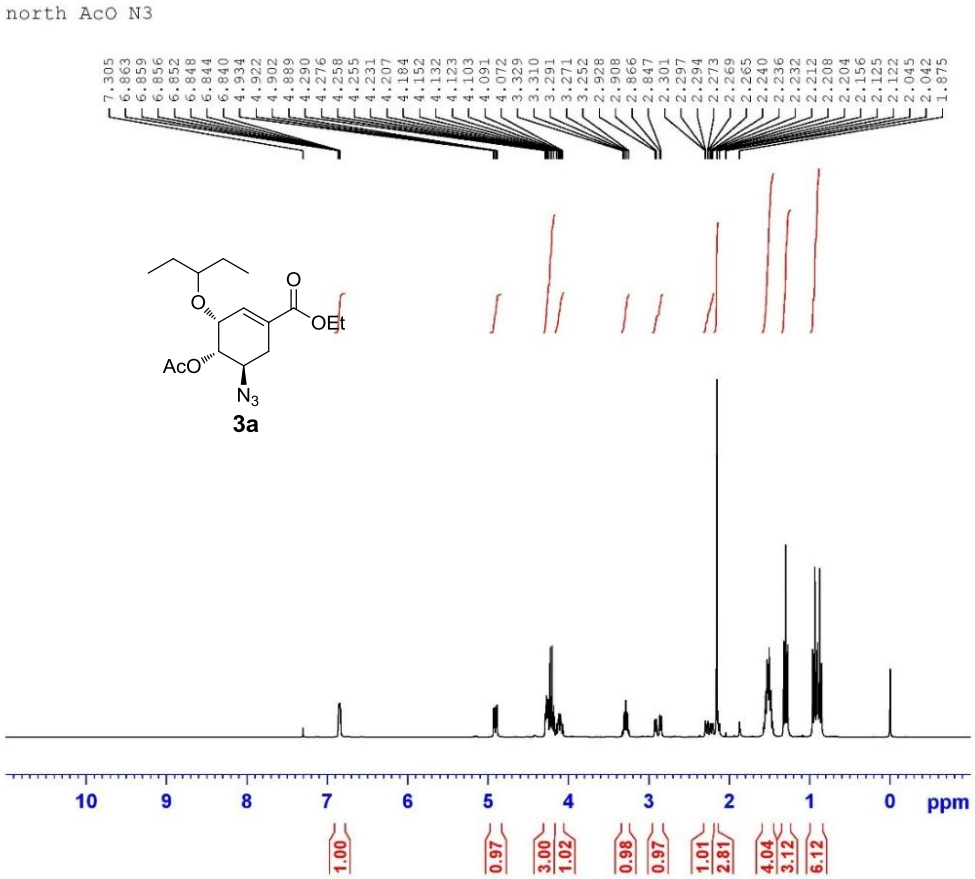
FTIR spectrum of compound **3**





3

Mass spectrum of compound **3**

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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140206
Time              16.09
INSTRUM           av300
PROBHD   5 mm BBO BB-1H
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            6172.839 Hz
FIDRES         0.094190 Hz
AQ             5.3084660 sec
RG                80.6
DW               81.000 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                11.80 usec
PL1               -1.50 dB
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                32768
SF           300.1299924 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```
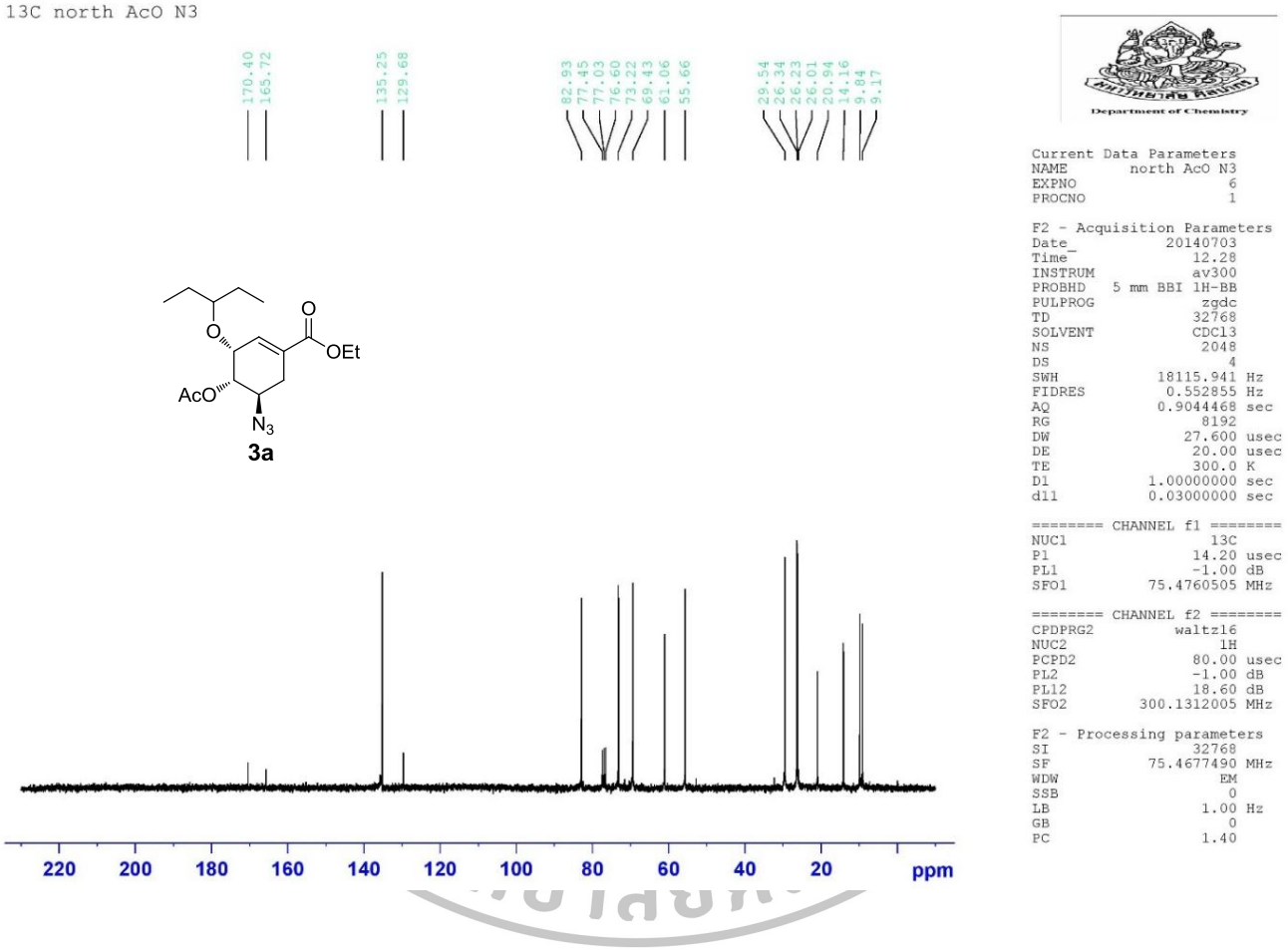
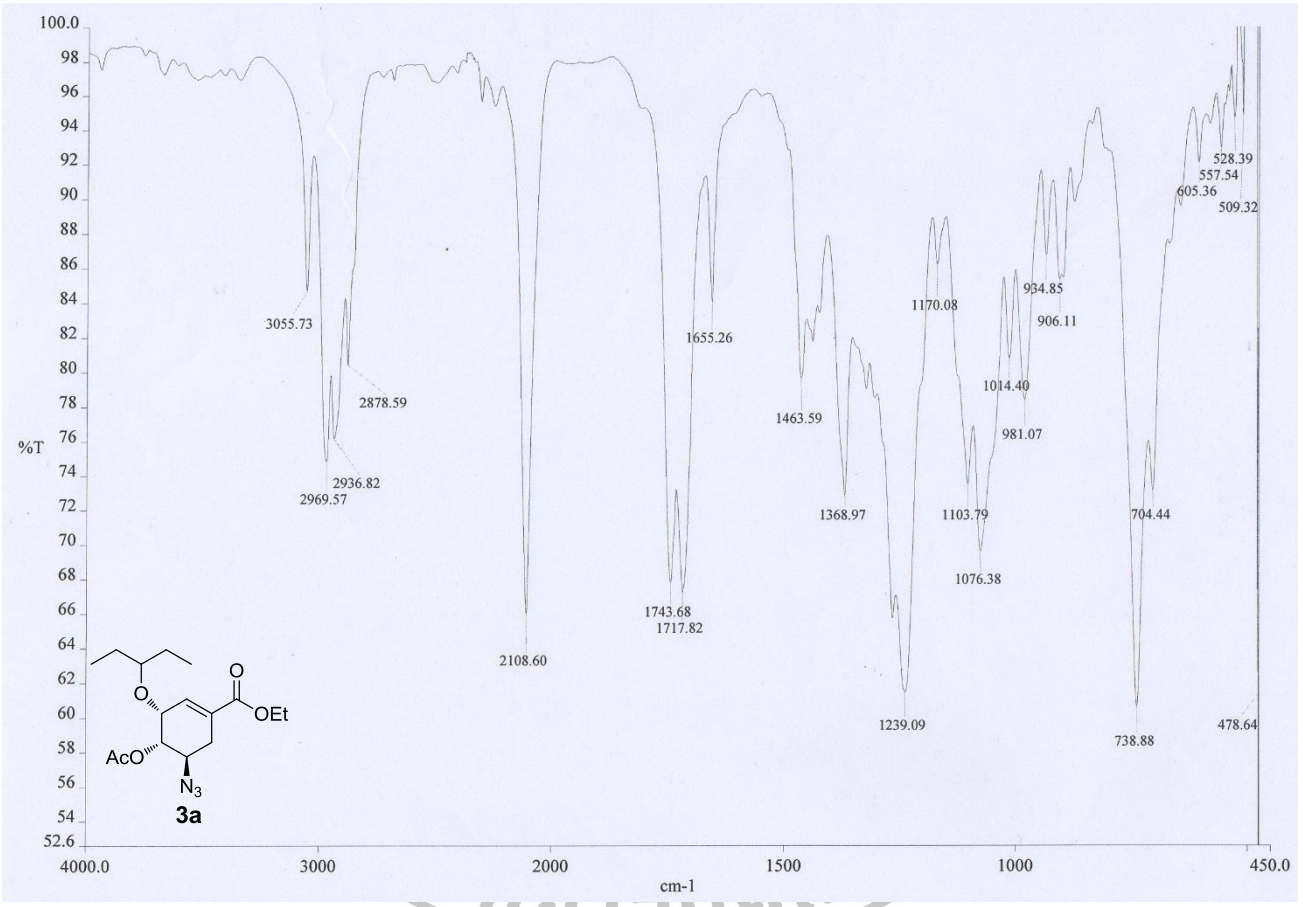

