DEVELOPMENT OF METHODOLOGY FOR THE SYNTHESIS OF 4-ARYLFLAVAN-3-OL LACTONES

Dissertation submitted in fulfilment of the requirements for the degree

Magister Scientiae

in the
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by

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External Co-Supervisors: **Prof. J.A. Steenkamp**
Dr. B.I. Kamara

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B. van Tonder

Contents

Abbreviations

The following abbreviations are used throughout the text:

A	=	acetone
T	=	toluene
B	=	benzene
THF	=	tetrahydrofuran
H	=	hexane
DMF	=	*N,N*-dimethylformamide
DCM	=	dichloromethane
Et_2O	=	diethyl ether
MeOH	=	methanol
EtOH	=	ethanol
EtOAc	=	ethyl acetate
TBAF	=	tetrabutyl ammonium chloride
TBSCl	=	*t*-butyldimethylsilyl chloride
PPTS	=	pyridinium *p*-toluenesulfonate
TFAA	=	trifluoroacetic anhydride
DMAP	=	4-dimethylaminopyridine
DBU	=	1,7-diazobicyclo-[5.4.0]-undec-7-ene

LITERATURE SURVEY

CHAPTER 1

Introduction

Flavonoids are phenolic secondary metabolites that are widely distributed throughout the plant kingdom. Research around flavonoids has increased dramatically over the last number of years. Flavonoids exhibit different properties that are beneficial to human health such as anti-fungal[1], anti-cancerous[2], anti-microbial[3] and anti-oxidant[4] activities. Great emphasis has been placed throughout the world on living healthy and preventing serious illnesses. 'You are what you eat' has become very applicable in recent times, thus leading to a renewed interest in the flavonoids consumed together with the food taken in.

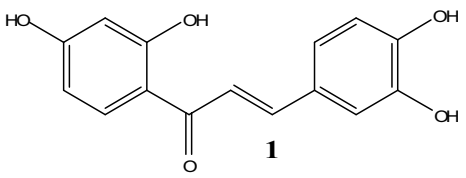
1.1 Structure variation

Flavonoids constitute a C_6-C_3-C_6 skeleton, where a C3 fragment joins two phenolic portions. Although some compounds with unsubstituted aromatic rings are found, these are rare, and most isolated compounds exhibit resorcinol, phloroglucinol, catechol or pyrogallol type hydroxylation patterns. While the resorcinol and pyrogallol oxygenation patterns may be present on either one or both aromatic rings, phloroglucinol type substitution is usually limited to one of the rings with the other one having *p*-oxygenation or a catechol substitution pattern.

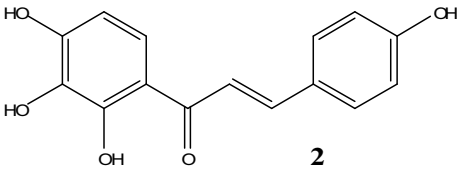
While O-methylation is a common feature on a large number of isolated flavonoids, alkylation is not limited to methyl groups and many flavonoids exhibit sugar moieties as part of their structure. These compounds, known as flavonoid glycosides, may contain a large variety of sugar molecules like glucose, galactose, arabinose and xylolose, which can be connected to the flavonoid unit *via* an oxygen bridge (O on the flavonoid) or by a direct bond between usually the anomeric carbon on the sugar and

a carbon on the flavonoid unit. Other O- or C-substituents on the aromatic rings include different alkyl groups, such as isopentenyl, which may or may not be involved in another heterocyclic ring system. More complex flavonoid structures include substituents like bigger ring systems on the phenolic portions.

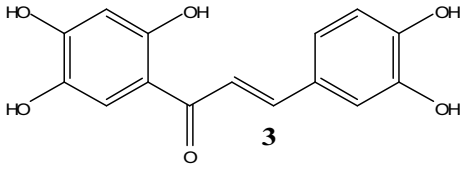
Scheme 1



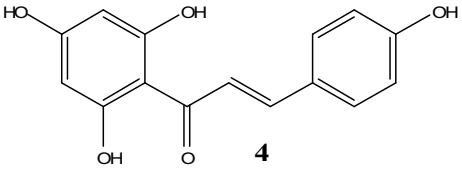
Resorcinol B-ring



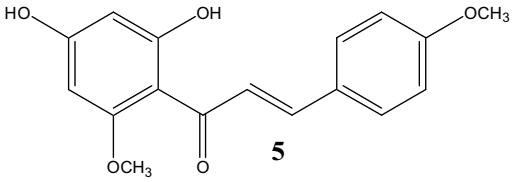
Pyrogallol B-ring



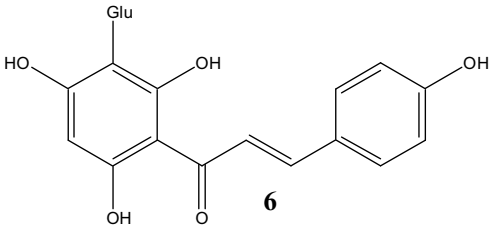
Catechol A-ring



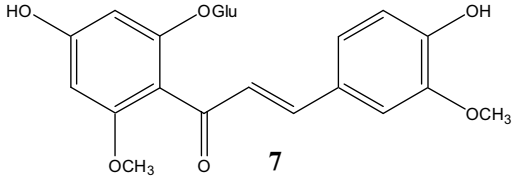
Phloroglucinol B-ring with *p*-hydroxy on A-ring



O-methylation on both rings



C-glycosylation on B-ring

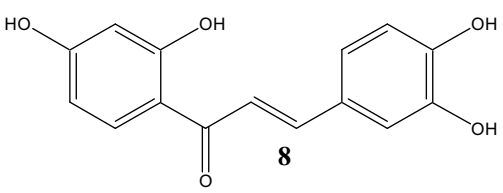


O-glycolsylation on B-ring

1.1.1 Acyclic flavonoids

The C_3 portion of the basic flavonoid skeleton provides another variation in the structure of these natural products. Chalcones (8), dihydrochalcones (9) and retrochalcones (10) contain an acyclic C_3 moiety that may vary in the level of oxidation state. While chalcones exhibit a α,β-unsaturated carbonyl system,

dihydrochalcones have a saturated C_2 unit and in retrochalcones the typical substitution pattern of the rings are inverted.



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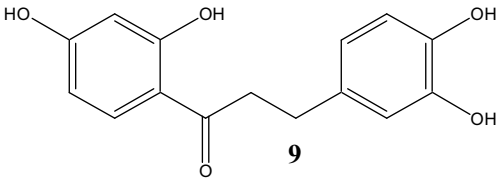
Chalcone



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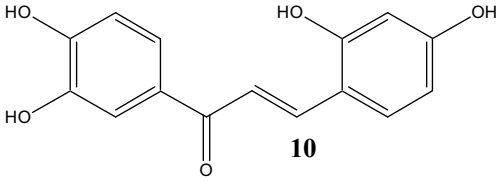
Dihydrochalcone



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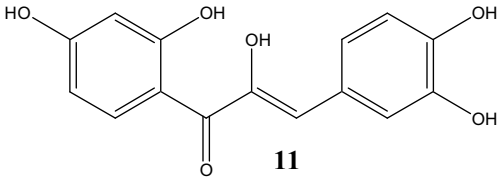
Retrochalcone

In some cases, substituents like hydroxyl groups may also be found on the bridging carbons of the chalcone structure (11 and 12).



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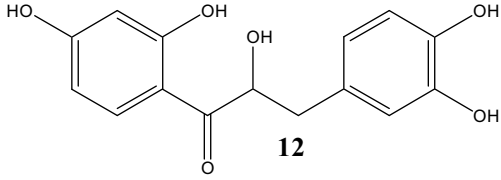
α-hydroxychalcone



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α-hydroxydihydrochalcone

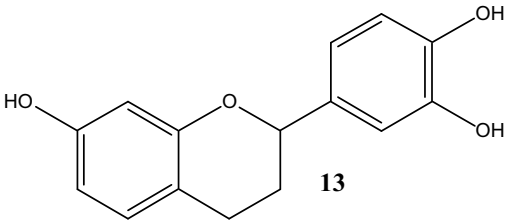
1.1.2 Cyclic flavonoids

Cyclic flavonoids are compounds that contain a heterocyclic ring between the two aromatic portions. These flavonoids can be divided into three major groups, i.e. the

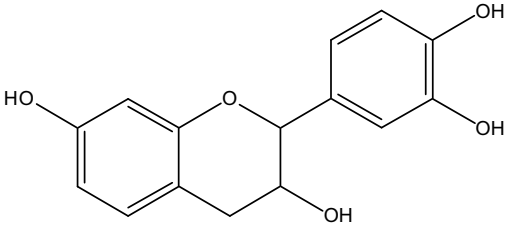
flavonoids with a basic 3-phenylchroman skeleton, commonly known as the flavonoids, the compounds with a basic 3-phenylchroman skeleton, the isoflavonoids, and the neoflavonoids with as 4-phenylchroman skeleton.

The different oxidation states of the heterocyclic ring of flavonoids lead to further grounds for classification. Flavans, with a fully saturated heterocyclic ring, may contain hydroxy substituents at C-3 (the flavan-3-ols) or both C-3 and 4 (the flavan-3,4-diols), while a carbonyl group at C-4 may give rise to the flavanone group of compounds. The carbonylgroup may again be accompanied by an OH at C-3, leading to the dihydroflavonols. In the absence of a C-4 carbonyl group, introduction of unsaturation between C-2 and C-3 or C-3 and C-4 would lead to flavenes, while the presence of a C=O group would give rise to flavones and flavonols depending on the absence or presence of a 3-hydroxy group.

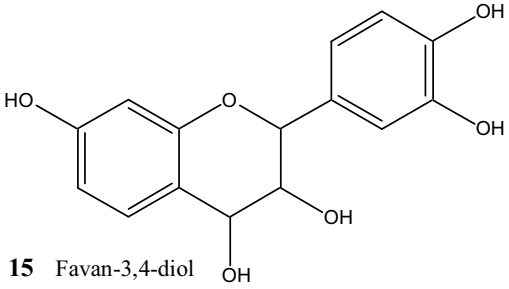
Scheme 2



13 Flavan



14 Favan-3-ol



15 Favan-3,4-diol

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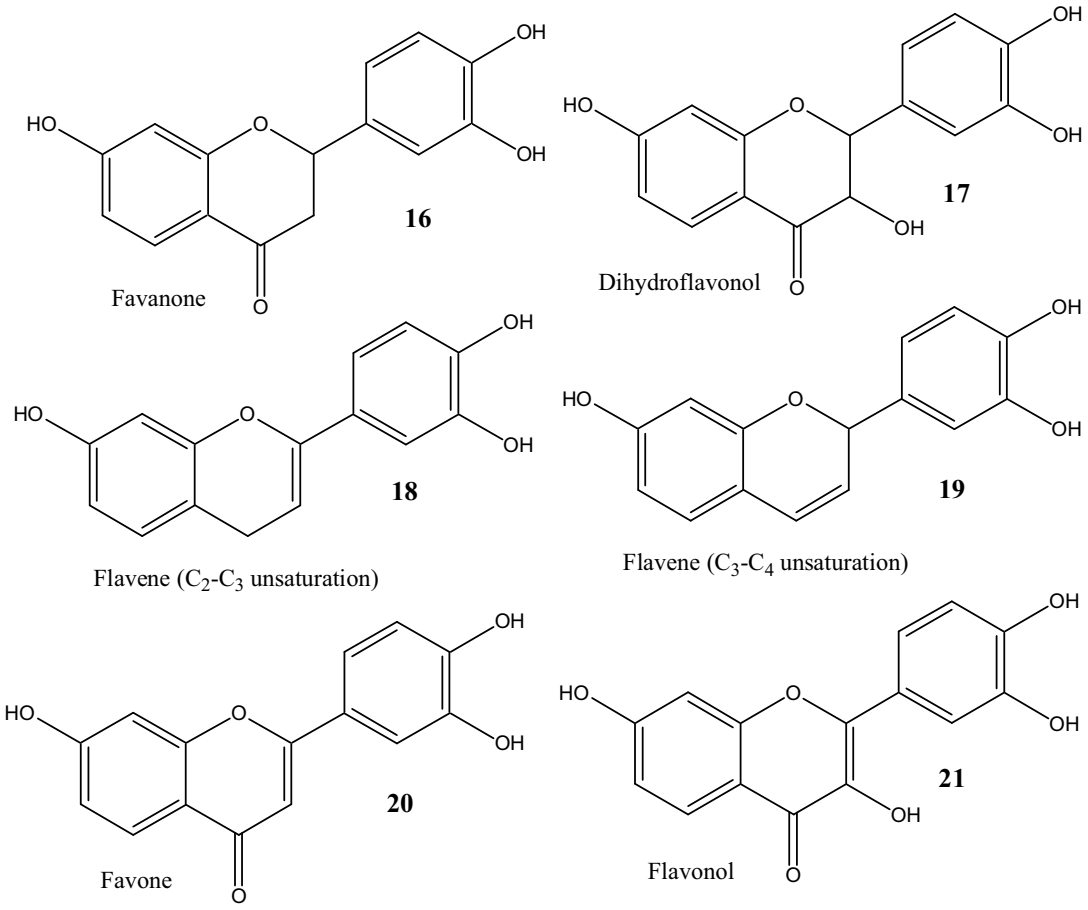
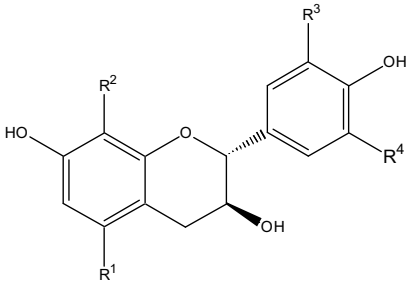
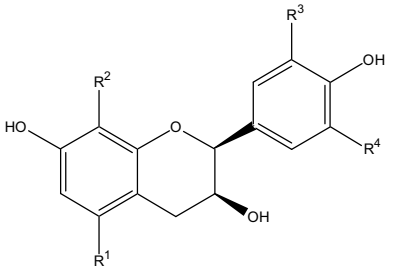
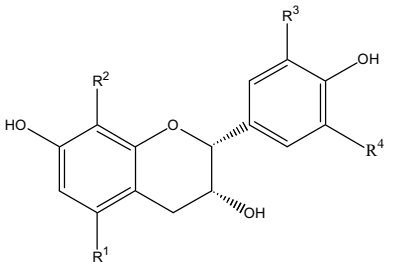
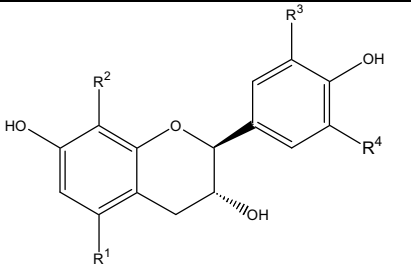
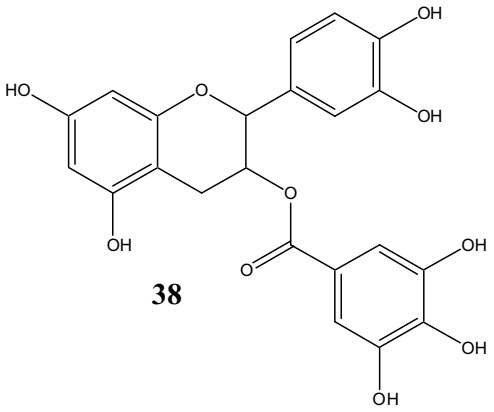


Depending on the oxidation state of the heterocyclic ring, the flavonoids may contain several chiral centres e.g. at C-2, C-3 and C-4. Relative as well as absolute stereochemistry is therefore important aspects of the nomenclature and structure of these compounds. In the case of flavan-3-ols definition of the stereochemistry of a particular compound was, in accordance with earlier conventions, included into the trivial name of that compound, which was based on the phenolic hydroxylation pattern of the specific compound. For example, the *cis*-diastereomer of one of the most common flavan-3-ols, catechin, is indicated by the prefix *epi*, while the enantiomer of the most abundant isomer of this compound is indicated by the prefix *ent*. The trivial names for the most common flavan-3-ols are given in table 1.

Table 1

Flavan-3-ol	Substitution pattern	Trivial name
	22: R^1 = OH, R^2 = R^3 = R^4 = H	Afzalechin
	23: R^1 = R^2 = R^3 = H, R^4 = OH	Fisetinidol
	24: R^1 = R^2 = H, R^3 = R^4 = OH	Robinetinidol
	25: R^1 = R^4 = OH, R^2 = R^3 = H	Catechin
	26: R^1 = R^3 = R^4 = OH, R^2 = H	Gallocatechin
	27: R^1 = R^2 = R^3 = R^4 = H	Guibouritnidol
	28: R^1 = R^3 = R^4 = H, R^2 = OH	Oritin
	29: R^1 = R^3 = H, R^2 = R^4 = OH	Mesquitol
	30: R^1 = OH, R^2 = R^3 = R^4 = H	Epiafzalechin
	31: R^1 = R^2 = R^3 = H, R^4 = OH	Ent-epifisetinidol
	32: R^1 = R^4 = OH, R^2 = R^3 = H	Ent-epicatechin
	33: R^1 = OH, R^2 = R^3 = R^4 = H	Ent-epiafzalechin
	34: R^1 = R^4 = OH, R^2 = R^3 = H	Epicatechin
	35: R^1 = R^3 = R^4 = OH, R^2 = H	Epigallocatechin
	36: R^1 = R^4 = OH, R^2 = R^3 = H	Entcatechin
	37: R^1 = R^2 = R^3 = H, R^4 = OH	Entfisetinidol

A structural feature commonly found among the flavan-3-ol flavonoids is the occurrence of gallate esters.[5] Both the 3-galloyl as well as the 3,5-digalloyl esters are commonly extracted from plant material.



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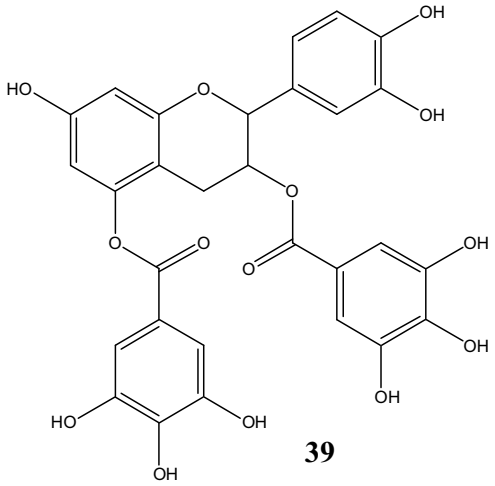
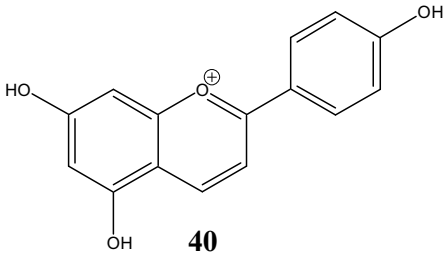
3-galloyl ester



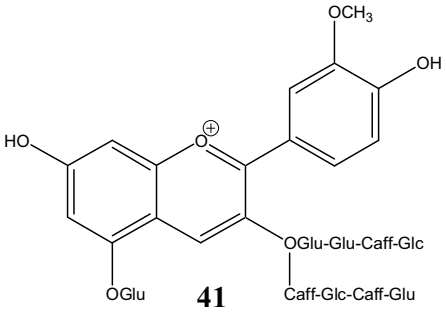
39

3,5-galloyl ester

The anthocyanidins exhibit a flavoniod skeleton with unsaturation between C-3 and C-4 as well as the heterocyclic atom and C-2 The substitution patterns of the anthocyanidins can be unique and complicated as is evident from (40) and (41).



40



41

Glu = glucose
Caff = Caffeic acid
Glc = galactose

Heavanly blue anthocyanidin

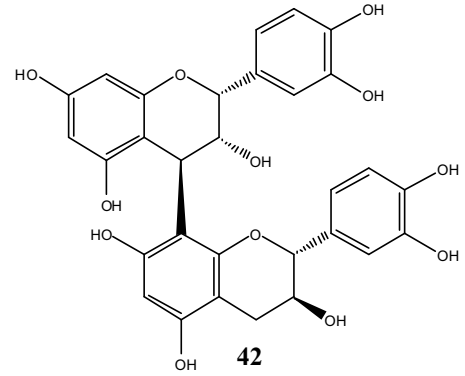
1.1.3 Oligomeric flavonoids

Oligomeric flavonoids (also known as proanthocyanidins) consist of two or more monomeric flavonoid building blocks. These compounds are classified as dimers, trimers, tetramers and even pentamers based on the number of monomeric units in the final structure. The flavan-3-ol building blocks may be the same or different in

hydroxylation pattern as well as stereochemistry. The position and stereochemistry of the linkage between the moieties are also of importance.

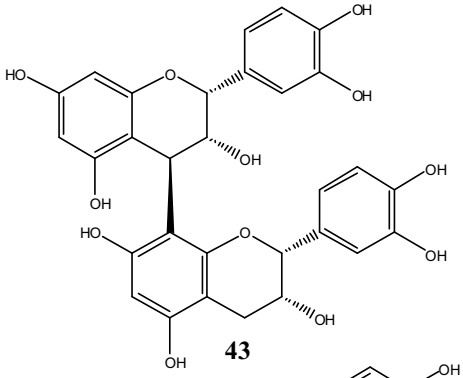
1.1.3.1 B-type oligomers

The dimeric B-type proanthocyanidins are characterized by a single interflavan bond between C-4 of the 'upper' unit and C-6 (**45**) or C-8[6] (**42** – **44**) of the extending or 'lower' unit. The stereochemistry of the two units and that of the interflavan bond determine the different types of proanthocyanindins.



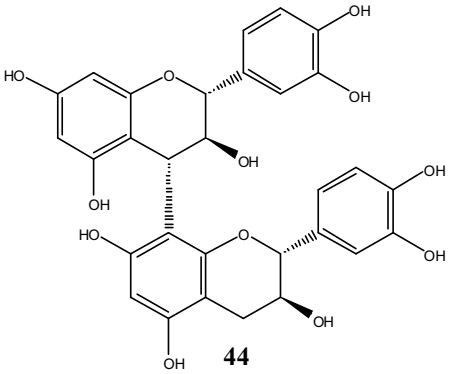
42

B6



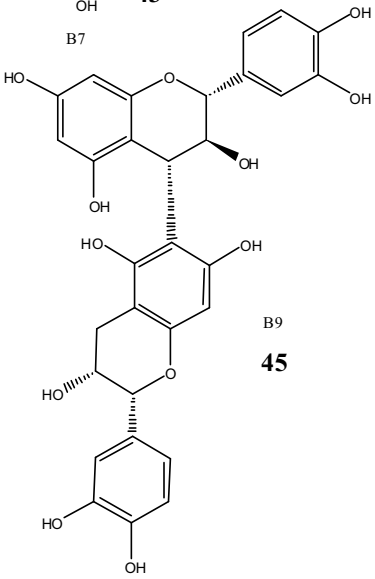
43

B7



44

B8

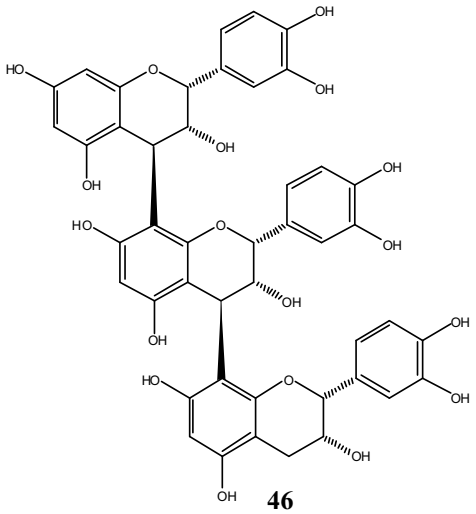


B9

45

Trimeric B-type proanthocyanidin structures are commonly isolated from plant material and can either be linear (4→8 bonding throughout the molecule) (**46**) or branched (at least one 4→6 linkage) (**47**). Electrophilic aromatic substitution reactions at the flavan-3-ol nucleus occur more readily at C-8 than the C-6 position thus forming linear polymer structures. Although the C-8 position is more

nucleophilic, substitution does take place as the C-6 position as well and this interflavanyl bond give rise to branched oligomers.[7]



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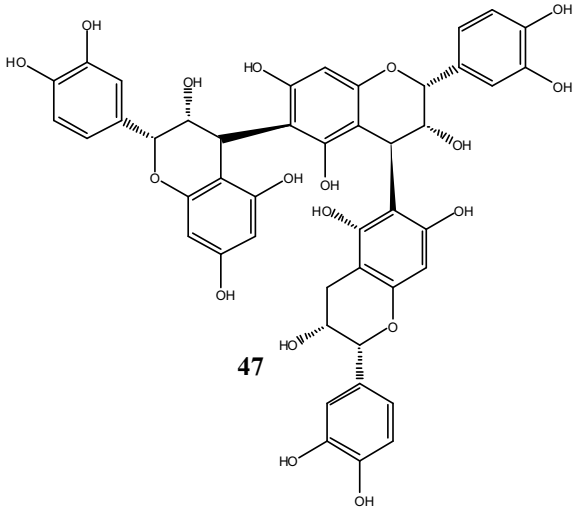
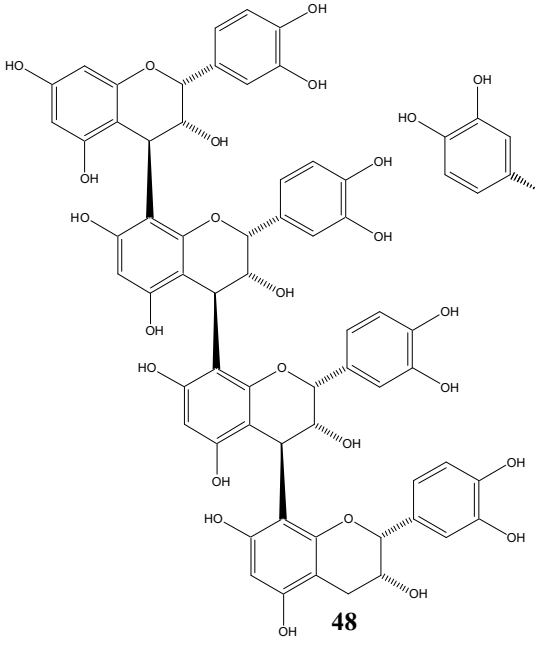
Linear trimer



47

Branched trimer

Tetrameric B-type proanthocyanidins are also a common feature of plant flavonoids and can constitute linear - or branched structures just like trimers.



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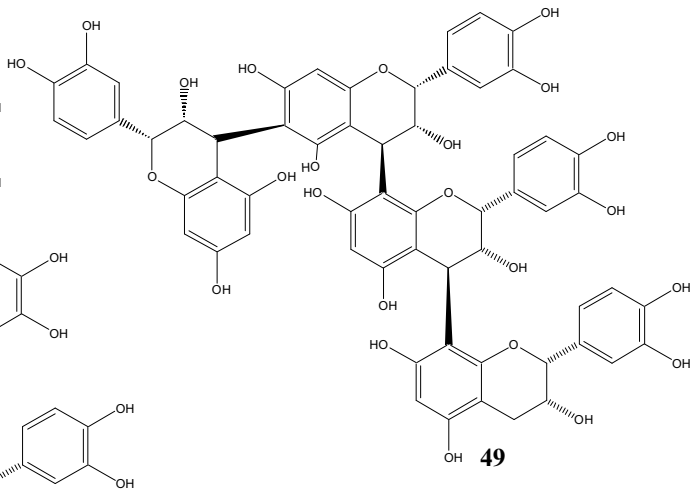
Linear tetramer



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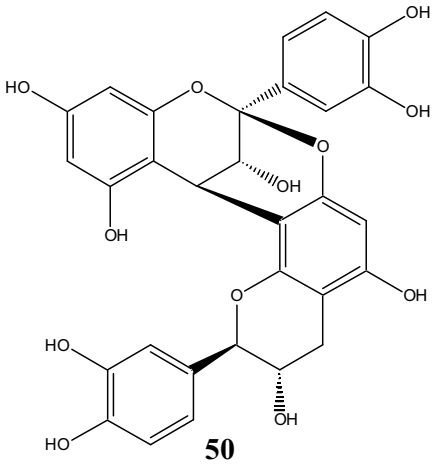
Branched tetramer

1.1.3.2 A-type oligomers

In addition to the one interflavan bond of the B-type oligomers, the A-type proanthocyanidins have a second ether linkage between an A-ring hydroxyl group of the extending unit and C-2 of the starting unit. As with the B-type oligomers, A-types may also exist as di-, tri-, or tertramers, while stereochemistry is of vital importance to distinguish between the different oligomers.



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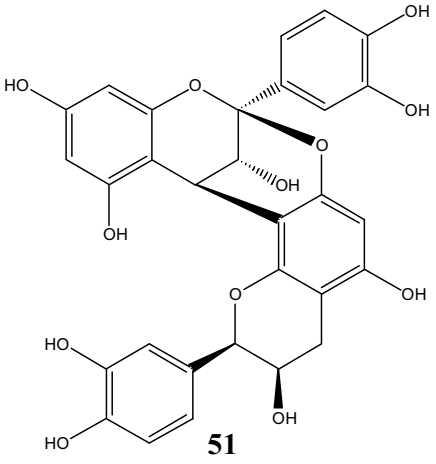
A1

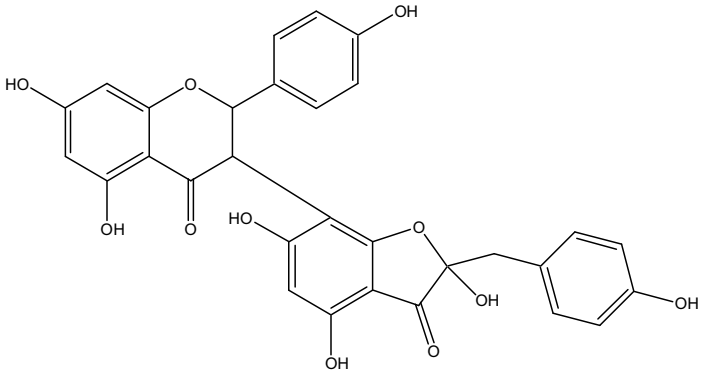


51

A2

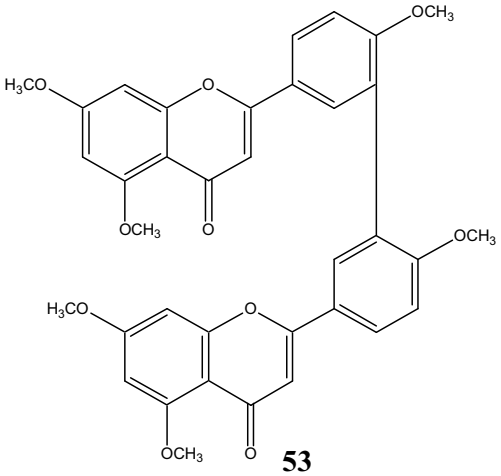
1.1.3.3 Other dimeric and oligomeric structures

Although the proanthocyanidins are the most abundant amongst the oligomeric flavonoids, structural diversity in this group of natural products is by no means limited to proanthocyanidin compounds. Other dimeric – (and higher oligomeric) flavonoids, collectively known as biflavonoids, are also known and may comprise of compounds, which are formed from other flavonoid classes such as flavones, isoflavones, aurones, dihydroflavonols, flavanones and chalcones.[8] These flavonoids usually also exhibit other interflavanoyl linkages such as linkages exclusively *via* the B-rings.



52

Zeyherin



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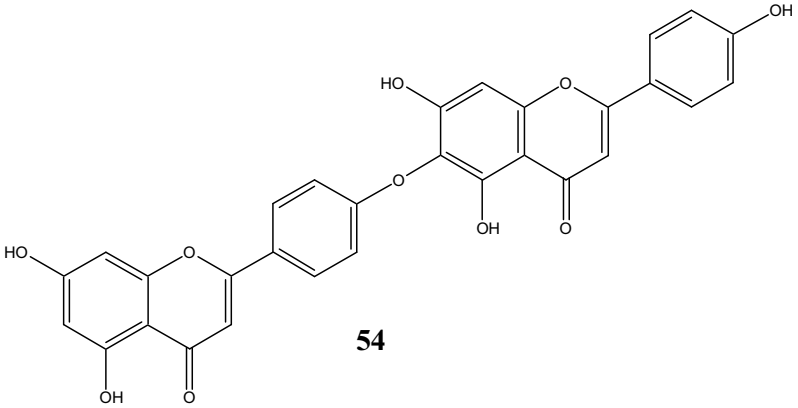

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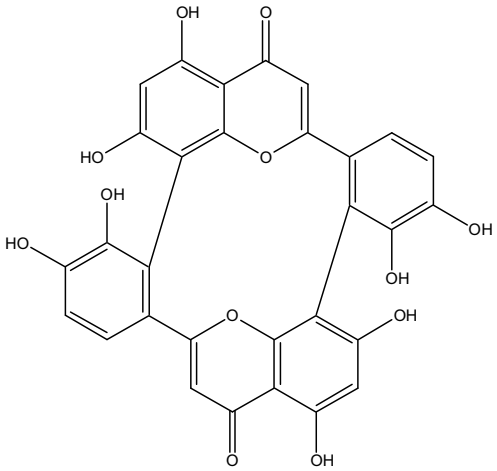

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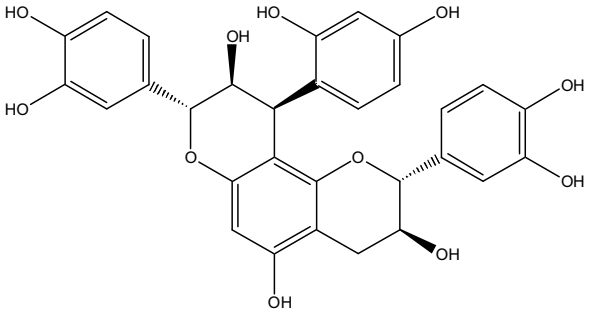

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1.2 The physiological activity of flavonoids

Free radicals[9] are molecules with unpaired electrons that are generated during oxidative metabolism and energy production in the body. These highly reactive species are involved in processes like enzyme-catalyzed reactions, electron transport in the mitochondria, signal transduction, gene expression, and the activation of nuclear transcription factors.[10] Radicals are, however, also involved in oxidative damage to molecules, cells, and tissue, as well as aging and certain diseases.

Normal metabolism is dependent on oxygen as it is the terminal electron acceptor for respiration. During respiration, reactive oxygen species such as $O^{\cdot-}_2$ and $OH^{\cdot}$ are formed, which are involved in the causes of aging, cancers, and cardiovascular and other diseases.[11] Damage to DNA, like specific oxidation of purin or pyrimidine bases and DNA strand breaks, may give rise to free radicals of oxygen, nitrogen, and sulphur, or other oxidizing agents, as well as ionization radiation and photo-oxidation of transition metal ions activated by peroxides.[12] The damage to DNA leads to mutations in the expression of the DNA, which can give rise to different diseases. Since flavonoids are known radical scavengers[4], it is in this regard that the beneficial health effects of flavonoids must be viewed.

It has been accepted that flavonoids in the body act mainly in their capacity as antioxidants and radical scavenging[4] species. Recent research pointed out that the antioxidant and radical scavenging capacity of flavonoids in the body are limited because of biotransformations[13] of the flavonoids that occur during uptake. Evidence presented indicates that flavonoids intervene by various ways in the metabolic processes in cells.

The evidence for flavonoid-rich food components as cardio- , neuro- , and chemoprotective agents is steadily accumulating. However, the exact mechanism of these actions still remains to be confirmed. Although there are hundreds of flavonoid molecules in dietary plants, the major components of current interest for their beneficial health effects, are flavonols[14], flavan-3-ols[15], flavanones[16], and anthocyanins[17]. A large number of *in vivo* studies have indicated flavonoids as being effective against both reactive oxygen species and active nitrogen species. It was also

found that flavonoids with the highest *in vitro* antioxidant potential were those containing a catechol B-ring[18] that readily donates a hydrogen/electron to stabilise a radical species. Until recently, the ability of flavonoids to act as classical H-donating antioxidants was believed to underlie many of their reported health effects. However, the extent of their antioxidant potential are dependent on the absorption, metabolism[18], distribution, and excretion within or from the body after ingestion, as well as the reducing properties of the resulting metabolites. An understanding of the processes involved in the absorption and distribution of polyphenols is essential for determining their bioactivities and *in vivo* significance. Since flavonoids are biotransformed[13] during metabolism it is very important to investigate their *in vivo* behaviour.

The application of flavonoids in direct health improvement has been demonstrated in the following instances: Application of flavonoids to the skin protects against the damaging effects of ultra violet (UV) light. The UVB wavelength of light has damaging effects on the body like DNA damage, premature aging of the skin, inflammation and cancer. The antioxidant properties of the flavonoids enable the molecules to combat the damaging effect of the UVB rays of the sun on the skin's surface. This property of flavonoids is utilised by the cosmetic industry to improve their products.

The moderate consumption of wine[4], especially red wine, has recently received more prominence due to a possible link with reduction in mortality from cardiovascular diseases. Wine polyphenols, which contribute to the colour of the wine as well as other sensorial properties, such as bitterness and astringency, comprise both flavonoids and non-flavonoids. Flavonoids included are flavonols (e.g. quercetin), flavan-3-ols (e.g. catechin), anthocyanins (e.g. malvadin-3-glucoside) and proanthocyanidins.

Another area where flavonoids are making a significant impact on human health is through the consumption of tea. Green, oolong and black tea[19] are products manufactured form the leaf of the tea plant *Cemellia sinensis*. Green tea is manufactured form the fresh leaves and buds that are pan-fried then rolled and dried.

Green tea is very rich in flavonoids of the catechin group (flavan-3-ols) with epigallocatechin-3-gallate[15] being the most abundant. Epigallocatechin gallate, epicatechin gallate and epicatechin make up nearly 40% of the total flavonoid content of tea. Oolong is produced by wilting the fresh leaves in the sun, then bruising them slightly followed by partial fermentation. The manufacturing of black tea includes an enzymatic step in which the slightly wilted leaves are fully fermented. During the fermentation process, the catechins are converted to complex condensed products, giving black tea its characteristic colour and flavour. Each tea has its own special properties. Green tea has the potential to fight skin, oesophageal, stomach and colon cancer and it can be used externally to stop or slow bleeding from cuts and scrapes and relieve the itching caused by insect bites. Some types of oolong tea have cholesterol-lowering properties. Black tea can be used to treat diarrhoea and can relieve certain types of headaches. Furthermore, damp black tea bags can be placed over tired red eyes or on insect bites to relieve itching and redness.

Some fruits like pomegranate and *Citrus*[16] are worth mentioning when it comes to flavonoid contents. Anthocyanindins are the major flavonoid component in pomegranate juice, while C*itrus* species accumulate substantial quantities of different flavonoids like flavonols, flavones and especially flavanones during their development. Research revealed that the antioxidant capacity of pomegranate juice exceeds that of red wine and green tea by three times. The consumption of *Citrus* is associated with improved immune responses.

Scientific research supports the role of cranberry[20] in the prevention of urinary tract infections. The A-type proanthocyanindin content of cranberries inhibits the adhesion of *Escherichia coli* to uroepithelial cells and thus prevents and fights the infection. Herbs like *Gingko biloba*[21] and Ginseng (*Panax ginseng*)[22], containing flavonols like quercetin and kaempferol, have been part of the Chinese medical remedies for centuries and is said to improve the short-term memory, alleviate the symptoms of mild to moderate Alzheimer-type dementia and to enhance the general well being of the consumer.

In plants, flavonoids play an important role in at least four areas of reproduction and growth: Most flavonoids that are synthesized in the leaves are found in the upper and lower epidermis of the leaves where they function as UV-protectors. Anthocyanidins and flavonol glycosides are, due to their intense colour, also involved in the reproduction of the plants by acting as pollinator attractants.[23] An essential role in plant reproduction has been established for flavonols. In this regard, pollen from maize and petunia plants lacking flavonols was unable to germinate.[24] Flavonoids produced in the roots of plants can act as signalling molecules between the root of the plant and the nitrogen-fixing rhizobial bacteria on the surface of the root itself to activate or deactivate the availability of nitrogen to the plant. During germination of seedlings and the lengthening process of roots, flavonoids are also released. Because the flavonoids associated with the growth and development are only needed at certain times in the lifecycle of the plant, the plant synthesizes the necessary flavonoids and stores them on the seed coat and inside the root cap respectively.

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CHAPTER 2

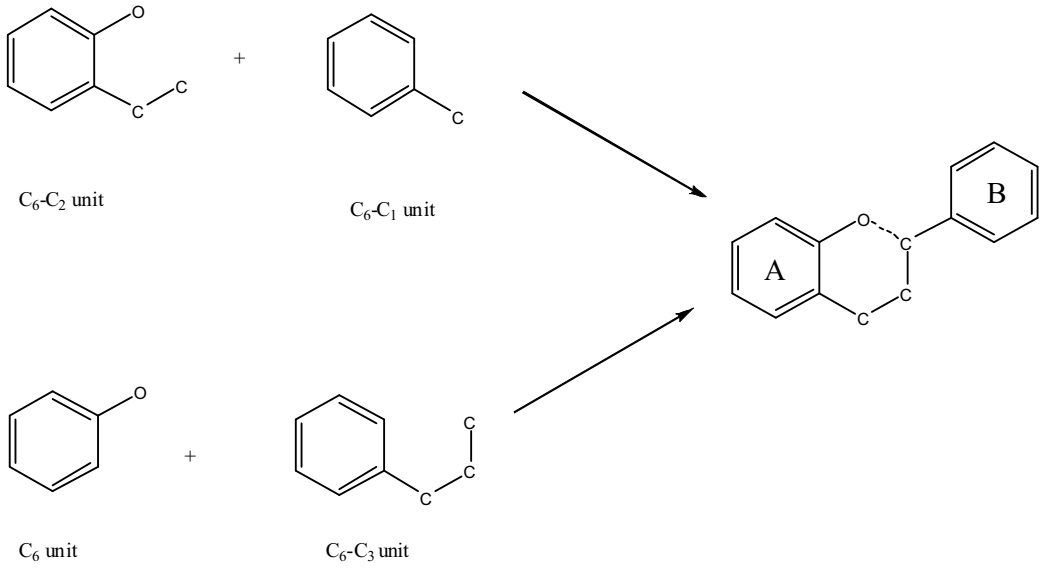
Flavonoid monomers

As the physiological importance of the flavonoids isolated from plant structures dawned on the scientist, methods were developed for the synthesis of the various isolated flavonoids. As synthetic endeavours grew over the years, vast improvements in technology development was experienced, but a simple and effective two- or three-step synthesis for all flavonoids is still to be developed.

2.1 Synthesis of different flavonoids

Since the basic C_6-C_3-C_6 skeleton of all monomeric flavonoids can be modified by oxidation, reduction, isomerization, O- and/or C-alkylation, glycosylation or hydrolysis to yield flavonoids of other classes, the construction of this basic unit received most of the attention and still plays a pivotal role in the synthesis of flavonoids. The general availability of C_6-C_2 units (2-hydroxyacetophenones) as well as C_6-C_1 units (aromatic aldehydes) of different oxygenation patterns led to the coupling of these units to be one of the favoured methods for putting together the basic carbon skeleton of flavonoids (Scheme 1). Another popular pathway entails the acylation of phenols (C_6 unit) with a cinnamic acid equivalent (C_6-C_3 unit) according to Scheme 3.[1] Since both these routes lead to a chalcone or 'chalcone type' intermediate, this route is encountered in almost all flavonoid synthetic procedures.

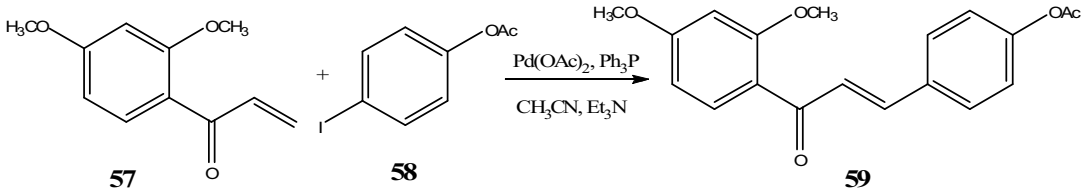
Scheme 3



2.1.1 Chalcones, dihydrochalcones, and flavanones

There are two well-established routes for synthesizing chalcones, which consist of either base - or acid catalized condensation between an appropriately substituted acetophenone and a benzaldehyde.[1] In the presence of a 2-hydroxy function on the acetophenone, the acid catalyzed condensation reaction tends to favour cyclized byproducts such as flavanones. This tendency led to the base catalized chalcone formation reaction to be the reaction of choice for the synthesis of this type of compound. The base most commonly used is a 50-60% potassium hydroxide solution, but precedence for the use of other bases such as sodium hydride and barium hydroxide is found in literature. Although not the reaction of choice, several protic acids, like dry HCl and Lewis acids like $AlCl_3$, $TiCl_4$ and BF_3 etherate have been utilised in the acid catalysed aldol reaction[2]. In most cases, the *trans*-isomer of the chalcone is produced, but it can be converted to the *cis*-isomer by means of UV irradiation. A novel synthesis of the chalcone involves the use of the Heck reaction where a α,β-unsaturated ketone is coupled to an aryl iodide (Scheme 4). This reaction proceeds in short time (about 4 hours) affording the chalcone in satisfactory yields (94%).[3]

Scheme 4



Dihydrochalcones, the saturated form of chalcones, can be obtained through selective catalytic hydrogenation (over Pd on C) of the corresponding chalcone or by zinc/glacial acetic acid hydrogenolysis of flavanones. A more direct method of synthesis would constitute the direct acylation of phenols with alkylated dihydrocinnamic acid derivatives. Several protic acids (like methane sulfonic acid, trifluoromethane sulfonic acid, Nafion H, zeolite and heteropoly salt) or Lewis acids ($AlCl_3$[4], $Hf(OTf)_4$, $Sc(OTf)_3$ and $Zr(OTf)_4$[5]) catalysts have been utilised in this Friedel-Crafts type acylation reaction.

α- or β-Hydroxydihydrochalcones can be synthesized from the corresponding chalcone epoxides by either catalytic hydrogenation (over either palladium on bariumsulphate or palladium on carbon)[6] or treatment with tributyltin hydride and azoisobutyronitrile (AIBN) under both photochemical and thermal conditions.[7] Chalcone epoxides are available through epoxidation of the chalcone skeleton. This can be achieved *via* a number of reactions with suitable reagents for the epoxidation of α,β-unsaturated systems. The most common method involves oxidation with hydrogen peroxide in a basic ethanolic medium in quantitative yields. The hydrogen peroxide can be substituted for other peroxides such as *t*-butyl hydroperoxide.[8] Other methods of epoxidation include DBU and *t*-butyl hydroperoxide[9], $NaBO_3$ and tetrahexylammonium hydrogen sulphate[10] and KF-Al_2O_3/*t*-butyl hydroperoxide[11]. Dioxiranes,[12]are excellent oxygen-transfer agents that can act under mild conditions to generate the required epoxides in excellent yields.

