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SYNTHESIS OF DIAMINO MOIETIES OF OSELTAMIVIR

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วรพล สุธีพิเชฐภัณฑ์ : การสังเคราะห์ส่วนของไดแอมิโนของโอเซลทามิเวียร์. (SYNTHESIS OF THE DIAMINO MOIETIES OF OSELTAMIVIR) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. ยงศักดิ์ ศรีธนาอนันต์, 64 หน้า.

งานวิจัยนี้ได้ทำการปรับปรุงกระบวนการสังเคราะห์โอเซลทามิเวียร์ 9 ของบริษัทโรชตามเส้นทางของเอไซด์ โดยใช้อีพอกไซด์พรีเคอร์เซอร์เป็นสารตั้งต้น 18 การสังเคราะห์ประกอบด้วยปฏิกิริยาจำนวน 5 ขั้นตอน ปฏิกิริยาขั้นแรกคือเปิดวงอีพอกไซด์ได้ ของผสมเรจิโอไอโซเมอร์ของไฮดรอกซิลเอไซด์ 19 และ 43 ในปริมาณ 88% จากนั้นนำของผสมดังกล่าว มาทำปฏิกิริยารวมขั้นโดยทำปฏิกิริยารีดักชัน (ขั้นที่ 2) ด้วยไตรฟีนิลฟอสฟีนและปิดวงเป็นเอซิริดีน 20 แล้วเปิดวงอีกครั้ง (ขั้นที่ 3) ได้อะมิโนเอไซด์ 21 ในปริมาณ 50% หลังจากนั้นนำอะมิโนเอไซด์ 21 ทำปฏิกิริยาอะเซทิเลชันด้วยอะเซทิลคลอไรด์ (ขั้นที่ 4) ได้อะเซทามิโดเอไซด์ 22 ในปริมาณ 97% อะเซทามิโดเอไซด์ 22 จะถูกรีดิวซ์ด้วยไตรฟีนิลฟอสฟีน (ขั้นที่ 5) ได้โอเซลทามิเวียร์ 9 ในปริมาณ 99% ซึ่งกระบวนการสังเคราะห์ทั้ง 4 ขั้นตอนนี้จะมีปริมาณโอเซลทามิเวียร์ 9 โดยรวมคือ 42% ส่วนการศึกษาปฏิกิริยารวมขั้น พบว่าการสังเคราะห์ที่รวมขั้นที่ 1-3 ได้อะมิโนเอไซด์ 21 ในปริมาณ 55% จากอิพอกไซด์ 18 การสังเคราะห์ที่รวมขั้นที่ 1-4 ได้อะเซทามิโดเอไซด์ 22 ในปริมาณ 49% จากอิพอกไซด์ 18 สุดท้ายการสังเคราะห์ที่รวมขั้นที่ 1-5 พบว่าได้โอเซลทามิเวียร์ 9 ในปริมาณ 18% เมื่อคำนวณจากอิพอกไซด์ 18

ภาควิชา……เคมี…… ลายมือชื่อนิสิต…….

สาขาวิชา……เคมี…… ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก……..

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This research aimed to improve the azide route of the Roche synthesis of oseltamivir **9** using epoxide precursor **18** as a starting reagent. The synthesis consists of five reaction steps. The first reaction step is the ring opening reaction to provide the mixture of regioisomers of hydroxyl azide **19** and **43** in 88% yield. Both regioisomer could be converted through one-pot operation by *in situ* reduction with triphenylphosphine and ring closing to aziridine **20** followed by ring reopening with sodium azide to provide amino azide **21** in 50% yield from hydroxyl azide **9**. The acetylation of amino azide **21** with acetyl chloride provided acetamido azide **22** in 97% yield. The reduction of acetamido azide **22** with triphenylphosphine was produced free base of oseltamivir **9** in 99%. The overall yield of these 4-steps process was 42%. The combined synthesis of steps 1-3 towards amino azide **21** could be achieved from epoxide **18** in 55% yield. Further combined synthesis of step 1-4 towards acetamido azide **22** could also be accomplished from epoxide **18** in 49% yield. Finally, the free base oseltamivir **9** could be obtained through one-pot procedure of step 1-5 from epoxide **18** in 18% yield.

Department: Chemistry Student's Signature.....
Field of Study: Chemistry Advisor's Signature.....
Academic Year: 2011

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LIST OF ABBREVIATIONS

^{13}C-NMR	: carbon-13 nuclear magnetic resonance spectroscopy
^{1}H-NMR	: proton nuclear magnetic resonance spectroscopy
TLC	: thin layer chromatography
$^{\circ}$C	: degree celsius
$CDCl_3$	: deuterated chloroform
EtOAc	: ethyl acetate
DMF	: *N*,*N*-dimethylformamide
FT-IR	: Fourier-transform infrared spectrophotometer
min	: minute
mL	: milliliter
ppm	: parts per million (unit of chemical shift)
THF	: tetrahydrofuran
EtOH	: ethanol
MeOH	: methanol
δ	: chemical shift
Hz	: Hertz
J	: coupling constant
s	: singlet
d	: doublet
dd	: double of doublet
t	: triplet
q	: quartet
µ	: micro
m	: multiplet
NA	: neuraminidase
HA	: hemagglutinin
Ac_2O	: acetic anhydride
AcCl	: acetyl chloride
py.	: pyridine

CHAPTER I

INTRODUCTION

1.1 Influenza pandemics and oseltamivir synthesis

Avian and Swine influenza virus strains were known to spread and kill many animals and humans worldwide, mostly in Asia. These influenza type A infectious diseases were caused by RNA viruses of the family *Orthomyxoviridae* [1]. The massive spreads of influenza A virus subtype have been recorded five times. The Spanish flu (1918-1919) caused by the H1N1 type killed about 40-50 million people worldwide. The Asian flu (1957-1958) caused by the H2N2 type killed 1-2 million victims. The Hong Kong flu (1968-1969) caused by the H3N2 type killed approximately 700,000. In 2004 to present, The Bird flu caused by the H5N1 type killed about 335 people less than other type [2]. In 2009 to present, The Swine flu caused by H1N1 type killed 14,337 people [3].

These influenza A virus consist of two surface proteins; Hemagglutinin (H or HA) with known 16 subtypes, and Neuraminidase (N or NA) with known 9 subtypes and M2 proton channel. HA has the ability to bind viruses to target cells at terminal sialic acid receptor of cell membrane. NA has the ability to break the glycosidic bond of sialic acid to release the virus from the host cell surface to other cells **(Figure 1.1)**. This process frees 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 [1].

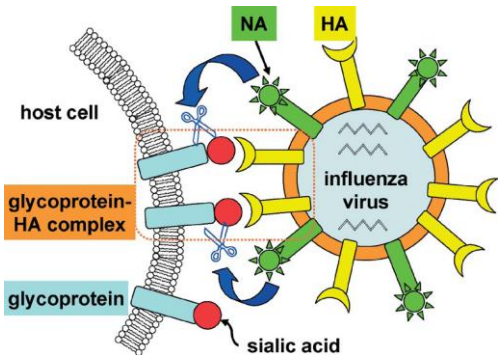


Figure 1.1 Schematic representation of an influenza virion budding from a host cell

1.2 Studies of Influenza Neuraminidase Inhibitor

NA is recognized as an important target for developing agents against influenza infection. NA can cleave sialic acid **1** via oxonium cation transition state **3** **(Figure 1.2)**

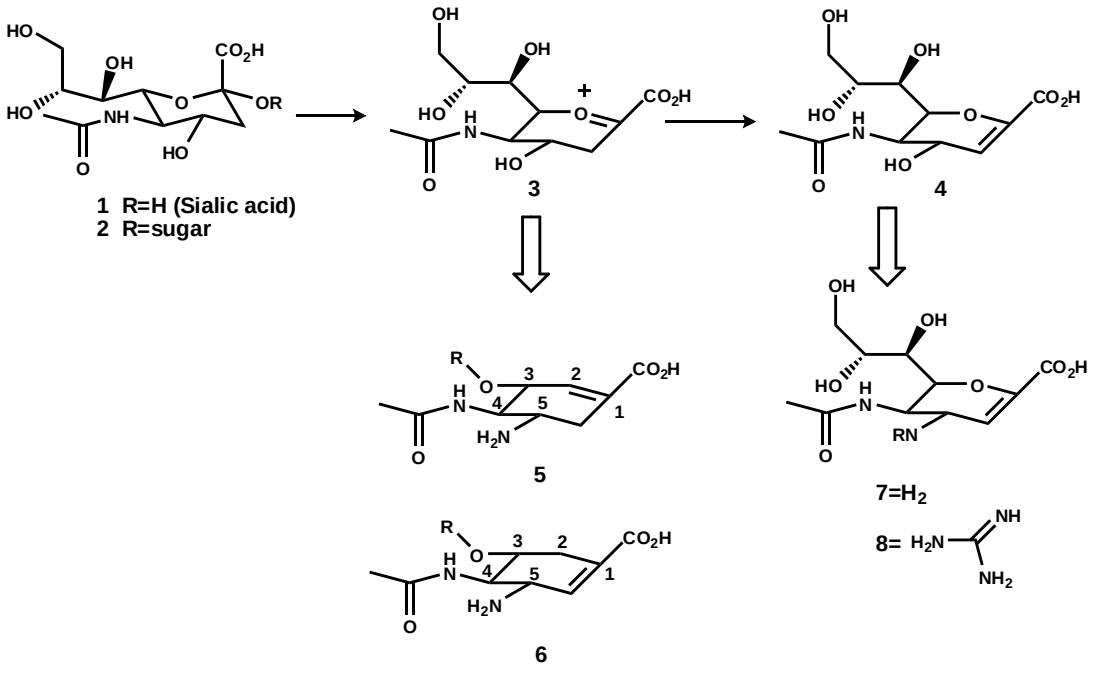


Figure 1.2 Rational design of carbocyclic transition state analog of sialic acid

On the basis of structural information generated from the X-ray crystallographic study of 2,3-dihydro-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 analouges, **5**, **6** and **9**, the 3-pentyloxy moiety have been identifield as an apparent optimal group at the C3 position as in **9 (Figure 1.3)** and shown in **Table 1.1**. [5]

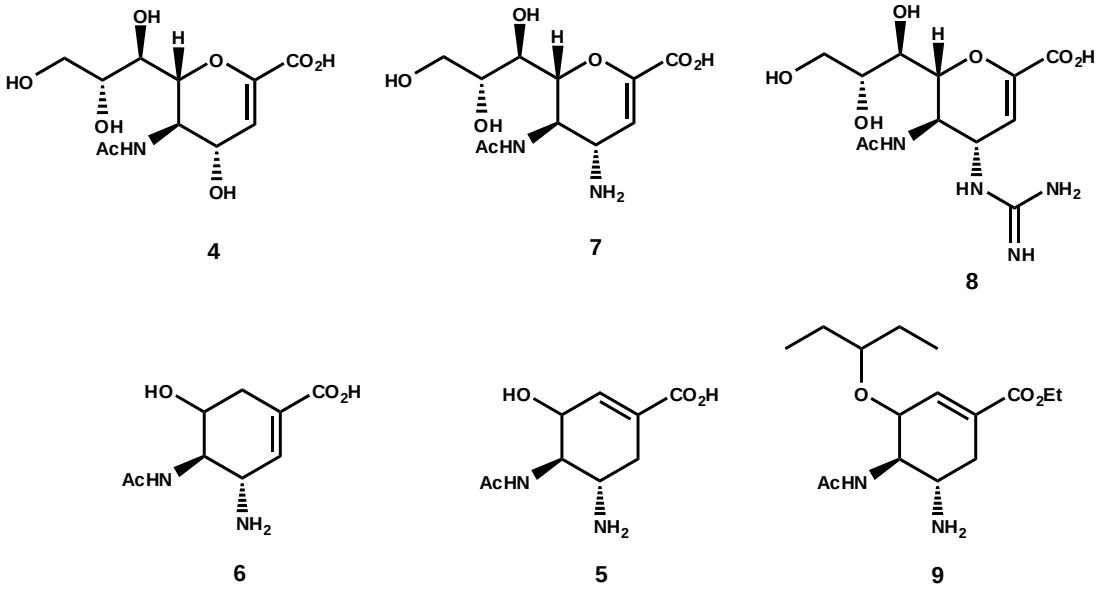


Figure 1.3 Structures of carbocyclic analogues

Table 1.1 Influenza NA inhibitory activity of carbocyclic analogues. [5]

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

In 1997, Kim and coworkers [5] had reported the design, structural analysis and synthesis of carbocyclic sialic acid analogues with potent anti-influenza activity, the crystal structure of the potent inhibitor **9** bound to NA was proved as shown in **Figure 1.4**

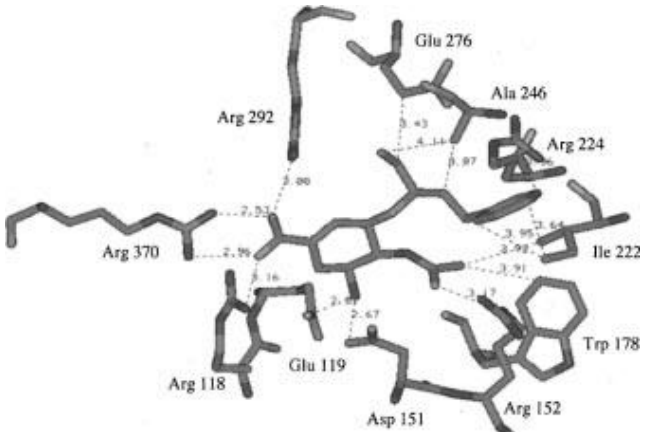


Figure 1.4 Interactions **9** to influenza neuraminidase in the x-ray structure

1.3 Treatment

Currently, two groups of anti-influenza drugs are classified based on its protein targets. Amantadine **11** and rimantadine **12** are M2 ion channel inhibitors. But they produce serious side effects such as hallucinations difficulty breathing, and seizures. They are not commonly used [1]. The neuraminidase inhibitors, including zanamivir (Relenza®) **8** and oseltamivir phosphate (Tamiflu®) **10** [6-7] have shown to be clinically effective against most strains of flu virus.

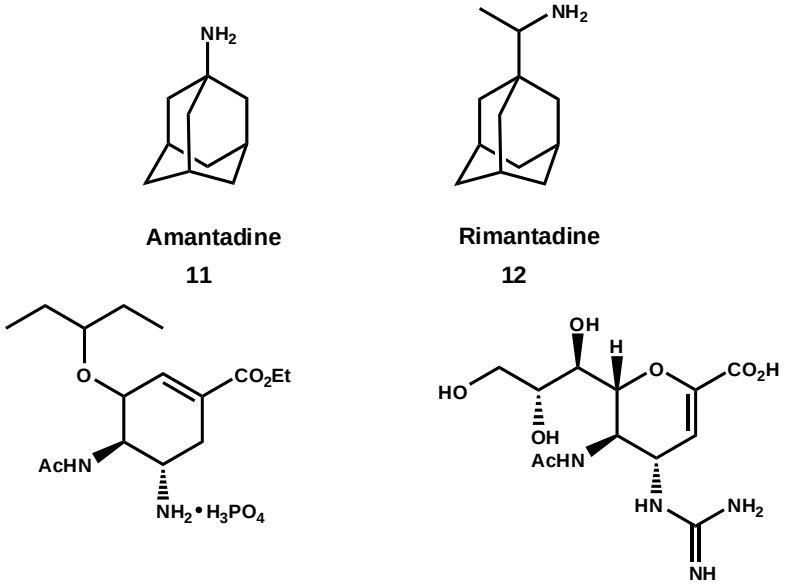


Figure 1.5 The four drugs available for treatment of influenza infections

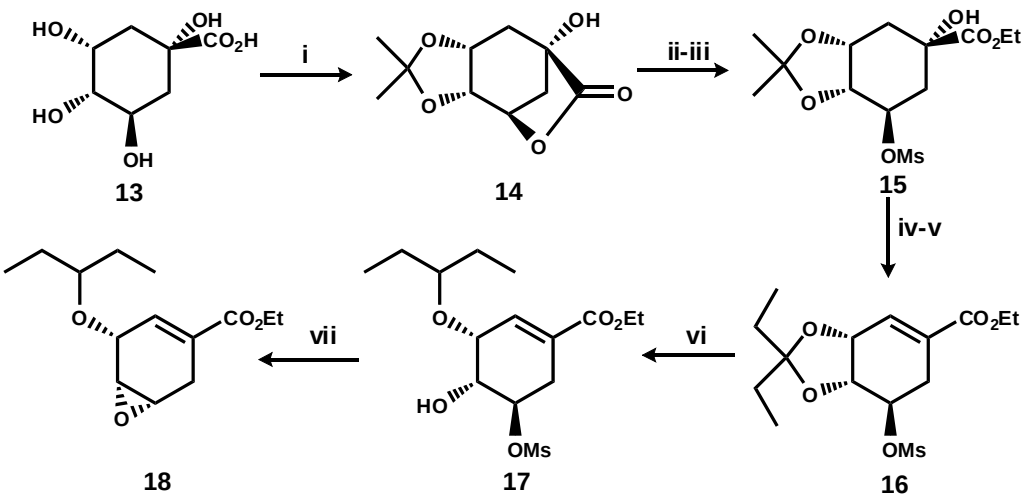
Zanamivir (Relenza®) **8** was discovered in 1990 by Glaxo Smith-Kline [6]. It has low bioavailability and must be used by inhalation, which is harmful to patients with respiratory disease. Oseltamivir phosphate (Tamiflu®) **10** is an orally administered antiviral drug. It was discovered at Gilead Sciences, co-developed with Roche, and approved into market in 1999, **(Figure 1.6)** [8].

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

Oseltamivir phosphate **10** is a neuraminidase inhibitor designed to bind with the neuraminidase protein, in which the protein receptor sites are almost identical in all common strains of influenza. Oseltamivir phosphate **10** was the first neuraminidase inhibitor in pill form that is effective in preventing the spread of both type 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 [8].

