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SYNTHETIC STUDIES TOWARDS BIOACTIVE FUNGAL METABOLITES

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DEDICATED TO MY BELOVED

PARENTS, WIFE &

SON (MAYANK)

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled **"SYNTHETIC STUDIES TOWARDS BIOACTIVE FUNGAL METABOLITES"** which is being submitted to the **University of Milan** for the award of **Doctor of Philosophy** in Chemistry, Biochemistry and Ecology of pesticides by **Santosh Vitthal Lahore** was carried out by him under my supervision at **University of Milan, Milan**. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other University.

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Candidate's Declaration

I hereby declare that the thesis entitled **"SYNTHETIC STUDIES TOWARDS BIOACTIVE FUNGAL METABOLITES" submitted** for the award of degree of **Doctor of Philosophy** in Chemistry, Biochemistry and Ecology of pesticides to the **University of Milan, Milan**. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other University. This work was carried out by me at **DeFENS, University of Milan, Milan, Italy**.

Santosh Vithal Lahore January , 2014

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General experimental methods

1. NMR Spectras

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions (where not otherwise stated) at room temperature on a Bruker AMX-300 spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts are reported as d values in parts per million (ppm), and are indirectly referenced to tetramethylsilane (TMS) via the solvent signal (7.26 for ^{1}H, 77.0 for ^{13}C) in $CDCl_3$. Coupling constant (*J*) are given in Hz.

2. Solvents

Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were obtained by distillation from sodium-benzophenone ketyl; dry methylene chloride (CH_2Cl_2) and toluene were obtained by distillation from $CaCl_2$. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and glassware was oven-dried.

3. Flash chromatography

Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230-400 mesh); when necessary deactivated silica gel was used.

4. Analytical thin-layer chromatography

Analytical thin-layer chromatography (TLC) was conducted on Fluka TLC plates (silica gel 60 F254, aluminium foil), and spots were visualized by UV light and/or by means of dyeing reagents.

5. Melting points

Melting points were determined on a Stuart Scientific SMP3 instrument and are uncorrected.

Abbreviations

Ac	-	Acetyl
Ac2O	-	Acetic anhydride
Ar	-	Aryl
Brs	-	Broad singlet
Boc	-	*tert*-Butoxy carbonyl
$(Boc)_2O$	-	Di-*tert*-butyl dicarbonate
Bu	-	Butyl
t-Bu	-	*tert*-Butyl
BuLi	-	Butyl Lithium
Cat.	-	Catalytic/Catalyst
$CDCl_3$	-	Deuterated chloroform
DCM	-	Dichloromethane
DME		1,2 dimethoxy ethane
dppe		1.2-Bis (diphenylphosphino)ethane
d	-	Doublet
dd	-	Doublet of doublet
DBU	-	1,8-Diazabicyclo [5.4.0] undec-7-ene

DCC	-	Dicyclohexylcarbodiimide
DIBAL-H	-	Diisobutylaluminium hydride
DMAP	-	*N, N'*-Dimethylaminopyridine
DMF	-	*N, N'*-Dimethylformamide
DMSO	-	Dimethyl sulfoxide
Et	-	Ethyl
Et_3N	-	Trimethyl amine
EtOAc	-	Ethyl acetate
EtOEt, Et_2O	-	Diethylether
EtOH	-	Ethanol
g	-	grams
h	-	hours
HPLC	-	High performance liquid chromatography
Hz	-	Hertz
Im	-	Imidazole
LAH	-	Lithium aluminium hydride
LDA	-	Lithiumdiisopropylamide

LiHMDS	-	Lithium bis(trimethylsilyl)amide
m	-	Multiplate
Me	-	Methyl
mg	-	Miligram
MIC	-	Minimum inhibitory concentration
min	-	Minutes
MeOH	-	Methanol
mmol	-	mmol
m.p	-	Melting point
MSA		Methanesulfonic acid
NBS		N-Bromosuccinimide
NIS		N- Iodosuccinimide
NMR	-	Nuclear magnetic resonance
NOESY	-	Nuclear overhauser effect spectroscopy
NMM	-	N- methyl morpholine
PAH		Polycyclic aromatic hydrocarbon
Ph	-	Phenyl

Pd/C	-	Palladium on carbon
PPA		Polyphosphoric acid
ppm	-	Parts per million
*p*TSA	-	*para*-Toluenesulfonic acid
Py	-	Pyridine
Py. HCl	-	Pyridine hydrochloride
rt	-	Room temperature
s	-	Singlet
TEA	-	Triethyl amine
THF	-	Tetrahydrofuran
TFA	-	Trifluoroacetic acid
TFFA	-	Trifluoroacetic anhydride
TLC	-	Thin layer chromatography
TPP	-	Triphenylposphine

CHAPTER 1

INTRODUCTION

1 Crop Protection

Since the dawn of time mankind has had two primary goals - obtaining enough food to survive and improving the quality of life. The single most important task facing a society is the production of food to feed its population. A country or society has to feed its people before it can devote resources to education, arts, technology or recreation. The need for producing more food within a particular area arose as nomadic populations of hunter-gatherers settled to form more permanent communities. For thousands of years, agricultural practices relied heavily on crop rotation or mixed crop planting to optimize natural pest control (such as predation, parasitism, and competition).

Feeding the world is an increasing challenge; on the other hand the size of arable land is limited and cannot be expanded without further inroads into vital natural habitats like the rain forests. As more people need more food per head from less land, the yields need to be increased by a combination of methods which is known as 'Integrated Crop Management'. This practice includes the selection of optimized seeds and the right locations, optimized sowing and harvesting techniques, crop rotation, fertilization and mechanical, biological or chemical crop protection.

Chemical crop protection products with activity against weeds, plant diseases, insects and mites help the farmer to assure or increase yields. In the most drastic cases crop protection products can prevent the total loss of a crop. Furthermore the quality of food is improved and the costs of production are cheaper.

1.1. Crop protection, a historical overview

The efforts to increase the agriculture production can be traced back to the prehistoric times. The concept of 'natural pesticides' arose early in the development of agriculture. A large number of volumes and reviews have been written about the use of natural products as pesticides.[1-9] Indeed, the Lithica poem (c. 400 B.C.) states 'All the pests that out of earth arise, the earth itself the antidote supplies'.[10] Greek and Roman scholars such as Theophrastus (371–287 B.C.), Cato the Censor (234–149 B.C.), Varro (116–27 B.C.), Vergil (70–19 B.C.), Columella (4–70 A.D.) and Pliny the elder (23–79 A.D.) published treaties on agricultural practices to minimize the negative effects of pests on crops. Methods such as mulching and burning, as well as the use of oils for pest control were mentioned. Chinese literature (ca. 300 A.D.) describes an elaborate system of biological control of caterpillar infestations in citrus orchards. Colonies of the predatory ants (*Oecophylla smaragdina*) were introduced in citrus groves, and bridges made of bamboo allowed the ants to move between trees. A survey of the Shengnong Ben Tsao Jing era (25–220 A.D.) shows that 267 plant species were known to have pesticidal activity.[11] Finally, the use of beneficial insects to control other insect pests was mentioned by Linnaeus as early as 1752, and he won a prize in 1763 for an essay describing the biological control of caterpillars. The European agricultural revolution that followed in the 19th century was accompanied by more extensive and international trade that resulted in the discovery of botanical insecticidal powders from *Chrysanthemum* flower heads and *Derris* root which contain pyrethrum and rotenone, respectively. The advent of extensive monoculture and intensive agricultural practices of the 20th century was accompanied by increases in yields. New cultivars were selected based on their higher yields, but many of these lines seem to have lower resistance to pests. This has resulted in greater pest pressure, which has mostly been addressed by the use of synthetic pesticides.

1.2 Pesticides

Plant protection products (PPPs), also known as pesticides, are the treatments used in both conventional and organic farming, to keep crops healthy by protecting them against pests and diseases. Pesticides are for plants the equivalent of medicines for humans. Pesticide companies manufacture synthetic products as well as products based on natural chemicals and minerals.[12-14]

The Food and Agriculture Organization (FAO) has defined pesticide as:

"Any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used for substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport."[4]

The development of modern pesticides began in the 1940s, when Europe faced food shortages and rationing in the wake of World War II and the protection of crop yields had heightened importance. Since the 1970s, this dynamic has shifted with more pressure to balance the need to increase food production with the need to ensure the safety of people, food and the environment.

1.2.1 Why we need pesticides?

By keeping crops free of pests and diseases, pesticides help to ensure a reliable and predictable food supply.[15] Crop losses due to pests and diseases are between 30 and 50% depending on the crop grown. For example, the losses of wheat yields could reach 50% if no pesticides were used;

for potatoes, the losses can reach up to 75%. Pesticides limit food losses and make significant contribution to food security.

1.2.2 Benefits of use of Pesticides

❖ **A healthy diet:**

✓ By protecting crops pesticides contribute to the production of a plentiful supply of high-quality and affordable food.

✓ They help the production of a choice of fruit & vegetables that are essential in a healthy diet.

✓ They reduce the exposure of consumers to naturally-produced toxins which plants develop if pests and diseases remain uncontrolled.

❖ **Economic positives:**

✓ Pesticide use increases yields and improves farm revenues. Without pesticides up to 50% of yields would be lost due to the presence of detrimental fungi, insects or weeds.

They contribute towards self-sufficiency of agriculture produce among nations in cereals and other crops.

Environmental protection:

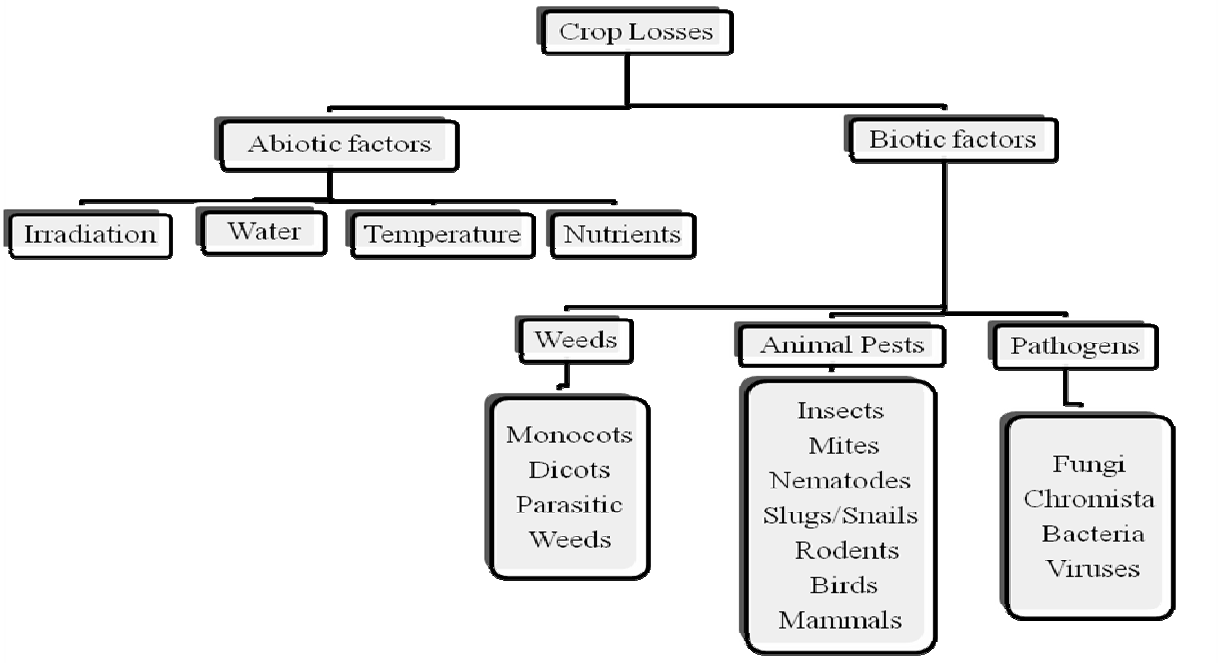
✓ Pesticide use allows more food to be produced on a given area of land, which reduces pressure to cultivate un-cropped land and therefore ensures the maintenance of important natural habitats and protected natural areas.

✓ They help to reduce greenhouse gas emissions by replacing mechanical crop protection.

✓ They promote low-tillage agricultural systems, which reduce the loss of soil nutrients and prevent soil erosion.

1.3 Factors responsible for crop losses

Losses can be classified according to two major factors, biotic and abiotic. Abiotic causes of crop losses are especially the lack or excess of water in the growth season, extreme temperatures, high or low irradiance (factors which can be controlled only within narrow limits) and nutrient supply. Biotic stressors have the potential to reduce crop production substantially. These organisms may be controlled by applying physical (cultivation, mechanical weeding, etc.), biological (cultivar choice, crop rotation, antagonists, predators, etc.) and chemical measures (pesticides).



1.4 Fungi

Fungi are microscopic plants with a basic, threadlike structure collectively called the mycelium. They have no chlorophyll and thus are unable to utilize carbon dioxide from the air for their nutrition. Instead, they utilize previously formed carbon compounds as a source of energy. They obtain these materials while growing saprophytically on the products or remains of plants and animals, or by parasitizing living plants and animals. In living, green plants, fungi usually degrade the host, producing visible damage, which, in vegetable crops, causes losses in yield and quality. As saprophytes, fungi are responsible for much of the natural breakdown of organic material and hence the recycling of essential elements and compounds in the environment. Mushrooms and toadstools are larger fungi that can be saprophytic, parasitic or, in many cases, symbiotic with green plants (mycorrhiza), living in plant roots to the mutual benefit of both fungus and host. Parasitic fungi fall into two broad groups: obligate parasites, which depend entirely on a living host for their nutrition and reproduction, and facultative parasites, which can do considerable damage to crop plants as parasites, but can also live indefinitely as saprophytes on plant remains. Obligate plant parasites include the rusts, powdery mildews and downy mildews, whose names broadly describe the symptoms of the diseases they cause. The ubiquitous gray mold fungus *Botrytis cinerea* is a facultative parasite. Virtually all fungi that cause plant diseases form microscopic spores that serve two basic functions: to act as dispersal and infective propagules to spread the disease, and to act as resistant structures permitting the pathogen to survive adverse environmental conditions. In addition, many fungi also form compact, hard structures called sclerotia. These, spores, are capable of resuming growth under favorable conditions to infect the host plant, sometimes after months or years. Spores are dispersed in various ways, for example by air, in water through the soil or irrigation systems, by

insects, or on hands, clothing and tools. Spores are the principal agents of plant infection. They germinate under suitable conditions, almost invariably in a water droplet or film or on a moist wound, to form a thread-like germ tube that can penetrate through the plant epidermis directly or through a stomatal pore. Once inside the plant tissue, the mycelium permeates the host tissues, sometimes blocking the water-conducting system, as in the wilt diseases. As the food supply for the fungus diminishes, more spores are formed to spread the pathogen through the crop. By this time the host is either severely damaged or dead. Spores can be produced by a sexual process, which imparts genetic variability to the fungus and can give rise to pesticide resistance or overcome host resistance, or they can be produced in huge numbers by an asexual, vegetative process. Some fungi form two or more types of spores that often do not much resemble each other in the same fungus. The sexual state is called the teleomorph and gives the fungus its proper, scientific (Latin) name, while asexual states are called anamorphs and frequently have a different Latin name. For example, the gray mold fungus *Botryotinia fuckeliana* is the teleomorph name for a rare, tiny, toadstool-like fungus. However, it is better known as *Botrytis cinerea,* the name that describes its asexual, dispersive and infective spores (conidia), which are arranged in a grape-like cluster. *Botrytis cinerea* is derived from the Greek, meaning an ashy-colored bunch of grapes. The fungus also has anamorphic microconidia, which are not infectious but have a sexual function, and chlamydospores. The latter are durable, long-lived spores in nature.

Serious damage is done to crops each year by fungal infections of plants such as smuts, rusts, ergots, and mildews. Fungi are the number one cause of crop loss worldwide and cause about 70% of all major crop diseases. Although similar, Oomycetes are not fungi. However, they use the same mechanisms to infect plants. Some of the major fungal disease are as follows.

Table 1.4 Important Diseases of Crop Plants

Fungal Class	Pathogen	
	Scientific Name	**Common Name**
Phycomycetes subclass Oomycetes	*Phytophthora infestans* *Plasmopara viticola* *Pseudoperonospora* *Cubensis*	potato late blight downy mildew of grape cucumber downy mildew
Ascomycetes	*Pythium spp.* *Erysiphe graminis* *Gaeumannomyces graminis* *Podosphaera leucotricha* *Pyrenophora teres* *Pyricularia oryzae* *Rhynchosporium secalis* *Sclerotinia Spp.* *Sphaerotheca fuliginea* *Uncinula necator* *Venturia inaequalis* *Mycosphaerella fijiensis*	damping off diseases powdery mildew of wheat/barley take-all of oats and wheat apple powdery mildew net blotch of barley rice blast leaf scald of barley, brown rot of pome fruit Leaf Spot of brassicas and legumes cucurbit powdery mildew grape powdery mildew scab of apple sigatoka disease of bananas
Basidiomycetes	*Puccinia Spp.* *Rhizoctonia Spp.* *Tilletia Spp.* *Uromyces Spp.* *Ustilago Spp.*	Leaf rust of wheat and oats black scurf of potato Sheath blight of rice sharp eyespot of wheat Bunts of wheat Beans rust Smuts of wheat, barley, oat and maize, early blight of potato

Deuteromycetes	*Alternaria Spp.*	early blight tobacco brown spot leaf of brassicas
	Botrytis.Spp	grey mold of grape and other crops
	Cercospora Spp.	leaf spot of sugarbeet, brown eyespot of coffee
		wilts, broad range of hosts blight of wheat
	Fusarium Spp.	root and foot rot of wheat leaf spor of maize
		eye spot of wheat
	Helminthosporium Spp.	glume blotch of wheat
	Pseudocercosporella herpotrichoides	
	Septoria nodorum, Septoria tritici	

1.4.1 Fungicides

Fungicides are one of the classes of pesticides. Fungicides are chemical compounds, or biological organisms to kill or inhibit fungi or fungal spores. Fungicides are also used to fight fungal infections in animal tissues. Fungicides can be either contact or systemic. A contact fungicide kills fungi when sprayed on its surface; a systemic fungicide has to be absorbed by the plant. Although there are benefits to the use of fungicides, there are also drawbacks, such as potential toxicity to humans and other animals. Regarding these drawbacks, the agrochemical industry deserves for safer and more effective agrochemicals with reduced environmental and/or mammalian toxicity. Essential to these efforts is identification of new lead candidates possessing high levels of desirable biological activities, reduced unwanted toxicities, new structural types,

and perhaps different modes of action, thereby providing protection from cross-resistance to currently used agrochemicals.

1.4.2 Development of Fungicides.

1807-The first fungicide

Prevost observed that spores, which grew into tiny germinating "plants," caused wheat bunt. He then made the serendipitous observation that a weak copper solution (generated when he held the spore suspension in a copper vessel) prevented their growth. Through experimentation, he demonstrated that farmers could control bunt by wetting wheat kernels with a copper sulphate solution. Copper-based seed treatments remained popular in some countries, including France, through the end of the 20th century.

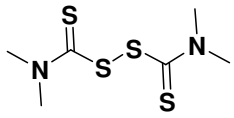
1885- The First Foliar Fungicide

In1885. P. M. A. Millardet, a farmer in the Bordeaux region of France, noticed that the vines sprayed with copper sulphate to discourage university students from pilfering grapes on their way to class retained their leaves while unsprayed plants had been defoliated by downy mildew.[16] After extensive experimentation, he described the effective use of a mixture of copper sulphate and lime for controlling the downy mildew on grapevines. The mixture is popularly known as Bordeaux mixture

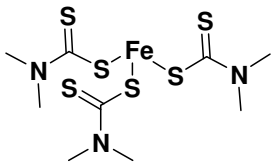
1915-Broad-Spectrum Control of Seed-Borne Disease.

The organomercurial seed treatment, chloro(2-hydroxyphenyl)mercury was introduced in Germany in 1913.[17] Research on organomercurials continued through the 1920s and 1930s, leading to commercialization of the 2-methoxyethyl silicate and acetate salt of 2-hydroxyphenyl mercury, among others.

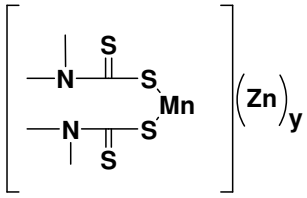
These seed treatments were a breakthrough for cereal farmers because the treatments had good seed safety and controlled mycelia of seed-borne fungi such as *Fusarium* and *Dreschlera* as well as bunt. They also provided protection against soil-borne *Fusarium* species, resulting in improved stand establishment[18] and their vapour activity helped overcome incomplete coverage of the seed surface.[19] However, despite their multi-site-mode of action and use only once per year, resistance eventually developed in some populations of *Dreschlera* on barley and oats. The use of organomercurials led to a increase in the concentration of mercury compounds in the environment. The toxicity and the persistence of mercury led most countries to ban the organomercurials when safer alternatives, e.g. new organic fungicides, such as such as **Thiram, Ferbam (Dithio-carbamate), Mancozeb (Dithiocarbamate),** became available. However, despite the availability of alternative treatments, organomercurial seed treatments were not banned in the UK until 1992.[18]



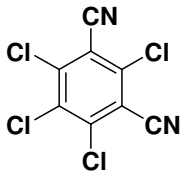
Thiram



Ferbam



Mancozeb

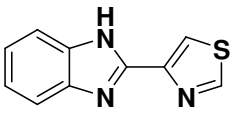


Chlorthalonil

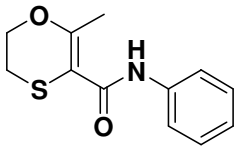
1969-The First Systemic Seed Treatment

The first systemic fungicide Carboxin, developed in 1966 and commercialized in 1969, not only controlled surface-borne bunts and smuts but also penetrated deeply in the seed embryo, where it eradicated loose smut infection. Carboxin also gave excellent control of early season rust and *Rhizoctonia* damping off. The utility of carboxin and its derivative of foliar application, oxycarboxin, are more effective against Basidiomycetous diseases. Additionally, carboxin had good plant safety as a seed treatment of row crops, particularly cotton and canola. It is less

effective on seed-borne *Fusarium* and *Dreschlera* disease than organomercurials.[20] Resistance development has been slow, although field resistance was eventually documented in some populations of *Ustilago nuda* after many years of continuous use[21]. However it is not a broad spectrum fungicide.



Thiabendazole



Carboxin

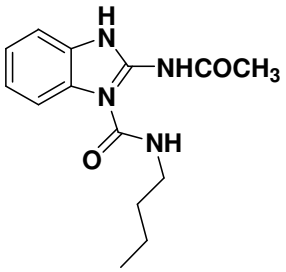
1970- The benzimidazole based fungicides :First Broad-Spectrum Foliar Systemic Fungicide.

The first fungicide with the broader spectrum typical of dithiocarbamates and the systemic activity of organophosphate insecticides was Benomyl, which was launched in 1970 and provided systemic and curative activity at low rates, with excellent plant and mammalian safety and extended intervals between sprays. These characteristics made Benomyl extremely popular from its introduction.[22] Most Ascomycetes with light-colored spores are controlled, including numerous types of leaf spots, fruit rots caused by *Botrytis* and *Penicillium*, powdery mildews, and stem diseases such as eyespot. Some Basidiomycetes, such as selected anastamosis groups of *Rhizoctonia solani*, are controlled. Diseases caused by Oomycetes and by Ascomycetes with dark spores (such as *Alternaria* and *Helminthosporium*) are also not controlled.[23]

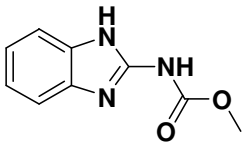
Repeated, and exclusive use on polycyclic diseases led to rapid development of resistant fungal populations. Within three years from introduction, resistance was reported in field and/or greenhouse populations of *Erysiphe*, *Botrytis*, *Penicillium*, and *Cercospora*.[22] This can be attributed to a single-site mode-of-action of Benomyl, which could be bypassed by the fungus

with a single mutation. Resistant strains could be equal in fitness to their susceptible counterparts, resulting in persistence of some resistant populations even when the benzimidazole fungicides were discontinued.[20] The agrochemical industry learned an important lesson about fungicides with specific modes-of-action from the benzimidazole experience, and now begins assessment of resistance risk early in fungicide development so that resistance management plans are in place at product launch.[24]

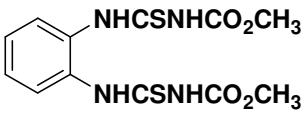
The benzimidazole fungicides were very successful on fruits and vegetables but had less utility for cereal diseases, since they gave no control of rusts or *Dreschlera* species. Further, the cereal diseases that were controlled, in particular the powdery mildews, rapidly became resistant.[22] A systemic, broad-spectrum fungicide with a new mode-of-action was still needed for foliar disease control in cereals.



Benomyl



Carbendazim



Thiophanate

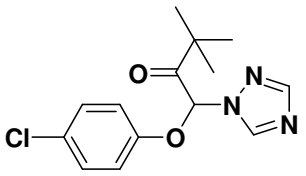
1976- Triazole fungicides : A Systemic, Curative Foliar Fungicide for Cereals.

The benzimidazole fungicides introduced in 1970 were very successful on fruits and vegetables but had less utility for cereal diseases. Hence there was a need of a fungicide which is effective on cereal disease management. Triadimefon, a triazole fungicide indroduced in 1976 by Bayer [25] provided curative as well as protectant activity, low application rates, and excellent redistribution in the plant. The spectrum of control covered all major cereal diseases and included most

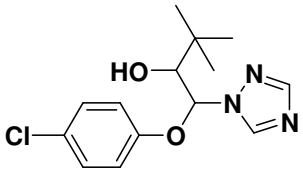
of Ascomycetes and Basidiomycetes (but not Oomycetes). Other triazole fungicides were introduced over the next two decades with improved potency and plant safety on cereals such as **epoxiconazole**, **propiconazole** and **tebuconazole** with a broader effective spectrum. Some specialized azoles such as **difenoconazole** and **triticonazole** were used only for seed treatment[25].

The use of triazole-based fungicides showed side effects on plants. Application to shoots and roots often reduces elongation and causes leaves to be smaller, thicker, and greener. Treated plants may be delayed in senescence, which can impede harvest or improve yields, depending on the crop.[26] Since the triazoles have many of the same properties as the benzimidazoles, they suffered from resistance development, (curative activity, single-site MOA, multiple applications per season). Resistance to the triazole fungicides (and other inhibitors of C14-demethylase in ergosterol biosynthesis) developed first in the powdery mildews and has been observed (but is less problematic) on other diseases.[25] Unlike resistance to the benzimidazoles, resistance to the triazoles involves multiple genes with intermediate levels of resistance and incomplete cross-resistance between different fungicides.[26] The use of mixtures has been remarkably successful in maintaining useful activity against most fungal targets for three decades.

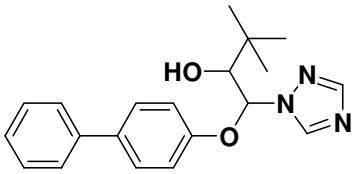
The benzimidazole and triazole fungicides proved potent systemic fungicide against Ascomycete and Basidiomycete diseases, but control of devastating Oomycete diseases such as potato late blight and grape downy mildew was limited to frequent sprays of protectant fungicides. Root rots of established plants (caused by *Phytophthora* and *Pythium*) and systemic downy mildews could not be controlled at all, and took an unknown toll on crop yield.



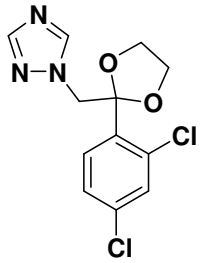
Triadimefon



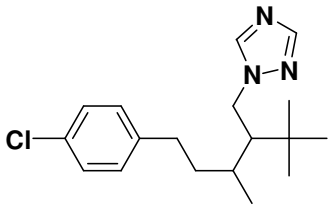
Triadimenol



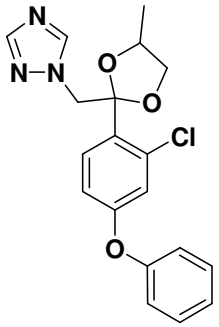
Bitertanol



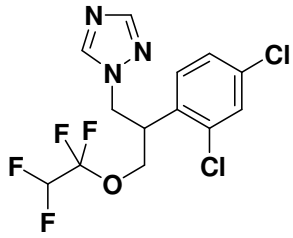
Propiconazole



Tebucoinazole



Difenoconazole

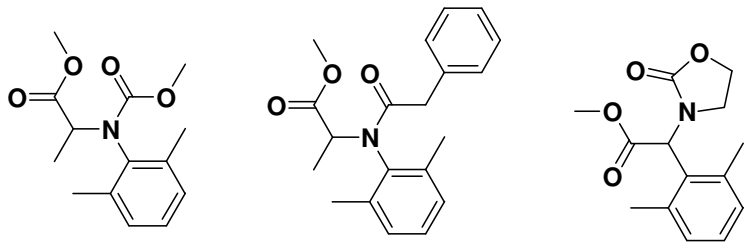


Tetraconazole

1977- Phenylamide fungicide : The First Systemic Oomycete Fungicides

The launch of the phenylamide fungicide **Metalaxyl** was an immediate success for control of Oomycete diseases[27] because of its outstanding properties such as high potency; excellent curative and protectant activity; excellent redistribution and protection of new growth; control of all members of the order Peronosporales (including *Pythium*); and flexible application methods including foliar spray, seed treatment, and root drench.[28] As with the previous fungicides such as

benzimidazoles, and triazoles the phenomenal success and overuse of the phenylamide fungicides led to rapid resistance development. Significant resistance to metalaxyl was first described in 1980 on cucumber downy mildew and late blight.[27] Resistance developed more rapidly where metalaxyl was used alone. The manufacturer Ciba-Geigy introduced fungicide prepacks containing metalaxyl and protectant fungicides, such as mancozeb, which extended the product life significantly.[27,28] The phenylamide experience was pivotal in the formation of the Fungicide Resistance Action Committee (FRAC), which developed a coordinated strategy across rival companies to limit the number of recommended phenylamide applications per season.[29] Despite a coordinated effort, susceptibility to phenylamides gradually eroded in populations of many foliar pathogens, and foliar uses of metalaxyl. Soil and seed applications of metalaxyl (or its active enantiomer, mefenoxam) have generally retained their effectiveness, particularly for control of *Pythium* and the root-infecting species of *Phytophthora*.

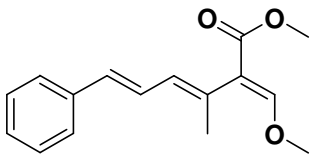


Metalaxyl **Benalaxyl** **Oxadixyl**

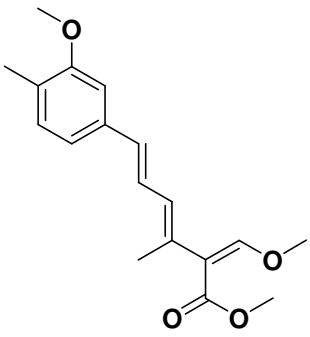
1996-Broad-Spectrum Fungicides with Novel Spectrum and New Mode-of-Action

The natural products **Strobilurin A** and **Oudemansin** were isolated from a saprophytic fungus in the late 1970s. They demonstrated excellent broad-spectrum control of fungal growth. Parallel research programs at ICI and BASF in the early 1980s were focused on invention of synthetic analogues with improved UV stability and spectrum.[29] These strobilurins differed from previous fungicides in combining an unusually broad spectrum (including control of Oomycetes,

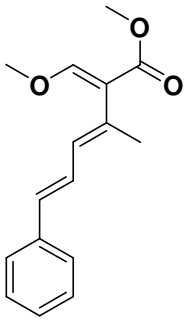
Ascomycetes, and Basidiomycetes) with a site-specific mode-of-action. The first strobilurin products were launched in 1996; Kresoxim-methyl had strong utility on cereals, and Azoxystrobin from Zeneca was suitable for a variety of crops due to its plant safety and strong redistribution. Additional strobilurins, including Trifloxystrobin, Picoxystrobin, and Pyraclostrobin, were launched. These compounds became popular in many markets because of their versatility at controlling diseases from different taxonomic classes, such as powdery and downy mildew on vines, and sheath blight and blast on rice.[30] The fungicide did not showed any side effects as with the triazoles, strobilurins often enhanced plant greening and delayed senescence, leading to improved yields even in the absence of significant disease pressure.[30,31] Some of the strobilurin fungicides commercialized after Azoxystrobin were tailored to the cereal market rather than the vegetable and fruit market, with attributes of long residual protection, vapor phase activity, and moderate redistribution. Widespread use of strobilurins resulted in the development of resistance for several diseases, including wheat, barley, and cucumber powdery mildew, grape and cucumber downy mildew, apple scab, black sigatoka on bananas,[31] and *Septoria* blotch on wheat.[32] Current recommendations for use of strobilurin fungicides limit the number of applications per season, suggest alternation of application with fungicides that have different modes-of-action[31]



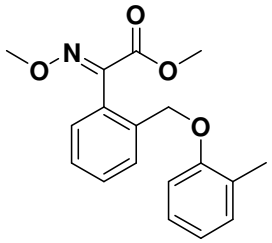
Strobilurin A



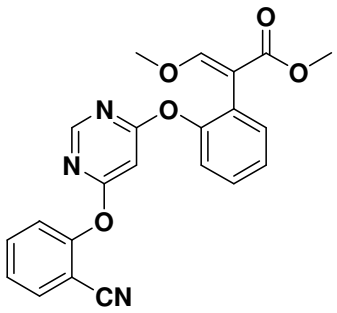
Strobilurin B



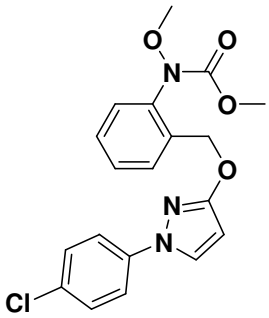
Strobilurin C



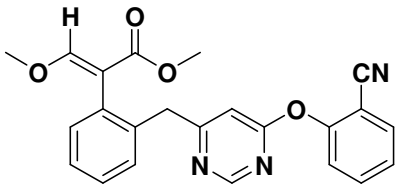
Kresoxim-methyl



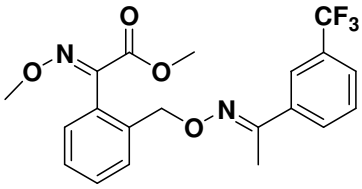
Azoxystrobin



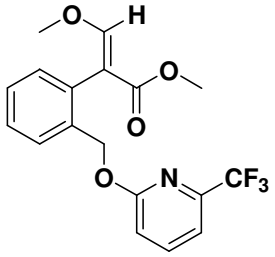
Pyraclostrobin



Azoxystrobin



Trifloxystrobin



Picoxystrobin

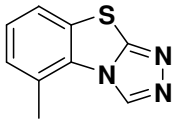
1976-1996-Fungicides with indirect Modes-of-Action

Increasing environmental and regulatory pressures resulted in developing fungicides that act on the plant-pathogen interaction rather than the fungus.[33] These compounds are not toxic to the isolated fungus, and should be more environmentally benign. These compounds inhibited one pathway or the other, for example the first compound developed was Tricyclazole, introduced in 1976. This systemic fungicide (as well as the newer Carpropamid) inhibits melanin biosynthesis, which is required for penetration of the leaf by the appressorium of some fungi.[34] Utility is limited mainly to rice blast. Quinoxyfen, is highly specific for powdery mildews; it also acts by inhibiting the fungus' ability to initiate infection. Molecular studies suggest that Quinoxyfen disrupts the infection process by inhibiting early fungus-plant signaling events and interfering with the fungus' ability to make the morphological changes necessary for infection.[35]

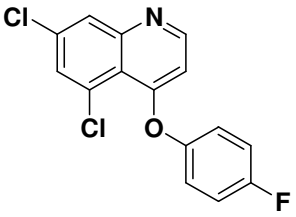
Other fungicides have been commercialized that act through stimulation of the plant's natural defence response. Probenazole is a systemic compound that indirectly controls rice blast and some bacterial rice diseases. It stimulates the accumulation of toxins and enzymes associated with systemic acquired resistance in rice, but is ineffective in other cereals.[18,36] Acibenzolar-S-methyl has the widest spectrum of activity among the non-fungitoxic compounds developed to date. It is active against various fungi, bacteria, and viruses and is highly mobile, with both acropetal and basipetal transport, but is rapidly metabolized.[30] It stimulates the plant's natural defense system, and must be applied as a protectant treatment several days before infection. It has been developed for use against powdery mildews in cereals, rice blast, sigatoka diseases of banana, and blue mold of tobacco.[30]

A challenge for treatments that elicit resistance responses in plants is the potential reduction of yield. Alteration of the production of secondary metabolites in plants has a demonstrated fitness cost in some cases, resulting in diminished plant growth.[37] This yield drag in the absence of disease may limit the future development of compounds that alter plant metabolism, especially if the compound must be applied before disease pressure is significant.

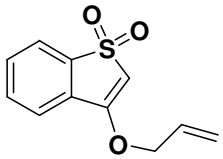
Because these compounds do not place selection pressure directly on fungal growth, they were expected to be more durable than conventional fungicides and unlikely to stimulate resistance development. Resistance has developed, however, for some compounds with indirect modes of action. Tricyclazole has remained effective for three decades, but resistance rapidly developed to Carpropamid,[34] despite both being inhibitors of melanin biosynthesis. Resistance to Quinoxyfen developed in the wheat powdery mildew population in Europe after more than five years of intensive use.[38] On the other hand, the compounds which act by stimulating host resistance have remained effective.



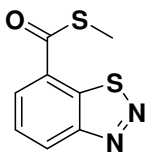
Tricyclazole



Quinoxyfen



Probenazole



Acibenzolar-s-methyl

The above historical perspective clearly reflects applications, advantages, and limitations of commercialized groups of fungicides. Clearly understanding of historical development of fungicides proves the major contribution of natural products in crop protection such as strobilurins. The orientation towards the natural products considers them in terms of safety of both, environment and human health. Additionally, the successful story of strobilurins in crop protection motivated us to work in the area of natural products synthesis, which should contribute in the research of crop protection.

1.5 Natural products as fungicides

1.5.1 Plant essential oils

Several plant essential oils are marketed as fungicides for organic farmers. These include jojoba (*Simmondsia californica*) oil (e.g., E-Rase™), rosemary (*Rosmarinus officinalis*) oil (Sporan™), thyme (*T. vulgaris*) oil (Promax™), clarified hydrophobic extract of neem (*A. indica*) oil (Trilogy™), and cottonseed (*Gossypium hirsutum*) oil with garlic (*Allium sativum*) extract (31% and 23%, respectively in GC-3™). Few scientific papers deal with these products and the actual active components, and their modes of action against individual plant pathogens are largely unknown.

Extract of giant knotweed

An extract of the giant knotweed (*Reynourtria sachalinensis*) (Milsana™) is used in Europe for the control of a wide spectrum of both fungal and bacterial plant diseases in both organic and non-organic agriculture. It is especially effective against powdery mildews and is used primarily on glasshouse and ornamental plants. It is sold as Regalia™ by Marrone Organic Innovations in the US for both food and non-food plants, but the current formulation is not yet accepted for organic agriculture. It apparently acts indirectly by induction of plant defenses.[39,40] Down-regulating chalcone synthase, a key enzyme of the flavonoid pathway, resulted in the nearly complete suppression of induced resistance by this product.[41] The main active elicitor compound(s) of this preparation are physcion and emodin,[42] a known antimicrobial compound.[43] While most of the activity seems to be associated with physcion, the photodynamic compound emodin can also generate reactive oxygen species in the presence of sunlight.[44] Therefore, emodin-dependent oxidative stress may also induce SAR to plant pathogens.[45]

1.5.2. Secondary compounds as source of Pesticides (Fungicides)

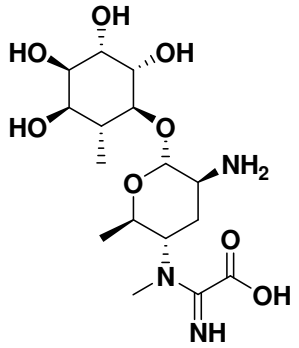
Without an immune system to combat pathogenic microorganisms, plants rely primarily on chemical protection with secondary compounds. Compounds that inhibit the establishment and growth of plant pathogens are termed phytoalexins. Many of these secondary compounds have been chemically characterized and proof is developing that these compounds have such a role in plant disease prevention and control. In fact, there is some evidence that certain synthetic fungicides used in plant protection act by inducing the production of phytoalexins in plants.

Several plant-derived compounds have been demonstrated to be strong elicitors of phytoalexins. For instance, certain oligosaccharide components of cell walls from stressed or dying higher plant cells will act as elicitors. Further knowledge of plant-derived phytoalexin elicitors could lead to their use as fungicides. Few examples are as follows.

1.5.2.1 Kasugamycin

Kasugamycin and kasugamycin hydrochloride hydrate, a systemic fungicide and bactericide with both protectant and curative properties were isolated form the soil actinomycete *Streptomyces kasuganensis* Hamada et al. and were first described by Umezawa et al. in 1965[46] The biological activity of these compounds was first described by Hamada et al.[47] in 1965. Kasugamycin is recommended for the control of rice blast (*P. oryzae*; perfect stage *M. grisea*) in the rice, leaf spot in sugar beet and celery (*Cercospora spp.*), bacterial disease in rice and vegetables and scab (*Venturia spp.*) in apples and pears. Resistance to Kasugamycin was detected within 3 years of its introduction in 1965, and by 1972 it had become a serious problem in Japanese rice field. Today, mixture of Kasugamycin with other fungicides with different mode of action are used. Kasugamycin inhibits hyphal growth of *P.oryzae* on rice. There has been evidence of slight phytotoxicity on crops such as peas, beans, soybeans, grapes, citrus and apples. No injury has

been found on rice, tomatoes, sugar beet, potatoes and many other vegetables. The use of Kasugamycin has declined due to the release of new, disease-specific chemical fungicides for rice blast control. As with other aminoglycoside antibiotics, Kasugamycin is not considered to be toxic to mammals and there is no evidence that it has had any adverse effects on non-target organisms or on the environment.

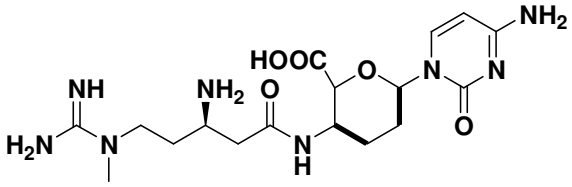


Kasugamycin

1.5.2.2 Blasticidin S

Blasticidin S was isolated from the soil actinomycete, *Streptomyces griseochromogenes* Fukunaga in 1955 by Fukunaga et al,[48] and later by Takeuchi et al,[49] in 1958. Its fungicidal properties were first described by Misato et al.[50]. Blasticidin S is used to control the rice blast, by foliar application. It inhibits protein biosynthesis by binding to the 50S ribosome in prokaryotes, leading to the inhibition of peptidyl transfer and protein chain elongation. It is a contact fungicide with protective and curative action, exhibiting a wide range of inhibitory activity on the growth of bacterial and fungal cells. It inhibits spore germination and mycelial growth of *P. oryzae* in the laboratory at rates below 1 mg mL^{-1}. Excessive application produces yellow spot on rice leaves. In recent years, its significance as a fungicide has decreased following the introduction of new lower-toxicity, pathogen-specific, synthetic rice blast products. Blasticidin-S

is relatively toxic to mammals, with the acute oral LD_{50} to rodents being below 100 mg kg^{-1} . Though a severe eye irritant, it is relatively non-hazardous to non-target organisms when used as labelled and has no deleterious effect on the environment.

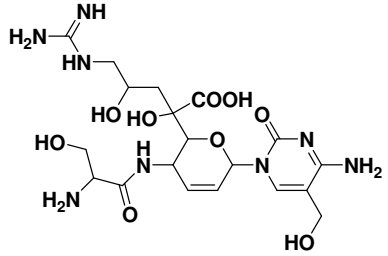


Blasticidin S

1.5.2.3 Mildiomycin

Mildiomycin was isolated from the soil actinomycete *Streptoverticillium rimofaciens* strain B-98 891 and first reported by Harada and Kishi;[51] its fungicidal properties were reported by Kusaka et al. in 1979.[52] Mildiomycin was used to control powdery mildews in ornamentals plants. The mode of action of Mildiomycin is believed to be inhibition of protein biosynthesis in fungi by blocking peptidyl-transferase.[53] In the field, it is effective as an eradicant, with some systemic activity.

