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NEW SYNTHETIC STRATEGIES TOWARDS OSELTAMIVIR PHOSPHATE

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มูฮำหมัด นิยมเดชา : แนวทางใหม่ในการสังเคราะห์โอเซลทามิเวียร์ฟอสเฟต. (NEW SYNTHETIC STRATEGIES TOWARDS OSELTAMIVIR PHOSPHATE) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. ยงศักดิ์ ศรีธนาอนันต์: อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ.ดร. ธีรยุทธ วิไลวัลย์, 124 หน้า

โอเซลทามิเวียร์ฟอสเฟต หรือทามิฟลู เป็นยาที่มีฤทธิ์ยับยั้งเอนไซม์นิวรามินิเดสของไวรัสไข้หวัด และเป็นยาที่กำลังนิยมใช้อย่างกว้างขวางในการรักษาไข้หวัดที่มีอาการรุนแรง งานวิจัยนี้ได้ทำการสังเคราะห์โอเซลทามิเวียร์ฟอสเฟต **10** และอนุพันธ์บางชนิดเพื่อปรับปรุงกระบวนการและเพิ่มโอกาสการค้นพบยาชนิดใหม่ในกลุ่มนี้ที่จะรองรับปัญหาการดื้อยาของเชื้อไวรัสในอนาคต การสังเคราะห์เริ่มต้นจากสารจากธรรมชาติ คือ ชิคิมิค แอซิด **32** ผ่านปฏิกิริยา 11 ขั้นตอน ได้สารตัวกลางอิพอกไซด์ แล้วตามด้วยการสังเคราะห์ผ่านเส้นทางเอไซด์ใน 6 ขั้นตอน ได้โอเซลทามิเวียร์ฟอสเฟต **10** ในปริมาณ 10% จากชิคิมิค แอซิด **32** อนุพันธ์โอเซลทามิเวียร์ฟอสเฟต 5 ชนิด คือ **56**, **124** และ **130-132** ซึ่งเป็นอนุพันธ์ในกลุ่มของไดแอสเตอริโอเมอร์ 4*R*,5*S* โดยผ่านปฏิกิริยาการแทนที่แบบ S_N2 หรือปฏิกิริยาของมิทสึโนบุ เป็นขั้นตอนที่สำคัญ ได้ปริมาณผลิตภัณฑ์ 18%, 18%, 40%, 40%, และ 29% จากชิคิมิค แอซิด **32** ตามลำดับ นอกจากนี้ยังสามารถสังเคราะห์อนุพันธ์ของโอเซลทามิเวียร์ฟอสเฟตในกลุ่มของไดแอสเตอริโอเมอร์ 4*S*,5*R* อีก 4 ชนิด คือ **121-123** และ **125a** ได้ปริมาณผลิตภัณฑ์ 41%, 35%, 37% และ 62% จากชิคิมิค แอซิด **32** ตามลำดับ โดยผ่านปฏิกิริยาการแทนที่อิพอกไซด์ **28** หรือไม่มีการแทนที่

ภาควิชา เคมี ลายมือชื่อนิสิต.....

สาขาวิชา เคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS: OSELTAMIVIR, TAMIFLU, INFLUENZA

MUHAMMAD NIYOMDECHA: NEW SYNTHETIC STRATEGIES TOWARDS OSELTAMIVIR PHOSPHATE. THESIS ADVISOR: ASST. PROF. YONGSAK SRITANA-ANANT, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. TIRAYUT VILAIVAN, D.Phil., 124 pp.

Oseltamivir phosphate or Tamiflu®, the inhibitor neuraminidase enzyme of influenza virus, is currently in use widely for acute influenza treatment. This research aimed to synthesize oseltamivir phosphate **10** and its derivatives to survey potentially improved process and enhance the possibility to discover new drugs in this family that could lead to future treatment of the emerging resistant strain. The synthesis started from naturally available (-)-shikimic acid **32**, through 11 steps yielding epoxide intermediate, followed by additional 6 steps in the azide route to provide oseltamivir phosphate **10** in overall yield of 10% from (-)-shikimic acid **32**. Five derivatives in the group of 4*R*,5*S* diastereomers, **56**, **124** and **130-132**, could be obtained via S_N2 substitution or Mitsunobu reaction as the key step in overall yields of 18%, 18%, 40%, 40%, and 29%, respectively from (-)-shikimic acid **32**. Another four derivatives in the group of 4*S*,5*R* diastereomers, **121-123** and **125a** could be obtained in 41%, 35%, 37% and 62%, respectively from (-)-shikimic acid **32** via substitution on epoxide **28** or none.

Department: Chemistry Student's signature.....
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CONTENTS

Page

Page

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

Page

Figure

Figure Page

LIST OF SCHEMES

Scheme Page

LIST OF ABBREVIATION

Ac_2O	: acetic anhydride
AcCl	: acetyl chloride
$AlCl_3$	: aluminun chloride
$BF_3.OEt_2$	: trifluoroboron diethyletherate
$(Boc)_2O$	: *tert*-butyl pyrocarbonate
t-BuOH	: *tert*-butanol
^{13}C-NMR	: carbon-13 nuclear magnetic resonance spectroscopy
Cbz	: benzyloxycarbonyl
CH_2Cl_2	: dichloromethane
$CDCl_3$	: deuterated chloroform
DMF	: *N, N*-dimethylformamide
DMSO	: dimethyl sulfoxide
D_2O	: deuterated water
DIAD	: diisopropylazodiimide
DPPA	: diphenyl phosphoryl azide
DDQ	: dichlorodicyano benzoquinone
EtOAc	: ethyl acetate
EtOH	: ethanol
Et_3SiH	: triethylsilane
FT-IR	: fourier-transform infrared spectrophotometer
HA or H	: hemaglutinin
^{1}H-NMR	: proton nuclear magnetic resonance spectroscopy
HN_3	: hydrazoic acid
IC_{50}	: inhibitory concentration
MeOH	: methanol
MOM	: methyl ether methylchloride
MsCl	: methaesulfonyl chloride
$NaHCO_3$	: sodium hydrogen carbonate or sodium bicarbonate
NMR	: nuclear magnetic resonance spectroscopy

$NaBH_4$	: sodium borohydride
$NaIO_4$	: sodium periodate
NA or N	: nuraminidase
NaN_3	: sodium azide
NH_4Cl	: ammonium chloride
NBA	: *N*-bromoacetamide
NANA	: nuraminic acid
py.	: pyridine
Ph_3P	: triphenylphosphine
SES	: 2-(trimethylsilyl)ethanesulfonamide
$SOCl_2$	: thionyl chloride
TBDPS	: *tert*-butyldipropylsilyl
$TMSN_3$	: trimethyl silyl azide
TfOH	: trifluoromethanesulfonic acid
$TiCl_4$	: titanium chloride
TMSCN	: trimethylsilylcyanide
TBAF	: tetrabutylamonium fluoride
THF	: tetrahydrofuran
TLC	: thin layer chromatography
d	: doublet
dd	: double of doublet
Hz	: Hertz
J	: coupling constant
mL	: milliliter
min	: minute
m	: multiplet
ppm	: parts per million (unit of chemical shift)
q	: quartet
δ	: chemical shift
μ	: micro
s	: singlet
t	: triplet

CHAPTER I

INTRODUCTION

1.1 Avian Influenza Virus

The advent of Avian influenza and the recent 2009 Swine flu have triggered another concern of pandemic in which the history has shown millions of people could have been killed worldwide. Since 1500s, the world has seen 22 influenza pandemics, one every 25 years on average. The most recent ones in the 20^{th} century were the Spanish flu (1918-1919), which killed 40-50 million people worldwide with H1N1 type. In 1957-1958, the Asian flu caused by the H2N2 type killed about 1-2 million casualties. The Hong Kong flu (1968-1969) caused by the H3N2 type killed approximately 700,000. It is estimated that if a similar event took place today, about 30% of the world's population could die. With very high fatality rate of over 50% from the H5N1 strain avian flu virus infection, an effective treatment of the infected patients in urgently needed [1].

The influenza virus is a type A virus in the *Orthomyxoviridae* species [2]. It is a RNA virus that has two types of protein antigen on its surface together with the important M_2 ion channel. The two types of protein are Hemagglutinin (H or HA) with known 16 subtypes, and Neuraminidase (N or NA) with known 9 subtypes. An influenza virion budding from an infectd cell binds to terminal sialic acid residue residue on the host cell surface glycoprotein bound with HA. NA hydrolytically cleaves the glycosidic bond of sialic acid to release the virus from the host cell surface **(Figure 1.1)**. This process liberates the budding virion from the infected cell and is essential for spreading the infection. As expected, the active site of NA is highly conserved across the influenza A and B virus strains. Therefore, an NA inhibitor is a prime candidate for broad spectrum anti-influenza drugs [2].

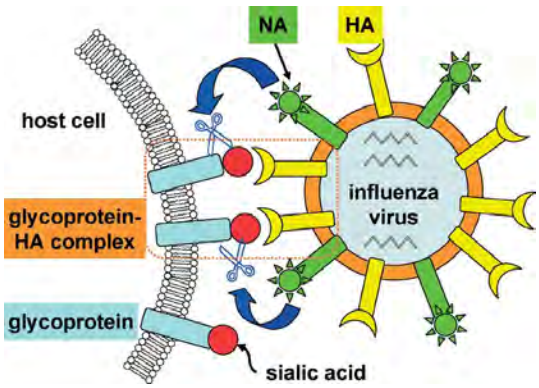


Figure 1.1 Schematic representation of an influenza virion budding from a host cell [38]

The avian H5N1 influenza virus, which originated in Hong Kong in 1997, has such characteristics. In Thailand were reported outbreaks of the H5N1 at Supanburi and Kanchanaburi provinces in January, 2004 [2]. Currently, the virus does not widely spread from human to human, although there are fears that it will soon gain the infectious ability to do that. Because of today's extensive global transport, a local influenza epidemic cannot be restricted to a specific area. Therefore, the number of patients could increase explosively in several remote places simultaneously.

1.2 Design, Synthesis and Structure Activity Relationship Studies of Influenza Neuraminidase Inhibitor [11]

NA is recognized as a potential target for developing agents against influenza infection. It has been proposed that sialic acid **1** cleaved by NA might progress via oxonium cation transition state **3** (**Figure 1.2**)

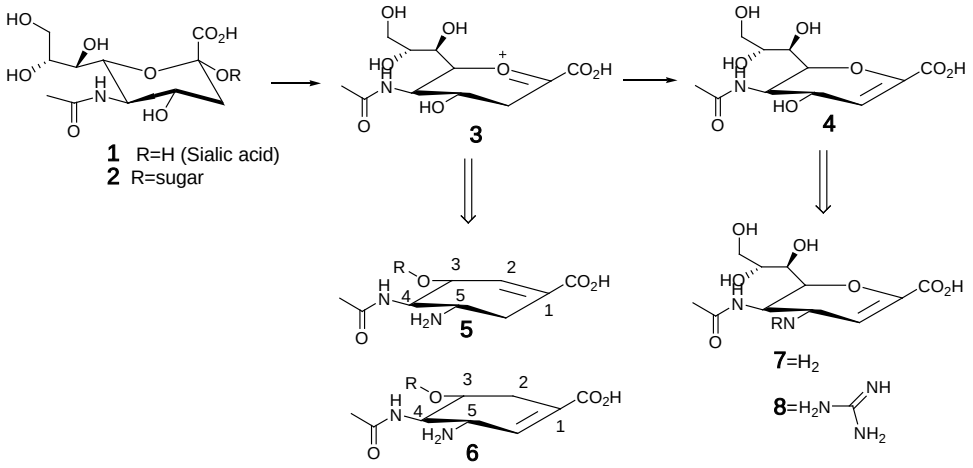


Figure 1.2 Rational design of carbocyclic transition state analogues

On the basis of structure information generated from the X-ray crystallographic study of 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (Neu5Ac2en, **4**), 2,3-dihydro-2,4-dideoxy-4-amino-*N*-acetylneuraminic acid (4-amino-Neu5Ac2en, **7**) and its guanidine analogue (4-guanidino-Neu5Ac2en, **8**), with NA, and comparison of potent NA inhibitors from structure-activity studies of series of carbocyclic analogues, **5**, **6** and **9**, the 3-pentyloxy moiety was identified as an apparent optimal group at the C3 position as in **9 (Figure 1.3)** and shown in **Table 1.1**. [11]

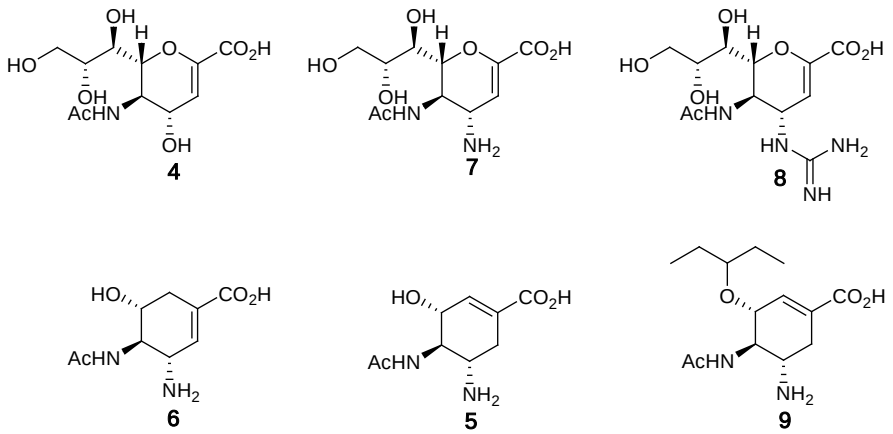


Figure 1.3 Structures of carbocyclic analogues

Table 1.1 Influenza NA inhibitory activity of carbocyclic analogues.

compound	Influenza NA inhibitory Activity (IC_{50},μM)
8	0.0001
4	4
7	0.001
6	>200
5	6.3
9	1

In 1997, Kim and coworkers [11] had reported the design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity, the crystal structure of the potent inhibitor **9** bound to NA was investigated as shown in **Figure 1.4.**

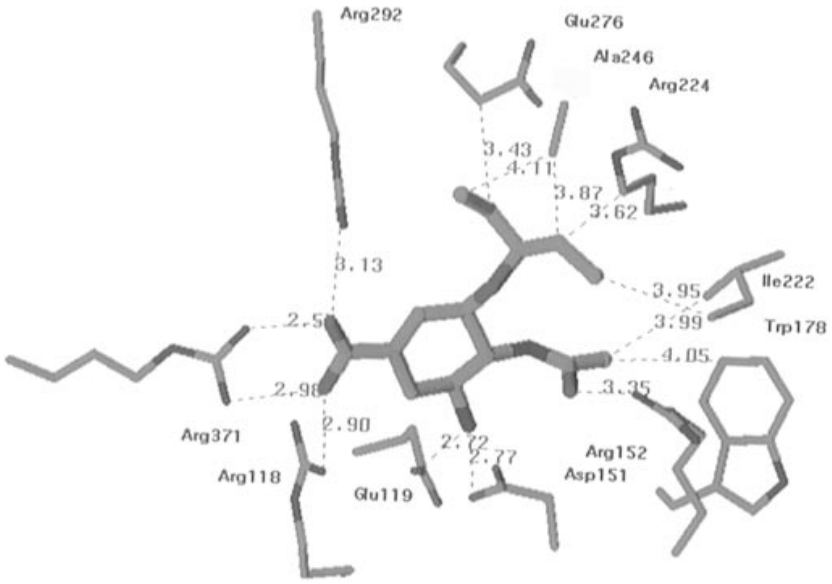
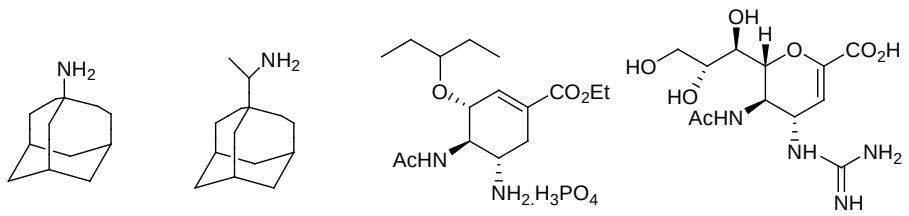


Figure 1.4 X-ray structure of **9** bound to influenza neuraminidase

1.3 Treatment

Currently, there are two groups of antiviral drugs available for the treatments of influenza infections. **(Figure 1.5)** The M_2 ion channel inhibitors, amantadine **11** and rimantadine **12**, are no longer recommended for most treatments due to the widespread resistance of the flu virus strains [2]. The newer drugs, the neuraminidase inhibitors, including oseltamivir phosphate (Tamiflu®) **10** and zanamivir (Relenza®) **8** [3-4], become the only group of drugs that are still effective against most strains of flu virus. Both influenza A and B showed fewer associated side effects with this group in comparison with those earlier adamantane-type drugs.



Amantadine **11** | Rimantadine **12** | Oseltamivir phosphate **10** | Zanamivir **8**

Figure 1.5 The four drugs available for treatment of influenza infections

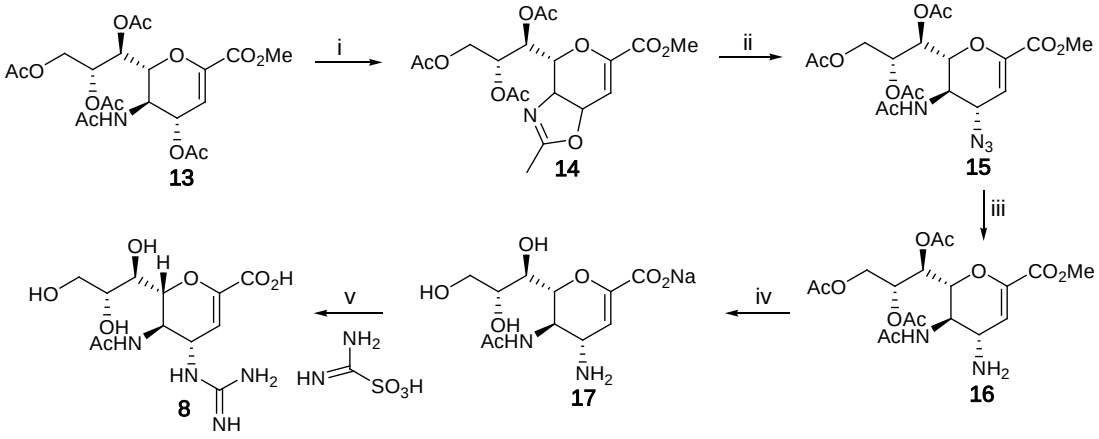
Zanamivir (Relenza®) **8** was discovered at Biota Holdings and further developed and patented by Glaxo Smith-Kline in 1990[4]. Its requirement of an inhaler makes it inappropriate for children or people suffering from asthma. Due to such inconvenience, the orally active oseltamivir phosphate (Tamiflu®) **10** became favored because of the usage. The latter drug was discovered at Gilead Sciences, co-developed with Roche, and approved into market in 1999, **(Figure 1.6)** [13].

Figure 1.6 Oseltamivir phosphate (Tamiflu®) **10**, zanamivir (Relenza®) **8** and rimantadine **12**.

The neuraminidase is a protein that will help spread the influenza virus into other cells. Oseltamivir phosphate **10** is a neuraminidase inhibitor designed to bind with the neuraminidase protein, one of the two identified major surface structures of the influenza virus. Since the protein receptor sites are nearly identical in all common strains of influenza, Oseltamivir phosphate **10** became the first neuraminidase inhibitor in pill form that is effective in preventing the spread of both types A and B strains of the virus within the body. This is in contrary to earlier drugs which were effective in treating only one strain [13].

1.4 Synthesis of zanamivir (Relenza®)

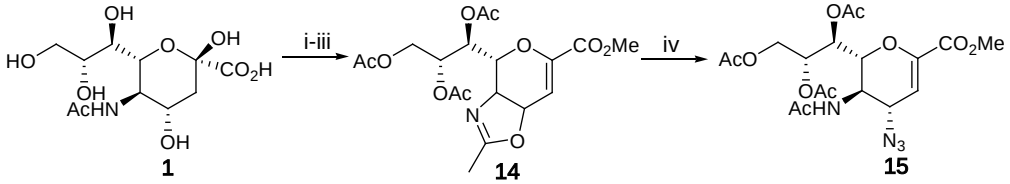
In 1994, Itstein and coworker [5] was first reported for the synthesis of zanamivir **8** **(Scheme 1.1)**, the Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-*D*-glycero-*D*-galacto-non-2-enoate (Neu4,5,7,8,9$Ac_5$2en1Me, **13**) was treated with $BF_3.OEt_2$ to give allylic oxazoline **14**, which was attacked by azide group to provide intermediate **15**. Hydrogenation of **15** with 10% Pd/C afforded amine **16**, ester hydrolysis with resin and aq. NaOH, followed by neutralization with H^+ provided sodium salt **17**. The zanamivir **8** was accomplished with aminoiminomethanesulfonic acid in a high yield of 30%.



i: $BF_3.OEt_2$, MeOH, CH_2Cl_2, 25-30 ^oC, 16h, 96%, ii: $TMSN_3$, *t*-BuOH, 80 ^oC, 4h, 83%, iii: H_2, Pd/C 10%, AcOH,MeOH,toluene, 1atm, 1h, 72%, iv: Amberlite IRA-400(OH^-), MeOH, rt, 3h, Dowex-50W X 8 (H^+), 91.6%, v: H_2O, K_2CO_3, 30-40 ^oC, 18h, 57%

Scheme 1.1 The synthesis of zanamivir **8**

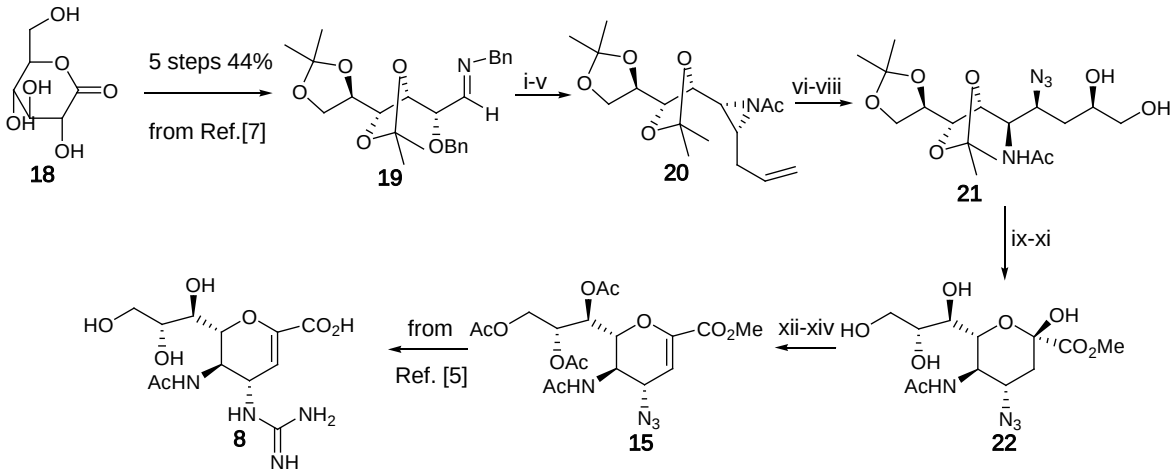
In 1995, Bamford et al. from Biota Holdings in Galaxo-Smith-Kline [37] synthezised and optimized resulting in improved yields of zanamivir **8** starting from commercially available *N*-acetyl-neuraminic acid **1** provided intermediate **15** (**Scheme 1.2**) and the completion of the synthesis of zanamivir **8** from **15** has been described in previous synthesis [5].



i:HCl gas, MeOH, 50 ^oC, 2.5h, 94%, ii: Ac_2O,DMAP, py, 0 ^oC to rt, 18h, iii: TMSOTf, EtOAc, 52 ^oC, 2.5h, 62%, 2 steps,
iv: $TMSN_3$, t-BuOH, 80 ^oC, 10.5h, 76%,

Scheme 1.2 Bamford's synthesis of intermediate **15**

In 2004, Yao and coworkers [6] reported the synthesis of zanamivir **8** from *D*-glucose. **(Scheme 1.3)** *D*-glucono-δ-lactone **18** was transformed to the imine **19** and treating with allyl magnesium bromide, Acetylation, deprotection, mesylation and aziridine **20** formation. The ring opening of aziridine **20**, with NaN_3, NH_4Cl in EtOH, followed by acetylation and subsequent dihydroxylation to give compound **21**. The intermediate **22** was completed via selective oxidation, esterification and cyclic formation by cleaved in the final step, which was acetylation with Ac_2O, followed by selective chloro substitution, and subsequent HCl elimination obtained azido compound **15**, respectively. The completion of the synthesis of zanamivir **8** from **15** has been described in previous synthesis [5].

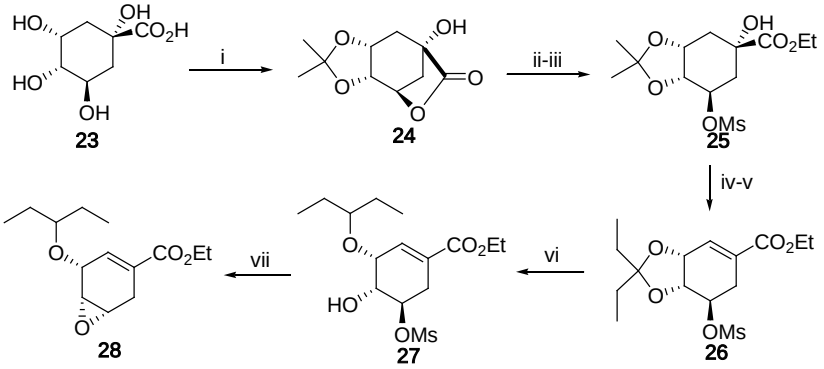


i: C_3H_5MgBr, Et_2O, 0-25 ^oC, 56%, ii: Ac_2O, TEA, CH_2Cl_2, 88%, iii: Li, NH_3, THF, -40 ^oC, 1h, 82%, iv: MsCl, TEA, CH_2Cl_2, 20 ^oC, 2h, 84%, v: NaH, THF, 40 ^oC, 24h, 87%, vi: NaN_3, NH_4Cl, EtOH/H_2O, reflux, 4h, 62%, vii: Ac_2O, TEA, DMAP, CH_2Cl_2, rt, 30 min, 88%, viii: cat..OsO_4, NMO.H_2O, t-BuOH, acetone, H_2O, rt, 14h, 96%, ix: KBr, TEMPO, TBAF, $Ca(ClO)_2$, 16-20 ^oC, MeI, K_2CO_3, DMF, 4h, rt, 80%, x: DMP, CH_2Cl_2, 0 ^oC, xi: 40% aq. HF in MeCN,1:19, 30 ^oC, 4h, 52%, 2 steps, xii: Ac_2O, py, 0 ^oC to rt, 12h, 65%, xiii: HCl, CH_2Cl_2, -40 ^oC to rt, 14h, 74%, xiv: DBU, CH_2Cl_2, 10 ^oC, 1h, 97%.

Scheme 1.3 Yao synthesis of zanamivir **8**

1.5 Synthesis of oseltamivir phosphate (Tamiflu®)

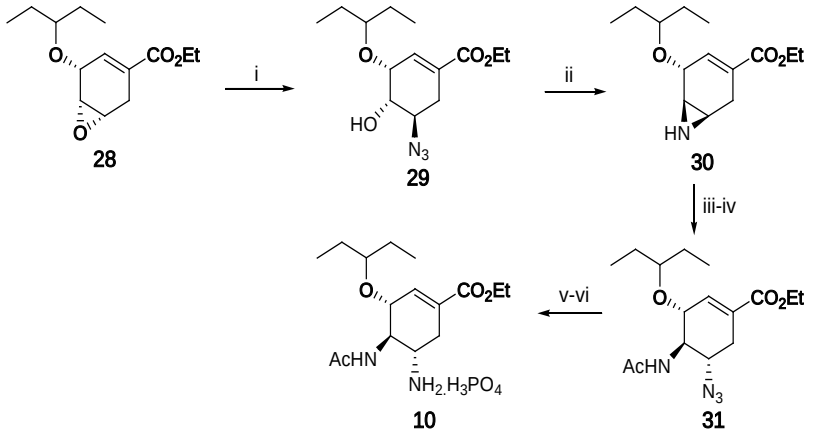
In 1997, Gilead's [10-11] first process route to oseltamivir phosphate or Tamiflu **10** started from (-)-quinic acid **23** [8-9], which was converted to the acetonide with concomitant lactonization to give **24**. (**Scheme 1.4**) The lactone was opened with sodium ethoxide and ethanol followed by mesylation with mesyl chloride to provide the ethyl ester **25**. Dehydration of **25** with thionyl chloride and pyridine and followed by transketalization with 3-pentanone in the presence of catalytic perchloric acid gave **26**. The 3,4-pentylidine ketal **26** was reduced by using trimethylsilyltriflate and borane dimethyl sulfide complex to give **27**. The product **27** was treated with potassium bicarbonate in aqueous ethanol to give epoxide **28** in 60% yield from **26**.



i: acetone dowex resin,PhH, DMF, reflux, 79%, ii: NaOEt, EtOH, 96%, iii: MsCl, DMAP, py, 92%, iv: $SOCl_2$, py, CH_2Cl_2, -20 ^oC to -30 ^oC, v: 3-pentanone, cat.$HClO_4$, 95%, vi: TMSOTf, BH_3-SMe_2, CH_2Cl_2, -20 ^oC, 75%, vii: $KHCO_3$, aq.EtOH, 1h, 96%.

Scheme 1.4 The preparation of epoxide intermediate **28** for synthesis of Tamiflu®**10**

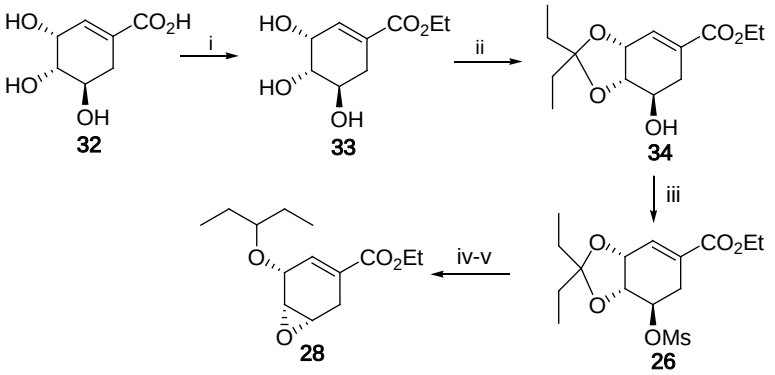
The first large scale synthesis was also reported in this group [12]. Epoxide **28** was heated with sodium azide and ammonium chloride in aq. ethanol to give azido alcohol **29** and reductive cyclization of **29** with trimethyl phosphine afforded aziridine **30**. (**Scheme 1.5**) Ring opening of aziridine **30** with sodium azide in the presence of ammonium chloride provided the azidoamine, which was directly acetylated with acetic anhydride to provide azidoacetamide **31** in 37% yield from epoxide **28**. Reduction of azide **31** using catalytic hydrogenation with Raney nickel in ethanol followed by salt formation with phosphoric acid provided the oseltamivir phosphate **10**.



i: NaN_3, NH_4Cl, EtOH,H_2O, 70-75 ^oC, 12-18h, 86%, ii: Me_3P, MeCN, 35 ^oC, 97%, iii: NaN_3, NH_4Cl, DMF, 70-80 ^oC, 12-18h, iv: Ac_2O, $NaHCO_3$, hexane, CH_2Cl_2, 1h, 44% 2 steps, v: H_2, Ra-Ni, EtOH,35^oC, 10-16h, vi: H_3PO_4, EtOH, 55-65 ^oC to rt 3-24h, 71%, 2 steps.

Scheme 1.5 The synthesis of Tamiflu®**10**

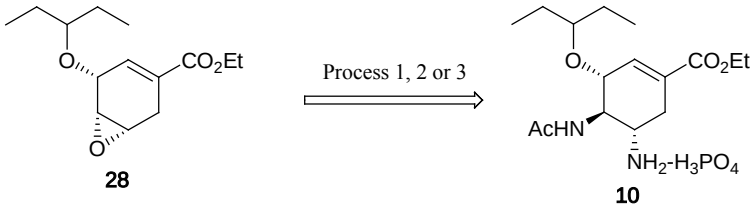
In 1999, Federspiel et al [13] from Roche research team had developed and optimize the synthesis tamiflu starting from (-)-shikimic acid **32**, either extracted from Chinese star anise or ginkgo leaves or from fermentation using a genetically engineered *E. coli* strain [14]. Esterification of (-)-shikimic acid **32** with $SOCl_2$ in EtOH provided ethyl shikimate **33**, which was treated with 3-pentanone in the presence of TfOH to give pentylidine ketal **34**. Mesylation of the hydroxyl group with mesyl chloride and Et_3N gave the intermediate **26**. The regioselective reduction the ring opening of the 3,4-pentylidine ketal **26** by triethyl silane and $TiCl_4$ at -32 ^oC to -36 ^oC followed by basic treatment with $NaHCO_3$ in EtOH gave epoxide intermediate **28** in 64% yield from **32**. (**Scheme 1.6**)



i: $SOCl_2$, EtOH, reflux, 97%, ii: 3-pentanone, TfOH, 98%, iii: MsCl, Et_3N, EtOAc, 89%, iv: Et_3SiH, $TiCl_4$, CH_2Cl_2, -32 to -36 ^oC, 87%, v: $NaHCO_3$, aq.EtOH, 96%.

Scheme 1.6 The preparation of epoxide intermediate **28**

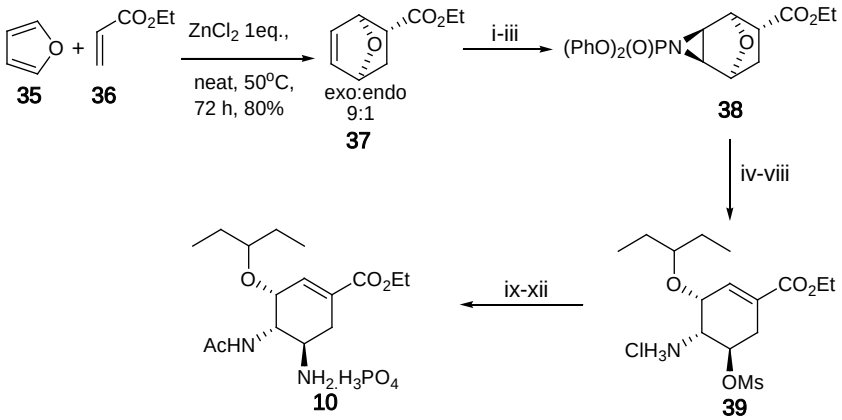
There are three processes developed by Roche's researchers for the synthesis of **10** from epoxide **28**. The shorter and economical azide route is currently used in the industrial production [15] while the allylamine and the tert-butylamine [16] routes were later reported as the alternatives to the use of hazardous azide reagents. (**Scheme 1.7**) The azide-free synthesis was reported by Karpf et al. (2001) [15].



1: azide route, 5 steps, 50-55%
2: allylamine route, 8 steps, 35-40%
3: *tert*-butylamine route, 6 steps, 60%

Scheme 1.7 Three Synthetic Routes of oseltamivir phosphate **10** from epoxide **28**

To avoid possible shortage of (-)-shikimic acid **32**, other precursors and synthesis have appeared in various attempts to synthesize the oseltamivir phosphate **10** without using the (-)-shikimic acid **32**. In 2004 [17], the zinc-catalyzed Diels-Alder reaction between furan **35** and ethyl acrylate **36** provided the thermodynamically major product **37**. Enzymatic resolution was achieved via enantioselective ester hydrolysis using Chirazyme L-2 giving the product in high yield. Further treatment with DPPA through [3+2] cycloaddition afforded the mixture of triazole compounds, which with continued heating at 70 ^{o}C and transesterification at the phosphate moiety resulted in an aziridine **38**. Base treatment to open the bicyclic system followed by O-mesylation and aziridine-opening with 3-pentanol produced a mesylated compound. Hydrolysis of the phophoryl amide and hydrochloride formation gave compound **39**, which can be converted into the oseltamivir phosphate **10** in another 4 steps with an overall yield of 17%. (**Scheme 1.8**)

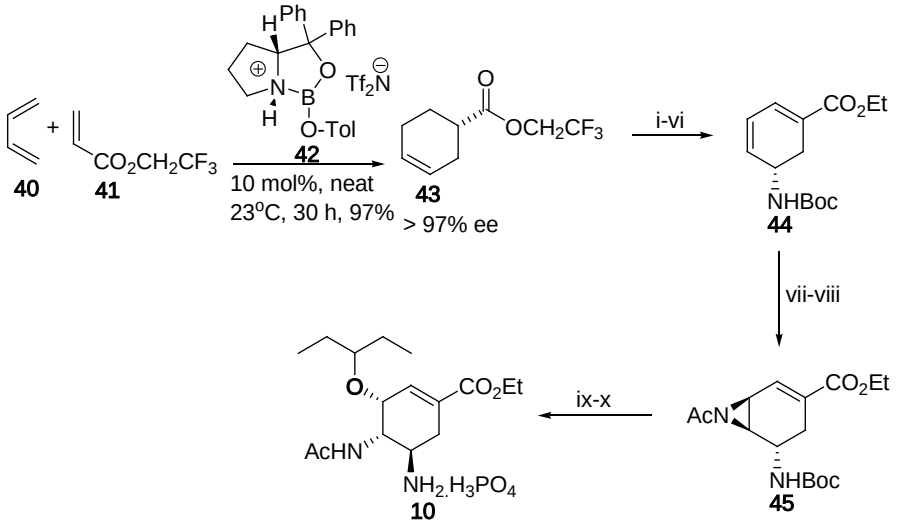


i: enzymatic resolution, Chirazyme L-2, ii: DPPA, toluene,70 ^{o}C, 18h. iii:NaOEt, EtOH i
v: NaHMDS, THF,-60 ^{o}C, 15h, v:MsCl, Et_3N, CH_2Cl_2, rt, vi: 3-pentanol, $BF_3.OEt_2$, CH_2Cl_2,
vii: 20%H_2SO_4, EtOH, 70 ^{o}C, viii: HCl, EtOH, ix: Allylamine, t-BuOMe, 110 ^{o}C, x: Ac_2O,
AcOH, $MeSO_3H$, t-BuOMe, rt, xi: 10% Pd/C, EtOH, xii: H_3PO_4, EtOH

Scheme 1.8 The Diels-Alder approach to oseltamivir phosphate **10**

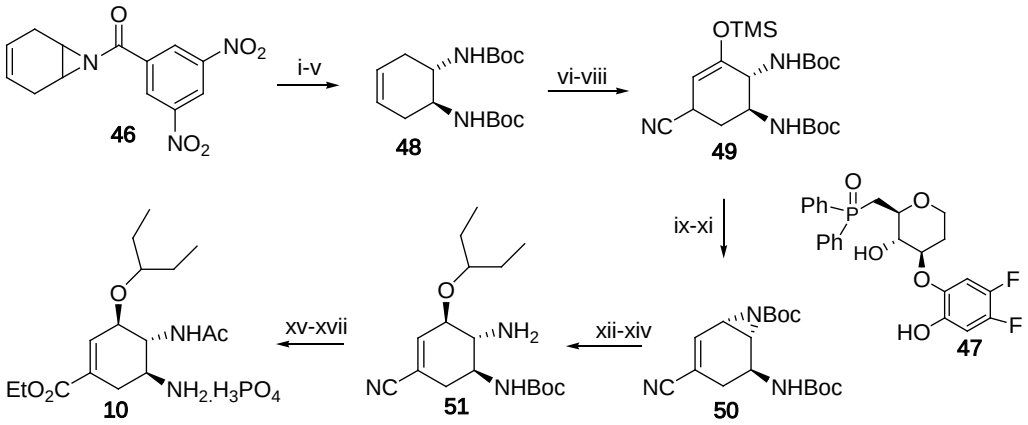
In 2006, Corey et al [18-19] used asymmetric Diels-Alder reaction between butadiene **40** and trifluoroethyl acrylate **41** in the presence of the (*S*)-proline-derived catalyst **42** [20] to make product **43** in excellent yield. Ammonolysis, iodolactamization using the Knapp protocol [65], *N*-protection, dehydroiodination, allylic bromination, and treatment of the bromo compound with cesium carbonate in

EtOH afforded the diene ethyl ester **44** in 72% yield. It was further converted to the oseltamivir phosphate **10** by bromoacetamidation with NBA, cyclization to the *N*-acetylaziridine **45** followed by pentyloxy ketal formation and subsequent removal of the Boc group and salt formation afforded the desired product. (**Scheme 1.9**)



Scheme 1.9 Corey's synthesis of oseltamivir phosphate **10**

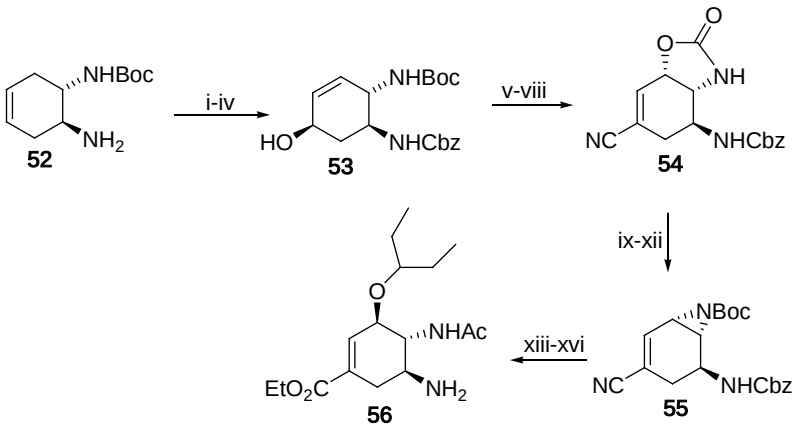
In 2006-2008, Shibasaki and coworkers have reported four different preparations of oseltamivir phosphate **10**. The first generation synthesis [21], used catalytic asymmetric ring-opening reaction of meso aziridine **46** with $TMSN_3$ in the presence of an yttrium catalyst to give azide, which was treated with $(Boc)_2O$, deprotected of the benzoyl group and followed by azide reduction and protection of the resulting amine providing *C2* symmetric dicarbamate **48**. The allylic group was oxidized with Dess-Martin periodinane, and addition of TMSCN to provide the nitrile compound **49**. Sequential oxidation, reduction and Mitsunobu reaction gave the aziridine compound **50**. Ring-opening of the aziridine with 3-pentanol followed by deprotection and reprotection of the Boc-groups yielding the intermediate **51**. The oseltamivir phosphate **10** was produced by acetylation, deprotection and conversion of the nitrile group of **51** to an ethyl ester with phosphate salt formation. (**Scheme 1.10**)



i: $Y(Oi\text{-}Pr)_3$ (2 mol%), **47** (4 mol%), $TMSN_3$ EtCN, rt, 48h, 96%, ii: Boc_2O, DMAP, MeCN, rt, 3h, iii: 4M NaOH, rt,2h, 98%, iv: Ph_3P, MeCN, 50 ^oC, 3 h, H_2O 40 ^oC, 2h, v: Boc_2O, TEA, CH_2Cl_2, rt, 2 h, 90%, vi: SeO_2, Dess-Martin periodinane, dioxane, 80 ^oC, 12h. vii: Dess-Martin periodinane, CH_2Cl_2, 4 ^oC, 62%, viii: $Ni(COD)_2$ (10 mol%), COD (10 mol%), TMSCN, THF, 60 ^oC, 65h. ix: NBS, THF, 20 min, TEA, 4 ^oC, 40 min, x: $LiAl(Ot\text{-}Bu)_3$, THF, 4 ^oC, 30 min, 60%, xi: DEAD, Ph_3P, THF, 4 ^oC, 1h, 87%, xii: 3-pentanol, $BF_3.OEt_2$, 4 ^oC, 1h, 52%, xiii: TFA, CH_2Cl_2, 4 ^oC to rt, 3 h, xiv: Boc_2O, TEA, CH_2Cl_2, 4 ^oC, 30 min, 87%, xv: Ac_2O, DMAP, py, rt, 1 h, 84%. xvi: 4.2 M HCl.EtOH, 60 ^oC, 4 h, 53%, xvii: 85% H_3PO_4, EtOH, 50%

Scheme 1.10 Shibasaki's first generation synthesis of oseltamivir phosphate **10**

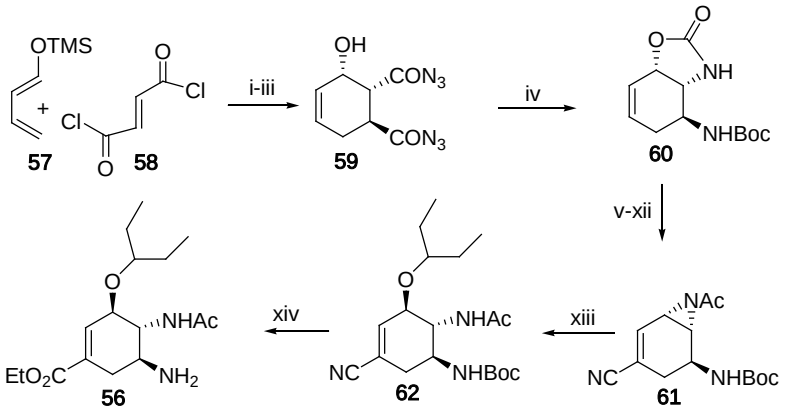
The second generation synthesis of oseltamivir **56** via allylic rearrangement [22] used the amine compound **52** obtained from the first generation. It was acetylated, iodocyclized and eliminated, protected and the resulting acetate was converted to alcohol **53**. Oxidation and treating with diethylphosphoryl cyanide gave the cyanophosphate, which was subjected to the key allylic rearrangement providing cyclic carbamate **54** via intramolecular S_N2 allylic substitution, reprotection and cleavage to give alcohol, followed by oxidation, reduction and subsequent Mitsunobu reaction provided Boc-protected aziridine **55**. The ring opening of aziridine group with 3-pentanol followed by deprotection of the Boc-group, acetylation, and deprotection of –Cbz group gave the free base of oseltamivir **56**. (**Scheme 1.11**)



i:Ac_2O, py, CH_2Cl_2, rt, 2h, 99%, ii: NIS, CH_2Cl_2, $CHCl_3$, 40-60 ^oC, DBU, rt, 12 h, iii: CbzCl, $NaHCO_3$, CH_2Cl_2, H_2O, rt, 2 h, 85%, iv, K_2CO_3, MeOH, rt, 2 h, 99%, v: Dess-Martin periodinane, CH_2Cl_2, rt, 16 h, 96%, vi: $(EtO)_2P(O)CN$, LiCN(17 mol%), THF, -20 ^oC, 1h, dr= 20:1, vii: PhMe, seal tube, 150 ^oC, 3 h, viii: Boc_2O, DMAP, py, rt, 10h, 72%, ix: Cs_2CO_3, (10 mol%) MeOH, rt, 3h, 97%, x: Dess-Martin periodinane, CH_2Cl_2, rt, 19h, 94%, xi: $LiAlH(Ot\text{-}Bu)_3$, THF, -20 to 0 ^oC, 2h, 91%, xii: DEAD, Ph_3P, THF, 0 ^oC, 3h, 87%, xiii: 3-pentanol, $BF_3.OEt_2$, -20 ^oC, 5h, xiv: TFA, CH_2Cl_2, 0 ^oC to rt, 3h, xv:, Ac_2O, TEA, CH_2Cl_2, 0 ^oC, to rt, 16h, 81%, xvi: conc,HCl, EtOH, rt, 24h, 25%,aq.NH_3, rt, 10h, 74%.

