The Synthesis of Enantiopure 1,3-dimethylisochroman and related Racemic Analogues Using Anacardic Acid as a Bio-renewable Starting Material

Tianqi Lu

A dissertation submitted to the Faculty of Science,

University of the Witwatersrand,

Johannesburg

In fulfilment of the requirements for the Degree of Master of Science

October 2019

Declaration

I declare that the work presented in this dissertation was carried out exclusively by myself under the supervision of Professor Charles B. de Koning and Dr. Kennedy J. Ngwira. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Tianqi Lu

13 May 2019

Abstract

Isochromans are a class of oxygenated heterocycles with a 3,4-dihydro-1*H*-benzo[*c*]pyran scaffold that are found in a variety of natural sources including bacteria, fungal, plants and insects. Isochroman-containing compounds display a wide range of biological activities including anti-bacterial, anti-tumour, anti-oxidation, anti-hypertension and herbicidal activities.

The dissertation involves research evaluating the use of lipase from *Candida rugosa* for the enzymatic kinetic resolution of an advanced synthetic intermediate *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethanol which eventually resulted in the enantiopure synthesis of (*S*)-5,8-dimethoxy-1,3-dimethylisochroman.

The synthesis of (*S*)-5,8-dimethoxy-1,3-dimethylisochroman commenced with the diallylation of 2,5-dihydroxybenzoic acid. The allylated compound allyl 5-(allyloxy)-2-hydroxybenzoate was then subjected to a thermal Claisen-rearrangement to afford allyl 2-allyl-3,6-dihydroxybenzoate which was then protected using dimethylsulphate. The dimethoxy compound allyl 2-allyl-3,6-dimethoxybenzoate was reduced to form alcohol (2-allyl-3,6-dimethoxyphenyl) methanol using lithium aluminium hydride followed by a PCC oxidation yielding aldehyde 2-allyl-3,6-dimethoxybenzaldehyde. The aldehyde was then treated with freshly made methylmagnesium iodide to yield the alcohol 1-(2-allyl-3,6-dimethoxyphenyl)ethanol as the racemate which was then acetylated to form *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate. Using lipase hydrolysis of the acetate racemate, we were able to obtain enantiopure (*S*)-1-(2-allyl-3,6-dimethoxyphenyl)ethanol which was then followed by potassium *tert*-butoxide ring closure to afford (*S*)-5,8-dimethoxy-1,3-dimethylisochroman with an overall yield of 1.31%

starting from 2,5-dihydroxybenzoic acid.

In this dissertation, we also demonstrated the synthesis of hydroquinone methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate using the bio-renewable substrate anacardic acid as starting material. Starting with the selective methylation of anacardic acid possessing a saturated side chain, methyl 2-hydroxy-6-pentadecyl benzoate was synthesized which was subjected to N-bromosuccinimide bromination to afford methyl 3,5-dibromo-2-hydroxy-6-pentadecyl benzoate. A Ceric(IV) ammonium nitrate oxidation followed by sodium dithionite reduction afforded methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate in an overall yield of 31% from anacardic acid.

Using anacardic acid as starting material and potassium *tert*-butoxide as a key step for the ring closure, we were able to synthesize another two isochroman; *rac*-8-methoxy-1,3-dimethylisochroman was synthesized over 9 steps with an overall yield of 13% and *rac*-8-methoxy-1-methyl-3-tridecylisochroman was synthesized over 7 steps with an overall yield of 16%.

Acknowledgement

I am truly indebted to my supervisor and mentor Professor Charles de Koning. This project would not have been possible without his support, guidance and most of all his understanding. You are truly an inspiration to me.

To my co-supervisor Dr. Kennedy J. Ngwira, for all his encouragement and suggestions throughout this project, thank you.

I would like to thank Dr. Hendrik Henning for the help with the NMR spectrometer.

I am very grateful for financial support from the University of the Witwatersrand and the National Research Foundation.

To all my fellow members of the Organic Research Group, thank you for a enjoyable working environment, and for all the useful advice as I found my feet in the lab.

To my family, thank you for all your support and encouragement, throughout everything.

Special thank you to Professor Aubury Parson, without you I wouldn't even be in South Africa. I will always treasure your guidance and friendship.

And finally, to my 'girl' Xinmin, I cannot express in words how grateful I am for having met you and having you in life. You are truly wonderful.

Table of contents

List of abbreviations

N-Bromosuccinimide NBS

t-butyldiphenylsilyl TBDPS

Cerium(IV) ammonium nitrate CAN

Cashew nut shell liquid CNSL

Dichloromethane DCM

N,N-dicyclohexylcarbodiimide DCC

4-dimethylaminopyridine DMAP

N,N-Dimethylformamide DMF

Enantiomeric excess ee

High Performance Liquid Chromatography HPLC

Isopropyl alcohol IPA

Lithium aluminium hydride LAH

Lithium diisopropylamide LDA

Methoxymethyl MOM

Minimal inhibitory concentration MIC

Pyridinium chlorochromate PCC

Tetrahydrofuran THF

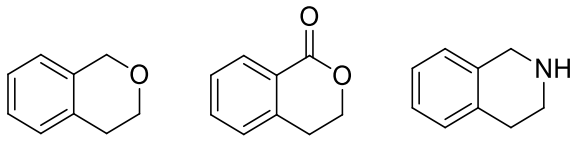
Thin layer Chromatography TLC

Chapter 1: Introduction and Aims

1.1 General introduction

In nature there are numerous oxygenated heterocycles which play an important role as pharmacologically active compounds. One such class of these oxygen-containing heterocycles are the isochromans, a class of compounds also known as 3,4-dihydro-1*H*-benzo[*c*]pyran derivatives. Since the 1970s, a large amount of research has been done on investigating the chemistry and biological activities of these isochroman containing compounds. As a result, isochromans have attracted the attention of scientists from a wide range of science disciplines including natural product chemists as well as, medicinal and synthetic organic chemists.

The isochroman containing compounds were found and isolated from a variety of natural sources which includes bacteria, fungal, plants and insects [1-2]. The basic structures of isochroman is depicted as **1**. However, there are derivatives such as those containing the isochromanone moiety **2** and even the nitrogen containing tetrahydroisoquinoline **3**, which will not be discussed in this dissertation.



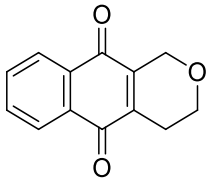
1 **2** **3**

1.2 Bioactivities of natural and synthetic isochromans

Isochroman-containing compounds display a wide range of biological activities which includes anti-bacterial, anti-tumor, anti-oxidation, anti-hypertension and herbicidal activities [3]. Some of these biological activities will be discussed in more detail below.

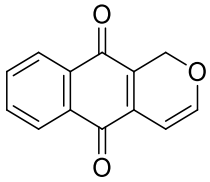
1.2.1 Natural antibiotics-pyranonaphthoquinones

A big class of natural isochroman containing compounds are the pyranonaphthoquinones which have a basic structure of naphtho[2,3-*c*]pyran-5,10-dione ring system as shown in structure **4** [4].



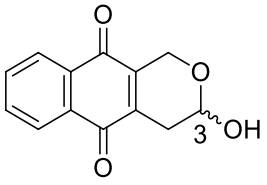
4

The simple pyranonaphthoquinone **5**, also named as pentalongin, has a structure closely related to **4**. It was isolated by Hari and coworkers form root bark of *Pentas longiflora* using hexane as the liquid chromatography solvent [5]. It was found to show significant antifungal and antiparasital activity. The Root bark of *Pentas longiflora* is traditionally used by the traditional medicine healers in Butare and Rwanda to treat the pityriasis versicolor [6].



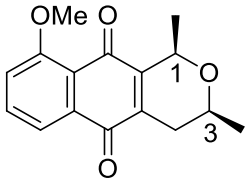
5

Psychorubin **±6** is another simple naturally occurring pyranonaphthoquinone containing the naphthopyrandione ring system possessing a hydroxy substituent at C-3, isolated by Hayashi and coworkers. A simple dehydration of quinone **±6** would result in pentalongin **5**. Psychorubin **±6** was found in *Psychotria rubra*, a traditional Chinese folk medicine. In Hayashi's paper, both pentalongin **5** and psychorubin **±6** were found to show cytotoxicity against KB cell assay with ED_{50} of 0.4 and 3.0 μg/mL, respectively [7].



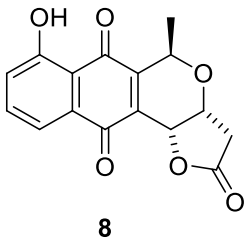
±6

Another natural isochroman containing compound, eleutherin **7**, possesses two methyl substituents at C-1 and C-3, was isolated from the tubers of *Eleutherine bulbosa* by Schmid and coworkers. It exhibits activity against Gram-positive bacteria *Pycococcus aureus* and *Streptococcus haemolyticus A,* and also weak activity against *Bacillus subtilis* with a minimum concentration of 62.5 μg/mL [8-9].



7

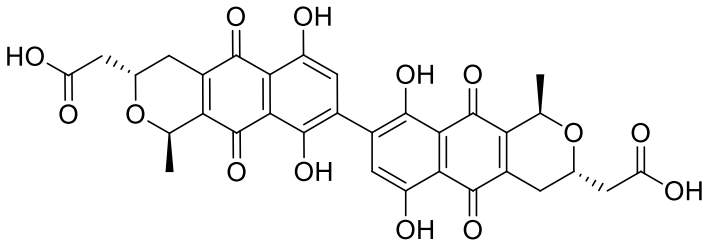
Kalafungin **8**, is another natural antibiotic containing an isochroman core with an additional γ-lactone ring. It was extracted from the fermentation broth of *Streptomyces tanashiensis* strain Kala [10]. In *vitro*, kalafungin inhibited gram-positive microorganisms with a minimal inhibitory concentration range of 1.0 to 16.0 μg/mL. Kalafungin was also found to inhibit a wide spectrum of human pathogenic fungi at a concentration range of 0.5 to 20 μg/mL [11].



8

There are also a number of dimeric pyranonaphthoquinones that show interesting biological activities. One such example is actinorhodin **9**, which is a red pigment that was first isolated by Brockmann and Pini from *Streptomyces*

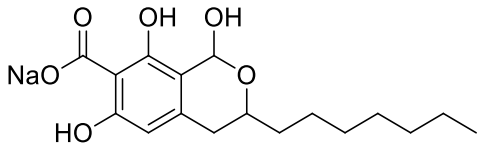
coelicolor A3. This dimeric compound showed activity against Gram-positive bacteria *Staphylococcus aureus* [12-13].



9

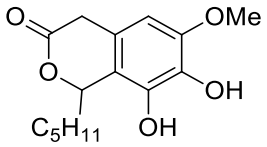
1.2.2 Anti-bacterial activity

The sodium salt of 3-heptyl-1,6,8-trihydroxyisochroman-7-carboxylate **±10**, a hemiacetal containing isochroman was isolated from a fungus *Penicillium* sp. strain which covered the cocoons of a leaf-rolling moth, *Dactylioglypha tonica*. The salt showed significant activity against gram-positive bacteria [14].



±10

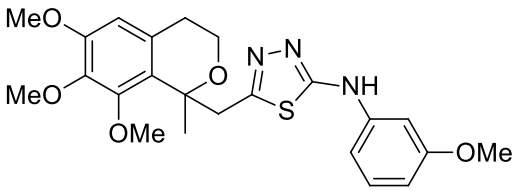
Cytosporone S **±11**, another natural fungus metabolite, was isolated from a culture broth of *Trichoderma* sp. FKI-6626 by Ishii and coworkers. The structure of the lactone-containing cytosporone S **±11** is shown below. The lactone showed broad-spectrum antimicrobial activity against various fungal strains. The most sensitive strain was *Saccharomyces cerevisiae* with an MIC value of 12.5 μg/mL.



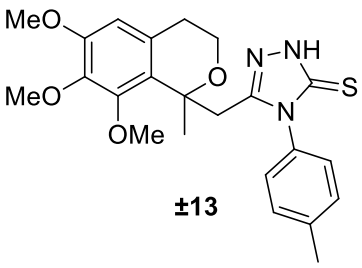
±11

Synthetic isochroman containing compounds also showed remarkable activities against bacteria and fungi. For example, Saeed and Mumtaz

successfully synthesized a range of 1-isochromanyl triazoles and thiadiazole hybrids. Among these synthetic isochroman-triazoles, compound **±12** and **±13**, were the most active against microbial growth of four bacterial strains, namely *Staphylococcus aureus* (ATTC-25923), *Escherichia coli* (ATTC-25922), *Pseudomonas aeruginosa* (ATTC-27853) and *Klebsiella pneumoniae* (recultured) [15].



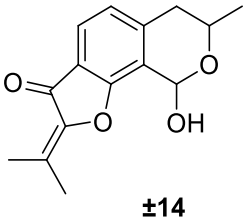
±12



±13

1.2.3 Anti-tumor activity

Another fungal metabolite 9-hydroxy-7-methyl-2-(methylethylidine)-furano[3,2-*H*]isochroman-3-one **±14**, was isolated from *Aspergillus pseudodeflectus*. This hemiacetal containing compound showed cytotoxicity against several human cancer cell lines from the stomach (NUGC-3), cervix (HeLa-S3), and peripheral blood (HL-60). The isochroman **±14** exhibited dose-dependent inhibitory effects where the concentration required for the LD_{50} of the three cancer cell line mentioned above were 49, 47, and 39 μg/L, respectively. [16].

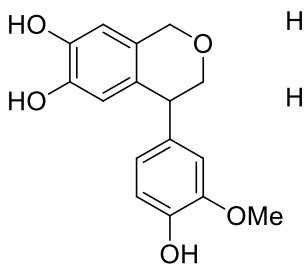


±14

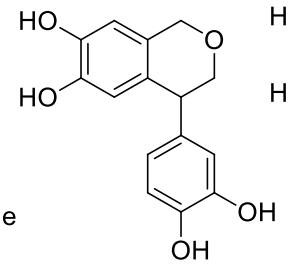
1.2.4 Anti-oxidant activity

Isochroman containing compounds have also been reported to be promising antioxidant agents. Lorenz and coworkers synthesized and tested the

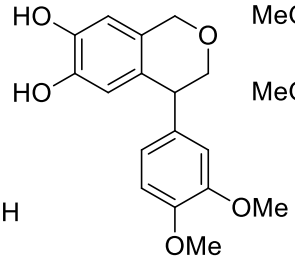
scavenging activities of naturally occurring isochroman **±15** and its derivatives **±16-±18** for the free radical scavenging activity. Isochroman **±16** was the best free radical scavenger, showing that the number of free phenols is probably directly related to the ability to combat oxidative/nitrosative stress caused by reactive oxygen/nitrogen species (ROS/RNS) [17].



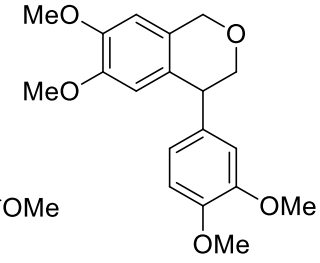
±15



±16

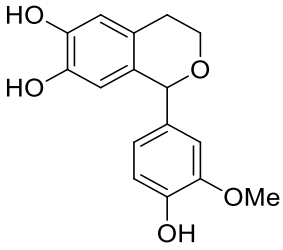


±17

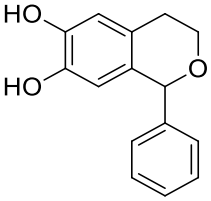


±18

Mediterranean cuisines have long been thought to be beneficial in the prevention of vascular diseases. Togna and coworkers were able to isolate two hydroxy-1-aryl-isochromans **±19** and **±20** from olive oil. These isochromans are effective against reactive free radical oxygen species and can also inhibit platelet aggregation and thromboxane release evoked by agonists that induce reactive oxygen species-mediated platelet activation [18].



±19

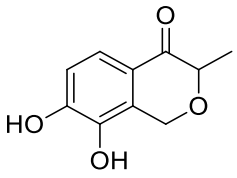


±20

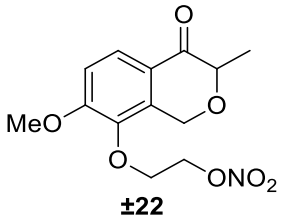
1.2.5 Anti hypertension activity

Another naturally occurring isochroman (±)-7, 8-dihydroxy-3-methyl-isochroman-4-one **±21**, isolated from banana (*Musa sapientum* L.) peels, was found to display antihypertensive activity. Using the natural isochroman **±21** as a lead, Bai and coworkers were able to synthesize

nitric oxide coupled analogs **±22**, which were able to reduce blood pressure by up to 40% in spontaneously hypertensive rats [19].



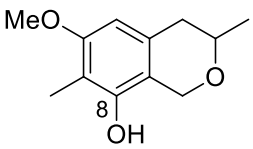
±21



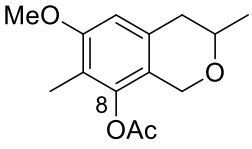
±22

1.2.6 Plant growth regulatory and herbicidal activities

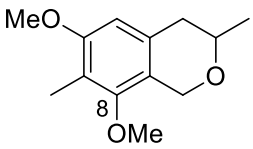
Cutler and coworkers were able to isolate 3,7-dimethyl-8-hydroxy-6-methoxyisochroman **±23**, from two different *Penicillium* species, and were also able to synthesize the C8 acetate derivative **±24** and the C8 methoxy derivative **±25** for biological testing. It was found that both the natural isochroman and the derivatives were able to inhibit etiolated wheat coleoptile growth and thus possess potential for agrochemical use [20].



±23

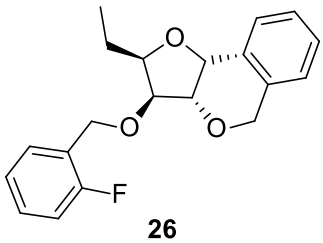


±24



±25

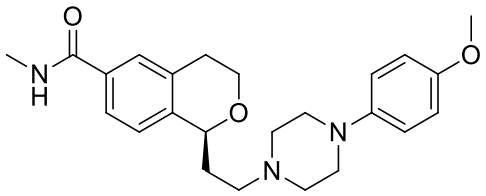
A group of isochromans containing a florinated benzyl ether moiety together with a chiral cyclic system was synthesized and evaluated by Kakimoto and coworkers. The herbicide, tricyclic 3,3a,5,9b-tetrahydro-2*H*-furo[3,2-c][2]benzopyran **26** (TFB) displayed herbicidally activity against annual paddy weeds [21].



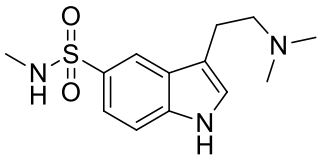
26

1.2.7 CNS receptor binding activities

Migraine headaches are a worldwide chronic disease that affects the lives of millions of people. Ennis and coworkers were able to synthesize (*S*)-1-(2-(4-(4-methoxyphenyl)piperazin-1-yl)ethyl)-*N*-methylisochroman-6-car boxamide **27** as a highly selective, non-indole 5-HT1D agonists which is more potent than sumatriptan **28**, a non-selective serotonergic agent which may cause cardiovascular side effects. The structure of isochroman carboxamide **27** and sumatriptan **28** are shown below [22]:



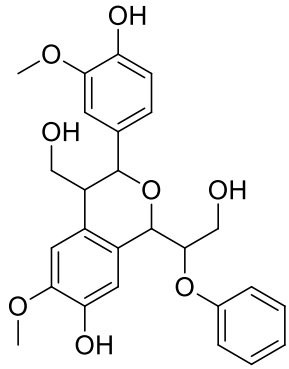
27



28

1.2.8 Biosynthesis-lignin synthesis

From softwood lignins, Ralph and his research team were able to isolate a new complex aryl isochroman **±29** which was proposed to be formed during *β*-1 path way of lignification biosynthesis [23].



±29

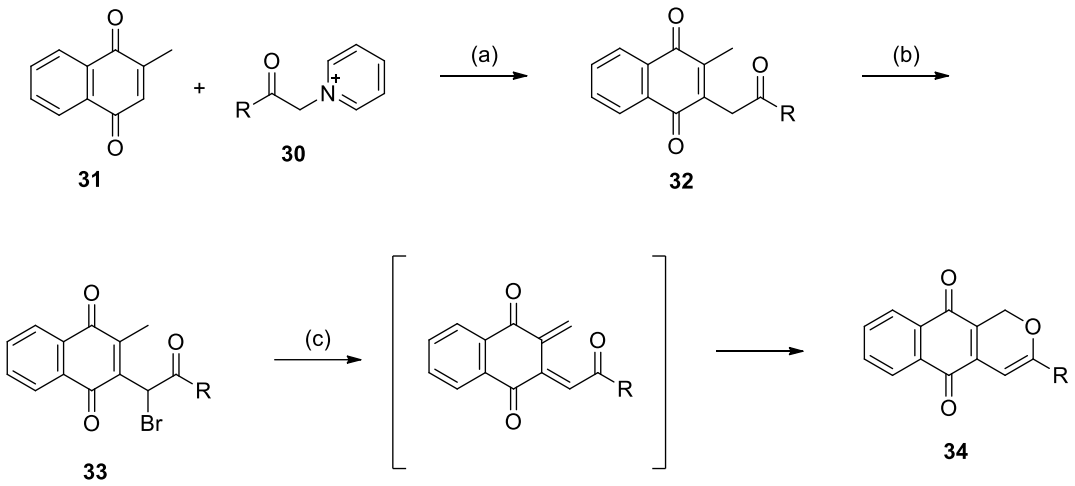
1.3 Synthetic strategies towards isochromans

As a result, in recent years, there has been significant interest in developing synthetic strategies for the assembly of isochroman containing compounds. This is partly due to the diverse biological activities which isochromans exhibit as has briefly been discussed. These compounds have shown good activities as potential antioxidants, antihypertensive agents as well as high affinity agonists for some receptors [24-25].

Despite some similarities in methodologies for the synthesis of isochromans, different substitution patterns, diverse starting materials and issues with the control of stereochemistry lead to a variety of different approaches. For these reasons there are numerous synthetic strategies that have been developed for the assembly of isochromans. Some of these will be discussed below.

1.3.1 Michael addition facilitated ring closure with *N*-ylides mediated acylation

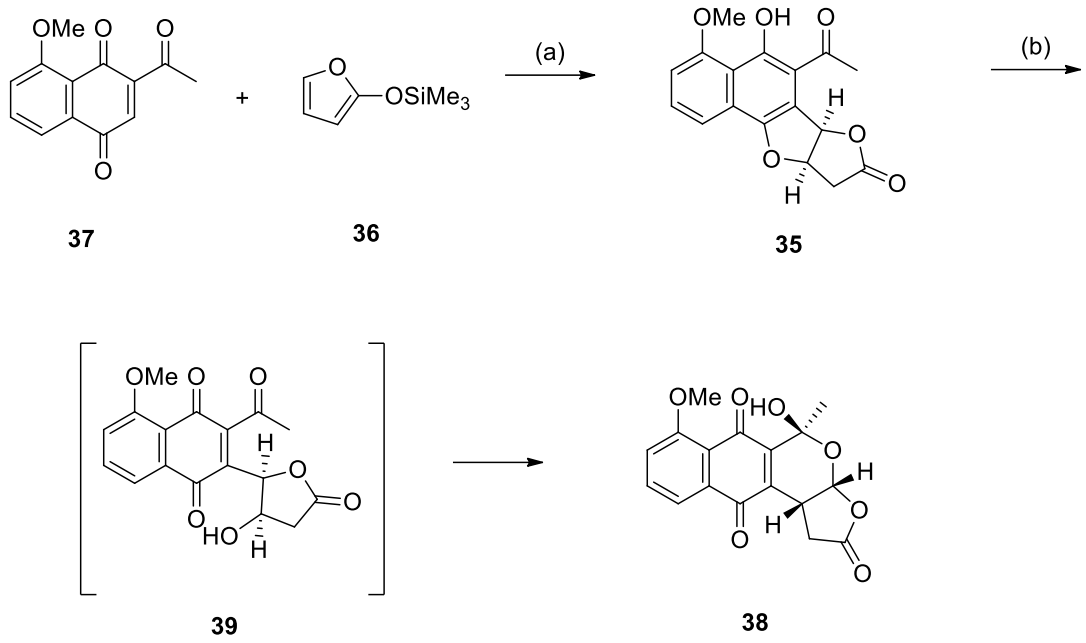
In 1983, Michael and coworkers reported that N-ylides generated carbanions can undergo nucleophilic addition reactions with quinones to form acetonylquinones [26], which was later applied in their synthesis of naphtho[2,3-*c*]pyran-5,10-diones. N-ylides **30** was synthesized by treating chloroketones or bromoketones with pyridine, which was then added to the quinone menaphthone **31** in acetonitrile containing triethylamine to yield the acylated quinones **32**. The cyclisation was achieved by firstly treating the quinone **32** with bromine in DCM in the dark to afford unstable intermediate **33**; and then this bromoketone was dissolved in DCM and treated with triethylamine to furnish naphthopyranquinones **34** (**Scheme 1**) [27].



Scheme 1. Reaction and conditions: (a) Triethylamine, methanol, rt, 1-24 h; (b) bromine, dichloromethane, dark, rt, 40 mins; (c) triethylamine, dichloromethane, dark, rt, 5-40 mins.

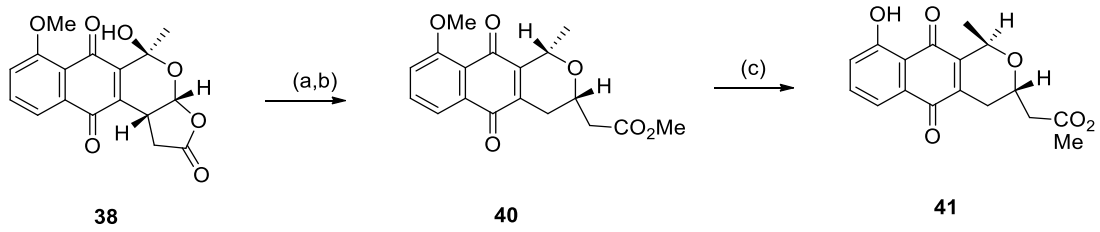
1.3.2 Cerium(IV) Ammonium Nitrate mediated oxidative rearrangements

Brimble and her group have reported the synthesis of kalafungin and related compounds using a cerium(IV) ammonium nitrate (CAN) mediated oxidative rearrangement as the key step to construct the naphtho[2,3-*c*]pyran-5,10-dione ring system. Synthesis of the *cis*-3a,8b-dihydrofuro[3,2-*b*]benzofuran-2(3*H*)-one **35** was achieved *via* the addition of 2-trimethylsilyloxyfuran **36** to 1,4-benzoquinones **37** containing an electron-withdrawing substituent at C-2. The dihydrofuran **35** was then transformed into the furo[3,2-*b*]benzopyran ring system **38** using CAN (**Scheme 2**). Brimble proposed that upon treated with CAN in aqueous acetonitrile, the cyclic ether **35** might undergo an analogous oxidative dealkylation reaction to give the β -hydroxylactone **39**. Subsequent nucleophilic attack of the hydroxy group on the methyl ketone would then afford the naphthopyranquinone **38** [28].



Scheme 2. Reagents and conditions: (a) MeCN, rt; then MeOH, 81%; (b) CAN, MeCN, H_2O, rt, 76%.

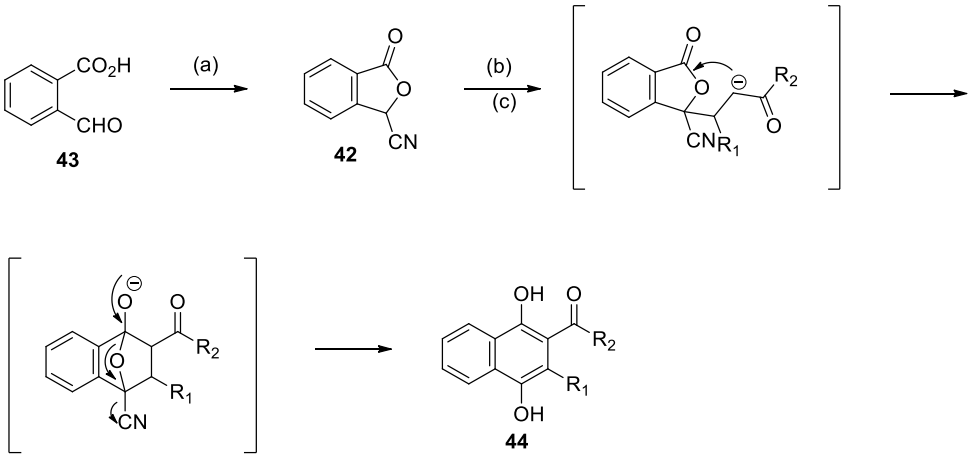
Later on, Brimble and Lynds reported the synthesis of Nanaomycin A as the hydrogenation of hemiacetal **38** followed by treatment with diazomethane leading to the reduction of the alcohol as well as the hydrogenolysis of the lactone to afford carboxylic ester **40**. These after the deprotection of the methyl ether also resulted in the *cis-trans* epimerization at C-1 to form Nanaomycin A **41** (**Scheme 3**) [29].



Scheme 3. Reagents and conditions: (a) H_2, Pd/C, EtOAc, (b) CH_2N_2, EtO_2, 55%; (c) BBr_3, DCM, -78 to 0 °C, 89%.

1.3.3 Phthalide annulation followed by one-pot cyclization

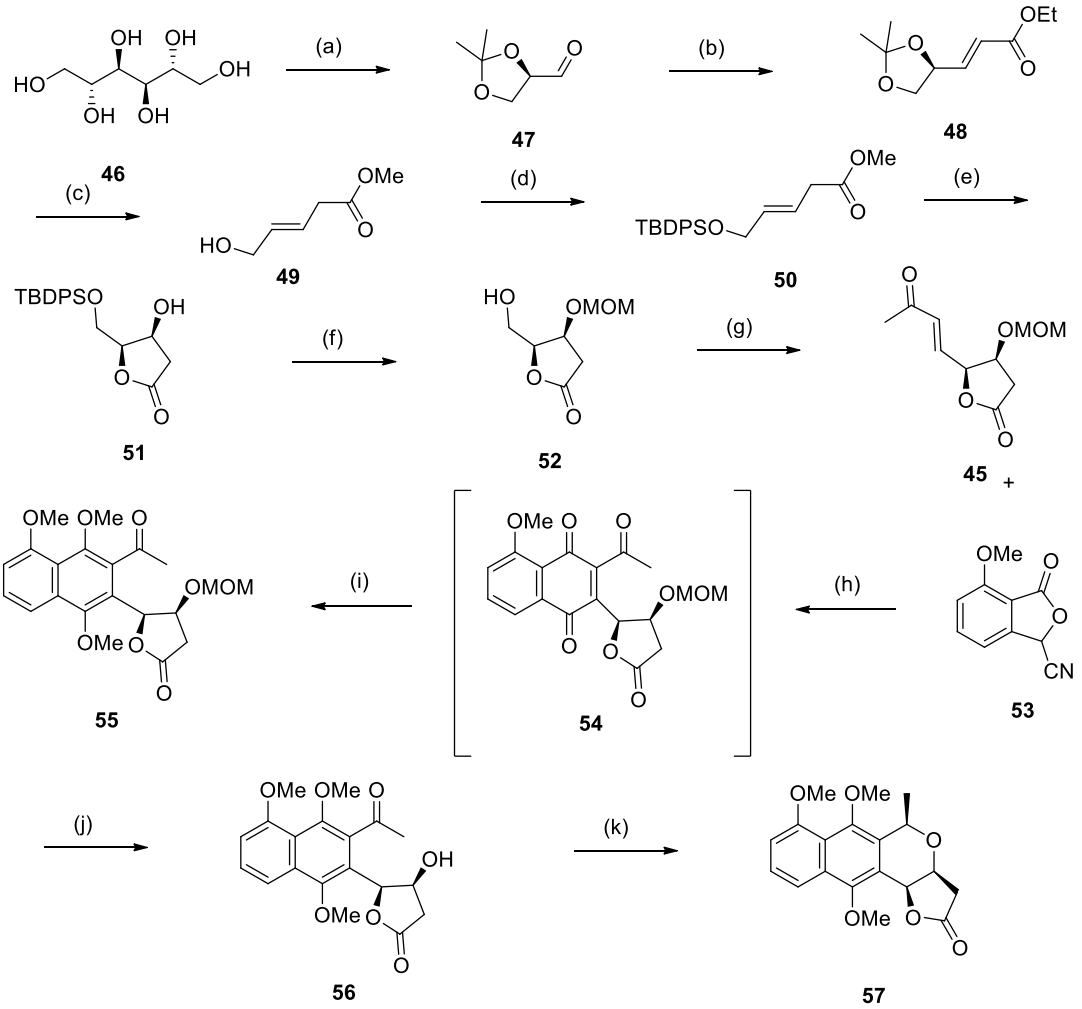
More than 30 years ago, Kraus and Sugimoto reported a general method for the synthesis of hydroquinones under mild reaction conditions. The phthalide **42** was easily prepared from 2-formylbenzoic acid **43** and HCN under acidic conditions. Phthalide **42** was then dissolved in THF and treated with lithiumdiisopropylamide in THF/HMPA; the reaction mixture was then treated with the Michael acceptor to afford hydroquinone **44** (**Scheme 4**) [30-31].



Scheme 4. Reagents and conditions: (a) KCN, aq. HCl, water, reflux, 40 min; (b) Lithiumdiisopropylamide, THF/HMPA, -78 to 0°C, 11 mins; (c) $R_1CHCHCOR_2$, *t*-BuOK, DMF, rt, 90 min.

Adopting this method, Najmah and coworkers were able to synthesize nanaomycin D using the chiral enone-lactone **45**. The chirality in the enone-lactone **45** was accomplished using a Sharpless asymmetric dihydroxylation. Starting with the commercially available D-mannitol **46**, acetonide protection followed by periodate cleavage gave aldehyde **47**; which was coupled with triethyl phosphonoacetate to afford E-alkene **48**. The β, γ-unsaturated ester **49** was then afforded by the reductive cleavage of alkene **48**. The primary alcohol of ester **49** was protected as a *t*-butyldiphenylsilyl (TBDPS) ether **50**, followed by the Sharpless asymmetric dihydroxylation and lactonization to yield hydroxy lactone **51**. The lactone was then protected as methoxymethyl ether and the TBDPS ether was deprotected to give alcohol **52**.