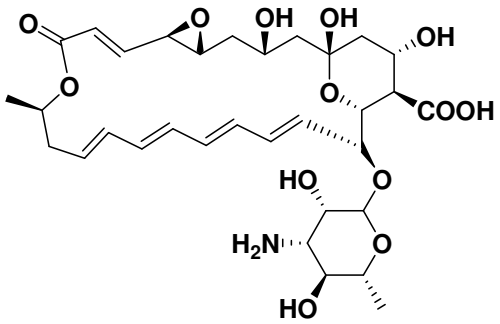
Mildiomycin has very low mammalian toxicity and it has not been shown to have any adverse effects on non-target organisms or the environment. However it has not been widely used for disease control outside Japan.



Mildiomycin

1.5.2.4 Natamycin

Natamycin is a secondary metabolite of the actinomycetes *Streptomyces natalensis.* It has a novel mode of action by binding ergosterol, an integral component of fungal cell membranes, thereby causing membrane dysfunction.[54] Its structure was established by Golding *et al*.[55] and Meyer,[56]and its stereochemistry was revised by Lancelin and Beau[57] and Duplantier and Masamune.[58] It is used to control various fungal diseases, but especially basal rots on ornamental bulbs such as daffodils that are caused by *Fusarium oxysporum* Schlecht. It is usually applied as a dip in combination with a hot water treatment prior to planting. Its precise mode of action is not known. Natamycin is not toxic to mammals or fish and is readily biodegradable.[59,60] No adverse effects have been observed on non-target organisms or on the environment.

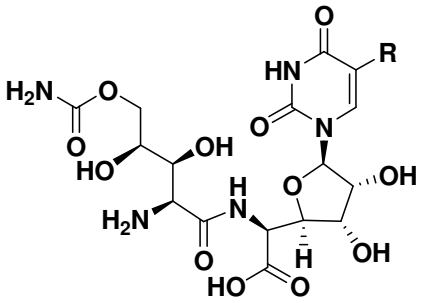


Natamycin

1.5.2.5 Polyoxins

The fungicidal polyoxins are polyoxin B and polyoxorim (BSI, pa ISO). Polyoxin B is one of the secondary metabolites produced by fermentation of the soil actinomycete *Streptomyces cocaoi* var *asoensis* Isono et al. It was first isolated by Isono et al,[61] in 1965 and was introduced as a commercial fungicide. Polyoxorim (polyoxin D) was isolated by Suzuki et al[62] and Isono et al.[63] Polyoxin B is used to control a range of plant pathogenic fungi such as *Sphaerotheca* spp. and

other powdery mildews, *Botrytis cinerea* Pers., *Sclerotinia sclerotiorum* De Bary, *Corynespora melonis* Lindau, *Cochliobolus miyabeanus* (Ito & Kuribay) Drechsler ex Dastur, *Alternaria alternate* (Fr.) Keissler and other *Alternaria* species in vines, apples, pears, vegetables and ornamentals. The primary use of Polyoxorim is for the control of rice sheath blight, *R. solani*. when applied as a foliar spray It is also effective against apple and pear canker [*Nectria galligena Bresadola* (*Diplodia pseudodiplodia* Fuckel)] and *Drechslera* spp., *Bipolaris* spp., *Curvularia* spp. and *Helminthosporium* spp. when applied as a paset, with the major use being in rice, although it also has application in pome fruit and turf. Polyoxins apparently exert their effects through inhibition of cell wall biosynthesis.[64, 65] They are systemic fungicides with protective action. Polyoxin B is ineffective against pear black spot and apple cork spot (*Alternaria* Spp.), grey moulds (*B. cinerea*) an other sclerotia-forming plant pathogens. Resistance to polyoxin B has been found in *A. alternata* in some orchards in Japan following intensive treatment. It is also ineffective against bacteria and yeasts.

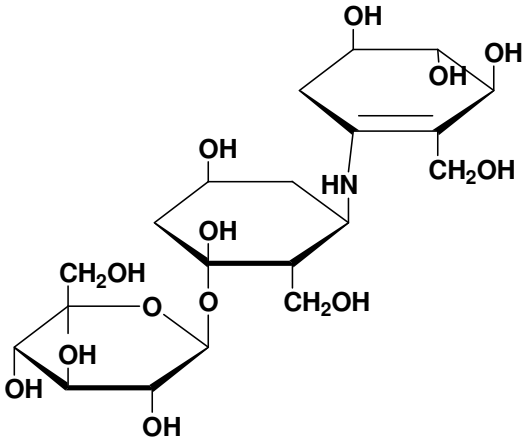


Polyoxin B, R =CH_2OH
Polyoxorim, R = COOH

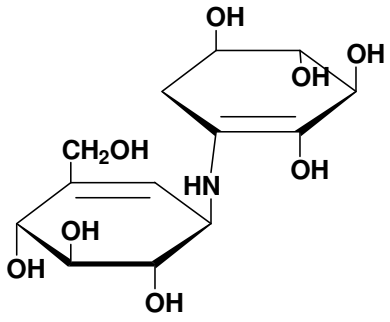
1.5.2.6 Validamycin

Validamycin (also known as validamycin A) was isolated from the soil actinomycete *Streptomyces hygroscopicus* (Jensen) Waskman & Henrici isolate *limoneus*. Its biological activity was described by Horii et al.[65] Validamycin is used for the control of *R. solani* and other *Rhizoctonia* species in rice, potatoes, vegetables, strawberries, tobacco, ginger, cotton, rice, sugar

beet and other crops. Validamycin is non-systemic, but has a fungistatic action, showing non fungicidal action against *R. solani*, but causing abnormal branching of the tips of pathogen followed by a cessation of further development.[66] Validamycin has a potent inhibitory activity against trehalase in *R. solani* AG-1, without any significant effects on other glycohydrolytic enzymes tested.[67] Very low rates of use give excellent control of *R. solani* in various crops, and rates of 0.3 g AIL^{-1} give effective control of rice sheath blight. It is applied as a foliar spray, a soil drench, a seed treatment or by soil incorporation. Validamycin continues to find wide range of application in a wide variety of crops, particularly in Japan. Validamycin is not considered to be toxic to mammals and has no adverse effects on non-target organisms or on the environment.



Validamycin A

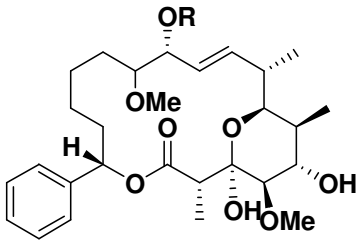


Validoxylamine A

1.5.2.7 Soraphen A

Soraphen A was discovered by the research groups of Reichenbach and Hofle at GBF[68], in their screening of extract form myxobacteria, a sample from *Sorangium cellulosum* strain So ce26. The new metabolite Soraphen A which was mainly responsible for the activity, showed broad antifungal *in vitro* activity. The total synthesis of Soraphen A was achieved by Giese and

coworkers.[69] Greenhouse tests revealed the high potential of soraphen as a plant protection agent against fungal pathogens. Field tests met the high hopes generated by the greenhouse results, and economical application rates seemed feasible.



Soraphen A R = CH_3

1.6. Herbicides

1.6.1 Introduction

Throughout the history of agriculture, more time, energy and money have been devoted to weed control than to any other agricultural activity. While there are records of ancient civilizations using chemicals such as common salt and wood ashes to control unwanted vegetation, in 1896 a french wine grower sprayed $CuSO_4$+CaO to control diseases on his vine. He also observed a beneficial side effect in that a weed, *Sinapis arvensis* L., exposed to the spray died. One year later another french grower discovered the herbicidal activity of H_2SO_4 that could selectively control some weeds without injuring the crops. These discoveries were in fact some of the first successful chemical weed control methods. Until recently, H_2SO_4 was used as a herbicide in many parts of Europe. Several other inorganic compounds were introduced for a shorter period. The first organic herbicide, DNOC, was patented by George Truffaut and K. Pastac in 1932 for use as a selective herbicide in cereals.

The rapid development of modern herbicides, experienced over the last 50 years, was stimulated by the need for increasing food production during World War II. The shortage of food in the UK

made agricultural researchers take advantage of previous discoveries of auxins. Auxins are growth substances that increase plant growth, primarily by promoting cell elongation. Unknown to each other, workers at Jealott's Hill Research Station and Rothamsted Experimental Station studied the possibilities to boost crop production with putative synthetic auxins, chlorinated phenoxyacetic acids. The researchers were capable of realizing when novel results were serendipitous. Both teams reported remarkable herbicidal activity. The substances appeared to act like an over-dose of auxin on the weeds, but did not harm the cereal crop.

With the return of peace the original findings, together with experiences of independent work from the USA, were published.[70] The phenoxyacetic acids were fundamentally different from the other organic compounds, for example DNOC; they were systemic in that they had to be translocated in the plant to exert their action. Soon after the release of MCPA and 2,4-D, 2,4,5-T was also released and a new era of weed control began. Within a few years, screening tests of literally thousands of putative herbicidal substances were set up that formed the basis for rapid development of new herbicides to control specific weed problems in specific crops. New important herbicide groups were developed. The s-triazines[71] were reported to have unique effects compared with existing classes of compounds in 1955, and three years before some substituted ureas were found to be potent inhibitors of photosynthesis.[72] For many years about half the commercialized herbicides were inhibitors of photosynthesis.

In 1971 glyphosate, a competitive inhibitor of an enzyme of the shikimic acid pathway, was developed as a herbicide, and this compound has played a pivotal role in changing the market of herbicides. Only a few years after their discovery, the site of action of sulfonylureas[73] was found to be inhibition of the enzyme acetolactate synthase that forms a part of the combined pathway responsible for the biosynthesis of valine, leucine and isoleucine. The absence of this enzyme in

man and other animals helps explain the low toxicity of the sulfonylureas. Because of their specific site of action and variety in molecular structures, sulfonylureas are potential candidates for tailoring chemistry to fit the crops. This specificity has also resulted in the unintentional development of sulfonylurea resistant biotypes of weeds after only a few years of use.

1.6.2 Classification of Herbicides

Herbicides can be classified in many ways such as application method (pre-emergence, post emergence or post direct), selectivity (selective or non selective), mode of action etc. Classifications based on the mode of action will be discussed here in details.

1.6.2.1 Acetyl CoA Carboxylase (ACCase) Inhibitors

Aryloxyphenoxypropionate (FOPs) and cyclohexanedione (DIMs) herbicides inhibit the enzyme acetyl-CoA carboxylase (ACCase), the enzyme catalyzing the first committed step in *de novo* fatty acid synthesis[74, 75] Inhibition of fatty acid synthesis presumably blocks the production of phospholipids used in building new membranes required for cell growth. Broadleaf species are naturally resistant to cyclohexanedione and aryloxyphenoxy propionate herbicides because of an insensitive ACCase enzyme. Similarly, natural tolerance of some grasses appears to be due to a less sensitive ACCase.[76] An alternative mechanism of action has been proposed involving destruction of the electrochemical potential of the cell membrane, but the contribution of this hypothesis remains in question.

1.6.2.2 Acetolactate Synthase (ALS) or Acetohydroxyacid Synthase (AHAS) Inhibitors

Imidazolinones, pyrimidinylthiobenzoates, sulfonylaminocarbonyltriazolinones, sulfonylureas, and triazolopyrimidines are herbicides that inhibit acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine[77] Plant death results from events occurring in

response to ALS inhibition and low branched-chain amino acid production, but the actual sequence of phytotoxic processes is unclear.

1.6.2.3 Photosystem II Inhibitors

Phenylcarbamates, pyridazinones, triazines, triazinones, uracils, amides, ureas, benzothiadiazinones, nitriles, and phenylpyridazines, are examples of herbicides that inhibit photosynthesis by binding to the QB-binding niche on the D1 protein of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at this protein location blocks electron transport from Q_A to Q_B and stops CO_2 fixation and production of ATP and $NADPH_2$ which are all needed for plant growth. However, plant death occurs by other processes in most cases. Inability to reoxidize Q_A promotes the formation of triplet state chlorophyll which interacts with ground state oxygen to form singlet oxygen. Both triplet chlorophyll and singlet oxygen can abstract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid peroxidation. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids and in leaky membranes which allow cells and cell organelles to dry and disintegrate rapidly. Some compounds in this group may also inhibit carotenoid biosynthesis (fluometuron) or synthesis of anthocyanins, RNA, and proteins (propanil), as well as effects on the plasmalemma (propanil).[78]

1.6.2.4 Photosystem I Inhibitors

Bipyridyliums are examples of herbicides that accept electrons from photosystem I and are reduced to form an herbicide radical. This radical then reduces molecular oxygen to form superoxide radicals. Superoxide radicals then react with themselves in the presence of superoxide dismutase to form hydrogen peroxides. Hydrogen peroxides and superoxides react to generate hydroxyl radicals. Superoxides and, to a lesser extent, hydrogen peroxides may oxidize

SH (sulfhydryl) groups on various organic compounds within the cell. Hydroxyl radical, however, is extremely reactive and readily destroys unsaturated lipids, including membrane fatty acids and chlorophyll. Hydroxyl radicals produce lipid radicals which react with oxygen to form lipid hydroperoxides plus another lipid radical to initiate a self-perpetuating chain reaction of lipid oxidation. Such lipid hydroperoxides destroy the integrity of cell membranes allowing cytoplasm to leak into intercellular spaces which leads to rapid leaf wilting and desiccation. These compounds can be reduced/oxidized repeatedly.[79]

1.6.2.5 Protoporphyrinogen Oxidase (PPG oxidase or Protox) Inhibitors

Diphenylethers, *N*-phenylphthalimides, oxadiazoles, oxazolidinediones, phenylpyrazoles, pyrimidinediones, thiadiazoles, and triazolinones are herbicides that appear to inhibit protoporphyrinogen oxidase (PPG oxidase or Protox), an enzyme of chlorophyll and heme biosynthesis catalyzing the oxidation of protoporphyrinogen IX (PPGIX) to protoporphyrin IX (PPIX). Protox inhibition leads to accumulation of PPIX, the first light-absorbing chlorophyll precursor. PPGIX accumulation apparently is transitory as it overflows its normal environment in the thylakoid membrane and oxidizes to PPIX. PPIX formed outside its native environment probably is separated from Mg chelatase and other pathway enzymes that normally prevent accumulation of PPIX. Light absorption by PPIX apparently produces triplet state PPIX which interacts with ground state oxygen to form singlet oxygen. Both triplet PPIX and singlet oxygen can abstract hydrogen from unsaturated lipids, producing a lipid radical and initiating a chain reaction of lipid peroxidation. Lipids and proteins are attacked and oxidized, resulting in loss of chlorophyll and carotenoids and in leaky membranes which allows cells and cell organelles to dry and disintegrate rapidly[80]

1.6.2.6 Carotenoid Biosynthesis Inhibitors

Amides, anilides, furanones, phenoxybutanamides, pyridazinones, and pyridines (F1(12)) are examples of compunds that block carotenoid biosynthesis by inhibition of phytoene desaturase[81,82] Carotenoids play an important role in dissipating the oxidative energy of singlet O_2 (^{1}O2). In normal photosynthetic electron transport, a low level of photosystem II reaction center chlorophylls in the first excited singlet state transform into the excited triplet state (^{3}Chl). This energized (^{3}Chl) can interact with ground state molecular oxygen (O_2) to form $^1O_2$. In healthy plants, the energy of $^1O_2$ is safely quenched by carotenoids and other protective molecules. Carotenoids are largely absent in fluridone-treated plants, allowing $^1O_2$ and ^{3}Chl to abstract a hydrogen from an unsaturated lipid (e.g. membrane fatty acid, chlorophyll) producing a lipid radical. The lipid radical interacts with O_2 yielding a peroxidized lipid and another lipid radical. Thus, a self-sustaining chain reaction of lipid peroxidation is initiated which functionally destroys chlorophyll and membrane lipids. Proteins also are destroyed by $^1O_2$. Destruction of integral membrane components leads to leaky membranes and rapid tissue desiccation.

Callistemones, isoxazoles, pyrazoles, and triketones are examples of herbicides that inhibit *p*-hydroxyphenyl pyruvate dioxygenase (HPPD), which converts *p*-hydroxymethyl pyruvate to homogentisate. This is a key step in plastoquinone biosynthesis and its inhibition gives rise to bleaching symptoms on new growth. These symptoms result from an indirect inhibition of carotenoid synthesis due to the involvement of plastoquinone as a cofactor of phytoene desaturase.

Recent evidence suggests that clomazone is metabolized to the 5-keto form of clomazone which is herbicidally active. The 5-keto form inhibits 1-deoxy-D-xyulose-5-phosphate synthase

(DOXP), a key component to plastid isoprenoid synthesis. Clomazone does not inhibit geranylgeranyl pyrophosphate biosynthesis[83, 84]

Amitrole inhibits accumulation of chlorophyll and carotenoids in the light,[85] although the specific site of action has not been determined. Precursors of carotenoid synthesis, including phytoene, phytofluene, carotenes, and lycopene accumulate in amitrole-treated plants[86] suggesting that phytoene desaturase, lycopene cyclase, imidazoleglycerol phosphate dehydratase, nitrate reductase, or catalase may be inhibited. Other research[87] however, indicates that the histidine, carotenoid, and chlorophyll biosynthetic pathways probably are not the primary sites of amitrole action. Instead, amitrole may have a greater effect on cell division and elongation than on pigment biosynthesis.

Aclonifen appears to act similar to carotenoid inhibiting/bleaching herbicides; but the exact mechanism of action in unknown.

1.6.2.7 Enolpyruvyl Shikimate-3-Phosphate (EPSP) Synthase Inhibitors

Glycines (glyphosate) are herbicides that inhibit 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase[88] which produces EPSP from shikimate-3-phosphate and phosphoenolpyruvate in the shikimic acid pathway. EPSP inhibition leads to depletion of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, all needed for protein synthesis or for biosynthetic pathways leading to growth. The failure of exogenous addition of these amino acids to completely overcome glyphosate toxicity in higher plants[89, 90] suggests that factors other than protein synthesis inhibition may be involved. Although plant death apparently results from events occurring in response to EPSP synthase inhibition, the actual sequence of phytotoxic processes is unclear.

1.6.2.8 Glutamine Synthetase Inhibitors

Phosphinic acids (glufosinate and bialophos) inhibit activity of glutamine synthetase,[91] the enzyme that converts glutamate and ammonia to glutamine. Accumulation of ammonia in the plant[92] destroys cells and directly inhibits photosystem I and photosystem II reactions.[93] Ammonia reduces the pH gradient across the membrane which can uncouple photophosphorylation.

1.6.2.9 Dihydropteroate Synthetase Inhibitors

The carbamate herbicide, asulam, appears to inhibit cell division and expansion in plant meristems, perhaps by interfering with microtubule assembly or function[94,95] Asulam also inhibits 7,8-dihydropteroate synthase, an enzyme involved in folic acid synthesis which is needed for purine nucleotide biosynthesis[96,97]

1.6.2.10 Mitosis Inhibitors

Benzamide, benzoic acid (DCPA), dinitroaniline, phosphoramidate, and pyridine herbicides are examples of herbicides that bind to tubulin, the major microtubule protein. The herbicide-tubulin complex inhibits polymerization of microtubules at the assembly end of the protein-based microtubule but has no effect on depolymerization of the tubule on the other end[98] leading to a loss of microtubule structure and function. As a result, the spindle apparatus is absent, thus preventing the alignment and separation of chromosomes during mitosis. In addition, the cell plate cannot be formed. Microtubules also function in cell wall formation. Herbicide-induced microtubule loss may cause the observed swelling of root tips as cells in this region neither divide nor elongate.

The carbamate herbicides, carbetamide, chlorpropham, and propham are examples of herbicides that inhibit cell division and microtubule organization and polymerization.

Acetamide, chloroacetamide, oxyacetamide, and tetrazolinone herbicides are examples of herbicides that are currently thought to inhibit very long chain fatty acid (VLCFA) synthesis[99, 100]. These compounds typically affect susceptible weeds before emergence, but do not inhibit seed germination.

1.6.2.11 Cellulose Inhibitors

Benzamides, nitriles, quinclorac, and triazolocarboxamides,are herbicides that inhibits cell wall biosynthesis (cellulose) in susceptible weeds[101]

1.6.2.12 Oxidative Phosphorylation Uncouplers

Dinitrophenols (dinoterb) are herbicides that uncouple the process of oxidative phosphorylation causing almost immediate membrane disruption and necrosis.

1.6.2.13 Fatty Acid and Lipid Biosynthesis Inhibitors

Benzofuran acids, phosphorodithioates, and thiocarbamates are examples of herbicides that are known inhibitors of several plant processes including: 1) biosynthesis of fatty acids and lipids which may account for reported reductions in cuticular wax deposition, 2) biosynthesis of proteins, isoprenoids (including gibberellins), and flavonoids (including anthocyanins), and 3) gibberellin synthesis inhibition which may result from the inhibition of kaurene synthesis. Photosynthesis also may be inhibited.[102] A currently viable hypothesis that may link all these effects involves the conjugation of acetyl coenzyme A and other sulfydryl-containing biomolecules by thiocarbamate sulfoxides[103, 104] The sulfoxide forms may be the active herbicides.[105]

1.6.2.14 Synthetic Auxins

Benzoic acids, phenoxycarboxylic acids, pyridine carboxylic acids, and quinoline carboxylic acids are herbicides that act similarly to endogenous auxin (IAA) although the true mechanism is

not well understood. The specific cellular or molecular binding site relevant to the action of IAA and the auxin-mimicking herbicides has not been identified. Nevertheless, the primary action of these compounds appears to affect cell wall plasticity and nucleic acid metabolism. These compounds are thought to acidify the cell wall by stimulating the activity of a membrane-bound ATPase proton pump. The reduction in apoplasmic pH induces cell elongation by increasing the activity of enzymes responsible for cell wall loosening. Low concentrations of auxin-mimicking herbicides also stimulate RNA polymerase, resulting in subsequent increases in RNA, DNA, and protein biosynthesis. Abnormal increases in these processes presumably lead to uncontrolled cell division and growth, which results in vascular tissue destruction. In contrast, high concentrations of these herbicides inhibit cell division and growth, usually in meristematic regions that accumulate photosynthate assimilates and herbicide from the phloem. Auxin-mimicking herbicides stimulate ethylene evolution which may in some cases produce the characteristic epinastic symptoms associated with exposure to these herbicides.

1.6.2.15 Auxin Transport Inhibitors

Phthalamates (naptalam) and semicarbazones (diflufenzopyr) are compounds that inhibit auxin transport. These compounds inhibit polar transport of naturally occurring auxin, indoleacetic acid (IAA) and synthetic auxin-mimicking herbicides in sensitive plants. Inhibition of auxin transport causes an abnormal accumulation of IAA and synthetic auxin agonists in meristematic shoot and root regions, disrupting the delicate auxin balance needed for plant growth. When diflufenzopyr is applied with dicamba, it focuses dicamba's translocation to the meristematic sinks, where it delivers effective weed control at reduced dicamba rates and across a wider range of weed species. Sensitive broadleaf weeds exhibit rapid and severe plant hormonal effects (e.g., epinasty) after application of the mixture; symptoms are visible within hours, and plant death

usually occurs within a few days. Symptomology, in sensitive annual grasses, is characterized by a stunted growth. Tolerance in corn occurs through rapid metabolism of diflufenzopyr and dicamba.

1.6.2.16 Potential Nucleic Acid Inhibitors or Non-descript mode of action

Several herbicides have been identified as having an unknown mode of action including the pyrazoliums, organic arsenicals, arylaminopropionic acids, and other non-classified herbicides.

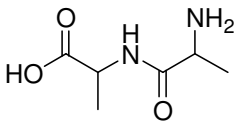
1.7. Natural Herbicides

Organic agriculture does not allow synthetic pesticides, including herbicides.[106,107] Weed management under organic agriculture practices is very problematic. While most methods rely on soil cultivation, hand hoeing, biocontrol, organic mulches, and ironically plastic (synthetic) ground cover, the use of some natural products is permitted (Table 1.1).**Table 1.1** Examples of commercial products containing natural products used for weed management in agriculture.

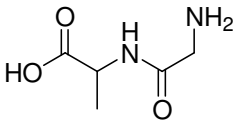
As opposed to synthetic herbicides, the available natural herbicides have little to no selectivity and they must be applied in relatively large quantities. Furthermore, little scientific literature is available on the use and environmental impact of natural products in organic agriculture.

1.7.1 Corn gluten meal

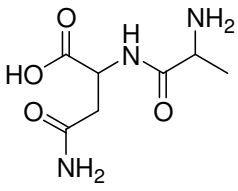
Corn (*Zea mays*, L.) gluten meal is a byproduct of corn milling. It is commercialized as both a fertilizer and a pre-emergence herbicide on lawns and high-value crops.[108-110]



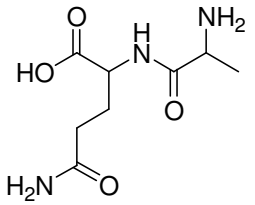
L-alanyl alanine



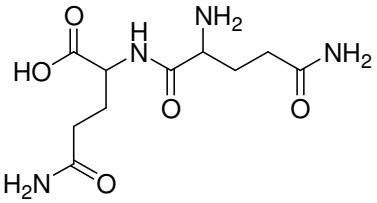
L-glucinyl alanine



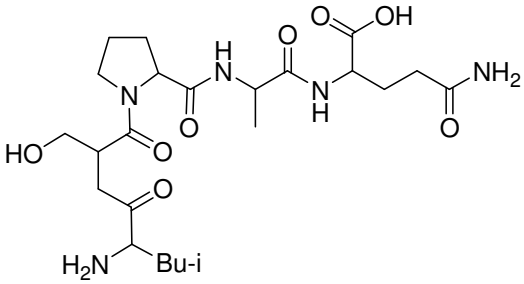
L-alaninyl-asparagine



L-alaninyl-glutamine



L-glutaminyl-glutamine



[(L-leucyl-L-seryl)-L-propyl]-L-alanyl]-L-glutamine

Fig.1.2.1 Structures of the phytotoxic oligopeptides in the hydrolysate of corn gluten meal used for weed management in organic agriculture.

The commercial products contain between 50% and 100% of corn gluten and are sold under a variety of trade names (Table 1.1). However, control of grasses and other weeds requires extremely high rates (e.g., 2 tons per hectare) and is often cost prohibitive. Corn gluten has no effect on existing weeds, but it has a broad-spectrum of activity on the germination and development of young emerging plants.[111, 112] Hydrolysis of corn gluten by soil microbes releases several phytotoxic dipeptides[113,114] and a phytotoxic pentapeptide (Fig. 1.2.1).[115] The exact mode of action of these oligopeptides is not known but they affect cell wall formation, membrane integrity, and nuclear development.[115] Corn gluten may be considered a slow-release proherbicide since it must be hydrolyzed to release the active ingredients.

Products	Components
WeedBan™	Corn gluten meal
Corn Weed Blocker™	
Bioscape	Corn gluten meal, soybean oil
Bioweed™	
Scythe™	Pelargonic acid (57%), related short chain fatty acids (3%), paraffinic petroleum oil (30%)
Burnout™	Clove oil (12–18%), sodium lauryl sulfate (8–10%), acetic acid, lecithin, citric acid (30%), mineral oil (80%)
Bioganic™	
Poison Ivy Defoliant™	
Bioorganic™ (4%)	Clove oil (5%), 2-phenethyl propionate (5%), sesame oil and sodium lauryl sulfate (0.5%)
AllDown™ (0.2%)	Citric acid (5%), acetic acid, yucca extracts, garlic oil
Interceptor™	10% pine oil
Weed Zap™	Clove oil or cinnamon oil (30%), vinegar (70%)
Weed-A-Tak™	Citric acid (32%), clove oil (8%), cinnamon oil (8%), 2-phenethyl propionate, lecithin. It may contain thyme oil, and wintergreen oil.
Repellex®	
Moss & Algae Killer™	Potassium salts of fatty acids (40%)
Naturell WK	

Herbicide™	
DeMoss™	
Mosskiller™	
Organic Weed & Grass Killer™	Citrus oil (70%)
GreenMatch O™	D-Limonene (70%), castor oil (1 to 4%), emulsifiers (18 to 23%)
Nature's Avenger™	
GreenMatch EX™	Lemongrass oil (50%) and a mixture of water, corn oil, glycerol esters, potassium oleate and lecithin
Matran II™	Clove oil (46%), wintergreen oil, butyl lactate, lecithin
Eco-Exempt™ Eco-Smart™	2-Phenethyl propionate (21.4%), clove oil (21.4%)

Table 1.1

1.7.2 Acetic acid

Acetic acid (Fig. 1.2.2) has been used as a weed control agent for several centuries. Acetic acid is a burndown, non-selective herbicide. Therefore, it is used for non-cropland areas, such as railway rights-of-way, golf courses, open space, driveways and industrial sites. Acetic acid solutions (10–20%) provide greater than 80% control of most small weeds.[116] However, the cost of applying acetic acid was more than ten times greater than the cost of using the more effective synthetic non-selective herbicide glyphosate (N-(phosphonomethyl) glycine) for roadside vegetation management. As is common with burn down herbicides, acetic acid kills the aerial portions of plants, but does not control the underground parts, and plants typically reemerge from

the root system after a few days or weeks. Oil adjuvants do not significantly increase the herbicidal activity of acetic acid. Although acetic acid is applied at relatively high concentrations, it does not have a long term negative influence on soil microorganisms.[117] Acetic acid can also be used to control invasive aquatic weeds. It kills propagules of hydrilla (*Hydrilla verticillata*), sago pondweed (*Stuckenia pectinatus*), and smooth cordgrass (*Spartina alterniflora*)[118,119] Careful treatments of lake sediments with acetic acid may have utility as an alternative to foliarly applied herbicides such as imazapyr and glyphosate.[119]

1.7.3 Fatty acids

The herbicidal activity of fatty acids has been known for many years [120,121] and some fatty acid salts are now marketed as non-selective herbicidal soaps. These are composed of fatty acids of various aliphatic length mixed with vinegar or acetic acid (Section 2.1.2) and emulsifiers such as organosilicones, saponified, methylated, and ethylated seed oil activator adjuvants. Herbicidal soaps act relatively rapidly and have no selectivity (broad-spectrum weed control). However, most weeds tend to recover because there is no residual activity after the initial burndown effect which takes place soon after application.[122] As such, these mixtures can be used as desiccants. Fatty acids with midrange aliphatic tails such as caprylic (C8, octanoic acid) and pelargonic acid (C9) are the most effective.[123] Pelargonic acid (Fig. 1.2.2) is a contact, broadspectrum commercial herbicide for control of annual weeds, mosses (*Bryum argenteum*) and liverwort (*Marcbantia polymorpha*).[124,125] It disrupts plant cell membranes, causing rapid loss of cellular function.[125] When saturated fatty acids from C6 to C14 were compared, the C9–C11 fatty acids were especially active, whereas the others were significantly less active.[126] C6 and C14 fatty acids had essentially no herbicidal activity. Pelargonic acid itself is considered a low toxicity and environmental impact herbicide.[120] It has no residual activity. Adding organic acids such as

succinic, lactic or glycolic acid enhance the efficacy of pelargonic acid formulations.[127] Potassium salts of fatty acid (up to 40%) preparations are commercialized, effective non-selective herbicides used for controlling mosses and liverworts (Table 1.1). Oleic acid is usually a major component of these mixtures, though the exact compositions of these products are not well publicized.

1.7.4 Essential oils

Essential oils have also shown some potential as herbicides. Surfactants, which are also limited in organic agriculture, are often required to assist in the spreading of the material. Since most essential oils commercialized for natural weed control consist of mixtures, it is difficult to cover the numerous formulations available. All commercialized essential oils act as non-selective, contact herbicides (burn down) that can provide good but transient weed control. The use of essential oils for weed control in organic agriculture seems promising, but these natural herbicides all act very rapidly and their efficacy is limited by the fact that they most likely volatilize relatively quickly. Alternative formulations, such as microencapsulation, are being developed to reduce the amounts applied, increase the duration of their effectiveness by reducing their volatilization, simplify the handling of material, and slow down the rate of degradation in the environment.[128]

1.7.4.1 Pine oil

Pine oil composed of terpene alcohols and saponified fatty acids is sold as a 10% aqueous emulsion for weed control (Table 1.1)[116] under the trade name of Interceptor by Certified Organics Ltd, Auckland, New Zealand. As with other natural product derived weed control, pine oil did not provide the level of control obtained with a single application of glyphosate.[116] It is considered largely non-toxic to humans and the environment, but may have minor effects on

aquatic organisms. It breaks down almost completely within 3 days of application. It is a fast-acting, non-selective contact herbicide that is not translocated.

1.7.4.2 Clove oil

The essential oil obtained by steam distillation of clove (*Eugenia caryophyllus* Spreng) leaves [CAS 8000-34-8] contains primarily eugenol (Fig. 1.2.2) together with several other terpenoids. Clove oil is commercialized for weed control under several forms (Table 1.1). For example, Matran® contains up to 50% clove oil and Burnout II® consists of a mixture of 12% clove oil with acetic acid. Clove oil has also been formulated for the control of poison ivy (*Rhus radicans* L.). Clove oil applied at concentrations of 1–5% controlled most small weeds,[129] but the relatively high rate required for control makes this treatment expensive, even in high-value vegetable production systems. Clove oil is a contact, non-selective foliar herbicide that will only control above-ground, green vegetation. It does not translocate. It causes rapid loss of cellular membrane integrity. Clove oil has low oral and dermal toxicity and has relatively little potential environmental effects.

1.7.4.3 Phenethyl propionate

2-Phenethyl propionate (Fig. 1.2.2) is a component of peppermint (*Mentha piperita*, L.) oil, which is also rich in menthol and menthone (Fig. 1.2.2).[130] 2-Phenethyl propionate has been patented as a herbicide[131] and can be found as a component of the formulations of natural herbicides (Table 1.1). This product must be diluted before application and its use recommendations are similar to those of clove oil or eugenol (see above Section 2.1.4.2). This compound is thought to be very safe to the environment and to human health, as it is used in food flavorings.

1.7.4.4 Lemongrass oil

Lemongrass (*Cymbopogon citratus* Stapf. or C. *flexuosus* D.C.) oil (Table 1.1) has recently been commercialized as an organic herbicide, but its potential use for weed control was first patented in England in 1924.[132] The main component (80%) of this oil is citral (Fig. 1.2.2).[133] A commercial product containing 50% lemongrass oil must be diluted to 7–15% before application. Lemongrass oil acts as a contact herbicide, and since the active ingredient (citral) does not translocate, only the portions of plants receiving the spray solution are affected. Application of lemongrass oil apparently provides weed control that is superior to that obtained with application of products containing technical grade D-limonene (Fig. 1.2.2) alone. D-Limonene is known to remove the waxy cuticular layer from the leaves of the plants treated, causing rapid dehydration and death of the tissues.

1.7.4.5 Citronella oil

Citronella oil, which is best known for its use as a mosquito repellent, has been tested as a herbicide in tree nurseries.[134] This oil is obtained from several sources, but *Cymbopogon* spp. are the most common. The primary components are citronellal (42%), geraniol (21%) and other terpenes (Fig. 1.2.2). Tests done in tree nurseries showed that citronella oil provided some weed control while not causing adverse effects on dormant broadleaf trees; however, conifer species were very sensitive to this treatment.

1.7.4.6 Other essential oils

Many other plant essential oils show potential as natural herbicides, but these remain to be commercialized. Eucalyptus oil extracted from *Eucalyptus citriodora* has been tested as a potential natural herbicide. This phytotoxic oil consists primarily of citronellal (77%) (Fig. 1.2.2) and other small terpenes.[135] In particular, eucalyptus proved to be effective as an alternative

control of little seed canarygrass (*Phalaris minor* Retz.).[136] Natural oils from neem (*Azadirachta indica* Juss.), coconut (*Cocos nucifera* L.), and sunflower (*Helianthus annuus* L.) controlled the parasitic weed within 2–3 days of application. Castor (*Ricinus communis* L.) and niger (*Guizotia abyssinica* (L. fil.) Cass.) oils killed the weed within 3–4 days, and mustard (*Brassica juncea* (L.) Czernjaew) oil required 5 days to kill the bud.[137] Essential oils of various varieties of oregano (*Origanum* spp.) and basil (*Ocimum basilicum*) have been tested against barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) and common lambsquarter (*Chenopodium album* L.) with some success.[138] These oils, which are composed primarily of *p*-cymene (20–25%), γ-terpinene (15–20%), thymol (10–35%), have been patented for moss control (Fig. 1.2.2).[139] Manuka oil is isolated from the leaves of *Leptospermum scoparium*.

It is composed of sesquiterpenes (up to 70%) [140] and is rich in ß-triketones.[141, 142] Leptospermone (Fig. 1.2.2), the most abundant triketone of these oils, causes bleaching of the foliage of grasses and broadleaf plants.[143, 144] These natural triketones are structurally similar to some synthetic herbicides (e.g., sulcotrione and mesotrione) and have the same molecular target site, namely *p*-hydroxyphenylpyruvate dioxygenase (HPPD).[145, 146]

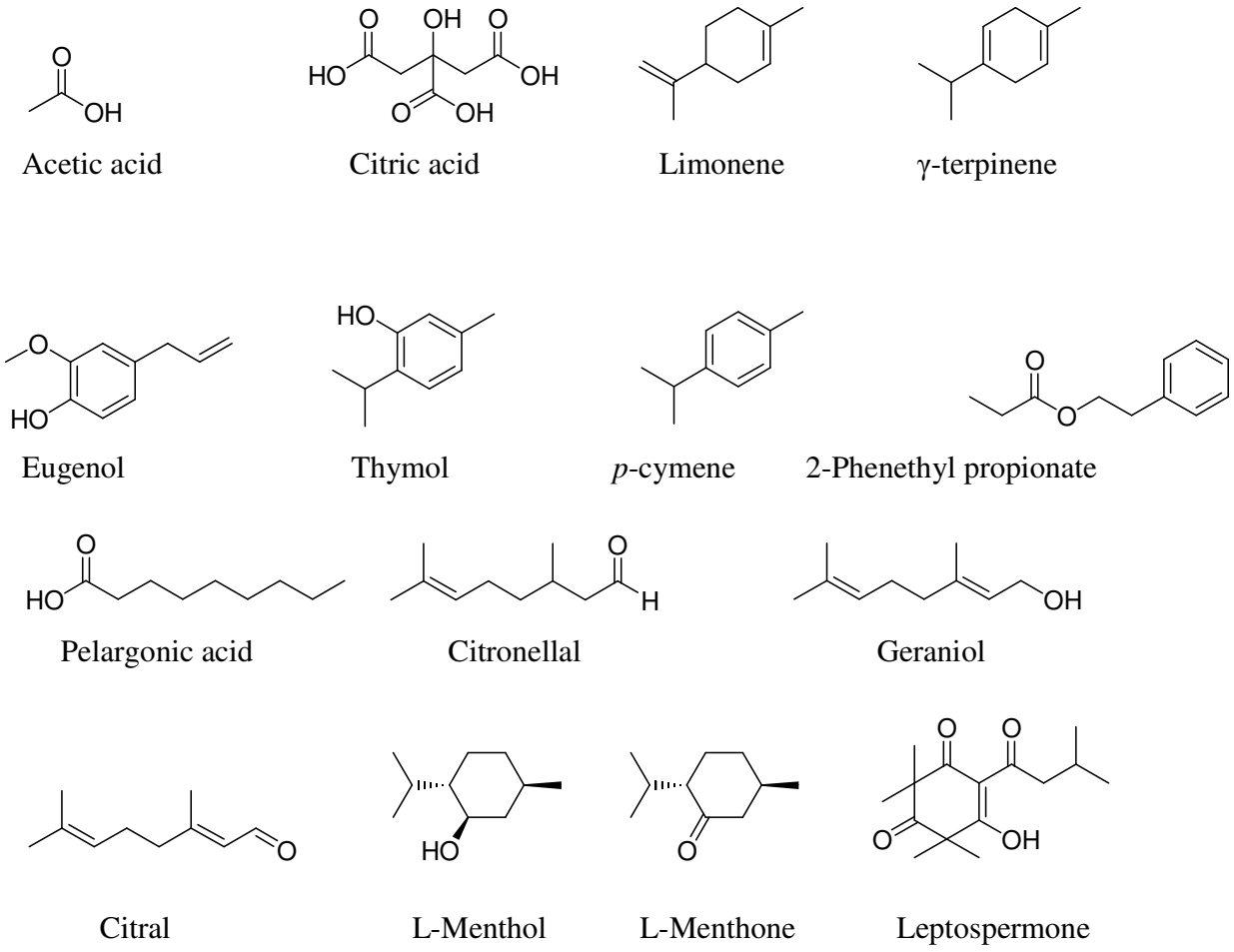


Fig. 1.2.2 Structures of the natural herbicides or main components of mixtures used for weed management in organic agriculture.

1.8 Allelopathy

Theophrastus (371–287 B.C.) wrote about the inhibitory effect of pigweed on alfalfa. As early as 1832, the Swiss botanist De Candolle suggested that 'soil sickness' associated with plants grown in some rotations was due to exudates of crops. The deleterious effect of black walnut trees on the growth of plants in the surrounding was reported fifty years later.[147] It took another fifty years to coin the term allelopathy, which was constructed from the two Greek words *allelo* and *pathy*, to mean mutual harm.[148] This definition was later expanded by Rice to include both inhibitory and stimulatory effects of one plant (or microorganism) upon another via a chemical

(allelochemical).[149] While allelopathy does not involve the direct application of natural products for weed management, and other factors such as competition for resources undoubtedly contribute to the overall control of weeds, this small section highlights instances where specific allelochemicals were identified as being the primary molecules involved in weed control by crops. For recent reviews see ref.[150, 151]

1.8.1 Momilactone B

A significant effort to generate highly allelopathic rice varieties is underway.[152, 153] Thousands of varieties have been screened for allelopathic potential, and up to 4% of varieties can suppress important paddy field weeds. While the level of weed management obtained to date is not equivalent to that obtained with herbicides, herbicide use rates can be substantially reduced in paddy fields planted with allelopathic varieties.[154] Momilactone B plays a key role in rice (*Oryza* spp.) allelopathy[155] (Fig. 1.2.4). Some rice varieties release up to 2–3 μg of momilactone/plant/day,[156] which is sufficient to inhibit the germination and growth of neighboring weeds.[157] Nothing is known about the mode of action of momilactone B.

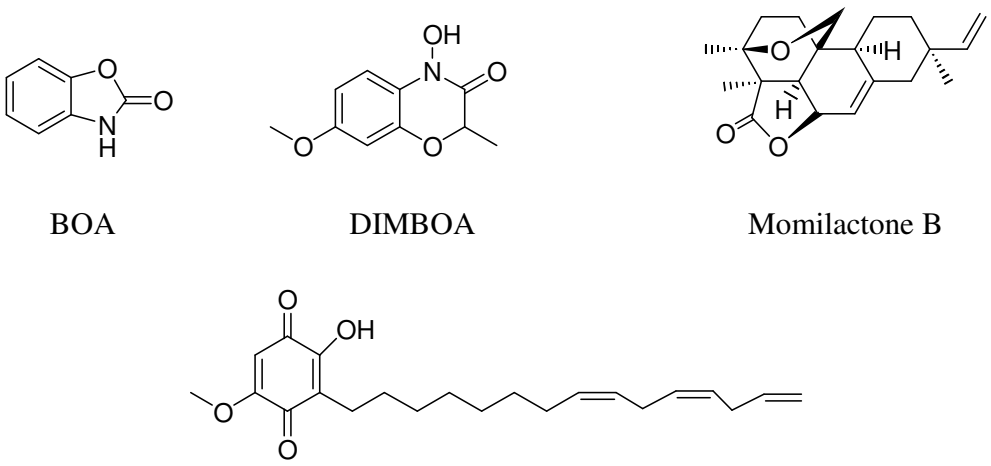
1.8.2 Sorgoleone

Sorgoleone, a lipid benzoquinone that exudes from the roots of sorghum (*Sorghum bicolor* L.) (Fig. 1.2.4),[157-161] suppresses the growth of a large number of plant species, but it is most active on small-seeded plants.[162–166] Sorghum accumulates sorgoleone and its analogues in mature root hairs,[167, 168] and the production (approximately 18 mg g^{-1} root dry weight) is optimum at temperatures ranging from 25 to 35 ^{o}C. The in vivo mechanism of action of sorgoleone and its analogs is unclear, but it is known to inhibit several physiological processes and enzymes in plants (e.g., photosynthetic and mitochondrial electron transport,[169, 170] *p*-hydroxyphenylpyruvate dioxygenase,[171] and root H+-ATPase and water uptake[172]). Sorgoleone applied to soil is easily

recovered within 1 h of application (85%). The recovery rate decreases over time, though low levels of sorgoleone are still extractable after 6 weeks. Sorgoleone degrades slowly to yet uncharacterized metabolites, but more research needs to be done to better characterize the fate of sorgoleone in soil.[173]

1.8.3 Benzoxazinoids

Allelopathic benzoxazinoids isolated in significant amount from many species within the *Poaceae* family have potential uses in agriculture as weed control agents.[174] Benzoxazinoids such as 2(3H)-benzoxazolone (BOA) and 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) (Fig. 1.2.4) exist mostly as glucosides within the producing plants.[175] However, these glucosides are subject to microbial degradation when released into the environment, which release the aglycone moieties. These unstable aglycones undergo further degradation (hydrolysis and ring contraction) into the corresponding benzoxazolinones with short half-lifes (1 day for DIMBOA).[176, 177] Benzoxazolinones might be further transformed, either chemically or by soil microbes, into more toxic degradation products.



