การสังเคราะห์สารมัธยันตร์สำคัญที่นำไปสู่ตัวยับยั้งเอนไซม์โปรทิเอสของเชื้อเอชไอวี

นางสาวประภาพรรณ โหมดหิรัญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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SYNTHESIS OF KEY INTERMEDIATES TOWARDS HIV PROTEASE INHIBITORS

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Thesis Title	SYNTHESIS OF KEY INTERMEDIATES TOWARDS HIV PROTEASE INHIBITORS
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Field of Study	Chemistry
Thesis Advisor	Assistant Professor Yongsak Sritana-anant, Ph.D.

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ประภาพรรณ โหมดหิรัญ: การสังเคราะห์สารมัธยันตร์สำคัญที่นำไปสู่ตัวยับยั้งเอนไซม์โปรทิเอสของเชื้อเอชไอวี (SYNTHESIS OF KEY INTERMEDIATES TOWARDS HIV PROTEASE INHIBITORS) อ.ที่ปรึกษา: ผศ. ดร. ยงศักดิ์ ศรีธนาอนันต์, 62 หน้า

ตัวยับยั้งเอนไซม์โปรทิเอสเป็นกลุ่มตัวยาที่สำคัญในการรักษาโรคเอดส์ ยาคาเลทราที่มีขายในท้องตลาดเป็นลิขสิทธิ์ของบริษัทแอ็บบอต ลาบอแรตอรีส จำกัด ซึ่งเป็นสูตรผสมของตัวยาที่มีประสิทธิภาพสูงสองชนิด คือ ริโทนาเวียร์และโลพินาเวียร์ โครงสร้างของยาทั้งสองนี้มีส่วนแกนกลางของโมเลกุลเหมือนกันคือ 2*S*,5*S*-ไดอะมิโน-1,6-ไดเฟนิล-3-เฮกซานอล และจากความซับซ้อนในการสังเคราะห์ส่วนแกนกลางตัวนี้ ทำให้ราคาของยาคาเลทราสูง และเป็นข้อจำกัดในการที่ผู้ป่วยจะเข้าถึงยา ดังนั้นจึงจำเป็นจะต้องหาวิธีการสังเคราะห์ที่สั้นและมีประสิทธิภาพมากกว่าวิธีเดิม วิธีที่ทดลองในงานวิจัยนี้เริ่มจาก แอล-เฟนิลอะลานีน เป็นสารตั้งต้น แล้วทำการเปลี่ยนหมู่ฟังก์ชั่นให้ได้ผลิตภัณฑ์เป็นอัลดีไฮด์ จากนั้นนำมาทำปฏิกิริยาเชื่อมต่อโดยใช้ปฏิกิริยาแมกเมอรี่ จะทำให้สามารถสังเคราะห์ไดอาสเตอริโอเมอริกไอโซเมอร์ของ 2*S*,5*S*-บิส[[(เทอร์เทียรีบิวทิวลอกซี)คาร์บอนิล]อะมิโน]-3,4-ไดไฮดรอกซี-1,6-ไดเฟนิลเฮกเซนได้ หลังจากขั้นตอนการแยกโดยใช้คอลัมน์โครมาโตกราฟีพบว่าสามารถแยก 2*S*,5*S*-บิส[[(เทอร์เทียรีบิวทิวลอกซี)คาร์บอนิล]อะมิโน]-3*S*,4*S*-ไดไฮดรอกซี-1,6-ไดเฟนิลเฮกเซนได้ใน 0.12% และ ไดอะสเตอริโอเมอร์ผสมระหว่าง 3*R*,4*R*-ไดไฮดรอกซี กับ 3*R*,4*S*-ไดไฮดรอกซี ในอัตราส่วน 1:1 ในปริมาณ 0.2% นอกเหนือไปจากการสังเคราะห์แกนกลางแล้ว ยังได้ทำการสังเคราะห์ส่วนซ้ายและขวาของยาโลพินาเวียร์โดย กรด 2,6-ไดเมทิลฟีนอกซีอะซิติก และ อนุพันธ์ของยูเรีย ได้ในปริมาณ 65% และ 5.1% ตามลำดับ

ภาควิชา.....เคมี.....ลายมือชื่อนิสิต.....ประภาพรรณ โหมดหิรัญ.....

สาขาวิชา.....เคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2550.....

##4872355523: MAJOR ORGANIC CHEMISTRY
KEYWORD: HIV PROTEASE INHIBITORS/KALETRA/LOPINAVIR

PRAPAPAN MODHIRAN: SYNTHESIS OF KEY INTERMEDIATES TOWARDS HIV PROTEASE INHIBITORS.THESIS ADVISOR: ASST. PROF. YONGSAK SRITANA-ANANT, Ph.D., 62 pp

HIV Protease inhibitors (PIs) are considered to be a highly potent class of drugs in AIDS therapy. The commercial anti-HIV drug Kaletra, patented by Abbott Laboratories, is the combination of two highly effective PIs; Ritonavir and Lopinavir. These two molecules contain the same 2*S*,5*S*-diamino-1,6-diphenyl-3-hexanol as their core structure. The rather complicate synthesis of this diamino alcohol core molecule effectively raises the price of Kaletra and limits the patients' access to this drug. Consequently, a shorter and more effective strategy is needed. In this research, a derivative of L-phenylalanine was used as the starting material. Functional group manipulations gave the *N*-[(*tert*-benzyloxy)carbonyl]-L-phenylalaninal as the main substrate. By using McMurry coupling as the key step, the diastereomeric mixture of 2,5-bis[[(*tert*-butyloxy)carbonyl]amino]-3,4-dihydroxy-1,6-diphenylhexane from the homocoupling of the starting aldehydes was obtained. After column chromatography gave 0.12% yield of *SSSS* isomer and 0.2% yield of diastereomeric mixture of *SRRS* and *SRSS* in 1:1 raito. In addition, the synthesis of the west and the east sides of Lopinavir, 2,6-Dimethylphenoxyacetic acid and urea derivative have been accomplished in 65% and 5.1% yield respectively.

Department……Chemistry……Student's signature……PRAPAPAN MODHIRAN……
Field of Study…Chemistry…….Advisor's signature……
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LIST OF ABBREVIATIONS

^{13}C-NMR	: carbon-13 nuclear magnetic resonance spectroscopy
^{1}H-NMR	: proton nuclear magnetic resonance spectroscopy
AcOH	: acetic acid
Boc_2O	: di-*tert*-butyl dicarbonate
CAN	: ceric ammonium nitrate
$CDCl_3$	: deuterated chloroform
d	: doublet (NMR)
dd	: double of doublet (NMR)
DMAc	: *N*,*N*'-dimethylacetamide
DME	: dimethoxyethane
DMSO	: dimethyl sulfoxide
DMSO-d_6	: hexadeuterated dimethyl sulfoxide
eq	: equivalent (s)
g	: gram (s)
h	: hour (s)
Hz	: hertz (s)
(-)-Ipc_2BCl	: diisopinocampheylchloroborane
J	: coupling constant
K_i	: inhibition constant
M	: molar (s)
m	: multiplet (NMR)
*m*CPBA	: *m*-chloroperoxybenzoic acid
m.p.	: melting point
MeOH	: methanol
mg	: milligram (s)
mL	: milliliter (s)
mM	: millimolar (s)
mmol	: millimole (s)
nM	: nanomolar (s)

MSA	: methanesulfonic acid
NaOCl	: sodium hypochlorite
Na_2SO_4	: sodium sulfate
NMR	: nuclear magnetic resonance spectroscopy
°C	: degrees Celsius
PCC	: pyridinium chlorochromate
PDC	: pyridinium dichromate
PI	: protease inhibitor
$PMPNH_2$	: 1-phenyl-3-methyl-5-pyrazolone
PMHS	: polymethylhydroxysilane
ppm	: part per million (unit of chemical shift)
py	: pyridine
q	: quartet (NMR)
rt	: room temperature
RT	: reverse transcriptase inhibitor
s	: singlet (s)
st	: stretching vibration (IR)
t	: triplet (NMR)
TEA	: triethylamine
TEMPO	: 2,2,6,6-tetramethyl-1-piperidinyloxy free radical
TEOA	: triethanolamine
TFA	: trifluoroacetic acid
THF	: tetrahydrofuran
$TiCl_4$	: titanium tetrachloride
TLC	: thin layer chromatography
TsCl	: *p*-toluenesulfonyl chloride
δ	: chemical shift

CHAPTER I

INTRODUCTION

1.1 Acquired immune deficiency syndrome (AIDS)

Acquired immune deficiency syndrome (AIDS) is a collection of symptoms and infections resulting from the specific damage to the immune system caused by the human immunodeficiency virus (HIV) in humans.[1] The spread of the AIDS has increasingly become one of the biggest social, economic and health problems. As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated that AIDS has killed more than 25 million people since it was first recognized in 1981, making it one of the most destructive pandemics in recorded history. In 2005 alone, AIDS claimed an estimated 2.4–3.3 million lives. It is estimated that about 0.6% of the world's living population is infected with HIV. In Thailand, it was estimated that 580,000 people were living with HIV.[2]

Table 1.1 Estimates of the global HIV&AIDS epidemic at the end of 2006

In 2006	Estimate (million)
People living with HIV/AIDS	39.5
People newly infected with HIV	4.5
AIDS deaths	2.9

1.2 Human immunodeficiency virus (HIV)

1.2.1 Structure of HIV

HIV was classified as a member of the retrovirus family. It is about 120 nm in diameter and roughly spherical. It is composed of two identical single-stranded RNAs

and enzymes needed for the development of the virion such as reverse transcriptase, protease, ribonuclease and integrase.[3,4]

1.2.2 Replication of HIV

HIV enters macrophages and CD4 T cells by the adsorption of glycoproteins on its surface to receptors on the target cell. Once HIV has bound to the target cell, its RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease and protease, are injected into the cell.[5] Then reverse transcriptase liberates the single-stranded RNA from the attached viral proteins and copies it into a complementary DNA and form a double-stranded viral DNA (vDNA) intermediate. This vDNA is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called integrase. The integrated provirus is copied to a full-length mRNA.[6] The polyprotein goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by protease and processed into the two HIV envelope glycoproteins. These are transported to the plasma membrane of the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins and enzymes. The various structural components then assemble to produce a mature HIV virion (**Figure 1.1**).[7]

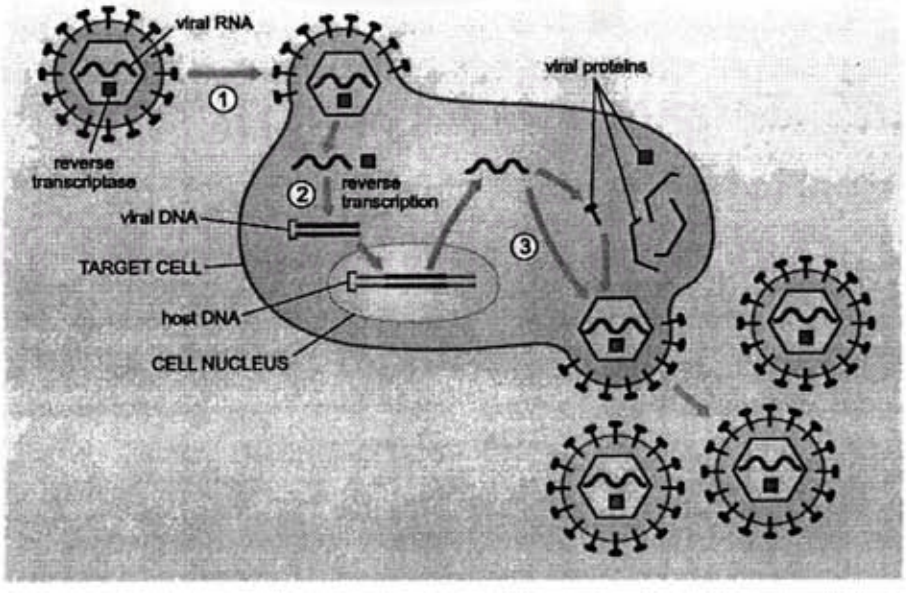


Figure 1.1 Schematic representation of the replication cycle of HIV

1.2.3 Treatment

There is currently no vaccine or cure for AIDS. The only known methods of prevention are based on avoiding exposure to the virus or, failing that, an antiretroviral treatment directly after a highly significant exposure, called post-exposure prophylaxis (PEP).[8] Currently, there have been five broad classifications of anti-retroviral drugs in development, reverse transcriptase inhibitors (RTs), protease inhibitor (PIs), integrase inhibitors, fusion inhibitors, entry inhibitors.

Reverse transcriptase inhibitor is the first class of drugs that FDA of the USA (Food and Drug Administration USA.) has approved; for example, zidovudine (AZT), didanozine (ddI) and zalzitabine (ddC). After the patients were treated with RTs drugs for a while, the drug resistance will occur.[9] So, the second class, protease inhibitors was later developed. For now, the optimal highly active antiretroviral therapy (HAART) is the best option for the patients. It consists of combinations (or "cocktails") of at least three drugs belonging to at least two classes of anti-retroviral agents.

In Thailand, the combination of drugs was selected by means of the side effect, when taken, and the price that the patients can afford. HIV treatment is highly cost because the patients have to take cocktail drugs for the rest of their lives. The 2 combinations of RTs will cost 3,500-5,000 Baht/person/month whereas the 3 combinations of RTs and PI will cost 20,000-25,000 Baht/person/month.[10] At this price of treatment, only some of the patients can access to the highly effective drugs like PIs. In order to provide the treatment for AIDS patients, the government added Efavirenz (RT) and Kaletra (PI) into the essential drug list in the year 2004.

Efavirenz is in the combination of a first line anti-retroviral treatment which is patented by Merck Sharp and Dohme Limited. Due to the high price, nevirapine, the RT which is locally produced in Thailand, is used instead. Approximately 20% of the patients using nevirapine will develop adverse drug reactions. Only when they become adverse drug reaction, they will be switched to Efavirenz based one. After taking the first line drugs for a few years, around 10% of the patients will develop drug resistance and will need second line anti-retroviral treatment, the combination that contain PIs; such as, Kaletra which is patented by Abbott Laboratories Limited.

Since October 2003, the government committed the policy of universal access to essential drugs for all Thais, the public health budget was raised in order to provide the anti-retroviral drugs for the patients. The budget to access anti-retroviral drugs alone is more than 3,800 million baht. This budget is to be the highest among the lower middle income developing countries. However the public health still cannot afford the universal access for AIDS patients. With the government compulsory use of Patent, the Efavirenz price will drop from 1,400 Baht/person/month to 650 Baht/person/month while the price of Kaletra will drop from 5,900 Baht/person/month to 4,800 Baht/person/month.[11]

1.3 HIV Protease

A protease is an essential enzyme for the viral replication that cleaves proteins to their component peptides. The HIV PR hydrolyzes viral polyprotein into functional protein products that are essential for viral assembly and subsequent activity. In 1989, the protease enzyme of HIV was crystallized and its three-dimensional structure was determined by Navia *et al.* [12]. Subsequently, a more accurate structure was reported by Kent *et al* [13]. The HIV protease enzyme is a homodimer composed of two identical 99-amino acid chains [14], with each chain containing the active site aspartic acid at positions 25 (Asp 25).[15] The structure can be separated into three parts: the flap, core and terminal domains.

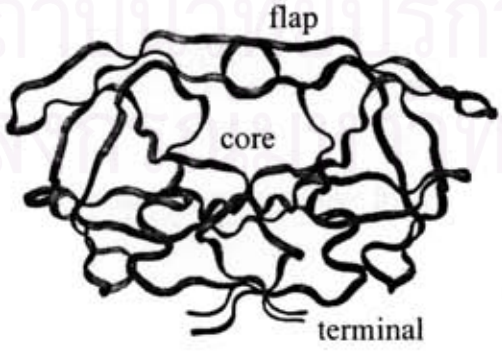


Figure 1.2 HIV-1 Protease enzyme

1.4 HIV Protease inhibitors

Protease inhibitors block the activity of the enzyme and prevent viral replication. The amino residues that involve in the major binding with inhibitor are the catalytic residue Asp25 and Asp25' in core domain. The minor binding residues are lle50, lle50' in flap domain. The presence of water molecule has been observed in most of the crystal structures of protease bound to different inhibitors.[16] The hydrogen bonds of this water molecule apply strain on the scissile amide bond, causing it to rotate out of the plane and lose its double bond character, which enhances its vulnerability towards hydrolysis (**Figure 1.5**).[17]

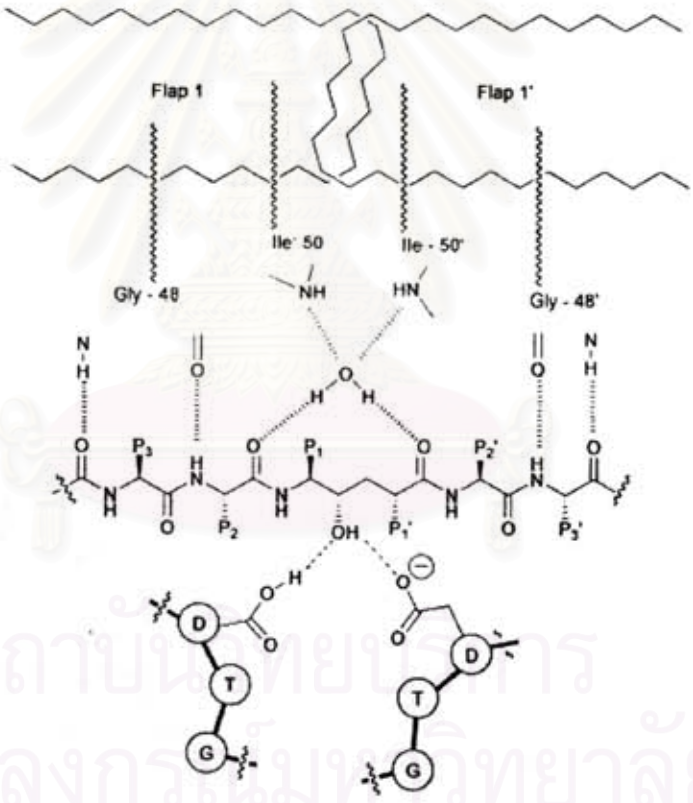


Figure 1.3 The water molecule makes four hydrogen bonds with the HIV protease and the inhibitor.

