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Synthesis of a Resveratrol Glycinate Derivative.

Shelley Marie Van Cleve
East Tennessee State University

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Synthesis of a Resveratrol Glycinate Derivative

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A thesis

presented to

the faculty of the Department of Chemistry

East Tennessee State University

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In partial fulfillment

of the requirements for the degree

Master of Science in Chemistry

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by

Shelley Marie Van Cleve

May 2011

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Dr. Yu Lin Jiang, Ph. D Chair

Dr. Kady, Ph. D

Dr. Vasiliev, Ph. D

ABSTRACT

Synthesis of a Resveratrol Glycinate Derivative

by

Shelley Marie Van Cleve

Recently, the compound resveratrol has had media attention as an anti carcinogen. However, the bioavailability of resveratrol is low in the human system due to its hydrophobic nature. Therefore, it must be administered in high dosages to be effective. A plethora of derivatives have been synthesized that have the potential of resveratrol but sadly share low bioavailability. The first effort of this research was an attempt to produce a more hydrophilic ester of resveratrol. Failing this, the final product was synthesized using a glycine derivative to produce 4-[(1E)-2-(3,5-diacetyloxyphenyl)ethenyl]phenyl N-[(1,1-dimethylethoxy)carbonyl]-glycinate.

DEDICATION

In loving memory of my grandfather, John Edward "Buck" Van Cleve Jr.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

"Wine is the most healthful and most hygienic of beverages"[1].

Louis Pasteur (1822-1895)

Fast forward 100 years to 1939, resveratrol was first discovered by M. Takaoka in the roots of the white hellebore. Eventually the compound was found in roughly 70 different plant species, paramount among them is the grape [2]. Resveratrol is the compound responsible for the advantageous characteristic of wine [3]. By the 1990s resveratrol had been studied by many research groups for its vast potential in several medicinal treatments and pharmaceutics. Due to its substantive antioxidant characteristic, it has been targeted as the key compound responsible for the French Paradox, the surprisingly epicurean yet non-detrimental lifestyle [4]. The compound has been found in several different plant genres (Table 1).

Table 1. A list of various foodstuffs containing resveratrol [5]

Foodstuffs	Reference
Grape	Jeandet *et al.* 1995a, Wang *et al.* 2005, Liew *et al.* 2005, Gonzalez-Barrio *et al.* 2005, Yilmaz *et al.* 2004, Roldan *et al.* 2003
Wine	Cantos *et al.* 2003, Wang *et al.* 2002, Vian *et al.* 2005. Shu *et al.*, 2005 Gambuti *et al.*, 2004
Bilberry	Ehala *et al.* 2005, Lyons *et al.* 2003, Rimando *et al.* 2004
High-Bush, Low Bush, Rabbit eye, Elliot's Blueberry	Lyons *et al.* 2003., Rimando et al. 2004
Cranberry	Ehala *et al.* 2005, Wang *et al.* 2002, Rimando *et al.* 2004
Deerberry	Rimando *et al.* 2004
Ligonberry	Rimando *et al.* 2004
Sparkleberry	Rimando *et al.* 2004
Partridgeberry	Rimando *et al.* 2004
Strawberry	Ehala *et al.* 2005
Red Currant	Ehala *et al.* 2005
Rhubarb	Kageura *et al.* 2001
Pistachio	Tokusoglu *et al* 2005
Peanut	Liu *et al.* 2003, Tokusoglu *et al.* 2005, Wang *et al.* 2005, Frank *et al.* 2003, Chen *et al.* 2002, Chung *et al.* 2000, Sanders *et al.* 2000, Sobolev *et al.* 1999, Schoppner *et al.* 1984
Peanut Butter	Sobolev *et al.* 1999, Ibern-Gomez *et al.* 2000

<center>Botanical Classification of Resveratrol</center>

Phytoalexin

Resveratrol is categorized as a phytoalexin. Phytoalexin is a compound that contains two or more benzene rings each having at least one hydroxyl group, also called a polyphenol [6]. This specific phytoalexin is a genetic feature of plants that are high in disease-resistant genotypes and low in disease-susceptible genotypes. The compound is produced to cope with environmental stresses such as UV exposure and fungal infections [7].

Stilbene

Resveratrol is subcategorized as a stilbene [6]. Stilbene is a compound with two benzene rings connected to each other by a conjugated single, double, single bond. Resveratrol is synthesized from two precursor molecules, malonyl-coenzyme A and p-coumaroyl-coenzyme A [4]. The malonyl-coenxyme A is derived from the elongation of acetyl CoA units, while the p-coumaroyl-coenzyme A is derived from phenylalanine [8]. Stilbene synthase enzyme converts one molecule of p-coumaroyl-coenzyme A for every three molecules of malonyl-coenzyme A to resveratrol [9] (Figure 1).
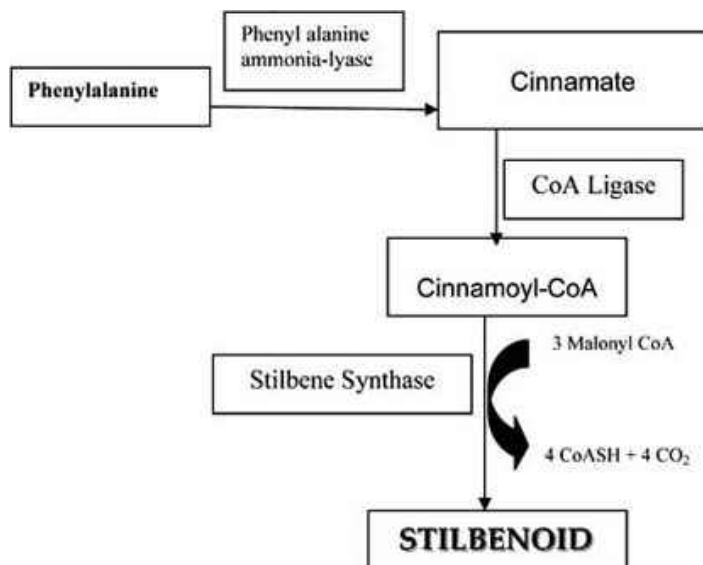
Figure 1. The synthetic route of resveratrol in plants [5]

Once an environmental threat has occurred, the plant host activates a phenylpropanoid pathway upon which stilbene structures are produced [5]. Resveratrol, being an induced stilbene, is secreted from soft tissue such as leaves or fruit to defend the plant [7]. The specific stilbene produced is dependent upon the host, the region of origin, and the environmental stimuli. The most well-known and well-characterized stilbene is resveratrol [5].

Chemical Classification of Resveratrol

Functional Group Activity

A closer look at the chemical aspects of the structure clarifies the compound's efficiencies and deficiencies as a pharmaceutical drug. Chemically resveratrol is described as a 3, 4', 5-trihydroxystilbene [6]. The functional groups are assessed based on their antioxidant capabilities. The hydroxyl functional groups associated with phenolic compounds are the most common and the most effective free radical scavengers in foods. It can donate an electron or the hydrogen to capture and convert free radicals to a more stable molecule [10 & 11]. The hydroxyl group at the 4' position is more involved in antioxidant activity and radical scavenging than

hydroxyl groups at the 3 or 5 positions. This is because of the electronic structure and the formation enthalpy of the three different phenoxyl radicals. The phenol at the 4' position has more resonance structures than either 3 or 5 and is therefore more stable as shown in Figure 2 [12].
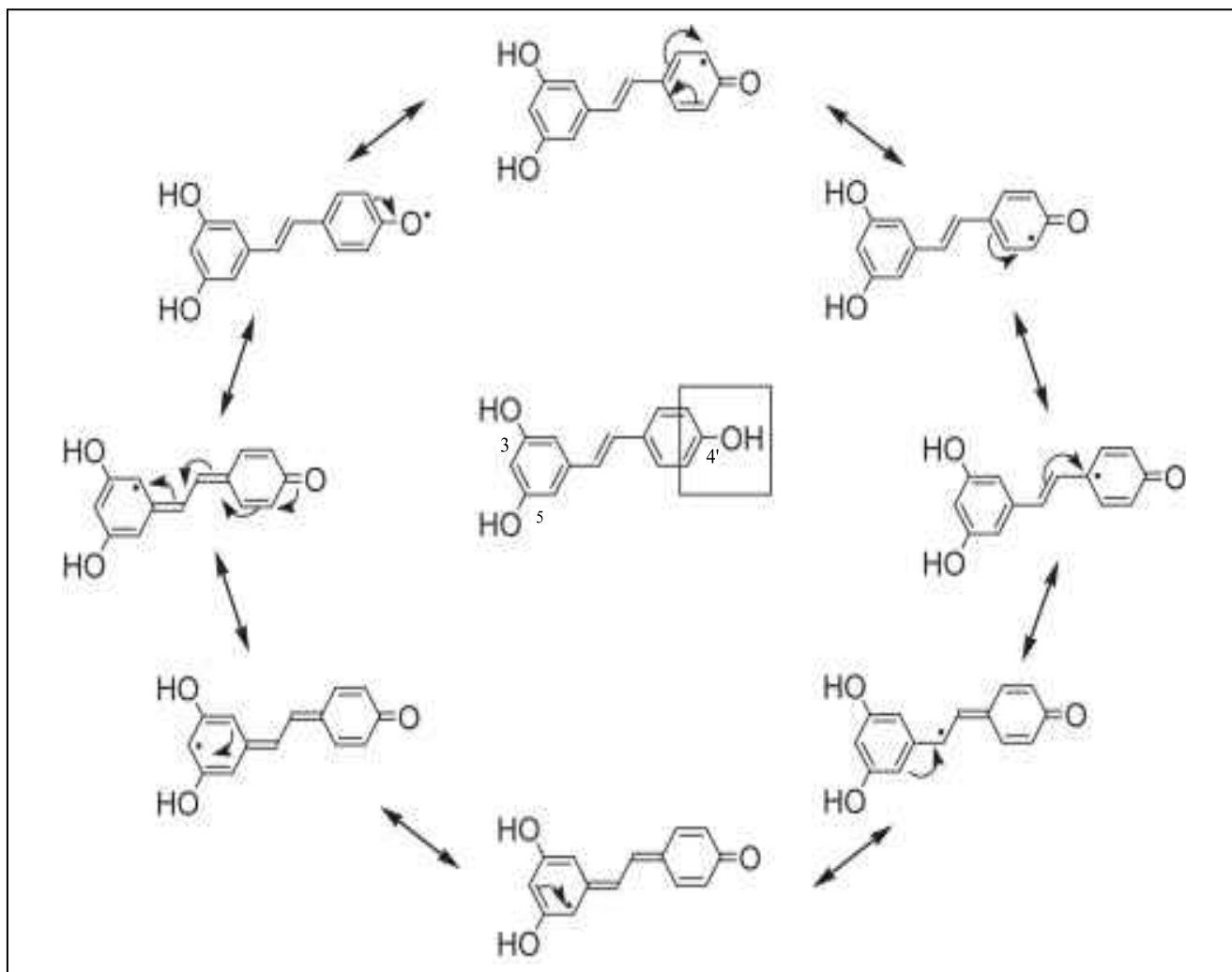
Figure 2. The resonance structure of resveratrol resulting from the loss of hydrogen from the 4' (para) position [13]

The 3, 5 hydroxyl groups do contribute to resveratrol antioxidant activity as shown in Figure 3 [14].
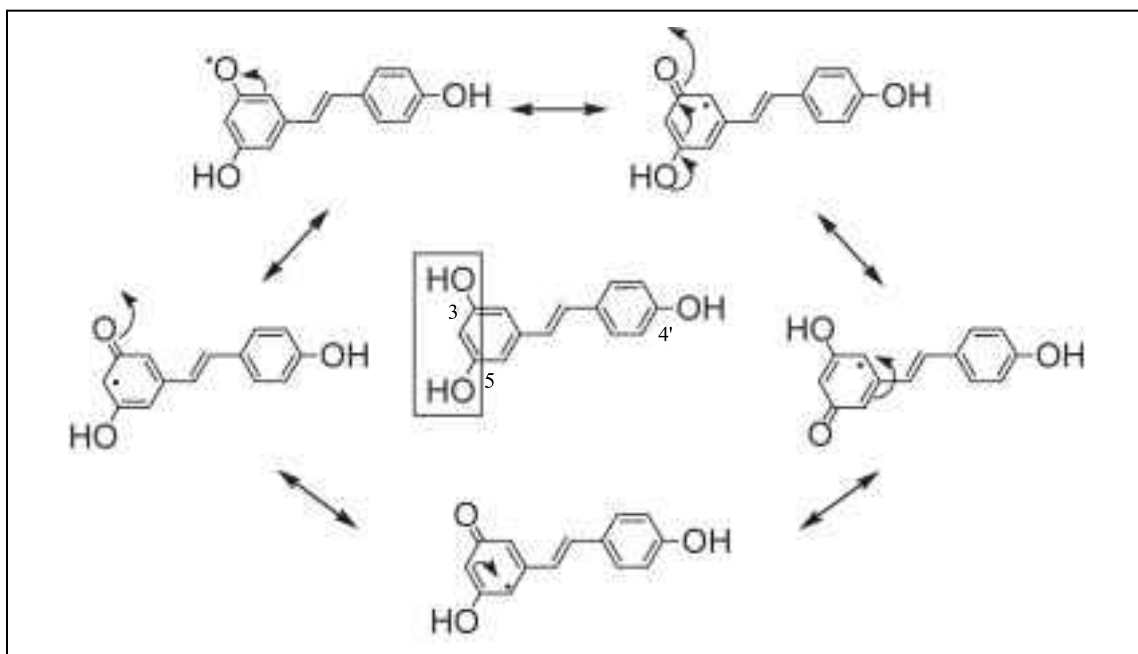
Figure 3. The resonance structures of resveratrol resulting from the loss of hydrogen from the 3, 5 positions [13]

However, the hydroxyl group in the para position and the double bonds of resveratrol play a larger part in the overall decrease in HOMO, IP, and BDE, values, and to the increase of change in E_{iso} value. These values conclude that the antioxidant activity of resveratrol is related to the stabilization energy of 4-hydroxystilbene [13].

<u>Isomerism</u>

Resveratrol has two isomers, shown in Figure 4, dependent upon the rotation of the alpha beta double bond connecting the two phenols.
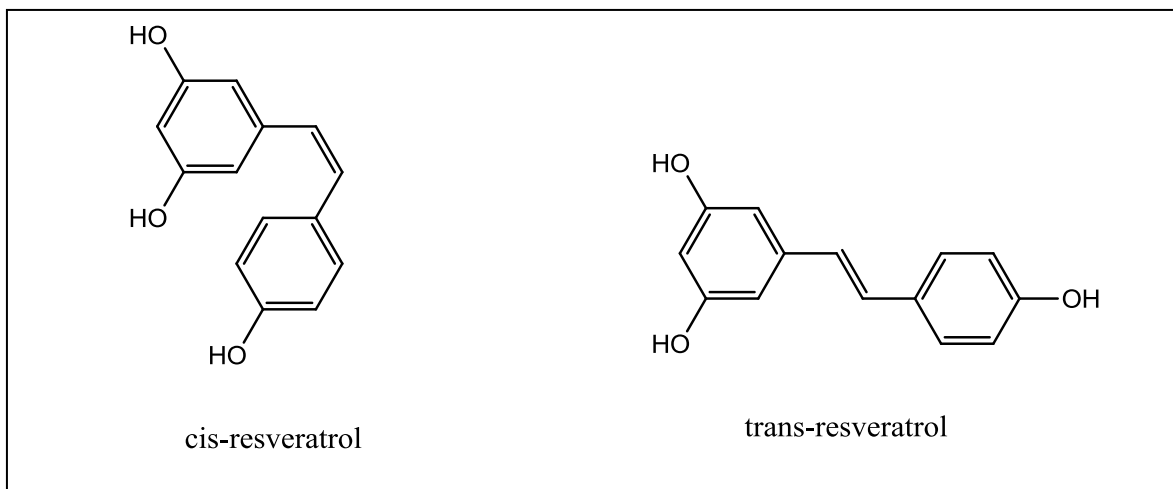
Figure 4. The molecular structure of both cis- and trans-resveratrol [15]

The trans isomer is more commonly seen in nature due to thermodynamics. The hydrogen abstraction from the 4'- position is expected to be favored for the trans isomer because it is more exothermic and therefore has a greater resonance stabilization energy [12]. Cis-resveratrol has a protective effect against oxidative damage and inflammation but not as qualitative as the trans isomer; therefore, it hasn't been studied as profusely [16].

<u>Resveratrol as a Pharmaceutical Drug</u>

<u>Dietary Supplement</u>

Resveratrol was considered worthwhile for mass production as a dietary supplement [5]. The compound had to be synthesized because it was too expensive to extract the small amounts of natural resveratrol from plants [17 & 18]. The largest amount of resveratrol was found in the skins of grapes measuring up 1.25 mg per cup serving [4].

The French Paradox

In 1992 resveratrol was speculated to be the therapeutic molecule responsible for the French Paradox. The French Paradox is an anomaly associated with southern French citizens. These people smoke regularly and eat a high-fat diet yet have a very low mortality rate from coronary heart disease. Scientists have attributed this unlikely relationship to the "moderate consumption of the anti-inflammatory and anti-oxidant polyphenolic compounds" such as resveratrol in red wine. The mechanism for this relationship is speculated to be comparable to calorie restriction [4]. It inhibits the molecular expression of vascular cell adhesion as well as the cell proliferation of vascular smooth muscles. The quantity of red wine intake stimulates the endolethelial nitric oxide synthase (eNOS) activity and inhibits platelet aggregation and LDL peroxidation [19].