BOA DIMBOA Momilactone B

Sorgoleone

Fig. 1.2.4 Structures of natural products involved in alleopathic interactions.

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CHAPTER 2

SCOPE OF THE THESIS

It can be concluded form Chapter 1 that the tremendous increase in crop yield, which resulted in the production of high quality food to meet the demands of ever increasing population of the world, would not have been achieved without the contribution of synthetic pesticides. However, the widespread and uncontrolled use has resulted in rapid resistance in pest making them even more difficult to control than before. Also as their use increased, dramatic declines of beneficial insects and other animals has been reported. Concerns over the potential impact of pesticides on the environment has now become more pressing and more stringent pesticide registration procedures, such as the Food Quality Protection Act in the United States, have been introduced. These new regulations have reduced the number of synthetic pesticides available in agriculture. Therefore, the current paradigm of relying almost exclusively on chemicals for pest control may need to be reconsidered.

New pesticides, including natural product based pesticides, are being discovered and developed to replace the compounds lost due to the new registration requirements. Natural products with herbicidal, insecticidal or fungicidal activity have been shown to be an important source of lead compounds in the past and we can probably expect more new interesting compounds to be discovered in the future. Good lead structures are uncommon and hard to find, but the search is worthwhile as the best ones can bring novelty, in terms of structure and mode of action, and can lead to highly successful crop protection products. Also a strategy implementing effective combination of natural and synthetic pesticides would prove to be more benefitting.

Small molecules produced in biological contexts have been, and still are, a large reservoir of new biologically active substances, which can become scaffolds for the discovery of new agrochemicals. Hence the aim of this PhD work was to synthesize naturally occurring potential antifungal and herbicidal compounds and to test their biological activity.

Efforts mainly focused towards the total synthesis of Bulgarein, a fungal metabolite produced by the fungus *Bulgaria inquinans.* Bulgarein possesses a benzo[j]fluoranthene skeleton that can be found in a number of polyketide-derived fungal metabolites endowed with significant biological activity, in particular inhibition of Topoisomerase I. As no attempt to synthesize any of these compounds has been reported in literature so far, a synthetic sequence to the benzo[j]fluoranthene nucleus has been studied.

As a second topic in this research, attention was dedicated to another natural compound, Farinomalein A, a structurally rather simple maleimide isolated in 2009 from the entomopathogenic fungus *Paecilomyces farinosus*. Farinomalein shows potent inhibition of *Phytophthora sojae*, a plant pathogen that every year causes enormous damage to soybean crops. Recently, three new farinomalein derivatives (Farinomalein C, D & E) were isolated from an endophyte from the mangrove plant *Avicennia marina,* growing in Oman. Due to the interesting antifungal activity of this class of compounds, a practical synthesis of farinomalein A was developed, which may have value in the large-scale preparation of the natural compound. Starting from farinomalein A, all the three derivatives were successfully synthesized. The antifungal activity of the derivatives was evaluated against *Cladosporium cladosporioides* and other pathogenic fungi.

An approach to the synthesis of Ascaulitoxin, a phytotoxic metabolite produced by the fungus *Ascochyta caulina*, was also developed.

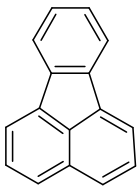
CHAPTER 3

SYNTHESIS OF OXYGENATED BENZO[J]FLUORANTHENES

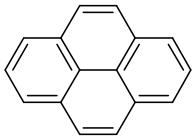
3.1 Introduction

Small molecules produced in biological contexts have been, and still are, a large reservoir of new biologically active substances, which can become scaffolds for the discovery of drugs, agrochemicals or can be utilized in other applications.[1]

The fluoranthene carbon skeleton with annelated rings is of interest for different areas of chemistry, including medicinal chemistry and material science for organic electronics as well as sensing.[2]



fluoranthene



pyrene

Fig. 3.1

Fluoranthene is a polycyclic aromatic hydrocarbon (PAH) consisting of naphthalene and a benzene unit connected by a five-membered ring. It is a member of the class of PAHs known as non-alternant PAHs because it has rings other than those with six carbon atoms. It is a structural isomer of the alternant PAH pyrene. It is not as thermodynamically stable as pyrene because its electrons cannot resonate throughout the complete structure like the corresponding ones in pyrene. Fluoranthene is found in many combustion products, along with other PAHs. Its presence is an indicator of less efficient or lower-temperature combustion, since non-alternant PAHs are less preferred in formation than alternant PAHs.

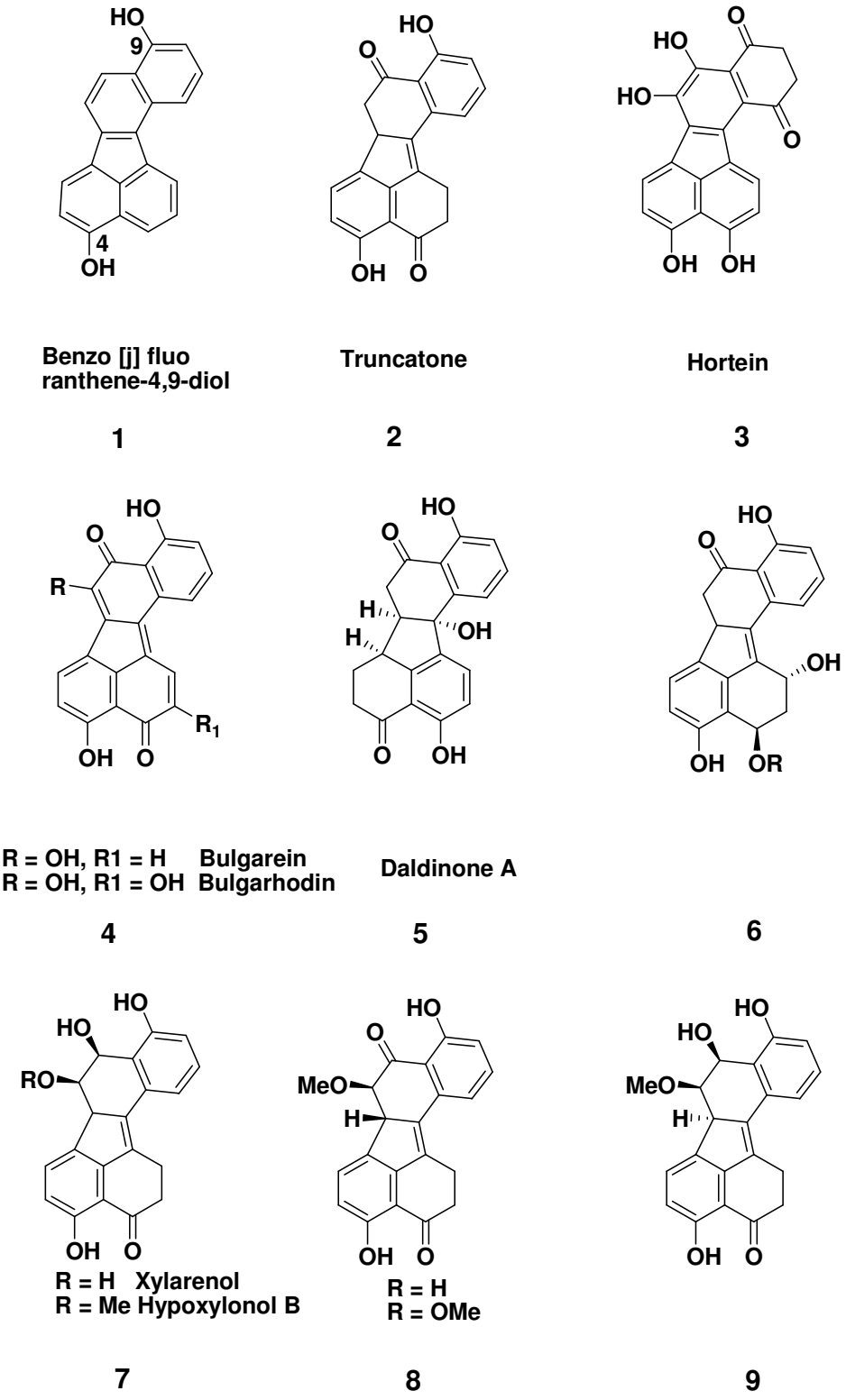


Fig. 3.2. Polyketide-derived fungal metabolites with benzo[j]fluoranthene nucleus

A number of polyketide-derived fungal metabolites, mostly from xylariaceous fungi[3], possess a differently oxygenated and/or reduced benzo[j]fluoranthene nucleus. Examples are bulgarein[4] **(4) (R = OH, R^1 = H),** bulgarhodin[4] **(R = OH, R^1 = OH),** hortein[5] **(3),** daldinones A-D[6,7]**(5),** truncatone [8] **(6)**, hypoxylonols A,B[9], **(7) (R = Me)** and xylarenol.[10] **(7) (R = H)** There are also some unnamed analogues,[11] among which benzo[j]fluoranthene-4,9-diol **(1),** recently isolated by Tan and coworkers[12] as one of the metabolites of Mantis-associated *Daldinia eschscholzii* fungus. **(Figure 3.2)** These compounds have interesting biological activities, e.g. topoisomerase I inhibition for bulgarein,[13] **(4) (R = OH, R^1 = H)** cytotoxicity for daldinone C and D,[7] **(5),** immunosuppressive activity for benzo[j]fluoranthene-4,9-diol[12] **(1)** and tyrosine kinase inhibition[10] for other compounds.

3.2 Biomimetic Pathways

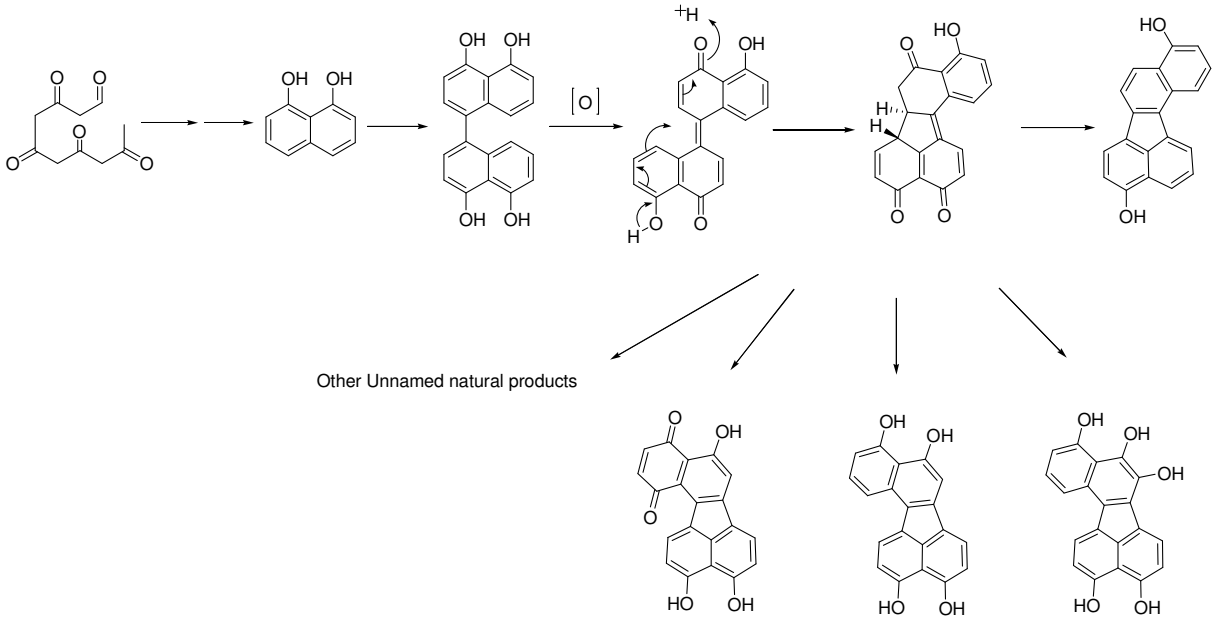


Figure 3.3

It is quite likely that these compounds derive from an oxidative phenol coupling of two hydroxylated naphthalene units, followed by further oxidative condensation and redox reactions.[7] This hypothesis is supported by the fact that in most of the metabolites, the carbon atoms 3, 4, 9, 10 are oxygenated.

To the best of our knowledge, no attempt to synthesize any of these compounds has been reported in literature so far. Therefore we deemed to approach a total synthesis of benzo[j]fluoranthene itself and of some oxygenated analogues. The availability of enough of these compounds would allow testing them for biological activities.

3.3 Literature Survey

Even if there is great interest in different fields of chemistry, the benzo[j]fluoranthene nucleus has been little studied from a synthetic point of view. Apart from the parent hydrocarbon, which can be obtained by gas phase high temperature reactions[14], few syntheses of substituted benzo[j]fluoranthenes have been reported in the literature so far. Ring closures with Friedel-Crafts reactions according to disconnections ***a***,[15] ***b***,[16, 17] and ***c***[15,16] (Figure **3.4**) or with a Suzuki reaction (disconnection ***d***[18]) have been reported to prepare fluoro, methoxy and methylthio derivatives by Rice[15,16,18] and Panda.[17]

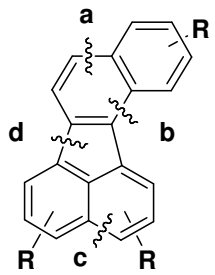


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Fig. 3.4

The recent synthesis of 10,11-dihydrobenzo[j]fluoranthen-12-one by Chang[19] mimics the last steps of the putative biosynthetic scheme.[7] (Figure **3.5**) The details about the known synthetic strategies are as follows.

3.3.1 Chang's Approach (*Org. Lett.* **2012**, *14*, 2198−2201)

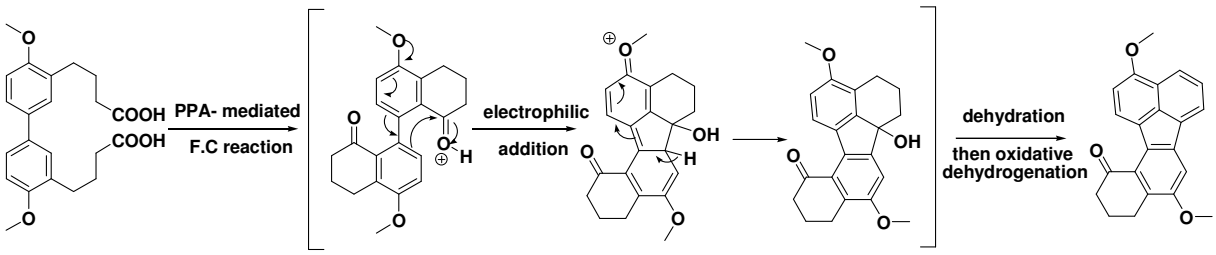

Fig. 3.5

3.3.2 Disconnection 'a'

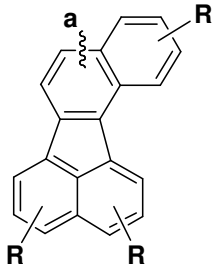

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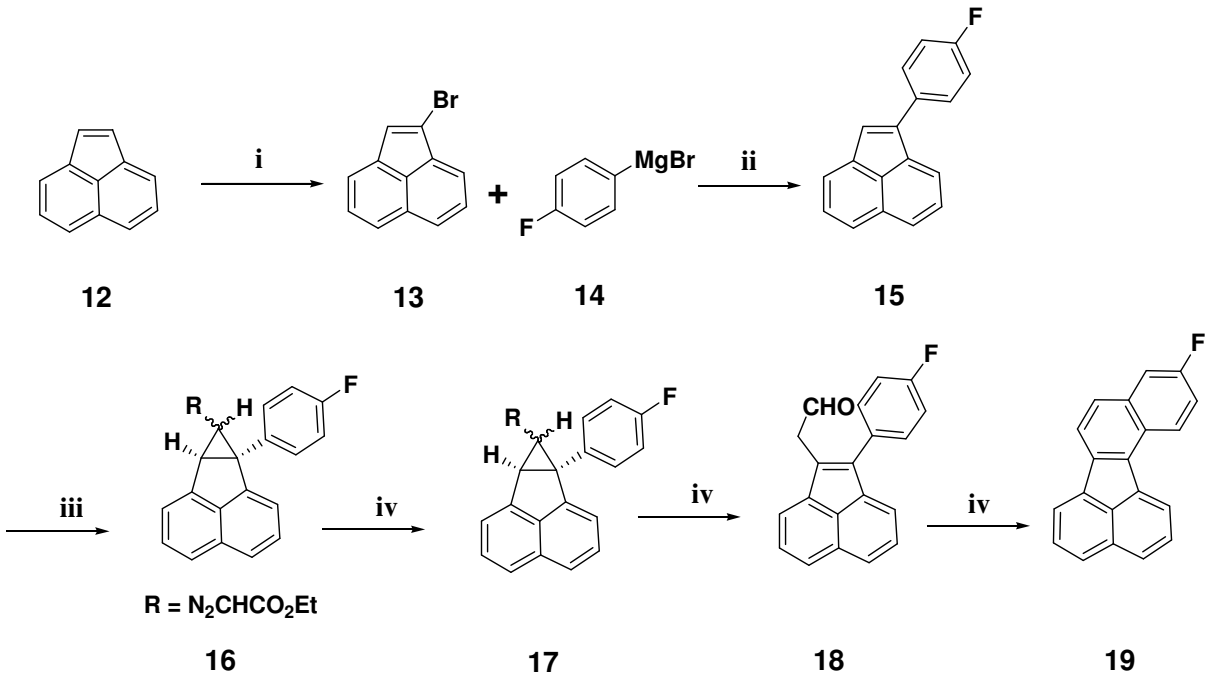
Fig. 3.6

Rice Approach . (*J. Org. Chem*. **1992**, *57*, 1784-1789.).

Approach I



Scheme 3.1

Reagents and conditions.

(i) a) Br_2, CCl_4 b) KOH, EtOH, reflux, 48h, 95 %

(ii) Ni(dppe)Cl_2, 0 ºC– rt, 5h, 72%.

(iii) Cu- bronze, DME, 80 – 85 ºC, then ethyl diazoacetate, 6h, 68%

(iv) a)DIBAL-H dry Toluene , - 23 ºC, 2h, 96% b) Swern oxidation, 84%

(v) PPA, 90–100 ºC, 3h, 91%

3.3.3 Disconnection ‘b’

Ila’s Approach . (*Eur. J. Org. Chem*. **2005**, 2045−2055)

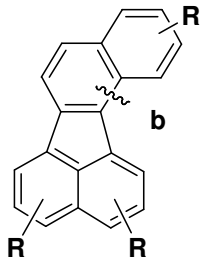


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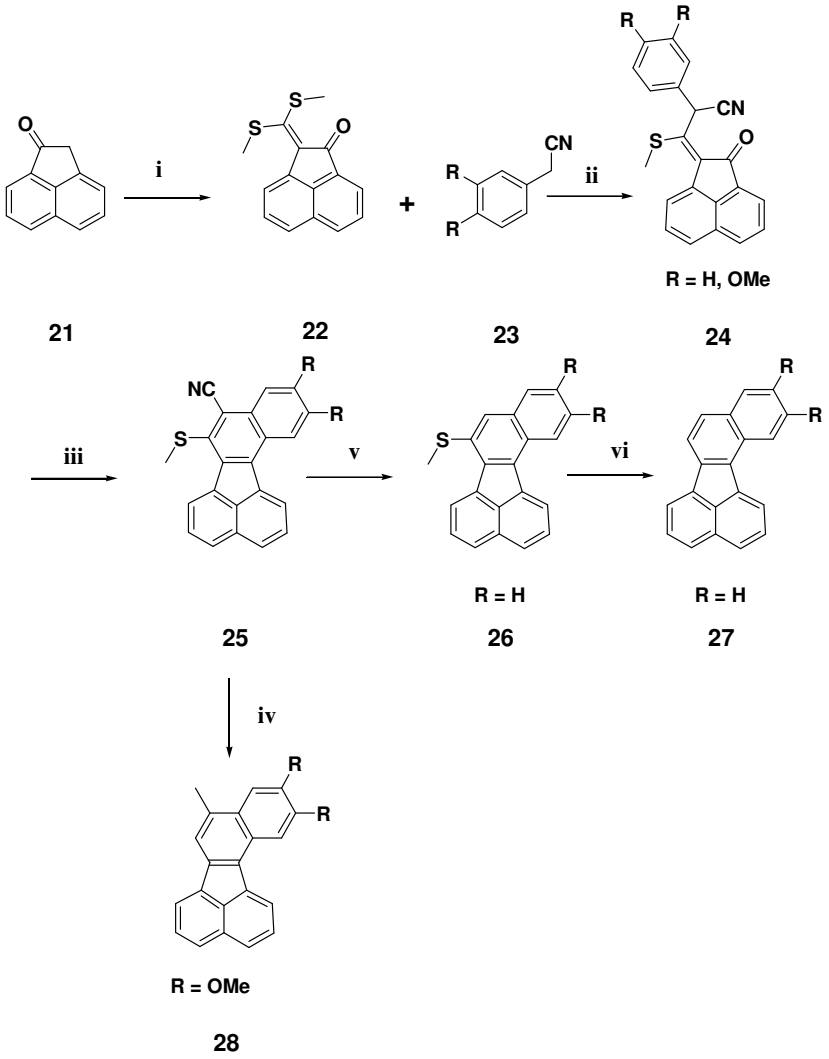
Fig. 3.7



Scheme 3.2

Reagents and conditions.

(i) a) NaH, CS_2, benzene, DMF, 0ºC b) dimethyl sulfhate, 72%

(ii) a) NaH, DMF, 0ºC, 45min, 72%. b) then **(23)**, 8h−10h, 68%

(iii) a)**for R= H**, PPA, 100 ºC, 5h, 54%. b) **for R= OMe**, H_3PO_4, 90 ºC, 4h, 86%

(iv) **for R= OMe** Raney Ni (W_2), EtOH, reflux, 62%, **for R= H** , H_2SO_4 / AcOH, 180 ºC, 3h, 61%

(v) **for R= H** Raney Ni (W_2), EtOH, reflux, 57%

3.3.4 Disconnection 'c'

Rice Approach (*J. Org. Chem.* **1990**, 55, 5490–5494.)

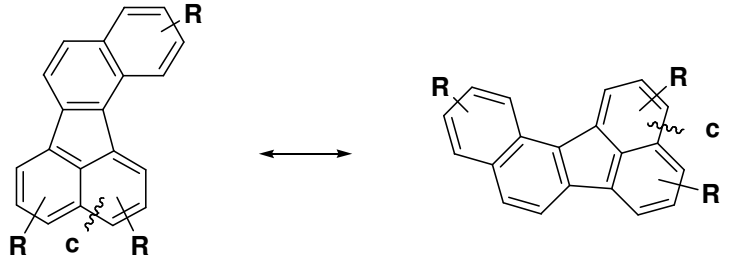


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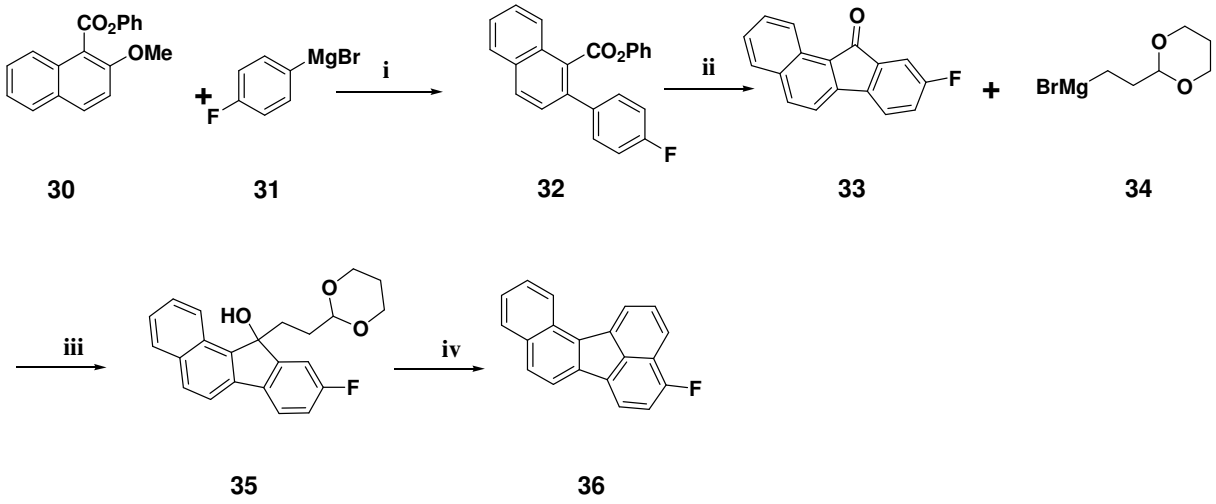
Fig. 3.8



Scheme 3.3

Reagents and conditions.

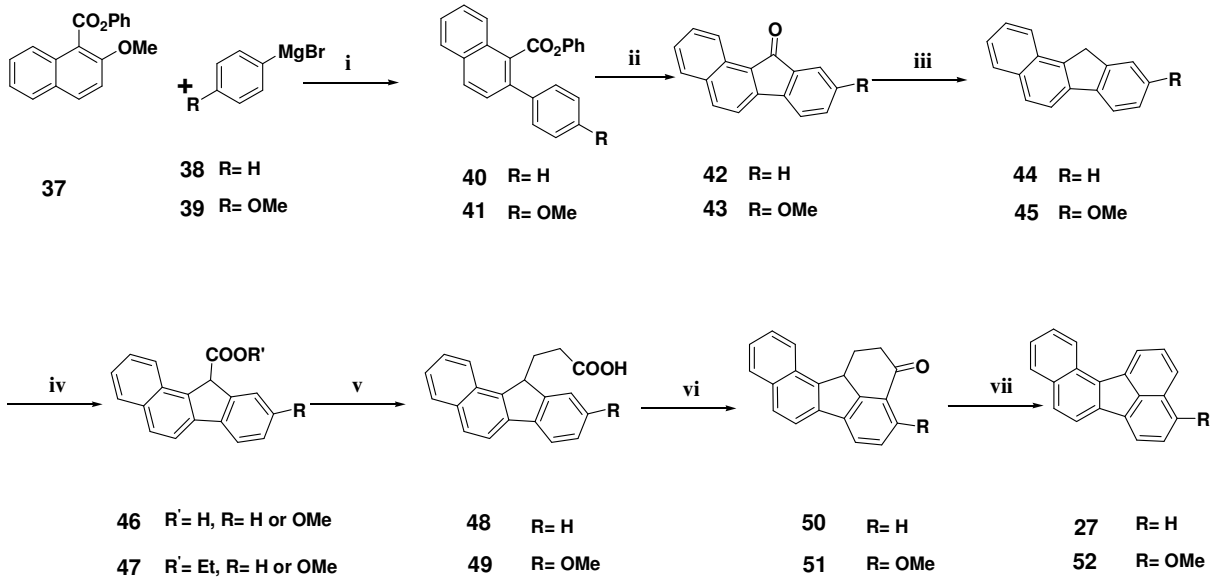
(i) 1:1 (diethyl ether : benzene), **(31)**, reflux, 12h, 77%

(ii) MSA, 12h, 59%

(iii) Mg turnings, THF, ethylenedibromide, **(33)**, 55 ºC, then **(34)**, 12h, 98%

(iv) **(35)** in $CHCl_3$, 60 ºC, 12h, 35%

Rice Approach (*J. Org. Chem.* **1987**, 52, 849–855.)



Scheme 3.4

Reagents and conditions.

(i) 1:1 (diethyl ether : benzene), **(38 or 39)**, reflux, 12h, 77%

(ii) MSA, 90min, rt, 100%

(iii) Zn amalgam, conc. HCl, **(42 or 43)** in toluene, reflux, 23h, 59%

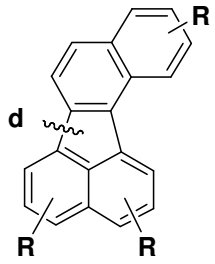
(iv) a) **(46 or 47)** in diethyl ether, −78 ºC b) nBuLi, − 30 ºC, 1h c) dry ice d) EtOH, benzene, con. H_2SO_4, 69%

(v) **(48 or 49)** in pyridine, 0 ºC, acrylonitrile, 10 N NaOH, 0 ºC – rt, 97%

(vi) MSA, rt, 19h, 70%

(vii) 1-methylnaphthalene, 10% Pd/C, reflux, N_2 gas bubbling, 7h, 100%

3.3.5 Disconnection 'd'

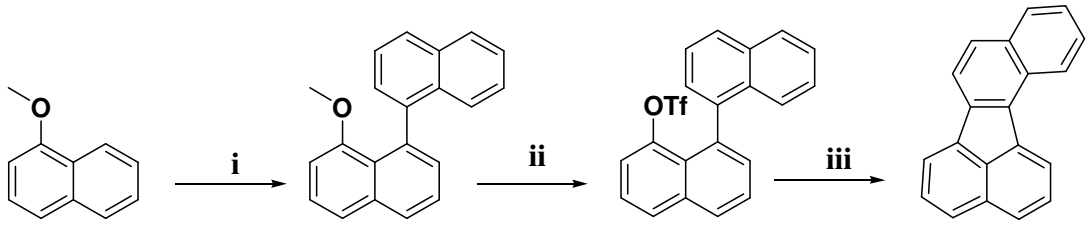


53

.Fig. 3.9

Intramolecular Arene – Triflate Coupling Using Palladium catalyst

Rice Approach (J. Org. Chem**. 1993**,*58*, 1415-1424)



54 **55** **56** **27**

Scheme 3.5

Reagents and conditions.

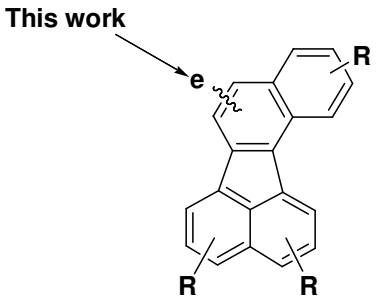
(i) a) tBuLi, $MgBr_2$ b) 1- bromo naphthalene**,** 64%

(ii) BBr_3, Tf_2O, 84%

(iii) $Pd(PPh_3)_2Cl_2$, LiCl, DBU, DMF, heat, 93%

Despite the successes cataloged above, it is clear that in spite of the considerable efforts expended by numerous research groups over many years on the synthesis of these type of cores, total synthesis of natural products containing oxygenated benzo[j]fluoranthene nucleus remains particularly challenging for synthetic chemists. We have tried to develop a synthetic strategy towards the synthesis of some of these natural products. In this I chapter will discuss our progress towards this endeavor.

3.3.6 Present work: disconnection 'e'



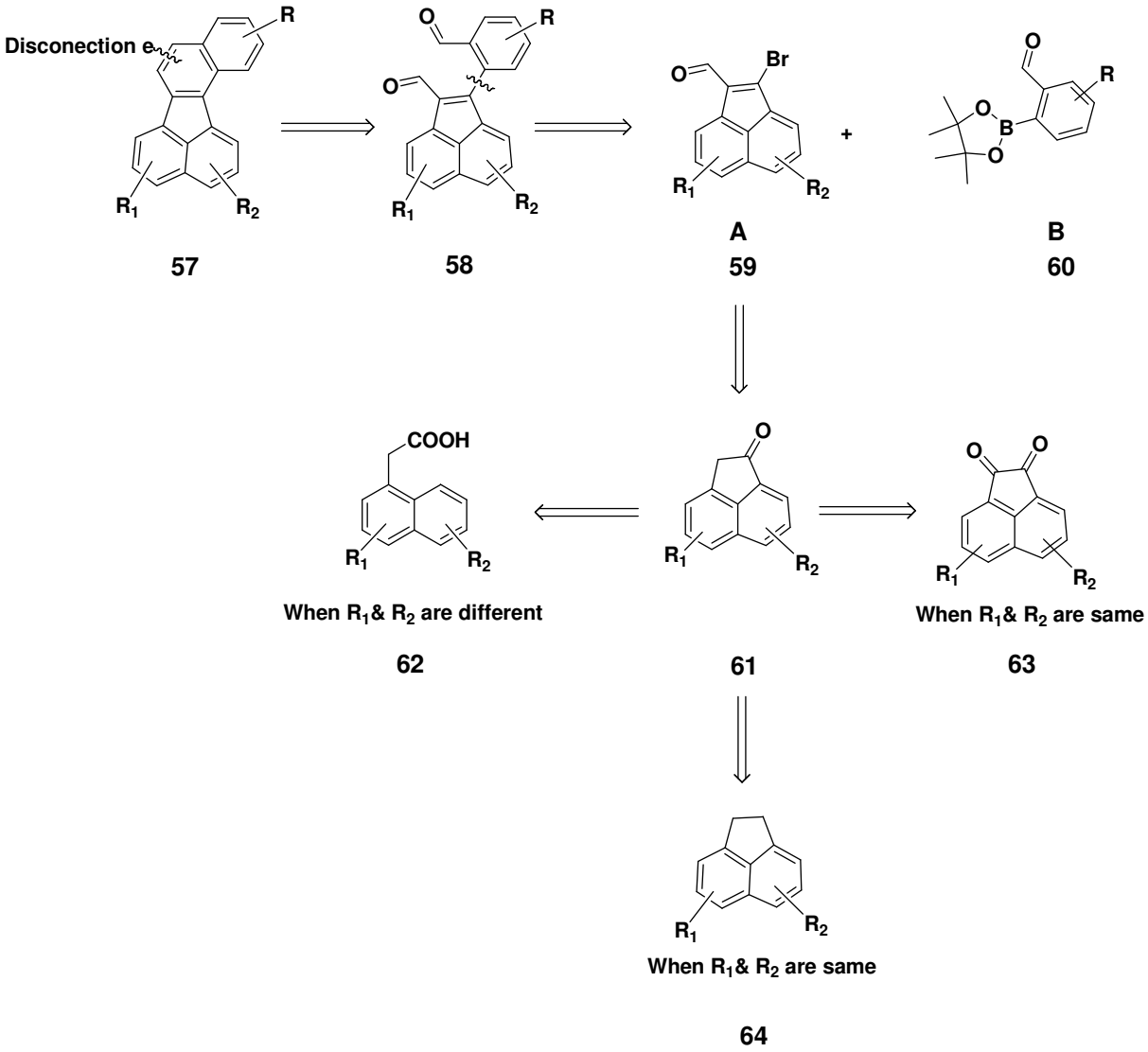
57

Fig. 3.10

Present retrosynthetic analysis for benzo[j]fluoranthene

These molecules possess deceptively simple looking molecular structure that present a number of challenges to the capabilities of the contemporary organic synthesis. We viewed our new synthetic approach for **(57)** through the retrosynthetic route as outlined in the **Scheme 6**. The key step envisaged in our approach was the intramolecular McMurry coupling reaction of a dialdehyde **(58),** which would allow the construction of the pentacyclic system.[20] Compounds **(58)** could be obtained by Suzuki reaction of a boronate **(60)** with a bromoacenaphthylenecarbaldehyde **(59)**, which in turn could be prepared from **(61).** If the substituents on the **(61)** were the same it could be prepared from either **(63)** or **(64)** depending on

the availability of the precursor and / or feasibility of synthesizing it. When the substituents on **(61)** were dissimilar it could be synthesized form the general reactant **(62).** (**Scheme 3.6.**)

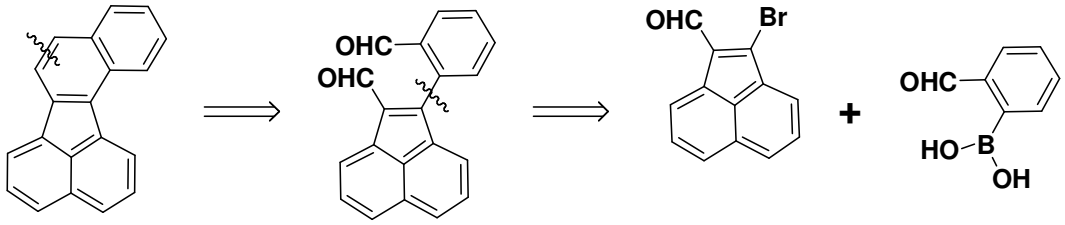


Scheme 3.6

3.4.Synthesis of benzo[j]fluoranthene

To test the feasibility of our approach we first synthesized the unsubstituted benzo[j]fluoranthene **(27)**, The strategy was then subsequently applied for the synthesis of other oxygenated compounds.

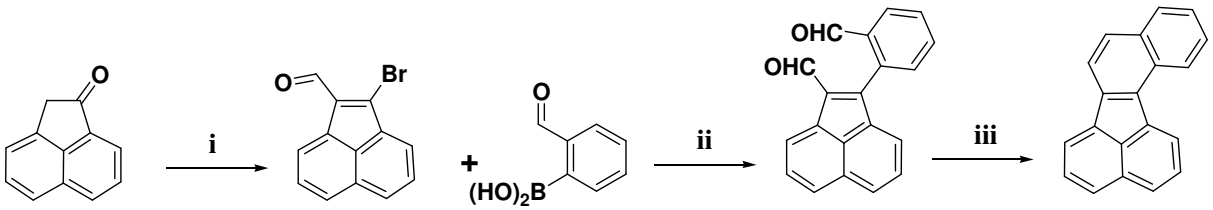
3.4.1 Retrosynthesis



27 **65** **66** **67**

Scheme 3.7

3.4.2 Synthesis



21 **66** **67** **65** **27**

Scheme 3.8

Reagents and conditions.

(i) PBr_3: DMF, $CHCl_3$, 0°-r.t., 70%

(ii) $Pd(PPh_3)_4$, K_2CO_3, Dioxane:water, 73%

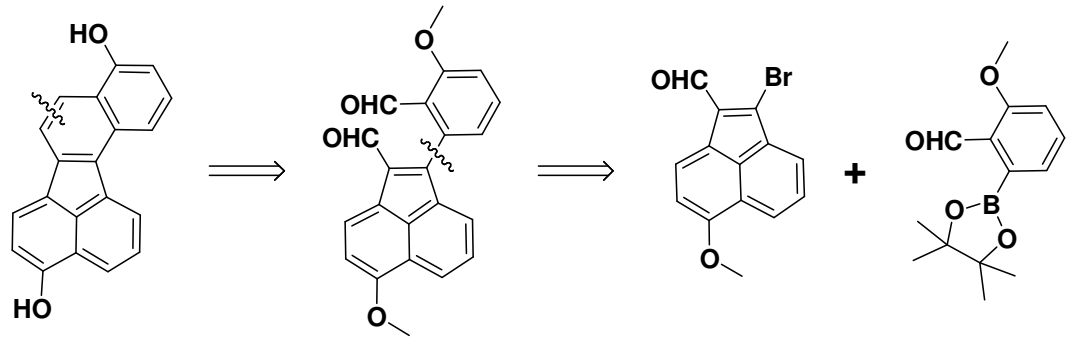
(iii) $TiCl_4$, Zn, THF, reflux, 83%;

Compound **(66)** was prepared starting from acenaphthone **(21)**, according to a reported procedure,[21] by utilizing Arnold modified Vismeier-Haack-Arnold reaction. Several attempts were made to couple model intermediates **66** and **67** by using various bases and solvents. Use of $Pd(PPh_3)_4$ as a palladium source and aq. Na_2CO_3, KOAc, or CsF as a base in DMF, toluene, DMSO, or DME gave undesirable hydrolytic deboronation products and the homocoupled product of β-bromovinyl aldehyde. A satisfactory conversion, though, was finally obtained by $Pd(PPh_3)_4$ with K_2CO_3 in 1,4-dioxane:water medium. The dialdehyde thus prepared was subjected to McMurry conditions to obtain Benzo[j]fluoranthene **(27).** These conditions were optimized in our own group. **65** was subjected to McMurry[20] conditions to obtain Benzo[j] fluoranthene **(27).**

3.5 Synthesis of the natural product Benzo[j]fluoranthene-4,9-diol (1)

Benzo[j]fluoranthene-4,9-diol **(1)** was recently isolated from scaled-up fermentations of the mantis-associated fungus *Daldinia. eschscholzii* by Tan et al.[12] It shows immunosuppressive as well as cytotoxic activity. We found it as a good target to apply our approach for the synthesis of oxygenated Benzo[j]fluoranthenes.

3.5.1 Retrosynthetic analysis of (1)

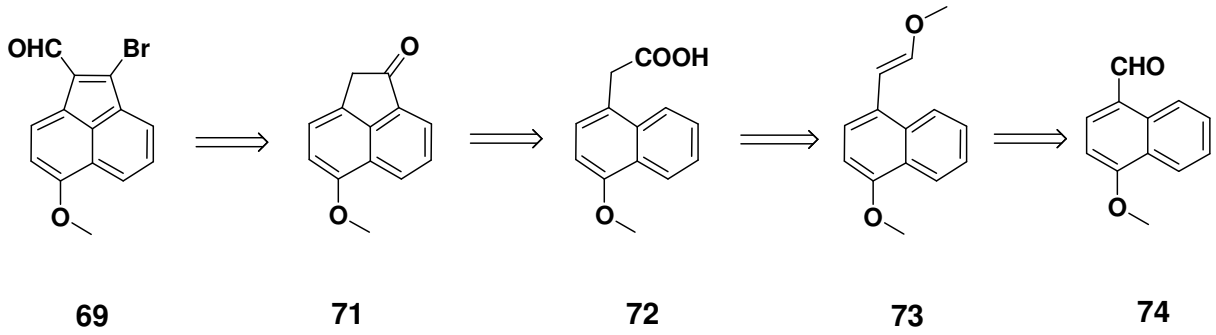


1 **68** **69** **70**

Scheme 3.9

As described earlier in our general approach towards the synthesis of these framework (scheme **6**), we designed the retrosynthesis of the molecule as shown in scheme **9.** The natural product **1** can be synthesised by intramolecular McMurry coupling of compound **68,** which can be synthesised by the Suzuki coupling of compounds **69** and **70** respectively.

3.5.2 Retrosynthetic analysis of (69)

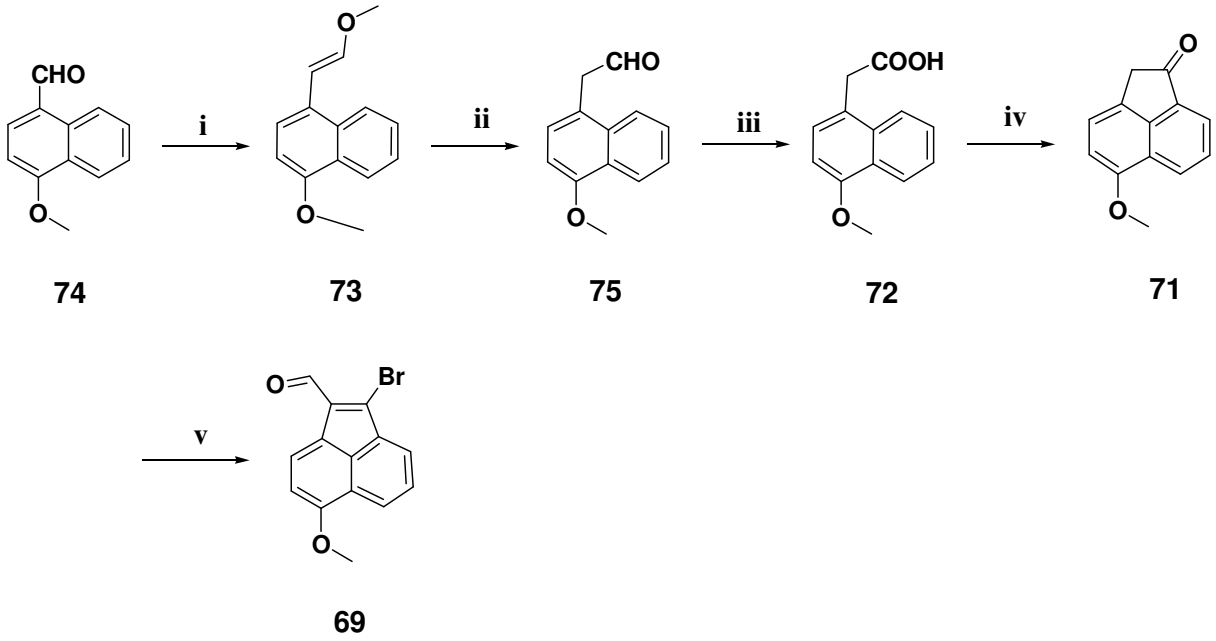


Scheme 3.10

Bromovinylaldehyde **69** can be prepared from cyclic ketone **71**, which in turn can be prepared from intramolecular Fridel-Craft acylation of acid **72** obtained from commercially available 4-methoxynaphthaldehyde after one carbon homologation using a Wittig reaction..