1.4 Synthesis of oseltamivir phosphate (Tamiflu®)

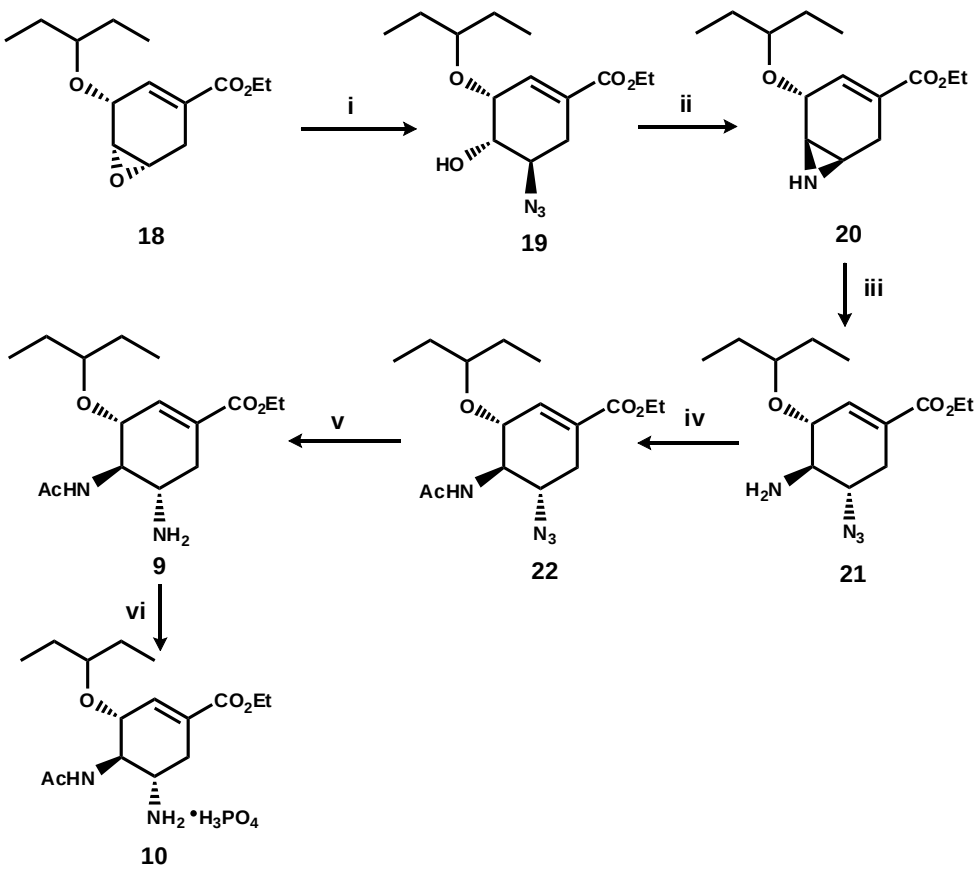
Oseltamivir phosphate was discovered in 1995 and revealed the details of synthetic process in 1997 by Gilead Science researchers [5,9]. This synthesis route started from (-)-quinic acid **13** that was converted to the acetonide with concomitant lactonization to give **14** [10-11]. The lactone was opened with sodium ethoxide and ethanol followed by mesylation with mesyl chloride to give the ethyl ester **15**. Dehydration of **15** with thionyl chloride and pyridine were followed by transketalization with 3-pentanone in the presence of catalytic perchloric acid to give **16**. The 3,4-pentylidine ketal **16** was reduced by trimethylsilyltriflate and borane dimethyl sulfide complex to give **17**. The product **17** was treated with potassium bicarbonate in aqueous ethanol to produce epoxide **18** in 60% yield from **16 (Scheme 1.1)**.



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: $KHCO_3$, ap.EtOH, 1h, 96%.

Scheme 1.1 The preparation of epoxide intermediate **18** for synthesis of Tamiflu® **10**

The proceeding toward large scale synthesis was also reported by this group [12]. Epoxide **18** was heated with sodium azide and ammonium chloride in aq. ethanol to produce azido alcohol **19** which was subjected to reductive cyclization with trimethyl phosphine to provide aziridine **20**. Ring opening of aziridine **20** with sodium azide, in the presence of ammonium chloride to produce an azidoamine. Afterwards, it was acetylated by acetic anhydride to produce acetamido azide **22** in 37% yield from epoxide **18**. Reduction of azide **22** by Raney nickel in ethanol followed by salt formation with phosphoric acid gave oseltamivir phosphate **10 (Scheme 1.2)**. This process can produce oseltamivir phosphate, but has too many steps and is inappropriate in mass production.

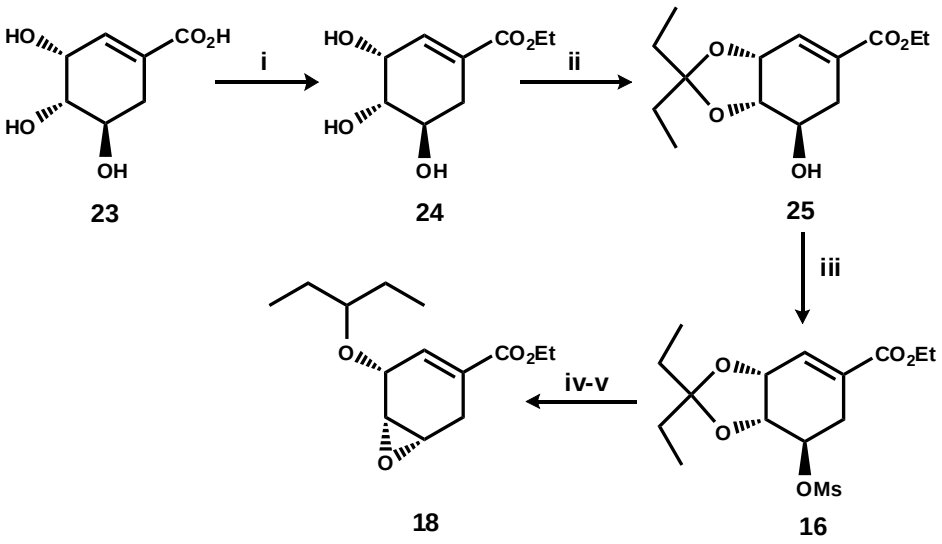


i: NaN_3, NH_4Cl, EtOH, H_2O, 70-75 ^oC, 12-18h, 86%, ii: Me_3P, MeCN, 35 ^oC, 97%, iii: NaN_3, NH_4Cl, DMF, 70-80 ^oC, 12-18h, iv: Ac_2O, $NaHCO_3$, hexane, CH_2Cl_2, 1h, 44% 2 steps, v: H_2, Ra-Ni, EtOH, 35 ^oC, 10-16h, vi: H_3PO_4, EtOH, 55-56 ^oC to rt 3-24h, 71%, 2 steps.

Scheme 1.2 The synthesis of Tamiflu® **10**

In 1999, the synthesis of oseltamivir phosphate has been developed and improved by Roche research team [8]. This synthetic route starts from (-)-shikimic acid **23**, either extracted from Chinese star anise or ginkgo leaves or from fermentation using a genetically engineered *E. coli* strain [13]. (-)-Shikimic acid **23** was refluxed with $SOCl_2$ in EtOH to produce ethyl shikimate **24**, which was treated with 3-pentanone in the presence of TfOH to give pentylidine ketal **25**. Mesylation of the hydroxy group with mesyl chloride and Et_3N produce the intermediate **16**. The epoxide intermediate **18** was occured from ring opening of 3,4-pentylidine ketal **16** by

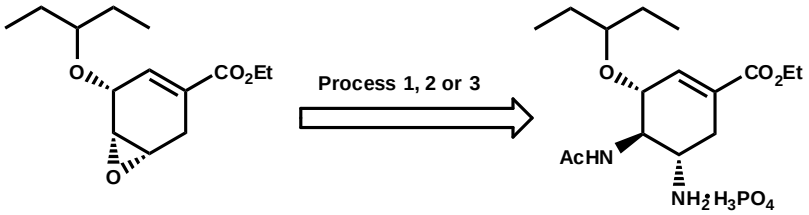
triethyl silane and $TiCl_4$ at -32 to -36 ^oC followed by basic treatment with $NaHCO_3$ in EtOH in 64% yield from **23**. **(Scheme 1.3)**



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 1.3 The preparation of epoxide intermediate **18**

Roche's researchers developed three processes for the synthesis of **10** from epoxide **18**. They used the same principle of changing epoxide **18** to aziridine **20**, followed by ring opening by a nitrogen nucleophile that is compatible to produce oseltamivir phosphate **10**, with correct stereochemistry. The first and shorter azide route was reported by Karpf et al. [14], which is currently used in the industrial production while the allylamine and tert-butylamine [15] routes were later reported as the alternative to the use of hazardous azide reagents **(Scheme 1.4)**.



18 **10**

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

Scheme 1.4 Three Synthetic Routes of oseltamivir phosphate **10** from epoxide **18**

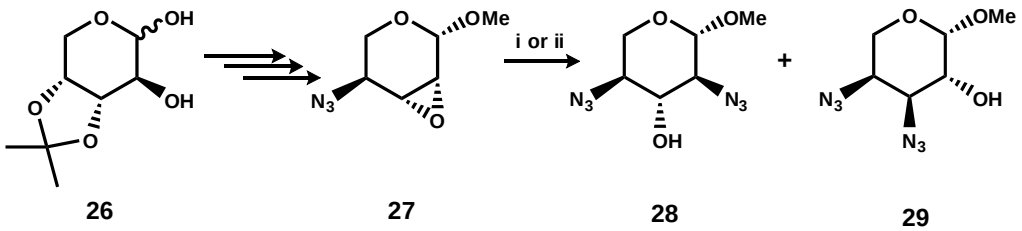
In previous important reports to optimize the synthesis of oseltamivir phosphate, there has been other methods of synthesis without using (-)-shikimic acid **23.** In 2004, Karpf and coworkers [16] used Diels-Alder reaction between furan and ethyl acrylate as key step in synthesis of oseltamivir phosphate **10**, which has overall yield of 17%. Corey et al [17-18] used Diels-Alder reaction between butadiene and trifluoroethyl acrylate to produce core structure, which has 10 steps to oseltamivir phosphate **10**. Shibasaki and coworkers have reported four different processes by four substrates: ring-opening reaction of meso-aziridine [19], allylic rearrangement with amine compound [19], Diels-Alder reaction and Curtius rearrangement with 1,3-butadiene and fumaryl chloride [21-22], Diels-Alder reaction with 1,3-butadiene and dimethylfumarate by barium-catalyzed [23]. In 2007, Fang and coworkers [24] used sugar compound D-xylose as the substrate to synthesize oseltamivir phosphate **10** in 15% yield from 16 steps. In 2008, Trost and coworkers [25] reported a short synthesis of oseltamivir **9** in 8 steps starting from lactone via Pd-catalyzed, gave oseltamivir **9** in 30% overall yield. In 2009, Shi and coworkers [26] reported two synthetic routes to oseltamivir phosphate **10** that relied on (-)-shikimic acid **23** as the substrate. The first route have 13 steps with 40% overall yield, which was optimized into the second route in 47% overall yield of 8 steps. In 2010, Osato and coworkers [27] used D-ribose as the substrate and Bernet-Vasella reaction as the key step to synthesize oseltamivir phosphate **10** in 7 steps. In 2011, Shi and coworkers [28] used thionyl

chloride to produce 3,4-cyclic sulfite intermediate to optimize the synthesis of oseltamivir phosphate **10** in 64% overall yield in 7 steps.

1.5 The azide route synthesis of oseltamivir phosphate 10

The synthesis of oseltamivir phosphate **10** have used green chemistry as well. From Roche's synthetic routes toward oseltamivir phosphate **10** started from epoxide **18** [14-15], the azide route was shorter than allylamine and tert-butylamine routes, which saved time and decreased auxiliary substances in separation of products.

In 2005, Popsavin and coworkers [30] studied the regiochemistry of epoxide ring opening. They used 3,4-*O*-isopropylidene-D-arabinose **26** as substrate to produce epoxide intermediate **27**, which reacted with sodium azide in dimethyl formamide. The substitution gave **28** as the major product and **29** as the minor product. This experiment showed that the use DMF as a solvent in azide substitution gave good yield, but it give minor product, which the attack of azide at the other side of epoxide **27 (Scheme 1.5)**.



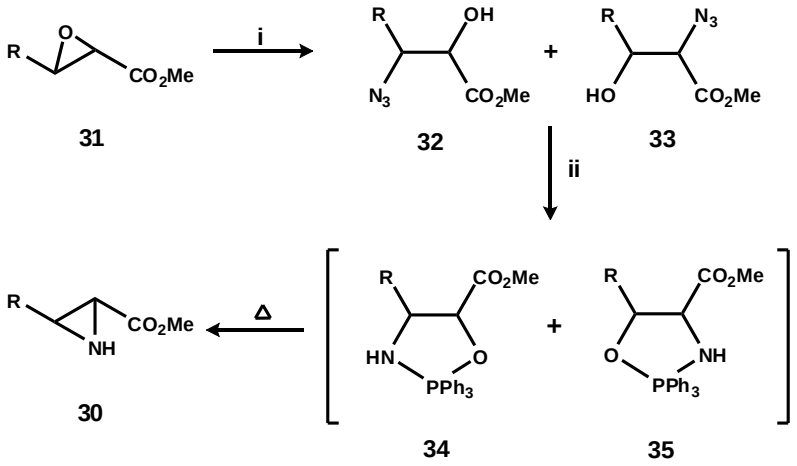
26 **27** **28** **29**

i: NaN_3, DMF, 105 ^oC, 21 h, 48% of 28, 8% of 29.
ii: Me_3SiN_3, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 23 h, 40% of 28, 8% of 29.

Scheme 1.5 Epoxide ring opening by azide substitution

In 1989, Zwanenburg and coworkers [31] reported a convenient method for the synthesis of optically active 1*H*-aziridine-2-carboxylic acids (ester) **30** starting from glycidic esters **31**. The glycidic esters were treated with sodium azide for S_N2-

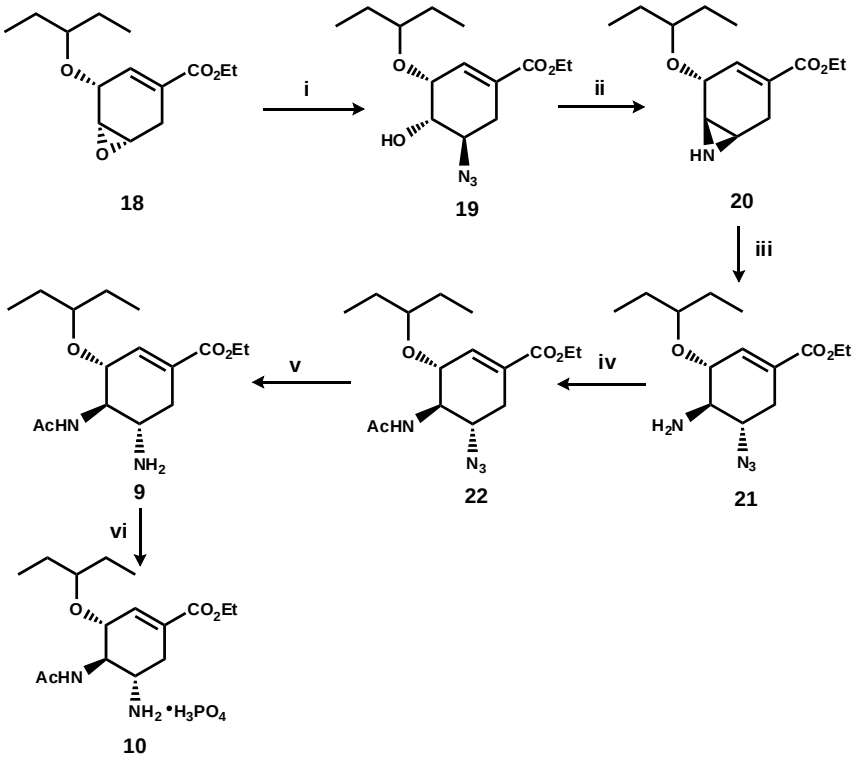
type reaction that led to mixtures of the isomeric azido alcohols **32** and **33**. In the later step the azido alcohols **32** and **33** were treated with triphenylphosphine to produce **34** and **35** via the Staudinger reaction. The reaction was performed in DMF or acetonitrile as the solvent and heating to 80 ^{o}C for a few hours to provide the aziridines **30** in good yields without isolation of the intermediate oxaphospholidines **34**, **35 (Scheme 1.6)**.



Scheme 1.6 Synthesis of 1H-aziridine-2-carboxylic acids (ester) **30**

There are two important reports on the conversion of the epoxide **18** to oseltamivir phosphate **10** by azide process. Both works converted epoxide **18** to azido alcohol **19** by ring opening with NaN_3 and NH_4Cl in EtOH **(Scheme 1.7)**. For the following reductive cyclization of **19**, Rohloff and coworkers [12] used trimethylphosphine, but Kent and coworkers [32] used triphenylphosphine to provided the aziridine **20**. The intermediate **20** was reacted with NaN_3 and NH_4Cl in DMF to give aminoazide **21**. Acetylation of aminoazide **21** with acetic anhydride, produced acetylaminoazide **22**. For the reduction of acetylaminoazide, Rohloff and

coworkers used Raney Ni, but Kent and coworkers used Lindlar catalyst with hydrogen gas to provide oseltamivir **9**. The crystallization of oseltamivir phosphate **10** was occurred by addition of 85% phosphoric acid. Triphenylphosphine is more readily available, stable non-volatile and easier to use than trimethylphosphine.



Rohloff i: NaN_3, NH_4Cl, EtOH, H_2O, 70-75 ^oC, 12-18h, 85%, ii: Me_3P, MeCN, 50 ^oC, 2h, 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, 10-16h, vi: H_3PO_4, EtOH, 55-56 ^oC to rt 3-24h, 71%, 2 steps.