^{1}H NMR spectrum of compound **3a**



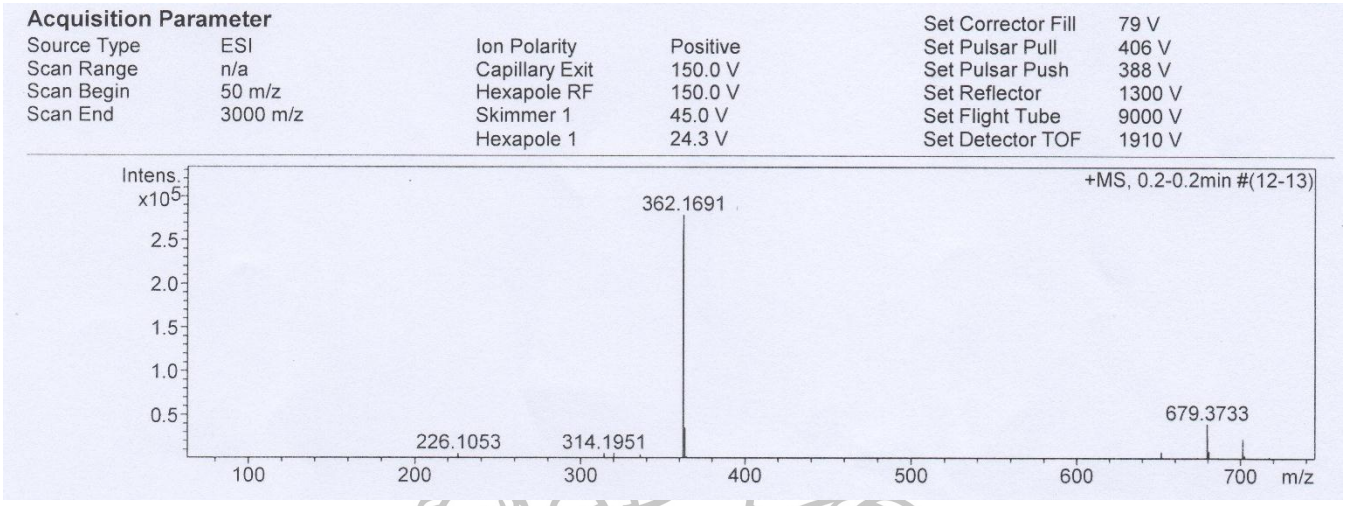
Department of Chemistry

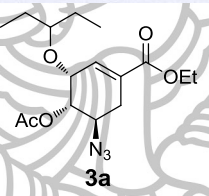
```
Current Data Parameters
NAME        north AcO N3
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140703
Time_              12.28
INSTRUM            av300
PROBHD   5 mm BBI 1H-BB
PULPROG             zgdc
TD                 32768
SOLVENT            CDCl3
NS                  2048
DS                     4
SWH            18115.941 Hz
FIDRES         0.552855 Hz
AQ             0.9044468 sec
RG                  8192
DW                27.600 usec
DE                20.00 usec
TE                300.0 K
D1            1.00000000 sec
d11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                14.20 usec
PL1               -1.00 dB
SFO1          75.4760505 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -1.00 dB
PL12              18.60 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4677490 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

^{13}C NMR spectrum of compound **3a**



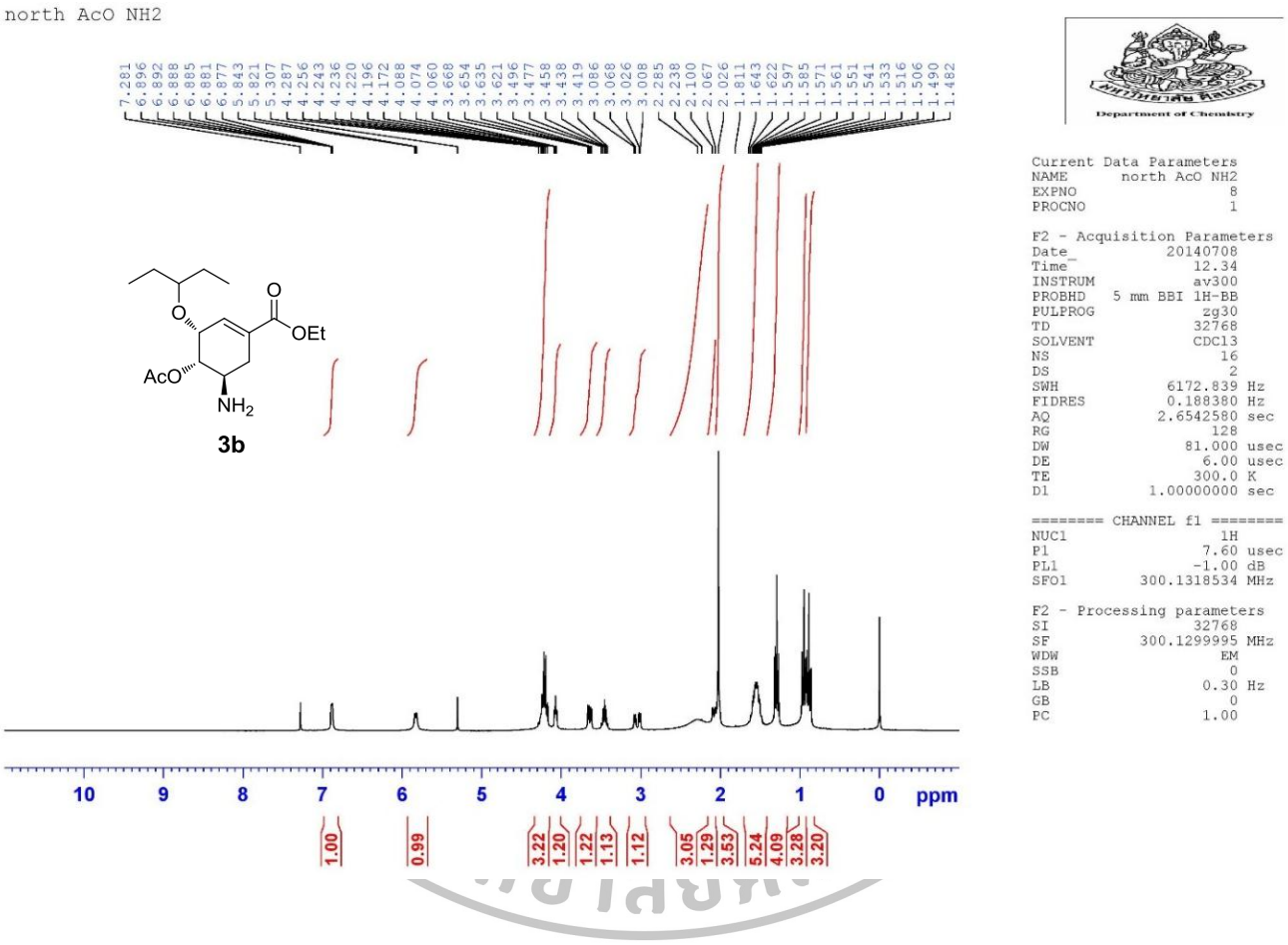
FTIR spectrum of compound **3a**





3a

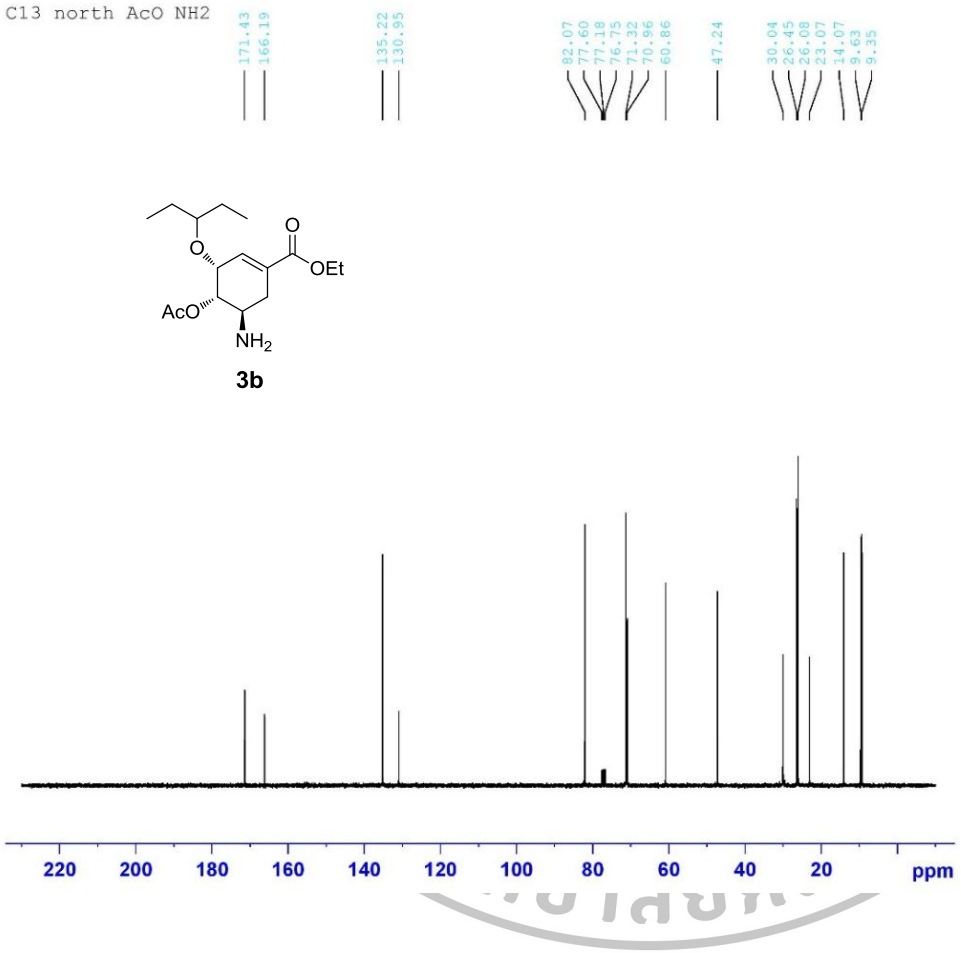
Mass spectrum of compound **3a**



Department of Chemistry

```
Current Data Parameters
NAME      north AcO NH2
EXPNO                 8
PROCNO                1

F2 - Acquisition Parameters
Date_          20140708
Time              12.34
INSTRUM           av300
PROBHD   5 mm BBI 1H-BB
PULPROG           zg30
TD               32768
SOLVENT          CDCl3
NS                  16
DS                   2
SWH           6172.839 Hz
FIDRES        0.188380 Hz
AQ            2.6542580 sec
RG                 128
DW              81.000 usec
DE               6.00 usec
TE              300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1               7.60 usec
PL1             -1.00 dB
SFO1       300.1318534 MHz

F2 - Processing parameters
SI               32768
SF         300.1299995 MHz
WDW                 EM
SSB                  0
LB              0.30 Hz
GB                   0
PC                1.00
```

^{1H} NMR spectrum of compound **3b**



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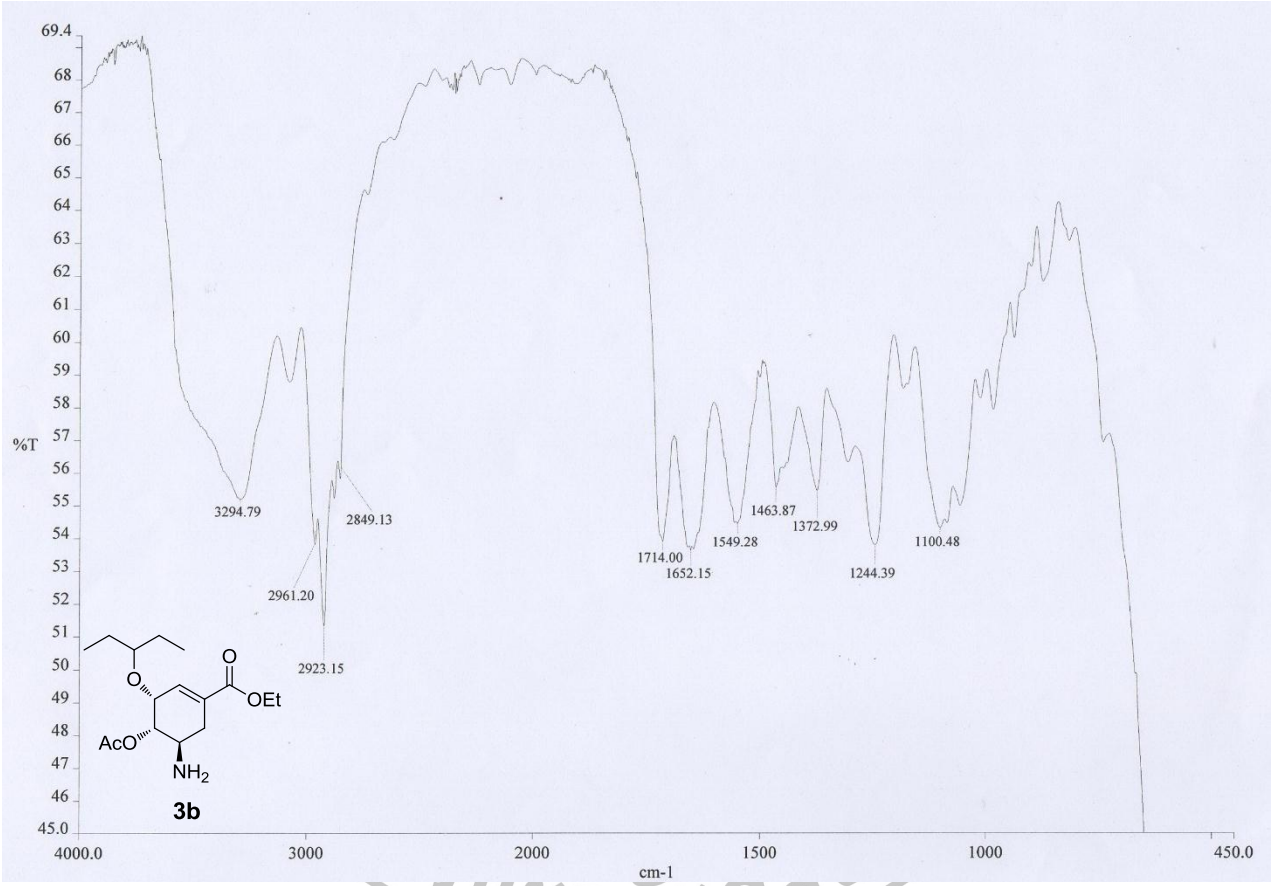
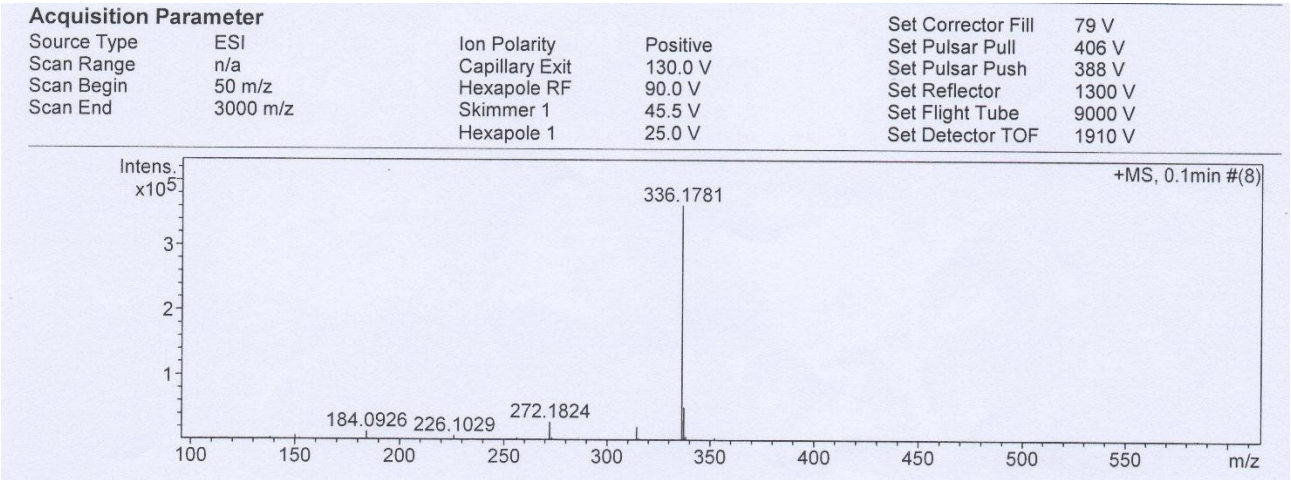
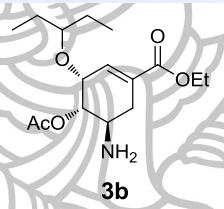
```
Current Data Parameters
NAME       north AcO NH2
EXPNO                  6
PROCNO                 1

