FLAVANOIDS FROM *PRUNUS AVIUM* AND SYNTHESIS OF POLYHYDROXYLATED PYRROLIDINES AND ANILINES

ISOLATION OF FLAVONOIDS FROM *PRUNUS AVIUM* AND SYNTHESIS OF POLYHYDROXYLATED PYRROLIDINES AND ANILINES AS POTENTIAL ANTIBACTERIAL AGENTS

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ABSTRACT

This thesis describes the isolation and structural determination of flavonoids from the heart wood of *Prunus avium* as well as synthesis of polyhydroxy pyrrolidine derivatives and aniline core structures as potential antibacterial agents. Nitrogen-synthons containing saturated heterocyclic systems and aniline core structures are important synthons in organic chemistry because of their presence in many biologically-active natural products. Mycobacterial viability is dependent upon the ability of the organism to produce an intact cell wall. Therefore, compounds that interfere with the biosynthesis of the cell wall complex glycons have the potential to become new drugs for the treatment of mycobacterial infections. The oligosaccharide galactan is one of the major structural components of the outer wall of the micro-organism. Galactofuranose is essential for cell growth and survival and therefore, its biosynthesis constitutes a new drug target. The biosynthetic process involves several enzymes having Uridine-diphosphogalacto furanose (UDP-Gal$f$) as the substrate; uridine 5^1-diphosphogalacto pyranose mutase which catalyzes the interconversion of UDP-galacatopyranose to UDP-galactofuranose as well as Gal$f$-transferase. We are seeking and designing molecules that could be mechanistic probes and/or inhibitors of efflux pumps to potentially combat multidrug resistance.

The isolation and structure-determination of six naturally occurring Flavan-type Natural products was performed. Such derivatives are known to reverse multiple-drug-resistance (MDR) in persistent microbial infections. The synthesis of pyrrolidine-based antibacterial agents was attempted using two different approaches from tartaric acid.

These derivatives were designed as potential transition-state mimics of a carbohydrate processing enzyme specific to TB. A synthetic approach to the aromatic core structure of the antibacterial agent Platensimycin was also investigated. The synergistic use of cytotoxic agents in conjunction with efflux-pump modulators is an emerging area of research in the MDR field; our efforts to make available materials for high-throughput screening in this area will be described.

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TABLE OF CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

Ac	acetyl
COSY	Correlation Spectroscopy
BOC	t-butyloxy carbonyl
Bu	butyl
CDCl$_3$	deuterated chloroform
CD$_3$OD	deuterated methanol
DCM	dichloromethane
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
Et	ethyl
EWG	electron withdrawing group
FTIR	Fourier Transform Infrared
HMBC	Heteronuclear Multiple Bond Correlation
KHMDS	Potassium bis (trimethylsiyl) amide
LiHMDS	Lithium bis (trimethylsiyl) amide
LiAlH$_4$	Lithium aluminium hydride
MDR	multidrug-resistance
MS	Mass Spectrometry
NaHMDS	Sodium bis (trimethylsiyl) amide
Na$_2$SO$_4$	sodium sulphate
NMR	Nuclear Magnetic Resonance
OPA	Oxaphosphetane
OMes	Methane sulfonyloxy
Pd/C	Palladium on carbon
PhMe	toluene
TBDMS	tertiary-butyldimethyl silyl
TBAB	tetrabutylammonium bromide
THF	tetrahydrofuran
TEA	triethylamine
TLC	thin layer chromatography

INTRODUCTION

Infectious diseases are as old as life itself, for example the battle with the pathogen *Mycobacterium tuberculosis*, the causative agent of Tuberculosis (TB), dates back to antiquity and this organism has resulted in over 1 billion human deaths in the last two centuries.[1a] In the case of such bacterial infections, a major breakthrough occurred in 1929 when Fleming observed that the growth of a *Staphylococcal* bacterial colony was inhibited when serendipitously contaminated with adventitious colonies of the *Penicillium notatum* mold.[1b] The accelerating pace of antimicrobial drug discovery since the 1930's has resulted in thousands of small molecules being screened for activity. These compounds range from natural products and their synthetic analogues to purely synthetic compounds. More recently the techniques of combinatorial chemistry have pushed the synthetic number considerably higher. Over the last decade it has become clear that the traditional approach undertaken in the discovery and development of chemotherapeutic treatments of various human diseases suffers from an underlying fundamental problem. This approach has centered upon the identification of an active "lead" compound followed by structure-activity studies to optimise the features of the small molecule against the target of interest. The optimised structure, as a single chemical entity (SCE), progresses from pre-clinical testing and further onwards into the clinical pipeline. Providing that the overall pharmacological profile is met, the SCE enters general usage as a treatment for the particular ailment. Such is the case in the development of antibacterial, anticancer, antiviral, antifungal and antiparasitic agents.

In all of these cases, the fundamental problem that arises over a more or less short period of time is the phenomenon known as multiple drug resistance (MDR). MDR can be defined as the ability of cells to develop resistance to multiple structurally and functionally non related drugs.[2] Such development renders the initially potent molecule (and along with similar structures) much less effective in treating the affliction. Thus, this is a very generic problem that has significance across a wide range of areas in the development of new chemotherapeutic treatments. Several mechanisms are known to be responsible for inducing MDR in the cell. The mechanisms include reduced uptake and/or increased efflux of the drug from the cell, modification of the drug target, inactivation of the drug by modification or conjugation, over expression of the drug target, and biosynthesis of a related drug resistant enzyme. The uptake of a drug into a cell is reduced as a consequence of a change in the structure of the cell membrane, which includes the loss (or mutations) of porins in the cell membrane. The outer membrane of a cell contains proteins called porins, which allow the influx of nutrients by providing channels across the membrane. The loss, downsizing, or mutation of porins constitute another mechanism through which bacterial cell permeability is lowered and resistance is provided.[3]