Acid or base catalyzed ring closure of chalcones containing a 2'-hydroxy group would give the corresponding flavanone. Reaction conditions for successful ring closure is dependent on the substitution pattern of the chalcone's two aromatic rings, thus cyclization of compounds with a 6'-hydroxy group is much easier than that of their 6'deoxy counterparts.

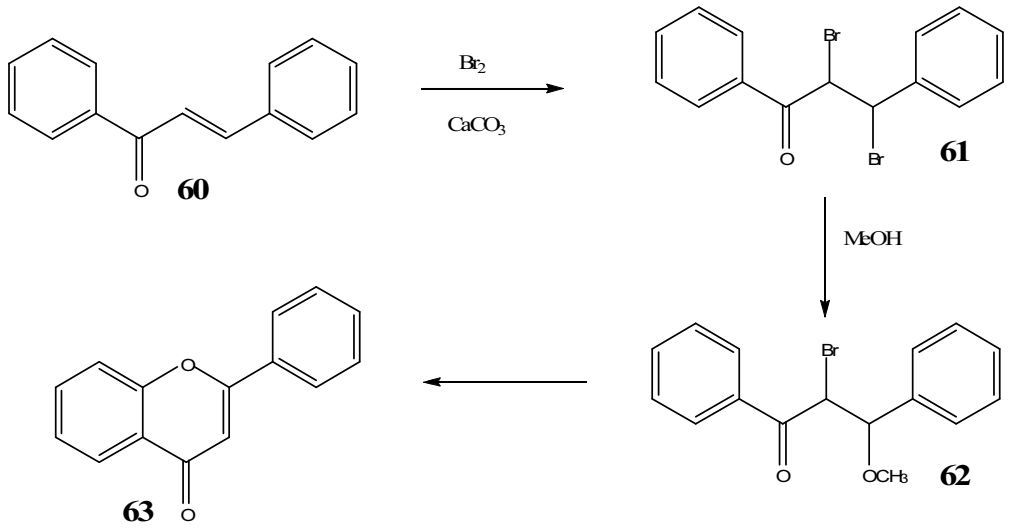
Flavanones can also be synthesized from simpler precursors by the condensation methods of Baker and Venkataraman[13] or Allan and Robinson[14] (*vide infra*).

2.1.2 Flavones, Flavonols, and Dihydroflavonols

(a) Formation by transformation of other flavonoids

Flavones can be formed by dehydrogenation of flavanones or through the modification of chalcones. In the case of the chalcone it can be brominated in the presence of calcium carbonate to form the dibromo derivative, which can then be boiled with methanol to yield the α-bromo-β-methoxychalcone[15] intermediate that cyclizes thermally, or it can just be cyclized thermally (Scheme 4). Simultaneous dehydrogenation and cyclization of 2'-dihydrochalcones over Pd/C will also yield the flavone.[16]

Scheme 5

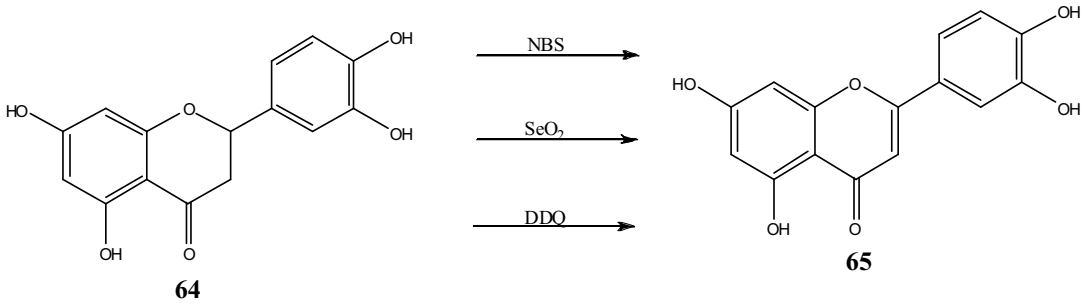


60 61 62 63

Several methods for the dehydrogenation of flavanones to flavones have been described in literature. Thus flavanones can be treated with NBS (N-bromosuccinimide) followed by acid hydrolysis or iodine in glacial acetic acid with the addition of sodium acetate and acetic anhydride followed by saponification with sodium methalate[17] or dehydrogenation by selenium dioxide. Dehydrogenation can also be achieved by means of refluxing flavanone with DDQ (2,3-dichloro-5,6-

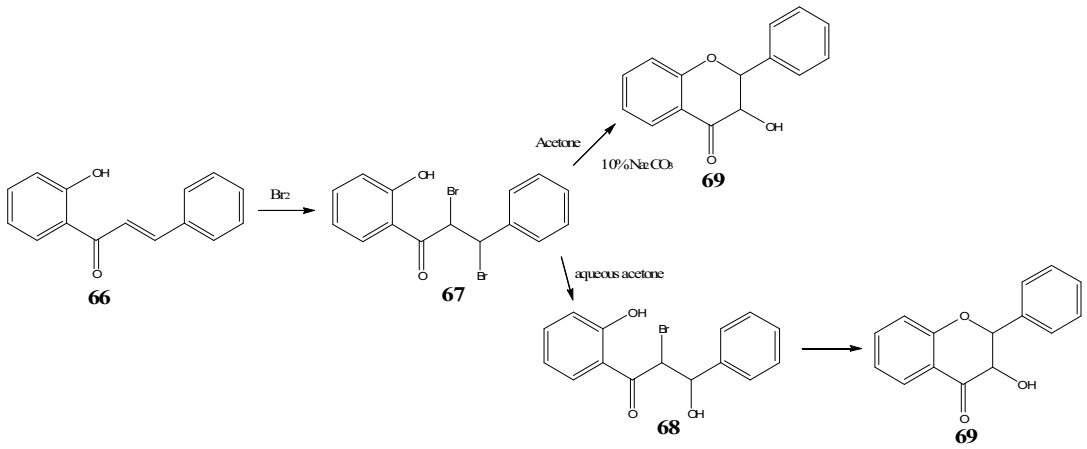
dicyano-1,4-benzoquinone) in 1,4-dioxane.[18,19] These routes are illustrated in the scheme below.

Scheme 6



Dihydroflavonols can be obtained by application of the AFO-reaction (Algar-Oyamada-Flynn reaction)[20] which involves the one step oxidation/cyclization of 2-hydroxychalcones with hydrogen peroxide in usually alkaline medium. Depending on the substitution patterns on the two aromatic rings of the chalcone and the reaction conditions, the major product from the reaction might, however, be the aurone, (2-benzyl-2-hydroxydihydrobenzofuranone) or 2-aryl benzofuran-3-carboxilic acid. Recent improvements to the reaction conditions entails that the reaction is conducted in a buffered medium at pH 9.4 with sodium tungstanate as catalyst.[21] Another mild method involves the oxidation and subsequent cyclization of the chalcone with hydrogen peroxide and diethyl amine at low temperatures[22] Tetrabutylammonium hydroxide proved to be the most efficient base for the formation of dyhydroflavonols as only a small number of by product were observed.[23] Acid catalyzed cyclization (hydrochloric acid or hydrogen chloride in glacial acetic acid or boron trifluoride etherate[24]) of the epoxide to dihydroflavonol is hampered by the formation of byproducts such as isoflavones and coumaranones. These reactions were improved by using trifluoroacetic acid/2,2,2-trifluoroethanol or *p*-toluenesulphonic acid/2,2,2-triflouroethanol instead.[25] The chalcone can also be brominated and then treated with acetone and 10% sodium carbonate to yield the dihydroflavonol. The dibromocompound can be converted by aqueous acetone into α-bromo-β-hydroxyl dihydrochalcones, which can cyclize to the dihydroflavonol in better yield than the dihalide chalcone (Scheme 7).[26]

Scheme 7



Dehydrogenation of dihidroflavonols or modification of chalcones can produce flavonols. By exposing the chalcone to AFO-type reaction conditions (16% aqueous sodium hydroxide, 15% aqueous hydrogen peroxide at 0°C for 1 hour and at -20°C for 46 hours) the flavonol is obtained in moderate to high yields depending on the substituents as well as te substitution patterns. Chalcones containing resorcinol type A-rings and halide substituents on the B-ring produced the highest yields.[27]

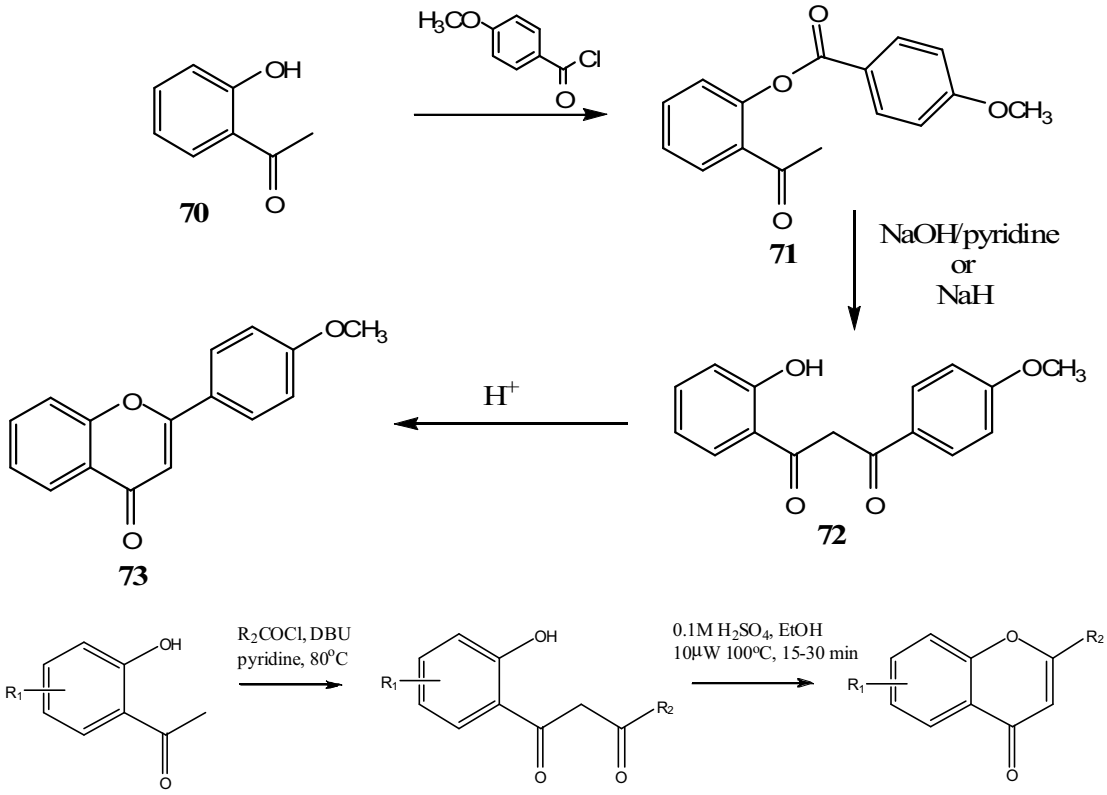
(b) Formation from non-flavonoid precursors

Baker-Venkataraman rearrangement

Flavones are also available from non-flavonoid precursors by application of the Baker-Venkataraman rearrangement. In this reaction a 2'-hydroxyacetophenone is acylated with an aromatic acid chloride and the resulting ester converted to the diketone by treatment with sodium hydroxide in pyridine or sodium hydride. Subsequent ring closure is obtained by treating the resulting diketone with ethanolic sulphuric acid, glacial acetic acid or anhydrous sodium acetate (Scheme 8).

The Baker-Venkataraman rearrangement of 2-hydroxyacetophenones have also be effected through the application of microwave-technology (65-85% yields) to close the heterocyclic ring.[28] Only flavones with limited hydroxylation patterns were synthesized in this way (Scheme 8).

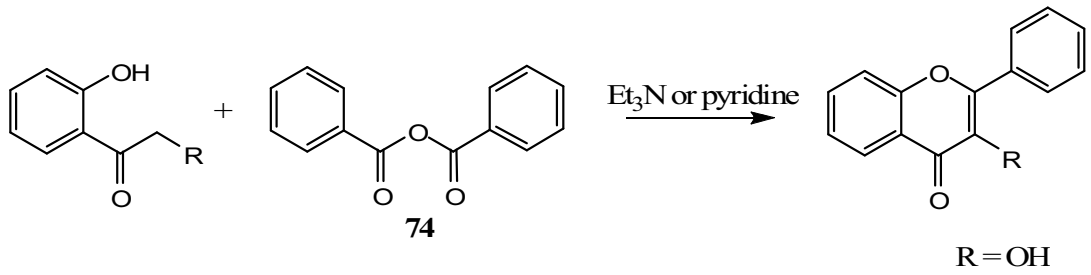
Scheme 8



Allan-Robinson condensation

One of the most frequently used synthetic methods for synthesizing 3-hydroxyflavones (flavonols) comprises the Allan-Robinson condensation reaction. This is a one step condensation reaction where 2'-hydroxyacetophenones are reacted with aromatic anhydrides in the presence of the salt of the same aromatic acid or in the presence of a base such as triethylamine or pyridine (Scheme 9).

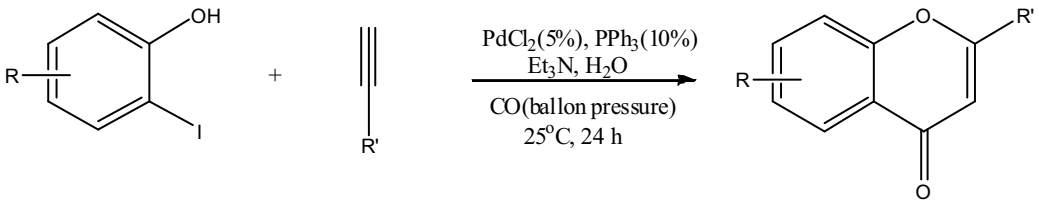
Scheme 9



Sonogashira type coupling

The carbonylative Sonogashira reaction of *o*-iodophenol derivatives with terminal acetylenes followed by intramolecular cyclization comprises a promising new route to flavones in acceptable yields (35-95%) in a one-pot operation. Only limited substitution patterns were tested.[29] This route is illustrated in the scheme below.

Scheme 10

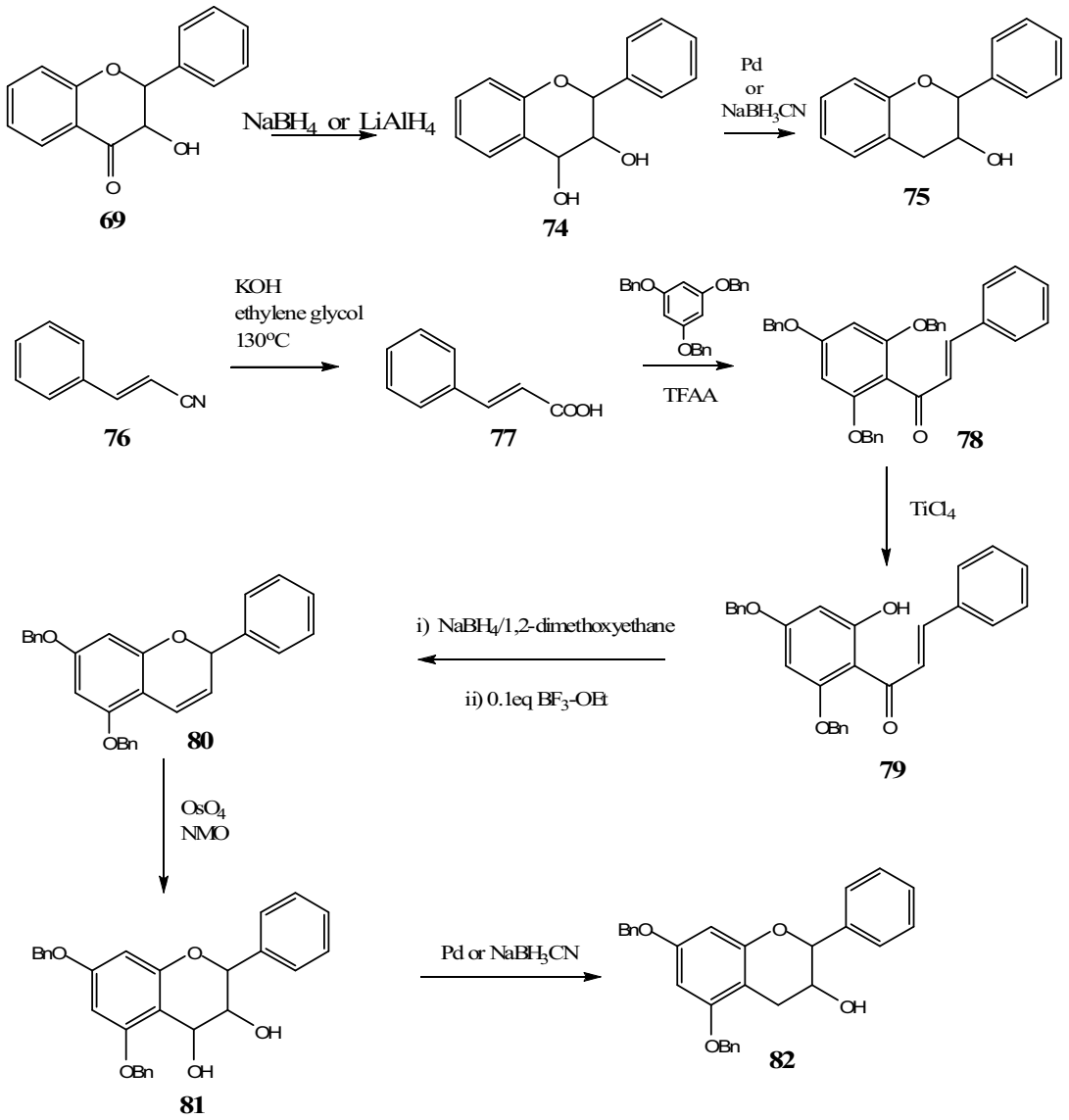


2.1.3 Flavan-3-ols

The synthesis of flavan-3-ols is of great importance to flavonoids research as these molecules are the building blocks for the condensed tannins or proanthocyanidins. Similar to the preparation of flavones, flavonols and dihydroflavonols, flavan-3-ols can also either be synthesized by manipulating an existing flavonoid skeleton or by a multistep synthetic protocol from other aromatic precursors.

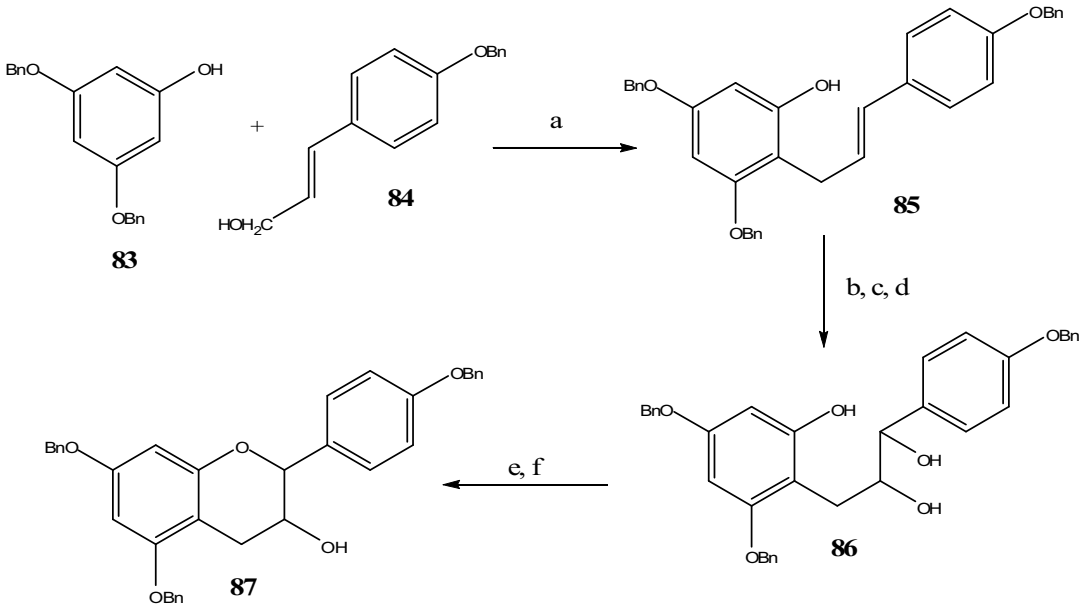
Since dihydroflavonols are easily available in a one step reaction through application of the Allan-Robinson condensation reaction, these compounds are very popular as starting materials for the synthesis of flavan-3-ols *via* flavan-3,4-diols. Reduction of the dihydroflavonol with sodium borohydride or lithium aluminum hydride produces the flavan-3,4-diol. Reductive dehydration by either palladium or sodium cyanoborohydride leads to the corresponding flavan-3-ol. Another tedious route includes the formation of a cinnamonitrile[30], which is *via* the chalcone converted to the flavene. This intermediate product can then be dihydroxilated with osmiumtetraoxide[31] and cyclized to the flavan-3,4-diol, which is treated as before to give the desired final product . These routes are illustrated in the scheme below.

Scheme 11



An alternative route to obtain the flavan-3-ol includes the Friedel-Craft alkylation of an appropriately substituted phenol under strictly controlled conditions, followed by protection, Sharpless dihydroxylation with AD-mix, deprotection and cyclization under the orthoformate/acidic conditions and base hydrolysis of the formate ester (Scheme 12). This route gave an 80% yield of the flavan-3-ol, afzalechin.[32]

Scheme 12



(a) $H_2SO_4(SiO_4)$; (b) TBSCl/imidazole/DMF; (c) AD-mix/$CH_3SO_2NH_2$/H_2O/*t*-BuOH; (d) TBAF/THF; (e) $CH(OEt)_3$/PPTS($CH_2Cl)_2$; K_2CO_3/MeOH/DME

2.1.4 Flavans

A method for synthesizing flavans involves dihydroflavonols as starting materials. The dihydroflavonol can be converted to a flavone-ethylene dithioketal with the aid of 1,2-ethyanedithiol and boron trifluoride after which the dithioketal is hydrogenated with Raney nickel.

2.2 Stereoselective synthesis

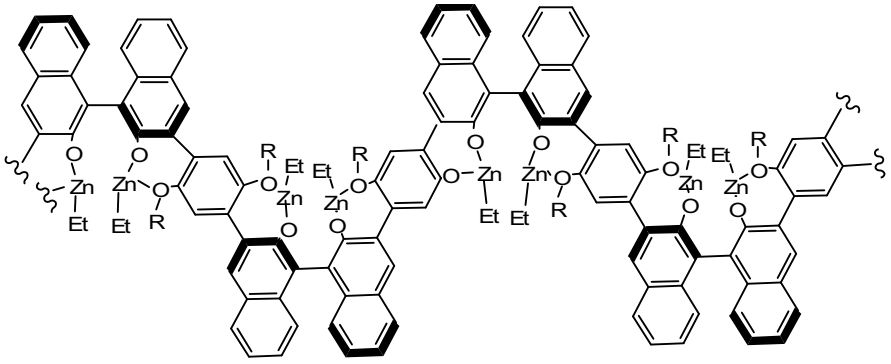
Like many other natural products the physiological properties of flavonoids are to a large extent determined by the stereochemistry present in the molecule. It is therefore important to have the compounds to be studied available in enantiomerically pure form. Since isolation of enantiomerically pure flavonoids can be a tedious and time-consuming process and many substitution patterns are not available in quantities sufficient for preparative purposes, attempts have been made to synthesize flavonoids stereoselectively.[33]

2.2.1 Asymmetric epoxidation

Due to the pivotal role played by chalcones and chalcone epoxides in the synthesis of many other types of flavonoids (*vide supra*), it was realised early on in the development of methodology for the stereoselective synthesis of flavonoids that control over the stereochemistry of the epoxide would be the key step in the stereoselective synthesis of many types of flavonoids. Asymmetric epoxidation can be achieved by different protocols.

One protocol by which asymmetric epoxides can be obtained includes the use of metal catalysis. Katsuki and Sharpless[34] discovered a asymmetric epoxidation method utilizing titanium tetraisopropoxide, *t*-butyl hydroperoxide and (+)- or (-)-diethyl tartrate as epoxidizing agents. Altough this method gave fairly good yields (70-81%) and enatiomeric excess (90-95%) it was unfortunately not extended to include phenolic substrates. Other methods included the use of diethyl zinc and (R,R)-N-methylpseudoephedrine[35] as epoxidation agents which gave fairly good yields (94%) but low ee (61%). Diehtyl zinc was later incorporated into a polybinaphtyl polymer chain (Figure 1) and used as epoxidation agent. It was thought that the use of the polymerbound metal would enhance the entantioselectivity of the epoxidation reaction, but although the ee increased the change was not dramatic.

Figure 1

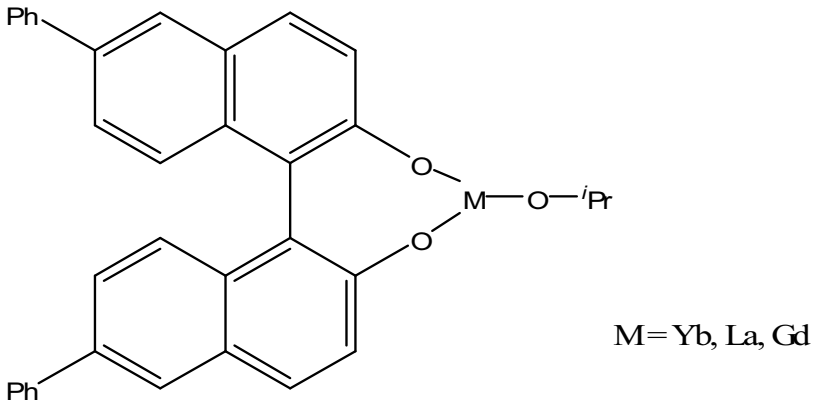


R = n-C_6H_{13}

Modified metal alkyl peroxides with (+)diethyl tartrate as chiral auxiliary was also investigated. Lithium peroxides were utilized with moderate yields (71-75%) but the ee was fairly low (62%) on the chalcone substrate. The lithium was substituted for magnesium to better the ee (78%) but the yield decreased dramatically (51%).[36]

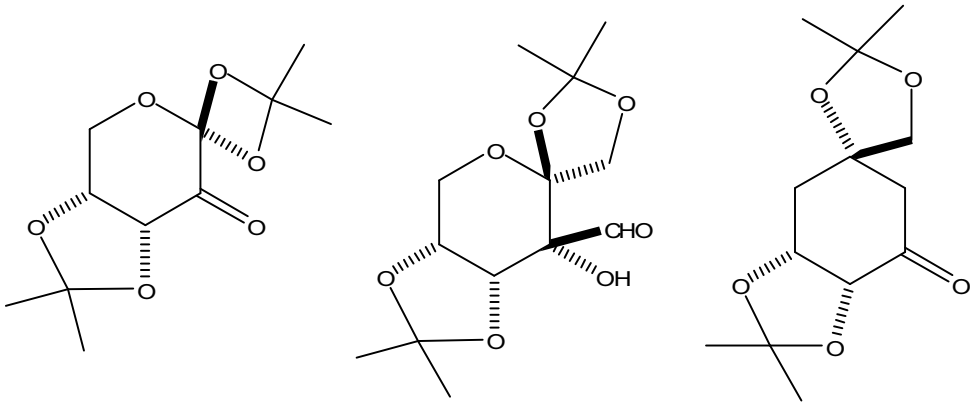
Binaphtyl complexes (Figure 2) with ytterbium, lanthanum and gadolinium as metal centres were investigated.[37,38] The yield and ee's obtained with these complexes ranged from 92-95% (yield) and 85-96% (ee) on the chalcone substrate. With mono-hydroxylated chalcones, however a decrease in the yield (78%) as well as the ee (83%) was observed.

Figure 2



An alternative approach involved the use of chiral dioxiranes to achieve stereoselective epoxidation. To introduce chirality to the reaction, the dioxiranes are generated *in situ* from Oxone and chiral ketones.[39, 40, 41] (Figure 3). The chiral dioxiranes formed in this way, introduces chirality to the substrate and yields a chiral epoxide in 80% yield and 94% ee.[42]

Figure 3



A third approach to introduce chirality into a molecule is to utilize phase-transfer[43] catalysts. A number of different phase-transfer catalysts exist, many of which is quaternary ammonium salts derived from cinchonine and quinine alkaloids (Table 2 Epoxidation of chalcone with Quinine benzylchloride).[44, 45] Modifications of these catalysts include, among others, the incorporation of a 9-anthracenylmethyl group.[46, 47]

Scheme 13

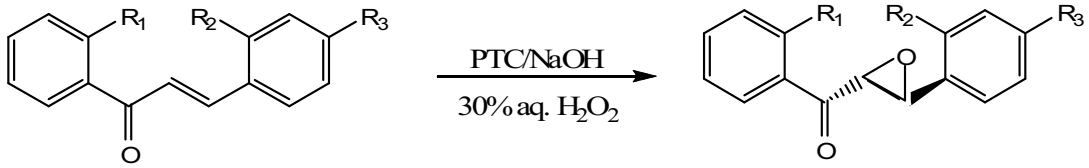
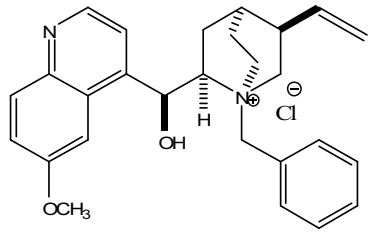
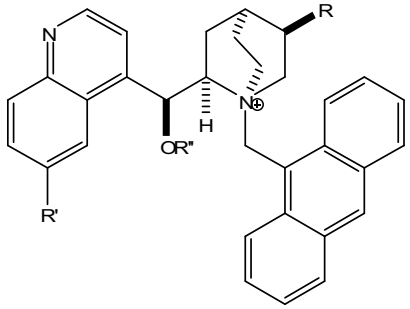


Table 2 Epoxidation of chalcone with Quinine benzylchloride

No	R_1	R_2	R_3	% Yield	% ee
60	H	H	H	92	31
88	H	OMe	H	92	48
89	OMOM	H	H	38	26



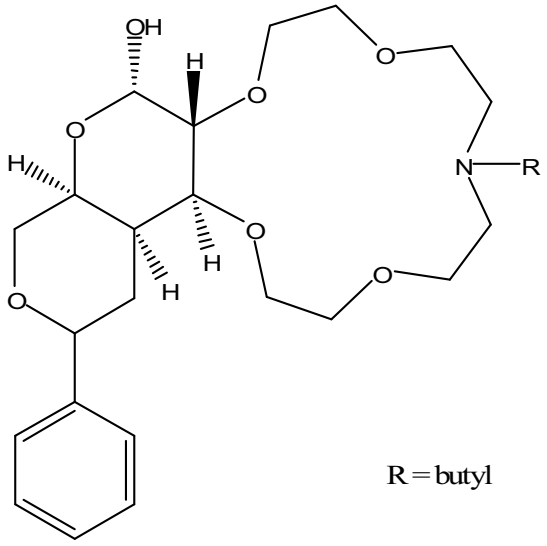
PTC : Quinine benzylchloride



Cinchonine alkaloid

Another reaction that utilizes a phase transfer catalyst to obtain a stereoselective epoxide involves the asymmetric Darzen condensation of phenacyl chloride and benzaldehyde with a chiral crown catalyst (Figure 4) in 49-90% yield and 42-71% ee. The chalcones used as substrates had only single substitution on the A-ring. Modifications to the catalyst include crown ethers derived from different sugar moeities such as D-glucose, D-galactose and D-mannitol.[48] These catalysts, however, proved to be less effective as epoxidation catalysts regarding both yield and ee.

Figure 4



All of the above mentioned protocols were tested on chalcones with no or limited substitution patterns. The only protocol that was tested on chalcones with a range of hydroxylation patterns includes the use of polyamino acids in the epoxidation reaction. These polyamino acids are added as chiral auxilliaries to introduce stereochemistry to a normal epoxide reaction where the substrate is epoxidized by hydrogen peroxide and a base. The Juliá-Colonna[49] asymmetric epoxidation reaction is conducted in a three-phase system comprising of alkaline hydrogen peroxide, and organic solvent and an insoluble poly-amino acid (for example poly-L-alanine or poly-L-leucine).[50] The yields of the reaction ranged from 53-77% and the ee form 38-96% (Table 2 Epoxidation of chalcone with Quinine benzylchloride). The original reaction were modified several times to increase yield and entantiomeric excess and to better reaction times. The three-phase system were modified to a non-aqueous

reaction by substituting the alkaline hydrogen peroxide with urea-hydrogen peroxide complex and a non-nucleophilic base such as 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[51, 52] This reduced the reaction times immensely (days to hours) while maintaining high yields (85%) as well as high ee (95%). Other modifications involved changes of the polyamino acid catalyst. The polyamino acids that are immobilized on silica[53] proved to be an improvement on the catalyst design. Furthermore, the silicon supported catalyst can withstand a range of temperatures and pressures retaining full catalyst activity.[54] Other support materials like polymers[55] were also found to be useful structural modifications to the polyamino acid catalyst, which also provided improved recyclization of the catalyst. Different polyamino acids also showed different epoxidizing acitivities with L-alanine and L-leucine giving the highest yield and ee.[56]

Scheme 14

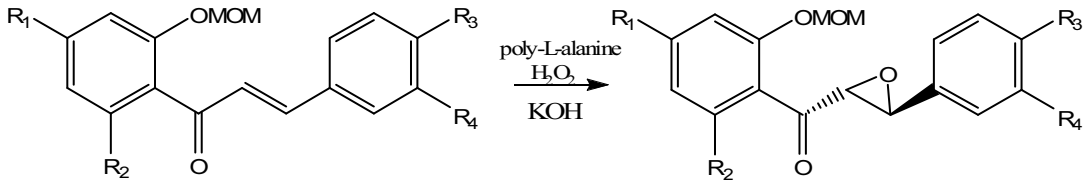


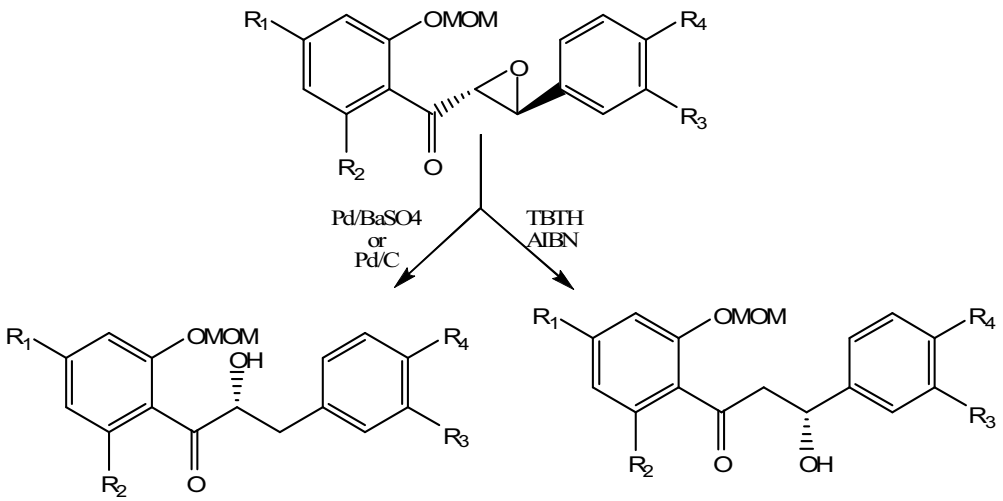
Table 3 Synthesis of chalcone epoxides

No.	R_1	R_2	R_3	R_4	% Yield	% ee
89	H	H	H	H	65	38
90	H	H	H	OMe	64	66
91	OMe	H	H	OMe	74	84
92	OMe	H	OMe	OMe	46	62
93	OMOM	H	H	OMe	43	70

2.2.2 Dihydrochalcones

Since chalcone epoxides are easily transformed into the corresponding α- or β-hydroxy dihydrochalcones by reductive cleavage of either the C_α- or C_β-oxygen bond (*vide supra*), this route was also followed in the asymmetric synthesis of these compounds[57] without loss of stereoselectivity (Scheme 15).

Scheme 15



TBTH: Tributyltin hydride
AIBN: azobutyronitrile

Table 4 Synthesis of α-hydroxy dihydrochalcones

No.	R_1	R_2	R_3	R_4	Catalyst	% Yield	% ee
89	H	H	H	H	$Pd/BaSO_4$	92	27
90	H	H	H	OMe	$Pd/BaSO_4$	51	61
91	OMe	H	H	OMe	$Pd/BaSO_4$	88	76
92	OMe	H	OMe	OMe	10% Pd/C	42	61
93	OMOM	H	H	OMe	$Pd/BaSO_4$	50	65

Table 5 Synthesis of β-hydroxydihydrochalcones

No.	R_1	R_2	R_3	R_4	% Yield	% ee
90	H	H	H	OMe	73	85
91	OMe	H	H	OMe	83	91
92	OMe	H	OMe	OMe	78	84
94	OMe	OMe	H	OMe	79	55
95	OMOM	OMe	OMe	OMe	83	48

Chiral flavanones can be synthesized from the acid catalyzed cyclization of chiral β-hydroxy dihydrochalcones.

2.2.3 Dihydroflavonols and flavan-3,4-diols

The introduction of stereoselectivity into the dihydroflavonol skeleton may also come from the epoxide precursor in the synthesis. In order to prevent unwanted cyclization

during the epoxidation step, the 2'-OH of the chalcone needs to go through a very challenging protection/deprotection sequence during this approach. Since the epoxide ring can easily be opened during the deprotection step, a very sensitive protecting group is required. Acid catalysed removal of a 2'-methoxymethyl group was accompanied by racemization and epimerization of the dihydroflavonol product as well as B-ring aryl migration followed by cyclization leading to the isolfavone. In order to improve the deprotection reaction several Lewis acids like $MgBr_2$ and BF_3-OEt_2 were evaluated, but while the optical purity of the molecule stayed intact, chemical yields were very poor. The focus then shifted to opening the oxirane ring prior to deprotection and cyclization. The benzylmercaptan/tin tetrachloride system selectively cleaved the C_β-O bond at -20°C and effectively deprotected the 2-methoxymethyl group at 0°C. Cyclization was achieved by treating the intermediate thio-ether compound with and thiophilic Lewis acid, i.e. $AgBF_4$. This protocol achieved the best yield and enantiomeric purity (Table 6 Synthesis of dihydroflavonols).[58, 59]

Scheme 16

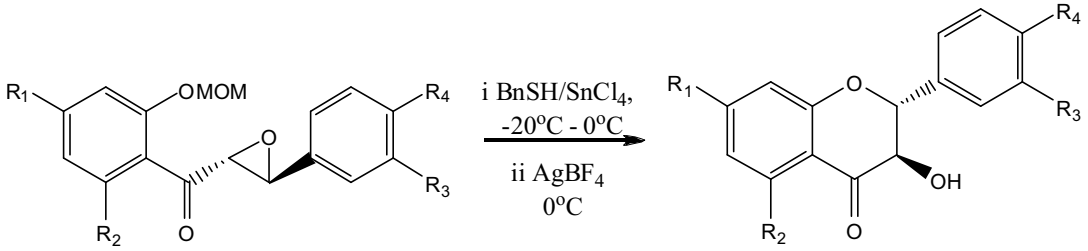
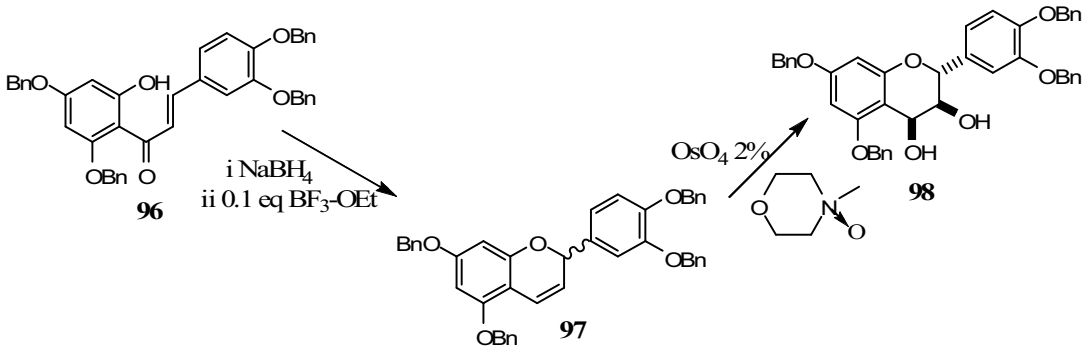


Table 6 Synthesis of dihydroflavonols

No.	R_1	R_2	R_3	R_4	% Yield	% ee	*trans:cis*
90	H	H	H	OMe	86	83	93:7
91	OMe	H	H	OMe	71	84	79:21
92	OMe	H	OMe	OMe	81	68	85:15
94	OMe	OMe	H	OMe	65	69	78:22
95	OMe	OMe	OMe	OMe	61	47	82:18

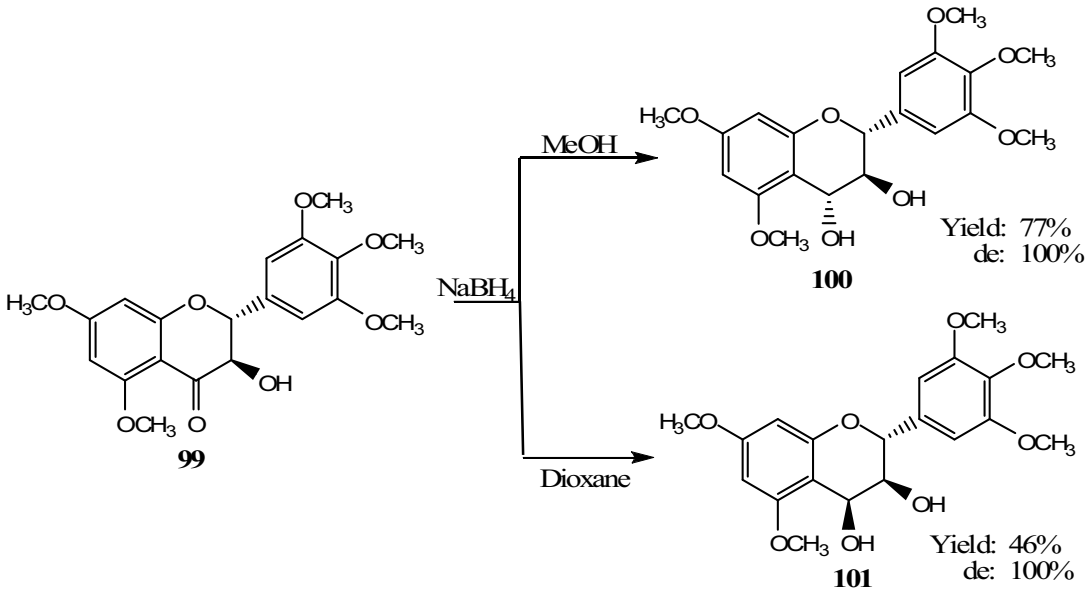
The flavan3,4-diol can be obtained in three steps from a 2'-OH chalcone by the Clark-Lewis method[60] which involves a borohydride reduction of the chalcone followed by a Lewis acid-catalyzed cyclization to a racemic flavene. The flavene is then transformed by a osmium catalyzed dihydroxylation to the 2,3-*trans*-3,4-*cis*-isomer in 58% yield (Scheme 17).

Scheme 17



A lot of research has been done to enhance the hydroxylation properties of osmium tetraoxide. The development of the AD-mix (asymmetric dihydroxylation) formulation simplified matters considerably. This mixture (a yellow salt that is quite stable in the absence of moisture) consists out of trace amounts of osmium salt and the appropriate chiral ligand, which are blended into the bulk ingredients that are ferricyanide and potassium carbonate. Depending on the chirality of the ligand, the salts are called AD-mix-α and AD-mix-β and these mixtures are frequently used in synthesis in a two phase system (*t*-BuOH : H_2O, 1:1) to introduce chirality *via* dihydroxilation.[61,62] An alternative route to obtain the flavan-3,4-diol involves a borohydride reduction of the dihydroflavonol (Scheme 18). The stereochemistry of the product is determined by the solvent used during the reaction.

Scheme 18



2.2.4 Flavan-3-ols

Although several approaches to the stereoselective synthesis of flavan-3-ols are described in literature, most of these involve a multitude of reaction steps and only gives moderate yields[60]. The easiest way to synthesize the desired enantiomer of a flavan-3-ol still remains reductive modification of an optically pure dihydroflavonol as described previously. (Scheme 18)

Since the cyclization step in the formation of dihydroflavonols remained very challenging, another approached towards the stereoselective synthesis of flavan-3-ols utilising the Sharpless asymmetric dihydroxylation, were developed. During this approach 2'-methoxymethylated dihydroretrochalcones are reduced to the 1,3-diarylpropenes, which can then be dihydroxylated (AD-mix) and subsequently cyclised to the desired flavan-3-ol. Catalytic hydrogenation (Pd on C) or $NaBH_4$ reduction would lead to the propanol, which can then be transformed into the corresponding propene by substitution of the hydroxyl group by a chloride ($SOCl_2$) followed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Asymetric dihydroxilation and acid catalysed cyclization would then lead to the desired flavanol (Scheme 19).