Scheme 1.11 Shibasaki's second generation synthesis of oseltamivir **56**

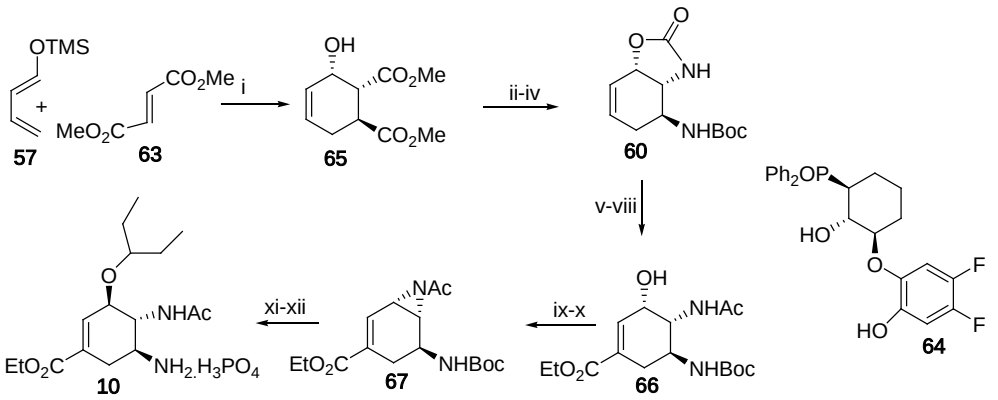
The third generation synthesis of oseltamivir **56** was carried out via Diels-Alder reaction and Curtius rearrangement. [23-24] The synthesis started with the cycloaddition of 1,3-butadiene **57** and fumaryl chloride **58**, which was then treated with $TMSN_3$, followed by the acidic cleavage of the trimethylsilyl ether to give alcohol **59**. Curtius rearrangement [57] key step yielded the product **60**, which was hydrolyzed to amine compound, acetylated, oxidized and subjected to Michael addition of cyanide with TMSCN. The enol ether product was brominated, eliminated and subjected to Mitsunobu reaction to prepare aziridine **61**. Finally, Ring opening of aziridine and then conversion of **62** to oseltamivir **56** were accomplished. (**Scheme 1.12**)



i: THF, rt, 2h, ii: $TMSN_3$, DMAP, rt, 2h, iii: 1N HCl, 4 ^oC, 10min, 55%, 3 steps, iv: t-BuOH, reflux, v: LiOH, vi: Ac_2O, TEA, vii: isobutyric anhydride, DMSO, 53%,4 steps, viii: Chiral HPLC, ix: TMSCN, $Ni(cod)_2$ (50 mol%), x: NBS, TEA, xi: $LiAlH(Ot\text{-}Bu)_3$, 44%, 3 steps, xii: DEAD, Ph_3P, 66%, xiii: 3-pentanol, $BF_3.OEt_2$, 56%, xiv: HCl, EtOH, 60%.

Scheme 1.12 Shibasaki' third generation synthesis of oseltamivir **56**

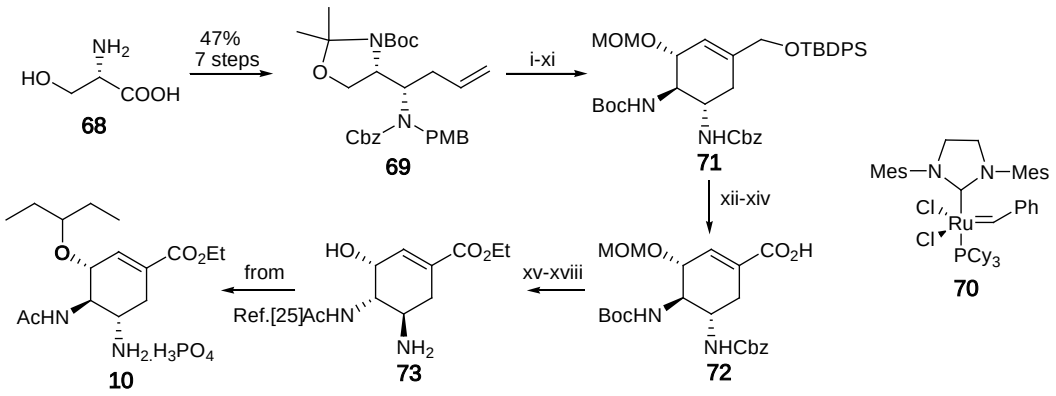
The fourth generation synthesis of oseltamivir phosphate **10** [25], via a barium-catalyzed asymmetric Diels-Alder reaction started from 1,3-butadiene **57** and dimethylfumarate **63** to give the mixture of products **65**, which was treated with DPPA and TEA generating diacyl azide and heated in anh. *tert*-BuOH to give carbamate **60** via a Curtius rearrangement. The acetylation, protection, epoxidation and opening of epoxide provided intermediate **66**. Double Mitsunobu reaction generated aziridine **67**, which was one of the intermediate found in Corey's synthesis of oseltamivir phosphate **10**. Final ring opening, deprotection and salt formation obtained the desired product. (**Scheme 1.13**)



i: $Ba(Oi\text{-}Pr)_2$, (2.5 mol%) **64** (2.5 mol%), CsF (2.5 mol%), THF, -20 ^oC, 36-96h, 1M HCl, 91%, ii: 2 M NaOH, MeOH, 60 ^oC, 10h, iii: DPPA, TEA, THF, 0 ^oC, 21h, 95%, 2 steps, iv: tert-BuOH, 80 ^oC, 13h, v: Ac_2O, TEA, DMAP, (10 mol%), CH_2Cl_2, rt, 2.5h, 80%, 2 steps, vi: acetyloxy malononitrile, $[Pd_2(dba)_3].CHCl_3$ (2 mol%), dppf (4 mol%), PhMe, 60 ^oC, 30 min, 85%, vii: Trifluoroperacetic acid, urea, H_2O, Na_2HPO_4, CH_2Cl_2, 4 ^oC, 2h, viii: K_2CO_3, EtOH, rt, 5h, ix: DEAD, Ph_3P, p-nitrobenzoic acid, THF, -20 ^oC, 1.5h, LiOH, EtOH, 20 ^oC, 15 min, 65%, 3 steps, x: DIAD, Me_2PPh, TEA, CH_2Cl_2, 4 ^oC, 10 min, 76%, xi: 3-pentanol, $BF_3.OEt_2$, -20 ^oC, 15 min, 75%, xii: TFA, H_3PO_4, 73%.

Scheme 1.13 Shibasaki's fourth generation synthesis of oseltamivir phosphate **10**

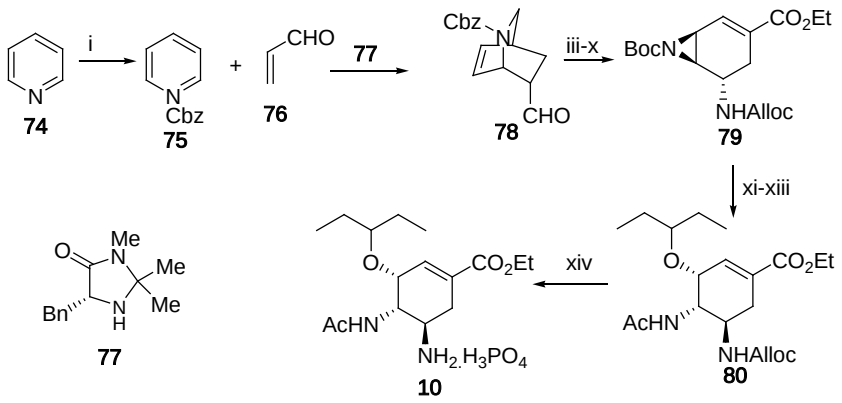
In 2006, Yao and coworkers [26] reported the synthesis of the cyclohexene compound **71** via a ring closing metathesis, which was started from L-serine **68** in 18 steps. (**Scheme 1.14**) The TBDPS protecting group of compound **71** was removed with TBAF and the hydroxyl group was oxidized to acid **72**. Esterification, removal of the MOM-group, followed by acetylation of the amine group and subsequent removal of the Cbz-protecting group under Pd-catalyzed reductive condition provided **73** in 19% yield from **68**. The completion of the synthesis toward **10** has been described in previous synthesis [25].



i: OsO_4, NMO, acetone, H_2O, 5:1, 89%, ii: H_2, $Pd(OH)_2$, MeOH, 35 ^oC, iii: CbzCl, $NaHCO_3$, H_2O, EtOAc, 1:1, 86%, 2 steps, iv: TBDPSCl, imidazole, CH_2Cl_2, rt, 96%, v: $(COCl)_2$, DMSO, CH_2Cl_2, TEA, -78 ^oC, vi: Ph_3PCH_3Br, n-BuLi, THF, -78 ^oC to rt, 86% 2steps, vii: $BiBr_3$, (20 mol%), MeCN, rt, 89%, viii: $(COCl)_2$, DMSO, CH_2Cl_2, TEA, -78 ^oC, ix: VinylMgBr 3eq., $ZnBr_2$ 1 eq., THF, -78 ^oC to -30 ^oC, 75%, x: MOMCl, DIPEA, CH_2Cl_2, rt, 98%, xi: **70** (10 mol%), CH_2Cl_2, rt, 98%, xii: TBAF, THF, rt, 96%, xiii: PCC, 4A^o molecularsieves, CH_2Cl_2, rt, xiv: $NaClO_2$, K_2HPO_4, 2,3-dimethylbuta-1,3-diene, t-BuOH,THF,H_2O, 4:1:1, 10 ^oC to rt, 88% 2 steps, xv: EtOH, HOBt, EDCI, DIPEA, CH_2Cl_2, rt, 85%, xvi: 5% HCl,EtOH, 0 ^oC to rt, xvii: AcCl, Na_2CO_3, EtOH, 0 ^oC to rt, 83% 2 steps, xviii: $Pd(OAc)_2$, Et_3SiH, TEA, CH_2Cl_2, 0 ^oC to rt, 92%.

Scheme 1.14 Yao's synthesis of oseltamivir phosphate **10**

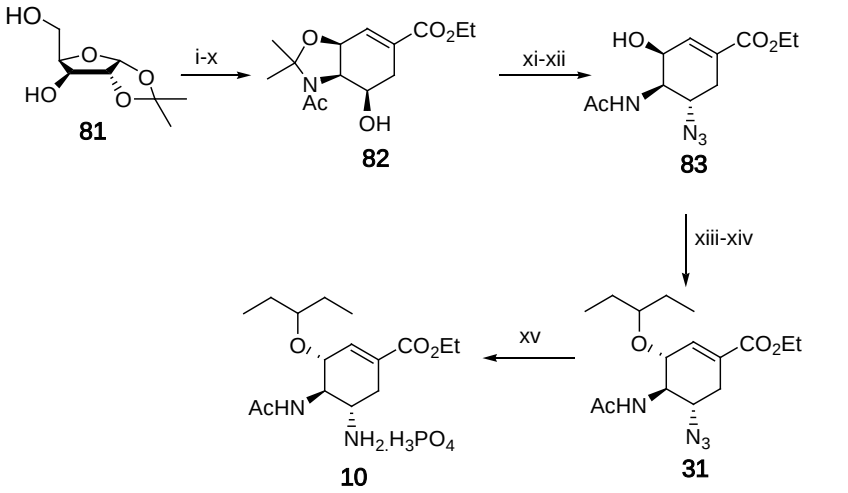
In 2007, Fukuyama and coworkers [27] reported a synthesis of oseltamivir phosphate **10**, through the intermediate aziridine **78** that was synthesized in 10 steps from pyridine **74**. Opening of aziridine ring with 3-pentanol, followed by the removal of the Boc-protecting group and acetylation of the resulting amine with Ac_2O, led to formation of acetamide **79**. Finally, the construction of oseltamivir phosphate **10** was completed in overall yield of 5.6% from pyridine **74**, as shown in **Scheme 1.15**.



i: $NaBH_4$, CbzCl, MeOH, -50 ^oC to -35 ^oC, 1h, ii: Acrolein, **77** (10 mol%), MeCN, H_2O, rt, 12h, iii: $NaClO_2$, $NaHPO_4.2H_2O$, 2-methyl-2-butene, t-BuOH, H_2O, 0 ^oC to rt, 1h, iv: Br_2, $NaHCO_3$, CH_2Cl_2, H_2O, rt, 26% 4 steps, v: H_2, Pd/C, Boc_2O, EtOH, THF, rt, 2h, 92%, vi: RuO_2nH_2O (10 mol%), $NaIO_4$, $ClCH_2CH_2Cl$, H_2O, 80 ^oC, 1.5h, vii: NH_3, t-BuOH, THF,rt, 0 ^oC, 95%, viii: MsCl, TEA, CH_2Cl_2, rt, 1h, 91%, ix: allyl alcohol, $PhI(OAc)_2$, sieves 4A^o, toluene, 60 ^oC 10h, 88%, x: NaOEt, EtOH, 0 ^oC 87%, xi: 3-pentanol, $BF_3.OEt_2$, -20 ^oC, 62%, xii: TFA, CH_2Cl_2, 0 ^oC to rt, xiii: Ac_2O, py, 88%2 steps, xiv: Pd/C, Ph_3P, 1,3-dimethylbarbituric acid, EtOH, reflux, 40 min, H_3PO_4, 76% 2 steps.

Scheme 1.15 Fukuyama's synthesis of oseltamivir phosphate **10**

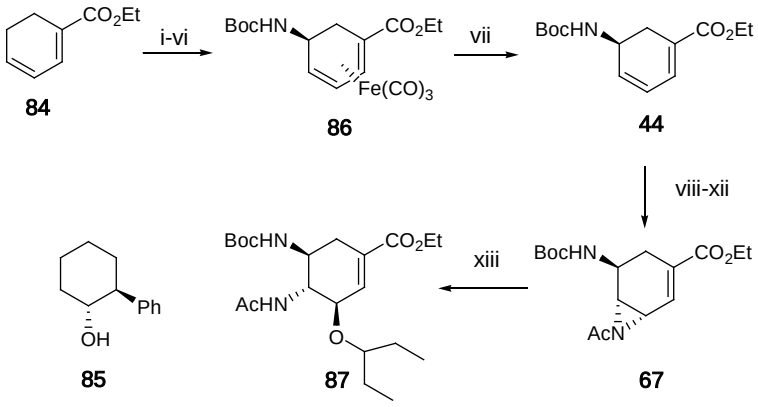
In 2007, Fang and coworker [28], has reported the synthesis of oseltamivir phosphate **10** from *D*-xylose. 1,2-*O*-isopropylidne-α-*D*-xylofuranose **81** was transformed to the cyclohexene compound **82** in 10 steps in high yield. A Mitsunobu reaction was introduced the azide group, and subsequent acid treatment deprotected the amino and hydroxyl group to generate azide **83**, after inverting the stereochemistry of the C3 hydroxy group, the resulting was treated with 3-pentyl trichloroacetimidate, the pentyl ether was formed to compound **31** and finally azide reduction and addition of H_3PO_4 to provided the tamiflu **10** in 15% yield of 16 steps, as shown in **Scheme 1.16**



i: PivCl, py, 0 ^{o}C, 8h, 89%, ii: PDC, Ac_2O, reflux, 1.5h, iii: $HONH_2.HCl$, py, 60 ^{o}C, 24h, 82%, iv: $LiAlH_4$, THF, 0 ^{o}C to reflux, 1.5 h, 88%, v: Ac_2O, py, 25 ^{o}C, 3h, vi: Benzyl alcohol, 4M HCl in dioxane, PhMe, 0 to 25 ^{o}C, 24h, 85% 2 steps, vii: 2,2-dimethoxypropane, p-TsOH, PhMe, 80 ^{o}C, 4h, 90%, viii: Tf_2O, py, CH_2Cl_2, -15 ^{o}C, 2h, triethylphosphonoacetate, NaH, 15-crown-5, DMF, 25 ^{o}C, 24h, 80%, ix: H_2, Pd/C, EtOH, 25 ^{o}C, 24h, NaH, THF, 25^{o}C, 1h, 83%, x: DPPA, DIAD, Ph_3P, THF, 25 ^{o}C, 48h, xi: HCl, EtOH, reflux, 1h, 93% 2 steps, xii: Tf_2O, py, CH_2Cl_2, -15 ^{o}C to -10 ^{o}C, 2h, xiii: KNO_2, 18-crown-6, DMF, 40 ^{o}C, 24h, 70%, xiv: $Cl_3C(=NH)OCHEt_2$, CF_3SO_3H, CH_2Cl_2, 25 ^{o}C, 24h, 78%, xv: H_2, Lindlar's catalyst, EtOH, rt, 16h, H_3PO_4, EtOH, 40 ^{o}C, 1h, 91% 2 steps.

Scheme 1.16 Fang's synthesis of oseltamivir phosphate **10**

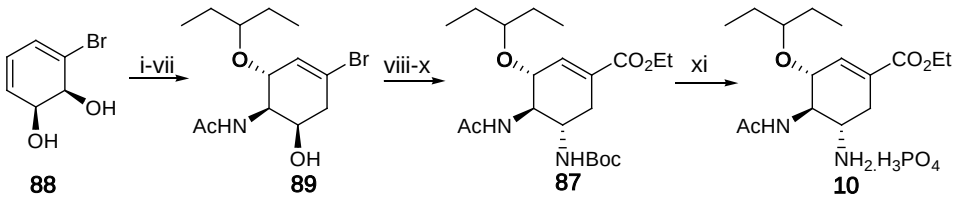
In 2007, Kann and coworkers [29] synthesized the oseltamivir derivative **86** based on cationic iron-carbonyl complex. The starting cyclohexadienecarboxylate **83** was complexed with carbonyl iron to provide the diastereomeric mixture **84**, which was later decomplexed to provide the Boc-compound **44**. Selective epoxidation, followed by azide opening of the epoxide group, hydroxyl group mesylation and subsequent reduction and acetylation of the azido group gave the aziridine compound **67**, which was converted to oseltamivir derivative **87 (Scheme 1.17)**.



i: $Fe_2(CO)_9$, PhMe, 55 ^oC, 86%, ii: Ph_3CPF_6, CH_2Cl_2, rt, 94%, iii: **85**, DIPEA, CH_2Cl_2, 0 ^oC, 75%, iv: preparative HPLC, 47%, v: HPF_6, Et_2O, 0 ^oC, 94%, vi: Boc-NH_2, DIPEA, CH_2Cl_2, 0^oC, 86%, vii: H_2O_2, NaOH, EtOH, 0^oC, 95%, viii: m-CPBA, CH_2Cl_2, -70 ^oC to rt, 95%, ix: NaN_3, NH_4Cl, DME, EtOH, H_2O, 0 ^oC, 95%, x: MsCl, TEA, CH_2Cl_2, 0 ^oC, xi: Ph_3P, TEA, THF, H_2O, rt, xii: Ac_2O, CH_2Cl_2, 0 ^oC, 65% 2 steps, xiii: 3-pentanol, $Cu(OTf)_2$, 0 ^oC, 48%.

Scheme 1.17 Kann's synthesis of oseltamivir phosphate **10**

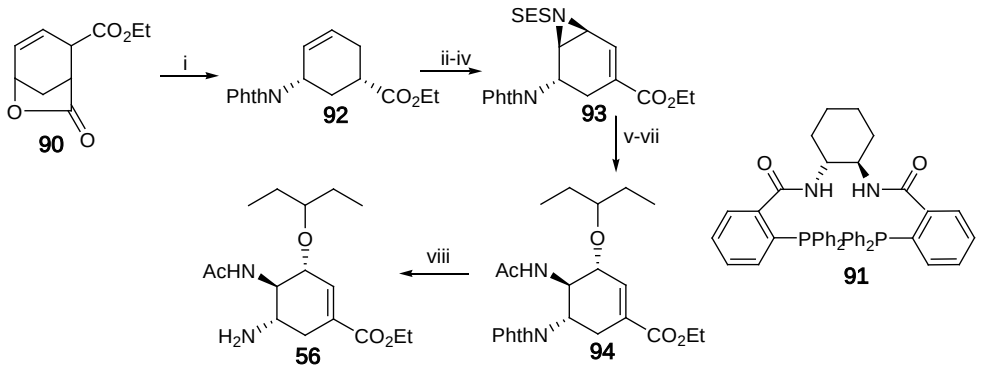
Later in 2008, Fang and coworkers [30] reported their second approach to oseltamivir phosphate **10** from commercially available enantiopure bromodiol **88** (**Scheme 1.18**). The bromodiol **88** was converted to the alcohol intermediate **89** in 7 steps. The amine was introduced through the reaction with tetrabutylammonium cyanate in the presence of Ph_3P and DDQ, followed by treatment with *t*-BuOH and the bromide exchange to the iodide and subsequent ethyl ester formation to give the **87**. The one-pot Boc-deprotection and salt formation provided the **10** in 22% yield.



i: Dimethoxy propane, p-TsOH.H_2O, acetone, 0 ^oC to rt, 30 min, ii: $SnBr_4$, (cat.), N-Bromoacetamide, MeCN, H_2O, 0 ^oC, 8h, 75% 2 steps, iii: LiHMDS, THF, -10 ^oC to rt, 30 min, iv: 3-pentanol, $BF_3.OEt_2$, -10 ^oC to 0 ^oC, 6h, 73%, 2 steps, v: conc.HCl, MeOH, 50 ^oC, 6h, 94%, vi: $AcOCMe_2COBr$, THF, 0 ^oC to rt, 3.5h, vii: $LiBHEt_3$, THF, 0 ^oC to rt, 2h, 82%, 2 steps, viii: DDQ, PPh_3, n-Bu_4NOCN, MeCN, rt, 18h, t-BuOH, reflux, 24h, 78%, 2 steps, ix: CuI, KI, N,N-dimethylethylenediamine, n-BuOH, 120 ^oC, 24h, x: $Pd(OAc)_2$, CO, NaOAc, EtOH, rt, 24h, 82% 2 steps, xi: H_3PO_4, EtOH, 50 ^oC, 6h, 81%.

Scheme 1.18 Fang's second approach synthesis of oseltamivir phosphate **10**

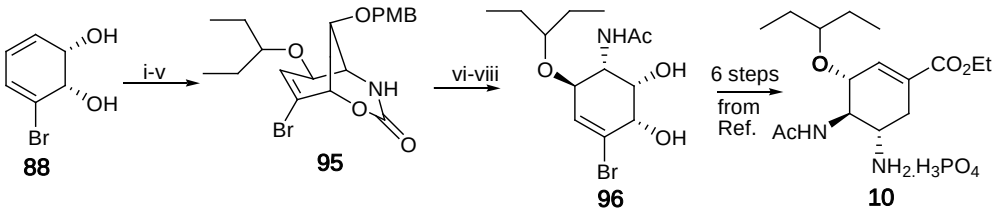
In 2008, Trost and coworkers [31] reported a short synthesis of oseltamivir **56** via Pd-catalyzed asymmetric allylic alkylation. The ethyl ester **92** was produced from a commercially available lactone **90**. Sulfenylation, oxidation, elimination and formation of aziridine **93** were completed from compound **92**. Compound **93** was reacted with 3-pentanol followed by acetylation using a microwave reactor, and removal of the SES protecting group by treatment with TBAF to give the oseltamivir derivative **94**. The final step involved the cleavage of phthalimido group with hydrazine to give oseltamivir **56** in 30% overall yield, as shown in **Scheme 1.19**



i: [(n_3-$C_3H_5PdCl)_2$ 2.5 mol%, **91**, 7.5 mol%, trimethylsilylphthalimide, THF, 40 ^oC, $TsOH.H_2O$, EtOH, reflux, 84%, ii: KHMDS, $PhSSO_2Ph$, THF, -78 ^oC to rt, 94%, iii: m-CPBA, $NaHCO_3$, 0 ^oC, DBU, PhMe, 60 ^oC, 85%, iv: rhodium catalyst, 2 mol%, 2-(trimethylsilyl)ethanesulfonamide($SESNH_2$), $PhI(O_2CCMe_3)_2$, MgO, PhCl, 0 ^oC to rt, 86%, v: 3-pentanol, $BF_3.OEt_2$, 75 ^oC, 65%, vi: DMAP, py, Ac_2O, Microwave,150 ^oC, 1h, 84%, vii: TBAF, THF, rt, 95%, viii: NH_2NH_2, EtOH, 68 ^oC, 100%.

Scheme 1.19 Trost's synthesis of oseltamivir **56**

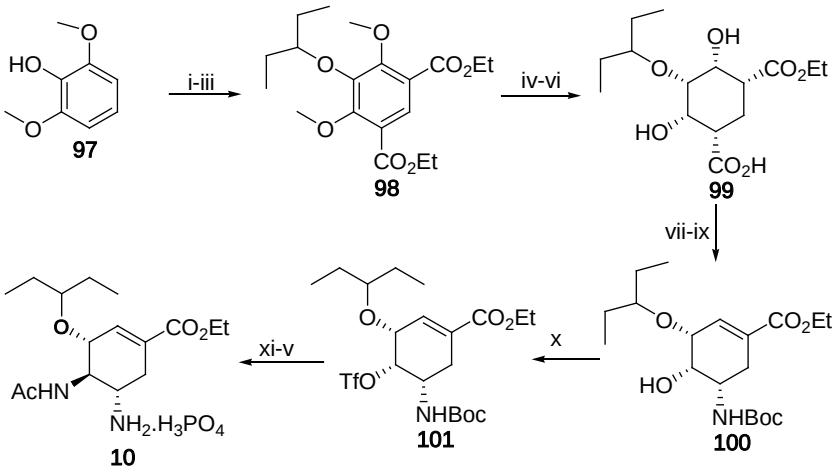
Bawell and coworkers [32] reported a chemoenzymatic formal synthesis of oseltamivir phosphate **10 (Scheme 1.20)**. The synthesis started with the reaction between **88** and 4-methoxybenzaldehyde dimethyl acetal in the presence of (+)-camphorsulfonic acid. Reduction, *N*-hydroxycarbamate formation, tosylation followed by copper-catalyzed intramolecular aziridination provided carbamate **95**. Bromodiol **96** was obtained from carbamate **95** by acetylation and deprotection, which was then converted to oseltamivir phosphate **10** as has been previously reported [30].



i: 4-methoxybenzaldehyde dimethyl acetal, (+)-camphorsulfonic acid, PhMe, 0 ^{o}C, 1.5h, ii: DIBAL-H, TEA, PhMe, -78 ^{o}C to -30 ^{o}C, 5h, 85%, 2 steps, iii: CDI, MeCN, 0 ^{o}C, 1h, $NH_2OH.HCl$, imidazole, 0 ^{o}C to 18 ^{o}C, 16h, 56%, iv: p-TsCl, TEA, Et_2O, 0 ^{o}C to 18 ^{o}C, 16h, 79%, v: $Cu(MeCN)_4PF_6$, K_2CO_3, MeCN, 3-pentanol, 0 ^{o}C to 18 ^{o}C, 16h, 43%, vi: LiOH, 1,4-dioxane,H_2O, 100 ^{o}C, 48h, 85%, vii: AcCl, TEA, 0 ^{o}C to 18 ^{o}C, 1h, 99%,viii: HCl, MeOH, 35 ^{o}C, 16h, 90%.

Scheme 1.20 Bawell's synthesis of oseltamivir phosphate **10**

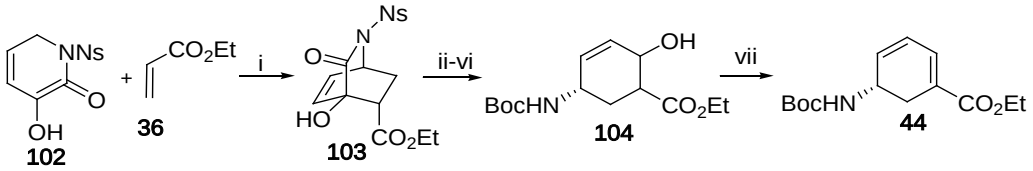
In 2008, Zutter and coworkers [33] reported a new enantioselective synthesis of **10** via enzymatic hydrolytic desymmetrization starting from 2,6-dimethoxyphenol **97**. Key steps of this approach were the *cis*-hydrogenation of trihydroxyisophthalic acid derivative **98** and then desymmetrization of the dihydroxy-meso-diester **98** by enantioselective hydrolysis with pig liver esterase, affording the *(S)*-monoacid **99**. Subsequent Shioiri-Yamada-Curtius degradation followed by a unique decarboxylative elimination reaction of Boc-oxazolidinone provided **100**. Substitution of the corresponding triflate **101** with NaN_3, azide reduction, N-acetylation, and deprotection of the Boc group and salt formation afforded **10**, as shown in **Scheme 1.21**.



i:3-pentylmesylate, KOtBu, DMSO, 50 ^{o}C, ii: NBS, DMF, 0 ^{o}C-rt, 90% iii: CO(10 bar) 0.5% $Pd(OAc)_2$, dppp, KOAc, EtOH, 110 ^{o}C, 20h, 95% iv; H_2, Ru-Al_2O_3, 82% v: TMSCl, NaI, MeCN,cat H_2O 97%, vi: PLE, pH 8 buffer, 96% vii: DPPA, Et_3N, DCM,40 ^{o}C, 81% viii: $(Boc)_2O$, DMAP, ix; NaH,toluene, x: Tf_2O, pyidine, CH_2Cl_2, -10 ^{o}C, 83% xi: NaN_3, rt, acetone-H_2O, 78% xii: (Bu_3P-H_2O); xiii: Ac_2O, Et_3N, xiv: HBr-AcOH, EtOAc, xv: H_3PO_4/EtOH, 83%

Scheme 1.21 Zutter's synthesis of oseltamivir phosphate **10**

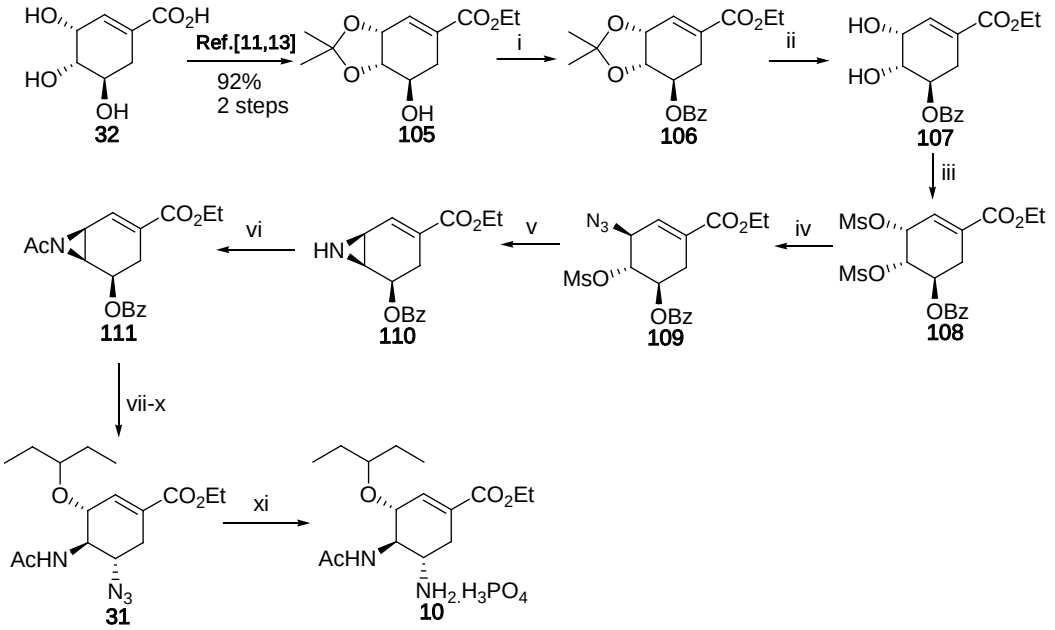
In 2008, Okamura and coworkers [34] reported base-catalyzed Diels-Alder reaction between N-nosyl-3-hydroxy-2-pyridone **102** and ethyl acrylate **36** in water to give bicyclolactam adduct **103**. Chemoselective reduction with $NaBH_4$, deprotection and reprotection with the Boc-group, and the diol was cleaved with $NaIO_4$ and reduced to give compound **104**. Mesylation of the resulting alcohol and elimination provided the racemic compound **44** with overall yield of 12% in 7 steps as shown in **Scheme 1.22**. This intermediated **44** could be converted to oseltamivir phosphate **10** in 4 steps using the previously reported procedure [18].



i: NaOH, H_2O, rt, 24 h, 83% ii: $NaBH_4$, THF, 0 ^{o}C, 2 h, 77% iii: PhSH, K_2CO_3, MeCN, rt, 3 h, iv: $(Boc)_2O$, H_2O, 24 h, 55%, v:$NaIO_4$, H_2O, THF, 0 ^{o}C, 3 h, vi: $NaBH_4$, EtOH, vii: MsCl, TEA, DMAP, CH_2Cl_2, 33%

Scheme 1.22 Okamura's synthesis intermediated **44**

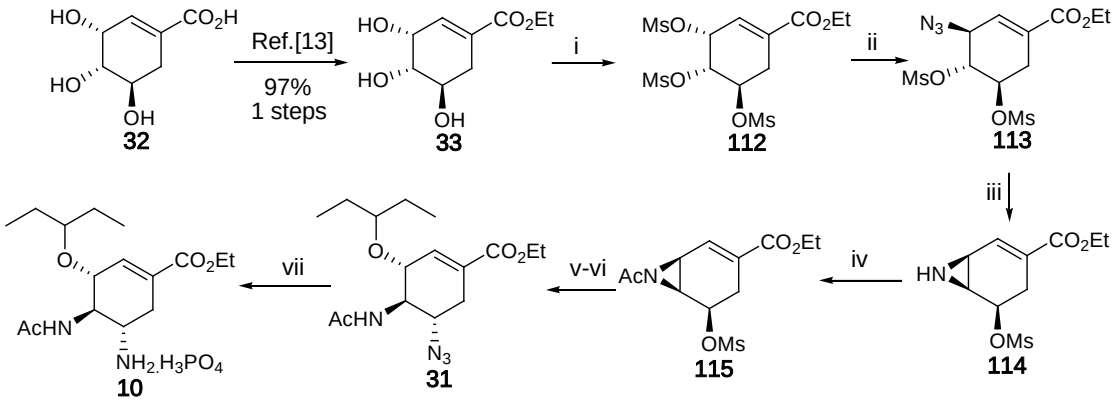
In 2009, Shi and coworkers [35] reported two synthetic approaches to oseltamivir phosphate **10** that relied an (-)-shikimic acid **32** a starting material. The first route accomplished in 13 steps with an overall yield of 40% (**Scheme 1.23**).



i: BzCl, TEA, cat.DMAP, CH_2Cl_2, 0 ^oC to rt, 5h, 98%, ii: cat.HCl, EtOAc, H_2O, 4:1, 6h, 94%, iii: MsCl, cat.TEA,, EtOAc, 0 ^oC, 1h, 97%, iv: NaN_3, DMF, H_2O, 5:1, -5 ^oC, 1.5h, 95%, v: Ph_3P, THF, rt, 2h, TEA, THF:H_2O= 10:1, rt, overnight, 88%, vi: Ac_2O, TEA, EtOH, rt, 6h, 90%, vii: 3-pentanol, $BF_3.OEt_2$, -5 ^oC to 0 ^oC, 30min, 92%,viii: K_2CO_3, EtOH, rt, 6h, 90%, ix: MsCl, TEA, CH_2Cl_2, 0 ^oC, 1h, 95%, x: NaN_3, DMF:H_2O= 5:1, 90 ^oC, 3h, 84%, xi: H_2, Lindlar catalyst, EtOH, rt, 16h, H_3PO_4, EtOAc:EtOH= 1:1, 50 ^oC, 30 min, 91%, 2 steps.

Scheme 1.23 Shi's first approach synthesis of the oseltamivir phosphate **10**

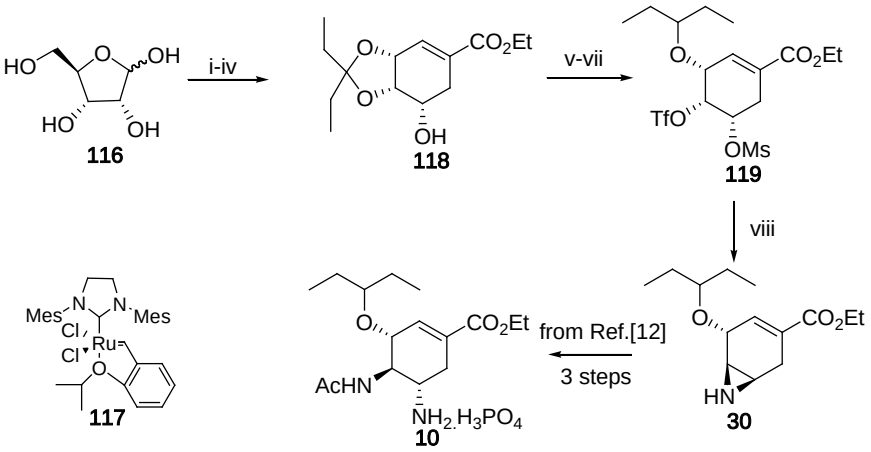
And the optimized second synthetic process required only 8 steps with an overall yield of 47%, respectively, as shown in **Scheme 1.24**



i: MsCl, TEA, cat.DMAP, EtOAc, 0 ^{o}C, 1h, 93%, ii: NaN_3, acetone:H_2O = 5:1, 0 ^{o}C, 4h, 92%, iii: Ph_3P, THF, rt, 30 min, TEA, H_2O, rt, 24h, 84%, iv: Ac_2O, TEA, EtOAc, 0 ^{o}C, 30 min, 98%, v: 3-pentanol, $BF_3.OEt_2$, -8 ^{o}C to 0 ^{o}C, 1h, 86%, vi: NaN_3, EtOH:H_2O = 5:1, reflux, 8h, 88%, vii: H_2, Lindlar catalyst, EtOH, rt, 16h, H_3PO_4, EtOAc,EtOH,1:1, 50 ^{o}C, 30 min, 91%, 2 steps.

Scheme 1.24 Shi's second approach synthesis of the oseltamivir phosphate **10**

In 2010, Osato and coworkers [36] reported an efficient formal synthesis of oseltamivir phosphate **10** in 12 steps using *D*-ribose **116** as the starting material. After protection, iodo substitution, followed by Bernet-Vasella reaction and subsequent ring closing olefin metathesis compound **118** was obtained. Stereoselective reduction of pentylidine ketal group and conversion into the triflate with trifluoromethanesulfonyl anhydride gave the mesyloxy triflate **119**. Aziridine compound **30** was then obtained from substitution of the triflate group followed by reduction, respectively (**Scheme 1.25**) [12].



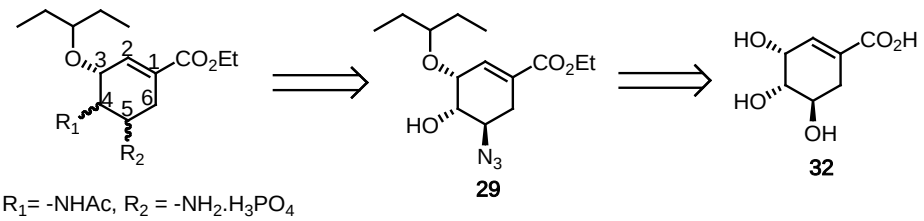
i: 3-pentanone, MeOH, HCl, $HC(OMe)_3$, reflux, 89%, ii: I_2, imidazole., PPh_3, CH_3CN:PhMe, 1:1, reflux, 90%, iii: Zn, THF:H_2O= 2:1, reflux, 3h, then ethyl 2-(bromomethyl)acrylate, reflux, 78%, **117**, (2 mol%), $(ClCH_2)_2$, reflux, 99%, iv: $AlCl_3$, $CHCl_3$, Et_3SiH, -50 ^oC-0 ^oC, 67%, v: MsCl, Et_3N, -20^oC, 92%, Tf_2O, py, -10 ^oC-0 ^oC, vi: NaN_3, acetone, H_2O, 86%, 2 steps, vii: n-Bu_3P, THF, then Et_3N, H_2O, 84%.

Scheme 1.25 Osato's synthesis of the oseltamivir phosphate **10**

1.6 Retrosynthesis of this research

Easier and shorter ways to synthesize the oseltamivir phosphate **10**, oseltamivir **56** or other related oseltamivir analogs remain the focus in this research. Similar intermediates **29** and **118** reported in **Scheme 1.5** and **Scheme 1.25** will be considered as the key of the synthesis.

The first plan of the retrosynthetic analysis to **10**, **56** or oseltamivir derivatives in this work is shown in **Scheme 1.26**

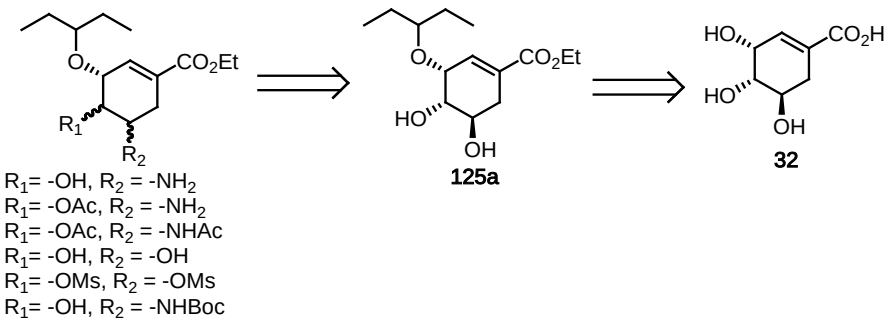


R_1= -NHAc, R_2 = $-NH_2.H_3PO_4$
R_1= -NHAc, R_2 = -NHAc
R_1= -NHAc, R_2 = $-NH_2$
R_1= -OH, R_2 = $-NH_2$
R_1= -OAc, R_2 = $-NH_2$
R_1= -OAc, R_2 = -NHAc

Scheme 1.26 Retrosynthesis of tamiflu and its derivatives of route 1

The substituents –OH, -NH_2, -OAc, –NHAc and –$NH_2.H_3PO_4$ on C4 and C5 of **10**, **56** or oseltamivir derivatives could be synthesized from intermediate compound **29** via application of key S_N2 substitution, reduction of the azide group, followed by acetylation, respectively. The intermediate compound **29** was derived from commercially available (-)-shikimic acid **32** through esterification, pentylidine ketal formation, followed by regioselective reduction, subsequent ring opening epoxidation and azide substitution, respectively.