The structure and mechanism of HIV protease and its inhibitor have paved the way towards the development of effective drugs for this enzyme. In the commercially available drugs and some of the inhibitors that are being or have been tested clinically, contain the core of hydroxyethylene isosteres, diaminoalcohols, and other

related molecules with more than one stereocenters (**Figure 1.6**).[19-22] The core is a good isostere replacement at the scissile bond that is believed to mimic the tetrahedral transition state of the proteolytic reaction. There are now ten FDA approved protease inhibitors in clinical use. They are Saquinavir, Amprenavir, Indinavir, Nelfinavir, Ritonavir, Lopinavir, Atazanavir, Tipranavir, Fosamprenavir and Darunavir (**Table 1.2**).

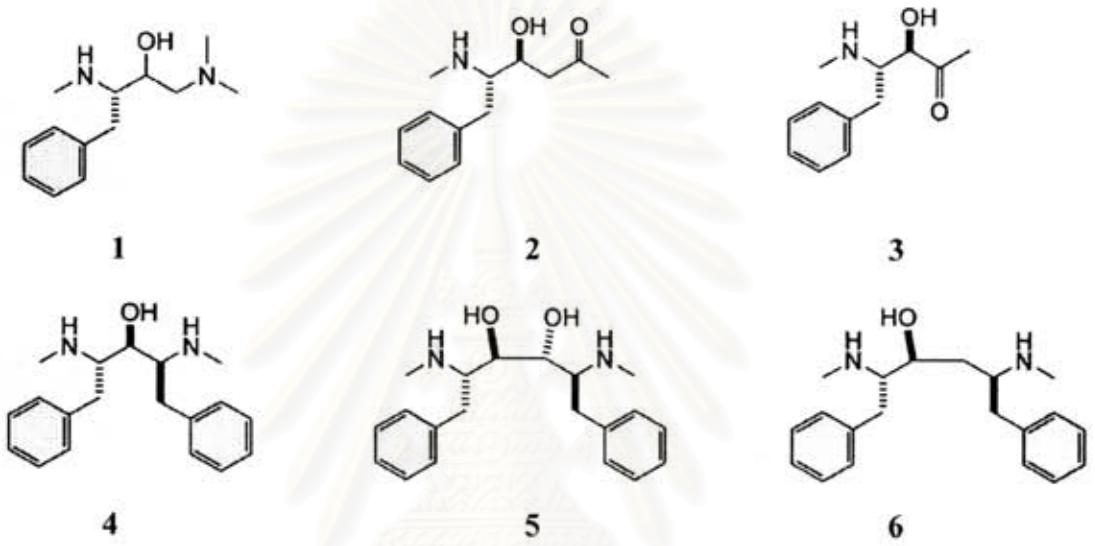


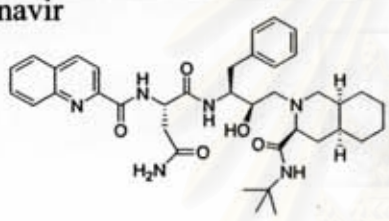
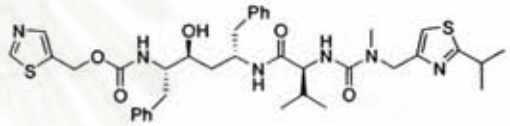
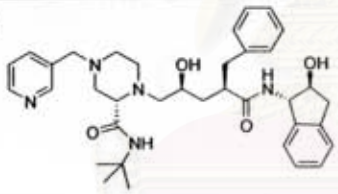
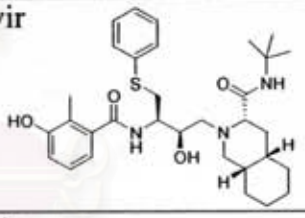
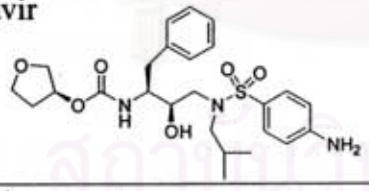
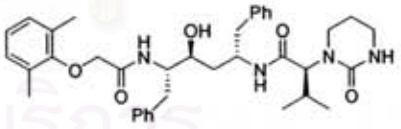
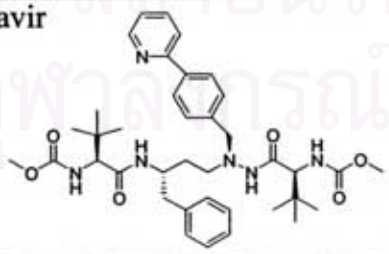
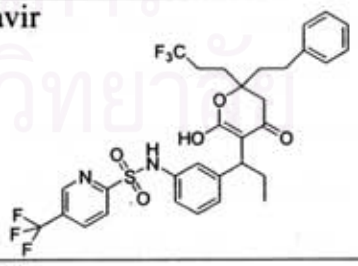
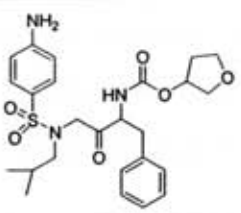
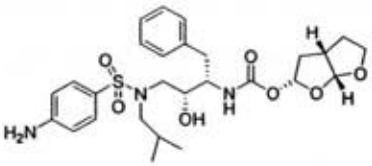
Figure 1.4 Typical cores of the substrate models of PIs

Saquinavir,[23] which was discovered by Hoffmann-La Roche, is the first FDA approved drug in 1995 for the treatment of AIDS. This potent inhibitor (K_i = 0.12 nM) has the hydroxyethylamine isostere replacement for the Phe-Pro cleavage site. The structure of this inhibitor was quite interesting because it had the *R* stereochemistry at the carbon bearing the central hydroxy group, which was unique among aspartic protease inhibitors that were known at that time. Both Amprenavir (K_i = 0.6 nM), which was discovered by Vertex Pharmaceuticals [24] and approved by the FDA in 1999, and Nelfinavir (K_i = 2 nM), which was discovered by a collaborative effort between Lilly and Agouron [25] and approved by the FDA in 1997, have similar hydroxyethylamine isostere replacement to that found in Saquinavir.

Research group at Merck discovered a novel variation of the hydroxyethylene transition state analog in their new drug, Indinavir (K_i = 0.56 nM),[26] which was

approved by the FDA in 1996. Abbott Laboratories have used the (*S*,*S*,*S*)-amino alcohol to prepare two of the commercially available drugs: Ritonavir (K_i = 0.01 nM),[27] which was approved by the FDA in 1996, and Lopinavir (K_i = 0.003 nM),[28] which is amomg the latest approved HIV protease inhibitors in 2000. Lopinavir is contained in a protease inhibitor formulation (Kaletra®) which includes Ritonavir. Ritonavir is known to inhibit cytochrome P-450 3A, the enzyme responsible for the metabolism of Lopinavir. Therefore this combination allows for increased plasma levels of Lopinavir.

Table 1.2 HIV-1 protease inhibitors

Saquinavir 	Ritonavir 
Indinavir 	Nelfinavir 
Amprenavir 	Lopinavir 
Atazanavir 	Tipranavir 
Fosamprenavir 	Darunavir 

The similarity between Ritonavir and Lopinavir allowed us to take an advantage in synthesizing their mutual intermediate (**Figure 1.7**). When one considers the retrosynthesis, both Ritonavir and Lopinavir can be divided into three parts. The general synthesis is to synthesize each part individually as carboxylic acid derivatives and a diamino compound, then use the peptide coupling reactions to connect them. The most difficult part to synthesize is the middle part, the core diamino alcohol **8**.

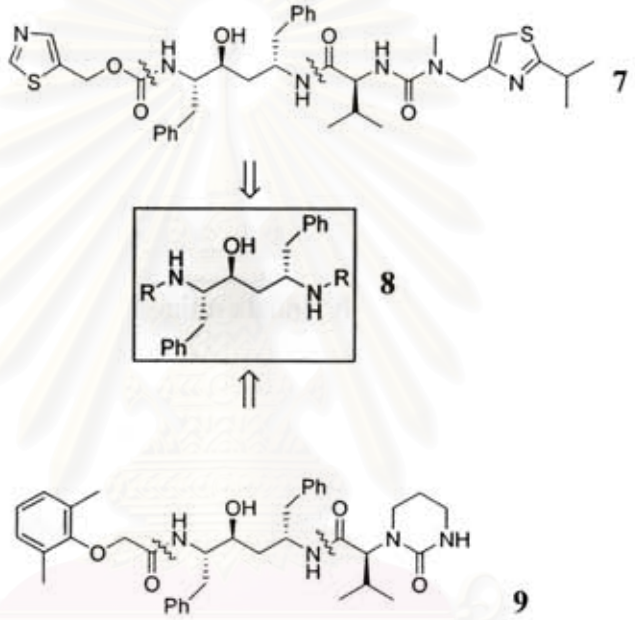
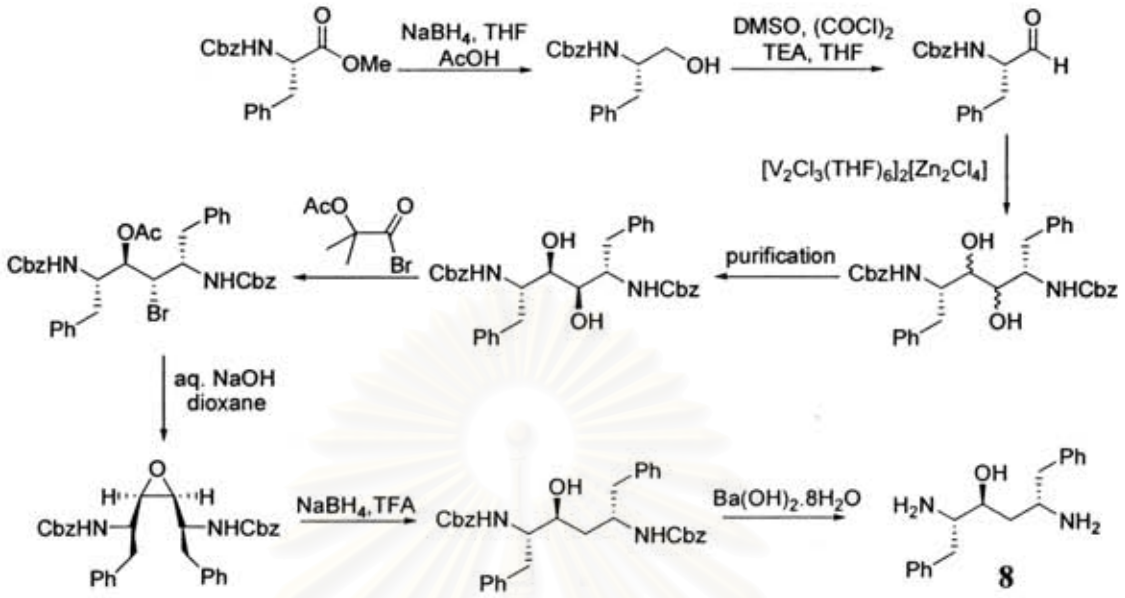


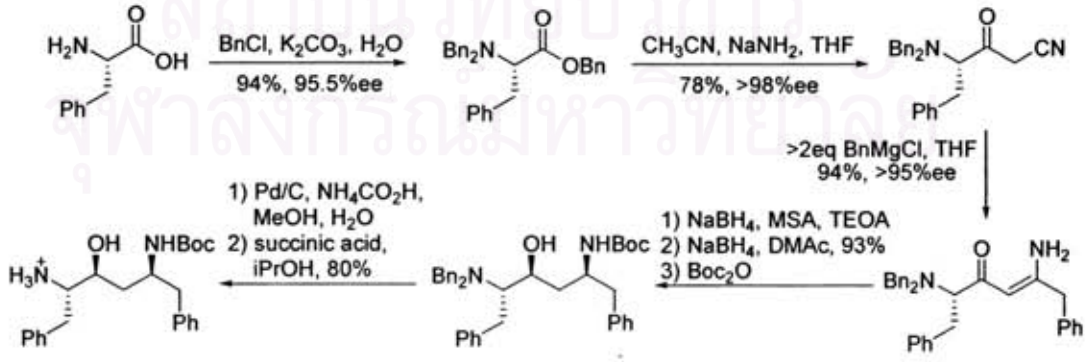
Figure 1.5 Retrosynthesis of Ritonavir and Lopinavir

In 1992, the early phase of development of Ritonavir and Lopinavir, L-phenylalanine methyl ester was first used as the starting material. *N*-Cbz-phenylalaninol, the reduction product, was subjected to Swern oxidation to give *N*-Cbz-phenylalaninal. The homocoupling of the resulting aldehyde was carried out with Pedersen's procedure to give the diastereoisomer of diols. After the purification step, the desired diol was then converted to bromohydrin acetal and epoxide, respectively. The core molecule was obtained after deprotection of the Cbz group (**Scheme 1.1**). This process took advantage of the physical and chemical properties of each intermediate to obtain the pure product.[29,30]



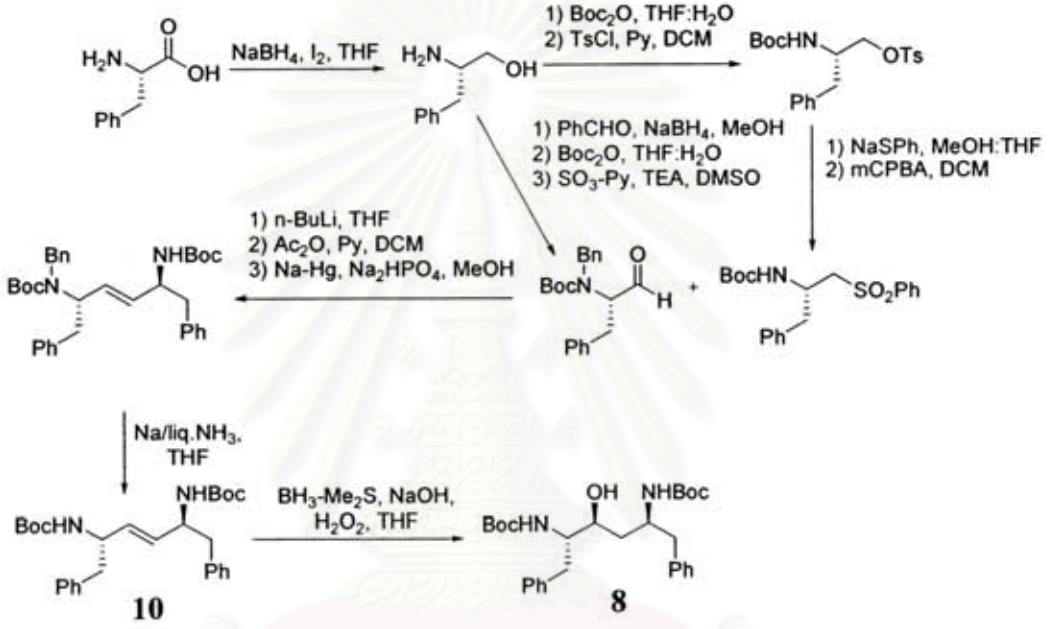
Scheme 1.1 Synthesis of the diamino alcohol **8** reported by Dale *et al.*[29,30]

In 1994, Stuk and coworkers published an efficient strategy, which was reported in U.S. patent,[31] to synthesize the core of Ritonavir and other related protease inhibitors. L-Phenylalanine was trialkylated with benzyl chloride followed by the nucleophilic acyl substitution by α-deprotonated acetonitrile anion and then allowed to react with benzyl Grignard reagent. The enaminone intermediate was reduced in two steps and then protected and deprotected amino groups. The pure monoprotected diamino alcohol was obtained by crystallization in the succinate form (**Scheme 1.2**).[32-34]



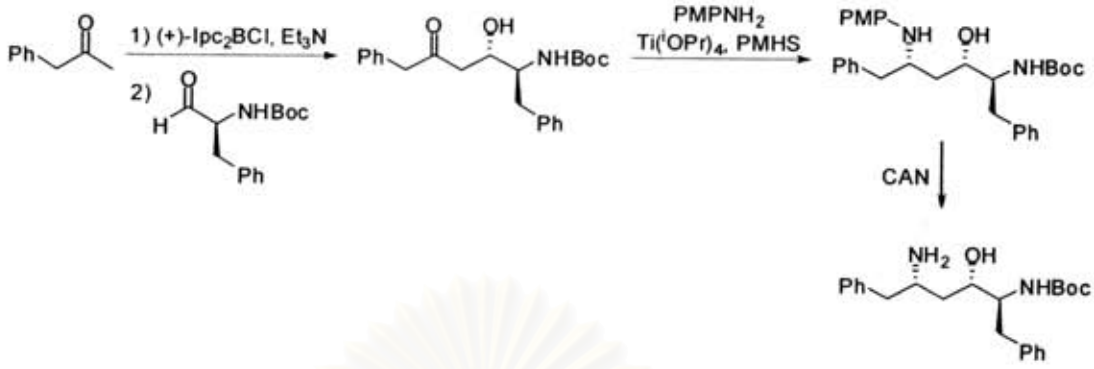
Scheme 1.2 Synthesis of the protected diamino alcohol **8** reported by Stuk *et al.*[32-34]

Later in 1995, Rao and coworkers reported a Julia's olefination strategy toward the synthesis of diaminoalcohol **8**. The same starting material, L-phenylalanine, was used to construct the diaminoalcohol core in thirteen steps. The key step is to synthesize C2 symmetric alkene **10,** then asymmetric hydroboration reaction was used to generate the last chiral center needed in this diaminoalcohol (**Scheme 1.3**).[35,36]



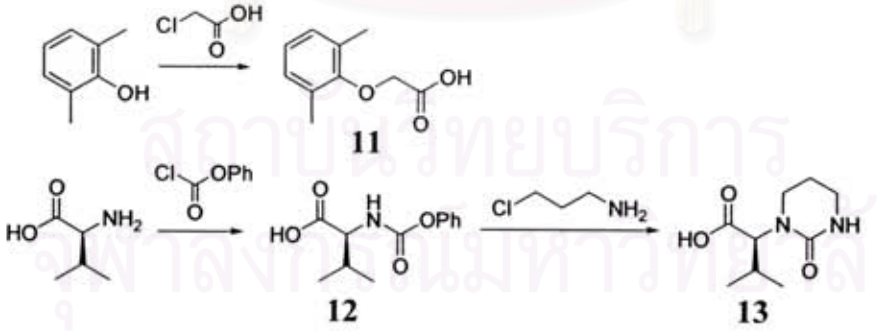
Scheme 1.3 Synthesis of the protected diamino alcohol **8** reported by Rao *et al.*[35,36]

Recently in 2007, Menche *et al.* reported the shortest route to synthesize core intermediate **8**. After the stereoselective aldol condensation by chiral Ipc-boron-mediated enolate methodology, β-hydroxy ketone was obtained. Then it was submitted to a one-pot-type reaction for a directed amination. After deprotection of the PMP group, gave the desired diamino alcohol was obtained in 56% yield (**Scheme 1.4**).[37]



Scheme 1.4 Synthesis of the protected diamino alcohol **8** reported by Menche *et al.*[37]

The other two parts of Lopinavir (**9**), the west and east side, can be synthesized as Hayes *et al.* and Stoner *et al.* reported, respectively.[38,39] The west side was synthesized as 2,6-dimethylphenoxyacetic acid **11** by nucleophilic substitution of chloroacetate anion by 2,6-dimethylphenoxide in one step. While the east side, cyclic urea derivative **13**, can be synthesized in three steps. The nucleophilic acyl substitution of L-valine and phenylchloroformate gave the intermediate which was reacted with 3-chloropropylamine followed by cyclization to give cyclic urea **13** (**Scheme1.5**). Finally, the three intermediates were connected together by using peptide coupling reaction as stated above.



