

# **SYNTHESIS OF ANALOGUES OF NORDIHYDROGUAIARETIC ACID AND THEIR OXIDATIVE METABOLISM**

A Thesis Submitted to the College of  
Graduate Studies and Research  
in Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
in the College of Pharmacy and Nutrition  
University of Saskatchewan  
Saskatoon

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## ABSTRACT

In order to investigate the structural features responsible for the cytotoxicity of the naturally occurring lignan nordihydroguaiaretic acid, the synthesis of four structural analogues of NDGA was proposed for the purpose of studying their oxidative metabolism. One analogue in particular (**1**), a mono-catechol analogue, was successfully synthesized employing a double Stobbe condensation approach. Following synthesis of this compound a series of oxidation experiments was performed consisting of: incubation in rat liver microsomes with and without the trapping agent glutathione (GSH), oxidation with mushroom tyrosinase, oxidation with silver oxide, and oxidation with horseradish peroxidase. Results were analyzed via HPLC and UPLC-MS. It was found that **1** does not autoxidize at pH 7.4 as NDGA does. Two products were produced during incubation of **1** in rat liver microsomes with UPLC-ESI(-)-MS results giving  $m/z$  of 879.23 and 574.18. This is consistent with **1** plus 2 GSH and **1** plus 1 GSH respectively; confirming **1** will oxidize to an electrophilic moiety. Oxidation with mushroom tyrosinase was found to produce high levels of the product with  $m/z$  574.18. Oxidation with horseradish peroxidase is found to produce high levels of the  $m/z$  879.23 product. Silver Oxide produced multiple products rather than the expected one major product, but most were found to be inconsistent with the products seen during rat liver microsomal incubation, and were not pursued.

## **ACKNOWLEDGEMENTS**

Many thanks to my supervisor Dr. Ed S. Krol, and to the members of my advisory committee Dr. Marek Majewski and Dr. David Palmer. Thank you to my external examiner Dr. Mark Berry, and to the chair of my committee Dr. Jane Alcorn. Thank you to Dr. Keith Brown of the Saskatchewan Structural Science Centre, Dr. Jennifer L. Billinsky and Brian M. Fahlman of Dr. Krol's research group, and Dr. Alistair Muir and Krista Thompson at Agriculture Canada. My appreciation also goes to my lab mates, fellow students, friends and family.

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## LIST OF ABBREVIATIONS

NDGA : nordihydroguaiaretic acid

LD<sub>50</sub>: median lethal dose

QM: quinone methide

GSH: glutathione

NADPH: nicotinamide adenine dinucleotide phosphate

ASECO: anhydrosecoisolariciresinol

TLC: thin layer chromatography

PTLC: preparative thin layer chromatography

HPLC: high performance liquid chromatography

UPLC: ultra high performance liquid chromatography

ESI: electrospray ionization

MS: mass spectrometry

NMR: nuclear magnetic resonance

*m/z*: mass to charge ratio

UV: ultraviolet

P450: cytochrome P450

SECO: secoisolariciresinol

## 1.0 INTRODUCTION

### 1.1 Nordihydroguaiaretic Acid

#### 1.1.1 Natural Occurrence: the Creosote Bush

Nordihydroguaiaretic acid is a naturally occurring lignan produced by the creosote bush, *Larrea tridentata* (Figure 1-1). This bush is a common flowering shrub found in arid regions of the south-western United States and northern Mexico. Considered to be the most abundant secondary metabolite of *L. tridentata*<sup>1</sup>, NDGA has been found in amounts between 5-15% by dry weight in the leaves and twigs of the bush<sup>2, 3</sup>.

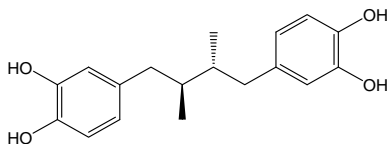


Figure 1-1: Structure of Nordihydroguaiaretic Acid (NDGA)

#### 1.1.1.1 Medicinal Uses of the Creosote Bush

The creosote bush has a long, varied history of medicinal uses, in particular among the native peoples indigenous to the area<sup>2</sup>. More than 50 documented illnesses and afflictions are treated with different preparations of the creosote bush<sup>1-5</sup>, including gallbladder and kidney stones, urinary tract infections and type II diabetes. It is most commonly administered as an aqueous extract prepared by boiling the leaves and twigs, which is then sipped throughout the day. Externally, prepared salves are administered to treat burns, chicken pox, and snakebite pain. These external preparations are thought to be particularly useful due to the reported antibiotic, antiviral, and antifungal properties of the creosote bush extract<sup>2, 6</sup>. More recently, creosote bush extract has been made available in tablet form as the natural supplement Chaparral. It was also reported as a component of the herbal product Herp-Eeze<sup>TM</sup>, marketed as a treatment for *Herpes simplex* infection<sup>1</sup>.

### **1.1.2 Pharmacological Properties of NDGA**

NDGA has been found to possess a number of potentially useful pharmacological properties. In 2004, Lambert et al.<sup>1</sup> reviewed a number of these biological activities, including NDGA as an effective cancer chemopreventative agent, NDGA's ability to inhibit the growth of a number of cancer types *in vitro* and *in vivo*, the fertility-regulating effects of NDGA, and the hypoglycemic activity of NDGA in both rat and mouse models of type II diabetes. The NDGA product Actinex® was also used as a topical medication for the treatment of the skin condition actinic keratoses for a short period of time. However, following the emergence of skin hypersensitivity in patients, it was removed from the market<sup>3, 7</sup>.

### **1.1.3 Other Properties of NDGA**

NDGA is a known antioxidant, preventing the propagation of lipid peroxy radical reactions by forming stable radicals and by-products. It is also a known inhibitor of the lipoxygenase and cyclooxygenase pathways<sup>2, 6, 8</sup>. Its antioxidant capacity was exploited for a short time in preserving fats and butter, but is currently only used as an antioxidant in the storage of natural and synthetic rubber<sup>2, 3, 9, 10</sup>.

### **1.1.4 Toxicity of NDGA**

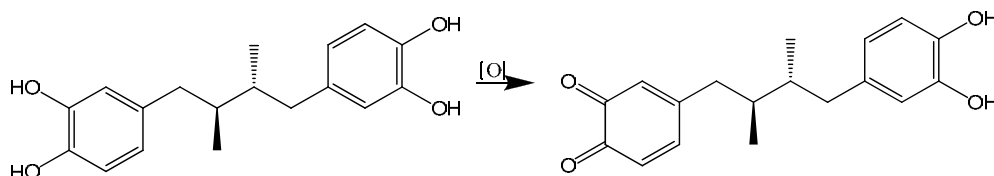
The withdrawal of NDGA as a food preservative was a result of a number of studies released in the late 1960s and early 1970s linking the ingestion of NDGA to the formation of cysts in the kidney<sup>9, 10</sup>. Goodman et al. reported that a 3% NDGA diet fed to 24 rats over a 15 day period resulted in the death of 7 animals. Significant weight loss and cystic structures were found over the entire surface of the kidneys in the animals studied. In 2002, Lambert et al.<sup>3</sup> determined the lethal intraperitoneal dose of NDGA in mice to be LD<sub>50</sub>=75 mg/kg. Significant weight loss in the test animals was observed, and on high dosing with NDGA (100 and 500 mg/kg), 100% mortality was observed within 30h of administration.

Hepatotoxicity has also been associated with prolonged overexposure to creosote bush-based products such as chaparral. Chronic consumption of chaparral in humans has resulted in patients suffering from toxic or drug-induced hepatitis<sup>2, 5</sup>. Upon cessation of ingestion of these products, the damage is most often found to be reversible and non-permanent. In some cases, the damage progressed to cirrhosis of the liver<sup>2, 5</sup>, and in fewer cases, total hepatic failure was

reported and liver transplantation was necessary<sup>2, 5, 8</sup>. In one particular case, a patient not only suffered hepatic failure, but also renal failure which was attributed to the prolonged consumption of creosote bush products<sup>2, 5</sup>.

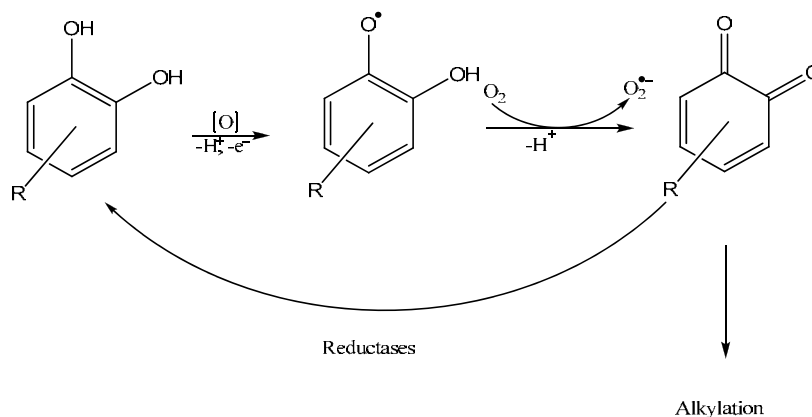
### 1.1.5 NDGA Quinones

The cytotoxicity attributed to NDGA is thought to be due to its oxidized *ortho*-quinone form (Scheme 1-1), a reactive intermediate that can be produced *in vivo* on metabolism of NDGA<sup>10, 11</sup>.



Scheme 1-1: Oxidation of NDGA from the Catechol to the Ortho-Quinone

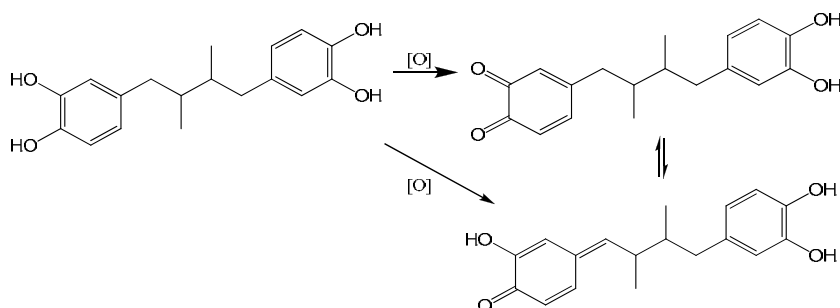
The toxicity of the *o*-quinone has two mechanisms: first, alkylation of cellular macromolecules via conjugate addition, which can result in alteration of nucleotide sequence in the case of DNA, or the disruption of tertiary and quaternary structure in the case of proteins; second, redox cycling, which produces reactive oxygen species that can oxidize essential cellular components (Scheme 1-2)<sup>12</sup>.



Scheme 1-2: Oxidation of a Catechol to an *o*-Quinone and Resulting Mechanism of Cytotoxicity

One other product is possible in the oxidation of catechols which possess at least one benzylic proton, and that is the *para*-quinone methide (QM). The *p*-QM can be formed directly from the catechol itself, or from the *o*-quinone as a tautomer<sup>11, 13</sup> (Scheme 1-3). The rate at which the *o*-quinone tautomerizes to the *p*-QM is impeded by increased substitution at the benzylic position<sup>12</sup>. This subsequently leads to the prediction that NDGA, possessing a methylene group

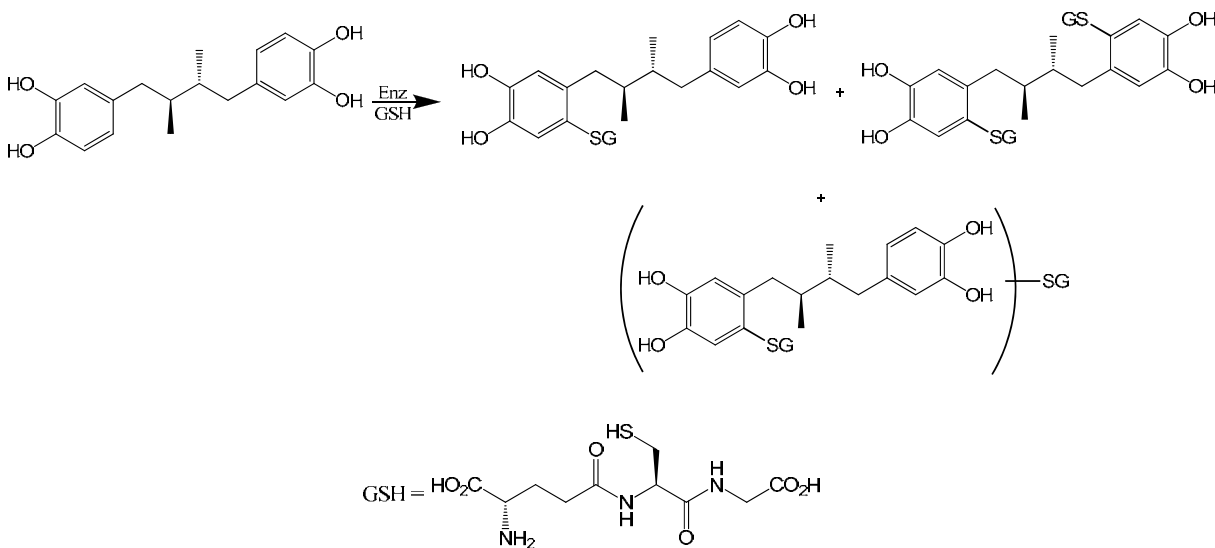
at the benzyl position, would isomerize to the *p*-QM slowly<sup>11</sup>. The *p*-QM is known to be much more electrophilic than the *o*-quinone, and consequently participates in the alkylation of cellular nucleophiles<sup>14, 15</sup>, and redox reactions are found to be uncommon<sup>12, 14</sup>.



Scheme 1-3: Conversion of NDGA to the *p*-QM.

### 1.1.5.1 NDGA Oxidation Experiments

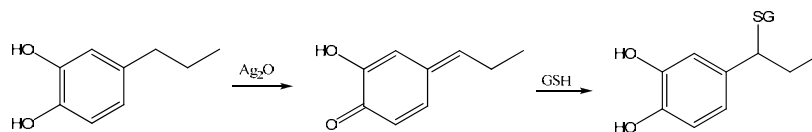
Oxidizing a catechol with tyrosinase as a catalyst is a common method of producing the *o*-quinone moiety<sup>14</sup>. It has been found that NDGA can be successfully oxidized to its *o*-quinone form using the enzyme mushroom tyrosinase, with the resulting products trapped as adducts with glutathione (GSH). Three NDGA-GSH adducts were isolated and identified (Scheme 1-4)<sup>11</sup>.



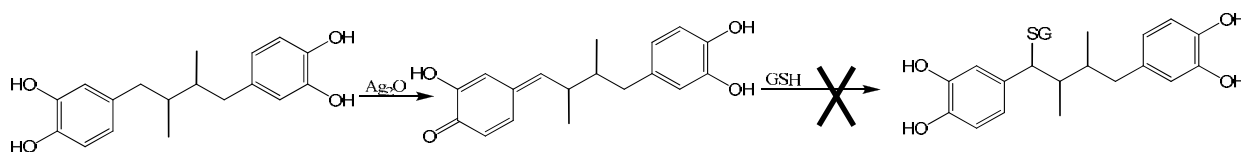
Scheme 1-4: Trapping the *o*-Quinone of NDGA with Glutathione

A common method of producing the *p*-QM form of a catechol is to oxidize with  $\text{Ag}_2\text{O}$ , and trap the resulting electrophilic entity with GSH as per the example shown in Scheme 1-5<sup>12</sup>. In order to assess the possibility of the *p*-QM contributing to the toxicity of NDGA, attempts were made to produce the *p*-QM via oxidation using  $\text{Ag}_2\text{O}$ , trapping the resulting product with

GSH. The UV spectrum ( $\lambda_{\text{max}}=360$  nm) was consistent with the formation of quinone products; however, this method failed to produce GSH-QM adducts, and no GSH active products were produced (Scheme 1-6)<sup>11</sup>. It is unclear why oxidation of NDGA with  $\text{Ag}_2\text{O}$  did not result in the formation of GSH-QM adducts.



Scheme 1-5: Oxidation of 4-Propyl Catechol to the *p*-QM using  $\text{Ag}_2\text{O}$



Scheme 1-6: Attempted Oxidation of NDGA to the *p*-Quinone Methide

The products obtained in these enzymatic and chemical oxidation experiments were compared to the products obtained when NDGA was incubated in rat liver microsomes for 60 min at pH 7.4, an *in vitro* experiment often used to investigate the potential formation of reactive metabolites for a given compound. It was determined that the *o*-quinone formed, and in the presence of GSH, all three adducts as seen in Scheme 1-4 were produced. Control microsomal incubations performed in the absence of NADPH, or in the presence of heat inactivated microsomes were also found to produce the same GSH adducts. This result suggests that the formation of the *o*-quinone at pH 7.4 is not cytochrome-P450 (P450) dependent, and that NDGA is undergoing autoxidation, which is an observation consistent with previously reported research on NDGA<sup>16</sup>. On further investigation, it was determined that the autoxidation of NDGA is a base-catalyzed process from pH 6.0 to 9.0, and the half-life of NDGA at pH 7.4 is 3.1 h<sup>11</sup>. This rate of autoxidation is faster than that for a number of catechols, such as catechol and dopamine<sup>11</sup>.

When NDGA was incubated at pH 7.4 in the absence of the trapping agent GSH, a small amount of the *o*-quinone was observed via LC-MS, along with a hydroxylated form of NDGA. The major product however was found to be a schisandrin-like dibenzocyclooctadiene lignan (Figure 1-2), likely formed by intramolecular cyclization of NDGA, possibly via a radical intermediate<sup>11</sup>. Given that many systems used to investigate the biological activity of NDGA are performed at or near pH 7.4, and furthermore that native healers traditionally extract the leaves

and twigs of the creosote bush by boiling in water, it is possible that a significant number of the pharmacological properties attributed to NDGA are in part due to the stable dibenzocyclooctadiene lignan<sup>17</sup>. This is speculation on the part of the authors.

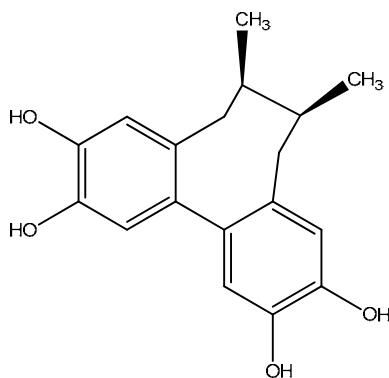


Figure 1-2: NDGA Derived Dibenzocyclooctadiene Lignan

## 1.2 Synthetic Analogues of NDGA

In an attempt to better understand these oxidative properties of NDGA, such as why NDGA does not form *p*-QM-GSH adducts, and what factors contribute to the rapid autoxidation of NDGA, the synthesis of four NDGA analogues (Figure 1-3) has been proposed for the purpose of studying their oxidative metabolism.

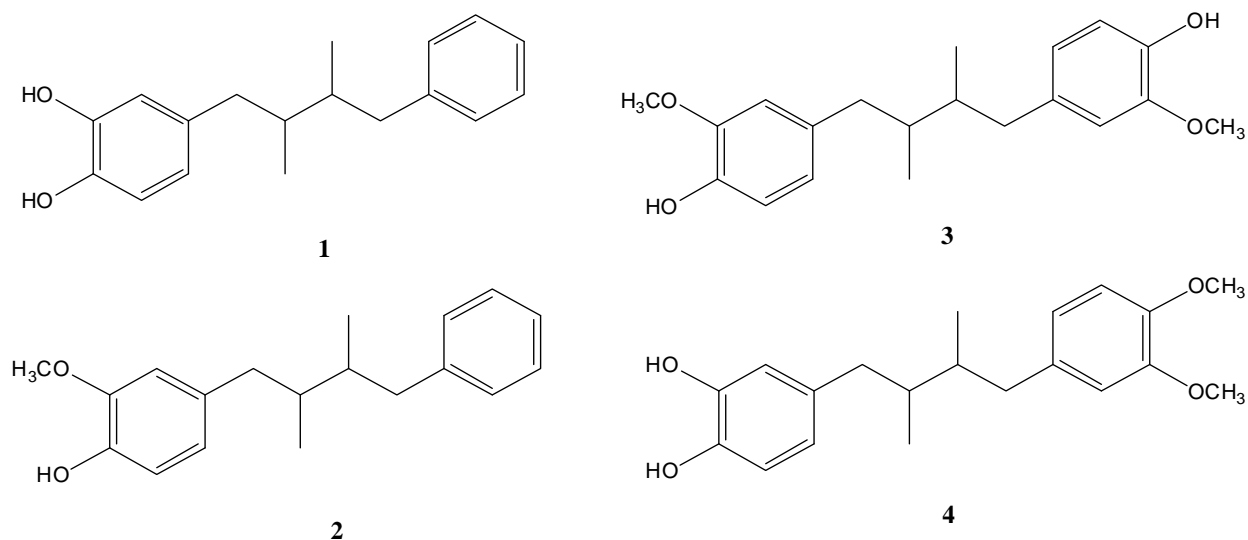


Figure 1-3: Proposed Synthetic Analogues of NDGA

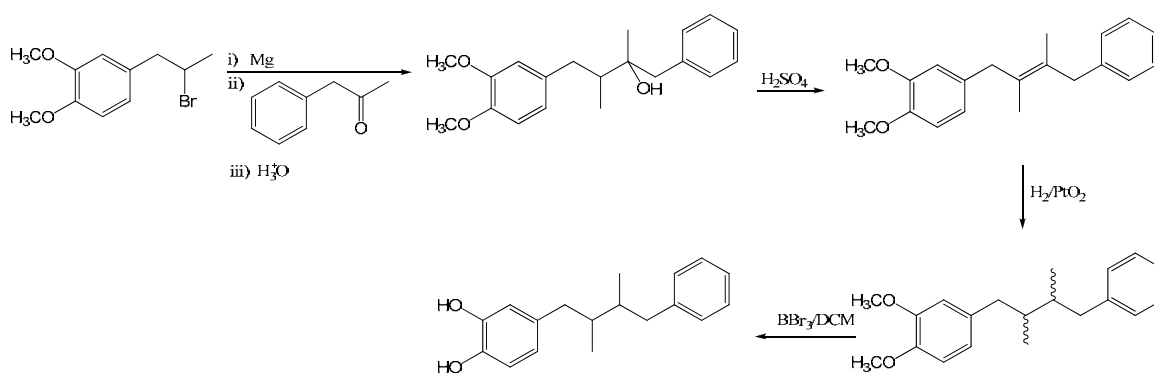


## 1.2.1 Synthetic Methods

A number of methods for synthesizing lignan analogues exist in the literature. Six possibilities were reviewed and considered as potential pathways to the desired analogues. Four of the methods involved coupling two phenylpropanoid units; a fifth involved an imprecise methylation of existing aromatic hydroxyl groups. Ultimately, the method chosen to pursue involved two consecutive Stobbe condensation reactions to form the lignan skeleton.

### 1.2.1.1 Method 1: Grignard Reaction

It was expected that all four analogues could be prepared via Grignard reaction (Scheme 1-7)<sup>18</sup>.



Scheme 1-7: Preparation of Analogue 1 via Grignard Reaction

The alkene produced in the dehydration step would theoretically result in a mixture of *Z* and *E* isomers (*E* isomer shown). Following hydrogenation of this mixture of alkenes, the resulting products would be a mixture of stereoisomers<sup>19</sup>. From the reaction for each of analogues **1**, **2** and **4**, four stereoisomers should result, consisting of two pairs of enantiomers (Figure 1-4). From the reaction for analogue **3**, three stereoisomers should be present consisting of one pair of enantiomers and one diastereomer (Figure 1-4). Chromatographic separation of diastereomers should be possible<sup>19</sup>.