Scheme 19

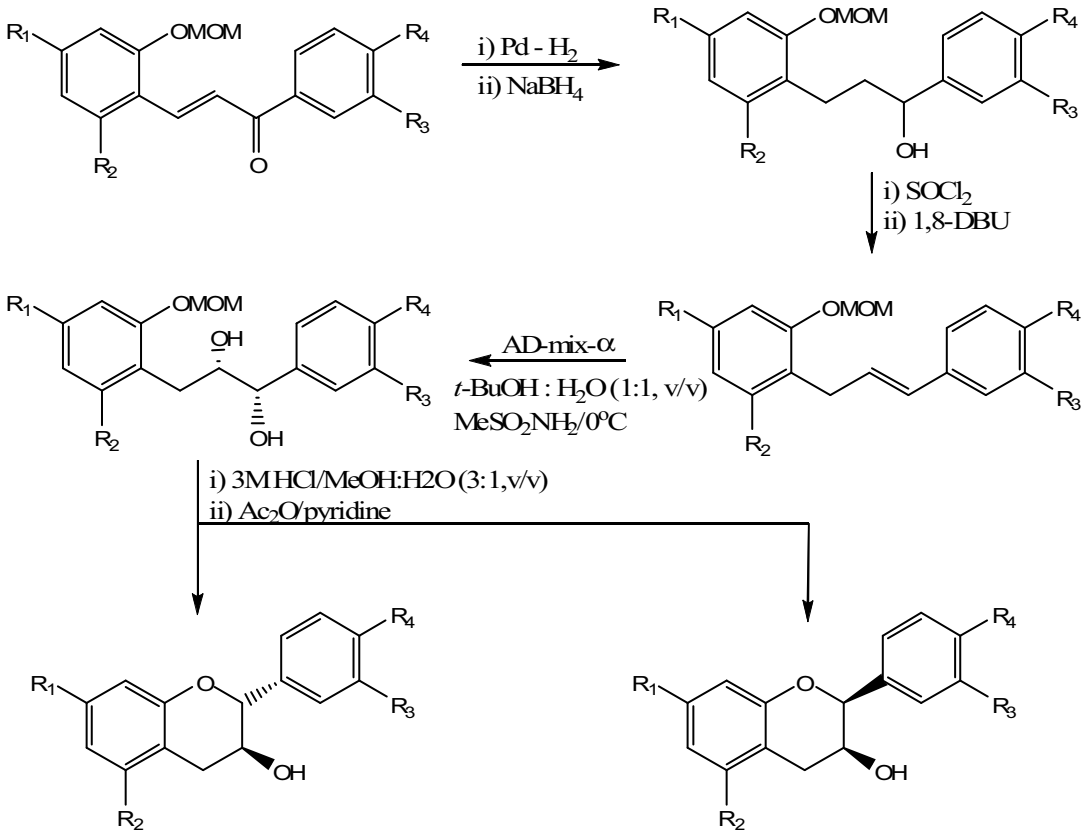


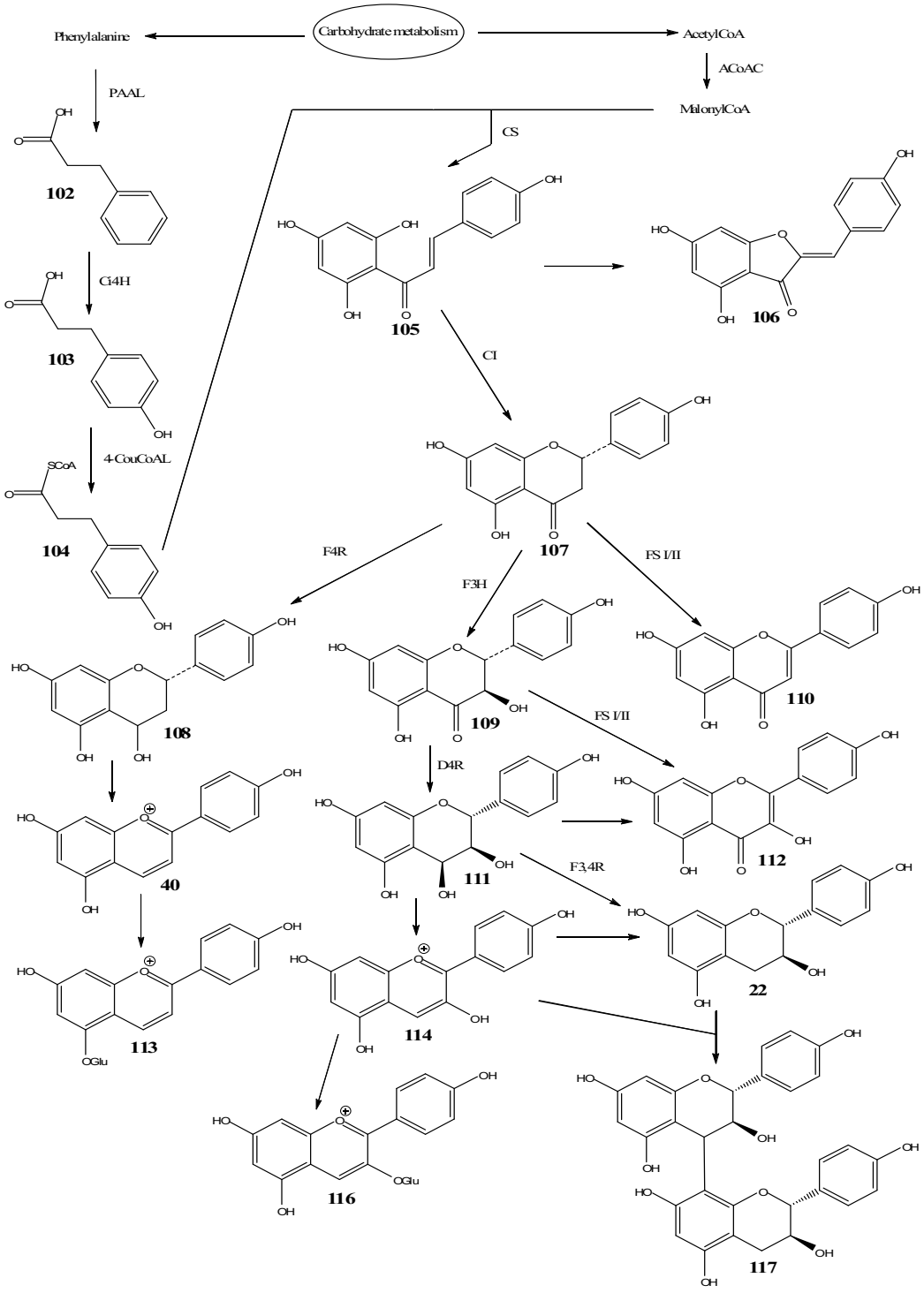
Table 7 Synthesis of *trans*-flavan-3-ols

No.	R_1	R_2	R_3	R_4	% Yield	% ee	*trans:cis*
90	H	H	H	OMe	87	99	1:0.33
91	OMe	H	H	OMe	88	89	1:0.36
92	OMe	H	OMe	OMe	82	99	1:0.32
94	OMe	OMe	H	OMe	71	99	1:0.32
95	OMe	OMe	OMe	OMe	66	99	1:0.34

2.3 Biosynthesis

A lot of research has been done to establish the biosynthetic pathways by which the flavonoids are synthesized in the plants. Enzymes catalize all of the reactions and in some instances, metals are utilized as co-catalysts in the synthesis.

Scheme 20 Biosynthesis of flavonoids



The building blocks of the flavonoid skeleton come from the carbohydrate metabolism in the plant. The aromatic amino acid phenylalanine and acetyl coenzyme A (acetyl CoA) are carbohydrate metabolites and the building blocks of the B- and A-rings of the flavonoid skeletons respectively. Phenylalanine is deaminated by the enzyme phenylalanine ammonia lyase (PAAL) to yield *trans*-cinnamate, which is hydroxylated to 4-coumarate by cinnamate-4-hydroxylase (Ci4H). The 4-coumarate is then esterified with CoA by 4-coumarate:CoA ligase (4CouCoAL). This 4-coumaroyl-CoA provides the B-ring as well as part of the heterocyclic ring of the flavonoid. Acetyl CoA and carbondioxide is converted to malonyl CoA in the presence of ATP (adenosine triphosphate) and magnesium by acetyl CoA carboxilase (ACoAC). The A-ring of the flavonoid skeleton therefore originates from malonyl CoA.

Chalcone synthase (CS) is a key enzyme in the biosynthetic pathway and catalyzes the stepwise condensation of three malonyl CoA acetate units with 4-coumaroyl-CoA to form the C_{15} skeleton of the flavonoid. The chalcone is subsequently cyclized stereospecifically by means of chalcone isomerase (CS) to form the flavanone, which serves as the main precursor to a variety of flavonoids including flavones, isoflavones, flavan-4-ols and dihydroflavonols. Flavone synthase I (FS I) and flavone synthase II (FS II) are the enzymes responsible for the introduction of a double bond between C-2 and C-3 of the flavanone to give the flavone. Reduction of the carbonyl of the flavanone by flavanone 4-reductase (F4R), yields the flavan-4-ol, which serves as precursor for anthocyanin molecules.

Dihydroflavonols are synthesized by hydroxylating flavanones at C-3 by flavanone 3-hydroxylase (F3H)[63,64] and are the precursor for the formation of a number of other 3-hydroxylated compounds like flavonols, flavan-3-ols, flavan-3,4-diols, anthocyanidins and proanthocyanidins. Flavone synthase (FS) are again responsible for introduction of the double bond between C-2 and C-3 of the dihydroflavonol skeleton leading to flavonols, while the enzyme, dihydroflavonol 4-reductase (D4R), is responsible for reduction of the carbonyl of the dihydroflavonol to from the flavan-3,4-diol. Further reduction of the flavan-3,4-diol by flavan-3,4-diol reductase (F3,4R) gives rise to flavan-3-ols. The anthocyanidins are most probably also derived from the flavan-3,4-

diol skeleton, but because of the unstable nature of anthocyanidins, this hypothesis still needs to be confirmed. Condensation of flavan-3-ol and anthocyanidin units give rise to the proanthocyanidins, but the exact mechanism for this reaction is still under debate.

Flavonoids with simple hydroxylation patterns can be modified to give rise to the overwhelming diversity of individual flavonoids isolated from natural products. Most of the enzymes involved in the modification process has high substrate specificity and usually work on the end product substrates. Hydroxylation can either be introduced in the chalcone formation step by substituting the starting material 4-coumaroyl-CoA with caffeoyl-CoA or feruloyl-CoA[65] or by means of flavonoid hydroxylases, which hydroxylates specific positions on the flavonoid skeleton. O-methyltransferase is the enzyme responsible for transferring a methyl group from S-adenosylmethionine to any position on the A-, B- and C-ring of the flavonoid skeleton. UDP sugars and UDP-glucoronic acid act as glycoside donors for the O-glycosylation of flavonoids through the activity of flavoniod O-glycosyl transferases, which are position specific. Acyltransferases are the enzymes responsible for acylation of the flavonoids. Acylation is important as it protects the molecule from degradation.

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RESULTS AND DISCUSSION

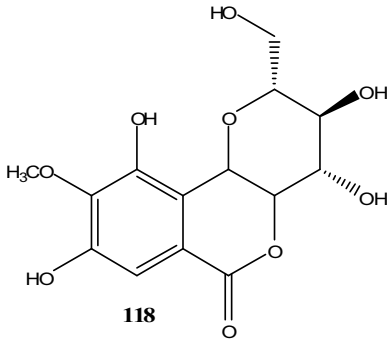
CHAPTER 3

Synthesis of 4-arylflavan-3-ol lactones

3.1 Introduction

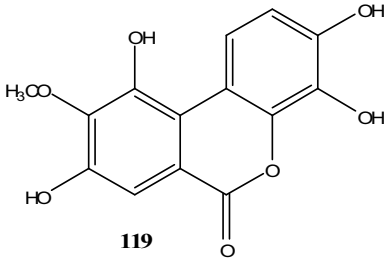
3.1.1 The heartwood composition of the African Wattle

Peltophorum africanum (or African Wattle) is the only species of the *Peltophorum* genus that is indigenous to Southern Africa. While the phenolic content of the heartwood of the African Wattle includes a wide variety of compounds such as flavonoids, dibenzopiranones (**119**), and dichromans, the flavonoids isolated are made up of only fisetinidol (**23**), fisetin (**213**), B-type proanthocyanidins (like **122**), and interestingly 4-arylflavan-3-ols (**121**). The heartwood also contained unique compounds exhibiting ether linkages between heterocyclic rings like (**123**) as well as the recently isolated 4-aryl-flavan-3-ol lactone (**124**). A nearly similar type of 4-arylflavan-3-ol lactone (**125**), differing only in the hydroxylation pattern of the B-ring, was also isolated form *Burkea africana* (Red Syringa)[1].



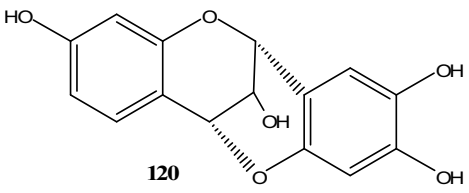
118

Bergenin



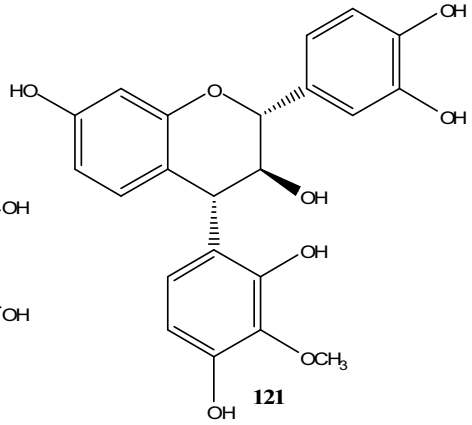
119

Dibenzopiran-6-one



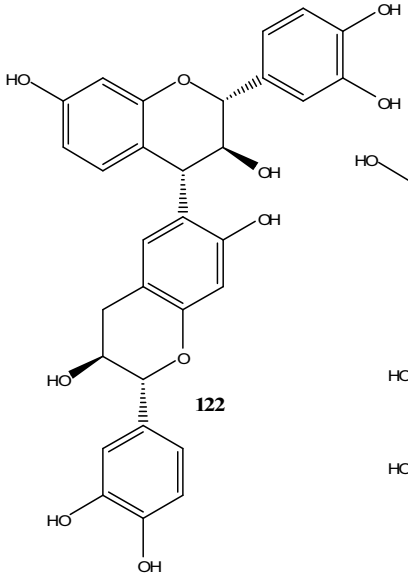
120

Dioxozine



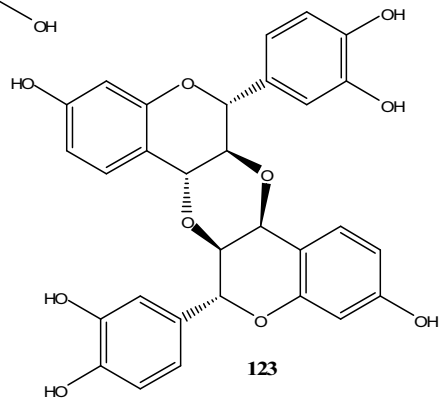
121

4-arylflavan-3-ol



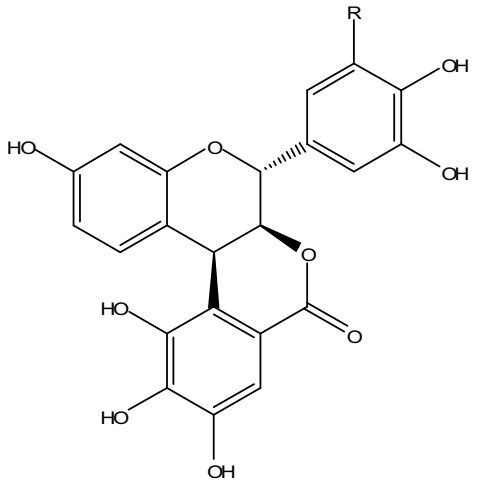
122

B-type branched dimer



123

Dioxane coupled dimer



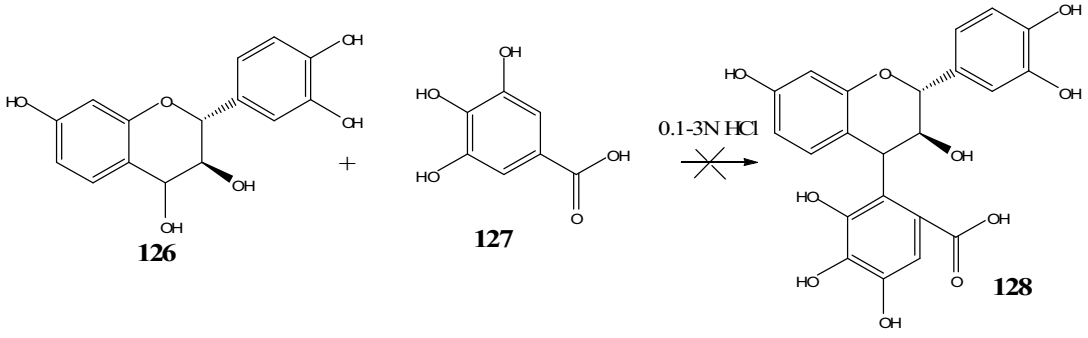
R = H (**124**) or OH (**125**)

In order to prove the structures of these two compounds and especially give unambiguous proof of the stereochemistry at the three chiral centres, several attempts at the synthesis of these compounds evolved over the past decade.

3.1.2 Previous synthetic attempts

Since the obvious way of synthesizing the 4-arylflavan-3-ol lactones would be to follow the standard methodology for the synthesis of 4-arylflavan-3-ols[2], this was the strategy in all of the initial attempts with only different ways of coupling the aryl unit to the flavan-3-ol being investigated. Bam[3] approached this coupling through the acid catalyzed reaction between mollisacacidine (**126**) and gallic acid (**127**), but no reaction could be effected (Scheme 21).

Scheme 21

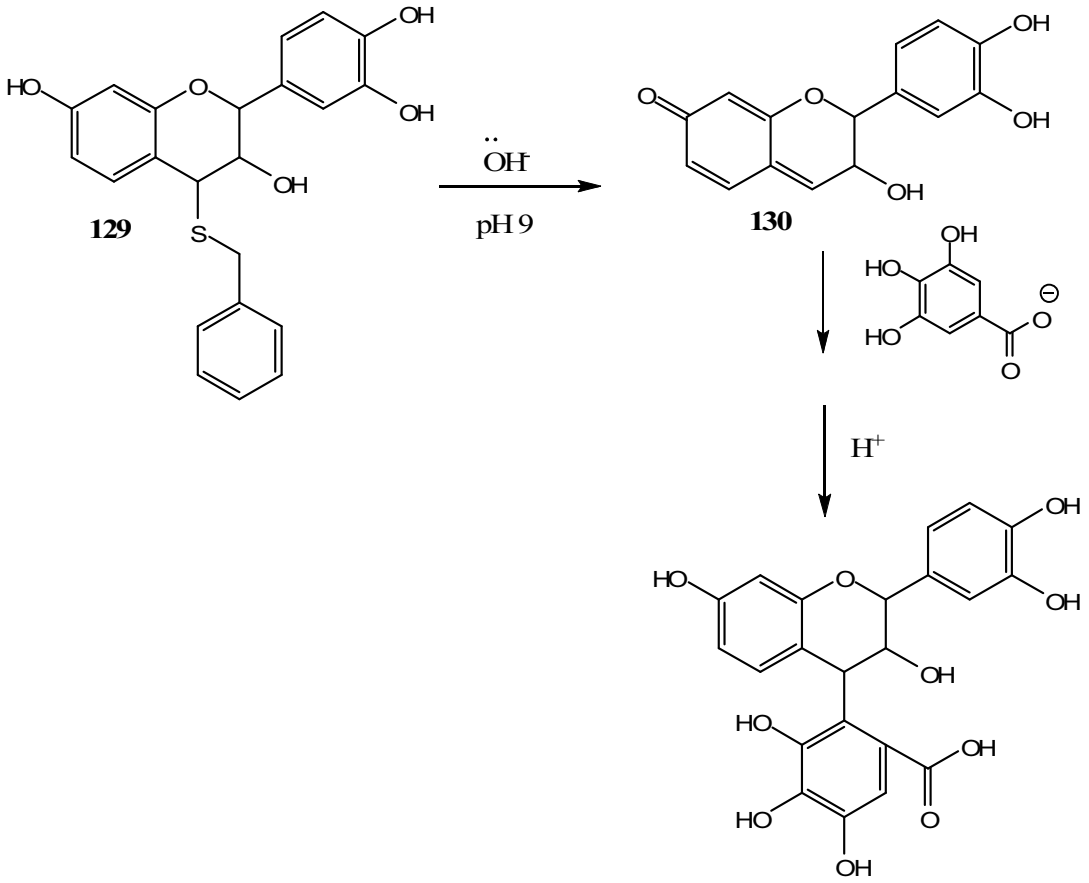


126 **127** **128**

The deactivation of the aromatic ring by the carbonyl group of the gallic acid, as well as possible protonation of the carboxylic acid function by the acid catalyst probably caused the reaction to fail. In a second approach these workers decided to enhance

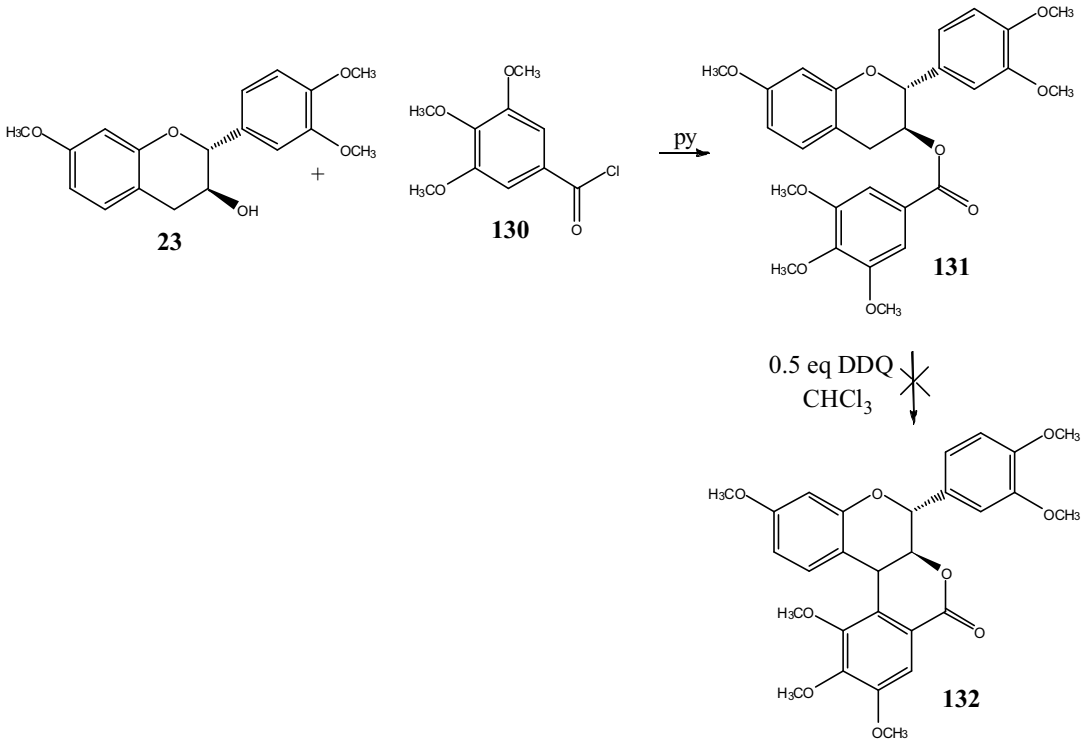
the nucleophilicity of the gallic acid entity by formation of the anion and effect the coupling through base catalysis, using an intermediate quinone methide as electrophile. Reaction between gallic acid and mollisacacidine phenylsulfide (**129**) at pH 9, however, again failed to produce any product (Scheme 22).

Scheme 22



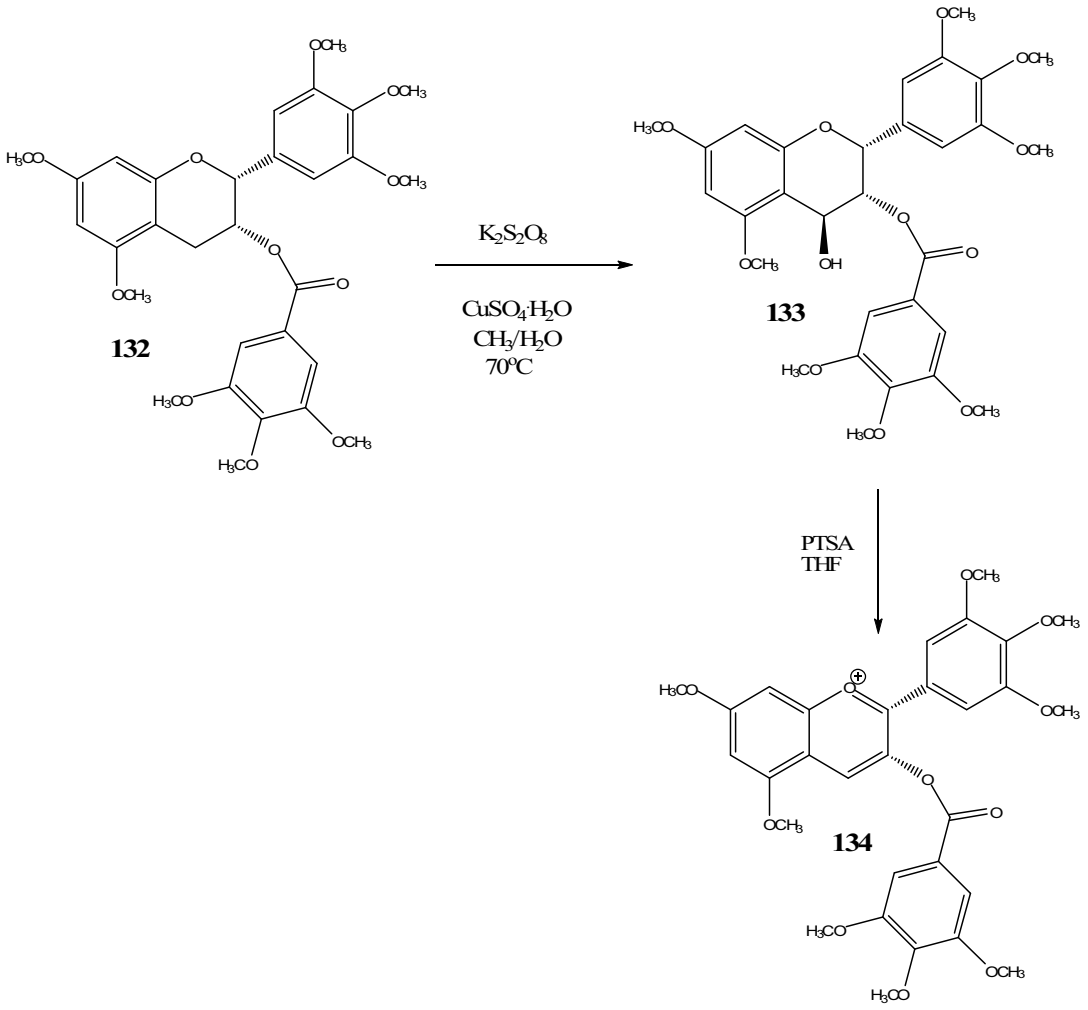
Bam's third approach at effecting the lactone formation centered around changing the intermolecular coupling to an intra molecular reaction (Scheme 22). After methylation of all the phenolic hydroxy groups on both the flavan-3-ol and gallic acid moieties, the 3-OH function of fisetinidol (**23**) could be esterified with the acid chloride of tri-O-methyl gallic acid (**130**) in 6 % yield. Subsequent generation of a C-4 carbocation on the esterified flavan-3-ol (**131**) was envisaged as the method of initiating attack by the galloyl aromatic ring on the flavan-3-ol unit. DDQ oxidation, under dry conditions, however, led to no identifiable product from the reaction.

Scheme 23

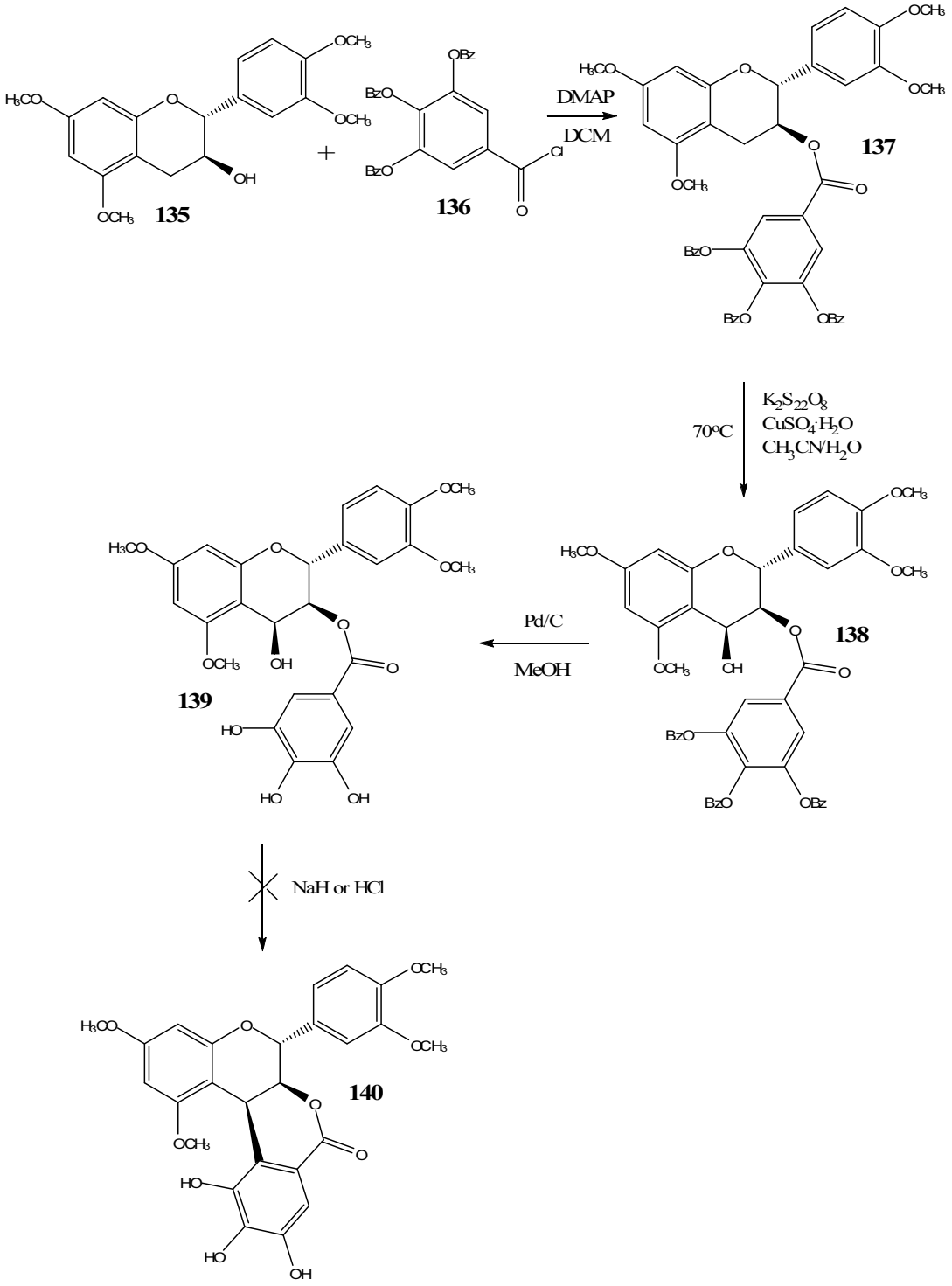


In a subsequent investigation, Botha[4] also attempted the synthesis of 4-arylflavan-3-ol lactones (**124**) and (**125**). In a model reaction, epigallocatechin gallate (**132**) was functionalised successfully at C-4 with $K_2S_2O_8$ – $CuSO_4$ in water/acetonitrile (Scheme 24). Only anthocyanidin formation was, however, observed, when cyclization with acid was attempted. Since poor nucleophilicity of the methylated pyrogallol ring was again assumed to be the cause of the failure, a free phenolic gallic acid moiety was used in the next attempt (Scheme 25). Acid catalyzed cyclization again afforded anthocyanidin or the 4-methyl ether of the substrate as only product, while base catalyzed reaction gave inseparable mixtures of highly polar compounds.

Scheme 24



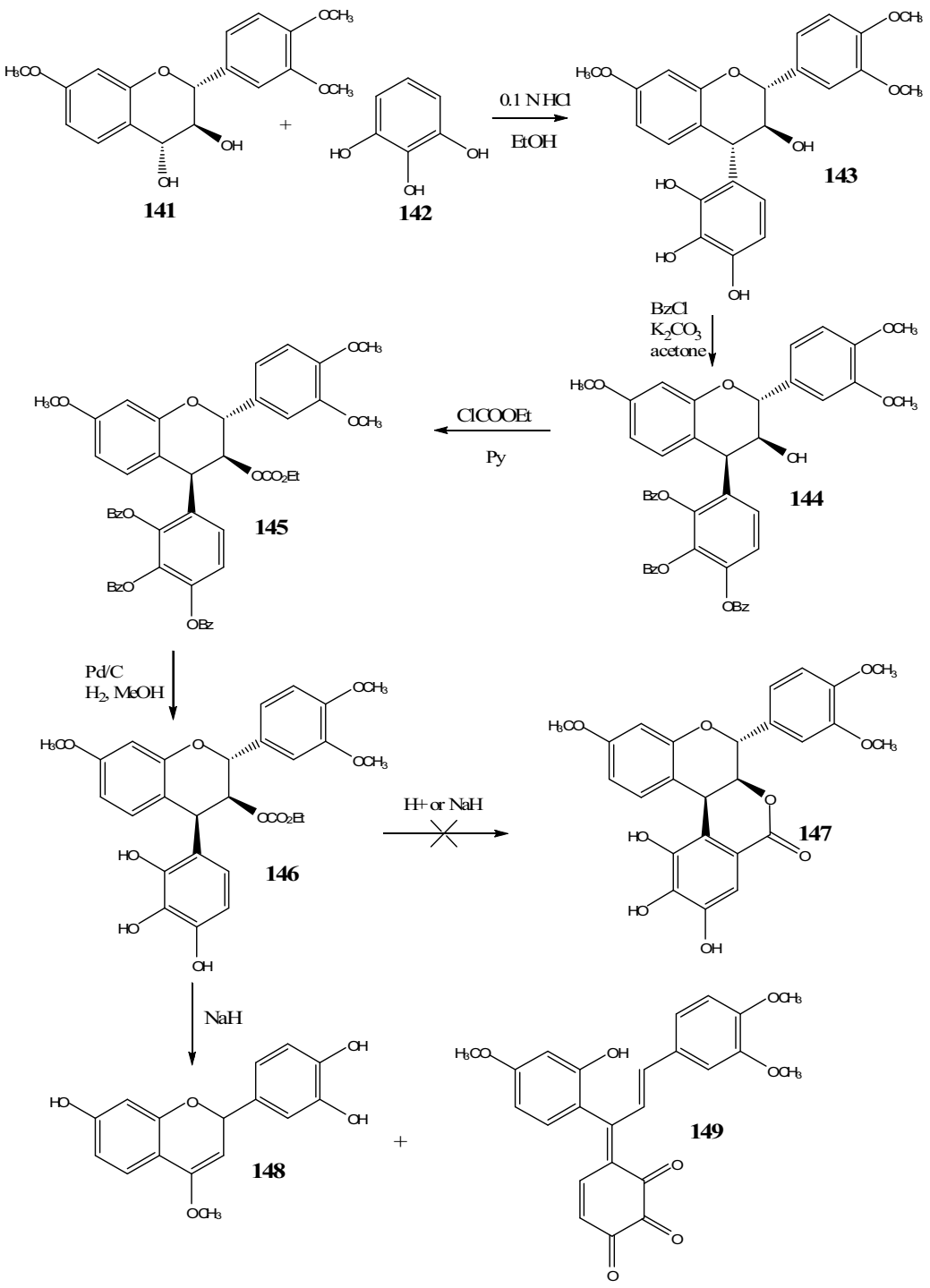
Scheme 25


135
136
DMAP
DCM
137
70°C
$K_2S_{22}O_8$
$CuSO_4 \cdot H_2O$
CH_3CN/H_2O
138
Pd/C
MeOH
139
NaH or HCl
140

Since no cyclization could be effected even with the highly nucleophylic free phenolic pyrogallol moiety, it became clear that the carbonyl function in the ester reduced the nucleophylicity of the pyrogallol ring to such an extent that nucleophylic attack became impossible. In Botha's last attempt, the carboxylic function was therefore removed from the aryl ring and it was envisaged that it could be introduced at a later stage once the pyrogallol ring has been coupled to a flavan-3,4-diol unit. Acid catalyzed coupling between pyrogallol and 3,4-dihydroxy-3',4',7-trimethoxyflavan (**141**) gave the 4-aryl flavan-3-ol **(143**), which was benzylated before reaction with ethylchloroformate introduced the ester moiety at C-3 (Scheme 26). In order to restore the nucleophylicity of the pyrogallol ring, it was debenzylated again before the formation of the lactone ring was attempted.

Acid catalyzed cyclization of (**146**) under mild conditions led to no observable product to be formed, while the anthocyanidin was again obtained when forcing conditions were employed. Base catalized reaction on the other hand led to elimination of the ethoxycarbonyl entity and opening of the heterocyclic (product **149**), as well as elimination of both the pyrogallol ring and the ethoxycarbonyl moieties (product **148**) (Scheme 26).

Scheme 26


0.1 N HCl
EtOH
BzCl
K_2CO_3
acetone
ClCOOEt
Py
Pd/C
H_2, MeOH
H+ or NaH
NaH

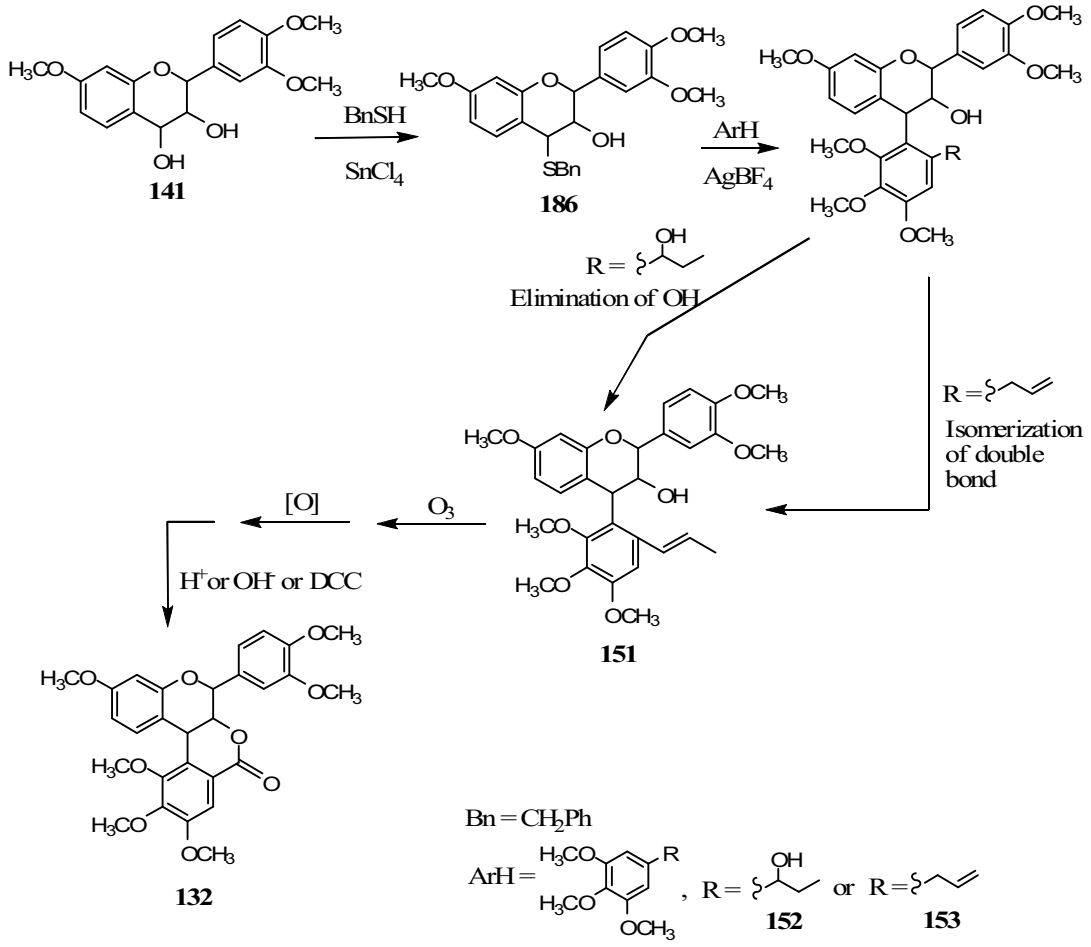
3.2 Current nucleophylic approach

Introduction and motivation

From the previous attempts at the synthesis of the 4-aryl-flavan-3-ol lactones two definitive conclusions could be arrived at: (i) The starting materials or products were sensitive to acid as well as basic reaction conditions with the consequence that the desired product(s) could not obtained. (ii) Even with three hydroxy - or methoxy groups attached to it, the electron density on the pyrogallol ring is reduced to such an extent by the carbonyl group attached, that it cannot act effectively as a nucleophile under the mild reaction conditions required to prevent unwanted decomposition of possible products. Protonation of the carbonyl group during acid catalysed processes would enhance the deactivating effect of the carbonyl group even further.

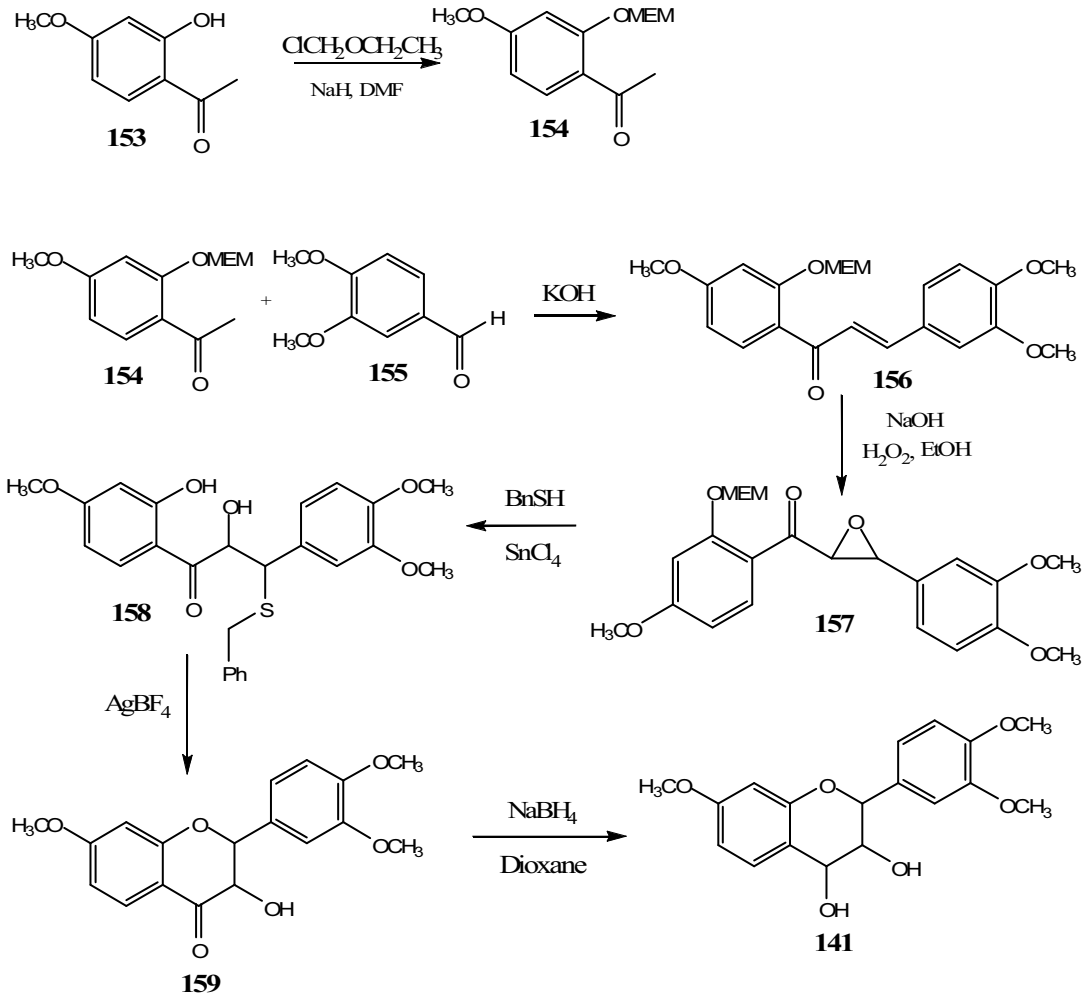
It was therefore decided to opt for a synthetic pathway, which would not include a nucleophile with a carbonyl attached to it; thus changing the substituent on the pyrogallol ring to a 1-hydroxypropyl - or even better an allyl group. If the hydroxypropyl substituent is used, the required carbonyl could be generated by elimination of the OH, followed by ozonolysis of the double bond to afford the aldehyde, which could then be oxidized and subjected to the esterification using mild and/or neutral conditions. In the case of an allyl substituent it was envisaged that isomerisation of the double bond to C-1 followed by the same reaction sequence would lead to the desired product. In order to eliminate the use of acid, it was decided to employ a thiophylic Lewis acid together with activation of C-4 (C-ring of the flavan unit) by means of a mercaptan leaving group. This methodology is illustrated in Scheme 27 below.

Scheme 27



Since the starting materials for this synthetic strategy was not available, both the flavan-3,4-diol as well as the 3,4,5-trimethoxyphenylpropan-1-ol had to be synthesized. Due to the fact that the methodology developed by Bezuidenhoudt and Ferreira[5] has the potential of giving the flavan-3,4-diol in enantiomerically enriched form, it was decided to follow this route towards the required flavan-3,4-diol (**141**) (Scheme 28).

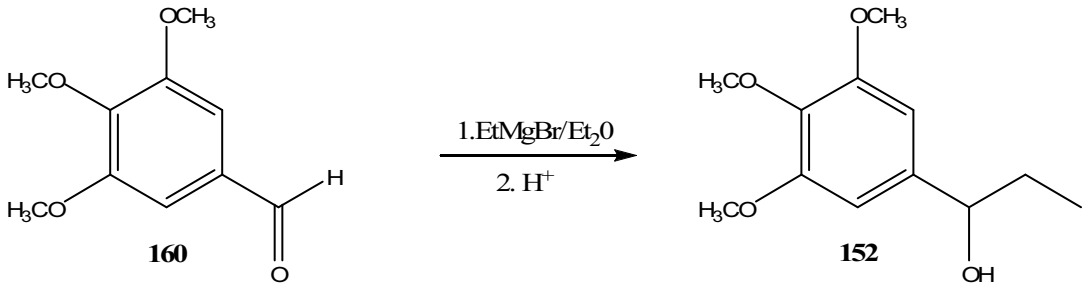
Scheme 28



3.2.1 Synthesis of 1-(3',4',5'-trimethoxyphenyl)-propan-1-ol

The phenylpropanol fragment (**152**) required for the synthesis was easily obtained from 3,4,5-trimethoxybenzaldehyde (**160**) by Grignard reaction with ethylmagnesium bromide in 64% yield (Scheme 29). Apart from the appropriate aromatic resonance [δ 6.46 (2 H, s, H-2,6)], the ^{1}H NMR spectrum (Plate 1) of (**152**) displayed signals from three methoxy groups [δ 3.76 and 3.73 (each s)] as well as a 1-hydroxypropyl group, [δ 4.40 (1 H, t, J = 6.6 Hz, H-1'), 2.70 (1 H, br s, 1'-OH), 1.66 (2 H, m, 2'-CH_2), and 0.84 (3 H, t, J = 7.4 Hz, 3'-CH_3)]; thus confirming the product as 1-(3',4',5'-trimethoxyphenyl)propan-1-ol.