The most important mechanism for MDR is the activation of the so-called "efflux pumps". Active drug efflux systems in bacteria can be divided into four families on the basis of mechanism, supramolecular assembly, and sequence of homology. 1. Major facilitator family (MF). 2. Resistant modulation division family, which includes transporters that pump out Cadmium, Cobalt or Nickel cations.[3] Staphylococcal multi-drug resistant family (Smr), consisting of small transporters that contain four trans-

membrane helices. 4. Adenosine triphosphate-binding cassette (ABC) transporter family.[4]

Drugs that have been shown to be susceptible to MDR do not share common mechanisms of action; for example some affect microtubules, some inhibit DNA, RNA or protein biosynthesis, and others may be more specific enzyme inhibitors. This type of resistance was identified from drug transport studies showing lower drug accumulation in MDR cells than in similar untreated cells. Thus, subsequent treatments with the drug would prove to be less effective. Clinical examples are well known in treating bacterial and viral infections as well as cancer. Drug entry appears to be normal but the cells have acquired the capacity to recognise and pump out the drug. The transporters are GPCR (G-protein-coupled receptors) trans-membrane proteins involved in binding and transporting small molecules across the plasma membrane. These proteins include the now well studied P-glycoprotein (Pgp) and multiple drug resistance proteins (MDRPs) of which there are now more than seven types recognized (i.e. MDRP 1 to MDRP 7).[5,6] These proteins are ATP-dependent, belonging to the ABC transporter family defined above. They contain ATP and small molecule binding domains[7] and utilize the energy liberated from ATP to efflux the drugs out of the cell. When a foreign body binds to the activated protein a conformational change in the backbone causes the body to be transported across the membrane and out of the cell. These proteins are "normal" efflux pumps, since they are not the result of oncogene expression, for example, they reside across the cell membrane but normally are only present in limited amounts. The normal physiological functions of these protein pumps are assisting the efflux of potentially toxic molecules out of the cell and they are consequently over expressed in the liver and other such tissue.[8]

MDR may be classed as either intrinsic or acquired. There may be *intrinsic* resistance to a particular SCE penetrating certain tissue upon an initial dosage resulting in its rapid net efflux, such as in the blood-brain barrier. Intrinsic MDR is caused by a synergy between a low permeability in the outer membrane and the expression of a number of broadly specific multidrug efflux systems.[9, 10] A SCE that enters the cell is recognized as a foreign body and in this way may be effluxed as part of the normal function within the host cell. In addition, *acquired* resistance is the major problem, resulting in overexpression of the genes encoding the particular efflux pump which leads to a decrease in the active concentration of the SCE in the cell during prolonged treatment, as is required in many chemotherapeutic regimens. The SCE concentration inside the cell would eventually reach some equilibrium value as a consequence of the normal diffusion into the cell and the efflux pushing out. The acquired resistance problem arises when the host cell, having become sensitized to the presence of a particular foreign body (for example delivered as a SCE) over expresses the Pgp or MDRP genes. In bacteria, acquired resistance manifests itself through incorporation of plasmids that encode for such proteins from other bacteria or by mutation. Other molecules structurally related to the SCE can also be effluxed in this manner when the cell is sensitized to the original SCE. The resulting higher concentration of the efflux pump in the membrane tends toward net efflux of the SCE and therefore a lower overall effective concentration of the SCE is seen within the cell. Several elegant experiments have documented and confirm the above descriptions. As an example, chemotherapy (e.g. taxol) frequently fails in cancer patients due to the presence of acquired multidrug resistance making subsequent treatment less effective. Unfortunately the first generation antibacterial drugs

such as Verapamil or Cyclosporine A are successful in vitro as patients could not benefit from these chemosensitizers, due to their clinically relevant doses exceeding safety limits and resulting in adverse effects such as toxicity.[11] The search for effective efflux pump modulators or inhibitors is now a very active area of research. In the course of these discovery efforts, we have observed that certain classes of active natural products continually prove to be valuable. These have included flavonoid derivatives, diterpenes and alkaloids incorporating an isoquinoline core.

In addition, one of the key issues that characterize the current state of MDR drug discovery is the ongoing race in academic and corporate labs to solve the MDR problem. Inhibition of efflux pumps is expected to decrease the level of intrinsic resistance, and significantly reverse acquired resistance.[12] In addition, it is widely accepted that the increased availability and use of antibacterial and antifungal agents in recent years has resulted in the control and even eradication of diseases, but that it has also led to the development of resistant strains to these drugs. Much effort is being expended to not only develop new drugs faster than resistance develops, but also to understand the basics of resistance itself.

Over the past few years work in the McNulty laboratory has become highly focussed as a result of the above fundamental problem. In conjunction with our interest in natural products we are developing a new concept based upon the screening of dual chemical entities (DCEs) as a platform for the discovery of potential chemotherapeutic treatments, which we have branded SYNPICT (Synergistic Natural Product Inspired Combination Therapy). Synergy is a popular concept in the field of herbal medicine. Two different compounds can act in synergy, with one compound disabling the resistance

mechanism and potentiating the antimicrobial or anticancer activity. This platform involves the identification of a cytotoxin (anticancer, antibacterial agent) in conjunction with an efflux pump inhibitor. The synergistic effect of these DCEs should allow for identification of effective treatments that are not as susceptible to the development of MDR.[13]

Examples of such singly paired synergistic effects are already well documented in the literature. It has been shown that the antibacterial alkaloid berberine, in conjunction with a flavanoid, namely Nor A efflux pump inhibitor (EPI), restores antibacterial activity against MDR *S. aureus*.[14] In addition, the use of Pgp inhibitors co-administered with anticancer drugs such as flavopyridol and doxorubicin, administered to MDR cancer cells restores high levels of cytotoxicity again.[7,10] The general platform that we are developing is shown schematically below and involves the use of a cell-based (MDR bacteria or human tumor cell lines) 96-well plate MTR-type assay (Fig. 1.1). The general concept is also applicable to higher density assay systems.