Scheme 1.5 Synthesis of 2,6-dimethylphenoxyacetic acid **10** and cyclic urea **13** [38,39]

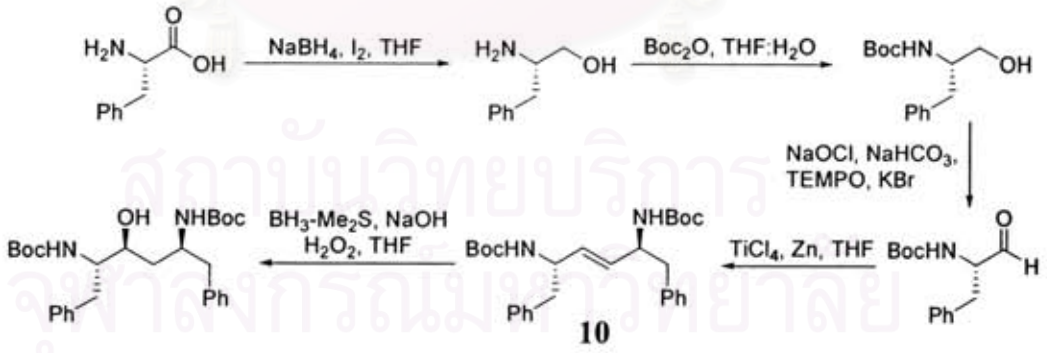
In Thailand, there is no report on the synthesis of any protease inhibitors. All these potent inhibitors must be imported in the form of ready-made drugs or active

ingredients. This raises the price and cost of the drugs and makes them inaccessible for AIDS patients. The ability to make the active compounds domestically would lower such high import expenses, increase access to healthcare systems and improve the quality of lives of the patients and the society. Among all the protease inhibitors and anti HIV drugs, Kaletra, the mixture of Ritonavir and Lopinavir, was selected for the reason that they are highly effective against both wild type and mutant HIV protease. Both active inhibitors in Kaletra can be assembled from the same core structure, the diamino alcohol **8**.

The strategy that is used in large scale synthesis of diamino alcohol **8** is the one reported in the patent (**Scheme 1.2**). Other strategies that have been developing will provide the easier and shorter way to synthesize the core **8**. In this research, the similar intermediate **10**, as Rao *et al.* reported (**Scheme 1.3**) [35,36], will be considered as the key step of synthesis.

1.5 Objectives

This research is aimed to use the new strategy (**Scheme 1.6**) to synthesize diamino alcohol core through McMurry coupling. The designed strategy is less involved with the asymmetric synthesis and shorter than the strategy described above.



Scheme 1.6 The new synthetic route in this research

The main challenge would be the development of effective strategies to synthesize the core molecule together with their two side wings and then connect them, to obtain the desired drugs. To this end, Lopinavir was chosen to be the target compound in this research.

CHAPTER II

EXPERIMENTS

2.1 Instruments and Equipments

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) using Varian Mercury NMR spectrometer operated at 400.00 MHz for ^{1}H and 100.00 MHz for ^{13}C nuclei (Varian Company, USA). The mass spectra were recorded on Mass Spectrometer: Waters Micromass Quatto micro API ESCi (Waters, USA).

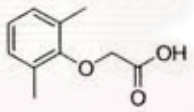
2.2 Chemicals

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 or Scharlau Chemie S. A., Barcelona, Spain). Solvents used in the synthesis were reagent or analytical grades. Solvents used in column chromatography were distilled from commercial grade solvents prior to used. Other reagents were purchased from the following venders:

- Labscan (Bangkok, Thailand): chloroform, concentrated hydrochloric acid, *n*-heptane, potassium hydroxide, toluene, tetrahydrofuran, dimethylsulfoxide, acetonitrile
- Acrös Organic (USA): pyridine dichlorochromate, 2,2,6,6-tetramethylpiperidinooxy, L-valine, phenyl chloroformate, lithium hydroxide monohydrate, 3-chloropropylamine hydrochloride, 2,6-dimethylphenol, chloroacetic acid, sodium amide

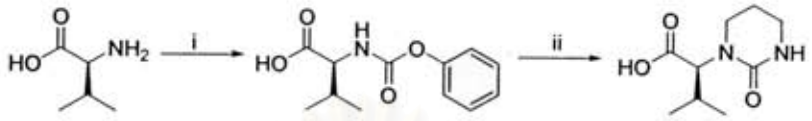
- Carlo Erba Reagent (Milan, Italy): iodine, potassium bromide, sodium hypochlorite, lithium chloride, aluminum oxide, benzyl chloride
- Fluka Chemical Company (Buchs, Switzerland): sodium borohydride, triethylamine, oxalyl chloride, pyridinium chlorochromate, di-*tert*-butyl dicarbonate, potassium *tert*-butoxide
- Merck Co. Ltd. (Darmstadt, Germany): ethanol, anhydrous sodium hydrogencarbonate, sodium hydroxide, sodium chloride, titanium tetrachloride, zinc dust
- Riedel-de Haën: anhydrous sodium sulfate
- BDH Chemicals (Poole, England): potassium iodide, sodium thiosulfate
- Wilmad (New Jersey, USA.): deuterated chloroform, hexadeuterated dimethylsulfoxide

2.3 Synthesis of 2,6-dimethylphenoxyacetic acid (11)



2,6-Dimethylphenol (0.6788 g, 5.56 mmol), chloroacetic acid (0.63 g, 6.67 mmol) and NaOH (0.45 g, 11.25 mmol) were dissolved in water (6 mL) and refluxed under nitrogen. After 6 hours, chloroacetic acid (0.42 g, 4.45 mmol) was added and the reaction was continued to reflux for another 3 hours. The reaction mixture was acidified with 10% aqueous HCl and extracted with diethyl ether. The organic layer was then washed with $NaHCO_3$ solution and the aqueous extracts were collected. This aqueous layer was re-acidified with 10% HCl and re-extracted with diethyl ether. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed. Recrystallization gave a pale yellow solid (0.6490 g, 65% yield). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.04 (d, 2H), 7.01 (t, 1H), 4.51 (s, 2H), 2.27 (s, 6H) (**Figure A.1**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 172, 154, 130, 129, 123, 68, 16 (**Figure A.2**) [38]

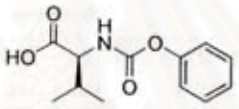
2.4 Synthesis of intermediate 13



12 **13**

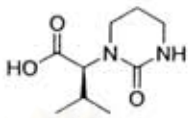
i) PhOCOCl, LiCl, Al_2O_3, LiOH
ii) 3-chloropropylamine, NaOH

Synthesis of *N*-phenoxycarbonyl-L-valine (**12**)



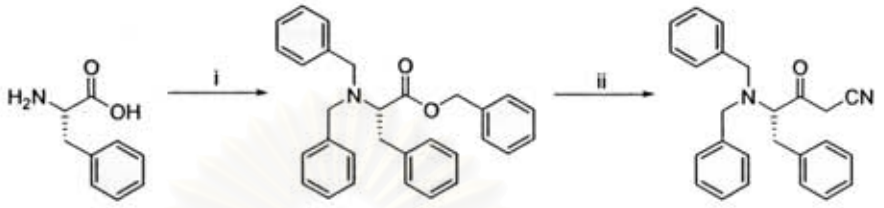
To the solution of L-valine (2.0019 g, 17.1 mmol) in water (20 mL) was added LiCl (3.99 g, 94.1 mmol) and Al_2O_3 (0.64g, 5.4 mmol) and cooled to -10 °C in an ice-salt bath. The pH value of the suspension mixture was adjusted to 10 using 3.2 M solution of LiOH. Phenyl chloroformate (2.2 mL, 17.5 mmol), precooled to -20 °C, was added. Additional 3.2 M solution of LiOH was added slowly to maintain the pH of the mixture constant between 9.8 and 10.0 and the temperature less than -5 °C. After 3 hours of adding LiOH solution into the stirred reaction, the white suspension was filtered then washed with water. The combined aqueous phase was washed twice with diethyl ether. After neutralized to pH 2 with concentrated H_2SO_4, the organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed (at below 50°C) to give the colorless gel product (0.2924 g, 11% yield). ^{1}H-NMR (CDC_{l3}): δ (ppm) = 7.0-7.2 (m, 5H), 5.4 (d, 1H, J = 9.0 Hz), 4.25 (dd, 1H, J = 4.2, 9.1 Hz), 2.2 (m, 1H), 1.0 (dd, 6H, J = 7.6, 29.0 Hz) (**FigureA.3**).

Synthesis of (*S*)-tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetic acid (**13**)



Compound **12** (0.2324 g, 0.981 mmol) and 3-chloropropylamine hydrochloride (1.64 g, 1.18 mmol) were dissolved in THF (5 mL) and cooled to 0 °C. NaOH (0.12 g, 3.17 mmol) was added to the stirring suspension. The reaction was stirred at less than 10 °C for 2 hours. A solution of potassium *tert*-butoxide (0.30 g, 2.67 mmol) in THF (5 mL) was slowly added to the reaction mixture. After stirred for 18 hours, water (8 mL) was added to quench the reaction then acidified to pH 9 with concentrated HCl. The aqueous layer was separated and added ethanol (3.2 mL). This aqueous layer was acidified to pH 3 with concentrated HCl and extracted twice with EtOAc. The combined organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed (at below 50 °C) to give the residue solid. The solid was dissolved in absolute ethanol and added charcoal to remove color. After filtration, the solvent was evaporated approximately one-third of the total volume. The suspension was then cooled to 0 °C for 1 hour. The light yellow solid was isolated by filtration and dried at less than 45 °C (0.1019 g, 45% yield). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.40 (s, 1H), 4.6 (d, 1H, J = 10.5 Hz), 3.1-3.3 (m, 4H), 2.1 (m, 1H), 1.8 (m, 2H), 0.8-1.0 (dd, 6H, J = 7.3, 43 Hz) (**Figure A.4**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 173, 158, 62, 42, 27, 22, 20, 19 (**Figure A.5**)

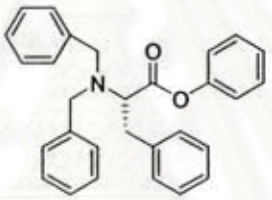
2.5 Synthesis of L-phenylanine derivatives (route 1)



14 **15**

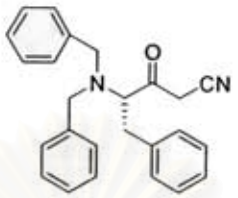
i) BnCl, K_2CO_3
ii) CH_3CN, $NaNH_2$

Synthesis of *N*,*N*-dibenzyl-(L)-phenylalanine benzyl ester (**14**)



A solution containing L-phenylalanine (2.5 g, 15.2 mmol), K_2CO_3 (6.91 g, 45.6 mmol), water (15 mL), ethanol (8 mL) and benzyl chloride (5.75 mL, 45.6 mmol) was heated to reflux for 10 hours. The reaction mixture was cooled to 50 °C. Hexane (5 mL) and water (2 mL) were added, stirred and the organic layer was separated. It was washed once with 1:1 water/methanol mixture and dried over anhydrous Na_2SO_4. The solvent was removed to give the product as colorless oil (0.6063 g, 92% yield). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.05-7.45 (m, 20H), 5.26 (d, 1H, *J* = 19.7 Hz), 5.14 (d, 1H, *J* = 13.2 Hz), 3.80 (d, 2H, *J* = 14.0 Hz), 3.75 (t, 1H, *J* = 8.5, 16.4 Hz), 3.57 (d, 2H, *J* = 14.0 Hz), 3.17 (dd, 1H, *J* = 7.1, 14.0 Hz), 3.04 (dd, 1H, *J* = 9.4, 14.6 Hz) (**Figure A.6**) ^{13}C-NMR ($CDCl_3$): δ (ppm) = 172, 139, 138, 136, 130, 129, 127, 126, 66, 62, 54, 36 (**Figure A.7**).

Synthesis of (4*S*)-4-(*N*,*N*-dibenzylamino)-3-oxo-5-phenylpentanonitrile (**15**)

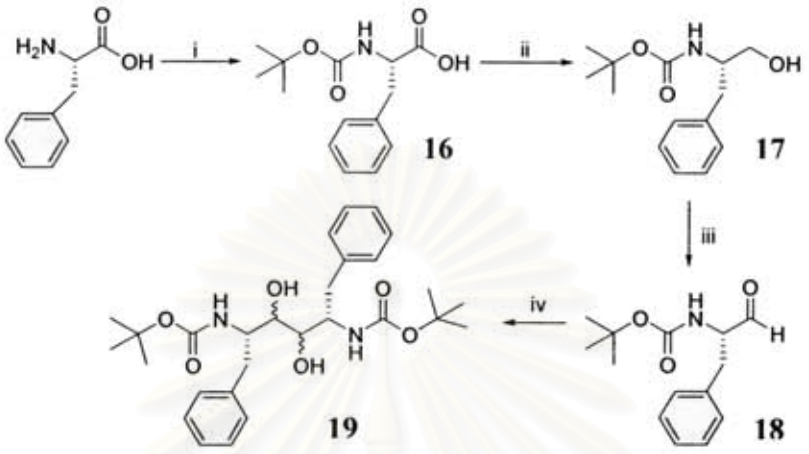


A solution containing sodium amide in dry solvent was cooled and then acetonitrile was added and the resulting solution was stirred at low temperature for more than 15 minutes. To a cooled solution containing compound **14** (1.044 g, 2.4mmol) in dry solvent and acetonitrile, under nitrogen, was then slowly added the sodium amide/acetonitrile solution at low temperature. The reaction mixture was stirred at low temperature for one hour and then quenched with 25% citric acid solution. The reaction was warm to room temperature and the organic layer was separated. After the organic layer was washed three times with a 25% sodium chloride solution, 15% methanolic water solution and brine, the organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed to give the crude product as an yellow oil. The characterization of the crude product showed that the starting material still remained as mixture with the unidentified mixtures (will be discussed in chapter III).

Table 2.1 Conditions for the synthesis of **15**

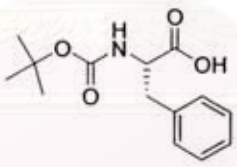
Batch (no.)	Reagent (eq.)		Solvent	Temperature (°C)
	CH_3CN	$NaNH_2$		
1	3.2	2.8	THF	-40
2	4.5	4.0	THF	-40
3	4.5	4.0	THF/Hexane (1:1)	-10
4	3.2	3.0	MTBE	<0

2.6 Synthesis of L-phenylanine derivatives (route 2)



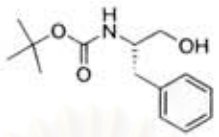
i) Boc_2O, H_2O/EtOH
ii) $NaBH_4/I_2$, THF
iii) Oxidizing agent
iv) $TiCl_4$, Zn, THF

Synthesis of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalanine (**16**)



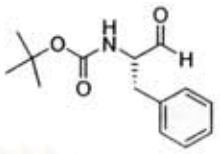
L-Phenylalanine (0.165 g, 1 mmol) and $NaHCO_3$ (0.252 g, 3 mmol) were dissolved in water (2 mL). To the stirred solution, Boc_2O (0.262 g, 1.2 mmol) in THF (2 mL) was added. The reaction mixture was stirred for 6 hours. After removing most of THF, the leftover solution was acidified to pH 2 with 10% solution of HCl and extracted twice with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed to give colorless oil. (0.1558 g, 59% yield) ^{1}H-NMR (CDC_{l3}): δ (ppm) = 7.2-7.4 (m, 1H), 6.20 (broad, 1H), 4.98 (d, 1H, J = 8.0 Hz), 4.60 (d, 1H, J = 7.2 Hz), 4.40 (broad, 1H), 3.21 (m, 2H), 3.10 (dd, 1H, J = 5.2, 14 Hz), 2.90 (m, 1H), 1.4 (s, 9H) 1.3 (s, 9H) (**Figure A.7**) [49]

Synthesis of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalaninol (**17**)



A solution of compound **16** (0.3114 g, 1.17 mmol) in THF (3 mL) was added dropwise to a suspension of $NaBH_4$ (0.13 g, 3.51 mmol) in THF (3 mL). The flask was cooled to 0 °C in an ice bath. A solution of iodine (0.48 g, 1.76 mmol) dissolved in THF (3 mL) was added dropwise into the reaction mixture, resulting in vigorous evolution of hydrogen gas. After addition of the iodine was complete and gas evolution ceased, the flask was heated to reflux till the reaction was complete and then cooled to room temperature. Methanol (5 mL) was added cautiously until the mixture became clear. After stirring for another 30 minutes, the solvent was removed and 20% KOH was added and the mixture was stirred for 4 hours. The reaction was extracted twice with CH_2Cl_2. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed to give the crude product. Purification by column chromatography gave white solid product (0.0292 g, 10% yield). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.10-7.30 (m, 5H), 3.81 (broad, 1H), 3.65 (m, 1H), 3.55 (dd, 1H, J = 5.1, 11.0 Hz), 2,85 (d, 2H, J = 7.6 Hz), 1.40 (s, 9H) (**Figure A.8**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 156, 138, 129, 128, 126, 80, 62, 52, 38, 24 (**Figure A.9**).