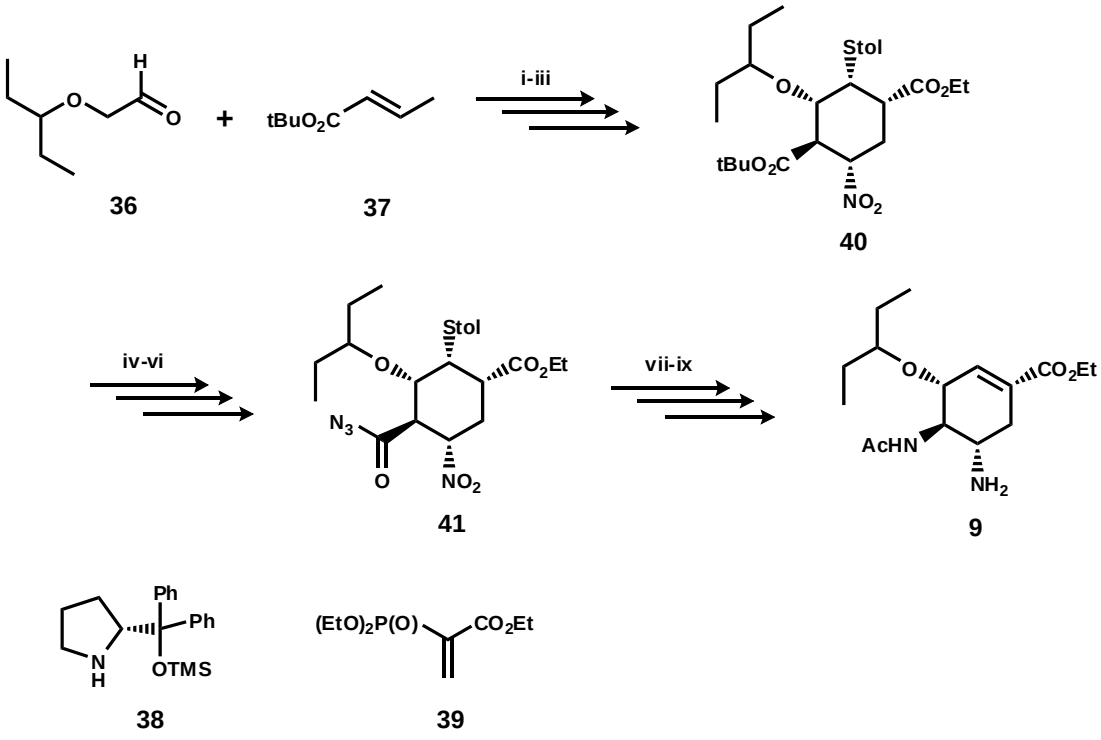
Kent i: NaN_3, NH_4Cl, EtOH, H_2O, 70-75 ^oC, 8h, 100%, ii: PPh_3, MeCN, reflux, 96%, iii: NaN_3, NH_4Cl, DMF, 80-85 ^oC, 5h, iv: Ac_2O, $NaHCO_3$, hexane, CH_2Cl_2, 1h, 55% 2 steps, v: H_2, Lindlar catalyst, EtOH, 18h, 98%, vi: H_3PO_4, EtOH, 0 ^oC,12h, 75%.

Scheme 1.7 Two reports on the Synthesis of oseltamivir phosphate **10** by azide route

1.6 One-pot synthesis

The one-pot synthesis is a procedure to enhance the efficiency of a chemical reaction whereby a reactant is subjected to several consecutive chemical reactions within just one reactor. The obvious advantage of one-pot synthesis is to avoid a lengthy separation and purification of the intermediate compounds, saving time and resources.

In 2009, Hayashi and coworkers [33] reported the three one-pot operations of oseltamivir **9** synthesis, starting from alkoxyaldehyde **36** and nitroalkene **37**. The first one-pot operation started from alkoxyaldehyde **36** and nitroalkene **37** reacted with diphenylprolinol silyl ether **38** and vinylphosphonate **39** to provided ethylcyclohexanecarboxylate **40** in 70% yield. The second one-pot operation was to covert **40** to acyl azide **41** without purification. The third one-pot operation transformed **41** via Curtius rearrangement and retro-Michael reaction to oseltamivir **9** in 82% yield from ethylcyclohexane- carboxylate **40 (Scheme 1.8)**.

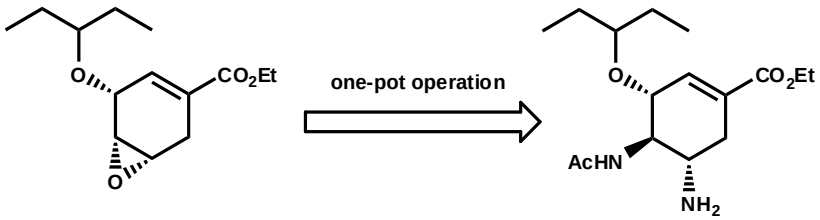


i: 38, $ClCH_2CO_2H$, CH_2Cl_2, rt, 40min, ii: 39, Cs_2CO_3, 0 ^oC, 3h, evaporation, then EtOH, rt, 15min, iii: tolSH, EtOH, -15 ^oC, 36h, 70% from 3steps iv: TFA, CH_2Cl_2, 2h, evaporation, v: $(COCl_2)$ cat, DMF, CH_2Cl_2, 1h, evaporation, vi: NaN_3, H_2O/acetone, 0 ^oC, 20min, vii: AcOH, Ac_2O, rt, 49h, evaporation, viii: Zn, TMSCl, EtOH, 70 ^oC, 2h, ix: NH_3, then K_2CO_3, EtOH, 6h, 82% from 40

Scheme 1.8 Synthesis of oseltamivir **9** in three one-pot operations

1.7 Objective

The goal of this work is to decrease the steps of the synthesis of oseltamivir **9** from epoxide procedure **18** by combining somw reactions together into one-pot synthesis **(Scheme 1.9)**, modifying from the existing azide route synthesis.



18 **9**

Scheme 1.9 One-pot operation of oseltamivir **9** started from epoxide **18**

CHAPTER II

EXPERIMENTAL SECTION

2.1 Instrumentation

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

The ^{1}H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ 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).

Melting point (m.p.) were determined with a Stuart Scientific Melting Point SMP1 (Bibby Sterlin Ltd., Staffordshire, UK).

The mass spectra were recorded on Mass Spectrometer: Bruker Daltonik GmbH micrOTOF-Q II. Samples were dissolved in a solvent and directly injected into the Mass Spectrometer.

2.2 Chemicals

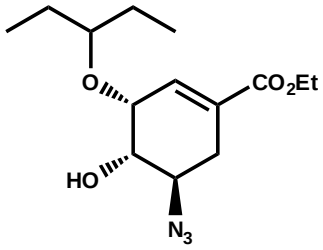
Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 F_{254}) (Merck KgaA, Darmstadt, Germany).

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). Solvents used as mobile phase for column chromatography and reaction were distilled from commercial grade solvents.

All other chemicals and solvents were used as purchased unless otherwise noted.

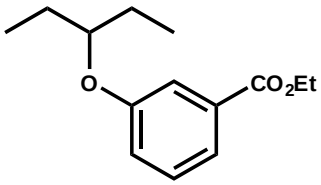
2.3 Synthetic procedures

2.3.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 19



19

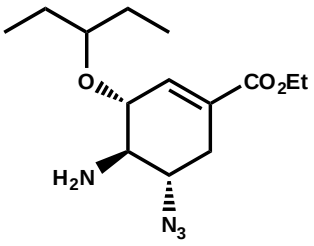
Many synthetic conditions were surveyed including equivalence of sodium azide (1.4-6.5), temperature (50-85 ^{o}C), solvent (acetone:H_2O (5:1), CH_3CN:H_2O (5:1), DMF, EtOH), reaction time (24-96 h) and extraction procedure. The optimum condition was as follow : a solution of ethyl (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **18** (0.50 g, 1.96 mmol) in EtOH (12 mL) was added sodium azide (0.64 g, 9.83 mmol) and ammonium chloride (0.53 g, 9.83 mmol). The reaction mixture was heated at 50 ^{o}C for 18 h and then added hexane (12 mL) to precipitate the remaining sediment and filtered. The filtrate was concentrated in vacuo. The product is brown oil (0.54 g, 88%) [12, 32], R_f on TLC chromatogram = 0.57 (40% ethyl acetate in 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 (-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).



42

In the experiments using DMF as the solvent, one of the by-products were found to be the aromatic compound **42** (yellow oil) [34]. ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.88 (t, *J*=8.0 Hz, 6H, -C($CH_2C\underline{H}_3$)$_2$, 1.31 (t, *J*=8.0 Hz, 3H, -$CH_2C\underline{H}_3$), 1.61 (m, 4H, -C($C\underline{H}_2CH_3$)$_2$, 4.11 (quint, *J*=6.0 Hz, 1H, -C$\underline{H}$(CH_2CH_3)$_2$), 4.29 (q, *J*=8.0 Hz, 2H, -$C\underline{H}_2CH_3$), 7.00 (dd, J_1=1.24 Hz, J_2=1.24 Hz, 1H, -CH=C$\underline{H}$-C-, 7.23 (t, *J*=7.92 Hz, 1H, =CH-C$\underline{H}$=CH-), 7.49 (s, 1H -C$\underline{H}$=C-), 7.52 (dd, J_1=0.88 Hz, J_2=0.92 Hz, -C=C$\underline{H}$-CH=).

2.3.2 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 21

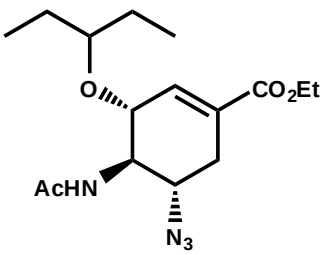


21

Many synthetic conditions were surveyed including equivalence of triphenylphosphine (0.8-1.05), temperature (50-80 ^{o}C), reaction time (2-24 h) and extraction procedure. The optimum condition was as follow: a solution mixture of regioisomers of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclo hexene-1-carboxylate **19** (0.97 g, 3.30 mmol) in DMF (12 mL) was added triphenylphosphine (0.907 g, 3.45 mmol), ammonium chloride (0.016 g, 0.29 mmol). The reaction mixture was heated at 70 ^{o}C for 4 h to provide aziridine intermediate **20**.

Sodium azide (0.302 g, 4.60 mmol) and ammonium chloride (0.208 g, 3.90 mmol) were added to the mixture at reflux condition for 3 h. The reaction mixture was extracted with ethyl acetate (30 mL) and water (4x20 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude mixture of brown oil and white solid was purified by column chromatography on silica gel, eluting with 40% ethyl acetate in hexane to provide compound **21** as yellow oil (0.493 g, = 50.4 %) [12, 32], R_f on TLC chromatogram = 0.375 (40% ethyl acetate in hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.91 (t, *J*=7.4 Hz, 6H, -C($CH_2C\underline{H}_3)_2$), 1.28 (t, *J*=7.2 Hz, 3H, -$CH_2C\underline{H}_3$), 1.54 (m, 4H, -C($C\underline{H}_2CH_3)_2$), 1.69 54 (br, 2H, -NH_2), 2.21-2.37 (m, 1H, -$C\underline{H}_2$-), 2.82-2.89 (m, 3H, -$C\underline{H}_2$- and –$C\underline{H}NH_2$), 3.37 (quint, *J*=5.8 Hz, 1H, -$C\underline{H}(CH_2CH_3)_2$), 3.40-3.52 (m, 1H, -$C\underline{H}$-$N_3$), 3.90 (m, 1H, -$C\underline{H}$-O-), 4.20 (q, *J*=7.2 Hz, 2H, -$C\underline{H}_2CH_3$), 6.82 (m, 1H, -$C\underline{H}$=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 (-$CH_2\underline{C}H_3$), 25.6 (-C($\underline{C}H_2CH_3)_2$), 26.4 (-C($\underline{C}H_2CH_3)_2$), 29.2 (-$\underline{C}H_2$-), 56.1 (-$\underline{C}$H-N_3), 61.0 (-$\underline{C}H_2CH_3$), 61.3 (-$\underline{C}$H-NH_2), 78.1 (-$\underline{C}$H-O-), 81.0 (-$\underline{C}H(CH_2CH_3)_2$), 130.3 (-CH=$\underline{C}$-), 135.0 (-$\underline{C}$H=C-), 165.9 (-$\underline{C}$=O).

2.3.3 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 22

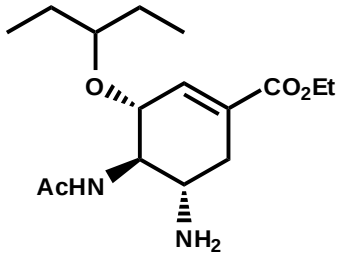


22

Many synthetic conditions were surveyed including reagent (AcO_2, AcCl), equivalence of Ac_2O (1.5-3.0), solvent (CH_2Cl_2, DMF), reaction time (2-24 h) and

extraction procedure. The optimum condition was as follow : a solution of ethyl (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **21** (0.1623 g, 0.54 mmol) in DMF (12 mL) was added acetyl chloride (1.16 mL) and pyridine (0.43 mL) and stirred at room temperature for 2 h. The reaction was extracted with ethyl acetate (30 mL) and water (5x30 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The product is white solid of acetamido azide compound **22** as white solid (0.18 g, 97%), mp = 136-137 ^{o}C [12, 32], R_f = 0.25 (40% ethyl acetate in hexane). ^{1}H NMR ($CDCl_3$) (δ, ppm): 0.91 (t, *J*=7.4 Hz, 3H, -C($CH_2C\underline{H}_3)_2$), 0.93 (t, *J*=7.3 Hz, 3H, -C($CH_2C\underline{H}_3)_2$), 1.30 (t, *J*=7.2 Hz, 3H, -C($CH_2C\underline{H}_3)_2$), 1.48-1.54 (m, 4H, -C($C\underline{H}_2CH_3)_2$), 2.06 (s, 3H, -C(O)C$\underline{H}_3$), 2.11-2.37 (m, 1H, -C$\underline{H}_2$-), 2.89 (dd, J_1=5.6 Hz, J_2=17.1 Hz, 1H, -C$\underline{H}_2$-), 3.37 (m, 2H, -C$\underline{H}(CH_2CH_3)_2$, -C$\underline{H}$-O-, 4.20 (q, *J*=7.2 Hz, 2H, -C$\underline{H}_2CH_3$), 4.27-4.34 (m, 1H, -C$\underline{H}$-N_3), 4.57-4.59 (m, 1H, -C$\underline{H}$-NHAc), 6.00 (d, *J*=7.3 Hz, 1H, -N$\underline{H}$-(C=O)-CH_3), 6.80 (dd, J_1=2.2 Hz, J_2=2.3 Hz, 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($CH_2\underline{C}H_3)_2$), 14.1 (-$CH_2\underline{C}H_3$), 23.5 (-NH-CO-CH3), 25.6 (-C($\underline{C}H_2CH_3)_2$), 26.3 (-C($\underline{C}H_2CH_3)_2$), 30.6 (-$\underline{C}H_2$-), 57.0 (-$\underline{C}$H-N_3), 58.2 (-$\underline{C}$HNHAc), 61.0 (-$\underline{C}H_2CH_3$), 73.31 (-$\underline{C}$H-O-), 82.0 (-$\underline{C}H(CH_2CH_3)_2$), 128.2 (-CH=$\underline{C}$-), 137.9 (-$\underline{C}$H=C-), 165.8 (-$\underline{C}$=O), 171.1 (-NH($\underline{C}$O)$CH_3$).

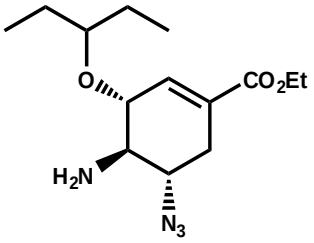
2.3.4 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (oseltamivir) 9



9

Many synthetic conditions were surveyed including reagent (Zinc dust, triphenylphosphine), equivalence of zinc dust (50-100), equivalence of triphenylphosphine (1.0-1.1), equivalence of ammonium chloride (0-20), solvent (EtOH, DMF, THF:H_2O (10:1)), temperature (0-70 ^oC), reaction time (1-24 h), and extraction procedure. The optimum condition was as follow : acetamido azide **22** (0.13 g, 0.39 mmol) was added THF (10 mL), water (1 mL) and triphenylphosphine (0.11 g, 0.43 mmol). The solution was stired at reflux for 15 h and then extracted with ethyl acetate (3x20 mL) and water (20 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The crude yellow oil product was purified by column chromatography on silica gel, eluting with 10:15:75 MeOH:Et_3N:EtOAc to provide free base of oseltamivir **9** (0.12 g, 99%) [12, 32], R_f = 0.05 (5% methanol in ethyl acetate). ^1H 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-). ^{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), 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)-$CH_3$).

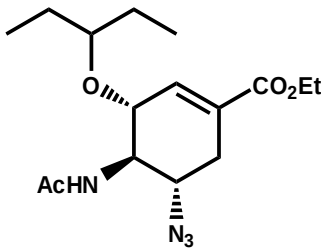
2.3.5 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 21 from epoxide 18



21

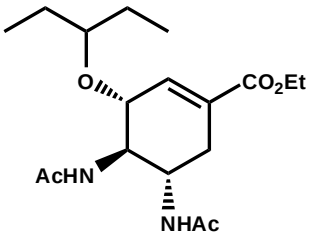
This procedure combined from the 2 previous processes (2.3.1 and 2.3.2). A solution of epoxide **18** (0.25 g, 0.98 mmol) in EtOH (12 mL) was added sodium azide (0.32 g, 4.90 mmol) and ammonium chloride (0.26 g, 4.90 mmol). The reaction mixture was heated at 50 ^{o}C for 18 h and then added hexane to precipitate the reagents. The sediment was filtered and the filtrate was evaporated to remove EtOH. The resulting mixture was added DMF (12 mL), triphenylphosphine (0.257 g, 0.98 mmol) and ammonium chloride (0.0047 g, 0.085 mmol). It was heated at 70 ^{o}C for 4 h to probably provide aziridine intermediate **20**. Sodium azide (0.095 g, 1.47 mmol) and ammonium chloride (0.068 g, 1.27 mmol) were added to the mixture and brought it back to reflux condition for 3 h**.** The reaction mixture was then added ethyl acetate (30 mL), washed with water (4x20 mL), dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The resulting mixture of brown oil and white solid were purified by column chromatography on silica gel, eluting with 40% ethyl acetate-hexane to provide the compound **21** (0.1623 g, 55%), R_f = 0.375 (40% ethyl acetate in hexane).