Various Medicinal Applications

Then in 1997, it was shown that resveratrol is able to exert cancer therapeutic activity at all three major stages of carcinogenesis, i.e. anti-initiation activity, anti-promotion activity, and anti-progression activity without noxious side effects [20]. As a polyphenol, resveratrol can interfere with the cellular membrane and intracellular receptors, modulate signaling cascades, interact with the basic enzymes involved in tumor promotion and metastasis, and interact with oncogene and oncoproteins. The known capabilities of Resveratrol have expanded from an antioxidant and anti inflammatory agent to antiviral and anti diabetic. It has shown cardioprotective, neuroprotective, and chemoprotective activities [21]. And lastly resveratrol has been shown to protect against infections as well as ischemia, reduce obesity, and prevent aging [2]. Resveratrol has potential as a pharmaceutical drug in a vast array of medical areas.

A proper pharmaceutical drug must target a specific pathway without a large dosage [22]. However, because resveratrol is capable of affecting so many symptoms targeting a specific pathway becomes difficult. The broad spectrum of activity exhibited by resveratrol can lead to possible side effects. Resveratrol also has limited bioavailability [3]. It's quickly metabolized by the liver to resveratrol 3-sulfate and resveratrol 3-glucuronide which are ineffective for any health benefit [16]. A possible solution to these problems is to develop resveratrol analogues that exhibit selectivity for only one target.

Research Effort

The analogues synthesized for this research effort must have high water solubility and a binding affinity toward human serum albumin. Resveratrol needs to have an increase in hydrophilicity to be more soluble in protic solvents such as water. Hydrophobicity can be enhanced by chemical or enzymatical lipophilization, esterification of the carboxylic acid group with a fatty alcohol. The obtainable product is an amphiphilic molecule that can easily penetrate a cellular membrane [10, 22, & 23]. The synthesis of the analogues is an attempt towards anti-cancer drugs. These attributes are to be tested at the pharmacology department.

Three synthetic pathways for a resveratrol analogue were attempted. The first was a simple multi-step chemical synthesis. The scheme included a Jones oxidation, esterification, Finkelstein reaction, and lastly a Williamson's ether synthesis (Scheme 1).

$$Cl(CH_2)_2O(CH_2)_2O(CH_2)_2OH \xrightarrow[\text{Acetone}]{CrO_3 \text{ in } 1.5\text{ M } H_2SO_4} Cl(CH_2)_2O(CH_2)_2OCH_2\underset{\underset{O}{\|}}{C}OH$$
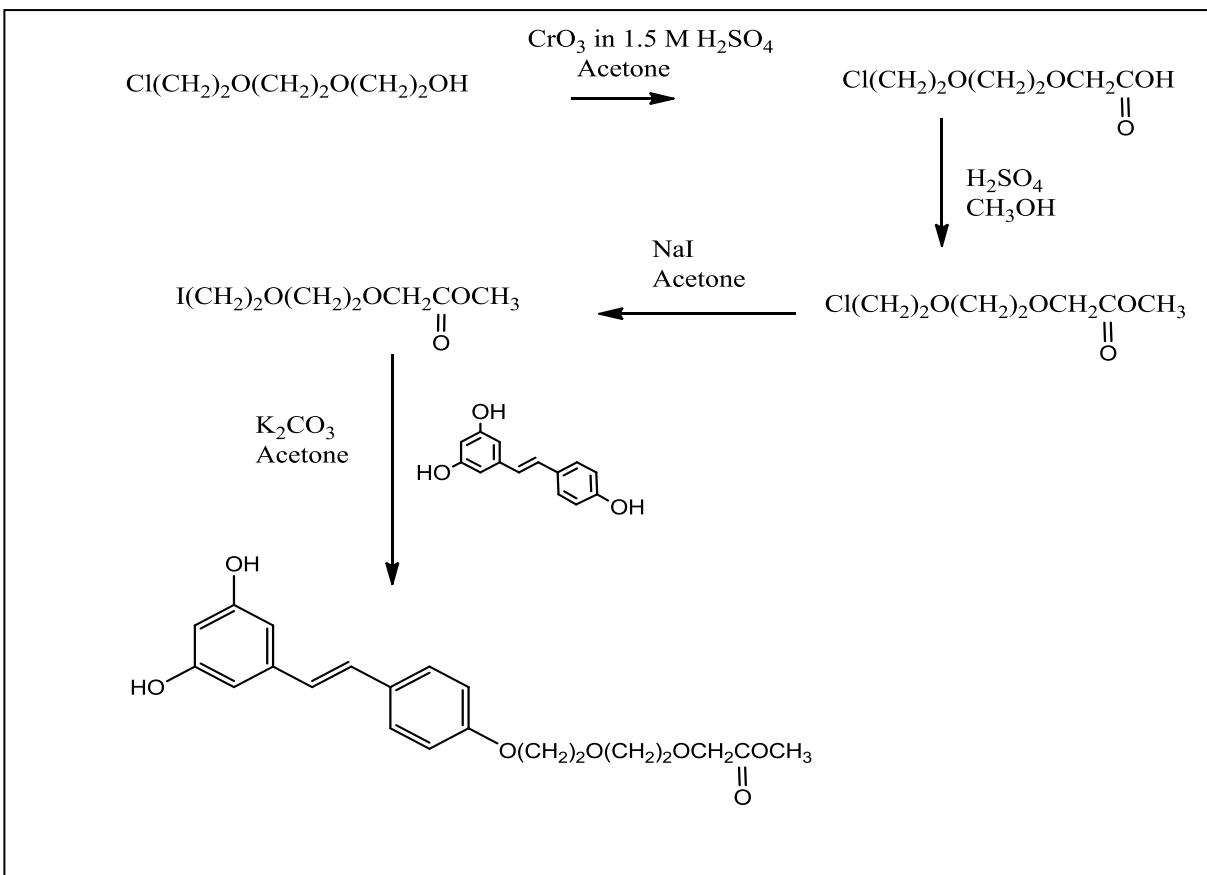
Scheme 1. The synthetic route using method 1

The last step is thought to have failed due to side reactions. The final product was only 92 % pure and had a 2.4 % product yield.

The second method using another ester was more expensive because it consisted of an enzymatic reaction. Resveratrol was first acytelated at each of the three phenol sites. Then the 4' position was hydrolyzed by the enzyme. Finally, the ester replaced the hydrogen of the phenol. The final product could not be separated from impurities on a TLC plate and therefore was inseparable using column chromatography (Scheme 2).
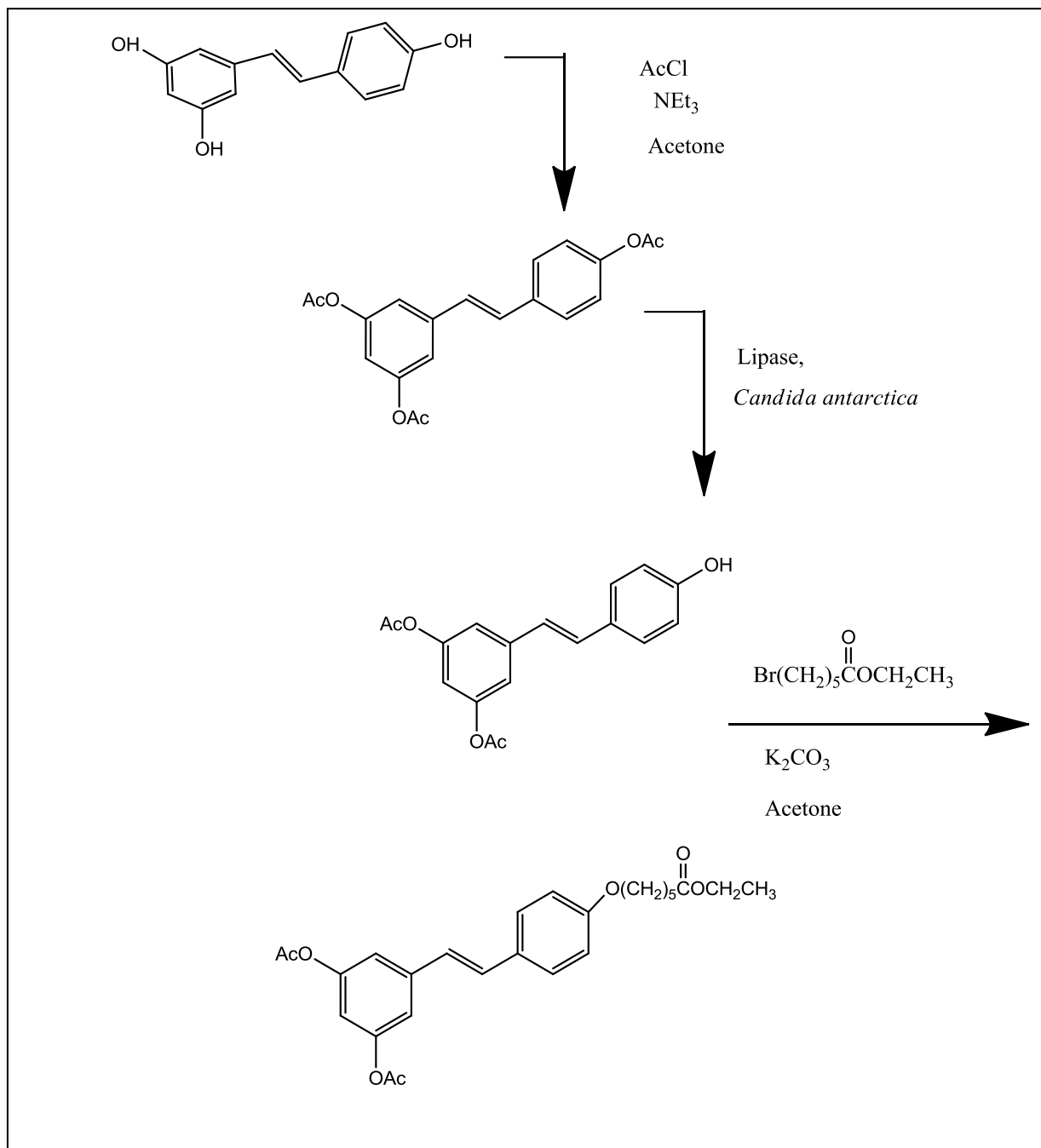
Scheme 2. The synthetic route using method 2

The final method was to attach a glycine derivative to resveratrol at the 4' position by forming an ester (Scheme 3). The final product was pure according to the NMR, CNMR, Mass Spectrometry, and IR spectrum. The final yield was 55 %.
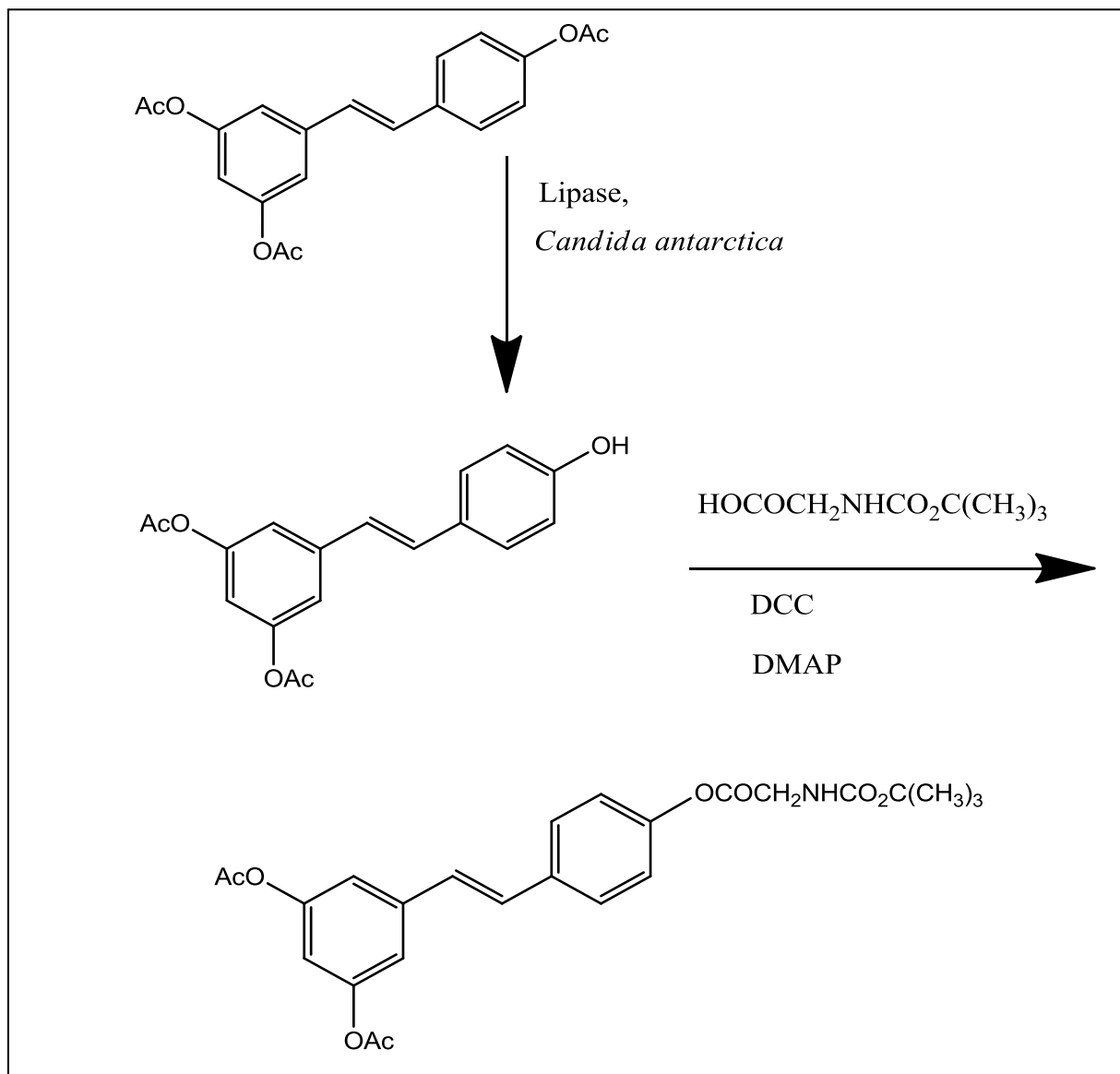
Scheme 3. The synthetic route using method 3

CHAPTER 2

EXPERIMENTAL

<u>General Methods</u>

All commercial reagents were purchased from Sigma (St. Louis, MO, USA). These reagents were used without further purification unless otherwise stated. All organic solvents were evaporated under a fumehood or by a rotovap.

The proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on JEOL-NMR Eclipse spectrometer operating at 400 MHz and 1000 MHz for proton and carbon nuclei respectively. The chemical shifts were recorded at delta values in parts per million (ppm) relative to TMS. The multiplicity of signals is reported as follows: s, singlet; d, doublet; m, multiplet. The Mass spectral analysis was carried out using a Shimadzu GCMS-QP2010 Plus instrument and the Infrared Spectra were obtained using the Shimadzu IR Presctige-21. Thin layer chromatography (TLC) and Large TLC 500 μM were performed with silica gel plates and visualized under a UV fluorescent. The stationary phase for column chromatography was silica gel. Melting points were recorded using a Cambridge MEL-TEMP instrument and were not corrected.

Synthesis of 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid

$$\text{HOCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl}$$
$$\overset{\overset{\displaystyle O}{\|}}{}$$
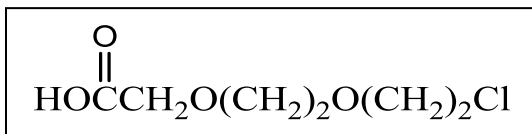
2

      1.5 M solution of sulfuric acid was chilled to 0˚C. Chromium VI oxide   (5.79 g, 38 mmol) was dissolved in this solution before adding, dropwise, the solution of 2-(2-(2 chloroethoxy)ethanol (1.69 g, 10 mmol) in acetone (50 mL). Once all of the acetone solution was added, the mixture was allowed to warm to room temperature before stirring for 18 hours. After the allotted time gravity filtration was performed to remove the chromium salts. Then the solution, freed of the chromium salts, was concentrated using a rotovapor, evaporating the acetone. The crude product was then removed from the concentrated solution using dicholormethane (3×100 mL). Magnesium sulfate (2 g) was used to dry the combined extracts. The extract was then gravity filtered to remove the magnesium sulfate. The extract was concentrated using a rotovapor. The product, 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid, (1.7 g,) was a colorless oil. ^{1}H NMR (400 MHz, CDCl$_3$, ppm) δ 3.85-3.6 (m, 8 H), δ 4.18 (d, J = 2 H), δ 7.9 (s, 1 H) [24].

Synthesis of Methyl 2-(2-(2-Chloroethoxy)ethoxy)acetate

$$CH_3OCCH_2O(CH_2)_2O(CH_2)_2Cl$$

(with carbonyl O above the second C)
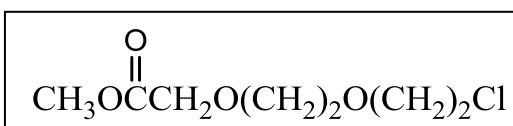
3

A reflux was prepared using a 15-mL round bottom flask. The product from the first synthesis, 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid reacted with concentrated H_2SO_4 (1 drop) in a methanol solution (5 mL). The reflux was carried out for 16 hours. After the allotted time, the solution was evaporated in the fumehood to dryness. The crude product was then dissolved in dichloromethane (10 mL). This solution was washed with 5% $NaHCO_3$ (2×15 mL). The organic, bottom layer was extracted then dried with $MgSO_4$. The cloudy solution became clear upon addition of the drying agent. The solution was gravity filtered, removing the $MgSO_4$, then dried for characterization. ^1H NMR (400 MHz, $CDCl_3$, ppm) δ 3.8-3.55 (m, 8 H), δ 4.12 (s, 5 H).