Synthesis of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalaninal (**18**)



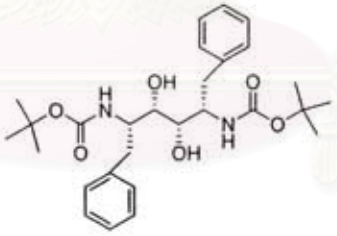
Method 1 To a stirred -40 °C solution of oxalylchloride (0.7 mL, 8 mmol) in CH_2Cl_2 1 mL was added DMSO (0.28 mL, 4 mmol) in CH_2Cl_2 (2 mL) dropwise by syringe. After stirring for 10 minutes, compound **17** (0.25 g, 1 mmol) was added. Over 30 minutes was added triethylamine (2.78 mL, 10 mmol) and the reaction mixture was then stirred for 5 minutes and then allowed to warm to room temperature. Cold water was then added and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined and were washed with 10% citric acid, $NaHCO_3$ solution, saturated NaCl solution, respectively. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to give the crude product (0.0992 g, 40% yield). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 9.63 (s, 1H), 7.31 (t, 2H, J = 7.0, 14.3 Hz), 7.26 (t, 2H, J = 4.3, 15.9 Hz), 7.17 (d, 1H, J = 8.4 Hz), 5.04 (br, 1H), 4.40 (dd, 1H, J = 7.0, 13.8 Hz), 3.10 (d, 2H, J = 5.0 Hz), 1.40 (s, 9H) (**Figure A.11**); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 199, 155, 136, 129, 128, 127, 80, 61, 36, 18 (**Figure A.12**).

Method 2 Compound **17** (0.16 g, 0.64 mmol) was dissolved in CH_2Cl_2 (2 mL). PCC (0.21 g, 0.96 mmol) was added, and the mixture was stirred at room temperature for 2 hours. Following filtration, the filtrate was dried with anhydrous Na_2SO_4, and was evaporated. Then the residue was purified by column chromatography (70:30 hexane/EtOAc). The white solid product (0.0520 g, 69% yield) was obtained.

Method 3 The procedure in method 2 was similarly followed except using PDC as the oxidizing agent in place of PCC and running the reaction overnight. The residue was purified by column chromatography (70:30 hexane/EtOAc). The white solid product (0.0470 g, 52% yield) was obtained.

Method 4 Compound **17** (0.16 g, 0.64 mmol), TEMPO (0.010 g, 0.064 mmol), and KBr (0.071 g, 0.96 mmol) were dissolved in CH_2Cl_2 (2 mL) and then cooled to 0 °c in an ice bath. To the stirred solution, an aqueous solution of NaOCl (1 mL, 0.096 mmol) containing $NaHCO_3$ (0.24 g, 2.86 mmol) is added dropwise over 5 minutes and then stirred for an additional 10 minutes. The aqueous layer was separated and washed with EtOAc, The combined organic layers were washed with a solution of KI (0.25 g) dissolved in 10% aqueous $KHSO_4$ (5 mL). The iodine-colored organic layer was then washed successively with 10% aqueous $Na_2S_2O_3$, and finally with saturated brine. Drying by anhydrous Na_2SO_4 gave the crude product. After purified by column chromatography (70:30 hexane/EtOAc) and recovered the unreacted starting material (0.067 g, 0.267 mmol), the white solid product (0.0720 mg, 96% yield based on the reactant used) was obtained.

Synthesis of (2*S*,5*S*-bis[[(*tert*-butyloxy)carbonyl]amino]-3*S*,4*S*-dihydroxy-1,6-diphenylhexane (**19A**)



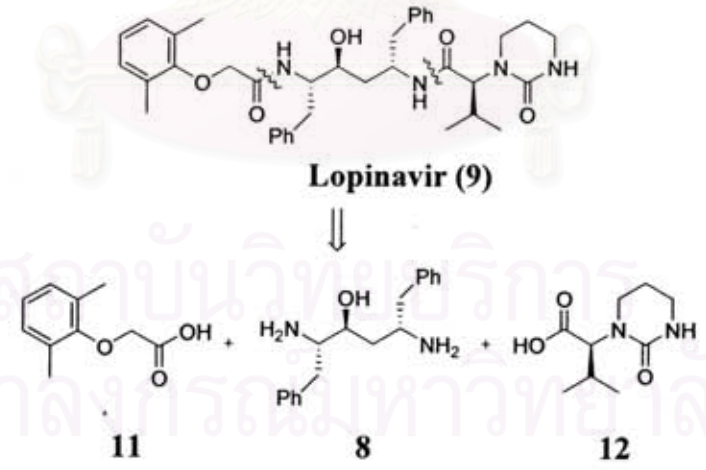
McMurry reagent was prepared right before used starting from feeding $TiCl_4$ under N_2 atmosphere into a dried two-necked round bottomed flask. Dry THF (10 mL) was added dropwise followed by activated Zn dust cautiously. The reaction was refluxed under nitrogen for 2 hours to yield the desired dark solution of McMurry reagent. To the cooled solution of compound **18** (0.100 g, 0.398 mmol) in dry THF (2 mL) was added the preformed McMurry reagent above (5 mL) and the reaction mixture was stirred for 2 hours and 30 minutes then allowed to warm to room temperature for 20 minutes. Water was added to quench the reaction and EtOAc was used to wash the aqueous layer at least

3 times. The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was removed to give the crude product, which was then seperated by flash column chromatography (70:30 hexane/EtOAc). ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.20-7.30 (m, 5H), 4.40 (d, 4H, J = 9.1 Hz), 4.40 (s(br), 2H), 3.20 (d, 4H, J = 10.0 Hz), 2.80 (dd, 2H, J = 4.4, 14.0 Hz) (**Figure A.13**).

CHAPTER III

RESULTS AND DISCUSSION

According to the retrosynthesis (**Scheme 3.1**), Lopinavir can be divided into three parts, the west side, the core, and the east side which are 2,6-dimethylphenoxyacetic acid **11**, diamino alcohol **8**, and cyclic urea derivatives **12**, respectively. Each part can be connected by peptide coupling reaction after the two carboxylic acids (**11** and **12**) are converted into its acid chloride derivatives and then reacting with the two amino groups of diamino alcohol **8**. The synthesis of **11** and **12** can be done in one or two steps. The more complicated part to synthesize is diamino alcohol **8** which contains three stereogenic centers. In this research, both east and west sides of Lopinavir were synthesized following the method of Hayes *et al.* and Stoner *et al.* [38,39]. The synthesis of diamino alcohol **8** followed the patented strategy [40] and compared the efficiency of the reaction to the new strategy proposed in chapter 1 (**Scheme 1.6**). The results of these syntheses are discussed in this chapter.

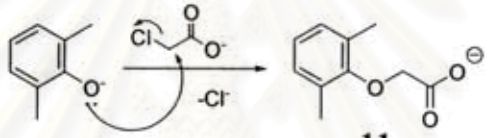


Scheme 3.1 Retrosynthesis of Lopinavir

3.1 Synthesis of 2,6-dimethylphenoxyacetic acid (11)

The synthesis of 2,6-dimethylphenoxyacetic acid **11** was reported by Hayes *et al.* [38] By following this method, using the nucleophilic substitution of chloroacetate

anion by 2,6-dimethylphenoxide in an S_N2 fashion (**Scheme 3.2**) and water as the solvent, 65% yield of the desired product was obtained after 3 hours of reflux. The reference paper did not report the yield of this reaction. Our result may be instead compared to the work by Beaulieu *et al.* [40] who reported another method to synthesize this compound from a two-step reaction. The nucleophilic substitution of ethyl bromoacetate by 2,6-dimethylphenoxide and subsequent hydrolysis to eliminate the ethoxide group in acetone solvent over 60 hours gave 62% yield of the product. The direct substitution method used in this research is more convenient and more effective in many ways. Firstly, less reaction time was required because of the one step reaction. Secondly, the solvent used was water rather than organic solvent and finally, the yield was comparable.

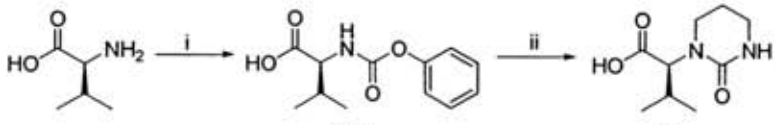


11

Scheme 3.2 Mechanism of nucleophilic substitution of chloroacetate anion by 2,6-dimethylphenoxide

For the reaction with an S_N2 mechanism, the rate of reaction depends on the concentration of the starting material and the reagent. Most phenolic derivatives are easily oxidized by air. In this case, too long of a reaction time would instead lower the concentration of 2,6-dimethylphenol. Optimization of the reaction time maybe needed to help improve the yield of the reaction.

3.2 Synthesis of (*S*)-tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetic acid (13)

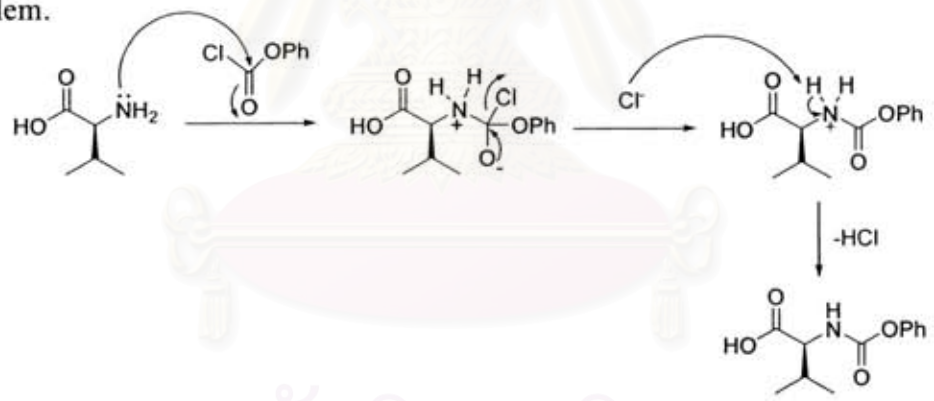


12 **13**

i) phenylchloroformate, LiCl, Al_2O_3, LiOH
ii) 3-chloropropylamine, KO^{t}Bu

Scheme 3.3 The synthesis of cyclic urea **13**

Following the method of Stoner *et al.* [39], cyclic urea **13** could be synthesized in two steps. The first step was the nucleophilic acyl substitution of phenylchloroformate by L-valine in water (**Scheme 3.4**). LiCl was added in order to lower the freezing point of water since the reaction was carried out below 0 °C. Al_2O_3 addition was to prevent gumming and emulsion formation during the course of the reaction which aided in accurate pH monitoring. The addition of LiOH solution in the reaction was to prevent dimerization of L-valine and to neutralize HCl by-product because the intermediate **12** was reactive and may under go further hydrolysis reaction in acid condition.[39] After 3 hours of pH controlling, gave a colorless gel product was obtained in 11% yield, which was much less than the original reported data (93%) [39]. The reason may be the difficulty to control the highly reactive phenylchloroformate reagent that could be quickly decomposed during the period of reaction. Moreover, the recrystallized product **12** was like a colorless gel and made it easily lost in the filtration process. Scaling up the reaction could have alleviated this problem.

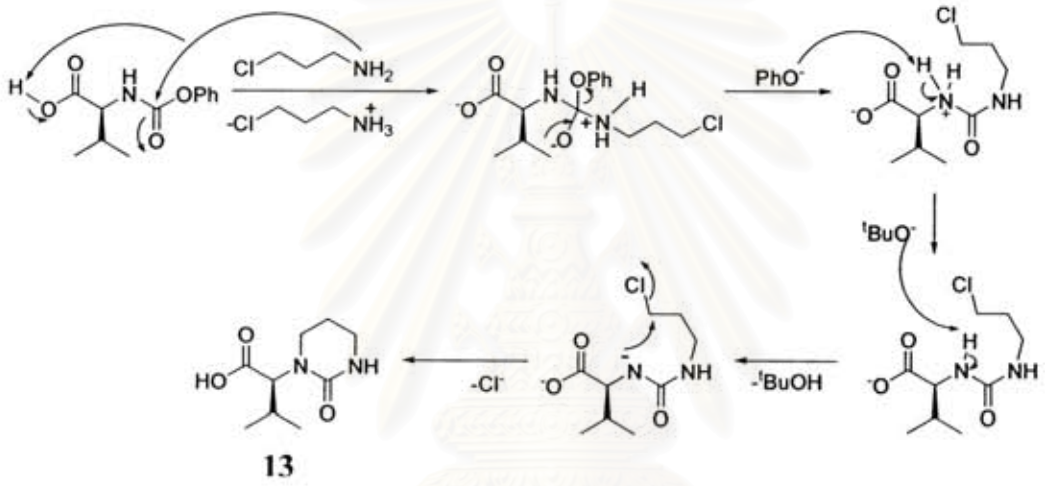


12

Scheme 3.4 Mechanism of nucleophilic acyl substitution in the synthesis of intermediate **12**

The second step consists of two consecutive reactions of nucleophilic acyl substitution and intramolecular nucleophilic substitution (**Scheme 3.5**). The intermediate **12** was attacked by the nucleophile, 3-chloropropylamine in THF. The low temperature is required to prevent dimerization of 3-chloropropylamine or cyclization to give an azitidine compound as the by-products. After completion of reaction, the next step was to add KO^{t}Bu base in order to generate the internal

nucleophile that cyclized intromolecularly to give the desired product. The phenol by-product from the first step could not be washed or extracted from the product **13**. However after subtracting out the signals of phenol in the ^{1}H-NMR of the crude product, the calculation showed that 45% yield of the product was obtained, which was lower than the reported yield (77%). [39] Most of the product may be lost during purification steps. The overall yield from two steps of the reaction is only 5.1%. The inefficiency was mainly from the first step of the synthesis.



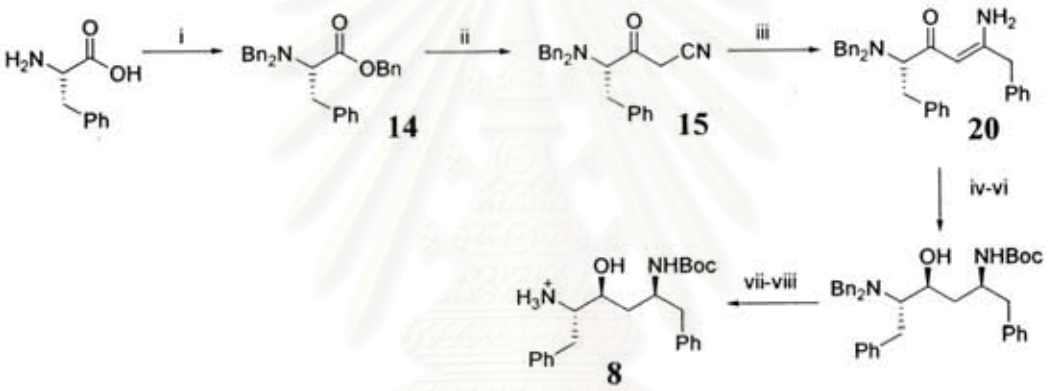
Scheme 3.5 Mechanism of the nucleophilic acyl substitution and intramolecular acyl substitution in an S_N2 fashion in the synthesis of cyclic urea **13**

3.3 Synthesis of L-phenylalanine derivatives

The synthesis of the core molecule **8** was divided into two routes. Route 1 (**Scheme 1.2**) was to follow the patented strategy [32] and route 2 (**Scheme 1.6**) was to follow the proposed strategy. The similarity of the two routes of synthesis is the use of L-phenylalanine as their starting materials. On the other hand, the difference is the concept of the method. The first route involved adding the required carbon chain onto the L-phenylalanine and manipulating the functional groups. The latter relied on the convergent coupling reaction of two similar pieces derived from L-phenylalanine. The detail of each route is discussed below.

