Total synthesis of eranthin acetate, ptaeroxylinol acetate and derivatives thereof

by

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Declaration

I, Modibo Sharleen Malefo declare that the dissertation, which I hereby submit for the degree of magister scientiae at the department of chemistry, University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other university.

Signature: _____

Date: 08-04-2021

Summary

In most countries, fungal infections represent an overwhelming problem, particularly in hospitals. *Candida albicans* is considered the most virulent among other species responsible for the fungal infection, candidiasis. Candidiasis has developed resistance against most of the current antifungal drugs. The 12-*O*-acetyl derivative of eranthin named obliquumol, isolated from a South African plant, *Ptaeroxylon obliquum*, was found to exhibit *in vitro* activity against *C. albicans* standard strain (ATCC10231). Ptaeroxylinol acetate, the linear isomer of eranthin acetate was also previously isolated from the *P. obliquum*. The aim of this study was two-fold. The first aim was to synthesise the 12-*O*-acetyl derivative of eranthin (obliquumol), its linear isomer ptaeroxylinol acetate, and their derivatives, in order to investigate their biological activities. The second aim of study was to synthesise the carbon-14 labelled eranthin acetate and its structural analogues for the *in vivo* tissue distribution studies.

Two approaches were investigated for the synthesis of the oxepinochromones eranthin acetate and ptaeroxylinol acetate. The first approach involved preparation of the oxepinochromones by employing the Kostanecki-Robinson reaction to synthesise the chromone intermediate, noreugenin, from 2',4',6'-trihydroxyacetophenone. Allylation and Claisen rearrangement resulted in two isomers, 8-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one and 6-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one. The resultant isomers led to the concurrent synthesis of eranthin acetate and ptaeroxylinol acetate from the 8-allyl isomer and the 6-allyl isomer, respectively. Consequently, the oxepine ring was constructed through a sequence of steps that involved alkylation, ring-closing metathesis (RCM) and a successive deprotection to afford eranthin and ptaeroxylinol, which were successfully acetylated to complete the synthesis of eranthin acetate and ptaeroxylinol acetate in modest yields.

The second approach entailed the construction of oxepine ring from 2',4',6'-trihydroxyacetophenone followed by the synthesis of the chromone scaffold to allow for the incorporation of a C-14 labelled precursor at a more stable position. To achieve the construction of the oxepine ring, various protection and deprotection protocols were investigated. The optimum protocol employed involved the partial protection of phloroacetophenone with MOM protecting groups, allylation and Claisen rearrangement

followed by the protection of the hydrogen-bonded hydroxy group with a methyl group. Selective deprotection of one of the MOM groups, a subsequent protection with methyl group, and removal of the remaining MOM group furnished 3-allyl-4-hydroxy-2,6-dimethoxyacetophenone that was alkylated and subjected to RCM reaction to afford the expected oxepine ring and a subsequent deprotection which gave the benzoxepine, 2,5-dihydro-3-(hydroxymethyl)-6,8-dimethoxybenzo[b]oxepin-7-yl)ethanone in 32% yields.

The RCM-deprotection step resulted in decomposition of the product and consequently, loss of material. The procedure was halted. This approach will be optimised in future to assemble the chromone scaffold and ultimately the target compounds. Furthermore, two derivatives were prepared via base-catalysed prenylation from two intermediates prepared during the investigation of the two above-mentioned strategies. Nine of the synthesised compounds were screened for antifungal activity against two genera of fungi, *C. albicans* and *Cryptococcus neoformans*. Among the tested compounds, eranthin acetate was found to have best activity against *C. albicans* while ptaeroxylinol acetate showed best activity against *C. neoformans*.

Publications

The thesis is based on the work reported in the following manuscript:

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Abbreviations

AcCl	Acetyl chloride
Ac_2O	Acetic anhydride
AcOH	Acetic acid
$CDCl_3$	Deuterated chloroform
°C	Degree Celsius
DBU	1,8-Diazabycyclo[5.4.0]undec-7-ene
DIPEA	*N*,*N*-Diisopropylethylamine
DMSO-d_6	Deuterated dimethyl sulfoxide
DMF	*N*,*N*-Dimethylformamide
EtOAc	Ethyl acetate
Et_3N	Triethylamine
HRMS	High-resolution mass spectrometry
h	Hour
IR	Infrared
min	minutes
m/z	Mass/charge ratio
NMR	Nuclear Magnetic Resonance
N,*N*-DEA	*N*,*N*-Diethylaniline
p-TsOH	*para*-Toluenesulfonic acid
PTLC	Preparative Thin-Layer Chromatography
RCM	Ring-closing Metathesis
rt	Room temperature

TBAF	*tert*-Butylammonium fluoride
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TBDPSCl	*tert*-Butyldiphenylsilyl chloride
TsCl	*para*-Toluenesulfonyl chloride

Chapter 1: Background and project overview

Introduction

1.1. The impact of fungal infections

In developed countries, there has been outstanding breakthrough in the fight against most terminal illnesses such as cancer and HIV/AIDS.[1] However, the treatments of these illnesses pose dire consequences to developing countries as they involve expensive immunosuppressing therapy, cancer chemotherapy, prolonged use of antibiotics, hospital overstay and lack of highly active anti-retroviral therapy, which lead to complications such as secondary infections.[1,2] Studies have shown that immunocompromised HIV-positive patients are most susceptible to secondary infections, particularly fungal infections. It is important to note that, in the early days, HIV infection was detected by fungal manifestation.[3,4]

Fungal infections are difficult to cure and are considered a great health threat to humankind worldwide as the mortality rate continues to rise despite the available antifungal treatments.[5] In developing countries such as South Africa, fungal infections are significantly driven by the incidence of HIV together with tuberculosis and poverty.[6] Furthermore, the epidemiological data for fungal infections is notably poor due to misdiagnosis and lack of standards for reporting, regardless of the extensive research in fungal infections.[7] A 2013 study estimated an annual burden of approximately 3.2 million cases of fungal infections in South Africa, indicating that they cause a significant morbidity and concluded that South Africa has a high burden of HIV-related fungal infections compared to other Sub-Saharan countries. As a result, fungal infections should be considered a public health concern in South Africa.[6]

There are two types of fungal infections, the true pathogenic fungal infections, and the opportunistic fungal infections. The true pathogenic infections occur mostly in healthy individuals. They are mild and are of short duration. Individuals infected with pathogenic fungi can fully recover and have resistance to reinfection. Diseases such as blastomycosis, coccidioidomycosis, and histoplasmosis are mostly associated with pathogenic infections. In contrast, opportunistic fungi infect immunocompromised individuals due to the underlying illness and can exacerbate the illness. These infections are difficult to cure, and their

treatment does not guarantee reinfection free effect. The most common opportunistic fungal infections are candidiasis, cryptococcosis, aspergillosis, mucormycosis, and pneumocystis pneumonia.[8,9] Moreover, they are at the forefront of fungal infections accounting for approximately 90% of human mortality cases.[10]

1.2. Candidiasis

Candidiasis is a fungal infection caused by the *Candida* species, which is a member of the human microflora.[11] *Candida* species are commonly found in places such as the skin, nails, oral cavity, gastrointestinal tract, and female genital tract. Generally, *Candida* species are harmless in nature. However, abnormal conditions, an overgrowth of the fungi or an imbalance between the fungi and the host's environment can turn the fungi into an opportunistic fungus that causes diseases from superficial infections to systemic infections.[11,12] *Candida* species are ranked fourth among other pathogens isolated from bloodstream infections after other bacterial pathogens.[13] Although *Candida albicans* is the most prevalent and isolated *Candida* species in candidiasis cases, non-*albican* species such as *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *C. auris* in other cases of candidiasis are reported with increasing incidence.[12]

C. albicans is considered the most virulent among the other species. Owing to its diverse morphology, *C. albicans* can adhere to the host's tissues and to the indwelling medical devices and can induce systemic infections in immunocompromised individuals.[14] The commensal fungi can adapt to the host's environment, hence they differ from host to host and geographic regions accounting for the difference in strain transmissions.[1,14] In 2005, Blignaut *et al.*[1] reported that five clades of *C. albicans* were identified (clade I, II, III, E, and SA) in different geographic regions, clade E was isolated from Europe and clade SA in South Africa.

The spectrum of candidiasis caused by *C. albicans* ranges from vulvovaginal candidiasis, oropharyngeal candidiasis (OC), oesophageal candidiasis to a few cases of fungal keratitis. Vulvovaginal candidiasis is a common infection that affects two out of every three healthy females at least once in their lifetime without any symptoms.[15] Furthermore, an underlying illness can induce systemic vulvovaginal candidiasis.[2,15,16] OC has been associated with HIV

infection, low CD4 count and was thus used as a guide to initiate Anti-Retrovirus (ARV) therapy in resource-restricted communities.[3,4] During their investigation of the effect of OC and Body Mass Index (BMI) on treatment outcome in patients with HIV infection, Evans *et al.*[4] found that poor treatment outcome was associated with low BMI with the occurrence of OC.

South Africa, considered to have one of the largest populations infected with HIV globally, still lacks highly active ARVs. People depend on government hospitals for health services. Consequently, the incidence of OC continues to increase with the increase of HIV-positive individuals,[1,17] which ultimately elevates the burden of fungal infections in the South African health system.[6]

Table 1.1. The study of *Candida* count (cfu/mL) in the colonised groups of patients from the Charlotte Maxeke Johannesburg Academic Hospital, South Africa.[14]

Candida colonised group	No. of patients with range of *Candida* counts (cfu/mL)		
	1-100 (%)	101-1000 (%)	>1000 (%)
Normal subject n = 21	14 (66.66%)	7 (33.34%)	0
Denture wearers n = 26	1 (3.85%)	10 (38.46%)	15 (57.69%)
Cancer patients n = 16	1 (6.25%)	3 (18.75%)	12 (75%)

As mentioned above, *C. albicans* has been mostly isolated in oral cavities[18] of most healthy individuals and in HIV-positive individuals. The oral manifestation of *C. albicans* is also common in babies and patients who wear removable dentures.[7] The fungus is also found in the oral cavities of head and neck cancer (HNC) patients who wear prostheses than healthy individuals, as shown in Table 1.[14] In a study conducted to determine the effect of OC on the severity of dysphagia related to mucositis in HNC patients, Saito and colleagues [19] concluded that the occurrence of OC actually aggravated the pain and the severity of dysphagia in HNC

patients undergoing chemotherapy. *C. albicans* is believed to be one of the causative agents for fungal infections of the cornea (fungal keratitis) in young males involved in agricultural activities.[20]

1.3. Predisposing factors to candidiasis

Apart from HIV infections, predisposing factors for candidiasis include overstaying in intensive care units, the use of broad-spectrum antibiotics, solid organ transplants and the use of invasive surgical apparatus.[21] Patients who undergo immunosuppressive radiotherapy, chemotherapy and those using prostheses such as central venous catheters are also at high risk of contracting candidiasis.[22] Abdominal surgery trauma, diabetes mellitus,[23] translocation of the fungi from the gut to bloodstream,[24] cross-contamination within in-patients through hospital staff, blood transfusions and hyperglycaemia, as well as diseases and treatments related to the central nervous system have also been considered as factors predisposing humans to candidiasis.[2,25,26,27]

1.4. Cryptococcosis, Aspergillosis, Mucormycosis and Pneumocystis pneumonia

The second most prevalent fungal infection is cryptococcosis, with an estimated yearly global burden of 1 million cases accounting for 62000 deaths in Sub-Saharan Africa[7,9] and an occurrence of 8357 cases per year in South Africa.[6] Cryptococcosis is caused by *Cryptococcus neoformans* and *C. gatti*, and in contrast to *Candida* species, *Cryptococcus* species are distributed worldwide in the soil and trees (*C. gatti*).[2,28] Infection of the non-commensal fungi occurs through inhalation into the lungs leading to systemic infections.[7]

Pneumocystis pneumonia is another respiratory infection caused by *Pneumocystis jirovecii*. Pneumocystis pneumonia infection occurs via contact with individuals who carry the fungi in their lungs, and through contact with infected aerosols.[7] Although, pneumocystis pneumonia has been mostly isolated in patients living with HIV, its global burden has not been fully determined. Immunocompromised individuals are at high risk of contracting cryptococcosis, pneumocystis pneumonia, aspergillosis and mucormycosis. *Aspergillus fumigatus* and *A. flavus* are the causative agents of aspergillosis and are distributed worldwide. Invasive

aspergillosis has 50% mortality rate even when treated and it is 100% fatal if untreated.[7] The least prevalent fungal infection, mucormycosis, is caused by mucormycotina.[2] Table 1.2 shows the above-mentioned fungal infections together with their common predisposing factors. The unacceptable high mortality associated with these fungal infections clearly reflects gaps in the current antifungal therapy.

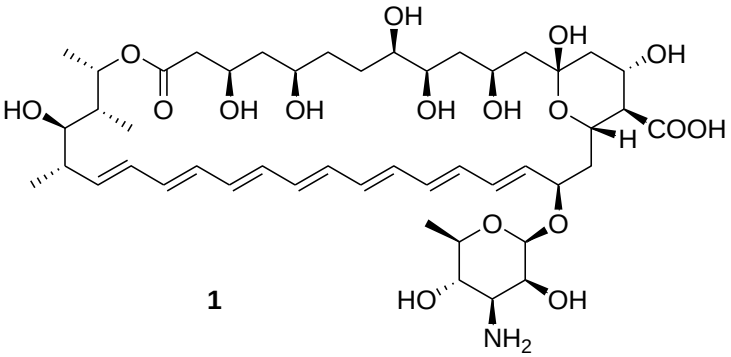
Table 1.2. Common fungal infections with their risk factors. Data is from Garbee *et al*.[2]

Type of fungal infection	**Risk factors**
Candida	Very low CD4 count Hyperglycaemia Antibiotic
Aspergillus	Chronic pulmonary disease Asthma, chronic bronchitis, Cystic fibrosis, contamination of air system
Cryptococcus	HIV/AIDS Exposure to soil with bird droppings
P. jirovecii	HIV/AIDS Chemotherapy patients Solid organ transplant recipients Rheumatologic disease
Histoplasmosis	Immunocompromised patients
Coccidioidomycosis	Immunocompromised patients Diabetic, pregnant.

1.5. Current antifungal agents

Owing to the close relatedness between fungi and humans, there are only three classes of antifungals, the polyenes (amphotericin B), the azoles (fluconazole), and the echinocandins.[5,10,13] Amphotericin B (**1**) was introduced in the 1950s and was used for approximately thirty years before the azoles were approved in the late 1980s.[28] Polyenes, including **1**, bind to ergosterol in the fungus cell membrane creating pores that render the cell

membrane highly permeable.[1] Antifungal activity of polyenes is unfortunately hindered by the nephrotoxicity associated with them and the emergence of natural resistance in *Candida* species. Hence, amphotericin B (**1**) is not available to more than 25% of the countries, and it is reserved for severe cases of cryptococcosis in South Africa.[5,29]



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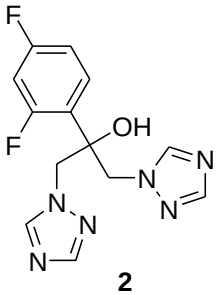

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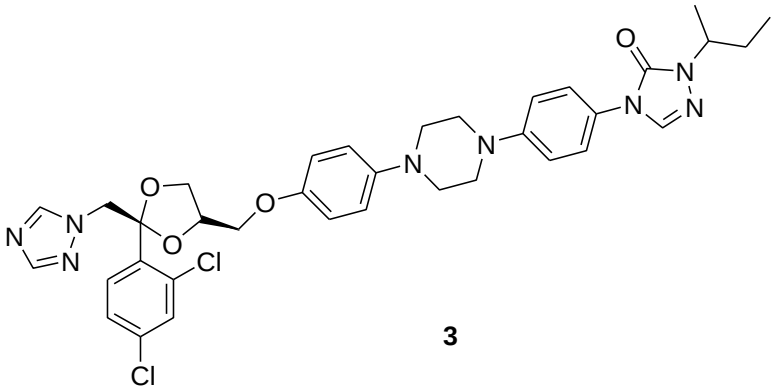

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Figure 1.1. Structures of the current commonly used antifungal drugs.

Since their approval in the late 1980, azoles, particularly fluconazole (**2**), and itraconazole (**3**) have been routinely administered for candidiasis in HIV-positive patients in Africa.[30] Due to the extensive use of fluconazole (**2**), long-term ARV therapy and overuse of antibiotics, *Candida* species have developed resistance to amphotericin B (**1**) and cross-resistance within the azole group.[11] The naturally derived Echinocandins inhibit the synthesis of 1,3-β-D-glucan, which compromise the fungal cell wall integrity and ultimately lead to cell death.[30] There is no known toxicity associated with echinocandins. Consequently, several guidelines recommend the use of echinocandins as the primary antifungal drugs for invasive candidiasis. The drawbacks associated with echinocandins include resistance of *Cryptococcus* species and that they can only be administered intravenously.[10,31]

Owing to the high mortality rate and the rapid development of drug resistance of fungal pathogens, there is an urgent need for the development of new drugs with reduced toxicity and reduced susceptibility to drug resistance.[32,33] Notably, drug resistance is not the only limiting factor in the battle against fungal infections. Drug discovery takes long and only a few drugs hit the market or rather the clinics. Moreover, there is a major access barrier to antifungals such as fluconazole in South Africa, as they are only available through the South African health products regulatory authority (SAHPRA) application, which can take few weeks. The US-FDA approved fluconazole can only be accessible through US-based companies, which can be costly.[34] Because of the challenges highlighted in this chapter, finding affordable and efficacious alternative medicines for treating fungal infections is vital. In Africa, the use of medicinal plants as remedies is common and widespread.[35]

1.5.1. Medicinal plants

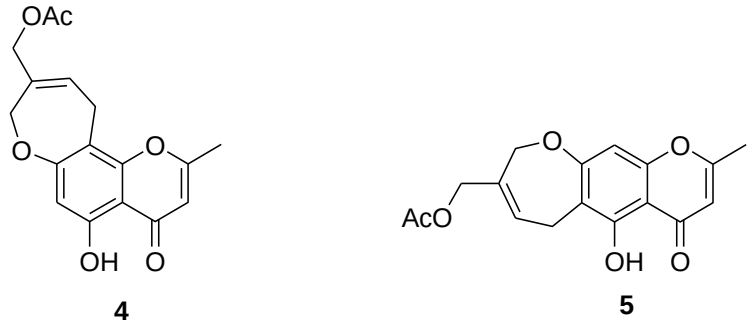
In South Africa, approximately 80% of people living in rural areas depend on medicinal plants for their primary healthcare needs.[36] Due to the higher number of HIV-infected individuals in rural areas, and the high cost of western medicine, most of the medicinal plants are traded as concoctions in local market outlets at a much cheaper cost.[37] Additionally, medicinal plants have been used for many years by traditional healers and general people who have acquired and transferred indigenous knowledge from one generation to the other.[37] Research on the screening of plants for antimicrobial activity has proven that a significant number of plants contains compounds which have antibacterial and antifungal activities.[38]

More than 120 South African plants are used for the treatment of oral diseases.[18] Masevhe *et al.*[39] investigated plants used by local traditional practitioners in Venda, South Africa, for management of candidiasis and related fungal diseases. They reported 12 new plants used by the traditional practitioners that show antifungal activity *viz. Acacia caffra*, *Clerodendrum glabrum*, *Croton gratissimus*, *Elaeodendron transvaalense*, *Faurea saligna*, *Hippocratea longipetiolata*, *Osyris lanceolata*, *Richardia brasiliensis*, *Schkuhria pinnata*, *Schotia brachypetala*, *Spilanthes acmella*, *Strychnos potatorum*, *Vangueria infausta* subsp. *infausta* and *Withania somnifera.*[39] In another study, the Phytomecidine group at University of Pretoria investigated several plant species for antifungal activity.[40-42] These included *Bidens*

pilosa, *Dichrostachys cinerea*, *Harpephyllum caffrum*, *Ptaeroxylon obliquum*, *Rapanea melanophloeos*, *Sclerocarya birrea*, *Securidaca longepedunculata* and *Ziziphus mucronate*. The bioassay results showed that *P. obliquum*, had the highest activity against *C. albicans*.[41,42]

P. obliquum (Sneezewood), is the only species in the genus *Ptaeroxylon*.[43] The powdered wood of *P. obliquum* is used to relieve headache and is also used to manage heart diseases and arthritis.[40] Phytochemical investigations of *P. obliquum* led to isolation of several compounds that included chromones, coumarins and terpenoids.[40,42,44-46] Among the isolated compounds, the 12-*O*-acetyl derivative of eranthin **4**, named obliquumol, was reported to be the most potent antifungal compound by Van Wyk and colleagues (Figure 1.2).[40-42] In their study, the novel compound **4** was found to be more active (MIC = 0.004 mg/mL) against *C. albican* ATCC 10231 than the standard antifungal agent amphotericin B (**1**) (MIC = 0.11 mg/mL). Moreover, obliquumol (**4**) exhibited a much lower cytotoxicity (LC_{50} = 7.23 µg/mL) than amphotericin B (**1**) (LC_{50} = 1.46 µg/mL).[40,41] It is important to note that the linear isomers of eranthin and the acetyl derivative, namely ptaeroxylinol and ptaeroxylinol acetate (**5**), respectively, were previously reported from the same plant, by other independent studies.[44-46]

Owing to the high potency of obliquumol (**4**) against several fungal strains, including candida strains, the oxepinochromone moiety could represent a new anticandidal scaffold.[40] Success in the total synthesis of the oxepinochromones **4**, **5** and their derivatives will help with the unambiguous confirmation of the structures of the two compounds. It will also allow for further investigations of their biological activities, structure-activity relationship studies and tissue distribution studies. Structural modification of the oxepinochromones may render more active derivatives against most of the clinical isolates of candida and increase selectivity.



4 **5**

Figure 1.2. The antifungal compound eranthin acetate/obliquumol (**4**) and its linear isomer ptaeroxylinol acetate (**5**) isolated from *P. obliquum*.

1.6. Aims and objectives

The aim of the study was to synthesise eranthin acetate/obliquumol (**4**), ptaeroxylinol acetate (**5**), and their derivatives, in order to investigate their biological activities and biodistribution. The objectives were to:

- Synthesise eranthin acetate/obliquumol (**4**), ptaeroxylinol acetate (**5**), and derivatives.
- Develop a robust synthetic route that will be used in the total synthesis of carbon-14 labelled eranthin acetate (**4**) and its structural analogues.
- Investigate the anticandidal and antifungal activities of the synthesised oxepinochromones and their derivatives.

1.7. Thesis outline

The dissertation is comprised of five chapters.Chapter 1 introduces the aim and objectives of the project. Chapter 2 examines the nature of chromones, and their biological activities followed by the review of the different synthetic routes employed in the preparation of chromones. Also included in the chapter is the biological activities of different types of oxepines and a detailed review on their synthesis. This is followed by the results and discussions in Chapter 3. This chapter is divided into two sections. The first section deals with the total synthesis of eranthin acetate (**4**), ptaeroxylinol acetate (**5**) and their structural derivatives. The second section reports on the development of a robust synthetic route that will allow for the late-stage incorporation of the carbon-14 isotope into both eranthin acetate

and ptaeroxylinol acetate. Chapter 4 concludes the overall study and the proposed future work. Lastly, experimental procedures are presented in Chapter 5.

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Chapter 2: Literature review

2.1. Introduction

The dependence on natural products for cheaper health care is unfortunately associated with uncertain toxicity, safety, efficacy, and quality. Therefore, this has led to the isolation and total synthesis of pure active compounds by scientists and pharmaceutical companies.[1] The current study focuses on the total synthesis of oxepinochromones obliquumol/ eranthin acetate (**4**) and ptaeroxylinol acetate (**5**). Eranthin acetate (**4**) has gained much interest owing to its antifungal activity.[2,3] Besides its anticandidal activity, eranthin acetate (**4**) is comprised of two chemically important moieties: chromone and oxepine moieties. Therefore, it presents enhanced biological properties (hybrid drug concept).[4]

2.2. Chromones and their Biological Activities

The name chromone is derived from a Greek word *chroma* which means colour and was first used by Kostanecki and Block to describe coloured natural oxygen-containing heterocycles with the benzopyran-4-one scaffold.[5,6] Chromones are structural isomers of coumarins (Figure 2.1) and only differ in the position of the carbonyl group and the double bond.[7] The rigid heterocycles are naturally occurring molecules abundant in plants and occur in notable quantities in other species.[8] Moreover, they are present in our everyday diet such as fruits, tea and olive oil.[9,10] These secondary metabolites are known to contribute to plant pigmentations responsible for attractive coloration for pollinators. In addition, they provide protection in plants against fungal pathogens or UV-radiation and are also reported to be involved in physiological processes such as photosynthesis, photosensitisation and growth regulations.[11]

The benzopyran-4-one system is a core fragment in several pharmacologically important molecules such as flavones, isoflavones, and xanthones as depicted in Figure 2.1. Chromones can be classified into two classes namely, simple chromones and fused chromones. Simple chromones constitute simple substituents such as a methyl group at C-2, or just hydroxy groups at C-5/C-7 positions and sometimes a phenyl group at C-2 (flavone) or at C-3

(isoflavone).[12,13] The fused chromones class includes chromones that are fused to other heterocycles such as furanochromones, pyranochromones and oxepinochromones as well as other ring systems.[13]

There is a huge number of discovered chromone based molecules with desirable pharmacological effects which did not reach clinical trials or are yet to be approved by the SAHPRA or FDA.[14] Chromones play an important role in medicinal chemistry and are featured in half of the clinically used drugs.[5,15] Owing to their structural versatility, chromones are considered privileged scaffolds and are defined as "a single framework able to provide ligands for diverse receptors".[9,16,17] The structural features such as the substituent pattern and the number of substituents attached to chromones determine their large variety of biological and pharmacological activities.[3,18]

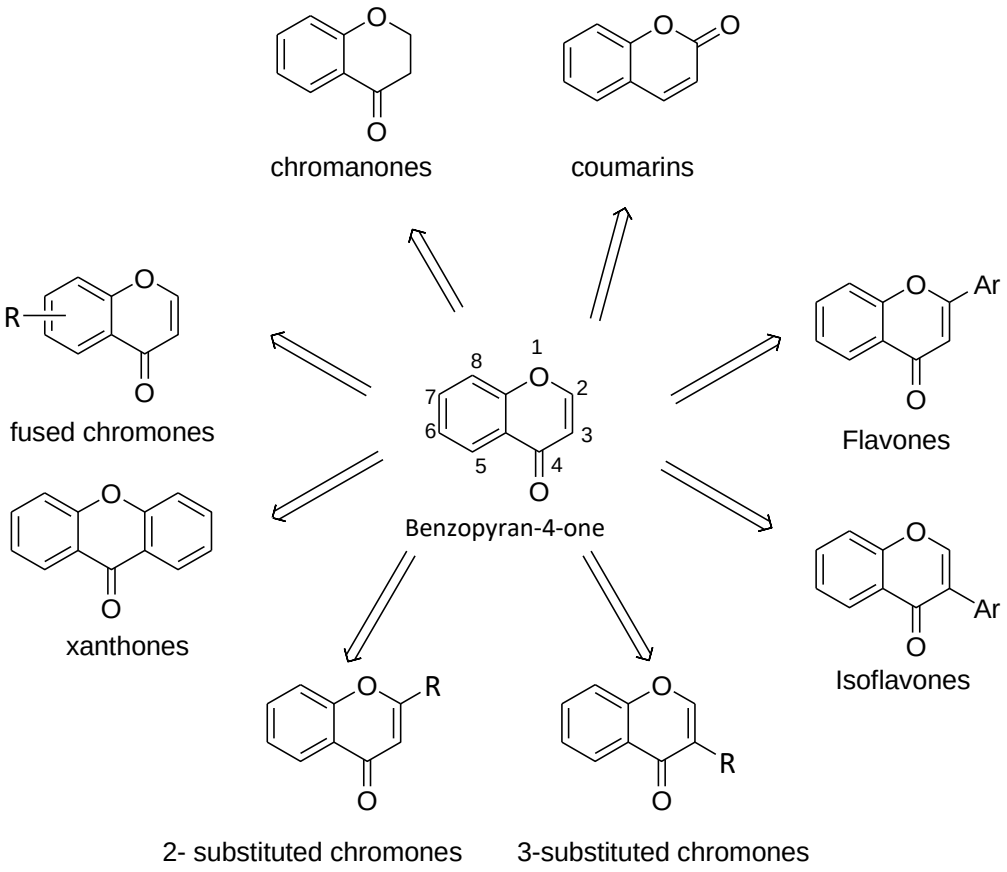
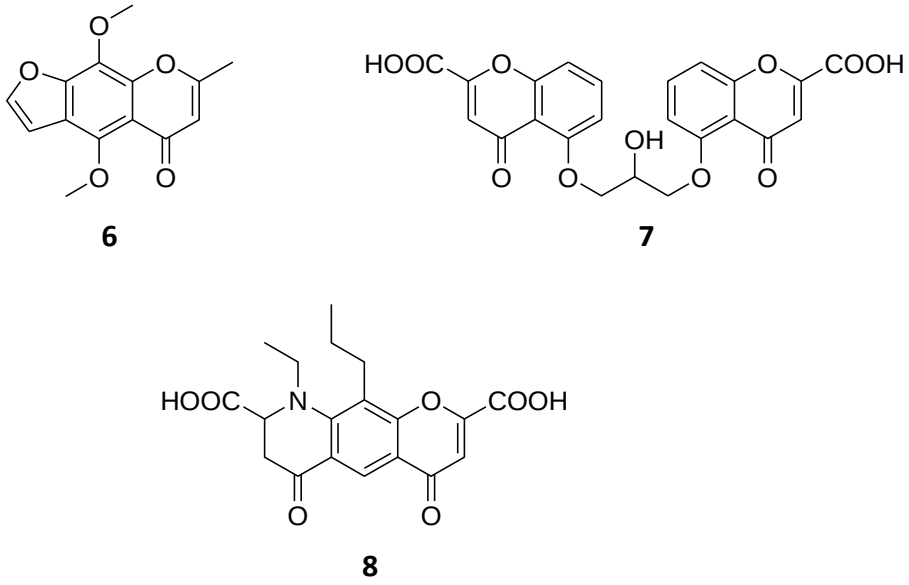


Figure 2.1. Classes of chromone derivatives.

The pharmacological effects attributed to chromones and their derivatives include anticancer, antiviral, antimicrobial, antitobacco mosaic virus, antifungal, anti-inflammatory, antidiabetic

and antioxidant activities.[15,19-23] Additionally, it has been reported that some chromone derivatives exhibit activities such as neuroprotective effect, radical scavenging activity, cardiovascular protective, hepatoprotective and cytotoxic activities.[3,24,25] Other biological activities that have been associated with chromones and derivatives include antiallergenic, anti-tubulin, anti-hypertensive,[1] immunostimulating effect, anti-ulcer and wound healing properties.[14] Several other researches have reported that some chromones show enzymatic inhibitory properties towards different systems including oxidoreductase and kinase.[9]



6 **7** **8**

Figure 2.2. Examples of clinically used chromone based molecules.

The chemistry of chromones and their derivatives has been studied considerably and has led to the development of novel chromones for medicinal use (Figure 2.2). Khellin (**6**), a natural furanochromone isolated from the fruits and seeds of *Ammi visnaga*, was the first chromone to be used clinically as a muscle relaxant in the treatment of angina pectoris, asthma and urinary bladder spasms.[9,11,12,14,16] However, Khellin (**6**) was found to show unfavourable side effects. Further studies to find its replacement led to the discovery of more chromone-based molecules including cromoglycate (**7**). Cromoglycate (**7**) was used in the treatment and as prophylaxis of asthma and other allergic conditions. Cromoglycate (**7**), when administered as a disodium salt,[16] works as a direct mast cell stabiliser in asthma, allergic conjunctivitis and rhinitis.[9] The effect of cromoglycate (**7**) was limited by its short half-life. Nedocromil (**8**) was also one the earliest discovered chromones used to prevent wheezing, for breath shortness

and other breathing difficulties.[26]

Gonzalez *et al.* reported the isolation of several chromones from *Cneorum tricoccum* and *C. pulverulentrum* species. They reported that, among the chromones isolated, ptaeroglycol (**9**), pulverochromenol (**10**), and prenylspathelia chromene **11**, among the chromones isolated, exhibited strong HeLa cells inhibitory activity with ID_{50} ranging between 1-5 µg/mL.[27] The ethanolic extract of the Chinese eaglewood, *Aquilaria sinensis* Gilg showed neuroprotective properties against glutamate induced and corticosterone induced neurotoxicity in P12 pheochromocytoma and human U251 glioma cells. From this extract, a new compound **12** was identified (Figure 2.3).[28] Tanaka *et al.* isolated more than 27 chromones from the methanolic extract of *Hypericum sikokumontanum* and found that compound **13** showed activity against *Helicobacter pyroli* and cytotoxic activity against human cancer cell lines.[29] It has been reported that chromones with free hydroxy groups can have inhibitory activity against HIV-1 protease, an important enzyme in the AIDS virus responsible for viral replication.[12] This is exemplified by quercetagetin (**14**), a flavone first isolated in 1994 from *Sculletaria baicarensis,* known to have HIV-1 integrase inhibitory activity due its many hydroxy groups (Figure 2.3).[5] Boonpong *et al.*[30] isolated chromone derivatives that were found to possess antitubercular and anti-plasmodial activities from the roots of *Artocarpus altilis*.

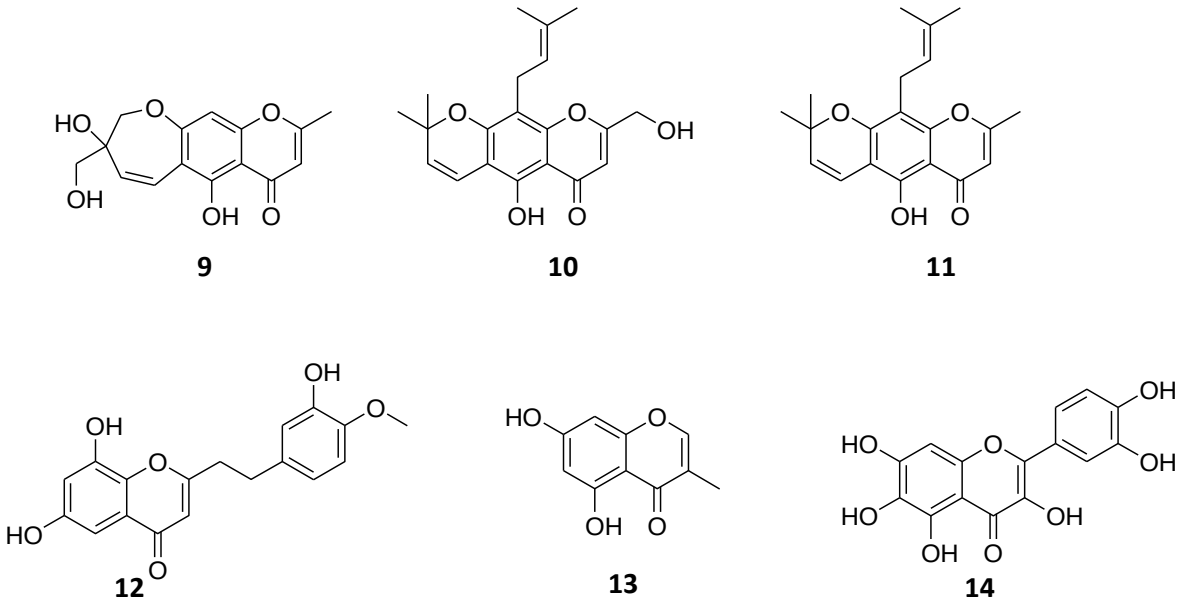
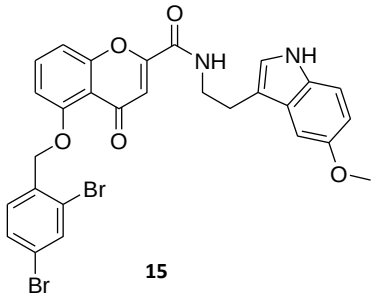
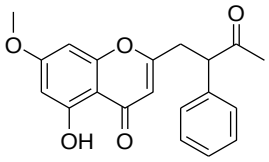


Figure 2.3. Examples of biologically active chromone derivatives.



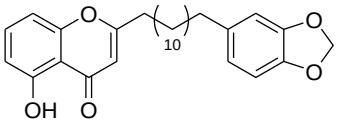
15

BCRP (cancer cell membrane protein) inhibitor



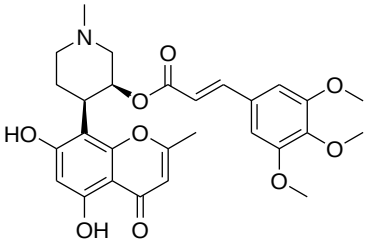
16

Whitening agent: tyrosinase inhibitor



17

Traditionally used against sterility



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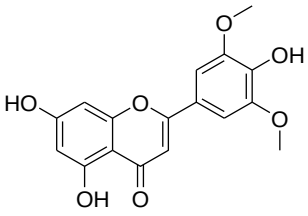
Dose dependent osteoclat differentiaton inhibitor



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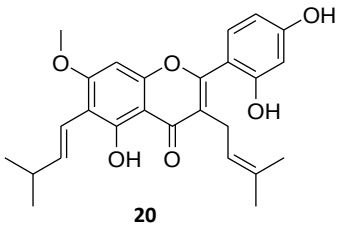
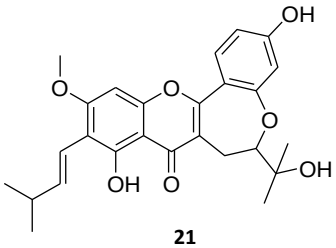
Antioxidant, antiviral, antihistamic, and anticancer



20

Antitubercular and antiplasmodial



21

Antitubercular and antiplasmodial

Figure 2.4. Discovered chromones with their biological activities.

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Recent studies have reported that the chromone skeleton was found to be a promising scaffold in the development of novel ABCG2 (cancer cell membrane protein) inhibitors.[31] Roussel *et al.* conducted quantitative structure-activity relationship (QSAR) studies on a set of chromone derivatives, and subsequently synthesised the most potent chromones. They found chromone **15** (synthesised and simulated form) to be the most potent ABCG2 inhibitor among the compounds synthesised.[31] Figure 2.4 shows more of the interesting chromone derivatives with their biological activities. Compound **16** was found to possess higher tyrosinase inhibitory activity than other whitening agents used in the cosmetic industry.[32] Mbah *et al*. isolated the chromone **17** as an active compound from *Peperomia vulcanica* Baker & C. H Wright, a plant used traditionally in Cameroon against sterility.[33] Chrotacumine G (**18**), isolated from the barks of *Dysoxylum acutangulum*, showed dose-dependent osteoclast

differentiation inhibitory activity, Morita and co-workers suggested that it may play an important role in the treatment of bone diseases.[34] Tricin (**19**) is a natural flavone that occurs as a glycoside in *Poaceae* species including rice, oats, and wheat. It has been reported to exhibit activities such as antioxidant, anticancer, anti-inflammatory, antiviral and antihistamic.[35] Compounds **20** and **21**, natural chromones isolated from the roots of *Artocarpus altilis*, were found to exhibit antitubercular and antiplasmodial activities.[30]

2.3. Synthetic approaches to chromones

Generally, the synthesis of simple chromones can be accomplished using 2-hydroxyacetophenones and phenols as starting materials. Despite the modifications and advances in the synthetic routes, the use of 2-hydroxyacetophenones and phenols is still the most cited in literature. There are three different classic synthetic routes that have been used for the synthesis of chromones *viz.*, Claisen condensation (Classic Claisen-Schmitt condensation, Baker-Venkataraman, and Kostanecki-Robinson), benzopyrylium salts and *via* Vilsmeier–Haack reactions illustrated in Figure 2.5 below.

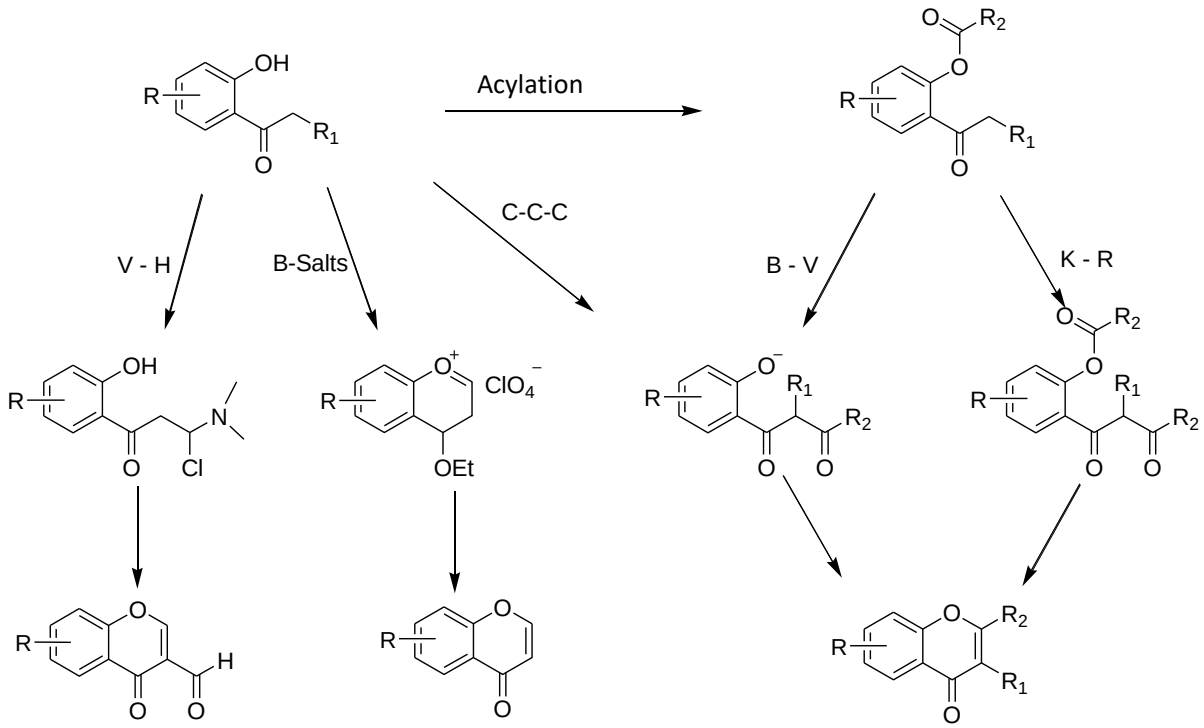
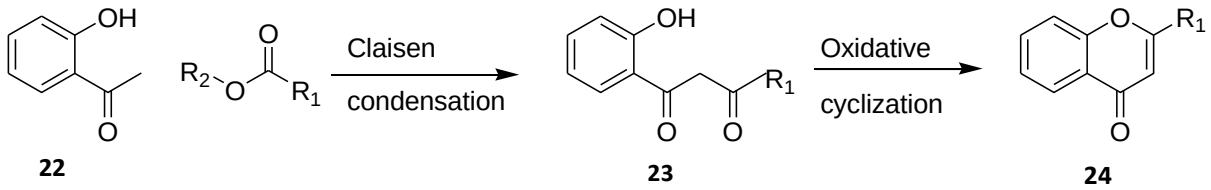


Figure 2.5. Schematic summary of the widely used classic synthetic routes for the preparation of chromones. V-H; Vilsmeier–Haack, B-Salts; Benzopyrylium salts, C-C-C; Classic Claisen condensation, B-V; Baker–Venkataraman, K–R; Kostanecki–Robinson reaction.

2.3.1. Classic Claisen condensation

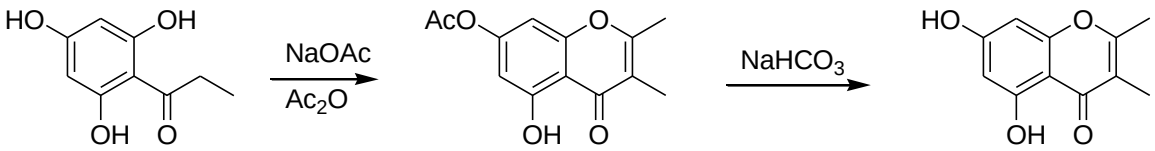


22 **23** **24**

Scheme 2.1. Typical Claisen-Schmidt condensation.

The Claisen condensation reaction is a well-known method.[36] It was first used for the synthesis of the chromone, 7-ethoxychromone-2-carboxylic acid by Kostanecki, Paul, and Tambo by constructing chromones over two steps from 2-hydroxyacetophenones (**22**). Scheme 2.1 outlines a typical Claisen-Schmidt condensation whereby the first step entails the reaction of a 2-hydroxyacetophenone (**22**) and a carboxylic ester in the presence of a strong base to afford a 1,3-diketone intermediate **23**, and subsequent cyclisation under acidic conditions using conventional heating to afford a chromone **24**.[1,6] Acid catalysts that are usually used include H_2SO_4, HCl and hydriodic acid.[37] More recently, microwave assisted Claisen condensation has been employed in the synthesis of chromone derivatives to conveniently reduce reaction time and the amount of solvents used.[38]

Kostanecki, Robinson, and co-workers modified the Claisen condensation method by reacting 2-hydroxyacetophenone with an aliphatic acid anhydride in the presence of a base such as K_2CO_3 without the acidification for ring closure. This route is called the Kostanecki-Robinson reaction and typically occurs at temperatures over 160 °C. Mild reaction conditions have been reported where K_2CO_3 in acetone is used under reflux for 24 h.[6,12] This type of a method is depicted in Scheme 2.2, where compound **27** was prepared in 83% yield, from the reaction of 2,4,6-trihydroxypropiophenone (**25**) with acetic anhydride and sodium acetate under reflux to give **26** in 56% yield. Deacetylation of 26 yielded the desired product.[39]

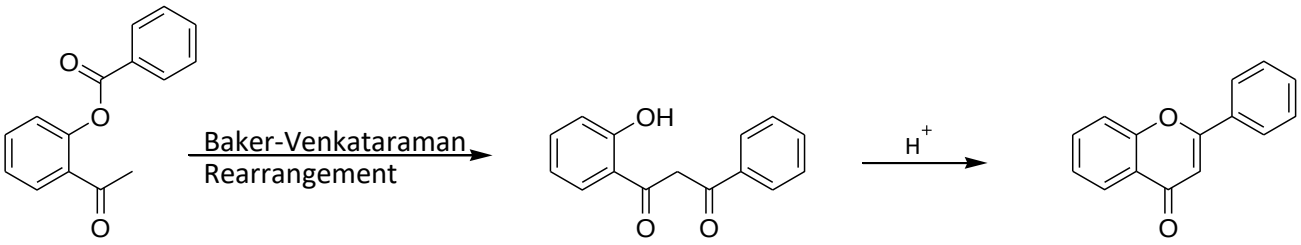


25 **26** 56% **27** 83%

Scheme 2.2. Synthesis of chromone 27 *via* Kostanecki-Robinson reaction.

2.3.2. The Baker-Venkataraman reactions

The Baker-Venkataraman reaction has received numerous citations over the years due to its use in the formation of carbon-carbon bonds *via* O→C acyl migration. Baker and Venkataraman independently reported the isolation of a 1,3-diketone following the .rearrangement of a 2-aroyloxyacetophenone and this method has since been explored by many scientists.[40-42] This method is another variation of a Claisen condensation as it involves the acylation of *ortho*-hydroxy acetophenone in a basic condition to form a 1,3-diketone intermediate.[12,37] The acyl group can be aliphatic or aryl leading to the production of an array of chromone derivatives. In contrast to classic Claisen condensation, the Baker-Venkataraman involves intramolecular condensation of a ketone and an ester, *ortho* to each another.[12,37,41] Other hydroxy groups on the aromatic ring may be acetylated during acetylation and hydrolysed back during work up.

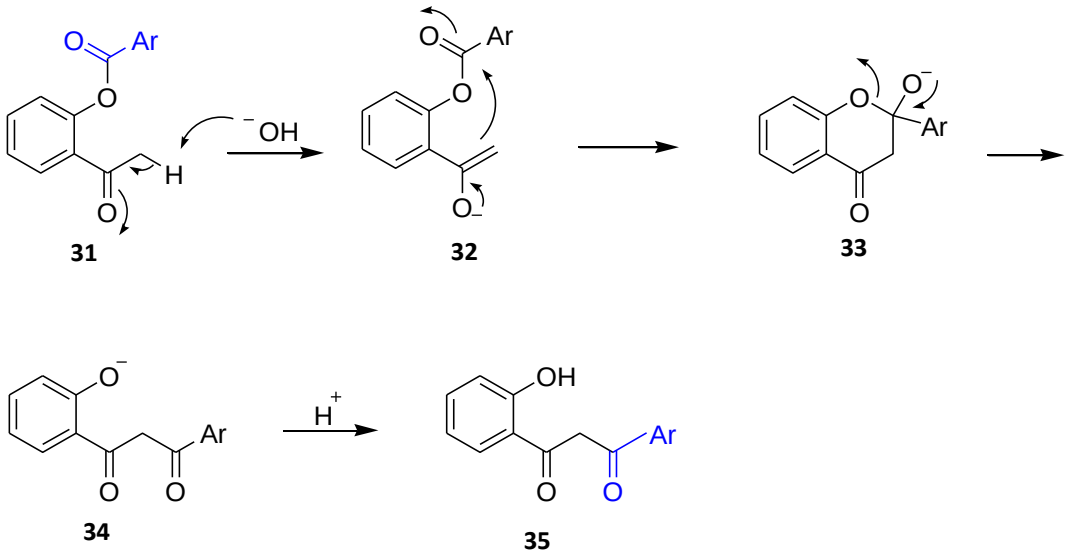


28 **29** **30**

Scheme 2.3. The initial Baker-Venkataraman rearrangement application in the synthesis of flavone **30**.

This method was originally used in the synthesis of flavone **30** from *ortho*-benzoyloxy acetophenone **28** which undergoes rearrangement to afford *ortho*-hydroxy dibenzoyl methane intermediate **29** as outlined in Scheme 2.3.[6] Contrary to the *in situ* cyclisation that occurs in Konstanecki-Robinson reaction, the Baker-Venkataraman rearrangement involves

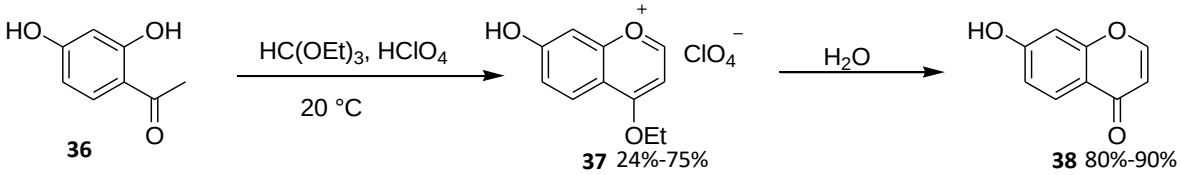
isolation of the intermediate before cyclisation occurs.[40] Several modifications have been proposed to this method in the attempt to perform the cyclisation step without isolating the 1,3-diketone intermediate.[6]



Scheme 2.4. The mechanism of Baker-Venkataraman rearrangement.

The proposed mechanism for the Baker-Venkataraman rearrangement is outlined in Scheme 2.4. The reaction proceeds with a base abstraction of the α-hydrogen of the acetophenone **31** resulting in the formation of an enolate **32**. An intramolecular attack of **32** onto the ortho-ester carbonyl affords a charged cyclic hemiacetal **33**. The charged hemiacetal subsequently tautomerizes into a more stable phenolate **34** which, during acid work-up, affords the 1,3-diketone **35** that can be isolated and subjected to cyclisation under acid conditions.[41]

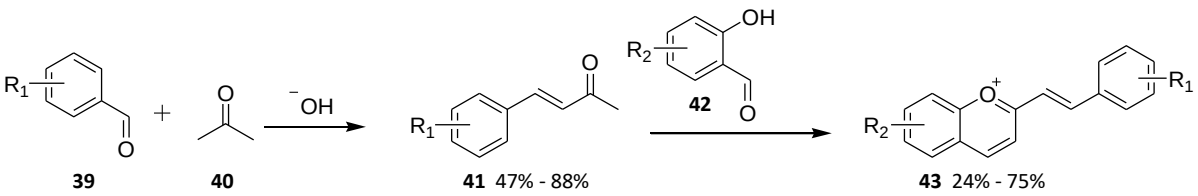
2.3.3. Synthesis of chromones *via* benzopyrylium salts



Scheme 2.5. The formation of benzopyrylium salts in the preparation of 2-non substituted chromone derivatives.

This methodology is usually used for the synthesis of 2-non-substituted chromone derivatives

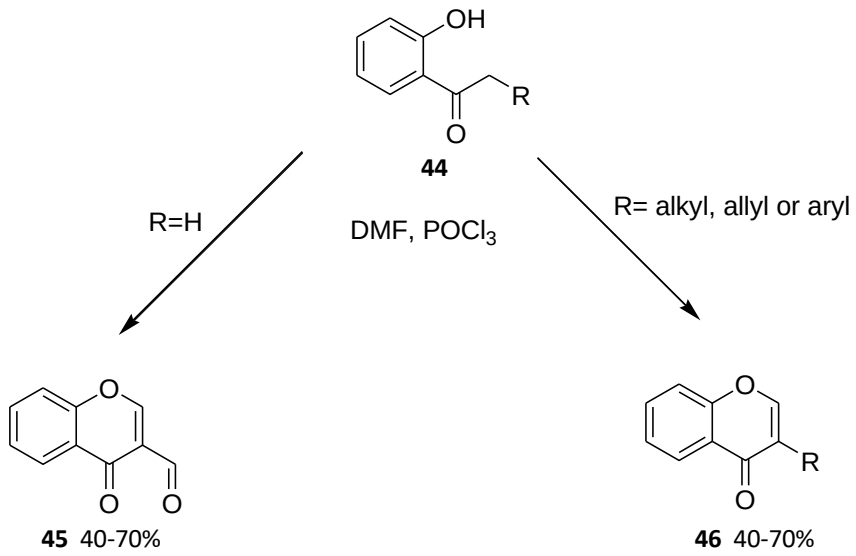
as depicted in Scheme 2.5. 2-Hydroxyacetophenone **36** reacts with triethyl orthoformate and a strong acid such as perchloric acid affording a benzopyrylium salt **37**, which is subsequently converted to the chromone **38** in good yields by heating in water.[43] Most of 3-substituted chromone derivatives such as 3-hydroxychromones and 3-methylchromones have been obtained through this route.[6] Scheme 2.6 illustrates the synthesis of a series of 2-styryl-1-benzopyrylium compounds **43** by condensation of 2-hydroxybenzaldehyde **42** and styrylmethylketones **41** reported by Gomez *et al*. The precursors, styrylmethylketones **41** were prepared by condensation of benzyladehydes **39** and acetone (**40**).[44]



Scheme 2.6. Condensation of chalcones and styrylmethylketones to afford a benzopyrylium salt.

2.3.4. Vilsmeier-Haack reactions

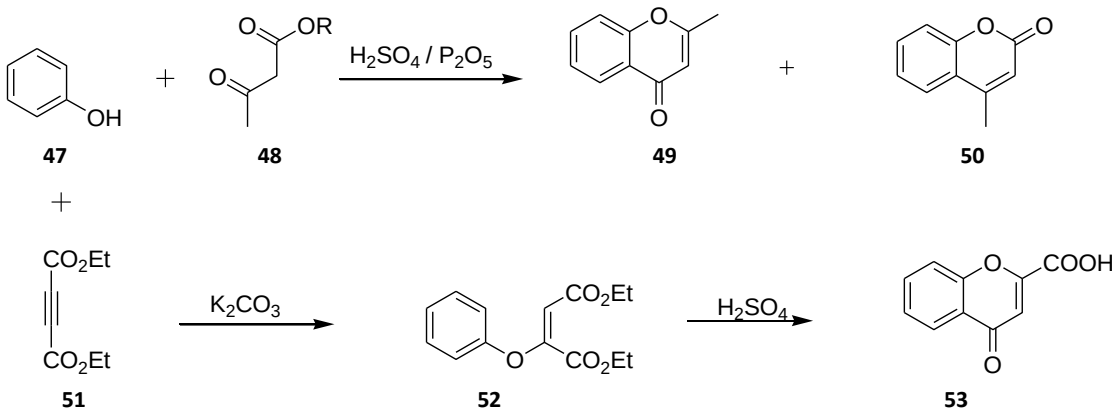
Reactions *via* Vilsmeier-Haack methodology involves the reaction of an *ortho*-hydroxy acetophenone **44** and phosphorous oxychloride in DMF (Scheme 2.7). This one pot procedure was first applied in 1973 for the synthesis of 3-alkyl (**46**) or formyl (**45**) chromones in modest to good yields (40-70%).[6,45] Ramanjaneyulu *et al*.[8] synthesised 3-formyl chromone from 2-hydroxy acetophenone using this method in dry DMF.



Scheme 2.7. Synthesis of 3-alkyl/formal chromones *via* the Vilsmeier-Haack method.

2.3.5. Synthesis of chromone derivatives *via* the Simonis reaction

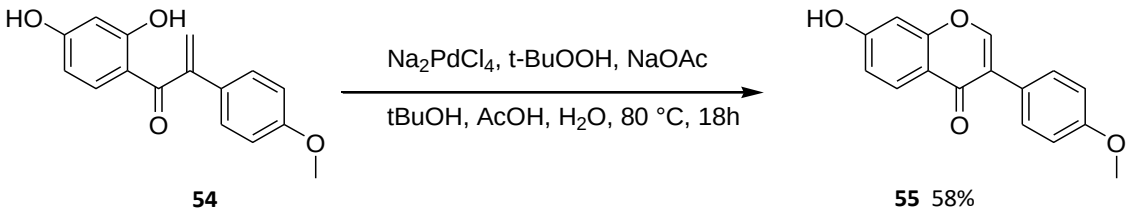
Besides 2-hydroxyacetophenones, chromones can be synthesised from a variety of starting materials such as phenols, salicylic acids, and derivatives. The Simonis reaction is an example of as method that has been used to obtain chromones from phenols.[22] In this reaction, a phenol **47** reacts with a β-ketoester **48** to give either 2-substituted chromones **49**, coumarin derivatives **50** or a mixture of both. Formation of chromones is favoured when P_2O_5 is used as a catalyst and when either the phenol contains deactivating groups such as halogen or nitro groups, or when the ketoester is α-substituted. The use of H_2SO_4 favours the formation of coumarins (Pechman condensation).[46] This method has also been used in the synthesis of chromone-2-carboxylic acids **53** through the ether **52**, when phenol reacts with diethyl acetylene dicarboxylate **51** as shown in Scheme 2.8**.**



Scheme 2.8. Synthesis of chromone derivatives from phenols *via* Simonis or Pechman reactions.

2.3.6. Wacker-Cook oxidation

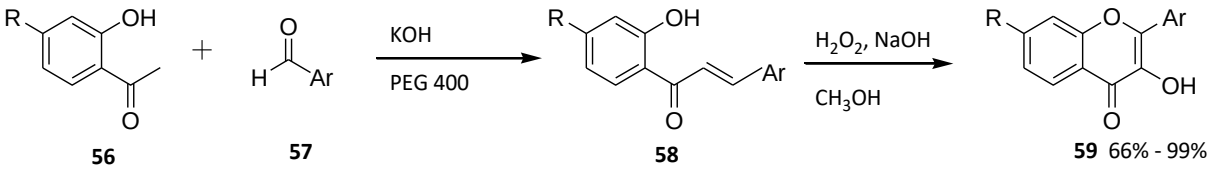
Wacker-Cook oxidation has become an efficient route to obtain a versatile class of functionalised molecules found in drug discovery. This method involves the intramolecular oxidative cyclisation of internal/terminal alkenes/alkynes under a wide variety of reaction conditions. Reports show that the deprotection-cyclisation-oxidation steps can be done in one step.[47] In 2009, Granados-Covarrubias and Maldonado employed this method to synthesise an isoflavone, formononetin **55**, using a palladium complex (Na_2PdCl_4) and t-BuOOH in a buffered (AcOH-NaOAc) aqueous *t*-BuOH media at 80 °C from the alkene **54** (Scheme 2.9).[48]



Scheme 2.9. Wacker-Cook oxidation to synthesise isoflavone.

2.3.7. The Algar-Flynn-Oyamada reaction

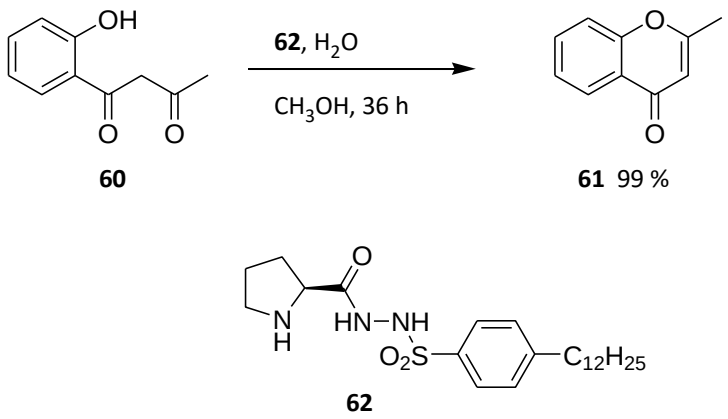
Another frequently used method for the synthesis of chromone derivatives is by the alkaline hydrogen peroxide oxidation of chalcones or 1,3-diketones **58**, known as the Algar-Flynn-Oyamada reaction.[49] Recently, Kamble *et al.* synthesised a series of 3-hydroxy chromones **59** by the Algar-Flynn-Oyamada method where the chalcones **58**, prepared *via* Claisen condensation of 2-hydroxy acetophenones **56** with aldehyde **57**, were subjected to oxidative cyclisation in basic solution by H_2O_2 (Scheme 2.10).[18]



Scheme 2.10. Synthesis of 3-hydroxychromones from chalcones by the Algar-Flynn-Oyamada pathway.

2.3.8. Other reactions applied to synthesise chromone derivatives.

Various other methods have been reported for the synthesis of chromone derivatives in literature with more advances and modifications. In 2014, Wen and co-workers developed a facile and versatile organocatalytic approach to synthesise 2-substituted and 2,3-disubstituted chromone derivative under mild conditions with yields up to 99%. Additionally, they synthesised and employed a novel catalyst **62** for the first time. In their method, when using a novel catalyst **62** in CH_3OH (Scheme 2.11), a diketone **60** was successfully cyclised to afford a chromone **61** in 99% yield.[50]



Scheme 2.11. A versatile organocatalytic approach for the synthesis of chromone derivatives.

As demonstrated above, several synthetic routes for the preparation of chromone derivatives have been established with varying reaction conditions from a variety of starting material. The 2-hydroxyacetophenone is the most utilised molecule as the starting material for the synthesis of functionalised chromones. Apart from the widely explored synthetic routes, the Kostanecki-Robinson and Baker-Venkataraman reactions are mostly employed, owing to the employment of the readily accessible bases and acids such as K_2CO_3 and HCl. No metal complexes and ligands are utilised in these strategies. Despite the good yields, the drawback associated with these methods is the high temperatures needed for the preparation of the chromone moiety and the long reaction time. Thus, a more robust strategy with short reaction time is needed for the preparation of chromone derivatives.

2.4. Oxepine-containing compounds and their biological activities

Oxepine derivatives are not as ubiquitous and extensively studied as chromone derivatives, although their prevalence in natural products has been observed. The terms oxepine, oxepene, and oxepin have been used to describe any oxygen-containing seven-membered heterocycles in literature.[51] As illustrated in Figure 2.6, oxepines can be classified into four categories according to their level of saturation *viz.*, Oxepines as fully unsaturated seven-membered ring bearing three C-C double bonds, dihydrooxepines bear two double bonds, tetrahydrooxepines as heterocycles bearing only one double bond and lastly, oxepanes as fully saturated seven-membered ring system bearing no double bond.[51] Nonetheless, the

current study will use the term oxepines to collectively describe seven-membered oxygen-containing heterocycles. Comparable to chromone derivatives, the level of saturation and substitution pattern on the oxepine ring system determine their vast range of pharmacological properties.

Figure 2.6. Classification of oxygen-containing seven-membered heterocycles.

Natural oxepines are generally flanked by aromatic rings forming benzo or dibenzo oxepines.[52] Natural products containing oxepine scaffold are rare and yet they possess interesting pharmacological activities such as antidepressant, anticholinergic, antipsychotic, analgesic and anti-inflammatory properties.[53,54] Moreover, oxepine bearing molecules were reported to exhibit angiotensin II receptor antagonistic properties,[55] cardiovascular, oral hypotensive, antiulcer, antimalarial activities and cancer cell growth inhibitory effects.[56,57] These aforementioned properties prompted the interest in synthesising molecules with oxepine rings and combining the ring system with other biologically active molecules to enhance their activity in drug design.

Fused oxepine ring systems have been seen in pharmaceutically relevant natural molecules such as the fungal metabolite pterulone (**63**), which was isolated from various fungus species and was first synthesised by Grubbs and co-workers.[58-61] Pterulone (**63**) was reported to show antifungal and antibiotic properties. In addition, pterulone (**63**) prevents the eukaryotic respiratory chain at the NADH: ubiquinone oxidoreductase.[60] Radulanin H (**64**) and E (**65**) isolated from the liverwort *Radula perrottetii* and R*. variabilis* are also reported to be some of the earliest discovered oxepine containing molecules with inhibitory activities towards calmodulin and cyclooxygenase.[60] Pacharin (**66**) from the *Bauhinia purpurea* possess cell growth inhibitory properties (Figure 2.7).[60]

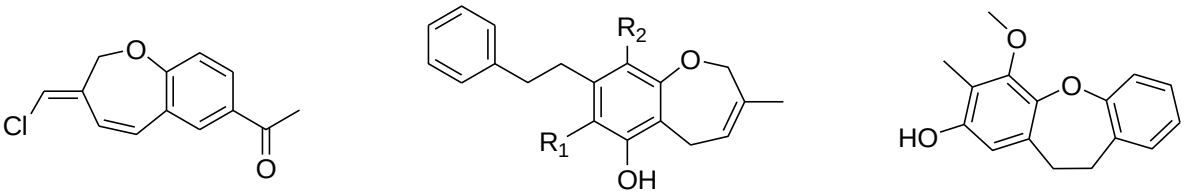


Figure 2.7. Earliest discovered Natural Oxepine derivatives.

