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Synthetic Design Of Dopa Mimetics To Inhibit Melanogenesis

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SYNTHETIC DESIGN OF DOPA MIMETICS TO INHIBIT MELANOGENESIS

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Biographical Sketch

Sharmista Prattipati was born on September 18, 1986 in Hyderabad, Andhra Pradesh, India. She received her Bachelor's degree in Pharmacy from Jawaharlal Nehru Technological University, India in 2008. She joined the Master's program in Chemistry at North Carolina Agricultural and Technical State University in the fall of 2009. She conducted research on the synthesis of 3, 4-dihroxy phenylalanine homologs for treating malignant melanomas.

Dedication

This thesis is dedicated to my husband, Karshak Kosaraju, my parents, Chandrababu Prattipati and Sridevi Prattipati, and my brother, Ranadhir Prattipati, for their love, support and encouragement.

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List of Schemes

Nomenclature

CD	Cysteinyl dopa
DBH	Dopamine β-hydroxylase
DEAM	Diethyl acetamidomalonate
DHICA	Dihydroxyindole carboxylic acid
DHI	5, 6-dihydroxy indole
DOPA	3, 4-dihydroxy phenylalanine
DMSO	Dimethyl solfoxide
DQ	Dopaquinone
FTIR	Fourier transform infrared spectroscopy
GC/MS	Gas chromatography/Mass spectrometry
NMR	Nuclear magnetic resonance
PMT	Phenolethanolamine-N-methyl transferase
Tyr 1	Tyrosinase related protein-1
Tyr 2	Tyrosinase related protein-2
Tyr-ase	Tyrosinase
UV	Ultraviolet

Abstract

The primary objective of this research involves the synthetic design of homologs of 3, 4-dihydroxyphenylalanine (dopa) in order to inhibit melanin formation, melanogenesis. These synthetic homologs are structured to have variations within their side chains and unto their aromatic ring when compared to the structure of dopa. Strategic structural variations are incorporated within the molecular framework of the homologs such that their dopa mimicking features are enhanced, and consequently inhibit the formation of melanins. The structural variations of the homologs are designed to exhibit specificity in the regulations of phase-I and phase –II of melanogenesis. Many studies have suggested a strong correlation between enhanced rates of melanin formation and many forms of malignant melanomas (e.g., skin cancers). In this work, a variety of dopa precursors and dopa homologs were successfully synthesized. The synthetic methods developed for the preparation of the dopa homologs were also designed for efficient entry into a variety of new amino acids and precursors. Structural validation of dopa and precursors were aided by the use of a variety of instrumentations; inclusive of GC/MS, FTIR and NMR. The ultimate success of this work is expected to lead to the development of a more comprehensive profile between strategic molecular designs and melanin inhibition.

CHAPTER 1

Introduction

Melanin, a redox biopolymer pigment plays an important role in the pigmentation of skin in mammals (Sulaimon & Kitchell, 2003). Melanin is produced in melanocytes and catecholaminergic neurons by a process called melanogenesis (Solano, Hearing, & García-Borrón, 1999). Melanocytes are the type of cells present in the skin, eyes, hair, ear, mucous membranes and in the central nervous system (Jimbow, 1998). Melanin in melanocytes is synthesized within highly specialized membrane bound intracellular organelles called melanosomes. In the epidermis of the skin, melanocytes deliver melanin to keratinocytes providing skin pigmentation, hair and feather coloration (Sulaimon & Kitchell, 2003). Skin complexion is related to the density of the melanocytes, the number, size and dispersion of melanosomes, the nature of the pigment and its degradation rate. Melanosomes in darker individuals are known to be larger in size (Solano, et al., 1999).

Mammalian melanin is composed of two pigments, eumelanin and pheomelanin respectively (Ito, 2003). Eumelanins are dark brown to black in color and are characterized as insoluble nitrogenous pigments responsible for skin coloration (Potterf et al., 1999). Eumelanin consists of 5, 6- dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid units in reduced or oxidized states (Ito, 2003). Pheomelanin is yellow to red colored alkali soluble chromophore responsible for feather coloration and some shades of hair (Potterf, et al., 1999). Pheomelanin mainly consists of benzothiazole units (Ito, 2003). The interaction between eumelanins and pheomelanins gives rise to the most common type of melanins called mixed melanins (Park et al., 2004).

Melanogenesis is a biochemical pathway for the melanin production that occurs in melanosomes (Sulaimon & Kitchell, 2003). During the development of melanin, melanosomes acquire three metallozymes in its production. These metallozymes are tyrosinase (tyr-ase), tyrosinase-related protein 1 (Tyr 1) and tyrosinase-related protein 2 (Tyr 2) (Hartmeyer et al., 1997). Of the three enzymes, tyr-ase plays the most critical role in melanogenesis (Tsukamoto, Jackson, Urabe, Montague, & Hearing, 1992).

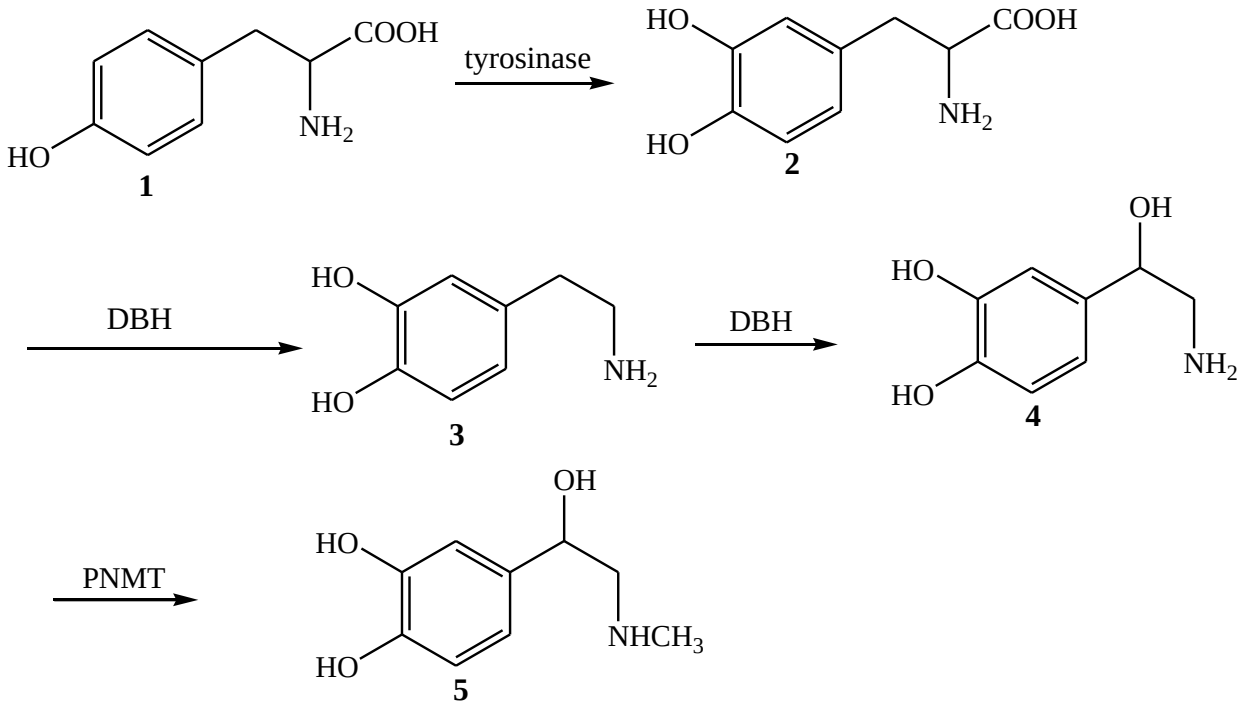
Tyrosinase is a bifunctional copper containing glycoprotein composed of 511 amino acids with a molecular mass of 60-70 kDa (Nishioka, 1978). The synthesis of tyr-ase occurs in the ribosome en route to the golgi complex, and is subsequently delivered to the melanosomes (V. J. Hearing & Ekel). Tyrosinase consists of an inner domain that resides inside the melanosome, a transmembrane domain and a cytoplasmic domain (Vincent J. Hearing & JimÉNez, 1989). The cytoplasmic domain facilitates tyr-ase transport from the ribosome to the golgi complex (Vijayasaradhi, Xu, Bouchard, & Houghton, 1995).

1.1 Biosynthesis of catecholamine neurotransmitters

The diphenol amino acid, L-dihydroxyphenylalanine (L-dopa) is a significant intermediate in the biological synthesis of three catecholaminergic neurotransmitters in neurons (Solano, et al., 1999). Dopa **2** is generated from tyrosine **1** by the action of tyr-ase. Dopamine, **3** an essential neurotransmitter is formed by the metabolic breakdown of L-dopa in the presence of dopa β decarboxylase (DBH, Scheme 1.1). The deficiency of dopamine in the human brain leads to Parkinson's disease. Norepinephrine, **4** is an important intermediate involved in the catecholamines biosynthesis and is formed by the action of DBH on dopamine (Scheme 1.1). Epinephrine **5,** a catecholamine is formed by the action of phenol ethanolamine-N-methyl transferase (PMT) on norepinephrine (White, Handler, & Smith, 1964).

Scheme 1.1. Biosynthesis of catecholamine transmitters.

(http://www.ncbi.nlm.nih.gov/books/NBK27988/)



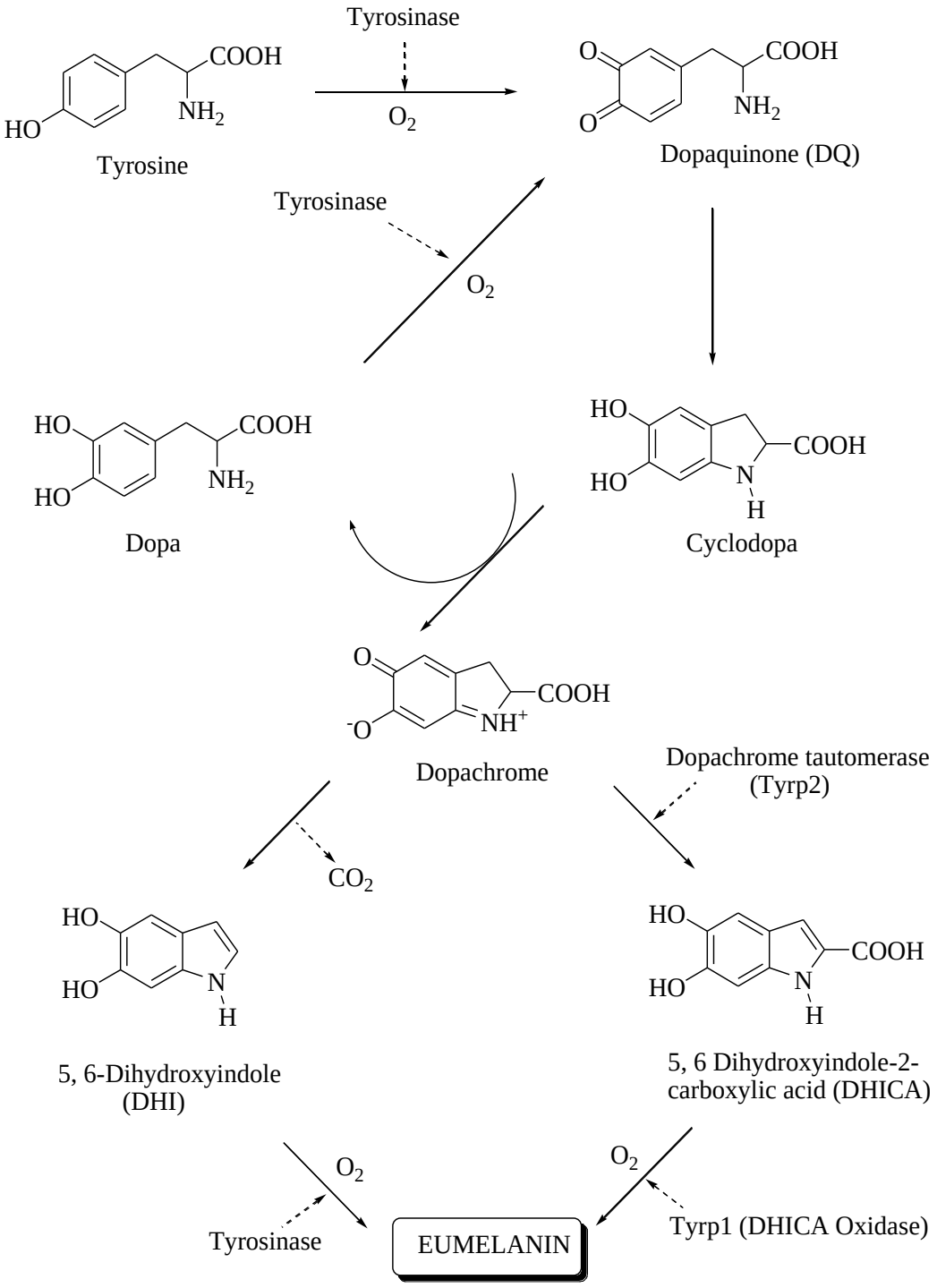
1.2 Biosynthetic pathway of melanin

In melanogenesis, tyr-ase catalyzes the first two rate determining steps in the biological process (Scheme 1.2). The metabolic precursor of melanin, tyrosine is hydroxylated to form dopa (Furumura, Sakai, Abdel-Malek, Barsh, & Hearing, 1996). Subsequently, dopa oxidizes to form dopaquinone (DQ); which is then processed into either eumelanin or pheomelanin (Slominski, Zmijewski, & Pawelek, 2012).

1.2.1 Pathway of eumelanogenesis. During eumelanogenesis in the presence of tyr-ase, the amino group of DQ undergoes an intramolecular 1, 4-addition to give rise to the leukodopachrome (Scheme 1.2, Park, et al., 2004). The rearrangement of leukodopachrome catalyzed by Trp 2 produces DHI as a major product and DHICA as a minor product

(Tsukamoto, et al., 1992). Finally, the dihydroxyindoles are oxidized to form eumelanin (Ito, 2003). The velocity of the process depends on tyr-ase related proteins (Slominski, et al., 2012).

Scheme 1.2. Shosuke Ito, pathway of eumelanogenesis (Ito, 2003).

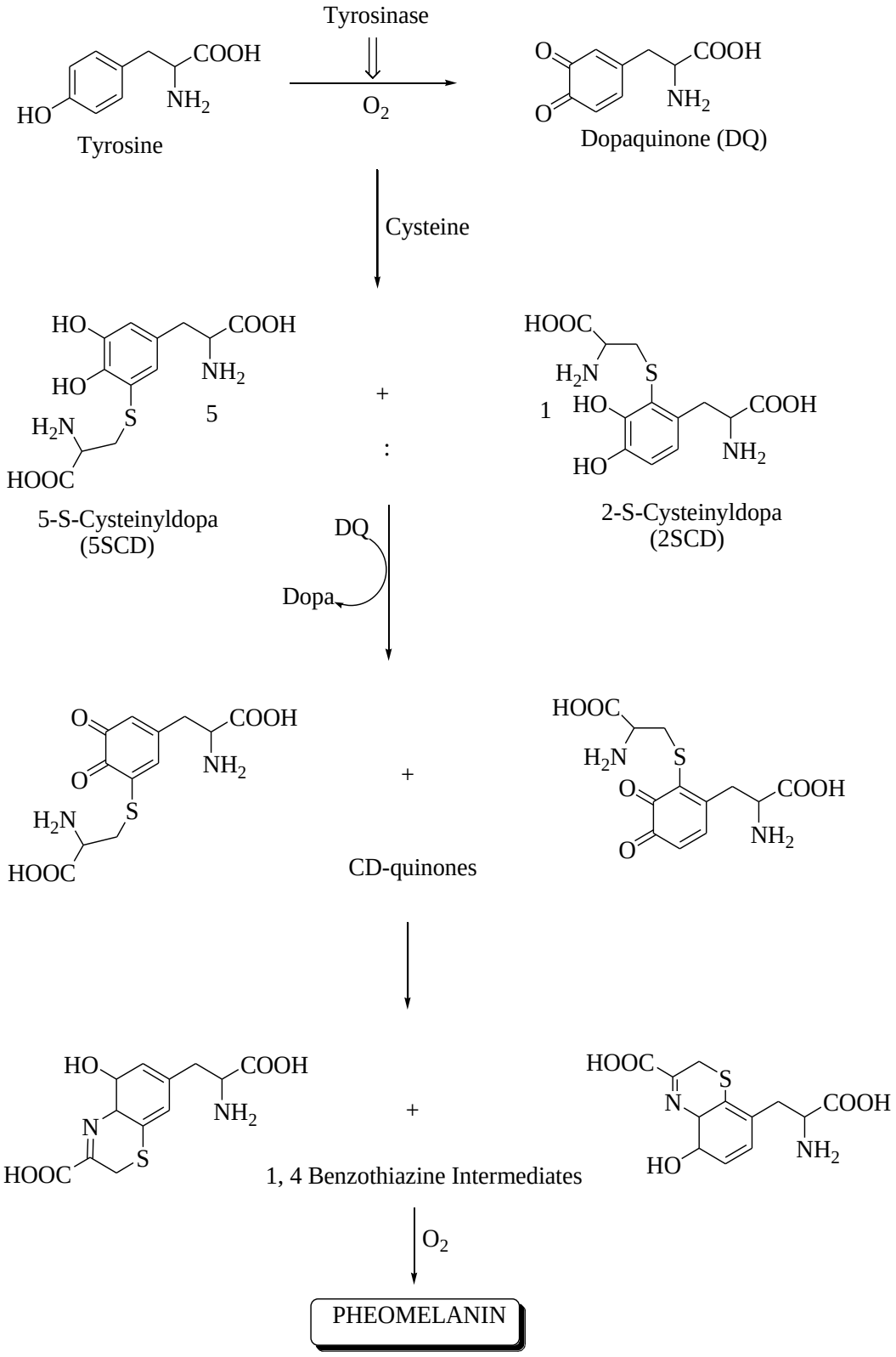


1.2.2 Pathway of pheomelanogenesis. The pheomelanins are present in certain types of reddish hair and feathers. Pheomelanins usually occur together with biogenetically related trichochromes. The trichochromes are commonly found in feathers and red human hair. Trichochromes are of four types, B, C, E and F respectively. Trichochromes B and C are yellow to orange colored, and E and F trichochromes are violet. The basic structural unit of trichochromes consists of a 1, 4 benzothiazine ring system existing in 2H and 4H tautomeric forms.

Pheomelanins are zwitterionic in nature, soluble in both dilute alkali and in certain acidic solvents. This character is due to the presence of alanine side chains that form the backbone of the pigment by attaching to the subunits. The general structure of pheomelanin is not clearly known. They appear to be irregular polymers or a mixture of polymers lacking well defined spectral and physical characteristics (Prota, 1992).

During the process of pheomelanogenesis, the thiol group of cysteine covalently attaches to the DQ to form cysteinyl dopa (CD, Scheme 1.3). In the presence of tyr-ase, cysteine may covalently incorporate at the 2 or 5 ring positions of DQ although the 5-CD is favored as a key intermediate in the biosynthesis of pheomelanin (Prota, 1992). The compound, 5-CD may be involved in the oxidative ring closure of the cysteine side chain leading to the formation of an unstable o-quinone-imine intermediate in significantly less yields. The quinone intermediate may give rise to dihydrobenzothiazine or 1, 4 benzothiazine. The CD is oxidized to form the CD-quinone, which upon cyclization leads to 1, 4 benzothiazine. The intermediate 1, 4 benzothiazine undergoes oxidation to form pheomelanin (Park, et al., 2004).

Scheme 1.3. John Simon's pathway of pheomelanogenesis (Ito, 2003).



1.3 Tyrosinase in the biosynthesis of melanin

Melanin plays a crucial role in the absorption of free radicals generated within the cytoplasm and in protecting the human skin from the harmful effects of solar UV radiation (Prota, 1992). The increased rate of tyrosine hydroxylation and dopa oxidation leads to increased melanin formation. Although melanin has a photo-protective function, the accumulation of abnormal melanin in the skin can cause hyperpigmentation disorders such as melanoma, freckles, melasma, ephelides, senile lentigo and age spots (Park, et al., 2004). Hence, the knowledge of melanocyte biology and the process underlying melanin synthesis had made remarkable progress over the few years (Prota, 1992).

The major cause of melanoma results from exposure to ultraviolet (UV) radiation. There are three types of UV radiations. For example, UV-A represents wavelength ranging from 400 nm to 315 nm, UV-B from 315 nm-280 nm and UV-C ranging from 100-200 nm. Of these radiations, UV-B is dangerous and directly damages the DNA; whereas UV-A targets cellular photo sensitizers (V. J. Hearing & Tsukamoto, 1991).

The enzyme, tyr-ase contains three binding sites; which include two active sites and one allosteric site. The active sites are where chemical conversion take place and tyrosine and dopa bind. The L form of dopa not only binds to the active or the substrate site, but also to the allosteric or the cofactor site. Since L-dopa can bind at both sites, studies have shown that the addition of external L-dopa increases the levels of tyrosine hydroxylation and L-dopa oxidation which subsequently leads to the enhancement of melanogenesis (Chang, 2009). As try-ase is an essential enzyme in the regulation of melanogenesis, this enzyme becomes a prime substrate/cofactor target for a variety of exogenous molecules in the regulation of melanogenesis

(Sander, Hamm, Elsner, & Thiele, 2003). There are three inhibitory mechanisms associated with the enzyme, tyr-ase. Of these inhibitory mechanisms, competitive inhibition is the process where the compound binds to the free enzyme and thus inhibits the binding of endogenous substrate to the enzyme. Non-competitive inhibition is the binding of compound to the enzyme-substrate complex and thus inhibiting the formation of melanin. A mixed type of inhibition is where the compound acts as both competitive and non-competitive inhibitor (Prota, 1992).