3.3.1 Route 1: synthesis of L-phenylalanine derivatives

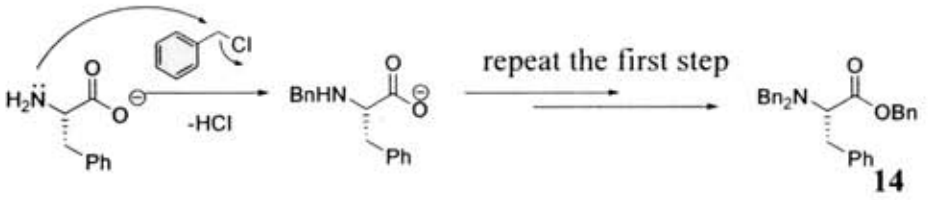
The synthesis of diamino alcohol core as reported in the patent [32] was carried out in 5 steps with two reactions involving induced asymmetric reduction (**Scheme 1.2**). The use of asymmetric induction of the one stereocenter existed in the molecule gave the desired configurations of the other stereocenters in the diamino alcohol derivative. The last three steps were the protection and deprotection of amino groups in order to get the final diamino alcohol **8**. In this report unfortunately only the first step could be done. Attempts to carry out the next two steps were not accomplished (**Scheme 3.6**).



i) BnCl, K_2CO_3 ii) CH_3CN, $NaNH_2$, iii) BnMgCl, THF vi) $NaBH_4$, MSA, TEOA
v) $NaBH_4$, DMAc, 93% vii) Boc_2O viii) Pd/C, NH_4CO_2H, MeOH, H_2O
viii) succinic acid, $^{i}PrOH$, 80%

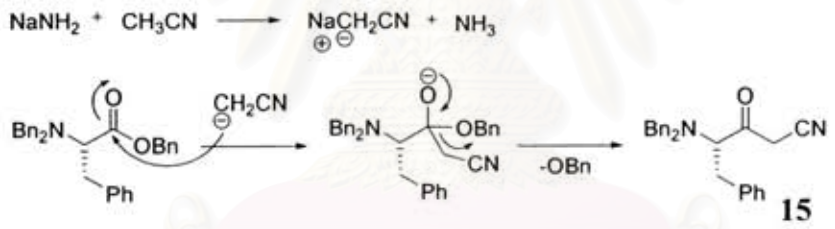
Scheme 3.6 Synthesis of diamino alcohol **8**

The first step of reaction has two purposes. The first one is to convert the carboxylic acid into its corresponding ester for the subsequent substitution. The other purpose is to protect the amino group. The tribenzylated compound **14** could be synthesized by nucleophilic substitution of benzyl chloride by L-phenylalanine in water/ethanol solvent (**Scheme 3.7**). The dibenzylated product is often observed as the intermediate. The reaction time should be at least approximately 10 hours in order to complete the tribenzylated reaction. After solvent extracting process, the colorless oil product was obtained in 92% yield, which is close to the reported yield by Stuk *et al.* (94%).[41] The product was sufficiently clean and was carried on to the next step without further purification.



Scheme 3.7 Mechanism of the nucleophilic tri-substitutions in the synthesis of tribenzylester **14**

The second step was the nucleophilic acyl substitution of tribenzyl ester **14** by acetonitrile anion, generated from the deprotonation of acetonitrile by sodium amide under nitrogen purge to prevent from a contact with moisture (**Scheme 3.8**). This condition raised the concern of the possibility of racemization at the stereo center in the tribenzylated L-phenylalanine precursor. In this case, the racemization process was minimized by running the reaction at low temperature.



Scheme 3.8 Mechanism of nucleophilic acyl substitution by acetonitrile anion

The solvents used in this reaction were dried over Na/benzophenone distillation or molecular sieves. By following the procedure by Haight *et al.* [33] with varying some conditions (**Table 3.1**), the starting material was still detected in the reaction mixture in all cases. At least 5 more products were observed on TLC. However, the results showed the characteristic signals that would correspond to the desired cyanomethyl ketone **15** in ^{1}H-NMR spectrum of the crude mixture. It was possible that the product underwent further reaction because as a ketone it is more reactive than its starting material, benzyl ester **14**.

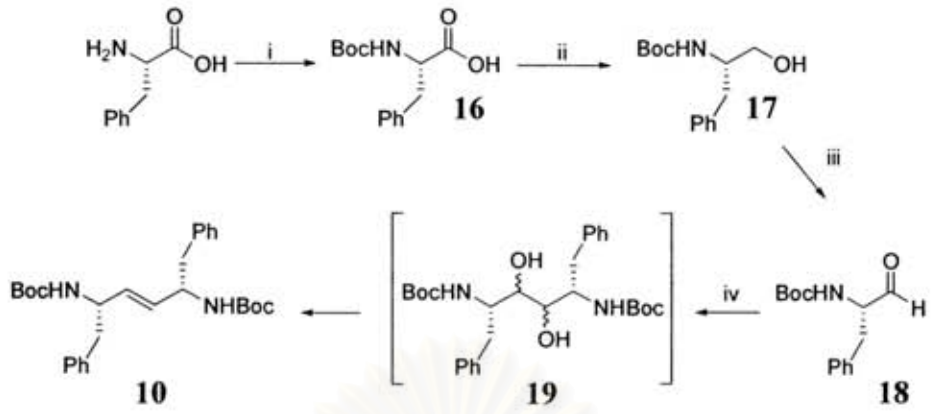
Table 3.1 Conditions for the substitution by acetonitrile anion

Entry	Reagent (eq)		Solvent	Temperature (°C)
	CH_3CN	$NaNH_2$		
1	3.2	2.8	THF	-40
2	4.5	4.0	THF	-40
3	4.5	4.0	THF/hexane (1:1)	-10
4	3.2	3.0	MTBE	~0

Haight *et al.* [33] also reported a one-pot synthesis of enaminone **20** by addition of benzyl Grignard reagent into the reaction mixture after the completion of the second step. This method was also attempted in this work. Unfortunately apart from obtaining the similar unidentified mixture as the above two-steps condition, the dimerized product of the Grignard reagent from usual Wurtz coupling was observed.

3.3.2 Route 2: Synthesis of L-phenylalaninal and its homocoupling study

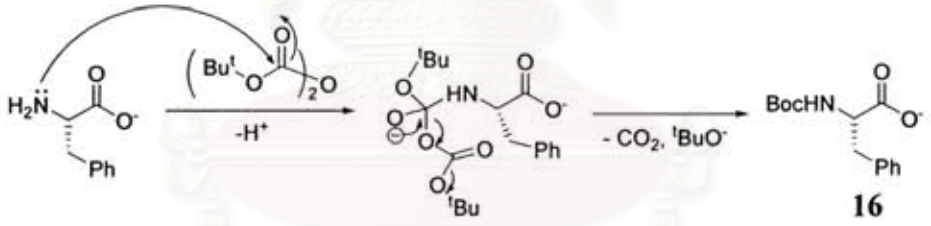
The idea of this new synthetic strategy is similar to what was reported by Rao *et al.* [35,36]. Their strategy to synthesize diamino alcohol **8** was required 13 steps. The key step was to synthesize the C2 symmetric alkene intermediate **10** from the homocoupling of L-phenylalanine derivatives (**Scheme 1.3**). The last and the only asymmetric synthesis was the asymmetric oxidation of alkene **10**. The proposed strategy in this research could in principle shorten the synthesis of alkene **10** from 11 steps to 4 steps. In addition, most intermediates synthesized are known or commercially available. The key step of this strategy is to use McMurry coupling to connect the two L-phenylalaninal derivatives (**Scheme 3.9**).



i) Boc_2O, H_2O/EtOH ii) $NaBH_4/I_2$, THF iii) Oxidizining agent iv) $TiCl_4$, Zn, THF

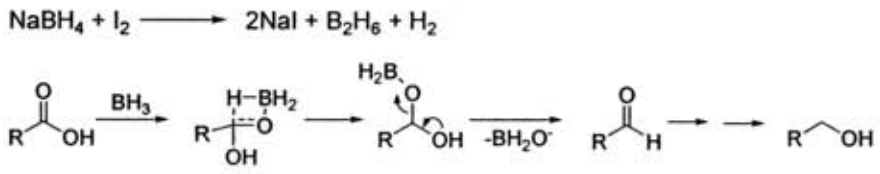
Scheme 3.9 The proposed homocoupling strategy

The first step of this route was the protection of amino group because of the possible incompatibility with the McMurry reagent. The nucleophilic acyl substitution of di-*tert*-butyl dicarbonate (Boc_2O) by L-phenylalanine in 1:1 water/THF at room temperature gave Boc-phenylalanine **16** in 59% yield (**Scheme 3.10**).



Scheme 3.10 Mechanism of nucleophilic acyl substitution in the synthesis of Boc-phenylalanine **16**

The second step was the functional group transformation of **16** into Boc-phenylalaninol **17** using sodium borohydride, and iodine in dry THF [41] (**Scheme 3.11**).



Scheme 3.11 Mechanism of the reduction for the synthesis of Boc-phenylalaninol **17**

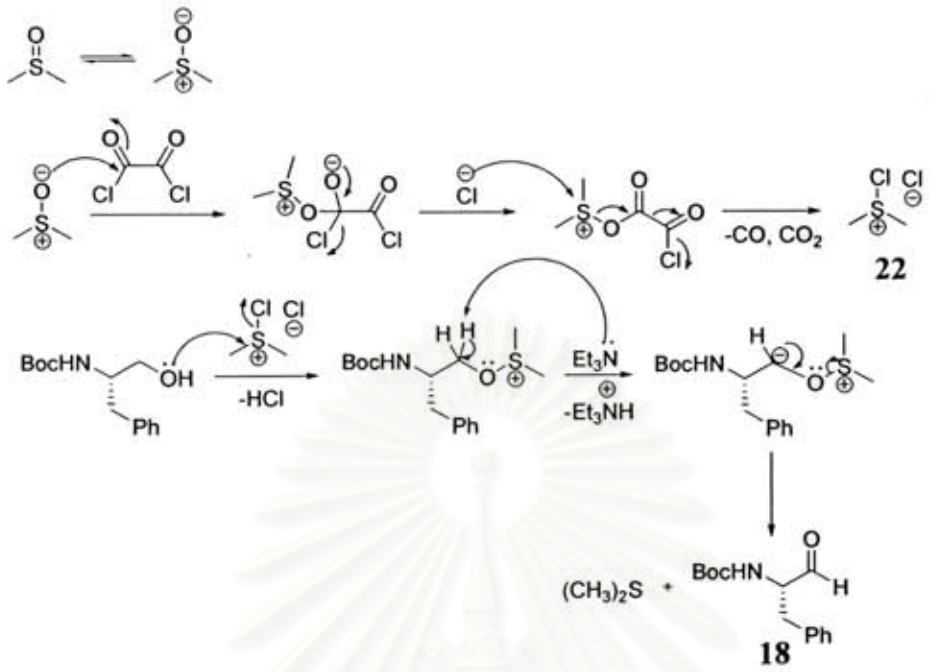
The third step was to synthesize aldehyde **18** by an oxidation reaction. Three groups of oxidizing agents were used in this research (**Table 3.2**). Swern oxidation is widely used in oxidizing primary and secondary alcohols to aldehydes and ketones.[42] The reagents in this group are oxalyl chloride-DMSO (Entry 1, the original Swern) and sulfur trioxide-pyridine complex with DMSO (Entry 2). The chromium metal based oxidations, pyridinium chlorochromate (PCC) and pyridine dichlorochromate were the second group. The last group of reagents, TEMPO-mediated oxidation, has been mostly used in large scale oxidation of primary alcohols.

Table 3.2 Aldehyde **18** obtained from different oxidizing agent

Entry	Oxidizing agents	Product yields
1	SO_3, pyridine, DMSO, TEA	No reaction
2	$(COCl)_2$, DMSO, TEA	40%
3	PCC, CH_2Cl_2	69%*
4	PDC, CH_2Cl_2	52%*
5	NaOCl/$NaHCO_3$, TEMPO, KBr	96%*

* Reaction was not complete

The first choice of oxidizing agent was the original Swern oxidation. The sulfoniumchloride intermediate **22** was generated *in situ* during the course of the reaction. The activation of DMSO by oxalyl chloride gave the sulfonium **22** together with the evolution of CO and CO_2 gas. After the addition of alcohol **17**, triethylamine was added to deprotonate the proton of the intermediate and finally gave the desired aldehyde **18** in 40% yield (**Scheme 3.12**).

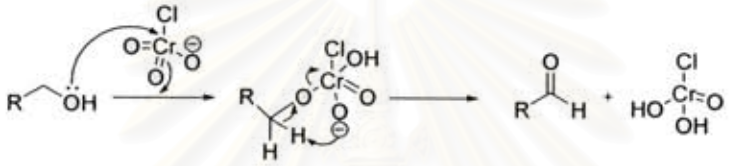


Scheme 3.12 Mechanism of Swern oxidation to aldehyde **18**

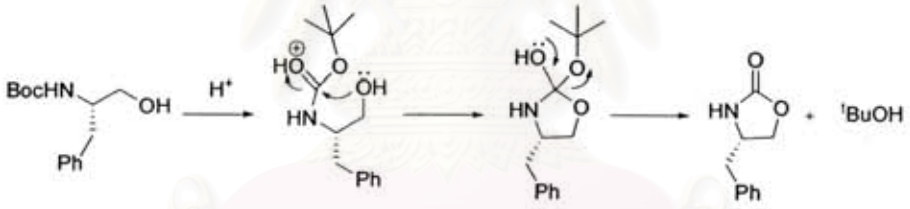
At the end of the reaction, some triethylamine was still found in the crude product, even after washing with 10% citric acid solution. To eliminate triethylamine, the crude mixture then had to be purified by a short column chromatography. This reagent can complete the reaction but its by-product, the stench odorus dimethylsulfide, was needed to be purged off by nitrogen for about 1 hour. During this period, aldehyde **18** might have partially decomposed. The second activating reagent used was SO_3·pyridine complex which is mostly used in large scale synthesis of aldehyde **18**.[44,45] Surprisingly, after following the reported method, there was no reaction observed. The solid of SO_3·pyridine may contain more moisture than the liquid oxalyl chloride, so the active oxidizing SO_3 was lost into sulfuric acid.

The metal-based oxidations, PCC and PDC, have been investigated. Through formation of chromate ester, the Cr(VI) was eventually converted into Cr(III) (**Scheme 3.13**).[46] The procedures were relatively simple because both PCC and PDC are solid and the reactions could be carried out at room temperature. The more acidic and reactive PCC oxidized the alcohol faster than PDC did.[46] When PCC was used (**Table 3.2**, entry 3) and the reaction was left for 2 hours, the result showed that the starting material could not be completely consumed. Prolonged reaction time

did not seem to improve this incompleteness. Finally, the reaction could give up to 69% of aldehyde **18**. The acidity of PCC could also promote the intramolecular cyclization of the starting alcohol to give the by-product, oxazolidinone **23** (**Scheme 3.14**). The reaction with PDC as oxidizing agent had to be run overnight in order to observe a clear sign as the TLC spot of the product. The reaction finally gave the aldehyde **18** in 52% yield, less than that obtained from using PCC reagent, it is possible that a longer reaction time could lead to the decomposition of the aldehyde **18** product. The same oxazolidine by-product **23** was also observed with the PDC oxidation.



Scheme 3.13 Mechanism of chromium based oxidation reaction

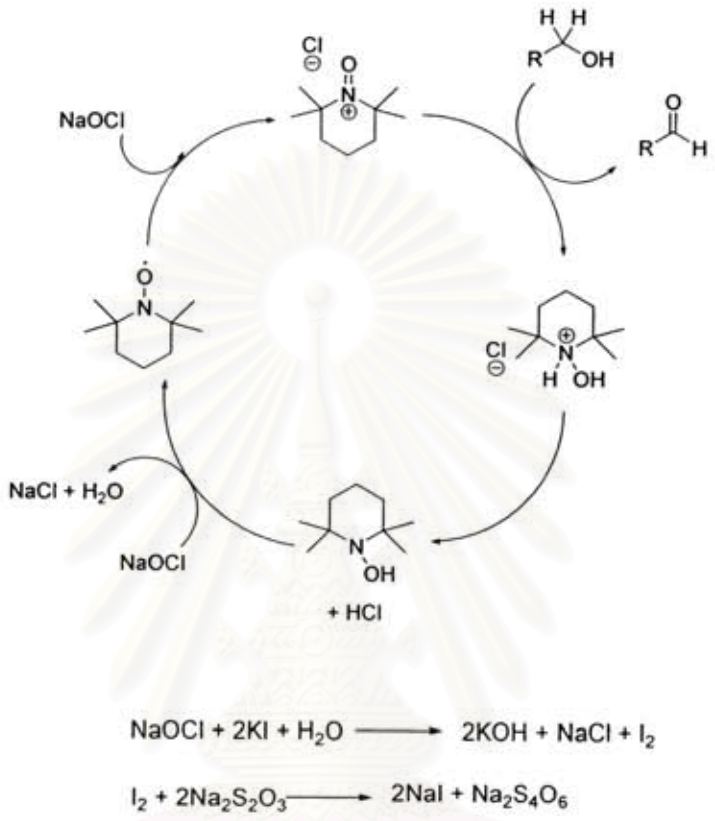


23

Scheme 3.14 The intramolecular cyclization to give oxazolidinone **23**

The last oxidizing agent was TEMPO-mediated oxidation. The oxoammonium derivatives, 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) radical, [46] had been used as the catalyst in the large scale oxidation of primary alcohols. TEMPO was used at 1-2% mol with bleach (NaOCl) as the stoichiometric oxidant and potassium bromide as an additive to help accelerate the reaction. The reaction was biphasic and needed to be vigorously stirred. Its mechanism was through the catalytic cycle of TEMPO (**Scheme 3.15**). The reaction was run at 0 °C for 10 minutes and quenched by KI solution to eliminate the leftover oxidants, and finally, sodium thiosulfate was used to bleach off the I_2 color (**Scheme 3.15**). Leanna *et al.* [46] reported that the *N*,*N*-dibenzyl protected L-phenylalaninol could be converted into its corresponding aldehyde in excellent yield, without further purification. By using their reported condition,

however only approximately 50% of the starting alcohol **17** could be converted into aldehyde **18**. The other half remained unchanged in the reaction.