Yuan *et al*. isolated a new oxepine molecule 6-methoxy-3-methyl-8-(2-oxopropyl) benzo[b]oxepin-5(2*H*)-one (**67**) (Figure 2.8) from the stems of *Cassia fistula* and confirmed its anti-tobacco mosaic virus activity with 22.5% inhibition rate.[62] In 2003, Reddy *et al*.[63] isolated and identified a new oxepine molecule, 5,6-dihydro-1,7-dihydroxy-3,4-dimethoxy-2-methyldibenz[*b,f*]oxepin (**68**). When Haoxin Li and co-workers were investigating the plant species *Empetrum nigrum* L. in 2015, they isolated and determined the structure of a new dibenz[*b,f*]oxepine **69**, a derivative of **68**, which showed significant inhibitory activity towards *Mycobacterium tuberculosis* H37Ra.[64] The oxepino[2,3-*b*]chromen-6-one molecules **70**-**72** were isolated from the endophytic fungus *Microsphaeropsis* species by Krohn *et al*. together with other derivatives. Compound **71** was then prepared by oxidation of the allylic alcohol **70** with MnO_2. They reported that the compounds showed strong antibacterial, fungicidal, and algicidal properties based on preliminary studies.[65]

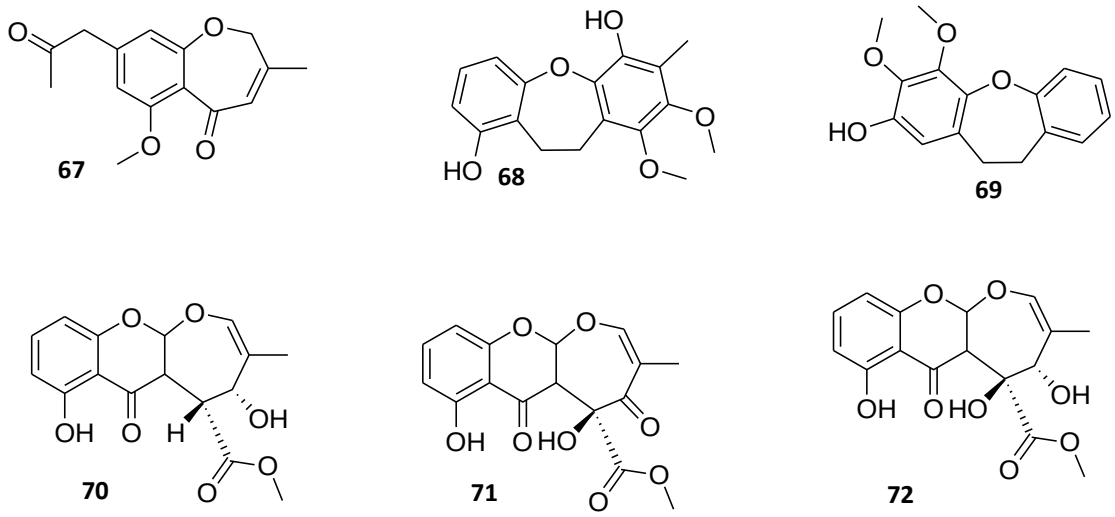


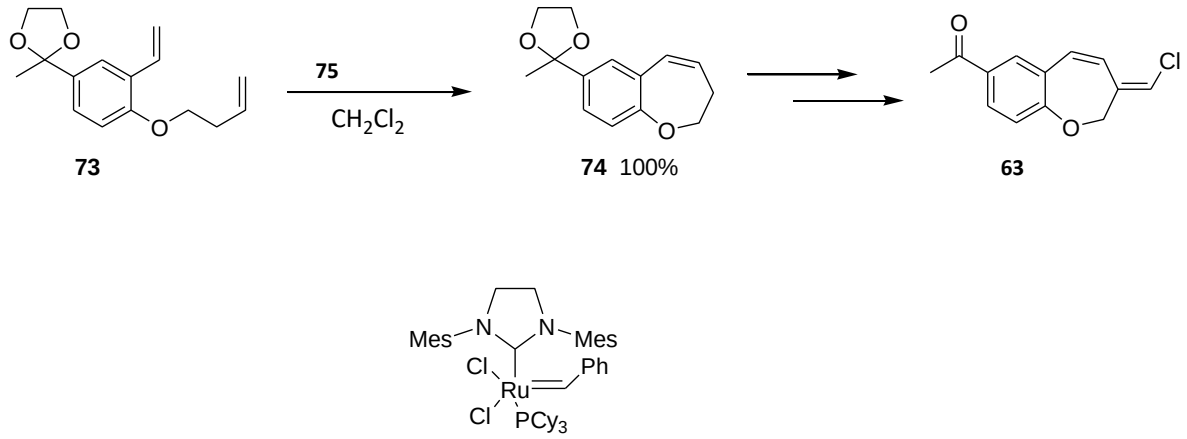
Figure 2.8. Structures of some of the isolated oxepine derivatives.

2.5. General synthetic routes to oxepines

The biological versatility displayed by oxepine containing natural products, as exemplified above, render these medium-sized ring systems as attractive targets in synthetic chemistry.[53] Accordingly, seven-membered rings are more challenging to synthesise relative to chromones and other small-sized ring systems due to enthalpic and entropic restraints.[53] Nevertheless, synthetic approaches for the preparation of oxepine rings have been pleasingly reviewed in recent years.[59,66-68] The most common reported synthetic routes for the synthesis of oxepine derivatives are: C-C bond formation (Claisen rearrangement, intramolecular cyclisation, annulation), C-O bond formation (Williamson ether or Ullmann-type), and ring expansion.[4,51,69]

2.5.1. Carbon-carbon (C-C) bond formation

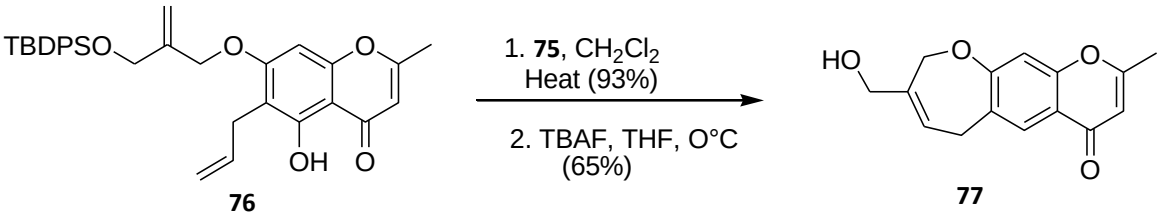
The type of C-C bond formation methodology that has expanded most drastically over the years, since the original reports by Grubbs and Fu, is ring-closing metathesis (RCM).[68] Grubbs and Fu demonstrated the application of RCM reactions to synthesise medium-sized ring systems using molybdenum catalysts. The method has been applied and optimised to synthesise oxepine derivatives.[59] In recent years, ruthenium catalysts have been used as an alternative to molybdenum complexes. Grubbs and co-workers demonstrated this by synthesising the natural compound pterulone (**63**) through the protected benzoxepine **74** from the diene **73** under the second-generation Grubbs catalyst **75** as depicted in Scheme 2.12.[59,61] Generally, ring formation by RCM reactions is favoured by the presence of functional groups that induce conformational constraints. These include additional ring system such as a benzene ring, a *gem*-dimethyl group or *N*-tosyl group.[59,68] An example of the application of the RCM reaction is the synthesis of ptaeroxylinol (**77**), a natural compound isolated from *P. obliquum*, as reported by Bruder *et al.* in 2010. The diene **76** was treated with Grubbs II catalyst **75** and heated in CH_2Cl_2 for 2 h to afford the oxepinochromone **77** (Scheme 2.13).[70]



73 **74** 100% **63**

Grubbs II **75**

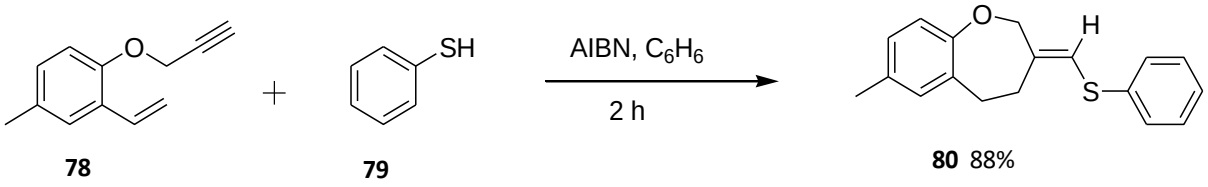
Scheme 2.12. Synthesis of pterulone (**63**) *via* ring closing metathesis



76 **77**

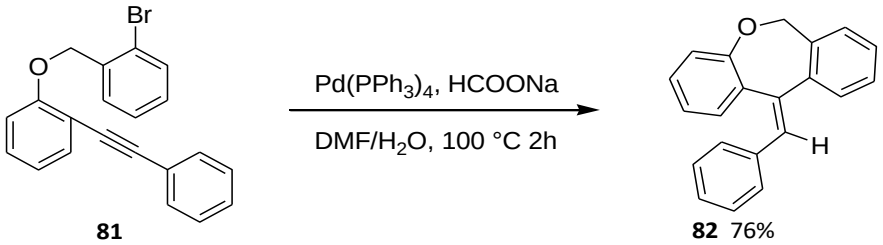
Scheme 2.13. Application of RCM reaction in the synthesis of ptaeroxylinol (**77**)

Majumda *et al.* discovered an efficient and economic synthetic route, a thiophenol-mediated 7-*endo* radical cyclisation, for the preparation of benzo[*b*]oxepine framework **80** from readily available terminal alkynes **78** in the presence of a thiophenol **79** and AIBN in benzene. They proposed that this method can be used to prepare various benzoxepine derivatives from readily available terminal alkynes as seen in Scheme 2.14.[54] A more recent advance of C-C bond formation is seen in Majumda's optimisation of the reductive Mizaroki-Heck approach by preparing dibenzo[*b,e*]oxepine derivatives. A pre-synthesised alkyne, 1-((3-bromobenzyl)oxy)-2(phenylethynyl)-benzene (**81**) (Scheme 2.15) was regioselectively cyclised by subjecting it to heat in an aqueous DMF in the presence of $Pd(PPh_3)_4$ and HCOONa for 2 h to afford the dibenzo[*b,e*]oxepine **82** in 76% yield.[60]



78 **79** **80** 88%

Scheme 2.14. Synthesis of a benzoxepine from a terminal alkyne.

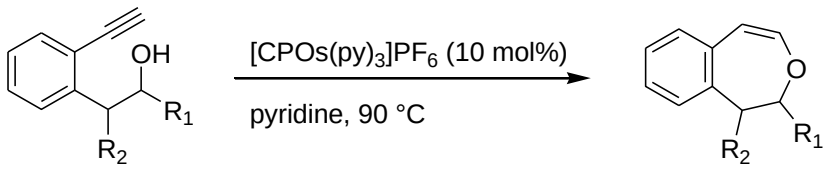


81 **82** 76%

Scheme 2.15. The synthesis of dibenzoxepine *via* an optimised reductive Mizaroki-Heck approach

2.5.2. Carbon-Oxygen bond formation

The metal-catalysed cycloisomerisation of alkynols has been used for the synthesis of oxepine derivatives. An example of C-O bond formation is found in the osmium-mediated synthesis of oxepine **84** from aromatic alkynols **83** (Scheme 2.16).[51,71] Cyclisation of hydroxy epoxides to synthesise oxepine derivatives has also generated interest in synthetic chemistry.[66]

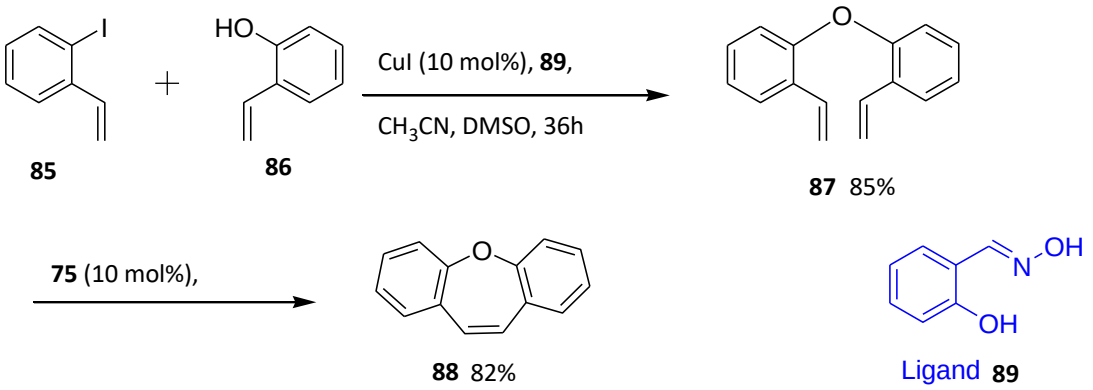


83 **84** 56% - 69%

Scheme 2.16. Synthesis of benzoxepine from an alkynol.

Bharath *et al.* developed an efficient synthetic route involving a tandem C-O and C-C bond formation methods to prepare dibenzo[*b,f*]oxepine molecules as illustrated in Scheme 2.17. The route proceeds *via* Ullmann-type etheration where iodo-2-vinylbenzene **85** reacts with 2-vinyl phenol **86** in the presence of CuI in CH_3CN and DMSO using $CsCO_3$ as a base and ligand

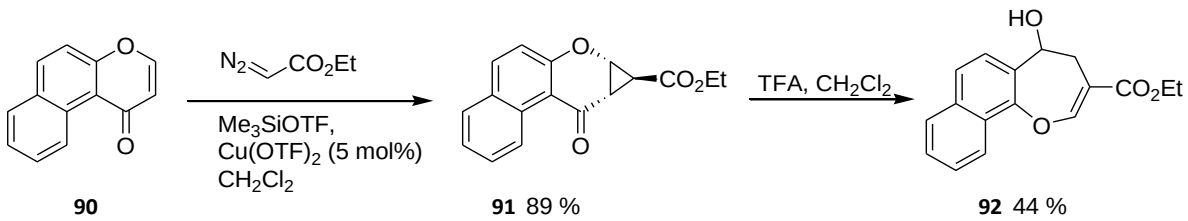
89 to afford the desired diene **87** in 85% yield. A subsequent RCM reaction of the diene **87** with Grubbs II catalyst **75** (10 mol %) furnished the target dibenzo[*b,f*]oxepine **88** in 82% yield under reflux for 10-12 h.[72]



Scheme 2.17. A tandem Ullman-type etheration and RCM approach in the synthesis of a dibenzoxepine.

2.5.3. Ring-expansion reactions

The ring-expansion reaction is one other method that has been applied in the synthesis of oxepine derivatives from small rings such as pyrans and epoxides.[66] This method has proven to be highly efficient and has been used in the preparation of natural products. Rotzoll *et al.* described the convenient synthesis of functionalised 2,3-benzoxepines **92** by cyclopropanation of benzopyrilium triflates with diazoesters and the subsequent ring expansion from benzopyran-4-one **90** (Scheme 2.18).[73]



Scheme 2.18. Synthesis of benzoxepine derivative *via* the expansion of a pyran ring

A variety of synthetic routes to prepare oxepine derivatives has been explored, from C-C bond formation to C-O bond formation to ring expansion reactions. The ring-closing metathesis

reaction was the most utilised. This could be due to the simplicity of the intramolecular ring closure reaction conditions required such as the employment of only Grubbs II catalyst in CH_2Cl_2 from a diene. Despite the simplified reaction conditions involved in the RCM reactions, this methodology requires an inert environment and utilises an expensive catalyst. Therefore, new robust and cost-sufficient strategies or optimisation protocols are needed to overcome the shortcomings accompanied by this synthetic approach.

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Chapter 3: Results and Discussions

3.1. Introduction

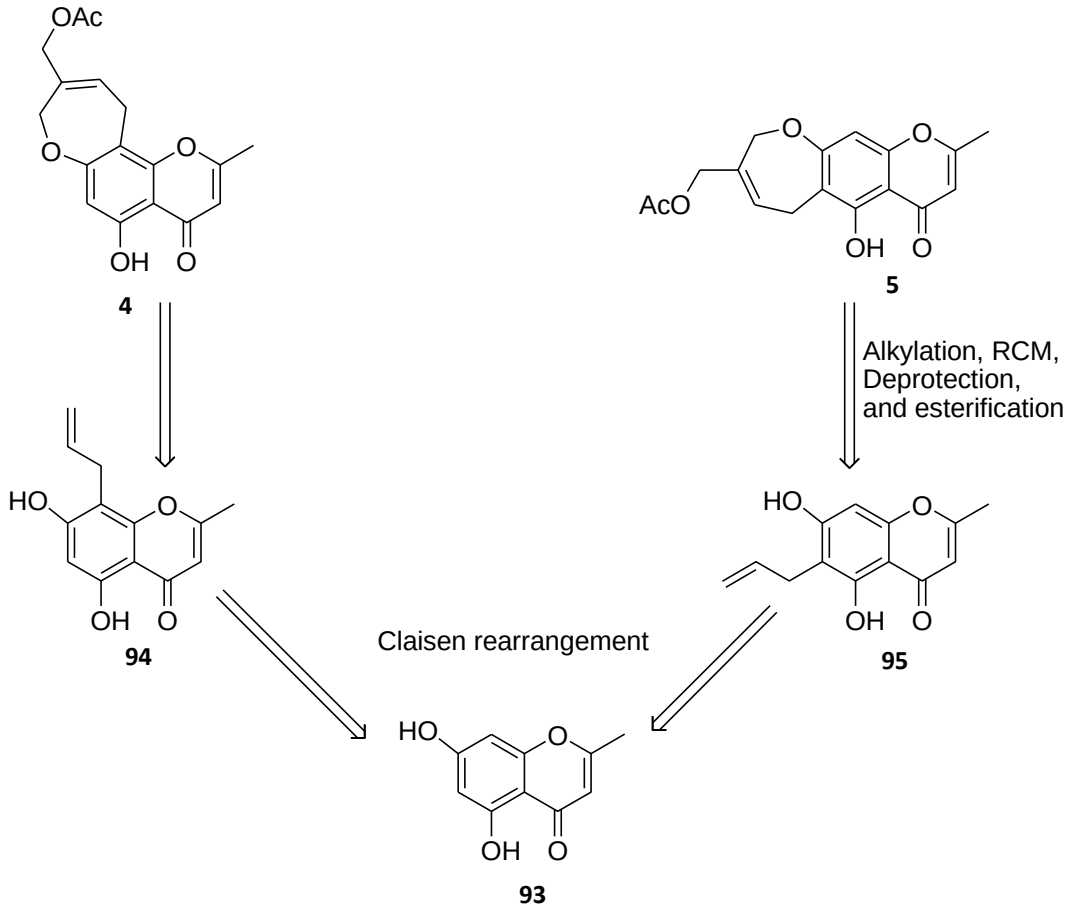
The first aim of our project was to synthesise the 12-*O*-acetyl derivative of eranthin **4**, named obliquumol based on the promising *in vitro* studies reported by van Wyk and co-workers.[1,2] In the event of Claisen rearrangement of the allyl group, a mixture of the requisite 8-allyl chromone and 6-allyl chromone precursors was obtained, which led to the parallel synthesis of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**).

The second aim of the project was to synthesise the carbon-14 labelled eranthin acetate and ptaeroxylinol acetate in order to facilitate their tissue distribution studies. Carbon-14 radioisotope is usually used for drug absorption, distribution, metabolism, and excretion (ADME) studies due to its stability, long half-life and the smaller quantity of material required.[3,4] For successful radiolabelling, it is important to incorporate the radiolabelled molecule at a more stable position to prevent losing the ^{14}C label in vivo and also the ^{14}C label must be installed as close as possible towards the end of the synthetic route to avoid generating loads of radioactive waste that is expensive to treat. Hence the objective was to design a robust synthetic route that would allow for incorporation of ^{14}C isotope to the targeted molecules at the later stage.

Accordingly, the discussion that follows will mainly focus on two synthetic approaches towards the total synthesis and the ^{14}C labelling of eranthin acetate (**4**), ptaeroxylinol acetate (**5**) and their derivatives. The first approach (Section 3.2) entails the synthesis of the chromone scaffold from phloroacetophenone (**94**) followed by the construction of an oxepine ring *via* a combined Claisen rearrangement – ring-closing metathesis (RCM) protocol. The second approach (Section 3.3) involves the construction of the oxepine moiety analogous to the first approach from **94** followed by the synthesis of the chromone scaffold. Section 3.4 provides the discussion on the synthesis of the derivatives with benzopyran instead of the oxepine ring. The last section 3.5 entails discussions on biological activities of the synthesised oxepinochromones **4** and **5** and the derivatives.

3.2. The first synthetic approach

The retrosynthetic analysis for the synthesis of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) is outlined in Scheme 3.1. It was envisaged that eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) would arise from noreugenin (**93**) based on a synthetic approach reported by Bruder *et al.* in 2010, which involved allylation, Claisen rearrangement, Williamson etherification, RCM and a subsequent desilylation.[5] Finally, the resulting penultimate oxepinochromone derivatives would undergo esterification to render the final compounds **4** and **5**.



Scheme 3.1. Retrosynthetic analysis for the synthesis of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) from noreugenin (**93**)

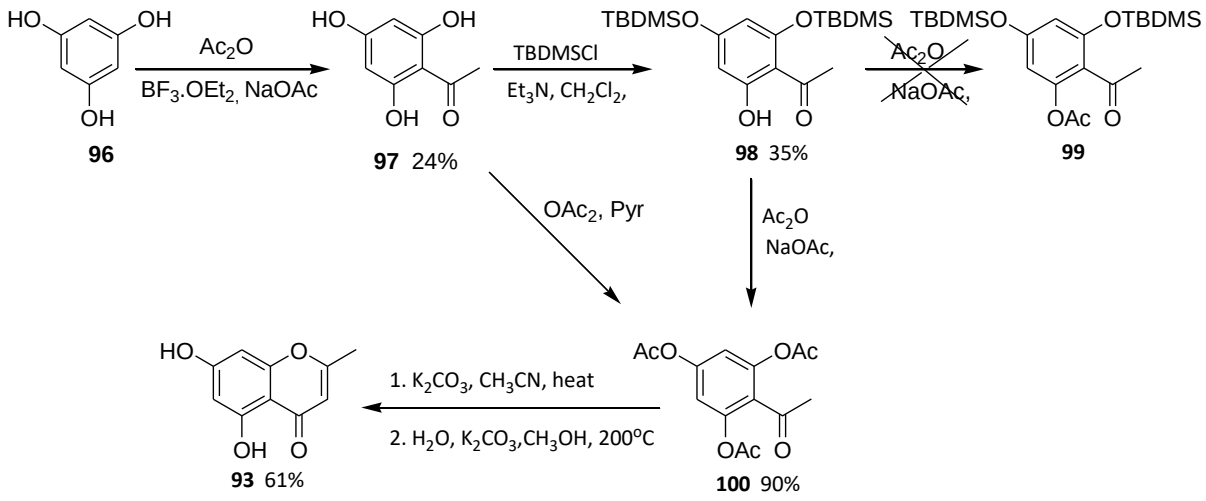
3.2.1. Preparation of noreugenin (93)

The synthesis of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) was initiated from phloroglucinol (**96**) as outlined in Scheme 3.2. The Friedel-Crafts acylation[6] of **96** with 1 mol

equivalent Ac_2O, $BF_3.OEt_2$ and NaOAc gave the requisite phloroacetophenone (**97**) in 24% yield, which was subsequently treated with triethylamine and TBDMSCl in CH_2Cl_2 to afford **98** in 35% yield.[7] It is worth noting that the plan was to convert the tri-protected acetophenone **99** into noreugenin (**93**). Unfortunately, when **98** was treated with acetic anhydride and NaOAc,[8] the intermediate **99** was not formed. When the TBDMS protecting groups were substituted with the acetyl groups, the 2,4,6-triacetoxyacetophenone (**100**) was obtained instead. The structure of **100** was confirmed by the disappearance of the TBDMS signals, but with the appearance of methyl of the acetoxy group at δ_H 2.28 (3H, s, OCOC$\underline{H}_3$) and 2.27 (6H, s × OCOC$\underline{H}_3$) in the ^{1}H NMR spectrum. In the spectrum there was also present two aromatic protons resonating at 6.92 (2H, s,) corresponding to H-3 and H-5 and the methyl resonance at δ_H 2.46 (3H, s, ArCOC$\underline{H}_3$). The ^{13}C NMR spectrum showed the carbonyl signal of the ketone at δ_C 197.6 (Ar$\underline{C}$OCH$_3$), carbonyl signals of the acetoxy group at δ_C 168.3 (2 × O$\underline{C}$OCH$_3$) and 168.2 (O$\underline{C}$OCH$_3$) and the methyl signals of the acetoxy group at 21.1 (OCO$\underline{C}$H$_3$) and 21.0 (2 × OCO$\underline{C}$H$_3$). In addition, three oxygen linked aromatic carbons were observed at δ_C 151.7 (C, C-4), 148.2 (2 × C, C-2, C-6), carbon linked aromatic carbon at δ_C 125.0 (C, C-1), three aromatic C-H carbons resonating at δ_C 114.1 (2 × CH, C-3, C-5) and methyl signal at δ_C 31.2 (CO$\underline{C}$H$_3$).

As the silyloxy group fell off during the acetylation to give the triacetoxyacetophenone **100**, the next plan was to prepare **100** directly from **97** following the procedure reported by Morita et al.[9] The naturally occurring noreugenin **93**, was then prepared in two steps from phloroacetophenone (**97**) *via* Kostanecki-Robinson reaction.[5,10] Using a known procedure,[9] **100** was achieved in high yields (90%) by reacting **97** with Ac_2O in pyridine at room temperature for 3 h. This was used in the next step without further purifications. Treatment of **100** with K_2CO_3 in acetonitrile under reflux for 3 h, and a subsequent heating at 110 °C in a mixture of saturated aqueous K_2CO_3 and CH_3OH (1:1) for a further 4 h furnished the target chromone **93** in a 61% yield.[10] The structure of compound **93** was assigned on the basis of ^{1}H NMR and ^{13}C NMR data. The ^{1}H NMR spectrum of **93** displayed two one-proton singlets at δ_H 12.87 (1H, s) and 10.85 (1H, s) corresponding to phenolic OH-5 and OH-7, a *meta* doublet at 6.36 (1H, d, J = 2.0 Hz, H-8), overlapping protons at 6.21 (2H, s) representing H-6 and H-3 and a three-proton singlet at δ_H 2.38 (3H, s) for the methyl group. The ^{13}C NMR spectrum of noreugenin **93** showed the presence of 10 carbon signals, with the carbonyl carbon (C-4) resonating at δ_C 181.7 and the alkene carbon (C-3) resonating at 107.9, which indicates the

chromone formation. In addition, there was one quaternary aromatic carbon resonating at δ_C 103.4 (C, C-4a), three tertiary oxygen linked aromatic carbons appearing at δ_C 164.1 (C, C-7), 161.5 (C, C-5) and 157.8 (C, C-8a), an oxymethine carbon resonating at δ_C 167.7 (C, C-2) and two aromatic carbons at δ_C 98.7 (CH, C-6) and 93.7 (CH, C-8) and a methyl carbon resonating at 19.9 (CH_3, CH_3-2) were observed in the carbon spectrum.



Scheme 3.2. The preparation of noreugenin (**93**)

Having accomplished the synthesis of noreugenin (**93**), the next step involved converting the OH-7 to the corresponding allyl ether via the Williamson ether synthesis. Thus, the allylation of **93** with allyl bromide in DMF in the presence of K_2CO_3 afforded the target 7-allyloxychromone **101** in a 63% yield (scheme 3.3). It is worth noting that this is a selective allylation to OH-7 of **93**, due to the hydrogen-bond formation between the OH-5 and the carbonyl group, which slightly reduces the reactivity of OH-5.[5] The structure of ether **101** was confirmed by the ^{1}H NMR analysis. The signals for the vinylic methines were displayed at δ_H 6.08- 5.98 (1H, m, H-2'), 5.42 (1H, *J* = 17.3 and 1.3 Hz, H-3'a), and 5.33 (1H, *J* = 10.5 and 1.3 Hz, H-3'b), and the signals for the oxymethylene protons were displayed at δ_H 4.58 (2H, d, *J* = 5.3 Hz, H-1'). The two resonances for the aromatic protons appeared at δ_H 6.36 (1 H, d, *J* = 2.2 Hz, H-8) and 6.34 (1 H, d, *J* = 2.2 Hz, H-6). The sharp singlet for the olefinic methine appeared at δ_H 6.02 (1 H, s, H-3) and the methyl signal resonated at δ_H 2.28 (3H, s, CH_3-2). Regioselectivity of the OH-7-allylation was confirmed by the correlation between the allylic methylene (H-1') at δ_H 4.58 and the carbon signal at δ_C 164.2 (C-7) in the HMBC spectrum as depicted in Figure 3.1

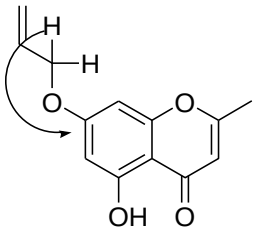
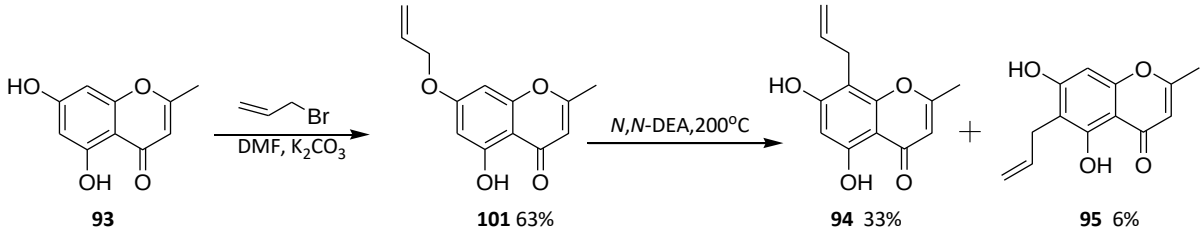


Figure 3.1. Characteristic HMBC correlations for **101**

When 7-allyloxychromone **101** was subjected to Claisen rearrangement, a mixture of the desired 8-allyl chromone **94** and the 6-allyl chromone isomer **95** was obtained as outlined in Scheme 3.3 (vide supra). Several methods to achieve the conversion of allyl ether **101** to compounds **94** and **95** were investigated. Initially, allyl ether **101** was subjected to heating at 160 °C in DMF for 48 h, which gave compound **94** and **95** in very low yields (2.7% and 2.3%, respectively) plus other unknown compounds as well as the unreacted starting material. In the attempt to achieve full conversion of **101** into the target compounds **94** and **95**, the next option was to apply the microwave irradiation as per Bruder *et al*.'s protocol[5] with slight modifications. Thus, the allyl ether **101** was absorbed on silica gel and exposed to solvent-free microwave irradiation for approximately 2 h. It was envisaged that the silica gel would retain the heat and effect Claisen rearrangement at a reduced reaction time with high yields. As expected, the reaction went into completion within 2 h as opposed to the initial conventional method and resulted in a mixture of the rearrangement products **94** and **95**. Chromatographic separation of the mixture gave the compounds **94** and **95** plus other inseparable mixture of unknown compounds, similar to the conventional Claisen rearrangement reaction (vide supra).

While this method was effective in converting 7-allyloxychromone **101** into the C-allyl compounds **94** and **95**, its effect was hindered by the difficulty and tediousness in extracting the products from the silica gel and the decomposition of material due to the high temperature, resulting in lower yields. Repeating the microwave-assisted reaction in DMF led to complete conversion of allyl ether **101** within five minutes of irradiation. However, this resulted in the evaporation of the solvent and consequently decomposition of most of the material. The outcome implied that a solvent with a higher boiling point would be ideal modification for this method to achieve Claisen rearrangement with complete conversion of

the starting material and without decomposition of the target compounds, therefore, *N,N*-Diethylaniline was considered to be a suitable solvent due to its high boiling point.[11] As a result, the allyl ether **101** was heated in *N,N*-Diethylaniline at 220 °C for 12 h to afford the rearranged products **94** and **95** in 33% and 6% yields, respectively.



Scheme 3.3. Preparation of **94** and **95** via Claisen rearrangement.

The ^{1}H NMR data of the C-8 allyl chromone **94** displayed two one-proton signals at δ_H 12.81 (1H, s, OH-5) and 10.81 (1H, s, OH-7), an aromatic proton signal at δ_H 6.31 (1H, s, H-6). In addition, a shielded resonance at δ_H 3.38 (2H, d, *J* = 6.3 Hz, H-1') was observed indicating a migration from C-O to C-C linkage. ^{13}C NMR data displayed six aromatic carbon signals consisting of three oxygen-linked carbon signals at δ_C 161.6 (C, C-7), 159.4 (C, C-5), and 155.2 (C, C-8a), one CH carbon signal at δ_C 98.2 (CH, C-6), and two tertiary carbon signals at δ_C 103.9 (C, C-8), 103.3 (C, C-4a). In addition, a methylene signal was present at δ_C 26.2 (CH_2, C-1'), and two vinylic carbon signals resonated at δ_C 135.8 (CH, C-2') and 114.8 (CH_2, C-3'). The rearrangement was further confirmed by HMBC analysis, which showed correlations between the aromatic proton H-6 (δ_H 6.31)/ C-5 and C-4a as illustrated in Figure 3.2a. Other correlations that substantiate rearrangement were observed between H_2-1' (δ_H 3.38)/ C-8a, C-7 and C-8. In addition, Figure 3.2b shows that correlations between OH-5 (δ_H 12.81)/C-5, C-6, C-7, and C-4 were observed. The correlation between H-6 (δ_H 6.31)/ C-5 together with the correlation between OH-5 (δ_H 12.81)/C-5 further confirm the rearrangement of the allyl group to the C-8 position.

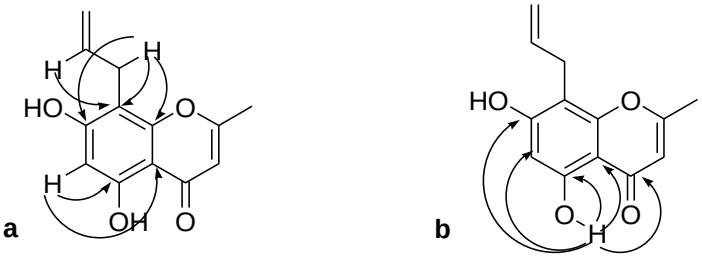


Figure 3.2. Key correlations for the 8-allyl isomer, **94** from HMBC.

The ^{1}H NMR data for allyl ether **95** displayed two one-proton signals at δ_H 13.13 (1H, s, OH-5) and 10.85 (1H, s, OH-7), an aromatic proton signal at δ_H 6.44 (1H, s, H-8). In addition, a shielded resonance at δ_H 3.29 (2H, d, *J* = 6.1 Hz, H-1') was observed indicating a migration from C-O to C-C linkage. ^{13}C NMR data displayed six aromatic carbon signals consisting of three oxygen-linked carbon signals at δ_C 161.9 (C, C-7), 158.6 (C, C-5), and 155.8 (C, C-8a), one CH carbon signal at δ_C 92.9 (CH, C-8), and two tertiary carbon signals at δ_C 108.9 (C, C-6), 103.1 (C, C-4a). In addition, a methylene signal was present at δ_C 26.0 (CH_2, C-1'), and two vinylic carbon signals resonated at δ_C 135.7 (CH, C-2') and 114.6 (CH_2, C-3'). The 6-allyl isomer **95** was confirmed by the HMBC correlations between the methylene proton H_2-1' (δ_H 3.29)/ C-5, C-6, and C-7 as outlined in Figure 3.3a. Another correlation was indicated between the aromatic proton signal H-8 (δ_H 6.44)/ C-7, C-8a and C-4a attesting to attachment of the allyl at C-6. More correlations are illustrated in Figure 3.3b between OH-5 (δ_H 13.13)/C-5, C-6, C-4, and C-4a. Attachment of the allyl group to the C-6 position is further validated by the correlations between H_2-1' (δ_H 3.29)/ C-5 and OH-5 (δ_H 13.13)/C-5 and C-6.

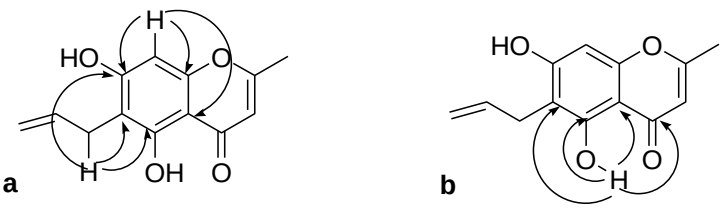
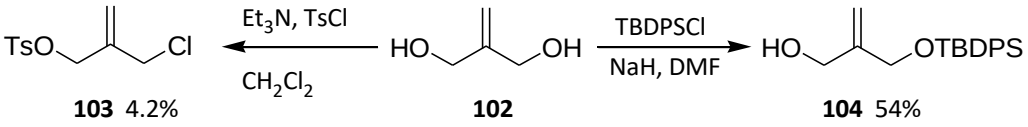


Figure 3.3. Characteristic correlations for 6-allyl isomer **95** observed from HMBC analysis.

3.2.2. Preparation of the alkylating agent 105c

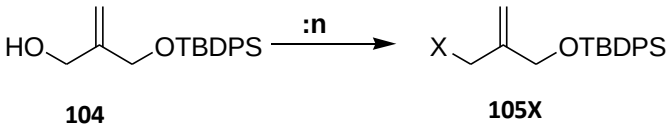
Synthesis of an alkylating compound was pivotal in the RCM reaction to afford the targeted oxepinochromones. Scheme 3.4 below demonstrates the proposed synthetic approach for the preparation of the alkyl group with a good leaving group from a commercially available 2-methylenepropane-1,3-diol (**102**). Considering the reactivity of both the alkene and the two hydroxy groups present in **102**, a protocol that would selectively convert one of the hydroxy groups into a good living group was essential. As a result, a couple of methods that would deliver a good leaving group were investigated. The first method that was attempted was the conversion of the diol **102** into the tosylate **103.** Treatment of diol **102** with 1 mole equivalent of TsCl and Et_3N as a base in CH_2Cl_2 generated the alkyl tosylate **103** in an unsatisfactory yield of 4.2%. The ^1H NMR spectrum of **103** displayed the presence of two aromatic protons appearing as AABB spin system at δ_H 7.81 (2H, *J* = 8.4 Hz) and 7.35 (2H, *J* = 8.1 Hz) and a three-proton singlet resonating at δ_H 2.45 for the aliphatic protons. In addition, the spectrum displayed one oxymethylene resonance at δ_H 4.62 (2H, s, C$\underline{H}_2$OTs) and vinylic methines at δ_H 5.35 (1H, s, C=C$\underline{H}_2$) and 5.28 (1H, s, C=C$\underline{H}_2$). The spectrum also displayed a further upfield two-proton signal at δ_H 4.02, suggesting a chloro-linked methylene resonance. These results implied that not only did tosylation take place, but the other hydroxy group was substituted with chloride. The ^{13}C NMR spectrum showed the aromatic carbons at δ_C 145.0 (C, C-Ar), 132.6 (C, C-Ar), 129.9 (2 × CH, C-Ar), 127.9 (2 × CH, C-Ar), an oxymethylene resonance at δ_C 69.5 (CH_2, $\underline{C}H_2$OTs), and a chloro-linked methylene resonance at δ_C 44.1 (CH_2, $\underline{C}H_2$Cl). It further displayed vinylic signals at δ_C 138.1 ($\underline{C}$=CH_2), and 120.1 (C=$\underline{C}H_2$) confirming the structure.



Scheme 3.4. Preparation of the alkyl compounds with different substituents.

Owing to the low yields obtained during the tosylation of diol **102**, there was a need to introduce a more stable protecting group. *tert*-Butyldiphenylsilyl chloride (TBDPSCl) was the preferred one, as it could later be easily cleaved easily at a later stage during its deprotection without interfering with the vinyl group present in the diol **102**. Treatment of the diol **102**

with 0.95 mol equivalent of TBDPSCl and NaH (60% suspension in oil) in THF afforded the monosilylated alcohol **104** in 54% yield as outlined in Scheme 3.4 above. The next step was to convert the hydroxy group in **104** into a good leaving group, which could be utilised in the Williamson ether synthesis. The initial plan was to convert **104** into alkyl bromide using PBr_3 as the brominating agent however, despite the common usage of PBr_3 to convert primary alcohols into alkyl bromides in literature, our concern was the lability of both the alkene and the alcohol to strong acids. Consequently, sulfonylation was explored as a viable option (Table 1 and scheme 3.5). An attempt to tosylate **104** with TsCl using Et_3N as a base in CH_2Cl_2 unexpectedly gave the alkyl chloride **105a** in 2% yield and a mixture of unidentified products. Analysis of the ^1H NMR spectrum of **105a** showed absence of the tosyl group resonance. However, it showed one oxymethylene signal at δ_H 4.28 (2H, s, $C\underline{H}_2OSi$) and one halogen linked methylene signal at δ_H 4.12 (2H, s, $ClC\underline{H}_2$). This implied that the expected product **105b** was in fact not formed. Additionally, comparison of the ^{13}C NMR spectra of **104** and **105a** showed absence of an oxygen-linked methylene signal, instead, a more upfield signal at δ_C 45.0 (CH_2, $Cl\underline{C}H_2$) was observed suggesting a possible halogenation rather than sulfonylation.



104 **105X**

Scheme 3.5. Preparation of the alkyl compounds with different substituents

Table 3.1. Showing reaction conditions for the synthesis of a stable alkyl group

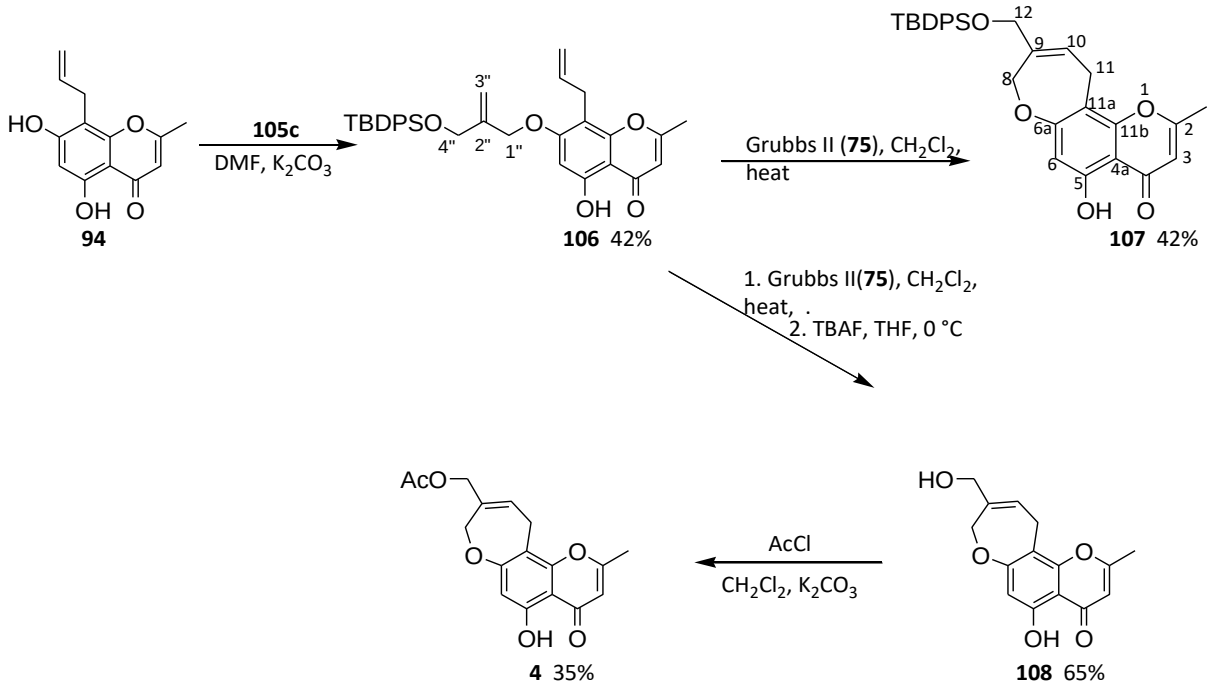
:n	**X**	**Product**	**% Yield**
Et_3N, TsCl, CH_2Cl_2, 24 h, rt	Cl	**105a**	3%
Pyr, TsCl, DMF, 3 h, rt	TsO	**105b**	11%
DBU, TsCl, DMF	Cl + TsO	**105a + 105b**	3.4% + 1.2%
PBr_3, Et_2O, 3 h, rt	Br	**105c**	42%

Kim *et al*.[12] reported that chlorination using sulfonyl chlorides such as MsCl and TsCl with a base is possible. When 4-bromobenzyl alcohol was reacted with sulfonyl chloride and DBU as base, a mixture of a chlorinated product and sulfonylation product was reported. They stipulated that TLC monitoring of the reaction showed that chlorination occurred after sulfonylation. With this in mind, it was decided to tosylate **104** with TsCl and pyridine in DMF. The expected alkyl tosyl **105b** was obtained in 11% yield. Evidence of tosylation was confirmed by the presence of the two characteristic *ortho*-coupled doublets at δ_H 7.53 (J = 8.2 Hz) and 7.27 (J = 8.9 Hz) and the singlet at δ_H 2.4 for the methyl group in the ^{1}H NMR spectrum.

Although, this approach produced the expected product, the yields were too low to continue with the total synthesis. Therefore, the use of DBU as a base was explored. Following the Kim *et al*., methodology, treatment of **104** with TsCl and DBU in DMF at room temperature afforded a mixture of the alkyl chloride **105a** and alkyl tosylate **105b** in 3.4 and 1.2% yields, respectively.[12] Due to the unsatisfying results obtained from the attempted approaches, the idea to brominate with PBr_3 was revisited. Treatment of monosilylated alcohol **104** with PBr_3 in diethyl ether at 0 °C for 3 h at room temperature furnished the alkyl bromide **105c** in modest yields (42%) after separation with silica gel column chromatophy.[5] Although the yields were not as good as in literature, converting the alcohol to alkyl bromide was successful in contrast to tosylation. The ^{1}H NMR spectrum of **105c** showed an upfield signal at δ_H 3.03 integrating for two protons which confirmed successful halogenation. In the ^{13}C NMR spectrum, the presence of an upfield signal at δ_C 32.7 attested to the successful bromination.

3.2.3. Preparation of the oxepinochromones 4 and 5

Having successfully prepared the precursors **94** and **105c** required for synthesis of eranthin acetate (**4**), the subsequent step involved the synthesis of the advanced intermediate **106** needed for RCM reaction. This was achieved by coupling of the allyl chromone **94** with the alkyl bromide **105c** in DMF using K_2CO_3 as a base at room temperature. The ether **106** was obtained in 42% yield as outlined in Scheme 3.6. With the ether **106** in hand, ring closure using second generation Grubbs catalyst (**75**) was refluxed in CH_2Cl_2 for 3 h. The desired oxepine **107** was achieved in 42% yield.



Scheme 3.6. The synthesis of eranthin acetate **4** via Claisen rearrangement and RCM method.

Confirmation that RCM was a success was evident in the ^{1}H NMR spectrum for the oxepine **107** with the appearance of three methylene units resonating at δ_H 4.67 (2H, d, J = 1.1 Hz, H-8), 4.06 (2H, s, H-12), 3.58 (2H, d, *J* = 5.5 Hz, H-11) and a characteristic vinylic methine at δ_H 5.88-5.84 (1H, m, H-10), in contrast to the four methylene units present in the proton NMR spectrum of RCM precursor **106**. This confirmed that ethene group was lost during ring closure. Apart from the characteristic aromatic proton and the upfield aliphatic signals corresponding to the TBDPS ether, the oxepinochromone gave a protonated molecular ion $[M + H]^+$ at *m/z* 513.2066 in the positive-ion HRMS-ESI, consistent with a molecular formula $C_{31}H_{33}O_5Si$ (calcd. 513.2099).

It is noteworthy to mention that intermediate **107** was decomposing upon purification on silica gel and also when exposed to air for more than a day. Therefore, it was decided to proceed to the next step without purification in order solve the stability problems**.** Thus, when the ether **106** was subjected to RCM and a subsequent removal of the silyl protecting groups with TBAF in THF for 1 h, the natural alcohol, eranthin (**108**) was achieved in a 65% yield. The ^{1}H NMR spectrum of **108** did not show the TBDPS ether signals. Instead, the spectrum displayed a vinylic proton resonance at δ_H 5.99 (1H, t, *J* = 5.3 Hz, H-10), an allylic methylene

resonating at δ_H 3.61 (2H, d, *J* = 5.3 Hz, H-11), and two oxymethylene proton signals at δ_H 4.72 (2H, s, H-8), and 4.03 (2H, s, H-12). ^{13}C NMR analysis displayed fifteen signals and were allocated by means of HSQC, HMBC and DEPT-135 NMR data. The spectrum displayed a ketocarbonyl signal at δ_C 183.1 (C, C-4), six aromatic carbons consisting of three C-O resonances at δ_C 164.4 (C, C-6a), 160.1 (C, C-5), and 153.8 (C, C-11b), two quaternary carbon signals at δ_C 111.0 (C, C-11a), and 107.0 (C, C-4a) and a CH carbon resonating at δ_C 104.6 (CH, C-6). In addition, two oxymethylene carbon resonances at δ_C 70.8 (CH_2, C-8), and 65.4 (CH_2, C-12), two vinylic group signals at δ_C 138.5 (C, C-9), and 124.2 (CH, C-10), and an allylic methylene carbon at δ_C 21.4 (CH_2, C-11) were observed. The molecular formula of eranthin was determined to be $C_{15}H_{14}O_5$ on the basis of HRMS-ESI *m/z* $[M + H]^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$, 275.0921)

Finally, the alcohol **108** was esterified to obtain the expected oxepinochromone **4** (Scheme 3.6). This was easily achieved by stirring the alcohol and acetyl chloride in CH_2Cl_2 in the presence of K_2CO_3 as a base for 4 h at room temperature. The oxepines **4** was obtained in a modest yield of 35%. The low yield could be due to the presence of two reactive hydroxy groups which could lead to a mixture of products including, monoacetylation at the targeted hydroxy group, acetylation at the OH-5 position or a di-acetylation on both the hydroxy groups. The molecular formular of the oxepinochromone **4** was determined to be $C_{17}H_{16}O_6$ based on the HRMS-ESI *m/z* $[M + H]^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1019). The ^{1}H NMR spectrum analysis of **4** displayed a signal at δ_H 2.05 indicative of the methyl of the acetoxy group. It further displayed three one-proton signals at δ_H 12.66 (1H, s, OH-5), 6.49 (1H, s, H-6), and 6.06 (1H, s H-3), vinylic proton resonance at δ_H 6.06-6.03 (1 H, m, H-10), an allylic methylene resonating at δ_H 3.63 (2H, d, *J* = 5.5 Hz, H-11), and two oxymethylene proton signals at δ_H 4.65 (2H, d, *J* = 1.4 Hz, H-8), and 4.46 (2H, s, H-12), and a methyl group at δ_H 2.38 (3H, s, CH_3-2). ^{13}C NMR data displayed signals at δ_C 170.7 and 20.9 indicative of the presence of the acetoxy carbonyl group and the methyl of the acetoxy group, respectively. The spectrum further displayed a ketocarbonyl signal at δ_C 183.1 (C, C-4), six aromatic carbons consisting of three C-O resonances at δ_C 164.3 (C, C-6a), 160.3 (C, C-5), and 153.7 (C, C-11b), two quaternary carbon signals at δ_C 111.4 (C, C-11a), and 107.2 (C, C-4a) and a CH carbon resonating at δ_C 104.7 (CH, C-6). In addition, there was present two oxymethylene carbon resonances at δ_C 71.0 (CH_2, C-8), and 66.5 (CH_2, C-12), two vinylic group signals at δ_C 133.8 (C, C-9), and 127.4

(CH, C-10), and an allylic methylene carbon at δ_C 21.6 (CH_2, C-11). The oxygen linked olefinic carbon signal at δ_C 166.8 (C, C-2), the olefinic carbon signal at δ_C 108.8 (CH, C-3), and signal of the methyl group at 20.5 (CH_3, CH_3-2) were characteristic of the chromone moiety.

Analysis of the HMBC spectrum of **4** indicated correlation between H-6 (δ_H 6.49)/ C-4a, C-5, C-6a, and C-11a as illustrated in Figure 3.4a. Furthermore, a correlation between H_2-11 (δ_H 3.63-3.62)/ C-11a, C-11b, C-10, C-9 and C-6a indicated the presence of an oxepine ring which confirms that the oxepine ring is attached at C-11a consistent with the structure of the angular isomer, **4**. Figure 3.4b shows more correlations that were observed between OH-5 (δ_H 12.66)/C-5, C-6, C-6a, and C-4a. The combined correlations between H-6 (δ_H 6.49)/ C-5 and OH-5 (δ_H 12.66)/C-5 and C-6 substantiate assignment of the aromatic proton signal at δ_H 6.49 in the ^{1}H NMR spectrum of **4** to H-6. Successful esterification was confirmed by a correlation between H_2-12 and the ester carbonyl carbon at δ_C 170.6.

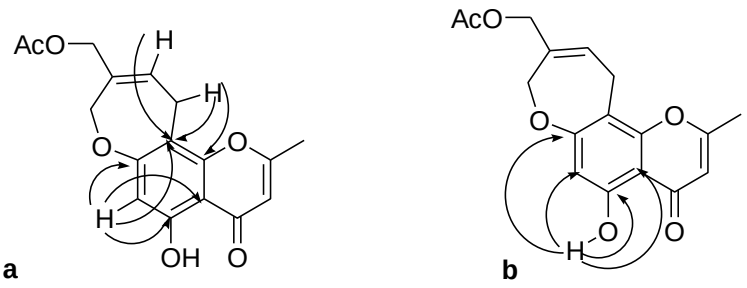
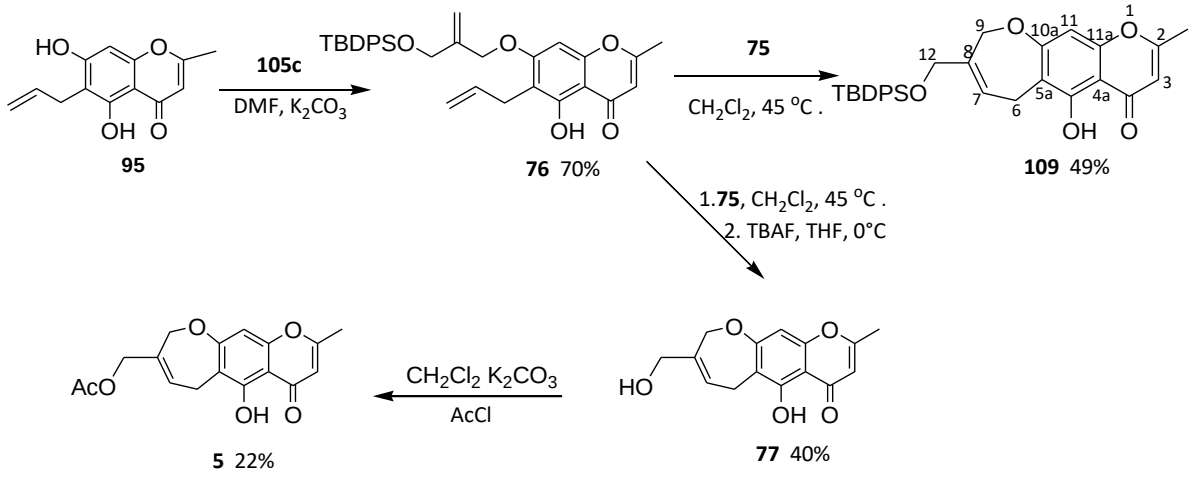


Figure 3.4. HMBC correlations for the angular isomer, eranthin acetate **4**.

Similarly, the preparation of ptaeroxylinol acetate (**5**) proceeded in the same manner as that of eranthin acetate (**4**) as outlined in scheme 3.7 above. This was achieved by coupling the allyl chromone **95** with the alkyl bromide **105c** in DMF using K_2CO_3 as a base at room temperature. The ether **76** was obtained in 70% yield. The ether **76** was subjected to ring closure using second generation Grubbs catalyst (**75**) in CH_2Cl_2 at reflux for 3 h. The expected oxepine **109** was obtained in 49% yield.



Scheme 3.7. Preparation of ptaeroxylinol acetate **5**

The ^{1}H NMR spectrum for the oxepine **109** showed three methylene units resonating at δ_H 4.70 (2H, d, *J* = 0.8 Hz, H-9), 4.06 (2H, s, H-12), 3.52 (2H, d, *J* = 5.6 Hz, H-6) and a characteristic vinylic methine at δ_H 5.85-5.82 (1H, m, H-7). The molecular formular was confirmed to be $C_{31}H_{32}O_5Si$ based on the HRMS-ESI *m/z* $[M + H]^+$ 513.2066 (calculated for $C_{31}H_{33}O_5Si$ 513.2091).

Owing to the instability of the oxepine **109**, it was decided to subject the diene **76** to a successive RCM and deprotection to afford the alcohol **77** as displayed in Scheme 3.7. The ^{1}H NMR spectra analysis of **77** did not show the TBDPS ether signals (Table 3.3). In addition, the spectrum displayed a vinylic proton resonance at δ_H 6.00 (1H, td, *J* = 5.6, 1.1 Hz, H-7), an allylic methylene resonating at δ_H 3.57 (2H, brd, *J* = 5.6 Hz, H-6), and two oxymethylene proton signals at δ_H 4.74 (2H, d, *J* = 1.1 Hz, H-9), and 4.03 (2H, s, H-12). ^{13}C NMR analysis displayed fifteen signals and were allocated by means of HSQC, HMBC and DEPT-135 NMR data. The spectrum displayed six aromatic carbons consisting of three C-O resonances at δ_C 164.6 (C, C-10a), 158.1 (C, C-5), and 155.9 (C, C-11a), two quaternary carbon signals at δ_C 115.8 (C, C-5a), and 106.7 (C, C-4a), and a CH carbon resonating at δ_C 99.3 (CH, C-11). In addition, there were two oxymethylene carbon resonances at δ_C 71.0 (CH_2, C-9), and 65.7 (CH_2, C-12), two vinylic group signals at δ_C 138.0 (C, C-8), and 125.4 (CH, C-7), and an allylic methylene carbon at δ_C 21.1 (CH_2, C-6). The molecular formula of eranthin was determined to be $C_{15}H_{14}O_5$ on the basis of HRMS-ESI *m/z* $[M + H]^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$, 275.0921).

Esterification of the alcohol **77** afforded the target oxepinochromone **5** in 22% yield. The molecular formular of **5** was similar to that of **4**, determined to be $C_{17}H_{16}O_6$ based on HRMS-ESI *m/z* $[M + H]^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1019). Similar to that of **4,** the ^{1}H NMR spectrum analysis of **5** displayed a signal at δ_H 2.05 indicative of the methyl of the acetoxy group. It further displayed three one-proton signals at δ_H 13.08 (1H, s, OH-5), 6.54 (1H, s, H-11) and 6.05 (1H, brs, H-3), a vinylic proton resonance at δ_H 6.05-6.08 (1H, m, H-7), an allylic methylene resonating at δ_H 3.59 (2H, d, *J* = 5.5 Hz, H-6), and two oxymethylene proton signals at δ_H 4.65 (2H, d, *J* = 1.5 Hz, H-9), and 4.46 (2H, s, H-12), and a methyl group at δ_H 2.36 (3H, s, CH_3-2). ^{13}C NMR data displayed signals at δ_C 170.7 and 20.9 indicative of the presence of the acetoxy carbonyl group and the methyl of the acetoxy group, respectively. The spectrum further displayed a ketocarbonyl signal at δ_C 182.8 (C, C-4), six aromatic carbons consisting of three C-O resonances at δ_C 164.5 (C, C-10a), 158.2 (C, C-5), and 155.9 (C, C-11a), two quaternary carbon signals at δ_C 116.0 (C, C-5a), and 106.9 (C, C-4a), and a CH carbon resonating at δ_C 99.4 (CH, C-11).

The aromatic proton signal at δ_H 6.54 in the ^{1}H NMR spectrum of **5** was assigned to H-11 based on the correlation observed in the HMBC spectrum between H-11/ C-10a, C-11a, C-5a, and C-4a as illustrated in Figure 3.5a. Additional correlation was established between H_2-6 (δ_H 3.59)/ C-5, C-8, C-7, C-5a. These established correlations imply that the oxepine ring is attached at C-5a, consistent with the linear structure of **5**. Correlations between OH-5 (δ_H 13.08)/ C-5 and C-5a (Figure 3.5b) together with the correlations between H-6 (δ_H 3.59)/ C-5, and C-5a confirm the assignment of the allylic methylene signal at δ_H 3.59 to H-6. Successful esterification was also confirmed by a correlation between H_2-12 and the ester carbonyl carbon at δ_C 170.7

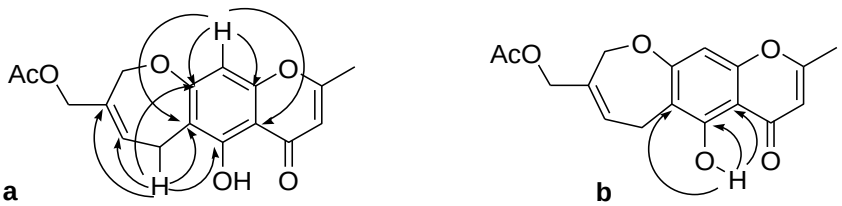


Figure 3.5. HMBC correlations for the linear isomer, ptaeroxylinol acetate **5**.

Having established which isomer is linear and which is angular, it was only essential to compare analytical data of the synthesised oxepinochromones **4** and **5** to the natural

oxepinochromones, obliquumol and ptaeroxylinol acetate (**5**), respectively. Table 3.2 below shows NMR data comparison between **108**, **5**, and obliquumol (reported by van Wyk et al). The NMR data shows a significant difference between eranthin acetate (**4**) and obliquumol, therefore, it could not be confirmed that **4** is in fact similar to the natural product, obliquumol despite the similarity in the proposed structures for both **4** and obliquumol. The difference is observed in C-6, C-11a, and C-11b carbon signals wherein the signals in **4** resonated at δ_C 104.7, 111.4, and 157.7, whilst those of the natural product obliquumol resonated at δ_C 99.29, 115.82, and 158.06, respectively. Rather, these carbon signals are comparable with those of the synthesised ptaeroxylinol acetate (**5**) resonating at δ_C 99.4, 116.0, and 158.2 corresponding to C-11, C-5a, and C-5, respectively. As illustrated in Table 3.3, there is insignificant difference between the NMR data of the synthesised **5** and the natural ptaeroxylinol acetate (**5**). These results suggest that the originally proposed structure of obliquumol is inconsistent with that of the angular isomer eranthin acetate (**4**) but correlates more with that of the linear structure **5**.

Table 3.2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of the synthesized compounds **108** and **4**, and comparison with obliquumol in $CDCl_3$.

	108		**4**		**Obliquumol**[2,1]	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2		166.8		166.8		167.14
3	6.04, s	108.7	6.06, s	108.8	5.99, q (0.7)	108.66
4		183.1		183.1		182.68
4a		107.0		107.2		106.72
5		160.1		160.3		155.79
6	6.45, s	104.6	6.49, s	104.7	6.47, s	99.29
6a		164.4		164.3		164.39
8	4.72, s	70.8	4.65, d (1.4)	71.0	4.61, tt (1.6, 1.6)	71.04
9		138.5		133.3		133.23
10	5.99, t (5.3)	124.2	6.05-6.03, m	127.4	6.02, tt (5.5, 1.2)	128.30
11	3.61, d (5.3)	21.4	3.63, d (5.5)	21.6	3.52, d (5.6, 1.2, 1.6)	21.14
11a		111.0		111.4		115.82

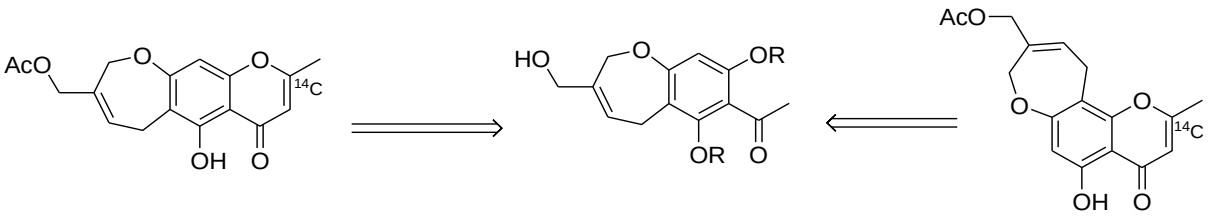
11b		153.8		153.7		158.06
12	4.03, s	65.4	4.46, s	66.5	4.41, brs	66.45
13				170.6		170.61
14			2.05, s	20.9	2.01, s	20.42
2-Me	2.38, s	20.5	2.38, s	20.5	2.34, d (0.7)	20.78
OH-5	12.61, s		12.66, s	-	12.94, brs	

Table 3.3. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of the synthesized compounds **77** and **5**, and comparison with the Isolated 12-*O*-Acetylptaeroxylinol in $CDCl_3$

	77		**5**		**12-*O*-Acetylptaeroxylinol** [13]	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2	-	167.2		167.1		167.2
3	6.04, s	108.7	6.05, brs	108.8	6.07, s	108.8
4	-	182.8		182.8		182.5
4a		106.7		106.9		114.1
5		158.1		158.2		158.2
5a		115.8		116.0		133.2[b]
6	3.57, d (5.6)	21.1	3.59, d (5.5)	21.2	3.60, d (5.5)	21.3
7	6.00, td (5.6, 1.1)	125.4	6.05-6.08, m	128.1	6.09, t (5.5)	128.4
8		138.0		133.2		116.1[b]
9	4.74, d (1.1)	71.0	4.65, d (1.5)	71.2	4.48,[b] s	66.1[c]
10a		164.6		164.5		167.2
11	6.52, s	99.3	6.54, s	99.4	6.56, s	99.4
11a		155.9		155.9		154.3
12	4.03, s	65.7	4.46, s	66.6	4.67,[b] s	71.2[c]
13		-		170.7		170.8
14		-	2.05 s	20.9	2.08 s	20.9
2-Me	2.35, s	20.5	2.36, s	20.5	2.38, s	20.6
OH-5	13.08, s	-	13.08, s	-	13.10, s	-

3.3. The second synthetic approach

The ultimate rationale to synthesise compound **4** and **5** was to perform further pharmacological studies and distribution studies using radiochemistry. The first approach is essential for accessing the compounds in their natural form and for further biological studies. However, it is not ideal for the synthesis of ^{14}C-labelled compounds as the late incorporation of the labelled isotope would be at the ester site, which is prone to metabolic degradation. This led to developing a synthetic route that would allow insertion of a ^{14}C-labelled molecule at a at a stable position, later stage. It was then envisaged that the chromone scaffold would be more stable than the ester. A sequence that starts with construction of the oxepine ring, followed by synthesis of the chromone moiety (Scheme 3.8) to ultimately allow for the incorporation of a ^{14}C-labelled molecule at a stable position was envisioned. In addition, this approach would prevent working with radioactive materials for many steps and thus minimise the amount of radioactive waste to be generated. This is costly to treat. This section focuses on the development of a synthetic approach for the synthesis of non-radiolabelled oxepinochromones **4** and **5** to be used for the synthesis of ^{14}C-labelled **4** and **5** in the future.