F2 - Acquisition Parameters
Date_           20140315
Time              12.09
INSTRUM            av300
PROBHD   5 mm BBO BB-1H
PULPROG             zgdc
TD                 32768
SOLVENT            CDCl3
NS                   560
DS                     4
SWH           18115.941 Hz
FIDRES         0.552855 Hz
AQ             0.9044468 sec
RG               10321.3
DW                27.600 usec
DE                20.00 usec
TE                300.0 K
D1           1.00000000 sec
d11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                  7.00 usec
PL1                -2.00 dB
SFO1         75.4760505 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2               -1.50 dB
PL12              16.30 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF          75.4677490 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.40
```

^{13}C NMR spectrum of compound **3b**



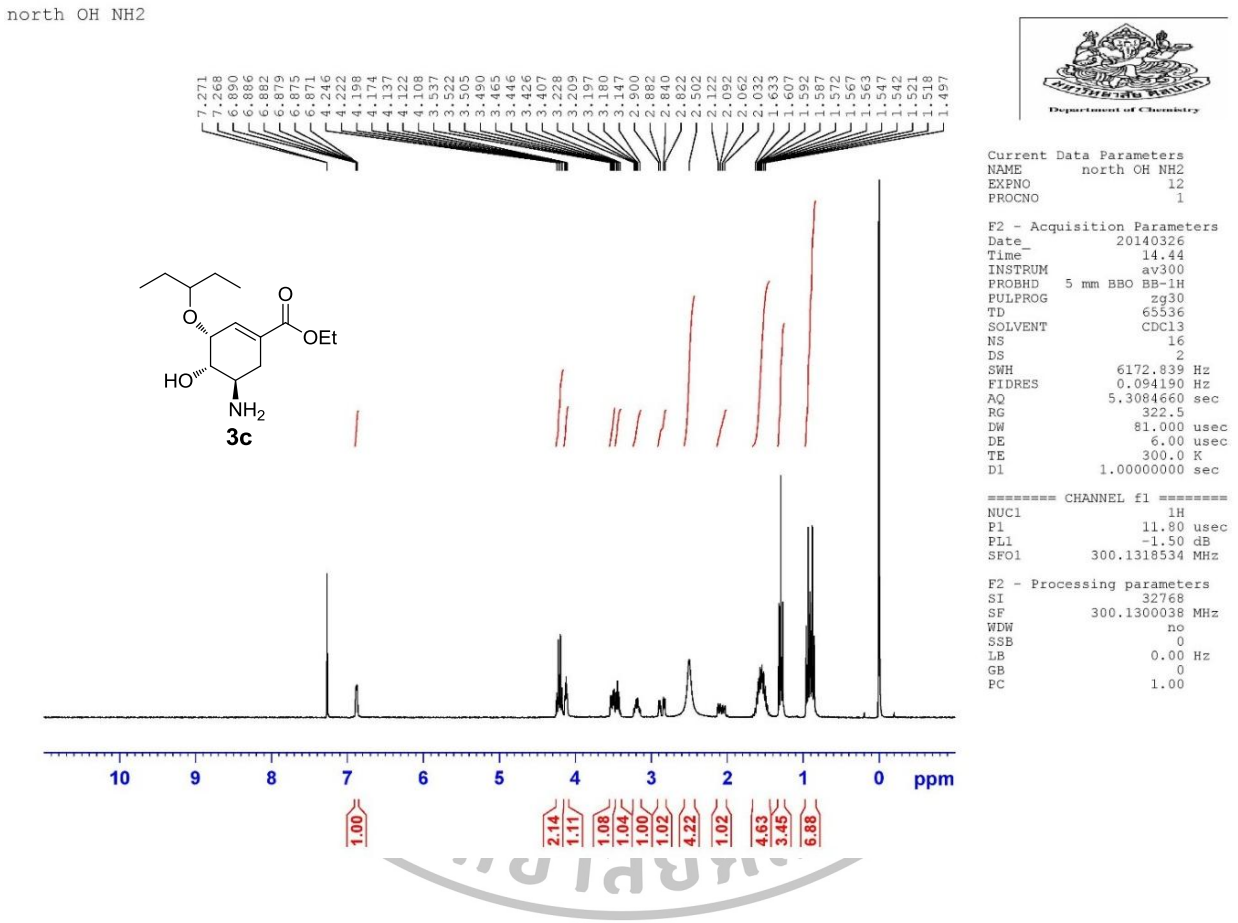
FTIR spectrum of compound **3b**



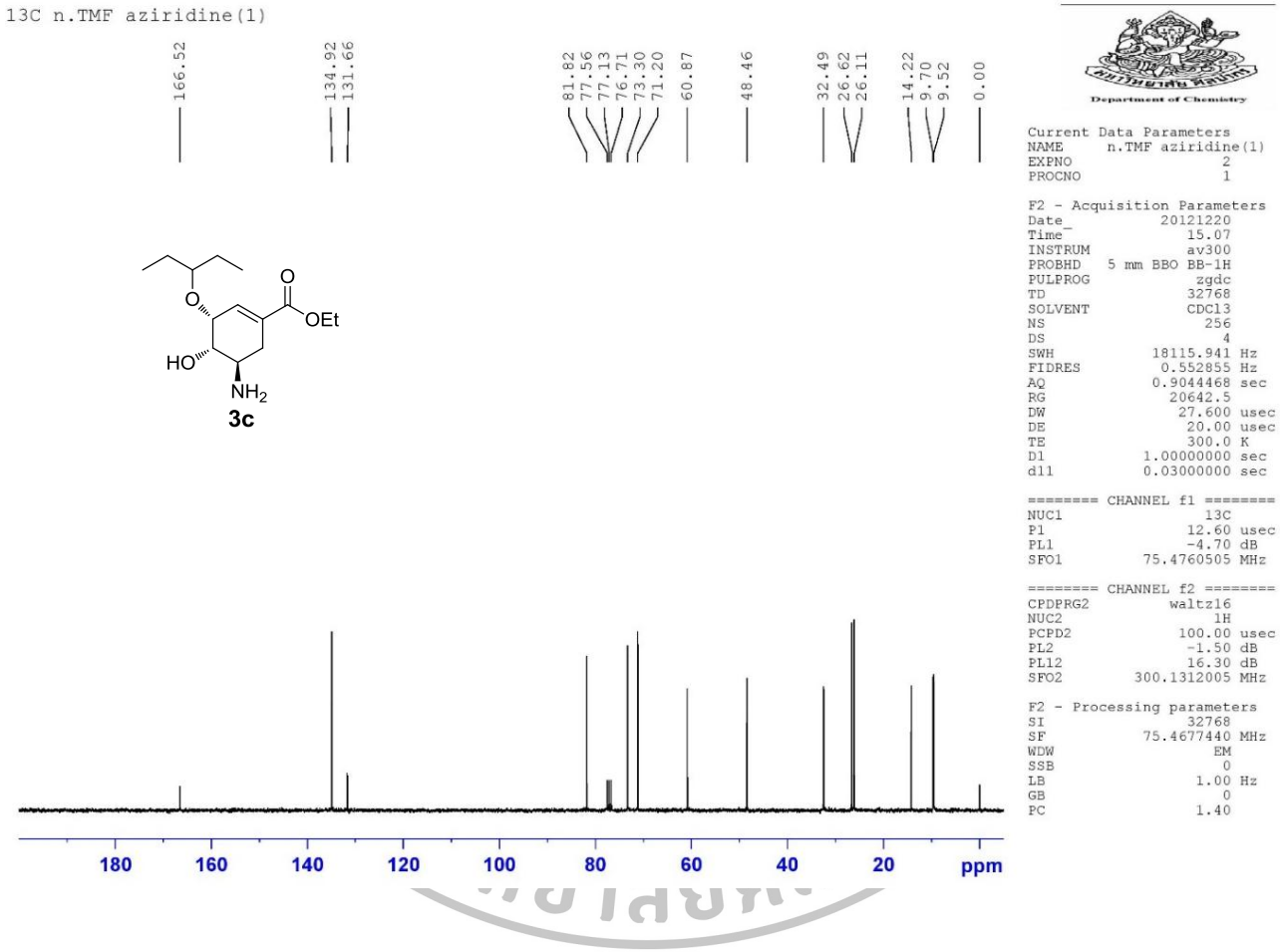


3b

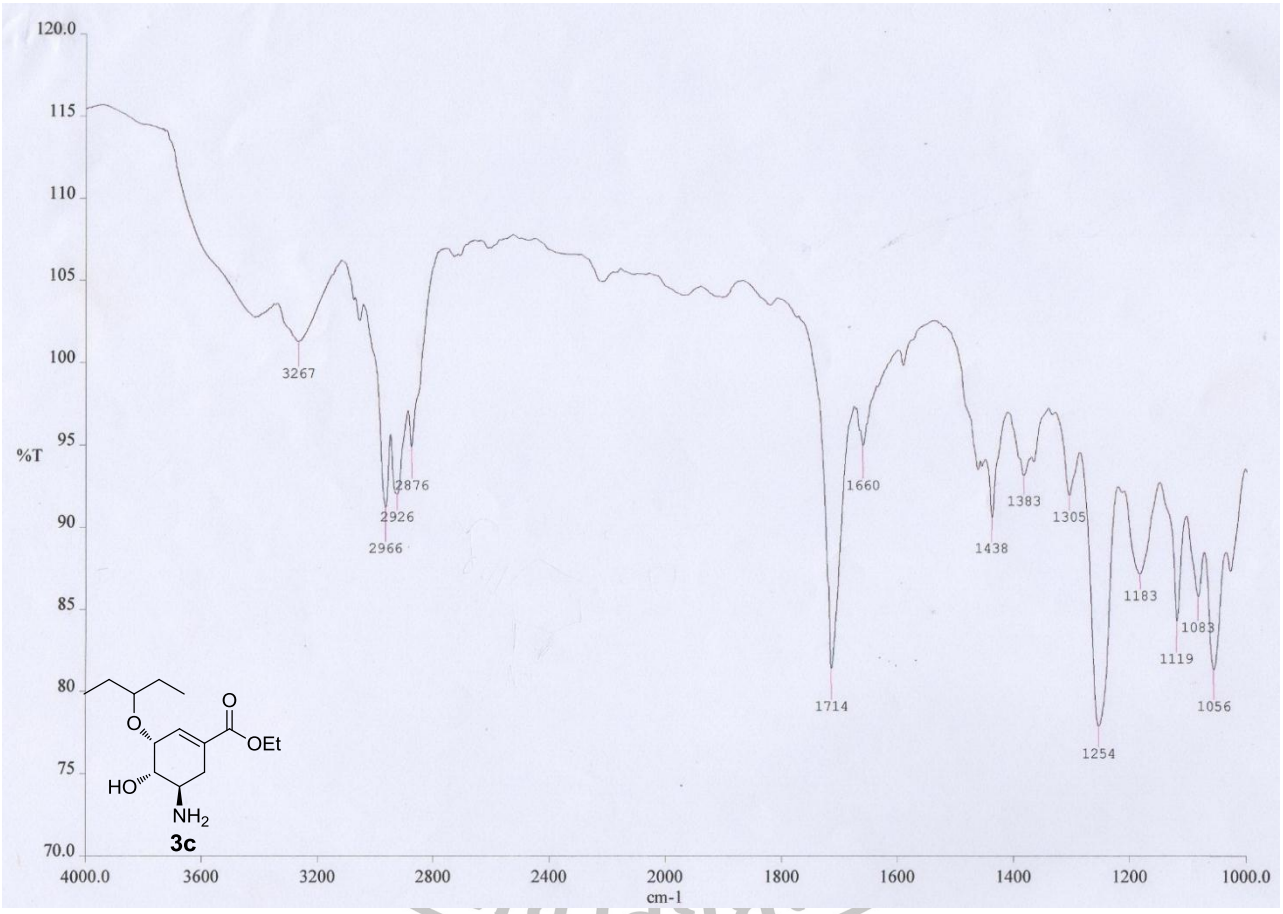
Mass NMR spectrum of compound **3b**



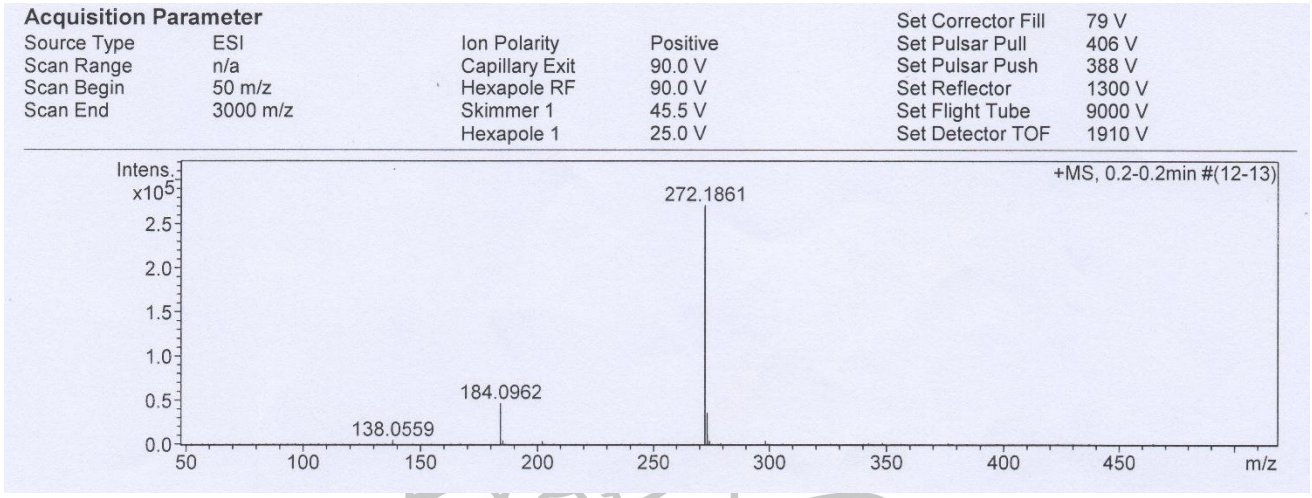
^{1}H NMR spectrum of compound **3c**

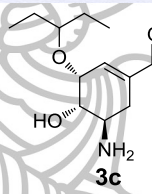


^{13}C NMR spectrum of compound **3c**



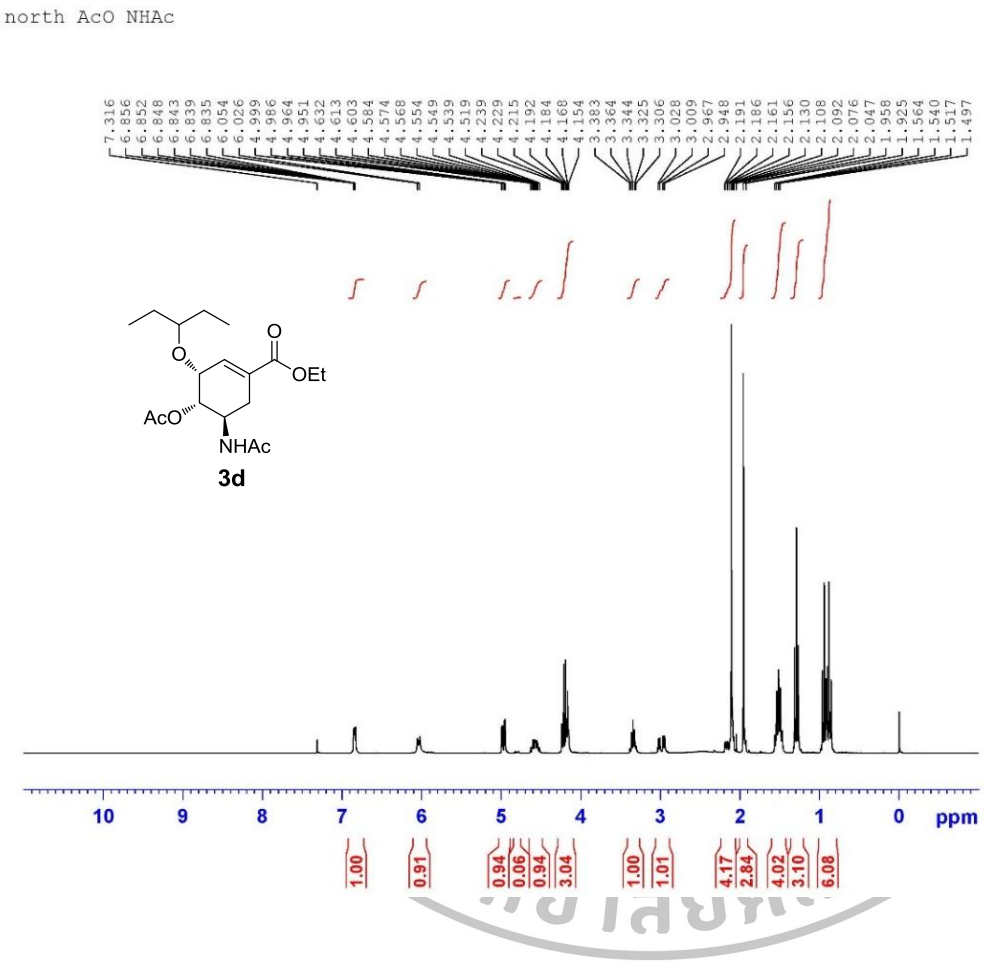
FTIR spectrum of compound **3c**





3c

Mass spectrum of compound **3c**



Department of Chemistry

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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
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Time              14.13
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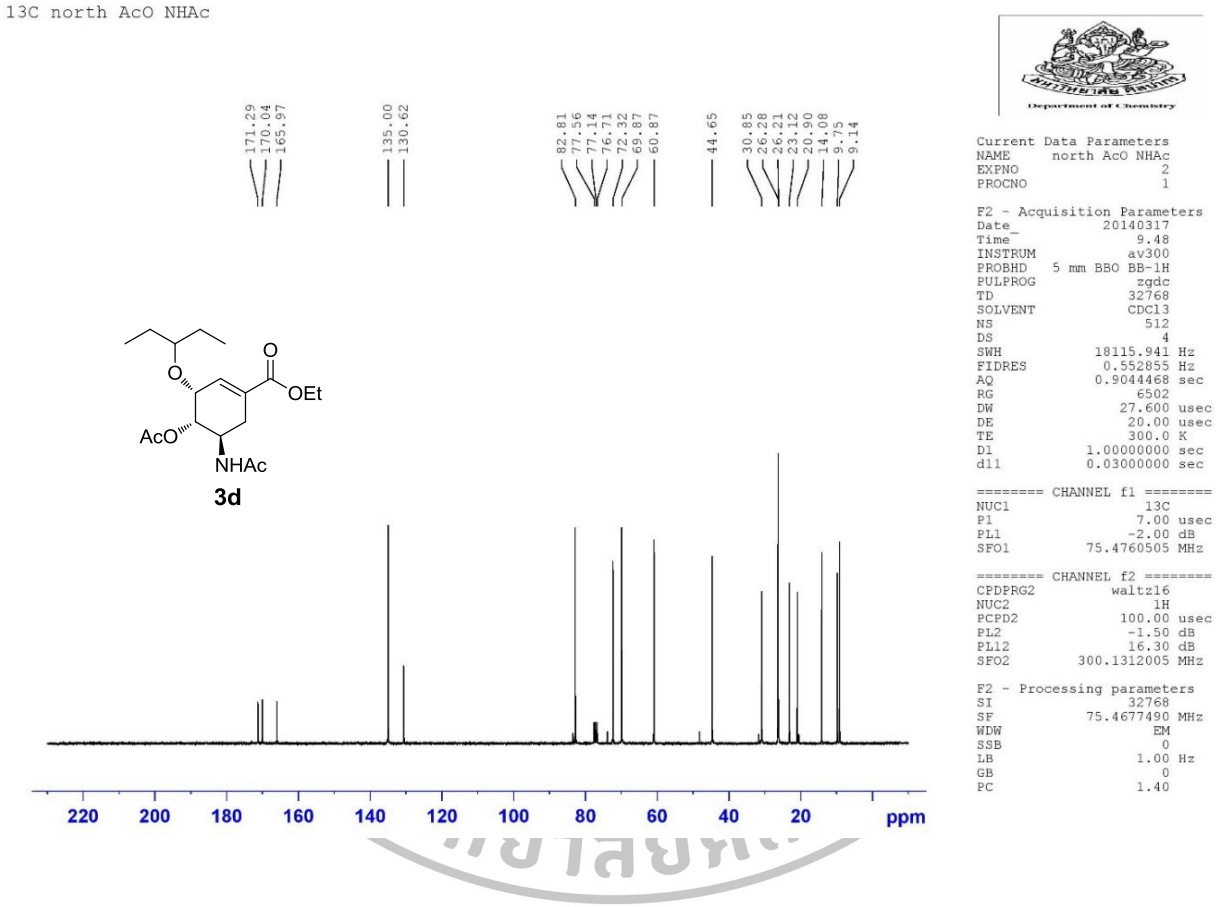
PROBHD   5 mm BBO BB-1H
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            6172.839 Hz
FIDRES         0.094190 Hz
AQ             5.3084660 sec
RG                90.5
DW               81.000 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec

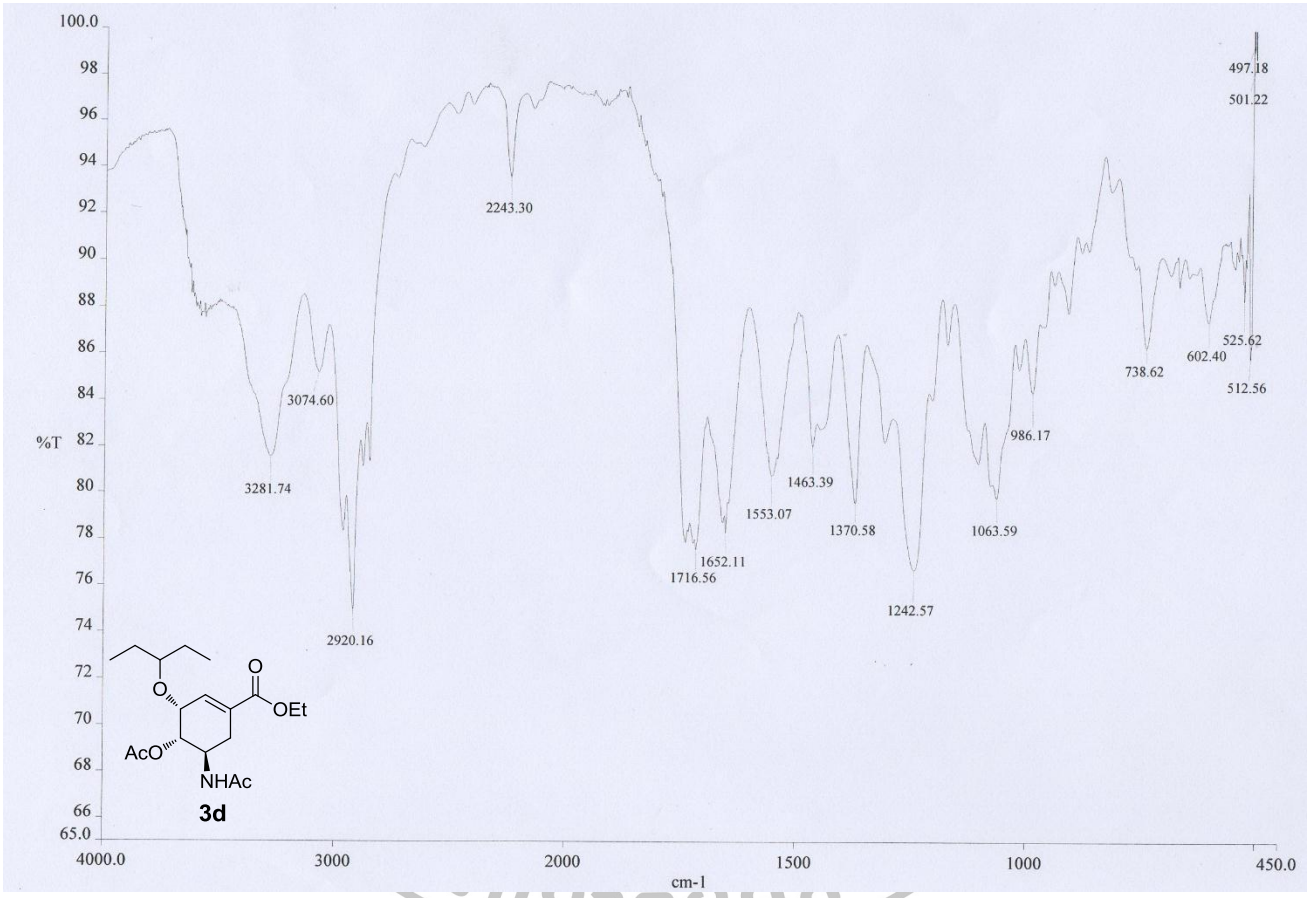
======== CHANNEL f1 ========
NUC1                 1H
P1                11.80 usec
PL1               -1.50 dB
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                32768
SF           300.1299898 MHz
WDW                  no
SSB                   0
LB                 0.00 Hz
GB                    0
PC                 1.00
```

