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Synthesis of Resveratrol Esters and Aliphatic Acids

A thesis

presented to

the faculty of the Department of Chemistry

East Tennessee State University

In partial fulfillment

the requirements for the degree

Master of Science in Chemistry

by

Stanley Mofor Jing

December 2011

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Keywords: phytoalexin, stilbenoid, bioavailabilty, biological activities

ABSTRACT

Synthesis of Resveratrol Esters and Aliphatic Acids

by

Stanley Mofor Jing

Resveratrol (RV) is a naturally occurring phytoalexin of the stilbenoid family produced by some plant species, and present in grape skin, peanuts, and red wine. It has been found to exhibit anti-cancer, anti-inflammatory, anti-viral, anti-aging, cardio protective, and anti-oxidant properties. Bioavailability is a huge setback that limits the potentials of RV. As a result, efforts have been made to design and synthesize RV esters and aliphatic acids in an attempt to increase its bioavailability, solubility in water, and possibly improving its biological activities. Resveratrol esters, 3,5,4'-triacyloxystilbene (**2**) and Methyl 1,1',1''-(3,4',5-stilbenyl)-1,6-hexanedioate (**3**) have been synthesized. Compound **3** is a new compound, synthetic yield is 88%, and purity is above 95% based on NMR integration. Both **2** and **3** are good candidates for biological evaluation. **3** was used as a precursor in the synthesis of resveratrol aliphatic acid, 8-(3',5'-dihydroxystilbene-4''-oxy)-3,6-dioxocotanoic acid(**9**). First, **2** was hydrolyzed to resveratrol diester, 3,5-diacetyloxystilbene (**4**). Mitsunobu reaction of **4** and methyl 8-hydroxy-3,6-dioxooctanoate (**7**) was then carried out to afford methyl 8-(3',5'-diacetyoxystilben-4''-oxy)-3,6-dioxooctanoate(**5**), which was then hydrolyzed to afford **9** in total 43.6 % yield. Structures of all newly synthesized compounds were confirmed by ¹H and ¹³C NMR spectroscopy.

DEDICATION

In loving memory of my late sister, Sally Sisuh Jing.

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
Fig	Figure
UV	Ultraviolet
h	Hours
R _f	Retention factor
Rpm	Rounds per minute
Temp	Temperature
M.p	Melting point
TMS	Tetramethylsilane
NMR	Nuclear Magnetic Resonance
Lit	Literature
THF	Tetrahydrofuran
Hex	Hexane
RV	Resveratrol

CHAPTER 1

INTRODUCTION

Within the past few centuries, scientists have been able to extract important compounds with biological activities from plants. Scientists believed it was impossible to totally synthesize these complex molecules in the laboratory. They encountered difficulty in understanding the exact structure of these molecules in the first place, let alone attempt to synthesize them. With the advent of good electronics, scientists could deduce the exact structure of most natural compounds with biological activities. Hence they could synthesize these compounds and study their structure activity relationship (SAR). Resveratrol (RV) is a bioactive compound produced by some plants in response to pathogen attack or stress. This compound has received increased attention within the past decade and it was commercialized as a dietary supplement as a result of several health benefits that have been reported. Examples of such health benefits includes antifungal¹, anti-cancer², antibacterial³, inhibition of platelet aggregation⁴, just to name a few. RV is currently in clinical trial phase two as an anti-cancer agent for colon cancer⁵⁻⁷. RV has shown anti-cancer activities that really attracted more researches to study it. However, there is a big setback associated with the health benefits of resveratrol; it has a low bioavailability. As a result, efforts to synthesize resveratrol derivatives with better bioavailability and hopefully improvement in some of its activities such as antioxidant activity, and anti-cancer activity were considered as objectives. This thesis discusses the biology of cancer, natural compounds with bioactivities, and details of the health benefits of resveratrol, and presents details of the design and synthesis of resveratrol derivatives.

Cancer

Cancer is any malignant growth or tumor caused by uncontrolled cell growth.⁸ Normal cells grow, divide, and die in an orderly manner (apoptosis). During the early years of our life, normal cells grow rapidly, but as we approach adulthood, most cells divide to replace older ones. Cancer cells don't die; they continue to grow, producing more abnormal cells. Cancer cells can travel to other parts of the body and grow into other tissues via blood and lymph, a process known as metastasis. Normal cells become cancer cells as a result of damage to DNA. Mistakes during normal cell division usually give rise to cancer cells. There are two main causes of DNA damage, environmental and hereditary. Cancer is a group of diseases that are usually named after the organ or tissue that it originates from; there are over 100 categories of cancer.

Nomenclature and Types of Cancer

Most cancers are named after the organ or region from which they originate. Below are some of the most popular cancer categories.

Leukemia: This is cancer that originates from blood-forming tissues, such as bone marrow, and causes a large number of abnormal cells to be produced into the blood stream.

Breast Cancer: This type of cancer usually originates from two tissues of the breast; tubes which carry milk to the nipples known as ducts and glands that make milk known as lobules. This type of cancer is common in both men and women; however, it is more frequent in females.

Colon cancer: This type of cancer originates from tissues of the colon. The colon is the longest part of the large intestine. The most common type originates in the mucus-making and releasing tissues known as adenocarcinomas.

Kidney Cancer: Originates from kidney tissues, two main tissues are involved in this type of cancer; renal cell carcinomas which originates from the lining of small tubes (nephrons) that filter blood and remove toxins, and renal pelvis carcinomas which originate from the center of the kidney where urine collects.

Lung Cancer: Cancer that begins in lung tissues and there are two main types namely small and non-small lung cancer. They differ based on how they appear under a microscope.

Melanomas: Cancer that begins in melanocytes which are cells producing the pigment melanin. It can originate from the mole (skin melanoma) or from other pigmented tissue such as the eye and intestines.

Prostate Cancer: This type of cancer originates from tissues of the prostate gland found in the male reproductive system. It is situated below the bladder in front of the rectum.

Causes and Prevention of Cancer

Cancer results from normal cells, which are programmed to divide when they need to and die when the body does not need them or when they are instructed to die. Cancer may result when cells grow out of control or when they forget to die. There are several factors that may cause a cell to grow out of control, which are usually related to DNA damage, a few of them are listed below

- Drinking excess alcohol
- Exposure to excessive sunlight
- Environmental toxins such as poisonous mushrooms
- Benzene and other carcinogenic chemicals (polyaromatics)

- Obesity
- Radiation Exposure
- Smoking
- Viruses

However, the cause of many cancers is still unknown. It is difficult to completely prevent cancer, but it is possible to reduce the risk of developing cancerous tumors. A few things to do in other to reduce risk of getting cancer is listed below

- Eating a healthy diet.
- Regular Exercising.
- No smoking or chewing tobacco.
- Minimizing exposure to radiation and toxic chemicals.
- Maintaining a healthy weight.
- Limiting alcohol consumption.
- Reducing sunlight exposure especially if skin is susceptible to getting burns.

In the Unites States, the most cancer-related deaths are due to lung cancer¹². Lung cancer and colon cancer are most common amongst males and females, while prostate cancer is very common with men and breast cancer with females.³⁴

Treatment Options

There are various treatment options that are currently being used to alleviate, treat, and control the symptoms of cancer. The most popular of these options will be discussed.

Chemotherapy. This type of therapy involves the administration of medications that treat cancer. It is the mainstay treatment of malignancies and it dates back as early as 1900s when

bone marrow suppression effect of nitrogen mustard was observed. It has evolved from relief of symptoms to cancer cure. Tumor cells reproduce at abnormally high rates compared to normal cells because their stop mechanism is faulty. Classic chemotherapy involves compounds that destroy RNA or DNA in cells. The more rapidly cells divide, the more effective these compounds perform their task of killing cells. The cell replication process consists of four phases; G₁, S, G₂, and M phase which stands for growth phase 1, synthesis (DNA replication), growth phase 2, and mitosis respectively. Chemotherapy medications that kill cells at any of the above phases are referred to as cycle nonspecific, whereas those that kill cells in a specific phase are known as cycle specific. When a cell is not in any of the above phases, it is said to be in a resting phase denoted by G₀. Medications can be administered orally (pill form), or injected directly into body cavity (bladder, abdominal cavity, or intra-arterially). Chemotherapy medications are usually administered as regimens. A regimen is a mixture of specific and nonspecific compounds. Chemotherapy is a systematic form of therapy because its effect is spread throughout the body. Chemo substances also kill normal cells in the body, thus the objective is to kill cancer cells and spare normal cells. It is important that traits specific to cancer cells be identified and chemo substances that target these cells are administered. Most chemo treatments target fast growing cells, unfortunately, there are also some fast growing normal cells and are thus killed as well. Hair follicles, skin, gastrointestinal track cells are victims as hair loss, rashes, and diarrhea respectively are common side effects of chemo. There are different kinds of chemotherapy that include: alkylating agents, antimetabolites, anthracyclines, plant alkaloids, antitumor antibodies, taxanes, and monoclonal antibodies.

Radiation Therapy. This is the use of high energy X-rays to kill cancer cells. It is a form of local therapy because it is region specific. There are two modes of application, externally or

internally. External radiation, the cancer region is exposed to X-ray from outside the body; it is also referred to as X-ray therapy. For internal radiation therapy, the source of radiation is surgically placed inside the body near the tumor. Such sources include Radium, Cesium, Iodine and Phosphorous. It is also known as Brachy or implant.

Surgical Oncology. This involves the surgical treatment of a variety of tumors. In 1809, Ephrain Mcdowell first reported resection of an ovarian tumor. However, ancient Egyptians described techniques for removing breast tumors back in the 7th century. Surgical Oncology is the oldest form of cancer treatment.

Bioactive Natural Products

These are chemical compounds in living organisms that exert a biological effect on other organisms. Such biological effects includes: therapeutic activity on other organisms, toxic activity that causes disease and biodegradable toxicity. The use of herbal and other natural medicine has a long history. Herbal medicine involves either the use of a whole plant or crude preparations for therapeutic and experimental reasons. This method of administration poses a number of disadvantages outlined below.

- Variation in the amount of active components with region. This is as a result of season, plant parts, morphology, climate, and ecological aspects.
- The presence of undesirable compounds that can cause drug synergistic, antagonistic, and unpredictable modulations of bioactivity.
- Changes or lose of bioactivity of the herbs due to variability in collection, storage, and preparation of the raw material.

As a result, isolation of bioactive natural products is essential to combat the above cons. Some advantages of isolation of bioactive products include:

- The administration of pure bioactive compounds is possible in reproducible and accurate doses.
- Development of analytical assays
- Because bioactive compounds are isolated, their structure can be determined. This will spur synthesis production, incorporation of structure modification, and rationalization of mechanisms of action.
- Reduced dependency on plants and saving the environment.
- Investigation of structure/activity relationship is facilitated.
- Development of new compounds with similar or more desirable bioactivities.

A very broad range of natural products has been identified, extracted, and synthesized within the last century; this established the basis of the pharmacological industry and drug discovery. This dependency on natural products is evident; approximately 60% of anticancer compounds and 70% of drugs for infectious diseases are natural products or their derivatives⁹. Natural products can be classified according to their sources. There are four main sources of natural products; plant kingdom, marine world, animal sources, and microbial world.

Plant kingdom

The plant kingdom is a very rich source of lead compounds. Examples include alkaloids, morphine, cocaine, digitalis, tubocurarine, quinine, nicotine, and muscarine. Some of these lead compounds are drugs themselves such as alkaloids, morphine, and cocaine¹⁰. Compounds derived from the plant kingdom forms the basis for synthetic drugs, such as local anaesthetics

developed from cocaine. A clinically useful drug isolated from the Yew tree is paclitaxel (brand name Taxol) a very effective anticancer agent⁹. An antimalarial agent called artemisinin has been isolated from *Artemisia annua*. The plant kingdom provides a large source of rich, complex, and highly diverse structures, some of which are unlikely to be synthesized in the laboratory. Studies of plants revealed that they produce protective compounds to deter animals and microbes. These types of compounds are classified as phytoalexin¹¹⁻¹⁵. Resveratrol is one of such compounds produced by the skin of grapes and certain plants. There are over 300,000 species of higher plants⁹, about 1% have been used for food of which about 150 species is commercially cultivated. About 10,000 plant species have documented medicinal use. This still represents a very small percentage of higher plants. Thus, there are potentially more important compounds in plant species that could have pharmacological applications that are yet to be discovered.

Microbial World

Microbial world represents organisms that are unicellular or live in a colony of cells. They are also known as microbes and include bacteria and fungi¹⁰. These organisms are invaluable for discovery of drugs and have been a reliable source of lead compounds. Microbes produce a large variety of anti-microbial agents, which have evolved to give their host an advantage over their competitors. The discovery of penicillin by Alexander Fleming in 1928 motivated the screening of soil and water samples for new microbes and strains. This became highly popular, and as a result an impressive arsenal of antibacterial agents were discovered. This includes cephalosporins, tetracycline, aminoglycosides, rifamycins, and chloramphenicol. A significant number of drugs derived from microbes are used as antibacterial agents. Microbial metabolites have also contributed to the medicinal world. Some metabolites have provided lead

compounds; example is asperlicin which was isolated from *Aspergillus alliaceus*. Lovastin (a fungal metabolite) was a lead compound in a drug series that lowers cholesterol levels.

Cyclosporine suppresses immune response after organ transplant operations. Thus the microbial world has been very useful in the medicinal world

Marine World

In recent years, great interest has been developed to explore the potentials of organism living in the marine world as a source of bioactive compounds as drugs or lead compounds.

Marine sources include coral, sponges, marine microbes, and fish. Bioactive compounds with inflammatory, antiviral, and anticancer properties are common. Curacin A obtained from cyanobacteria exhibits antitumor activity. Other examples include eleutherothin, discodermolide, bryostatins, dolostatins, and cephalostatins.

Animal Sources

Animals have sometimes been an important source of new drugs or lead compounds. A series of antibiotic peptides have been extracted and isolated from the skin of an African claired dog. A very potent analgesic compound called epibatidine has been isolated from the Ecuadorian poison frog.

Antioxidants

An antioxidant, as the name implies, is a molecule that has the ability of inhibiting the oxidation of other molecules. Oxidation is the transfer of electrons or hydrogens to an oxidizing agent. Oxidation processes can produce free radicals that in turn can start chain reactions. In biological tissues, biochemical reactions related to infections, exposure to radiation, and energy

production processes are examples of processes that generate free radicals ^{1, 16-18}. Free radicals are thus naturally generated in the form of endogenous reactive species ¹⁹. There are two types of reactive species, reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species include hydroxyl radical (HO[·]), superoxide (O₂^{·-}), hypochlorite ion (ClO⁻), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), and peroxy radical. RNS includes nitric oxide (NO) and peroxynitrite (ONOO⁻), the latter is formed when NO reacts with superoxide.

Antioxidants inhibit the oxidation of other molecules by getting oxidized themselves; they are thus reducing agents such as thiols, ascorbic acid, and polyphenols. These species are very reactive and are associated to a very short life time that ranges from 10⁻⁹ – 10⁻¹s. The hydroxyl radical (HO⁻¹) has the shortest lifetime of 10⁻⁹s and is the most reactive ²⁰. These species react via three major pathways; radical transfer, charge transfer, and recombination. Our body has a natural antioxidant enzymatic system that involves enzymes like superoxide dismutase (SOD), catalases, and peroxidase. These enzymes control the concentration of these species (production and destruction) by acting in synergy with dietary antioxidants such as vitamin C, vitamin E, β-carotene, and glutathione.¹⁹ There is a number of factors that may induce excessive production of these species such that the natural control process becomes rapidly inefficient and leads to a condition called oxidative stress. Oxidative stress is a physiological condition in which there is an imbalance between production and manifestation of ROS and RNS and the body's natural system to control their concentrations ^{16, 19, 20}. Such factors include exposure to UV radiation, pollution, smoking, alcohol, and other chemicals. Oxidative stress leads to lipid peroxidation and as a result rapid cell structural damage, tissue injury, and gene mutation follows. This ultimately leads to development of various health disorders; Alzheimer's disease, cancer, atherosclerosis, diabetes mellitus, hypertension, and ageing.^{17, 18, 20, 21}

Antioxidants are classified according to their solubility; water soluble (hydrophilic) that reacts with oxidants in cytosol and blood plasma and lipid soluble (hydrophobic) that protects cell membranes from lipid peroxidation. Antioxidants function in three ways

- Getting oxidized by reactive species
- Chelating transition metals and prevents them from catalyzing the production of free radicals in cells
- Sequestration of iron. This involves iron binding proteins such as transferrin and ferritin.

