การสังเคราะห์ (2เอส)-2-(9เอช-9-ฟลูออรีนิลเมทอกซีคาร์บอนิลอะมิโน)-3-(9-ฟีแนนทรีนิล)โพรพิออนิกแอซิด

นายแมน ผิวเหลืองดี

SYNTHESIS OF

(2*S*)-2-(9*H*-9-FLUORENYLMETHOXYCARBONYLAMINO)-3-
(9-PHENANTHRENYL)PROPIONIC ACID

Mr. Man Phewluangdee

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By	Mr. Man Phewluangdee
Department	Chemistry
Thesis Advisor	Associate Professor Supawan Tantayanon, Ph.D.
Thesis Co-advisor	Professor Stephen J. Weininger, Ph.D.

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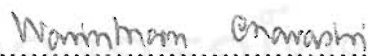
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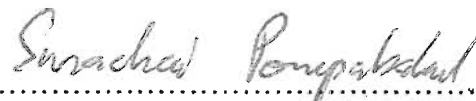
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การสังเคราะห์ (2เอส)-2-(9เอช-9-ฟลูออรีนิลเมทอกซีคาร์บอนิลอะมิโน)-3-(9-ฟีแนนทรี-นิล)โพรพิออนิกแอซิด เสร็จสมบูรณ์ใน 8 ขั้นตอน ให้โมเลกุลเป้าหมาย 35% จากฟีแนนทรีน-9-คาร์บอกซาลดีไฮด์ ของผสมราซีมิก (อาร์/เอส)-อะมิโนแอซิดเตรียมได้จากไดเอทิลแอซทามิโด-มาโลเนตกับ 9-(โบรโมเมทิล)ฟีแนนทรีน โดยวิธีของ Sörensen ที่ได้รับการปรับแปรแล้ว ปฏิกิริยาเอสเทอริฟิเคชันของกรดอะมิโนนี้ให้อะมิโนแอซิดเอสเทอร์ ต่อจากนั้นแยกอะมิโนแอซิดเอสเทอร์ให้อยู่ในรูปออพติคัลลีแอกทีฟ คือ (2เอส)-2-อะซีทิลอะมิโน-3-(9-ฟีแนนทรีนิล)โพรพิออนิกแอซิด โดยการไฮโดรไลส์หมู่เอสเทอร์ด้วยซับทิไลซิน CLECs-BL การรีโซลูชันด้วยเอนไซม์นี้ให้ (เอส)-อะมิโนแอซิด 45% คอนเวอร์ชัน (>98% ee) หลังจากฟักตัวในของผสมอะซีโตน-สารละลายฟอสเฟตบัฟเฟอร์ (pH 7.5-8.0) ที่อุณหภูมิ 37-40 องศาเซลเซียสเป็นเวลา 24 ชั่วโมง ค่าความบริสุทธิ์ของอีแนนชิโอเมอร์ (ee) ของสารผลิตภัณฑ์ที่แยกแล้วคำนวณได้จากโปรตอน NMR สเปกตรัมของอนุพันธ์ MTPA ของกรดอะมิโนที่แยกได้ แทนที่หมู่อะซีทิลของกรดอะมิโนแยกได้ด้วยหมู่ปกป้อง 9เอช-9-ฟลูออรีนิลเมทอกซีคาร์บอนิล (เอฟมอก) เพื่อนำไปใช้ในการสังเคราะห์เพปไทด์ต่อไป

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MAN PHEWLUANGDEE : SYNTHESIS OF (2*S*)-2-(9*H*-9-FLUORENYL-METHOXYCARBONYLAMINO)-3-(9-PHENANTHRENYL)PROPIONIC ACID. THESIS ADVISOR : ASSOC. PROF. SUPAWAN TANTAYANON, Ph.D., THESIS CO-ADVISOR : PROF. STEPHEN J. WEININGER, Ph.D., 91 pp. ISBN 974-346-757-2.

The synthesis of (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid was completed in 8 steps, which gave the target molecule in 35% overall yield from phenanthrene-9-carboxaldehyde. The racemic (*R/S*)-amino acid was prepared from diethyl acetamidomalonate and 9-(bromomethyl)phenanthrene by a modification of the Sörensen method. The esterification of this amino acid afforded amino acid ester, which was later resolved into its optically active form, (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid, by enzymatic hydrolysis of the ester group with subtilisin CLECs-BL. The enzymatic resolution gave (*S*)-amino acid in 45% conversion (>98% ee) after incubation in mixed acetone-aqueous phosphate buffer (pH 7.5-8.0) at 37-40 °C for 24 hours. An enatiomeric excess (ee) of resolved product was obtained from ^{1}H NMR spectrum of MTPA derivative of resolved amino acid. The acetyl group of the resolved amino acid was replaced with 9*H*-9-fluorenylmethoxycarbonyl- (Fmoc-) protecting group for further peptide synthesis.

Department of..Chemistry.......... Student's signature....................
Field of study...Organinc Chemistry Advisor's signature....................
Academic year..2000.................. Co-advisor's signature....................

ACKNOWLEDGEMENT

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ABBREVIATIONS

lit.	literature
de	diastereomeric excess
ee	enantiomeric excess
mg	milligram
MHz	megahertz
rpm	rounds per minute
cm^{-1}	units per centimeter (wave number)
$^{\circ}C$	degree Celsius
Fmoc	9H-9-fluorenylmethoxycarbonyl
Boc	t-butoxycarbonyl
Z	benzyloxycarbonyl
Ts	4-toluenesulphonyl
Ar	an unspecified aryl group
R, R^1...R^n	unspecified alkyl group
CLECs	cross-linked enzyme crystals
MTPA	α-methoxy-α-trifluoromethylphenyl acetic acid; Mosher's acid
PPL	porcine pancreatic lipase
DIPT	diisopropyl tartrate
Binap	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
DMF	dimethylformamide
DMSO	dimethylsulfoxide
THF	tetrahydrofuran
TLC	thin-layer chromatography
NMR	nuclear magnetic resonance spectrometry
FT-IR	fourier transform infrared spectrophotometry
GLPC	gas-liquid partition chromatography

CHAPTER I

INTRODUCTION

Introduction

Photoinduced intramolecular electron and energy transfer in organic molecules composed of several chromophores, electron donors and electron acceptors, has become a very interesting subject in recent years [1-8]. For the most part, previous works had focused on the rate of transfer processes and effects of various factors on them, such as interchromophore distance [2], stereochemistry or molecular conformation [2b-c, 3], solvent, temperature, electric field or dipole-dipole interaction [4], and other external factors. Their study led to the proposal of through-bond and through-space transfer mechanisms. Although the through-bond mechanism was recognized as long as 40 years ago by McConnell [5] and won acceptance by most investigators over the years, the through-space mechanism has been recently reported by Zimmt and co-workers as giving a more accurate description of the photochemistry of 'C-clamp' dichromophores [6].

In addition, polychromophores larger than dichromophores have been studied and have received increasing emphasis recently [7]. Usually, peptides have been used as models for investigation of these systems [2a, c, 3-4, 8], because they allow us to fix and vary the interchromophore distance [2c], Furthermore, incorporation of different chromophores into the peptide backbone is not complicated. In order to investigate the photoinduced intramolecular electron and energy transfer mechanisms in polychromophoric helical peptides, which have recently been the subject of increasing interest, here is a need for a number of optically active unnatural amino acids, which contain various chromophores for incorporation into peptide backbones.

Usually, the synthesis routes to amino acids that contain a chiral center proceed to the free racemic amino acids, which then have to be resolved. This is often accomplished by an enzymatic reaction [9]. Many kinds of enzymes such as

proteases, lipases, and acylases, were used by most of the previous workers to resolve both natural and unnatural amino acids. Bommarius and co-workers [9a] used acylase from *Aspergillus oryzae* to produce a variety of non-proteinogenic chiral amino acids. Chenault and co-workers [9b] used acylase I to prepare a wide-range of chiral amino acids. Bosshard [9c], Nestor and co-workers [10] used protease to resolve alanine derivatives. Leanna and Morton [9d] used both protease and acylase to prepare highly enantiopure unnatural amino acids, and so on. However, the resolution of amino acids is accomplished by other methods. Egusa and co-workers [11] prepared enantiopure amino acids by using *l*-ephedrine to form diastereomeric salts with amino acid derivatives and Nájera and co-workers used chiral template to prepare alanine derivatives [12], In this study, subtilisin *Carlsberg* has been chosen as the enzymatic catalyst, because it gave much faster resolution of the more hydrophobic amino acids than acylase or α-chymotrypsin. Also, it specifically hydrolyzes the (*S*)-ester [10], for this reason, the (*R*)-ester can be separated from the (*S*)-amino acid after the enzymatic hydrolysis has finished [9].

Objective and Scope of the research

In the recent years, photoinduced intramolecular electron and energy transfer in organic molecules has been studied and received increasing interest from many researchers. The need of this investigation is the organic molecules that consist of various chromophores. In most cases, the researchers used peptide backbone to study this subject. The optically active amino acids are therefore the appropriate molecule for incorporation into peptide backbone. The goal of this research is thus to synthesize an optically active amino acid, which is (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid (**a**). In the synthesis procedure, phenanthrenyl-9-carboxaldehyde will be used as starting material. The racemate of 2-amino-3-(9-phenanthrenyl)propionic acid will be produced after the several steps of synthesis have done. The racemate then will be enzymatically resolved with subtilisin protease and the resolved amino acid will be confirmed optical purity by its enantiomeric excess (ee). This parameter will be determined by NMR technique. In order to decrease activity of the amino acid product, the amino group of product will be protected with 9*H*-9-fluorenyl-

methoxycarbonyl (Fmoc)-protecting group, which will give a more stable molecule (b).

(a)

(b)
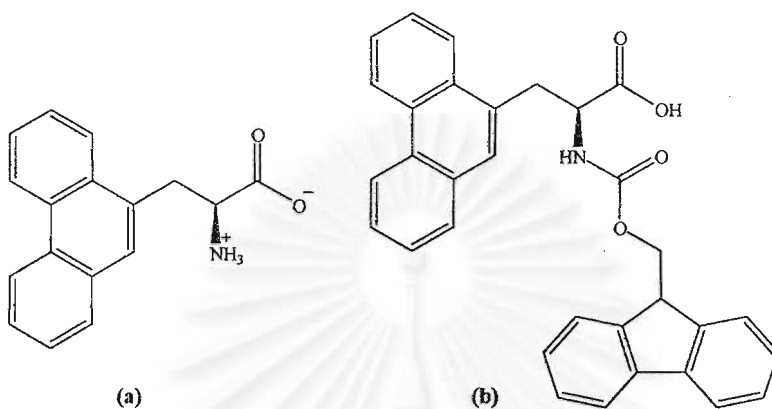
Figure 1: Structure of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid (a) and (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl) propionic acid (b)

CHAPTER II

THEORY

2.1 α-Amino Acid Synthesis [13, 14]

The proteinogenic α-amino acids can be produced on an industrial scale by fermentation methods and by chemical synthesis. Their principal application is food additives, but they are incidentally cheap starting materials for laboratory work. The synthesis of α-amino acids at the bench is nevertheless an active field because of the demand for specifically labeled, unnatural and unusual amino acids. The need is almost always for a homochiral product such as the (L)-enantiomers, which in most cases corresponds to the (*S*)-configuration. So assembly of the target without regard to α-chirality must be followed by resolution; alternatively an asymmetric synthesis must be employed; or an enantiospecific conversion of a freely available homochiral compound to the required α-amino acid must be achieved. Furthermore, suitable derivatives of most common amino acids allow the direct separation of their racemates without the need of a chiral auxiliary for the formation of diastereomeric derivatives [15].

2.1.1 General Synthetic Methods

The several general methods for the synthesis of α-amino acids, including displacement reactions on α-halo acids (e.g. *Scheme 2.1*), the Strecker synthesis (e.g. *Scheme 2.2*) [16], and approaches through hydantoins (e.g. *Scheme 2.3*) [17], via oxazolones (e.g. *Scheme 2.4*) [8c], were developed in the early days of amino acids chemistry, but still retain their importance. Although ammonia works well enough in conversion of very simple α-halo acids to α-amino acids, potassium phthalimide

(followed by strong acid hydrolysis of the intermediate phthalimido derivative: the Gabriel synthesis) or azide ion (followed by reduction) is superior reagents.
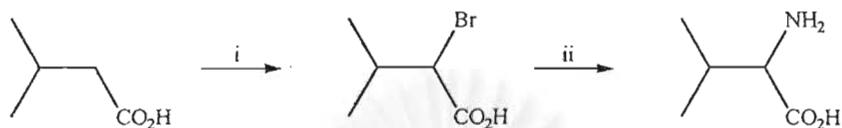
Scheme 2.1 α-Halo acids displacement reaction; condition: i) Br_2/PCl_3; ii) NH_3.
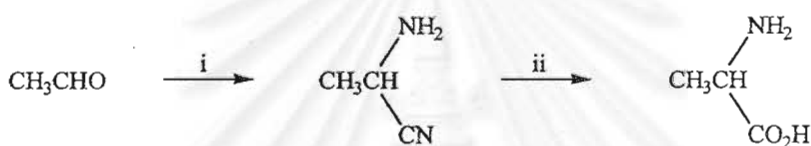
Scheme 2.2 The Strecker synthesis; condition: i) $NaCN/NH_4Cl$; ii) H_3O^+.
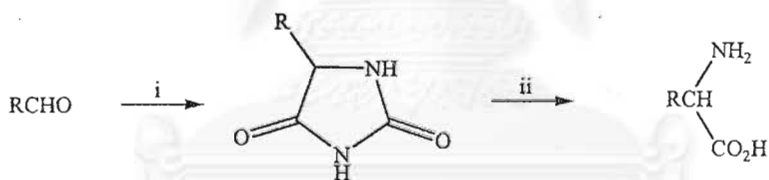
Scheme 2.3 Synthesis of amino acid approaches through hydantoins; condition: i) $KCN/(NH_4)_2CO_3$ (there are several alternatives for this ring-formation); ii) H_3O^+ or OH^-.
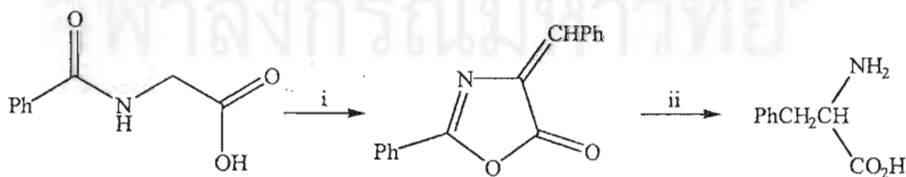
Scheme 2.4 The amino acid synthesis via oxazolones; condition: i) $PhCHO/Ac_2O/NaOAc$; ii) aq. HI/P/heat.

However, none of the above procedures is as frequently employed as that involving acylaminomalonates (e.g. *Scheme 2.5*). Even if aminomalonic acid and its

$$AcNHCH(CO_2Et)_2 \xrightarrow{\text{i}} \underset{\underset{(CH_2)_5CO_2Et}{|}}{AcNHC(CO_2Et)_2} \xrightarrow{\text{ii}} \underset{\underset{(CH_2)_5CO_2H}{|}}{NH_2CHCO_2H}$$
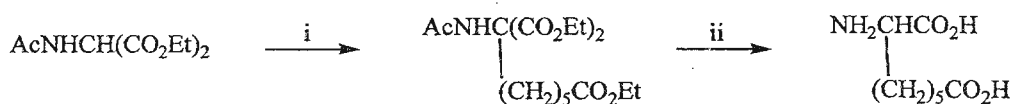
Scheme 2.5 The amino acids synthesis from diethyl acetamidomalonate; condition: i) NaOEt, then $Br(CH_2)_5CO_2Et$; ii) H_3O^+.

α-alkyl derivatives are isolable, they are unstable with respect to decarboxylation, so that α-amino acids are produced directly under the vigorous conditions of the last stage. Diethyl acetamidomalonate is easily obtained as in *Scheme 2.6*, and a range of other acylaminomalonates can be made and applied analogously (see *Scheme 2.10*).

$$CH_2(CO_2Et)_2 \xrightarrow{\text{i}} HON{=\!=}C(CO_2Et)_2 \xrightarrow{\text{ii}} AcNHCH(CO_2Et)_2$$
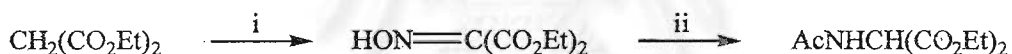
Scheme 2.6 Diethyl acetamidomalonate synthesis; condition: i) $NaNO_2/AcOH$; ii) $H_2/$ Pd(C), then Ac_2O.

Two further general strategies are illustrated in Scheme 2.7 and 2.8.

$$PhCH_2CN \xrightarrow{\text{i}} \underset{\underset{CN}{|}}{PhCHLi} \xrightarrow{\text{ii}} \underset{\underset{NH_2}{|}}{PhCHCO_2H}$$
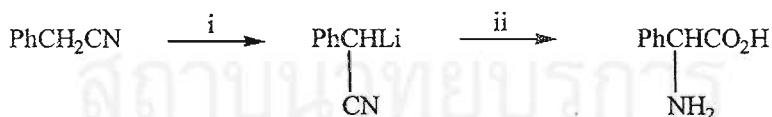
Scheme 2.7 The general strategy for the amino acids synthesis; condition: i) *n*-BuLi, ii) CO_2 at $-80\,^{\circ}C$, then H_3O^+.

$$RCH_2CO_2H \xrightarrow{\text{i}} RCH(Li)CO_2Li \xrightarrow{\text{ii}} RCH(NH_2)CO_2H$$
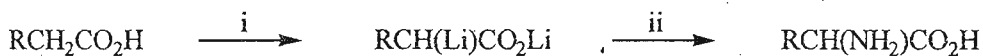
Scheme 2.8 The general strategy for the amino acids synthesis; condition: i) *i*-Pr$_2$NLi; ii) NH_2OMe.

2.1.2 Resolution

The reactions illustrated in Section **2.1.1** all inevitably lead to racemic products. The traditional general approach to the resolution of racemates of all kinds is to derivatize with and optically active reagent, separate the diastereomers, and then reverse the derivatization. *Scheme 2.9* shows an example which works well with

Z-DL-Ala + L-TyrNHNH$_2$ $\xrightarrow{\text{i}}$ Z-D-Ala, L-TyrNHNH$_2$ + Z-L-Ala,L-TyrHNH$_2$

Precipitate   Remain in solution

| ii | | iii |

Z-D-Ala   Z-L-Ala, Dcha
Optically pure   Optically pure
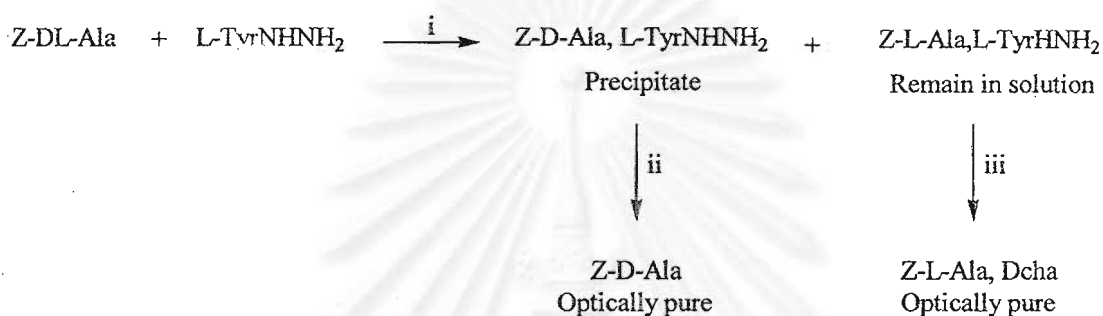
Scheme 2.9 The resolution of racemates by the diastereomers derivatization method; condition: i) mix equimolar amounts in MeOH; ii) filter off, recrystallize once and neutralize (recovery of resolving agent); iii) neutralize liquors (recovery of resolving agent), add dicyclohexylamine (Dcha), and recrystallize once.

benzyloxycarbonylamino acids: the D-L and L-L diastereomeric salts differ so much in solubility and ease of crystallization that the former separates practically quantitatively. Enzymes provide an alternative approach, which is especially appropriate for α-amino acids. If an enzyme catalyses a conversion at all, it is usually highly enantioselective, and a suitable enzyme and conversion can be found for most cases. *Scheme 2.10* shows a convenient combination of the acylaminomalonate synthesis with an enzymatic method of resolution; *Scheme 2.11* outlines a different tactic with a different enzyme, which is particularly valuable because although the enzyme used is enantioselective, it works with a wide range of side chains.

For more information about the resolution, see *section 2.2* (Kinetic Resolution of Racemates).

$$\text{AcNHCH(CO}_2\text{Et)}_2 \xrightarrow{\quad i \quad} \text{AcNHCR(CO}_2\text{Et)}_2 \xrightarrow{\quad ii \quad} \text{AcNHCR} \begin{matrix} \nearrow \text{CO}_2\text{H} \\ \\ \searrow \text{CO}_2\text{Et} \end{matrix}$$

$$\xrightarrow{\quad iii \quad} \begin{matrix} \text{AcNHCHRCO}_2\text{Et} \\ \text{DL} \end{matrix} \xrightarrow{\quad iv \quad} \begin{matrix} \text{AcNHCHRCO}_2\text{H} \\ \text{L} \end{matrix} + \begin{matrix} \text{AcNHCHRCO}_2\text{Et} \\ \text{D} \end{matrix}$$

Scheme 2.10 The resolution of racemates by using protease; condition: i) NaOEt, then RX; ii) KOH until one group saponified; iii) heat in dioxane; iv) protease (subtilisin *Carlsberg*) [10].

Scheme 2.11 The resolution of racemates by using acylase; condition: acylase I (aminoacylase) from porcine kidney [9a, c].

2.1.3 Asymmetric Synthesis

For a reaction which creates a new chiral center to lead to more of one form than the other, i.e. for asymmetric synthesis to take place, at least one of the reagents must be optically active, but very high enantiomeric excesses (ee) can sometimes be obtained. Several ingenious techniques have been devised for the efficient asymmetric synthesis of α-amino acids. All are of relatively recent invention: *Scheme 2.12* and *2.13* illustrate two of particular interest and importance.

Scheme 2.12 Asymmetric synthesis of amino acid; condition: i) MeCOCO$_2$Me; ii) NaOMe/heat; iii) Al(Hg)/H$_2$O; iv) H$_2$/Pd(C), followed by recrystallization to remove traces of the minor diastereomer; v) H$_3$O$^+$.
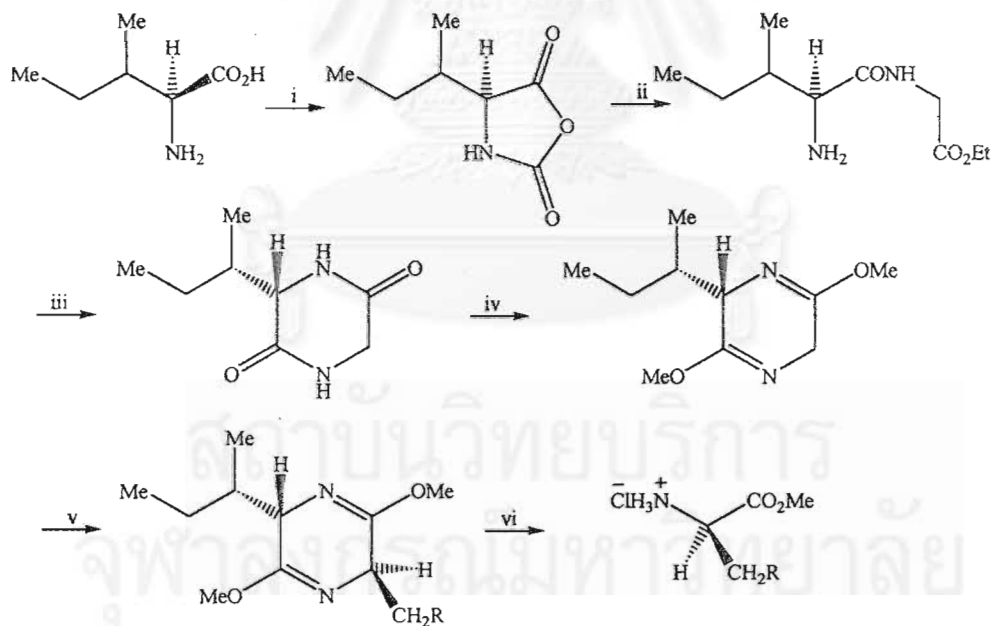
Scheme 2.13 Asymmetric synthesis of amino acid; condition: i) COCl$_2$; ii) NH$_2$CH$_2$CO$_2$Et; iii) heat; iv) Me$_3$O$^+$BF$_4^-$; v) n-BuLi, then RCH$_2$X; vi) 0.25N HCl. Products with 70-95% ee were obtained, depending on R.

2.1.4 Enantiospecific Synthesis

In principle, the selective chemical modification of the side chains of cheap proteinogenic amino acids offers attractive access to rarer or unnatural L-α-amino acids, provided the original chirality can be preserved. For example, L-vinylglycine can be obtained from α-diprotected L-methionine or L-glutamic acid (Scheme 2.14).
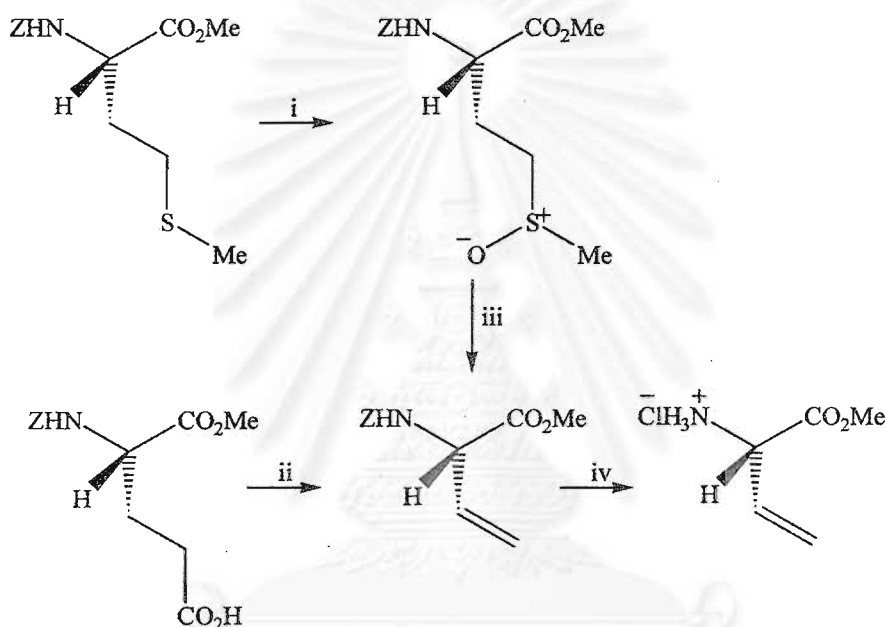
Scheme 2.14 Enantiospecific synthesis of amino acids; condition: i) $NaIO_4/MeOH/$ $H_2O/0\ ^\circ C$; ii) $Pb(OAc)_4/Cu(OAc)_2/PhH/reflux$; iii) pyrolysis 148 $^\circ C/3$ mm Hg; iv) 6N HCl/reflux.

2.2 Kinetic Resolution of Racemates [14]

In contrast to the classical resolution by crystallization or chromatographic separation of diastereomeric derivatives, and as opposed to the direct separation by preferential crystallization, chromatography using chiral stationary phases, or enantioselective transport through chiral membranes, kinetic resolution makes use of enantiomer selective reactions. A racemate is thereby chemically transformed by a

chiral reagent or catalyst. The optical purities of product and remaining starting material are a function of the different rates for the enantiomers. In ideal cases, the reaction stops after 50% conversion. If concomitant racemization of only the starting material is possible, a complete transformation to one enantiomer can be achieved.

2.2.1 Chemical Methods

Cyclic amino acids can be racemized smoothly by heating in carboxylic acids with an aldehyde as catalyst. Protonated Schiff's bases are proposed as intermediates of this epimerization. In combination with enantioselective salt precipitation using (R,R)- or (S,S)-tartaric acid, Shiraiwa and coworkers have applied this principle for the deracemization of amino acids. As shown in *Scheme 2.15* (R/S)-cysteine (**1**) was transformed with acetone/AcOH to 2,2-dimethylthiazolidine-4-carboxylic acid (**2**). The salt **3** was precipitated in high yield by heating with one equivalent of (R,R)-tartaric acid in presence of salicyladehyde and hydrolysis of this salt gave (S)-cysteine (**1**) of 98% ee in 80% overall yield.
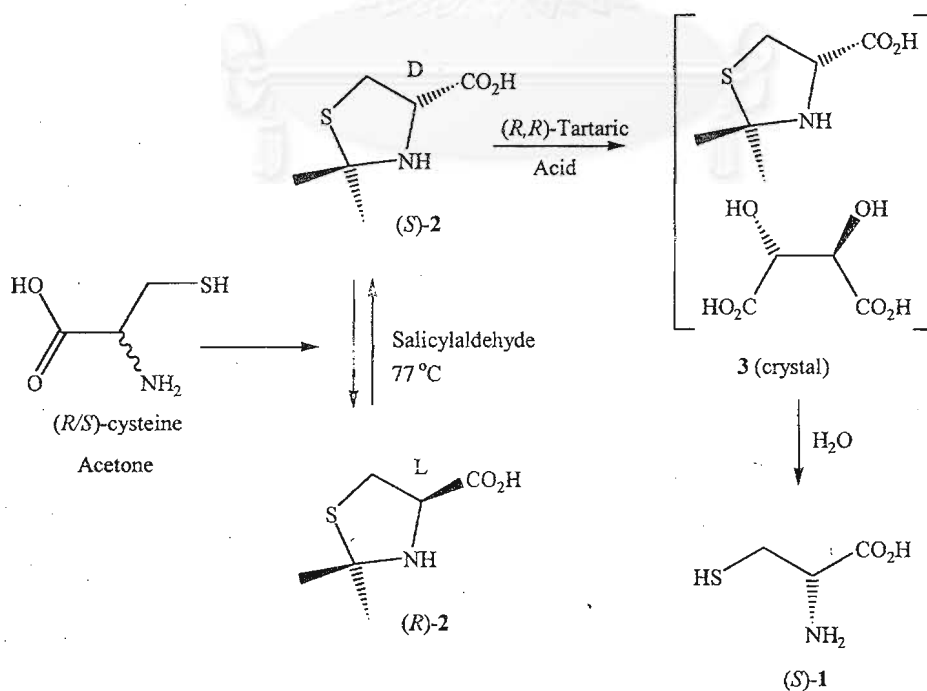
Scheme 2.15 Deracemization of amino acids by using tartaric acid.

Not surprisingly, the catalytic oxidation system, which was developed by Sharpless and coworkers for enantioselective epoxidation [18], has also been applied for kinetic resolution of amino acid precursors (*Scheme 2.16*). The (*R*)-enantiomer of the racemic allyl alcohol **4** was selectively oxidized to the epoxide **5** by using (D)-(-)-DIPT (diisopropyl tartrate), which formed hydroxy-L-prolinol **6** by Ti(O-i-Pr)₄ assisted ring closure. Amidomercuration of (*S*)-4 (→7) followed by *O*-protection and oxidative demercuration gave the enantiomeric prolinol **8**, selectively protected at O-C(3). By *O*-silylation and RuO₂-mediated oxidation of C(5), the diol **6** was transformed into an intermediate for *threo*-3-hydroxyglutamate. Swern oxidation of **8** affords 3-hydroxyprolinal.
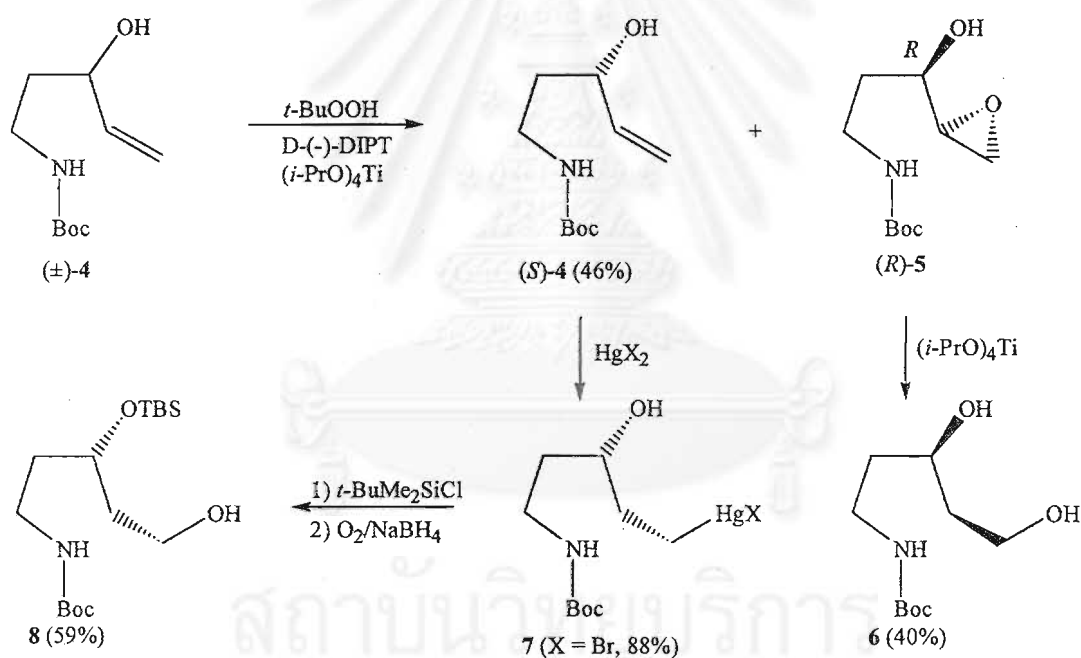
Scheme 2.16 Kinetic resolution of amino acid precursors by Sharpless epoxidation.

The α-furyltosylamides **9** are prepared from *N*-tosylfurfuralimine. Sharpless epoxidation of **9** afforded the unstable dihydropyridones **10** and the (*S*)-enantiomers of **9** (R: Me, Et, *n*-Pr, *n*-Bu, *n*-Hex) in high optical purity (90-98% ee). Oxidative degradation of two representatives (R: Me, *i*-Bu) with O₃ or RuCl₃/NaIO₄ afforded the *N*-tosyl amino acids **11** (*Scheme 2.17*).
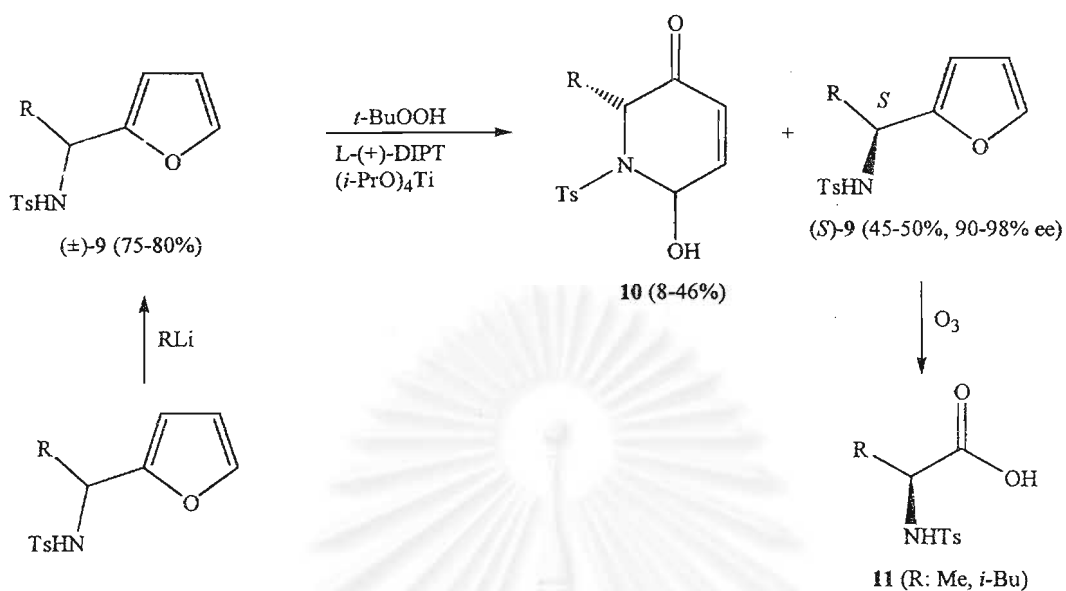
Scheme 2.17 Deracemization of amino acids by Sharpless epoxidation and followed with oxidative degradation.

The enantiomers of α-amido-β-ketoesters are in rapid equilibrium, due to the acidity of the α-hydrogen. Reduction of the keto-function generates a new stereocenter and the facile racemization is stopped simultaneously, an ideal situation for kinetic resolution. The Ru/Binap hydrogenation catalyst developed by Noyori and
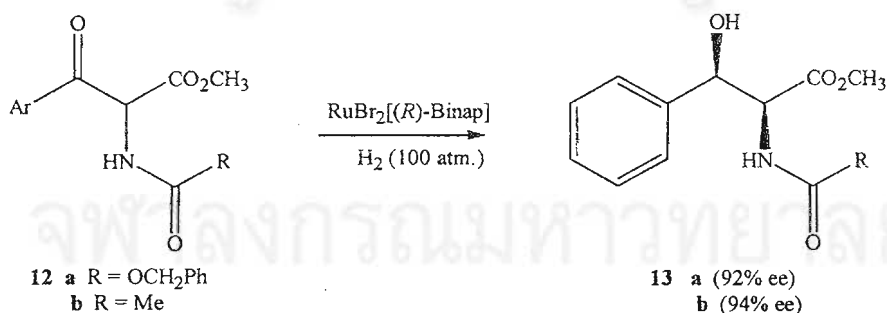
Scheme 2.18 Reductive resolution of amino acids by the Ru/Binap hydrogenation catalyst.

associates is ideally suited for this transformation and the arylketone **12** is converted quantitatively to the (L)-*threo*-β-hydroxyphenylalanine derivative **13** with excellent

induction (92-94% ee, *Scheme 2.18*). While cationic Rh-complexes turned out to be less discriminating, the Ru/Binap system was successfully applied for the reductive resolution of various α-acylamino-β-oxocarboxylates as well, e.g. acetoacetate **14a**. However, in this case 23% of *erythro*-epimer **15a** was formed in addition to the major
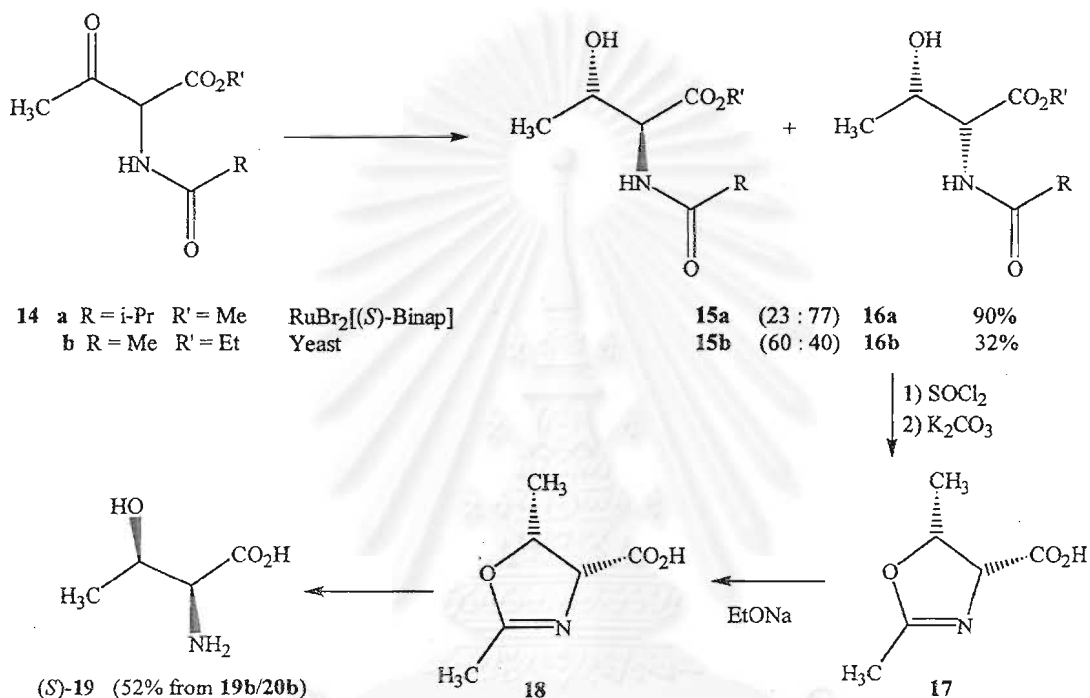
Scheme 2.19 Reductive resolution of amino acid by the Ru/Binap hydrogenation catalyst and microbial reduction using Baker's yeast.

diastereomer **16a**. The same transformation can also be effected by microbial reduction (*Saccharomyces rouxii*, baker's yeast). The 3:2 mixture of **15b** and **16b** could be transformed into the oxazolines **17** and **18**, involving an inversion of C(3). Equilibration at C(4) and oxazoline cleavage gave L-threonine **19** in 52% overall yield from **15b/16b** (*Scheme 2.19*). *Cis*-3-Hydroxy-(*R*)-proline and the six-membered analog can be produced by microbial reduction of the corresponding cyclic ketoesters as well. D-Configurated phenylalanines **20** with various substituents on the aromatic ring have been obtained in excellent yield and with high optical purity by subjecting the racemates to L-amino acid oxidase (*Scheme 2.20*). The L-enantiomers are thereby transformed into the α-ketoacids **21**.
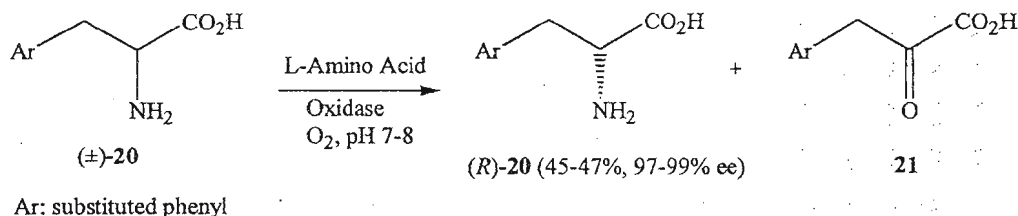
Scheme 2.20 The kinetic resolution of amino acids by L-amino acid oxidase.