Scheme 3.15 Mechanism of TEMPO-mediated oxidation

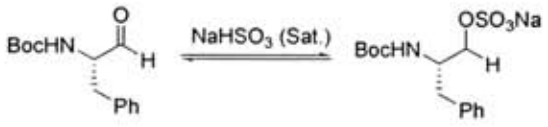
The condition of the reaction was varied in order to drive the reaction toward completion (**Table 3.3**). The addition of more bleach and TEMPO (entries 3 and 4) and longer reaction time (entry 2), both made the occurrence of an unidentified by-product, indicating that TEMPO could promote other reactions at longer time. In entries 6 and 8, changing buffers in the bleach solution and adding no KBr additive respectively, made no reaction at all. Anelli *et al.* [47] reported that high pH of commercial bleach could slow down the reaction unless the pH value of the reaction was controlled in the range of 8-9. Attempts to adjust the condition according to this report nevertheless made no improvement to the yield of the product (entry 6).

Table 3.3 Conditions for TEMPO mediated-oxidation

Entry	TEMPO (eq)	Time (min.)	Results
1	0.01	10	Incomplete reaction
2	0.01	60	Incomplete reaction[e]
3	0.01[a]	10	Incomplete reaction
4	0.10	10	Incomplete reaction[e]
5	0.01[b]	10	No reaction
6	0.01[c]	10	No reaction
7	0.01[d]	10	Incomplete reaction

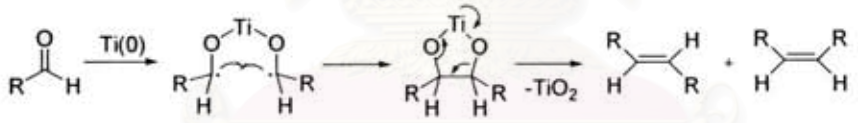
[a] 3 eq of NaOCl was added. [b] no KBr was added. [c] NH_4Cl was used in the same amount and in place of $NaHCO_3$. [d] HCl was used in the same amount and in place of $NaHCO_3$. [e] by product observed.

From all reagents used in the synthesis of aldehyde **18**, TEMPO-mediated oxidation was chosen to be the best oxidizing agent in this research with the original condition (**Table 3.3**, entry 1). The reason came from the fact that there was no by-product observed, the reaction time was the shortest and the procedure was relatively simple. The crude product from oxidation reaction had to be separated from the remaining starting material alcohol **17**. Attempts to use a saturated bisulfite solution to precipitate the aldehyde were not very effective, perhaps due to the rather small amount of the product. (**Sheme 3.16**). The aldehyde was then obtained in pure form using flash chromatography (30% EtOAc/hexane). The pure aldehyde **18** was kept at -20 °C.



Scheme 3.16 The saturated bisulfite addition onto aldehyde **18**

The next step of the reaction was the homocoupling reaction of the two aldehyde **18** molecules by McMurry coupling reaction (**Scheme 3.17**). McMurry coupling was typically used to synthesize olefins from carbonyl compounds on treatment of low valent titanium reagents (Ti(0)). The reagents used for generating Ti(0) are $TiCl_3/LiAlH_4$, $TiCl_3$/Zn-Cu, $TiCl_4$/Zn and some other combinations in THF or DME.[47] In this research, activated Zn dust was used as the reducing agent to reduce $TiCl_4$ into their reactive states, such as Ti(0), Ti(I) or Ti(II). The presence of any easily reducible functional groups is not compatible with this reaction while amide groups such as that in aldehyde **18** could react only slowly. The mechanism consists of three steps, the electron transfer from Ti(0) to create the radicals, the pinacol coupling to give the titanium-diol complex and the deoxygenation to give the final alkene compound (**Scheme 3.17**). If the reaction is carried out at low temperature the pinacol coupling products would be obtained while at the solvent reflux temperature, the reaction would provide the alkene products. Mostly, the *trans*-alkenes were the major product from the coupling reaction due to the steric effect.[48]



Scheme 3.17 McMurry homocoupling of aldehydes

Although high temperature at solvent reflux was required in order to obtain alkene compound, at solvent reflux condition, aldehyde **18** unfortunately appeared to decompose. At room temperature, the reaction completed within 15 minutes after adding aldehyde **18**, the result ^{1}H-NMR showed no characteristic peak of Boc-group showing that the product appeared to decompose. So the low temperature reaction was needed, moreover, the quantity of reagent was also effect the reaction. From the varying conditions (**Table 3.4**), 5 equivalent of $TiCl_4$ in THF and 10 equivalent of Zn to generate McMurry reagent for 2 hours of reaction time seemed to be the most suitable (**Table 3.4**, entry 3). Then the reagent was carefully transferred into the aldehyde **18** solution in THF. After 2 hours and 30 minutes of reaction at 0 °C and 30 minutes at room temperature, the reaction was completed. The pinacol coupling

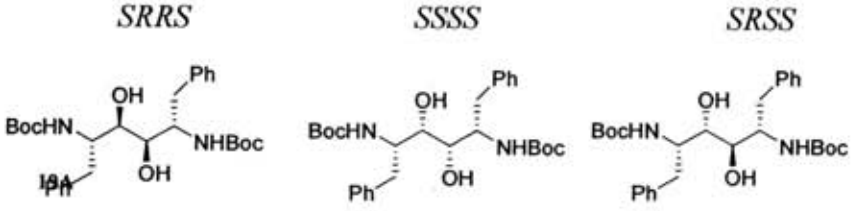
products was obtained (**Figure 3.1**). The less equivalent of $TiCl_4$ than 5 (entries 1 and 2), at the same period of reaction time, would lead to incompletion of reaction and the more equivalent would lead to the decomposition of the product.

Table 3.4 Conditions for titanium induced carbonyl coupling

Entry	Reagent (eq)		Results
	$TiCl_4$	Zn	
1	1	2	Incomplete reaction
2	3	6	Incomplete reaction
3	5	10	Complete reaction
4	7	14	Product decomposed

The four diamino diols from coupling reaction are the intermediates in synthesizing alkene **21**, which was expected to obtain after deoxygenations. The elimination reactions usually occurred at high temperature. So after the completion of the coupling reaction, the temperature was raised to approximately 60 °C. From ^{1}H-NMR of the crude product however, there was no characterestic signal of the desired alkene **21**. The diamino diols themselves also seemed to partially decompose.

The coupling could lead to four different diastereomer of diamino diols **19**, *SRRS*, *SSSS*, *SRSS* and *SSRS* isomer (the latter two isomers are meso compounds). From entry 3 (**Table 3.4**) the three diamino diols obtained from the coupling reaction were diastereomers with the configurations assigned to be *SRRS*, *SSSS* and *SRSS* (**19A**, **19B** and **19C** respectively) (**Figure 3.1**). Using flash column chromatography, the *SSSS* isomer could be purely separated but the other two isomers could not. The pure **19B** was obtained in 0.12% yield and the mixture of diastereomeric mixture **19A**:**19C** in approximately 1:1 was obtained in 0.2% yield.



19A **19B** **19C**

Figure 3.1 The three diastereomeric isomers of diamino diols **19**

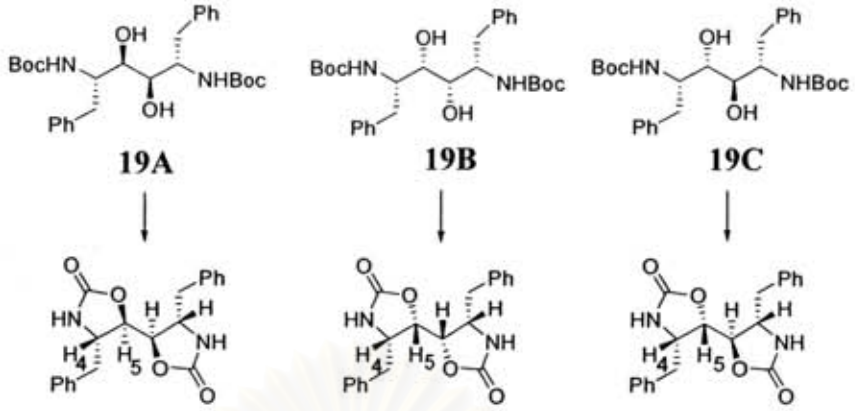
The results from entries 1 and 2 showed that both reactions were not complete. The diamino diol obtained from entry 2 were **19A** and its two diastereomeric mixture. The ^{1}H-NMR characterizations were compared to that from Kempf *et al.* [29] in which they synthesized the diamino diol **19** by using pinacol coupling reaction using different reagents and condition. In their case, the more reactive titanium (III) and the Zn-Cu as the reducing agent were used as the McMurry reagent and DME as the solvent (**Table 3.4**).

Table 3.4 Comparison between the two McMurry couplings

Entry	Reagent (eq)	Reducing agent (eq)	Solvent	Reaction time	Results		
					19A	19B	19C
1	$TiCl_4$ (5)	Zn (10)	THF	0°C 2.5 h. RT 30 mins	2	1	1
2*	$TiCl_3(DME)_2$ (5)	Zn-Cu (7.75)	DME	0°C 1 h.	1	1	2

* Ref. 29

They reported that three diastereoisomers were obtained in approximately 1:1:2 ratio (**19A**:**19B**:**19C**) and their configurations can be confirmed by converted them into their corresponding bis(oxazolidinones) and calculate the coupling constant between H_4 and H_5 (**Scheme 3.18**). The signal of the three diaminodiols obtained from this research, after comparing the ^{1}H-NMR signals were match.



Scheme 3.18 Conversion of diamino diols into bis(oxazolidinones) to determine their configuration

Comparing with the reported diols [29], the yields of the products were much lower. McMurry coupling reaction is sensitive to air; a little leak of air in transferring process may destroy some of the McMurry reagents. The starting material, aldehyde **18** may be decomposed because of the long period reaction time. The larger scale of synthesis may raise the yield of the desired products. The dehydroxylation of **19A** would give the desired diamino alcohol **8**. The new strategy tends to be accomplished if its key step is further developed.

CHAPTER IV

CONCLUSION

The synthesis of the two side wings of Lopinavir followed the strategy of Stoner *et al.* [39], gave 2,6-dimethylphenoxyacetic acid **11** and **13** were obtained in 65% and 5.1% yield respectively. The first route synthesis of the core, diamino alcohol **8** followed the reported route in patent by Stuk *et al.* [30] was accomplished only in the first reaction, the benzylation step, the tribenzylated ester was obtained in 92% yield. The second step of the reaction, the synthesis of cyanomethylketone compound, gave more than 5 unidentified mixtures and the reaction could not be completed. The result from adding benzyl Grignard reagent follow the report of Haight *et al.* as one-pot reaction to obtain the next intermediate was not accomplished, the similar unidentified mixtures were observed.

The second route to synthesize diamino alcohol **8** was success in the first three steps. The 59% yield of protected L-phenylalanine from the first step was obtained. The reduction of **16** gave boc-phenylalaninol in 10% yield. The proper condition for the oxidation of alcohol **17** was to use NaOCl, TEMPO and KBr as oxidizing agent, catalyst and an additive respectively, and run the reaction for 10 minutes. The result boc-phenylalaninal **18** was obtained in 96% based on the reactant used while other groups of oxidizing agent gave the undesired by-products. The McMurry coupling by using 5 equivalent of $TiCl_4$ and 10 equivalent of activated Zn dust at 0 °C for 2 hours and 30 minutes and at room temperature for 20 minutes gave the pinacol coupling product **19** in four diastereomers. The **19A** isomer was obtained only in 0.12% yield whereas **19B** and **19C** were obtained in 0.2% yield of diastereomeric mixture in 1:1 ratio. Further deoxygenation and reduction steps should be made in order to obtain the desired diamino alcohol **8**.

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APPENDIX

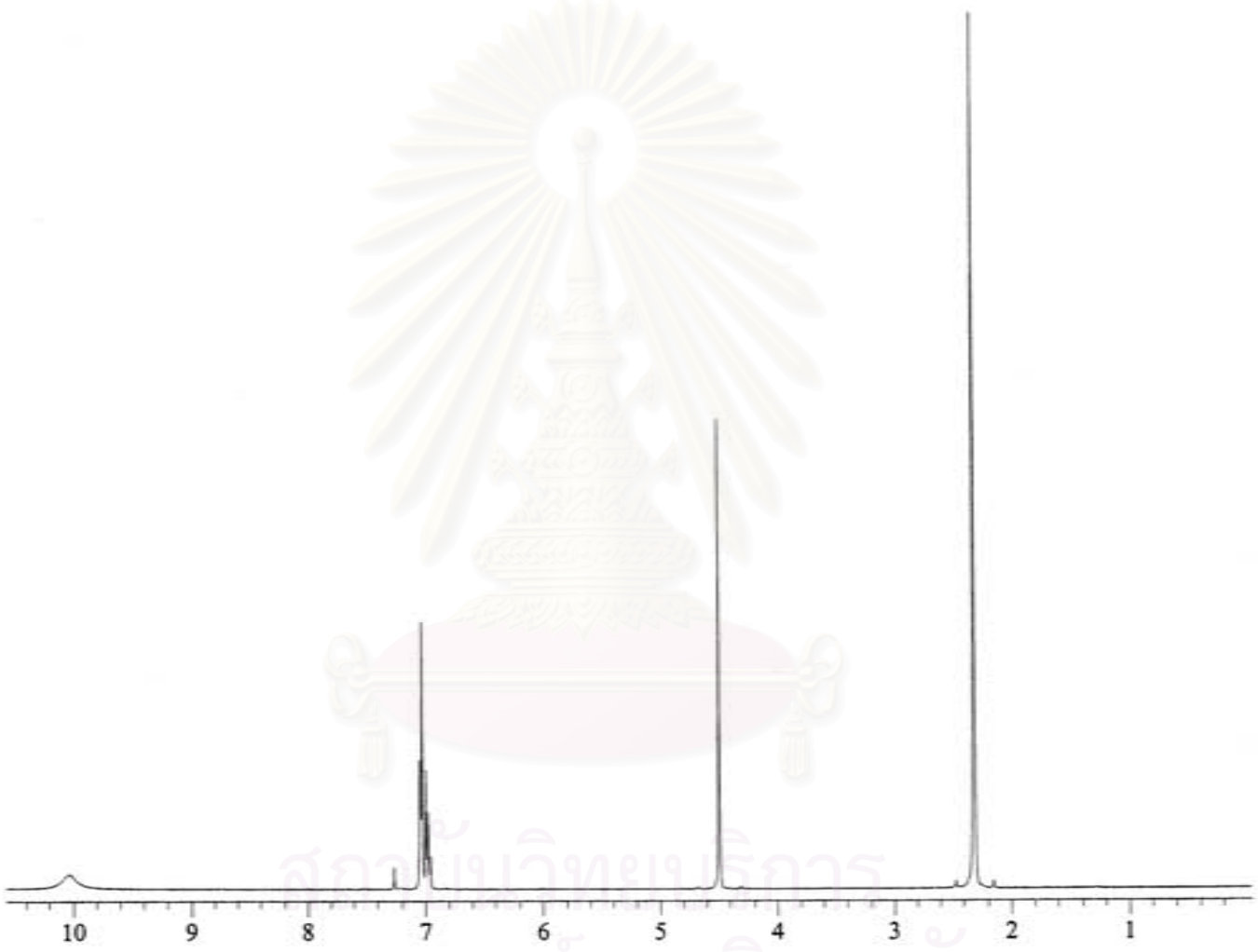

Figure **A.1** ^{1}H-NMR ($CDCl_3$) Spectrum of 2,6-dimethylphenoxyacetic acid (**11**)

49

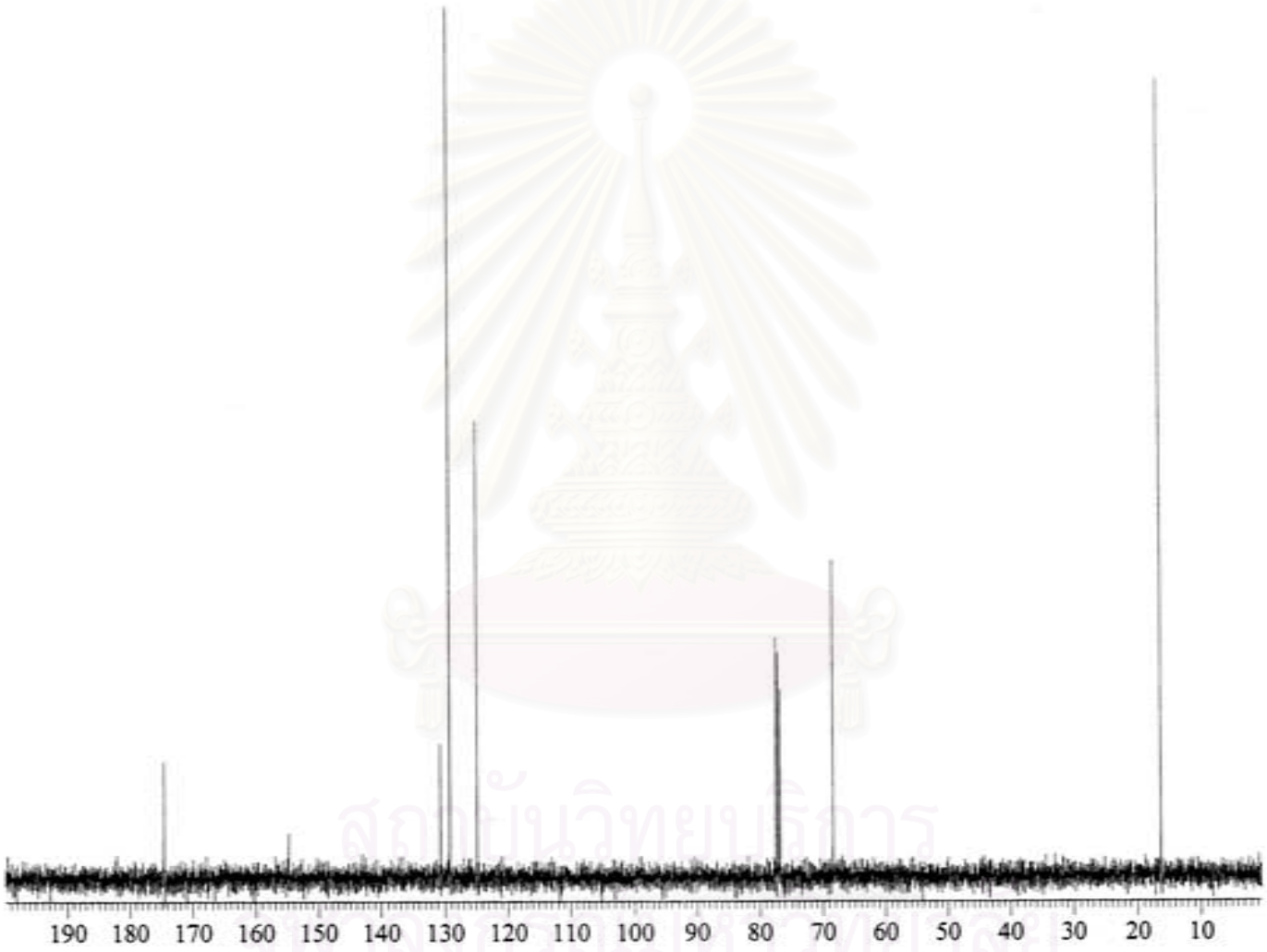

Figure **A.2** ^{13}C-NMR ($CDCl_3$) Spectrum of 2,6-dimethylphenoxyacetic acid (**11**)