2.3.6 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 22 from epoxide 18



22

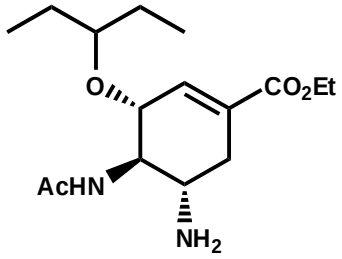
This procedure combined from the 2 previous processes (2.3.3 and 2.3.5). A solution of epoxide **18** (0.25 g, 0.98 mmol) in EtOH (12 mL) was added sodium azide (0.32 g, 4.90 mmol) and ammonium chloride (0.26 g, 4.90 mmol). The reaction mixture was heated at 50 ^{o}C for 18 h and then added hexane to precipitate the reagents. The sediment was filtered and the filtrate was evaporated to remove EtOH. The resulting mixture was added DMF (12 mL), triphenylphosphine (0.257 g, 0.98 mmol) and ammonium chloride (0.0047 g, 0.085 mmol). It was heated at 70 ^{o}C for 4 h to probably provide aziridine intermediate **20**. Sodium azide (0.095 g, 1.47 mmol) and ammonium chloride (0.068 g, 1.27 mmol) were added to the mixture and brought it back to reflux for 3 h. Acetyl chloride (2.1 mL, 29.53 mmol) and pyridine (0.79 mL, 9.80 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was then added ethyl acetate (30 mL), washed with water (5x30 mL), dried over anhydrous magnesium sulfate, filtered and concentrated in vacuo. The resulting mixture of yellow oil and white solid were purified by column chromatography on silica gel, eluting with 40% ethyl acetate-hexane to provide the acetamido azide compound **22** (0.151 g, 49%), mp = 135-136 ^{o}C, R_f = 0.25 (40% ethyl acetate in hexane).



45

If the reaction have not cool to room temperature before the addition of acetyl chloride, one of the by-products are find to be the diamide **45** (yellow oil). ^{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, 1H, -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)-$CH_3$), HRMS (ESI/methanol) m/z calcd for $C_{18}H_{30}N_2O_5$ $(M+Na)^+$ 377.2052, found 377.2034.

2.3.7 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (free base oseltamivir) 9 from epoxide 18



9

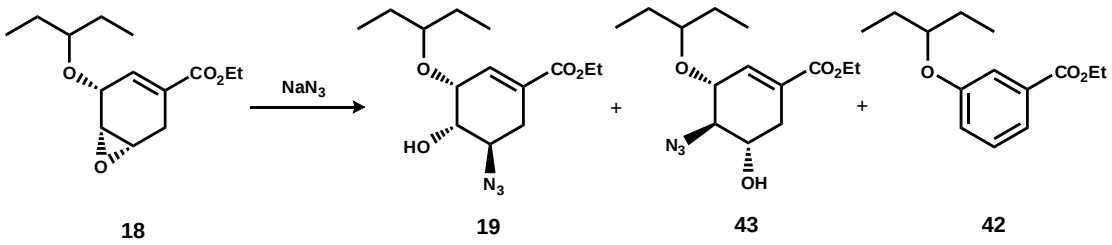
This procedure combined the 2 previous processes (2.3.4 and 2.3.6). A solution of epoxide **18** (0.25 g, 0.98 mmol) in EtOH (12 mL) was added sodium azide (0.32 g, 4.90 mmol) and ammonium chloride (0.26 g, 4.90 mmol). The reaction mixture was heated at 50 ^{o}C for 26 - 28 h and then added hexane to precipitate the reagents. The sediment was filtered and the filtrate was evaporated to remove EtOH. The resulting mixture was added DMF (12 mL), triphenylphosphine (0.257 g, 0.98 mmol) and ammonium chloride (0.0047 g, 0.085 mmol). It was heated at 70 ^{o}C for 4 h to probably provide aziridine intermediate **20**. Sodium azide (0.095 g, 1.47 mmol) and ammonium chloride (0.068 g, 1.27 mmol) were added to the mixture and brought it back to reflux for 3 h. Acetylchloride (2.1 mL, 29.53 mmol) and pyridine (0.79 mL, 9.80 mmol) were added and stirred at room temperature for 2 h. The reaction mixture was then added EtOH (12 mL) and sodium hydrogen carbonate (0.5 g) to neutralize acetyl chloride. The reaction mixture was filtered and concentrated in vacuo. The mixture was added triphenylphosphine (0.257 g, 0.98 mmol). It was stirred at room temperature for 1 h. The reaction mixture was then added 1% of hydrochloric acid (30 ml) to convert **9** to salt form in water layer and then washed with ethyl acetate (30 mL) for remove other impurities. The water layer was added the solution of 1 M sodium hydroxide (30 mL) for convert **9** to base form and then added ethyl acetate (3x20 mL) to extract **9** to organic layer. The organic layer was dried over anhydrous

magnesium sulfate, filtered and concentrated in vacuo. The resulting yellow oil was purified by column chromatography on silica gel, eluting with 10:15:75 MeOH:Et_3N:EtOAc to provide the isomer mixture of free base of oseltamivir **9** (0.055 g, 18%), R_f = 0.05 (5% methanol in ethyl acetate).

CHAPTER III

RESULTS AND DISCUSSION

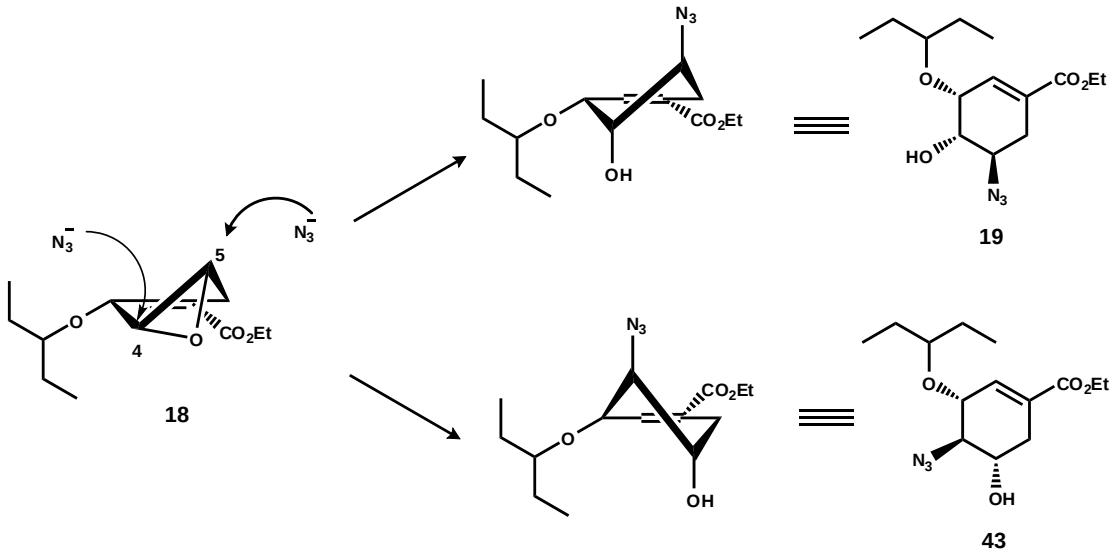
3.1 Synthesis of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 19



18 19 43 42

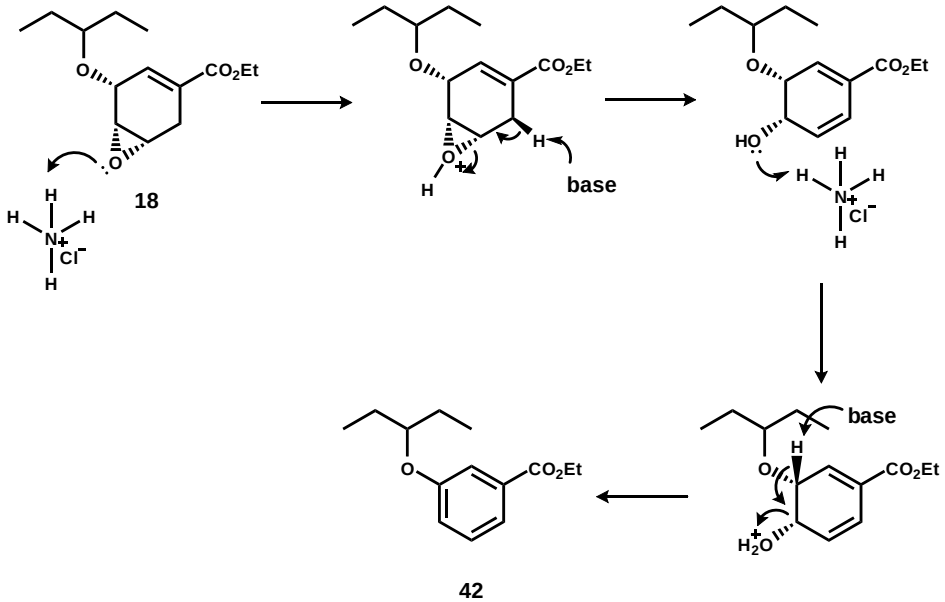
Scheme 3.1 Synthesis of hydroxyl azide **19** and **43**

The ring opening of epoxide **18** was achieved by substitution reaction using azide as the nucleophile. The products were mostly obtained as a mixture of two regioisomers **19** and **43** from the two possible substituted positions of epoxide **18** (**Scheme 3.1**). The major product was **19**, from which the azide reacted at the probably less steric 5-position of **18** (**Scheme 3.2**). The obtained ratios of **19** and **43** were usually around 10:1 [12].



Scheme 3.2 The mechanism of the formation of regioisomers **19** and **43**

In some cases, the thermodynamically favored aromatic compound **42** was also observed as one of the by-products, possibly from a series of elimination reactions. The mechanism of the elimination reactions is shown in **Scheme 3.3**.



Scheme 3.3 The mechanism of the elimination reactions to obtain **42**

The aromatic compound **42** is a product of side reaction of epoxide **18** [12]. It's difficult to isolate and may interfere in the following steps. Therefore, the appropriate conditions which avoided the occurrence of aromatic compound **42** was surveyed as shown in **Table 3.1**.

Table 3.1 A attempts on the synthesis of **19** and **43**

Entry	Solvent	NaN_3 (eq)	NH_4Cl (eq)	Temp (^0C)	Time (h)	**42**	%Yield*
1	Acetone:H_2O (5:1)	6.5	-	60	72	✓	-
2	CH_3CN:DMF (5:1)	5.0	-	60	96	✓	-
3	DMF	5.0	-	80	96	✓	-
4	DMF	2.18	0.04	60	27	✓	-
5	DMF	5.0	1.2	55	24	✓	-
6	DMF	5.0	5.0	50	24	✓	97.2
7	EtOH	1.4	1.2	55	50	×	-
8	EtOH	5.0	1.2	60	27	×	-
9	EtOH	5.0	5.0	50	28	×	-
10	EtOH	5.0	5.0	70	27	×	92
11**	EtOH	5.0	5.0	50	48	×	88

* Combined yield of both regioisomers **19** and **43**
** Filtration of products was used instead of extraction

Table 3.1 reported the attempts to prepare **19** from azide substitution of **18** in various conditions. The reactions were monitored until no more starting precursor **18** was detected by TLC.

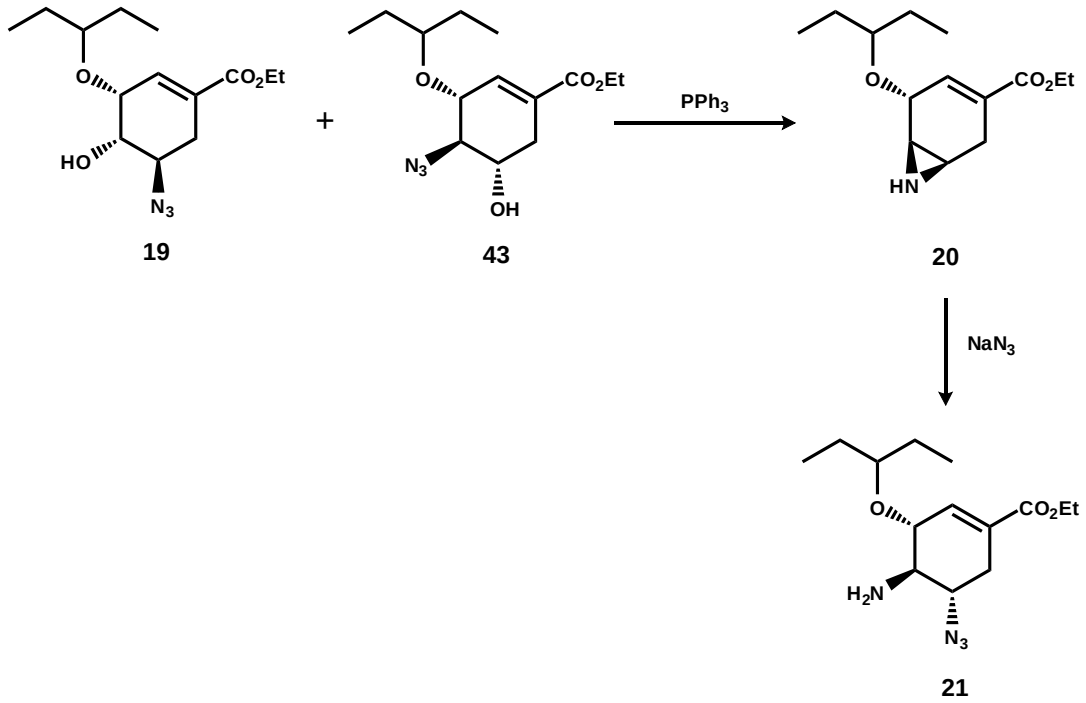
The use of water mixed with acetone (Entry 1) could dissolve sodium azide and lead it azide to organic phase. But the undesired aromatic compound **42** was detected. The solvent mixture of acetonitrile and DMF (Entry 2) also found the aromatic compound **42**. The use of DMF following the method reported by Popsavin and coworkers [30], could decrease time of reaction but still gave some of the

aromatic compound **42** (Entry 6). The use of EtOH following the method reported by Rohloff and Kent [12, 32], could also decrease time of reaction without giving aromatic compound **42** (Entries 7-11). It could be concluded that EtOH was the suitable solvent choice for clean result with no **42**.

It secured that the concentration of sodium azide did not affect the formation of aromatic compound **42**. But it did increase the rate of reaction (Entry 7 v.s. 8). Ammonium chloride was also required to decrease reaction time. Large amount (about 5 equiv) of both azide and ammonium chloride was used eventually to ensure the completion of the reduction at a convenient time frame.

Entry 6 showed the highest yield of 97.2% after a chromatographic separation to remove small amount of aromatic compound **42**. The slightly lower yield of Entry 10 was perhaps due to the less from extraction, but no **42** was present and the hurdles of chromatography could be avoided. Yet, large amount of solvent was still needed and made it not suitable for industrial scale synthesis. To solve this problem, hexane was added to precipitate off the inorganic salts, which was conveniently filtered (Entry 11). Pure **19** and **43** was obtained in 88% yield which was comparable to the previous entry and those of Rohloff and Kent [12, 32]. The regioisomers **19** and **43** were not needed to be separated because both of them would be converted to the same compound in the next step. Nevertheless, the compound **19** could be purely isolated by chromatographic separations and characterized with ^{1}H-NMR spectroscopy (**Figure A.3** in Appendix)

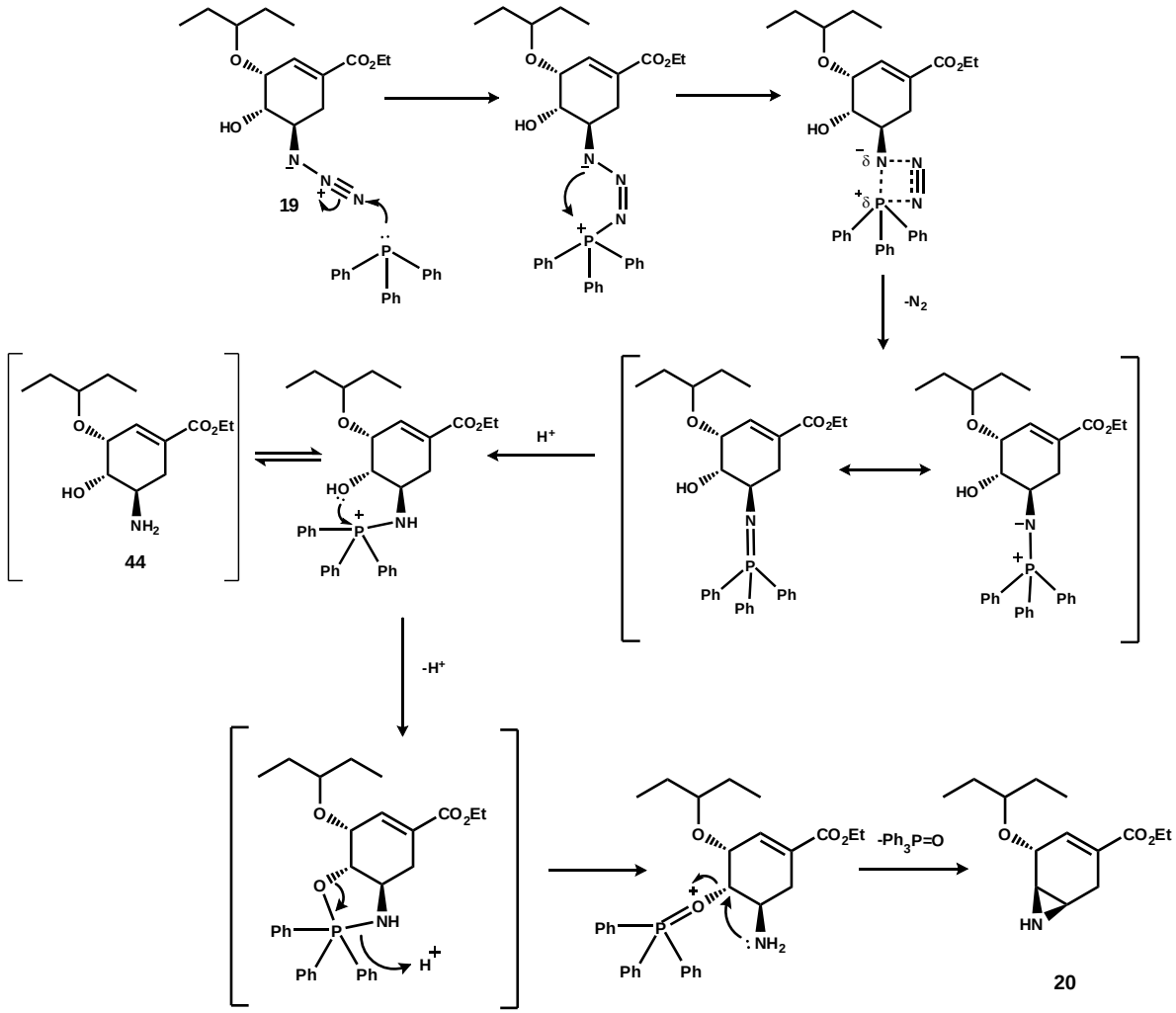
3.2 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 21



Scheme 3.4 Synthesis of amino azide **21**

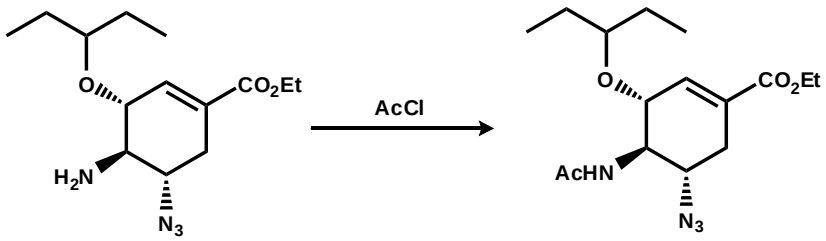
This process consists of 2 steps: the conversion of the azide group on **19** and **43** to amino group by Staudinger Reduction with subsequent ring closing to aziridine **20**. It was then reopened to amino azide **21**. From the method reported by Rohloff and coworkers [12], they used trimethylphosphine as the reducing reagent to give aziridine **20** in 97.3% from regioisomers **19** and **43**. Kent and coworkers [32] used triphenylphosphine that formed aziridine **20** in 96.8% from regioisomers **19** and **43**. Triphenylphosphine is cheap, non-volatile and easier to use than trimethylphosphine. Although the resulted triphenylphosphineoxide was harder to isolate than trimethylphosphineoxide. The mechanism of Staudinger reaction is shown in **Scheme 3.5**.