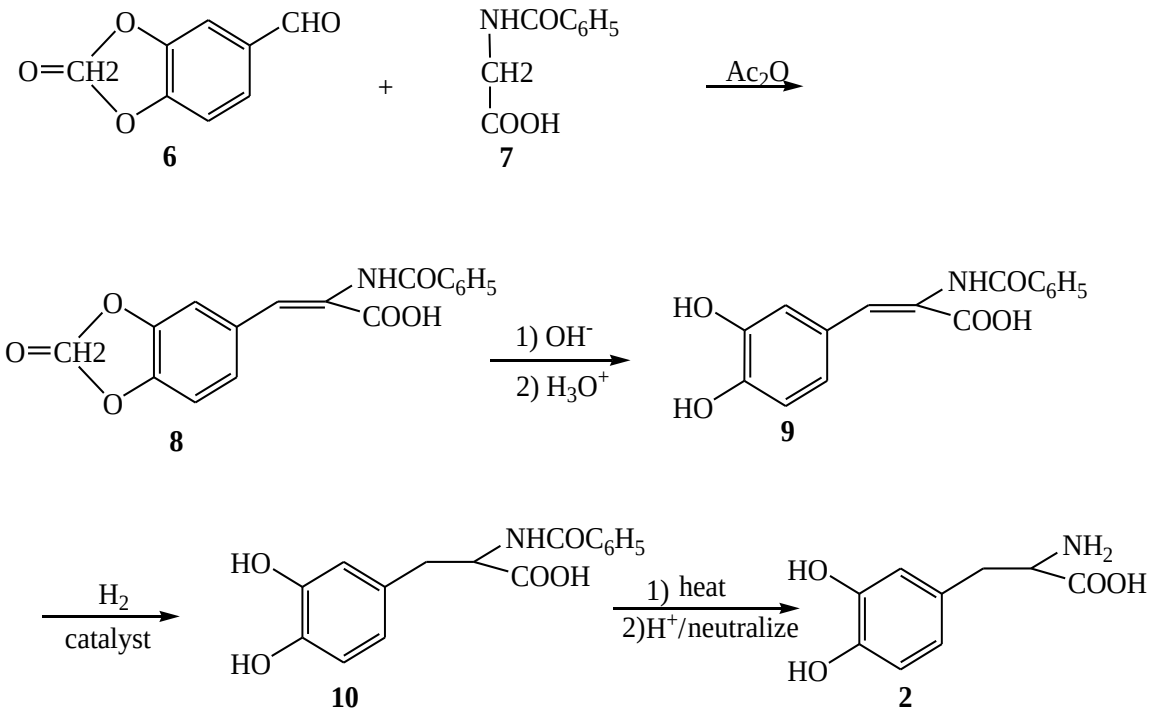
CHAPTER 2

Literature Review

2.1 Modified Erlenmeyer synthesis of dopa via azalactone

Various methods are available for the synthesis of α-amino acids, as the amino acids can act as homologs of dopa to be utilized as therapeutic molecules in the regulation of melanogenesis. Casimir Funk developed a modified Erlenmeyer synthesis of dopa by protecting the catechol group (Scheme 2.1). The compound carbonyldihydroxybenzaldehyde, **6** was subjected to condensation with hippuric acid **7** to yield azalactone **8**. The product, **8** was subjected to hydrolysis to give benzamido-3, 4-dihydroxycinnamic acid **9** which upon treating with hydrogen in the presence of a catalyst gave benzoyl-3, 4-dihydroxyphenylalanine **10**. The final product, dopa **2**, was obtained by refluxing **10** with aqueous hydrochloric acid (HCl) and then neutralizing with sodium hydroxide; NaOH (Funk, 1911).

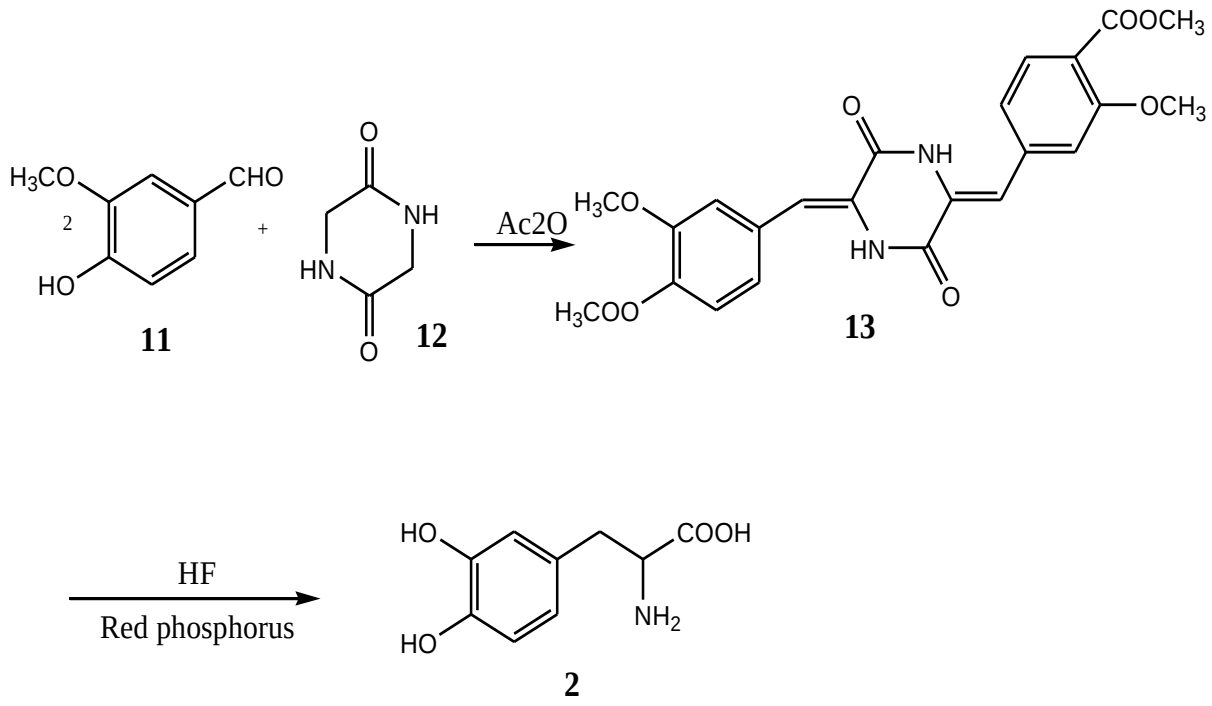
Scheme 2.1. Casimir Funk's Erlenmeyer synthesis of dopa (Funk, 1911).



2.2 Modified Erlenmeyer synthesis of dopa via vanillin

The Erlenmeyer syntheses of the racemic mixture of dopa lead to valuable procedures in the synthesis of dopa and its homologs (Harington & Randall, 1931). Hirai synthesized racemic dopa by condensing vanillin **11** with acetic anhydride (Scheme 2.2). The acetic anhydride was used to condense the cyclic diamide **12** with aldehyde **11** to yield adduct **13** by replacing the hydroxyl group with O-acetyl group. The resulting mixture containing the adduct **13** was refluxed with concentrated HF in the presence of red phosphorus to give rise to dopa (Waser & Lewandowski, 1921).

Scheme 2.2. Hirai's synthesis of dopa (Waser & Lewandowski, 1921).

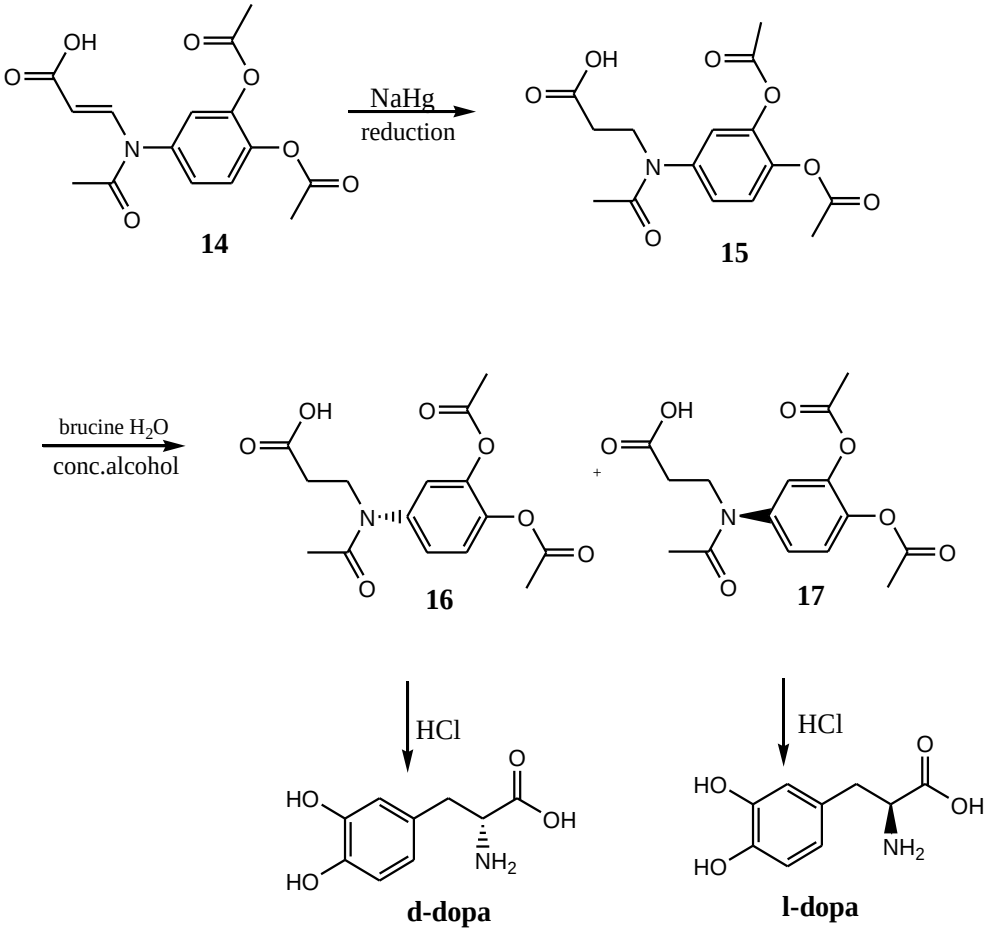


2.3 Synthesis of d and l forms of dopa

Stephen and Weizmann used an alternating method by utilizing alkylated sodio pthalimidomalonate with methylenedioxybenzylbromide to synthesize a racemic mixture of dopa (Stephen & Weizmann, 1914). In 1913, Guggenheim was the first to isolate L-dopa from a plant

called Vicia faba (Harington & Randall, 1931).In 1931, Harrington and Randall synthesized d and l forms of dopa separately from *dl*-β-3, 4-diacetoxyphenyl-α-acetaminoacrylic acid, **11** (Scheme 2.3). Reduction of compound **14** with sodium amalgam gave *dl*-β-3, 4-diacetoxyphenyl-α-acetaminopropionic acid **15**. The resulting product, **15** was treated with one equivalent of hydrated brucine (alkaloid) in concentrated alcohol; which resulted in crystallization. The crystallized species, the salt of brucine *l*-β-3, 4-dihydroxyphenyl-α-acetaminopropionate **17** was separated, and the filtrate was concentrated under reduced pressure to yield brucine *d*-β-3, 4-dihydroxyphenyl-α-acetaminopropionate **16**. The resulting products **17** and **16**, were hydrolyzed with hydrochloric acid to give d-dopa and l-dopa respectively (Harington & Randall, 1931).

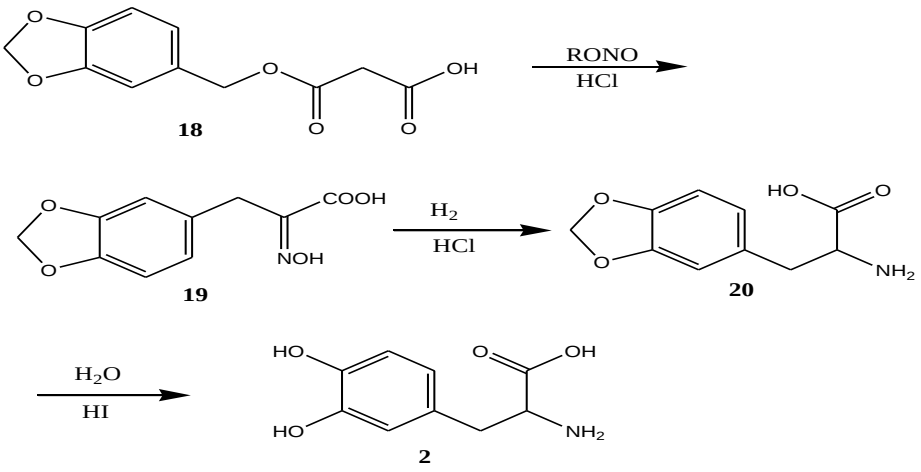
Scheme 2.3. Harrington and Randall's synthesis of dopa (Harington & Randall, 1931).



2.4 Synthesis of dopa via α oxamino acid

Throughout the nineteenth century, a host of methods have been developed and applied toward the synthesis of dl-dopa (Stephen & Weizmann, 1914). Hamlin proved that α-amino acids can be prepared by the reduction of α-oxamino acids (Barry, Mattocks, & Hartung, 1948). In 1948, Richard et. al were quite successful in synthesizing a variety of intermediates enroute to the production of dopa (Scheme 2.4). These intermediates consist of α-oxamino acids from acetoacetic and malonic esters. The nitrosation reaction of 3, 4-methylenedioxybenzyl malonic acid **18** with alkyl nitrite in the presence of HCl gave α-oxamino-β-(3, 4-methylenedioxyphenyl)-propionic acid **19**. The catalytic reduction of **19** with HCl yielded 3, 4-methylendioxy phenylalanine **20**, a very stable product. The product **20,** can be hydrolyzed with HI to yield dopa (Barry, et al., 1948).

Scheme 2.4. Richard's synthesis of dopa using malonic acid (Barry, et al., 1948).

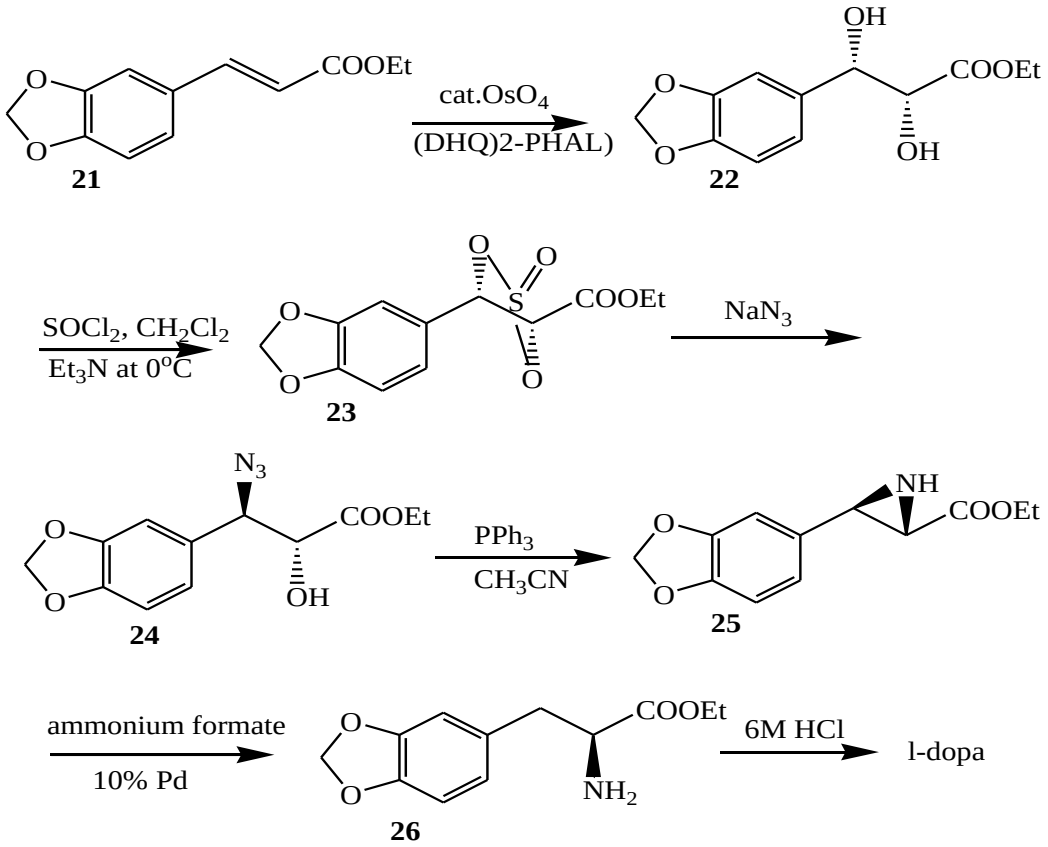


2.5 Synthesis of dopa by asymmetric dihydroxylation

There has been a variety of selective methods developed for the synthesis of dopa. An important method involved asymmetric dihydroxylation (AD) reaction of olefins (Kolb, VanNieuwenhze, & Sharpless, 1994). In 2004, Iliyas synthesized dopa using an AD reaction

involving reductive ring opening of aziridines (Scheme 2.5). Styrene (**21**) was subjected to the AD reaction in the presence of a catalytic amount of osmium tetraoxide (OsO_4) and hydroquinine-1, 4-pthalazinediyldiether to give vicinal diol **22**. Thionyl chloride ($SOCl_2$) and methylene chloride were added to **22** in the presence of triethylamine and dichloromethane at 0^oC to yield cyclic sulfite **23**, which upon treating with sodium azide gave azido alcohol **24**. Triphenylphosphine (PPh_3) in acetonitrile was added to **24** to give 3-phenylaziridine-2-carboxylic ester **25**, which was then, treated with ammonium formate and 10% palladium to give amine **26**. The amine, **26** was subjected to hydrolysis with 6M HCl to obtain l-dopa (Sayyed & Sudalai, 2004).

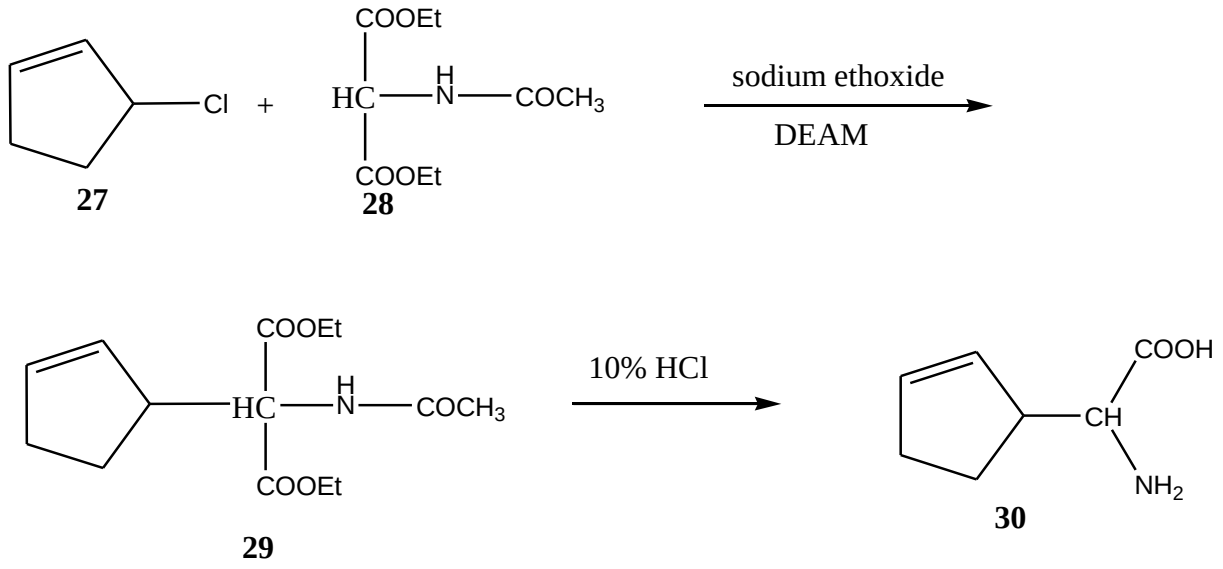
Scheme 2.5. Iliyas's dopa synthesis by asymmetric dihydroxylation (Sayyed & Sudalai, 2004).



2.6 Synthesis of α amino acid by hydrolysis

Since the 50's, a wide variety of methods have been developed for the synthesis of α-amino acids. Dennis and Sutherland developed a convenient amino acid synthesis which employed 2-amino-2-(cyclopent-2-enyl) acetic acid **30** as a glycine equivalent (Scheme 2.6). This method has shown that the 3-chlorocyclopentene **27** is formed from cyclopentadiene and hydrogen chloride. The condensation of **27** with diethyl acetamidomalonate (DEAM), **28** resulted in the formation of the diethyl acetamido-2-cyclopentene-1-malonate **29**. The glycine equivalent, **30** was obtained upon hydrolysis and decarboxylation of **29** in the presence of 10% HCl (Dennis, Plant, Skinner, Sutherland, & Shive, 1955).

Scheme 2.6. Dennis and Sutherland's synthesis of amino acid.

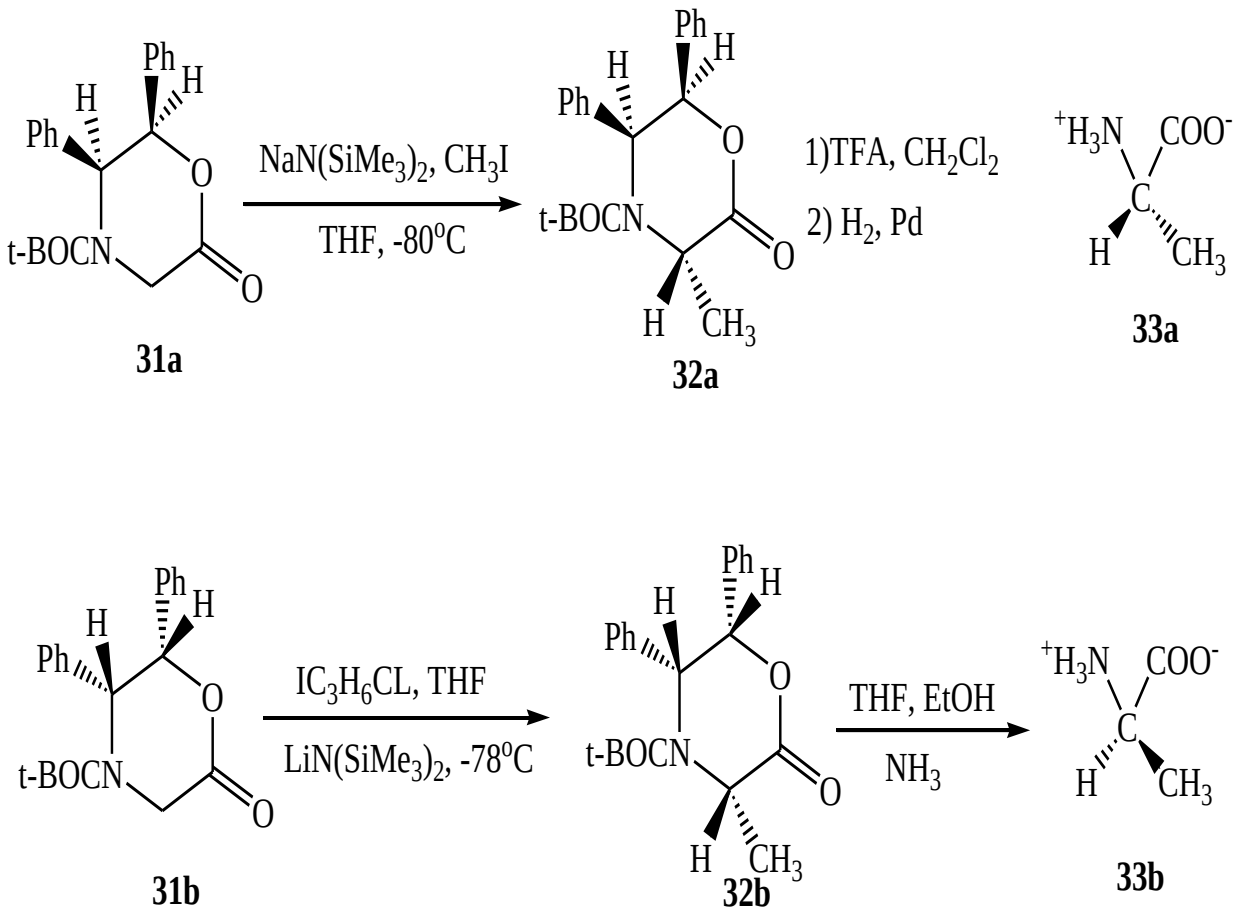


2.7 Synthesis of α amino acid by stereoselective enolate alkylation

Williams et. al have developed and applied asymmetric syntheses towards the construction of non-proteinogenic α-amino acids by employing nucleophilic and electrophilic bond formations in diphenyloxazinones (Williams & Im, 1991). William's method involved the reactions of D and L erythro forms of oxazinone **31a** and **31b** with sodium and lithium

bis(trimethylsilyl)amide in THF and methyl iodide to yield monoalkylated oxazinone **32a** and **32b** respectively (Scheme 2.7). The boc protected amide, **32a** was first treated in dichloromethane with trifloroacetic acid, and subsequently in the presence of palladium to yield L-amino acid **33a**. A similar method was applied in the synthesis of the isomeric form of **33b**, with the exceptions as shown in Scheme 2.7. The methyl-glycine products formed through nucleophilic carbanion alkylation were isolated in 95% enantiomeric excess. The anionic form of the same methyl-glycine product can undergo a second alkylation reaction; thus resulting in a dialkylated product.