Oxazolin-5-ones (azlactones), derivatives of *N*-acyl-amino acids, are especially prone to racemization and therefore suited for kinetic resolution with concomitant isomerization. Miyazawa and coworkers used α-amino-esters for the enantiomer-selective aminolysis of such oxazolinones (*Scheme 2.21*). 2-Phenyl-5-oxazolinones 27 gave thereby better results than the corresponding azlactones derived

R	% de (CH$_2$Cl$_2$)	% de (DMF)
i-Pr	84	72
i-Bu	90	72
s-Bu	79	83
t-Bu	86	80
Ph	89	68
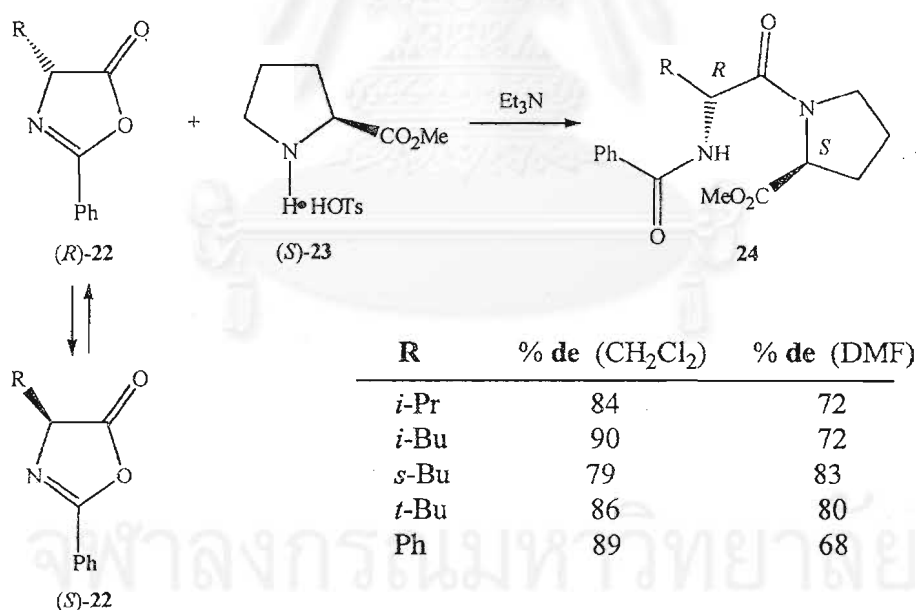
Scheme 2.21 The enantiomer-selective aminolysis of oxazolinones using α-aminoesters

from formic, acetic, pivalic, or trifluoroacetic acid; and proline methyl ester 23 was more selective in forming the (*R,S*)-configurated *N*-benzoyl dipeptides 24 than other

α-amino-esters. The diastereomeric excess is generally better in non-polar solvents like CH_2Cl_2 or xylene than in DMF and can be increased by lowering the temperature. In a recent report, Sih and coworkers demonstrated that the same 2-phenyloxazolinones (±)-**22** can also be hydrolyzed enzymatically with high enantiomer selectivity (*Scheme 2.22*). By testing ten different lipase, it was found that

% ee	R	% ee
83	$MeS(CH_2)_2$	80
37	$4\text{-}HOC_6H_4CH_2$	19
99	$C_6H_5CH_2$	99
34	$Indol\text{-}3\text{-}CH_2$	55
6	*i*-Bu	87
80	C_6H_5	76
15	$4\text{-}HOC_6H_4$	67
20	$C_6H_5CH_2SCH_2$	20
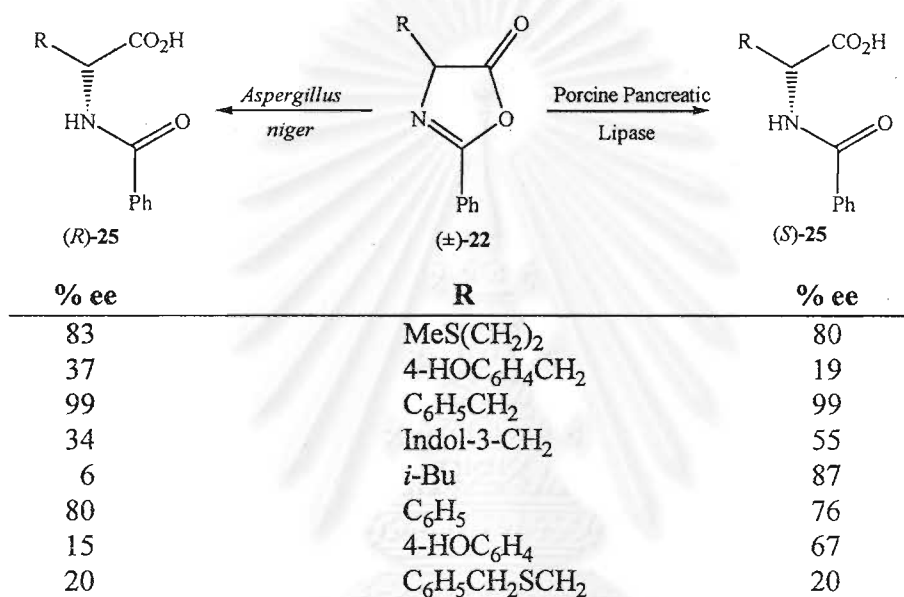
Scheme 2.22 The kinetic resolution of amino acids by enzymatic hydrolysis.

porcine pancreatic lipase (PPL) affords the (*S*)-enantiomers of the *N*-benzoylamino acids **25**, while the *Aspergillus niger* enzyme is (*R*)-selective. In some cases like phenylalanine, the induction is impressive. However, for some substrates, uncatalyzed hydrolysis appears to compete with the enzymatic process. In a recent report, a more general methods for the enantiomer-selective hydrolysis of (±)-**22** is described: in a first step methanolysis catalyzed by *Pseudomonas cepacia* lipase gives *N*-benzoyl methyl esters in 46-91% yield and with 66-95% ee. Further treatment with *Protease N* or *Prozyme 6*, two commercially available enzyme preparations, affords (*S*)-**25** of > 99% ee and often in more than 50% overall yield. The same proteases also catalyzed the hydrolysis of C(4)-substituted 2-phenylthiazolin-5-ones to L-*N*-thiobenzoylamino acids (14-98%, 57-98% ee). The alcoholysis (*n*-BuOH) of (±)-**22** has also been catalyzed by a fungal lipase (*Mucorniehei*). However, the induction is not as high (43-69% ee, *S*) and the racemization too slow to allow a complete conversion.

2.2.2 Use of Hydrolytic Enzymes

Hydrolytic enzymes are especially well suited for kinetic resolution of racemic amino acid derivatives, so this method has found numerous industrial applications and has also recently been reviewed. The different approaches are best classified according to the bond cleaved by enzymatic assistance. The major processes are amide or nitrile hydrolysis by *aminopeptidases* or *nitrilases*, cleavage of *N*-acyl groups by *acylases*, and ester hydrolysis by *lipases* or *proteases*. A disadvantage of enzymatic methods is often the narrow substrate tolerance; the determination of scope and limitation is therefore of crucial importance. As most enzymes selectively process the L-configurated enantiomers, recent effort has been directed towards finding D-selective enzymes. The availability of the enzymes from natural sources is no longer a major issue, as sequencing, cloning, and expression can now be done routinely. Enzymes with improved properties, e.g. higher stability, become available from site-specific mutations.

R:			
R:	*i*-Pr	31% (98% ee)	26% (46% ee)
R:	*c*-Pent.	42% (98% ee)	27% (74% ee)
R:	Allyl	21% (95% ee)	
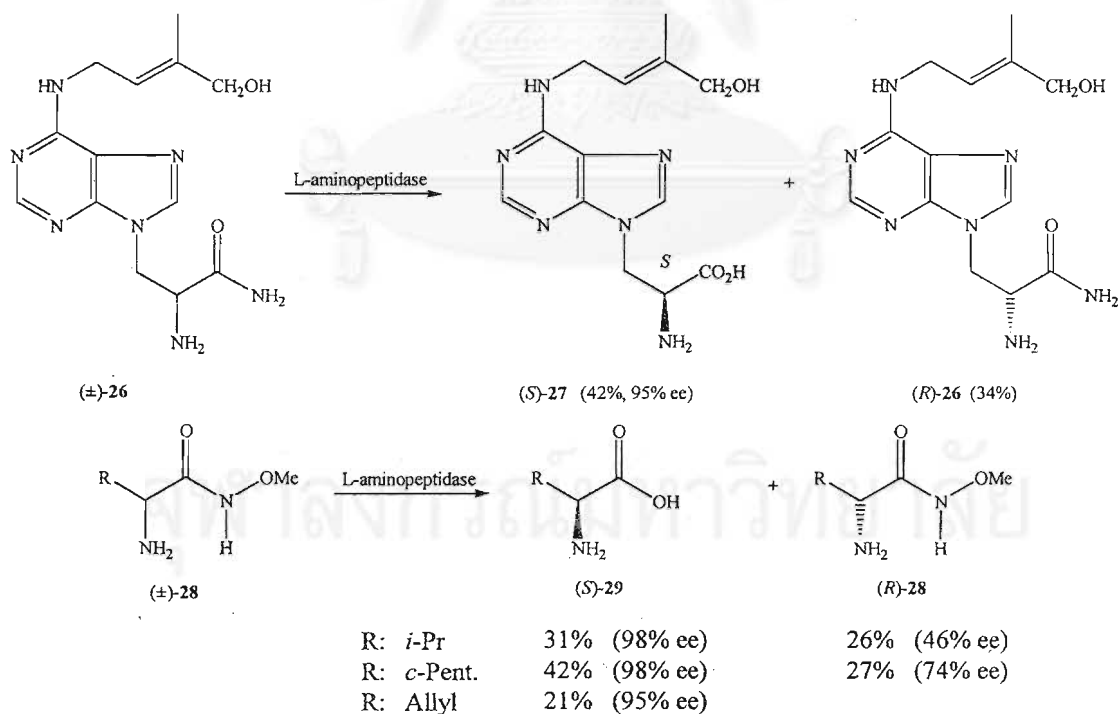
Scheme 2.23 The kinetic resolution of amino acids by L-specific aminopeptidase.

The versatility of the L-specific aminopeptidases from *Pseudomonas putida* has been illustrated in several recent review and articles. Complex substrates such as amide **26** are smoothly converted into (*S*)-lupinic acid **27** leaving the D-enantiomer (*R*)-**26** untouched (*Scheme 2.23*). *N*-Methoxy-amides **28** are more soluble than the unsubstituted counterparts and are therefore easier to handle. Their successful kinetic resolution by the L-specific aminopeptidase affording L-amino acids **29** is therefore of high practical value. With the aid of the D-specific aminopeptidase from *Ochrobacterium anthropi* aminolysis of racemic amino acid esters **30** in aprotic solvents affords selectively the (*R*)-configurated amides, e.g. alanine-3-pentylamide (*R*)-**31** (*Scheme 2.24*). The substrate tolerance of this thiol-peptidase is, however, rather narrow, and branched-chain amino acids as well as serine, threonine, and methionine are not processed. While 3-pentylamine can be replaced by *n*-butylamine, neopentylamine, or benzylamine (slow), laurylamine and aniline are not tolerated.
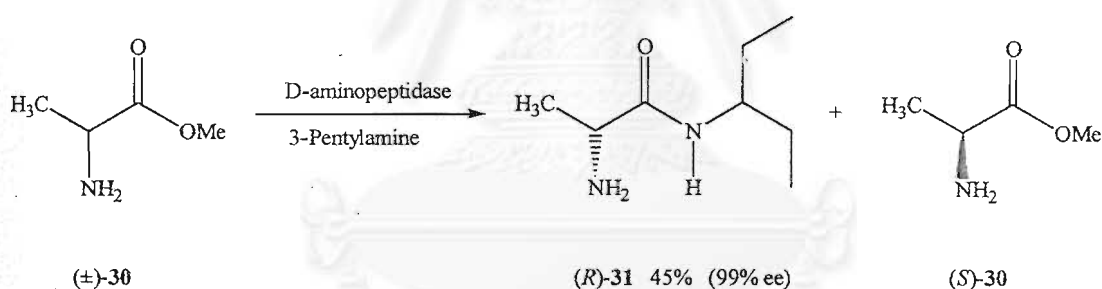
Scheme 2.24 The kinetic resolution of amino acids by D-specific aminopeptidase.

Racemic α-aminonitriles **32** are efficiently prepared by the Strecker synthesis. Their enantiomer-selective hydrolysis to α-amino amides or amino acids **33** is therefore of great practical value. The nitrilase from *Rhodococcus rhodochrous* converts several substrates **32** with high L-selectivity to (*S*)-configurated acids **33** (*Scheme 2.25*). In the case of R = CH_3, however, D-alanine of 57% ee is produced. The enantiomer-selective α-aminonitrile hydrolysis has also been attempted with chiral ketones as catalysts. For phenylalanine amide, the maximal enantiomeric excess obtained was 42%. With the aid of a new D-selective hydantoinase (EC 3.5.2.2) from *Agrobacterium radiobacter* racemic hydantoins with an ω-ureido function **34** can be

cleaved to (R)-configurated acids **35**. Due to *in situ* racemization of **34**, the yields exceed 50% (*Scheme 2.25*) [19]. Thiazoline **36**, a synthetic intermediate for racemic cystein, can be transformed quantitatively to L-cysteine (**1**) by fermentation with *Pseudomonas thiazophilum* under carefully optimized conditions [20].

R:		
i-Pr	>99% ee	
n-Pr	92% ee	
i-Bu	89% ee	
n-Bu	>99% ee	
MeS(CH$_2$)$_2$	96% ee	

X:		
CH$_2$	69%	98% ee
(CH$_2$)$_2$	73%	96% ee
(CH$_2$)$_3$	79%	96% ee
(CH$_2$)$_4$	70%	20% ee
(CH$_2$)$_2$SCH$_2$	71%	96% ee
(CH$_2$)$_2$S(CH$_2$)$_2$	78%	96% ee
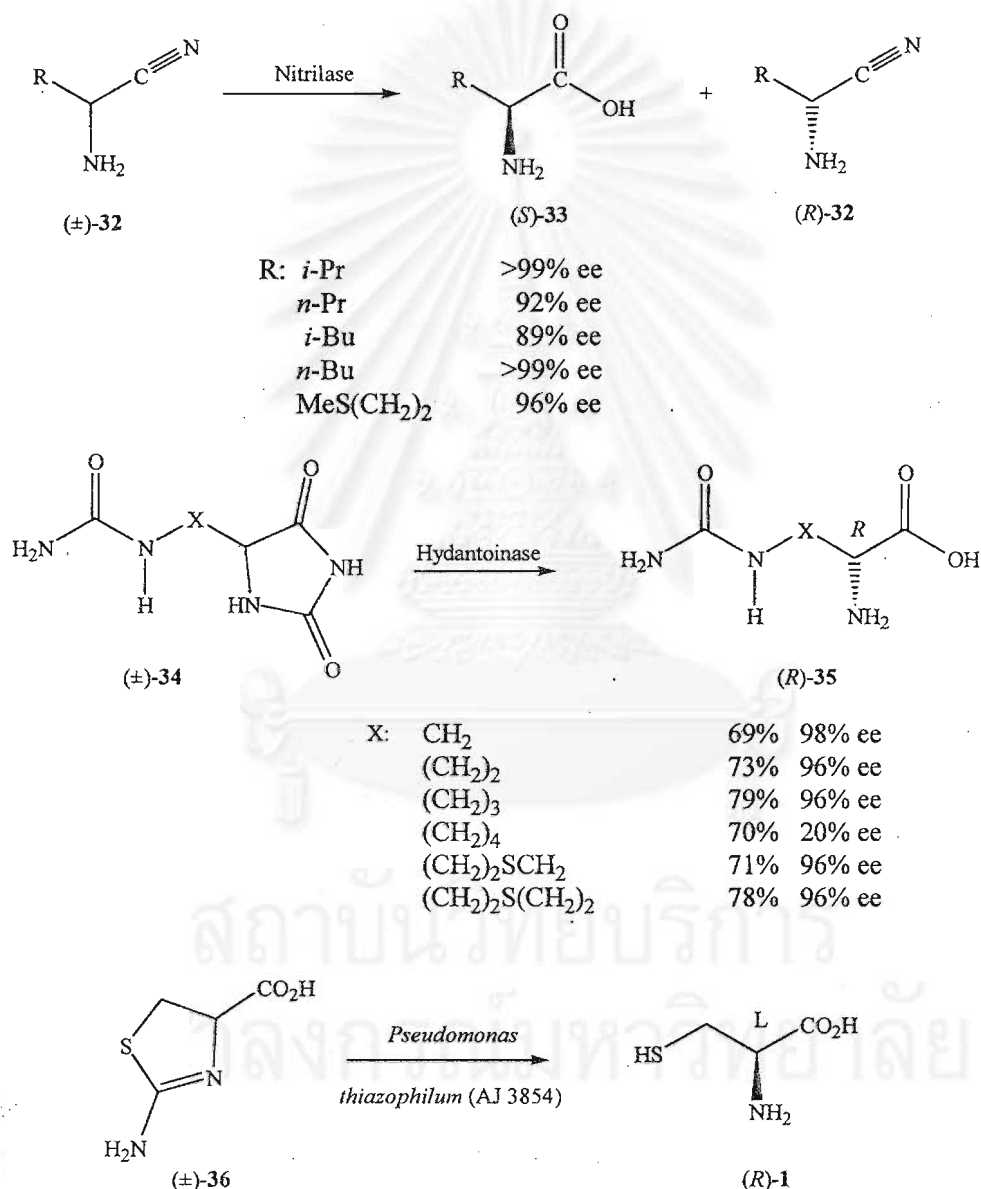
Scheme 2.25 The amino acids synthesis by enzymatic resolution.

Table 1 Enantiomer selective hydrolysis of racemic N-acetyl or N-(chloroacetyl) amino acids with acylase I (EC 3.5.1.14) [21].
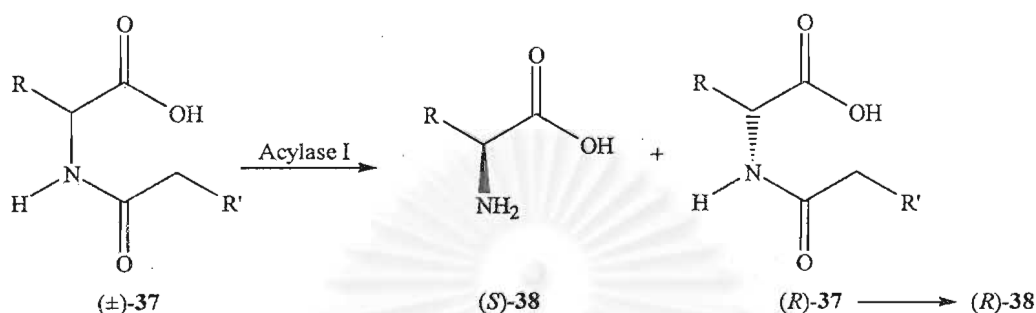
R	R'	Enzyme	(S)-42 Yield (% ee)	(R)-42 Yield (% ee)
Et	H	Porcine kidney	40% (99.5)	32% (99.5)
Et	Cl	Porcine kidney	40% (99.5)	41% (99.5)
n-Pr	H	Aspergillus	33% (99.5)	32% (99.5)
Allyl	Cl	Porcine kidney	41% (99.5)	33% (99.5)
trans-Butenyl	H	Aspergillus	33% (99)	38% (93)
cis-Butenyl	H	Porcine kidney	44% (99.5)	47% (99.5)
cyclo-Pr	Cl	Porcine kidney	37% (99)	42% (84)
cyclo-PrCH$_2$	H	Aspergillus	50% (95)	50% (98)
2-FurylCH$_2$	H	Aspergillus	45% (99)	41% (-)[a]
MeS(CH$_2$)$_2$	Cl	Porcine kidney	51% (93)	31% (-)[a]
PhCH$_2$	Cl	Porcine kidney	43% (91)	46% (80)
4-HOC$_6$H$_4$CH$_2$	Cl	Porcine kidney	17% (95)	64% (47)

a) The free amino acid (R)-38 was not isolated.

One of the most versatile acylases for the L-selective cleavage of N-acetyl, chloroacetyl, or methoxyacetyl groups is acylase I (EC 3.5.1.14), obtained either from *porcine kidney* or *Aspergillus oryzae*. Whiteside and associates have reported an exhaustive study involving over 50 substrates (±)-37; some of their reports are summarized in *Table 1*. The selectivity is generally excellent, and after separation of L-38, D-37 can often be hydrolyzed chemically, affording the enantiomer D-38 in high optical purity as well. The two enzymes show some complementarity, as only the fungal enzyme tolerates α-methyl-α-amino acids, while the O$_2$-sensitive acylase from

porcine kidney is to be preferred for aromatic and β-branched sidechains. Amino acids with additional functionalities (lysine, histidine, arginine) are poor substrates; aspartic acid and secondary amino acids (proline, pyroglutamate) are not tolerated.

It is therefore not surprising, that this method has found rather broad application, and further structured resolved with acylase I are show in *Figure 2*. Enzymatic hydrolysis is probably the best method for obtaining the L-enantiomers of delicate β,γ-unsaturated amino acids like 39-44, used for labeling with ^{3}H (39, 40) or as enzyme inhibitors and antimetabolites (40-44). Trifluoro-norvaline (45), the bornyl-alanine (46), used for artificial sweeteners, and cyclooctatetraenyl-alanine (47), designed as a metal ligand, have been resolved with acylase I as well as furyl- and thienyl-alanine.
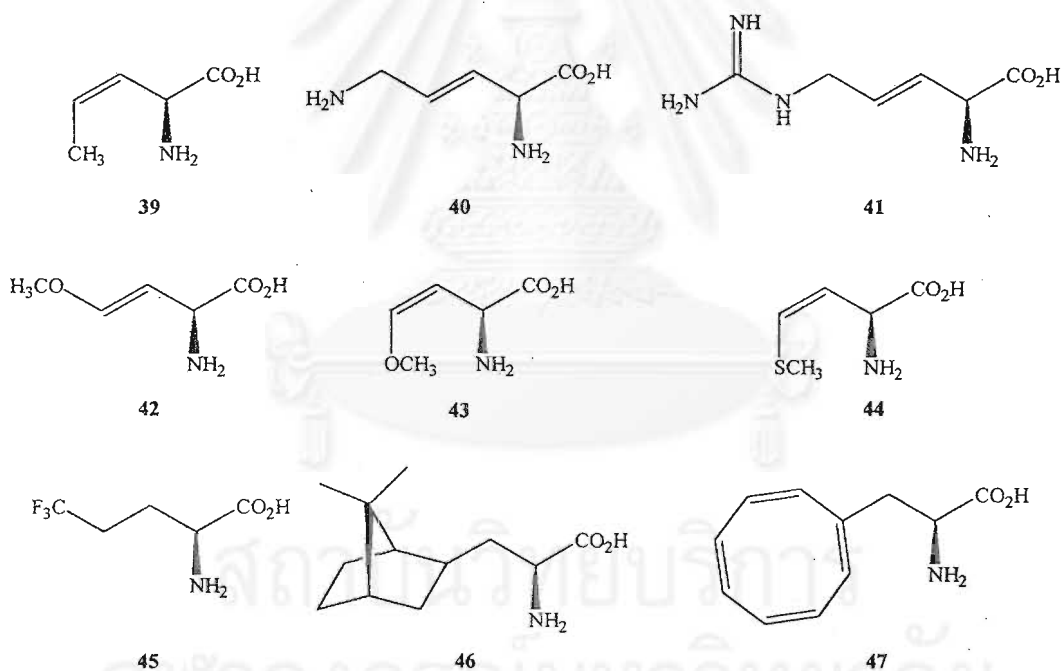
Figure 2: Amino acids resolved by acylase I (EC 3.5.1.14)

While acylase I does not process proline or other cyclic amino acids, an L-selective proline-acylase was found in *Bacterium comamonas testosteroni* (DSM 5416). As shown in *Scheme 2.26*, this enzyme not only converts L-configurated *N*-acetyl or *N*-chloroacetyl-proline 48a,b into L-proline (49), but can also be used to resolve azetidine-carboxylic acid (50), pipecolic acid (51), and some *N*-methyl-α-

amino acids (**52**). *Penicillin acylase* (EC 3.5.1.11) is another enzyme, readily available from bacterial sources, which cleaves *N*-pheylacetyl derivatives of α-amino acids with high L-selectivity. This enzyme has also found use for selective deprotection in peptide and carbohydrate chemistry, and was successfully applied for the resolution of 5-phosphono-α-amino-pentenoate (**53**) *via* the phenylacetamide (±)-**54**. Chemical hydrolysis of (*R*)-**54**, not processed by the enzyme, gave (*R*)-**53** of high optical purity,
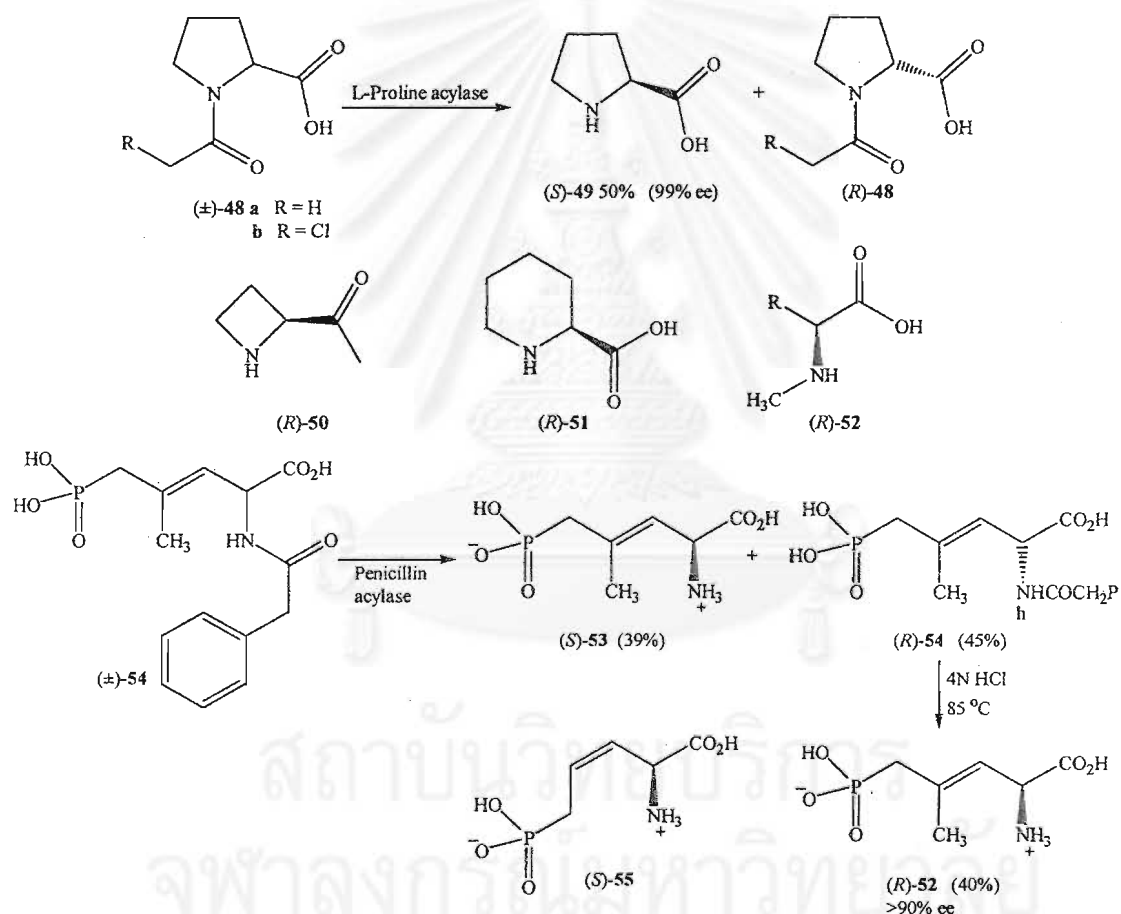
Scheme 2.26 The kinetic resolution of amino acid by acylase.

a potent glutamate antagonist. The (*S*)-enantiomer of cis-2-amino-5-phosphono-3-pentanoic acid (**55**) was obtained by the same method. The specific rotation of (*S*)-**55** ($[\alpha]_D = 198$, c: 0.5/H$_2$O) is much higher than reported for the material obtained by acidic hydrolysis of the peptidic antibiotics plumbemycine or rhizocticin. Moreover,

by applying the Clough-Lutz-Jirgensoa rule to **55** the wrong absolute configuration was deduced; the correct configuration of (S)-(+)-**55** was confirmed by an independent synthesis from (R)-serine. The fact that the original erroneous rotation value for (S)-**55** is reported in connection with a recently claimed total synthesis of (S)-**55**, adds to doubts over this work and related publications.

Table 2 L-Selective ester hydrolysis of racemic N-benzyloxycarbonyl protected amino acid methyl esters (±)-**56** with microbial protease [22].
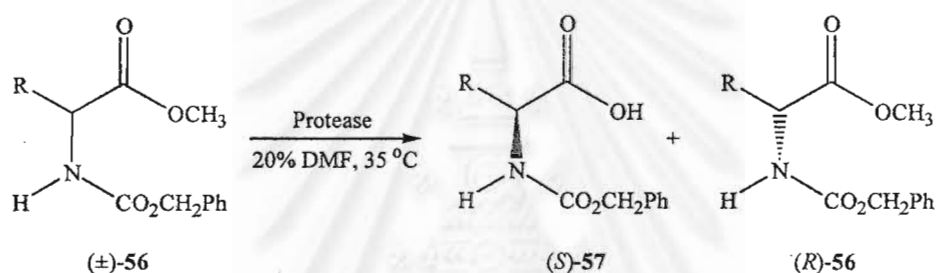
R	Aspergillus oryzae		Bacillus subtilis	
	Conversion	% ee (57)	Conversion	% ee (57)
Et	45%	78	40%	95
n-Pr	40%	83	40%	91
n-Bu	40%	98	40%	99
i-Bu	40%	93	40%	97
n-Pentyl	33%	94	40%	98
n-Hexyl	30%	97	38%	99
$HOCH_2$	38%	21	40%	78
$ZNH(CH_2)_4$	21%	98	40%	93
$C_6H_5CH_2$	40%	94	45%	98
$4-FC_6H_4CH_2$	17%	75	40%	85
$4-ClC_6H_4CH_2$	40%	98	40%	94
$4-BrC_6H_4CH_2$	32%	97	40%	90
i-Pr	-	-	40%	98
$MeS(CH_2)_2$	-	-	40%	99
C_6H_5	-	-	25%	41
$C_6H_5(CH_2)_3$	-	-	40%	99

The third method for enzymatic resolution of amino acids makes use of proteolytic enzymes for enantiomer-selective ester hydrolysis of *N*-acyl amino acid esters. Among the best-studied enzymes are the microbial proteases of *Bacillus subtilis* and *Aspergillus oryzae*. The scope of these enzymes has recently been surveyed and some of the results are collected in *Table 2*. These hydrolytic enzymes apparently have a broader substrate tolerance than the acylases, and benzyloxycarbonyl-protected derivatives (±)-**56** with unusual residues are cleaved L-selectively to the (*S*)-configurated acids **57**. The protease from *Bacillus subtilis* gives generally better results. Low optical purity is observed with serine and phenylglycine, and amino acids with long aliphatic sidechains are processed at slow rates.

Solvent	v_L/v_D (initial rates)
CH$_3$CN	7.1 : 1
Toluene	1 : 3.8
Octane	1 : 4.2
CCl$_4$	1: 5.2

59 a X = CO$_2$CH$_3$
 b X = CONHOH
 c X = SO$_3$Na
 d X = PO(OEt)$_2$
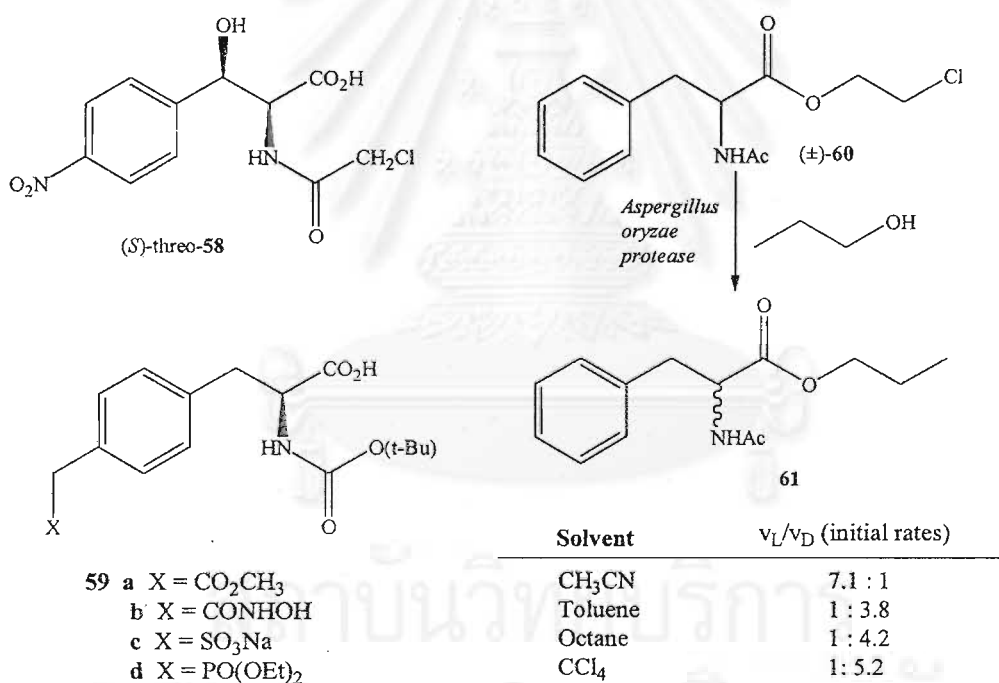
Scheme 2.27 The application of protease for amino acids synthesis.

Site-directed mutation has yielded a subtilisin mutant (8350) with 100-fold enhancement of stability in H$_2$O and 50-fold higher stability in DMF, exhibiting similar or even moderately better catalytic activity. The *Bacillus subtilis* protease was the only enzyme, which allowed resolution of the chloroamphenicol precursor **58**, and the *O*-phosphotyrosine analogs **59** have also been obtained by L-selective hydrolysis

of the corresponding methyl or ethyl esters with this enzyme (*Scheme 2.27*). In aprotic media, the transesterification of β-chloroethyl ester (±)-**60** with n-propanol (→**61**) is catalyzed by the *Aspergillus oryzae* protease. Interestingly, the preference of the enzyme changes from moderate L-selectivity in polar solvents to moderate D-seclectactivity in a polar media. It has recently been reported that bovine carbonic anhydrase (CE 4.2.2.1), a Zn-enzyme, cleaves methyl ester of *N*-acetyl-phenylalanine, -aspartate, and -glutamate with high D-selectivity.

Besides α-chymotrypsin, papain, and bromelain, *porcine pancreatic lipase* (PPL) is successfully used for enantiomer-selective ester hydrolysis, if trifluoroethyl esters are used. In *Table 3*, the results with PPL (EC 3.1.1.3) are compared with lipases from *Aspergillus niger*, *Pseudomonas fluorescens*, and *Candida cylindracea*. Substrates are the benzyloxycarbonyl protected 2,2,2-trifluoroethyl esters (±)-**62a** in the case of PPL and the corresponding β-chloroethyl esters for the other lipase. With the exception of alanine, valine, n-hexylglycine PPL is more selective than the three microbial enzymes. In sharp contrast to the *Bacillus subtilis* protease (*Table 2*), the conversion to L-phenylglycine proceeds well and with excellent selectivity.

The alkaline protease preparation "Alcalase" from *Bacillus licheniformis* (mainly *subtilisin Carlsberg*) is emerging as another useful enzyme for L-selective ester cleavage (±)-**56** to (*S*)-configurated acid **57** and (*R*)-**56** (*Scheme 2.28*). While unprotected esters are best hydrolyzed in water without buffer by adjusting the pH to 7 with 5N NaOH, a cosolvent (acetone or dioxane) is advisable for the lipophilic *N*-benzyloxycarbonyl protected derivatives (±)-**56**. In this case the enzyme should be stabilized by immobilization on *Amberlite XAD-8*. After L-selective ester-cleavage with subtilisin, it is advantageous to cleave *N*-acetyl derivatives with acylase I, resulting in a further upgrading of enantiomeric purity. Alcalase was also used for the liberation of (*S*)-**63**, for the regioselective monohydrolysis of *meso*-2,7-diaminoheptanedioic acid monoester (→ **64**) and for the resolution of the bipyridyl-alanines (*S*)-**65** and (*S*)-**66**. The enzyme *pronase* (EC 3.4.24.4) converted the dibenzyl esters of α-amino diacids regioselectively to the monoesters **67**. Treatment of racemic *N,O*-diacetyl-4-difluorothreonine (±)-**68** as the 4,4,4-trifluoro-analog with a *cellulase* from *Trichoderma viride* gave the monoprotected derivatives, *e.g.* **69**, with moderate

Table 3 L-Selective hydrolysis of α-amino esters (±)-**61** with Different lipases [23].