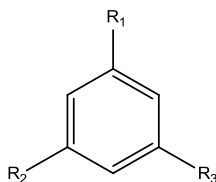
The plant kingdom is an invaluable source of antioxidants ¹⁷, the most popular of which is a group of compounds referred to as polyphenols (flavonoids and proanthocyanidins). Phenolic antioxidants have been recognized as an important class of food ingredients as additives or novel ingredients to introduce extra health benefits. ¹⁸ The next section examines examples of polyphenols in terms of their properties, classes, and sources.

Phenolic Compounds and Polyphenols

Phenolic compounds are those that have one or more hydroxyl groups attached directly to an aromatic ring. However, polyphenols are those compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings. Polyphenols represents a large group of diverse chemical compounds. Polyphenols can be classified into several structural classes of which a few are outlined below.

Simple Phenolic

These are hydroxyl substituted phenols at the ortho, Meta, or para positions. Examples include Resorcinol (1,3-dihydroxybenzen) and phloroglucinol(1,3,5-trihydroxybenzene).

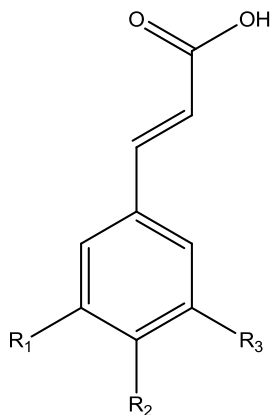


$R_1 = R_2 = R_3 = \text{OH}$: Phloroglucinol
 $R_1 = \text{H}, R_2 = R_3 = \text{OH}$: Resorcinol

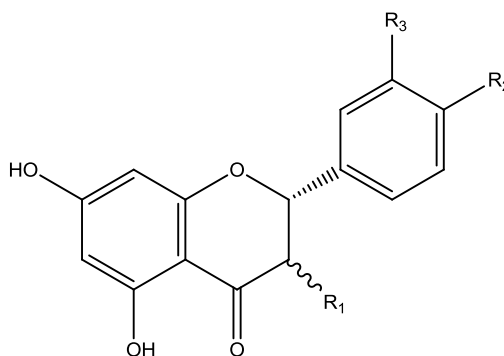
Fig 1. Structure of simple phenolic

Cinnamic acids

This class of polyphenols is derivatives of cinnamic acid; examples are caffeic acid and sinapic acid, Fig. 2



$R_1 = \text{H}, R_2 = R_3 = \text{OH}$: Caffeic Acid
 $R_1 = R_2 = \text{OH}, R_3 = \text{OCH}_3$: Sinapic Acid



$R_1 = R_3 = \text{H}, R_2 = \text{OH}$: Naringenin
 $R_1 = R_2 = R_3 = \text{OH}$: Taxifolin

Fig 2. Structure of selected cinnamic acids and flavones

Flavones

In addition to the ketone group, a hydroxyl group is present. They are also known as dihydroxylflavonols. A good example is taxifolin that occurs naturally in association with tannins in heartwood, Fig 2.

Napthaquinones and Anthraquinone

Napthaquinones are very rare polyphenols of which juglone is the most popular and has been well studied. Anthraquinones are found in walnut; a good example is emodin Fig 3.

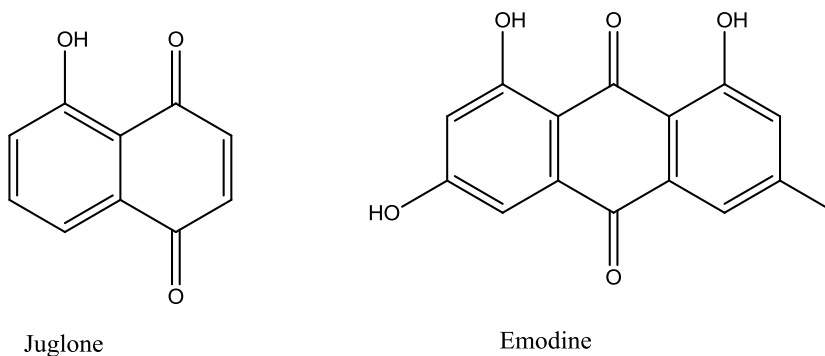


Fig 3. Structure of naphthaquinones

Stilbene and its Derivatives

Stilbene is 1,2-diphenylethylene that is a highly conjugated compound. It exists as the trans and cis isomers of which the latter is thermodynamically less stable due to steric interactions with the two phenyl rings.

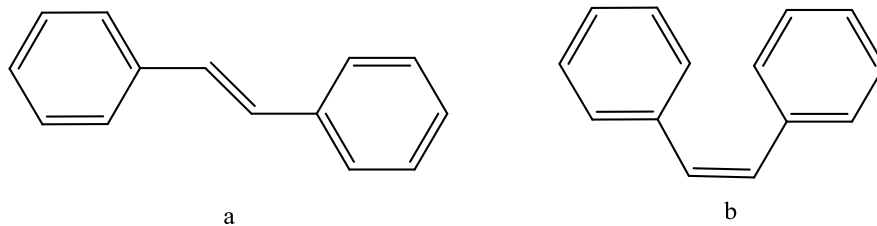


Fig 4. Structure of cis and trans stilbene

Isomerism

The trans isomer, (E)-1,2-diphenylethene is a solid that melts around 120 °C and is thermodynamically stable (Fig 4). Other names includes, (E)-Stilbene, trans-stilbene, and trans-1,2-diphenylethene. Cis Isomer :(Z)-1,2-diphenylethene is a liquid that is thermodynamically less stable (Fig 4) due to steric interactions involving the aromatic rings that forces them out of plane and prevents conjugation. Other names include (Z)-Stilbene and cis-1,2-diphenyl ethane. Stilbene is used in the manufacture of dyes and optical brighteners, gain medium in dye lasers, and scintillator. The Z-isomer is widely used in the study of electrocyclic reactions.

Derivatives of Stilbene

Any compound that contains the stilbene skeleton is considered a derivative of stilbene. Generally, all compounds produced by plants containing the stilbene skeleton are referred to as stilbenes. One of the most common class of stilbenes produces by plants is known as stilbenoids. Stilbenoids are hydroxylated derivatives of stilbenes. Stilbenoids are a small class of compounds that belong to a family of phenylpropanoids. Phenylpropanoids are compounds synthesized by plants from amino-acid phenylalanine. Stilbenoids are produced by plants in response to pathogen infection or stress and are thus called phytoalexins. Phytoalexins are compounds produced by some plants in response to infections or stress. Examples include piceatonol,

pinosylvin, resveratrol, and its cousin pterostilbene . Piceatannol, pinosylvin, and pterostilbene are discussed briefly below, while resveratrol is discussed in great details later.

Piceatannol.5-[(E)-2-(3,4'-dihydroxyphenyl)vinyl]benzene-1,3-diol, also called 3',4',3,5-tetrahydroxy-trans-stilbene, is a natural occurring metabolite of resveratrol previously identified as the active ingredient in herbal preparations in folk medicine. It is present in rhubarb, berries, peanuts, sugar cane, wine, and skins of grape. It is an inhibitor of protein tyrosine kinase^{16, 17}, and other enzymes. Recent studies show that it inhibits gastric H⁺, K⁺-ATPase Enzyme²². It also exhibits strong antioxidant and antilukaemic activities due to the presence of the catechol moiety.

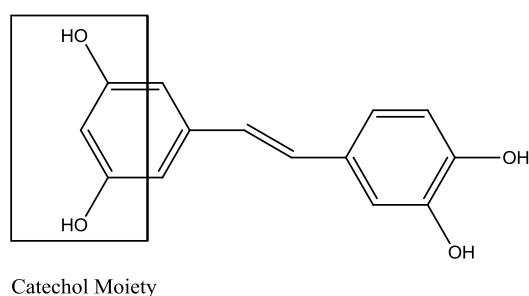


Fig 5. Structure of piceatannol

Pinosylvin.5-[(E)-2-phenylethenyl]benzene-1,3-diol is a pre-infectious stilbenoid present in heartwood (*Pinus densiflora*) and leaf of pine (*pinus densiflora*)²³. It is produced prior to infection and is thus referred to as a pre-infectious stilbenoid. It protects the woods from fungal infections. Pinosylvin possesses antibacterial and antifungal activities when tested with *Staphylococcus aureus* and *Escherichia coli* and also inhibits yeast growth.

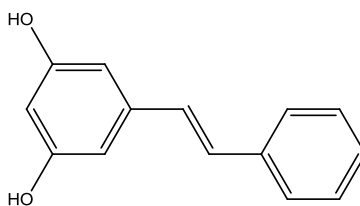


Fig 6. Structure of pinosylvin

Pterostilbene. 4-[(E)-2-(3,5-Dimethoxyphenyl)ethenyl]phenol is a stilbenoid that was initially isolated from sandalwood tree. It is chemically related to resveratrol and is present in blueberries and the skin of grapes just like RV²⁴. Animal studies reveal anti-cancer, anti-hypercholesterolemia, anti-hypertriglyceridemia, and anti-diabetic properties and its ability to fight and reverse cognitive decline. Pterostilbene possesses a higher bioavailability when compared to resveratrol and as a result it is easily transported into cells, more resistant to degradation and elimination. It also possesses strong anti-oxidant properties thought to be through modulation of gene expression and enzyme activity. Beneficial effects on cognitive and neuronal function during aging have been observed. Anti-inflammatory, anti-neoplastic, antifungal and anti-viral activities have also been reported.

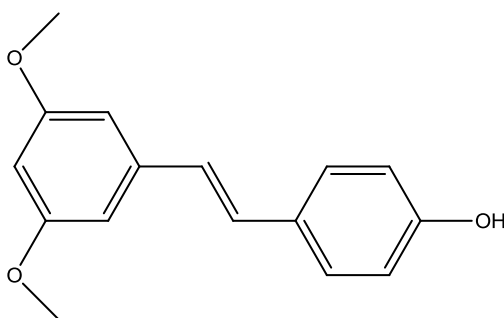


Fig 7. Structure of pterostilbene

Resveratrol

Resveratrol is a natural polyphenol produced by some plants, and it possesses several biological activities that have been exploited in recent years. The research efforts of this thesis are to synthesize new derivatives of RV so as to enhance some of their biological activities. It is thus important to outline the characteristics of this compound, its history, sources, biological activities, synthesis, and chemical and physical properties. The next section discusses most of the aspects of resveratrol mentioned above.

Nomenclature and Structure

The structural formula of RV is shown in Fig. 7, including the numbering of the carbon atoms according to IUPAC. Other names includes trans-3,5,4'-trihydroxystilbene, 3,4',5-stilbenetriol, and trans-resveratrol.

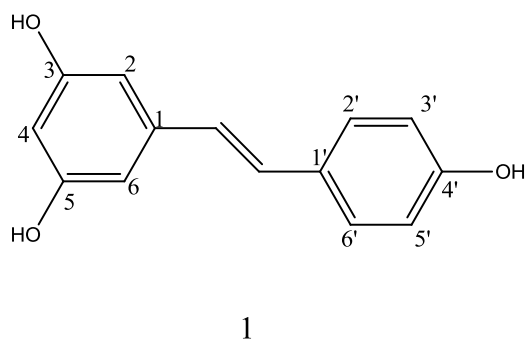


Fig 8. Structure of resveratrol, IUPAC numbering giving.

Isomerism

RV exists as two isomers, the cis and the trans isomers, Fig.8. The trans isomer is thermodynamically more stable just like the trans stilbene. The Cis isomer is thermodynamically less stable due to steric interactions that forces the phenyl rings out of plane and prevents proper conjugation. However, the Cis isomer possesses antioxidant and anti-inflammatory properties but has not been thoroughly studied.

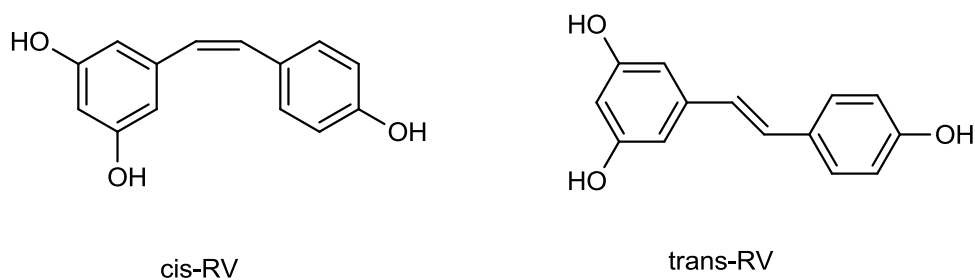


Fig 9. Structure of RV isomers

Chemical and Physical Properties

RV is a white powder with a slightly yellow cast and very low water solubility (0.03g/L). However, it is more soluble in DMSO and ethanol, 16 g/L, and 50 g/L respectively. RV is a derivative of stilbene and thus exists in cis and trans forms of which the trans is most stable. The trans form can undergo isomerization to the cis form upon exposure to UV light. RV has the phenol unit and will thus undergo most chemical reactions of phenols such as oxidation. RV shows anti-oxidant properties and thus easily oxidized.

History and Sources

Resveratrol (RV) was first isolated in 1940 as an ingredient of the roots of white hellebore (*Veratrum gradiflorum* O. Loes)²⁵. In 1963, it was identified as the active ingredient in

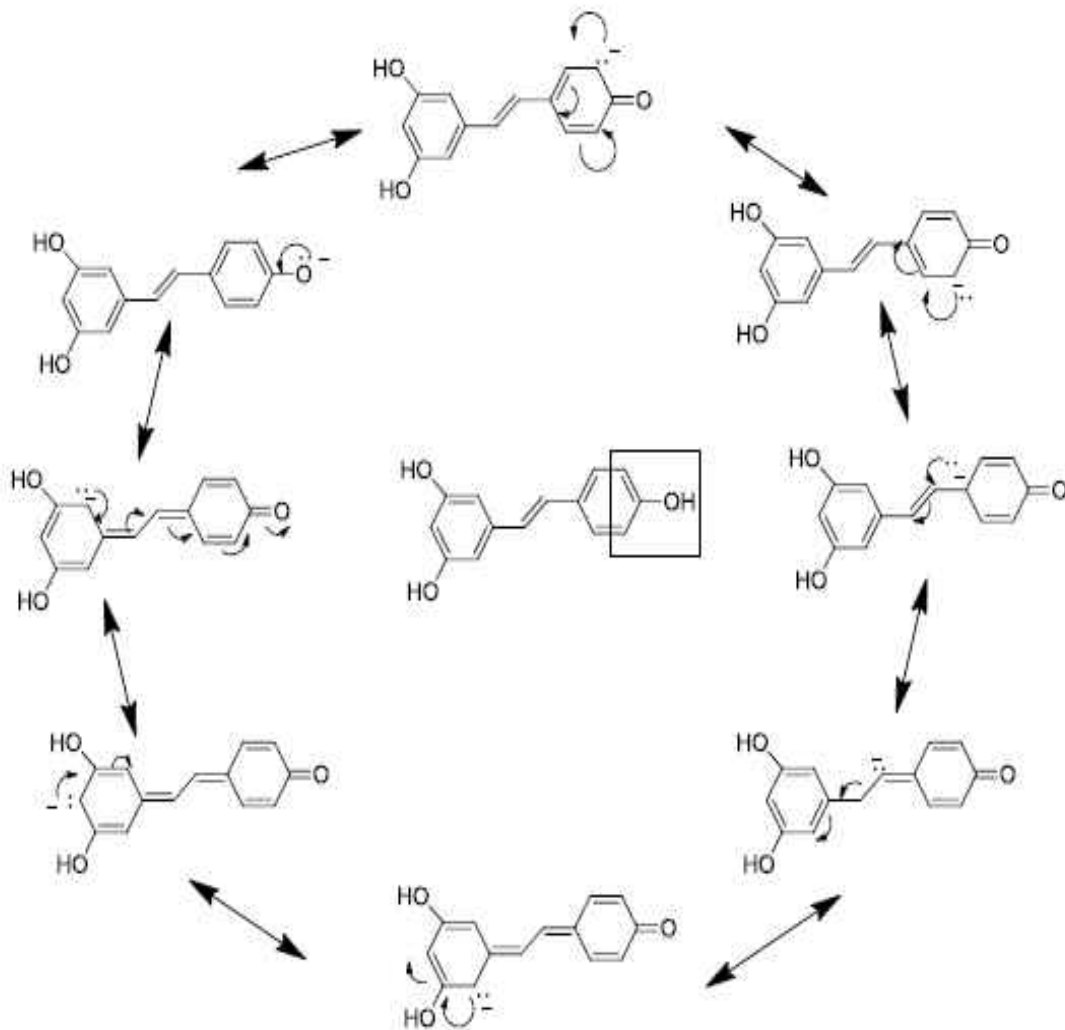
dried roots of *Polygonum cuspidatum* also referred to as Ko-jo-kon in Japanese. RV was first detected in grape vines in 1972 and wine in 1992²⁵⁻²⁸. It was detected in grape skin especially when infected with *Botrytis cinerea*. RV is synthesized in leaf epidermis and grape skin. Fresh grape skin contains about 50-100 mg per g of dry weight. The concentration of RV in red wine ranges from 2-40 μ M. RV is present in peanut butter and many types of berries^{2, 8, 29-31}

The French Paradox

Within the past few decades, the French population has been identified as a regular consumer of red wine. The so called 'French Paradox' is the low prevalence of coronary heart disease related death in the French population compared to the rest of Europe despite high intake to dietary cholesterol and saturated fats^{11, 12, 25, 32}. The incidence of heart infarction in France is about 40% lower than the rest of Europe.²⁵ Epidemiological Studies reveal an inverse relationship between the consumption of wine and the incidence of heart infarction²⁵. All these observations led to the suggestion that RV might be the active ingredient in red wine responsible for cardio protection.