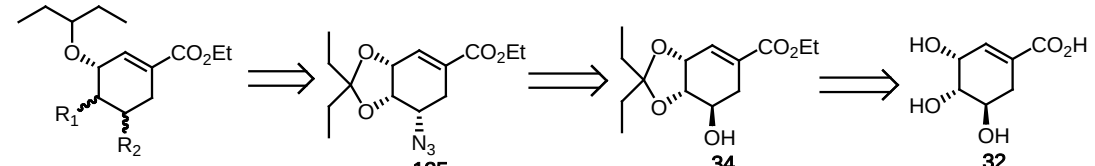
The second plan of the retrosynthetic analysis for the synthesis of oseltamivir derivatives in this work is shown in **Scheme 1.27**



Scheme 1.27 Retrosynthesis of oseltamivir derivatives of route 2

The substituents –OH, -OMs, -NH_2, -OAc, –NHAc and -NHBoc on C4 and C5 of the oseltamivir derivatives can be synthesize from *trans*-diol compound via application of the key Mitsunobu reaction, reduction of the azide group and followed by acetylation, respectively. The intermediate compounds was derived from commercially available (-)-shikimic acid **32** through esterification, followed by pentylidine ketal formation and subsequent regioselective reduction, respectively.

The third plan of the retrosynthetic analysis for the synthesis of the monoalcohol intermediate towards the synthesis of oseltamivir derivatives in this work, as shown in **Scheme 1.28**



R_1= -OH, R_2 = -NH_2
R_1= -OAc, R_2 = -NH_2
R_1= -OAc, R_2 = -NHAc
R_1= -OMs, R_2 = -NH_2
R_1= -OMs, R_2 = -NHAc
R_1= -NH_2, R_2 = -NHAc
R_1= -NHAc, R_2 = -NH_2
R_1= -NHAc, R_2 = -NHAc

Scheme 1.28 Retrosynthesis of oseltamivir derivatives of route 3

The substituents –OH, -OMs, -NH_2, -OAc, –NHAc and –$NH_2.H_3PO_4$ on C4 and C5 of the oseltamivir derivatives can be synthesize from monoalcohol intermediate via application of Mitsunobu reaction, reduction and followed by acetylation, respectively, which was derived from commercially available (-)-shikimic acid **32** through esterification, and pentylidine ketal formation, respectively.

1.7 Objective

This work aimed to carry out the three described plans in **Scheme 1.26-1.28** to synthesize new intermediates, oseltamivir phosphate **10** and its derivatives through the key S_N2 substitution or Mitsunobu reaction. Many analogs resulted from these syntheses could be used for structure-activity relationship study of new neuraminidase inhibitor of the various influenza strains including the emerging resistant strains currently encounter.

CHAPTER II

EXPERIMENTAL

2.1 Instrumentation

The following analytical methods were used throughout this work unless otherwise indicated.

The FT-IR spectra were recorded on a Perkin-Elmer FT-IR, spectrum RXI spectrometer (Perkin Elmer Instruments LLC., Shelton., U.S.A.). Samples were dissolved in dichloromethane or ethyl acetate and then dropped on potassium bromide crystal cell.

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in $CDCl_3$, DMSO-d_6 or D_2O using Varian Mercury NMR spectrometer which operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, CA, USA).

The mass spectra were recorded on Mass Spectrometer: Waters Micromass Quatto micro API ESCi (Waters, MA, USA). Samples were dissolved in a solvent and directly injected 100 μL of the solution into the Mass Spectrometer.

2.2 Chemicals

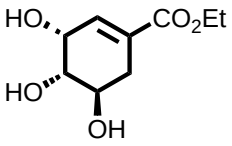
Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany).

Column chromatography was performed using silica gel (0.06-0.2 mm or 70-230 mesh ASTM), Merck Kieselgel 60 G (Merck KgaA, Darmstadt, Germany).

Chemicals and solvents were used as purchased unless otherwise noted.

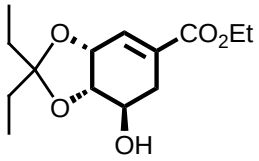
2.3 Methods

2.3.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate (ethyl shikimate) 33



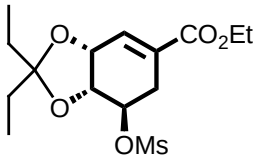
Thionyl chloride (0.42 g, 5.75 mmol) was added dropwise over to the stirring and ice-cooled solution of (-)-shikimic acid **32** (2.00 g, 11.50 mmol) in ethanol (10 mL) for 15 min. The reaction was refluxed for 3.0 h, then cooled to room temperature and concentrated in vacuo to give the brown oil of ethyl shikimate **33** (3.50 g, quantitative yield), R_f on TLC chromatogram = 0.125 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 1.20 (t, *J*=6.2 Hz, 3H, -C<u>H</u>$_3$), 2.11 (m, 1H, -C<u>H</u>$_2$-), 2.75 (m, 1H, -C<u>H</u>$_2$-), 3.61 (t, *J*=6.3 Hz, 1H, -C<u>H</u>-OH), 3.96 (br-s, 1H, -C<u>H</u>-OH), 4.10 (m, 2H, -C<u>H</u>$_2$-CH$_3$), 4.35 (br-s, 1H, -C<u>H</u>-OH), 5.48 (br-s, -O<u>H</u>), 6.73 (m, 1H, -C<u>H</u>=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 14.1 (-CH$_2$<u>C</u>H$_3$), 18.1 (-<u>C</u>H$_2$-), 31.9 (-<u>C</u>H-OH), 61.2 (-<u>C</u>H$_2$CH$_3$), 66.1 (-<u>C</u>H-OH), 66.7 (-<u>C</u>H-OH), 130.6 (-<u>C</u>H=C-), 136.0 (-CH=<u>C</u>-), 166.7 (-<u>C</u>=O); IR (neat, cm^{-1}): 3360 (-OH), 2910 (-C=C-H), 1701 (C=O), 1380, 1253 (C=C), 1081(C-O).

2.3.2 Synthesis of ethyl (3a*R*,7*R*,7a*S*)-2,2-diethyl-7-hydroxy-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-hydroxy shikimate) 34



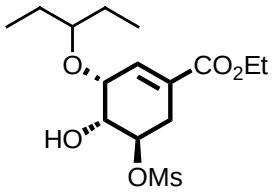
Trifluoromethane sulfonic acid (0.70 mL, 0.74 mmol) was added dropwise with syringe to the stirring and ice-cooled solution of (-)-ethyl shikimate **33** (3.00 g, 14.85 mmol) in 3-pentanone (50 mL). After stirring for 3.0 h at room temperature, unreacted 3-pentanone was distilled off with hexane as azeotropic 2:1 mixture to give the brown oil, which was redissolved in CH_2Cl_2 (25 mL), washed with water (2x25 mL), saturated $NaHCO_3$ solution (25 mL), and dried over anhydrous Na_2SO_4. The filtered solution was then concentrated in vacuo to provide the brown oil **34** (2.24 g, 83 %), R_f on TLC chromatogram = 0.50 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.0 Hz, 3H, -C($CH_2C\underline{H}_3$)$_2$), 0.92 (t, *J*=7.8 Hz, 3H, -C($CH_2C\underline{H}_3$)$_2$), 1.30 (t, *J*=7.0 Hz, 3H, -$CH_2C\underline{H}_3$), 1.74 (m, 4H, -C($C\underline{H}_2CH_3$)$_2$), 2.24 (dd, J_1=8.6 Hz, J_2=17.2 Hz, 1H, -$C\underline{H}_2$-), 2.78 (dd, J_1=4.9 Hz, 1H, -$C\underline{H}_2$-), 3.91(m, 1H, -C<u>H</u>-OH), 4.11 (t, *J*=7.0 Hz, 1H, -C<u>H</u>-O-), 4.22 (q, *J*=14.9, 2H, -$C\underline{H}_2CH_3$), 4.76 (m, 1H, -C<u>H</u>-O-), 6.93 (m, 1H, -C<u>H</u>=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 7.8 (-C($CH_2\underline{C}H_3$)$_2$, 8.5 (-C($CH_2\underline{C}H_3$)$_2$, 14.1 (-$CH_2\underline{C}H_3$), 29.0 (-$C\underline{H}_2$-), 29.2 (-C($\underline{C}H_2CH_3$)$_2$, 29.6 (-C($\underline{C}H_2CH_3$)$_2$, 61.0 (-$\underline{C}H_2CH_3$), 68.6 (-<u>C</u>H-OH), 72.2 (-<u>C</u>H-O-), 77.6 (-<u>C</u>H-O-), 113.5 (-<u>C</u>(CH_2CH_3)$_2$, 130.2 (-CH=<u>C</u>-), 134.1 (-<u>C</u>H=C-), 166.2 (-<u>C</u>=O); IR (neat, cm^{-1}): 3468 (-OH), 2976, 2932 (-C=C-H), 1712, 1650 (C=O), 1460, 1246 (C=C), 1067 (C-O).

2.3.3 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-methanesulphonyl-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-methanesulphonyl-shikimate) 26



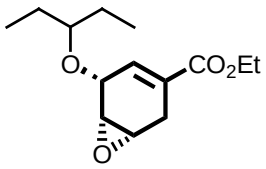
Methanesulfonylchloride (0.86 mL, 11.11 mmol) was added dropwise to the stirring solution of **34** (2.00 g. 7.40 mmol) in EtOAc (10 mL). The reaction was stirred for 15 minutes and then added Et_3N (2.00 mL, 14.8 mmol). After stirring at room temperature for 6.0 hours, the solution was filtered and washed with H_2O (2x10 mL), with 1 M $NaHCO_3$ (2x10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil **26** (2.30 g, 89% yield), R_f on TLC chromatogram = 0.65 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.83 (t, *J*=5.5 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.85 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.24 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.62 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.43 (dd, J_1=8.6 Hz, J_2=17.2 Hz, 1H, -$C\underline{H}_2$-), 2.91 (dd, J_1=4.7 Hz, J_2=17.2, 1H, -$C\underline{H}_2$-), 3.05(s, 3H, -O$\underline{Ms}$), 4.16 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.25 (t, *J*=7.0, 1H, (-C$\underline{H}$-O-)), 4.75 (m, 2H, (-C$\underline{H}$-O-), -C$\underline{H}$-OMs), 6.90 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 7.8 (-C($CH_2\underline{C}H_3$)$_2$, 8.6 (-C($CH_2\underline{C}H_3$)$_2$, 14.2 (-$CH_2\underline{C}H_3$), 27.9 (-$C\underline{H}_2$-), 28.9 (-C($\underline{C}H_2CH_3$)$_2$, 29.6 (-C($\underline{C}H_2CH_3$)$_2$, 38.7 (-O-SO_2-O-$\underline{C}H_3$), 61.2 (-$\underline{C}$H-OH), 72.3 (-$\underline{C}$H-O-), 75.0 (-$\underline{C}$H-O-), 79.1 (-$\underline{C}$H-O-), 114.4 (-$\underline{C}$(CH_2CH_3)$_2$, 129.3 (-CH=$\underline{C}$-), 134.0 (-$\underline{C}$H=C-), 165.3 (-$\underline{C}$=O).

2.3.4 Synthesis of ethyl (3*R*,4*R*,5*R*)-3-(1-ethyl-propoxy)-4-hydroxy-5-methanesulfonyloxy-1-cyclohexene-1-carboxylate 27



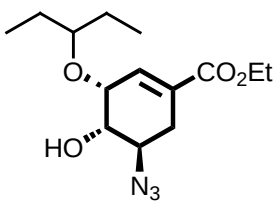
Compound **26** (2.00 g, 5.75 mmol) in CH_2Cl_2 (5 mL) was added to the stirring, ice-cooled mixture of $AlCl_3$ (0.92 g, 6.90 mmol) in CH_2Cl_2 (30 mL) followed by an addition of Et_3SiH (1.37 mL, 8.62 mmol). The reaction was left at 0 ^oC for 5.0 h and then quenched by pouring into iced water. The organic layer was separated and washed with aqueous $NaHCO_3$, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The obtained brown oil was purified by silica gel column chromatography, eluting with 10% ethyl acetate–hexane to provide the ethyl 4-hydroxy-5-methansulfonyl-3-pentylideneketal-1-cyclohexene-1-carboxylate **27** (1.50 g, 75%), R_f on TLC chromatogram = 0.60 (50% ethyl acetate:hexane). ^1H NMR ($CDCl_3$) (δ, ppm): 0.86 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.90 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.27 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.52 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.49 (dd, J_1=6.2 Hz, J_2=18.3 Hz, 1H, -$C\underline{H}_2$-), 2.97 (dd, J_1=5.5 Hz, J_2=17.9 Hz, 1H, -$C\underline{H}_2$-), 3.08(s, 3H, -O<u>Ms</u>), 3.41(quint, *J*=5.5 Hz, 1H, (-C<u>H</u>(CH_2CH_3)$_2$), 3.91(m, 1H, -C<u>H</u>-O-), 4.19 (m, 2H, (-$C\underline{H}_2CH_3$)), 4.94 (m, 2H, -C<u>H</u>-OMs, -C<u>H</u>-OH), 6.82 (m, 1H, -C<u>H</u>=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.4 (-C($CH_2\underline{C}H_3$)$_2$, 9.6 (-C($CH_2\underline{C}H_3$)$_2$, 14.2 (-$CH_2\underline{C}H_3$), 26.1 (-C($\underline{C}H_2CH_3$)$_2$, 26.4 (-C($\underline{C}H_2CH_3$)$_2$, 29.3 (-$C\underline{H}_2$-), 38.7 (-O-SO_2-O-$\underline{C}H_3$), 61.2 (-<u>C</u>H-OH), 68.6 (-<u>C</u>H-OH), 70.0 (-<u>C</u>H-O-), 71.2 (-<u>C</u>H-OMs), 82.1 (-<u>C</u>H(CH_2CH_3)$_2$, 129.2 (-CH=<u>C</u>-), 135.0 (-<u>C</u>H=C-), 165.7 (-<u>C</u>=O).

2.3.5 Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 28



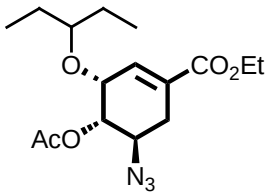
A mixture of the brown oil of ethyl 5-mesyl-4-hydroxy-5-pentylidene ketal compound **27** (1.00 g, 2.86 mmol), EtOH (20 mL) and 7.5% $NaHCO_3$ solution, was heated at 60 ^oC for 3.0 hours. The reaction mixture was extracted with n-hexane (4x20 mL), washed with water (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to light yellow oil. The residue was purified by recrystalization with hexane at 0 ^oC to give the white crystalline solid **28** (0.70 g, 96.4%), R_f on TLC chromatogram = 0.63 (50% ethyl acetate:hexane). ^1H NMR ($CDCl_3$) (δ, ppm): 0.94 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.96 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.26 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.57 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$)), 2.40 (dd, J_1=6.2 Hz, J_2=19.5 Hz, 1H, -$C\underline{H}_2$-), 3.04 (d, *J*=19.5 Hz, 1H, -$C\underline{H}_2$-), 3.46 (m, 3H, 2-C$\underline{H}$-O-, (-C$\underline{H}$(CH_2CH_3)$_2$), 4.17 (m, 2H, (-$C\underline{H}_2CH_3$)), 4.36 (m, 1H, -C$\underline{H}$-O-), 6.69 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 24.5 (-$C\underline{H}_2$-), 26.5 (2x-C($\underline{C}H_2CH_3$)$_2$, 50.7 (-$\underline{C}$H-O-), 53.4 (-$\underline{C}$H-O-), 60.8 (-$\underline{C}H_2CH_3$), 71.3 (-$\underline{C}$H-O-), 81.6 (-$\underline{C}$H(CH_2CH_3)$_2$, 126.9 (-CH=$\underline{C}$-), 135.5 (-$\underline{C}$H=C-), 166.1 (-$\underline{C}$=O).

2.3.6 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 29



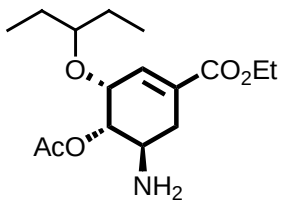
A solution of epoxide **28** (0.78 g, 3.07 mmol) in EtOH (3 mL) was added dropwise to the mixture of sodium azide (0.40 g, 6.14 mmol), ammonium chloride (0.329 g, 6.14 mmol), water (2 mL) and EtOH (10 mL). The reaction mixture was heated at 70 ^{0}C for 18 hours. The residue was extracted with EtOAc (20 mL), washed with sodium bicarbonate (10 mL), water (2x10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The resulting brown oil was purified by column chromatography on silica gel, eluting with 10% ethyl acetate–hexane to provide the ethyl 5-azido-4-hydroxy-3-pentylidene ketal compound **29** (0.84 g, 92.72 %), R_f on TLC chromatogram = 0.75 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.92 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$)), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.54 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.24 (dd, J_1=7.0 Hz, J_2=18.7 Hz, 1H, -C$\underline{H}_2$-), 2.74 (br-s, 1H, -OH), 2.87 (dd, J_1=5.5 Hz, J_2=17.9 Hz, 1H, -C$\underline{H}_2$-), 3.42 (quint, *J*=5.5 Hz, 1H, (-C$\underline{H}$(CH_2CH_3)$_2$)), 3.74 (m, 1H, -C$\underline{H}$-N_3), 3.85 (q, *J*=7.0, 1H, -C$\underline{H}$-OH), 4.11 (m, 1H, -C$\underline{H}$-O-), 4.20 (q, *J*=7.0 Hz, 2H, (-C$\underline{H}_2CH_3$)), 6.82 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.0 (-C($\underline{C}H_2CH_3$)$_2$), 26.5 (-C($\underline{C}H_2CH_3$)$_2$), 28.2 (-$\underline{C}H_2$-), 58.8 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 70.3 (-$\underline{C}$H-OH), 71.0 (-$\underline{C}$H-O-), 81.8 (-$\underline{C}$H(CH_2CH_3)$_2$)), 130.3 (-CH=$\underline{C}$-), 135.0 (-$\underline{C}$H=C-), 165.9 (-$\underline{C}$=O).

2.3.7 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 120



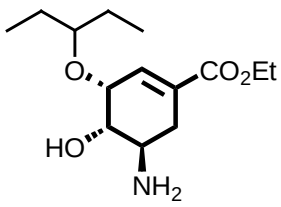
A mixture of compound **29** (0.20 g, 0.67 mmol), acetyl chloride (2 mL) and pyridine (0.5 mL) was refluxed for 3.0 hours. The reaction mixture was extracted into CH_2Cl_2 (5 mL) and dried the organic layer over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil of **120** (0.25 g, quantitative yield), R_f on TLC chromatogram = 0.71 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.81 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.23 (t, *J*=7.0 Hz, 3H , (-$CH_2C\underline{H}_3$)), 1.44 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$)), 2.09 (s, 3H, -C(O)C$\underline{H}_3$), 2.18 (dd, J_1=4.4 Hz, J_2=20.0 Hz, 1H, -C$\underline{H}_2$-), 2.82 (dd, J_1=5.5 Hz, J_2=18.7 Hz, 1H, -C$\underline{H}_2$-), 3.22 (quint, *J*=6.2 Hz, 1H, (-C$\underline{H}$(CH_2CH_3)$_2$)), 4.00 (q, *J*=9.3 Hz, 1H, -C$\underline{H}$-N_3), 4.15 (q, *J*=7.0 Hz, 2H, (-C$\underline{H}_2CH_3$)), 4.20 (m, 1H, C$\underline{H}$-O-), 4.84 (dd, J_1=4.0 Hz, J_2=9.4 Hz, 1H, -C$\underline{H}$-OAc), 6.78 (m, 1H, -C$\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.1 (-C($CH_2\underline{C}H_3$)$_2$), 9.8 (-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.1 (-(CO)-$\underline{C}H_3$), 24.2 (2x-C($CH_2\underline{C}H_3$)$_2$), 29.6 (-C$\underline{H}_2$-), 55.6 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 69.3 (-$\underline{C}$H-O-), 73.2 (-$\underline{C}$H-O(C=O)-CH_3), 83.0 (-$\underline{C}$H(CH_2CH_3)$_2$)), 129.6 (-CH=$\underline{C}$-), 135.2 (-$\underline{C}$H=C-), 165.6 (-CH-O($\underline{C}$=O)-CH_3), 170.5 (-$\underline{C}$=O).

2.3.8 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 121



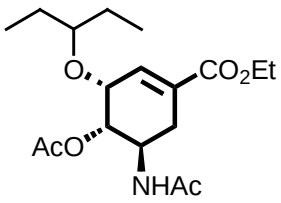
Compound **120** (0.20 g, 0.588 mmol) in CH_3CN (1 mL) was added dropwise over to the stirring and ice–cooled solution of triphenyl phosphine (0.30 g, 0.882 mmol) in CH_3CN-H_2O (5:1) (6 mL) and stirred the mixture for 15 minutes, and then at room temperature for 3.0 hours. The reaction mixture was evaporated to CH_3CN and then added EtOAc (10 mL). The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **121** (0.14 g, 70%), R_f on TLC chromatogram = 0.10 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.94 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.47-1.60 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$)), 2.02 (s, 3H, -C(O)$C\underline{H}_3$), 2.06 (d, *J*=8.58 Hz, 1H, (-$C\underline{H}_2$-), 3.04 (dd, J_1=4.7 Hz, J_2=18.0 Hz, 1H, -$C\underline{H}_2$-), 3.44 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}$(CH_2CH_3)$_2$), 3.63 (m, 1H, -$C\underline{H}$-NH_2), 4.06 (m, 1H, (-$C\underline{H}$-O(C=O)-CH_3)), 4.17-4.24 (m, 3H, (-$C\underline{H}_2CH_3$), -$C\underline{H}$-OAc), 5.80 (m, 1H, (-$N\underline{H}$-(C=O)-CH_3)), 6.88 (m, 1H, -$C\underline{H}$=C-); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.1 (-C($CH_2\underline{C}H_3$)$_2$), 9.8 (-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.1 (-(CO)-$\underline{C}H_3$), 24.2 (2x-C($CH_2\underline{C}H_3$)$_2$), 29.6 (-$C\underline{H}_2$-), 55.6 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 69.3 (-$\underline{C}$H-O-), 73.2 (-$\underline{C}$H-O(C=O)-CH_3), 83.0 (-$\underline{C}$H(CH_2CH_3)$_2$)), 129.6 (-CH=$\underline{C}$-), 135.2 (-$\underline{C}$H=C-), 165.6 (-CH-O($\underline{C}$=O)-CH_3), 170.5 (-$\underline{C}$=O).

2.3.9 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 122



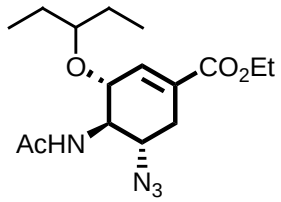
Compound **29** (0.10 g, 0.337mmol) in CH_3CN (1 mL) was added dropwise over to the stirring and ice–cooled solution of triphenyl phosphine (0.11g, 0.404 mmol) in CH_3CN-H_2O (3:1) (4 mL) and stirred the mixture for 15 minutes, and then at room temperature for 3 hours, the reaction mixture was remove CH_3CN to give the aqueous solution and then EtOAc (5 mL) was added, the mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate. Filtered and concentrated in vacuo and the residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **122** (0.75 g, 82%), R_f on TLC chromatogram = 0.28 (9:1 ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.78 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.82 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.20 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.44 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.37 (m, 1H, (-$C\underline{H}_2$-)), 3.01 (dd, J_1=4.7 Hz, J_2=17.9 Hz, 1H, (-$C\underline{H}_2$-)), 3.37 (quint, *J*=4.5 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.46 (m, 1H, (-$C\underline{H}$-$NH_2$)), 3.87 (m, 1H, (-$C\underline{H}$-OH)), 4.05 (m, 1H, (-$C\underline{H}$-O-)), 4.12 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.82 (m, 1H, (-$C\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.6 (2x-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.0 (2x-C($\underline{C}H_2CH_3$)$_2$), 26.5 (-$\underline{C}H_2$-), 48.5 (-$\underline{C}$H-NH_2), 61.2 (-$\underline{C}H_2CH_3$), 65.0 (-$\underline{C}$H-OH), 66.0 (-$\underline{C}$H-OH), 82.0 (-$\underline{C}H(CH_2CH_3)_2$)), 131.0 (-$\underline{C}$H=C-), 135.0 (-$\underline{C}$H=C-), 166.0 (-$\underline{C}$=O), MS (EI) $[M+H]^+$ = 272.367.

2.3.10 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 123



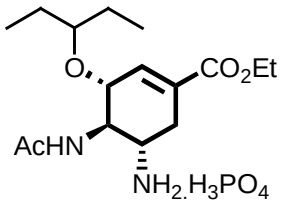
A mixture of compound **122** (0.05 g, 0.184 mmol), acytyl chloride (2 mL) and pyridine (0.5 mL) was refluxed for 3.0 hours. The reaction mixture was extracted into CH_2Cl_2 (5 mL), and dried the organic layer over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil **123** (0.60 g, 91%), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.87 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.93 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.27 (t, *J*=7.8 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.50 (quint, *J*=7.0 Hz, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 1.94 (s, 3H, (-C(O)$C\underline{H}_3$)), 2.10 (s, 3H, (-C(O)$C\underline{H}_3$)), 2.10 (m, 1H, (-$C\underline{H}_2$-)), 3.00 (dd, J_1=5.5 Hz, J_2=18.3 Hz, 1H, (-$C\underline{H}_2$-)), 3.33 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}$(CH_2CH_3)$_2$), 4.13 (m, 1H, (-$C\underline{H}$-O-)), 4.18(q, *J*=6.2 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.57 (quint, *J*=7.0 Hz, 1H, (-$C\underline{H}$-NHAc)), 4.95 (dd, J_1=3.1 Hz, J_2=11.3 Hz, 1H, (-$C\underline{H}$-OAc)), 5.68(d, *J*=9.4 Hz, 1H, (-$N\underline{H}$-(C=O)-CH_3)), 6.83 (m, 1H, (-$C\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.3 (-C($CH_2\underline{C}H_3$)$_2$), 10.0 (-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.3 (-O-(CO)-$\underline{C}H_3$), 23.5 (-NH-(CO)-$\underline{C}H_3$), 26.5 (2x-C($CH_2\underline{C}H_3$)$_2$), 31.4 (-$C\underline{H}_2$-), 44.9 (-$\underline{C}$H-O-), 61.0 (-$\underline{C}H_2CH_3$), 70.0 (-$\underline{C}$H-O-(C=O)-CH_3), 72.5 (-$\underline{C}$H-NH-(C=O)-CH_3), 83.0 (-$\underline{C}$H(CH_2CH_3)$_2$), 130.8 (-CH=$\underline{C}$-), 135.0 (-$\underline{C}$H=C-), 165.9 (-$\underline{C}$=O), 169.8 (-CH-O-($\underline{C}$=O)-CH_3), 171.7 (-CH-NH-($\underline{C}$=O)-CH_3), MS (EI) $[M+H+Na]^+$ = 378.50.

2.3.11 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 31



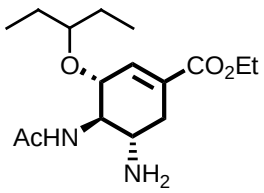
A solution of ethyl-5-azido-4-hydroxy-3-pentylidene ketal compound **29** (1.70 g. 5.70 mol) in DMF (5 mL) was added dropwise to the triphenylphosphin (1.70 g. 5.70 mol) in CH_3CN (2 mL). The mixture was heated at reflux for 6.0 hours, then concentrated in vacuo to dark brown oil and then the solution of crude in DMF (2 mL) was added dropwise to the mixture of sodium azide (1.70 g. 5.70 mol), ammonium chloride (1.70 g. 5.70 mol) in DMF (2 mL), the reaction mixture was heated at 80 ^{0}C for 18.0 hours, acetic anhydride (2 mL) and triethylamine (2 mL) in CH_2Cl_2 (5 mL) were added to the reaction and then refluxed for 3.0 hours. The reaction mixture was extracted with CH_2Cl_2 (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the brown oil, and purified by column chromatography on silica gel, eluting with 10% ethyl acetate–hexane to provide the acetamido azide compound 31 (0.691 g, 36%), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.92 (t, *J*=7.3 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.93 (t, *J*=7.3 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.32 (t, *J*=7.1 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.47-1.59 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.06 (s, 3H, (-C(O)$C\underline{H}_3$)), 2.10-2.31 (m, 1H, (-$C\underline{H}_2$-)), 2.88 (dd, J_1=5.7 Hz, J_2=17.1 Hz, 1H, (-$C\underline{H}_2$-)), 3.40 (m, 2H, (-$C\underline{H}(CH_2CH_3)_2$, (-$C\underline{H}$-O-)), 4.23 (q, *J*=7.1 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.27-4.34 (m, 1H, (-$C\underline{H}$-N_3)), 4.57-4.60 (m, 1H, (-$C\underline{H}$-NHAc)), 6.01 (d, *J*=7.4 Hz, 1H, (-$N\underline{H}$-(C=O)-CH_3)), 6.81 (dd, J_1=2.2 Hz, J_2=2.3 Hz, 1H, (-$C\underline{H}$=C-)).

2.3.12 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-*N*-acetamido-5-amino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate phosphate (oseltamivir phosphate) 10



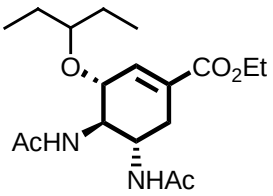
A solution of azido compound **31** (0.69 g. 2.04 mmol) in CH_3CN (5 mL) was added dropwise to the triphenylphosphine (0.532 g, 2.04 mmol) in CH_3CN-H_2O (3:1) (12 mL) and stirring was continued for 15 minutes. After the reaction mixture was stirred at the room temperature for 3.0 hours, the reaction mixture was evaporated to CH_3CN and then added EtOAc (10 mL). The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by column chromatography through siliga gel using MeOH–EtOAc (1:9) as eluent to give a yellow oil compound **56** and dissolve in abs.EtOH (10 mL) and added 85% H_3PO_4 (1 mL). Crystallization commenced immediately and after cooling to 0 ^oC for 12 hours the precipitate was collected by filtration to afford tamiflu **10** (0.462 g, 55%) [12], R_f on TLC chromatogram = 0.15 (9:1 ethyl acetate:MeOH). ^1H NMR ($CDCl_3$) (δ, ppm): 0.65 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.69 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.10 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.24-1.43 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 1.89 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.32 (m, 1H, (-$C\underline{H}_2$-)), 2.77 (dd, J_1=6.2 Hz, J_2=16.8 Hz, 1H, (-$C\underline{H}_2$-)), 3.35 (m, 2H, (-$C\underline{H}$(CH_2CH_3)$_2$), (-$C\underline{H}$-$NH_2.H_3PO_4$)), 3.86 (t, *J*=10.1 Hz, 1H, (-$C\underline{H}$-NHAc)), 4.06 (q, *J*=6.2 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.14 (d, *J*=9.4 Hz, 1H, (-$C\underline{H}$-O-)), 6.83 (m, 1H, (-$C\underline{H}$=C-)). ^{13}C NMR ($CDCl_3$) (δ, ppm): 8.4 (-C($CH_2\underline{C}H_3$)$_2$), 8.5 (-C($CH_2\underline{C}H_3$)$_2$), 13.3 (-$CH_2\underline{C}H_3$), 22.4 (-O-(CO)-$\underline{C}H_3$), 25.0 (-C($CH_2\underline{C}H_3$)$_2$), 25.4 (-C($CH_2\underline{C}H_3$)$_2$), 28.1 (-$C\underline{H}_2$-), 49.1 (-$\underline{C}$H-$NH_2.H_3PO_4$), 52.6 (-$\underline{C}$H-O-), 62.4 (-$\underline{C}H_2CH_3$), 75.1 (-$\underline{C}$H-NH-(C=O)-CH_3), 84.3 (-$\underline{C}$H(CH_2CH_3)$_2$), 127.6 (-CH=$\underline{C}$-), 137.9 (-$\underline{C}$H=C-), 165.0 (-$\underline{C}$=O), 176.0 (-CH-NH-($\underline{C}$=O)-CH_3), MS (EI) M^+ = 313.397.

2.3.13 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (oseltamivir) 56



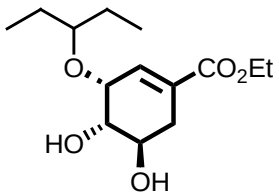
Oseltamivir phosphate **10** (0.010 g, 0.238 mmol) dissolved in CH_2Cl_2 (1 mL) was neutralized by shaking with saturated $NaHCO_3$ (3 mL) for 5 min. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give the free base of oseltamivir **56**. R_f on TLC chromatogram = 0.12 (1:4 ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.89 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.46-1.54 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 2.03 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.11-2.18 (m, 1H, (-$C\underline{H}_2$-)), 2.74 (dd, J_1=5.5 Hz, J_2=17.6 Hz, 1H, (-$C\underline{H}_2$-)), 3.22 (m, 1H, (-$C\underline{H}$-NH_2)), 3.33 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.53 (q, *J*=9.36 Hz, 1H, (-$C\underline{H}$-NHAc)), 4.19 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 5.78 (d, *J*=7.8 Hz, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.77 (s, 1H, (-$C\underline{H}$=C-)), MS (EI) $[M+H]^+$ = 313.397.

2.3.14 Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 124



Compound **56** (0. 010 g, 0.310 mmol) was dissolved in CH_2Cl_2 and acetyl chloride (3 mL) was added and followed by pyridine (1 mL). The reaction was stirred at reflux for 3.0 hours and the cooled mixture was extracted with CH_2Cl_2 (2x10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give yellow solid **124** (0.015 g, quantitative yield), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.82 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$)), 0.84 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$)), 1.23 (t, *J*=7.8 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.44 (quint, *J*=7.0 Hz, 2H, (-C($C\underline{H}_2CH_3)_2$)), 1.45 (quint, *J*=7.0 Hz, 2H, (-C($C\underline{H}_2CH_3)_2$)), 1.92 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 1.92 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.23 (dd, J_1=9.4 Hz, J_2=17.6 Hz, 1H, (-$C\underline{H}_2$-)), 2.69 (dd, J_1=4.7 Hz, J_2=17.9 Hz, 1H, (-$C\underline{H}_2$-)), 3.31 (quint, *J*=6.2 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$)), 4.01 (m, 3H, (-C$\underline{H}$-O-), (2x-C$\underline{H}$-NHAc)), 4.14 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.08 (br-s, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.63 (m, 1H, (-N$\underline{H}$-(C=O)-CH_3)), 6.73 (s, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.3 (-C($CH_2\underline{C}H_3)_2$), 9.5 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 23.3 (2x-NH-(CO)-$\underline{C}H_3$), 25.8 (-C($CH_2\underline{C}H_3)_2$), 26.2 (-C($CH_2\underline{C}H_3)_2$), 30.5 (-$C\underline{H}_2$-), 48.5 (-$\underline{C}$H-NH-), 53.7 (-$\underline{C}$H-NH-), 61.0 (-$\underline{C}H_2CH_3$), 75.4 (-$\underline{C}$H-O-)), 82.1 (-$\underline{C}$H($CH_2CH_3)_2$), 131.0 (-CH=$\underline{C}$-), 136.9 (-$\underline{C}$H=C-), 167.0 (-$\underline{C}$=O), 172.0 (-CH-NH-($\underline{C}$=O)-CH_3), 174.0 (-CH-NH-($\underline{C}$=O)-CH_3), MS (EI) $[M+H]^+$ = 355.477.

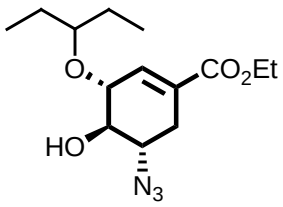
2.3.15 Synthesis of ethyl (3*R*,4*S*,5*R*)-3-(1-ethyl-propoxy)-4,5-dihydroxy-1-cyclohexene-1-carboxylate 125



$TiCl_4$ (0.60 g, 5.55 mmol) in CH_2Cl_2 (2 mL) was added to the stirring, ice-cooled mixture of compound **34** (1.0 g, 3.70 mmol) in CH_2Cl_2 (10 mL) followed by Et_3SiH (0.50 mL, 5.55 mmol). The reaction was left at 0 ^oC for 5.0 hours, and then quenched by pouring into iced water. The organic layer was separated and washed

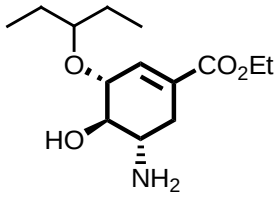
with aqueous $NaHCO_3$, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The obtained brown oil was purified by silica gel column chromatography, eluting with 30% ethyl acetate–hexane to provide the ethyl 4,5-dihydroxy-3-pentylidene ketal-1-cyclohexene-1-carboxylate **125a** (0.75 g, 75%), R_f on TLC chromatogram = 0.36 (50% ethyl acetate:hexane). and **125b** (0.10 g, 10%), R_f on TLC chromatogram = 0.83 (50% ethyl acetate:hexane). **125a**: ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.86 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.92 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.27 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.53 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.18 (dd, J_1=7.8 Hz, J_2=17.9 Hz, 1H, (-$C\underline{H}_2$-)), 2.52 (br-s, 2H, -O<u>H</u>), 2.88 (dd, J_1=4.7 Hz, J_2=18.3 Hz, 1H, (-$C\underline{H}_2$-)), 3.41 (quint, *J*=5.5 Hz, 1H, (-C<u>H</u>($CH_2CH_3)_2$), 3.59 (dd, J_1=4.7 Hz, J_2=9.4, 1H, (-C<u>H</u>-OH)), 3.94 (q, *J*=6.2 Hz, 1H, (-C<u>H</u>-OH)), 4.14 (t, *J*=4.7 Hz, 1H, (-C<u>H</u>-O-)), 4.19 (q, *J*=7.0 Hz, 1H, (-$C\underline{H}_2CH_3$)), 6.85 (m, 1H, (-C<u>H</u>=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.5(2), (2x-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.0 (-C($\underline{C}H_2CH_3)_2$), 26.6 (-C($\underline{C}H_2CH_3)_2$), 31.3 (-$\underline{C}H_2$-), 61.0 (-$\underline{C}H_2CH_3$), 67.7 (-<u>C</u>H-OH), 71.2 (-<u>C</u>H-O-), 72.2 (-<u>C</u>H-OH), 81.8 (-<u>C</u>H($CH_2CH_3)_2$), 130.9 (-CH=<u>C</u>-), 134.7 (-<u>C</u>H=C-), 166.6 (-<u>C</u>=O); IR (neat, cm^{-1}): 3428 (-O-H), 2965 (-C=C-H), 1708 (-C=O), 1602 (-C=C-), 1457 (-C=C-), 1089 (-C-O). MS (EI) $[M+Na]^+$ = 295.318. **125b**: ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (m, 6H, (-C($CH_2C\underline{H}_3)_2$), 1.25 (t, *J*=7.5 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.44 (m, 2H, (-C($C\underline{H}_2CH_3)_2$)), 1.52 (m, 2H, (-C($C\underline{H}_2CH_3)_2$), 2.29 (d, *J*=18.8 Hz, 1H, (-$C\underline{H}_2$-)), 2.60 (d, *J*=17.8 Hz, 1H, (-$C\underline{H}_2$-)), 2.63 (br-s, 2H, -OH), 3.24 (quint, *J*=5.6 Hz, 1H, (-C<u>H</u>($CH_2CH_3)_2$)), 3.42 (q, *J*=5.6 Hz, 1H, (-C<u>H</u>-OH)), 3.87 (m, 1H, (-C<u>H</u>-OH)), 4.17 (q, *J*=6.6 Hz, 2H, (-$C\underline{H}_2CH_3$)), 4.21 (m, 1H, (-C<u>H</u>-O-)), 6.70 (m, 1H, (-C<u>H</u>=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.4 (-C($CH_2\underline{C}H_3)_2$), 9.7 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.4 (-C($\underline{C}H_2CH_3)_2$), 26.6 (-C($\underline{C}H_2CH_3)_2$), 27.5 (-$\underline{C}H_2$-), 60.6 (-$\underline{C}H_2CH_3$), 68.4 (-<u>C</u>H-OH), 72.0 (-<u>C</u>H-O-), 73.1 (-<u>C</u>H-OH), 81.7 (-<u>C</u>H($CH_2CH_3)_2$), 129.4 (-CH=<u>C</u>-), 135.8 (-<u>C</u>H=C-), 166.6 (-<u>C</u>=O); IR (neat, cm^{-1}): 3428 (-O-H), 2965 (-C=C-H), 1708 (-C=O), 1249 (-C=C-), 1089 (-C-O).

2.3.16 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 126a



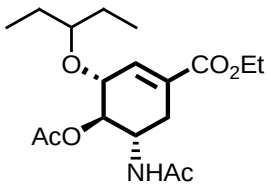
Hydrazoic acid (HN_3) was prepared by NaN_3, (0.80 g, 12.3 mmol) dissolved with H_2O:benzene (5:1, 6 mL) at 0 ^oC, conc.H_2SO_4 (1 mL) was added dropwise to the solution for 30 min. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered to obtaining the hydrazoic solution. Diisopropylazodicarboxylate (3.0 mL, 11.8 mmol) and hydrazoic acid (HN_3) (12.3 mmol, 4 mL) were added dropwise to the stirred solution of triphenylphospine (Ph_3P) (3.0 g, 11.8 mmol) in toluene (5 mL) at 0 ^oC for 10 minutes, and then the solution of compound **125a** (0.80 g, 2.95 mmol) in toluene (2 mL) was added dropwise. The stirring was continued at 0 ^oC for 6.0 hours and then for additional 24.0 hours to mixture solution. Evaporation of the solvent and purified the residue by silica gel column chromatography, eluting with 5% EtOAc–hexane to provide the product **126a** (0.370 g, 61%), R_f on TLC chromatogram = 0.60 (50% ethyl acetate:hexane). ^1H NMR ($CDCl_3$) (δ, ppm): 0.84 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 0.86 (t, *J*=5.0 Hz, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.24 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.46 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.07 (m, 1H, (-$C\underline{H}_2$-)), 2.84 (dd, J_1=4.0 Hz, J_2=18.0 Hz, 1H, (-$C\underline{H}_2$-)), 2.99 (s, 1H, -OH), 3.29 (quint, *J*=6.0 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$)), 3.64 (t, *J*=8.99 Hz, 1H, (-$C\underline{H}$-N_3)), 4.08 (m, 1H, (-$C\underline{H}$-OH)), 4.15 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.53 (t, *J*=3.0 Hz, 1H, (-$C\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) δ 9.4 (-C($CH_2\underline{C}H_3$)$_2$), 9.8 (-C($CH_2\underline{C}H_3$)$_2$), 14.1 (-$CH_2\underline{C}H_3$), 25.7 (-C($\underline{C}H_2CH_3$)$_2$), 26.6 (-C($\underline{C}H_2CH_3$)$_2$), 30.0 (-$\underline{C}H_2$-), 61.2 (-$\underline{C}H_2CH_3$), 63.2 (-$\underline{C}H$-N_3), 74.6 (-$\underline{C}H$-OH), 75.0 (-$\underline{C}H$-O-), 80.6 (-$\underline{C}H(CH_2CH_3)_2$), 130.0 (-CH=$\underline{C}$-), 134.0 (-$\underline{C}H$=C-), 165.7 (-$\underline{C}$=O); MS (EI) M^+ = 297.287.

2.3.17 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate 130



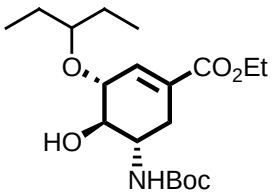
Compound **126** (0.10 g, 0.34 mmol) in CH_3CN (1 mL) was added dropwise to the stirring, cool solution of triphenyl phosphine (0.20 g, 0.76 mmol) in 2:1 CH_3CN-H_2O (6 mL) for 15 min. After stirring room temperature for 3.0 hours, most of the solvent was removed and EtOAc (10 mL) was added. The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography using 5% MeOH–EtOAc (1:9) as eluent to give yellow oil **130** (0.070 g, 91.1 %), R_f on TLC chromatogram = 0.30 (25% ethyl acetate:MeOH). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.83 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.85 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.22 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.43 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.13 (m, 1H, (-$C\underline{H}_2$-)), 2.78 (dd, J_1=4.9 Hz, J_2=17.2 Hz 1H, (-$C\underline{H}_2$-)), 3.28 (quint, *J*=5.5 Hz, 1H, (-C$\underline{H}$($CH_2CH_3)_2$)), 3.49 (m, 2H, (-C$\underline{H}$-OH), (-C$\underline{H}$-NH_2)), 3.58 (m, 1H, (-C$\underline{H}$-O-)), 4.13 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.66 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.3 (-C($CH_2\underline{C}H_3)_2$), 9.9 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 25.9 (-C($\underline{C}H_2CH_3)_2$), 26.6 (-C($\underline{C}H_2CH_3)_2$), 29.9 (-$\underline{C}H_2$-), 53.8 (-$\underline{C}$H-NH_2), 61.0 (-$\underline{C}H_2CH_3$), 74.8 (-$\underline{C}$H-OH), 75.0 (-$\underline{C}$H-O-), 80.8 (-$\underline{C}$H($CH_2CH_3)_2$), 129.4 (-CH=$\underline{C}$-), 136.8 (-$\underline{C}$H=C-), 166.2 (-$\underline{C}$=O); MS (EI) $[M+H]^+$ = 273.210.