Synthesis of Methyl 2-(2-(2-Iodoethoxy)ethoxy)acetate

$$CH_3OCCH_2O(CH_2)_2O(CH_2)_2I$$

(with carbonyl O above the second C)
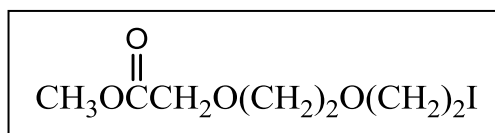
4

A solution was prepared of sodium iodine (2.862 g, 300 mmol) dissolved in acetone (37.55 mL) for a 32-hour reflux. Once the sodium iodine had completely dissolved methyl 2-(2-(2-chloroethoxy)ethoxy)acetate (0.751g, 60 mmol) was added to the solution. After 12 hours of reflux chloride began to precipitate out of the solution. Once 32 hours had passed the solution was evaporated to half of its original volume. The solution was then separated between chloroform and distilled water. The organic layer was extracted, washed with sodium thiosulfate, and then washed with distilled water. The product was dried using magnesium sulfate then

gravity filtered. Once the chloroform had evaporated in the fumehood, a yellow solution

remained. [1] H NMR (400 MHz, CDCl$_3$, ppm) δ 3.2 (m, 3 H), δ 3.8-3.5 (m, 5 H), δ 4.1 (d, J =

2.56 Hz, 5 H) [25].

Synthesis of 4'-(2{2-[Carboxymethoxy)ethoxy]ethoxy})- 3,5 hydroxystilbene
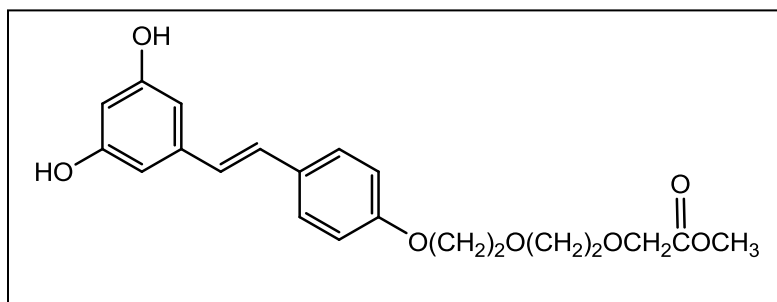
5

Resveratrol (0.198 g, 0.868 mmol) was dissolved in a 50-mL round bottom flask using

acetone (20 mL). Half of the, pulverized potassium carbonate (0.240 g, 1.73 mmol) and ester,

methyl 2-[2-(2-iodoethoxy) ethoxy] acetate, (0.25 g, 0.868 mmol) were added to the acetone

solution for the first eight hours of reflux. The temperature of the reflux was 65°C. After the first

eight hours the rest of the pulverized, potassium carbonate and the ester were added. Once the 16

hours had expired half of the acetone was evaporated. Saturated ammonium chloride (20 mL)

was poured over the solution to decompose the potassium carbonate and therefore neutralize the

solution. The organic layer was extracted using ethyl acetate (3×20 mL). The ethyl acetate was

evaporated under a fumehood before purification of the crude product using column

chromatography. The solvent system was ethyl acetate in hexane (40%-70% in fives) then a

smaller column using acetone in hexane (5%-40% in fives). The fractions were taken as 20 mL

aliquots. The last purification step was using the large silica TLC plate (500 mM). The solvent

system was 45% acetone in hexane. The last line was cut from the plate as the product. The

product was extracted from the silica using 100% acetone. 4'-(2{2-[Carboxymethoxy)ethoxy]ethoxy})- 3,5 hydroxystilbene was the remaining liquid. It was 4 mg and 92% pure. ^{1}H NMR (400 MHz, MeOD, ppm) δ 3.3 (t, J = 2.8 Hz, 2 H), δ 3.8 (t, J = 2 Hz, 4 H), δ 4.12 (t, J = 4 Hz, 4 H), δ 4.16 (d, J = 2 Hz, 3 H), δ 6.21(s, 1H), δ 6.26 (d, J = 6 Hz, 2 H), δ 6.37 (s, 1 H), δ 6.43 (s, 1 H), δ 6.89 (t, J = 1.2 Hz, 2 H), δ 6.9 (d, J = 4 Hz, 2 H), δ 7.3 (d, J = 8 Hz, 2 H), δ 7.4 (d, J = 8 Hz, 2 H).

Synthesis of 3, 5, 4'-Tri-O-acetyl resveratrol

6
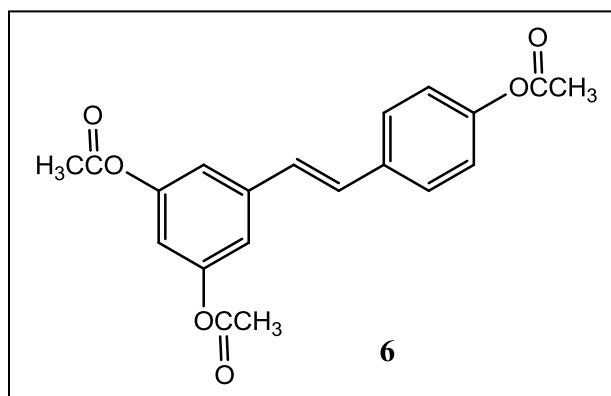
A solution of resveratrol (0.6 g, 2.629 mmol), acetyl chloride (0.5607 mL, 7.887 mmol), and triethylamine (1.096 mL, 7.887 mmol) was stirred in a 50-mL round bottom flask with acetone (20 mL) acting as the solvent. After the solution was stirred for 12 hours, it was acidified (≤5) using hydrochloric acid (2N). The crude product was extracted in the organic layer using ethyl acetate (3×20 mL). Sodium hydrocarbonate (20 mL) was used as a washing to remove excess hydrochloric acid. The product was dried using magnesium sulfate then filtered with gravity. ^{1}H NMR (400 MHz, MeOD, ppm) δ 2.2 (d, J = 5.88 Hz, 6 H), δ 6.8 (t, J = 0.36 Hz, 1 H), δ 7.10 (d, J = 8.8 Hz, 1 H), δ 7.1486 (s, 1 H), δ 7.2 (q, J = 1.12 Hz, 3 H), δ 7.6 (d, J = 8.44 Hz, 2 H) [26, 27, 28].

Synthesis of 3, 5-Di-O-acetyl-resveratrol
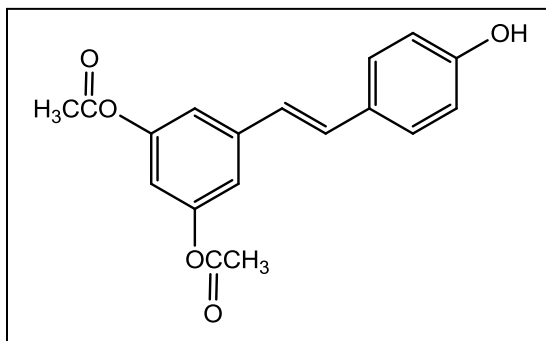
7

3, 5, 4'-Tri-O-acetylresveratrol (100 mg, 0.28 mmol) was put in a 25-mL round bottom flask with "t"-butylmethyl ether (5 mL, 42 mmol). *C. Antarctica* (100 mg) in n-butyl alcohol (0.4 mL) was pipette into the round bottom flask. The mixture was stirred using a rotovap (40 ˚C, 280 rpm) for 30 minutes. After the allotted time the enzyme was filtered and the filtrate evaporated leaving a residue. The crude product was purified using column chromatography. The solvent system was acetone in hexane (5 %- 30 %). ^{1}H NMR (400 MHz, CDCl$_3$, ppm) δ 2.32 (s, 6 H), δ 6.79 (m, 5 H), δ 6.81 (d, J = 1.84 Hz, 2 H), δ 6.86 (s, 1 H), δ 6.9 (s, 1H), δ 7.01 (s, 1 H), δ 7.08 (d, J = 2.2 Hz, 2 H), δ 7.36 (d, J = 8.4 Hz, 1 H) [26, 27, 28].

Synthesis of 4'-(Ethyl 6-oxyhexaneoate) 3, 5,-Di-O-acetyl-resveratrol
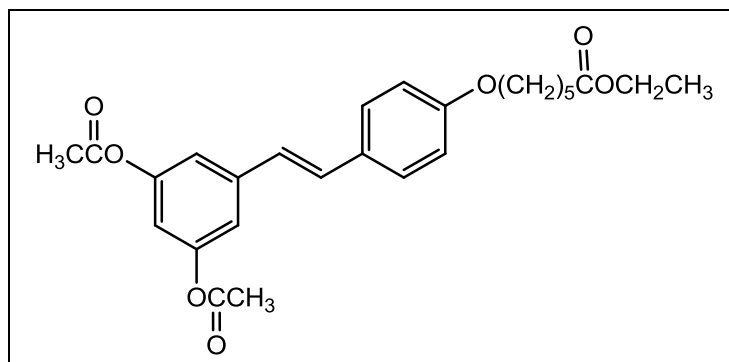
8

The compound 3, 5-di-O-acetyl-resveratrol (0.55 g, 1.757 mmol) was dissolved in acetone (20 mL) in a 25-mL round bottom flask. The potassium carbonate (0.97 g, 7.029 mmol) was not pulverized before addition and the ester (0.78 g, 3.514 mmol) was the last reagent to be added. The temperature was set at 50°C and the reflux time was 16 hours. The dissolving and extraction techniques were the same as for compound **5**. The solvent system for the column was acetone in hexane (5%-35% in fives). The fractions were collected as 20 mL aliquots. The fourth spot on TLC was further purified using a large TLC plate (500 mM). The solvent system was acetone in hexane (30%). The final product was extracted from the silica using acetone (100%). The final product was an oil, 6 mg, and had 33% purity. ^{1}H NMR (400 MHz, CDCl$_3$, ppm) δ 1.2 (t, J = 7.32 Hz, 2 H), δ 1.5 (q, J = 2.92 Hz, 2 H), δ 1.7 (t, J = 0.4 Hz, 2 H), δ 1.8 (t, J = 0.36 Hz, 2 H), δ 2.3 (t, J = 11.64 Hz, 2 H), δ 3.9 (t, J = 0.4 Hz, 2 H), δ 4.1 (q, J = 0.36 Hz, 3 H), δ 6.5 (t, J = 2.2 Hz, 2 H), δ 6.8 (t, J = 1.44 Hz, 2 H), δ 6.92 (s, 1 H), δ 6.96 (s, 1 H), δ 7.02 (s, 1 H), δ 7.0 (d, J = 8.8 Hz, 2 H), δ 7.4 (d, J = 8.8 Hz, 1 H), δ 7.4 (d, J = 6.96 Hz, 1 H).
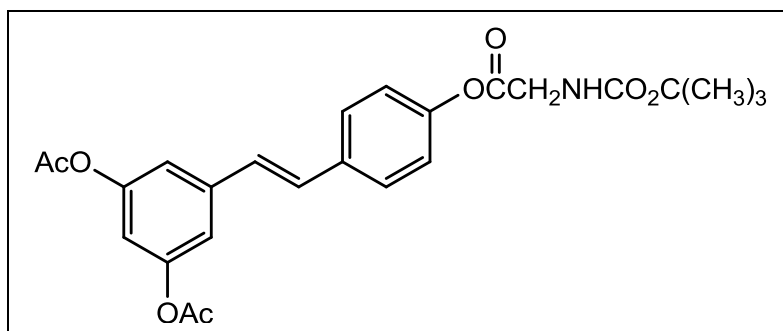
Synthesis of 4-[(1E)-2-(3,5-Diacetyloxyphenyl)ethenyl]phenyl N-[1,1-dimethylethoxy)carbonyl]-glycinate


9

The 3, 5-di-"O"-acetylresveratrol (0.048 g, 0.153 mmol), N-(tert-butoxycarbonyl) glycine (0.0269 g, 0.153 mmol), and 4-dimethylaminopyridine (0.0269 g, 0.220 mmol) were put in a 10-mL round bottom flask with acetonitrile (1 mL). The flask was heated until all of the compounds dissolved then allowed to cool to room temperature. Once cooled to room temperature, dicyclohexyl carbodiimide (0.038 g, 0.183 mmol) was added. The solution was stirred at room temperature for 18 hours. Once the time had elapsed the solution was filtered. The white precipitate was washed with acetonitrile (1 mL) to collect all of the solution. Purification was done with column chromatography. The solvent system was acetone in hexane (5-35% in fives). The fractions were collected in 20 mL aliquots. The compound weighed 15 mg and was 100 % pure according to characterization tests. The reaction had a 56% yield while the overall synthesis, method 3, had a 4% yield. M.p. 119-122°C. IR (neat, cm^{-1}) 1762.94, 163.86, 1506.41, 1371.39, 1197.79, 1165.00, 1126.43, 1024.20. ^{1}H NMR (400 MHz, CDCl$_3$, ppm) δ 1.46 (s, 1 H), δ 1.62 (d, J = 8.8 Hz, H), δ 2.3 (s, 1 H), δ 4.1 (d, J = 5.88 Hz, 1 H), δ 5.08 (d, J = 23.44 Hz 1 H), δ 6.81 (d, J = 2.2 Hz, 2 H) δ 6.9 (s, 1 H), δ 7.03 (s, 1H), δ 7.11 (t, J = 3.68 Hz, 2 H), δ 7.2 (s, 1 H), δ 7.49 (d, J = 8.8 Hz, 2 H). ^{13}C NMR (100 MHz, CDCl$_3$, ppm) 21.24, 28.40, 42.70, 76.79, 77.12, 77.43, 114.59, 117.05, 121.76, 127.47, 127.81, 129.59, 134.84, 139.54, 150.08, 151.37, 155.80, 169.15. GCMS calcd for C$_{25}$H$_{27}$NO$_8$ ($^+$M + H) 469, found 469.

CHAPTER 3

RESULTS AND DISCUSSION

Synthesis of 2-(2-(2-Chloroethoxy)ethoxy)ethanoic acid

$$\text{HOCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl}$$
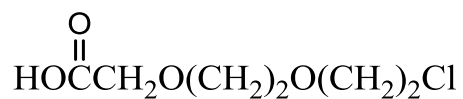
2

Compound **2** was obtained as a colorless oil after extraction with dichloromethane. The reaction is a Jones oxidation. The oxidizer, chromic acid, oxidizes an alcohol through an aldehyde to a carboxylic acid, in a two-step reaction. A chromate ester is believed to be formed before the aldehyde and carboxylic acid formations. Acetone precipitates the reduced chromate [29]. The final product 2-(2-(2-chloroethoxy)ethoxy)ethanoic acid had an experimental yield of 88% while the reported literature yield was 93% [24]. The synthetic route is shown in Scheme 4.
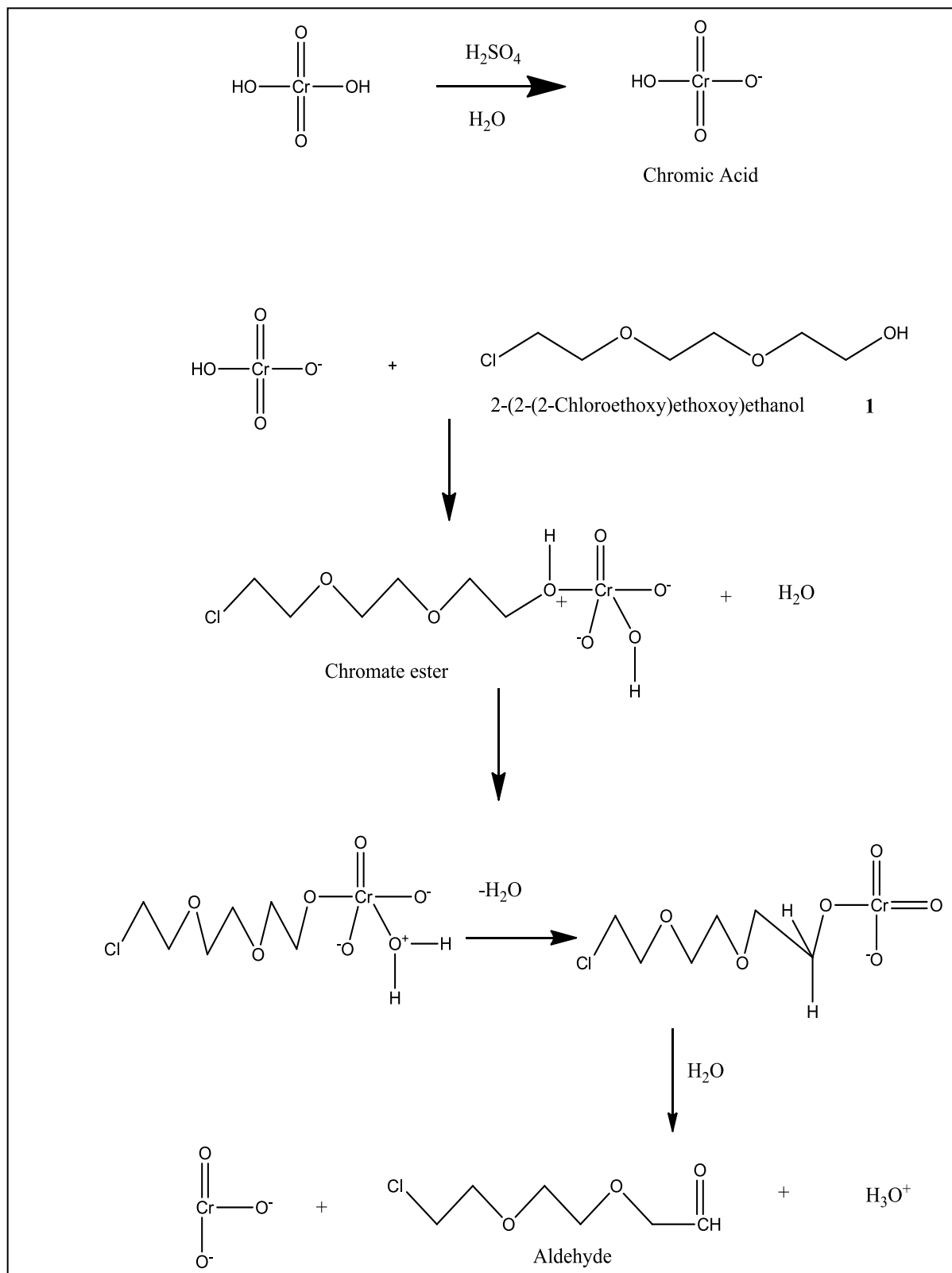
Scheme 4. Synthesis of a carboxylic acid **2** through a Jones oxidation (continued on the next page)
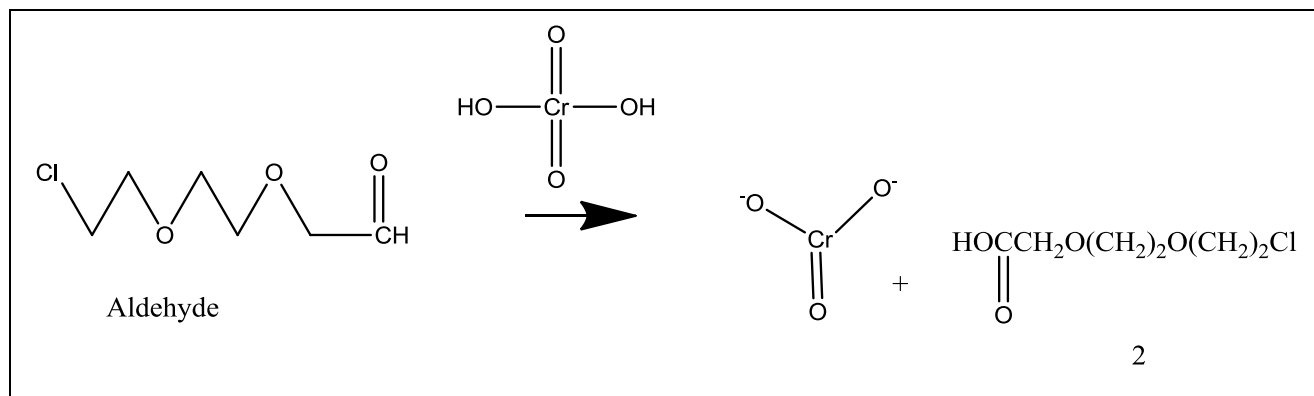
Synthesis of Methyl 2-(2-(2-Chloroethoxy)ethoxy)acetate
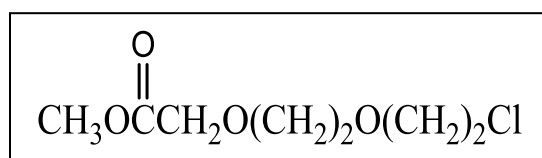
3