Reactivity

The stilbene skeleton is stable and the reactivity is associated with the hydroxyl functional group of the phenol units. RV is a good radical scavenger through electron capture or proton donation to convert free radicals to more stable molecules. From the structure of RV, the OH-group at the 4'-position is more acidic compared to the 3 and 5 position. Schemes 1 and 2 show the resonance structures involved with proton abstraction at the 4'-position and 3-position respectively. The 3-position and 5-position are equivalent due to rotation of the sigma bond between carbon-1 and the SP² hybridized carbon on the structure of RV.

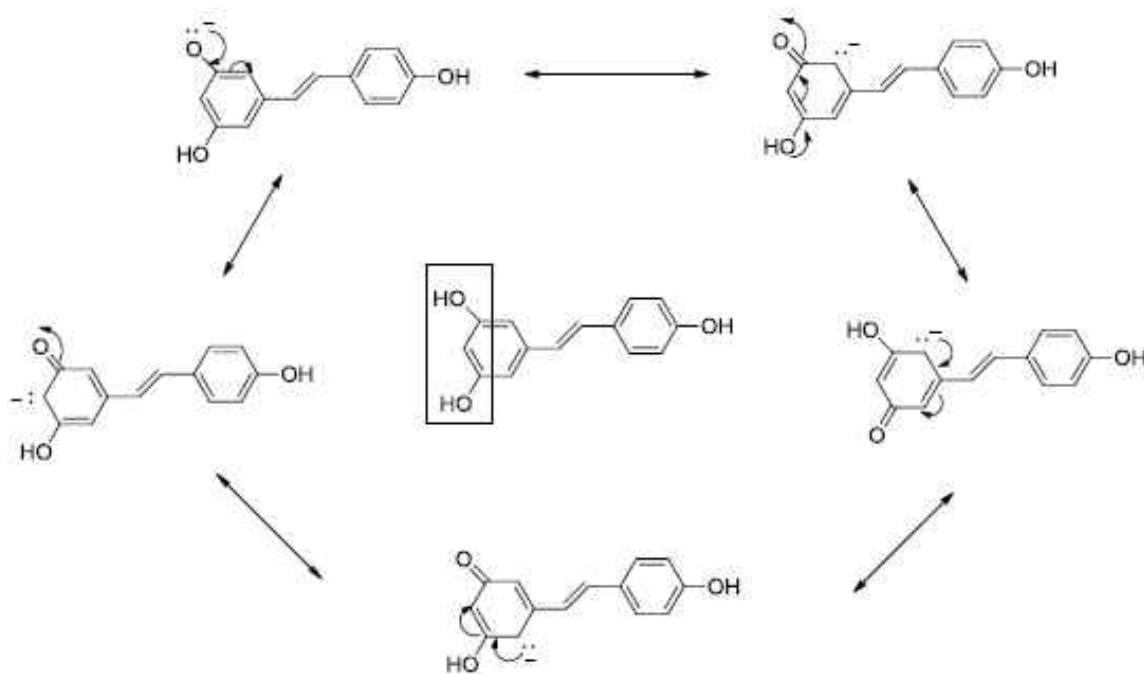


Scheme 1. Resonance structures from proton abstraction at 4'-hydroxyl group

Dietary supplement

RV has been shown to have many health benefits and has been available as a dietary supplement within the past 10 years²¹. Extensive media coverage greatly increased the sales of RV in 2006 although studies presented the fact that benefits to humans are yet to be proven²⁷. Many kinds of supplements are available in the market today and vary in terms of purity (50 to

99%). Many brands are unpurified extracts from Japanese knotweed tree, which also contains emodin¹⁶



Scheme 2. Resonance structures from proton abstraction at 3 or 5-hydroxyl group.

Reported Biological Activities

On the basis that RV is a phytoalexin and strong antioxidant, it has been subjected to a several pharmacological studies, and several biological activities have been reported. This includes antioxidant^{1, 16, 33-35}, coronary vasodilator, inhibitor of platelet aggregation, cardioprotective, anti-microbial, anti-fungal, anti-leukaemic, anti-diabetic, anti-inflammatory³⁶, cancer chemo-protective^{15, 26, 29}, anti-tumor^{29, 31}, anti-aging^{37, 38}, and several unreported biological studies. However, most of the biological activities of RV have been observed by in vitro studies

and more in vivo studies are in progress. This has led to increased interest in RV related research.

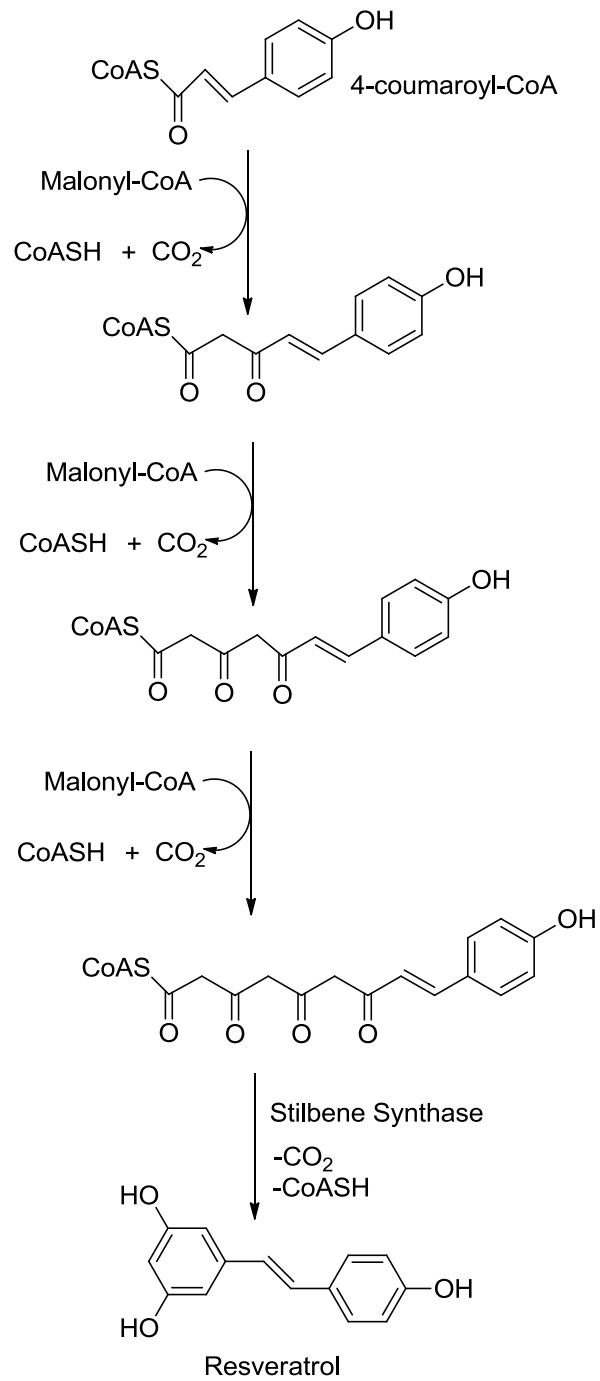
Recent Applications

In 2010, Kanti et al. explained the biological effects of RV are due to its anti-oxidant activities. In their review on the anti-oxidative activity of RV, they identified all studies relating to the anti-oxidant activity of RV and linked it with some biological activities. For example, based on a study that RV prevented formation of membrane protein carbonyls (PCO) in red blood cells and plasma under oxidative stress conditions, it is clear that RV prevents protein oxidation because PCO are products of protein oxidation. Also, based a study that confirmed the presence of PCO in artery plaque, Kanti et al. concluded that the prevention of coronary heart disease by resveratrol was due to its anti-oxidant activity.

In May 2011, Kumerz et al. discovered that RV had the potential to prevent migration of vascular smooth muscle cells (VSMC). Migration of VSMC can be induced by platelet-derived growth factor (PDGF), the wound healing process, or by Epidermal Growth factor (EGF). This migration is believed to be mediated by the Rac Activation. In their study, they found out that RV (50 μ M) reduced the migration of VSMC due to EGF but not PDGF³²

Biosynthesis of Resveratrol

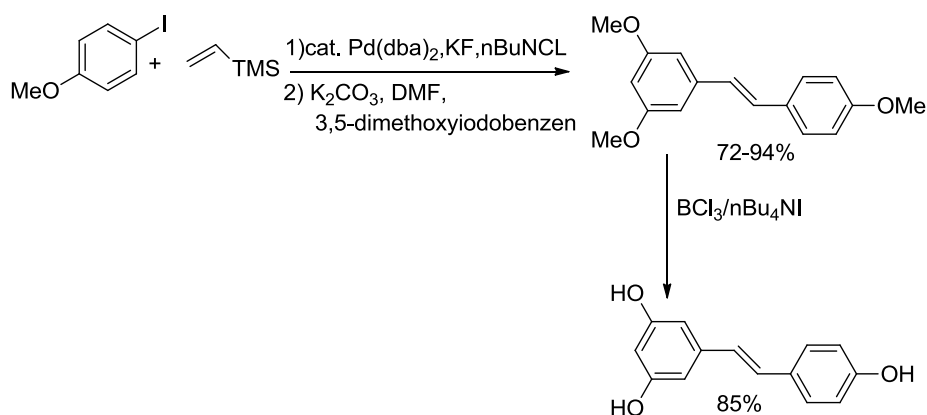
In biosystems, RV is synthesized through the same pathway as chalcone and Quercetin. All plants have the substrates (4-coumaroyl-CoA and Malonyl-CoA) necessary for the synthesis of resveratrol but lack the enzyme stilbene synthase (STS), a key enzyme in the synthesis. Scheme 3 shows the synthetic pathway in which 4-coumaroyl-CoA is used to perform three condensation reactions with Malonyl-CoA.



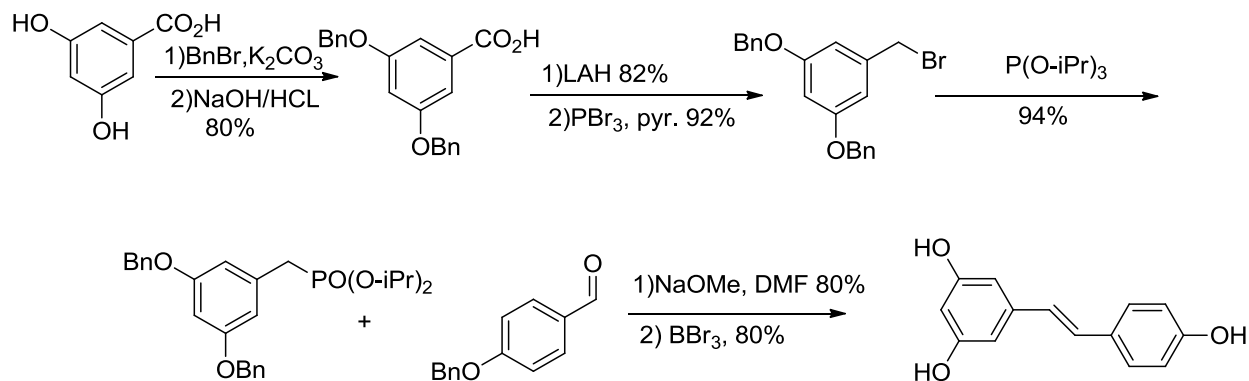
Scheme 3. Biosynthetic pathway of resveratrol

Chemical Synthesis of Resveratrol

RV has been extracted from plants; however, extraction processes are associated with high cost and low purity and thus a need for total synthesis of RV. Several synthetic pathways have been used to synthesize RV including the Wittig reaction, vinylsilane Heck reaction, and Optimized Horner–Emmons reaction. The reaction scheme for the last two synthetic pathways are shown below



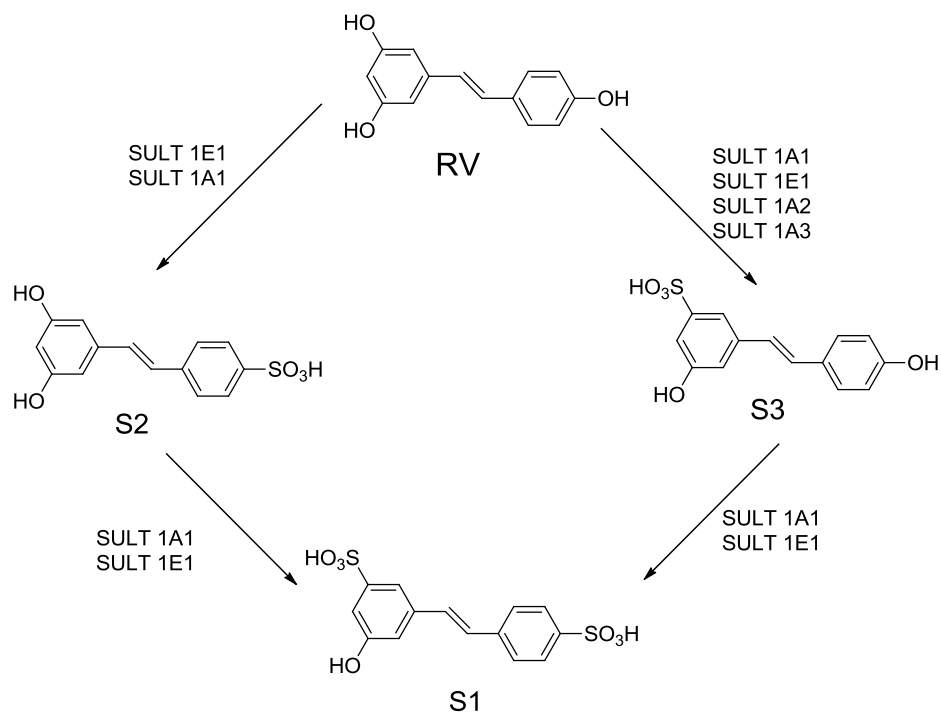
Scheme 4. Vinylsilane Heck Reaction for synthesis of RV



Scheme 5. Optimized Horner-Emmons synthesis of RV

Pharmacokinetics

Several *in vivo* studies in animals and humans reveal that intestinal uptake of RV is very low^{25,39}. There are only trace amounts in the blood stream due to extensive metabolism in the gut and liver. Oral administration and intravenous injection of RV is rapidly metabolized within 2 h reaching a peak at about 30mins⁴⁰. The bulk of the RV is converted to sulfate and glucuronide substrates²⁵. As a result of this fast metabolism, it has been suggested that the biological effects of RV might be associated with its metabolites or could act in synergy with other polyphenols²⁵. In humans, most of RV administered intravenously is metabolized to the sulfate conjugate within ≈ 30 mins. The scheme below shows the proposed metabolic pathway of RV in human cytosol *in vitro*. Human recombinant sulfotransferases (SULTs) are involved in the catalysis.