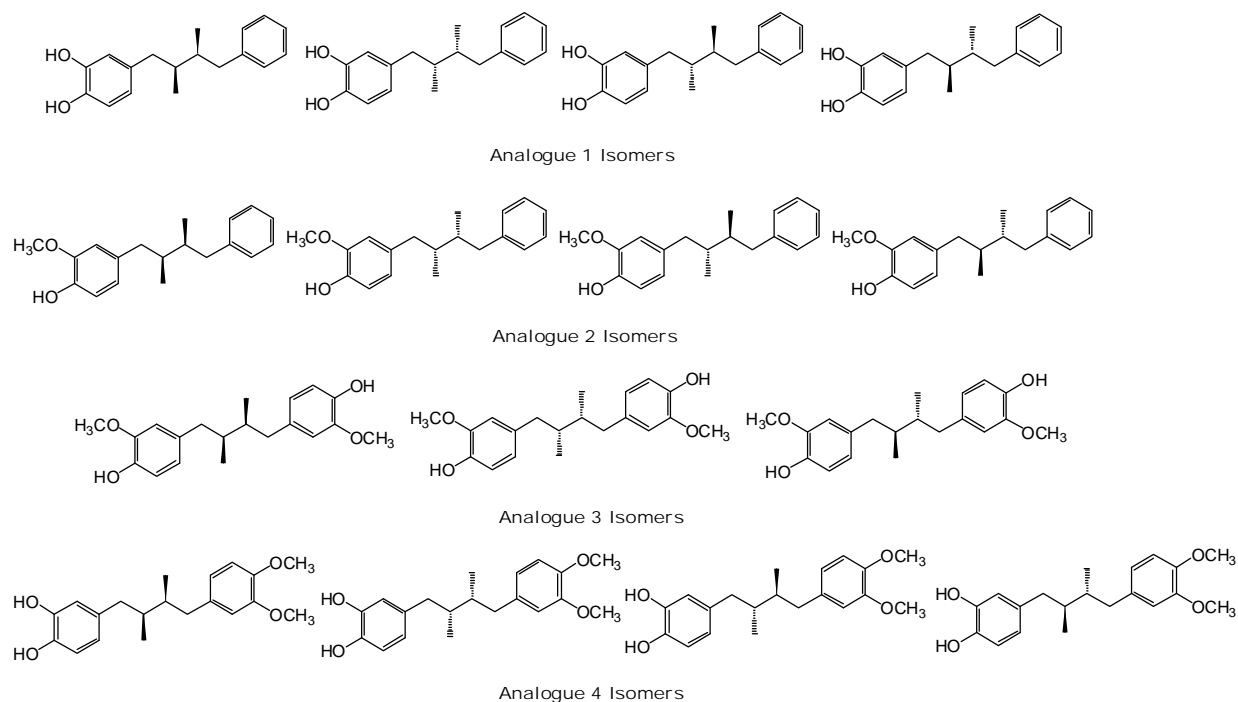
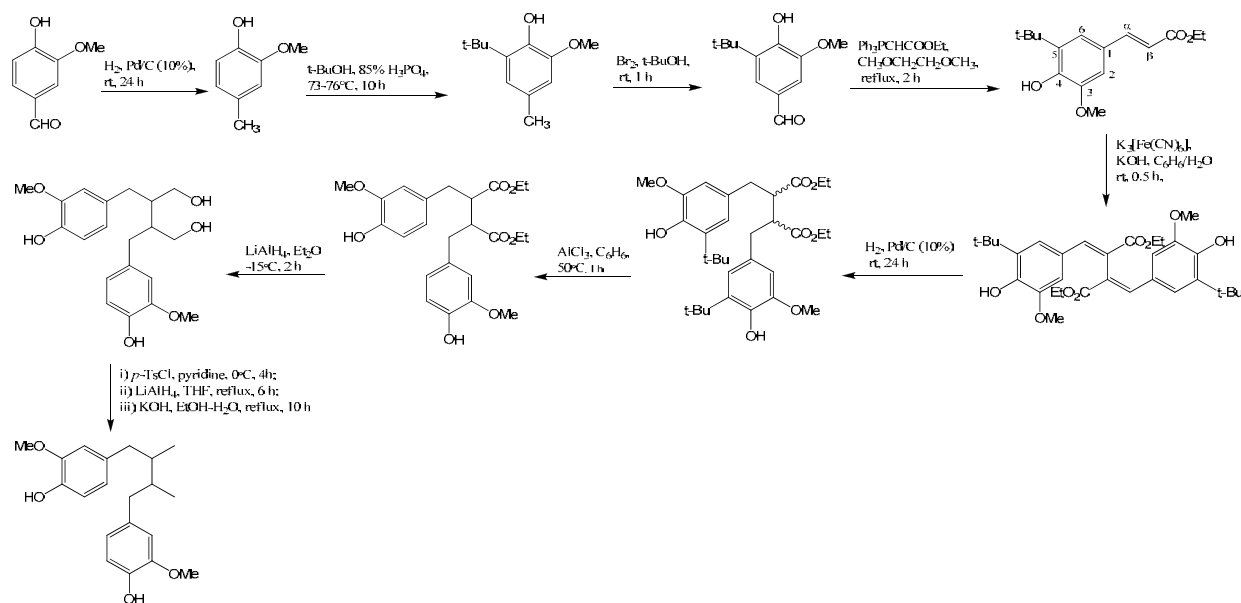


Figure 1-4: Possible Isomers for Analogues 1, 2, 3 and 4 Following Alkene Hydrogenation

Reaction yields reported in the literature from which this scheme was derived include individual reaction yields for each step between 70-85%, with an overall yield between 45-54%<sup>18</sup>. Previous unpublished work from the Krol group suggests that the first step in this particular method has a low yield for the synthesis of analogue **1**.

### 1.2.1.2 Method 2: $\beta$ - $\beta$ Coupling with Alkaline Potassium Ferricyanide

This particular coupling reaction would only be successful in the synthesis of the symmetrical analogue **3** (Scheme 1-8).



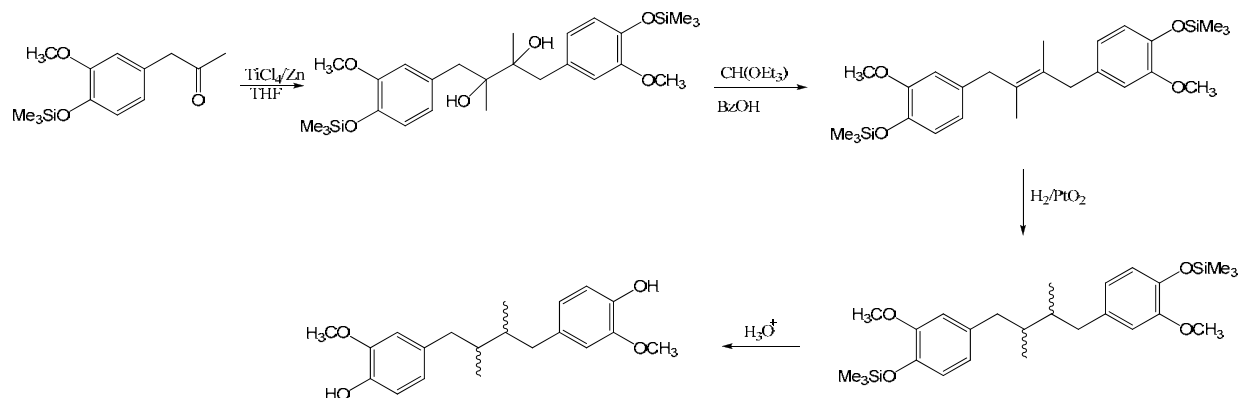
Scheme 1-8: Synthesis of Analogue 3 via  $\beta$ - $\beta$  Coupling Reaction

The key step to producing the lignan skeleton in this method is a  $\beta$ - $\beta$  coupling reaction performed with alkaline potassium ferricyanide in a two-phase benzene-water system<sup>19</sup>. Prior to this step, it is crucial to block the 5-position on the aromatic ring with a *t*-Bu group to eliminate the formation of the  $\beta$ -5 coupling product, which was reported to be the main product when the 5-position was left exposed<sup>19</sup>. Protection of the aromatic hydroxyl group does not appear to be necessary.

Yields reported in the literature for individual reactions range from 49%-99%<sup>19</sup>. The second hydrogenation performed on the diene-diester entity is anticipated to give the three stereoisomers as seen above in Figure 1-4.

### 1.2.1.3 Method 3: McMurry Coupling

The McMurry coupling reaction would only be successful in producing the symmetric analogue 3 (Scheme 1-9). This method was reported by Gezgin et al. in 2001<sup>7</sup> as a synthetic way to produce NDGA.



Scheme 1-9: McMurry Coupling Reaction, Analogue 3

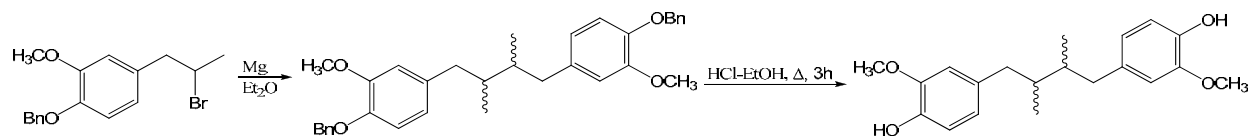
A similar reaction sequence was attempted by me as part of my University of Saskatchewan chemistry 483.6 project in 2005/06. At that time, the starting material employed was 4-benzyloxy-3-methoxyphenylacetone, utilizing a benzyl protecting group for the aromatic hydroxyl. The  $\text{TiCl}_4/\text{Zn}$  coupling reaction was not successful with this compound, and the expected diol was never obtained. Given that the 3,4-dimethoxyphenylacetone coupling reaction was indeed successful when performed by me, it was theorized that perhaps the benzyloxy group was too electron-withdrawing in comparison to the methoxy group, and a different protecting group may be required for a successful coupling reaction. Trimethylsilyl ether is shown as the protecting group in Scheme 1-9, but could be replaced by *tert*-butyldimethylsilyl ether as an alternate protecting group.

Both the *Z* and *E* isomers of the alkene would be expected to form on dehydrogenation of the diol, and the hydrogenation product is expected to consist of two enantiomers and one diastereomer.

Yields were not fully reported in the literature, and included a 4:6 mixture of the *Z*:*E* alkene mixture with an overall yield of 65%. The diol product yield was reported as 73%<sup>7</sup>.

#### 1.2.1.4 Method 4: Grignard Reagent Dimerization

Often considered to be the unwanted side-product in a Grignard reaction, the Grignard reagent dimerization reaction could provide a route to analogue **3** (Scheme 1-10). This reaction scheme was adapted from Lieberman et al.<sup>20</sup>.



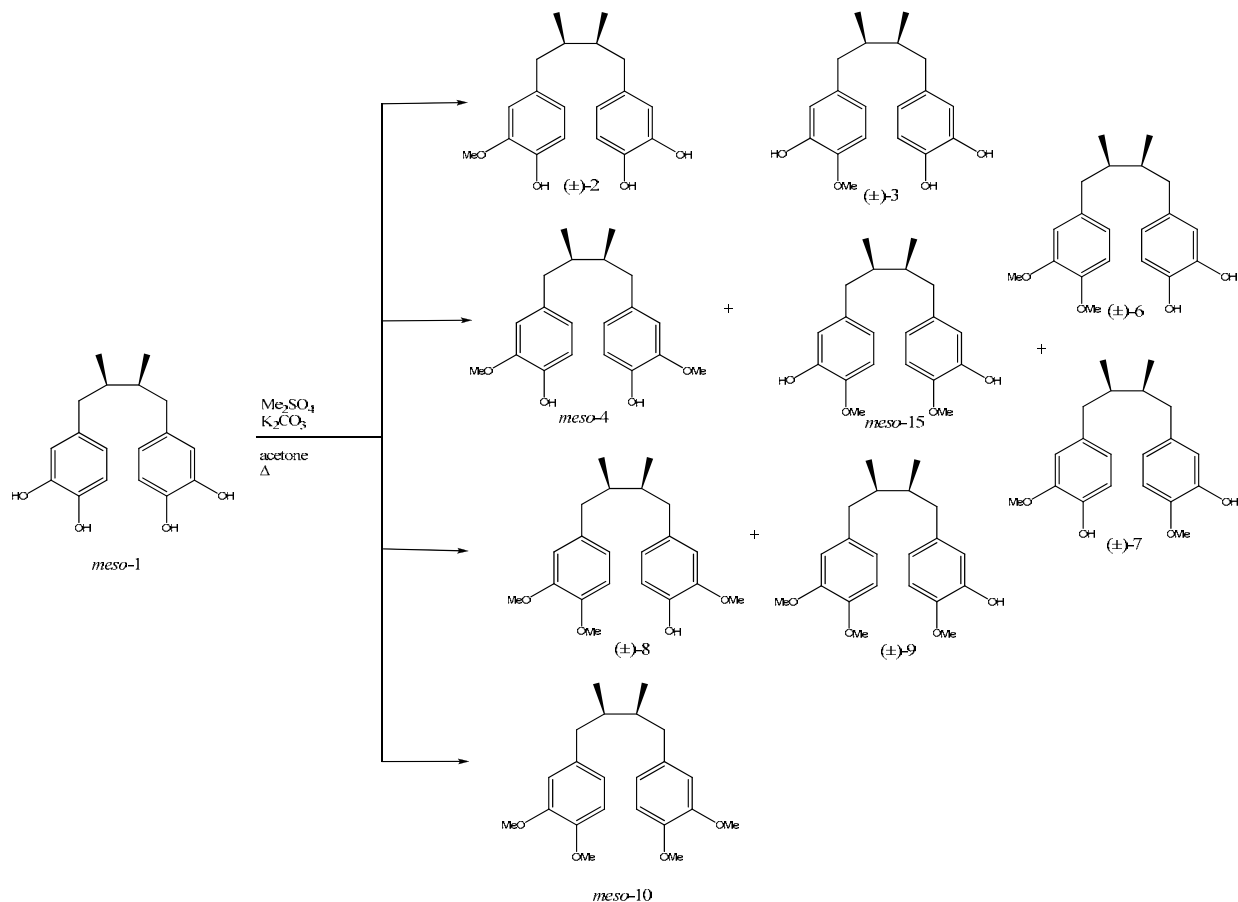
Scheme 1-10: Grignard Reagent Dimerization to Produce Analogue 3

A yield of only 30% was reported in the literature for this method<sup>20</sup>.

It is unclear what, if any, stereoselectivity may result from the Grignard reagent dimerization reaction. Three diastereomers, including a pair of enantiomers, could be possible.

### 1.2.1.5 Method 5: Methylation

The strategy of methylating pre-existing hydroxyl groups was reported in 1998 by Hwu et al.<sup>21</sup> (Scheme 1-11). This method could be successful in producing analogue 3 and 4. In the case that analogue 1 happens to be easily synthesized, analogue 2 could possibly be formed from analogue 1 with this method.

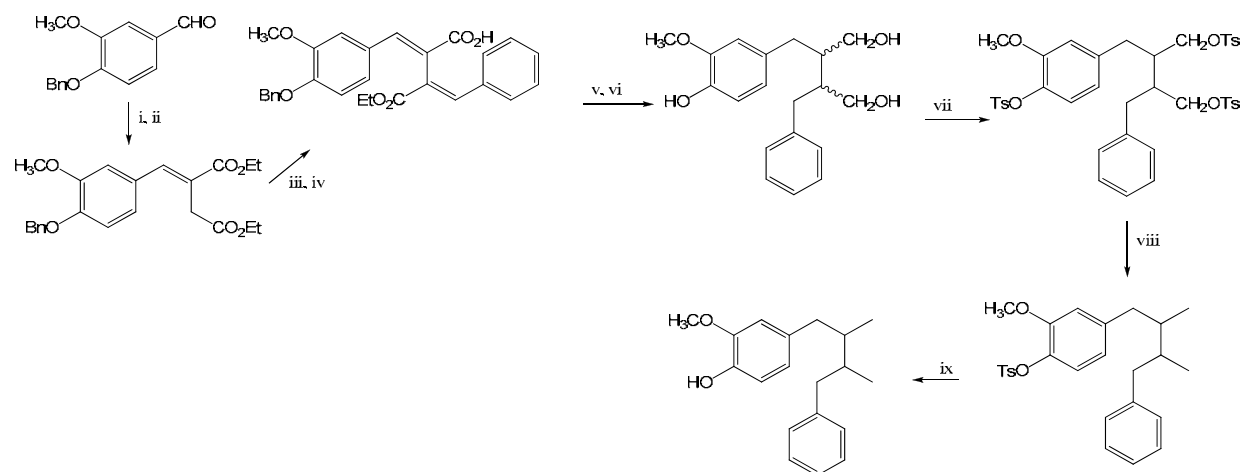


Scheme 1-11: Reaction of NDGA with Dimethyl Sulfate to give a Mixture of Nine Methylated Products<sup>21</sup>.

This method is not specific, and could possibly result in a mixture of products that may be difficult to separate. Of these nine products, only ‘meso-4’ and ‘(±)-6’ are desired compounds for this study. The maximum yield reported for all di-methylated products, 4, 5 and 6, is 28%<sup>21</sup>, with conditions consisting of 1.0 equivalent of Me<sub>2</sub>SO<sub>4</sub> and 4.9 equivalents of K<sub>2</sub>CO<sub>3</sub>.

### 1.2.1.6 Method 6: Stobbe Condensation

This synthetic scheme was adapted from Xia et al.<sup>22</sup>. It was selected based on the fact that all four analogues could theoretically be prepared via this method. The details shown in Scheme 1-12 outline the synthesis of Analogue 2.



Scheme 1-12: Preparation of Analogue 2 via Stobbe Condensation

i) a : EtONa, diethylsuccinate, reflux, 4h ; b : H<sub>2</sub>O, NaOH, reflux, 2h ; ii) EtOH, THF, reflux, 24h ; iii) a) EtONa, benzaldehyde, 0°C, 24h ; b : H<sub>2</sub>O, NaOH, reflux, 2h ; iv) EtOH, THF, reflux, 36h ; v) H<sub>2</sub>, Pd/C (10%) ; vi) LiAlH<sub>4</sub>, THF, reflux ; vii) TsCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub> ; viii) LiAlH<sub>4</sub>, THF, reflux ; ix) KOH, EtOH-H<sub>2</sub>O, reflux, 10 h.

Individual reaction yields reported in the literature range from 73-99%<sup>22</sup>.

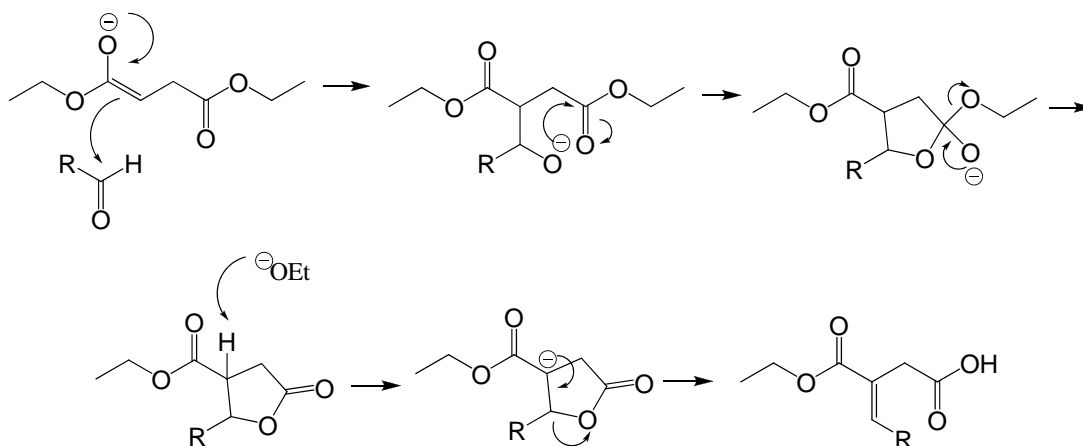
Hydrogenation of the double bonds (step v) is anticipated to result in a number of different stereoisomers, as per Figure 1-4 above: four isomers for analogues **1**, **2** and **4** consisting of two pairs of enantiomers, and three diastereomers including one pair of enantiomers for analogue **3**.

#### 1.2.1.6.1 The Mechanism and Stereoselectivity of the Stobbe Condensation

The specific case of the condensation of diester succinates with ketones was identified as unusual in 1893 by Hans Stobbe when the reaction between diethyl succinate and acetone gave a product that was inconsistent with the expected Claisen-type product. An extensive study of this

reactivity ultimately showed that aldehydes and ketones generally reacted in this manner with diester succinates<sup>23</sup>.

Diethyl succinate is found to react more readily with weaker bases such as sodium ethoxide, and is considered to have a higher reactivity than even that of malonates, as indicated by its ability to react with benzophenone, which is absent in malonates under similar conditions. This reactivity is thought to be due to the mechanism of the Stobbe condensation (Scheme 1-13). This mechanism is widely believed to include an intramolecular cyclization involving the intermediate oxyanion which results in the formation of a 5-membered lactone<sup>23-26</sup>. Evidence for the formation of this intermediate is supported by the isolation of the lactone product, particularly during shorter reaction times<sup>23</sup>. Furthermore, only the alkene is produced during the Stobbe condensation reaction via  $\beta$ -elimination on cleavage of the lactone, and the alcohol has never been isolated. This proposed mechanism also explains the formation of the carboxylic acid product rather than the di-ester product.



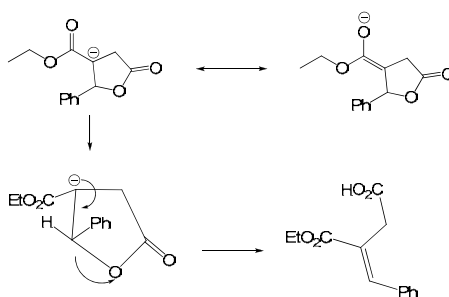
Scheme 1-13: Mechanism of the Formation of the 5-Membered Lactone and Subsequent  $\beta$ -Elimination During a Stobbe Condensation Reaction between Diethyl Succinate and an Aldehyde

It has been noted that in the case of the formation of the lignan skeleton by way of the double Stobbe condensation, as considered here, the mechanism of the second condensation reaction is also thought to proceed by way of the intermediate lactone, which is again supported by the isolation of this intermediate product at lower temperatures.

The stereochemistry of the double bond that is formed during the  $\beta$ -elimination step following the condensation of an aromatic aldehyde with diester succinate has been found by a number of groups to be stereoselective, giving only the *E* configuration<sup>25, 26</sup>. The *E* or *Z* configuration of these compounds can be differentiated by <sup>1</sup>H NMR studies. It has been found

that the olefinic proton in the *E* configuration appears beyond 7.5ppm, downfield from the *Z* proton by approximately 0.5-1.0ppm<sup>25, 26</sup>. This shift is due to the deshielding effect of the carbonyl group<sup>26</sup>.

The preference of the Stobbe condensation to form only the *E* isomer is due to a stereoelectronic effect originally described as the result of a mechanism involving ‘overlap control’<sup>26</sup>, as proposed by Zimmerman et al.<sup>27</sup> to explain the stereoselectivity of carbanionoid reactions, and in particular, the Perkin reaction. This overlap control mechanism includes the stabilization of the negative charge which is formed on the tertiary carbon following proton extraction by the alkoxide (Scheme 1-13), by delocalization to the oxygen atom of the ethyl ester<sup>26</sup>. This results in a favourable anti-periplanar transition state that does not result in any eclipsing effects between the aromatic system and the ester groups, and allows for maximum overlap of the developing  $\pi$ -orbitals (Scheme 1-14)<sup>25, 26</sup>.



Scheme 1-14: Conformation of the Stobbe Intermediate

The stereochemistry of the double bond formed during the second Stobbe condensation has not been reported in the literature as often as the first. Anjaneyulu et al.<sup>25</sup> reported the *E,E* configuration only in the synthesis of dibenzylidene succinic anhydrides via double Stobbe condensation. These structures were again determined by <sup>1</sup>H NMR, with both olefinic protons appearing downfield of what would be the *Z* proton. It is worth noting that in the case of a symmetrical compound, the olefinic protons appeared as a single peak<sup>25</sup>.



## 2.0 HYPOTHESES

1. Autoxidation of mono-catechol lignan **1** will be significantly slower than that of NDGA, a di-catechol.
2. Mono-catechol analogue **1** of NDGA can form an *o*-quinone.
3. Mono-catechol analogue **1** of NDGA can form a *p*-QM either directly, or as a result of *o*-quinone isomerization.

## **3.0 OBJECTIVES AND AIMS**

### ***3.1 Synthesis of NDGA Analogues***

Method 6, which included two consecutive Stobbe condensations to form the basic lignan skeleton, was chosen as the primary method for the synthesis of analogue **1**. This method provides the greatest flexibility for producing a variety of analogues beyond **1**, and also provides the option of isolating a diol intermediate which may have other applications within the lab group. A number of pilot reactions were performed on the compound eugenol in order to attempt the addition and removal of protecting groups which were to be used during the synthesis.