2.3.18 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-actamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 131



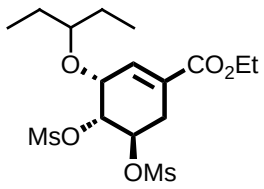
Compound **130** (0.030 g, 0.111 mmol) and acetyl chloride (5 mL) was stirred at room temperature and added by pyridine (1 mL). The reaction was brought to reflux for 3.0 hours and then quenched with water. The obtained mixture was extracted with CH_2Cl_2 (2x10 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give white solid **131** (0.040 g, 100%), R_f on TLC chromatogram = 0.75 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm); 0.84 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$)), 0.87 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$)), 1.28 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.44 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 1.95 (s, 3H, (-O-C(O)$C\underline{H}_3$)), 2.04 (s, 3H, (-NH-C(O)$C\underline{H}_3$)), 2.50 (m, 2H, (-$C\underline{H}_2$-)), 3.30 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$)), 3.87 (q, *J*=5.5 Hz, 1H, (-C<u>H</u>-O-)), 4.20 (m, 2H, (-$C\underline{H}_2CH_3$)), 4.75 (m, 1H, (-C<u>H</u>-NHAc)), 4.90 (t, *J*=4.7 Hz, 1H, (-C<u>H</u>-OAc)), 6.17 (d, *J*=8.6 Hz, 1H, (-N<u>H</u>-(C=O)-CH_3)), 6.72 (m, 1H, (-C<u>H</u>=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.2 (-C($CH_2\underline{C}H_3)_2$), 9.6 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 21.0 (-O-(CO)-$\underline{C}H_3$), 23.2 (-NH-(CO)-$\underline{C}H_3$), 25.7 (-C($CH_2\underline{C}H_3)_2$), 26.2 (-C($CH_2\underline{C}H_3)_2$), 27.6 (-$C\underline{H}_2$-), 47.7 (-<u>C</u>H-O-), 59.3 (-$\underline{C}H_2CH_3$), 71.0 (-<u>C</u>H-O-(C=O)-CH_3), 71.6 (-<u>C</u>H-NH-(C=O)-CH_3), 81.6 (-$\underline{C}H(CH_2CH_3)_2$), 128.6 (-CH=<u>C</u>-), 134.8 (-<u>C</u>H=C-), 166.4 (-<u>C</u>=O), 169.1 (-CH-O-(<u>C</u>=O)-CH_3), 170.5 (-CH-NH-(<u>C</u>=O)-CH_3); IR (neat, cm^{-1}): 3268 (-O-H), 2966 (-C=C-H), 1741 (-C=O), 1249 (-C=C-), 1056 (-C-O); MS (EI): $[M+Na]^+$=378.159.

2.3.19 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-*tert*-butoxycarbonylamino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate 132



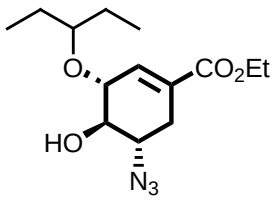
A solution of compound **130** (0.030 g, 0.112 mmol) in THF (1 mL) was added dropwise to the stirring, ice cooled solution of $NaHCO_3$ (0.012 g, 0.134 mmol) and $(Boc)_2O$ (0.030 g, 0.134 mmol) in 5:2 THF-H_2O (7 mL). After the reaction mixture was stirred at room temperature for 5.0 hours, the solvent was removed and then EtOAc (10 mL) was added. The mixture was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography using 10% EtOAc-hexane as eluent to give yellow oil **132** (0.030 g, 72.5%), R_f on TLC chromatogram = 0.65 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.86 (t, *J*=7.8 Hz, 6H, (2x-C($CH_2C\underline{H}_3)_2$), 1.26 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.43 (s, 9H, (-Boc)), 1.51 (m, 4H, (-C($C\underline{H}_2CH_3)_2$)), 2.20 (d, *J*=15.6 Hz, 1H, (-$C\underline{H}_2$-)), 2.75 (d, *J*=17.0 Hz, 1H, (-$C\underline{H}_2$-)), 3.32 (quint, *J*=5.5 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.60 (m, 2H, (-$C\underline{H}$-O-), (-$C\underline{H}$-OH)), 4.17 (m, 2H, (-$C\underline{H}_2CH_3$)), 4.28 (m, 1H, (-$C\underline{H}$-NH-Boc)), 4.98 (d, *J*=7.8 Hz, 1H, (-N$\underline{H}$-(C=O)-Boc)), 6.69 (s, 1H, (-$C\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.2 (-C($CH_2\underline{C}H_3)_2$), 9.8 (-C($CH_2\underline{C}H_3)_2$), 14.2 (-$CH_2\underline{C}H_3$), 25.8 (-C($CH_2\underline{C}H_3)_2$), 26.5 (-C($CH_2\underline{C}H_3)_2$), 28.3 (-NH-O-(CO)-($\underline{C}(CH_3)_3$), 28.9 (-$C\underline{H}_2$-), 53.0 (-$\underline{C}$H-NH-Boc), 60.8 (-$\underline{C}H_2CH_3$), 73.0 (-$\underline{C}$H-OH), 73.4 (-$\underline{C}$H-O-), 79.8 (-NH-O-(CO)-($\underline{C}(CH_3)_3$), 80.6 (-$\underline{C}H(CH_2CH_3)_2$), 137.0 (-CH=$\underline{C}$-), 138.0 (-$\underline{C}$H=C-), 155.6 (-NH-O-($\underline{C}$O)-($C(CH_3)_3$), 166.4 (-$\underline{C}$=O); MS (EI) $[M+Na]^+$=394.253.

2.3.20 Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-bis(mesyloxy)-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 133



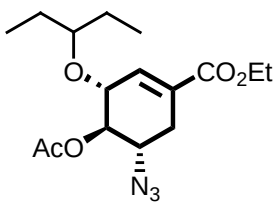
Methanesulfonyl chloride (0.30 mL, 2.768 mmol) was added dropwise to the stirring solution of **125a** (0.25 g, 0.923 mmol) in EtOAc (5 mL). The reaction was stirred for 15 minutes and then added Et_3N (0.60 mL, 3.69 mmol). After stirring at room temperature for 6.0 hours, the solution was filtered and washed with H_2O (2x10 ml), extracted with 1 M. $NaHCO_3$ (2x10 mL), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography using 10% EtOAc-hexane as eluent to give **133** (0.35 g, 89%), R_f on TLC chromatogram = 0.50 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.85-0.97 (m, 6H, (-C($CH_2C\underline{H}_3$)$_2$), 1.25-1.30 (m, 3H, (-C($CH_2C\underline{H}_3$)$_2$), 1.53 (m, 4H, (-C($C\underline{H}_2CH_3$)$_2$), 2.58 (m, 1H, (-C$\underline{H}_2$-)), 3.11 (s, 6H, -OMs), 4.20 (m, 2H, (-$C\underline{H}_2CH_3$)), 4.37 (m, 1H, (-C$\underline{H}$-O-)), 4.80 (m, 1H, (-C$\underline{H}$-OMs)), 5.19 (m, 1H, (-C$\underline{H}$-OMs)), 6.83 (m, 1H, (-C$\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 9.1 (-C($CH_2\underline{C}H_3$)$_2$), 9.8 (-C($CH_2\underline{C}H_3$)$_2$), 14.2 (-$CH_2\underline{C}H_3$), 26.0 (-O-(SO_2)-O$\underline{C}H_3$), 26.5 (-O-(SO_2)-O$\underline{C}H_3$), 30.4 (-C$\underline{H}_2$-), 38.2 (-C($\underline{C}H_2CH_3$)$_2$), 38.7 (-C($\underline{C}H_2CH_3$)$_2$), 61.2 (-$\underline{C}H_2CH_3$), 70.6 (-$\underline{C}$H-O-), 73.9 (-$\underline{C}$H-OMs), 77.5 (-$\underline{C}$H-OMs), 83.5 (-$\underline{C}$H(CH_2CH_3)$_2$), 128.9 (-CH=$\underline{C}$-), 134.5 (-$\underline{C}$H=C-), 165.3 (-$\underline{C}$=O).

2.3.21 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 126



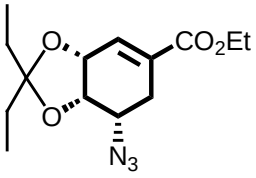
A solution of bis-mesyloxy compound **133** (0.350 g. 0.820 mmol) in DMF (2 mL) was added dropwise to the mixture of sodium azide (NaN_3) (1.0 g. 8.20 mmol), potassium fluoride (KF) (48 mg. 0.082mmol) 18-crown-6 (20 mg. 0.082 mmol) in DMF (2 mL), the reaction mixture was heated at 70^0C for 24.0 hours. The reaction residue was extracted with EtOAc (5 mL) and was washed with water (3x10 mL), dried over anhydrous sodium sulfate (anh.Na_2SO_4), filtered and concentrated in vacuo. The brown oil (0.250 g.) was purified by column chromatography on silica gel, eluting with 10% ethyl acetate–hexane to provide the ethyl 5-azido-4-hydroxy-3-pentylidine ketal compound **126a** (0.18 g. 74% yield), R_f on TLC chromatogram = 0.60 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.84 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 0.86 (t, *J*=5.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.24 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.46 (m, 4H, (-C($C\underline{H}_2CH_3)_2$), 2.07 (m, 1H, (-$C\underline{H}_2$-)), 2.84 (dd, J_1=4.0 Hz, J_2=18.0 Hz, 1H, (-$C\underline{H}_2$-)), 2.99 (s, 1H, -OH), 3.29 (quint, *J*=6.0 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.64 (t, *J*=8.99 Hz, 1H, (-$C\underline{H}$-N_3)), 4.08 (m, 1H, (-$C\underline{H}$-OH)), 4.15 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 6.53 (t, *J*=3.0 Hz, 1H, (-$C\underline{H}$=C-)); ^{13}C NMR ($CDCl_3$) δ 9.4 (-C($CH_2\underline{C}H_3)_2$), 9.8 (-C($CH_2\underline{C}H_3)_2$), 14.1 (-$CH_2\underline{C}H_3$), 25.7 (-C($\underline{C}H_2CH_3)_2$), 26.6 (-C($\underline{C}H_2CH_3)_2$), 30.0 (-$\underline{C}H_2$-), 61.2 (-$\underline{C}H_2CH_3$), 63.2 (-$\underline{C}$H-N_3), 74.6 (-$\underline{C}$H-OH), 75.0 (-$\underline{C}$H-O-), 80.6 (-$\underline{C}H(CH_2CH_3)_2$), 130.0 (-CH=$\underline{C}$-), 134.0 (-$\underline{C}$H=C-), 165.7 (-$\underline{C}$=O); MS (EI) M^+ = 297.287.

2.3.22 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 134



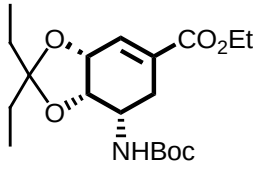
Compound **126** (0.010 g, 0.0337 mmol) and acetyl chloride (1 mL) was stirred at room temperature and added by pyridine (0.2 mL). The reaction was brought to reflux for 2.0 h and then quenched with water. The obtained mixture was extracted with CH_2Cl_2 (2x5 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give yellow oil **134** (0.012 g, 100%), R_f on TLC chromatogram = 0.70 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.80 (t, *J*=7.0 Hz, 6H, (-C($CH_2C\underline{H}_3)_2$)), 1.25 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.36-1.64 (m, 4H, (-$CH_2C\underline{H}_3$)), 2.05 (s, 3H, (-C(O)$C\underline{H}_3$)), 2.29-2.35 (m, 1H, (-$C\underline{H}_2$-)), 2.74 (dd, J_1=6.2 Hz, J_2=18.0 Hz, 1H, (-$C\underline{H}_2$-)), 3.20 (quint, *J*=4.7 Hz, 1H, (-$C\underline{H}(CH_2CH_3)_2$), 3.59 (m, 1H, (-$C\underline{H}$-$N_3$)), 4.00 (m, 1H, (-$C\underline{H}$-O-)), 4.17 (q, *J*=7.0 Hz, 2H, (-$C\underline{H}_2CH_3$)), 5.07 (t, *J*=8.6 Hz, 1H, (-$C\underline{H}$-OAc)), 6.62 (m, 1H, (-$C\underline{H}$=C-)), ^{13}C NMR ($CDCl_3$) (δ, ppm): 8.2 (-C($CH_2\underline{C}H_3)_2$), 8.6 (-C($CH_2\underline{C}H_3)_2$), 13.1 (-$CH_2\underline{C}H_3$), 20.0 (-(CO)-$\underline{C}H_3$), 24.9 (-C($\underline{C}H_2CH_3)_2$), 25.1 (-C($CH_2CH_3)_2$), 28.7 (-$\underline{C}H_2$-), 29.3 (-$\underline{C}$H-N_3), 59.9 (-$\underline{C}H_2CH_3$), 71.3 (-$\underline{C}$H-O-), 72.7 (-$\underline{C}$H-O(C=O)-CH_3), 80.8 (-$\underline{C}H(CH_2CH_3)_2$), 130.4 (-CH=$\underline{C}$-), 131.7 (-$\underline{C}$H=C-), 168.9 (-CH-O($\underline{C}$=O)-$CH_3$), 174.0 (-$\underline{C}$=O).

2.3.23 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-azido-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-azido-shikimate) 135



Hydrazoic acid (HN_3) was prepared by NaN_3, (0.20 g, 3.075 mmol) dissolved with H_2O:benzene (3:1, 4 mL) at 0^oC, conc.H_2SO_4 (1 mL) was added dropwise to the solution for 30 min. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered to obtaining the hydrazoic solution. Diisopropylazodicarboxylate (DIAD) (1.0 mL, 2.95 mmol) and hydrazoic acid (HN_3) (3.075 mmol, 2 mL) were added dropwise to the stirred solution of triphenylphospine (Ph_3P) (0.750 g, 2.95 mmol) in toluene (3 mL) at 0^oC for 10 minutes, and then the solution of compound **34** (0.20 g, 0.738 mmol) in toluene (1 mL) was added dropwise. The stirring was continued at 0^oC for 24.0 h. Evaporation of the solvent and purified the residue by silica gel column chromatography, eluting with 5% EtOAc–hexane to provide the product **135** (0.15 g, 69%), R_f on TLC chromatogram = 0.67 (50% ethyl acetate:hexane). ^1H NMR ($CDCl_3$) (δ, ppm): 0.77 (t, *J*=7.0 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$)), 0.86 (t, *J*=7.8 Hz, 3H, (-C($CH_2C\underline{H}_3)_2$), 1.25 (t, *J*=7.0 Hz, 3H, (-$CH_2C\underline{H}_3$)), 1.56 (q, *J*=8.6 Hz, 2H, (-$CH_2C\underline{H}_3$)), 1.61 (q, *J*=7.8 Hz, 2H, $CH_2C\underline{H}_3$)), 2.45 (m, 1H, (-$C\underline{H}_2$-)), 2.68 (dd, J_1=4.7 Hz, J_2=16.8 Hz, 1H, (-$C\underline{H}_2$-)), 3.46 (m, 1H, (-$C\underline{H}$-N_3)), 4.17 (q, *J*=7.0 Hz, 1H, (-$C\underline{H}_2CH_3$)), 4.43 (d, *J*=4.7 Hz, 1H, (-C<u>H</u>-O-)), 4.69 (m, 1H, (-C<u>H</u>-O-)), 6.69 (m, 1H, -C<u>H</u>=C-)); ^{13}C NMR ($CDCl_3$) (δ, ppm): 8.1 (-C($CH_2\underline{C}H_3)_2$, 8.3 (-C($CH_2\underline{C}H_3)_2$, 14.2 (-$CH_2\underline{C}H_3$), 23.5 (-C($\underline{C}H_2CH_3)_2$), 26.6 (-C($\underline{C}H_2CH_3)_2$), 30.1 (-$\underline{C}H_2$-), 37.3 (-$\underline{C}$H-N_3), 57.3 (-$\underline{C}H_2CH_3$), 61.2 (-<u>C</u>H-O-), 74.3 (-<u>C</u>H-O-), 117.0 (-<u>C</u>($CH_2CH_3)_2$, 135.2 (-<u>C</u>H=C-), 165.9 (-<u>C</u>=O).

2.3.24 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-*tert*-butoxycarbonylamino-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-*tert*-butoxycarbonylamino-shikimate) 137



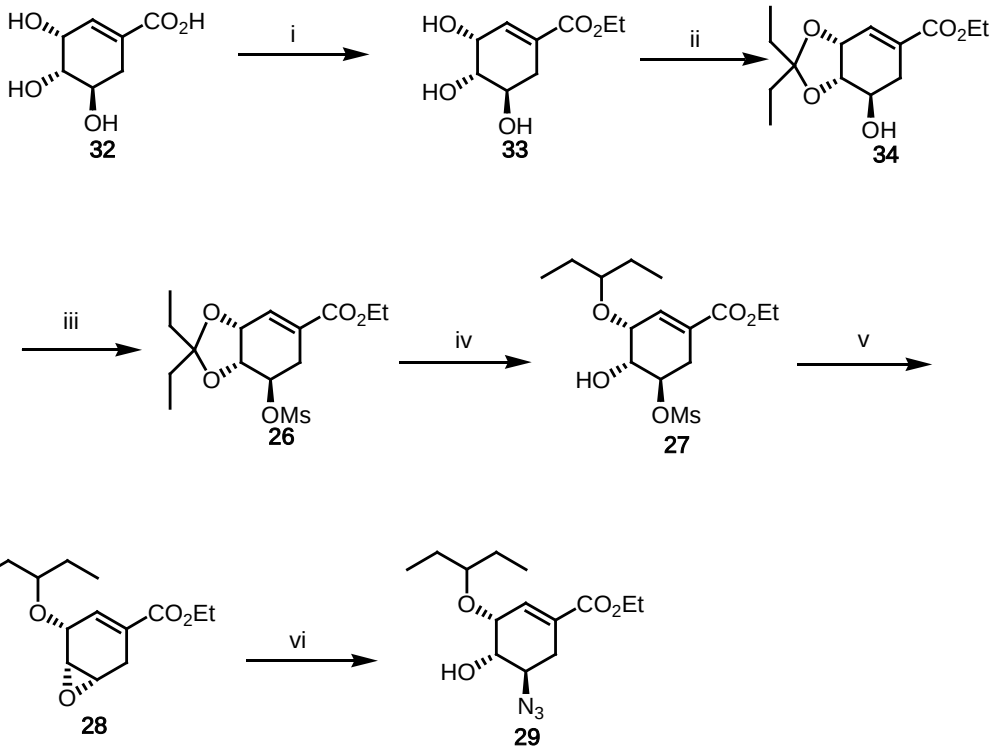
Compound **135** (0.10g, 0.372 mmol) in CH_3CN (1 mL) was added dropwise to the stirring, cool solution of triphenyl phosphine (0.10 g, 0.372 mmol) in 2:1 CH_3CN-H_2O (6 mL) for 15 min. After stirring room temperature for 3.0 h, most of the solvent was removed and EtOAc (10 mL) was added. The mixture was washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate. filtered and concentrated in vacuo to give crude 0.20 g, and then added dropwise of crude reaction in THF (1 mL) to the stirring ice–cool solution of $NaHCO_3$ (0.050 g, 0.558 mmol) and $(Boc)_2O$ (0.122 g. 0.558 mmol) in 5:2 THF-H_2O (7 mL). After the reaction mixture was stirred at room temperature for 5.0 h, the solvent was removed and then EtOAc (10 mL) was added. The mixture was washed with water, saturated sodium chloride solution, dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography using 10% EtOAc-hexane as eluent to give yellow oil **137** (0.10 g, 72%), R_f on TLC chromatogram = 0.73 (50% ethyl acetate:hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm); ^{1}H NMR ($CDCl_3$) (δ, ppm); 0.74 (t, *J*=7.0 Hz, 3H), 0.84 (t, *J*=7.8 Hz, 3H), 1.23 (t, *J*=7.0 Hz, 3H), 1.40(s, 9H), 1.49 (q, *J*=7.8 Hz, 2H), 1.57 (q, *J*=7.8 Hz, 2H), 1.65(br-s, 1H), 2.07-2.13(m, 1H), 2.62 (dd, J_1=5.5 Hz, J_2=17.2 Hz, 1H), 3.86 (m, 1H), 4.14 (q, *J*=7.0 Hz, 2H), 4.29 (m, 1H), 4.68 (m, 1H), 4.95(d, *J*=9.4 Hz, 1H), 6.69 (m, 1H).

CHAPTER III

RESULTS AND DISCUSSION

3.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 29

Compound **29** was an important intermediate for the synthesis of oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **121-124**. This compound was synthesized from (-)-shikimic acid **32**, as shown in **Scheme 3.1**

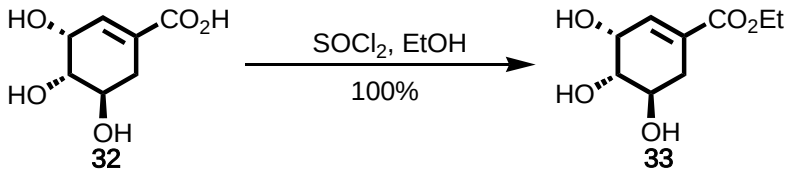


Reagents: i:$SOCl_2$, EtOH, heated to reflux, 3.0 h, ii: 3-pentanone, TfOH, rt, 3.0 h, iii: MsCl, Et_3N, EtOAc, rt, 6.0 h, iv: Et_3SiH, $AlCl_3$, CH_2Cl_2, 0 ^oC, 5.0 h, v: aq.$NaHCO_3$, EtOH-H_2O, 60 ^oC, 3.0 h, vi: NaN_3, NH_4Cl, EtOH, 70 ^oC, 18.0 h.

Scheme 3.1 Synthesis of intermediate **29**

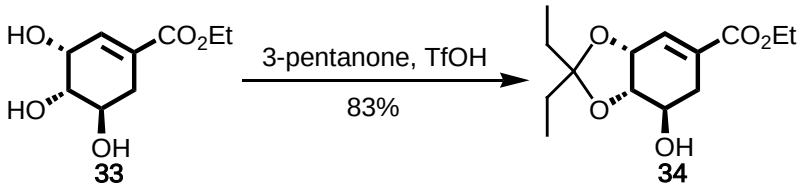
Synthesis of the 5-azido-4-hydroxy-3-pentylidene ketal **29** was accomplished in 6 steps with 50% overall yield from the commercially available chemicals (-)-shikimic acid **32**.

3.1.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexene-1-carboxylate (ethyl shikimate) 33

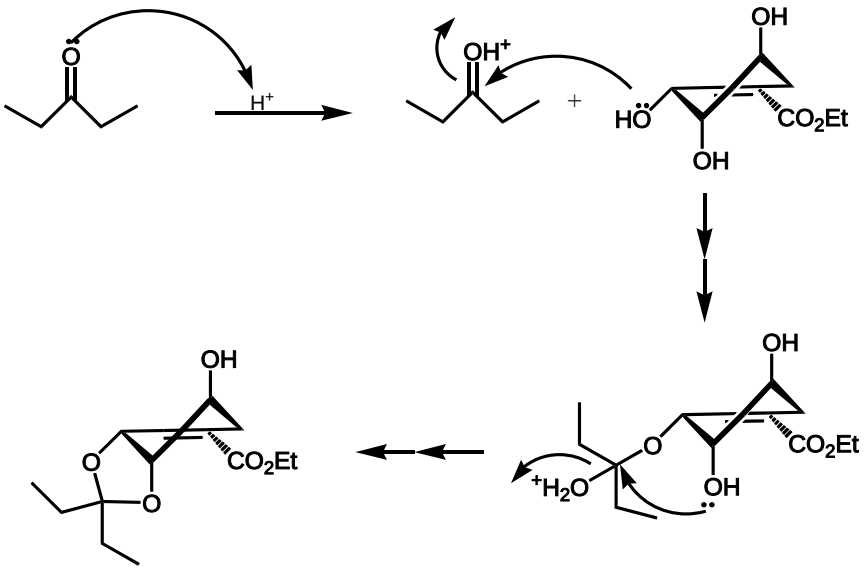


The (-)-shikimic acid **32** was esterified by thionylchloride ($SOCl_2$) in ethanol (EtOH) [41, 46]. This reaction was performed at reflux for 3.0 hours to afford the known ethyl shikimate **33** in quantitative yield, [13, 97%]. The ^{1}H-NMR spectrum of the compound **33** exhibited a characteristic peak of ethyl ester proton ($-CO_2C\underline{H}_2C\underline{H}_3$) as triplet and quartet at δ = 1.2 and 4.1 ppm, respectively, (**Figure A.3** in Appendix). ^{13}C-NMR spectrum revealed 9 different types of carbon corresponding to the structure of ethyl shikimate **33** (**Figure A.4** in Appendix)**.** The IR spectrum showed O-H, C=O and C-O stretching of compound **33** at 3360, 1701 and 1081 cm^{-1}, respectively. These spectra corresponded well to those reported in literature [13].

3.1.2 Synthesis of ethyl (3a*R*,7*R*,7a*S*)-2,2-diethyl-7-hydroxy-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-hydroxy shikimate) 34



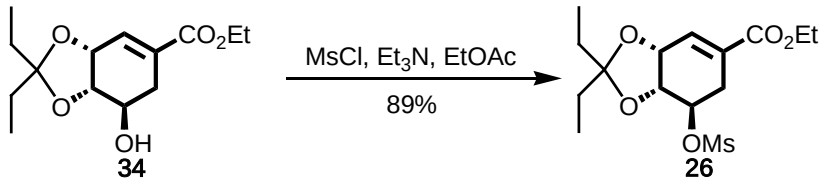
Pentylidene ketal formation of the two *cis*-hydroxy groups of the (-)-ethyl shikimate **33** with 3-pentanone in the presence of catalytic amount of trifluoromethansulfonic acid (TfOH) [45] afforded the known pentylidene ketal compound **34** in 83% yield, [13, 97%]. The mechanism of this pentylidne ketal formation is shown in **Scheme 3.2**



Scheme 3.2 The mechanism of pentylidene ketal formation

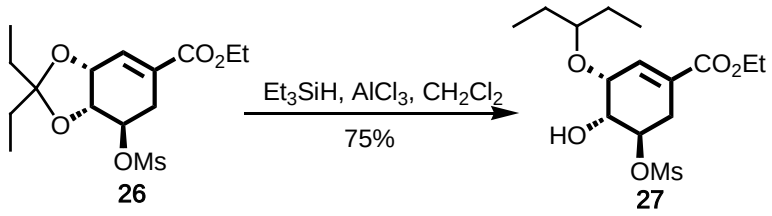
^{1}H-NMR spectrum showed two methyl and two methylene protons of the ethyl groups (-C<u>H</u>$_2$C<u>H</u>$_3$) of this compound as a triplet and multiplet appeared at δ = 0.88 (3H), 0.92 (3H) and 1.74 (4H) ppm, respectively (**Figure A.5** in Appendix). ^{13}C-NMR spectrum revealed the 13 different types of carbon that substantiated the pentylidene ketal **34** (**Figure A.6** in Appendix)**.** The IR spectrum showed O-H, C=O and C-O stretching of compound **34** at 3468, 1712 and 1067 cm^{-1}, respectively. These spectra corresponded well to those reported in literature [13].

3.1.3 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethy-l-7-methanesulphonyl-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-methanesulphonyl-shikimate) 26



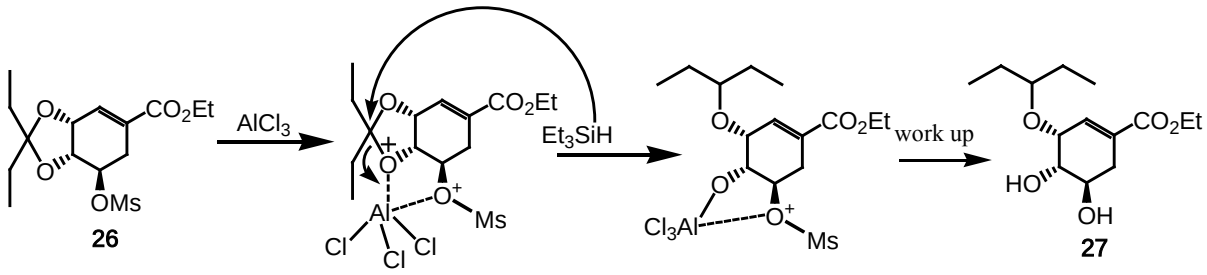
The hydroxyl of compound **34** was mesylated by using methanesulfonyl chloride and triethylamine in EtOAc at the room temperature for 3.0 hours [40, 47, 48]. The desired known mesylated ester **26** could be synthesized in 89% yield, [13, 89%]. ^{1}H-NMR spectrum showed singlet peak of mesyl group (-SO$_2$OC<u>H</u>$_3$) at 3.1 ppm and a change of (-C<u>H</u>OH) proton from 3.9 ppm to (-C<u>H</u>OMs) 4.8 ppm (**Figure A.7** in Appendix). ^{13}C-NMR spectrum revealed the 15 different types of carbon that substantiated the mesylated compound **26** (**Figure A.8** in Appendix). These spectra corresponded well to those reported in literature [13].

3.1.4 Synthesis of ethyl (3*R*,4*R*,5*R*)-3-(1-ethyl-propoxy)-4-hydroxy-5-methanesulfonyloxy-1-cyclohexene-1-carboxylate 27



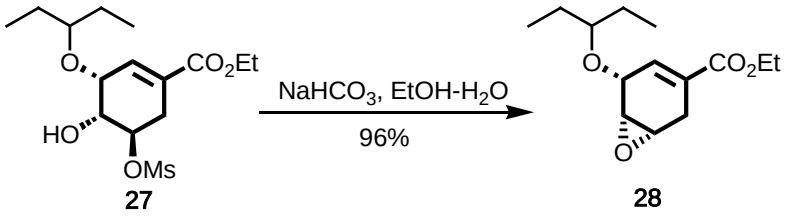
The regioselective reduction of pentylidene ketal compound **26** with triethylsilylhydride (Et_3SiH) and aluminum chloride ($AlCl_3$) in CH_2Cl_2 at 0 ^oC for 3.0 hours provided the isopentyl ether **27** in 75% yield, [12, 75%]. In the literature Et_3SiH and $TiCl_4$ in CH_2Cl_2 at -32 ^oC to -32 ^oC was used for the reduction. In this work, the much cheaper and more easily handled $AlCl_3$, was employed instead of $TiCl_4$. Anothe advantage is that the reaction can be carreid out at 0 ^oC instead of -32 ^oC or lower temperature. ^{1}H-NMR spectrum of compound **27** showed a quintet proton of the pentyl group (-C<u>H</u>(CH_2CH_3)) at 3.41 ppm which is different from that compound **26** (**Figure A.9** in Appendix). ^{13}C-NMR spectrum revealed the 15 different types of carbon that substantiated the hydroxyl mesylated compound **27** (**Figure A.10** in Appendix). These spectra corresponded well to those reported in literature [12].

The mechanism of the reduction of the pentylidene ketal group is shown in **Scheme 3.3.** The presence of additional OMs group may chelate the aluminium trichloride, which results in highly regioselective reduction of the pentylidene ketal.



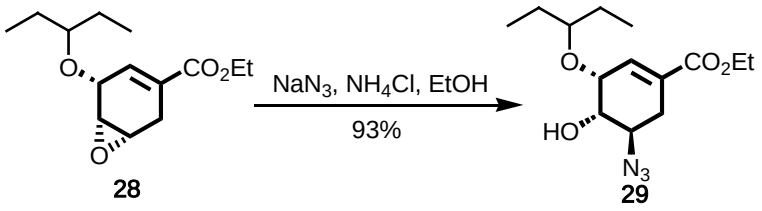
Scheme 3.3 The mechanism of regioselective reduction of the pentylidene ketal group

3.1.5 Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (epoxide) 28

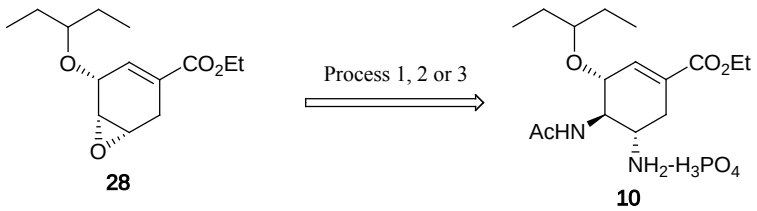


Treatment of the compound **27** with aq. $NaHCO_3$ in $EtOH/H_2O$, at 60 ^oC gave the known epoxide **28** in 96% yield, [12, 96%]. The epoxide **28** could be extracted with hexane and then recrystalized at 0 ^oC to give white crystals. ^{1}H-NMR spectrum of **28** exhibited characteristic peaks of methine protons of the epoxide group (-C<u>H</u>-C<u>H</u>-) at 3.46 ppm and the signal of mesyl group at 3.1 ppm disappeared (**Figure A.11** in Appendix). ^{13}C-NMR spectrum revealed the 12 different types of carbon that is in good agreement with the structure of the epoxide **28** (**Figure A.12** in Appendix)**.** These spectra also corresponded well to those reported in literature [12].

3.1.6 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 29



Three processes were developed by Roche's researchers for the synthesis of **10** from epoxide **28** as shown in **Scheme 3.4**.[15-16].



1: azide route, 5 steps, 50-55%
2: allylamine route, 8 steps, 35-40%
3: *tert*-butylamine route, 6 steps, 60%

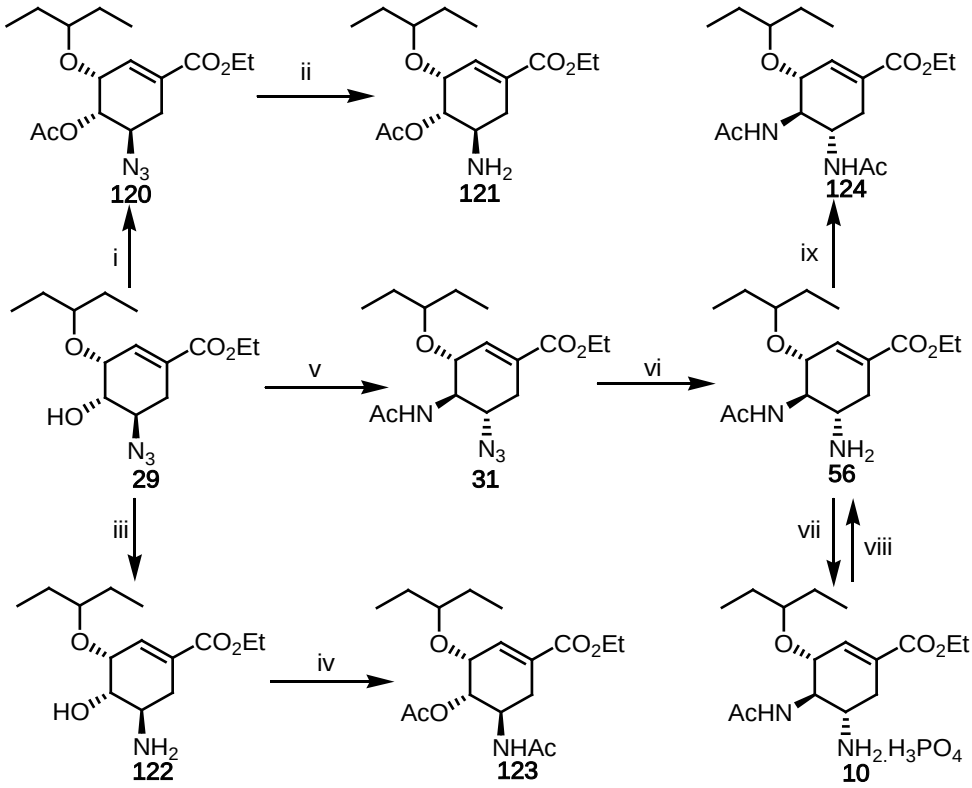
Scheme 3.4 Three Synthesis of **10** from **28**

The shorter and economical azide route [15] is currently used in the industrial production while the allylamine and the *tert*-butylamine routes [16] were later reported as the alternatives to the use of hazardous azide reagents.

In this work, the azide route was used for the ring opening of the epoxide **28** to the known hydroxyl azide intermediate **29**. NaN_3, NH_4Cl in EtOH-H_2O at 70-75 ^oC were used in this reaction to give yellow oil **29** with 93% chromatographic yield [15, 86%]. Analysis by ^{1}H-NMR showed that the ring opening of the epoxide **28** resulted in-downfield shift of the methine signals at 3.74-3.85 ppm. (**Figure A.13** in Appendix). ^{13}C-NMR spectrum revealed the 13 different types of carbon that substantiated the intermediate **29** (**Figure A.14** in Appendix)**.** These spectra corresponded well to those reported in literature [15].

3.2 Synthesis of oseltamivir phosphate 10, oseltamivir 56 and its derivatives 121-124

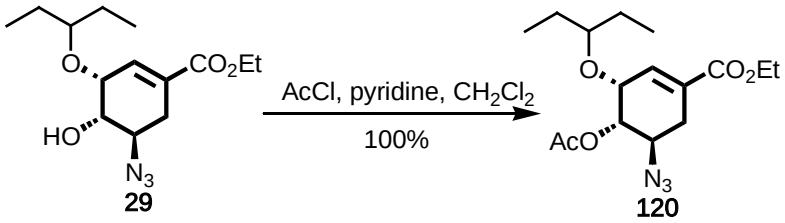
Oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **121-124** were synthesized from the 5-azido-4-hydroxy-3-pentyloxy intermediate **29**, as shown in **Scheme 3.5**



Reagents: i: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, ii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iv: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, v: a. Ph_3P, CH_3CN-H_2O, rt, 6.0 h, b. NaN_3, NH_4Cl, DMF, 70-75 ^oC, 18-20 h, c. Ac_2O, Et_3N, CH_2Cl_2, rt, 2.0 h, vi: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, vii: H_3PO_4, EtOH, rt, viii: sat.$NaHCO_3$, CH_2Cl_2, 5 min, ix: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h.

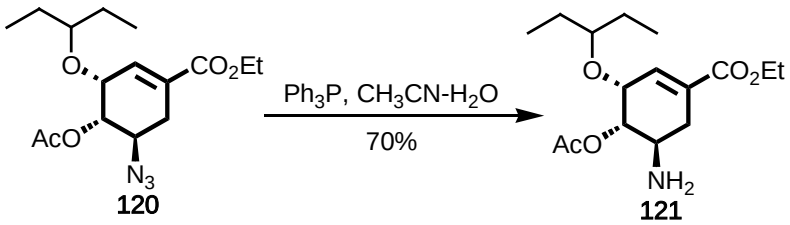
Scheme 3.5 Synthesis of oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **121-124**

3.2.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 120



Acetylation of the hydroxyl azide **29** using acetyl chloride and pyridine was carried out at reflux for 2.0 hours. The expected new acetyloxy azide compound **120** was obtained in quantitative yield. Characteristic ^{1}H-NMR signals include a singlet signal of the acetyl group [(-O(CO)C<u>H</u>$_3$)] at 2.09 ppm and a quartet signal of the methine proton next to the acetyloxy group (-C<u>H</u>-OAc) at 4.00 ppm (**Figure A.15** in Appendix). ^{13}C-NMR spectrum revealed the 16 different types of carbon that substantiated the compound **120** with two types of singlet signals of the acetyl carbonyl carbons appeared at δ = 165.6 and 170.5 ppm (**Figure A.16** in Appendix).

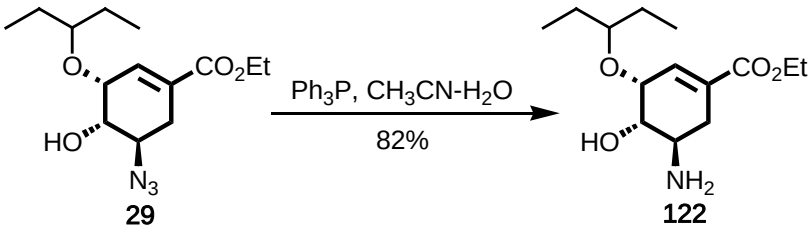
3.2.2 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 121



The new oseltamivir derivative **121** was obtained from Ph_3P reduction [56] of azido group of compound **120**. It was purified by column chromatography to afford the amino acetyloxy compound **121** in 70% yield. ^{1}H-NMR spectrum of the oseltamivir derivative **121** still show the acetyloxy peak [(-O(CO)C<u>H</u>$_3$)] as a singlet

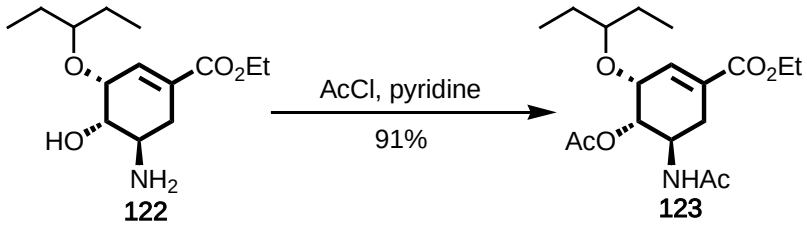
signal at 2.02 ppm, and showed multiplet signal of the next to nitrogen atom (-C<u>H</u>-NH_2) at 3.63 ppm (**Figure A.17** in Appendix). ^{13}C-NMR spectrum revealed the 16 different types of carbon that substantiated the oseltamivir derivative **121** with one type of singlet signal of the acetyl carbonyl carbons appeared at δ = 165.6 ppm (**Figure A.18** in Appendix).

3.2.3 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 122



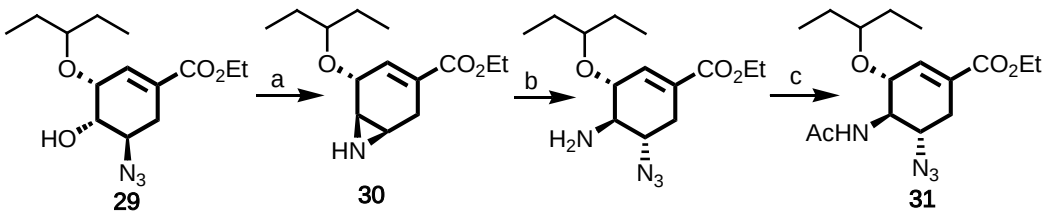
The oseltamivir derivative **122** [15] after purified by column chromatography, was obtained in 82% yield from triphenylphosphine reduction of the azido group of **29**. ^{1}H-NMR spectrum of the oseltamivir derivative **122** exhibited a characteristic peak of methine proton at C4 (-C<u>H</u>OH) and C5 (-C<u>H</u>NH_2) positions as multiplet signals at 3.46 ppm and 3.87 ppm, respectively (**Figure A.19** in Appendix). ^{13}C-NMR spectrum revealed the 14 different types of carbon that substantiated the new oseltamivir derivative **122** (**Figure A.20** in Appendix). The molecular weight was confirmed by ESI mass spectrometry showing the molecular ion peak at $[M+H]^+$ = 272.367 m/z.

3.2.4 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 123



The new diacetylated compound **123** was obtained from acetylations on the –OH and $–NH_2$ groups of **122** with AcCl and pyridine to give the oseltamivir derivative **123** in a high yield of 91%. ^{1}H-NMR spectrum showed singlet signals of two acetyl groups, -NHAc and –OAc, at 1.94 ppm and 2.10 ppm, respectively. Two methine protons next to oxygen atom (-C<u>H</u>-OAc) and nitrogen atom (-C<u>H</u>-NHAc) appeared as quintet at 4.57 ppm, J= 7.0 Hz and double of doublet at 4.95 ppm, J_1=3.1 and J_2 = 11.3 ppm, respectively (**Figure A.21** in Appendix). ^{13}C-NMR spectrum revealed the 18 different types of carbon that substantiated the oseltamivir derivative **123,** and three types of singlet signas of the carbonyl carbons appeared at δ = 165.9, 169.8 and 171.7 ppm, respectively (**Figure A.22** in Appendix). The molecular weight was confirmed by ESI spectrometry showing the molecular ion peak at $[M+Na]^+$ = 378.50 m/z.

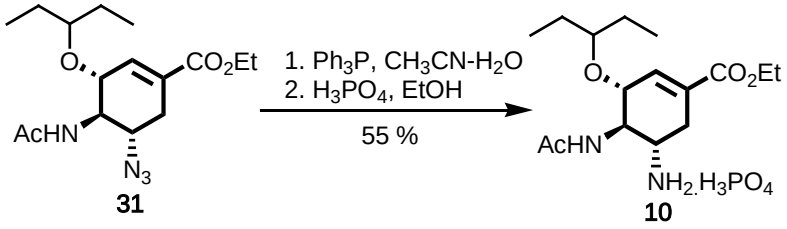
3.3.5 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 31



a: Ph_3P, DMF-HOAc, b: NaN_3, NH_4Cl, DMF, c: Ac_2O, Et_3N, CH_2Cl_2, 36%, 3 steps

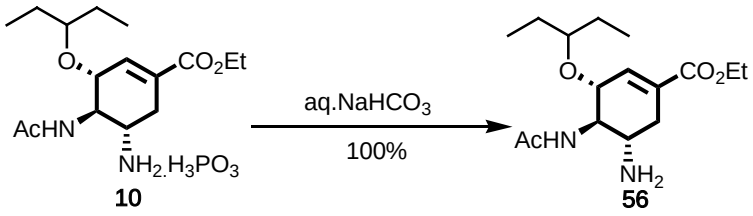
A one pot reaction was attempted for the synthesis of the acetyl amino azide. The intermediate compound **29** was reduced by Ph_3P in DMF followed by in situ aziridine **30** formation and then NaN_3 substitution that opened the aziridine to amino azide intermediate. Finally, acetylation of the amino group by Ac_2O and Et_3N gave the product after column chromatography in moderate yield (36%, 3 steps). ^{1}H-NMR spectrum of compound **31** showed singlet signal proton of acetyl group (-NHAc) at 2.00 ppm, and amide group (-NH-) at δ = 6.90 ppm, respectively (**Figure A.23** in Appendix) [12].