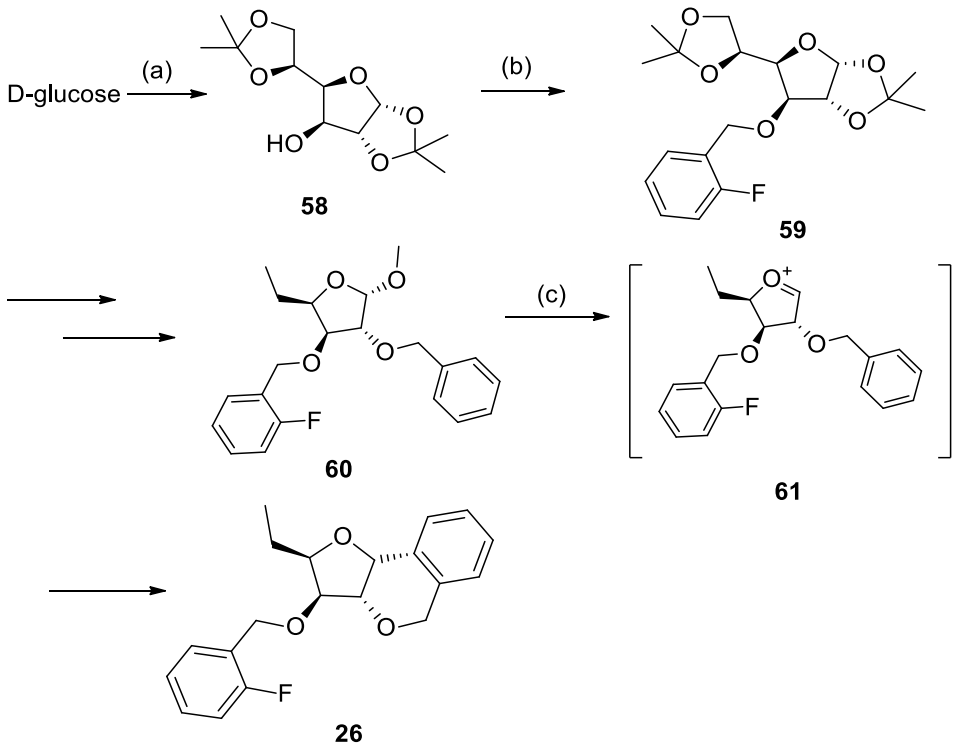
Oxidation of the alcohol **52** followed by olefination furnished the desired chiral enone-lactone **45**. The annulation proceeded by treating the cyanophthalide **53** with the chiral enone-lactone **45** using lithium diisopropylamide (LDA) as the base to afford the annulation product **54**; which was methylated immediately to afford trimethoxynaphthalene intermediate **55**. The methoxymethyl (MOM) group in intermediate **54** was first removed to afford hydroxyketone **56**; which was then cyclized giving pyranonaphthalenelactone **57** (**Scheme 5**) [32].



Scheme 5. Reagents and conditions: (a) 1) $ZnCl_2$, $(CH_3)_2CO$, 24 h then K_2CO_3 (71%); 2) $NaHCO_3$, $NaIO_4$, DCM (45%); (b) $EtO_2CCH_2PO(OEt_2)$, K_2CO_3, 89%; (c) Mg/MeOH, 80%; (d) TBDPSCl, Et_3N, DMAP, DCM, 99%; (e) $(DHQ)_2PHAL$, K_2CO_3, $K_3Fe(CN)_6$, OsO_4, $MeSO_4NH_2$, H_2O/*t*-BuOH, 0°C, 76%, 79%ee; (f) 1) $CH_2(OMe)_2$, P_2O_5, $CHCl_3$, 0°C to rt, 80%; 2) TBAF, AcOH, THF, 92%; (g) 1) DMP, $NaHCO_3$, DCM; 2) $MeCOCH_2PO(OMe)_2$, NaH, 0°C to rt, THF, 43%; (h) LDA, THF, -78% to rt, 30 min; (i) $Na_2S_2O_4$, Cs_2CO_3, MeI, rt, 15 min, 35%; (j) HCl(aq), THF, 40%, 2.5h, 55%; (k) BF_3OEt_2, Et_3SiH, DCM, -78% to rt, 5h, 26%.

1.3.4 Friedel–Crafts type intramolecular cyclization

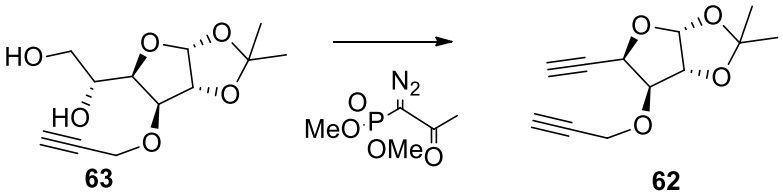
Kakimoto and coworkers were able to synthesize and evaluate a group of isochromans containing a benzyl ether moiety attached to a chiral cyclic system. Among which, compound **26** showed promising activities against *E crus-galli* with a MIC values of 0.2 mg/L. Starting from *D*-glucose, the hydroxy groups of the C-1, C-2, C-5 and C-6 positions were protected by the isopropylidene group to yield diacetonide **58**. The diacetonide was then treated with 2-fluorobenzylchloride to give the benzyl ether **59**, this was followed by a sequenced selective deprotection and benzylation to afford the dibenzyl ether compound **60** which was ready for the ring closure. By treating the dibenzyl ether with an acid catalyst, an oxonium cation intermediate **61** was formed, which was then cyclized by a Friedel-Crafts type intramolecular cyclization to afford only *cis*-fused 3,3a,5,9b-tetrahydro-2*H*-furo[3,2-c][2]benzopyran **21** [21] as shown in **Scheme 6**:



Scheme 6. Reagents and conditions: (a) cat. H_2SO_4/acetone, reflux, 10 h, 60%; (b) NaH, cat TBAI, 2-fluorobenzyl chloride/THF, reflux, 4h, 90%; (c) $BF_3.OEt_2/CH_2Cl_2$, 0°C to room temp, 2 h, 82%.

1.3.5 Carbohydrate templated [2+2+2]-cyclotrimerization

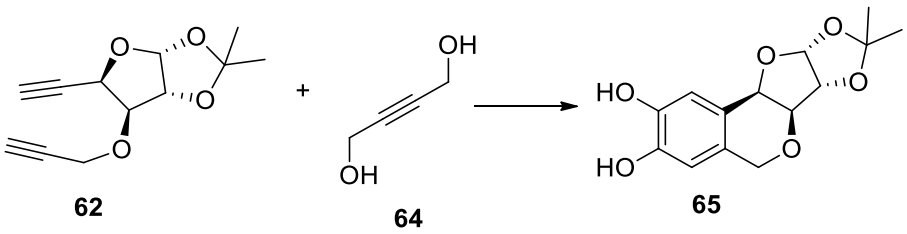
Developed by Suryawanshi and coworkers, this method also involved the use of carbohydrates in the synthesis to accomplish the assembly of enantiopure tricyclic containing compounds. Prepared carbohydrates diynes were treated with substituted alkynes under ruthenium catalysis to afford enantiopure isochromans. The preparation of carbohydrates-containing diyne **62** is shown in **Scheme 7**. The diyne was prepared from the diol **63** in 78% overall yield through sodium metaperiodate mediated cleavage and subsequent Ohirae-Bestmann alkynylation of the intermediate aldehyde.



63 **62**

Scheme 7. Reagents and conditions: a) $NaIO_4$, $MeOH/H_2O$; b) K_2CO_3, MeOH, rt, 6 h

Once the diyne **62** was synthesized, it was heated with but-2-yne-1,4-diol **64** and Wilkinson's catalyst ($[RhCl(PPH_3)_3]$) in toluene/ethanol at 80°C to afford isochroman **65** [33] as depicted in **Scheme 8**:

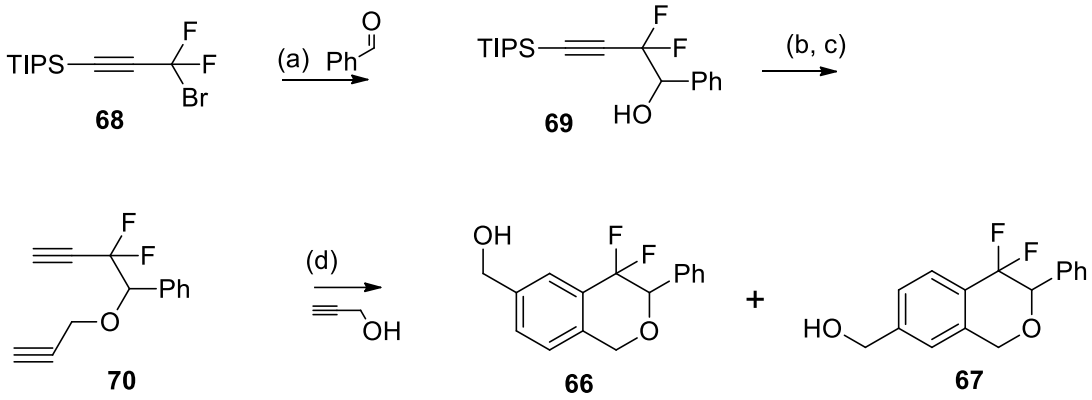


62 **64** **65**

Scheme 8. Reagents and conditions: $RhCl(PPh_3)_3$ (5 mol%), toluene/ethanol (4:1), 80°C, 8 h

Similar to the Suryawanshi approach, Arimitsu and research team were able to synthesize 4,4-difluoroisochromans **66** and **67** starting by a Barbier-type reaction of difluoropropargyl bromide **68** with benzaldehyde in aqueous media catalyzed by indium and lanthanide triflate to afford **69**. The synthesized alcohol **69** was then treated with propargyl bromide followed by desilylation to yield diyne **70**. The synthesis of racemic isohromans **66** and **67** was then

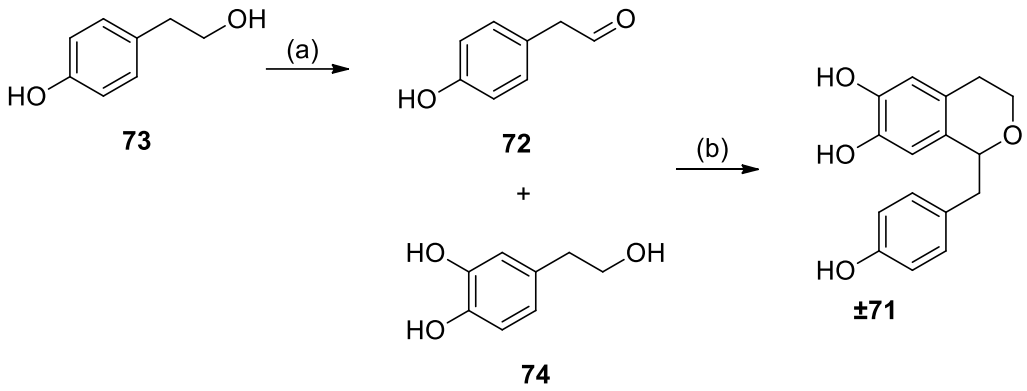
achieved by a $RhCl(PPh_3)_3$ catalyzed cyclization of diyne **70** and prop-2-yn-1-ol [34] (**Scheme 9**).



Scheme 9. Reagents and conditions: (a) In(1 eq.), $Eu(OTf)_3$ (5 mol%), H_2O/THF, 40°C, 20 h; (b) Propargyl bromide, NaH, THF, rt, 24 h; (c) TABF, AcOH, THF, rt, 5 h; (d) $RhCl(PPH_3)_3$ (5 mol%), toluene, reflux, 4 h.

1.3.6 Oxa-Pictet-Spengler condensation

Using a modified oxa-Pictet-Spengler reaction, Guiso and coworkers were able to synthesize the 6-hydroxy-isochromans **±71**. With the benefits of the one-step synthesis, no protecting groups were needed and this resulted in the product being formed in high yield. Aldehyde **72** was synthesized prior to the oxa-Pictet-Spengler reaction by treating 4-(2-hydroxyethyl)phenol **73** with PDC. Phenolic diol **74** and aldehyde **72** was dissolved in methyl alcohol using *p*-toluenesulfonic acid as a catalyst. This resulted in the isochroman **±71** being synthesized in high yield [35]. The reaction scheme is shown in **Scheme 10**.

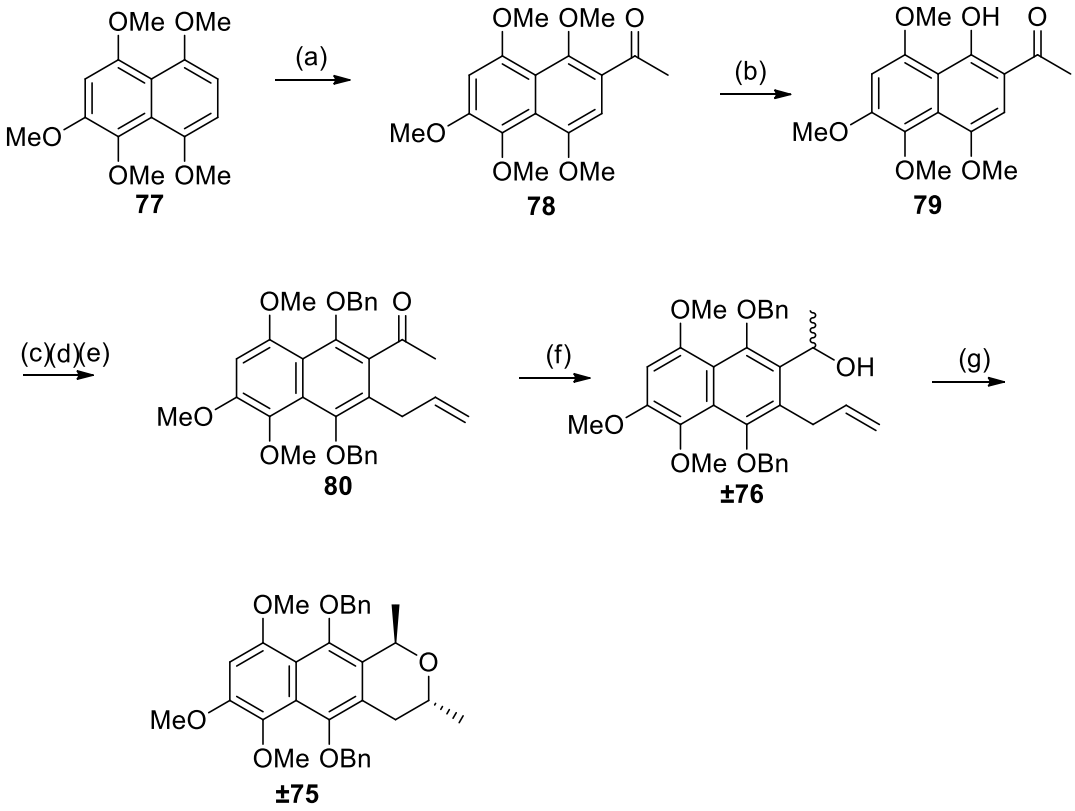


Scheme 10: Reagents and conditions: (a) PDC, CH_2Cl_2/EtOAc (2:1), 5h; (b) cat. p-toluenesulfonic acid, 4°C, 24 h.

1.3.7 Potassium *tert*-butoxide mediated ring closure as a key step in the synthesis of isochromans

de Koning and coworkers reported the synthesis of isomeric dimethylnaphthopyran **±75** mediated by potassium *tert*-butoxide as a key step to obtain exclusively the *trans* geometry of the desired naphthopyran **±75**.

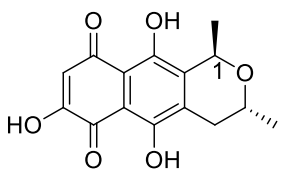
To assemble the precursor **±76**, this naphthalene was produced in a few steps using 2,3-dibromo-1,4,5-trimethoxybenzene as starting material. Regioselective acetylation of pentamethoxynaphthalene **77** gave solely 6-acetyl ketone **78**, which was treated with limited quantities of borontrichloride to afford *ortho*-acetylnaphthol **79**. The naphthol was oxidized with ceric ammonium nitrate to yield a quinone, which was reduced and then subjected to an *O*-allylation followed by a Claisen rearrangement and an *O*-benzylation to afford dibenzyl ether **80**. The ketone of the naphthalene dibenzyl ether **80** was reduced with lithium aluminium hydride to yield the alcohol **±76**, which was then treated with potassium *tert*-butoxide as the key step to give the isomeric *trans* 1,3-dimethylnaphthopyran **±75** [36-37], as shown in **Scheme11**.



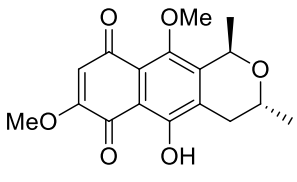
Scheme 11: Reagents and conditions: (a) Acetic acid, TFAA, DCM, rt, 21 h, 83%; (b) BCl_3, DCM, -78°C to rt, 30 min, 88%; (c) CAN, $MeCN/H_2O$, 15 min; (d)BCl_3, allyltrimethylstannane, DCM, -78°C, 1 h; (e) K_2CO_3, benzyl chloride, acetone, reflux, 12 h, 51%; (f) LAH, diethyl ether,rt, 5 min, 99%; (g) Potassium tert-butoxide, DMF, 70°C, 2 h, 92%.

1.4 Background, and aim of this MSc project

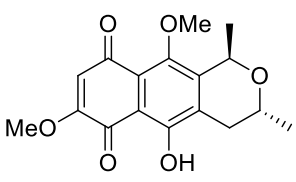
In our research laboratories the developed strategies for the synthesis of the isochroman core were first published in 1991 when my supervisor completed his PhD in the synthesis of ventiloquinones. The potassium *tert*-butoxide mediated pyran ring closure to synthesize racemic mixtures of a number of ventiloquinones: namely ventiloquinone G **±81**, ventiloquinone J **±82**, and a new ventiloquinone **±83** [36] was utilized as a key reaction.



±81



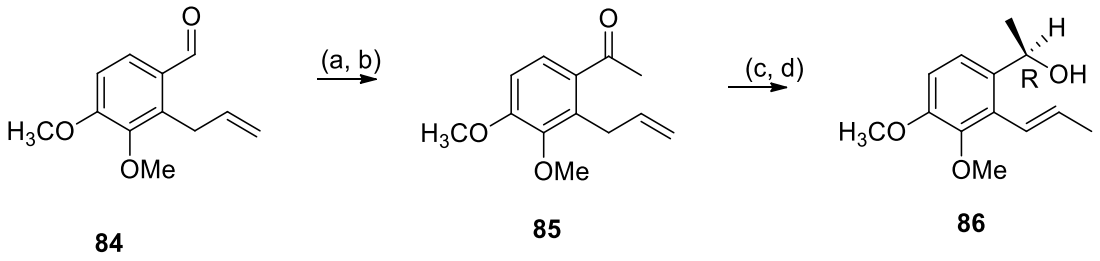
±82



±83

However, an enantioselective synthesis of the ventiloquinones was not possible until 2002, when Green, de Koning and coworkers reported on the introduction of the benzlic secondary alcohol using the Corey–Bakshi–Shibata (CBS) reduction during the synthesis of chiral isochroman-quinones. However, the enantiomeric excess for this reaction was mediocre.

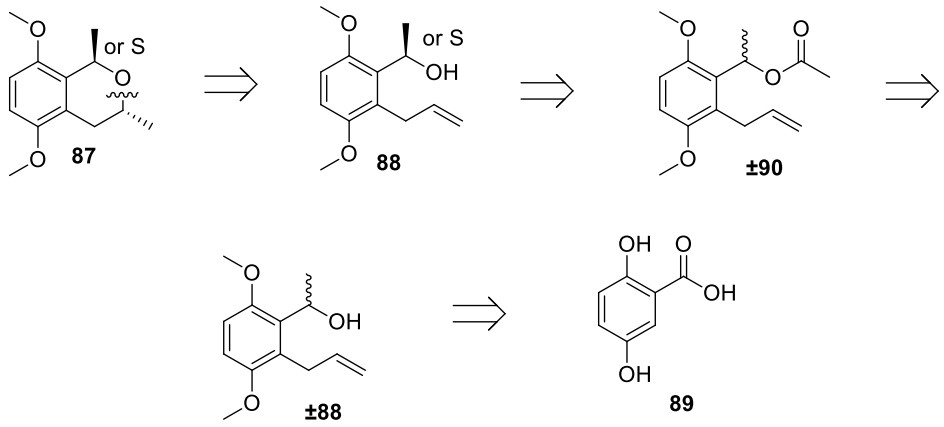
The aromatic precursor **84** was easily synthesized from isovanillin. The aldehyde **84** was then treated with methylmagnesium iodide followed by oxidation with manganese (IV) oxide to yield ketone **85**. The enantioselective ketone reduction was achieved by firstly conjugation of the olefinic bond of the allyl substituent using $PdCl_2(MeCN)_2$ to give the styrene, followed by addition of the borane dimethyl–sulfide complex and the Corey–Bakshi–Shibata catalyst to afford the enantiopure alcohol **86** [38] (**Scheme 12**).



84 **85** **86**

Scheme 12. Reagents and conditions: (a) CH_3MgI, Et_2O, 25°C, 40 min, 90%; (b) MnO_2, benzene, reflux, 45 min, 57%; (c) $PdCl_2(MeCN)_2$, CH_2Cl_2, 35°C, 72 h, 89%; (v) $BH_3{\cdot}Me_2S$, THF, CBS catalyst, 25°C, 30 min, 61%, ee 75%.

However, as the enantiomeric excess was moderate, other methods had to be developed to accomplish this transformation. Thus, the aim of this project, was to develop a method for the synthesis of enantio-pure 5,8-dimethoxy-1,3-dimethylisochroman **87** (R/S). Our retrosynthesis, as shown in **Scheme 13**, a key step would be to obtain the single enantiomer **88** (R/S) from *rac*-1-(2-allyly-3,6-dimethoxyphenyl) ethanol **±88**. We believed that using enzymatic kinetic resolution would provide a solution to this problem.



Scheme 13. Retrosynthesis of enantiopure 5,8-dimethoxy-1,3-dimethylisochroman **87**

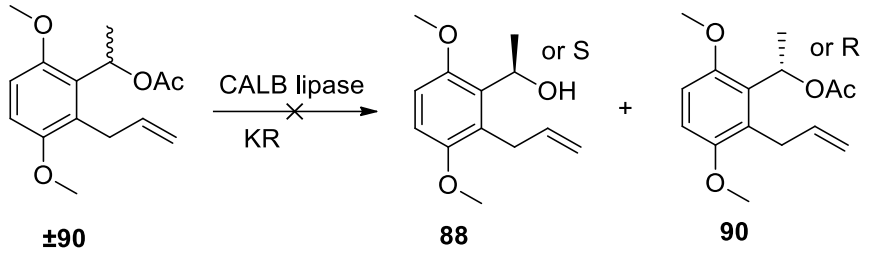
In essence, the synthesis would start with 2,5-dihydroxybenzoic acid **89**, and the racemic alcohol **±88** could be synthesized in a few steps. In order to perform the enzymatic kinetic resolution, the secondary alcohol **±88** had be acetylated to give ester **±90**. Using enzymatic kinetic resolution on the substrate **±90**, the synthesis of enantiopure alcohol **88** (R/S) could hopefully be achieved. The product **88** (R/S) would be ready for the pyran ring closure using potassium *tert*-butoxide methodology to form the desired *trans*-1,3-dimethyl isochroman **87**.

1.5 Enantiomeric discrimination

There are different approaches to enantioselectively synthesizing a stereogenic center. In this project, we envisaged using enzymatic routes for the preparation of the enantiopure alcohol **88**. During the previous synthesis of isochroman, the racemate of alcohol **±88** was obtained without separation which led to the formation of diastereomers in the synthetic route. We were therefore interested in producing the enantiopure alcohol **88** which would then potentially allow for the enantioselective synthesis of the desired isochroman **87**.

1.5.1 Background

In recent years, our lab has investigated the use of enzymes to kinetically resolve racemic mixtures of alcohols. Dr. Myron Johnson, one of our former PhD students, tried to apply enzymatic kinetic resolution as the key step to synthesize enantiopure kalafungin. In his attempt, CALB lipase was used to enantioselectively hydrolyze the racemic mixture of acetate **±90** prepared from alcohol racemate **±88**. In summary, CALB lipase was not able to perform the kinetic resolution satisfactorily even after a few days of vigorous stirring [39] (**Scheme 14**).



Scheme 14. Enzymatic kinetic resolution using lipase CALB

1.5.2 Introduction to biocatalysis

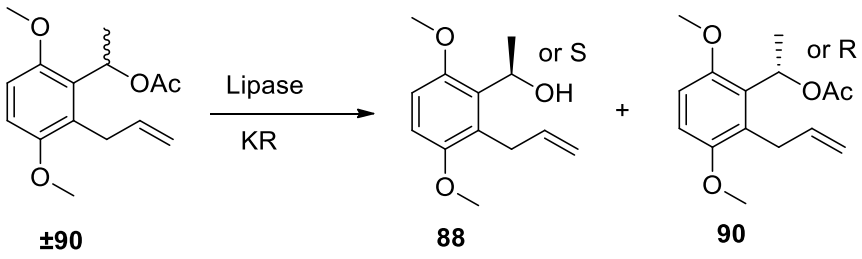
In recent years, aspects of green chemistry have become more and more important in organic synthesis. Application of biocatalysts is one of the most important ways of reducing hazardous wastes and achieving better atom economy [40]. The use of enzymes has become a valuable tool to organic chemists, especially in the synthesis of compounds containing stereogenic centers. This is because enzymes are remarkable catalysts that can display high chemo-, regio- and enantio-selectivity, but are also not capable of accepting a wide range of substrates.

Enzymes are macro-sized molecules with unique three-dimensional structure arising from the folding of a linear polypeptide chain. This polypeptide chain in

turn consists of a linear sequence of α–amino acids joined by amide bonds. Each enzyme processes unique catalytic activity on substrates sharing structural similarities because of the presence of an active site. Due to the chiral nature of the active site, it is often able to naturally bind one enantiomer of the substrate over the other. This high substrate selectivity is one of the reasons why enzymes allow for enantioselective transformation [41]. Our aim was to employ a lipase enzyme to kinetically resolve a racemic mixture of alcohols in order to isolate the individual optically active enantiomers.

1.5.3 Enzymatic kinetic resolution

In nature, two enantiomers can exhibit totally different bioactivities. In this project, we will use the selectivity of lipases to hydrolyze one enantiomer of the racemate of 1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90** to afford enantiopure methanol **88** (R/S) leaving the other enantiopure acetate **90** unreacted (**Scheme 15**). HPLC equipped with a chiral column will be used to monitor the progress of the reaction as one of acetate peak of **±90** will disappear and one peak for alcohol **88** will appear. However, a significant drawback of kinetic resolution is that it will only provide a maximum of 50% yield.



Scheme 15. Demonstration of enzymatic kinetic resolution

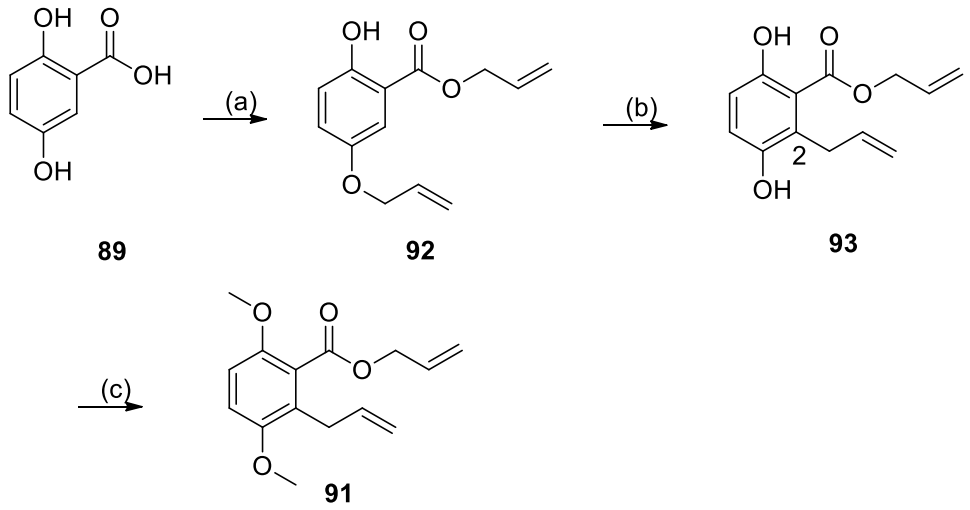
During my honors project [42], some preliminary research had been conducted and over 100 commercial lipases were screened for the transformation shown

in **Scheme 15**; only one lipase from *Candida Rogusa* was found to exhibit selectivity for our substrate 1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90**. The details of which will not be discussed in this MSc dissertation. This provided the hope that by building on these results the synthesis of enantiopure isochroman skeleton **87** could be accomplished.

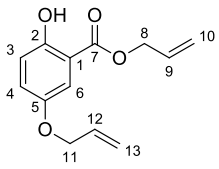
Chapter 2: Results and Discussion

2.1 Synthesis of allyl 2-allyl-3,6-dimethoxybenzoate 91

We believed that the synthesis of of the desired racemic alcohol **±88** could be achieved via the ester allyl 2-allyl-3,6-dimethoxybenzoate **91**. Hence as shown in **Scheme 16**, allyl 5-(allyloxyl)-2-hydroxybenzoate **92** was prepared using commercially available 2,5-dihydroxybenzoic acid **89** as starting material; which was then subjected to Claisen rearrangement conditions by heating neat at 170°C to yield **93** with the allyl group at C-2 position *ortho* to the ester group. The synthesis of allyl 2-allyl-3,6-dimethoxybenzoate **91** was achieved by exposure of **93** to dimethyl sulfate and potassium carbonate.

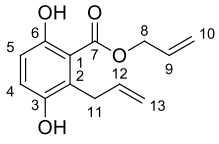


Scheme 16. Reagents and conditions: a) K_2CO_3, allyl bromide, acetone, reflux, 18 h, 74%; b) 170°C, 18 h, 90%; c) K_2CO_3, dimethyl sulfate, acetone, reflux, 24 h, 62%.



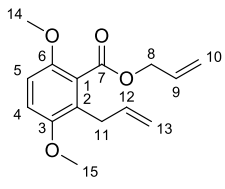
92

In detail, 2,5-dihydroxybenzoic acid **89** was initially treated with potassium carbonate and allyl bromide in acetone and heated under refluxed overnight. The resulting product after purification was isolated as white needle like crystals with a melting point of 46-48°C, close to the reported value of 43.5–44.5°C by Harwood [43]. The formation of compound **92** was confirmed by NMR spectroscopy. Compared to the starting material 2,5-dihydroxybenzoic acid **89**, we could easily see that between δ 6.14 and 5.25 ppm there were 6 newly formed alkene hydrogen signals corresponding to H_9, H_{10}, $H_{10'}$, H_{12} and H_{13}, $H_{13'}$. Signals at δ 4.84 and δ 4.49 ppm in the ^{1}H NMR spectrum corresponded to the two CH_2-H_8 and H_{11} signals. In addition to this, alkene carbons in the ^{13}C NMR spectrum at δ 133.55, 131.92, 118.11, 113.87 ppm proved that two allyl groups were indeed present. Hence compound **92** had been synthesized in a reasonable yield of 74%.



93

Having the diallylated product **92** in hand, the next step in the synthesis involved the thermally mediated Claisen rearrangement to furnish 2-allyl-3,6-dihydroxybenzoate **93** in an excellent yield of 90%. This was achieved by heating the di-allylated product **92** at 170 °C for 18 hours under a nitrogen atmosphere. The resulting product was a viscous orange oil. ^{1}H NMR, ^{13}C NMR and IR spectroscopy were used to confirm the structure of the resulting product. A strong broad signal appeared at 3414 cm^{-1} in the IR spectrum which was attributed to the new alcohol functional group. In the ^{1}H NMR, a new peak at δ 5.28 ppm also indicated the presence of a new OH signal. In addition, only two aromatic hydrogen signals that were *otho* coupled to each other were observed in the ^{1}H NMR spectrum. The NMR spectra obtained for compound **93** compared well with the one reported in literature [44].



91

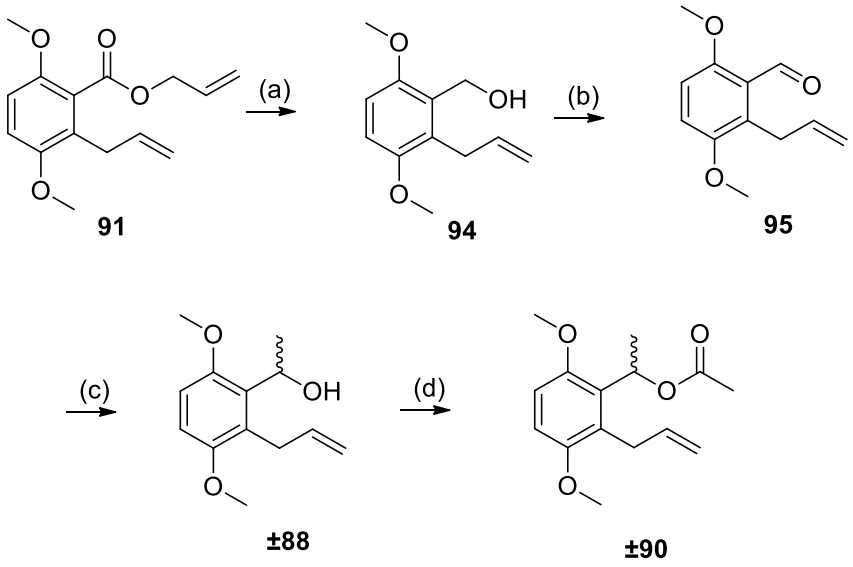
After the Claisen rearrangement, the next synthetic step was

to protect the hydroxyquinone as a methyl ether. To achieve this, the *o*-allyl 2-allyl-3,6-dihydroxybenzoate **93** was treated with potassium carbonate and dimethyl sulfate in dry acetone, resulting in a clear orange oil to afford the product **91** in a yield of 62%. Confirmation of the successful synthesis of **91** was again obtained from both the ^{1}H, ^{13}C NMR and IR spectroscopy. The IR spectrum clearly showed the absence of the alcohol O-H vibrations at 3414 cm^{-1} and only the C-H vibrations at 2940 cm^{-1} were observed, suggesting that the hydroquinone in compound **93** were replaced by two methoxy groups. The absence of the hydroquinone signals at δ 10.42 and 5.28 ppm observed in 2-allyl-3,6-dihydroxybenzoate **93**, together with the appearance of two singlet signals at δ 3.77 and 3.78 ppm was consistent with appearance of methoxy group protons, which proved we had synthesized of compound **91**. In addition to this information, two new carbon signals at δ 56.63 and 56.62 ppm corresponding to the formation of two methoxy groups were found in the ^{13}C NMR spectrum. The NMR spectra analyzed for compound **91** compared well with the literature data [44]

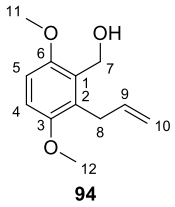
2.2 Synthesis of *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate ±90

With the allyl group in place, the next stage of the synthesis was to modify the ester group to allow the enantioselective hydrolysis with the lipase from *Candida Rugosa*. The dimethoxy-containing aromatic compound **91** was reduced to form alcohol **94** using lithium aluminium hydride (LAH). This was followed by a pyridinium chlorochromate (PCC) oxidation yielding aldehyde **95**. The aldehyde **95** was then treated with freshly made methylmagnesium iodide to yield alcohol as the racemate **±88**. *Rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90** was then synthesized by acetylation of **±88** using acetic anhydride

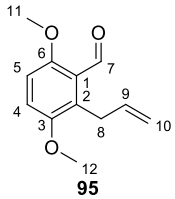
to prapare for the enzymatic kinetic resolution step (**Scheme 17**).