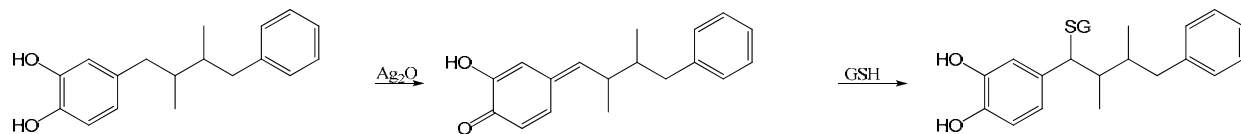
### ***3.2 Determination of the Metabolites of the Lignan Analogues: In Vitro Microsomal Incubation***

In vitro microsomal studies are a common method used in metabolic studies of new drug candidates. In order to determine the likelihood of synthetic analogue **1** forming reactive intermediates, the lignan is incubated at 37°C for one hour in rat liver microsomes with an NADPH-generating system and GSH to study the products of P450-mediated metabolism. Control reactions include lignan in buffer, lignan in buffer with GSH, a system without NADPH, a system which includes only heat inactivated microsomes, and a system without GSH. The products are identified via HPLC and electrospray mass spectrometry.

### ***3.3 Oxidation of the Lignan Analogues using Chemical and Enzymatic Methods***

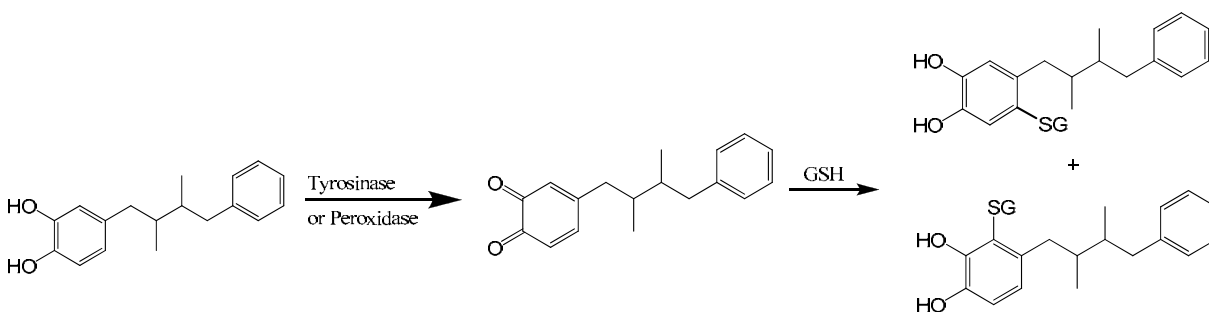
In order to accurately determine which of the possible metabolites are formed during microsomal incubations, preparation of the reactive quinone intermediates is necessary. Analogue **1** can be oxidized to the corresponding *p*-QM using silver oxide in acetonitrile

(Scheme 3-1)<sup>12</sup>. The reactive quinone is trapped with GSH in buffer, and the GSH adduct analyzed via LC-MS.



Scheme 3-1: Formation of Quinone Methide and Potential GSH Product of Analogue 1

Analogue **1** is also oxidized using the enzyme tyrosinase, and the enzyme horseradish peroxidase, and the resulting *o*-quinone is trapped using GSH (Scheme 3-2). LC-MS can be used for product identification.



Scheme 3-2: Formation of *o*-Quinone and Potential GSH Products of Analogue 1

## 4.0 EXPERIMENTAL

### 4.1 Materials

The following chemicals were obtained from Sigma-Aldrich (St. Louis, MO): eugenol, 3,4-dimethoxy benzaldehyde, benzaldehyde, vanillin, 3-hydroxy-4-methoxybenzaldehyde, diethyl succinate, Pd/C, DMSO  $d_6$ , anhydrous DMSO, benzyl bromide, 3,4-dihydroxybenzaldehyde,  $\text{BBr}_3$  (1 M in DCM),  $\text{LiAlH}_4$ , anhydrous pyridine,  $\text{MgCl}_2$ , GSH, NADPH, salicylamide, mushroom tyrosinase, horseradish peroxidase,  $\text{Ag}_2\text{O}$ , *p*-toluenesulfonyl chloride, Mg turnings, anhydrous THF, anhydrous DCM, molecular sieves 3A, iodomethane. Ethanol, which was distilled and stored over sieve under  $\text{N}_{2(g)}$  prior to use, was obtained from Commercial Alcohols Inc. (Brampton, ON).  $\text{CDCl}_3$ , MeOD, and DMSO  $d_6$  were obtained from Cambridge Isotope Laboratories Inc. (Andover, MA). All other solvents were of the highest grade purity, obtained from EMD (Gibbstown, NJ). Celite® 545 and silica gel 60 (0.040-0.063 mm) used in flash columns was also obtained from EMD. Benzyl bromide, NaCl and NaOH were obtained from Fluka Chemika (Switzerland).  $\text{K}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ , HCl,  $\text{H}_2\text{SO}_4$ ,  $\text{Na}_2\text{HPO}_4$ , KOH, and perchloric acid were all obtained from BDH Chemicals (Toronto).  $\text{H}_3\text{PO}_4$  and the sea sand used in the flash columns were obtained from Fisher Scientific (Fairlawn, NJ).  $\text{MgSO}_4$  was obtained from JT Baker Inc. (Phillipsburg, NJ). Anhydrosecoisolariciresinol (ASECO) was prepared and provided by J. L. Billinsky (University of Saskatchewan) as outlined below. NaOEt solution in ethanol was prepared by addition of  $\text{Na}_{(s)}$  to dry ethanol.

#### 4.1.1 Preparation of Rat Hepatic Microsomes

Male Sprague-Dawley (S-D) rats were purchased from Charles River Laboratories, Inc. (Saint-Constant, PQ) at approximately 200-250g or 13 weeks of age (~150g). Rats were housed in a temperature, humidity and 12 hour light-dark controlled facility at the College of Pharmacy and Nutrition, University of Saskatchewan. Rats were allowed to acclimatize in the facility for at least one week and were fed standard laboratory rat chow, *ad libitum* and had free access to

water. All procedures were approved by the University Committee on Animal Care and Supply at the University of Saskatchewan. Rats (n=4) were anesthetized with isoflurane and a midline incision made to expose the abdominal cavity. The portal vein was isolated and cannulated with a 20g 1¼” Terumo Surflo I.V. catheter. The vena cava was nicked to allow blood and perfusate outflow. The livers were perfused via the portal vein with room temperature phosphate buffered saline (PBS) until the liver cleared and no blood was visible exiting the vena cava. Livers were removed and rinsed in PBS. Livers were weighed into 3 or 6 g pieces<sup>33</sup> and immediately flash frozen in liquid nitrogen and stored at -80°C until microsomal preparation. Microsomes were prepared by differential centrifugation according to the method of Iba et al.<sup>28</sup>. Briefly, tissues were homogenized with a teflon pestle in a glass sleeve in homogenization buffer (0.1 mM phenylmethylsulfonylfluoride, 50mM Tris buffer, 150 mM KCl, 0.1 mM dithiothreitol, 1 mM ethylenediamine tetracetic acid and 20% glycerol) until homogeneous. The mixture was transferred to a Beckman (Beckman Coulter Canada, Inc. Mississauga, ON) ultracentrifuge tube and spun in a Beckman L8-55 Ultracentrifuge with a Ti-70 rotor at 9000g for 30 minutes at 4°C. The supernatant, which contains both cytosolic and endoplasmic reticulum components, was transferred into clean ultracentrifuge tubes and spun at 100 000g for 30 minutes at 4°C. The pellet was washed with 150 mM KCl then similarly spun for an additional 30 minutes at 4°C. The pellet was reconstituted in 0.25 M sucrose and passed through a syringe. The microsomes were pooled (n = 4) at this point and stored at -80°C. Samples were only used after one thaw.

#### **4.1.1.1 Determination of Hepatic Microsomal Protein and Cytochrome P450 Content**

Microsomal protein content was determined by the method of Lowry et al.<sup>29</sup>. Briefly, microsomes were diluted 1:50 in 0.5 M NaOH. To samples containing 0, 0.2, 0.4, 0.6 0.8 or 1.0 mL diluted microsomes (final volume 1 mL with 0.5 M NaOH) 5 mL of copper reagent (0.01% copper sulphate, 0.02% sodium potassium tartrate, 99.97% sodium carbonate) was added. After 10 minutes, 0.5 mL of Folin’s reagent (1:2 dilution with H<sub>2</sub>O) was added and samples stood for an additional 30 minutes. Absorbance was determined in a UV/Vis spectrophotometer (#8453E, Agilent Technologies, Palo Alto, 34 CA) at 750 nm. Samples were compared to a similarly prepared bovine serum albumin standard curve to determine protein content. Cytochrome P450 content was determined using the method of Omura and Sato<sup>30</sup>. Microsomes were thawed on ice

and diluted to 1 mg/mL in a quartz cuvette. A few mg of hydrosulphite was added and CO gas bubbled at a rate of three bubbles per second for 30 seconds. From absorbance measurements at 420, 450 and 490 nm in an UV/Vis spectrophotometer, the CYP content was determined.

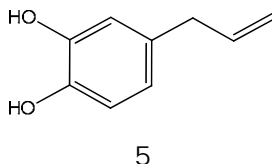
## **4.2 Equipment**

TLC aluminum sheets used were silica gel 60 F<sub>254</sub> purchased from EMD Chemicals Inc. (Gibbstown, NJ). Rotary evaporator systems used were Büchi Rotavapor R-200, and Büchi v700 vacuum pump with attached v850 vacuum controller. The Eppendorf Concentrator 4301 with Büchi Vac V-500 with attached v850 vacuum controllers was used to concentrate samples in tubes. Trace amounts of solvent and moisture were removed using an Edwards High Vacuum Pump. Vacuum filtration and flask evacuation for hydrogenation was performed using a Gast pump (Benton Harbor, MI). Vortexed reactions utilized a Janke & Kunkel IKA-Vibrax VXR with VX2 attachment. Incubations took place in a Boehler Grant ORS 200 shaking heating water bath. All water used was purified via a Millipore Milli-Q system with a Quantum EX Cartridge (Mississauga, ON). Samples were centrifuged in an Eppendorf centrifuge 5417C. HPLC system used was the Waters Alliance 2695. Analytical column was 250mm x 4.6mm Allsphere ODS-2 5u Alltech column (Deerfield, IL). The HPLC system was controlled by, and data interpreted with the Waters Empower software. The UPLC-MS system was a Waters Acquity Synapt HDMS system with Waters Acquity PDA and Synapt Q-TOF. The Column was a Waters BEH C18 1.7 micron 2.1 x 50 mm column, with the data analyzed on Masslynx 4.2 software. All NMR experiments were performed on a Bruker AVANCE DPX-500 spectrometer, with all data processed by X-WIN NMR 3.5 software. All compounds were named using ChemDraw Ultra.

## 4.3 Methods

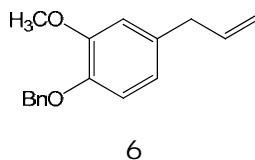
### 4.3.1 Pilot Reactions: Eugenol

#### 4.3.1.1 1-Allyl-3,4-dihydroxybenzene (5)<sup>14</sup>



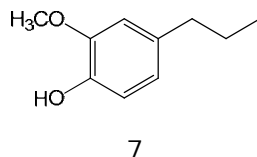
To a stirring solution of  $(\text{CH}_3)_2\text{S}\cdot\text{BBr}_3$  (3.8 g, 12.2 mmol) in DCM (35 mL) under  $\text{N}_{2(\text{g})}$  was added dropwise 0.50 g (3.0 mmol) of eugenol, and the reaction mixture refluxed 2 h. The reaction was hydrolyzed with 10 mL  $\text{H}_2\text{O}$  and diluted with ether. The organic phase was separated and washed with  $\text{NaHCO}_3$ , and extracted with 1 M  $\text{NaOH}$ . The combined  $\text{NaOH}$  extracts was acidified, extracted with ether, and dried over  $\text{MgSO}_4$ . The resulting solvent was concentrated under vacuum, and the residue chromatographed over Si gel (25:1  $\text{CHCl}_3$ : $\text{MeOH}$ ) (0.13 g, 0.84 mmol, 28%).

#### 4.3.1.2 4-Allyl-1-(benzyloxy)-2-methoxybenzene (6)<sup>31</sup>



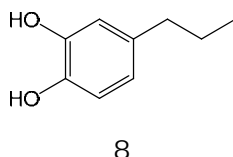
To a stirring solution of eugenol (0.50 g, 3.0 mmol) in  $\text{EtOH}$  (12 mL) under  $\text{N}_{2(\text{g})}$  was added  $\text{K}_2\text{CO}_3$  (0.63 g, 4.6 mmol) and  $\text{BnBr}$  (0.52 g, 3.0 mmol). The reaction was stirred overnight for 20 h. The mixture was filtered through celite, and the filtrate diluted with  $\text{EtOAc}$  (50 mL). The organic layer was then washed with 1 M  $\text{H}_3\text{PO}_4$  (60 mL) and saturated  $\text{NaCl}$  (3 x 50 mL) solution, and dried over  $\text{MgSO}_4$ . The resulting solution was concentrated under vacuum, and the residue chromatographed over Si gel (8:1 hexane: ethyl acetate) via flash column. Final product was a yellow liquid (0.62 g, 2.4 mmol, 80%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.37(d, 2H), 3.91(s, 3H), 5.11 (m, 2H), 5.17 (s, 2H), 6.00 (m, 1H), 6.71 (d, 1H), 6.78 (s, 1H), 6.85 (d, 1H), 7.33 (t, 1H), 7.40 (t, 2 H), 7.48 (d, 2H).

#### 4.3.1.3 2-Methoxy-4-propylphenol (**7**)<sup>12</sup>



To a stirring solution of **6** (0.54 g, 2.1 mmol) in ethanol (20 mL) was added Pd/C (10%) and 3 drops of TFA. The flask was evacuated and flushed with H<sub>2(g)</sub> several times once it had been stoppered and sealed tightly. The mixture was stirred under H<sub>2(g)</sub> at room temperature for 26 h. The reaction mixture was then filtered through Celite and concentrated under vacuum. Final product was a dark yellow oil (0.35 g, 2.10 mmol, 99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>), δ 0.96 (t, 3H), 1.63 (sextet, 2H), 2.54 (t, 2H), 3.89 (s, 3H), 6.70 (d, 2H), 6.85 (d, 1H).

#### 4.3.1.4 4-Propylbenzene-1,2-diol (**8**)<sup>18, 32</sup>

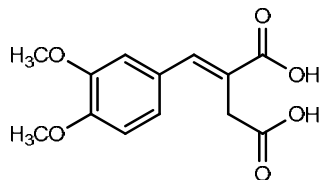


A stirring mixture of **7** (0.22 g, 1.34 mmol) in anhydrous DCM (13 mL) under N<sub>2(g)</sub> was cooled to -78°C. BBr<sub>3</sub> (3.35 mL of 1 M in DCM) was then added to the solution via syringe and stirred for 2 h. The solution was then warmed to room temperature, and the reaction quenched with H<sub>2</sub>O (66 mL) and extracted with EtOAc (1 x 30 mL, 2 x 20 mL). The combined organic layers were washed with 10% NaHCO<sub>3</sub> (30 mL), 1 M HCl (30 mL), and H<sub>2</sub>O (30 mL). The resulting solution was dried over MgSO<sub>4</sub> and concentrated under vacuum. Final product was a black/yellow oil (0.20 g, 1.30 mmol, 97%). <sup>1</sup>H NMR (CDCl<sub>3</sub>), δ 0.95 (t, 3H), 1.58 (sextet, 2H), 2.47 (t, 2H), 5.91 (br d, 2 H), 6.63 (d, 1H), 6.73 (s, 1H), 6.80 (d, 1H).



### 4.3.2 Partial Synthesis of Analogues 1 and 4

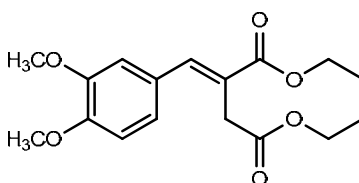
#### 4.3.2.1 2-(3,4-Dimethoxybenzylidene)succinic acid (**9**)<sup>33</sup>



9

To a stirring mixture of 3,4-dimethoxybenzaldehyde (5.01 g, 30.1 mmol) and diethyl succinate (10.5 g, 60.2 mmol) in ethanol (42 mL) was added 28% NaOEt solution in ethanol (12 mL). The reaction mixture was refluxed 20 h under N<sub>2(g)</sub>. 2 M NaOH was added, and the resulting mixture refluxed a further 5 h. The reaction mixture was then cooled and concentrated under vacuum. H<sub>2</sub>O (42 mL) and conc. HCl (42 mL) was added, and extracted with ethyl acetate (84 mL + 3 x 42 mL). Combined organic extracts were washed with H<sub>2</sub>O (100 mL) and dried over MgSO<sub>4</sub>. Solution was concentrated under vacuum, and the resulting residue washed with ethyl acetate. The final product, a pale orange powder, was vacuum filtered and rinsed with small amounts of ethyl acetate (4.95 g, 18.5 mmol, 61%). <sup>1</sup>H NMR (MeOD), δ 3.54 (s, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 7.01 (s, 2H), 7.05 (s, 1H), 7.84 (s, 1H). <sup>13</sup>C NMR (MeOD), δ 29.96, 56.55, 56.76, 112.85, 113.94, 124.23, 126.14, 129.46, 143.10, 150.54, 151.56, 175.37, 176.31.

#### 4.3.2.2 Diethyl 2-(3,4-dimethoxybenzylidene)succinate (**10**)<sup>33</sup>



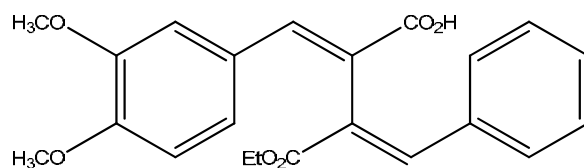
10

To a stirring mixture of **9** (4.95 g, 18.5 mmol) in ethanol (70 mL) was added concentrated H<sub>2</sub>SO<sub>4</sub> (2.1 mL) and refluxed 42h. The reaction mixture was cooled and concentrated under vacuum. H<sub>2</sub>O (61 mL) was added to the residue, and extracted with ethyl acetate (92 mL + 2 x 37 mL). The combined organic extracts were washed with H<sub>2</sub>O (80 mL) and dried over MgSO<sub>4</sub>. The solution was concentrated under vacuum, and the resulting residue chromatographed over Si

gel (4:1 petroleum ether: ethyl acetate) via flash column. Final product was a yellow oil (5.67 g, 17.6 mmol, 95%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$  1.24 (t, 3 H), 1.30 (t, 3H), 3.54 (s, 2H), 3.88 (s, 3H), 3.90 (s, 3H), 4.14 (q, 2H), 4.23 (q, 2H), 6.82 (d, 1H), 6.97 (m, 2H), 7.84 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ),  $\delta$  14.08, 14.18, 33.83, 55.74, 55.80, 60.83, 60.91, 111.01, 112.14, 122.51, 124.43, 127.69, 141.56, 148.78, 149.69, 167.42, 171.25.

### 4.3.3 Synthesis of Analogue 1

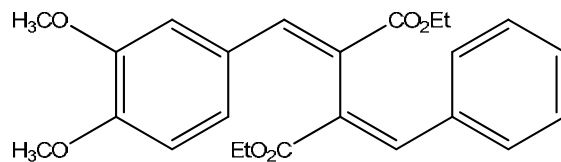
#### 4.3.3.1 2-(3,4-Dimethoxybenzylidene)-3-(ethoxycarbonyl)-4-phenylbut-3-enoic acid (**11**)<sup>33</sup>



11

To a stirring mixture of benzaldehyde (0.880 g, 8.30 mmol) and **10** (2.43 g, 7.54 mmol) in ethanol (31 mL) was added 28% NaOEt solution in ethanol (6.2 mL) and refluxed 5 h under  $\text{N}_2(\text{g})$ . Reaction mixture was cooled, and  $\text{H}_2\text{O}$  (12 mL) and conc. HCl (3.0 mL) were added. The resulting mixture was extracted with ethyl acetate (26 mL + 2 x 15 mL), and the combined organic extracts washed with  $\text{H}_2\text{O}$  (25 mL) and dried over  $\text{MgSO}_4$ . The solution was concentrated under vacuum, and the resulting residue chromatographed over Si gel (2:1 petroleum ether: ethyl acetate) via flash column, flushing column with methanol following collection of fractions. Final product was an extremely viscous yellow oil (2.59 g, 7.16 mmol, 95%).  $^1\text{H}$  NMR (MeOD),  $\delta$  1.13 (t, 3H), 3.73 (s, 3H), 3.84 (s, 3H), 4.15 (q, 2H), 6.75 (d, 1H), 7.06 (m, 2H) 7.23 (s, 3H), 7.48 (d, 2H), 7.88 (s, 1H), 7.92 (s, 1H).

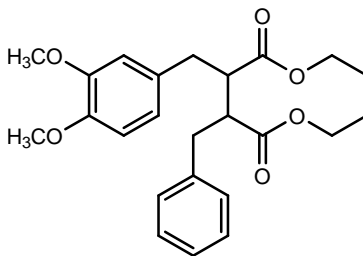
### 4.3.3.2 Diethyl 2-benzylidene-3-(3,4-dimethoxybenzylidene)succinate (**12**)<sup>33</sup>



12

To a stirring mixture of **11** (3.28g, 9.06 mmol) in ethanol (35 mL) was added concentrated H<sub>2</sub>SO<sub>4</sub> (0.50 mL), and the resulting solution refluxed 16 h. The reaction mixture was cooled and concentrated under vacuum. H<sub>2</sub>O (30 mL) was added to the residue, and extracted with ethyl acetate (45 mL + 2 x 18 mL). The combined organic extracts were washed with H<sub>2</sub>O (40 mL) and dried over MgSO<sub>4</sub>. The solution was concentrated under vacuum and the resulting residue chromatographed over Si gel (4:1 petroleum ether: ethyl acetate) via flash column. Final product is a very pale yellow non-viscous liquid (1.75 g, 4.26 mmol, 47%). <sup>1</sup>H NMR (CDCl<sub>3</sub>), δ 1.10 (t, 3H), 1.15 (t, 3H), 3.79 (s, 3H), 3.89 (s, 3H), 4.16 (m, 4H), 6.82 (d, 1H), 7.11 (d, 2H), 7.30 (m, 3H), 7.49 (m, 2H), 7.87 (s, 1H), 7.94 (s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ 14.26, 14.33, 55.88, 56.05, 61.20, 61.37, 111.04, 112.03, 124.48, 124.84, 127.89, 128.01, 128.76, 129.71, 129.84, 135.18, 142.56, 142.72, 148.89, 150.50, 167.16, 167.32.

### 4.3.3.3 Diethyl 2-benzyl-3-(3,4-dimethoxybenzyl)succinate (**13**)<sup>34</sup>

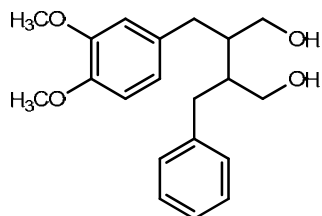


13

To a stirring solution of **12** (1.75 g, 4.26 mmol) in ethanol (913 mL) was added Pd/C (21%). The flask was evacuated and flushed with H<sub>2(g)</sub> several times once it had been stoppered and sealed tightly. The mixture was stirred under H<sub>2(g)</sub> at atmospheric pressure at room temperature 68 h. The reaction mixture was filtered through Celite and concentrated under vacuum. Resulting product was a yellow oil (1.74 g, 4.26 mmol, 99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>), δ 1.12 (m, 6H), 2.92 (m, 6H), 3.65 (s, 3H), 3.78 (s, 3H), 4.02 (m, 4H), 6.68 (m, 2H), 6.83 (d, 1H),

7.14 (m, 5H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.09, 14.14, 35.24, 35.64, 47.86, 48.14, 55.78, 55.96, 60.71, 60.73, 111.18, 112.14, 121.24, 126.53, 128.43, 128.98, 129.12, 131.23, 138.73, 147.68, 148.83, 173.50.