CY = Cytotoxin; e.g. beriberine, methicillin, etc.
EP = Efflux pump inhibitor; e.g. flavonoids

0	CY 11	CY 10	CY 9	CY 8	CY 7	CY 6	CY 5	CY 4	CY 3	CY 2	CY 1	
												EP 1
												EP 2
												EP 3
					X							EP 4
												EP 5
												EP 6
												EP 7
												0

96 well cell based screen MRSA

Fig. 1.1. MTR type Assay

At present, the short-term goal is to identify 11 cytotoxins and 7 efflux inhibitors in order to conduct a proof-of-principle assay of the new SYNPICT forum. Thus in one plate, all possible 77 combinations of cytotoxin/EPI's would be assessed for synergy in addition to single and double blank controls. We hope that this will evolve to a new general strategy for the discovery of new synergistic chemotherapeutic treatments that will allow the rapid screening of many different pairs of compounds against any whole cell target.

The ever expanding literature on both cytotoxic compounds and the various classes of EPI's led us to consider sources of naturally occurring flavanoids as the EPI dimension in this program. The potential benefits of broad-spectrum efflux pump inhibitors prompted us to screen natural products and synthetic libraries to search for potential EPI's. The goal therefore became the isolation of up to 7 different flavanoids of various structures as one part of this screening. We are also considering a new general synthesis of a combinatorial library of flavanoids (which will not be discussed in this thesis) given the seemingly ubiquitous appearance of flavans as EPI's in the recent literature. Such compounds have been shown to restore activity of cytotoxins to both MDR antibacterial and drug resistant tumour cell lines.

CHAPTER 1. Isolation of Flavanoid

Natural Products

1.1. Isolation of Flavanoids from *Prunus avium:*

The first part of this thesis details our undertakings in the isolation and structure determination of such a family of compounds from the bark and heartwood of sweet cherry *Prunus avium*, a known source of flavonoids. The second dimension of the project involves the identification of up to 11 different cytotoxins, again either as natural products or synthetic derivatives. The second part of this thesis details our approach to the synthesis of a family of potential cytotoxins in the antibacterial arena. These cytotoxins should be selective to prokaryotes over higher order organisms as they are designed to inhibit the oligosaccharide biosynthetic pathway unique to these organisms (such as *Mycobacterium tuberculosis*). New chemical entities with novel mechanisms of action will most likely possess activity against MDR pathogens. However, these entities alone will not provide the breakthrough that is needed. The key to improving therapy is to develop new agents in combination with potent sterilizing activity that will lead to a shortening of the duration of chemotherapy and counteract the tendency towards MDR.

Plants are one of the most important sources of biologically active compounds. From the dawn of civilization men have been utilizing plants for their personal benefits. Several active constituents of plants are associated with interesting medicinal and pesticidal properties. With advances made in natural product chemistry it has now become possible to identify the bioactive molecules from different plants. Ancient Asian traditional systems of medicine include Ayurveda, Siddha, and Unani. These traditional systems of medicine use mostly plant extracts which are available in the local regions to cure several diseases. The chemistry of these medicinal plants has been a subject of research for many organic chemists. It is therefore necessary to study the past

literature of any medicinal plant before undertaking a chemical investigation. A literature survey can be undertaken either from its botanical name or on its main active principle.

Phenolic constituents of higher plants have been shown to be potentially useful markers in botanical research. The large diversity of these compounds, especially the flavonoids, make them particularly suitable for taxonomic studies in spite of their within-plant variation and their susceptibility to environmental and developmental change. The flavonoid pigments, one of the most numerous and widespread groups of natural constituents, is of interest to a wide variety of physiological and biological scientists. These scientists work on flavonoid chemistry, occurrence and natural distribution but studies of flavonoid biological function also continue unabated. The study of flavonoid chemistry has emerged, like that of most natural products, from the search for new compounds with useful physiological properties. In addition to the multitude of industrial applications, the oligo- and polymeric proanthocyanidins are now also credited for the profound health-promoting effects of tea, fruit juices and red wine. This is mainly due to their in vitro antioxidant and radical scavenging properties, while the polyflavonoids in red wine have recently been implicated in protection against cardiovascular disorders.[15-19]

It has been observed that *Prunus avium L.* is resistant to bark canker induced by the fungus. This observation led us to study the flavonoid pattern of the heartwood of this species.

1.2. Results:

Isolation and determination of flavonoids from the heartwood of *P. avium*.

The heartwood of *Prunus avium* was obtained from a felled tree and air-dried chips (10 g) were ground and extracted at room temperature (rt) overnight with chloroform (75 mL) and then for 24 h with methanol (75 mL). The chloroform solvent was evaporated under reduced pressure to yield a suspension which was applied to a gravity Silica column and eluted with an *n*-hexane/ethyl acetate gradient. The purities of compounds were verified by thin layer chromatography (TLC).

Scheme: 1.1:

Prunus avium (Heartwood)