3.2.6 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-*N*-acetamido-5-amino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate phosphate (oseltamivir phosphate) 10



The acetamido azide intermediate **31** was converted to **10** by reduction of the azide group with Ph_3P in CH_3CN-H_2O followed by converting the free base into the oseltamivir phosphate **10** in 55% yield from 2 steps. ^{1}H-NMR in D_2O showed two methine protons next to nitrogen atoms (-C<u>H</u>-NHAc) and (-C<u>H</u>-$NH_2.H_3PO_4$) appeared as multiplet at 3.35 ppm and 3.86, respectively (**Figure A.25** in Appendix). ^{13}C-NMR spectrum of **10** correctly revealed the 16 different types of carbon that substantiated the oseltamivir phosphate **10** (**Figure A.26** in Appendix). These spectra corresponded well to those reported in literature [12].

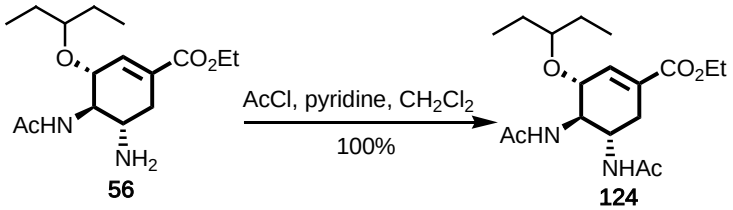
3.2.7 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-diacetylamino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (oseltamivir) 56



10 56

The known oseltamivir **56** was isolated from the salt **10** by shaking with saturated aqueous $NaHCO_3$ (3 mL) for 5 min in quantitative yield. ^{1}H-NMR spectrum showed singlet signal of acetyl group (-NHAc) at 1.92 ppm and two methine protons next to nitrogen atoms (-C<u>H</u>-NHAc, -C<u>H</u>-NH_2) appeared as multiplet at 3.22 ppm and quartet at 3.53 ppm, respectively and amide group (-NH-) at δ = 5.78 ppm (**Figure A.27** in Appendix). ^{13}C-NMR spectrum of **56** correctly revealed the 16 different types of carbon that substantiated the oseltamivir **56** (**Figure A.28** in Appendix). The molecular weight was confirmed by ESI mass spectrometry showing the molecular ion peak at $[M+H]^+$ = 313.397 m/z.

3.2.8 Synthesis of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 124

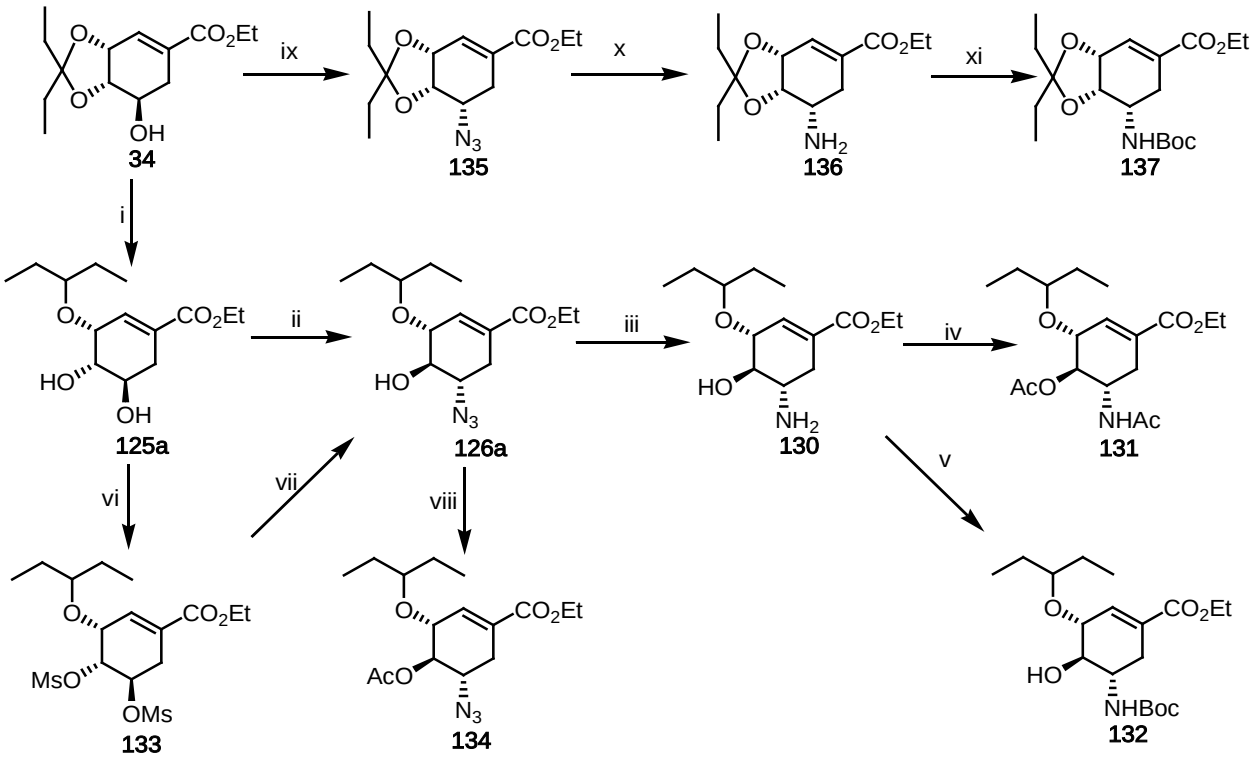


56 124

The new diacetylated compound **124** was obtained by acetylation on the $-NH_2$ group of **56** with AcCl and pyridine to give **124** in quantitative yield. ^{1}H-NMR spectrum showed singlet signal of two acetyl group (-NHAc) at 1.89 ppm, and 1.92 ppm, respectively and two methine protons next to nitrogen atoms (-C<u>H</u>-NHAc) appeared as multiplets at 4.01 ppm and amide group (-NH-) at δ = 6.08 and 6.63 ppm (**Figure A.29** in Appendix). The molecular weight was confirmed by mass spectrometry showing the molecular ion peak at $[M+H]^+$ = 355.477.

3.3 Synthesis of oseltamivir derivatives **125a**, **130-135** and **137**

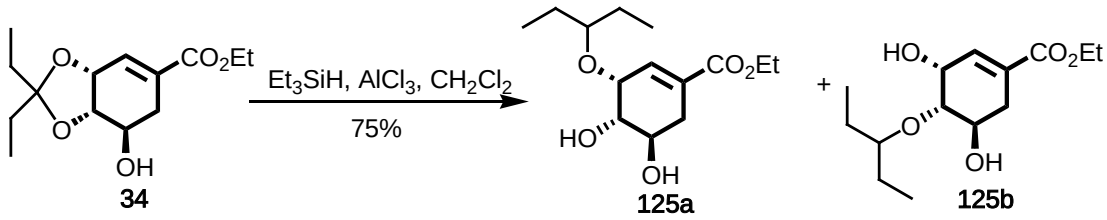
Compound **34** and **125a** were important intermediates for the synthesis of oseltamivir derivatives **125a** and **130-133**. Intermediate **125a** was obtained from stereoselective reduction of compound **34**, as shown in **Scheme 3.6**.



Reagents: i: Et_3SiH, $TiCl_4$, CH_2Cl_2, 0 ^oC, 5.0 h, ii: DIAD, Ph_3P, HN_3, toluene, 0 ^oC 6.0 h and then rt. 24.0 h, iii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iv: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, v: $(Boc)_2O$, aq.$NaHCO_3$, EtOH-H_2O, rt, 5.0 h, vi: MsCl, Et_3N, EtOAc, rt, 6.0 h, vii: $TMSN_3$, KF, 18-crown-6, DMF, reflux, 24.0 h, viii: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h ix: DIAD, Ph_3P, HN_3, toluene, 0 ^oC 6.0 h, rt. 24.0 h, x: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, xi: $(Boc)_2O$, aq.$NaHCO_3$, EtOH-H_2O, rt, 5.0 h.

Scheme 3.6 Synthesis of oseltamivir derivatives **125a**, **130-135** and **137**.

3.3.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-3-(1-ethyl-propoxy)-4,5-dihydroxy-1-cyclohexene-1-carboxylate 125



The reductive ring opening of pentylidene ketal **34** was accomplished with the reducing agent Et_3SiH in the presence of a Lewis acid ($AlCl_3$ or $TiCl_4$) in CH_2Cl_2 at 0 ^oC for 5.0 hours to provide the two 4,5-trans-diols, **125a** (major) and its regioisomer, **125b** (minor product).

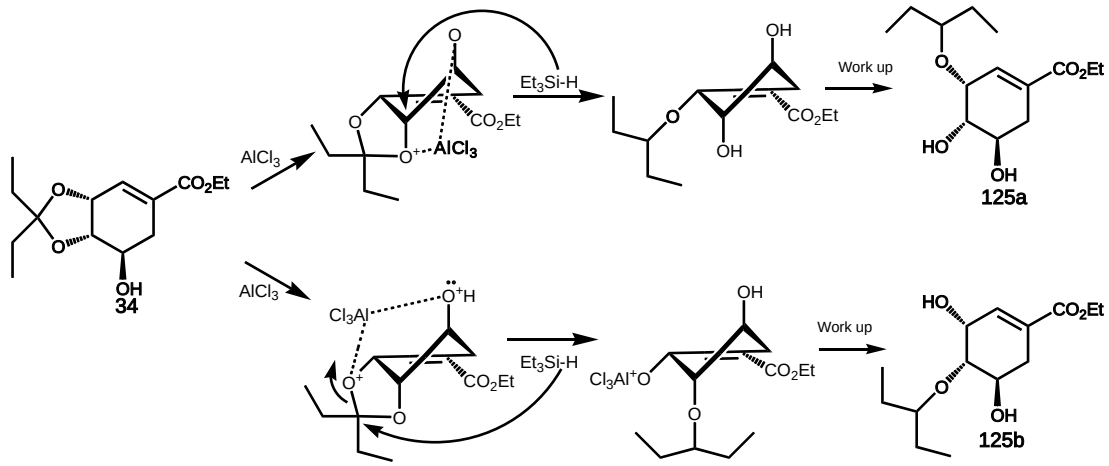
A selective reduction [59] was observed by Rolf and et al. [12], screening from different reducing agents with or without acid. They found that BH_3.THF, $NaBH_4/CF_3COOH$, $NaCNBH_3/BF_3.OEt_2$, DIBAH and Et_3SiH/Nafion-H gave unsatisfactory results. However, $Et_3SiH/TiCl_4$ in CH_2Cl_2 at -32 ^oC to -36 ^oC gave good yields and regioselectivity for this reduction.

Using Et_3SiH in CH_2Cl_2 and $TiCl_4$ or $AlCl_3$, and various conditions including the report above resulted in **Table 3.1**

Table 3.1 Reductive ring opening of pentylidene ketal compound **34**

Entry	Reagents and Reaction conditions	% yields of **125a** : **125b**
1	$TiCl_4$, -32 ^oC to -36 ^oC	75 : 10
2	$TiCl_4$, 0 ^oC	70 : 10
3	$AlCl_3$, -10 ^oC to -32 ^oC	70 : 18
4	$AlCl_3$, 0 ^oC	67 : 20

In our hands, it appeared that $Et_3SiH/TiCl_4$ in CH_2Cl_2 at low temperature was a better choice for this selective reduction. On the other hand, while $AlCl_3$ was more convenient to handle in comparison with $TiCl_4$ and gave a comparable yield of **125a**, the regioselectivity was not as good. The mechanism of this pentylidene ketal reduction is shown in **Scheme 3.7**.

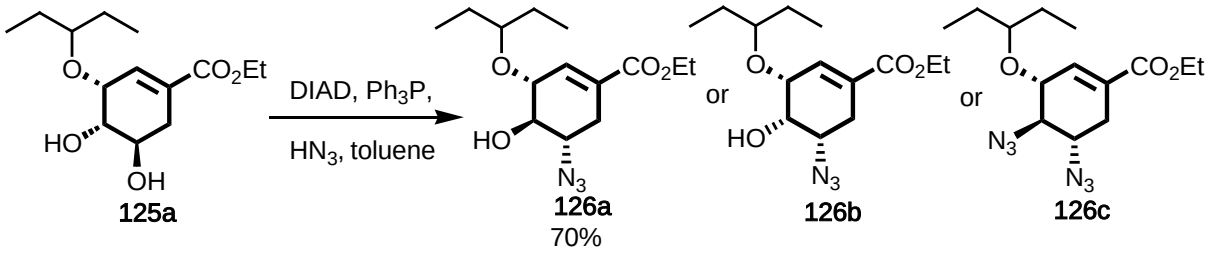


Scheme 3.7 The mechanism of selective reduction of ketal

The ^{1}H-NMR spectrum of compound **125a** showed a broad singlet signal of hydroxyl group at δ = 2.52 ppm and methine proton next to oxygen atom at the pentyloxy group (-O(C<u>H</u>$CH_2CH_3)_2$) as quintet at δ = 3.41 ppm. The two methine protons at C4 and C5 (-C<u>H</u>-OH) appeared as a double of doublet at δ = 3.59 ppm, J_1=4.7 Hz, J_2=9.4 Hz and a multiplet at δ = 3.94 ppm, respectively. The methylene protons at C6 of this compound showed at δ = 2.18 ppm as a double of doublet, J_1=7.8 Hz, J_2=17.9 Hz, and at δ = 2.88 ppm as a double of doublet, J_1=4.7 Hz, J_2=18.3 Hz (**Figure A.31** in Appendix). ^{13}C-NMR spectrum matched well with the compound **125a** (**Figure A.32** in Appendix)**.** The IR spectrum of compound **125a** exhibited O-H stretching of hydroxyl group absorption at 3428 cm^{-1}, C=O stretching of ester group absorption at 1708 cm^{-1} and C=C stretching absorption at 1602 cm^{-1} and C-O stretching absorption at 1089 cm^{-1}. The molecular weight was confirmed by mass spectrometry showing the molecular ion peak at $[M+Na]^+$ = 295.318. ^{1}H-NMR spectrum of compound **125b** showed methine proton next to oxygen atom (-

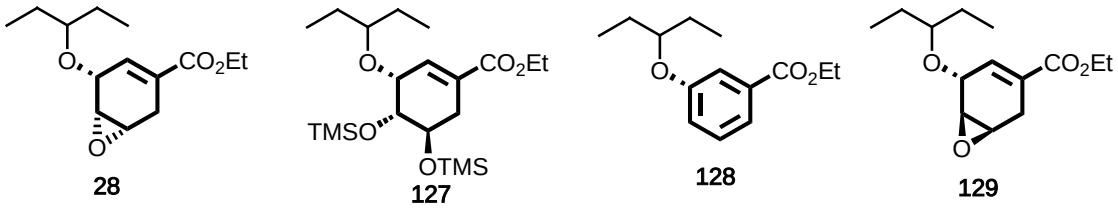
O(C<u>H</u>$CH_2CH_3)_2$) as quintet at δ = 3.24 ppm and two methine protons at C3 and C5 (-C<u>H</u>-OH) of this compound as a quartet at δ = 3.42 ppm, *J* = 5.6 Hz, and broad singlet at δ = 3.87 ppm and methylene protons at C6 (-C<u>H_2</u>-) of this compound showed at δ = 2.29 ppm as doublet *J*=18.8 Hz and at 2.60 Hz as a doublet, *J*=17.8 Hz (**Figure A.33** in Appendix). ^{13}C-NMR spectrum matched well with the compound **125b** (**Figure A.34** in Appendix)**.**

3.3.2 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 126a



At the beginning, it was attempted to convert the dihydroxy compound **125a** to the corresponding diazido compound **126c** with concomitant inversion of the two stereogenic centers. This compound will be just a few steps from oseltamivir. The compound **125a** was therefore reacted with various azide reagents under Mitsunobu conditions [49-54] as shown in **Table 3.2**

DPPA [55] and $TMSN_3$ proved to be ineffective azide sourses for the attempted Mitsunobu reactions. When running the reaction at low temperature, (entries 1, 2 and 5) either no product was obtained or yielding only derivatives **127** of the starting material, (**Figure A.35** in Appendix)**.** At high temperature, dehydration of the hydroxyl groups dominated yielding only the aromatized product **128**, at δ = 7.0-7.6 ppm, (**Figure A.36** in Appendix), as shown in **Scheme 3.8**.

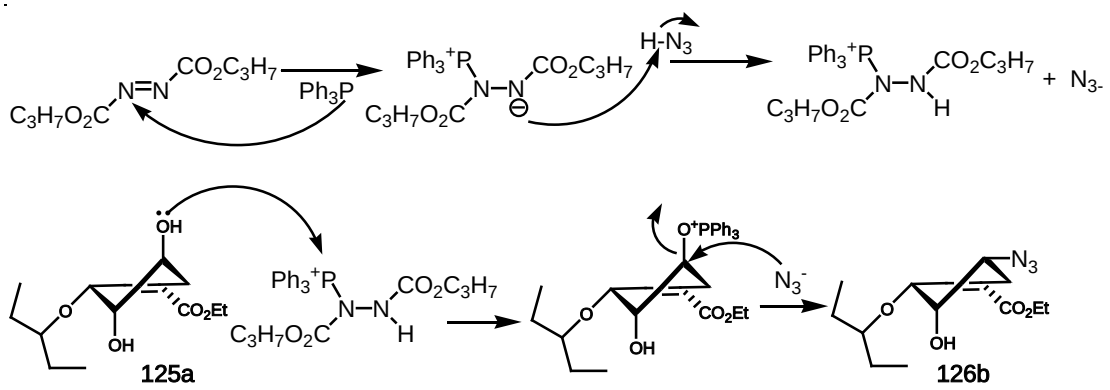


Scheme 3.8 By products from Mitsunobu conditions

Table 3.2 Conditions of the Mitsunobu reaction for the synthesis of hydroxyl azide **125a**

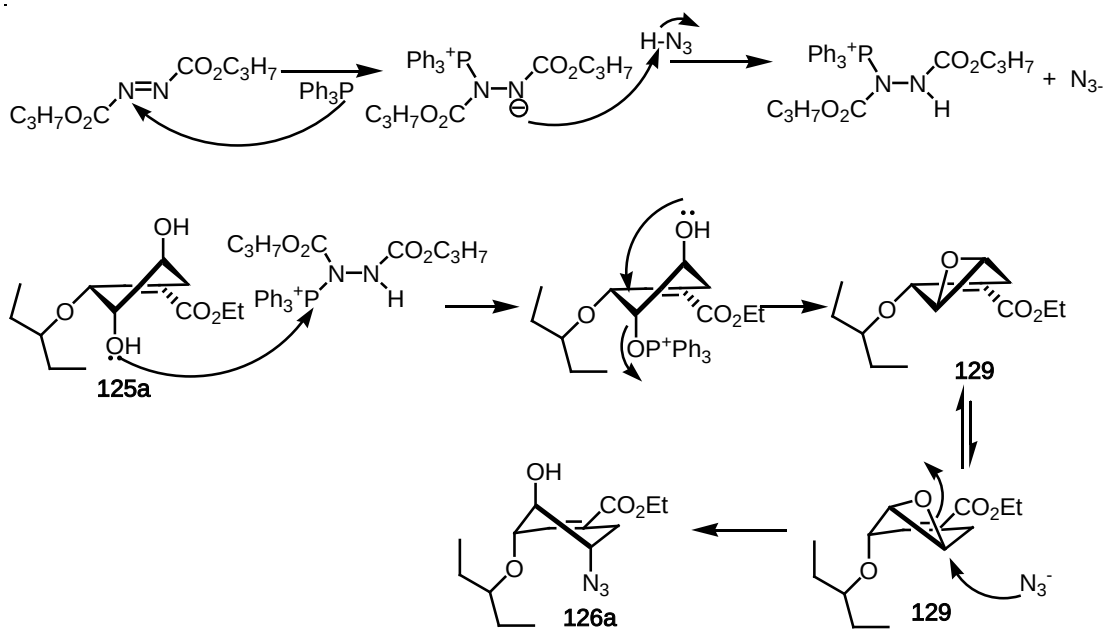
Entry	Azide reagent and Solvents	Reaction Conditions	Product (% yield)
1	DPPA, THF	0 ^{o}C, 6 h, then rt, 24 h.	No reaction
2	DPPA, toluene	0 ^{o}C, 6 h, then rt, 24 h.	No reaction
5	$TMSN_3$, toluene	0 ^{o}C, 6 h, then rt, 24 h.	**127** (60%)
6	HN_3, toluene	0 ^{o}C, 6 h, then rt, 24 h.	**126a** (70%)
7	HN_3, toluene	-78 ^{o}C, 6h, then rt, 24 h.	**28** (80%)
8	HN_3, toluene	reflux	**128 (**50%)

We had hoped that the Mitsunobu reaction would proceed via nucleophilic attack of the the oxophosphonium ion by azide ion (N_3^-) twice. Unfortunately the azide substitution took place only at the C-5 positions. This procedure resulted in a new mono azide, 5-azido-4-hydroxy compound **126a** as a major product with DIAD, Ph_3P and HN_3 in toluene at 0^{o}C for 6.0 hours and room temperature for 24.0 hours at in 70%. At first it was proposed that the reaction proceeded as shown in **Scheme 3.9**



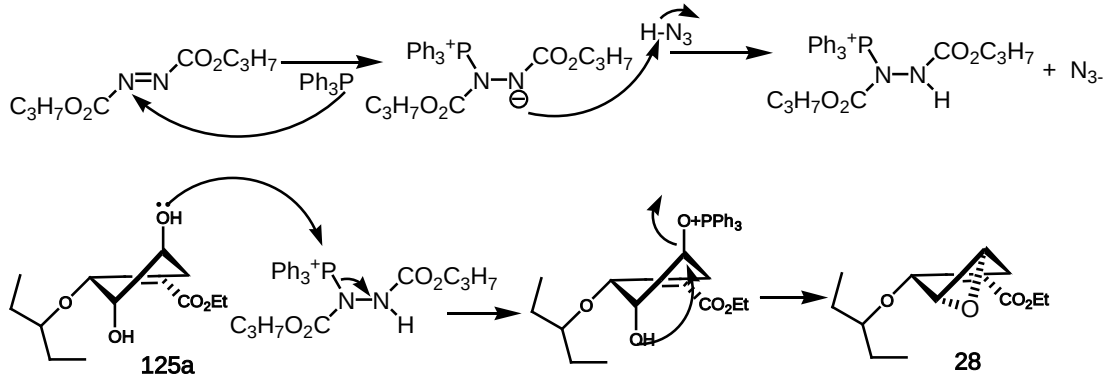
Scheme 3.9 The mechanism of Mitsunobu reaction **1** [50, 66]

The ^{1}H-NMR spectrum of the isolated compound **126a** showed broad singlet signal of hydroxyl group at δ = 2.99 ppm and methine proton (C-3) next to oxygen atom (-O(C<u>H</u>$CH_2CH_3)_2$) as quintet at δ = 3.29 ppm and two methine protons at C4 and C5 (-C<u>H</u>-OH), (-C<u>H</u>-N_3) of this compound as a sextet at δ = 3.43 ppm , *J*=5.0 Hz, and triplet at δ = 3.64 ppm, *J*=9.0 Hz, respectively and methylene proton at C6 (-C<u>H_2</u>-) of this compound showed at δ = 2.07 ppm as multiplet and at δ = 2.84 ppm as double of doublet, J_1=4.0 Hz, J_2=18.0 Hz and high field peak of methine proton at C-2 (-C<u>H</u>=C-) is 6.53 as triplet, *J*=3.0 Hz, (**Figure A.37** in Appendix). ^{13}C-NMR spectrum revealed the 14 different types of carbon that substantiated the compound **126** (**Figure A.38** in Appendix)**.** The molecular weight was confirmed by ESI mass spectrometry showing the molecular ion peak at M^+ = 297.29 m/z. The absolute configuration of this compound was later shown by X-ray structure of its acetyl derivative to be 3*R*,4*R*,5*S* as shown in the structure of **126a**. The mechanism of the formation of **126a** was believed to go through an epoxide intermediate **129** as depicted in **Scheme 3.10**.



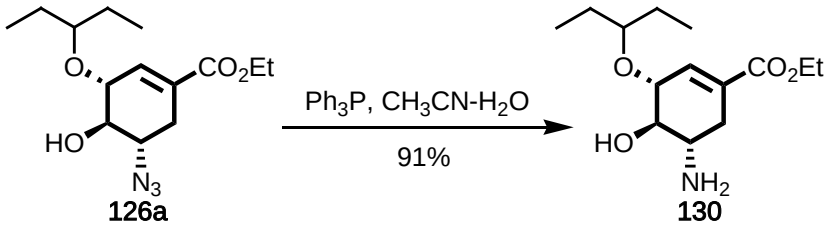
Scheme 3.10 The mechanism of the formation of **126a** via **129**

Although HN_3 was the suitable reagent that successfully gave the substitution product, the condition of the reaction still required low temperature to avoid the dehydration to the aromatic compound **128**. (entry 8). Interestingly, at lower temperature (entry 7) the epoxide **28** was obtained instead of the azide. The mechanism of this reaction of Mitsunobu, as shown in **Scheme 3.11**



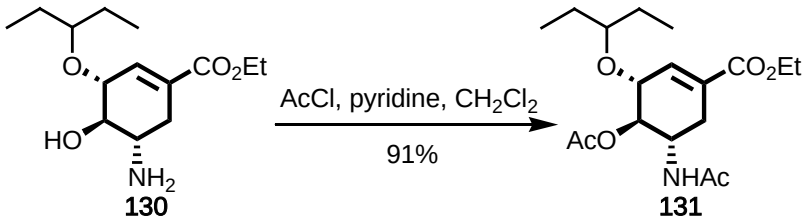
Scheme 3.11 The mechanism of Mitsunobu reaction **2**

3.3.3 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate 130



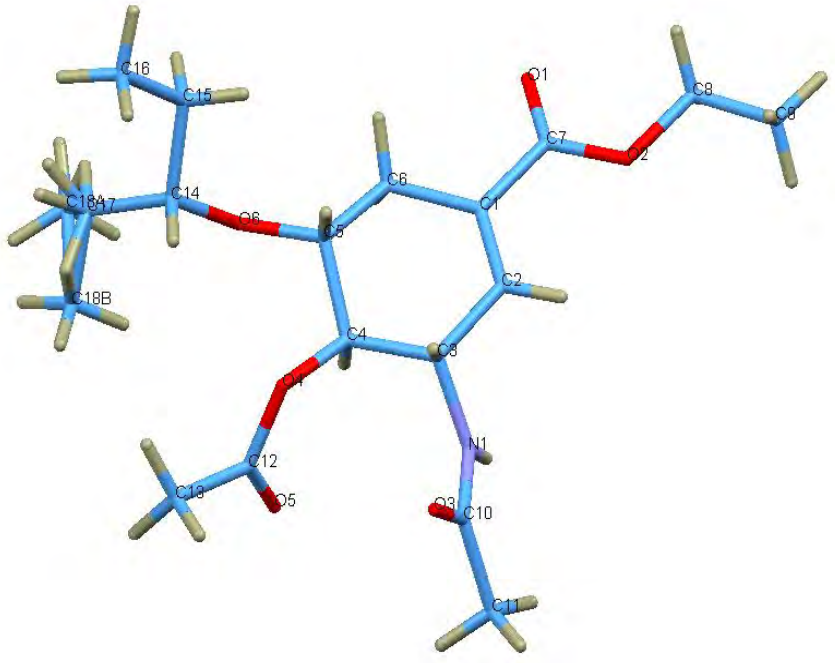
The new oseltamivir derivative **130** was obtained from reduction of the azido group of compound **126a** [56, 58]. Purification by column chromatography afforded the new amino hydroxyl compound **130** in 91% yield. ^{1}H-NMR spectrum of **130** exhibited a characteristic proton signals next to the oxygen and nitrogen atoms (-C<u>H</u>-OH), (-C<u>H</u>-NH_2) as multiplet signal at 3.49 ppm and showed broad singlet signals of -OH and NH_2 at δ = 4.65 ppm, (**Figure A.39** in Appendix). ^{13}C-NMR spectrum revealed the 14 different types of carbon that substantiated the oseltamivir derivative **130** (**Figure A.40** in Appendix). The molecular weight was confirmed by mass spectrometry showing the molecular ion peak at $[M+H]^+$ = 273.210.

3.3.4 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 131



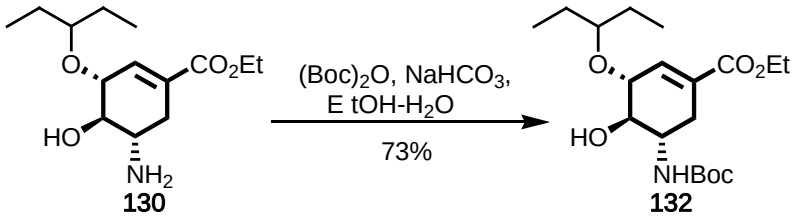
The new diacetylated compound **131** was obtained from acetylation [60, 61] of the –OH and –NH_2 groups with AcCl in pyridine (91%). ^{1}H-NMR spectrum showed singlet signal of methylene C6 (-C<u>H</u>$_2$-) at δ = 2.50 ppm, and singlet signals of two

acetyl groups (-NHAc, -OAc) at δ = 1.95 ppm, and δ = 2.04 ppm, respectively. Two methine protons next to oxygen atom (-C<u>H</u>-OAc) and nitrogen atom (-C<u>H</u>-NHAc) appeared as multiplet at δ = 4.75 ppm, and triplet at δ = 4.90 ppm, J_1=4.7, respectively, (**Figure A.41** in Appendix). ^{13}C-NMR spectrum revealed the 18 different types of carbon that substantiated the oseltamivir derivative **131**. The three types of singlet signals of the carbonyl carbons appeared at δ = 166.4, 169.1 and 170.5 ppm, (**Figure A.42** in Appendix). The IR spectrum of compound **131** exhibited C=O stretching of ester group absorption at 1741 cm^{-1}, and C-O stretching absorption at 1056 cm^{-1}. The molecular weight was confirmed by ESI mass spectrometry showing the molecular ion peak at $[M+Na]^+$ = 378.16 m/z. Recrystallization of the white solid from **131** gave crystals that was suitable for X-ray crystallographic analysis, which confirmed the absolute configuration of compound **131** is (3*R*,4*R*,5*S*), as shown in **Scheme 3.12**, (**Figure A. 43** and **A. 44** in Appendix).



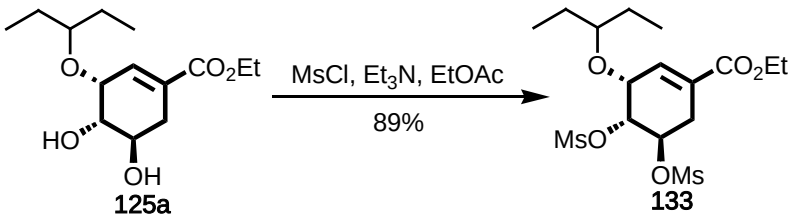
Scheme 3.12 The structure from X-ray crystallographic analysis of compound **131**

3.3.5 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-*tert*-butoxycarbonylamino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate 132



A solution of compound **130** was treated with $(Boc)_2O$ in aqueous $NaHCO_3$ [62] to give the Boc-compound **132** in 73% yield after purified by column chromatography. The ^{1}H-NMR spectrum exhibited a characteristic peak of *tert*-butoxycarbonylamino group (-Boc) as singlet at 1.43 ppm, and methine proton next to nitrogen atom (-C<u>H</u>-NHBoc) and next to oxygen atom (-C<u>H</u>-OH) appeared as multiplet at 3.60 ppm, and amide group (-NH-) as shown an exchangeable proton depend on concentrated at δ = 4.98 ppm (**Figure A.45** in Appendix). ^{13}C-NMR spectrum revealed the 15 different types of carbon that substantiated the compound **132,** and two types of singlet signal of the carbonyl carbon were appeared at δ = 155.6 ppm and 166.4 ppm, respectively (**Figure A.46** in Appendix). The molecular weight was confirmed by ESI mass spectrometry showing the molecular ion peak at $[M+Na]^+$ = 394.253 m/z

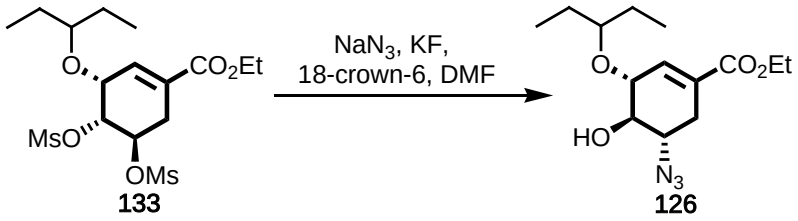
3.3.6 Synthesis of ethyl (3*R*,4*R*,5*R*)-4,5-bis(mesyloxy)-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 133



The residue from a reaction of **125a**, excess of MsCl and Et_3N was purified by silica gel column chromatography to give the bismesylated compound **133** in 89%

yield, ^{1}H-NMR spectrum showed singlet signal of two mesylate groups (-OMs) at δ = 3.11 ppm, and two methine proton next to oxygen atom (-C<u>H</u>-OMs) appeared as multiplet at δ = 4.80 ppm, and δ = 5.19 ppm, respectively (**Figure A.47** in Appendix). ^{13}C-NMR spectrum revealed the 15 different types of carbon that substantiated the oseltamivir derivative **133** (**Figure A.48** in Appendix).

3.3.7 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 126a



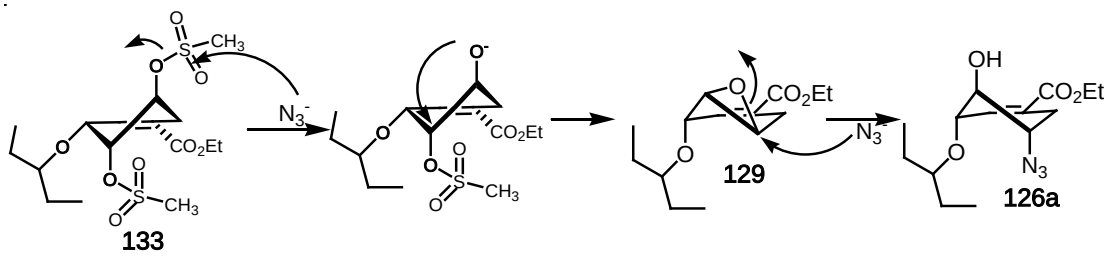
In the initial plan, it was attempted to do double S_N2 substitutions of the two mesylate groups to give a diazide intermediate of oseltamivir. However, the hydroxy azide compound **126a** was obtained from substitution of compound **133** with azide. (**Table 3.3**).

Table 3.3 Substitution of the bis-mesylated compound **133**

Entry	Reagent and Solvent	Reaction conditions	Product (%yield)
1	NaN_3, Acetone-H_2O,	rt. 24 h to 70 ^{o}C, 24 h	No reaction
2	$TMSN_3$, KF, 18-crown-6, DMF	rt. 24 h to 70 ^{o}C, 24 h	**126a** (74%)

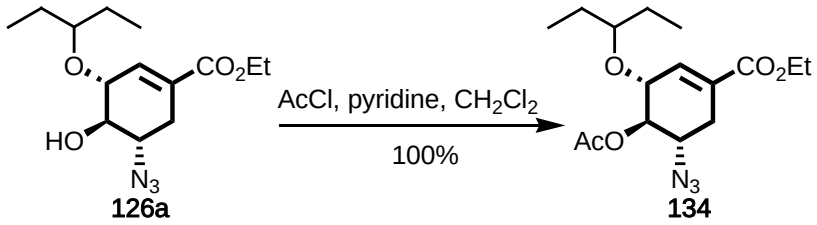
The previously reported procedure of S_N2 substitution of mesylated compounds toward azide products have failed to give the desired diazide in the first attempt. (entry 1 **Table 3.3**). Changing the solvent to aprotic solvent and modifying the condition to use catalytic amount of KF and 18-crown-6 in DMF (entry 2) obtained another mono-azide product **126a** after column chromatography in 72% yield, which was presumably formed via yet another epoxide **129**, **(Scheme 3.13**).

The product was assumed to initiate starting from the unexpected loss of one of the mesyl group followed by the fast intramolecular ring closing to form epoxide. Reopening of the epoxide ring by azide ion yielded **126a**. Spectroscopic characterizations of this compound matched well with the corresponding hydroxyazides obtained by other methods.



Scheme 3.13 The mechanisms of the substitutions of dimesylated compound **133**

3.3.8 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate 134



Acetylation of the new hydroxyl azide compound **126a** at the hydroxyl group was achieved by using acetyl chloride and pyridine. The reaction was heated to reflux for 2.0 hours to give acetyloxy azide compound **134** in quantitative yield. ^{1}H-NMR spectrum showed singlet signal of acetyl group (-O(CO)C$\underline{H}_3$) appeared at δ = 2.05 ppm, the triplet signal of methine proton next to acetyloxy group (-C$\underline{H}$-OAc) at δ = 5.07 ppm, *J*=8.6 Hz and the multiplet signal of methine proton next to azido group (-C$\underline{H}$-N_3) at δ = 3.59 ppm (**Figure A.49** in Appendix). ^{13}C-NMR spectrum revealed the 16 different types of carbon that substantiated the compound **134,** and two types of

singlet signal of the carbonyl carbon were appeared at δ = 168.9 and 174.0, respectively (**Figure A.50** in Appendix).

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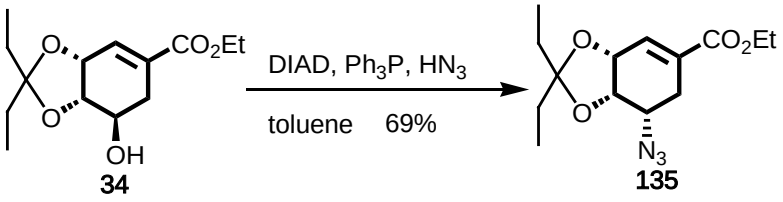
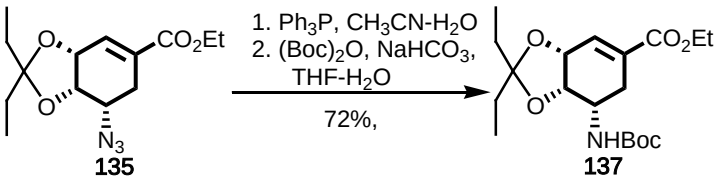
3.3.9 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-azido-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-azido-shikimate) 135



The hydroxyl pentylidene ketal compound **34** was converted to the **135** in 69% by Mitsunobu using the same conditions that had been reported previously. (Section 3.3.2). The ^{1}H-NMR spectrum of compound **135** showed the two methine protons (C3, C4) next to oxygen atoms (-C<u>H</u>-O-) as bord doublet at δ = 4.43 ppm and multiplet at δ = 4.69 ppm. The methine protons at C5 (-C<u>H</u>-N_3) was shown as broad doublet at δ = 3.46 ppm (**Figure A.51** in Appendix). ^{13}C-NMR spectrum revealed the 14 different types of carbon that substantiated the compound **135,** (**Figure A.52** in Appendix).

3.4.10 Synthesis of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-*tert*-butoxycarbonylamino-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-*tert*-butoxycarbonylamino shikimate) 137



Azido compound **135** was reduced by Ph_3P in CH_3CN-H_2O. After work up, the crude product was treated with $(Boc)_2O$ in THF-H_2O to give the Boc protected compound **137** in 72% yield in two steps after purified by column chromatography. The ^{1}H-NMR spectrum exhibited a characteristic peak of *tert*-butoxycarbonylamino

group (Boc) as singlet at δ = 1.40 ppm, and methine proton next to the nitrogen atom (-C<u>H</u>-NHBoc) as a multiplet at δ = 3.86 ppm, and methine protons next to the oxygen atoms (-C<u>H</u>-O-) as multiplets at δ = 4.68 and δ = 4.69 ppm, and amide proton (-NH-CO-) at δ = 4.95 ppm (**Figure A. 53** in Appendix).

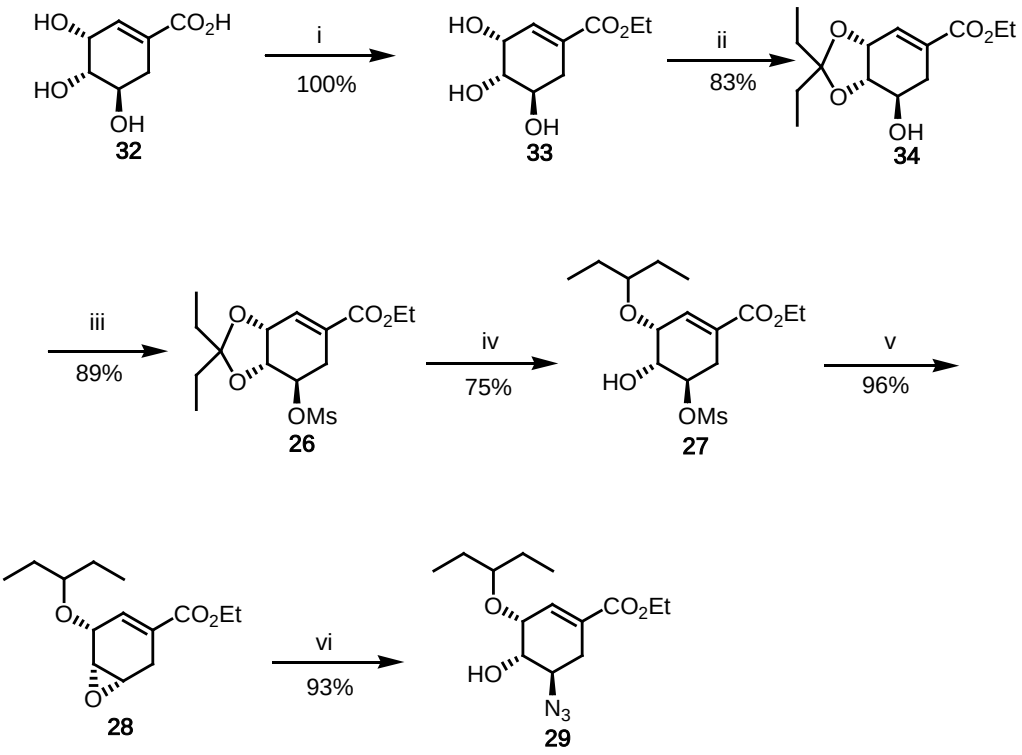
In principle, the ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29**, ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **122**, ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **126a** and ethyl (3*R*,4*R*,5*S*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **130** are all could be converted into a diazide, which would eventually be reduced to the diamino groups of oseltamivir **56** and its related derivatives, especially that from compounds **126a** and **130** which has not yet been reported. However, more investigation are required to evaluate the feasibility and practicality of this approach.

Compounds **10**, **56**, **121-124**, **125a** and **130-134**. These eight (**121**, **123**, **124**, **125a** and **130-133**) new derivatives synthesized in this work could expand the scope and varieties of neuraminidase inhibitors based on the core structure of oseltamivir. This may help expand the range of drug to fight against various strains of flu virus, especially the emerging oseltamivir resistant strains [63-64, 67-70].