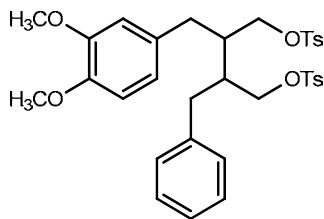
#### 4.3.3.4 2-Benzyl-3-(3,4-dimethoxybenzyl)butane-1,4-diol (**14**)<sup>35</sup>



14

A stirring mixture of  $\text{LiAlH}_4$  (0.17 g, 4.61 mmol) in THF (9 mL) under  $\text{N}_{2(g)}$  was cooled to  $0^\circ\text{C}$ . **13** (0.87 g, 2.10 mmol) dissolved in THF (7 mL) was slowly added via syringe to the reaction vessel. The reaction was warmed to room temperature and stirred for 2 h. The reaction was then cooled to  $0^\circ\text{C}$  and quenched with  $\text{H}_2\text{O}$  (0.17 mL), added dropwise very slowly, at a ratio of 1 mL  $\text{H}_2\text{O}$ /1 g LAH. Following 5 min of stirring, 15% w/v NaOH solution (0.17 mL) was added at a ratio of 1 mL/1 g LAH. Reaction was then warmed to room temperature with continued stirring. A further aliquot of  $\text{H}_2\text{O}$  (0.51 mL) was added at a ratio of 3 mL/1 g LAH. The resulting mixture was filtered through Celite by vacuum filtration, and the residue rinsed with ethyl acetate. The solution was concentrated under vacuum and the resulting residue chromatographed over Si gel (1:1 petroleum ether: ethyl acetate) via flash column (0.59 g, 1.65 mmol, 78%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.07 (m, 2H), 2.78 (m, 4H), 3.50 (m, 4H), 3.83 (s, 3H), 3.87 (s, 3H), 6.66 (s, 1H), 6.72 (m, 1H), 6.78 (d, 1H), 7.16 (d, 1H), 7.22 (t, 2H), 7.28 (t, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  35.98, 36.38, 44.16, 44.21, 56.00, 56.11, 60.62, 60.72, 111.30, 112.22, 121.20, 126.20, 128.58, 129.25, 133.30, 140.82, 147.47, 149.05.

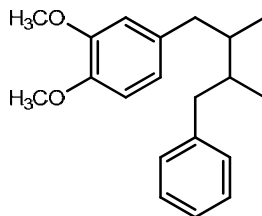
#### 4.3.3.5 2-Benzyl-3-(3,4-dimethoxybenzyl)butane-1,4-diyl bis(4-methylbenzenesulfonate) (**15**)<sup>19</sup>



15

A stirring solution of **14** (0.63 g, 1.9 mmol) in pyridine (2.5 mL) under  $N_{2(g)}$  was cooled to  $0^{\circ}C$  for 20 min. *p*-TsCl (1.19 g, 6.3 mmol) was added, and the mixture then stirred at  $0^{\circ}C$  for 4 h. The reaction was quenched with 2 M HCl (14 mL) and extracted with EtOAc (30 mL). The resulting mixture was then vacuum filtered, isolating the filtrate. It was then extracted a further two times with EtOAc (2 x 15 mL), and the combined organic layers dried with  $MgSO_4$ . The resulting solution was concentrated under vacuum, and the resulting residue chromatographed over Si gel (2:1 hexane: ethyl acetate) via flash column, isolating a white crystalline solid (0.49 g, 0.76 mmol, 40%).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  2.09 (m, 2H), 2.47 (s, 6H), 2.51 (m, 2H), 2.69 (m, 2H), 3.80 (s, 3H), 3.87 (s, 3H), 3.94 (m, 4H), 6.49 (d, 1H), 6.55 (s, 1H), 6.67 (d, 1H), 6.90 (m, 2H), 7.17 (m, 3H), 7.33 (d, 4H), 7.72 (m, 4H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  21.89, 33.94, 34.29, 40.18, 40.29, 56.01, 56.10, 69.68, 69.76, 111.26, 112.06, 121.13, 126.59, 128.14, 128.71, 129.08, 130.15, 131.36, 132.77, 132.79, 138.88, 145.18, 147.76, 149.21.

#### 4.3.3.6 4-(2,3-Dimethyl-4-phenylbutyl)-1,2-dimethoxybenzene (**16**)<sup>19, 35</sup>

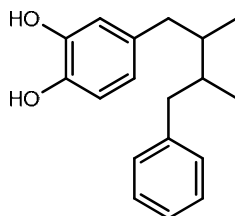


16

A stirring mixture of  $LiAlH_4$  (0.22 g, 5.8 mmol) in THF (16 mL) under  $N_{2(g)}$  was cooled to  $0^{\circ}C$ . **15** (0.74 g, 1.2 mmol) dissolved in THF (8 mL) was slowly added via syringe to the reaction vessel. The reaction was warmed to room temperature and stirred for 6 h. The reaction was then cooled to  $0^{\circ}C$  and quenched with  $H_2O$  (0.2 mL), added dropwise very slowly, at a ratio

of 1 mL H<sub>2</sub>O/1 g LAH. Following 5 min of stirring, 15% w/v NaOH solution (0.2 mL) was added at a ratio of 1 mL/1 g LAH. The reaction was then warmed to room temperature with continued stirring. A further aliquot of H<sub>2</sub>O (0.6 mL) was added at a ratio of 3 mL/1 g LAH. The resulting mixture was filtered through Celite by vacuum filtration, and the residue was rinsed with ethyl acetate. The resulting solution was concentrated under vacuum and the residue chromatographed over Si gel (95:5 hexane: ethyl acetate) via flash column. Final product was a yellow oil (0.34 g, 1.1 mmol, 92%) <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.87 (m, 6H), 1.82 (m, 2H), 2.46 (2 x dd, 2H), 2.65 (2 x dd, 2H), 3.85 (s, 3H), 3.89 (s, 3H), 6.60 (s, 1H), 6.67 (m, 1H), 6.80 (d, 1H), 7.13 (d, 2H), 7.21 (m, 2H), 7.30 (t, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.08, 14.12, 37.94, 38.03, 41.18, 41.61, 55.94, 56.09, 111.10, 112.17, 121.08, 138.31, 129.22, 129.31, 134.40, 141.86, 147.20, 148.86.

#### 4.3.3.7 4-(2,3-Dimethyl-4-phenylbutyl)benzene-1,2-diol (**1**)<sup>18,32</sup>

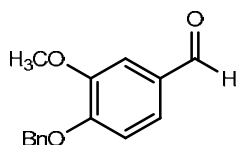


1

A stirring mixture of **16** (0.30 g, 1.0 mmol) in anhydrous DCM (10 mL) under N<sub>2(g)</sub> was cooled to -78°C. BBr<sub>3</sub> (1 M in DCM, 5 mL) was added to the solution via syringe and the resulting mixture was stirred for 2 h. The solution was warmed to room temperature, quenched with H<sub>2</sub>O (10 mL), and extracted with EtOAc (20 mL + 2 x 10 mL). The combined organic layers were washed with 10% NaHCO<sub>3</sub> (15 mL), 1 M HCl (15 mL), and H<sub>2</sub>O (15 mL). The final solution was dried over MgSO<sub>4</sub> and concentrated under vacuum. Final product was a dark yellow/black oil (0.25 g, 0.92 mmol, 92%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.86 (m, 6H), 1.80 (m, 2H), 2.35 (dd, 1H), 2.46 (dd, 1H), 2.56 (dd, 1H), 2.66 (dd, 1H), 5.09 (br s, 2H), 6.56 (d, 1H), 6.60 (s, 1H), 6.78 (d, 1H), 7.14 (d, 2H), 7.22 (m, 1H), 7.29 (m, 2H). <sup>13</sup>C NMR 14.14, 16.43, 38.20, 39.48, 40.83, 41.60, 115.39, 116.19, 121.70, 125.79, 128.33, 129.30, 135.04, 141.53, 141.94, 143.42. MS-ESI(-) *m/z* 269.13.

### 4.3.4 Partial Synthesis of Analogues 2 and 3

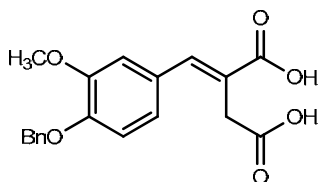
#### 4.3.4.1 4-Benzyloxy-3-methoxybenzaldehyde (**17**)<sup>31</sup>



17

To a stirring solution of vanillin (10.0 g, 65.8 mmol) in EtOH (258 mL) under N<sub>2(g)</sub> was added K<sub>2</sub>CO<sub>3</sub> (13.6 g, 62.2 mmol) and BnBr (7.81 mL, 98.7 mmol). The mixture was stirred overnight, approximately 22 h. The reaction mixture was then filtered through Celite, and the filtrate concentrated under vacuum to approximately 25% the original volume. The resulting solution was diluted with EtOAc, and was washed with 1 M H<sub>3</sub>PO<sub>4</sub>, saturated NaCl solution, and dried over MgSO<sub>4</sub>. The final solution was concentrated under vacuum, and the resulting residue chromatographed over Si gel (5:1 hexane: ethyl acetate) via flash column. The final product was an off-white solid (11.6 g, 48.0 mmol, 73%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.88 (s, 3H), 5.18 (s, 2H), 6.95 (d, 1H), 7.29 (m, 1H), 7.37 (m, 3H), 7.33 (m, 3H), 9.80 (s, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 56.23, 71.03, 109.52, 112.56, 126.77, 127.38, 128.39, 128.90, 130.47, 136.19, 150.25, 153.77, 191.08.

#### 4.3.4.2 2-(4-(Benzyloxy)-3-methoxybenzylidene)succinic acid (**18**)<sup>33</sup>



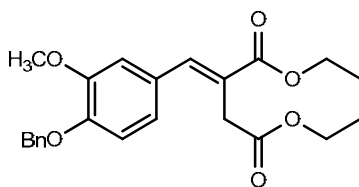
18

To a stirring mixture of **17** (9.53 g, 39.3 mmol) and diethyl succinate (20.6 mL, 123 mmol) in ethanol (55 mL) was added 28% NaOEt solution in ethanol (15 mL), and refluxed 5 h under N<sub>2(g)</sub>. 2 M NaOH (110 mL) was added and refluxed a further 15 h. The reaction mixture was cooled and concentrated under vacuum. H<sub>2</sub>O (55 mL) and conc. HCl (55 mL) were added, and extracted with ethyl acetate (110 mL + 2 x 55 mL). The combined organic extracts were washed with H<sub>2</sub>O and dried over MgSO<sub>4</sub>. The resulting solution was concentrated under vacuum, and the resulting residue washed with ethyl acetate. The final product was vacuum filtered and rinsed with a small amount of ethyl acetate to give a pale yellow powder (11.89 g,





#### 4.3.4.4 Diethyl 2-(4-(benzyloxy)-3-methoxybenzylidene)succinate (**19**)

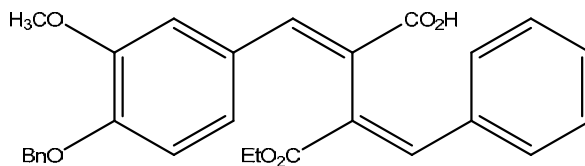


19

To a stirring mixture of **20** (4.6 g, 14.8 mmol) in EtOH (58 mL) under N<sub>2(g)</sub> was added K<sub>2</sub>CO<sub>3</sub> (3.07 g, 22.2 mmol) and BnBr (3.52 mL, 29.6 mmol). The mixture was stirred overnight, approximately 22 h. The reaction mixture was then filtered through Celite, and the filtrate concentrated under vacuum to approximately 25% the original volume. The resulting solution was diluted with EtOAc, and was washed with 1 M H<sub>3</sub>PO<sub>4</sub>, saturated NaCl solution, and dried over MgSO<sub>4</sub>. The final solution was concentrated under vacuum, and the resulting residue chromatographed over Si gel (6:1 hexane: ethyl acetate) via flash column. The final product was a yellow oil (5.07 g, 12.7 mmol, 86%). NMR as per **19** (4.3.4.3) above.

#### 4.3.5 Partial Synthesis of Analogue 2

##### 4.3.5.1 2-(4-(Benzyloxy)-3-methoxybenzylidene)-3-(ethoxycarbonyl)-4-phenylbut-3-enoic acid (**21**)<sup>33</sup>

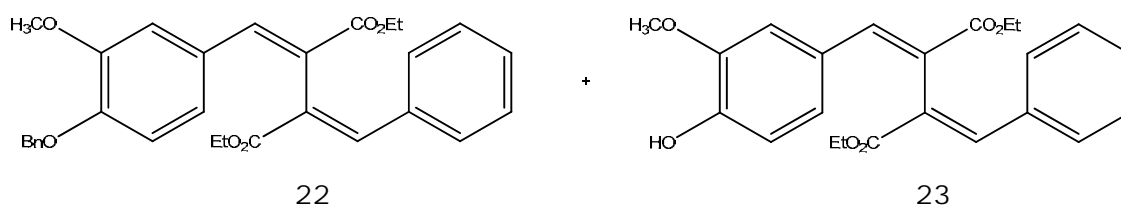


21

To a stirring mixture of benzaldehyde (1.50 mL, 14.8 mmol) and **19** (5.39 g, 13.5 mmol) in ethanol (56 mL) was added 28% NaOEt solution in ethanol (8.7 mL), and refluxed 5 h under N<sub>2(g)</sub>. The reaction mixture was cooled, and H<sub>2</sub>O (18.9 mL) and conc. HCl (18.9 mL) was added, and extracted with ethyl acetate (37.8 mL + 2 x 18.9 mL). The combined organic extracts were washed with H<sub>2</sub>O and dried over MgSO<sub>4</sub>. The resulting solution was concentrated under vacuum, and the resulting residue chromatographed over Si gel (2:1 petroleum ether: ethyl acetate) via flash column, flushing column with methanol following collection of fractions (6.58

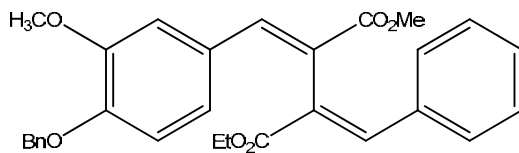
g, 14.4 mmol, 97%).  $^1\text{H}$  NMR (MeOD)  $\delta$  1.11 (t, 3H), 3.63 (s, 3H), 4.13 (q, 2H), 4.93 (s, 2H), 6.79 (d, 1H), 6.98 (d, 1H), 7.13 (s, 1H), 7.21 (m, 2H) 7.26 (t, 3H), 7.33 (m, 3H), 7.48 (m, 2H), 7.86 (s, 1H), 7.92 (s, 1H).  $^{13}\text{C}$  NMR (MeOD)  $\delta$  13.23, 48.61, 54.93, 61.04, 70.30, 112.25, 113.20, 123.99, 125.54, 127.34, 127.66, 128.10, 128.15, 128.20, 128.32, 128.61, 129.42, 129.48, 134.83, 136.77, 142.03, 142.06, 149.27, 149.59, 167.38, 169.24.

#### 4.3.5.2 Diethyl 2-benzylidene-3-(4-(benzyloxy)-3-methoxybenzylidene)succinate (**22**)<sup>33</sup> and diethyl 2-benzylidene-3-(4-hydroxy-3-methoxybenzylidene)succinate (**23**)



To a stirring mixture of **21** (3.76 g, 8.20 mmol) in ethanol (31.1 mL) was added concentrated  $\text{H}_2\text{SO}_4$  (0.46 mL) and refluxed 16 h. The reaction mixture was cooled and concentrated under vacuum.  $\text{H}_2\text{O}$  (27 mL) was added to the residue and extracted with ethyl acetate (41 mL + 2 x 16 mL). The combined organic extracts were washed with  $\text{H}_2\text{O}$  and dried over  $\text{MgSO}_4$ . The resulting solution was concentrated under vacuum and the final residue chromatographed over Si gel (4:1 petroleum ether: ethyl acetate) via flash column to give the final product, a colourless oil (0.34 g, 0.71 mmol, 8.6%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.11 (m, 6H), 3.79 (s, 3H), 4.17 (m, 4H), 5.15 (s, 2H), 6.81 (d, 1H), 7.03 (d, 1H), 7.14 (s, 1H), 7.28 (m, 4H), 7.36 (t, 2H), 7.41 (d, 2H), 7.48 (m, 2H), 7.86 (s, 1H), 7.94 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.08, 14.13, 55.77, 61.02, 61.18, 70.71, 112.34, 113.22, 124.08, 124.84, 127.22, 127.75, 127.99, 128.20, 128.56, 128.61, 129.53, 129.62, 135.03, 136.62, 142.42, 142.56, 149.26, 149.44, 166.94, 167.10. Also isolated was **23**, a colourless oil (0.37 g, 0.94 mmol, 11%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.08 (t, 3H), 1.11 (t, 3H), 3.78 (s, 3H), 4.14 (m, 4H), 6.84 (d, 1H), 7.04 (d, 1H), 7.09 (s, 1H), 7.28 (m, 3H), 7.47 (m, 2H), 7.83 (s, 1H), 7.91 (s, 1H).

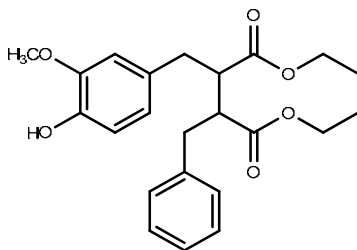
#### 4.3.5.3 1-Ethyl 4-methyl 2-benzylidene-3-(4-(benzyloxy)-3-methoxybenzylidene)succinate (**24**)<sup>36</sup>



24

To stirring DMSO (18.6 mL) was added powdered KOH (1.04 g, 18.6 mmol), and resulting mixture stirred for 5 min. **21** (4.27 g, 9.30 mmol) was then added, along with CH<sub>3</sub>I (18.6 mmol, 1.16 mL), and the final reaction mixture was stirred for 1 h. H<sub>2</sub>O was added to the mixture (186 mL), and was then extracted with DCM (3 x 186 mL). The combined DCM extracts were washed with H<sub>2</sub>O (5 x 93 mL), dried over MgSO<sub>4</sub>, and concentrated under vacuum. The resulting residue was chromatographed over Si gel (4:1 petroleum ether: ethyl acetate) via flash column to give the final product, a yellow oil (2.52 g, 5.33 mmol, 57%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.13 (t, 3H), 3.69 (s, 3H), 3.78 (s, 3H), 4.18 (q, 2H), 5.16 (s, 2H), 6.80 (d, 1H), 7.02 (d, 1H), 7.12 (d, 1H), 7.28 (m, 4H), 7.28 (m, 2H), 7.32 (m, 2H), 7.46 (m, 2H), 7.85 (s, 1H), 7.93 (s, 1H).

#### 4.3.5.4 Diethyl 2-benzyl-3-(4-hydroxy-3-methoxybenzyl)succinate (**25**)<sup>34</sup>

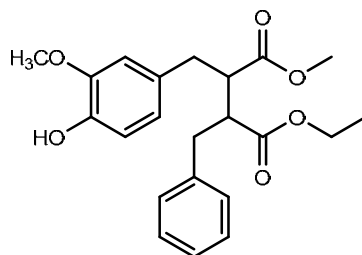


25

To a stirring solution of **22** and **23** (0.77 g and 0.36 g, 2.50 mmol total) in ethanol (537 mL) was added Pd/C (21%) and 3 drops of TFA. The flask was stoppered and sealed tightly, and evacuated and flushed with H<sub>2(g)</sub> several times. The mixture was stirred under H<sub>2(g)</sub> at room temperature 68 h. The resulting reaction mixture was filtered through Celite and concentrated under vacuum. Final product was a dark, black/purple oil (0.99 g, 2.47 mmol, 99%) <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.14 (m, 6H), 2.84 (m, 4H), 2.94 (m, 2H), 3.75 (s, 3H), 3.99 (m, 4H), 6.53 (d, 1H), 6.61 (s, 1H), 6.66 (d, 1H), 7.07 (d, 2H), 7.16 (d, 2H), 7.22 (t, 1H). <sup>13</sup>C NMR (MeOD) δ 14.54, 14.58,

36.65, 37.03, 50.18, 52.9, 56.45, 61.85, 61.88, 113.76, 116.20, 122.88, 127.71, 129.55, 129.59, 131.36, 140.14, 146.48, 149.00, 175.34, 175.47.

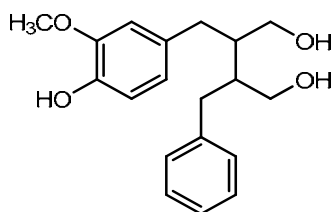
#### 4.3.5.5 1-Ethyl 4-methyl 2-benzyl-3-(4-hydroxy-3-methoxybenzyl)succinate (26)<sup>34</sup>



26

To a stirring solution of **24** (2.03 g, 4.30 mmol) in ethanol (950 mL) was added Pd/C (21%) and 3 drops of TFA. The flask was stoppered and sealed tightly, and evacuated and flushed with H<sub>2(g)</sub> several times. The mixture was stirred under H<sub>2(g)</sub> at room temperature 68 h. The resulting reaction mixture was filtered through Celite and concentrated under vacuum. Final product was a dark, black/purple oil (1.65 g, 4.27 mmol, 99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.26 (t, 3H), 3.00 (m, 6H), 3.64 (s, 3H), 3.74 (q, 2H), 3.86 (s, 3H), 6.55 (s, 1H), 6.63 (m, 1H), 6.81 (d, 1H), 7.10 (m, 2H), 7.21 (m, 2H), 7.29 (m, 1H).

#### 4.3.5.6 2-Benzyl-3-(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (27)<sup>35</sup>

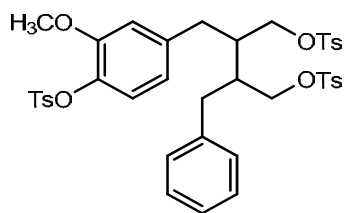


27

A stirring mixture of LiAlH<sub>4</sub> (0.29 g, 7.6 mmol) in THF (16 mL) under N<sub>2(g)</sub> was cooled to 0°C. **25** and **26** (1.39 g, 3.63 mmol) dissolved in THF (6 mL) was slowly added to the flask via syringe. The reaction was then allowed to warm to room temperature, and was stirred 2 h. The reaction was then cooled to 0°C, and quenched with H<sub>2</sub>O (0.3 mL), added dropwise very slowly, at a ratio of 1 mL H<sub>2</sub>O/1 g LAH. Following 5 mins. of stirring, 15% w/v NaOH solution (0.3 mL) was added at a ratio of 1 mL/1 g LAH. The reaction was warmed to room temperature

with continued stirring. A further aliquot of H<sub>2</sub>O (0.9 mL) was added at a ratio of 3 mL/1 g LAH, and the resulting mixture was filtered through Celite by vacuum filtration, rinsing the residue with ethyl acetate. The resulting solution was concentrated under vacuum and chromatographed over Si gel (3:1 ethyl acetate: petroleum ether) via flash column, flushing column with MeOH, isolating the final product as a colourless oil (0.49 g, 1.5 mmol, 41%). <sup>1</sup>H NMR (MeOD) δ 1.91 (m, 2 H), 2.59 (m, 2H), 2.68 (m, 2H), 3.34 (s, 3H), 3.56 (m, 4H), 6.55 (d, 1H), 6.63 (s, 1H), 6.68 (d, 1H), 7.13 (m, 5H). <sup>13</sup>C NMR (MeOD) δ 35.98, 36.37, 44.38, 44.47, 50.00, 56.36, 61.95, 61.99, 113.56, 116.00, 122.77, 126.89, 129.34, 130.29, 133.90, 142.55, 145.61, 148.90.