Compound **3** was the product of a Fischer esterification. The catalyst, sulfuric acid, protonated the double bonded oxygen of the carboxylic acid to form a positively charged carbonyl. The oxygen of methanol attacks the carbonyl by a nucleophilic acyl substitution reaction. Once water and a hydrogen atom have broken off an ester, methyl 2-(2-(2-chloroethoxy)ethoxy)acetate forms [29]. The final product was a yellow liquid with an experimental yield of 60% after a 16-hour reflux. The synthetic route is shown in Scheme 5.

$$\text{HOCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl} \qquad\qquad + \qquad\qquad \text{H}_2\text{SO}_4$$

with the O double bonded above the C (carboxylic acid).

2

$$\left[\text{Cl(CH}_2)_2\text{O(CH}_2)_2\text{OCH}_2\overset{+}{\text{C}}\text{OH} \right] \quad \text{with OH above}$$

$$\xleftarrow{\text{CH}_3\text{OH}}$$

$$\text{Cl(CH}_2)_2\text{O(CH}_2)_2\text{OCH}_2\overset{OH}{\underset{OCH_3}{\overset{+}{\text{C}}}}\text{OH}_2$$

$- \text{H}_2\text{O}$

$$\text{CH}_3\text{OCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl} \qquad (\text{with } ^+\text{OH above})$$

$$\xrightarrow{- \text{H}^+}$$

$$\text{CH}_3\text{OCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl} \qquad (\text{with O double bond above})$$

3
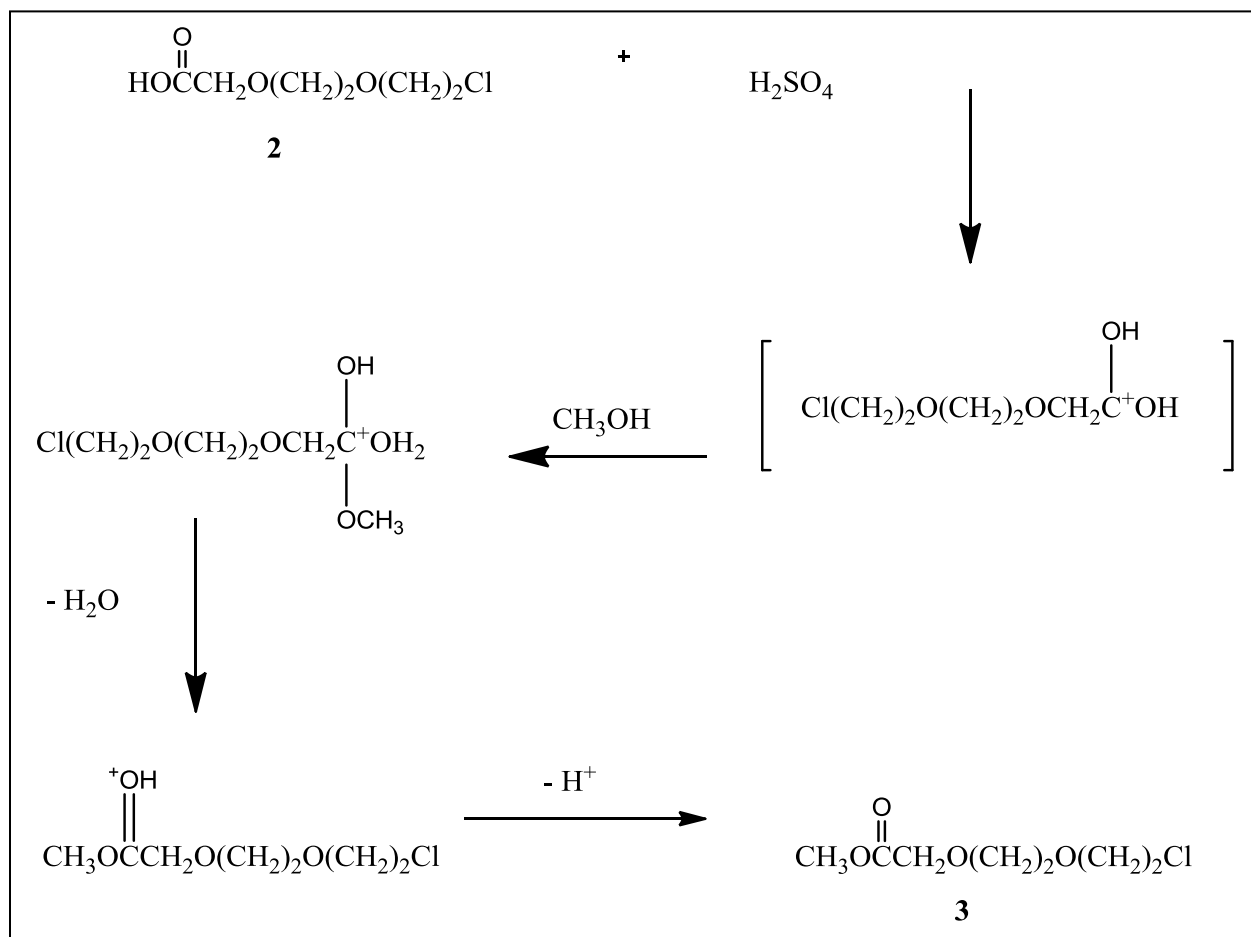
Scheme 5. The methylation of a carboxylic alcohol group using Fischer esterification

Synthesis of Methyl 2-(2-(2-Iodoethoxy)ethoxy)acetate

$$\text{CH}_3\text{OCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{I} \qquad (\text{with O double bond above the second C})$$
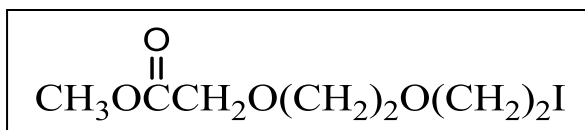
4

The Finkelstein synthesis is an S_N2 reaction producing compound **4**. The reaction is carried out in acetone to precipitate the chloride salt. After extraction using chloroform and water, the organic layer is washed with sodium thiosulfate to get rid of the iodine, which was

formed during the reaction due to oxidation of sodium iodide [30]. After drying and evaporation,

the pale yellow solution is methyl 2-(2-(2-iodoethoxy)ethoxy)acetate [29]. The experimental

yield was 54% while the literature yield was 73% [30]. The synthetic route is shown in Scheme

6.

$$CH_3OCCH_2O(CH_2)_2O(CH_2)_2Cl \xrightarrow[\text{acetone}]{\text{NaI}} CH_3OCCH_2O(CH_2)_2O(CH_2)_2I + NaCl$$

3   **4**
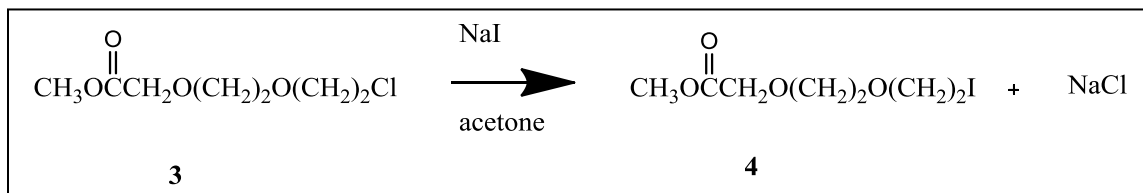
Scheme 6. Synthesis of an iodo compound through a Finkelstein Reaction

Synthesis of 4'-(2-{2-[Carboxymethoxy)ethoxy]ethoxy})-3,5-hydroxystilbene

5
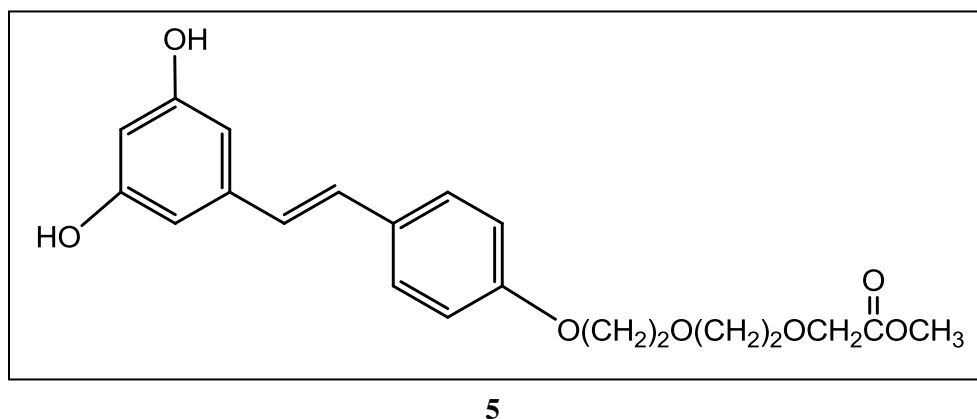
The final product, compound **5**, was based on a Williamson's ether synthesis. The mild

base, potassium carbonate, exchanges the hydrogen atom of resveratrol phenol with a potassium

ion [29 & 31]. The 4' hydroxyl group reacts first then the hydroxyl groups at positions 3 and 5.

Because the pKa values of the phenols at the 4' and 3 positions are so close in values, column

chromatography had to be used to separate the desired product from the di and tri-substituted

products [20]. The final product yield was 2.4% with 92% purity while the literature yield was

60% with 100% purity. The synthetic route is shown in Scheme 7.
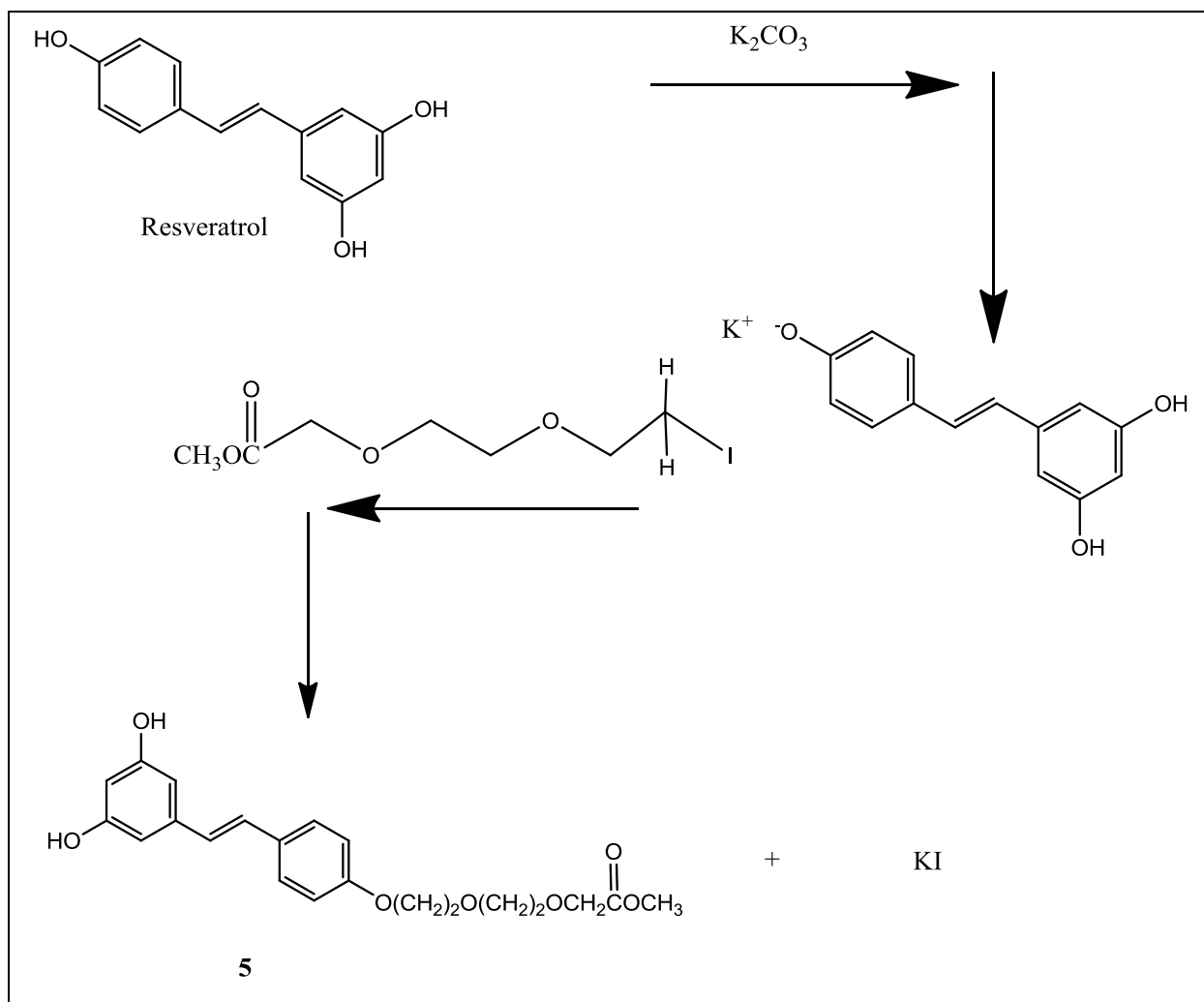
Scheme 7. Synthesis of final product, analogue of resveratrol, through Williamson etherification

The problem is thought to be self epoxification by the ether group once the halide ion broke off, as shown in Scheme 8.
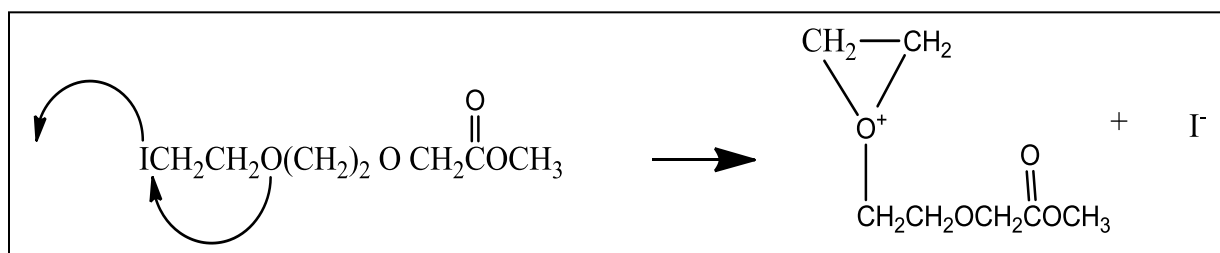
Scheme 8. The possible epoxide formation of ether ester group