Scheme 29



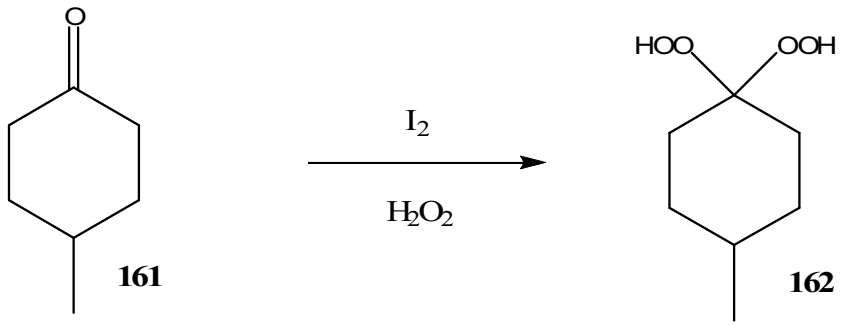
3.2.2 Synthesis of 2'-ethoxymethoxy-3,4,4'-trimethoxychalcone (156)

In order to prevent unwanted preliminary cyclization and side product formation during the synthesis of the 3-hydroxyflavanone (**159**) enroute to the flavan-3,4-diol, 2-hydroxy-4-methoxyacetophenone was protected as the ethoxymethyl ether using the sodium hydride/DMF (N,N-dimethylformamide) methodology[6] [^{1}H NMR (Plate 2) δ 7.80 (1 H, d, J = 8.76 Hz, H-6) 5.31 (2 H, s, O$\underline{CH_2}$OCH$_2$CH$_3$) 3.76 (2 H, q, J = 7.07 Hz, OCH$_2$O$\underline{CH_2}$CH$_3$) 1.25 (3 H, t, J = 7.07 Hz, OCH$_2$OCH$_2$$\underline{CH_3}$)]. Standard Claissen-Schmidt[7] condensation of the protected acetophenone with 3,4-dimethoxybenzaldehyde yielded the polyoxygenated chalcone (**156**) as yellow crystals (76 %). The ^{1}H-NMR spectrum (plate 3) of the chalcone showed the typical α- and β-proton resonances as doublets at δ 7.62 and 7.37 ppm, respectively. From the large coupling constants exhibited by these doublets, 15.75 Hz, it can be concluded that the *trans*-isomer was formed exclusively during the reaction, while the presence of the ethoxymethyl protecting group at C-2' was confirmed by the presence of a singulet, a quartet and a triplet at δ 5.31, 3.76, and 1.23 ppm respectively in the ^{1}H-NMR spectrum.

3.2.3 Epoxidation of chalcone (156)

Although the standard epoxidation conditions of H_2O_2 in a basic ethanolic medium usually works well for the epoxidation of a variety of chalcones, this reagent does not always work that well when stereoselective epoxidations is to be performed. This can probably be ascribed to the fact that chiral induction in these reactions is usually

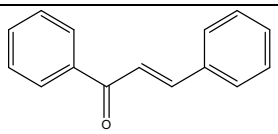
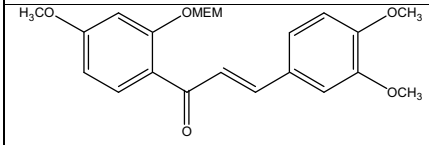
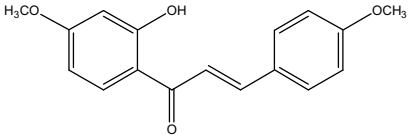
obtained from poly-amino acids in a tri-phasic system[8] consisting of a solid phase (the poly-amino acid), an organic phase containing the substrate, and the aqueous peroxide phase. It was therefore decided to investigate other epoxidizing agents, especially those that would be soluble in organic solvents and could be altered to induce chirality into the substrate molecule, and compare these to the standard H_2O_2 system. 4-Methylcyclohexylidene-bishydroperoxide[9], which could easily be prepared by reaction of 4-methylcyclohexanone with H_2O_2 in the presence of I_2, was identified from literature as a reagent with this potential and was therefore investigated for the epoxidation of a few chalcones with typical natural product substitution patterns.



161 **162**

Since epoxidations with 4-methylcyclohexylidenebishydroperoxide requires the presence of a base like KOH[9], the first objective in the quest for a fully homogeneous epoxidation system based on this reagent, was the identification of a suitable organic base that could replace the KOH in the reaction. Thus strong organic bases like DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene) and Protonsponge (1,8-bis-[dimethylamino]-naphthalene) was compared to KOH in the epoxidation of unsubstituted and two chalcones exhibiting typical natural product substitution patterns (Table 8).

Table 8

Substrate	H_2O_2 and KOH*	4-Methylcyclohexylidenebishydroperoxide		
		KOH#	DBU**	Protonsponge®
	Yield: 100%, 4 hours	Yield: 90%, 24 hours	Yield: 80%, 24 hours	-
	Yield: 90% 12 hours	Yield: 85% 24 hours	Yield: 60% 24 hours	
		Yield: 65% 24 hours		

* Solvent: EtOH # Solvent: 1,4-dioxane ** Solvent: DCM

From the results obtained, it is clear that the unsubstituted *trans*–chalcone gave the best results with all of the systems evaluated. As can clearly be seen from the KOH and 4-methylcyclohexylidenebishydroperoxide reactions the reactivity of the chalcones decreases with higher substitution on the aromatic rings or free phenolic substituents that would render the β-position less electrophilic. When compared to KOH, DBU gave lower yields for both the chalcones tested, which is probably explicable in terms of it being a weaker base than KOH and thus not deprotonating the reagent to the extent it would be possible with the stronger base. While both KOH and DBU gave acceptable yields in the epoxidation of unsubstituted chalcone with 4-methylcyclo-hexylidenebishydroperoxide as oxidant, reactions in the presence of Protonsponge completely failed. Since Protonsponge is known for its ability to mop up protons, this result probably is an indication of it not being such a good base in reactions where the removal of a proton is required.

Dimethyldioxirane (DMDO), another useful epoxidizing agent, that has been used for a variety of olefins including α,β-unsaturated compounds such as chalcones (even

highly substituted chalcones), also has the potential to be altered into a chiral epoxidizing agent. Added advantages of DMDO as epoxidizing agent are very mild reaction conditions as well as the end products being only the desired epoxide and acetone, that could easily be removed by distillation.

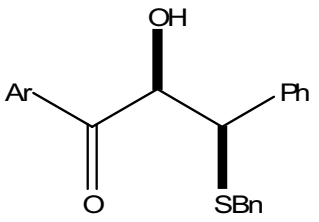
The 2'-ethoxymethyl-3,4,4'-trimethoxychalcone was therefore epoxidized with DMDO and the 2'-ethoxymethoxy-3,4,7'-trimethoxychalcone epoxide obtained in 98% yield, showing that this reagent indeed works well for highly substituted chalcones. The two large doublets of the α- and β-protons in the ^{1}H NMR spectrum of the chalcone was replaced by two small doublets at 4.29 and 3.91 ppm (J = 1.9 Hz) in the ^{1}H NMR spectrum (plate 4) of (**157**); thus confirming the structure of the product to be that of the epoxide.

3.2.4 Cyclization of epoxide (157) and formation of the flavan-3,4-diol

Initial attempts to cyclize chalcone epoxides to the corresponding dihydroflavonols (DHF) described in literature were hampered by low yields and the formation of various side products; isoflavone being the major product from the reaction. The formation of these side-products could be attributed to two key problems, i.e. (a) opening of the oxirane ring under mild conditions and (b) removal of the 2' protecting group which should happen before or at least simultaneously with the opening of the epoxide. Confronted with this problem, Van Rensburg *et al*[5] decided to attempt this synthetic sequence in a step wise manner rather than to try and tune the reaction to the point that both reactions would occur simultaneously. Since epoxides and especially chalcone analogues, are highly reactive and can easily be opened up by nucleophiles under acid catalysis, these workers decided to utilise the excellent nucleophilic - and nucleofugic properties of mercaptans in solving this problem. In order to be in a position to remove the 2' protecting group under acid catalysis and not effect rearrangement to the isoflavone, the epoxide was opened up with a sulphur nucleophile first. Once the 2' protecting group was removed, formation of the heterocyclic ring was effected by utilizing the nucleofugic properties of the mercaptan through treatment with a thiophilic Lewis acid. In utilizing tin tetrachloride as Lewis acid together with benzylmercaptan as nucleophile, these workers were able to

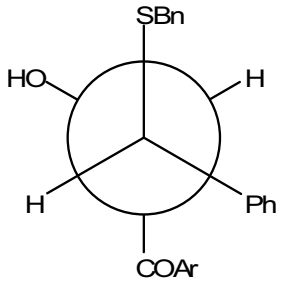
effectively cleave the C_β-O bond of the epoxide at -20°C, while removal of the 2'-methoxymethyl group followed by allowing the reaction mixture to warm up to 0 °C.,

Application of the Van Rensburg methodology to 2'-ethoxymethyl-3,4,4'-trimethoxychalcone epoxide led to smooth opening of the epoxide ring as well as removal of the ethoxymethyl group, but gave the α,2'-dihydroxy-3,4,4'-trimethoxy-β-benzylthioldihydrochalcone (**158**) as an inseprable diastereomeric mixture of the *syn* and *anti* isomers (ratio = 0.67:1) in 61% yield. The ^{1}H-NMR spectrum (plate 5A) of the diastereomeric mixture (indicated as A and B) showed two pairs of two ABX systems in the aromatic region {δ 7.30 [1H, d, J = 1.92 Hz, H-6'(A or B)], 7.06[1H, d, J = 2.18 Hz, H-6'(A or B)], 7.02 (1H, d, J = 1.42 Hz, H-2(B)], 6.87 [1H, dd, J = 1.42 and 8.17 Hz, H-6(B)] 6.82[1H,d, J = 8.17 Hz, H-5(B)] 6.77[1H,d, J = 1.92 Hz, H-2(A)] 6.71[1H,d, J = 8.23 Hz, H-5(A)], 6.53 [1H,dd, J = 1.92 and 8.23 Hz, H-6(A)] 6.48 [1H, d, J = 2.47 Hz, H-3'(B)] 6.42 [1H, d, J = 2.50 Hz, H-3'(A)] 6.35 [1H, dd, J = 2.47 and 8.99 Hz, H-5'(B)] and 6.30 [1H, dd, J = 2.50 and 8.99 Hz, H-5'(A)]} as well as two signals from the α – {δ 5.21 [1H, m, H-α(B)] and 5.21 [1H, d, J = 2.9 Hz, H-α(A)]} and β-protons {δ 4.02 (1H, d, J = 2.9 Hz, H-β(A)] and 4.00 (1H, d, J = 7.6 Hz, H-β(B)]}, respectively together with the required benzyl resonances. If it is assumed that the *syn* and *anti* products would have preferred conformations as indicated in fig nr with the thiobenzyl and A-rings in the *anti* periplanar orientation, it can be concluded that the *syn* product should display an H-α,β coupling constant of ca 8 Hz, while that for the *anti* product would be approximately 2 Hz. Since coupling constants of 2.9 and 7.6 Hz was in fact observed for the two isomeric products in the ^{1}H NMR spectrum, the major product from the reaction (indicated as A) should be the *anti* product with the minor one (B) being the *syn* isomer.

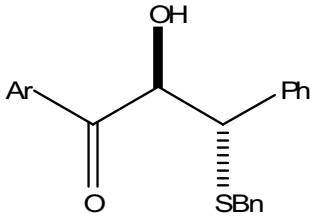


syn-product

≡

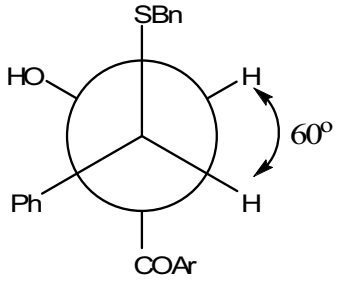


Dihedral angle = 180°
Coupling constant ~ 8 Hz



anti-product

≡

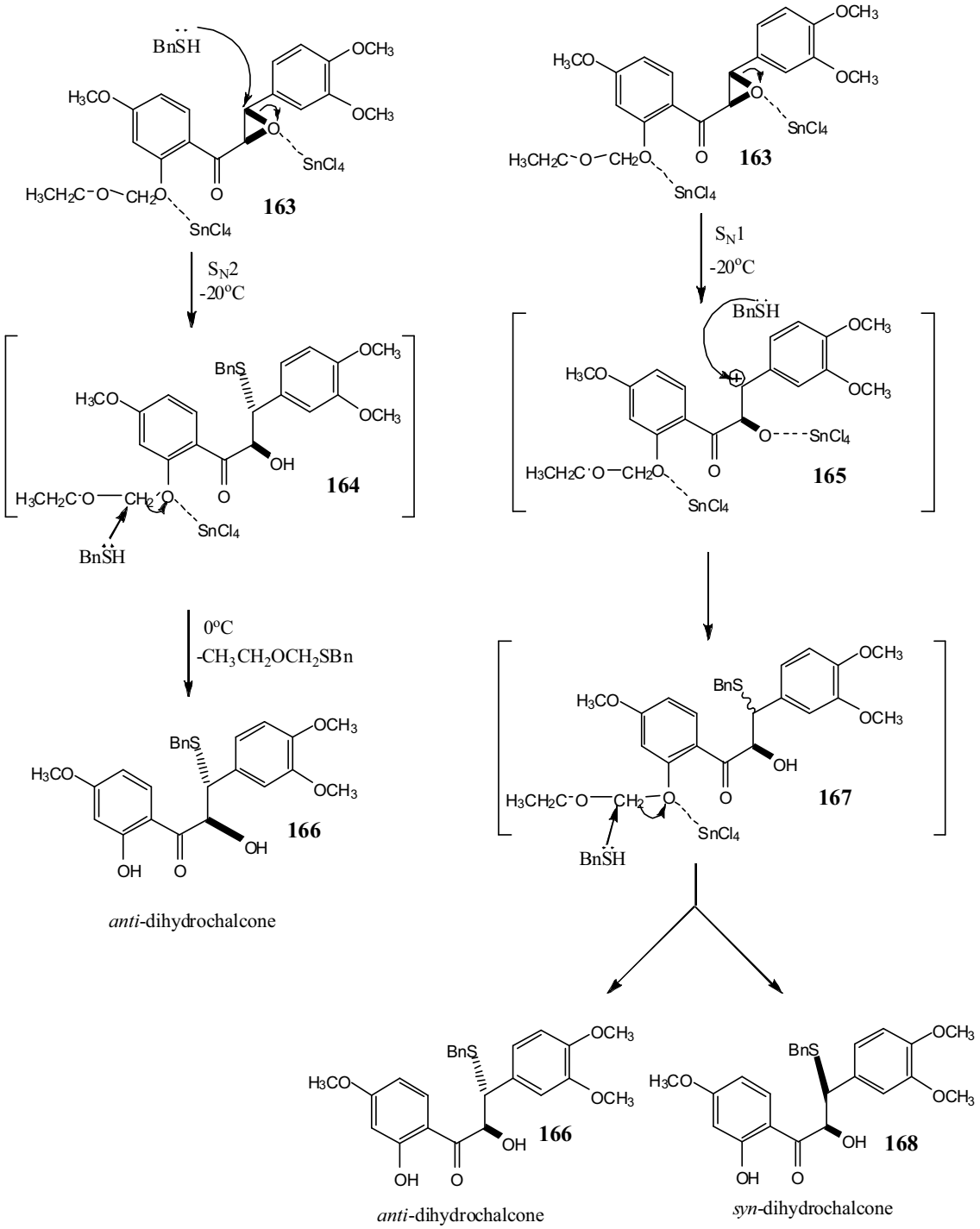


Dihedral angle = 60°
Coupling constant ~ 2 Hz

.

The observed ratio of the isomers indicates that a mixed S_N2/S_N1 reaction mechanism for the oxirane ring cleavage prevails during the reaction.

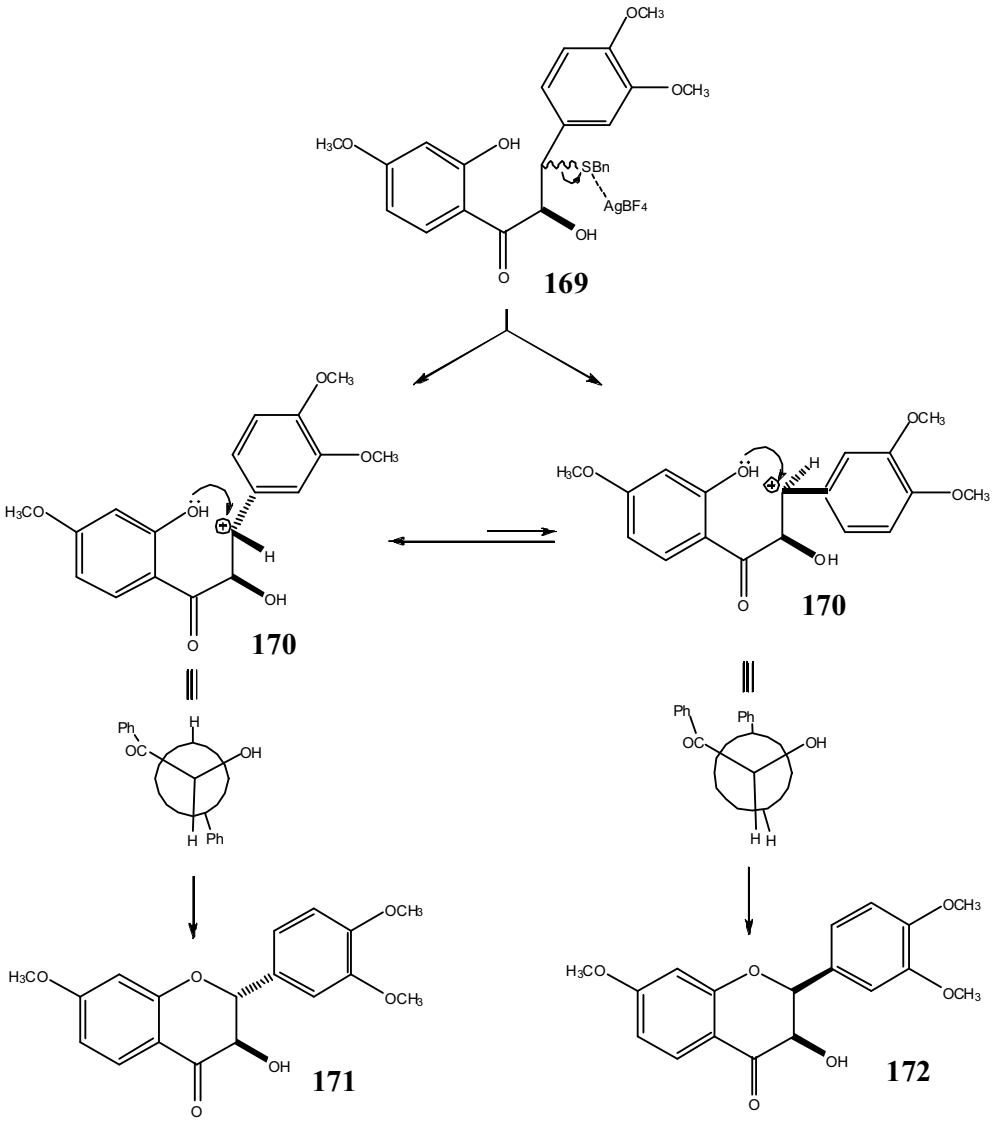
Scheme 30



The final step in the formation of the dihydroflavonol, i.e. cyclization of the mercaptodihydrochalcone (**158**) was effected by treatment with the thiophilic Lewis

acid, $AgBF_4$ in DCM. Intramolecular substitution at C_β gave only the *trans*-dihydroflavonol (**159**) in 83 % yield. The large coupling constant (J = 12.2 Hz) between H-2 and H-3 [δ 5.06 (1 H, d, J = 12.22 Hz, H-2) and 4.58 (1 H, dd, J = 1.53, 12.22 Hz, H-3)] in the ^1H NMR spectrum (plate 6) of (**159**) indicated the product to be the *trans*-isomer of the dihydroflavonol. The formation of only the *trans*-dihydroflavonol from both the *syn* - and *anti* isomers of the starting material, may be explained by an S_N1 mechanism where the preferred conformation of the carbocationic intermediate (**170**), which cyclizes to form the thermodynamically more stable 2,3-*trans*-dihydrflavonol, as illustrated in Scheme 31 below, plays the decisive role.

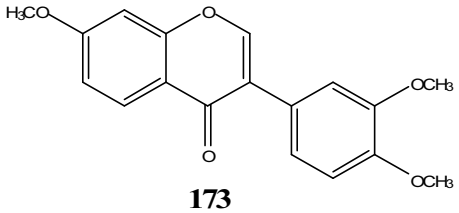
Scheme 31



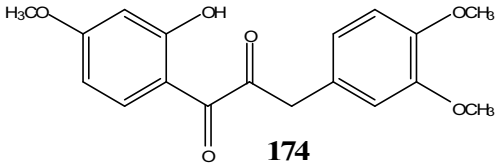
2,3-*trans*-dihydroflavonol 2,3-*cis*-dihydroflavonol

Despite all precautions for preventing the formation of side-products the dihydroflavonol was, however, accompanied by two minor products (**173**) and (**174**) in 8 and 7 % yield respectively. The ^{1}H NMR spectrum (plate 7) of the first product (**173**, R_f 0.2) showed, apart from the expected aromatic protons, a singulet at δ 7.95 ppm, which indicated it to be the corresponding isoflavone, while the spectrum (plate 8) of the second product (**174**, R_f 0.14) displayed a singulet integrating for two protons at δ 4.17 ppm. Since no other heterocyclic resonances were visible and the aromatic region only contained the expected two ABX systems [δ 7.77 (1 H, d, J =

8.83 Hz, H-6'), 6.85 (1 H, d, J = 8.07 Hz, H-5), 6.83 (1 H, dd, J = 1.65, 8.07 Hz, H-6), 6.46 (1 H, dd, J = 2.40, 8.83 Hz, H-5')] this side-product could only be the α-diketone (**174**)



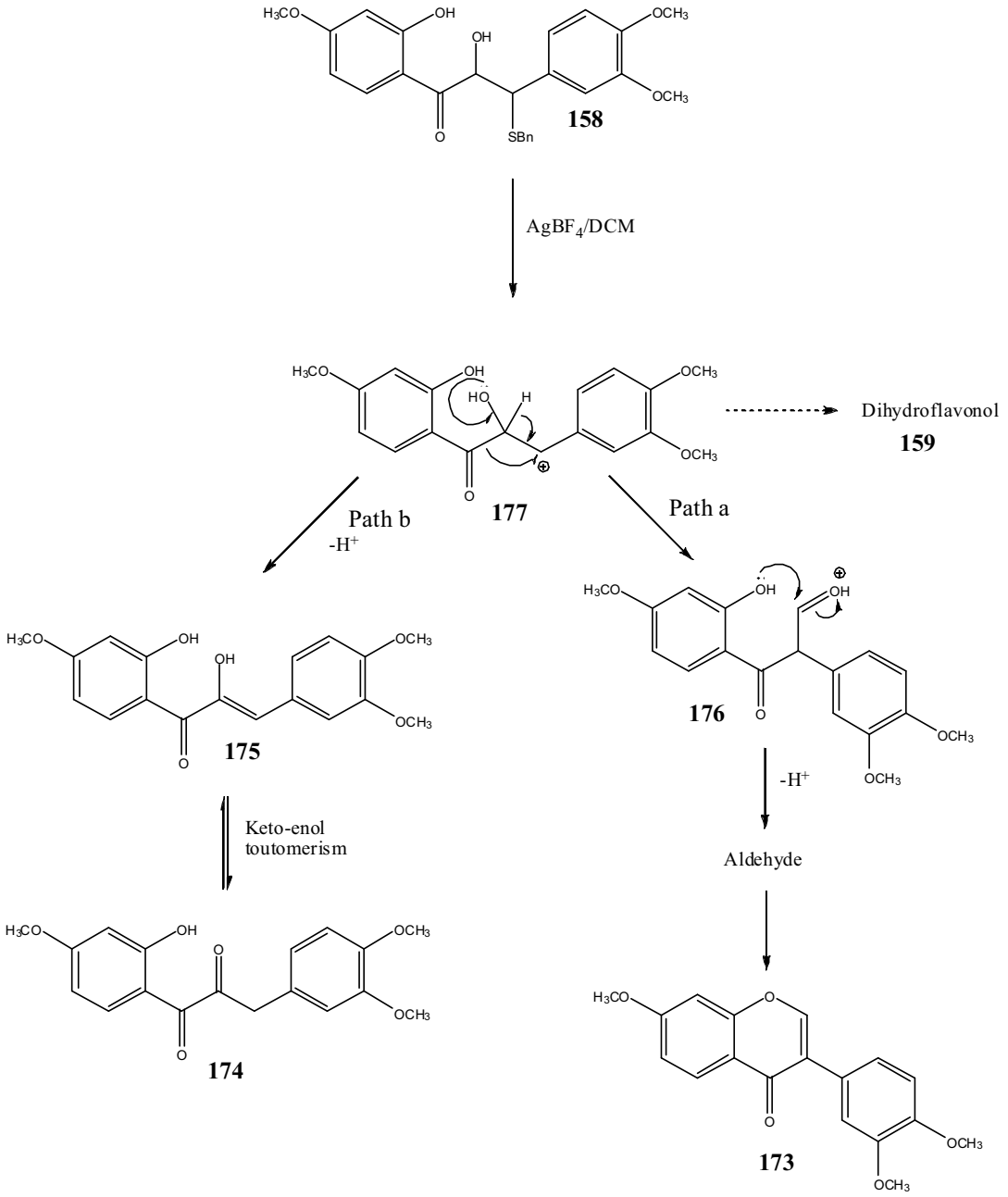
173



174

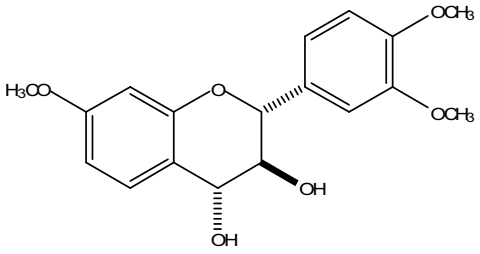
The transformation of the mercaptodihydrochalcone into the isoflavone and α-diketone is probably explicable in term of the mechanisms as indicated in Scheme 32. For both the isoflavone and α-diketone products it can be envisaged that the C_β-S bond is activated by $AgBF_4$ and a carbocation intermediate formed at C_β. Aroyl migration[10] (path a) followed by attack of the 2-hydroxy function of the A-ring on the incipient aldehyde and subsequent water elimination would then lead to the isoflavone. Formation of the α-diketone probably comes from the elimination of the acidic α-hydrogen form the carbocation intermediate (path b), which then leads to the α,β-unsaturated alcohol (**175**), the enol form of the α-diketone.

Scheme 32



The final flavonoid starting material for the formation of the lactone, the flavan-3,4-diol (**141**), was prepared in quantitive yield by reduction of the C-4 carbonyl by reaction with sodium borohydride in ethanolic solution. The ^{1}H NMR spectrum (plate 9) of 3',4',7-trimethoxyflavan-3,4-diol (**141**) displayed, apart from the expected aromatic protons, three signals for the heterocyclic part of the molecule. H-4

resonated as a doublet of doublets (J = 5.6 Hz and 7.9 Hz) at δ 4.87 ppm, while H-3 and H-2 were present as a multiplet (at δ 3.91 ppm) and doublet [at δ 4.81 (J = 9.91 Hz)] respectively. Addition of D_2O to the spectrum led to the resonance allocated to H-4 to change into a doublet (J = 7.9 Hz) thus indicating the observed second coupling to come from the OH group bound to the same carbon atom and the relative configuration of the product to be 2,3-*trans*-3,4-*trans*.

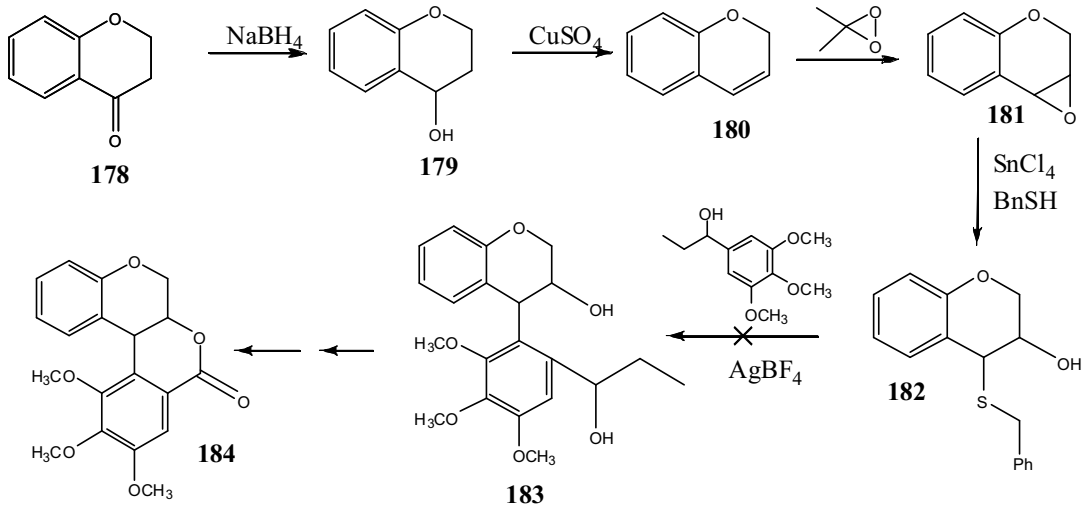


141

3.2.5 Coupling of the flavan-3,4-diol (141) to the pyrogallol moiety

Since the flavan-3,4-diol only became available after quite a number of steps, it was decided to investigate the coupling of the pyrogallol unit to a model compound, the chromanol (**179**), first (Scheme 33).

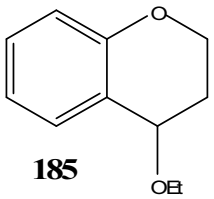
Scheme 33



Sodium borohydride reduction of commercial 4-chromanone yielded the chromanol (**179**) [^{1}H NMR (Plate 10) δ ppm 7.21 (1 H, dd, J = 1.71, 7.59 Hz, H-8), 7.14 (1 H, ddd, J = 0.58, 1.71, 6.93 Hz, H-7), 7.06 (1 H, dd, J = 1.49, 8.24 Hz, H-5), 6.88 (1 H,

ddd, J = 1.51, 6.92, 7.63 Hz, H-6), 4.42 (1 H, t, J = 4.23, H-4), 4.07 and 3.89 (2 H, ddd, J = 3.93, 4.71, 10.99 Hz, 2 H-2), 1.72-1.55 (2 H, m, 2 H-3)] in quantitative yield. The reduction step was followed by anhydrous copper sulphate dehydration to the the chromene[#] (**180**) [^{1}H NMR (Plate 11) δ ppm 7.10 (1 H, ddd, J = 1.80, 7.33, 8.02 Hz, H-7), 6.96 (1 H, dd, J = 1.80, 7.43 Hz, H-8), 6.83 (1 H, dt, J = 1.83, 7.40 Hz, H-6), 6.78 (1 H, td, J = 1.14, 8.05 Hz, H-5), 6.42 (1H, dtd, J = 0.81, 1.89, 9.83 Hz, H-4), 5.77 (1 H, dt, J = 3.56, 9.83 Hz, H-3), 4.83 (2 H, dd, J = 1.88, 3.57 Hz, 2 H-2)], which was obtained in 70% yield.

Despite numerous attempts with several epoxidizing agents like basic H_2O_2, 4-methyl-cyclohexylidenebishydroperoxide[9], and dimethyldioxirane[11], the formation of the chroman epoxide could, however, not be brought about successfully; the only decernable product being the 4-ethoxy ether (**185**).



185

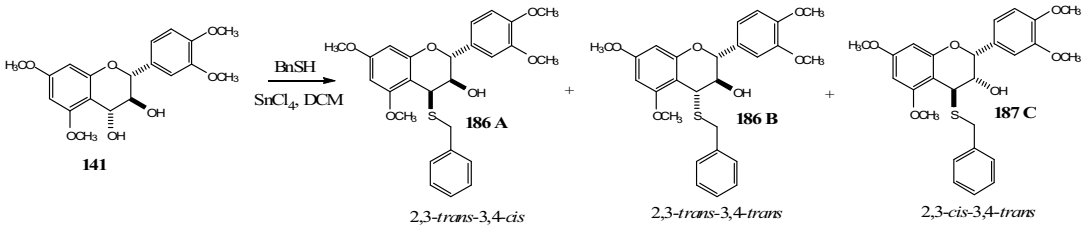
Direct synthesis of the chroman-3,4-diol, which could also be turned into the 4-benzylthio derivative (**182**), was therefore investigated as an alternative to the epoxidation strategy. Application of the Sharpless dihydroxylation methodology (potassium osmium tetraoxide and N-morpholine-N-oxide in *t*-butanol)[12], however, also failed to produce the chroman-3,4-diol. While the the failure of the chromene to undergo oxidation by both methods, remains inexplicable at this stage, it was decided not to spend more time on the model reaction, but to utilize the real target flavan-3,4-diol (**141**) in subsequent coupling reactions instead.

Due to the reasons mentioned earlier (*vide supra*) the flavan-3,4-diol was therefore converted into the 4-benzylthio derivative (**186**) by treatment with $SnCl_4$ in DCM leading to an inseparable diastereomeric mixture of the products in 57 % yield. In

[#] It has to be noted that the chromene shows a high tendency towards polymerisation and has to be handled with care.

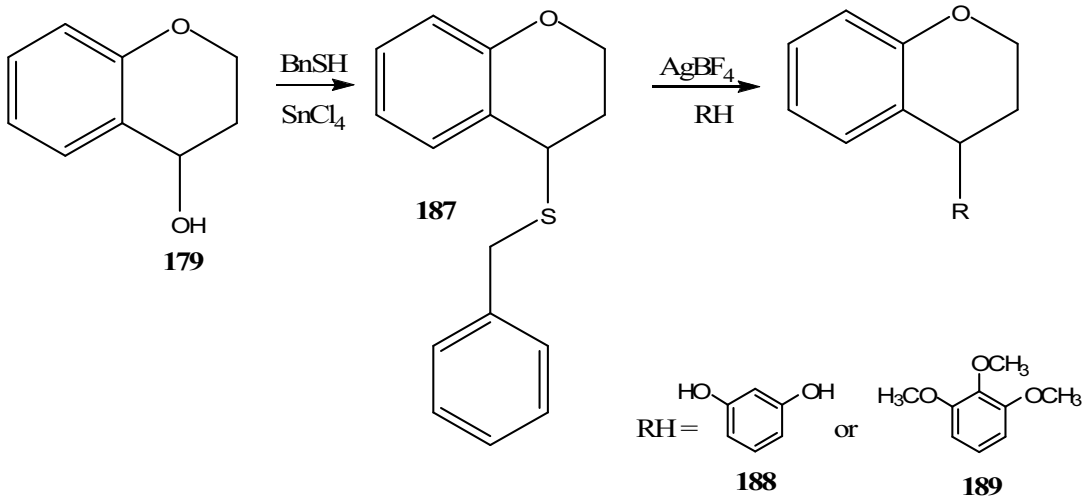
addition to six ABX systems, which could not be clearly identified, the ^{1}H NMR spectra (plate 13A) of the diastereomeric mixture displayed three sets of resonances from heterocyclic protons at δ 4.93 (d, J = 8.9 Hz), 4.67 (d, J = 9.5 Hz), and 3.96 (d, J = 3.0 Hz) respectively for the H-2 protons, δ 4.24 (dd, J = 4.64 and 8.91 Hz), 3.83 – 3..81 (m), and 4.27 (dd, J = 3.18 and 10.54 Hz) respectively, H-3 protons, and δ 4.12 (d, J = 4.64 Hz), 4.05 (d, J = 9.24 Hz), and 3.41 (d, J = 10.54 Hz) respectively for the H-4 protons; all of which being identifiable by correlations in the 2D COSY spectrum (plate 13C) of the mixture. From the coupling constants displayed by the H-2 and H-4 protons together with the integral values of these peaks it was concluded that the 2,3-*trans*-3,4-*cis* (**186 A**), 2,3-*trans*-3,4-*trans* (**186 B**), and 2,3-*cis*-3,4- *trans* (**186 C**) products were formed in a ca 1.3 : 0.9 : 0.5 ratio (Scheme 34). Since the reaction between the flavan-3,4-diol and the benzylmercaptan was performed on pure 2,3-*trans*-flavandiol, the formation of the 2,3-*cis* isomer of the products seems very strange and can only be explained in terms of the Lewis acid catalyst causing some opening and re-closing of the heterocyclic C-ring during the thioether formation process. Final coupling of the thioether (**186**) to the 2,3,4-trimethoxypropanol (**152**) through the action of the thiophilic Lewis acid, $AgBF_4$, could, however, not be effected, so attention was again reverted to some model studies.

Scheme 34



4-Chromanol was transformed into the 4-benzylmercapto derivative (**187**) [^{1}H NMR (Plate 14) δ ppm 7.58-7.43 (5 H, m, Ar-H), 7.35-7.18 (2 H, m, H-5 and H-8), 6.99-6.89 (2 H, m, H-6 and H-7), 4.56 (1 H, dt, *J* = 11.03, 2.29 Hz, H-2), 4.33 (1 H, dt, *J* = 10.92, 3.60 Hz, 1 H-2), 4.02 (1 H, t, *J* = 3.31 Hz, H-4), 3.88 (2 H, 2 d, *J* = 13.54 Hz, SC$\underline{H_2}$Ph), 2.38-2.26 (1 H, m, H-3), 2.14 (1 H, m, H-3)] through the action of tin tetrachloride in 93 % yield (Scheme 35).

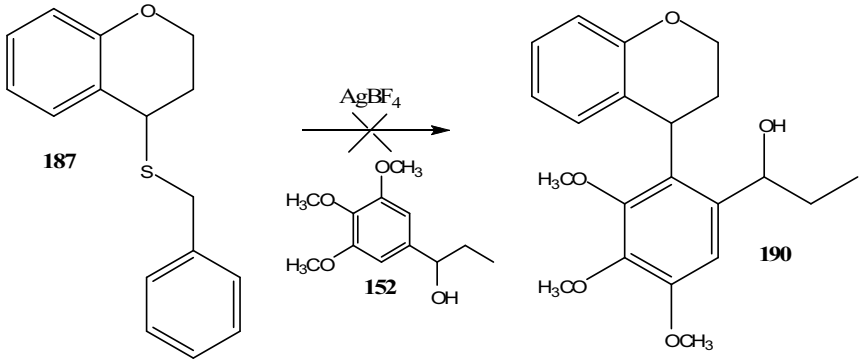
Scheme 35

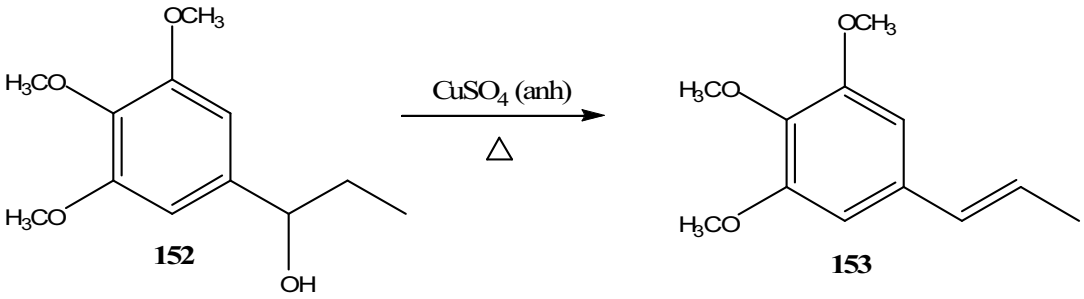


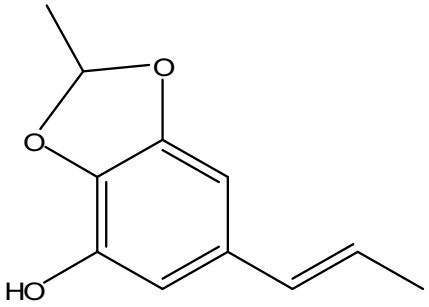
In order to varify that the 4-chroman thioether would indeed be prone to C-C coupling to an aromatic nucleophile under the prevailing conditions ($AgBF_4$, dichloromethane, -20^{o}C to room temp.), the potential electrophile was first reacted with resorcinol resulting in the 4-aryl derivative (**188**) [^{1}H-NMR spectrum (plate 15) δ ppm 7.13 – 6.1 (4 H, m, H-5,6,7,8), 6.38 (1 H, d, J = 1.98 Hz, H-6'), 6.66 (1 H, d, J = 8.29 Hz, H-3'), 6.32 (1 H, dd, J = 2.02, 8.28 Hz, H-5'), 4.40 (1 H, t, J = 5.95 Hz, H-4), 4.15 (1 H, t, *J* = 5.03 Hz, 2 H-2), 2.22-2.04 (2 H, m, 2 H-3)] being obtained in 62 % yield. When the nucleophile was changed to pyrogallol the reaction proved to be less successful, with only an inseparable mixture of highly polar products being obtained. Since the unwanted result obtained during this reaction could probably be attributed to the relatively high nucleophilicity of the pyrogallol ring, it was decided to reduce the reactivity of the pyrogallol by methylation of the free hydroxyl groups. This alteration in the reactivity of the pyrogallol ring had the desired affect and the coupled product, 4-(3',4',5'-trimethoxypheyl)chroman (**189**) could be isolated from the reaction in 72% yield. The ^{1}H-NMR spectrum (plate 16) of (**189**) displayed apart from the expected aromatic and heterocyclic protons, H-2' and H-6' (of the 'new' aromatic ring) as a singulet at δ 6.58 ppm; thus confirming the structure as that of the wanted product.

Since reaction between the 4-benzylmercaptan derivative and the model nucleophiles were successful, coupling with the real nucleophile (**152**) was attempted next with

confidence. Reaction between these two compounds could, however, not be brought about, despite the application of a variety of conditions. In previous work (*vide supra*), the low nucleophylicity of the pyrogallol moiety was ascribed to the presence of a carbonyl group attached to the aromatic ring and it was felt that the failure of the current coupling might also be the consequence of the electron withdrawing 4-hydroxy group, which renders the aromatic ring less electron rich through the negative inductive effect. In an attempt to enhance the nucleophilicity of the aromatic ring, it was therefore decided to dehydrate the 3,4,5-trimethoxyphenylpropanol (**152**), thus removing the electron withdrawing effect of the hydroxygroup. This was achieved in 73% yield by refluxing the 3,4,5-trimethoxyphenylpropanol in the presence of anhydrous copper sulphate. The coupling reaction, however, failed again, even under elevated reaction temperatures. Since the nucleophylicity of the pyrogallol system in this instance would be of the same order as that of the trimethoxybenzene entity (**189**) that could be coupled successfully, the failure of the reaction was ascribed to steric congestion brought about by the adjacent methoxy - and allyl groups in the nucleophile.







191

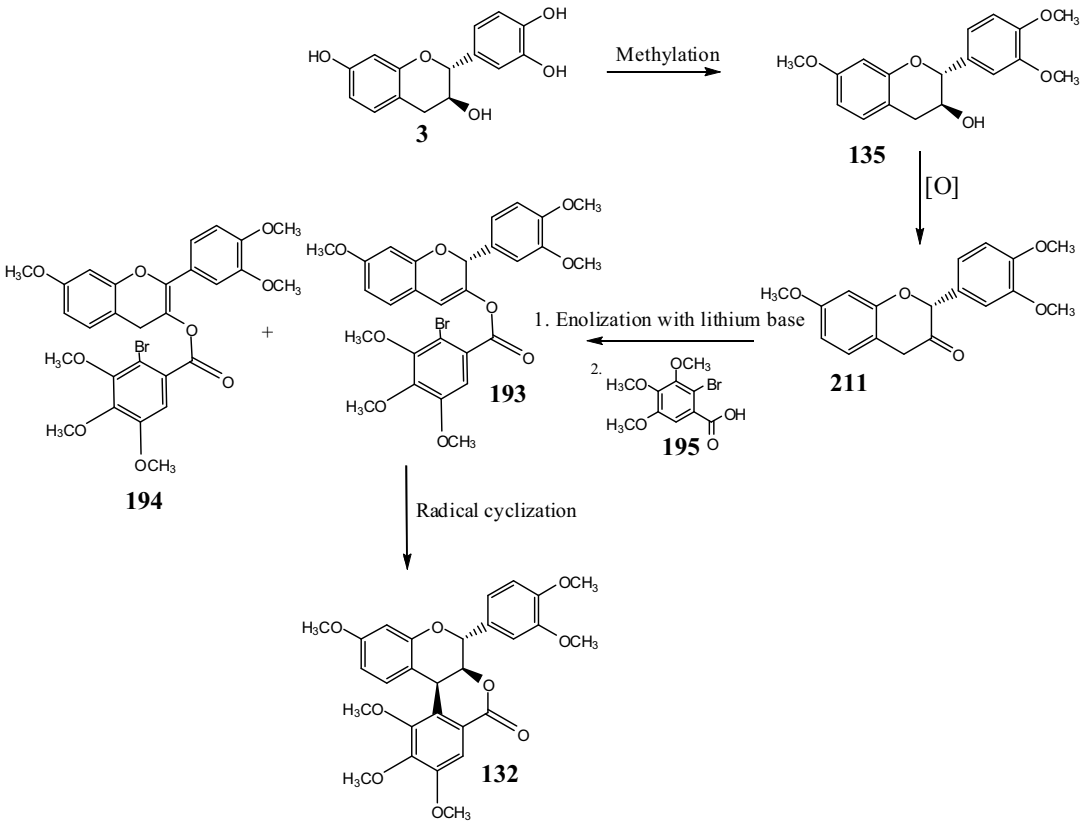
Although the steric effect of the methoxy group could be alleviated by changing the protecting group on the hydroxy functions of the pyrogallol moiety to a methylenedeoxy function as is indicated in structure (**191**), the steric effect of a free rotating allyl group on reaction at the adjacent aromatic carbon could not be removed and it was therefore decided to abandon this approach in favour of a completely different alternative.