CHAPTER IV

CONCLUSION

Compound **29** was an important intermediate for the synthesis of oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **121-124**. This compound was synthesized from (-)-shikimic acid **32**, as shown in **Scheme 4.1**



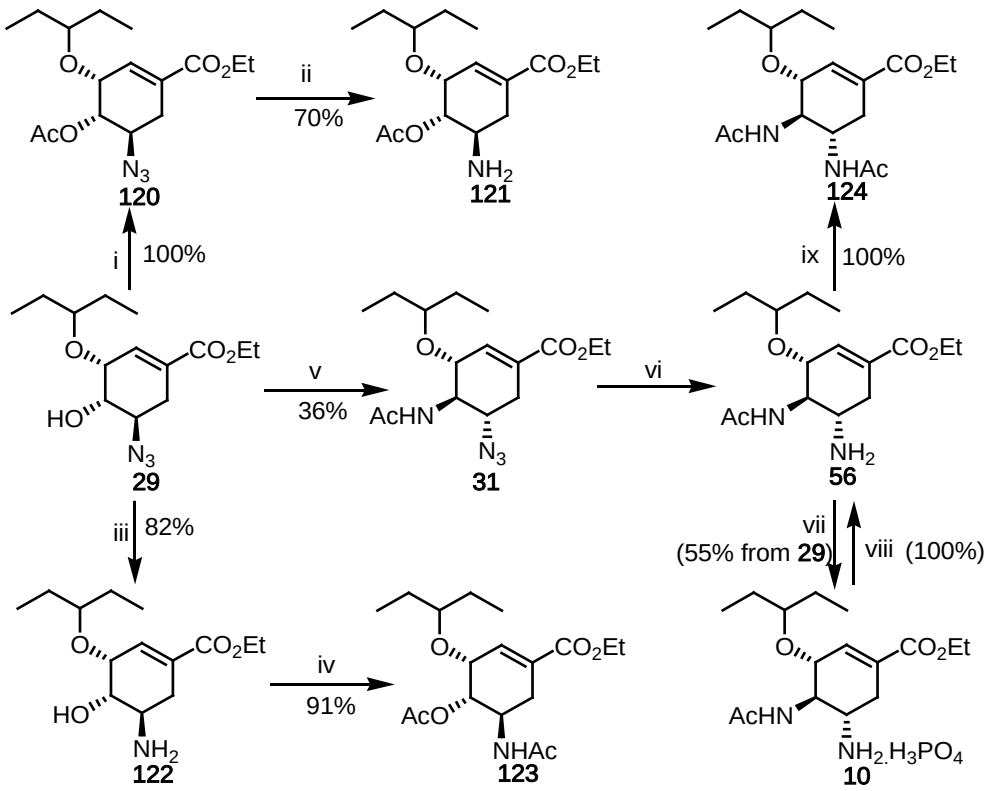
Reagents: i:$SOCl_2$, EtOH, heated to reflux, 3.0 h, ii: 3-pentanone, TfOH, rt, 3.0 h, iii: MsCl, Et_3N, EtOAc, rt, 6.0 h, iv: Et_3SiH, $AlCl_3$, CH_2Cl_2, 0 ^oC, 5.0 h, v: aq.$NaHCO_3$, EtOH-H_2O, 60 ^oC, 3.0 h, vi: NaN_3, NH_4Cl, EtOH, 70 ^oC for 18.0 h.

Scheme 4.1 Synthesis of intermediate **29**

The first part of synthesis started from commercially available shikimic acid **32**, esterified with $SOCl_2$ in ethanol followed by the condensation with 3-pentanone in the presence of TfOH, obtained the pentylidene ketal **34** in 83% yield. Mesylation of **34** was accomplished with MsCl and Et_3N in EtOAc to provided mesyl compound **26** in 89% yield. The reductive regioselective ring opening of the 3,4-pentylidene ketal **26** with Et_3SiH and $AlCl_3$ at 0 ^oC followed by treatment with $NaHCO_3$ in EtOH gave epoxide **27** in 72% yield from compound **32**, The epoxide-opening reaction with NaN_3 provided the azido hydroxyl compound **29** in 93% yield.

Overall, the synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29** was accomplished in 6 steps with 50 % overall yield from the commercially available (-)-shikimic acid **32**, which was 17% lower than the value reported in the literature [12].

Oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **121-124** were synthesized from the intermediate ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29**, as shown in **Scheme 4.2**



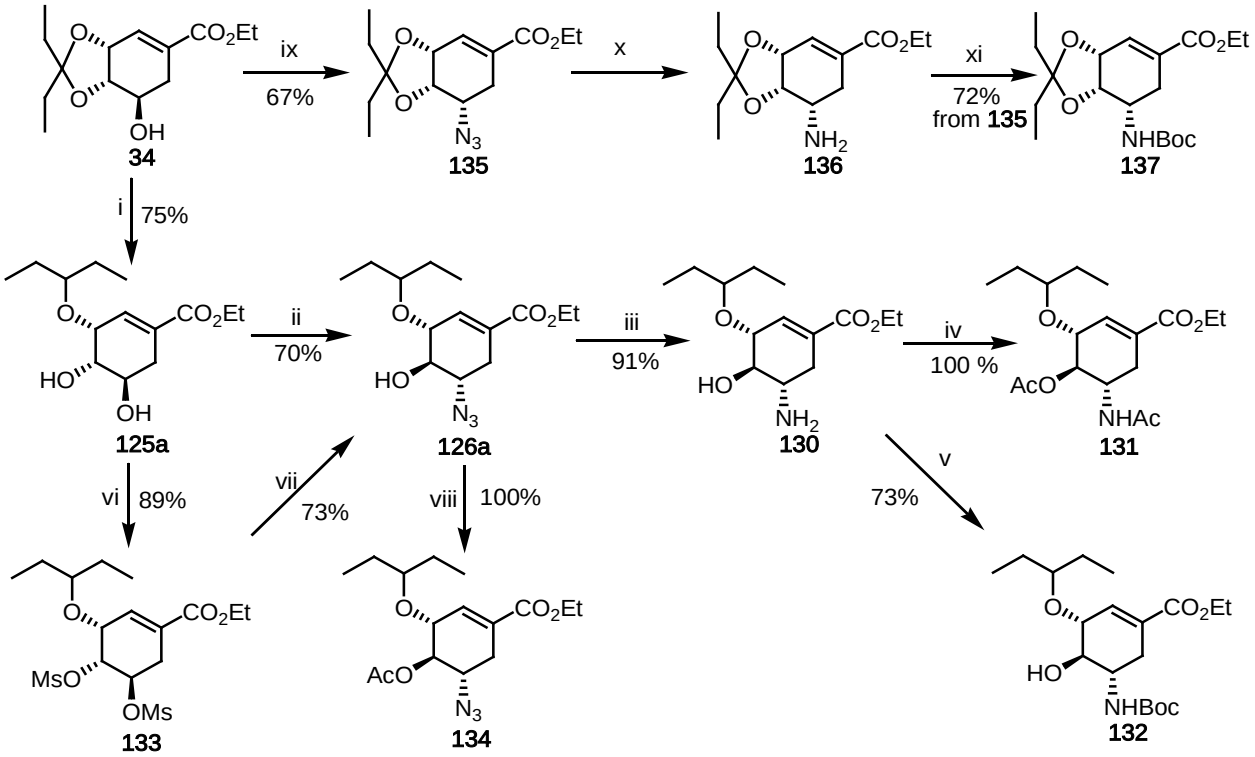
Reagents: i: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, ii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iv: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, v: a. Ph_3P, CH_3CN-H_2O, rt, 6.0 h, b. NaN_3, NH_4Cl, DMF, 70-75 ^oC, 18-20 h, c. Ac_2O, Et_3N, CH_2Cl_2, rt, 2.0 h, vi: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, vii: H_3PO_4, EtOH, rt, viii: sat.$NaHCO_3$, CH_2Cl_2, 5 min, ix: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h.

Scheme 4.2 Synthesis of oseltamivir phosphate **10**, oseltamivir **56** and its derivatives **120-123**

The acetylation of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29** using AcCl and pyridine in CH_2Cl_2 provided the acetyloxy azidide **120**, which was reduced with Ph_3P in CH_3CN-H_2O to obtain the oseltamivir derivative **121** in overall yield of 35%. A one pot reaction sequence on compound **29** that include reduction of azide group by Ph_3P, intramolecular aziridine formation, and opening of the aziridine ring with NaN_3 gave the amino azide in 73% yield from compound **29**, which was comparable to the 2-steps process reported in the literature [12]. Acetylation of the amino group, followed by reduction of the azide group obtained the oseltamivir **56** with an overall yield of 36% from compound **31**. Treatment of the free amine **56** with H_3PO_4 in EtOH eventually resulted in a 55% yield of oseltamivir phosphate **10**. In summary, oseltamivir phosphate **10** and oseltamivir **56** were successfully synthesized via the intermediate compound **29** in 11 and 10 steps with 10% and 18% overall yield, respectively from the starting material, (-)-shikimic acid **32**.

Oseltamivir derivative **122** was obtained in 82% from compound **29** by reduction with Ph_3P in CH_3CN-H_2O. The derivative **123** was also obtained from acetylation of the –OH and $–NH_2$ groups of **122** in 91% yield. In addition, the oseltamivir derivative **124** was synthesized through acetylation of the oseltamivir **56** in quantitative yield.

Compounds **34** and **125a** were important intermediates for the synthesis of oseltamivir derivatives **130-135** and **137**. The intermediate **125a** was obtained from reoselective reduction of compound **34**, as shown in **Scheme 4.3**.



Reagents: i: Et_3SiH, $TiCl_4$, CH_2Cl_2, 0 ^oC, 5.0 h, ii: DIAD, Ph_3P, HN_3, toluene, 0 ^oC 6.0 h and then rt. 24.0 h, iii: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, iv: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h, v: $(Boc)_2O$, aq.$NaHCO_3$, EtOH-H_2O, rt, 5.0 h, vi: MsCl, Et_3N, EtOAc, rt, 6.0 h, vii: $TMSN_3$, KF, 18-crown-6, DMF, reflux, 24.0 h, viii: AcCl, pyridine, CH_2Cl_2, reflux, 3.0 h ix: DIAD, Ph_3P, HN_3, toluene, 0 ^oC 6.0 h, rt. 24.0 h, x: Ph_3P, CH_3CN-H_2O, rt, 3.0 h, xi: $(Boc)_2O$, aq.$NaHCO_3$, EtOH-H_2O, rt, 5.0 h.

Scheme 4.3 Synthesis of oseltamivir derivatives **125a**, **130-135** and **137**.

The compound **34** and dihydroxy compound **125a** were important intermediates for the synthesis of derivatives **130-134, 135** and **137**.

The reductive ring opening of ethyl 3,4-*O*-isopentylidine-5-hydroxy shikimate **34** was accomplished with the reducing agent Et_3SiH in the presence of Lewis acid $AlCl_3$ or $TiCl_4$ in CH_2Cl_2 at 0 ^oC for 5.0 hours providing the two *trans*-4,5-diols **125a** in 75% yield. This compound was subjected to Mitsunobu reaction using DIAD, Ph_3P and HN_3 in toluene at 0 ^oC for 6.0 hours and followed by room temperature for 24.0 hours giving ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **126** in 70% yield. The hydroxyl amino compound **130** was obtained from reduction of **126** with Ph_3P in CH_3CN-H_2O in 91% yield. The oseltamivir derivative **131** was then obtained from the acetylation of **130** in 91% yield. Furthermore, the amino compound **130** was transformed with di-*tert*-butyl-di-carbonate $(Boc)_2O$ and $NaHCO_3$ that led to the Boc protected compound **132** in a moderated yield of 73%. The bismesylate **133** was obtained in high yield of 89% from the diol **125a** by mesylation with MsCl and Et_3N.

The azide compound **135** was obtained from compound **34**, which was reduced and protected with $(Boc)_2O$ to provide the compound **137** in 72% yield.

In summary, four new oseltamivir derivatives **130-133** could be obtained in 40%, 40%, 29% and 55% from (-)-shikimic acid **32**, respectively.

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APPENDIX

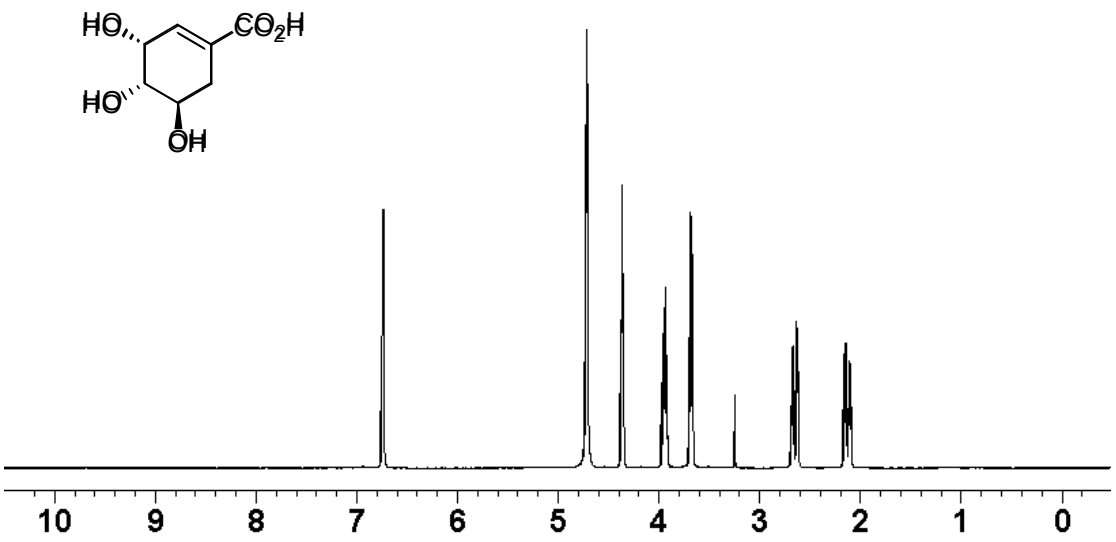

Figure A.1 ^{1}H-NMR (D_2O) Spectrum of (-)-shikimic acid **32**

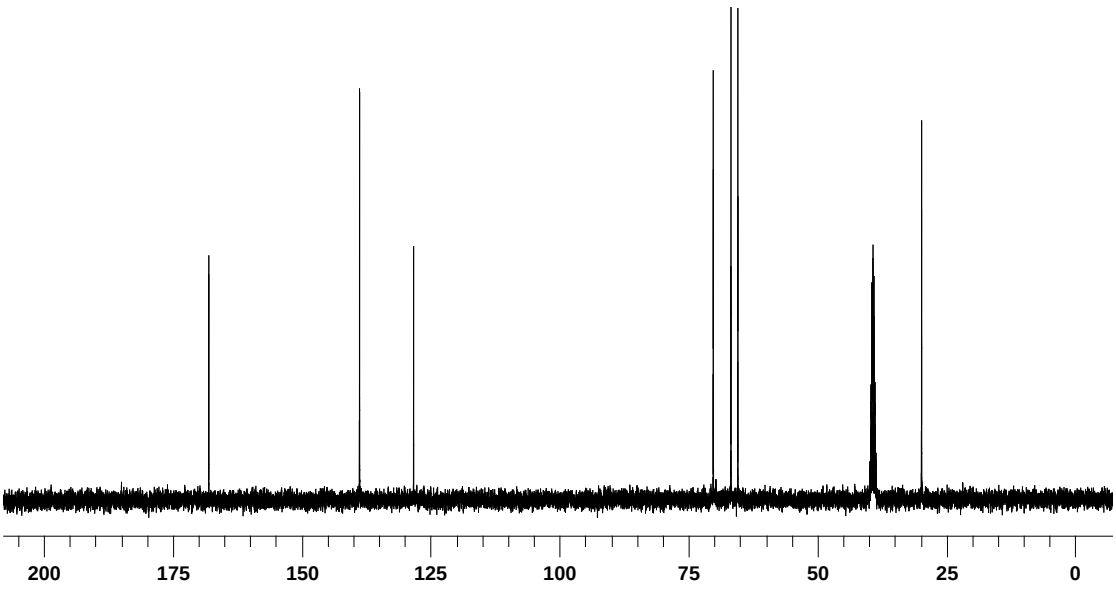

Figure A.2 ^{13}C-NMR (D_2O) Spectrum of (-)-shikimic acid **32**

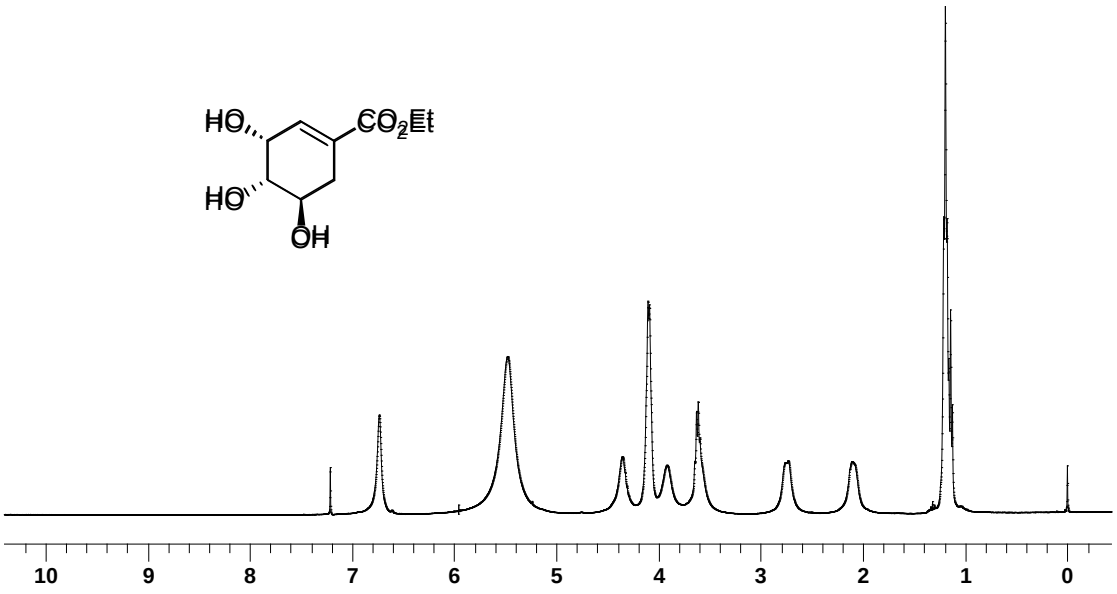

Figure A.3 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylate (ethyl shikimate) **33**

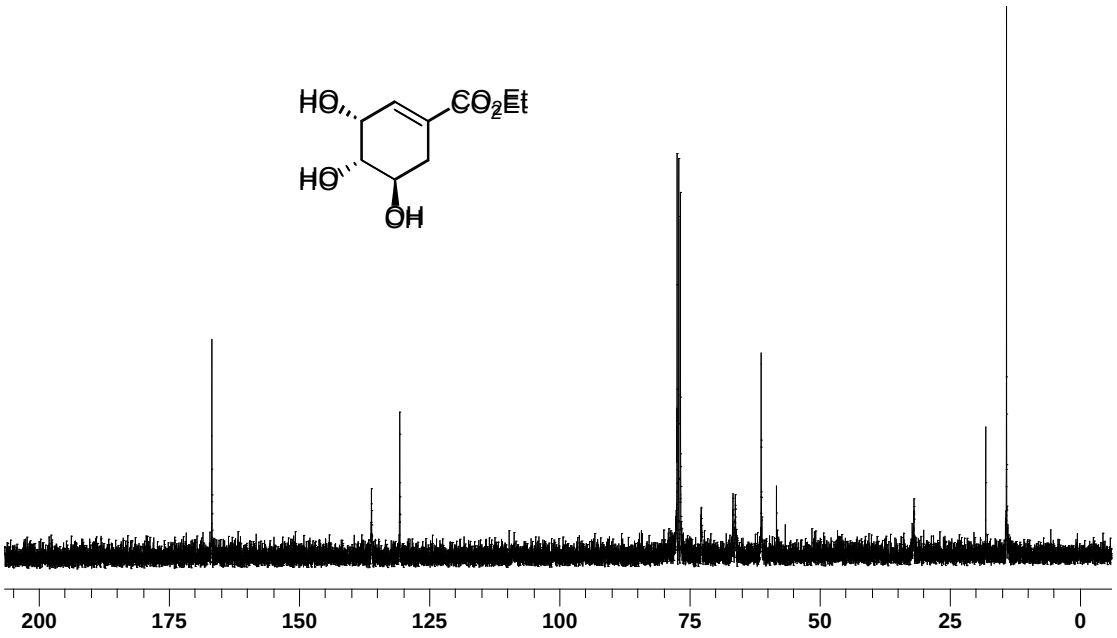

Figure A.4 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylate (ethyl shikimate) **33**

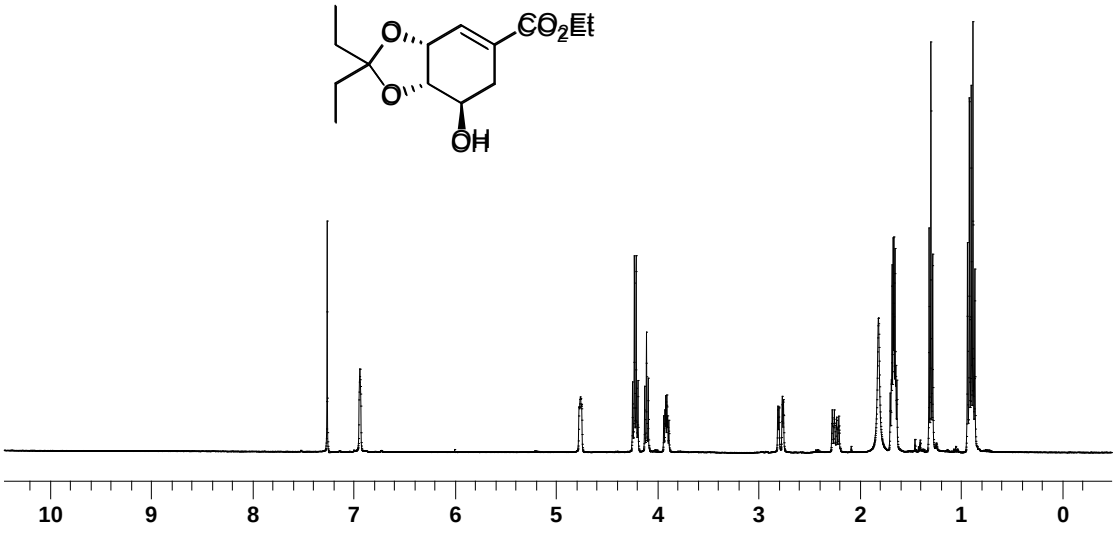

Figure A.5 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*S*)-2,2-diethyl-7-hydroxy-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-hydroxy shikimate) **34**

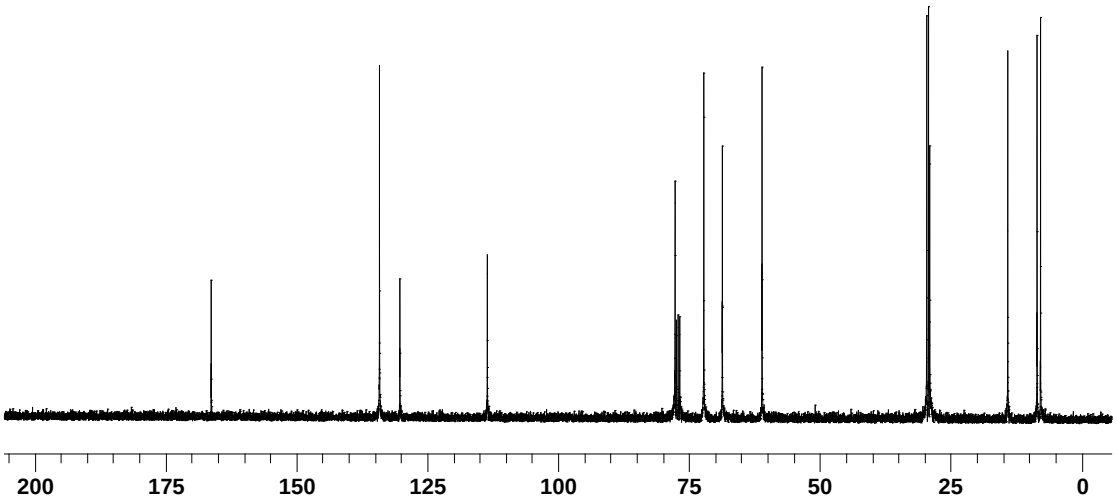

Figure A.6 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*S*)-2,2-diethyl-7-hydroxy-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-hydroxy shikimate) **34**

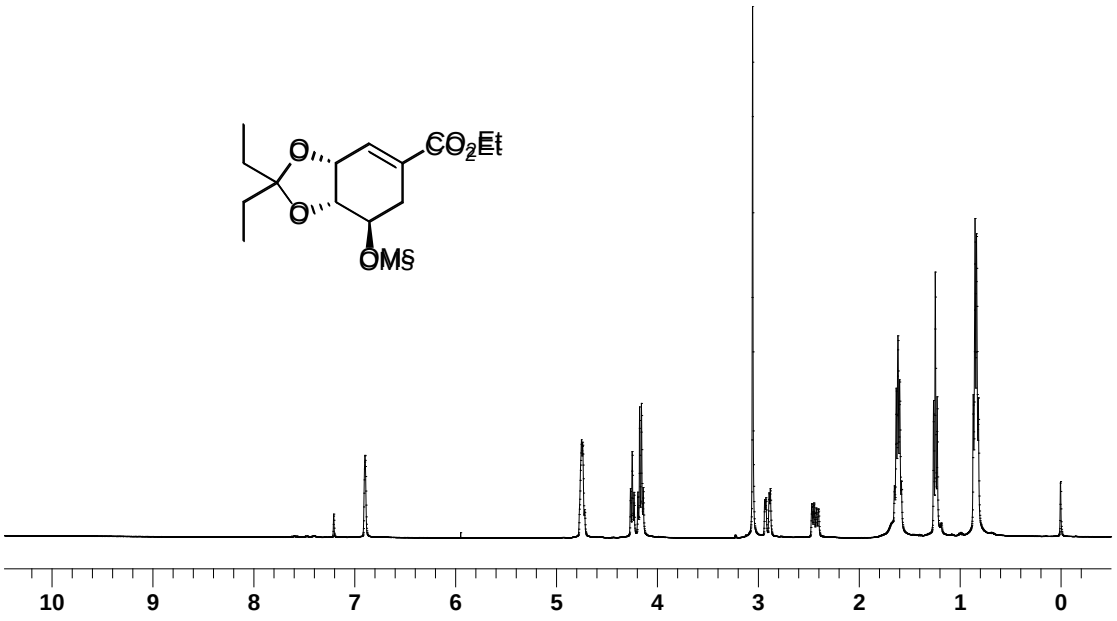

Figure A.7 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-methanesulphonyl-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-methansulphonyl-shikimate) **26**

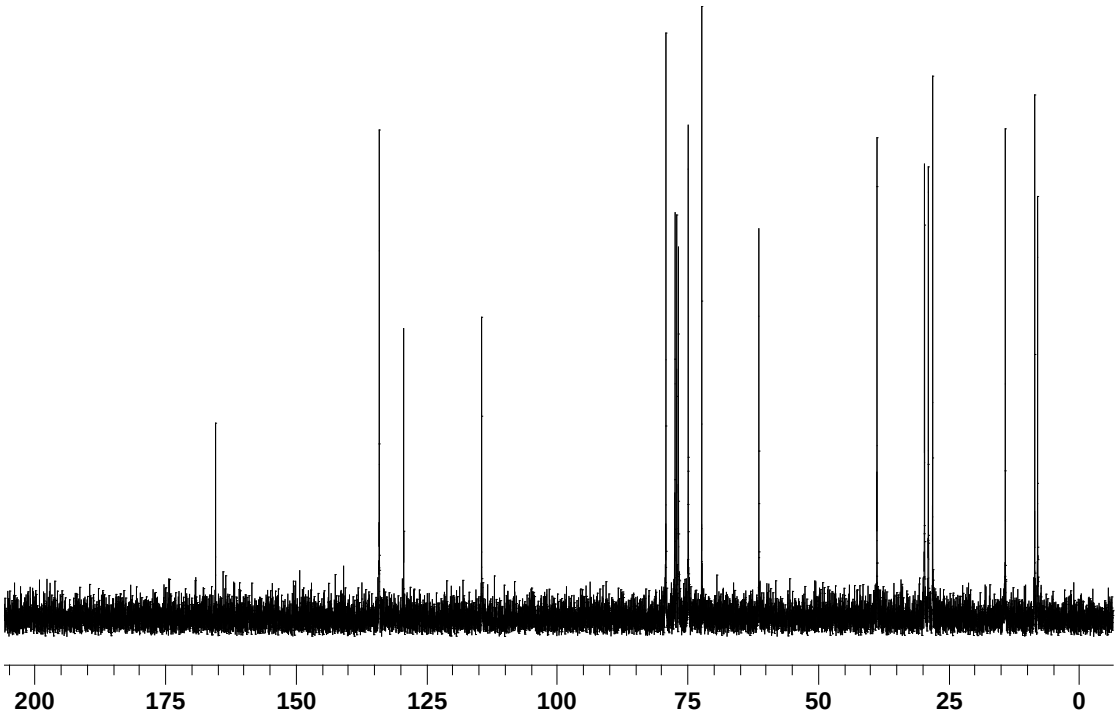

Figure A.8 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-methanesulphonyl-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-methansulphonyl-shikimate) **26**

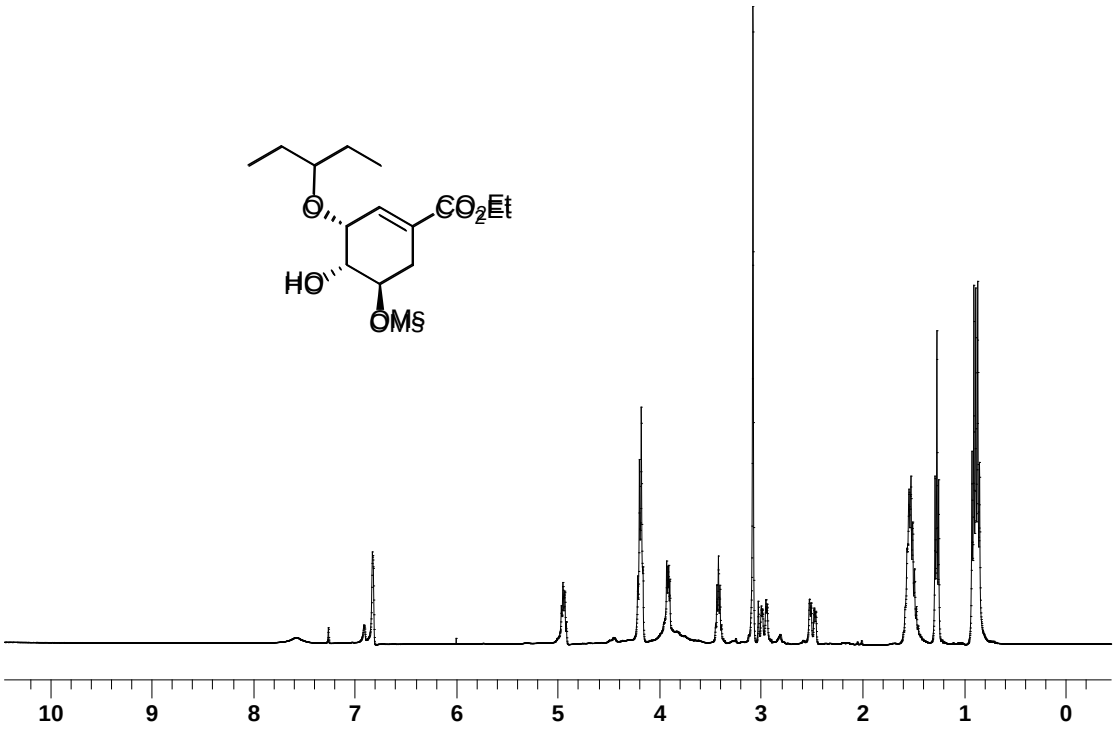

Figure A.9 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*R*)-3-(1-ethyl-propoxy)-4-hydroxy-5-methanesulfonyloxy-1-cyclohexene-1-carboxylate **27**

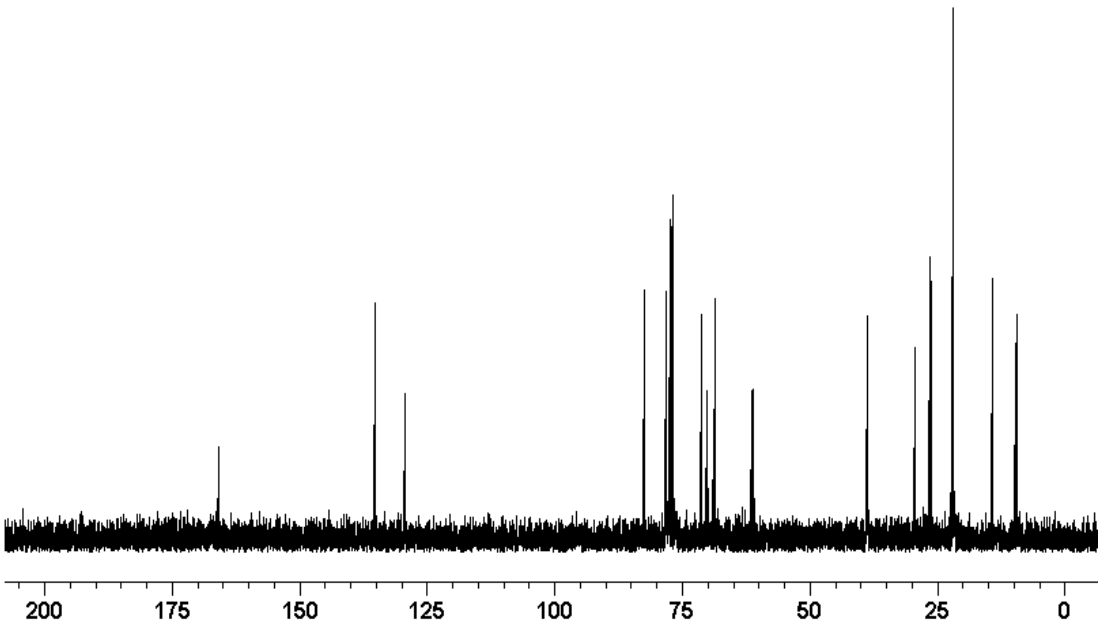

Figure A.10 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*R*)-3-(1-ethyl-propoxy)-4-hydroxy-5-methanesulfonyloxy-1-cyclohexene-1-carboxylate **27**

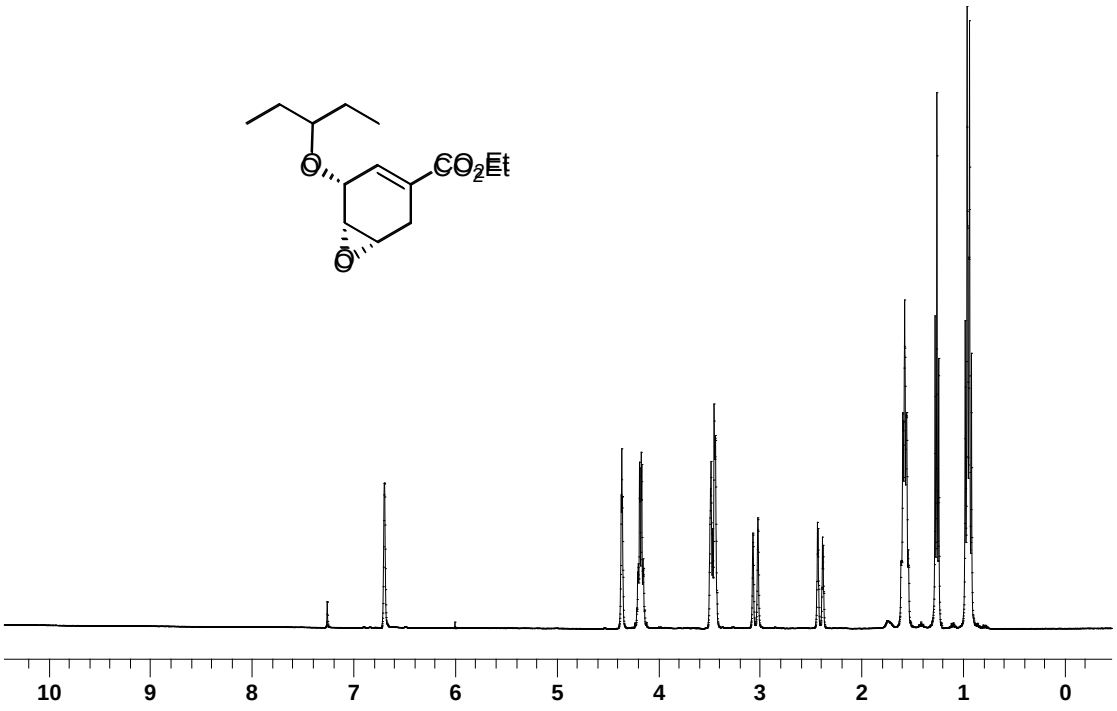

Figure A.11 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (epoxide) **28**

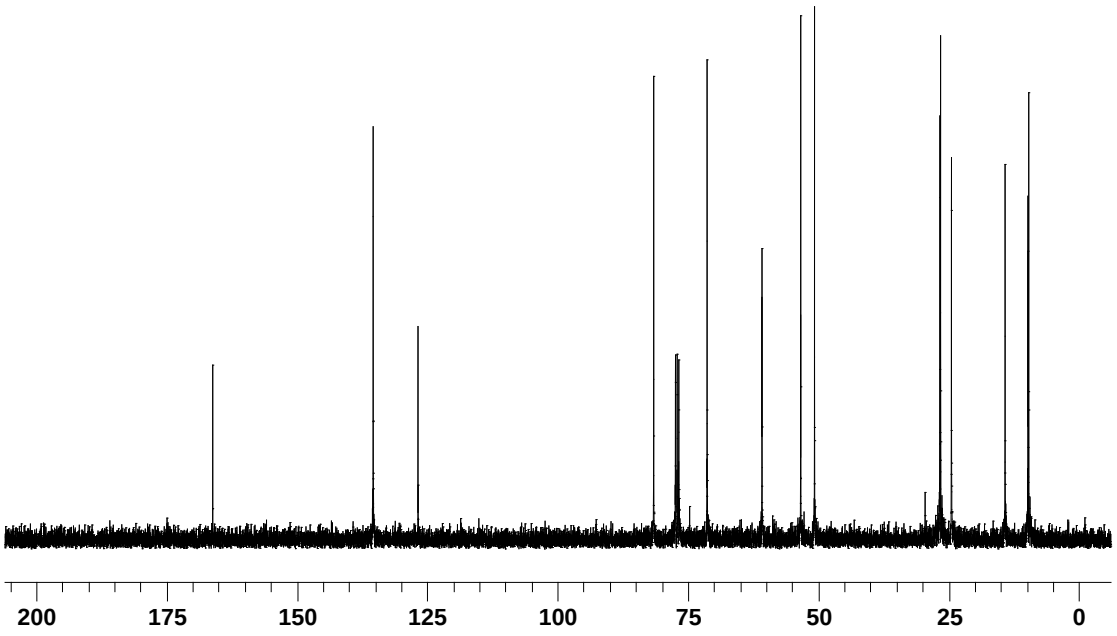

Figure A.12 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (epoxide) **28**