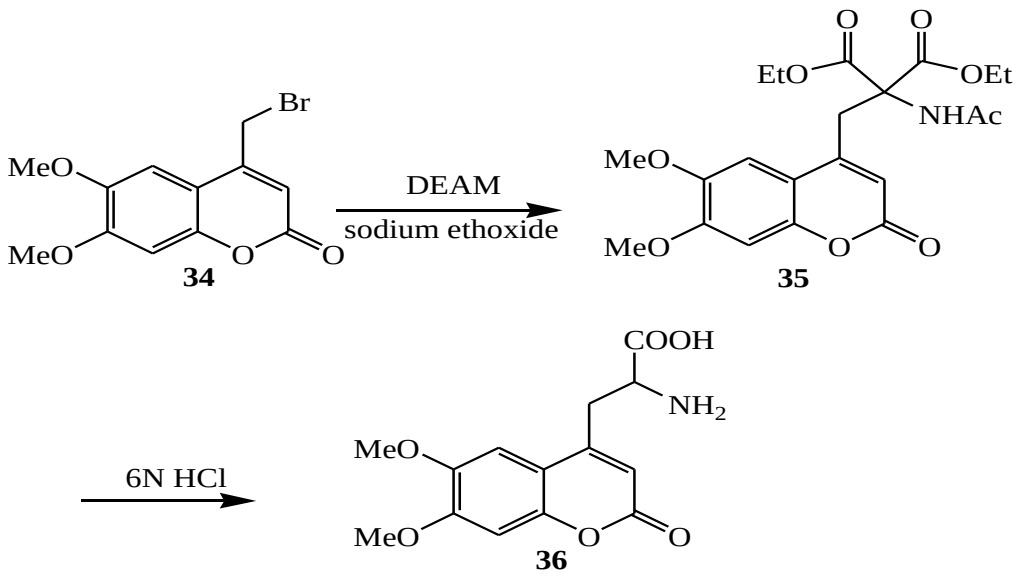
Scheme 2.7. William's synthesis of amino acids using oxazinones (Williams & Im, 1991).



2.8 Synthesis of fluorescent amino acid by hydrolysis

Guodong et. al developed a synthetic method for producing racemic coumarin based florescent amino acid, DL-2-amino-3-(6, 7-dimethoxy-4-coumaryl)-propionic acid (DL-Adp, Scheme 2.8). The sodio anion of DEAM was alkylated with the flourogenic broup 4-(bromo methyl)-6, 7 dimethoxycoumarin **34** to give 2-acetylamino-2-(6, 7-dimethyoxy-4-coumaryl methyl)-malonic acid diethyl ester **35** (Sui, Kele, Orbulescu, Huo, & Leblanc, 2001). The resulting diethyl ester (**35**) was hydrolyzed with 6N HCl to form racemic DL- Adp.

Scheme 2.8. Guodong's synthesis of fluorescent amino acid.



2.9 Statement of problem

The main objective of this work is to synthetically structure dopa mimetics (homologs and analogs) in order to regulate the process of melanogenesis by altering the activity of the enzyme, tyr-ase. These dopa mimetics may be structured as analogs or homologs. The ideal homolog differs from an analog by one carbon unit. A variety of structural designs of the dopa mimetics are proposed to prevent or slow down the process of melanogenesis; by acting as

competitive inhibitors. The dopa mimicking features of the homologs involve strategic synthetic designs of the molecular framework of the homologs that give rise to similar or identical dimensions as that of dopa. In addition to optimizing the dopa mimicking features, this work has led to the development of a variety of strategies in regulating tyr-ase. The validation of the structuring methods will be verifying based on their regulatory activity towards tyr-ase. In this work several dopa homologs were proposed (Figure 1.1). Out of the compounds proposed, three major amino acids and several key intermediates were synthesized.

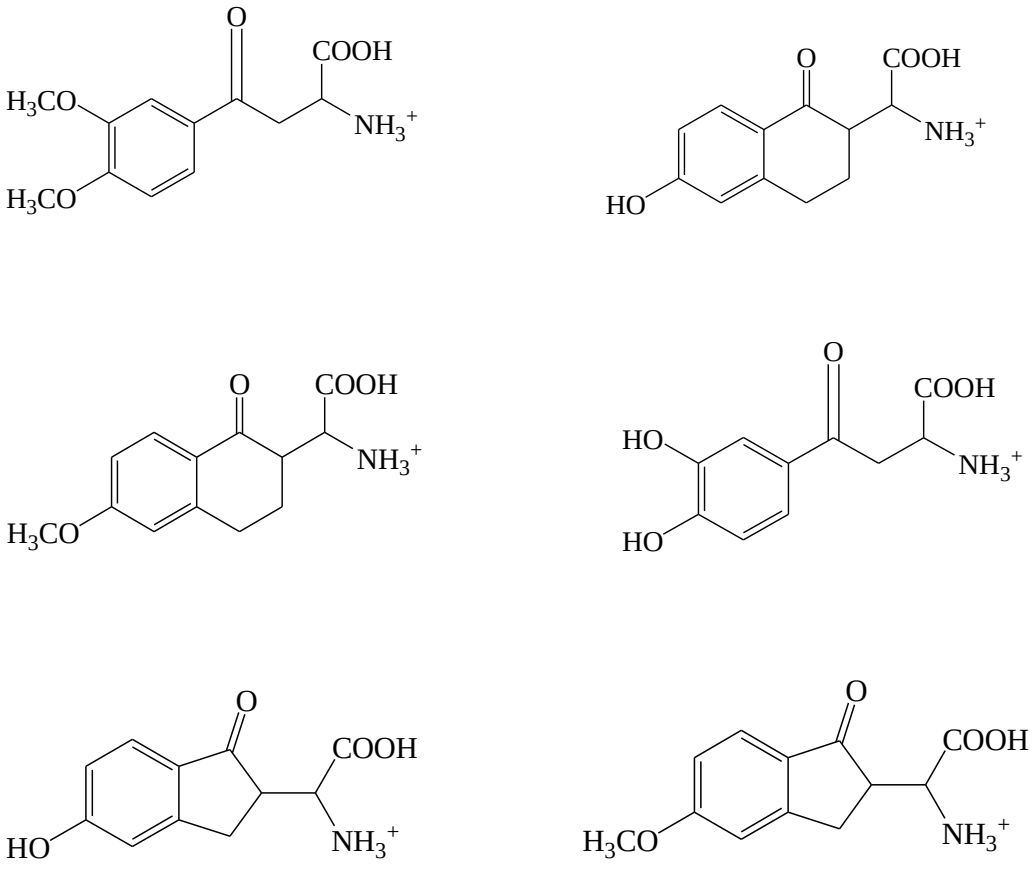


Figure 1.1. The proposed dopa homologs to inhibit melanogenesis.

CHAPTER 3

Experimental

3.1 Synthesis of 2-bromo-3, 4-dimethoxy acetophenone (38)

This reaction required the use of a reflux apparatus that was connected to a dropping funnel and a calcium chloride tube. To a stirring and refluxing solution of 62.40 g (94 mmol) copper II bromide ($CuBr_2$) in 225 ml of ethyl acetate was added 30.00g (56 mmol) of 3, 4-dimethoxy acetophenone (56 mmol) through a dropping funnel for 10 mins. The resulting greenish color solution was refluxed for 7hrs after which $CuBr_2$ (21.00 g, 31.65 mmol) was added. The resulting mixture was allowed to continue reflux overnight with stirring. The dark purple solution was cooled to room temperature and was vacuum filtered to get rid of the copper salts. The filtrate was concentrated using a rotary evaporator. The resulting product was completely dried under reduced pressure to form a solid of **38**.

For recrystallization, the product **38** (2.00 g) was dissolved in methanol (~ 9ml) and stirred until dissolved at room temperature for 10 mins resulting in a brown solution. Upon cooling, crystals were formed; which were vacuum filtered. For further purification, the procedure was repeated (92.6%), mp 76^0C; ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 3.98 (s, 3H), 4.43 (s, 2H), 6.95, 7.55, 7.64 (d, 1H, J=8.7Hz, d, 1H, J=2.7 Hz, dd, 1H, J=8.7 Hz, 2.6 Hz); MS, m/z 260 (M^++2), 258 (M^+), 165; IR (KBr) 3090, 3050, 2991, 2850, 1681, 1585, 1513, 1419, 1275, 1244, 1146, 1020, 619.

3.2 Synthesis of diethyl 2-acetamido-2-(2-(3, 4- dimethoxy phenyl)acetyl)malonate (39)

The reaction apparatus was set up for reflux in an oil bath under an atmosphere of nitrogen at 70^0C. Sodium hydride dispersed in 60% of mineral oil (2.27 g and 56.5 mmol) was washed with hexane to remove the mineral oil. The hexane was decanted and was neutralized

with anhydrous isopropanol. The activated sodium hydride was immediately added to the reaction flask. Residue of sodium hydride left in the beaker was rinsed with DMSO (81.6 ml) and added to the reaction flask. Solid anhydrous DEAM (8.47 g, 39 mmol) was added to the solution resulting in the liberation of hydrogen gas. The resulting reaction mixture was stirred at 70^0C for 3 hrs producing a yellowish solution. The bromo-ketone, **38** (10.00 g, 39 mmol) dissolved in 81.6 ml of toluene was added to the reaction flask using a dropping funnel, and the resulting reaction mixture was allowed to stir overnight under an atmosphere of nitrogen at 70^0C.

The resulting dark brown reaction mixture was cooled, and distilled water (150mL) was added to the reaction flask. The resulting mixture was added to a separatory funnel, and the organic layer (product) was separated. The aqueous layer was extracted three additional times with 60 ml portions of ethyl ether, and the organic extracts were combined and washed twice with 60 ml of distilled water and washed once with 60 ml of saturated sodium chloride. The organic layer was simultaneously dried and decolorized with anhydrous sodium sulfate and with activated carbon. The resulting mixture was filtered and concentrated using a rotary evaporator to yield crude brown crystals (87%). The crystals can be further purified by recrystallization using ethyl acetate and petroleum ether, mp 131^0C, ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 6H, J=7.6 Hz), 2.0 (s, 3H), 3.85; 3.95 (s, 3H; s, 3H), 4.25 (s, 2H), 4.32 (q, 4H, J=7.6 Hz), 6.88; 7.5; 7.63 (d, 1H, J=9.0 Hz; s, 1H, D, 1H), 7.25 (s, 1H); MS, m/z 395 (M^+), base peak 165; IR (KBr) 3344, 300, 2938, 2847, 1750, 1740, 1666, 1657, 1596, 1517, 1420, 1290, 1213, 1023.

3.3 Synthesis of 2-amino-4-(3, 4-dimethoxy phenyl)-3-oxobutanoic acid (40)

The reaction apparatus was set up for reflux under an atmosphere of nitrogen. The product **39** (15.00 g) was grinded using a mortar and pestle for easy dissolution. The pulverized

crystals were added into a round bottom flask and concentrated hydrochloric acid was added (112 ml). The reaction mixture was allowed to stir vigorously at room temperature for 30 min under an atmosphere of nitrogen. An equal volume of distilled water (112 ml) was added to dilute the acidic reaction mixture to give rise to a 50% hydrochloric acid mixture. The resulting reaction mixture was refluxed overnight. The dark brown solution was concentrated under reduced pressure to yield **40**. Isopropyl alcohol was added to the product **40b** with stirring until completely dissolved. Activated charcoal was added to the solution with stirring. The resulting mixture was vacuum filtered (i.e., 1X-2X) as needed, and subsequently concentrated to yield a dark brown/reddish solid. Using a reflux set-up, 70 ml ethyl acetate was added to the solid which was then stirred vigorously under reflux for 15 mins. The mixture was cooled, and the undissolved (product) crystals were vacuum filtered. This procedure was repeated a second time, and the filtered solid was concentrated to complete dryness under reduced pressure to give rise to a dark purple solid (45%). The filtrates can be saved, combined with future filtrates and concentrated for further use; ^{1}H NMR 3.64 (dd, 2H, J=20.1, 5.8 Hz), 3.80; 3.82 (s, 3H; s, 3H), 4.24 (q, 1H, J=4.9 Hz), 7.03 (m, 2H), 7.44 (ddd, 3H), 7.64 (d, 1H, J=8.4 Hz), 9.57 (s, 1H).

3.4 Synthesis of 2-bromo-6-methoxy tetralone (42)

This reaction required the use of a reflux apparatus connected to a dropping funnel and a calcium chloride tube. To an appropriate sized reaction flask was added, copper II bromide (25.50 g, 87.6 mmol) and 90 ml ethyl acetate. The stirred solution was allowed to come to reflux, and through a dropping funnel over a period of 10-15 mins was added, 6- methoxy tetralone **41** (12.00 g, 18.6 mol) dissolved in 120 ml of chloroform. The resulting mixture was refluxed for 8 hrs after which time was added a second portion of $CuBr_2$ (8.52 g). The resulting mixture was allowed to continue refluxing overnight with stirring. The dark purple resulting mixture was

cooled to room temperature and was vacuum filtered to get rid of the copper salts. If necessary, the filtrate can be filtered a second time in order to completely remove the copper salts. The yellow filtrate was concentrated from solvent using a rotary evaporator to yield the crude brown colored brominated product (**42**). The crude brominated product was completely concentrated using reduced pressure.

For recrystallization, methanol (7.6 ml) was added to **42** (2.00 g) at room temperature with stirring until the solid product dissolved. The resulting solution was cooled on ice to give rise to product crystals. The solution was vacuum filtered, and concentrated under reduced pressure in order to isolate product crystals of **42** (92.6%), ^{1}H NMR ($CDCl_3$) δ 2.16 (dt, 1H), 2.46 (m, 1H), 2.86 (dt, 1H, J=17.1, 4.5 Hz), 3.27 (dt, 1H, J= 15.8, 5.1 Hz), 4.68 (t, 1H, J=4.3 Hz), 6.81 (m, 2H), 8.03 (dd, 1H, J=8.8, 1.5 Hz); MS m/z 254 (M^+), 256 (M^++2), 148.

3.5 Synthesis of diethyl 2-amino-2-(1, 2, 3, 4-tetrahydro-6-methoxy-1-oxonaphthalen-2-yl)malonate (43)

The reaction apparatus was set up for reflux in an oil bath and under an atmosphere of nitrogen at 70^0C. Sodium hydride dispersed in 60% mineral oil (1.40 g, 34.2 mmol) was washed with hexane to remove the mineral oil. The hexane was decanted and was cautiously neutralized with anhydrous isopropanol. The activated sodium hydride residue was immediately added to the reaction flask. Residue of sodium hydride left in the beaker was rinsed with DMSO (49.3 ml) and added to the reaction flask. Solid anhydrous DEAM (5.23g, 24.1 mmol) was added to the solution resulting in the liberation of hydrogen gas. The resulting reaction mixture was stirred at 70^0C for 3 hrs producing a yellowish solution. The bromo-ketone, **42** (5.00 g, 19.69 mmol) dissolved in 49.3 ml of toluene was added to the reaction flask using a dropping funnel, and the resulting reaction mixture was allowed to stir overnight under an atmosphere of nitrogen at

70^0C. The resulting dark brown reaction mixture was cooled, and distilled water (150 ml) was added to the reaction flask. The resulting mixture was added to a separatory funnel, and the organic layer (product) was separated. The aqueous layer was extracted three additional times with 60 ml portions of ethyl ether, and the organic extracts were combined, washed twice with 60 ml of distilled water and washed once with 60 ml of saturated sodium chloride. The organic layer was simultaneously dried and decolorized with anhydrous sodium sulfate and with activated carbon. The resulting mixture was filtered and concentrated using a rotary evaporator to yield crude brown product crystals of **43** (76.4%). The crystals can be further purified using either, ethyl acetate/petroleum ether or methanol/hexane. ^{1}H NMR δ 1.24 (t, 6H), 1.88; 1.92 (m, 2H), 2.0 (s, 3H), 2.9 (dt, 1H), 3.15 (dt, 1H), 3.83 (s, 3H), 4.26 (tt, 4H, J=17.9, 10.7), 6.76 (m, 2H), 6.91 (d, 1H, J=6.2 Hz), 7.90 (d, 1H, J=8.8 Hz); MS m/z 391 (M^+), 175.

3.6 Synthesis of 2-amino-2-(1, 2, 3, 4-tetrahydro-6-hydroxy-1-oxonaphthalen-2-yl)acetic acid (44)

The reaction apparatus was set up for reflux under an atmosphere of nitrogen. The diester amide of tetralone **43** (4.00 g) was grinded using a mortar and pestle for easy dissolution. The pulverized crystals were added into a round bottom flask and concentrated hydrobromic acid was added (60 ml). The reaction mixture was allowed to stir overnight under an atmosphere of nitrogen. The resulting dark brown mixture was concentrated under reduced pressure to yield **44**. Isopropyl alcohol was added to the product **44** with stirring until completely dissolved. Activated charcoal was added to the solution with stirring. The resulting mixture was vacuum filtered (i.e., 1X-2X) as needed, and the filtrate was subsequently concentrated to yield a dark purple solid. Using a reflux set-up, 25-30 ml ethyl acetate was added to the solid which was then stirred vigorously under reflux for 15 mins. The mixture was cooled, and the un-dissolved

product crystals were vacuum filtered from the filtrate. This procedure was repeated a second time, and the resulting solid was concentrated completely from residual solvents under reduced pressure to give rise to a dark purple solid (60%). ^{1}H NMR (DMSO) δ 1.91; 2.14 (t, 2H), 2.85; 3.0 (t, 2H), 3.25-3.68 (t, 1H), 4.39 (s, 1H), 4.42 (s, 1H), 6.61; 6.71 (s, 2H), 6.96; 7.13; 7.30 (d, 3H), 7.76 (t, 1H, J=8.0 Hz), 10.49(s, 1H).

3.7 Synthesis of 2-amino-2-(1, 2, 3, 4-tetrahydro-6-methoxy-1-oxonaphthalen-2-yl)acetic acid (45)

The same method developed for the synthesis of compound **40** was also used in the synthesis of 6-methoxy tetralone amino acid, **45**. The resulting solid was concentrated completely from solvents under reduced pressure to give rise to a light brown colored solid. ^{1}H NMR (DMSO) δ 2.02; 2.23 (td, 2H), 3.01; 3.65 (d, 2H, J=16.6 Hz), 3.82 (s, 1H), 4.41 (t, 1H, J= 7.5 Hz), 6.72 (d, 1H), 6.90 (t, 1H, J= 10.0, 4.7, 3.5), 712; 7.29; 7.46 (d, 3H), 7.80 (dd, 1H, J=8.6, 6.1), 10.62 (s, 1H).

3.8 Synthesis of 2-bromo-5-methoxy indanone (47)

This reaction required the use of a reflux apparatus connected to a dropping funnel and a calcium chloride tube. To an appropriate sized reaction flask was added, $CuBr_2$ (13.30 g, 59.54 mmol) and 44 ml ethyl acetate. The stirred solution was allowed to come to reflux, and through a dropping funnel over a period of 10-15 mins was added, 5-methoxy indanone, **47** (5.75 g, 35.71 mmol) dissolved in 63 ml of chloroform. The resulting mixture was refluxed for 8 hrs after which time was added a second portion of $CuBr_2$ (4.43 g, 19.83 mmol). The resulting mixture was allowed to continue refluxing overnight with stirring. The yellow resulting mixture was cooled to room temperature and the copper salts were vacuum filtered to give rise to a reddish brown product solution. The resulting filtrate was concentrated using a rotary evaporator to yield

the crude brominated product **47**. The crude brominated product was completely concentrated using reduced pressure. The resulting product was recrystallized by the same method used for **42**. The resulting solution was vacuum filtered, and concentrated under reduced pressure in order to isolate product crystals of **47**. ^{1}H NMR ($CDCl_3$) δ 3.37 (d, 1H, J=1801 Hz), 3.78 (d, 1H), 3.91 (s, 3H), 4.64 (t, 1H), 6.86 (s, 1H), 6.96 (d, 1H, J=8.5 Hz), 7.7 (dd, 1H, J=8.3, 2.4 Hz); MS m/z 240 (M^+), 242 (M^++2), 161.

3.9 Synthesis of diethyl 2-acetamido-2-(2, 3-dihydro-5-methoxy-1-oxo-1H-inden-2-yl)malonate (48)

The same method developed for the synthesis of compound **43** was also used in the synthesis of compound **48**. The reaction apparatus was set up for the reflux in an oil bath under an atmosphere of nitrogen at 70^{0}C. Sodium hydride (1.76 g, 44 mmol) dispersed in 60% mineral oil was washed with hexane to remove the mineral oil. The hexane was decanted and was cautiously neutralized with anhydrous isopropanol. Residue of sodium hydride left in the beaker was rinsed with DMSO (63.5 ml) and added to the reaction flask. Solid anhydrous DEAM (6.52 g, 30 mmol) was added to the solution resulting in the liberation of hydrogen gas. The resulting reaction mixture was stirred at 70^{0}C for 3 hrs producing a yellowish solution. The bromo-ketone, **47** (6.00 g, 30 mmol) dissolved in 63.5 ml of toluene was added to the reaction mixture and was refluxed overnight under an atmosphere of nitrogen at 70^{0}C. The resulting reaction mixture was cooled and extracted by the same method of extraction used for product **43**. The resulting reaction mixture was filtered and concentrated using a rotary evaporator to yield crude orange product crystals of **48** (81.4%). MS m/z 377 (M^+), 262; ^{13}C NMR ($CDCl_3$) δ 13.9 (-OCH_3), 23.16 (-CH_3), 55.6 (-CH_2), 62.6 (-CH), 62.9 (-OCH_3), 66.1 (-CH_2), 111.5 (-CH), 129.7 (-CH), 147 (-CH), 164; 168.5; 169.6 (-C=O) 196.3 (-C=O).