The effect of the base, reaction temperature, and the addition order of reagents were investigated. Potassium carbonate was pulverized to a powder to allow the reaction to proceed more quickly. This resulted in more of the di substituted product and resveratrol as confirmed by thin layer chromatography (TLC). The structures of the di and tri substituted resveratrol are shown in Figure 5.
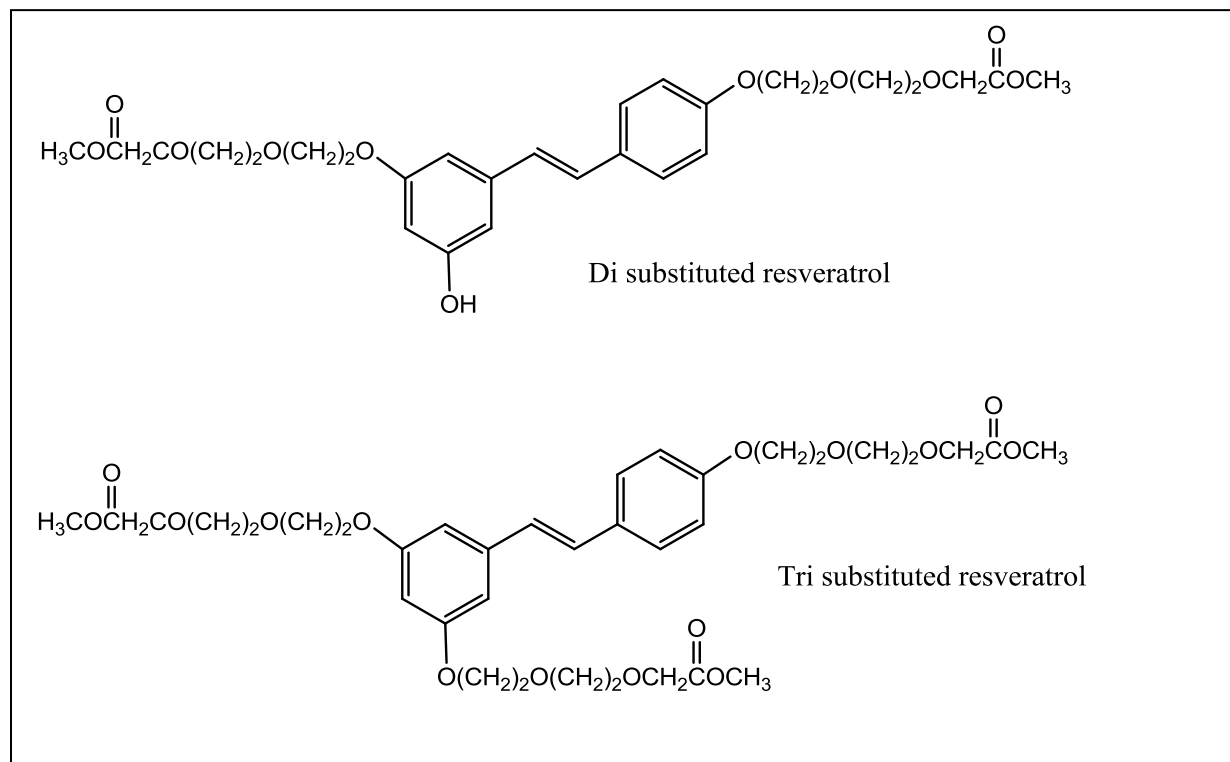

Figure 5. The structures of di and tri substituted resveratrol products

Then other bases were tested for a better exchange between their cations and the hydrogen of the 4' position. There was no reaction when sodium carbonate was used. There was overreaction when cesium carbonate was used. The reaction mixture stuck to the side of the glass cylinder. The various temperatures evaluated were room temperature, 50°C, 65°C, and 70°C. Both reactions at room temperature and 50°C showed no product. The best temperature for the reaction was 65°C. Over 70°C the product was mostly the di and tri substituted products. The last attempts to improve the yield were experimenting with the order of addition of reagents. Potassium carbonate was allowed to react with resveratrol for an hour before adding the ester. The di and tri substituted products were the most

dominant spots on the TLC. Further investigation of similar research suggested adding the ester slowly [17 & 20]. A little more of the mono substituted product was collected but was still very impure. The largest quantity of the final compound was collected when half of the ester and potassium carbonate were added within the first eight hours of the reflux. A large silica TLC plate was used for the final purification because Recrystallization was impossible with so little crude product. The final product was only 92% pure and weighed 5 mg. Another synthetic route was attempted with a different ester, ethyl 6-bromohexanoate, to improve the overall yield of the mono-substituted product. A summary of the experimental attempts are found in Tables 2 through 4.

Table 2. Variable bases used to react in Williamson etherification reaction

Base	Reacitivity	Yield (%)
Sodium Carbonate	No Reaction	0
Potassium Carbonate (pulverized)	Best Reaction	3.5
Cesium Carbonate	Gumm like substance stuck to Glass	0

Table 3. Experimental temperatures for the Williamson etherification reaction

Temperature of Reaction (˚C)	Reactivity	Yield (%)
50	No Reaction	0
65	4 mg 4'para	2.4
70	≤ 2 mg 4'para	1

Table 4. The addition of reagents in various experiments for the Williamson etherification

Reagent Addition	Reaction	Yield (%)
Base + Resveratrol for 1 hour	2 mg 4' product	1
60 % Ester + 70 % Base first 8 hrs	3 mg 4' product	1.5
50 % Ester + 50% Base first 8 hrs	4 mg 4' product	2

Synthesis of 3,5,4'-Tri-O-acetyl-resveratrol
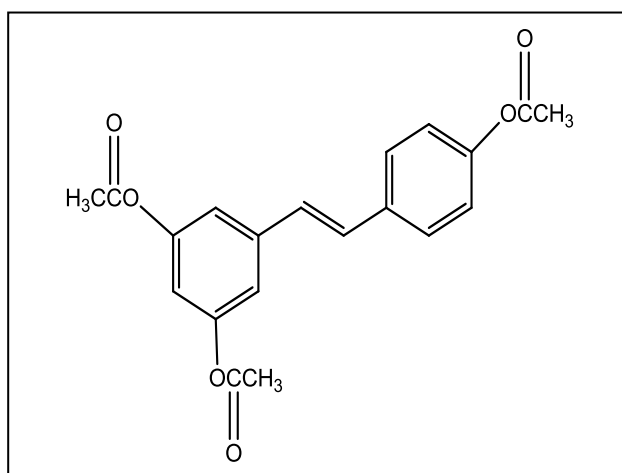
6

Compound **6** is the product of an acylation reaction, shown in Scheme 9. Triethylamine acts as a nucleophile attacking the acyl carbon. The carbonyl carbon then forms a bond with the 4' oxygen of resveratrol. Finally, the triethylamine breaks off to form triethylamine hydrochloride. After 12 hours the 4', 3, and 5 phenol positions are acylated, (Scheme 5). The final product, 3, 4', 5'-tri-O-acetyl resveratrol, was a white compound. The experimental yield was 76% while the literature yield was 90-95% [32, 33, & 34].
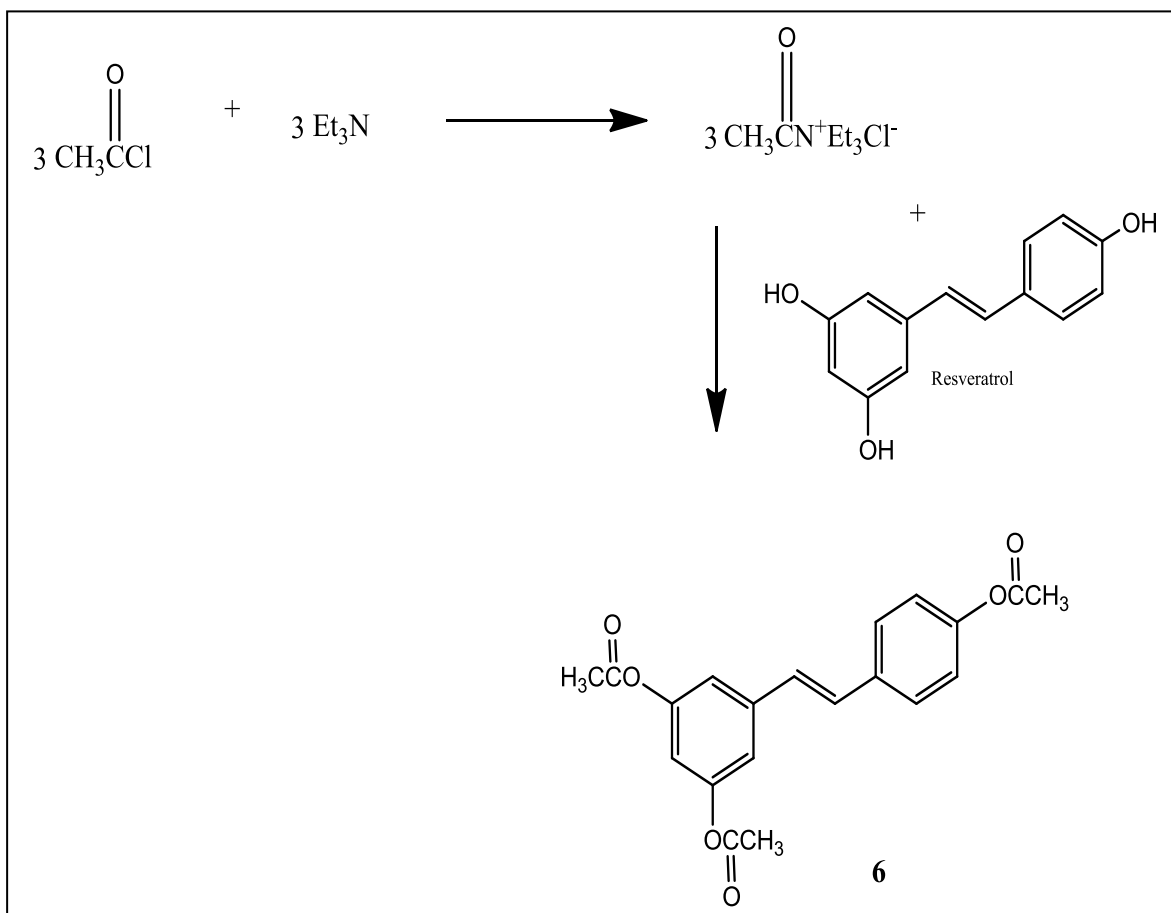
Scheme 9. Acetylation of resveratrol at each of the phenol groups

Synthesis of 3, 5-Di-O-acetyl-resveratrol
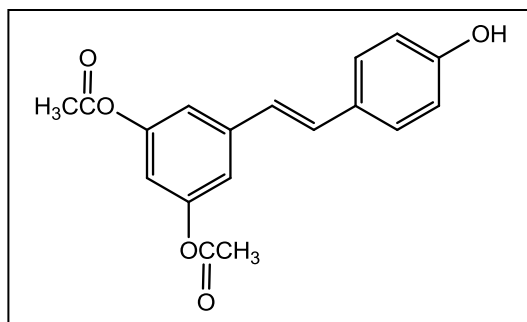
7

Compound **7** was obtained as an off white flakey solid. It was the product of an enzymatic alcoholysis, as shown in Scheme 10. Butanol acted as a nucleophile, attacking the carbonyl carbon. Once the double bonded oxygen reformed, the ether bond breaks leaving an oxygen anion. The hydrogen from the butanol group bonds to the oxygen anion producing 3, 5-di-O-acetyl-resveratrol and an ether (Scheme 6). Most of what was recovered from column chromatography was starting material. If the enzyme could have been recycled, and more time given for the reaction, then more of the product could have been separated. The experimental yield was 20% while the literature yield was 22% [32, 33, & 34].
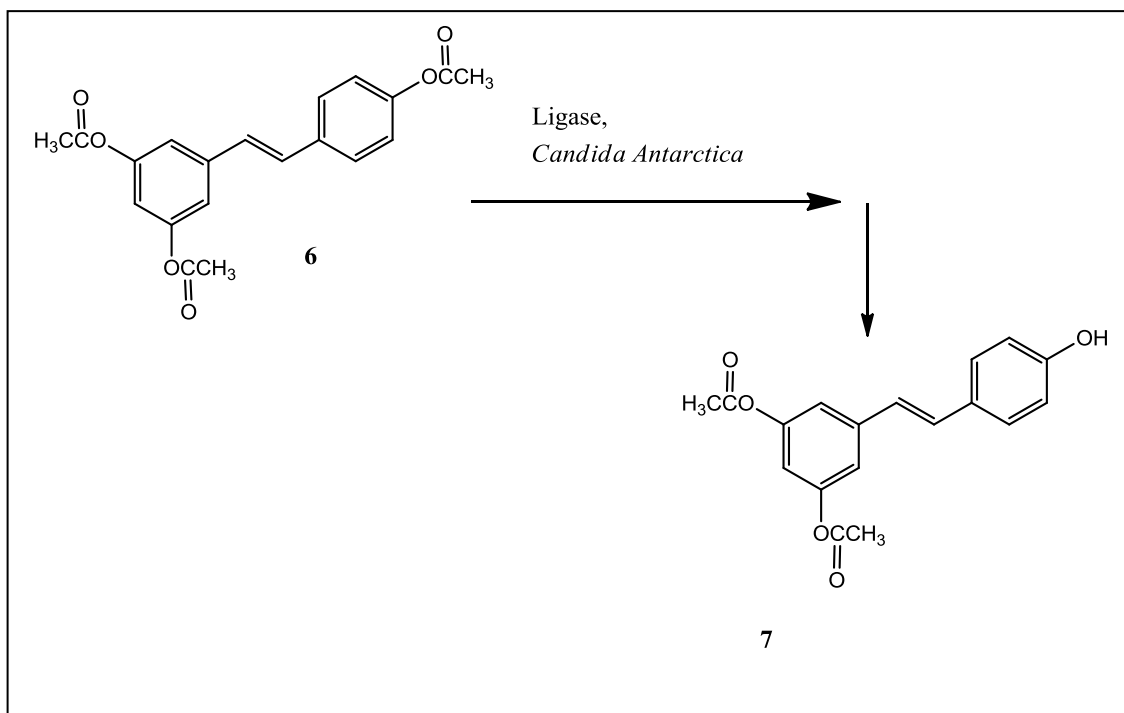
Scheme 10. Enzymatic hydroxylation at the 4' para position

Synthesis of 3.5-Di-acetyl(4'-ethyl 6-oxyhexanoate) stilbene
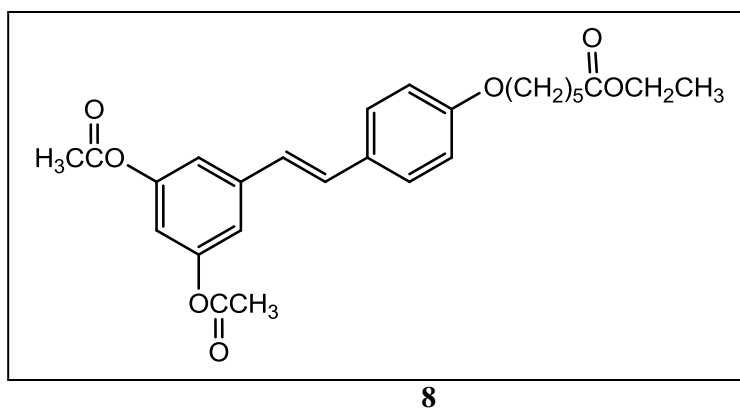
Selective alkylation of compound **7** afforded an impure **8**. The same Williamson ether

synthesis as compound **5** was employed for the selective deprotonation of the organic acid

followed by alkylation of the phenol, as shown in Scheme 11. After separation using column

chromatography one spot was seen on a TLC. However, an NMR spectrum showed impurity within the stilbenes area, 7.5-6.4 ppm. The compound could not be further purified and no other experimentation followed.
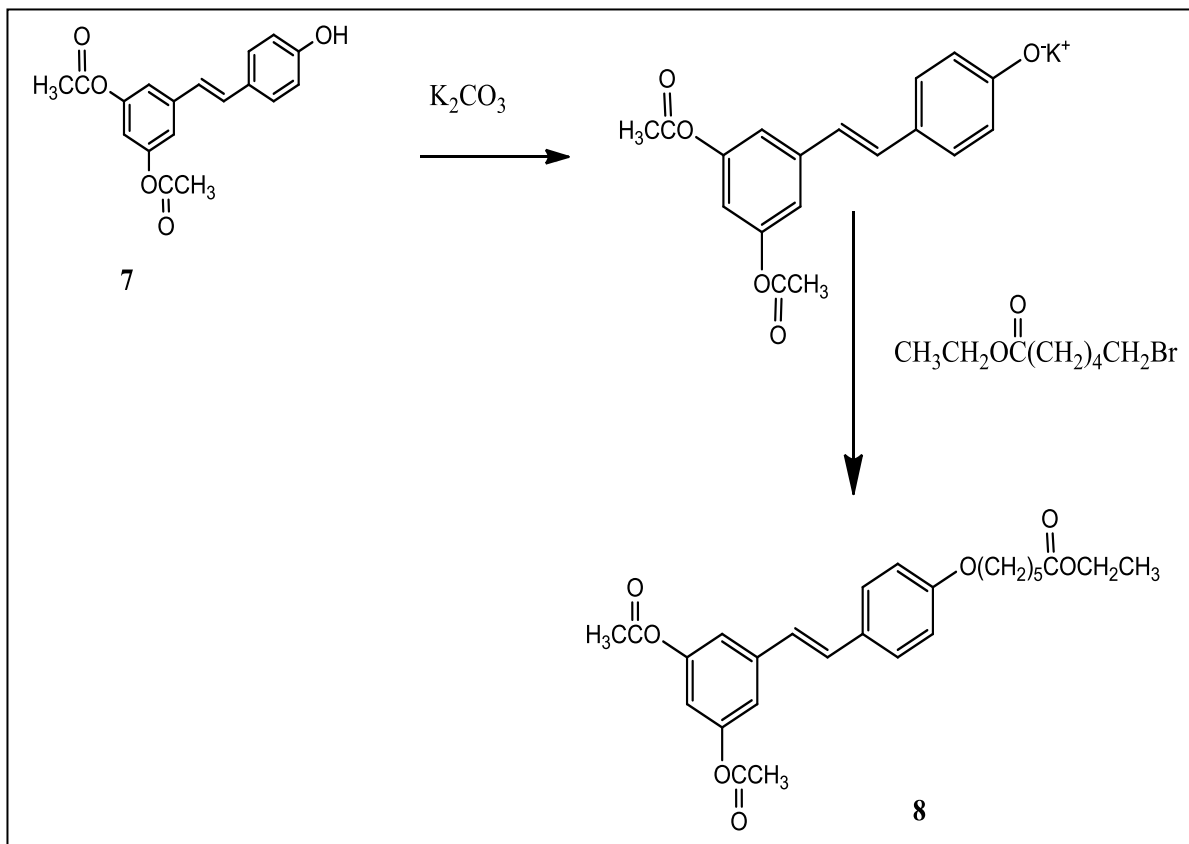