Scheme 17. Reagents and conditions: a) LAH, THF, 0°C to rt, 18 h, 95%; b) PCC, silica, DCM, rt, 18 h, 30%; c) Mg, MeI, diethyl ether/THF, rt, 18 h, 56%; d) DMAP, acetic anhydride, triethylamine, THF, rt, 18 h, 45%.

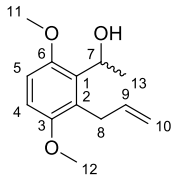

94

In detail, once the dimethoxy ester containing compound **91** was synthesized, it was treated with lithium aluminium hydride in THF in an ice bath. The resulting reaction mixture warmed up to room temperature and stirred overnight. The resulting product **94** was obtained as a light yellow oil. The IR spectrum clearly showed that there was a new broad peak at 3409 cm^{-1}, indicative of an alcohol group. Signals of H_8 at δ 4.81 ppm and alkene hydrogens H_9, H_{10} at δ 5.46–5.32 and 6.08 – 5.81 ppm observed in compound **91** were now absent indicating that ester group was reduced to an alcohol. The newly formed singlet signal at δ 2.57 ppm corresponded to the new alcohol hydrogen. In addition to those, three missing carbons signals at δ 132.31 (C_9), 112.70 (C_{10}) and 66.17 (C_8) ppm found in starting material also confirmed that the reduction of ester had taken place. The NMR spectra analysed for compound **94** compared well with the literature data [44].


95

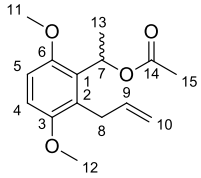
The next synthetic step involved treatment of

(2-allyl-3,6-dimethoxyphenyl)methanol **94** with pyridinium chlorochromate (PCC) and silica in dry DCM. The resulting product **95** was a clear yellow oil obtained in a yield of 70%. The formation of the compound was confirmed spectroscopically by examining the ^{1}H and ^{13}C NMR spectra and IR spectrum. The IR spectrum clearly showed that the O-H peak at 3409 cm^{-1} was no longer present, instead, a carbonyl (C=O) stretching signal appeared at 1684 cm^{-1}. A singlet signal appeared at δ 10.55 ppm in the ^{1}H NMR spectrum which corresponded to the formation of an aldehyde. The ^{13}C NMR spectrum also confirmed this assignment due to the presence of a peak at δ 192.91 ppm. In addition, the signal for an alcohol proton observed on the ^{1}H NMR spectrum of the starting material at δ 2.57 ppm was absent. The NMR spectroscopic results unambiguously confirmed that the compound was 2-allyl-3,6-dimethoxybenzaldehyde **95** [45].



±88

The benzylic alcohol **±88** was prepared as a racemate by treating 2-allyl-3,6- dimethoxybenzaldehyde **95** in dry THF with the Grignard reagent made from magnesium metal and methyl iodide in dry diethyl ether. The resulting product **±88** was obtained as a clear oil in a yield of 56%. ^{1}H NMR, ^{13}C NMR and IR spectroscopy were used to characterize the resulting product. The IR spectrum clearly showed the presence of a new hydroxyl stretching at 3546 cm^{-1}, as well as the disappearance of the carbonyl group (C=O) peak at 1684 cm^{-1} in the starting material **95**. In agreement with IR spectroscopy, two doublet signals at δ 4.04 ppm and δ 1.53 ppm in the ^{1}H NMR spectrum suggested that there was an alcohol hydrogen and an additional methyl group. The ^{13}C NMR spectroscopy also confirmed the synthesis as the signal at δ 192.91 ppm for the carbonyl of **95** was no longer observed. Moreover, a new peak was found at δ 24.03 ppm which corresponded to the methyl carbon. The NMR spectra analysed for compound **±88** was in good aggrement with the literature data [44].



±90

To perform the enzymatic kinetic resolution, the product of the Grignard reaction **±88** had to be converted into an ester by acetylation for the enzyme to selectively hydrolyze to the desired single enantiomer. The preparation of the acetate racemate **±90** was the crucial step of this part of the project and it was synthesized by the acetylation of 1-(2-allyl-3,6-dimethoxyphenyl)ethanol **±88** in dry THF using DMAP, acetic anhydride and trimethylamine. The resulting product **±90** was obtained as a clear oil in a disappointing yield of 45% due to the fact that the reaction didn't go complete. In the IR spectrum, the newly formed peak at 1732 cm^{-1} was an indication of presence of carbonyl of the ester of **±90**. A missing signal at δ 4.04 ppm in the ^{1}H NMR of compound **±88** suggested that the alcohol group found in the starting material was absent. The new peak appeared at δ 2.02 ppm corresponded to the methyl of the acetyl protons. In the ^{13}C NMR spectrum, the highly de-shielded signal at δ 170.85 ppm suggested the presence of ester carbonyl carbon. The additional carbon signal at δ 20.52 ppm was in agreement with the methyl protons from acetyl group. The NMR spectroscopic results unambiguously confirmed that the ester was 1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90** had been formed.

2.3 Preparation and characterization of enantiopure 1-(2-allyl-3,6-dimethoxyphenyl) ethanol 88

2.3.1 HPLC identification

With our racemic acetate **±90** in hand, we were now in the position to attempt the kinetic resolution. Before we could strat with our enzyme-catalysed reaction, we needed to determine the enantiomeric ratio of the ester racemate **±90** and the alcohol racemate **±88** which in theory should be 1:1. The Chiral OD chiral high pressure liquid chromatography chiral column showed the best

separation with a mixture of hexane and isopropyl alcohol as the mobile phase. After a series of test of mobile phase ratios, it was found that 20:80 isopropyl alcohol to hexane mixture was the best combination of solvent to achieve separation. The HPLC chromatograms for **±90** and **±88**, in **Figure 1** and **Figure 2** respectively showed that we had a 1:1 racemic mixture of the two enantiomers. We used these retention time data as the standard to monitor the enantioselective hydrolysis reaction.

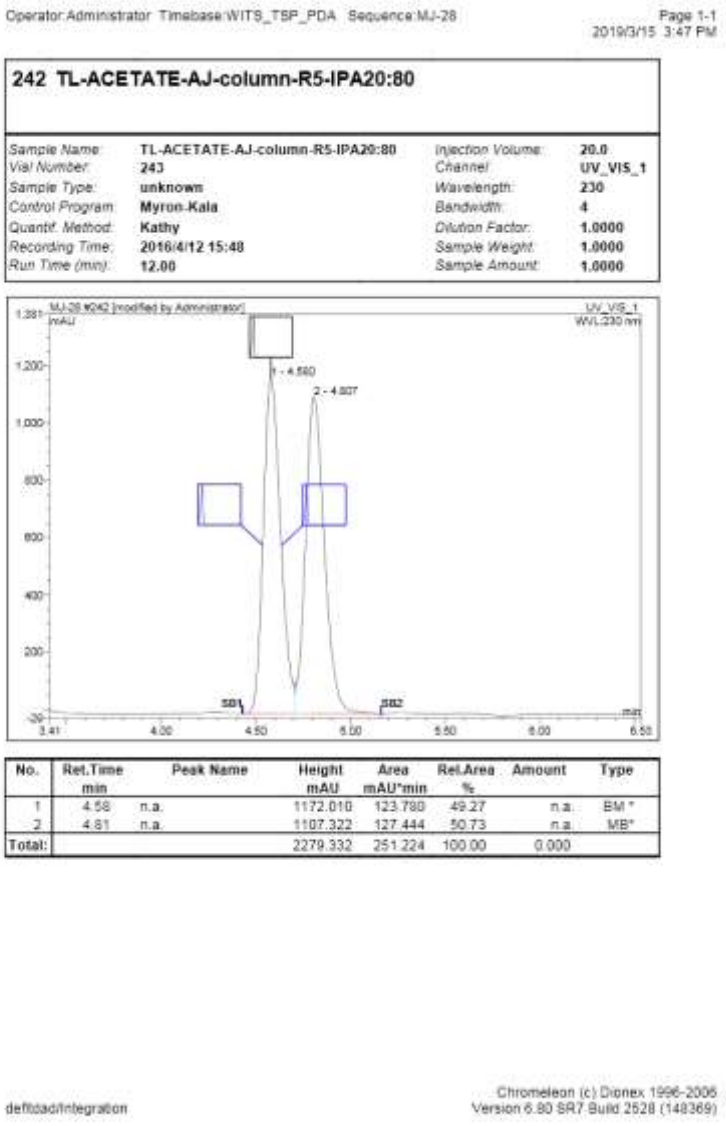


Figure 1: HPLC chromatogram of the acetylated product **±90**

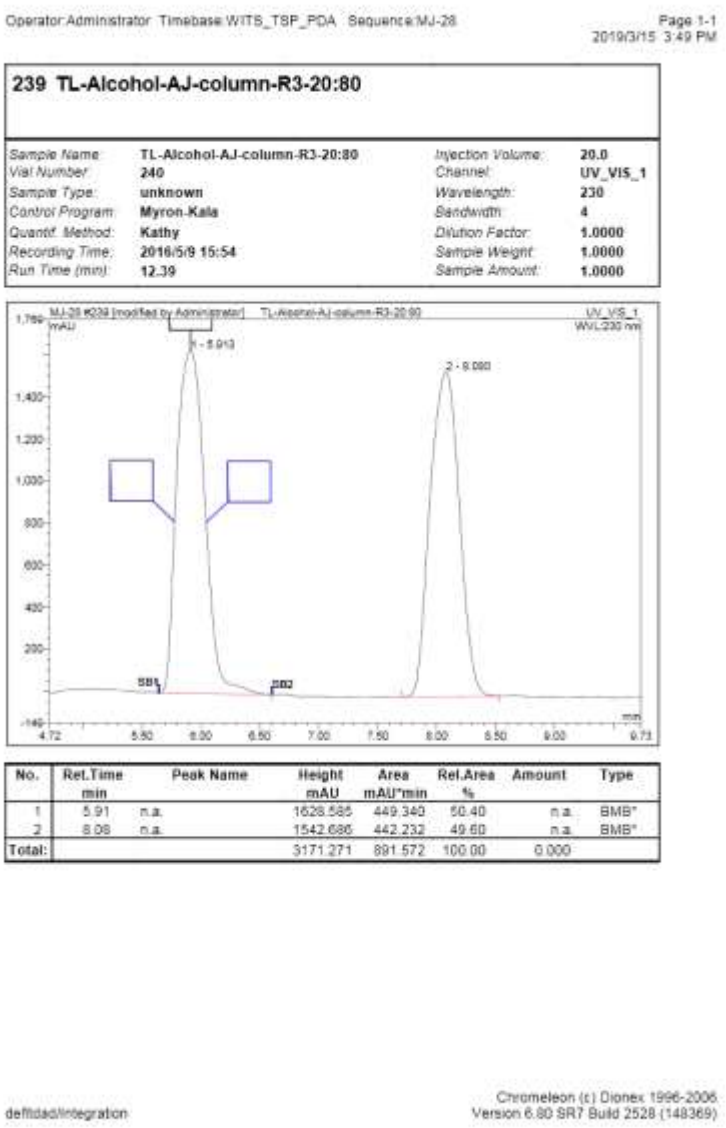
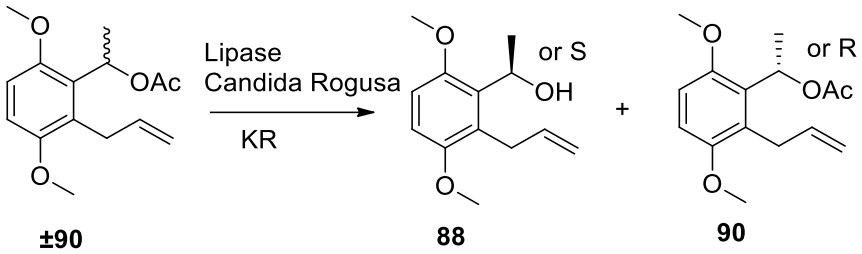


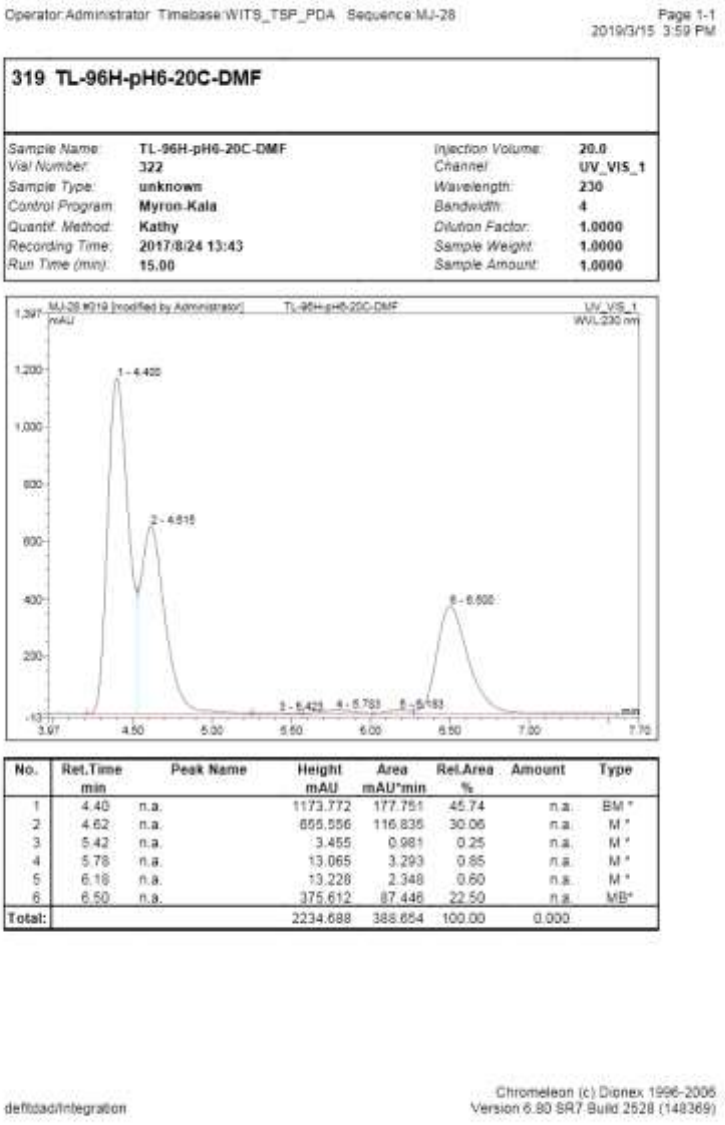
Figure 2: HPLC chromatogram of the Grignard product **±88**

2.3.2 Resolution of benzylic alcohol ±88

Once the racemic acetate **±90** was identified as a 1:1 mixture of two enantiomers using HPLC, it was subjected to the enzyme reaction as shown in **Scheme 18**. The reaction progress was monitored by TLC and HPLC. The progress of the enzymatic reaction after 96 hours at 20°C was monitored using chiral HPLC, as shown in **Figure 3**.



Scheme 18. Reagents and conditions: DMF, pH 7 buffer, 20°C, 96 h.



Operator:Administrator Timebase:WITS_TSP_PDA Sequence:MJ-28 Page 1-1
2019/3/15 3:59 PM

319 TL-96H-pH6-20C-DMF

Sample Name:	TL-96H-pH6-20C-DMF	Injection Volume:	20.0
Vial Number:	322	Channel	UV_VIS_1
Sample Type:	unknown	Wavelength:	230
Control Program:	Myron-Kala	Bandwidth:	4
Quantif. Method:	Kathy	Dilution Factor:	1.0000
Recording Time:	2017/8/24 13:43	Sample Weight:	1.0000
Run Time (min):	15.00	Sample Amount:	1.0000

No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	4.40	n.a.	1173.772	177.751	45.74	n.a.	BM *
2	4.62	n.a.	655.556	116.835	30.06	n.a.	M *
3	5.42	n.a.	3.455	0.981	0.25	n.a.	M *
4	5.78	n.a.	13.065	3.293	0.85	n.a.	M *
5	6.18	n.a.	13.228	2.348	0.60	n.a.	M *
6	6.50	n.a.	375.612	87.446	22.50	n.a.	MB*
Total:			2234.688	388.654	100.00	0.000	

defitdad/Integration

Chromeleon (c) Dionex 1996-2005
Version 6.80 SR7 Build 2528 (148369)

Figure 3: HPLC chromatogram of the enzyme hydrolysis reaction after 96h

One thing should be pointed out is that the retention time of the acetate **±90** and alcohol **±88** had shifted from that shown in **Figure 1** and **2**. We had to

change the mobile phase ratio to 10:90 (IPA : hexane) in order to obtain a better separation of the acetate **±90** peaks. However, it was clear that only one enantiomer of the alcohol **88** was formed as a result of the enzymatic hydrolysis of acetate **±90**. The enantiomeric excess (*ee*) of the reactant was calculated to be 20%, and the enantiomeric excess of the product was calculated to be 93%. At this point, we decided to quench the reaction and isolate the unreacted mixture of **±90** and the single enantiomer from the hydrolysis product **88**. After column chromatography, the alcohol **88** was subjected to polarimeter for the optical rotation measurement. At concentration of 9.6 mg/mL, the specific rotation was calculated to be $[\alpha]_D^{23}$ = -10.00 (methanol), suggested that we did indeed obtain the single enantiomer of the secondary alcohol **88** with an *ee* of 93%.

2.3.3 Determination of the absolute configuration of the benzylic alcohol 88

Since we found that from our enzymatic reaction the product was optically active, the next step was to determine the absolute configuration of the enantiopure alcohol **88**. Mosher acid methodology is a well-developed protocol that we can use to determine the absolute configuration of the stereogenic center. The enantio-pure alcohol **88** in DCM was treated with (*S*)-(-)-α-methoxy-α (trifluoromethyl) phenylacetic acid (*S*-Mosher acid), N,N-dicyclohexylcarbodiimide (DCC) and 4-dimethylamino pyridine (DMAP) to afford *S*-Mosher ester **96**. *R*-Mosher ester **97** was obtained using *R*-Mosher acid under the same reaction conditions. This approach was based on the anisotropic effect from NMR spectroscopy that the phenyl group of the chiral auxiliary MTPA exerts on the substituents (L1/L2) of the alcohol. **Figure 4** shown below explained the anisotropic effect.

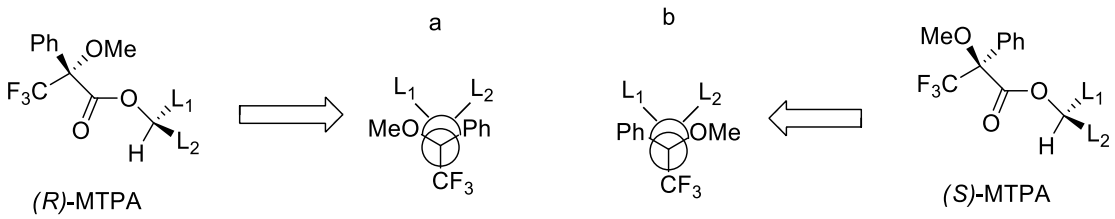


Figure 4

Comparing *R*-mosher ester **a** and *S*-mosher ester **b**, the protons of substituent L_2 are shielded by the phenyl ring in the ester **a** whereas those on L_2 remain unaffected in the ester **b**. On the other hand, the protons of substituent L_1 are shielded by the phenyl ring in the ester **b** whereas those on L_1 remain unaffected in the ester **a**. Therefore, the substituents L_1 will be more shielded in the *S*-mosher ester and the substituents L_2 will be more shielded in the *R*-mosher ester. These difference in shielding effects can be expressed as chemical shift on ^{1}H NMR spectra.

In our case, L_1 is the methyl group while L_2 is the 6-allyl-2,5-dimethoxy benzene moiety. There are two possible structures for each MTPA ester depending on the absolute configuration of the alcohol **88**, which are shown below (**Figure 5**).

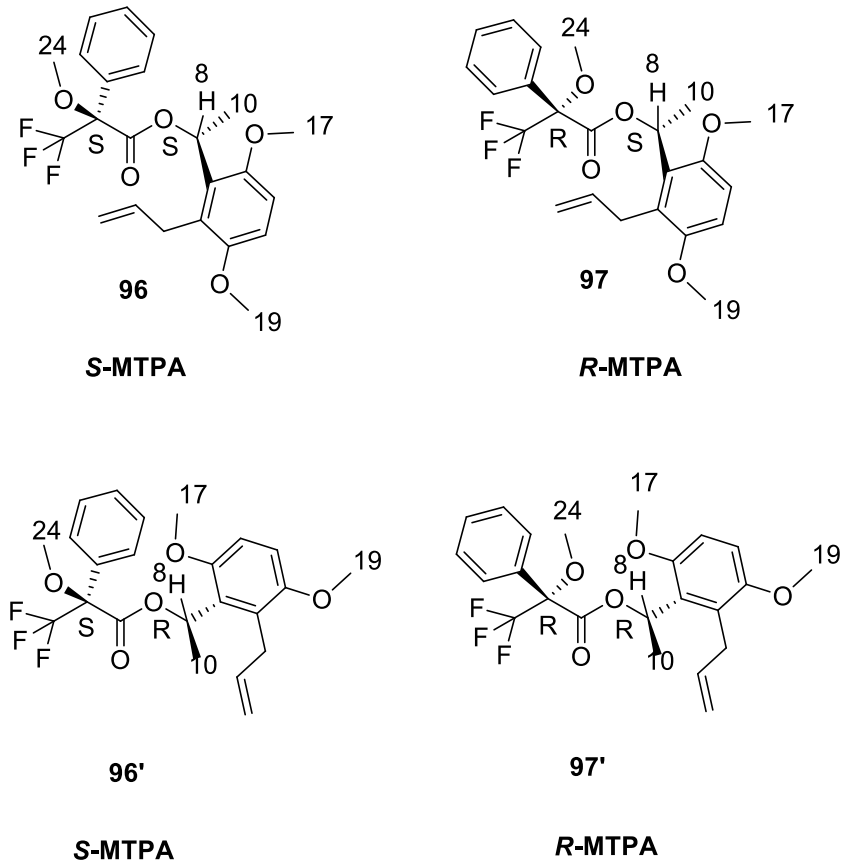


Figure 5

Two possible (*S*)-MTPA esters could be (*S, S*)-MTPA ester **96** or (*S, R*)-MTPA ester **96'** where two possible (*R*)-MTPA esters are (*R, S*)-MTPA ester **97** or (*R, R*)-MTPA ester **97'**. In the ^{1}H NMR spectrum of (*S*)-MTPA ester, we observed two singlet signals at δ 3.77 and 3.70 ppm which were corresponded to the two methyl ether protons H_{17} and H_{19}. Another doublet signal with *J* = 1.2 Hz at δ 3.48 ppm were corresponded to the other methyl ether protons H_{24}. The doublet signal could be a result from the interaction between H_{17} and H_8 through space. In the ^{1}H NMR spectrum of (*R*)-MTPA ester, we also observed two singlet signals but slightly shielded at δ 3.75 and 3.54 ppm which were assigned to protons H_{17} and H_{19}. Another more deshielded doublet signal with *J* = 1.2 Hz at δ 3.57 ppm were assigned to the other methyl ether protons H_{24}. Comparing chemical shifts of these methyl ether protons in the two ^{1}H NMR spectra, we observed that the methoxy hydrogen H_{19} (3.54 ppm) and H_{17} (3.75

ppm) in the (*R*)-MTPA ester were more shielded than that 3.77 and 3.70 ppm in the (*S*)-MTPA ester. Therefore, we can conclude that L_2 (the 6-allyl-2,5-dimethoxy benzene moiety) was shielded by the phenyl ring in (*R*)-MTPA ester **97**.

Comparison of the shielding effect of phenyl substituent on L_1 led us to the conclusion which was consistent with the (*R*)-MTPA ester **97**. On the one hand, in the ^{1}H NMR spectrum of (*S*)-MTPA ester, the observed doublet signal was at δ 1.63 ppm with J = 6.8 Hz, this doublet was corresponded to the methyl protons H_{10}; On the other hand, we observed the doublet signal with same coupling constant 8.8 Hz but at slightly deshielded chemical shift of δ 1.68 ppm. Therefore, L_1 was shielded by the phenyl ring in (*S*)-MTPA ester **96**. The observations on the shielding effects of the phenyl substituents were consistent, therefore, we could conclude that compound **96** (*S,S*)-MTPA ester and **97** (*R,S*)-MTPA ester have the structures shown in **Figure 6.**

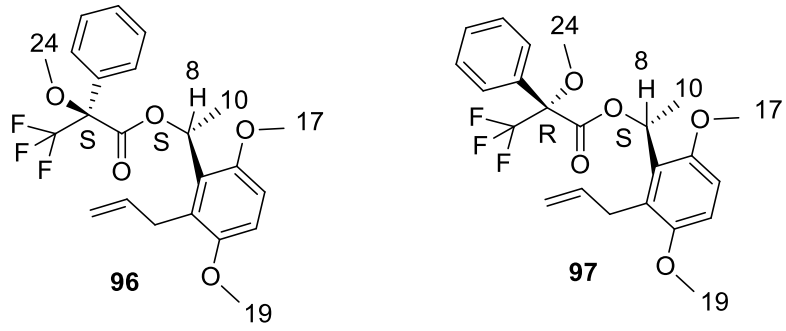
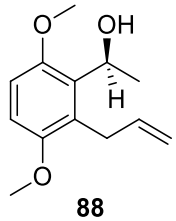


Figure 6

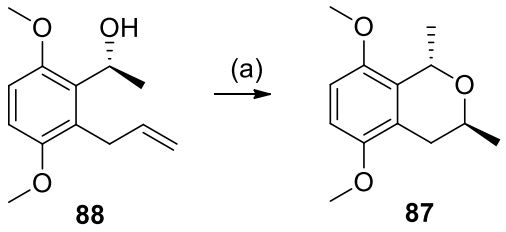
In summary, the Mosher acid protocol was a success to determine the absolute configuration; the absolute configuration of the enzymatic kinetic resolution product was (*S*)-1-(2-allyl-3,6-dimethoxyphenyl) ethanol **88**:



88

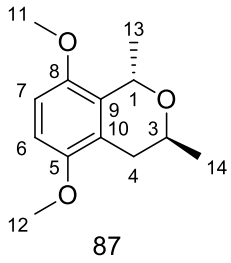
2.4 Preparation of (*S*)-5,8-dimethoxy-1,3-dimethylisochroman 87

The next step was to perform the pyran ring formation in order to synthesize the desired isochroman **87** (**scheme 19**).



Scheme 19. Reagents and conditions: a) Potasium tert-butoxide, DMF, 80°C, 2 h, 87%.

To achieve this, (*S*)-1-(2-allyl-3,6-dimethoxyphenyl) ethanol **88** was dissolved in DMF and treated with potassium *tert*-butoxide, which was heated to 80 °C for two hours. The resulting product **87** was obtained as a clear oil with a yield of 87%.



87

At concentration of 1 mg/mL, the specific rotation was calculated to be $[\alpha]_D^{23}$ = 55 (methanol). NMR spectroscopy were used to characterize the resulting product. Firstly, the alkene hydrogen signals that were observed at δ 5.92 ppm and between δ 5.12-4.78 ppm in the starting material had disappeared. Instead, we noticed two double of doublet signals at δ 2.77 and 2.32 ppm which suggested the formation of the pyran ring. Besides the existing doublet signal resulting from H_{13} protons, the new doublet signal at δ 1.34 ppm corresponded to the formation of methyl group as the pyran ring closure between the OH and the styrene. Two signals at δ 136.93 and 115.29 ppm which were assigned to the two alkene carbons in the allyl group were no longer observed in the ^{13}C NMR spectrum, instead, we noticed new signals at δ 62.22 and 19.56 ppm which corresponded to saturated carbon signals. The stereochemistry was assigned according to the literature [46]. For 1,3-cis methyl

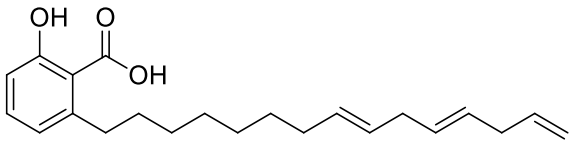
case, the signal of H_3 proton should be in the range of 3.70-3.80 ppm. In our case, the multiplet signal at δ 4.06 ppm was assigned to H_3, in the range of δ 3.9-4.3 ppm, suggesting the presence of *trans* isomer. The NMR spectroscopic results unambiguously confirmed that the compound was (*S)*-5,8-dimethoxy-1,3-dimethylisochroman **87**.

In summary, we successfully synthesized racemate acetate **±90** using 2,5-dihydroxy benzoic acid as starting material in 7 steps with an overall yield of 3%. Application of enzymatic kinetic resolution gave us enantiopure secondary alcohol **88**; using this alcohol, we were able to synthesize enantiopure isochroman **87**.

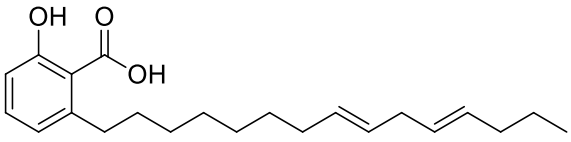
Chapter 3: Syntheses and biological activities of the renewable starting material anacardic acid

3.1 General introduction

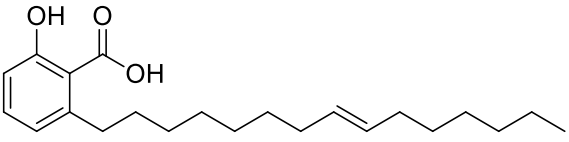
So far, our syntheses are based on 2,5-dihydroxy benzoic acid, a petroleum derived starting material. A green chemistry protocol was proposed to reduce or eliminate the use and generation of hazardous substances of our synthetic route. It is common knowledge that fossil oil is limited, and chemists are trying to look for alternative resources as starting material for chemical synthesis [47]. An interesting compound that attracted our attention is the aromatic compound anacardic acid **98**. Depending on the saturation of the 15-carbon alkyl chain, it can also be obtained with a diene **99**, a monoene **100** or a fully saturated alkyl chain **101**.



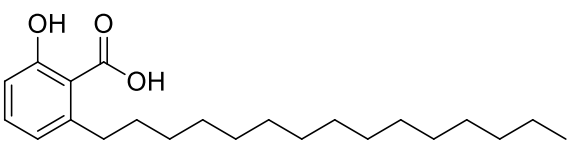
98



99



100



101

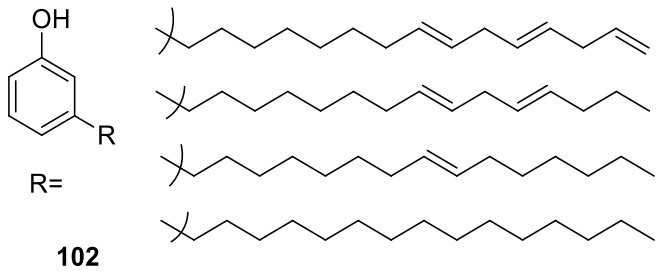
These four anacardic acids are one of the naturally occurring long chain phenolic compounds that are isolated from cashew nut shell liquid (*Anacardium occidentale L.*) [48]. Cashew nut shell liquid (CNSL) is a by-product of the cashew industry, which is obtained from the shell of a cashew nut. The cashew tree was originally grown in Brazil before it spreaded extensively into northern Africa and south-east Asian including India, Mozambique, the Malagasy Republic, Tanzania, Phillippines and other tropical countries[49].

Being a natural phenolic containing compound, anacardic acid resembles the structure of salicylic acid with an additional 15-carbon alkyl chain. A large amount of research has been conducted on anacardic acid and it has shown interesting bioactivities. We will discuss some of these bioactivities in the section below.

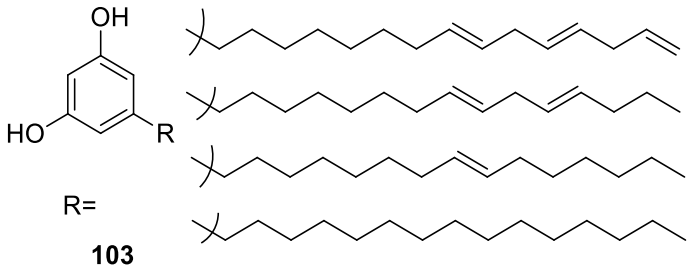
3.2 Bioactivities of phenolies from CNSL

3.2.1 Antibacterial activity

Himejima and Kubo have conducted research on the antibacterial activity on CNSL as long ago as 1991. In their preliminary screening, the CNSL exhibited biological activity against *Bacillus subtilis*, which is a Gram-positive bacterium. Therefore, they further examined CNSL against additional three Gram-positive bacteria and all of them were sensitive towards CNSL. After the isolation of a range of different phenolic compounds, they found that with additional hydroxy groups, cardols **102** had better antibacterial properties than cardanols **103**. With an addition of carboxylic group, anacardic acid exhibited a dramatic increase in antibacterial activity[50].



102



103

Other research conducted by Kubo found that the anacardic acid exhibited activities against methicillin-susceptible *Staph. Aureus.* With a minimal inhibitory concentration (MIC) value of 6.25 μg/mL [51].

Further research conducted by Kubo showed that anacardic acid exhibited antibacterial activity against Gram-negative bacterium *Helicobacter pylori*, isolated form the gastric mucosa of patients with duodenal-ulcer disease. This suggested that anacardic acid could be considered as potential treatment for ulcers [52].

3.2.2 Anti-tumor activity

Anacardic acid was also found to exhibit activity against tumors. Both BT-20 Breast and HeLa Epithelioid cervix carcinoma cells were used to study the cytotoxicity. It was found that number of saturation on the side alkyl chain affected the ED_{50}, as 2-hydroxy-6-((8*Z*,11*Z*)-pentadeca-8,11,14-trien-1-yl)benzoic acid had a lowest ED_{50} of 3.23 μg/mL for BT-20, while 2-hydroxy-6-(pentadec-8*Z*-en-1-yl)benzoic acid had a lowest ED_{50} of 2.69 μg/mL for HeLa [53].