3.3 Biomemetic strategy

Since the nucleophilic coupling strategy described above proved to be tedious and inconclusive, it was decided to consider a biomimetic approach towards the synthesis of the 4-arylflavan-3-ol lactones. From what is known of plant metabolism, it is evident that radicals play an important role in the *in vivo* synthesis of many natural products[13]. It was therefore envisaged that the generation of a radical adjacent to the carbonyl carbon on a flav-3-ene 3-gallate ester moiety (**193**) (Scheme 36) could result in the formation of the desired lactone after quenching of the resultant radical at C-3 with a suitable hydride reagent. The required flav-3-ene 3-gallate ester moiety would be obtainable through mild oxidation of the 3-OH of fisetinidol (**23**) followed by enolization with a strong lithium base and quenching of the resulting ester with the appropriate acid chloride. This strategy could however be compromised if the enolate is not formed in the right position for the radical cyclization to be effective. Since both hydrogens that could be abstracted by the base in the enolate formation are benzylic in nature and the conformation of the C-ring of the fisetinidol unit is such

that both the hydrogen atoms at C-2 and C-4 are susceptible to abstraction (enol **194** *vs* **193**), formation of the right enol ester was crucial to the whole strategy and had to be assessed beforehand.

Scheme 36



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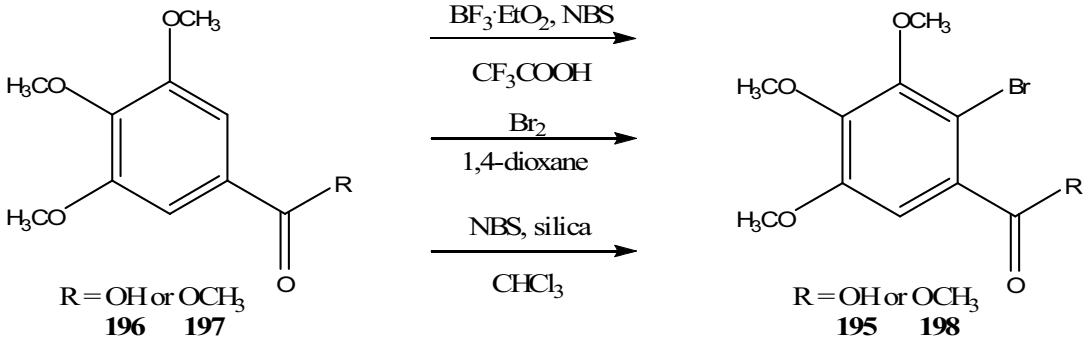
3.3.1 Synthesis of 2-bromo-3,4,5-trimethoxybenzoic acid (197)

In order to be able to prepare the enol ester required for the radical reaction, the appropriate acid chloride for trapping of the enolate was required. Since the acid chloride would not be achievable with free hydroxyl groups present on the aromatic ring, methylated gallic acid was subjected to bromination. Bromination with NBS (N-bromosuccinimide) and BF_3 etherate in CF_3COOH, however, gave very low yields of the required brominated product, while a solution of bromine in 46% yield. When the reactants was changed to 3,4,5-trimethoxybenzoic methyl ester, NBS and silica in

refluxing chloroform the desired 2-bromo-3,4,5-trimethoxybenzoic methyl ester was obtained in 51% yield. [^{1}H NMR (plate 18) δ ppm 7.17 (1 H, s, H-6), 3.94 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3)].

Scheme 37



Although the yield was still disappointingly low, the required product was obtained and it was decided to pay attention to the real reaction and come back to improving on the bromination methodology at a later stage.

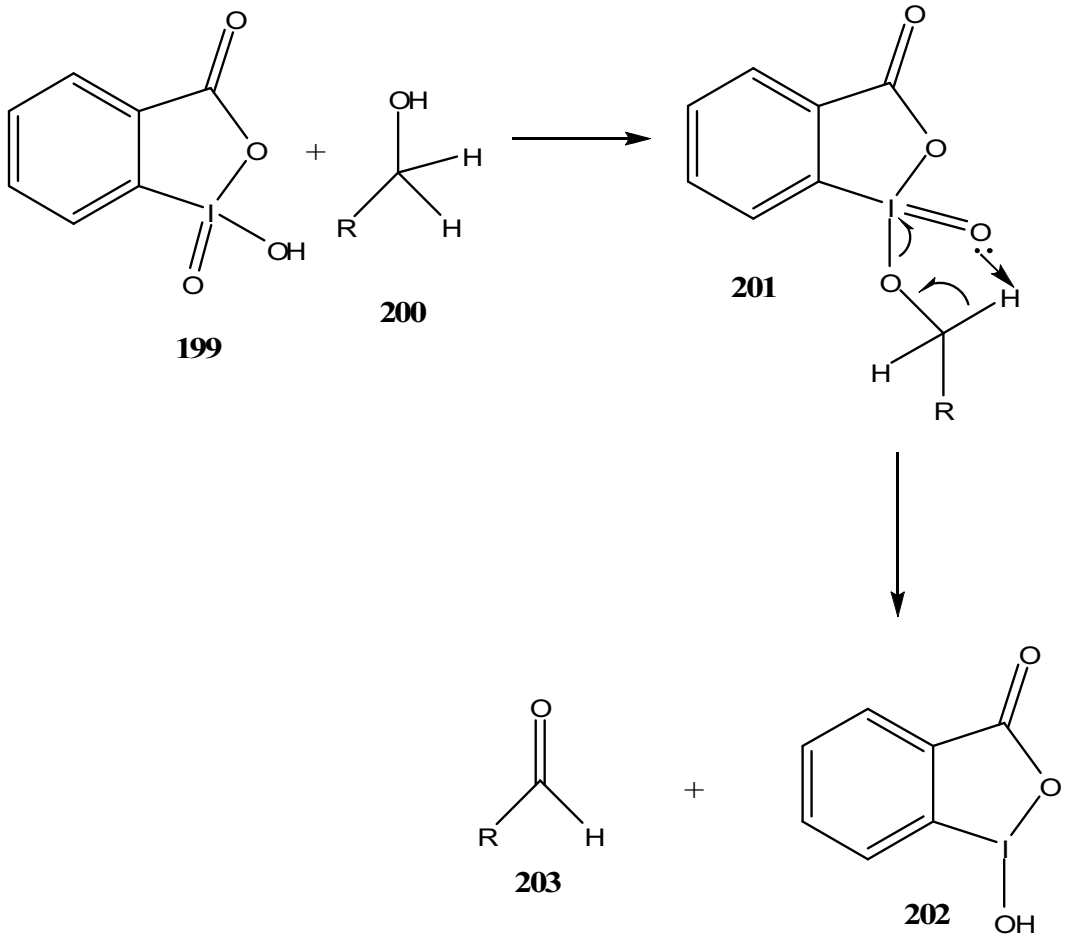
3.3.2 Attempted synthesis of 3',4',5,7-trimethoxy-4-arylflavan-3-ol lactone

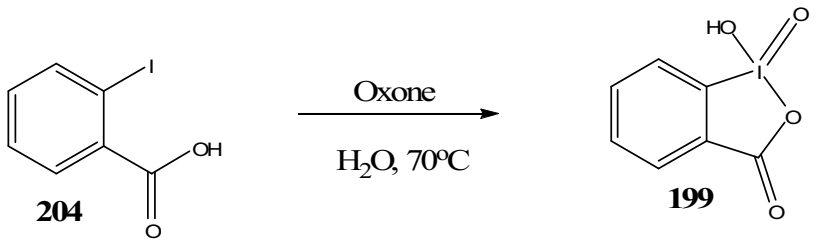
Since fisetinidol, the required flavan-3-ol starting material for the synthesis of the lactone, was not readily available, it was decided to utilise catechin as model substrate in the synthetic process. Although numerous methylating agents known in literature, many of these like methyl iodide and potassium carbonate or DMS (dimethylsulfide) are not selective and would lead to the methylation of all the hydroxyl groups present in the catechin molecule including the 3-OH that has to be utilised in the subsequent esterification reaction. Diazomethane, also a well known methylating reagent, has been used extensively and with great success for the selective methylation of phenols and enols without affecting the heterocyclic hydroxyl functions in flavan-3-ols. Diazomethane, however, represents a very hazardous and dangerous material to handle, because it is highly toxic, thermally labile and potentially explosive. A new reagent trimethylsilyl diazomethane (TMSDM), which is thermally stable, non-explosive, non-mutagenic and can safely be distilled at atmospheric pressure[14], was therefore investigated for the selective methylation of catechin.

Thus treatment of free phenolic catechin with TMSDM and tributylamine in methanol gave the derised permethylated product (**212**) in 81% yield [^{1}H-NMR (plate 19) δ ppm 7.00 (1 H, dd, J = 2.00, 8.12 Hz, H-6'), 6.97 (1 H, d, J = 1.94 Hz, H-2'), 6.89 (1 H, d, J = 8.14 Hz, H-5'), 6.14 (1 H, d, J = 2.34 Hz, H-8), 6.12 (1H, d, J = 2.35 Hz, H-6), 4.66 (1 H, d, J = 8.38 Hz, H-2), 4.14 - 3.91 (1 H, m, H-3), 3.89 (6 H, s, 2 OCH_3), 3.80 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3), 3.07 and 2.59 (2 H, dd, J = 5.74, 9.13 Hz, 2 H-4)].

With the methylated catechin in hand one of the key steps in the synthesis, i.e. selective oxidation of the 3-OH was subsequently embarked upon. Since IBX (*o*-iodoxybenzoic acid)[15] is known to be a very mild oxidizing agent that are able to oxidize primary alcohols to aldehydes with no over-oxidation to acids and it has been used for the oxidation of flavonoids like catechin[16] before, this compound was the reagent of choice for the required oxidation reaction. (The mechanism[17] for the oxidation of alcohols with IBX is shown in the scheme below.) The reagent (IBX) was prepared by the oxidation of 2-iodobenzoic acid with oxone[18], as illustrated below.

Scheme 38

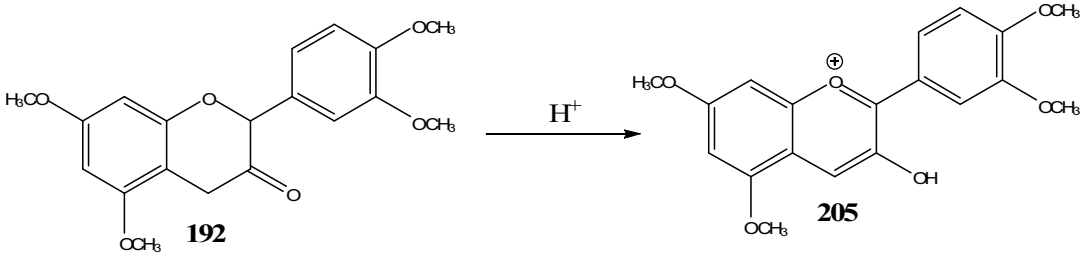




Thus oxidation of the 3-OH of methylated catechin was achieved by refluxing the catechin and prepared IBX in acetonitrile. Although only one product could be identified (TLC) in the reaction mixture before work-up and purification, yields of the desired product after preparative TLC were rather low (61 %). Since the silica displayed a pink/violet colour characteristic of anthocyanidins after the separation process, the low yields could probably be due to anthocyanidin formation (Scheme

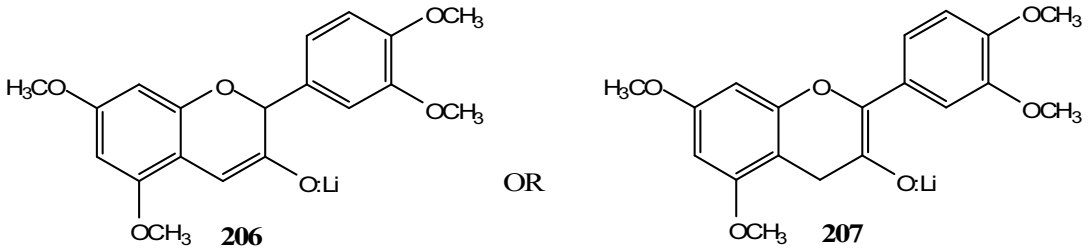
39) on the slightly acidic silica powder. Apart from the aromatic systems that stayed intact, the ^{1}H NMR spectrum (Plate 20) of the oxidized product (**192**) displayed a singulet at δ 5.29 (H-2) and two doublets at δ 3.60 and 3.47 ppm (each 1H, J = 21.91 Hz, 4-CH_2) in the heterocyclic region of the spectrum, thus confirming the isolated product to be the required catechin ketone.

Scheme 39



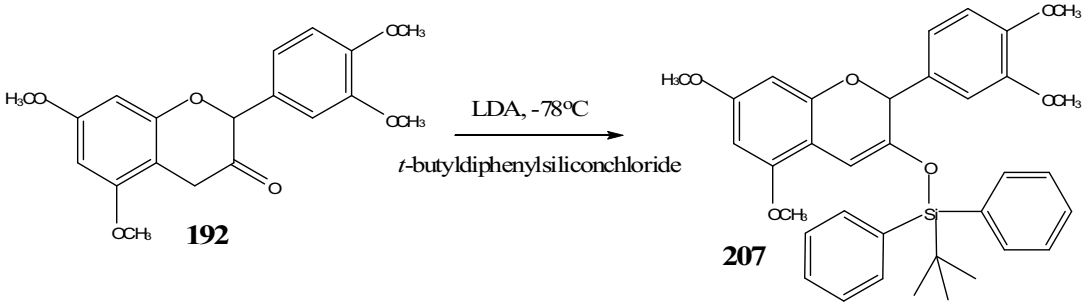
192 **205**

Since formation of the desired enol (**206**) was identified as one of the crucial steps in the successful application of this strategy to the synthesis of the flavan-3-ol lactones, the enol formation reaction was the next step to be investigated in the current methodology development.



206 **207**

Since trapping of enols by silylation has been established as one of the best methods of determining the geometry and position of the double bond in enol ethers[19], the position of the double bond was to be determined by quenching the lithium enolate with a rather stable silylgroup, i.e. *t*-butyldiphenylsilyl. Treatment of the catechin ketone (**192**) with a strong lithium base, LDA (lithiumdiisopropylamide), at -78^{o}C in the presence of one equivalent of HMPA (hexamethylphosphoramide) followed by *t*-butyldiphenylsilyl-chloride gave one product of R_f 0.57 (H:A, 7:3). The HMPA was added to the reaction mixture to minimize aggregation of the LDA base and thus enhance its reactivity towards enol formation and subsequent silylation.



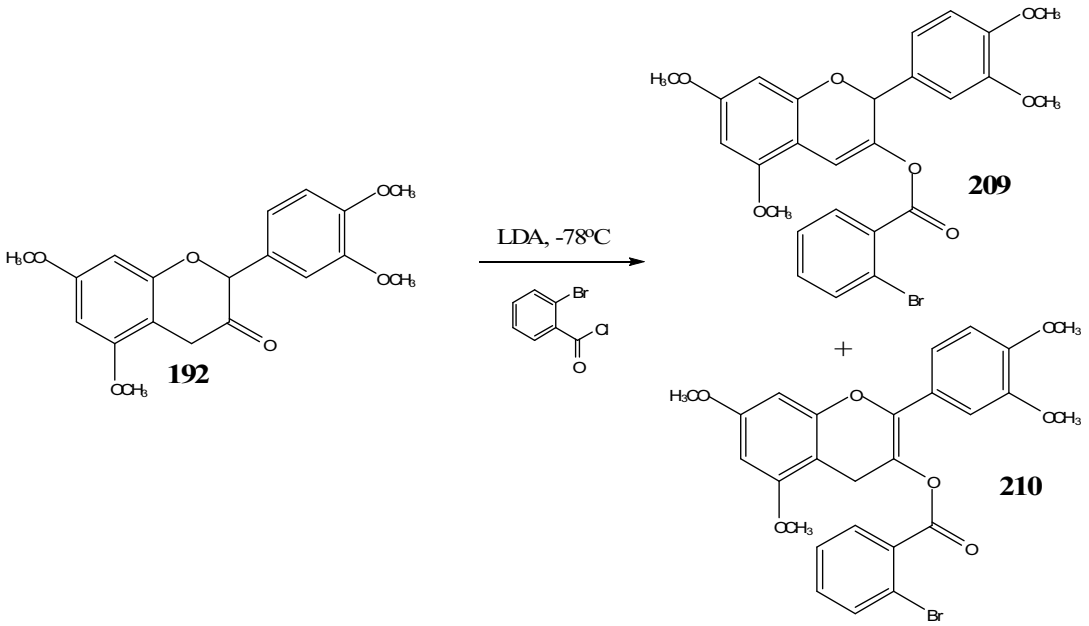
192 **207**

The ^{1}H-NMR (Plate 21) of the product (**207**), which was isolated in 52% yield, clearly showed the presence of the *t*-butyl group with a singulet resonance at δ 0.94 ppm as well as the protons of two additional phenyl groups in the aromatic region of the spectrum [δ ppm 7.72-7.29 (10 H, m, 2 Ar-H)]. Although the absence of a normal 4-methylene group was evident from the spectrum (no resonances at the normal position of a benzylic CH_2 group at δ 4-5 ppm), deciding if and where the double bond was, proved to be somewhat more problematic. The NMR spectrum contained only two singulet peaks at δ 5.62 and 5.69 ppm in addition to those of the methoxy groups and aromatic protons; thus indicating one hydrogen to be absent from the heterocyclic ring. Although the possibility of this proton appearing in the high field aromatic region was considered (it is both benzylic and allylic in nature), careful analysis of the integrals in the aromatic region showed no extra proton buried under the aromatic signals especially in the δ 6 ppm area. It was therefore concluded that a double bond was indeed present in the heterocyclic C-ring and the position of such a double bond remained the only unresolved issue. If the double bond was formed between C-2 and C-3, the protons at C-4 would show geminal coupling unless they are chemically equivalent, which would result in them resonating as a two-proton singulet. Since neither of these possibilities appeared to be the case, it was concluded that the double bond was situated between C-3 and C-4 with (**207**) being the structure of the isolated product. The peculiar absence of any allylic coupling between the designated protons (H-2 and H-4), however, brought some doubt as to whether (**207**) in fact was the structure of the isolated compound. This issue was resolved by careful investigation of a model of (**207**), which indicated the dihedral angle between the two hydrogens to be 90^o, thus explaining why no coupling was observed in the ^{1}H-NMR spectrum. Another interesting feature in the ^{1}H-NMR spectrum of the silyl enol ether (**207**) was

the even spacing of the four methoxy groups in contrast to what was observed in the spectra (plates 19 and 20) of both catechin and it's ketone derivative. This is probably explicable in terms of restricted rotation of the B-ring due to the steric bulk of the *t*-butyldiphenylsilyloxy group attached to C-3 of the heterocyclic ring.

Once it was established that the enolate could indeed be formed with the double bond in the desired position, the reaction was repeated with 2-bromobenzoyl chloride as electrophile (Scheme 40).

Scheme 40



Two products of R_f 0.51 (22 %) and R_f 0.61 (43 %) were isolated from the reaction mixture by preparative TLC (H:A, 7:3). Apart for the expected aromatic resonances of the catechin unit [δ 6.96 (1 H, d, *J* = 1.92 Hz, H-2'), 6.94 (1 H, dd, *J* = 8.27, 1.92 Hz, H-6'), 6.84 (1 H, d, *J* = 8.27 Hz, H-5'), 6.28 (1 H, d, *J* = 2.30 Hz, H-6), 5.87 (1 H, d, *J* = 2.30 Hz, H-8)] and three methoxy signals [δ 3.871, 3.869, 3.74, (each s)] the ^{1}H NMR spectrum (plate 22) of the first product (R_f 0.51) contained a singulet peak at δ 5.49 (1 H) as well as an up-field three proton singulet (δ 3.10) and five extra protons in the aromatic region [δ 7.67-7.65 (1 H, m, Ar-H), 7.24-7.16 (4 H, m, Ar-H and H-4)]. Although the spectrum looked very unfamiliar at first it was realised that the bromobenzoyl group was indeed attached to the catechin unit as was indicated by the extra proton signals in the aromatic region. The presence of an extra proton (5

instead of the expected 4) in the aromatic region of the spectrum, as well as only one heterocyclic proton, however, pointed towards one heterocyclic proton being deshielded to the aromatic region and is probably explicable in term of H-4 being both benzylic and in the β-position wrt the electron withdrawing ester moiety. The remaining heterocyclic resonance (δ 5.49) could therefore be allocated to H-2 and structure (**209**) assigned to the first enolate from the reaction. The fact that no coupling was observed between H-4 and H-2 (as would be expected) in the spectrum can be attributed to the fact that these two protons are at right angles to each other, while the up-field position (δ 3.10) of one methoxy resonance is probable due to the relative orientation of the B- and the ester aromatic rings, where one of the B-ring methoxy groups is situated above the ester aromatinc ring in some conformations.

The ^{1}H NMR spectrum (plate 23) of the other compound (R_f 0.61.), which was contaminated with some of the previous product (**209**), exhibited two doublets (each 1 H) at δ 3.28 and 2.84 ppm respectively with a large coupling constant (J = 17.12 Hz) typical of geminal coupling together with all the expected aromatic resonances [δ ppm 7.64-7.61 (1 H, m, Ar-H), 7.60-7.58 (1 H, m, Ar-H), 7.33-7.31 (2 H, m, Ar-H), 7.10 (1 H, d, *J* = 1.91 Hz, H-2'), 7.07 (1 H, dd, *J* = 8.26, 1.91 Hz, H-6'), 6.84 (1 H, d, *J* = 8.26 Hz, H-5'), 6.20 (1 H, d, *J* = 2.25 Hz, H-6), 6.14 (1 H, d, *J* = 2.25 Hz, H-8)]. Since the aromatic region of the spectrum clearly indicated the presence of the ester functionality that was not attached to either the A- or B-rings, and the C-3 enolate was already identified as product, this compound was identified as the C-2 enolate (**210**). The fact that the C-2 enolate (**210**) was contaminated by some of the C-3 isomer and not *vice versa* is probably explicable in terms of the C-3 enolate being the thermodynamically favoured product. It is therefore assumed that some of the C-2 isomer was transformed into the more stable C-3 isomer by the slightly acidic silica environment during the TLC separation process. The isomerisation probably happened on the dry silica powder before the product could be recovered from it.

3.4 Conclusion

Although the synthesis of the 4-arylflavan-3-ol lactones isolated from the African wattle and Red syringa trees has not been completed successfully during the current

investigation, two new approaches towards the formation of these metabolites have been evaluated. Since low nucleophilicity of the attacking pyrogallol ring was identified as the cause of failure of previous attempts, this problem was circumvented by a new strategy of removing the carbonyl deactivating group form the nucleophile and replacing it with a allyl type substituent that could be turned into a carbonyl containing unit at a later stage. Although the issue of low nucleophilicity of the attacking moiety was resolved in this way, a second problem i.e. that of steric congestion around the point of electrophilic attach on the pyrogallol ring, led to this methodology also not being successful.

The second biomimetic approach investigated during the current study, centred around two potential problems, i.e. oxidation of the 3-hydroxy group of a flavan-3-ol moiety without affecting the rest of the molecule and formation of an enolate ester at C-3 that could be utilised in a radical coupling reaction for the formation of the lactone ring. Both these problems were addressed successfully, so the final step in the synthesis of the target lactones can now be attempted with confidence during the candidate's PhD study.

3.5 References

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EXPERIMENTAL

CHAPTER 4

STANDARD EXPERIMENTAL TECHNIQUES

4.1 CHROMATOGRAPHY

4.1.1 Thin layer chromatography

Qualitative thin layer chromatography (TLC) was conducted on "Merck TLC-aluminium plates: Silica Gel F_{254}" (0.2 mm layer) divided into strips of *ca.* 3 x 5 cm. R_f values are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 x 20 cm) coated with a layer (1.0 mm) of unactivated Merck Kieselgel 60 PF_{254} and which were dried overnight at room temperature. After development in the appropriate eluent the plates were dried in a fast stream of air and the bands distinguished by UV-light (254 nm). The bands were eluted with acetone and the acetone evaporated under reduced pressure at *ca.* 40 ^{o}C.

4.1.2 Flash column chromatography (FCC)

A glass column was charged with 100 g of Merck "Kieselgel 60 (230-400 mesh) for column chromatography" for every 1 g of crude product. Air was disposed of by elution with the appropriate solvent under N_2-pressure (*ca.* 50 kPa). The crude product was dissolved in a minimum amount of the eluent and applied to the top of the column. The purified product was recovered by elution under N_2-pressure with the appropriate solvent system and collected in fractions.

4.2 Development of chromatograms with ferrichloride-perchloric acid

Thin layer chromatograms were gently sprayed with a mixture of a 35% (v/v) aqueous perchloric acid (100 ml) and 0.5 M ferrichloride (5 ml) and gently heated at 110 °C until maximum color development were obtained.

4.3 Development of chromatograms with palladium chloride-hydrochloric acid

All chromatograms involving thio derivatives were sprayed with a 0.02 M $PdCl_2$ solution in 6% HCl which developed a unique yellow spot for divalent sulfur compounds only.

4.4 Abbreviations

The following abbreviations for solvents are used throughout the experimental section:

A	=	acetone
T	=	toluene
B	=	benzene
THF	=	tetrahydrofuran
H	=	hexane
DMF	=	*N,N*-dimethylformamide
DCM	=	dichloromethane
Et_2O	=	diethyl ether
MeOH	=	methanol
EtOH	=	ethanol
EtOAc	=	ethyl acetate

4.5 Anhydrous solvents and reagents

4.5.1 Solvents

Acetone was left over dry K_2CO_3 (oven-dried, 24 hours, 200°C) for 24 hours. The solvent was distilled under Ar prior to use.

Benzene, diethyl ether, dioxane and THF were refluxed over sodium/benzophenone under Ar until a dark blue colour persisted with subsequent fresh distillation under Ar prior to use.

Dichloromethane were refluxed over sodium under Ar for 12 hours with subsequent fresh distillation under Ar before use.

4.5.2 Distillation of hexamethylphosphoramide (HMPA)

The HMPA was left over CaO for 2 weeks after which it was distilled under vacuum (2 mmHg) at 93^{o}C.

4.6 Spectroscopical and spectrometrical methods

4.6.1 Nuclear magnetic resonance spectroscopy (NMR)

NMR-spectroscopy were performed on a Bruker AM 300 FT- or a Bruker AM 600 FT-spectrometer at 296 K with, unless specified to the contrary, deuterochloroform ($CDCl_3$) as solvent. Chemical shifts are reported in parts per million (ppm) with the solvent residual peak at 7.26 ppm for proton spectra and 77.16 ppm for carbon spectra on the δ-scale and coupling constants are given in Hz.

4.7 Melting points

Melting points were determined with a Barloworld Scientific Stuart Melting Point Apparatus SMP3.

4.8 Chemical methods

4.8.1 Standard work-up procedure

Unless specified to the contrary, water was added to the reaction mixture and the aqueous phase extracted with diethyl ether. The organic extract was washed with water, dried (Na_2SO_4) and the ether removed under reduced pressure at *ca*. 40°C. Purification *via* PLC or FCC or crystallization afforded the product.

4.8.2 Preparation of dimethyldioxirane[1]

Acetone (192 ml) was added to an aqueous $NaHCO_3$ solution (58 g in 245 ml) and the mixture cooled to 5°C before solid oxone (120 g) was added in five portions at 3 min intervals while the reaction mixture was stirred vigorously. 3 Minutes after the last addition, vacuum (90 mmHg) was applied to the system and the reaction flask removed from the cooling bath. The dimethyldioxirane/acetone solution (0.09-0.11M) was distilled, collected in a cooled (-78°C) receiving flask and stored in the freezer (-20°C).

4.8.3 Preparation of 2-iodoxybenzoic acid (IBX)[2]

Oxone (18,2 g) was added to an aqueous 2-iodobenzoic acid solution (5 g in 65 ml). The mixture was slowly heated to 70°C and stirred at this temperature for 3 h after which it was cooled and stirred to 5°C and stirred for a further 1.5 h. The resulting white precipitate was filtered off, washed with water (6 x 10 ml) and acetone (2 x 10 ml) and left to dry at room temperature for 16 h.

4.8.4 2-Ethoxymethoxy-4-methoxy acetophenone (154)[3]

To a solution of 2-hydroxy-4-methoxy acetophenone (153) (500mg, 3mmol, 1eq) in dry DMF (20ml), was added chloromethyl ethylether (1.5eq, 4.5mmol, 0.42ml) followed by NaH (1.3eq, 3.9mmol, 94mg, 60% dispersion in oil). The reaction mixture was stirred for 2 hours at room temperature. The solvent was thus evaporated under reduced pressure and the residue extracted with diethyl ether (3 x 20ml). The

organic layers were combined, dried (Na_2SO_4) and evaporated under reduced pressure. The reaction yielded 2-ethoxymethoxy-4-methoxy acetophenone (154) as a yellow oil. Mass 583mg, 86.6% yield, R_f 0.42 (H:A, 8:2) ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.80 (1 H, d, J = 8.76 Hz, H-6), 6.72 (1 H, d, J = 2.33 Hz, H-3), 6.57 (1 H, dd, J = 2.33, 8.76 Hz, H-5), 5.31 (2 H, s, O$\underline{CH_2}$OCH$_2$CH$_3$), 3.84 (3 H, s, OCH$_3$), 3.76 (2 H, q, J = 7.07 Hz, OCH$_2$O$\underline{CH_2}$CH$_3$), 2.59 (3 H, s, CH$_3$), 1.25 (3 H, t, J = 7.07 Hz, OCH$_2$OCH$_2$$\underline{CH_3}$)

4.8.5 2'-Ethoxymethoxy-3,4,4'-trimethoxy chalcone (156)[4]

2-Ethoxymethoxy-4-methoxy acetophenone (154) (3.98g, 17.76mmol, 1eq) was dissolved in ethanol (50ml). Potassium hydroxide (60%, 75ml) was added and the reaction mixture was stirred at room temperature for 30 minutes. 3,4-Dimethoxybenzaldehyde (23.08mmol, 3.85g, 1.3eq) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was acidified (3M HCl) and extracted with ethyl acetate (3 x 100ml). The organic layers were combined, washed with sodium bicarbonate, dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was recrystallized form ethanol to give 2-ethoxymethoxy-3,4,4'-trimethoxy chalcone (156) as light yellow crystals. Mass 5.04g, 76.16% yield, R_f 0.15 (H:A, 8:2), Mp 92.1^{o}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.70 (1 H, d, J = 8.64 Hz, H-6'), 7.62 (1 H, d, J = 15.74 Hz, β-H), 7.37 (1 H, d, J = 15.74 Hz, α-H), 7.19 (1 H, dd, J = 1.84, 8.30 Hz, H-6), 7.14 (1 H, d, J = 1.84 Hz, H-2), 6.90 (1 H, d, J = 8.30, H-5), 6.77 (1 H, d, J = 2.30 Hz, H-3'), 6.65 (1 H, dd, J = 2.30, 8.64 Hz, H-5'), 5.31 (2 H, s, O$\underline{CH_2}$OCH$_2$CH$_3$), 3.94 (3 H, s, OCH$_3$), 3.93 (3 H, s, OCH$_3$), 3.87 (3 H, s, OCH$_3$), 3.76 (2 H, q, J = 7.06 Hz, OCH$_2$O$\underline{CH_2}$CH$_3$), 1.23 (3 H, t, J = 7.06 Hz, OCH$_2$OCH$_2$$\underline{CH_3}$)

4.8.6 2'-Ethoxymethoxy-3,4,4'-trimethoxy chalcone epoxide (157)[5]

2M NaOH (1 ml) was added to a solution of 2'-ethoxymethoxy-3,4,4'-trimethoxy chalcone (156) (200mg, 0.54mmol; 1eq) in THF (minimum) and methanol (20 ml). The reaction mixture was stirred for 10 min, whereafter 30% H_2O_2 (2.5 ml) was added and stirring continued for another 12 h (followed by TLC). Water (15 ml) was added to the reaction mixture and the aqueous phase was extracted with diethyl ether (3 x 20 ml), the organic extracts were combined and washed with water (2 x 20 ml), dried (Na_2SO_4) and the ether removed under reduced pressure. The crude product was

purified by recrystallization from ethanol to give 2'-ethoxymethoxy-3,4,4'-trimethoxy chalcone epoxide (157) as white neeldes. Mass 490mg, 90% yield, R_f 0.47 (H:A, 8:2), Mp 122.1°C, ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.85 (1 H, d, J = 8.77 Hz, H-5'), 6.95 (1 H, dd, J = 1.82, 8.24 Hz, H-6), 6.86 (1 H, d, J = 8.24 Hz, H-5), 6.82 (1 H, d, J = 1.82 Hz, H-2), 6.68 (1 H, d, J = 2.26 Hz, H-3'), 6.60 (1 H, dd, J = 2.26, 8.77 Hz, H-4'), 4.94 (1 H, d, J = 7.09 Hz, $O\underline{CH_2}OCH_2CH_3$), 4.87 (1 H, d, J = 7.09 Hz, $O\underline{CH_2}OCH_2CH_3$), 4.29 (1 H, d, J = 1.92 Hz, β-H), 3.91 (1 H, d, J = 1.89 Hz, α-H), 3.88 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 3.83 (3 H, s, OCH_3), 3.40-3.22 (2 H, m, $OCH_2O\underline{CH_2}CH_3$), 1.04 (3 H, t, J = 7.07 Hz, $OCH_2OCH_2\underline{CH_3}$)

An alternative synthesis with dimethyldioxirane: 2'-Ethoxymethoxy-3,4,4'-trimethoxy chalcone (156) (200mg, 0.54mmol; 1eq) was added to a solution of dimethyldioxirane (20ml, 1.1eq, 2eq) in acetone and stirred at 0°C for three hours (followed by TLC). The reaction mixture was left to warm to room temperature and the solvent removed under reduced pressure. The crude product was purified by recrystallization from ethanol to give 2'-ethoxymethoxy-3,4,4'-trimethoxy chalcone epoxide (157) as white needles. Mass 510mg, 98% yield.

4.8.7 General procedure for sulfanation of the 4-position[6]

To a solution of substrate (1.5g, 3.86mmol) in dry DCM (100ml) was added benzyl mercaptan (2ml, 15.54mmol) and the mixture stirred at -20°C for 10 minutes. Tin tetrachloride (0.14ml, 15.45mmol) was added and the stirring continued as the reaction mixture warmed to room temperature. After completion of the reaction (according to TLC), saturated sodium bicarbonate solution was added (5ml) and the products extracted into diethyl ether (3 x 50ml). The etherial layer was washed with water (2 x 50ml), dried ($NaSO_4$) and the solvent removed under reduced pressure. Purification by PLC followed.

4.8.7.1 4-Benzylsulfanyl chroman (187)

4-Chromanol (500mg, 3.33mmol, 1eq), benzyl mercaptan (1.71ml, 13.32mmol, 4eq) tin tetrachloride (0.12ml, 0.67mmol, 0.4eq). Purification by PLC gave 4-benzylsulfanyl chroman (157) as a off-white solid. Mass 790mg, 93% yield, R_f 0.84

(H:A, 8:2); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.58-7.43 (5 H, m, Ar-H), 7.35-7.18 (2 H, m, Ar-H), 6.99-6.89 (2 H, m, Ar-H), 4.56 (1 H, dt, *J* = 10.96, 2.29 Hz, H-2), 4.33 (1 H, dt, *J* = 10.96, 3.60 Hz, H-2), 4.02 (1 H, t, *J* = 3.31 Hz, H-4), 3.92 (1 H, d, 13.54 Hz, SC<u>H$_2$</u>Ph), 3.84 (1 H, d, *J* = 13.54 Hz, SC<u>H$_2$</u>Ph), 2.38-2.26 (1 H, m, H-3), 2.14 (1 H, m, H-3)

4.8.7.2 2'-Hydroxy-3,4,4'-trimethoxy-α-hydroxy-β-benzylsulfanyldihydrochalcone (158)

2'-Ethoxymethoxy-3,4,4'-trimethoxy chalcone epoxide (157) (100mg, 0.26mmol, 1eq), benzyl mercaptan (0.13ml, 1.09mmol, 4eq), tin tetrachloride (0.05ml, 0.052mmol, 0.2eq). Purification by PLC (H:B:A, 5:4:1) yielded 2'-hydroxy-3,4,4'-trimethoxy-α-hydroxy-β-benzylsulfanyldihydrochalcone (158) as a yellow powder. Mass 83.2mg, 61% yield; R_f 0.53; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.34-7.39 (m, Ar-H), 7.30 [1H, d, J = 1.92 Hz, H-6'(A or B)], 7.11-7.10 (m, Ar-H), 7.09-7.08 (m, Ar-H), 7.06[1H, d, J = 2.18 Hz, H-6'(A or B)], 7.02 [1H, d, J = 1.42 Hz, H-2(B)], 6.87 [1H, dd, J = 1.42 and 8.17 Hz, H-6(B)] 6.82[1H,d, J = 8.17 Hz, H-5(B)] 6.77[1H,d, J = 1.92 Hz, H-2(A)] 6.71[1H,d, J = 8.23 Hz, H-5(A)], 6.53 [1H,dd, J = 1.92, 8.23 Hz, H-6(A)] 6.48 [1H, d, J = 2.47 Hz, H-3'(B)] 6.42 [1H, d, J = 2.50 Hz, H-3'(A)] 6.35 [1H, dd, J = 2.47, 8.99 Hz, H-5'(B)] and 6.30 [1H, dd, J = 2.50 and 8.99 Hz, H-5'(A)], 5.21 [1H, m, H-α(B)], 5.21 [1H, d, J = 2.9 Hz, H-α(A)], 4.02 [1H, d, J = 2.9 Hz, H-β(A)], 4.00 [1H, d, J = 7.6 Hz, H-β(B)], 3.91 [3 H, s, OCH_3], 3.895 [3 H, s, OCH_3], 3.892 [3 H, s, OCH_3], 3.81 [3 H, s, OCH_3], 3.74[d, *J* = 13.94 Hz, S<u>CH$_2$</u>Ph], 3.56-3.49 [m, S<u>CH$_2$</u>Ph], 3.36 [d, *J* = 13.72 Hz, S<u>CH$_2$</u>Ph]

4.8.7.3 4-Benzylsulfany-3',4',7-trimethoxydihydroflavonol (186)

3',4',7-Trimethoxyflavan-3,4-diol (141) (250mg, 0.75mmol, 1eq) benzyl mercaptan (0.4ml, 3.02mmol, 4eq) tin tetrachloride (0.15ml, 0.15mmol, 0.2eq). Purification by PLC yielded 4-benzylsulfanyl-3',4',7-trimethoxydihydroflavonol (186) as a yellow powder. Mass 187mg; 57% yield; R_f 0.61 (H:B:A, 5:4:1); ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.58 (d, *J* = 8.96 Hz, Ar-H), 7.40-7.29 (m, Ar-HH), 7.27-7.18 (m, Ar-H), 7.15-7.09 (m, Ar-H), 6.99 (dd, *J* = 8.18, 1.82

Hz, Ar-H), 6.95-6.90 (m, Ar-H), 6.69 (d, *J* = 8.21 Hz, Ar-H), 6.65 (dd, *J* = 8.70, 2.54 Hz, Ar-H), 6.56 (d, *J* = 2.50 Hz, Ar-H), 6.51 (dd, *J* = 8.56, 2.50 Hz, Ar-H), 6.48 (d, *J* = 2.49 Hz, Ar-H), 6.42 (d, *J* = 2.51 Hz, Ar-H), 6.37 (dd, *J* = 8.20, 1.86 Hz, Ar-H), 6.23 (dd, *J* = 8.34, 2.58 Hz, Ar-H), 6.16 (d, *J* = 1.71 Hz, Ar-H), 6.08 (d, *J* = 8.37 Hz, Ar-H), 4.92 (d, *J* = 8.91 Hz, H-2{2,3t,3,4c}), 4.67 (d, *J* = 9.54 Hz, H-2{2,3t,3,4t}), 4.27 (dd, *J* = 10.54, 3.18 Hz, H-3{2,3c,3,4t}), 4.24 (dd, *J* = 8.91, 4.64 Hz, H-3{2,3t,3,4c}), 4.12 (d, *J* = 4.64 Hz, H-4{2,3t,3,4c}), 4.05 (d, *J* = 9.24 Hz, H-4{2,3t,3,4t}), 3.98 (d, *J* = 13.35 Hz, S$\underline{CH_2}$Ph), 3.96 (d, *J* = 3.18 Hz, H-2{2,3c,2,4t}), 3.95 (s, OCH_3), 3.92 (s, OCH_3), 3.89 (s, OCH_3), 3.86 (d, *J* = 13.04 Hz, S$\underline{CH_2}$Ph), 3.83 (dd, *J* = 9.54, 9.24 Hz, H-3{2,3t,3,4c}), 3.80 (s, OCH_3), 3.76 (s, OCH_3), 3.67 (d, *J* = 12.96 Hz, S$\underline{CH_2}$Ph), 3.62 (d, *J* = 12.46 Hz, S$\underline{CH_2}$Ph), 3.48 (d, *J* = 3.18 Hz, H-2{2,3c,3,4t}), 3.41 (d, *J* = 10.54 Hz, H-4{2,3c,3,4t})

4.8.8 Cyclization of 2'-hydroxy-3,4,4'-trimethoxy-α-hydroxy-β-benzylsulfanyldihydrochalcone[7]

A solution of 2'-hydroxy-3,4,4'-trimethoxy-α-hydroxy-β-benzylthioldihydrochalcone (158) (760mg, 1.5mmol, 1eq) in DCM (200ml) was stirred with $AgBF_4$ (564mg, 2.9mmol, 2eq) at room temperature for 12 hours (TLC). Water (50ml) was added to the mixture, which as then extracted into diethyl ether (3 x 50ml). The organic layer was washed with water (2 x 50ml), dried ($NaSO_4$) and the solvent removed under reduced pressure. Purification by FCC (H:T:A, 4:4:2) gave three products.

4.8.8.1 3',4',7-Trimethoxydihydroflavonol (159)

Mass 411mg; 83.2% yield; R_f 0.26 (H:T:A, 4:4:2); ^{1}H NMR (600 MHz, *CDCl*$_3$, δ ppm 7.86 (1 H, d, J = 8.82 Hz, H-5), 7.13 (1 H, dd, J = 1.84, 8.19 Hz, H-6'), 7.10 (1 H, d, J = 1.84 Hz, H-2'), 6.95 (1 H, d, J = 8.19 Hz, H-5'), 6.67 (1 H, dd, J = 2.30, 8.82 Hz, H-6), 6.50 (1 H, d, J = 2.30 Hz, H-8), 5.06 (1 H, d, J = 12.22 Hz, H-2), 4.58 (1 H, dd, J = 1.53, 12.22 Hz, H-3), 3.94 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.85 (3 H, s, OCH_3), 3.71 (1 H, d, J = 1.60 Hz, 3-OH)

4.8.8.2 3',4',7-Trimethoxyisoflavone (173)

Mass 39mg; 8.4% yield; R_f 0.2 (H:T:A, 4:4:2); ^{1}H NMR (600 MHz, *$CDCl_3$*, δ ppm 8.21 (1 H, d, J = 8.91 Hz, H-5), 7.95 (1 H, s, H-2), 7.22 (1 H, d, J = 1.87 Hz, H-2'), 7.06 (1 H, dd, J = 1.87, 8.23 Hz, H-6'), 7.00 (1 H, dd, J = 2.34, 8.91 Hz, H-6), 6.93 (1 H, d, J = 8.23, H-5'), 6.87 (1 H, d, J = 2.34, H-8), 3.93 (3 H, s, OCH_3), 3.92 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3)

4.8.8.3 1-(3,4-Dimethoxyphenyl)-3-(2-hydroxy-4-methoxyphenyl)-propane-1,2-dione (174)

Mass 36mg; 7.3% yield; R_f 0.14 (H:T:A, 4:4:2); ^{1}H NMR (600 MHz, *$CDCl_3$*, δ ppm 7.77 (1 H, d, J = 8.83 Hz, H-6'), 6.85 (1 H, d, J = 8.07 Hz, H-5), 6.83 (1 H, dd, J = 1.65, 8.07 Hz, H-6), 6.80 (1 H, d, J = 1.65 Hz, H-2), 6.46 (1 H, dd, J = 2.40, 8.83 Hz, H-5'), 6.45 (1 H, d, J = 2.40 Hz, H-3'), 4.17 (2 H, s, 2 α-H), 3.89 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 3.85 (3 H, s, OCH_3)

4.8.9 General procedure for reduction with $NaBH_4$[8]

Substrate was dissolved in EtOH:THF (1:1; 5ml/100mg substrate). $NaBH_4$ (1eq) was added and the solution was stirred at r.t. until completion. Acetone (10ml) was added to the reaction mixture and stirred for 30 min. The reaction mixture was evaporated to dryness. The crude product was extracted with Et_2O (3 x 10 ml per 100 mg substrate) and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure, where after the crude product was purified by FCC.

4.8.9.1 4-Chromanol (179)

4-Chromanone (178) (5g, 33.75mmol, 1eq) and $NaBH_4$ (388mg, 10.25mmol, 0.33eq). Purification with FCC yielded 4-chromanol (179) as a colourless oil as product. Mass 4.41g; 87.03% yield R_f 0.44 (T:A, 8:2); ^{1}H NMR (300 MHz, *$CDCl_3$*) δ ppm 7.26 $CDCl_3$, 7.21 (1 H, dd, J = 1.71, 7.60 Hz, H-8), 7.14 (1 H, dd, J = 1.71, 6.93 Hz, H-7), 7.06 (1 H, d, J = 1.50 Hz, H-5), 6.88 (1 H, ddd, J = 1.50, 6.93, 7.63 Hz, H-7), 4.42 (1

H, t, J = 4.23, H-4), 4.07 and 3.89 (each 1 H, ddd, J = 3.93, 4.71, 10.99 Hz, 2 H-2), 1.72-1.55 (2 H, m, 2 H-3)

4.8.9.2 3',4',7-Trimethoxyflavan-3,4-diol (141)

3',4',7-Trimethoxydihydroflavonol (159) (375mg, 0.759mmol, 1eq) and $NaBH_4$ (8.62mg, 0.228mmol, 0.33eq). The reaction yielded 3',4',7-trimethoxyflavan3,4-diol (141) as an off-white powder after purification. Mass 177mg; 70.6% yield; R_f 0.20 (H:T:A, 3:4:3); ^{1}H NMR (600 MHz, *CDCl*$_3$, δ ppm 7.43 (1 H, d, J = 8.61 Hz, H-5), 7.05 (1 H, dd, J = 1.49, 8.20 Hz, H-6'), 7.02 (1 H, d, J = 1.49 Hz, H-2'), 6.93 (1 H, d, J = 8.20 Hz, H-5'), 6.62 (1 H, dd, J = 2.25, 8.61 Hz, H-6), 6.44 (1 H, d, J= 2.25 Hz, H-8), 4.87 (1 H, dd, J = 5.51, 7.87 Hz, H-4), 4.81 (1 H, d, J = 9.91 Hz, H-2), 3.91 (1 H, m, H-3), 3.91 (3 H, s, OCH_3), 3.91 (3 H, s, OCH_3), 2.43 (d, J = 5.51 Hz, 4-OH), 1.95 (d, J = 2.51 Hz, 3-OH)

4.8.10 General procedure for dehydration[9]

Dry phenolic material (1g, 6.5mmol, 1eq) was dissolved in hexane (50 ml). Anhydrous $CuSO_4$ (1eq) was added and the reaction mixture refluxed for 24 h (monitored by TLC). After completion of the reaction, the mixture was extracted into hexane (3 x 20ml), the organic phase washed with water (3 x 20ml), dried (Na_2SO_4) and the solvent removed by evaporation. Purification was done by PLC.