This experiment, the reduction step using triphenylphosphine in the presence of catalytic ammonium chloride in DMF was combined with ring opening of aziridine **20** by sodium azide to generate amino azide **21** in 50% overall yield from mixture of **19** and **43** [12, overall yield ≥42.6%] [32, overall yield ≥52.8%], . The aziridine **20** was known to be degraded easily and difficult to purify. Therefore, it was not isolated during the reaction from regioisomers **19** and **43** to amino azide **21**. The intermediate **44** or its unhydrolyzed precursor was assumed to undergo ring closing to aziridine **20** via oxazaphospholidine intermediate, catalyed by the acidic ammonium chloride as shown in **Scheme 3.5**. The aziridine ring was then reopened by an insitu substitution with azide to amino azide **21**.



Scheme 3.5 The synthesis of aziridine **20** via Staudinger reaction

3.3 Synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 22



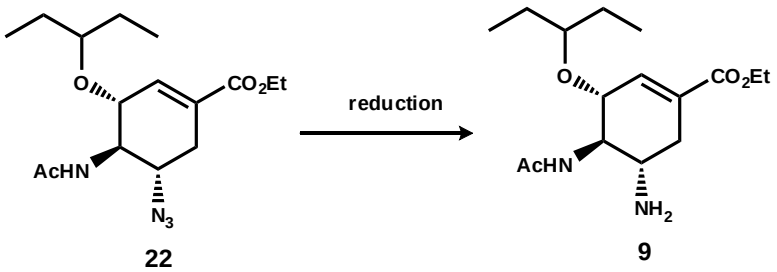
Scheme 3.6 Synthesis of acetamido azide **22**

The acetylation of amino azide **21** provided acetamido azide **22**. (**Table 3.2**) Acetic anhydride was first used for the acetylation together with triethylamine in dichloromethane at room temperature following the original reports (Entries 1 - 2). [12, 32] However, no reaction was observed in these cases. It's possible that the main reagent acetic anhydride, may have absorped too much moisture and was mostly hydrolyzed and inactive. Next, large excess of acetyl chloride and pyridine was used instead with DMF as the solvent at room temperature (Entry 3). This latter condition successfully provided acetamido azide **22** in 97% yield. Thus, the condition with acetyl chloride 30 eq and pyridine 10 eq in DMF at room temperature was taken as the optimum for this reaction without further survey and employed in all of the later synthesis of **22**.

Table 3.2 The study of acetylation of amino azide **21**

Entry	Reagent	Condition	Result
1	Ac_2O 1.5 eq, NEt_3 1.5 eq	CH_2Cl_2, 2 h	No reaction
2	Ac_2O 3.0 eq, NEt_3 3.0 eq	CH_2Cl_2, 24 h	No reaction
3	AcCl 30 eq, Pyridine 10 eq	DMF, 2 h	97% yield of **22**

3.4 Synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (free base of oseltamivir) 9



22 **9**

Scheme 3.7 Synthesis of free base of oseltamivir **9**

The acetamido azide **22** can be transformed into the free base form of oseltamivir **9** by reduction. Zinc dust and ammonium chloride in ethanol were the reagents in the early attempts [34] (**Table 3.3**).

Table 3.3 The reductions of acetamido azide **22** with reducer.

Entry	Reducer (eq)	Solvent	NH_4Cl (eq)	Temp (^oC)	Total time (h)	**22**	%yield
1	Zn (100)	EtOH	20	rt	2	✓	-
2	Zn (50)	EtOH	10	rt	24	✓	-
3	Zn* (100)	EtOH	20	rt	3	×	-
4	Zn* (50)	EtOH	10	0 to rt	1	×	76
5	Zn* (50)	EtOH	10	0 to rt	1.30	×	78
6	Zn* (50)	EtOH	10	0	1	×	68
7	PPh_3 (1.0)	DMF	-	rt	1	×	46
8	PPh_3 (1.1)	THF/H_2O	-	reflux	15	×	99

*Activated by washing with HCl

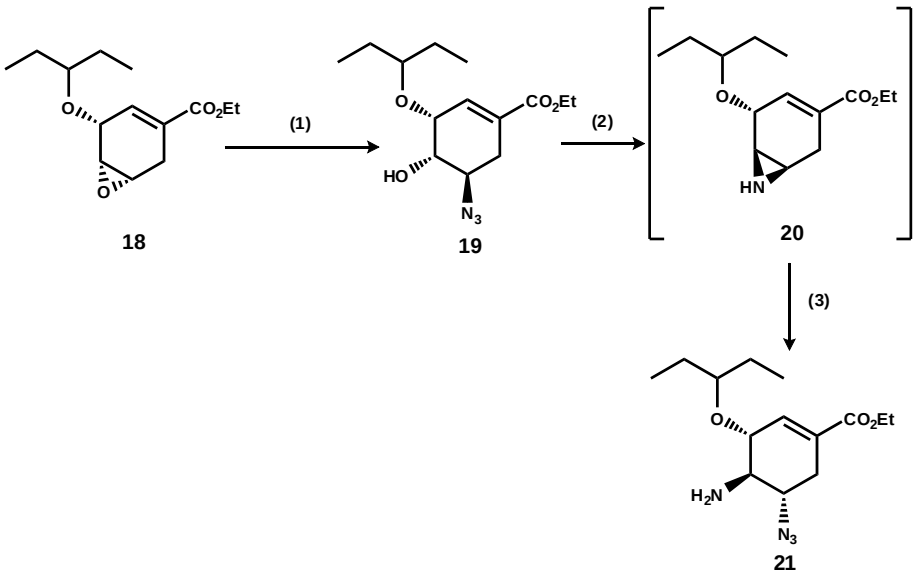
When the zinc dust reagent was used right out both, the starting material acetamido azide **22** still remained, even with large excess of reagent or prolonged reaction time to 24 h (Entries 1 - 2). It could be assumed that the normally used zinc dust is not quite active enough for the complete reduction of precursor **22**. This is probably, because the oxide built up during storage in air prevented zinc dust to expose its active surface. The zinc dust was then activated by washing with diluted of hydrochloric acid for 30 seconds and was reused with water and acetone. The activated zinc dust was successfully completed the conversion of acetaamido azide **22** within only a few hours (Entries 3 – 6).

In typical experiments, the activated zinc dust and ammonium chloride were cooled to 0 ^{o}C before adding the starting materials. In Entry 4, the reaction was kept at 0 ^{o}C for 15 minutes and then allowed to warm to room temperature for 45 minutes in Entry 5, it was left at 0 ^{o}C for 1 h and then warmed to room temperature for 30 minutes. These time variations seemed to have no effect on the reaction since the yield of Entry 4 was similar to that of Entry 5. When the reaction was kept at 0 ^{o}C without warming up, the yield of the product decreased slightly (Entry 6). It could be suggested that the reaction should be partly allowed to run at room temperature. The ^{1}H-NMR spectrum of the obtained reduced product from this method (**Figure A.12** in Appendix) surprisingly did not match with the free base of oseltamivir **9** (**Figure A.9** in Appendix) obtained from neutralization of standard oseltamivir phosphate drug. The results suggested that the obtained product from the reduction by zinc dust is not the desired free base of oseltamivir **9**. Although it is still unclear what isomer or compound this product would be.

Staudinger reaction [31] is another method for reducing azide group to amino group using triphenylphosphine, the same as that of section 3.2 (**Scheme 3.5**). In entry 7, triphenylphosphine in DMF could achieve the complete reduction of acetamido azide **22** within only 1 h and provided the correct, match up product of free base oseltamivir **9** in 46% yield based on ^{1}H-NMR after subtraction of triphenylphosphine oxide (**Figure A.13** in Appendix). Although the character of the product now matched that of **9**, the ^{1}H-NMR also showed small amount of an impurity which was possibly its diastereomer. These diastereoisomeric mixtures were

very similar in polarity and and could not be separated. Nevertheless, it has recently been reported that using 90% THF as the solvent and reflux for at least 12 h would solve the problem [36]. Following this procedure, free base oseltamivir **9** purely was obtained in nearly quantitative yield of 99% without any other isomers (Entry 8). This was clearly demonstrated that the use of triphenylphosphine in this step was more suitable than activated zinc dust. Although DMF solvent could accelerate the reduction to completion within just a few hours, the slower reaction in THF (THF:H_2O 9:1) at reflux would be the better choice that provided the clean, correct product in much better yield (**Figure A.14** in Appendix).

3.5 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 21 from epoxide 18



Reagents and Conditions : (1) NaN_3 5.0 eq, NH_4Cl 5.0 eq, EtOH, 50 ^oC, 48 h ; (2) PPh_3 1.0 eq, NH_4Cl 0.09 eq, DMF, 70 ^oC, 3 h ; (3) NaN_3 1.4 eq, NH_4Cl 1.2 eq, DMF, reflux, 3 h.

Scheme 3.8 The combined synthesis of amino azide **21** from **18**

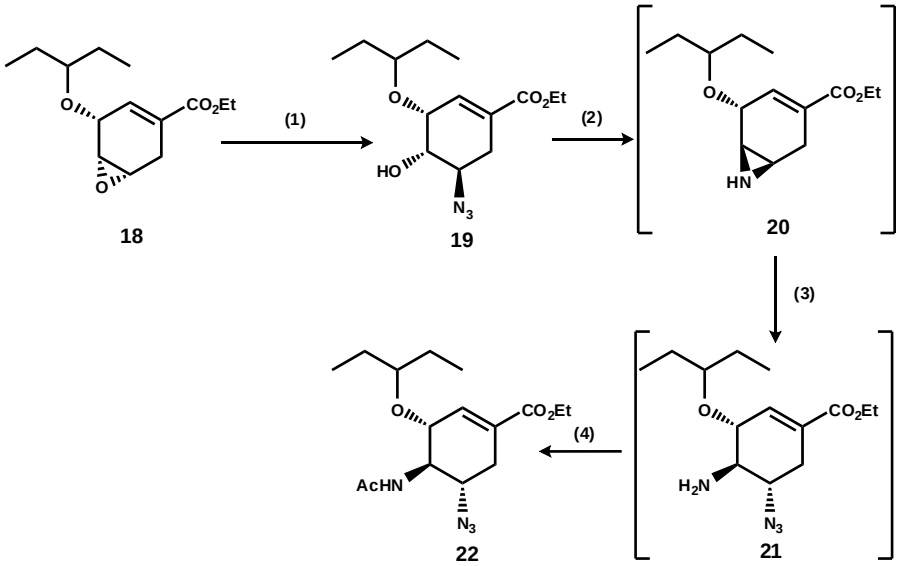
In section 3.1, the best condition to open the epoxide ring to hydroxyl azide **19** was found to be: using sodium azide as nucleophile in EtOH at 50 ^oC for 48 h (Entry 11, Table 3.1). This procedure gave high yields of the product and its regioisomer and could avoid product purification by column chromatography. While in section 3.2, **19**

and its isomer **43** could be converted to **21** without isolation of **20** using triphenylphosphine via Staudinger Reduction followed by ring closing and reopening with sodium azide and ammonium chloride in DMF (**Scheme 3.5**). Combination of both results would create a pathway that gave **21** directly from **18**. However, DMF has been found earlier that the undesired **42** would be present during the epoxide opening. Consequently, a solvent changing step was needed to avoid the formation of this impurity.

The combination of these 3 steps started from ring opening of epoxide **18** by sodium azide in EtOH to provide a mixture of **19** and **43** following the best condition in section 3.1. After step 1 was completed, excess salts were filtered off and EtOH was removed. Triphenylphosphine, ammonium chloride and DMF were added to the reaction for the reduction and ring closing to aziridine intermediate **20**, which immediately followed by the ring opening using another portion of added sodium azide and ammonium chloride in DMF at reflux temperature. Amino azide **21** was obtained from these sequenes in 55% yield from epoxide **18**.

To compare the results of this combined 3 steps with that of the separated steps, the overall yield of separated steps was 46% [12, ≥36.2%] [32, ≥52.8%] while the combined 3 steps was 55% yield. This combined steps process was clearly better than the separated steps in terms of yield, reaction time and convenience.

3.6 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate 22 from epoxide 18



Reagents and Conditions : (1) NaN_3 5.0 eq, NH_4Cl 5.0 eq, EtOH, 50 °C, 48 h ; (2) PPh_3 1.0 eq, NH_4Cl 0.09 eq, DMF, 70 °C, 3 h ; (3) NaN_3 1.4 eq, NH_4Cl 1.2 eq, DMF, reflux, 3 h ; (4) AcCl 30 eq, Pyridine 10 eq, DMF, rt, 2 h.

Scheme 3.9 The combined synthesis of acetamido azide **22**

In section 3.3, (Entry 3, Table 3.2) the amino azide **21** could be acetylated to provide pure acetamido azide **22** in high yield using large excess of acetyl chloride in DMF. While in section 3.5, the synthesis of **21** directly from **18** was achieved from combining the separated steps into a continuous process without intermediate isolations, which gave even better yield of product. In this section, attempts to further combine the acetylation were carried out.

The variation of combination of these 4 steps is shown in **Table 3.4**. In the early attempts, DMF was chosen to be solvent for all steps.

In entry 1, epoxide **18** reacted with high concentration of sodium azide and ammonium chloride in step 1, expecting the excess reagents to be left over to step 3. This expectation was not met, however. Only the byproduct **42** (Section 3.1, Scheme 3.3) was found. Addition of more sodium azide and ammonium chloride to step 3 (Entry 2) gave the aminoazide **21** together with **42**. Thus, the failure to get **21** in Entry

1 could be due to the unexpected reaction of triphenylphosphine with the leftover sodium azide during the section in step 2. Therefore, an experiment was setup by mixing sodium azide and triphenylphosphine in DMF for 3 h at 70 ^{o}C. During the reaction, the amount of triphenylphosphine was slowly decreased in the presence of dark solid polymer. The well soluble sodium azide in DMF was found to react with triphenylphosphine directly and interfere with the reaction in step 2-3 to give **21**. From this result and the usual presence of aromatic compound **42** during the reaction in step 1, it could be concluded that DMF should not be used as the sole solvent for continuous process of these combined 4-steps reaction. Besides, excess azide left from step 1 should be got rid of before moving on to step 2, and replenished back to carry on the reaction in step 3.

Table 3.4 The variation of combined 4-steps synthesis of acetamido azide **22** from **18**

Entry	Reagents (eq) and conditions				Product
	Step 1	Step 2	Step 3	Step 4	
1	NaN_3 (5.0), NH_4Cl (5.0) , DMF, 50 ^oC, 24 h	PPh_3 (1.05), DMF, 70 ^oC, 21 h	-	Ac_2O (1.5), NEt_3 (1.5), DMF, rt, 20 h	**42**
2	NaN_3 (5.0), NH_4Cl (1.2) , DMF, 50 ^oC, 24 h	PPh_3 (1.05), NH_4Cl (0.09), DMF, 70 ^oC, 5 h	NaN_3 (1.4), NH_4Cl (1.2), DMF, 70 ^oC, 24 h	Ac_2O (1.5), NEt_3 (1.5), DMF, rt, 5 h	**21, 42**
3	NaN_3 (5.0), NH_4Cl (1.2), EtOH, 50 ^oC, 29 h	PPh_3 (1.05), NH_4Cl (0.09), DMF, 70 ^oC, 4 h	NaN_3 (5.0), NH_4Cl (5.0), DMF, 70 ^oC, 24 h	Ac_2O (10.0), pyridine (5.0), DMF, rt, 24 h	**21**
4	NaN_3 (5.0), NH_4Cl (5.0), EtOH, 50 ^oC, 29 h	PPh_3 (1.05), NH_4Cl (0.09), DMF, 70 ^oC, 6 h	NaN_3 (1.4), NH_4Cl (1.2), DMF, 70 ^oC, 24 h	Ac_2O (10.0), NEt_3 (5.0), then AcCl (4.0), pyridine (4.0), DMF, rt, 24 h	**22**

In section 3.1, it has been demonstrated that the use of EtOH in step 1 could suppress the formation of aromatic compound **42**. Excess sodium azide could be eliminated before adding triphenylphosphine in step 2 by the addition of hexane and filtering out the salt and evaporating the solvent before switching to DMF as accomplished in section 3.5. Following this procedure (entries 3 – 4, Table 3.4) the amino azide moiety was successfully obtained without **42**. Although in entry 3, the resulted product **21** could not be acetylated by acetic anhydride to provide **22**. As shown in section 3.3, excess acetyl chloride was needed for the acetylation in step 4 (Entry 4).