(±)-**63 a** R : CF$_3$ (PPL)
     b R : CH$_2$Cl (other lipase)
(S)-**57**
(R)-**62**

R	PPL conv.	(% ee)	A. Niger conv.	(% ee)	P. fluorescens conv.	(% ee)	C. cylindracea conv.	(% ee)
Me	31%	(21)	36%	(89)	46%	(16)	38%	(7)
Et	40%	(97)	40%	(96)	49%	(52)	36%	(7)
n-Pr	40%	(97)	27%	(86)	43%	(58)		
n-Bu	38%	(92)	32%	(85)	53%	(60)	42%	(30)
i-Bu	40%	(98)						
n-Pentyl	40%	(87)	44%	(95)	25%	(80)	27%	(66)
i-Pentyl	40%	(95)						
n-Hexyl	33%	(61)	31%	(94)	47%	(87)		
n-Heptyl	21%	(52)						
i-Pr	a)		14%	(92)	8%	(53)		
Allyl	38%	(93)	34%	(86)	40%	(52)	31%	(27)
MeS(CH$_2$)$_2$	40%	(90)						
C$_6$H$_5$	44%	(97)						
C$_6$H$_5$CH$_2$	40%	(99)	32%	(94)	12%	(57)	35%	(63)
Thiazol-4-CH$_2$	44%	(89)	37%	(94)	40%	(70)	35%	(43)
C$_6$H$_5$(CH$_2$)$_2$	30%	(71)						
C$_6$H$_5$(CH$_2$)$_3$	17%	(36)						
2-FC$_6$H$_4$CH$_2$	40%	(97)						
4-FC$_6$H$_4$CH$_2$	40%	(90)						
4-ClC$_6$H$_4$CH$_2$	28%	(94)						

a) not a substrate

L-selectivity (*Scheme 2.28*). D-4-Trifluorothreonine and L-4-trifluoro-*allo*-threonine are obtained by *O*-deacetylation with lipase MY from *Candida cylindracea*.
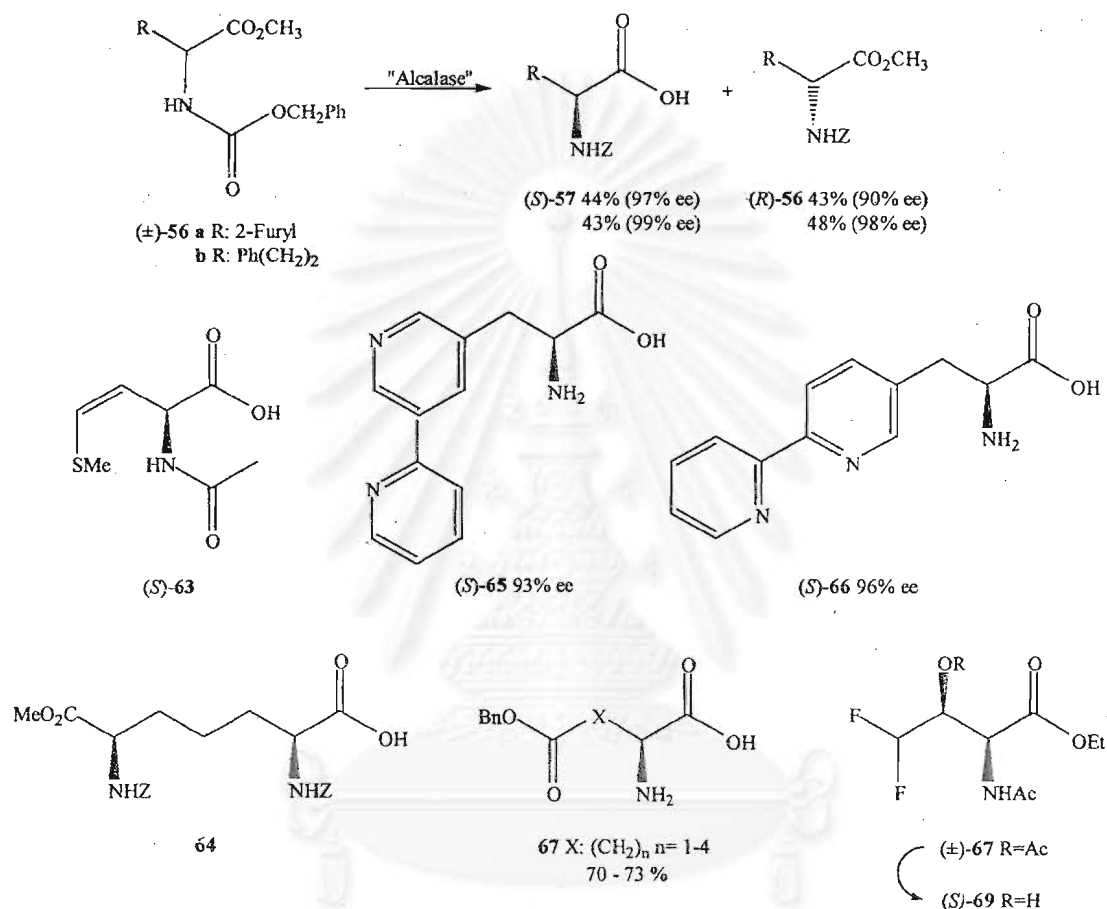
Scheme 2.28 The L-selective ester-cleavage with Alcalase for the kinetic resolution of amino acids.

2.3 α-Amino protection [13]

If an α-amino group is to be protected in the context of peptide synthesis, its nucleophilic reactivity must be suppressed, by draining its electron density away into an appropriate substituent, or by concealing it altogether behind a screen of gross steric hindrance. To be useful, protection according to one or both of these simple principles must be achieved easily at the outset; the protecting group should introduce

no problems of its own while in position; it must stay firmly at its post as long as it is needed; and when its job is done, it should slip quietly away on command, under conditions which have no adverse effects on the rest of the structure being assembled—all without jeopardizing the chiral integrity of nearby chiral centers. Except in the special case where, at the end of a synthesis, several protected functionalities have to be exposed simultaneously to give the completed target molecule, there may be the additional requirement that the amino-protecting groups which are in play.

There are demanding criteria, ruling out of serious consideration all simple alkanoyl groups, which in some respects might seem obvious candidates, Thus, nothing could be more straightforward than acetylation, which satisfies the fundamental requirement of suppressing nucleophilic reactivity very well, and is hydrolytically reversible, Unfortunately, the severe conditions required for hydrolytic deacetylation devastate most peptide chains too, This difficulty can be eased by building in the potential for mild intramolecular deprotection, as in *Scheme 2.29*, but the protecting group so derived is nevertheless still quite useless for α-amino protection in peptide synthesis on other counts, especially in relation to racemization.
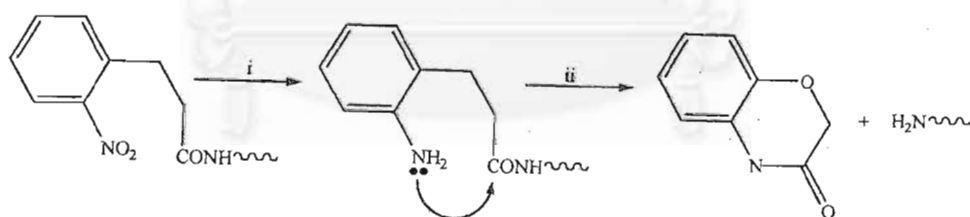
Scheme 2.29 Mild intramolecular deprotection of amino group; conditions: i) $H_2/PtO_2/aq.$ $NaHCO_3$; ii) $H_2O/100\ °C/1h$.

The benzoyl and 4-toluenesulphonyl ('tosyl') groups can be dismissed similarly— their introduction is trivial, and they are good electron-withdrawing groups, but they otherwise fail to fit the bill.

The development of α-amino-protecting groups, which do meet the necessary specifications, has entailed an extended investigation of a very large number of variations on a number of themes. Many subtly different groups have been proposed

such as benzyloxycarbonyl (Z, **70**), *t*-butoxycarbonyl (Boc, **71**), 2-(4-biphenylyl)-isopropoxycarbonyl (Bpoc, **72**), and *9H*-9-fluorenylmethoxycarbonyl (Fmoc, **73**) group (*Figure 3*) but only Fmoc group will be reviewed.
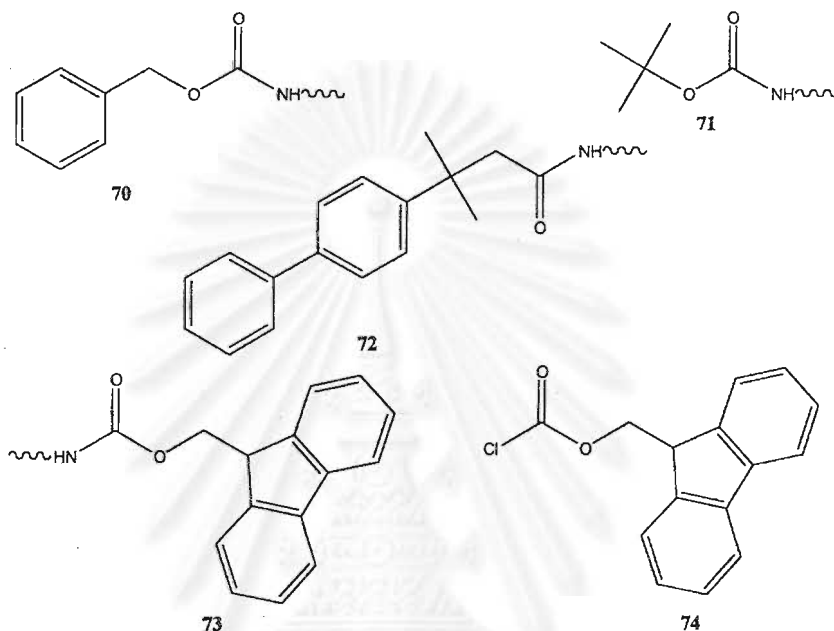
Figure 3 Some protecting groups for α-amino acid.

The Fmoc group is normally introduced in the Schotten-Baumann manner, using the rather stable chloroformate **74**. Dipeptide formation seems to have been found a more serious side-reaction in the preparation of Fmoc amino acids this way than it is with the corresponding reaction of benzyl chloroformate, and tripeptide by-products have been detected too, but this may be due to greater care having been taken to check up on the purity of more expensive intermediates, rather than any chemical difference. Several less reagents have been investigated and recommended. Alternatively, Fmoc amino acids may be prepared using **74** without oligomer formation by reaction with pertrimethylsilylamino acids and base in aprotic solvents.

The Fmoc group is very stable to acidic reagents, but is cleaved swiftly under certain basic conditions. Piperidine (20% in DMF) is the routine reagent. However, other systems (e.g. fluoride ion in DMF) are also effective. Deprotection with piperidine takes only a matter of seconds at room temperature. The mechanism of

cleavage is E1cb, via the stabilized dibenzocyclopentadienide anion; the dibenzofulvene produced reacts with piperidine, giving the adduct **75** as coproduct (*Scheme 2.30*). Peptide terminal amino groups, however, neither induce cleavage nor trap dibenzofulvene to a significant extent. This deprotective procedure does not affect Z or Boc groups, or indeed most other modern protecting groups. There was initially some confusion over the position with respect to catalytic hydrogenolysis, but it is now agreed that the Fmoc group is not inert to the usual conditions for carrying this out.
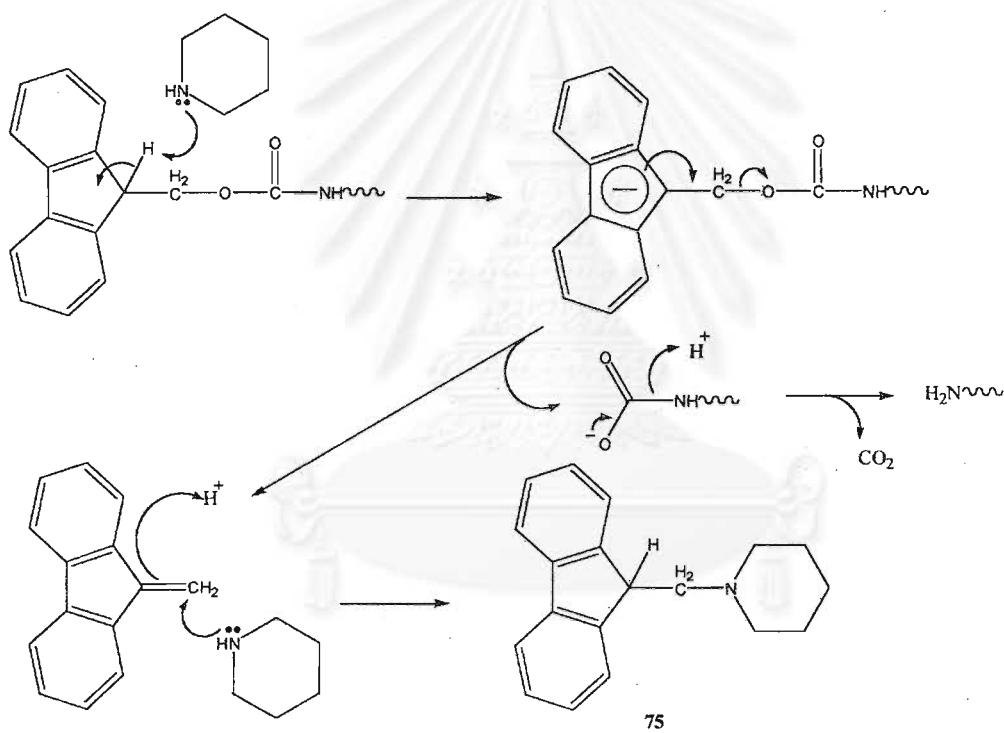
Scheme 2.30 Deprotection of Fmoc group with piperidine; conditions: 20% piperidine/DMF.

2.4 Determination of an enantiomeric excess [24]

The traditional method for determination of an enantiomeric excess in organic molecules containing chiral center is measurement of the rotation of polarized light. This method, however, is apt to be misleading if impurities rotate the plane of the polarized light substantially more than the compound under investigation.

Furthermore, it can only be used if the extent of rotation given by one of the pure enantiomers is already known. For these reasons, the modern methods, which can solve this problem and give higher accuracy and more reliable result, have been modified. Two popular methods are HPLC technique using specific chiral column and NMR technique. In this case of study, NMR technique will be used to determine the enantiomeric excess of the product. Therefore, only NMR technique will be reviewed.

The presence of asymmetric C atoms in a molecule many, of course, be indicated by diastereotopic shifts and absolute configurations may be determined empirically by comparison of diastereotopic shifts [25]. However, enantiomers are not differentiated in the NMR spectrum. The spectrum gives no indication as to whether a chiral compound exists in a racemic form or as a pure enantiomer.

Nevertheless, it is possible to convert a racemic sample with chiral reagents such as Mosher's acid or MTPA (76) [26], into diastereomers or simply to dissolve it in an enantiomerically pure solvent R or S; following this process, solvation diasteromers arise from the racemate (RP + SP) of the sample P, e.g. R:RP and R:SP, in which the enantiomers are recognizable because of their different shifts. Compounds with groups, which influence the chemical shift because of their anisotropy effect, are suitable for use as chiral solvents, e.g. 1-phenylethylamine and 2,2,2-trifluoro-1-phenylethanol. A reliable method of checking the enantiomeric purity by means of NMR uses europium(III) or praseodymium(III) chelates as chiral shift reagents. Besides these two chiral solvents, MTPA is a suited chiral reagent for determination of enantiomeric excess of either chiral alcohols or amines (amino acids). [26]
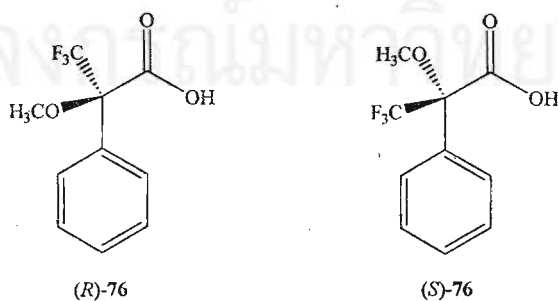

(R)-76   (S)-76

Figure 4 The structures of Mosher's acid or MTPA

The advantages of this reagent for the determination of enantiomeric excess of a chiral alcohol or a amino acid are:

1. The generally excellent separation of both proton and fluorine NMR signals of its diastereomers.

2. The presence of the trifluoromethyl group permitting the use of fluorine NMR, which occurs in an un-congested region of the spectrum.

3. Its marked stability toward racemization even under severe conditions of acidity, basicity, and temperature.

4. Its relative ease of preparation and resolution.

5. Its inherent volatility, which allows lower molecular weight derivatives to be purified, as well as analyzed, by GLPC.

It should be re-emphasized that this method is absolute and does not require that the carbinol or amine be previously resolved. Furthermore, the accuracy of the determination of enantiomeric excess by this methods is not dependent in any way on the magnitude of the optical rotation and yet one can calculate the maximum optical rotation from the rotation of a partially active sample and its enantiomeric excess as determined by this technique.

CHAPTER III

EXPERIMENTAL SECTION

3.1 Materials and Apparatus

The chemicals were mostly obtained from Aldrich Chemical Company, Inc. excepted sutilisin enzyme and (R)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid; (R)-(+)-MTPA, $R:S \sim$ 98.5:1.5, were obtained from Sigma and Fluka Chemical Companies, respectively. All chemicals were used as received and the prepared compounds from each step were used without further purification. ^{1}H NMR spectra were recorded at 400 MHz on Bruker AVANCE 400 NMR spectrometers excepted ^{1}H NMR spectra of MTPA derivative were recorded at 200 MHz on Bruker ACF-200. ^{13}C NMR spectra were recorded at 100 MHz on Bruker AVANCE 400 NMR spectrometer. FT-IR spectra were recorded on Nicolet Impact 410 spectrophotometer. The melting points (m.p.) were obtained from Electrothermal Digital Melting Point Apparatus.

3.2 Preparation of 9-(hydroxymethyl)phenanthrene [27]

Phenanthrene-9-carboxaldehyde (992.3 mg, 4.81 mmol) was dissolved in MeOH, swirl and slight warming until aldehyde dissolved completely. Then the solution was allowed to cool down to room temperature. The amount of NaBH$_4$ (80.2 mg) was added into the solution and stirred for 2 hours. The reaction was monitored by TLC (SiO$_2$, CH$_2$Cl$_2$ as mobile phase). After reaction period, the amount of 20 cm^3 of deionized water was added and heated to reflux at 63-65 °C for half an hours (magnetically stirred while refluxing). The reaction mixture was cooled down to room temperature and then poured into the separating funnel, which contained 150 cm^3 of cold water. The alcohol product was extracted with CH$_2$Cl$_2$. The organic layer was washed with 10% NaHCO$_3$ and dried over Na$_2$SO$_4$. The CH$_2$Cl$_2$ layer was evaporated

to afford white needle crystals of 9-(hydroxymethyl)phenanthrene in 99% yield (991.2 mg, 4.76 mmol): m.p. 148.5-149 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 5.05 (d, 2H, J = 5.4, O-CH_2), 5.48 (t, 1H, J = 5.4, OH), 7.67 (m, 4H, H-2, 3, 6 and 7), 7.9 (s, 1H, H-10), 7.98 (dd, 1H, J = 6.9 and 2.1, H-1), 8.13 (dd, 1H, J = 7.3 and 1.9, H-8), 8.78 (dd, 1H, J = 7.5 and 1.0, H-5), 8.85 (dd, 1H, J = 7.3 and 1.6, H-4) ppm; ^{13}C NMR (d_6-DMSO, 100 MHz) δ 61.8 (CH_2-OH), 123.1 (C-4), 123.6 (C-5), 124.6 (C-3 and C-6), 126.9 (C-2 and C-7), 127.1 (C-1), 127.2 (C-8), 128.7 (C-10), 129.8 (C-8a), 130.2 (C-10a), 130.3 (C-4a), 131.6 (C-4b), 136.4 (C-9) ppm; FT-IR (KBr) 750 (=C-H out of plane, 1015 (C-O), 1445 (CH$_2$), 1496 and 1603 (C=C aromatic), 2925 (-C-H), 3048 (=C-H), 3186 (OH) cm^{-1}.

3.3 Preparation of 9-(bromomethyl)phenanthrene [28]

To prepare the bromide derivative product, 9-(hydroxymethyl)phenanthrene (908.4 mg, 4.36 mmol) was dissolved in 20 cm^3 of CCl$_4$. Into this solution was added 0.45 cm^3 of PBr$_3$ (d 2.850 g/cm^3) by dropwise (10 minutes and kept stirring). Then the solution was heated to reflux for about 4 hours and the reaction was monitored by TLC (SiO$_2$, 2:1 CH$_2$Cl$_2$:CCl$_4$ as mobile phase). After the reaction period, the solution was transferred to another flask and evaporated to remove solvent. The absolute MeOH was added cautiously into the residue and stirred overnight to give crystals of 9-(bromomethyl)phenanthrene. The white crystals were collected over filter to yield 94%: m.p. 118-118.5 °C (lit. 118.5-119); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 4.89 (s, 2H, Br-CH_2), 7.52 (t, 1H, J = 7.3, H-2), 7.59 (t, 1H, J = 7.2, H-7), 7.63 (td, 2H, J = 6.1 and 1.0, H-3 and 6), 7.71 (s, 1H, H-10), 7.75 (d, 1H, J = 7.8, H-1), 8.14 (dd, 1H, J = 7.3 and 1.3, H-8), 8.55 (d, 1H, J = 8.2, H-5), 8.62 (dd, 1H, J = 7.4 and 1.2, H-4) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz) δ 32.5 (CH_2Br), 122.5 (C-4), 123.2 (C-5), 124.5 (C-10), 126.8 (C-3 and 6), 126.9 (C-2), 127.4 (C-7), 128.7 (C-1), 128.8 (C-8), 129.4 (C-8a), 130.8 (C-10a), 130.9 (C-4a), 131.0 (C-4b), 131.5 (C-9) ppm; FT-IR (KBr) 548 (C-Br), 748 (=C-H out of plane), 1443 (CH$_2$), 1489 and 1607 (C=C aromatic), 2972 (-C-H), 3059 (=C-H) cm^{-1}.

3.4 Preparation of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate [29]

Diethyl acetamidomalonate (1163.2 mg, 5.35 mmol) and NaH (139.9 mg, 5.83 mmol) were mixed in the three-necked round bottom flask stirring and saturating with nitrogen atmosphere and then slowly added 20 cm^3 of anhydrous THF. The reaction mixture was allowed to stand in ice bath. Then absolute EtOH (0.2 cm^3) was added into the solution (kept stirring and purging N$_2$) and the resulting mixture was allowed to stand at room temperature. A solution of 9-(bromomethyl)phenanthrene (1072.2 mg, 3.95 mmol) in anhydrous THF (10 cm^3) was added and refluxed overnight. The reaction progress was checked by TLC [SiO-C$_{18}$, H$_2$O:EtOH (1:4) as mobile phase]. The amount of THF was evaporated off. The residue was dissolved with absolute EtOH and then refluxed for an hour. The mixture was filtered and washed the precipitate with hot absolute EtOH. The filtrate was extracted with CH$_2$Cl$_2$/H$_2$O. The organic layer was dried over Na$_2$SO$_4$ and evaporated to give a white of white powder of the title compound in 96% yield: m.p. 137.5-138 °C; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.30 (t, 3H, J = 7.1, CH_3-CH$_2$-), 1.31 (t, 3H, J = 7.1, CH_3-CH$_2$-), 1.87 (s, 3H, CH_3-C=O), 4.19 (s, 2H, CH_2-C), 4.25 (q, 2H, J = 7.1,OCH_2CH$_3$), 4.26 (q, 2H, J = 7.1, OCH_2CH$_3$), 6.48 (s, 1H, NH), 7.45 (s, 1H, H-10), 7.55 (td, 2H, J = 7.1 and 1.0, H-2 and 7), 7.61 (m, 2H, H-3 and 6), 7.73 (d, 1H, J = 7.7, H-1), 8.06 (d, 1H, J = 8.1, H-8), 8.62 (d, 1H, J = 8.1, H-5), 8.68 (d, 1H, J = 8.2, H-4) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz) δ 14.0 (CH$_3$CH$_2$), 23.0 (CH$_3$C=O), 34.1 (CH$_2$CNHAc), 62.7 (OCH$_2$CH$_3$), 67.4 (CCH$_2$), 122.5 (C-4), 123.2 (C-5), 124.4 (C-10), 126.3 (C-3), 126.4 (C-6), 126.6 (C-2), 126.8 (C-7), 128.0 (C-1), 129.1 (C-8), 130.0 (C-4a), 130.3 (C-8a), 130.5 (C-10a), 131.2 (C-4b), 131.7 (C-9), 167.6 (NHC=O), 169.6 (CH$_2$OC=O) ppm; FT-IR (KBr) 743 (=C-H out of plane), 1188 (C-O), 1295 (C-N), 1366 (CH$_3$), 1443 (CH$_2$), 1525 (N-H deformation), 1638 (C=O amide), 1755 (C=O ester), 2977 (-C-H), 3028 (=C-H), 3243 (N-H) cm^{-1}.

3.5 Preparation of 2-acetylamino-3-(9-phenanthrenyl)propionic acid [9c]

The diethyl ester from **3.4** (1.5 g, 3.68 mmol) was added into a solution of 1M KOH in EtOH-H$_2$O (4:1) (15 cm^3, 15 mmol). After refluxing overnight, the reaction

mixture was evaporated to remove EtOH and then adjusted to pH 1-2 with 3N HCl. The resulting mixture was refluxed at 120 °C for 2 hours. Then the reaction mixture was adjusted to pH 10 with 3N aq. NaOH and the organic impurities was removed by extraction with CH_2Cl_2. The aqueous layer was adjusted to pH 4-5 and partitioned with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated to give a white powder of the title compound in 83% yield: m.p. 237-238 °C; ^1H NMR (d_6-DMSO, 400 MHz) δ 1.77 (s, 3H, CH_3-C=O), 3.31 (dd, 1H, J = 14.1 and 8.9, CH_2-CH), 3.61 (dd, 1H, J = 14.1 and 5.0, CH_2-CH), 4.62 (dt, 1H, J = 4.7 and 8.7, CH-CH$_2$), 7.65 (td, 2H, J = 7.6 and 1.7, H-2 and 7), 7.72 (s, 1H, H-10), 7.73 (t, 2H, J = 6.3, H-3 and 6), 7.89 (dd, 1H, J = 7.3 and 1.3, H-1), 8.22 (dd, 1H, J = 5.4 and 4.0, H-8), 8.40 (d, 1H, J = 8.0, NH), 8.81 (d, 1H, J = 7.9, H-5), 8.89 (dd, 1H, J = 5.3 and 4.2, H-4), 12.82 (br-s, 1H, HO-C=O) ppm; ^{13}C NMR (d_6-DMSO, 100MHz) δ 22.7 (CH$_3$C=O), 35.2 (CH$_2$CH), 52.9 (CHCH$_2$), 123.1 (C-4), 124.0 (C-5), 124.3 (C-10), 126.9 (C-3), 127.0 (C-6), 127.3 (C-2), 127.4 (C-7), 128.0 (C-1), 128.5 (C-8), 129.8 (C-4a), 130.5 (C-8a), 130.8 (C-10a), 131.4 (C-4b), 132.2 (C-9), 169.7 (O=C-NH), 173.6 (O=C-OH) ppm; FT-IR (KBr) 1121 (C-O), 1238 (C-N), 1438 (CH$_2$), 1551 (N-H deformation), 1622 (O=C-NH), 1714 (O=C-OH), 2931 (-C-H), 3059 (=C-H), 2700-3600 (broad OH carboxylic acid and N-H) cm^{-1}.

3.6 Preparation of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate

The amino acid from 3.5 (1.2 g, 3.90 mmol) was dissolved in dry MeOH (50 cm^3), which was containing 0.45 cm^3 of BF$_3$.OEt$_3$ (d 1.120 g/cm^3). Then the solution was refluxed for 3 hours. The reaction progress was checked with TLC [SiO$_2$-C$_{18}$, H$_2$O:EtOH (1:4) as mobile phase]. After refluxing, the reaction mixture was evaporated and the residue was extracted with CH$_2$Cl$_2$/H$_2$O. The organic layer was washed with the mixture of 5% NaHCO$_3$ (10 cm^3), H$_2$O (5 cm^3), and 4% NaHSO$_4$ (cm^3) and dried over Na$_2$SO$_4$. Then the solvent was evaporated off to give a white power of the title compound in 97% yield: m.p. 159-161 °C; ^1H NMR (CDCl$_3$, 400 MHz) δ 1.90 (s, 3H, CH_3C=O), 3.49 (dd, 1H, J = 14.1 and 6.6, CH_2CH), 3.58 (dd, 1H, J = 14.4 and 6.8, CH_2CH), 3.57 (s, 3H, CH_3O), 5.03 (dt, 1H, J = 7.4 and 6.8, CHCH$_2$), 6.27 (br-s, 1H, NH), 7.48 (s, 1H, H-10), 7.55 (t, 1H, J = 6.8, H-2), 7.59 (td, 1H, J =

6.8 and 1.2, *H*-7), 7.64 (dd, 2H, *J* = 6.2 and 3.2, *H*-3 and 6), 7.77 (dd, 1H, *J* = 6.7 and 1.1, *H*-1), 8.15 (dd, 1H, *J* = 6.2 and 3.4, *H*-8), 8.61 (d, 1H, *J* = 8.1, *H*-5), 8.71 (dd, 1H, *J* = 6.2 and 3.3, *H*-4) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz) δ 23.5 (*C*H$_3$C=O), 36.1 (*C*H$_2$CH), 52.7 (*C*H$_3$O), 53.4 (CH*C*H$_2$), 123.0 (*C*-4), 123.7 (*C*-5), 124.6 (*C*-10), 127.0 (*C*-3), 127.1 (*C*-6), 127.2 (*C*-2), 127.3 (*C*-7), 128.5 (*C*-1), 128.6 (*C*-8), 130.5 (*C*-4a), 131.1 (*C*-8a), 131.2 (*C*-10a), 131.4 (*C*-4b), 131.8 (*C*-9), 170.3 (CH$_3$*C*=O), 172.9 (O=*C*OCH$_3$) ppm; FT-IR (KBr) 1019 (C-O), 1280 (C-N), 1433 (CH$_2$), 1535 (N-H deformation), 1648 (O=C-NH), 1740 (O=COCH$_3$), 2952 (-C-H), 3069 (=C-H), 3310 (N-H) cm^{-1}.

3.7 Preparation of (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid: enzymatic resolution step [9g,10, 30]

The methyl ester from **3.6** (1.1 g, 3.42 mmol) was dissolved in acetone (20 cm^3). Then the amount of 20 cm^3 of 0.2M phosphate buffer (pH 7.5) was added into the solution and adjusted to pH 7.5 with 1N HCl. To the solution was added subtilisin (type *Carlsberg*) protease (15 mg). The resulting mixture was incubated on an orbital shaker at 220 rpm at 37 °C for 24 hours. After this period, the amount of acetone was evaporated off, the residue was poured into 5% aq. NaHCO$_3$ and extracted with CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$ and evaporated to give the unreacted material, which consisted of (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate and unreacted (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionate in 54% recovery yield: m.p. 161-162.5 °C; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.92 (s, 3H, C*H*$_3$C=O), 3.55 (dd, 1H, *J* = 14.2 and 6.8, C*H*$_2$CH), 3.58 (s, 3H, C*H*$_3$O), 3.59 (dd, 1H, *J* = 14.3 and 6.8, C*H*$_2$CH), 5.04 (dt, 1H, *J* = 7.5 and 6.7, C*H*CH$_2$), 6.19 (br-d, 1H, *J* = 7.5, N*H*), 7.50 (s, 1H, *H*-10), 7.57 (td, 1H, *J* = 6.8 and 1.2, *H*-2), 7.61 (td, 1H, *J* = 6.8 and 1.2, *H*-7), 7.65 (dd, 2H, *J* = 6.0 and 2.9, *H*-3 and 6), 7.79 (dd, 1H, *J* = 7.7 and 1.4, *H*-1), 8.16 (dd, 1H, *J* = 6.2 and 3.4, *H*-8), 8.64 (d, 1H, *J* = 8.1, *H*-5), 8.72 (dd, 1H, *J* = 6.2 and 3.4, *H*-4) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz) δ 23.1 (*C*H$_3$C=O), 35.6 (*C*H$_2$CH), 52.3 (*C*H$_3$O), 53.0 (CH*C*H$_2$), 122.5 (*C*-4), 123.3 (*C*-5), 124.2 (*C*-10), 126.5 (*C*-3), 126.6 (*C*-6), 126.8 (*C*-2), 126.9 (*C*-7), 128.0 (*C*-1), 128.1 (*C*-8), 130.1 (*C*-4a), 130.6 (*C*-8a), 130.7 (*C*-10a), 131.0 (*C*-4b), 131.3 (*C*-9), 169.9 (CH$_3$*C*=O),

172.5 (O=COCH$_3$) ppm; FT-IR (KBr) 743 (=C-H out of plane), 1188 (C-O), 1372 (C-N), 1433 (CH$_2$), 1535 (N-H deformation), 1638 (O=C-NH), 1755 (O=COCH$_3$), 2947 (-C-H), 3044 (=C-H), 3279 (N-H) cm^{-1}. The aqueous solution was adjusted to pH 3-4 and then extracted with EtOAc. The EtOAc solution was dried over Na$_2$SO$_4$ and evaporated off to give a white powder of (2S)-2-acetylamino-3-(9-phenanthrenyl) propionic acid in 45% conversion (>98% ee): m.p. 235.5-236.5 °C; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 1.72 (s, 3H, CH_3C=O), 3.27 (dd, 1H, J = 14.1 and 9.4, CH_2CH), 3.57 (dd, 1H, J = 14.2 and 4.9, CH_2CH), 4.56 (dt, 1H, J = 4.5 and 8.7, CHCH$_2$), 7.60 (td, 2H, J = 7.5 and 1.4, H-2 and 7), 7.69 (t, 2H, J = 4.0, H-3 and 6), 7.71 (s, 1H, H-10), 7.84 (d, 1H, J = 7.2, H-1), 8.17 (dd, 1H, J = 5.3 and 3.8, H-8), 8.45 (d, 1H, J = 8.0, NH), 8.76 (d, 1H, J = 7.9, H-5), 8.84 (dd, 1H, J = 5.3 and 4.0, H-4) ppm; ^{13}C NMR (d_6-DMSO, 100MHz) δ 22.7 (CH$_3$C=O), 35.1 (CH$_2$CH), 52.9 (CHCH$_2$), 123.1 (C-4), 124.0 (C-5), 124.3 (C-10), 126.9 (C-3), 127.0 (C-6), 127.3 (C-2), 127.4 (C-7), 128.0 (C-1), 128.5 (C-8), 129.7 (C-4a), 130.5 (C-8a), 130.8 (C-10a), 131.4 (C-4b), 132.2 (C-9), 169.8 (O=C-NH), 173.6 (O=C-OH) ppm; FT-IR (KBr) 745 (=C-H out of plane), 1243 (C-N), 1553 (N-H deformation), 1619 (O=C-NH), 1715 (O=C-OH), 2954 (-C-H), 3071 (=C-H), 3309 (N-H), 3431 (broad OH carboxylic acid) cm^{-1}.

3.8 Preparation of (2S)-2-amino-3-(9-phenanthrenyl)proionic acid hydrochloride [8c]

The (S)-acetylamino acid from **3.7** (450 mg, 1.46 mmol) was suspended in a mixture of acetic acid (15 cm^3) and 6N HCl (15 cm^3). The reaction mixture was refluxed overnight. After refluxing, the mixture was concentrated. Then the distilled water was added and the resulting mixture was allowed to stand to complete the precipitation of amino acid hydrochloride product. Then the white precipitates were collected by filtration to give the title compound in 88% yield: m.p. 230.5-231.5 °C; FT-IR (KBr pellet) 743 (=C-H out of plane), 1327 (C-N), 1394 (CH$_2$), 1511 (N-H deformation), 1603 (O=C-OH), 2930 (-C-H), 3063 (=C-H), 3288 (broad OH carboxylic acid), 3421 (N-H) cm^{-1}. ^{1}H NMR spectrum (*Figure H2*) used to confirm the deacylation.

3.9 Preparation of (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid [31]

The amino acid hydrochloride from **3.8** (380 mg, 1.26 mmol) was suspended in the mixture of dioxane and H_2O (2:1 v/v). Then 2.1 eq. of $NaHCO_3$ was added into the mixture under low temperature (in ice bath). Then the solution of 1.5 eq. of 9-fluorenylmethoxychloroformate in dioxane was added into the reaction mixture very slowly (drop by drop). The reaction mixture was stirred in ice bath for 2 hours and then at room temperature for 24 hours. The reaction progress was checked with TLC [SiO_2-C_{18}, H_2O:MeOH (1:4) as mobile phase]. After the reaction time, the amount of solvent was evaporated off, the residue was poured into 10% aq. $NaHCO_3$ and extracted with ether. The aqueous solution was adjusted to pH 2-3 and extracted with EtOAc, dried over Na_2SO_4 and evaporated to give a yellowish powder of the title compound in 85% yield: m.p. 210-211.5 °C; ^1H NMR (DMSO, 400 MHz) δ 3.31 (dd, 1H, *J* = 14.1 and 10.5, C*H*$_2$CH), 3.70 (dd, 1H, *J* = 14.1 and 4.1, C*H*$_2$CH), 4.06 (t, 1H, *J* = 6.3, *H*-9'), 4.11 (m, 2H, OC*H*$_2$CH), 4.38 (dt, 1H, *J* = 4.4 and 10.1, C*H*CH$_2$), 7.06 (td, 1H, *J* = 6.7 and 0.7, *H*-2'), 7.23 (td, 1H, *J* = 6.7 and 0.8, *H*-7'), 7.32 (t, 1H, *J* = 7.2, *H*-3'), 7.36 (t, 1H, *J* = 7.2, *H*-6'), 7.52 (d, 1H, *J* = 7.5, N*H*), 7.59 (d, 2H, *J* = 7.9, *H*-1' and 8'), 7.65 (td, 1H, *J* = 6.8 and 1.2, *H*-2), 7.72 (td, 2H, *J* = 6.9 and 1.4, *H*-3 and 7), 7.79 (s, 1H, *H*-10), 7.83 (dd, 2H, *J* = 7.7 and 3.1, *H*-4' and 5'), 7.85 (t, 1H, *J* = 7.7, *H*-6), 7.98 (d, 1H, *J* = 8.5, *H*-1), 8.20 (dd, 1H, *J* = 7.4 and 1.9, *H*-8), 8.80 (d, 1H, *J* = 8.1, *H*-5), 8.88 (dd, 1H, *J* = 7.5 and 1.9, *H*-4) ppm; ^{13}C NMR (acetone-d_6), 100MHz) δ 36.6 (*C*H$_2$CH), 48.2 (*C*-9'), 55.6 (*C*HCH$_2$), 67.6 (O*C*H$_2$CH), 121.2 (*C*-3' and 6'), 123.9 (*C*-4), 124.8 (*C*-5), 125.4 (*C*-10), 126.5 (*C*-3), 126.6 (*C*-6), 127.9 (*C*-2), 128.1 (*C*-7), 128.3 (*C*-2', 4', 5' and 7'), 128.9 (*C*-1' and 8'), 129.6 (*C*-1), 129.7 (*C*-8), 131.3 (*C*-4a), 132.1 (*C*-8a), 132.3 (*C*-10a), 132.9 (*C*-4b), 133.2 (*C*-9), 142.4 (*C*-4a' and 4b'), 145.3 (*C*-8a' and 9a'), 157.4 (O=*C*-NH), 174.2 (O=*C*-OH) ppm; FT-IR (KBr) 740 (=C-H out of plane), 1035 (C-O), 1265 (C-N), 1445 (CH$_2$), 1537 (N-H deformation), 1690 (O=C-OH), 1726 (O=C-NH), 2950 (-C-H), 3063 (=C-H), 3411 (broad OH carboxylic acid), 3549 (N-H) cm^{-1}.

3.10 Determination of an enantiomeric excess of (2S)-2-amino-3-(9-phenanthrenyl)propionic acid: preparation of MTPA amide derivative

The MTPA (14.2 mg, 0.06 mmol) and DCC (12.5 mg, 0.06 mmol) were dissolved in CHCl$_3$ (1 mL) in ice bath and under an N$_2$ atmosphere. Then, methyl (2S)-2-amino-3-(9-phenanthrenyl)propionate (14.1 mg, 0.05 mmol) and Et$_3$N (0.2 cm^3) were added respectively while maintaining inert atmosphere. Allowed the reaction mixture to stand and stir at room temperature for 18 hours. After the reaction period, the solution was diluted with CHCl$_3$ (5 mL) and washed with 6N HCl, saturated aqueous NaHCO$_3$, and brine. The organic phase was dried over Na$_2$SO$_4$ and evaporated to remove the solvent. The crude product was purified with column chromatography (SiO$_2$, CH$_2$Cl$_2$ as mobile phase) to yield 17.8 mg (71%). ^{1}H NMR spectrum was shown in *Figure J2*.