Scheme 6. Proposed metabolic pathway of RV in human liver cytosol *in vitro*

Interesting Resveratrol Analogues

Within the past few years, several resveratrol analogues have been synthesized and subjected to biological evaluation. Some very interesting results have been reported in which certain biological activities of the new analogues were better than RV. There are two main designs involved in the synthesis, conservation of the stilbene skeleton and modification of the skeleton by adding a nitrogen or sulfur in one or both rings. Three examples are discussed in this thesis, one for modification of the skeleton and two in which the stilbene skeleton is conserved.

In 2005, Chen et al.³⁶ synthesized and evaluated the anti-inflammatory activity 17 novel resveratrol derivatives on xylene-induced mouse ear edema. The pharmacological results showed that some compounds have potent anti-inflammatory activities. Compounds 3a and 4b below showed an edema reduction ranging from 30 to 35%.

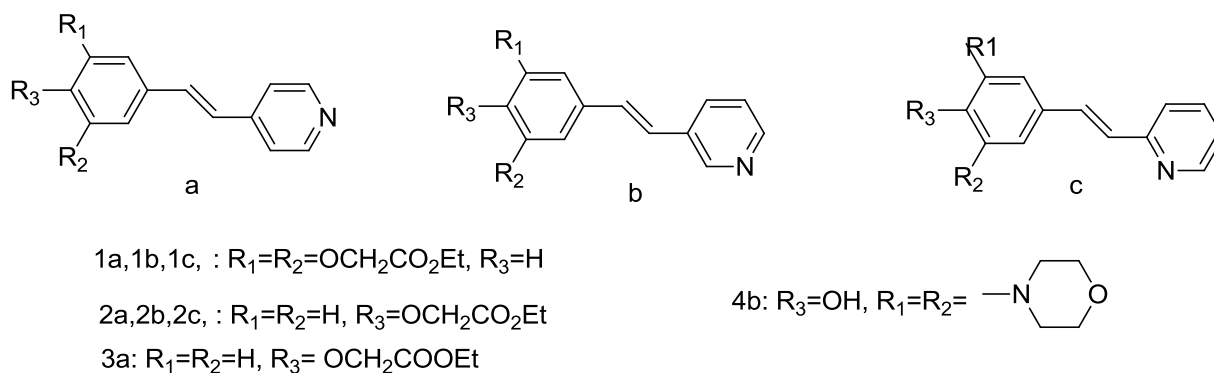


Fig 10. Structure of selected analogues by Chen et al

In 2008, Jiang³⁸ designed, synthesized, and did spectroscopic studies of resveratrol aliphatic acid ligands of human serum albumin (HSA). HSA is the most abundant serum protein in humans produced in the liver and has a life span up to 20 days. Jiang synthesized both aliphatic esters and aliphatic acids. The acids showed better solubility in pure water and

phosphate buffer (pH 7). Compound **4** in figure below was found to be a much better ligand for HSA than resveratrol and hence **5** should bound tightly to HSA.

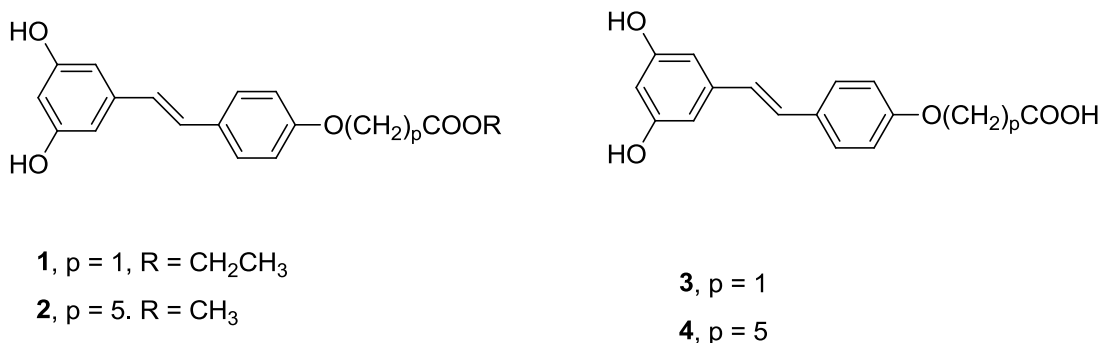


Fig 11. Resveratrol derivatives by Jiang

In 2009, Jiang et al.⁵ further investigated the role of compound **3** and **4** in Toll-like receptor 2 (TLR2)-mediated apoptosis. TLR2 are involved in innate immune system (non-specific immune system) and function to recognize pathogens, and thus aimed at defending the host. TLR recognizes and responds to pathogens in a generic way. They reported that compound **4** significantly inhibits the expression of TLR2 and also overexpression of TLR2 in human embryonic kidney (HEK293) cells caused significant decrease in apoptosis after treatment with compound **4**.

Recently, Biasutto et al.⁴¹ added glucosyl groups to the RV preserving the stilbene skeleton (Fig 11). When administered to rats, its blood concentration versus time curve shifted towards longer times compared to resveratrol. However, these derivatives were destroyed by blood esterases in less than 1 h. The compound was found to be water soluble and nearly stable in acidic environments similar to the stomach.

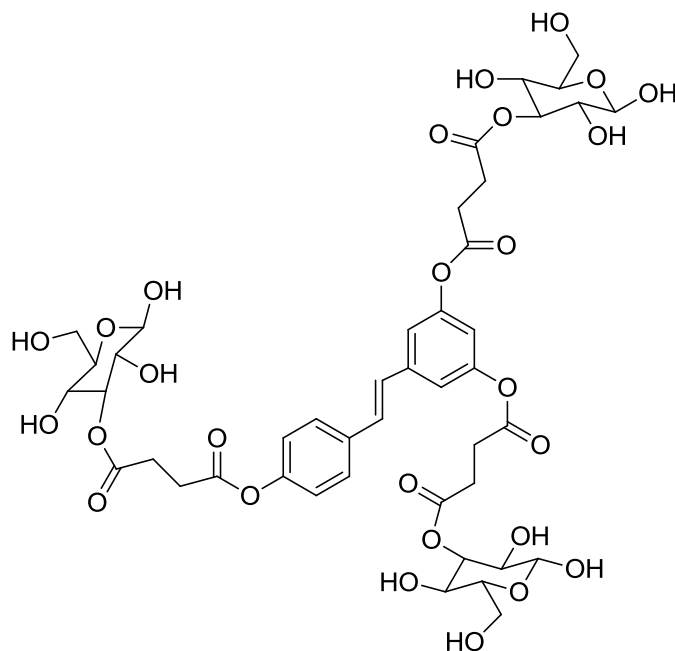
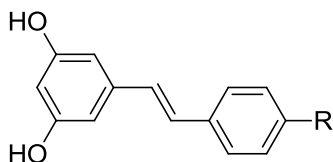


Fig 12. Resveratrol derivative by Biasutto et al. (3',4',5-tri(α -D-glucose-3-succinyl)

In 2007, Yang et al.³⁷ published in *Aging Cell*, a series of compounds that could extend yeast replicative lifespan. It is known that dietary restriction (DR) delays aging and extends the maximum lifespan of a wide range of organisms. In mammals, DR delays numerous age-associated diseases including cancer, neurodegeneration, atherosclerosis, and type II diabetes. An active area of research is the search for small molecule mimetics that can deliver the health benefits of DR. Since the identification of members of the family of Sirtuin genes (SIRT2, SIRT2L1, SIRT2 α) it has been hypothesized that they play a key role in an organism's response to stress and also responsible for the life-extension benefits of DR. It has been observed that increased expression of the SIRT2 gene in many simple organisms is associated to 30-50% life extension^{42, 43}. Yang et al. designed synthesis routes in which the stilbene skeleton was preserved as well as the 3 and 5 hydroxyl groups and modified the 4'-hydroxyl group by substituting the proton with thiomethyl, methyl, ethyl, methoxy, and acetoxy groups, Fig.12. Their design was

based on previous structure-activity relationship of SIRT1 activation by stilbenes from plant sources.



- 1, R = Thiomethyl
- 2, R = Methyl
- 3, R = Ethyl
- 4, R = Acetoxy

Fig 13. Derivatives by Yang et al

Compound 1 above was the most effective at stimulating SIRT1 with 18-fold increase in activation. RV, 2, 3, and 4 activated SIRT1 12-fold, 16-fold, 14-fold, and 11-fold respectively. Also, compounds 1, 3, and 4 extended the mean and maximum yeast life span.

Drawbacks of Resveratrol

The main drawback of resveratrol is its low bioavailability. Bioavailability is broadly defined as the absorption and use of a nutrient⁴⁰. The low bioavailability of RV is associated to its fast metabolism in the liver and gut and also its low serum solubility. Also, most in vivo studies involve administration of high doses of RV that could be toxic to cells. Hence the cytotoxicity of RV needs to be investigated properly.

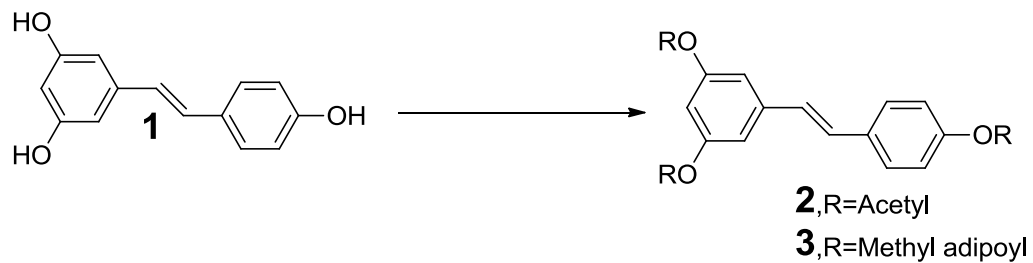
Objectives of Research

The main objectives of this research are to design and synthesize new resveratrol esters and acids derivatives to improve water solubility, serum transport by HSA, anti-cancer activity, and other biological activities. Another objective of this research is to carry out independent organic synthesis and structure elucidation of such compounds by spectral analysis.

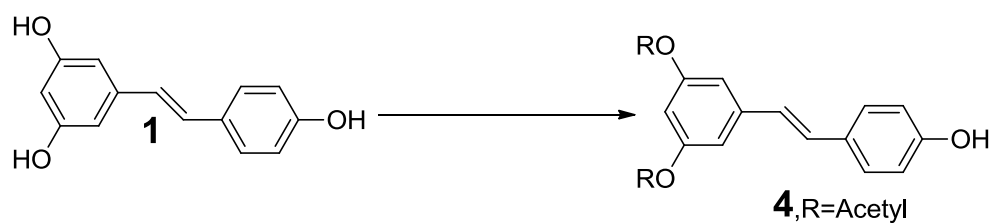
Synthesis Design

The types of RV derivatives to be synthesized were motivated by results reported by Jiang et al.³⁸ They reported increased binding of long chain RV ester derivatives to HSA and increase water solubility of RV aliphatic acids. Three methods were designed to synthesize RV esters and one for total synthesis of RV aliphatic acids. Generally, all the designs conserve the stilbene skeleton, and modifications were made on the hydroxyl groups. Scheme 7 below shows the types of derivatives to be synthesized.

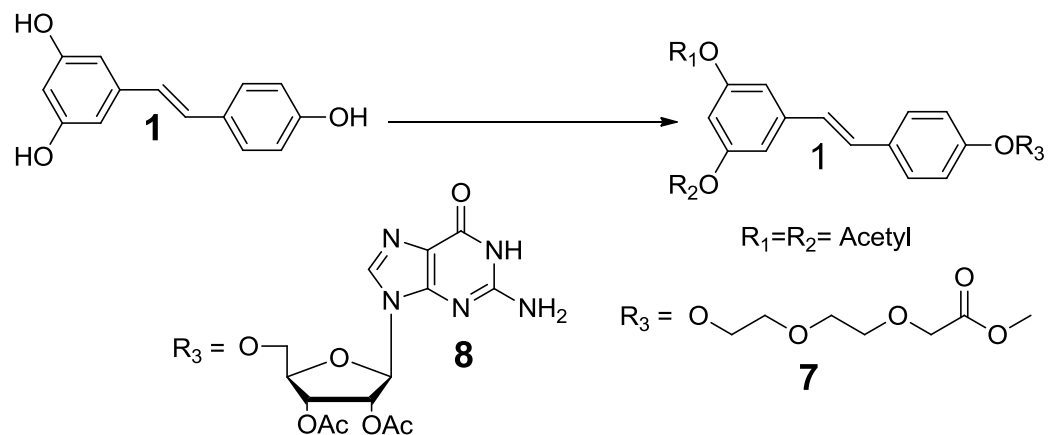
METHOD A :Synthesis of RV Tri-Esters



METHOD B: Synthesis of Di-Esters



METHOD C: Synthesis of Mixed Tri-Esters



Scheme 7. Synthesis Design for RV Esters

Method A

This method is designed to synthesize tri-esters such that all the hydroxyl groups undergo esterification. The objective is to have the same ester chain. This can easily be achieved by reacting RV with acid chlorides in the presence of a base.

Method B

This method is designed to synthesize di-esters at the 3 and 5-hydroxyl positions leaving the 4'-hydroxyl group available. This type of ester is designed as a precursor for synthesis of mixed ethers. This conversion can be achieved by subjecting a tri-ester to a regiospecific lipase enzyme to preferentially hydrolyze the 4' position.

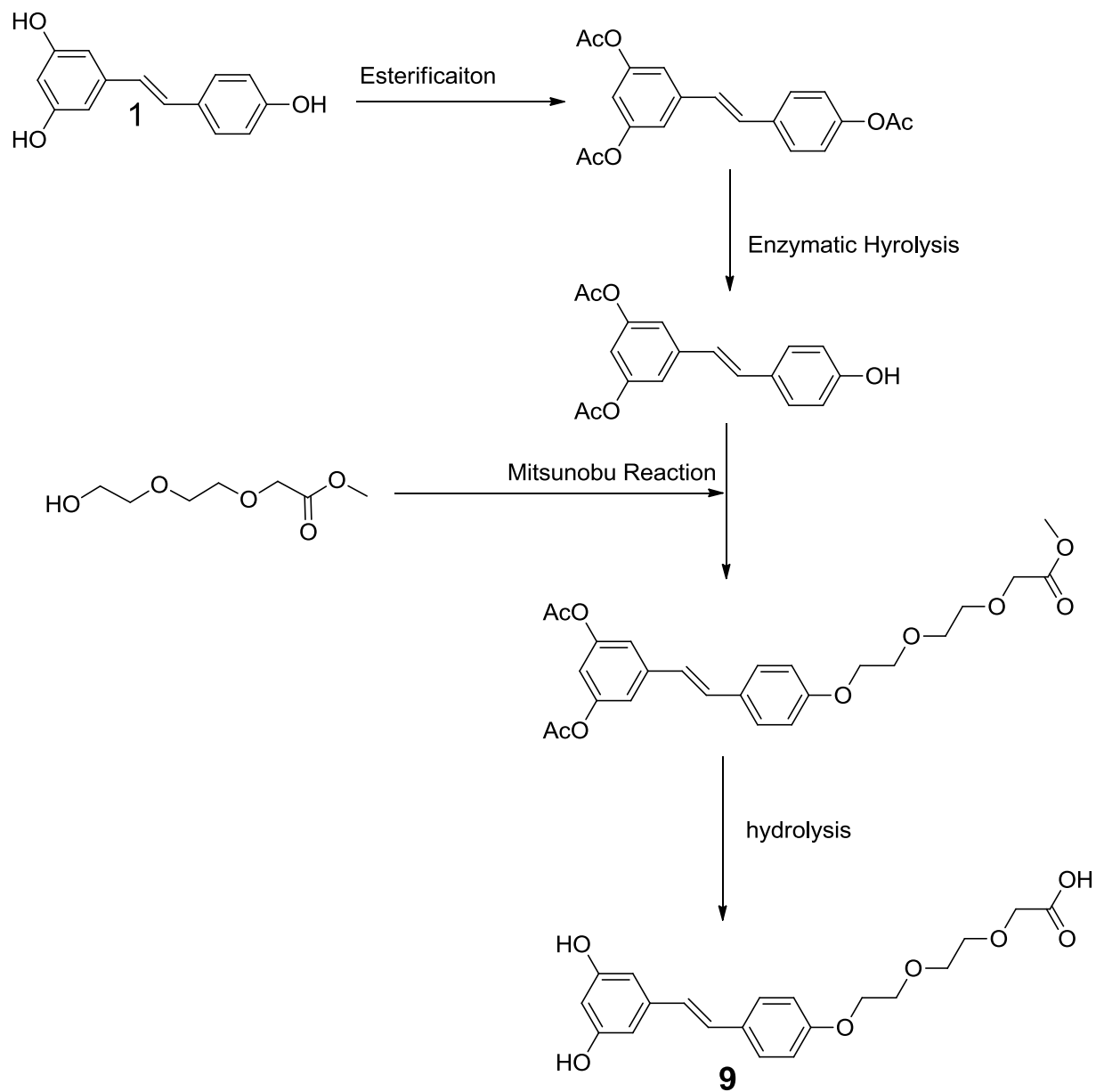
Method C

This method is designed to synthesize mixed esters by esterification of 4'-hydroxyl group while the 3 and 5-hydroxyl groups have already been esterified. A Mitsunobu reaction is employed to achieve the target compounds.