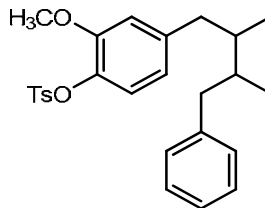
#### 4.3.5.7 2-Benzyl-3-(3-methoxy-4-(tosyloxy)benzyl)butane-1,4-diyl bis(4-methylbenzenesulfonate) (**28**)<sup>37</sup>



28

A stirring solution of **27** (0.49 g, 1.5mmol) in pyridine (1.8 mL) under N<sub>2(g)</sub> was cooled to 0°C for 20 min. *p*-TsCl (7.56 mmol) was added, and the mixture then stirred at 0°C for 8 h. The reaction was quenched with 2 M HCl (11 mL) and extracted with EtOAc, and the combined organic layers dried with MgSO<sub>4</sub>. The resulting solution was concentrated under vacuum, and the resulting residue washed with DCM isolating a white solid (0.43 g, 0.55 mmol, 37%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.08 (m, 2H), 2.46 (s, 3H), 2.47 (s, 6H), 2.52 (m, 2H), 2.71 (m, 2H), 3.48 (s, 3H), 3.90 (m, 4 H), 6.40 (d, 1H), 6.54 (s, 1H), 6.87 (m, 2H), 6.96 (d, 1H), 7.17 (d, 3H), 7.33 (m, 6H), 7.72 (m, 6H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 34.24, 40.17, 40.29, 55.72, 69.50, 69.63, 113.40, 121.04, 124.07, 126.70, 128.08, 128.78, 128.82, 129.04, 129.56, 130.21, 130.25, 132.60, 133.41, 137.11, 138.68, 139.23, 145.24, 145.32, 145.46, 151.95.

#### 4.3.5.8 4-(2,3-Dimethyl-4-phenylbutyl)-2-methoxyphenyl 4-methylbenzenesulfonate (29)<sup>19, 35</sup>

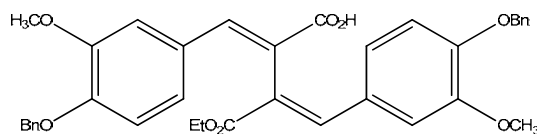


29

A stirring mixture of  $\text{LiAlH}_4$  (14.2 mg, 0.37 mmol) in THF (1.0 mL) under  $\text{N}_{2(g)}$  was cooled to  $0^\circ\text{C}$ . **28** (53.7 mg, 0.0689 mmol) dissolved in THF (0.5 mL) was slowly added via syringe to the reaction vessel. The reaction was warmed to room temperature, and then refluxed for 6 h. The reaction was then cooled to room temperature, and then to  $0^\circ\text{C}$  and quenched with  $\text{H}_2\text{O}$  added dropwise very slowly at a ratio of 1 mL  $\text{H}_2\text{O}$ /1 g LAH. Following 5 min of stirring, 15% w/v NaOH solution was added at a ratio of 1 mL/1 g LAH. The reaction was then warmed to room temperature with continued stirring. A further aliquot of  $\text{H}_2\text{O}$  was added at a ratio of 3 mL/1 g LAH. The resulting mixture was filtered through Celite by vacuum filtration, and the residue was rinsed with ethyl acetate. The resulting solution was concentrated under vacuum and the residue chromatographed over Si gel (8:1 petroleum ether: ethyl acetate) via PTLC. Final product was a yellow oil (21.8 mg, 0.0497 mmol, 72%)  $^1\text{H}$  NMR (MeOD)  $\delta$  0.80 (m, 6H), 1.71 (m, 2H), 2.38 (s, 3H) 2.41 (m, 2H), 2.53 (m, 2H), 3.34 (s, 3H), 6.52 (s, 1H), 6.58 (d, 1H), 6.96 (d, 1H), 7.00 (d, 2H), 7.10 (d, 1H), 7.17 (d, 2H), 7.29 (d, 2H), 7.61 (d, 2H).  $^{13}\text{C}$  NMR (MeOD)  $\delta$  14.29, 16.63, 21.74, 38.82, 42.28, 42.50, 55.99, 114.39, 122.08, 124.55, 126.86, 129.33, 129.94, 130.12, 130.55, 134.42, 137.77, 142.90, 143.75, 146.86, 152.83.

### 4.3.6 Partial Synthesis of Analogue 3

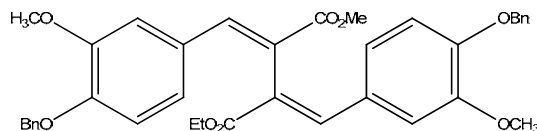
#### 4.3.6.1 2-(4-(Benzyloxy)-3-methoxybenzylidene)-4-(4-(benzyloxy)-3-methoxyphenyl)-3-(ethoxycarbonyl)but-3-enoic acid (**30**)<sup>33</sup>



30

To a stirring mixture of **17** (1.75 g, 7.24 mmol) and **19** (2.62 g, 6.58 mmol) in ethanol (27.3 mL) was added 28% NaOEt solution in ethanol (4.9 mL) and refluxed 5 h under N<sub>2(g)</sub>. The reaction mixture was then cooled, and H<sub>2</sub>O and conc. HCl (2.6 mL) were added. The resulting mixture was extracted with ethyl acetate, and the combined organic extracts washed with H<sub>2</sub>O and dried over MgSO<sub>4</sub>. The resulting solution was concentrated under vacuum, and the resulting residue washed with ethyl acetate. The final product, a pale yellow powder, was vacuum-filtered and rinsed with small amounts of ethyl acetate (1.62 g, 2.72 mmol, 41%). <sup>1</sup>H NMR (d<sub>6</sub>-DMSO) δ 1.06 (t, 3H), 3.64 (m, 6H), 4.09 (m, 2H), 5.06 (br s, 4H), 7.01 (m, 2H), 7.11 (m, 2H), 7.18 (s, 1H), 7.23 (s, 1H), 7.38 (br m, 10 H), 7.76 (s, 2H). <sup>13</sup>C NMR (d<sub>6</sub>-DMSO) δ 13.94, 55.27, 60.78, 70.06, 113.11, 123.56, 123.76, 125.35, 125.62, 127.45, 127.57, 127.83, 127.87, 127.94, 128.42, 136.69, 140.78, 148.67, 149.17, 149.31, 166.44, 167.79.

#### 4.3.6.2 1-Ethyl 4-methyl 2,3-bis(4-(benzyloxy)-3-methoxybenzylidene)succinate (**31**)<sup>36</sup>



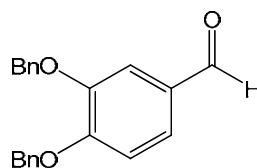
31

To DMSO (0.56 mL) was added powdered KOH (63.3 mg, 1.13 mmol), and stirred for 5 min. **30** (168 mg, 0.282 mmol) was then added, along with CH<sub>3</sub>I (0.035 mL, 0.564 mmol), and stirred for another 1 h. To this solution was added H<sub>2</sub>O (5.6 mL), and the resulting mixture extracted with three aliquots of DCM (3 x 5.6 mL). The combined organic extracts were then washed with 5 aliquots of H<sub>2</sub>O (5 x 2.8 mL), and dried over MgSO<sub>4</sub>. The resulting solution was

concentrated to give a yellow oil (165 mg, 0.270 mmol, 96%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.13 (t, 3H), 3.70 (s, 3H), 3.77 (s, 6H), 4.18 (q, 2H), 5.14 (s, 4H), 6.81 (d, 2H), 7.05 (d, 2H), 7.19 (m, 2 H), 7.30 (m, 2H), 7.35 (t, 4H), 7.41 (m, 4H), 7.90 (s, 2H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  14.39, 52.53, 55.94, 61.24, 70.90, 112.54, 113.34, 124.53, 124.93, 125.11, 127.41, 128.20, 128.26, 128.42, 128.82, 128.95, 136.79, 142.24, 124.44, 149.41, 149.74, 167.34, 168.03.

### 4.3.7 Partial Synthesis of Analogue 4

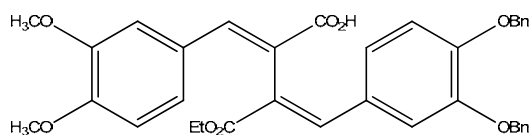
#### 4.3.7.1 3,4-Bis(benzyloxy)benzaldehyde (**32**)<sup>31</sup>



32

To a stirring solution of 3,4-dihydroxybenzaldehyde (5.03 g, 36.4 mmol) in ethanol (143 mL) under  $\text{N}_{2(\text{g})}$  was added  $\text{K}_2\text{CO}_3$  (15.1 g, 109.2 mmol) and  $\text{BnBr}$  (8.65 mL, 72.8 mmol). The reaction was stirred over night, and the resulting mixture filtered through Celite. The filtrate was diluted with  $\text{EtOAc}$ , and washed with 1M  $\text{H}_3\text{PO}_4$  and saturated  $\text{NaCl}$  solution. The organic extracts were dried over  $\text{MgSO}_4$ , and concentrated under vacuum. The crude product was recrystallized with  $\text{MeOH}$ , and the final product, a pale yellow solid, was collected via vacuum filtration (1.32 g, 4.15 mmol, 11%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.23 (s, 2H), 5.27 (s, 2H), 7.03 (d, 1H), 7.38-7.50 (br m, 12 H), 9.82 (s, 1H).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  71.06, 71.20, 112.61, 113.31, 126.90, 126.90, 127.53, 128.23, 128.34, 128.80, 128.88, 130.53, 136.45, 136.76, 149.42, 154.48, 191.04.

#### 4.3.7.2 4-(3,4-Bis(benzyloxy)phenyl)-2-(3,4-dimethoxybenzylidene)-3-(ethoxycarbonyl)but-3-enoic acid (**33**)<sup>33</sup>



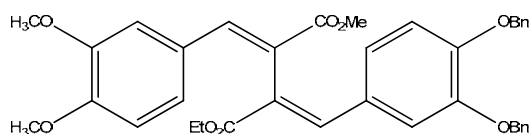
33

To a stirring mixture of **10** (1.19 g, 3.70 mmol) and **32** (1.30 g, 4.07 mmol) in ethanol (15.4 mL) was added 28%  $\text{NaOEt}$  solution in ethanol (4.3 mL), and the resulting solution



refluxed under  $N_{2(g)}$ . The reaction mixture was cooled, and conc. HCl (5.7 mL) and  $H_2O$  (5.7 mL) was added and extracted with EtOAc. The combined organic extracts were dried over  $MgSO_4$ , and the resulting solution concentrated under vacuum. The final residue was chromatographed over Si gel (2:1 petroleum ether: ethyl acetate), with the column flushed with methanol to give a yellow solid (1.16 g, 1.96 mmol, 53%).  $^1H$  NMR ( $d_6$ -DMSO)  $\delta$  1.05 (t, 3H), 3.72 (s, 3H), 3.75 (s, 3H), 4.11 (q, 2H), 5.10 (m, 2H), 5.18 (s, 2H), 6.91 (d, 1H), 7.02 (d, 1H), 7.08 (d, 1H), 7.10 (s, 1H), 7.13 (d, 1H), 7.28 (d, 1H), 7.32 (t, 3H), 7.36 (m, 5H), 7.40 (d, 2H), 7.40 (d, 2H).  $^{13}C$  NMR ( $d_6$ -DMSO)  $\delta$  14.09, 48.60, 55.13, 55.46, 60.35, 69.76, 69.85, 111.55, 113.65, 127.54, 127.80, 127.86, 128.35, 128.42, 136.79, 136.86, 147.55, 148.26.

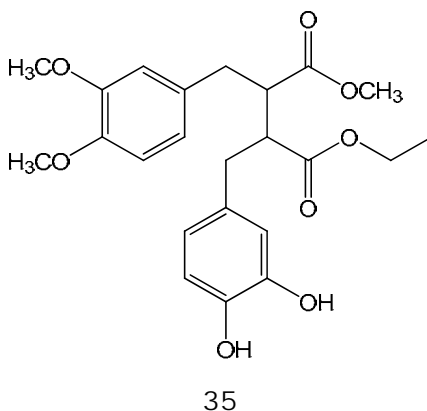
#### 4.3.7.3 1-Ethyl 4-methyl 2-(3,4-bis(benzyloxy)benzylidene)-3-(3,4-dimethoxybenzylidene)succinate (**34**)<sup>36</sup>



34

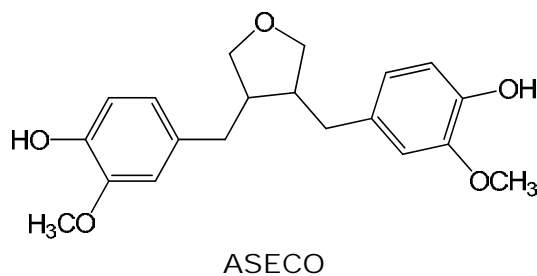
To DMSO (3.4 mL) was added powdered KOH (0.38 g, 6.79 mmol), and stirred for 5 min. **33** (1.01 g, 1.70 mmol) was then added, along with  $CH_3I$  (0.21 mL, 3.40 mmol), and stirred for another 1 h. To this solution was added  $H_2O$  (34 mL), and the resulting mixture extracted with three aliquots of DCM (3 x 34 mL). The combined organic extracts were then washed with 5 aliquots of  $H_2O$  (5 x 17 mL), and dried over  $MgSO_4$ . The resulting solution was concentrated, and the residue chromatographed over Si gel (5:2 hexane: ethyl acetate) via flash column. Final product was a yellow oil (0.58 g, 0.95 mmol, 56%).  $^1H$  NMR ( $CDCl_3$ )  $\delta$  1.13 (t, 3H), 3.68 (s, 3H), 3.72 (s, 3H), 3.89 (s, 3H), 4.15 (q, 2H), 5.09 (m, 2H), 5.18 (s, 2H), 6.80 (d, 1H), 6.86 (d, 1H), 7.07 (m, 3H), 7.20 (s, 1H), 7.29 (m, 4H), 7.37 (m, 4H), 7.42 (m, 2H), 7.83 (s, 2H).  $^{13}C$  NMR ( $CDCl_3$ )  $\delta$  14.41, 52.51, 55.84, 56.05, 61.24, 71.01, 71.03, 110.98, 111.97, 114.11, 115.28, 124.69, 124.71, 125.06, 125.25, 127.30, 127.33, 127.92, 127.94, 128.12, 128.28, 128.64, 128.75, 136.95, 137.03, 142.26, 142.62, 148.60, 148.86, 150.48, 150.57, 167.30, 167.92.

#### 4.3.7.4 1-Ethyl 4-methyl 2-(3,4-dihydroxybenzyl)-3-(3,4-dimethoxybenzyl)succinate (35)<sup>34</sup>



To a stirring solution of **34** (0.56 g, 0.91 mmol) in ethanol (196 mL) was added Pd/C (21%) and 3 drops of TFA. The flask was stoppered and sealed tightly, and evacuated and flushed with H<sub>2(g)</sub> several times. The mixture was stirred under H<sub>2(g)</sub> at room temperature 68 h. The resulting reaction mixture was filtered through Celite and concentrated under vacuum. Final product was a purple/black oil (0.39 g, 0.90 mmol, 99%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 1.20 (t, 3H), 2.85 (m, 4H), 2.99 (m, 2H), 3.63 (s, 3H), 3.85 (s, 6H), 4.10 (q, 2H), 6.49 (m, 2H), 6.56 (s, 1H), 6.76 (s, 1H), 7.41 (m, 2H). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 14.23, 35.13, 35.43, 47.93, 47.98, 55.99, 56.06, 58.68, 61.02, 109.60, 111.33, 112.21, 112.58, 115.41, 116.28, 121.47, 128.92, 131.04, 144.00, 147.67, 148.82, 174.04, 174.40.

#### 4.3.8 Synthesis of Anhydrosecoisolariciresinol (ASECO)



To secoisolariciresinol (SECO) (254 mg, 0.702 mmol) dissolved in methanol (30 mL) was added 15 drops of concentrated HCl, and the reaction mixture was refluxed for 20 h. The reaction was quenched with H<sub>2</sub>O (30 mL) and extracted with ethyl acetate (3 x 40 mL). The organic layer was washed with sodium bicarbonate (0.6 M, 80 mL), H<sub>2</sub>O (80 mL) and dried over

MgSO<sub>4</sub>. The organic layer was concentrated under vacuum and the residue chromatographed over Si gel (9:1 dichloromethane: ethyl acetate) via flash column. Resulting product was an off-white powder (0.267 mmol, 91.9 mg, 38%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.19 (m, 2 H), 2.56 (m, 4 H), 3.56 (dd, 2 H), 3.88 (s, 6 H), 3.95 (dd, 2 H), 6.51 (s, 2 H), 6.59 (d, 2 H), 6.81 (d, 2H), 7.27 (s, 2 H).

### 4.3.9 Oxidation Experiments

#### 4.3.9.1 Incubation of **1** in Rat Liver Microsomes<sup>11</sup>

0.5 mM **1** and 5.0 mM GSH were incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP (rat liver microsomes) and 5.0 mM MgCl<sub>2</sub> at 37°C for 60 min at a final reaction volume of 500 μL. Reaction was initiated by addition of 1 mM NADPH and terminated by addition of 50 μL ice cold 2 mM salicylamide in acetonitrile. Reaction was centrifuged at 14,000 rpm for 10 min, and supernatant was analyzed by HPLC and by UPLC-MS.

Simultaneously, 0.5 mM **1** was incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4, 0.5 mM **1** and 5.0 mM GSH were incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4, 0.5 mM **1** was incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP, 5.0 mM MgCl<sub>2</sub>, and 1 mM NADPH, 0.5 mM **1** and 5.0 mM GSH were incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL heat inactivated microsomes, 5.0 mM MgCl<sub>2</sub>, and 1 mM NADPH, and finally, 0.5 mM **1** and 5.0 mM GSH were incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP, and 5.0 mM MgCl<sub>2</sub>. All control reactions were performed at a final volume of 500 μL for 60 min and included the addition of salicylamide solution as per the active incubation. Each reaction was performed in triplicate.

#### 4.3.9.2 Mushroom Tyrosinase Oxidation **1**<sup>11</sup>

1.0 mM **1**, 0.23 U/μL mushroom tyrosinase, and 5.0 mM glutathione in Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 6.0) at a total volume of 500 μL were vortexed for 60 min at 22°C. Reaction was quenched with perchloric acid, and resulting mixture centrifuged 14,000 rpm for 10 min. Supernatant was analyzed by HPLC and UPLC-MS.

Control reactions were also performed in the absence of GSH, in the absence of mushroom tyrosinase, and with the addition of GSH after the 60 min vortex. Each reaction was performed in triplicate.

#### 4.3.9.3 Ag<sub>2</sub>O Oxidation 1<sup>11</sup>

1.0 mM **1** and 4.1 mM Ag<sub>2</sub>O in acetonitrile at a total volume of 500 µL were incubated at 37°C for 2 h. Resulting mixture was centrifuged at 14,000 rpm for 10 min, and the resulting supernatant was added to GSH, with a final concentration of 5.0 mM (pH 7.4). This solution was analyzed via HPLC.

One control reaction was also performed in the absence of GSH. Each reaction was performed in duplicate.

#### 4.3.9.4 Horseradish Peroxidase Oxidation 1<sup>38</sup>

0.5 mM **1**, 100 µg/mL horseradish peroxidase, and 0.5 mM H<sub>2</sub>O<sub>2</sub> was incubated with 1 mM GSH in 50 mM Na<sub>2</sub>HPO<sub>4</sub> buffer (pH=7.4) at 37°C. One set of data was collected after 60 min and one set after 120 min. Reactions were quenched with 25 µL perchloric acid, and resulting mixture centrifuged at 14,000 rpm for 10 min. Supernatant was analyzed via HPLC. Each of the two experiments with varying lengths was performed in triplicate.

#### 4.3.9.5 Incubation of ASECO in Rat Liver Microsomes<sup>11</sup>

0.5 mM ASECO and 5.0 mM GSH were incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP (rat liver microsomes) and 5.0 mM MgCl<sub>2</sub> to a volume of 500 µL at 37°C for 60 min. Reaction was initiated by addition of 1 mM NADPH and terminated by addition of 50 µL ice cold 2 mM salicylamide in acetonitrile. Reaction was centrifuged at 14,000 rpm for 10 min, and supernatant was analyzed by HPLC and UPLC-MS.

Simultaneously, the following control reactions were also performed: 0.5 mM ASECO was incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP, 5.0 mM MgCl<sub>2</sub> and NADPH; 0.5 mM ASECO was incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with heat inactivated microsomes, 5.0 mM GSH, 5.0 mM MgCl<sub>2</sub>, and NADPH; 0.5 mM ASECO was incubated in 50 mM Na<sub>2</sub>HPO<sub>4</sub> at pH 7.4 with 0.5 mg/mL CYP, 5.0 mM GSH, and 5.0 mM MgCl<sub>2</sub>. All control reactions were performed for 60 min at 37°C and included the addition of salicylamide as per the active incubation. Each reaction was performed in duplicate.

The HPLC method used for all oxidation experiments utilised a mobile phase of 0.1% formic acid in H<sub>2</sub>O “A”, and 0.1% formic acid in acetonitrile “B”, and was developed as follows: 95% A for 3 min, decrease to 80% A over 4 min, isocratic for 3 min, decrease to 65% A over 26

min, decrease to 10% A over 5 min, isocratic for 4 min, increase to 95% A over 5 min, ending run at 50 min. Volume injection was 150  $\mu$ L and flow rate was 1.5 mL/min.

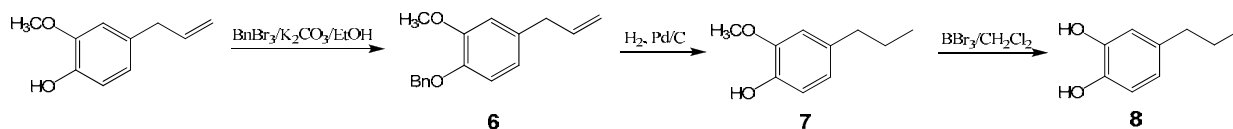
The UPLC-MS method used for oxidation experiments utilised a mobile phase of 1% formic acid in H<sub>2</sub>O “A”, and 1% formic acid in acetonitrile “B”, and was executed as follows: 95% A initially decreasing to 5% A over 2.60 min, increasing to 95% over 2.08 min, isocratic for 1.32 min ending run at 6 min. Volume injection was 10  $\mu$ L and flow rate was 0.600 mL/min. All HPLC and UPLC chromatograms were extracted at  $\lambda=280$  nm.

## 5.0 RESULTS AND DISCUSSION

The preparation of the lignan analogues for this study was based on the use of consecutive Stobbe condensation reactions<sup>22</sup>. The benzaldehyde, or benzaldehyde derivative, introduced the aromatic functionality, and diethyl succinate provided the alkyl chain of the lignan. Employment of this strategy allowed for the preparation of lignan analogues possessing different substituents on the aromatic portions, thus providing a general route to existing and novel lignans. Many of the reactions performed in the synthesis of the lignan analogues described in this thesis were carried out several times. Although small changes were made in an effort to improve yield and purity, no systematic optimization of the reaction conditions was carried out as the goal of the study was to prepare lignan analogues for *in vitro* metabolic studies.

### 5.1 Pilot Reactions: Eugenol

In order to assess the potential reactivity of the four analogues, a number of pilot reactions were performed using eugenol, specifically the addition and removal of protecting groups to be used during the synthesis of analogues **1** through **4** (Scheme 5-1). The benzylation and hydrogenation of eugenol proceeded as expected; <sup>1</sup>H NMR was consistent with the addition of a benzyl protecting group to the aromatic hydroxyl group of eugenol following the reaction with benzyl bromide. Hydrogenation of **6** resulted in removal of the benzyl protecting group along with saturation of the aliphatic double bond (**7**) as indicated by <sup>1</sup>H NMR. Finally, removal of the methyl ether by way of BBr<sub>3</sub> gave the final desired catechol **8**, as seen by <sup>1</sup>H NMR. Demethylation of similar compounds has previously been performed using either (CH<sub>3</sub>)<sub>2</sub>S·BBr<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> or BBr<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub><sup>14</sup>. In this case, demethylation of the aromatic hydroxyl group was originally attempted using the less reactive (CH<sub>3</sub>)<sub>2</sub>S·BBr<sub>3</sub>, but with limited success. The yield of this reaction was found to be 28%, in comparison to the much higher 97% yield obtained using BBr<sub>3</sub> for the same reaction. Based on these results, subsequent demethylation reactions were carried out using BBr<sub>3</sub>.