CHAPTER IV

RESULTS AND DISCUSSION

The optical antipodes of a hydrophobic unnatural amino acid, (2*S*)-2-acetylamino-3-(9-phenanthryl)propionic acid or known as *N*-acetyl-(L)-β-(9-phenanthrenyl)alanine, could be obtained from enzymatic resolution (*Table 4*) of its racemic methyl ester with subtilisin. For this compound, subtilisin gave a good resolution of 45% conversion (>98% ee) of the acetyl derivative of (*S*)-amino acid after incubation in mixed acetone-aqueous phosphate buffer (pH 7.5-8.0) at 37-40 °C for 24 hours [9g, 30]. Besides acetone, dimethyl sulfoxide was also used as solvent [10], which gave heterogeneous reaction mixture, but subtilisin gave lower conversion (38%) in longer incubation time (48 hours). These results indicated that the substrate should be dissolved in the reaction mixture to provide homogeneous solution in order to obtain a good and fast resolution. Acetone-aqueous mixture was therefore an appropriate solvent in this study. After enzymatic resolution, the acetyl derivative of (*S*)-amino acid could be dissolved in pH 8-9 aqueous solution which then the remaining unhydrolyzed D-ester could be easily removed by extraction with CH_2Cl_2. In resolution procedure, subtilisin could be used both as native enzyme and cross-linked enzyme crystals (CLECs) forms. CLECs forms, however, gave better and faster resolution and it was also more stable than native enzyme in both aqueous and mixed aqueous-organic solution [9g]. By the way, in case of *acylase* we tried on this enzyme with 2-acetylamino-3-(9-phenanthrenyl)propionic acid, anyhow, it failed to resolve this molecule after incubated for 48 hours. The resolved the acetyl derivative of (*S*)-amino acid was later deacylated and replaced with *9H*-9-fluorenylmethoxycarbonyl- (Fmoc-) protecting group, which gave an overall yield of 35% of target product from phenanthrene-9-carboxaldehyde, (2*S*)-2-(*9H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenan-threnyl)propionic acid, for further peptide synthesis.

Table 4 The enzymatic resolution of 2-acetylamino-3-(9-phenanthrenyl)propionic acid

Enzymes	Condition	pH-controlling method	Incubation time (h)	%Conversion
Acylase I	aq. KOH/CoCl$_2$.6H$_2$O, 25-30 °C [9b]	Adding 0.1N KOH	48	0
Protease *Carlsberg*	DMSO/H$_2$O/0.01M KCl (6:5:1), 37-40 °C [10]	Adding 0.1N NaOH	48	38
Protease *Carlsberg*	Acetone/Phosphate buffer (1:1), 37-40 °C [30]	Phosphate buffer	24	44
Protease CLEC-BL	Acetone/Phosphate buffer (1:1), 37-40 °C	Phosphate buffer	24	45

4.1 Preparation of 9-(hydroxymethyl)phenanthrene
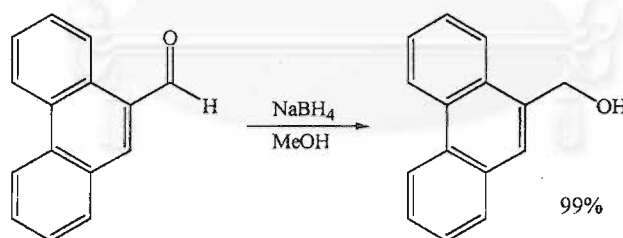
Scheme 4.1 Preparation of 9-(hydroxymethyl)phenanthrene

9-(Hydroxymethyl)phenanthrene was prepared by well known procedure. When phenanthrene-9-carboxaldehyde was reduced with NaBH$_4$ in MeOH solution. It was white needle crystals, which was melted at 148.5-149 °C (lit. [32] 149-149.5) and obtained in an excellent 99% yield.

From IR spectrum of the product in *Figure A1* demonstrated the signals of OH group at 3186 cm^{-1}, Ar-H (stretching) at 3048 cm^{-1}, saturated-H (CH$_2$, stretching) at 2925 cm^{-1}, C=C (stretching) at 1603 and 1496 cm^{-1}, CH$_2$ (bending) at 1445 cm^{-1}, C-O

(stretching) at 1015 cm^{-1} and Ar-H (out of plane) at 750 cm^{-1}. From ^{1}H NMR spectra of the product in *Figure A2-A4*, there were doublet and triplet peaks due to –OCH$_2$ and –OH protons at 5.05 and 5.48, respectively. In addition, the signals of the aromatic protons appeared at 7.67-8.85 ppm and ^{13}C NMR spectra (*Figure A5-A8*) showed characteristic peak of –OCH$_2$ carbon at 61.8 ppm. The spectroscopic data therefore indicated that the aldehyde starting material was converted to alcohol product.

4.2 Preparation of 9-(bromomethyl)phenanthrene
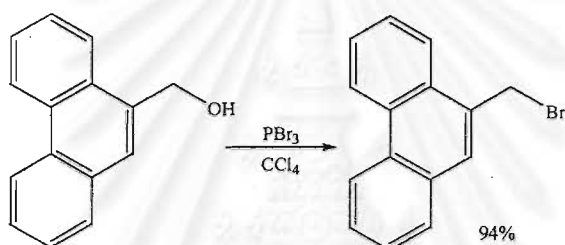
Scheme 4.2 Preparation of 9-(bromomethyl)phenanthrene

9-(Bromomethyl)phenanthrene was prepared by previously described procedure. 9-(Hydroxymethyl)phenanthrene was treated with PBr$_3$ in CCl$_4$ solution. The white powder product was obtained in an excellent yield (94%), which was melted at 118-118.5 °C (lit.[33] 118.5-119).

The IR spectrum of the product (*Figure B1*) demonstrated that the signals of OH group at 3186 cm^{-1} and C-O (stretching) at 1015 cm^{-1}, shown in *Figure A1*, disappeared while the other signals still remained and a new signal due to C-Br stretching appeared at 548 cm^{-1}. From this incident depicted that the alcohol derivative was converted to new compound, which was 9-(bromomethyl) phenanthrene. Furthermore, the depiction was supported by NMR data (*Figure B1-B7*), which showed the important peak due to CH$_2$-Br group at 4.89 ppm in ^{1}H NMR spectrum (*Figure B2*) and 32.5 ppm in ^{13}C NMR spectrum (*Figure B4*). The change of chemical shifts of CH$_2$-Br both proton and carbon was a good evidence to confirm the conversion of the alcohol derivative.

4.3 Preparation of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate
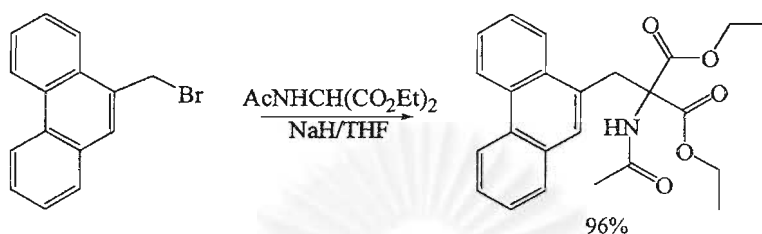
Scheme 4.3 Preparation of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate

Diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate was prepared as the same previous reported procedure. The bromide derivative was reacted with diethyl acetamidomalonate in the presence of NaH as base and small amount of EtOH in THF. The white powder of coupling product was obtained in an excellent yield (96%) and melted at 137.5-138 °C.

The IR spectrum of the product in *Figure C1* illustrated the signals of NH group at 3243 cm^{-1}, Ar-H (stretching) at 3028 cm^{-1}, saturated-H (CH$_2$ and CH$_3$, stretching) at 2977 cm^{-1}, C=O of ester at 1755 cm^{-1}, C=O of amide at 1638 cm^{-1}, NH (deformation) at 1525 cm^{-1}, CH$_2$ and CH$_3$ (bending) at 1445 and 1366 cm^{-1}, CN (stretching) at 1295 cm^{-1}, C-O (stretching) at 1188 cm^{-1} and Ar-H (out of plane) at 743 cm^{-1}. From ^{1}H NMR spectra of the product in *Figure C2-C4*, there were two triplet and two quartet peaks due to the protons of the ethyl groups of the malonate part at 1.30, 1.31, 4.25 and 4.26 ppm, respectively while CH$_3$ protons of acetyl group and CH$_2$ protons of Ar-CH$_2$-C showed singlet peaks at 1.87 and 4.17 ppm, respectively. In addition, the signals of the NH proton showed singlet peak at 6.48 and the aromatic protons appeared at 7.45-8.68 ppm. The ^{13}C NMR spectra (*Figure C5-C8*) supported ^{1}H NMR data, which showed peaks of CH$_2$ and CH$_3$ carbons of the ethyl group at 62.7 and 14.0 ppm respectively, CH$_3$ carbon of acetyl group at 23.0, CH$_2$ and quaternary carbons of Ar-CH$_2$-C at 34.1 and 67.4 ppm respectively, the aromatic carbons at 112.5-131.7 ppm, and two carbonyl carbons at 167.6 and 169.6 ppm. From the spectroscopic data therefore depicted that the product was the desired compound, which was diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate.

4.4 Preparation of 2-acetylamino-3-(9-phenanthrenyl)propionic acid
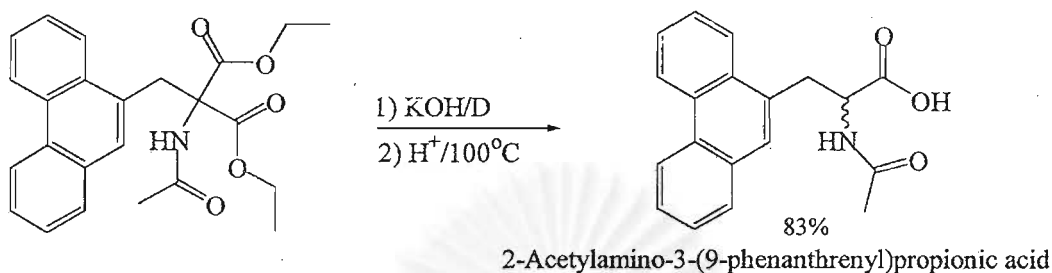
Scheme 4.4 Preparation of 2-acetylamino-3-(9-phenanthrenyl)propionic acid

2-Acetylamino-3-(9-phenanthrenyl)propionic acid was synthesized as the usual way. Diethyl 2-acetylamino-2-(9-phenanthrenylmethyl)malonate was basically hydrolyzed with the solution of KOH in EtOH in the presence of 20% of water to produce dicarboxylic acid, which then was monodecarboxylated. The white powder of monocarboxylic acid was obtained in high yield (83%) and melted at 237-238 °C.

The IR spectrum of the amino acid product in *Figure D1* showed the signals of NH group at 3315 cm^{-1}, Ar-H (stretching) and OH of carboxylic acid at 3059 cm^{-1}, saturated-H (CH_2 and CH_3, stretching) at 2931 cm^{-1}, C=O of acid at 1714 cm^{-1}, C=O of amide at 1622 cm^{-1}, NH (deformation) at 1551 cm^{-1}, CH_2 and CH_3 (bending) at 1438 and 1370 cm^{-1}, CN (stretching) at 1238 cm^{-1} and Ar-H (out of plane) at 748 cm^{-1}. The important evidences, which indicated the acid was produced, were the decreasing of the frequency of C=O signal from 1755 to 1714 cm^{-1} and the disappearance of C-O signal of malonate at 1188 cm^{-1}. In addition, the appearing of CH proton signal at 4.62 ppm and –COOH proton signal at 12.82 ppm on ^1H NMR spectra (*Figure D2-D4*) supported IR data that the malonate derivative was converted to carboxylic acid. The ^1H NMR spectra of the product (*Figure D2-D4*) indicated that the product was 2-acetylamino-3-(9-phenanthrenyl)propionic acid because of the absence of the signals of -OCH_2CH_3 protons, which was the part of malonate derivative. Moreover, the appearance of CH proton illustrated that the decarboxylation was successful and the split of CH_2 protons signal (at 3.31 and 3.61 ppm) also indicated that the new adjacent chiral center (CH) was generated which herein proton signal at 3.31 ppm (^2J = 14.1 Hz, ^3J = 8.9 Hz) due to a CH_2 proton that

was *trans* position with the proton at chiral center. On the other hand, another CH_2 proton, *cis* position, showed signal at 3.61 ppm ($^2J = 14.1$ Hz, $^3J = 5.0$ Hz). The depiction was also supported by ^{13}C NMR data (*Figure D5-D8*), in which the signal of CH carbon at 52.9 ppm was present while the signals of $-OCH_2CH_3$ and quaternary carbons, present in *Figute C5*, was absent.

4.5 Preparation of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate

Scheme 4.5 Preparation of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate

Methyl 2-acetylamino-3-(9-phenanthrenyl)propionate was prepared by the general method. 2-Acetylamino-3-(9-phenanthrenyl)propionic acid was reacted with MeOH in the presence of the catalytic amount of $BF_3.OEt_2$. The methyl ester was obtained in an excellent yield (97%) and melted at 159-161 °C.

The IR spectrum of the product (*Figure E1*) demonstrated that the signals of OH group of carboxylic acid at 3059 cm^{-1}, shown in *Figure D1*, disappeared. On the other hand, the C-O signals appeared at 1208 cm^{-1} and the C=O was shift from 1714 to 1740 cm^{-1}, which depicted that the methyl ester was generated. Besides IR data, the ^1H NMR spectra (*Figure E2-E4*) also showed the signal of $-OCH_3$ protons at 3.57 ppm while ^{13}C NMR spectra (*Figure E5-E8*) showed the signals of $-OCH_3$ carbon at 52.7 ppm and C=O of ester at 172.9 ppm. The spectroscopic data thus indicated that the product was methyl 2-acetylamino-3-(9-phenanthrenyl)propionate. By the way, its ^{13}C NMR spectra showed the interesting phenomenon, which was the couple signal of each carbon due to the diisomerization effect (racemate). However, in case of 2-acetylamino-3-(9-phenanthrenyl)propionic acid, this event did not appear and the reason was not investigated yet.

4.6 Preparation of (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid: enzymatic resolution step
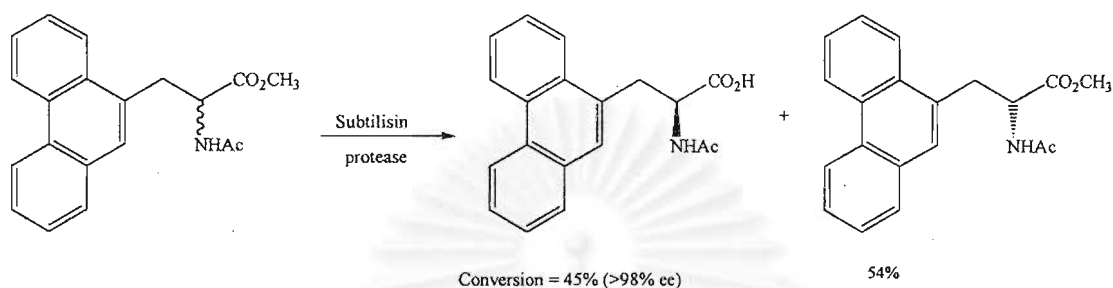
Scheme 4.6 The enzymatic resolution of methyl 2-acetylamino-3-(9-phenanthrenyl)-Propionate.

(2*S*)-2-Acetylamino-3-(9-phenanthrenyl)propionic acid was prepared by well known the enzymatic resolution method. The mixture of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate and subtilisin protease was incubated in the mixture of acetone and phosphate buffer (pH 7.5) for 24 hours. The (*R*)-ester and unreacted (*S*)-ester was recovered in 54% yield and melted at 161.5-162.5 °C while the (*S*)-amino acid was obtained in 45% yield and melted at 235.5-236.5 °C.

Both IR spectrum (*Figure F1*) and ^{1}H NMR spectra (*Figure F2-F4*) of the recovered material demonstrated the signals as same as the spectra of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate (*Figure E1-E4*). While the ^{13}C NMR spectra (*Figure F5-F8*) showed the difference, which was the decreasing of the diisomerization effect after compared to the spectra of unresolved methyl 2-acetylamino-3-(9-phenanthrenyl)propionate (*Figure E5-E8*). From this incident, the recovered material was therefore (*R*)-ester mixing with the small amount of unreacted (*S*)-ester and the resolution was successful.

Besides the recovered material, the spectra of the resolved product (*Figure G1-G8*) also showed the signals as same as that of unresolved 2-acetylamino-3-(9-phenanthrenyl)propionic acid. The resolved product was thus (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid.

4.7 Preparation of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid hydrochloride
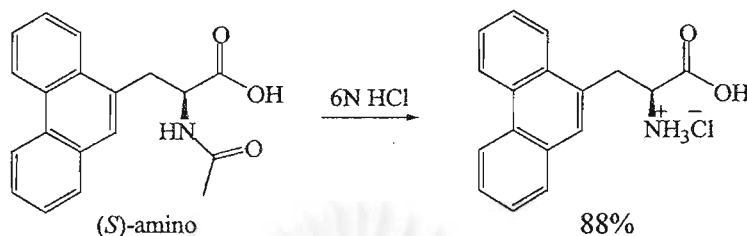
Scheme 4.7 Preparation of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid hydrochloride

(2*S*)-2-Amino-3-(9-phenanthrenyl)propionic acid hydrochloride was prepared by hydrolysis with 6N HCl in AcOH media. The (*S*)-amino acid hydrochloride was obtained in high yield (88%) and melted at 230.5-231.5 °C.

The absence of a C=O signal at 1715 cm^{-1} in IR spectrum (*Figure H1*) indicated that the acetyl group was successfully removed. The ^{1}H NMR spectrum (*Figure H2*) confirmed the indication of IR data with the disappearance of the acetyl protons at 1.90 ppm.

4.8 Preparation of (2*H*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid
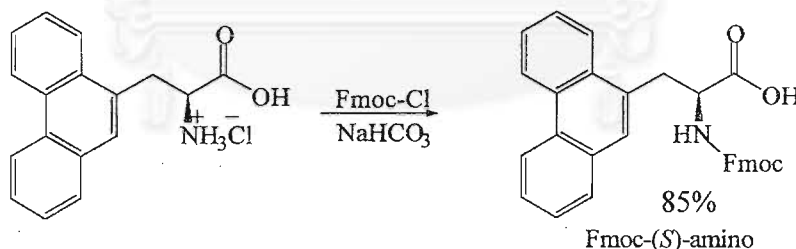
Scheme 4.8 Preparation of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid

(2*S*)-2-(9*H*-9-Fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid was prepared in the general way. (2*S*)-2-Amino-3-(9-phenanthrenyl)propionic acid hydrochloride was reacted with fluorenylmethoxychloroformate in present of NaHCO$_3$ as base in the mixture of dioxane and water. The yellowish powder was produced with high yield (85%) and melted at 210-211.5 °C.

The IR spectrum of the product (*Figure I1*) showed the signals of NH group at 3549 cm^{-1}, OH group at 3411 cm^{-1}, Ar-H (stretching) at 3063 cm^{-1}, saturated-H (CH,

CH$_2$, stretching) at 2950 cm^{-1}, two C=O group at 1726 and 1690 cm^{-1}, Ar-H (deformation) at 1600 cm^{-1}, NH (deformation) at 1573 cm^{-1}, CH$_2$ (bending) at 1445 cm^{-1}, C-N (stretching) at 1265 cm^{-1}, C-O (stretching) at 1035 cm^{-1}, and Ar-H (out of plane) at 740 cm^{-1}. From ^{1}H NMR spetra (*Figure I2-I4*) illustrated the signals of the protons of (9-phenanthrenyl)amino acid part at 3.31 (C*H$_2$*-CH proton), 3.70 (C*H$_2$*-CH proton), 4.38 (C*H*CH$_2$ proton), 7.52 (N*H* proton), and 7.65-8.88 (*phenanthrenyl* protons) while the signals of the Fmoc protons was shown at 4.06 (C*H*CH$_2$O proton), 4.11 (CHC*H$_2$*O protons), and 7.06-7.83 (*fluorenyl* protons) ppm. The ^{13}C NMR spectrum demonstrated the characteristic signals of (9-phenanthrenyl)amino acid part at 36.6 (CH$_2$CH carbon) and 55.6 (CHCH$_2$ carbon) ppm while the characteristic signals of Fmoc part were shown at 48.2 (CHCH$_2$O carbon) and 67.6 (OCH$_2$CH carbon) ppm. All spectroscopic data thus depicted that (2*S*)-2-(9*H*-9-fluorenyl-methoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid was successfully produced.

4.9 Determination of an enantiomeric excess of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid

From ^{1}H NMR spectrum of MTPA amide derivative of methyl (2*S*)-2-amino-3-(9-phenanthrenyl)propionate (*Figure J2*) showed high enantiomeric purity of (*S*)-amino acid from the enzymatic resolution compared to ^{1}H NMR spectra of MTPA amide derivatives of both racemate (*Figure J1*) and recovered material (*Figure J3*). In case of the recovered material, ^{1}H NMR spectrum of its MTPA amide derivative showed the lower enantiomeric purity, which compared to ^{13}C NMR spectrum of recovered material (*Figure F5-6*). This incident could be effected of the racemization, via direct enolization or the oxazolone mechanism [13], in the preparation of MTPA amide derivative of methyl (2*R*)-2-amino-3-(9-phenanthrenyl)propionate involved several steps, including base-hydrolysis of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl)propionate.

An enantiomeric excess could be calculated by using the following equation.

$$\% ee = \frac{R - S}{R + S} \times 100$$

From ^{1}H NMR spectrum in *Figure J2*, considered the signals at 3.12 (*S*) and 3.30 (*R*) ppm, which their integral values were 4.617 and 0.034, respectively. From the equation and both integral values, the %ee could be obtained as following.

Where *R* and *S* in the equation are the integral values of the signal of *R*- and *S*-form, respectively.

$$\%ee = \frac{4.617 - 0.034}{4.617 + 0.034}$$

$$\%ee = 98.6$$

Thus, an enantiomeric excess of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid from the enzymatic resolution is >98%.

CHAPTER V

CONCLUSION

In this work, a powerful method for the synthesis of hydrophobic unnatural amino acid, (2S)-2-acetylamino-3-(9-phenanthrenyl)propionic acid or known as N-acetyl-(L)-β-(9-phenanthrenyl)alanine, was studied. A useful modification of the Sö rensen method of synthesis of amino acids was used to prepare the racemate of amino acid. Diethyl acetamidomalonate was alkylated with 9-(bromomethyl) phenanthrene and the product was converted to the racemate of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate or known as N-acetyl-(D,L)-β-(9-phenanthrenyl)alanine methyl ester. Attempt to resolved the racemate with subtilisin protease (CLECs-BL) was successful after incubation in mixed acetone-aqueous phosphate buffer (pH 7.5-8.0) at 37-40 °C for 24 hours. The resolution gave 45% conversion of (S)-amino acid and 54% of unreacted ester, comprising of (R)- and (S)-ester. The (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl) propionic acid or known as Fmoc-(L)-(9-phenanthrenyl)alanine was synthesized with an overall yield of 35% after 8 steps of synthesis were done.

An enantiomeric excess of the resolved amino acid was determined with NMR technique by measurement of ^{1}H NMR spectrum of MTPA derivative of methyl (2S)-2-animo-3-(9-phenanthrenyl)propionate. The resulting spectrum showed high enantiomeric purity of (S)-amino acid with >98% ee.

SUGGESTION FOR FURTHER WORK

Further work can be focused on;

1. Synthesize opically pure of other unnatural hydrophobic amion acids, which comprise of the different aromatic chromophore group such as pyrenyl, or substitute at the different position such as 1, 2, 3, and so on.

2. Synthesize the dipeptide of this compound by coupling with another alanine, which comprising of different chromophore and then study its photoinduced electron and energy transfer properties.