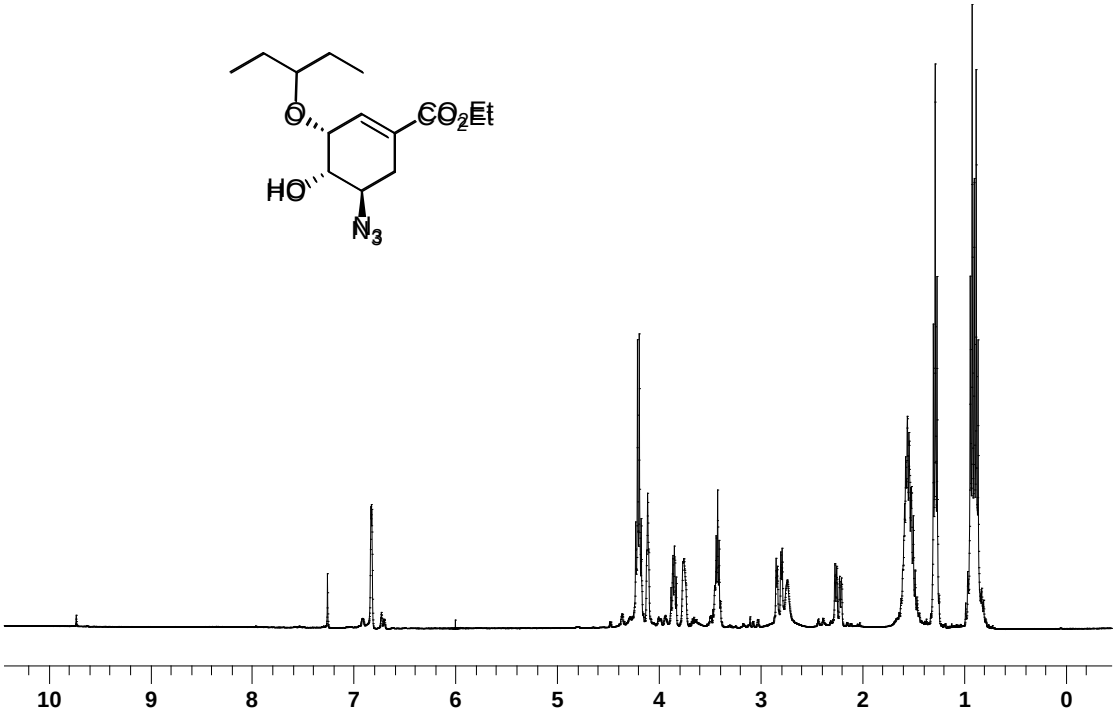

Figure A.13 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29**

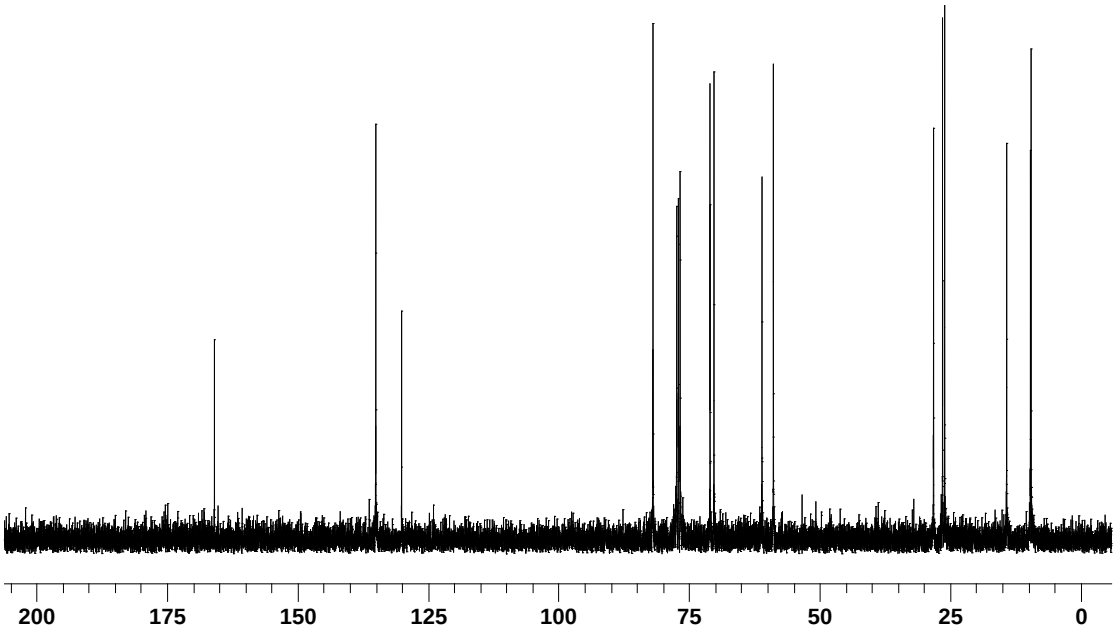

Figure A.14 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **29**

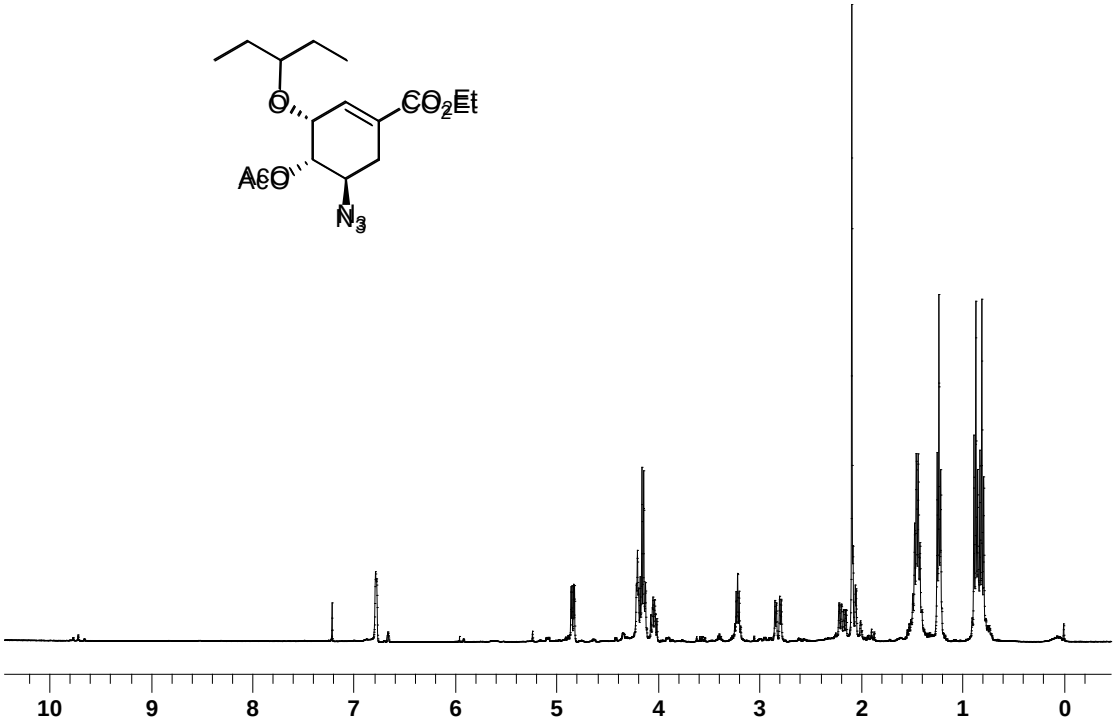

Figure A.15 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **120**

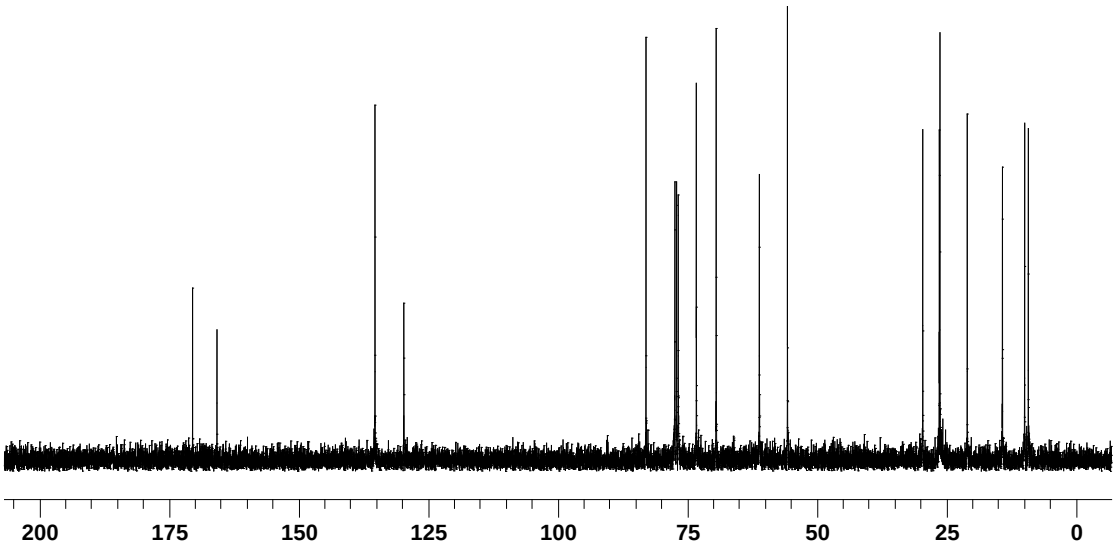

Figure A.16 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **120**

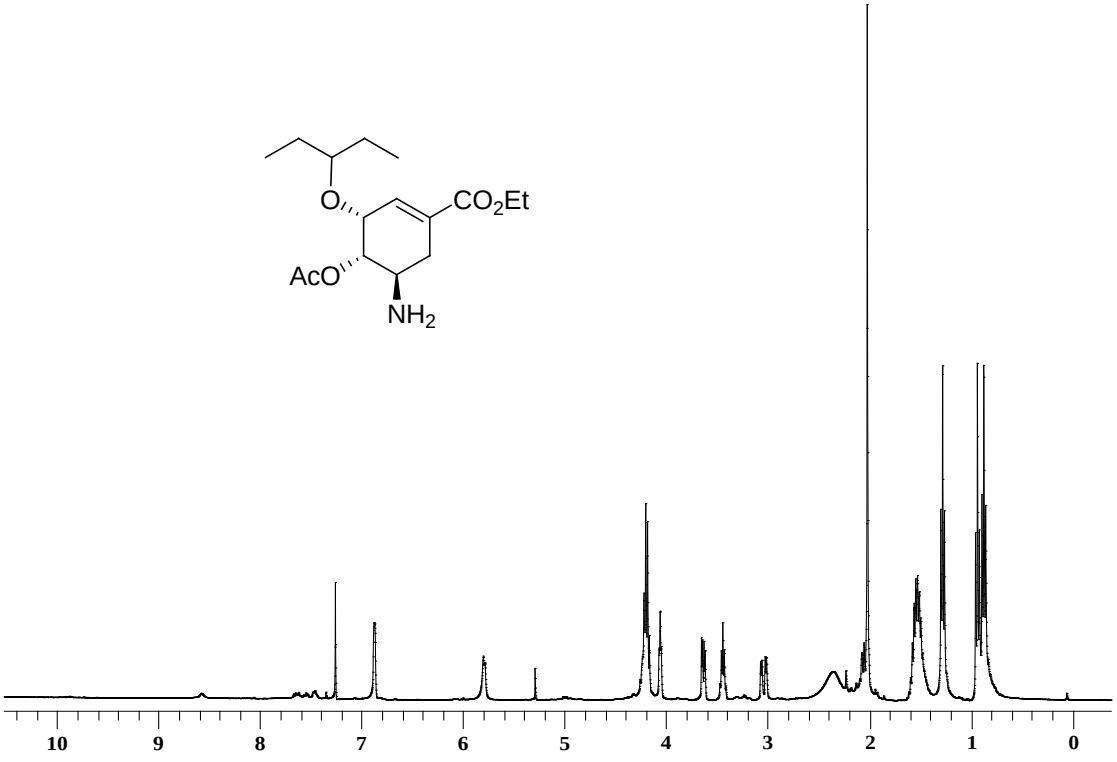

Figure A.17 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **121**

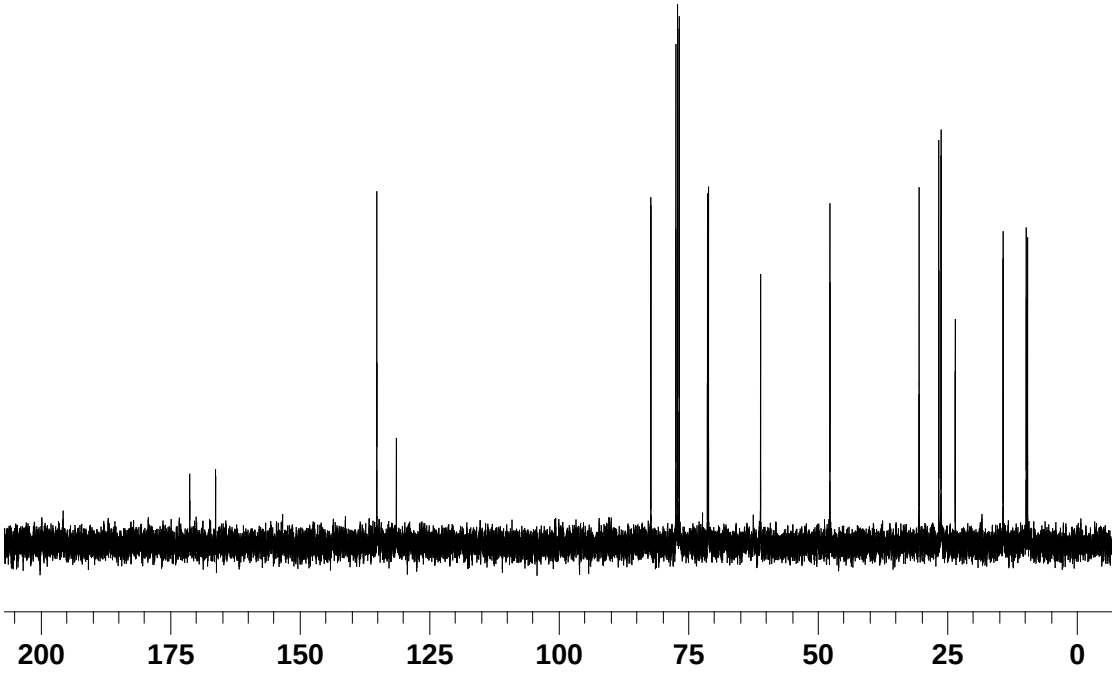

Figure A.18 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **121**

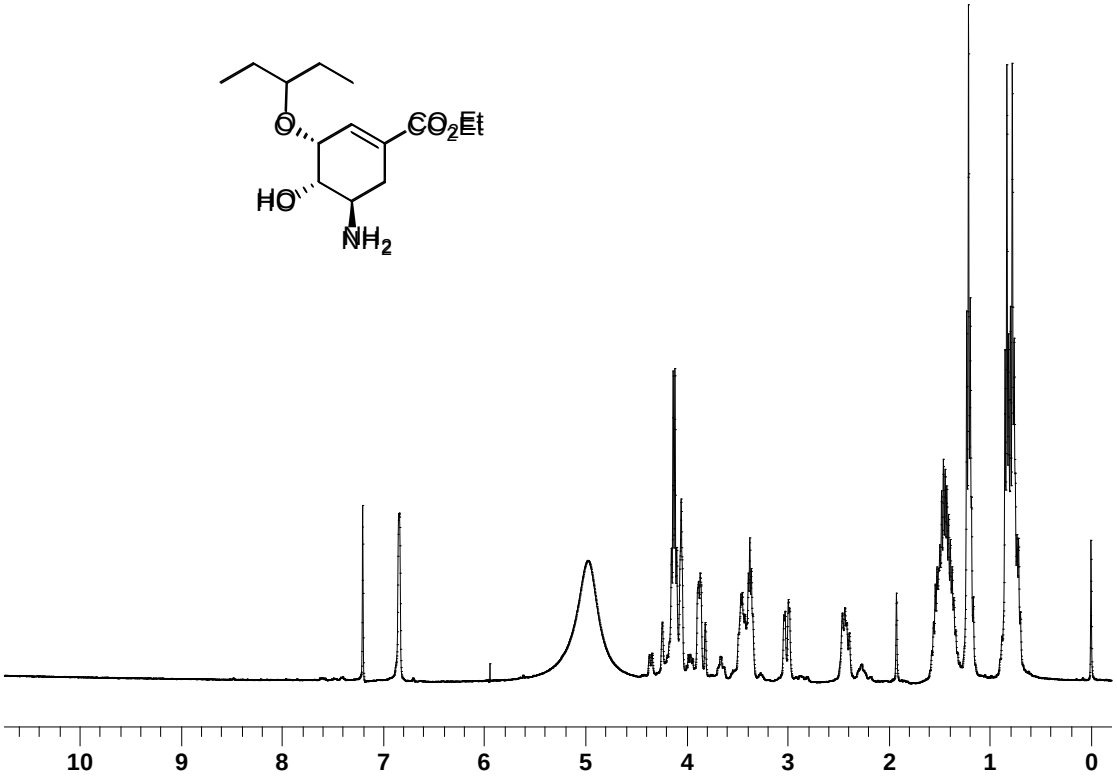

Figure A.19 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **122**

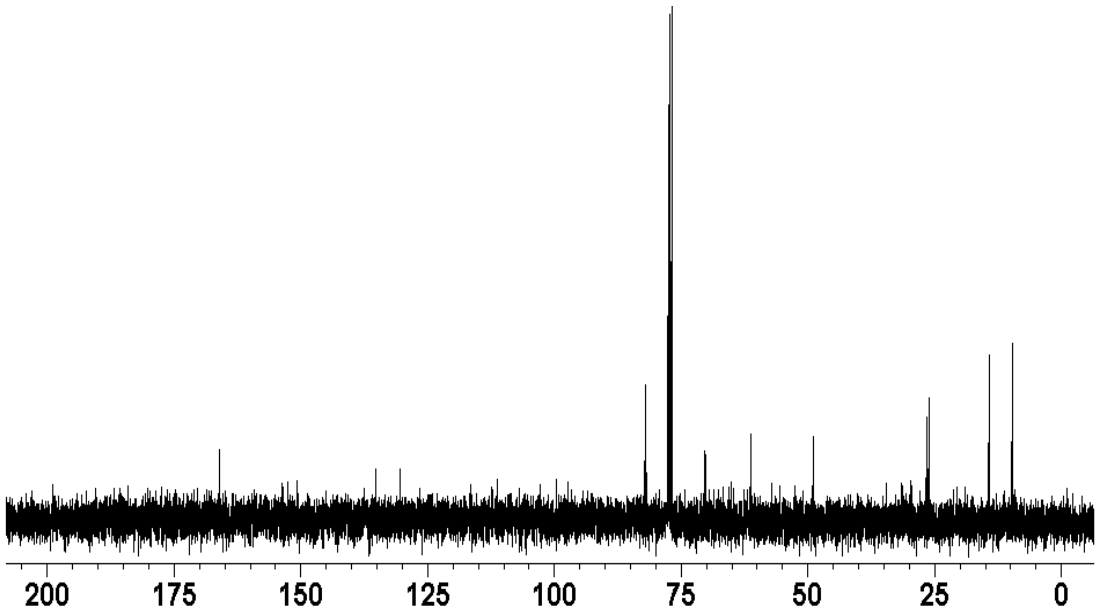

Figure A.20 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **122**

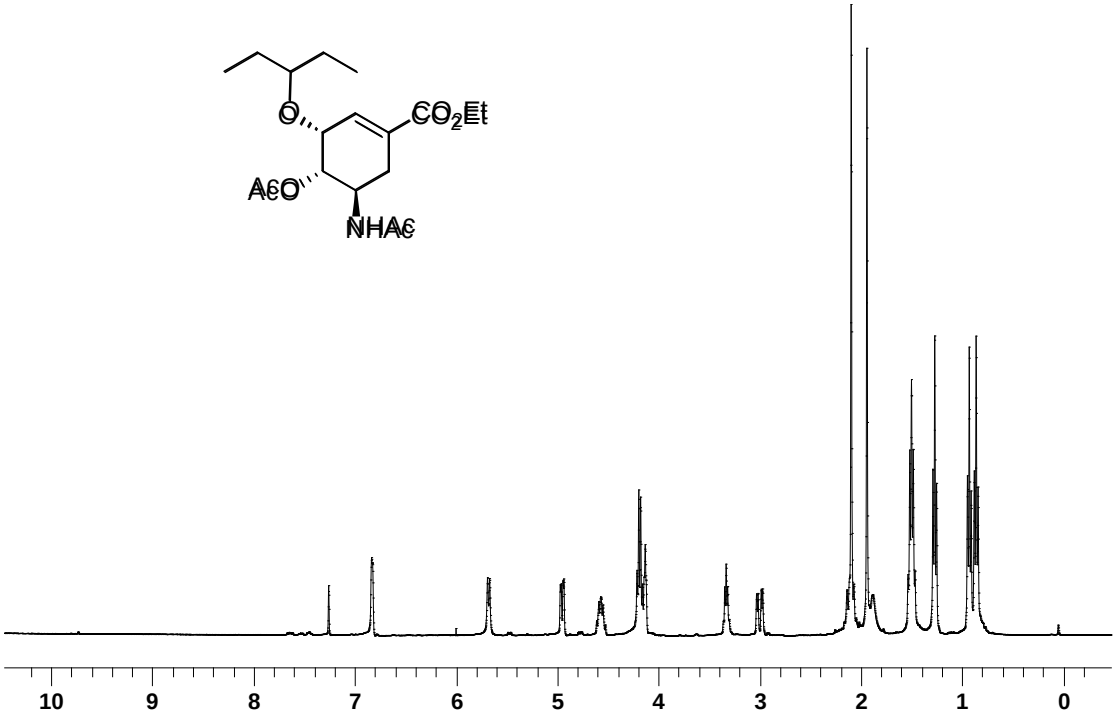

Figure A.21 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **123**

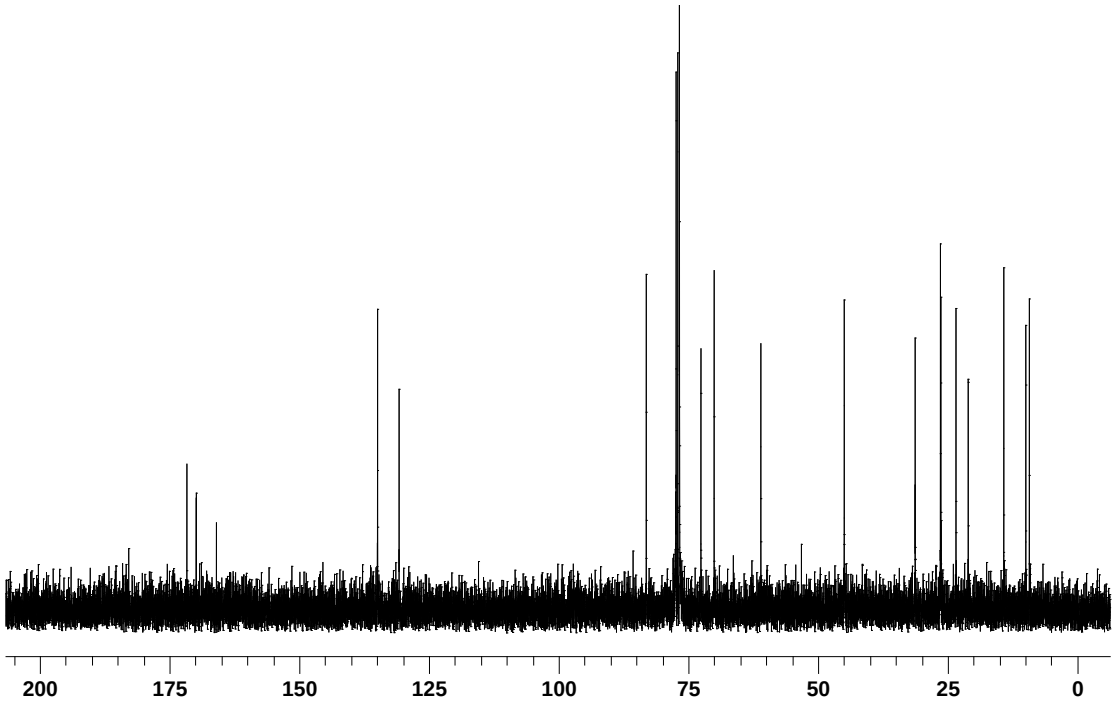

Figure A.22 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **123**

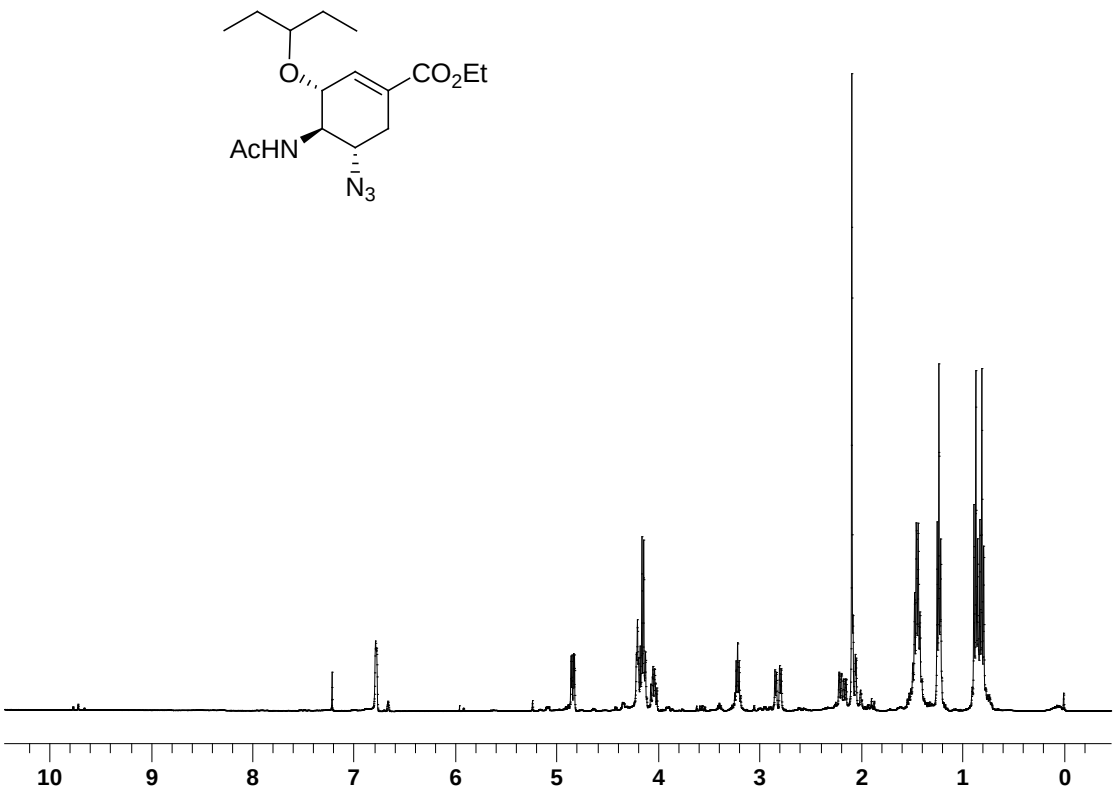

Figure A.23 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **31**

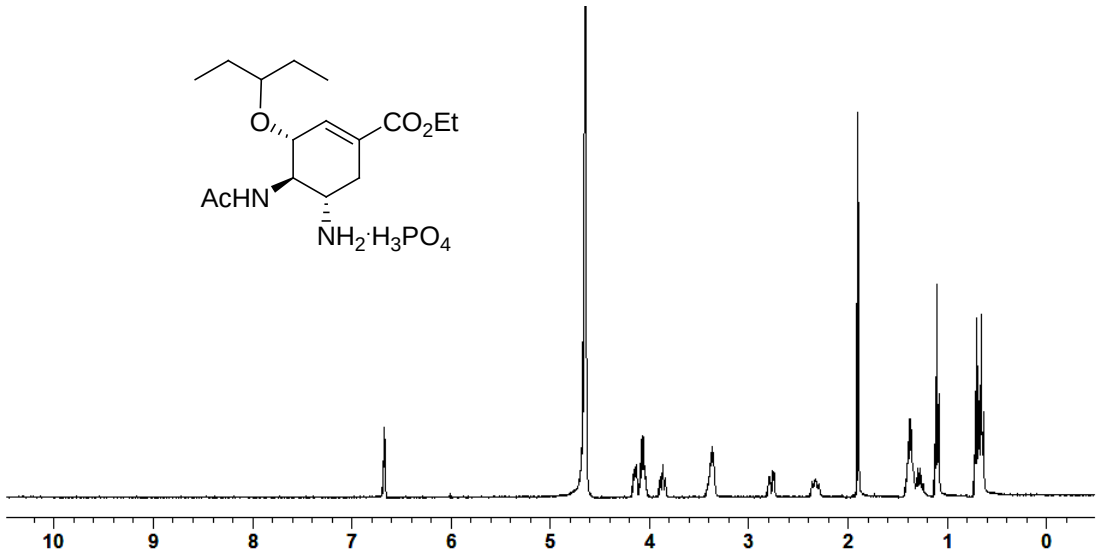

Figure A.25 ^{1}H-NMR (D_2O) Spectrum of ethyl (3*R*,4*R*,5*S*)-4-*N*-acetamido-5-amino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate phosphate (oseltamivir phosphate) **10**

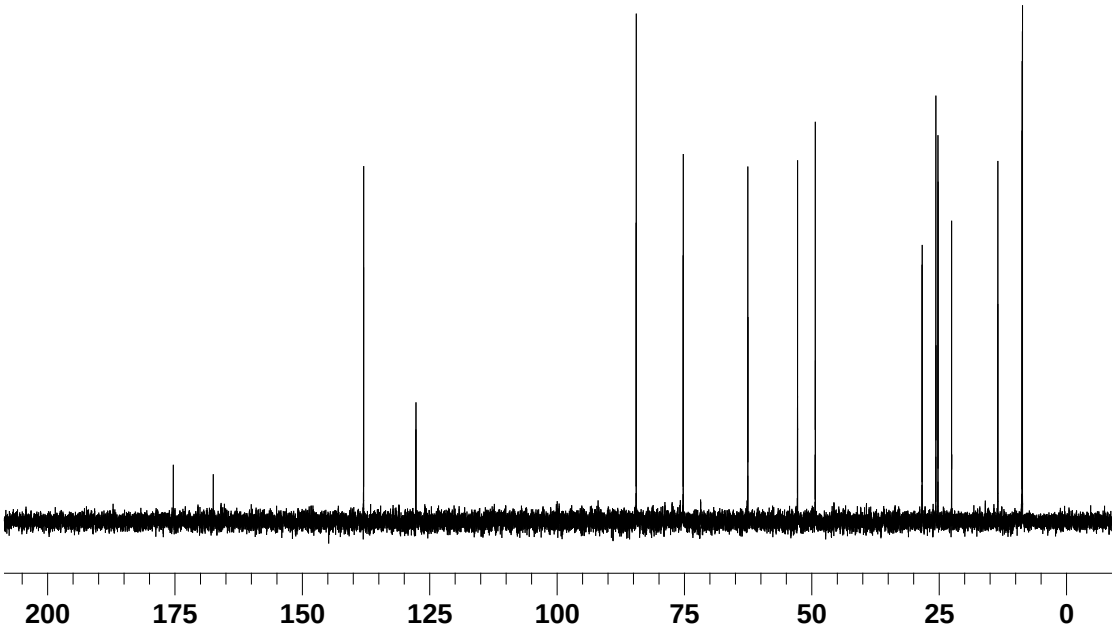

Figure A.26 ^{13}C-NMR (D_2O) Spectrum of ethyl (3*R*,4*R*,5*S*)-4-*N*-acetamido-5-amino-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate phosphate (oseltamivir phosphate) **10**

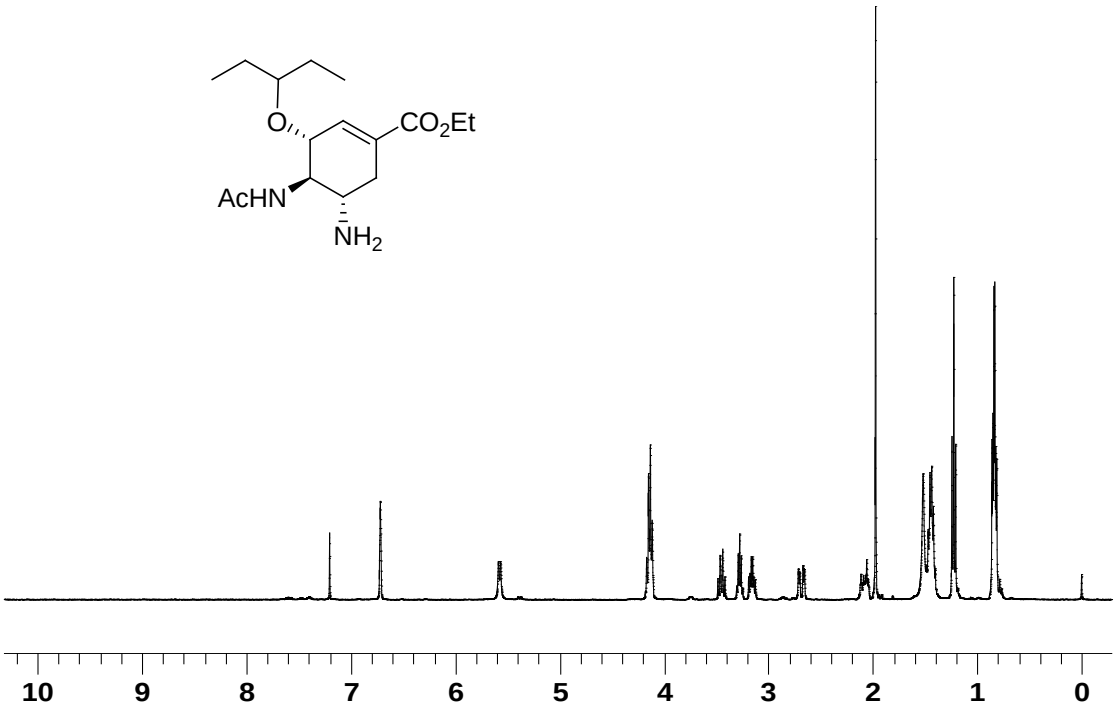

Figure A.27 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-4-hydroxy cyclohex-1-ene-1-carboxylate (oseltamivir) **56**

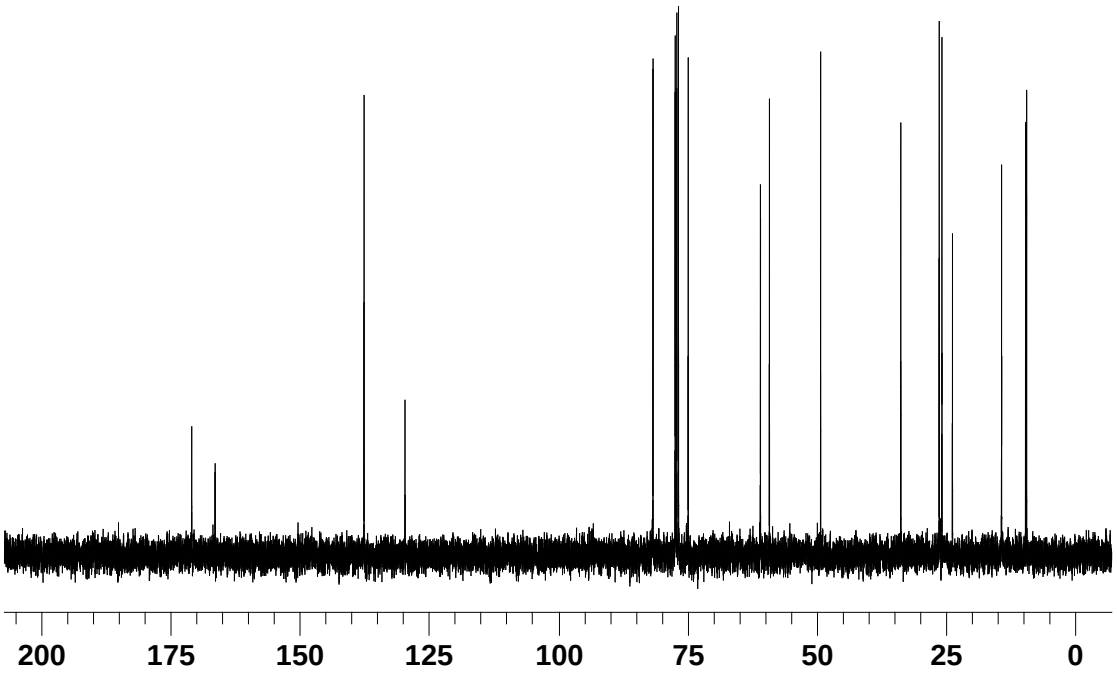

Figure A.28 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-4-hydroxy cyclohex-1-ene-1-carboxylate (oseltamivir) **56**

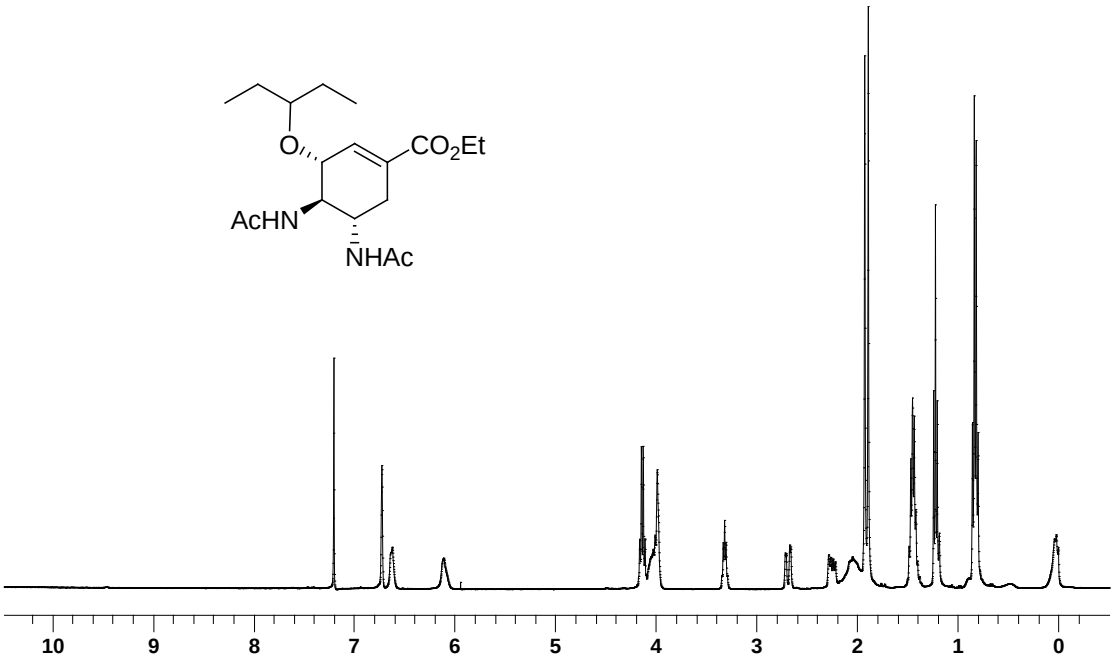

Figure A.29 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **124**

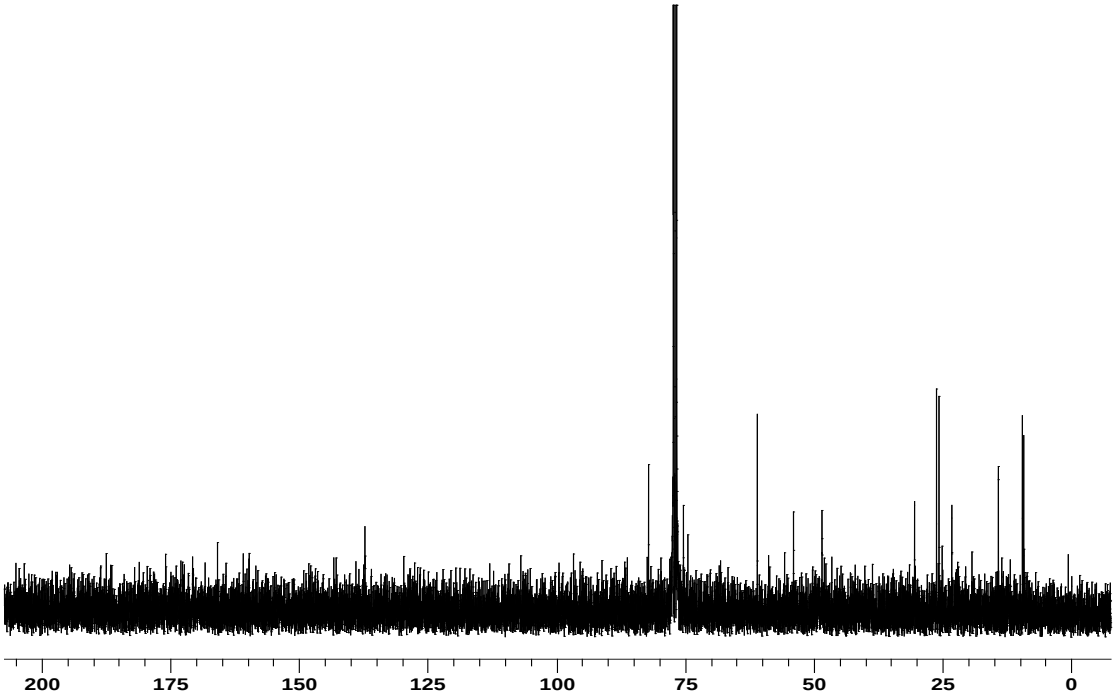

Figure A.30 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **124**

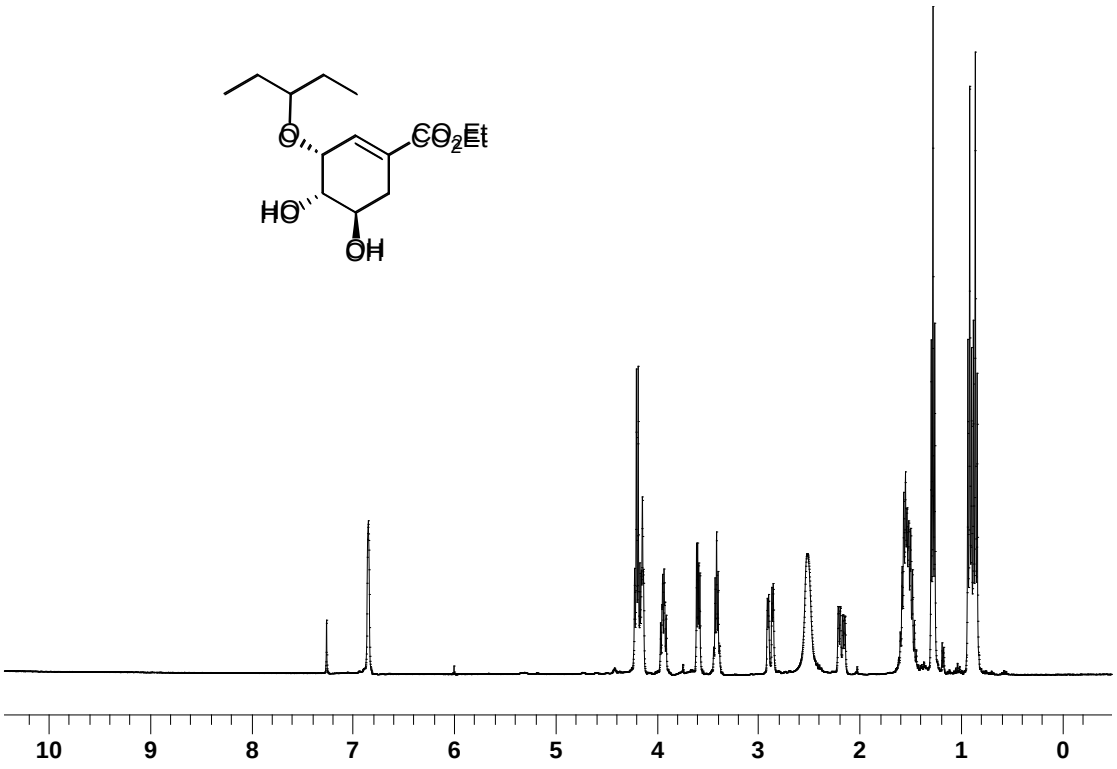

Figure A.31 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-3-(1-ethyl-propoxy)-4,5-dihydroxy-1-cyclohexene-1-carboxylate **125a**

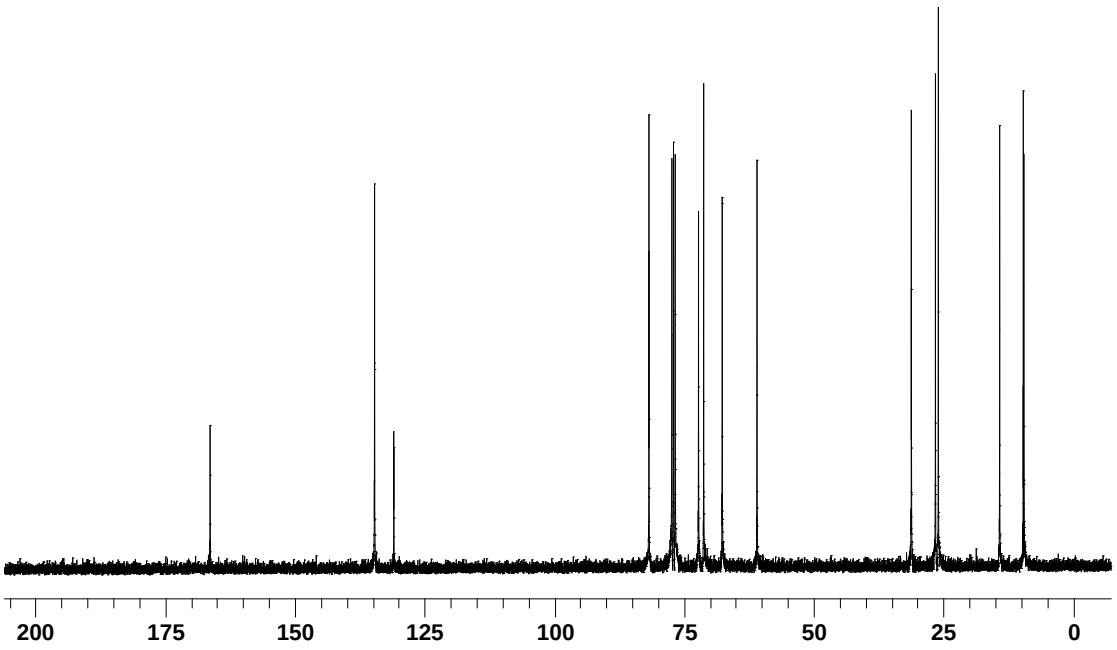

Figure A.32 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-3-(1-ethyl-propoxy)-4,5-dihydroxy-1-cyclohexene-1-carboxylate **125a**

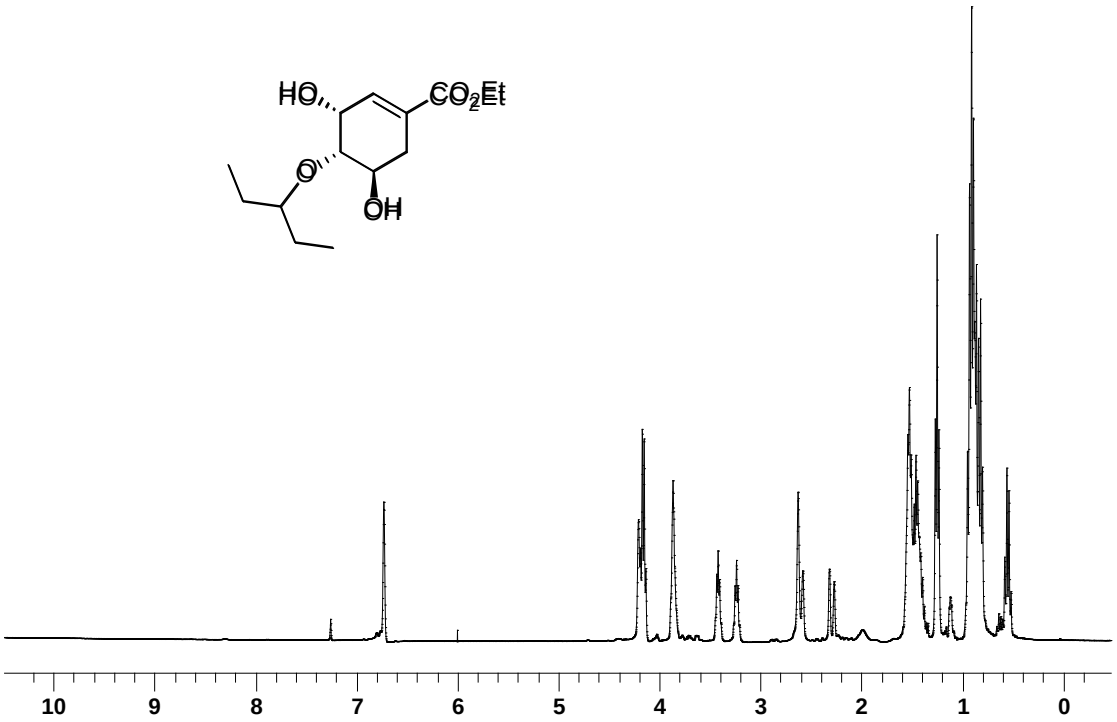

Figure A.33 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-4-(1-ethyl-propoxy)-3,5-dihydroxy-1-cyclohexene-1-carboxylate **125b**

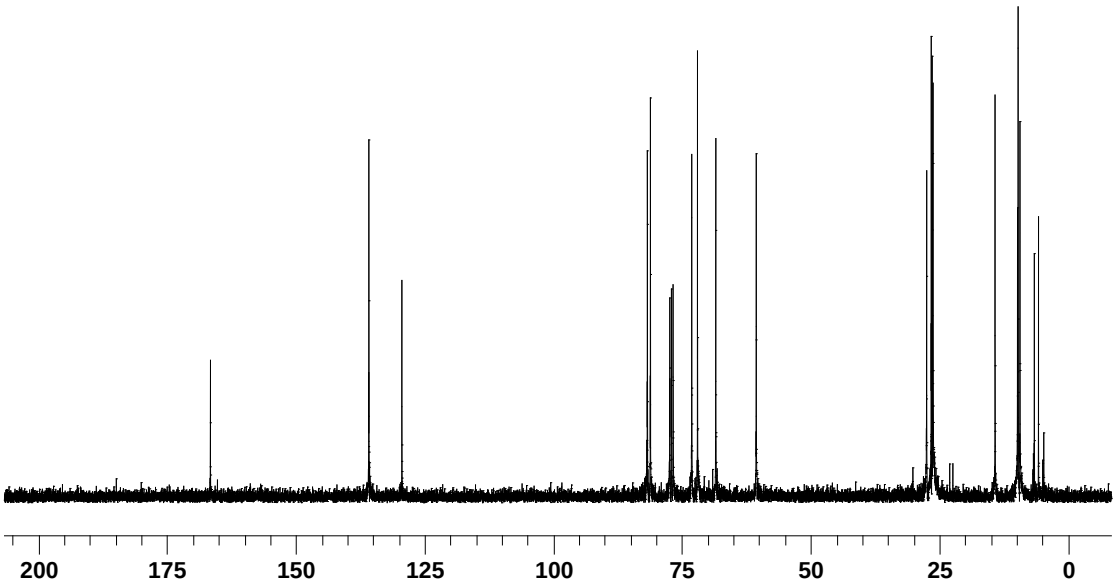

Figure A.34 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-3-(1-ethyl-propoxy)-4,5-dihydroxy-1-cyclohexene-1-carboxylate **125b**