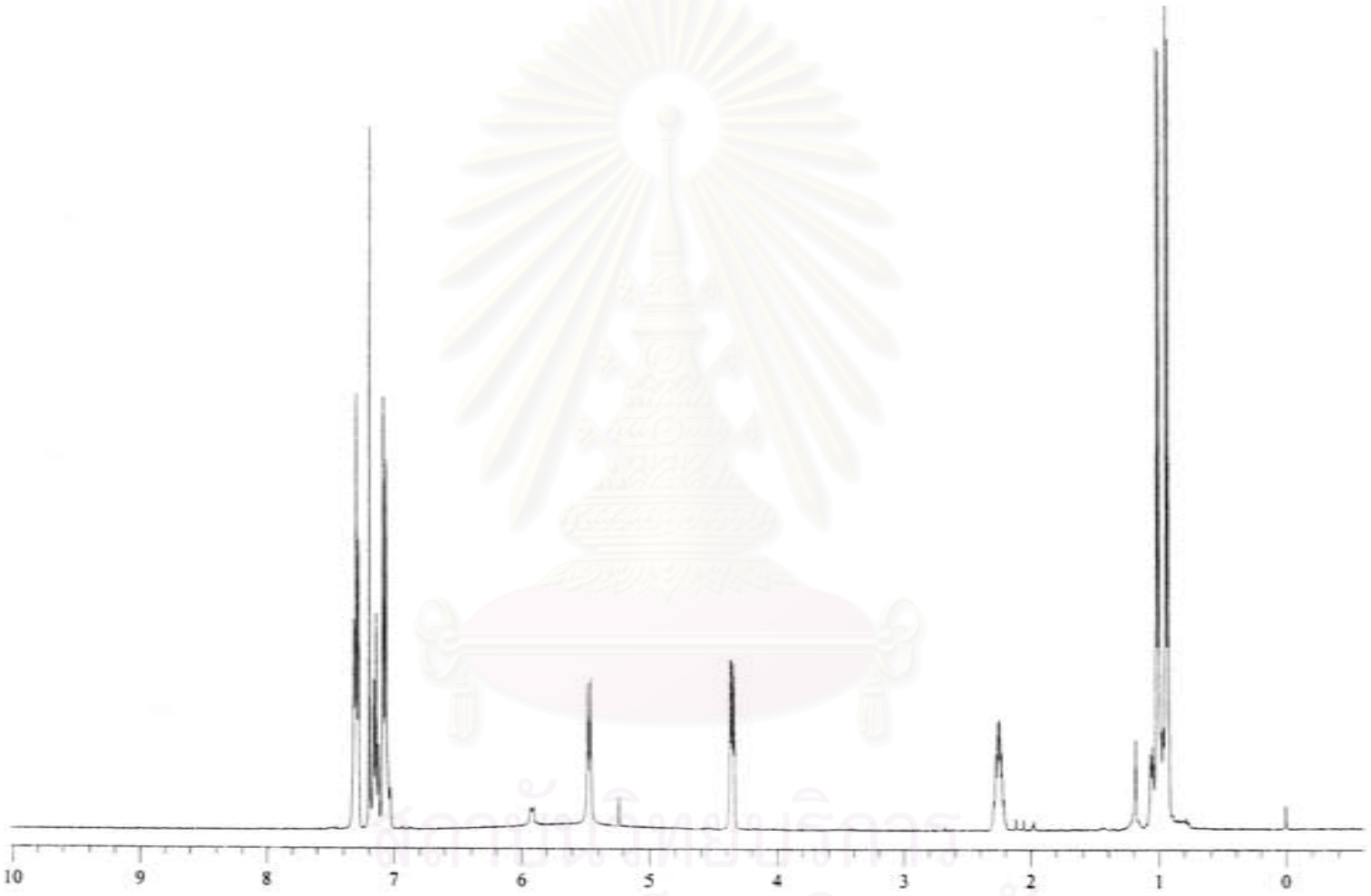

Figure **A.3** ^{1}H-NMR ($CDCl_3$) Spectrum of *N*-phenoxycarbonyl-L-valine (**12**)

51

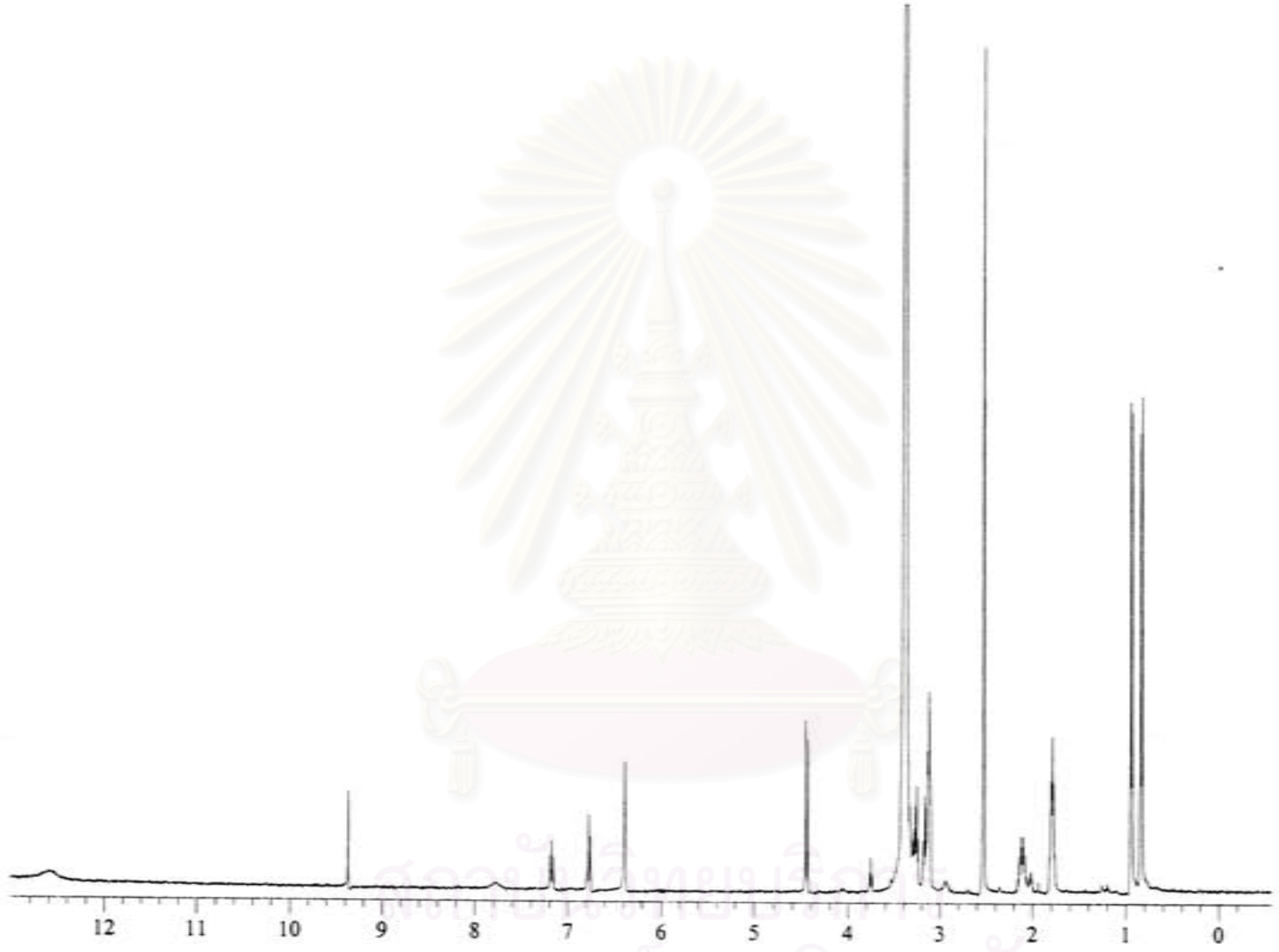

Figure **A.4** ^{1}H-NMR ($CDCl_3$) Spectrum of (*S*)-Tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetic acid (**13**)

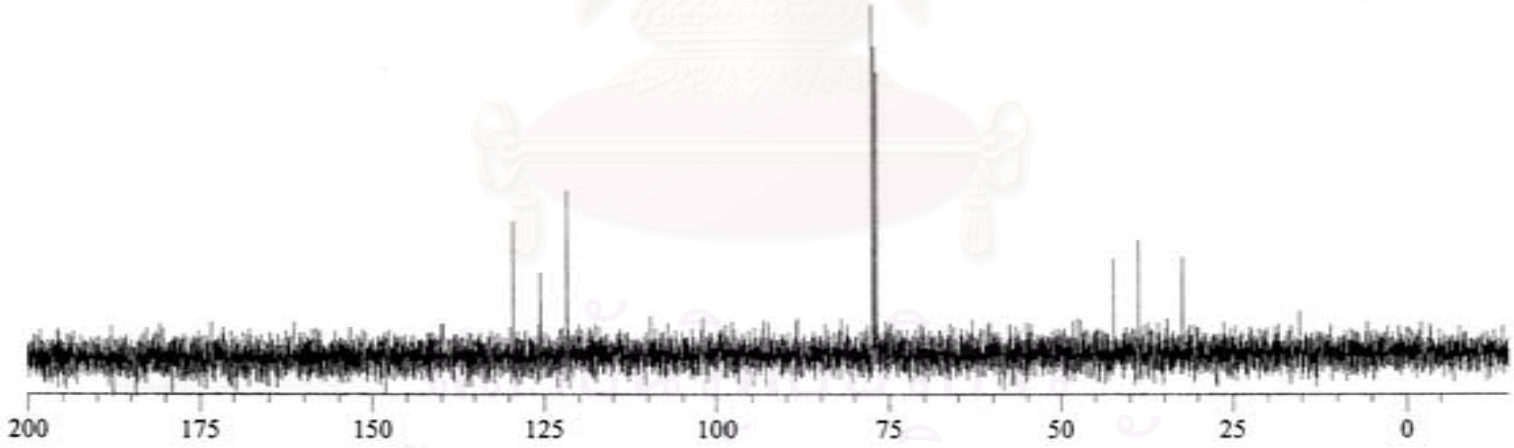

Figure **A.5** ^{13}C-NMR ($CDCl_3$) Spectrum of (*S*)-Tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetic acid (**13**)

53

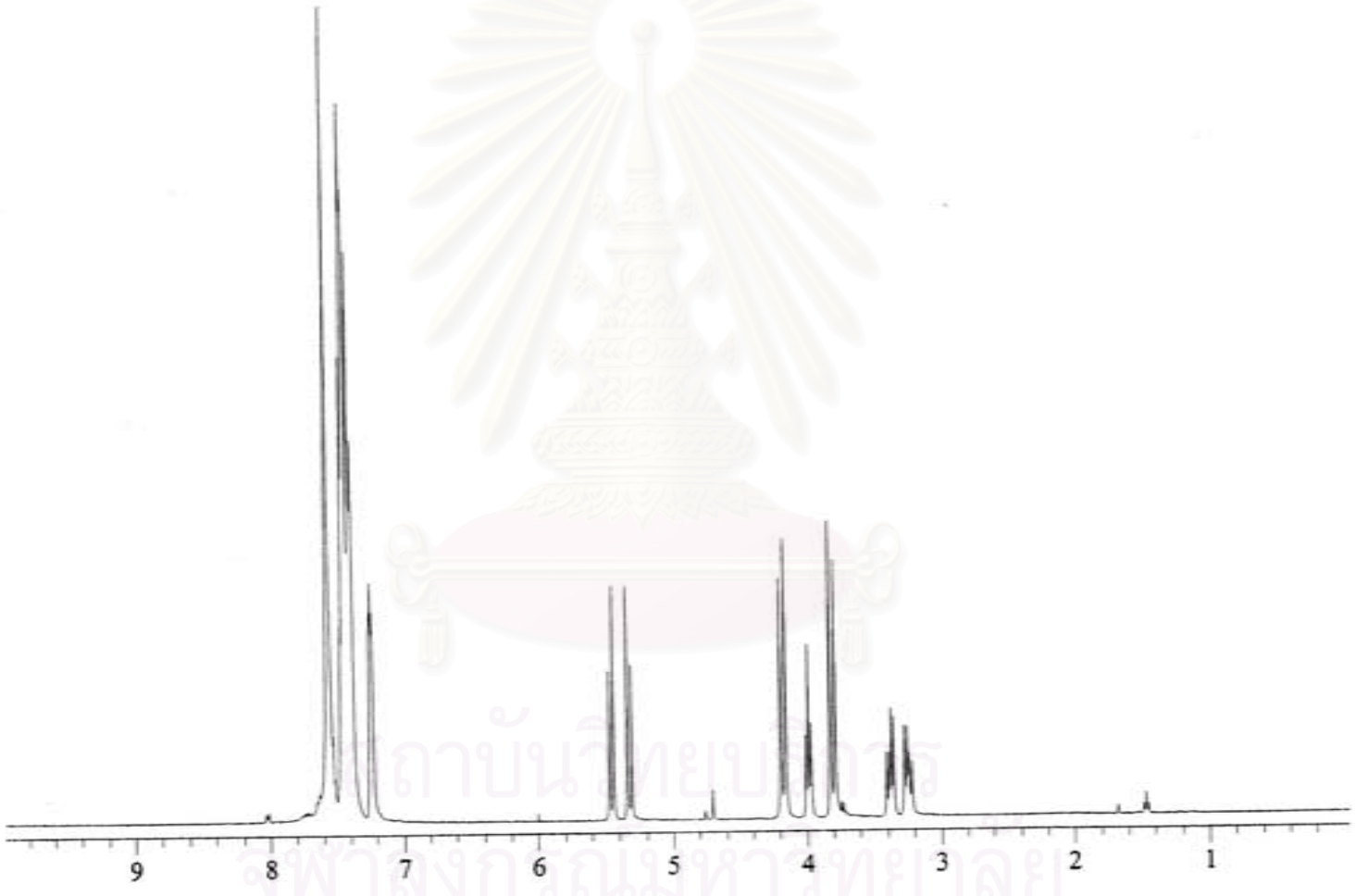

Figure **A.6** ^{1}H-NMR ($CDCl_3$) Spectrum of *N*,*N*-dibenzyl-(L)-phenylalanine benzyl ester (**14**)

54

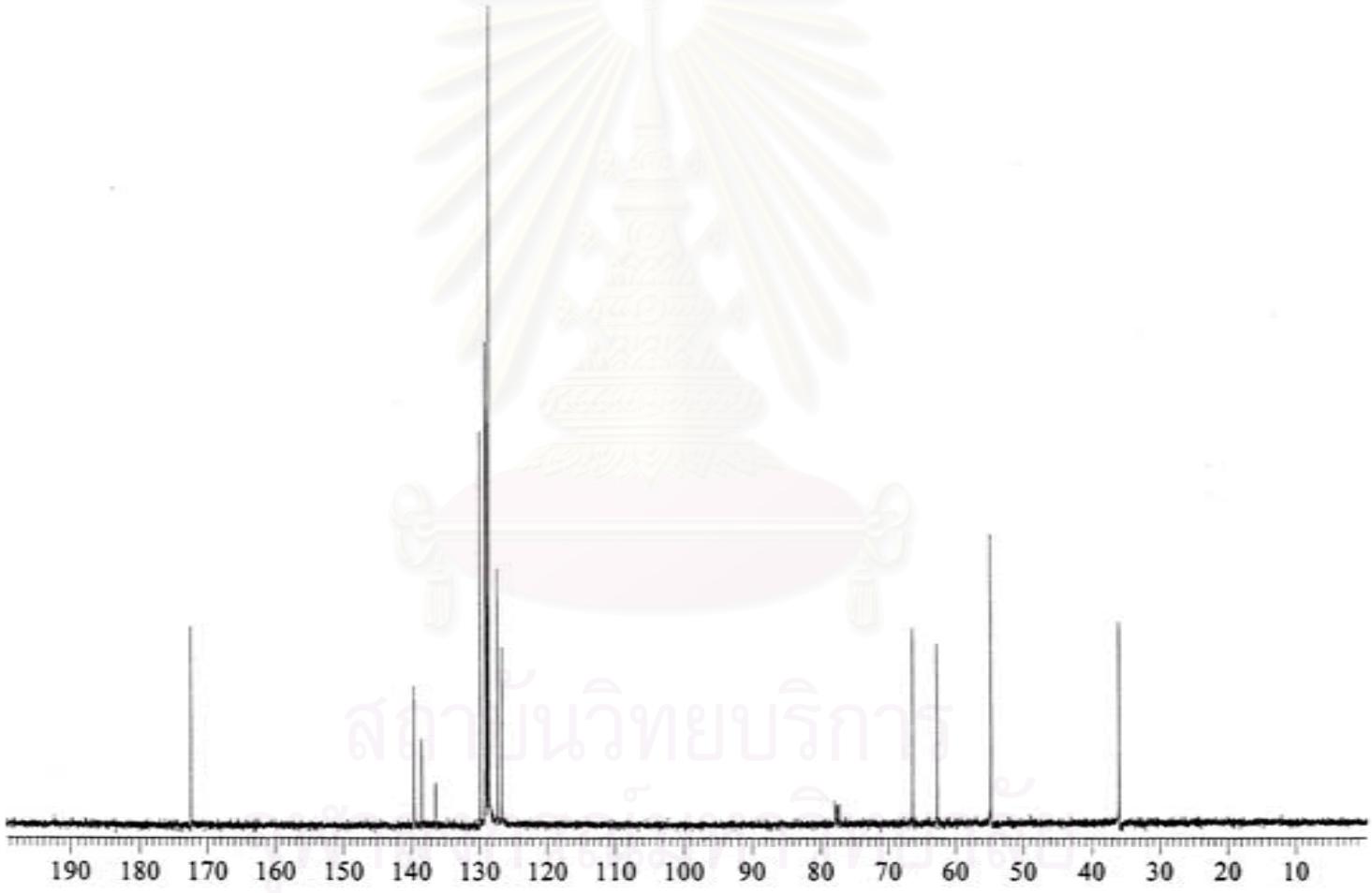

Figure **A.7** ^{13}C-NMR ($CDCl_3$) Spectrum of *N*-[(*tert*-benzyloxy)carbonyl]-L-phenylalaninol (**14**)