3. Incorporate this amino acid in to the peptide backbone and then study on this peptide about photochemistry.

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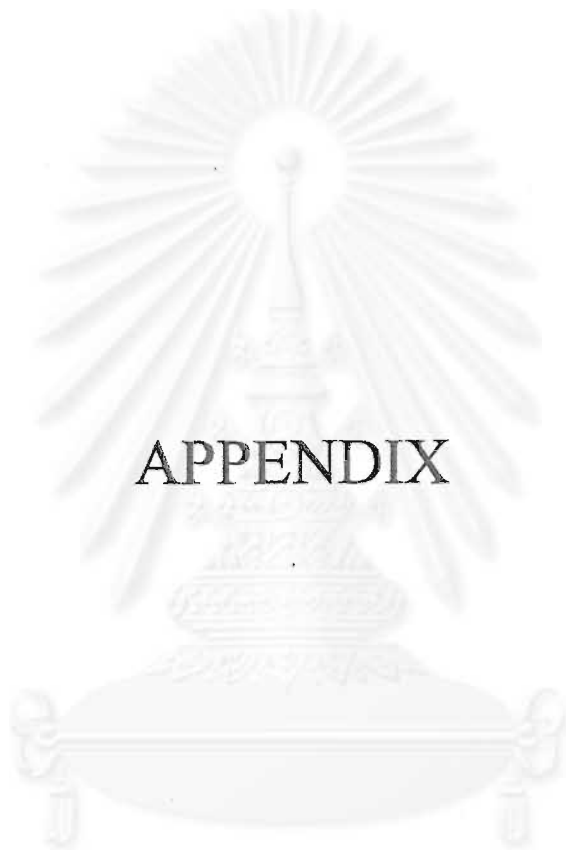
APPENDIX
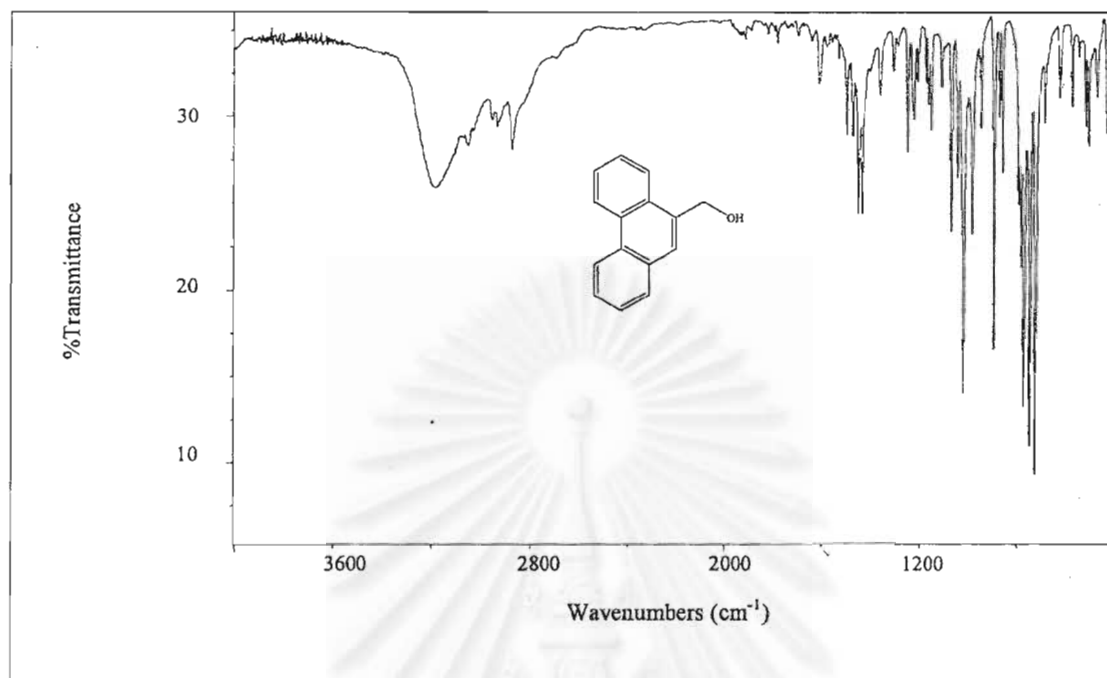
Figure A1: FT-TR spectrum of 9-(hydroxymethyl)phenanthrene
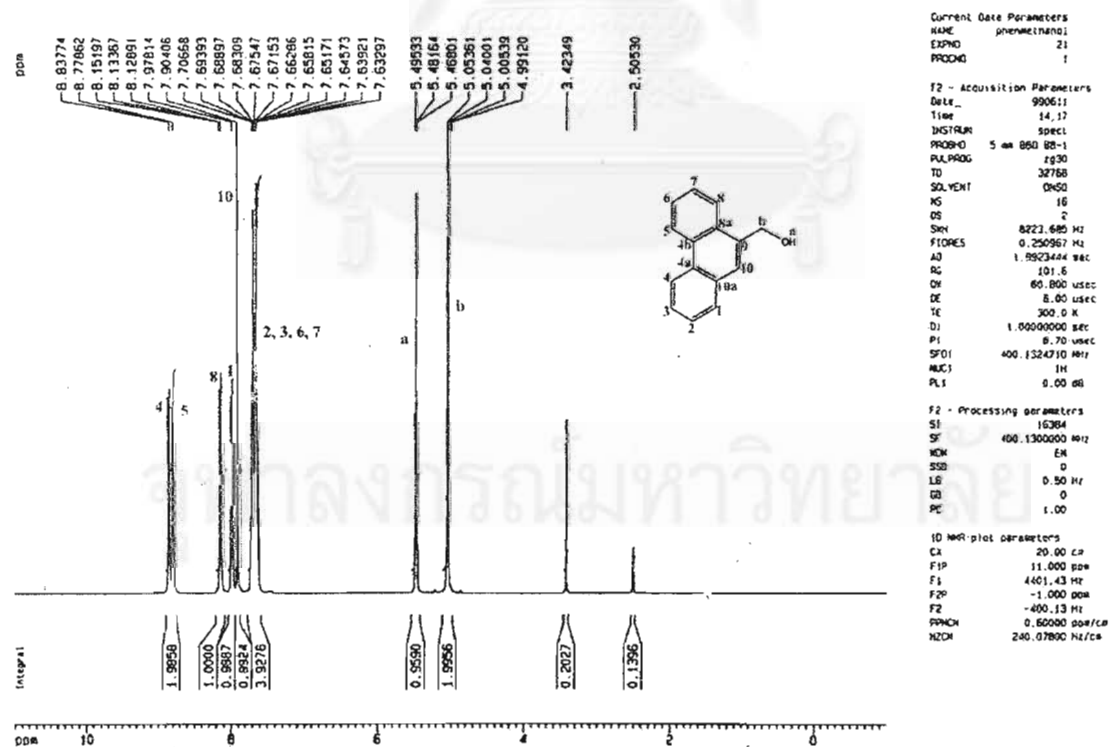
Figure A2: ¹H NMR spectrum of 9-(hydroxymethyl)phenanthrene
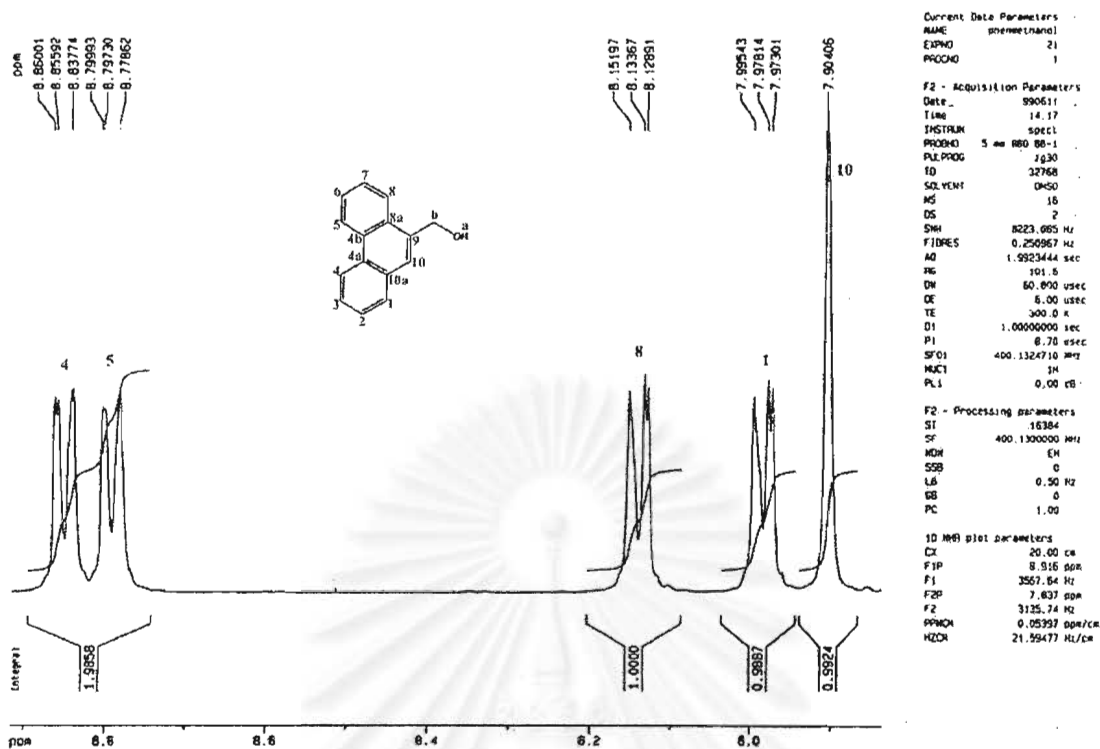
Figure A3: ^{1}H NMR spectrum of 9-(hydroxymethyl)phenanthrene (blow up at 7.8-8.9 ppm)
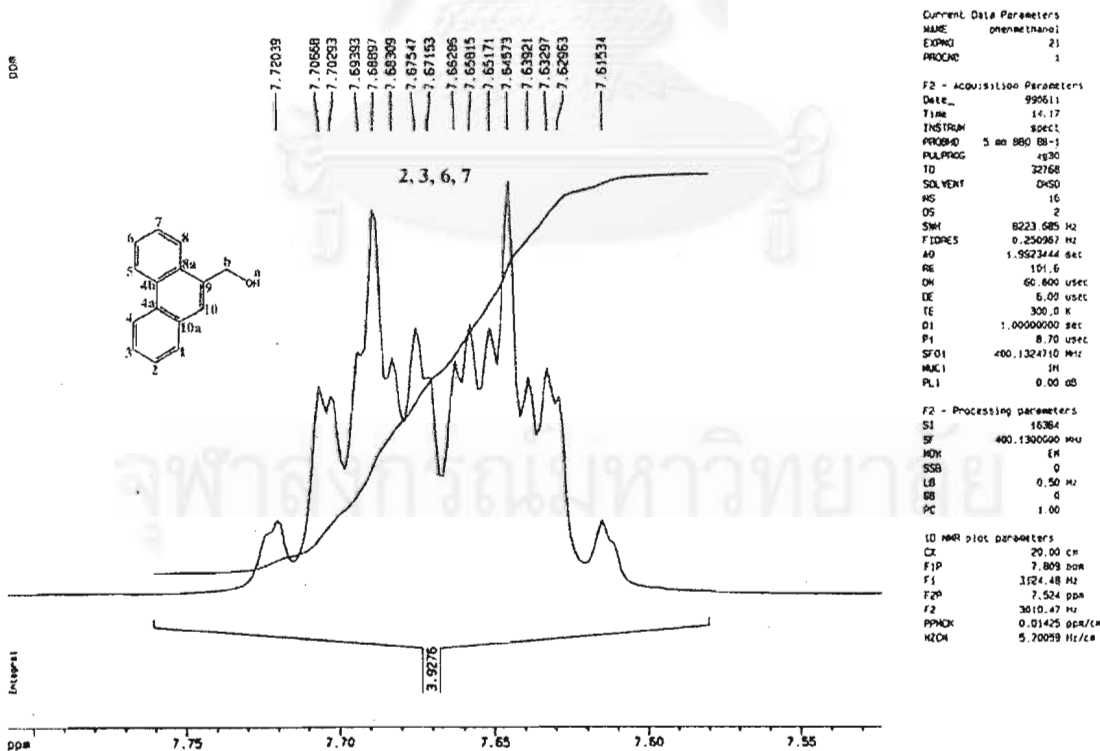
Figure A4: ^{1}H NMR spectrum of 9-(hydroxymethyl)phenanthrene (blow up at 7.55-7.80 ppm)
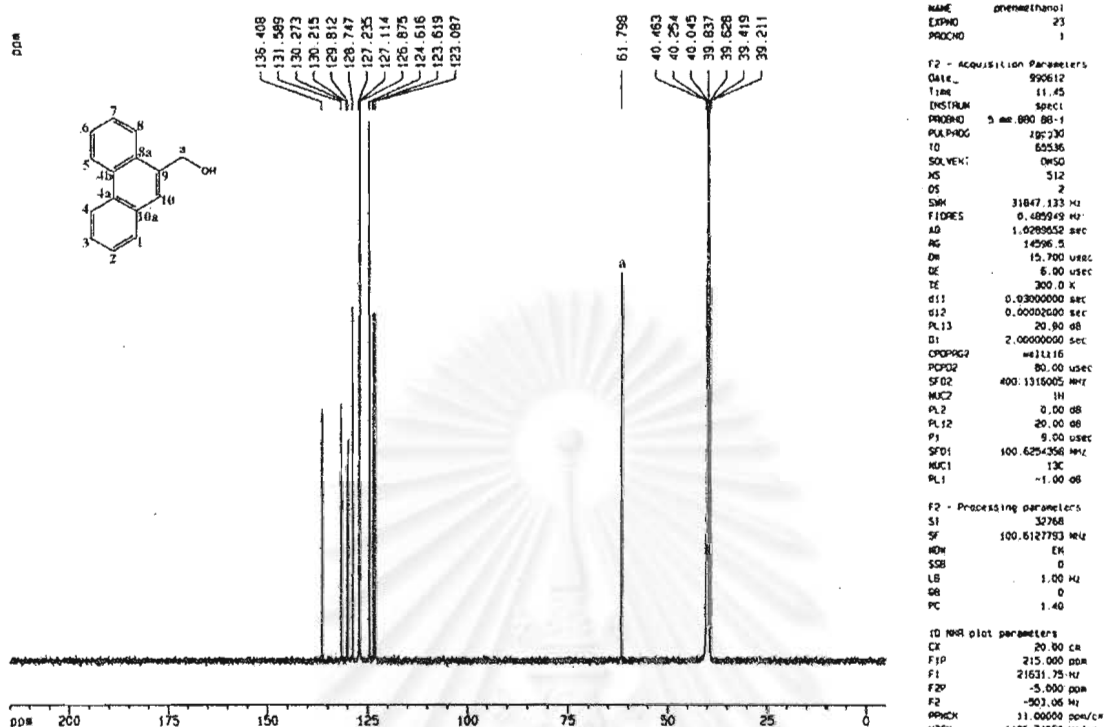
Figure A5: ^{13}C NMR spectrum of 9-(hydroxymethyl)phenanthrene
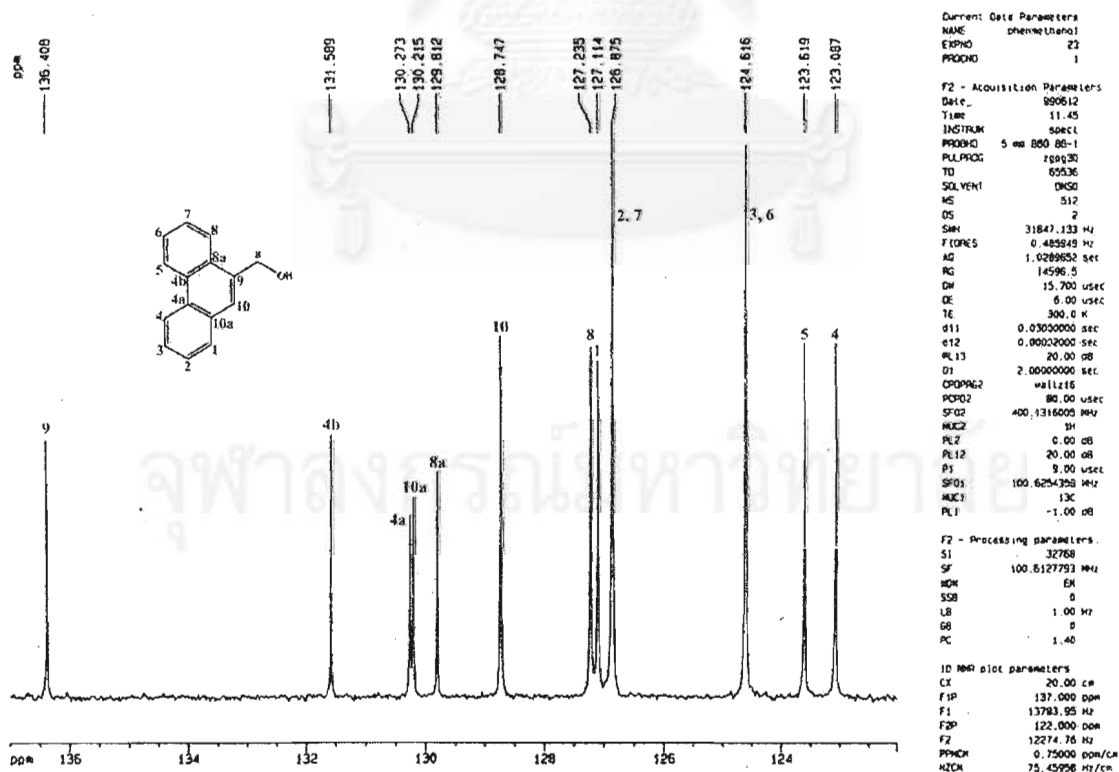
Figure A6: ^{13}C NMR spectrum of 9-(hydroxymethyl)phenanthrene (blow up at 122-137 ppm)
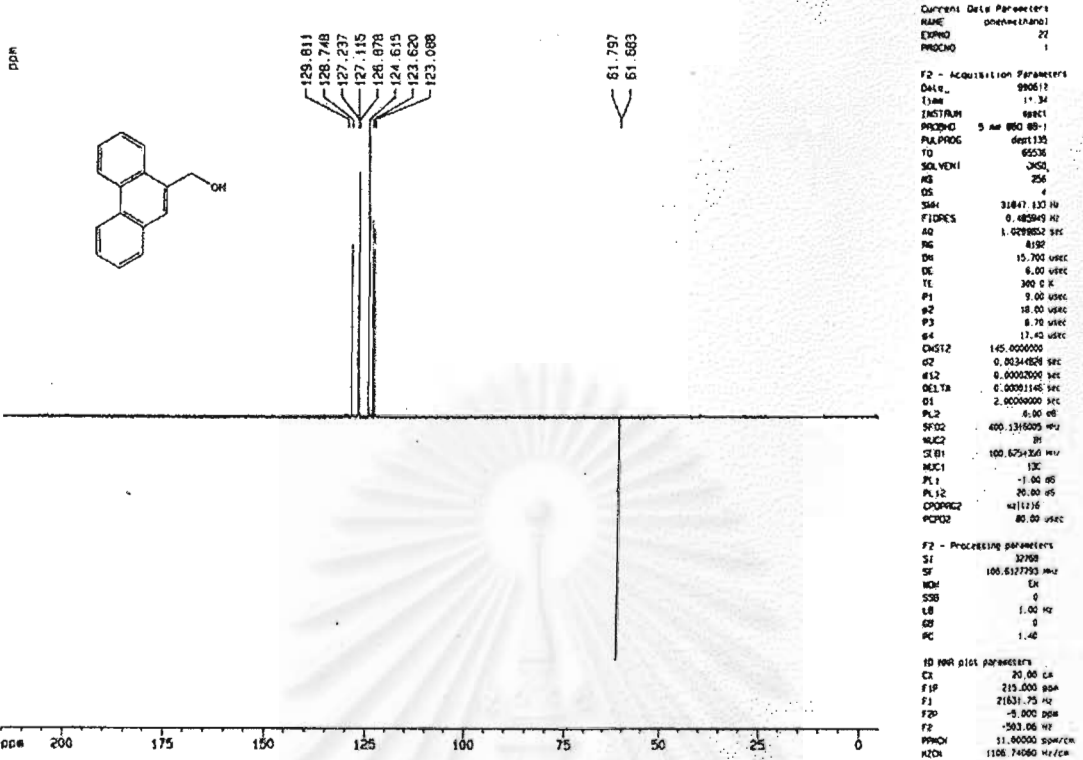
Figure A7: DEPT-135 spectrum of 9-(hydroxymethyl)phenanthrene
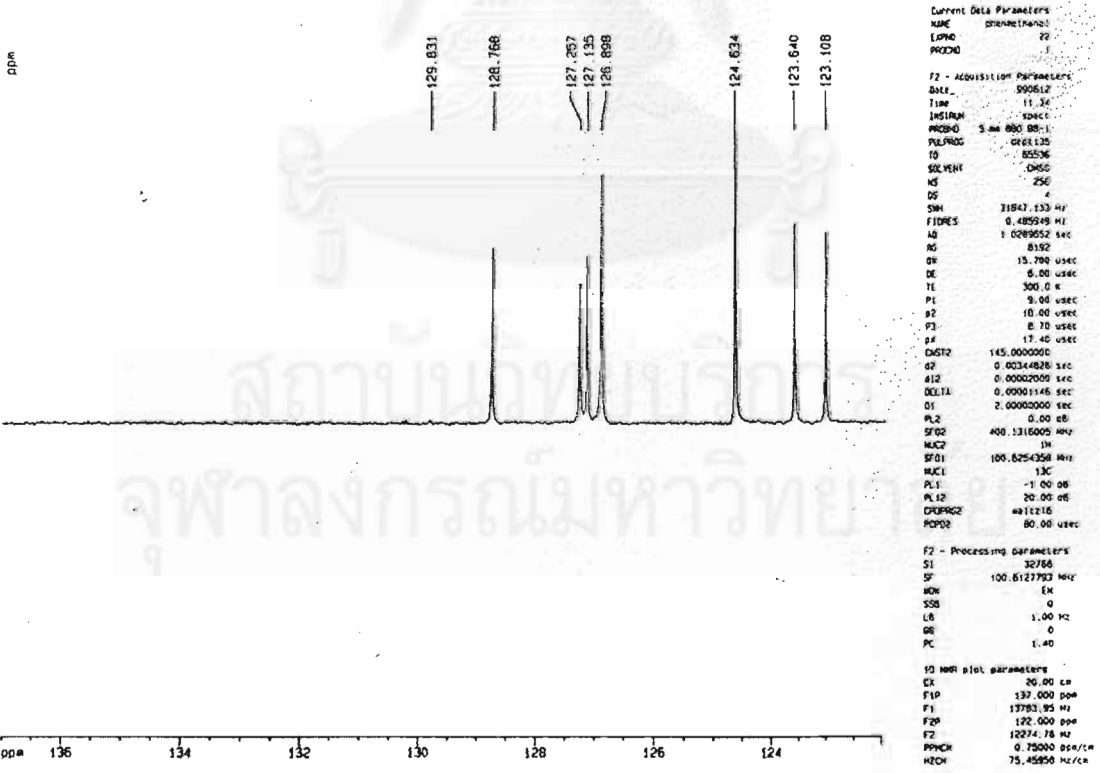
Figure A8: DEPT-135 spectrum of 9-(hydroxymethyl)phenanthrene (blow up at 122-137 ppm)
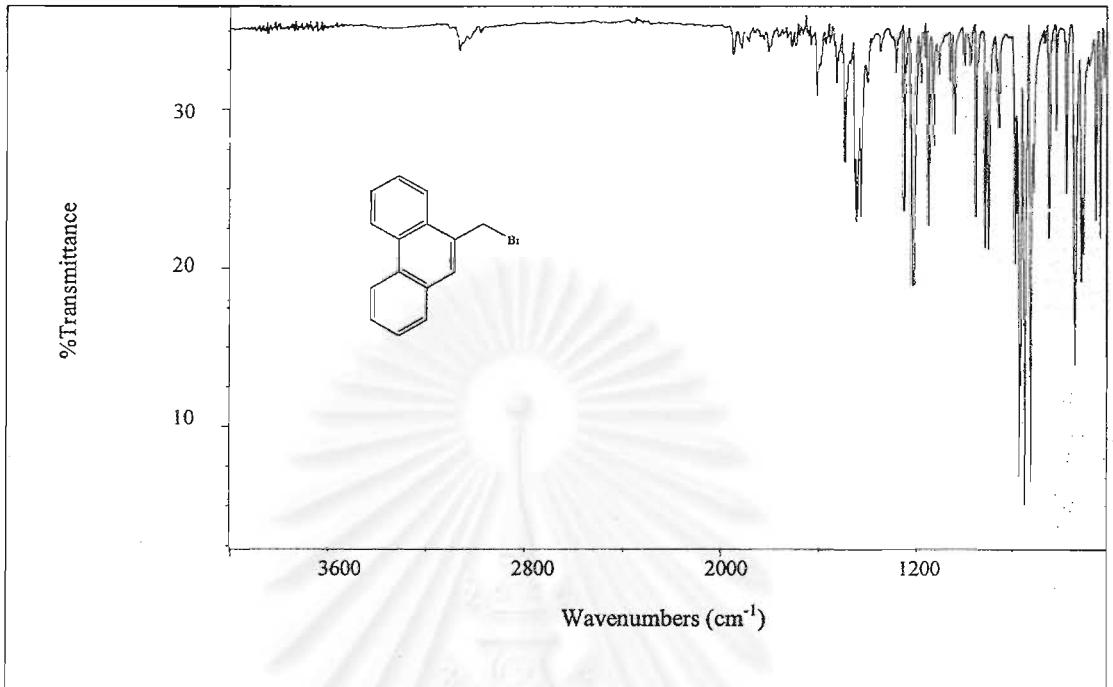
Figure B1: FT-IR spectrum of 9-(bromomethyl)phenanthrene
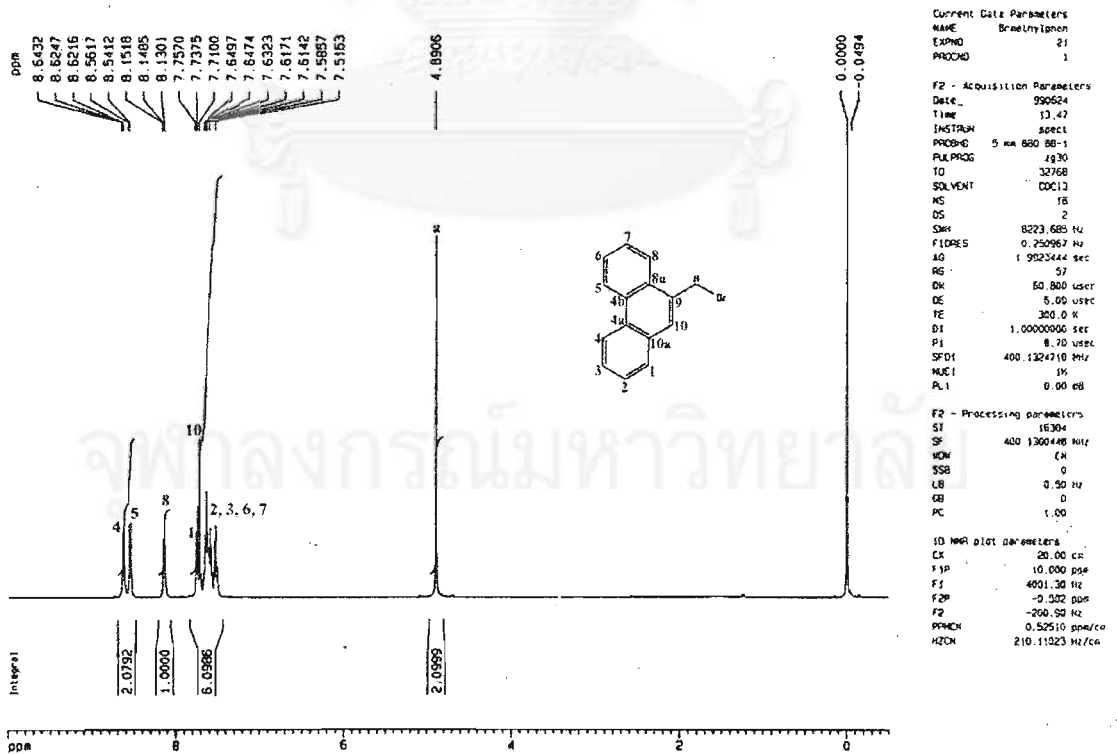
Figure B2: ^{1}H NMR spectrum of 9-(bromomethyl)phenanthrene
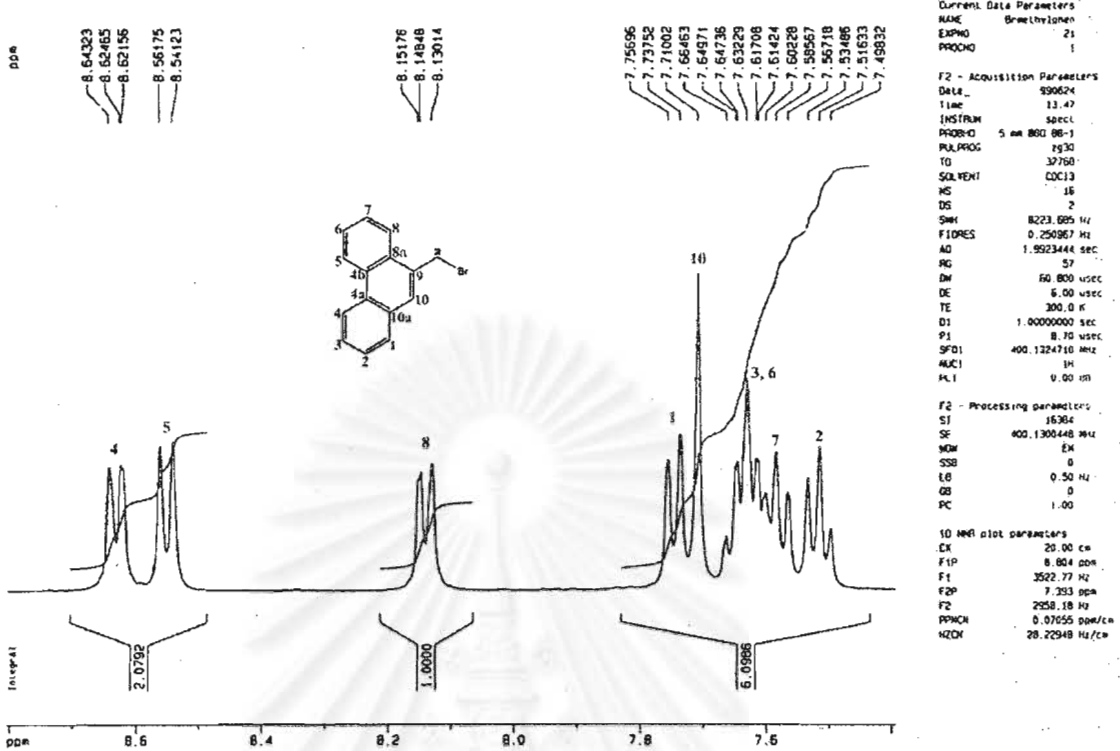
Figure B3: ¹H NMR spectrum of 9-(bromomethyl)phenanthrene (blow up at 7.64-8.88 ppm)
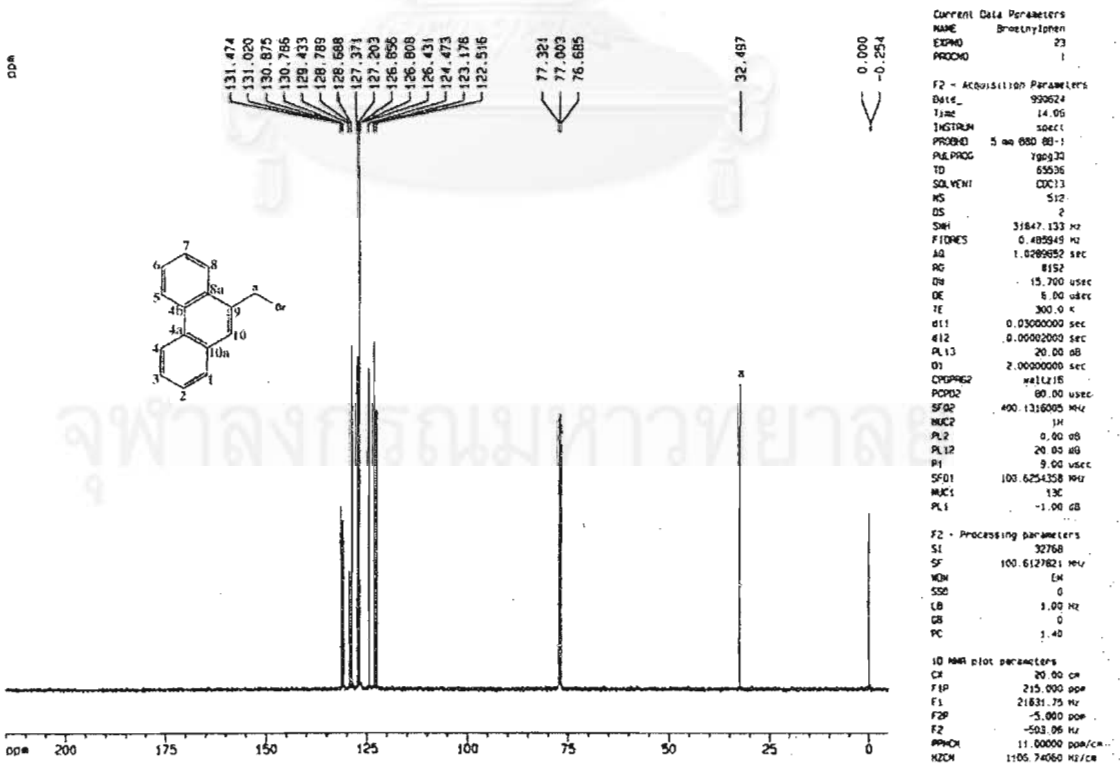
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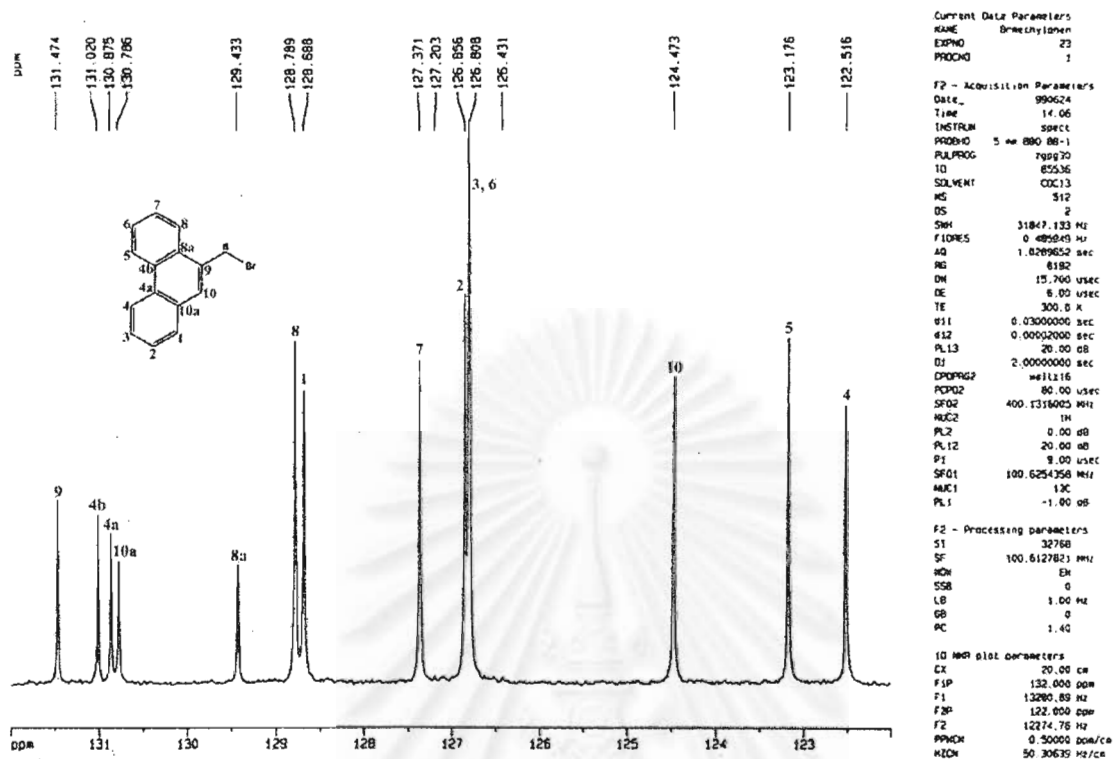
Figure B5: ^{13}C NMR spectrum of 9-(bromomethyl)phenanthrene (blow up at 122-132 ppm)
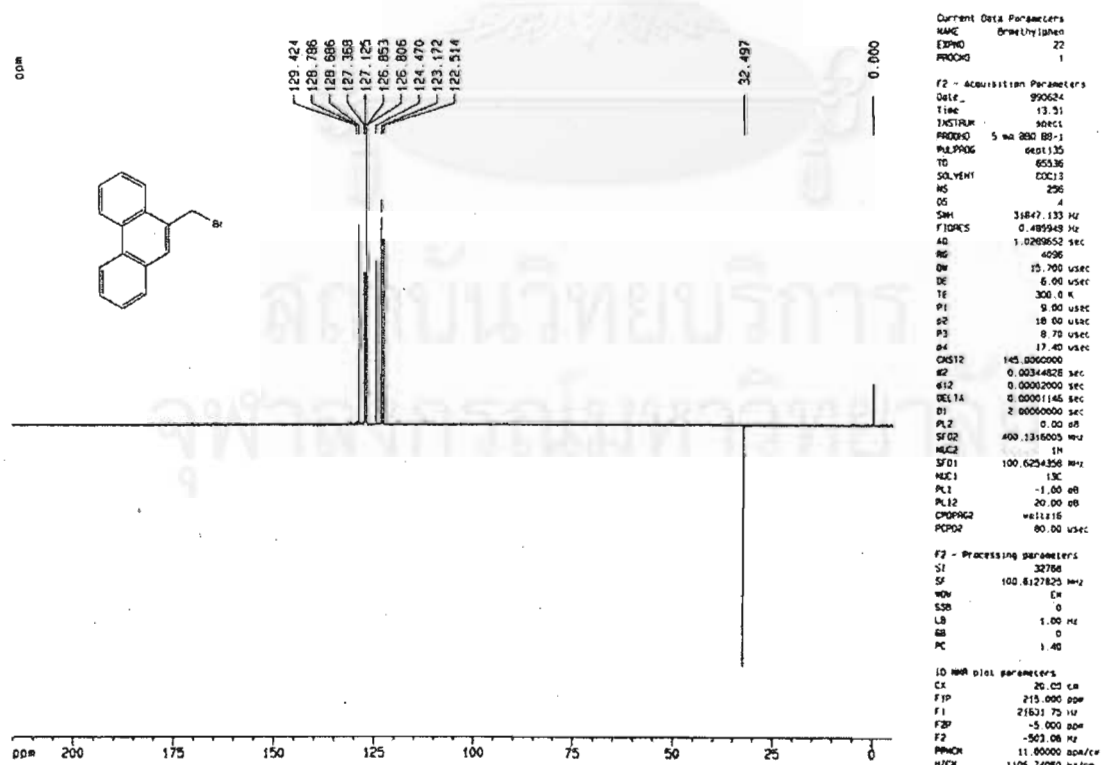
Figure B6: DEPT-135 spectrum of 9-(bromomethyl)phenanthrene
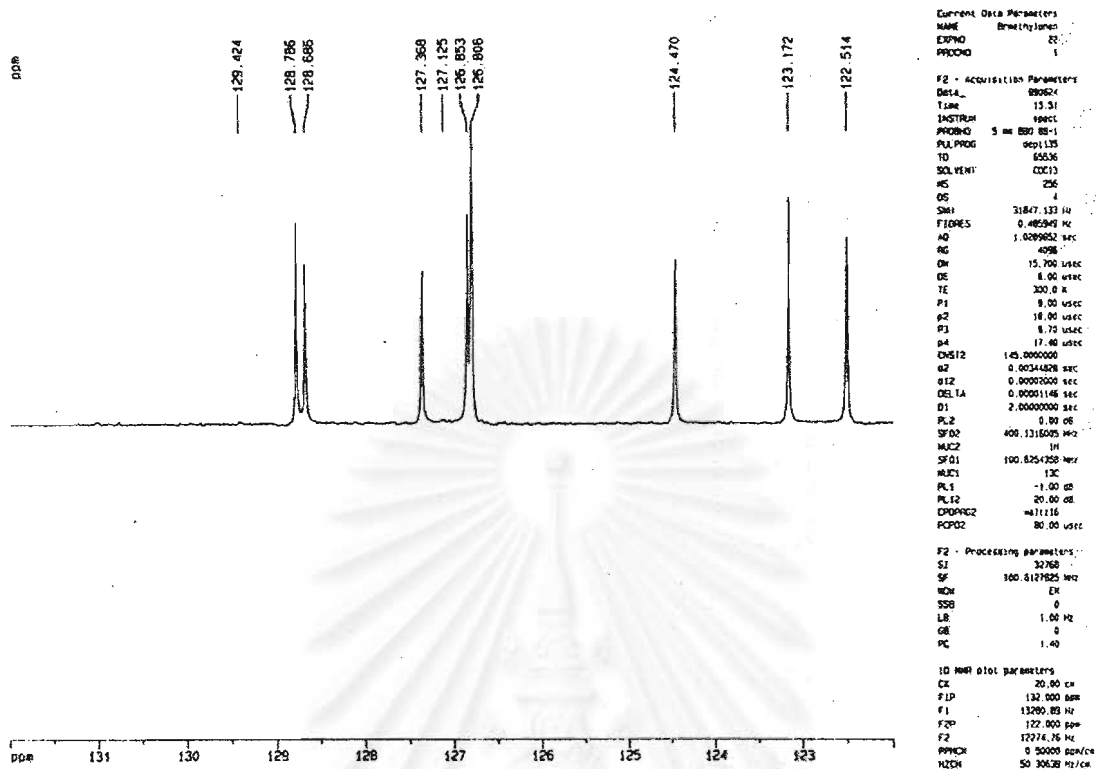
Figure B7: DEPT-135 spectrum of 9-(bromomethyl)phenanthrene (blow up at 122-132 ppm)
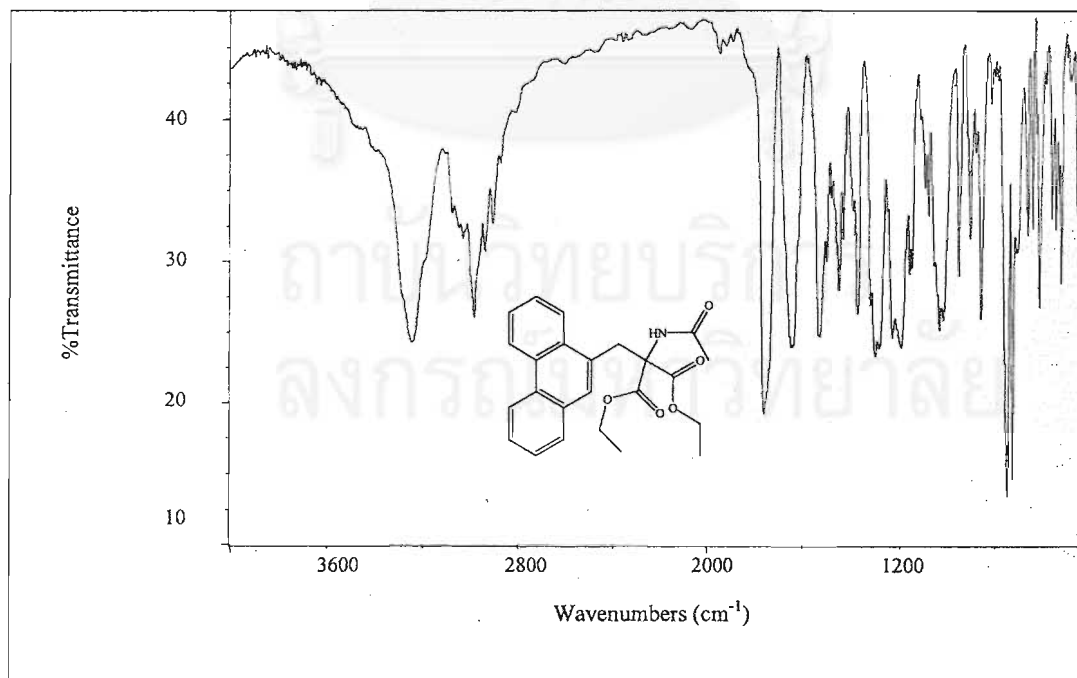
Figure C1: FT-IR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate
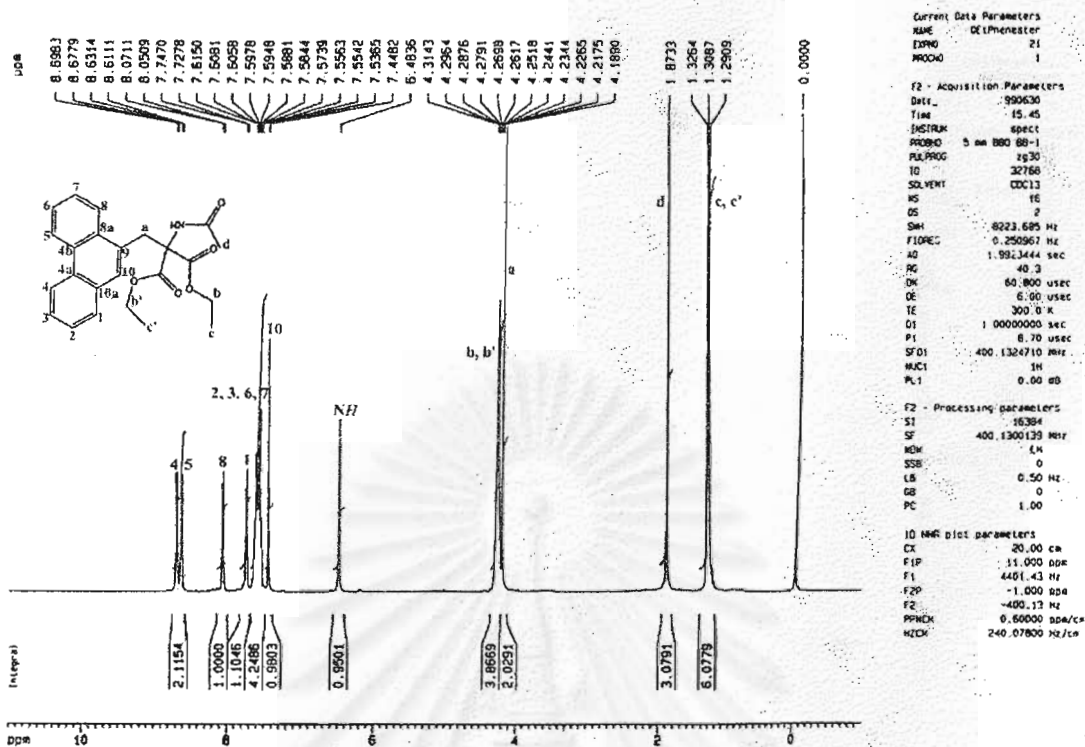
Figure C2: ^{1}H NMR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate
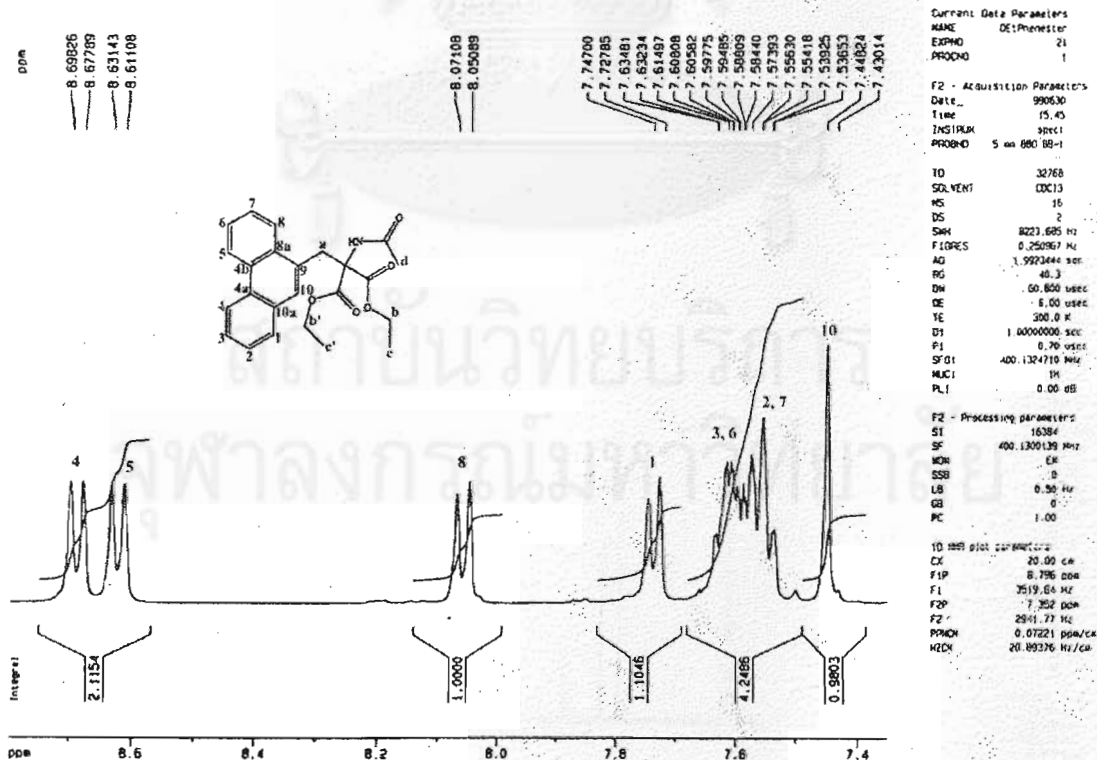
Figure C3: ^{1}H NMR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate (blow up at 7.4-8.8 ppm)

Figure C4: ¹H NMR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate (blow up at 3.9-4.4 ppm)