[^{14}C]Ptaeroxylinol acetate (**5**) [^{14}C]Eranthin acetate (**4**)

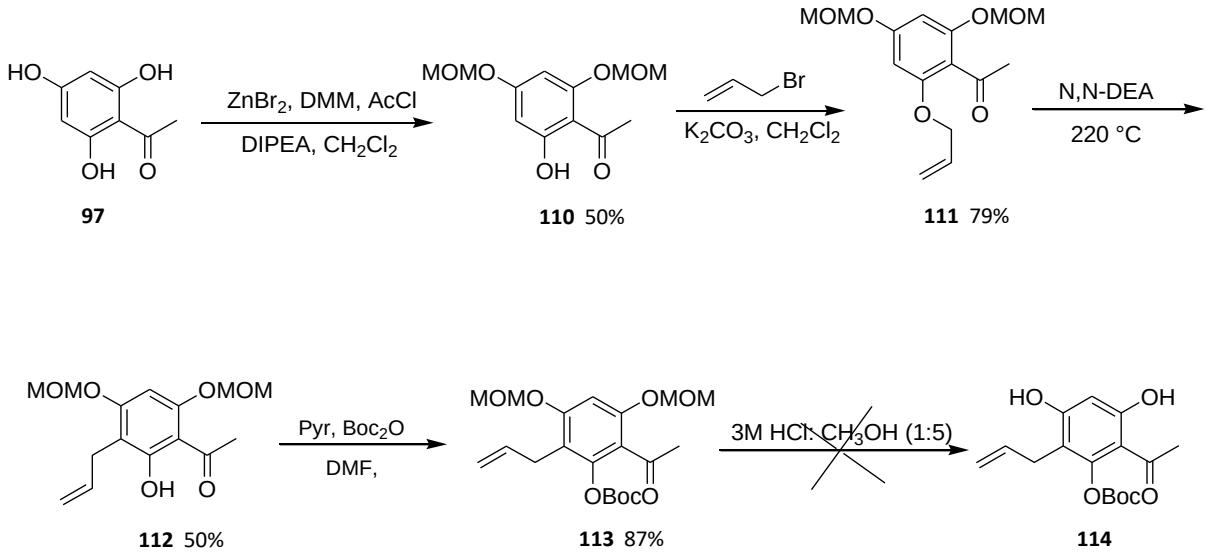
Scheme 3.8. Retrosynthetic analysis for C-14 radiolabelled eranthin acetate and ptaeroxylinol acetate

3.3.1. Preparation of benzoxepines from phloroacetophenone

As mentioned above, the second approach involves construction of the oxepine ring from 2',4',6'-trihydroxyacetophenone (**97**) followed by the synthesis of the chromone scaffold. Synthesis of the oxepine ring in this approach is analogous to the first approach as it involves allylation, Claisen rearrangement, Williamson etheration and finally, RCM. It was crucial to protect the 4'- and 6'-hydroxy groups of the phloroacetophenone **97** before the coupling with

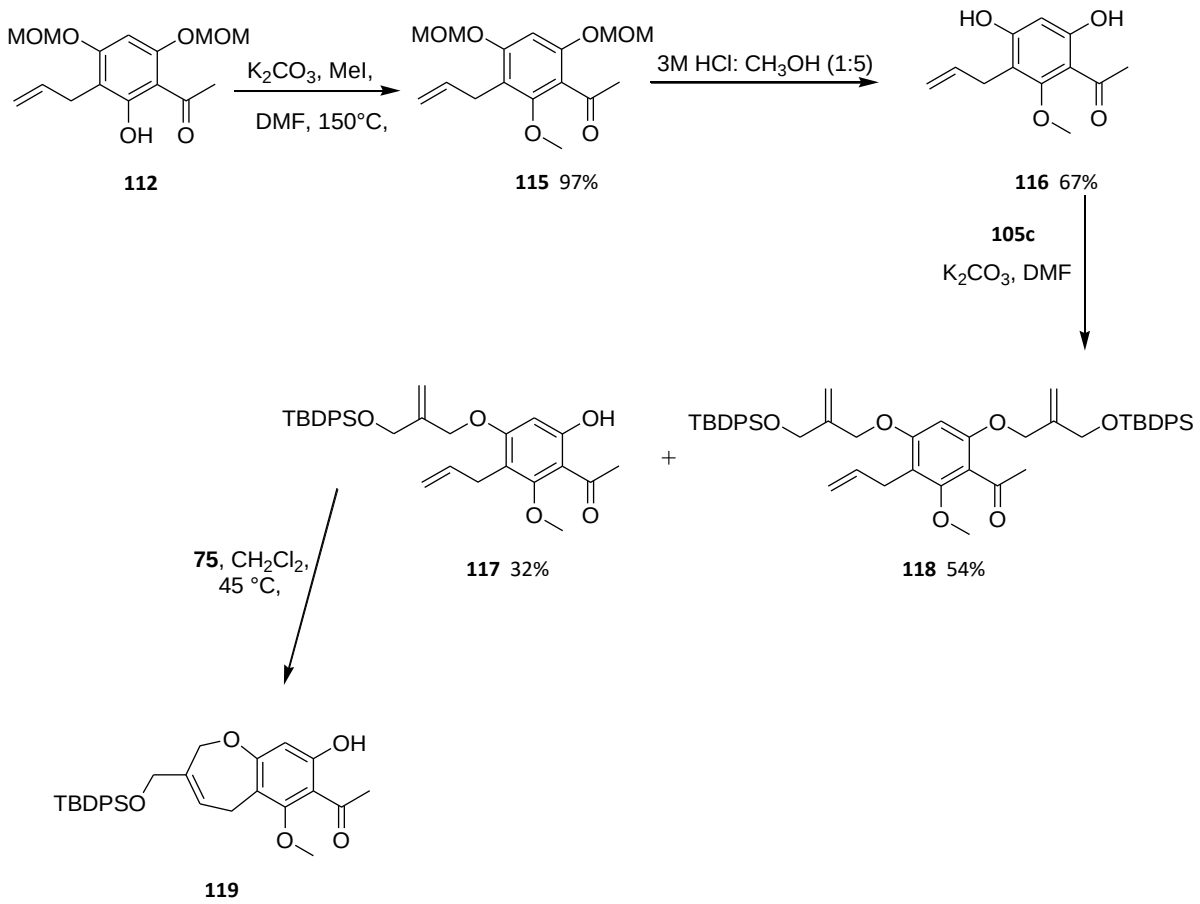
allyl bromide to prevent side reactions with the other hydroxy groups which may ultimately result in obtaining the desired product in low yields. The MOM protecting group was selected for its robustness under basic conditions and facile cleavage under acidic conditions.[14]

This was achieved by partially protecting 2',4',6'-trihydroxyacetophenone (**97**) with MOMCl generated *in situ* from the reaction of dimethoxymethane, acetyl chloride, and $ZnBr_2$ in CH_2Cl_2.[14,15] The di-protected acetophenone **110** was obtained in modest yields (50%) (Scheme 3.9). Confirmation of the protection was evident in the ^{1}H NMR spectrum which showed two *meta*-coupled doublets at δ_H 6.26 (1H, d, *J* = 2.4 Hz, H-3) and 6.24 (1H, d, *J* = 2.4 Hz, H-5) corresponding to the two aromatic protons H-3 and H-5, respectively. The hydrogen-bonded hydroxy signal was displayed at δ_H 13.71 (1H, s, OH). The resonances for the oxymethylene groups of the MOM ether pair signals appeared at δ_H 5.25 (2H, s, OC<u>H</u>$_2$O)and 5.17 (2H, s, OC<u>H</u>$_2$O), while the signals for the methyl groups of the MOM protecting group appeared at δ_H 3.52 (3H, s, OC<u>H</u>$_3$), and 3.47 (3H, s, OC<u>H</u>$_3$). The reaction of **110** with allyl bromide in the presence of K_2CO_3 afforded the allyl ether **111** in a 79% yield. Analysis of the ^{1}H NMR data displayed the absence of the hydrogen-bonded hydroxy signal that was observed in **110**. Evidence for allylation was given by the appearance of the vinylic signals at δ_H 6.03-5.93 (1H, m, H-2'), 5.37-5.33 (1H, m, H-3'a) and 5.27-5.26 (1H, m, H-3'b), as well as an oxymethylene signal appearing at δ_H 4.51 (2H, dt, *J* = 5.2 and 1.5 Hz, H-1').



Scheme 3.9. Attempted selective protection and deprotection using MOM ether and Boc protecting groups

Following the allylation of **110**, the allyl ether, **111** was subjected to Claisen rearrangement to obtain the C-allyl **112** following the same methodology in the first approach as outlined in Scheme 3.9. The allyloxy-acetophenone **111** was heated in *N*,*N*-DEA at 220 °C overnight to afford the C-allyl ether **112** in a 50% yield. The ^{1}H NMR spectrum of C-allyl ether **112** displayed an upfield shift for the methylene signal at δ_H 3.36 (2H, dt, *J* = 6.1 and 1.5 Hz, H-1'), relative to the signal at δ_H 4.51 (2H, dt, *J* = 5.2 and 1.5 Hz, H-1') observed in the ^{1}H NMR spectrum of the O-allyl **111**. Appearance of the characteristic hydrogen-bonded hydroxy signal at δ_H 13.86 (1H, s, 2-OH) and a single aromatic singlet at δ_H 6.39 (1H, s, H-5) was observed. The ^{13}C NMR spectrum displayed the same upfield shift at δ_C 26.5 (CH_2, C-1') for **112**, relative to the signal at δ_C 69.3 (CH_2, C-1') in the ^{13}C NMR spectrum of **111** indicating the absence of an ether linkage. The next step would be to introduce the alkyl bromide **105c** at the OH-4- in preparation for the RCM precursor, which would require subsequent deprotection of the MOM protecting groups and protection of the 2-hydroxy group to prevent side reactions that may occur. Initially, we opted to use the Boc-protecting group as it would be relatively easier to remove. Thus, treatment of **112** with Boc_2O in the presence of pyridine using DMF as a solvent gave **113** in an 87% yield. However, the targeted product **114,** was unfortunately not obtained when **113** was treated with HCl in CH_3OH at 65 °C. It was suspected that the Boc protecting group may have been hydrolysed in the presence of HCl and heat.



Scheme 3.10. The alternative route to be followed for radiolabelling.

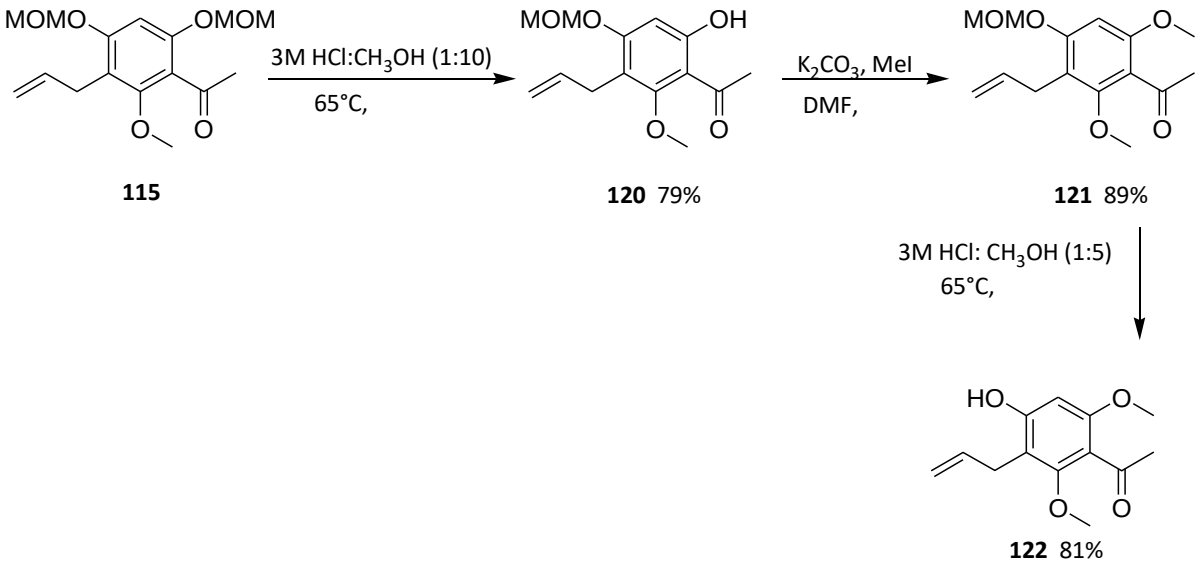
Consequently, we settled for a more stable methyl ether protecting group (Scheme 3.10). Gratifyingly, refluxing of the C-ally **112** with K_2CO_3 and iodomethane in DMF furnished the tri-protected acetophenone **115** in a 97% yield. Subsequent deprotection of the MOM ether protecting groups with 3M HCl in CH_3OH proceeded to give the 3-allyl-4,6-dihydroxy-2-methoxyacetophenone (**116**) in a 67% yield.[16] The structure of **116** was confirmed by the absence of the MOM signals and the appearance of the three-proton singlet at δ_H 3.74 (3H, s, OCH_3) resonating for the 2-methoxy protons, a one-proton signal at δ_H 5.83 (1H, s, OH-4) and a singlet at δ_H 13.20 (1H, s, OH-6). ^{13}C NMR data displayed a methoxy group signal at δ_C 63.0 (CH_3, OCH_3) and three oxygen-linked aromatic carbon signals at δ_C 164.4 (C, C-6), 161.9 (C, C-4), and 161.8 (C, C-2).

Coupling of the C-allyl acetophenone **116** with alkyl bromide **105c** to afford the di-allyl acetophenone **117** was an important step in the synthesis of the requisite intermediate **119**. Initially the C-allyl acetophenone **116** and alkyl bromide **105c** were stirred in DMF in the

presence of K_2CO_3 at room temperature for 3 h. This gave the di-alkylated product **118** instead of the desired product **117**. It was suspected that these unexpected results were because of the long reaction time, therefore, the reaction was repeated and was monitored after 30 minutes of the reaction start time. However, this time around the mixture of the mono-alkylated **117** and the di-alkylated **118** was observed. When the reaction was conducted for the third time, TLC monitoring after the first 5 minutes revealed that the unwanted product **118** was still generated as a major product (54%) whereas the desired product **117** was generated as the minor product (32%). The ^{1}H NMR spectrum of the desired mono-alkylated product **117** exhibited one set of the TBDPS ether signals at δ_H 7.67-7.65 (4H, m, SiArH), 7.44-7.35 (6H, m, SiArH), 1.07 (9H, s, $(CH_3)_3CSi$), two vinylic methine signals at δ_H 5.38 (1H, s, H-3"a), 5.25 (1H, d, *J* = 1.2 Hz, H-3"b), and two oxymethylene signals at δ_H 4.53 (2H, s, H-1") and 4.26 (2H, s, H-4"). In addition, there was present a one-proton signal at δ_H 13.36 (1H, S, OH-6) indicating monoprotection. ^{13}C NMR data displayed the TBDPS ether signals at δ_C 135.5 (4 × CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 26.8 (3 × CH_3, $(CH_3)_3CSi$), and 19.3 (C, $(CH_3)_3CSi$). In addition, the spectrum showed two oxymethylene signals at δ_C 69.1 (CH_2, C-1") and 64.5 (CH_2, C-4") and two vinylic carbon signals at δ_C 112.8 (CH_2, C-3") and 142.7 (C, C-2").

Despite the undesired results that were obtained, an attempt was made to convert **117** to the benzoxepine **119** *via* RCM using Grubbs II catalyst in CH_2Cl_2. However, the target compound **119** could not be completely characterised due to decomposition during purification. Considering the unwanted results, it was evident that a new synthetic strategy with favourable outcome needed to be developed. It was later discovered that, instead of removing both of the MOM protecting groups from **115**, a mono-deprotection at the OH-6 and subsequent protection with a robust protecting group that is stable to HCl followed by the removal of the 4-MOM protecting group could render a regioselective alkylation with improved yields. Thus, mono-deprotection of **115** with 3M HCl: CH_3OH (1:10) gave **120** in 79% yield as outlined in Scheme 3.11. The regiochemistry of **120** was confirmed by the characteristic hydrogen-bonded hydroxy signal at δ_H 13.19 (1H, s, OH-6) in the ^{1}H NMR spectrum of **120** indicating the removal of a single MOM protecting group at the OH-6 instead of the OH-4. The ^{1}H NMR data also revealed resonance of a single set of MOM ether signals at δ_H 5.20 (2H, s, OCH_2O) and 3.45 (3H, s, OCH_3) relative to resonance of a pair of MOM ether

signals displayed in the ^{1}H NMR spectrum of **115**. Treatment of **120** with K_2CO_3 and iodomethane in DMF gave **121** in 89% yield, and hydrolysis of the MOM protecting group with 3M HCl:CH_3OH (1:5) afforded the intermediate product **122** in good yields (81%). In the ^{1}H NMR spectrum of **122**, no MOM signals were displayed, implying successful deprotection. Other diagnostic signals displayed in the ^{1}H NMR were resonances for two methoxy groups at δ_H 3.73 (3H, s, OCH_3) and 3.69 (3H, s, OCH_3). ^{13}C NMR displayed two methoxy group signals at δ_C 63.4 (CH_3, OCH_3) and 55.7 (CH_3, OCH_3).

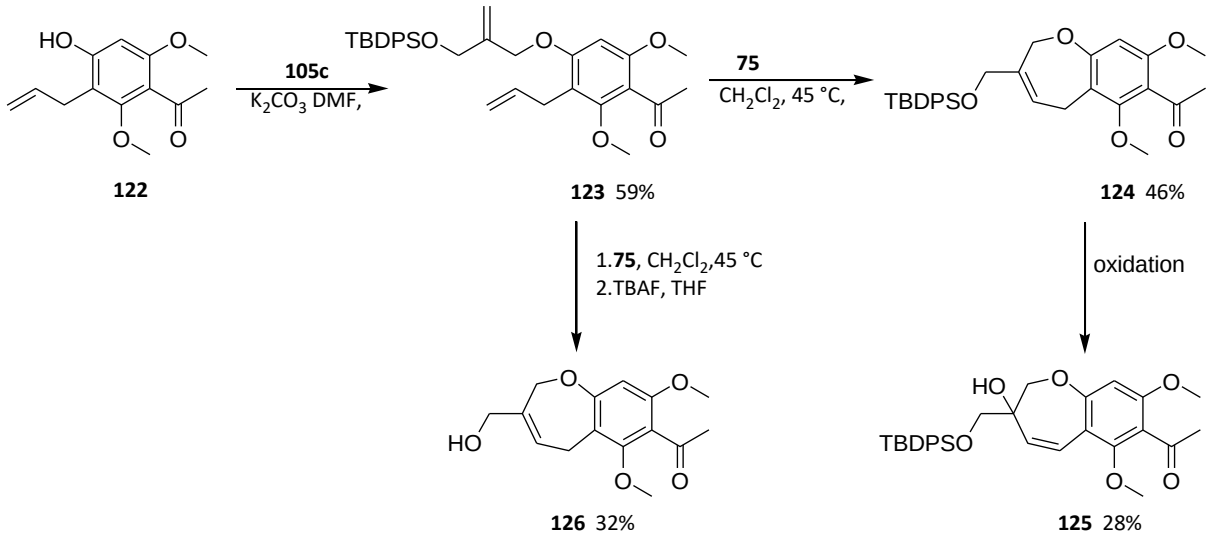


Scheme 3.11. Preparation of **122** by selective protection and deprotection using MOM and methyl protecting groups.

With **122** at hand, the synthesis of the RCM precursor was performed by stirring **122** and the alkyl bromide **105c** in DMF for 5 minutes at room temperature in the presence of K_2CO_3 (Scheme 3.12). The diene **123** was obtained in a 59% yield, an improvement from the 32% yield obtained from the preparation of **117**. ^{1}H NMR analysis of the diene **123** revealed the occurrence of two vinylic methine signals at δ_H 5.39 (1H, d, *J* = 0.8 Hz, H-3"a) and 5.26 (1H, d, *J* = 1.1 Hz, H-3"b), two oxymethylene signals at δ_H 4.56 (2H, s, H-1") and 4.29 (2H, s, H-4"). In addition, there were TBDPS ether signals at δ_H 7.68-7.66 (4H, m, SiAr<u>H</u>), 7.45-7.35 (6H, m, SiAr<u>H</u>), 1.00 (9H, s, (C<u>H</u>$_3$)$_3$CSi), and the allylic signals consisting of a vinylic methine at δ_H 5.87-5.77 (1H, m, H-2'), a vinylic methylene at δ_H 4.87-4.82 (2H, m, H-3') and a methylene at δ_H 3.25 (2H, d, *J* = 6.0 Hz, H-1'). ^{13}C NMR data displayed the TBDPS ether signals at δ_C 135.4 (4 ×

CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 26.8 (3 × CH_3, $(CH_3)_3CSi$), and 19.3 (C, $(CH_3)_3CSi$). In addition, the spectrum showed two vinylic carbon signals at δ_C 112.3 (CH_2, C-3") and 142.4 (C, C-2"), and two oxymethylene signals at δ_C 69.1 (CH_2, C-1"), and 64.5 (CH_2, C-4").

Ring closing metathesis of the diene **123** in CH_2Cl_2 gave the benzoxepine **124** in modest yields (46%). The benzoxepine **124** showed a molecular formula of $C_{31}H_{36}O_5Si$ based on HRMS-ESI (*m/z* $[M + H]^+$ 517.2403; calculated for $C_{31}H_{37}O_5Si$ 517.2404). The ^1H NMR spectrum displayed two oxymethylene protons at δ_H 4.63 (2H, d, *J* = 1.6 Hz, H-2), 4.01 (2H, s, H-1'), a methylene signal at δ_H 3.40 (2H, d, *J* = 5.5 Hz, H-5), and an olefinic resonance at δ_H 5.81-5.77 (1H, m, H-4). Additionally, the TBDPS ether signals at δ_H 7.65-7.63 (4H, m, SiArH), 7.45-7.35 (6H, m, SiArH), 1.04 (9H, s, $(CH_3)_3CSi$) and two methoxy signals at δ_H 3.78 (3H, s, OCH_3) and 3.67 (3H, s, OCH_3) were observed. ^{13}C NMR data displayed the TBDPS ether signals at 135.5 (4 × CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 26.8 (3 × CH_3, $(CH_3)_3CSi$, and 19.2 (C, $(CH_3)_3CSi$). In addition, there was a ketocarbonyl signal at δ_C 202.5 (C, $ArCOCH_3$) and a signal for the methyl of the acetyl group at δ_C 32.5 (CH_3, $ArCOCH_3$). Further analysis of the spectrum showed two oxymethylene signal at δ_C 71.6 (CH_2, C-2) and 66.2 (CH_2, C-1'), two olefinic carbon signals at δ_C 137.5 (C, C-3) and 121.9 (CH, C-4), and an allylic methylene signal at δ_C 21.9 (CH, C-5). Two methoxy signals resonated at δ_C 63.3 (CH_3, OCH_3) and 55.8 (CH_3, OCH_3).



Scheme 3.12. Preparation of **126** through alkylation and the classic RCM.

Similar to the previous RCM products, **124** decomposed during purification and when exposed to air. Fortunately, in this case, the corresponding decomposition product was successfully isolated and assigned to the structure of **125** based on NMR analysis. ^{1}H NMR spectrum of **125** exhibited four signal of the two diastereotopic oxymethylene protons at [δ_H 4.41 (1H, dd, *J* = 11.6 and 1.5 Hz, H-2a) and 4.00 (1H, d, *J* = 11.6 Hz, H-2b)], [δ_H 3.84 (1H, d, *J* = 10.2 Hz, H-1'a) and 3.60 (1H, d, *J* = 10.2 Hz, H-1'b)], two olefinic signals at δ_H 6.62 (1H, d, *J* = 12.1 Hz, H-5) and 5.74 (1H, dd, *J* = 12.1, and 1.4 Hz, H-4). Additionally, the TBDPS ether signals at δ_H 7.70-7.66 (4H, m, SiArH), 7.45-7.38 (6H, m, SiArH), and 1.09 (9H, s, $(CH_3)_3CSi$) were observed. The ^{13}C NMR spectrum revealed an upfield shift at δ_C 74.1 (C, C-3) for the quaternary carbon C-3, instead of the signal at δ_C 137.5 (C, C-3) observed in the ^{13}C NMR spectrum of **124** indicating the oxygen linkage. Another chemical shift was observed at δ_C 120.9 (CH, C-5), relative to the CH_2 signal at δ_C 21.9 (CH, C-5) observed in the ^{13}C NMR spectrum of **124**. The downfield shift is consistent with the double bond migration displayed in the structure of **125**. The structure of **125** was further elucidated from HMBC correlations (Figure 3.6). HMBC showed a correlation between the olefinic proton H-5 (δ_H 6.62)/ C-9a, C-6, C-4, and C-1', while key correlation in COSY spectrum was established between the two olefinic protons H-5/H-4. Hence the structure of **125** was assigned as 1-(2,3-dihydro-3-hydroxy-3-(tert-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone.

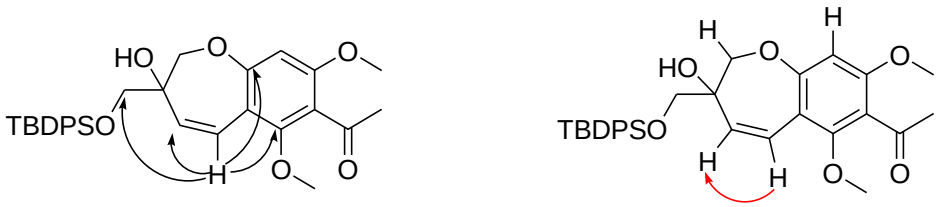
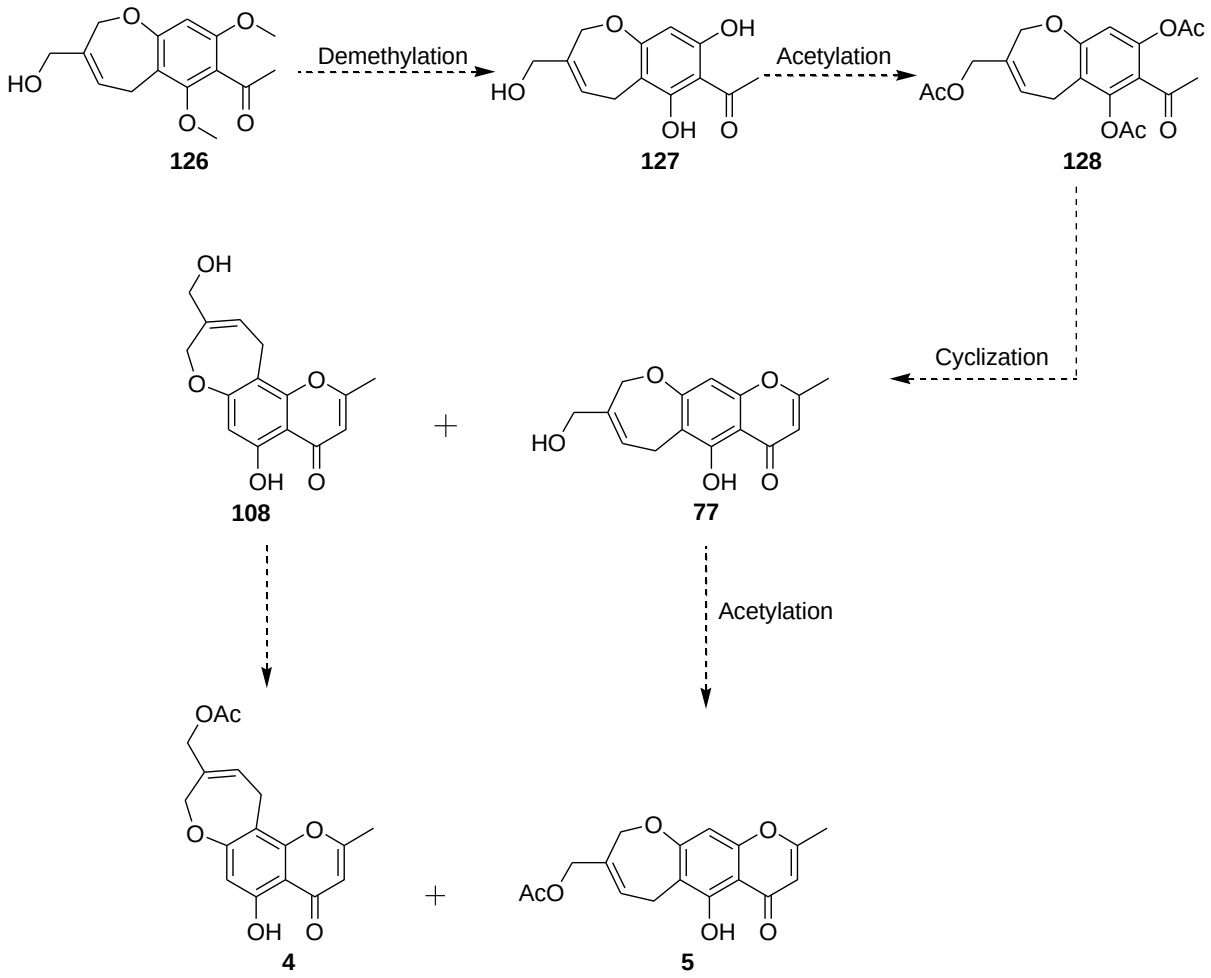


Figure 3.6. Key HMBC and COSY correlations of **125**.

As shown in Scheme 3.12, subjecting the diene **123** to RCM and subsequent removal of the silyl protecting group afforded the unprotected benzoxepine **126** in a 32% yield. The benzoxepine **126** showed a molecular formula of $C_{15}H_{18}O_5$ based on HRMS-ESI (ESI *m/z* $[M + H]^+$ 279.1214 (calculated for $C_{15}H_{19}O_5$ 279.1234). The structure of **126** was confirmed by the absence of the TBDPS ether signals and the appearance of two oxymethylene signals at δ_H 4.67 (2H, s, H-2) and 3.98 (2H, s, H-1'), a methylene signal at δ_H 3.44 (2H, d, *J* = 5.5 Hz, H-5), and an olefinic proton signal at δ_H 5.93-5.91 (1H, t, *J* = 5.3 Hz, H-4). ^{13}C NMR data displayed

signals at δ_C 202.4 (C, Ar<u>C</u>OCH$_3$) and δ_C 32.4 (CH$_3$, ArCO<u>C</u>H$_3$) for the acetyl group. Further analysis of the spectrum showed two oxymethylene signal at δ_C 71.6 (CH$_2$, C-2) and 65.7 (CH$_2$, C-1'), two olefinic carbon signals at δ_C 138.2 (C, C-3) and 123.9 (CH, C-4), and an allylic methylene signal at δ_C 22.0 (CH$_2$, C-5). The two methoxy signals resonated at δ_C 63.3 (CH$_3$, OCH$_3$), 55.8 (CH$_3$, OCH$_3$).



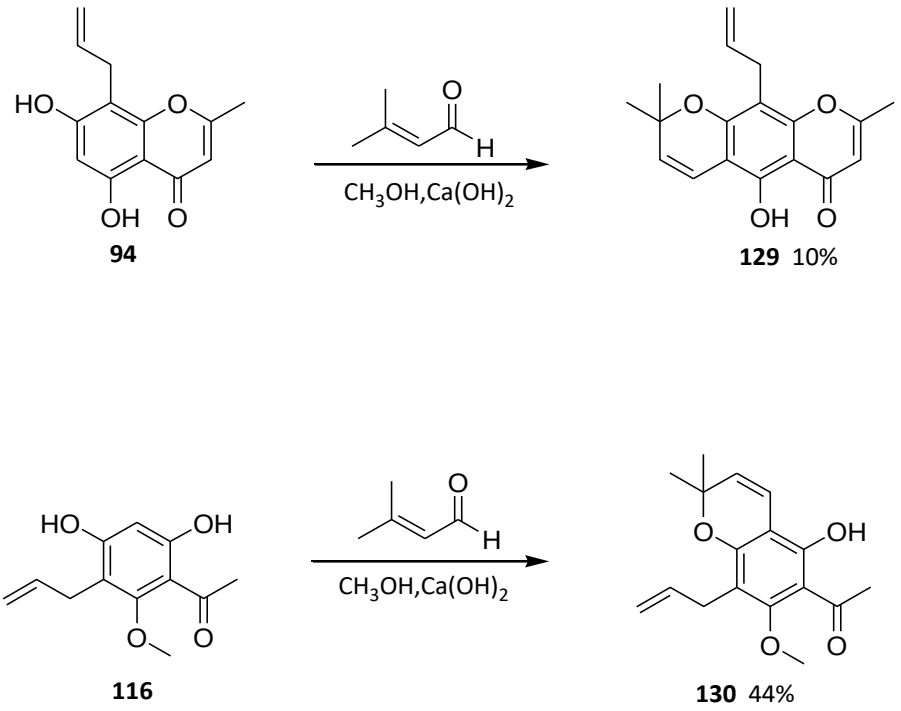
Scheme 3.13. The proposed synthetic route for the preparation of eranthin acetate and ptaeroxylinol acetate from compound **126**

With the successful synthesis of compound **126**, the next step was to convert it to **127** via demethylation as outlined in Scheme 3.13. However, the benzoxepine **126** was not stable to air and was decomposing during purification on column chromatography. Therefore, the demethylation of compound **126** to **127** was not realised, due to the above drawbacks. It is also noteworthy to mention that to arrive at **126** several attempts were made which resulted into material loss for further starting materials and intermediates, thus the synthesis

culminated at the preparation of **126**. It was anticipated that this step would be followed by acetylation with excess acetic anhydride in order to prevent production of multiple products such as monoacetylated or diacetylated products. Oxidative cyclisation of the tri-acetylated benzoxepine **128** would proceed to give a mixture of the oxepinochromones **114** and **77**, which would be subjected to esterification with acetyl chloride to afford the desired oxepinochromones **4** and **5**. The halt to the preparation of the oxepinochromones **4** and **5** through this approach was due to the complications presented by the individual steps explained above and time constraints. It is necessary to optimise this approach in future to prevent the decomposition of products and intermediates.

3.4. Preparation of derivatives

Attention was then shifted to the synthesis of derivatives as per one of the objectives. Prenylation has been found to stimulate the biological activity of natural products. Generally, prenylated compounds are mostly prepared by Mitsunobu *O*-prenylation and a subsequent Claisen rearrangement. An alternative route to synthesising prenylated compounds is through *C*-prenylation followed by 6-*endo* cyclization.[11,17] Therefore, we planned to prepare **129** and **130** from readily available derivatives **94** and **116**, respectively as outlined in Scheme 3.14. Synthesis of **129** was achieved by condensation of **94** with prenal in the presence of calcium hydroxide in CH_3OH.[18] The dimethylpyranochromone **129** was obtained in a 10% yield. The ^{1}H NMR results showed the dimethylpyran protons as singlet integrating for six protons at δ_H 1.44 (6H, s, CH_3-3') and two olefinic proton resonances at δ_H 6.72-6.69 (1H, d, *J* = 10.0 Hz, H-1'), and 5.61-5.58 (1H, d, *J* =10.0 Hz, H-2'). The hydrogen-bonded hydroxy proton appeared at δ_H 12.97 (1H, s, OH) suggesting that *o*-cyclization occurred at the OH-7 instead of the hydrogen-bonded OH-5. The allylic signals consisting of a methylene, vinylic methine, and vinylic methylene appeared at δ_H 3.41 (2H, dt, *J* = 6.3 and 1.4 Hz, H-1"), 5.93-5.83 (1H, m, H-2"), and 5.04-4.94 (2H, m, H-3"), respectively. ^{13}C NMR exhibited methyl signals at δ_C 28.2 (2 × CH_3), two olefinic carbon signals at δ_C 127.8 (CH, C-2'), and 115.8 (CH, C-1'), and a quaternary carbon signal at δ_C 77.8 (C, C-3') indicating successful cyclization.



Scheme 3.14. Synthesis of derivatives via prenylation

Similarly, the benzopyran **130** was prepared from **116** *via* by base-catalysed condensation with prenal in CH_3OH. The structure of **130** was confirmed by the appearance of the hydrogen-bonded OH-2 in the ^1H NMR spectrum of **130** at δ_H 13.55 (1H, s, OH-5), and subsequently, confirming the regioselectivity of the *o*-cyclisation. The dimethylpyran proton signals appeared at δ_H 1.43 (6H, s, 2 × CH_3), and two olefinic proton signals appeared at δ_H 6.68 (1H, d, *J* = 10.0 Hz, H-4), and 5.51 (1H, d, *J* = 10.0 Hz, H-3). The allylic signals consisting of a methylene, vinylic methine, and vinylic methylene appeared at δ_H 3.31 (2H, dt, *J* = 6.0 and 1.6 Hz, H-1'), 5.99-5.92 (1H, m, H-2'), and 5.04-4.98 (2H, m, H-3'), respectively. ^{13}C NMR exhibited methyl signals at δ_C 28.4 (2 × CH_3, CH_3-2), two olefinic carbon signals at 126.7 (CH, C-3) and 116.0 (CH, C-4) and a tertiary carbon signal at δ_C 78.0 (C, C-2), indicating successful cyclization

3.5. Biological studies for the synthesised compounds

Biological activities of the synthesised compounds, **108**, **77**, **4**, **5**, **93**, **94**, **126**, **129**, and **130** were studied against two strains of fungi, *Candida albican* and *Cryptococcus neoformans* with Amphotericin B used as a positive control. Table 3.4 shows the minimum inhibitory concentrations (MIC, µM) of all the tested compounds. Compared to the nine tested

compounds, the angular oxepinochromone **4** showed equipotent activity against both of the tested fungi regardless of incubation time with MIC value of 9.9 µM. In addition, **4** showed the highest activity against C. albicans relative to the other screened compounds. The linear oxepinochromone **5** had the highest activity against C. neoformans with an MIC value of 4.9 µM. Among the two oxepinochromones, the linear isomer **5** showed interesting selectivity between C. albicans and C. neoformans with MIC values of 19.8 and 4.9 µM, respectively. The only difference between the isomers is the position of the oxepine ring on the chromone scaffold, to which selectivity may be attributed to.

Table 3.4. MIC (µM) values of *Ptaeroxylon obliquum* derivatives against *Candida albicans* and *Cryptococcus neoformans* at different incubation times.

	MIC (µM)			
	Candida albicans		***Cryptococcus neoformans***	
Compound	**24 h**	**48 h**	**24 h**	**48 h**
108	11.4	11.4	5.7	5.7
77	11.4	11.4	5.7	5.7
4	9.9	9.9	9.9	9.9
5	19.8	19.8	4.9	4.9
93	16.3	16.3	16.3	16.3
94	13.5	13.5	13.5	13.5
126	11.2	22.5	11.2	11.2
129	41.9	83.8	10.5	10.5
130	86.7	86.7	21.7	43.3
Amp-B	0.42	0.84	0.42	0.42

The corresponding alcohols, **108** and **77** showed equipotency for the fungi strains, *C. albicans* (11.4 µM) and *C. neoformans* (5.7 µM) in both 24 and 48 hours of incubation. These results

imply that the removal of the acetyl group slightly reduces the antifungal activity as seen in Table 3.4. The MIC values for the benzoxepine **126** was relatively lower than those of the chromone derivatives **93**, **94**, and **129**, and those of the acetophenone **130**, which all lack the oxepine ring, thus indicating the significance of the oxepine moiety for the antifungal activity of the compounds. The MIC values for the compounds with dimethylpyran moiety, **129** and **130**, indicates the insignificance of the dimethylpyran moiety on antifungal activity. Although compared to amphotericin B, all of the screened compounds showed lower antifungal activity against *C. albicans* and *C. neoformans*, the oxepine moiety derivatives proved promising than the compounds lacking the oxepine moiety.

3.6. Conclusions

Two synthetic approaches for the preparation of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) were explored. The first approach involved the construction of the natural chromone, noreugenin (**93**) in modest yields via Kostanecki-Robinson reaction from phloroacetophenone (**97**) followed by the synthesis of oxepine moiety in five key sequential steps, which were *O*-allylation of noreugenin, Claisen rearrangement into the *C*-allyl chromones **94** and **95**, alkylation with the alkyl bromide, ring closing metathesis and subsequent deprotection to give naturally occurring eranthin (**108**) and ptaeroxylinol (**77**). Esterification of the alcohols finally afforded the corresponding acetates, eranthin acetate (**4**) and ptaeroxylinol acetate (**5**). This approach presented challenges during Claisen rearrangement and decomposition of the TBDPS-protected oxepinochromones which were avoided by application of subsequent RCM-deprotection protocol, wherein the RCM products were not isolated before deprotection. Overall, this synthetic procedure resulted in the first total synthesis of two natural compounds eranthin acetate (**4**) and ptaeroxylinol acetate (**5)** in six key steps from phloroacetophenone (**97**).

The second synthetic approach involved the synthesis of **4** and **5** by constructing the oxepine ring from phloroacetophenone (**97**) followed by the construction of the chromone moiety. Synthesis of the oxepine ring from phloroacetophenone was based on protection of OH-4 and OH-6 with MOM protecting group followed by allylation and Claisen rearrangement. The rearrangement was followed by the selective protection and deprotection of the hydroxy

groups and alkylation at the OH-4 to give the RCM precursor **123**. RCM and deprotection gave the benzoxepine **126** which also was prone to decomposition during column chromatography purification. This route gave access to an unexpected benzoxepine **125** with a migrated double bond. This benzoxepine resembles a natural occurring oxepinochromone, ptaeroglycol (**9**), a natural compound isolated from *P. obliquum.*[13] Due to problems associated with this route as mentioned above, and time constraints, this approach was halted and more investigations and optimization to achieve the development of a robust synthetic route for the synthesis of ^{14}C-labelled oxepinochromones **4** and **5** will be performed in the future. Two derivative intermediates **128** and **129** were successfully prepared in one step from readily available intermediates **94** and **116**, respectively via prenylation with commercially available prenal. Of the nine compounds screened for antifungal activity against *C. albicans* and *C. neoformans*, oxepinochromones **4** and **5** showed promising activity.

3.7. References

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Chapter 4: Conclusions and future work

4.1. Conclusion

In this study, four naturally occurring oxepinochromones including eranthin (**108**), ptaeroxylinol (**77**) and their corresponding acetates, eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) were successfully synthesised. Eranthin acetate (**4**) and ptaeroxylinol acetate (**5**) were prepared by employing the first synthetic approach, which was based on construction of the chromone scaffold followed by the construction of the oxepine ring. The chromone scaffold was obtained by employing a slightly modified Kostanecki-Robinson reaction using 2',4',6'-trihydroxyacetophenone (**97**) as a starting material. Subjecting the resulting natural chromone, noreugenin (**93**) to allylation and a subsequent Claisen rearrangement using *N,N*-Diethylaniline as a solvent afforded two isomers, 8-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**94**) and 6-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**95**) that led to the concurrent synthesis of eranthin acetate (**4**) and ptaeroxylinol acetate (**5**). The main challenge encountered with the synthetic route was the decomposition of the RCM products, which prompted employment of the one-pot RCM-deprotection protocol to afford the penultimate alcohols eranthin (**108**) and ptaeroxylinol (**77**). The corresponding acetates, eranthin acetate (**4**) and ptaeroxylinol acetate (**5**), were successfully prepared from the alcohols via esterification with acetyl chloride in modest yields of 35% and 22%, respectively. This approach follows a previously reported synthesis of eranthin (**108**) and ptaeroxylinol (**77**) with slight modifications. This six-key step synthetic route was the most viable approach for the preparation of the target naturally occurring oxepinochromones from phloroacetophenone (**97**). Through detailed NMR analysis of the synthesised eranthin acetate (**4**) and ptaeroxylinol acetate (**5**), the structure of the antifungal agent obliquumol that was previously assigned as eranthin acetate (**4**) was corrected to ptaeroxylinol acetate (**5**).

The second approach to prepare the oxepinochromones by first constructing the oxepine ring from 2',4',6'-trihydroxyacetophenone (**97**) followed by the construction of the chromone scaffold was investigated. The construction of the oxepine ring was analogous to the first approach which included allylation, Claisen rearrangement, alkylation and RCM-deprotection steps. However, due to the reactivity of the phloroacetophenone (**97**), it was essential to

develop optimum protection and deprotection protocol to prevent multiple side reactions. Due to this, the oxepine ring could be prepared from phloroacetophenone in multiple steps, which involved partial protection of the starting material with MOM protecting group, allylation, Claisen rearrangement, protection of the hydrogen-bonded hydroxy group with acid-stable methyl protecting group, selective removal of one MOM group followed by protection with a methyl group and lastly, the removal of the remaining MOM group. The oxepine ring could eventually be constructed by alkylation followed by RCM reaction. Decomposition of the RCM product led to the discovery of an unexpected benzoxepine molecule **125**, with a migrated double bond in the oxepine ring. Similar to the first approach, one-pot RCM-deprotection protocol gave the expected benzoxepine, 2,5-dihydro-3-(hydroxymethyl)-6,8-dimethoxybenzo[b]oxepin-7-yl)ethanone **126** in 32% yields. Unfortunately, decomposition of the synthesised products, resulting in material loss, and time constraints, did not allow construction of the desired chromone scaffold and thus this approach was halted. Two derivative intermediates **129** and **130** were successfully prepared in one step from readily available intermediates **94** and **116**, respectively in via geranylation with commercially available prenal. Compounds **4**, **5**, **108**, **77**, **93**, **94**, **126**, **129**, and **130** were screened for antifungal activity against C. *albicans* and C. *neoformans*. These results showed that the oxepin ring plays an important role in the antifungal activity of the compounds since the compounds with the oxepine ring showed relatively higher activity compared to the compounds lacking the oxepine moiety. The oxepinochromone **4** had higher activity against C. *albicans* whilst **5** had best activity against C. *neoformans*.

4.2. Future work

Although the oxepinochromones, **4** and **5**, could be prepared, the yields were unsatisfactory, therefore, future research is expected to focus on optimising key steps in the first approach to improve yields. Different reaction conditions will be investigated to optimise the Claisen rearrangement step. Cost-effective strategies will be investigated for the construction of the oxepine ring. In addition, methods for purifying the RCM products will be optimised to obtain more stable products with improved yields. The second synthetic approach will be completed to obtain the targeted oxepinochromones, **4** and **5**. Other protection and deprotection protocols with fewer steps will be investigated to reduce the number of synthetic steps

followed to prepare the oxepine ring in acceptable yields. Several structural derivatives will be prepared and further biological activities of the synthesised products and key intermediated will be studied. Upon complete development of the second approach, ^{14}C labelled eranthin acetate, ptaeroxylinol acetate and more bioactive derivatives will be prepared and their tissue distribution studies will be conducted.

Chapter 5: Experimental procedures

5.1. General experimental procedures

All reactions that require anhydrous conditions were carried out under argon in oven-dried glassware. Reagent grade solvents and chemicals used for syntheses were purchased from Sigma-Aldrich or Merck and were used without further purification.

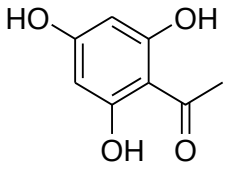
Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminum plates and were observed under UV light (254 nm). The plates were developed in vanillin/ potassium permanganate stain followed by heating. Preparative TLC was carried out on Merck silica gel glass plates and visualised under UV light (254 nm). Column chromatographic purifications were carried out on silica gel (230-400 mesh) using the eluent system detailed in each procedure.

Synthesised compounds were characterized by infrared spectroscopy, mass spectrometry and Nuclear Magnetic Resonance (NMR) spectroscopy. The IR spectra were recorded on an Alpha Bruker Optics FT-IR spectrometer and all data were reported in wavenumbers. HRMS were recorded on a Waters UPLC coupled to QTOF Synapt G2 spectrometer using electrospray ionisation in the positive or negative mode.

NMR spectra were recorded at room temperature on Bruker Avance III 300 or 400 MHz spectrometer. ^{1}H and ^{13}C NMR chemical shifts were referenced to residual protonated solvents peaks; which are δ_H 7.26, δ_C 77.0 for $CDCl_3$; δ_H 2.54, δ_C 39.5 for DMSO-d_6. Chemical shifts are reported in ppm (δ) and spin-spin coupling constants (*J*) in Hertz (Hz). The multiplicities of ^{1}H and ^{13}C resonances are expressed by abbreviations: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), quartet (q), m (multiplet). 1D NMR (^{1}H, ^{13}C and DEPT-135) and 2D NMR (HSQC, HMBC, NOESY and COSY) spectra were used for the complete assignment of NMR signals.

5.2. Experimental data for the first approach

1-[2,4,6-tris(hydroxy)phenyl]ethanone (97)



To the mixture of phloroglucinol (**96**) (2.50 g, 19.8 mmol) and Ac_2O (1.90 mL, 19.8 mmol), $BF_3.OEt_2$ (7.50 mL, 60.9 mmol) was added dropwise under an argon atmosphere. The solution was left to stir for 12 h. Thereafter 10% NaOAc solution was added, and the mixture was stirred for another 12 h. The precipitate formed was filtered off and washed with H_2O. The precipitate was fixed on silica gel and purified on silica gel column chromatography using petroleum ether: EtOAc (8:2) as the eluent to afford the target compound **97** (0.810 g, 4.82 mmol, 24%, Rf = 0.26) as a white powder, and 2,4-diacetylphloroglucinol (2.08 g, 9.90 mmol, 50%, Rf = 0.59) as a pink powder.

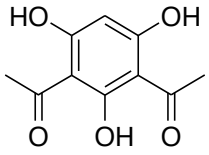
IR: ν_{max} 3020, 2968, 1740, 1437, 1370, 1222, 515 cm^{-1}

^1H NMR (DMSO-d_6, 300 MHz) δ: 12.26 (2H, s, OH-2, OH-6), 10.40 (1H, s, OH-4), 5.83 (2H, s, H-3, H-5), 2.58 (3H, s, $ArCOCH_3$)

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 202.5 (C, $ArCOCH_3$), 164.8 (C, C-4), 164.3 (2 × C, C-2, C-6), 104.0 (C, C-1), 94.5 (2 ×CH, C-3, C-5), 32.4 (CH_3, $ArCOCH_3$)

HRMS-ESI *m/z* $[M + H]^+$ 169.0525 (calculated for $C_8H_9O_4$ 169.0495)

2,4-Diacetylphloroglucinol



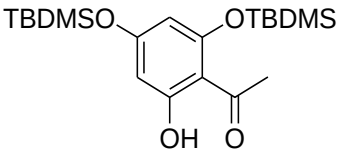
IR: ν_{max} 2589, 1567, 1361, 1281, 1189, 1109, 969, 811, 600, 557cm^{-1}

^1H NMR (DMSO-d_6, 300 MHz) δ: 13.27 (2H, s, OH-2, OH-6), 5.92 (1H, s, H-5), 2.64 (6H, s, $ArCOCH_3$)

^{13}C NMR (DMSO-d_6, 75 MHz) δ: 203.6 (2 × C, ArCOCH$_3$), 171.1 (C, C-2), 168.6 (2 × C, C-4, C-6), 103.6 (2 × C, C-1, C-3), 94.6 (CH, C-5), 32.5 (2 × CH$_3$, ArCOCH$_3$)

HRMS-ESI *m/z* [M + H]$^+$ 211.0597 (calculated for $C_{10}H_{11}O_5$ 211.0601)

1-[4,6-bis(*tert*-butyldimethylsiloxy)-2-hydroxyphenyl]ethanone(98)

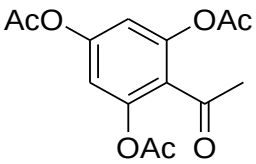


To a stirred suspension of phloroacetophenone (**97**) (1.60 g, 9.75 mmol) in dry CH_2Cl_2 (15 mL), Et_3N (4.49 mL, 32.2 mmol) was added at once resulting in a formation of a clear reddish-yellow solution. TBDMSCl (3.23 g, 21.5 mmol) in dry CH_2Cl_2 (20 mL) was added and the solution was stirred at rt for 2 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and 2 M H_2SO_4 (5 mL) was added dropwise. The reaction mixture was further quenched with H_2O (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography using hexanes: EtOAc (20:1) as the eluent to afford the target compound **98** (1.09 g, 2.76 mmol, 35%, Rf = 0.77) as a brownish-red oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ: 13.52 (1H, s, OH-2), 6.02 (1H, d, *J* = 2.3 Hz, H-5), 5.83 (1H, d, *J* = 2.3 Hz, H-3), 2.62 (3H, s, ArCOCH$_3$), 1.00 (9H, s, (CH$_3$)$_3$CSi), 0.96 (9H, s, (CH$_3$)$_3$CSi), 0.34 (6H, s, Si(CH$_3$)$_2$), 0.23 (6H, s, Si(CH$_3$)$_2$)

^{13}C NMR ($CDCl_3$, 75 MHz) δ: 203.4 (C, ArCOCH$_3$), 166.3 (C, C-6), 162.4 (C, C-4), 159.5 (C, C-2), 109.0 (C, C-1), 102.7 (CH, C-3), 101.7 (CH, C-5), 32.8 (CH$_3$, ArCOCH$_3$), 26.1 (CH$_3$, (CH$_3$)$_3$CSi), 25.5 (CH$_3$, (CH$_3$)$_3$CSi), 18.8 (C, (CH$_3$)$_3$CSi), 18.2 (C, (CH$_3$)$_3$CSi), -3.5 (CH$_3$, Si(CH$_3$)$_2$), -4.4 (CH$_3$, Si(CH$_3$)$_2$)

1-[2,4,6-tris(acetyloxy)phenyl]ethanone (100)



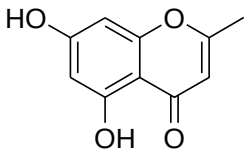
To a stirred solution of phloroacetophenone **97** (3.00 g, 16.1 mmol) in pyridine (6.0 mL, 74 mmol), Ac_2O (6.40 mL, 68.8 mmol) was added at rt and the reaction was stirred for 4 h. The product was precipitated out by adding crushed ice into the reaction mixture and shaking. The precipitate was filtered out and washed with ice cold H_2O. It was collected as a white solid (4.30 g, 90%, 14.6 mmol) and used in the next step without further purification.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.92 (2H, s, H-3, H-5), 2.46 (3H, s, ArCOC$\underline{H}_3$), 2.28 (3H, s, OCOC$\underline{H}_3$), 2.27 (6H, s, OCOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 197.6 (C, ArC$\underline{O}$CH_3), 168.3 (2 × C, O$\underline{C}$OCH_3), 168.2 (C, O$\underline{C}$OCH_3) 151.7 (C, C-4), 148.2 (2 × C, C-2, C-6), 125.0 (C, C-1), 114.1 (2 × CH, C-3, C-5), 31.2 (CH_3, ArCO$\underline{C}H_3$), 21.1 (CH_3, OCO$\underline{C}H_3$), 21.0 (2 × CH_3, OCO$\underline{C}H_3$)

HRMS-ESI *m/z* $[M + Na]^+$ 317.0637 (calculated for $C_{14}H_{14}O_7$ Na 317.0632)

5,7-Dihydroxy-2-methyl-4*H*-chromen-4-one, Noreugenin (93)



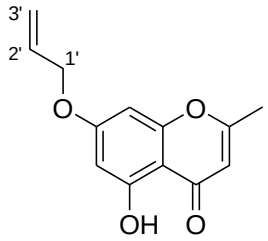
To a solution of 2,4,6-triacetoxyacetophenone (**100**) (8.58 g, 29.2 mmol) in CH_3CN (35 mL), K_2CO_3 (20.0 g, 146 mmol) was added, and the reaction mixture was refluxed for 2 h. Upon completion of the starting material, a 1:1 mixture of CH_3OH and a saturated aqueous K_2CO_3 solution (20 mL) was added to the mixture and was heated to reflux for 3 h. After the mixture has cooled down, it was acidified with 3 M HCl and the precipitate that formed was filtered and washed with cold 3 M HCl to give the product **93** (3.53 g, 18.4 mmol, 61%) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.87 (1H, s, OH-5), 10.85 (1H, s, OH-7), 6.36 (1H, d, *J* = 2 Hz, H-8), 6.21 (2H, s, H-6, H-3), 2.38 (3H, s, CH_3-2).

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 181.7 (C, C-4), 167.7 (C, C-2), 164.1 (C, C-7), 161.5 (C, C-5), 157.8 (C, C-8a), 107.9 (CH, C-3), 103.4 (C, C-4a), 98.7 (CH, C-6), 93.7 (CH, C-8), 19.9 (CH_3, CH_3-2).

HRMS-ESI *m/z* $[M + H]^+$ 193.0517 (calculated for $C_{10}H_9O_4$ 193.0495)

7-Allyloxy-5-hydroxy-2-methyl-4*H*-chromen-4-one (101)



To a solution of noreugenin (**93**) (1.60 g, 8.52 mmol) in DMF (26 mL), K_2CO_3 (1.80 g, 12.8 mmol) was added. The mixture was stirred for 10 min under argon before allyl bromide (0.81 mL, 9.37 mmol) was added. The reaction mixture was stirred at rt for 3 h. Upon completion, the mixture was filtered and washed with EtOAc (25 mL). The filtrate was washed with saturated NH_4Cl solution (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexanes: EtOAc (8:2) to afford compound **101** (1.20 g, 5.34 mmol, 63%, Rf = 0.4) as a light-yellow solid.

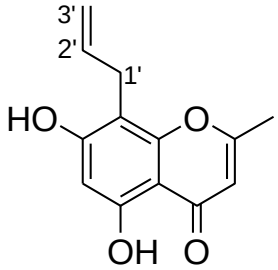
IR: ν_{max} 1739, 1664, 1624, 1368, 1342, 1203, 1167cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.36 (1 H, d, *J* = 2.2 Hz, H-8), 6.34 (1 H, d, *J* = 2.2 Hz, H-6), 6.08-5.98 (1 H, m, H-2'), 6.02 (1 H, s, H- 3), 5.42 (1 H, dd, *J* = 17.3 Hz and 1.3 Hz, H-3'a), 5.33 (1H, dd, *J* = 10.5 Hz and 1.3 Hz, H-3'b), 4.58 (2H, d, *J* = 5.3 Hz, H-1'), 2.28 (3H, s, CH_3-2);

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.4 (C, C-4), 166.8 (C, C-2), 164.2 (C, C-7), 162.1 (C, C-5), 158.0 (C, C-8a), 132.1 (CH, C-2'), 118.4 (CH_2, C-3'), 108.8 (CH, C-3), 105.3 (C, C-4a), 98.5 (CH, C-6), 93.1 (CH, C-8), 69.2 (CH_2, C-1'), 20.5 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 233.0848 (calculated for $C_{13}H_{13}O_4$ 233.0808)

8-Allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (94)



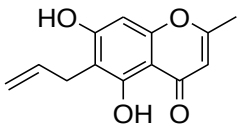
The allyl ether **101** (1.0 g, 4.3 mmol) was refluxed in *N*,*N*-DEA (5 mL) at 220 °C for 12 h. After cooling the reaction mixture to rt, EtOAc (30 mL) was added, and the mixture was then washed with 1 M HCl (10 mL). A saturated NH_4Cl solution (30 mL) was added, and the product was extracted with EtOAc (3 × 30 mL), washed with brine, and dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was filtered through a short pad of silica gel using hexanes: EtOAc (9:1) to remove the excess *N*,*N*-diethyl aniline and the solvent was evaporated in vacuo. The product was then purified by silica gel column chromatography using hexanes: EtOAc (8:2) as the eluent to afford **94** (0.32 g, 1.4 mmol, 32%, Rf = 0.44) as a yellow solid, and **95** (60 mg, 0.27 mmol, 6.19%, Rf = 0.29), as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.81 (1H, s, OH-5), 10.81 (1H, s, OH-7), 6.31 (1H, s, H-6), 6.19 (1H, s, H-3), 5.95-5.86 (1H, m, H-2'), 5.02-4.96 (2H, m, H-3'), 3.38 (2H, d, *J* = 6.3 Hz, H-1'), 2.39 (3H, s, CH_3-2)

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 182.1 (C, C-4), 167.6 (C, C-2), 161.6 (C, C-7), 159.4 (C, C-5), 155.2 (C, C-8a), 135.8 (CH, C-2'), 114.8 (CH_2, C-3'), 107.7 (CH, C-3), 103.9 (C, C-8), 103.3 (C, C-4a), 98.2 (CH, C-6), 26.2 (CH_2, C-1'), 19.9 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 233.0819 (calculated for $C_{13}H_{13}O_4$ 233.0808)

6-Allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (95)

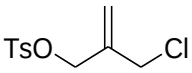


^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.13 (1H, s, OH-5), 10.85 (1H, s, OH-7), 6.44 (1H, s, H-8), 6.19 (1H, s, H-3), 5.93-5.87 (1H, m, H-2'), 5.99-4.94 (2H, m, H-3'), 3.29 (2H, d, *J* = 6.1 Hz, H-1'), 2.38 (3H, s, CH_3-2)

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 181.8 (C, C-4), 167.4 (C, C-2), 161.9 (C, C-7), 158.6 (C, C-5), 155.8 (C, C-8a), 135.7 (CH, C-2'), 114.6 (CH_2, C-3'), 108.9 (C, C-6), 107.8 (CH, C-3), 103.1 (C, C-4a), 92.9 (CH, C-8), 26.0 (CH_2, C-1'), 19.9 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 233.0819 (calculated for $C_{13}H_{13}O_4$ 233.0808)

2-(Chloromethyl)allyl-4-methylbenzenesulfonate (103)

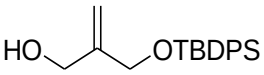


To a solution of 2-methylenepropane-1,3-diol (**102**) (0.20 g, 2.3 mmol) in CH_2Cl_2 (10 mL), Et_3N (0.41 mL, 3.0 mmol) was added. The solution was stirred for 10 min and TsCl (0.43 g, 2.3 mmol) was added. The reaction mixture was stirred at rt for 3 h. The mixture was quenched with excess H_2O and was extracted with CH_2Cl_2 (3 × 10 mL) and washed with H_2O and concentrated on rotary evaporator. The residue was purified by silica gel column chromatography by gradient elution using hexanes: EtOAc (9:1- 6:4) as the eluent to give **103** (25 mg, 4.2%, 0.096 mmol, Rf = 0.71) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.81 (2H, d, *J* = 8.4 Hz, ArH), 7.35 (2H, d, *J* = 8.4 Hz, ArH), 5.35 (1H, s, C=CH_2), 5.28 (1H, s, C=CH_2), 4.62 (2H, s, CH_2OTs), 4.02 (2H, s, CH_2Cl), 2.45 (3H, s, Ar-CH_3)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 145.0 (C, C-Ar), 138.1 (C=CH_2), 132.6 (C, C-Ar), 129.9 (2 × CH, C-Ar), 127.9 (2 × CH, C-Ar), 120.1 (C=CH_2), 69.5 (CH_2, CH_2OTs), 44.1 (CH_2, CH_2Cl), 21.6 (CH_3, Ar-CH_3).

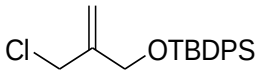
2-(*tert*-Butyldiphenylsiloxy)methylprop-2-en-1-ol (104)



Sodium hydride (NaH) (60% dispersion in mineral oil; 1.80 g, 45.4 mmol) was added to a solution of 2-methylenepropane-1,3-diol (**102**) (4.00 g, 45.4 mmol) in dry THF/ DMF (30 mL) under argon at 0 °C. After stirring for 70 min at rt, the solution was cooled to 0 °C, and TBDPSCl (11.2 mL, 43.1 mmol) was added dropwise. The reaction mixture was stirred for 16 h at rt. The reaction was quenched with H_2O (20 mL) and the mixture was extracted with diethyl ether (5 × 30 mL). The diethyl ether layer was washed with brine, dried over anhydrous Na_2SO_4, and was concentrated in vacuo. The mixture was purified by silica gel chromatography using hexanes: EtOAc (9:1) as the eluent to give the target compound **104** (10.1 g, 30.9 mmol, 68%, Rf = 0.51) as a colorless oil.