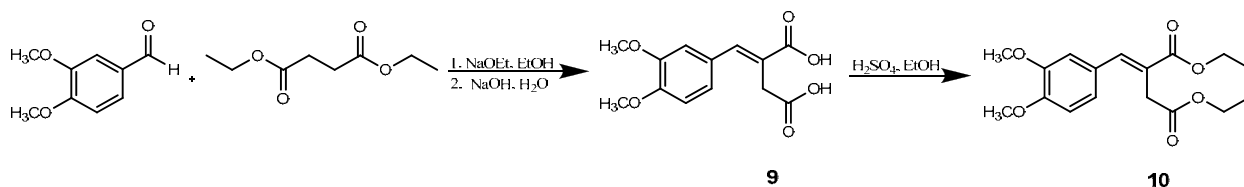


Scheme 5-1: Pilot Reactions As Performed with Eugenol

## 5.2 Synthesis of Analogue 1

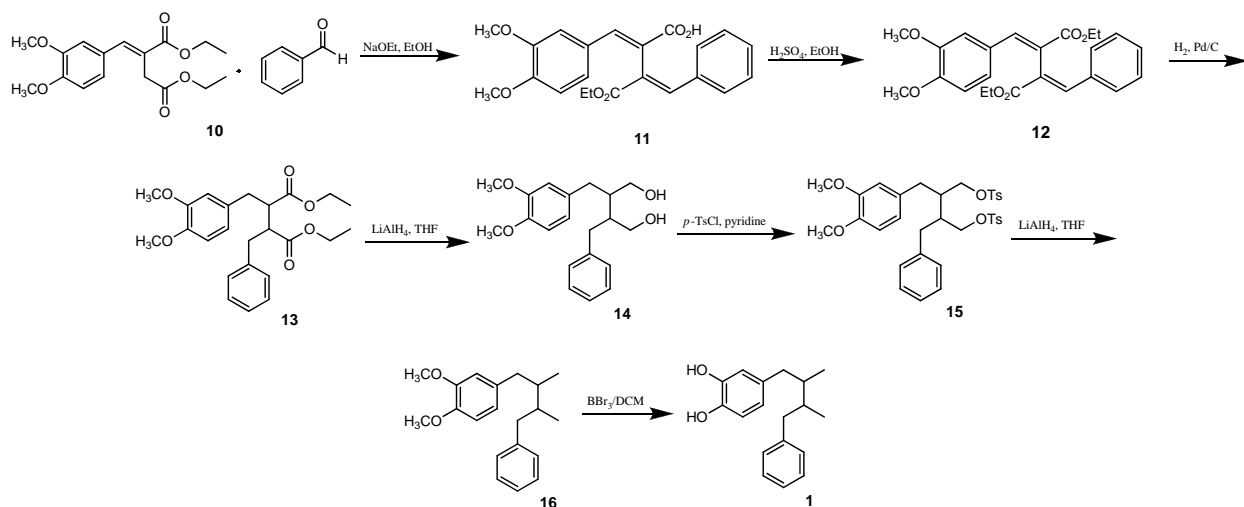
The first Stobbe condensation reaction was carried out in order to prepare the dimethoxy phenyl intermediate **9** which would become a component of both analogues **1** and **4** (Scheme 5-2). This reaction was successfully performed on five different amounts, ranging from 1 g of starting material to 5 g of starting material. The highest yield (62%) was performed using 5 g of starting material. It was anticipated that the benzylic proton of the desired alkene would serve as a diagnostic for the formation of the first Stobbe condensation product.  $^1\text{H}$  NMR showed the presence of a benzylic position proton attached to an  $\text{sp}^2$  hybridized carbon atom at 7.84 ppm, which was consistent with the expected alkene.

Esterification of the first Stobbe di-carboxylic acid for the synthesis of analogue **1** (Scheme 5-2) was successfully performed in amounts ranging from 0.4 g to 5 g. Yields of about 70% were achieved at smaller scale reactions, but the highest yield (95%) was achieved when the reaction was performed using approximately 5 g of starting material.  $^1\text{H}$  NMR data showed the presence of the ethyl groups (1.24, 1.30, 4.14, 4.23 ppm), giving the desired diester.



Scheme 5-2: Partial Synthesis of Analogues 1 and 4

The second Stobbe condensation reaction (Scheme 5-3), giving an ethyl ester-carboxylic acid containing product, was performed on amounts ranging from 0.2 g to 2.4 g. Yields ranged from 58% to 95%, with amounts of 1.4 g of starting material and 2.4 g of starting material giving 95% yield. NMR experiments showed the addition of the second aromatic ring, along with the presence of a proton attached to the  $\text{sp}^2$  hybridized carbon atom at the benzylic position of the second ring (7.88, 7.92 ppm). Only one ethyl ester group was present in the NMR spectrum (1.13, 4.15 ppm) suggesting that the predicted carboxylic acid **11** was produced.



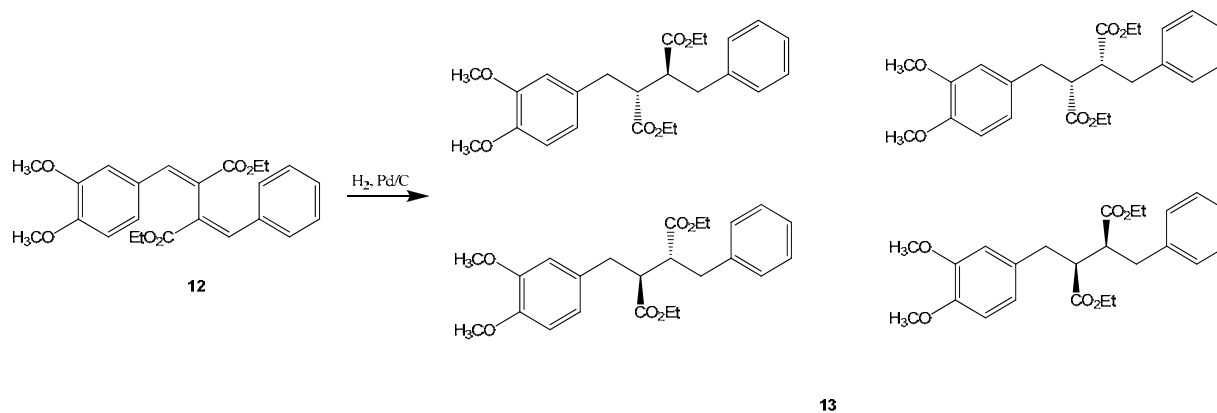
Scheme 5-3: Synthesis of Analogue 1

Esterification of the carboxylic acid produced from the second Stobbe condensation reaction was performed on amounts ranging from 0.20 g to 3.3 g. Yields ranged from 47% to 95%, with the yield of 95% performed on 1.8 g of starting material. NMR showed the presence of two ethyl ester groups following this esterification reaction (1.10, 1.15, 4.16 ppm; 10 H).

The use of two Stobbe condensation reactions was ultimately found to be a successful method for producing the basic lignan skeleton. The configuration of the two double bonds that were formed during the synthesis of **1** was not investigated. However, the  $^1\text{H}$  NMR shifts of the two protons attached to the  $\text{sp}^2$  hybridized carbon atoms of the double bonds were within less than 0.1 ppm of each other. This is consistent with the double bonds being of the same configuration<sup>25</sup>. Furthermore, the chemical shift of these olefinic protons were found to agree with literature values for similar lignan-like compounds reported as having an *E,E* configuration<sup>19, 25, 33, 39</sup>, which are typically reported as being downfield of *Z* olefinic protons in similar compounds.

Hydrogenation of the two double bonds following esterification was performed in amounts ranging from 0.2 g to 1.8 g. Yields were consistently in the range of 95-98% for all reactions. The absence of the diagnostic downfield  $\text{sp}^2$  benzylic protons, in addition to the presence of a further six protons upfield indicated the hydrogenation was successful. Hydrogenation of the diene diester **12** was expected to give four stereoisomers consisting of two pairs of enantiomers (Scheme 5-4). It was expected that some separation of the stereoisomers via traditional chromatographic techniques would be possible, however, no separation of the stereoisomers of **13** was observed.





Scheme 5-4: Hydrogenation of **12** and Potential Stereoisomers

Attempts to selectively synthesize one specific stereoisomer at the point of hydrogenation were beyond the scope of this project. The use of a chiral catalyst could however allow for the production of only one stereoisomer at this stage<sup>40</sup>. It has been reported that some rhodium and ruthenium complexes with chiral phosphines, such as DIPAMP and BINAP are effective for the purpose of enantioselective hydrogenation of alkenyl carboxylates, such as **10**<sup>40</sup>. It is unclear what effect if any two such reactive sites in one compound, such as **12**, would have on the asymmetric hydrogenation reaction.

LiAlH<sub>4</sub> mediated reduction of the diester to the diol was performed in amounts ranging from 0.4 g to 0.8 g. Yields ranged from 26% to 78%, with the highest yield performed at the scale of 0.8 g. The absence of both ethyl ester groups along with the addition of four protons consistent with attachment to carbon atoms adjacent to hydroxyl groups (3.50 ppm) suggests the desired diol was produced. Silica gel flash column chromatography resulted in a small degree of stereoisomer separation, which was anticipated to be due to two pairs of racemic mixtures, as indicated in Scheme 5-4. Subsequent analysis using reverse phase HPLC showed two product peaks that were not baseline separated, thus maintaining a significant amount of overlap. Further attempts to separate the stereoisomers were not carried out at this stage. Since the lignan diol is an analogue to the flaxseed lignan secoisolariciresinol, a small amount of the diol was maintained for future studies on secoisolariciresinol analogues.

This isomeric mixture then underwent tosylation of the diol to give **15**. Addition of tosyl groups to the aliphatic hydroxyl groups was performed in amounts ranging from 0.29 g to 0.6 g. Yields ranged from 16% to 40%, with the highest yield of 40% performed at the scale of 0.6 g. The addition of two tosyl groups to the diol is seen in the <sup>1</sup>H NMR by the presence of eight more

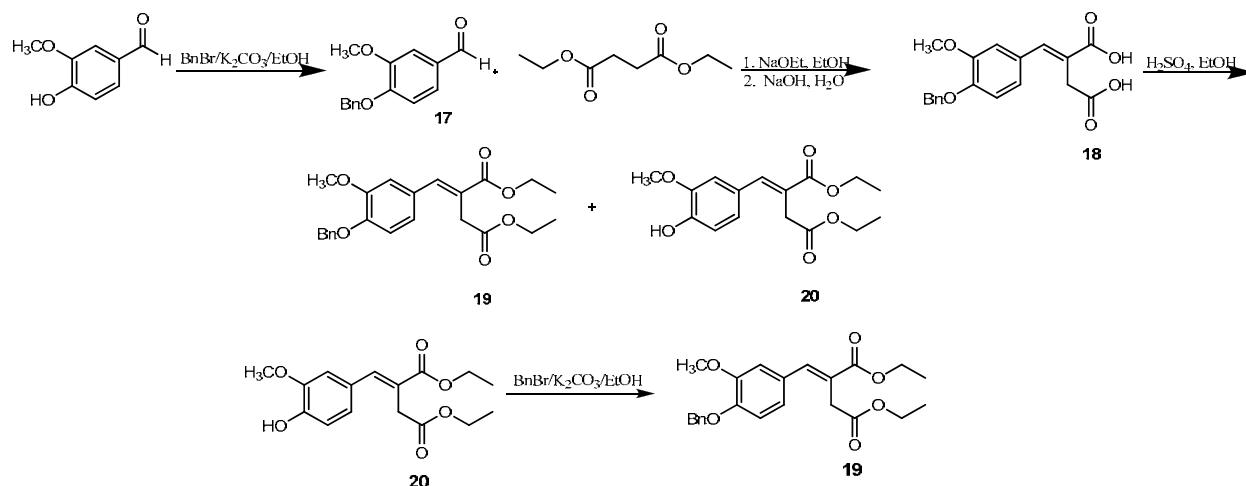
protons in the aromatic region, and six protons present (2.47 ppm) as the aromatic methyl group of the tosyl group. It is notable that following chromatographic purification, the tosylated product was found to crystallize within the mobile phase. The white, needle-like crystals were subsequently found to precipitate out of a number of solvent systems readily, including petroleum ether, hexane, and ethyl acetate mixtures.  $^1\text{H}$  NMR data suggest that the crystals represent one of the expected racemic mixtures. This stereochemical resolution was not anticipated, but may provide a general method for separation of stereoisomers for other lignan analogues. Whether this is a single enantiomer or a single racemic mixture is not known, and attempts are currently underway through a collaborator to determine the crystal structure of this tosylated product. For the subsequent steps in the synthesis of analogue **1**, only these isolated crystals were used.

Removal of the tosylate via  $\text{LiAlH}_4$  was performed at 0.7 g and 0.09 g, with yields of 95% and 90% respectively. The absence of the aromatic protons attributed to the tosylate groups, along with the presence of the aliphatic methyl groups on the connecting carbon chain suggested the tosylate groups had been successfully removed.

The final step required removal of the methyl groups from the aromatic hydroxyl groups. This was performed at the scales of 0.04 g and 0.3 g, with yields of 87% and 92% respectively.  $^1\text{H}$  NMR showed the absence of both methyl ether groups indicating that the deprotection was successful. UPLC-MS data was consistent with the preparation of analogue **1**. In summary, analogue **1** was successfully synthesized in quantities large enough to continue with the desired oxidation reactions.

### **5.3 Attempted Synthesis of Analogue 2**

The first step in the synthesis of analogue **2** required the preparation of 4-benzyl-3-methoxy benzaldehyde, an intermediate that is also required for the preparation of analogue **3**. Below is a discussion of the results for the preparation of analogue **2**, although some of the initial Stobbe condensation product was reserved for use in the preparation of analogue **3** (Scheme 5-5).

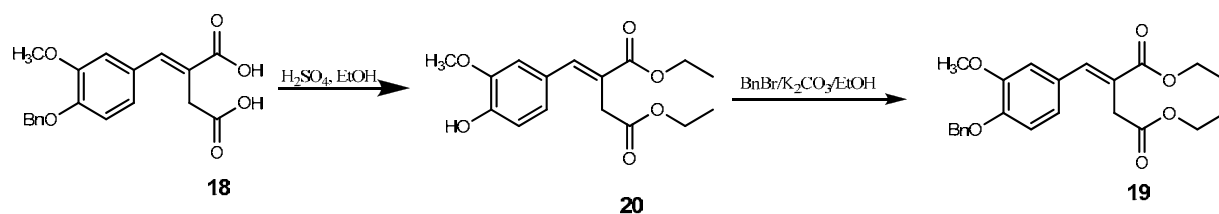


Scheme 5-5: Partial Synthesis of Analogues 2 and 3

The protected benzaldehyde **17** was prepared by the addition of the benzyl protecting group to the aromatic hydroxyl group of vanillin in amounts ranging from 6.3 g to 10 g. Yields of 72% to 78% were attained, with the largest yield of 78% achieved at the 6.6 g scale.  $^1\text{H}$  NMR of the final compound showed the addition of five extra aromatic protons, along with the two extra benzylic protons (5.18 ppm).

The first Stobbe condensation reaction in the synthesis of analogues **2** and **3** was performed in amounts ranging from 2.7 g to 9.5 g. Yields ranged from 63% to 95%, with the yield of 95% achieved at the scale of 6.5 g of starting material.  $^1\text{H}$  NMR showed the presence of the diagnostic benzylic proton attached to an  $\text{sp}^2$  hybridized carbon atom at 7.85 ppm, which was consistent with the expected alkene.

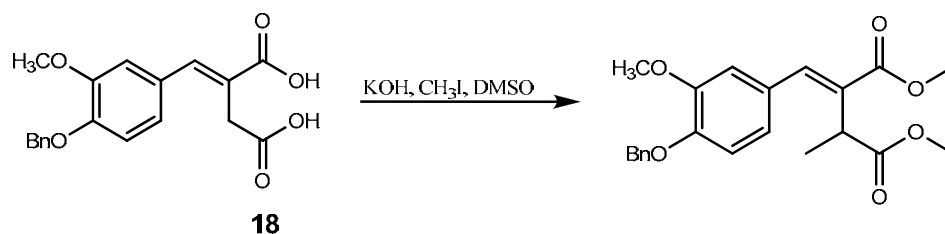
Esterification of the first Stobbe dicarboxylic acid in the synthesis of analogues **2** and **3** proceeded in amounts ranging from 3.79 g to 11.9 g. It was predicted that loss of the benzyl protecting group may also result during the acid-catalyzed esterification of **18**. This was found to be the case, and both the desired diester **19** and the de-benzylated product **20** were isolated from the reaction mixture (Scheme 5-6). Total yield of both products from the acid-catalyzed esterification ranged from 59% to 73%, with the yield of 73% achieved at the scale of 11.9 g of starting material. Benzylation of **20** following the esterification also gave **19** at a yield of 86% when starting with 4.6 g of starting material (Scheme 5-6).  $^1\text{H}$  NMR showed the presence of two ethyl ester groups following esterification (1.27, 1.34, 4.19, 4.28 ppm), and five additional aromatic protons when the benzyl protecting group was present.



Scheme 5-6: Debenzylation of 18 and Subsequent Benzylation of 20

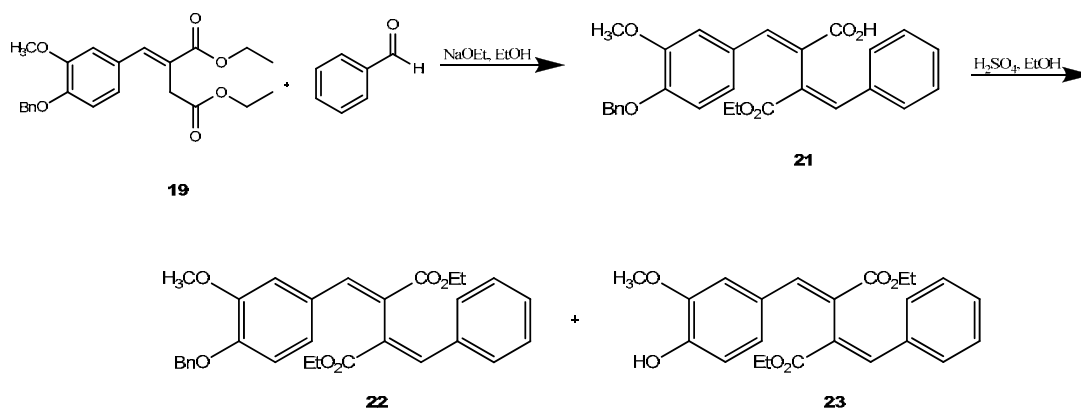
During the scale-up of the synthesis of analogue **2**, a base-catalyzed esterification reaction was attempted for the conversion of the first Stobbe condensation product **18** to the diester (Scheme 5-7). This reaction was not expected to result in the loss of the benzyl protecting group, and was assessed as a faster reaction with a generally higher yield in comparison to the acid-catalyzed esterification which would not require the subsequent re-benzylation step.

It was found after purification of the major product that the esterification of the two carboxylic acid groups was successful, but an additional methylation reaction took place at one of the  $\alpha$  carbons. The protons on the saturated  $\alpha$ -carbon are sufficiently acidic to result in proton abstraction, likely after ester formation has occurred. The carbanion would then undergo a substitution reaction with iodomethane to form the methylated intermediate. Unfortunately this product could not be used in the synthesis of analogue **2**, but was deemed to be potentially useful for the preparation of lignan analogues to SECO and NDGA containing only one aromatic ring. Analogues of this nature are of interest to the lab research program and thus the intermediate was kept for future use. As a result of this side-reaction, further attempts to use the base-catalyzed esterification with iodomethane at that particular stage of the reaction were not pursued. However, it may be that carrying out the reaction under less basic conditions, for example using potassium carbonate in place of KOH, may avoid removal of the acidic  $\alpha$ -carbon and provide a facile route to the esterified intermediate. Ultimately, the acid-catalyzed esterification coupled with the extra benzylation step was left in the reaction scheme.



Scheme 5-7: Attempted Base-Catalyzed Esterification of 18 and Resulting Major Product

The second Stobbe condensation reaction for synthesizing analogue **2** was performed in amounts ranging from 1.8 g to 5.4 g of starting materials (Scheme 5-8). Reaction yields ranged from 60% to 97%, with the 97% yield obtained at the scale of 5.4 g. NMR experiments showed the addition of the second aromatic ring, along with the presence of a proton attached to the  $sp^2$  hybridized carbon atom at the benzylic position of the second ring (7.84, 7.91 ppm). Only one ethyl ester group was present in the NMR spectrum (1.12, 4.13 ppm) consistent with the formation of the predicted carboxylic acid.

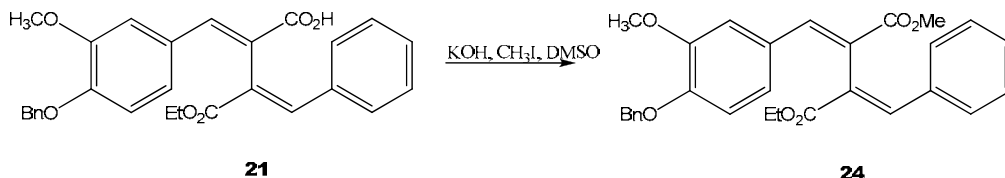


Scheme 5-8: Synthesis of Analogue 2 - Compounds **21**, **22** and **23**

Acid catalyzed esterification of the carboxylic acid produced during the second Stobbe condensation in the synthesis of analogue **2** gave the benzyl protected diester **22**, as well as the de-benzylated diester **23**. The reaction was performed with 1.32 g and 3.76 g of starting material. Total yields at these amounts of **22** and **23** combined were 48% and 20% respectively.  $^1\text{H}$  NMR of **22** showed the addition of the second ethyl ester following the reaction ( $\delta$  1.11, 6H; 4.17, 4H); compound **23** also showed the addition of the second ethyl ester, along with the absence of the benzyl protecting group present in **22**. Given that the following step, hydrogenation, would result in the loss of the benzyl protecting group, the mixture of compounds **22** and **23** were put forward together.

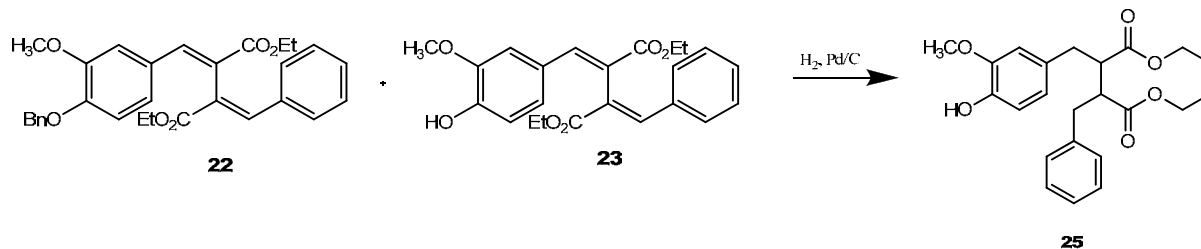
As a result of the continued low yields in the acid-catalyzed esterification of **21**, a base-catalyzed esterification reaction of the carboxylic acid with iodomethane as the methyl source was attempted, and was found to be more efficient in that it was a faster reaction, and resulted in a higher yield (Scheme 5-9). The base catalyzed esterification was performed in amounts ranging from 4.26 g to 6.51 g, with the highest yield produced at the 4.26 g scale at a value of 59%. The lowest yield at 6.51 g was found to be 29%.  $^1\text{H}$  NMR showed the addition of the

methyl ester group ( $\delta$  3.67, 3H) indicating that the desired product had formed. The base-catalyzed esterification had the advantage of the benzyl protecting group remaining intact, which allowed for purification to take place on only one compound (**24**), rather than a mixture of the benzylated **24** and the de-benzylated diester **23**.



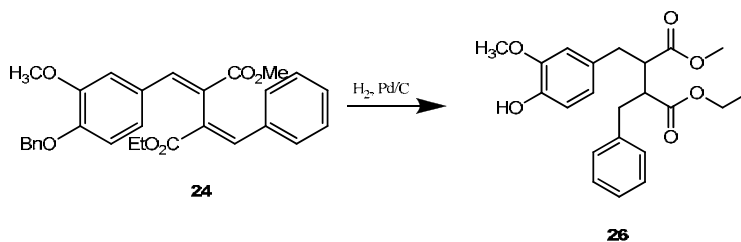
Scheme 5-9: Synthesis of Analogue 2 - Compound 24

Hydrogenation of the diester products was anticipated to result in conversion to an alkene and hydrogenolysis of the benzyl protecting group (Scheme 5-10). Hydrogenation of the acid catalyzed diethyl diester products **22** and **23** was performed twice in the combined amounts of 0.65 g and 1.13 g. Both reactions gave yields of 99%, and the final desired product **25** was indicated by the absence of the protons attached to the  $sp^2$  hybridized carbon atoms present in the diene entity, as well as the absence of the benzyl protecting group present in compound **22**.