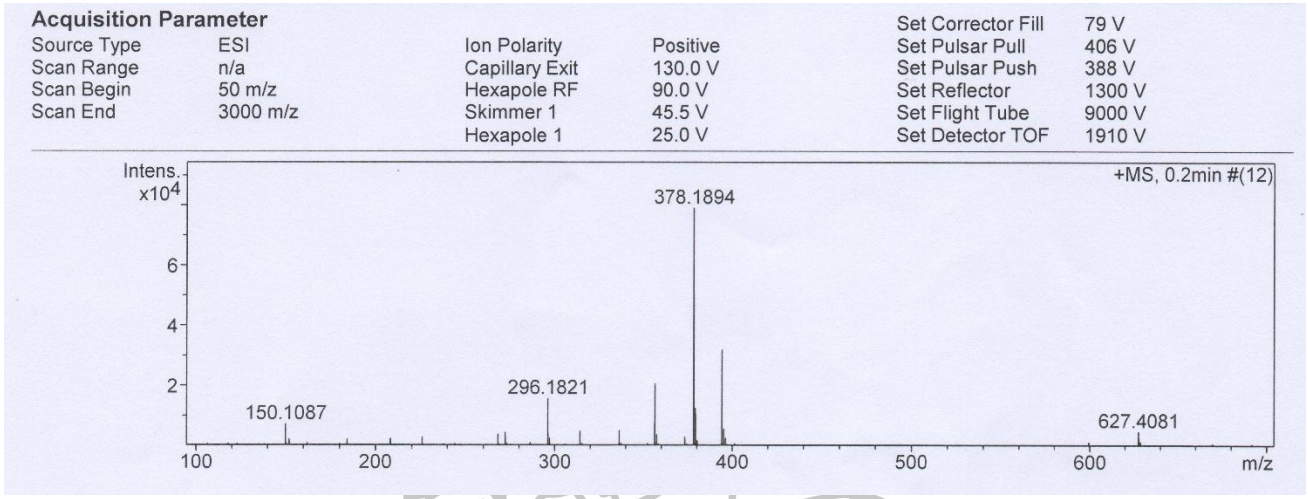
^{1}H NMR spectrum of compound **3d**

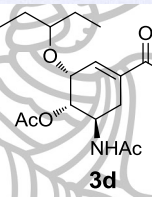


^{13}C NMR spectrum of compound **3d**



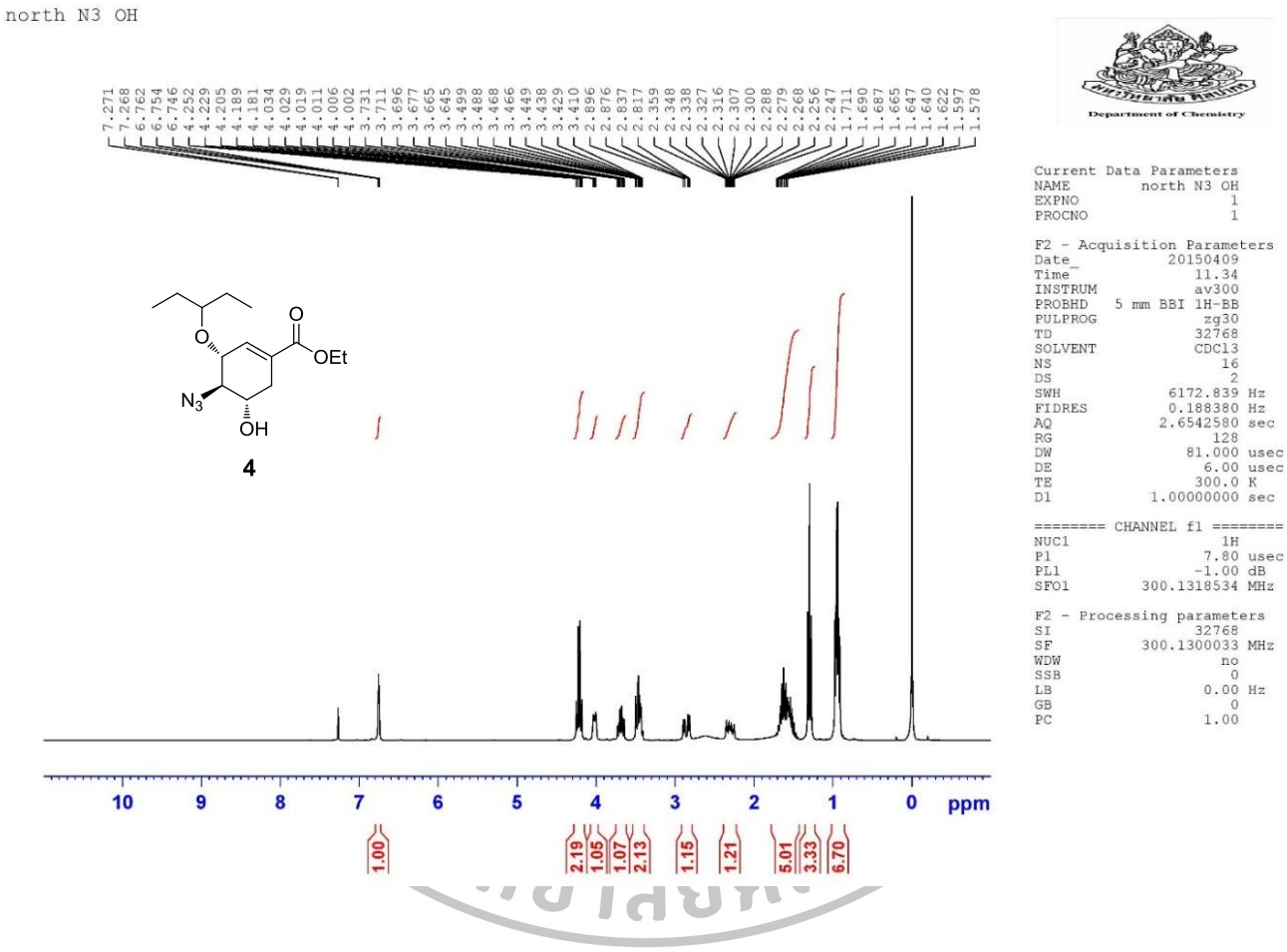
FTIR spectrum of compound **3d**



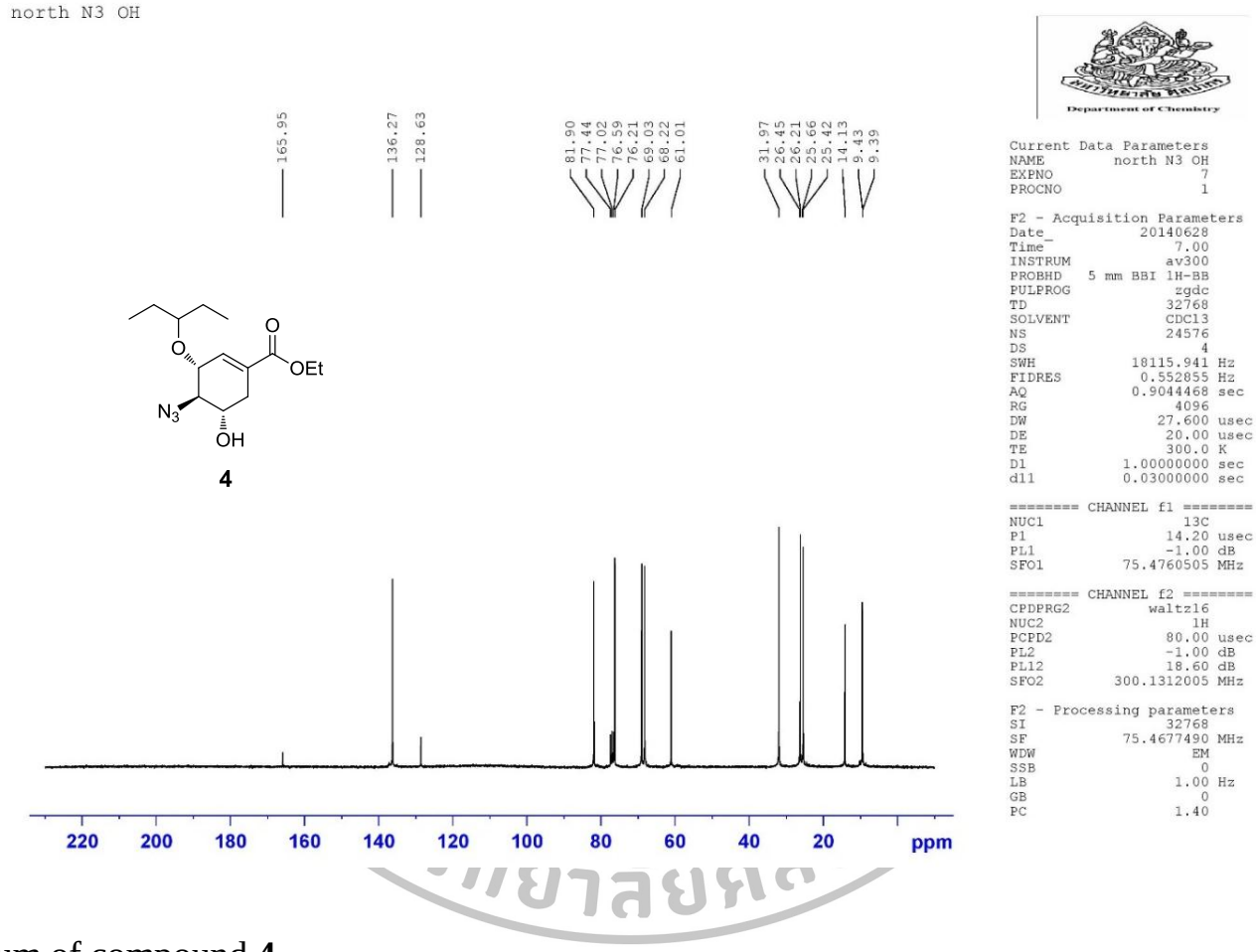


3d

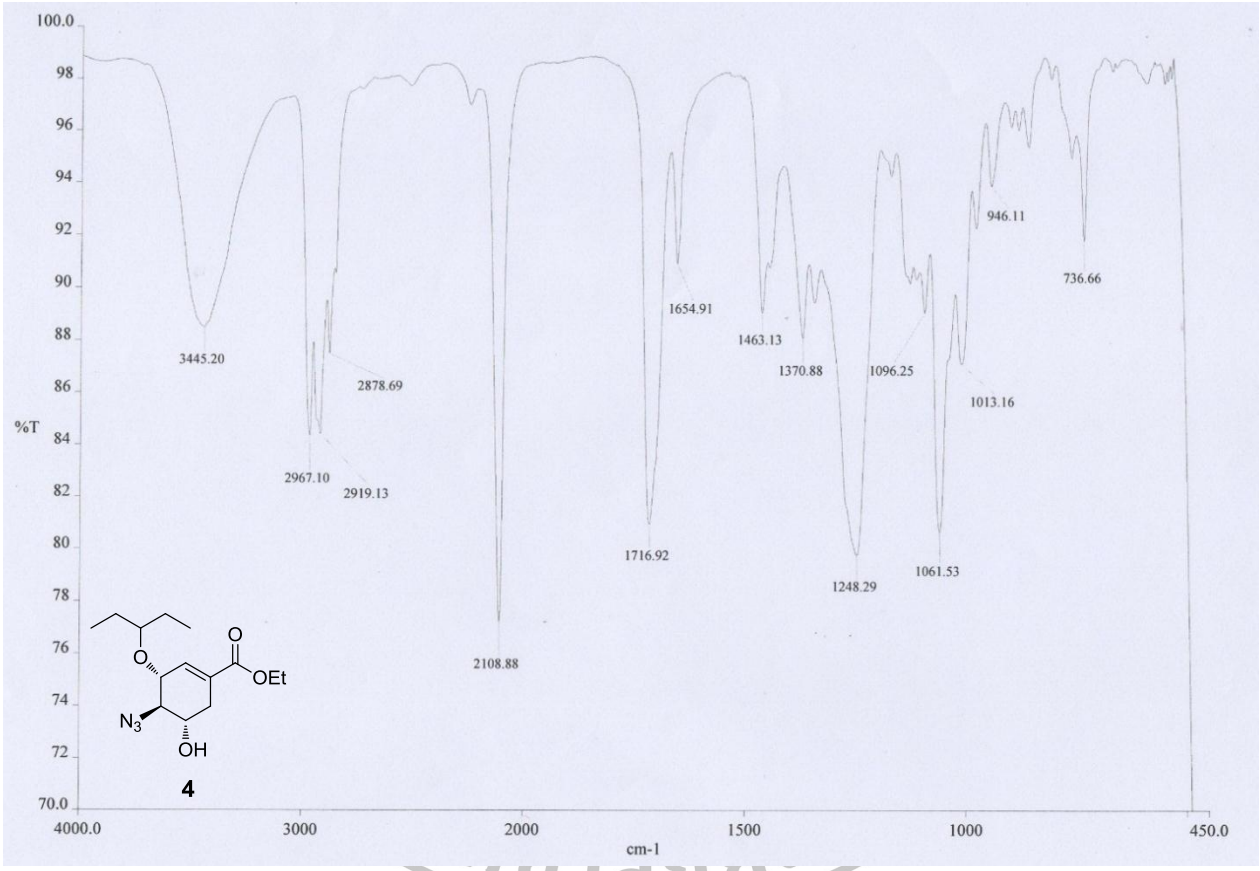
Mass spectrum of compound **3d**



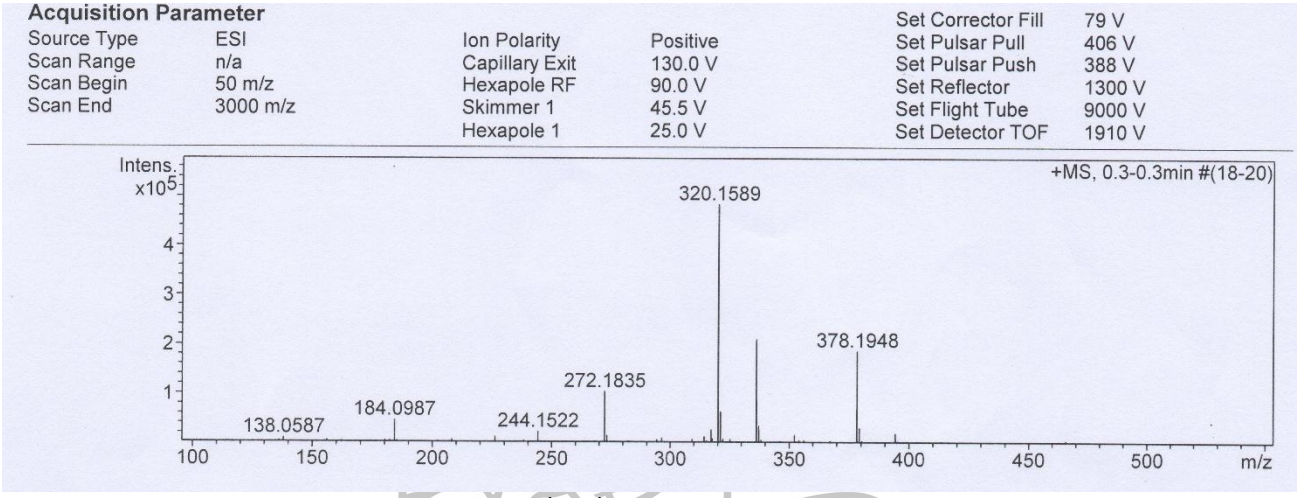
^{1}H NMR spectrum of compound **4**

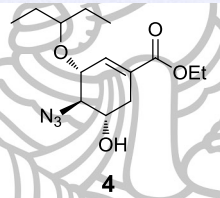


^{13}C NMR spectrum of compound **4**



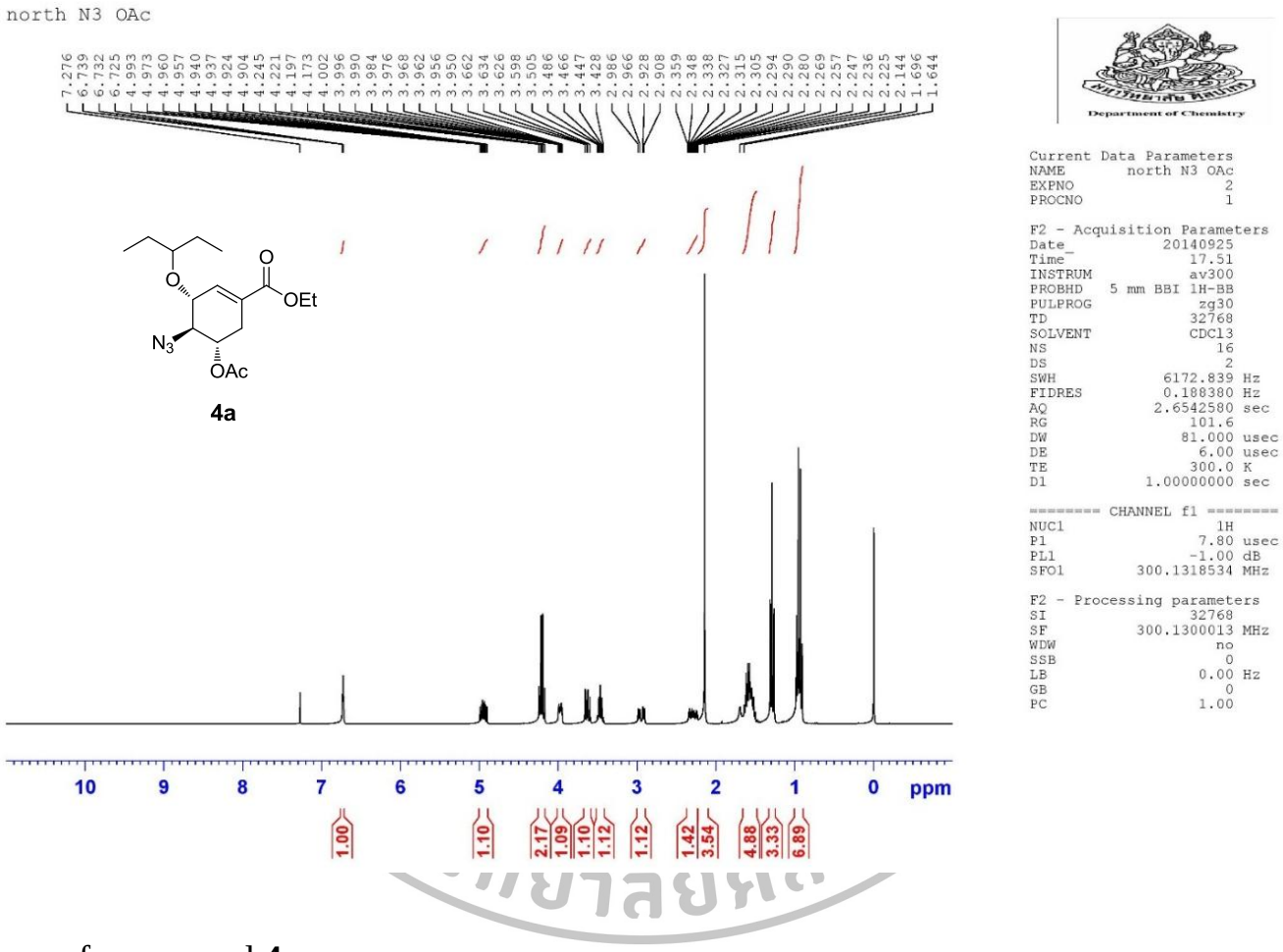
FTIR spectrum of compound **4**





4

Mass spectrum of compound **4**



Department of Chemistry

```
Current Data Parameters
NAME         north N3 OAc
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
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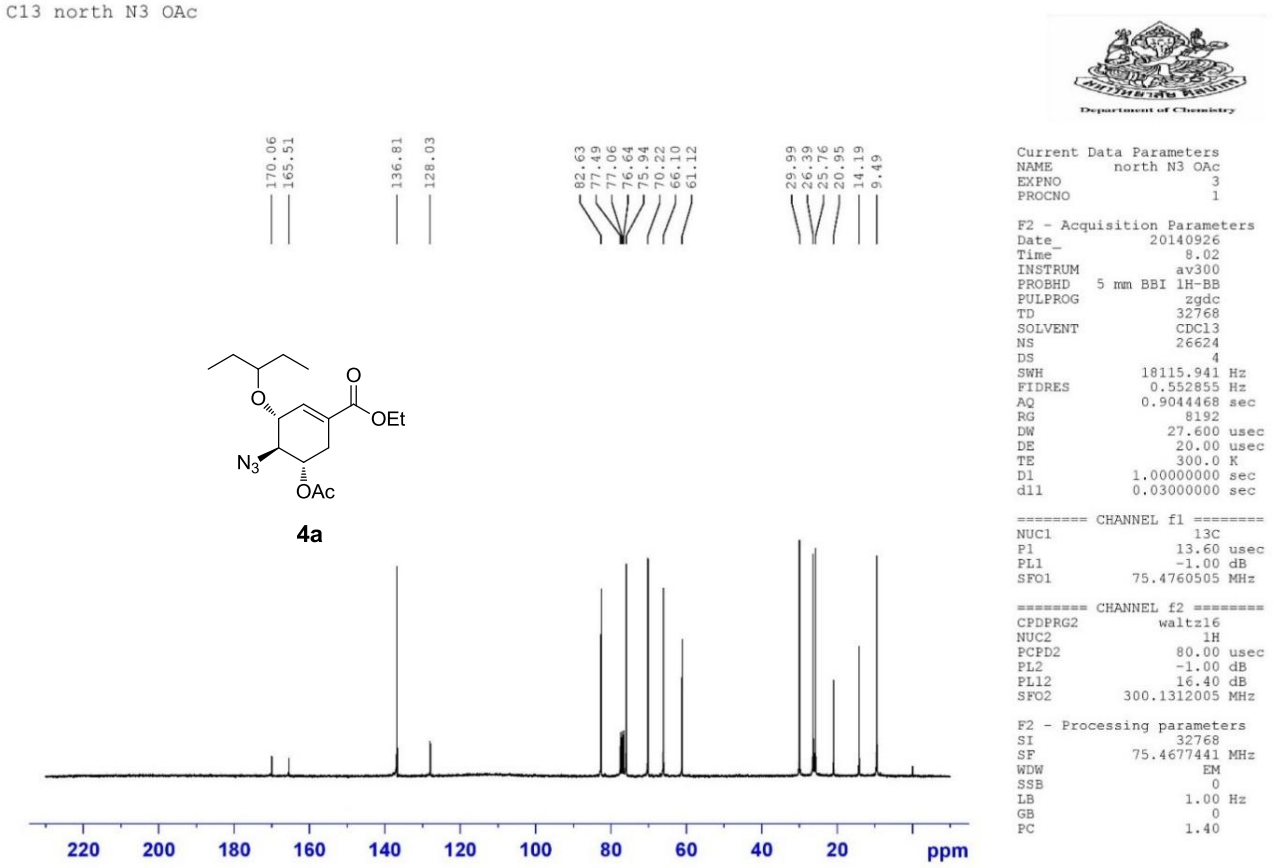
Time               17.51
INSTRUM            av300
PROBHD   5 mm BBI 1H-BB
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6172.839 Hz
FIDRES             0.188380 Hz