3.2.3 Antifungal properties

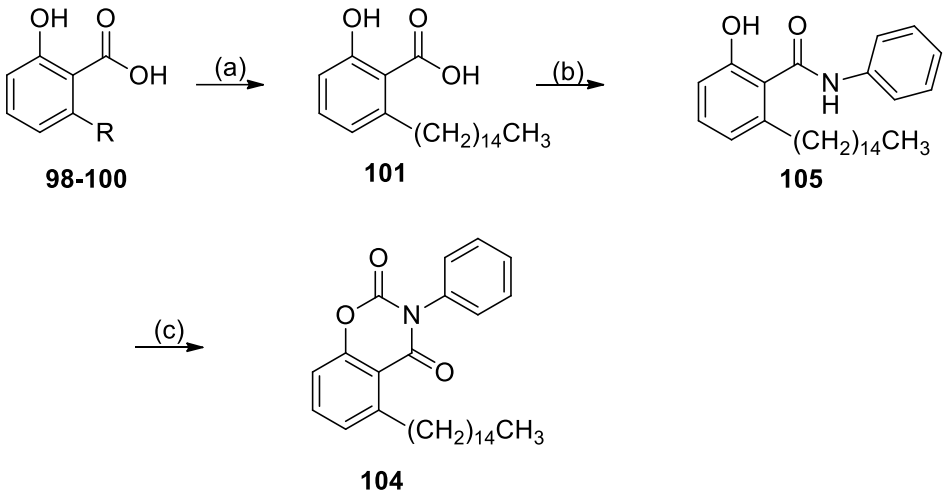
Prithiviraj and coworkers have compared the antifungal activity of anacardic acid and two of its derivatives against plant pathogenic fungi *Colletotrichum capsici* spore germination with that of salicylic acid. Anacardic acid was found to be most effective with a minimal inhibitory concentration value of 125–150 µg/mL. [54]

3.3 Chemical modifications of anacardic acid

Because of the interesting biological properties of anacardic acid, it has attracted researchers, especially synthetic organic chemists to modify the structure of anacardic acid to further explore their biological activities. Below are some examples of anacardic derivatives, synthesized using anacardic acid as the starting material.

3.3.1 Synthesis of substituted 3-aryl-1,3-benzoxazine-2,4-dione 104

Resck was able to synthesize substituted 3-aryl-1,3-benzoxazine-2,4-dione **104** using anacardic acid with unsaturated side chain (**98-100**) as starting material. Benzoxazines exhibits promising antibacterial and antifungal activities yet not much research has been done on the synthesis of this class of compounds. In this synthesis, triphosgene was used for one-step cycloaddition. The side chain of starting material was first treated with Pd/C with hydrogen gas followed by PCl_3 and anilines to afford an anilide **105**, which was treated with triphosgene to afford substituted 3-aryl-1,3-benzoxazine-2,4-dione **104** [55]. The reactions are depicted in **Scheme 20**:

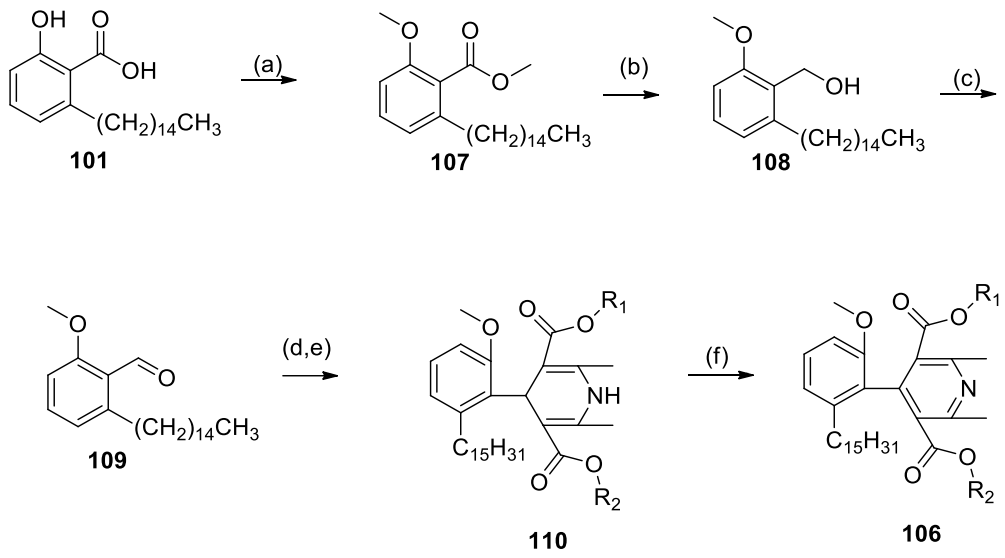


98-100 **101** **105** **104**

Scheme 20. Reagents and conditions: a) H_2, Pd/C, EtOH, rt, 2 h; b) PCl_3, PhCl, reflux, aniline; c) triphosgene, pyridine, CH_2Cl_2, rt, 12 h

3.3.2 Synthesis of dihydropyridine derivative 106

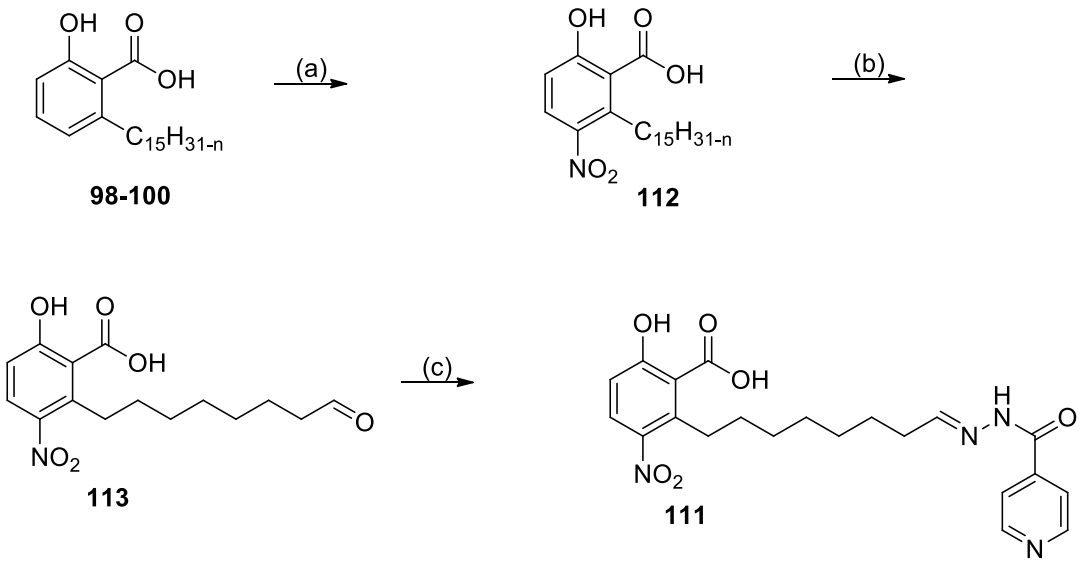
Kumar and has reported synthesis of nifedipine analogs which were found to possess activity to block T-type calcium channels with an IC_{50} values ranging between 1 to 40 μM. The calcium channels are involved in causes of hypertension, congestive heart failure and peripheral vascular disorders. Starting with the anacardic acids (**98-100**), the alkyl side chains were first saturated with H_2 gas and Pd/C to form **101**, this was followed by *O*-alkylation with dimethyl sulfate. The alkylated product **107** was treated with lithium aluminium hydride to afford the alcohol **108** which was followed by treatment of PCC to yield the aldehyde **109**. The aldehyde was then converted to 1,4-dihydropyridine **110** using the Hantzsch procedure, which eventually was converted to pyridine **106** [56], as shown in the **Scheme 21**.



Scheme 21. Reagents and conditions: a) $(CH_3)_2SO_4/K_2CO_3$, acetone, reflux 3 h; b) $LiAlH_4$, Tetrahydrofuran, reflux 3 h; c) PCC, dichloromethane, rt. 3 h; d) $CH_3COCH_2COOR_1$/piperidine/acetic acid, n-butanol, rt. 3 h ; e) $(CH_3)(NH_2)C=CH(COOR_2)$, n-butanol, reflux 30 h.

3.3.3 Synthesis of isonicotinoylhydrazone 111

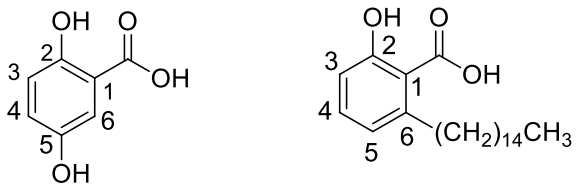
Again, starting with the anacardic acids (**98-100**), Swamy and coworkers have synthesized isonicotinoylhydrazone **111**, which exhibited biological activity against *Mycobacterium smegmatis.* Starting with the ene mixture of anacardic acids, 5-nitroanacardic acids **112** were prepared using HNO_3 in acetic acid. Oxidative cleavage of the side alkyl chain afforded C_8 aldehyde **113**, which was then coupled with isonicotinylhydrazide to afford isonicotinoylhydrazones **111** [57]. The reactions are shown in the **Scheme 22**:



Scheme 22. Synthesis of isonicotinoylhydrazones **111.** a) HNO_3/CH_3COOH, 65 °C, 15 min; (b) 1)Hydrogen peroxide/formic acid (3/4) 40 °C, 10 min; 2) $NaIO_4$, 30 min; (c) isoniazid, methanol, 70 °C 1 h.

3.4 Aims

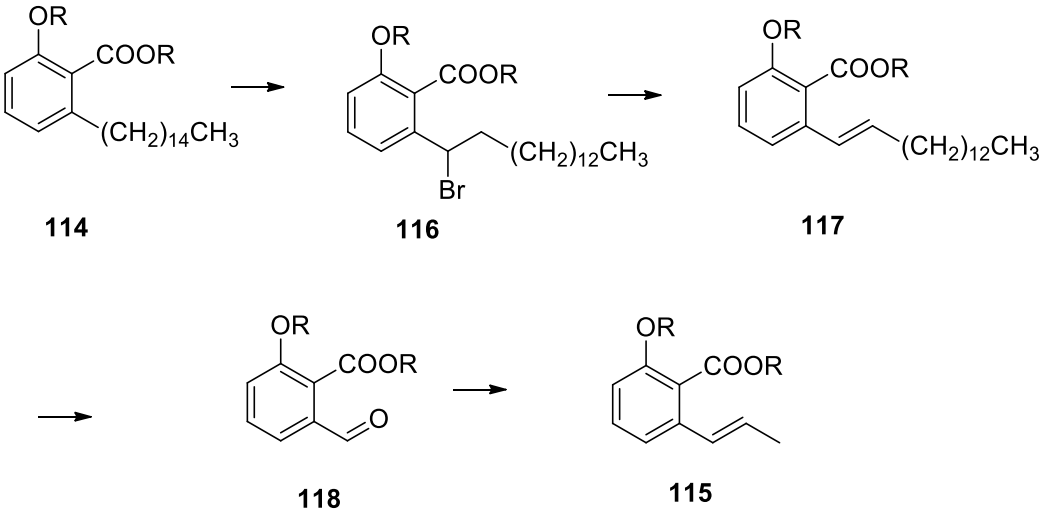
As a major aim of this part of the MSc project, we decided to also examine the possible use of bio-renewable chemicals, instead of petroleum derived chemicals that we had used to synthesize our isochroman. After examining the molecular structures of the isochromans, we chose anacardic acid as our starting material. Comparing the structures of 2,5-dihydroxy benzoic acid **89** and anacardic acid **101**, the similarities are obvious. They both have hydroxy groups on C-2 and carboxy groups on C-1.



89 **101**

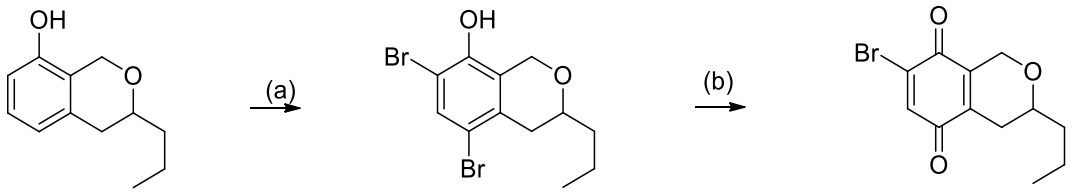
The additional alkyl side chain on the anacardic ester **114** can be easily

transformed into the styrene functional group **115**. The saturated alkyl side chain could first be brominated to form the benzylic bromide **116**, which after elimination can form the long chain alkene **117**. Alkene **117** can be oxidatively cleaved to form the aldehyde **118** which when followed by a Wittig reaction could form the styrene **115** (**Scheme 23**).



Scheme 23. Transformation of alkyl side chain

In order to introduce the remaining phenol at C-4 of anacardic acid **101** we believe that this can be accomplished by using the methodologies of Donner [58] and Kim [59]. In Donner's work, an oxygen was successfully inserted onto the benzene in the para position to a phenol, as shown in **Scheme 24**. The isochroman **119** was initially brominated using NBS in DMF to form the dibrominated aromatic compound **120**, which was then subjected to a CAN oxidation to form the quinone **121**.



Scheme 24. Reagents and conditions: (a) NBS, DMF, 18 h (82%); (b) CAN, MeCN, THF, H_2O, 30 min (91%).

The research group of Kim has proposed a mechanism for the NBS facilitated oxidative demethylation, shown below in **Figure 4**.

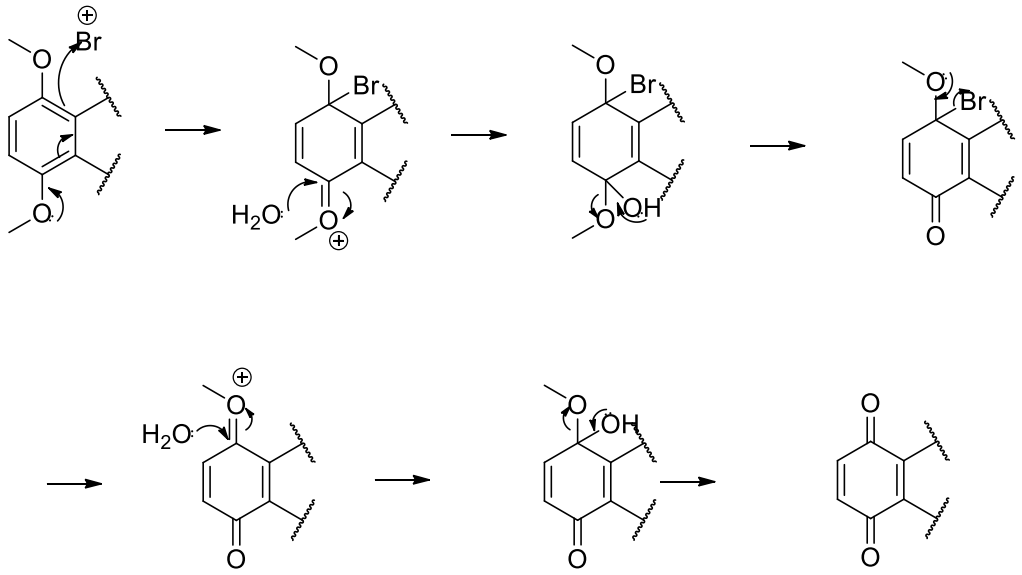
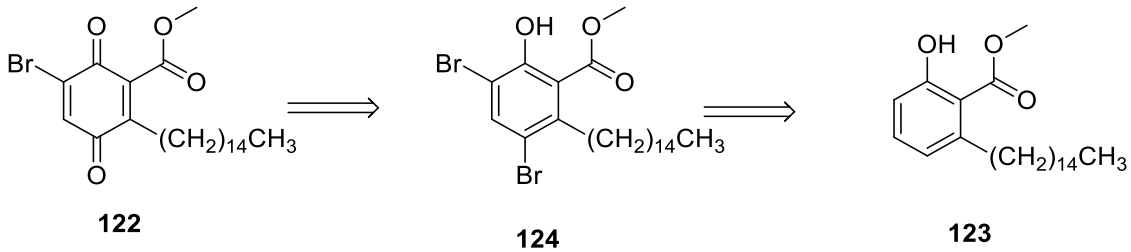


Figure 4

Therefore, as one possibility we reasoned that in order to synthesize the quinone **122**, we could brominate the anacardic ester **123** to initially form brominated anacardic ester **124**, oxidation of **124** would afford **122** resulting in the addition of the extra oxygen substituent to the aromatic ring (**Scheme 25**).



122 **124** **123**

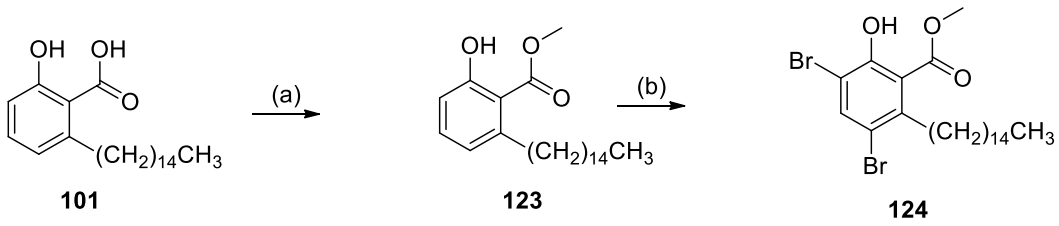
Scheme 25. Retrosynthesis of bromo quinone **122**

Thus, the aim was to achieve the synthesis of the 1,3-dimethyl isochroman skeleton using anacardic acid as the starting material. Once the synthesis of isochroman was achieved, we would also look into the expanded synthetic applications of the isochroman core.

Chapter 4: Results and Discussion

As a starting point for this aspect of the project we wished to see if the aromatic dibromination followed by oxidation to yield the quinone would be possible on an anacardic acid derivative.

We envisaged that the synthesis of the dibromo benzoate **124** could be achieved by the saturation of the side alkyl chains of the anacardic acid ene mixtures **98-100** to followed by methyl iodide esterification to form ester **123**. NBS bromination should then give the desired compound **124** (**Scheme 26**).

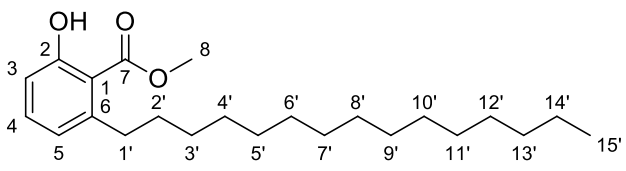


101 **123** **124**

Scheme 26. Reagents and conditions: a) Sodium bicarbonate, methyl iodide, DMF, 40°C, 5 h, 91%; b) NBS, DMF, dark, rt, 18 h, 75%

4.1 Preparation of methyl 3,5-dibromo-2-hydroxy-6-pentadecylbenzoate 124

Saturated anacardic acid **101** was treated with sodium bicarbonate and methyl iodide in dry DMF, resulting in a white solid of ester **123** in a yield of 91%. The white crystals were measured to have a melting point of 40-43°C which is quite close to the reported value of 39-41°C by Zehnter [60].

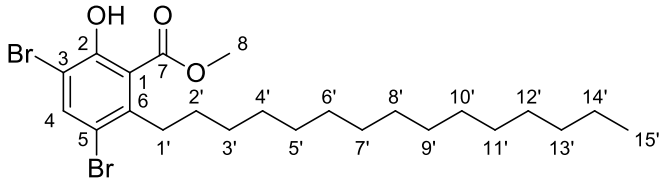


123

In the ^{1}H NMR spectrum, we could easily see that the broad

carboxylic acid proton signal at δ 11.02 ppm was no longer present, compared to the starting material anacardic acid. Instead, a singlet signal was observed at δ 3.95 ppm which integrated for three protons, indicating that the salicylic acid was transformed into a methyl ester. In addition, newly appeared carbon signals at δ 52.45 ppm suggesting the presence of a carbon next to an electron rich atom which was consistent with the formation of a methyl ester. Combining all the information obtained from the NMR spectra we can conclude that the resulting white crystal was methyl 2-hydroxy-6-pentadecyl benzoate **123**.

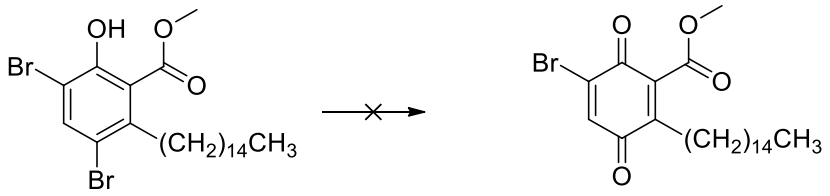
With the ester **123** in hand, it was then treated with NBS in DMF, resulting in a clear yellow oil with a yield of 75%.



124

NMR spectroscopy was used to characterize the product. Looking at the ^{1}H NMR spectrum in the aromatic region, we could clearly see a singlet signal at δ 7.87 ppm suggesting that benzene ring was now penta-substituted. Using NMR spectra, we can confirm that the resulting clear yellow oil was methyl 3,5-dibromo-2-hydroxy-6-pentadecylbenzoate **124**.

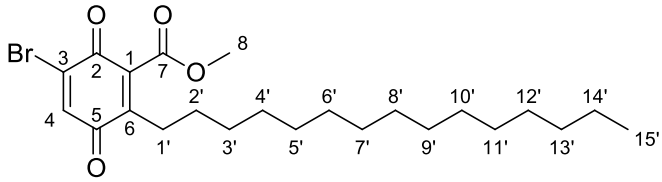
Excited that we now had our dibrominated compound **124**, the next key step was to oxidize it to a quinone using CAN mediated protocol. Unfortunately, the reaction did not work as most of the starting material did not dissolve in the reaction medium of water and acetonitrile (**Scheme 27**).



124 **122**

Scheme 27. Reagents and conditions: CAN, MeCN/THF/H_2O, 18 h

As the reaction was not successful, we thought we could perform the oxidation under harsher conditions. Silver (II) oxide with conc. nitric acid is a common protocol for this purpose. As we followed the protocol and monitored the reaction using TLC, we did see new spot that having higher Rf value than starting material. Initially, we could not purify the resulting mixture using normal column chromatography as this product tended to decompose on the silica gel. As we increased the reaction scale and shorten the silica gel column, we however managed to isolate a small amount of product **122** and subject it to NMR spectroscopy.

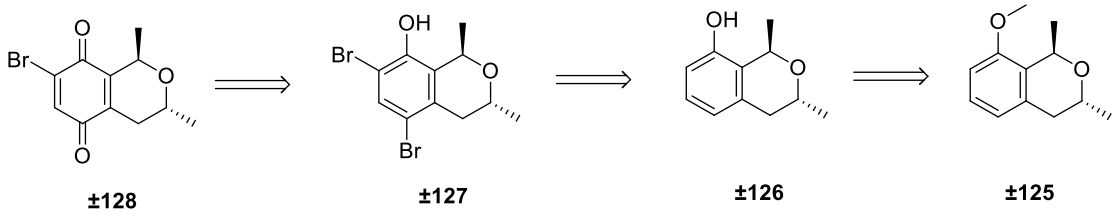


122

In the aromatic region of ^1H NMR spectrum, we could observe a singlet signal appeared at δ 7.31 ppm; where in the starting material, the singlet signal was at δ 7.87 ppm. In addition, the phenol signal observed at δ 10.99 ppm in the starting material was no longer seen. These observations were consistent with the formation of a quinone. Unfortunately, we were not able to further characterize the compound due to the fact that the quinone was unstable and it was quickly oxidized.

As an alternative strategy and examination of the research of Donner, we thought that instead of inserting the needed oxygen at this early stage of the

synthesis, we envisaged that the oxygen could be introduced onto the *para*-position of the benzene once we formed the isochroman skeleton, the retrosynthesis is shown in **Scheme 28**.

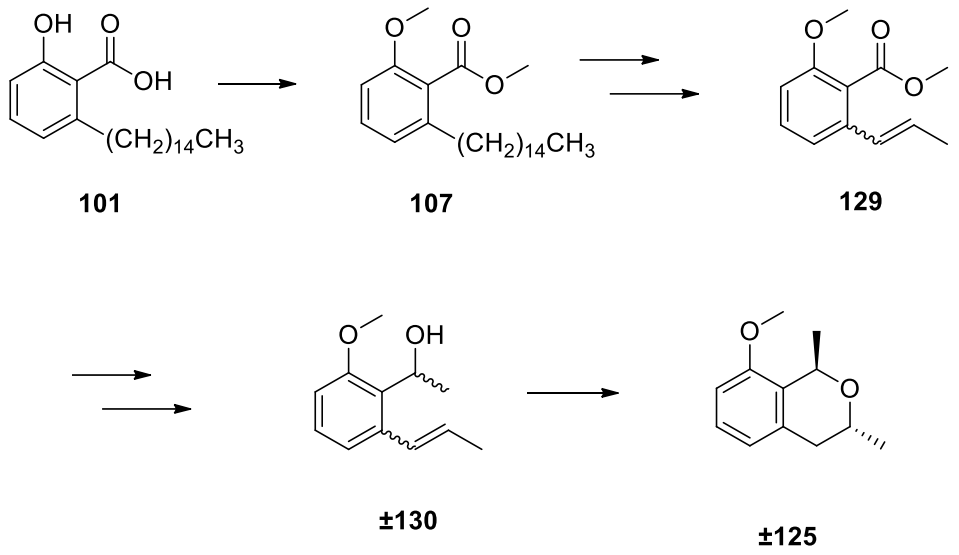


Scheme 28: Retrosynthesis of *rac*-7-bromo-1,3-dimethyl-3,4-dihydro-1*H*-isochromene-5,8-dione **±128**

Once we had synthesized isochroman **±125**, boron tribromide can be used to deprotect the methylether to yield phenol **±126**, which could then be brominated to afford dibromo compound **±127**. The synthesis of the desired isochromene-dione **±128** should then be achieved by exposure to silver (II) oxide in the presence of nitric acid.

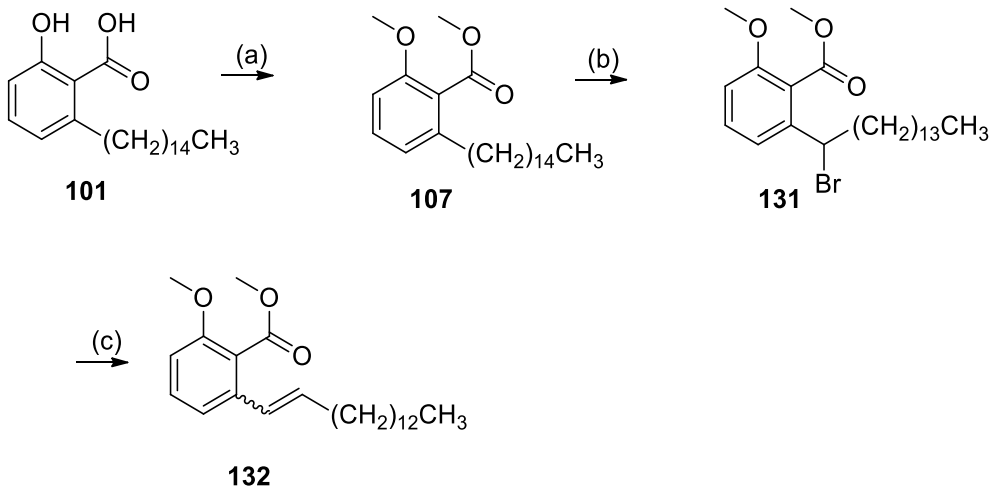
4.2 Preparation of methyl 3,6-dimethoxy-2-(pentadec-1-en-1-yl)benzoate 132

In summary, since we changed the synthesis, we rethought the strategy to achieve the synthesis of isochroman **±125** using anacardic acid **101** as the starting material. As discussed in the previous section, the alkyl side chain of benzoate **107** can be modified into a styrene in a few steps to afford styrene **129**. The conversion of the ester group of **129** to the secondary alcohol **±130** can be achieved using the same LAH reduction followed by PCC oxidation and then a Grignard reaction with methyl magnesium iodide. The isochroman **±125** could then be synthesized using *tert*-butoxide mediated ring closure from styrene **±130** (**Scheme 29**).



Scheme 29: Synthesis plan towards *rac*-8-methoxy-1,3-dimethylisochroman **±125**

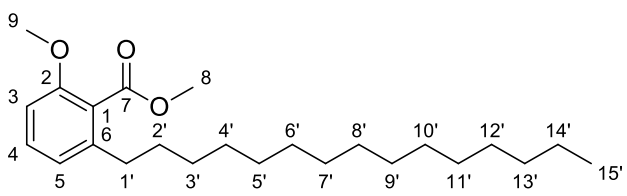
With anacardic acid **101** in hand, it was first treated with potassium carbonate and dimethyl sulfate to afford methylated anacardic ester **107**, this was followed by a NBS benzylic bromination to yield product **131**. The brominated compound **131** was treated with DBU to afford the elimination product **132** (**Scheme 30**).



Scheme 30. Reagents and conditions: a) Potassium carbonate, dimethyl sulfate, acetone, reflux, 18 h, 96%; b) NBS, AIBN, CCl_4, reflux, 18 h, 76%; c) DBU, toluene, 95°C, 18 h, 80%.

The detailed synthesis used anacardic acid **101** as starting material, which

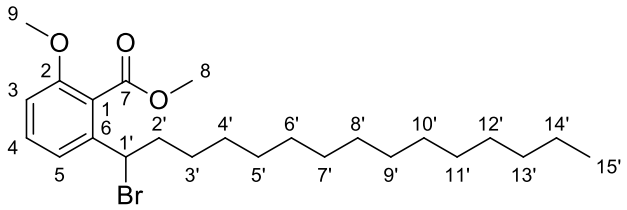
was treated with potassium carbonate and dimethyl sulfate in dry acetone, resulting in white crystals with a melting point of 35-37 °C (*lit* [61] 36-37°C).



107

^{1}H NMR and ^{13}C NMR spectroscopy were used to characterize the product. Comparing with the ^{1}H NMR spectrum of the starting material anacardic acid, we could easily see that the broad acidic proton signal at δ 11.02 ppm was no longer present. Instead, two new singlet signals were observed at δ 3.90 and 3.81 ppm. They both integrated for three protons, which corresponded to the formation of the two methoxy substituent. In addition, new carbon signals at δ 55.85 and 52.10 ppm in the ^{13}C NMR spectrum suggesting the presence of two carbons next to electron rich oxygen. The analytical data for compound **107** was in good agreement with the literature [61].

Once the phenol and carboxylic acid were protected, the next step was to place a bromine at the benzylic position of the side chain. This reaction has been investigated by our group in recent times where alternative solvents to carbon tetrachloride were evaluated. However, the reaction proceeded best by treating methyl(2-methoxy-6-pentadecyl) benzoate **107** with NBS and AIBN in the non-green solvent carbon tetrachloride. The resulting product after purification was a pale-yellow solid with a yield of 76%. The measured melting point of the resulting solid was 56-59 °C (*lit* [62] 57-58°C).

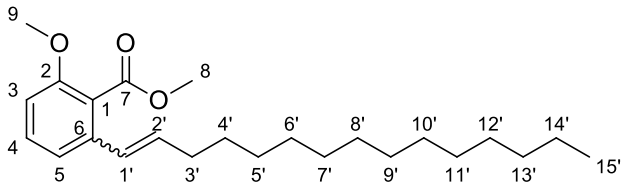


131

^{1}H and ^{13}C NMR spectroscopy were again used to determine the identity of the product. The triplet signal appearing at δ 7.21 ppm suggested that the

bromination at C-1 position was successful. The ^{13}C NMR spectrum corresponded to the theory as we could see that the signal of C-1' had shifted from δ 33.50 ppm to δ 52.43 ppm, indicating substitution reaction had occurred on that C-1' carbon. The NMR spectra data for the compound **131** was in good agreement with the literature [62].

The next step was elimination to afford the alkene **132**; which was achieved by treating the brominated product **131** with DBU in toluene to provide the product **132** in a yield of 80%. The resulting product after purification was light yellow solid with a melting point of 35-36 °C (*lit* [62] 33-34°C).



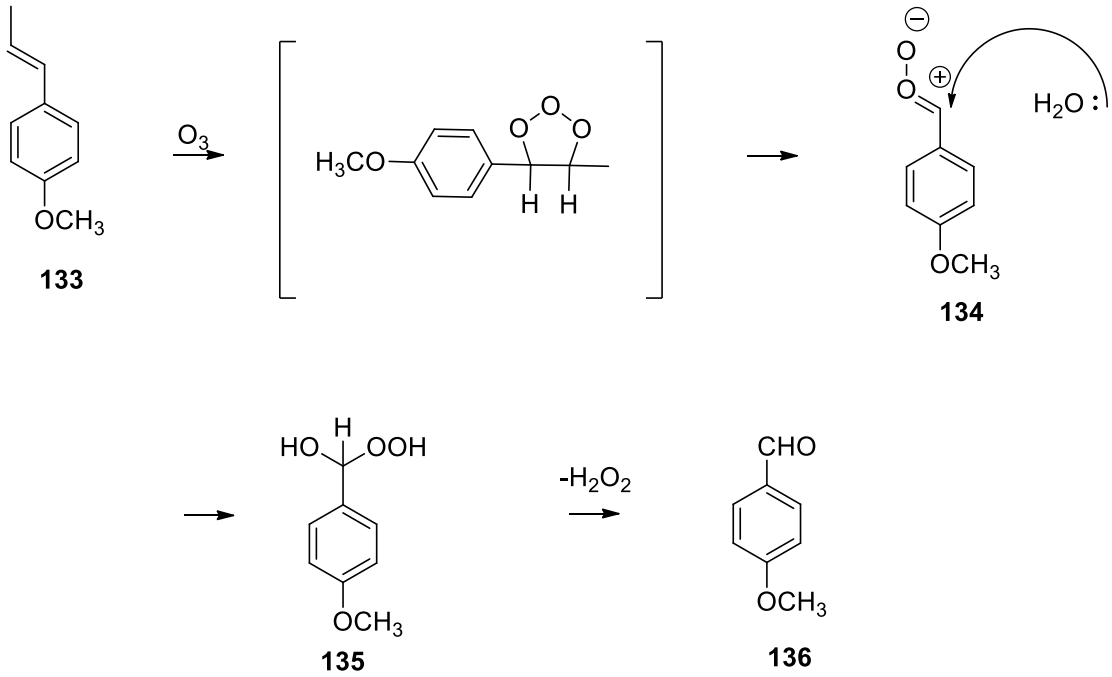
132

The triplet signal resulting from the H-1' at δ 7.21 ppm in the ^{1}H NMR spectrum of alkene **132** was no longer observed. Instead, we observed a multiplet at δ 6.35 – 6.11 ppm integrated for two protons; which are typical alkene hydrogens. The ^{13}C NMR spectrum showed two new signals at δ 134.75 and 126.07 ppm and the C-1' signal at δ 52.43 ppm in the starting material was no longer observed. The ^{13}C NMR spectra data suggesting that the bromine was absent a double bond had formed. Combining all the information obtained from NMR spectroscopy, we could conclude that the resulting pale yellow oil is methyl 2-methoxy-6-(pentadec-1-en-1-yl)benzoate **132**.