Total Synthesis of Resveratrol Aliphatic Acid

Jiang et al.³⁸ reported the increased water solubility of RV aliphatic acids, thus, a different type of aliphatic acid similar to that which they synthesized could have better biological evaluation data. The design for total synthesis is shown in the scheme below. It generally involves synthesis of esters followed by hydrolysis, scheme 8.

Total Synthesis of Resveratrol Aliphatic Acid



Scheme 8. Synthesis of resveratrol aliphatic acid

The Mitsunobu Reaction

This reaction was discovered by Oyo Mitsunobu (shown below); it converts an alcohol into a variety of functional groups such as esters using triphenylphosphine and an azodicarboxylate such as diethyl azodicarboxylate (DEAD). This reaction requires an acidic nucleophile because DEAD must be protonated during the reaction; typically, the nucleophile must have a pKa lower than 15. The reaction proceeds with inversion of configuration on the alcohol carbon as shown on the scheme below. The order of addition of reagents is crucial, typically the alcohol, acidic nucleophile, triphenylphosphine are dissolved in THF, then DEAD added slowly to the mixture and stirred for about 12 h.

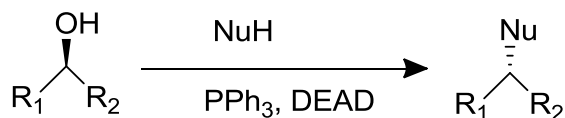
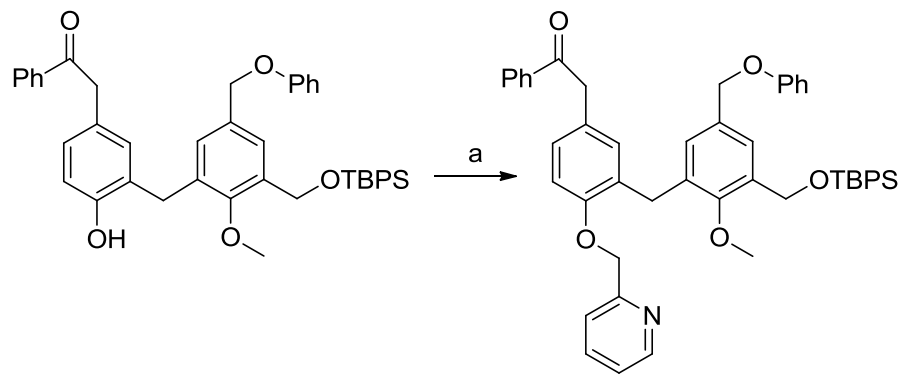


Fig 14. Mitsunobu reaction

Example of Mitsunobu Reaction

Bruno et al. in 1997 made use of the Mitsunobu reaction to synthesize functionalized aromatic oligomers⁴⁴. A phenol functional group was used as the acidic nucleophile and 2-(2-hydroxyethyl) pyridine as the primary alcohol to afford the desired product in high yield (84%).

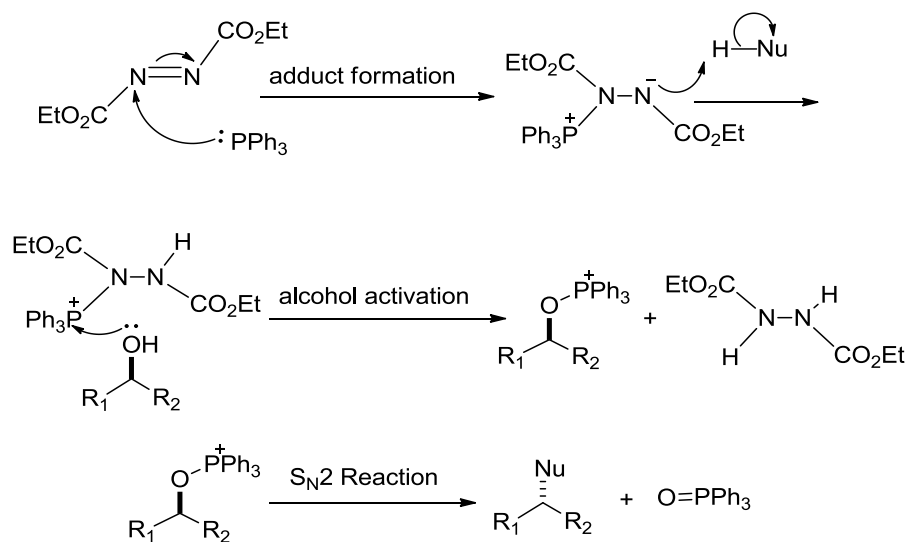


(a) 2-(2-hydroxyethyl)pyridine/DEAD, Ph₃P, r.t., 84%

Scheme 9. Mitsunobu reaction by Bruno et al.

Mechanism of Mitsunobu reaction

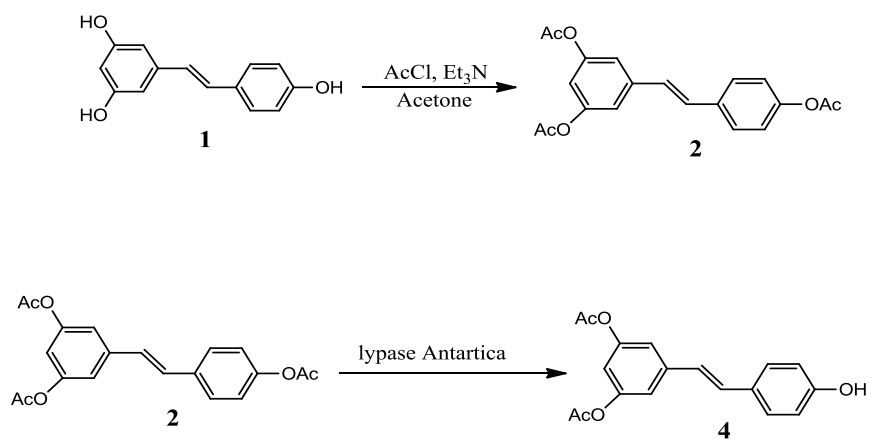
There has been much debate on the intermediates involved in the Mitsunobu reaction, and it has been a long debate. Generally, the phosphine makes a nucleophilic attack on DEAD to form betaine intermediate that abstracts a proton from the acidic nucleophile to form an ionic intermediate. The DEAD abstracts a proton from the alcohol resulting in the production of the oxyphosphonium ion and a diamide by-product. The nucleophile then attacks the oxyphosphonium ion to form the product and triphenyloxide. The scheme below shows the detailed mechanism.



Scheme 10. Mechanism of Mitsunobu reaction

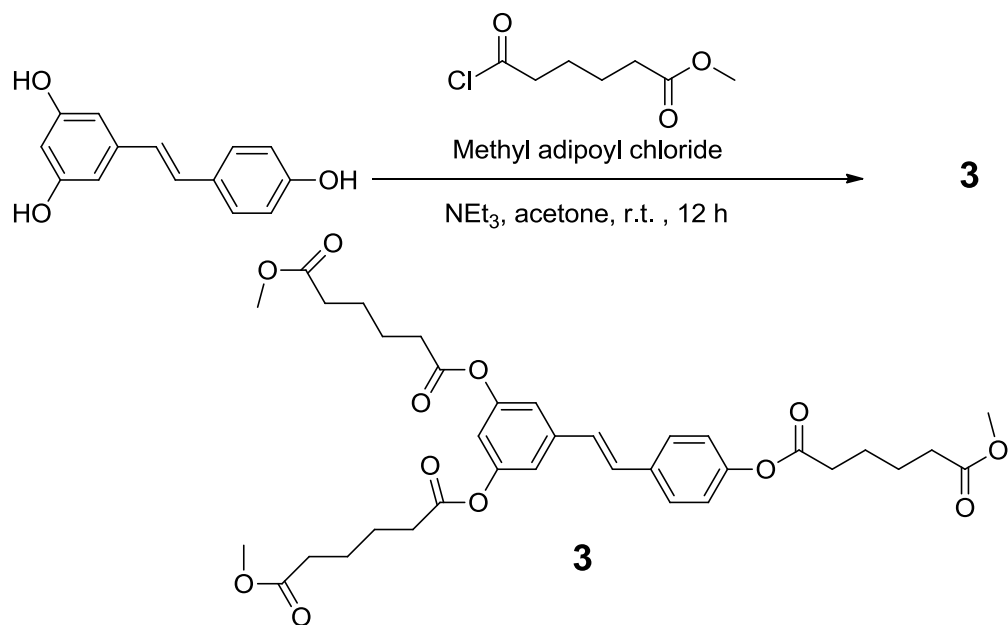
Proposed Synthetic Pathway for Compound 2 and 4

Compounds 2 and 3 have been synthesized before; they are to be used as precursors for compound 5.



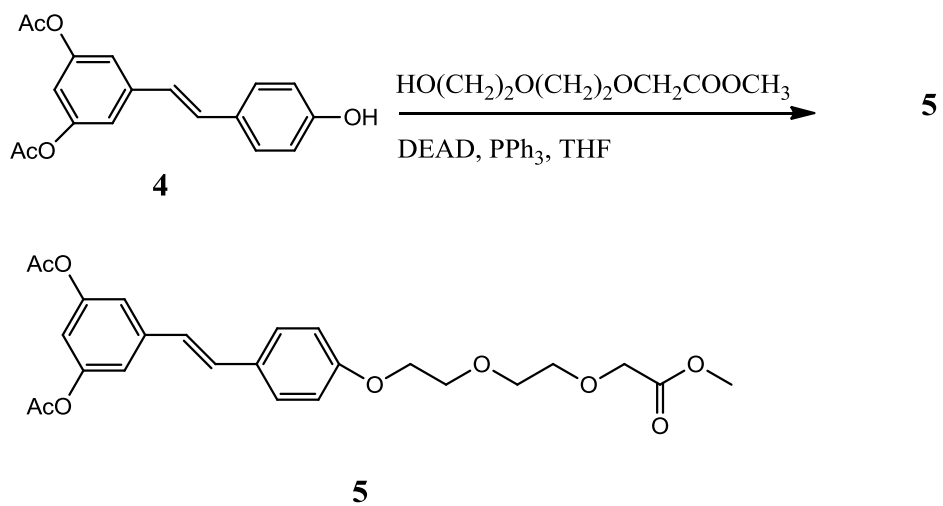
Scheme 11. Synthesis of tri and di acetyl esters of RV.

Proposed Synthetic Pathway for Compound 3



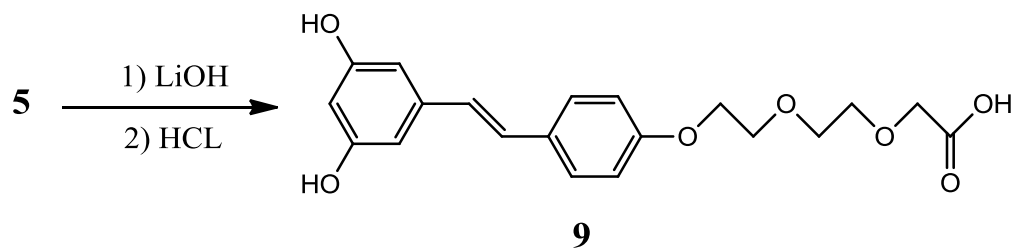
Scheme 12. Synthesis of long chain tri-ester of RV

Proposed Synthetic Pathway for Compound 5



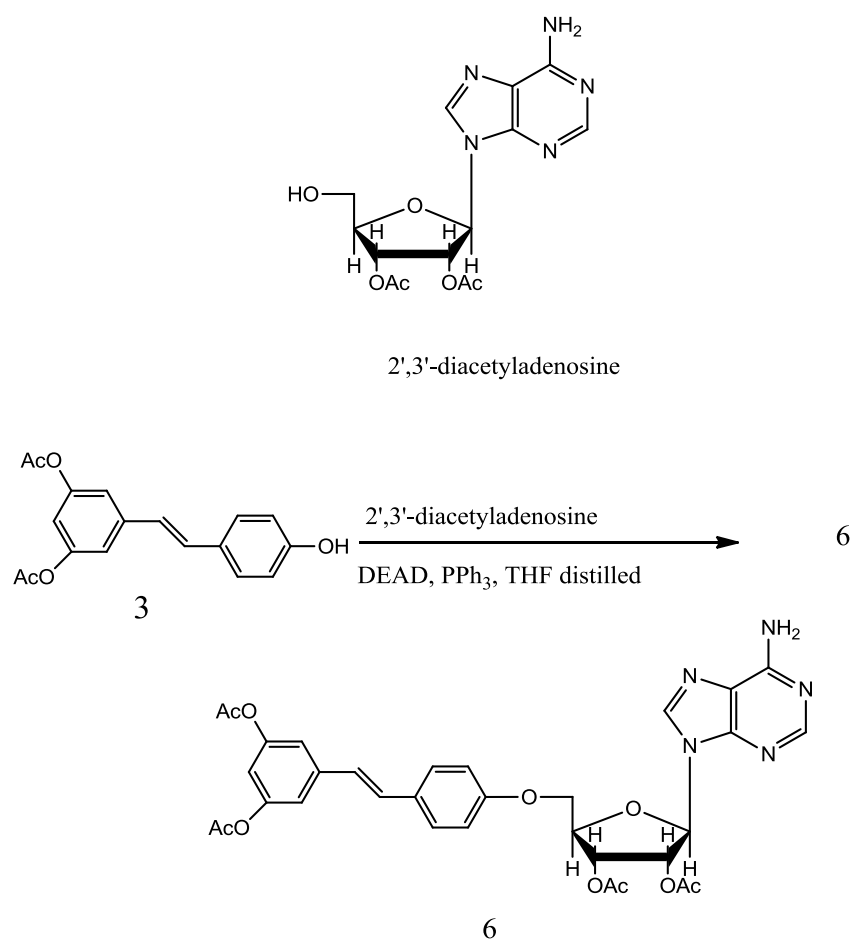
Scheme 13. Mitsunobu Reaction for synthesis of 5