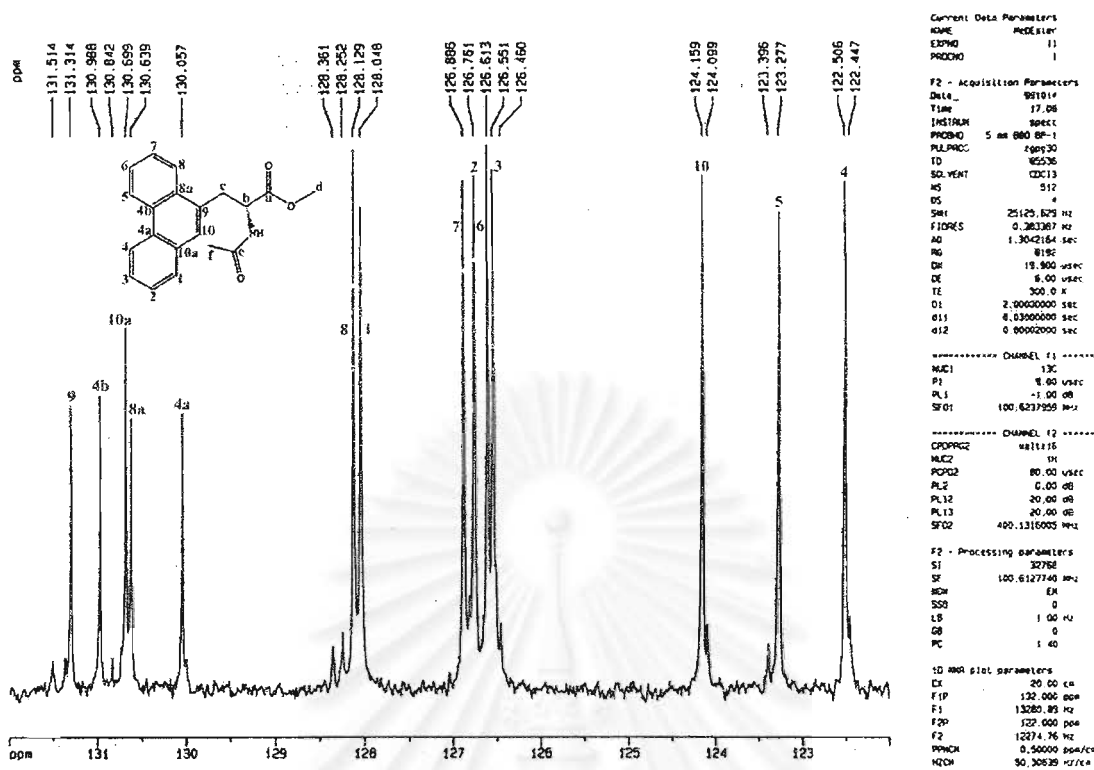**Figure C5:** ¹³C NMR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate
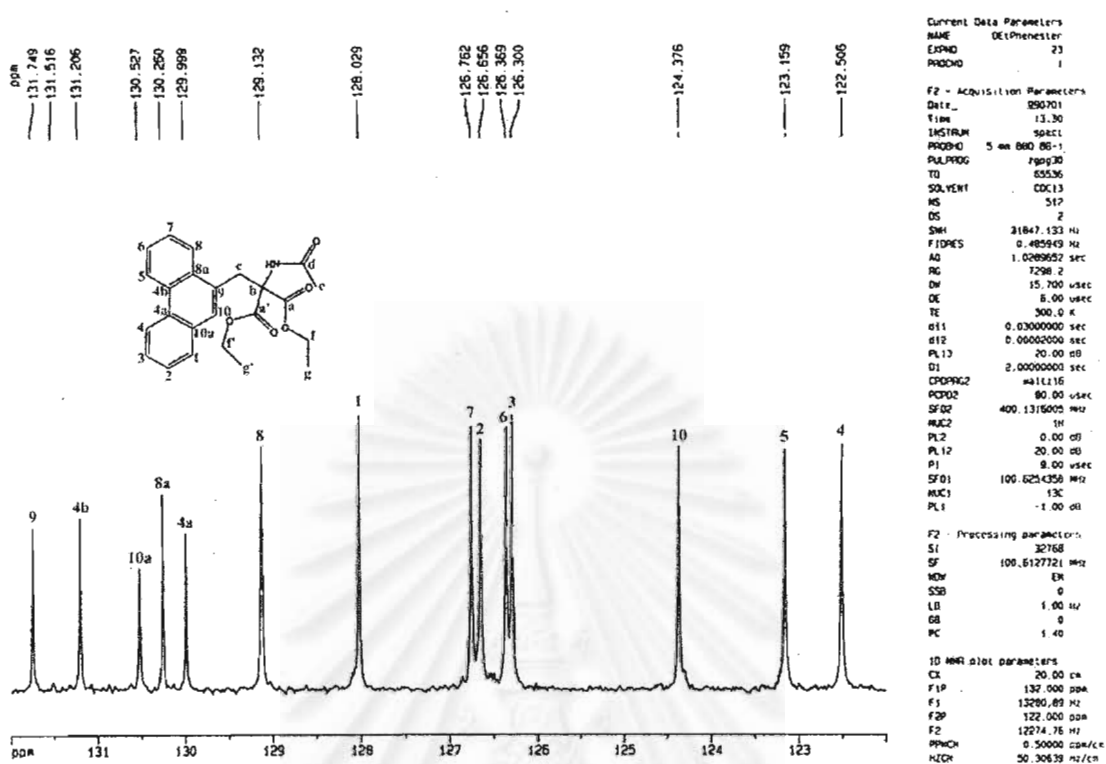
Figure C6: ^{13}C NMR spectrum of diethyl 2-acetylamino-2-(9-phenanthrenylmethyl) malonate (blow up at 122-132 ppm)
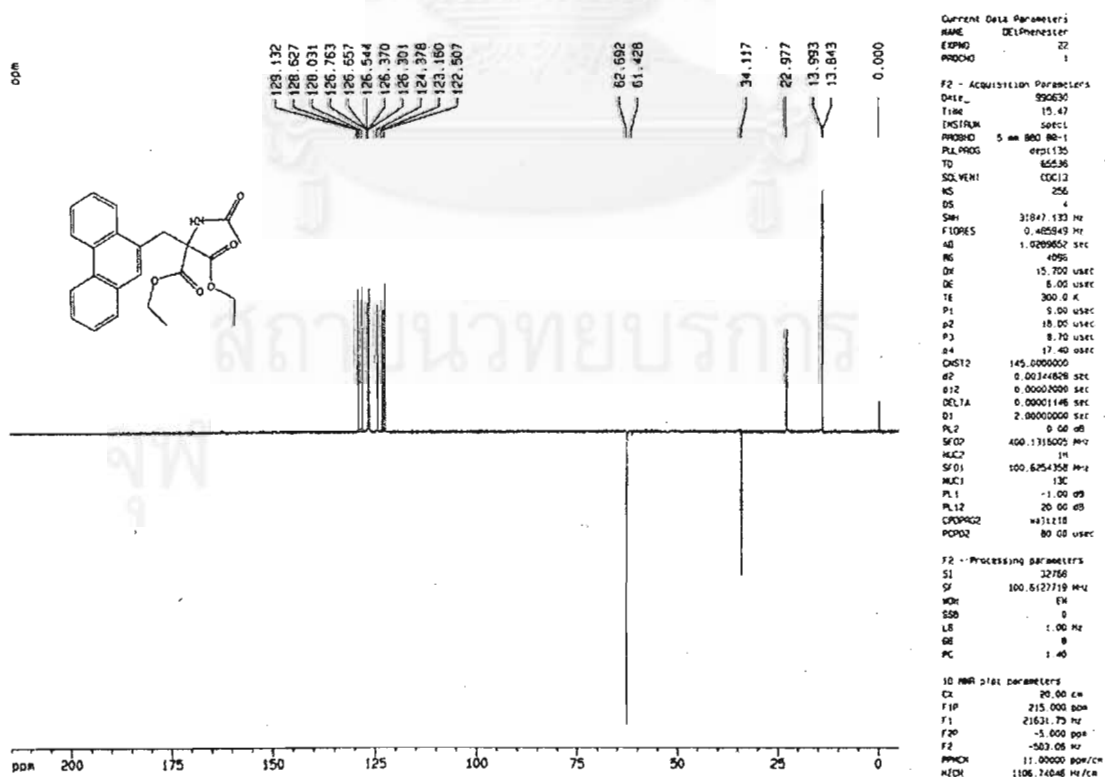
Figure C7: DEPT-135 spectrum of diethyl 2-acetylamino-2-(9-phenanthrenyl-methyl)malonate
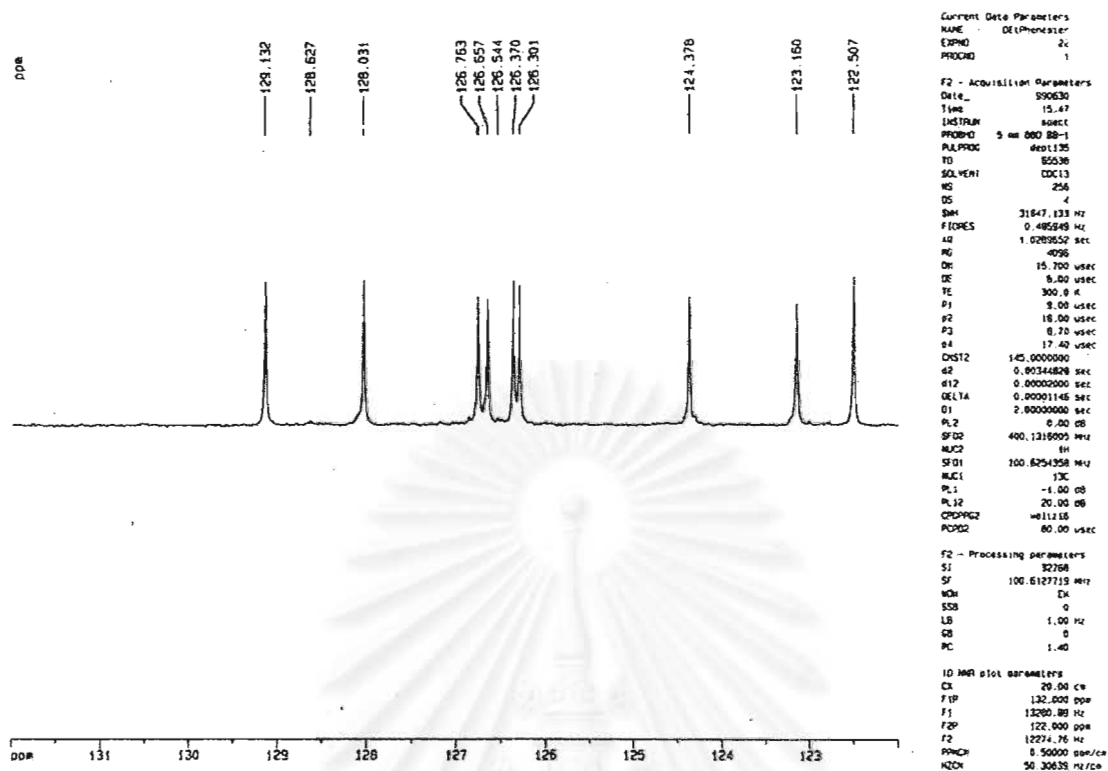
Figure C8: DEPT-135 spectrum of diethyl 2-acetylamino-2-(9-phenanthrenyl-methyl)malonate (blow up at 122-132 ppm)
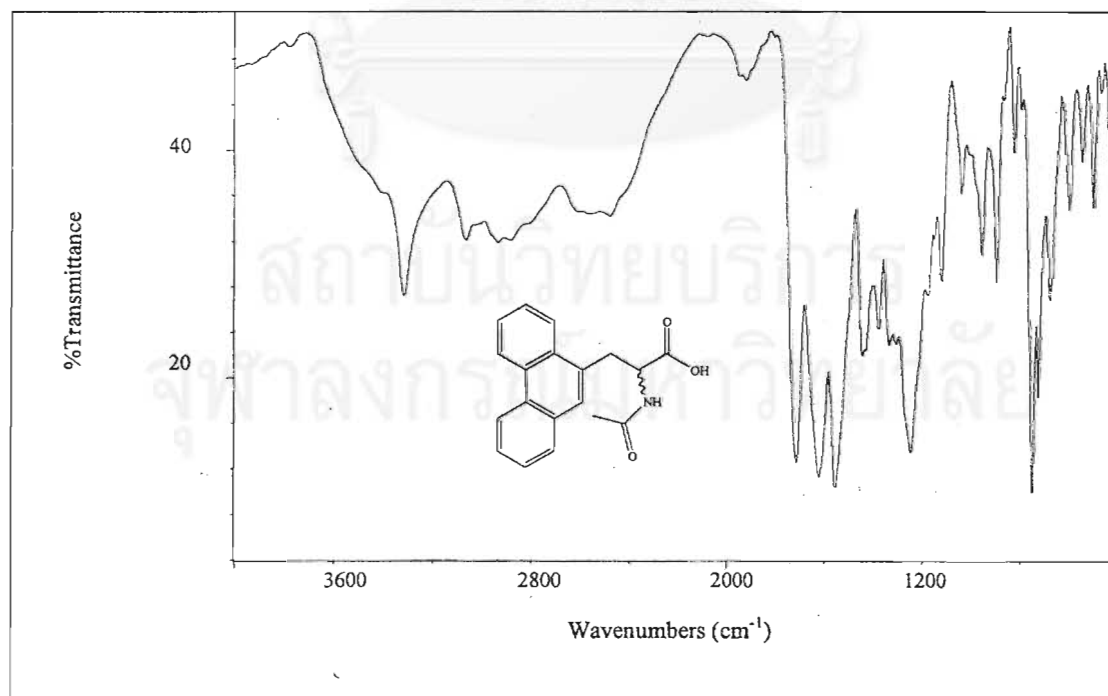
Figure D1: FT-IR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid
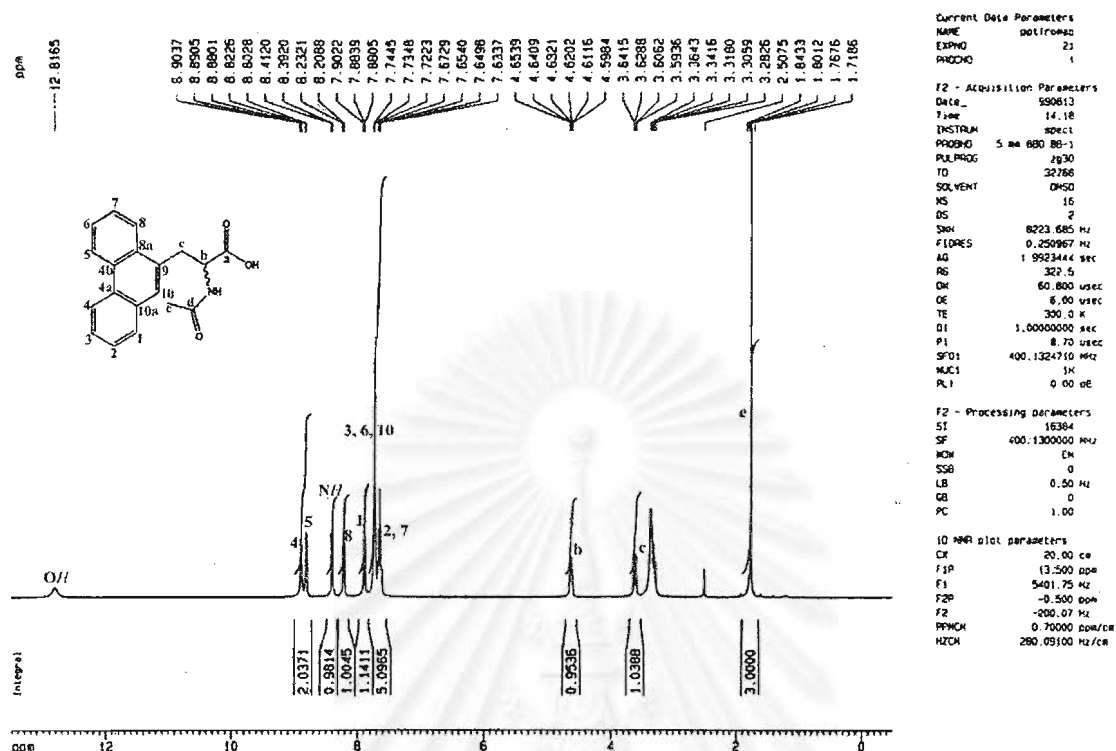
Figure D2: ^{1}H NMR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid
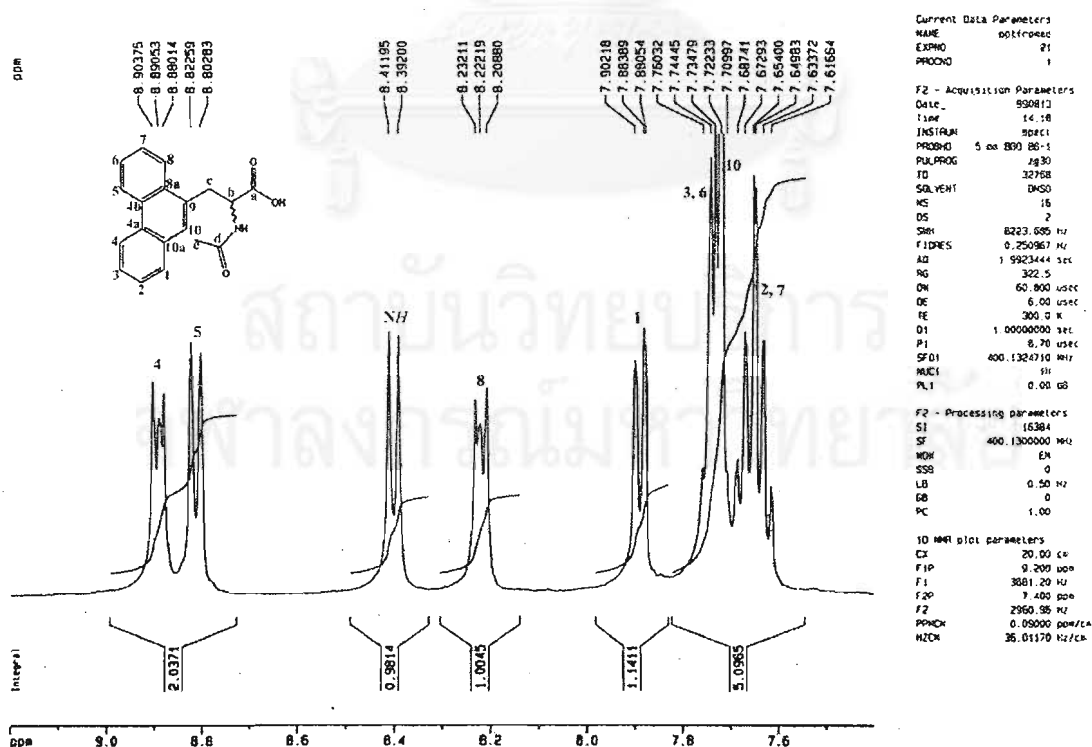
Figure D3: ^{1}H NMR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 7.5-9.0 ppm)

Figure D4: ^{1}H NMR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 3.2-4.7 ppm)