3.5.3 Synthesis of (69)

Reaction of commercially available 4-hydroxy-1-naphthaldehyde **74** with $Ph_3P{=}CHOCH_3$ gave enol ether[22] **73** and subsequent acidic hydrolysis gave the homologous aldehyde[23] **75**. Aldehyde **75** was oxidized to the corresponding acid by employing Pinnick oxidation[24,25] conditions. Acid **72** was converted to its acid chloride followed by intramolecular Friedel–Crafts acylation gave ketone[26] **71** in good yield. Compound **71** was converted into the bromoaldehydes **69** by an Arnold-modified Vilsmeier–Haack reaction[21].



Scheme 3.11

Reagents and conditions.

(i) t-BuLi, $CH_3CH_2P(Ph_3)_3^+Cl^-$, THF, - 78ºC- rt,12h, 70%.

(ii) HCl, THF, reflux, 3h, 70%

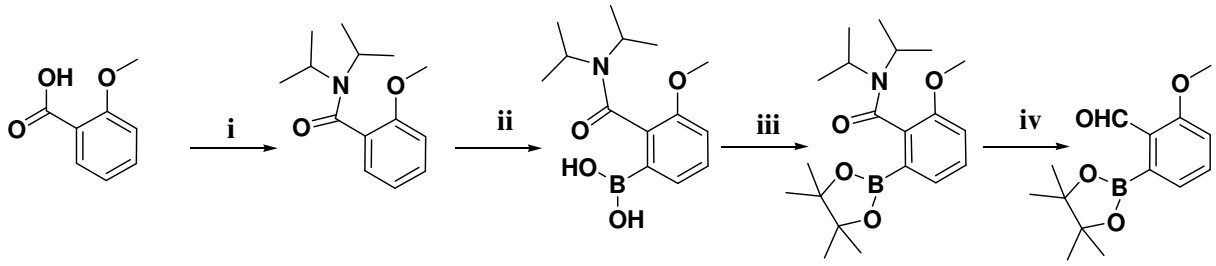
(iii) KH_2PO_4, $NaClO_2$,rt, 40 min, 93%

(iv) a) Oxalyl chloride, CH_2Cl_2, 25 ºC, 3h. b) $AlCl_3$, - 78 ºC-rt, 12h, 65%

(v) PBr_3: DMF, $CHCl_3$, 0-RT^{0}C, 80%

Since boronate **70** was not commercially available it was synthesised by DIBAL-H reduction from the corresponding boronate **75.** The boronic acid was synthesised from the amide **77** using Neighbouring group participation strategy.

3.5.4 Synthesis of (70)



78 77 76 75 70

Scheme 3.12

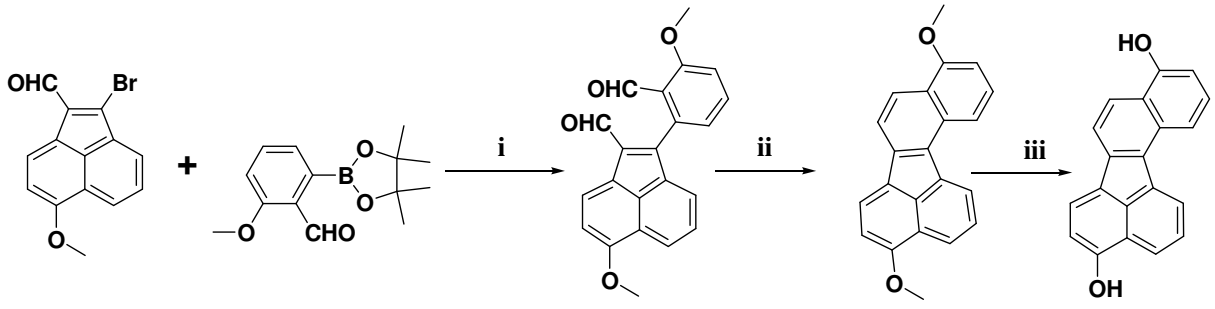
(i) a) $SOCl_2$, 50 ºC, b) $(iPr_2)NH$, Et_3NH, 0 ºC, 95%

(ii) a) t-BuLi, 78 ºC, 2h b) $B(OCH_3)_3$, dil.HCl, 70%

(iii) Pinacol, toluene, 120 ºC, 5h, quantative

(iv) DIBAL-H, 0 ºC, 30 min., 73%

Boronate **70** was synthesized starting from 2-methoxybenzoic acid **78**, which was converted into the N,N diisopropylamide[27] **77** via the acid chloride. The conversion to the amide group allowed both directed ortho-metalation to insert the boronic acid group, and the synthesis of the aldehyde by reduction. Thus, generation of a carbanion ortho to the N,N-diisopropyl amide group of amide **77,** by tert-BuLi in THF at −78 °C for 2 h, followed by quenching with trimethyl borate and acidic workup, gave regiospecifically N,N′-diisopropylcarbamoyl-3-methoxy-1-phenylboronic acid[28] **76.** This was protected by reaction with pinacol in toluene under azeotropic distillation conditions, to give pinacolate[29] **75**, which afforded aldehyde[30] **70** by treatment with DIBAL-H at 0°C.



69 **70** **68** **79** **1**

Scheme 3.13

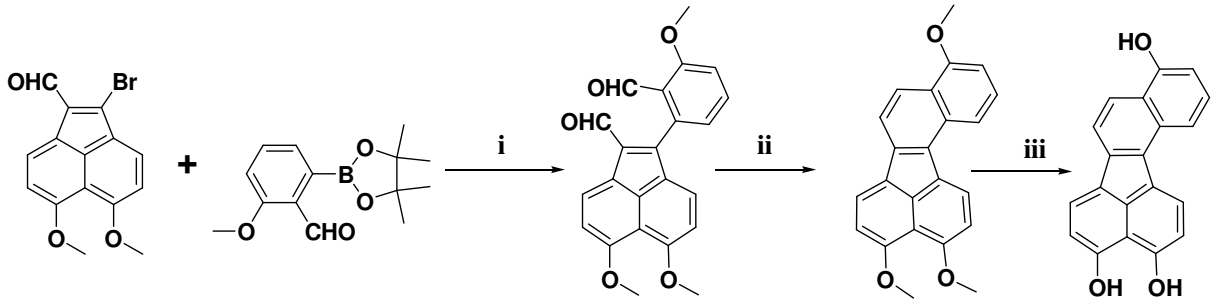
(i) $Pd(Ph_3)_4$, K_2CO_3, dioxane/water, 80 ºC, 4h, 80%

(ii) Zn, $TiCl_4$, THF, reflux, 7h, 89%

(iii) Py.HCl, 140-150 ºC, 20 min, 65%

The two building blocks **69** and **70** were subjected to a Suzuki condensation by $Pd(PPh_3)_4$ with K_2CO_3 in 1,4-dioxane:water medium.to give dialdehyde **68**. Intramolecular McMurry coupling[20] of **68** gave compound **79**, followed by deprotection of hydroxy groups using pyridine hydrochloride at 140–150°C gave **1.** The ^1H and ^{13}C NMR spectra of **1** completely matched the spectra reported for the natural compound[12]

3.6 Synthesis of trihydroxy compound (89)



86 **70** **87** **88** **89**

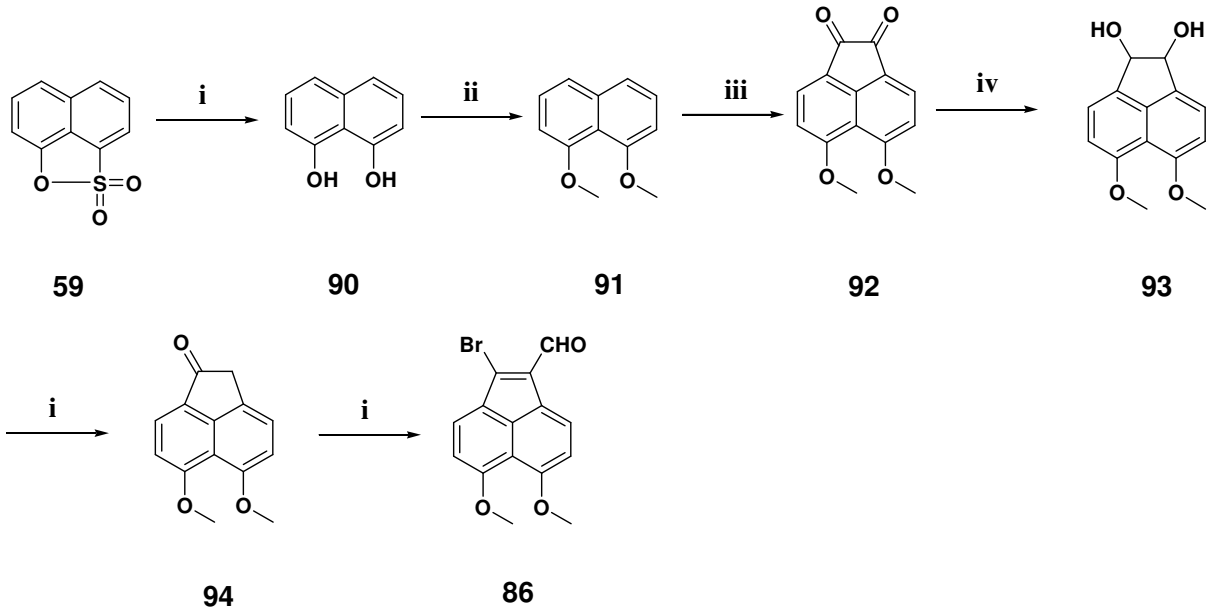
Scheme 3.14

(i) $Pd(Ph_3)_4$, K_2CO_3, dioxane/water, 80 ºC, 4h, 89%

(ii) Zn, $TiCl_4$, THF, reflux, 7h, 80%

(iii) Py.HCl, 140-150 ºC, 20 min, 69%

The trihydroxy compound **89** was synthesised similarily from bromovinylaldehyde **86** bromovinylaldehyde **86** was synthesised by the following sequence,



Scheme 3.15

(i) KOH, 300^0C, 30 min, 80%

(ii) Aq.NaOH, PTC, MeI, 60-70^0C, 70%

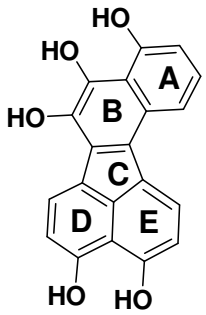
(iii)$AlCl_3$, $(COCl)_2$, DCM, 0-RT^0C, 80%

(iv)$NaBH_4$, EtOH: H_2O , rt, 21h, 92%

(v) pTSA, Toluene, 80^0C, 75%

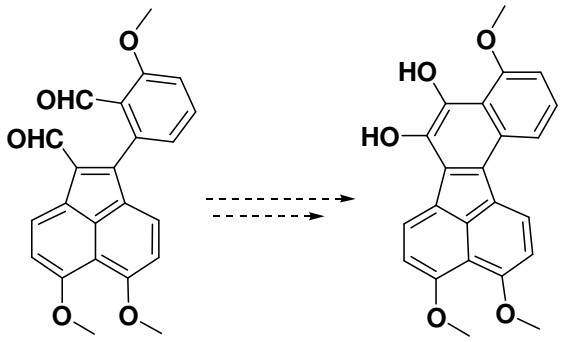
(vi)PBr_3: DMF, $CHCl_3$, 0-RT^0C, 70%

3.7 Synthesis of benzo[j]fluoranthenes with oxygens on ring B



Bulgarein (94)

As in many naturally occurring compounds with benzo[j]fluoranthene backbone there are oxygens on ring B, we planned to extend our synthetic stratergy towards the synthesis of Bulgarein and related other natural products. Since during Mc Murry coupling the carbonyl oxygens are lost, we thought of replacing this step by a reaction which would result in the construction of ring 'B' without the elimination of oxygen atoms.



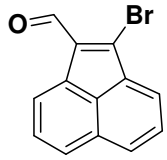
87 **95**

To harness the possibility of synthesizing benzo[j]fluoranthenes with oxygen atom at ring B, a literature survey was done and it was found that Benzoin and Pinacol condensations were extensively employed to couple the sp^2 carbons resulting in a vicinal diol.

A number of conditions[31-34] and reagents were tried to achieve the desired compound **(95).** However, all attempts were unsuccessful, resulting in either no reaction or to decomposition of the reactant when harsh conditions such as high temperature and stronger bases were utilized. Hence, an alternative strategy to synthesize this highly oxygenated compounds was developed and it will be discussed in detail in chapter **4.**

3.8. Experimental

2-bromo-1,2-dihydroacenaphthylene-1-carbaldehyde (66)

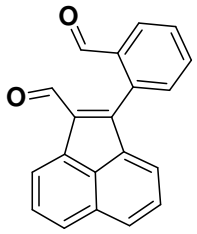


PBr_3 (0.651 g, 0.228 mL, 2.40 mmol) was added dropwise at 0 °C to a solution of dry DMF (66 mg, 0.206 mL, 2.67 mmol) and dry chloroform (10 mL) and the resulting white suspension was stirred at room temperature for 30 min. A solution of **(21)** (150 mg, 0.891 mmol) in anhydrous chloroform (10 mL) was added dropwise over 10 min at 0 °C. After stirring at room temperature for 12 h, the solution was poured into ice cold water. Sodium bicarbonate was carefully added to neutralize the acid and the aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were washed with water (2 × 20 mL), dried to give a residue which was purified by flash column chromatography to afford **(66)** (185 mg, 80% yield) as a orange red solid.

^{1}H NMR ($CDCl_3$) δ: 7.59(m,1H), 7.7(m, 1H), 7.86(1H, d, J= 8.4Hz), 7.96(1H, d, J = 6.8Hz), 8.07(1H, d, J = 8Hz), 8.33(d, 1H, J = 6.8Hz), 10.33 (1H, s).

m.p : 137-138 °C

2-(2-formylphenyl)acenaphthylene-1-carbaldehyde (65).



A solution of 2-formylboronic acid **(67)** (58 mg, 0.39 mmol), bromoaldehyde **(66)** (100 mg, 0.386 mmol) and K_2CO_3 (319 mg, 2.31 mmol) in 1,4-dioxane/water (4/1) was degassed by purging N_2 for 30 min. $Pd(PPh_3)_4$ (8.8 mg, 0.0076 mmol) was added, and the resulting reaction mixture was refluxed at 80 °C for 4 h under N_2 atmosphere, and then cooled to room temperature, diluted with diethyl ether (10 mL) and filtered through a small silica gel bed. The bed was washed with diethyl ether (3 × 10 mL). The resulting mixture was dried and evaporated. Purification by chromatography gave dialdehyde **(65)** (73 mg, 73%)as a bright orange colored powder

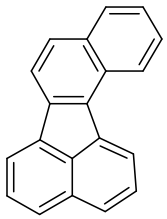
NMR ($CDCl_3$) δ: 10.08 (1H, s), 10.05 (1H, s), 8.49 (1H, d, *J* = 7.1 Hz), 8.19 (1H, dd, *J* = 1,5, 8.2 Hz), 8.09 (1H, m), 7.96 (1H, d, *J* = 8.2 Hz), 7.81−7.57 (6H, m).

^{13}C NMR (CDCl3) δ:190.9, 188.7, 151.4, 139.4, 136.1, 135.7, 135.6, 135.1, 134.0, 132.23, 132.17, 129.9, 129.1, 128.9, 128.74, 128.69, 128.51, 128.48, 128.0, 127.8.

Anal. Calcd for $C_{20}H_{12}O_2$: C, 84.49; H, 4.25. Found: 84.62; H, 4.27.

mp.: 150-151.4 °C

benzo[j]fluoranthene (27).

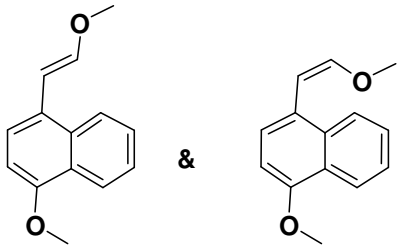


$TiCl_4$ (55 mg, 316 µl, 2.87 mmol) was carefully added under N_2 atmosphere to dry THF (10 mL) at 0 °C, giving a bright-yellow mixture of $TiCl_4$.2 THF complex. The solution was refluxed for 20 min and cooled to room temperature. Zinc (406 mg, 6.21 mmol) was added and the mixture was refluxed for 2 h during which the colour changed from yellow to dark greenish blue. The mixture was cooled and pyridine (227 mg, 231 µl, 2.88 mmol) was added. Refluxing continued for 30 min.A solution of dialdehyde **(65)** (41 mg, 0.145 mmol) in dry THF (10 mL) was added and the reaction mixture was refluxed for a further 4h, then it was cooled. Saturated aqueous K_2CO_3 solution was added till the aqueous layer became almost colourless and transparent, then the aqueous layer was extracted with ethyl acetate (2× 20 mL). Evaporation of the solvent and purification by flash column chromatography on silica gel column provided fluoranthene **(27)** (30 mg, 89 % yield). The analytical data completely matched with those reported in the literature

^{1}H NMR ($CDCl_3$) *δ*: 8.70 (1H, d, J = 8.3), 8.42 (1H, d, J = 7.0), 8.02 (1H, d, J = 8.4), 7.97 (1H,d, J = 6.9), 7.95 (1H, m), 7.86(2H, d, J=7.4), 7.72-7.59(3H, m), 7.51(lH, dd, J= 8.3, 6.9)

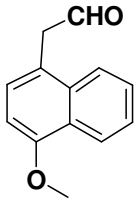
mp : 164- 165 °C

(E&Z)-1-methoxy-4-(2-methoxyvinyl)naphthalene (73)



t-Butyl lithium (1.7M in hexane, 4 mL, 6.80 mmol) was added dropwise to a suspension of methoxymethyltriphenylphosphonium chloride (2.20 g, 6.50 mmol) in dry THF (60 mL) at -78 °C in a round bottom flask under nitrogen. The colour of solution turned to red. After stirring for 30 min, 4-methoxy-naphthalene-1-carbaldehyde **(74)** (1 g, 5.35 mmol) was added in a single lot. The solution was allowed to gradually warm to room temperature and the colour changed to pale yellow. The reaction was stirred at room temperature for 12 h, then it was quenched by dilution with diethyl ether (100 mL). The aqueous layer was extracted with ether (2× 25 mL). Evaporation of solvent and purification by flash column chromatography provided a viscous oil. NMR analysis showed it was a 1:1 mixture of (E) and (Z) 1-methoxy-4-(2-methoxyvinyl)naphthalene **(73)** isomers (0.815 g, 70 %) which were not separated and were directly used for the next step.

(4-Methoxy-naphthalen-1-yl)acetaldehyde(75)



(E/Z) 1-methoxy-4-(2-methoxyvinyl)naphthalene **(73)** (0.50 g, 2.30 mmol) was dissolved in 50 mL of THF. A 2.0 M HCl (2.3 mL, 4.60 mmol) solution was added and the reaction was refluxed for 3 h. The reaction was diluted with ethyl acetate (75 mL), washed with a satd. solution of $NaHCO_3$ (50 mL × 2) water (25 mL), and finally with brine (25 mL). The organic layer was evaporated to give a crude that was purified by flash column chromatography to provide (4-Methoxy-naphthalen-1-yl)acetaldehyde **(75)** (0.320 g, 70% yield) as a white solid.

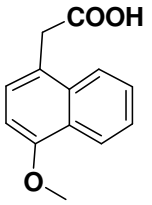
^{1}H NMR ($CDCl_3$) δ: 9.76 (1H, s), 8.37 (1H, d, *J* = 7.9 Hz), 7.82 (1H, d, *J*= 7.9 Hz), 7.64–7.50 (2H, m), 7.32 (1H, d, *J* = 7.9 Hz), 6.82 (1H, d, *J*= 7.9 Hz), 4.03 (3H, s)

^{13}C NMR ($CDCl_3$) δ: 200.4, 155.7, 133.2, 128.7, 127.3, 126.2, 125.5, 123.5, 123.0, 120.3, 103.6, 55.7, 48.1.

Anal. Calcd for $C_{13}H_{12}O_2$: C, 77.98; H, 6.04. Found: C, 77.84; H, 6.06.

mp= 55–56 ° C

2-(4-methoxynaphthalen-1-yl)acetic acid (72)



Compound **(75)** (0.30 g, 1.50 mmol) was dissolved in a 1:1 solution of H_2O : *t*-BuOH (0.1 M). To this suspension 2-methyl-2-butene (2.10 g, 30 mmol), KH_2PO_4 (0.41 g, 3.0 mmol) and sodium chlorite (0.34 g, 3.75 mmol) were added in sequence and the mixture was stirred for 40 min at room temperature.[34] The reaction mixture was diluted with ethyl acetate (50 mL), the aqueous layer was re-extracted by ethyl acetate (2 × 15 mL) and the combined organic layers were then treated with saturated aq. $NaHCO_3$ (2 × 20 mL). The basic layer was then washed with ethyl acetate (2 × 15 mL), then cooled and carefully acidified by dil HCl. The precipitate formed was filtered, then dissolved in ethyl acetate and washed with water and with brine. The organic layer was dried and concentrated to give compound 2-(4-methoxynaphthalen-1-yl)acetic acid **(72)** (0.30 g, 93% yield) as a white solid.

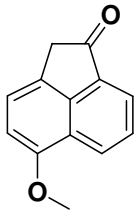
^{1}H NMR ($CDCl_3$) δ: 8.32 (1H, d, *J* = 8.4 Hz), 7.88 (1H, d, *J* = 8.4 Hz), 7.59–7.46 (2H, m), 7.31 (1H, d, *J* = 7.8 Hz), 6.76 (1H, d, *J* = 7.8 Hz), 3.99 (5H, s)

^{13}C NMR ($CDCl_3$) δ: 178.5, 155.6, 133.0, 128.4, 127.2, 126.1, 125.4, 123.7, 122.9, 121.9, 103.5, 55.7, 38.5.

Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.10; H, 5.61.

mp= 147–148 ° C

5-methoxyacenaphthylen-1(2H)-one(71)



Oxalyl chloride (38 mg, 0.30 mmol) was added dropwise to a solution of **(72)** (50 mg, 0.23 mmol) in dry CH_2Cl_2 (1.5 mL) at 0 °C under nitrogen. The solution was stirred at 25 °C for 3 h. The solvent was evaporated to give a colourless oil that was added with dry CH_2Cl_2 (1.5 mL) under nitrogen. The solution was cooled to -78 °C, then pulverized $AlCl_3$ (52 mg, 0.39 mmol) was added portionwise and stirring was continued for 12 h at room temperature. The reaction was quenched at -78 °C by dropwise addition of 4 N HCl (0.24 mL), then it was diluted with CH_2Cl_2 (15 mL) and the aqueous layer was extracted by CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried to give a crude that was purified by flash column chromatography to provide **(71)** (30 mg, 65 % yield) as a colourless off-white solid.

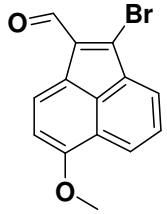
^{1}H NMR ($CDCl_3$) δ: 8.32 (1H, d, *J* = 7.9 Hz), 7.96(1H, d, *J* = 7.1 Hz), 7.66 (1H, dd, *J* = 7.1, 7.9 Hz), 7.33 (1H, d, *J* = 7.5 Hz), 6.85 (1H, d, *J* = 7.5 Hz), 4.02 (3H, s), 3.73 (2H, s)

^{13}C NMR ($CDCl_3$) δ: 204.0, 154.2, 144.2, 134.5, 127.3, 127.2, 126.5, 123.9, 122.1, 121.5, 105.8, 55.8, 41.7

Anal.Calcd for $C_{13}H_{10}O_2$: C, 78.77; H, 5.09. Found: C, 78.96; H, 5.07.

mp = 163−164 °C

2-bromo-6-methoxyacenaphthylene-1-carbaldehyde (69)



PBr_3 (0.22 g, 0.07 mL, 0.81 mmol) was added dropwise at 0 °C to a solution of dry DMF (66 mg, 0.07 mL, 0.91 mmol) and dry chloroform (3 mL) and the resulting white suspension was stirred at room temperature for 30 min. A solution of **(71)** (60 mg, 0.30 mmol) in anhydrous chloroform (1.5 mL) was added dropwise over 10 min at 0 °C. After stirring at room temperature for 12 h, the solution was poured into ice cold water. Sodium bicarbonate was carefully added to neutralize the acid and the aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed with water (2 × 10 mL), dried to give a residue which was purified by flash column chromatography to afford **(69)** (70 mg, 80% yield) as a orange red solid.

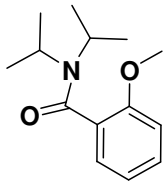
^{1}H NMR ($CDCl_3$) *δ*: 10.28 (1H, s), 8.28 (1H, d, *J* = 8.2 Hz), 8.18 (1H, d, *J* = 7.9 Hz), 7.91 (1H, d, *J* = 7.2 Hz), 7.62 (1H, dd, *J* = 7.2, 8.2 Hz), 6.76 (1H, d, *J* = 7.9 Hz)

^{13}C NMR ($CDCl_3$) *δ*: 188.1, 158.1, 135.5, 133.3, 130.8, 128.5, 128.2, 127.1, 126.6, 126.5, 126.2, 121.4, 105.5, 55.6

Anal.Calcd for $C_{14}H_9BrO_2$: C, 58.16; H, 3.14. Found: C, 58.31, H, 3.12.

mp = 187−188 °C

N,N-diisopropyl-2-methoxybenzamide(77)

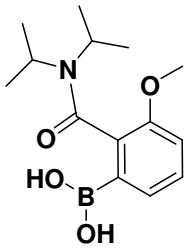


To a round bottom flask containing *ortho*-anisic acid (3.00 g. 19.7 mmol) was added excess $SOCl_2$ (7.19 ml, 98.6 mmol) under nitrogen. A catalytic quantity of dry DMF was added and the reaction mixture was heated at 50^0C for 4h under nitrogen. The excess $SOCl_2$ was removed under reduced pressure. The resulting clear to slightly brown oil was dissolved in dry DCM (20ml) and was cooled to 0^0C. A solution of N,N-diisopropyl amine (23.7 mmol , 1.2 eq.) and triethylamine(4.12 ml, 29.6 mmol, 1.5 equiv.) in dry DCM was added dropwise to the acid chloride solution, resulting in a cream colour precipitate. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The resulting mixture was quenched with water (6ml), extracted with ethyl acetate (2 x 40ml), and successively washed with dil HCl (2 x 40ml) and saturated $NaHCO_3$(2 x 40ml). The organic layer was dried over anhydrous Na_2SO_4. After solvent evaporation under reduced pressure, the resulting residue was recrystallized by Ethyl acetate: Hexane to give pale yellow crystals of N,N-Di-isopropyl-2-methoxybenzamide **(77)** in 92% yield.

^{1}H NMR ($CDCl_3$) δ: 7.28 (1H, ddd, *J* = 1.91, 7.63, 9.16 Hz) ; 7.13 (1H, dd, *J*= 1.91, 7.63 Hz); 6,94 (1H, ddd, *J* = 7.63, 1.14, 7.63 Hz); 6.87 (1H, dd, *J* = 9.16, 1,14Hz); 3.80 (3H, s); 3.86 (1H, sept, *J* = 6.87 Hz); 3.48 (1H, sept, *J* = 6.48 Hz) ; 1.55 (3H, d, *J* = 6.87 Hz) ; 1.53 (3H, d, *J* = 6.87 Hz) ; 1.14 (3H, d, *J* = 6.48); 1.02 (3H, d, *J* = 6.48)

mp = 88–89 °C

2-(diisopropylcarbamoyl)-3-methoxyphenylboronic acid (76)



In an oven-dry flask, N,N-di-isopropyl-2-methoxybenzamide **(77)** (2.0 g, 8.51 mmol) was dissolved in dry THF (50 ml) under Ar atmosphere and the resulting solution was cooled to -78^{0}C. To the solution *t*-BuLi (1.7M solution in Hexane, 8.34 ml, 14.13 mmol) was added dropwise over 30 min to avoid rise in temperature. The yellowish colour reaction mixture was stirred at -78^{0}C for an additional 2 hours resulting in a thick slurry. The carbanion was quenched by the addition of trimethyl borate (3.42 g, 3.74 ml , 32.87 mmol). The solution was warmed to 25^{0}C and the THF was removed under reduced pressure.. The residue was rinsed with DCM (100 ml), then washed with 10% HCl (3 x 30 ml). The aqueous washings were back extracted with DCM (2 x 30 ml). The combined organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure, and the resulting residue was purified by acid-base extraction, to give **(76)** (1.66 g, 70%) a white coloured title compound.

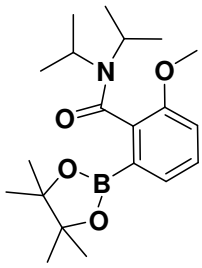
^{1}H NMR ($CDCl_3$) *δ*: 7.47 (1H, d, *J* = 7.3 Hz), 7.33 (1H, dd, *J* = 7.3, 8.2 Hz), 6.96 (1H, d, *J* = 8.2 Hz), 6.40 (2H, brs), 3.81 (3H, s), 3.62 (1H, sept, *J* = 6.7 Hz), 3.52 (1H, sept, *J* = 6.7 Hz), 1.57 (6H, d, *J* = 6.7 Hz), 1.08 (6H, d, *J* = 6.7 Hz)

^{13}C NMR ($CDCl_3$) *δ*: 171.5, 154.6, 132.0, 129.3, 127.7, 127.2, 112.8, 55.5, 51.7, 46.4, 20.7, 20.5, 20.4

Anal.Calcd for $C_{14}H_{22}BNO_4$: C, 60.24; H, 7.94. Found: C, 60.11; H, 7.96.

mp=149−150°C.

N,N-diisopropyl-2-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide(75)



In a flask fitted with azeotropic distillation condenser under N_2 atmosphere, a suspension of boronic acid **(76)** (1 g, 3.58 mmol) and pinacol (466 mg, 3.94 mmol) in dry toluene (50 mL) was heated to 120 °C for 5h. Toluene was removed to give the boronate **(75)** as a white solid (1.23 g, 95% yield).

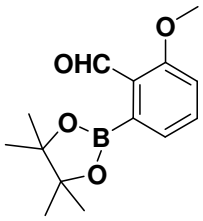
^{1}H NMR ($CDCl_3$) δ: 7.40 (1H, d, *J* = 7.3 Hz), 7.27 (1H, dd, *J* = 7.3 Hz), 6.96 (1H, d, *J* = 8.2 Hz), 3.79 (3H, s), 3.62 (1H, sept., *J* = 6.7 Hz), 3.49 (1H, sept., *J* = 6.7 Hz), 1.59 (6H, d, *J* = 6.7 Hz), 1.31 (12H, s), 1.11 (6H, d, *J* = 6.7 Hz)

^{13}C NMR ($CDCl_3$) δ: 168.3, 155.0, 134.7, 128.4, 128.1, 113.8, 84.1, 55.7, 51.1, 45.8, 25.0, 20.6, 20.4

Anal.Calcd for $C_{14}H_{32}BNO_4$: C, 66.49; H, 8.93. Found: C, 66.65; H, 8.90.

mp = 136–137 °C

2-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde (70)



Under argon atmosphere, to a precooled solution of boronate **(75)** (430 mg, 1.19 mmol) in anhydrous THF (20 mL) at 0 °C, a solution of DIBAL-H (1M in hexane, 170 mg, 1.2 mL, 1.19 mmol) was added slowly at 0 °C over 30 min to avoid rise in temperature. The reaction was quenched with 1M HCl (1.5 mL), pH 7 buffer (10 mL). The organic layer was separated and the aqueous layer was extracted with saturated brine: Ether: DCM (2 × 100 mL). The combined organic layer was dried on Na_2SO_4 and concentrated. The obtained residue was dissolved in minimum amount of hot *n*-hexane, filtered, and concentrated under reduced pressure to give **(70)** as a white powder (230 mg, 73% yield)

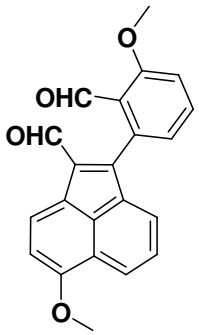
^{1}H NMR ($CDCl_3$) δ: 10.43 (1H, s), 7.52 (1, dd, *J* = 7.6, 8.8 Hz), 7.05 (1H, d, *J* = 7.6 Hz), 6.96 (1H, d, *J* = 8.8 Hz), 3.90 (3H, s)

^{13}C NMR ($CDCl_3$) δ: 191.0, 161.7, 135.6, 128.3, 124.6, 112.4, 84.2, 55.8, 29.9, 25.0

Anal.Calcd for $C_{14}H_{19}BNO_4$: C, 64.15; H, 7.31. Found: C, 64.32; H, 7.28.

mp = 107−108 °C

2-(2-formyl-3-methoxyphenyl)-6-methoxyacenaphthylene-1-carbaldehyde(68)



A solution of boronic acid **(70)** (55 mg, 0.16 mmol), bromoaldehyde **(69)** (55 mg, 0.19 mmol) and K_2CO_3 (319 mg, 2.31 mmol) in 1,4-dioxane/water (4/l) was degassed by purging N_2 for 30 min. $Pd(PPh_3)_4$ (8.8 mg, 0.0076 mmol) was added and the resulting reaction mixture was refluxed at 80 °C for 4 h under N_2 atmosphere, then cooled to room temperature, diluted with diethyl ether (10 mL) and filtered through a small silica gel bed. The bed was washed with diethyl ether (3 × 10 mL). The resulting mixture was dried and evaporated. Purification by chromatography using EtOAc: *n*-hexane (4:96) as an eluent gave dialdehyde **(68)** as a bright orange coloured powder. (50 mg, 75% yield);

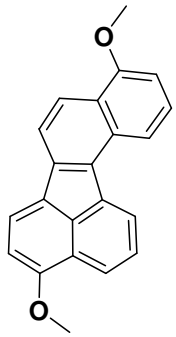
^{1}H NMR ($CDCl_3$) *δ*: 10.41 (1H, s), 9.96 (1H, s), 8.30 (1H, d, *J* = 7.7 Hz), 8.25 (1H, m), 7.61 (1H, m), 7.58-7.49 (2H, m), 7.15 (1H, d, *J* = 8.6 Hz), 7.07 (1H, d, *J* = 7.7 Hz), 6.85 (1H, d, *J* = 7.7 Hz), 4.04 (3H, s), 4.01 (3H, s)

^{13}C NMR ($CDCl_3$) *δ*: 190.0, 189.2, 162.1, 158.8, 152.5, 138.1, 135.8, 134.7, 134.3, 129.64, 129.58, 127.8, 127.14, 127.11, 126.5, 124.8, 124.6, 122.3, 112.6, 106.3, 56.3, 56.2

Anal.Calcd for $C_{22}H_{16}O_4$: C, 76.73; H, 4.68. Found: C, 76.89; H, 4.66

mp = 82–83 °C

3,10-dimethoxybenzo[j]fluoranthene (79)



$TiCl_4$ (55 mg, 316 µl, 2.87 mmol) was carefully added under N_2 atmosphere to dry THF (10 mL) at 0 °C, giving a bright-yellow mixture of $TiCl_4$.2 THF complex. The solution was refluxed for 20 min and cooled to room temperature. Zinc (406 mg, 6.21 mmol) was added and the mixture was refluxed for 2 h during which the colour changed from yellow to dark greenish blue. The mixture was cooled and pyridine (227 mg, 231 µl, 2.88 mmol) was added. Refluxing continued for 30 min. A solution of dialdehyde **(68)** (50 mg, 0.145 mmol) in dry THF (10 mL) was added and the reaction mixture was refluxed for a further 4h, then it was cooled. Saturated aqueous K_2CO_3 solution was added till the aqueous layer became almost colourless and transparent, then the aqueous layer was extracted with ethyl acetate (2× 20 mL). Evaporation of the solvent and purification by flash column chromatography on silica gel column provided fluoranthene **(79)** (40 mg, 89 % yield),

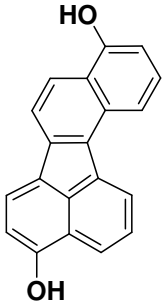
^{1}H NMR ($CDCl_3$) δ: 8.44 (1H, d, *J* = 7.2 Hz), 8.32 (1H, d, *J* = 8.6 Hz), 8.28 (1H, d, *J* = 8.6 Hz), 8.13 (1H, d, *J* = 8.6 Hz), 7.98 (1H, d, *J* = 8.6 Hz), 7.90 (1H, d, *J* = 7.6 Hz), 7.67 (1H, m), 7.52 (1H, m), 6.88 (1H, d, *J* = 7.6 Hz), 6.81 (1H, d, *J* = 7.6 Hz), 4.07 (3H, s), 4.05 (3H, s)

^{13}C NMR ($CDCl_3$) δ: 158.0, 156.5, 138.3, 137.5, 133.4, 133.3, 131.8, 129.7, 127.4, 127.2, 125.4, 124.5, 122.7, 122.3, 122.2, 121.9, 118.8, 116.7, 105.7, 103.2, 56.0, 55.7

mp = 164–165 °C

Anal.Calcd for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16. Found: C, 84.73; H, 5.14

Benzo[j]fluoranthene-3,10-diol (1)



A mixture of 3,10-dimethoxybenzo[j]fluoranthene **(79)** (40 mg, 0.128 mmol) and pyridine hydrochloride (537 mg, 4.65 mmol) was heated under N_2 at 140-150 °C for 30 min during which the solid mixture became homogeneous. After complete conversion, the reaction was allowed to cool to room temperature. The reaction was poured in ice cold water and was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with water (2 × 10 mL), dried over sodium sulfate to give a residue which was purified by flash column chromatography to afford **1** as a dark greenish yellow solid **(1)** (24 mg, 65 % yield)

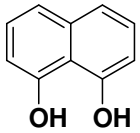
^{1}H NMR (acetone-d_6) δ: 9.62 (1H, brs), 9.17 (1H, brs), 8.63 (1H, d, *J* = 7.0 Hz), 8.38 (1H, d, *J* = 8.6 Hz), 8.33 (1H, dd, *J* = 8.5, 0.8 Hz), 8.25 (1H, d, *J* = 8.0 Hz), 8.09 (1H, d, *J* = 8.6 Hz), 8.03 (1H, d, *J* = 7.5 Hz), 7.78 (1H, dd, *J* = 8.0, 7.0 Hz), 7.50 (1H, dd, *J* = 8.5, 7.5 Hz), 7.08 (1H, d, *J* = 7.5 Hz), 6.96 (1H, dd, *J* = 7.5, 0.8 Hz)

^{13}C NMR (acetone-d_6) δ: 157.0, 155.1, 139.2, 138.2, 134.1, 133.3, 133.0, 129.3, 128.6, 127.8, 125.3, 123.9, 123.4, 122.9, 122.7, 119.0, 116.2, 111.2, 108.2

Anal.Calcd for $C_{20}H_{12}O_2$: C, 84.49; H, 4.25. Found: C, 84.65; H, 4.27

mp = 164–165 °C

1,8-Dihydroxynaphthalene (90)

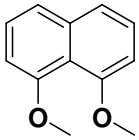


Commercially available 1,8-naphthosultone (10 g, 0.048 mol) and KOH (41 g, 0.73 mol) were heated together in a stainless steel beaker at 300^0C with a Bunsen burner, and the temperature was kept at 300^0C for 30 min until the mixture became a homogeneous black liquid. It was then cooled down to RT and diluted with 400 ml ice-cold water, then hydrochloric acid (conc. HCl-H_2O, 1:2) added with stirring until neutral pH was obtained. Then EtOAc (400 ml) was added and whole mixture was stirred for 30 min for extraction of diol. The two layers were separated and the aqueous layer was extracted with EtOAc (3 x 100ml). The combined organic layer was dried (Na_2SO_4) and the solvent removed under reduced pressure. The black residue was purified by chromatography (EtOAc: Hexane. 1:9) to obtain 1,8-dihydroxynaphthalene as white solid in 80% yield.

^{1}H NMR ($CDCl_3$) *δ*: 7.92 (2H, br, s), 7.36 (2H, dd, *J*= 0.8, 8.4Hz), 7.28 (2H, t, *J*= 8.0), 6.80 (2H, dd, *J*= 0.8, 7.5Hz),

mp: 142 °C

1,8-Dimethoxynaphthalene (91)

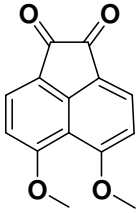


To a suspension of 1,8-dihydroxynaphthalene **(90)** (3.63 g, 22.69 mol), in 28.33 ml of 5M aq. NaOH solution and phase transfer catalyst aliquat®336 (0.97 g, 0.1 equiv.), methyl iodide (9.67 g, 4.3 ml, 0.068 mol) was added .Then the reaction mixture was heated to 60-70^{0}C temperature for 2h. Then, the reaction mixture was cooled to room temperature, filtered, the residue was washed with water (50 ml) and after 30 min with hexane (50 ml). The obtained product was a faint pink coloured powder, which was recrystallized from hot ethanol (50 ml). Then, the reaction mixture was cooled to room temperature, the obtained precipitate was filtered and dried in vacuo to give a buff white powder of ether **(91)** (2.9 g, 70%).

^{1}H NMR ($CDCl_3$) δ: 7.45 -7.28 (4H, m), 6.85 (2H, d, *J*= 8.09 Hz), 3.95 (3H, s).

mp.: 160-160.8 °C

5,6-Dimethoxy-acenaphthylene-1,2-dione (92)



In an ovendried flask, 1,8-dimethoxynaphthalene **(91)** (2 g, 10.64 mmol) was dissolved in dry dichloromethane (35 mL) under argon atmosphere. The resulting solution was cooled to 0−5 °C. After 30 min anhydrous $AlCl_3$ (1.71 g, 12.80 mmol) was added in a single lot, followed by dropwise addition of oxalyl chloride (0.68 g, 480 μL, 5.30 mmol). The reaction mixture was allowed to reach room temperature overnight, and then poured slowly into ice−water (200 mL) containing 40 mL of conc. HCl under stirring. After addition of 50 mL of n-hexane, the above mixture was stirred for further 35−40 min. The resultant mixture was filtered through a sintered funnel, and the residue was washed with water (100 mL) and 2% EtOAc/n-Hexane (30 mL). gave diketone **(92)** (2.06 g, 80%) as an orange solid

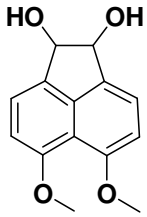
^{1}**H NMR (CDCl$_3$ + TFA)** δ**:** 8.06 (2H, d, J = 8.4 Hz), 7.03 (2H, d, J = 8.4 Hz); 4.12 (6H, s)

^{13}**C NMR (CDCl$_3$ + TFA)** δ**:**188.3, 163.4, 152.9, 127.1, 120.3, 114.4, 107.6, 57.0.

Anal. Calcd for $C_{14}H_{10}O_4$: C, 69.42; H, 4.16. Found: C, 69.25; H, 4.18.

mp : 262-265 °C (dec.)

5,6-Dimethoxy-acenaphthene-1,2-diol (93)



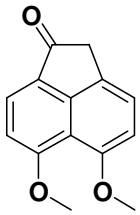
To a suspension of diketone **(92)** (2 g, 8.26 mmol) in ethanol (334 mL) and water (71 mL), $NaBH_4$ (6.25 g, 165.30 mmol) was added in portions with stirring at room temperature. The reaction was stirred for further 21 h at room temperature, and then poured into ice-cold water (400 mL), and treated with solid NH_4Cl (16.70 g). DCM (400 mL) was added, and the mixture stirred for 30 min. The organic layer was separated, and the aqueous layer was extracted with DCM (3 × 100 mL). The combined organic layers were dried and evaporated. The obtained residue was washed with EtOAc:hexane (1:99) and filtered through a sintered funnel to give diol **(93)** (1.87 g, 92% yield). The dried product was enough pure for further reaction.

1H NMR (DMSOd_6) *δ*:Mixture of diastereomers 2:1, Major δ 7.30 (2H, d, *J* = 7.9 Hz), 6.90 (2H, d, *J* = 7.9 Hz), 5.60 (2H, d, *J* = 5.8 Hz, exchanges with D_2O), 5.03 (2H, d, *J* = 5.8 Hz), 3.84 (6H, s), minor δ 7.46 (2H, d, *J* = 7.9 Hz), 6.95 (2H, d, *J* = 7.9 Hz), 5.89 (2H, s), 3.87 (6H, s)

13C NMR (DMSO-d6) *δ*: Major δ 155.0, 132.7, 121.2, 107.4, 82.1, 56.0, minor δ 156.1, 139.0, 135.3, 123.0, 80.0, 56.0.

Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.45; H, 5.76.

5,6-Dimethoxy-2H-acenaphthylen-1-one (94)



To a suspension of diol **(93)** (1.5 g, 6.10 mmol) in dry toluene (150 mL), ptoluenesulfonic acid (0.25 g, 1.31 mmol) was added under N_2atmosphere. The reaction mixture was heated at 80 °C for 2 h. and then cooled to room temperature and washed with aq. satd. $NaHCO_3$ (100 mL). The separated organic layer was sequentially washed with brine (2 × 100 mL) and water (2 × 100 mL). The separated organic layer was dried, and the solvent evaporated to a crude product that was purified by chromatography using acetone:DCM (1:99) as an eluent to give ketone **(94)** (1.04 g, 75% yield) as an orange colored powder.

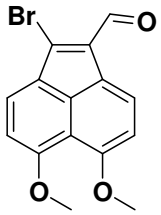
^{1}H NMR ($CDCl_3$) *δ*: 7.94 (1H, d, *J* = 7.9 Hz), 7.34 (1H, d, *J* = 7.9 Hz), 7.01 (1H, d, *J* = 7.9 Hz), 6.87 (1H, d, *J* = 7.9 Hz), 4.10 (3H, s), 4.01 (3H, s), 3.71 (2H, s);

^{13}C NMR (CDCl3) *δ*: δ 201.8, 161.2, 154.8, 146.1, 129.3, 127.1, 125.9, 125.7, 123.7, 122.9, 121.8, 114.1, 107.0, 106.2, 56.2, 55.8, 41.5.

Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.89; H, 5.27.

mp = 133 °C

2-bromo-5.6-dimethoxyacenaphthylene-1-carbaldehyde (86)



To a precooled solution of dry DMF (0.91 g, 957 μL, 12.30 mmol) in dry chloroform (13.75 mL) under argon atmosphere, PBr_3 (3.03 g, 1.06 mL, 11.19 mmol) was added dropwise at 0 °C over 20 min. The reaction was stirred for 1 h at the same temperature, and then the solution of ketone **(94)** (0.85 g, 3.73 mmol) in anhydrous chloroform (12.2 mL) was added over 30 min maintaining the temperature to 0 °C. The reaction mixture was allowed to reach room temperature overnight, and then was poured slowly into ice−water mixture (100 g to 100 mL) to avoid emulsion, and neutralized by solid $NaHCO_3$ to p^H 7. The organic layer was separated, and the aqueous layer was again extracted with EtOAc (2 × 100 mL). The combined organic layers were dried on Na_2SO_4. Evaporation of solvent gave a crude product that was purified by chromatography using EtOAc:n-hexane (30:70) as an eluent to give **(86)** (0.22 g, 79% yield) as an orange colored powder.

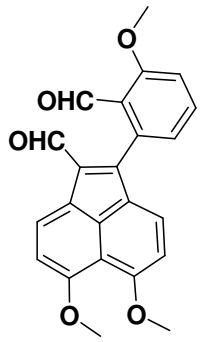
NMR ($CDCl_3$ + TFA) δ: 9.91 (1H, s), 8.29 (1H, d, *J* = 8.2 Hz), 8.05 (1H, d, *J* = 8.2 Hz), 7.11 (1H, d, *J* = 8.2 Hz), 6.96 (1H, d, *J* = 8.2 Hz), 4.16 (3H, s), 4.09 (3H, s)

^{13}C NMR ($CDCl_3$ + TFA) δ : 190.6, 165.1, 159.2, 138.0, 132.1, 131.4, 130.6, 129.6, 129.0, 126.0, 112.8, 109.2, 108.2, 57.2, 56.9.

Anal. Calcd for $C_{15}H_{11}BrO_3$: C, 56.45; H, 3.47. Found: C, 56.63; H, 3.45.

mp = 132−136 °C

2-(2-formyl-3-methoxyphenyl)-5,6-dimethoxyacenaphthylene-1-carbaldehyde (87)



To a solution of boronate **(70)** (166 mg, 0.634 mmol), bromo compound **(86)** (200 mg, 0.627 mmol) and K_2CO_3 (518 mg, 3.746 mmol) in 1,4-dioxane/water (11ml/2.6 ml), degassed by purging N_2 for 30 min, $Pd(PPh_3)_4$ (14.5 mg, 0.013 mmol) thewas added and resulting reaction mixture was refluxed at 80^0C for 4h under N_2 atmosphere. Then, the reaction was cooled to room temperature and diluted with diethyl ether (10 ml), the resulting diluted reaction mixture was filtered through a short silica gel bed. The silica gel bed was washed with diethyl ether (3 x 20ml), the resulting mixture was dried on Na_2SO_4, and after evaporation of solvent under reduced pressure gave a crude product. The crude product was purified by flash column chromatography using EtOAc: DCM (2:98) as an eluent, to gave dialdehyde **(87)** (210 mg, 89%) as a red coloured powder.

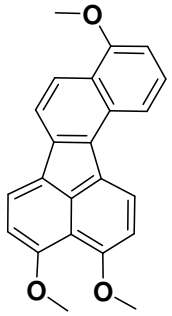
^{1}H NMR ($CDCl_3$) *δ*: δ 10.34 (1H, s), 9.92 (1H, s), 8.42 (1H, d, J = 7.9 Hz), 7.64 (1H, dd, J = 7.9, 8.2 Hz), 7.53 (1H, d, J = 7.9 Hz), 7.17 (1H, d, J = 8.2 Hz), 7.11 (1H, d, J = 7.9 Hz), 6.98 (1H, d, J = 7.9 Hz), 6.90 (1H, d, J = 7.9 Hz), 4.10 (3H, s), 4.09 (3H, s), 4.04 (3H, s)

^{13}C NMR ($CDCl_3$) *δ*: 190.3, 188.7, 162.4, 161.6, 159.4, 151.0, 136.7, 134.5, 132.7, 131.3, 30.9, 129.8, 129.7, 127.4, 124.9, 124.8, 112.4, 107.7, 107.5, 107.1, 56.8, 56.7, 56.3.

Anal.Calcd for $C_{23}H_{18}O_5$: C, 73.79; H, 4.85. Found: C, 73.92; H, 4.82.

mp = 128 – 129 °C

3,4,10-trimethoxybenzo[j]fluoranthen (88)



To a solution of zerovalent titanium prepared from $TiCl_4$ (1.408 g, 810 µl 7.417 mmol) was carefully added under N_2 atmosphere, dry THF (50 mL) at 0^0C, thus affording a bright-yellow mixture due to the formation of $TiCl_4$.2THF complex. After stirring the mixture at 0^0C for 5 min, zinc (1.116 g, 16.02 mmol) was added and the mixture was refluxed for 2 h (the colour changes from yellow to dark blue), after which pyridine (0.586 g, 597.4 µl, 7.417 mmol) was added and the refluxing continued for another 30 min. After cooling the mixture at room temperature, a solution of dialdehyde **(87)** (0.140 g, 0.374 mmol) in dry THF (15 mL) was added and the reaction mixture was refluxed for a further 7 h. Then reaction mixture was cooled and quenched with saturated aqueous K_2CO_3 solution (50 ml). The precipitated inorganic material was removed by filtration. The precipitate was thoroughly washed with THF. Then organic layer was separated and aqueous layer from the filtrate was extracted with ethyl acetate (2 x 30 ml). The combined organic layer was dried on Na_2SO_4 , the solvent was removed under reduced pressure. The obtained residue was then purified by column chromatography using DCM: Hexane (50:50) as an eluent, to give arene **(88)** (103 mg, 80%) as a fluorescent yellow coloured powder.

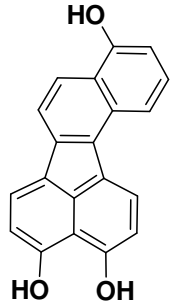
^{1}H NMR ($CDCl_3$) δ: 8.37 (1H, d, J = 7.8 Hz), 8.31–8.20 (2H, m), 8.02–7.93 (2H, m), 7.50 (1H, dd, J = 7.8, 7.8 Hz), 7.03–6.92 (2H, m), 6.81 (2H, d, J = 7.3 Hz), 4.10 (3H, s), 4.09 (3H, s), 4.04 (3H, s)

^{13}C NMR ($CDCl_3$) δ: 158.7, 158.0, 156.5, 137.3, 135.2, 132.8, 131.3, 130.3, 129.3, 127.0, 25.8, 125.3, 122.6, 120.9, 118.7, 116.7, 114.4, 107.0, 106.8, 103.2, 56.7, 56.6, 55.7.

Anal.Calcd for $C_{23}H_{18}O_3$: C, 80.68; H, 5.30. Found: C, 80.84; H, 5.32.

mp = 164 – 165 °C

benzo[j]fluoranthene-3,4,10-triol (89)



A mixture of compound **(88)**(104 mg, 0.304 mmol) and pyridine hydrochloride (1.277 g, 11.05 mmol) was placed in a stoppered round bottom flask and subjected to microwave irradiation at 210W for 20 min. After complete conversion the reaction mixture was decomposed using ice-water (50 ml) and extracted with diethyl ether (3 x 30 ml). The whole organic layer was washed with water (20 ml), then the organic layer was dried on Na_2SO_4. Then, the solvent was evaporated under reduced pressure, to give trihydroxy compound **(89)** (75 mg, 69%) as a dark green coloured powder.

^{1}H NMR (acetone d_6) δ: δ 9.97 (1H, brs), 9.06 (1H, brs), 8.43 (1H, d, J = 7.8 Hz), 8.25 (1H, d, J = 8.5 Hz), 8.21 (1H, d, J = 8.5 Hz), 8.07−8.02 (2H, m), 7.42 (1H, d, J = 7.6 Hz), 7.39 (1H, d, J = 7.6 Hz), 7.05 (1H, d, J = 7.8 Hz), 7.00 (1H, d, J = 7.6 Hz), 6.89 (1H, dd, J = 7.6, 0.8 Hz);

^{13}C NMR (acetone d_6) δ: δ156.8, 156.2, 155.1, 137.9, 132.5, 129.9, 128.1, 128.0, 127.5, 127.4, 124.4, 124.3, 121.7, 121.3, 119.0, 116.4, 111.2, 110.9, 108.2, 104.1.

Anal.Calcd for $C_{20}H_{12}O_3$: C, 79.99; H, 4.03. Found: C, 80.16; H, 4.00.

mp = 175 °C

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CHAPTER 4

APPROACHES TO THE SYNTHESIS OF BULGAREIN

4.1 Introduction

As discussed in the Chapter **1**, serious damage is done to crops each year by fungal infections, which are the number one cause of crop loss worldwide. The losses estimated are about 70% of all major crop diseases. The historical perspective discussed in first chapter clearly reflects applications, advantages, and limitations of commercialized groups of fungicides. With the increasing environmental and regulatory pressures there is increasing interest to develop fungicides that are safe to the environment and end-user. Natural products with fungicidal activity have emerged as an important source of lead compounds. These natural compounds provide diversity in terms of structure and mode of action, and can lead to highly successful crop protection products. The products can be directly used or can be modified chemically to increase their fungicidal properties.

4.2 Topoisomerase Inhibitors as Antifungal compounds

DNA topoisomerase I could be a potential target for therapeutic antifungal agents predicted to have a fungicidal mode of action.

DNA topoisomerases are enzymes which modulate the topological structure of DNA, being associated with the processes of DNA transcription, replication, and recombination[1-3].Topoisomerases are distinguished by their reaction mechanism as either type I topoisomerases which introduce a transient nick in one single strand of DNA backbone during catalysis or type II topoisomerases which make concerted breaks on both strand of DNA[4-6]

Since topoisomerases are found in both the host cell and the invading pathogen, agents with selectivity for the fungal topoisomerases over the human enzyme are anticipated to reduce side effects on the host cells. Studies done on varying degrees of selectivity for the fungal topoisomerase I compared to the human enzyme showed that there are sufficient structural

differences between the topoisomerase I from *Candida albicans* and human cells to allow selective targeting of the fungal topoisomerase I over its human counterpart.[7] For example the aminocatechol A-3253 is active against several pathogenic fungi, including *Candida albicans*, *Cryptococcus albidus*, and *Aspergillus niger*. A-3253 interferes with both the in vitro biosynthesis of (1,3)-β-glucan and the activity of topoisomerases I isolated from *Candida* spp.

Another example which illustrates the potential of Topoisomerase I enzyme inhibitors is the alkaloid Eupolauridine[8]. Eupolauridine shows in vitro antifungal activity against *C. albicans*, *C. neoformans*, *A. fumigatus*, *A. flavus*, and *T. mentagrophytes.* The activity of eupolauridine against *C. albicans* has also been reported. Interestingly Eupolauridine showed no cytotoxicity to any of the mammalian cell lines tested at concentrations up to 60 µg/ml.

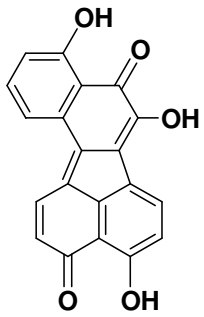
A-3253 **Eupolauridine**

Fig. 4.1

The above examples show topoisomerase I inhibitors as a potential means to control the opportunistic fungal infection. Although all the studies mentioned in literature are carried out against the fungal pathogen infecting humans, it is also possible that topoisomerase I be a useful target against the fungi infesting agriculture crops.

4.3 Bulgarein

Bulgarein is a fungal metabolite produced by the fungus *Bulgaria inquinans*. The structure of Bulgarein was first reported by Edwards and Lockett[9,10] Fuji isolated again bulgarein from a fungus *Heteroconium sp.* and reported its catalytic activity in inhibiting Topoisomerase I *in vitro*. Acivity of Bulgarein was comparable to that of camptothecin at a drug concentration range 0.025 - 5μM. No further report has appeared in the literature on the topoisomerase inhibiting activity of bulgarein, and no synthesis of this compound has been reported so far.



1

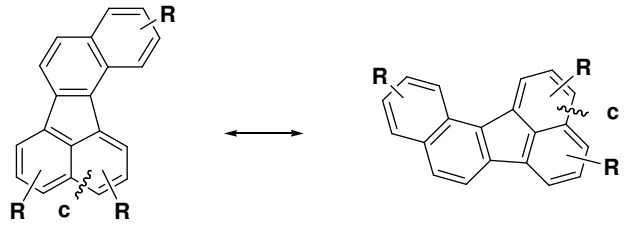
Fig. 4.2

As Bulgarein has Topoisomerase I inhibiting activity and its total synthesis has not yet been reported, we found it to be a challenging target that can be synthesised and derivatised to test its activity as a potential antifungal compound.

As discussed in Chapter 3, attempts to obtain benzo[j]fluoranthenes oxygenated at ring B following the procedure developed by us failed (**Chapter 3. section 3.7**). Thus, we decided to study a different approach, inspired by the synthesis reported by Rice.[11]

The literature survey of approaches towards the synthesis of benzo[j]fluoranthenes has been discussed in detail in Chapter **3**, the first retrosynthetic attempt towards the total synthesis of **(1)** was inspired by Rice et.al **(Scheme 4.1)**

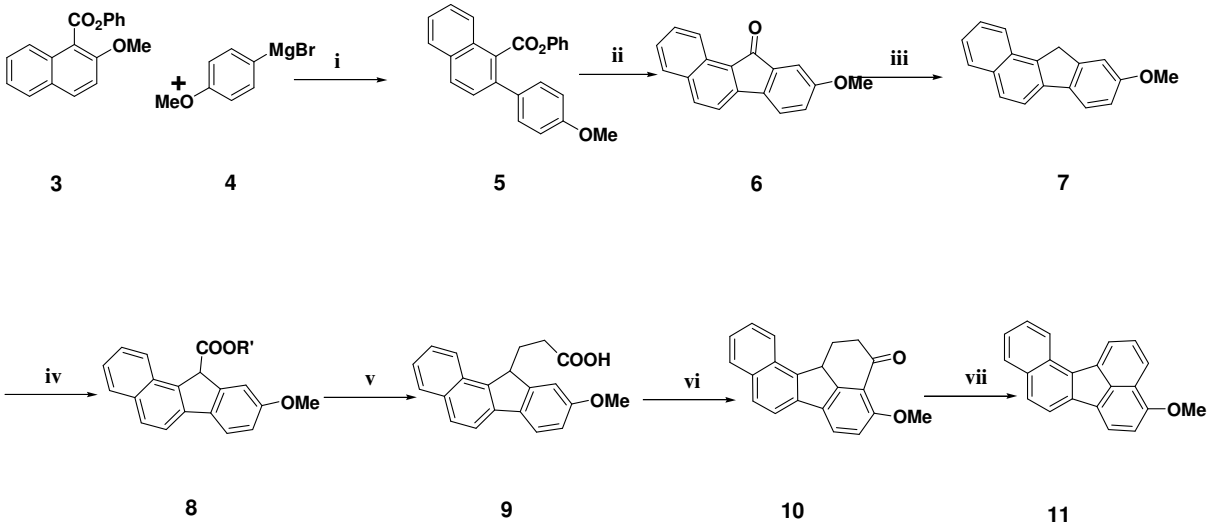
4.3.1 Disconnection 'c'



2

Fig. 4.3

Rice Approach (*J. Org. Chem.* **1987**, 52, 849–855.)



Scheme 4.1

Reagents and conditions.

(viii) 1:1 (diethyl ether : benzene), reflux, 12h, 77%;

(ix) MSA, 90min, rt, 100%

(x) Zn amalgam, conc. HCl, in toluene, reflux, 23h, 59%

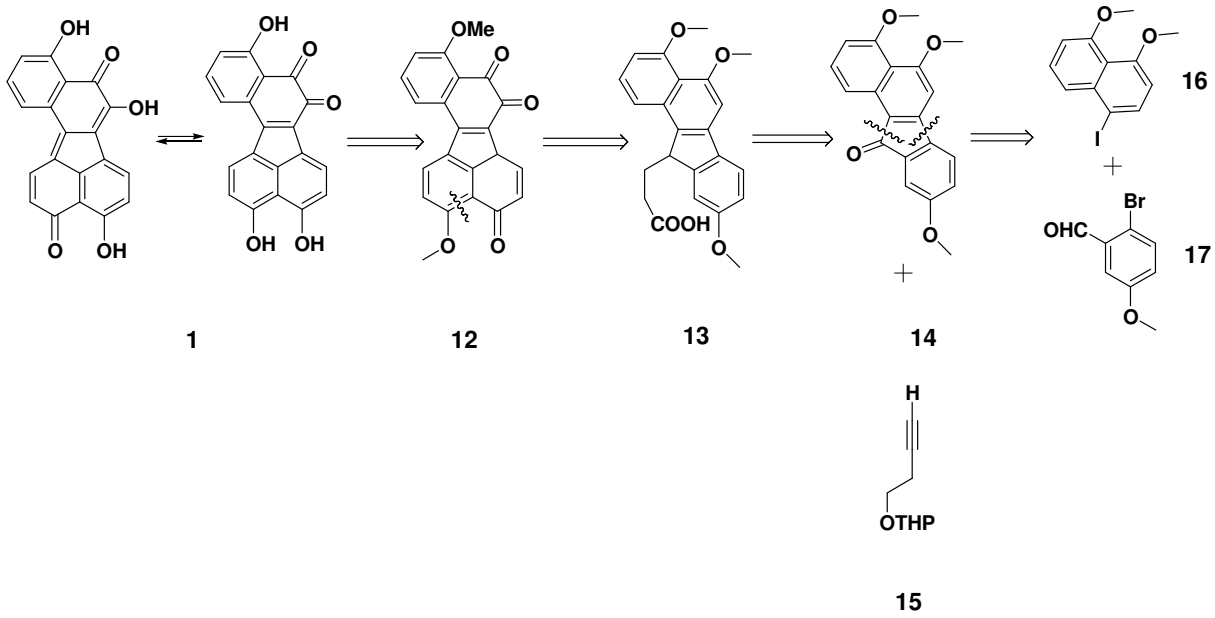
(xi) a) diethyl ether, –78 ºC b) nBuLi, – 30 ºC, 1h c) dry ice d) EtOH, benzene, con. H_2SO_4, 69%

(xii) pyridine, 0 ºC, acrylonitrile, 10 N NaOH, 0 ºC – rt, 97%

(xiii) MSA, rt, 19h, 70%

(xiv) 1-methylnaphthalene, 10% Pd/C, reflux, N_2 gas bubbling, 7 h, 100%

Since the reported synthetic strategy is very lengthy, we thought of an alternative retrosynthesis involving Domino reaction which is as follows,



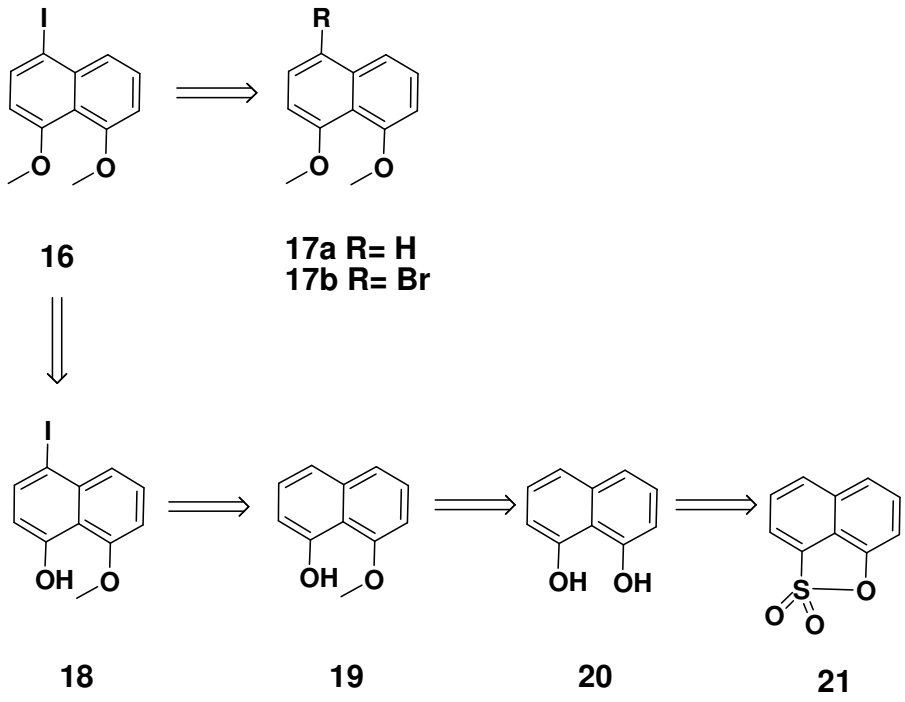
Scheme 4.2

4.4 Domino reaction approach

Domino reactions are defined as processes of two or more bond-forming reactions under identical conditions, in which the subsequent transformation takes place at the functionalities obtained in the former transformation. They allow the efficient synthesis of complex molecules from simple substrates in an ecologically (not always!!) and economically favorable way[12].

Palladium intermediates provide an avenue by which this strategy can be realized. It has been well established that palladium intermediates possess electrophilic character; however, it has recently been demonstrated that nucleophilic behavior is also possible.[13-15]

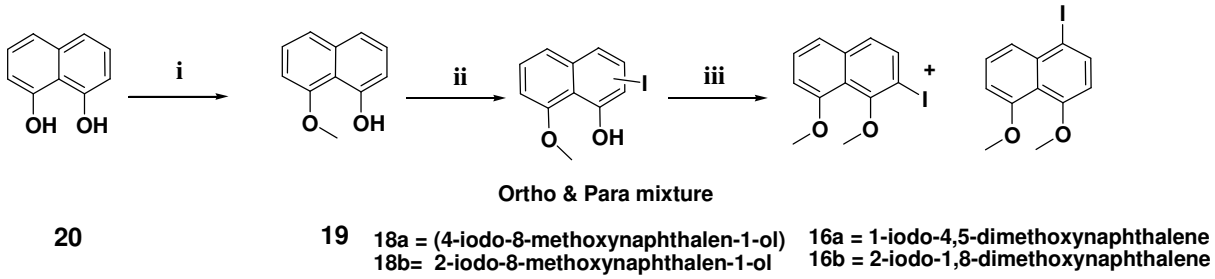
Lautens et al[16].reported the synthesis of a number of 9H-fluoren-9-one derivatives under the optimized conditions and in moderate to good yield. Based on this report we planned to synthesize the desired intermediate **(14).** For this we required the compound **(16).**



Scheme 4.3

As there are no reports for the iodination of compound **(17a)** several different conditions were tried,[17] for example halogen exchange of brominated compound **(17b)** using various conditions which resulted in either no halogenations exchange or debrominated[18,19] 1,8-dimethoxy naphthalene. Hence attempts were made towards iodination of compound **(19)** assuming to obtain **(18)** exclusively.[20] However, iodination of compound **(19)** resulted in a mixture of compounds with iodination at *ortho* and *para* to the free hydroxyl group, which were inseparable on TLC **(18a)** and **(18b)**. When the mixture was subjected to methylation using MeI and NaH in DMF[19] to our delight the two isomers were separated on TLC. Purification by flash column chromatography gave the desired product **(16a).**

The obtained compound was reacted with 2-bromo-5-methoxybenzaldehyde using the conditions reported[16], to give the desired fluorenone **(14).**



Scheme 4.4

Reagents and conditions.

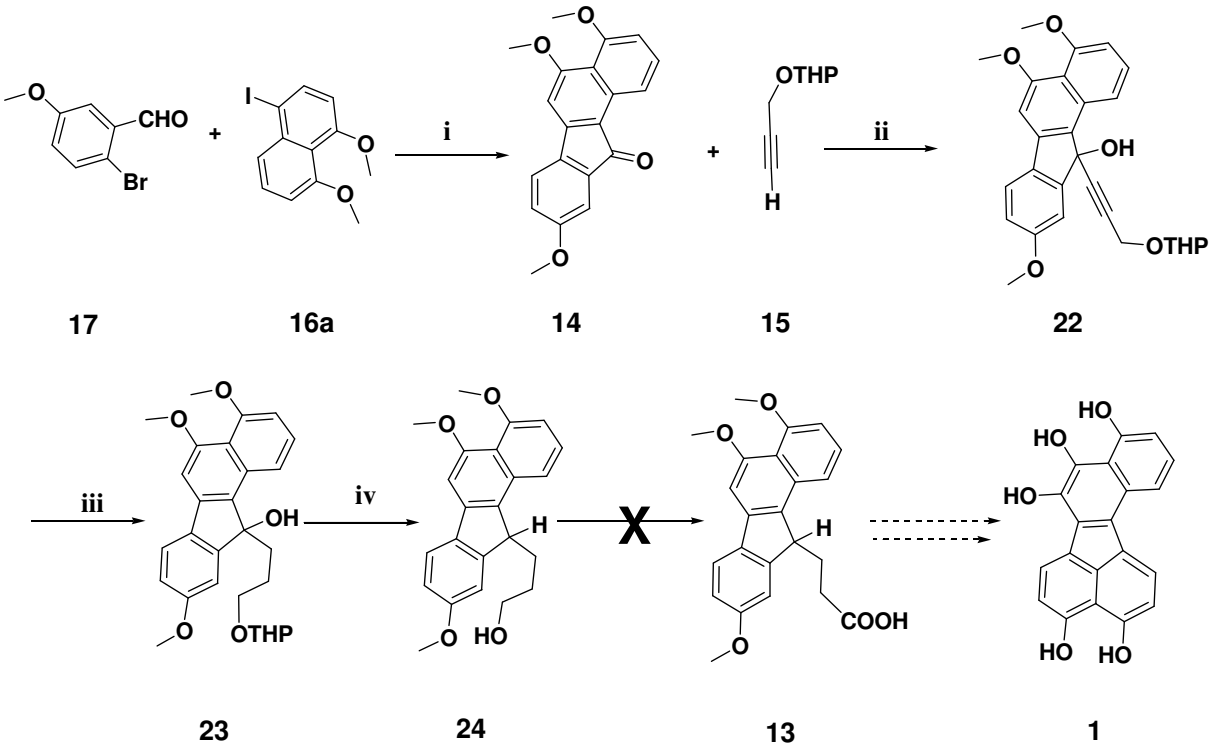
(i) K_2CO_3, MeI, acetone, 12h,

(ii) pTSA, NIS, Acetonitrile, 0 ºC, 4-6h,

(iii) MeI, NaH, DMF, 12h,

The three carbon chain was introduced as protected propargyl alchol[21] **(15).** It was reacted with compound **(18)** to give compound **(22).** [21] The triple bond was reduced using Pd/C in ethyl acetate to give compound **(23).**

.Deoxygenation[22] was carried out using triethylsilane and TFA which resulted in both deoxygenation of tertiary hydroxy group and deprotection of OTHP group to give desired compound **(24)**. Various methods to oxidize the primary alcohol of compound **(24)** to either aldehyde or acid failed.



Scheme 4.5

Reagents and conditions.

(i) TPP, Norbornene, $CsCO_3$, dimethoxyethane, $Pd(Ac)_2$, reflux, 10 h,

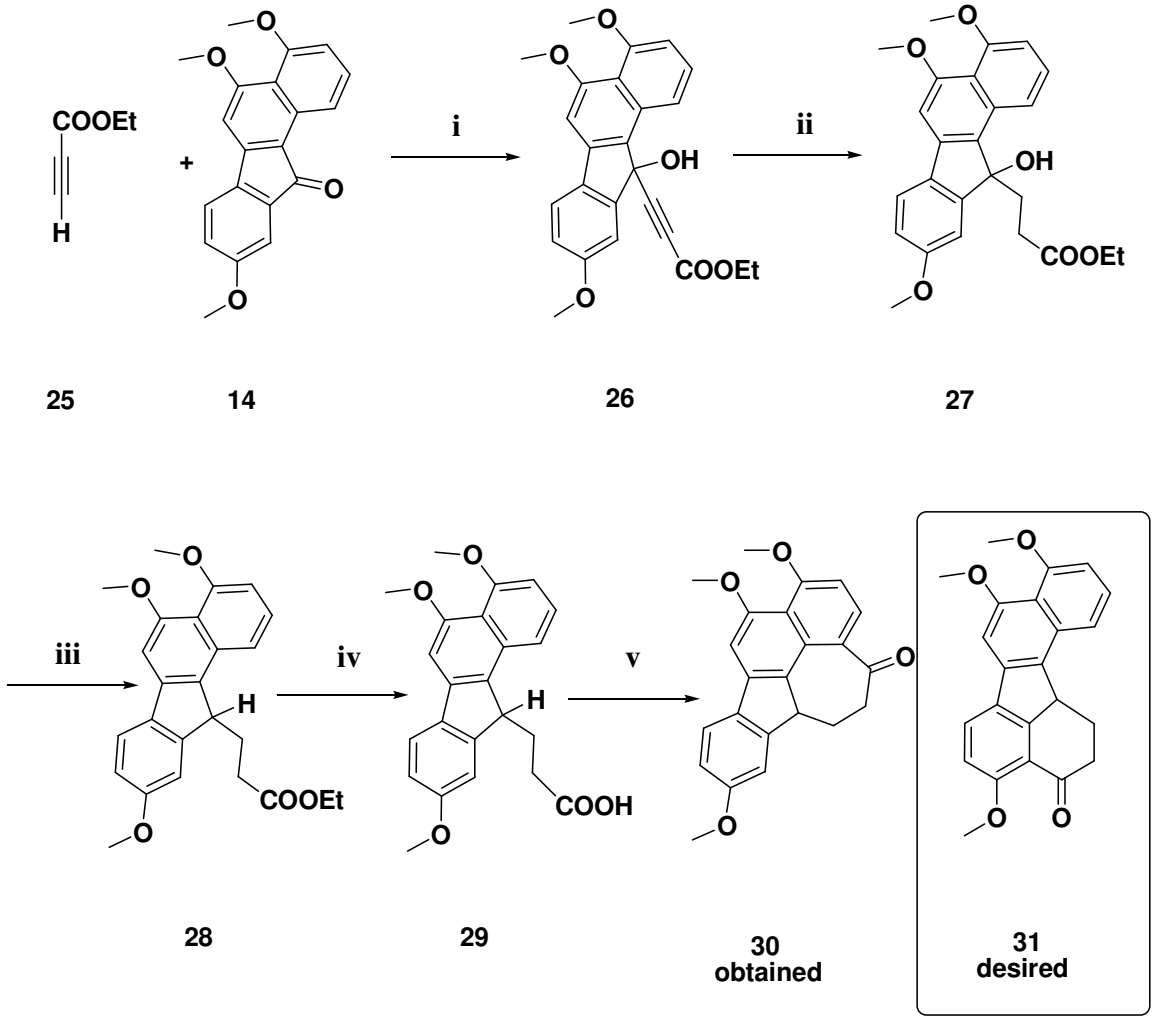
(ii) Potassium *tert*-butoxide, DMF, −55 - −65 ºC

(iii) 10% Pd/C, H_2 gas, 4h,

(iv) Et_3SiH, TFA, 1h.

Hence, instead of using compound **(15),** we decide to use ethyl propiolate **(25)**. Reaction of ethyl propiolate **(25)** with compound **(14)** resulted in compound **(26).** Reduction of this compound followed by deoxygenation gave compound **(28).** [22] Hydrolysis of **(28)** using LiOH[23] gave acid **(29).** Acid **(29)** was subjected to intramolecular acylation using MSA.[24] Disappointingly, the product obtained was not a six- but a seven-membered compound **(30).**

Various conditions were tried, eg. Fridel-Crafts acylation using $AlCl_3$, PPA, but none of the methods gave the desired six-member cyclised compound.



Scheme 4.6

Reagents and conditions.

(i) Potassium *tert*-butoxide, DMF, −55 - −65 ºC

(ii) 10% Pd/C, H_2 gas, 4h.

(iii) Et_3SiH, TFA, 1h.

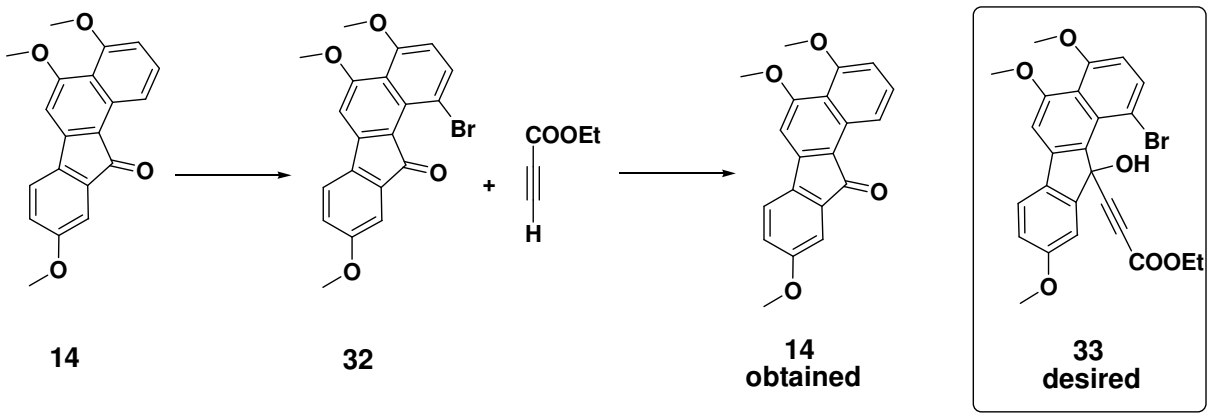
(iv) THF:H_2O (1:1), LiOH, 12h, rt.

(v) MSA, rt, 12h

With this result in hand we thought of blocking the position using bromine, so that the only possible cyclised product would be the six-membered compound **(31).**

Hence compound **(14)** was brominated using NBS in dry DMF. The major product formed was brominated at the desired carbon[25] **(32).**

However reaction of ethyl propiolate with **(32)** gave debrominated compound **(14)** instead of compound **(33).** This result led us to a possible conclusion that perhaps the carbonyl carbon is too sterically hindered for a nucleophilic attack. Also there should be other unknown electronic reasons behind the failure to obtain the desired product.

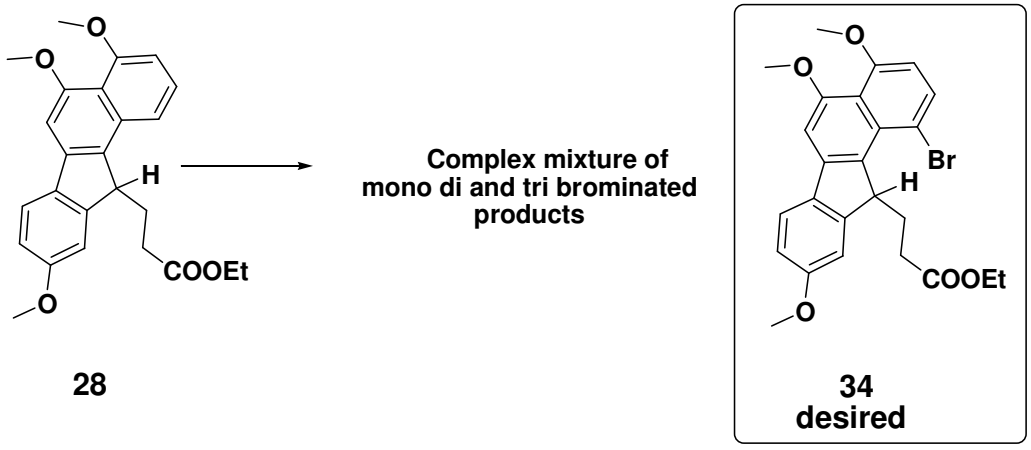


Scheme 4.7

Reagents and conditions.

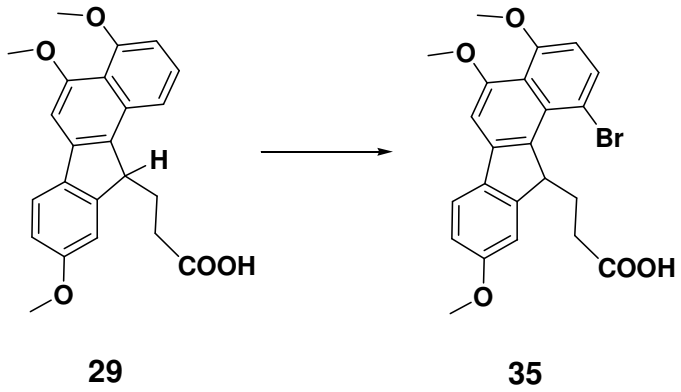
(i) Potassium *tert*-butoxide, DMF, −55 - −65 ºC

So we decided to do bromination in the late stages of the synthetic scheme. Bromination[25] of **(28)** was carried out using NBS in DMF, however a mixture of mono-, di- and tribrominated compounds was obtained.



Scheme 4.8

Hence in order to get a better selectivity, bromination of compound **(29)** was tried using the same conditions, and the desired product with bromination[25] at correct carbon was obtained as a major product **(35)**.

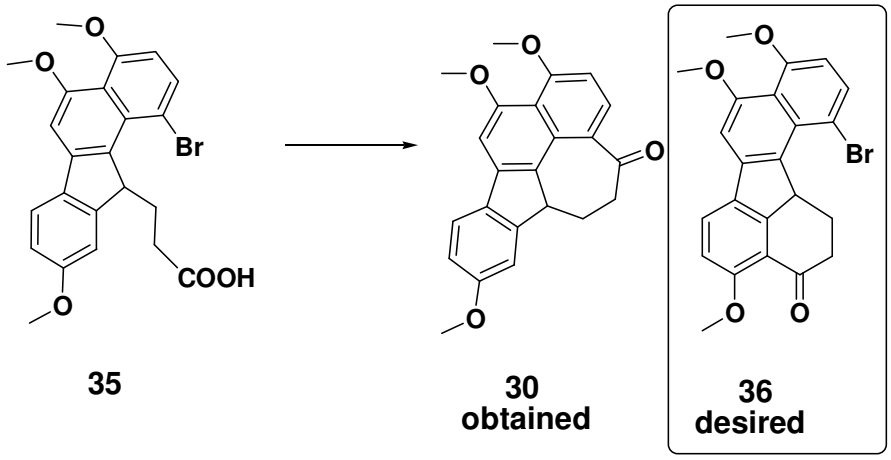


Scheme 4.9

Reagents and conditions.

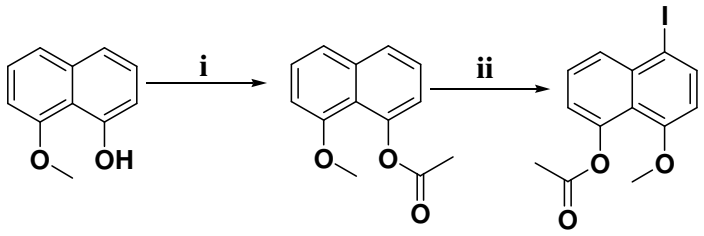
(i) NBS, DMF, rt, 12 h

Attempts to cyclise compound **(35)**.using various conditions for cyclisation did not yield the desired product. On the contrary, debromination followed by cyclisation to seven-membered compound gave one of the products.



Scheme 4.10

Another possibility was the deactivation of the ring by changing the protecting group at the hydroxyl from methyl to acetate.group.[26] For this purpose the monomethoxy compound was protected with an acetate group. This compound was subjected to iodination which gave a single isomer, where iodine was[27] para to the methoxy group **(38).**



Scheme 4.11

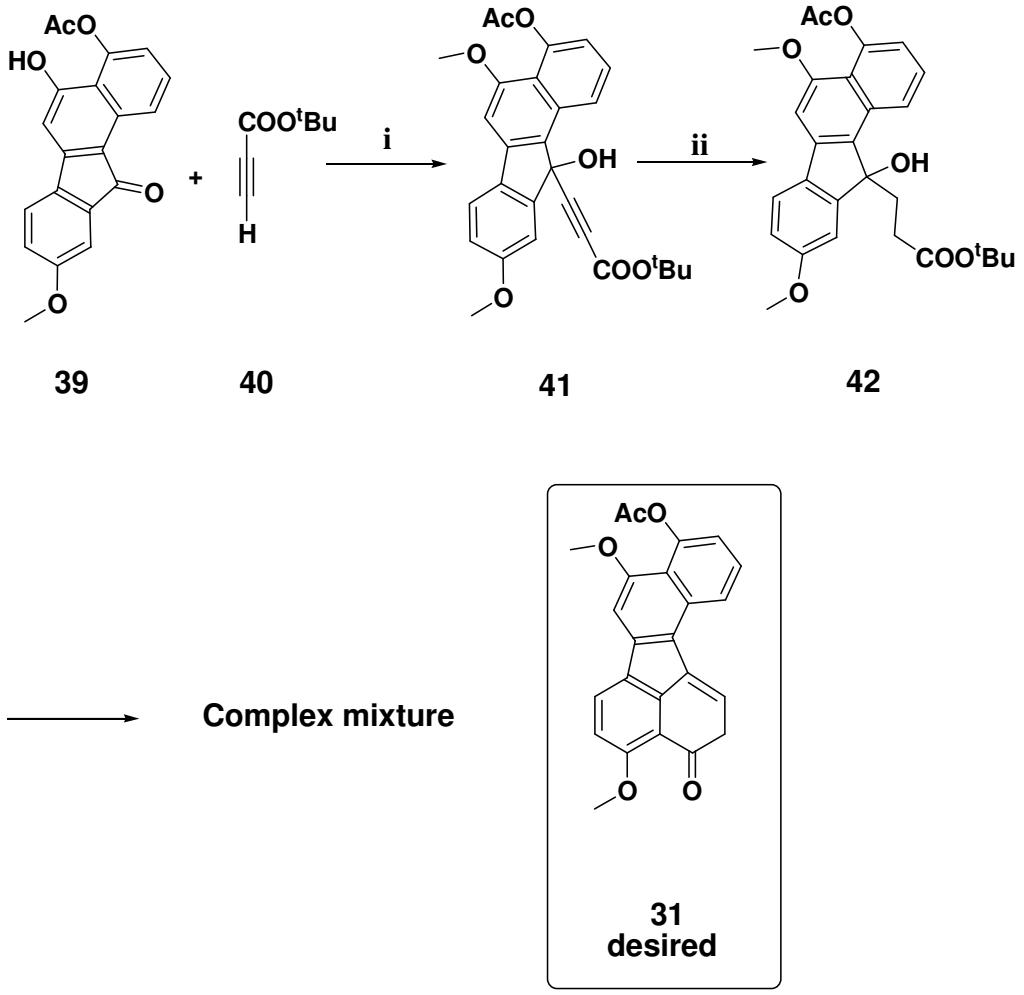
Reagents and conditions.