AQ              2.6542580 sec
RG                 101.6
DW                81.000 usec
DE                 6.00 usec
TE                300.0 K
D1             1.00000000 sec

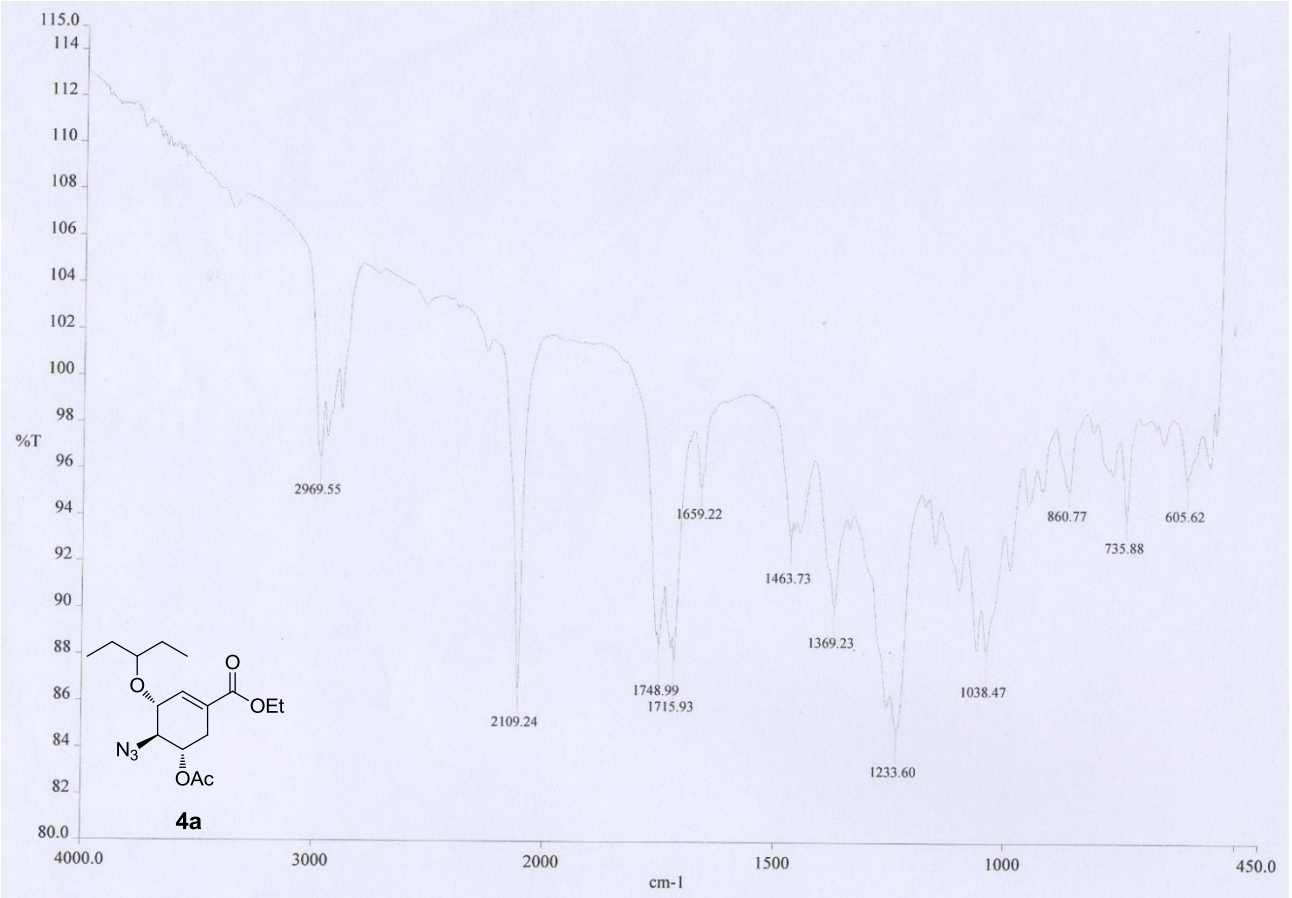
======== CHANNEL f1 ========
NUC1                   1H
P1                  7.80 usec
PL1                -1.00 dB
SFO1         300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF           300.1300013 MHz
WDW                   no
SSB                    0
LB                  0.00 Hz
GB                     0
PC                  1.00
```

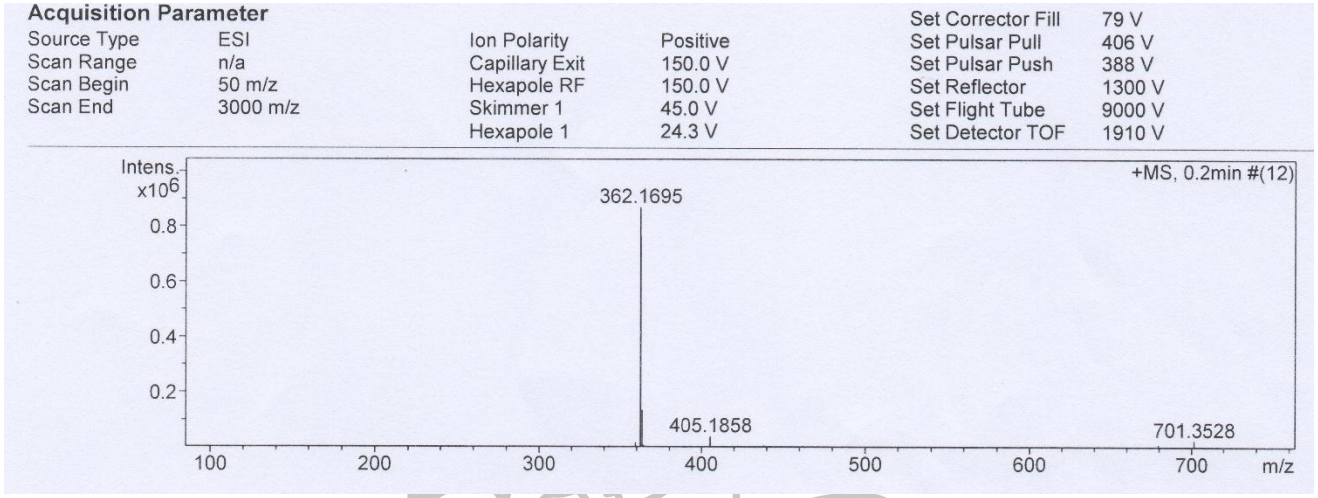
^{1}H NMR spectrum of compound **4a**

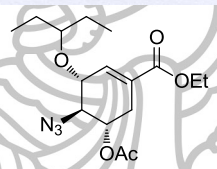


^{13}C NMR spectrum of compound **4a**



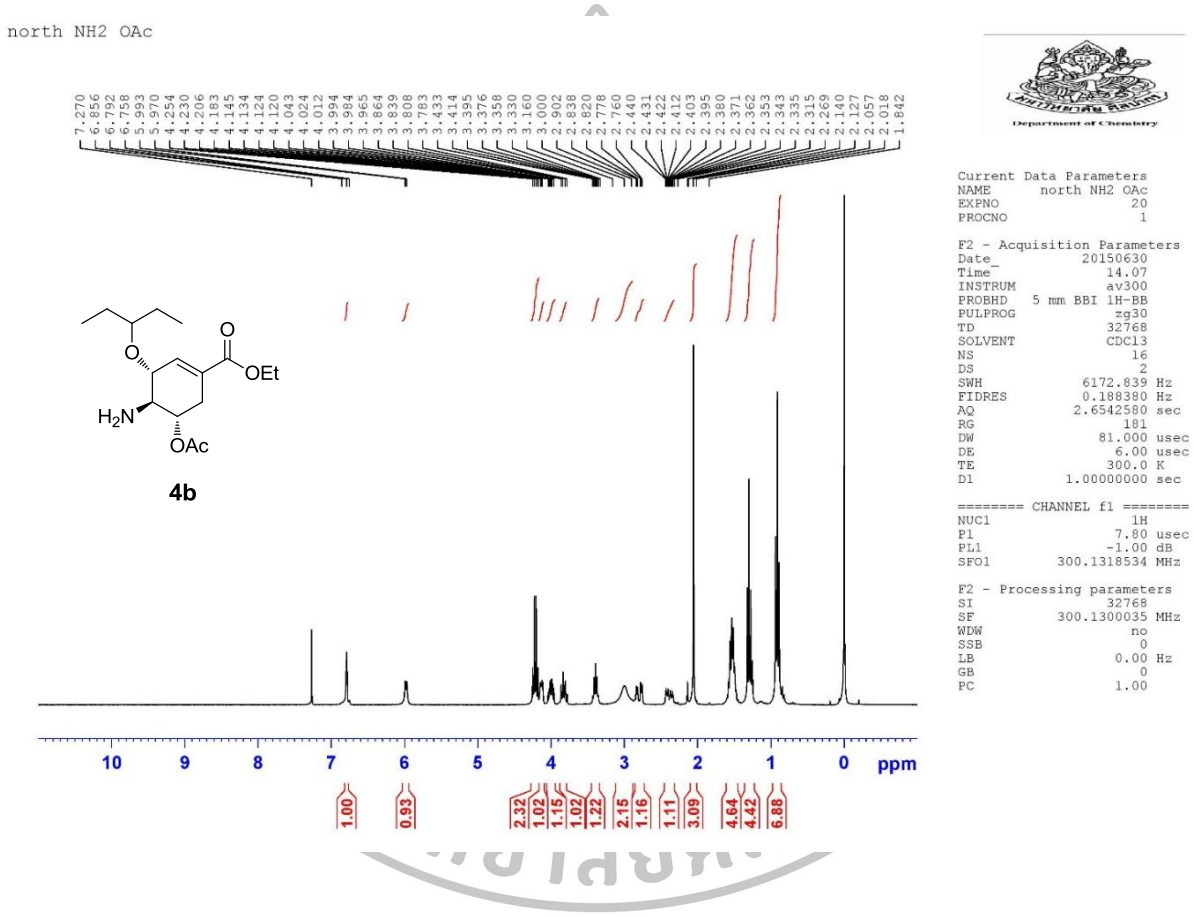
FTIR spectrum of compound **4a**



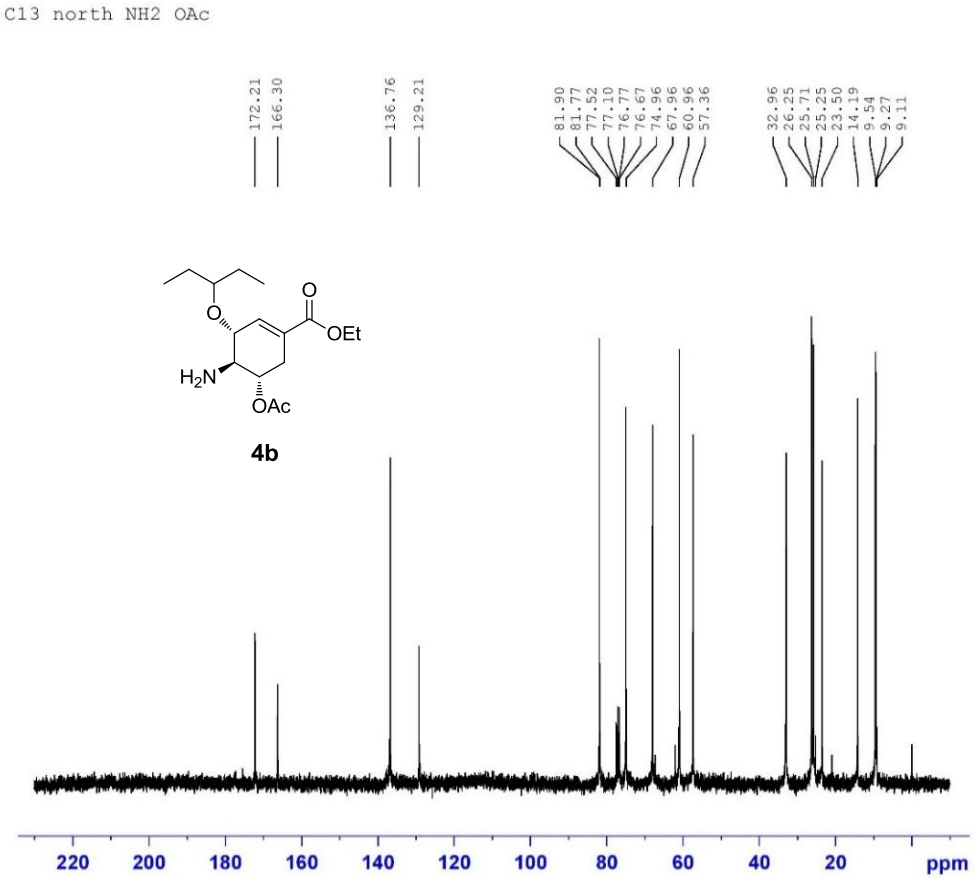


4a

Mass spectrum of compound **4a**



^{1}H NMR spectrum of compound **4b**

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EXPNO                 17
PROCNO                 1