CHCl₃

MeOH

Tectochrysin

Sakuranetin

Dihydrowagonin

Naringenin

Dihydrokaempferol

Catechin
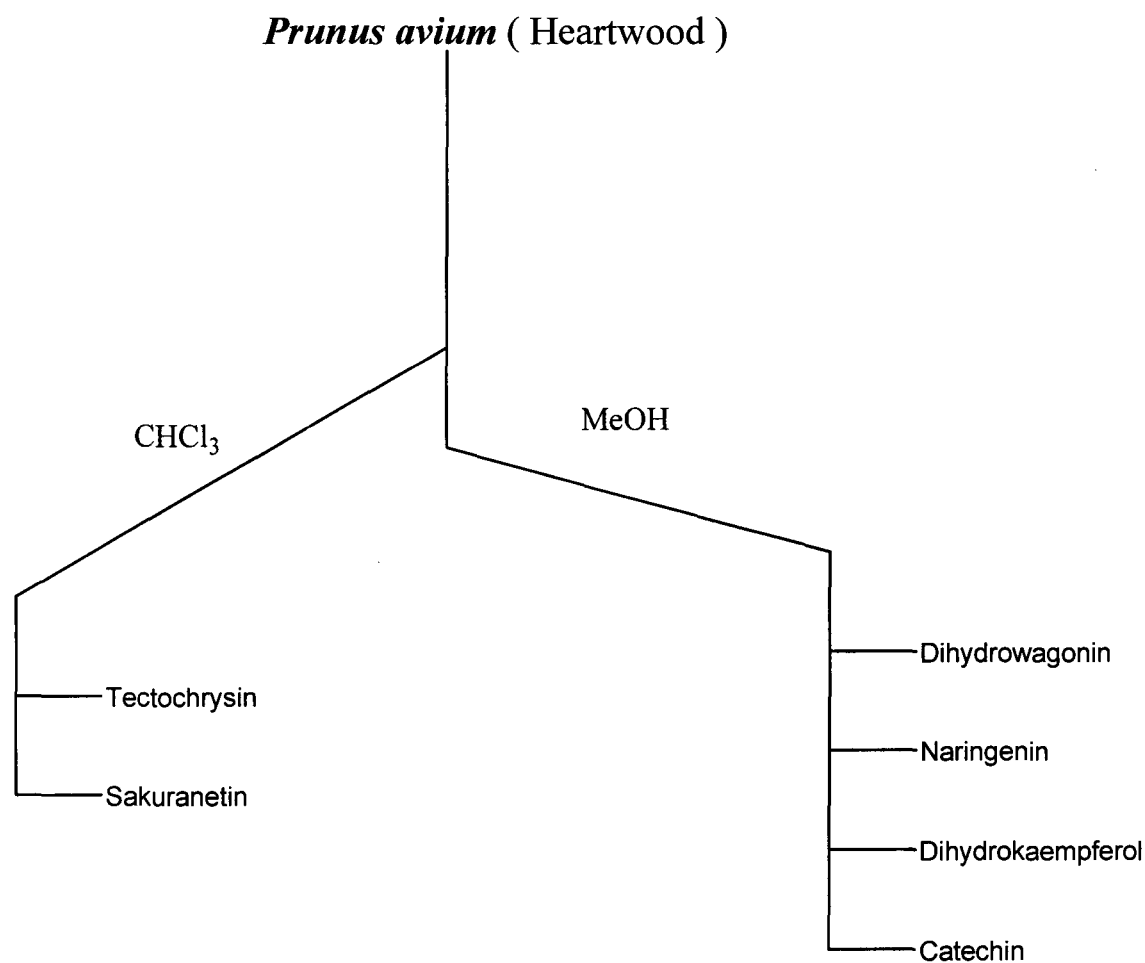
The compounds outlined in scheme 1.1. have previously been isolated from different plant sources and some of these compounds exhibit biological activity. Table 1.1 below shows the compounds isolated, as % dry weight. Structures of all these compounds were confirmed by NMR, Mass, and IR spectroscopy. The chirality of these compounds was confirmed by optical rotation and the data is compared with literature values (see experimental).
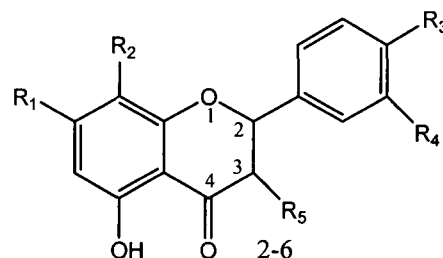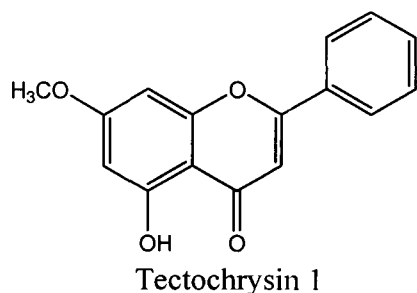
Tectochrysin 1

Table. 1.1

Compound	Isolation	Biological activity	Weight	% dry weight
Tectochrysin	*Prunus cerasus*	Anti oxidant	2 mg	0.02%
Sakuranetin R_1 R_2 R_3 R_4 R_5 OCH_3 H OH H OH	Leaves of *Ribes nigrum*	Anti microbial	5 mg	0.05%
Naringenin OH H OCH_3 H H	*Choerospondias axillaris*	Anti inflammatory	2 mg	0.02%
Dihydrowagonin OCH_3 OH H H H	Leaves of *Prunus padus*	Anti fungal	3 mg	0.03%
Catechin ($C_4 = CH_2$) OH H OH OH OH	*Green tea*	Anti tumor	10 mg	0.1%
Dihydrokaempferol OH H OH H OH	*Riesling wine*		3 mg	0.03 %