CHAPTER 4

Results and Discussion

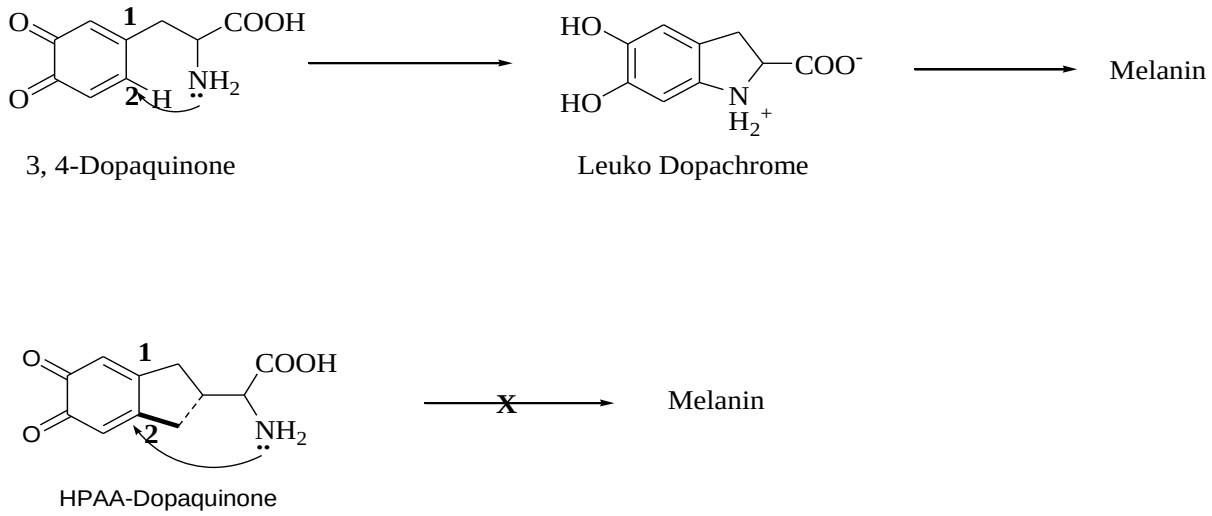
The overall strategies employed in the synthesis of dopa homologs involved the strategic synthetic construction of compounds that highly mimic the structure of dopa, and when these same compounds are acted upon by the enzyme tyr-ase, they are subsequently able to utilize incorporated structural features that favorably regulate the process of melanogenesis. It is also expected that the intrinsic binding strength and the regulatory ability of the proposed substrates will be most influential towards the enzyme, tyr-ase when compared to the endogenous substrates.

4.1 Expected mechanism of action of dopa homologs

The structural designs of the dopa mimetics are likely to exhibit inhibitory activity on melanin formation within malignant melanocytes. Once the dopa homolog has displayed activity within the active site of tyr-ase, this would suggest that the C-4 framework of the homolog has not exceeded the binding domains of the active site of the enzyme. Once the binding domain for try-ase is established, the exogenous amino acids are expected to utilize their structured features to inhibit melanin formation.

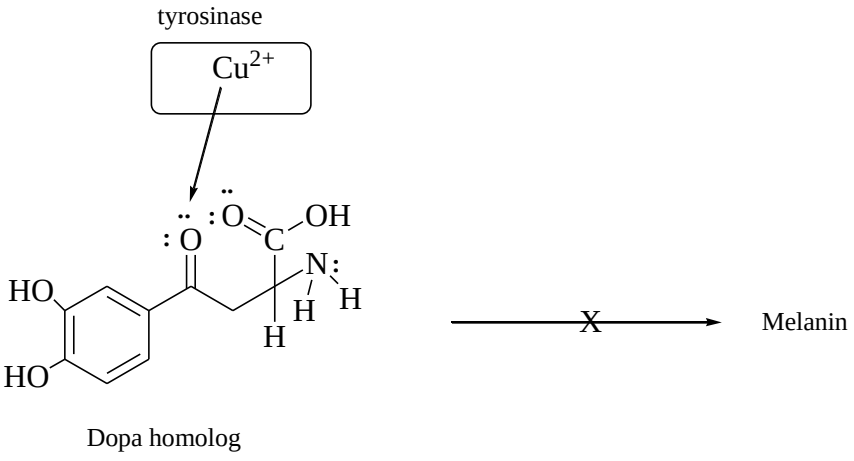
4.1.1 Amino cyclization prevention. Amino cyclization prevention is a feature synthetically incorporated into the structural frame-work of the dopa homologs that inhibits the formation of melanin (Scheme 4.1). In this strategy, an inert group is incorporated at the 2-position of the 3, 4-dopaquinone which prevents the nucleophilic attack of the lone pair of electrons on nitrogen; which subsequently prevents the formation of the requisite intermediate for melanin formation, the leukodopachrome.

Scheme 4.1. Melanin inhibition via amino cyclization prevention.



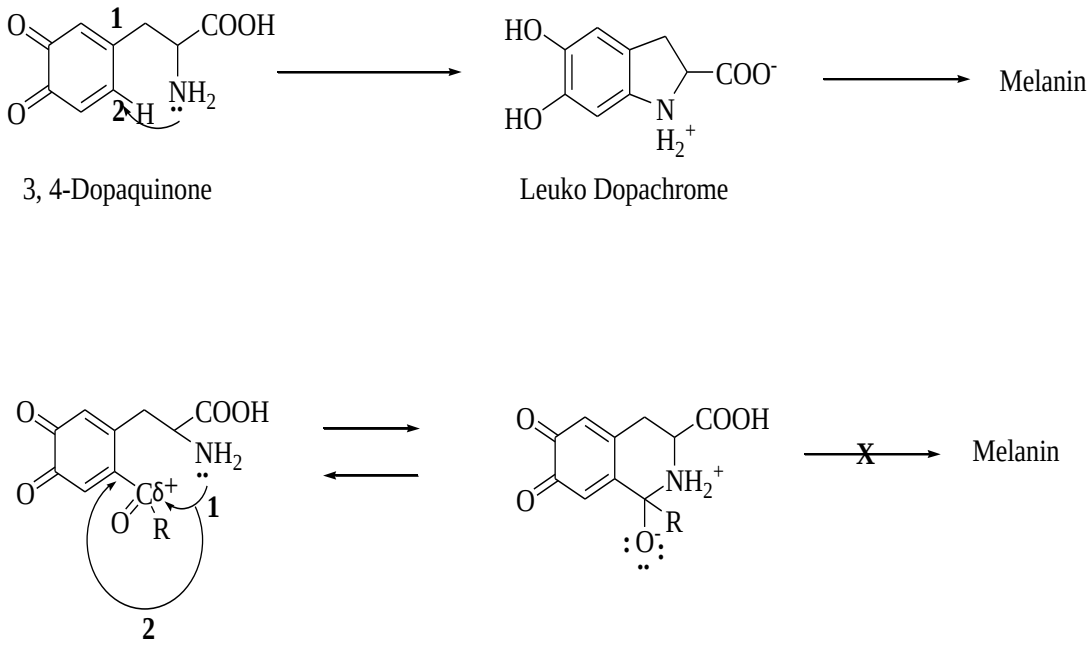
4.1.2 Chelation functionalization. Chelation functionalization is another strategy used for inhibiting melanogenesis (Scheme 4.2). It occurs by incorporating functional groups within the molecular framework of relevant molecules that can bind at the copper active site of tyr-ase. The lone pair of electrons on hetero atoms are prime candidates in chelating with copper at the active site of tyr-ase. This results in the inhibition of the endogenous dopa binding to the active site of tyr-ase, thus preventing the formation of melanin.

Scheme 4.2. Melanin inhibition by chelation functionalization.



4.1.3 Polarized functionalization. Polarized functionalization was a method developed for the inhibition of melanogenesis (Scheme 4.3). In this methodology, a polar group is incorporated at the 2-position of the 3, 4-Dopaquinone. The polarized group undergoes nucleophilic attack to give rise to equilibrium between the precursory DQ and the intermediate which inhibits melanin production and therefore, inhibits phase II of melanogenesis. The exogenous DQ as displayed below in Scheme 4.3, can undergo two modes of futile attack, at the 2-aromatic position or at the carbonyl carbon of the 2-aromatic position respectively.

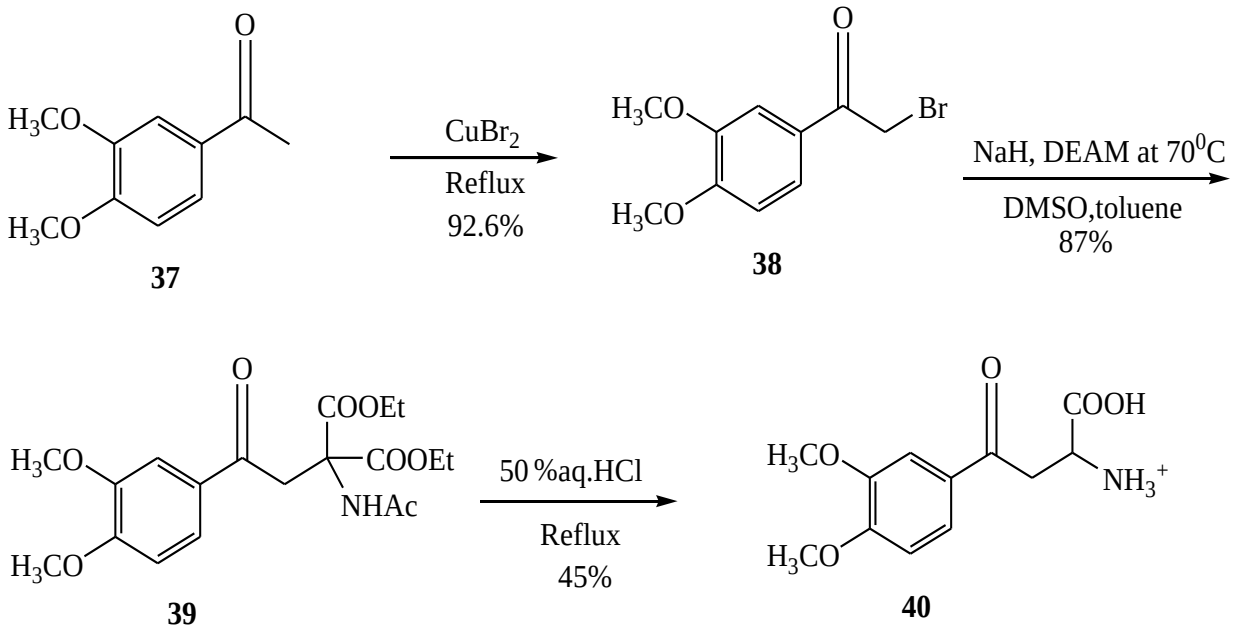
Scheme 4.3. Melanin inhibition via polarized functionalization.



4.2 2-amino-4-(3, 4-dimethoxyphenyl)-3-oxobutanoic acid (40)

The synthesis of 2-amino-4-(3, 4dimethoxyphenyl)-3-oxobutanoic acid **40** involved the utility of 3, 4 dimethoxy acetophenone **37** (Scheme 4.4)**.** The bromo ketone **38** was condensed with DEAM to give rise to the keto diester amide **39**, which was then subsequently hydrolyzed to form the glycine equivalent, **40.**

Scheme 4.4. Synthesis of amino acid of 3, 4-dimethoxy acetophenone.



4.2.1 2-bromo-3, 4-dimethoxy acetophenone (38). A considerable effort was made to develop an efficient and inexpensive synthetic route to produce α- bromo ketones by employing different brominating agents. In the process of obtaining a brominating agent, we attempted to use pyridinium tribromide as a brominating agent. This method did not yield the desired product in a sizable yield based on GC/MS analysis (Siegel & Graefe, 1953). In response to the inability to produce a significant amount of bromo ketone, an alternative brominating method was used involving copper (2) bromide; $CuBr_2$ (Scheme 4.4). This procedure involved two separate addition of $CuBr_2$, in which the initial duration continued for 3 hrs and the second duration was for 4 hrs (King & Ostrum, 1964). The chromatogram of the GC/MS of the resulting product gave an indication of an abundant amount of starting material. The most effective procedure involved reacting **37** with the first portion of $CuBr_2$ for 14 hrs and then the second portion for 8 hours to give 92.6% yield of the expected product (Figure 4.1). The brominated product obtained was identified and evaluated using GC/MS, FTIR and proton NMR analyses.

The gas chromatogram as featured in Figure 4.1 displays peaks for the starting material (13.21min), the bromoketone **38** (15.89 min) and the di-bromoketone (18.51 min). The synthesis using $CuBr_2$ gave rise to an abundance of the bromoketone, **38** (15.89 min) as displayed by the GC-MS (Figure 4.1-4.2). The mass spectrum reveals the base peak of **38** at 165 (m/z) and molecular ion peak at 258 (Figure 4.2). The occurrence of the base peak of bromo ketone **38** is indicative of α-cleavage of methylene bromo moiety to give rise to its respective acylium cation. The synthesis of bromo ketone **38** with $CuBr_2$ was determined to be the method of choice, due to its purity and percentage yield.

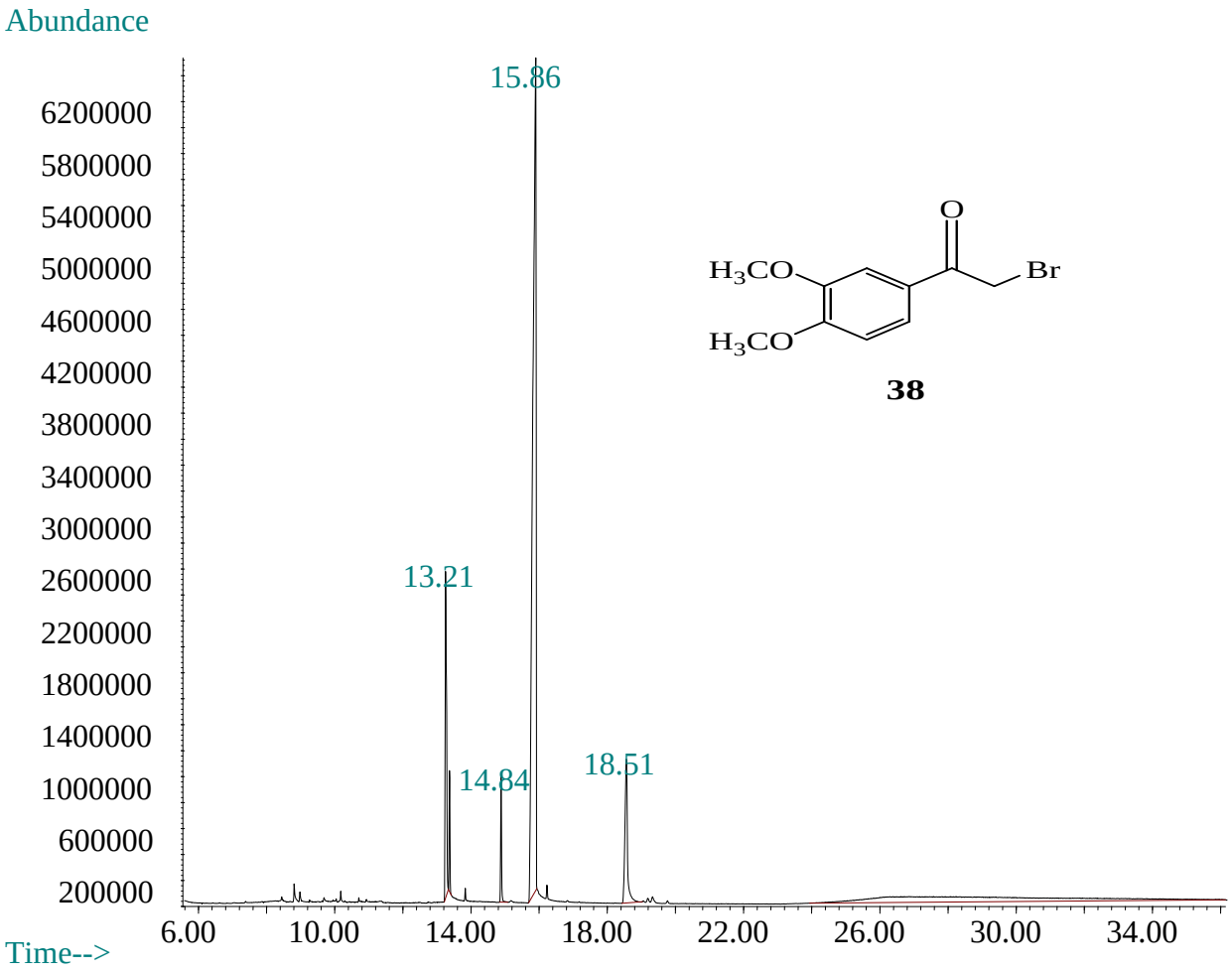


Figure 4.1. Gas chromatogram of 2-bromo-3, 4-dimethoxy acetophenone.

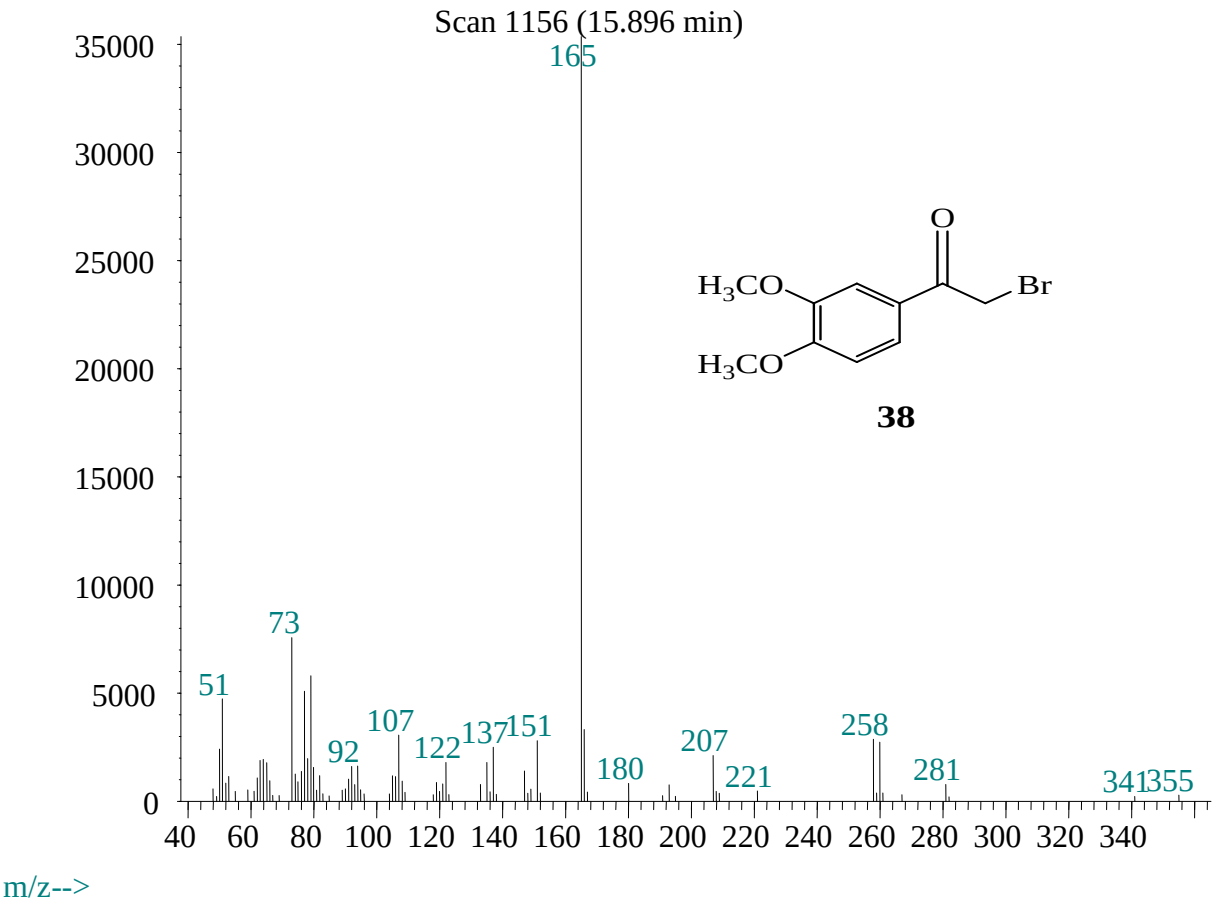


Figure 4.2. Mass spectrum of 2-bromo-3, 4-dimethoxy acetophenone.

4.2.2 2-chloro-3, 4-dimethoxy acetophenone. The GC/MS for the chloro ketone is shown in Figures 4.3 and 4.4. The method developed for the synthesis of chloroketone, employed sulfuryl chloride as the chlorinating agent. The GC analysis indicated the starting material as a major component at 13.38 min and the product peak as being a minor component at 14.62 min. The mass spectrum indicates the molecular ion peak at 214 (M^+) and 216 (M+2) with intensity ratios of 3:1 respectively, which represents the monochlorinated product.

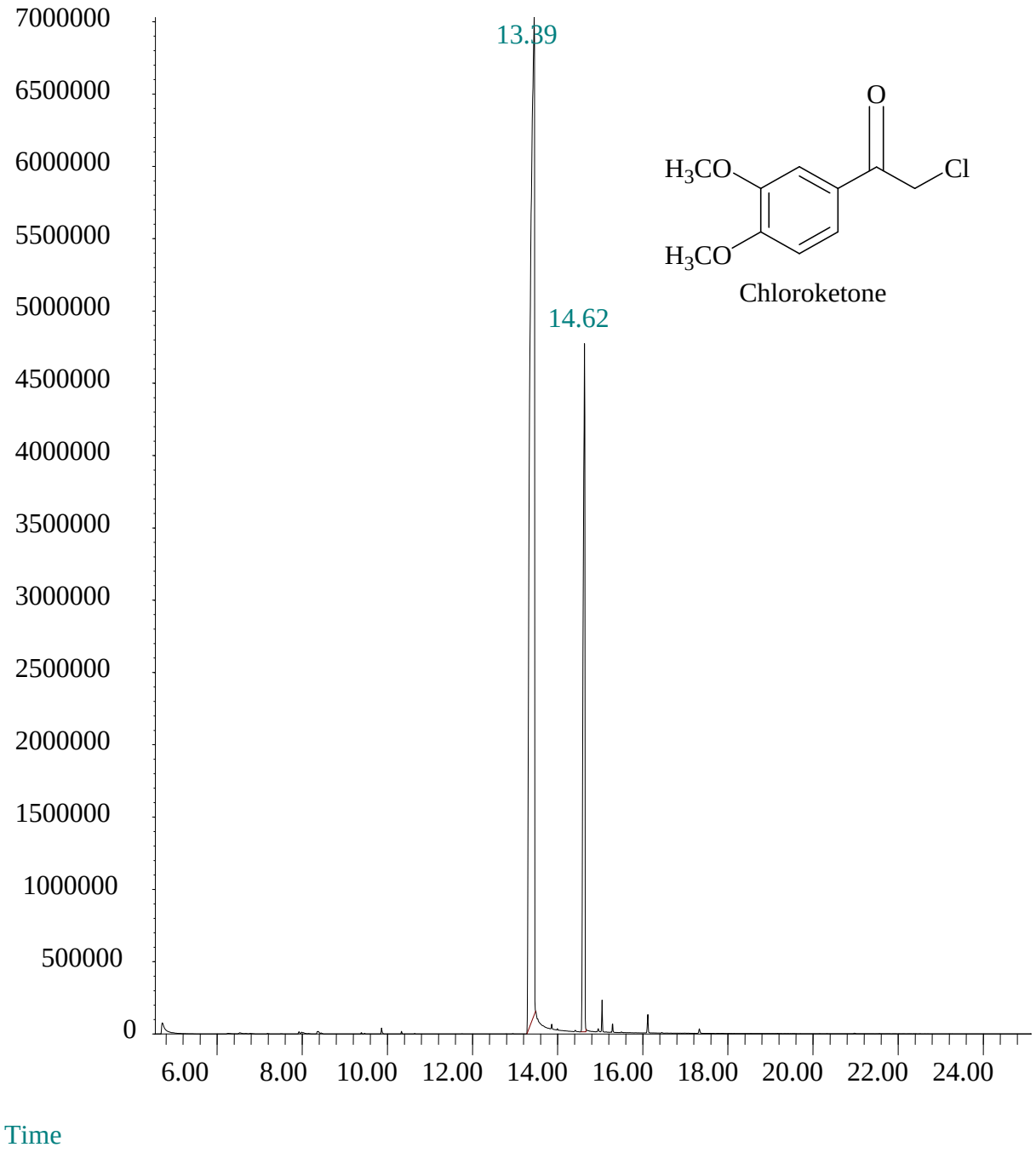


Figure 4.3. Gas chromatogram of 2-chloro-3, 4-dimethoxy acetophenone.