Scheme 11. The selective alkylation of 4' para hydroxyl using a Williamson etherification reaction

Synthesis of 4-[(1E)-2-(3, 5-Diacetyloxyphenyl)ethenyl] phenyl N-[(1,1-

dimethylethoxy)carbonyl]-glycinate
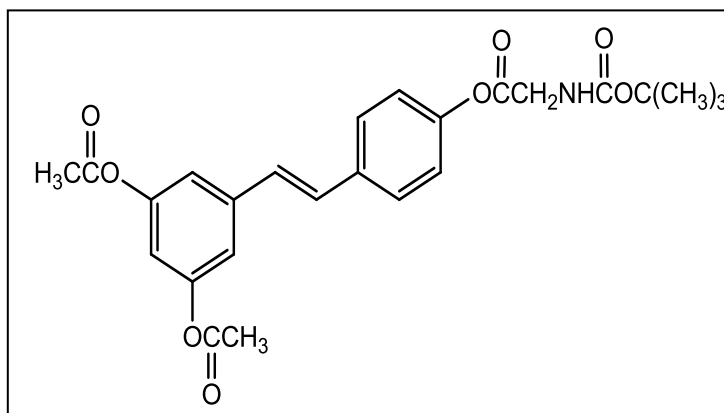
9

The final compound synthesized was compound **9**. A glycine molecule was attached to resveratrol through an esterification reaction, as shown in Scheme 12. Dicyclohexylcarbodiimide (DCC) first attaches to the alcohol functional group of the glycine. Then the anion of another glycine attacks the carbonyl carbon of the carboxylic group forming an anhydride and dicyclohexyl urea. An ester anion of the anhydride is removed when the compound reacts with 4-dimethylaminopyridine. Finally the 4' deprotonated oxygen attacks the carbonyl carbon of the amide breaking the nitrogen bond and producing the final ester (Scheme 8). The final product was a white solid. The literature product yield was 70% while the experimental yield was 56%. The compound was characterized using ^{1}H NMR, ^{13}C NMR, IR, MS, and melting point.

.
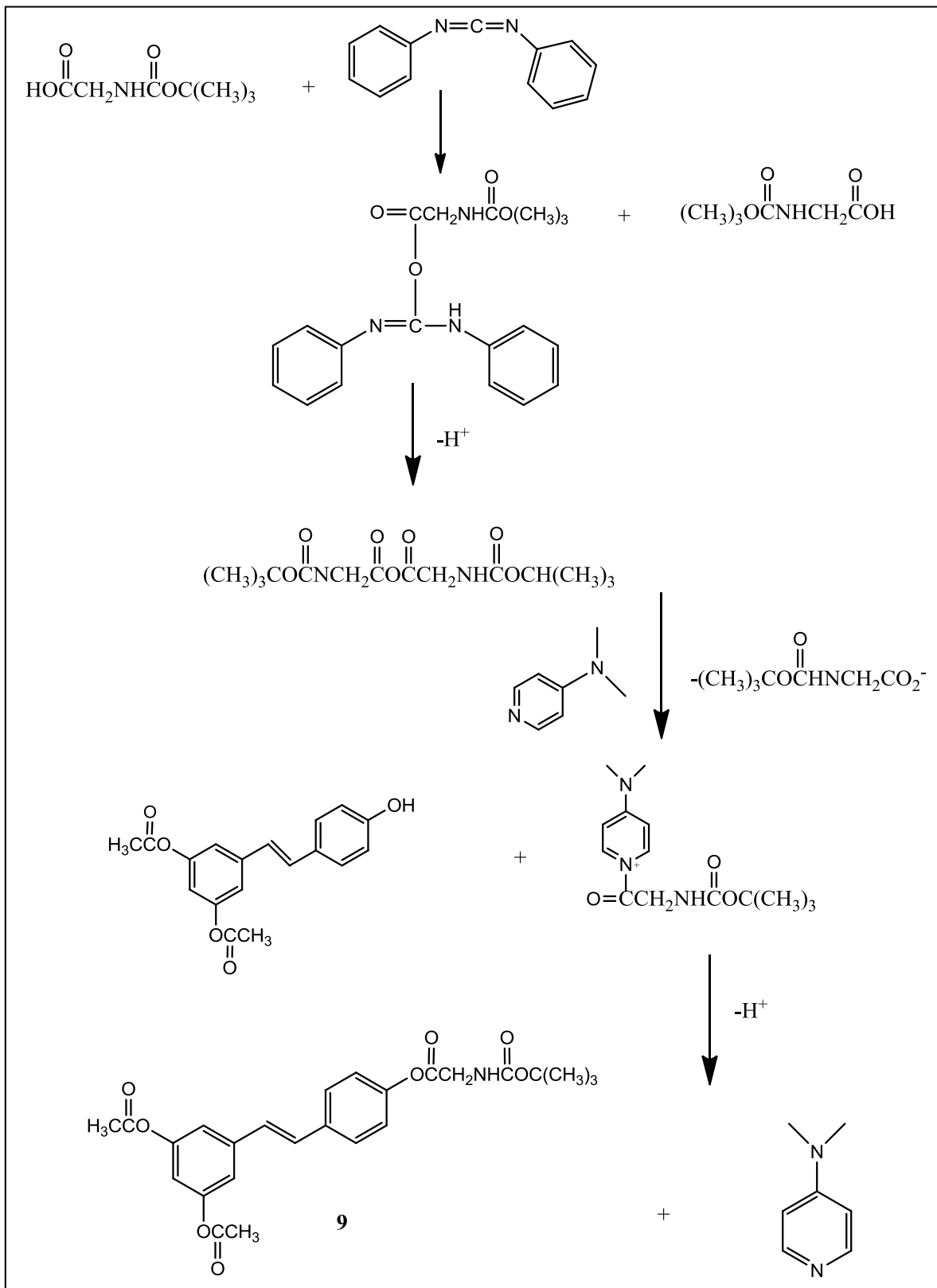
Scheme 12. Synthesis of final analoge of resveratrol

46

CHAPTER 3

CONCLUSION

The objective of this research has partially been fulfilled with the synthesis of the pure

compound **9,** 4-[(1E)-2-(3,5-diacetyloxyphenyl)ethenyl]phenyl N-[1,1-dimethylethoxy)carbonyl]-

glycinate. Two synthetic routes were attempted each using a different ester to link to the 4'

position of resveratrol. The objective of both was to esterify resveratrol at the 4' position. This

molecule can be further converted to resveratrol glycinate with a free amino group, which would

enhance the water solubility of resveratrol.

The major synthetic routes are the chemical and enzymatic esterification of phenolic

compounds. The chemical synthesis proved to be difficult because of the heat sensitivity of

phenolic acids as well as its susceptibility to oxidation in alkaline media. When using the

iodoester the reaction was carried out at 65˚C, and at 50˚C in the case of the bromoester. The

melting point of resveratrol is 253-255˚C, but side reactions could occur because chemical

synthesis is relatively unselective. It also involves many intermediate stages and purification

steps to remove byproducts and catalyst residues. The Williamson ether synthesis is carried out

in acetone using anhydrous potassium carbonate as the base [10, 35-39]. Once the reaction time

has expired, the potassium carbonate is decomposed using ammonium chloride. This is to avoid

an alkaline media that can over oxidize resveratrol [5 & 17]. Other research groups prepared

their derivatives under N_2 atmostphere to avoid secondary reactions [17]. The enzymatic

esterification of phenolic compounds has several advantages over chemical synthesis. This

includes: mild reaction conditions, minimization of side reactions and byproducts, selectivity, a

wider variety of pure synthetic substrates, fewer intermediary and purification steps, and more

environmentally friendly process [10, 26, 40]. An enzymatic process was seen within the second

method of synthesis, enzymatic alcoholysis, and although there was no side reactions very little

product was obtained. The literature ratio of product formed to recovered reactant was 30:70 [34]. The enzyme, *C. Antarctica,* is a relatively expensive reactant that was disposed of after each reaction. The last step was a Williamson ether synthesis but did not give any of the desired product. The product showed one spot on the TLC but the NMR spectrum was inconclusive and showed impurities. The compound could not be further separated using column chromatography. There was so little product from the previous reaction, enzymatic alcoholysis, the amount of final product was only 4-5 mg. This amount was too small for column chromatography and because the spot could not be separated on a TLC plate the product and impurity cannot be distinguished using a large TLC plate. The factors that need to be considered for good chemical esterification yields include solubility of substrates and products, solvent polarity, temperature, and water content [10].

An acceptable analogue of resveratrol was synthesized using an esterification method with a glycine derivative. The compound had to be separated from the starting material using column chromatography. The characterization tests showed a pure compound, 15 mg of **9**, 4-[(1E)-2-(3,5-diacetyloxyphenyl)ethenyl]phenyl N-[1,1-dimethylethoxy)carbonyl]-glycinate .
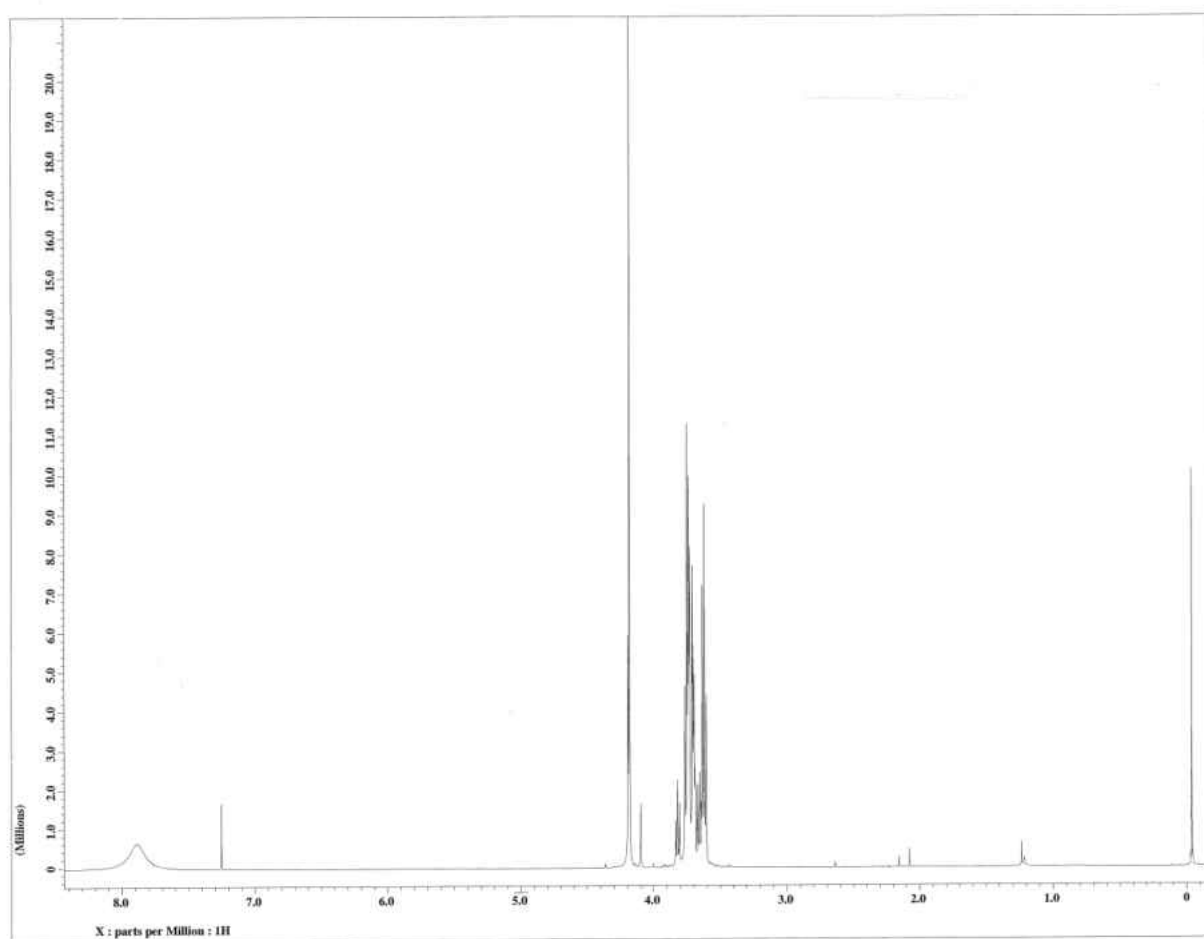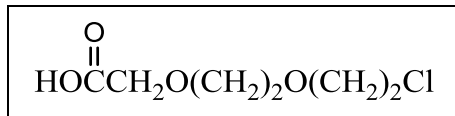
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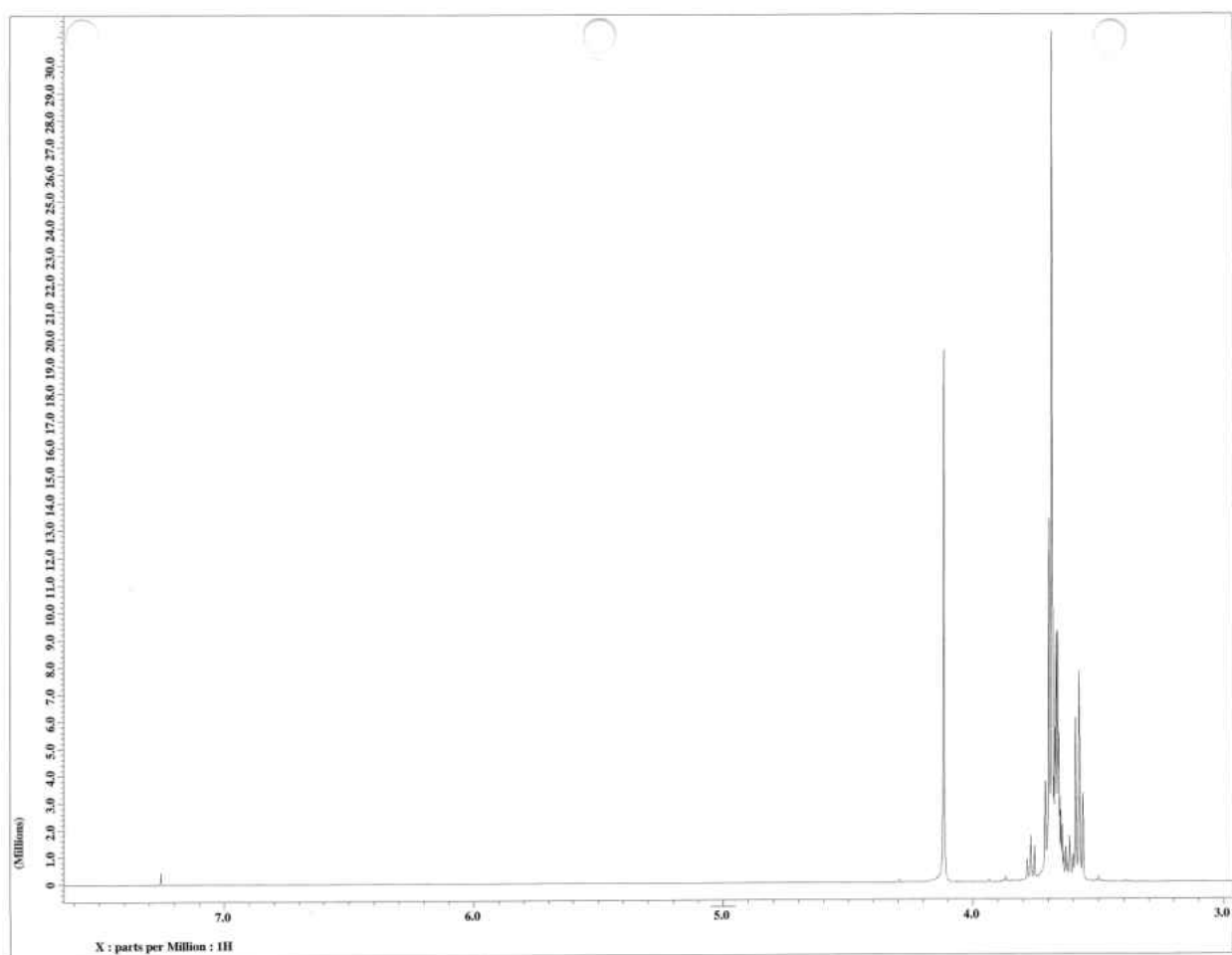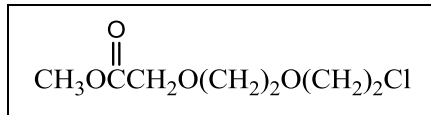
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APPENDICES