Kent and coworkers [32], had indicated that the yields of acetamido azide **22** were mostly depended on the performances of step 3-4, especiallythe handing of reactive aziridine **20**. Thus, the experiments were performed with variation of conditions in step 3 and step 4 as shown in **Table 3.5**, keeping the conditions of step 1 and step 2 as in Entry 4 of Table 3.4.

Table 3.5 The variation of steps 3 and 4 for the synthesis of **22** from **18**

Entry	Reagents (eq) and conditions		%yield
	Step 3	Step 4	
1	NaN_3 (5.0), NH_4Cl (5.0), DMF, 70 ^oC, 24 h	AcCl (50), pyridine (10), DMF, rt, 24 h	16
2	NaN_3 (5.0), NH_4Cl (5.0), DMF, 70 ^oC, 24 h	AcCl (50), pyridine (10), DMF, rt, 3 h	24
3	NaN_3 (1.5), NH_4Cl (1.3), DMF, 100 ^oC, 18 h	AcCl (50), pyridine (10), DMF, rt, 2 h	31.2
4	NaN_3 (1.5), NH_4Cl (1.3), DMF, 130 ^oC, 4 h	AcCl (50), pyridine (10), DMF, rt, 2 h	35
5	NaN_3 (1.5), NH_4Cl (1.3), DMF, 130 ^oC, 19 h	AcCl (30), pyridine (10), DMF, rt, 5 h	32.8
6	NaN_3 (1.5), NH_4Cl (1.3), DMF, 130 ^oC, 6 h	AcCl (30), pyridine (10), DMF, rt, 2 h	32.2
7	NaN_3 (1.5), NH_4Cl (1.3), DMF, reflux, 2 h	AcCl (30), pyridine (10), DMF, rt, 24 h	18
8	NaN_3 (1.5), NH_4Cl (1.3), DMF, reflux, 3 h	AcCl (30), pyridine (10), DMF, rt, 24 h	30
9*	NaN_3 (1.5), NH_4Cl (1.3), DMF, reflux, 4 h	AcCl (30), pyridine (10), DMF, rt, 2 h	49

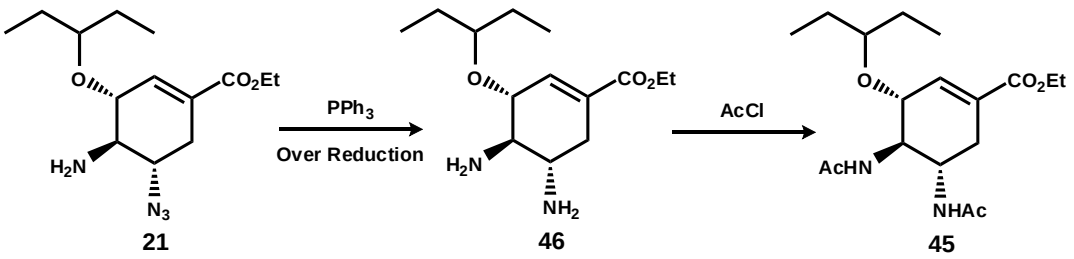
*use ethyl acetate for the extraction instead of hexane

From Table 3.5 entry 1, **22** could be obtained in 16% yield. The yield was unexpectedly increased with shortened reaction time of step 4 (Entry 2). The resulted product may be degraded when left in the reaction for too long. Decreasing the concentration of sodium azide and ammonium chloride in step 3, but increasing temperature of reaction to 100 ^oC, the yield of **22** was increased (Entry 3). It could be noted that higher temperature in the reaction of step 3 would improve the yield of **22** produced while excess of azide added in this step was not necessary.

Increasing the reaction temperature of step 3 kept the comparably good yield of **22** with shorter reaction time (Entry 4). Entries 5 - 6 showed that longer reaction time of step 3 did not improve the yield of **22** while the amount of acetyl chloride could be reduced to achieve the acetylation of amino azide **21** to acetamido azide **22**. Raising the reaction temperature in step 3 to reflux with less reaction time worsened the result (Entry 7 v.s. 8). The reaction might be incomplete at shorter time.

In all cases, large amount of hexane was added followed by several aqueous washes during the final workup to extract **22**. It was speculated that part of the product may be lost due to insufficient solubility in hexane. To reduce waste and increase the extraction efficiency, EtOAc was used instead (Entry 9). The result did improve the product yield with less solvent used as expected.

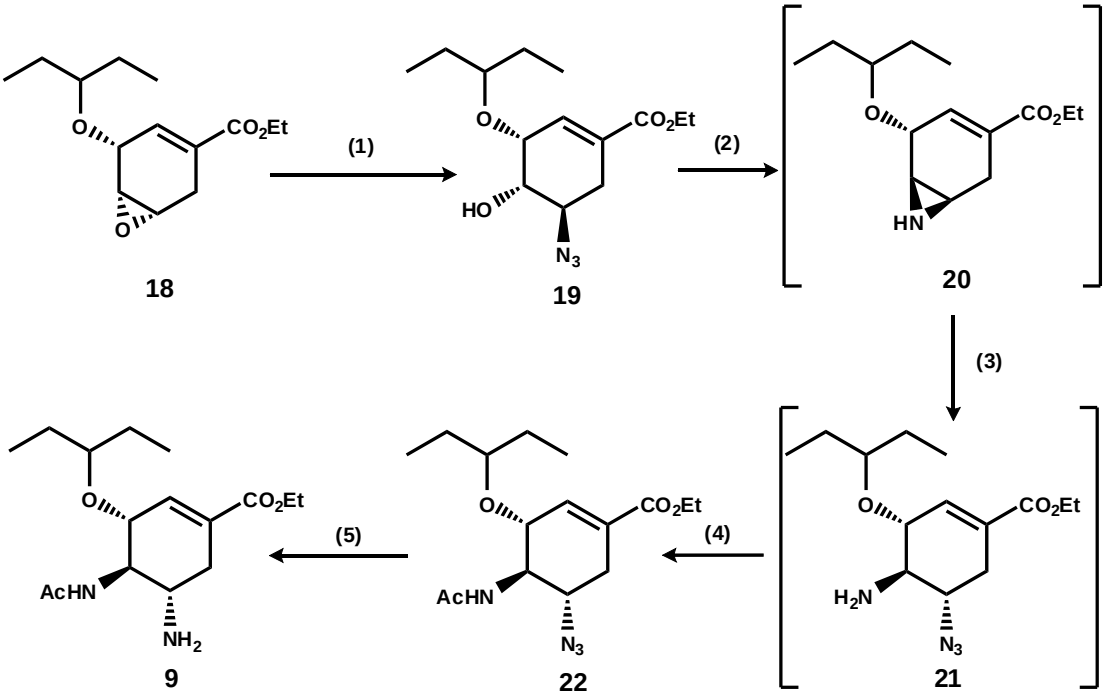
In some cases, especially when larger amount of phosphine was used, a by-product was observed with **22** after the acetylation. This side product was identified to be the diamide **45** (**Scheme 3.10** and **Figure A.15** and **Figure A.16** in Appendix). The formation of **45** was assumed to occur through the acetylations of diamino **46**, which was probably over reduced from **21** from the leftover triphenylphosphine during the reactions in step 3, induced by strong acidic condition from the subsequent addition of acetyl chloride. Thus, when the reaction of step 3 was left to cool to room temperature before the addition of acetyl chloride in step 4, diamide **45** was no longer observed.



Scheme 3.10 The tentative formation of diamide **45**

In comparison, the result from the process when these 4 steps reactions were separated could be calculated to be the overall yield of 44% [12, 36.2%] [32, 52.8%], comparing to 49% yield when they were combined. Therefore, this combined process is considered better than the separated steps.

3.7 Combined synthesis of ethyl (3*R*,4*R*,5*S*)-5-amino-4-acetamido-3-(1-ethyl-propoxy)-1-cyclohexene-1-carboxylate (oseltamivir) 9 from epoxide 18



Reagents and Conditions : (1) NaN_3 5.0 eq, NH_4Cl 5.0 eq, EtOH, 50 ^oC, 48 h ; (2) PPh_3 1.0 eq, NH_4Cl 0.09 eq, DMF, 70 ^oC, 3 h ; (3) NaN_3 1.4 eq, NH_4Cl 1.2 eq, DMF, reflux, 3 h ; (4) AcCl 30 eq, Pyridine 10 eq, DMF, rt, 2 h ; (5) PPh_3 1.0 eq, DMF, rt, 1 h.

Scheme 3.11 The combined synthesis of free base oseltamivir **9** from **18**

In section 3.4, Staudinger reduction by triphenylphosphine was the method of choice to convert acetamido azide **22** to free base oseltamivir **9** in mild condition and

short reaction time. While in section 3.6, the synthesis of **22** directly from **18** was achieved from combining the separated steps into a continuous process without intermediate isolations, which gave even better yield of product. In this section, attempts to further combine the reduction were carried out. The variation of combination of these 5 steps is shown in **Table 3.6**. The conditions of steps 1 – 4 followed those from section 3.6.

Table 3.6 The variation of steps 4 and 5 for the synthesis of free base oseltamivir **9** from **18**

Entry	Reagents (eq) and conditions			%yield*
	Work up step 4	Reaction Step 5	Work up step 5	
1	EtOAc + H_2O	Activated Zn (50), NH_4Cl (10), EtOH, 0 ^oC to rt, 1 h	NEt_3 + EtOAc + H_2O	-
2	EtOAc + H_2O	PPh_3 (2.0), DMF, rt, 1 h	NEt_3 + EtOAc + H_2O	11.8%*
3	EtOH + $NaHCO_3$	PPh_3 (1.0), DMF, rt, 1 h	NEt_3 + EtOAc + H_2O	26%*
4	EtOH + $NaHCO_3$	PPh_3 (0.8), DMF, rt, 3 h	HCl, then NaOH + EtOAc	18%

*Yield of a mixture of free base of oseltamivir **9** and its isomer

From the results from section 3.4, it was found that the use of activated zinc dust could not provide free base oseltamivir **9**. The result was confirmed in this series of experiments (Entry 1). Only the same isomer of the free base oseltamivir **9** was once again observed.

The use of triphenylphosphine in DMF was next applied in the reduction to successfully provide free base oseltamivir **9** and small amount of its diastereoisomer as observed earlier (Table 3.3). These isomers could not be separated, because of their closely related polarity. The isolated product was a mixture of **9** and this isomer

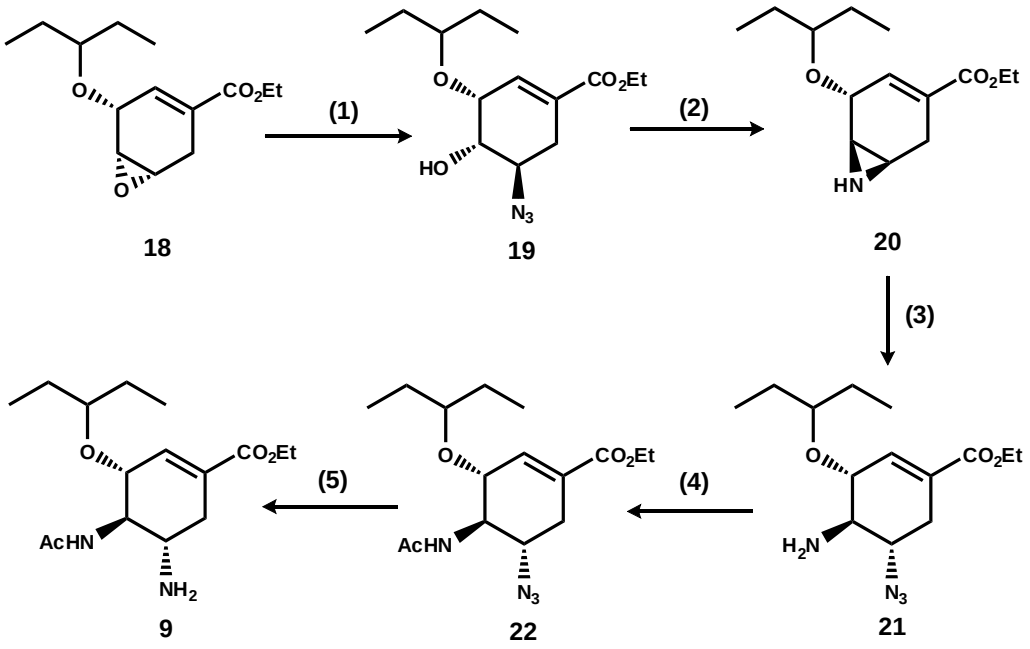
combined to a yield of 11.8% from epoxide **18**. The work up procedure in step 4 was then modified to use EtOH to react with the remaining acetyl chloride followed by addition of diluted sodium hydrogen carbonate for neutralization (Entry 3). This modification could increase the yield of the mixture product to 26% from **18** even with lower equivalence of triphenylphosphine. Although in this case, column chromatography was needed for the purification.

Because oseltamivir **9** could be extracted into aqueous phase in acidic condition, the work up of step 5 was changed to use 1% hydrochloric acid to extract **9** out from most other impurities that remained in organic phase (Entry 4). Diluted sodium hydroxide then converted the salt form of **9** back to the free base, which partitioned into organic phase. This extraction helped cleaning off the impurities without using chromatographic technique. However, free base oseltamivir **9** and its isomer were obtained in only 18% yield from epoxide **18**. It was possible that attempts to wash off the DMF with water several times during the extraction resulted in the observed low yield.

In comparison, the overall yield of the separated steps in the synthesis of **9** from **18** was 42% [12, 36.3%] [32, 51.7%] which was much better than the process that combined all 5 steps continuously. Therefore, step 5 should not be included. The combined process up to step 4 with a separated last step seemed to be the best method for the synthesis of oseltamivir **9** from epoxide **18**.

CHAPTER IV

CONCLUSIONS



18 19 20 21 22 9

Scheme 4.1 Synthesis of oseltamivir **9** from epoxide **18**

In step (1), the ring opening of epoxide **18** with sodium azide in EtOH at 50 ^{o}C for 26-28 h, provided the regioisomers of hydroxyl azide **19** and **43** in 88% yield without isolation. In step (2)-(3), the regioisomers of hydroxyl azide **19** and **43** were converted to aziridine **20** together by ring closing with triphenylphosphine in DMF at 70 ^{o}C for 4 h and then ring opening with sodium azide at reflux for 3 h to provide amino azide **21** in 50% yield from hydroxyl azide **9** without isolate aziridine **20**. Step (4), the acetylation of amino azide **21** with acetyl chloride in DMF at room temperature for 2 h, was provided acetamido azide **22** in 97% yield. In step (5), the reduction of acetamido azide **22** with triphenylphosphine in mixture solvent of THF

and water 10:1 at reflux for 15 h, was produced free base of oseltamivir **9** in 99%. The overall yield of step (1)-(5) is 42%.

The one-pot synthesis was referred the method from separation steps to combine them without isolate intermediate in each steps.

The combine of step (1)-(3) was converted epoxide **18** to amino azide **21** in 55% yield which based on the method of separation steps without isolate regioisomers of hydroxyl azide **19** and **43** and aziridine **20**. This combine steps has higher yield of amino azide **21** than separate steps which have 46% overall yield.

The combine of step (1)-(4) was converted epoxide **18** to acetamido azide **22** in 49% yield which based on the method of separation steps without isolate regioisomers of hydroxyl azide **19** and **43**, aziridine **20** and amino azide **21**. This combine steps has higher yield of acetamido azide **22** than separate steps which have 44% overall yield.

The combine of step (1)-(5) was converted epoxide **18** to free base oseltamivir **9** which based on the method of separation steps without isolate regioisomers of hydroxyl azide **19** and **43**, aziridine **20**, amino azide **21** and acetamido azide **22**, but the reduction of acetamido azide **22** was used triphenylphosphine in DMF instead of mixture solvent THF and water 10:1 at room temperature for 1 h. The reduction of acetamido azide **22** in DMF was provided free base oseltamivir **9** in 18% yield from epoxide **18**. This combine steps has lower yield of free base oseltamivir **9** than separate steps which have 42% overall yield.

Therefore, the one-pot synthesis should combine steps (1)-(4) only into process, because step (5) in separation step can be provided free base oseltamivir **9** in 42% overall yield but in combine step which use DMF as solvent instead of mixture solvent THF and water 10:1 was decreased yield of **9** from 42% to 18% overall yield.

Suggestion of the solution of step (5) for increase yield is the addition of THF and water to reaction in the presence of DMF. When the reaction complete, add phosphoric acid for crystallization to provide oseltamivir phosphate **10**. This

suggestion may force oseltamivir **9** to oseltamivir phosphate **10** without lose oseltamivir **9** in extraction.