F2 - Acquisition Parameters
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Time              12.45
INSTRUM           av300
PROBHD   5 mm BBI 1H-BB
PULPROG           zgdc
TD               32768
SOLVENT          CDCl3
NS                1840
DS                   4
SWH           18115.941 Hz
FIDRES        0.552855 Hz
AQ            0.9044468 sec
RG               5160.6
DW               27.600 usec
DE               20.00 usec
TE               300.0 K
D1           1.00000000 sec
d11          0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1               13.60 usec
PL1              -1.00 dB
SFO1         75.4760505 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2               1H
PCPD2            80.00 usec
PL2              -1.00 dB
PL12             16.40 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI               32768
SF          75.4677445 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
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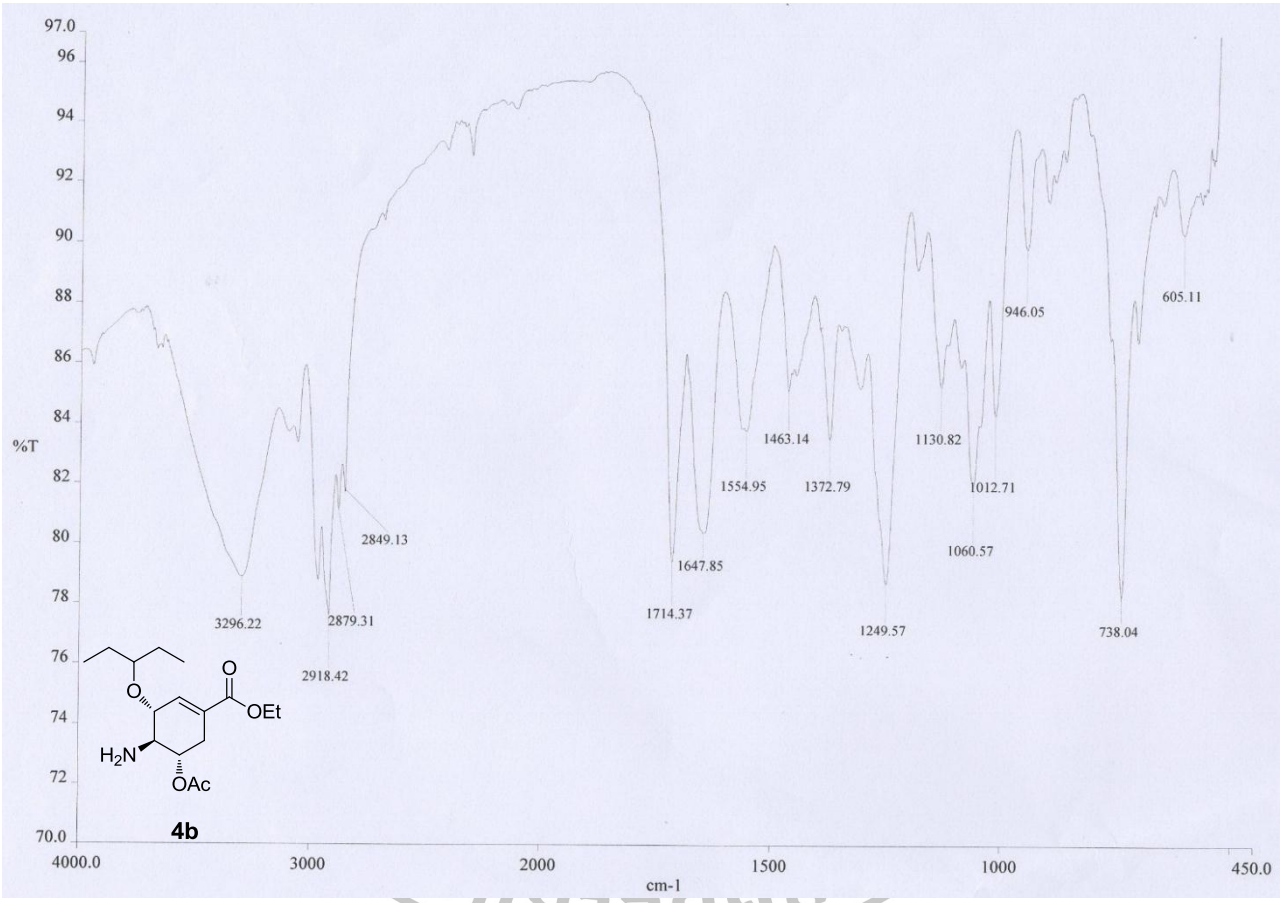
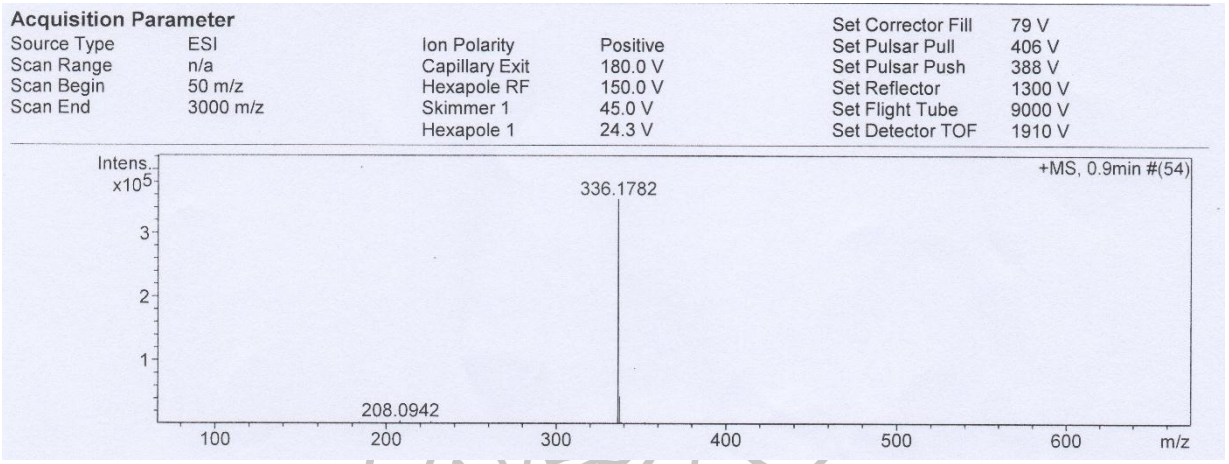
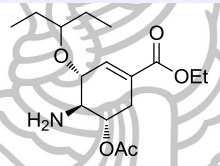
^{13}C NMR spectrum of compound **4b**