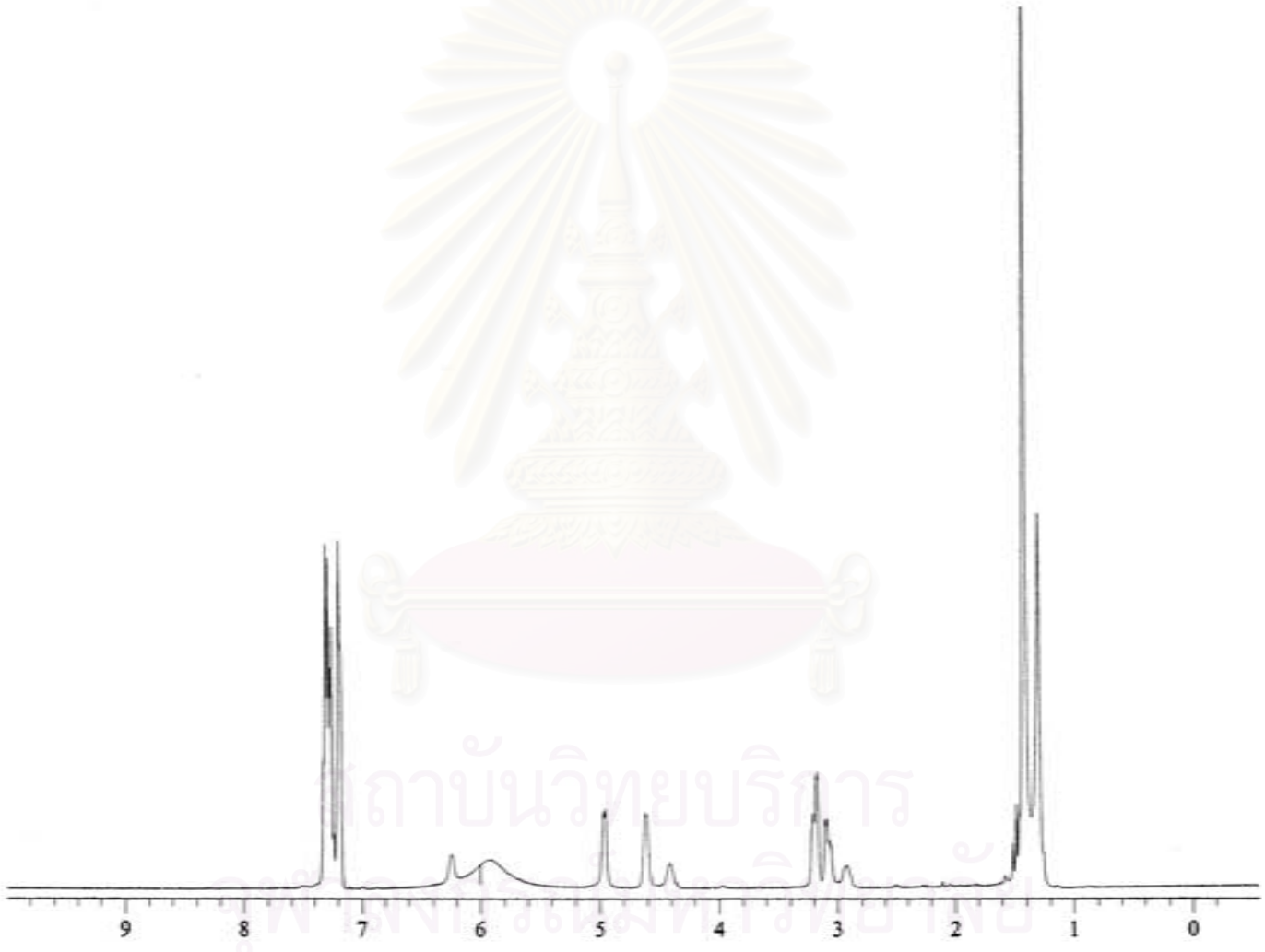

Figure **A.8** ^{1}H-NMR ($CDCl_3$) Spectrum of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalaninol (**16**)

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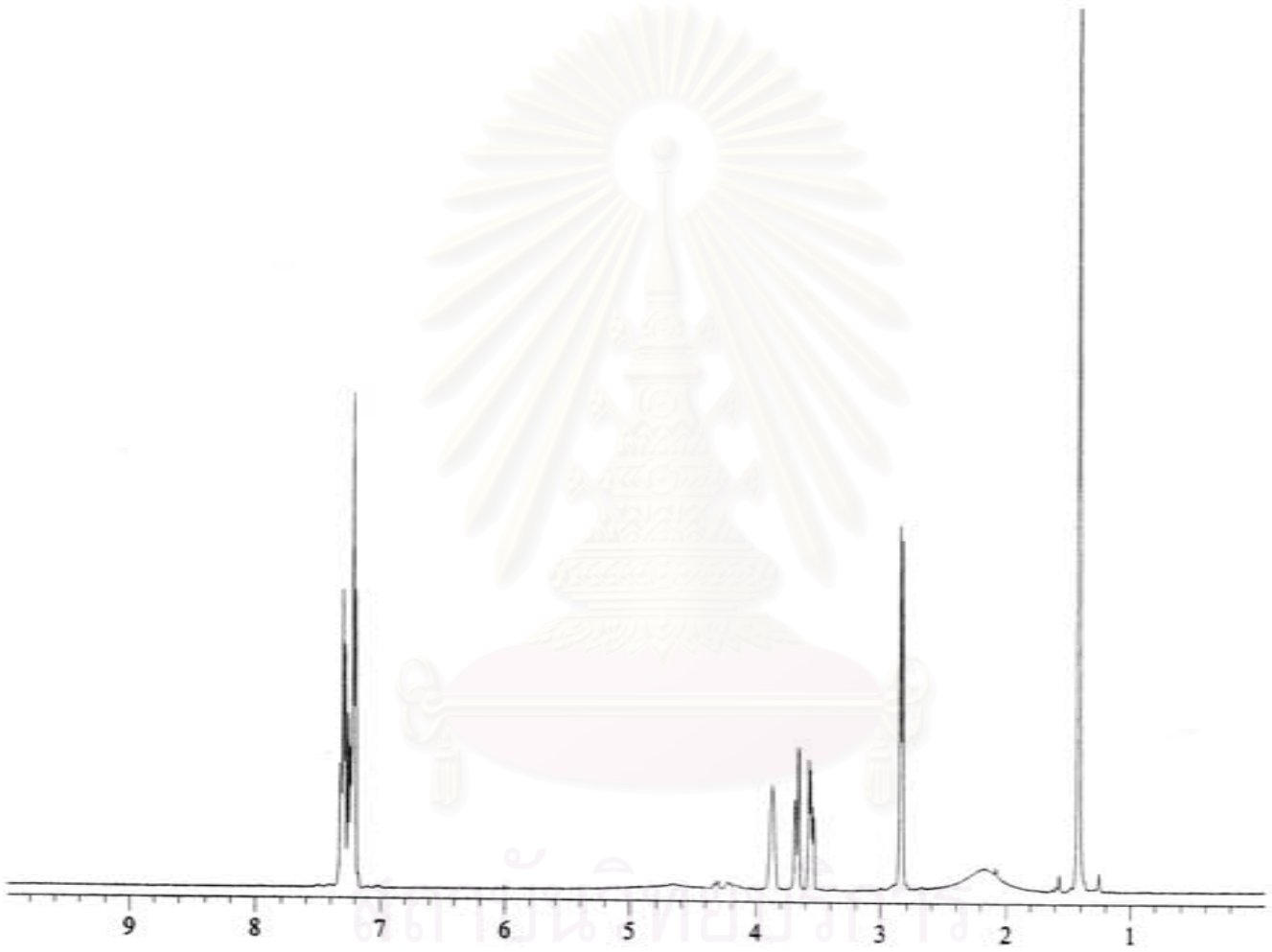

Figure **A.9** ^{1}H-NMR ($CDCl_3$) Spectrum of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalaninol (**17**)

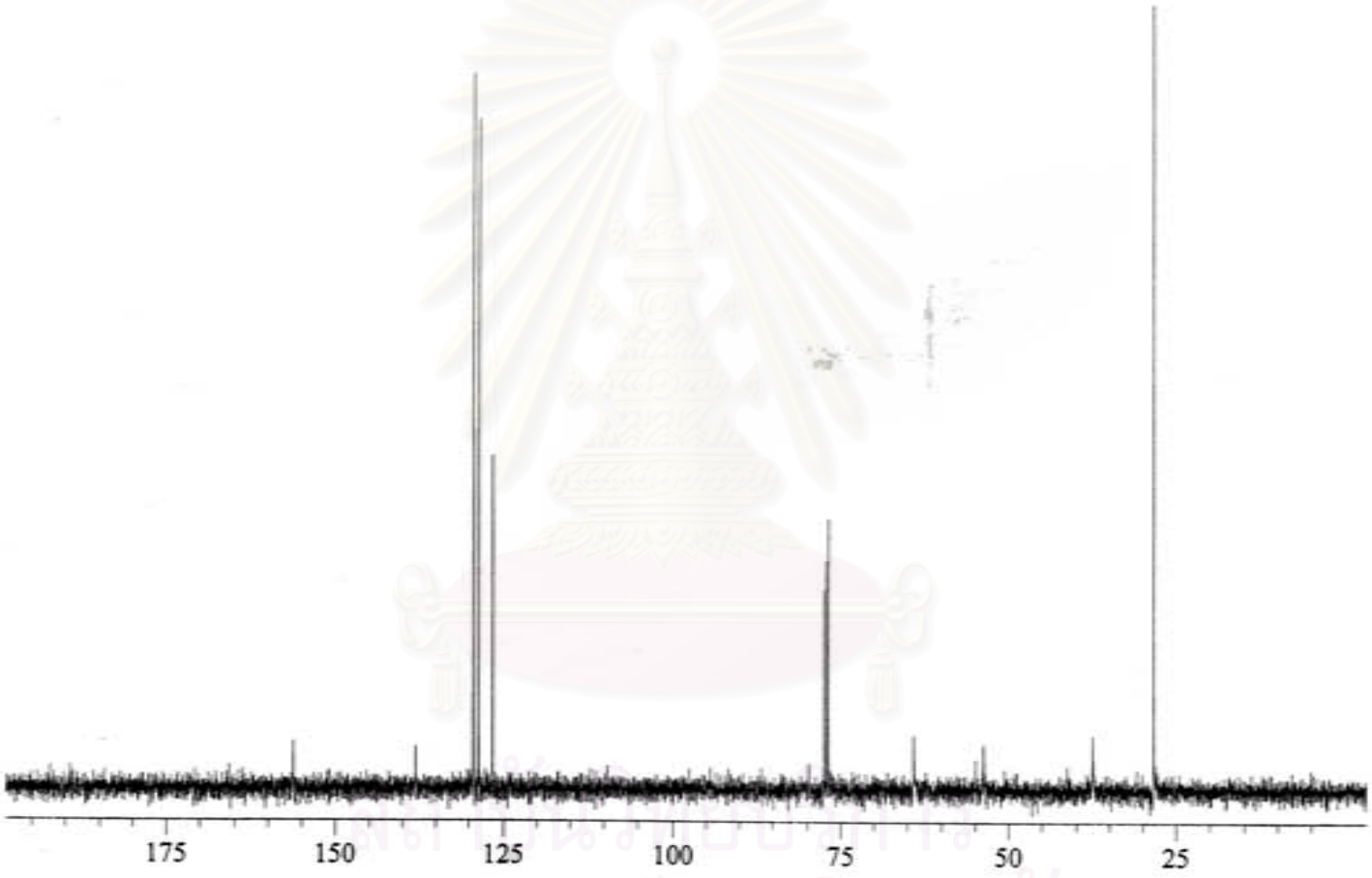

Figure **A.10** ^{13}C-NMR ($CDCl_3$) Spectrum of *N*-[(*tert*-benzyloxy)carbonyl]-L-phenylalaninol (**14**)

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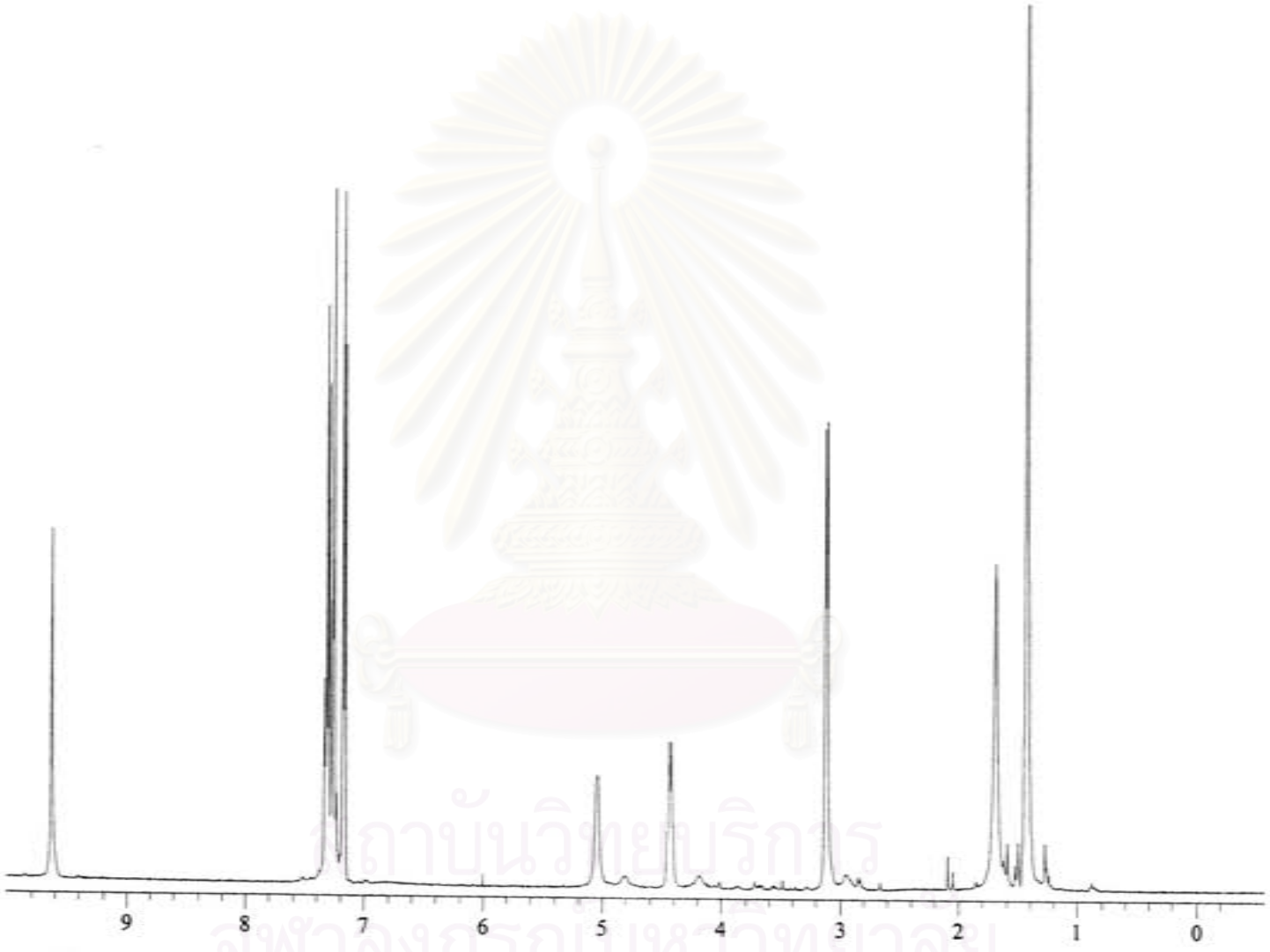

Figure **A.11** ^{1}H-NMR ($CDCl_3$) Spectrum of *N*-[(*tert*-butyloxy)carbonyl]-L-phenylalaninal (**18**)

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VITA

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