(i) Ac_2O, DMAP, DCM, Pyridine, rt, 12h

(ii) NIS, Acetonitrile, TFA, rt., 4-6 h.

Hence it was decided to use *tert*-butyl propiolate **(40)** instead of ethyl propiolate to try a cyclisation without deoxygenation of the tertiary hydroxy group.

Hence compound **(41)** was prepared by nucleophilic attack of **(40)** on the carbonyl carbon of **(39).** Compound[21] **(41)** was subjected to hydrogenation using Pd/C to give **(42).** Cyclisation of **(42)** resulted in a complex mixture.



Scheme 4.12

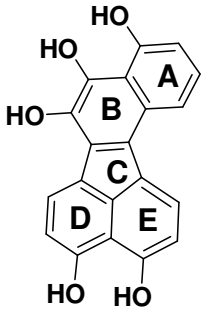
Reagents and conditions.

(i) Potassium *tert*-butoxide, DMF, −55 - −65 ºC

(ii) 10% Pd/C,H_2 gas, 4h.

In conclusion, deactivation or blocking of the position to avoid the seven-membered cyclisation did not result in the formation of the desired product. Hence it might be concluded that the structure favors seven-membered ring over six-membered due to reasons unknown.

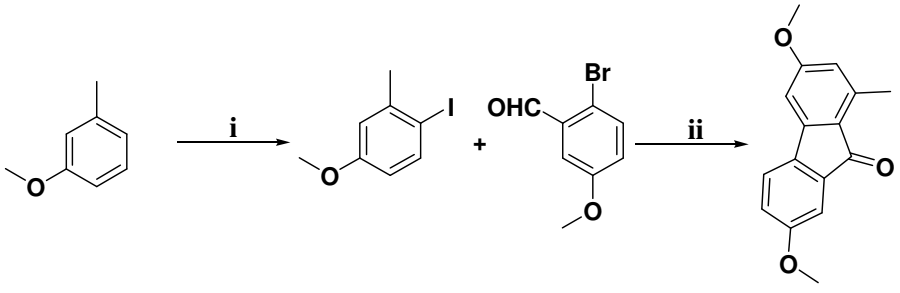
To understand the reactivity of the substrate in the absence or ring **A (fig 4.4)** the same reaction sequence without ring **(A)** was carried out.



1

Fig. 4.4

Thus compound **(43)**, which contains a methyl substituent, was chosen. Iodination[28] of **(43)** gave **(44).** Palladium catalysed domino reaction[16] was done to obtain compound **(45).**



43 **44** **17** **45**

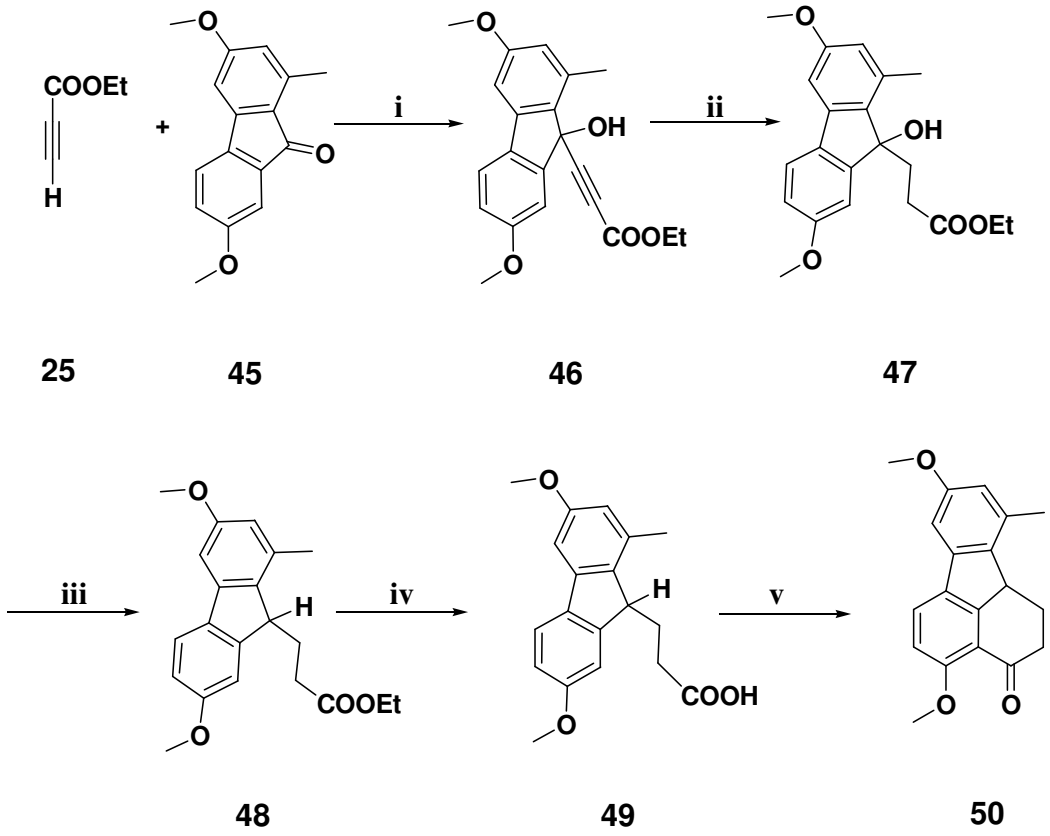
Scheme 4.13

Reagents and conditions.

(i) NIS, Acetonitrile, 82 °C, 2h

(ii) TPP, Norbornene, $CsCO_3$, dimethoxyethane, $Pd(Ac)_2$, reflux, 10 h,

The same sequence of reactions was repeated to achieve the desired acid[21-23] **(49).** Cyclisation was carried out using MSA, which after usual workup gave compound **(50)** as the only product. This result suggests that electronic factors, e.g. the effect of the methoxy group on ring A, might be responsible for the failure in case of previous attempts on substrates with additional ring **A.**



Scheme 4.14

Reagents and conditions.

(i) Potassium *tert*-butoxide, DMF, −55 - −65 ºC

(ii) 10% Pd/C, H_2 gas, 4h.

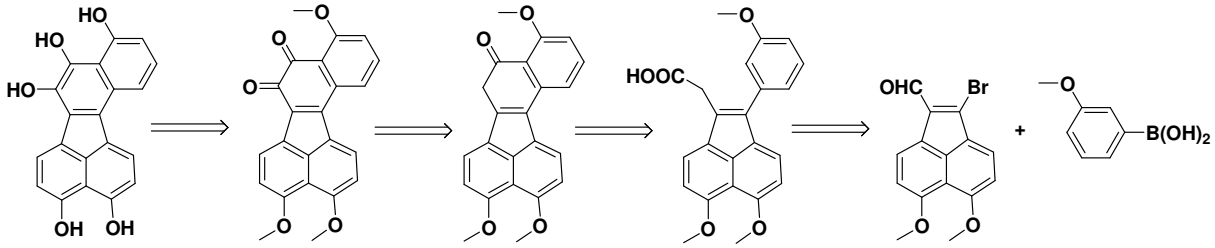
(iii) Et_3SiH, TFA, 1h.

(iv) THF:H_2O (1:1), LiOH, 12h, rt.

(v) MSA, rt, 12h

4.5 Future perspective

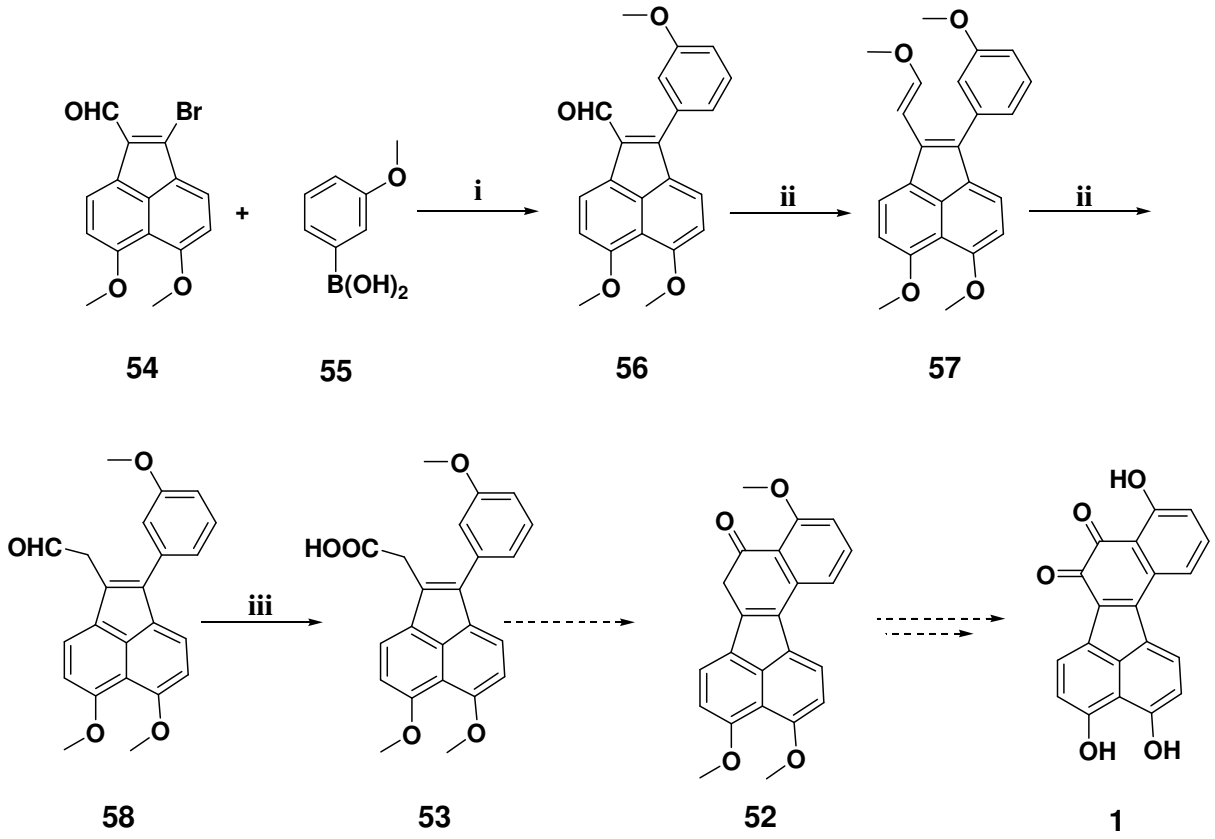
This result made us think of another disconnection strategy. The alternative is shown as in Scheme 4.15



1 51 52 53 54 55

Scheme 4.15

Bromovinyl aldehyde **(54)** was synthesized as described previously in chapter **3** It was coupled with commercially available boronic acid **(55)** to give coupled product[29] **(56)**. It was homologated using one-carbon Wittig reagent $Ph_3P{=}CHOCH_3$ to give[30] vinylether **(57),** which was deprotected to give the homologated aldehyde[31] **(58).** The aldehyde thus obtained was oxidized[32] to the corresponding acid **(53).** Cyclisation, oxidation to introduce oxygen and deprotection should result in Bulgarein. **(1)**



Scheme 4.16

Reagents and conditions.

(iv) $Pd(Ph_3)_4$, K_2CO_3, dioxane/water, 80 ºC, 4h

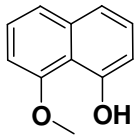
(v) Potassium *tert*-butoxide, $CH_3CH_2P(Ph_3)_3^+Cl^-$, diethyl ether, rt,4-6 h,.

(vi) HCl, THF, reflux, 3h

(vii) KH_2PO_4, $NaClO_2$, rt, 40 min

4.6 Experimental

8-methoxynaphthalen-1-ol (19)



To a suspension of naphthalene-1,8-diol (2 g, 12.48 mmol) and K_2CO_3 (1.72 g, 12.48 mmol) in acetone (12 ml) was added MeI (0.852 ml, 13.7 mmol). After the reaction mixture was stirred at 18 ^oC for 9 h, another batch of K_2CO_3 (0.172 g, 1.2 mmol) was added. Upon stirring at 18 ^oC for 10 h, the reaction mixture was concentrated, acidified with 1N HCl aqueous solution, extracted with Et_2O (5x100 ml), dried over Na_2SO_4, filtered, concentrated, and purified by flash chromatography, to afford 8-methoxynaphthalen-1-ol as a white solid (2 g, 92.16%).

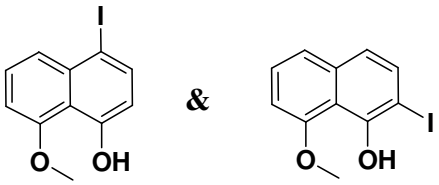
^{1}H NMR ($CDCl_3$) δ: 9.32 (s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.38-7.29 (m, 3H), 6.89 (d, J = 7.2 Hz, 1H), 6.78 (d, J = 7.6 Hz, 1H), 4.06 (s, 3H)

mp : 48 ºC

4-iodo-8-methoxynaphthalen-1-ol (18a)

&

2-iodo-8-methoxynaphthalen-1-ol (18b)



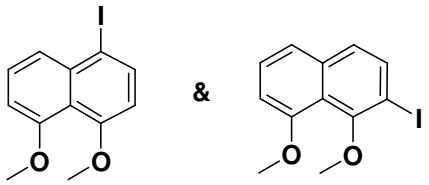
pTsOH (0.339 g, 1.78 mmol) was added to a stirred solution of 8-methoxynaphthalen-1-ol **(19)** (0.310 g, 1.78 mmol) in acetonitrile (36 ml) cooled to 0 ^{o}C. After 5 min. N-iodosuccinimide (0.400 g, 1.78 mmol) was added and stirred at the same temperature for 8-10 hrs. under inert atmosphere of nitrogen. The completion of reaction was checked by TLC. After the completion, the reaction was quenched by adding hexane (30 ml) and then adding sat. aq $Na_2S_2O_3$ till the solution was almost colourless. Ethyl acetate (50 ml) was added and stirred for another 10 min. The reaction mixture was washed with water (50 ml), dried over Na_2SO_4, filtered, concentrated, and purified by flash chromatography, to afford (0.300 g, 56%). a mixture of 4-iodo-8-methoxynaphthalen-1-ol **(18a)** and 2-iodo-8-methoxynaphthalen-1-ol **(18b)** as a viscous oil.

^{1}H NMR ($CDCl_3$) δ: 10.28 (1H, s), 9.54 (1H, s), 7.94 (1H, d, J = 8.2 Hz), 7.73 (1H, d, J= 8.8 Hz), 7.72 (1H, d, J= 8.8 Hz), 7.48–7.31 (3H, m), 7.09 (1H, d, J = 8.8 Hz), 6.89 (1H, d, J= 8.2 Hz), 6.84 (1H, d, J= 8.2 Hz), 6.69 (1H, d, J= 8.2 Hz), 4.09 (3H, s), 4.08 (3H, s)

1-iodo-4,5-dimethoxynaphthalene (16a)

&

2-iodo-1,8-dimethoxynaphthalene (16b)



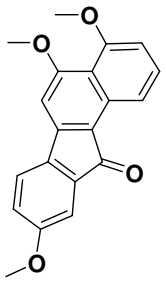
16a **16b**

To a solution of compounds **(18a)** and **(18b)** (0.313 g, 1.04 mmol) and MeI (0.222 g, 1.56 mmol) in DMF (15 mL) was added NaH (60% in mineral oil) (0.050 g, 1.25 mmol) at 0 ^{o}C. After the reaction mixture was stirred at 0 ^{o}C for 1 h, it was stirred at rt for 10 h, then the reaction mixture was cooled to 0 ^{o}C and quenched with cold water (20 mL), extracted with EtOAc (3x20 mL), The organic layer was washed with brine (2 x 15 mL), dried over Na_2SO_4, filtered, concentrated, and purified by flash chromatography to give **(16a),** as a low-melting white solid **16a** (0.112 g).and **16b** (0.130 g)

^{1}H NMR (16a) ($CDCl_3$) δ: 7.98 (1H, dd, J = 8.2, 1.5 Hz), 7.73 (1H, d, J= 7.9 Hz), 7.48 (1H, dd, J = 7.9, 8.2 Hz), 6.96 (1H, d, J= 7.9 Hz), 6.64 (1H, dd, J= 7.9, 1.5 Hz), 4.00 (3H, s), 3.98 (3H, s)

^{1}H NMR (16b) ($CDCl_3$) δ: 7.80 (1H, d, J = 8.8 Hz), 7.44-7.38 (2H, m), 7.33 (1H, d, J = 8.8 Hz), 6.98 (1H, d, J= 8.8 Hz), 4.03 (3H, s), 3.88 (3H, s)

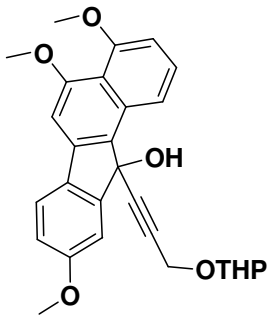
4,5,9-trimethoxy-11H-benzo[a]fluoren-11-one (14)



To a 100 ml round bottom flask previously dried in an oven at 120 °C for 10 h containing a magnetic stir bar was added palladium acetate (57 mg, 0.25 mmol, 0.1, eq.), triphenylphosphine (146 mg, 0.56 mmol, 0.22, eq.), norbornene (715 mg, 7.6 mmol, 3.0, eq.), aryl iodide **(16a)** (796 mg, 2.53 mmol, 1.0, eq.), bromoaldehyde **(17)** (817 mg, 3.8 mmol, 1.5 eq.) and cesium carbonate (2.476 g, 0.25 mmol, 3.0, eq.). The flask was sealed and flushed with argon for 1 minute by venting through a needle after which dimethoxyethane (55 ml) was added and stirring was begun. Nitrogen was then bubbled through the solvent for 5 minutes. The resulting mixture was then stirred at room temperature for 5 minutes and then placed in an oil bath at 90 °C (the oil bath was preheated to 90 °C) for 10- 12 hrs. The crude was then allowed to reach room temperature and was diluted with dichloromethane (100 ml). The organic layer was washed with brine (1x 30 ml) and the aqueous phase was extracted back with dichloromethane (2 x 25 ml). The extracts were dried over sodium sulfate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(14)** as **a** red semisolid (498 mg, 61.7 %)

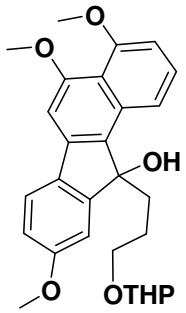
^{1}H NMR ($CDCl_3$) *δ*: 8.58 (1H, d, J= 8.2 Hz), 7.45 (1H, dd, J = 8.2, 8.2 Hz), 7.3 (1H, d, J= 8.2 Hz), 7.13 (1H, d, J= 1.8 Hz), 6.88 (1H, s), 6.84 (1H, dd, J= 8.2, 1.8 Hz,), 6.75 (1H, d, J= 8.2 Hz), 4.09 (3H, s), 3.94 (3H, s), 3.85 (3H, s)

4,5,9-Trimethoxy-11-(3-(tetrahydro-2H-pyran-2-yloxy)prop-1-ynyl)-11H-benzo[a]fluoren-11-ol (22)



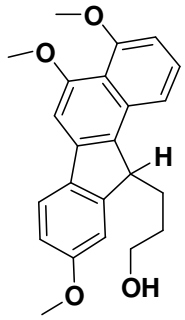
A solution of fluoreneone **(14)** (50 mg, 0.156 mol) and (*RS*)-2-(prop-2-yn-1-yloxy) tetrahydropyran **(15)** (24 mg, 0.163 mol) in DMF (2 ml) was cooled to −25 °C, and potassium *tert*-butoxide (36.82 mg, 0.330 mol) was added at −25 °C. The reaction was stirred at this temperature for 30 min. The completion of reaction was checked by TLC. After the completion of reaction it was quenched by adding water at −25 °C. The reaction mixture was extracted using ethyl acetate (20 ml). The organic layer was washed with brine (2x 30 ml) and the aqueous phase was extracted back with ethyl acetate (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(22)** as red viscous semisolid (60 mg, 84 %)

4,5,9-Trimethoxy-11-(3-(tetrahydro-2H-pyran-2-yloxy)propyl)-11H-benzo[a]fluoren-11-ol (23)



To a suspension of alkyne **(22)** (375 mg, 0.815 mol) in ethyl acetate (60 ml) under nitrogen was added 5% palladium on carbon (520 mg). The air in the reaction vessel was evacuated by applying vacuum till bubbles appeared in the solution, and then flushed with hydrogen gas. The procedure is repeated 2-3 times and then the reaction is allowed to stir for 5-6 hrs. The reaction course is followed by TLC with intervals of 2 hrs. After the completion of reaction judged by TLC, reaction is filtered through a column of Celite to remove palladium and washed with ethyl acetate, concentrated under reduced pressure and purified by using column chromatography to give **(23)** (247 mg, 65 %)

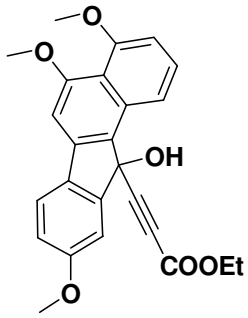
3-(4,5,9-Trimethoxy-11H-benzo[a]fluoren-11-yl)propan-1-ol (24)



Et_3SiH (0.530 mL, 3.32 mmol) was added to the solution of (**23**) (200 mg, 0.431 mmol) in CH_2Cl_2–trifluoroacetic acid (25 mL, 10:1, v/v). The colorless solution was stirred for 30 min at room temperature, the progress of reaction was monitored by TLC. After completion of reaction the reaction mixture was poured into a saturated NaCl aqueous solution. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with a saturated Na_2CO_3 aqueous solution followed by a saturated NaCl aqueous solution, and dried over Na_2SO_4. After evaporating the solvent, the resulting oil was purified by column chromatography (silica gel, hexane) to give **(24)** as a colorless oil. (150 mg, 95.5 %)

^{1}H NMR (DMSO-d_6) δ: 7.85 (1H, d, J= 8.2 Hz), 7.53 (1H, dd, J = 8.2, 1.5 Hz), 7.44 (1H, dd, J= 7.9, 8.2 Hz), 7.40 (1H, s),7.20 (1H, d, J= 2.1 Hz), 6.98 (1H, dd, J= 8.2, 2.1 Hz,), 6.88 (1H, dd, J= 7.9, 1.5 Hz), 4.36 (1H, m), 4.08 (2H, m), 3.95 (3H, s), 3.86 (3H, s), 3.83 (3H, s), 2.29 (2H, m), 0.97 (2H, m)

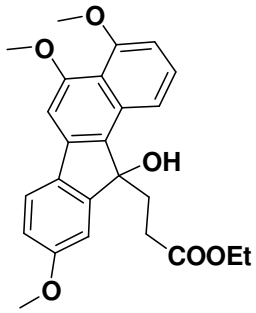
Ethyl 3-(11-hydroxy-4,5,9-trimethoxy-11H-benzo[a]fluoren-11-yl)propiolate (26)



A solution of fluorenone **(14)** (55 mg, 0.171 mol) and ethyl propiolate **(15)** (20.3 mg, 21 µL., 0.206 mol) in DMF (2 ml) was cooled to – 65 °C, The temperature was maintained between – 55 to – 65 °C. the reaction was stirred for 15 min., and potassium *tert*-butoxide (41 mg, 0.365 mol) was added as a single lot. The reaction was stirred at this temperature for 30 min. The completion of reaction was checked by TLC. After the completion of reaction it was quenched by adding water at –25 °C. The reaction mixture was extracted using ethyl acetate (20 ml). The organic layer was washed with brine (2x 30 ml) and the aqueous phase was extracted back with ethyl acetate (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(26)** as an off-white viscous semisolid (46 mg, 64 %)

^{1}H NMR ($CDCl_3$) δ: 7.89 (1H, d, J = 8.2 Hz), 7.46–7.33 (2H, m), 6.97 (1H, dd, J= 8.2, 2.4 Hz), 6.41 (1H, d, J = 8.2 Hz), 6.29 (1H, s), 4.13 (2H, q, J= 7.0 Hz), 3.94 (3H, s), 3.83 (3H, s), 3.21 (3H, s), 1.23 (3H, t, J= 7.0 Hz)

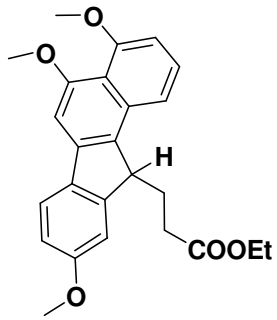
Ethyl 3-(11-hydroxy-4,5,9-trimethoxy-11H-benzo[a]fluoren-11-yl)propanoate (27)



To a suspension of alkyne **(27)** (134 mg, 0.320 mol) in ethyl acetate (35 ml) under nitrogen was added a catalytic amount of 10% palladium on carbon (50 mg). The air in the reaction vessel was evacuated by applying vacuum till bubbles appeared in the solution, and then flushed with hydrogen gas. The procedure is repeated 2-3 times and then the reaction is allowed to stir for 5-6 hrs. The reaction course is followed by TLC with intervals of 2 hrs. After the completion of reaction judged by TLC, reaction is filtered through a column of Celite to remove palladium and washed with ethyl acetate, concentrated under reduced pressure and purified by using column chromatography to give **(27)** (134 mg, 99 %)

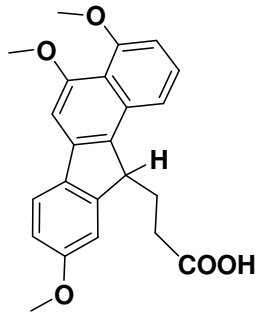
^{1}H NMR ($CDCl_3$) δ: 7.90 (1H, d, J = 8.2 Hz), 7.46 (1H, d, J = 8.2 Hz),7.36 (1H, dd, J = 8.2, 8.2 Hz), 7.19 (1H, d, J = 1.8 Hz), 6.93 (1H, dd, J= 8.2, 1.8 Hz), 6.56 (1H, s), 6.52 (1H, d, J = 8.2 Hz), 3.92 (6H, s), 3.83 (2H, q, J= 7.0 Hz), 3.42 (3H, s), 2.83 (1H, m), 2.66 (1H, m), 1.54 (2H, m), 1.04 (3H, t, J= 7.0 Hz)

Ethyl 3-(4,5,9-trimethoxy-11H-benzo[a]fluoren-11-yl)propanoate (28)



Et_3SiH (60.86 µL, 0.381 mmol) was added to the solution of (**27)** (134 mg, 0.317 mmol) in CH_2Cl_2–trifluoroacetic acid (4 mL, 10:1, v/v). The colorless solution was stirred for 30 min at room temperature, the progress of reaction was monitored by TLC. After completion of reaction the reaction mixture was poured into a saturated NaCl aqueous solution. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with a saturated Na_2CO_3 aqueous solution followed by a saturated NaCl aqueous solution, and dried over Na_2SO_4. After evaporating the solvent, the resulting oil was purified by column chromatography (silica gel, hexane) to give a colorless oil.(103 mg, 80 %)

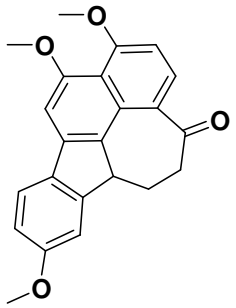
3-(4,5,9-Trimethoxy-11H-benzo[a]fluoren-11-yl)propanoic acid11-ol (29)



The ethyl ester **(28)** (178 mg, 0.438 mmol) was dissolved in THF (5 ml). LiOH. H_2O (55.2 mg, 1.31 mmol) was dissolved in deionised water (5 ml), and was added to the THF solution containing the ester. The solution was vigorously stirred for 16 h at rt. THF was removed under reduced pressure. The residue was diluted with water (10 ml), extracted with EtOAc:Hexane (30:70) mixture (15 ml X 2) to remove the organic impurities. The aqueous layer was cooled and acidified with dilute hydrochloric acid to precipitate the acid. The acid was dissolved in ethyl acetate, organic layer was dried over Na_2SO_4 and concentrated to give acid **(29)** (150 mg, 91 %)

^{1}H NMR ($CDCl_3$) δ: 7.65 (1H, d, J = 8.2 Hz), 7.57 (1H, d, J = 8.2 Hz),7.44 (1H, dd, J = 8.2, 8.2 Hz), 7.18 (1H, s),7.12 (1H, d, J = 2.4 Hz), 6.95 (1H, dd, J= 8.2, 2.4 Hz), 6.84 (1H, d, J = 8.2 Hz), 4.38 (1H, t, J = 4.9 Hz), 4.08 (3H, s), 4.00 (3H, s), 3.89 (3H, s), 2.59 (2H, m), 1.69 (2H, m)

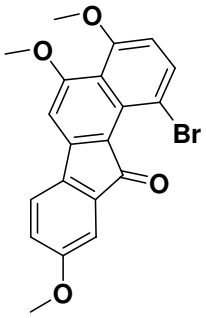
3,4,8-Trimethoxy-10,11-dihydro-9bH-benzo[a]naphtho[2,1,8-cd]azulen-12-one (30)



MSA (2 ml) was added to compound **(29)** (30 mg, 0.080 mmol) under nitrogen. The dark purple solution was allowed to stir for 12 h at rt. The reaction was quenched by adding ice cold water. The aqueous solution was extracted with ethyl acetate (10 ml). The organic layer was washed with brine (20 ml) and the aqueous phase was extracted back with ethyl acetate (10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **30** as a lightly fluorescent oil (15 mg, 52.57 %)

^{1}H NMR ($CDCl_3$) *δ*: 7.77 (1H, d, J = 8.2 Hz), 7.69 (1H, d, J = 8.2 Hz), 7.26 (1H, s),7.05 (1H, d, J = 1.8 Hz), 6.96 (1H, dd, J = 8.2, 1.8 Hz), 6.80 (1H, d, J = 8.2 Hz), 4.09 (3H, s), 4.05 (3H, s), 3.90 (3H, s), 3.15 (1H, m), 2.93 (1H, m), 2.35 (1H, m), 2.03 (1H, m)

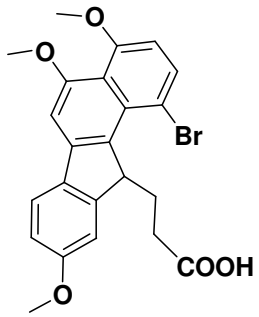
1-Bromo-4,5,9-trimethoxy-11H-benzo[a]fluoren-11-one(32)



To a solution of fluorenone **(14)** (137 mg, 0.428 mmol) in DMF (3 mL) was added NBS (80.5 mg, 0.450 mmol) in a single portion. The resulting solution was stirred for 12 h, and the reaction was quenched by ice pieces. The aqueous solution was extracted with ethyl acetate (10 ml). The organic layer was washed with brine (2 X 20 ml) and the aqueous phase was extracted back with ethyl acetate (10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified using flash column chromatography, to give **(32)** (120 mg, 70 %)

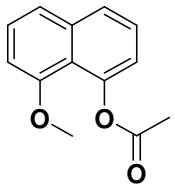
^{1}H NMR ($CDCl_3$) δ: 7.74 (1H, d, J= 8.5 Hz), 7.37 (1H, d, J= 8.2 Hz),7.19 (1H, d, J= 1.8 Hz), 6.95 (1H, s), 6.89 (1H, dd, J = 8.2, 1.8 Hz), 6.66 (1H, d, J= 8.5 Hz), 4.11 (3H, s), 3.95 (3H, s), 3.88 (3H, s)

3-(1-Bromo-4,5,9-trimethoxy-11H-benzo[a]fluoren-11-yl)propanoic acid (35)



To a solution of fluorenone **(35)** (26 mg, 0.069 mmol) in DMF (3 mL) was added NBS (13.5 mg, 0.075 mmol) in a single portion. The resulting solution was stirred for 12 h, and the reaction was quenched by ice pieces. The aqueous solution was extracted with ethyl acetate (10 ml). The organic layer was washed with brine (2 X 20 ml) and the aqueous phase was extracted back with ethyl acetate (10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(35)** (19 mg, 60 %)

8-Methoxynaphthalen-1-yl acetate (37)

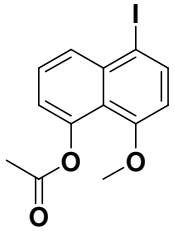


To a solution of naphthol **(19)** (400 mg, 2.30 mmol) in CH_2Cl_2 (25 ml) was added triethylamine (698 mg, 961 µL, 6.890 mmol), acetic anhydride (469 mg, 435 µL, 4.59 mmol), and DMAP (28 mg, 0.229 mmol, 10 mol %). The resultant mixture was stirred under nitrogen atmosphere at room temperature for 12 h. When the reaction was considered complete, as determined by TLC analysis, the reaction mixture was washed with water, brine, dried over Na_2SO_4 and filtered. Solvents were evaporated under reduced pressure. The residue was purified by chromatography on silica gel to afford **(37)** (496 mg, 99%)

^{1}H NMR ($CDCl_3$) δ: 2.38 (s, 3 H), 3.92 (s, 3 H), 6.84 (1 H, dd, J = 7.6 Hz, 0.9 Hz), 7.07 (1 H, dd, J= 7.6 Hz, 0.9 Hz), 7.38 (1 H, t, J = 7.9 Hz), 7.42 (1 H, t, J = 8.2 Hz), 7.45 (1 H, dd, J = 8.2 Hz, 0.9 Hz), 7.69 (1 H, dd, J = 8.2 Hz, 0.9 Hz).

mp : 82ºC

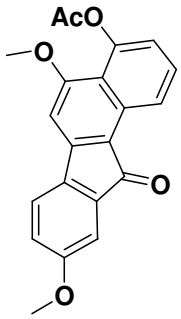
5-Iodo-8-methoxynaphthalen-1-yl acetate (38)



TFA (78 mg, 53 µL, 0.688 mmol) was added to a stirred solution of **37** (496 mg, 2.296 mmol) and N-iodosuccinimide (568 mg, 2.52 mmol) in acetonitrile (20 ml) at 25 °C and stirred at the same temperature for 20-25 min. The completion of reaction was checked by TLC. After the completion of reaction, ice cold water was added. The reaction mixture was extracted using ethyl acetate (50 ml), organic layer was washed with water (100 ml), dried over Na_2SO_4, filtered, concentrated, and purified by flash chromatography, afforded **(38)** (0.500 g, 63.7%). as a white solid.

^{1}H NMR ($CDCl_3$) *δ*: 8.04 (1H, d, J = 8.2 Hz), 7.99 (1H, d, J= 8.2 Hz), 7.55 (1H, dd, J = 8.2, 8.2 Hz), 7.17 (1H, d, J= 8.2 Hz), 6.63 (1H, d, J= 8.2 Hz), 3.93 (3H, s), 2.39 (3H, s)

5,9-Dimethoxy-11-oxo-11H-benzo[a]fluoren-4-yl acetate (39)

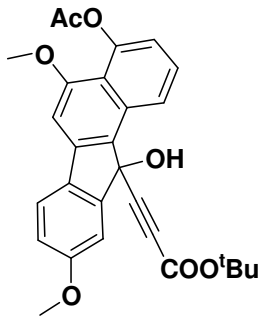


To a 25 ml R.B previously dried in an oven at 120 °C for 10 h containing a magnetic stir bar was added palladium acetate (6.6 mg, 0.030 mmol, 0.1, equivalents), triphenylphosphine (17 mg, 0.064 mmol, 0.22, equivalents), norbornene (83 mg, 0.87 mmol, 3.0, equivalents), aryl iodide **(38)** (100 mg, 0.29 mmol, 1.0, equivalent), bromoaldehyde **(17)** (94 mg, 0.44 mmol, 1.5, equivalents) and cesium carbonate (285.8 mg, 0.87 mmol, 3.0, equivalents). The R.B was the sealed and flushed with argon for 1 minute by venting through a needle after which dimethoxyethane (10 ml) was added and stirring was begun. Nitrogen is then bubbled through the solvent for 5 minutes The resulting mixture was then stirred at room temperature for 5 minutes and then placed in an oil bath at 90 °C (the oil bath was preheated to 90 °C) for 10- 12 hrs. The crude was then allowed to reach room temperature and was diluted with dichloromethane (15 ml). The crude was washed with brine (1x 10 ml) and the aqueous phase was extracted back with dichloromethane (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(39)** as a red semisolid (66.2 mg, 65 %)

^{1}H NMR ($CDCl_3$) δ: 8.89 (1H, d, J= 8.2 Hz), 7.53 (1H, dd, J = 8.2, 8.2 Hz), 7.31 (1H, d, J= 8.2 Hz), 7.16 (1H, d, J= 2.4 Hz), 6.98 (1H, d, J= 8.2 Hz,), 6.90 (1H, s), 6.87 (1H, dd, J= 8.2, 2.4 Hz), 4.07 (3H, s), 3.88 (3H, s), 2.38 (3H, s)

tert-Butyl 3-(4-acetoxy-11-hydroxy-5,9-dimethoxy-11H-benzo[a]fluoren-11-yl)propiolate

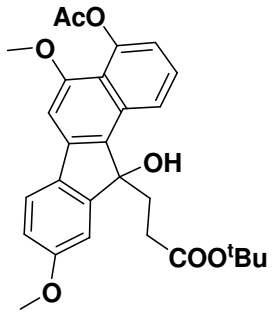
(41)



A solution of fluoreneone **(39)** (50 mg, 0.143 mol) and *tert*-butyl propiolate **(40)** (22 mg, 23 µL., 0.172 mol) in DMF (2 ml) was cooled to −65 °C, The temperature was maintained between −55 to −65 °C. the reaction is stirred for 15 min., and potassium *tert*-butoxide (33.8 mg, 0.301 mol) was added as a single lot. The reaction was stirred at this temperature for 30 min. The completion of reaction was checked by TLC. After the completion of reaction it was quenched by adding water at −25 °C. The reaction mixture was extracted using ethyl acetate (20 ml). The organic layer was washed with brine (2x 30 ml) and the aqueous phase was extracted back with ethyl acetate (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(41)** as **an** off white viscous semisolid (32 mg, 60 %)

tert-Butyl3-(4-acetoxy-11-hydroxy-5,9-dimethoxy-11H-benzo[a]fluoren-11-yl)propanoate

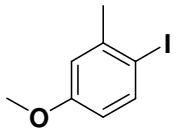
(42)



To a suspension of alkyne **(41)** (25 mg, 0.052 mol) in ethyl acetate (15 ml) under nitrogen was added a catalytic amount of 5% palladium (20 mg) on carbon. The air in the reaction vessel was evacuated by applying vacuum till bubbles appeared in the solution, and then flushed with hydrogen gas. The procedure is repeated 2-3 times and then the reaction is allowed to stir for 5-6 hrs. The reaction course is followed by TLC with intervals of 2 hrs. After the completion of reaction judged by TLC, reaction is filtered through a column of Celite to remove palladium and washed with ethyl acetate, concentrated under reduced pressure and purified by using column chromatography to give **(42)** (17 mg, 65 %)

^{1}H NMR ($CDCl_3$) δ: 8.30 (1H, d, J= 8.4 Hz), 7.57-7.41 (2H, m), 7.15 (1H, d, J= 1.9 Hz), 7.03-6.95 (2H, m), 6.91 (1H, dd, J= 8.4, 1.9 Hz,), 3.99 (3H, s), 3.89 (3H, s), 2.66 (2H, m), 2.37 (3H, s), 1.57 (2H, m), 1.28 (9H, m)

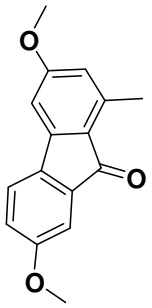
1-Iodo-4-methoxy-2-methylbenzene (44)



To a solution of 3-methylanisole (185 mg, 1.514 mmol) in acetonitrile (10 ml) was added NIS (510 mg, 2.27 mmol) and the reaction was stirred at 82 °C for 2h After the completion of reaction, the solvent was evaporated and ether was added. The organic phase was washed with aqueous $NaHSO_3$ solution and water. The organic layer was dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave **(44**).(300 mg, 80%) as an oil

^{1}H NMR ($CDCl_3$) δ: 7.67 (1H, d, J= 8.5 Hz), 6.83 (1H, d, J= 1.8 Hz), 6.50 (1H, dd, J= 8.5, 1.8 Hz), 3.79 (3H, s), 2.41 (3H, s)

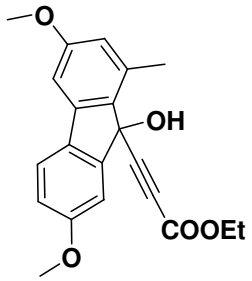
3,7-Dimethoxy-1-methyl-9H-fluoren-9-one (45)



To a 25 ml R.B previously dried in an oven at 120 °C for 10 h containing a magnetic stir bar was added palladium acetate (9 mg, 0.040 mmol, 0.1, equivalents), triphenylphosphine (23.3 mg, 0.088 mmol, 0.22, equivalents), norbornene (114 mg, 1.2 mmol, 3.0, equivalents), aryl iodide **(44)** (100 mg, 0.40 mmol, 1.0, equivalent), bromo aldehyde **(17)** (130 mg, 0.60 mmol, 1.5, equivalents) and cesium carbonate (394.2 mg, 1.2 mmol, 3.0, equivalents). The R.B was the sealed and flushed with argon for 1 minute by venting through a needle after which dimethoxyethane (10 ml) was added and stirring was begun. Nitrogen was then bubbled through the solvent for 5 minutes The resulting mixture was then stirred at room temperature for 5 minutes and then placed in an oil bath at 90 °C (the oil bath was preheated to 90 °C) for 10- 12 hrs. The crude was then allowed to reach room temperature and was diluted with dichloromethane (15 ml). The crude was washed with brine (1x 10 ml) and the aqueous phase was extracted back with dichloromethane (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(45)** as red semisolid (25 mg, 24.4 %)

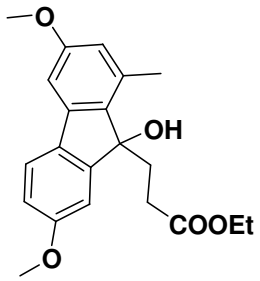
^{1}H NMR ($CDCl_3$) *δ*: 7.35 (1H, d, J= 8.2 Hz), 7.15 (1H, d, J= 2.3 Hz), 6.93 (1H, dd, J= 8.2, 2.3 Hz), 6.77 (1H, d, J= 2.3 Hz), 6.40 (1H, d, J= 2.3 Hz), 3.87 (3H, s), 3.85 (3H, s), 2.58 (3H, s)

Ethyl 3-(9-hydroxy-3,7-dimethoxy-1-methyl-9H-fluoren-9-yl)propiolate (46)



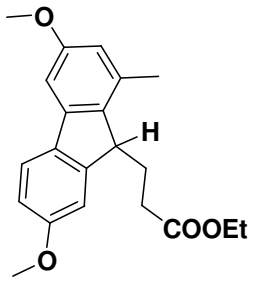
A solution of fluorenone **(39)** (20 mg, 0.078 mol) and ethyl propiolate **(15)** (9.25 mg, 9.6 µL., 0.094 mol) in DMF (2 ml) was cooled to −65 °C, The temperature was maintained between −55 to −65 °C. the reaction was stirred for 15 min., and potassium *tert*-butoxide (18.71 mg, 0.166 mol) was added as a single lot. The reaction was stirred at this temperature for 30 min. The completion of reaction was checked by TLC. After the completion of reaction it was quenched by adding water at −25 °C. The reaction mixture was extracted using ethyl acetate (20 ml). The organic layer was washed with brine (2x 30 ml) and the aqueous phase was extracted back with ethyl acetate (2 x 10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified via flash column chromatography, to give **(46)** as viscous semisolid (25 mg, crude weight) which was used without further purification for the next step.

Ethyl 3-(9-hydroxy-3,7-dimethoxy-1-methyl-9H-fluoren-9-yl)propanoate (47)



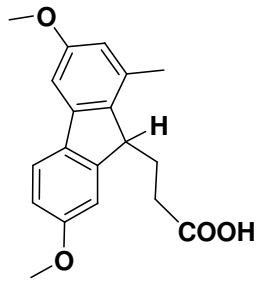
To a suspension of alkyne **(46)** (20 mg, 0.056 mol) in ethyl acetate (15 ml) under nitrogen was added a catalytic amount of 10% palladium on carbon (20 mg). The air in the reaction vessel was evacuated by applying vacuum till bubbles appear in the solution, and then flushed with hydrogen gas. The procedure is repeated 2-3 times and then the reaction is allowed to stir for 5-6 hrs. The reaction course is followed by TLC with intervals of 2 hrs. After the completion of reaction judged by TLC, reaction is filtered through a column of Celite to remove palladium and washed with ethyl acetate, concentrated under reduced pressure and purified by using column chromatography to give **(47)** (20 mg crude) which was used without further purification.