4.3 Preparation of methyl 2-methoxy-6-(prop-1-en-1-yl)benzoate 129

Satisfied that we had the eliminated product **132**, the next stage was to synthesize the styrene compound **129** that no longer containing the lipophilic

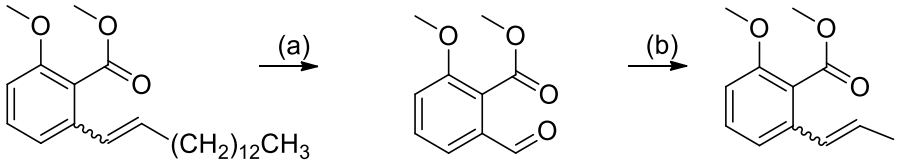
chain. The styrene functional group was achieved by oxidative cleavage of alkyl side chain followed by Wittig carbon-carbon bond formation. Oxidative cleavage is traditionally done using osmium tetraoxide or ozone as cleaving agents. In order to make our synthesis greener and produce fewer toxic wastes, we decided to proceed using ozone. Ozonolysis is known to be performed at -78 °C using dimethyl sulfide as a reducing agent. Jing Yu and coworkers, however, reported a rather unusual approach which only used water as reducing agent and reaction was carried out at room temperature [63]. The proposed mechanism is shown below (**Scheme 31**). Anethole **133** first reacted with ozone to generate carbonyl oxide **134**, which was then reacted with water to form gem-hydroperoxy **135**. Anisaldehyde **136** was afforded once hydroperoxy decomposed.



Scheme 31. Mechanism of water facilated ozonolysis

Thus, to synthesize the target molecule **129**, ozone methodology was adopted to yield aldehyde **137**. In our case, the use of ozone in a mixture of ethyl

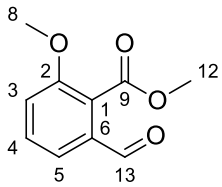
acetate and water proved to work best. The mechanism for this transformation is shown above in **Scheme 31**. This was followed by treatment with ethyl triphenylphosphine bromide which facilited the Wittig reaction to afford **129** (**Scheme 32**).



132 **137** **129**

Scheme 32. Reagents and conditions: a) O_3, ethyl acetate/water, rt, 5 min, 60%; b) NaH, $EtPPh_3Br$, rt to reflux, 4 h, 70%.

To achieve this, the benzoate **132** dissolved in ethyl acetate with presence of H_2O was treated with a flow of O_2/O_3. The reaction was fast and the aldehyde **137** was produced with a yield of 60%. The resulted product was white solid with a melting point of 58-61°C which was different to the reported value of 70.5°C by Bohlmann [64].

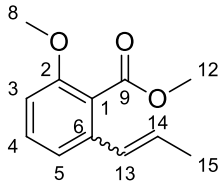


137

^1H NMR and ^{13}C NMR spectroscopy were used to characterize the product. The peaks between δ 0.88 to 2.17 ppm were no longer seen suggesting the long alkyl chain was no longer present. At δ 9.96 ppm, we observed a highly deshielded signal suggesting the presence of an aldehyde. The ^{13}C NMR spectrum suggested the formation of desired product as the number of signals decreased by 14 and we could see a signal at δ 190.32 ppm consistent with aldehyde carbon. On the basis of these signals from NMR data, we could conclude that the resulting white solid was methyl 2-formyl-6-methoxybenzoate **137** [64].

The final stage was to transform the formyl group into a styrene substituent.

This was achieved by utilizing the Wittig reaction. Aldehyde **137** was treated with sodium hydride in the presence of ethyl triphenyl phosphonium bromide to afford a clear yellow oil in a yield of 70%.

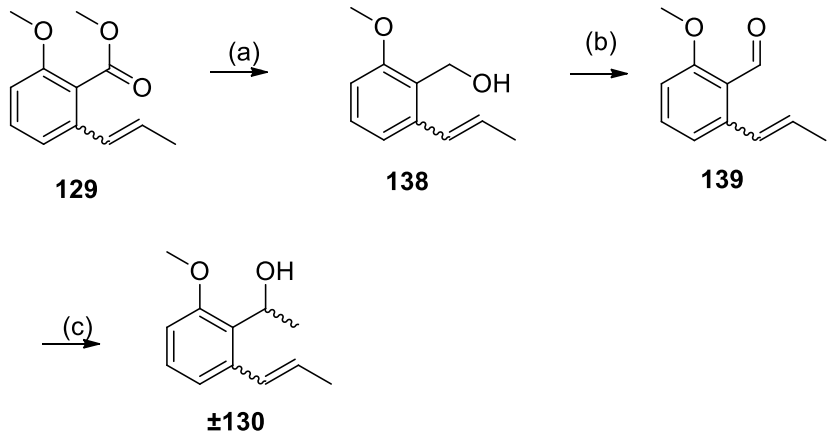


129

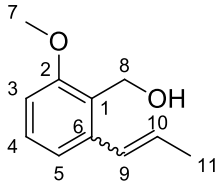
In the ^{1}H NMR spectrum, the disappearance of the signal at δ 9.96 ppm suggested that the conversion of formyl group had taken place. Also, we observed new multiplets at δ 5.96-5.75 ppm integrated as two, which were in the region of sp^2 carbon hydrogen consistent with alkene hydrogens. At δ 1.90-1.71 ppm, there were two sets double of doublets, which integrated for three hydrogens when counted as a whole. The presence of two sets of signals indicated that we had both (*E*) and (*Z*) isomers. The presence of both the (*E*) and (*Z*) isomers would be carried through all the reactions until we eventually formed the pyran ring. The disappearance of the signal at δ 190.32 ppm in the ^{13}C NMR spectrum corresponded to the conversion of a formyl group into a styrene. All the above data unambiguously proved that the desired styrene **129** was successfully synthesized.

4.4 Preparation of *rac*-1-(2-methoxy-6-(prop-1-en-1-yl)phenyl)ethanol ±130

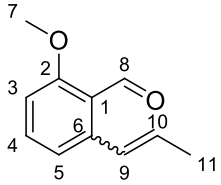
Now that we had accomplished the desired modification of the long alkyl chain, the reduction of the methyl ester **129** was done using standard protocol. Styrene compound **129** was first reduced using LAH to form the alcohol **138**, which was then oxidized with pyridinium chlorochromate to afford aldehyde **139**. The additional methyl group of the secondary alcohol **±130** was introduced as before using the Grignard reaction (**Scheme 33**).



Scheme 33. Reagents and conditions: a) LAH, THF, 0 °C to rt, 5 h, 86%; b) PCC, DCM, rt, 5 h, 93%; c) Mg, MeI, diethyl ether/THF, rt, 18 h, 74%.

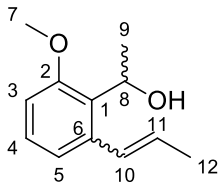


In detail, methyl 2-methoxy-6-(prop-1-en-1-yl)benzoate **129** was treated with LAH to afford white crystals of **138** in a yield of 86%. ^{1}H NMR, ^{13}C NMR and IR spectroscopy was used to characterize the product. In the IR spectrum, the appearance of a board peak at 3411 cm^{-1} suggested the formation of an alcohol. The ^{1}H NMR spectrum now showed the absence of a signal at δ 3.90 ppm suggested that the methoxy group from the ester was no longer present. A new signal appeared at δ 4.72 ppm were integrated for two protons, consistent with the reduction of the carbonyl carbon. Another signal observed at 2.46 ppm corresponded to the alcohol hydrogen. In the ^{13}C NMR spectrum the carbonyl carbon signals which was observed at δ 168.99 and 168.74 ppm for the two geometric isomers in the starting material were no longer present. The NMR spectroscopic results unambiguously confirmed that the resulting white crystal was 2-methoxy-6-(prop-1-en-1-yl) phenyl methanol **138**.



The next synthetic step involved treatment of (2-methoxy-6-(prop-1-en-1-yl)phenyl)methanol **138** with PCC in dry DCM. The resulting product was obtained as a pale

yellow oil with a yield of 93%. The IR spectrum clearly showed that the O-H peak at 3411 cm^{-1} was gone, instead, a carbonyl group (C=O) appeared at 1684 cm^{-1}. A singlet signal appeared at δ 10.58 ppm in the ^{1}H NMR spectrum corresponded to the formation of an aldehyde. The ^{13}C NMR spectrum also agreed to the formation of the aldehyde with signals at δ 192.38 and 191.71 ppm (E and Z isomers) for the carbonyl. In addition, the peak for alcohol in the starting material at δ 2.46 ppm was no longer evident. Combining all the information obtained from NMR and IR spectroscopy, we can conclude that the resulting pale yellow oil was 2-methoxy-6-(prop-1-en-1-yl)benzaldehyde **139**.

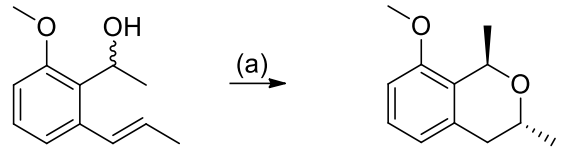


±130

Once we had the aldehyde **139** in hand, the next step was to perform the Grignard reaction. Since methyl iodide was used, the preparation of methylmagnesium iodide was successful. Starting material benzaldehyde **139** in dry THF was treated with the freshly prepared Grignard reagent made from magnesium and methyl iodide in dry diethyl ether. Again, after the reaction the IR spectrum clearly showed that a new O-H peak at 3438 cm^{-1}, in addition, the carbonyl group (C=O) appeared at 1684 cm^{-1} in the starting material was now absent. A doublet signal at δ 1.51 ppm in the ^{1}H NMR spectrum integrated for 3 protons suggesting that there was an additional methyl group. The ^{13}C NMR supported this observation as the aldehyde peaks at δ 192.38, 191.71 ppm in starting material were no longer seen. Moreover, new peaks were found at δ 23.43, 23.34 ppm which were consistent with a methyl substituent to the (E) and (Z) isomers. The NMR spectroscopic results unambiguously confirmed that the *rac*-1-(2-methoxy-6-(prop-1-en-1-yl)phenyl)ethanol **±130** was successfully synthesized with a yield of 74%.

4.5 Preparation of *rac-tans*-8-methoxy-1,3-dimethylisochroman ±125

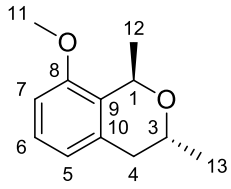
Once having the racemic ethanol **±130**, the next step was to test if the *tert*-butoxide mediated pyran ring closure could also work with the styrene **±130**. The same protocol as described before was used as shown (**Scheme 34**).



±130 **±125**

Scheme 34. Reagents and conditions: a) Potasium tert-butoxiode, DMF, 80°C, 2 h, 90%.

Rac-1-(2-methoxy-6-(prop-1-en-1-yl)phenyl)ethanol **±130** was dissolved in DMF and treated with potassium *tert*-butoxide. The resulting product afforded from this reaction was a transparent solid in a yield of 90% with a melting point of 34-36 °C which is quite close to the reported value of 35-36°C by Bringmann [65].



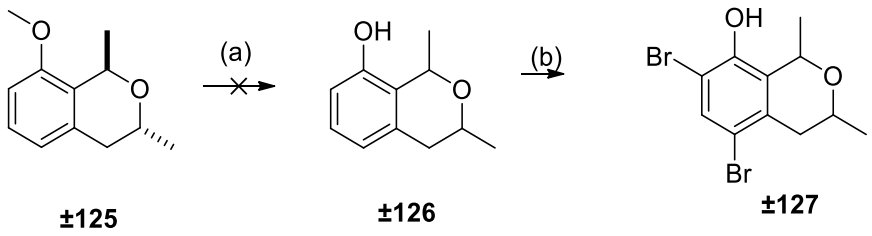
±125

In the ^{1}H NMR spectrum, two multiplet signal at δ 6.70 – 6.46 and 6.07 – 5.77 ppm were no longer present, indicating the alkene had reacted. Instead we observed one multiplet signal at δ 4.22-4.05 ppm which integrated for one hydrogen and another signal at δ 2.77-2.52 ppm which integrated for two hydrogen. The observation was consistent with the pyran ring closure, where signal at δ 4.22-4.05 ppm corresponded to the 3-H and the signal at δ 2.77-2.52 ppm is also characteristic for the 4-H in a pyran ring [66]. Signals at δ 129.63,128.47 and 128.26, 128.09 ppm which corresponded to the alkene carbons were no longer observed in the ^{13}C NMR spectrum, instead, we saw new signals at δ 62.59 and 35.89 ppm, which were consistent with the saturated carbons. The

stereochemistry was assigned according to predicted from the literature [46]. The multiplet signal at δ 4.22-4.05 ppm was assigned to H_3; since these are in the range of δ 3.9-4.3 ppm, suggesting the presence of the *trans* isomer of **±125**. Combining all the information obtained from NMR and IR, we can conclude that the resulting clear oil was *rac-trans*-8-methoxy-1,3-dimethylisochroman **±125**.

4.6 Preparation of 5,7-dibromo-1,3-dimethylisochroman-8-ol ±127

In order to introduce an oxygen para to the methoxy on molecule **±125**, we needed to first brominate the isochroman followed by oxidation to the quinone. The synthetic strategy was to initially deprotect the aromatic methoxy substituent in compound **±125** in order to allow for the dibromination of phenol isochroman **±126** and in the following step a debromination to afford target compound **±127** (**Scheme 35**).

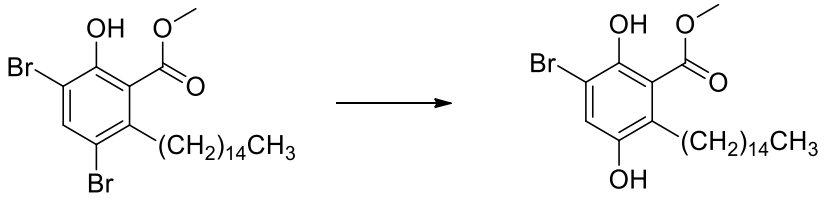


Scheme 35. Reagents and conditions: a) BBr_3, DCM, -78°C, 18h; (b) NBS, DMF, dark, rt, 18 h.

However, unfortunately the demethylation did not occur as we thought, even though we tried to vary the reaction conditions. Thus, at this point, we were happy enough to have the isochroman core synthesized from a bio renewable resource.

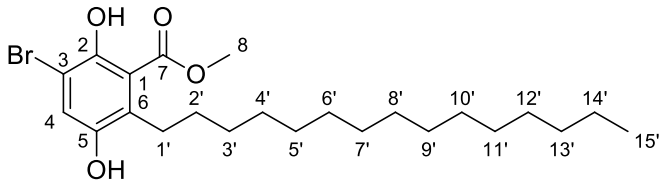
4.7 Preparation of methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate 140

At this stage of the synthesis, we now had time to revisit the synthesis of methyl 5-bromo-3,6-dioxo-2-pentadecylcyclohexa-1,4-dienecarboxylate **122**. As discussed before, the quinone **122** was unstable thus the synthesis could not continue. However, we thought we could reduce the quinone **122** to hydroquinone **140** which should be more stable. In addition, we thought that the of addition of THF in MeCN might solve the solubility problem we previously encountered (**Scheme 36**).



Scheme 36. Reagents and conditions: **1)** CAN, MeCN/THF/H_2O, 18 h; 2) NaO_4S_2, DMF/H_2O, 2 h.

Therefore, the dibromo benzoate **124** was treated with CAN in a mixture of THF/MeCN and stirred overnight. The crude dark orange oil was treated with sodium dithionite in DMF/H_2O without purification. The resulting product was obtained as a light yellow oil with an overall yield of 46% over two steps.



140

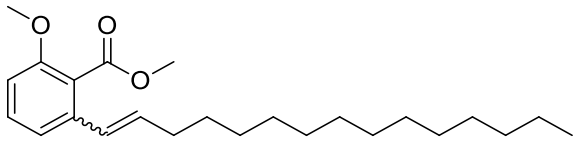
A highly deshielded signal at δ 10.65 ppm in the ^1H NMR spectrum integrated for 1 proton suggesting the presence of phenol which was hydrogen bonding to the ester carbonyl group. Another singlet signal at δ 4.93 ppm was consistent with the formation of a second phenol OH. The NMR spectroscopic results unambiguously confirmed that the methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate **140** was successfully synthesized with a yield of 46%. Further research needs to be

done on this synthetic route to allow for the production of quinone **±128**.

Thus, we have successfully demonstrated the transformation of anacardic acid **101** to the hydroquinone **140** over 4 steps with an overall yield of 31%. In the meantime, we were also able to synthesize the isochroman **±125** using anacardic acid as starting material over 9 steps with an overall yield of 13%.

4.8 Preparation of 1-(2-methoxy-6-(pentadec-1-en-1-yl)phenyl)ethanol ±141

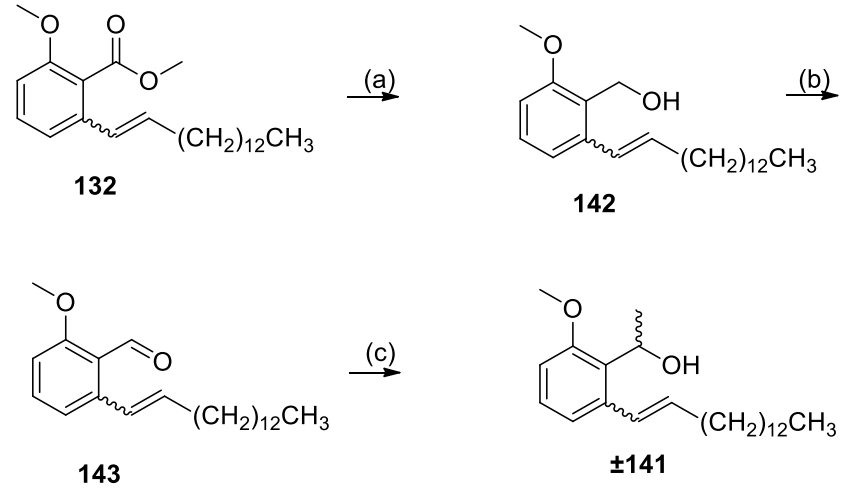
During the synthesis of *rac-tans*-8-methoxy-1,3-dimethylisochroman **±125**, we had not preserved the 15-carbon alkyl chain. This is considered not to be atomic economical as during the conversion of **132** to **129** via the aldehyde **137**, we introduced an extra carbon by means of the Wittig reaction. Hence, we also explored the synthesis of ischroman analogs with the aliphatic side chain attached.



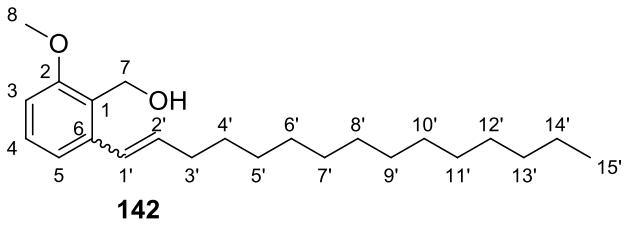
132

The substrate **132** could potentially also be used in a potassium *tert*-butoxide protocol to afford the pyran ring with a 13 carbon chain attached at C-3 of the pyran. The reduction of the methyl ester was done using standard protocols as described before (**Scheme 37**). The aromatic ester **132** was first reduced using LAH to form the primary alcohol **142**, which was then oxidized using PCC to yield aldehyde **143**. The aldehyde was finally treated with methylmagnesium iodide to afford the secondary alcohol as a racemic mixture

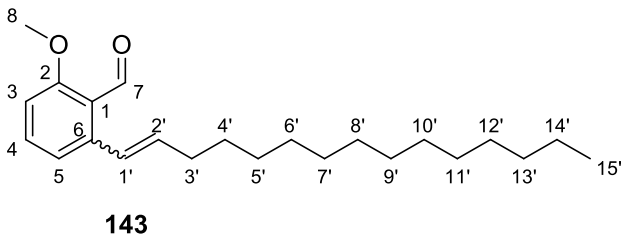
±141.



Scheme 37. Reagents and conditions: a) LAH, THF, 0 °C to rt, 5 h, 80%; b) PCC, DCM, rt, 5 h, 90%; c) Mg, MeI, diethyl ether/THF, rt, 18 h, 70%.



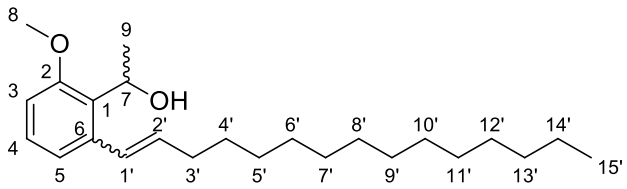
In detail, methyl 2-methoxy-6-(pentadec-1-en-1-yl)benzoate **132** was treated was treated with LAH in THF to afford white crystal with a melting point of 44-46°C. The ^{1}H NMR and ^{13}C NMR spectroscopy were used to determine the identity of the product. The appearance of a doublet signal at δ 4.79 ppm corresponded to the CH_2 of a primary alcohol. In the ^{13}C NMR spectrum of compound **132**, the carbonyl carbon signal at δ 168.93 ppm together with carbon signal of O-CH_3 at 52.1 ppm were no longer observed which indicated the reduction of the ester. Thus we concluded that the compound 2-methoxy-6-(pentadec-1-en-1-yl)phenyl methanol **142** was successfully synthesized with a yield of 80%.



The next synthetic step involved treatment of 2-methoxy-6-(pentadec-1-en-1-yl)phenyl methanol **142** with pyridinium chlorochromate

(PCC) in dry DCM. The resulting product **143** was a pale yellow oil produced in a yield of 90%. A singlet signal appeared at δ 10.60 ppm was found in the ^{1}H NMR spectrum corresponded to the formation of an aldehyde. The ^{13}C NMR NMR spectrum also agreed as a signal at δ 192.30 ppm was observed. In addition, the disappearance of doublet signal at δ 4.79 ppm corresponded to the oxidation of a primary alcohol. The NMR analysis confirmed that the resulting pale yellow oil was 2-methoxy-6-(pentadec-1-en-1-yl) benzaldehyde **143**.

The secondary alcohol containing compound **±141** was formed from the starting material 2-methoxy-6-(pentadec-1-en-1-yl)benzaldehyde **143** by again treating it with Grignard reagent made from magnesium and methyl iodide in dry diethyl ether. The resulting product **±141** was formed as a clear pale yellow oil in a yield of 70%.

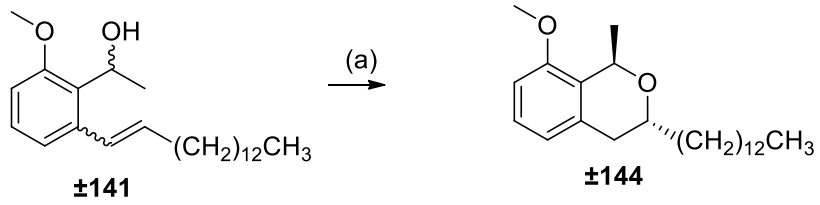


±141

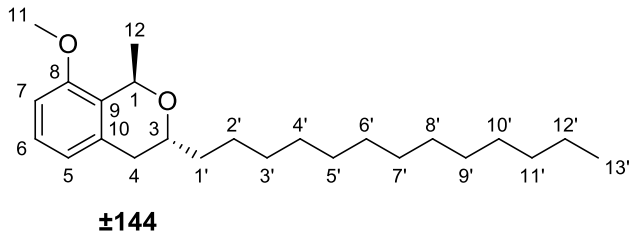
The IR spectrum clearly showed that a new O-H peak at 3340 cm^{-1}; furthermore, the carbonyl group (C=O) which appeared at 1575 cm^{-1} was now absent. In agreement with the IR spectrum, signals in the in ^{1}H NMR spectrum confirmed that the reaction was successful. The doublet signal at δ 1.53 ppm suggested that there was an additional methyl group that was introduced to the starting material. The ^{13}C NMR NMR spectrum supported this observation as the peak of carbonyl carbon at δ 192.30 ppm was no longer seen. In addition, a new signal was found at δ 22.71 ppm in the ^{13}C NMR NMR spectrum corresponded to the methyl at the newly formed secondary alcohol. Combining all the information obtained from NMR and IR spectroscopy, we concluded that the resulting pale yellow oil were *rac*-1-(2-methoxy-6-(pentadec-1-en-1-yl)phenyl)ethanol **±141**.

4.9 Preparation of *rac-tans*-8-methoxy-1-methyl-3-tridecylisochroman ±144

With the racemic alcohol **±141** made, the next step was to see if the *tert*-butoxide mediated pyran ring closure could also work on the scaffold with 15-carbon chain substituent. Using the same protocol as described before, the reaction was not successful even after 18 hours' stirring. Therefore, we resorted to use of microwaves as the new energy source. *Rac*-1-(2-methoxy-6-(prop-1-en-1-yl)phenyl)ethanol **±141** was dissolved in DMF and treated with potassium *tert*-butoxide. The mixture was irradiated with a microwave which led to a clear light yellow oil in a yield of 53%. The reaction is shown below in **Scheme 38**.



Scheme 38. Reagents and conditions: a) Potasium *tert*-butoxiode, DMF, 100 W, 120°C, 20 min, 53%.



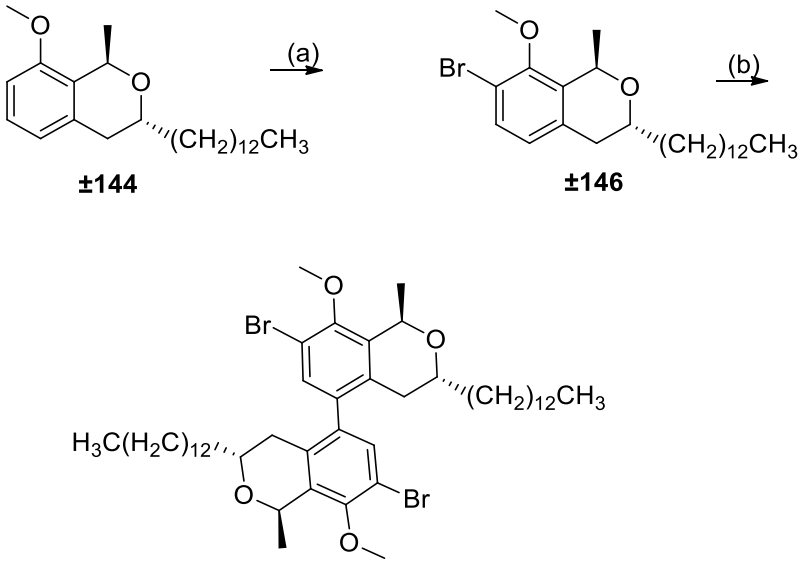
±144

^{1}H NMR and ^{13}C NMR spectroscopy were used to characterize the resulting product **±144**. The alkene signals of starting material **±141** showed one doublet signal at δ 6.63 ppm and a doublet of triplet signal at δ 5.97 ppm which were no longer observed suggesting the saturation of pentadec-1-en-1-yl substitution. Instead we observed one multiplet signal at δ 2.73-2.55 ppm which integrated for two hydrogens, which is characteristic signal for the pyran ring H-4 position [66]. Signals at δ 127.60 and 127.17 ppm

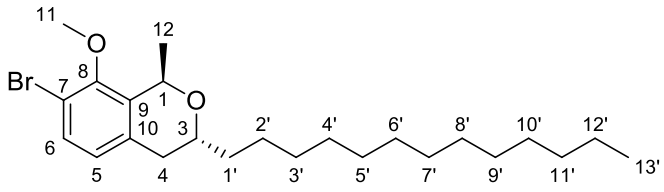
which corresponded to the alkene two carbons were no longer seen in the ^{13}C NMR spectrum, instead, new peaks were observed at δ 73.83, 68.69 and 36.44, 36.30 ppm which are consistent with the saturated sp^3 carbons. The stereochemistry was assigned according to precedent found in the literature [46]. The multiplet signal at δ 3.97-3.76 ppm was assigned to H_3, in the range of δ 3.9-4.3 ppm, suggesting the presence of *trans* isomer. Combining all the data obtained from NMR, we concluded that the resulting clear light yellow oil was *rac-trans*- 8-methoxy-1-methyl-2-tridecylisochroman **±144**.

4.10 Preparation of 8,8'-dimethoxy-1,1'-dimethyl-3,3'-ditridecyl-7,7'-biisochroman ±145

Having successfully synthesized the isochroman with the long aliphatic side chain, we decided to explore the expansion of the isochroman core to possibly assemble biaryl compound. We thus did the bromination on the methoxy isochroman **±144** resulted in the product which was characterized as *ortho* substituted bromide isochroman **±146**. Since there are quite a lot of natural dimerized isochromans that exhibit biological activities, we decide to see if we can dimerize our isochroman **±146**. Due to the fact that the *ortho* position was already substituted, the dimerization could occur exclusively at the *para* position (**Scheme 39**). Isochroman **±144** was first treated with NBS to form mono bromine substituted product **±146**, which was then subjected to iron(III) chloride mediated dimerization to yield compound **±145**.



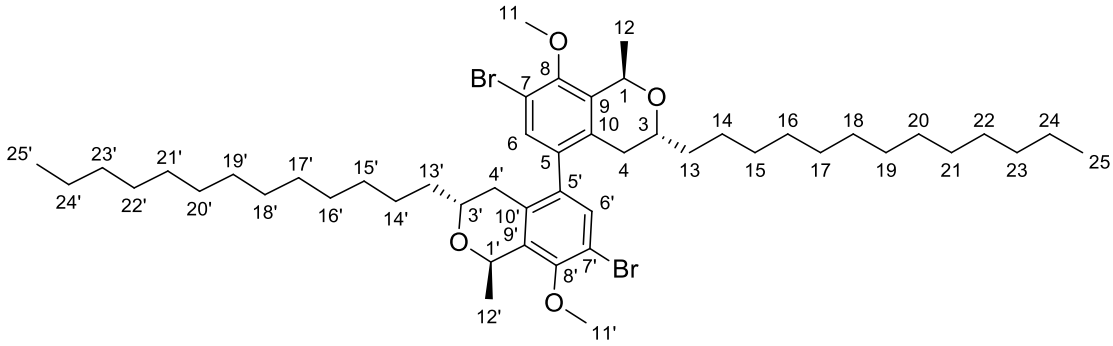
Scheme 39. Reagents and conditions: a) NBS, DMF, reflux, 72 h, 85%; b) $FeCl_3 \cdot 6H_2O$, toluene, 85°C, 24 h.



8-methoxy-1-methyl-2-tridecy lisochroman more specifically **±144** was treated with NBS in DMF and refluxed for 72 hours resulting in a pale-yellow solid with a melting point of 38-40°C. NMR spectroscopy was used to characterize the resulting product. Two *ortho* coupling doublet signals were observed at δ 7.37 and 6.59 ppm integrated for two hydrogens in the ^{1}H NMR spectrum suggesting the benzene ring was substituted during the reaction. Using NMR spectrum, we had enough evidence to conclude that the resulting pale-yellow solid was 7-bromo-8-methoxy-1-methyl-3-tridecylisochroman **±146**.

Excited that we were able to synthesize the mono brominated isochroman, the next step was the dimerization to achieve the final product **±145**. Bromonated compound **±146** was treated with $FeCl_3 \cdot 6H_2O$ in toluene and heated at 85°C

for 24 hours resulting in a pale yellow solid. However, the resulting product was so little that we only obtained useful information from the ^{1}H NMR spectrum.



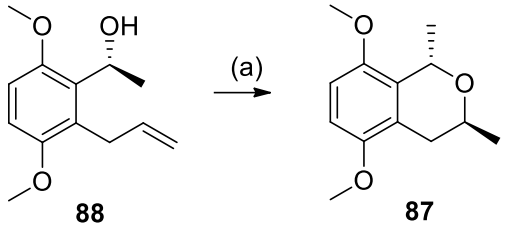
±145

As we expected, only a singlet signal integrated for two hydrogens appeared in the aromatic region at δ 7.51 ppm, suggested that the presence of two penta-substituted benzene ring. The methoxy signal at δ 3.88 ppm was integrated for 6 hydrogens also an indication of dimerization. As a matter of fact, all the signals were showing the double amount of integration. Therefore, we presumed that the dimerization was successful.

Chapter 5: Conclusion and Future work

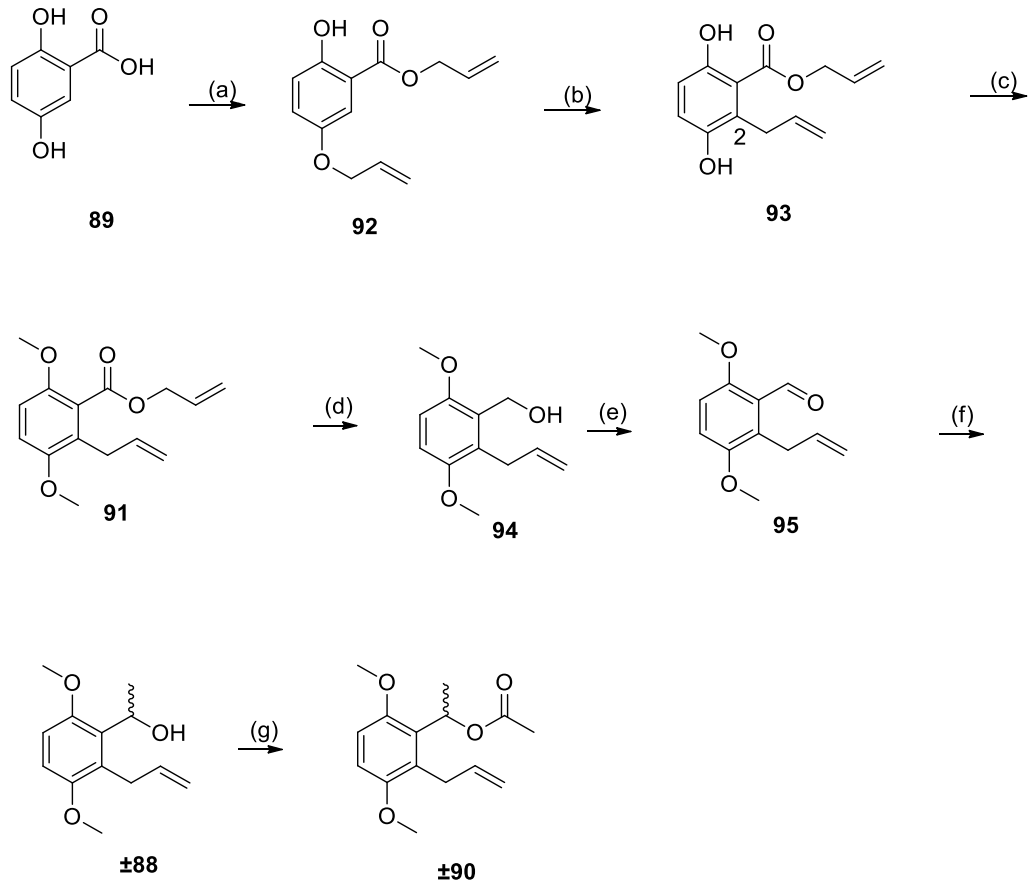
5.1 Summary of results achieved

The primary aim of this project was the synthesis of enantiopure isochroman (*S*)-*trans*-5,8-dimethoxy-1,3-dimethylisochroman **87** prepared from (*S*)-1-(2-allyl-3,6-dimethoxyphenyl) ethanol **88** using *tert*-butoxide in a yield of 87% (**Scheme 40**).