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APPENDIX

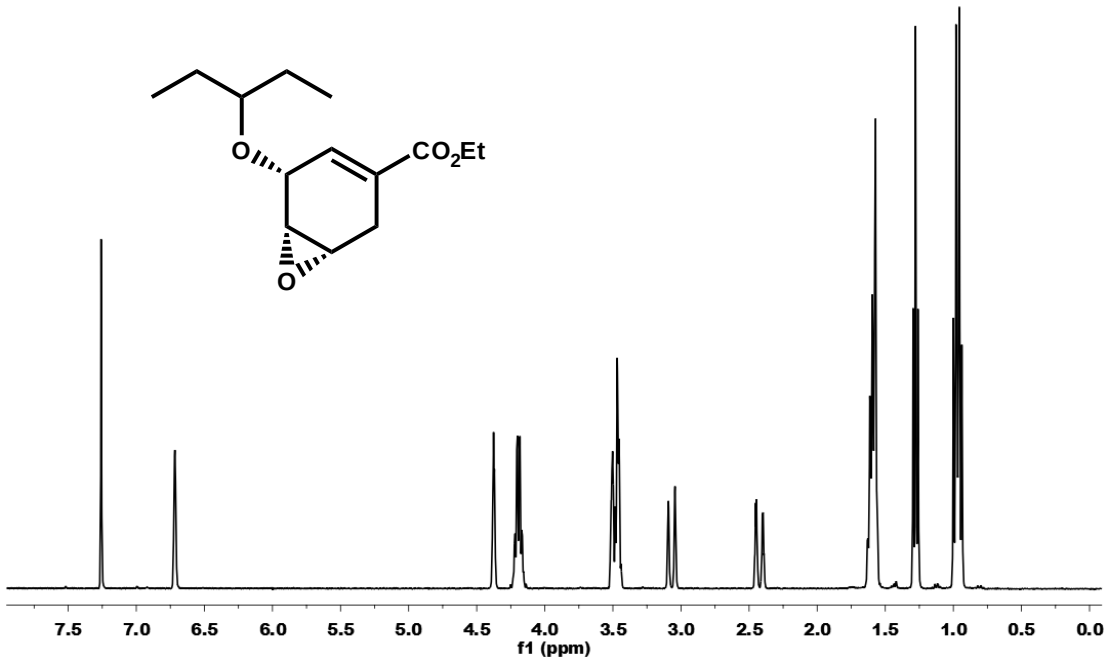


Figure A.1 ^{1}H-NMR ($CDCl_3$) Spectrum of (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (epoxide) **18**

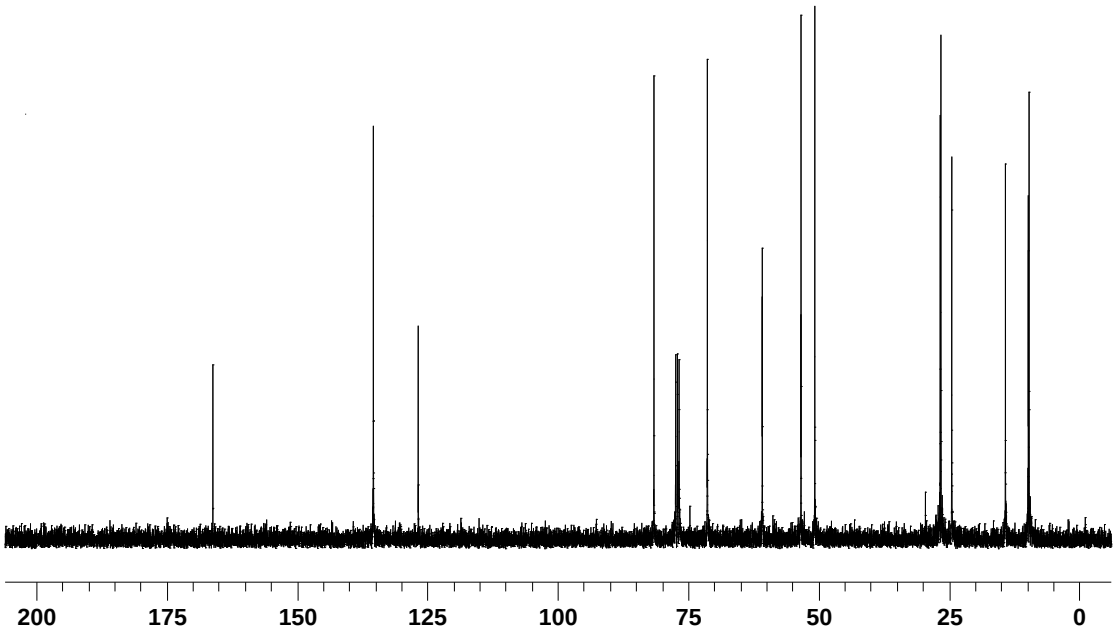


Figure A.2 ^{13}C-NMR ($CDCl_3$) Spectrum of (3*R*,4*R*,5*S*)-4,5-epoxy-3-(1-ethyl propoxy)-1-cyclohexene-1-carboxylate (epoxide) **18**

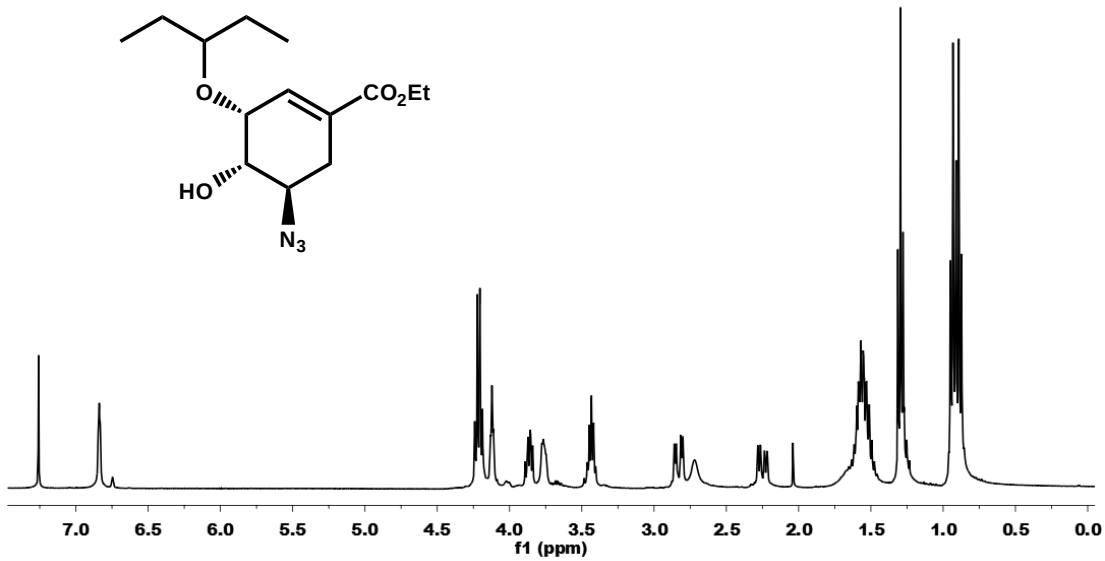


Figure A.3 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **19**

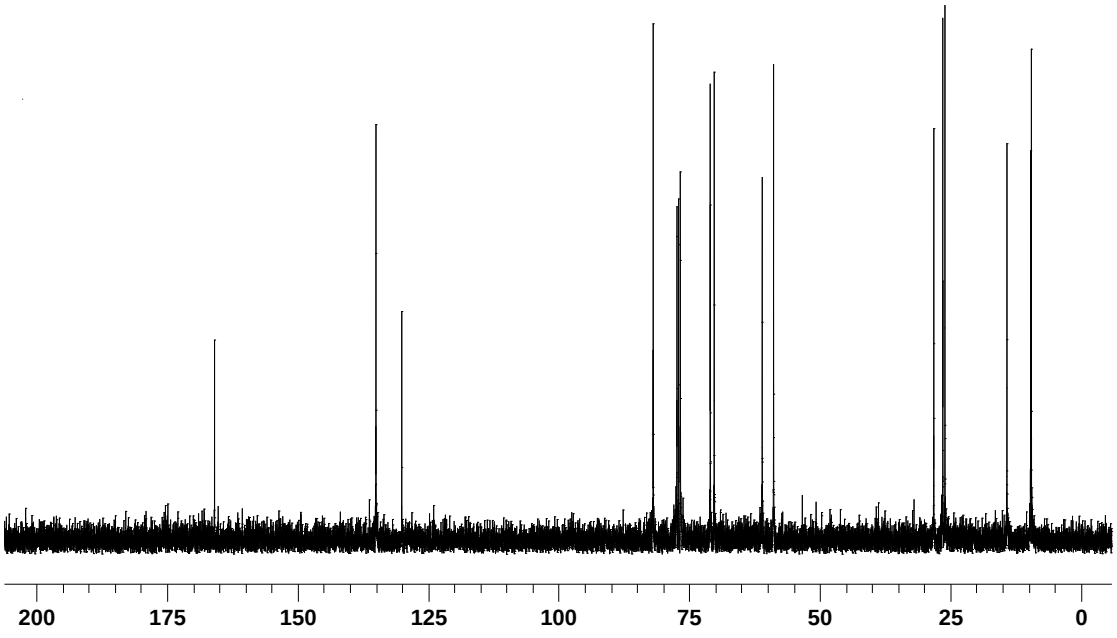


Figure A.4 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*S*,5*R*)-5-azido-4-hydroxy-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **19**

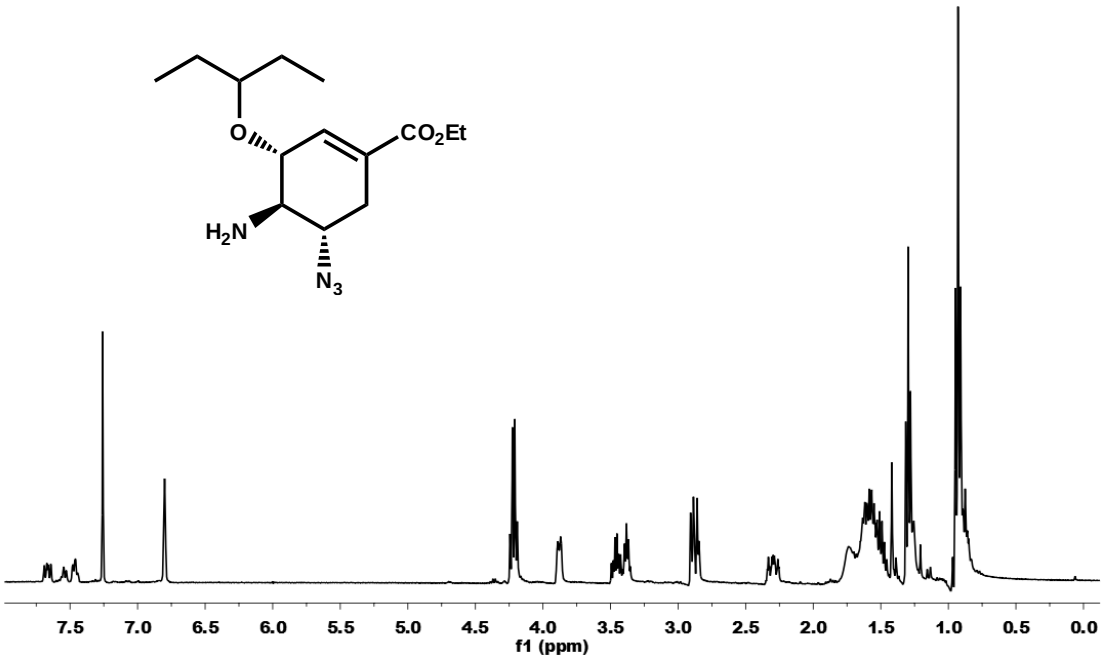


Figure A.5 ^{1}H-NMR ($CDCl_3$) Spectrum of (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **21**

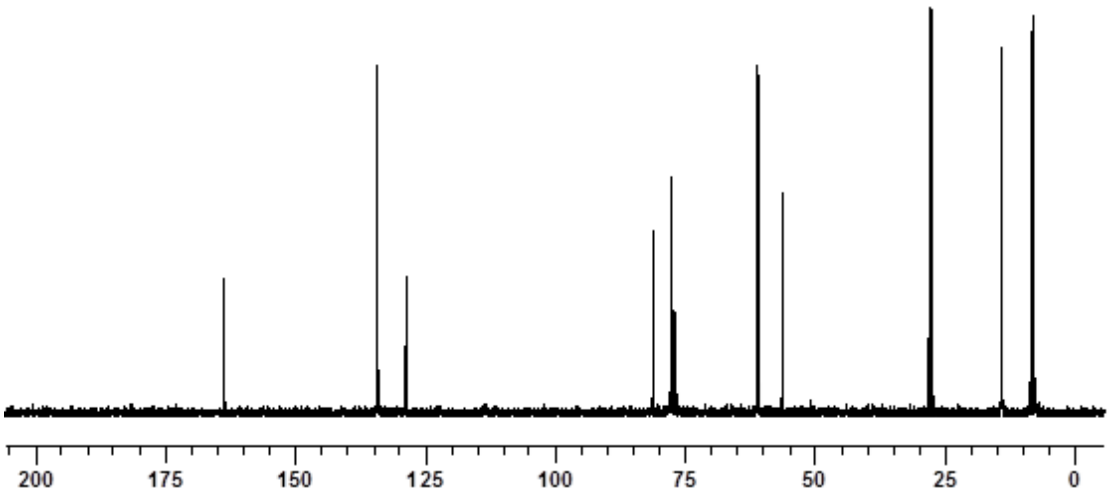


Figure A.6 ^{13}C-NMR ($CDCl_3$) Spectrum of (3*R*,4*R*,5*S*)-4-amino-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **21**

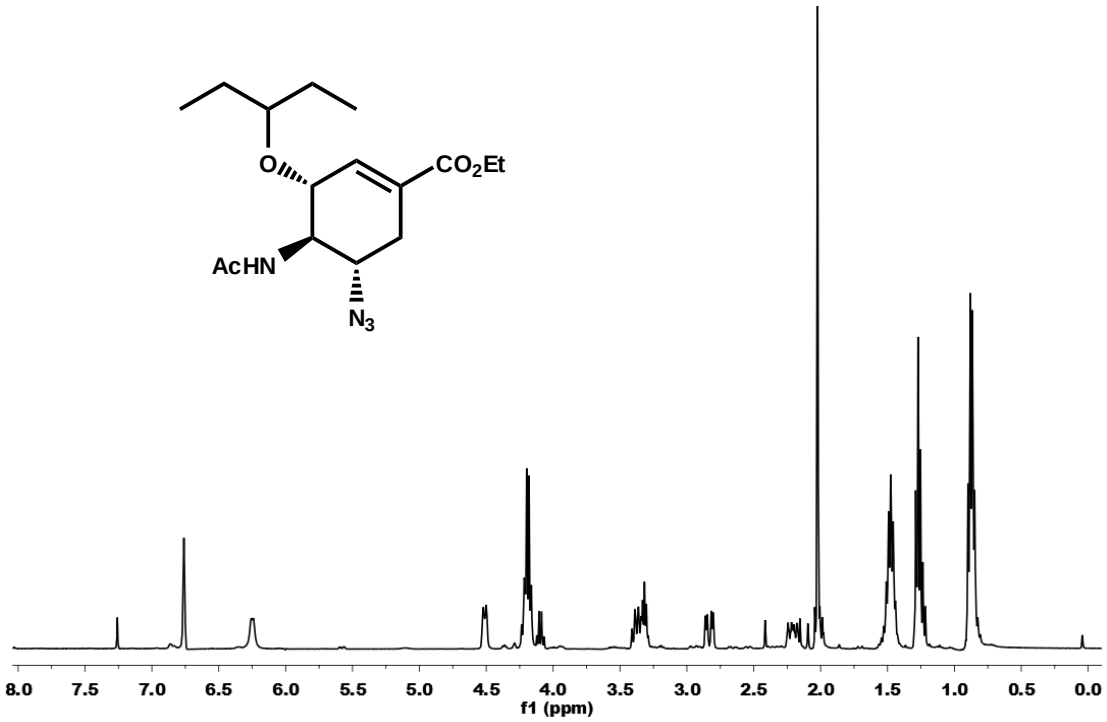


Figure A.7 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **22**

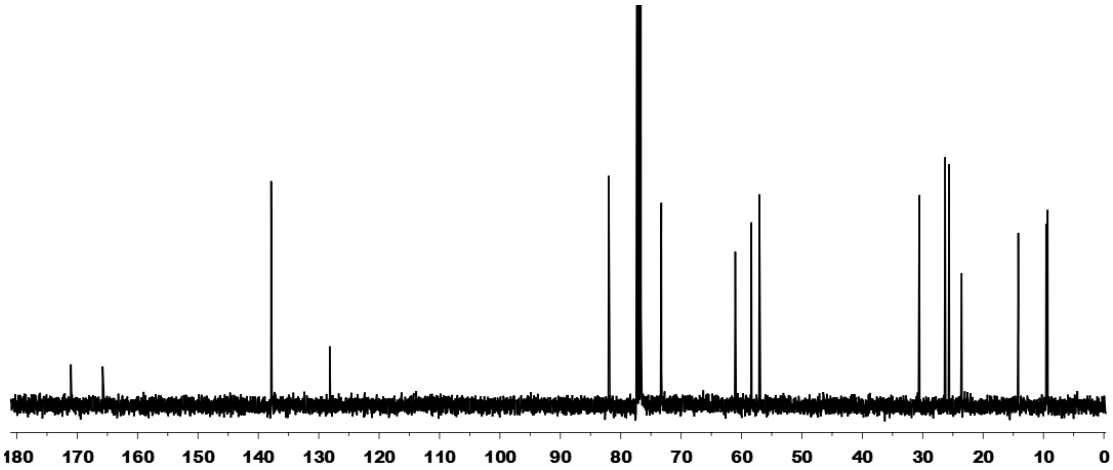


Figure A.8 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate **22**

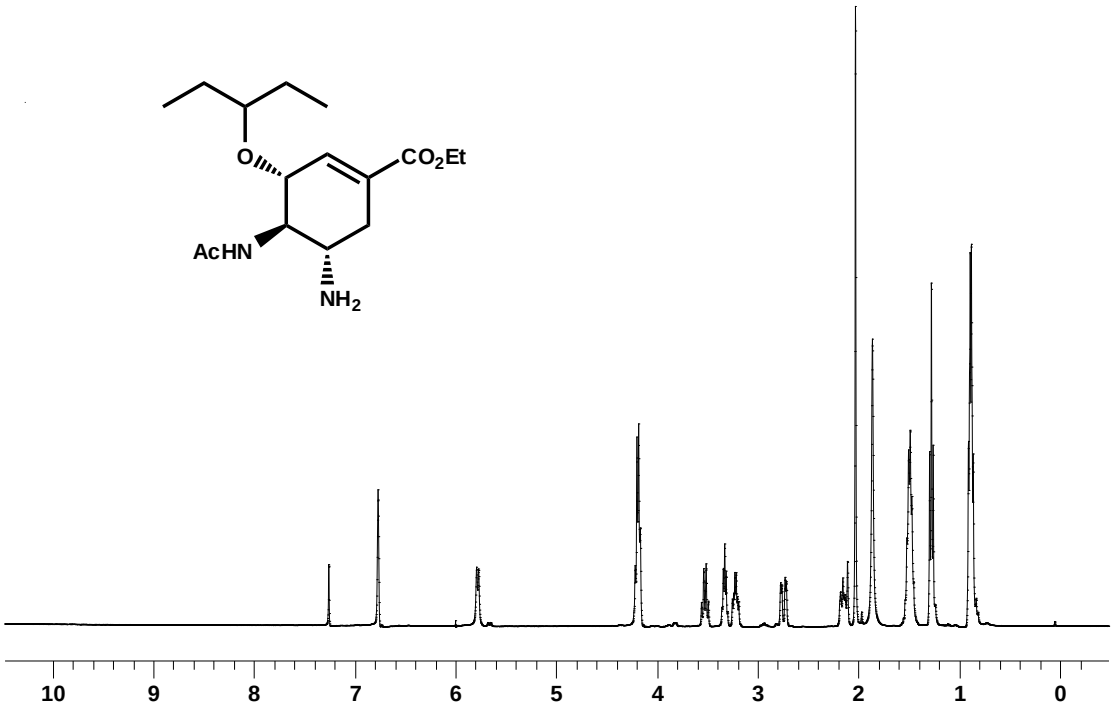


Figure A.9 ^{1}H-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetylamino-3-(1-ethyl-propoxy)-4-hydroxy cyclohex-1-ene-1-carboxylate (oseltamivir) **9**