1.3. Structural interpretation of Sakuranetin (4):
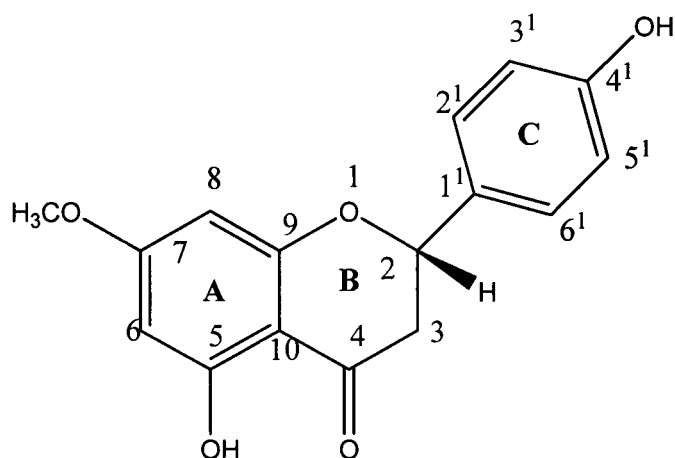
4. Sakuranetin

The ^{1}H NMR spectrum of Sakuranetin showed signals characteristic of a flavanone; a signal at δ 5.35 for H-2 as a doublet of doublets with coupling constants 13.2 Hz and 3.0 Hz with H-3$_{trans}$ and H-3$_{cis}$ respectively. The H-3 protons each presented a doublet of doublets at δ 3.09 (H-3$_{trans}$) and 2.78 (H-3$_{cis}$) with coupling constants of 13.2 and 17.4 Hz for H-3$_{trans}$ and 3.0 and 17.4 Hz for H-3$_{cis}$. The substitution at 4^1 of ring B was confirmed by the presence of two doublets each integrating for two protons at δ 7.37 and 6.88, corresponding to H-2^1, H-6^1 and H-3^1, H-5^1 respectively; with a coupling constant of 8.4 Hz. At δ 6.15 a doublet appeared corresponding to two protons, H-6 and H-8 with a coupling constant of 2.4 Hz, indicative of meta coupling.

The ^{13}C NMR spectrum showed a signal due to the methoxy group at δ 56.2 and signals at δ 44.0 and 80.6 characteristic for C-3 and C-2 respectively. This spectrum also had a signal at δ 198.2 for the carbonyl group and four oxygenated aromatic carbons (δ 159.1, 164.7, 165.2, 169.5) corresponding to C-4^1, C-9, C-5 and C-6.

The mass spectrum had the molecular ion peak M+1 at m/z 287 corresponding to $C_{16}H_{14}O_5$ as the base peak. The fragments at m/z 193, 180, 167 confirmed the structure of ring A with one hydroxy and one methoxy group. The fragment ion at m/z 120 is typical of a tropylium ion.

The COSY spectrum showed H-3_b and H-3_a correlating with H-2. H2[1], H6[1] at (δ 7.37) and H-3[1], H5[1] at (δ 6.88) correlation was also established between H-5[1] and H-6[1] as well as H-2[1] and H-3[1].

The HSQC spectrum connected H-3_b (δ 3.09) and H-3_a (δ 2.78) to C-3 (δ 44.0) as well as H-2 (δ 5.3) to C-2 (δ 80.6).

The HMBC spectrum revealed two-bond correlation between H-3_a (δ 3.09) and H-3_b (δ 2.78) to C-4 (δ 198.2). Similarly, a two-bond correlation between H-2 (δ 5.35) and C-1[1] (δ 130.2) as well as a four-bond correlation between H-2 to C-3[1] and C-5[1] were identified. Based on this structural identification of sakuranetin (±), all the other flavonoid structures were elucidated.

1.4. Conclusions:

A common misconception has been that natural product research has not kept pace with other drug discovery techniques and as a consequence, has become uncompetitive for lead discovery. However, improvements in instrumentation, robotics and bioassay technology have increased the speed of bioassay-guided isolation and structure elucidation of natural products considerably and these improvements have allowed natural product research to be more competitive with synthetic compound screening. Another misconception has been that natural product research has failed to deliver many new compounds that have undergone clinical evaluation over the last few

years. However, in reality, 15 natural product derived drugs have been launched in the key markets of the USA, Europe and Japan over the past 6 years; additionally, some 15 natural product derived compounds were in phase III clinical trials at the end of 2003. These natural product drugs also contribute considerably to the profitability of many pharmaceutical and biotech companies. These factors and others, such as the inadequate number of lead compounds in many therapeutic areas and the unique chemical space occupied by natural products, have led to a renewed interest in natural product research. However, this renewed interest can be sustained only if natural product research can continue to be competitive with other drug discovery techniques. Key factors for remaining competitive include continual improvements in isolation, structure elucidation, the speed of dereliction, the compound supply process and prudent selection of drug targets for the screening of natural product libraries.

In summary, several flavonoids have been isolated from *Prunus avium*. It is to be hoped that a therapeutically useful drug will soon emerge from the clinical assays currently in progress.

CHAPTER 2. Synthesis of Potential

Selective Glycosidase Inhibitors

2.1 Synthesis of polyhydroxylated pyrrolidine alkaloids as potential glycosidase inhibitors

The great advances in antibacterial chemotherapy since the introduction of sulphanilamides and then penicillin have been created in large part by the pharmaceutical industry. The industry has an impressive track record of successful product introductions that are in response to increasing medical need and to increasing resistance of bacteria to both individual classes of antibiotics and especially across different classes[23]. The successful identification, research and development of penicillins, cephalosporins, carbapenems, macrolides, glycopeptides, amino glycosides and quinolones has relied heavily upon the scientific ability, technical expertise, investment, planning, project-managing abilities and courage of the people in the pharmaceutical industry, as well as others in academia. Antibiotics have proved remarkably effective in controlling bacterial infections.[24] The companies that continued to work in the field of antibacterial research found it increasingly difficult to identify new compounds with known existing modes of action that could be progressed commercially because their advantages over existing, increasingly generic, antibiotics were largely incremental. The introduction of totally new antibiotics with novel mechanisms has been sporadic over the past 15 years. The emergence of multidrug resistant (MDR) pathogens has changed this view considerably over the past decade and new strains of common pathogens such as *S. aureus, S. pneumoniae* and TB resistant to last line of defence agents, such as vancomycin and methicillin, have emerged.

Glycosylation reactions conducted by glycosyltransferases are fundamental biological processes for the modification of lipids and proteins. In addition to

greatly influencing the physical properties of these constructs, glycosides are crucial to various molecular recognition phenomena including those involved in bacterial and viral recognition and infection, cell adhesion in inflammation, the immune response, cellular differentiation, development, regulation, intercellular communication and signal transduction to name a few. Interference with such processes could therefore have disastrous consequences in the normal life-processes of the cell. Of relevance also is the fact that pathogenically relevant prokaryotes such as *Mycobacterium tuberculosis* and *S. aureus* utilize monosaccharide building blocks that have little relevance to the metabolics of higher order mammalian cells. Interference with the construction of such glyco-conjugates therefore in theory offers the potential for the development of selective cytotoxins. D-Galactans are an example of such a construct that are key components of the mycobacterial cell wall.[25] The main building blocks are D-galactofuranose monosacharides which have no relevance in mammalian cellular metabolism.