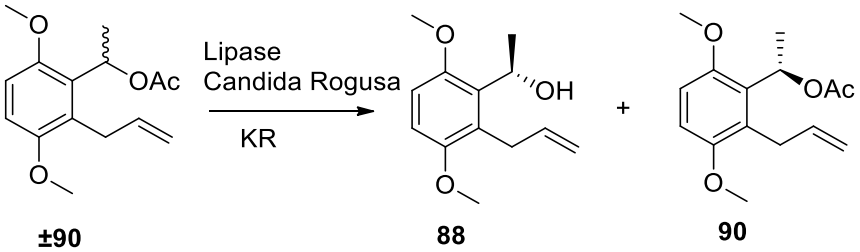
Scheme 40. Reagents and conditions: a) Potasium tert-butoxide, DMF, 80°C, 2 h, 87%.

The synthesis commenced with the preparation of the substrate for the enzymatic kinetic resolution *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90**. Allyl 5-(allyloxyl)-2-hydroxybenzoate **92** was prepared using commercially available 2,5-dihydroxybenzoic acid **89** as starting material; which was then subjected to Claisen rearrangement conditions by heating neat at 170°C to yield **93**. The synthesis of allyl 2-allyl-3,6-dimethoxybenzoate **91** was achieved by exposure of **93** to dimethyl sulfate. The dimethoxy compound **91** was reduced to form alcohol **94** using lithium aluminium hydride followed by a pyridinium chlorochromate oxidation yielding aldehyde **95**. The aldehyde **95** was then treated with freshly made methylmagnesium iodide to yield alcohol as the racemate **±88**, which was then acetylated to afford *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90** with an overall yield of 8% over 7 steps (**Scheme 41**).



Scheme 41. Reagents and conditions: a) K_2CO_3, allyl bromide, acetone, felux, 18 h, 74%; b) 170°C, 18 h, 90%; c) K_2CO_3, dimethyl sulfate, acetone, reflux, 24 h, 62%; d) LAH, THF, 0°C to rt, 18 h, 95%; e) PCC, silica, DCM, rt, 18 h, 78%; f) Mg, MeI, diethyl ether/THF, rt, 18 h, 56%; g) DMAP, acetic anhydride, triethylamine, THF, rt, 18 h, 45%.

The use of bio-catalyst lipase from *Candida rugosa* was used for the enzymatic kinetic resolution of *rac*-1-(2-allyl-3,6-dimethoxyphenyl)ethyl acetate **±90** which resulted in the synthesis of (*S*)-1-(2-allyl-3,6-dimethoxyphenyl) ethanol **88** (**Scheme 42**).

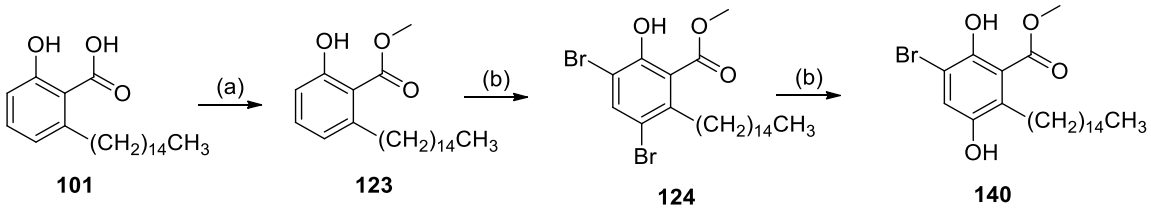


Scheme 42. Reagents and conditions: DMF, pH 7 buffer, 20°C.

As another major aim of this project, we decided to also examine the possible

use of bio-renewable chemicals, instead of petroleum derived chemicals that we had used to synthesize our isochroman. In this dissertation, we successfully synthesized hydroquinone methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate from anacardic acid (**Scheme 43**).

Started with the selective methylation of anacardic acid **101**, methyl 2-hydroxy-6-pentadecyl benzoate **123** was synthesized which was then subjected to NBS bromination to afford methyl 3,5-dibromo-2-hydroxy-6-pentadecyl benzoate **124**. A CAN oxidation followed by sodium dithionite reduction gave us methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate **140** in an overall yield of 31%.



101 **123** **124** **140**

Scheme 43. Reagents and conditions: a) Sodium bicarbonate, methyl iodide, DMF, 40°C, 5 h, 91%; b) NBS, DMF, dark, rt, 18 h, 75%; c) 1) CAN, MeCN/THF/H_2O, 18 h; 2) NaO_4S_2, DMF/H_2O, 2 h, 46%.

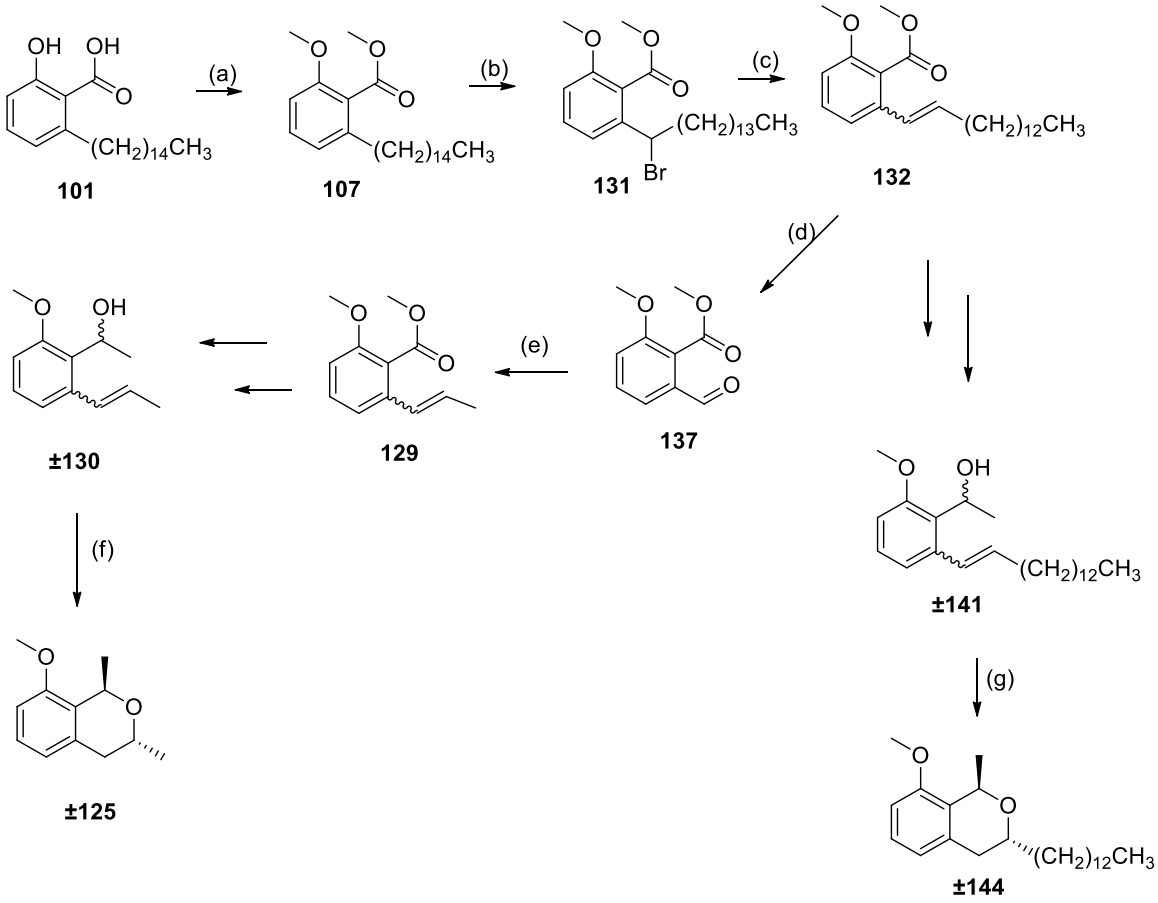
Anacardic acid was successfully transformed into two styrene containing compounds methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate **132** and methyl 2-methoxy-6-(prop-1-en-1-yl) benzoate **129**, which in turn led to the synthesis of two isochromans 8-methoxy-1,3-dimethylisochroman **±125** and 8-methoxy-1-methyl-3-tridecylisochroman **±144** (**Scheme 44**).

Anacardic acid was first methylated to form methyl 2-methoxy-6-pentadecylbenzoate **107**, which underwent benzylic bromination to afford methyl 2-(1-bromopentadecyl)-6-methoxybenzoate **131**. Methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate **132** was synthesized from elimination of **131** with an overall yield of 58% over 3 steps.

Using the same procedure detailed above, styrene **132** was transformed into

rac-1-(2-methoxy-6-(pentadec-1-en-1-yl)phenyl)ethanol **±141** over three steps and *tert*-butoxide ring closure resulted in the formation of 8-methoxy-1-methyl-3-tridecylisochroman **±144** in an overall yield of 16%.

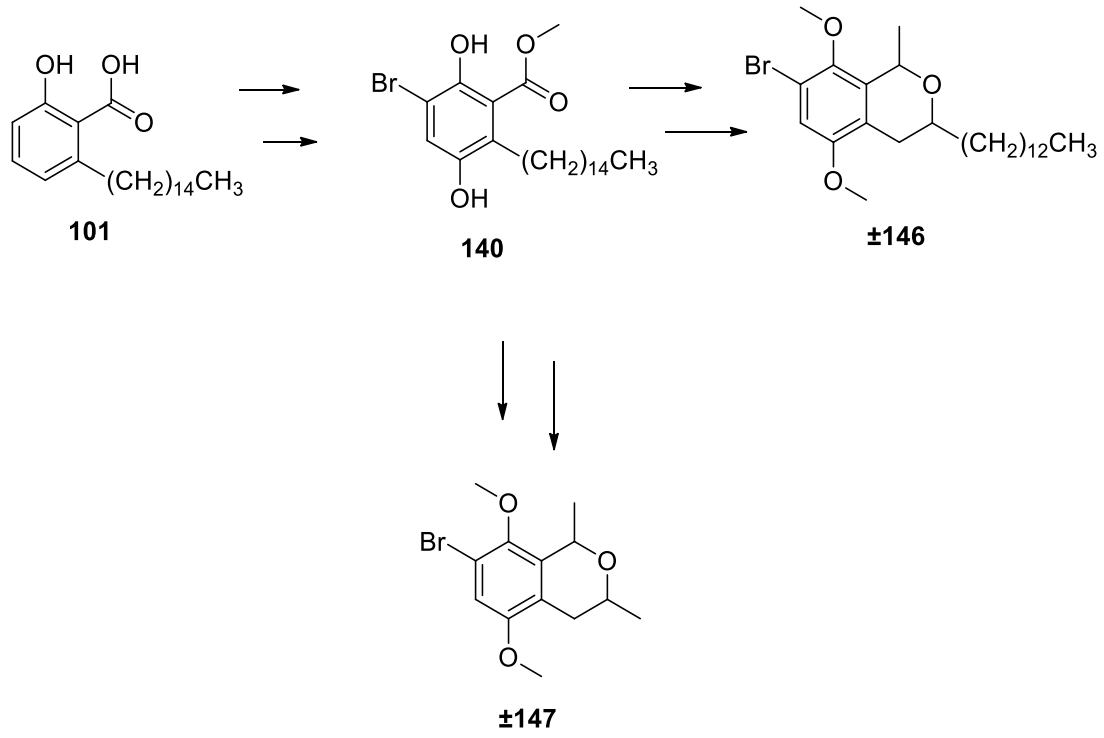
Styrene **129** was synthesized from **132** by ozone oxidative cleavage of the double bond followed by a Wittig reaction in two steps. Again, using the same procedure, 1-(2-methoxy-6-(prop-1-en-1-yl) phenyl) ethanol **±130** was prepared from styrene **129**. 8-methoxy-1,3-dimethylisochroman **±125** was synthesized with an overall yield of 13% over 9 steps from anacardic acid.



Scheme 44. Reagents and conditions: a) Potassium carbonate, dimethyl sulfate, acetone, reflux, 18 h, 96%; b) NBS, AIBN, CCl_4, reflux, 18 h, 76%; c) DBU, toluene, 95°C, 18 h, 80%; d) O_3, ethyl acetate/water, rt, 5 min, 60; e) NaH, $EtPPh_3Br$, rt to reflux, 4 h, 70%; f) Potasium tert-butoxiode, DMF, 80°C, 2 h, 90%; g) Potasium *tert*-butoxiode, DMF, 100 W, 120°C, 20 min, 53%.

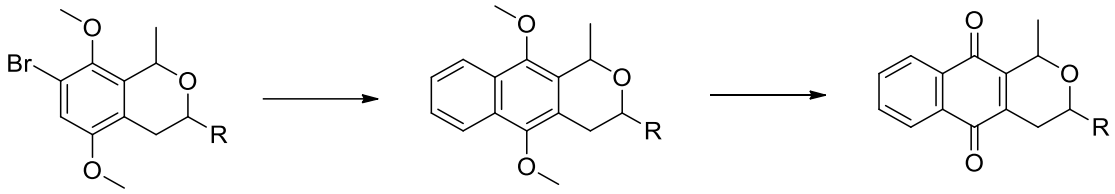
5.2 Future prospects for isochroman synthesis

The results from this research project demonstrate the use of bio-renewable source anacardic acid as the starting material for the synthesis of isochromans. The use of *tert*-butoxide as a key step of the ring closure for both allyl and styrene system showed its versatility. We were not able to synthesize 5,8-dimethoxy-1,3-dimethylisochroman **±87** from anacardic acid as we could not oxidatively insert the oxygen in the beginning of the synthesis, however, with the hydroquinone methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate **140** in hand, we believe it is now possible to achieve the synthesis of isochroman **±146** and **±147** using anacardic acid as the starting material (**Scheme 45**).



Scheme 45

With the bromine substitution of the ring, there would no difficulty to introduce another benzene ring which could eventually lead to the synthesis of pyrannonaphthaquinones **±148** (**Scheme 46**).



±146/±147 **±148**

Scheme 46

In conclusion, the aims of the project were achieved. The synthetic route developed using anacardic acid as starting material and *tert*-butoxide as a key reagent in the cyclisation step provides an alternative route to the synthesis of isochromans. This methodology could therefore be extended to the synthesis of naturally occurring pyrannonaphthaquinones

.

Chapter 6: Experimental

6.1 General procedure

Solvents such as hexane and ethyl acetate used for silica gal chromatography were distilled prior to use by conventional distillation procedure. Solvents such as dry THF and diethyl ether used for reaction purposes were first distilled and then dried using appropriate drying agents. Diethyl ether were distilled from sodium wire using benzophenone as the indicator. All reagents were obtained from commercial suppliers and used without further purification.

Normal silica (particle size 0.063-0.200 mm) was used for standard column chromatography.

TLC analysis was done using TLC on aluminium-backed Macherey-Nagel ALUGRAM Sil G/UV254 plates pre-coated with 0.25 mm silica gel 60. Crude products were checked by putting the TLC plate under UV light or by dipping plates with various TLC staining solutions.

^{1}H NMR spectra were recorded on a Bruker AVANCE 300 at 300 MHz.

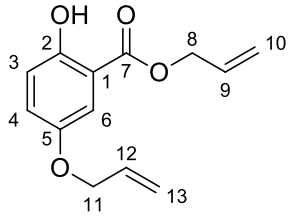
^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 at 125 MHz.

Infra-red spectra were recorded using a Bruker Tensor 27 Fourier Transform spectrometer equipped with a diamond ATR attachment. Measurements were made by loading the sample directly onto the diamond cell. All signals are reported on the wavenumber scale (v/cm^{-1}).

High pressure liquid chromatography (HPLC) was performed on a Dionex HPLC at a flow rate of 1.0 mL/min. A Chiralcel OJ 10µ 250 x 4.6 was used to monitor reactions using mobile phases consisting of isopropyl alcohol and hexane. Detection of the eluted analytes was achieved using a TSP variable wavelength UV detector at 215 nM. All calculations were based on peak area.

6.2 Preparation of allyl 5-(allyloxyl)-2-hydroxybenzoate 92

2,5-dihydroxybenzoic acid (5.99 g, 38.93 mmol, 1.0 equiv.) and potassium carbonate (11.84 g, 85.65 mmol, 2.2 equiv.) were transferred into a 1000 mL conical flask containing acetone (650 mL) equipped with reflux condenser. Allyl bromide (7.4 ml, 85.65 mmol, 2.2 equiv.) was added to the mixture. The reaction mixture was refluxed for 18 hours under nitrogen atmosphere, during which time it became a cream paste. The reaction was then cooled to room temperature, filtered through cotton wool and the acetone removed in vacuo. The residue was purified by silica gel column chromatography (10% Ethyl acetate/Hexane) to yield the diallylated product as white needle-like crystals (6.29 g, 74%).



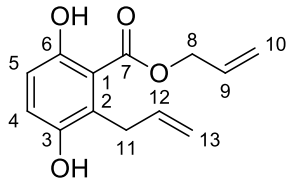
92

R_f = 0.843 (20% EtOAc/Hexane); **mp** 46-48°C, (*lit* 43.5–44.5°C); **IR**: ν_{max} (cm^{-1}): 3204.86 (OH), 1670.73 (C=O), 1650.20 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 10.35 (1H, s, OH), 7.35 (1H, d, *J* = 3.0 Hz,H_6), 7.10 (1H, dd, *J* = 9.0, 3.0 Hz, H_4), 6.91 (1H, d, *J* = 9.0 Hz, H_3), 6.14– 5.93 (2H, m, H_9 and H_{12} overlapped), 5.41 (2H, ddq, *J* = 17.2, 5.1, 1.6 Hz, H_{13}), 5.35 – 5.25 (2H, m, H_{10}), 4.84 (2H, dt, *J* = 6.0, 1.5 Hz, H_8), 4.49 (2H, dt, *J* = 6.0, 1.5 Hz, H_{11}); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 169.83 (C_7), 156.65 (C_2), 151.29 (C_5), 133.55(C_9), 131.92 (C_{12}), 125.01 (C_6), 119.26 (C_3), 118.84 (C_4), 118.11 (C_{10}), 113.87 (C_{13}), 112.27 (C_5), 70.01 (C_8), 66.20 (C_{11}).

6.3 Preparation of allyl 2-allyl-3,6-dihydroxybenzoate 93

In a 250 mL RB flask equipped with a reflux condenser, allyl 5-(allyloxy)-2-hydroxybenzoate (13.49 g, 57.59 mmol, 1 equiv.) was heated at 170°C over night, under nitrogen atmosphere. The white powder melted to first form a

yellow oil and then turned to a dark orange liquid. Upon completion of the reaction, the crude product was cooled to room temperature before purifying by silica gel column chromatography (10% Ethyl acetate/Hexane) to yield 2-allyl-3,6-dihydroxybenzoate as a viscous orange oil (12.20 g, 90%).



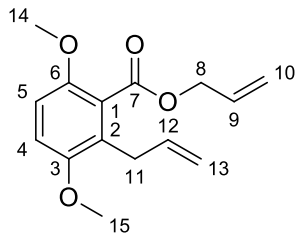
93

R_f = 0.436 (20% EtOAc/Hexane); **IR**: ν_{max} (cm^{-1}): 3409.40 (OH), 1661.04 (C=O), 1609.62 and 1459.27 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 10.42 (1H, s, OH-6), 6.97 (1H, d, *J* = 9.0 Hz, H_5), 6.79 (1H, d, *J* = 9.0 Hz, H_4), 6.00 (2H, dtt, *J* = 17.1, 9.7, 5.9 Hz, H_9,H_{12}), 5.46 – 5.29 (2H, m, H_{10}), 5.28 (1H, s, OH-3), 5.10 – 4.94 (2H, m, H_{13}), 4.85 (2H, dt, *J* = 6.0, 1.3 Hz, H_8), 3.73 (2H, dt, *J* = 5.9, 1.8 Hz, H_{11}); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.95 (C_7), 156.38 (C_1), 147.69 (C_5), 136.72 (C_{12}), 131.57 (C_9), 126.89 (C_6), 123.78 (C_4), 120.08 (C_3), 116.88 (C_{13}), 115.83 (C_{10}), 113.38 (C_2), 66.89 (C_8), 32.69 (C_{11}).

6.4 Preparation of allyl 2-allyl-3,6-dimethoxybenzoate 91

In a 250 mL RB flask equipped with a reflux condenser, potassium carbonate (17.99 g, 130.20 mmol, 2.5 equiv.) and allyl 2-allyl-3,6- dihydroxybenzoate (12.20 g, 52.08 mmol, 1 equiv.) were added to dry acetone (100 mL). The solution turned brown immediately. Dimethyl sulfate (12.8 mL, 17.08 g, 135.40 mmol, 2.6 equiv.) was then added and the resulting mixture was refluxed for 24 hours. The crude reaction mixture was filtered through cotton wool and the acetone removed *in vacuo*. Once the crude product was cooled, diethyl ether (100 mL) was added. The ether layer was washed with 25% aqueous ammonia solution (80 mL). The aqueous layer was thoroughly extracted with diethyl ether and dichloromethane. The organic extracts were combined and dried with anhydrous magnesium sulphate before filtering. The solvent was removed *in vacuo*. The residue was purified by silica gel column

chromatography (15-20% Ethyl acetate /Hexane) to afford 2-allyl-3,6-dimethoxybenzoate as an orange oil (8.41 g, 62%).

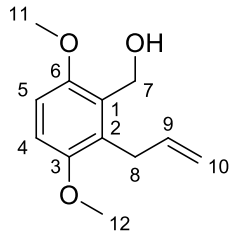


91

R_f = 0.686 (20% EtOAc/Hexane); **IR**: ν_{max} (cm^{-1}): 3080.04 (C-H), 1727.61 (C=O), 1638.30 and 1481.30 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 6.83 (1H, d, *J* = 9.0 Hz, H_5), 6.73 (1H, d, *J* = 9.0 Hz, H_6), 6.08 – 5.81 (2H, m, H_9,H_{12}), 5.46 – 5.23 (2H, m, H_{10}), 5.04 – 4.92 (2H, m, H_{13}), 4.81 (2H, dt, *J* = 5.8, 1.5 Hz, H_8), 3.76 (3H, s, H_{14}), 3.76 (3H, s, H_{15}), 3.35 (2H, dt, *J* = 6.4, 1.6 Hz, H_{11}); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 167.77 (C_7), 151.97 (C_1), 150.60 (C_5), 136.25 (C_{12}), 132.31 (C_9), 127.38 (C_6), 125.48 (C_4), 118.98 (C_3), 115.68 (C_{13}), 112.70 (C_{10}), 110.19 (C_2), 66.17 (C_8), 56.70 (C_{14}), 56.56 (C_{15}), 31.71 (C_{11}).

6.5 Preparation of (2-allyl-3,6-dimethoxyphenyl) methanol 94

In a 500 mL RB flask fitted with a reflux condenser, 2-allyl-3,6-dimethoxybenzoate (8.41 g, 32.08 mmol) was added to THF (400 mL). The solution was cooled in the ice bath until reach 0°C. Lithium aluminium hydride (4.90 g, 128.32 mmol, 4 equiv.) was added slowly. The reaction mixture was left stirring overnight. The mixture was cooled to 0°C and aqueous HCl solution (10%, 15 mL) was added slowly. Lithium aluminium hydride residue was filtered off using cotton wool. THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether (4 × 30 mL). The organic extracts were combined and dried with anhydrous magnesium sulphate before filtering through cotton wool. The solvent was then removed *in vacuo*. The residue was purified by silica gel column chromatography (20% Ethyl acetate/Hexane) to afford 2-allyl-3,6- dimethoxyphenyl as a light yellow oil (6.69 g, 95%).

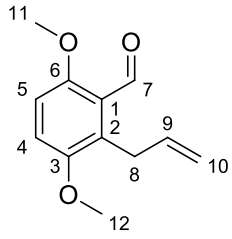


94

R_f = 0.237 (20% EtOAc/Hexane); **IR**: ν_{max} (cm^{-1}): 3404.50 (OH), 1637.07 and 1479.19(C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 6.79 (1H, d, *J*=9.0 Hz, H_5), 6.73 (1H, d, *J* = 9.0 Hz, H_4), 5.98 (1H, ddt, *J* = 17.1, 10.1, 5.8 Hz, H_9) , 5.04–4.81 (2H, m, H_{10}), 4.70 (2H, s, H_7), 3.80 (3H, s, H_{11}), 3.76 (3H, s, H_{12}), 3.54 (2H, dt, *J* = 5.9, 1.8 Hz, H_8), 2.57 (1H, s, OH); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 152.68 (C_9), 152.17 (C_{10}), 137.76 (C_2), 129.17 (C_3), 128.72 (C_6), 115.05 (C_1), 111.07 (C_5), 109.24 (C_4), 57.58 (C_{11}), 56.37 (C_{12}), 56.18 (C_7), 30.27 (C_8).

6.6 Preparation of 2-allyl-3,6-dimethoxybenzaldehyde 95

In a 500 mL RB flask, pyridium chlorochromate (PCC) (13.74 g, 63.74 mmol, 2.1 equiv.) and 2-allyl-3,6-dimethoxyphenyl (6.32 g, 30.35 mmol) were added together with normal silica (13.74 g) to dry DCM (350 mL). The reaction mixture was allowed to stir at room temperature for 18 hours under nitrogen. The mixture was filtered through celite and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (15–20% Ethyl acetate/Hexane) to afford 2-allyl-3,6-dimethoxybenzaldehyde as a clear yellow oil (1.90 g, 70%).

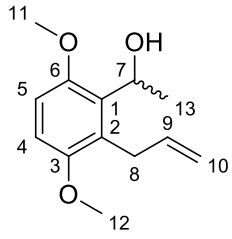


95

R_f = 0.453 (20% EtOAc/Hexane); **IR**: ν_{max}(cm^{-1}): 2939.94 (C-H), 1683.73 (C=O) ; **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 10.55 (1H, s, H_7), 7.05 (1H, d, *J* = 9.0 Hz, H_5), 6.81 (1H, d, *J* = 9.0 Hz, H_4), 5.95 (1H, ddt, *J* = 17.1, 10.1, 6.1 Hz, H_9), 5.02-4.89 (2H, m, H_{10}), 3.84 (3H, s, H_{11}), 3.79 (3H, s, H_{12}), 3.75 (2H, dt, *J* = 6.0, 1.5 Hz, H_8); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 192.91 (C_7), 157.26 (C_6), 152.12 (C_3), 137.05 (C_9), 131.84 (C_1), 124.36 (C_2), 117.62 (C_5), 115.12 (C_{10}), 110.13 (C_4), 56.96 (C_{11}), 56.49 (C_{12}), 29.73 (C_8).

6.7 Preparation of *rac*-1-(2-allyly-3,6-dimethoxyphenyl) ethanol ±88

Magnesium metal (0.97g, 40.19 mmol, 1.8 equiv.) was added to an oven dried 250 mL three-necked RB flask equipped with a condenser, a dropping funnel and a stopper under a nitrogen atmosphere. Dry diethyl ether (60 mL) followed by methyl iodide (4.75g, 2.8 mL, 33.50 mmol, 1.5 equiv.) was added to the flask. The reaction mixture became cloudy. Once the magnesium was consumed, a solution of 2-allyl-3,6- dimethoxybenzaldehyde (4.60g, 22.33 mmol, 1 equiv.) in dry THF (65 mL) was added dropwise to the freshly prepared Grignard reagent. The mixture was stirred at room temperature for 18 hours. Upon completion, the reaction was cooled using an ice-bath and water (20 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (2 × 50 mL) and the organic layer was washed with brine (100 mL). The organic extracts were combined, dried over anhydrous magnesium sulphate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (20% Ethyl acetate/Hexane) to afford *rac*-1-(2-allyly-3,6-dimethoxyphenyl) ethanol as a clear oil (2.78 g, 56%).



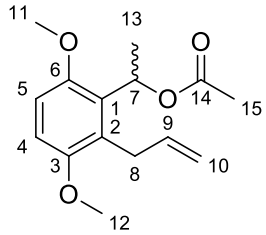
±88

R_f = 0.343 (20% EtOAc/Hexane); **IR**: ν_{max}(cm^{-1}): 3548.49 (OH), 2991.58 (C-H), 1637.45 and 1477.66(C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 6.77 (1H, d, *J* = 9.0 Hz, H_5), 6.73 (1H, d, *J* = 9.0 Hz, H_4), 5.92 (1H, ddt, *J* = 17.1, 10.1, 5.8 Hz, H_9), 5.12-4.78 (3H, m, H_{10} and H_7 overlapping), 4.04 (1H, d, *J* = 11.0 Hz, OH), 3.84 (3H, s, H_{11}), 3.76 (1H, s, H_{12}), 3.46 (2H, qdt, *J* = 15.6, 5.6, 1.8 Hz, H_8), 1.53 (3H, d, *J* = 6.7 Hz, H_{13}); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 152.29 (C_6), 152.18 (C_3), 136.93 (C_9), 133.05 (C_5), 126.48 (C_4), 115.29 (C_{10}), 109.84 (C_2),

109.78 (C_1), 67.64 (C_7), 56.54 (C_{12}), 55.90 (C_{11}), 30.13 (C_8), 24.03 (C_{13}).

6.8 Preparation of *rac*-1-(2-allyl-3,6-dimethoxyphenyl) ethyl acetate ±90

1-(2-allyl-3,6-dimethoxyphenyl) ethanol (2.78g, 12.48 mmol) and dry THF (60 mL) was added to a 200 mL two necked RB flask fitted with a rubber septum and a condenser under nitrogen. DMAP (0.0765 g, 0.624 mmol, 5 mol%), acetic anhydride (5.10 g, 4.8 mL, 49.92 mmol, 4 equiv.) and triethylamine (12.5 g, 17.8 mL, 124.8 mmol, 10 equiv.) were added to the flask. The reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was then quenched with water (50 mL) and the organic material extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, dried with anhydrous magnesium sulphate, filtered through cotton wool and the solvent removed *in vacuo*. The residue was purified by flash silica gel column chromatography (15% Ethyl acetate/Hexane) to afford *rac*-1-(2-allyl-3,6-dimethoxyphenyl) ethyl acetate as a clear oil (1.48 g, 45%).



±90

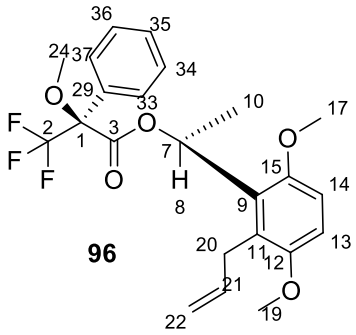
R_f = 0.486 (20% EtOAc/Hexane); **IR**: ν_{max}(cm^{-1}): 2937.91 and 2835.25 (C–H stretch), 1728.35 (C=O), 1596.55 and 1478.18 (C=C), 1238.94 (C–O); **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.77 (2H, d, *J* = 0.9 Hz, H_4,H_5), 6.41 (1H, q, *J* = 4.0 Hz, H_7), 5.98 (1H, ddt, *J* = 17.1, 10.1, 5.6 Hz, H_9), 6.02-4.83 (2H, m, H_{10}), 3.81 (3H, s, H_{11}), 3.76 (3H, s, H_{12}), 3.66 (2H, ddt, *J* = 7.6, 4.6, 1.8 Hz, H_8), 2.02 (3H, s, H_{15}), 1.58 (3H, d, *J* = 6.9 Hz, H_{13}); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.85 (C_{14}), 152.62 (C_6), 152.29 (C_3), 137.78 (C_9), 129.71 (C_5), 128.52 (C_4), 114.77 (C_{10}), 110.96 (C_2), 110.69 (C_1), 67.87 (C_{13}), 56.82 (C_{11}), 56.45 (C_{12}), 30.89 (C_8), 21.83 (C_7), 20.52 (C_{15}).

6.9 Preparation of enantiopure 1-(2-allyly-3,6-dimethoxyphenyl) ethanol 88

The enzyme selected to hydrolyze *rac*-1-(2-allyl-3,6-dimethoxyphenyl) ethyl acetate was initially screened by myself during honors project. In a 100ml two necked round bottom flask, 0.2 g (0.76 mmol, 1 equiv.) of substrate was dissolved in DMF (2.5 mL). Lipase *Candida Rougosa* (0.2g) dissolved in 47.5ml pH 6 phosphorus buffer was added to the flask. The reaction was allowed to stir at 30°C for 2 weeks. The reaction mixture was then quenched with water (20 mL) and the organic material extracted with ethyl acetate. The organic extracts were combined, dried with anhydrous magnesium sulphate, filtered through cotton wool and the solvent removed *in vacuo*. The residue was purified by flash silica gel column chromatography (15% Ethyl acetate/Hexane) to afford enantiopure 1-(2-allyly-3,6-dimethoxyphenyl) ethanol as a light yellow oil (0.076g, 45%).

6.10 Preparation of (*S*)-(*S*)-1-(2-allyl-3,6-dimethoxyphenyl) ethyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 96

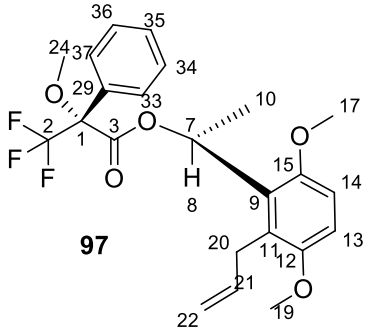
To a solution of enantiopure 1-(2-allyl-3,6-dimethoxyphenyl) ethanol (0.050g, 0.225 mmol, 1.0 equiv.) in DCM (15 mL), (S)-(-)-α-methoxy-α (trifluoromethyl) phenylacetic acid (S-Mosher) (0.106g, 0.45 mmol, 2 equiv.), N,N-dicyclohexylcarbodiimide (DCC) (0,102g, 0.45 mmol, 2 equiv.) and 4-dimethylamino pyridine (DMAP) (0.033g, 0.027 mmol, 0.12 equiv.) were added and stirred at room temperature overnight. The reaction mixture was filtered and concentrated. The crude material was purified by silica gel column chromatography (10% Ethyl acetate/Hexane) to afford (S)-(R)-1-(2-allyl-3,6-dimethoxyphenyl) ethyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate as a white solid (0.024g, 24%).