4.8.10.1 Chromene (180)

4-Chromanol (179) (200 mg, 1.3 mmol, 1eq) and $CuSO_4$ (210 mg, 1.3 mmol, 1eq). After purification with PLC chromene (180) was obtained as a yellow oil. Mass 120mg; 70% yield; R_f 0.60 (H:A, 98:2); ^{1}H NMR (300 MHz, *CDCl*$_3$) δ ppm 7.10 (1 H, ddd, J = 1.80, 7.33, 8.02 Hz, H-7), 6.96 (1 H, dd, J = 1.80, 7.41 Hz, H-8), 6.83 (1 H, ddd, J = 1.53, 7.33, 7.41 Hz, H-6), 6.78 (1 H, td, J = 1.53, 8.02 Hz, H-8), 6.42 (1H, m, H-4), 5.77 (1 H, dt, J = 3.56, 3.56, 9.83 Hz, H-3), 4.83 (2 H, dd, J = 1.88, 3.57 Hz, 2 H-2)

4.8.10.2 (*Trans*)-1-(3',4',5'-trimethoxyphenyl)-1-propene (153)

2,3,4-trimethoxyphenylpropan-1-ol (152) (2.5g, 11mmol, 1eq) and $CuSO_4$ (1.75g, 11mmol, 1eq). Purification gave (*trans*)-1-(3,4,5-trimethoxyphenyl)-1-propene as a colourless oil. Mass 1.68g; 73% yield; R_f 0.48 (H:A, 8:2); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 6.55 (2 H, s, H-2' and H-6'), 6.34 (1 H, d, *J* = 15.78 Hz, H-1), 6.17 (1 H, m, H-2), 3.88 (6 H, s, 3'-OCH3 and 5'-OCH3), 3.85 (3 H, s, 4'-OCH3), 1.96-1.87 (3 H, m, 3 H-3)

4.8.11 1-hydroxy-(3',4',5'-trimethoxyphenyl)-propan-1-ol (152)[10]

Ethylmagnesium bromide (4ml, 10.02mmol, 1.1eq) was added to 12ml of anhydrous ether and cooled to -5°C. 3,4,5-Trimethoxybenzaldehyde (160) (2g, 10mmol, 1eq) was dissolved in anhydrous ether (8ml) and was added drop wise to the Grignard solution over a period of 30 minutes. The reaction mixture was left to stir for 2 hours (monitored by TLC). The reaction mixture was poured over crushed ice and extracted into diethyl ether (3 x 25ml). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. After purification by PLC 1-hydroxy-(3',4',5'-trimethoxyphenyl)-propan-1-ol (152) was obtained as an off-white oil. Mass 1.45g; 64% yield; R_f 0.62 (T:H:A, 6:3:1); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 6.46 (2 H, s, H-2',6'), 4.40 (1 H, t, J = 6.56 Hz, H-1), 3.76 (6 H, s, 3',5'-OCH_3), 3.73 (3 H, s, 4'-OCH_3), 2.70 (1 H, br s, 1-OH), 1.66 (2 H, m, 2 H-2), 0.84 (3 H, t, J = 7.41 Hz, H-3)

4.8.12 General procedure for the nucleophilic coupling with a thiophilic Lewis Acid

To a solution of substrate (610mg, 2.38mmol, 1eq) and nucleophile (23.85mmol, 3g, 10eq) in dry THF (100ml) cooled to 0°C, solid silver tetrafluoroborate (5.95mmol, 1.16g, 2eq) was added in one batch and the reaction was stirred for 12 hours (monitored by TLC). After the addition of water (20ml), the reaction mixture was extracted with ethyl acetate (3 x 25ml), the organic layer dried (Na_2SO_4) and evaporated under reduced pressure. Purification by PLC followed.

4.8.12.1 4-(2,3,4-Trimethoxyphenyl)-chroman (189)

4-Benzylsulfanyl chroman (187) (75mg, 0.30mmol, 1eq), trimethoxybenzene (1.98mg, 1.18mmol, 4eq) and $AgBF_4$ (117mg, 0.59mmol, 2eq). Purification by PLC yielded 4-(2,3,4-trimethoxyphenyl)-chroman (189) as a white powder. Mass 64.6mg; 72% yield; R_f 0.51 (H:A, 8:2); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.17-6.83 (4 H, m, H-5,6,7,8), 6.58 (2 H, s, H-2' and H-6'), 4.47 (1 H, t, *J* = 6.32 Hz, H-4), 4.22 (2 H, dd, *J* = 5.39 Hz, 2 H-2), 3.92 (3 H, s, OCH3), 3.90 (3 H, s, OCH3), 3.85 (3 H, s, OCH3), 2.33-2.20 (1 H, m, H-3), 2.09-2.04 (1 H, m, H-3)

4.8.12.2 4-(2,4-Dihydroxyphenyl)-chroman (188)

4-Benzylsulfanyl chroman (200mg; 1.332mmol, 1eq), resorcinol(176mg, 1.33mmol, 1eq) and $AgBF_4$ (132mg, 0.67mmol, 0.5eq). Purification by PLC yielded 4-(2,4-dihydroxyphenyl)-chroman (188) as a white powder. Mass 195mg; 62% yield; R_f 0.35 (T:A, 7:3), ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.13 – 6.1 (4 H, m, H-5,6,7,8), 6.66 (1 H, d, J = 8.29 Hz, H-3'), 6.38 (1 H, d, J = 1.98 Hz, H-6'), 6.32 (1 H, dd, J = 1.98, 8.29 Hz, H-5'), 4.40 (1 H, t, J = 5.95 Hz, H-4), 4.15 (1 H, dd, *J* = 5.03, 5.01 Hz, 2 H-2), 2.22-2.04 (2 H, m, 2 H-3)

4.8.13 General procedure for the methylation of phenolic compounds[11]

The phenolic substrate (2 g, 9.4 mmol, 1eq), tributyl amine (3.2 ml, 6eq), MeOH (5 ml) and trimethylsilyldiazomethane (6.5 ml, 6eq) was stirred at room temperature in the dark for 12 h (monitored by TLC), after which the reaction mixture was acidified (3 M HCl) and extracted with EtOAc (30 ml). The organic layer was washed with water (2 x 20 ml), dried ($NaSO_4$), the solvent removed under reduced pressure and the crude product purified by FCC or PLC.

4.8.13.1 3',4',5,7-Tetra-O-methyl catechin (135)

Catechin (25) (290 mg, 1 mmol, 1eq), tributyl amine (4.3 ml, 6eq) and trimethylsilyldiazomethane (8.3 ml, 6eq). FCC of the crude product yielded 3',4',5,7-

tetra-o-methyl catechin (135) as a white powder. Mass 286mg; 83% yield; R_f 0.85 (T:A, 8:2); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.26 $CDCl_3$, 7.00 (1 H, dd, J = 2.00, 8.12 Hz, H-6'), 6.97 (1 H, d, J = 2.00 Hz, H-2'), 6.89 (1 H, d, J = 8.12 Hz, H-5'), 6.14 (1 H, d, J = 2.34 Hz, H-8), 6.12 (1H, d, J = 2.34 Hz, H-6), 4.66 (1 H, d, J = 8.38 Hz, H-2), 4.14 - 3.91 (1 H, m, H-3), 3.89 (6 H, s, 2 OCH_3), 3.80 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3), 3.07 and 2.59 (2 H, dd, J = 5.74, 9.13 Hz, 2 H-4)

4.8.14 3',4',5,7-tetramethoxydihydroflav-3-one (192)[2]

In minimum acetonitrile, 3',4',5'7-tetra-O-methyl catechin (135) (1g, 2.89mmol, 1eq) and IBX (1.2g, 4.34mmol, 1.5eq) was refluxed for 6.5 hours (monitored by TLC). After completion, the reaction mixture was filtered and the filtrate extracted with Et_2O (30 ml). The organic layer was washed with water (3 x 10ml), dried (Na_2SO_4) and the solvent removed under reduce pressure. Purification of the crude product with PLC, yielded 3',4',5,7-tetramethoxydihydroflav-3-one (192) as a white powder. Mass 605mg; 61% yield; R_f 0.51 (H:A, 7:3); ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.26 $CDCl_3$, 6.93-6.89 (2 H, m, H-2',6'), 6.85 (1 H, d, J = 8.26 Hz, H-5'), 6.32 (1 H, d, J = 2.21 Hz, H-8), 6.20 (1 H, d, J = 2.21 Hz, H-6), 5.29 (1 H, s, H-2), 3.87 (3 H, s, OCH_3), 3.86 (3 H, s, OCH_3), 3.80 (6 H, s, 2 OCH_3), 3.60 and 3.47 (2 H, each d, J = 21.91 Hz, 4-CH_2)

4.8.15 t-butyl[2-(3',4'-dimethoxyphenyl)-5,7-dimethoxychrom-3-en-3-yloxy]-diphenylsilane (209)[12]

A solution of 3',4',5,7-tetramethoxydihydroflav-3-one (192) (200mg, 0.58mmol; 1eq) in dry THF (4ml) was added slowly to a solution of lithium diisopropylamide (0.45ml, 0.87mmol, 1.5eq) in dry THF (24ml) at -78°C under argon atmosphere. Hexamethylphosphoramide (0.15ml, 0.87mmol, 1.5eq) was added followed by the dropwise addition of *t*-butyldiphenylsiliconchloride (0.24ml, 0.93mmol, 1.6eq). The reaction mixture was stirred at -78°C for 1 hour and left to warm to 0°C after which saturated ammonium chloride solution (2ml) was added and the reaction mixture extracted with diethyl ether (3 x 10ml). The organic layers were combined, washed with saturated sodium bicarbonate solution (10ml), dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification of the crude product by PLC gave t-

butyl[2-(3',4'-dimethoxyphenyl)-5,7-dimethoxychrom-3-en-3-yloxy]-diphenylsilane (209) as a white powder. Mass 275mg; 84% yield; R_f 0.57 (H:A, 7:3); ^{1}H NMR (300 MHz, CDCl3) δ ppm 7.72-7.29 (10 H,m, Ar-H), 6.98 (1H, dd, J = 1.83, 8.20 Hz, H-6'), 6.95 (1 H, d, J = 1.83 Hz, H-2'), 6.81 (1 H, d, J = 8.20 Hz, H-5'), 6.02 (1 H, d, H = 1.91 Hz, H-8), 5.94 (1 H, d, H = 1.91 Hz, H-6), 5.69 (1 H, s, H-2), 5.62 (1 H, s, H-4), 3.88 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3), 3.68 (3 H, s, OCH_3), 3.56 (3 H, s, OCH_3), 0.94 (9 H, s, t-butyl-H)

4.8.16 Methyl-2-bromo-3,4,5-trimethoxybenzoate (198)[13]

To a solution of methyl-3,4,5-trimethoxybenzoate (195) (700mg, 2.73mmol, 1eq) in chloroform (20ml) was added silika (2g) and NBS (672mg, 3mmol, 1.1eq), whereafter the reaction mixture was stirred in the dark for 21 hours (TLC). After filtration, the filtrate was washed with $Na_2S_2O_3$ (10%, 3 x 10ml), brine (10 ml) and the organic layer extract concentrated removed under reduced pressure. Purification of the crude product by PLC yielded methyl-2-bromo-3,4,5-trimethoxybenzoate (198) as a light yellow powder. Mass 425mg; 51% yield, R_f 0.6, (T:H:A, 4:4:2), ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.17 (1 H, s, H-6), 3.94 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 3.90 (6 H, s, 2 OCH_3).

4.8.17 Attempt to synthesize chroman-3,4-diol[14]

m-Chloroperbenzoic acid (3.13g, 18.16mmol, 1.2eq) in DCM (35ml) was added dropwise to a chromene (180) (2g, 15.13mmol, 1eq) solution over a period of 10 minutes and the mixture was stirred for a further 1 hour after which the excess acid was destroyed with sodium sulfite (10% solution), washed with water (3 x 20 ml), dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by PLC yielded only the 4-ethoxychroman (185). Mass 1.51g, 56% yield, R_f 0.32 (H:A, 98:2) ^{1}H NMR (300 MHz, *CDCl$_3$*) δ ppm 7.29 (1 H, dd, *J* = 7.44, 1.55 Hz, H-5), 7.23 (1 H, ddd, *J* = 8.20, 1.55 Hz, H-7), 6.93 (1 H, ddd, *J* = 1.03, 7.44, 8.20 Hz, H-6), 6.87 (1 H, dd, *J* = 8.20, 1.03 Hz, H-8), 4.41 (1 H, t, *J* = 3.83 Hz, H-4), 4.39-4.23 (2 H, m, 2 H-2), 3.75-3.58 (2 H, m, 2 H-2'), 2.21-2.01 (2 H, m, 2 H-3), 1.29 (3 H, t, *J* = 6.98 Hz, 3 H-1')

4.8.18 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-2H-chrom-3-en-3-yl 2-bromo- benzoate (209)1[12]

A solution of 3',4',5,7-trimethoxydihydroflavan-3-one (192) (500mg, 1.46mmol, 1eq) in dry THF (5ml) was added slowly to a solution of lithium diisopropylamide (1.1ml, 2.18mmol, 1.5eq) in dry THF (25ml) at -78°C under argon atmosphere. Hexamethylphosphoramide (0.38ml, 2.18mmol, 1.5eq) was added followed by the dropwise addition of 2-bromobenzoyl chloride (0.19ml, 2.33mmol, 1.6eq). The reaction mixture was stirred at -78°C for 1 hour and left to heat up to 0°C after which wet ether was added and the reaction mixture extracted with diethyl ether (3 x 10ml). The organic layers were combined, washed with water (3 x10ml), dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification of the crude product by PLC gave 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-2H-chrom-3-en-3-yl 2-bromo-benzoate (209) as light orange powder together with 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4H-chromen-3-yl 2-bromobenzoate (nr). Mass 169mg, 22% yield, R_f 0.51 (H:A, 7:3) ^{1}H NMR (600 MHz, *CDCl$_3$*) δ ppm 7.67-7.65 (1 H, m, Ar-H), 7.24-7.16 (4 H, m, Ar-H and H-4), 6.96 (1 H, d, *J* = 1.92 Hz, H-2'), 6.94 (1 H, dd, *J* = 8.27, 1.92 Hz, H-6'), 6.84 (1 H, d, *J* = 8.27 Hz, H-5'), 6.28 (1 H, d, *J* = 2.30 Hz, H-6), 5.87 (1 H, d, *J* = 2.30 Hz, H-8), 5.49 (1 H, s, H-2), 3.871 (3 H, s, OCH_3), 3.869 (3 H, s, OCH_3), 3.74 (3 H, s, OCH_3), 3.10 (3 H, s, OCH_3)

4.8.18.1 2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4H-chrom-2-en-3-yl 2-bromobenzoate (210)

Mass 330mg, 43% yield, R_f 0.61 (H:A, 7:3) ^{1}H NMR (600 MHz, *CDCl3*) δ ppm 7.64-7.61 (1 H, m, Ar-H), 7.60-7.58 (1 H, m, Ar-H), 7.33-7.31 (2 H, m, Ar-H), 7.10 (1 H, d, *J* = 1.91 Hz, H-2'), 7.07 (1 H, dd, *J* = 8.26, 1.91 Hz, H-6'), 6.84 (1 H, d, *J* = 8.26 Hz, H-5'), 6.20 (1 H, d, *J* = 2.25 Hz, H-6), 6.14 (1 H, d, *J* = 2.25 Hz, H-8), 3.88 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 3.61 (3 H, s, OCH_3), 3.28 (1 H, d, *J* = 17.12 Hz, H-4), 2.84 (1 H, d, *J* = 17.12 Hz, H-4)

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APPENDIX

REPRESENTATIVE NMR SPECTRA

Plate 1; ^{1}H NMR [$CDCl_3$]:

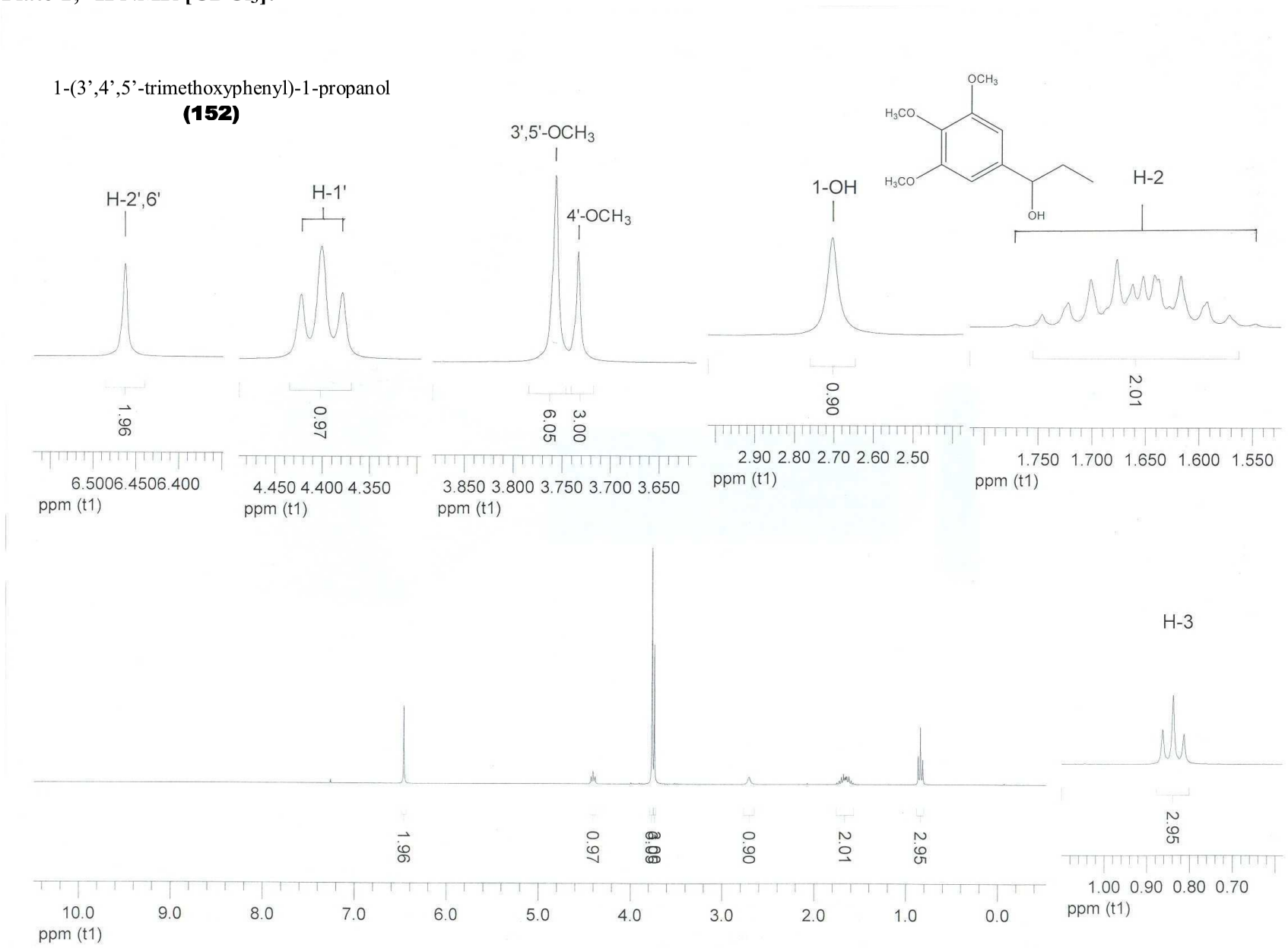


Plate 2; ^{1}H NMR [$CDCl_3$]:

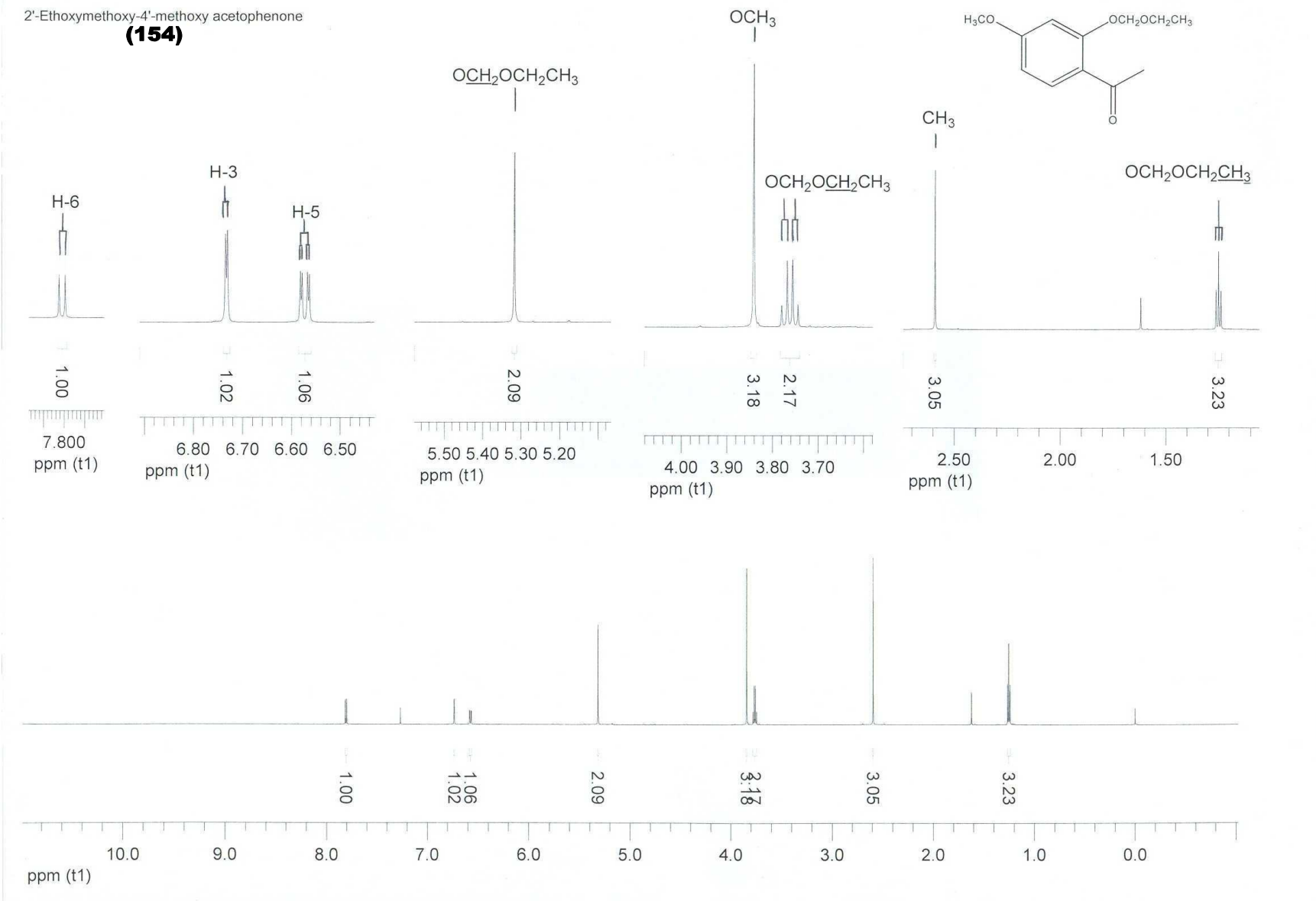


Plate 3; ^{1}H NMR [$CDCl_3$]:

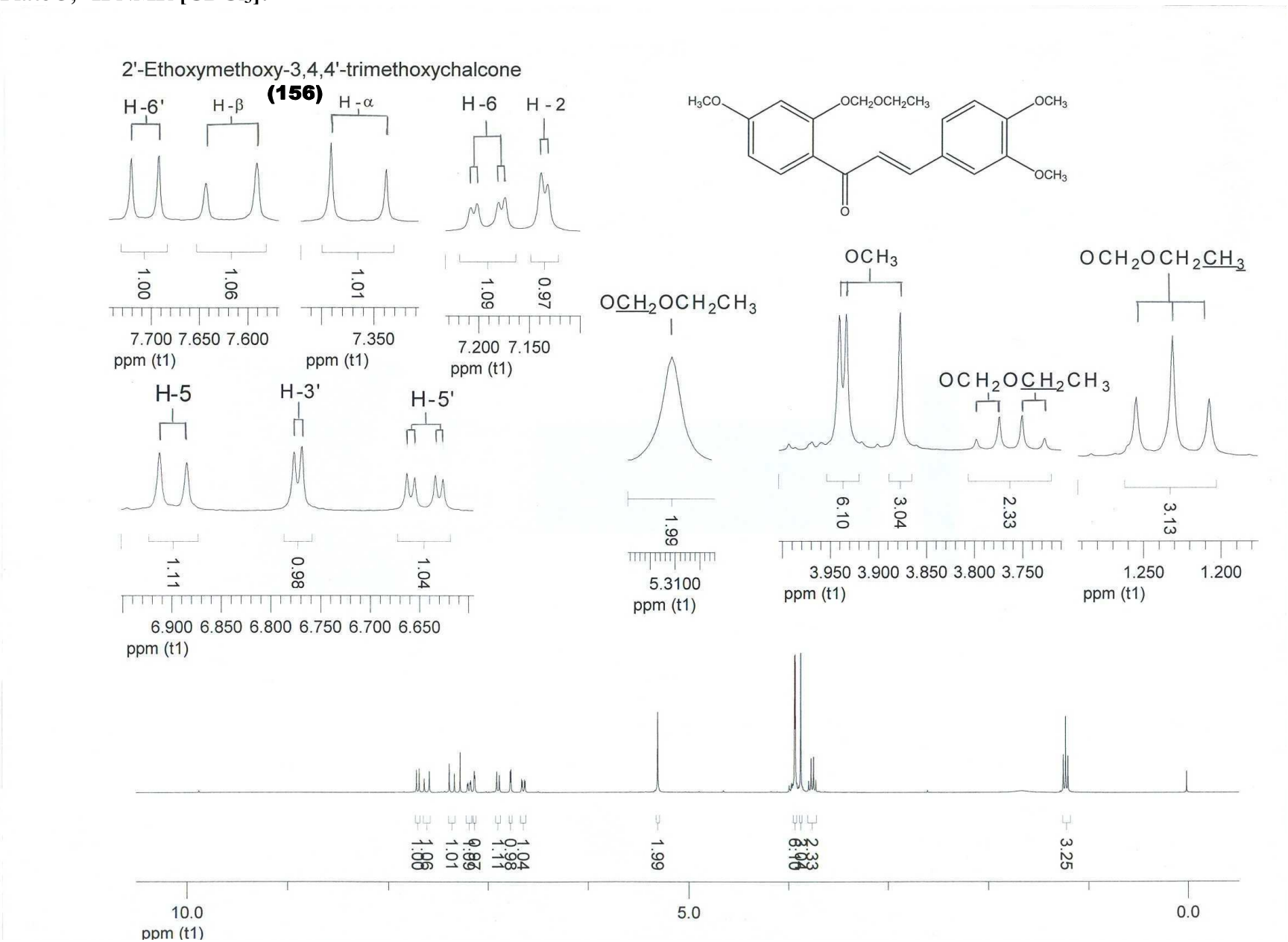
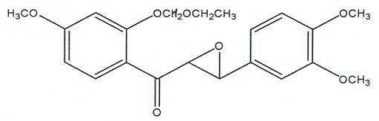


Plate 4; ^{1}H NMR [$CDCl_3$]:

2-Ethoxymethoxy-4',3,4-trimethoxychalcone epoxide
(157)



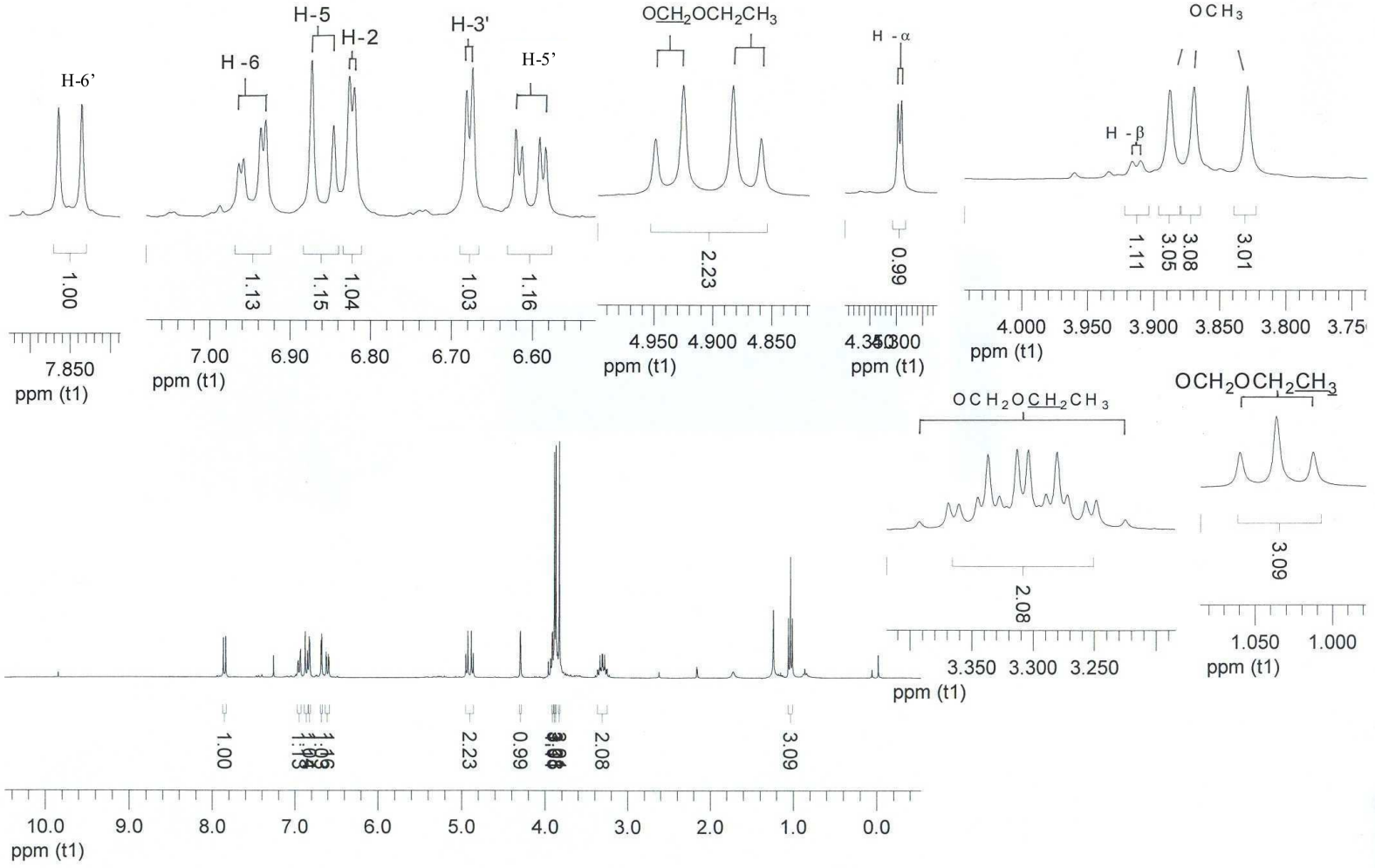


Plate 5A; ^{1}H NMR [$CDCl_3$]:

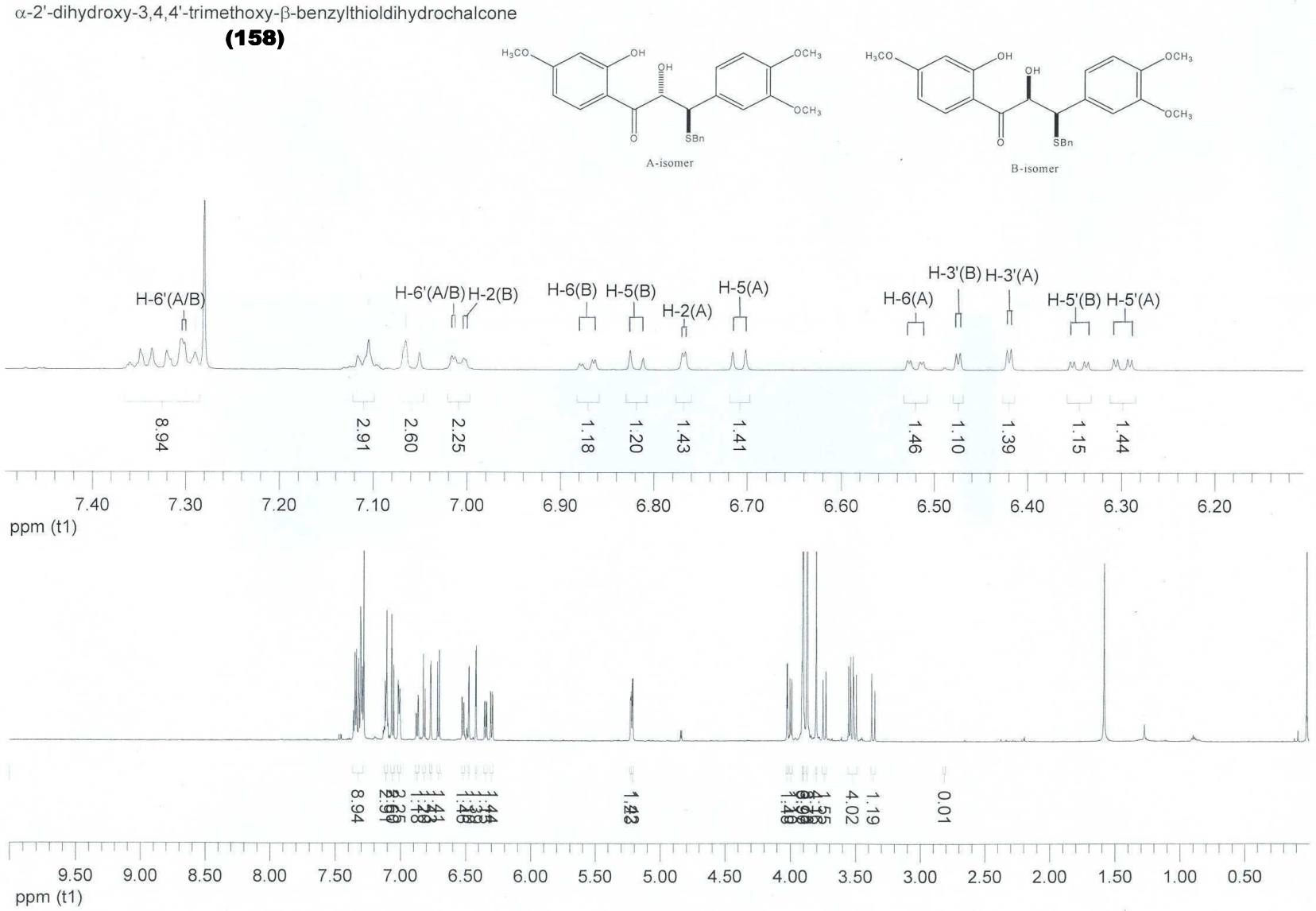


Plate 5A continued

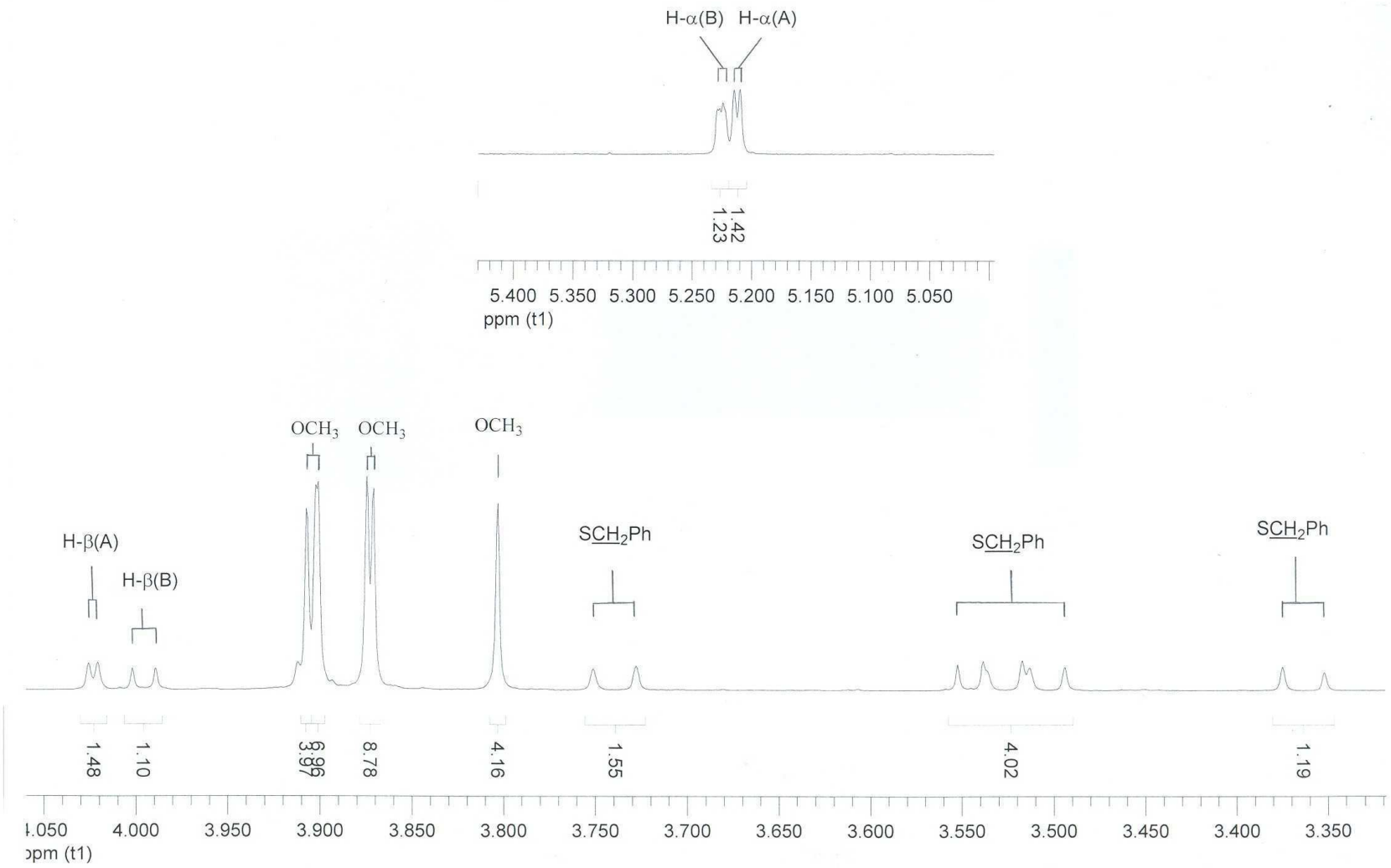


Plate 5B; ^{13}C NMR [$CDCl_3$]:

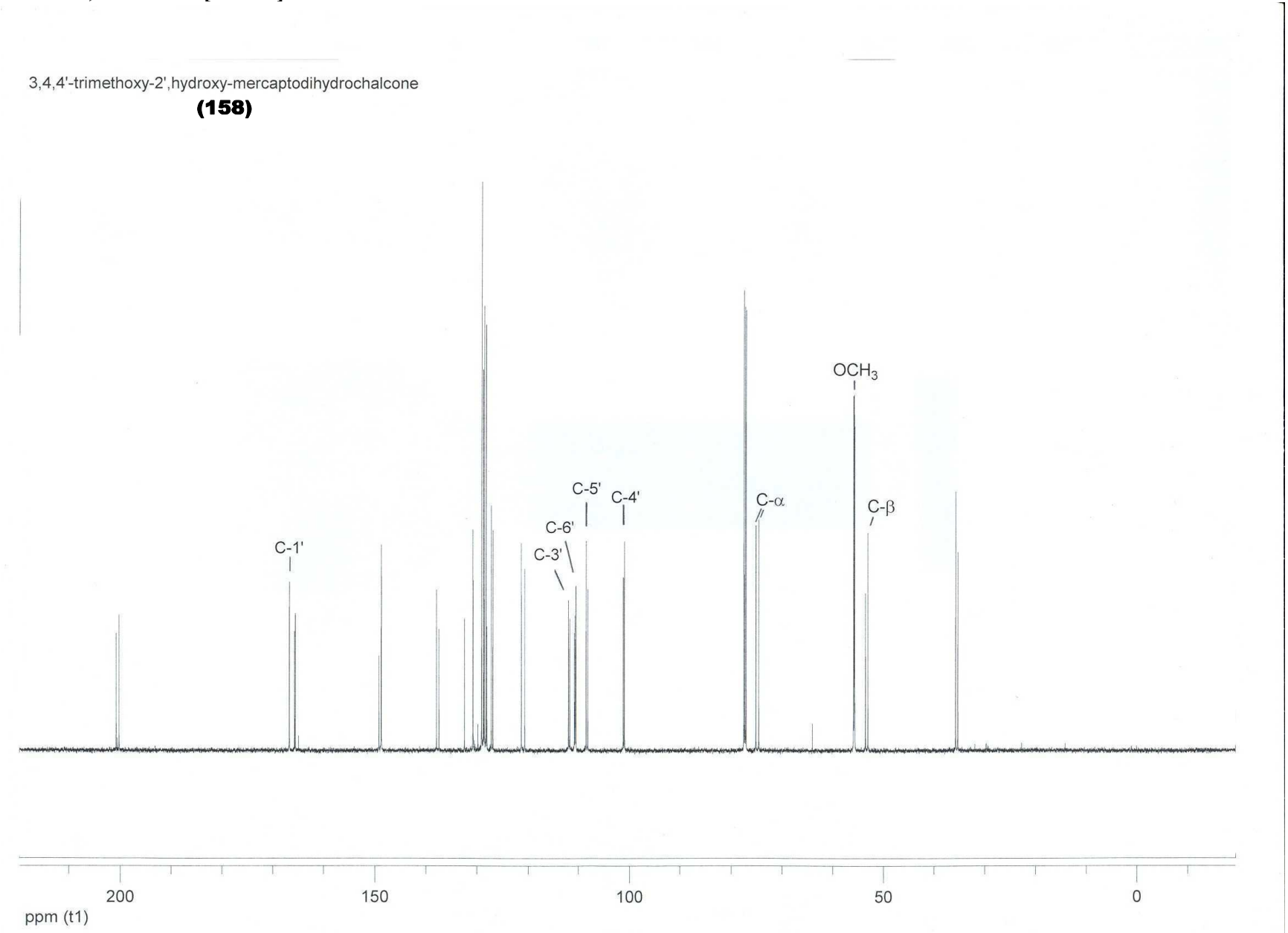


Plate 5C; COSY NMR [$CDCl_3$]: _____

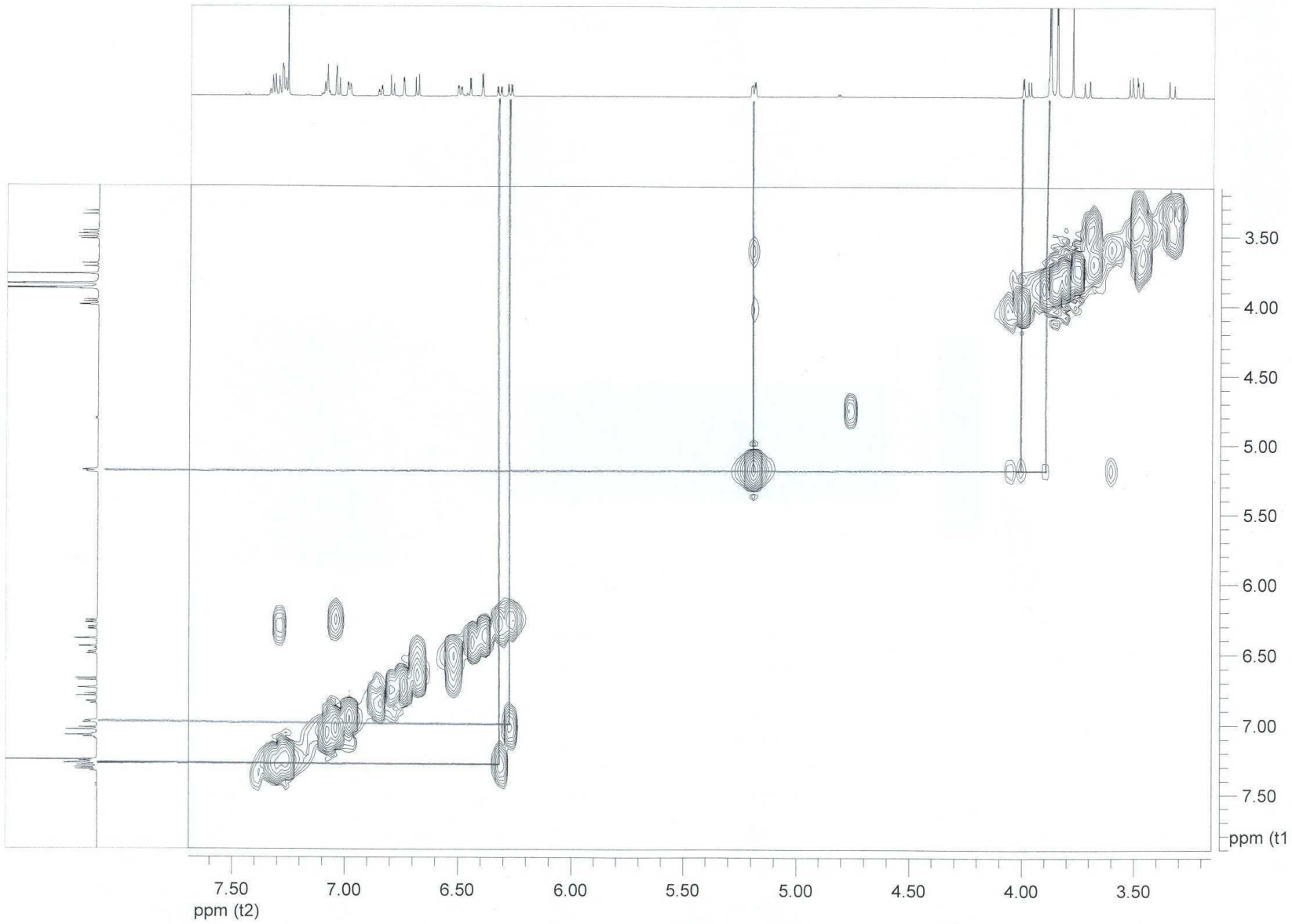


Plate 6; ^{1}H NMR [$CDCl_3$]:

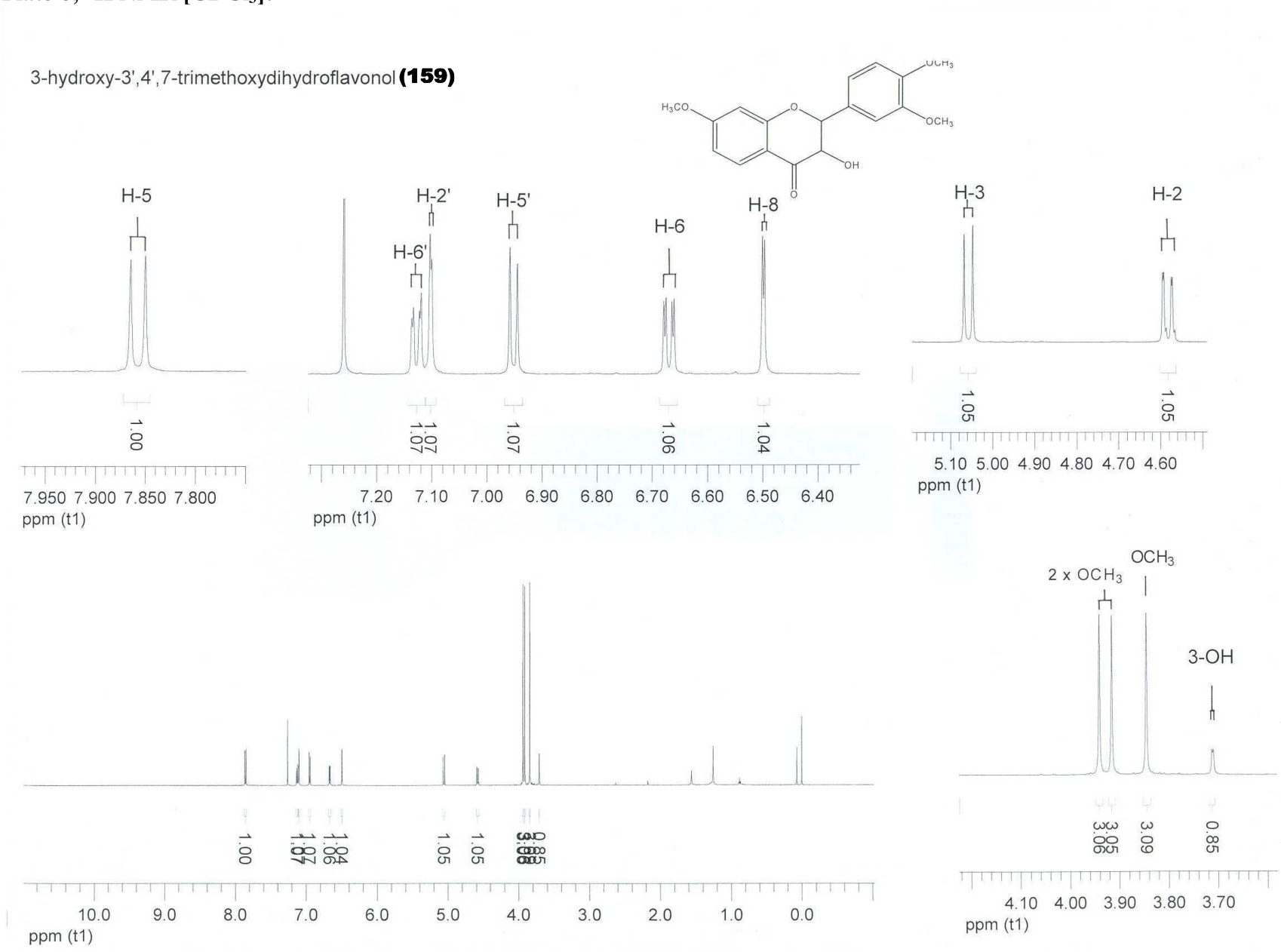


Plate 7; ^{1}H NMR [$CDCl_3$]:

3',4',7-trimethoxyisoflavone **(173)**

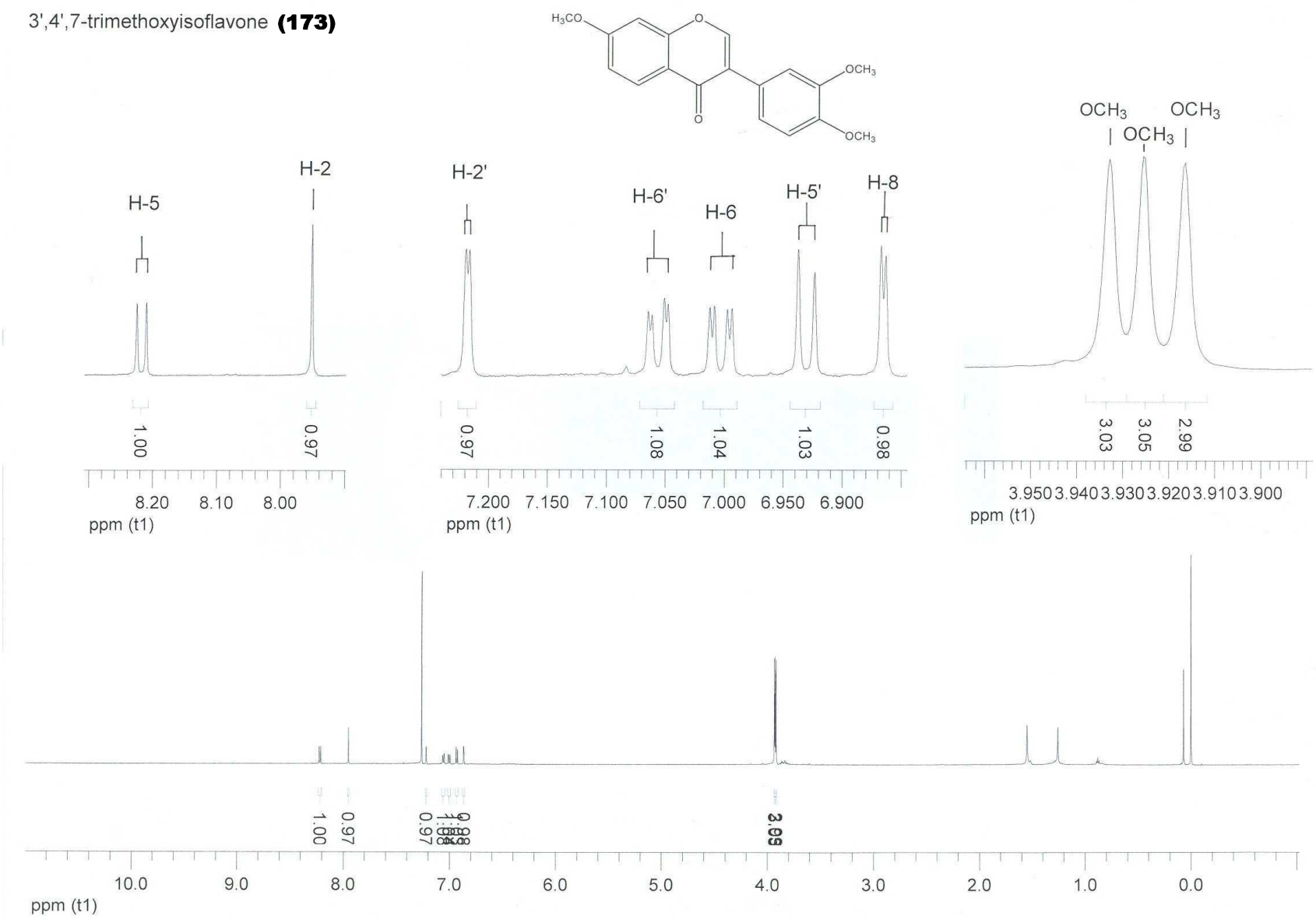


Plate 8; ^{1}H NMR [$CDCl_3$]:

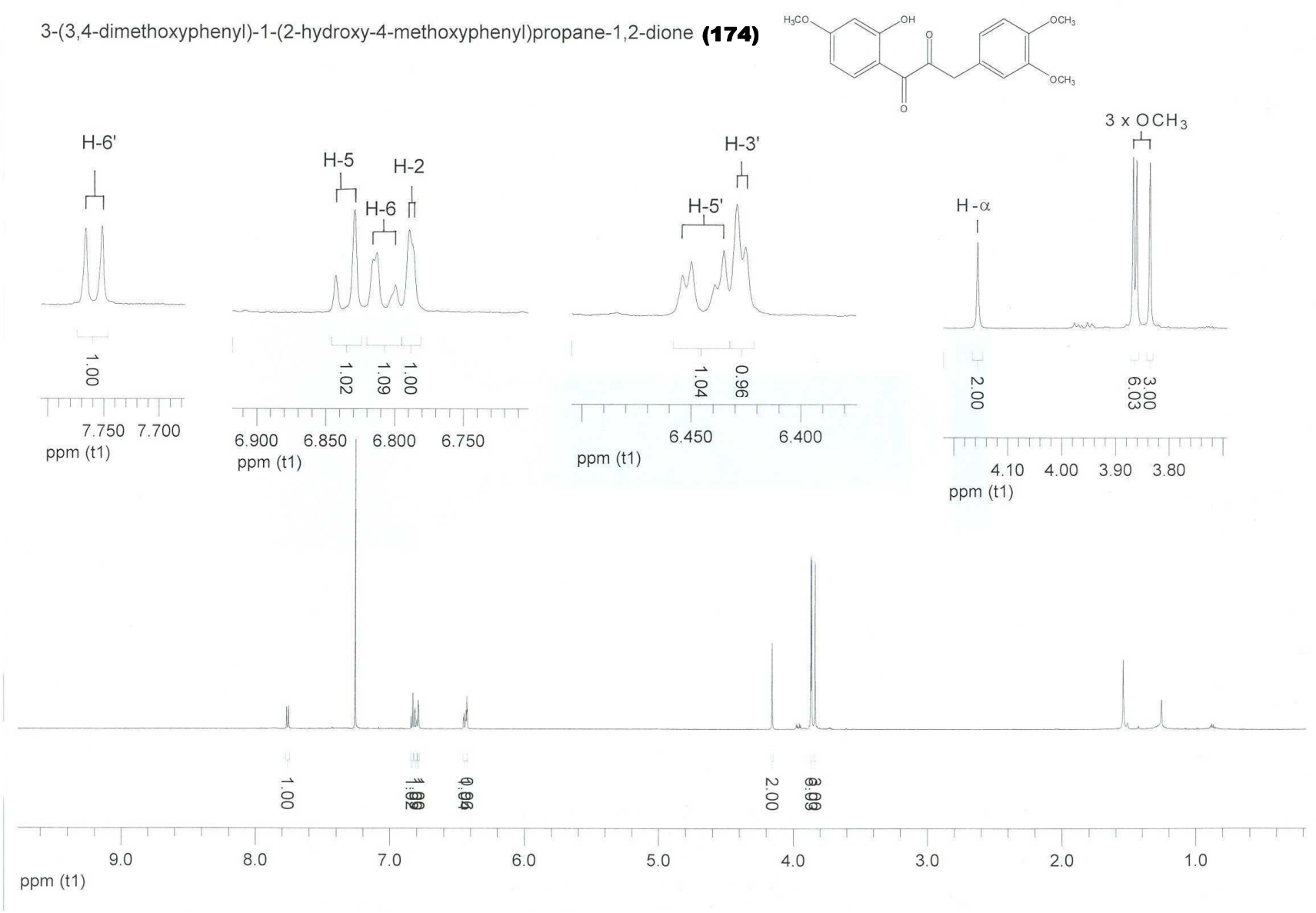


Plate 9; ^{1}H NMR [$CDCl_3$]:

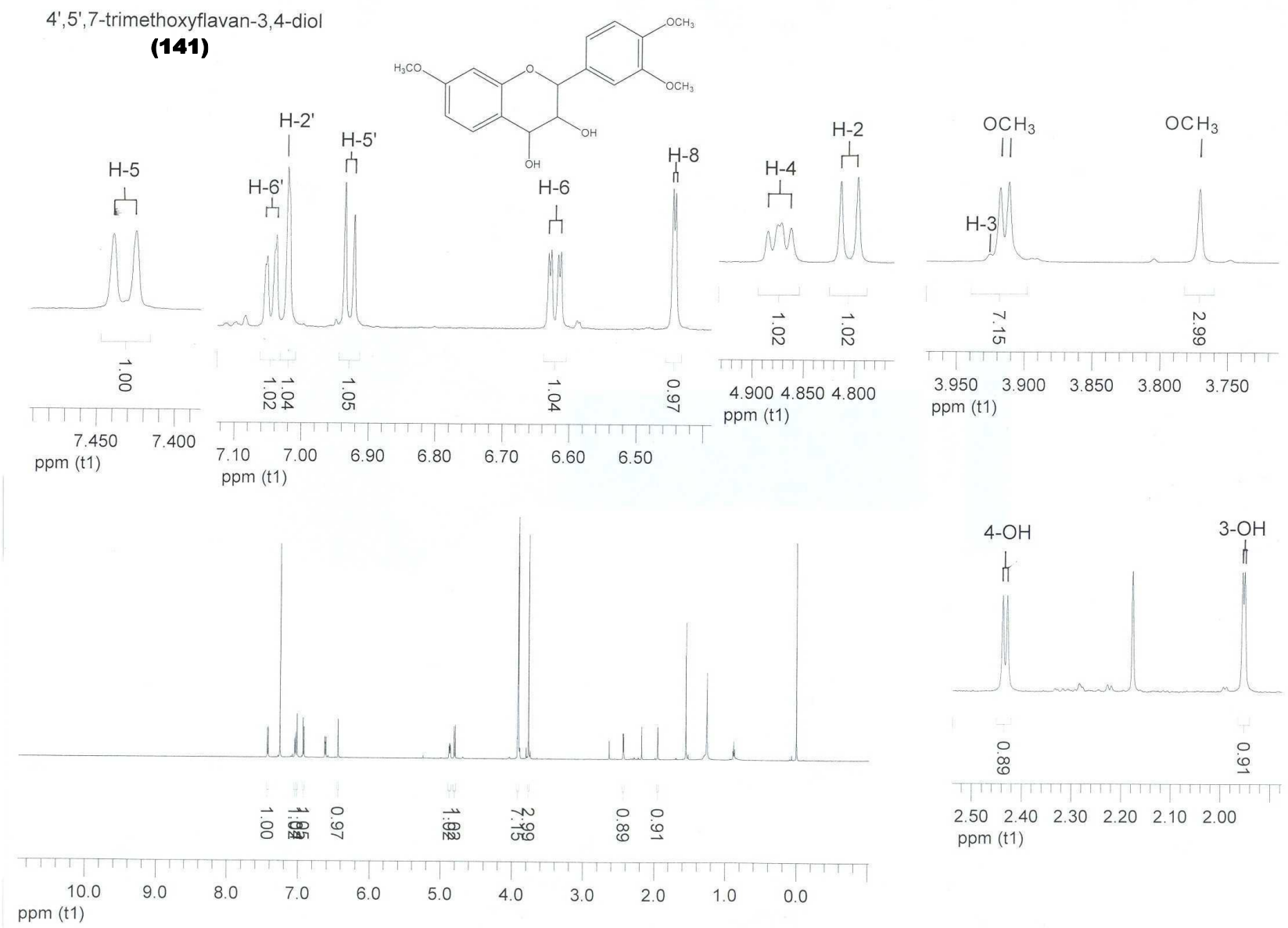


Plate 10; ^{1}H NMR [$CDCl_3$]:

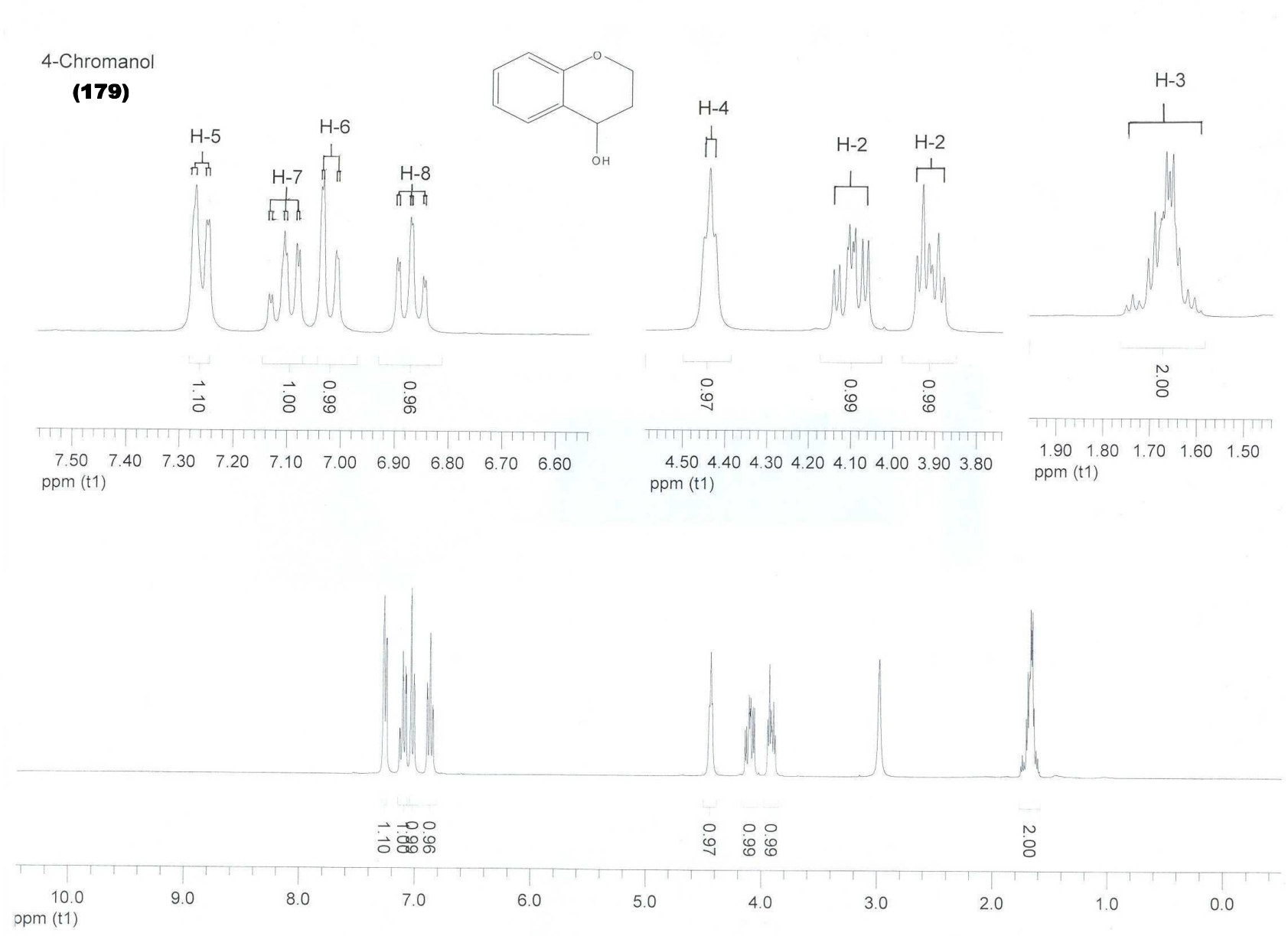


Plate 11; ^{1}H NMR [$CDCl_3$]:

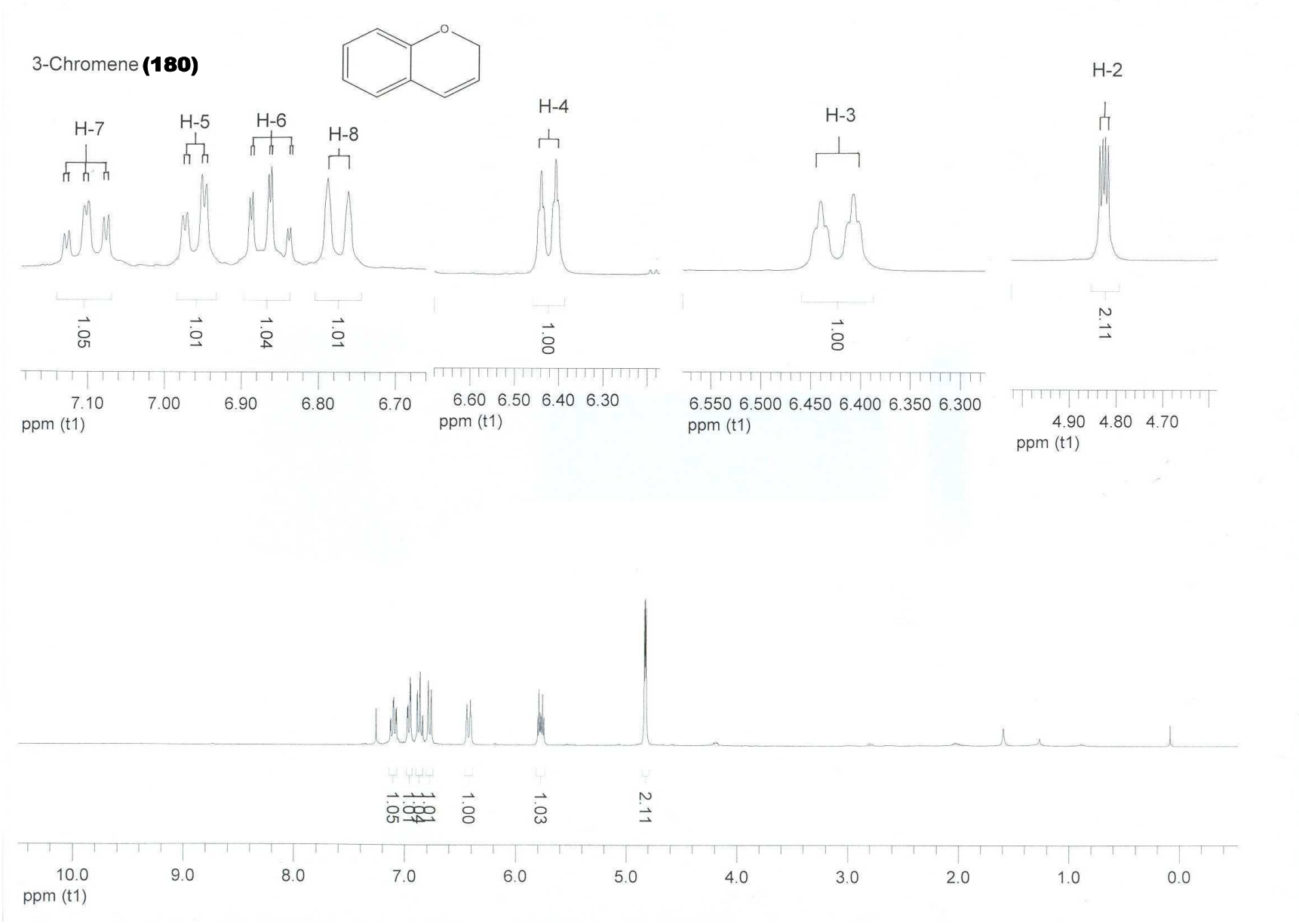


Plate 12; ^{1}H NMR [$CDCl_3$]:

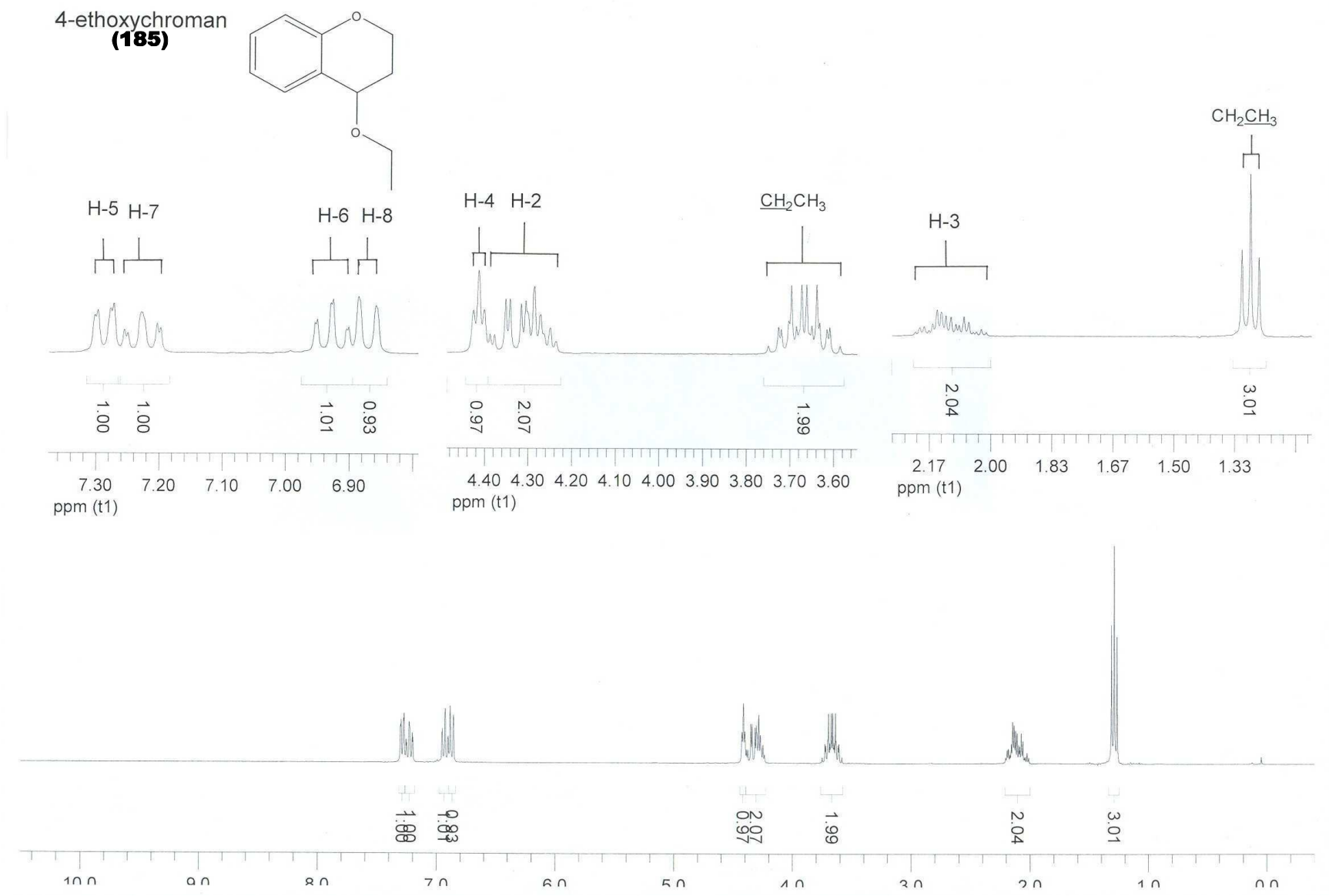
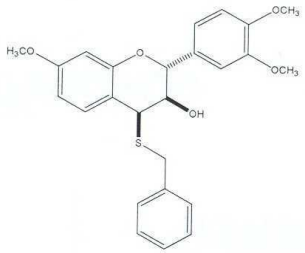


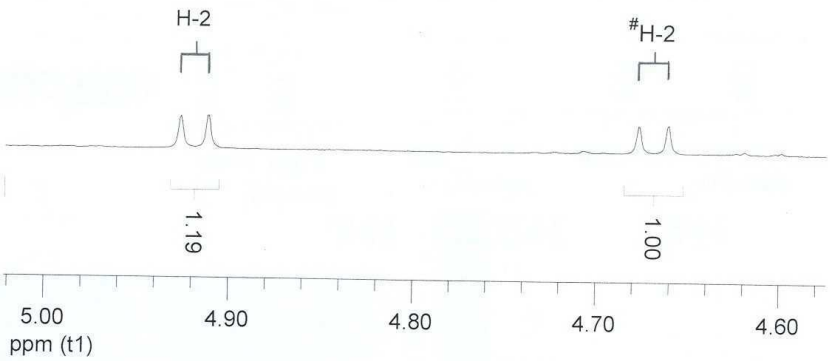
Plate 13A; ^{1}H NMR [$CDCl_3$]:

3',4',7-Trimethoxy-3-hydroxy-benzylthioflavan

(186)



= 2,3-*trans*, 3,4-*cis*
* = 2,3-*cis*, 3,4-*trans*
\# = 2,3-*trans*, 3,4-*trans*



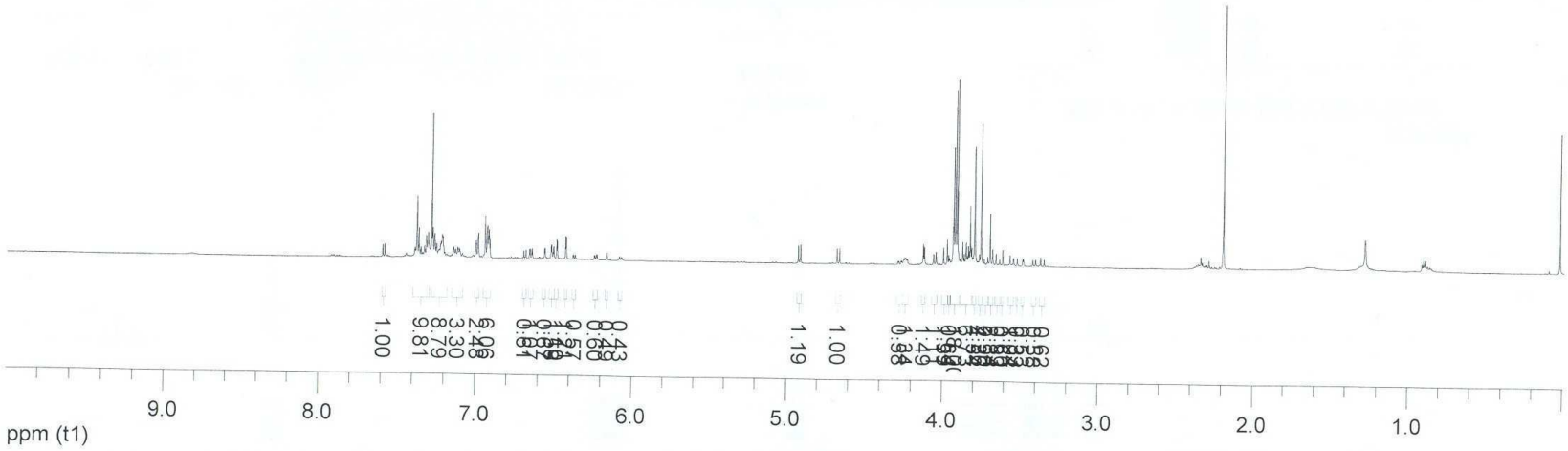
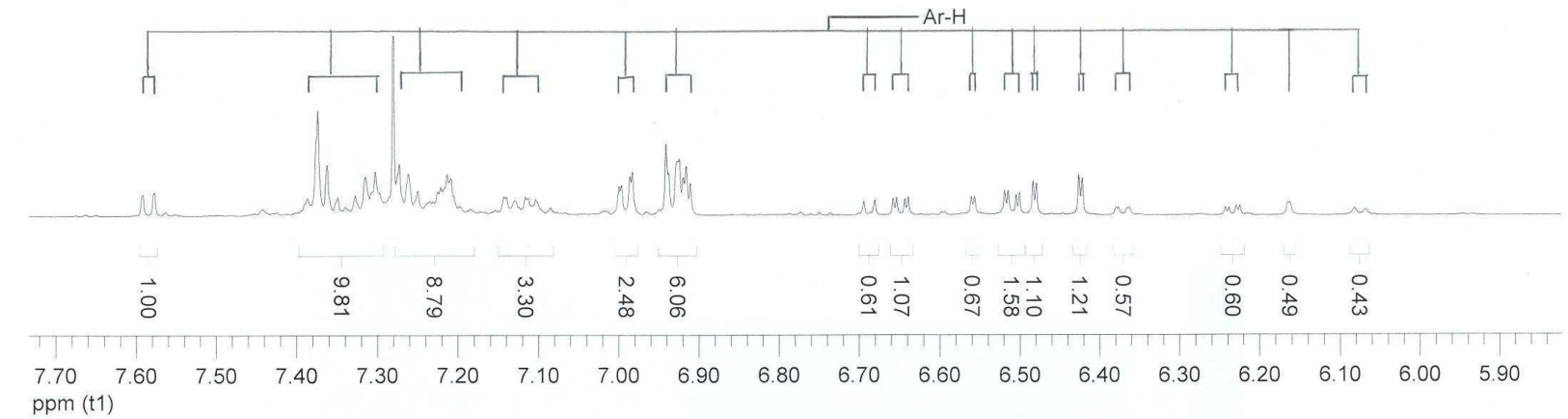


Plate 13A continued



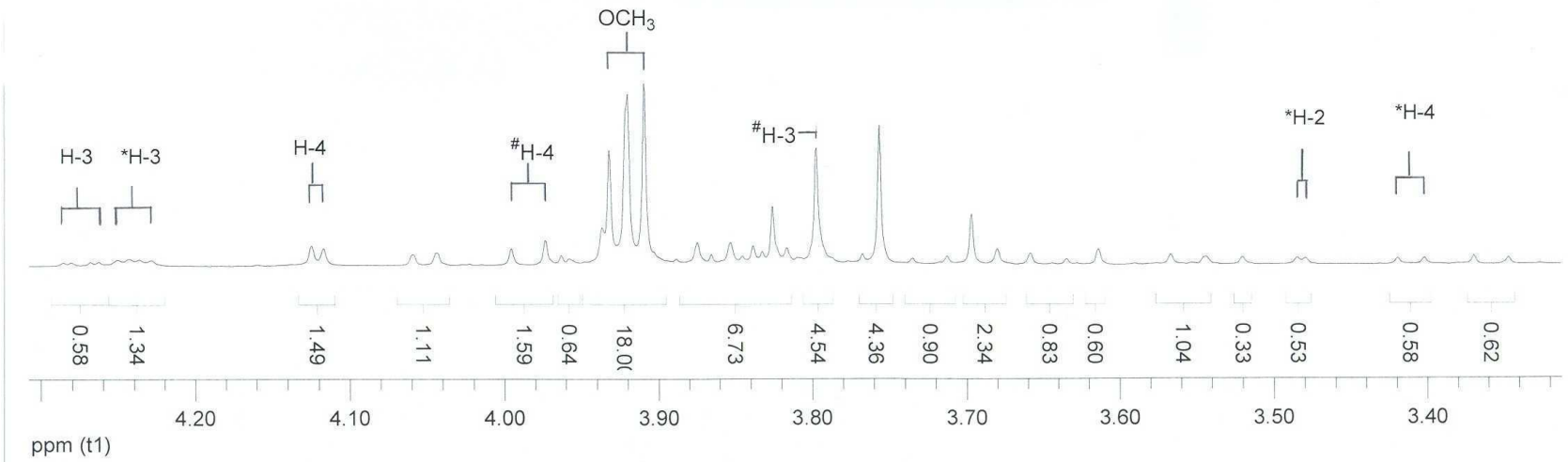


Plate 13B; ^{13}C NMR [$CDCl_3$]:

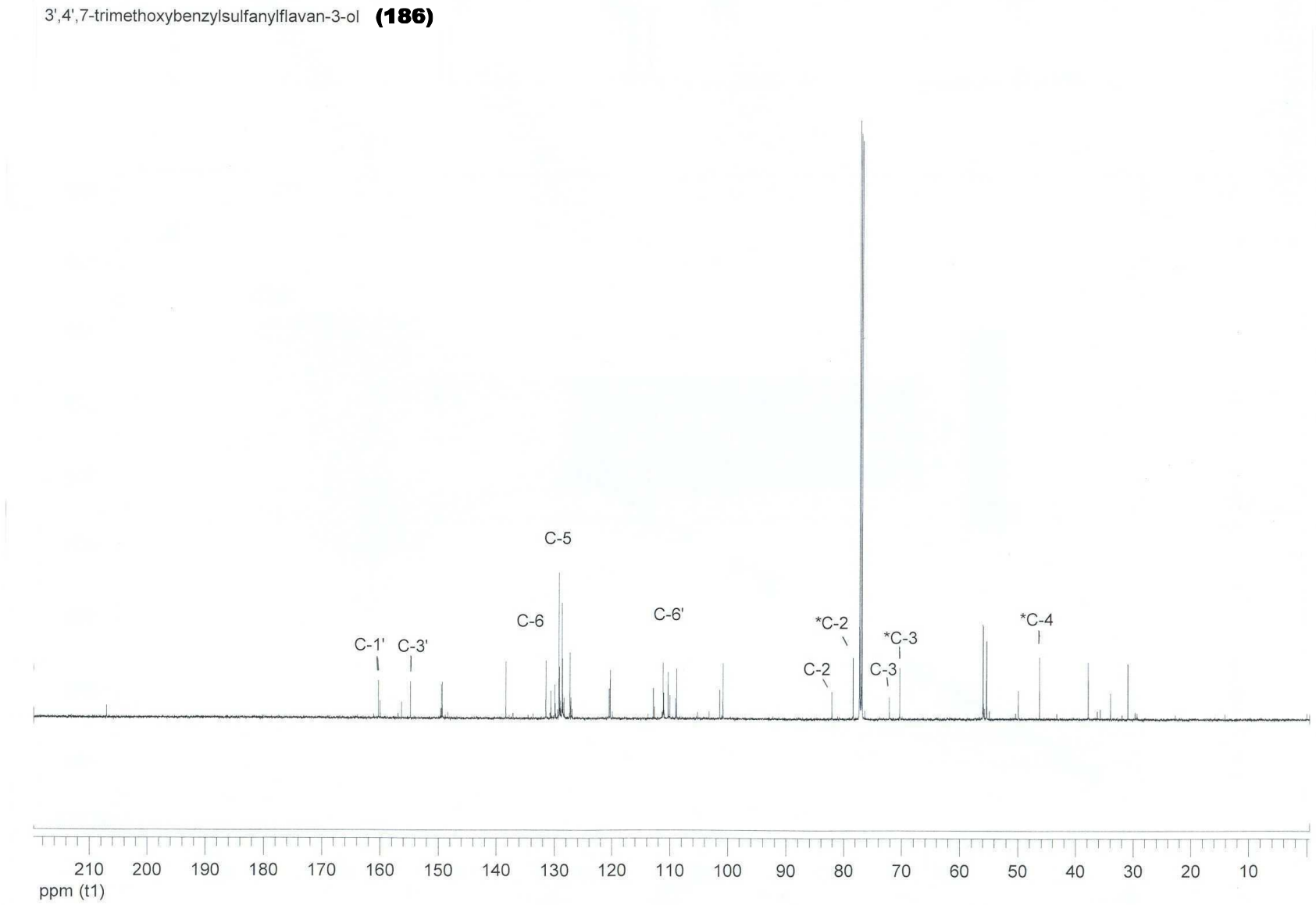


Plate 13C; COSY NMR [$CDCl_3$]:

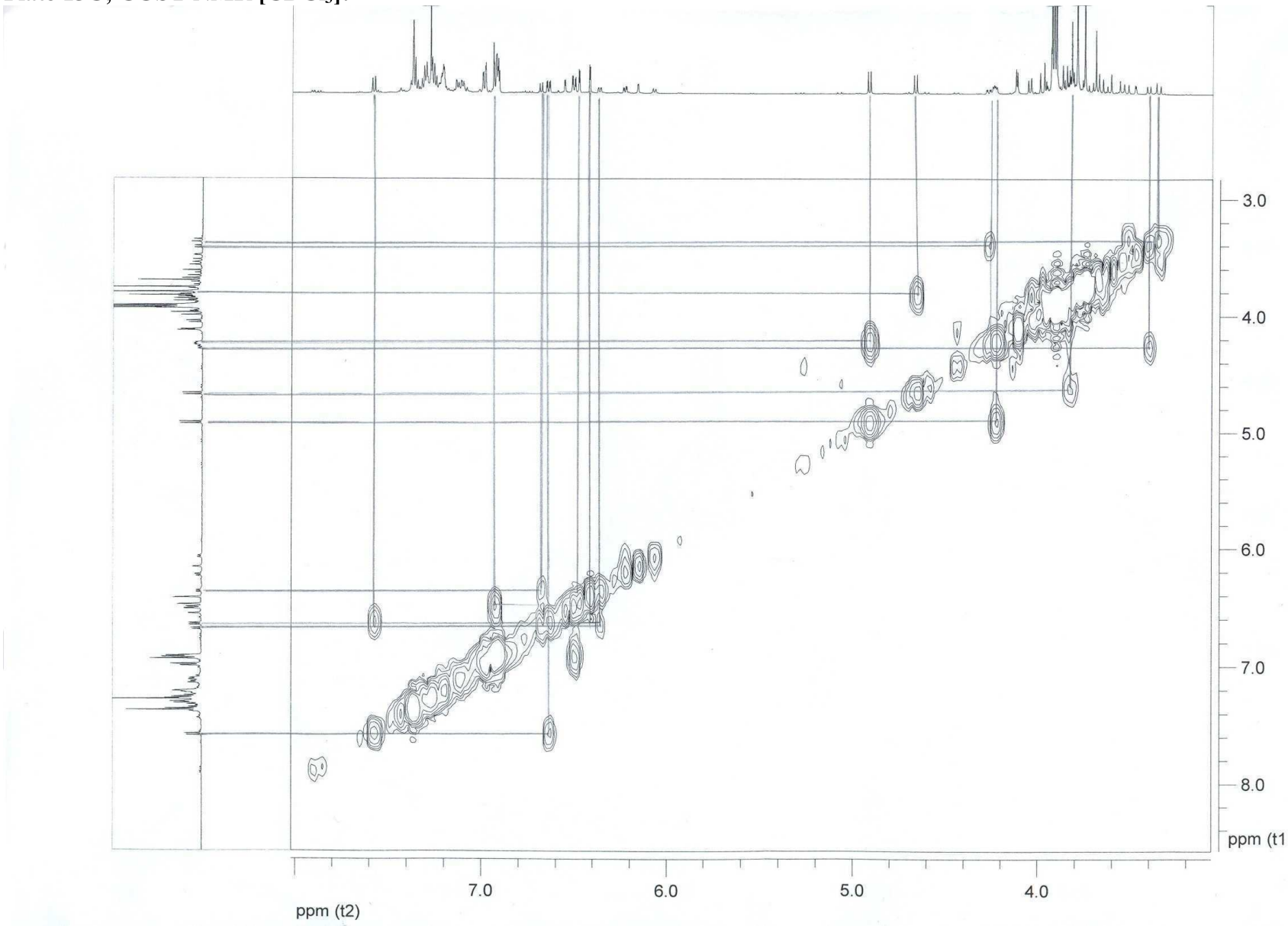


Plate 14; ^{1}H NMR [$CDCl_3$]:

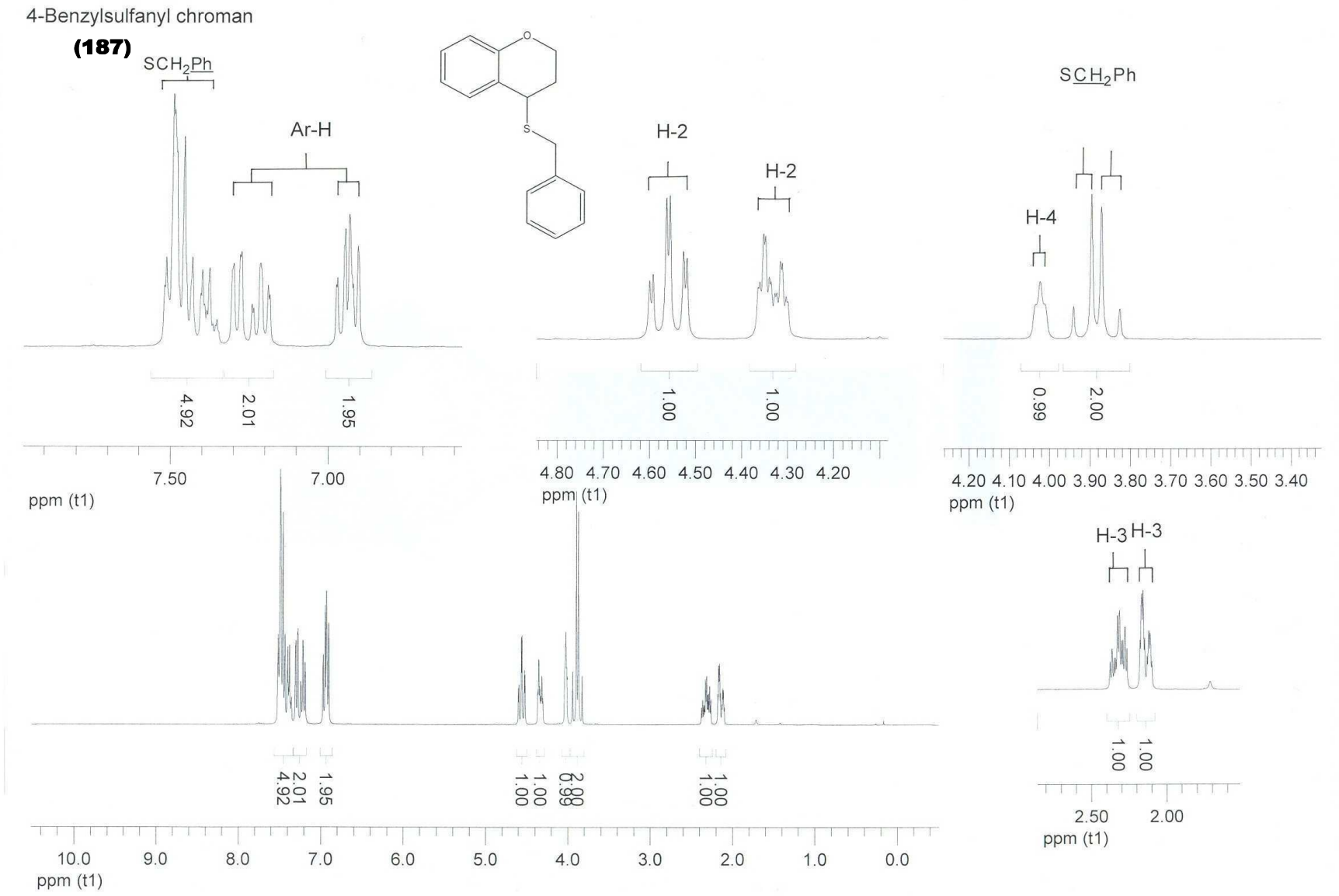


Plate 15; ^{1}H NMR [$CDCl_3$]:

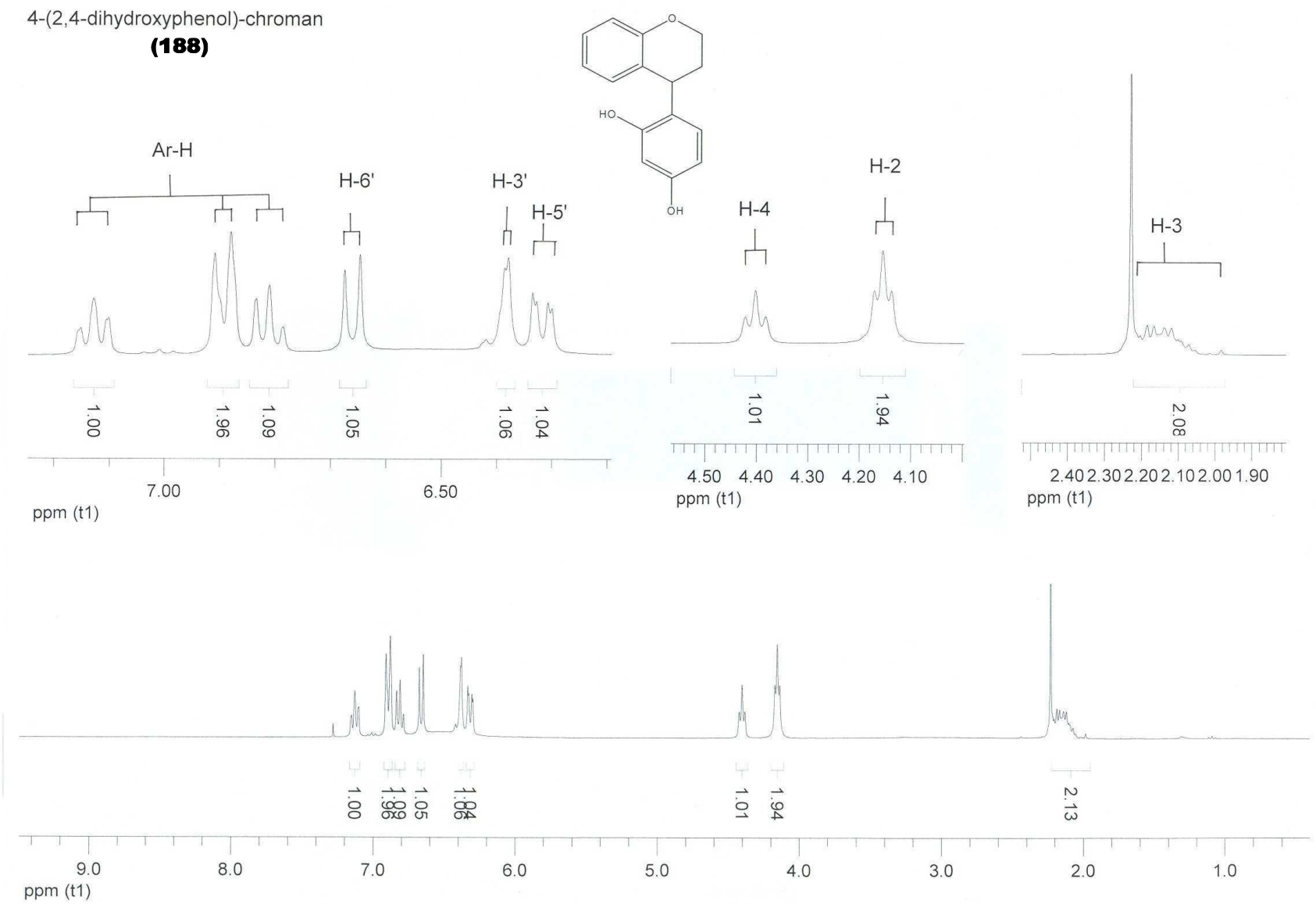


Plate 16; ^{1}H NMR [$CDCl_3$]:

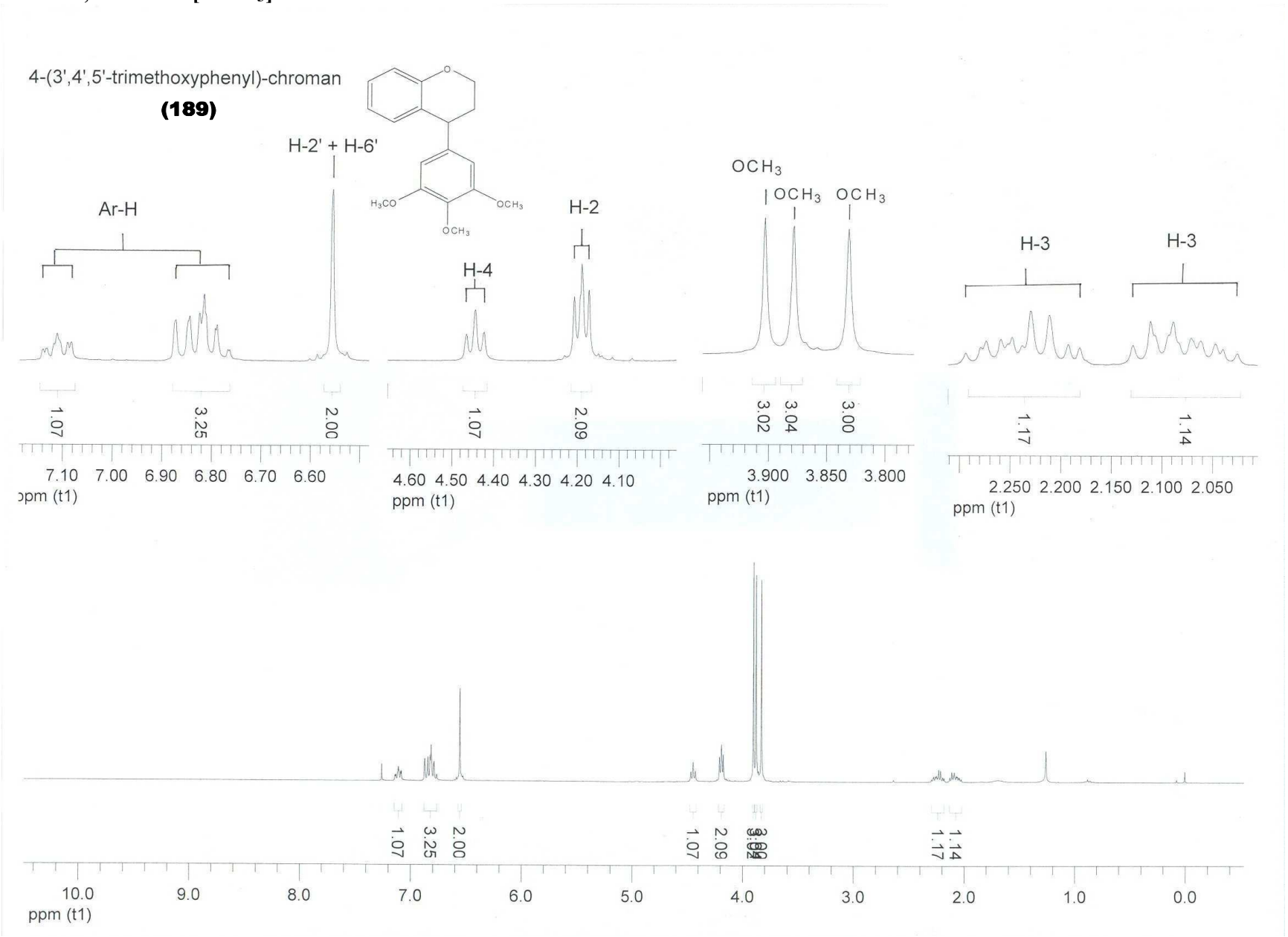


Plate 17; ^{1}H NMR [$CDCl_3$]:

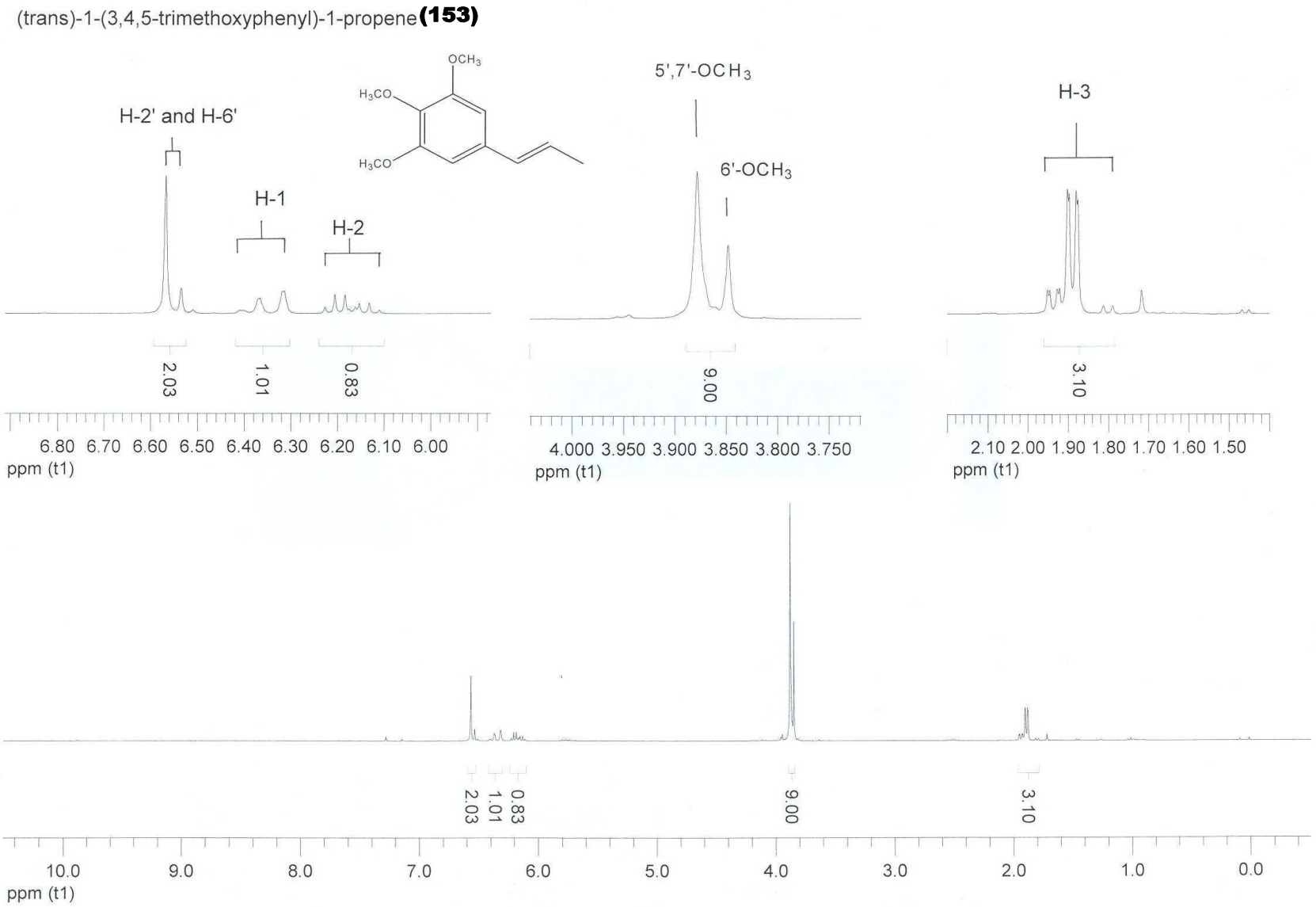


Plate 18; ^{1}H NMR [$CDCl_3$]:

2-Bromo-3-4-5-trimethoxybenzioc methyl ester

(198)

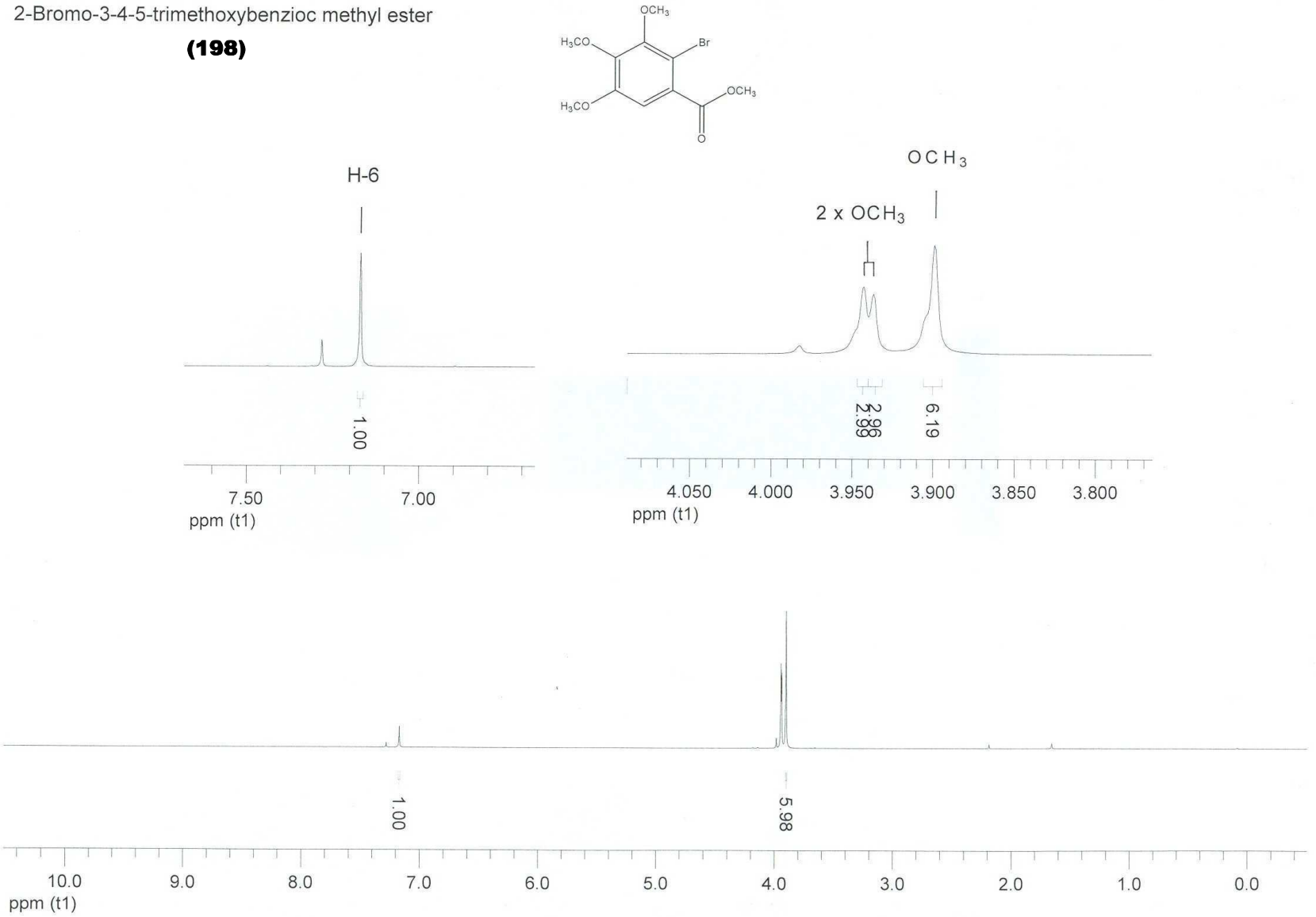


Plate 19; ^{1}H NMR [$CDCl_3$]:

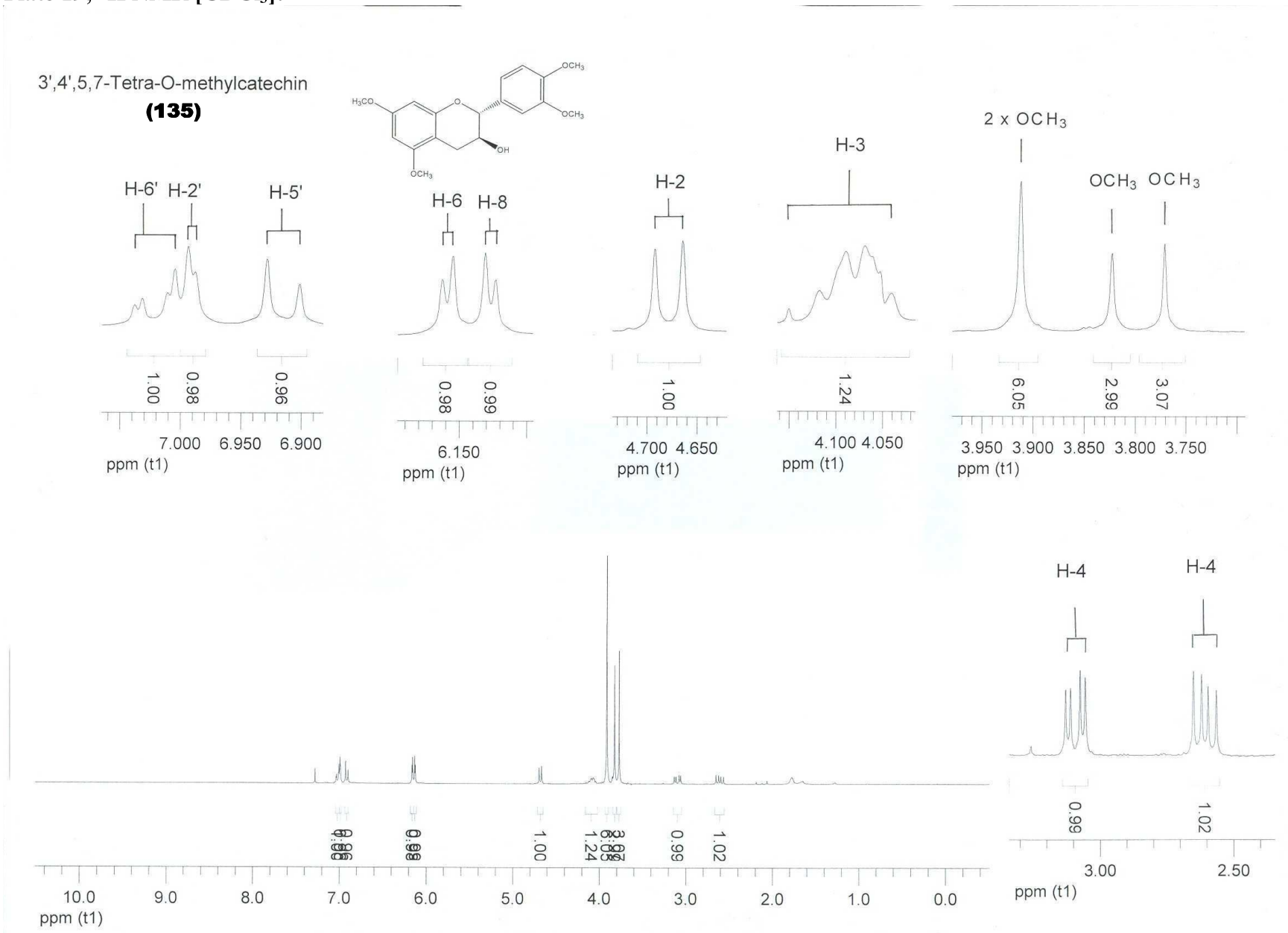


Plate 20; ^{1}H NMR [$CDCl_3$]:

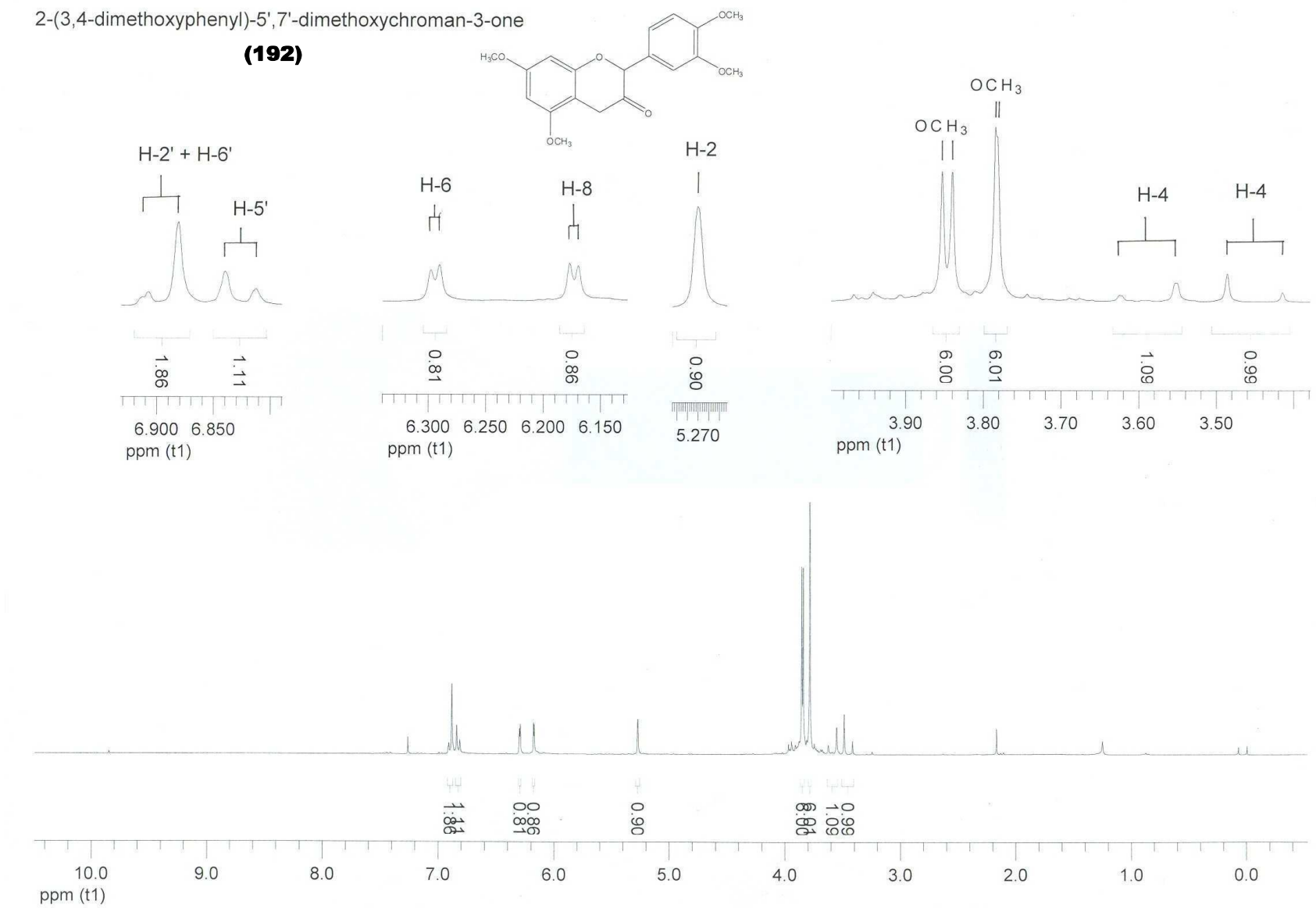


Plate 21; ^{1}H NMR [$CDCl_3$]:

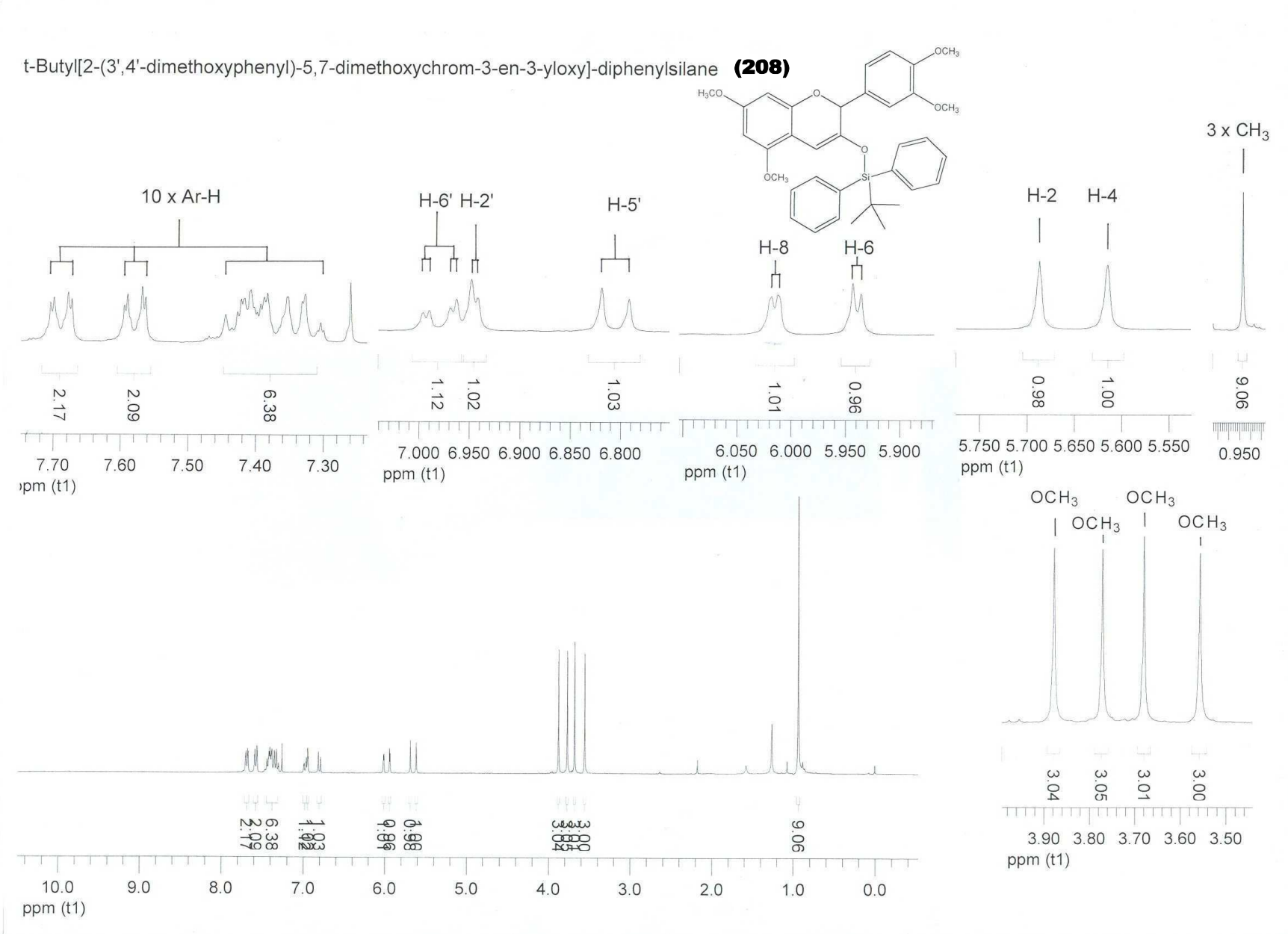


Plate 22; ^{1}H NMR [$CDCl_3$]:

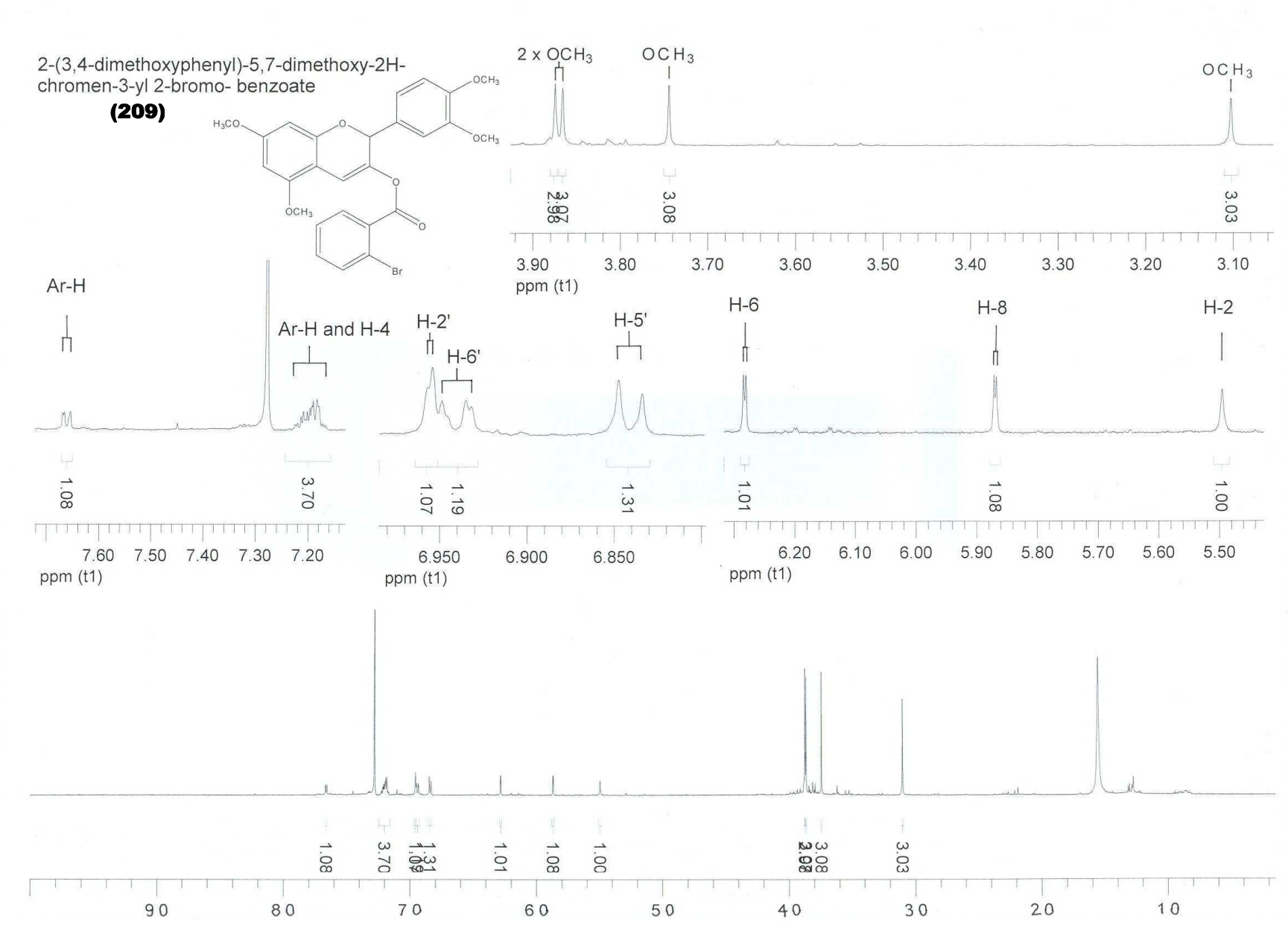
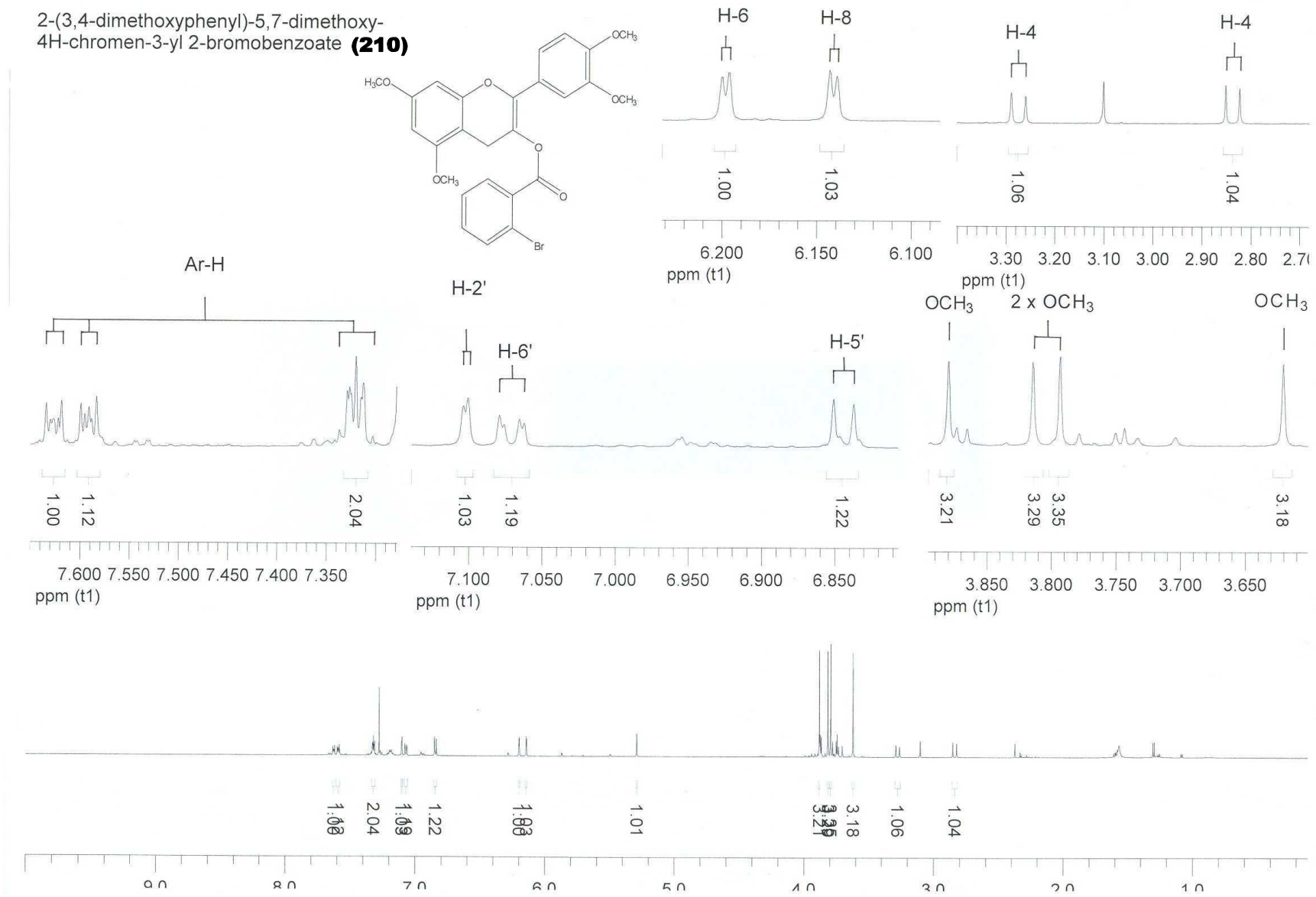


Plate 23; ^{1}H NMR [$CDCl_3$]:



SUMMARY

Summary

The phenolic content of the heartwood of *Peltophorum africanum* (African wattle) and *Burkea africanum* (Red syringa) proved to contain a variety of compounds including the novel 4-arylflavan-3-ol lactones, 6-(3,4-dihydroxyphenyl)-6,6a,8,12b-tetrahydroiso-chromeno[3,4-c]chromene-3,8,10,11,12-pentaol and 6-(3,4,5-tri-hydroxylphenyl)-6,6a,8,12b-tetrahydroisochromeno[3,4-c]chromene-3,8,10,11,12-pentaol respectively. Several attempts at the synthesis of these compounds to give final proof of the structure including the stereochemistry at the three chiral centres, failed due to the fact that the nucleophilicity of the pyrogallol ring of the gallic acid analogue (both protected and fee phenolic) is reduced to such an extent by the presence of the carbonyl group that it cannot effectively react with a C-4 electrophile generated on the flavan-3-ol starting material. In addition it became evident that the starting materials and products are sensitive to more drastic acid and basic reaction conditions to the extent that no desired products could be isolated from the attempted coupling of gallic acid analogues to C-4 functionalised flavan-3-ols.

In order to alleviate these problems, it was envisaged to utilize a nucleophile without the carbonyl attached to it, thus changing the substrate to a pyrogallol entity containing a 1-hydroxypropyl - or allyl substituent. After coupling at C-4 of the flavan-3-ol the required carbonyl could then be introduced by consecutive water elimination and isomerization (1-hydroxylpropyl substituent) or isomerization (allyl substituent) followed by ozonolysis (with non-reductive work-up) and subsequent esterification. Since the starting materials for the synthetic strategy were not available commercially, both the flavan-3,4-diol and 1-(3',4',5'-trimethoxyphenyl)propan-1-ol had to be synthesized. While the phenylpropan-1-ol analogue was obtained in 64 % yield through utilisation of a Grignard reaction between 3,4,5-trimethoxybenzaldehyde and ethylmagnesium bromide, the required 3',4',7-trimethoxyflavan-3,4-diol became available through synthesis and subsequent manipulation of the appropriate chalcone. Thus *trans*-2'-ethoxymethoxy-3,4,4'-trimethoxychalcone, obtained by standard Claisen-Schmidt condensation of 2-ethoxymethoxy-4-methoxyacetophenone and 3,4-dimethoxybenzaldehyde, was epoxidized with dimethyldioxirane to give the chalcone epoxide in 98 % yield. Deprotection and cyclization to the dihydroflavonol were accomplished in 67.5 % overall yield *via* treatment of the chalcone epoxide with

benzylmercaptan and tin(iv)chloride, followed by reaction of the subsequent α-hydroxy-β-benzylmercaptodihydrochalcone with silver tetrafluoroborate. Altough the *syn*- and *anti*-isomers of the mercaptodihydrochalcone were observed, both of these isomers led to only the 2,3-*trans*-dihydroflavonol. Finally, $NaBH_4$ reduction of the dihydroflavonol gave the flavan-3,4-diol 70 % yield.

Since the starting materials for - and products from the coupling reaction are known to be acid/base sensitive, it was decided to functionalise the flavan-3,4-diol through a mercaptan leaving unit that could be activated by thiophilic Lewis acid in order to induce coupling with the pyrogallol moiety. Silver tetrafluoroborate catalysed model reactions between 4-benzylmercaptochroman and the aromatic nucleophiles, resorcinol and methylated pyrogallol, gave the 4-arylchromans in 62 and 72% yield respectively. When the nucleophile was changed to 1-(3',4',5'-trimethoxyphenyl)propan-1-ol or the dehydrated version, 1-(3',4',5'-trimethoxy-phenyl)-1-propene, however, no couple could be detected. Since no apparent change in nucleophilicity could be identified as the cause of the reaction not giving any product, the failure can probably be ascribed to steric congestion brought about by the methoxy- and allyl - or propyl groups adjacent to the required point of reaction.

As it is known from literature that radicals play an important role in the *in vivo* synthesis of many natural products, a biomimetic approach towards the synthesis of the 4-arylflavan-3-ol lactones was considered as next alternative. It was therefore envisaged that generation of a phenolic radical at C-2 of the pyrogallol ring of a flav-3-ene 3-gallate ester moiety could result in the formation of the desired lactone. Thus tetra-O-methyl catechin was converted into the 3-keto compound by mild IBX (2-iodoxybenzoic acid) oxidation in 61 % yield. Treatment of the catechin derivative with LDA followed by quenching of the enolate with *t*-butyldiphenylchlorosilane led to 3-*tert*-butyldiphneylsilyloxy-3',4',5,7-tetramethoxyflav-3-ene in 84 % yield; thus proving that the double bond was indeed in the right position (between C-3 and C-4 and not C-2 and C-3) for the lactone to be formed. With the position of the double bond established, the enolization reaction was repeated with 2-bromobenzoyl chloride as model electrophile and the enol ester obtained in 22 % yield. In this instance, however, the desired enol ester was accompanied by the 2,3-unsaturated isomer (43 %). With all the uncertainties round the oxidation and enol formation sorted out, the final step in this strategy for the

synthesis of the target lactones can now attempted with confidence. AIBN initiated radical cyclization of 3-*O*-(3",4",5"-trimethoxybenzoyl)-3',4',7-trimethoxyflav-3-ene and 3-*O*-(3",4",5"-trimethoxybenzoyl)-3',4',5',7-tetramethoxyflav-3-ene will, however, receive attention in a subsequent PhD study.

Keywords:

Flavonoids; lactone; pyrogallol; catechin; benzylmercaptan; chalcone; epoxidation; enolization; natural; product

---

SAMEVATTING

Samevatting

Howel ‘n groot verskeidenheid fenoliese verbindings reeds oor die jare uit die kernhout van *Peltophorum africanum* (“African wattle”) en *Burkea africanum* (Rooisering) geïsoleer is, is twee nuwe unieke 4-arielflavan-3-ol laktone, naamlik 6-(3,4-dihidroksielfeniel)-6,6a,8,12b-tetrahidroisochromeno[3,4-c]chromeen-3,8,10,11,12-pentaol en 6-(3,4,5-trihidrok-sielfeniel)-6,6a,8,12b-tetrahidroiso-chromeno-[3,4-c]chromeen-3,8,10,11,12-pentaol onderskeidelike, onlangs ook uit die hout van hierdie twee bome verkry. Ten einde finale struktuurbewys, veral tov die absolute konfigurasie van die drie chirale sentrums teenwoordig op die C-ring van hierdie verbindings, te lewer, is verskeie pogings tot die sintese van hiedie laktone reed aangewend. Die teenwoordigheid van ‘n karbonielgroep in die gallusuur eenheid wat as nukleofiel tydens vorige sinteses benut is, het egter weens verlaagde nukleofiliteit van die pirogallol ring daartoe gelei dat reaksie met ‘n karbokatioon gegenereer by C-4 van die flavan-3-ol eenheid nie plaasgevind nie. Voorts is bevind dat meer drastiese suur - en basisiese reaksiekondisies, wat toegepas is ten einde die reaksie te forseer, tot die ontbinding van produkte en/of uitgangstowwe aanleiding gegee het.

Om genoemde probleme aangaande verlaagde nukleofiliteit van die pirogallol ring te vermy, is besluit om van ‘n alternatiewe nukleofiel, sonder ‘n karboniel-funksionaliteit, gebruik te maak en is die substraat vir die koppelingsreaksies na ‘n pirogallol entiteit met ‘n 1-hydroksipropiel- of alliel substituent, verander. Laasgenoemde groepe kan dan na koppeling deur isomerisasie (allielgroep) or water eliminasie (propanolgroep) gevolg deur osonolise met nie-reduktiewe opwerk kondisies en esterifikasie in die laktoon omgeskakel word. Aangesien beide uitgangstowwe vir die nuwe benadering nie kommersieel beskikbaar is nie, moes beide die 1-(3’,4’,5’-trimetoksifeniel)propan-1-ol en die flavan-3,4-diol self gesintetiseer word. Toepassing van ’n Grignardreaksie tussen 3,4,5-trimetoksibensaldehied en etielmagnesiumbromied het vervolgens die 1-feniel-1-propanol in 64 % opbrengs gelewer, terwyl die flavan-3,4-diol deur manipulasie van die gepaste chalkoon, verkry is. Epoksidasie van *trans*-2’-etoksimetoksi-3,4,4’-trimetoksiechalkoon, verkry deur die standaard Claisen-Schmidt kondensasie van 2-etoksimetoksi-4-metoksiasetofenoon en 3,4-dimetoksibensaldehied, met dimetiel-

dioksiraan het vervolgens die chalkoonepoksied in 98% opbrengs gelewer. Omsetting van die chalkoonepoksied na die verlangde 3',4,4'-trimetoksieflavan-3,4-diol is mbv 'n twee stap proses bewerkstellig: Eerstens is die dihidroflavonol (67.5 % algehele opbrengs) verkry deur die chalkoonepoksied met bensielmerkaptaan en tin(iv)chloride te behandel, waarna die α-hydroksi-β-bensielmerkaptodihidrochalkoon met behulp van silwertetrafluoroboraat gesikliseer is. Alhoewel 'n mengsel van beide die *syn*- en *anti*-isomere van die merkaptodihidrochalkoon tydens die sikliserings reaksie benut is, is slegs die *trans*-dihidroflavonol as produk uit hierdie reaksie verkry. Die flavan-3,4-diol is vervolgens in 70% opbrengs deur $NaBH_4$ reduksie van die dihidroflavonol daargestel.

Aangsien die produkte sowel as die substrate van die koppelingsreaksie suur/basis sensitief is, is daar besluit om die flavan-3,4-diol met 'n merkaptaan verlatende groep te funksionaliseer, wat dan weer deur 'n tiofiliese Lewis suur geaktiveer kan word om koppeling met die pirogallol eenheid te induseer. $AgBF_4$ gakataliseerde model reaksies tussen 4-bensielmerkaptochromaan en die nukleofiele reagense, resorsinol en gemetileerde pirogallol, het die 4-arielchromane in 62 en 72 % opbrengs onderskeidelik, opgelewer. Die gebruik van 1-(3',4',5'-trimetoksifeniel)propan-1-ol asook die gedehidrateerde weergawe daarvan, 1-(3',4',5'-trimetoksifeniel)-1-propeen, het egter onder dieselfde kondisies geen koppeling tot gevolg gehad nie. Aangesien gee radikale verskil in die nukleofiliteit van die substrate wat reaksie getoon het en die wat nie gereageer het nie, verwag kan word nie, kan die mislukking van die fenielpropeen en fenielpropanol reaksies waarskynlik aan steriese faktore toegeskryf word.

Weens die feit dat die vorige benadering nie suksesvol was nie, en dit bekend is dat radikale 'n belangrike rol speel in die *in vivo* sintese van baie natuurprodukte, is 'n biomimetiese benadering vir die sintese van die 4-arielflavan-3-ol laktone as alternatief oorweeg. Die moontlikheid dat die verlangde laktoon verkry kan word deur die skepping en reaksie van 'n radikaal op C-2 van die pirogallol ring van 'n flav-3-een 3-gallaat ester entiteit, is dus voorts ondersoek. Tetra-O-metielkatesjien is gevolglik omgeskakel na die 3-keto analoog in 70% opbrengs deur milde IBX (*2*-iodoksibensoësuur) oksidasie. Deur die katesjien derivaat met LDA (litiumdiisopropielamied) te behandel en die gevormde enolaat as 'n *t*-

butieldifenielchlorosilaan derivaat in 84 % opbrengs te isoleer, is bepaal dat die dubbelbinding inderdaad op die regte plek vir latere laktoon vorming, tussen C-3 en C-4 (en nie tussen C-2 en C-3 nie), gevorm word. Die enolaat reaksie is vervolgens met 2-bromobenzoielchloried as model elektrofiel herhaal en die enol ester in 22 % opbrengs verkry. Laasgenoemde reaksie het egter ook die termodinamies minder stabiele 2,3-enolester (43 %) as produk opgelewer. Met al die onsekerhede mbt die oksidasie van die flavan-3,4-diol eenheid en vorming van die verlangde enolaat ester opgeklaar, kan die finale stap in die sintese van die flavan-3-ol laktone nou met vertroue aangepak word.. Radikaal geïnduseerde (AIBN) siklisering van 3-*O*-(3”,4”,5”-trimetoksibenzoïel)-3’,4’,7-trimetoksiflav-3-een en 3-*O*-(3”,4”,5”-trimetoksibenzoïel)-3’,4’,5’-tetrametoksiflav-3-een behoort die metieleters van die geïsoleerde flavan-3-ol laktone te lewer en sal in ‘n komende PhD ondersoek aandag geniet.