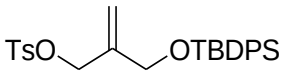
^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.69-7.67 (4H, m, SiArH), 7.46-7.37 (6H, m, SiArH), 5.15 (1H, m, C=CH_2), 5.12 (1H, m, C=CH_2), 4.26 (2H, s, HOCH_2), 4.12 (2H, s, CH_2OSi), 1.07 (9H, s, (CH_3)$_3$CSi)
^{13}C NMR ($CDCl_3$, 100 MHz) δ: 147.1 (C, C=CH_2), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 111.1 (CH_2, C=CH_2), 65.5 (CH_2, CH_2OSi), 64.5 (CH_2, HOCH_2C), 26.8 (3 × CH_3, (CH_3)$_3$CSi), 19.2 (C, (CH_3)$_3$CSi)
HRMS-ESI *m/z* $[M + Na]^+$ 349.1627 (calculated for $C_{20}H_{26}O_2SiNa$ 349.1594)

3-Chloro-2-(*tert*-butyldiphenylsiloxy)methylpropene (105a)



To a solution of 2-((tert-butyldiphenylsilyloxy)-methyl)-prop-2-en-1-ol (**104**) (0.60 g, 1.8 mmol) in CH_2Cl_2 (10 mL), Et_3N (0.38 mL, 2.8 mmol) was added. The solution was allowed to stir for 10 min and TsCl (0.42 g, 2.2 mmol) was added. The reaction mixture was stirred at rt for 24 h. The mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the organic layer was washed with excess H_2O and dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified by PTLC using hexanes: EtOAc (9:1) as the eluent to afford **105a** (20 mg, 3%, 0.058 mmol, Rf = 0.8) as a yellow oil.
^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.70-7.67 (4H, m, SiArH), 7.46- 7.37 (6H, m, SiArH), 5.31 (1H, m, C=CH_2), 5.25 (1H, m, C=CH_2), 4.28 (2H, s, CH_2OSi), 4.12 (2H, s, ClCH_2), 1.08 (9H, s, (CH_3)$_3$CSi)
^{13}C NMR ($CDCl_3$, 100 MHz) δ: 144.1 (C, C=CH_2), 135.5 (4 × CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 114.5 (CH_2, C=CH_2), 64.1 (CH_2, CH_2OSi), 45.0 (CH_2, ClCH_2), 26.8 (3 × CH_3, (CH_3)$_3$CSi), 19.3 (C, (CH_3)$_3$CSi)

2-(*tert*-Butyldiphenylsiloxymethyl)allyl-4-methylbenzenesulfonate (105b)



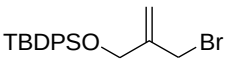
To a stirred solution of the protected alcohol **104** (0.20 g, 0.61 mmol) in DMF (2 mL), pyridine (0.10 mL, 1.2 mmol) was added, and the reaction was stirred for 15 min before TsCl (0.14 g, 0.73 mmol) was added. The reaction mixture was left to stir at rt for 3 h. The mixture

containing the product was extracted into EtOAc (3 × 10 mL), washed with H_2O, then brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The mixture was purified by silica chromatography using hexanes: EtOAc (9:1) as the eluent to provide the compound **105b** (0.054 g, 0.11 mmol, 11%, Rf = 0.46) as a light-yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.65-7.62 (4H, m, SiArH), 7.53 (2H, d, *J* = 8.2 Hz, SiArH), 7.44-7.34 (6H, m, SiArH), 7.27 (2H, d, *J* = 8.9 Hz, SiArH), 5.31 (1H, d, *J* = 1.3 Hz, C=CH_2), 5.15 (1H, d, *J* = 1.3 Hz, C=CH_2), 4.52 (1H, d, *J* = 12.2 Hz, CH_2OSi), 4.14 (2H, m, CH_2OTs), 4.11 (1H, d, *J* = 12.2 Hz, CH_2OSi), 2.40 (3H, s, Ar-CH_3), 1.03 (9H, s, (CH_3)$_3$CSi)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 142.8 (C, C-Ar), 142.7 (C, C=CH_2), 141.4 (C, C-Ar), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 129.7 (2 × CH, C-Ar), 127.7 (4 × CH, SiAr), 125.2 (2 × CH, C-Ar), 114.1 (CH_2, C=CH_2), 64.5 (CH_2, CH_2OTs), 64.2 (CH_2, CH_2OSi), 26.7 (3 × CH_3, (CH_3)$_3$CSi), 21.5 (CH_3, Ar-CH_3), 19.2 (C, (CH_3)$_3$CSi)

(2-(Bromomethyl)allyloxy)(*tert*-butyl)diphenylsilane (105c)

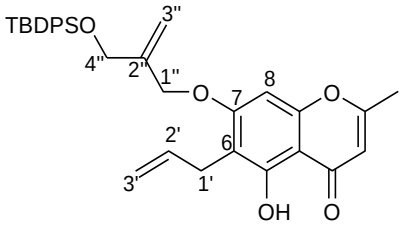


To a solution of the protected alcohol **104** (2.0 g, 6.1 mmol) in diethyl ether (31 mL), PBr_3 (0.70 mL, 7.4 mmol) was added dropwise at 0 °C under argon. The reaction mixture was stirred for 3 h at rt, and cold H_2O (50 mL) was added slowly. The organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexanes: EtOAc (9:1) as the eluent to give the target compound **105c** (0.25 g, 0.64 mmol, 42%, Rf = 0.90) as a yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68 (4H, m, SiArH), 7.44-7.39 (6H, m, SiArH), 5.31-5.28 (2H, m, C=CH_2), 4.30 (2H, s, CH_2OSi), 3.03 (2H, s, CH_2Br), 1.07 (9H, s, (CH_3)$_3$CSi)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 144.4 (C, C=CH_2), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 114.9 (CH_2, C=CH_2), 64.1 (CH_2, CH_2OSi), 32.7 (CH_2, CH_2Br), 26.7 (3 × CH_3, (CH_3)$_3$CSi), 19.2 (C, (CH_3)$_3$CSi)

7-(2''-(*tert*-Butyldiphenylsiloxymethyl)allyloxy)-6-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (76)



To a solution of ally chromone **95** (0.18 g, 0.76 mmol) in DMF (5 mL), K_2CO_3 (0.160 g, 1.14 mmol) was added, and the mixture was stirred for 15 min at rt under argon. Alkyl bromide **105c** (0.39 g, 0.92 mmol) was added to the mixture which was then stirred for 5 h at rt. The reaction mixture was filtered, diluted with EtOAc (20 mL) and washed with a saturated NH_4Cl solution (20 mL). The product was then extracted with EtOAc (3 × 20 mL), washed with brine, dried over anhydrous Na_2SO_4 and was concentrated in vacuo. The mixture was purified by PTLC using hexanes: EtOAc (9:1) to afford the target compound **76** (0.029 g, 0.54 mmol, 70%, Rf = 0.56) as colorless oil.

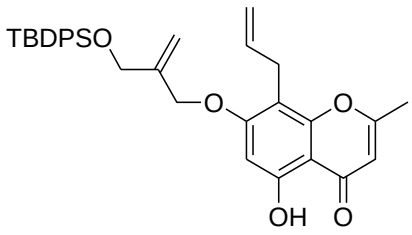
IR: ν_{max} 2928, 2857, 1740, 1660, 1451, 1374, 1343, 1267, 1229, 1204, 1109, 819, 706cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.68-7.66 (4H, m, SiArH), 7.43-7.36 (6H, m, SiArH), 6.37 (1H, s, H-8), 6.03 (1H, s, H-3), 5.87-5.81 (1H, m, H-2'), 5.36 (1H, s, H-"a), 5.26 (1H, s, H-3"b), 4.92 (1H, dd, *J* = 17.1 and 1.6 Hz, H-3'a), 4.85 (1H, dd, *J* = 10.0 and 1.6 Hz, H-3'b), 4.61 (2H, s, H-1"), 4.28 (2H, s, H-4"), 3.34 (2H, d, *J* = 6.1 Hz, H-1'), 2.33 (3H, s, CH_3-2), 1.08 (9H, s, $(CH_3)_3CSi$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.4 (C, C-4), 166.4 (C, C-2), 161.9 (C, C-7), 158.6 (C, C-5), 156.6 (C, C-8a), 142.1 (C, C-2"), 135.7 (CH, C-2'), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.8 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 114.5 (CH_2, C-3'), 112.9 (CH_2, C-3"), 111.1 (C, C-6), 108.8 (CH, C-3), 105.2 (C, C-4a), 90.4 (CH, C-8), 68.9 (CH_2, C-1"), 64.6 (CH_2, C-4"), 26.8 (3 × CH_3, $(CH_3)_3CSi$), 26.4 (CH_2, C-1'), 20.4 (CH_3, CH_3-2), 19.3 (C, $(CH_3)_3CSi$)

HRMS-ESI *m/z* $[M + H]^+$ 541.2401 (calculated for $C_{33}H_{37}O_5Si$ 541.2404)

7-(2''-(*tert*-Butyldiphenylsiloxymethyl)allyloxy)-8-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (106)



To a solution of allyl chromone **94** (0.30 g, 1.3 mmol) in DMF (10 mL), K_2CO_3 (0.27 g, 1.9 mmol) was added, and the mixture was stirred for 15 min at rt under argon. Alkyl bromide **105c** (0.65 g, 1.7 mmol) was added to the mixture which was then stirred at rt for 5 h. The reaction mixture was filtered, diluted with EtOAc (20 mL) and washed with a saturated NH_4Cl solution (20 mL). The mixture was extracted with EtOAc (3 × 20 mL), washed with brine, dried over anhydrous Na_2SO_4 and was concentrated in vacuo. The mixture was purified by PTLC using hexanes: EtOAc (9:1) to afford the target compound **106** (0.29 g, 0.54 mmol, 42%, Rf= 0.48) as colorless oil.

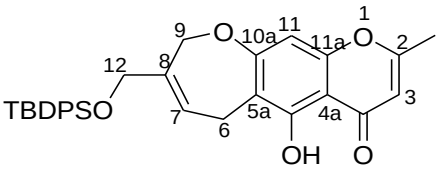
IR: ν_{max} 2933, 2859, 1741, 1659, 1467, 1422, 1382, 1325, 1268, 1237, 1193, 1109, 820, 743, 704, 613, 502cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.77 (1H, brs, OH-5), 7.68-7.66 (4H, m, SiArH), 7.44-7.36 (6H, m, SiArH), 6.34 (1H, s, H-6), 6.02 (1H, s, H-3), 5.82-5.72 (1H, m, H-2'), 5.38 (1H, brs, H-3''a), 5.26 (1H, d, *J* = 1.2 Hz, H-3''b), 4.90-4.83 (2H, m, H-3'), 4.59 (2H, s, H-1''), 4.28 (2H, s, H-4''), 3.37 (2H, d, *J* = 6.2 Hz, H-1'), 2.35 (3H, s, CH_3-2), 1.07 (9H, s, $(CH_3)_3CSi$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.9 (C, C-4), 166.8 (C, C-2), 161.6 (C, C-7), 160.7 (C, C-5), 154.9 (C, C-8a), 142.7 (C, C-2''), 135.6 (CH, C-2'), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 114.6 (CH_2, C-3'), 112.8 (CH_2, C-3''), 108.3 (CH, C-3), 105.9 (C, C-8), 104.7 (C, C-4a), 95.8 (CH, C-6), 69.2 (CH_2, C-1''), 64.4 (CH_2, C-4''), 26.8 (3 × CH_3, $(CH_3)_3CSi$), 26.6 (CH_2, C-1'), 20.5 (CH_3, CH_3-2), 19.2 (C, $(CH_3)_3CSi$)

HRMS-ESI *m/z* $[M + H]^+$ 541.2401 (calculated for $C_{33}H_{37}O_5Si$ 541.2404)

5-Hydroxy-8-(*tert*-butyldiphenylsiloxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-*g*]chromen-4-one (109)



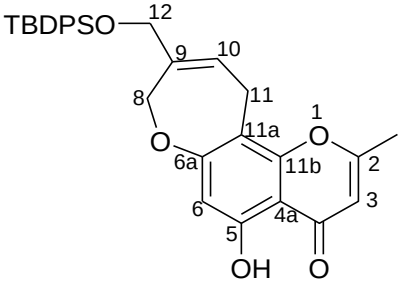
To a solution of diene **76** (19.2 mg, 0.0354 mmol) in dry CH_2Cl_2 (18 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene](tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (3.12 mg, 3.68 µmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h and evaporated after cooling. The resulting oil was purified by PTLC using hexanes: EtOAc (8:2) to give the target compound **109** (9.0 mg, 0.017 mmol, 49%, Rf = 0.7) as a green oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.65-7.63 (4H, m, SiArH), 7.42-7.36 (6H, m, SiArH), 6.52 (1H, s, H-11), 6.05 (1H, s, H-3), 5.85-5.82 (1H, m, H-7), 4.70 (2H, d, *J* = 0.8 Hz, H-9), 4.06 (2H, s, H-12), 3.52 (2H, d, *J* = 5.6 Hz, H-6), 2.36 (3H, s, CH_3-2), 1.04 (9H, s, $(CH_3)_3CSi$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.8 (C, C-4), 167.1 (C, C-2), 164.8 (C, C-10a), 158.1 (C, C-5), 155.8 (C, C-11a), 137.1 (C, C-8), 135.5 (4 × CH, SiAr), 133.2 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 123.5 (CH, C-7), 116.1 (C, C-5a), 108.7 (CH, C-3), 106.6 (C, C-4a), 99.2 (CH, C-11), 71.0 (CH_2, C-9), 66.2 (CH_2, C-12), 26.7 (3 × CH_3, $(CH_3)_3CSi$), 21.0 (CH_2, C-6), 20.5 (CH_3, CH_3-2), 19.2 (C, $(CH_3)_3CSi$)

HRMS-ESI *m/z* $[M + H]^+$ 513.2066 (calculated for $C_{31}H_{33}O_5Si$ 513.2091)

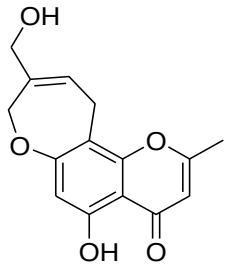
5-Hydroxy-9-(*tert*-butyldiphenylsiloxymethyl)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (107)



To a solution of diene **106** (97.9 mg, 0.181 mmol) in dry CH_2Cl_2 (20 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene](tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (16.0 mg, 18.8 µmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h and evaporated after cooling. The resulting oil was purified by PTLC using hexanes: EtOAc (8:2) to give the target compound **107** (38.4 mg, 0.0761 mmol, 42 % Rf = 0.45) as green oil.

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.66 (1H, s, OH-5), 7.66-7.63 (4H, m, SiArH), 7.42-7.36 (6H, m, SiArH), 6.47 (1H, s, H-6), 6.07 (1H, s, H-3), 5.88-5.84 (1H, m, H-10), 4.67 (2H, d, J = 1.1 Hz, H-8), 4.06 (2H, s, H-12), 3.58 (2H, d, *J* = 5.5 Hz, H-11), 2.39 (3H, s, CH_3-2), 1.05 (9H, s, $SiC(CH_3)_3$). HRMS-ESI *m/z* $[M + H]^+$ 513.2066 (calculated for $C_{31}H_{33}O_5Si$ 513.2091)

5-Hydroxy-9-(hydroxymethyl)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (eranthin) (108)



To a solution of diene **106** (0.29 g, 0.54 mmol) in dry CH_2Cl_2 (30 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene](tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (0.05 g, 0.06 mmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h and the solvent was evaporated after cooling. The residue was dissolved in THF (5 mL) and TBAF solution (1 M in THF; 0.59 mL, 0.59 mmol) was added dropwise at 0 °C under argon. After stirring the reaction mixture for 1 h at 5 °C, EtOAc (20 mL) was added, followed by a saturated aqueous NH_4Cl solution (20 mL). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The resulting oil was purified by silica gel column chromatography using hexanes: EtOAc (3:7) as the eluent to afford **108** (95.0 mg, 0.346 mmol, 65%, Rf = 0.4) as a green solid.

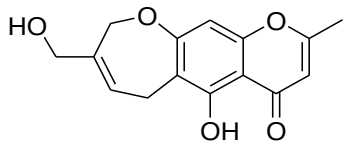
IR: ν_{max} 3446, 2923, 2853, 1655, 1615, 1586, 1486, 1414, 1265, 1180, 1158, 1101, 1073, 1024, 846, 640, 554cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.61 (1H, s, OH-5), 6.45 (1H, s, H-6), 6.04 (1H, s, H-3), 5.99 (1H, t, *J* = 5.3 Hz, H-10), 4.72 (2H, s, H-8), 4.03 (2H, s, H-12), 3.61 (2H, d, *J* = 5.3 Hz, H-11), 2.38 (3H, s, CH_3-2).

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 183.1 (C, C-4), 166.8 (C, C-2), 164.4 (C, C-6a), 160.1 (C, C-5), 153.8 (C, C-11b), 138.5 (C, C-9), 124.2 (CH, C-10), 111.0 (C, C-11a), 108.7 (CH, C-3), 107.0 (C, C-4a), 104.6 (CH, C-6), 70.8 (CH_2, C-8), 65.4 (CH_2, C-12), 21.4 (CH_2, C-11), 20.5 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$ 275.0921)

5-Hydroxy-8-(hydroxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-*g*]chromen-4-one (ptaeroxylinol) (77)



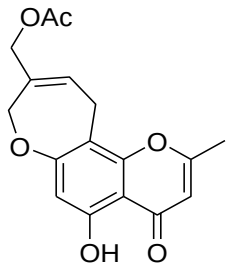
To a solution of diene **76** (0.28 g, 0.51 mmol) in dry CH_2Cl_2 (30 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene](tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (0.045 g, 0.053 mmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h and the solvent was evaporated after cooling. The residue was then dissolved in THF (5 mL) and TBAF solution (1 M in THF; 0.56 mL, 0.56 mmol) was added dropwise at 0 °C under argon. The reaction mixture was stirred for 1 h at 5 °C, then EtOAc (20 mL) and a saturated aqueous NH_4Cl solution (20 mL) were added. The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The resulting oil was purified by silica gel column chromatography using hexanes: EtOAc (3:7) as the eluent to afford **77** (0.056 g, 0.20 mmol, 40%, Rf = 0.18) as a light-green crystal.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 13.08 (1H, s, OH-5), 6.52 (1H, s, H-11), 6.04 (1H, s, H-3), 6.00 (1H, tt, *J* = 5.7, 1.1 Hz, H-7), 4.74 (2H, d, *J* = 1.1 Hz, H-9), 4.03 (2H, s, H-12), 3.57 (2H, brd, *J* = 5.6 Hz, H-6), 2.35 (3H, s, CH_3-2),

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.8 (C, C-4), 167.2 (C, C-2), 164.6 (C, C-10a), 158.1 (C, C-5), 155.9 (C, C-11a), 138.0 (C, C-8), 125.4 (CH, C-7), 115.8 (C, C-5a), 108.7 (CH, C-3), 106.7 (C, C-4a), 99.3 (CH, C- 11), 71.0 (CH_2, C-9), 65.7 (CH_2, C-12), 21.1 (CH_2, C-6), 20.5 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$ 275.0921).

5-Hydroxy-9-(methylacetate)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (4)



K_2CO_3 (0.088 g, 0.28 mmol) was added to a stirred mixture of eranthin **108** (0.025 g, 0.092 mmol) and AcCl (6.6 µL, 0.092 mmol) in CH_2Cl_2 (10 mL) under argon atmosphere. The reaction mixture was stirred at rt for 4 h. Saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by PTLC using hexanes: EtOAc (3:7) to give **4** (10.0 mg, 35%, 0.0316 mmol, Rf = 0.58) as white solid.

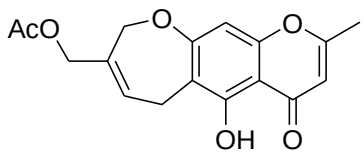
IR: ν_{max} 3463, 3018, 2968, 1740, 1662, 1434, 1369, 1222, 1103, 900, 782, 518 cm^{-1}

^1H NMR ($CDCl_3$, 400 MHz) δ: 12.66 (1H, s, OH-5), 6.49 (1H, s, H-6), 6.06 (1H, s H-3), 6.06-6.03 (1 H, m, H-10), 4.65 (2H, d, *J* = 1.4 Hz, H-8), 4.46 (2H, s, H-12), 3.63-3.62 (2H, d, *J* = 5.5 Hz, H-11), 2.38 (3H, s, CH_3-2), 2.05 (3H, s, $COCH_3$),

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 183.1 (C, C-4), 170.7 (C, $COCH_3$), 166.8 (C, C-2), 164.3 (C, C-6a), 160.3 (C, C-5), 153.7 (C, C-11b), 133.8 (C, C-9), 127.4 (CH, C-10), 111.4 (C, C-11a), 108.8 (CH, C-3), 107.2 (C, C-4a), 104.7 (CH, C-6), 71.0 (CH_2, C-8), 66.5 (CH_2, C-12), 21.6 (CH_2, C-11), 20.9 (CH_3, $COCH_3$), 20.5 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1019)

5-Hydroxy-8-(methylacetate)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-*g*]chromen-4-one (5)



K_2CO_3 (40 mg, 0.29 mmol) was added to a stirred mixture of **77** (40 mg, 0.15 mmol) and AcCl (10 µL, 0.15 mmol) in CH_2Cl_2 (10 mL) under argon atmosphere. The reaction mixture was

stirred at rt for 4 h. Saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The product was purified by PTLC using hexanes: EtOAc (3:7) to give **5** (10.0 mg, 23%, 0.0345 mmol, Rf = 0.68) as colorless crystals.

IR: ν_{max} 3467, 2924, 2853, 1739, 1650, 1619, 1484, 1447, 1398, 1327, 1235, 1169, 1110, 1083, 1037, 966, 845, 796, 578, 544, 505 cm^{-1}

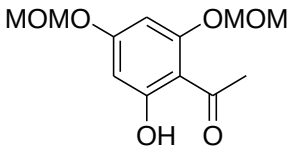
^1H NMR ($CDCl_3$, 400 MHz) δ: 13.08 (1H, s, OH-5), 6.54 (1H, s, H-11), 6.08-6.05 (1H, m, H-7), 6.05 (1H, brs, H-3), 4.65 (2H, d, *J* = 1.5 Hz, H-9), 4.46 (2H, s, H-12), 3.59 (2H, d, *J* = 5.5 Hz, H-6), 2.36 (3H, s, CH_3-2), 2.05 (3H, s, $COCH_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.8 (C, C-4), 170.7 (C, $COCH_3$), 167.2 (C, C-2), 164.5 (C, C-10a), 158.2 (C, C-5), 155.9 (C, C-11a), 133.2 (C, C-8), 128.1 (CH, C-7), 116.0 (C, C-5a), 108.8 (CH, C-3), 106.9 (C, C-4a), 99.4 (CH, C-11), 71.2 (CH_2, C-9), 66.6 (CH_2, C-12), 21.2 (CH_2, C-6), 20.9 (CH_3, $COCH_3$), 20.5 (CH_3, CH_3-2)

HRMS-ESI *m/z* $[M + H]^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1019)

5.3. Experimental data for the second approach

1-[2-Hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (110)



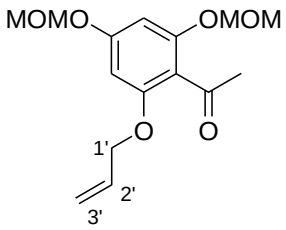
$ZnBr_2$ (3.00 mg, 0.0131 mmol) was dissolved in dimethoxymethane (0.59 mL, 6.7 mmol) under an argon atmosphere, and then AcCl (0.47 mL, 6.7 mmol) was added dropwise to the stirred solution. The solution was stirred at rt for an additional 2 h and transferred into an ice-cold solution of the pre-dried phloroacetophenone (**97**) (0.50 g, 2.7 mmol) and DIPEA (0.93 mL, 5.4 mmol) in CH_2Cl_2 (30 mL) under an argon atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution (30 mL) and stirred for an additional 15 min. The two phases were partitioned, and the aqueous phase was extracted with CH_2Cl_2 (5 × 30 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give a yellow oil which was purified by silica gel column chromatography using hexanes: EtOAc (5:1) to afford **110** (0.35 g, 1.4 mmol, 50%, Rf = 0.84) as a colorless oil which solidified at room temperature.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 13.71 (1H, s, OH), 6.26 (1H, d, *J* = 2.4 Hz, H-3), 6.24 (1H, d, *J* = 2.4 Hz, H-5), 5.25 (2H, s, OC$\underline{H}_2$O), 5.17 (2H, s, OC$\underline{H}_2$O), 3.52 (3H, s, OC$\underline{H}_3$), 3.47 (3H, s, OC$\underline{H}_3$), 2.65 (3H, s, ArCOC$\underline{H}_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.2 (C, Ar$\underline{C}$OCH$_3$), 166.8 (C, C-2), 163.4 (C, C-4), 160.3 (C, C-6), 106.9 (C, C-1), 97.5 (CH, C-3), 94.4 (CH_2, OCH_2O), 94.0 (CH_2, OCH_2O), 94.0 (CH, C-5), 56.7 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 33.0 (CH_3, ArCO$\underline{C}$H$_3$).

HRMS-ESI *m/z* $[M + H]^+$ 257.1034 (calculated for $C_{12}H_{17}O_6$ 257.1019)

1-[2-Allyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (111)



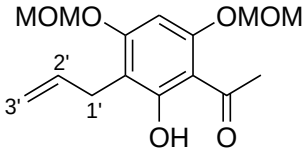
To a solution of 2-hydroxy-4,6-dimethoxymethoxyacetophenone (**110**) (0.10 g, 0.39 mmol) in DMF (5 mL), K_2CO_3 (0.081 g, 0.59 mmol) was added. The mixture was stirred for 10 min under argon before allyl bromide (0.37 mL, 0.429 mmol) was added, and stirring was continued at rt for 3 h. Upon completion, the mixture was filtered and washed with EtOAc (30 mL). The filtrate was washed with saturated NH_4Cl solution (30 mL) and the product was extracted with EtOAc (3 × 30 mL), washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexanes: EtOAc (8:2) to give **111** (0.092 g, 0.31 mmol, 79%, Rf = 0.41) as a light-yellow oil.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.45 (1H, d, *J* = 2.0 Hz, H-5), 6.30 (1H, d, *J* = 2.0 Hz, H-3), 6.03-5.93 (1H, m, H-2'), 5.37–5.33 (1H, m, H-3'a), 5.27-5.26 (1H, m, H-3'b), 5.14 (2H, s, OC$\underline{H}_2$O), 5.13 (2H, s, OC$\underline{H}_2$O), 4.51 (2H, dt, *J* = 5.2 and 1.5 Hz, H-1'), 3.47 (3H, s, OCH_3), 3.45 (3H, s, OC$\underline{H}_3$), 2.48 (3H, s, ArCOC$\underline{H}_3$);

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 201.5 (C, ArC$\underline{O}CH_3$), 159.5 (C, C-4), 156.8 (C, C-2), 155.3 (C, C-6), 132.6 (CH, C-2'), 117.7 (CH_2, C-3'), 116.2 (C, C-1), 96.2 (CH, C-5), 95.0 (CH, C-3), 94.8 (CH_2, O$\underline{C}H_2$O), 94.4 (CH_2, O$\underline{C}H_2$O), 69.3 (CH_2, C-1'), 56.3 (CH_3, O$\underline{C}H_3$), 56.1 (CH_3, O$\underline{C}H_3$), 32.5 (CH_3, ArCO$\underline{C}H_3$).

HRMS-ESI *m/z* $[M + H]^+$ 297.1338 (calculated for $C_{15}H_{21}O_6$ 297.1332)

1-[3-Allyl-2-hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (112)



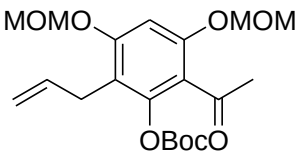
A solution of 2-allyloxy-4,6-dimethoxymethoxyacetophenone (**111**) (0.20 g, 0.67 mmol) was refluxed in *N*,*N*-DEA (5 mL) at 220 °C for 12 h. After cooling the reaction mixture to rt, EtOAc

(30 mL) and 1 M HCl (10 mL) were added. The mixture was partitioned, and the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography using hexanes: EtOAc (5:1) as the eluent to give **112** (0.10 g, 0.33 mmol, 50%, Rf = 0.42) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.86 (1H, s, OH), 6.39 (1H, s, H-5), 5.99-5.89 (1H, m, H-2'), 5.26 (2H, s, OCH_2O), 5.23 (2H, s, OCH_2O), 5.03−4.93 (2H, m, H-3'), 3.52 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.36 (2H, dt, *J* = 6.1 and 1.5 Hz, H-1'), 2.66 (3H, s, $ArCOCH_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.5 (C, $ArCOCH_3$), 163.6 (C-4), 163.6 (C, C-2), 160.9 (C, C-4), 159.1 (C, C-6), 136.3 (CH, C-2'), 114.1 (CH_2, C-3'), 109.6 (C, C-3), 106.8 (C-1), 94.5 (CH_2, OCH_2O), 93.8 (CH_2, OCH_2O), 91.2 (CH, C-5), 56.7 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 33.2 (CH_3, $ArCOCH_3$), 26.5 (CH_2, C-1')

1-[3-Allyl-2-*tert*-butoxycarbonyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (113)

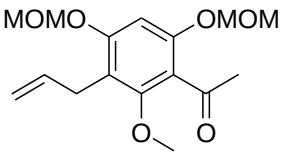


To a solution of 3-allyl-2-hydroxy-4,6-dimethoxymethoxyacetophenone (**112**) (100 mg, 0.330 mmol) in DMF (4 mL), pyridine (40 µL, 0.97 mmol) and di-*tert*-butyl dicarbonate (88.4 mg, 0.400 mmol) were added under argon. The mixture was stirred at rt for 4 h. Upon completion, the reaction was quenched with cold saturated NH_4Cl solution (15 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford **113** (0.117 g, 0.295 mmol, 87 %, Rf = 0.6) as a red-yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.85 (1H, s, H-5), 5.87−5.80 (1H, m, H-2'), 5.20 (2H, s, OCH_2O), 5.19 (2H, s, OCH_2O), 5.04−4.92 (2H, m, H-3'), 3.48 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.30 (2H, d, *J* = 6.3 Hz, H-1'), 2.51 (3H, s, $ArCOCH_3$), 1.53 (3H, s, $CO_2C(CH_3)_3$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 199.6 (C, $ArCOCH_3$), 157.5 (C, C-4), 154.8 (C, C-6), 151.3 (C, $CO_2C(CH_3)_3$), 147.3 (C, C-2), 135.5 (CH, C-2'), 116.3 (C, C-3), 115.1 (CH_2, C-3'), 118.7 (C, C-1), 99.0 (CH, C-5), 95.1 (CH_2, OCH_2O), 94.4 (CH_2, OCH_2O), 83.6 (C, $CO_2C(CH_3)_3$), 56.5 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 32.1 (CH_3, $ArCOCH_3$), 28.0 (CH_2, C-1'), 27.6 (CH_3, $CO_2C(CH_3)_3$).

1-[3-Allyl-2-methoxy-4,6-bis(methoxymethoxy)phenyl]ethanone (115)



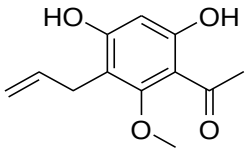
Iodomethane (0.59 mL, 9.5 mmol) was added to a solution of 3-allyl-2-hydroxy-4,6-dimethoxymethoxyacetophenone (**112**) (2.16 g, 7.30 mmol) and K_2CO_3 (5.05 g, 36.5 mmol) in DMF (10 mL). The reaction mixture was heated to reflux for 1 h, cooled to rt and diluted with EtOAc (50 mL) and NH_4Cl solution (50 mL). The mixture was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified by silica gel column chromatography using hexanes: EtOAc (10:1) as the eluent to give target compound **115** (1.83 g, 5.90 mmol, 97.7%, Rf = 0.29) as yellow oil.

IR: ν_{max} 2939, 1741, 1691, 1594, 1474, 1395, 1314, 1256, 1201, 1147, 1109, 1064, 1040, 997, 920, 832, 557cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.71 (1H, s, H-5), 5.99−5.91 (1H, m, H-2'), 5.18 (2H, s, OCH_2O), 5.14 (2H, s, OCH_2O), 5.01−4.96 (2H, m, H-3'), 3.71 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 3.36 (2H, dt, *J* = 6.0 and 1.6 Hz, H-1'), 2.51 (3H, s, ArCOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.1 (C, Ar$\underline{C}$OCH_3), 157.3 (C, C-4), 156.3 (C, C-2), 153.3 (C, C-6), 136.8 (CH, C-2'), 120.7 (C, C-1), 116.4 (C, C-3), 114.6 (CH_2, C-3'), 97.8 (CH, C-5), 95.0 (CH_2, OCH_2O), 94.3 (CH_2, OCH_2O), 63.4 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 56.2 (CH_3, OCH_3), 32.6 (CH_3, ArCO$\underline{C}H_3$), 27.7 (CH_2, C-1').

1-[3-Allyl-2-methoxy-4,6-bis(hydroxy)phenyl]ethanone (116)



To a solution of 3-allyl-2-methoxy-4,6-dimethoxymethoxyacetophenone (**115**) (0.320 g, 1.04 mmol) in CH_3OH (3.5 mL), 3 M HCl (1 mL) was added. The mixture was heated to reflux for 8 h. The reaction was quenched with saturated NH_4Cl solution (30 mL) and product was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine, dried over

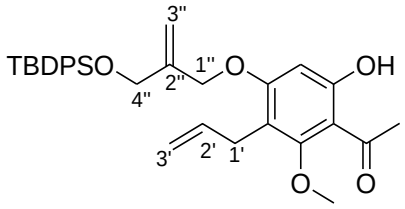
anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified by silica gel column chromatography using hexanes: EtOAc (8.5: 1.5) as the eluent to give the target compound **116** (0.16 g, 0.70 mmol, 67%, Rf = 0.44) as a light-yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.20 (1H, s, OH-6), 6.24 (1H, s, H-5), 6.09−5.99 (1H, m, H-2'), 5.83 (1H, s, OH-4), 5.18−5.12 (2H, m, H-3'), 3.74 (3H, s, OCH_3), 3.41 (2H, dt, *J* = 5.7 and 1.8 Hz, H-1'), 2.69 (3H, s, ArCOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.5 (C, Ar$\underline{C}$OCH_3), 164.4 (C, C-6), 161.9 (C, C-4), 161.8 (C, C-2), 136.2 (CH, C-2'), 116.3 (CH_2, C-3'), 111.0 (C, C-3), 109.8 (C, C-1), 100.5 (CH, C-5), 63.0 (CH_3, OCH_3), 31.0 (CH_3, ArCO$\underline{C}H_3$), 27.8 (CH_2, C-1').

HRMS-ESI *m/z* $[M + H]^+$ 223.0965 (calculated for $C_{12}H_{15}O_4$ 223.0964

1-(4-{2"-[(*tert*-Butyldiphenylsiloxy)methyl]allyloxy}-3-allyl-6-hydroxy-2-methoxyphenyl)ethanone (117)



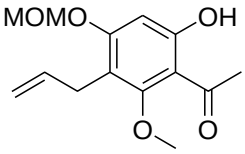
To a solution of ally ether **116** (35 mg, 0.15 mmol) in DMF, K_2CO_3 (20 mg, 0.15 mmol) was added, and the mixture was stirred at rt for 15 min under argon. (2-(Bromomethyl)allyloxy)(tert-butyl)diphenyl silane (**105c**) (60 mg, 0.15 mmol) was added to the mixture which was then stirred for 15 min at rt. The reaction mixture was diluted with EtOAc and filtered. The filtrate was washed with saturated NH_4Cl solution (30 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and was concentrated in vacuo. The mixture was purified by PTLC using hexanes: EtOAc (8:2) to afford the target compound **117** (26 mg, 0.049 mmol, 32%, Rf =0.49) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.36 (1H, S, OH-6), 7.67-7.65 (4H, m, SiAr$\underline{H}$), 7.44-7.35 (6H, m, SiAr$\underline{H}$), 6.23 (1H, s, H-5), 5.87-5.77 (1H, m, H-2'), 5.38 (1H, s, H-3"a), 5.25 (1H, d, *J* = 1.2 Hz, H-3"b), 4.88-4.82 (2H, m, H-3'), 4.53 (2H, s, H-1") 4.26 (2H, s, H-4"), 3.71 (3H, s, OCH_3), 3.25 (2H, dt, *J* = 5.8 and 1.5 Hz, H-1'), 2.68 (3H, s, ArCOC$\underline{H}_3$), 1.07 (9H, s, $(C\underline{H}_3)_3$CSi).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.3 (C, Ar<u>C</u>OCH_3), 164.8 (C, C-6), 163.4 (C, C-4), 161.1 (C, C-2), 142.7 (C, C-2''), 136.7 (CH, C-2'), 135.5 (4 × CH, Si<u>Ar</u>), 133.3 (2 × C, Si<u>Ar</u>), 129.7 (2 × CH, Si<u>Ar</u>), 127.7 (4 × CH, Si<u>Ar</u>), 114.6 (CH_2, C-3'), 113 (C, C-3), 112.8 (CH_2, C-3''), 109.2 (C, C-1), 96.9 (CH, C-5), 69.1 (CH_2, C-1''), 64.5 (CH_2, C-4''), 63.0 (CH_3, OCH_3), 30.9 (CH_3, ArCO<u>C</u>H_3), 27.6 (CH_2, C-1'), 26.8 (3 × CH_3, (<u>C</u>H_3)$_3$CSi), 19.3 (C, (CH_3)$_3$<u>C</u>Si).

HRMS-ESI *m/z* $[M + H]^+$ 531.2784 (calculated for $C_{32}H_{39}O_5$ Si 531.2561)

1-[3-Allyl-6-hydroxy-2-methoxy-4-(methoxymethoxy)phenyl]ethanone (120)



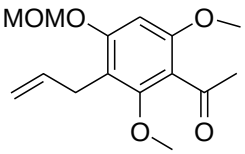
A solution of compound **115** (1.47 g, 4.73 mmol) in CH_3OH (30 mL), and 3 M HCl (1.5 mL) was heated at 60 °C for 8 h. The reaction was quenched with saturated NH_4Cl solution (30 mL) and the product was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography using hexanes: EtOAc (8.5:1.5) as the eluent to give target compound **120** (1.00 g, 3.75 mmol, 79%, Rf = 0.58) as light-yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.19 (1H, s, OH-6), 6.47 (1H, s, H-5), 6.01−5.94 (1H, m, H-2'), 5.20 (2H, s, OCH_2O), 5.01−4.96 (2H, m, H-3'), 3.74 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.36 (2H, d, *J* = 5.7 Hz, H-1'), 2.69 (3H, s, ArCO<u>C</u>H_3).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.5 (C, Ar<u>C</u>OCH_3), 164.4 (C, C-6), 161.8 (C, C-4), 161.1 (C, C-2), 136.9 (CH, C-2'), 114.6 (CH_2, C-3'), 114.0 (C, C-3), 109.9 (C, C-1), 98.7 (CH, C-5), 93.9 (CH_2, OCH_2O), 63.1 (CH_3, OCH_3), 56.6 (CH_3, OCH_3), 30.9 (CH_3, ArCO<u>C</u>H_3), 27.7 (CH_2, C-1').

HRMS-ESI *m/z* $[M + H]^+$ 267.1259 (calculated for $C_{14}H_{19}O_5$ 267.1224)

1-[3-Allyl-2,6-dimethoxy-4-(methoxymethoxy)phenyl]ethanone (121)



The mixture of **120** (1.00 g, 3.77 mmol), K_2CO_3 (2.61 g, 18.8 mmol) and iodomethane (0.31 mL, 4.9 mmol) in DMF (5 mL) was heated to reflux for 1 h. The reaction mixture was cooled to rt and diluted with EtOAc (20 mL) and saturated NH_4Cl solution (30 mL). The organic and the aqueous phases were partitioned, and the aqueous phase was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was filtered through a short pad of silica gel using hexanes: EtOAc (8.5:1.5) as the eluent to give target compound **121** (0.94 g, 3.4 mmol, 89%, Rf = 0.55) as yellow oil.

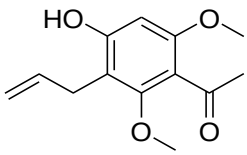
IR: ν_{max} 2967, 1740, 1691, 1597, 1446, 1373, 1206, 1148, 1104, 1053, 1012 cm^{-1}

^1H NMR (400 MHz, $CDCl_3$) δ: 6.53 (1H, s, H-5), 6.01−5.91 (1H, m, H-2'), 5.19 (2H, s, OCH_2O), 4.99−4.94 (2H, m, H-3'), 3.79 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.34 (2H, dt, J = 5.9, and 1.6 Hz, H-1'), 2.49 (3H, s, $ArCOCH_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.4 (C, $ArCOCH_3$), 157.6 (C, C-4), 156.5 (C, C-2), 156.0 (C, C-6), 136.9 (CH, C-2'), 119.5 (C, C-1), 114.9 (CH_2, C-3'), 114.5 (C, C-3), 94.3 (CH, C-5), 94.3 (CH_2, OCH_2O), 63.4 (CH_3, OCH_3), 56.1 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.5 (CH_3, $ArCOCH_3$), 27.6 (CH_2, C-1')

HRMS-ESI m/z $[M + H]^+$ 281.1367 (calculated for $C_{15}H_{21}O_5$ 281.1385)

1-(3-Allyl-4-hydroxy-2,6-dimethoxyphenyl)ethanone (122)



A solution of **121** (0.92 g, 3.3 mmol) in CH_3OH (20 mL), and 3 M HCl (4 mL) was heated to reflux for 5 h. The reaction was quenched with saturated NH_4Cl solution (30 mL) and the mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The product was purified on silica gel column chromatography using hexanes: EtOAc (8.5: 1.5) as the eluent to give target compound **122** (0.63 g, 2.7 mmol, 81%, Rf = 0.19) as a light-yellow oil.

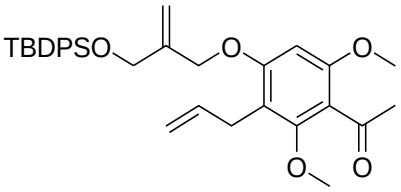
IR: ν_{max} 3316, 2940, 1740, 1668, 1593, 1493, 1408, 1323, 1257, 1200, 1146, 1001, 912, 819, 562, 497cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.26 (1H, s, H-5), 6.05−5.95 (1H, m, H-2'), 5.16-5.11 (2H, m, H-3'), 3.73 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.38 (2H, dt, *J* = 5.8 and 1.7 Hz, H-1'), 2.50 (3H, s, ArCOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.7 (C, ArC$\underline{O}CH_3$), 157.5 (C, C-4), 156.8 (C, C-2), 156.4 (C, C-6), 136.2 (CH, C-2'), 118.3 (C, C-1), 116.2 (CH_2, C-3'), 110.9 (C, C-3), 95.9 (CH, C-5), 63.4 (CH_3, OCH_3), 55.7 (CH_3, OCH_3), 32.5 (CH_3, ArCO$\underline{C}H_3$), 27.6 (CH_2, C-1').

HRMS-ESI *m/z* $[M + H]^+$ 237.1097 (calculated for $C_{13}H_{17}O_4$ 237.1121)

1-(4-{2"-[(*tert*-Butyldiphenylsiloxy)methyl]allyloxy}-3-allyl-2,6-dimethoxyphenyl)ethanone (123)



To a solution of **122** (0.60 g, 2.6 mmol) in DMF (5 mL), K_2CO_3 (0.35 g, 2.6 mmol) was added, and the mixture was stirred for 15 min at rt under argon. (2-(bromomethyl)allyloxy)(*tert*-butyl)diphenyl silane (**105c**) (0.79 g, 2.1 mmol) was added to the mixture which was then stirred for 1 h at rt. The reaction mixture was diluted with EtOAc (20 mL) and filtered. The filtrate was washed with saturated NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and was concentrated in vacuo. The mixture was purified by PTLC using hexanes: EtOAc (9:1-8:2) to afford the target compound **123** (0.82 g, 1.5 mmol, 58.9%, Rf = 0.5) as a light-yellow oil.

IR: ν_{max} 3016, 2970, 2944, 1738, 1436, 1366, 1228, 1216, 1206, 1111, 538cm^{-1}

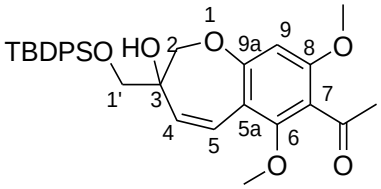
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68-7.66 (4H, m, SiArH), 7.45-7.35 (6H, m, SiArH), 6.24 (1H, s, H-5), 5.87-5.77 (1H, m, H-2'), 5.39 (1H, d, *J* = 0.8 Hz, H-3"a), 5.26 (1H, d, *J* = 1.1 Hz, H-3"b), 4.87-4.82 (2H, m, H-3'), 4.56 (2H, s, H-1") 4.29 (2H, s, H-4"), 3.75 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.25 (2H, d, *J* = 6.0 Hz, H-1'), 2.49 (3H, s, ArCOC$\underline{H}_3$), 1.00 (9H, s, $(C\underline{H}_3)_3CSi$)

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.3 (C, ArC$\underline{O}CH_3$), 158.8 (C, C-4), 156.7 (C, C-2), 156.0 (C, C-6), 142.4 (C, C-2"), 136.8 (CH, C-2'), 135.4 (4 × CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 118.5 (C, C-1), 114.4 (CH_2, C-3'), 114.4 (C, C-3), 112.3 (CH_2, C-3"), 92.3

(CH, C-5), 69.1 (CH_2, C-1''), 64.5 (CH_2, C-4''), 63.3 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.5 (CH_3, $ArCOCH_3$), 27.5 (CH_2, C-1'), 26.8 (3 × CH_3, $(CH_3)_3CSi$), 19.3 (C, $(CH_3)_3CSi$).

HRMS-ESI *m/z* $[M + H]^+$ 545.2715 (calculated for $C_{33}H_{41}O_5Si$ 545.2717)

1-(2,3-Dihydro-3-hydroxy-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl) ethanone (125)



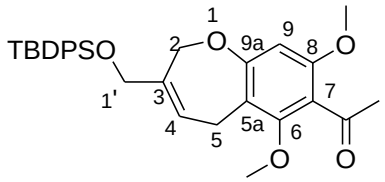
To a solution of diene **123** (0.40 g, 0.73 mmol) in CH_2Cl_2 (30 mL), [1,3-*bis*(2,4,6-trimethylphenyl)-2-imidazolidinylidene] (tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (0.064 g, 0.076 mmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h. The solvent was evaporated, and the resulting oil was purified by silica gel column chromatography using hexanes: EtOAc (8.6:1.4) as eluent to give the compound **125** (0.11 g, 0.21 mmol, 28%, Rf = 0.33) as a solid.

IR: ν_{max} 3449, 2932, 2857, 1738, 1687, 1597, 1463, 1387, 1328, 1245, 1196, 1141, 1100, 928, 819, 743, 701, 611, 567, 501, 430 cm^{-1}

^1H NMR (400 MHz, $CDCl_3$) δ: 7.70-7.66 (4H, m, SiArH), 7.45-7.38 (6H, m, SiArH), 6.62 (1H, d, *J* = 12.1 Hz, H-5), 6.38 (1H, s, H-9), 5.74 (1H, dd, *J* = 12.1, and 1.4 Hz, H-4), 4.41 (1H, dd, *J* = 11.6 and 1.5 Hz, H-2a), 4.00 (1H, d, *J* = 11.6 Hz, H-2b), 3.84 (1H, d, *J* = 10.2 Hz, H-1'a), 3.60 (1H, d, *J* = 10.2 Hz, H-1'b), 3.81 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 2.47 (3H, s, $ArCOCH_3$), 1.09 (9H, s, $(CH_3)_3CSi$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 201.9 (C, $ArCOCH_3$), 161.4 (C, C-9a), 157.2 (C, C-6), 156.7 (C, C-8), 135.5 (4 × CH, SiAr), 132.7 (2 × C, SiAr), 130.0 (CH, C-4), 129.9 (2 × CH, SiAr), 127.9 (4 × CH, SiAr), 121.0 (C, C-7), 120.9 (CH, C-5), 112.3 (C, C-5a), 99.0 (CH, C-9), 74.1 (C, C-3), 73.4 (CH_2, C-2), 66.5 (CH_2, C-1'), 63.9 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.4 (CH_3, $ArCOCH_3$), 26.9 (3 × CH_3, $(CH_3)_3CSi$), 19.4 (C, $(CH_3)_3CSi$).

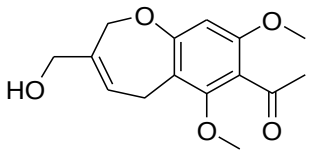
1-(2,5-Dihydro-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone (124)



To a solution of diene **123** (0.40 g, 0.73 mmol) in dry CH_2Cl_2 (30 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene](tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (0.064 mg, 0.0070 mmol) was added in one portion under argon. The reaction mixture was heated at 45 °C for 2 h. The solvent was evaporated, and the resulting oil was purified on silica gel column chromatography using hexanes: EtOAc (8.6:1.4) as eluent to give the target compound **124** (0.17 g, 0.34 mmol, 46.6%, Rf = 0.33) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$ δ: 7.65-7.63 (4H, m, SiArH), 7.45-7.35 (6H, m, SiArH), 6.46 (1H, s, H-9), 5.81-5.77 (1H, m, H-4), 4.63 (2H, d, *J* = 1.6 Hz, H-2), 4.01 (2H, s, H-1'), 3.78 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 3.40 (2H, d, *J* = 5.5 Hz, H-5), 2.50 (3H, s, $ArCOCH_3$), 1.04 (9H, s, $(CH_3)_3CSi$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.5 (C, $ArCOCH_3$), 161.1 (C, C-9a), 155.5 (C, C-8), 154.6 (C, C-6), 137.5 (C, C-3), 135.5 (4 × CH, SiAr), 133.3 (2 × C, SiAr), 129.7 (2 × CH, SiAr), 127.7 (4 × CH, SiAr), 121.9 (CH, C-4), 121.9 (C, C-7), 120.9 (C, C-5a), 101.1 (CH, C-9), 71.6 (CH_2, C-2), 66.2 (CH_2, C-1'), 63.3 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.5 (CH_3, $ArCOCH_3$), 26.8 (3 × CH_3, $(CH_3)_3CSi$, 21.9 (CH, C-5), 19.2 (C, $(CH_3)_3CSi$).

HRMS-ESI *m/z* $[M + H]^+$ 517.2403 (calculated for $C_{31}H_{37}O_5Si$ 517.2404)

1-(2,5-Dihydro-3-(hydroxymethyl)-6,8-dimethoxybenzo[b]oxepin-7-yl)ethanone (126)



To a solution of diene **123** (1.00 g, 1.84 mmol) in CH_2Cl_2 (30 mL), [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene] (tricyclohexylphosphine) benzylideneruthenium (IV) dichloride (Grubbs' II catalyst) (0.162 g, 0.191 mmol) was added in one portion under argon.

The reaction mixture was heated at 45 °C for 2 h. The solvent was evaporated, and the residue was dissolved in THF. TBAF (0.520 mL, 2.02 mmol) was added at 0 °C under argon atmosphere and the mixture was stirred for 2 h at 0 °C. The mixture was diluted with EtOAc (30 mL) and saturated NH_4Cl solution. The mixture was extracted with EtOAc (3 × 30 mL) and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting dark brown oil was purified through a short pad of silica gel using hexanes: EtOAc (9:1- 5:5) as eluent gradient to give the target compound **126** (0.16 g, 32%, 0.59 mmol, Rf = 0.45) as a light-yellow oil.

IR: ν_{max} 3381, 2941, 2600, 2492, 1690, 1596, 1471, 1394, 1322, 1253, 1197, 1145, 1098, 1031, 843, 806, 747, 567, 466cm^{-1}

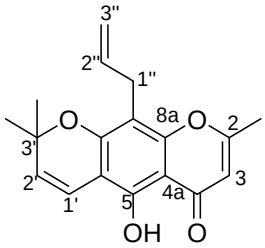
^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.47 (1H, s, H-9), 5.93-5.91 (1H, t, *J* = 5.3 Hz, H-4), 4.67 (2H, s, H-2), 3.98 (2H, s, H-1'), 3.78 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.44 (2H, d, *J* = 5.5 Hz, H-5), 2.48 (3H, s, ArCOC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 202.4 (C, Ar$\underline{C}$OCH$_3$), 160.9 (C, C-9a), 155.6 (C, C-8), 154.6 (C, C-6), 138.2 (C, C-3), 123.9 (CH, C-4), 121.9 (C, C-7), 120.6 (C, C-5a), 101.1 (CH, C-9), 71.6 (CH_2, C-2), 65.7 (CH_2, C-1'), 63.3 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.4 (CH_3, ArCO$\underline{C}$H$_3$), 22.0 (CH_2, C-5).

HRMS-ESI *m/z* $[M + H]^+$ 279.1214 (calculated for $C_{15}H_{19}O_5$ 279.1234)

5.4. Experimental data for the synthesised derivatives

8-Allyl-5-hydroxy-3',3'-dimethylpyrano[7,6]chromone (129)



To a solution of **94** (0.20 g, 0.86 mmol) in CH_3OH (10 mL), $Ca(OH)_2$ (0.120 g, 1.72 mmol) was added followed by prenal (0.40 mL, 4.3 mmol). The mixture was stirred under argon atmosphere for 3 days at rt, then diluted with EtOAc (15 mL) and 1 M HCl (15 mL). The two phases were partitioned, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The

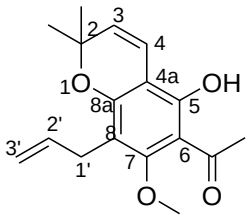
combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude product was purified by silica gel column chromatography using Hexanes: EtOAc (7:3) to give the product as white solid (26.0 mg, 10% 0.872 mmol, Rf = 0.7).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.97 (1H, s, OH), 6.72-6.69 (1H, d, *J* = 10.0 Hz, H-1'), 6.00 (1H, s, H-3), 5.93-5.83 (1H, m, H-2"), 5.61-5.58 (1H, d, *J* =10.0 Hz, H-2'), 5.04-4.94 (2H, m, H-3"), 3.41 (2H, dt, *J* = 6.3 and 1.4 Hz, H-1"), 2.34 (3H, s, CH_3-2), 1.44 (6H, s, CH_3-3').

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.9 (C, C-4), 166.4 (C, C-2), 156.8 (C, C-7), 154.9 (C, C-8a), 154.7 (C, C-5), 135.9 (CH, C-2"), 127.8 (CH, C-2'), 115.8 (CH, C-1'), 114.7 (CH_2, C-3"), 108.5 (CH, C-3), 105.3 (C, C-8), 105.2 (C, C-6), 104.8 (C, C-4a), 77.8 (C, C-3'), 28.2 (2 × CH_3, CH_3-3'), 26.5 (CH_2, C-1"), 20.4 (CH_3, CH_3-2).

HRMS-ESI *m/z* $[M + H]^+$ 299.1285 (calculated for $C_{18}H_{19}O_4$ 299.1285)

1-(8-Allyl-5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-6-yl)ethanone (130)



To a solution of **116** (30.0 mg, 0.135 mmol) in CH_3OH (5 mL), $Ca(OH)_2$ (20.0 mg, 0.270 mmol) was added followed by prenal (64.0 µL, 0.675 mmol). The mixture was stirred under argon atmosphere for 3 days at rt, the reaction mixture diluted with EtOAc (15 mL) and 1 M HCl (15 mL). The two phases were partitioned, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The crude product purified by PTLC using Hexanes: EtOAc (7:3) to give a light-yellow oil (17 mg, 44%, 0.059 mmol, Rf = 0.76).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 13.55 (1H, s, OH-5), 6.68 (1H, d, *J* = 10.0 Hz, H-4), 5.99-5.92 (1H, m, H-2'), 5.51 (1H, d, *J* = 10.0 Hz, H-3), 5.04-4.98 (2H, m, H-3'), 3.74 (3H, s, OCH_3), 3.31 (2H, dt, *J* = 6.0 and 1.6 Hz, H-1'), 2.67 (3H, s, $CO\underline{C}H_3$), 1.43 (6H, s, CH_3-2).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.5 (C, $\underline{C}OCH_3$), 161.2 (C, C-7), 159.2 (C, C-5), 158.5 (C, C-8a), 136.9 (CH, C-2'), 126.7 (CH, C-3), 116.0 (CH, C-4), 114.8 (CH_2, C-3'), 112.9 (C, C-8), 109.0 (C, C-

6), 105.8 (C, C-4a), 78.0 (C, C-2), 63.1 (CH_3, OCH_3), 31.0 (CH_3, $CO\underline{C}H_3$), 28.4 (2 × CH_3, CH_3-2), 27.5 (CH_2, C-1').

HRMS-ESI *m/z* $[M + H]^+$ 289.1489 (calculated for $C_{17}H_{21}O_4$ 289.1442)

Appendix I: copies of NMR spectra

Plate 1a. ^{1}H NMR spectrum of 1-[2,4,6-tris(hydroxy)phenyl]ethanone (**97**) in DMSO-d_6

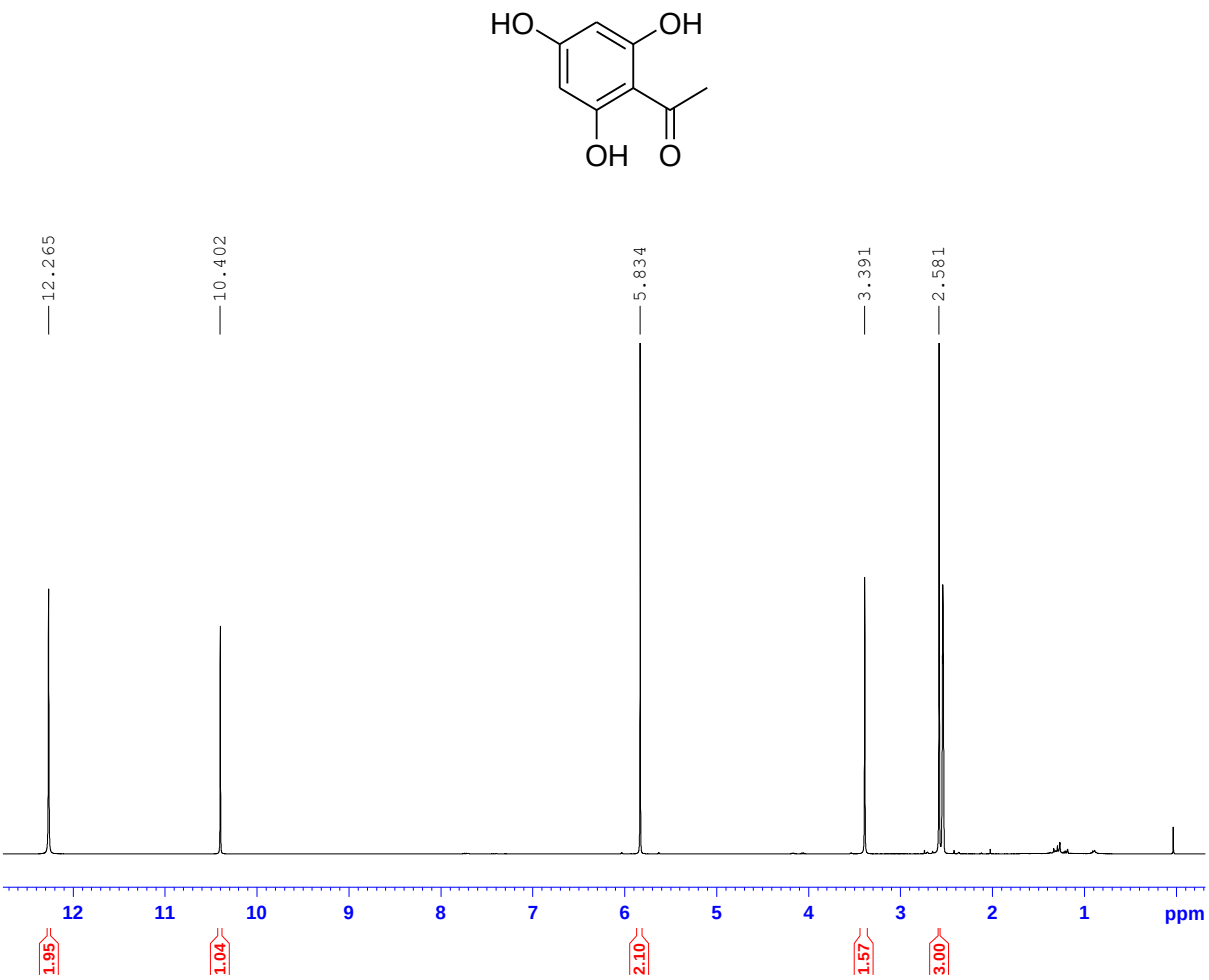


Plate 1b. ^{13}C NMR spectrum of 1-[2,4,6-tris(hydroxy)phenyl]ethanone (**97**) in DMSO-d_6

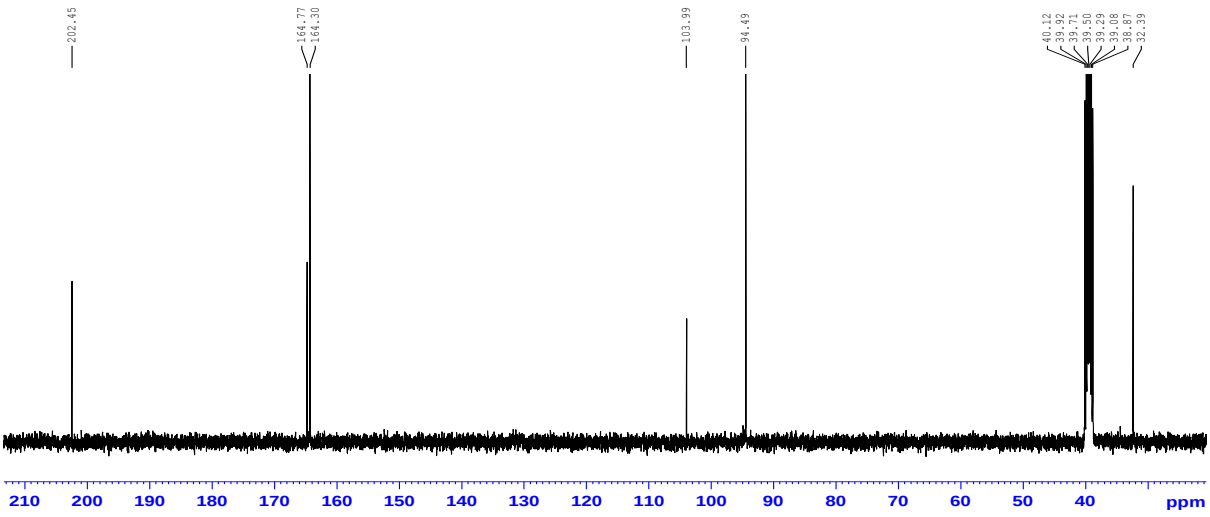


Plate 2a. ^{1}H NMR spectrum of 1-[4,6-bis(*tert*-butyldimethylsiloxy)-2-hydroxy-phenyl]ethanone (**98**) in $CDCl_3$

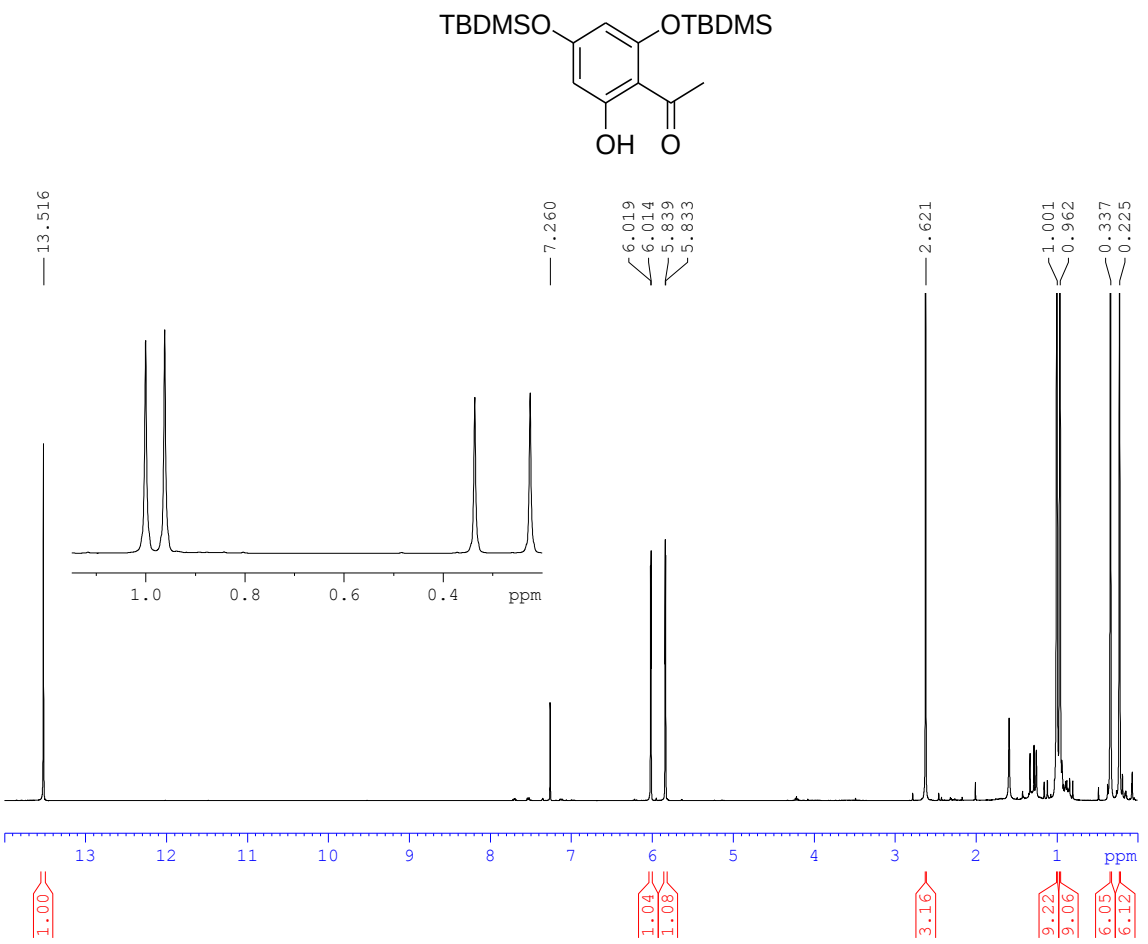


Plate 2b. ^{13}C NMR spectrum of 1-[4,6-bis(*tert*-butyldimethylsiloxy)-2-hydroxy-phenyl]ethanone (**98**) in $CDCl_3$

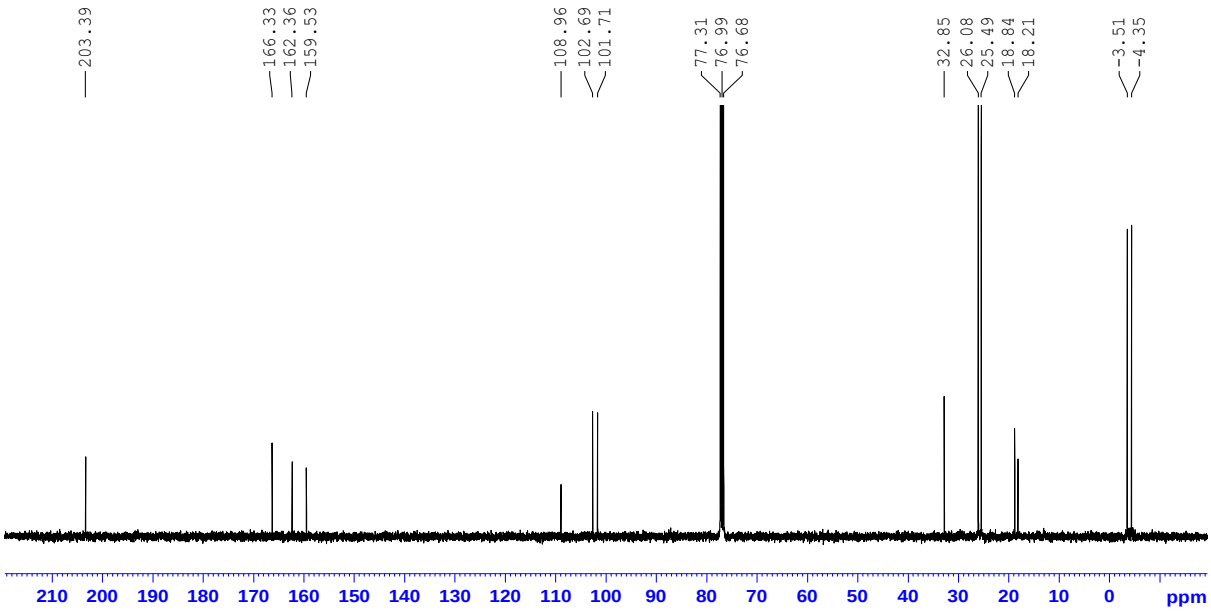
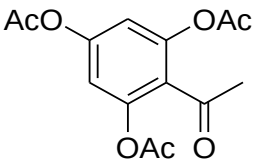