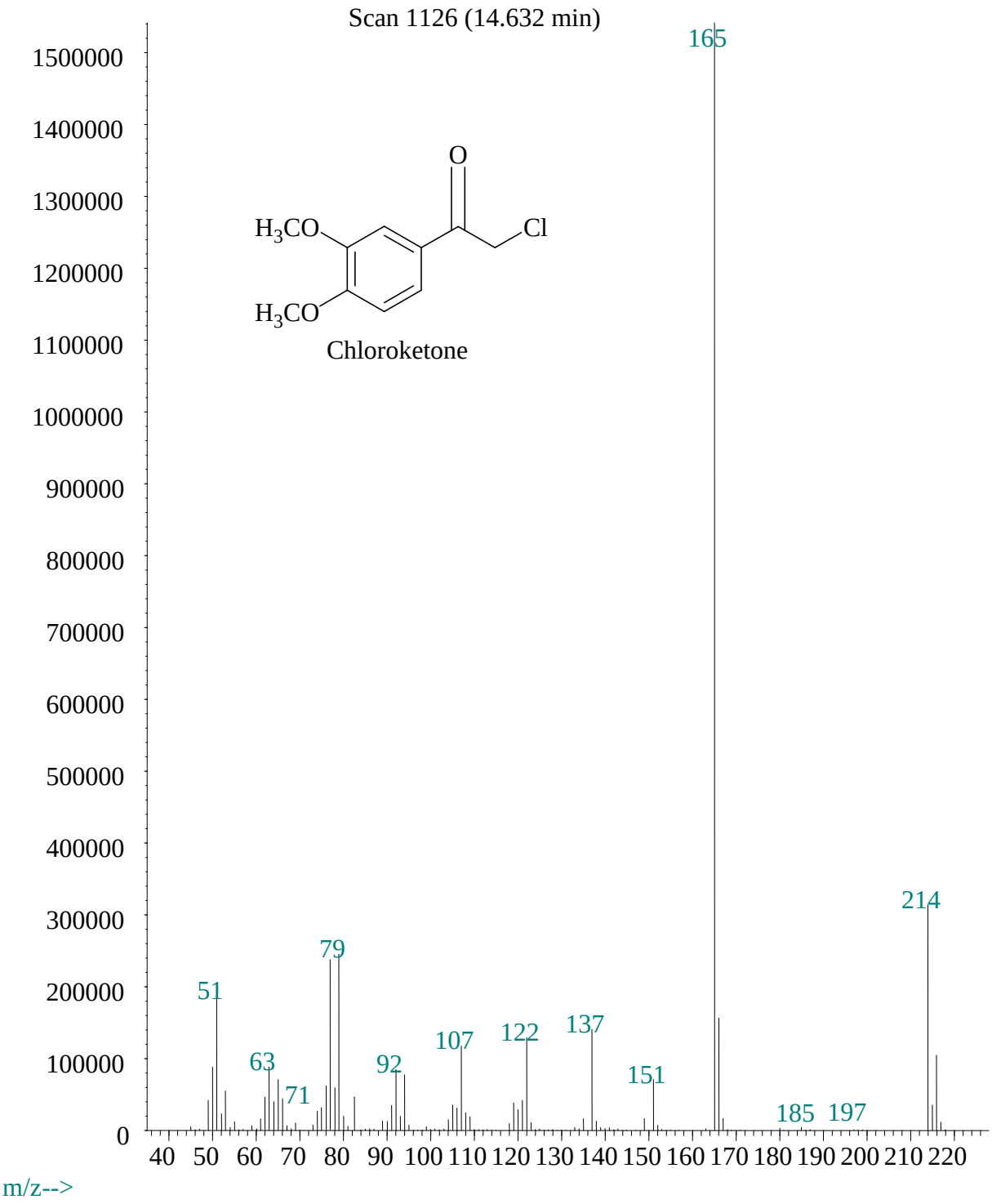


Figure 4.4. Mass spectrum of 2-chloro-3, 4-dimethoxy acetophenone.

4.2.3 Diethyl 2-acetamido-2-(2-(3, 4-dimethoxy phenyl)acetyl)malonate (39). With respect to the keto diester amide **39**, the synthetic method is shown in Scheme 4.4 on page 30. The bromoketone was condensed with the sodio anion of DEAM, which was generated in a refluxing medium consisting of anhydrous sodium hydride (NaH) in a 50:50 solvent mixture of DMSO and toluene to give the diester amide, **39** (King & Ostrum, 1964).

The produced yields of the diester amide were compared using 1.05 eq and 1.15 eq of sodium hydride. This reaction was most efficient using 1.15 eq of NaH. The keto diester amide **39** is expected to be the most important intermediate regarding the dopa homolog because of its versatile functionality.

The ^{1}H NMR of product **39** indicated chemical shifts consistent with the expected compound. Evaluation of product **39** utilizing GC/MS gave a variety of fragmentation ions when a gradient temperature profile was used. There were two major peaks displayed in the GC, indicating the starting material and the expected product. A second method was used to analyze the keto diester amide **39** by running the sample isothermally, due to its thermolability. The resulting GC displayed peak was indicative of product **39** (Figure 4.5). The mass spectrum of compound **39** indicated the molecular ion peak at 395 m/z (Figure 4.6). The second most abundant peak in the mass spectrum is indicative of compound **39** undergoing demethylation. The base peak indicates keto α cleavage and formation of the respective acylium ion. In summary, the synthetic route using 1.15 eq of NaH was determined to be the method of choice; as there are no peaks for starting material or impurities.

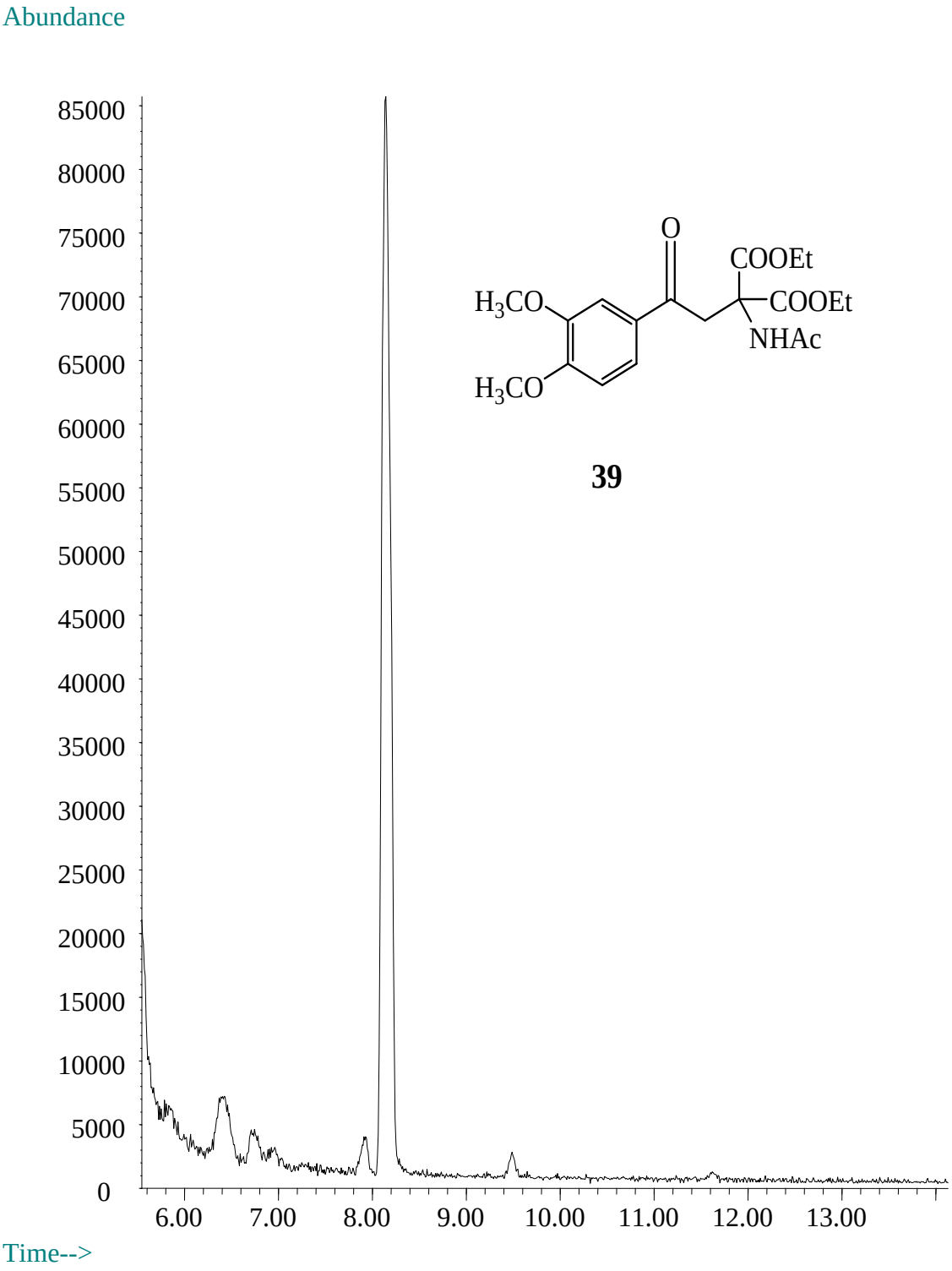
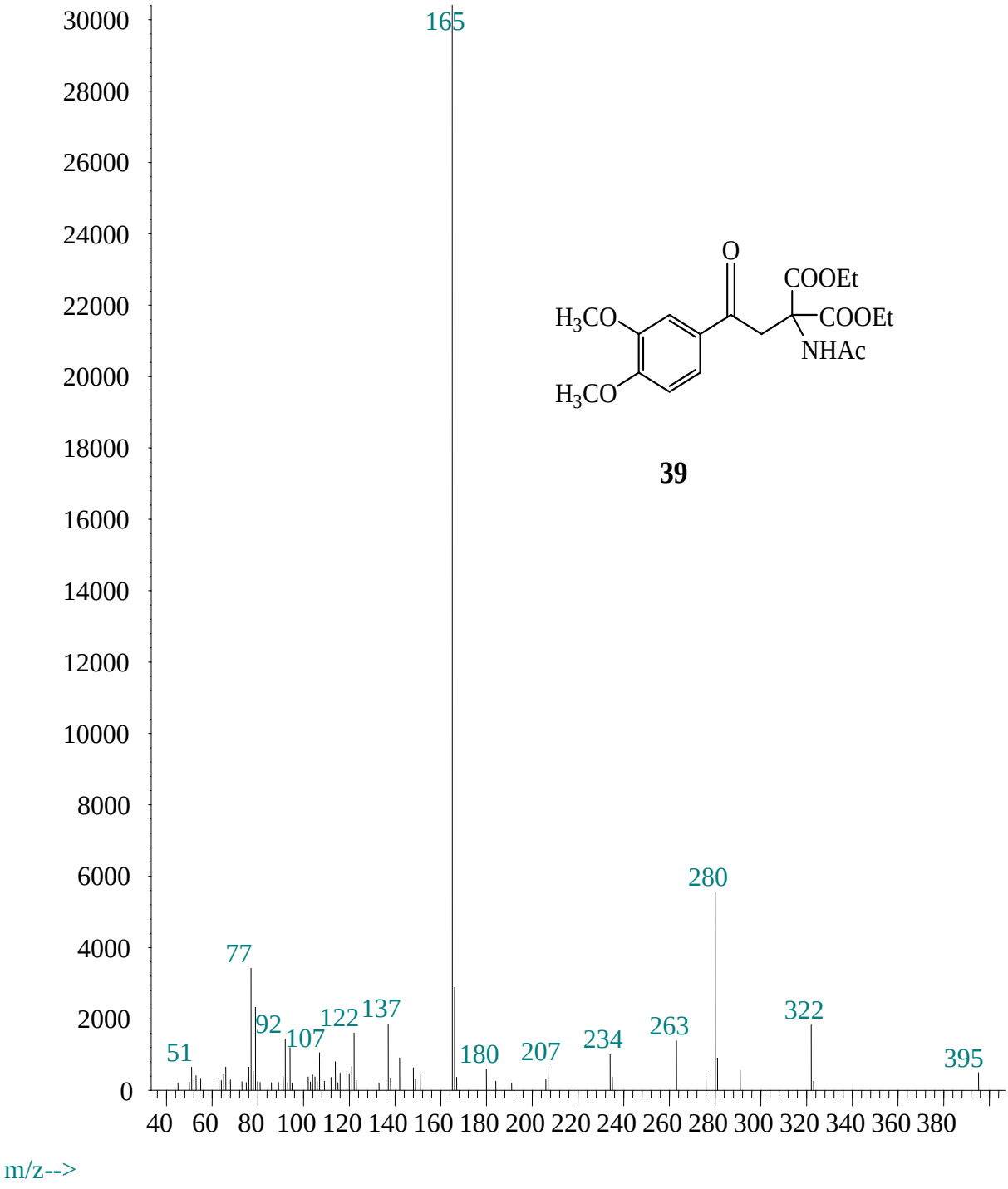


Figure 4.5. Gas chromatogram of diester amide of acetophenone.



39

Figure 4.6. Mass spectrum of diester amide of acetophenone.

4.2.4 2-amino-4-(3, 4-dimethoxy phenyl)-3-oxobutanoic acid (40). Several methods were employed in the synthesis of the dopa homolog, **40**. These methods involved the acid hydrolysis and decarboxylation reactions of **39** using two different acid concentrations in isolating either the amine or the ammonium salt. In utilizing 25% HCl refluxed for 8 hrs, the product was basified with ammonium hydroxide to an isoelectric point of 5.6 (Herr, Enkoji, & Dailey, 1957). Uncommonly, the expected neutral amine product did not precipitate at its isoelectric point.

The experience gained in the acid-base hydrolysis, suggested a different methodology. The second method was carried out using 33% HCl (Dennis, et al., 1955). The resulting solution was concentrated to dryness, an appropriate amount of distilled water was added, and the aqueous layer was washed with ethyl acetate. The resulting aqueous wash was concentrated to give rise to the ammonium salt. The overall procedure for acid hydrolysis was carried out by adding the required amount of concentrated hydrochloric chloric acid to most effectively dissolve the keto diester amide. The desired concentration of hydrolysis was established by adding the appropriate amount of distilled water. Various final percentages of HCl (i.e, 30%, 50%) were evaluated and it was determined that 50% HCl was most effective in giving rise to the highest percentage yield.

In evaluating the acid hydrolysis using 50% HCl, the ^{1}H NMR spectrum was consistent in the characterization of the dopa homolog, **40** (Figure 4.7). The peak at δ 9.57 indicates the presence of the hydroxyl proton of the carboxylic acid and the peaks at δ 7.36, 7.44 and 7.64 indicate the protons of aromatic ring. The dimethoxy protons resonated at δ 3.79 and 3.82. The -NH_3Cl-CH proton-proton coupling give rise to three doublets for the NH_3Cl moiety which

overlaps the aromatic protons at δ between 7-7.5 ppm. The broad peak at δ 8.40 represents the waters of hydration.

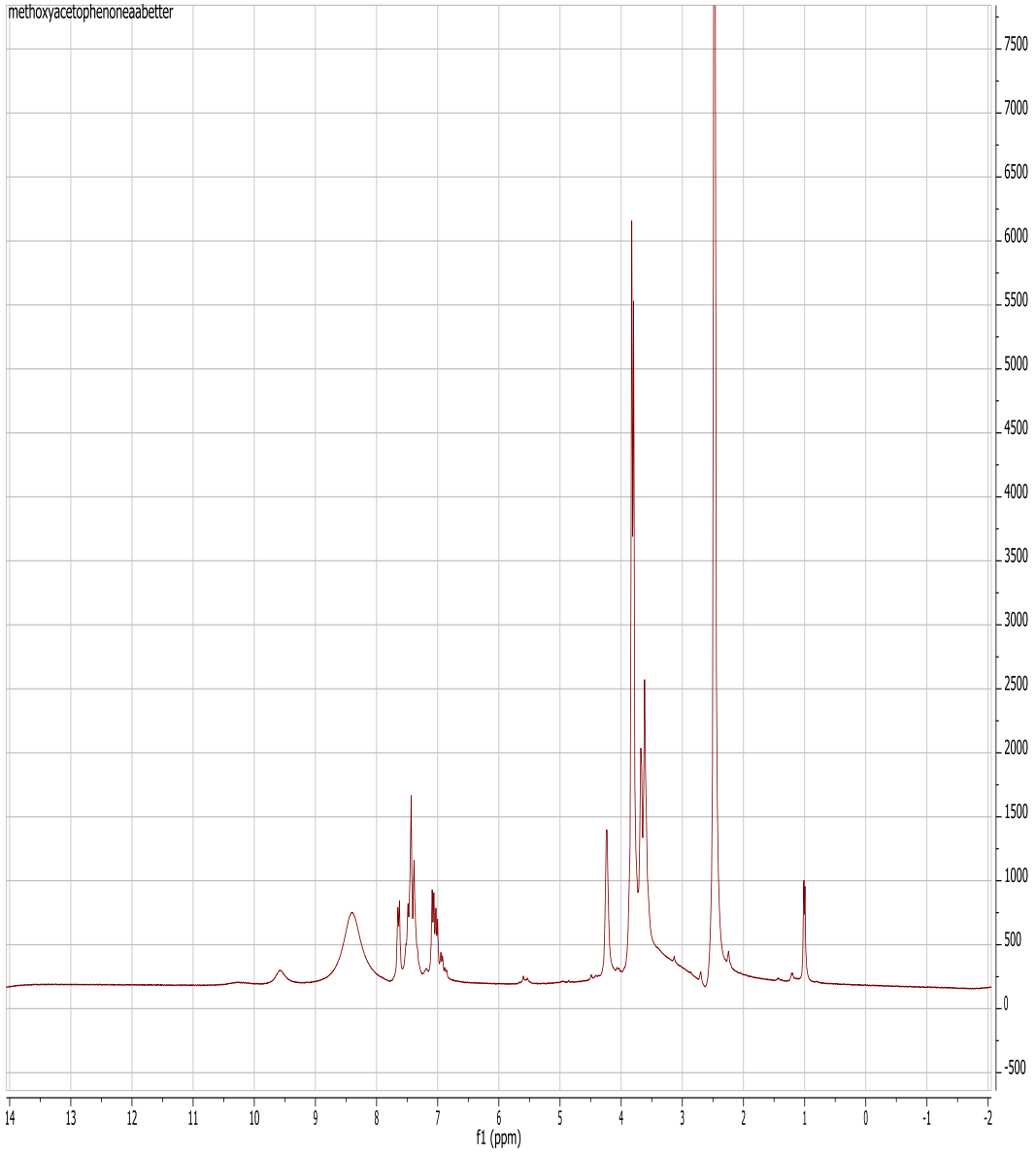


Figure 4.7. Proton NMR spectrum of amino acid of 3, 4-dimethoxy acetophenone.

The keto moiety of dopa homolog **40** is a prime functionality in promoting side chain chelation at the copper active site of the enzyme, tyr-ase (Scheme 4.2, page 30). Thus, the keto function and the amino acid moiety are expected to bind copper at the active site of tyr-ase and

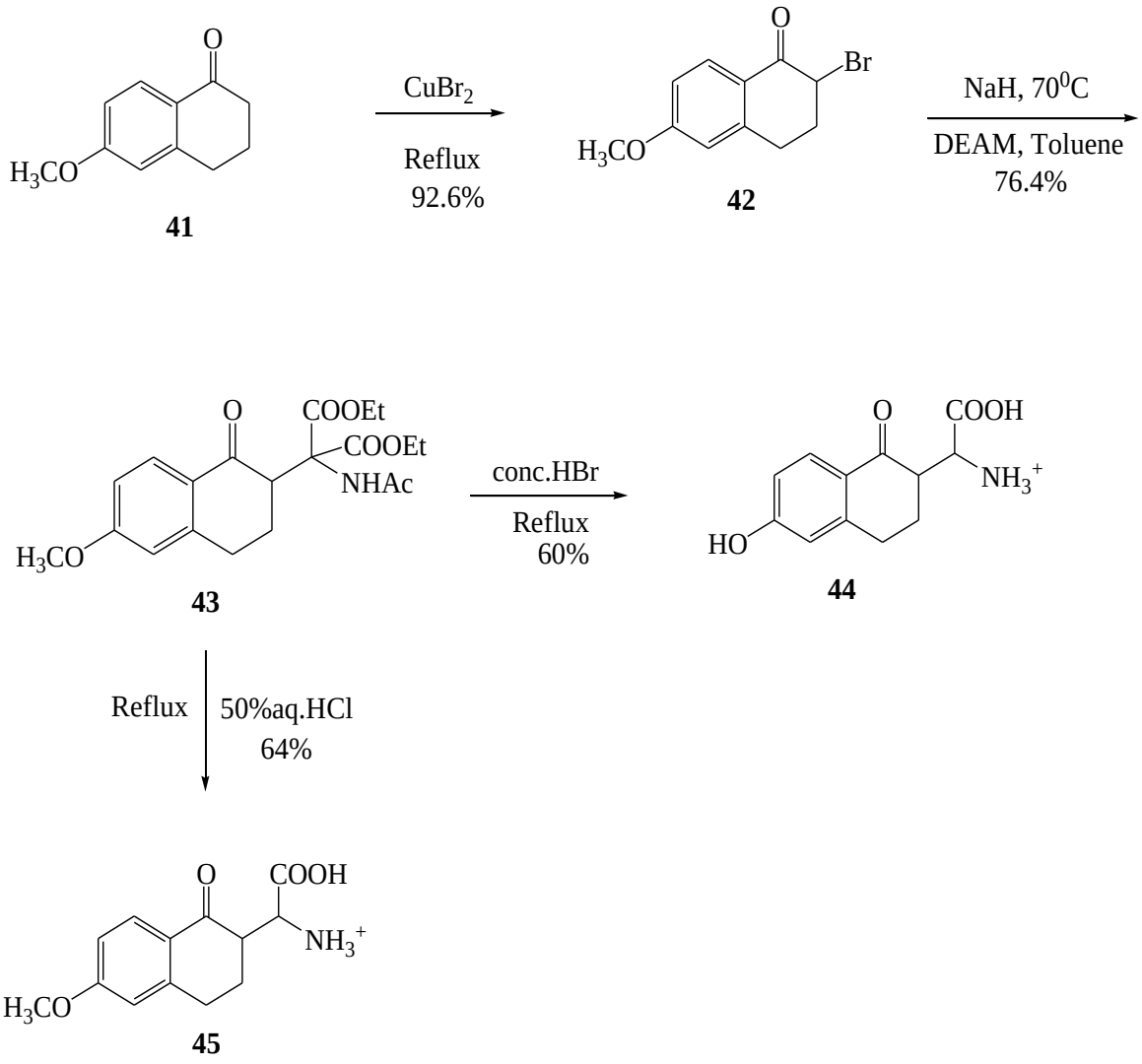
exclude the substrate conversion of endogenous dopa (Kahn & Andrawis, 1985). This type of exogenous chelation binding is capable of exhibiting significant inhibition of melanogenesis. The extended C-4 side chain homolog **40** may also act as a competitive substrate, and upon enzymatic conversion give rise to lower order oligomers rather than higher order melanin polymers, this activity is melanogenic inhibitory. Alternatively, the enzymatically converted side chain homolog **40** may give rise to a proportion of C-4 amino units incorporated into the resultant melanin(s) that give rise to a less destructive form of melanin as compared to the harmful form of rapidly produced C-3 melanins.