Figure D5: ^{13}C NMR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid
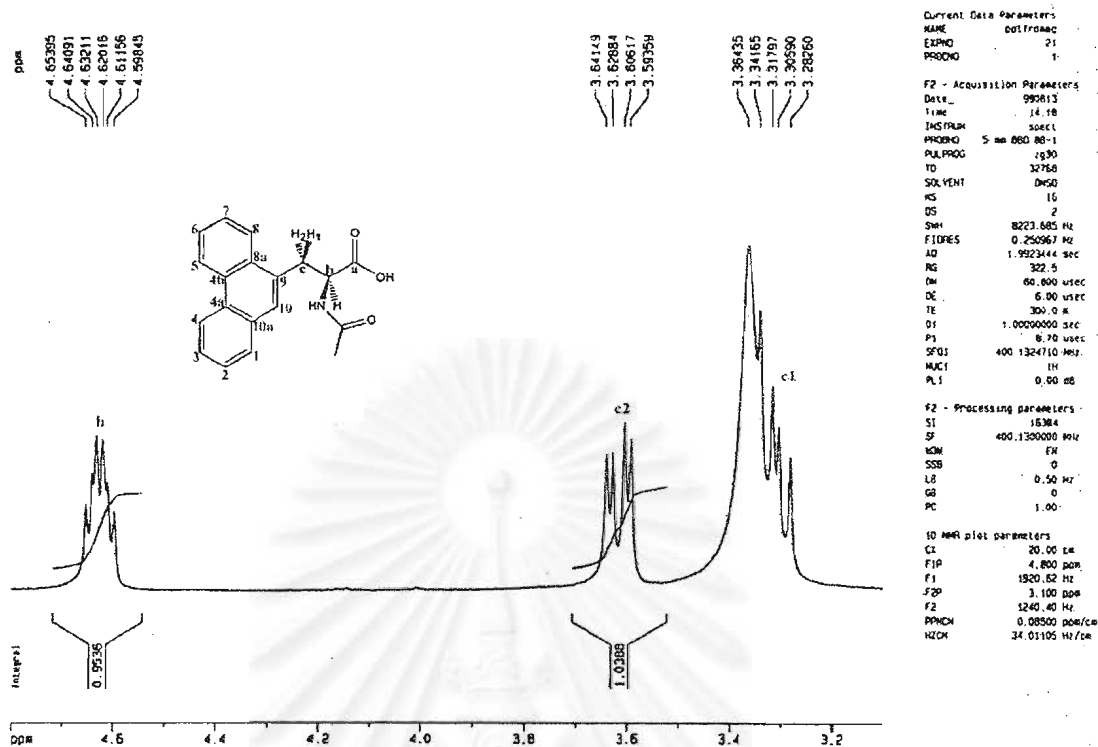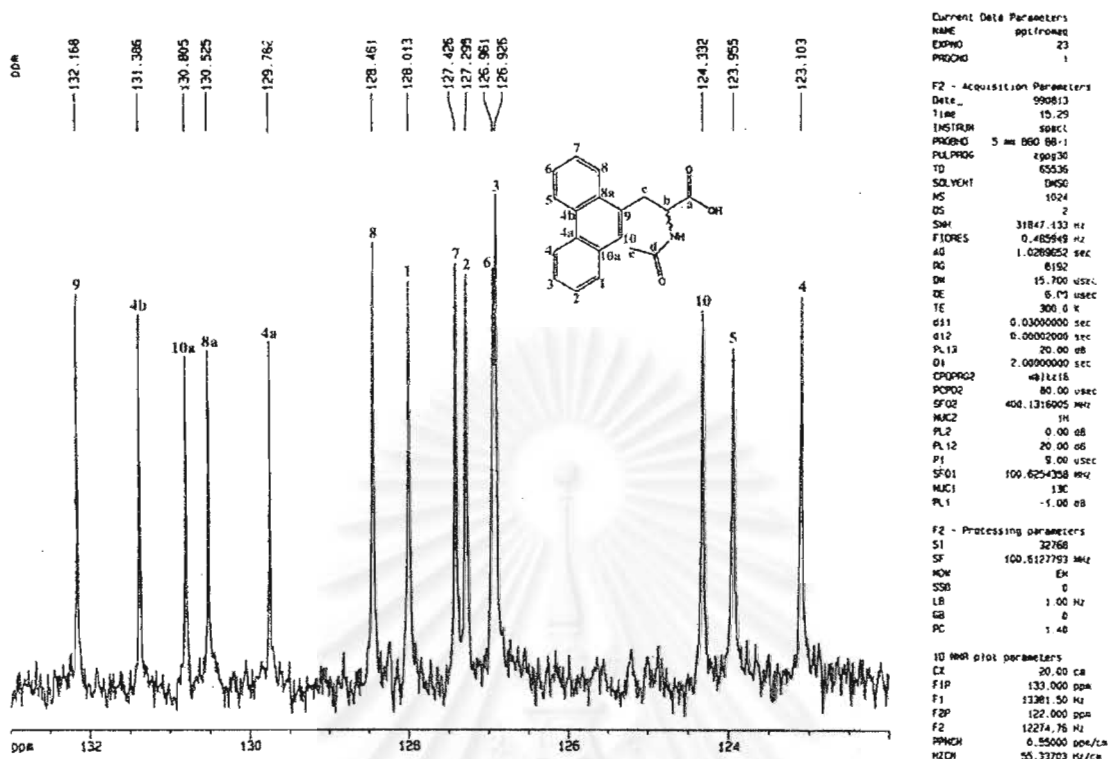
Figure D6: ^{13}C NMR spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at (122-133 ppm)
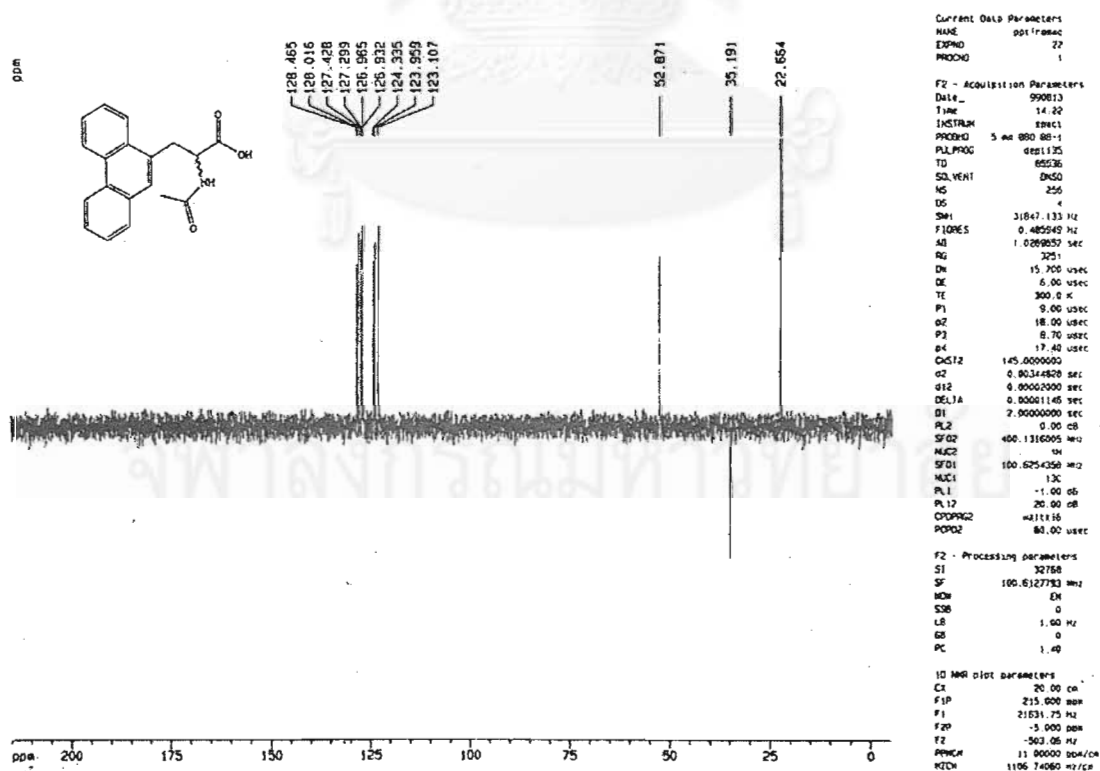
Figure D7: DEPT-135 spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid
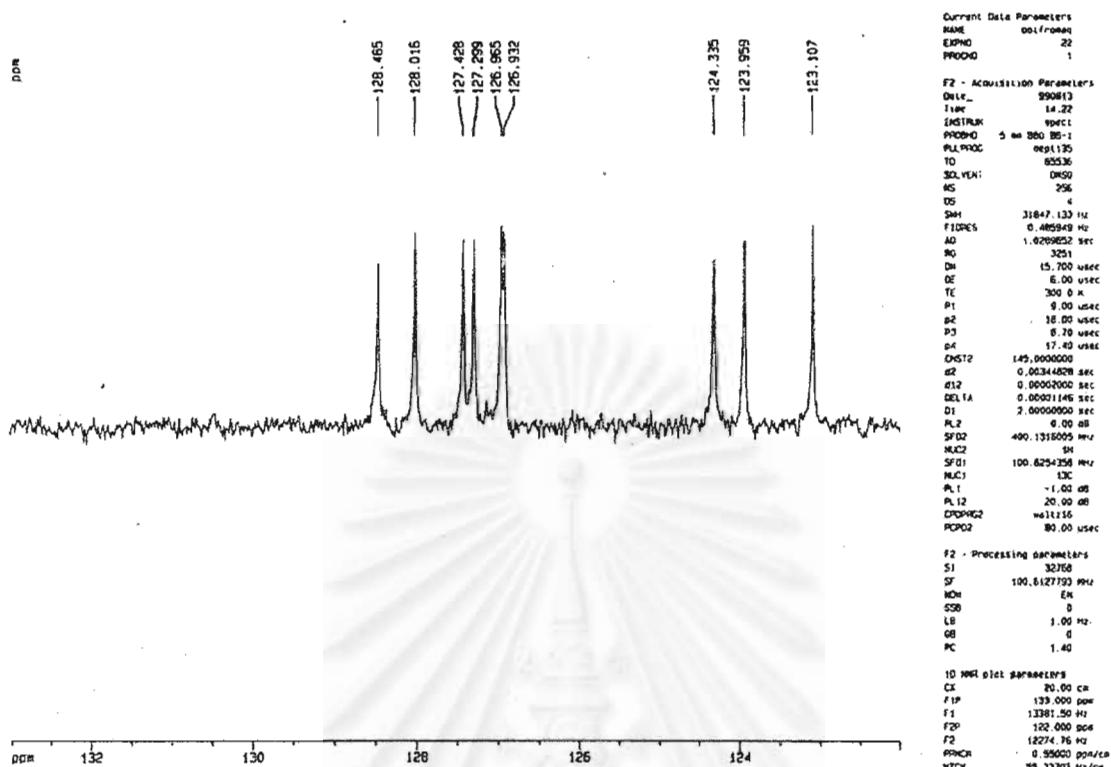
Figure D8: DEPT-135 spectrum of 2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 122-133 ppm)
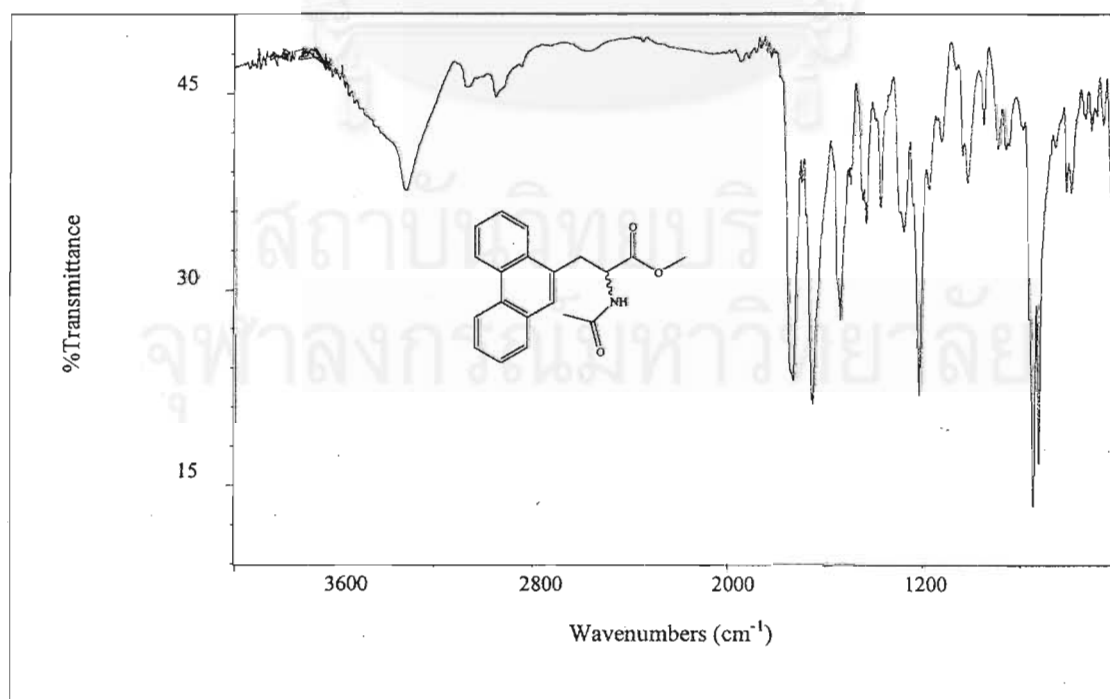
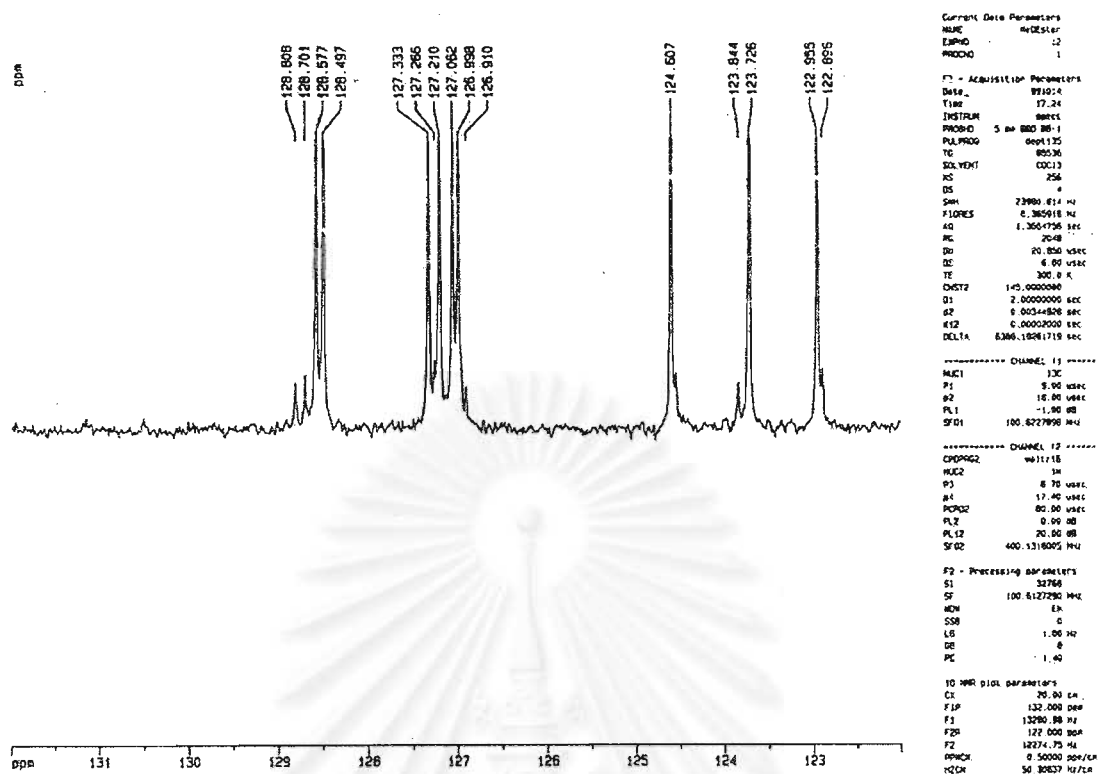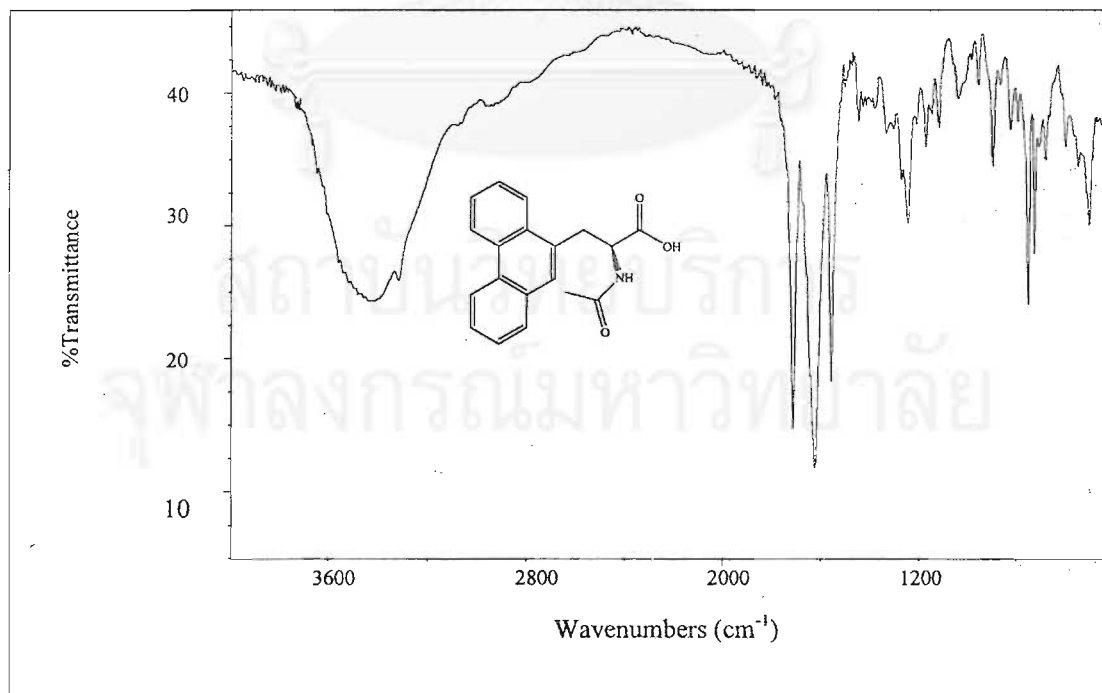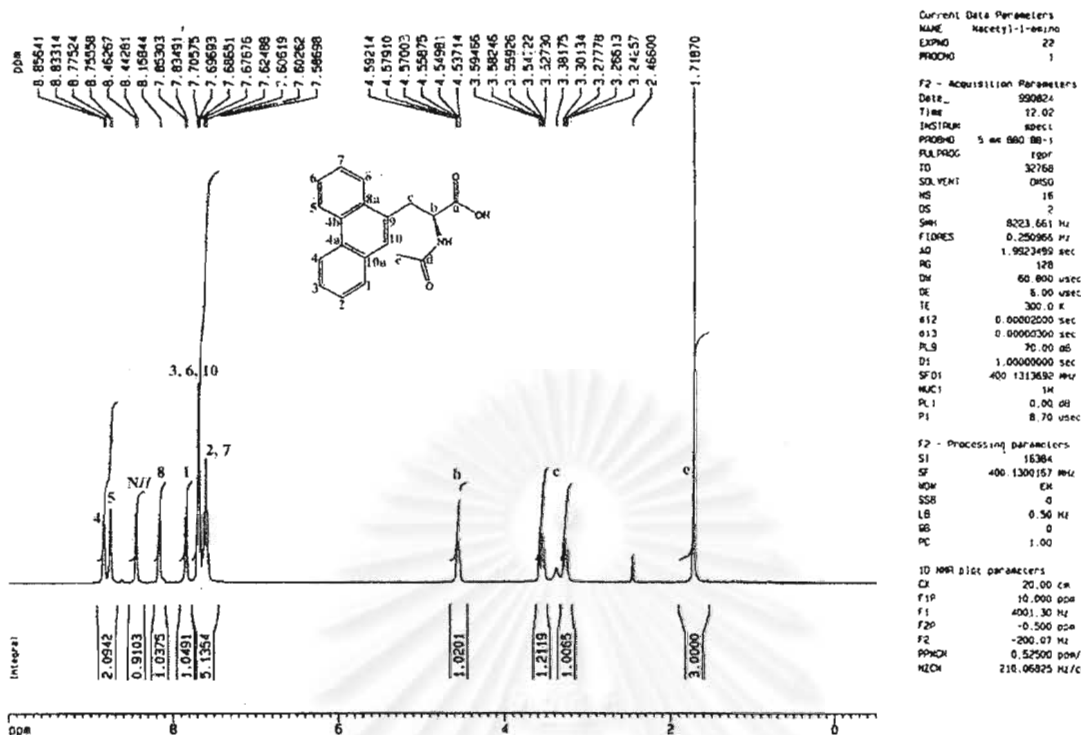**Figure E1:** FT-IR spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl)propionate
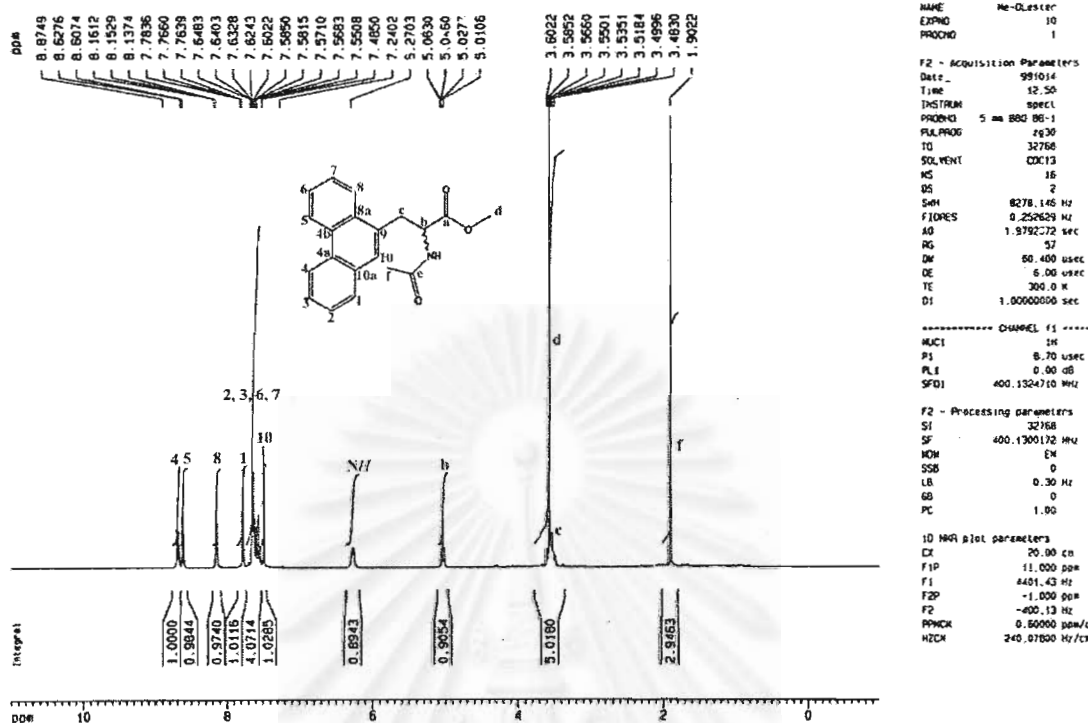
Figure E2: ¹H NMR spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate
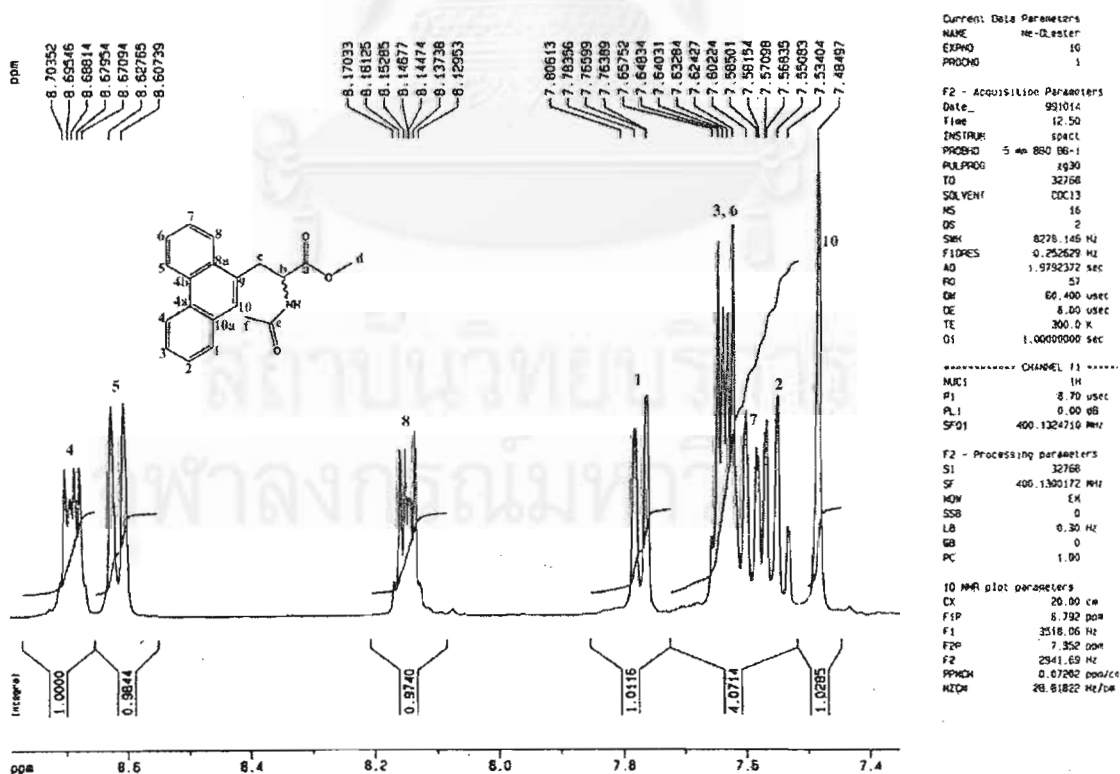
Figure E3: ¹H NMR spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 7.4-8.6 ppm)
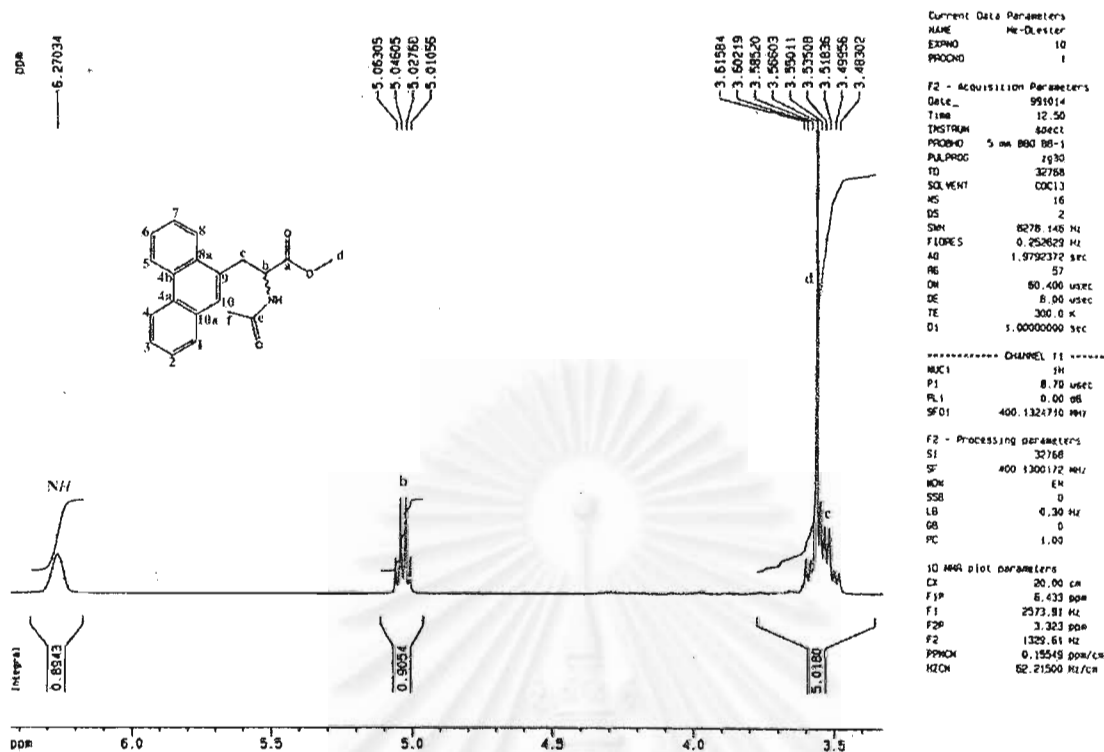
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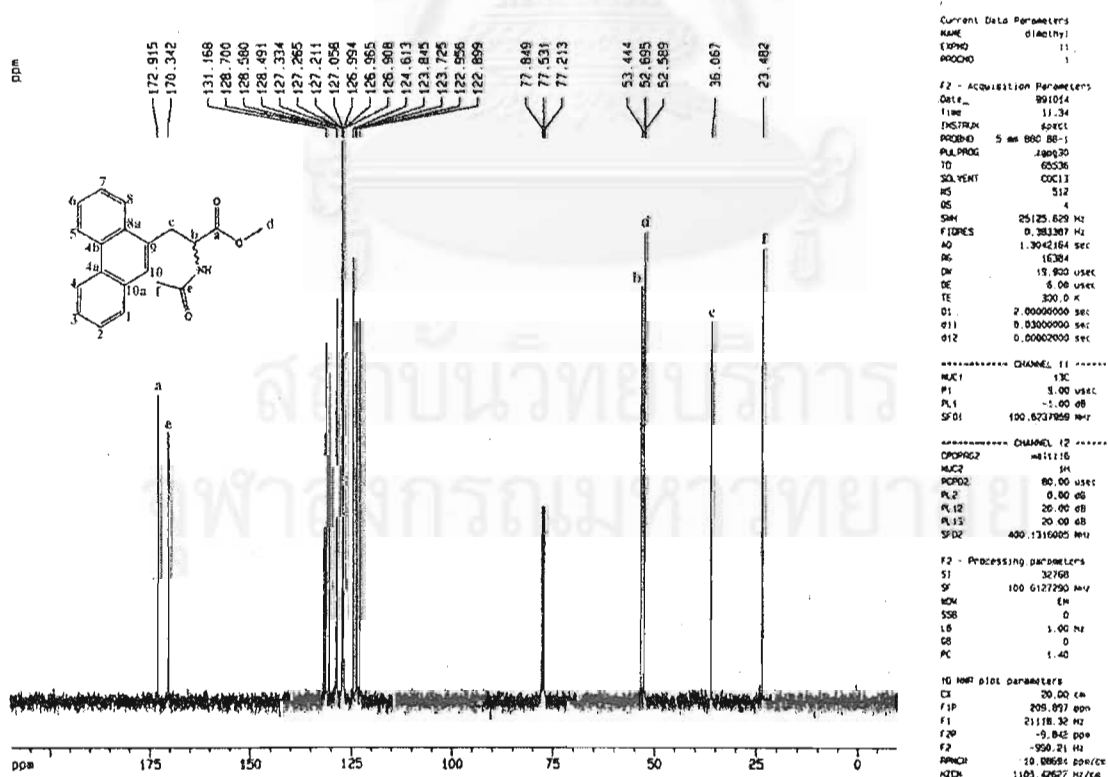
Figure E5: ¹³C NMR spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate
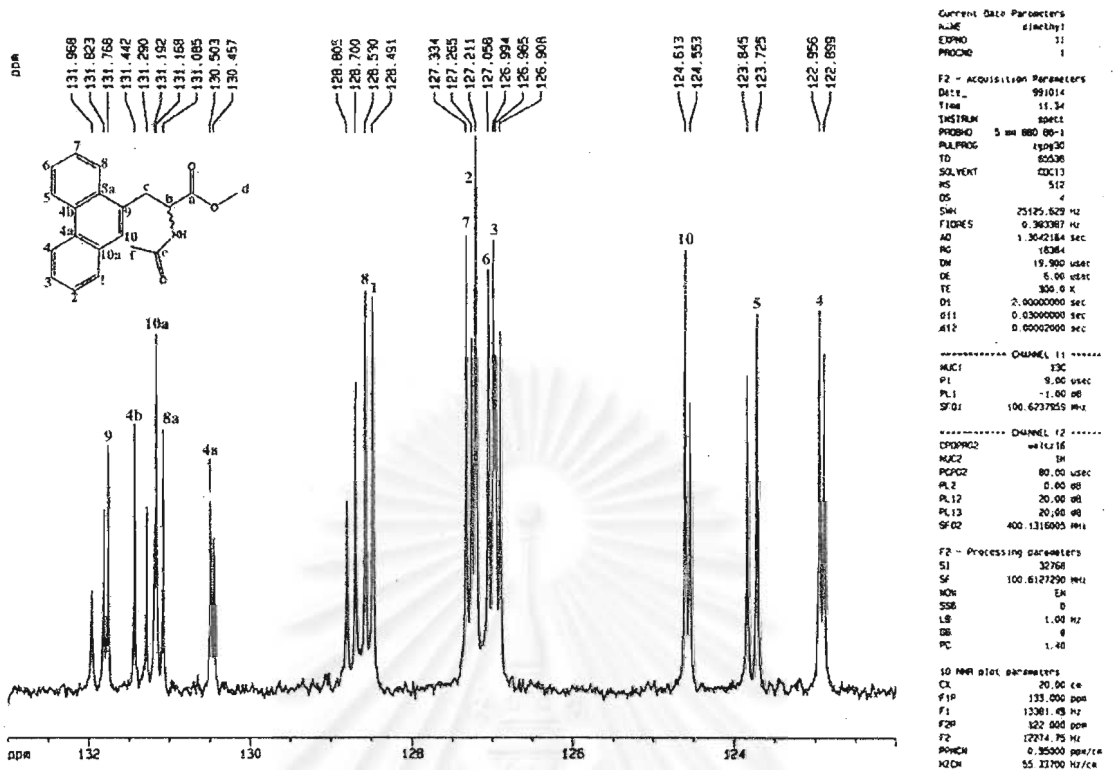
Figure E6: ^{13}C NMR spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 122-133 ppm)
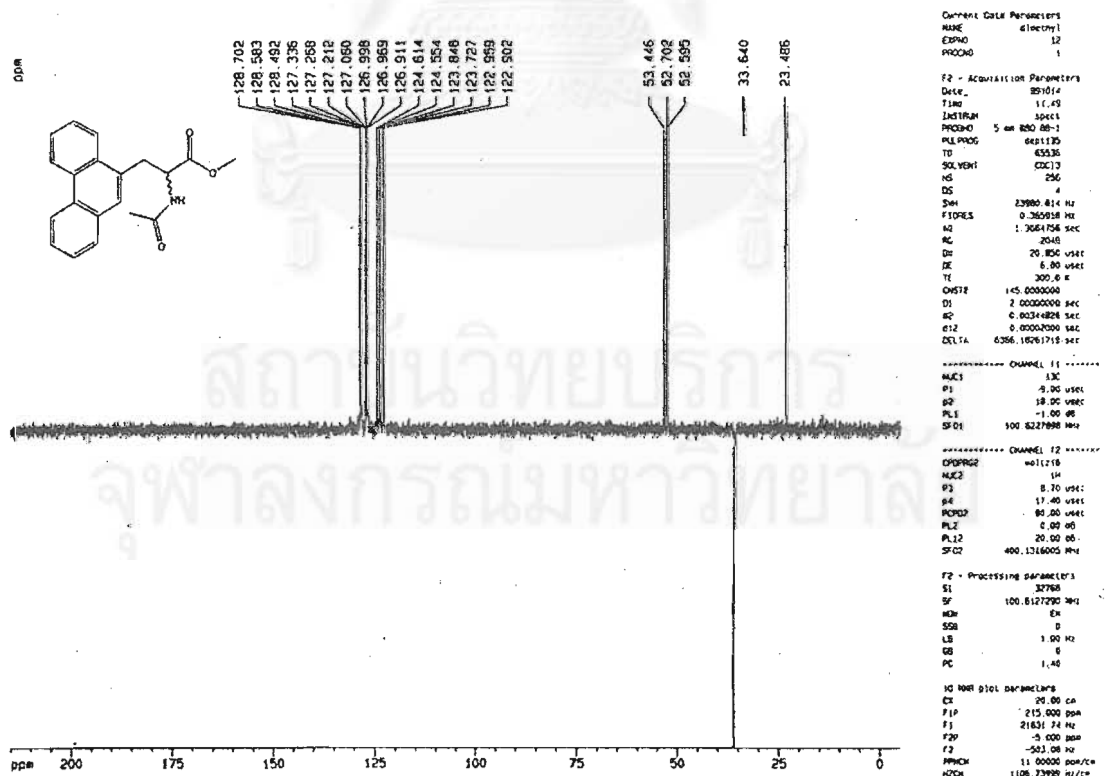
Figure E7: DEPT-135 spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate
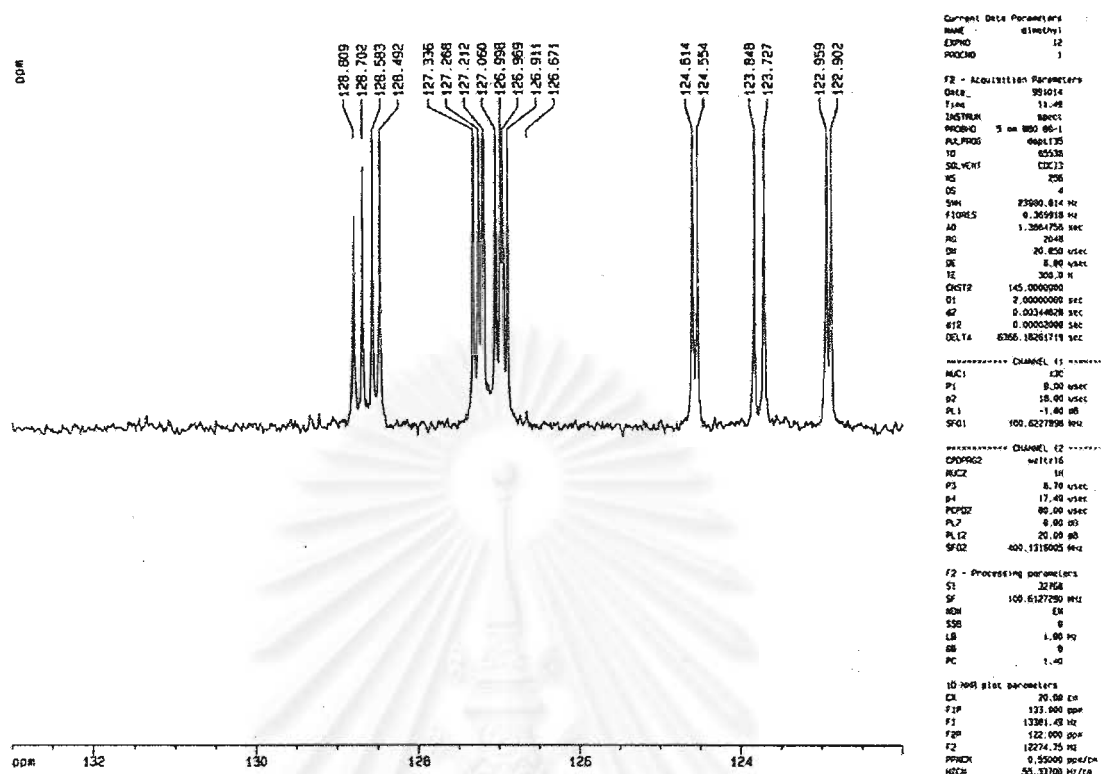
Figure E8: DEPT-135 spectrum of methyl 2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 122-133 ppm)
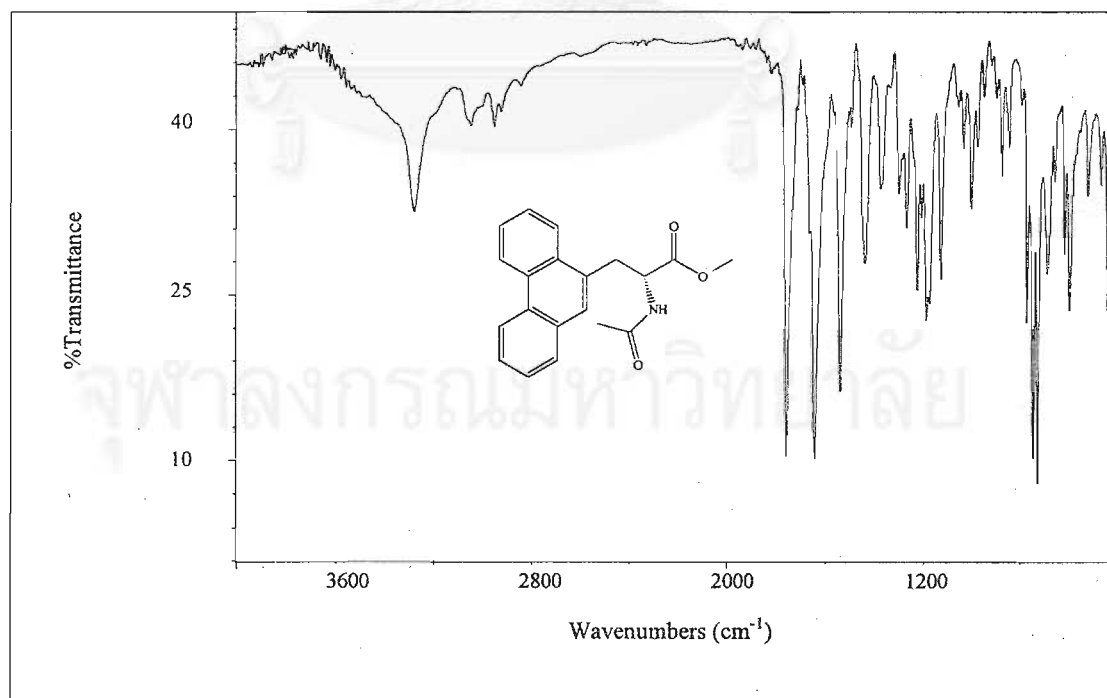
Figure F1: FT-IR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate
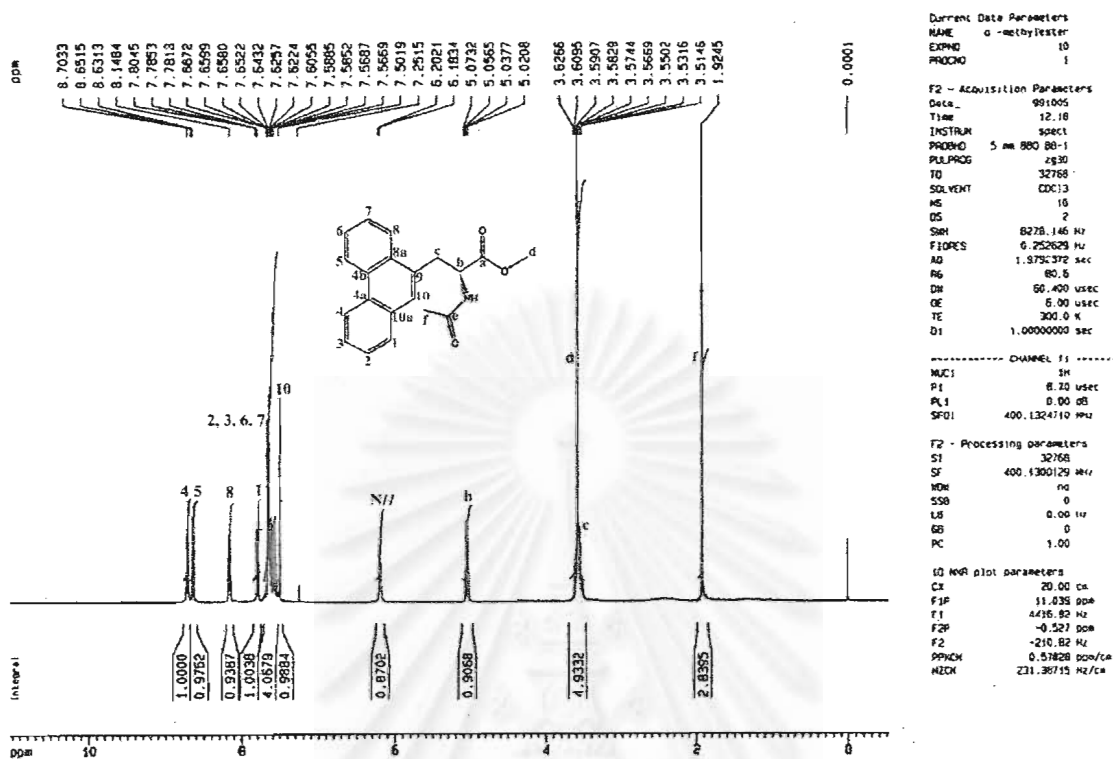
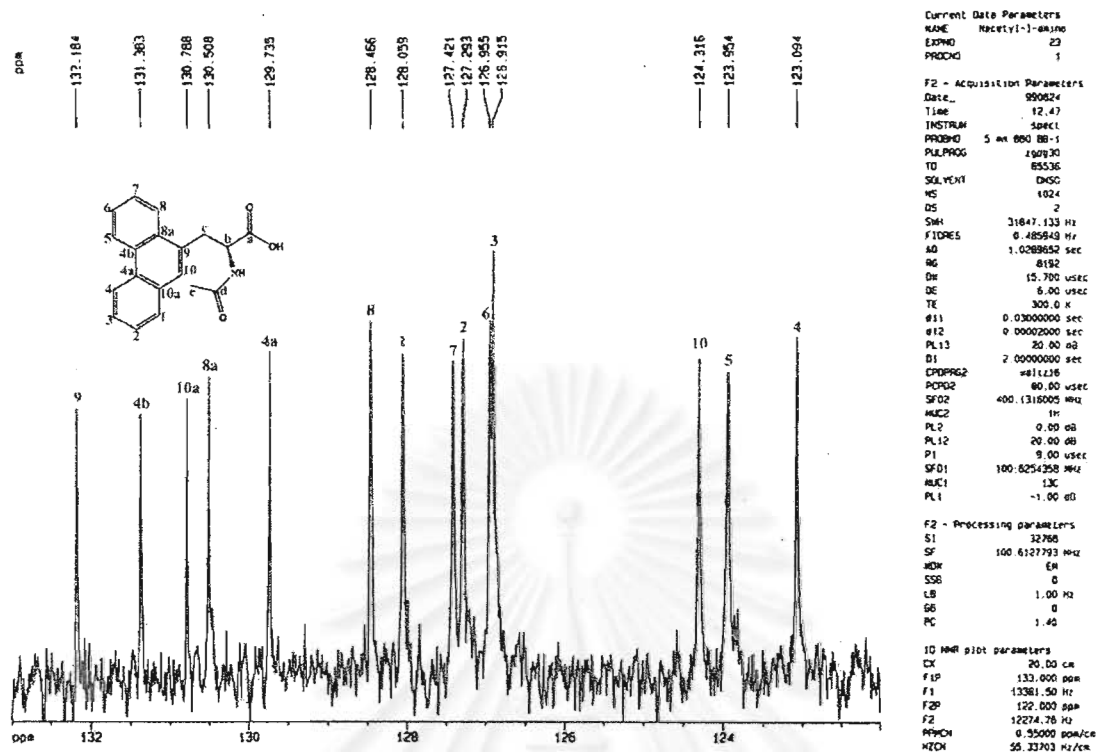**Figure F2:** ^{1}H NMR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate
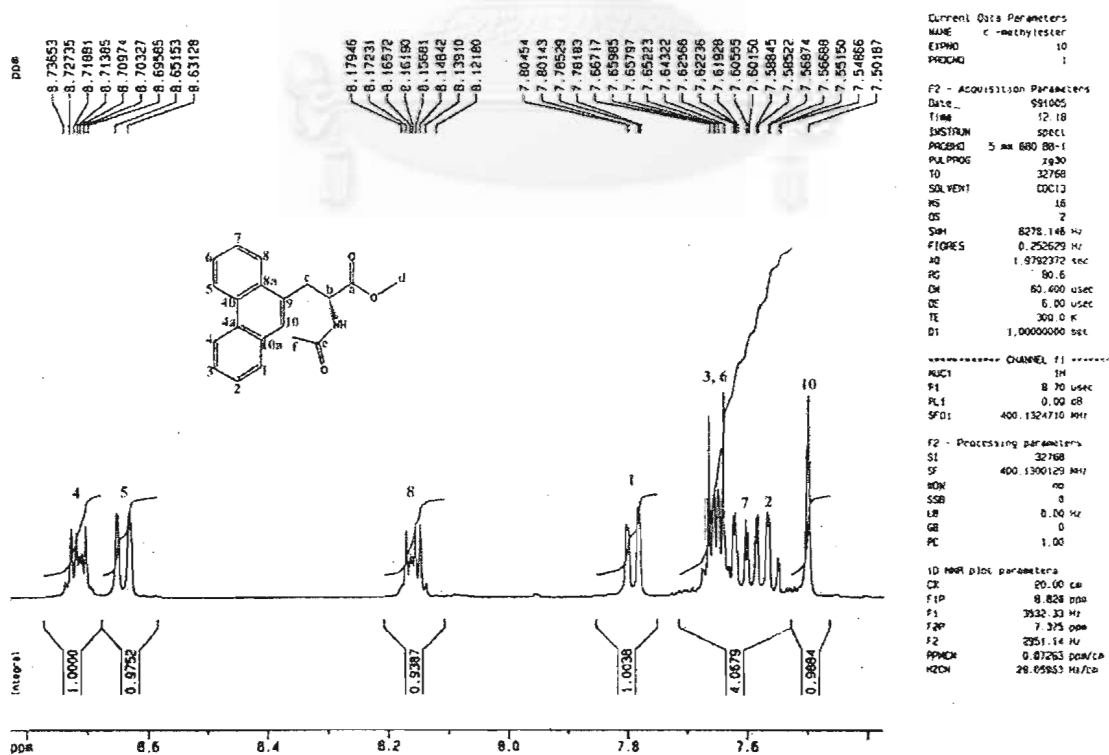
Figure F3: ^{1}H NMR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 7.4-8.8 ppm)
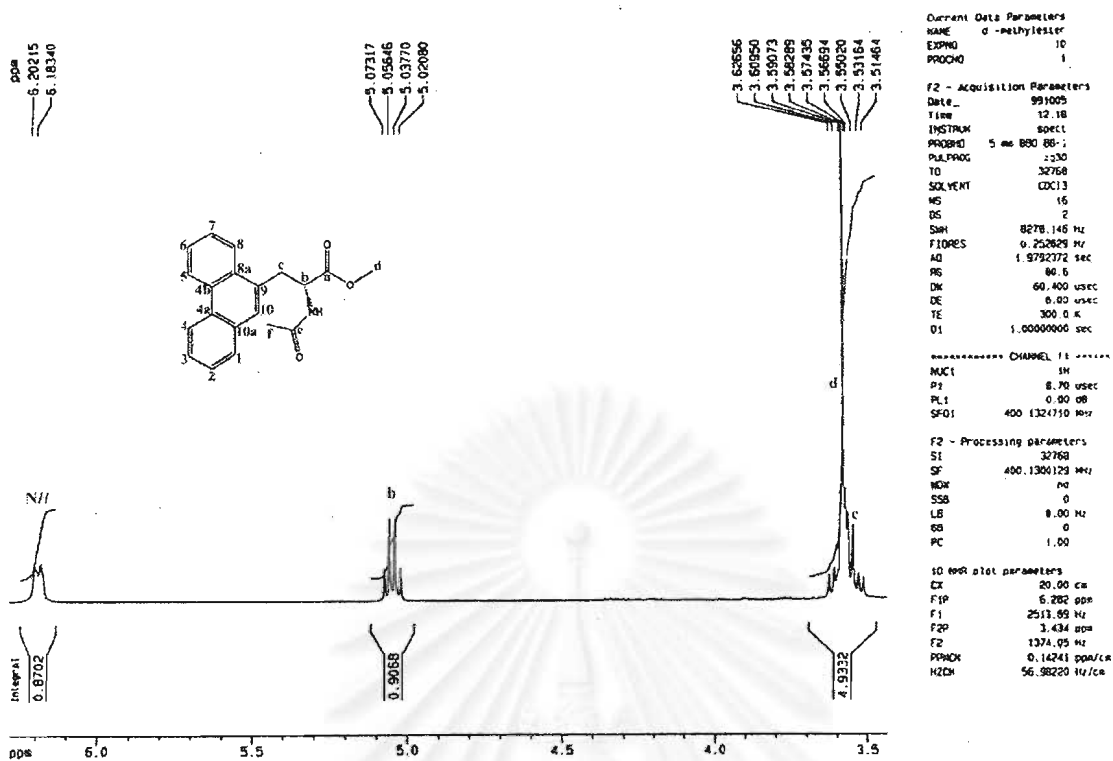
Figure F4: ^{1}H NMR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 3.4-6.3 ppm)
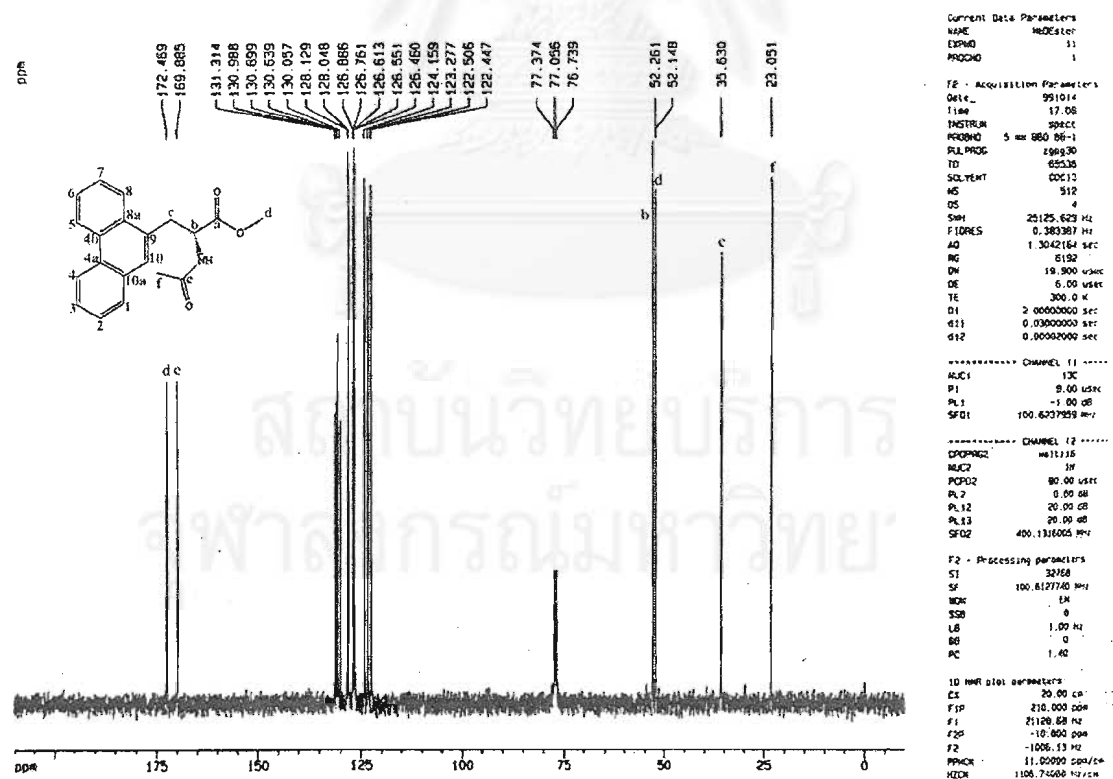
Figure F5: ^{13}C NMR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate

Figure F6: ^{13}C NMR spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 122-132 ppm)
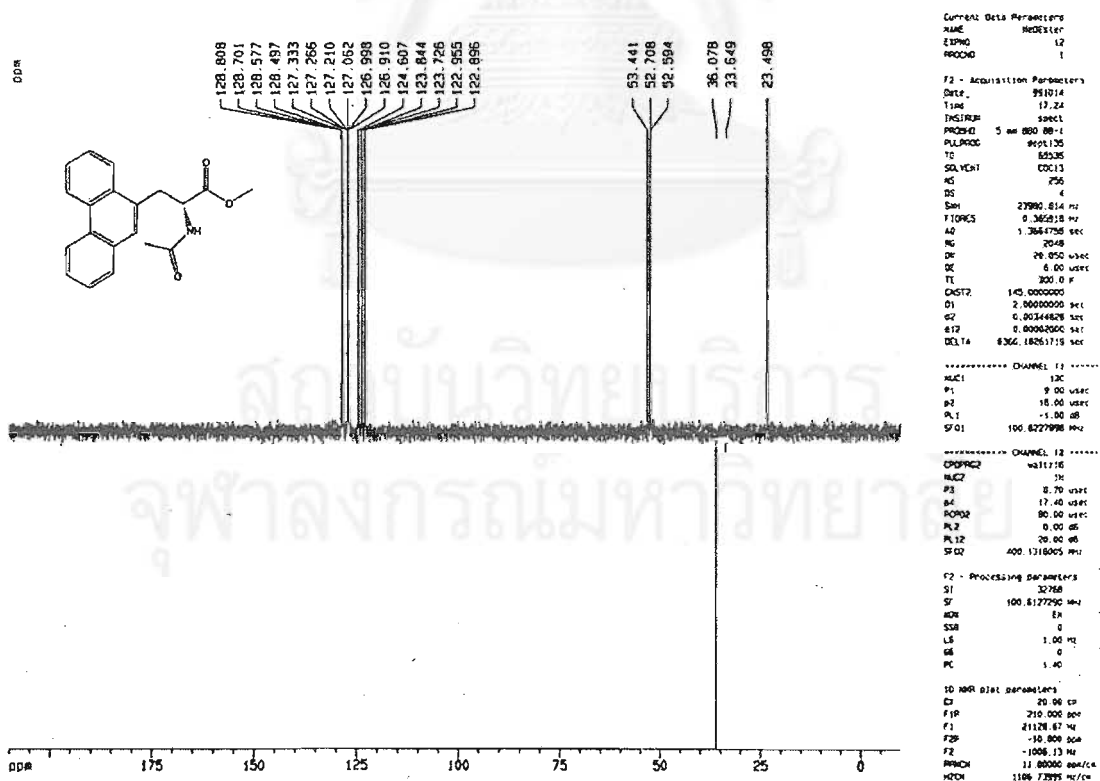
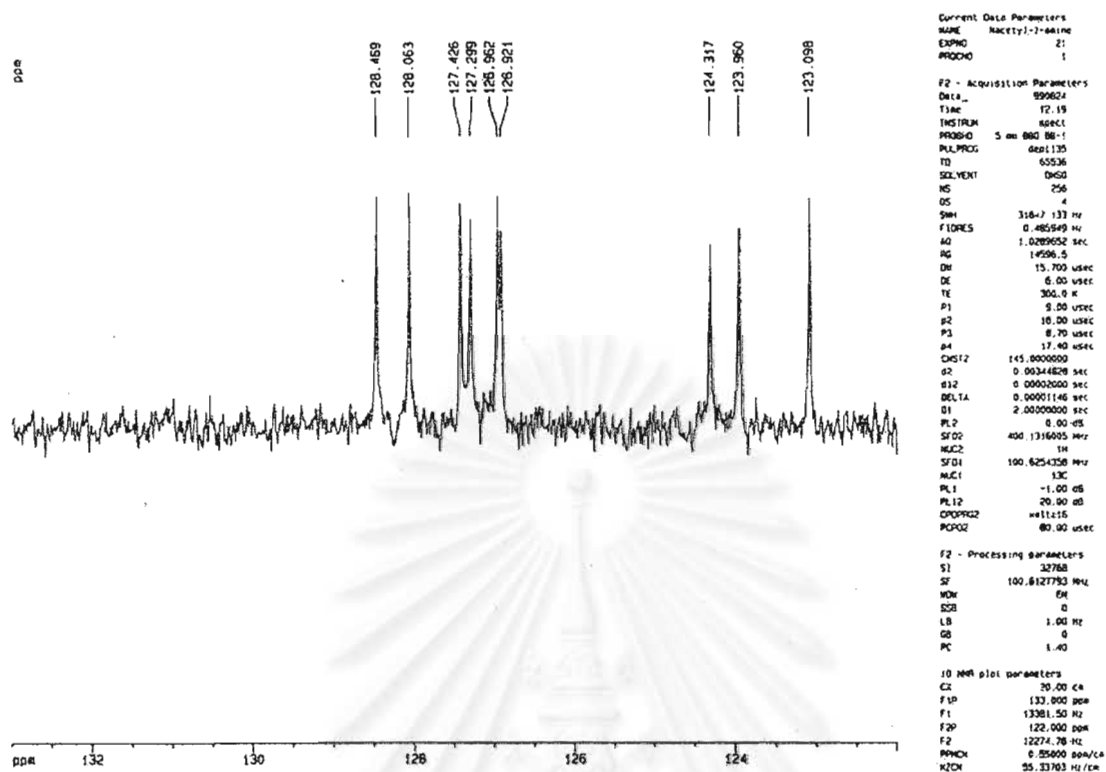**Figure F7**: DEPT-135 spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate

Figure F8: DEPT-135 spectrum of methyl (2*R*)-2-acetylamino-3-(9-phenanthrenyl) propionate (blow up at 122-132 ppm)
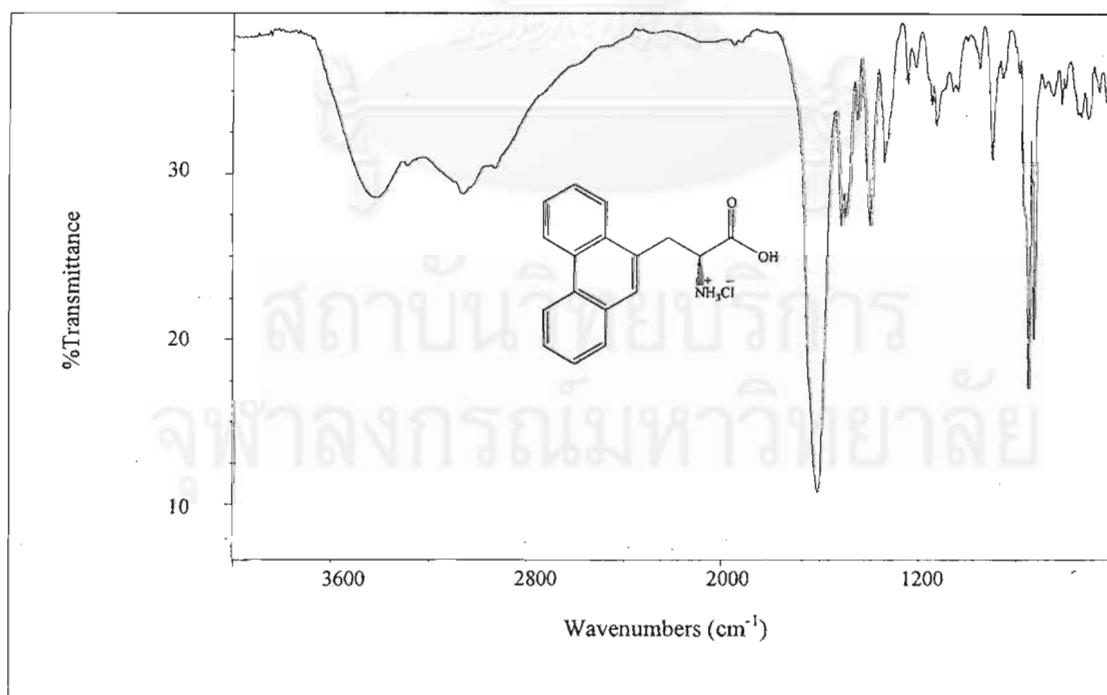

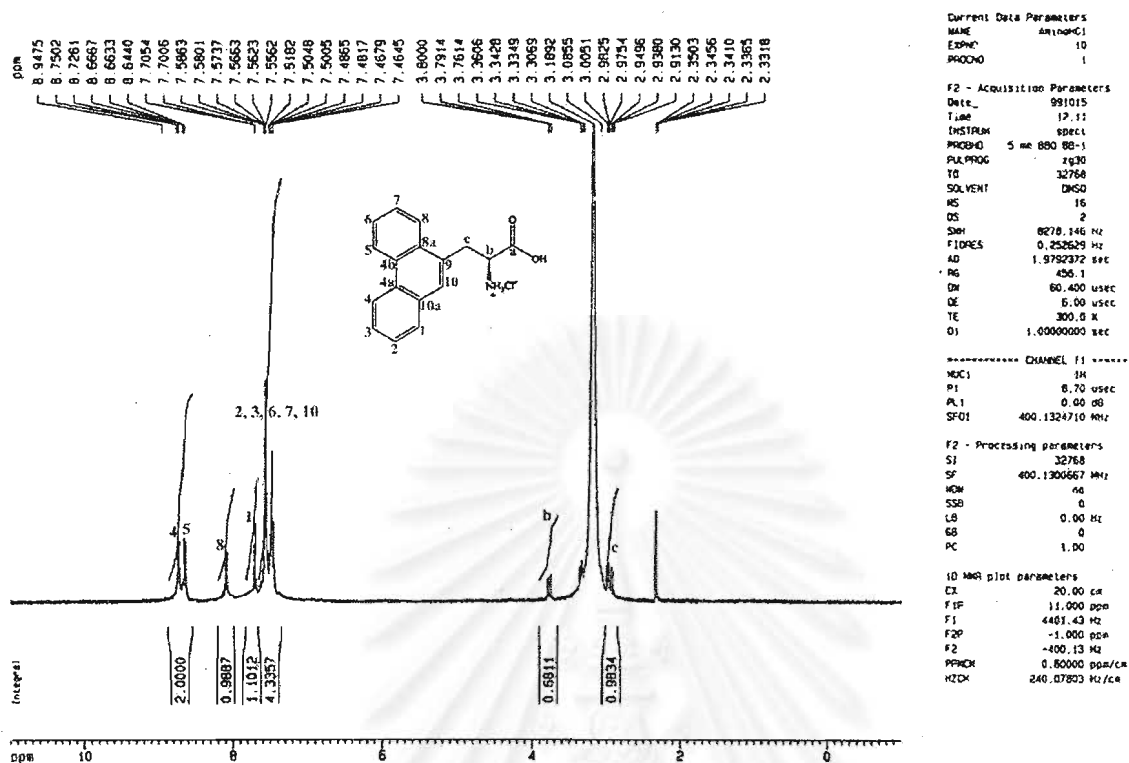**Figure G1**: FT-IR spectrum of (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid

Figure G2: ¹H NMR spectrum of (2S)-2-acetylamino-3-(9-phenanthrenyl)propionic acid
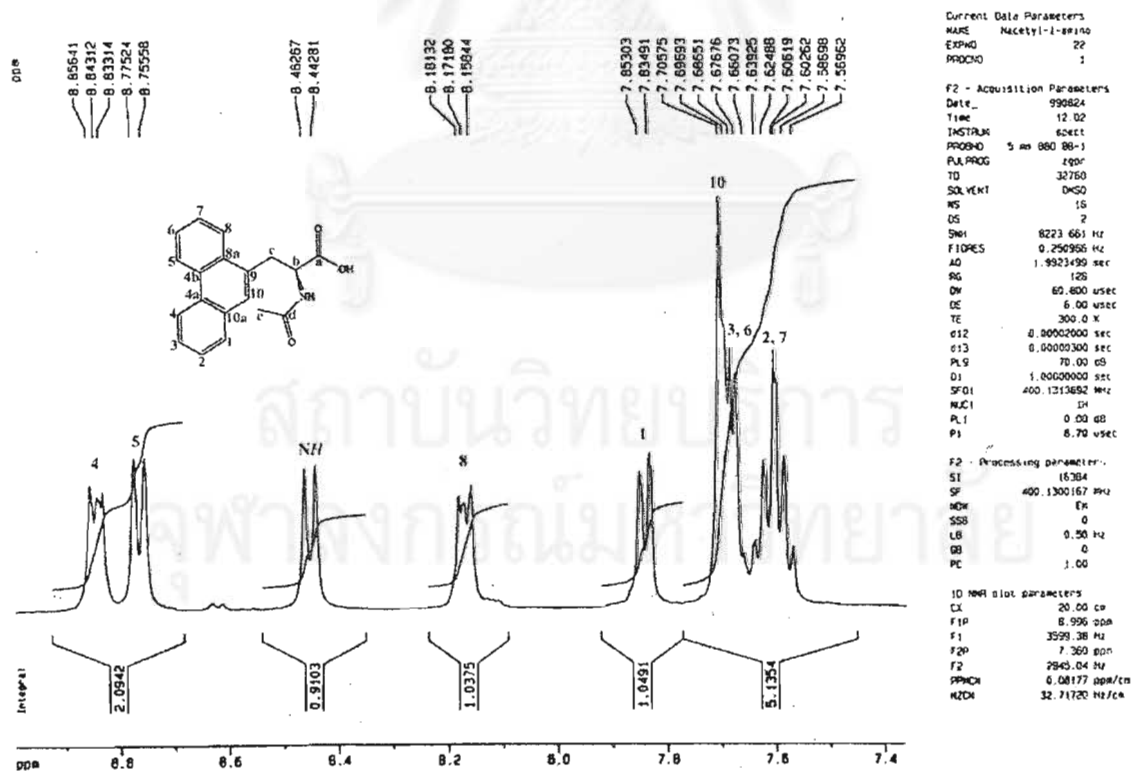
Figure G3: ¹H NMR spectrum of (2S)-2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 7.5-8.9 ppm)

Figure G4: ¹H NMR spectrum of (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 3.1-4.5 ppm)

Figure G5: ¹³C NMR spectrum of (2*S*)-2-acetylamino-3-(9-phenanthrenyl)propionic acid
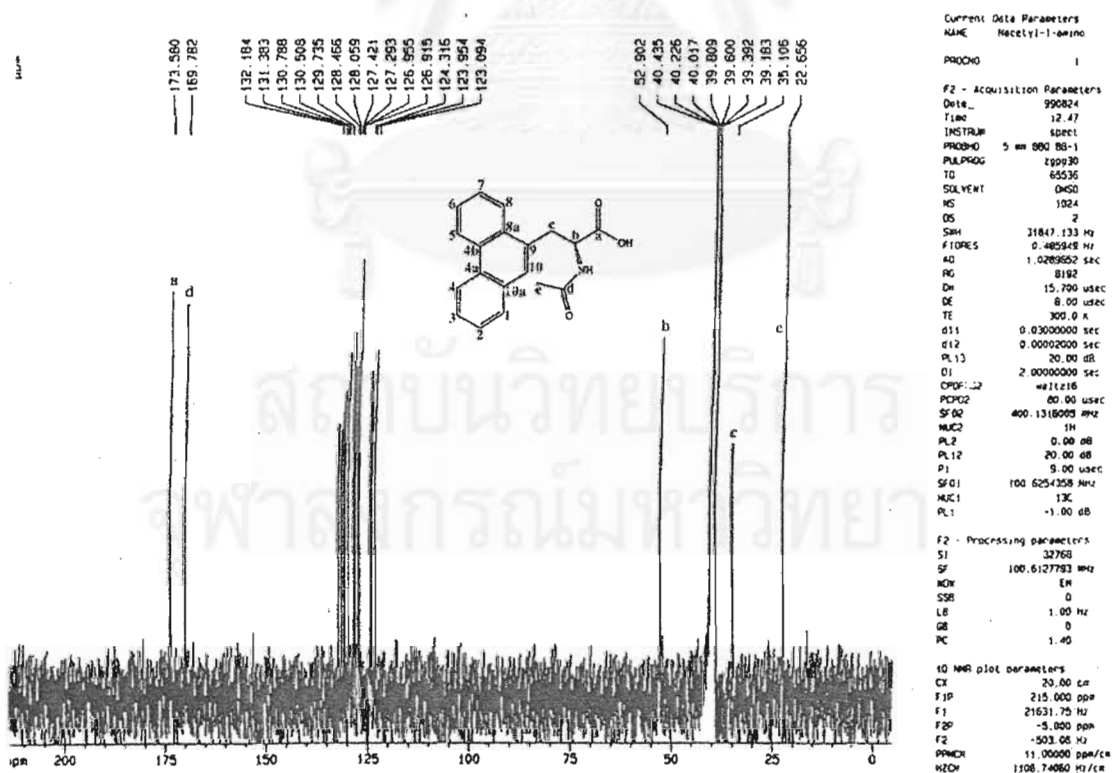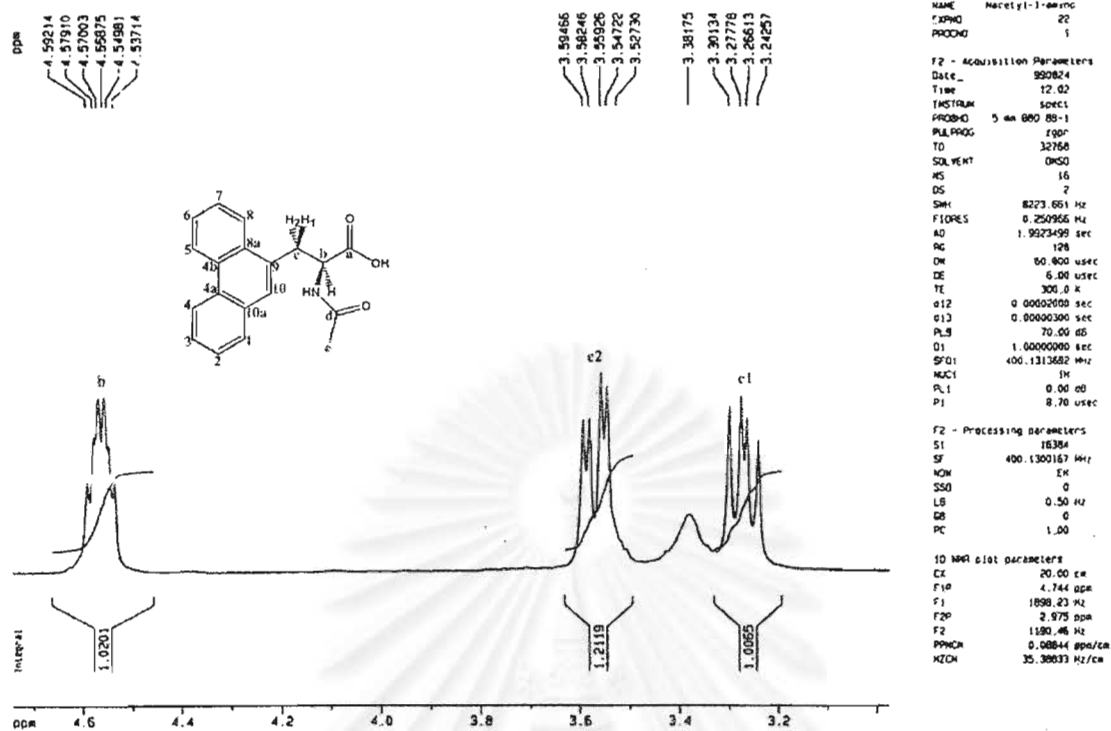
Figure G6: ^{13}C NMR spectrum of (2S)-2-acetylamino-3-(9-phenanthrenyl)propionic acid (blow up at 122-133 ppm)
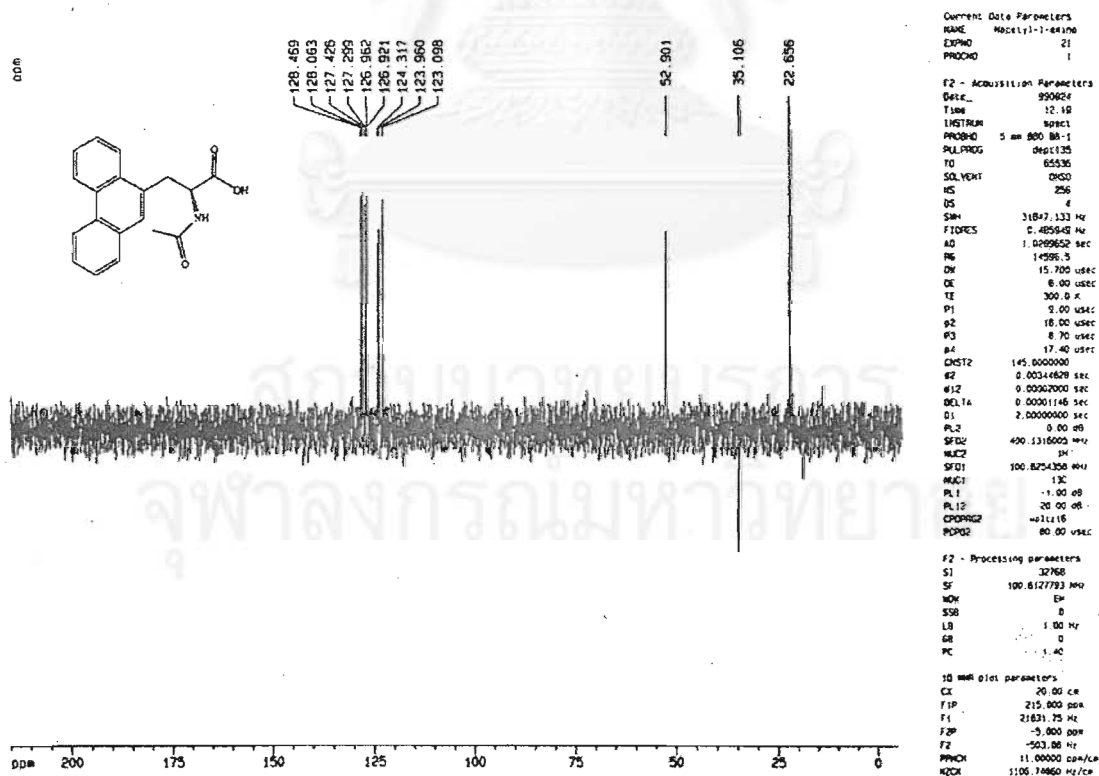
Figure G7: DEPT-135 spectrum of (2S)-2-acetylamino-3-(9-phenanthrenyl) propionic acid

Figure G8: DEPT-135 spectrum of (2*S*)-2-acetylamino-3-(9-phenanthrenyl) propionic acid (blow up at 122-133 ppm)

Figure H1: FT-IR spectrum of (2*S*)-2-amino-3-(9-phenanthrenyl)propionic acid hydrochlorid

Figure H2: ^{1}H NMR spectrum of (2S)-2-amino-3-(9-phenanthrenyl)propionic acid hydrochlorid

Figure I1: FT-IR spectrum of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid
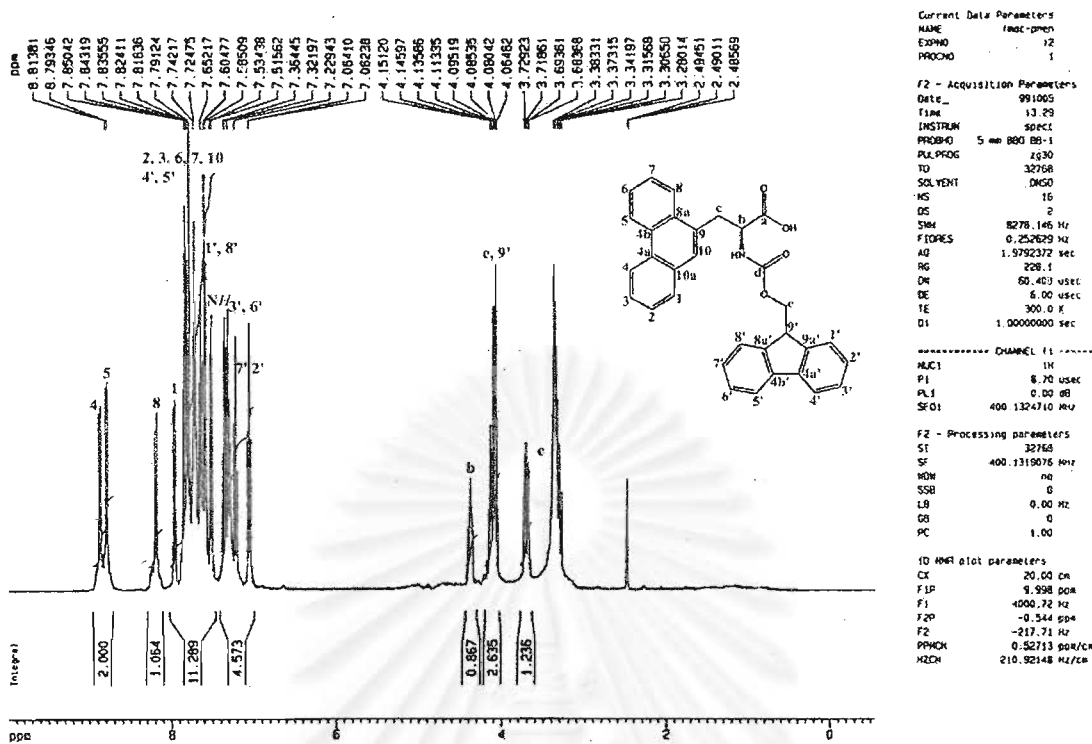
Figure I2: ^{1}H NMR spectrum of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid
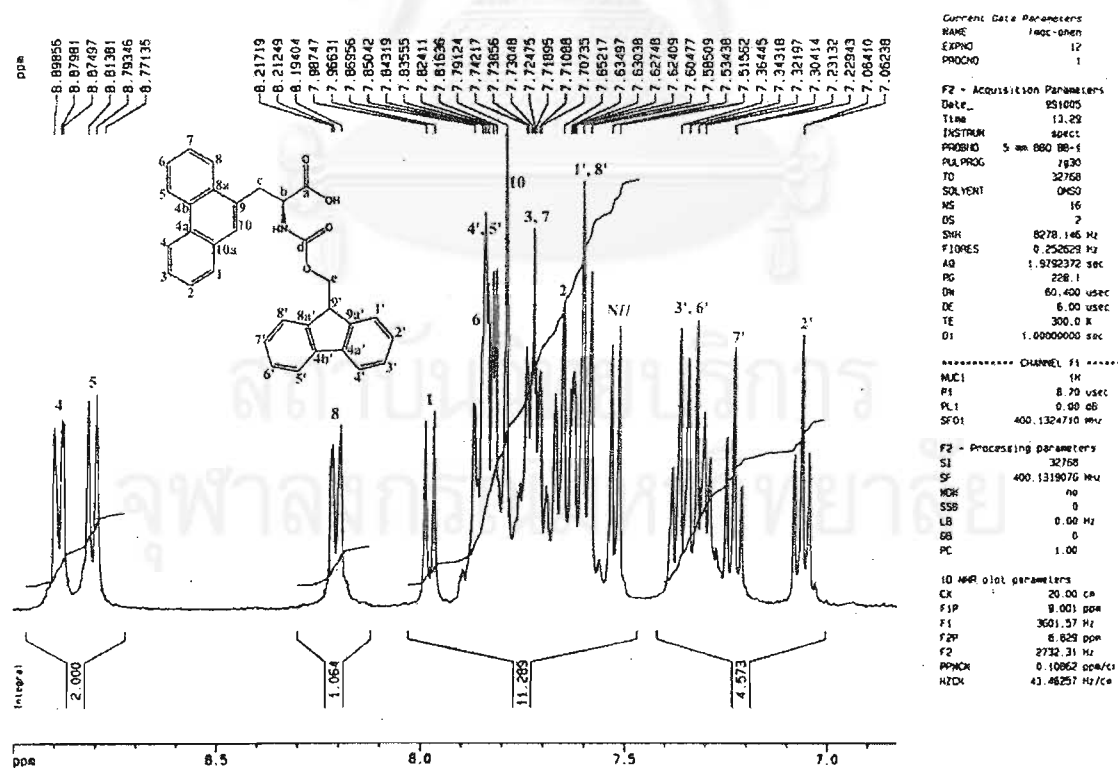
Figure I3: ^{1}H NMR spectrum of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid (blow up at 6.9-9.0 ppm)
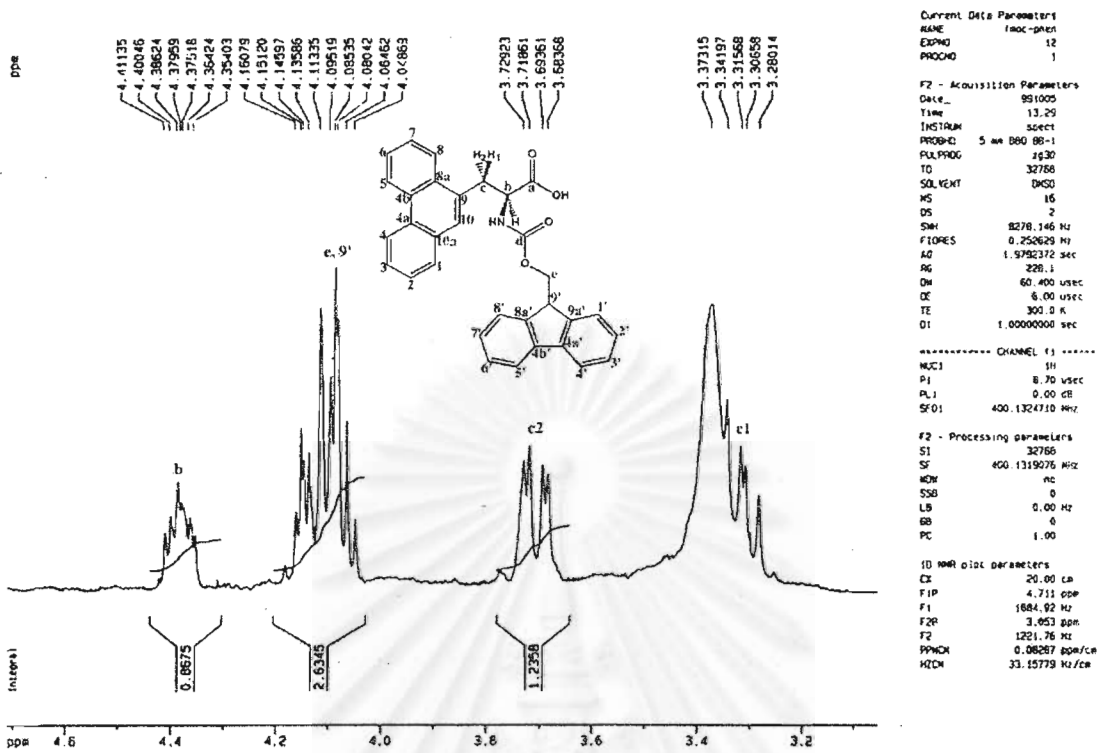
Figure I4: ^{1}H NMR spectrum of (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid (blow up at 3.2-4.5 ppm)
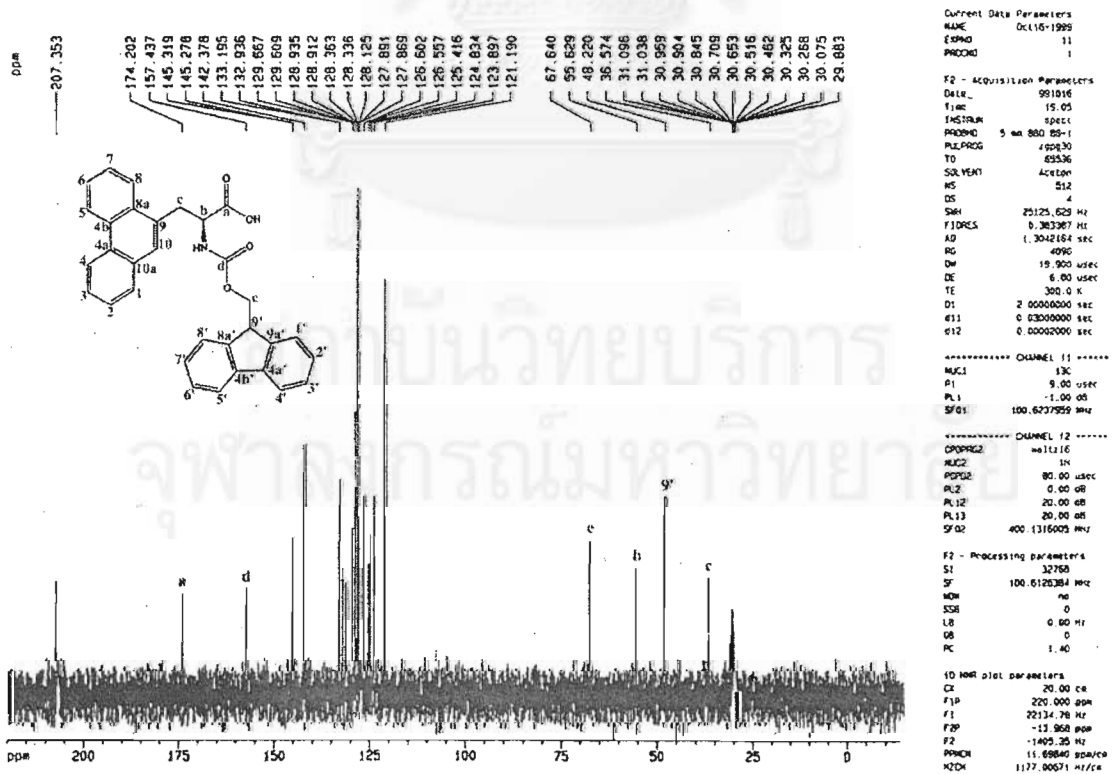

Figure I5: ^{13}C NMR spectrum of (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid
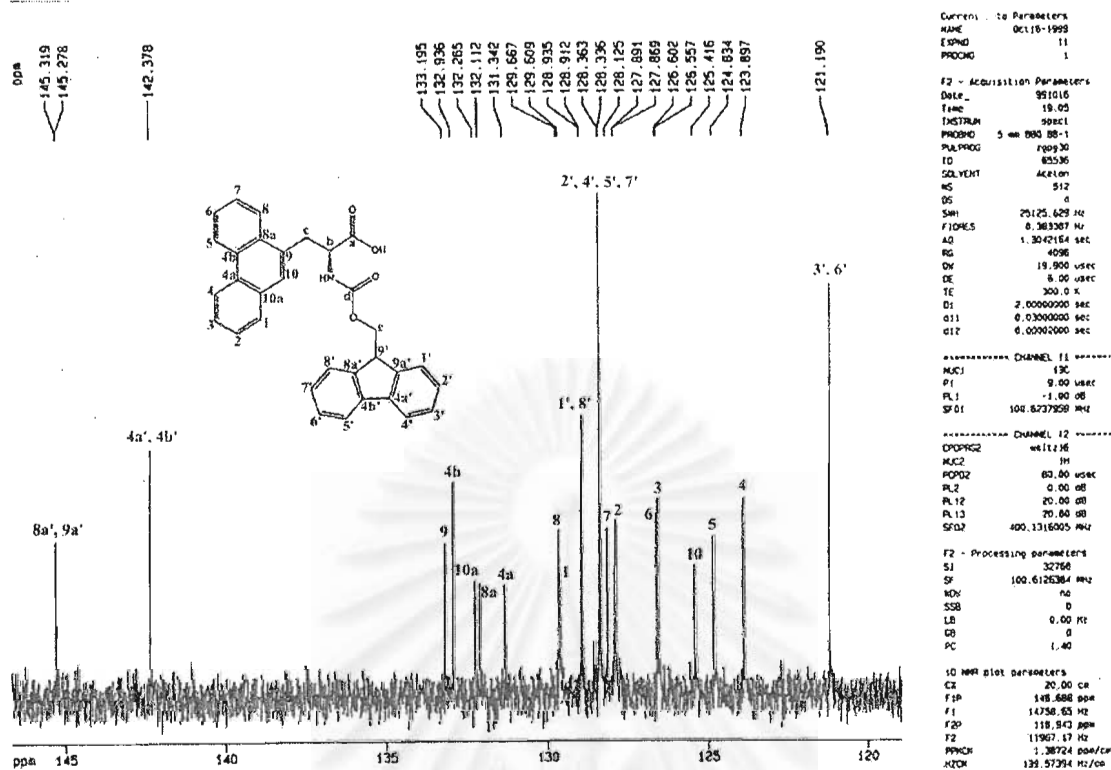
Figure I6: ^{13}C NMR spectrum of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid (blow up at 120-146 ppm)
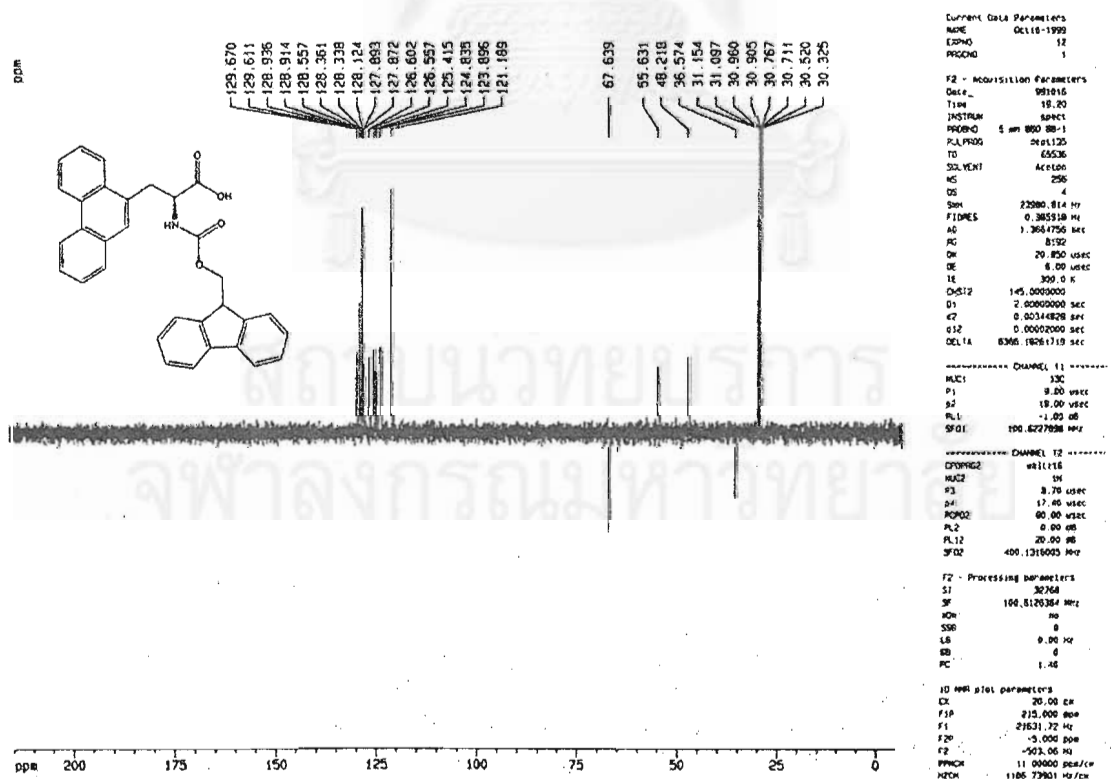

Figure I7: DEPT-135 spectrum of (2S)-2-(9H-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid
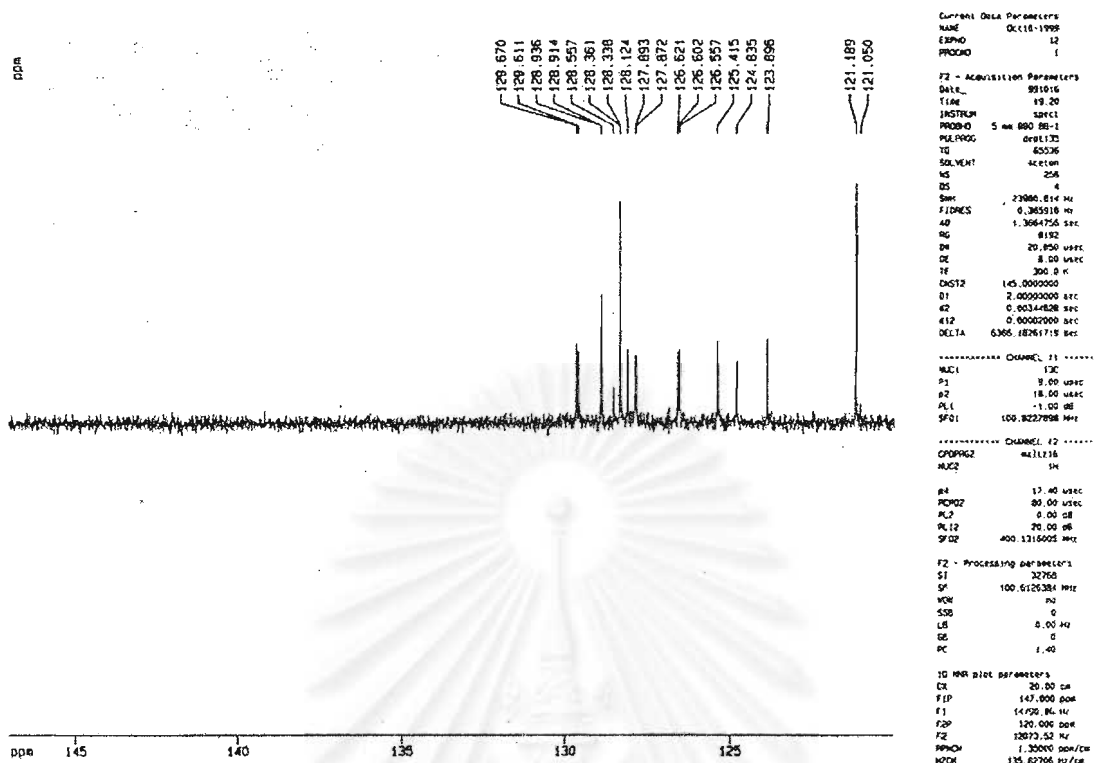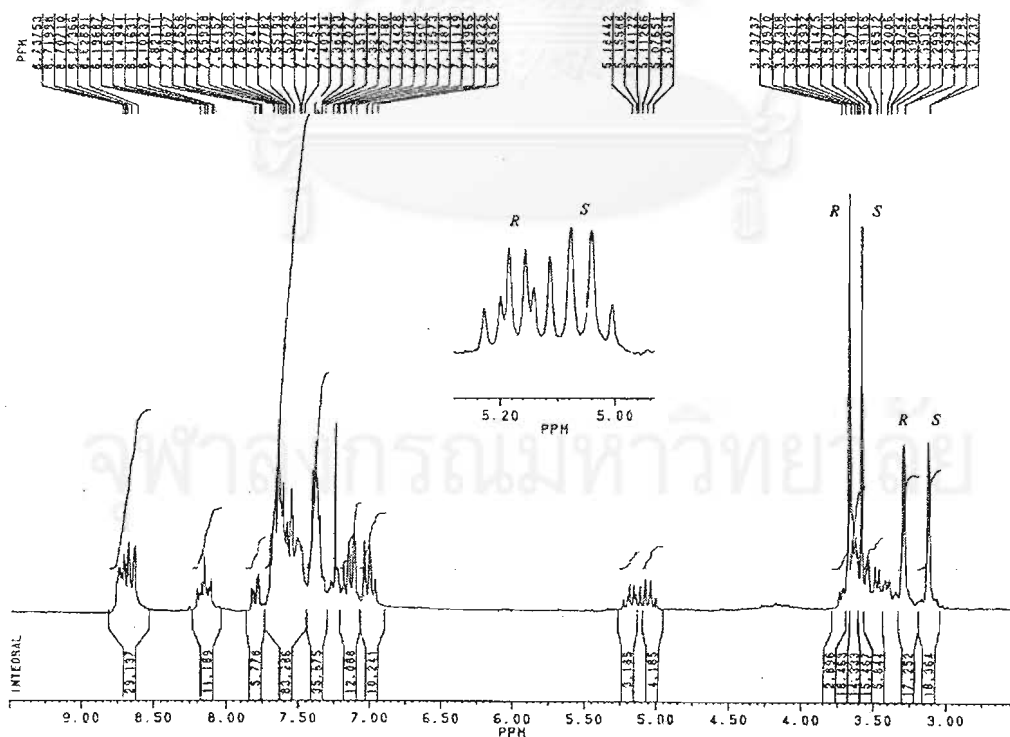
Figure I8: DEPT-135 spectrum of (2*S*)-2-(9*H*-9-fluorenylmethoxycarbonylamino)-3-(9-phenanthrenyl)propionic acid (blow up at 120-146 ppm)
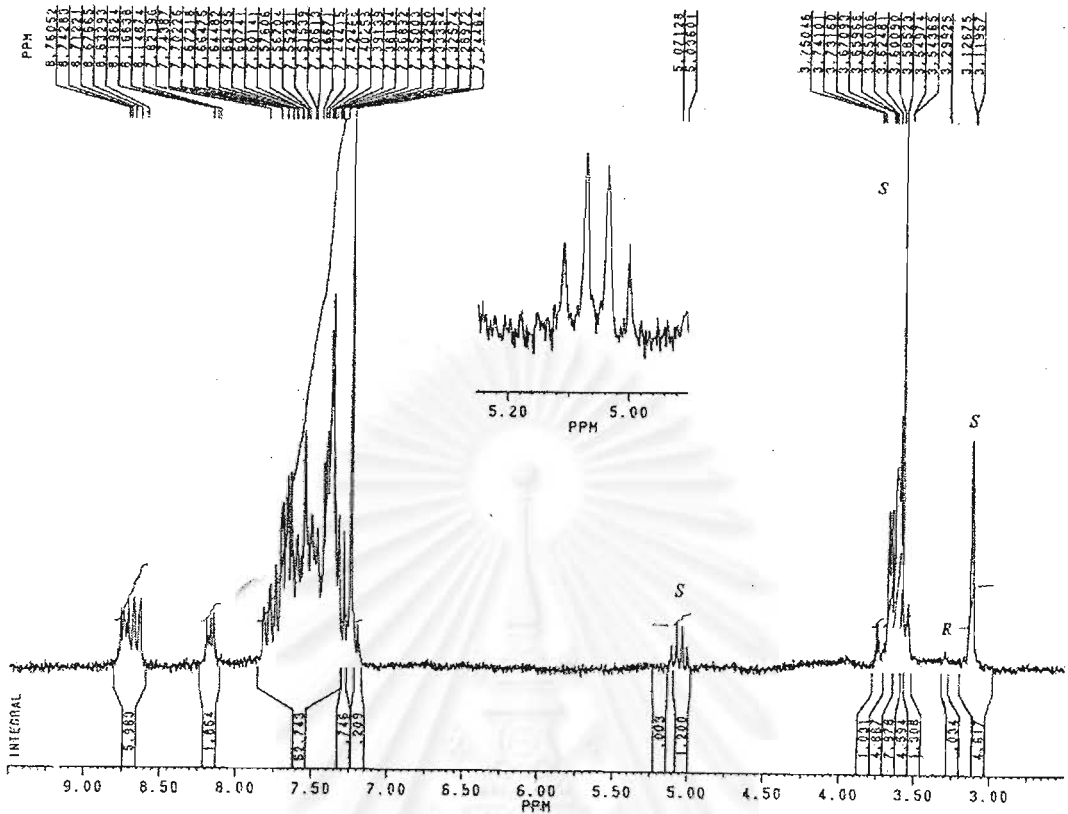

Figure J1: ¹H NMR spectrum of MTPA derivative of methyl 2-amino-3-(9-phenanthrenyl)propionate

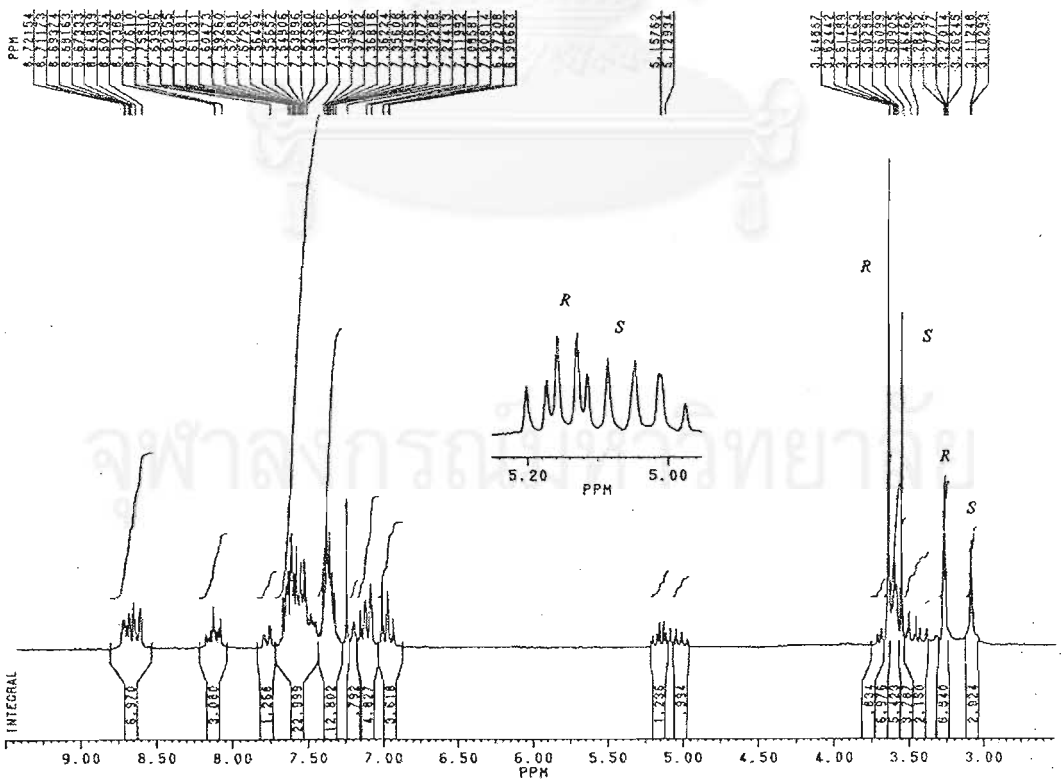**Figure J2:** ^{1}H NMR spectrum of MTPA derivative of methyl (2*S*)-2-amino-3-(9-phenanthrenyl)propionate

Figure J3: ^{1}H NMR spectrum of MTPA derivative of methyl (2*R*)-2-amino-3-(9-phenanthrenyl)propionate

VITA

Mr. Man Phewluangdee was born on March 5, 1975 in Suphan Buri. He received his Bachelor Degree of Science in Chemistry from Chulalongkorn University, in 1998. Since 1998 he has been a graduate student at the Department of Chemistry, Chulalongkorn University, studying in the field of Organic Chemistry leading to a Master of Science degree.