Plate 3a. ^{1}H NMR spectrum of 1-[2,4,6-tris(acetyloxy)phenyl]ethanone (**100**) in $CDCl_3$



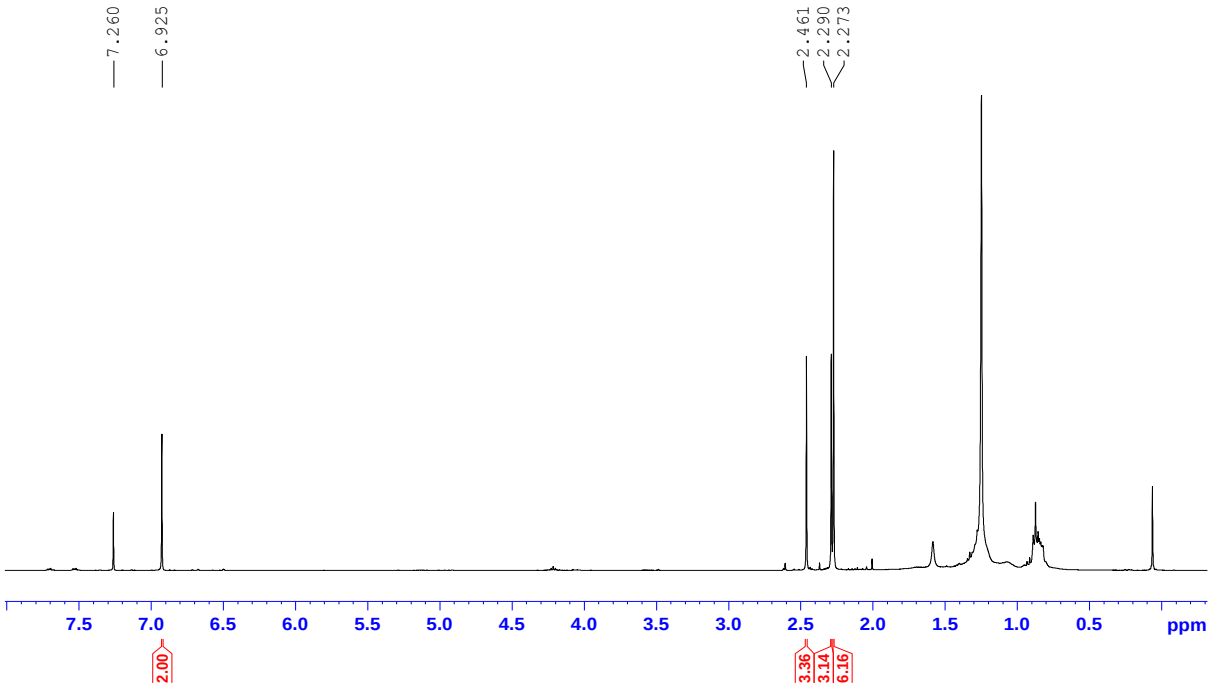


Plate 3b. ^{13}C NMR spectrum of 1-[2,4,6-tris(acetyloxy)phenyl]ethanone (**100**) in $CDCl_3$

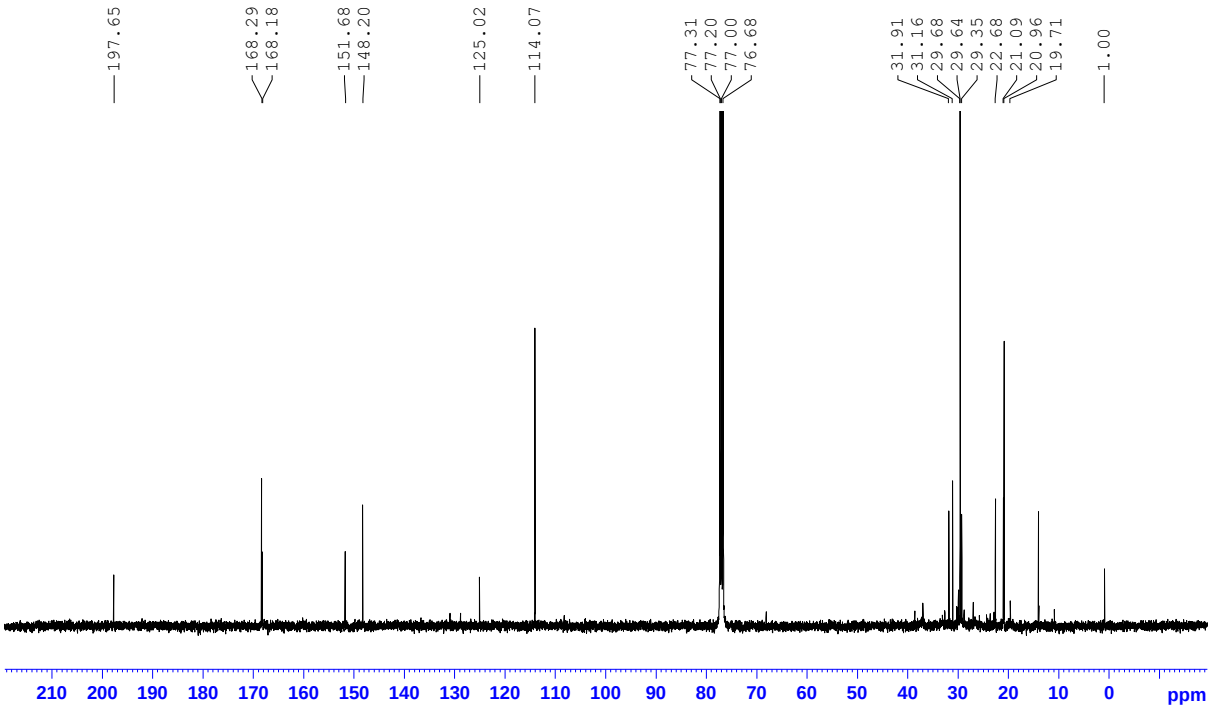


Plate 4a. ^{1}H NMR spectrum of 5,7-Dihydroxy-2-methyl-4*H*-chromen-4-one, noreugenin (**93**) in DMSO-d_6

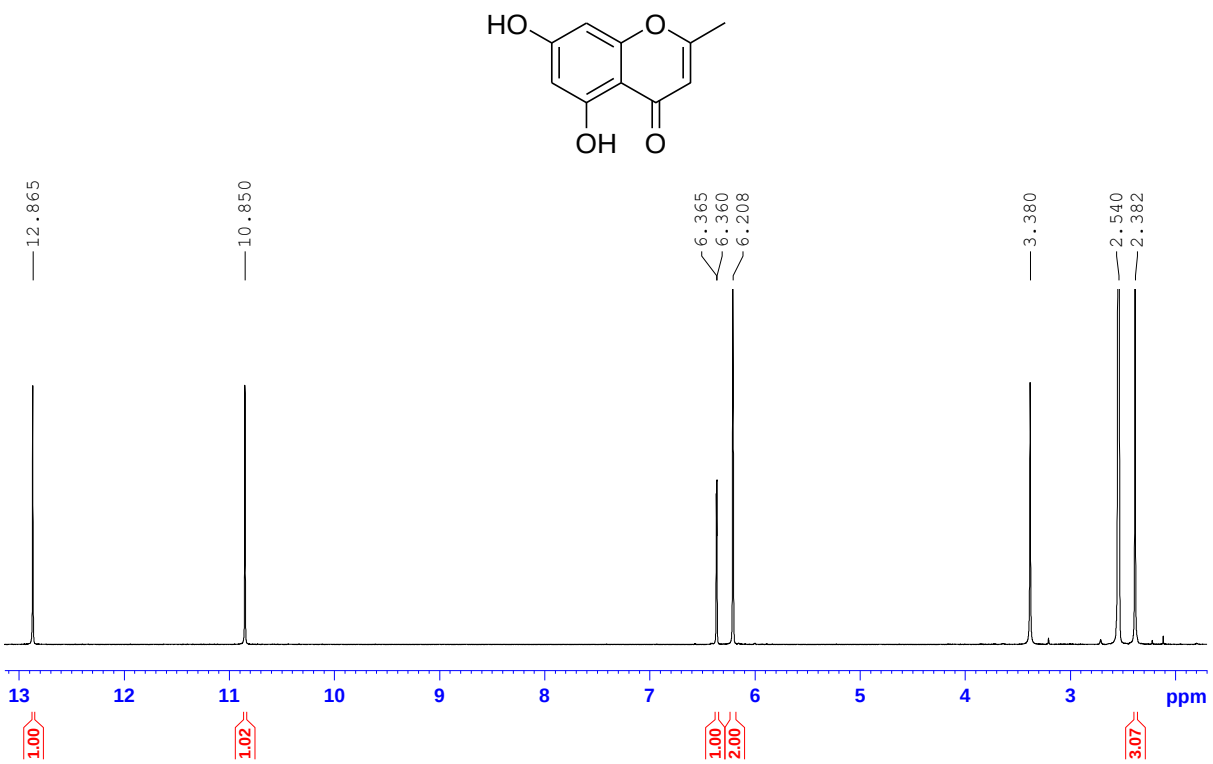


Plate 4b. ^{13}C NMR spectrum of 5,7-Dihydroxy-2-methyl-4*H*-chromen-4-one, noreugenin (**93**) in DMSO-d_6

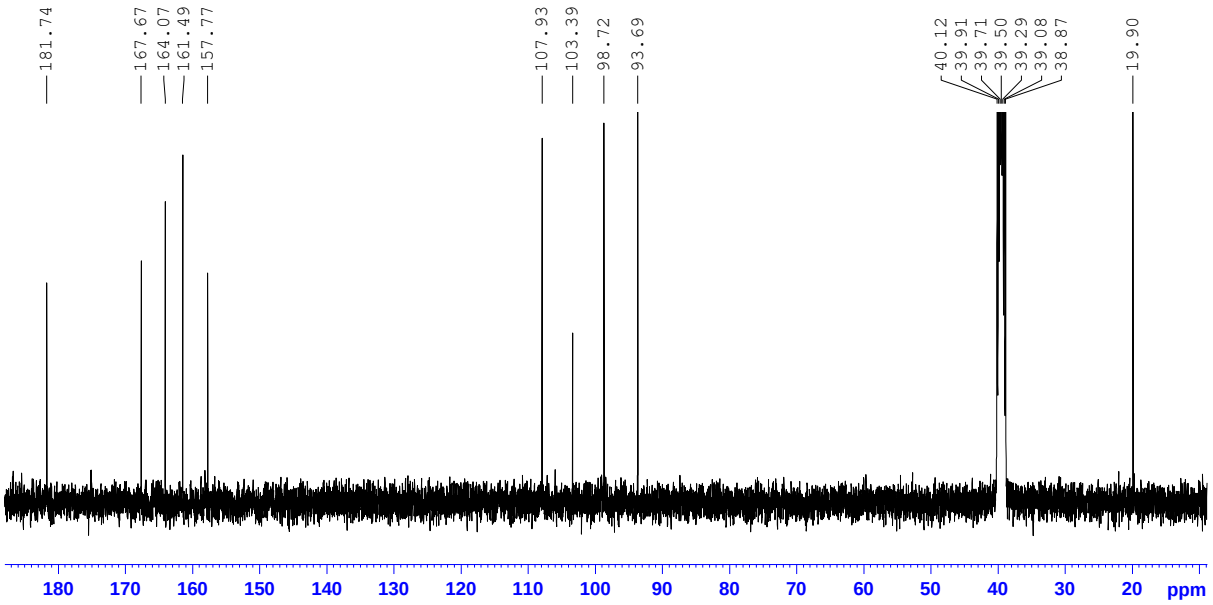


Plate 5a. ^{1}H NMR spectrum of 7-allyloxy-5-hydroxy-2-methyl-4*H*-chromen-4-one (**101**) in $CDCl_3$

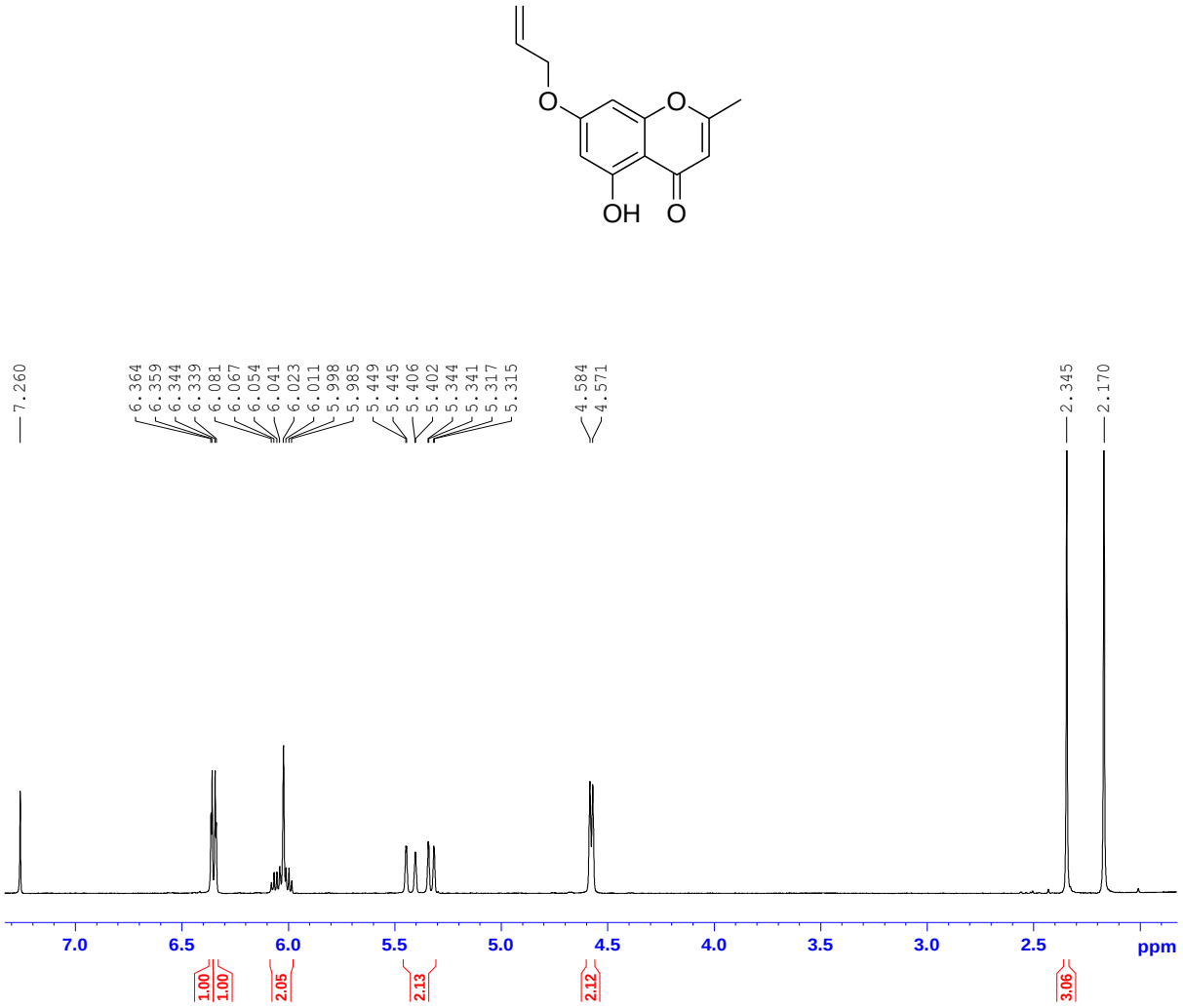


Plate 5b. ^{13}C NMR spectrum of 7-allyloxy-5-hydroxy-2-methyl-4*H*-chromen-4-one (**101**) in $CDCl_3$

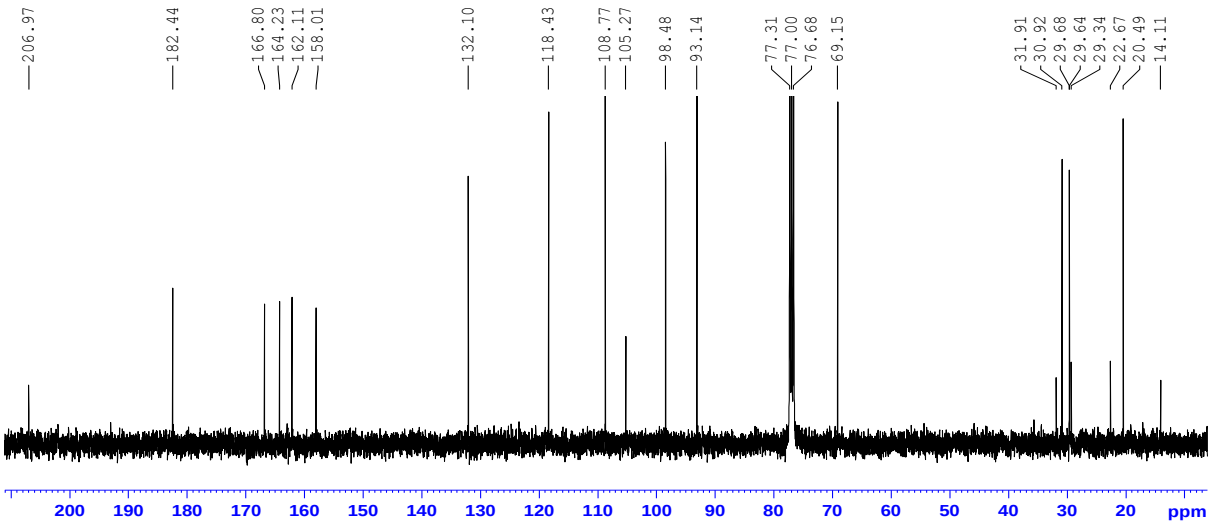


Plate 6a. ^{1}H NMR spectrum of 8-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**94**) in DMSO-d_6

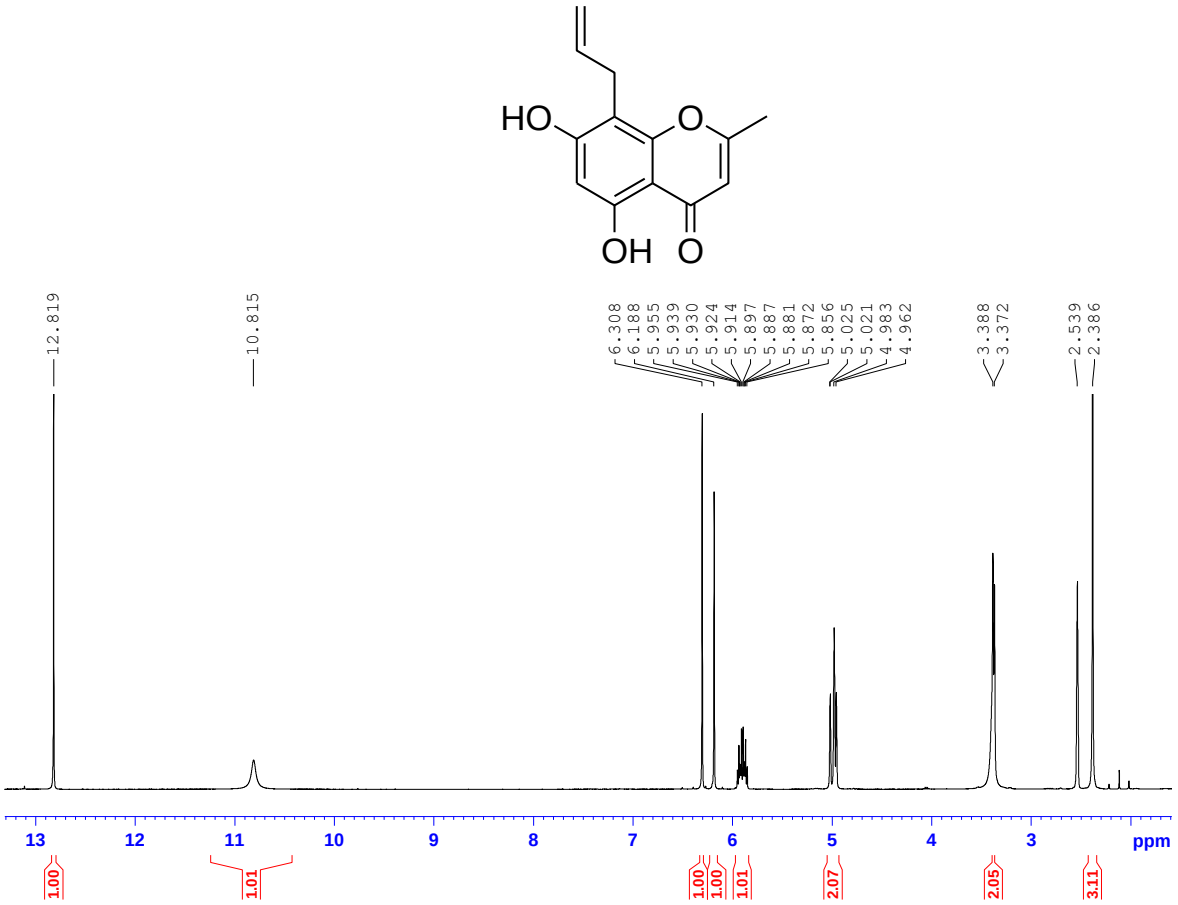


Plate 6b. ^{13}C NMR spectrum of 8-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**94**) in DMSO-d_6

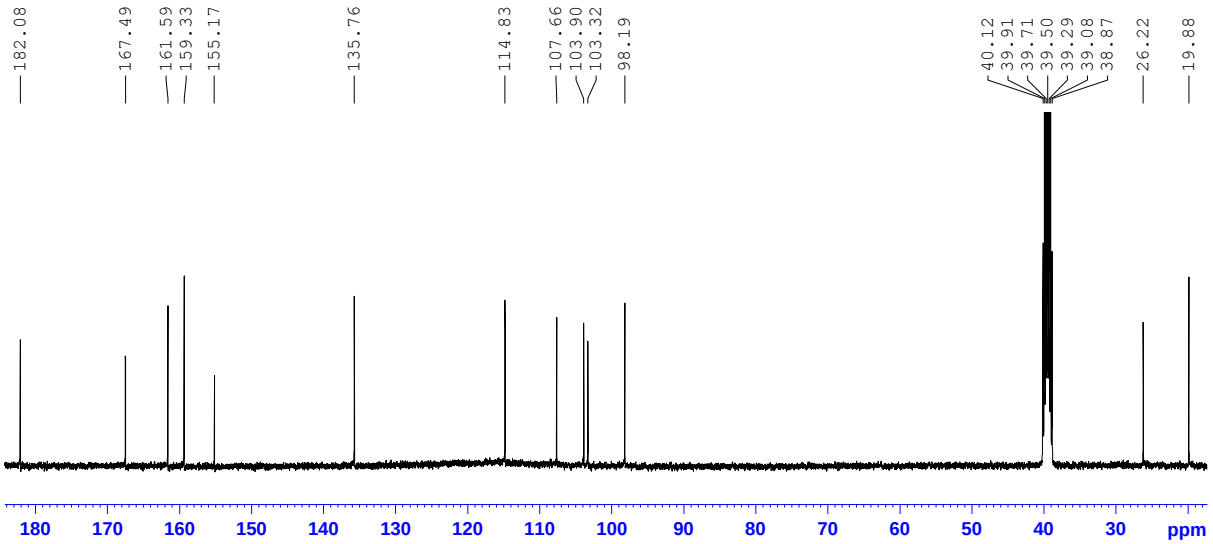


Plate 7a. ^{1}H NMR spectrum of 6-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**95**) in DMSO-d_6

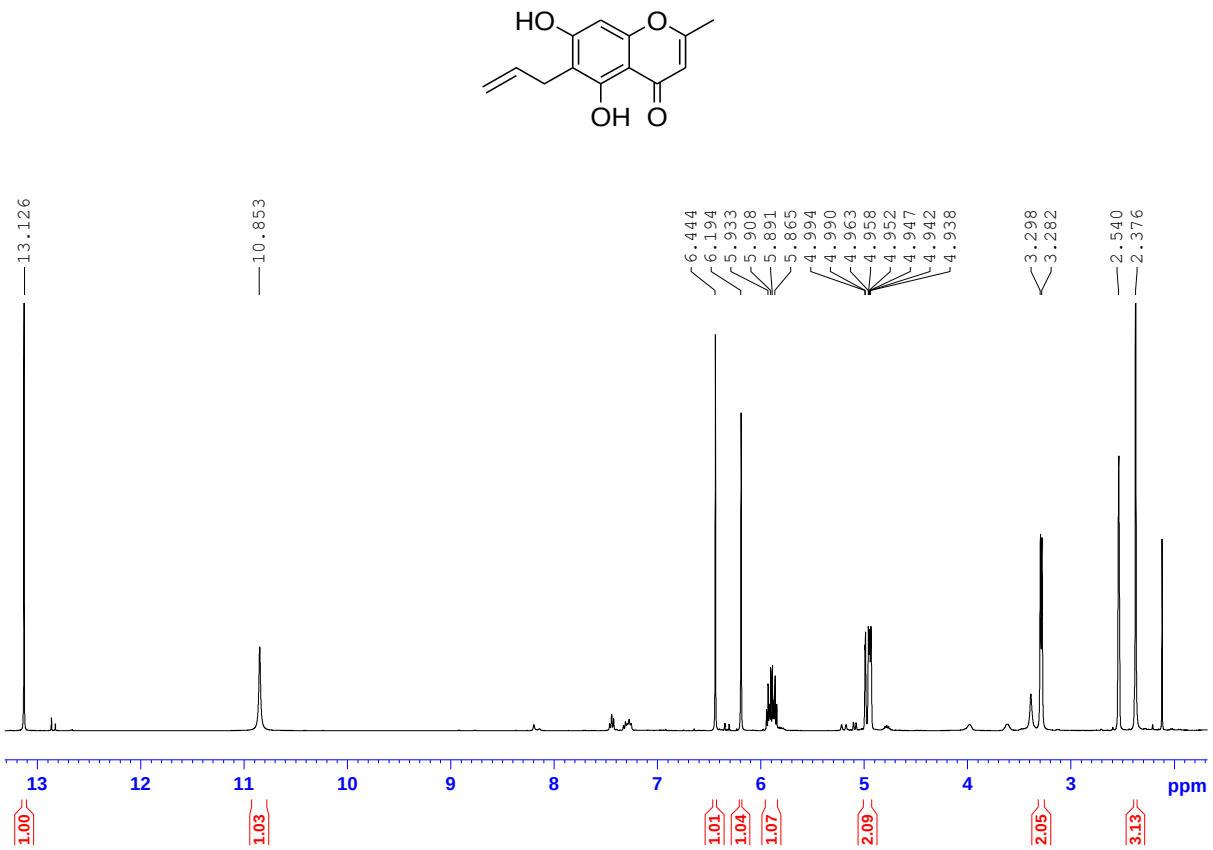


Plate 7b. ^{13}C NMR spectrum of 6-allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (**95**) in DMSO-d_6

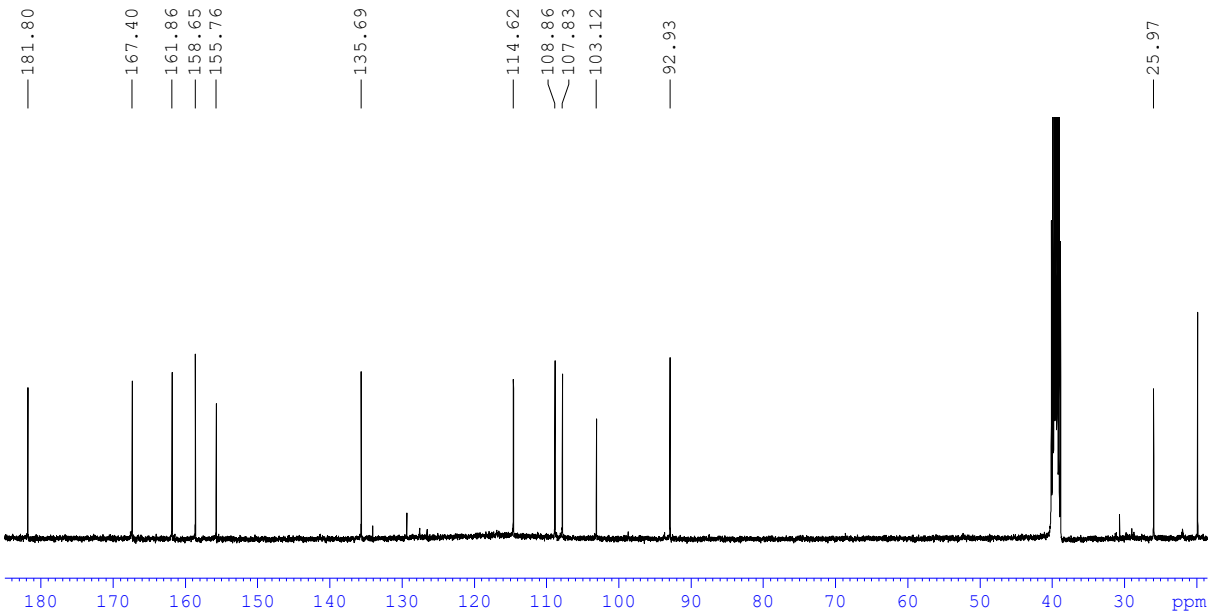


Plate 8a. ^{1}H NMR spectrum of 2-(chloromethyl)allyl-4-methylbenzenesulfonate (**103**) in $CDCl_3$

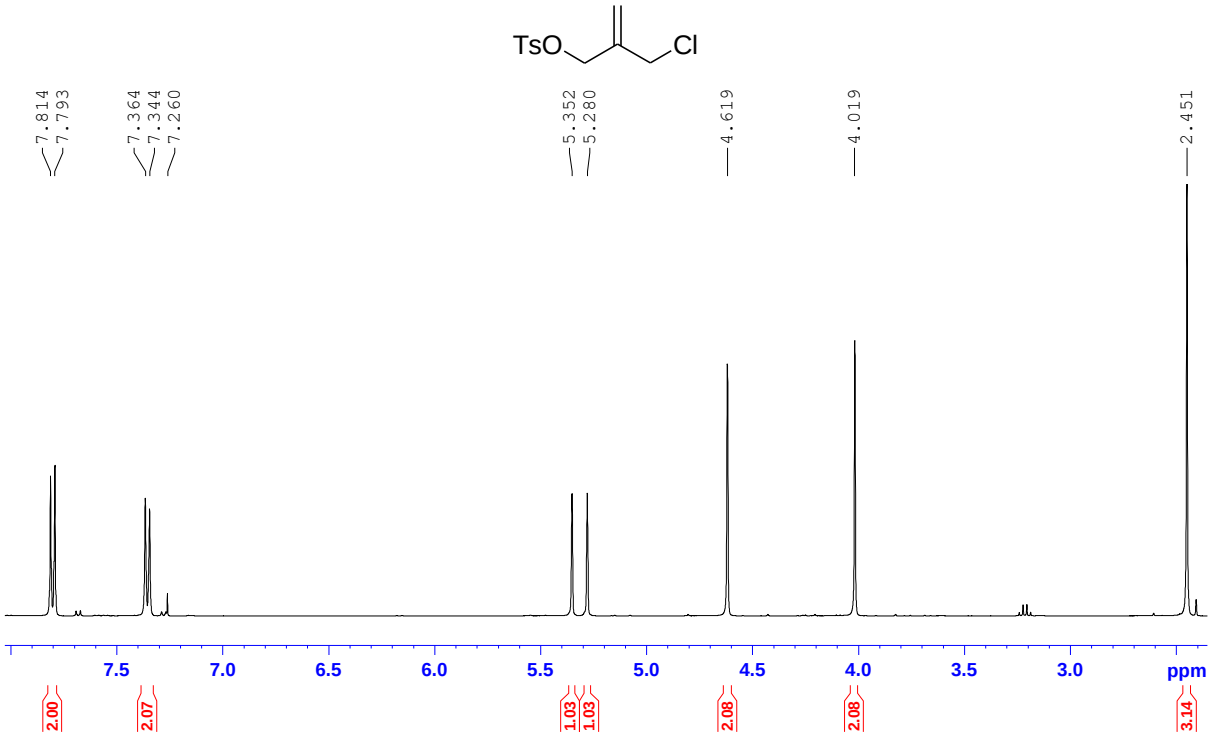


Plate 8b. ^{13}C NMR spectrum of 2-(chloromethyl)allyl-4-methylbenzenesulfonate (**103**) in $CDCl_3$

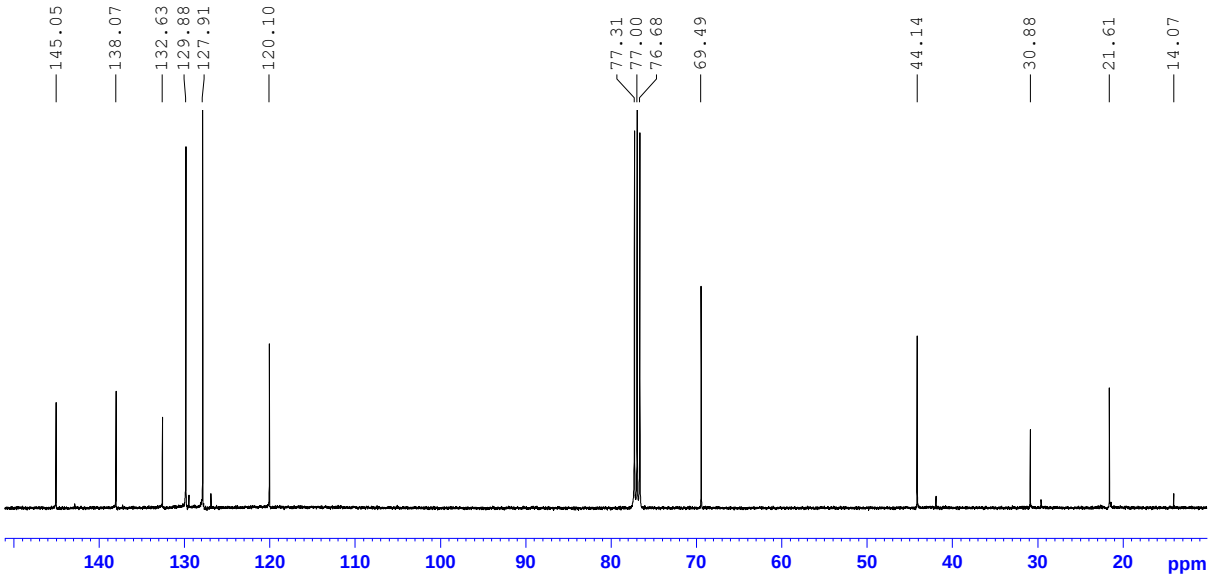


Plate 9a. ^{1}H NMR spectrum of 2-(*tert*-butyldiphenylsiloxy)methylprop-2-en-1-ol (**104**) in $CDCl_3$

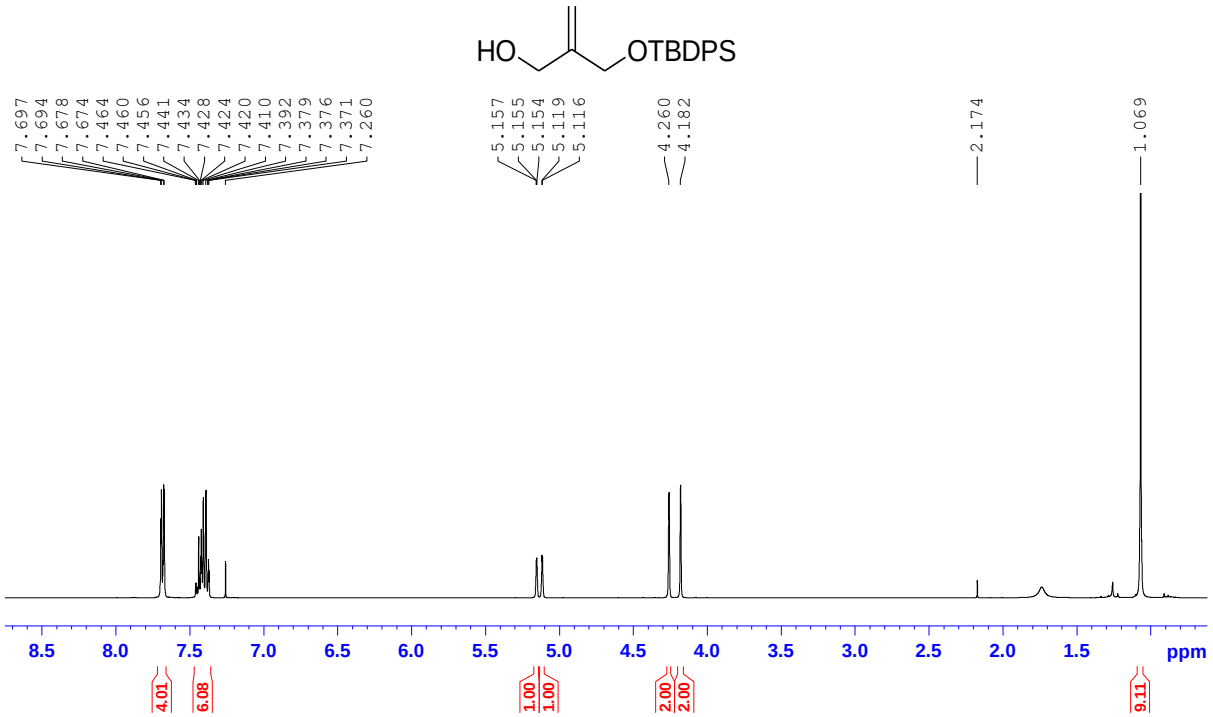


Plate 9b. ^{13}C NMR spectrum of 2-(*tert*-butyldiphenylsiloxy)methylprop-2-en-1-ol (**104**) in $CDCl_3$

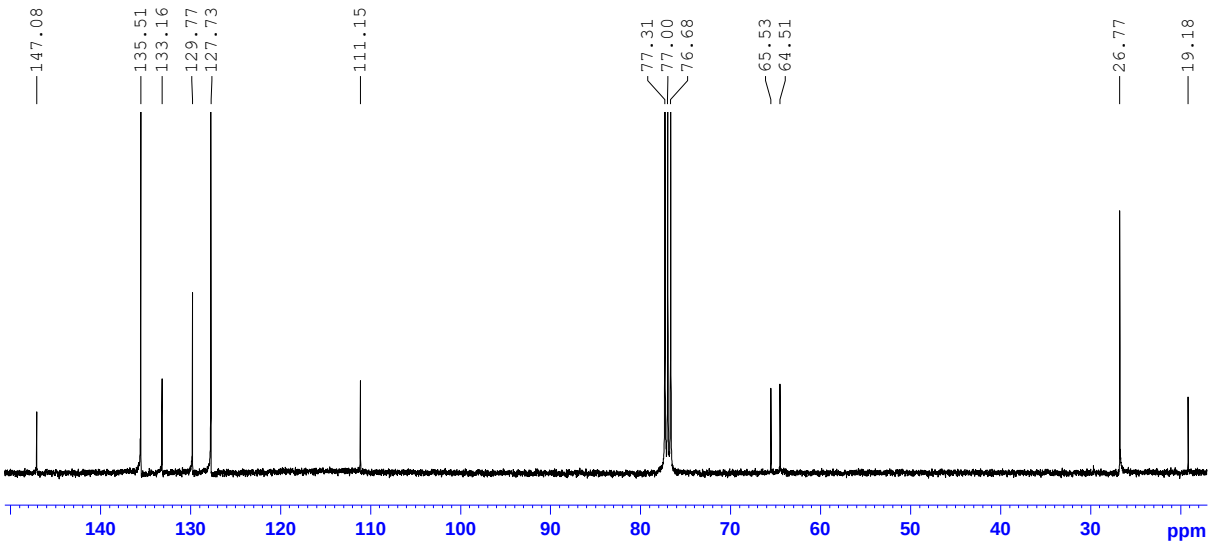


Plate 10a. ^{1}H NMR spectrum of 3-chloro-2-(*tert*-butyldiphenylsiloxy)methylpropene (**105a**) in $CDCl_3$

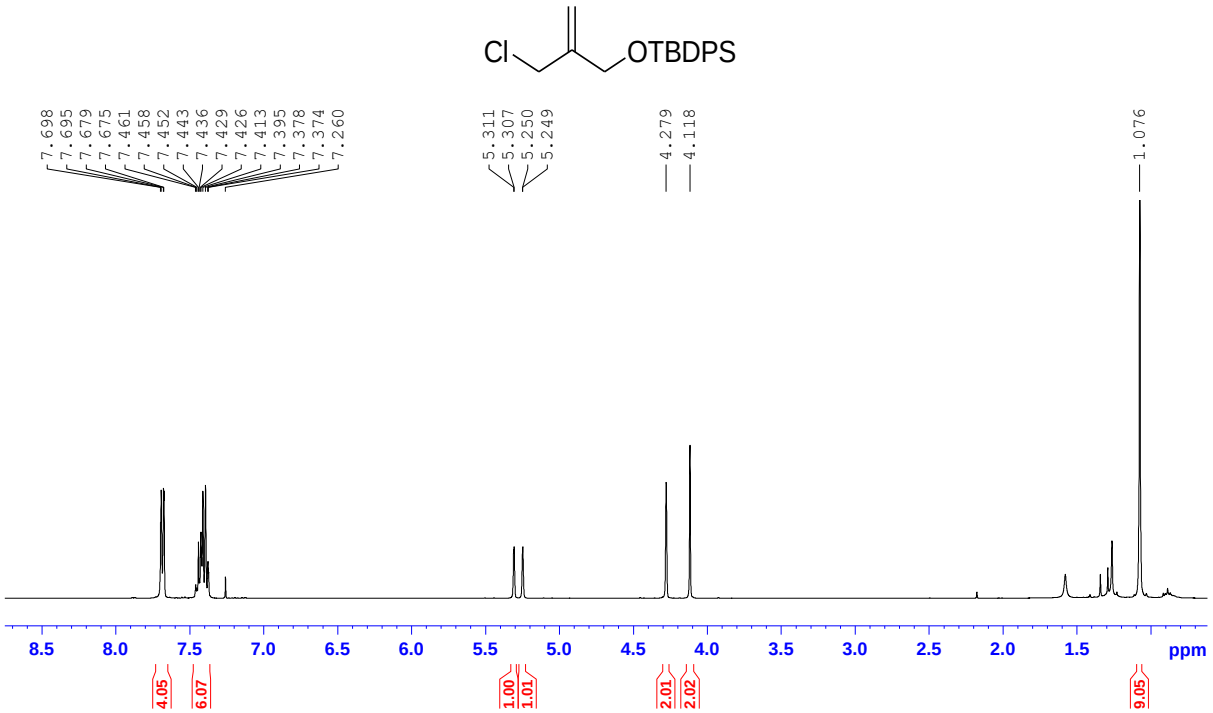


Plate 10b. ^{13}C NMR spectrum of 3-chloro-2-(*tert*-butyldiphenylsiloxy)methylpropene (**105a**) in $CDCl_3$

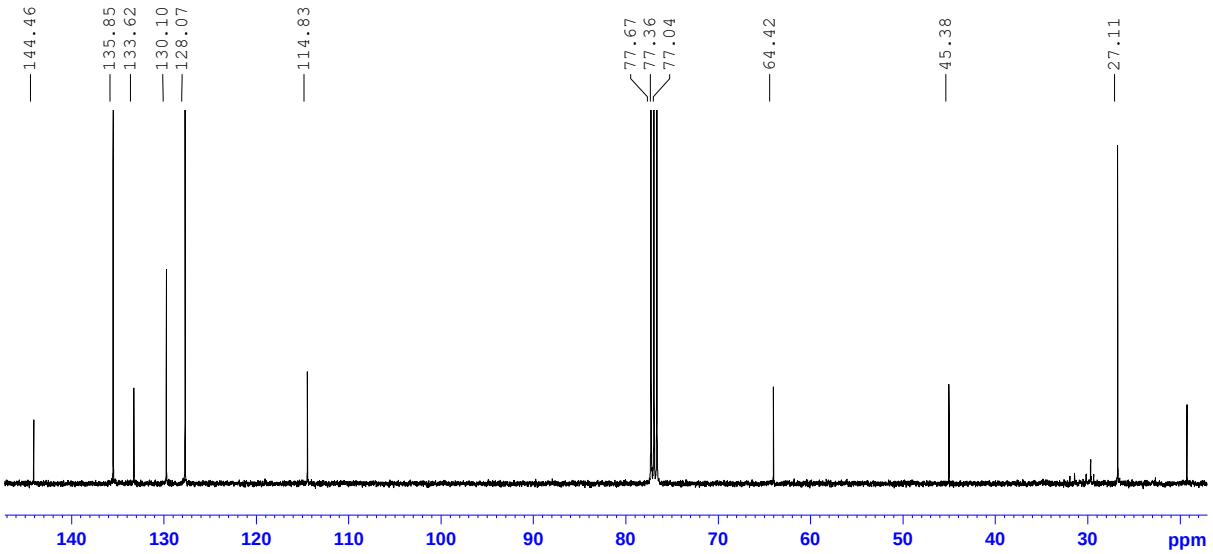


Plate 11a. ^{1}H NMR spectrum of 2-(*tert*-butyldiphenylsiloxymethyl)allyl-4-methylbenzenesulfonate (**105b**) in $CDCl_3$

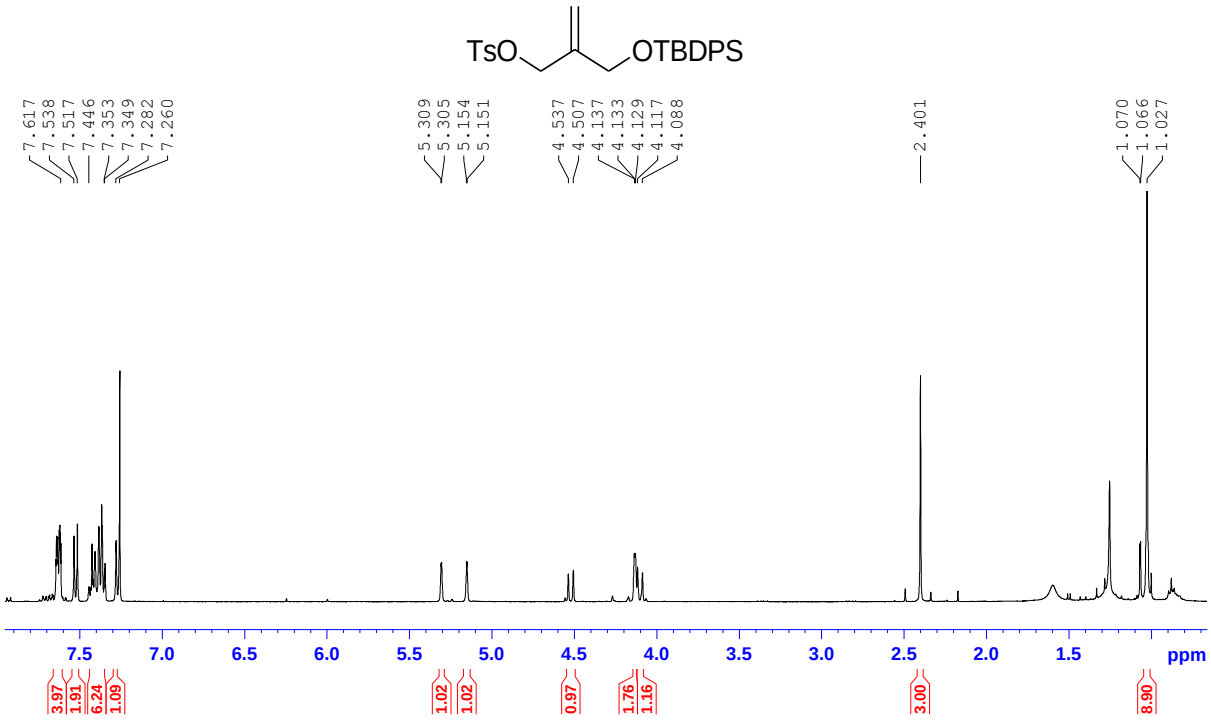


Plate 11b. ^{13}C NMR spectrum of 2-(*tert*-Butyldiphenylsiloxymethyl)allyl-4-methylbenzenesulfonate (**105b**) in $CDCl_3$

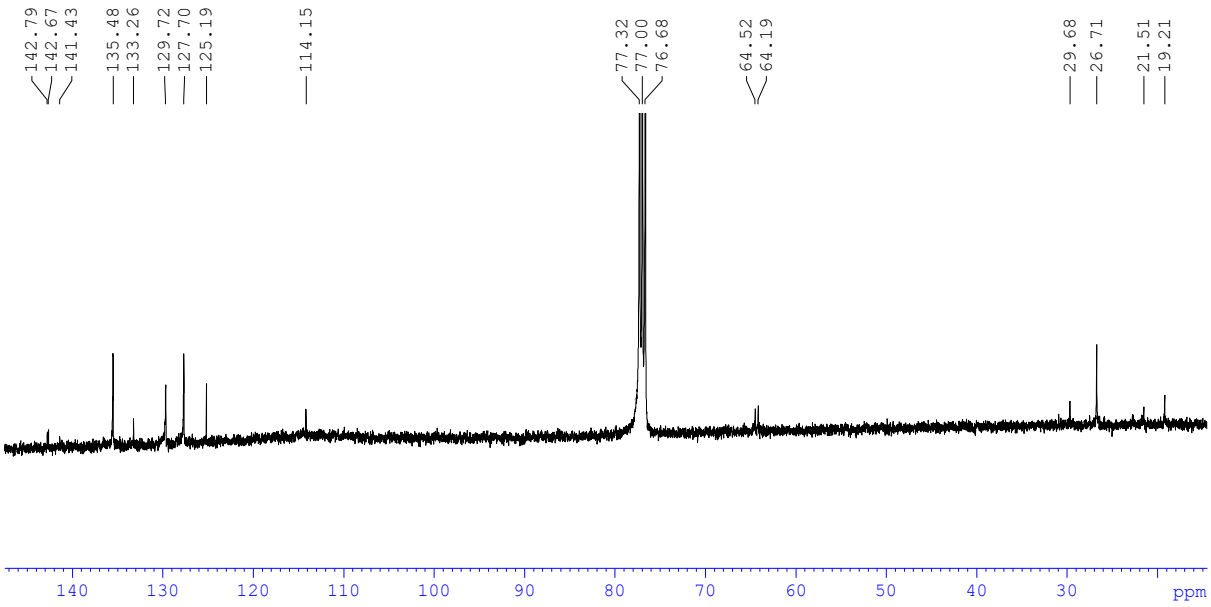


Plate 12a. ^{1}H NMR spectrum of (2-(bromomethyl)allyloxy)(*tert*-butyl)diphenylsilane (**105c**) in $CDCl_3$

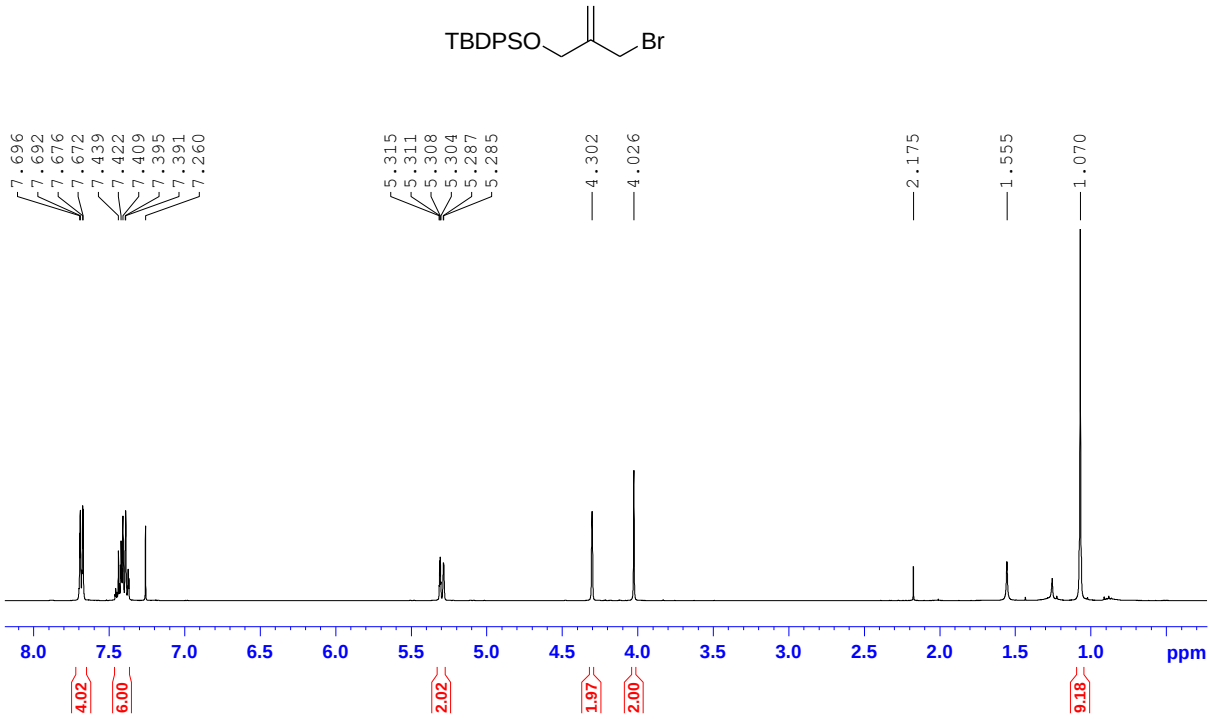


Plate 12b. ^{13}C NMR spectrum of (2-(Bromomethyl)allyloxy)(*tert*-butyl)diphenylsilane (**105c**) in $CDCl_3$

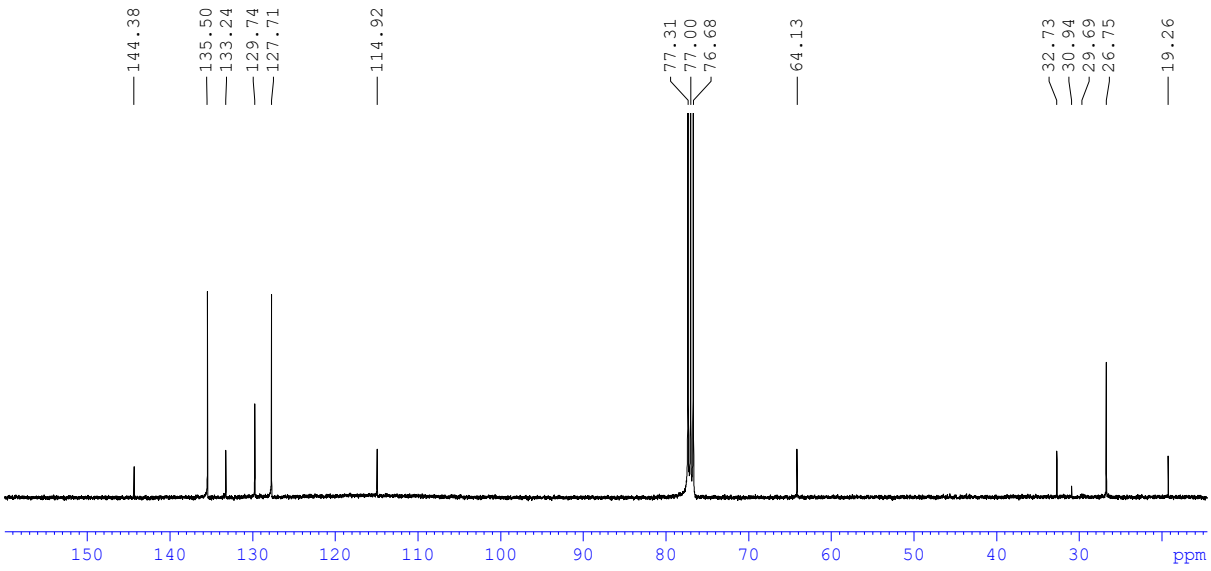


Plate 13a. ^{1}H NMR spectrum of 7-(2"-(*tert*-butyldiphenylsiloxymethyl)allyloxy)-6-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (**76**) in $CDCl_3$

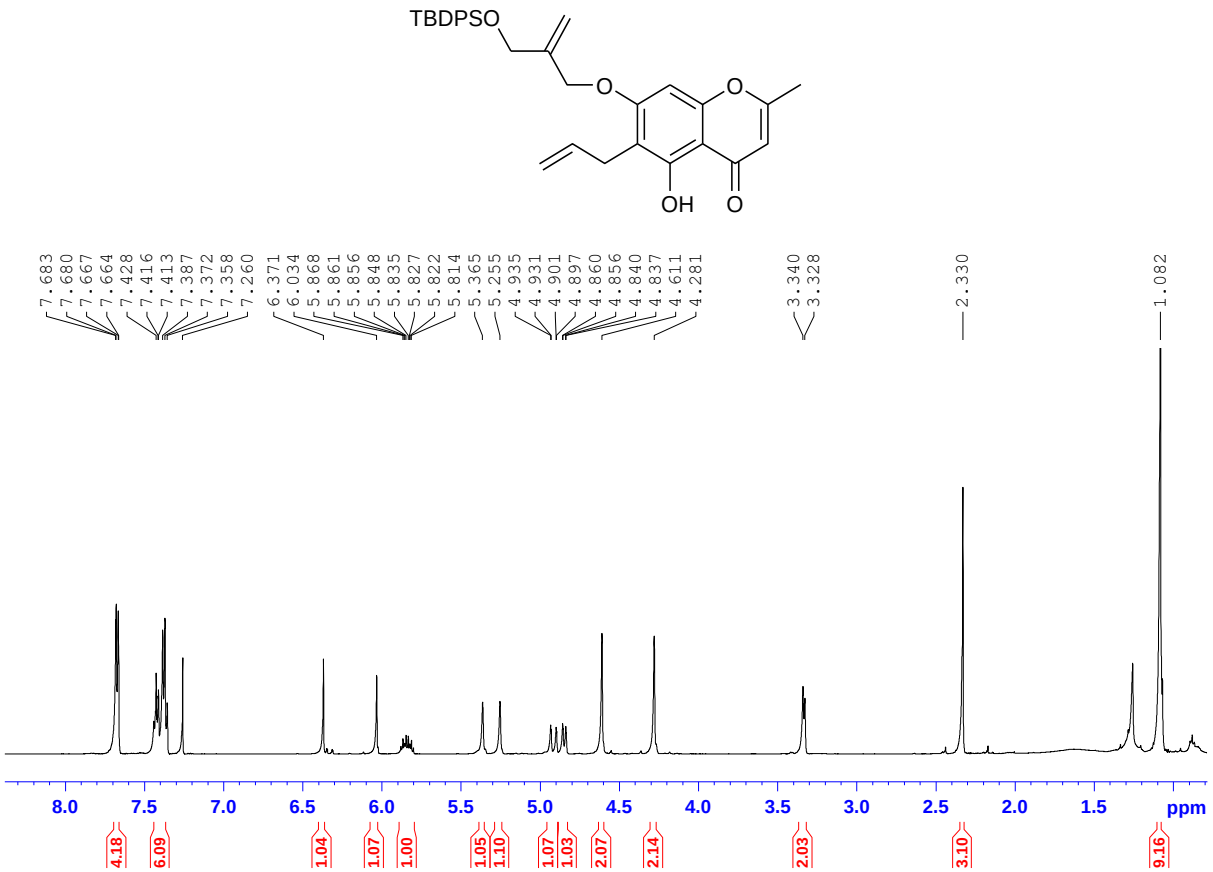


Plate 13b. ^{13}C NMR spectrum of 7-(2"-(*tert*-butyldiphenylsiloxymethyl)allyloxy)-6-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (**76**) in $CDCl_3$

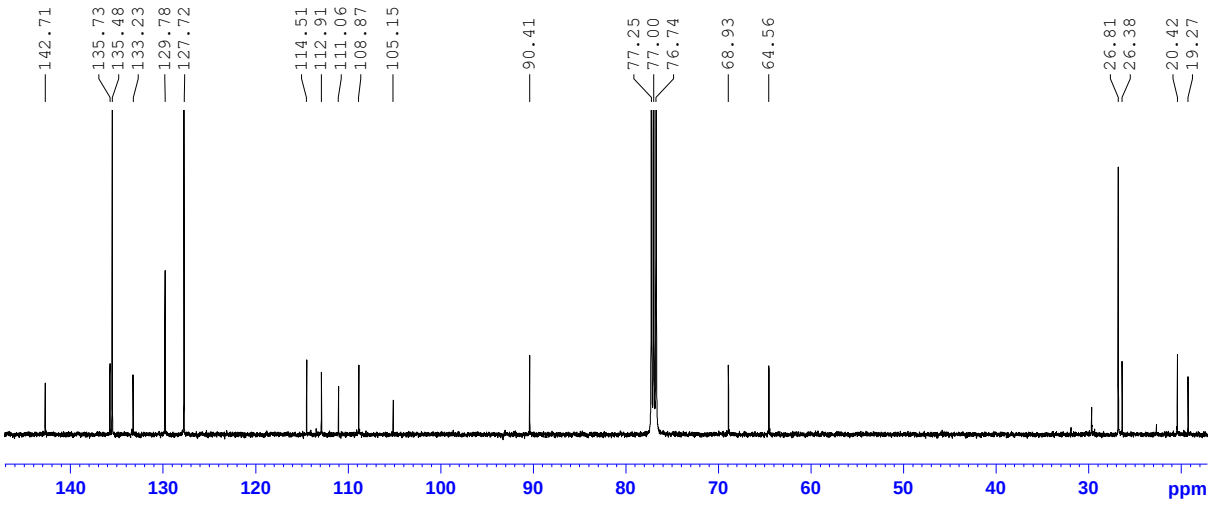


Plate 14a. ^{1}H NMR spectrum of 7-(2"-(*tert*-butyldiphenylsiloxymethyl)allyloxy)-8-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (**106**) in $CDCl_3$

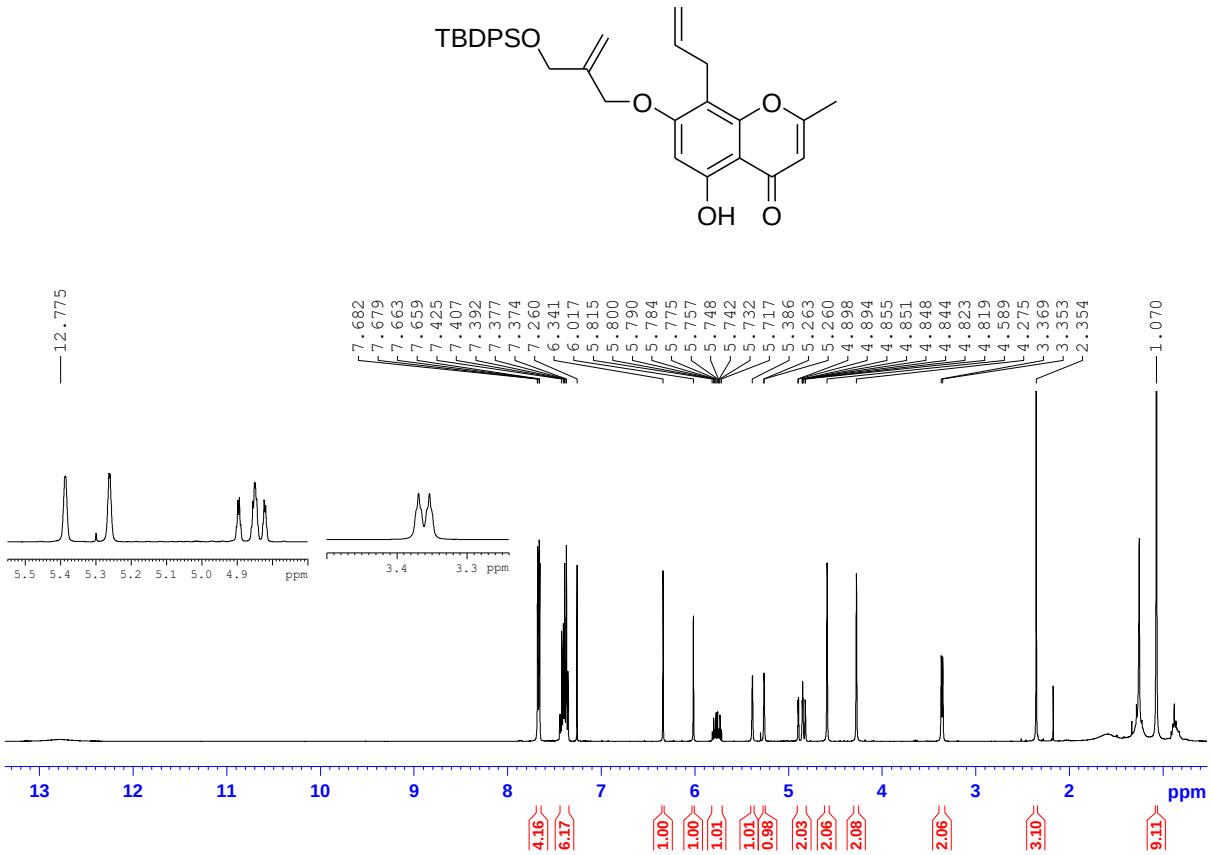


Plate 14b. ^{13}C NMR spectrum of 7-(2"-(*tert*-butyldiphenylsiloxymethyl)allyloxy)-8-allyl-5-hydroxy-2-methyl-4*H*-chromen-4-one (**106**) in $CDCl_3$