R_f = 0.886 (20% EtOAc /Hexane); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.49 – 7.29 (5H, m, $H_{33,34,35,36,37}$), 6.82 (1H, d, *J* = 9.0 Hz, H_{13}), 6.75 (1H, d, *J* = 8.9 Hz, H_{14}), 6.48 (1H, s, H_8), 5.97 – 5.85 (1H, m, H_{21}), 4.96 (1H, dq, *J* = 10.3, 1.8 Hz, H_{22}), 4.84 (1H, dt, *J* = 17.0, 1.9 Hz, $H_{22'}$), 3.77 (3H, s, H_{24}), 3.70 (3H, s, H_{19}), 3.68 – 3.55 (2H, m, H_{20}), 3.48 (3H, d, *J* = 1.2 Hz, H_{17}), 1.63 (3H, d, *J* = 6.8 Hz, H_{10}).

6.11 Preparation of (*R*)-(*S*)-1-(2-allyl-3,6-dimethoxyphenyl)ethyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 97

To a solution of enantiopure 1-(2-allyl-3,6-dimethoxyphenyl) ethanol (0.050g, 0.225 mmol, 1.0 equiv.) in DCM (15 mL), (R)-(-)-α-methoxy-α (trifluoromethyl) phenylacetic acid (R-Mosher) (0.106g, 0.45 mmol, 2 equiv.), N,N-dicyclohexylcarbodiimide (DCC) (0,102g, 0.45 mmol, 2 equiv) and 4-dimethylamino pyridine (DMAP) (0.033g, 0.027 mmol, 0.12 equiv) were added and stirred at room temperature overnight. The reaction mixture was filtered and concentrated. The crude material was purified by silica gel column chromatography (10% Ethyl acetate/Hexane) to afford (R)-(R)-1-(2-allyl-3,6-dimethoxyphenyl)ethyl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate as a white solid (quantative).

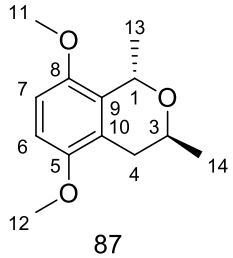


R_f = 0.886 (20% EtOAc /Hexane); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 7.46 – 7.27 (5H, m, $H_{33,34,35,36,37}$), 6.79 (1H, d, *J* = 9.0 Hz, H_{13}), 6.67 (1H, d, *J* = 9.0 Hz, H_{14}), 6.49 – 6.35 (1H, m, H_8), 6.00 – 5.80 (1H, m, H_{21}), 4.94 (1H, dq, *J* = 10.1, 1.8 Hz,

H_{22}), 4.82 (1H, dq, *J* = 17.2, 1.9 Hz, $H_{22'}$), 3.75 (3H, s, H_{24}), [3.74 – 3.58 and 3.49 – 3.45] (2H, m, H_{20}), 3.57 (3H, d, *J* = 1.2 Hz, H_{17}), 3.54 (3H, s, H_{19}), , 1.68 (3H, d, *J* = 6.8 Hz, H_{10}).

6.12 Preparation of (*S*)-5,8-dimethoxy-1,3-dimethylisochroman 87

In a 100ml two necked round bottom flask equipped with a condenser, (R)-1-(2-allyl-3,6-dimethoxyphenyl) ethanol (0.090g, 0.40 mmol, 1 equiv.) was dissolved in dry DMF (15ml). After stirring for 10 minutes, potassium *tert*-butoxide (0.50g, 4.50 mmol, 11 equiv.) was added into the reaction mixture. The reaction was heated to 80°C and stirred for 2 hours under nitrogen. Upon completion, the reaction was cooled using an ice-bath and water (50 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (3 × 50 mL) and the organic layer was washed with brine. The organic extracts were combined, dried with anhydrous magnesium sulfate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (10% Ethyl acetate/Hexane) to afford (1R)-5,8-dimethoxy-1,3-dimethylisochroman as a clear oil (0.077g, 87%).

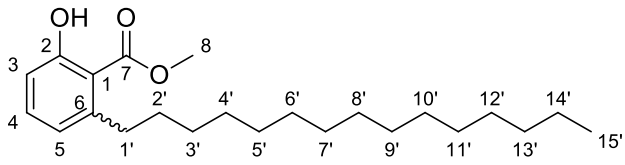


87

R_f = 0.436 (20% EtOAc/Hexane); **IR**: ν_{max}(cm^{-1}): 2970 (C-H), 1603 and 1463 (C=C), 1147 (C-O); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ: 6.76 – 6.59 (2H, m, H_6,H_7), 5.10 (1H, q, *J* = 6.6 Hz, H_1), 4.06 (1H, ddd, *J* = 11.1, 6.0, 3.6 Hz, H_3), 3.79 (3H, s, H_{12}), 3.77 (3H, s, H_{11}), 2.77 (1H, dd, *J* = 17.2, 3.5 Hz, H_4), 2.41 – 2.22 (1H, m, $H_{4'}$), 1.52 (3H, d, *J* = 6.6 Hz, H_{13}), 1.34 (3H, d, *J* = 6.1 Hz, H_{14}); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 150.85(C_8), 149.47(C_5), 129.16(C_9), 123.47(C_{10}), 107.49(C_7), 107.20(C_6), 68.29(C_1), 62.22(C_3), 55.58(C_{11}), 55.41(C_{12}), 30.38(C_4), 22.03(C_{13}), 19.56(C_{14}).

6.13 Preparation of methyl 2-hydroxy-6-pentadecyl benzoate 123

In a two necked 100 mL round bottom flask equipped with a reflux condenser, sodium bicarbonate (0.13 g, 1.52 mmol, 1.1 equiv.) and anacardic acid (2-hydroxy-6-pentadecylbenzoic acid) (0.49 g, 1.38 mmol, 1 equiv.) were added to dry DMF (5 ml). After stirring for 10 minutes, methyl iodide (0.12 mL, 1.80 mmol, 1.3 equiv.) was then added and the resulting mixture was heated to 40°C for 5 hours. Upon completion water (50 mL) was added to quench the reaction. The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10% ethyl acetate/Hexane) to yield methyl 2-hydroxy-6-pentadecyl benzoate as white solid (0.457 g, 91%).

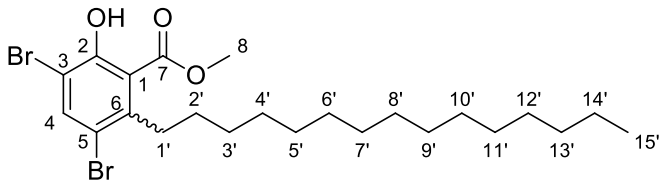


123

R_f = 0.914 (20% EtOAc /Hexane); **mp** 40-43°C, (*lit* 39–41°C); **IR**: $\nu_{max}(cm^{-1})$: 2923 (C-H), 1666 (C=O), 1609 and 1449 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 11.12 (1H, s, C_2-OH), 7.37 – 7.19 (1H, m, H_4), 6.83 (1H, dd, *J* = 8.4, 1.3 Hz, H_3), 6.72 (1H, dd, *J* = 7.5, 1.3 Hz, H_5), 3.95 (3H, s, H_8), 2.93 – 2.83 (2H, m, $H_{1'}$), 1.62 – 1.47 (2H, m, $H_{2'}$), 1.26 (24H, s, $H_{3'\text{-}14'}$), 0.88 (3H, t, *J* = 6.6 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 172.30(C_7), 162.92(C_1), 146.53(C_6), 134.51(C_4), 122.77(C_5), 115.92(C_3), 112.19(C_1), 52.45(C_8), 36.99($C_{1'}$), 32.48($C_{2'}$), 32.28($C_{13'}$), [30.25, 30.11, 30.05, 30.02, 29.99, 29.88, 29.72]($C_{3'\text{-}12'}$), 23.05($C_{14'}$), 14.46($C_{15'}$).

6.14 Preparation of methyl 3,5-dibromo-2-hydroxy-6-pentadecyl benzoate 124

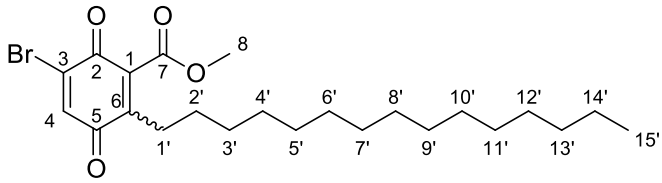
In a two necked 50 mL round bottom flask equipped with a stopper, starting material methyl 2-hydroxy-6-pentadecyl benzoate (0.10 g, 0.28 mmol, 1 equiv.) was dissolved in 5 ml DMF. NBS (0.10 g, 0.59 mmol, 2.1 equiv.) was dissolved in 1 mL DMF and added to the flask dropwise. The reaction was stirred in the dark under a nitrogen atmosphere for 18 hours at room temperature. Water (30 mL) was added to quench the reaction. The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10% ethyl acetate/Hexane) to yield methyl 3,5-dibromo-2-hydroxy-6-pentadecylbenzoate as clear yellow oil (0.109 g, 75%).



124

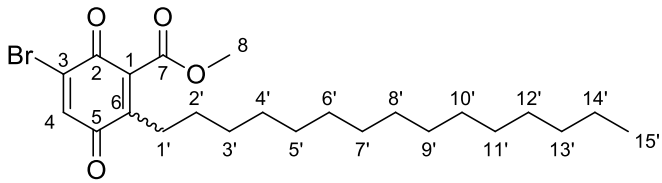
R_f = 0.829 (20% EtOAc /Hexane); **mp** 45-47°C; **IR**: $\nu_{max}(cm^{-1})$: 2923 (C-H), 1666 (C=O), 1465 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 10.99 (1H, s, C_2-OH), 7.87 (1H, s, H_4), 4.00 (3H, s, H_8), 3.02 – 2.86 (2H, m, $H_{1'}$), 1.62 – 1.49 (3H, m, $H_{2'}$), 1.26 (24H, d, *J* = 2.3 Hz, $H_{3'-14'}$), 0.88 (3H, t, *J* = 6.5 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 170.84(C_7), 157.35(C_2), 143.71(C_6), 140.82(C_4), 116.16(C_3), 115.92(C_5), 109.88(C_1), 53.36(C_8), 35.77($C_{13'}$), 32.28($C_{1'}$), [30.31, 30.04, 30.01, 29.96, 29.71, 29.66]($C_{2'-12'}$), 23.04($C_{14'}$), 14.47($C_{15'}$).

6.15 Preparation of methyl 3-bromo-2,5-dioxo-6-pentadecylcyclohexa-1,4-dienecarboxylate 122



122

Sliver oxide (0.14 g, 1.16 mmol, 2 equiv.) was added to the stirring solution of methyl 3,5-dibromo-2-hydroxy-6-pentadecylbenzoate (0.3g, 0,58 mmol, 1 equiv.) in 1,4-dioxane (5 mL). The reaction mixture was stirred for 10 minutes at room temperature after which 55% aqueous nitric acid was added drop-wise until all sliver oxide dissolved. The resultant solution was stirred for a further 5 minutes which was followed by addition of dichloromethane (20ml) and water (20ml). The aqueous phase was extracted with dichloromethane (3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by a short silica gel column chromatography (10% ethyl acetate/Hexane) to yield methyl 3-bromo-2,5-dioxo-6-pentadecylcyclohexa-1,4-dienecarboxylate as dark orange oil.

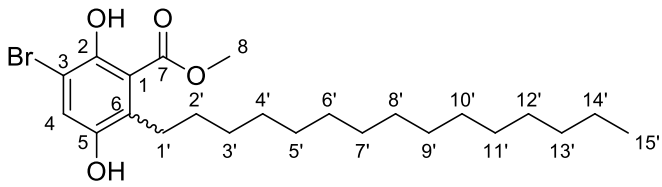


122

R_f = 0.921 (20% EtOAc /Hexane); **^{1}H NMR** ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.31 (1H, s, H_4), 3.92 (3H, s, H_8), 2.45 – 2.35 (2H, m, $H_{1'}$), 1.57 (4H, m, $H_{2',3'}$), 1.25 (22H, s, $H_{4'\text{-}14'}$), 0.88 (3H, t, *J* = 6.5 Hz, $H_{15'}$).

6.16 Preparation of methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate 140

In a two necked 50 mL round bottom flask equipped with a stopper, starting material methyl 3,5-dibromo-2-hydroxy-6-pentadecylbenzoate (0.05 g, 0.19 mmol, 1 equiv.) was dissolved in 1 mL THF and 15 mL MeCN. CAN (0.14 g, 0.25 mmol, 2.6 equiv.) was dissolved in 1 mL H_2O and added to the flask dropwise. The reaction was stirred under a nitrogen atmosphere for 18 hours at room temperature. Water (15 mL) was added to quench the reaction. The aqueous phase was extracted with ethyl acetate (3 × 10 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The solvent was removed *in vacuo*. The dark orange oil was subjected to sodium dithionite reduction immediately by dissolving the resulting crude oil (0.05 g, 0.11 mmol, 1 equiv.) in 5 mL DMF in a 25 mL round bottom flask. Sodium dithionite (0.02 g, 0.11 mmol, 1 equiv.) was dissolved in 1 mL H_2O and added to the flask. The reaction mixture was allowed to stirred at room temperature for 2 hours under a nitrogen atmosphere. Water (30 mL) was added to quench the reaction. The aqueous phase was extracted with ethyl acetate (3 × 10 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The solvent was removed *in vacuo*. The residue was purified by silica gel column chromatography (20% ethyl acetate/Hexane) to yield methyl 3-bromo-2,5-dihydroxy-6-pentadecylbenzoate as light yellow oil (46% over two steps).



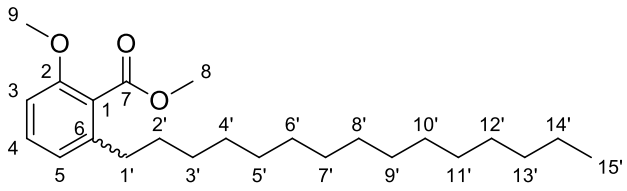
140

R_f = 0.436 (20% EtOAc /Hexane); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 10.65 (1H, s, OH_2), 7.30 (1H, s, H_4), 4.93

(1H, s, OH_5), 4.01 (3H, s, H_8), 2.93 – 2.81 (2H, m, $H_{1'}$), 1.70 – 1.46 (4H, m, $H_{2'}$ and $H_{3'}$), 1.29 (22H, s, $H_{4'\text{-}14'}$), 0.96 – 0.87 (3H, m, $H_{15'}$).

6.17 Preparation of methyl(2-methoxy-6-pentadecyl) benzoate 107

In a two necked 250 mL round bottom flask equipped with a reflux condenser, potassium carbonate (0.99 g, 7.18 mmol, 2.5 equiv.) and anacardic acid (2-hydroxy-6-pentadecylbenzoic acid) (1.04 g, 2.87 mmol, 1 equiv.) were added to dry acetone (100 mL). After stirring for 10 minutes, dimethyl sulfate (0.71 ml, 7.46 mmol, 2.6 equiv.) was then added and the resulting mixture was stirred under flux refluxed for 18 hours. The crude reaction mixture was filtered through cotton wool and the acetone removed *in vacuo*. Once the crude product was cooled, diethyl ether (100 mL) was added. The ether layer was washed with 25% aqueous ammonia solution (80 mL). The aqueous layer was thoroughly extracted with dichloromethane (3 × 30 mL). The organic extracts were combined and dried over anhydrous magnesium sulfate before filtering. The residue was purified by silica gel column chromatography (15-20% ethyl acetate/Hexane) to afford methyl(2-methoxy-6-pentadecyl) benzoate as transparent crystal (1.04 g, 96%).



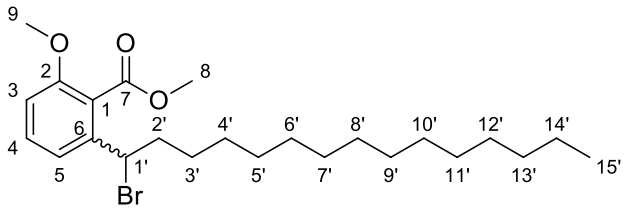
107

R_f = 0.857 (20% EtOAc /Hexane); **mp**: 35-57°C, (*lit* 36-37°C); **IR**: $\nu_{max}(cm^{-1})$: 2916 (C-H), 1730 (C=O), 1583 and 1465 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 7.26 (1H, t, *J* = 8.0 Hz, H_4), 6.81 (1H, d, *J* = 7.7 Hz, H_5), 6.75 (1H, d, *J* = 8.3 Hz, H_3), 3.90 (3H, s, H_8), 3.81 (3H, s, H_9), 2.53 (2H, dd, *J* = 9.2, 6.6 Hz, $H_{1'}$), 1.57 (2H, t, *J* = 7.6 Hz, $H_{2'}$), 1.26 (24H, d, *J* = 6.6 Hz, $H_{3'\text{-}14'}$ 0.88 (3H, t, *J* = 6.4 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz,

$CDCl_3$): δ 168.93(C_7), 156.25(C_2), 141.39(C_6), 130.22(C_4), 123.49(C_1), 121.49(C_5), 108.34(C_3), 55.85(C_9), 52.10(C_8), 33.50($C_{1'}$), 31.95($C_{2'}$), 31.18($C_{13'}$), [29.72, 29.69, 29.61, 29.58, 29.53, 29.48, 29.44, 29.39, 29.18]($C_{3'\text{-}12'}$), 22.71($C_{14'}$), 14.13($C_{15'}$).

6.18 Preparation of methyl 6-(1-bromopentadecyl)-2-methoxybenzoate 131

Methyl(2-methoxy-6-pentadecyl) benzoate (5.00 g, 13.28 mmol, 1.0 equiv.), NBS (3.07 g,17.26 mmol, 1.3 equiv.) and AIBN (0.22 g,1.33 mmol, 0.1 equiv.) were transferred into a 250 mL two necked round bottom flask equipped with reflux condenser containing carbon tetrachloride (70 mL). The reaction mixture was refluxed for 18 hours under a nitrogen atmosphere. The reaction was then cooled to room temperature and quenched with saturated aqueous sodium bicarbonate (20 mL). The aqueous layer was thoroughly extracted with dichloromethane (3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10% ethyl acetate/Hexane) to yield the methyl 6-(1-bromopentadecyl)-2-methoxybenzoate as pale-yellow oil (4.59 g, 76%).



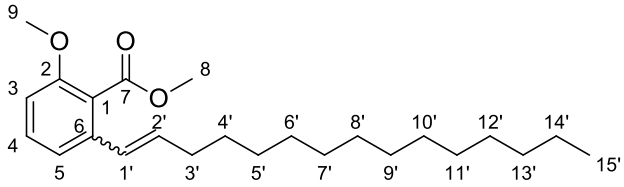
131

R_f = 0.829 (20% EtOAc /Hexane); **mp**: 56-59°C, (*lit* 57-58°C); **IR**: $\nu_{max}(cm^{-1})$: 2913 (C-H), 1764 (C=O), 1603 and 1469 (C=C), 776 (C-Br); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 7.37 (1H, t, *J* = 8.1 Hz, H_4), 7.21 (1H, d, *J* = 7.9 Hz, H_3), 6.83 (1H, d, *J* = 8.3 Hz, H_5), 4.92 (1H, t, *J* = 7.4 Hz, $H_{1'}$), 3.93 (3H, s, H_8), 3.81 (3H, s, H_9), 2.39 – 2.05 (2H, m, $H_{2'}$), 1.58 – 1.38 (2H, m, $H_{3'}$), 1.25 (22H, d, *J* = 3.1 Hz, $H_{4'\text{-}14'}$), 0.88 (3H, t, *J* = 6.5 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz,

$CDCl_3$): δ 167.75(C_7), 155.94(C_2), 140.61(C_6), 130.98(C_4), 122.48(C_3), 119.68(C_5), 110.46(C_1), 56.02(C_9), 52.43($C_{1'}$), 50.63(C_8), 39.95($C_{2'}$), [31.93, 29.69, 29.66, 29.61, 29.54, 29.37, 28.85, 28.00])($C_{3'\text{-}13'}$), 22.70($C_{14'}$), 14.13($C_{15'}$).

6.19 Preparation of methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate 132

Methyl 2-(1-bromopentadecyl)-6-methoxy benzoate (5.95 g, 13.06 mmol, 1.0 equiv.) was dissolved in toluene (150 mL) in a 500 mL two necked round bottom flask equipped with reflux condenser. While stirring, DBU (2,9 mL ,19.60 mmol,1.5 equiv.) was added to the flask. The reaction mixture was heated up to 95°C for 18 hours under a nitrogen atmosphere. The reaction was then cooled to room temperature and quenched with 10% aqueous HCl solution. The aqueous phase was extracted with ethyl acetate (3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10% ethyl acetate/Hexane) to yield the methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate as pale-yellow oil (3.91 g, 80%).



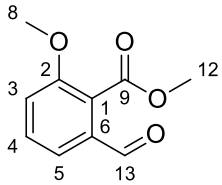
132

R_f = 0.811 (20% EtOAc /Hexane); **mp**: 35-36°C, (*lit* 33-34°C);**IR**: ν_{max}(cm^{-1}): 2922 (OH), 1733 (C=O), 1599 and 1468 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 7.26 (1H, t, *J* = 8.1 Hz, H_4), 7.09 (1H, d, *J* = 7.9 Hz, H_5), 6.76 (1H, d, *J* = 8.2 Hz, H_3), 6.35 – 6.11 (2H, m, $H_{1'}$,$H_{2'}$), 3.91 (3H, s, H_8), 3.80 (3H, s, H_9), 2.17 (2H, q, *J* = 6.9 Hz, $H_{3'}$), 1.43 (2H, t, *J* = 7.1 Hz, $H_{4'}$), 1.27 (20H, s, $H_{7'\text{-}14'}$), 0.88 (3H, t, *J* = 6.5 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 168.71(C_7), 156.37(C_2), 136.68(C_6), 134.75($C_{2'}$),

130.22(C_4), 126.07($C_{1'}$), 122.20(C_1), 117.75(C_5), 109.08(C_3), 55.92(C_9), 52.24(C_8), 33.23($C_{3'}$), 31.95($C_{13'}$), [29.86, 29.72, 29.68, 29.65, 29.61, 29.54, 29.39, 29.25, 29.21, 29.17]($C_{4'-12'}$), 22.71($C_{14'}$), 14.13($C_{15'}$).

6.20 Preparation of methyl 6-formyl-2-methoxybenzoate 137

In a long necked 100 mL round bottom flask, methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate (0.50 g, 1.33 mmol, 1 equiv.) and water (0.024 mL, 1.33 mmol, 1 equiv.) were added to ethyl acetate (20 mL). The reaction mixture was cool to 0°C before pass a flow of O_3/O_2. After 5 minutes, the reaction mixture was diluted with ethyl acetate (10 mL) and dried over anhydrous magnesium sulfate. The residue was purified by silica gel column chromatography (20-30% ethyl acetate/Hexane) to yield the methyl 6-formyl-2-methoxybenzoate as white powder (0.16 g, 60%).



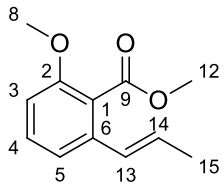
137

R_f = 0.143 (20% EtOAc /Hexane); **mp**: 58-61°C; **IR**: ν_{max}(cm^{-1}): 1734 and 1696 (C=O), 1605 and 1470 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ 9.96 (1H, s, H_{13}), 7.62 – 7.51 (1H, t, H_4), 7.46 (1H, dd, *J* = 7.6, 1.1 Hz, H_5), 7.21 (1H, dd, *J* = 8.3, 1.1 Hz, H_3), 3.97 (3H, s, H_{12}), 3.89 (3H, s, H_8). **^{13}C NMR** (75 MHz, $CDCl_3$) δ: 190.32(C_{13}), 167.22(C_9), 156.70(C_2), 134.29(C_6), 131.06(C_4), 123.58(C_1), 123.15(C_5), 116.97(C_3), 56.36(C_8), 52.84(C_{12}).

6.21 Preparation of methyl 2-methoxy-6-(prop-1-en-1-yl) benzoate 129

To a mixture of acetonitrile (30 mL) and tetrahydrofuran (150 mL) was added sodium hydride (60%, 0.89 g, 37.08 mmol, 2.4 equiv.) at room temperature. After stirring at 50°C for 1 hour, the reaction mixture was cooled to room temperature. To this cooled mixture was added ethyl triphenyl phosphonium bromide (7.05 g, 18.54 mmol, 1.2 equiv.) and the mixture was stirred at room

temperature for 1 hour. Upon completion, a solution of methyl 2-formyl-6-methoxybenzoate (2.95 g, 15.45 mmol, 1 equiv.) in 10ml THF was added dropwise. The reaction was then heated under reflux for 4 hours. The reaction was quenched water (50 mL), followed by an addition of 10% aqueous HCl (5 mL). The aqueous phase was extracted with ethyl acetate (3 × 40 mL). The organic extracts were combined and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10-15% ethyl acetate/Hexane) to yield the methyl 2-methoxy-6-(prop-1-en-1-yl) benzoate as yellow oil (2.23 g, 70%).



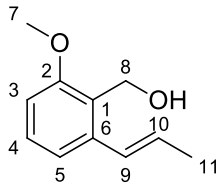
129

R_f = 0.686 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 3011 (C-H), 1728 (C=O), 1596 and 1470 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*): δ [7.34 – 7.27 (m), 7.25 – 7.15 (m), 7.09 (d, *J* = 7.7 Hz), 6.92 – 6.73 (m)](3H, H_3,H_4,H_5), [6.43 – 6.14 (m), 5.96 – 5.75 (m)](2H, H_{13},H_{14}), 3.90 (3H, d, *J* = 14.3 Hz, H_{12}), 3.80 (3H, d, *J* = 5.3 Hz, H_8), 1.90 – 1.71 (3H, m, H_{15}). ^{13}C NMR (75 MHz, $CDCl_3$): δ [168.99, 168.74](C_9), [156.35, 156.00](C_2), [136.58, 136.39](C_6), [130.32, 130.03](C_{14}), [129.49, 129.37](C_{13}), [127.27, 126.64](C_4), [121.63, 120.27](C_5), [117.66, 115.32](C_3), [109.34, 109.12](C_1), [55.92, 55.89](C_8), [52.39, 52.30](C_{12}) [18.75, 14.54](C_{15}).

6.22 Preparation of {2-methoxy-(6-prop-1-en-1-yl)phenyl} methanol 138

In a 500 mL RB flask fitted with a reflux condenser, methyl 2-methoxy-6-(prop-1-en-1-yl) benzoate (0.27 g, 1.31 mmol, 1 equiv.) was added to THF (20 mL). The solution was cooled to until 0°C in an ice bath. Lithium aluminium hydride (0.21 g, 5.24 mmol, 4 equiv.) was added slowly. The resulting reaction mixture was allowed to warm up to room temperature and stirred for 5 hours. The mixture was again cooled to 0°C and aqueous HCl solution (10%, 15 mL) was added slowly. Lithium aluminium hydride residue

was filtered off using cotton wool and THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether (4 × 30ml). The organic extracts were combined and dried with anhydrous magnesium sulfate before filtering through cotton wool. The solvent was then removed *in vacuo*. The residue was purified by silica gel column chromatography (15-20% Ethyl acetate/Hexane) to afford {2-methoxy-(6-prop-1-en-1-yl)phenyl} methanol as a white crystal (0.20 g, 86%).



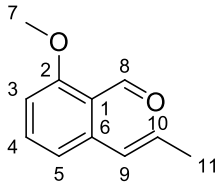
138

R_f = 0.286 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 3411 (OH), 1597 and 1470 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ: 7.26 – 7.17 (1H, m, H_4), [7.03, 6.86 – 6.75] (2H, m, H_3,H_5), 6.75 – 6.52 (1H, m, H_9), 6.18 – 5.79 (1H, m, H_{10}), 4.72 (2H, d, *J* = 22.6 Hz, H_8), 3.83 (3H, d, *J* = 8.0 Hz, H_7), 2.46 (1H, d, *J* = 40.5 Hz, C_8-OH), 1.78 (3H, ddd, *J* = 64.8, 6.8, 1.8 Hz, H_{11}). ^{13}C NMR (75 MHz, $CDCl_3$) δ [158.07, 157.98](C_2), [138.84, 137.77](C_6), [129.21, 128.48](C_9), [128.70, 128.19](C_4), [128.07, 127.90](C_{12}), [126.91, 125.68](C_1), [57.95, 56.69](C_8), [55.55, 55.46](C_7), [18.79, 14.34](C_{11}).

6.23 Preparation of 2-methoxy-6-(prop-1-en-1-yl) benzaldehyde 139

In a 500 mL round bottom flask, pyridium chlorochromate (PCC) (0.38 g, 1.76 mmol, 2.1 equiv.) and (2-methoxy-6-(prop-1-en-1-yl)phenyl)methanol (0.15 g, 0.84 mmol) were added together with normal silica (0.38 g) to dry DCM (40 mL). The reaction mixture was stirred at room temperature for 5 hours under a nitrogen atmosphere. The mixture was filtered through celite and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (15–20% Ethyl acetate /Hexane) to afford 2-methoxy-6-(prop-1-en-1-yl) benzaldehyde as a pale yellow solid (0.14g,

93%).



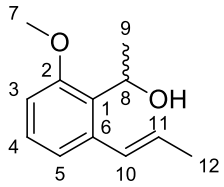
139

R_f = 0.629 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 1763 (C=O), 1593 and 1471 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ: 10.58 (1H, d, *J* = 16.6 Hz, H_8), 7.46 (1H, dd, *J* = 8.4, 7.7 Hz, H_4), [7.09 (d, *J* = 7.9 Hz, 0H), 6.89 (dd, *J* = 8.1, 3.9 Hz, 2H)](2H, H_3,H_5), 6.86 – 6.78 (1H, m, H_9), [6.20 (d, *J* = 6.7 Hz, 0H), 5.96 – 5.81 (m, 1H)](1H, H_{10}), 3.91 (3H, d, *J* = 7.4 Hz, H_7), 1.82 (3H, ddd, *J* = 57.8, 6.9, 1.8 Hz, H_{11}). ^{13}C NMR (75 MHz, $CDCl_3$) δ: [192.38, 191.71](C_8), [162.35, 161.74](C_2), 140.52(C_6), [134.38, 134.11](C_9), [130.22, 128.94](C_{10}), [129.29, 127.50](C_4), [123.11, 122.84](C_5), 119.61(C_1), [109.95, 109.49](C_3), 55.86(C_7), [18.82, 14.49](C_{11}).

6.24 Preparation of *rac*-1-{2-methoxy-6-(prop-1-en-1-yl) phenyl}ethanol ±130

Magnesium metal (0,03 g, 1.38 mmol, 1.8 equiv.) was added to an oven dried 100 mL three-necked round bottom flask equipped with a condenser, a dropping funnel and a stopper under a nitrogen atmosphere. Dry diethyl ether (30 mL) followed by methyl iodide (0.07 mL,1.16 mmol, 1.5 equiv.) was added to the flask. The reaction mixture became cloudy. Once the magnesium was consumed, a solution of 2-methoxy-6-(prop-1-en-1-yl) benzaldehyde (0.13 g, 0.77 mmol, 1 equiv.) in dry THF (10ml) was added dropwise to the freshly prepared Grignard reagent. The mixture was stirred at room temperature for 18 hours. Upon completion, the reaction was cooled using an ice-bath and water (10 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (3 × 30 mL) and the organic layer was washed with brine. The organic extracts were combined, dried over anhydrous magnesium sulfate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (20% Ethyl acetate/Hexane) to afford

rac-1-{2-methoxy-6-(prop-1-en-1-yl)phenyl}ethanol as a clear pale yellow oil (0.11g, 74%).



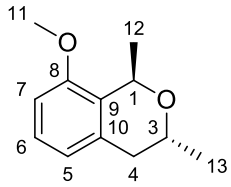
±130

R_f = 0.486 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 3438 (OH), 1597 and 1470 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ: 7.16 (1H, td, *J* = 8.0, 6.7 Hz, H_4), 7.00 – 6.81 (1H, m, H_5), 6.81 – 6.74 (1H, m, H_3), 6.70 – 6.46 (1H, m, H_{10}), 6.07 – 5.77 (1H, m, H11), 5.10 (1H, dd, *J* = 44.1, 6.7 Hz, H_8), 3.88 (3H, d, *J* = 5.1 Hz, H_7), 1.77 (3H, ddd, *J* = 68.6, 6.8, 1.8 Hz, H_{12}), 1.51 (3H, dd, *J* = 14.1, 6.7 Hz, H_9). ^{13}C NMR (75 MHz, $CDCl_3$) δ; [157.50, 157.38](C_2), [137.40, 136.23](C_6), [130.79, 129.27](C_1),[129.63,128.47](C_{10}), [128.26 , 128.09](C_{11}), [127.63, 127.23](C_4), [122.78, 120.31](C_5), [109.88, 109.80](C_3), [67.82, 66.97](C_8), [55.42, 55.35](C_7), [23.43, 23.34](C_9), [18.76, 14.23](C_{12}).

6.25 Preparation of *rac-trans*-8-methoxy-1,3-dimethylisochroman ±125

In a 100 mL two necked round bottom flask equipped with a condenser, *rac*-1-{2-methoxy-6-(prop-1-en-1-yl)phenyl}ethanol (0.11 g, 0.57 mmol, 1 equiv.) was dissolved in dry DMF (15 mL). After stirring for 10 minutes, potassium *tert*-butoxide (0.70 g, 6.27 mmol, 11 equiv.) was added into the reaction mixture. The reaction was heated to 80°C and stirred for 2 hours under a nitrogen atmosphere. Upon completion, the reaction was cooled to 0°C in an ice-bath and water (60 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (3 × 30 mL) and the organic layer was washed with brine. The organic extracts were combined, dried with anhydrous magnesium sulfate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (10% Ethyl acetate/Hexane) to afford

rac-trans- 8-methoxy-1,3-dimethylisochroman as a clear oil (0.098g, 90%).

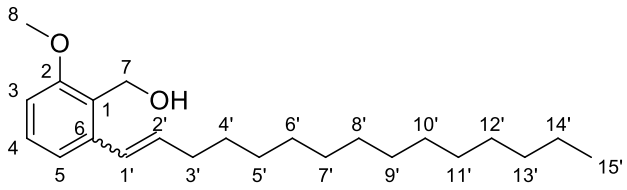


±125

R_f = 0.886 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 2969 (C-H), 1586and 1471 (C=C); ^{1}H NMR (300 MHz, Chloroform-*d*) δ: 7.15 (1H, t, *J* = 7.9 Hz, H_6), 6.77 – 6.65 (2H, m, H_9,H_5), 5.14 (1H, q, *J* = 6.6 Hz, H_1), 4.22 – 4.05 (1H, m, H_3), 3.82 (3H, s, H_{11}), 2.77 – 2.52 (2H, m, H_4), 1.54 (3H, d, *J* = 6.6 Hz, H_{12}), 1.34 (3H, d, *J* = 6.1 Hz, H_{13}). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.37(C_8), 134.46(C_{10}), 127.87(C_9), 126.87(C_6), 120.96(C_5), 107.57(C_7), 68.40(C_1), 62.59(C_3), 55.06(C_{11}), 35.89(C_4), 21.84(C_{13}), 19.61(C_{12}).