Proposed Synthetic Pathway for Compound 9



Scheme 14. Hydrolysis of ester to acid

Proposed Synthetic Pathway for compound 6

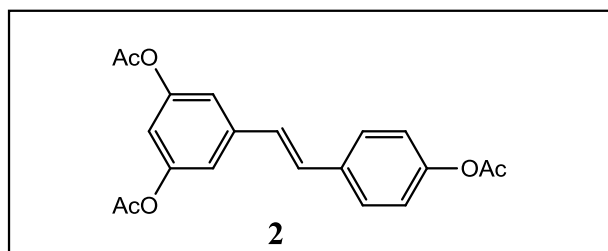


Scheme 15. Mitsunobu Reaction with 2', 3'-di-O-Acetyl adenosine

CHAPTER 2

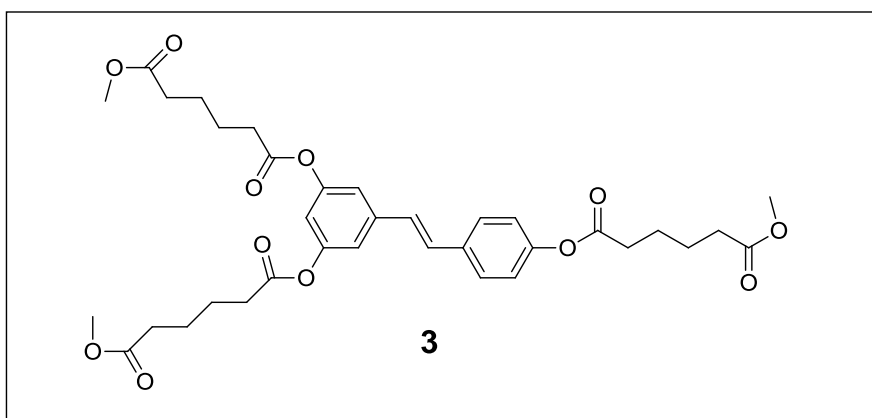
RESULTS AND DISCUSSION

Synthesis of 3,4',5-triacetyloxystilbene, 2



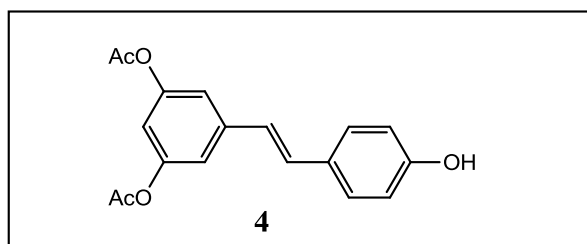
Compound **2** was obtained as a white solid after recrystallization from a 1: 1 hexane/acetone solvent mixture with Mp 120-121 °C (lit 121-122) °C. This compound has been synthesized before and was used as a precursor in the proposed synthetic pathway. A yield of 74% was obtained versus 94% reported in lit⁴⁵. The triethylamine reacts with the acetyl chloride to produce a salt which is a stronger electrophile and will be attacked by the hydroxyl groups of RV according to their acidity starting with the 4'-hydroxyl group.

Synthesis of methyl 1,1',1''-(3,4',5-stilbenyl)-1,6-hexanedioate, 3



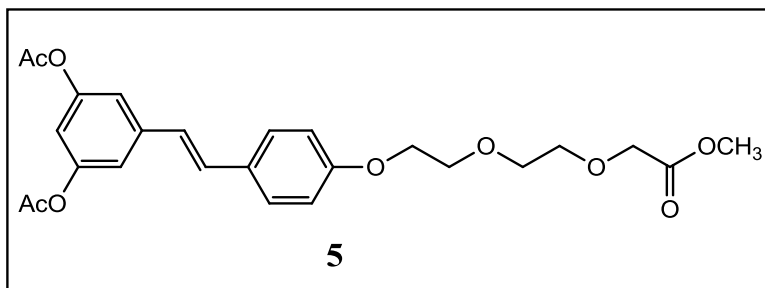
Compound **3** was obtained in high yield (88%) as a dense colorless liquid. ^1H NMR was very clean and purity above 95% was calculated based on the ratio of the most intense peak and sum of impurity peaks. This is a new compound and will be subjected to biological evaluation.

Synthesis of 3,5-diacetyloxystilbene, 4



Compound **4** was obtained as a white solid after recrystallization from hexane/acetone (4:1, v/v). Compound **2** was subjected to enzymatic alcoholysis with *lipase Antarctica* which is regiospecific to the 4' position. The overall yield of the above reaction was up to 95% as the starting material could be recovered and subjected to further hydrolysis. However, the reaction yield for single enzymatic alcoholysis was 18% compared to 25% reported in lit. Compound **4** was synthesized as a precursor to compound **5**.

Synthesis of methyl 8-(3',5'-diacetyloxystilbene-4''-oxy)-3,6-dioxooctanate, 5



Compound **4** was subjected to the Mitsunobu reaction to serve as the acid to yield compound **5** as a greasy brown oil. However, both ^1H NMR and ^{13}C NMR revealed the presence of a by-product of the Mitsunobu reaction that appears to be closely associated to compound **4**. Several purification attempts did not remove the impurity; however, the amount of impurity was reduced to obtain **4** with about 90% purity. The impurity was identified as the structure in Fig below.

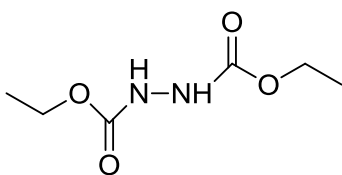
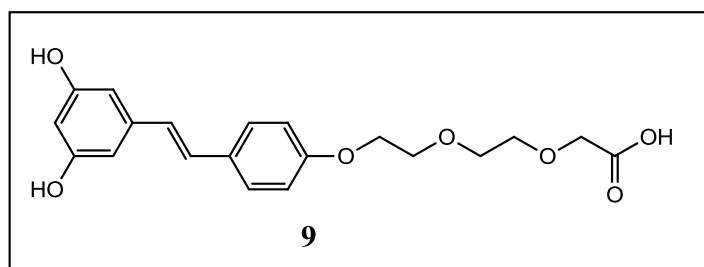


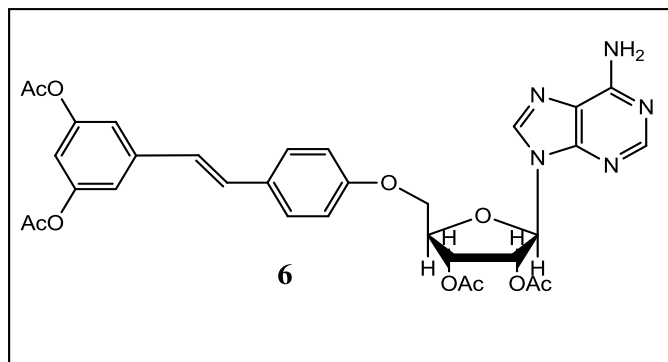
Fig 15. By-product of Mitsunobu reaction

Synthesis of 8-(3',5'-dihydroxystilbene-4''-oxy)-3,6-dioxooctanoic acid, **9**



Compound **9** was obtained as a brown solid, 43.6% yield. However, ^1H NMR spectra reveal the presence of the same impurity that was present in **5**. Attempt to further purify it involved further hydrolysis with a strong base, KOH, for two days and column purification hoping that all the by-products will be hydrolyzed. But the impurity was still present, leaving the compound only about 90% pure.

Synthesis of 4'-(2',3'-di-O-acetyladenosine)-3,5-Di-O-acetylstilbene, **6**



Compound **6** was obtained as a white solid. However, the ¹H NMR spectrum was not good. Compound **6** was very basic and difficult to separate from starting material, even after several column purification with different solvent systems. Further work on this compound was abandoned.

Conclusions

The objectives of this research were fulfilled with the synthesis of new RV esters (**3**, **5**, and **6**) and aliphatic acid (**9**) derivatives. However, only one of these new compounds (**3**) is good enough for publication per Journal of American Chemical Society, and can be subjected to biological evaluation because it was obtained in high purity and yield. Compounds **2** and **4** have been synthesized before; however, they were not subjected to biological evaluation. Hence, they can be subjected to biological evaluation

Method A

This method was designed to synthesize tri-esters of RV by esterification of the three hydroxyl groups of RV. Acid chlorides were used because they are more reactive than the corresponding carboxylic acid. A new compound, **3** was obtained through this method in high yield and purity. This compound is a good candidate for biological testing and could have better bioavailability compared to RV. The long hydrophobic chain and hydrophilic heads could be very interesting. A similar kind of triester had been synthesized before and biological evaluation revealed it had better bioavailability⁴¹ than RV. The Purity of **3** was determined to be more than 95% pure based on ¹H NMR integration. Compound **2**, which had been synthesized before, was obtained through this method as a precursor that was used for total synthesis of the aliphatic acid.

Method B

This method was designed to synthesize di-esters of RV with esterification at the 3 and 5-hydroxyl group. Compound **4** obtained through this method and used as a precursor to compound **5** and **6**. The synthesis of this compound involves enzymatic hydrolysis of compound **2** using *lipase Antarctica* that was selective for hydrolysis at the 4'-position. A yield of 22% (lit. 60%⁴⁵

30%⁴⁶) was obtained and starting material was recovered and reused. For future work, this conversion can be increased to 94% by using a different lipase enzyme, *P.cepacia lipase*⁴⁶.

Method C

This method was designed to obtain long chains of esters at the 4'-position. Two mixed esters were obtained through the Mitsunobu reaction of compound **4** to afford compounds **5** and **6**. These two compounds were associated to impurities that could not be completely removed and purity was assessed to be below 90%. Future work will involve improving the purity of the compounds by trying out different reaction schemes to synthesize the compounds.

Total Synthesis of RV aliphatic acid

Compound **9** was obtained by hydrolysis of **5**. However, the same impurity present in compound **5**, which was identified to be the by-product of the Mitsunobu reaction, was present. Further hydrolysis for 2-days in an attempt to hydrolyze the product was attempted, but still final compound was only about 90% pure. Future work will involve synthesizing the compound through a method that high purity can be achieved.

A new RV triester has been synthesized in high yield and purity and would be subjected to biological evaluation in future work. Future work will involve synthesis of the di- and mono-ester derivatives by enzymatic hydrolysis and Mitsunobu reaction respectively.

CHAPTER 3

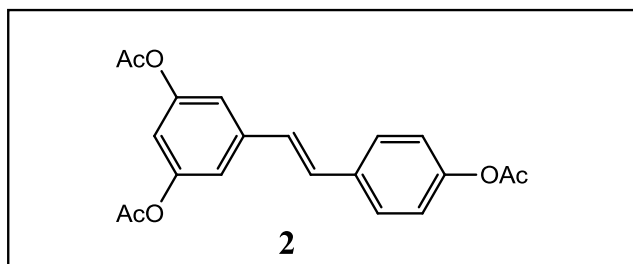
EXPERIMENTAL

General Methods

All commercial reagents including RV were purchased from Sigma (St.Louis, MO, USA) and used without further purification. Solvents were distilled when necessary and mentioned in the experimental procedures. All proton (^1H) and carbon (^{13}C) NMR spectra were recorded on JEOL-NMR Eclipse spectrometer operating at 400 MHz. Chemical shifts were recorded as delta values in parts per million (ppm) relative to TMS. The multiplicity of signals is reported as follows: s, singlet; d, doublet; m, multiplet. Column chromatograph was performed using silica gel as stationary phase and different mixtures of solvents as eluents. Thin layer chromatography (TLC) was performed on silica gel plates using appropriate solvents and visualized under a UVGL-58 UV lamp using short wave. All weighings were done using Adventurer Pro mass scale. Melting points were determined using MEL-TEMP and recorded without correction.

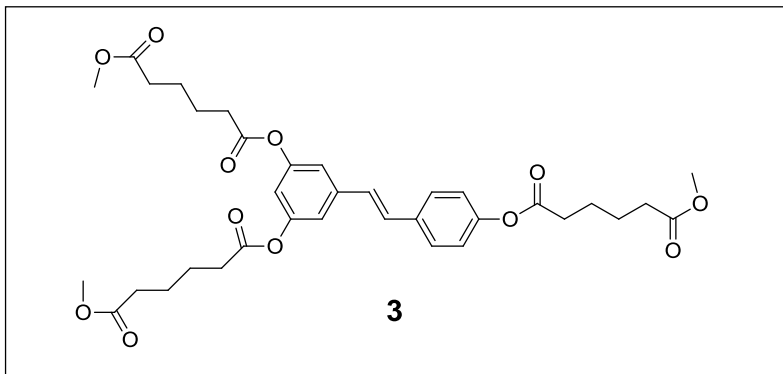
Experimental Procedures

Synthesis of 3,4',5-triacetyloxystilbene, 2



To a one-neck 50 ml-round-bottomed flask were added resveratrol (0.60 g, 2.63 mmol), acetone (20 mL), acetyl chloride (0.561 mL, 7.89 mmol), and triethylamine (1.096 mL, 7.89 mmol). The mixture was stirred (350 rpm) at r.t for 16 h. The mixture was acidified using 2N HCl. The resulting mixture was extracted with ethyl acetate (2 × 20 mL), washed with a saturated solution of sodium bicarbonate (3 × 10 mL), and dried with anhydrous magnesium sulfate. The magnesium salt was filtered by gravity and after removal of the solvent in the hood, the residue was purified with column chromatography using a mixed solvent (10-40% of acetone in hexane in volume) to give compound **2** (0.67 g, 74%). R_f value, 0.26 (Acetone/Hexane, 30:70, V/V), Mp 120-121 °C. $^1\text{H NMR}$ (CDCl_3 , δ ppm) , 2.2(s, 3 H, acetyl) 2.3 (s, 6 H, acetyl), 6.8 (t, $J = 0.36$, 1 H), 7.1 (d, $J = 8.8$, 1 H), 7.2 (q, $J = 1.12$ Hz, 3 H), 7.6 (d, $J = 8.4$ Hz, 2 H)

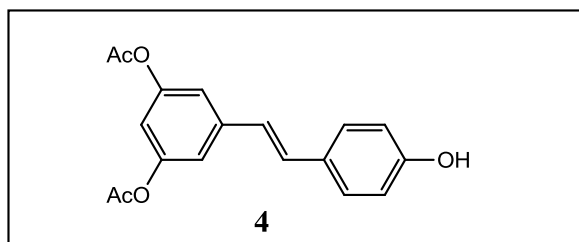
Synthesis of 1,1',1''-(3,4',5-stilbenyl) 1,6-hexanedioate, 3



To a one-neck 50 ml-round-bottomed flask were added resveratrol (0.60 g, 2.63 mmol), acetone (20 mL), methyl adipoyl chloride (1.643 g, 9.20 mmol), and triethylamine (1.286 mL, 9.20 mmol). The mixture was stirred (350 rpm) at r.t. for 16 h. The mixture was acidified using 2N HCl. The resulting mixture was extracted with ethyl acetate (2 × 20 mL), washed with a saturated solution of sodium bicarbonate (3 × 10 mL) and dried with anhydrous magnesium

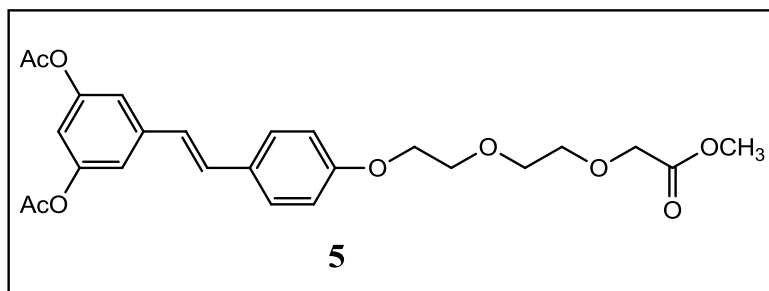
sulfate. The magnesium salt was filtered by gravity and after removal of the solvent in the hood, the oily residue was purified with column chromatography using a mixed solvent (10-40% of acetone in hexane in volume) to give compound **7** (1.51 g, 88%). R_f value, 0.32 (Acetone/Hexane, 30:70, V/V), $^1\text{H NMR}$ (400 MHz, CDCl_3 , δ , ppm), 6.7-7.4 (stilbene, 9 H) 3.7 (s, 9 H, CH_3), 2.4 (t, 6 H, CH_2), 2.6 (t, 6 H, CH_2), 1.8 (m, 12 H, CH_2CH_2 , 2 H), $^{13}\text{C NMR}$ (100Mz, CDCl_3 , δ , ppm), 23.1, 23.2, 33.1, 33.2, 51.4, 114,156.5, 122, 128, 129,130,134,140,150,151.5,172, 172.5, 173.