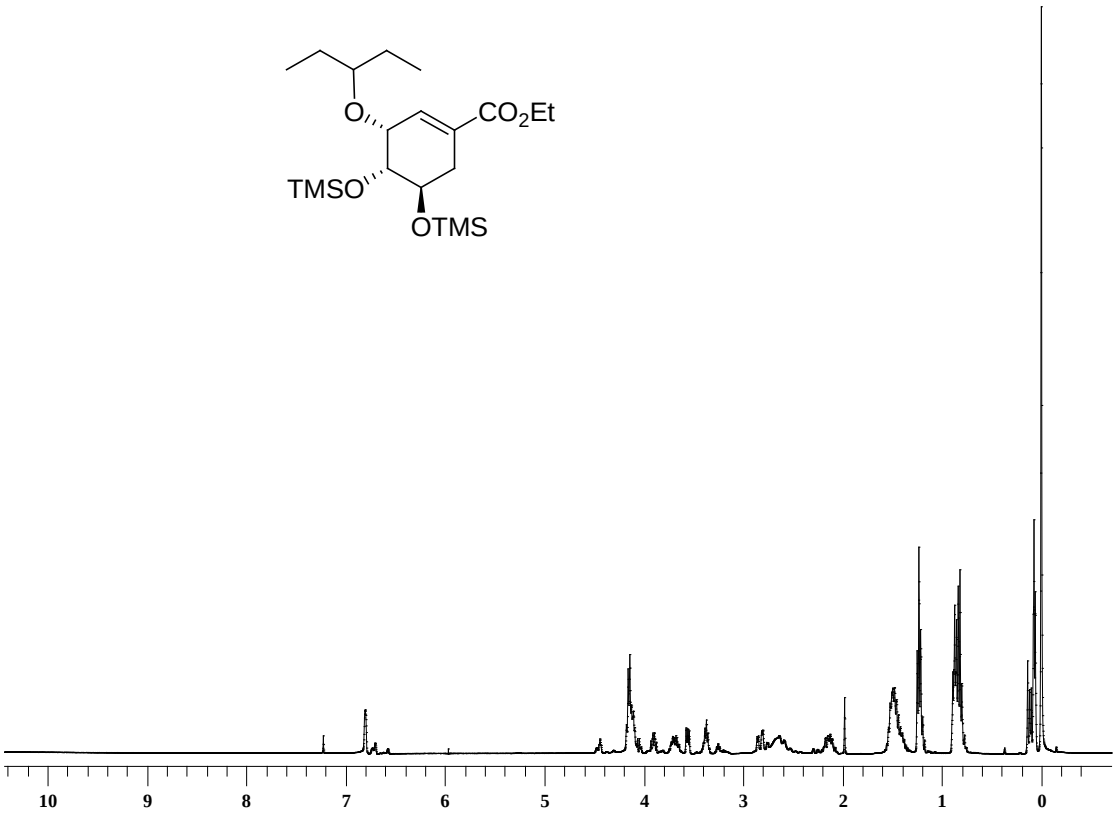

Figure A.35 ^{1}H-NMR ($CDCl_3$) Spectrum of compound **127**

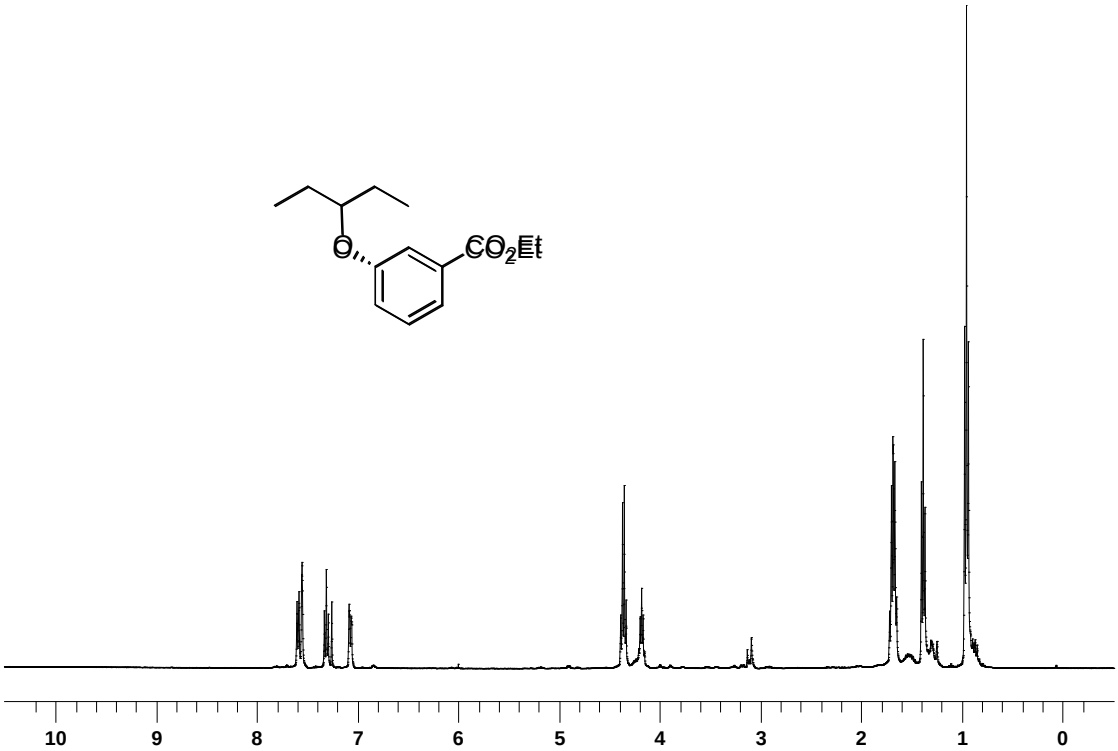

Figure A.36 ^{1}H-NMR ($CDCl_3$) Spectrum of compound **128**

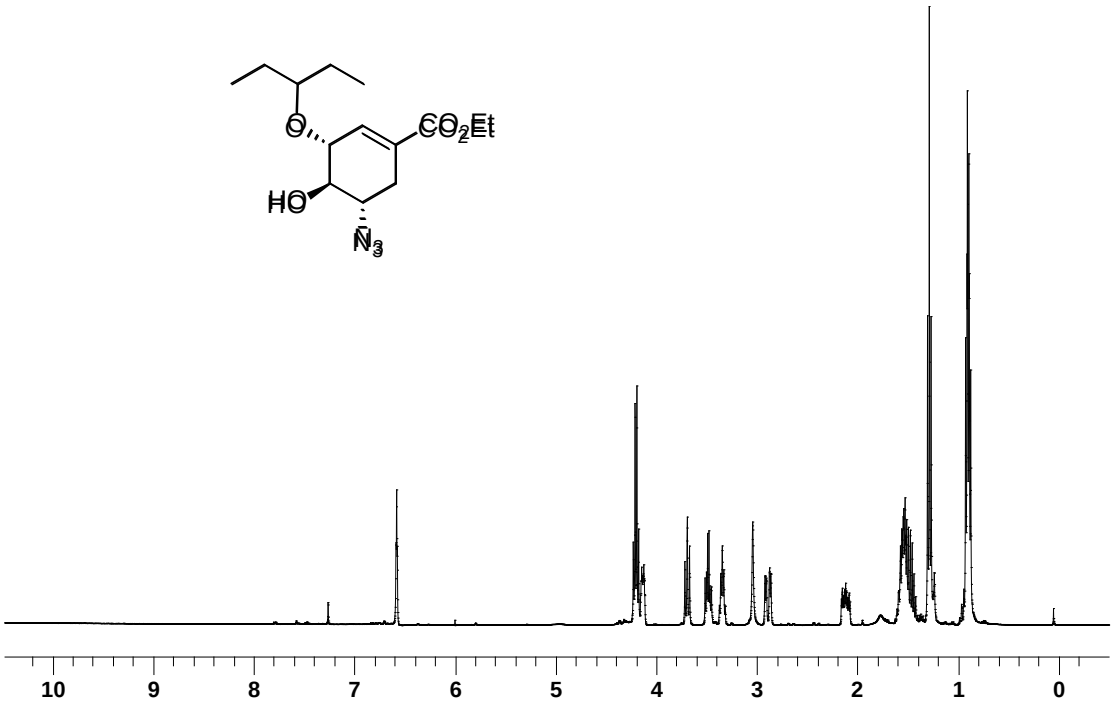

Figure A.37 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **126a**

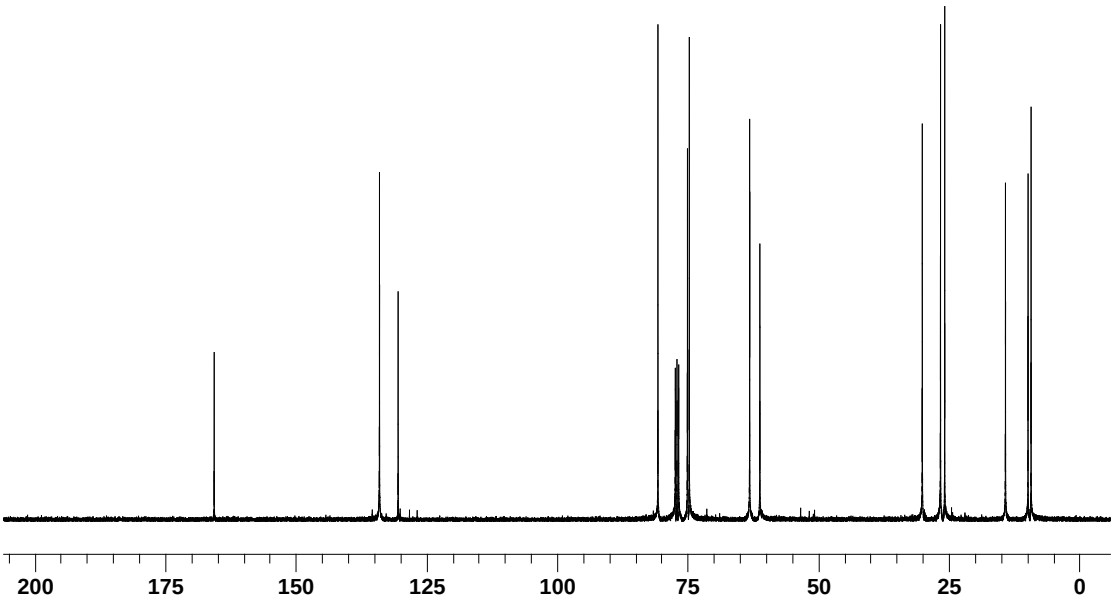

Figure A.38 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-azido-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **126a**

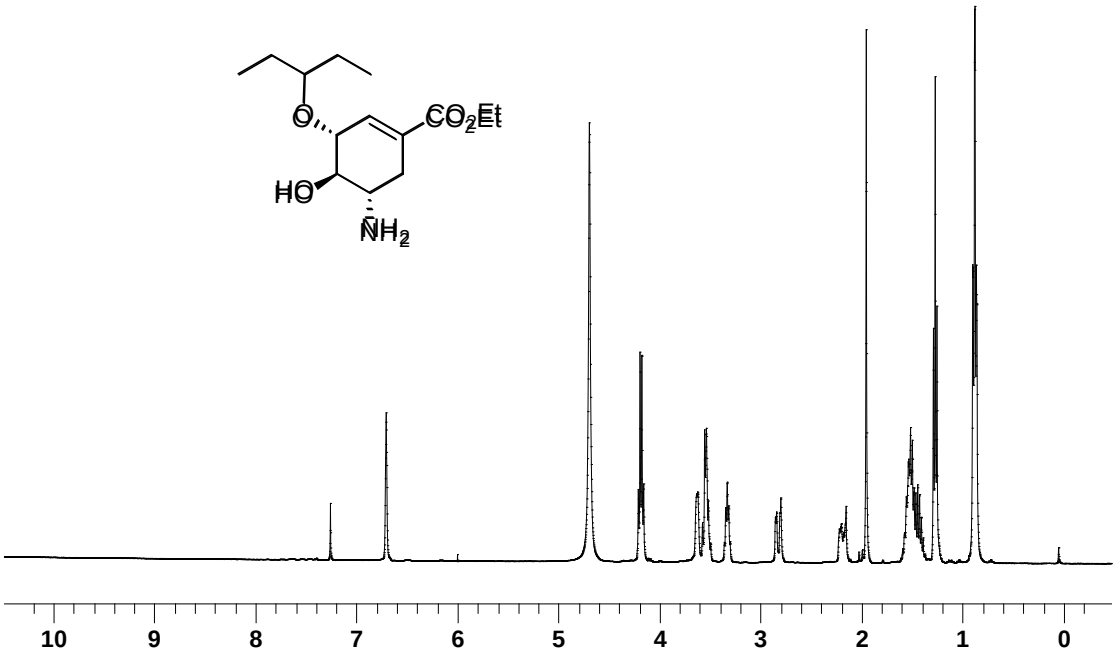

Figure A.39 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **130**

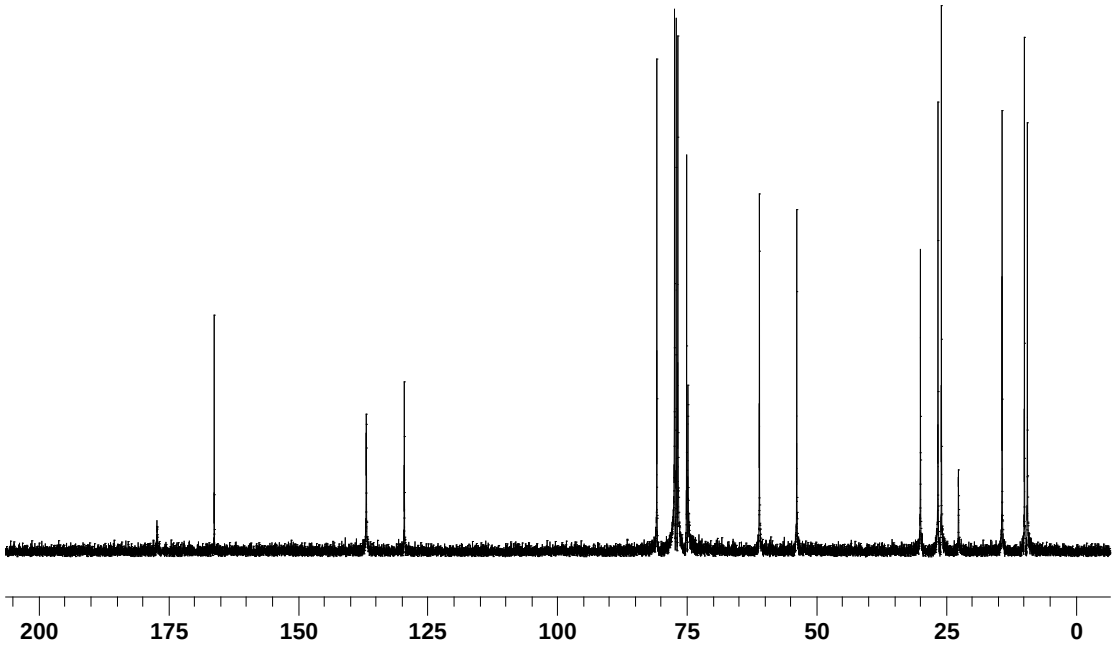

Figure A.40 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-amino-4-hydroxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **130**

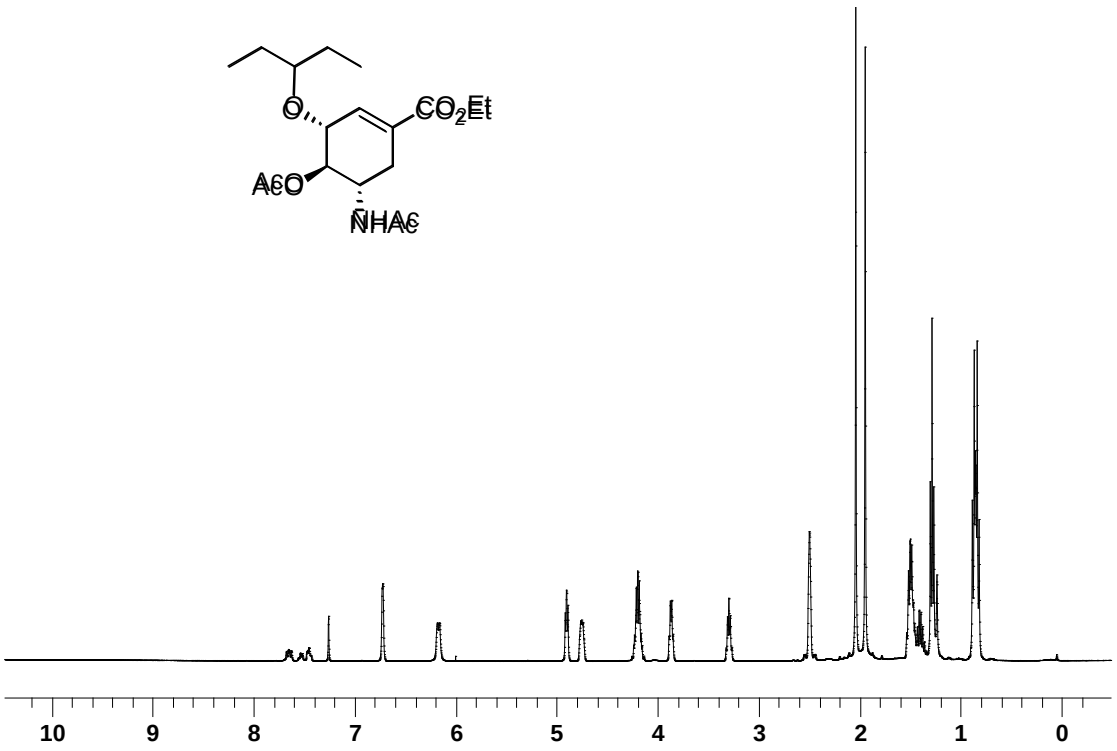

Figure A.41 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **131**

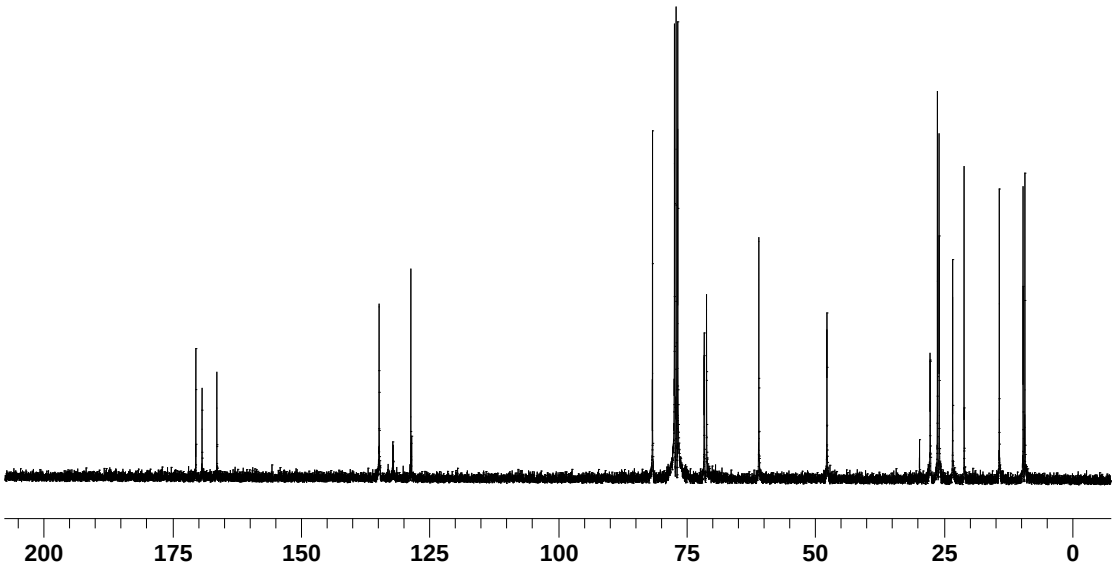

Figure A.42 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **131**

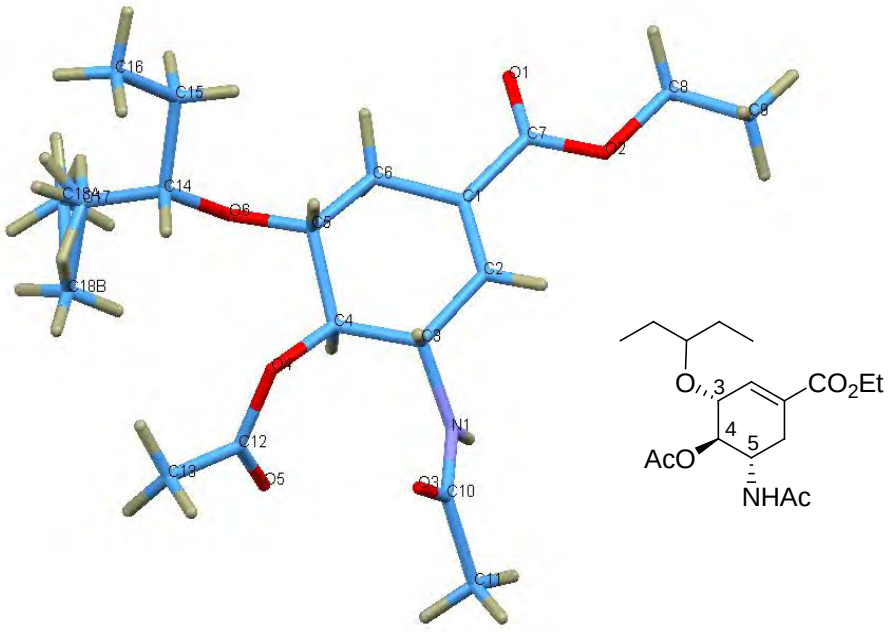

Figure A.43 X-ray crystallography of ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **131**

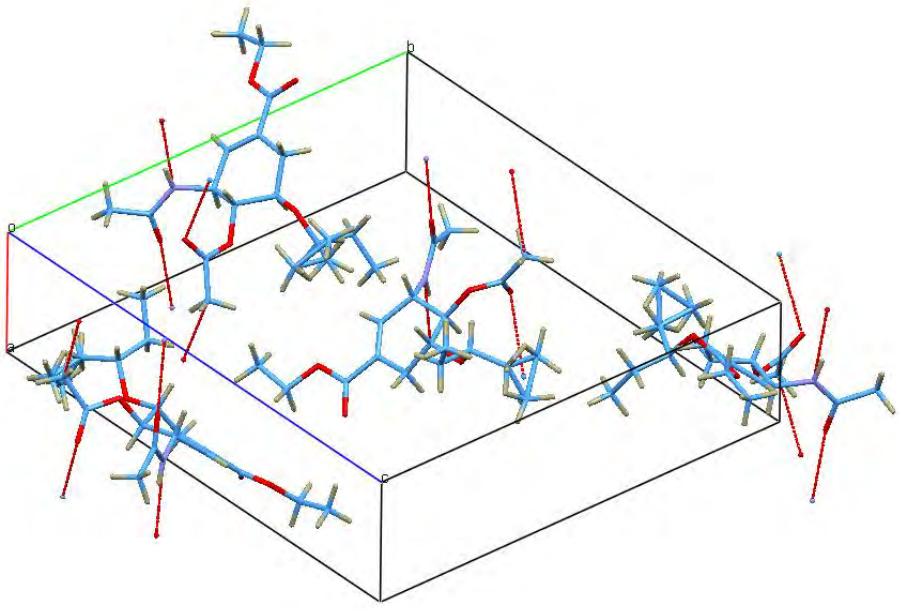

Figure A.44 X-ray crystallography of ethyl (3*R*,4*R*,5*S*)-5-acetamido-4-acetyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **131**

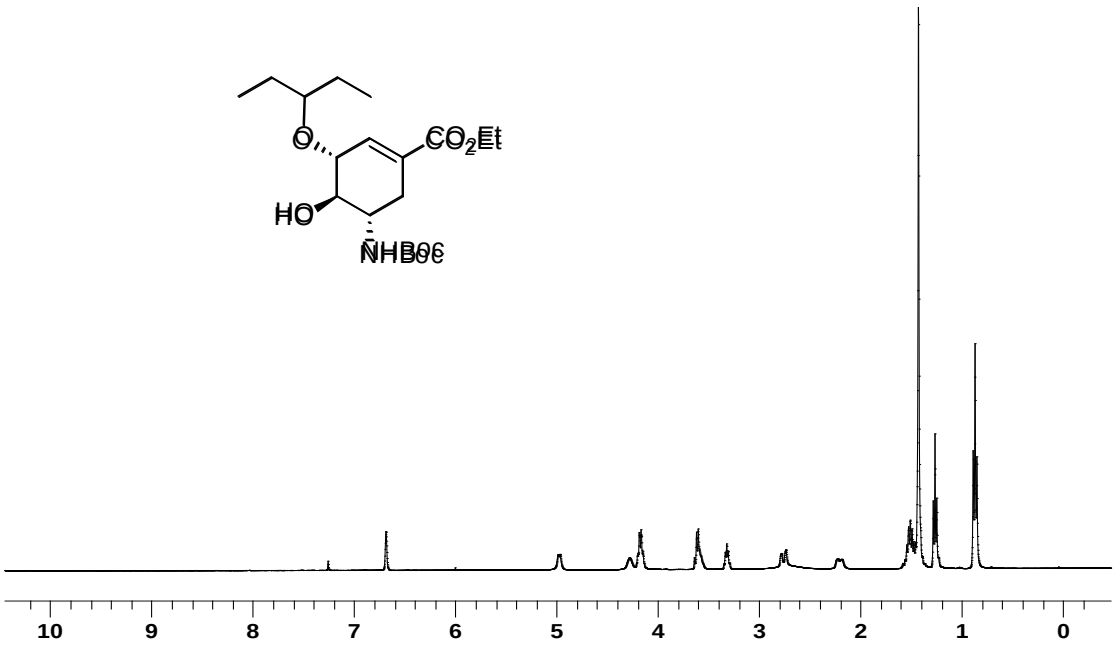

Figure A.45 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*R*)-5-*tert*-butoxycarbonylamino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate **132**

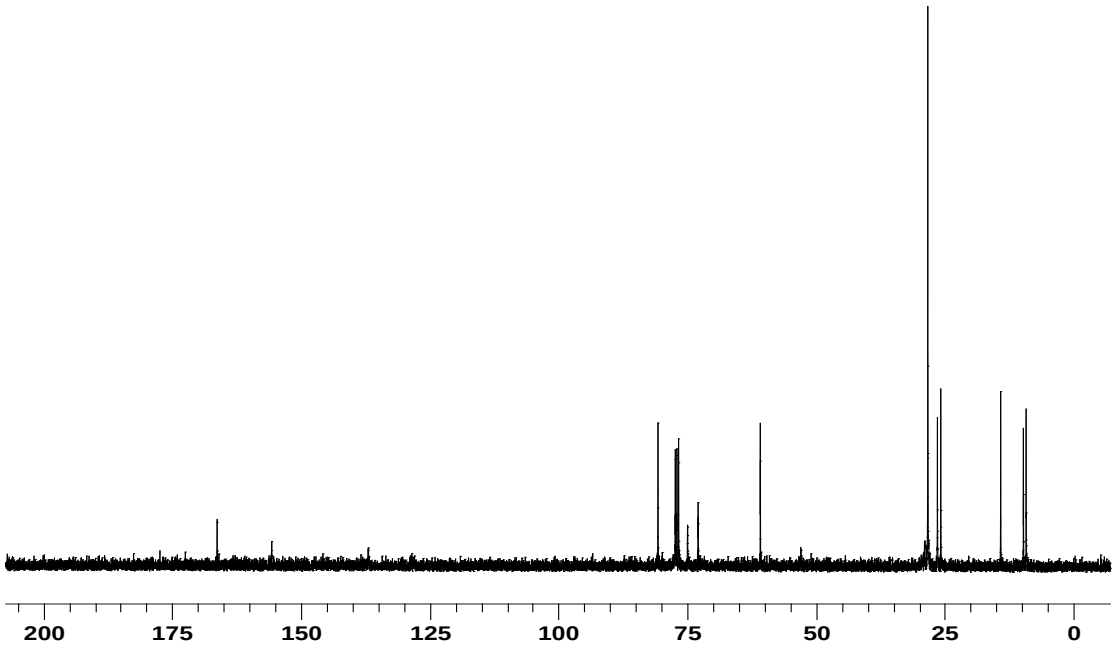

Figure A.46 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*R*)-5-*tert*-butoxycarbonylamino-3-(1-ethyl-propoxy)-4-hydroxy-1-cyclohexene-1-carboxylate **132**

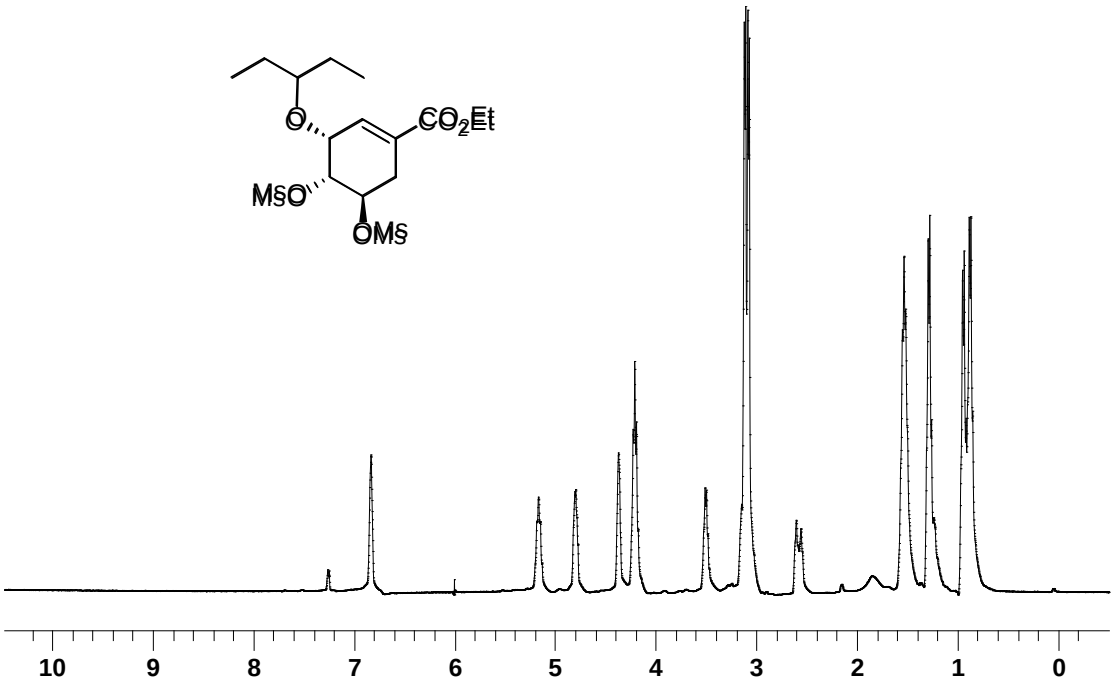

Figure A.47 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*S*)-4,5-bis(mesyloxy)-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **133**

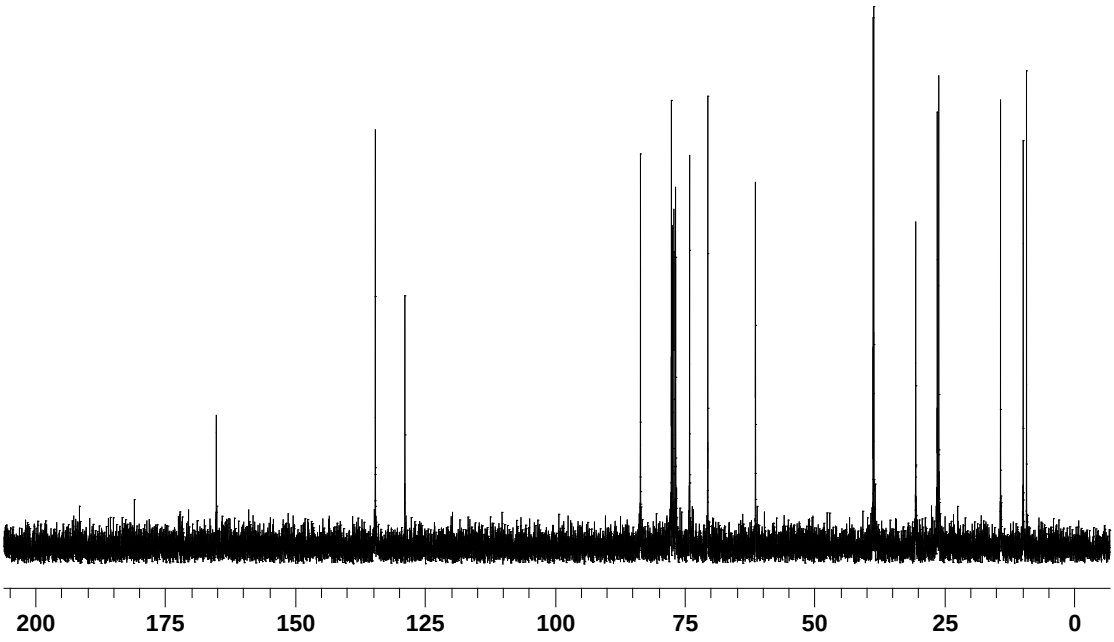

Figure A.48 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*S*)-4,5-bis(mesyloxy)-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **133**

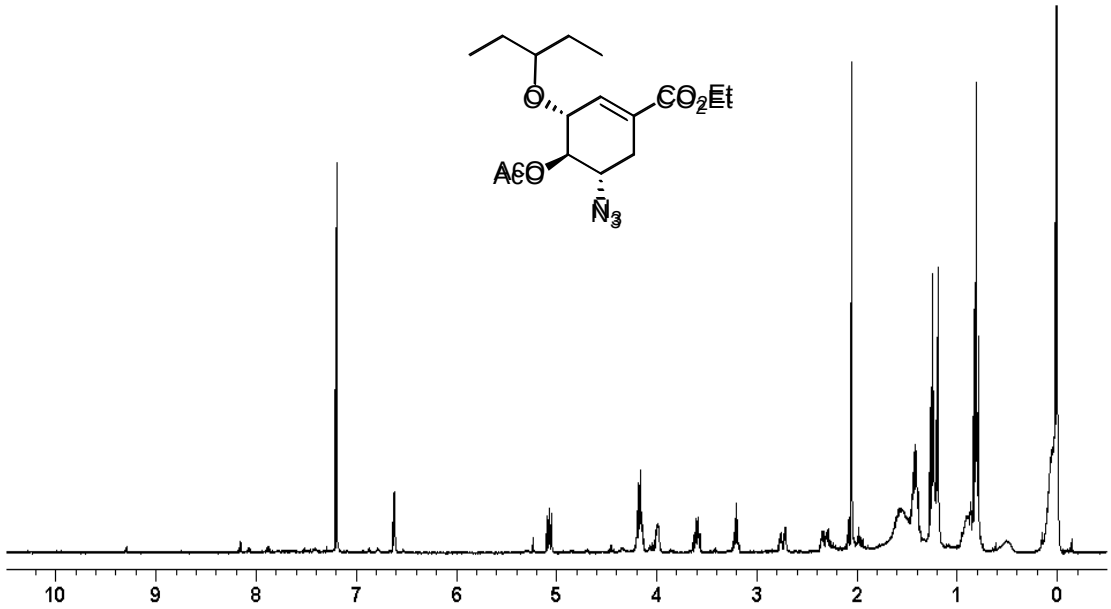

Figure A.49 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-dimesyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **134**

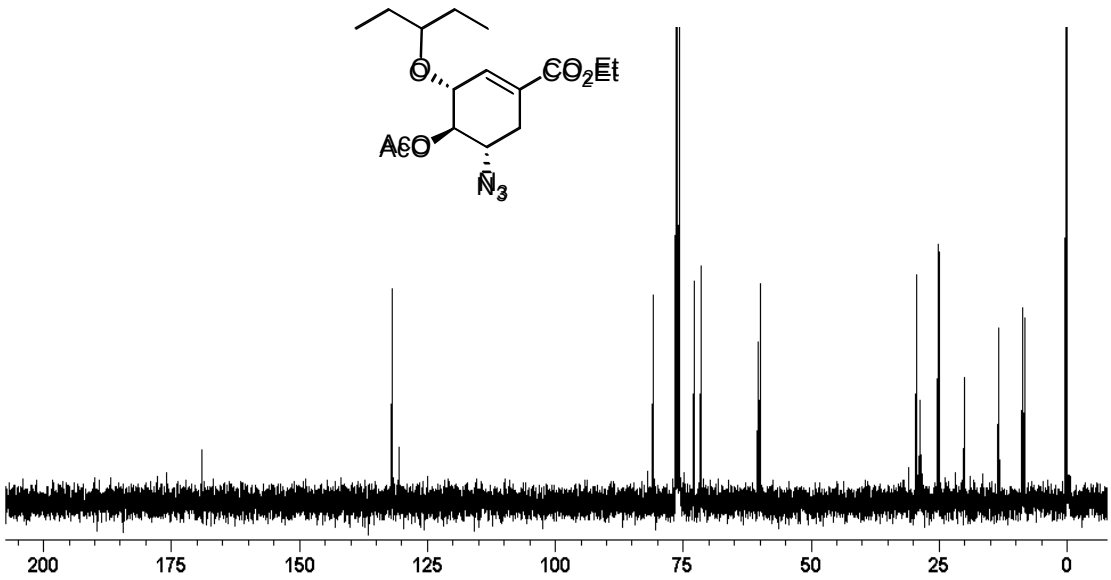

Figure A.50 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-dimesyloxy-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate **134**

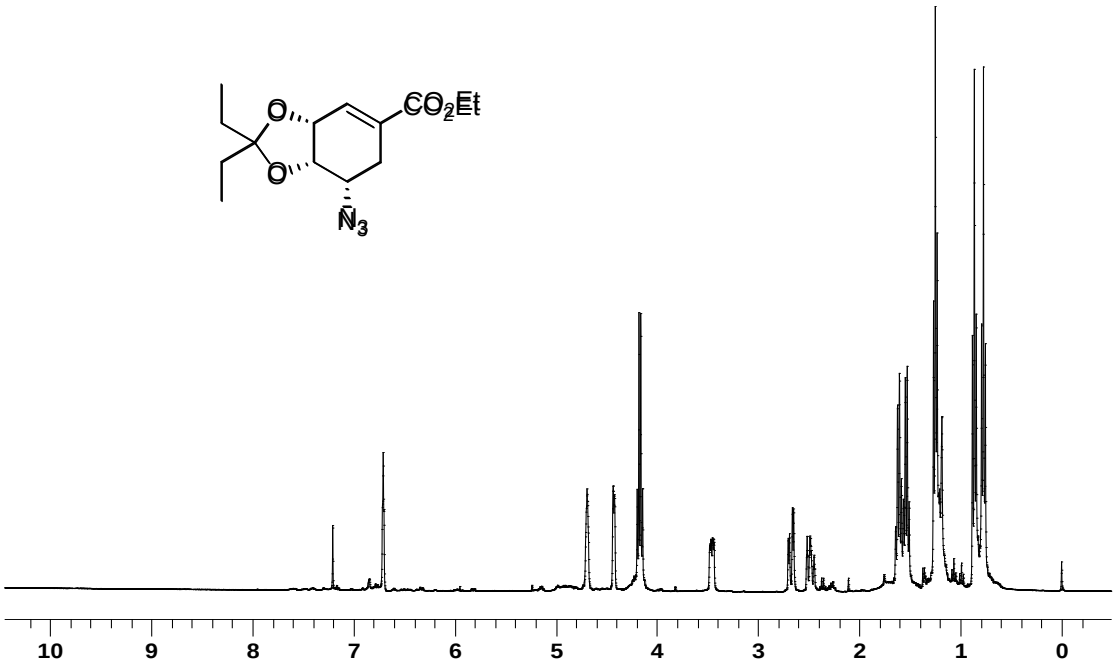

Figure A.51 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-azido-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-azido-shikimate) **135**

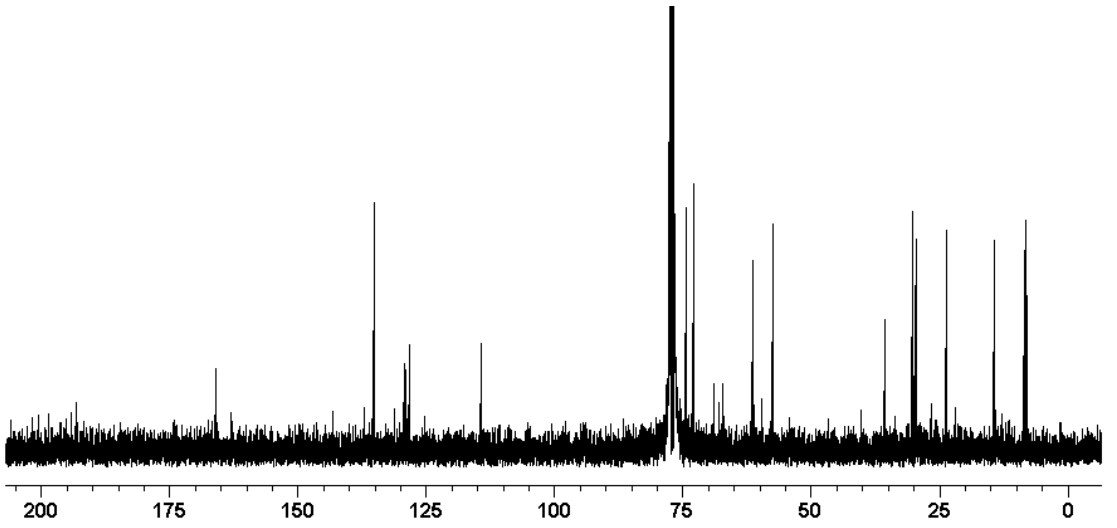

Figure A.52 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-azido-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-azido-shikimate) **135**

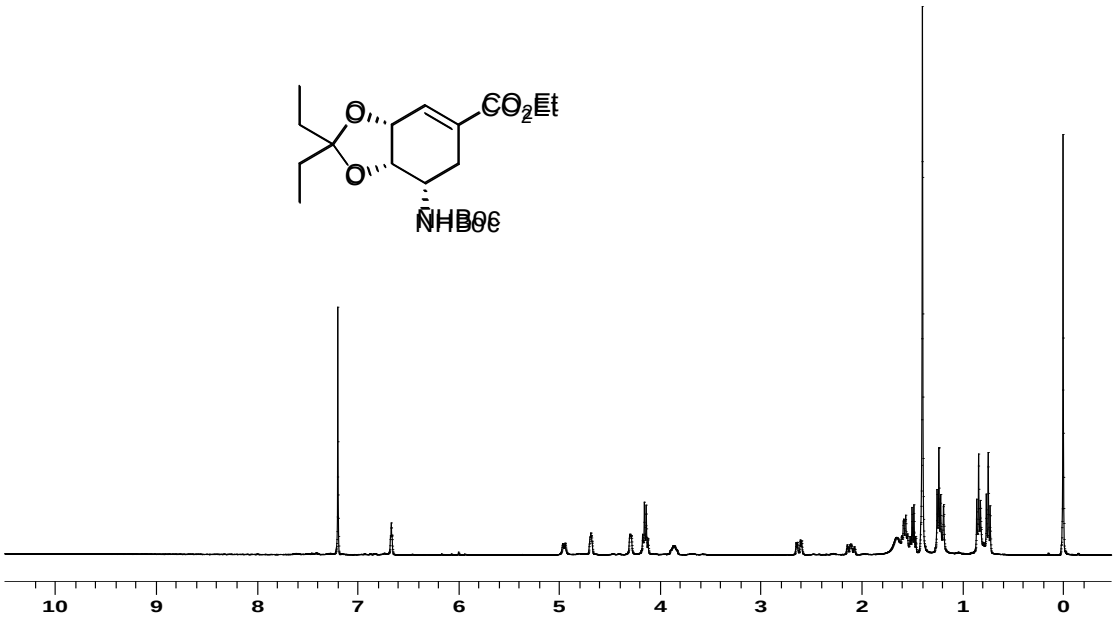

Figure A.53 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3a*R*,7*R*,7a*R*)-2,2-diethyl-7-*tert*-butoxycarbonylamino-3a,6,7,7a-tetrahydrobenzo[1,3]dioxole-5-carboxylate (ethyl 3,4-*O*-isopentylidene-5-*tert*-butoxycarbonylamino-shikimate **137**

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Presentation in Conference:

1. Niyomdecha M, Pattarapongdilok N, Sritana-anant Y, Vilaivan T, *Application of Mitsunobu reaction in the introduction of amino substituent of Oseltamivir phosphate,* 34st Congress on Science and Technology of Thailand, 31October-2 November, 2008, Queen Sirikit National Convention Center, Bangkok, Thailand.

2. Niyomdecha M, Sritana-anant Y, Vilaivan T, *Application of Mitsunobu reaction in the introduction of amino substituent of Oseltamivir phosphate,* The 4th Mathematics and Physical Sciences Graduate Congress**,** 17-19 December 2008, Faculty of Science, National University of Singapore.

3. Niyomdecha M, Sritana-anant Y, Vilaivan T, *Application of Mitsunobu reaction in the introduction of amino substituent of Oseltamivir phosphate,* Organic synthesis research unit mini-symposium 2009**,** 12 February 2009, Faculty of Science, Chulalongkorn University, Bangkok.