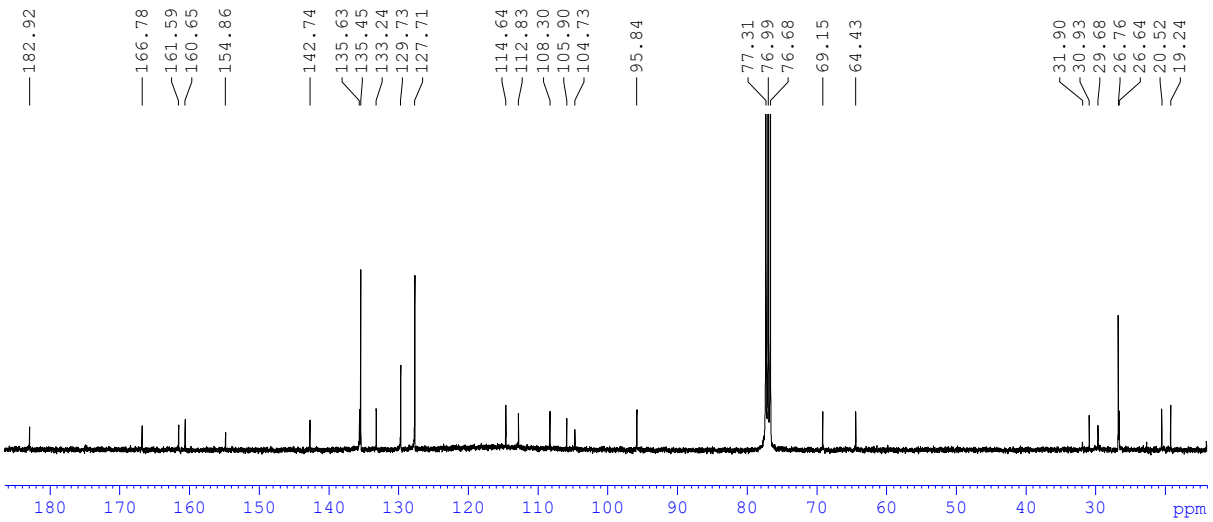


Plate 15a. ^{1}H NMR spectrum of 5-hydroxy-8-(*tert*-butyldiphenylsiloxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (**109**) in $CDCl_3$

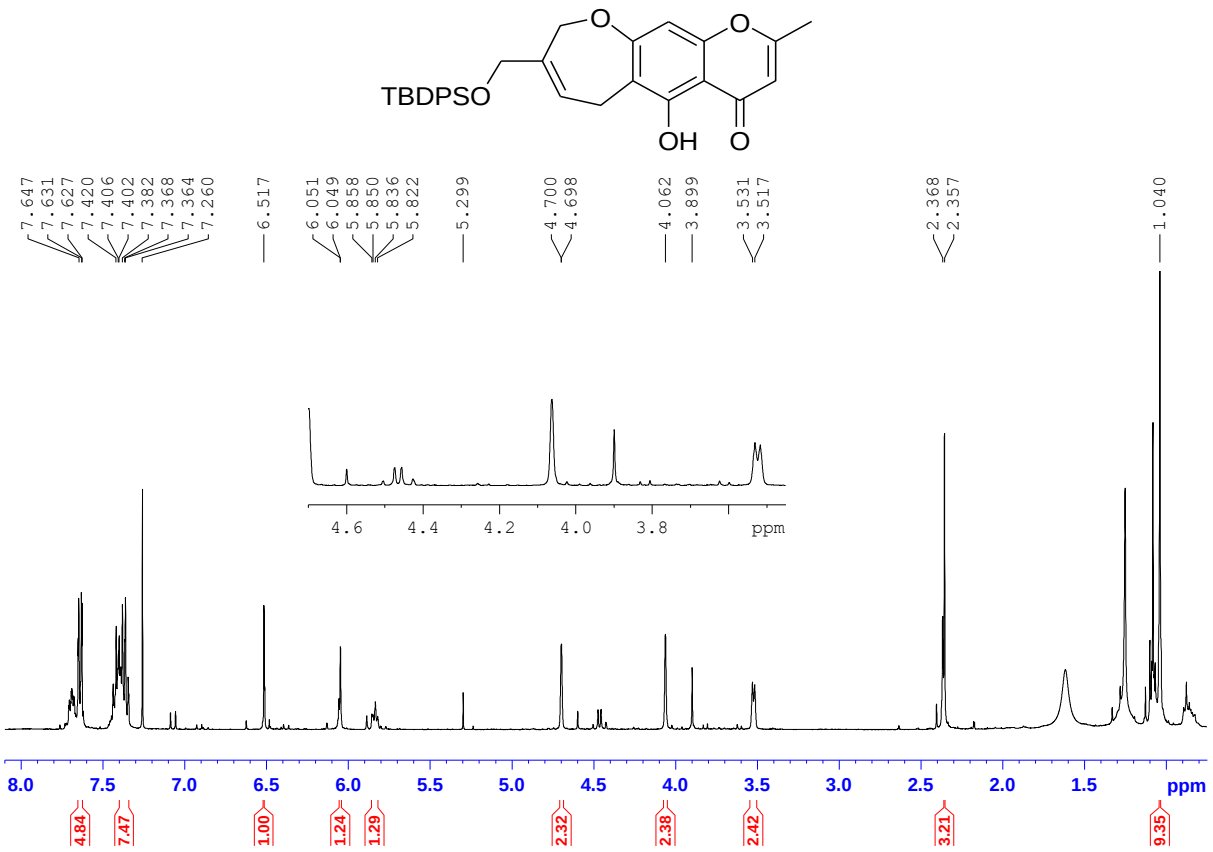


Plate 15b. ^{13}C NMR spectrum of 5-hydroxy-8-(*tert*-butyldiphenylsiloxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (**109**) in $CDCl_3$

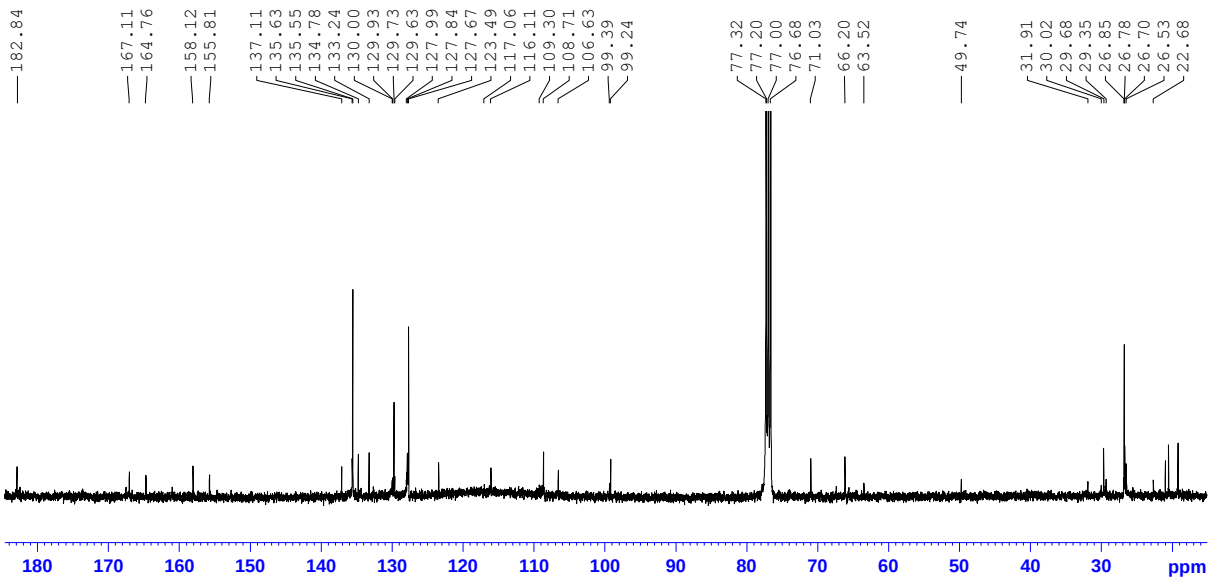


Plate 16a. ^{1}H NMR spectrum of 5-hydroxy-9-(*tert*-butyldiphenylsiloxymethyl)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (**107**) in $CDCl_3$

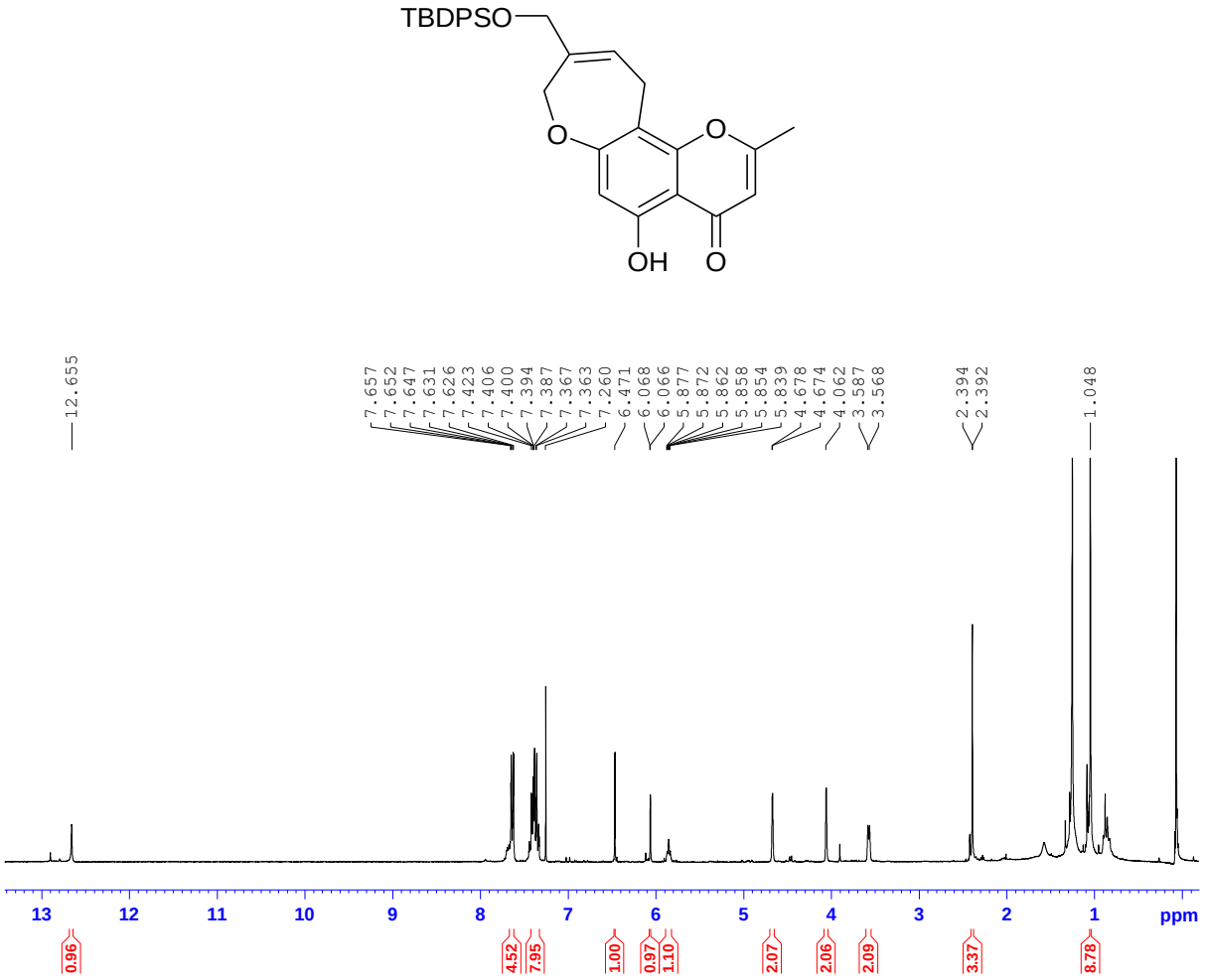


Plate 17a. ^{1}H NMR spectrum of 5-hydroxy-9-(hydroxymethyl)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (eranthin) (**108**) in $CDCl_3$

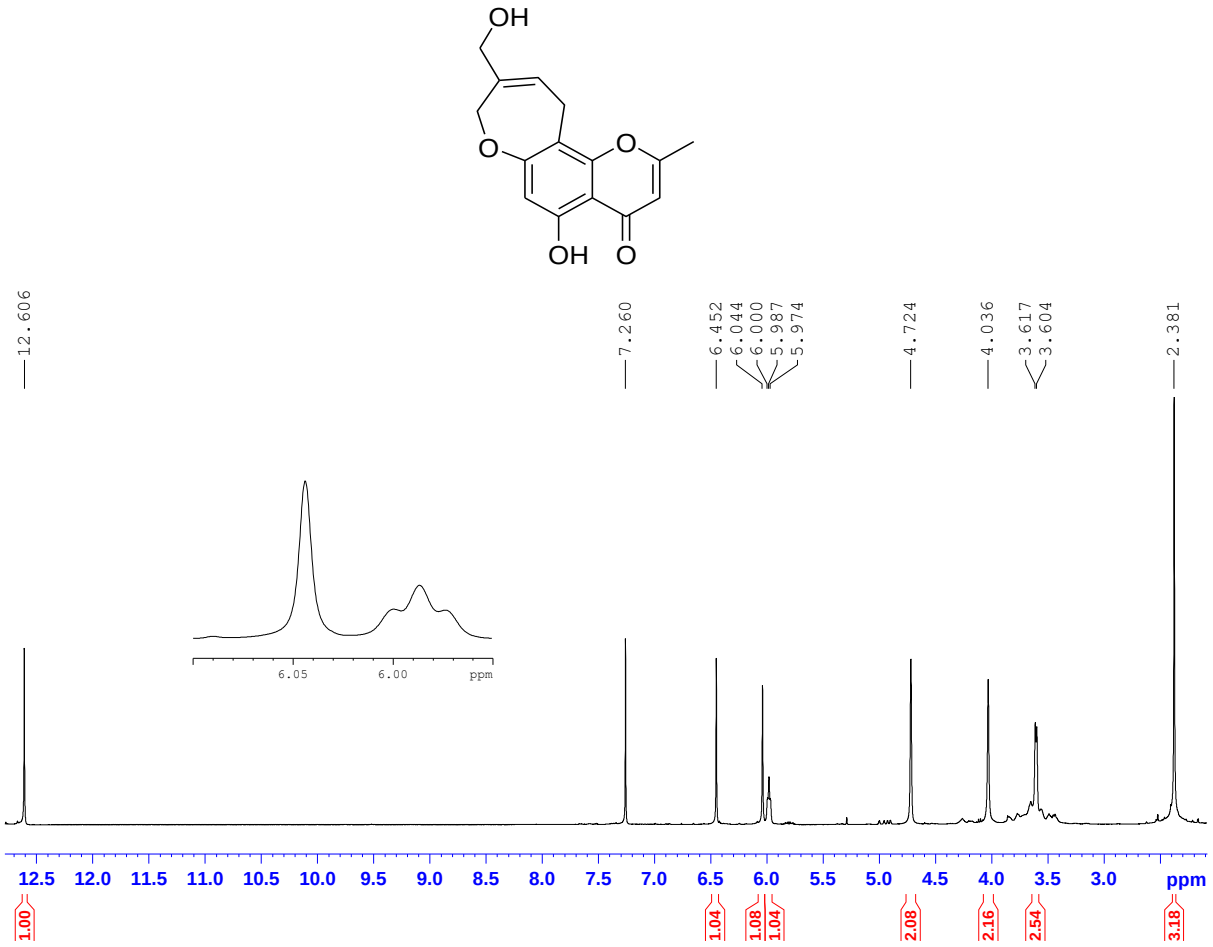


Plate 17b. ^{13}C NMR spectrum of 5-hydroxy-9-(hydroxymethyl)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (eranthin) (**108**) in $CDCl_3$

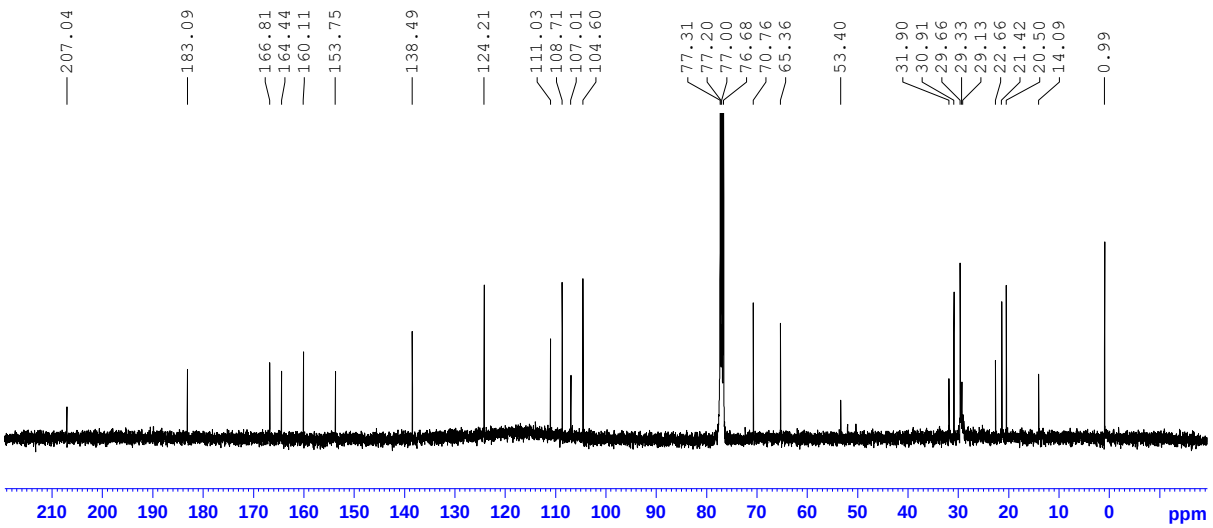
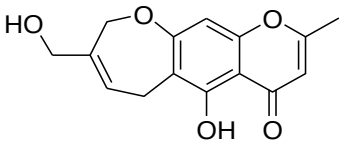


Plate 18a. ^{1}H NMR spectrum of 5-hydroxy-8-(hydroxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (ptaeroxylinol) (**77**) in $CDCl_3$



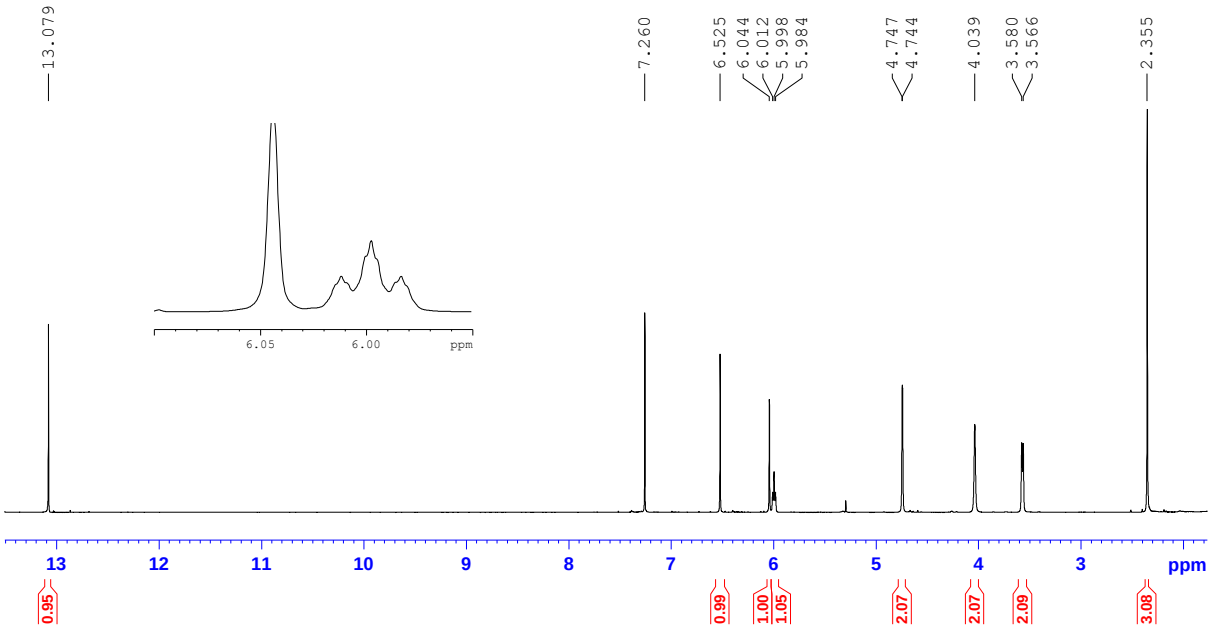


Plate 18b. ^{13}C NMR spectrum of 5-hydroxy-8-(hydroxymethyl)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (ptaeroxylinol) (**77**) in $CDCl_3$

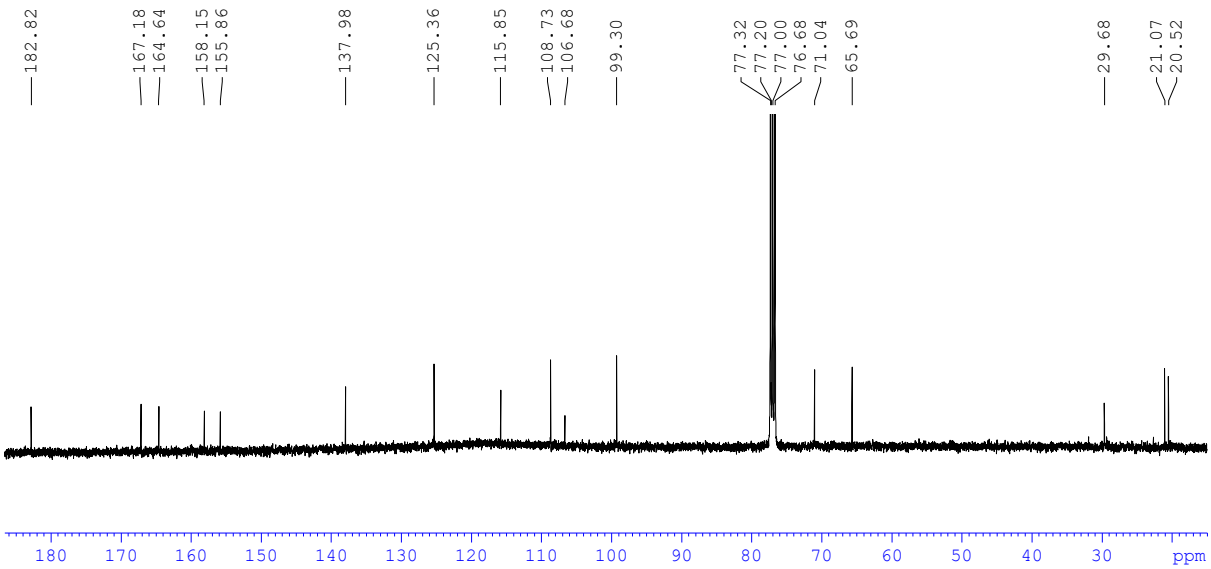


Plate 19a. ^{13}C NMR spectrum of 5-hydroxy-9-(methylacetate)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (**4**) in $CDCl_3$

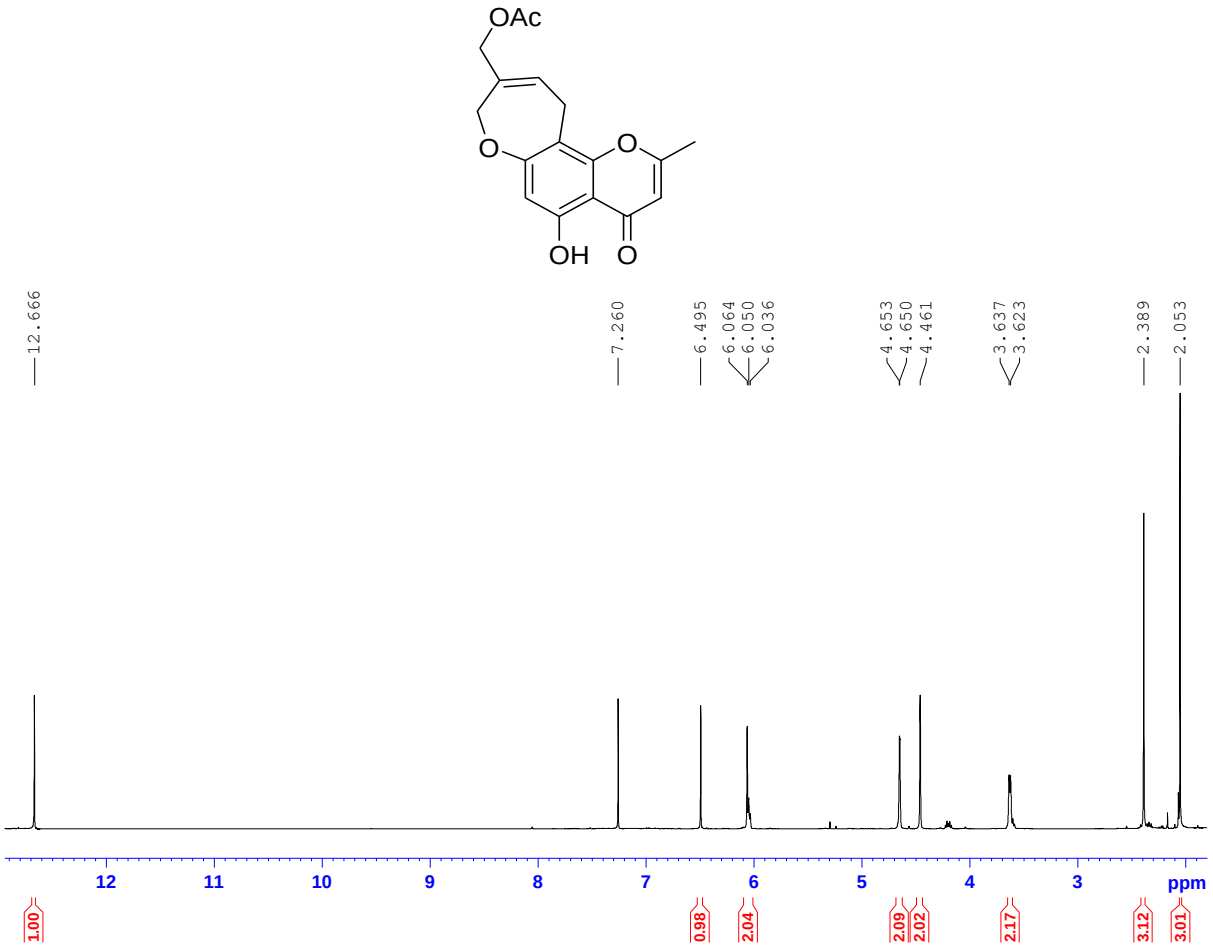


Plate 19b. ^{13}C NMR spectrum of 5-hydroxy-9-(methylacetate)-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one (**4**) in $CDCl_3$

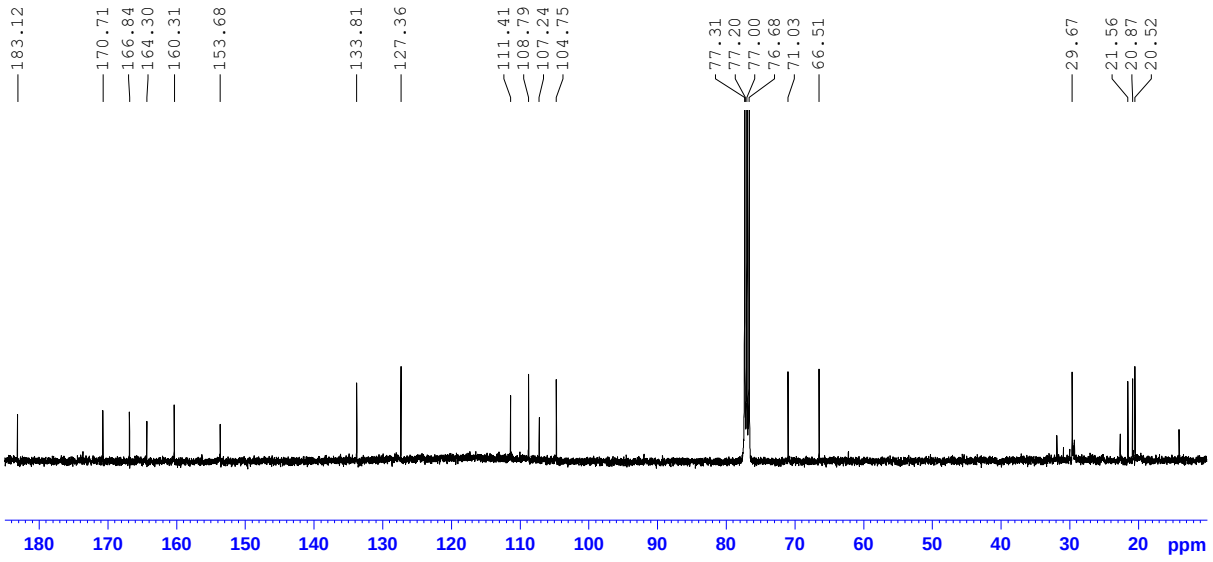


Plate 20a. ^{1}H NMR spectrum of 5-hydroxy-8-(methylacetate)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (**5**) in $CDCl_3$

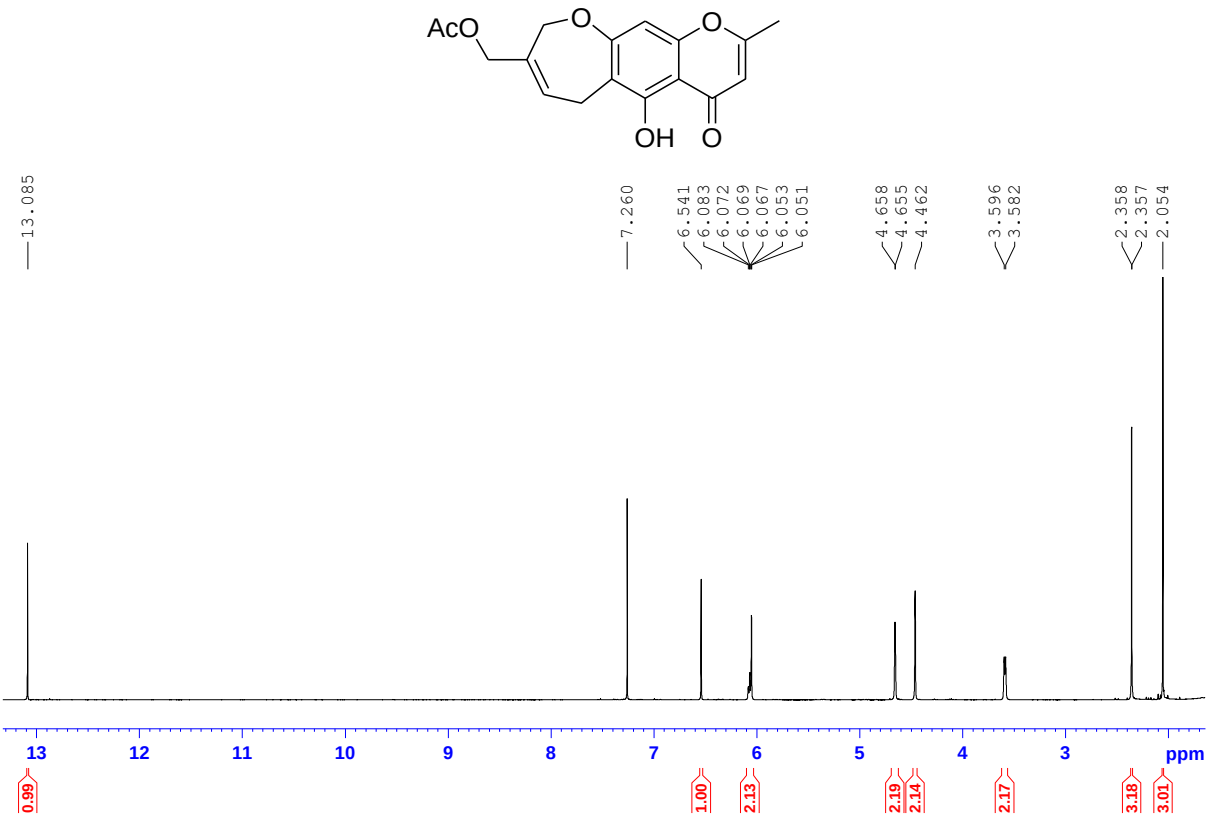


Plate 20b. ^{13}C NMR spectrum of 5-hydroxy-8-(methylacetate)-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-g]chromen-4-one (**5**) in $CDCl_3$

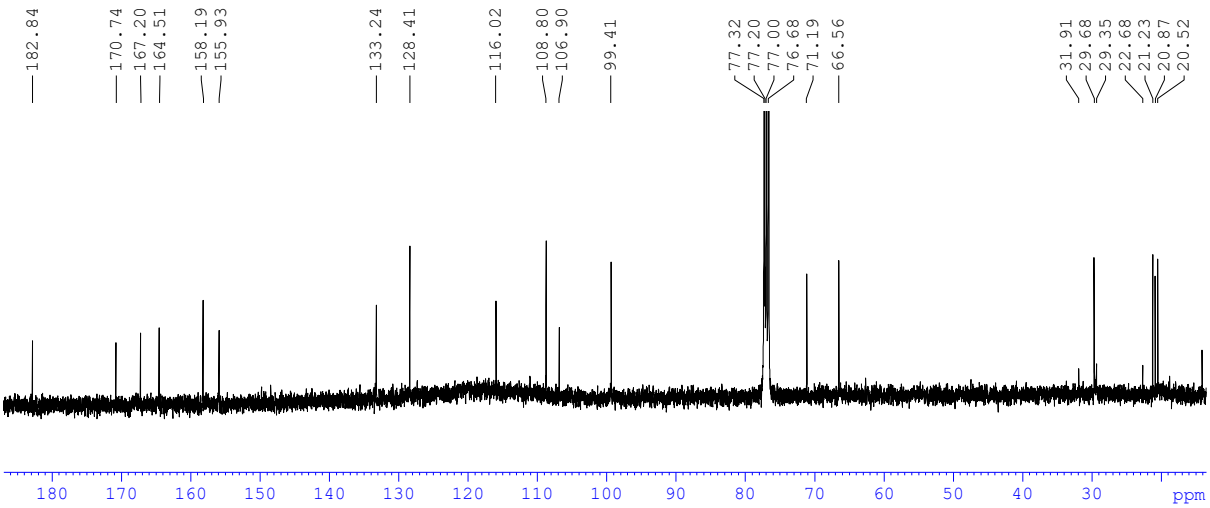


Plate 21a. ^{1}H NMR spectrum of 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**110**) in $CDCl_3$

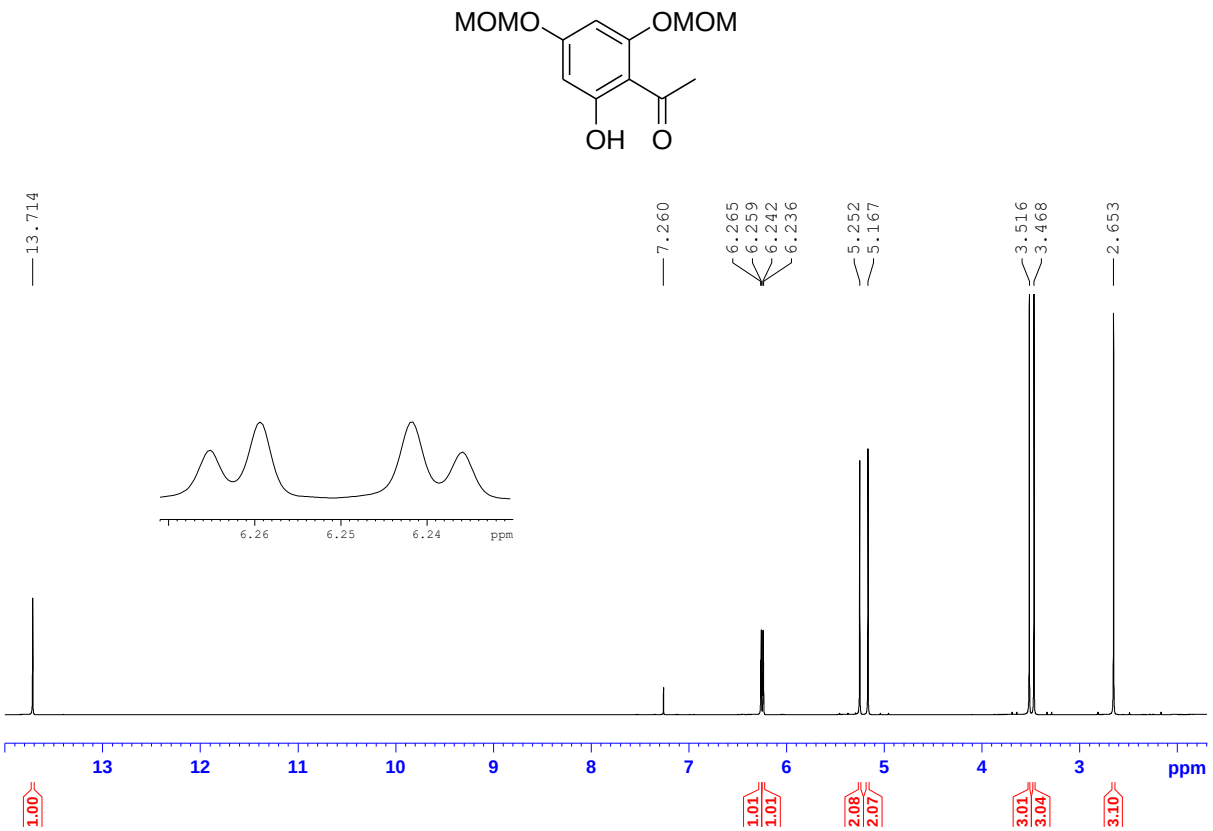


Plate 21b. ^{13}C NMR spectrum of 1-[2-hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**110**) in $CDCl_3$

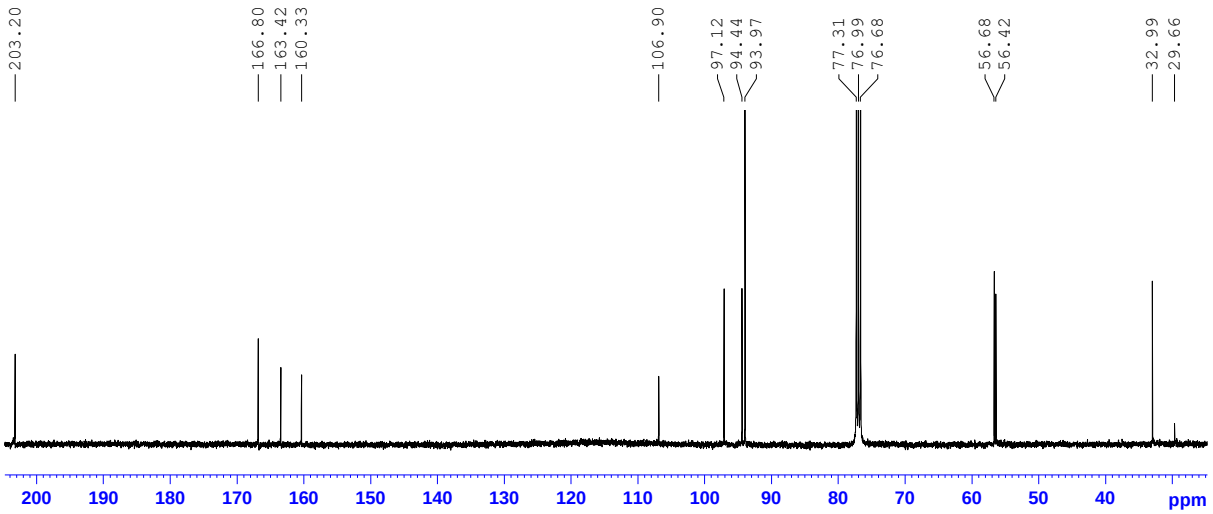


Plate 22a. ^{1}H NMR spectrum of 1-[2-allyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**111**) in $CDCl_3$

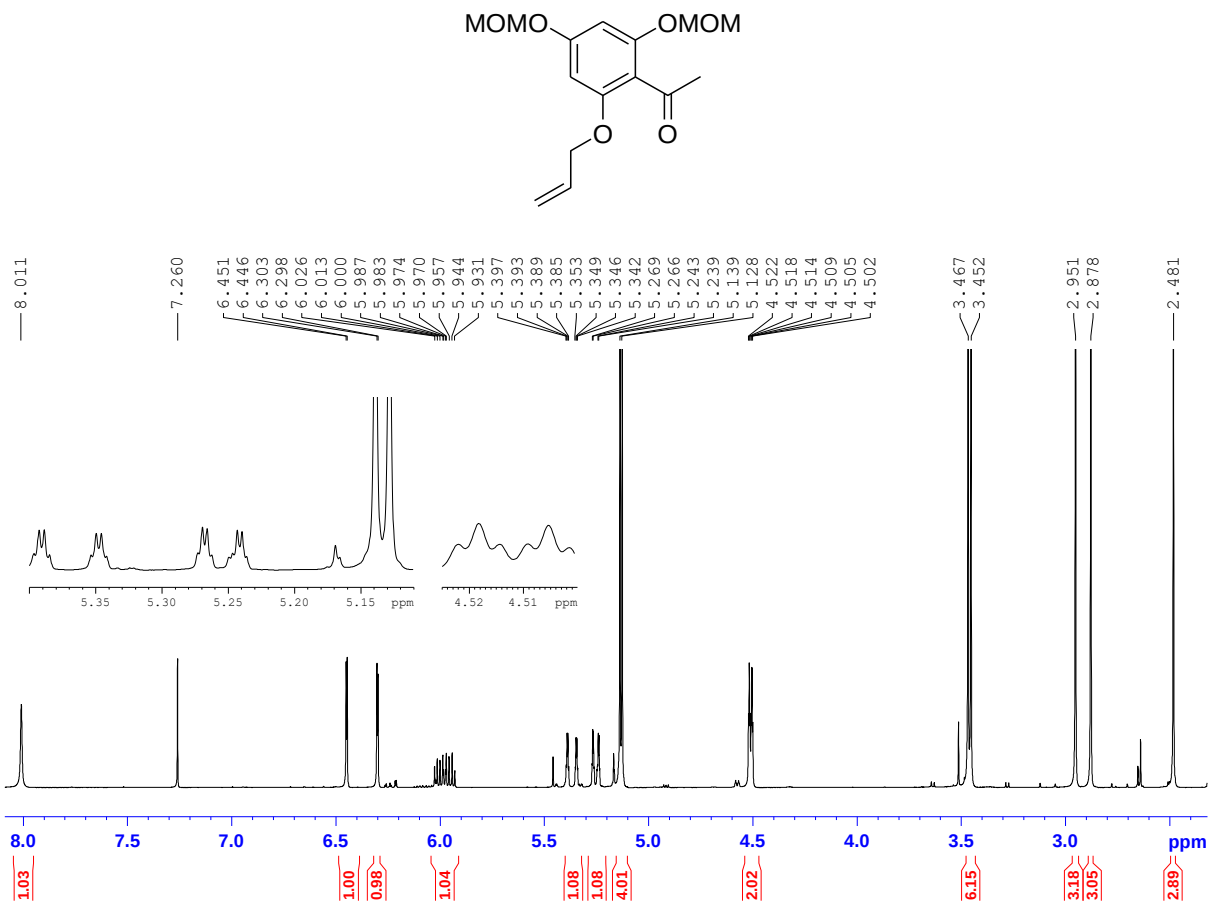


Plate 22b. ^{13}C NMR spectrum of 1-[2-allyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**111**) in $CDCl_3$

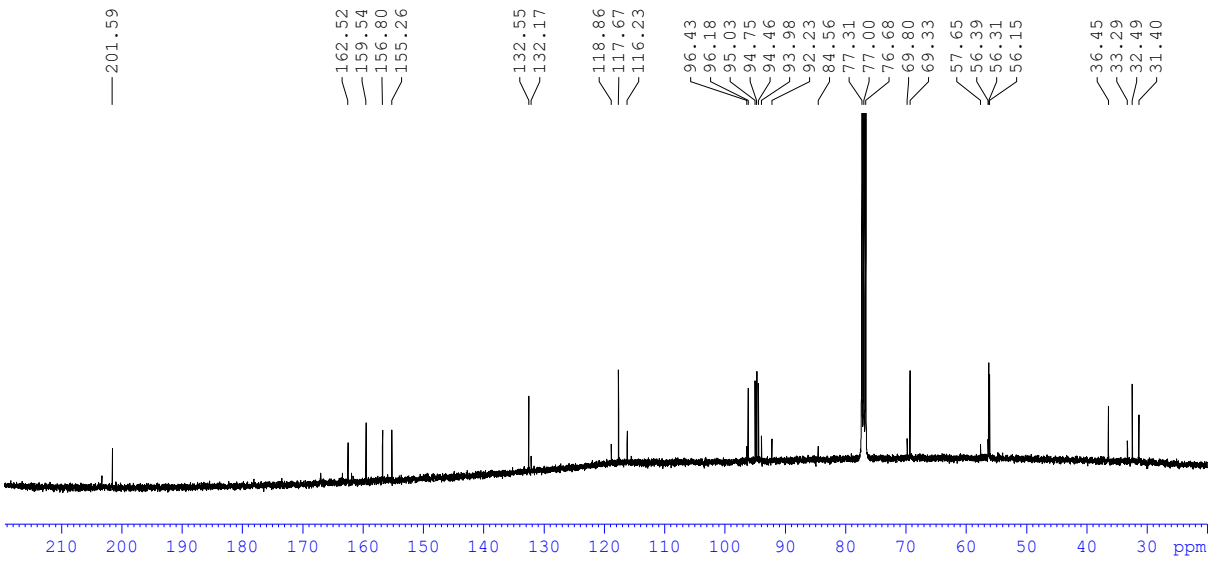


Plate 23a. ^{1}H NMR spectrum of 1-[3-allyl-2-hydroxy-4,6-bis(methoxymethoxy)phenyl] ethanone (**112**) in $CDCl_3$

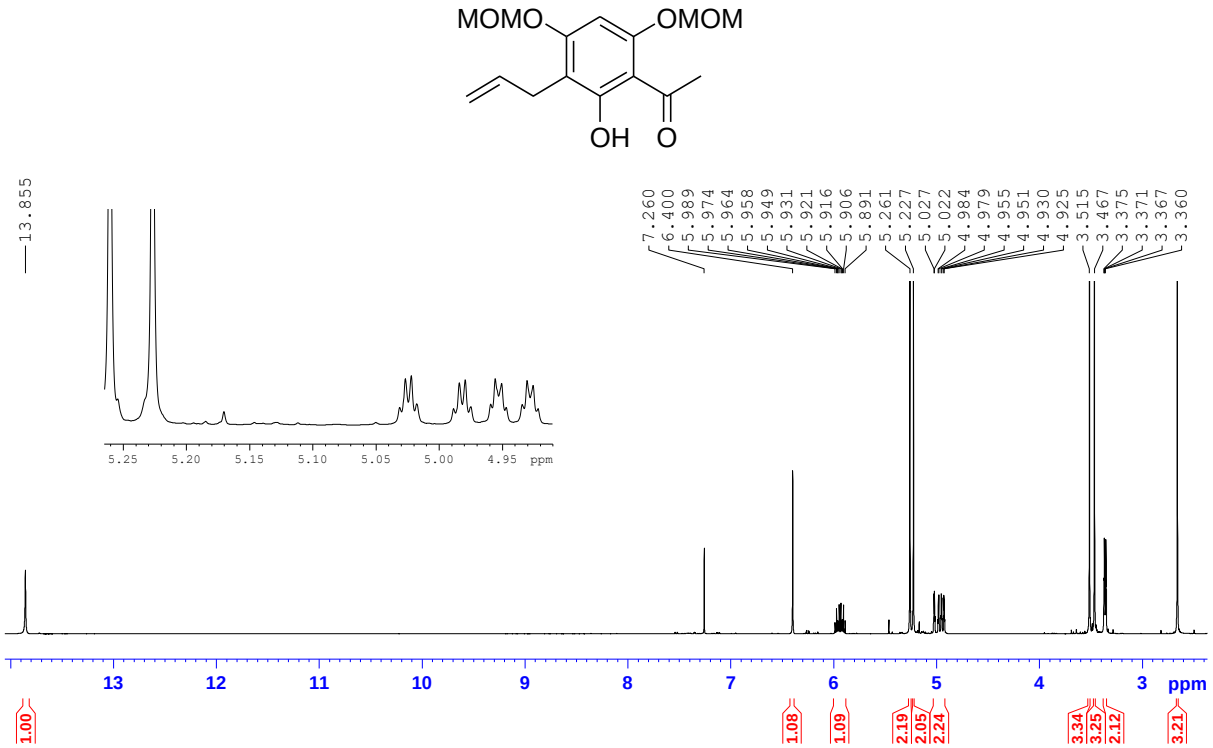


Plate 23b. ^{13}C NMR spectrum of 1-[3-allyl-2-hydroxy-4,6-bis(methoxymethoxy)phenyl] ethanone (**112**) in $CDCl_3$

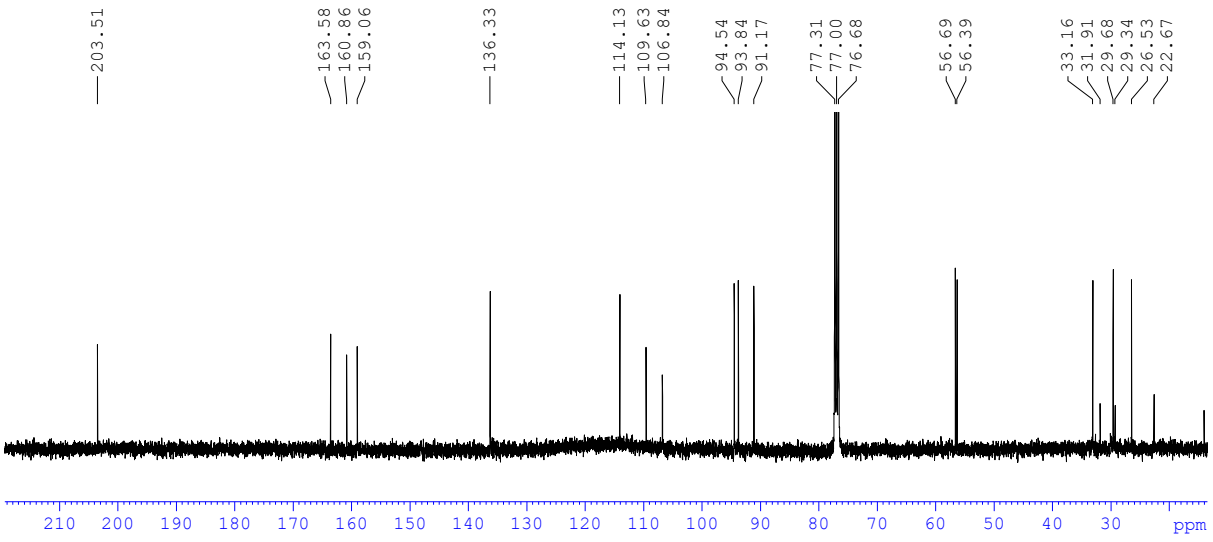


Plate 24a. ^{1}H NMR spectrum of 1-[3-allyl-2-*tert*-butoxycarbonyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**113**) in $CDCl_3$

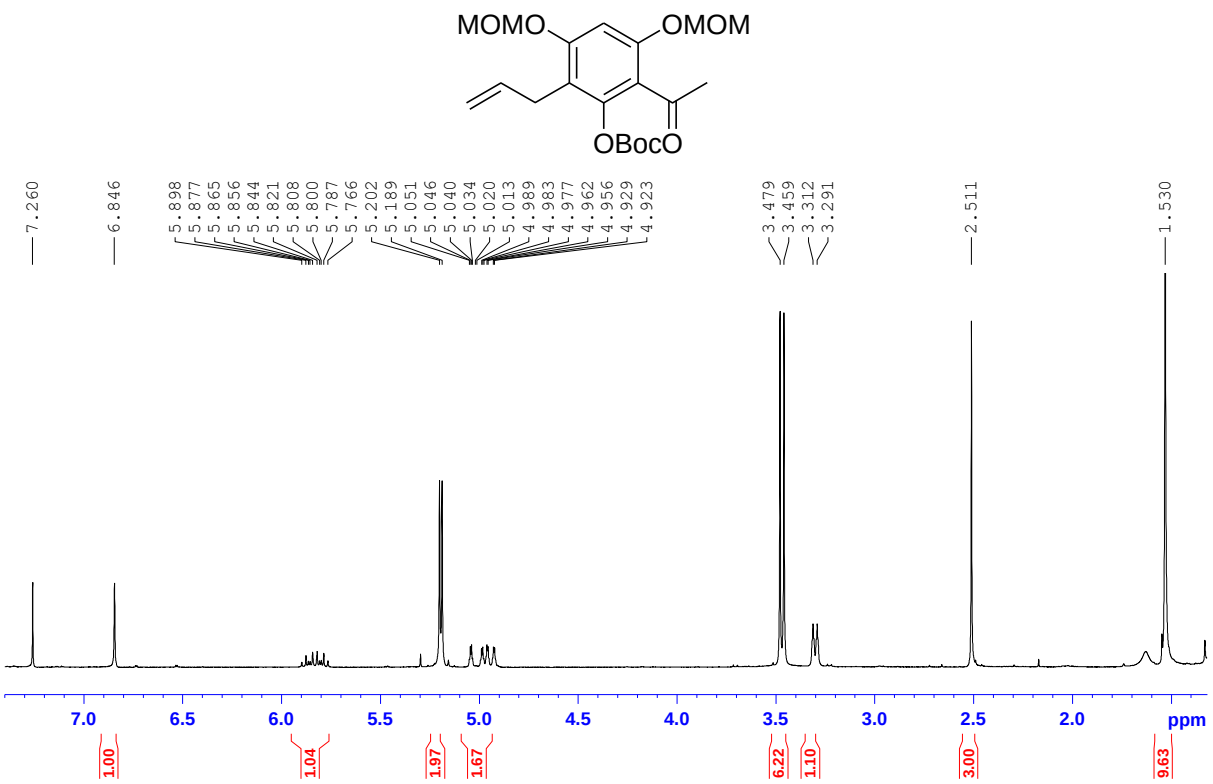


Plate 24b. ^{1}H NMR spectrum of 1-[3-allyl-2-*tert*-butoxycarbonyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**113**) in $CDCl_3$

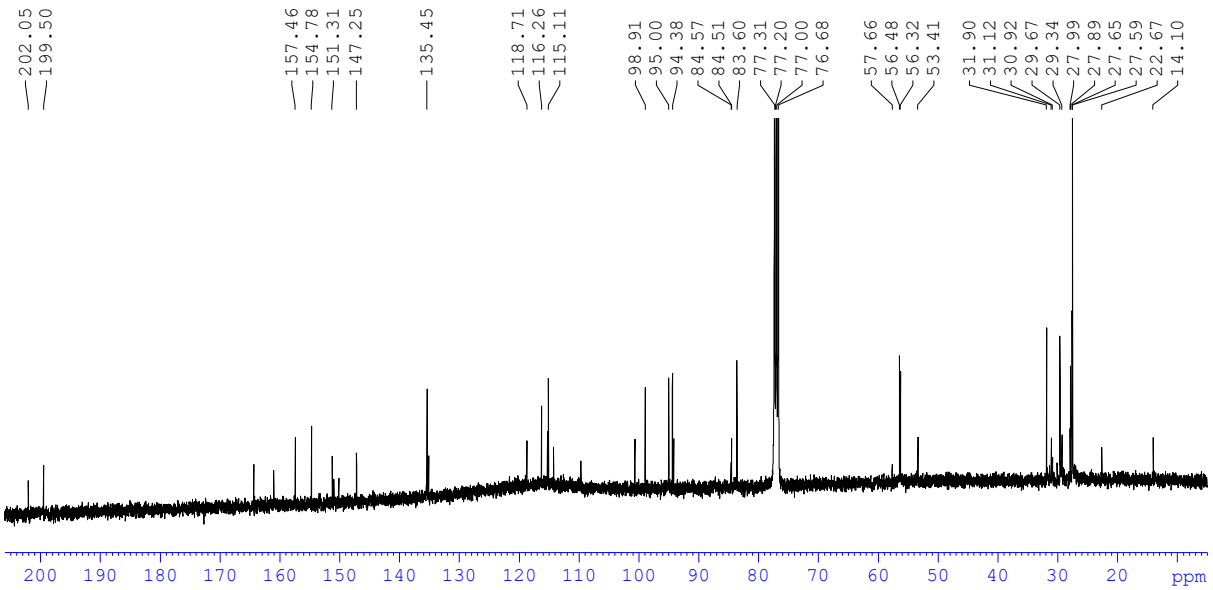


Plate 25a. ^{1}H NMR spectrum of 1-[3-allyl-2-methoxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**115**) in $CDCl_3$

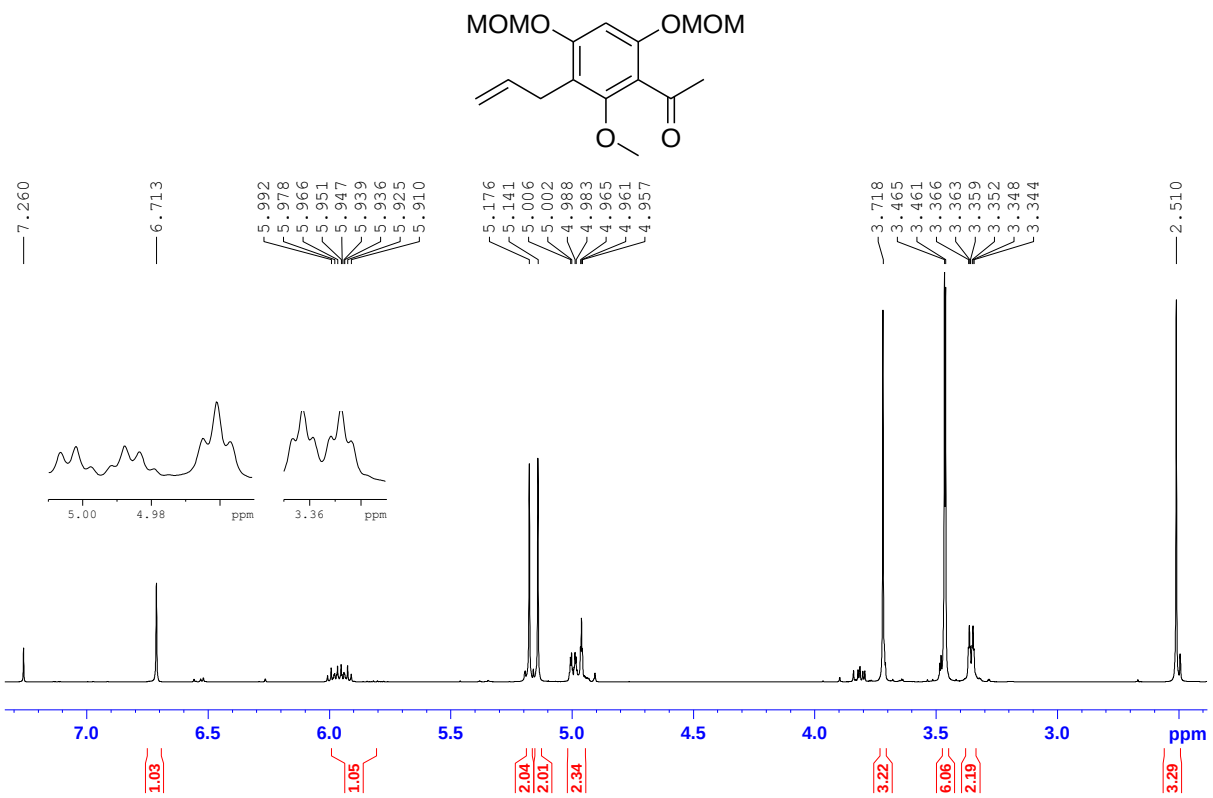


Plate 25b. ^{13}C NMR spectrum of 1-[3-allyl-2-methoxy-4,6-bis(methoxymethoxy)phenyl]ethanone (**115**) in $CDCl_3$

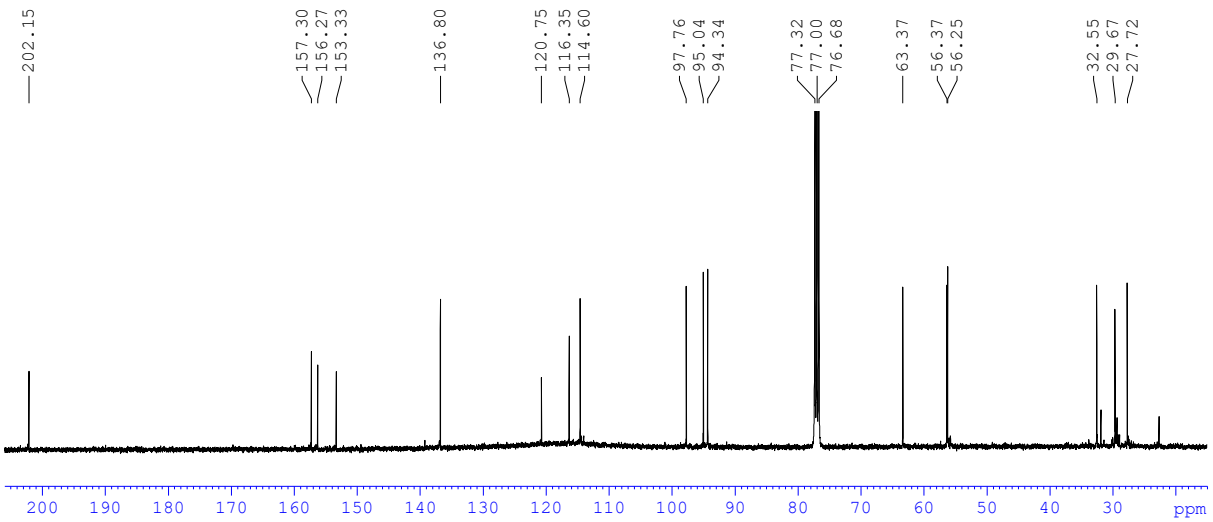


Plate 26a. ^{1}H NMR spectrum of 1-[3-allyl-2-methoxy-4,6-bis(hydroxy)phenyl]ethanone (**116**) in $CDCl_3$

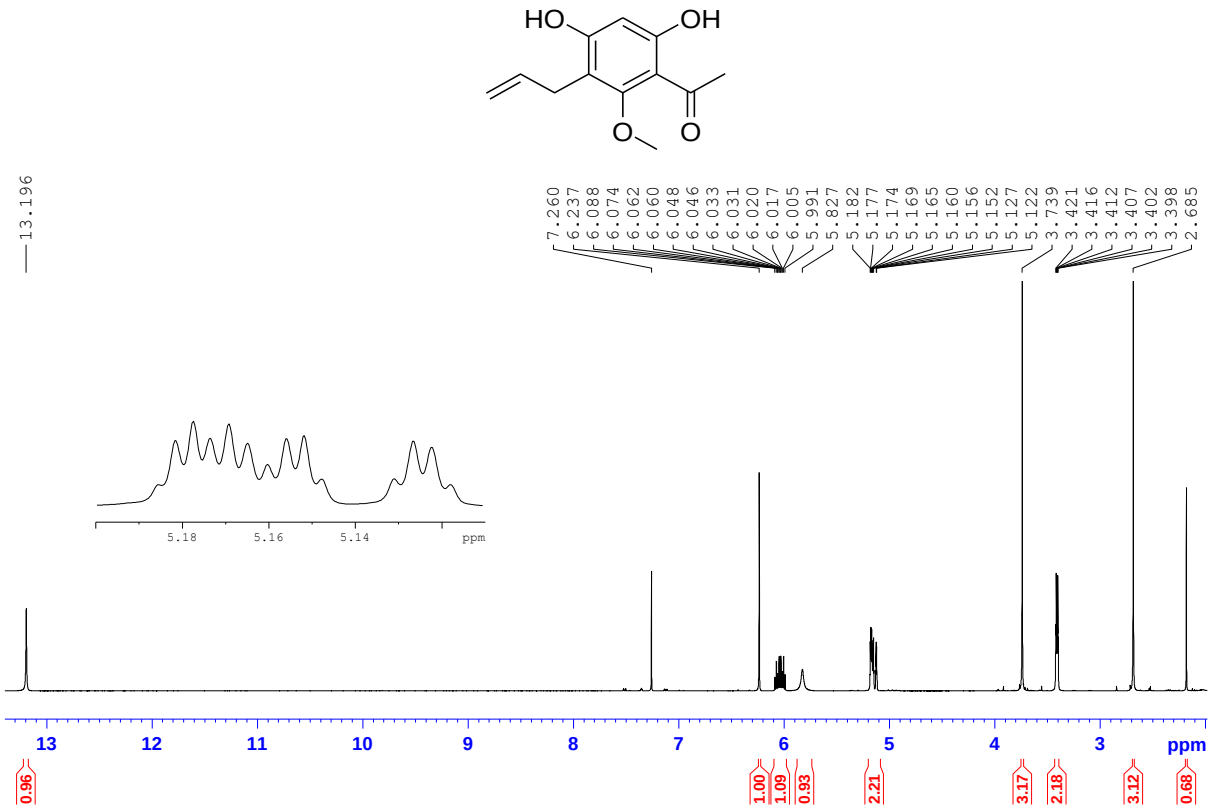


Plate 26b. ^{13}C NMR spectrum of 1-[3-allyl-2-methoxy-4,6-bis(hydroxy)phenyl]ethanone (**116**) in $CDCl_3$