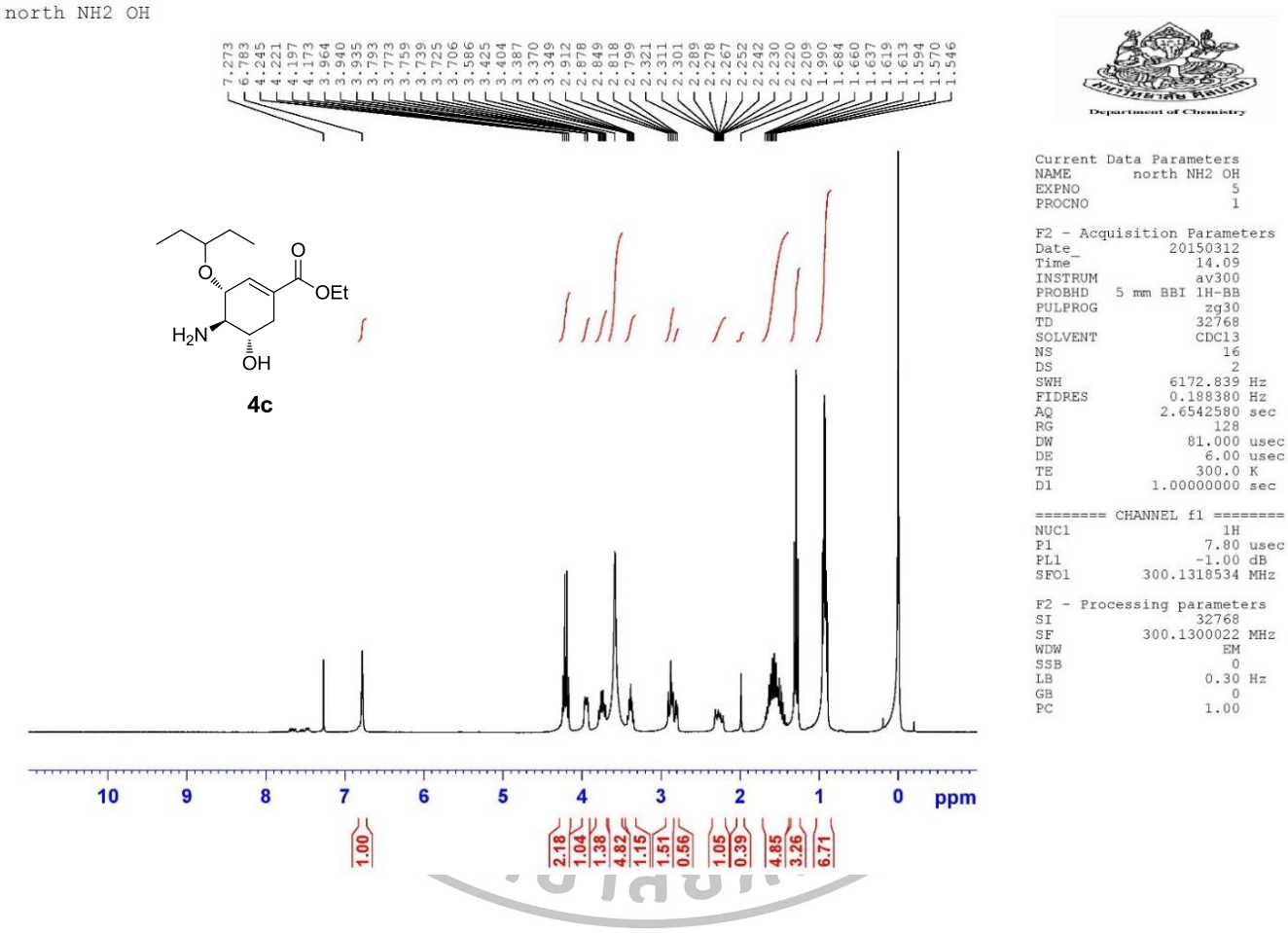
FTIR spectrum of compound **4b**



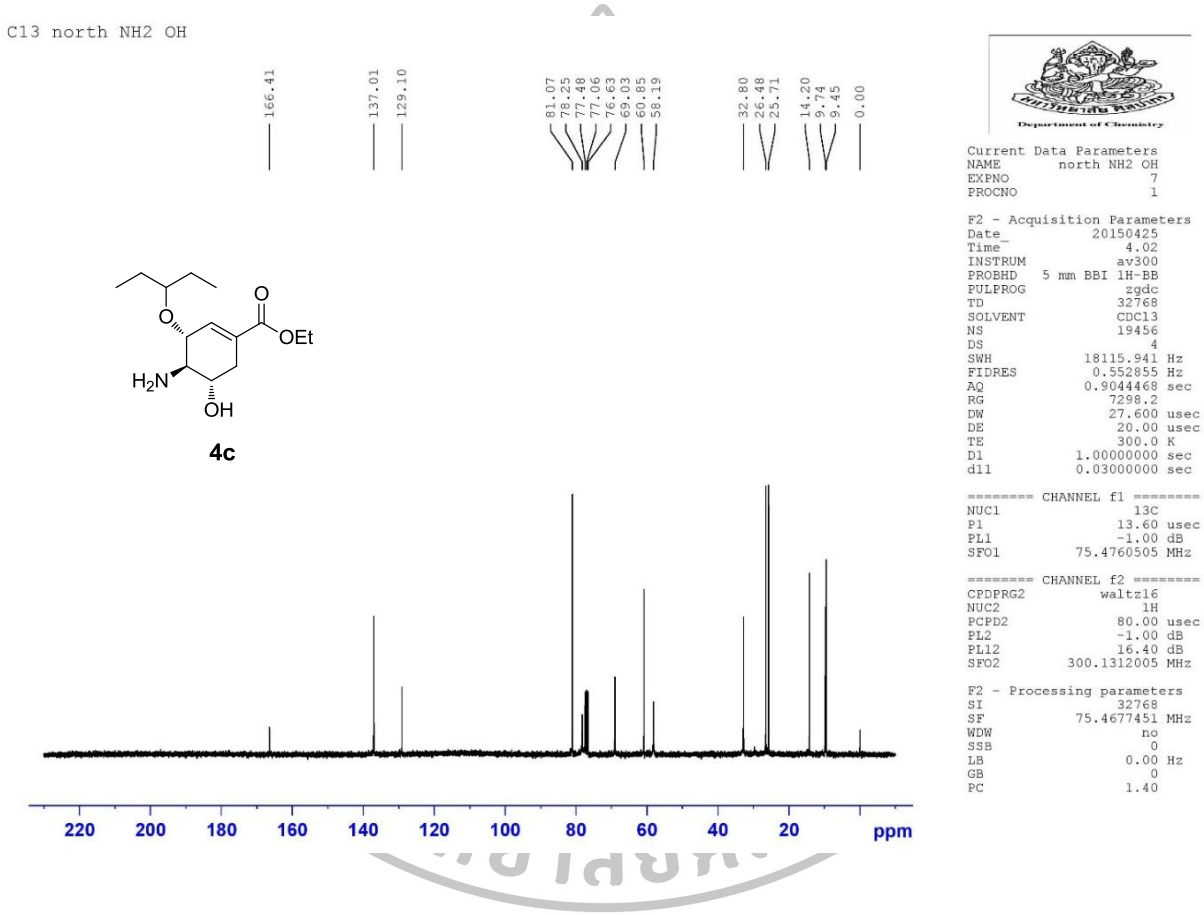


4b

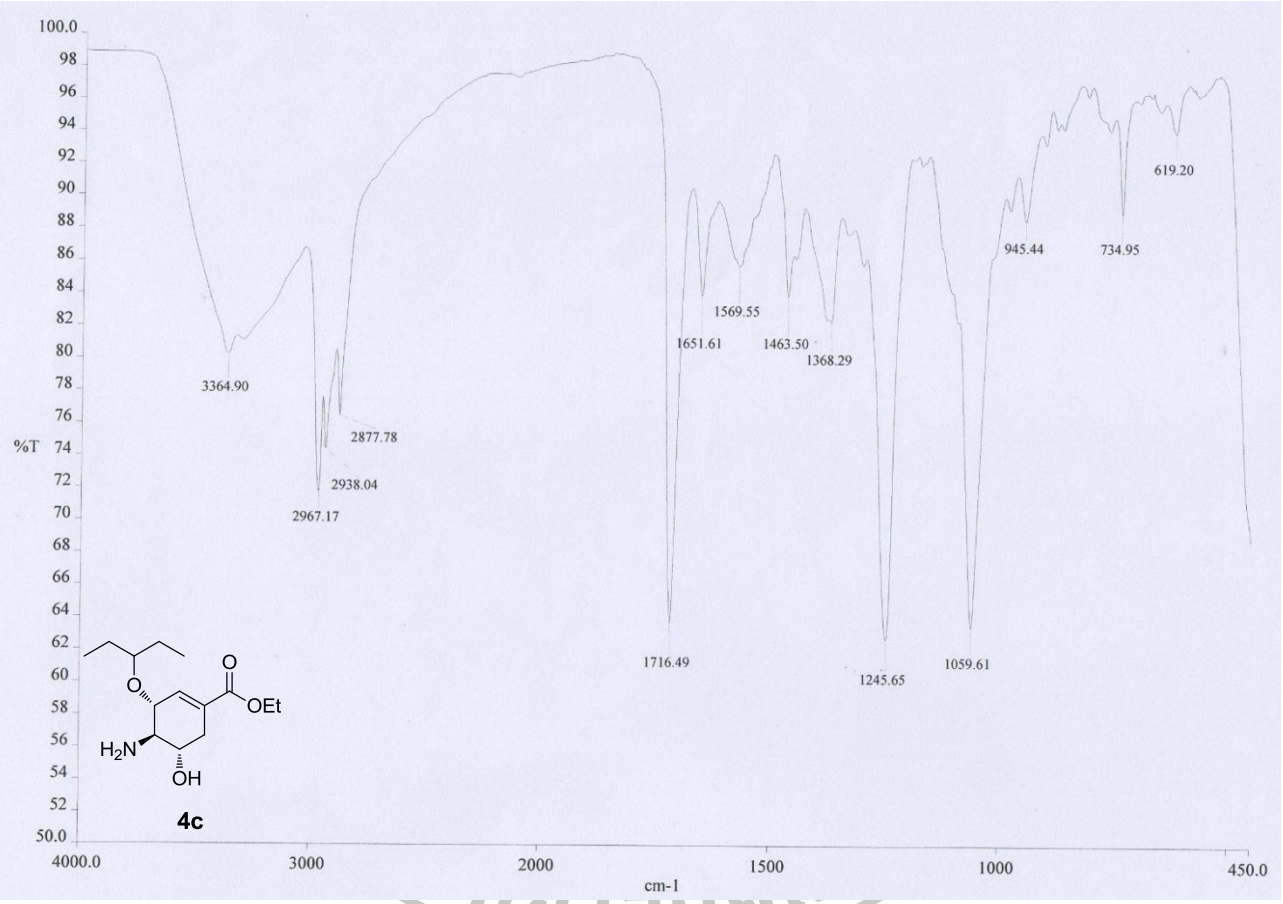
Mass spectrum of compound **4b**



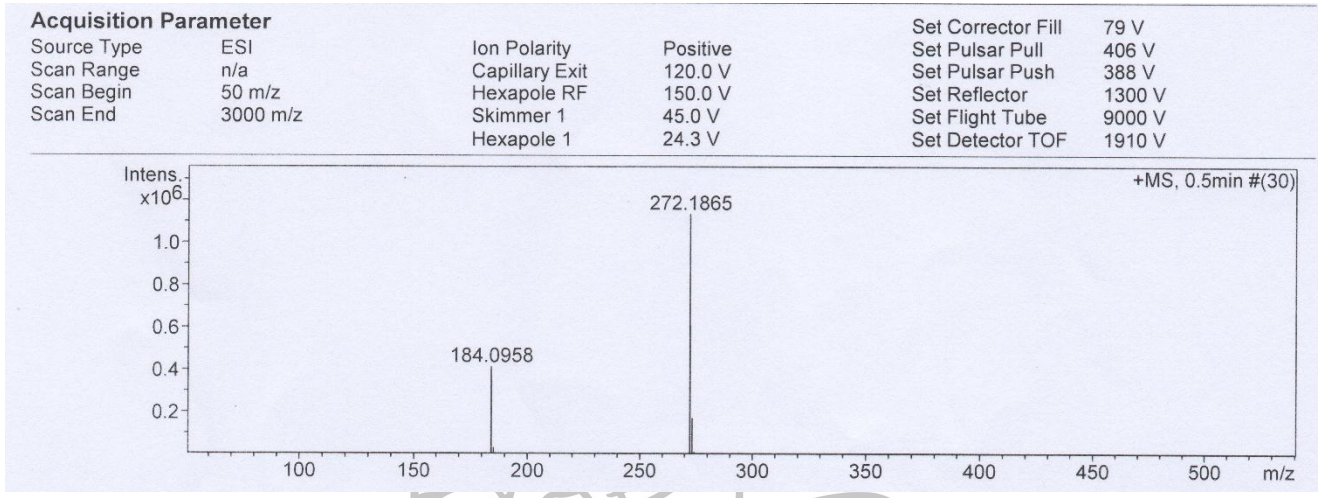
^{1}H NMR spectrum of compound **4c**

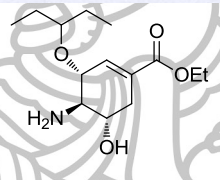


^{13}C NMR spectrum of compound **4c**



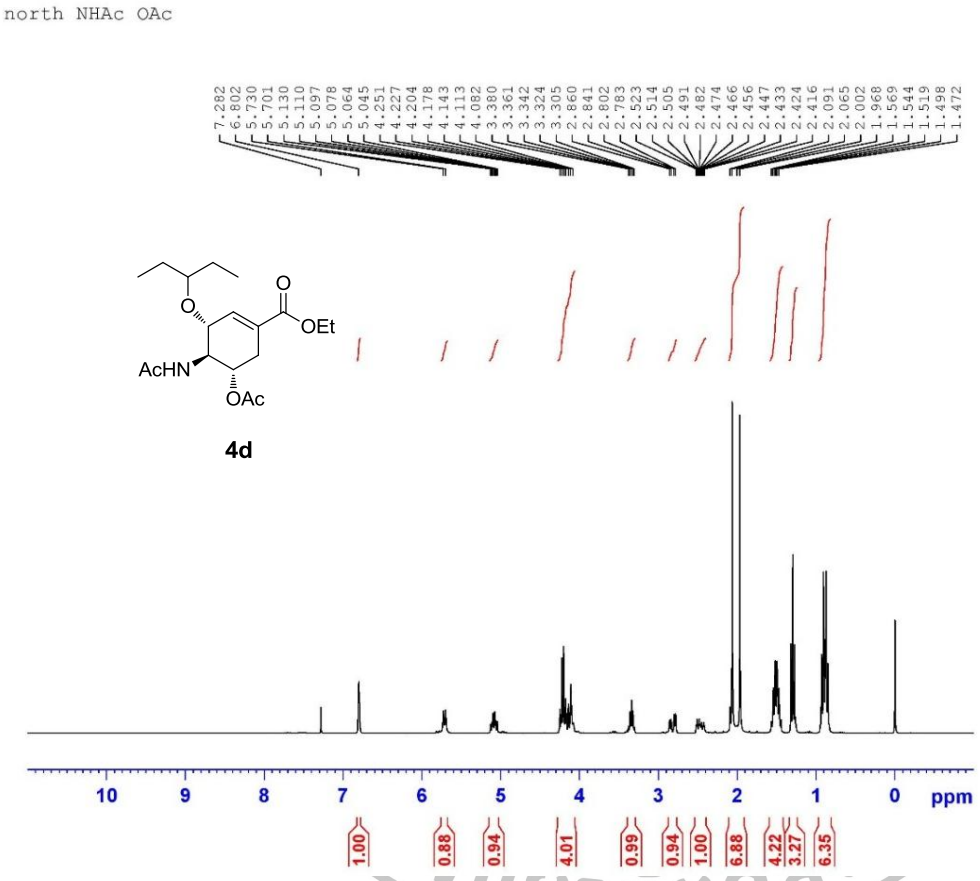
FTIR spectrum of compound **4c**





4c

Mass spectrum of compound **4c**



Department of Chemistry

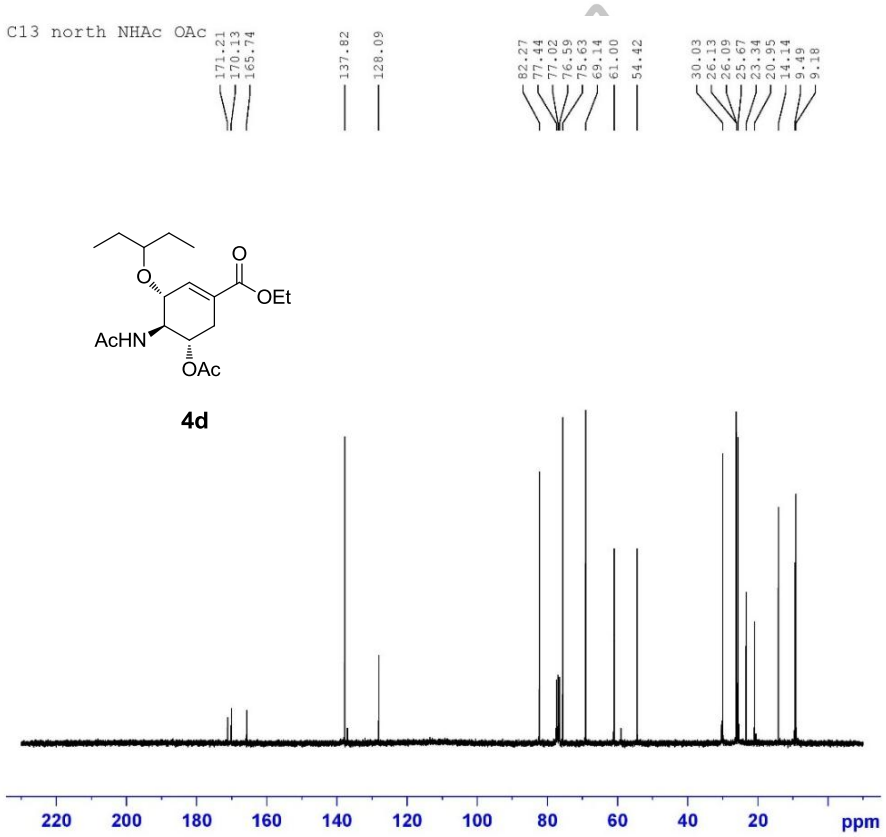
```
Current Data Parameters
NAME      north NHAc OAc
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150511
Time_              17.24
INSTRUM            av300
PROBHD   5 mm BBI 1H-BB
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    16
DS                     2
SWH             6172.839 Hz
FIDRES          0.188380 Hz
AQ              2.6542580 sec
RG                   128
DW                81.000 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 7.80 usec
PL1               -1.00 dB
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                 32768
SF          300.1299992 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{13}C NMR spectrum of compound **4d**



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Current Data Parameters
NAME      north NHAc OAc
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150509
Time                4.25
INSTRUM            av300