6.26 Preparation of {2-methoxy-6-(pentadec-1-en-1-yl)phenyl}methanol 142

In a two necked 100 mL round bottom flask fitted with a reflux condenser, methyl 2-methoxy-6-(pentadec-1-en-1-yl) benzoate (2.01 g, 5.15 mmol, 1 equiv.) was added to THF (20 mL). The solution was cooled to 0°C in an ice bath. Lithium aluminium hydride (0.78 g, 20.59 mmol, 4 equiv.) was added slowly. The resulting reaction mixture was allowed to warm up to room temperature and stirred for 5 hours. The mixture was again cooled to 0°C and aqueous HCl solution (10%, 15 mL) was added slowly. Lithium aluminium hydride residue was filtered off using cotton wool. THF was removed *in vacuo* and the aqueous layer was extracted with diethyl ether (3 × 30 mL). The organic extracts were combined and dried over anhydrous magnesium sulfate before filtering through cotton wool. The solvent was then removed *in vacuo*. The residue was purified by silica gel column chromatography (15-20% Ethyl acetate/Hexane) to afford {2-methoxy-6-(pentadec-1-en-1-yl)phenyl}methanol as a white crystal (1.43 g, 80%).

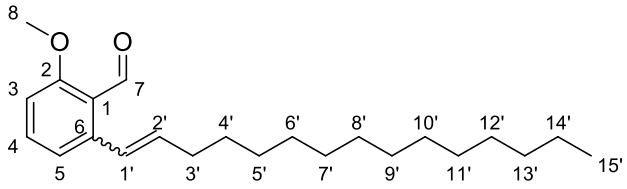


142

R_f = 0.657 (20% EtOAc /Hexane); **mp**: 44-46°C**; IR**: ν_{max}(cm^{-1}): 3409 (OH), 1609 and 1459 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ: 7.21 (1H, t, *J* = 8.0 Hz, H_4), 7.06 (1H, dd, *J* = 7.9, 1.1 Hz, H_5), 6.78 (1H, dd, *J* = 8.1, 1.1 Hz, H_3), 6.72 (1H, d, *J* = 15.7 Hz, $H_{1'}$), 6.11 (1H, dt, *J* = 15.6, 6.9 Hz, $H_{2'}$), 4.79 (2H, d, *J* = 6.1 Hz, H_7), 3.86 (3H, d, *J* = 1.5 Hz, H_8), 2.29 – 2.13 (2H, m, $H_{3'}$), 1.47 (2H, t, *J* = 7.5 Hz, $H_{4'}$), 1.27 (20H, s, $3H_{5'-14'}$), 0.90 (3H, d, *J* = 6.4 Hz, $H_{15'}$); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 158.03(C_2), 138.87(C_6), 134.99(C_4), 128.70($C_{1'}$), 126.65($C_{2'}$), 125.75(C_1), 119.18(C_5), 108.84(C_3), 56.96(C_7), 55.57(C_8), 33.33($C_{3'}$), 31.94($C_{13'}$), [29.70, 29.67, 29.65, 29.54, 29.38, 29.33, 29.27]($C_{4'-13'}$), 22.71($C_{14'}$), 14.13($C_{15'}$).

6.27 Preparation of 2-methoxy-6-(prop-1-en-1-yl)benzaldehyde 143

In a 100 mL round bottom flask, pyridium chlorochromate (PCC) (0.26g, 1.21 mmol, 2.1 equiv.) and {2-methoxy-6-(pentadec-1-en-1-yl)phenyl}methanol (0.20g, 0.58 mmol) were added together with normal silica (0.26 g) in dry DCM (30ml). The reaction mixture was stirred at room temperature for 5 hours under a nitrogen atmosphere. The mixture was filtered through celite and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (15–20% Ethylacetate/Hexane) to afford 2-methoxy-6-(pentadec-1-en-1-yl)benzaldehyde as a pale yellow oil (0.18g, 90%).



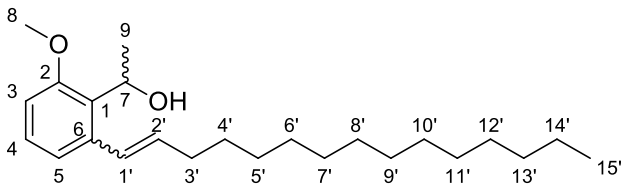
143

R_f = 0.771 (20% EtOAc /Hexane); **IR**: ν_{max}(cm^{-1}): 1711 (C=O), (C=O), 1688 and 1469 (C=C); **^{1}H NMR** (300 MHz,

Chloroform-*d*) δ 10.60 (1H, d, *J* = 0.5 Hz, H_7), 7.41 (1H, t, *J* = 8.1 Hz, H_4), 7.28 – 7.19 (1H, m, H_5), 7.14 – 7.08 (1H, m, H_3), 6.83 (1H, dd, *J* = 8.4, 0.9 Hz, $H_{1'}$), 6.17 (1H, dt, *J* = 15.7, 6.9 Hz, $H_{2'}$), 3.89 (3H, s, H_8), 2.30 – 2.18 (2H, m, $H_{3'}$), 1.47 (2H, d, *J* = 7.3 Hz, $H_{4'}$), 1.26 (20H, s, $H_{5'\text{-}14'}$), 0.92 – 0.83 (3H, m, $H_{15'}$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ 192.30(C_7), 162.58(C_2), 141.32(C_6), 135.78($C_{1'}$), 134.30($C_{2'}$), 127.93(C_4), 121.81(C_1), 119.59(C_5), 109.44(C_3), 55.85(C_8), 33.24($C_{3'}$), 31.94($C_{13'}$), [29.72, 29.70, 29.67, 29.64, 29.55, 29.38, 29.30, 29.25]($C_{4'\text{-}12'}$), 22.71($C_{14'}$), 14.13($C_{15'}$).

6.28 Preparation of *rac*-1-{2-methoxy-6-(pentadec-1-en-1-yl)phenyl}ethanol ±141

Magnesium (0,025 g, 1.05 mmol, 1.8 equiv.) was added to an oven dried 100 mL three-necked round bottom flask equipped with a condenser, a dropping funnel and a stopper under nitrogen. Dry diethyl ether (30 mL) followed by methyl iodide (0.05 mL, 0.87 mmol, 1.5 equiv.) was added to the flask. The reaction mixture became cloudy. Once the magnesium was consumed, a solution of 2-methoxy-6-(pentadec-1-en-1-yl)benzaldehyde (0.20 g, 0.58 mmol, 1 equiv.) in dry THF (10ml) was added dropwise to the freshly prepared Grignard reagent. The mixture was stirred at room temperature for 18 hours. Upon completion, the reaction was cooled to 0°C in an ice-bath and water (10 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (3 × 20 mL) and the organic layer was washed with brine. The organic extracts were combined, dried over anhydrous magnesium sulfate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (20% Ethyl acetate/Hexane) to afford *rac*-1-(2-methoxy-6-(pentadec-1-en-1-yl)phenyl)ethanol as a clear pale yellow oil (0.15g, 70%).

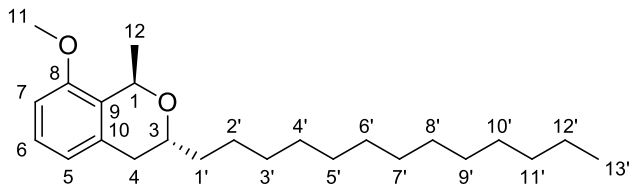


±141

R_f = 0.686 (20% EtOAc /Hexane); **mp**: 51-54°C; **IR**: ν_{max}(cm^{-1}): 3541 (OH), 1599 and 1470 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 7.16 (1H, t, *J* = 8.0 Hz, H_4), 6.98 (1H, dd, *J* = 7.8, 1.1 Hz, H_5), 6.81 (1H, dd, *J* = 8.3, 1.2 Hz, H_3), 6.63 (1H, d, *J* = 15.6 Hz, $H_{1'}$), 5.97 (1H, dt, *J* = 15.5, 6.9 Hz, $H_{2'}$), 5.24 – 5.12 (1H, m, H_7), 3.88 (3H, s, H_8), 2.27 – 2.15 (2H, m, $H_{3'}$), 1.53 (3H, d, *J* = 6.7 Hz, H_9), 1.47 (2H, dd, *J* = 8.6, 6.7 Hz, $H_{4'}$), 1.27 (20H, s, $H_{5'-14'}$), 0.93 – 0.81 (3H, m, $H_{15'}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.38(C_2), 137.50(C_6), 134.94(C_4), 129.70(C_1), 127.60($C_{1'}$), 127.17($C_{2'}$), 120.37(C_5), 109.78(C_3), 67.03(C_7), 55.42(C_8), 33.25($C_{3'}$), 31.94($C_{13'}$), [29.70, 29.68, 29.66, 29.54, 29.38, 29.32, 29.21]($C_{4'-12'}$), 23.44($C_{14'}$), 22.71(C_9), 14.13($C_{15'}$).

6.29 Preparation of *rac-trans*-8-methoxy-1-methyl-3-tridecylisochroman ±144

In a 100 mL long necked round bottom flask equipped with a condenser, was added *rac*-1-{2-methoxy-6-(pentadec-1-en-1-yl)phenyl}ethanol (0.50 g, 1.39 mmol, 1 equiv.) in dry DMF (20 mL). Potassium *tert*-butoxide (1.70 g, 15.25 mmol, 11 equiv.) was added into the reaction mixture. The flask was placed in microwave reactor with settings of following: reflux mode, 100 W, 120°C. Upon completion, the reaction was cooled to room temperature and water (50 mL) was added. The reaction mixture was transferred to a separation funnel and washed with ethyl acetate (3 × 50 mL) and the organic layer was washed with brine. The organic extracts were combined, dried over anhydrous magnesium sulfate, filtered through cotton wool and the solvent removed *in vacuo*. The crude material was purified by silica gel column chromatography (10% EtOAc/Hexane) to afford *rac-trans*-8-methoxy-1-methyl-3-tridecylisochroman as a clear light yellow oil (0.27g, 53%).



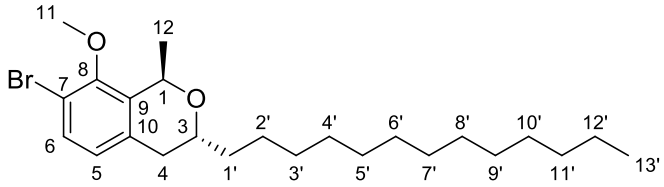
±144

R_f = 0.914 (20% EtOAc /Hexane); **IR**: $\nu_{max}(cm^{-1})$: 3409.40 (OH), 1661.04 (C=O), (C=O), 1587 and 1469 (C=C); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 7.13 (1H, t, *J* = 7.9 Hz, H_6), 6.70 (2H, t, *J* = 8.2 Hz, H_5,H_7), 5.05 (1H, dq, *J* = 34.3, 6.5 Hz, H_1), 3.97 – 3.76 (4H, m, H_{11},H_3), 2.73 – 2.55 (2H, m, H_4), 1.53 (3H, dd, *J* = 10.9, 6.4 Hz, H_{12}), 1.27 (24H, s, $H_{1'-12'}$), 0.89 (3H, t, *J* = 6.6 Hz, $H_{13'}$); **^{13}C NMR** (75 MHz, $CDCl_3$) δ [156.53, 155.77](C_9), [136.90, 135.06](C_{10}), 128.61(C_8), [127.17, 127.06](C_6), 121.45(C_5), [108.52, 107.90](C_7), [73.83, 68.69](C_3), [71.33, 66.93](C_1), [55.45, 55.37](C_{11}), [36.44, 36.30](C_4), [36.00, 34.74]($C_{1'}$), [32.29, 29.73]($C_{11'}$), [30.07, 30.04, 30.01]($C_{4'-10'}$), [25.93, 25.89]($C_{2'}$), 23.06(C_{12}), 22.14($C_{3'}$), 19.88($C_{12'}$), 14.48($C_{13'}$).

6.30 Preparation of *rac-trans*-7-bromo-8-methoxy-1-methyl-3-tridecylisochroman ±146

Rac-trans-8-methoxy-1-methyl-3-tridecylisochroman (0.13 g, 0.36 mmol, 1.0 equiv.) and NBS (0.07 g, 0.40 mmol, 1.1 equiv.) were transferred into a 100 ml two necked round bottom flask equipped with reflux condenser containing 25 ml DMF. The reaction mixture was refluxed for 72 hours under a nitrogen atmosphere. The reaction was then cooled to room temperature and quenched with saturated sodium bicarbonate (10 mL). The aqueous layer was thoroughly extracted with DCM(3 × 30 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate before filtering through celite. The residue was purified by silica gel column chromatography (10% ethyl acetate/Hexane) to yield the

rac-trans-7-bromo-8-methoxy-1-methyl-3-tridecylisochroman as pale-yellow solid (0.134g, 85%).



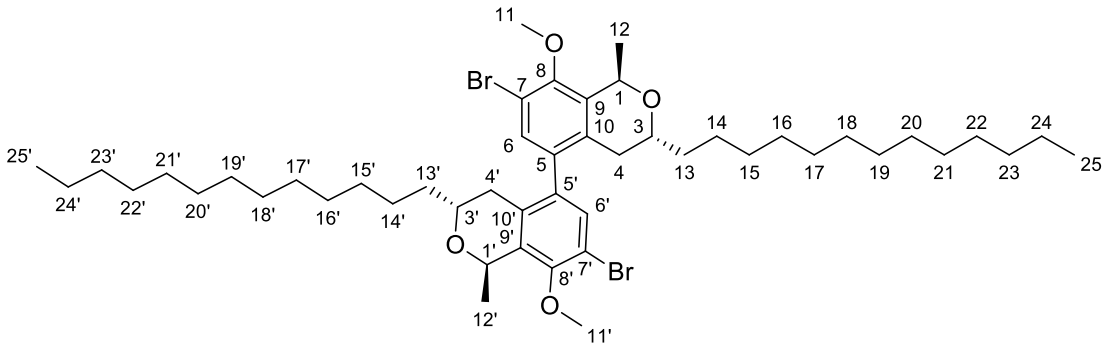
±146

R_f = 0.943 (20% EtOAc /Hexane); **mp**: 49-52°C; **IR**: $\nu_{max}(cm^{-1})$: 1469 (C=C), 788 (C-Br); **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 7.37 (1H, d, *J* = 8.7 Hz, H_6), 6.59 (1H, d, *J* = 8.7 Hz, H_5), 5.07 (1H, q, *J* = 6.6 Hz, H_1), 3.98 – 3.80 (1H, m, H_3), 3.79 (3H, s, H_{11}), 2.74 (1H, dd, *J* = 17.1, 3.5 Hz, H_4), 2.40 (1H, dd, *J* = 17.1, 10.9 Hz, $H_{4"}$), 1.68 – 1.52 (2H, m, $H_{1'}$), 1.48 (3H, d, *J* = 6.6 Hz, H_{12}), 1.26 (22H, s, $H_{2'-12'}$), 0.87 (3H, d, *J* = 7.0 Hz, $H_{13'}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.54(C_8), 133.93(C_{10}), 130.87(C_9), 130.26(C_6), 115.80(C_7), 109.24(C_5), 68.14(C_3), 66.41(C_1), 55.35(C_{11}), 36.09(C_4), 35.35($C_{1'}$), 31.94($C_{11'}$), [29.72, 29.69, 29.66, 29.64, 29.38]($C_{3'-10'}$), 25.48($C_{2'}$), 22.71(C_{12}), 19.29($C_{12'}$), 14.13($C_{13'}$)

6.31 Preparation of *rac-trans*-7,7'-dibromo-8,8'-dimethoxy-1,1'-dimethyl-3,3'-ditridecyl-5,5'-biisochroman ±145

Rac-trans-7-bromo-8-methoxy-1-methyl-2-tridecylisochroman (0.05 g, 0.11 mmol, 1.0 equiv.) and $FeCl_3 \cdot 6H_2O$ (0.074 g, 0.28 mmol, 2.5 equiv.) were transferred into a 50 mL two necked round bottom flask equipped with stopper containing 5 ml Toluene. The reaction mixture was heated at 85°C for 24 hours under a nitrogen atmosphere. The reaction was then cooled to room temperature and quenched with water (10 mL). The aqueous layer was thoroughly extracted with DCM(3 × 5 mL). The organic extracts were combined and washed with brine and dried over anhydrous magnesium sulfate

before filtering through celite. The resulting product was in really low yield; thus, it was subjected to characterization without purification.



±145

R_f = 0.925 (20% EtOAc /Hexane); **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.51 (2H, s, H_6, $H_{6'}$), 5.12 (2H, q, *J* = 6.7 Hz, H_1, $H_{1'}$), 3.88 (6H, s, H_{11}, $H_{11'}$), 3.84 – 3.79 (2H, m, H_3, $H_{3'}$), 2.74 (2H, dd, *J* = 17.2, 3.5 Hz, H_4), 2.43 – 2.27 (m, $H_{4'}$), 1.64 (ddd, *J* = 11.5, 5.2, 2.9 Hz, H from long chain), 1.56 (9H, d, *J* = 6.7 Hz, H_{12}, $H_{12'}$), 1.27 (d, *J* = 19.3 Hz, H from long chain), 0.96 – 0.81 (m,H_{25}, $H_{25'}$).

Chapter 7: References

1. Markaryan, E.; Samodurova, A., Advances in the chemistry of isochroman. *Russian Chemical Reviews* **1989,** *58* (5), 479.
2. Suzuki, T.; Tanemura, K.; Horaguchi, T.; Kaneko, K., Synthesis and structure of 8-hydroxy-6-methoxy-3, 7-dimethylisochromane and its analogues. *Tetrahedron* **2006,** *62* (15), 3739-3751.
3. Larghi, E. L.; Kaufman, T. S., The oxa-Pictet-Spengler cyclization: Synthesis of isochromans and related pyran-type heterocycles. *Synthesis* **2006,** *2006* (02), 187-220.
4. Brimble, M. A., Nairn, M. R.; Prabaharan, H., Synthetic strategies towards pyranonaphthoquinone antibiotics. *Tetrahedron* **2000,** *56* (14), 1937-1992.
5. Hari, L.; De Buyck, L.; De Pootert, H., Naphthoquinoid pigments from Pentas longiflora. *Phytochemistry* **1991,** *30* (5), 1726-1727.
6. De Kimpe, N.; Van Puyvelde, L.; Schripsema, J.; Erkelens, C.; Verpoorte, R., Complete proton and ^{13}C NMR spectral assignments of pentalongin. *Magnetic resonance in chemistry* **1993,** *31* (4), 329-330.
7. Hayashi, T.; Smith, F. T.; Lee, K. H., Antitumor agents. 89. Psychorubrin, a new cytotoxic naphthoquinone from Psychotria rubra and its structure-activity relationships. *Journal of Medicinal Chemistry* **1987,** *30* (11), 2005-2008.
8. Bianchi, C.; Ceriotti, G., Chemical and pharmacological investigations of constituents of Eleutherine bulbosa (Miller) Urb.(Iridaceae). *Journal of Pharmaceutical Sciences* **1975,** *64* (8), 1305-1308.
9. Brimble, M.; Nairn, M.; Duncalf, L., Pyranonaphthoquinone antibiotics—isolation, structure and biological activity. *Natural Product Reports* **1999,** *16* (3), 267-281.
10. Bergy, M., Kalafungin, a new broad spectrum antibiotic. *The Journal of Antibiotics* **1968,** *21* (7), 454-457.
11. Johnson, L. E.; Dietz, A., Kalafungin, a new antibiotic produced by Streptomyces tanashiensis strain Kala. *Appl. Environ. Microbiol.* **1968,** *16* (12), 1815-1821.
12. Brockmann, H.; Pini, H., Actinorhodin, ein roter Farbstoff aus Actinomyceten. *Naturwissenschaften* **1947,** *34* (6), 190-190.
13. Brockmann, H.; Pini, H.; v. Plotho, O., Über Actinomycetenfarbstoffe, I. Mitteil.: Actinorhodin, ein roter, antibiotisch wirksamer Farbstoff aus Actinomyceten. *Chemische Berichte* **1950,** *83* (2), 161-167.
14. Imamura, N.; Ishikawa, T.; Ohtsuka, T.; Yamamoto, K.; Dekura, M.; Fukami, H.; Nishida, R., An antibiotic from Penicillium sp. covering the cocoon of the leaf-rolling moth, Dactylioglypha tonica. *Bioscience,*

Biotechnology, and Biochemistry **2000,** *64* (10), 2216-2217.
15. Saeed, A.; Mumtaz, A., Novel isochroman-triazoles and thiadiazole hybrids: Design, synthesis and antimicrobial activity. *Journal of Saudi Chemical Society* **2017,** *21* (2), 186-192.
16. Ogawa, A.; Murakami, C.; Kamisuki, S.; Kuriyama, I.; Yoshida, H.; Sugawara, F.; Mizushina, Y., Pseudodeflectusin, a novel isochroman derivative from Aspergillus pseudodeflectus a parasite of the sea weed, Sargassum fusiform, as a selective human cancer cytotoxin. *Bioorganic & Medicinal Chemistry Letters* **2004,** *14* (13), 3539-3543.
17. Lorenz, P.; Zeh, M.; Martens-Lobenhoffer, J.; Schmidt, H.; Wolf, G.; Horn, T. F., Natural and newly synthesized hydroxy-1-aryl-isochromans: a class of potential antioxidants and radical scavengers. *Free Radical Research* **2005,** *39* (5), 535-545.
18. Togna, G. I.; Togna, A. R.; Franconi, M.; Marra, C.; Guiso, M., Olive oil isochromans inhibit human platelet reactivity. *The Journal of Nutrition* **2003,** *133* (8), 2532-2536.
19. Bai, R.; Yang, X.; Zhu, Y.; Zhou, Z.; Xie, W.; Yao, H.; Jiang, J.; Liu, J.; Shen, M.; Wu, X., Novel nitric oxide-releasing isochroman-4-one derivatives: Synthesis and evaluation of antihypertensive activity. *Bioorganic & Medicinal Chemistry* **2012,** *20* (23), 6848-6855.
20. Cutler, H. G.; Arrendale, R. F.; Cole, P. D.; Davis, E. E.; Cox, R. H., 3, 7-Dimethyl-8-hydroxy-6-methoxy-isochroman from Penicillium corylophilum: plant growth regulatory activity. *Agricultural and Biological Chemistry* **1989,** *53* (7), 1975-1977.
21. Kakimoto, T.; Koizumi, F.; Hirase, K.; Banba, S.; Tanaka, E.; Arai, K., Novel 3, 3a, 5, 9b - tetrahydro - 2*H* - furo [3, 2 - *c*]benzopyran derivatives: synthesis of chiral glycol benzyl ether herbicides. *Pest Management Science: formerly Pesticide Science* **2004,** *60* (5), 493-500.
22. Ennis, M. D.; Ghazal, N. B.; Hoffman, R. L.; Smith, M. W.; Schlachter, S. K.; Lawson, C. F.; Im, W. B.; Pregenzer, J. F.; Svensson, K. A.; Lewis, R. A., Isochroman-6-carboxamides as highly selective 5-HT1D agonists: potential new treatment for migraine without cardiovascular side effects. *Journal of Medicinal Chemistry* **1998,** *41* (13), 2180-2183.
23. Ralph, J.; Peng, J.; Lua, F., Isochroman structures in lignin: a new β-1 pathway. *Tetrahedron Letters* **1998,** *39* (28), 4963-4964.
24. TenBrink, R. E.; Bergh, C. L.; Duncan, J. N.; Harris, D. W.; Huff, R.; Lahti, R. A.; Lawson, C. F.; Lutzke, B. S.; Martin, I. J.; Rees, S. A., (S)-(-)-4-[4-[2-(Isochroman-1-yl) ethyl] piperazin-1-yl] benzenesulfonamide, a Selective Dopamine D4 Antagonist. *Journal of Medicinal Chemistry* **1996,** *39* (13), 2435-2437.
25. Bury, P. S.; Christiansen, L. B.; Jacobsen, P.; Jørgensen, A. S.; Kanstrup, A.; Nærum, L.; Bain, S.; Fledelius, C.; Gissel, B.; Hansen, B. S., Synthesis and pharmacological evaluation of novel cis-3,

4-diaryl-hydroxychromanes as high affinity partial agonists for the estrogen receptor. *Bioorganic & Medicinal Chemistry* **2002,** *10* (1), 125-145.
26. Aldersley, M. F.; Dean, F. M.; Nayyir-Mazhir, R., Alkylation of quinones by carbanions: use of pyridinium ylides to insert phenacyl, acetonyl and related groups. *Journal of the Chemical Society, Perkin Transactions 1* **1983**, 1753-1757.
27. Aldersley, M. F.; Dean, F. M.; Hamzah, A. S., Derivatives of naphtho [2, 3-*c*] pyran-5,10-dione; a simple synthesis and a note of their chromogenic properties. *Tetrahedron Letters* **1986,** *27* (2), 255-258.
28. Brimble, M. A.; Stuart, S. J., Synthesis of 5-epi-7-deoxykalafungin and 5-epi-7-O-methylkalafungin. *Journal of the Chemical Society, Perkin Transactions 1* **1990,** 881-885.
29. Brimble, M. A.; Lynds, S. M., A short synthesis of deoxyfrenolicin. *Journal of the Chemical Society, Perkin Transactions 1* **1994,** 493-496.
30. Kraus, G. A.; Sugimoto, H., An annelation route to quionones. *Tetrahedron Letters* **1978,** *19* (26), 2263-2266.
31. Perkin, W. H.; Rây, J. N.; Robinson, R., CIII.—A synthesis of oxyberberine. Part I. *Journal of the Chemical Society, Transactions* **1925,** *127*, 740-744.
32. Hassan, N. P.; Naysmith, B. J.; Sperry, J.; Brimble, M. A., Formal synthesis of nanaomycin D *via* a Hauser–Kraus annulation using a chiral enone-lactone. *Tetrahedron* **2015,** *71* (39), 7137-7143.
33. Suryawanshi, S. B.; Dushing, M. P.; Gonnade, R. G.; Ramana, C., The isochroman-and 1, 3-dihydroisobenzofuran-annulation on carbohydrate templates via [2+ 2+ 2]-cyclotrimerization and synthesis of some tricyclic nucleosides. *Tetrahedron* **2010,** *66* (32), 6085-6096.
34. Arimitsu, S.; Hammond, G. B., Lanthanide triflate-catalyzed preparation of β, β-difluorohomopropargyl alcohols in aqueous media. Application to the synthesis of 4, 4-difluoroisochromans. *The Journal of Organic Chemistry* **2006,** *71* (22), 8665-8668.
35. Guiso, M.; Bianco, A.; Marra, C.; Cavarischia, C., One-Pot Synthesis of 6-Hydroxyisochromans: The Example of Demethyl-oxa-coclaurine. *European Journal of Organic Chemistry* **2003,** *2003* (17), 3407-3411.
36. de Koning, C. B.; Giles, R. G.; Green, I. R., Naphthopyranquinones. Confirmation of the structures of the ventiloquinones E, G and J by synthesis. *Journal of the Chemical Society, Perkin Transactions 1* **1991,** 2743-2748.
37. de Koning, C. B.; Giles, R. G.; Engelhardt, L. M.; Whitet, A. H., Convenient syntheses of the naturally occurring benzo[*b*]xanthen-12-one bikaverin. X-Ray crystallographic confirmation of the product regiochemistry. *Journal of the Chemical Society, Perkin Transactions 1* **1988,** 3209-3216.
38. de Koning, C. B.; Giles, R. G.; Green, I. R.; Jahed, N. M., Towards

the synthesis of chiral isochromanquinones. The use of Corey–Bakshi–Shibata reductions. *Tetrahedron Letters* **2002,** *43* (23), 4199-4201.
39. Johnson, M. M. The development of novel synthetic methodology for the synthesis of oxygenated heterocycles. PhD, University of the Witwatersrand, 2014.
40. Anastas, P. T.; Kirchhoff, M. M.; Williamson, T. C., Catalysis as a foundational pillar of green chemistry. *Applied Catalysis A: General* **2001,** *221* (1-2), 3-13.
41. Bugg, T. D., *Introduction to enzyme and coenzyme chemistry*. John Wiley & Sons: 2012.
42. Lu, T., Towards the Synthesis of (+)-kalafungin Using Enzymatic Kinetic Resolution. Honours Project, University of the Witwatersrand, **2016.**
43. Harwood, L. M., Access to phenolic fungal metabolites via the acid-catalysed Claisen rearrangement. The total synthesis of (±)-mellein, aurocitrin, and 5′, 6′-dihydroaurocitrin. *Journal of the Chemical Society, Perkin Transactions 1* **1984,** 2577-2582.
44. Giles, R.; Green, I. R.; Hugo, V. I.; Oosthuisen, F. J.; Van Eeden, N., Convenient synthetic route to antimicrobial benzo[*c*]pyranquinones. *South African Journal of Chemistry* **1995,** *48* (1-2), 15-22.
45. Vargas, D. F.; Larghi, E. L.; Kaufman, T. S., Synthesis of Polysubstituted 3-Methylisoquinolines through the 6π-Electron Cyclization/Elimination of 1-Azatrienes derived from 1, 1-Dimethylhydrazine. *European Journal of Organic Chemistry* **2018,** *2018* (40), 5605-5614.
46. Giles, R. G.; Green, I. R.; Hugo, V. I.; Mitchell, P. R.; Yorke, S. C., Naphthopyrans by ring-closure of substituted naphthalenes using potassium *t*-butoxide in dimethylformamide. *Journal of the Chemical Society, Perkin Transactions 1* **1983,** 2309-2313.
47. Anastas, P. T.; Kirchhoff, M. M., Origins, current status, and future challenges of green chemistry. *Accounts of Chemical Research* **2002,** *35* (9), 686-694.
48. Paramashivappa, R.; Kumar, P. P.; Vithayathil, P.; Rao, A. S., Novel method for isolation of major phenolic constituents from cashew (*Anacardium occidentale L.*) nut shell liquid. *Journal of Agricultural and Food Chemistry* **2001,** *49* (5), 2548-2551.
49. Gedam, P.; Sampathkumaran, P., Cashew nut shell liquid: extraction, chemistry and applications. *Progress in Organic Coatings* **1986,** *14* (2), 115-157.
50. Himejima, M.; Kubo, I., Antibacterial agents from the cashew Anacardium occidentale (*Anacardiaceae*) nut shell oil. *Journal of Agricultural and Food Chemistry* **1991,** *39* (2), 418-421.
51. Muroi, H.; Kubo, I., Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillin

resistant *Staphylococcus aureus*. *Journal of Applied Bacteriology* **1996,** *80* (4), 387-394.
52. Kubo, J.; Lee, J. R.; Kubo, I., Anti-Helicobacter pylori agents from the cashew apple. *Journal of Agricultural and Food Chemistry* **1999,** *47* (2), 533-537.
53. Kubo, I.; Ochi, M.; Vieira, P. C.; Komatsu, S., Antitumor agents from the cashew (*Anacardium occidentale*) apple juice. *Journal of Agricultural and Food Chemistry* **1993,** *41* (6), 1012-1015.
54. Prithiviraj, B.; Singh, U.; Manickam, M.; Ray, A., Antifungal activity of anacardic acid, a naturally occurring derivative of salicylic acid. *Canadian Journal of Botany* **1997,** *75* (1), 207-211.
55. Resck, I. S.; Santos, M. L. d.; Soares Romeiro, L. A., New application of triphosgene in a convenient synthesis of 3-aryl-1, 3-benzoxazine-2, 4-diones from anacardic acids. *Heterocycles-Sendai Institute of Heterocyclic Chemistry* **2005,** *65* (2), 311-318.
56. Kumar, P. P.; Stotz, S. C.; Paramashivappa, R.; Beedle, A. M.; Zamponi, G. W.; Rao, A. S., Synthesis and evaluation of a new class of nifedipine analogs with T-type calcium channel blocking activity. *Molecular Pharmacology* **2002,** *61* (3), 649-658.
57. Swamy, B. N.; Suma, T.; Rao, G. V.; Reddy, G. C., Synthesis of isonicotinoylhydrazones from anacardic acid and their in vitro activity against Mycobacterium smegmatis. *European Journal of Medicinal Chemistry* **2007,** *42* (3), 420-424.
58. Donner, C. D.; Cuzzupe, A. N.; Falzon, C. L.; Gill, M., Investigations towards the synthesis of xylindein, a blue-green pigment from the fungus Chlorociboria aeruginosa. *Tetrahedron* **2012,** *68* (13), 2799-2805.
59. Kim, D. W.; Choi, H. Y.; Lee, K.-J.; Chi, D. Y., Facile Oxidation of fused 1, 4-dimethoxybenzenes to 1, 4-quinones using NBS: Fine-tuned Control over Bromination and Oxidation Reactions. *Organic Letters* **2001,** *3* (3), 445-447.
60. Zehnter, R.; Gerlach, H., Synthesis of anacardic acids. *Liebigs Annalen* **1995,** *1995* (12), 2209-2220.
61. Rambabu, N.; Dubey, P.; Ram, B.; Balram, B., Synthesis, Characterization and Antimicrobial Evaluation of (*E*)-N'-[(1-(2-methoxy-6-pentadecylbenzyl)-
1*H*-1, 2, 3-triazol-4-yl]-methylene) benzohydrazide Derivatives. *Asian Journal of Chemistry* **2016,** *28* (1), 175.
62. Lograd o, L. P.; Santos, C. O.; Romeiro, L. A.; Costa, A. M.; Ferreira, J. R.; Cavalcanti, B. C.; de Moraes, O. M.; Costa-Lotufo, L. V.; Pessoa, C.; dos Santos, M. L., Synthesis and cytotoxicity screening of substituted isobenzofuranones designed from anacardic acids. *European Journal of Medicinal Chemistry* **2010,** *45* (8), 3480-3489.
63. Yu, J.; Shen, M.; Deng, L.; Gan, L.; Ha, C., Facile one-pot synthesis of anisaldehyde. *Chemistry of Natural Compounds* **2012,** *48* (4), 541-543.

64. Bohlmann, F.; Prezewowsky, K., Polyacetylenverbindungen, LV. Synthese des Frutescinollactons. *Chemische Berichte* **1964,** *97* (4), 1176-1178.
65. Bringmann, G.; Jansen, J. R., Acetogenine Isochinolin - Alkaloide, 7. Einfache Synthesen nützlicher Diketo - Bausteine für biomimetische Isochinolin - und Naphthalin - Synthesen. *Liebigs Annalen der Chemie* **1985,** *1985* (11), 2116-2125.
66. Robin, G.; Lee Son, V. R., Stereoselective base-induced conversion of naphthalenic precursors into naphthopyrans related to the aphin-derived glucoside B. *Journal of the Chemical Society, Perkin Transactions 1* **1994,** 859-864.