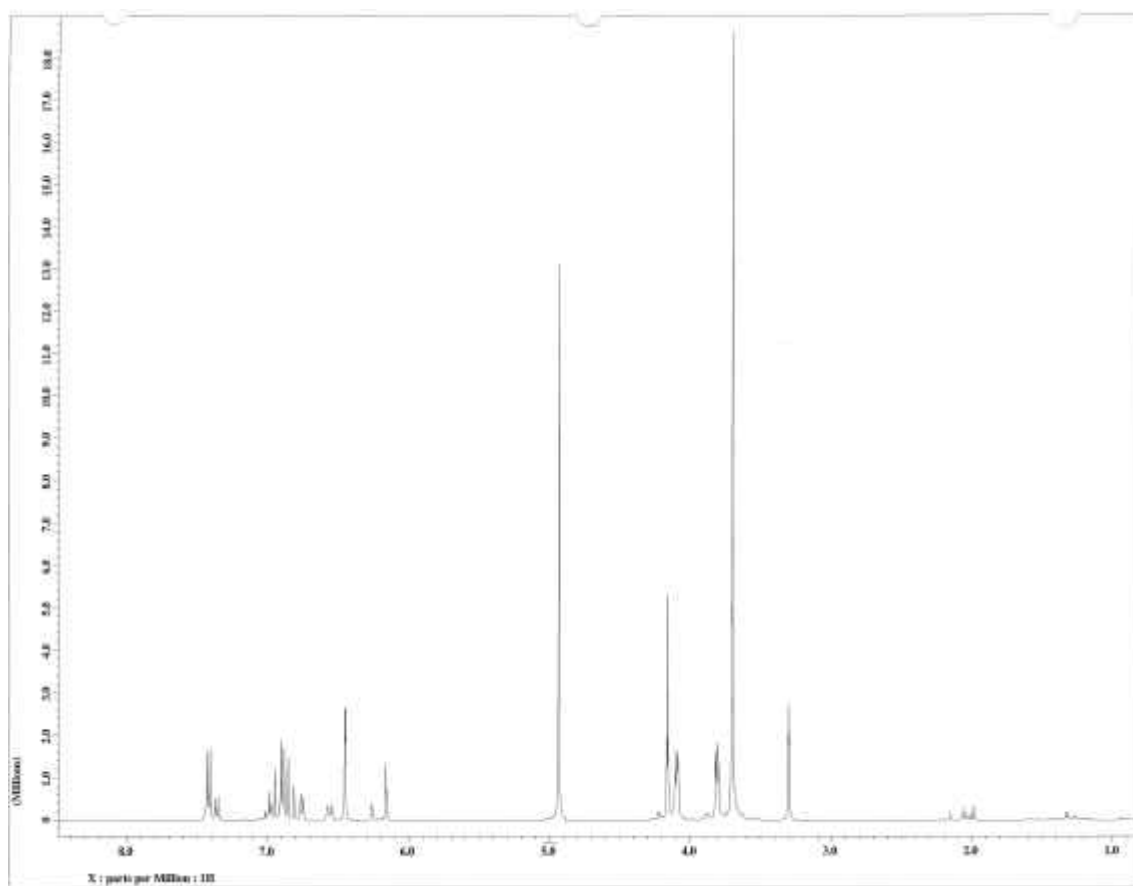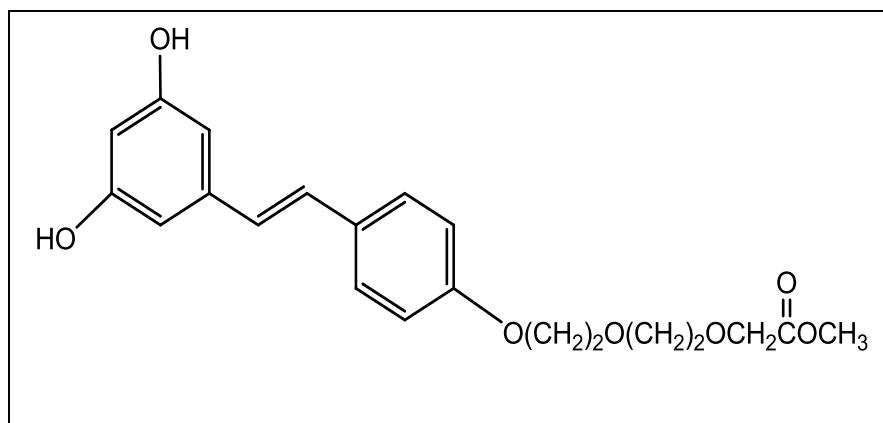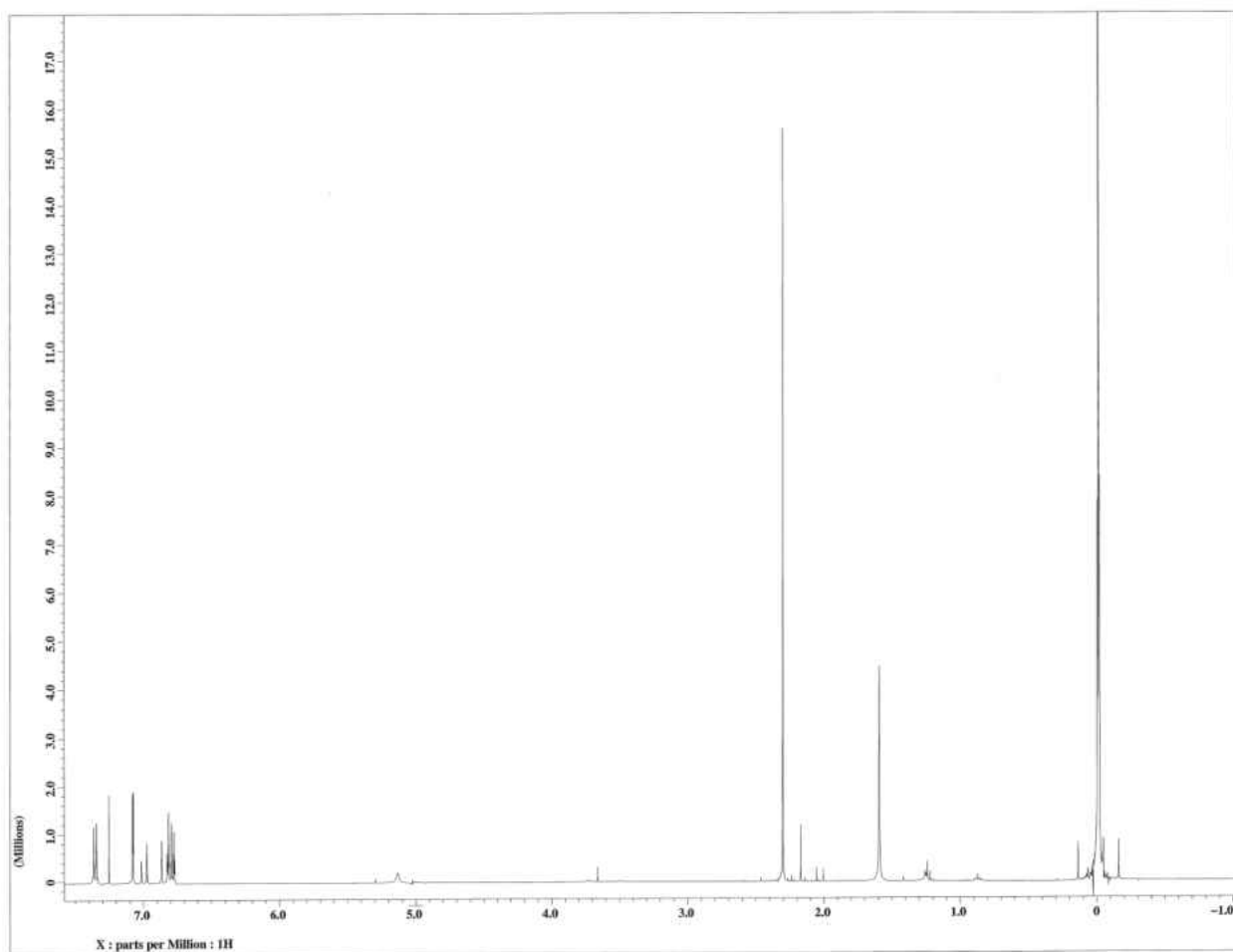## APPENDIX A. ^{1}H NMR Spectrum of Compound **2** in CDCl$_3$

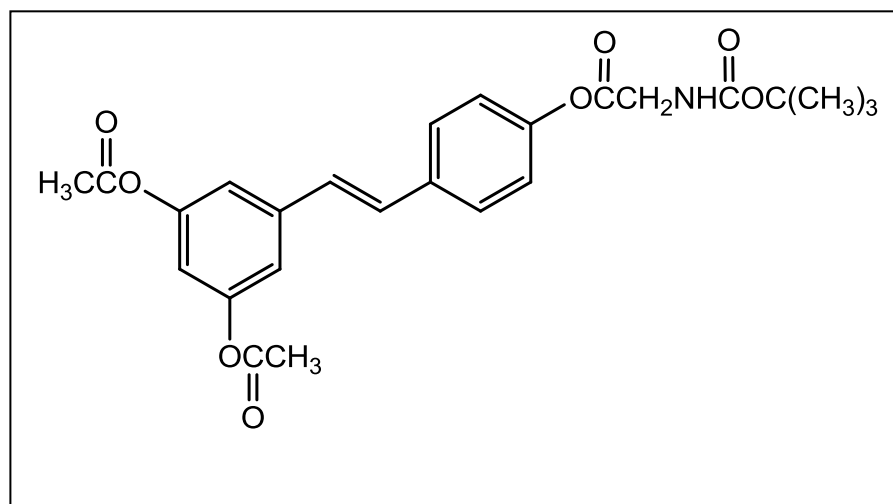$$\overset{\displaystyle \overset{O}{\|}}{HOCCH_2O(CH_2)_2O(CH_2)_2Cl}$$

X : parts per Million : 1H

$$\underset{\substack{\|\\ O}}{\text{CH}_3\text{OCCH}_2\text{O(CH}_2)_2\text{O(CH}_2)_2\text{Cl}}$$

(Millions)

X : parts per Million : 1H

$$\underset{O}{\overset{\parallel}{CH_3OCCH_2O(CH_2)_2O(CH_2)_2I}}$$

X : parts per Million : 1H
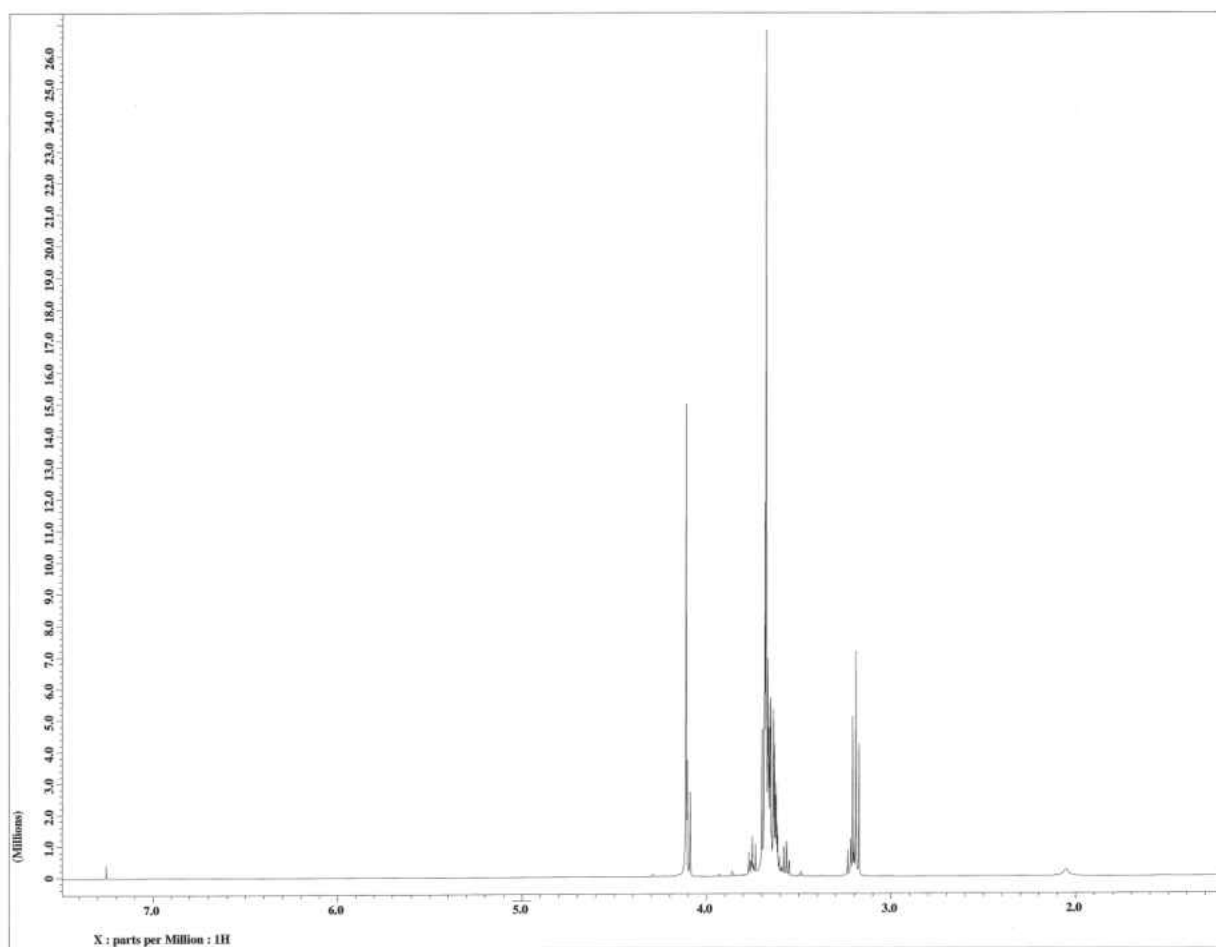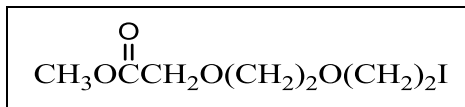
APPENDIX D. ^{1}H NMR Spectrum of Compound **5** in CD$_3$OD