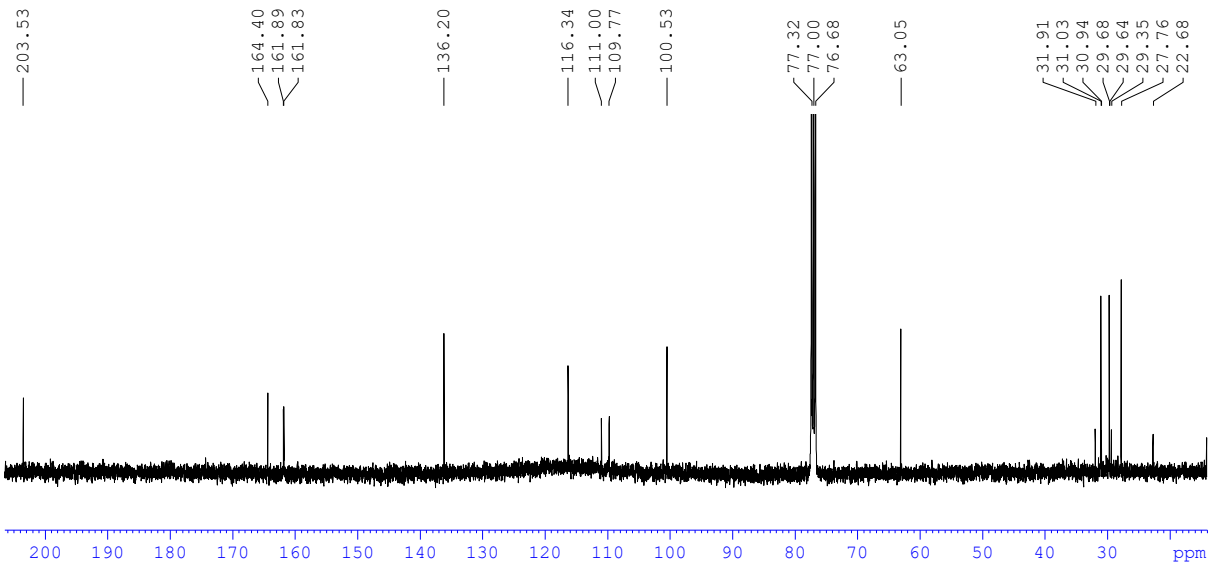


Plate 27a. ^{1}H NMR spectrum of 1-(4-{2"-[(*tert*-butyldiphenylsiloxymethyl)allyloxy)-3-allyl-6-hydroxy-2-methoxyphenyl)ethanone (**117**) in $CDCl_3$

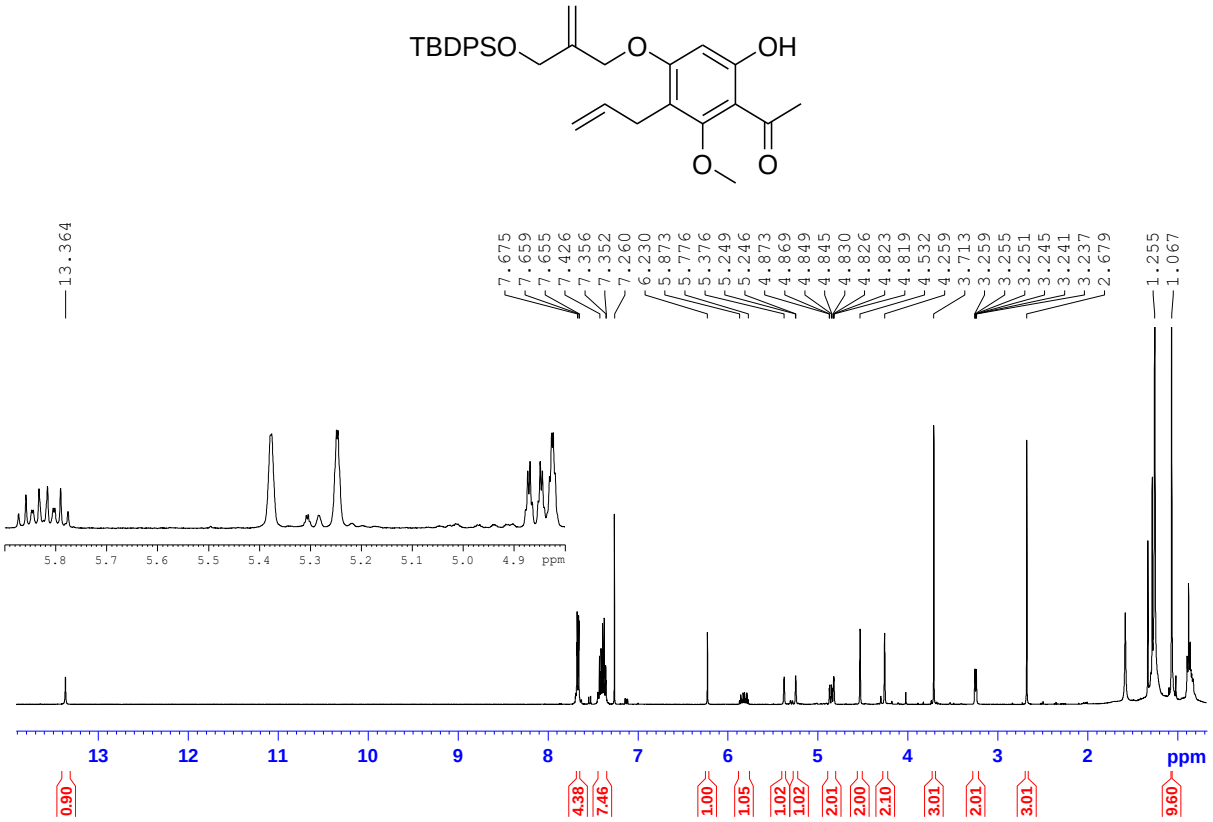


Plate 27b. ^{13}C NMR spectrum of 1-(4-{2"-[(*tert*-butyldiphenylsiloxymethyl)allyloxy)-3-allyl-6-hydroxy-2-methoxyphenyl)ethanone (**117**) in $CDCl_3$

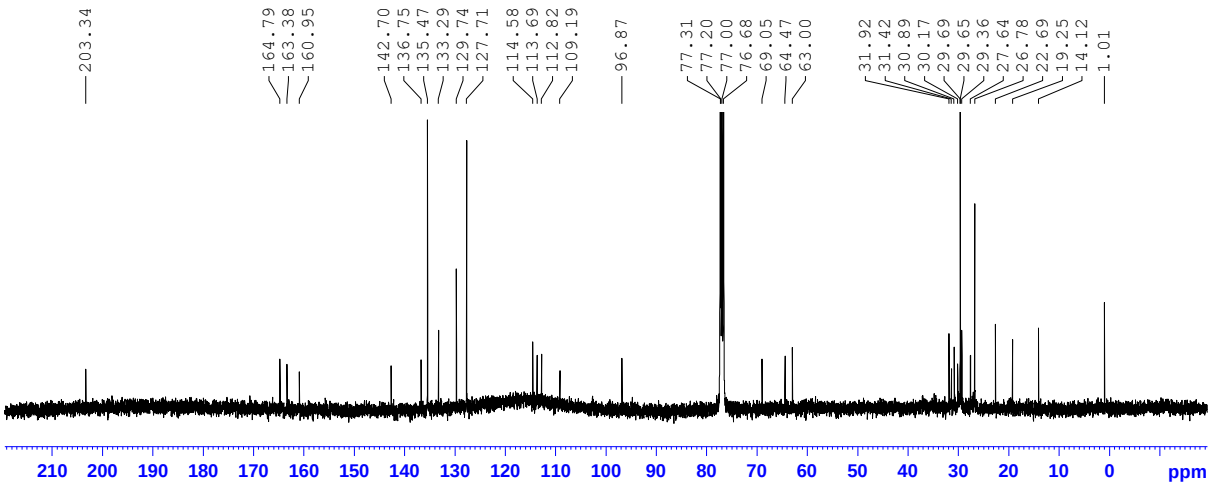


Plate 28a. ^{1}H NMR spectrum of 1-[3-allyl-6-hydroxy-2-methoxy-4-(methoxymethoxy)phenyl] ethanone (**120**) in $CDCl_3$

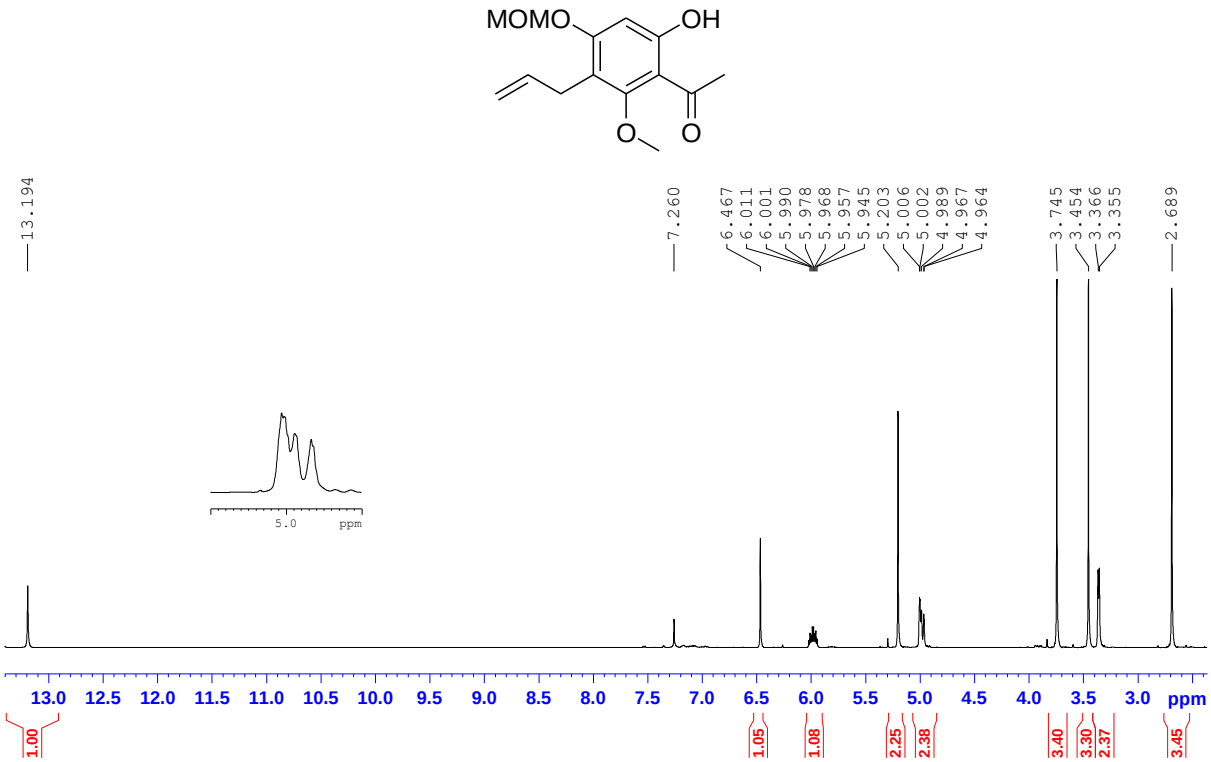


Plate 28b. ^{13}C NMR spectrum of 1-[3-allyl-6-hydroxy-2-methoxy-4-(methoxymethoxy)phenyl] ethanone (**120**) in $CDCl_3$

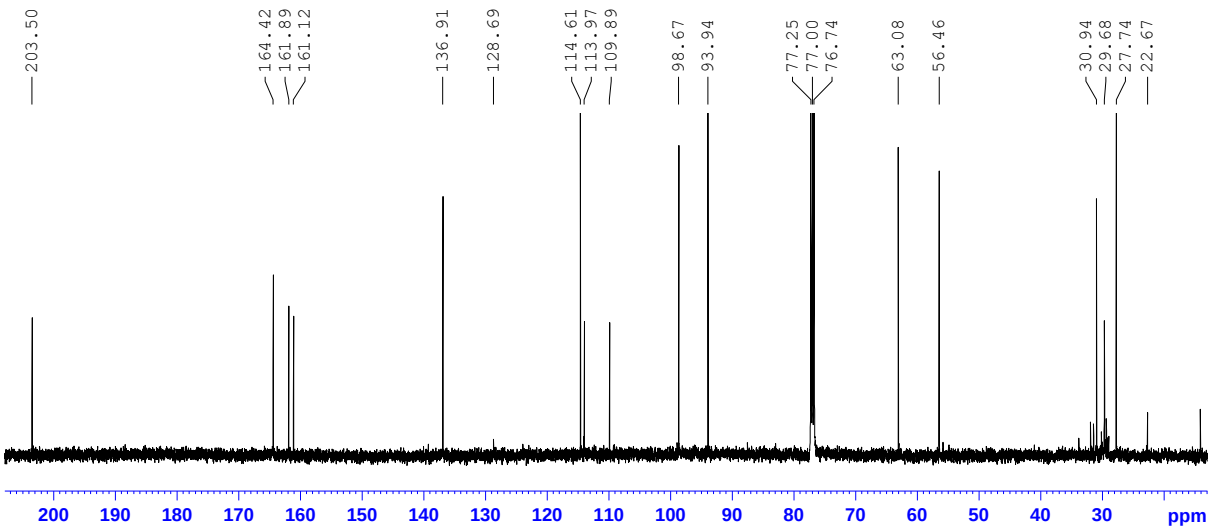


Plate 29a. ^{1}H NMR spectrum of 1-[3-allyl-2,6-dimethoxy-4-(methoxymethoxy)phenyl] ethanone (**121**) in $CDCl_3$

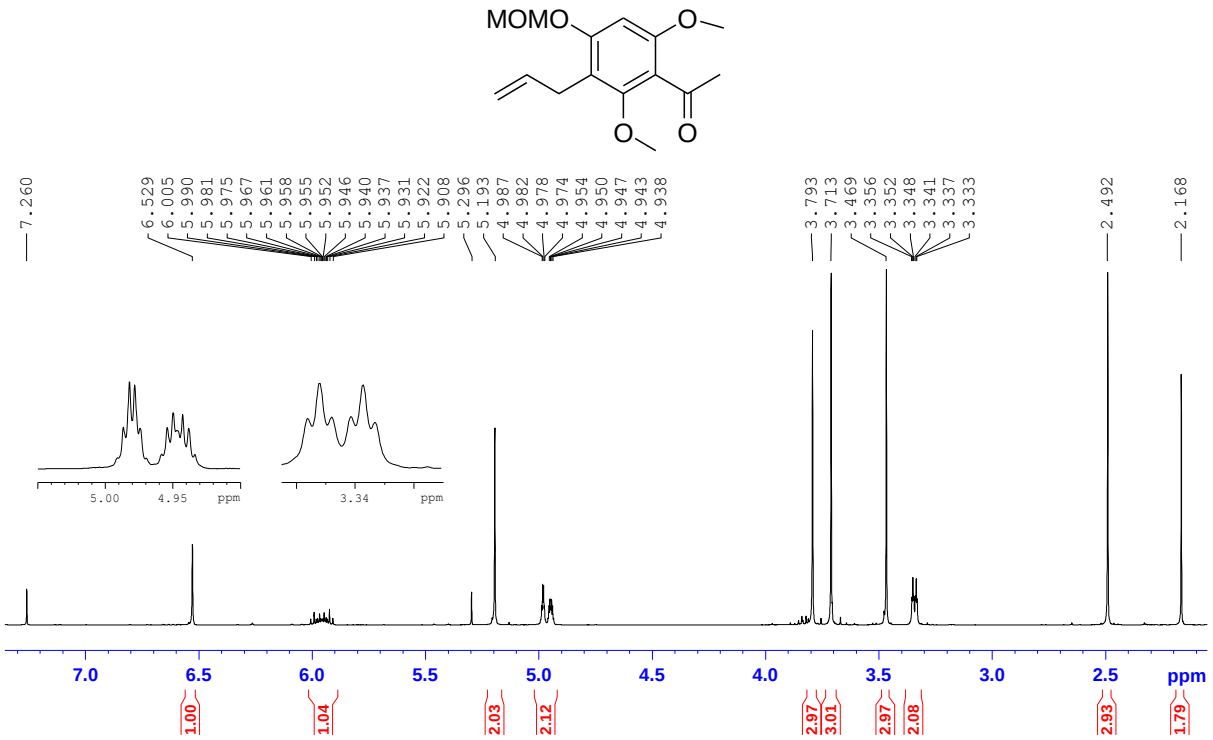


Plate 29b. ^{13}C NMR spectrum of 1-[3-allyl-2,6-dimethoxy-4-(methoxymethoxy)phenyl] ethanone (**121**) in $CDCl_3$

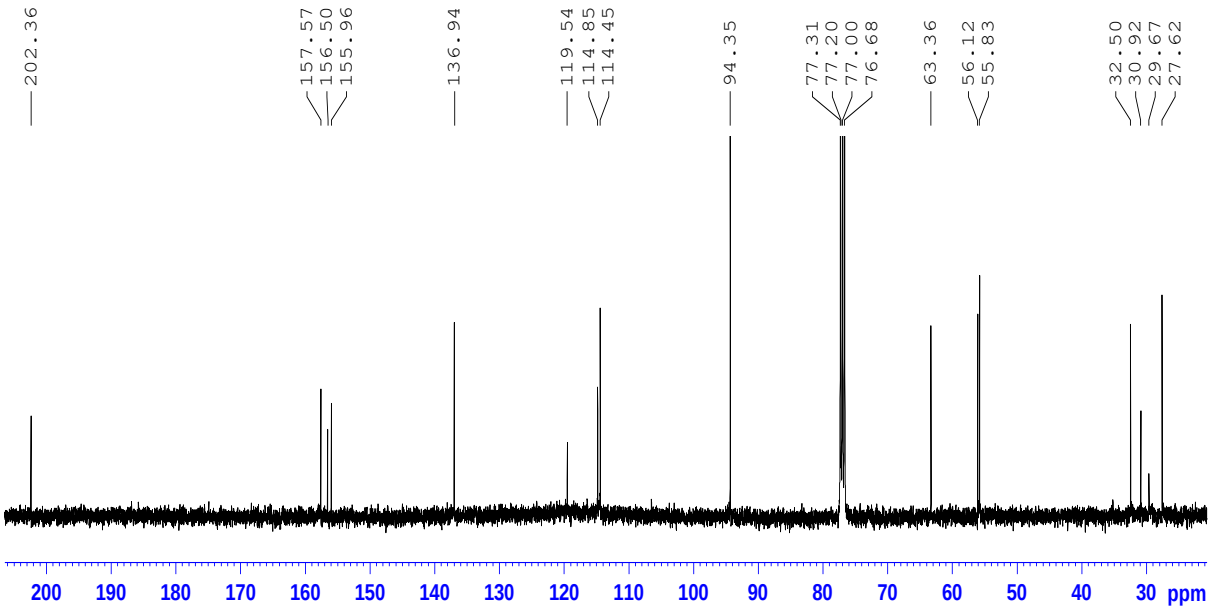


Plate 30a. ^{1}H NMR spectrum of 1-(3-allyl-4-hydroxy-2,6-dimethoxyphenyl)ethanone (**122**) in $CDCl_3$

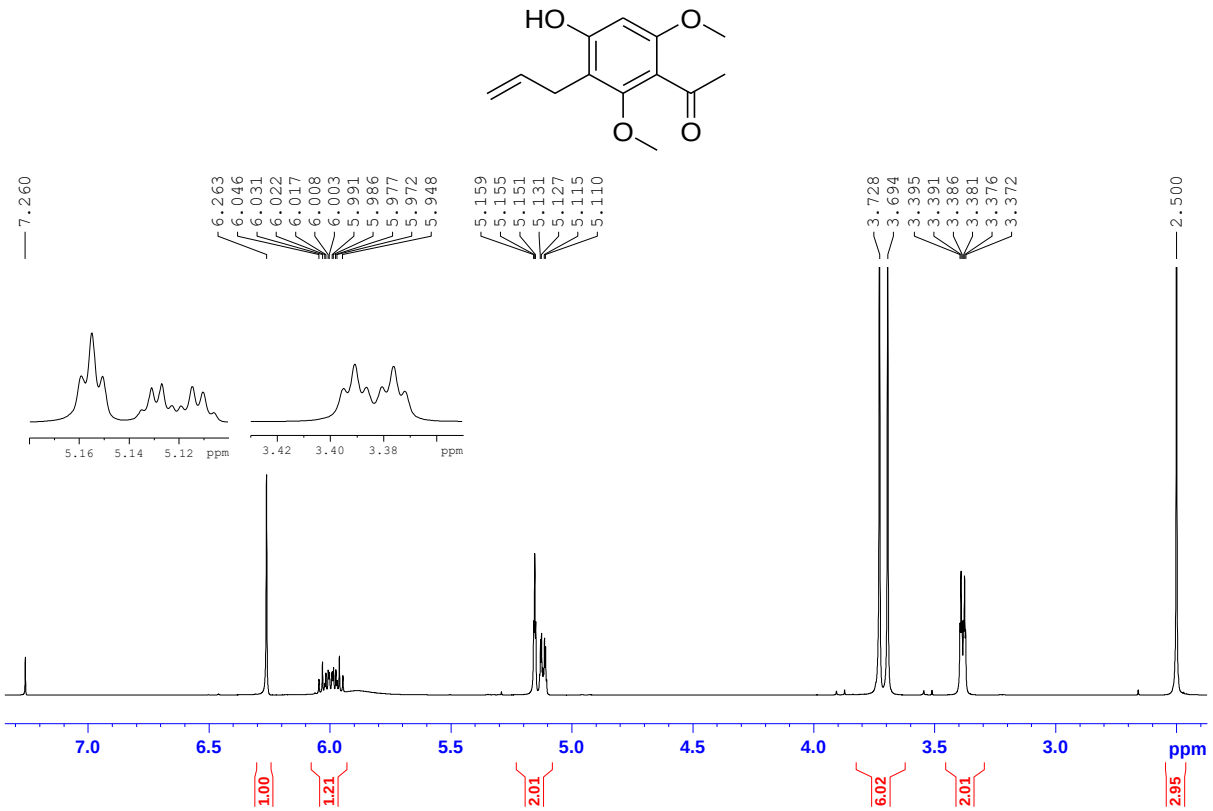


Plate 30b. ^{13}C NMR spectrum of 1-(3-allyl-4-hydroxy-2,6-dimethoxyphenyl)ethanone (**122**) in $CDCl_3$

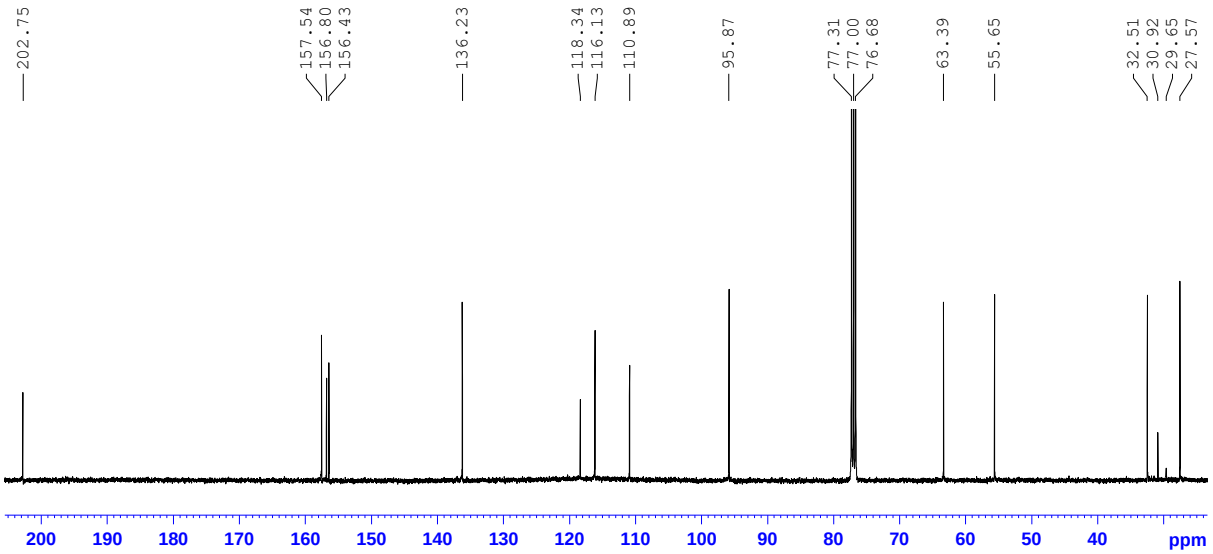


Plate 31a. ^{1}H NMR spectrum of 1-(4-{2"-[(*tert*-butyldiphenylsiloxy)methyl]allyloxy}-3-allyl-2,6-dimethoxyphenyl)ethanone (**123**) in $CDCl_3$

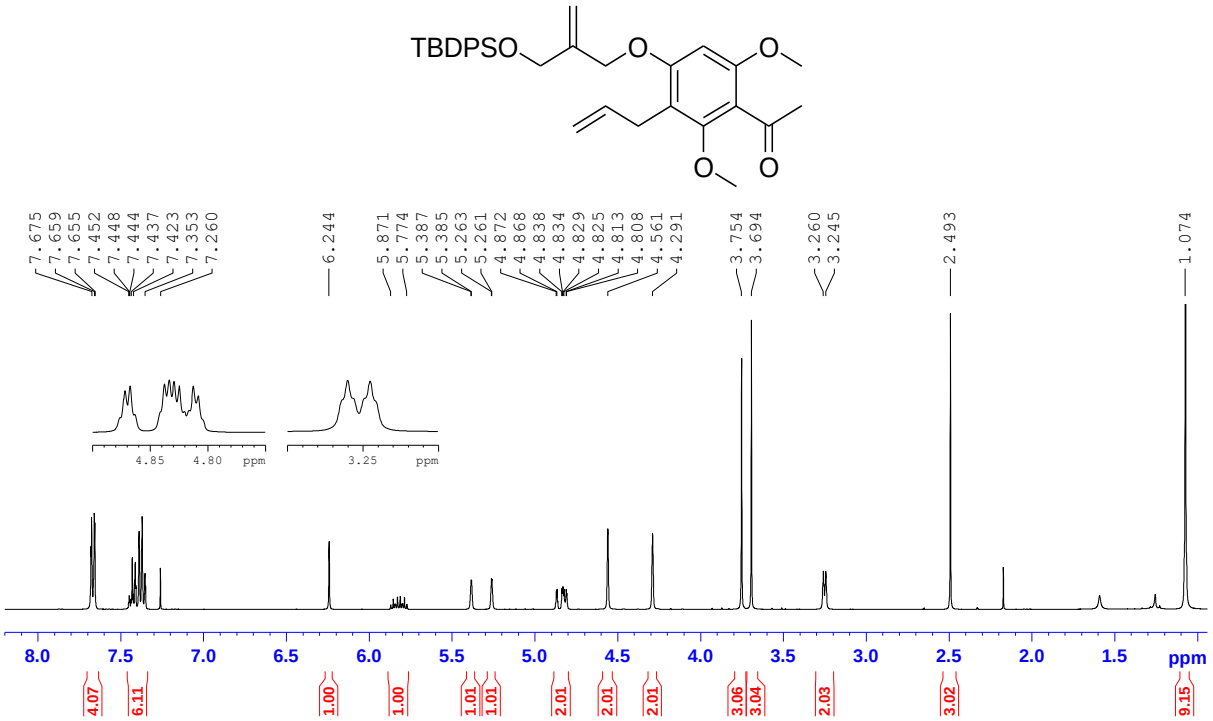


Plate 31b. ^{13}C NMR spectrum of 1-(4-{2"-[(*tert*-butyldiphenylsiloxy)methyl]allyloxy}-3-allyl-2,6-dimethoxyphenyl)ethanone (**123**) in $CDCl_3$

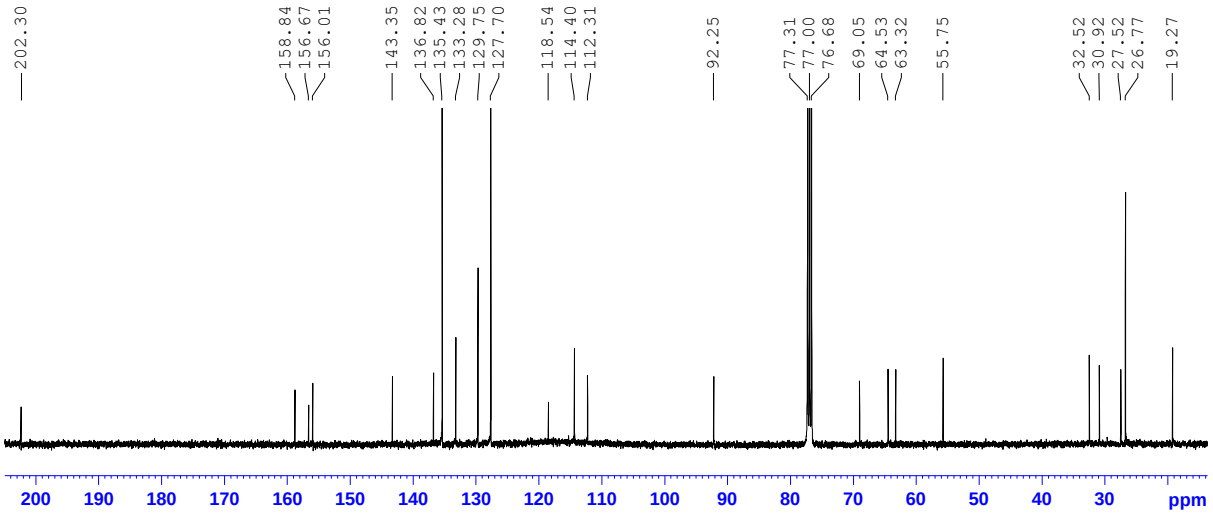


Plate 32a. ^{1}H NMR spectrum of1-(2,3-dihydro-3-hydroxy-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone (**125**) in $CDCl_3$

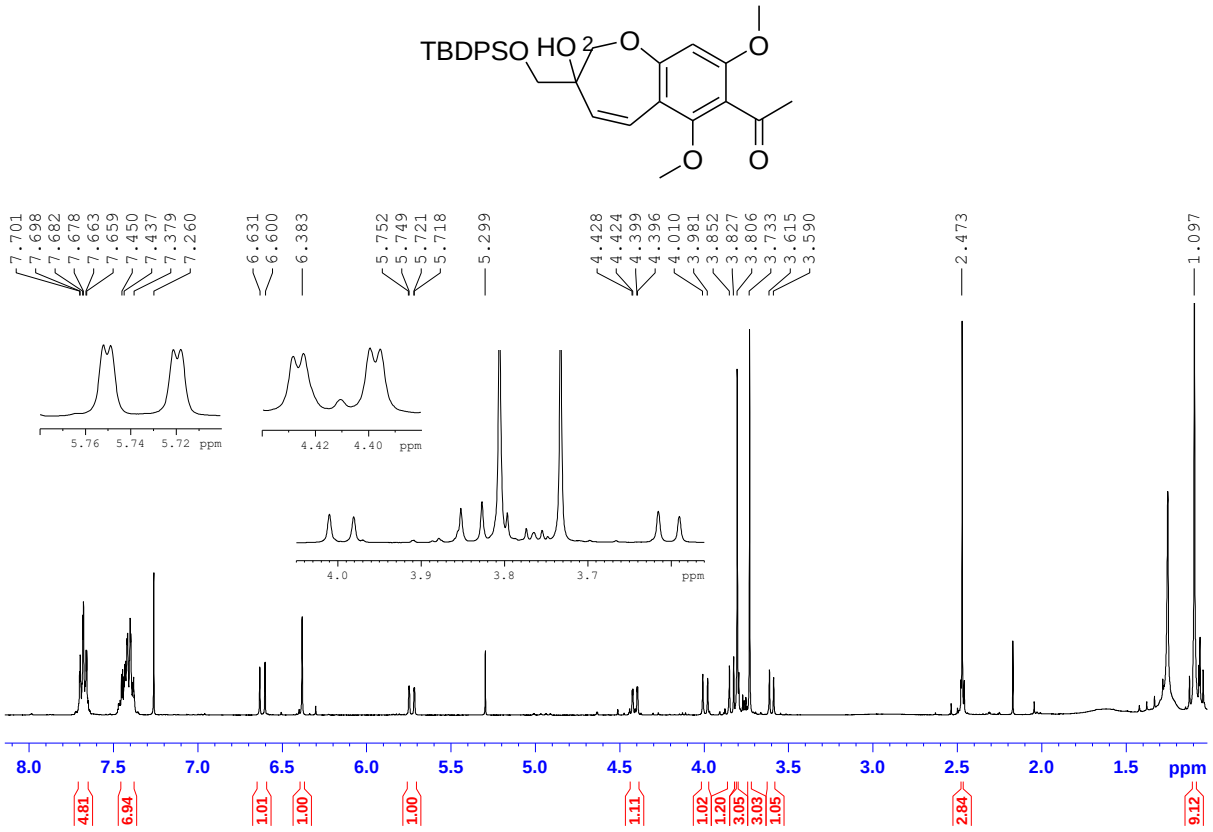


Plate 32b. ^{13}C NMR spectrum of1-(2,3-dihydro-3-hydroxy-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone (**125**) in $CDCl_3$

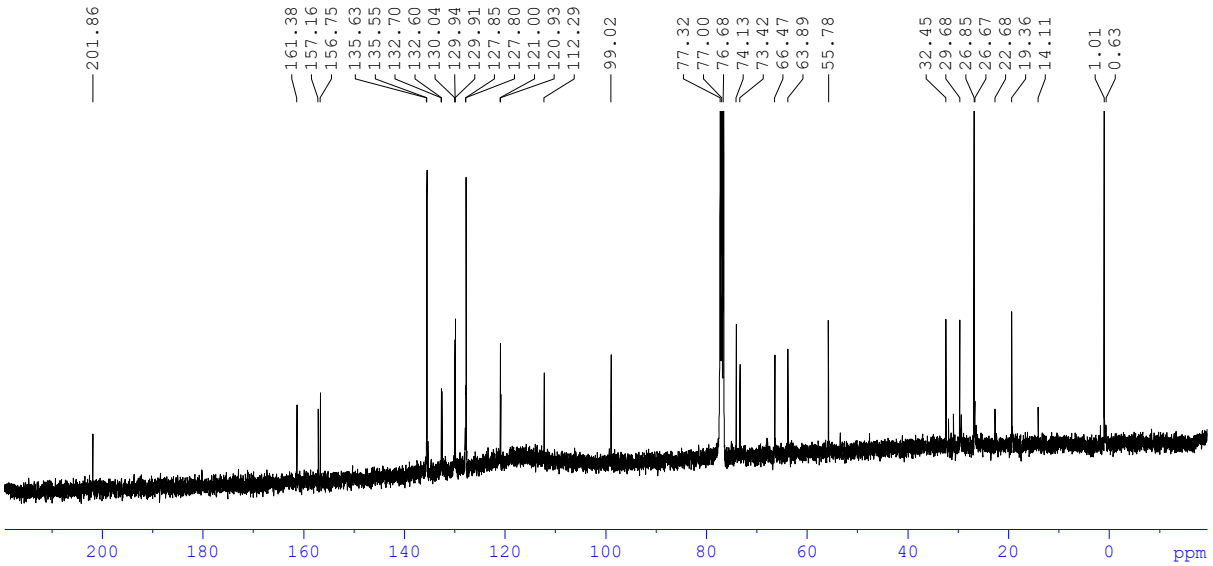


Plate 33a. ^{1}H NMR spectrum of 1-(2,5-dihydro-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone (**124**) in $CDCl_3$

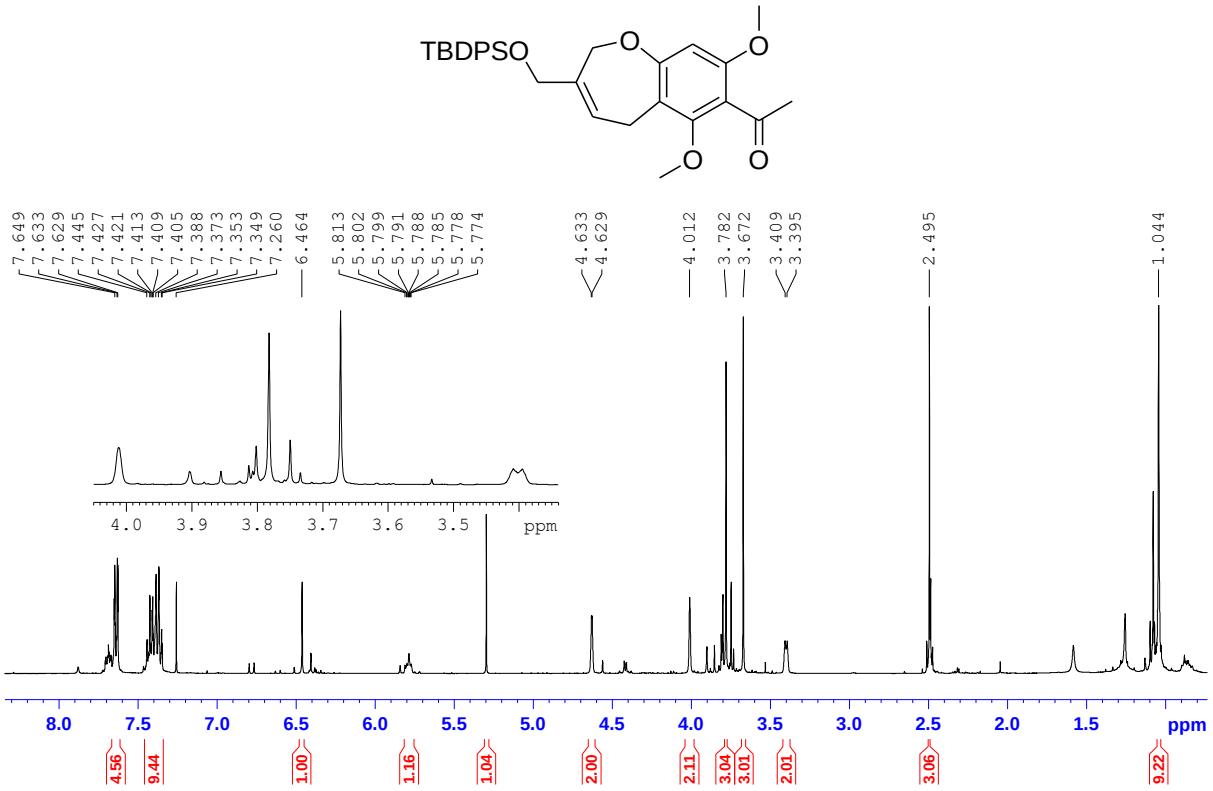


Plate 33b. ^{13}C NMR spectrum of 1-(2,5-dihydro-3-(*tert*-butyldiphenylsiloxymethyl)-6,8-dimethoxybenzo[*b*]oxepin-7-yl)ethanone (**124**) in $CDCl_3$

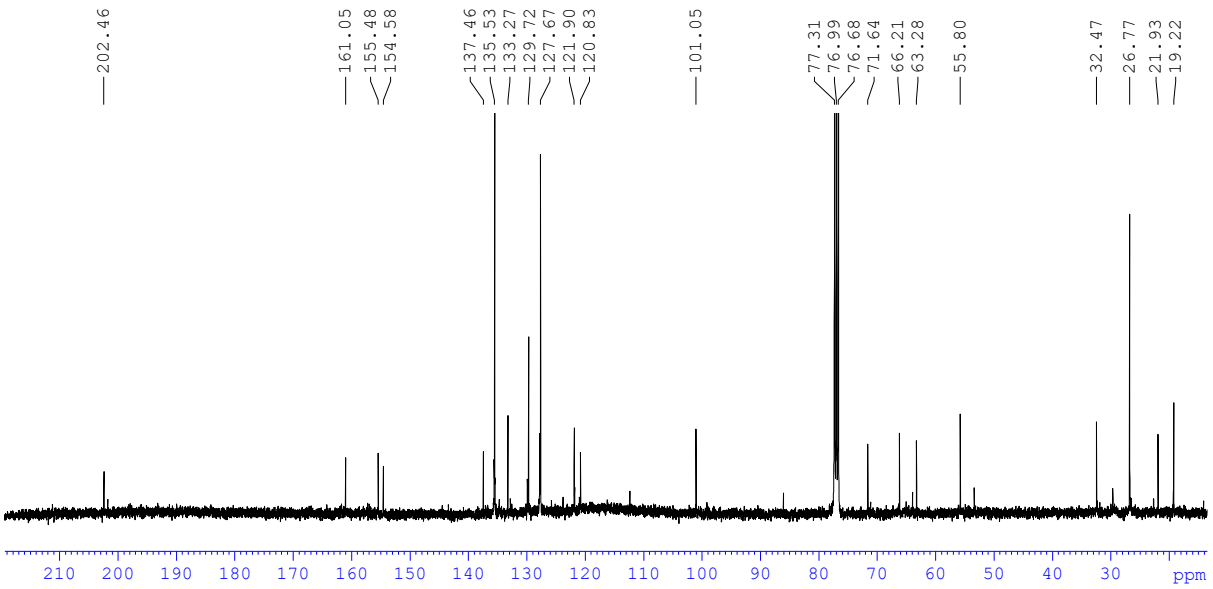
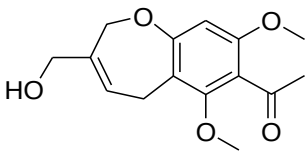


Plate 34a. ^{1}H NMR spectrum of 1-(2,5-dihydro-3-(hydroxymethyl)-6,8-dimethoxybenzo[b]oxepin-7-yl) ethanone (**126**) in $CDCl_3$



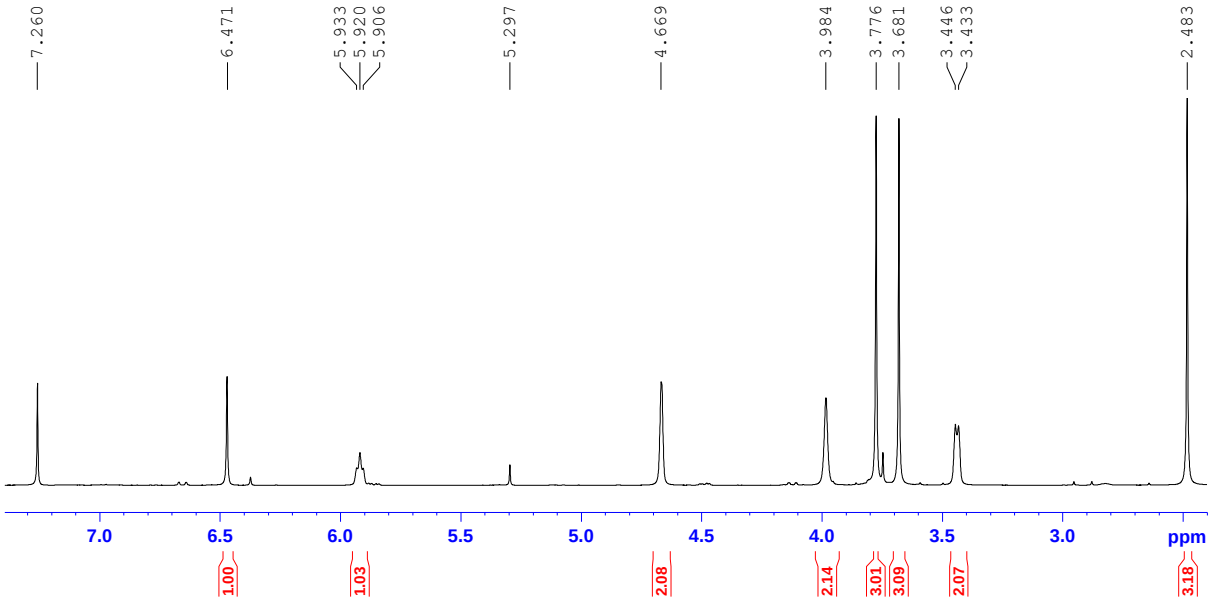


Plate 34b. ^{13}C NMR spectrum of 1-(2,5-dihydro-3-(hydroxymethyl)-6,8-dimethoxybenzo[b]oxepin-7-yl) ethanone (**126**) in $CDCl_3$

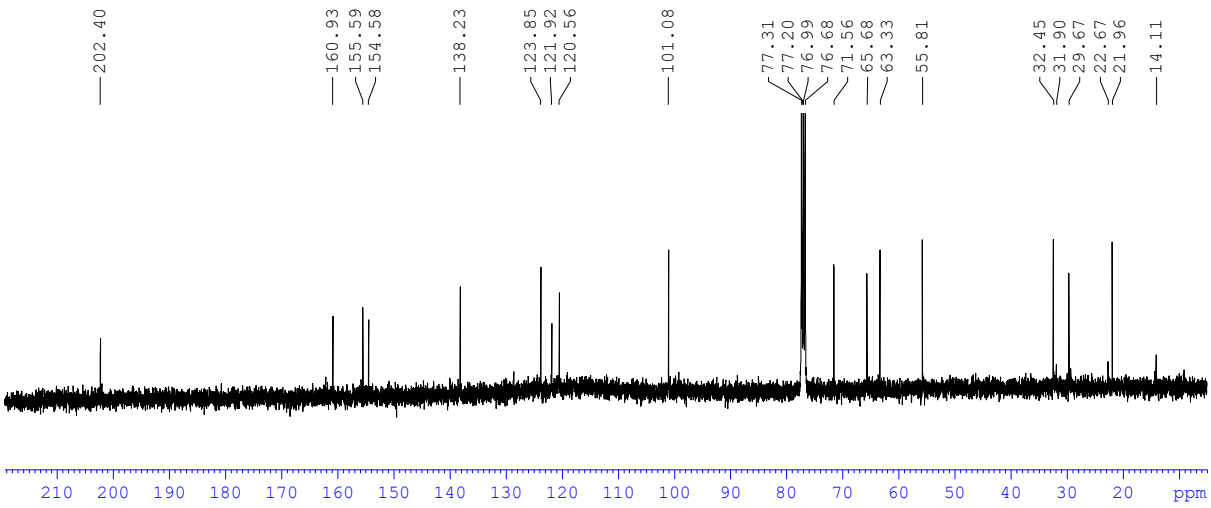


Plate 35a. ^{1}H NMR spectrum of 8-allyl-5-hydroxy-3',3'-dimethylpyrano[7,6]chromone (**129**) in $CDCl_3$

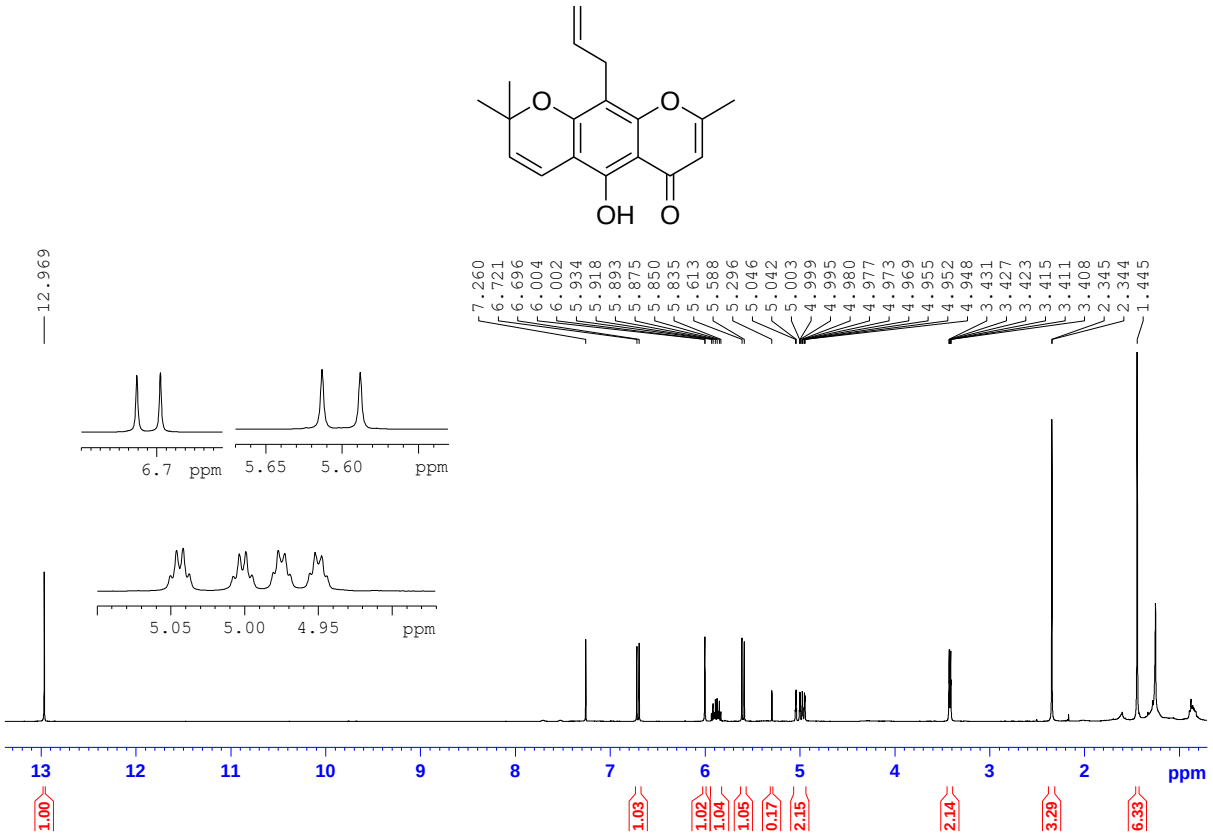


Plate 35b. ^{13}C NMR spectrum of 8-allyl-5-hydroxy-3',3'-dimethylpyrano[7,6]chromone (**129**) in $CDCl_3$

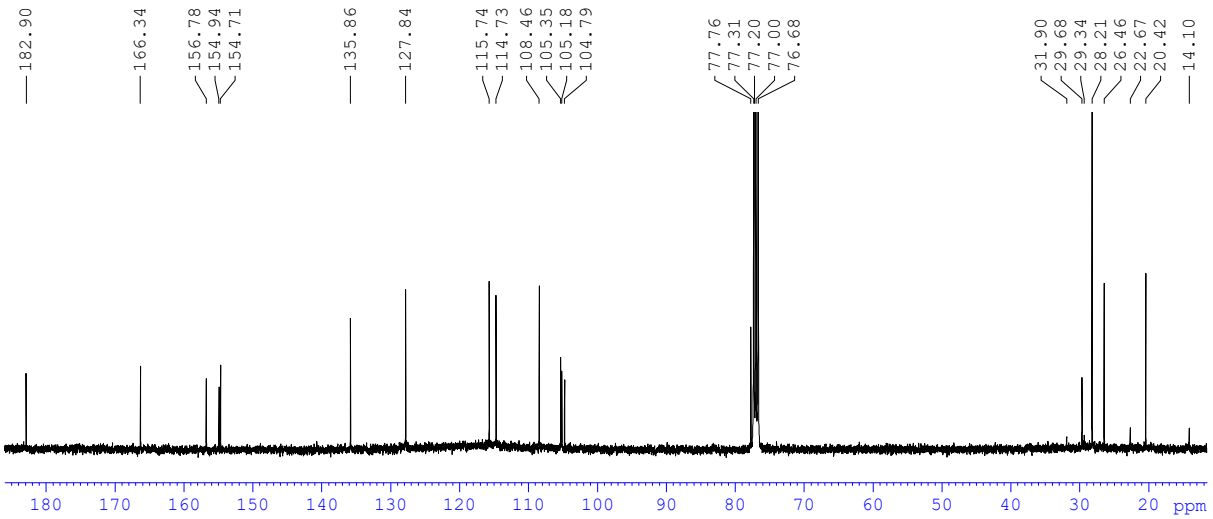


Plate 36a. ^{1}H NMR spectrum of 1-(8-allyl-5-hydroxy-7-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)ethanone (**130**) in $CDCl_3$

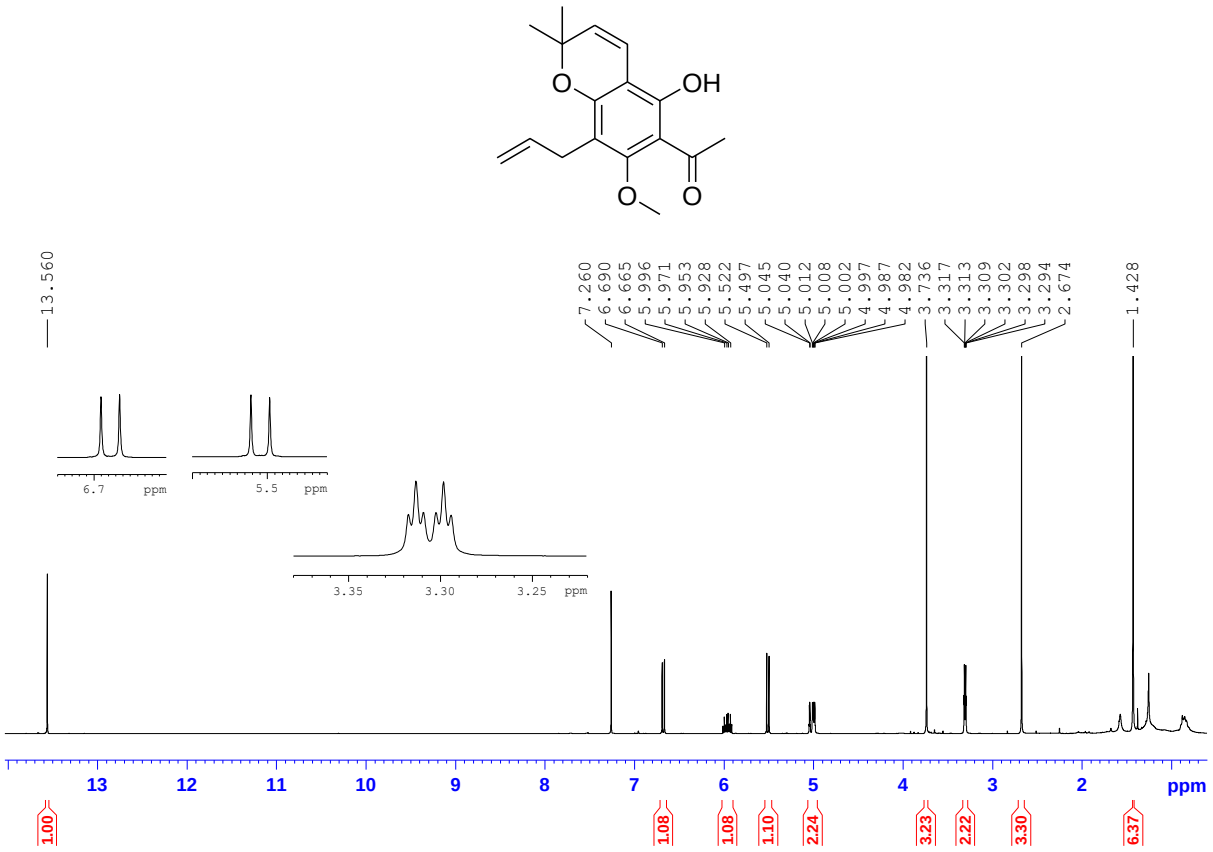
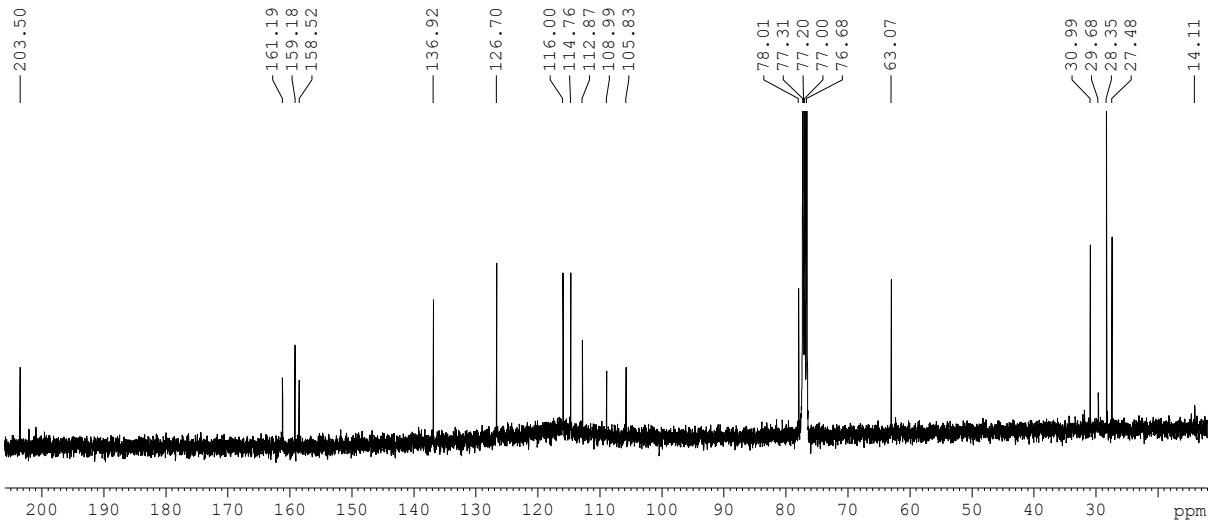


Plate 36b. ^{13}C NMR spectrum of 1-(8-allyl-5-hydroxy-7-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)ethanone (**130**) in $CDCl_3$



Appendix II: Copy of published manuscript

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Article

Synthesis and Antifungal Activity of Chromones and Benzoxepines from the Leaves of *Ptaeroxylon obliquum*

Modibo S. Malefo, Thanyani E. Ramadwa, Ibukun M. Famuyide, Lyndy J. McGaw, Jacobus N. Eloff, Molahlehi S. Sonopo, and Mamoalosi A. Selepe*

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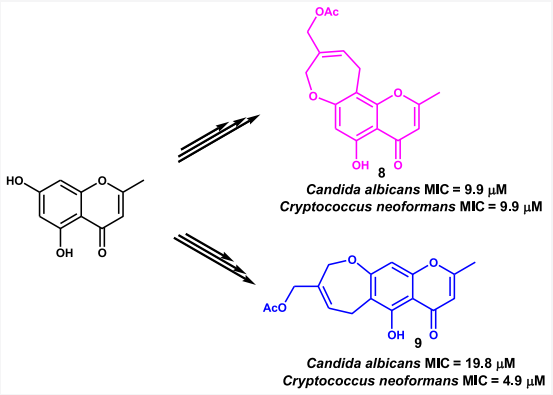
ABSTRACT: This study reports the first total synthesis of the bioactive oxepinochromones 12-*O*-acetyleranthin (**8**) (angular isomer) and 12-*O*-acetylptaeroxylinol (**9**) (linear isomer). The antifungal activity of these compounds and their derivatives was determined against *Candida albicans* and *Cryptococcus neoformans*. Most compounds had good selectivity between the two fungi and showed moderate to good activity. 12-*O*-Acetyleranthin (**8**) had the highest activity against *C. albicans*, with an MIC value of 9.9 μM, while 12-*O*-acetylptaeroxylinol (**9**), the compound present in *Ptaeroxylon obliquum*, had the highest activity against *C. neoformans*, with an MIC value of 4.9 μM.



Infections caused by pathogenic or opportunistic fungi are difficult to cure and are considered a great health threat to humankind worldwide,[1] mainly due to the continuous spread of invasive fungal infections and the associated high mortality rate, which surpasses one and a half million annually.[1−4] These infections and deaths mostly affect people with underlying conditions such as cancer or HIV/AIDS.[5] Species of *Cryptococcus*, *Candida*, *Aspergillus*, *Mucormycosis*, and *Pneumocystis* are at the forefront of invasive fungal infections.[6,7] Although there are a number of agents available that can be used to treat fungal infections, only a few are approved for clinical use. The antifungals that are currently used are divided into four classes on the basis of their targets and mechanism of action: the polyenes, e.g., amphotericin B (**1**), the azoles, e.g., fluconazole (**2**), the echinocandins, e.g., caspofungin (**3**), and the pyrimidine analogues, e.g., olorofim (**4**) (Figure 1).[1−4,6−8]

The azoles have been used as the first-line treatment for candidiasis infection for many years.[9,10] However, very recently there have been reports that show that candidiasis is showing reduced susceptibility toward the azoles.[11] This led to the use of the echinocandins as the mainstay treatment for invasive *Candida* infections, particularly to patients with compromised immune systems since they display favorable tolerability and a reduced toxicity profile.[12] However, some candidiasis-causing species such as *Candida albicans*, *C. glabrata*, and *C. parapsilosis* are resistant to these drugs when used for a prolonged duration, and they can only be administered intravenously.[6,13,14] Owing to the high mortality rate, high costs, and the rapid development of drug resistance of fungal pathogens against the current antifungal treatment, there is an urgent need for the development of new drugs with new mechanisms of action, reduced toxicity profiles, and reduced susceptibility to drug resistance.[3,15,16] Current investigational antifungal agents with different skeletons from the existing ones include a triterpenoid analogue, ibrexafungerp (**5**), derived from enfumafungin (Figure 1).[17,18] The two triterpenoids act as specific inhibitors of glucan synthesis.[17,19]

One of the most frequently explored classes of natural products is the chromones. Chromone scaffolds are found in many compounds with a variety of biological activities including antifungal, anti-inflammatory, antiallergenic, antiviral, antitubulin, antihypertensive, antimalarial, neuroprotective, and anti-HIV activity.[16,20−22] The benzoxepinochromones represent a rare class of chromone compounds that were first isolated by Dean et al. from the sneezewood tree in 1966.[23] Few compounds carrying the benzoxepinochromone architecture have been isolated since, mostly from the species *Ptaeroxylon obliquum* (Rutaceae) (Thunb.) Radlk. and *Eranthus hiemalis* (Ranunculaceae) Salisb. The isolated benzoxepinochromones from *P. obliquum* include desokarenin,

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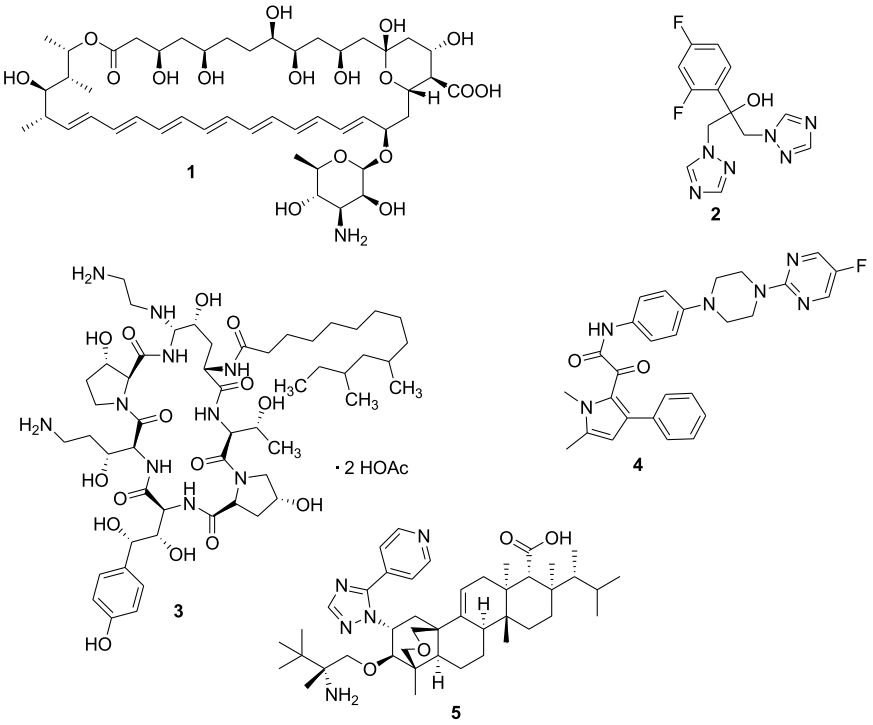
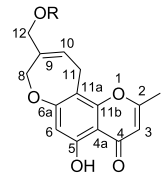
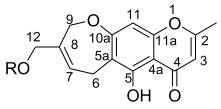


Figure 1. Structures of the current commonly used antifungal drugs and the investigational triterpenoid ibrexafungerp (**5**).

karenin, ptaeroxylinol (**7**), 12-*O*-acetylptaeroxylinol (**9**), and ptaeroglycol.[23−27] Those isolated from *E. hiemalis* include eranthin (**6**) (Figure 2), its glucoside, and eranthinglycol.[28,29]



6 R = H, Eranthin



7 R = H, Ptaeroxylinol

8 R = Ac, 12-*O*-Acetyleranthin

9 R = Ac, 12-*O*-Acetylptaeroxylinol

Figure 2. Structures of oxepinochromones **6**, **7**, **8**, and **9**.

Important to note is that the benzoxepinochromones isolated from *P. obliquum* are mostly linear isomers, while those isolated from *E. hiemalis* are angular isomers.

Continuation of the work on *P. obliquum* led to the isolation of the *O*-acetyl derivative of eranthin **8**, named obliquumol, and other known terpenoids.[26,27] The isolated compounds were tested against various fungal species. Obliquumol emerged as the most potent antifungal compound as reported by Van Wyk and colleagues.[26,27,30] Therefore, the oxepinochromone moiety could represent a new scaffold of antifungal leads.[27] Inspired by the high potency of obliquumol against several fungal strains, including the *Candida* species, it was decided to prepare the natural oxepinochromones and their derivatives in order to provide a sufficient amount of material for further evaluation of their antifungal properties and establish the scaffolds responsible for the antifungal activity.

RESULTS AND DISCUSSION

The synthesis of the two oxepinochromones 12-*O*-acetyleranthin (**8**) and 12-*O*-acetylptaeroxylinol (**9**) was undertaken as shown in Scheme 1 following the reported procedure by Bruder et al.[31] The key intermediate noreugenin (**11**) was prepared from phloroacetophenone (**10**) via the Kostanecki−Robinson reaction on a gram scale (2−4 g). Compound **11** was allylated with allyl bromide and subsequently subjected to Claisen rearrangement conditions at 220 °C in *N*,*N*-diethylaniline to give a mixture of regioisomers **12** and **13** in a ratio of 11:2. Purification by column chromatography gave 8-allyl isomer **12** in a 33% yield and 6-allyl isomer **13** in a 6% yield. The structures of the regioisomers **12** and **13** were confirmed by 1D and 2D NMR data analysis. Other conditions that were tested for the Claisen rearrangement such as using DMF as a solvent or conducting the reaction in a microwave oven or neat still led to lower yields of 8- and 6-allyl isomers **12** and **13**. Having successfully prepared regioisomers **12** and **13**, access to the total synthesis of 12-*O*-acetylptaeroxylinol (**9**) and 12-*O*-acetyleranthin (**8**) could be achieved rapidly. Thus, the 7-*O*-alkylation of **12** and **13** with 3-bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene followed by ring-closing metathesis (RCM) with the Grubbs second-generation (Grubbs II) catalyst in dry CH_2Cl_2 and subsequent desilylation with TBAF afforded the requisite eranthin (**6**) and ptaeroxylinol (**7**), respectively.[31] Finally, acetylation of **6** and **7** afforded 12-*O*-acetyleranthin (**8**) and 12-*O*-acetylptaeroxylinol (**9**) in 22% and 35% yields, respectively.