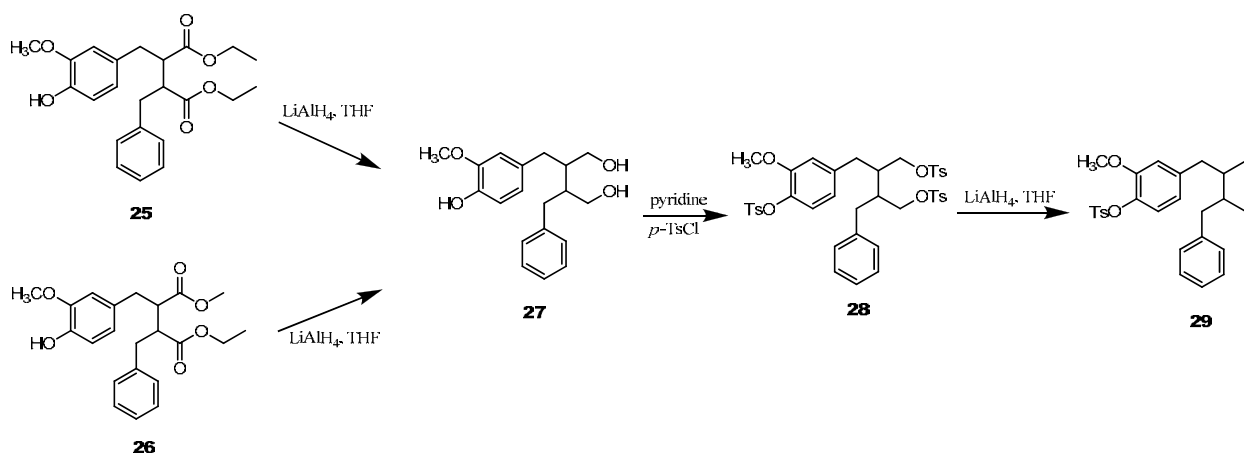
Scheme 5-10: Synthesis of Analogue 2 - Compound 25

Hydrogenation of the base catalyzed ethyl-methyl diester product **24** was performed twice, both times at a scale of 2.03 g (Scheme 5-11). Yields were 99% for both reactions.  $^1\text{H}$  NMR again showed the loss of the distinctive downfield protons attached to the  $sp^2$  hybridized carbon atoms present in the diene **24**, as well as the absence of the benzyl protecting group also present in compound **24**. As was the case with analogue **1**, no separation of the four expected stereoisomers via traditional chromatographic techniques was seen following the hydrogenation of the diene entities to compounds **25** or **26**.



Scheme 5-11: Synthesis of Analogue 2 - Compound 26

Reduction of both esters **25** and **26** with  $\text{LiAlH}_4$  gave diol **27** as indicated by the absence of the ester groups in the  $^1\text{H}$  NMR of the final product (Scheme 5-12). The diethyl ester **25** was reduced to the diol twice at amounts of 0.66 g and 0.83 g, with yields of 46% and 25% respectively. The ethyl-methyl ester **26** was reduced to the desired diol twice at amounts of 1.42 g and 1.39 g. Yields for these reactions were 23% and 42% respectively. As was the case for analogue **1**, the mixture of stereoisomeric diols (**27**) could not be adequately separated via flash column chromatography and the mixture was carried forward for subsequent reactions.



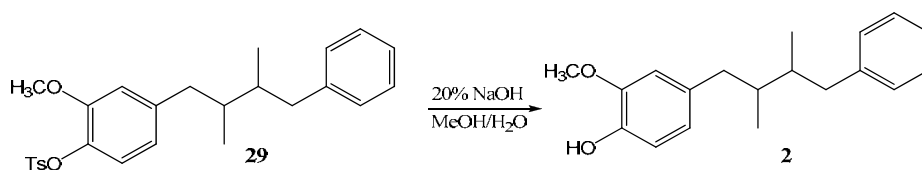
Scheme 5-12: Synthesis of Analogue 2 - Compounds 27, 28, and 29

Tosylation of the hydroxyl groups of **27** (three in total) was performed in amounts of 0.26 g and 0.49 g, with yields of 30% and 36 % respectively. The formation of the tosylated lignan once again resulted in the formation of a precipitate that allowed for the separation of what appeared to be one pair of enantiomers. This assessment was made based mainly on  $^1\text{H}$  NMR evidence. The mixture obtained prior to the flash column contained what appears to be two sets of aliphatic  $^1\text{H}$  NMR signals that are consistent with the aliphatic portion of the lignan; however the purified white solid contains only one of these sets of signals.

Reduction of the aliphatic tosylate groups using  $\text{LiAlH}_4$  was performed once in the amount of 53.7 mg of starting material giving a yield of 72%.  $^1\text{H}$  NMR confirmed the loss of

two tosylate groups from **28**, showing the expected aliphatic methyl groups, but indicated the aromatic tosylate group remained, as shown by the expected four aromatic and three methyl protons. A second attempted detosylation reaction performed at the scale of approximately 440 mg was unsuccessful as a large number of side products were formed during this reaction.  $^1\text{H}$  NMR spectra obtained from the results of this reaction showed signals, integrals and chemical shifts with both starting material and desired product. Virtually no usable product was isolated.

One attempt was made to remove the aromatic tosylate group (Scheme 5-13), which would have given the final compound **2**. This was performed by refluxing approximately 20 mg of **29** in 20% NaOH solution and methanol<sup>37</sup>.  $^1\text{H}$  NMR of the crude product showed that the tosylate group had been successfully removed from the aromatic ring. It was expected that one signal would be present at approximately 3.8 ppm with an integral of 3 corresponding to the aromatic methyl ether present in compound **2**. It was found, however, that two signals were present at 3.85 ppm and 3.89 ppm, both integrating to approximately 2.



Scheme 5-13: Attempted Synthesis of Final Product Analogue 2

TLC showed what appeared to be two products. An attempted separation of the two products was performed via PTLC.  $^1\text{H}$  NMR of the two separated products showed some degree of purification; in particular, one spectrum showed a higher intensity signal at 3.85 ppm compared to 3.89 ppm, and the other spectrum showed a higher intensity signal at 3.89 ppm compared to 3.85 ppm. It was also noted that the one of the two separated products (lower  $R_f$ ) turned yellow prior to being scraped off the PTLC plate.

On comparing the  $^1\text{H}$  NMR spectrum of the crude product with compound **16**, the dimethoxy compound in the synthesis of **1**, it was found that they were virtually identical. In particular, the two aromatic methyl ether signals of compound **16** were found at 3.85 ppm and 3.89 ppm in the  $^1\text{H}$  NMR spectrum. Based on this evidence, it was theorized that an intramolecular methyl transfer reaction had potentially taken place, and the final product of the attempted detosylation reaction was a mixture of methoxy phenol products (Figure 5-1). It was also thought that the compound possessing the lower  $R_f$  value was likely the *para* phenol due to



the observation that quinones often turn yellow on oxidation (personal communication, E.S. Krol)

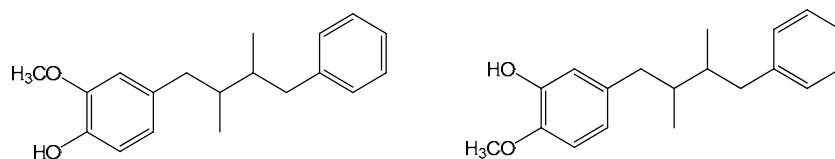
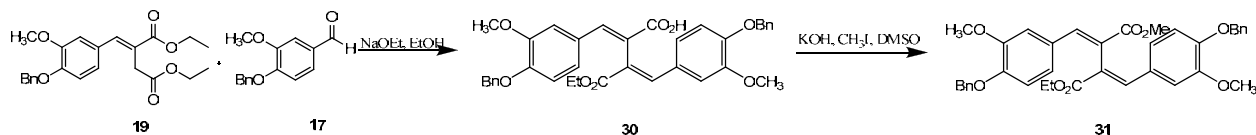


Figure 5-1: Potential Product Mixture following Attempted Detosylation of 29

It is unclear exactly what caused this unusual result. Following the unsuccessful second attempt to remove the alkyl tosylate groups with  $\text{LiAlH}_4$ , it was determined that further investigation was warranted, and the solutions to these issues were likely beyond the scope of this project. One possible future direction for solving the problem of removing the aromatic tosylate could involve adding a tosyl group to a eugenol analogue such as compound **7** and attempting to remove it using NaOH solution and methanol as per literature procedures.

#### 5.4 Attempted Synthesis of Analogue 3

The second Stobbe condensation for the synthesis of **3** was performed once at the scale of 2.62 g of starting material **19**, giving a yield of 41% (Scheme 5-14).  $^1\text{H}$  NMR showed the addition of the second benzaldehyde unit, including the eight additional protons found in the aromatic region of the spectrum, and the integration of the signal corresponding to the protons attached to the  $\text{sp}^2$  hybridized carbon atoms as two protons ( $\delta$  7.76).



Scheme 5-14: Partial Synthesis of Analogue 3

The carboxylic acid **30** produced from the second Stobbe condensation reaction in the synthesis of analogue **3** was found to be highly insoluble in most common solvents, including ethanol. This made the acid-catalyzed esterification step performed on analogues **1** and **2** essentially impossible, and another method was required to produce the ester. After determining that the acid was soluble in DMSO, which was utilized for obtaining  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, a second esterification reaction was identified in the literature as potentially useful<sup>36</sup>. This reaction was performed in DMSO, along with KOH and  $\text{CH}_3\text{I}$ . This method would give the methyl ester as opposed to the ethyl ester, but the diol produced following hydrogenation and  $\text{LiAlH}_4$

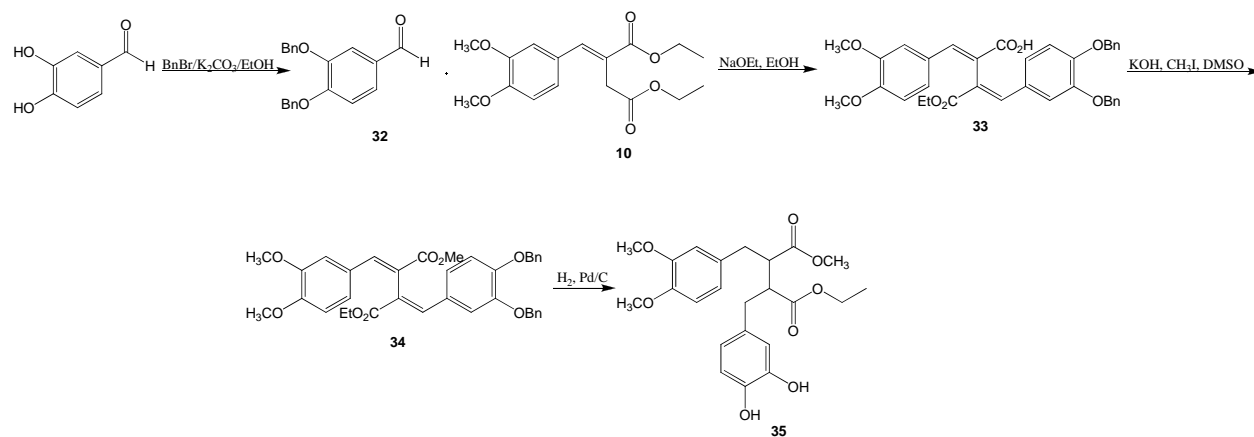
reduction of the diester would be identical. The base-catalyzed esterification of the carboxylic acid was performed once in the amount of 168 mg and produced a higher yield (96%) with fewer impurities and a shorter reaction time when compared to its analogue **1** and **2** counterparts.  $^1\text{H}$  NMR showed the successful esterification of the carboxylic acid by the addition of the methyl group at  $\delta$  3.70. The base-catalyzed esterification was incorporated into the schemes for the synthesis of analogues **1** and **2** during scale-up of these products and has been outlined in previous sections.

$^1\text{H}$  NMR of compounds **30** and **31** showed only one signal integrated to two protons, corresponding to the olefinic hydrogen atoms. Despite the presence of the carboxylic acid group in compound **30**, this is likely due to the symmetrical nature of analogue **3**. Given that it has been reported in the literature that protons in similar compounds (i.e. alkenes or dienes that are the result of a single or double Stobbe condensation reaction) show significantly different chemical shifts in an *E* configuration versus a *Z* configuration<sup>25, 33, 39</sup>, it is expected that these two double bonds have the same configuration.

Due to time constraints, analogue **3** was never pursued beyond this diester entity.

## 5.5 Attempted Synthesis of Analogue 4

The benzylation of dihydroxybenzaldehyde was performed once in the amount of 5.03 g of starting material (Scheme 5-15). The final yield was found to be 11%.  $^1\text{H}$  NMR showed ten extra signals in the aromatic region of the spectrum indicating the reaction was successfully performed.



Scheme 5-15: Partial Synthesis of Analogue 4

The second Stobbe condensation reaction in the synthesis of analogue **4** was performed once with 1.30 g of benzaldehyde. The yield for this reaction was found to be 53%. <sup>1</sup>H NMR showed the expected carboxylic acid from a Stobbe condensation reaction, including the two protons attached to the sp<sup>2</sup> hybridized carbon atoms (δ 7.40).

The carboxylic acid **33** formed during the second Stobbe condensation reaction in the synthesis of analogue **4** was found to be as equally insoluble as its analogue **3** counterpart. Following the success of the base-catalyzed esterification of **30** performed in DMSO in the synthesis of analogue **3**, the base-catalyzed esterification of **33** was performed once on a scale of 1.01 g of starting material, giving a yield of 56%. <sup>1</sup>H NMR showed the addition of the methyl group to the carboxyl group at δ 3.89.

Hydrogenation of the diene diester entity **34** was performed once with 0.56 g of starting material. The final yield of this reaction was found to be 98%. The loss of the downfield sp<sup>2</sup>-attached proton signals, along with the absence of the ten aromatic region protons indicative of benzyl protecting groups in the <sup>1</sup>H NMR indicated the reaction was performed successfully. No attempts were made to separate the stereoisomers that presumably formed during the reaction. Due to time limitations, the synthesis of analogue **4** was not pursued beyond the hydrogenation step.

## **5.6 Incubation of 1 in Rat Liver Microsomes**

The incubation of analogue **1** in rat liver microsomes was performed on two separate occasions. The first set of experiments was analyzed by HPLC only. Analysis of the resulting data showed that analogue **1** eluted at R<sub>t</sub>=42.4 min, and salicylamide was found to elute at R<sub>t</sub>=11.3 min. The presence of a small amount of impurity present in the analogue **1** sample, which eluted at R<sub>t</sub>=43.2 min, was also observed.

Given that the di-catechol NDGA autoxidizes at pH 7.4 over the one hour incubation time in the presence of active microsomes in phosphate buffer alone<sup>11</sup>, the possibility of similar autoxidation activity in the mono-catechol **1** was not discounted. The fact that NDGA autoxidizes faster than a number of other catechols, such as catechol and dopamine<sup>11</sup>, does suggest however that autoxidation rates would be significantly lower for analogue **1**.

In the absence of active microsomes, no product formation occurred (Figure 5-2), and there was no apparent decrease in the area of the HPLC peak for **1**. This was the case in only

buffer, in the presence of heat inactivated microsomes, and in the absence of NADPH. The absence of autoxidation implies that rapid autoxidation of a lignan such as NDGA requires substitution on both aromatic rings.

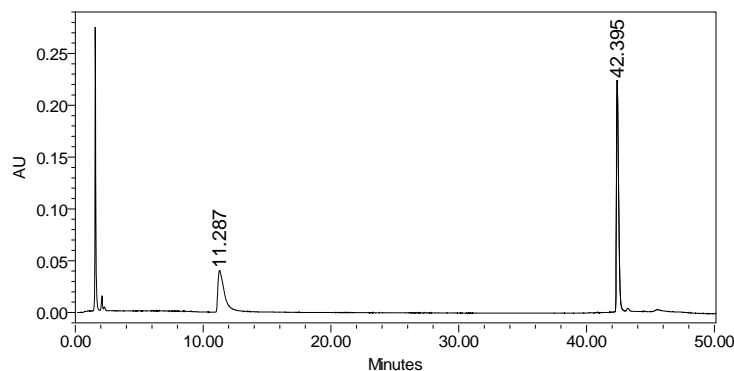


Figure 5-2: HPLC-UV (280 nm) Chromatogram of **1** Following 1 h Incubation in the Absence of Active Microsomes

The incubation of analogue **1** in active microsomes was expected to result in the formation of an *o*-quinone. In the presence of glutathione, it follows that the electrophilic *o*-quinone would be trapped by the nucleophilic tripeptide.

Incubation of **1** without GSH resulted in the formation of one product eluting at  $R_t=40.7$  min with the analytical method developed for the Waters Alliance HPLC system (Figure 5-3). Incubation in the presence of GSH also gave the 40.7 min product, as well as another product eluting at  $R_t=35.4$  min (Figure 5-4). The major component of the mixture following the incubation experiments continued to be analogue **1**, but of the two products formed, the 35.4 min peak was estimated to be present in larger amounts based on the absorbance units measured by the HPLC.

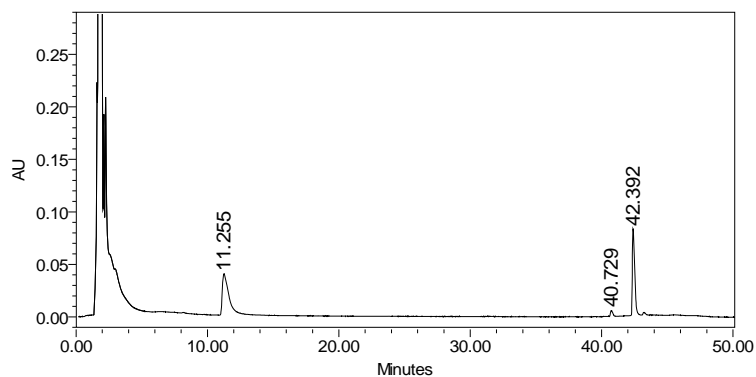


Figure 5-3: HPLC-UV (280 nm) Chromatogram of **1** Following 1 h Incubation in the Presence of Active Microsomes; no GSH.

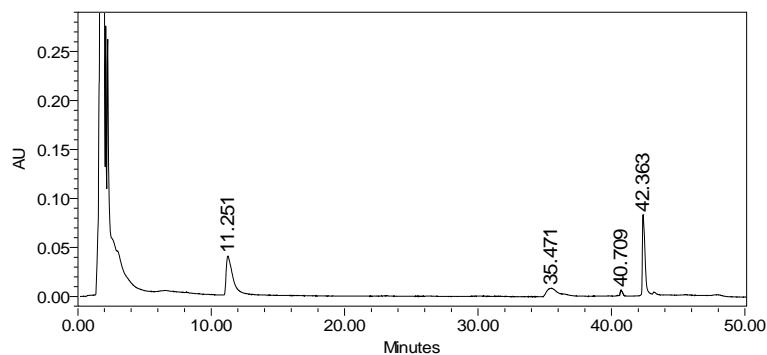


Figure 5-4: HPLC-UV (280 nm) Chromatogram of **1** Following 1 h Incubation in the Presence of Active Microsomes plus GSH

The second set of incubation data obtained was analyzed by HPLC and by UPLC-ESI-MS(-). When the incubation performed in the presence of GSH was analyzed via UPLC-MS, the product peaks were found to elute at 1.2 min with  $m/z$  879.2, and 1.5 min with  $m/z$  574.2. The experimental mass of 574.2 corresponds to analogue **1** plus one unit of GSH. This result is unexpected as this product was observed in the absence of GSH. It is possible, however, that some endogenous GSH was present in the isolated rat liver microsomes. The experimental mass of 879.2 corresponds to analogue **1** plus two units of GSH.

The addition of GSH to analogue **1** suggests the formation of an electrophilic entity, such as an *o*-quinone, via oxidation by P450 enzymes present in the rat liver microsomes.

### 5.7 Oxidation of **1** with Mushroom Tyrosinase

The oxidation of analogue **1** using mushroom tyrosinase was expected to result in the formation of the *o*-quinone form of the catechol<sup>11, 12, 14</sup>. Addition of GSH to the oxidation reaction would result in the *o*-quinone being trapped with GSH. Similar experiments performed on NDGA resulted in the formation of the *o*-quinone, and three different NDGA-GSH adducts were isolated and identified<sup>11</sup>.

The oxidation experiment of **1** with mushroom tyrosinase in the absence of glutathione showed no product formation. In the presence of glutathione, added at  $t=0$  min of the reaction, and at  $t=60$  min of the reaction, three product peaks were observed via HPLC as follows:  $R_t=34.9$  min,  $R_t=40.3$  min, and  $R_t=41.0$  min (Figure 5-5).

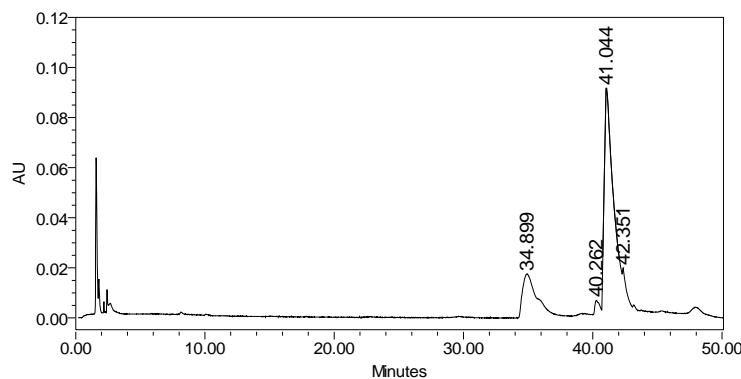


Figure 5-5: Oxidation of **1** with Mushroom Tyrosinase; GSH Present

The major product was found by UPLC-ESI-MS(-) to have a  $m/z$  574.1 eluting at 1.5 min. This corresponds to analogue **1** plus one GSH, and also corresponds to one of the products formed during the incubation of **1** in rat liver microsomes.

One of the minor products formed in the oxidation of **1** via mushroom tyrosinase was found to elute at 1.2 min with  $m/z$  879.2. This corresponds to **1** plus two GSH, and is consistent with the product formed during the incubation of **1** in rat liver microsomes. A third product was formed during the tyrosinase oxidation which eluted at 1.4 min with  $m/z$  731.3. Based on the recorded UV absorbance units, it appears that only a small amount of this product formed. The mass does not correspond to any of the products formed during the microsomal incubation.

It appears that the major product  $m/z$  574.2 is formed in sufficient quantities to allow for the collection of this product, however it is unlikely that mushroom tyrosinase oxidation would allow for the collection of the secondary product  $m/z$  879.2. Investigation into the structure of this **1**-GSH adduct via NMR may provide more information as to the nature of the electrophilic moiety formed on mushroom tyrosinase and P450-mediated oxidation. The position at which the GSH bonds with the compound could potentially confirm the formation of the *o*-quinone.

## 5.8 Oxidation of **1** with Silver Oxide

The oxidation of analogue **1** with silver oxide was predicted to give a *p*-QM, as is commonly seen in catechols<sup>12, 14, 38, 41</sup>. In the case of NDGA, Ag<sub>2</sub>O failed to produce the *p*-QM, and no GSH reactive products were ever isolated. The oxidation of analogue **1** with Ag<sub>2</sub>O both in the absence (Figure 5-6) and presence (Figure 5-7) of GSH showed multiple product peaks via HPLC. The major product in the presence of GSH appears to be consistent with the major peak

formed during oxidation with mushroom tyrosinase, which was ultimately found to correlate with **1**-GSH. Given the multiple product peaks that were present which did not correspond to peaks present in either the mushroom tyrosinase oxidation, or the microsomal incubation, the oxidation of **1** via Ag<sub>2</sub>O was not pursued any further.