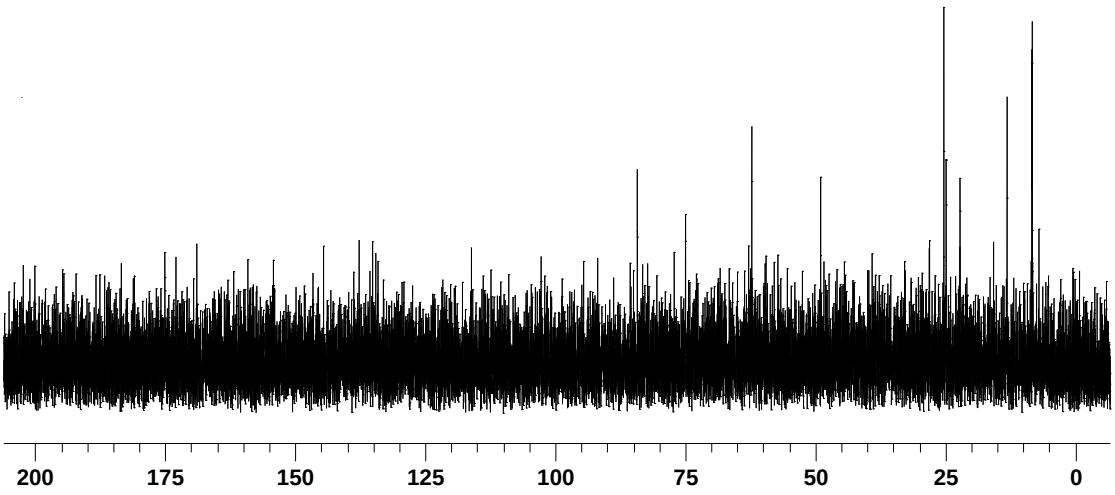


Figure A.10 ^{13}C-NMR ($CDCl_3$) Spectrum of ethyl (3*R*,4*R*,5*S*)-4,5-diacetylamino-3-(1-ethyl-propoxy)-4-hydroxy cyclohex-1-ene-1-carboxylate (oseltamivir) **9**

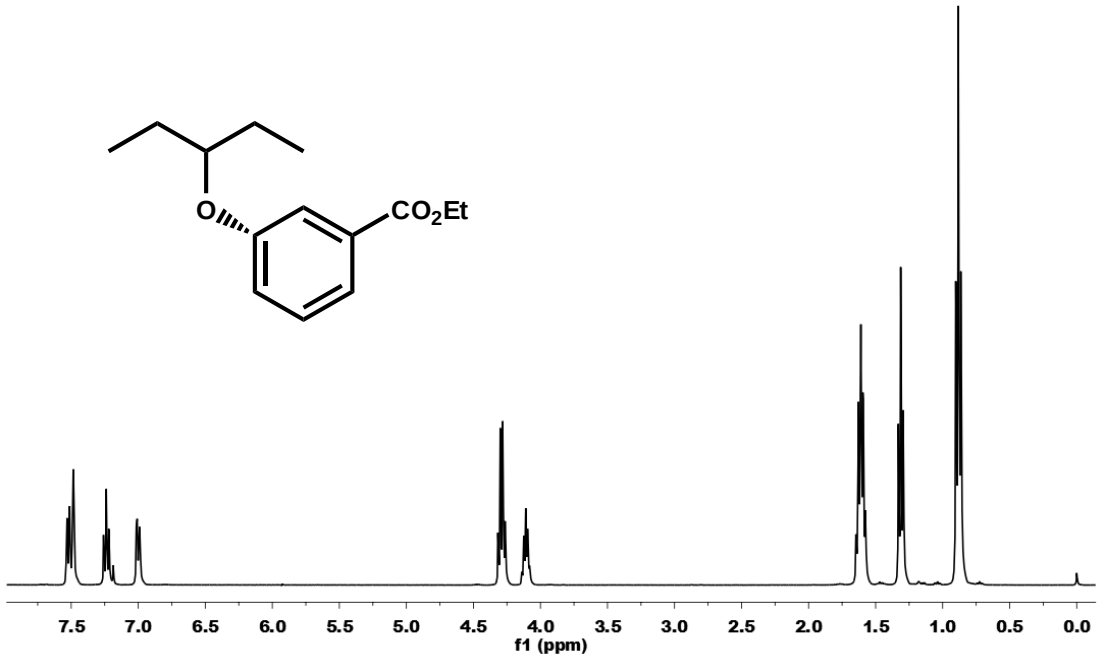


Figure A.11 ^{1}H-NMR ($CDCl_3$) Spectrum of compound **43**

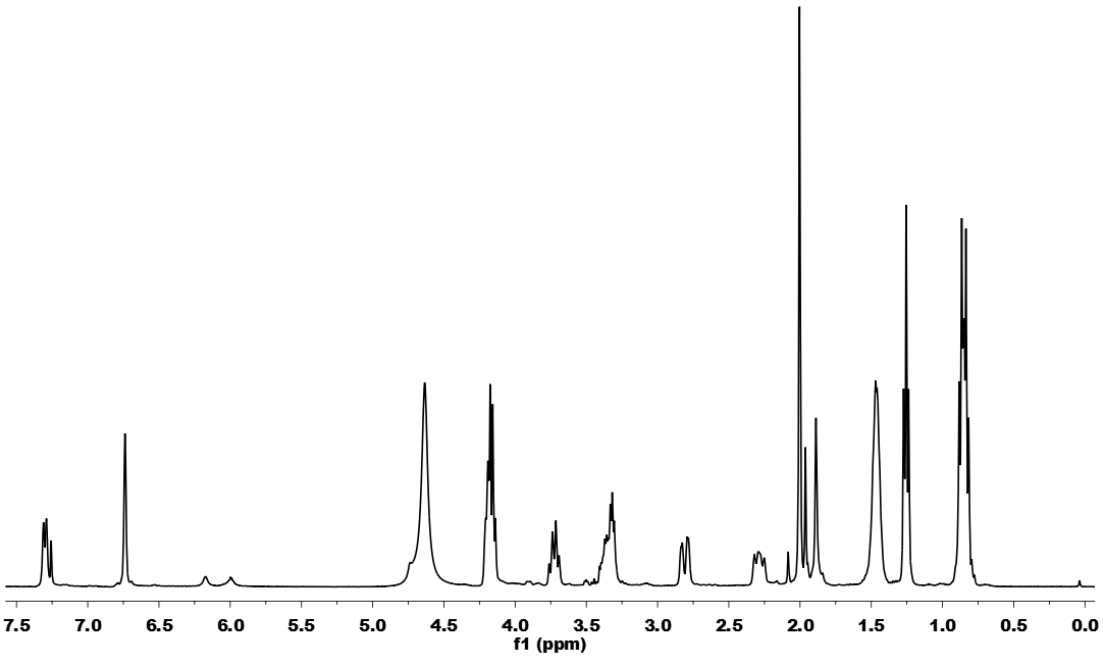


Figure A.12 ^{1}H-NMR ($CDCl_3$) Spectrum of unknown compound

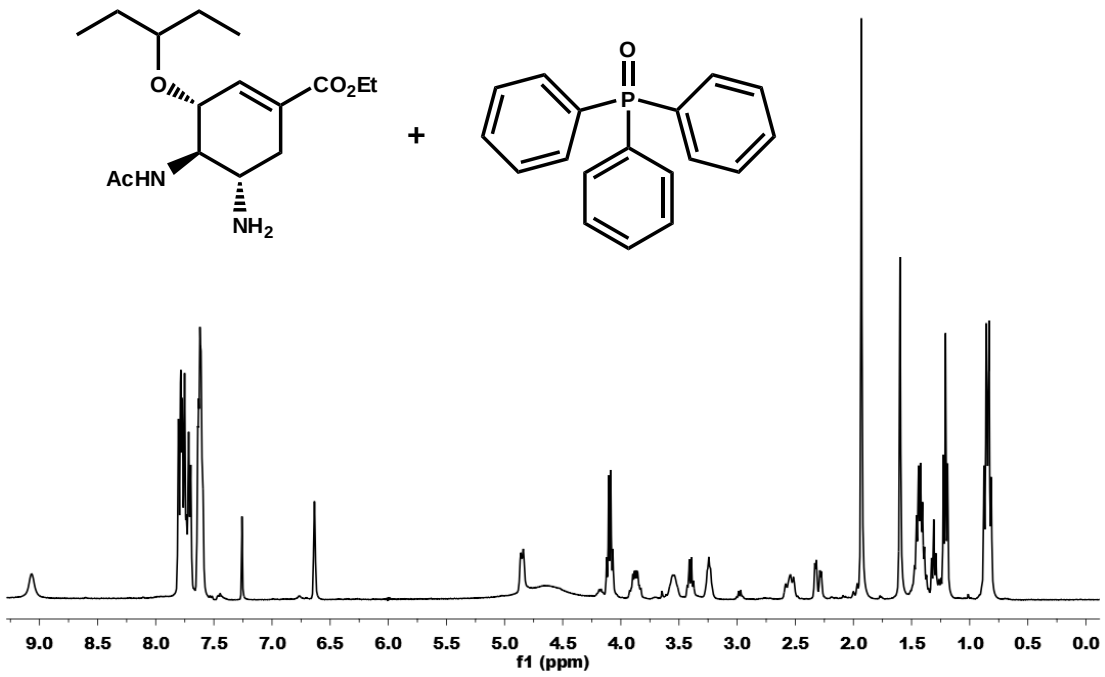


Figure A.13 ^{1}H-NMR ($CDCl_3$) Spectrum of mixture compound free base oseltamivir **9** and triphenylphosphineoxide

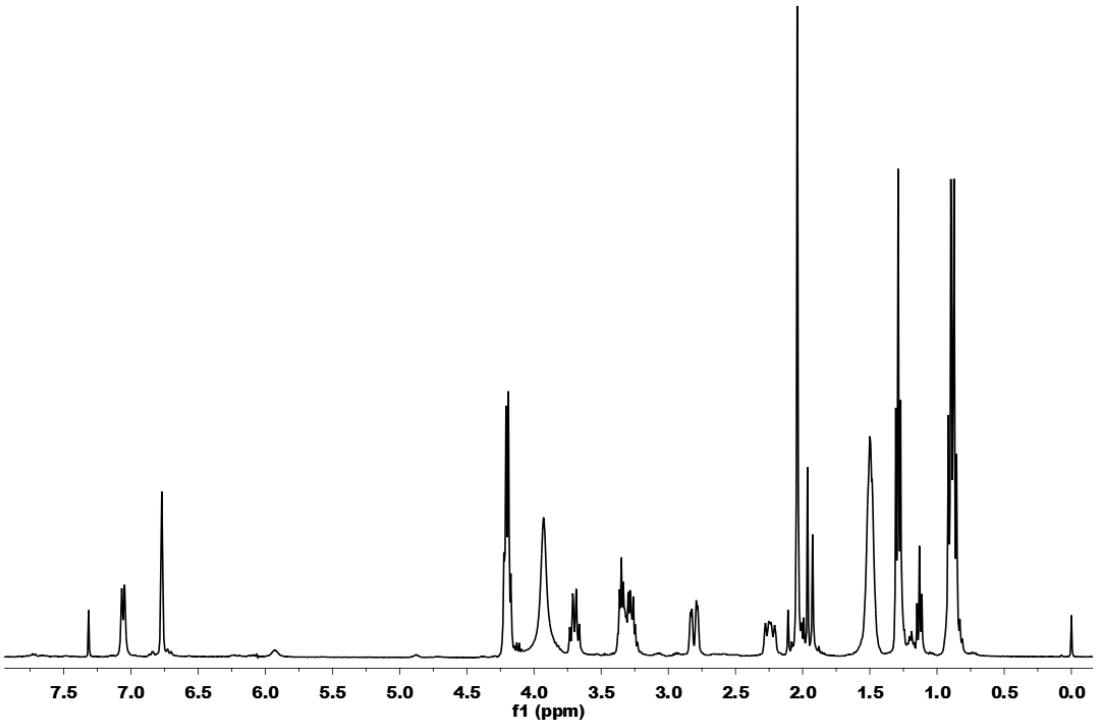


Figure A.14 ^{1}H-NMR ($CDCl_3$) Spectrum of free base oseltamivir **9** from the reduction by triphenylphosphine in THF and water 10:1

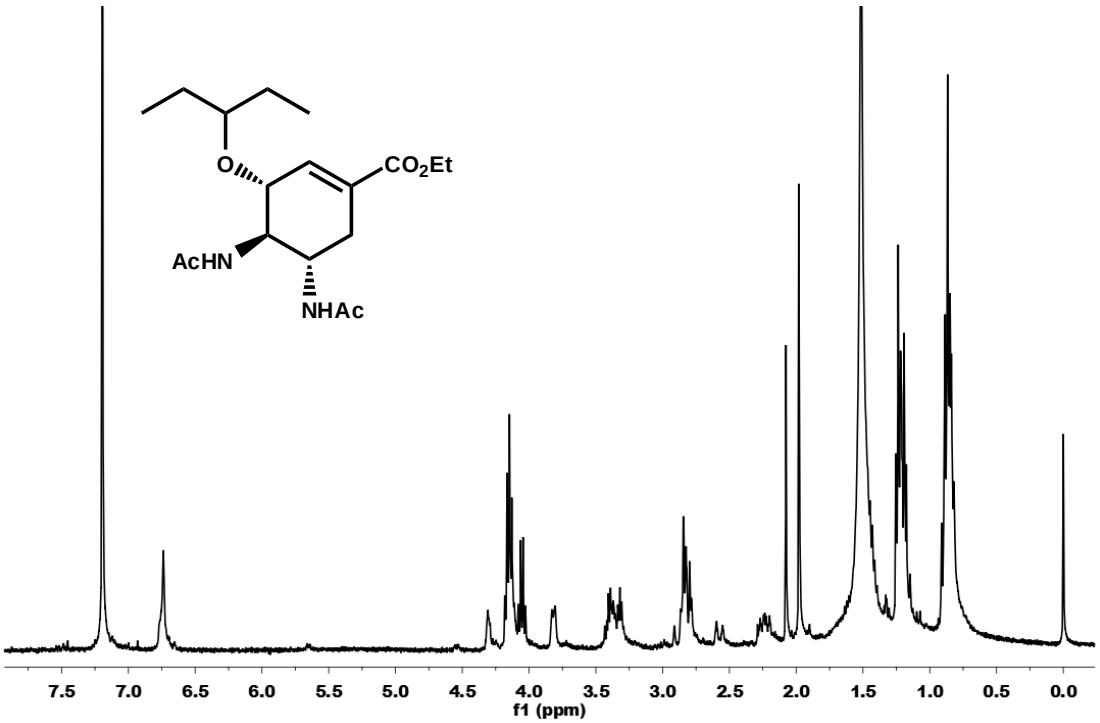
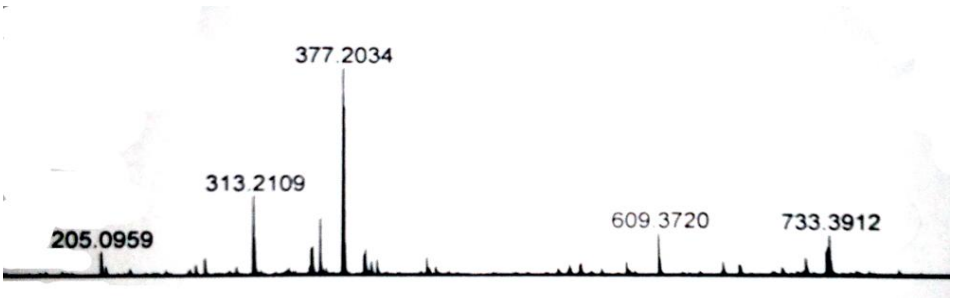


Figure A.15 ^{1}H-NMR ($CDCl_3$) Spectrum of diamide **45**



#	m/z	Res.	S/N	I	FWHW
1	205.0959	6402	2981.5	104783	0.0320
2	313.2109	7655	1713.4	374804	0.0409
3	377.2034	7171	2559.1	991907	0.0526
4	609.3720	9165	459.0	198138	0.0655
5	733.3912	9168	259.4	191180	0.0800

Figure A.16 High resolution mass spectrum of diamide **45**

VITAE

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