2. 2. The resistance problem

The use of antimicrobial drugs for prophylactic or therapeutic purposes in humans, or for veterinary or agricultural purposes, has provided the selective pressure favouring the survival and spread of resistant organisms[25]. As a result of more intensive antibiotic use in hospitals as compared with the community, higher rates of resistance are noted in hospital pathogens, especially in the intensive care unit (ICU) where infections caused by Gram-positive bacteria are increasing. Over 50% of hospital bloodstream infections are currently caused by *Staphylococcus aureus*, coagulase-negative staphylococci and *Enterococcus* species, with methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE) causing particular problems.

The syntheses of aza-sugars, sugar mimics in which the ring oxygen has been substituted by a nitrogen, have been the subject of much continued interest over the last 25 years;[26] they have proved to be highly potent enzyme inhibitors, especially of carbohydrate-processing enzymes, and have been used as invaluable probes of the nature and mechanism of action of many enzymes.[27] Most syntheses of aza-sugars have focused on logical designs based on the stereochemistry of the functional groups around the heterocyclic ring of the putative carbohydrate mimic, and this approach has yielded potent inhibitors. For example, deoxynojirimycin (DNJ), the direct configurational counterpart of 1-deoxy-D-glucopyranose is a potent β-glucosidase inhibitor.[28] Interestingly; however, this type of approach does not always result in a high amount of inhibition.

Glycosidases and glycosyltransferases belong to an important group of enzymes involved in the biosynthesis and processing of the glycoconjugate components of all organisms.[29] The discovery of specific inhibitors of these enzymes has allowed for important advances in the control of cellular functions. Some inhibitors of glycosidase and glycosyltransferases have shown promising chemotherapeutic applications against diabetes,[30] cancer,[31] and viral infections including AIDS.[32] Many glycosidase inhibitors mimic the configuration, shape, and charge distribution of the cation liberated during the enzyme-catalyzed processes. Among the most powerful glycosidase inhibitors are 1, 5-dideoxy-1, 5-iminoalditols,[33] which are protonated under physiological conditions. 1,4-Dideoxy-1,4- iminoalditols are also an important class of glycosidase inhibitors, although their higher conformational flexibility reduces, in some instances, their selectivity. Imino- *C*-disaccharides have emerged as a possible solution to increase selectivity in

enzyme inhibition because they contain not only the information of the glycosyl moiety that is cleaved during the enzymatic hydrolysis, but also that of the aglycon.[34] Several approaches, which generally imply a coupling reaction between a pyrrolidine or piperidine moiety and a sugar compound or a precursor, have been attempted in order to synthesize the above compounds On the other hand, homochiral polyhydroxylated pyrrolidines and piperidines joined to polyhydroxy chains through C-C links (iminoalditols) can be considered as acyclic imino-*C*-disaccharide analogues and therefore, are potential specific inhibitors of glycosidases. A number of iminoalditols with short-polyolic side chains have been described and shown to be inhibitors of glycosidases; Fig. 2.1. e.g., iminohexitols such as 1, 4-dideoxy-1, 4-imino-D-mannitol (7) [36], -D-talitol (8) [37], and -L-allitol (9)[38] are inhibitors of R-mannosidases, and the related 1, 4-dideoxy-1, 4-imino- L-iditol (10) is a potent inhibitor of R-D- galactosidases. Iminoheptitols such as 1, 4-imino-L-*glycero*-D-*ido* (11) and 1, 4-imino-L-*glycero*-D-*gluco*heptitols (12)[39] or 2, 5-imino-L*glycero*- L-*gluco*heptitol (13) and -D-*glycero*-D-*manno*octitols (14)[40] have proved to be good and specific inhibitors of R- and α-D-glycosidase. Several 1, 5-dideoxy-1, 5-iminoheptitols[41] and 1, 5-dideoxy-1, 5-imino-octitols[42] have been synthesized and evaluated as glycosidase inhibitors. Most of them showed weak or a complete lack of inhibition toward glycosidases, except 1, 5, -Dideoxy-1, 5-iminooctitol (15), [42] which was found to be a strong inhibitor of yeast R-glucosidase.
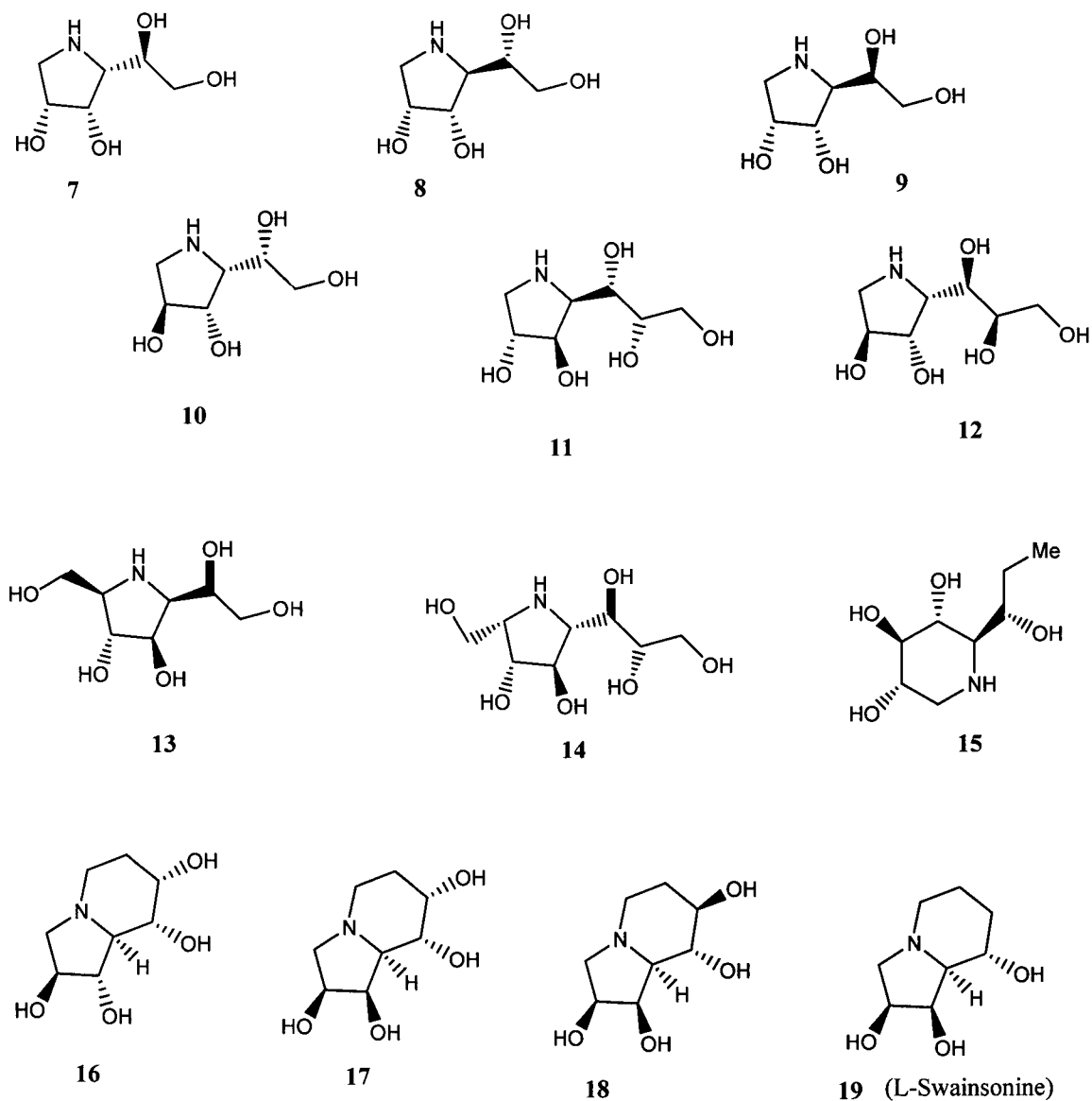
Fig. 2.1