Since both the angular and linear oxepinochromones **8** and **9** were previously reported from *P. obliquum*,[24,26,27,32] distinction between the two natural compounds can be a challenge and may lead to misassignment due to the similarities of the two structures. Therefore, it was important to characterize the two synthesized compounds using different analytical methods that include HRMS and 1D and 2D NMR spectroscopy and use the data as reference for confirming the structures of the isolated compounds unequivocally. The molecular formula of compound **8** was determined to be $C_{17}H_{16}O_6$ on the basis of the HRESIMS (*m*/*z* 317.1052 $[M + H]^+$; calcd for $C_{17}H_{17}O_6$, 317.1020) and the ^{13}C NMR data. The ^1H NMR data (Table

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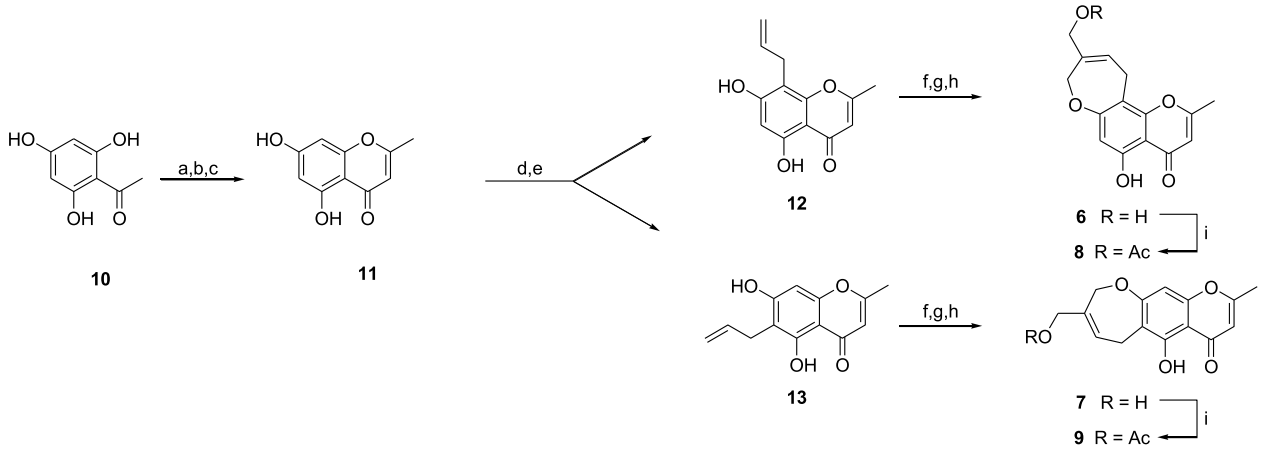
Scheme 1. Total Synthesis of 12-*O*-Acetyleranthin (8) and 12-*O*-Acetylptaeroxylinol (9)[a]



[a]Reagents and conditions: (a) Py, Ac_2O, 90% (**10a**); (b) K_2CO_3, CH_3CN, heat; (c) H_2O, K_2CO_3, CH_3OH, reflux, 61% (**11**) over 2 steps; (d) allyl bromide, K_2CO_3, DMF, 63% (**11a**); (e) *N*,*N*-diethylaniline, reflux, 33% (**12**) and 6% (**13**); (f) 3-bromo-2-(*tert*-butyldiphenylsiloxy)-methylpropene, K_2CO_3, DMF, 42% for 8-allyl (**12a**) and 70% for 6-allyl (**13a**); (g) Grubb's II, CH_2Cl_2, 40 °C; (h) TBAF, THF, 0 °C, 65% (**6**) and 40% (**7**) over 2 steps; AcCl, K_2CO_3, CH_2Cl_2, 35% (**8**) and 22% (**9**).

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of the Synthesized Compounds 6 and 8 and Comparison with Obliquumol in $CDCl_3$.

	6		8		obliquumol[26,27]	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2		166.8		166.8		167.14
3	6.05, s	108.7	6.06, s	108.8	5.99, q (0.7)	108.66
4		183.1		183.1		182.68
4a		107.0		107.2		106.72
5		160.1		160.3		155.79
6	6.46, s	104.6	6.49, s	104.7	6.47, s	99.29
6a		164.4		164.3		164.39
8	4.72, s	70.8	4.65, d (1.4)	71.0	4.61, tt (1.6, 1.6)	71.04
9		138.5		133.8		133.23
10	5.99, t (5.3)	124.2	6.05−6.03, m	127.4	6.02, tt (5.5, 1.2)	128.30
11	3.61, d (5.3)	21.4	3.63, d (5.5)	21.6	3.52, d (5.6, 1.2, 1.6)	21.14
11a		111.0		111.4		115.82
11b		153.8		153.7		158.06
12	4.03, s	65.4	4.46, s	66.5	4.41, brs	66.45
13				170.7		170.61
14			2.05, s	20.9	2.01, s	20.42
2-Me	2.38, s	20.5	2.38, s	20.5	2.34, d (0.7)	20.78
HO-5	12.61, s		12.66, s		12.94, brs	

1) displayed three one-proton singlets at δ_H 12.66 (HO-5) (representing the hydrogen-bonded hydroxy group), δ_H 6.49 (H-6) in the aromatic region, and δ_H 6.06 (H-3) characteristic of a 2-substituted benzopyran-4-one moiety, while the signal at δ_H 6.05−6.03 (H-10, m) indicated the presence of a vinylic proton. The ^{1}H NMR data also indicated the presence of two sets of oxymethylene protons at δ_H 4.65 (d, *J* = 1.4, H-8) and δ_H 4.46 (s, H-12); an allylic methylene group at δ_H 3.63 (d, *J* = 5.5, H-11); a methyl group at δ_H 2.38 (3H, s, 2-Me); and a signal for the methyl of the acetoxy group at δ_H 2.05 (3H, s, $CO_2C\underline{H}_3$). Seventeen signals were observed in the ^{13}C NMR spectrum and allocated by means of HSQC, HMBC, and DEPT-135 NMR data (Table 1), with the signal of the ketocarbonyl carbon appearing at δ_C 183.1 and that of the acetoxy carbonyl appearing at δ_C 170.7. The six aromatic carbon signals consisted of three C−O signals at δ_C 164.3, 160.3, and 153.7; two tertiary carbons at δ_C 111.4 and 107.2, and an aromatic CH signal at δ_C 104.7. The oxygenated olefinic carbon at δ_C 166.8 and the olefinic carbon at δ_C 108.8 were indicative of the presence of the chromone moiety. The signals at δ_C 71.0 and 66.5 were indicative of the presence of the oxymethylene carbons, and the signal at δ_C 21.6 indicated the allylic methylene group. The signals at δ_C 133.8 and 127.4 were indicative of a vinylic group, and those at δ_C 20.9 and 20.5 indicated the presence of two methyl groups. Analysis of the COSY spectrum showed correlations of H-10/H_2-11. The HMBC spectrum indicated the H-6 (δ_H 6.49)/C-4a, C-5, C-6a, and C-11a correlations as illustrated in Figure 3. The additional HMBC correlations between H_2-11 (δ_H 3.63)/C-6a, C-9, C-10, C-11a, and C-11b confirmed the presence of the oxepine ring. This was further corroborated by the correlation of H-10 (δ_H 6.05−6.03)/C-8, C-9, and C-11a. These correlations imply that the oxepine ring is attached at C-11a, consistent with the angular structure of **8**. On the other hand,

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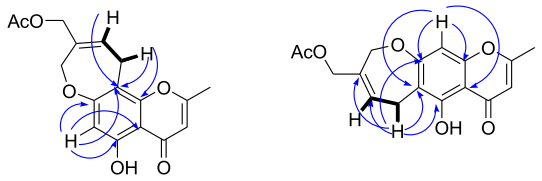

Figure 3. Key correlations, HMBC (blue arrow) and COSY (thick black line), observed for compounds **8** and **9**.

H-3 (δ_H 6.06, s) correlated to Me-2, indicating that the methyl group was linked to C-2 of the benzopyran system. The presence of the acetyl group was identified by the strong HMBC correlation between H_2-12 and the ester carbonyl carbon at δ_C 170.7.

Similarly, the molecular formula for compound **9** was identical to that of compound **8** from the HRESIMS (317.1052 $[M + H]^+$; calcd for $C_{17}H_{17}O_6$, 317.1020) and ^{13}C NMR data (Table 2). The NMR data of compound **9** were similar to those of compound **8**, except for the deshielded signal at δ_H 6.54 in the aromatic region. This signal (6.54 ppm) was assigned to H-11 based on its long-range HMBC correlations to C-10a, C-11a, C-5a, and C-4a as illustrated in Figure 3. The correlation of H_2-6 (δ_H 3.59) to C-5, C-8, C-7, and C-5a confirmed the presence of the oxepine ring and its attachment at C-5a. The data of the synthesized compound **9** were consistent with those of the natural compound 12-*O*-acetylptaeroxylinol (**9**) reported by Agostinho et al., except for a few signals that were interchanged.[24]

Comparison of the ^1H and ^{13}C NMR data of the isolated natural compound obliquumol with those of the synthesized 12-*O*-acetyleranthin (**8**) showed some inconsistencies (Table 1). There was a clear deviation of the C-6, C-11a, and C-11b carbon signals. These resonances for the synthesized **8** occurred at δ_C 104.7, 111.4, and 153.7 and for the natural product obliquumol at δ_C 99.29, 115.82, and 158.06, respectively.[26,27] The carbon signals for the natural product obliquumol compared rather favorably with those of the synthesized compound **9** (Table 2) at δ_C 99.4, 116.0, and 158.2, representing C-11, C-5a, and C-5, respectively. These findings suggest the structure of obliquumol correlated with that of the linear isomer 12-*O*-acetylptaeroxylinol (**9**) and not the angular isomer 12-*O*-acetyleranthin (**8**). Although eranthin (**6**) has been isolated and synthesized previously,[28,31] it is noteworthy that its *O*-acetyl derivative **8** has not previously been synthesized nor isolated.

Having successfully synthesized the angular and linear oxepinochromones **6**, **7**, **8**, and **9**, the next task was to prepare derivatives of these compounds in order to evaluate their biological activities and determine the scaffolds responsible for the antifungal activities. First, the benzoxepine **22**, devoid of the chromone moiety, was prepared. As shown in Scheme 2, the synthesis of **22** began with the selective protection of **10** with MOMCl to give 2-hydroxy-4,6-dimethoxymethoxyacetophenone (**14**) in 50% isolated yield.[33] The allylation of **14** with allyl bromide via the Williamson ether synthesis proceeded smoothly to give the allyl ether **15** in a 79% yield, which set the stage for the Claisen rearrangement reaction. Upon thermally heating the allyl ether **15** at 220 °C in *N*,*N*-diethylaniline for 24 h, compound **16** was obtained in 50% yield. Treatment of **16** with CH_3I in the presence of K_2CO_3 generated **17** in 98% yield, which was followed by the deprotection with 3 M HCl/CH_3OH (1:10) to give phenol **18** in 79% yield. Methylation of phenol **18** afforded dimethoxyacetophenone **19** in 89% yield, followed by the removal of the MOM protecting group with 3 M HCl/CH_3OH (1:5) to afford **20** in 81% yield. Treating compound **20** with 3-bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene afforded the fully protected **21** in 59% yield.[31] Subjecting compound **21** to RCM with Grubbs II catalyst, followed by removal of the TBDPS group with TBAF, gave the benzoxepine **22** in 32% yield.[31] It is worth mentioning that attempts to purify the product formed after RCM were hampered by instability when the mixture was exposed to silica gel and air; hence it was

Table 2. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) Data of the Synthesized Compounds 7 and 9 and Comparison with the Isolated 12-*O*-Acetylptaeroxylinol in $CDCl_3$[a]

	7		9		12-*O*-acetylptaeroxylinol[24]	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
2		167.2		167.1		167.2
3	6.04, s	108.7	6.05, brs	108.8	6.07, s	108.8
4		182.8		182.8		182.5
4a		106.7		106.9		114.1
5		158.1		158.2		158.2
5a		115.8		116.0		133.2[b]
6	3.57, d (5.6)	21.1	3.59, d (5.5)	21.2	3.60, d (5.5)	21.3
7	6.00, td (5.6, 1.1)	125.4	6.05−6.08, m	128.4	6.09, t (5.5)	128.4
8		138.0		133.2		116.1[b]
9	4.74, d (1.1)	71.0	4.65, d (1.5)	71.2	4.48,[b] s	66.1[c]
10a		164.6		164.5		167.2
11	6.52, s	99.3	6.54, s	99.4	6.56, s	99.4
11a		155.9		155.9		154.3
12	4.03, s	65.7	4.46, s	66.6	4.67,[b] s	71.2[c]
13				170.7		170.8
14			2.05 s	20.9	2.08 s	20.9
2-Me	2.35, s	20.5	2.36, s	20.5	2.38, s	20.6
HO-5	13.08, s		13.08, s		13.10, s	

[a−c]Signals with the same supercripted b and c letters in the same column are interchangeable.

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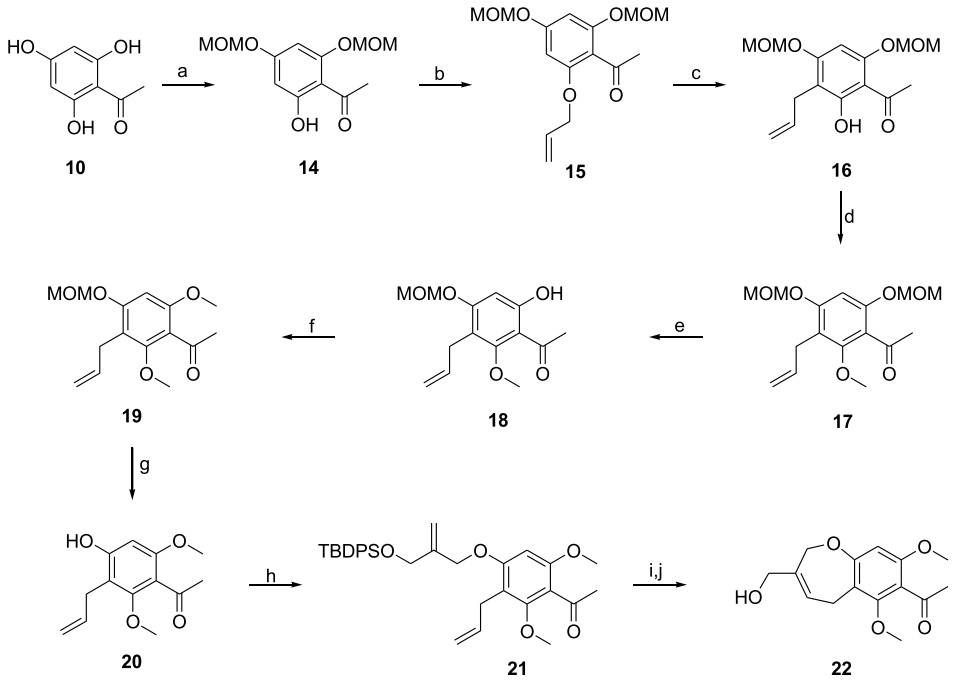
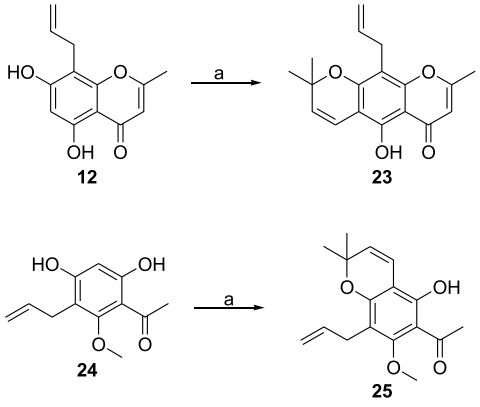
Scheme 2. Synthesis of Benzoxepine 22[a]



[a]Reagents and conditions: (a) $ZnBr_2$, dimethoxymethane (DMM), AcCl, DIPEA, CH_2Cl_2, 50%; (b) allyl bromide, K_2CO_3, DMF, 79%; (c) *N*,*N*-diethylaniline, 220 °C, 50%; (d) MeI, K_2CO_3, DMF, 150 °C, 98%; (e) 3 M HCl/CH_3OH (1:10), reflux, 79%; (f) MeI, K_2CO_3, DMF, 150 °C, 89%; (g) 3 M HCl/CH_3OH (1:10), reflux, 81%; (h) 3-bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene, K_2CO_3, DMF, 59%; (i) Grubb's II, CH_2Cl_2, 40 °C; (j) TBAF, THF, 0 °C, 32%.

desilylated without any further purification. Derivatives **23** and **25** were prepared as shown in Scheme 3, by reacting compounds **12** and **24** with 3-methylbut-2-enal (prenal) under $Ca(OH)_2$, respectively.[33]

Scheme 3. Synthesis of Benzopyrans 23 and 25[a]



[a]Reagents and conditions: (a) 3-methylbut-2-enal, $Ca(OH)_2$, 10% for **23** and 44% for **25**.

The synthesized compounds were tested in vitro against the fungi *Candida albicans* and *Cryptococcus neoformans*. The minimum inhibitory concentrations (MICs, μM) of all tested compounds are collated in Table 3. In this study amphotericin B was used as a positive control. Of the nine screened compounds, compound **25** was least potent against both fungi, and compound **23** was ineffective against *C. albicans*. Benzoxepinochromone **8** was the most active compound against *C. albicans* with an MIC value of 9.9 μM, while its

Table 3. MIC (μM) Values of *Ptaeroxylon obliquum* Derivatives against *Candida albicans* and *Cryptococcus neoformans* Incubated for Different Times

	MIC (μM)			
	Candida albicans		*Cryptococcus neoformans*	
compound	24 h	48 h	24 h	48 h
6	11.4	11.4	5.7	5.7
7	11.4	11.4	5.7	5.7
8	9.9	9.9	9.9	9.9
9	19.8	19.8	4.9	4.9
11	16.3	16.3	16.3	16.3
12	13.5	13.5	13.5	13.5
22	11.2	22.5	11.2	11.2
23	41.9	83.8	10.5	10.5
25	86.7	86.7	21.7	43.3
Amp-B	0.42	0.84	0.42	0.42

regioisomer **9** had the best activity against *C. neoformans* with an MIC value of 4.9 μM. Interestingly, oxepinochromone **9** showed selectivity between the *C. albicans* and *C. neoformans* fungi (Table 3). This selectivity may be attributed to the position of the oxepine ring. However, oxepinochromone **8** showed equipotency across the strains, thus failing to show selectivity between the two strains. Given the results, it was decided to explore other benzoxepinochromones (**6** and **7**) devoid of the acetoxy group. Removing the acetyl group in **8** and **9** was found to slightly decrease the activity as seen in compounds **6** and **7** (Table 3). Both compounds **6** and **7** showed equipotent activity (11.4 μM) for *C. albicans* and *C. neoformans* (5.7 μM). The benzoxepine **22** lacking the chromone moiety also had activity comparable to those of **6** and **7** against *C. albicans*, which indicates the importance of the

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oxepine moiety for the antifungal activity of the compounds. However, the compounds with a chromone scaffold, **11** and **12**, lacking the oxepine moiety, showed low potency. This trend was also observed with the compounds carrying the dimethylpyran moiety, **23** and **25**, which had lower activity against both fungal genera. In most cases the activity appears to be fungicidal and not fungistatic.

In conclusion, the present work resulted in the total synthesis of 12-*O*-acetylptaeroxylinol (**9**) and the new compound 12-*O*-acetyleranthin (**8**). These compounds were synthesized in a seven-step reaction sequence, involving Kostanecki−Robinson reaction, Williamson etherification, Claisen rearrangement, ring-closing metathesis, desilylation, and acetylation. The structure of the antifungal compound obliquumol occurring naturally in *Ptaeroxylon obliquum* that was previously assigned as 12-*O*-acetyleranthin (**8**) is corrected to 12-*O*-acetylptaeroxylinol (**9**). The oxepinochromones **8** and **9** and their derivatives were tested for antifungal activity against *C. albicans* and *C. neoformans*. Most tested compounds had good to moderate activity against these pathogens. Compounds carrying the oxepine moiety had good activity and selectivity between the two fungal genera, while those with the dimethylpyran ring were less potent. Therefore, the benzoxepine compounds are earmarked for further development.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions that require anhydrous conditions were carried out under argon in oven-dried glassware. Reagent grade solvents and chemicals used for syntheses were purchased from Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa) or Merck Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa) and were used without further purification. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminum plates and were observed under UV light (254 nm). The plates were developed in vanillin/$KMnO_4$ stain followed by heating. Preparative TLC was carried out on Merck silica gel glass plates and visualized under UV light (254 nm). Column chromatographic purifications were carried out on silica gel (230−400 mesh) using the eluent system detailed for each procedure. The synthesized compounds were analyzed by IR spectroscopy, mass spectrometry (MS), and NMR spectroscopy. The IR spectra were recorded on an Alpha Bruker Optics FT-IR spectrometer, and all data were reported in wavenumbers. HRMS data were acquired on a Waters UPLC coupled to QTOF Synapt G2 spectrometry using electrospray ionization in the positive or negative mode. The NMR spectra were recorded at rt on a Bruker Avance III 300 or 400 MHz spectrometer. ^{1}H and ^{13}C NMR chemical shifts were referenced to residual protonated solvents peaks: δ_H 7.26, δ_C 77.0 for $CDCl_3$; δ_H 2.50, δ_C 39.5 for DMSO-d_6. Chemical shifts are reported in ppm (δ), and spin−spin coupling constants (J) in hertz (Hz). The multiplicities of ^{1}H and ^{13}C resonances are expressed by the following abbreviations: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), dt (doublet of triplet), quartet (q), m (multiplet). 1D NMR (^{1}H, ^{13}C, and DEPT-135) and 2D NMR (HSQC, HMBC, NOESY, and COSY) spectra were used for the complete assignment of NMR signals.

1-[2,4,6-Tris(acetyloxy)phenyl]ethanone (10a). To a stirred solution of phloroacetophenone (**10**) (3.00 g, 16.1 mmol, 1.0 equiv) in pyridine (6.0 mL, 74 mmol, 4.6 equiv) at rt was added Ac_2O (6.40 mL, 68.8 mmol, 4.3 equiv), and the reaction mixture was stirred for 4 h. The product was precipitated by adding crushed ice into the reaction mixture. The precipitate was filtered and washed with ice cold H_2O. The white solid (4.30 g, 90%, 14.6 mmol) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (2H, s, H-3, H-5), 2.46 (3H, s, $ArCOC\underline{H}_3$), 2.28 (3H, s, $OCOC\underline{H}_3$), 2.27 (6H, s, $OCOC\underline{H}_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.6 (C, $ArC\underline{O}CH_3$), 168.3 (2 × C, $OC\underline{O}CH_3$), 168.2 (C, $OC\underline{O}CH_3$) 151.7 (C, C-4), 148.2 (2 × C, C-2, C-6), 125.0 (C, C-1), 114.1 (2 × CH, C-3, C-5), 31.2 (CH_3, $ArCOC\underline{H}_3$), 21.1 (CH_3, $OCOC\underline{H}_3$), 21.0 (2 × CH_3, $OCOC\underline{H}_3$); HRMSESI m/z $[M + Na]^+$ 317.0637 (calculated for $C_{14}H_{14}O_7$ Na 317.0632).

5,7-Dihydroxy-2-methyl-4*H*-chromen-4-one, Noreugenin (11). To a solution of 2,4,6-triacetoxyacetophenone (**10a**) (8.58 g, 29.2 mmol, 1.0 equiv) in CH_3CN (35 mL) was added K_2CO_3 (20.0 g, 144.7 mmol, 4.9 equiv), and the reaction mixture was refluxed for 2 h. Upon complete consumption of the starting material, a 1:1 mixture of CH_3OH and a saturated aqueous K_2CO_3 solution (20 mL) was added, and the mixture was heated at reflux for 3 h. After the mixture had cooled, it was acidified with 3 M HCl, and the precipitate that formed was filtered and washed with cold 3 M HCl to give the product **11** as a beige solid (3.53 g, 18.4 mmol, 61%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (1H, s, HO-5), 10.81 (1H, s, HO-7), 6.32 (1H, d, J = 2 Hz, H-8), 6.17 (2H, s, H-6, H-3), 2.34 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.7 (C, C-4), 167.7 (C, C-2), 164.1 (C, C-7), 161.5 (C, C-5), 157.8 (C, C-8a), 107.9 (CH, C-3), 103.4 (C, C-4a), 98.7 (CH, C-6), 93.7 (CH, C-8), 19.9 (CH_3, CH_3-2); HRMSESI m/z $[M + H]^+$ 193.0517 (calculated for $C_{10}H_9O_4$ 193.0495). All spectroscopic data were in agreement with the reported data.[31]

7-Allyloxy-5-hydroxy-2-methyl-4*H*-chromen-4-one (11a). To a solution of noreugenin (**11**) (1.60 g, 8.52 mmol, 1.0 equiv) in DMF (26 mL) was added K_2CO_3 (1.80 g, 12.8 mmol, 1.5 equiv). The mixture was stirred for 10 min under argon, allyl bromide (0.81 mL, 9.37 mmol, 1.1 equiv) was added, and the mixture was stirred at rt for 3 h. Upon complete consumption of material, the mixture was filtered and washed with EtOAc (25 mL). The filtrate was washed with saturated NH_4Cl solution and extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography using hexanes/EtOAc (8:2) to afford compound **11a** as a light yellow solid (1.20 g, 5.34 mmol, 63%, R_f = 0.4); IR ν_{max} 1739, 1664, 1624, 1368, 1342, 1203, 1167 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (1 H, d, J = 2.2 Hz, H-8), 6.34 (1 H, d, J = 2.2 Hz, H-6), 6.08−5.98 (1 H, m, H-2′), 6.02 (1 H, s, H- 3), 5.42 (1 H, dd, J = 17.3 and 1.2 Hz, H-3′a), 5.33 (1H, dd, J = 10.5 and 1.2 Hz, H-3′b), 4.58 (2H, d, J = 5.3 Hz, H-1′), 2.28 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4 (C, C-4), 166.8 (C, C-2), 164.2 (C, C-7), 162.1 (C, C-5), 158.0 (C, C-8a), 132.1 (CH, C-2′), 118.4 (CH_2, C-3′), 108.8 (CH, C-3), 105.3 (C, C-4a), 98.5 (CH, C-6), 93.1 (CH, C-8), 69.2 (CH_2, C-1′), 20.5 (CH_3, CH_3-2); HRMSESI m/z $[M + H]^+$ 233.0848 (calculated for $C_{13}H_{13}O_4$ 233.0808). All spectroscopic data were in agreement with the reported data.[31]

8-Allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (12). The allyl ether **11a** (1.0 g, 4.3 mmol, 1 equiv) was refluxed in *N*,*N*-diethylaniline (5 mL) at 220 °C for 12 h. After cooling the reaction mixture to rt, EtOAc (30 mL) was added and the mixture was washed with 1 M HCl (10 mL). A saturated NH_4Cl solution (30 mL) was added, and the product was extracted with EtOAc (3 × 30 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was filtered through a short pad of silica gel using hexanes/EtOAc (9:1) to remove the excess *N*,*N*-diethylaniline, and the solvent was evaporated in vacuo. The product was purified by silica gel column chromatography using hexanes/EtOAc (8:2) as the eluent to afford **12** (0.32 g, 1.4 mmol, 32%, R_f = 0.44) as a yellow solid and **13** (60 mg, 0.27 mmol, 6.19%, R_f = 0.29) as a yellow oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (1H, s, HO-5), 10.78 (1H, s, HO-7), 6.23 (1H, s, H-6), 6.15 (1H, s, H-3), 5.91−5.82 (1H, m, H-2′), 4.98−4.92 (2H, m, H-3′), 3.34 (2H, d, J = 6.3 Hz, H-1′), 2.34 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.1 (C, C-4), 167.5 (C, C-2), 161.6 (C, C-7), 159.4 (C, C-5), 155.2 (C, C-8a), 135.8 (CH, C-2′), 114.8 (CH_2, C-3′), 107.7 (CH, C-3), 103.9 (C, C-8), 103.3 (C, C-4a), 98.2 (CH, C-6), 26.2 (CH_2, C-1′), 19.9 (CH_3, CH_3-2); HRMS-ESI m/z $[M + H]^+$ 233.0819 (calculated for $C_{13}H_{13}O_4$ 233.0808).

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6-Allyl-5,7-dihydroxy-2-methyl-4*H*-chromen-4-one (13). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.08 (1H, s, HO-5), 10.81 (1H, s, HO-7), 6.40 (1H, s, H-8), 6.15 (1H, s, H-3), 5.91−5.81 (1H, m, H-2′), 4.95−4.90 (2H, m, H-3′), 3.25 (2H, d, J = 6.1 Hz, H-1′), 2.34 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.8 (C, C-4), 167.4 (C, C-2), 161.9 (C, C-7), 158.6 (C, C-5), 155.8 (C, C-8a), 135.7 (CH, C-2′), 114.6 (CH_2, C-3′), 108.9 (C, C-6), 107.8 (CH, C-3), 103.1 (C, C-4a), 92.9 (CH, C-8), 26.0 (CH_2, C-1′), 19.9 (CH_3, CH_3-2); HRMSESI m/z [M + H]$^+$ 233.0819 (calculated for $C_{13}H_{13}O_4$ 233.0808). All spectroscopic data were in agreement with the reported data.[31]

7-{2″-[(*tert*-Butyldiphenylsiloxy)methyl]allyloxy}-8-allyl-5-hydroxy-2-methyl-4H-chromen-4-one (12a). To a solution of allyl ether **12** (0.30 g, 1.3 mmol, 1.0 equiv) in DMF (10 mL) was added K_2CO_3 (0.27 g, 1.9 mmol, 1.5 equiv), and the mixture was stirred for 15 min at rt under argon. 3-Bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene (0.65 g, 1.7 mmol, 1.3 equiv) was added to the mixture, which was then stirred at rt for 5 h. The reaction mixture was filtered, diluted with EtOAc (20 mL), and washed with a saturated NH_4Cl solution (20 mL). The mixture was extracted with EtOAc (3 × 20 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The mixture was purified by PTLC using hexanes/EtOAc (9:1) to afford the target compound **12a** (0.29 g, 0.54 mmol, 42%, R_f = 0.48) as a colorless oil: IR ν_{max} 2933, 2859, 1741, 1659, 1467, 1422, 1382, 1325, 1268, 1237, 1193, 1109, 820, 743, 704, 613, 502 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.79 (1H, brs, HO-5), 7.68−7.66 (4H, m, Si<u>Ar</u>$_2$), 7.44−7.36 (6H, m, Si<u>Ar</u>$_2$), 6.34 (1H, s, H-6), 6.02 (1H, s, H-3), 5.82−5.72 (1H, m, H-2′), 5.38 (1H, brs, H-3″a), 5.26 (1H, d, J = 1.2 Hz, H-3″b), 4.90−4.83 (2H, m, H-3′), 4.59 (2H, s, H-1″), 4.28 (2H, s, H-4″), 3.37 (2H, d, J = 6.2 Hz, H-1′), 2.35 (3H, s, CH_3-2), 1.07 (9H, s, (<u>CH</u>$_3$)$_3$CSi); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9 (C, C-4), 166.8 (C, C-2), 161.6 (C, C-7), 160.7 (C, C-5), 154.9 (C, C-8a), 142.7 (C, C-2″), 135.6 (CH, C-2′), 135.5 (4 × CH, Si<u>Ar</u>$_2$), 133.2 (2 × C, Si<u>Ar</u>$_2$), 129.7 (2 × CH, Si<u>Ar</u>$_2$), 127.7 (4 × CH, Si<u>Ar</u>$_2$), 114.6 (CH_2, C-3′), 112.8 (CH_2, C-3″), 108.3 (CH, C-3), 105.9 (C, C-8), 104.7 (C, C-4a), 95.8 (CH, C-6), 69.2 (CH_2, C-1″), 64.4 (CH_2, C-4″), 29.7 (3 × CH_3, (<u>CH</u>$_3$)$_3$CSi), 26.6 (CH_2, C-1′), 20.5 (CH_3, CH_3-2), 19.2 (C, (CH$_3$)$_3$<u>C</u>Si); HRMSESI m/z [M + H]$^+$ 541.2401 (calculated for $C_{33}H_{37}O_5Si$ 541.2404). All spectroscopic data were in agreement with the reported data.[31]

5-Hydroxy-9-hydroxymethyl-2-methyl-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-4-one, Eranthin (6). To a solution of diene **12a** (0.29 g, 0.54 mmol, 1.0 equiv) in dry CH_2Cl_2 (30 mL) was added [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]-(tricyclohexylphosphine) benzylideneruthenium(IV) dichloride (Grubbs' II catalyst) (0.05 g, 0.06 mmol, 0.1 equiv) in one portion under argon. The reaction mixture was heated at 45 °C for 2 h, and the solvent was evaporated after cooling. The residue was dissolved in THF (5 mL), and a TBAF solution (1 M in THF; 0.59 mL, 0.59 mmol, 1.1 equiv) was added dropwise at 0 °C under argon. After stirring the reaction mixture for 1 h at 5 °C, EtOAc (20 mL) was added, followed by a saturated aqueous NH_4Cl solution (20 mL). The mixture was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The resulting oil was purified by silica gel column chromatography using hexanes/EtOAc (3:7) as the eluent to afford **6** (95.0 mg, 0.346 mmol, 65%, R_f = 0.4) as a green solid: IR ν_{max} 3446, 2923, 2853, 1655, 1615, 1586, 1486, 1414, 1265, 1180, 1158, 1101, 1073, 1024, 846, 640, 554 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.61 (1H, s, OH-5), 6.45 (1H, s, H-6), 6.04 (1H, s, H-3), 5.99 (1H, t, J = 5.3 Hz, H-10), 4.72 (2H, s, H-8), 4.03 (2H, s, H-12), 3.61 (2H, d, J = 5.3 Hz, H-11), 2.38 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1 (C, C-4), 166.8 (C, C-2), 164.4 (C, C-6a), 160.1 (C, C-5), 153.8 (C, C-11b), 138.5 (C, C-9), 124.2 (CH, C-10), 111.0 (C, C-11a), 108.7 (CH, C-3), 107.0 (C, C-4a), 104.6 (CH, C-6), 70.8 (CH_2, C-8), 65.4 (CH_2, C-12), 21.4 (CH_2, C-11), 20.5 (CH_3, CH_3-2); HRMSESI m/z [M + H]$^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$ 275.0921). All spectroscopic data were in agreement with the reported data.[31]

(5-Hydroxy-2-methyl-4-oxo-8,11-dihydro-4*H*-oxepino[2,3-*h*]chromen-9-yl)methyl acetate, 12-*O*-Acetyleranthin (8). K_2CO_3 (0.088 g, 0.28 mmol, 3.0 equiv) was added to a stirred mixture of eranthin **6** (0.025 g, 0.092 mmol, 1.0 equiv) and AcCl (6.6 μL, 0.092 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) under an argon atmosphere. The reaction mixture was stirred at rt for 4 h. A saturated NH_4Cl solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by PTLC using hexanes/EtOAc (3:7) to give **8** (10.0 mg, 35%, 0.0316 mmol, R_f = 0.58) as white solid: IR ν_{max} 3463, 3018, 2968, 1740, 1662, 1434, 1369, 1222, 1103, 900, 782, 518 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.66 (1H, s, HO-5), 6.49 (1H, s, H-6), 6.06 (1H, s H-3), 6.06−6.03 (1 H, m, H-10), 4.65 (2H, d, J = 1.4 Hz, H-8), 4.46 (2H, s, H-12), 3.63 (2H, d, J = 5.5 Hz, H-11), 2.38 (3H, s, CH_3-2), 2.05 (3H, s, CO<u>C</u>H$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1 (C, C-4), 170.7 (C, <u>C</u>OCH$_3$), 166.8 (C, C-2), 164.3 (C, C-6a), 160.3 (C, C-5), 153.7 (C, C-11b), 133.8 (C, C-9), 127.4 (CH, C-10), 111.4 (C, C-11a), 108.8 (CH, C-3), 107.2 (C, C-4a), 104.7 (CH, C-6), 71.0 (CH_2, C-8), 66.5 (CH_2, C-12), 21.6 (CH_2, C-11), 20.9 (CH_3, CO<u>CH</u>$_3$), 20.5 (CH_3, CH_3-2); HRMSESI m/z [M + H]$^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1020)

7-{2″-[(*tert*-Butyldiphenylsiloxy)methyl]allyloxy}-6-allyl-5-hydroxy-2-methyl-4H-chromen-4-one (13a). Compound **13a** was obtained as a colorless oil (0.029 g, 0.54 mmol, 70%, R_f = 0.56) after purification with PTLC using hexanes/EtOAc (9:1) from the treatment of allyl ether **13** (0.18 g, 0.76 mmol, 1.0 equiv) with 3-bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene (0.39 g, 0.92 mmol, 1.2 equiv) in the presence of K_2CO_3 (0.160 g, 1.14 mmol, 1.5 equiv) in DMF (5 mL) following the procedure for synthesizing **12a** above. IR ν_{max} 2928, 2857, 1740, 1660, 1451, 1374, 1343, 1267, 1229, 1204, 1109, 819, 706 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68−7.66 (4H, m, Si<u>Ar</u>$_2$), 7.43−7.36 (6H, m, Si<u>Ar</u>$_2$), 6.37 (1H, s, H-8), 6.03 (1H, s, H-3), 5.87−5.81 (1H, m, H-2′), 5.36 (1H, s, H-3″a), 5.26 (1H, s, H-3″b), 4.92 (1H, dd, J = 17.1 and 1.6 Hz, H-3′a), 4.85 (1H, dd, J = 10.0 and 1.6 Hz, H-3′b), 4.61 (2H, s, H-1″), 4.28 (2H, s, H-4″), 3.34 (2H, d, J = 6.1 Hz, H-1′), 2.33 (3H, s, CH_3-2), 1.08 (9H, s, (<u>CH</u>$_3$)$_3$CSi); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.4 (C, C-4), 166.4 (C, C-2), 161.9 (C, C-7), 158.6 (C, C-5), 156.6 (C, C-8a), 142.7 (C, C-2″), 135.7 (CH, C-2′), 135.5 (4 × CH, Si<u>Ar</u>$_2$), 133.2 (2 × C, Si<u>Ar</u>$_2$), 129.8 (2 × CH, Si<u>Ar</u>$_2$), 127.7 (4 × CH, Si<u>Ar</u>$_2$), 114.5 (CH_2, C-3′), 112.9 (CH_2, C-3″), 111.1 (C, C-6), 108.8 (CH, C-3), 105.2 (C, C-4a), 90.4 (CH, C-8), 68.9 (CH_2, C-1″), 64.6 (CH_2, C-4″), 26.8 (3 × CH_3, (<u>CH</u>$_3$)$_3$CSi), 26.4 (CH_2, C-1′), 20.4 (CH_3, CH_3-2), 19.3 (C, (CH$_3$)$_3$<u>C</u>Si); HRMSESI m/z [M + H]$^+$ 541.2401 (calculated for $C_{33}H_{37}O_5Si$ 541.2404). All spectroscopic data were in agreement with the reported data.[31]

5-Hydroxy-8-hydroxymethyl-2-methyl-6,9-dihydro-4*H*-oxepino[3,2-*g*]chromen-4-one, Ptaeroxylinol (7). The treatment of diene **13a** (0.28 g, 0.51 mmol, 1.0 equiv) with Grubbs II catalyst (0.045 g, 0.053 mmol, 0.1 equiv) in dry CH_2Cl_2 (30 mL), followed by TBAF solution (1 M in THF; 0.56 mL, 0.56 mmol, 1.1 equiv) in THF (5 mL), afforded the target compound **7** (0.056 g, 0.20 mmol, 40%, R_f = 0.18) as light green crystals, after purification via silica gel column chromatography using hexanes/EtOAc (3:7) as the eluent while following the procedure for **6** above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.08 (1H, s, HO-5), 6.52 (1H, s, H-11), 6.04 (1H, s, H-3), 6.00 (1H, tt, J = 5.6, 1.1 Hz, H-7), 4.74 (2H, d, J = 1.1 Hz, H-9), 4.03 (2H, s, H-12), 3.57 (2H, d, J = 5.6 Hz, H-6), 2.35 (3H, s, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.8 (C, C-4), 167.2 (C, C-2), 164.6 (C, C-10a), 158.1 (C, C-5), 155.9 (C, C-11a), 138.0 (C, C-8), 125.4 (CH, C-7), 115.8 (C, C-5a), 108.7 (CH, C-3), 106.7 (C, C-4a), 99.3 (CH, C-11), 71.0 (CH_2, C-9), 65.7 (CH_2, C-12), 21.1 (CH_2, C-6), 20.5 (CH_3, CH_3-2); HRMSESI m/z [M + H]$^+$ 275.0898 (calculated for $C_{15}H_{15}O_5$ 275.0921). All spectroscopic data were in agreement with the reported data.[31]

(5-Hydroxy-2-methyl-4-oxo-6,9-dihydro-4*H*-oxepino[3,2-*g*]-chromen-8-yl)methyl acetate, 12-*O*-Acetylptaeroxylinol (9). The treatment of **7** (40 mg, 0.15 mmol, 1.0 equiv) with K_2CO_3 (40 mg, 0.29 mmol, 1.9 equiv) and AcCl (10 μL, 0.15 mmol, 1.0 equiv) in

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CH_2Cl_2 (10 mL) under an argon atmosphere afforded oxepinochromone **9** (10.0 mg, 23%, 0.0345 mmol, R_f = 0.68) as colorless crystals after purification by PTLC using hexanes/EtOAc (3:7) as the eluent while following the procedure for **8** above. IR ν_{max} 3467, 2924, 2853, 1739, 1650, 1619, 1484, 1447, 1398, 1327, 1235, 1169, 1110, 1083, 1037, 966, 845, 796, 578, 544, 505 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 13.08 (1H, s, HO-5), 6.54 (1H, s, H-11), 6.08−6.05 (1H, m, H-7), 6.05 (1H, brs, H-3), 4.65 (2H, d, J = 1.5 Hz, H-9), 4.46 (2H, s, H-12), 3.59 (2H, d, J = 5.5 Hz, H-6), 2.36 (3H, s, CH_3-2), 2.05 (3H, s, CO$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.8 (C, C-4), 170.7 (C, $\underline{C}OCH_3$), 167.2 (C, C-2), 164.5 (C, C-10a), 158.2 (C, C-5), 155.9 (C, C-11a), 133.2 (C, C-8), 128.4 (CH, C-7), 116.0 (C, C-5a), 108.8 (CH, C-3), 106.9 (C, C-4a), 99.4 (CH, C-11), 71.2 (CH_2, C-9), 66.6 (CH_2, C-12), 21.2 (CH_2, C-6), 20.9 (CH_3, CO$\underline{CH_3}$), 20.5 (CH_3, CH_3-2); HRMSESI m/z $[M + H]^+$ 317.1052 (calculated for $C_{17}H_{17}O_6$ 317.1020).

1-[2-Hydroxy-4,6-bis(methoxymethoxy)phenyl]ethanone (14). $ZnBr_2$ (3.00 mg, 0.0131 mmol, 0.0049 equiv) was stirred in dimethoxymethane (0.59 mL, 6.7 mmol, 2.5 equiv) under an argon atmosphere, and then AcCl (0.47 mL, 6.7 mmol, 2.5 equiv) was added dropwise to the stirred solution. The solution was stirred at rt for an additional 2 h and transferred into an ice-cold solution of the predried phloroacetophenone (**10**) (0.50 g, 2.7 mmol, 1.0 equiv) and DIPEA (0.93 mL, 5.4 mmol, 2.0 equiv) in CH_2Cl_2 (30 mL) under an argon atmosphere. The mixture was stirred for 3 h, diluted with saturated NH_4Cl solution (30 mL), and stirred for an additional 15 min. The two phases were partitioned, and the aqueous phase was extracted with CH_2Cl_2 (5 × 30 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give a yellow oil, which was purified by silica gel column chromatography using hexanes/EtOAc (5:1) to afford **14** (0.35 g, 1.4 mmol, 50%, R_f = 0.84) as a colorless oil that solidified at rt. ^1H NMR (400 MHz, $CDCl_3$) δ 13.71 (1H, s, HO-2), 6.26 (1H, d, J = 2.4 Hz, H-3), 6.24 (1H, d, J = 2.4 Hz, H-5), 5.25 (2H, s, OC$\underline{H_2}$O), 5.17 (2H, s, OC$\underline{H_2}$O), 3.52 (3H, s, OC$\underline{H_3}$), 3.47 (3H, s, OC$\underline{H_3}$), 2.65 (3H, s, ArCOC$\underline{H_3}$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 203.2 (C, Ar$\underline{C}$OCH$_3$), 166.8 (C, C-2), 163.4 (C, C-4), 160.3 (C, C-6), 106.9 (C, C-1), 97.5 (CH, C-3), 94.4 (CH_2, OCH_2O), 94.0 (CH_2, OCH_2O), 94.0 (CH, C-5), 56.7 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 33.0 (CH_3, ArCO$\underline{C}H_3$); HRMSESI m/z $[M + H]^+$ 257.1034 (calculated for $C_{12}H_{17}O_6$ 257.1019). All spectroscopic data were in agreement with the reported data.[33]

1-[2-Allyloxy-4,6-bis(methoxymethoxy)phenyl]ethanone (15). Compound **15** was obtained as a light yellow oil (0.092 g, 3.1 mmol, 79%, R_f = 0.41) after purification by silica gel column chromatography using hexanes/EtOAc (8:2) as eluents from the treatment of 2-hydroxy-4,6-dimethoxymethoxyacetophenone (**14**) (0.10 g, 0.39 mmol, 1.0 equiv) with allyl bromide (0.37 mL, 0.429 mmol, 1.1 equiv) and K_2CO_3 (0.081 g, 0.59 mmol, 1.5 equiv) in DMF (10 mL) following the procedure for preparing **11a**. ^{1} H NMR (400 MHz, $CDCl_3$) δ 6.45 (1H, d, J = 2.0 Hz, H-5), 6.30 (1H, d, J = 2.0 Hz, H-3), 6.03−5.93 (1H, m, H-2′), 5.37−5.33 (1H, m, H-3′a), 5.27−5.26 (1H, m, H-3′b), 5.14 (2H, s, OC$\underline{H_2}$O), 5.13 (2H, s, OC$\underline{H_2}$O), 4.51 (2H, dt, J = 5.2 and 1.5 Hz, H-1′), 3.47 (3H, s, OC$\underline{H_3}$), 3.45 (3H, s, OC$\underline{H_3}$), 2.48 (3H, s, ArCOC$\underline{H_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5 (C, Ar$\underline{C}$OCH$_3$), 159.5 (C, C-4), 156.8 (C, C-2), 155.3 (C, C-6), 132.6 (CH, C-2′), 117.7 (CH_2, C-3′), 116.2 (C, C-1), 96.2 (CH, C-5), 95.0 (CH, C-3), 94.8 (CH_2, O$\underline{C}H_2$O), 94.4 (CH_2, O$\underline{C}H_2$O), 69.3 (CH_2, C-1′), 56.3 (CH_3, O$\underline{C}H_3$), 56.1 (CH_3, O$\underline{C}H_3$), 32.5 (CH_3, ArCO$\underline{C}H_3$); HRMSESI m/z $[M + H]^+$ 297.1338 (calculated for $C_{15}H_{21}O_6$ 297.1332)

1-[3-Allyl-2-hydroxy-4,6-bis(methoxymethoxy)phenyl]-ethanone (16). Compound **16** was obtained as a yellow oil (0.10 g, 0.33 mmol, 50%, R_f = 0.42) after purification by silica gel column chromatography using hexanes/EtOAc (5:1) as eluent from the Claisen rearrangement reaction of **15** (0.20 g, 0.67 mmol, 1.0 equiv) in *N*,*N*-diethylaniline (5 mL) at 220 °C for 12 h while following the procedure for the preparation of **12**. ^1H NMR (400 MHz, $CDCl_3$) δ 13.86 (1H, s, HO-2), 6.39 (1H, s, H-5), 5.99−5.89 (1H, m, H-2′), 5.26 (2H, s, OCH_2O), 5.23 (2H, s, OCH_2O), 5.03−4.93 (2H, m, H-3′), 3.52 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.36 (2H, dt, J = 6.1 and 1.5 Hz, H-1′), 2.66 (3H, s, ArCOC$\underline{H_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.5 (C, Ar$\underline{C}$OCH$_3$), 163.6 (C-4), 163.6 (C, C-2), 160.9 (C, C-4), 159.1 (C, C-6), 136.3 (CH, C-2′), 114.1 (CH_2, C-3′), 109.6 (C, C-3), 106.8 (C-1), 94.5 (CH_2, O$\underline{C}H_2$O), 93.8 (CH_2, O$\underline{C}H_2$O), 91.2 (CH, C-5), 56.7 (CH_3, O$\underline{C}H_3$), 56.4 (CH_3, O$\underline{C}H_3$), 33.2 (CH_3, ArCO$\underline{C}H_3$), 26.5 (CH_2, C-1′). All spectroscopic data were in agreement with the reported data.[34]

1-[3-Allyl-2-methoxy-4,6-bis(methoxymethoxy)phenyl]-ethanone (17). Iodomethane (0.59 mL, 9.50 mmol, 1.3 equiv) was added to a solution of 3-allyl-2-hydroxy-4,6-dimethoxymethoxy-acetophenone (**16**) (2.16 g, 7.30 mmol, 1.0 equiv) and K_2CO_3 (5.05 g, 36.5 mmol, 5 equiv) in DMF (10 mL). The reaction mixture was heated at reflux for 1 h, cooled to rt, and diluted with EtOAc (50 mL) and NH_4Cl solution (50 mL). The mixture was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was purified by silica gel column chromatography using hexanes/EtOAc (10:1) as the eluent to give target compound **17** (1.83 g, 5.90 mmol, 97.7%, R_f = 0.29) as a yellow oil: IR ν_{max} 2939, 1741, 1691, 1594, 1474, 1395, 1314, 1256, 1201, 1147, 1109, 1064, 1040, 997, 920, 832, 557 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.71 (1H, s, H-5), 5.99−5.91 (1H, m, H-2′), 5.18 (2H, s, OCH_2O), 5.14 (2H, s, OCH_2O), 5.01−4.96 (2H, m, H-3′), 3.71 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.46 (3H, s, OCH_3), 3.36 (2H, dt, J = 6.0 and 1.6 Hz, H-1′), 2.51 (3H, s, CO$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.1 (C, $\underline{C}OCH_3$), 157.3 (C, C-4), 156.3 (C, C-2), 153.3 (C, C-6), 136.8 (CH, C-2′), 120.7 (C, C-1), 116.4 (C, C-3), 114.6 (CH_2, C-3′), 97.8 (CH, C-5), 95.0 (CH_2, OCH_2O), 94.3 (CH_2, OCH_2O), 63.4 (CH_3, OCH_3), 56.4 (CH_3, OCH_3), 56.2 (CH_3, OCH_3), 32.6 (CH_3, CO$\underline{CH_3}$), 27.7 (CH_2, C-1′).

1-[3-Allyl-6-hydroxy-2-methoxy-4-(methoxymethoxy)-phenyl]ethanone (18). A solution of compound **17** (1.47 g, 4.73 mmol, 1.0 equiv) in CH_3OH (30 mL), and 3 M HCl (1.5 mL) was heated at 60 °C for 8 h. The reaction was quenched with a saturated NH_4Cl solution (30 mL), and the product was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by silica gel column chromatography using hexanes/EtOAc (8.5:1.5) as the eluent to give target compound **18** (1.00 g, 3.75 mmol, 79%, R_f = 0.58) as a light yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 13.19 (1H, s, HO-6), 6.47 (1H, s, H-5), 6.01−5.94 (1H, m, H-2′), 5.20 (2H, s, OCH_2O), 5.01−4.96 (2H, m, H-3′), 3.74 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 3.36 (2H, d, J = 5.7 Hz, H-1′), 2.69 (3H, s, CO$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.5 (C, $\underline{C}OCH_3$), 164.4 (C, C-6), 161.8 (C, C-4), 161.1 (C, C-2), 136.9 (CH, C-2′), 114.6 (CH_2, C-3′), 114.0 (C, C-3), 109.9 (C, C-1), 98.7 (CH, C-5), 93.9 (CH_2, OCH_2O), 63.1 (CH_3, OCH_3), 56.6 (CH_3, OCH_3), 30.9 (CH_3, CO$\underline{CH_3}$), 27.7 (CH_2, C-1′); HRMSESI m/z $[M + H]^+$ 267.1259 (calculated for $C_{14}H_{19}O_5$ 267.1224).

1-[3-Allyl-2,6-dimethoxy-4-(methoxymethoxy)phenyl]-ethanone (19). A mixture of **18** (1.00 g, 3.77 mmol, 1.0 equiv), K_2CO_3 (2.61 g, 18.8 mmol, 5.0 equiv), and CH_3I (0.31 mL, 4.9 mmol, 1.3 equiv) in DMF (5 mL) was heated at reflux for 1 h. The reaction mixture was cooled to rt and diluted with EtOAc (20 mL) and saturated NH_4Cl solution (30 mL). The organic and aqueous phases were partitioned, and the aqueous phase was extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The product was filtered through a short pad of silica gel using hexanes/EtOAc (8.5:1.5) as the eluent to give target compound **19** (0.94 g, 3.4 mmol, 89%, R_f = 0.55) as a yellow oil: IR ν_{max} 2967, 1740, 1691, 1597, 1446, 1373, 1206, 1148, 1104, 1053, 1012 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.53 (1H, s, H-5), 6.01−5.91 (1H, m, H-2′), 5.19 (2H, s, OCH_2O), 4.99−4.94 (2H, m, H-3′), 3.79 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.47 (3H, s, OCH_3), 3.34 (2H, dt, J = 5.9, and 1.6 Hz, H-1′), 2.49 (3H, s, CO$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4 (C, $\underline{C}OCH_3$), 157.6 (C, C-4), 156.5 (C, C-2), 156.0 (C, C-6), 136.9 (CH, C-2′), 119.5 (C, C-1), 114.9 (CH_2, C-3′), 114.5 (C, C-3), 94.3 (CH, C-5), 94.3 (CH_2, OCH_2O), 63.4 (CH_3, OCH_3), 56.1 (CH_3,

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OCH_3), 55.8 (CH_3, OCH_3), 32.5 (CH_3, $CO\underline{C}H_3$), 27.6 (CH_2, C-1′); HRMSESI m/z $[M + H]^+$ 281.1367 (calculated for $C_{15}H_{21}O_5$ 281.1385).

1-(3-Allyl-4-hydroxy-2,6-dimethoxyphenyl)ethanone (20). Compound **20** was obtained as a light yellow oil (0.63 g, 2.7 mmol, 81%, R_f = 0.19) after purification by silica gel column chromatography using hexanes/EtOAc (8.5:1.5) as eluent from MOM deprotection of **19** (0.92 g, 3.3 mmol, 1.0 equiv) in CH_3OH (20 mL), and 3 M HCl (4 mL) at 70 °C following the procedure for the preparation of **18**. IR ν_{max} 3316, 2940, 1740, 1668, 1593, 1493, 1408, 1323, 1257, 1200, 1146, 1001, 912, 819, 562, 497 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.26 (1H, s, H-5), 6.05−5.95 (1H, m, H-2′), 5.16−5.11 (2H, m, H-3′), 3.73 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.38 (2H, dt, J = 5.8 and 1.7 Hz, H-1′), 2.50 (3H, s, $CO\underline{C}H_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.7 (C, $\underline{C}OCH_3$), 157.5 (C, C-4), 156.8 (C, C-2), 156.4 (C, C-6), 136.2 (CH, C-2′), 118.3 (C, C-1), 116.2 (CH_2, C-3′), 110.9 (C, C-3), 95.9 (CH, C-5), 63.4 (CH_3, OCH_3), 55.7 (CH_3, OCH_3), 32.5 (CH_3, $CO\underline{C}H_3$), 27.6 (CH_2, C-1′); HRMSESI m/z $[M + H]^+$ 237.1097 (calculated for $C_{13}H_{17}O_4$ 237.1121).

1-(4-{2″-[(*tert*-Butyldiphenylsiloloxy)methyl]allyloxy}-3-allyl-2,6-dimethoxyphenyl)ethanone (21). Compound **21** was obtained as a light yellow oil (0.82 g, 1.5 mmol, 58.9%, R_f = 0.5) after purification by PTLC using hexanes/EtOAc (9:1−8:2) from the treatment of **20** (0.60 g, 2.6 mmol, 1.0 equiv) with 3-bromo-2-(*tert*-butyldiphenylsiloxy)methylpropene (0.79 g, 2.1 mmol, 0.86 equiv) and K_2CO_3 (0.35 g, 2.6 mmol, 1.0 equiv) in DMF (10 mL) following the procedure used to synthesize **12a**. IR ν_{max} 3016, 2970, 2944, 1738, 1436, 1366, 1228, 1216, 1206, 1111, 538 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.68−7.66 (4H, m, $Si\underline{Ar}_2$), 7.45−7.35 (6H, m, $Si\underline{Ar}_2$), 6.24 (1H, s, H-5), 5.87−5.77 (1H, m, H-2′), 5.39 (1H, d, J = 0.8 Hz, H-3″a), 5.26 (1H, d, J = 1.1 Hz, H-3″b), 4.87−4.82 (2H, m, H-3′), 4.56 (2H, s, H-1″) 4.29 (2H, s, H-4″), 3.75 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.25 (2H, d, J = 6.0 Hz, H-1′), 2.49 (3H, s, $COCH_3$), 1.00 (9H, s, $(C\underline{H}_3)_3CSi$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3 (C, $\underline{C}OCH_3$), 158.8 (C, C-4), 156.7 (C, C-2), 156.0 (C, C-6), 143.4 (C, C-2″), 136.8 (CH, C-2′), 135.4 (4 × CH, $Si\underline{Ar}_2$), 133.3 (2 × C, $Si\underline{Ar}_2$), 129.7 (2 × CH, $Si\underline{Ar}_2$), 127.7 (4 × CH, $Si\underline{Ar}_2$), 118.5 (C, C-1), 114.4 (CH_2, C-3′), 114.4 (C, C-3), 112.3 (CH_2, C-3″), 92.3 (CH, C-5), 69.1 (CH_2, C-1″), 64.5 (CH_2, C-4″), 63.3 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.5 (CH_3, $CO\underline{C}H_3$), 27.5 (CH_2, C-1′), 26.8 (3 × CH_3, $(\underline{C}H_3)_3CSi$), 19.3 (C, $(CH_3)_3\underline{C}Si$); HRMSESI m/z $[M + H]^+$ 545.2715 (calculated for $C_{33}H_{41}O_5Si$ 545.2717).

1-(2,5-Dihydro-3-hydroxymethyl-6,8-dimethoxybenzo[*b*]-oxepin-7-yl)ethanone (22). The treatment of diene **21** (1.00 g, 1.84 mmol, 1.0 equiv) with (Grubbs II catalyst) (0.162 g, 0.191 mmol, 0.10 equiv) in dry CH_2Cl_2 (30 mL), followed by TBAF solution (0.520 mL, 2.02 mmol, 1.1 equiv) in THF, afforded the target benzoxepine **22** (0.16 g, 32%, 0.59 mmol, R_f = 0.45) as a light yellow oil, after purification through a short pad of silica gel using hexanes/EtOAc (9:1−5:5) as the eluent while following the procedure for preparing **6**. IR ν_{max} 3381, 2941, 2600, 2492, 1690, 1596, 1471, 1394, 1322, 1253, 1197, 1145, 1098, 1031, 843, 806, 747, 567, 466 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.47 (1H, s, H-9), 5.91 (1H, t, J = 5.3 Hz, H-4), 4.67 (2H, brs, H-2), 3.98 (2H, brs, H-1′), 3.78 (3H, s, OCH_3), 3.68 (3H, s, OCH_3), 3.44 (2H, d, J = 5.5 Hz, H-5), 2.48 (3H, s, $CO\underline{C}H_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.4 (C, $\underline{C}OCH_3$), 160.9 (C, C-9a), 155.6 (C, C-8), 154.6 (C, C-6), 138.2 (C, C-3), 123.9 (CH, C-4), 121.9 (C, C-7), 120.6 (C, C-5a), 101.1 (CH, C-9), 71.6 (CH_2, C-2), 65.7 (CH_2, C-1′), 63.3 (CH_3, OCH_3), 55.8 (CH_3, OCH_3), 32.4 (CH_3, $CO\underline{C}H_3$), 22.0 (CH_2, C-5); HRMSESI m/z $[M + H]^+$ 279.1214 (calculated for $C_{15}H_{19}O_5$ 279.1234).

8-Allyl-5-hydroxy-2-methyl-3′,3′-dimethylpyrano[7,6]-chromone (23). To a solution of **12** (0.20 g, 0.86 mmol, 1.0 equiv) in CH_3OH (10 mL) was added $Ca(OH)_2$ (0.120 g, 1.72 mmol, 2.0 equiv) followed by prenal (0.40 mL, 4.3 mmol, 5 equiv). The mixture was stirred under an argon atmosphere for 3 days at rt and diluted with EtOAc (15 mL) and 1 M HCl (15 mL). The two phases were partitioned, and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H_2O and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude product was purified by silica gel column chromatography using hexanes/EtOAc (7:3) to give **23** as a white solid (26.0 mg, 10% 0.872 mmol, R_f = 0.7): ^1H NMR (400 MHz, $CDCl_3$) δ 12.97 (1H, s, HO-5), 6.71 (1H, d, J = 10.0 Hz, H-1′), 6.00 (1H, s, H-3), 5.93−5.83 (1H, m, H-2″), 5.60 (1H, d, J = 10.0 Hz, H-2′), 5.04−4.94 (2H, m, H-3″), 3.42 (2H, dt, J = 6.3 and 1.4 Hz, H-1″), 2.34 (3H, s, CH_3-2), 1.44 (6H, s, CH_3-3′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9 (C, C-4), 166.4 (C, C-2), 156.8 (C, C-7), 154.9 (C, C-8a), 154.7 (C, C-5), 135.9 (CH, C-2″), 127.8 (CH, C-2′), 115.8 (CH, C-1′), 114.7 (CH_2, C-3″), 108.5 (CH, C-3), 105.3 (C, C-8), 105.2 (C, C-6), 104.8 (C, C-4a), 77.8 (C, C-3′), 28.2 (2 × CH_3, CH_3-3′), 26.5 (CH_2, C-1″), 20.4 (CH_3, CH_3-2); HRMSESI m/z $[M + H]^+$ 299.1285 (calculated for $C_{18}H_{19}O_4$ 299.1285).

1-(3-Allyl-4,6-dihydroxy-2-methoxyphenyl)ethanone (24). Compound **24** was obtained as a light yellow solid (0.16 g, 0.70 mmol, 67%, R_f = 0.44) after purification by silica gel column chromatography using hexanes/EtOAc (8.5:1.5) as the eluent from MOM deprotection of **17** (0.320 g, 1.04 mmol, 1.0 equiv) in CH_3OH (3.5 mL) and 3 M HCl (1 mL) at 70 °C following the procedure for the preparation of **18**. ^1H NMR (400 MHz, $CDCl_3$) δ 13.20 (1H, s, HO-6), 6.24 (1H, s, H-5), 6.09−5.99 (1H, m, H-2′), 5.83 (1H, s, HO-4), 5.18−5.12 (2H, m, H-3′), 3.74 (3H, s, OCH_3), 3.41 (2H, dt, J = 5.7 and 1.8 Hz, H-1′), 2.69 (3H, s, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.5 (C, $\underline{C}OCH_3$), 164.4 (C, C-6), 161.9 (C, C-4), 161.8 (C, C-2), 136.2 (CH, C-2′), 116.3 (CH_2, C-3′), 111.0 (C, C-3), 109.8 (C, C-1), 100.5 (CH, C-5), 63.0 (CH_3, OCH_3), 31.0 (CH_3, $CO\underline{C}H_3$), 27.8 (CH_2, C-1′); HRMSESI m/z $[M + H]^+$ 223.0965 (calculated for $C_{12}H_{15}O_4$ 223.0964).

1-(8-Allyl-5-hydroxy-7-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)ethanone (25). Compound **25** was obtained as a light yellow oil (17 mg, 44%, 0.059 mmol, R_f = 0.76) after purification by PTLC using hexanes/EtOAc (7:3) as the eluent from the reaction of phenol **24** (30.0 mg, 0.135 mmol, 1.0 equiv) with prenal (64.0 μL, 0.675 mmol, 5.0 equiv) and added $Ca(OH)_2$ (20.0 mg, 0.270 mmol, 2.0 equiv) in CH_3OH according to the procedure used to prepare **23**. ^1H NMR (400 MHz, $CDCl_3$) δ 13.55 (1H, s, HO-5), 6.68 (1H, d, J = 10.0 Hz, H-4), 6.01−5.91 (1H, m, H-2′), 5.51 (1H, d, J = 10.0 Hz, H-3), 5.04−4.98 (2H, m, H-3′), 3.74 (3H, s, OCH_3), 3.31 (2H, dt, J = 6.0 and 1.6 Hz, H-1′), 2.67 (3H, s, $CO\underline{C}H_3$), 1.43 (6H, s, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.5 (C, $\underline{C}OCH_3$), 161.2 (C, C-7), 159.2 (C, C-5), 158.5 (C, C-8a), 136.9 (CH, C-2′), 126.7 (CH, C-3), 116.0 (CH, C-4), 114.8 (CH_2, C-3′), 112.9 (C, C-8), 109.0 (C, C-6), 105.8 (C, C-4a), 78.0 (C, C-2), 63.1 (CH_3, OCH_3), 31.0 (CH_3, $CO\underline{C}H_3$), 28.4 (2 × CH_3, CH_3-2), 27.5 (CH_2, C-1′); HRMSESI m/z $[M + H]^+$ 289.1489 (calculated for $C_{17}H_{21}O_4$ 289.1442).

Antifungal Assay. *Fungal Test Organisms.* The test fungi, *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (isolated from a cheetah), were obtained from the culture collection of the Phytomedicine programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria. The fungi were maintained on Sabouraud dextrose agar (Oxoid, Basingstoke, UK).

Microdilution Assay. A reported microdilution assay with tetrazolium violet added as growth indicator was used to determine the MIC values for the compounds.[35,36] Compounds were dissolved in DMSO and added to a sterile 96-well microtiter plate in triplicate. A serial dilution was done along the ordinate of the wells with sterile distilled water. Fungal cultures diluted in Sabouraud dextrose broth were then added to each well. The final concentrations of each compound ranged between 25 and 0.2 μg/mL. Amphotericin B and DMSO served as positive and negative controls, respectively. Forty microliters of 0.2 mg/mL *p*-iodonitrotetrazolium violet (Sigma) dissolved in sterile water was added to each well of the microplate. The plates were incubated for 1−2 days at 35 °C and 100% relative humidity. The MIC was recorded as the lowest concentration of the compound that inhibited fungal growth after 24 and 48 h.

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■ ASSOCIATED CONTENT

Supporting Information

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^{1}H NMR and ^{13}C NMR spectra for the synthesized compounds (PDF)

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Notes

The authors declare no competing financial interest.

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