4.3 Homologs of 6-hydroxy (44) and 6-methoxy tetralone (45)

The synthesis of the tetralone homolog of dopa (**44**) is shown in Scheme 4.5. This synthetic route allows for the efficient synthetic entry to the equivalent methoxy dopa homolog, **45**. The first step in the synthesis of amino acids **44** and **45** involved the universal α-bromination reaction applied to 6-methoxy tetralone (**41**) by employing $CuBr_2$ to give rise to **42**. The transformation of the remaining intermediates in route to the respective amino acids employed the same method used to synthesize dopa homolog **38** (Scheme 4.4). The cyclohexyl frame-work incorporated into the structures of dopa homologs **44** and **45** may give an indication of its molecular contracting influence as an effective means of enhancing the dopa mimicking abilities of **44** and **45**. Thus, the cyclohexyl ring represents a C-4 side chain unit that can be compared in molecular length to the C-3 side chain of dopa. The methylene carbon of the cyclohexyl unit at the 2-position of aromatic rings of **44** and **45** prevents amino cyclization in forming leukodopachrome; as **44** and **45** will be evaluated for their aminocyclization inhibitory influence on melanogenesis (Scheme 4.1). As aforementioned, the keto-amino acid side chains of **44** and

45 are expected to act in an inhibitory manner by chelating at the Cu^{+2} active site of the enzyme, tyr-ase (Kahn & Andrawis, 1985).

Scheme 4.5. Synthesis of 6-hydroxy tetralone amino acid.



4.3.1 2-bromo-6-methoxy tetralone (42). The GC/MS and ^{1}H NMR spectra were consistent in the characterization of **42** (Figure 4.8). The gas chromatogram revealed the expected product peak at 20.98min and the less abundant peak for starting material at 17.23min. The mass spectrum displays the isotopic molecular ion peaks at 254 (M^{+}) and 256 (M+2); which

confirms the monobrominated ketone, **42** (Figure 4.9). The base peak at 148 represents α-keto cleavage which gives rise to the respective acylium cation. Gas chromatography–mass spectrometry analysis of the α-bromo ketone **42** gave validation to the effectiveness of the α-bromination method employing $CuBr_2$; as this method was used in all subsequent syntheses involving α-bromination of ketones.

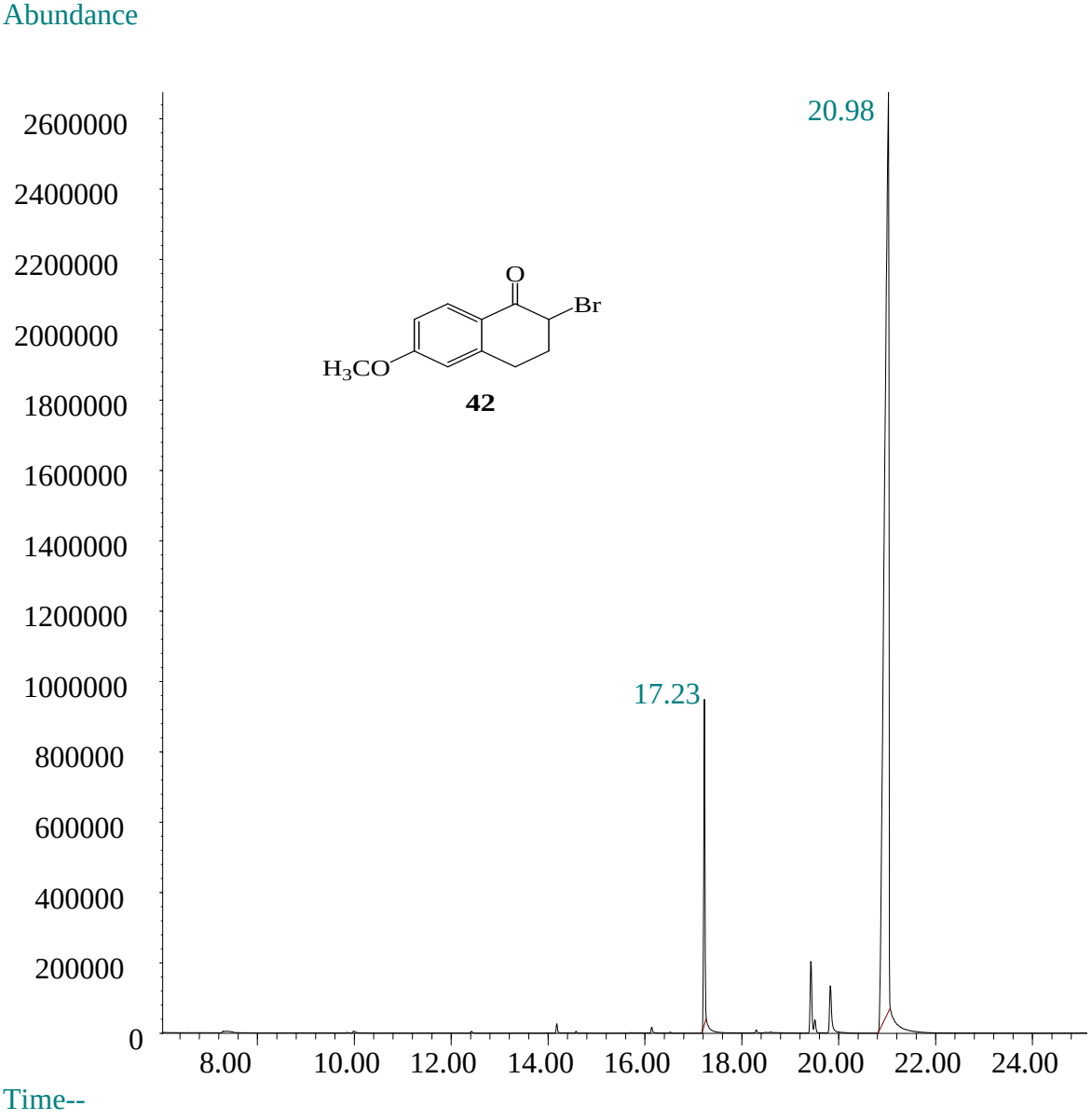


Figure 4.8. Gas chromatogram of bromo tetralone.

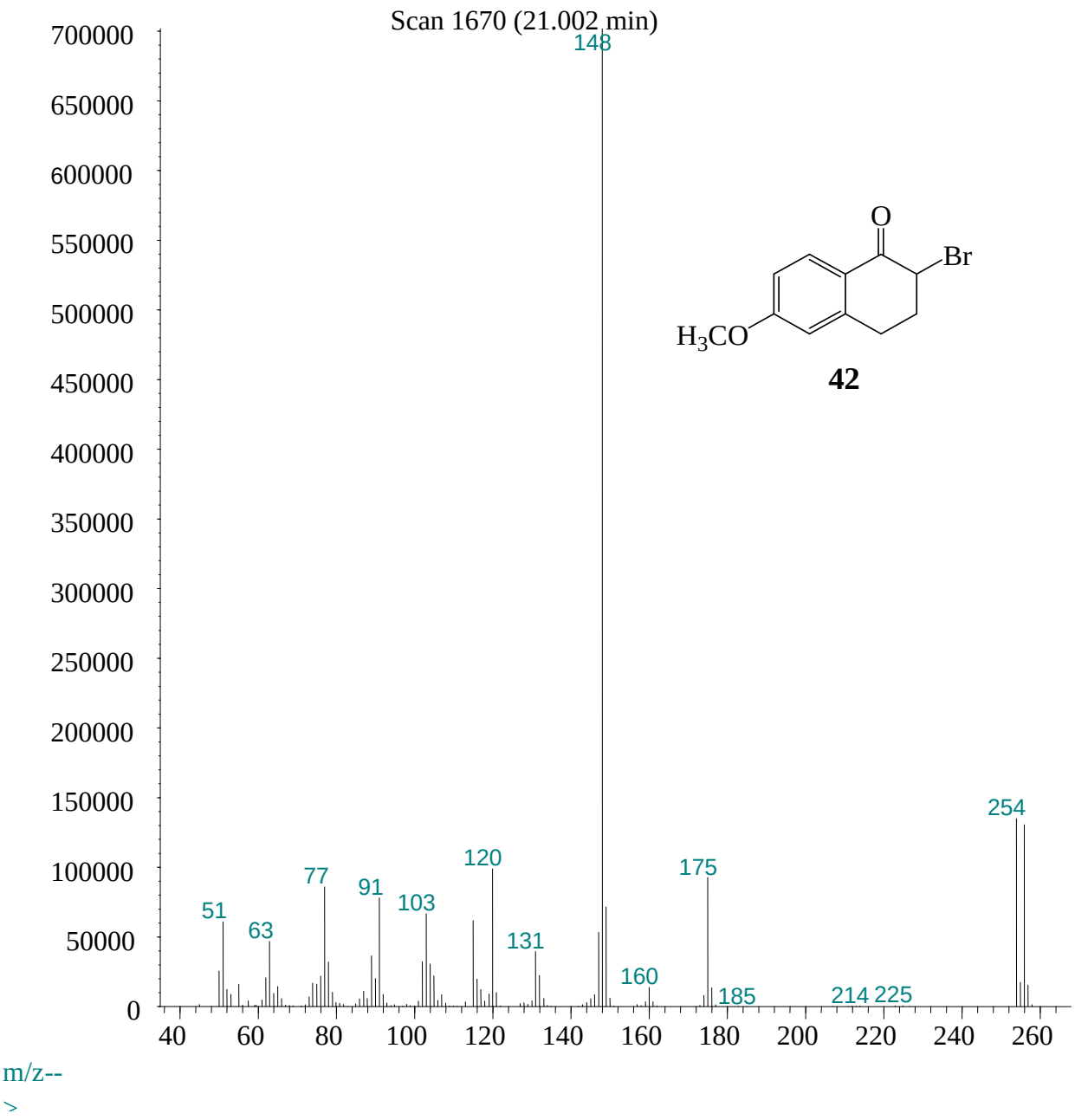


Figure 4.9. Mass spectrum of bromo tetralone.

4.3.2 Keto-diester amide of tetralone (43). The keto-diesteramide of tetralone **43** was synthesized by applying the same method used in the synthesis of **39** (Scheme 4.5). The GC/MS and ^{1}H NMR spectra were consistent in the characterization of the keto-diesteramide, **43**. The

GC/MS of the keto-diesteramide **43** was run isothermally to give rise to a single chromatogram peak at 11.64 min (Figure 4.10). The sample was run isothermally in order to preserve the molecular ion and therefore the appearance of the corresponding peak at m/z 391 (Figure 4.11). The mass spectrum revealed the base peak at m/z 175 which resulted from the loss of the diethyl acetamido malonate moiety group. The ^{1}H NMR spectrum was also consistent with the desired product, **43**.

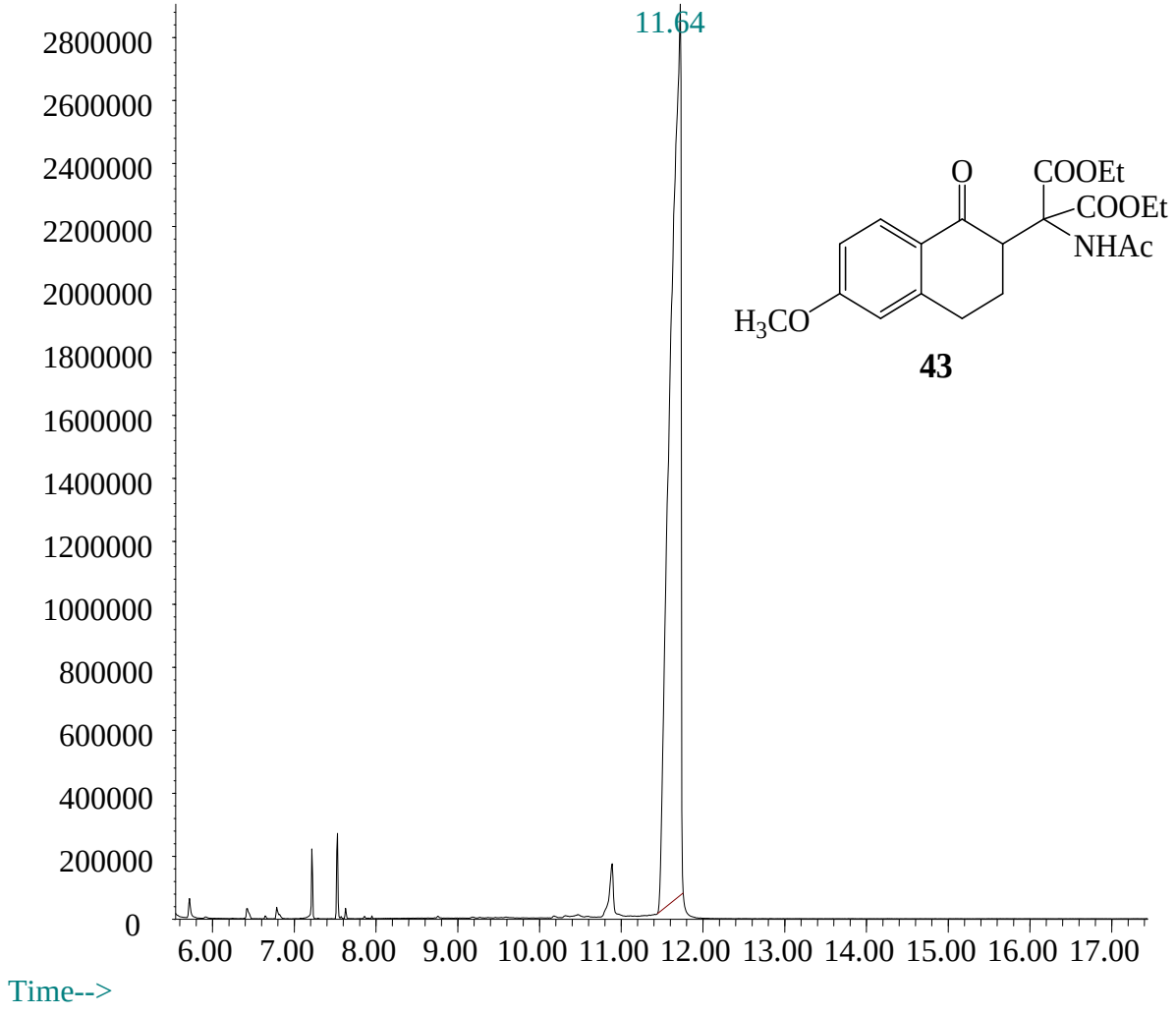


Figure 4.10. Gas chromatogram of diester amide of tetralone.

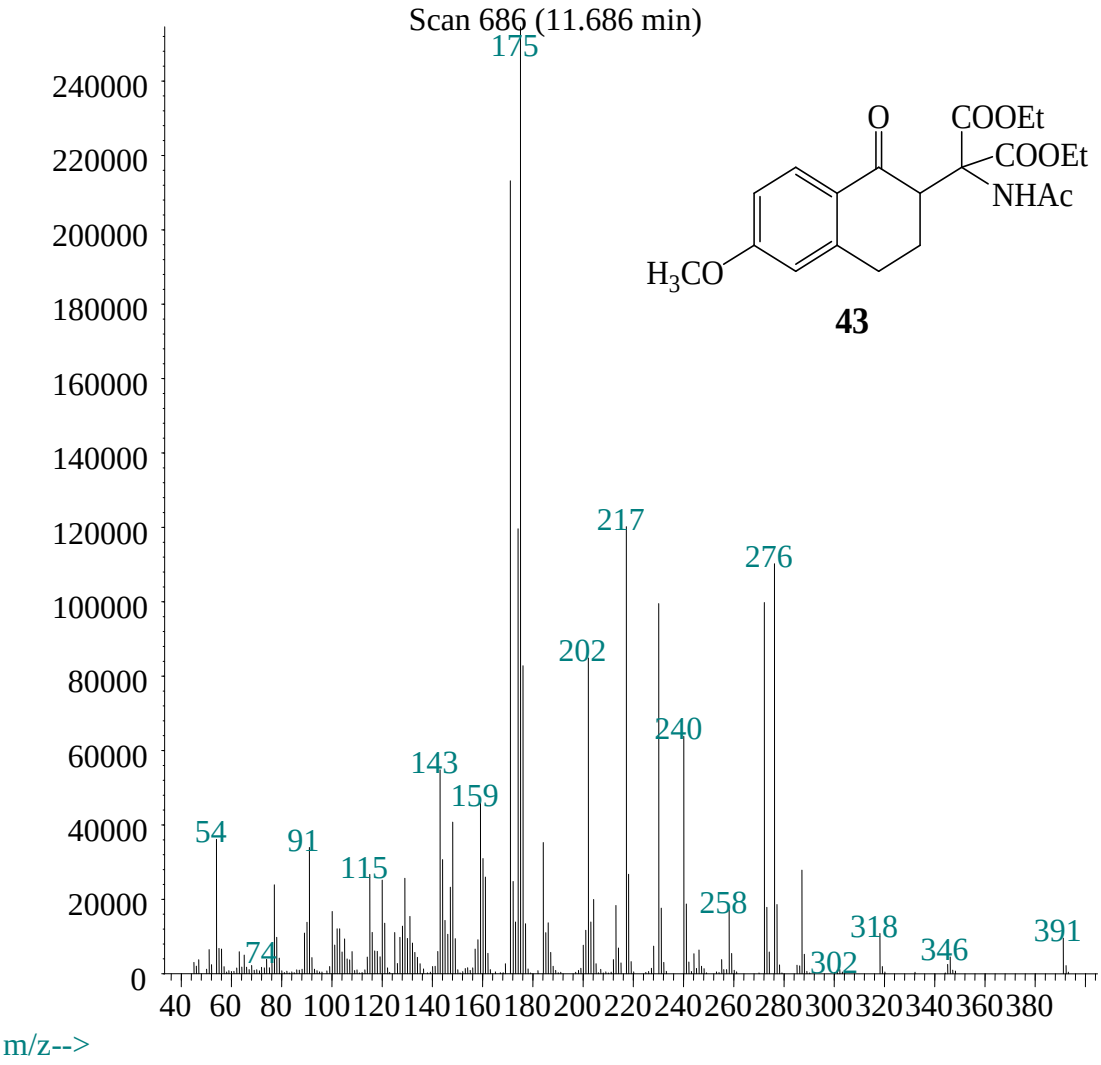


Figure 4.11. Mass spectrum of diester amide of tetralone.

4.3.3 6-hydroxy tetralone homolog of dopa (44). Synthesis of the tetralone homolog of dopa involved complete deprotection and decarboxylation using 50% hydrobromic acid (HBr) to give rise to 60% yield of the desired product, **44**. The resulting product **44** was confirmed using NMR analysis (Figure 4.12). The peak at δ 10.49 (s, 1H) represents the carboxyl proton, the peak at δ 4.39 represents the α-proton of the chiral carbon, and the phenolic proton associates with

external water, resonates at δ 4.41. External hydrogen bonding (i.e., exchange) with the NH_3Br protons generally give rise to a peak near a δ of 7; whereas internal molecular association of the NH_3Br protons allow for effective CH-NH proton-proton coupling to give rise to the 3 doublets at δ 6.96, 7.13 and 7.30. (Donald L. Pavia, 2001). The singlet at δ 8.25 normally represents internal waters as hydrates.