The need for precise probes of carbohydrate-processing enzymes continues to build enormously. The burgeoning field of chemical genetics[43] demands broad-ranging probes and inhibitors of key enzymes to provide information on central biosynthetic glycosylation processes, often where little or no functional or structural information on putative protein targets exists.

As a solution to the need for more varied potential carbohydrate-processing probes, we have sought to design a general method that will allow the generation and screening of a broad range of potential inhibitors. This method retains three key structural features that have typically generated inhibitors: a five-membered ring with a nitrogen heterocyclic core, polyhydroxylation and a hydrophobic group at or near putative aglycone binding sites. By varying hydroxyl stereo chemistries and functional groups within this central aza-sugar scaffold design we sought to investigate their potential for inhibition of a number of carbohydrate-processing enzymes. This required a synthetic approach that would lend itself easily to the synthesis of diverse arrays of aza-sugars and allow the potential introduction of a wide range of substituents to an aza-sugar scaffold.

The iminosugars have been designed and synthesized as potential inhibitors of glycosidase, which is involved in the biosynthesis of bacterial cell walls. Imino sugars are analogues of monosaccharides where the ring oxygen has been replaced by a nitrogen to constitute a class of promising inhibitors of carbohydrate enzymes. In particular, they have been demonstrated to be potent inhibitors of glycosidases and glycosyltransferases. Based on glycosyltransferase studies a possible transition state structure for the transferase reaction is proposed Fig. 2.2[43a].
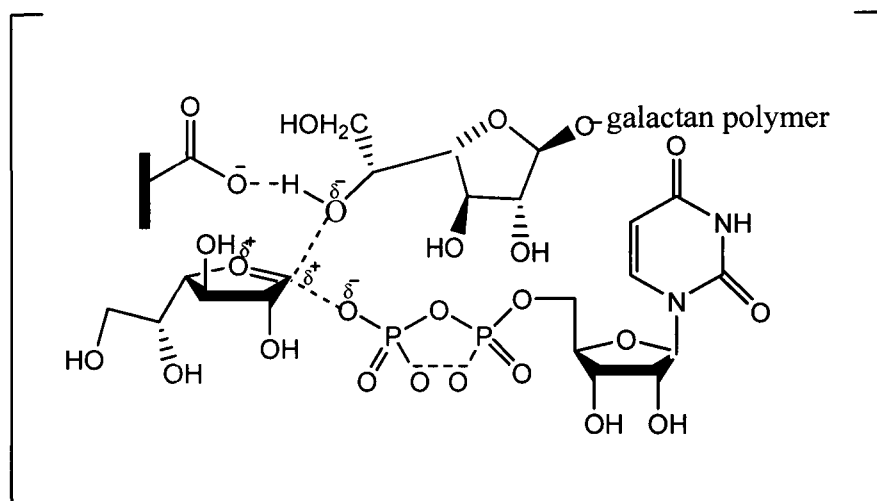
Fig. 2. 2. Proposed transition state for UDP-Gal*f* transferase reaction.