PROBHD   5 mm BBI 1H-BB
PULPROG             zgdc
TD                 32768
SOLVENT            CDCl3
NS                 19456
DS                     4
SWH           18115.941 Hz
FIDRES         0.552855 Hz
AQ             0.9044468 sec
RG                5160.6
DW                27.600 usec
DE                20.00 usec
TE                300.0 K
D1            1.00000000 sec
d11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                13.60 usec
PL1               -1.00 dB
SFO1          75.4760505 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             80.00 usec
PL2               -1.00 dB
PL12              16.40 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4677490 MHz
WDW                   no
SSB                    0
LB                 0.00 Hz
GB                     0
PC                 1.40
```
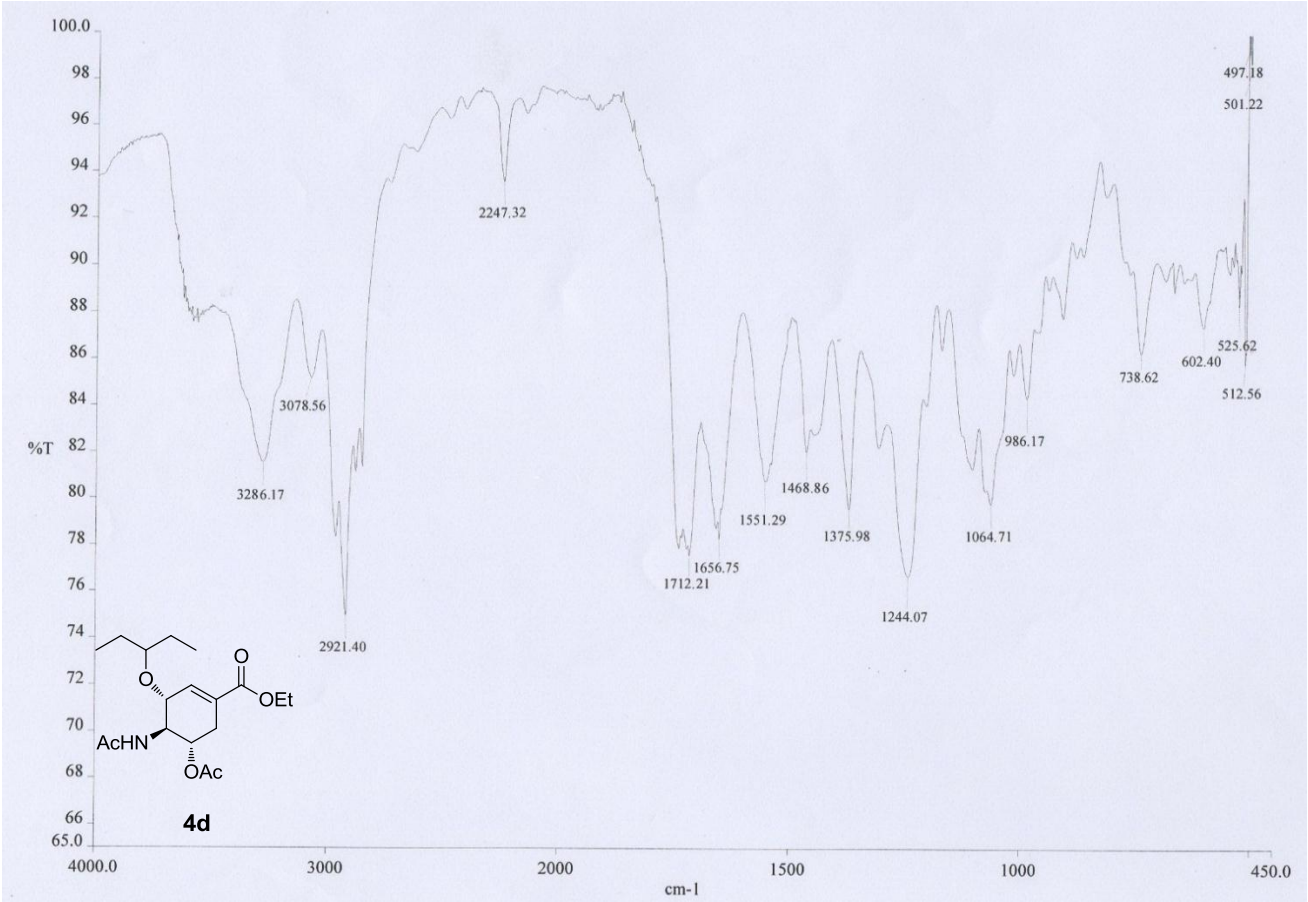
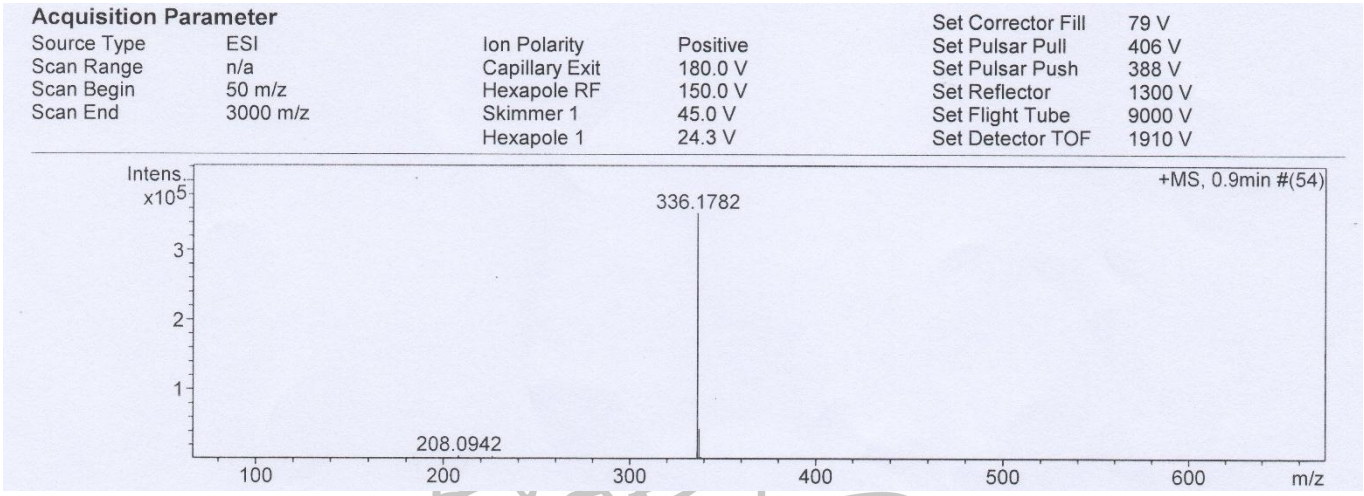
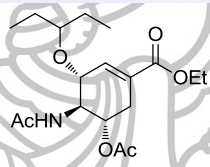

^{13}C NMR spectrum of compound **4d**



FTIR spectrum of compound **4d**





4d

FTIR spectrum of compound **4d**

Biography

Name	Mr.Kittiwat Srikittiwanna
ชื่อ-สกุล	**นายกิตติวัฏ ศรีกิตติวรรณา**
Email address	kittiwat.sri@gmail.com
Address	97, Soi Kanchanapisek 4/1, Ratchada Arcadian Village, Bangon, Bangkok, Thailand 101502

Education

2009-2013	Bachelor of Science (Chemistry), Silpakorn University, Thailand
2013-2016	Master of Science (Organic Chemistry),Silpakorn University, Thailand