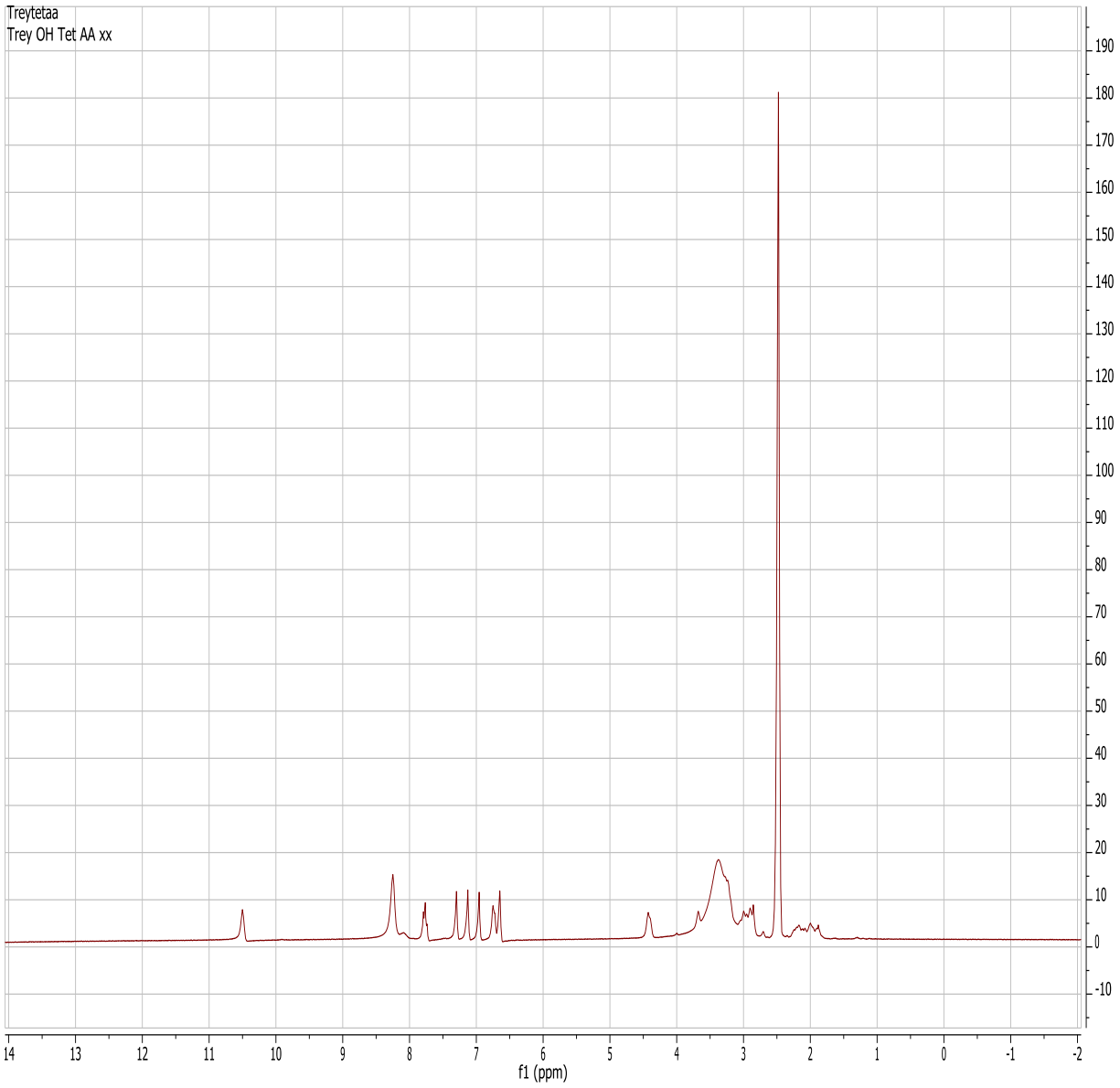


Figure 4.12. Proton NMR spectrum of 6-hydroxy tetralone amino acid.

4.3.4 6-methoxy tetralone homolog of dopa (45). Synthesis of the methoxy-tetralone homolog of dopa involved partial deprotection and decarboxylation using 50% hydrochloric acid (HCl) to give rise to 64% yield of the desired product **45**. The resulting product **45** was confirmed using NMR analysis (Figure 4.13). The peak at δ 3.82 represents the uncleaved -OCH_3 group on the aromatic ring. The peak at δ 10.49 (s, 1H) represents the carboxyl proton and the resonance peaks at δ 6.72, 6.90 and 7.80 represent the aromatic protons. The peaks at δ 7.12, 7.29 and 7.46 represent effective CH-NH proton-proton coupling.

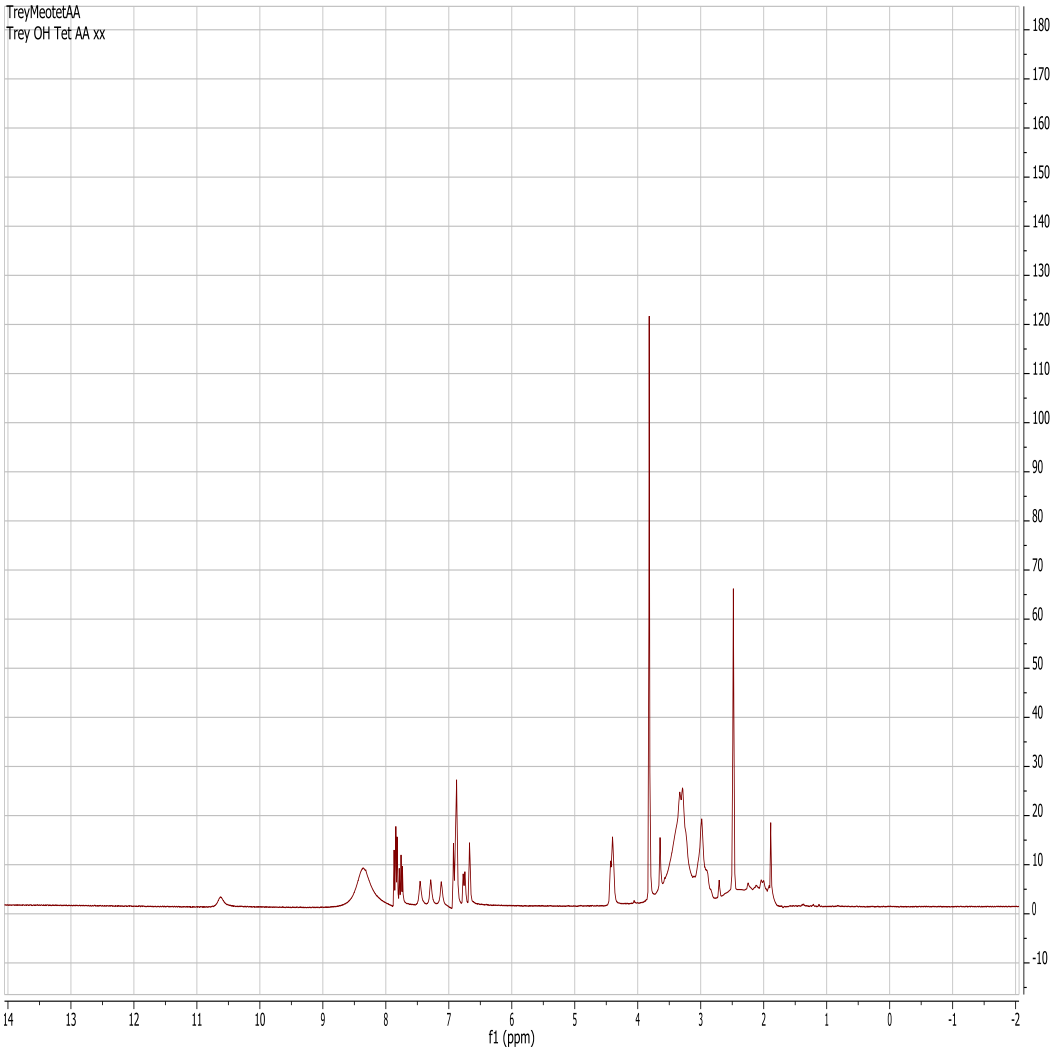
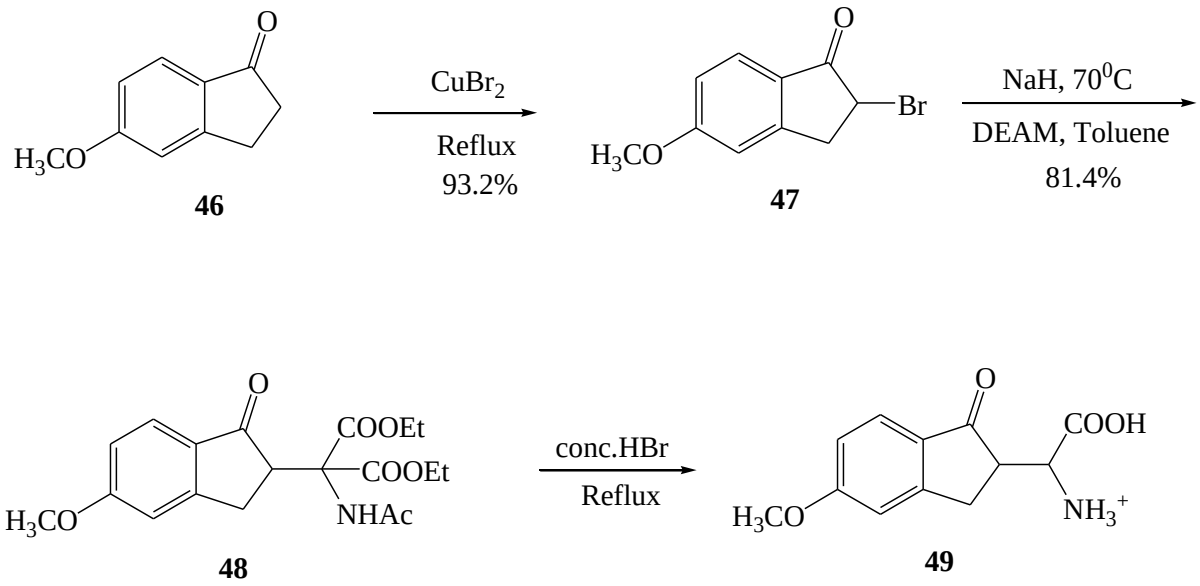


Figure 4.13. Proton NMR spectrum of 6-methoxy tetralone amino acid.

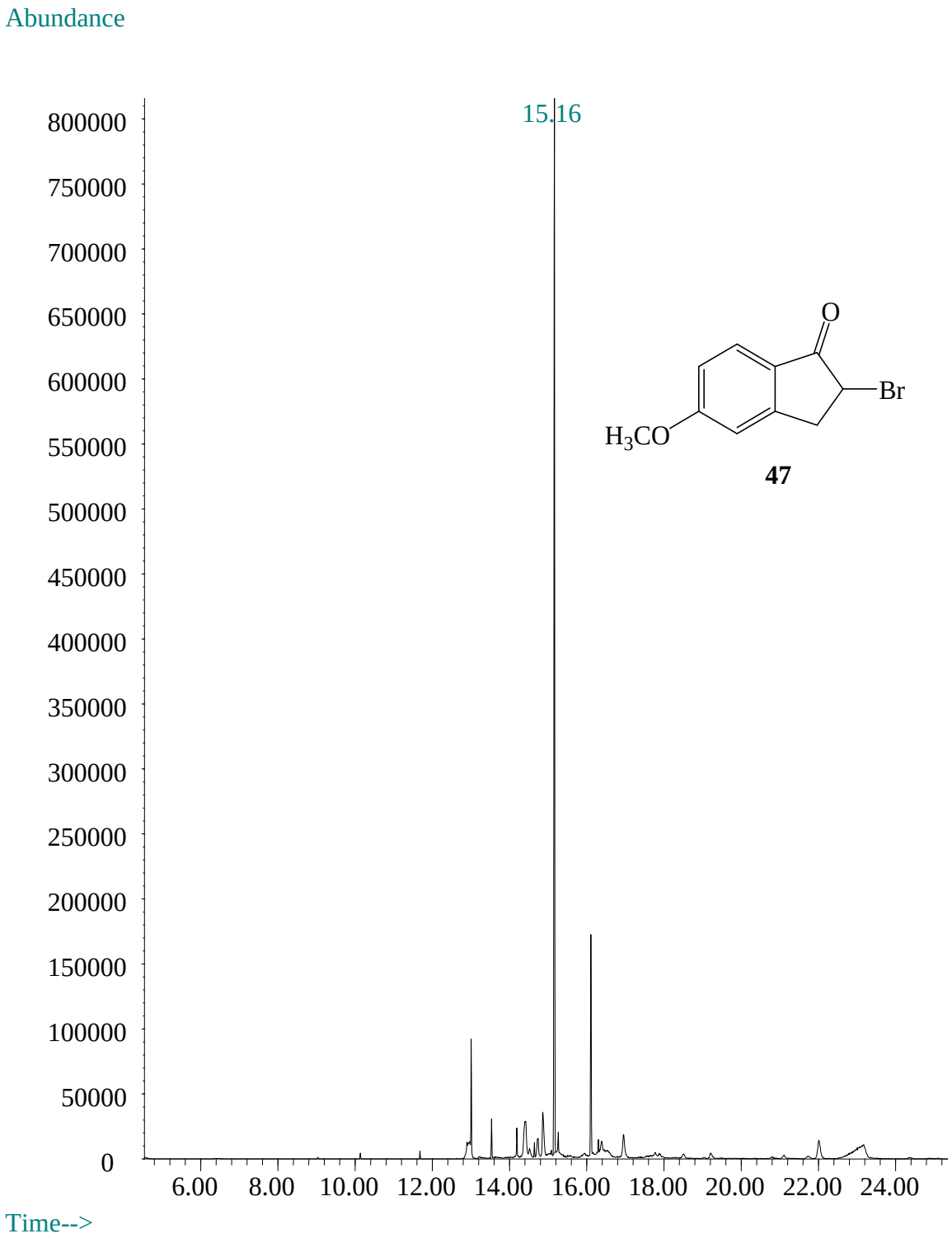
4.4 2-amino-2-(2, 3-dihydro-5-hydroxy-1-oxo-1H-inden-2-yl)acetic acid (49)

The keto-dopa homolog **49** is structured to exhibit dopa mimicking features as well as melanogenic inhibition via preventive amino cyclization. The multi step synthesis of this compound is described in Figure 4.19. The overall method used in the synthesis of **49** is identical to the method used in the production of **44** (Scheme 4.6).

Scheme 4.6. Synthesis of 5-hydroxy indanone amino acid.



4.4.1 2-bromo-5-methoxy indanone (47). The mass spectrum/NMR is consistent in the characterization of α-bromo ketone, **47**. The gas chromatogram analyses suggest minor starting material and little evidence of the di-bromonated product. The GC peak for the expected bromo ketone is displayed at 15.16 min (Figure 4.14). The mass spectrum displays the isotopic molecular ion peaks at 240 (M^+) and 242 (M+2); which confirms monobrominated ketone, **47** (Figure 4.15). The base peak at 161 represents α-keto cleavage which gives rise to the respective acylium cation.



47

Figure 4.14. Gas chromatogram of bromo indanone.

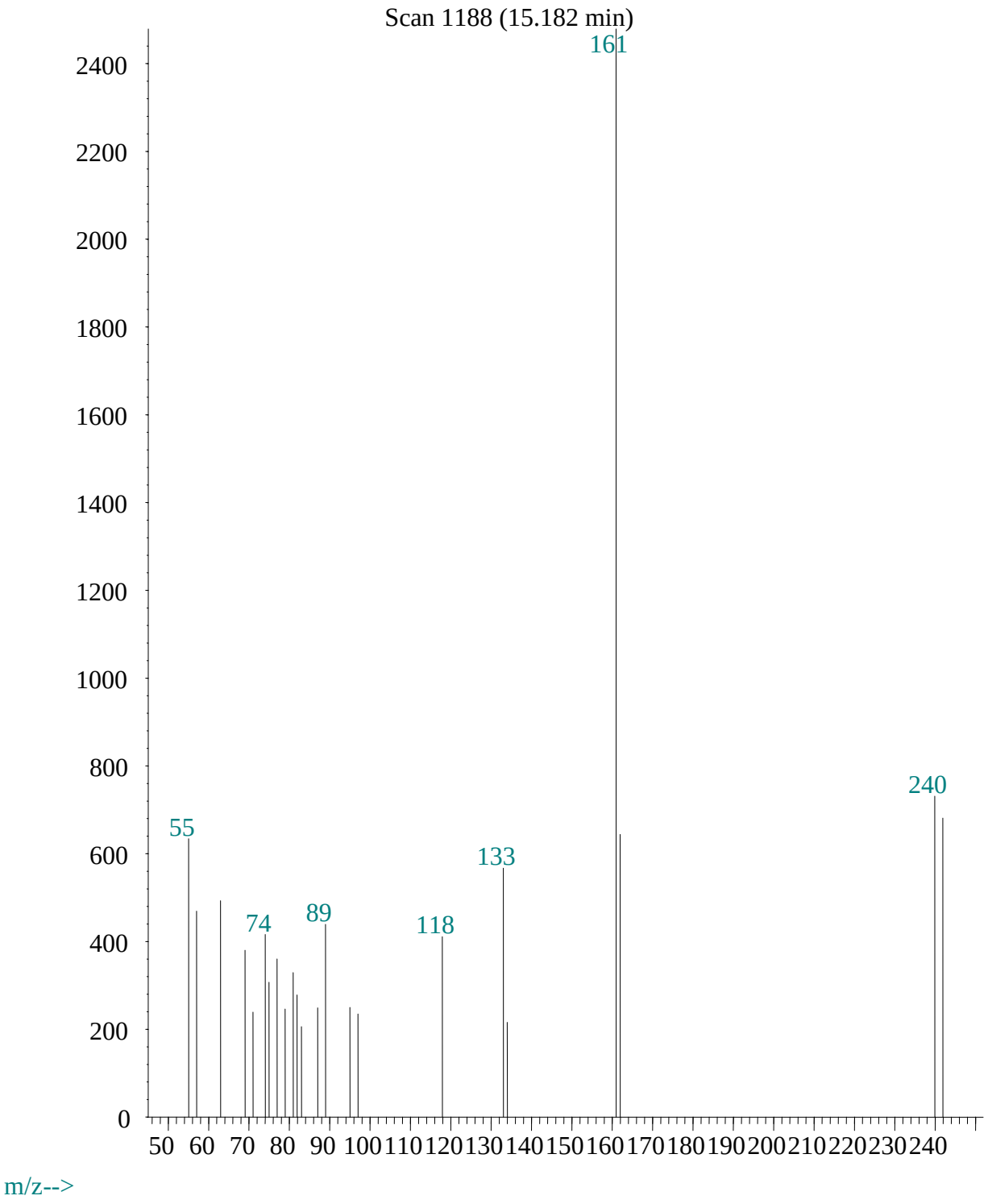


Figure 4.15. Mass spectrum of bromo indanone.

4.4.2 Diester amide of 5-methoxy indanone (48). The keto-diesteramide of indanone **47** was synthesized by applying the same method used in the synthesis of **39** (Scheme 4.6). The GC/MS and ^{1}H NMR spectra were consistent in the characterization of the keto-diesteramide, **47**.The GC/MS of the keto-diesteramide of indanone **47** was run isothermally to give rise to a single chromatogram peak at 11.64 min (Figure 4.16). The sample was run isothermally in order to preserve the molecular ion and therefore the appearance of the corresponding peak at m/z 377 (Figure 4.17). The base peak at 262 min indicated the formation of acylium ion by losing the amide group first and then by deethylation.

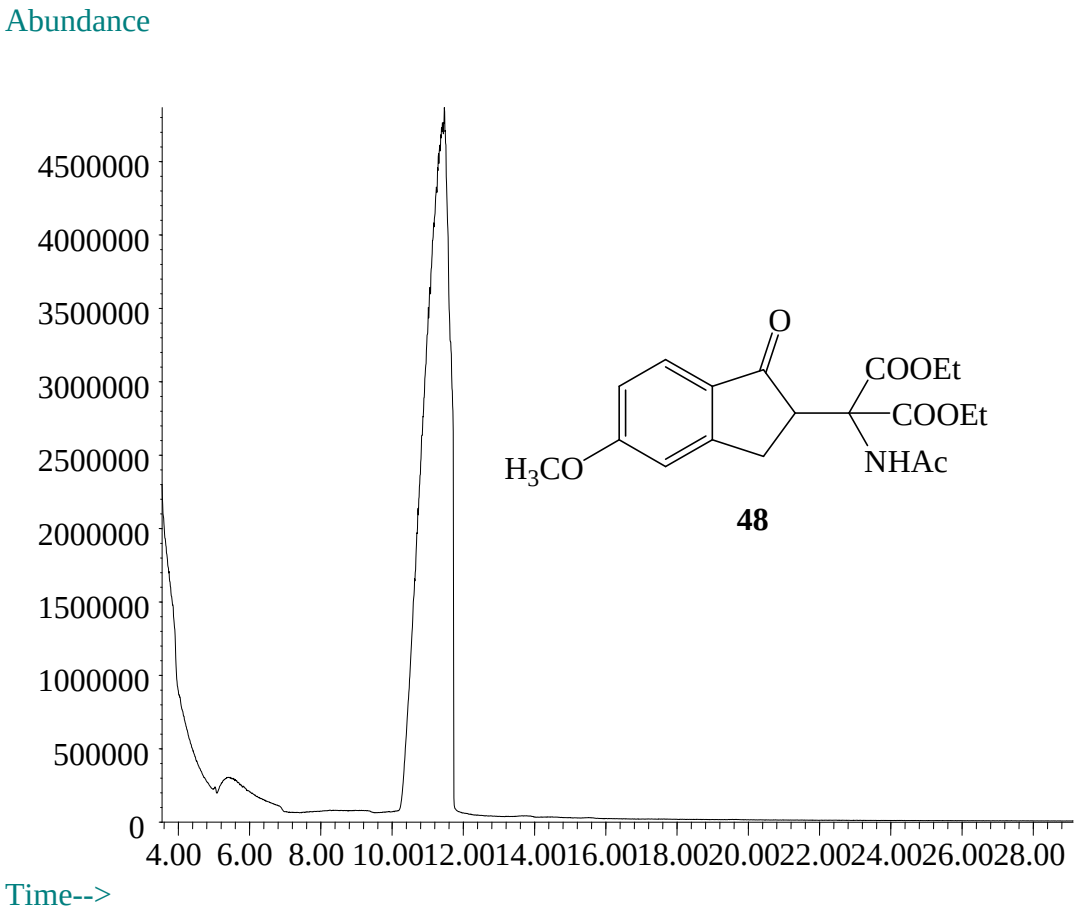


Figure 4.16. Gas chromatogram of diester amide of indanone.

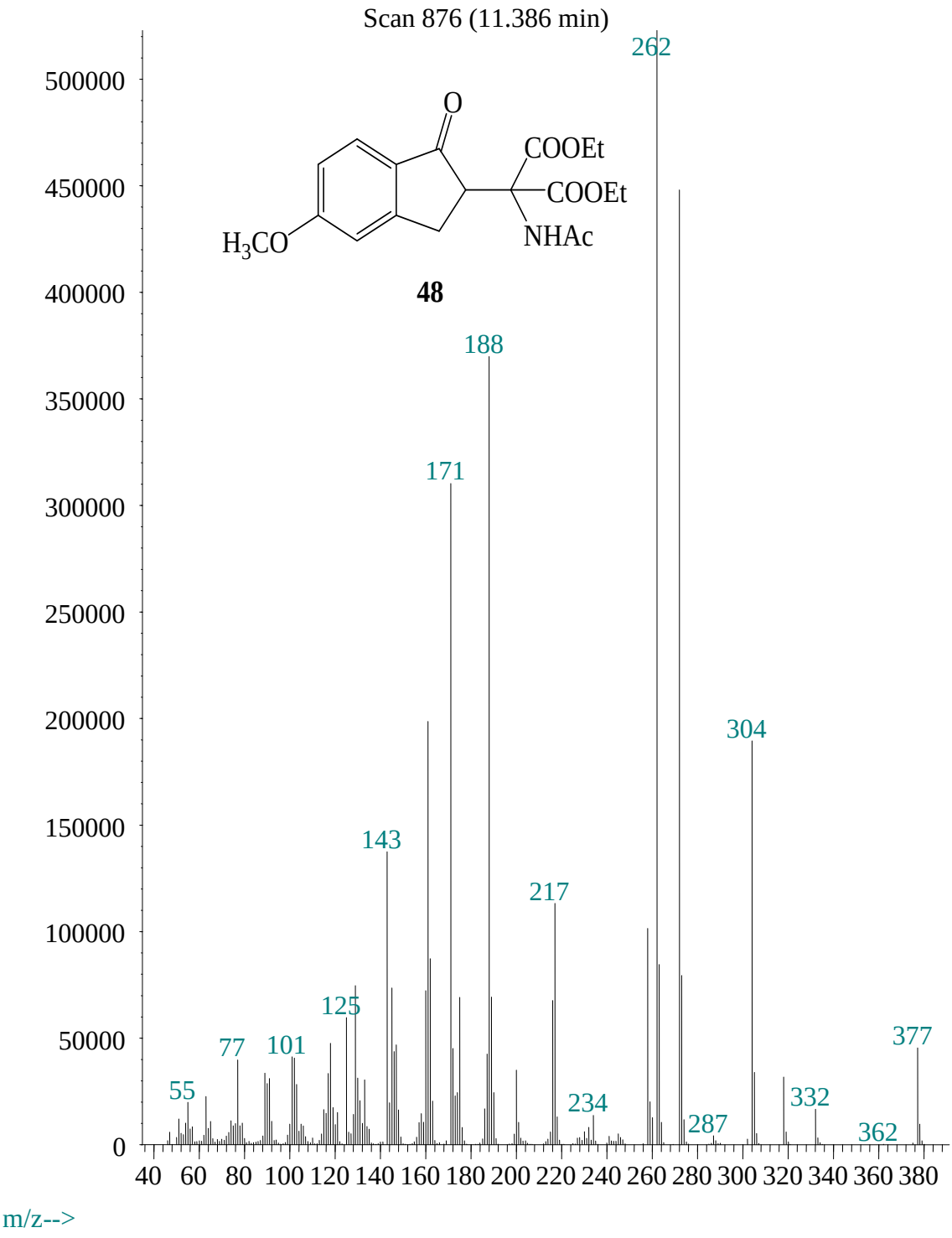


Figure 4.17. Mass spectrum of diester amide of indanone.