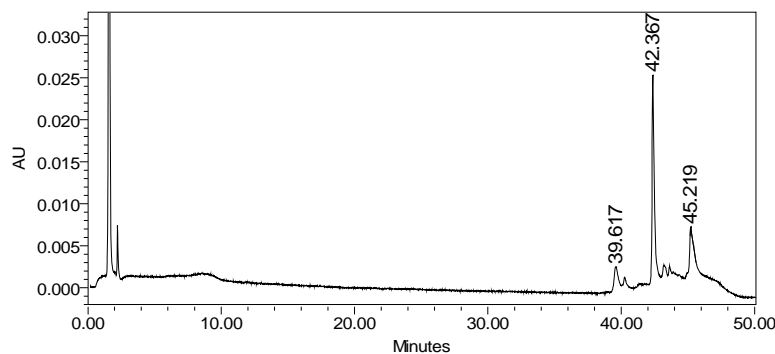


Figure 5-6: Ag<sub>2</sub>O Oxidation of **1** in the Absence of GSH

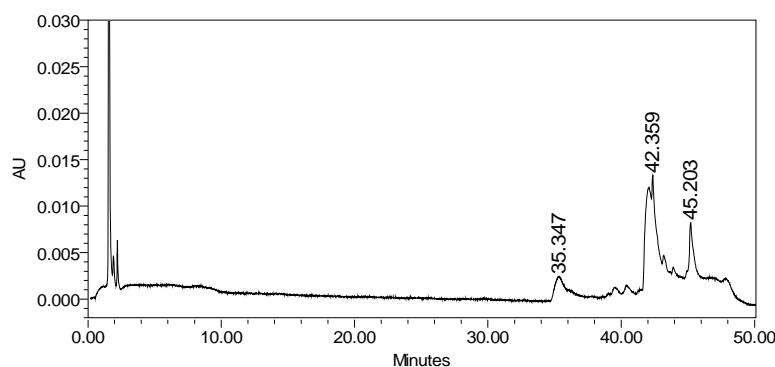


Figure 5-7: Ag<sub>2</sub>O Oxidation of **1** in the Presence of GSH

## 5.9 Oxidation of **1** with Horseradish Peroxidase

In an effort to find a method which would allow for the isolation of product *m/z* 879.2, analogue **1** was oxidized with horseradish peroxidase. It was found that the major product was in fact the desired peak at *R*<sub>t</sub>=35.3 min (Figure 5-8), and HPLC chromatograms showed excellent baseline separation between the desired product, and the other components of the reaction mixture. A further six products were seen via HPLC, none of which appeared to correspond to any of the products seen in the other oxidation experiments.

Isolation of the major product from the horseradish peroxidase oxidation experiment would allow for the investigation of the structure of this adduct via NMR. Determination of the

location of the GSH molecules on the lignan would provide information regarding the nature of the electrophilic entity produced during oxidation of **1** via rat liver microsomes, mushroom tyrosinase, and horseradish peroxidase.

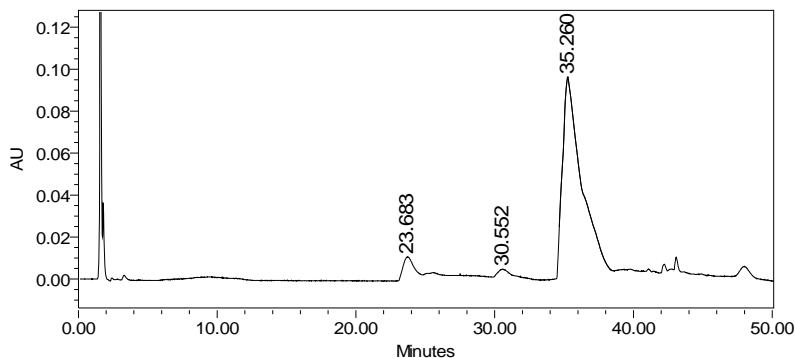


Figure 5-8: Oxidation of **1** with Horseradish Peroxidase with GSH

### 5.10 Incubation of ASECO in Rat Liver Microsomes

The structure of ASECO closely resembles that of analogue **3**. Consequently, ASECO was incubated in the presence of rat liver microsomes in an effort to better predict the formation of an electrophilic entity from analogue **3**. Control reactions performed at pH 7.4, including heat inactivated microsomes and microsomes with no NADPH present showed no product formation. HPLC results showed product formation as a peak at  $R_t=22.2$  min for the active incubation in the presence of GSH (Figure 5-9). The peak present at  $R_t=32.8$  was identified as ASECO. UPLC-MS showed product at  $R_t=1.1$  min, with a  $m/z$  648.2 in MS-ESI(-). This corresponds to ASECO plus one GSH.

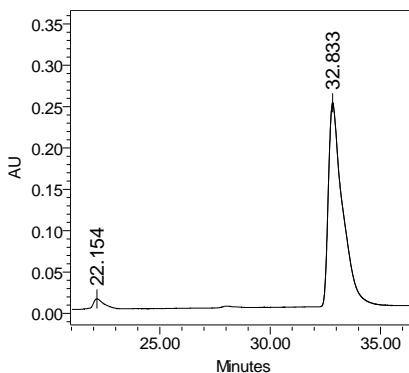
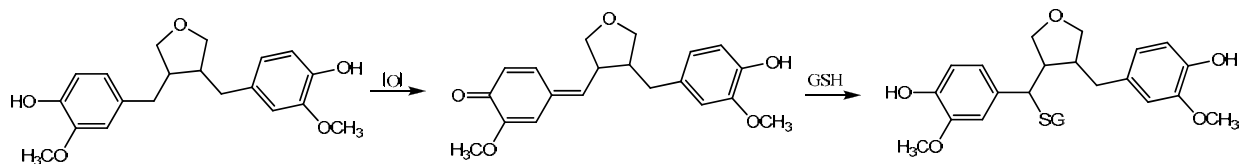


Figure 5-9: Close-up of HPLC Results from ASECO Incubation in Rat Liver Microsomes with GSH



Given the structure of ASECO, it is likely that it was oxidized to the *p*-QM, which was then trapped with GSH (Scheme 5-16). This theory could be tested by oxidizing ASECO with Ag<sub>2</sub>O in the presence of GSH. In the case that the same product was found as that present in the microsomal incubation, the reaction could be performed in sufficient quantities to collect the product for NMR.



Scheme 5-16: Oxidation of ASECO and Subsequent Trapping with GSH

The fact that ASECO formed an electrophilic product during the microsomal incubation suggests that analogue **3** could potentially form a similar product under similar conditions. The similar substitution pattern of one of the aromatic rings (i.e. *para*-phenol) of **2** also suggests the possibility for the formation of an electrophilic intermediate on oxidation of analogue **2**.

## 6.0 SUMMARY AND CONCLUSIONS

The attempted synthesis of the four structural analogues of NDGA ultimately consumed the majority of the time devoted to this project. The double Stobbe condensation reaction was found to provide sufficient flexibility for the synthesis of a variety of substituted lignan skeletons. Furthermore, the reactions reported by Xia et al.<sup>22</sup> in the synthesis of NDGA were found to be successful in the synthesis of the desired mono-catechol analogue **1**.

Following the successful synthesis of **1**, the determination of the metabolites formed during incubation in rat liver microsomes was investigated. Oxidation of analogue **1** in the presence of rat liver microsomes produced an electrophilic metabolite which reacted with GSH. UPLC-ESI-MS(-) found the products to have  $m/z$  574.2, and  $m/z$  879.2, which is consistent with **1** + GSH, and **1** + 2GSH respectively. The absence of product formation during control incubations confirmed that the autoxidation of **1** at pH 7.4 is significantly slower than that of NDGA.

In order to determine the identities of the products formed during the microsomal incubation, the oxidation of analogue **1** using enzymatic and chemical methods was carried out. Mushroom tyrosinase, which is known to produce *o*-quinone moieties in catechols<sup>14</sup> gave a major product with  $m/z$  574.2, apparently identical to that of the product found with incubation in rat liver microsomes. A small amount of product with  $m/z$  879.2 was also found. It is likely that the *o*-quinone did form, however further investigation is required to confirm.

Oxidation of analogue **1** with silver oxide resulted in some product corresponding to  $m/z$  879.2, but most products were inconsistent with the results from the rat liver microsomes oxidation. It is unclear whether this reaction produced a *p*-QM. Oxidation of analogue **1** with horseradish peroxidase was found to produce significant amounts of the product possessing a retention time consistent with that of the product with  $m/z$  879.2. Finally, incubation of ASECO in rat liver microsomes was found to give a product of  $m/z$  648.2, which is consistent with ASECO + GSH, indicative of *p*-QM formation.

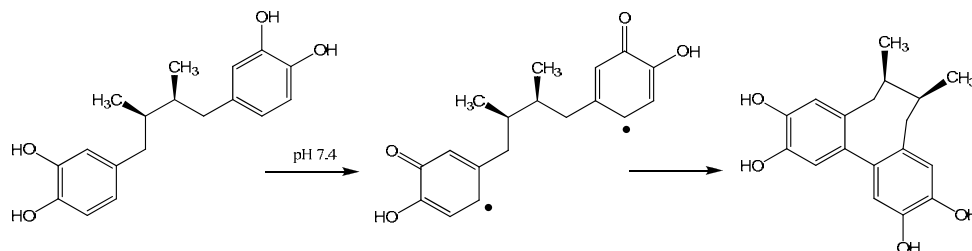
## 7.0 FUTURE DIRECTIONS

Completion of the synthesis of analogues **2**, **3**, and **4** will be needed to obtain a complete set of oxidation data for these structural analogues.

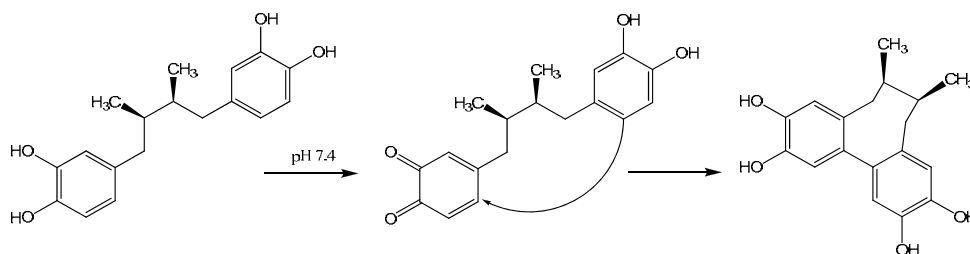
With the major metabolites of analogue **1** during incubation in rat liver microsomes identified via HPLC and UPLC-MS, the next challenge is the synthesis of these metabolites, potentially via mushroom tyrosinase and horseradish peroxidase. Once sufficient quantities of these products have been produced, determination of the structure of these GSH adducts can then be performed via NMR studies. The position at which GSH binds to analogue **1** should help in identifying the nature of the electrophilic entity which formed during the metabolism experiments. Investigation into the autoxidation potential of **1** could also be performed by way of a pH rate profile.

Other potential directions for this project include the synthesis of other analogues of NDGA and naturally occurring lignans, such as ASECO. Potential biological or pharmacological activity of these compounds, such as anti-viral activity or *in vitro* inhibition of P450 could also be assessed.

Another interesting question that may be addressed is the mechanism of the NDGA autoxidation reaction. The previous observation of NDGA autoxidation to the dibenzocyclooctadiene was suggested to occur via a radical reaction (Scheme 8-1)<sup>11</sup>, but could also occur as the result of an electrophilic addition (Scheme 8-2)<sup>42</sup>.

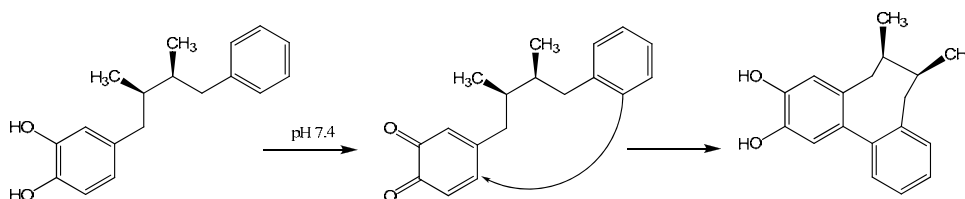


Scheme 8-1: Cyclization of NDGA to the Dibenzocyclooctadiene via a Radical-Mediated Reaction



Scheme 8-2: Cyclization of NDGA to the Dibenzocyclooctadiene via an Electrophilic Addition Reaction

Analogue **1** could only undergo a similar type of cyclization via an electrophilic addition since it does not possess a readily ionizable -OH on the unsubstituted ring (Scheme 8-3). Thus, analogue **1** could undergo autoxidation, although it must occur at a greatly reduced rate. The Hammett  $\sigma$  value for H = 0.0, and for OH is -0.37 (*para*) and 0.12 (*meta*)<sup>43</sup>, which would suggest that the catechol-substituted aromatic ring in NDGA is more nucleophilic than the unsubstituted ring in analogue **1**. If autoxidation were to occur through an electrophilic addition reaction as described in schemes 8-2 and 8-3, then analogue **4** (OCH<sub>3</sub>, Hammett  $\sigma$  = -0.27 (*para*) and 0.12 (*meta*)) would be anticipated to undergo autoxidation at a rate closer to that observed for NDGA. Analogue **4** cannot undergo a radical-mediated reaction as outlined in scheme 8-1, therefore the absence of autoxidation in analogue **4** could imply that a radical-mediated process more accurately describes NDGA oxidative cyclization.



Scheme 8-3: Cyclization of Analogue 1 to a Dibenzocyclooctadiene via an Electrophilic Addition Reaction

## 8.0 REFERENCES

1. Lambert, J. D.; Dorr, R. T.; Timmermann, B. N. Nordihydroguaiaretic Acid: A Review of Its Numerous and Varied Biological Activities. *Pharmaceutical Biology* **2004**, *42*, 149-158.
2. Arteaga, S.; Andrade-Cetto, A.; Cardenas, R. Larrea tridentata (Creosote bush), an abundant plant of Mexican and US-American deserts and its metabolite nordihydroguaiaretic acid. *J. Ethnopharmacol.* **2005**, *98*, 231-239.
3. Lambert, J. D.; Zhao, D.; Meyers, R. O.; Kuester, R. K.; Timmermann, B. N.; Dorr, R. T. Nordihydroguaiaretic acid: hepatotoxicity and detoxification in the mouse. *Toxicol* **2002**, *40*, 1701-1708.
4. Stickel, F.; Egerer, G.; Seitz, H. K. Hepatotoxicity of botanicals. *Public Health Nutrition* **2000**, *3*, 113-124.
5. Sheikh, N. M.; Philen, R. M.; Love, L. A. Chaparral-Associate Hepatotoxicity. *Archives of Internal Medicine* **1997**, *157*, 913-919.
6. Kauma, H.; Koskela, R.; Mäkisalo, H.; Autio-Harmanen, H.; Lehtola, J.; Höckerstedt, K. Toxic Acute Hepatitis and Hepatic Fibrosis after Consumption of Chaparral Tablets. *Scand J. Gastroenterol* **2004**, *39*, 1168-1171.
7. Gezgin, M. H.; Timmermann, B. N. A short synthetic route to nordihydroguaiaretic acid (NDGA) and its stereoisomer using Ti-induced carbonyl-coupling reaction. *Tetrahedron Letters* **2001**, *42*, 6083-6085.
8. Gordon, D. W.; Rosenthal, G.; Hart, J.; Sirota, R.; Baker, A. L. Chaparral ingestion. The broadening spectrum of liver injury caused by herbal medications. *JAMA* **1995**, *273*, 489-490.
9. Goodman, T.; Grice, H. C.; Becking, G. C.; Salem, F. A. A cystic nephropathy induced by nordihydroguaiaretic acid in the rat. Light and electron microscopic investigations. *Lab Invest* **1970**, *23*, 93-107.
10. Grice, H. C.; Becking, G.; Goodman, T. Toxic properties of nordihydroguaiaretic acid. *Food Cosmet. Toxicol* **1968**, *6*, 155-161.
11. Billinsky, J.; Marcoux, M. R.; Krol, E. S. Oxidation of the Lignan Nordihydroguaiaretic Acid. *Chem. Res. Toxicol.* **2007**, *20*, 1352-1358.

12. Iverson, S. L.; Hu, L. Q.; Vukomanovic, V.; Bolton, J. L. The influence of the p-alkyl substituent on the isomerization of o-quinones to p-quinone methides: potential bioactivation mechanism for catechols. *Chem. Res. Toxicol.* **1995**, *8*, 537-544.
13. Aptula, A. O.; Roberts, D. W.; Cronin, M. T. D.; Schultz, T. W. Chemistry-Toxicity Relationships for the Effects of Di- and Trihydroxybenzenes to *Tetrahymena pyriformis*. *Chem Res. Toxicol* **2005**, *18*, 844-854.
14. Bolton, J. L.; Acay, N. M.; Vukomanovic, V. Evidence that 4-allyl-o-quinones spontaneously rearrange to their more electrophilic quinone methides: potential bioactivation mechanism for the hepatocarcinogen safrole. *Chem. Res. Toxicol.* **1994**, *7*, 443-450.
15. Monks, T. J.; Hanzlik, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. Contemporary Issues in Toxicology: Quinone Chemistry and Toxicity. *Toxicology and Applied Pharmacology* **1992**, *112*, 2-16.
16. Wagner, P.; Lewis, R. A. Interaction Between Activated Nordihydroguaiaretic Acid and Deoxyribonucleic Acid. *Biochem. Pharmacol.* **1980**, *29*, 3299-3306.
17. Billinsky, J. L.; Krol, E. S. Nordihydroguaiaretic Acid Autoxidation Produces a Schisandrin-like Dibenzocyclooctadiene Lignan. *J. Nat. Prod.* **2008**, *71*, 1612-1615.
18. Son, J.-K.; Lee, S. H.; Nagarapu, L.; Jahng, Y. A Simple Synthesis of Nordihydroguaiaretic Acid and Its Analogues. *Bull. Korean Chem. Soc.* **2005**, *26*, 1117-1120.
19. Wang, Q.; Yang, Y.; Li, Y.; Yu, W.; Hou, Z. J. An efficient method for the synthesis of lignans. *Tetrahedron* **2006**, *62*, 6107-6112.
20. Lieberman, S. V.; Mueller, G. P.; Stiller, E. T. A Synthesis of Nordihydroguaiaretic Acid. *J. Org. Chem* **1947**, *69*, 1540-1541.
21. Hwu, J. R.; Tseng, W. N.; Gnabre, J.; Giza, P.; Huang, R. C. C. Antiviral Activities of Methylated Nordihydroguaiaretic Acids. 1. Synthesis, Structure Identification, and Inhibition of Tat-Regulated HIV Transactivation. *J. Med. Chem.* **1998**, *41*, 2994-3000.
22. Xia, Y. M.; Cao, X. P.; Peng, K.; Ren, X. F.; Pan, X. F. An Efficient Synthetic Method of Nordihydroguaiaretic Acid (NDGA). *Chinese Chemical Letters* **2003**, *14*, 359-360.
23. Johnson, W. S.; McCloskey, A. L.; Dunnigan, D. A. The Mechanism of the Stobbe Condensation. *J. Am. Chem. Soc.* **1950**, *72*, 514-517.

24. Robinson, R.; Seijo, E. Experiments on the Synthesis of Substances related to the Sterols. Part XXXVIII. Ethyl *cyclo*Hexane-1:4-dione-2-carboxylate and Other Intermediates. *J. Am. Chem. Soc.* **1941**, 582-586.
25. Anjaneyulu, A. S. R.; Raghu, P.; Ramakrishna Rao, K. V.; Sastry, Ch. V. M.; Umasundari, P.; Satyanarayana, P. On The Stereoselectivity of Stobbe Condensation with Ortho Substituted Aromatic Aldehydes: (E, Z) Configuration of Monobenzylidenesuccinates and Dibenzylidenesuccinic Anhydrides. *Curr. Science* **1984**, 53, 239-243.
26. Hart, R. J.; Heller, H. G. Overcrowded Molecules. Part VII. Thermal and Photochemical Reactions of Photochormic (E)- and (Z)-Benzylidene(diphenylmethylene)-succinic Anhydrides and Imides. *J. C. S. Perkin I* **1972**, 1321-1324.
27. Zimmerman, H. E.; Ahramjian, L. Overlap Control of Organic Reactions. II. The Stereochemistry of the Perkin Reaction and Related Condensation Reactions . *J. Am. Chem. Soc.* **1959**, 81, 2086-2091.
28. Iba, M.M.; Soyka, L.F.; Schulman, M.P. Characteristics of the liver microsomal drug-metabolizing enzyme system of newborn rats. *Mol. Pharmacol.* **1977**, 13, 1092-1104.
29. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, 193, 265.
30. Omura, T.; Sato, R. The carbon monoxide binding pigment of liver microsomes. II Solubilization, purification, and properties. *J. Biol. Chem.* **1964**, 239, 2379-2385.
31. Quideau, S.; Pouységu, L.; Looney, M. A. Novel preparation of orthoquinol acetates and their application in oxygen heterocyclization reactions. *J. Org. Chem* **1998**, 63, 9597-9600.
32. Bolton, J. L.; Zhang, F. Synthesis of the equine estrogen metabolites 2-hydroxyequilin and 2-hydroxyequilenin. *Chem Res. Toxicol* **1999**, 12, 200-203.
33. Mizufune, H.; Nakamura, M.; Mitsudera, H. Process research on aryl $n$ aphthalene lignan aza-analogues: a new palladium-catalyzed benzannulation of  $\alpha,\beta$ -bisbenzylidenesuccinic acid derivatives. *Tetrahedron* **2006**, 62, 8539-8549.
34. Datta, P. K.; Yau, C.; Hooper, T. S.; Yvon, B. L.; Charlton, J. L. Acid-catalyzed cyclization of 2,3-dibenzylidenesuccinates: synthesis of Lignans ( $\pm$ )-cagayanin and ( $\pm$ )-galbulin. *J. Org. Chem* **2001**, 66, 8606-8611.
35. Ward, D. E.; Sales, M.; Man, C. C.; Shen, J.; Sasmal, P. K.; Guo, C. Influence of the  $\beta$ -alkoxy group on the diastereoselectivity of aldol reactions of tetrahydro-4H-thiopyran-4-one with 4-alkoxytetrahydro-2H-thiopyran-3-carboxaldehydes. *J. Org. Chem* **2002**, 67, 1618-1629.

36. Johnstone, R. A. W.; Rose, M. E. A Rapid, Simple, and Mild Procedure for Alkylation of Phenols, Alcohols, Amides, and Acids. *Tetrahedron* **1979**, *35*, 2169-2173.
37. Reddy, M. V. R.; Mallireddigari, M. R.; Cosenza, S. C.; Pallela, V. R.; Iqbal, N. M.; Robell, K. A.; Kang, A. D.; Reddy, E. P. Design, Synthesis, and Biological Evaluation of (*E*)-Styrylbenzylsulfones as Novel Anticancer Agents. *J. Med. Chem.* **2008**, *51*, 86-100.
38. Iverson, S. L.; Shen, L.; Anlar, N.; Bolton, J. L. Bioactivation of Estrone and Its Catechol Metabolites to Quinoid-Glutathione Conjugates in Rat Liver Microsomes. *Chem Res. Toxicol* **1996**, *9*, 492-499.
39. White, J. D.; Hrnčiar, P.; Stappenbeck, F. Asymmetric Total Synthesis of (+)-Codeine via Intramolecular Carbenoid Insertion. *J. Org. Chem* **1999**, *64*, 7871-7884.
40. Ohkuma, T.; Kitamura, M.; Noyori, R. Asymmetric Hydrogenation. In *Catalytic Asymmetric Synthesis*, 2nd ed.; Ojima, I. Ed.; Wiley-VCH: 2000; pp 1-110.
41. Yu, L.; Liu, H.; Li, W.; Zhang, F.; Luckie, C.; van Breemen, R. B.; Thatcher, G. R.; Bolton, J. L. Oxidation of Raloxifene to Quinoids: Potential Toxic Pathways via a Diquinone Methide and *o*-Quinones. *Chem Res. Toxicol* **2004**, *17*, 879-888.
42. Krafczyk, N.; Heinrich, T.; Porzel, A.; Glomb, M. A. Oxidation of the Dihydrochalcone Aspalathin Leads to Dimerization. *J. Agric. Food Chem.* **2009**, *57*, 6838-5843.
43. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979.