APPENDIX E. ^{1}H NMR Spectrum of Compound **6** in MeOD
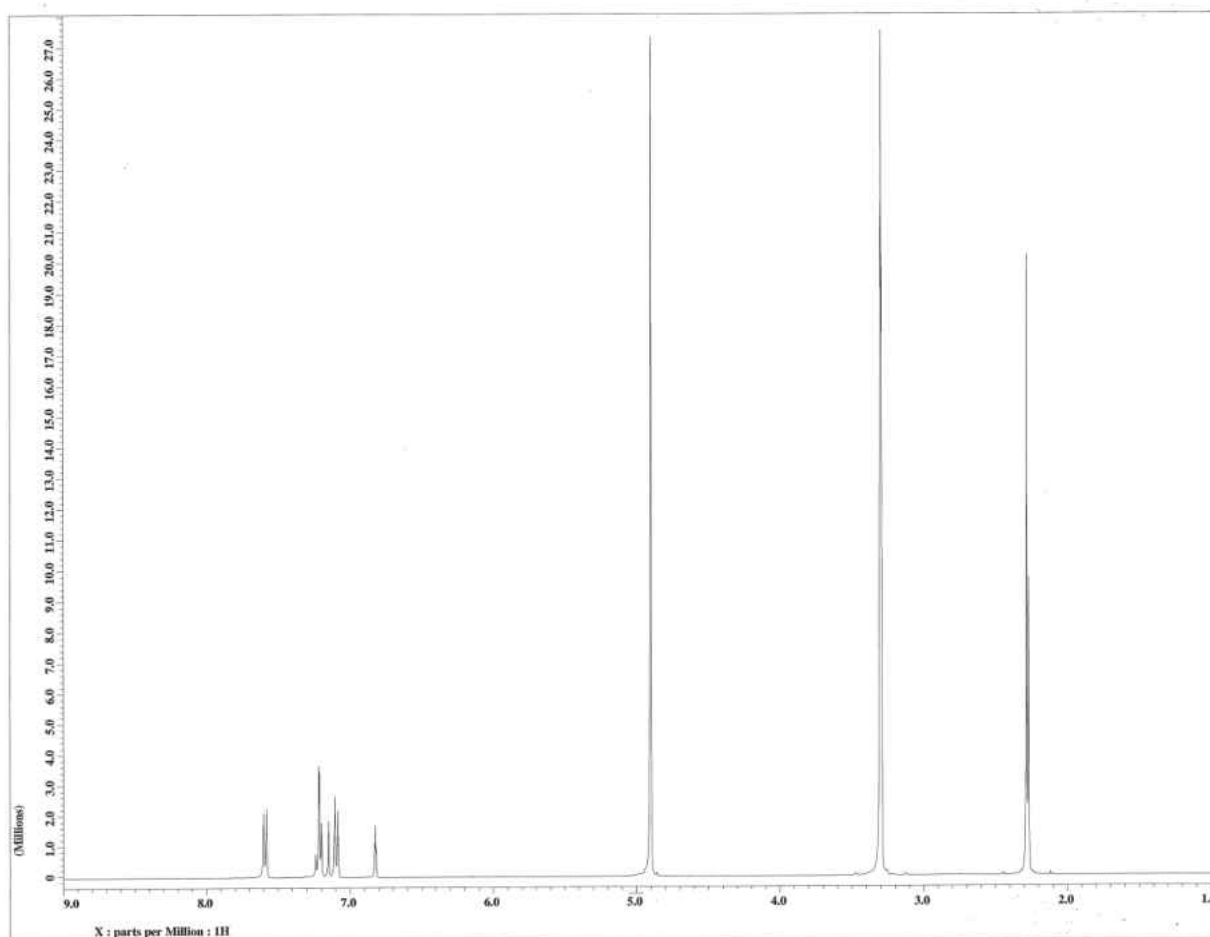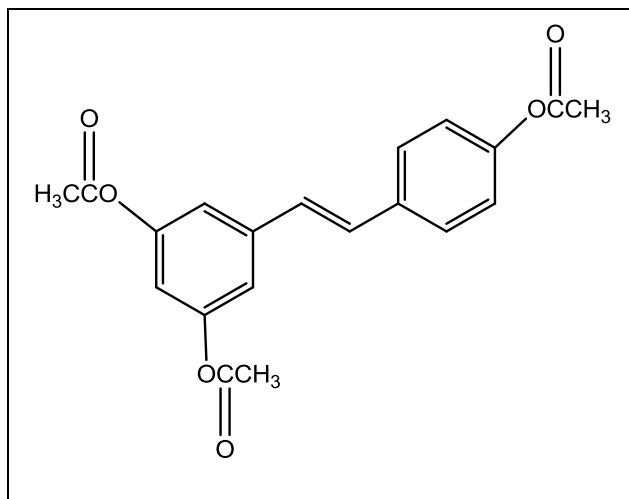
APPENDIX F. ^{1}H NMR Spectrum of Compound **7** CDCl$_3$
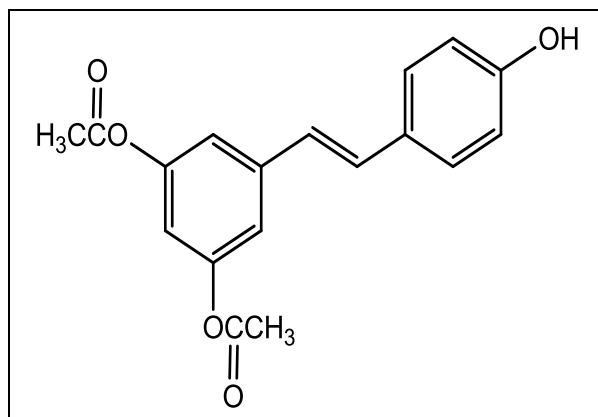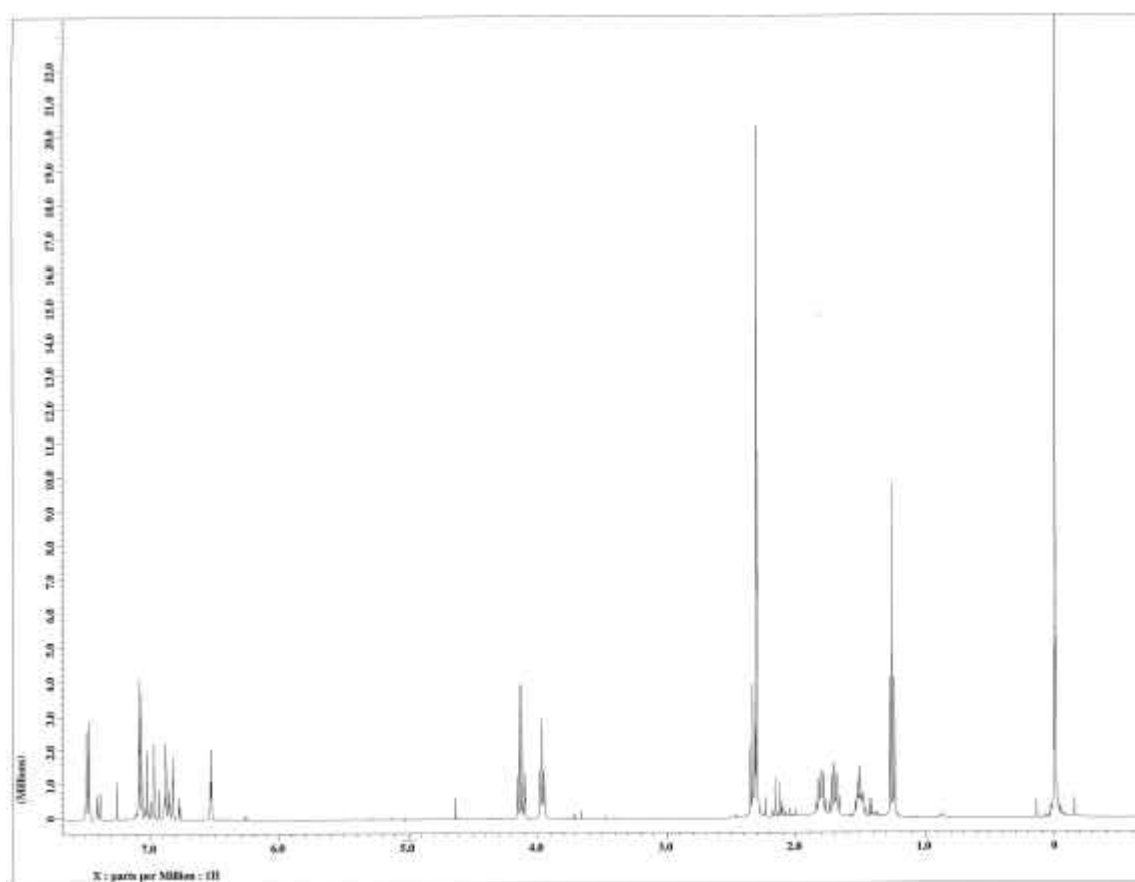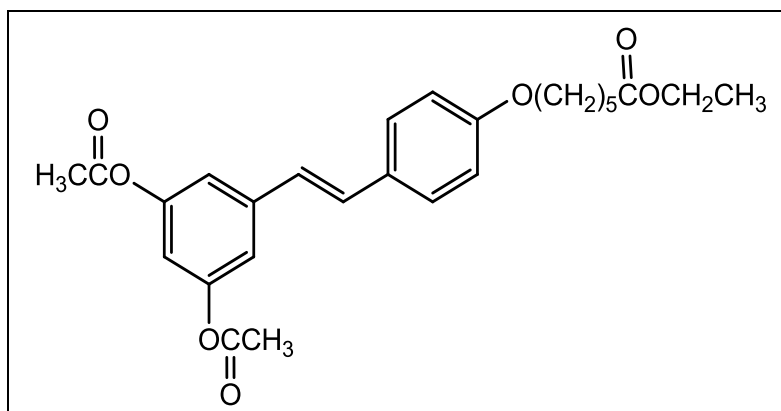
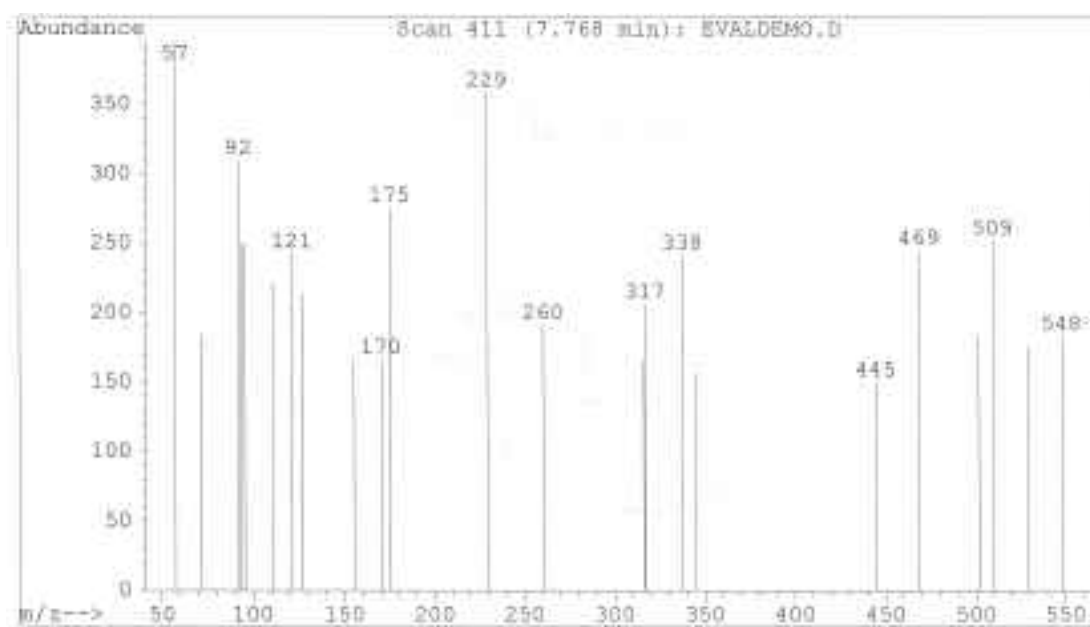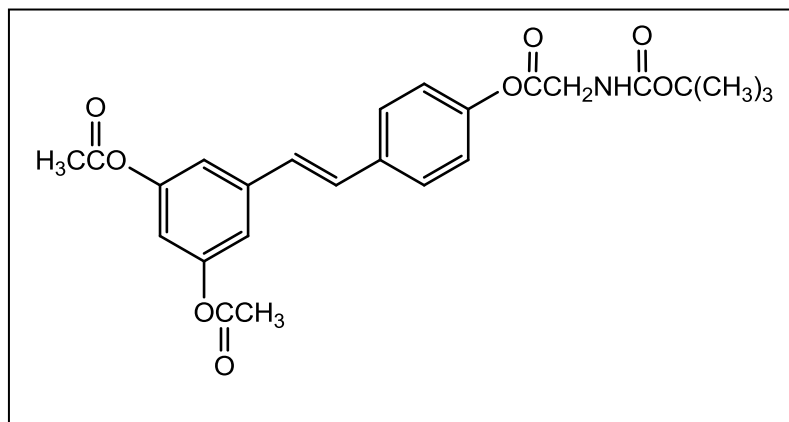APPENDIX I. ^{13}C NMR Spectrum of Compound **9** in CDCl$_3$
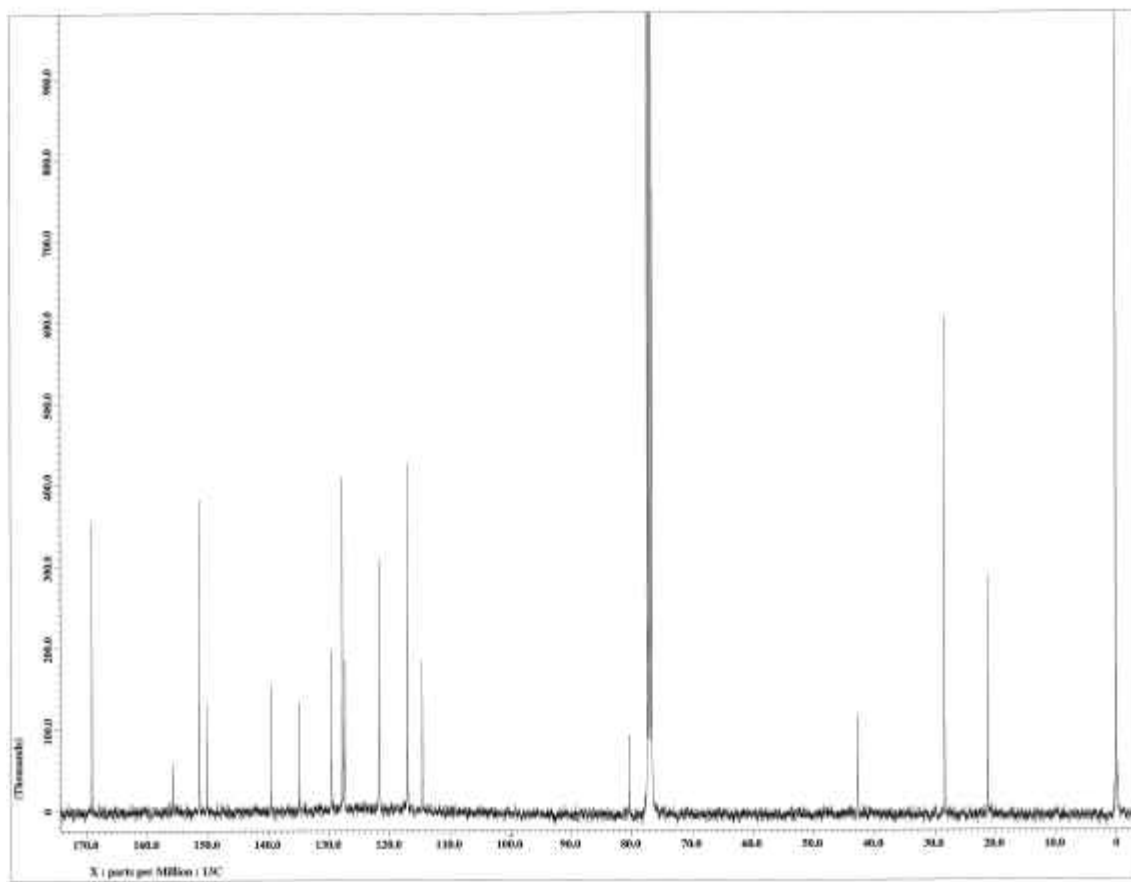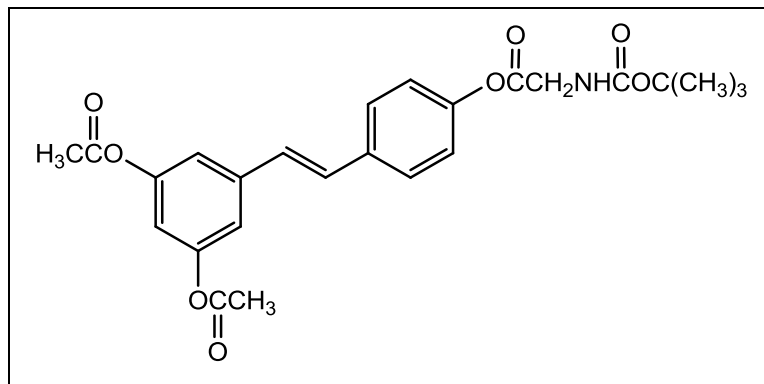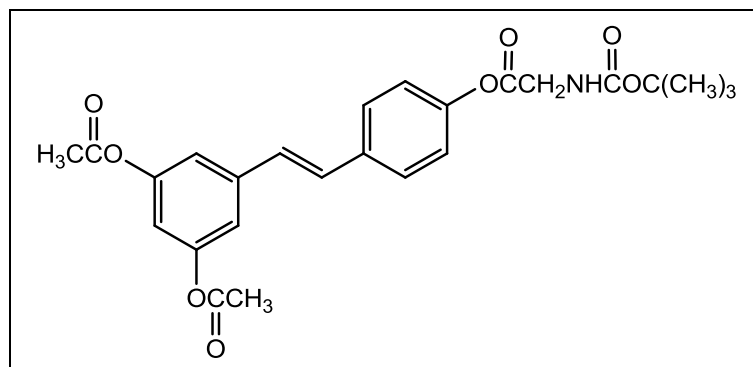
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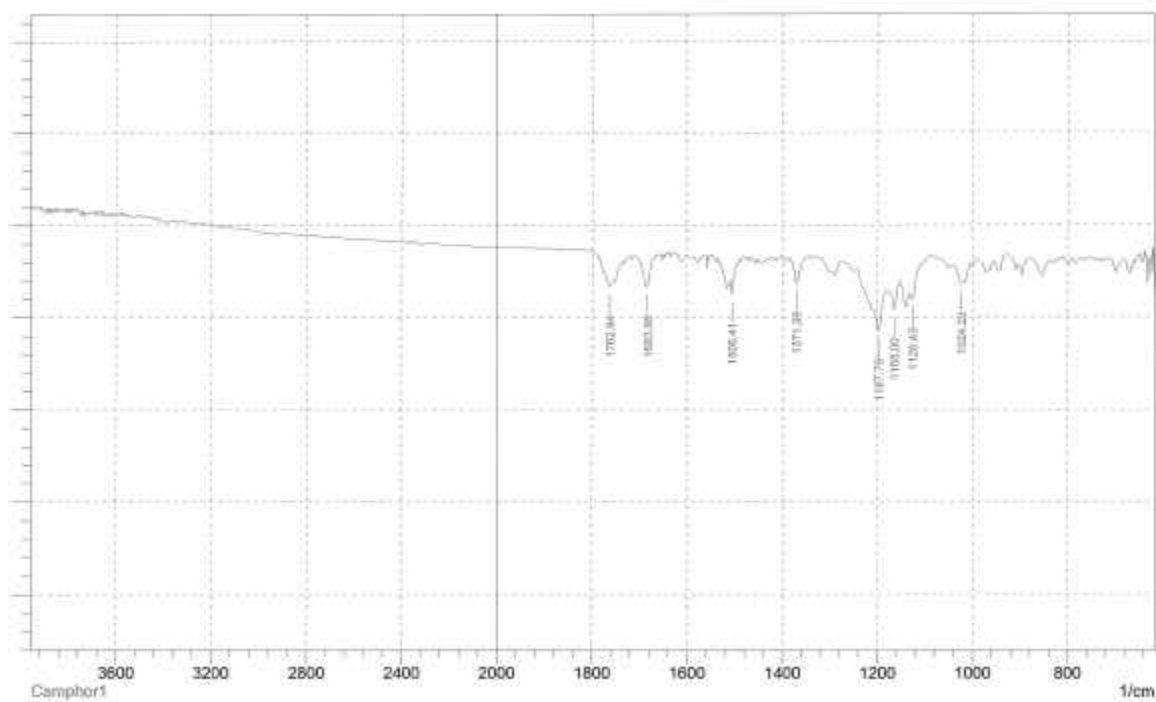
Camphor1

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APPENDIX K. GCMS Spectrum of Compound **9**

VITA

SHELLEY MARIE VAN CLEVE

Personal Data: Date of Birth: March 21, 1985

Place of Birth: Columbia, Tennessee

Marital Status: Single

Education: East Tennessee State University (ETSU)

Johnson City, Tennessee, Chemistry, B.S. 2008

East Tennessee State University (ETSU)

Johnson City, Tennessee, Chemistry, M.S. 2011

Professional Experience: Graduate Teaching Assistant at ETSU

Johnson City, Tennessee, 2008-2010