4.4.3 Amino acids of 5-methoxy indanone (49) and 5-hydroxy indanone (50). The synthesis of the methoxy-indanone homolog of dopa **49** involved partial deprotection and decarboxylation using 50% aqueous hydrochloric acid (Figure 4.19). The synthesis of hydroxyl-indanone homolog **50** involves the deprotection of methyl group and decarboxylation using conc. hydrobromic acid. The resulting products, **49** and **50** were analyzed using NMR analysis. The NMR spectrum did not show peaks consistent with the expected products.

CHAPTER 5

Conclusion and Recommendations

Many reaction conditions were applied in the synthesis of a variety of dopa homologs. In this work, all of the proposed dopa homologs were successfully synthesized with the exception of the methoxy (**49**) and hydroxy forms (**50**) of the indanone amino acids (Figure 1). Numerous attempts were made in synthesizing **49** and **50**; as these compounds were found to be quite labile within the acid mediums. All precursors to the proposed dopa homologs of the diester amides were successfully synthesized with good-excellent percentage yields, and were all confirmed using GC/MS, FTIR and NMR. In summary, six precursors to the proposed dopa homologs as well as three proposed dopa homologs were successfully synthesized.

Throughout this research, experimental evidence has led to the proper use of acid concentrations in giving rise to the greatest quantities of product yields of the desired amino acids. As a result, it has been determined that 50% hydrochloric acid and concentrated hydrobromic acid were most effective in establishing the most effective/stable reaction medium in giving rise to the amino acids; with the exception of **49** and **50**.

Future prospectives of this work will involve the stereoselective synthesis, and enzymatic resolution of the D and L forms of relevant amino acids. The dopa homologs that were synthesized are expected to be tested for their inhibitory activity on melanogenesis. These same homologs can also be tested on brain and evaluated for their activity on the release of dopamine in patients suffering from psychological disorders. Additional functionalized dopa homologs are to be synthesized and tested for other important biological activity.

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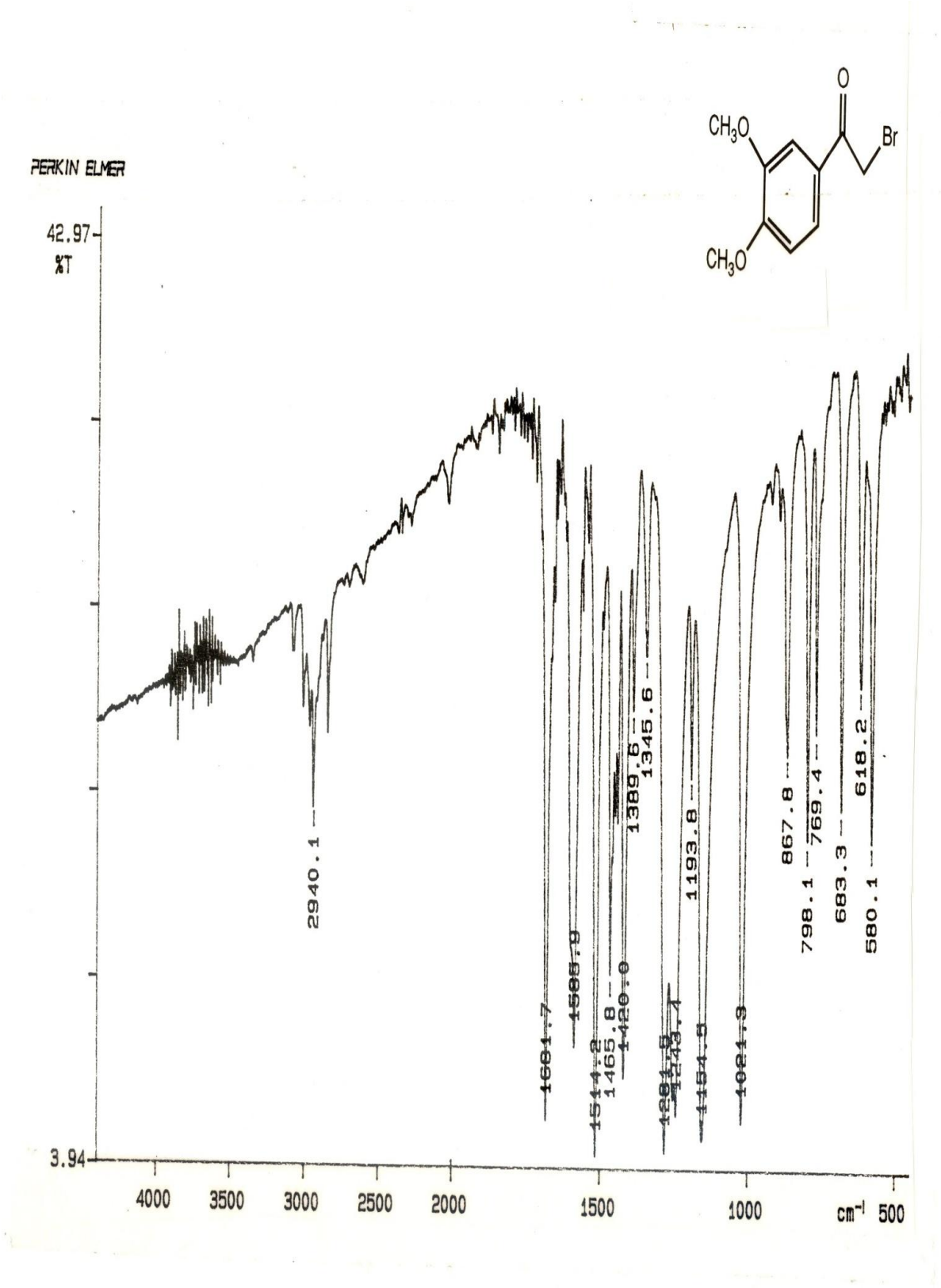
Appendix

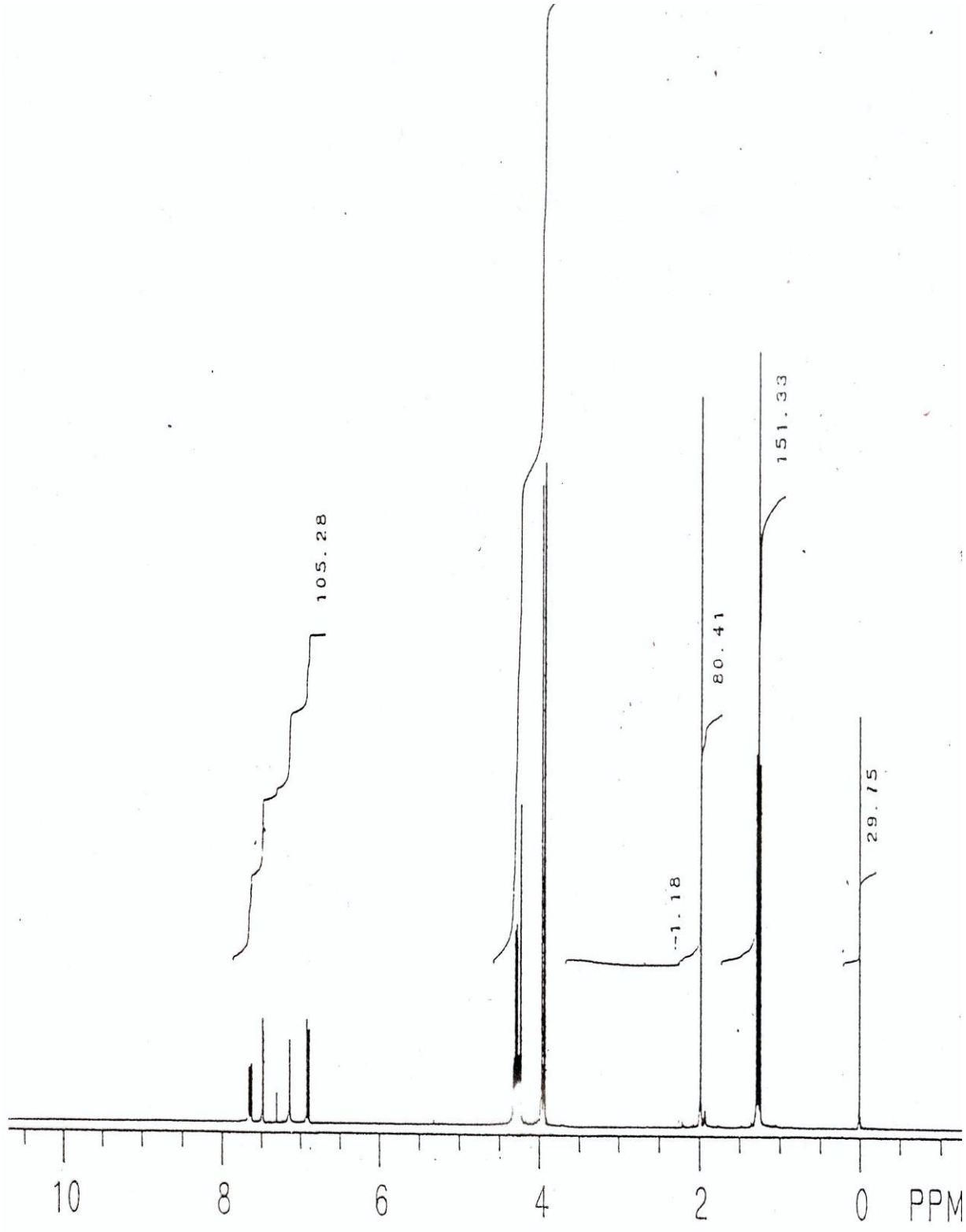
Spectra of compounds

1. ^{1}H NMR of 2-Bromo 3, 4-dimethoxy acetophenone (**38**)

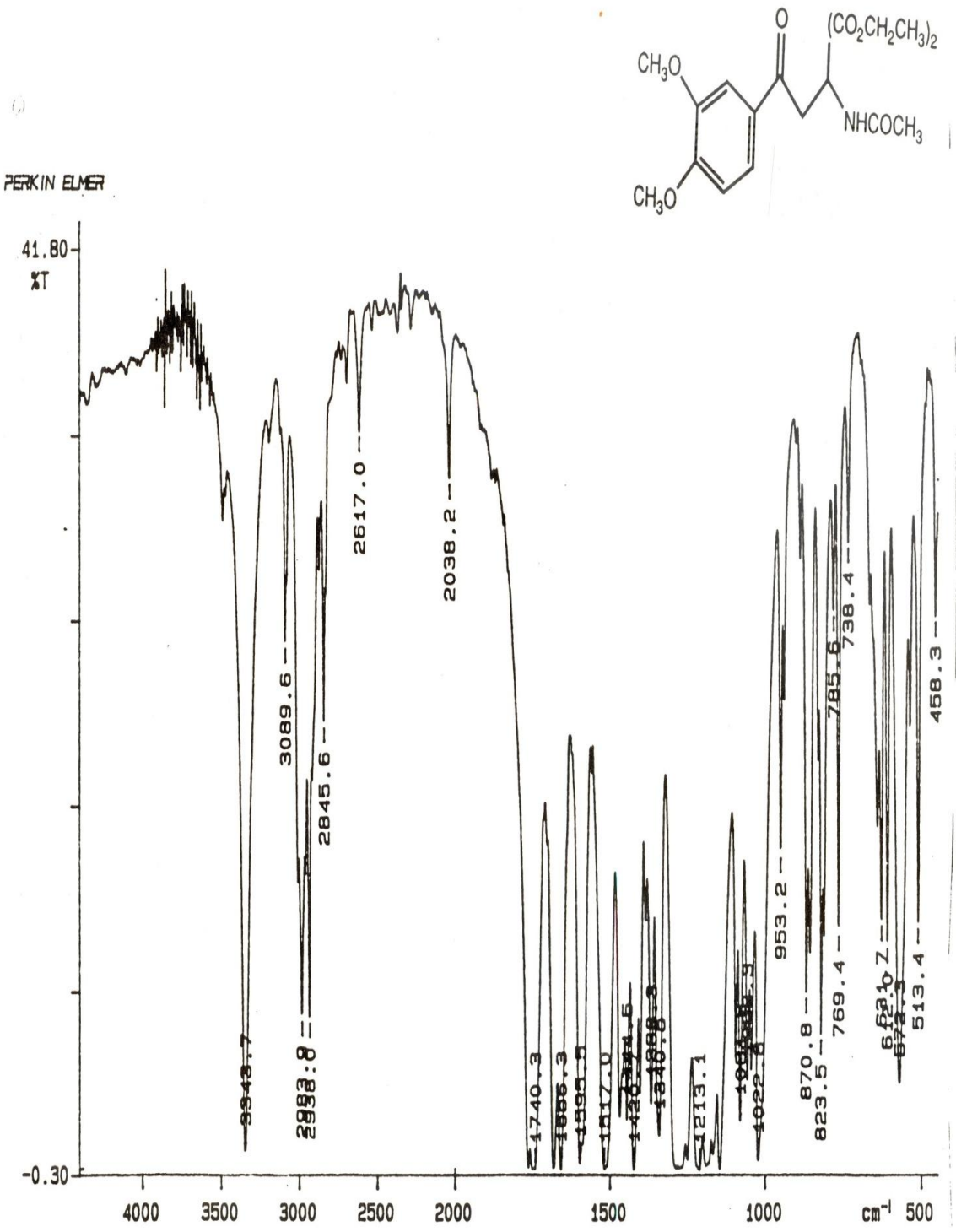
2. FTIR of 2-Bromo 3, 4-dimethoxy acetophenone (**38**)



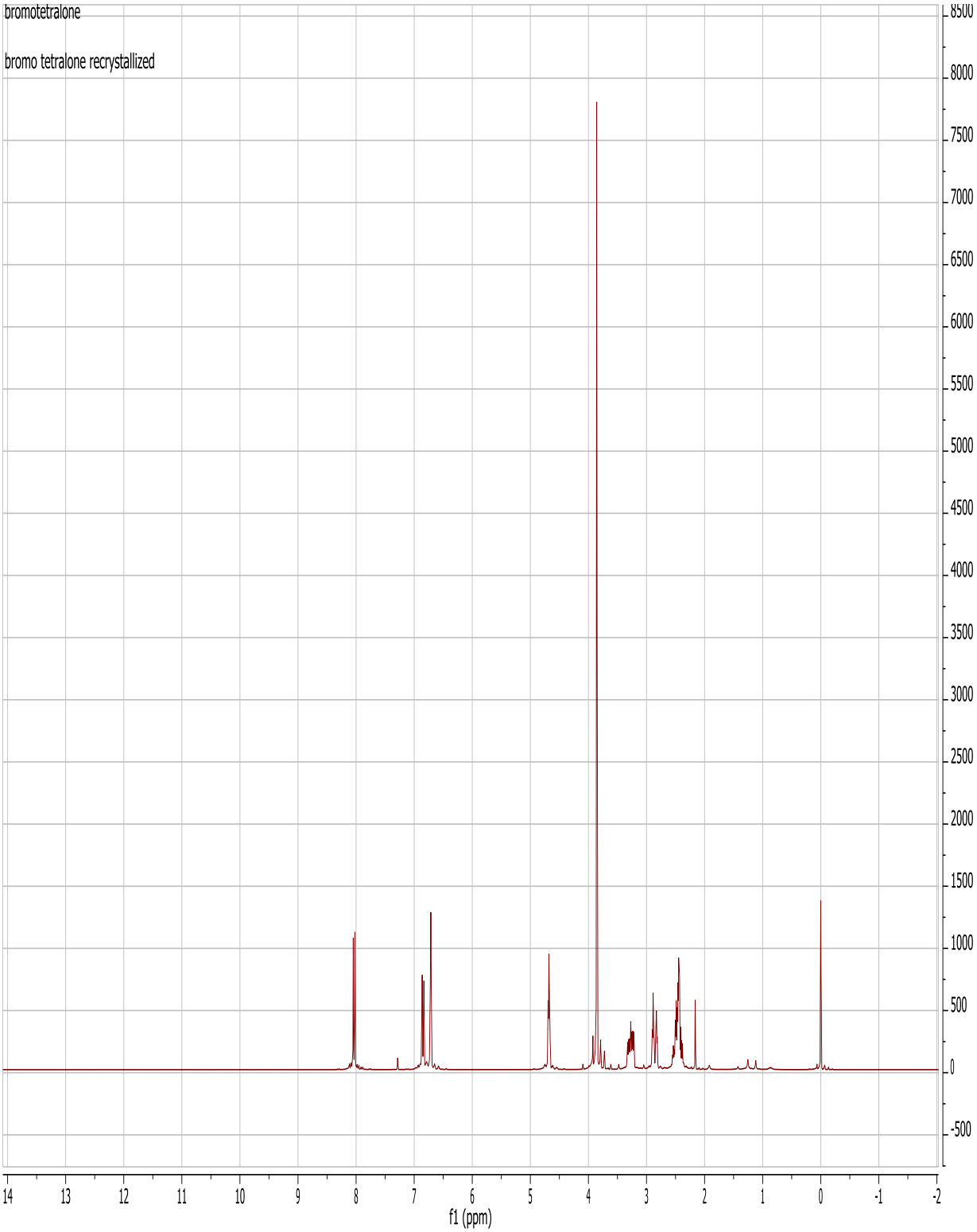
3. ^{1}H NMR of Diethyl 2-acetamido-2-(2-(3,4- dimethoxy phenyl)acetyl) malonate (**39**)



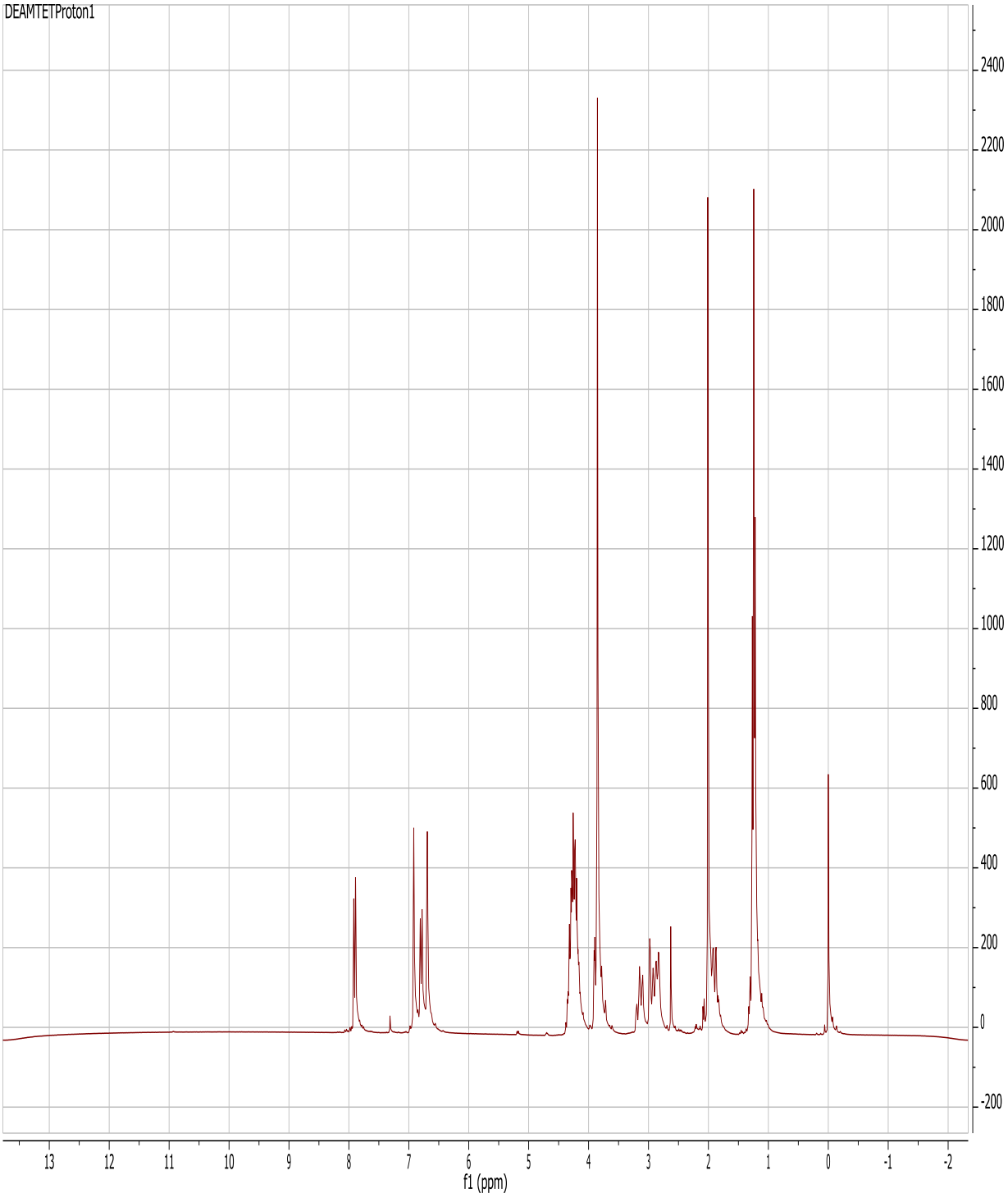
4. FTIR of Diethyl 2-acetamido-2-(2-(3,4- dimethoxy phenyl)acetyl) malonate (**39**)



5. ^{1}H NMR of 2-bromo 6-methoxy tetralone (**42**)



6. ^{1}H NMR of Diester amide of 6-methoxy tetralone (**43**)



7. ^{1}H NMR of 2-bromo 5-methoxy indanone (**47**)

8. ^{13}C NMR of Diester amide of 5-methoxy indanone (**48**)