Ethyl 3-(3,7-dimethoxy-1-methyl-9H-fluoren-9-yl)propanoate (48)



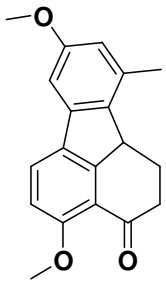
Et_3SiH (8.07 µL, 0.0505 mmol) was added to the solution of **(47)** (15 mg, 0.0420 mmol) in CH_2Cl_2–trifluoroacetic acid (5 mL, 10:1, v/v). The colorless solution was stirred for 30 min at room temperature, the progress of reaction was monitored by TLC. After completion of reaction the reaction mixture was poured into a saturated NaCl aqueous solution. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with a saturated Na_2CO_3aqueous solution followed by a saturated NaCl aqueous solution, and dried over Na_2SO_4. After evaporating the solvent, the resulting oil (15mg of crude) was used as such for further step.

3-(3,7-Dimethoxy-1-methyl-9H-fluoren-9-yl)propanoic acid (49)



The crude ethyl ester **(48)** (15 mg, 0.044 mmol) was dissolved in THF (2 ml). LiOH. H_2O (5.5 mg, 0.133 mmol) was dissolved in deionised water (2 ml), and was added to the THF soloution containing the ester. The solution was vigorously stirred for 16 h at rt. THF was removed under reduced pressure. The residue was diluted with water (5 ml) was extracted with EtOAc:Hexane (30:70) mixture (5 ml X 2) to remove the organic impurities. The aqueous layer was cooled and acidified with dilute hydrochloric acid to precipitate the acid. The acid was dissolved in ethyl acetate, organic layer was dried over Na_2SO_4 concentrated to give acid **(49)** (10 mg)

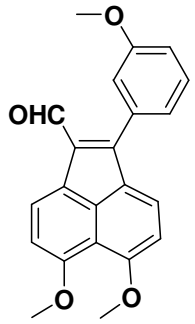
4,8-Dimethoxy-10-methyl-1,2-dihydrofluoranthen-3(10bH)-one (50)



MSA (1 ml) was added to compound **(49)** (10 mg, 0.0320 mmol) under nitrogen. The dark purple solution was allowed to stir for 12 h at rt. the reaction was quenched by adding ice cold water. The aqueous solution was extracted with ethyl acetate (10 ml). The organic layer was washed with brine (10 ml) and the aqueous phase was extracted back with ethyl acetate (10 ml). The extracts were dried over sodium sulphate and filtered. Evaporation of the volatiles by rotary evaporation under reduced pressure gave a residue which was purified TLC preparative chromatography, to give **(50)** as lightly fluorescent blue oil (7 mg)

^{1}H NMR ($CDCl_3$) δ: 7.80 (1H, d, J= 8.4 Hz), 7.07 (1H, d, J= 1.8 Hz), 6.99 (1H, d, J= 8.4 Hz), 6.64 (1H, d, J= 1.8 Hz), 3.98 (3H, s), 3.87 (3H, s), 2.93-2.72 (2H, m), 2.45 (3H, s), 1.73-1.54 (2H, m)

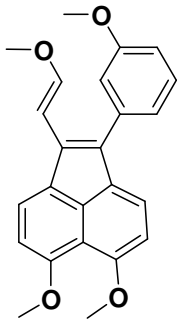
5,6-Dimethoxy-2-(3-methoxyphenyl)acenaphthylene-1-carbaldehyde (56)



A solution of boronic acid **55** (24 mg, 0.158 mol), bromoaldehyde **54** (50 mg, 0.156 mmol) and K_2CO_3 (127.6 mg, 0.924 mmol) in 1,4-dioxane/water (4/l) (14 ml) was degassed by purging with N_2 for 30 min. $Pd(PPh_3)_4$ (7 mg, 0.0062 mmol) was added and the resulting reaction mixture was refluxed at 80 °C for 6 h under N_2 atmosphere, then cooled to room temperature, diluted with diethyl ether (15 mL) and filtered through a small silica gel bed. The bed was washed with diethyl ether (3 × 10 mL). The resulting mixture was dried and evaporated. Purification by chromatography using EtOAc: *n*-hexane as an eluent gave aldehyde **56** as a bright yellow colored semisolid. (50 mg, 92 %)

^{1}H NMR ($CDCl_3$) δ: 10.15 (1H, s), 8.45 (1H, d, J= 8.1 Hz), 7.91 (1H, d, J= 8.1 Hz), 7.46 (1H, dd, J= 8.1, 7.8 Hz), 7.22 (1H, d, J= 7.8 Hz), 7.17 (1H, d, J= 2.2 Hz), 7.05 (1H, dd, J= 8.1, 2.2 Hz), 6.98 (1H, d, J= 8.1 Hz), 6.97 (1H, d, J= 8.1 Hz), 4.11 (3H, s), 4.09 (3H, s), 3.89 (3H, s)

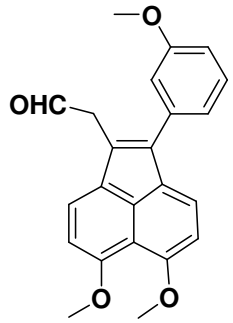
(E)-5,6-Dimethoxy-1-(3-methoxyphenyl)-2-(2-methoxyvinyl)acenaphthylene (57)



Potassium *tert*-butoxide (30.5 mg, 0.271 mmol) was added to a suspension of methoxymethyltriphenylphosphonium chloride (93 mg, 0.271 mmol) and aldehyde **(56)** (47 mg, 0.136 mmol) in dry diethyl ether (50 mL) at rt in a round bottom flask under nitrogen. The colour of solution turned to yellowish orange. After stirring for 3h, the completion of reaction was checked by TLC. The reaction was quenched adding water (50 mL). The aqueous layer was extracted with ether (2× 25 mL). Evaporation of solvent and purification by flash column chromatography provided **57** (45 g, 88.5 %) as an orange foam, with trans alkene as the major product.

^{1}H NMR ($CDCl_3$) *δ*: 7.82 (1H, d, J= 7.9 Hz), 7.52 (1H, d, J= 7.9 Hz), 7.39 (1H, d, J= 12.8 Hz), 7.46-7.11 (4H, m), 6.98-6.86 (2H, m), 6.18 (1H, d, J= 12.8 Hz), 4.09 (3H, s), 4.06 (3H, s), 3.88 (3H, s), 3.71 (3H, s)

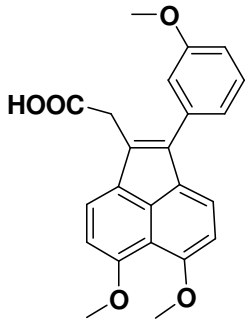
2-(5,6-Dimethoxy-2-(3-methoxyphenyl)acenaphthylen-1-yl)acetaldehyde (58)



(E/Z) alkene **(57)** (70 mg, 0.187 mmol) was dissolved in 15 mL of THF. A 2.0 M HCl (0.121 mL, 0.243 mmol) solution was added and the reaction was refluxed for 3 h. The reaction was diluted with ethyl acetate (20 mL), washed with a satd. solution of $NaHCO_3$ (5 mL × 2) water (20 mL), and finally with brine (10 mL). The organic layer was evaporated to give a crude that was purified by flash column chromatography to provide **(58)** (47.2 mg, 70%) as a yellow viscous oil.

^{1}H NMR ($CDCl_3$) δ: 9.83 (1H, t, J= 2.35 Hz), 7.63-7.54 (2H, m),7.41 (1H, dd, J= 8.1, 8.1Hz), 7.08 (1H, dd, J= 8.1, 1.5 Hz), 7.04 (1H, dd, J= 1.5, 1.5 Hz), 6.95 (1H, ddd, J= 8.1, 1.5, 1.5 Hz),6.91-6.84 (2H, m), 4.07 (3H, s), 4.06 (3H, s), 3.92 (2H, d, J= 2.35 Hz),3.87 (3H, s)

2-(5,6-dimethoxy-2-(3-methoxyphenyl)acenaphthylen-1-yl)acetic acid (53)



Compound **(58)** (70 mg, 0.194 mmol) was dissolved in a 1:1 solution of H_2O : *t*-BuOH (0.1 M). To this suspension 2-methyl-2-butene (273 mg, 411.96 µL, 3.88 mmol), KH_2PO_4 (53 mg, 0.388 mmol) and sodium chlorite (44 mg, 0.486 mmol) were added in sequence and the mixture was stirred for 40 min at room temperature. The reaction mixture was extracted with EtOAc:Hexane (20:80) mixture (5 ml X 2) to remove the organic impurities. The aqueous layer was cooled and acidified with dilute hydrochloric acid to precipitate the acid. The acid was dissolved in ethyl acetate, organic layer was dried over Na_2SO_4 concentrated to give acid **(53)** (40 mg, 55 %) as viscous yellow oil.

^{1}H NMR ($CDCl_3$) δ: 7.73 (1H, d, J= 7.9 Hz), 7.60 (1H, d, J= 7.9 Hz), 7.41 (1H, dd, J= 8.2, 8.2 Hz), 7.22-7.13 (2H, m), 6.94 (1H, dd, J= 8.2, 1.5 Hz), 6.89 (1H, d, J= 7.9 Hz), 6.84 (1H, d, J= 7.9 Hz), 4.06 (3H, s), 4.04 (3H, s), 3.85 (3H, s)

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CHAPTER 5

SYNTHESIS OF FARINOMALEIN C-E AND THEIR ANTIFUNGAL ACTIVITY

5.1 Introduction

Entomopathogenic fungi are well known for their ability to produce various compounds during infection and proliferation in insects[1-3] and thus are considered as potential sources of novel bioactive compounds.[4-6] Those belonging to the genus *Paecilomyces* have been the source of wide range of bioactive metabolites, including the antimalarial and antitumor cyclohexadepsipeptide paecilodepsipeptide A from *P.cinnamomeus*,[7] an antibiotic containing a tetramic acid moiety (paecilosetin from *P. farinosus*[8]) and neuritogenic pyridine alkaloids (farinosones A-C and militarinones A-D form *P.militaris,* respectively[9,10]). Farinomalein **(1),** 3-(3-isopropyl-2,5-dioxo-2,5-dihydropyrrol-1-yl)-propionic acid is a relatively structurally simple maleimide, recently isolated in 2009 from the entomopathogenic fungus *Paecilomyces farinosusn* HF599, by Nihira et.al.[11]

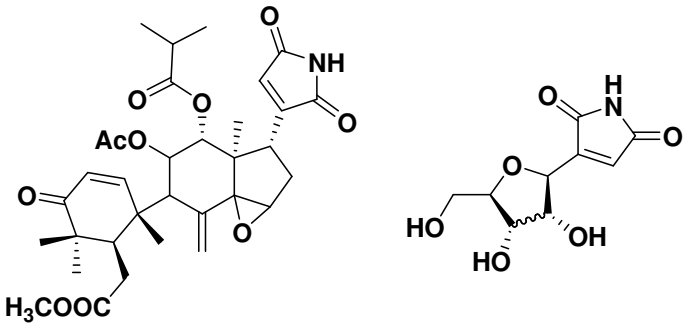
5.2 Natural compounds bearing maleimide rings.

Reports of natural compounds bearing maleimide rings are very limited. Examples include showdomycin from *Streptomyces showdoensis*, pencolide from *Penicillium multicolor,* turrapubesin from the twigs and leaves of *Turraea pubescens*.[12-14]and farinomalein[11].

5.2.1 Pencolide from *Penicillium multicolor*

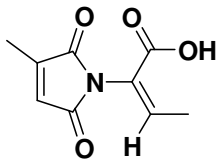
A new nitrogen-containg product, pencolide,[15] obtained from the culture fluid of a strain of *Penicillium multicolor* Grigorieva Manilova and Poradielova, grown on Raulin Thom medium, was reported by Birkinshaw[13]et al. in 1963 (**A**). The *E* configuration was subsequently assigned to the double bond on the basis of NMR arguments (**B**); however Olsen and collaborators have revised the geometry to *Z* in a recent publication. It was suggested (**A**) that biosynthetic precursor of pencolide might be citraconic acid and threonine or a related amino acid.

Pencolide was first synthesized by Strungz and Ren[16] in 1975 from citraconic anhydride and threonine. Evidence is presented which corroborates the Z configuration.

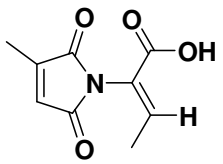


Turrapubesin B

Showdomycin



(A)



(B)

Fig. 5.1

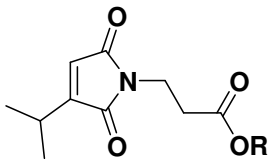
5.2.2 Turrapubesin B

Turrapubesin B,[17] (fig 5.1), a maleimide bearing limonoid, was isolated from the twigs and leaves of *Turraea pubescens* by Yue et al, in 2006. Limonoids are a class of highly oxygenated nortriterpenoids, either containing or derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. They present a wide range of biological activities, such as insect antifeeding, antibacterial, antifungal, antiviral, antimalarial and anticancer properties.[18] The plants belonging to the families of Meliaceae and Rutaceae are rich sources of these fascinating metabolites. Previous studies on the genus *Turraea* have afforded a series of protolimonoids and limonoids.[19] The plant material of *T. pubescens* has been used in the remedies of dysentery,

pharyngolaryngitis, and traumatic hemorrhage[20]. Turrapubesin B is the first example of a maleimide bearing limonoid in nature.

5.2.3 Showdomycin

Showdomycin[21] (fig. 5.1) is a broad spectrum antibiotic, first isolated from *Streptomyces showdoensis* by Nishimura and his coworkers[22], in 1964 (1). The antibiotic has been found to exhibit definite activity against Ehrlich ascites tumor in vivo and against cultured HeLa cells. The structure has now been elucidated as a new class of C-nucleoside. Showdomycin consist of a maleimide portion and a furanose group bearing two cis vicinal hydroxyl groups. Both the sugar and the base moieties are linked with C-C bond.



1. R=H
2. R=CH_3

Farinomalein (1) R=H

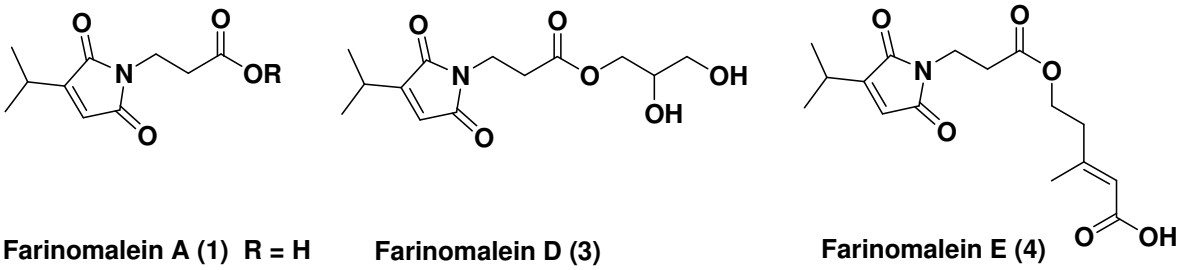
Fig. 5.2

5.2.4 Farinomalein

During a screening for a compound active against plant pathogenic oomycetes, the EtOAc extract of *P. farinosus* HF599 by Nihira et.al.[11]showed strong inhibitory activity against *Phytophthora sojae*.[23] Detailed investigation of the fungal extract led tot the identification of the new maleimide compound farinomalein (**1**).

Recently, Proksch et.al[24] have isolated three new farinomalein derivatives, farinomaleins C, D, and E, (Fig. 5.3) in addition to one new isoindoline from an unidentified endophytic fungus of

the mangrove plant *Avicennia marina* from Oman. The distinctive structural framework prompted us to undertake the synthesis of these new molecules.



Farinomalein A (1) R = H **Farinomalein D (3)** **Farinomalein E (4)**

Farinomalein C (2) R = C_4H_9

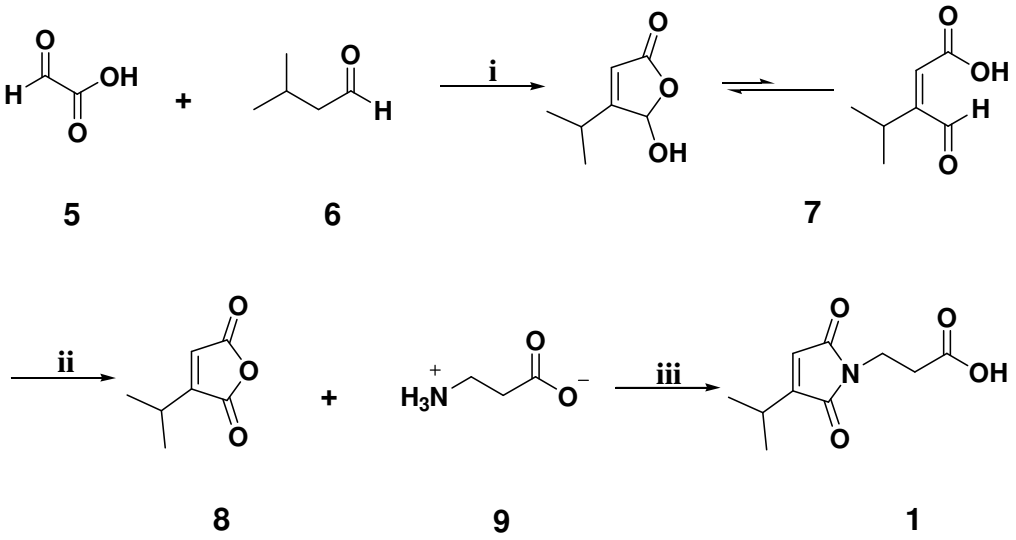
Fig. 5.3

5.3 Biological activity

Farinomalein shows potent inhibition of *Phytophthora sojae*, a plant pathogen that causes every year enormous damage to soybean crops,[25] with an MIC value of 5µg/disk, whereas the MIC of the antifungal agent amphotericin B is 10µg/disk. Recently, maleimides such as N-methylmaleimide, N-ethylmaleimide, and phenylmaleimide PM-20 have attracted the interest of many researchers due to their cytotoxicity towards tumor cell lines through the inhibition of human topoisomerase II[26] or the inhibition of Cdc25A.[27] These facts suggested that, in addition to its potent antioomycete activity, farinomalein could also possess other biological functions, such as cytotoxicity toward tumor cell lines.

5.4 Reported Synthesis of farinomalein

A three-step synthesis of farinomalein has been reported by Miles and Yan.[28]



Scheme 5.1

Reagents and conditions.

(vi)(5), powdered morpholinium hydrochloride, dioxane then **(6)** rt, 1h, reflux, 24h, 70 %

(vii) DMP, DCM

(viii) AcOH, β-alanine, reflux, 2h, 64%

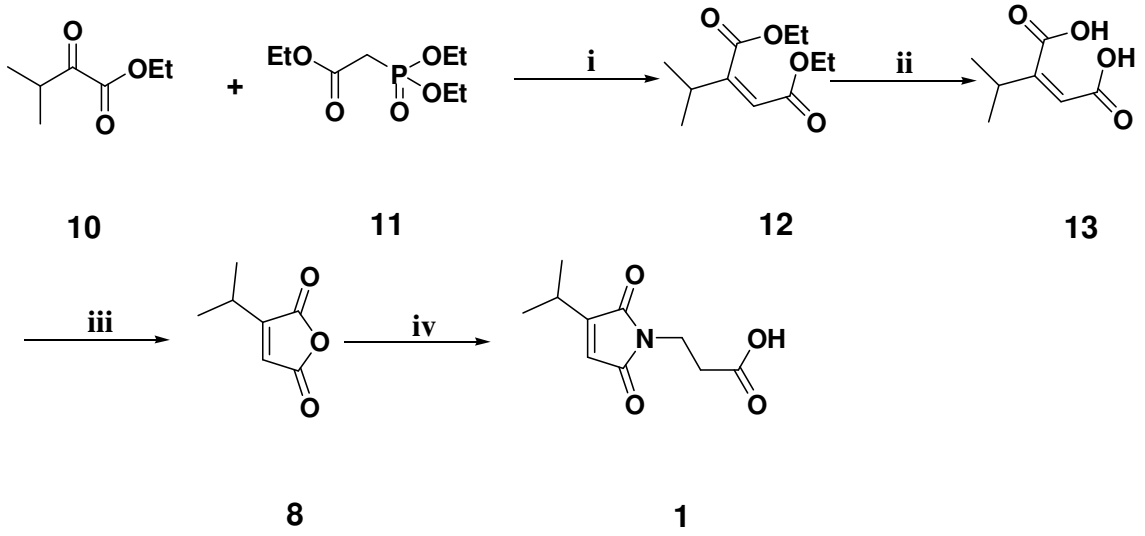
However, the Authors themselves state that their synthesis is difficult to scale-up.[28] More recently, an alternative strategy for the preparation of farinomalein has been reported by our group.[29]

5.5 Synthesis of Farinomalein C - E

As seen in Scheme, the synthesis reported by us was developed in four steps with high yield and without using any hazardous chemical (Scheme 5.2).

We envisioned that **Farinomaleins C-E** could be synthesized simply by coupling farinomalein A, obtained following the previously reported procedure, with suitable alcohols. Thus,

Farinomalein C was obtained in 92% yield by reacting farinomalein A with 1-butanol using DCC, DMAP in DCM (Scheme 5.3).



Scheme 5.2

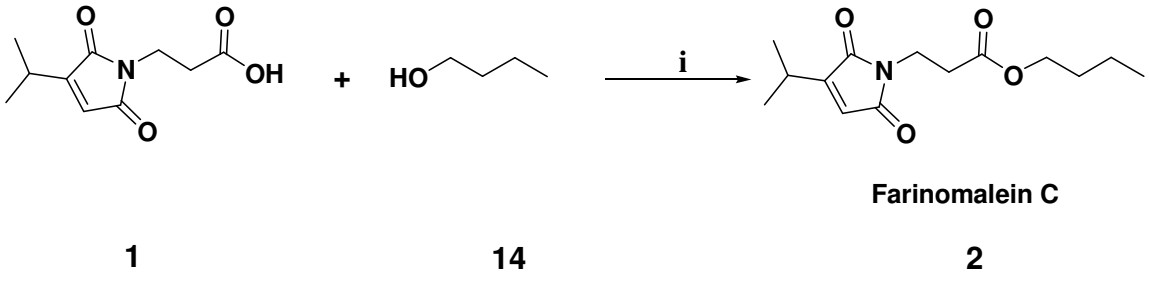
Reagents and conditions.

(i) NaH, THF, reflux, 0 ºC then 50 ºC 2 h, 79 %

(ii) 2N LiOH, THF, 0 ºC - rt, 8h, 96 %.

(iii) TFAA,rt, 12h

(iv) AcOH, β-alanine, reflux, 2h, 60%

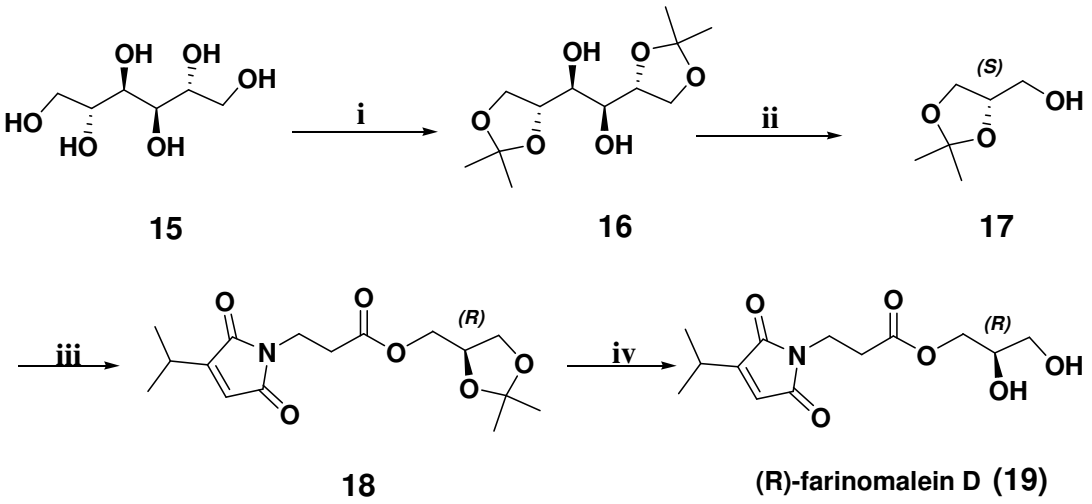


Scheme 5.3

Reagents and conditions.

(i) n-butanol, DCC, DMAP, 0 ºC - r t, 12h, 92%.

Similarly, a synthesis of farinomalein D was carried out. As the absolute configuration at C-2' was not assigned by the Authors, we decided to synthesize both the enantiomers along with racemic farinomalein D. **(Scheme 4.4-6)** summarizes the synthesis of (*R*)-farinomalein D **(19)** from D-mannitol **(15)** by acetonide formation[30] **(16)** and oxidative cleavage of the vicinal diol using sodium periodate[31], followed by reduction with $NaBH_4$[32] **(17)** After coupling with farinomalein A using DCC and DMAP, deprotection of **(18)** using samarium chloride gave (*R*)-farinomalein D. **(19)**



Scheme 5.4

Reagents and conditions.

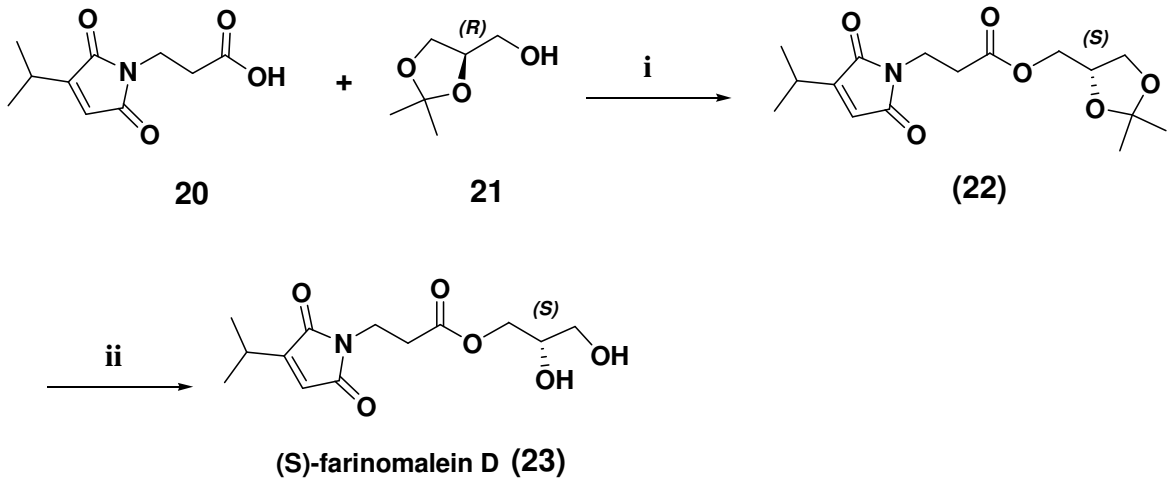
(i) 2,2 dimethoxypropane, p-TSA, DMSO, rt, 16 h, 61%

(ii) a) $NaIO_4$, $NaHCO_3$, rt, 1.5h, 72%. b) $NaBH_4$, EtOAc, 0 ºC – rt, 1h, then 0 ºC , 3*N* HCl, 95%

(iii) DCC, DMAP, 0 ºC– rt, 12h, 77%.

(iv) $SmCl_2$, ethanol, reflux, 8h, 20%

The same reaction pathway was followed to prepare (*S*)-farinomalein D **(23)** starting from (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol **(21)** in 58% overall yield. Racemic Farinomalein D **(27)** was also prepared, starting from DL-1,2 isopropylideneglycerol[33] **(25).**

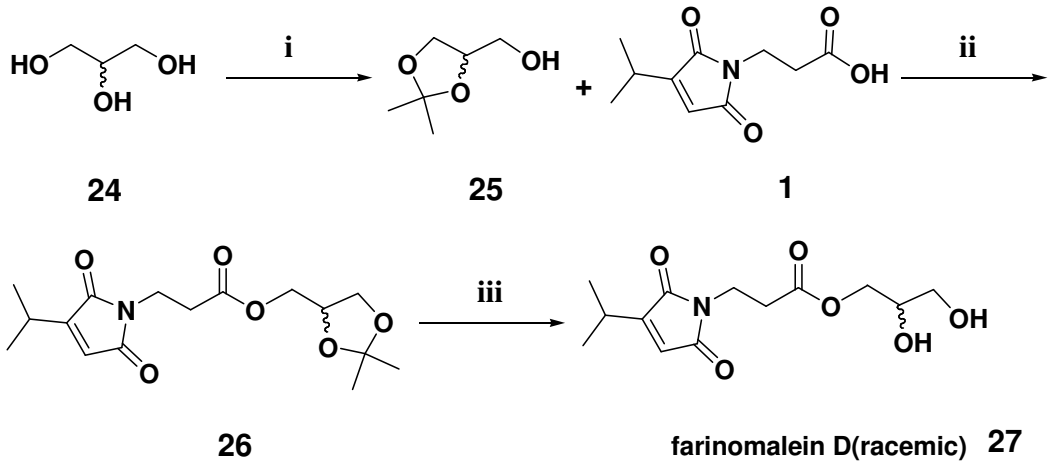


Scheme 5.5

Reagents and conditions.

(i) DCC, DMAP, 0 ºC– rt, 12h, 73%.

(ii) $SmCl_2$, ethanol, reflux, 8h, 28%



Scheme 5.6

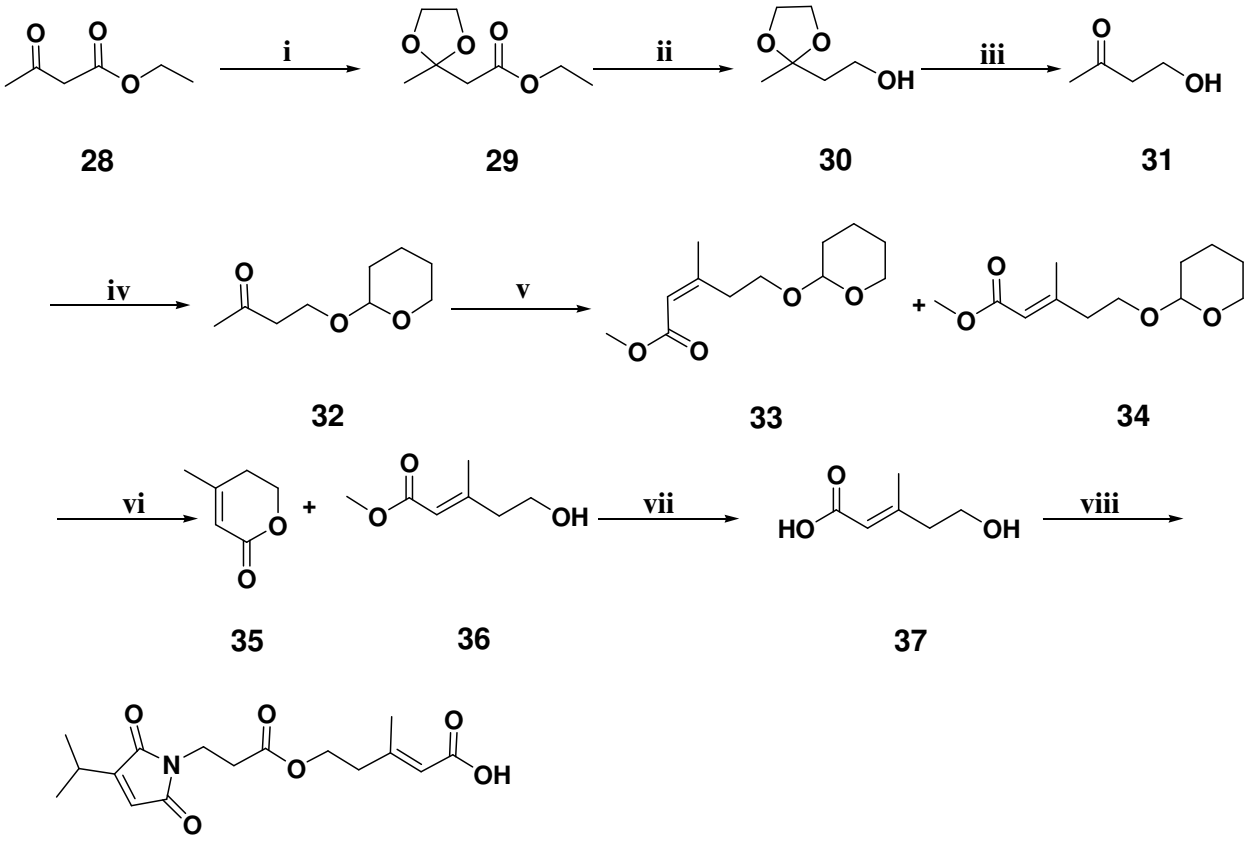
Reagents and conditions.

(i) glycerol, acetone:pentane, (3:7), p-TSA, dean stark, 41h, 96 %

(ii) DCC, DMAP, 0 ºC– rt, 12h, 71%.

(iii)$SmCl_2$, ethanol, reflux, 8h, .35%

For the synthesis of the side chain of farinomalein E **(Scheme 5.7)** the reaction sequences described by White et.al.[34] and Albizati et.al.[35] were followed. The carbonyl group of *β*-keto ester **(28)** was protected using standard methodology providing ketal **(29)**. Reduction of **(29)** with lithium aluminium hydride gave the primary alcohol **(30)** in high yield. In the key transformation, treatment of *β*-hydroxy ketal **(30)** with oxalic acid and wet silica gel according to the procedure by Conia et al.[36] provided the *β*-hydroxy ketone **(31)** in high yield without elimination products. Compound **(31)** was protected as tetrahydropyranyl derivative,[34] **(32)** and this latter was condensed with methyl (triphenylphosphoranylidene)acetate via benzoic acid[37] catalyzed Wittig reaction. A 3:2 mixture of *E* and *Z* *α,β*-unsaturated esters **(33)** and **(34)** was produced. The mixture was treated with methanolic HCl without separation of the stereoisomers. Following removal of the THP group, the *Z* isomer **(33)** underwent spontaneous lactonization to **(35)** and could thus be removed in a facile chromatographic separation from the desired hydroxy ester **(36)**. Finally, saponification with sodium hydroxide in water followed by acidification gave the acid **(37)** in quantitative yield. **Farinomalein E (4)** was then obtained by coupling with farinomalein A **(1)** with **(37)** in 21% yield.



Farinomalein E (4)

Scheme 5.7

Reagents and conditions.

(i) cat. p-TsOH, ethylene glycol/benzene, reflux 73%

(ii) $LiAlH_4$/THF, rt, 91%

(iii) silica, DCM, aq. soln. of oxalic acid, rt, 12 h, 84%

(iv) DHP, p-TSA, DCM, 0 ºC-rt, 1h, then $NaHCO_3$, 70%.

(v) methyl (triphenylphosphoranylidene)acetate, benzoic acid, Toluene, reflux, 48 h, 42%

(vi) 2M HCl, Methanol, 1h, rt.

(vii) 1N NaOH, reflux, 10 min, then 1 N HCl, 88%

(viii) DCC, DMAP, 0 ºC– rt, 12h, 21%.

5.6 Evaluation of Fungicidal Activity

The antifungal activity of the synthesized natural compounds (Farinomaleins A,C-E) was investigated on *Cladosporium cladosporioides*, a fungal plant pathogen that affects wheat, using the method of bioautography.[37, 38]

For the antifungal assay 10.0 µL of solutions corresponding to 50.0, 40.0, 30.0, 20.0, 10.0, 5.0, 1.0, 0.5, and 0.1 µg were applied to precoated Si gel TLC plates. The chromatograms were sprayed with a spore suspension of *Cladosporium cladosporioides* in CZAPEK broth and incubated for 72h in darkness in a moistened chamber at 25 ^{o}C, following a previously reported procedure.[39, 40] Fungal growth inhibition appeared as clear zones against a dark background (fig 8), indicating the minimum amount of compounds required for it (table). Prochloraz was used as control.

Compound	Antifungal activity (μg)[a] *C. cladosporioides*
Farinomalein A	5
Farinomalein C	0.5
Farinomalein D	20
Farinomalein E	20
prochloraz	0.1

Table 5.1

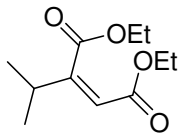
The results confirmed that **Farinomalein A** is endowed with antifungal activity on this pathogen, the minimum amount required for the inhibition of fungal growth on thin-layer chromatography (TLC) plates being 5 µg. Interestingly, **Farinomalein C** showed a ten-fold increased potency, inhibiting the fungal growth at 0.5µg. **Farinomalein E** and (R)-/ (S)- / racemic **Farinomaleins D** showed all a decreased activity (20 µg), thus confirming that the stereochemistry did not affect the antifungal activity **(Table 5.1)**.

The results clearly indicate that the introduction of different chains to the carboxylic group and the esterification with polar moieties was detrimental for activity. Conversely, a lipophilc chain such as that of farinomalein C led to an increase of activity, comparable with that of reference compound prochloraz. These data confirm that farinomaleins can be considered promising

Current efforts are directed towards the synthesis of new analogues. Notwithstanding the fact that the molecular mechanism of action of the reported compounds remains to be defined, structure-activity information inferred from our study could provide a basis for rational design of new structurally simple antifungal agents and the starting point for their optimization.

5.7 Experimental

2-Isopropyl-but-2-enedioic acid diethyl ester (9)

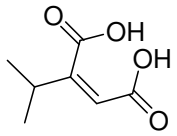


9

To a stirred solution of NaH (60 %, 0.138 g 3.4 mmole 1eq) in THF (5 mL), a solution of triethyl phosphonoacetate **8** (0.77 g 3.4 mmol, 1 eq) in THF(15.5 mL) was added at 0 ^{o}C, stirred for 30 min at 0 ^{o}C. To this reaction mixture ethyl 3-methyl-2-oxobutyrate **7** (0.51 ml 3.4 mmole) was added at 0 ^{o}C. The solution was stirred for 5 min at 0 ^{o}C, then it was slowly warmed to 50 ^{o}C for 2 h. Saturated aqueous NH_4Cl (10 mL) was added and the aqueous phase was extracted with diethyl ether (3 x 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash column chromatography to afford **9** (0.590 g 79.%) as a faint yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.80 (d, J = 1.2 Hz, 1H), 4.12 (q, J = 7.2 Hz, 2H), 4.31 (q, J = 7.2 Hz, 2H), 2.65 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H), 1.25 (t, J = 7.2 Hz, 3H), 1.15 (d, J = 7.0 Hz, 6H).

2-Isopropyl-but-2-enedioic acid (6)

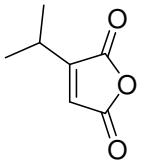


6

To the stirred solution of **9** (0.405 g, 1.8 mmole) in THF (21 mL) was added 2N LiOH (16 eq, 0.634 g in 7 mL H_2O) at 0 ^oC, then the reaction mixture was stirred for 8 h at RT. THF was removed under vacuum, the aqueous reaction mixture was acidified with 1N HCl (~30 mL) at 0 ^oC. The aqueous phase was extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated, to obtain **6** as a colourless oil (0.286 g, 96 %). This crude product was used for the next reaction. R*f* = 0.43 (EtOAc / hexane 50:50),

^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.60 (brs, 2H), 5.85 (s, 1H), 2.65-2.80 (m, 1H), 1.20 (d, J = 7.0 Hz, 6H).

3-Isopropyl-furan-2,5-dione (5)

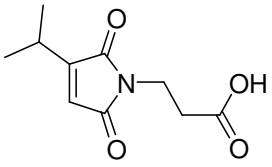


5

In trifluoroacetic anhydride (2.56 mL, 10 eq), **6** (0.289 g) was stirred overnight at rt. The resulting solution was evaporated under vacuum to provide **5** as a blackish brown oil (0.245 g, 98 %), The crude product was used for the next reaction.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 1H), 2.80-3.00 (m, 1H), 1.2 (d, J = 7.0 Hz, 6H).

3-(3-Isopropyl-2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-propionic acid (Farinomalein, 1)



1

To the stirred solution of **5** (0.100 g 0.71 mmol) in AcOH (0.5 mL) β-alanine (0.063 g, 0.71 mmol) was added at room temperature. The reaction mixture was refluxed for 2 h, then cooled to 60 ^{o}C and the solvent was evaporated under vacuum. To the crude product EtOAc (10 mL) was added and the organic layer was washed with 2N HCl (5 mL) and brine, then dried over Na_2SO_4 and concentrated. Purification by flash column chromatography afforded **1** as a white solid (0.089 g, 60 %).

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.25 (d, J = 1.8 Hz, 1H), 3.80 (t, J = 7.0 Hz, 2H), 2.80-2.95 (m, 1H), 2.70 (t, J = 7.0 Hz, 2H), 1.21 (d, J = 6.9 Hz, 6H).

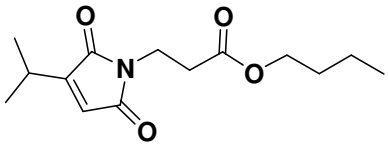
^{13}C NMR (CD_3OD)) δ: 173.2, 171.0, 170.8, 155.7, 124.5, 33.3, 32.2, 25.6, 19.7.

Anal. Calcd for $C_{10}H_{13}NO_4$: C 56.86; H 6.20; N 6.63. Found: C 56.98; H 6.10; N 6.51.

m.p. = 76 °C

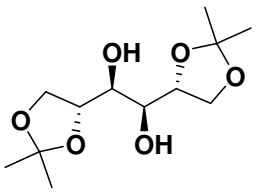
Butyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (2)

Farinomalein C



To a stirred solution of farinomalein A (0.1 g, 0.47 mmol) in DCM (5 mL), DMAP (0.86 g, 0.70 mmol) was added and the mixture was stirred at rt under nitrogen for 10 min. After addition of DCC (0.146 g, 0.70 mmol) stirring was continued for further 30 min at rt, then 1-butanol (0.053 g, 0.70 mmole) was added at 10°C. The reaction mixture was stirred at rt overnight, then it was filtered through cotton and purified by column chromatography to give Farinomalein C **(2)** (0.117 g, 92 %). The spectroscopic data of the compound completely matched with those reported in the literature

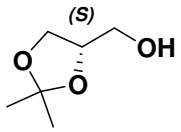
(1S,2S)-1,2-Bis((R)-2,2-dimethyl-1,3-dioxolan-4-yl)ethane-1,2-diol (16)



To a solution of D-Mannitol **(15)** (2.0 g, 10.96 mmol) in 15 mL of dry DMSO and p-TSA (catalytic) was added 2,2-dimethoxypropane (3 mL, 24.16 mmol) at room temperature for16h. The reaction mixture was diluted with ethyl acetate (30 mL) and washed with 5% $NaHCO_3$ (15 mL), dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography to remove the undesired 1,2:3,4:5,6-triisopropylidene-D-mannitol afforded **(16)** (1.76 g, 61 % yield).

^{1}H NMR (300 MHz, $CDCl_3$) δ: d 4.09 (m, 4H), 4.00 (dd, *J* = 8.1 Hz, 5.7 Hz, 2H), 3.77 (t, *J* = 7.1 Hz, 2H), 2.60 (d, J = 6.7 Hz, 2H), 1.44 (s, 6H), 1.38 (s, 6H).

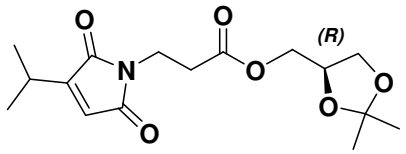
(S)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methanol (17)



To a solution of aqueous $NaHCO_3$ (0.402 mL) and **(16)** (1 g, 3.81 mmol) in dichloromethane (10 mL) was added $NaIO_4$ (1.62 g, 7.6 mmol) and the mixture was stirred for 1.5 h at room temperature. After decantation, dichloromethane was removed and the residue was washed with more dichloromethane (15 mL). The solvent was evaporated to give (R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (0.900 g, 98%) as a colourless viscous oil which was sufficiently pure to be used directly for the next reaction.