Synthesis of 3,5-diacetyloxy-4'-hydroxyl stilbene, **4**



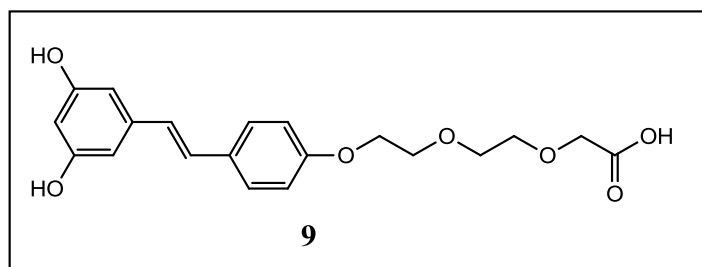
To a one-neck 25 mL-round-bottomed flask were added **2** (100 mg, 0.28 mmol), tert-butyl methyl ether (10 mL) and 1-butanol(0.4 mL). The mixture was stirred in a rotavop at 280 rpm for 5 minutes to allow **2** to completely dissolve, then *lipase Antarctica* (100 mg) was added and the mixture stirred for 25 minutes. The enzyme was filtered and the resulting mixture concentrated and submitted for column chromatography using a mixed solvent (10-40% acetone in hexane in volume) to give compound **3** (0.020 g, 22%). R_f value, 0.32 (Acetone/Hexane, 40:60, V/V), Mp 120-121 $^{\circ}\text{C}$. $^1\text{H NMR}$ (400MHz, CDCl_3 , δ , ppm), 2.3 (s, 6 H, acetyl), 6.8 (t, J = 0.36, 1 H), 7.1 (d, J = 8.8, 1 H), 7.2 (q, J = 1.12 Hz, 3 H) 7.6 (d, J = 8.4 Hz, 2 H)

Synthesis of methyl 8-(3',5'-diacetyloxystilbene-4''-oxy)-3,6-dioxooctanoate, 5



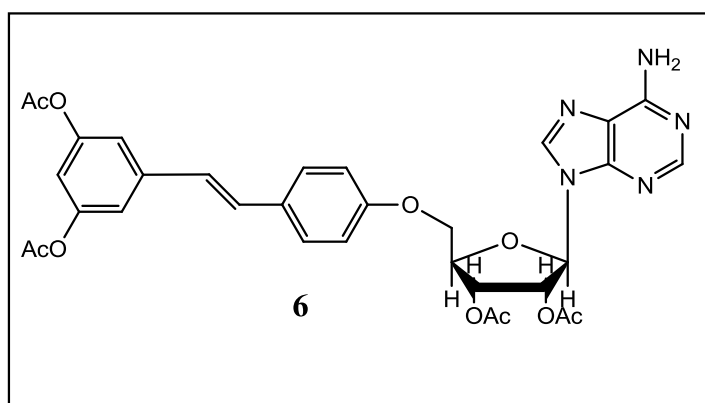
To a one-neck 10 mL round-bottomed flask were added **4** (0.060 g, 0.20 mmol), THF freshly distilled (1 mL), compound **9** (110uL, 0.60mmol), triphenylphosphine (0.162 g, 0.60 mmol) , and diethylazodicarboxylate (DEAD) (100 uL, 0.60 mmol). The resultant mixture was stirred at r.t. under N₂ atmosphere for 12 h and then concentrated with an evaporator equipped with an oil pump. The residue was purified with flash column chromatography (SiO₂, 50-60% EtOAc/Hexane) to afford **4** as a brown greasy solid (0.067 g, 71.2%). ¹HNMR(400 MHz,CDCl₃ δ ,ppm), 6.7-7.4 (stilbene, 9 H), 2.3 (s, 6 H), 3.7 (m,4 H), 3.8 (s, 2 H),4.1 (s, 3 H) 4.2 (t, 4 H). ¹³C NMR (100 MHz, acetone-d₆, δ, ppm), 10.4, 20.1, 52.2, 63.4, 67.8, 68.5, 69.5, 70.25, 115.6, 116.3, 117.2, 125.8, 127.23, 130.0, 131.9, 140.2, 151.2, 157, 159, 169, 171.

Synthesis of 8-(3',5'-dihydroxystilben-4''oxy)-3,6-dioxooctanoic acid, 9



To a three neck 50 mL round- bottomed flask was added compound **4** (0.050 g, 0.11 mmol), THF (5 mL), methanol (5 mL), and 0.6 M lithium hydroxide (2.0 mL). The resultant mixture was stirred for 12 h and then acidified with 2M HCL to pH 2-3. The organic phase was extracted with ethyl acetate (3 × 5 mL), then concentrated and purified with flash column chromatography (80%/18%/2% acetone/hexane/HOAc, v/v/v) to afford **5**, as a brown solid (0.017 g, 43.6%) R_f 0.32 (80%/18%/2% acetone/hexane/HOAc, v/v/v). ^1H NMR (400 MHz, acetone- d_6 , δ , ppm), 6.7-7.4 (stilbene, 10 H), 3.7 (m, 4 H), 3.8 (s, 2 H) 4.2 (t, 4 H).

Synthesis of 4'-oxo-(2'',3'')-di-O-acetyladenosine)-3,5-diacetyloxystilbene, **6**



To a one-neck 10 mL round-bottomed flask were added **4** (0.050 g, 0.16 mmol), THF freshly distilled (1mL), diacetyl adenosine (0.168 g, 0.48 mmol), triphenylphosphine (0.125 g, 0.48mmol), and diethylazodicarboxylate (DEAD) (75uL, 0.48mmol). The resultant mixture was stirred at r.t under N_2 atmosphere for 12 h and concentrated with an evaporator equipped with an oil pump. The residue was purified with flash column chromatography (SiO_2 , 90% $\text{CH}_2\text{Cl}_2/\text{MeOH}$) to afford **6** as a white solid. But ^1H NMR spectra was not clean, compound was very impure despite a single TLC spot.

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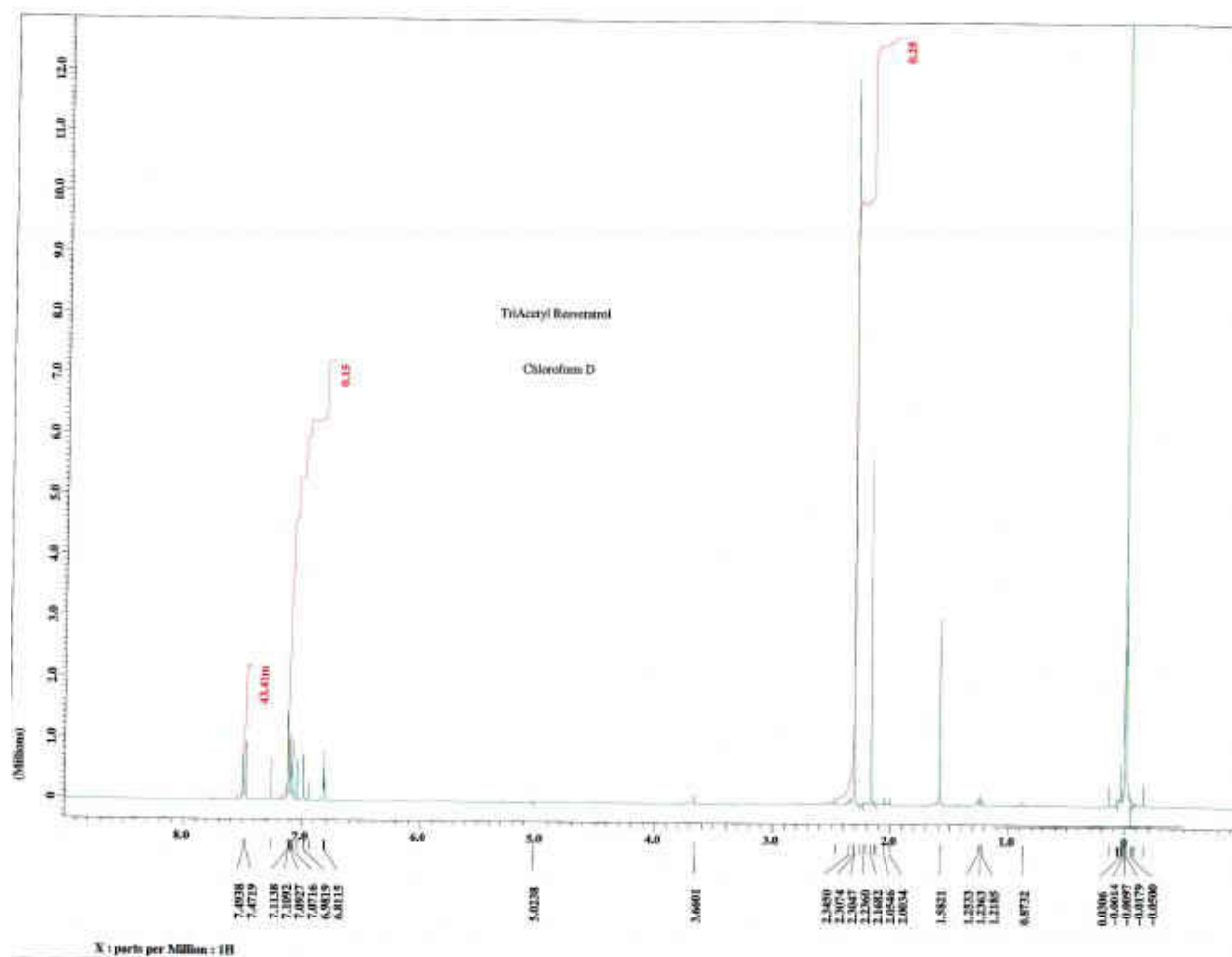
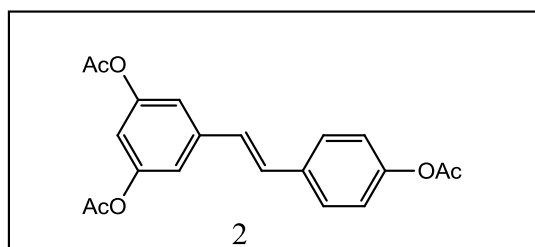
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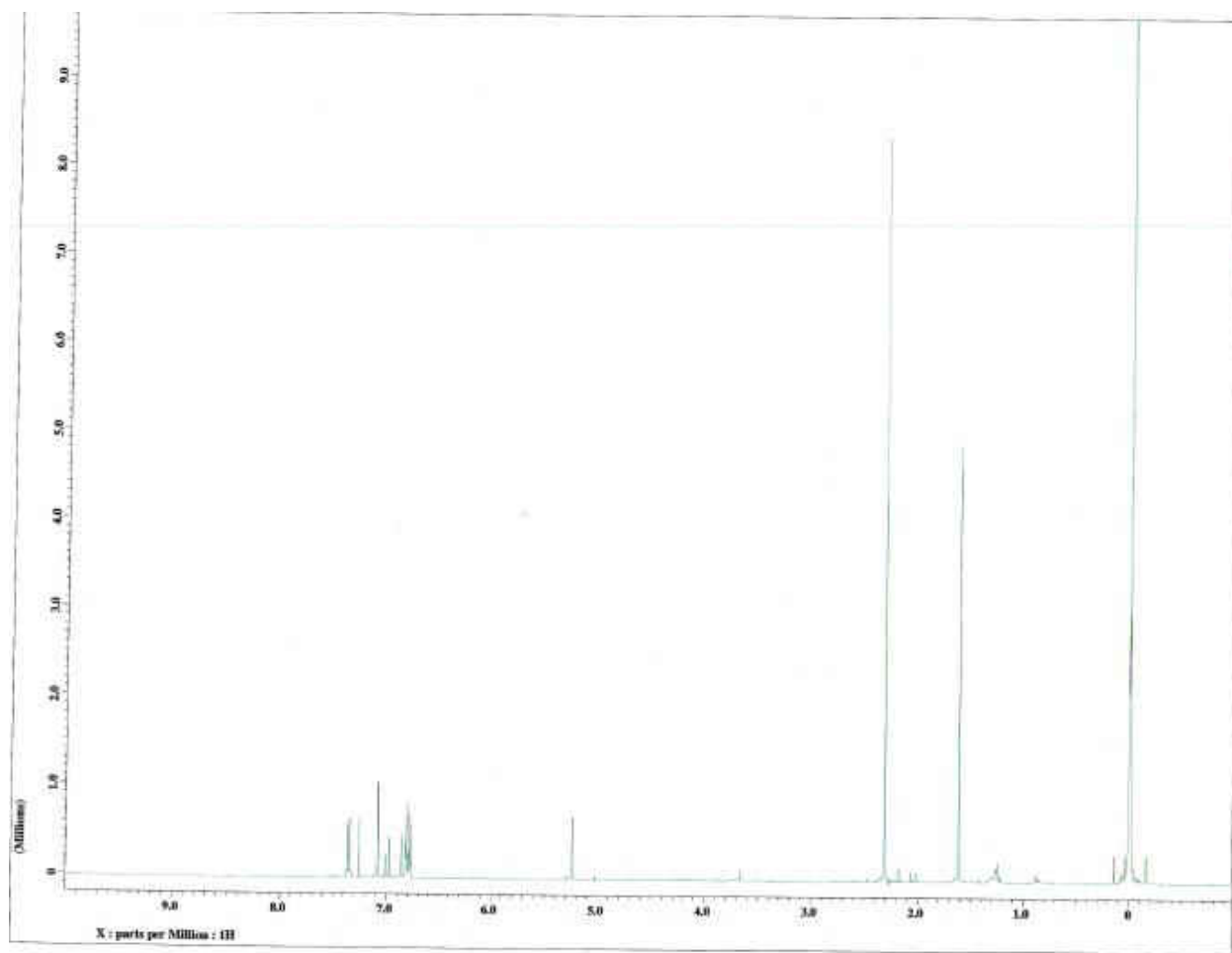
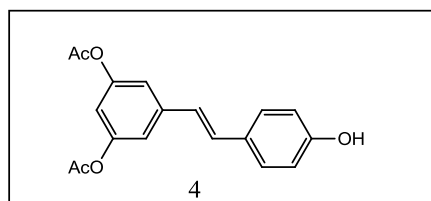
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APPENDICES