To a solution of (R)-2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (0.500 g, 3.83 mmol) in 5 ml ethyl acetate was added ethanol (0.5 ml) followed by gradual addition of sodium borohydride (0.116 g, 3 mmol) at 0 °C. This reaction mixture was allowed to stir at room temperature for 1 h, then acetone (4 mL) was added, and the mixture was stirred at room temperature for further 15 min. It was then cooled to 0 °C and 3*N* HCl was added dropwise till there was no more evolution of hydrogen gas. The layers were separated and the aqueous layer was extracted with diethyl ether twice. The combined organic layers were washed with brine, dried over Na_2SO_4, conc. *in vacuo* to obtain crude product. The crude alcohol obtained was subjected to short-path silica gel column chromatography to give **(17)** (0.480 g, 95 %). The spectroscopic data of the compound completely matched with those reported in the literature

(R)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl) propanoate (18)



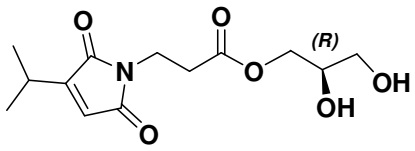
To a stirred solution of Farinomalein A **(1)** (0.2 g, 0.94 mmol) in DCM (10 mL), DCC (0.292g, 1.4 mmol), DMAP (0.172g, 1.41 mmol) and alcohol **(17)** (0.186g, 1.41 mmol) were added at 0°C. The resulting mixture was stirred at rt overnight under nitrogen, then it was filtered through cotton and purified by column chromatography to give the title compound (0.223 g, 73 %)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.22 (1H, s); 4.30 (1H, m); 4.19-4.02 (3H, m); 3.80 (2H, t, J = 7.3 Hz); 3.73 (1H, m); 2.82 (1H, m); 2.66 (2H, t, J = 7.3 Hz); 1.42 (3H, s); 1.35 (3H, s); 1.20 (6H, d, J = 6.7 Hz).

^{13}C NMR (CD_3OD)) δ: 170.4, 170.2, 170.0, 155.5, 124.2, 109.4, 73.0, 65.9, 64.7, 33.1, 32.5, 26.3, 25.4, 24.9, 20.4 (x2).

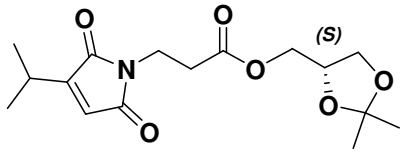
(R)-2,3-Dihydroxypropyl -3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (19)

(R)-farinomalein D



To a solution of compound **18** (0.200 g, 0.613 mmol) in 3 mL of ethanol, $SmCl_2$ (10 mol%) was added and the mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel to obtain (*R*)-farinomalein D **(19)** (0.050 g, 28 %) The spectroscopic data of the compound completely matched with those reported in the literature.

(S)-(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (22)



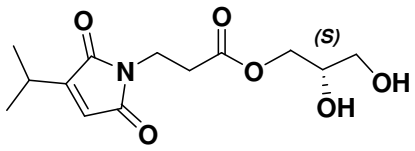
To a stirred solution of Farinomalein A **(1)** (0.2 g, 0.947 mmol) in DCM (10 mL), DCC (0.292 g, 1.4 mmol), DMAP (0.172 g, 1.4 mmol) and alcohol **(21)** (0.187 g, 1.4 mmol) were added at 0°C. The resulting mixture was stirred at rt overnight under nitrogen, then it was filtered through cotton and purified by column chromatography to give the title compound **(22)**(0.236 g, 77 %)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.22 (1H, s); 4.30 (1H, m); 4.19-4.02 (3H, m); 3.80 (2H, t, J = 7.3 Hz); 3.73 (1H, m); 2.82 (1H, m); 2.66 (2H, t, J = 7.3 Hz); 1.42 (3H, s); 1.35 (3H, s); 1.20 (6H, d, J = 6.7 Hz).

^{13}C NMR (CD_3OD)) δ: 170.4, 170.2, 170.0, 155.5, 124.2, 109.4, 73.0, 65.9, 64.7, 33.1, 32.5, 26.3, 25.4, 24.9, 20.4 (x2).

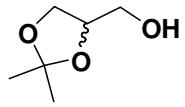
(S)-2,3-Dihydroxypropyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (23)

(S)-Farinomalein D



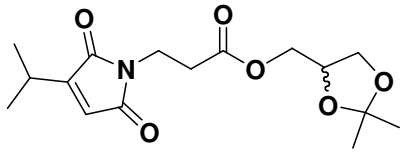
To a solution of compound **22** (0.200 g, 0.613 mmol) in 2 mL of ethanol, $SmCl_2$ (10 mol%) was added and the mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel to obtain (S)-farinomalein D **(23)** (0.036 g, 20 %). The spectroscopic data of the compound completely matched with those reported in the literature.

(2,2-dimethyl-1,3-dioxolan-4-yl)methanol (25)



Glycerol (10 mmol, 1 g), acetone (3 ml), pentane (10 ml) and p-toluenesulfonic acid (0.16 mmol, 30 mg) were successively introduced into a vessel fitted with a Dean–Stark device. The mixture was then stirred at reflux for 41 h. After cooling, sodium acetate (0.18 mmol, 15 mg) was added. The mixture was filtered and the solvents were evaporated under reduced pressure to give **(25)** (1.3 g, 96%) as a light yellow oil. The spectroscopic data of the compound completely matched with those reported in the literature.

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (26)

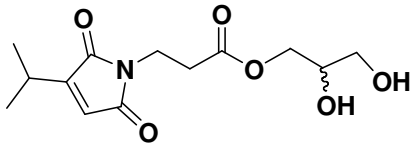


To a stirred solution of Farinomalein **A (1)** (0.100 g, 0.473 mmol) in DCM (5 mL), DCC (0.146 g, 0.707 mmol), DMAP (0.086, 0.703 mmol) and alcohol **(25)** (0.093 g, 0.704 mmol) were added at 0°C. The resulting mixture was stirred at rt overnight under nitrogen, then it was filtered through cotton and purified by column chromatography to give the compound **(26)** (0.110 g, 71%)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.22 (1H, s); 4.30 (1H, m); 4.19-4.02 (3H, m); 3.80 (2H, t, J = 7.3 Hz); 3.73 (1H, m); 2.82 (1H, m); 2.66 (2H, t, J = 7.3 Hz); 1.42 (3H, s); 1.35 (3H, s); 1.20 (6H, d, J = 6.7 Hz).

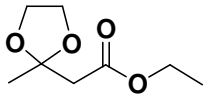
^{13}C NMR (CD_3OD)) δ: 170.4, 170.2, 170.0, 155.5, 124.2, 109.4, 73.0, 65.9, 64.7, 33.1, 32.5, 26.3, 25.4, 24.9, 20.4 (x2).

2,3-Dihydroxypropyl 3-(3-isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoate (27)



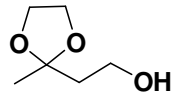
To a solution of glycerol acetonide **26** (0.100 g, 0.306 mmol) in 2 mL of ethanol, $SmCl_2$ (10 mol%) was added and the mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel to obtain (rac)-farinomalein D **(27)** (0.033 g, 35 %). The spectroscopic data of the compound completely matched with those reported in the literature.

Ethyl 2-(2-methyl-1,3-dioxolan-2-yl)acetate (29)



To a solution of Ethyl 3-oxobutanoate **(28)** (15.0 g, 115.5 mmol) in toluene (30 mL) were added a solution of ethylene glycol (21.45 g, 345.8 mmol) in toluene (15 mL) and a catalytic amount of p-TsOH at room temperature. After the mixture was stirred for 20 h under reflux, the reaction was quenched with satd aq. $NaHCO_3$ solution at 0°C. The organic layer was washed with satd aq. $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After evaporation under reduced pressure, a colorless oil **(29)** (15g, 73%) was obtained, which was used as such for next reaction. The spectroscopic data of the compound completely matched with those reported in the literature.

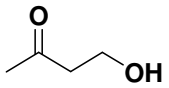
2-(2-Methyl-1,3-dioxolan-2-yl)ethanol (30)



To a suspension of $LiAlH_4$ (15.0 g, 86.2 mmol) in THF (150 mL) was added a solution of **(29)** (3.30 g, 86.2 mmol) in THF (90 mL) at 0°C. The mixture was stirred for 1 h. The reaction was quenched slowly with H_2O (3.3 mL), 15% NaOH (3.3 mL), H_2O (6.6 mL) at 0°C., and the mixture was stirred for 24 h. Filtration through a Celite pad and evaporation under reduced pressure, gave **(30)** as a colorless oil (10.3 g, 91%)

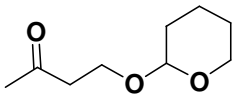
^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.0 (4H, s), 3.76 (2H, dt, J = 5.5, 2 Hz), 2.94 (1H, t, J = 5.5Hz), 1.95 (1H, t, J = 5.5Hz), 1.37 (s, 3H).

4-Hydroxybutan-2-one (31)



To a stirred suspension of silica gel (40.57 g) in DCM (250 ml) 10 % aq. oxalic acid (11.5 ml) was added. The suspension was vigorously stirred for 30 min. till the suspension became homogenous. Alcohol **(30)** (4.62 g, 35.0 mmol) in DCM (50 ml) was added and the reaction was stirred for 12 h. After the completion of reaction, which was monitored by TLC, the suspension was filtered, through silica bed using a glass-centered funnel. The bed was washed with DCM (25 ml x 2). The organic layer was dried over Na_2SO_4 .The organic layer was carefully evaporated under reduced pressure at low temperature as the product was volatile to give compound **(31)** (2.2 g, 71.4 %) The spectroscopic data of the compound completely matched with those reported in the literature.

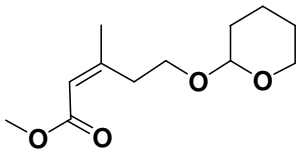
4-(Tetrahydro-2H-pyran-2-yloxy)butan-2-one (32)



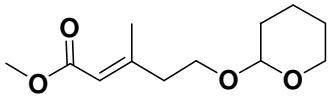
A solution of **(31)** (0.350 g, 3.97 mmol), dihydropyran (0.434 g, 5.1 mmol) and p-toluenesulfonic acid (20 mg) in dichloromethane (5 ml) was stirred at 0°C under nitrogen for 1 h. Solid sodium bicarbonate (0.270 g) was added, and the mixture was stirred at room temperature for 0.5 h. The mixture was filtered through Celite, the filtrate was evaporated, and the residue was purified by column chromatography to give **(32)** (0.400 g, 80 %). The spectroscopic data of the compound completely matched with those reported in the literature.

(Z)-Methyl 3-methyl-5-(tetrahydro-2H-pyran-2-yloxy)pent-2-enoate (33)

(E)-Methyl 3-methyl-5-(tetrahydro-2H-pyran-2-yloxy)pent-2-enoate (34)



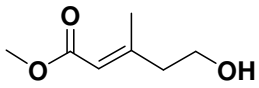
33



34

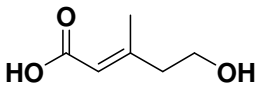
A solution of **(32)** (0.400 g, 2.56 mmol) and methyl (triphenylphosphoranylidene)acetate (0.856 g, 02.56 mmol)**,** and benzoic acid (5 mg) in toluene (3 ml) was heated at reflux for 48 h. The solvent was removed in vacuo, and the solid residue was triturated with hexane. The hexane extract was filtered, and the filtrate was evaporated to leave a viscous oil which was chromatographed on silica gel to afford (0.254 g, 42 %) of a mixture of **(33)** and **(34).** which was used as such for the next step. The spectroscopic data of the compound completely matched with those reported in the literature.

(E)-Methyl 5-hydroxy-3-methylpent-2-enoate (36)



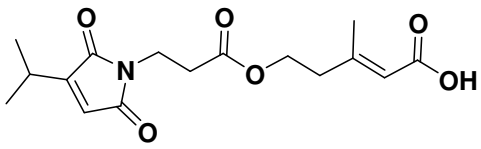
A solution of a mixture of E and Z alkene **(33)** and **(34)** (0.127 g, 0.556 mmol) and HCl (30µl, 2 M) in methanol (1.5ml) was stirred at rt for 1 h. The methanol was distilled off under reduced pressure. The residue was diluted by adding ethyl acetate (15 ml). The organic layer was washed with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and purified by column chromatography to give compound **(36)** (40 mg, 50%) .The spectroscopic data of the compound completely matched with those reported in the literature.

(E)-5-Hydroxy-3-methylpent-2-enoic acid (37)



To a stirred solution of ester **(36)** (211 mg, 1.46 mmol) in THF : H_2O (3:1) (4 ml) was added aq NaOH (280 mg, 7 mmol) and the reaction was vigorously stirred at rt for 12h. THF from the reaction was removed under vacuum. The aqueous layer was treated with HCl (2 N) solution till it was acidic The reaction mixture was extracted by EtOAc (15 ml). The organic layer was washed with brine. The organic layer was dried over Na_2SO_4 to give acid **(37).** (167 mg, 88%) The spectroscopic data of the compound completely matched with those reported in the literature.

(E)-5-(3-(3-Isopropyl-2,5-dioxo-2H-pyrrol-1(5H)-yl)propanoyloxy)-3-methylpent-2-enoic acid (4) (Farinomalein E)



To a stirred solution of **Farinomalein A (1)** (0.2 g, 0.94 mmol) in DCM (5 mL) was added DMAP (0.115 g, 0.94 mmol) and the solution was stirred for 10 min. EDCI. HCl (0.181 g, 0.94 mmol) was added to the above solution and it was allowed to stir for additional 30 min. at rt. The solution was cooled to 10°C. A solution of alkene **(37)** (0.123 g, 0.94 mmol) in DCM (2 ml) was added and the reaction was allowed to stir at rt. for 12 h. under nitrogen. The reaction was quenched by adding water, then it was extracted by DCM (15 ml). The organic layer was washed with brine. The organic layer was dried over Na_2SO_4 and purified by column chromatography to give compound **(4) (Farinomalein E)** (35 mg, 21%) The spectroscopic data of the compound completely matched with those reported in the literature.

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CHAPTER 6

APPROACHES TO THE SYNTHESIS OF ASCAULITOXIN AGLYCONE

6.1 Introduction

A key objective for modern herbicide research is to discover new products that ideally control the widest possible range of weed species, and can be applied pre- and post-emergence, at low application rates. In addition, selective herbicides need to be safe to the target crop, and safe to the environment and end-user. The biological agents offer the advantage of being fully compatible with the environment, often with high specificity, and represent a long term solution to control weeds, particularly those resistant to chemical herbicides. Therefore, many efforts have been made for weed biocontrol using their natural antagonists, mainly pathogens[1] and insects. Fungi appear to be the most appropriate agents as mycoherbicides and some of them have also been commercialized.[2] The phytotoxins produced by pathogenic weed fungi can also be used as pure compounds to develop new natural and safe herbicides.[3]

6.2 Herbicides with amino acid functional group.

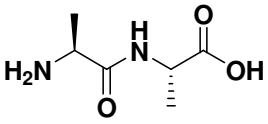
Herbicides with chemical structure containing amino acid functional group have shown promising activity against many common weeds. Some of the synthetic and naturally occurring herbicides used commercially are described here.

The dipeptide, **L-alanyl-alanine**, can be found in hydrolysed corn gluten meal, a byproduct of corn wetmilling. It shows activity against *Lolium perenne* (perennial ryegrass).[4]

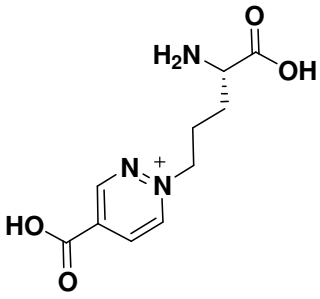
Pyridazocidin was isolated from a *Streptomyces* sp. strain and is one of the very rare examples of naturally occurring pyridazines.[5] It shows significant postemergence activity against *Setaria faberi* (giant foxtail).

The cyclic tetrapeptide **tentoxin**, is produced by the fungus *Alternaria alternata*. It induces chlorosis on a variety of soybean and corn weeds, such as *Ipomoea hederacea* (morning glory),

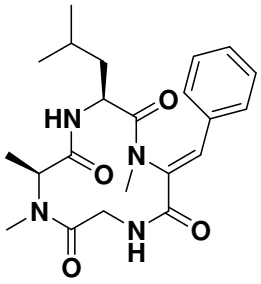
Cassia obtusifolia (sicklepod) and *Sorghum halepense* (Johnson grass) without affecting the corresponding crops.[6]



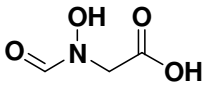
L-alanyl-alanine



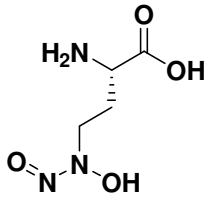
Pyridazocidin



tentoxin



hadacidin



homoalanosine

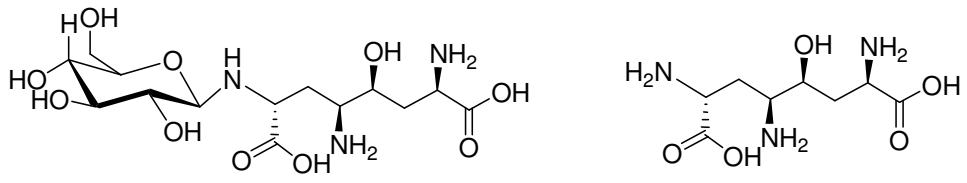
Fig.6.1

The simple glycine derivative, **hadacidin**, from *Penicillium purpurescens*, inhibits purine biosynthesis by blocking the conversion of IMP into AMP at the site of adenylosuccinate synthetase. It demonstrates herbicidal activity against *Panicum crusgalli* (Japanese millet) and *Digitaria sanguinalis* (crabgrass).[7]

The herbicide arylaminopropionic acid derivatives **flamprop-M-isopropyl** and **flamprop-M-methyl** were first reported by Jeffcoat and Harries in 1975.[8,9] Herbicidal activity depends on hydrolytic conversion of the esters within the plant to the corresponding acid, preferentially for the selective, post-emergence control of wild oats (*Avena* spp.) and blackgrass (*Alopecurus myosuroides* Huds.) in barley and wheat[8, 9]

Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with commercial crops grown around the globe. It was discovered to be a herbicide by Monsanto chemist John E. Franz in 1970. Plants treated with glyphosate translocate the systemic herbicide to their roots, shoot regions and fruit, where it interferes with the plant's ability to form aromatic amino acids necessary for protein synthesis.

6.3 Ascaulitoxin, Ascaulitoxin aglycone and their biological activity



1 **2**

Ascaulitoxin **Ascaulitoxin Aglycone(AscA)**

Fig 6.2

Ascaulitoxin **(1)** is a new phytotoxic *bis*-aminoacid *N*-glucoside, which was isolated by Evidente and coworkers from the culture filtrate of *Ascochyta caulina* (P. Karst.) v.d. Aa and v. kest,[10,11] the causal agent of leaf and stem necrosis of *Chenopodium album* (also known as lambsquarter

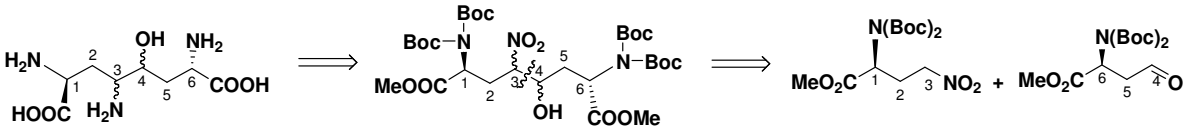
or fat hen, a common world-wide weed of many arable crops such as sugar beet and maize[12]), and that has become a promising microherbicide for the biological control of this common noxious weed. Ascaulitoxin with its bis-amino acid side chain contains four stereogenic centers, two of which with nitrogen substituents. The relative configuration of its four stereocenters was determined by the *J*-based NMR configurationally analysis by Bassarello and co-workers.[11]

6.3.1 Ascaulitoxin aglycone (AscA) (2)

The aglycone of ascaulitoxin **(AscA) (2)** was found to be a more potent phytotoxin than Ascaulitoxin as per preliminary studies[13]. The experiments carried out to investigate the mode of action by using metabolite feeding and metabolite profiling suggest that the most probable biochemical pathways that might be directly affected by AscA were those involved in amino acids metabolism.

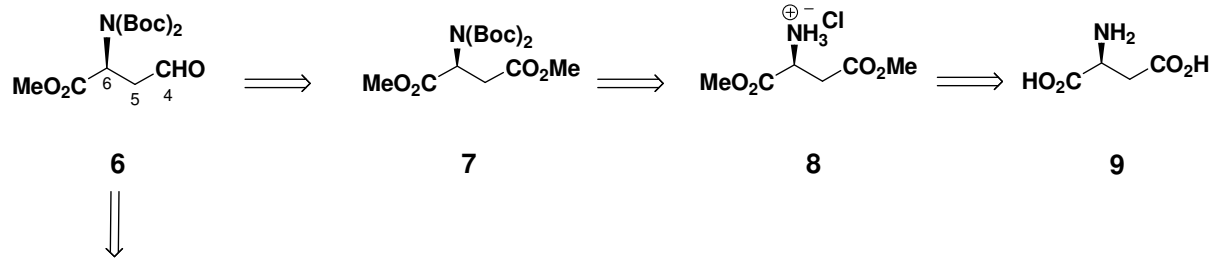
6.3.2 Retrosynthesis of Racemic AscA

As the absolute configuration of natural Ascaulitoxin aglycone **(2)** is not known, and the relative configuration suggests that the configuration at carbons **(C_3)** and **(C_4)** is opposite to that at carbons **(C_1)** and **(C_6)** **(Fig. 6.2),**[11] we supposed that most probably the configuration at the two stereogenic centers **(C_1)** and **(C_6)** is that of natural amino acids, i.e. 'S'. Therefore fragment **5** and **6** could be prepared from commercially available L-aspartic acid with known configuration i.e. 'S'. The two fragments **5** and **6** could then be connected under Henry reaction condition to give racemic **4.** The corresponding nitro derivative could be subsequently reduced to amine **(3).** Initially, to check the feasibility of our approach, we wished to synthesize the racemic AscA at carbon **(C_3)** and **(C_4)** starting from compounds enantiomerically pure at carbon **(C_1)** and **(C_6).** The retro synthetic strategy is shown in **Scheme 6.1.**



3 **4** **5** **6**

Scheme 6.1

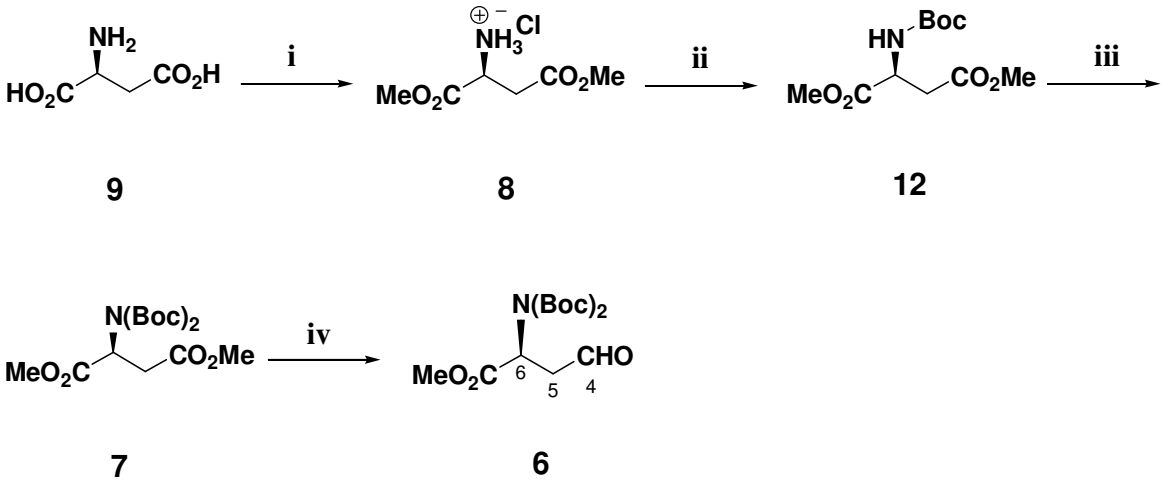


6 **7** **8** **9**

10 **11** **5**

Scheme 6.2

6.3.3 Synthesis of fragment (6)



Reagents and conditions.

(xv) $SOCl_2$, MeOH, 0ºC - rt, 45h, 98%;

(xvi) $(Boc)_2O$, TEA, THF, rt, 2.5h, 100%

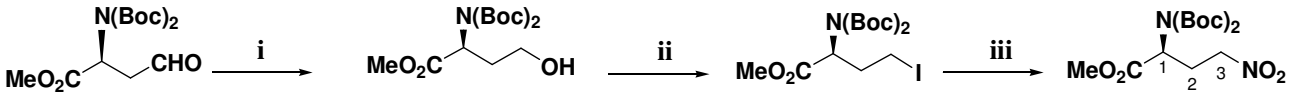
(xvii) $(Boc)_2O$, DMAP, THF, rt, 22h, 81%

(xviii) DIBAL-H, Et_2O, – 78 ºC, 15 min, 82%

Scheme 6.3

Commercially available, inexpensive L-aspartic acid **(9)** was found to be a common precursor for both the fragments **5** and **6.** The aspartic acid ester **(8)** was prepared from L-aspartic acid by using $SOCl_2$/MeOH.[14] Dimethyl L-aspartate **(8)** was successively treated with $(Boc)_2O$ /triethyl amine to give compound **(12)** and $(Boc)_2O$ in the presence as DMAP to give bis-Boc compound **(7)** in excellent yield. The diester **(7)** was selectively reduced to give aldehyde **(6)** using DIBAL-H.[15]

6.3.4 Synthesis of fragment 5



6 **10** **11** **5**

Reagents and conditions.

(i) $NaBH_4$, MeOH, 0ºC, 45 min, 100 %;

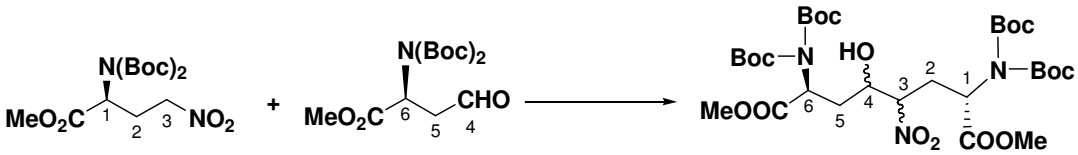
(ii) I_2, PPh_3, Imidazole, rt, 2h, 92%

(iii) $NaNO_2$, DMF, rt, 2h, 55%

Scheme 6.4

Further reduction of aldehyde **(6)** with $NaBH_4$ gave the primary alcohol **(10)** which was converted to the iodide **(11)** in good yield by using standard procedures using triphenylphosphine, imidazole and iodine in THF.[15-17] Subsequent treatment of iodide (*S*)-(-)-**11** with sodium nitrite in DMF afforded the nitro compound (*S*)-(-)-**5**.[15]

Treatment of nitro compound **(5)** with aldehyde **(6)** in the presence of DMAP in CH_2Cl_2[18] allowed smooth condensation (Henry reaction) to give the hydroxynitro compound **(4)** in 81% yield as a mixture of diastereomers because of the two newly generated stereogenic centers at the carbons bearing NO_2 and OH groups at **(C_3)** and **(C_4)** respectively.



5 **6** **4**

Reagents and conditions.

(i) DMAP, DCM, rt, 4 days, 28.7%

Scheme 6.5

With this positive result in hand we planned to synthesize the enantiomerically enriched Ascaulitoxin aglycone using the Shibasaki asymmetric Nitro Aldol reaction.

6.4 Shibasaki asymmetric Nitro Aldol reaction.

The reaction between an *in situ* generated nitronate species and a carbonyl compound, known as Henry (nitroaldol) reaction, is an important carbon-carbon bond-forming method in organic synthesis [19]. This process represents a powerful and useful tool for the synthesis of valuable β-nitroalcohols,[20] providing, after further transformation of the β-nitroalcohols, efficient access to interesting and highly functionalized intermediates like nitroalkenes, 1,2-amino alcohols and α-hydroxycarboxylic acids.[21,22] Various methods for catalytic enantioselective synthesis of β-amino alcohols have been developed over the past decade,[23] and the catalytic asymmetric nitroaldol (Henry) reaction is an efficient method for providing chiral β-amino alcohols by reduction of the nitro moiety in nitroaldol adducts.[24]

In 1992, Shibasaki et al. reported for the first time an application of chiral heterobimetallic lanthanoid complexes (LnLB) as chiral catalysts in asymmetric catalysis, namely in the catalytic asymmetric nitroaldol reaction (Henry reaction). using the concept of *multifunctional* catalysis[25] wherein the catalysts exhibit both Lewis acidity and Brønsted basicity.[26] The synergistic effects of the two functions enable transformations that have never been possible using conventional catalysts employing only Lewis acidity. Furthermore, a variety of enantioselective transformations has been realized by carefully choosing the metal elements according to the type of the reaction, consistent with the above-mentioned concept.

The proposed mechanism for the asymmetric nitroaldol reaction catalyzed by heterobimetallic lanthanoid complexes is shown in **Fig. 6.3.**[27] In the initial step, the nitroalkane component is deprotonated and the resulting lithium nitronate coordinates to the lanthanoid complex under

formation of the intermediate I.[28] Subsequent addition of the aldehyde by coordination of the C=O double bond to the lanthanoid (III) center ion leads to intermediate II, in which the carbonyl function should be attacked by the nitronate via a six-membered transition state (in an asymmetric environment). A proton exchange reaction step will then generate the desired optically active nitroalkanol adduct with regeneration of the "free" rare earth complex LnLB

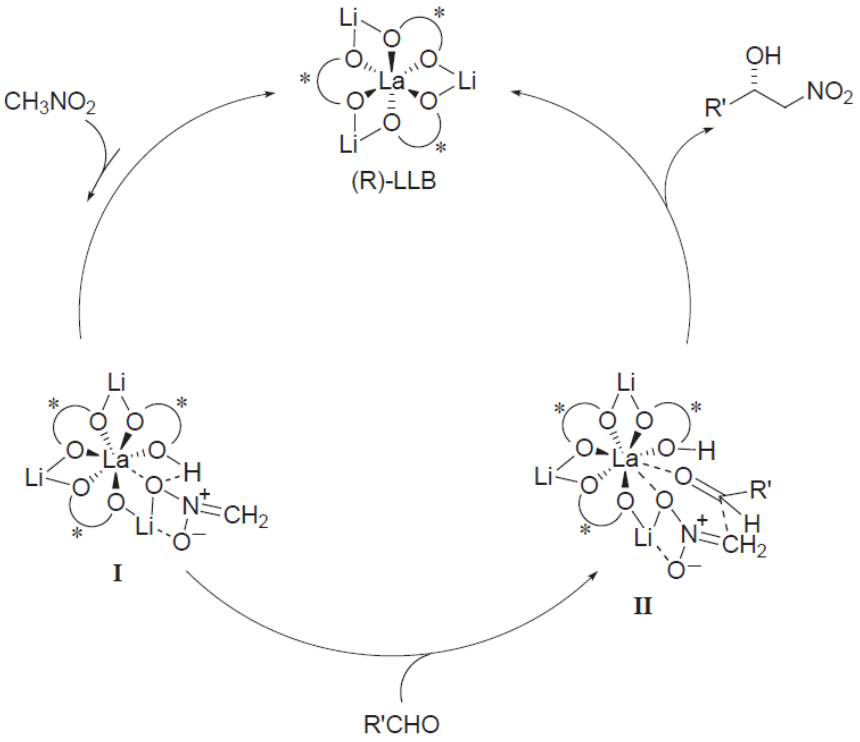


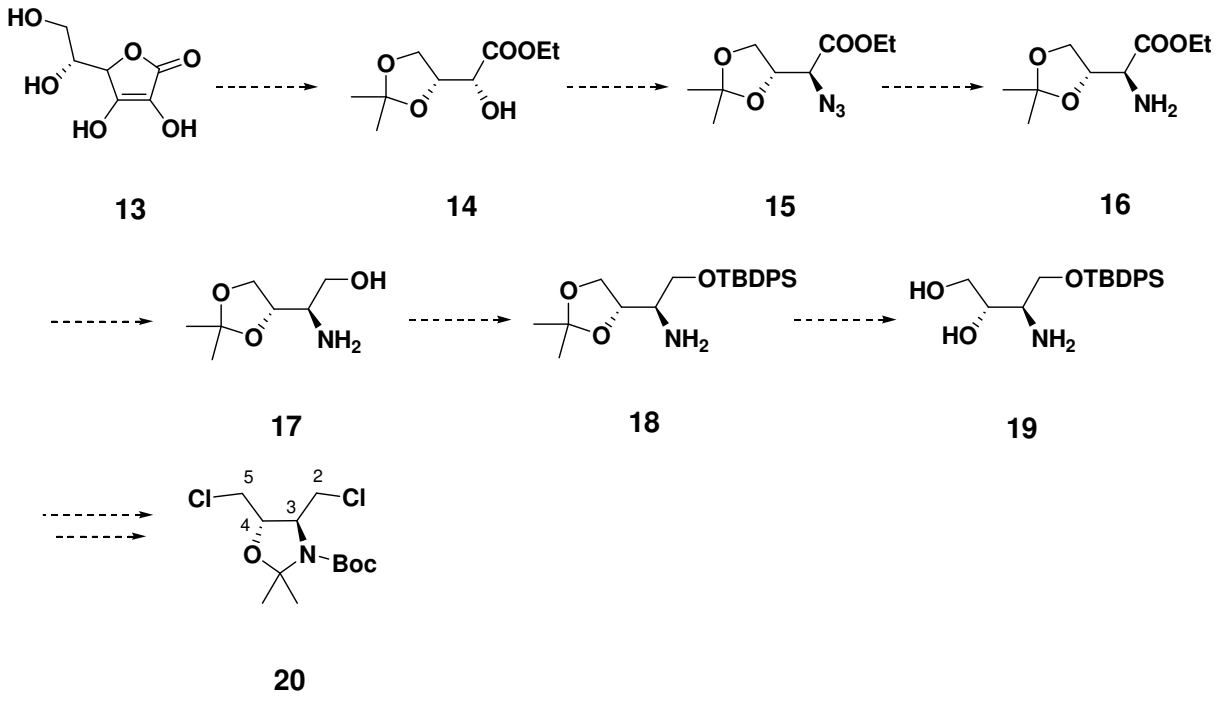
Fig. 6.3

The LnLB catalyst was synthesized according to literature procedure.[29] The two reactants were prepared according to scheme **(3)** and **(4),** were reacted using 10 eq of nitro compound **(5)** and 1 eq of aldehyde **(6)** using the LnLB catalyst in dry THF at – 40 °C for 24h. Unfortunately the reaction was not successful as no new spot was observed in the TLC of the reaction. One of the reason for the failure may be that the diastereoselective nitro aldol reactions are very substrate specific and are carried out at a lower temperature. As already stated the nitro aldol reaction

using DMAP in DCM took 4 days to complete with still some unreacted starting material at 25 °C.

6.5 Future perspective

A possible alternative synthetic strategy is the one in which the carbon $\mathbf{C_3}$ and $\mathbf{C_4}$ are synthesized first and the amino acid groups at the two ends are introduced during the later stages of the synthesis.

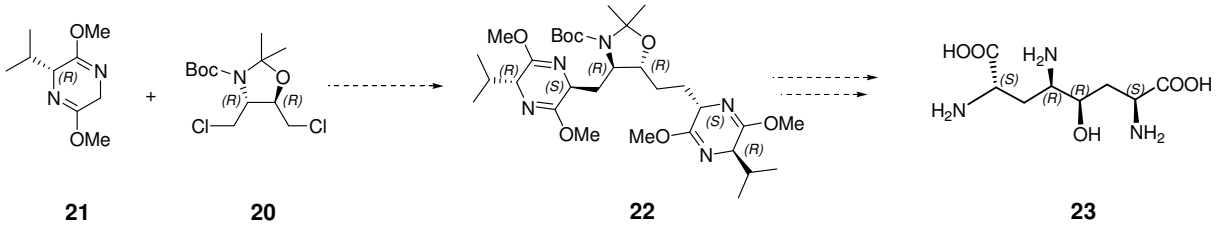


Scheme 6.6

For this purpose compound **(20)**, which contains the four central carbons of ascaulitoxin can be synthesized form D-ascorbic acid using Merrer's approach[30] **(Scheme 6.6)**.With compound **(20)** in hand the amino acid precursor can be introduced by Schöllkopf bis-lactim amino acid synthesis protocol using (R)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine **(21)** as Schöllkopf

reagent.[31, 32, 33] Compound **(22)** after deprotection would give the desired aglycone **(23)** (**Scheme 6.7**).

Work is now in progress to develop this new synthetic strategy.

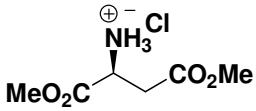


21 **20** **22** **23**

Scheme 6.7

6.6 Experimental

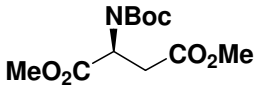
2-Amino-succinic acid dimethyl ester (8)



Thionyl chloride was added dropwise to a suspension of L-aspartic acid (1.05 g, 7.89 mmol) in MeOH (6.0 mL) at 0 ^{o}C. The bath was removed and the solution was allowed to stir at room temperature for 45 h and then concentrated. The residual oil was triturated with ether and the resulting white crystalline solid was filtered, washed with cold ether and dried to afford **8** (1.53 g, 98%) as a white solid.

mp =114-115 ^{o}C.

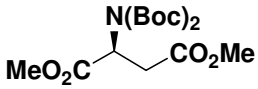
2-*tert*-Butoxycarbonylamino-succinic acid dimethyl ester (12)



Triethyl amine (1.16 mL, 8.35 mmol) and $(Boc)_2O$ (1.82 g, 8.35 mmol) were added sequentially to a suspension of dimethyl-L-aspartate HCl **5** (1.5 g, 7.59 mmol) in THF at room temperature under nitrogen. The mixture was stirred for 2.5 h and concentrated, then the residue was dissolved in ethyl acetate, washed with water, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude compound was purified by flash column chromatography (Ethyl acetate/petroleum ether 2:8) to afford **6** as colorless viscous oil (1.98 g, 100%).

^{1}H NMR ($CDCl_3$) *δ*: 5.49 (d, J = 7.34 Hz, 1H), 4.58 (m, 1H), 3.76 (s, 3H), 3.69 (s, 3H), 3.01 (dd, J = 16.87, J = 4.40 Hz, 1H), 2.82 (dd, J = 16.87, J = 4.70 Hz, 1H), 1.45 (s, 9H).

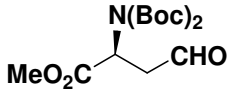
(-)-Dimethyl *N,N*-bis(*tert*-butoxycarbonyl)-L-aspartate (7)



Dimethyl *N*-(*tert*-butoxycarbonyl)-L-aspartate **(12)** (1.98 g, 7.58 mmol) was dissolved in THF (20.0 mL) followed by addition of DMAP (0.185 g, 1.52 mmol). To this reaction mixture a solution of $(Boc)_2O$ (2.48 g, 11.37 mmol) in THF (5.0 mL) was added at room temperature under nitrogen atmosphere. After stirring the mixture for 22 h, it was concentrated to dryness and purified by flash column chromatography to afford **(7)** as a colorless thick oil which solidified on standing (2.22 g, 81%).

^{1}H NMR ($CDCl_3$) δ: 5.44 (dd, J = 6.60, J = 7.04 Hz, 1H), 3.72 (s, 3H), 3.70 (s, 3H), 3.25 (dd, J = 16.58, J = 7.04 Hz, 1H), 2.73 (dd, J = 16.58, J = 6.60 Hz, 1H), 1.50 (s, 18H).

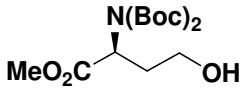
(S)-(-)-methyl-2-[bis(tert-butoxycarbonyl)amino]-4-oxobutanoate (6)



DIBAL-H (1M solution in THF, 6.6 mL, 6.57 mmol) was added to a -78 ^{o}C cooled solution of **(7)** (2.16 g, 5.98 mmol) in diethyl ether (60.0 mL) over a 10 min period under nitrogen. After having stirred for 15 min, water was added at -78 ^{o}C and the mixture was allowed to warm to room temperature. The resulting white precipitate was filtered through Celite powder and washed with diethyl ether. The filtrate was concentrated. The crude compound was purified by flash column chromatography (Ethyl acetate/petroleum ether 2:8) to afford **(6)** as a colorless viscous oil (1.63 g, 82%).

^{1}H NMR ($CDCl_3$) δ: 9.79 (m, 1H) 5.53 (dd, J = 6.90 Hz, J = 5.72 Hz, 1H), 3.73 (s, 3H), 3.42 (dd, J = 6.90 Hz, J = 13.2 Hz, J = 17.75 Hz, 1H), 2.81(ddd, J = 5.92 Hz, J = 11.7 Hz, J = 17.75 Hz, 1H), 1.50 (s, 18H).

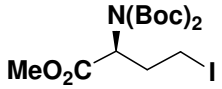
(*S*)-(-)-methyl-2-[bis(*tert*-butoxycarbonyl)amino]-4-hydroxybutanoate (10)



Sodium borohydride (0.365 g, 9.66 mmol) was added to a 0°C cooled solution of **(7)** (1.6 g, 4.83 mmol) in MeOH (24.0 mL) under nitrogen. The mixture was stirred for 45 min, quenched with water (40.0 mL) and extracted with EtOAc (3 x 30 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, filtered and concentrated. The crude compound was purified by flash column chromatography (Ethyl acetate/petroleum ether 4:6) to afford **(10)** as a colorless viscous oil (1.61 g, 100%).

^{1}H NMR ($CDCl_3$) δ: 5.00 (dd, J = 4.70 Hz, J = 9.83 Hz, 1H), 3.73 (s, 3H), 3.58-3.70 (m, 2H), 2.42 (m, 1H), 2.04 (m, 1H), 1.50 (s, 18H).

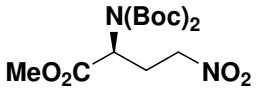
(*S*)-(-)-methyl-2-[bis(*tert*-butoxycarbonyl)amino]-4-iodobutanoate (11)



Triphenylphosphine (1.475 g, 5.62 mmol), imidazole (0.408 g, 5.99 mmol) and iodine (1.43 g, 5.62 mmol) were added sequentially to a solution of (S)-(-)-methyl-2-[bis(tertbutoxycarbonyl) amino]-4-hydroxybutanoate **(10)** (1.250 g, 3.75 mmol) in THF (20.0 mL) at room temperature under nitrogen. After stirring the mixture for 2 h, it was diluted with 20% aqueous NaCl and extracted with EtOAc (3 x 30 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude compound was purified by flash column chromatography (Ethyl cetate /petroleum ether 10:90) to afford **(11)** as a pale yellow viscous oil (1.53 g, 92%).

^{1}H NMR ($CDCl_3$) *δ*: 4.99 (dd, J = 5.58 Hz, J = 8.22 Hz, 1H), 3.72 (s, 3H), 3.24 (m, 2H), 2.77 (m, 1H), 2.39 (m, 1H), 1.50 (s, 18H).

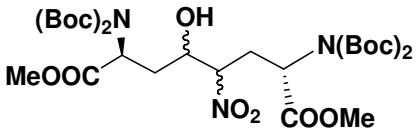
(*S*)-(-)-methyl-2-[bis(*tert*-butoxycarbonyl)amino]-4-nitrobutanoate (5)



Sodium nitrite (0.467 g, 6.77 mmol) was added to a solution of (*S*)-(-)-iodide **(11)** (1.5 g, 3.38 mmol) in DMF (18.0 mL) at room temperature under nitrogen. After having stirred the mixture for 2 h, it was diluted with 20% aqueous NaCl and extracted with EtOAc (3 x 30 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, filtered and concentrated. The crude compound was purified by flash column chromatography to afford **(5)** as a colorless viscous oil (0.674 g, 55%).

^{1}H NMR ($CDCl_3$) *δ*: 5.00 (dd, J = 5.28 Hz, J = 8.80 Hz, 1H), 4.50 (m, 2H), 3.74 (s, 3H), 2.88 (m, 1H), 2.53 (m, 1H), 1.50 (s, 18H).

(6S,11S)-dimethyl 5,12-bis(tert-butoxycarbonyl)-8-hydroxy-2,2,15,15-tetramethyl-9-nitro-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-6,11-dicarboxylate (4)



A mixture of (*S*)-(-)-methyl-2-[bis(*tert*-butoxycarbonyl)amino]-4-nitrobutanoate **(5)** (0.200 g, 0.551 mmol) and (S)-(-)-methyl-2-[bis(tert-butoxycarbonyl)amino]-4-oxobutanoate **(6)** (0.201 g, 0.607 mmol) were dissolved in CH_2Cl_2 (3 mL), and 4-(dimethylamino)pyridine (DMAP, 0.269 g, 2.204 mmol) was added at room temperature under nitrogen. After the reaction mixture was stirred for 2 days, an additional amount of CH_2Cl_2 (1.5 mL) was added, and the mixture was stirred for 2 days. The crude reaction mixture was then directly purified by silica gel column chromatography to afford of _-hydroxy nitro compound **4** (0.110 g, 28.7%) as a mixture of diastereomers. A mixture of reactants was also obtained.

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