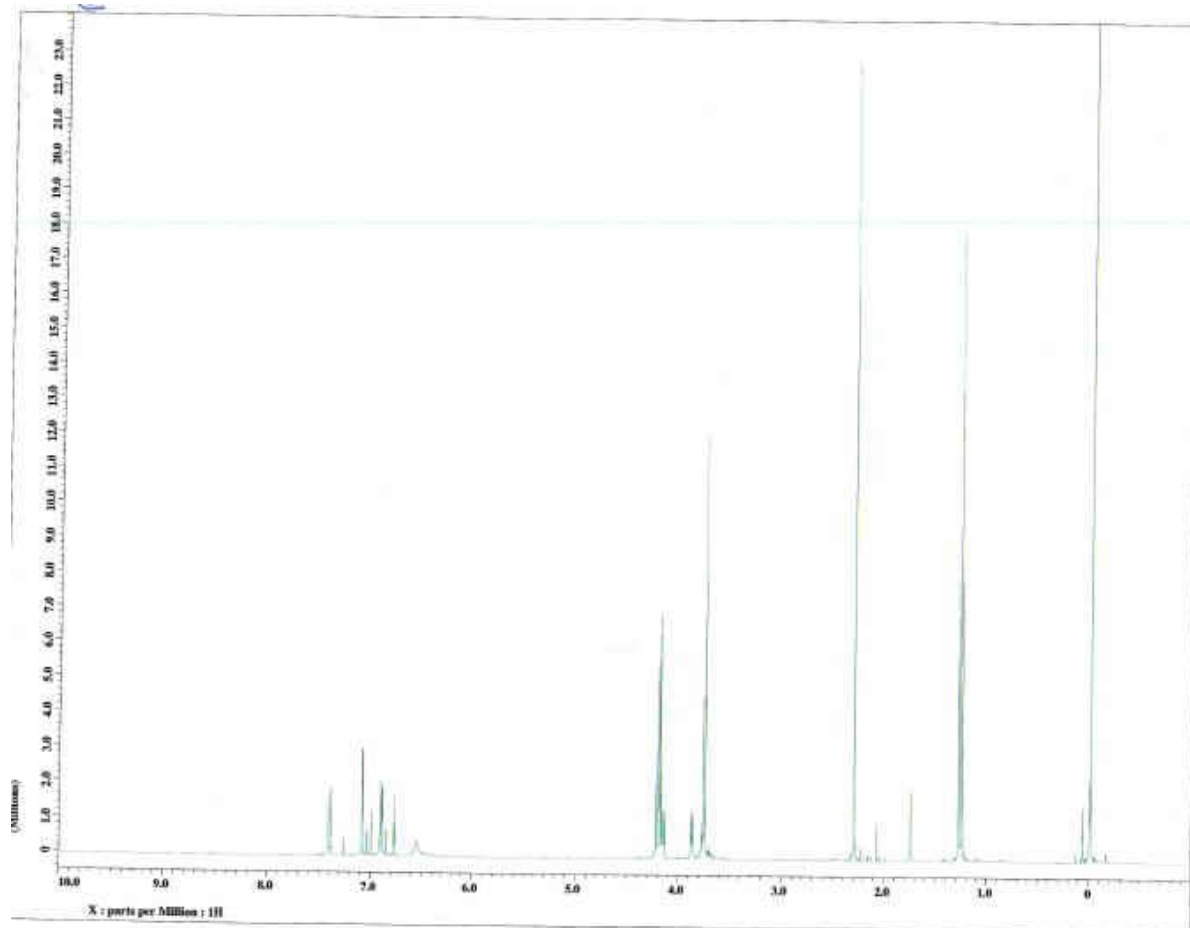
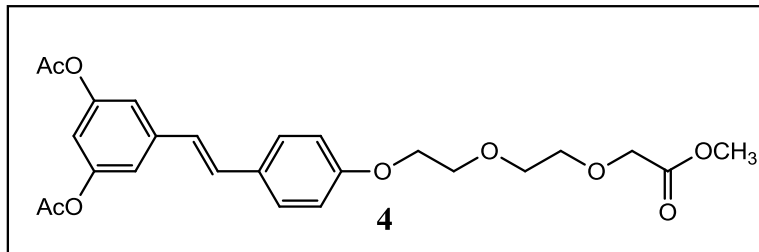
Appendix A: ^1H NMR spectrum of Compound 2 in CCl_3D



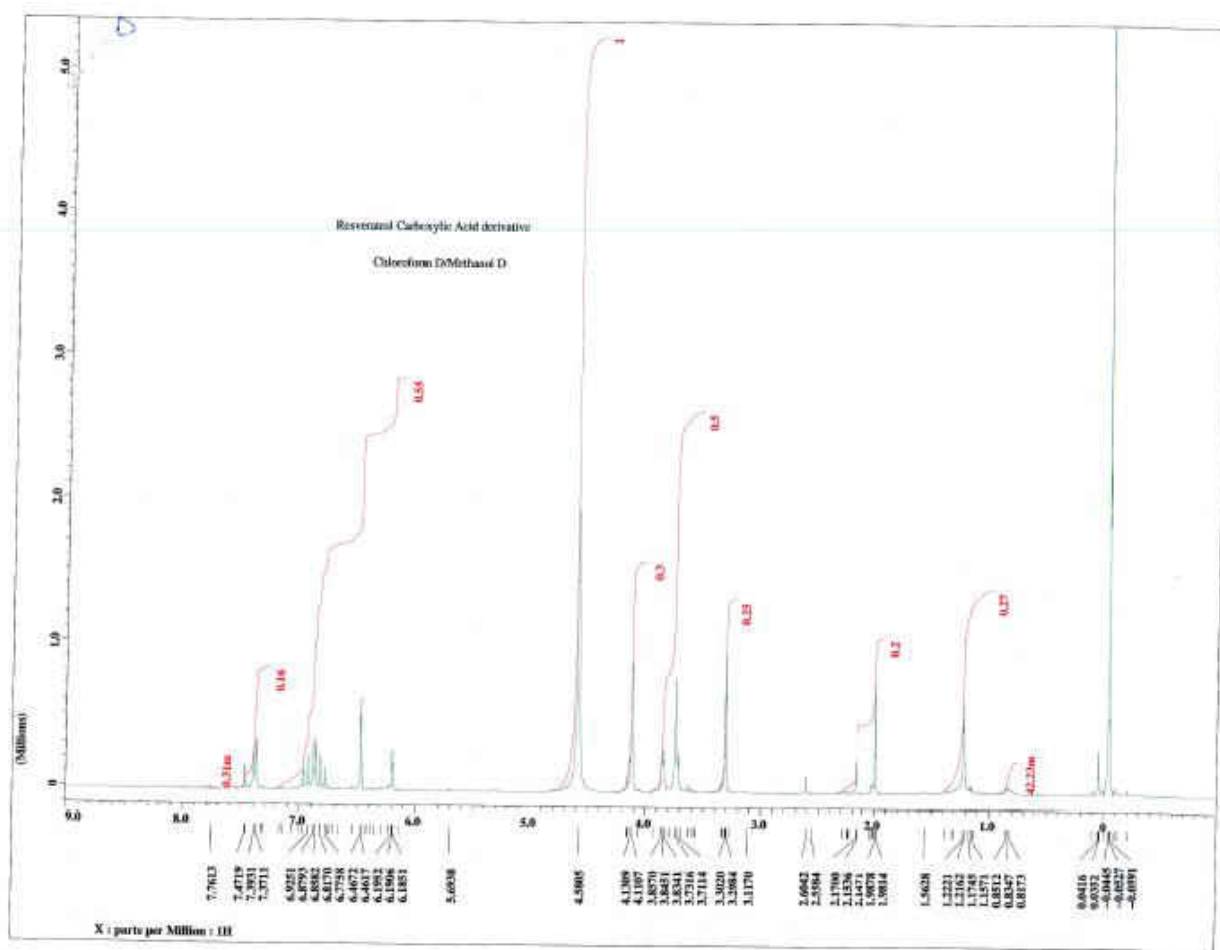
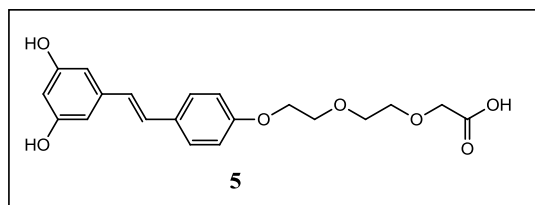
Appendix B: ^1H NMR spectrum of Compound 4 in CCl_3D



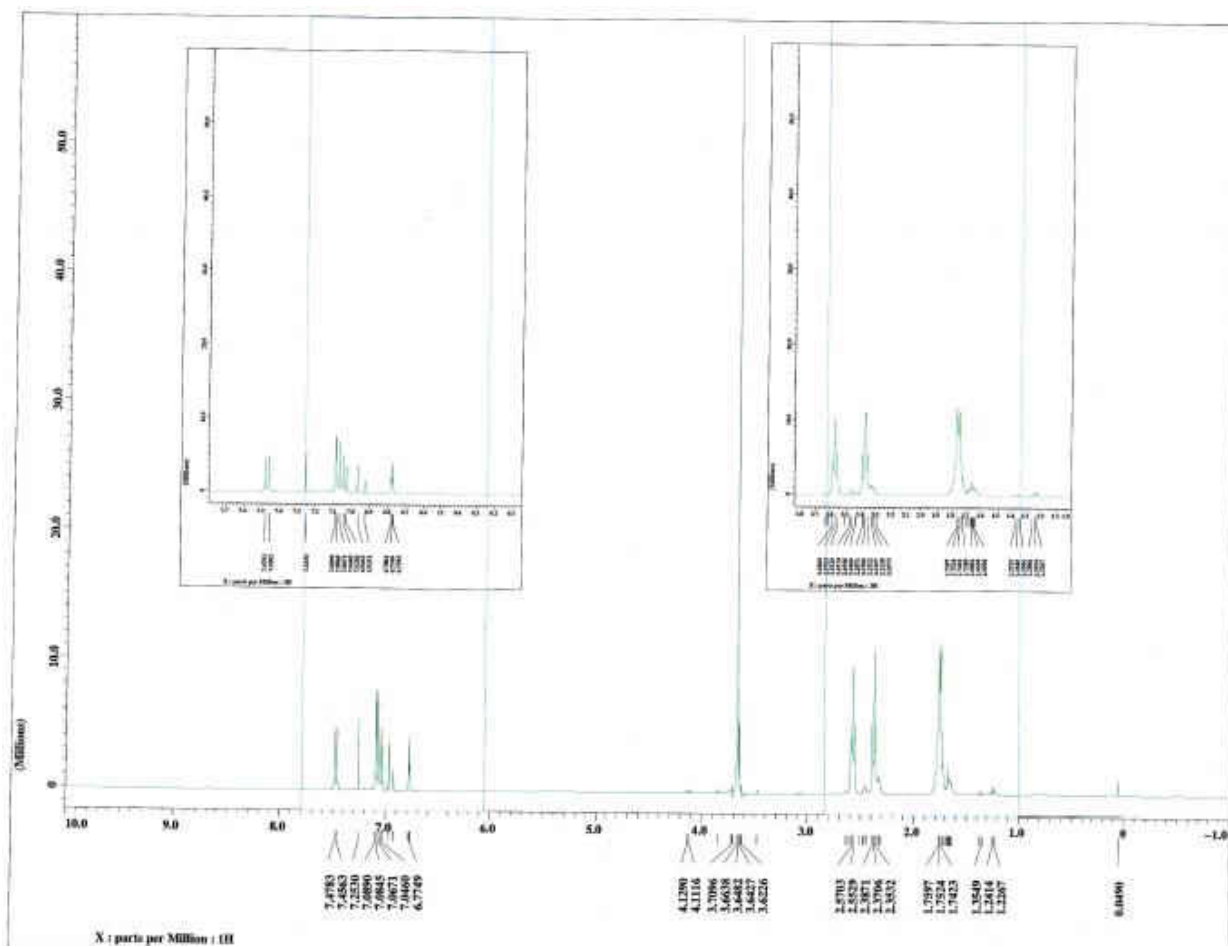
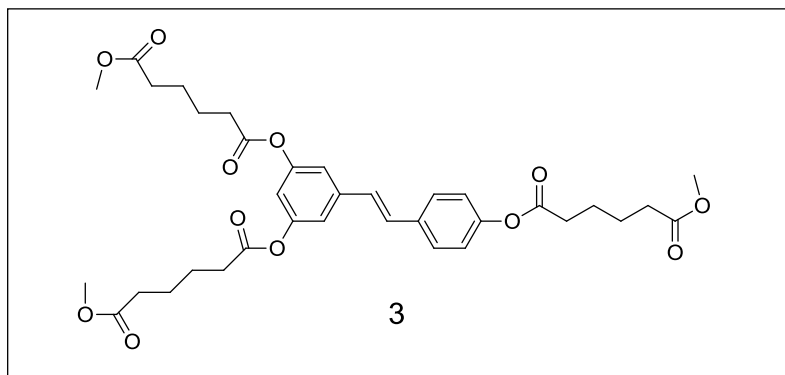
Appendix C: ^1H NMR spectrum of Compound 5 in CCl_3D



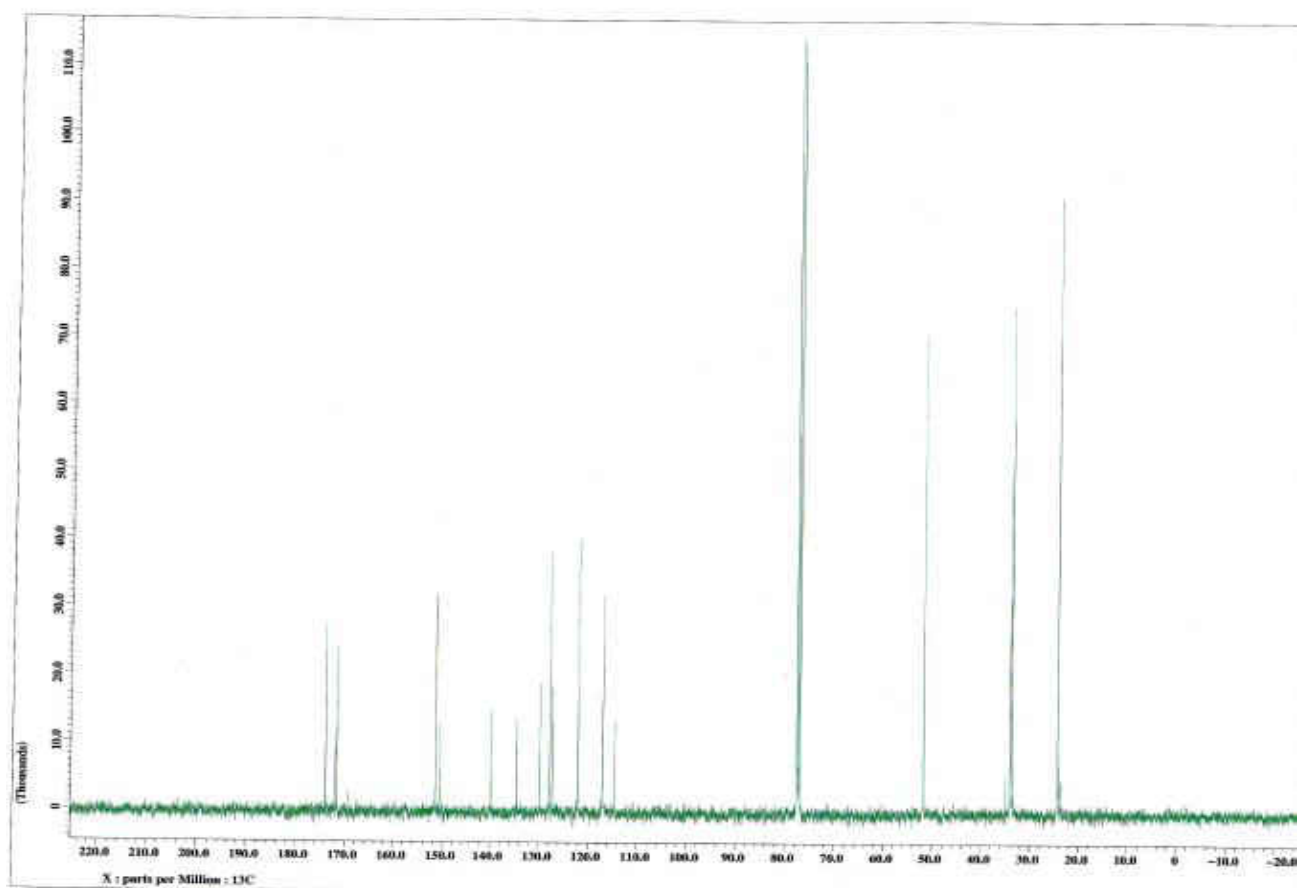
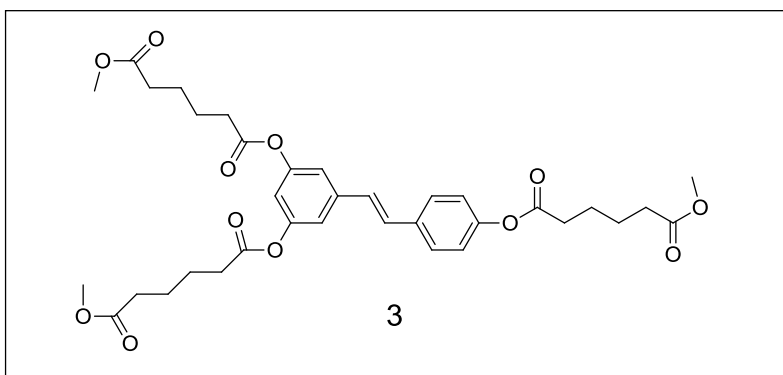
Appendix C: ^1H NMR spectrum of Compound 5 in acetone- d_6 /MeOD



Appendix D: ^1H NMR spectrum of Compound 3 in CCl_3D



Appendix E: ^{13}C NMR spectrum of Compound 3 in CCl_3D



VITA

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