There are two different approaches to mimic the transition state with imino sugars. The ring oxygen of galactofuranose can be replaced by a nitrogen atom, which by protonation could mimic the oxocarbenium ion-like transition state. Alternatively, the anomeric carbon could be replaced by nitrogen and the ring oxygen by a methylene group to give a 1-*N*-imino sugar. It is believed that these compounds could inhibit monosaccharide enzymes, which are involved in the biosynthesis of bacterial cell walls.[43b]

A key challenge to the antibiotics industry is that constant innovation is necessary not only because of resistance, but also because of side effects. For example, erythromycin is a well-known macrolide antibiotic used as an alternative for patients who are allergic to penicillins.[44] The development of these analogs was driven essentially by the need for drugs with reduced side effects. It is often possible to develop multiple analogs of successful antibiotics that achieve the same results with less adverse side effects. In considering antibiotic development and application, it is important to look at

the larger context of the total clinical picture, as antibiotic treatment of infection is only one of many issues. A direct corollary of this is that interference between drugs used for different purposes becomes a major problem, in the body. As a result, every new antibiotic is developed while taking into account as many scenarios as possible where it would be used in the presence of other drugs. [45]

2.3. Results:

Scheme 2.1:

First approach:

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In our synthesis, we followed two approaches. The first approach commenced with commercially available L-(-)-tartaric acid (25) which was converted into the corresponding dimethyl-2,3,O-isopropylidene tartrate (26).[46] L-tartaric acid was refluxed with 2,2-dimethoxypropane in the presence of p-toluenesulfonic acid to yield the corresponding isopropylidine dimethyl ester in one pot conversion with excellent yield. Subsequently lithium aluminium hydride reduction in diethyl ether gave L-threitol acetonide (27),[47] which was treated with sodium hydride in THF and *tert*-butyldimethylsilyl chloride at rt to afford the monosilylated derivative (28).[48] Swern oxidation of (28) in the presence of oxalyl chloride–DMSO in dry CH_2Cl_2 at -78 °C, followed by treatment with triethylamine, gave aldehyde (29) (76%) which was immediately homologated with the lithium salt of trimethoxy phosphonoacetate in THF at -78 °C to provide (30) in 72% yield. I tried with different reagents under different reaction conditions (1.Benzyl amine, DCM, RT-50 °C, 2. benzyl amine, no solvent, RT-65 °C, 3. NH_3, EtOH, 0 °C-RT) to afford the desired cyclized product (33). I couldn't get the cyclized product. We believe that stereochemistry at the carbons 3 and 4 is playing a key role in preventing the cyclization.

SCHEME 2.2:

Second Approach:
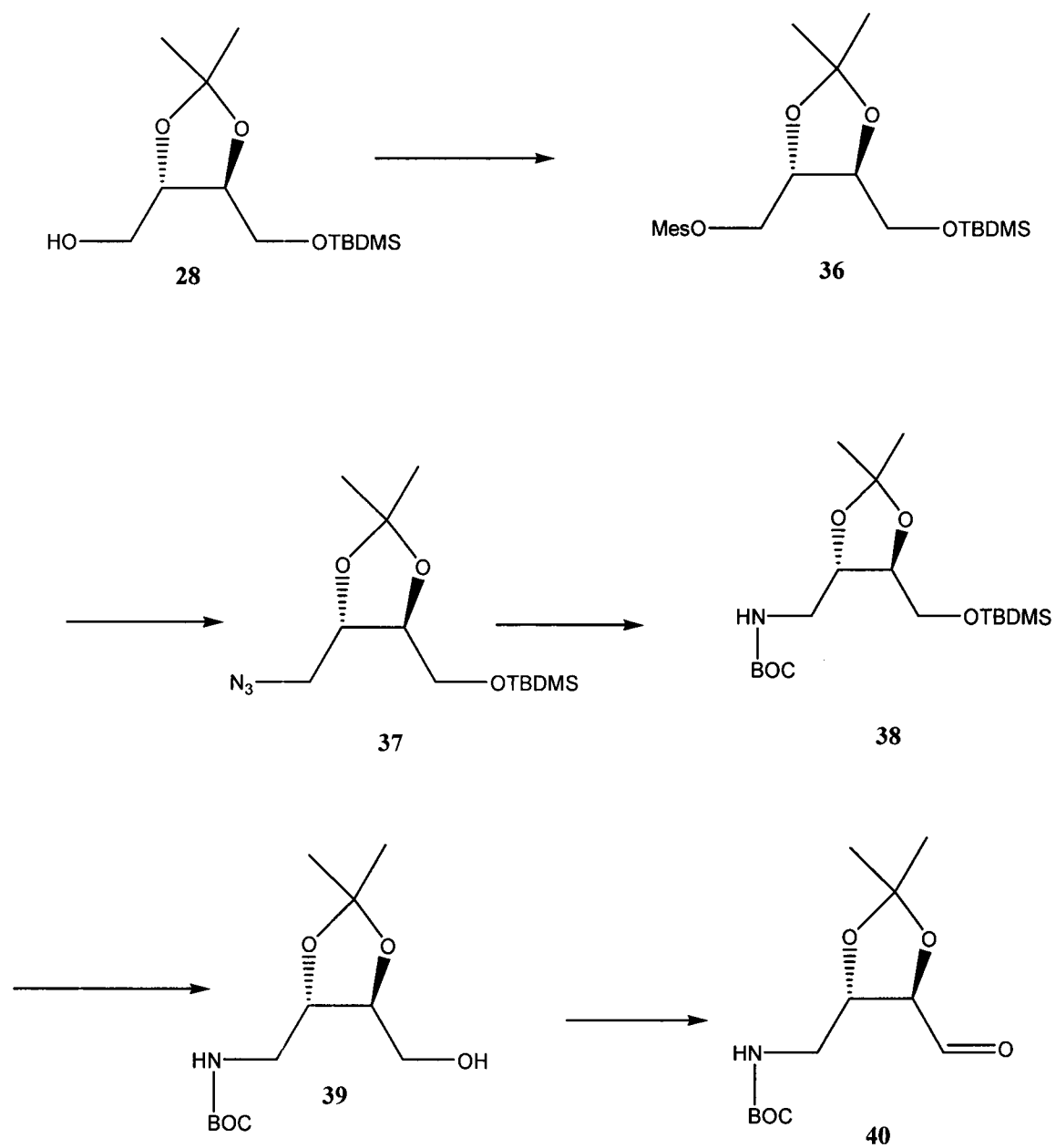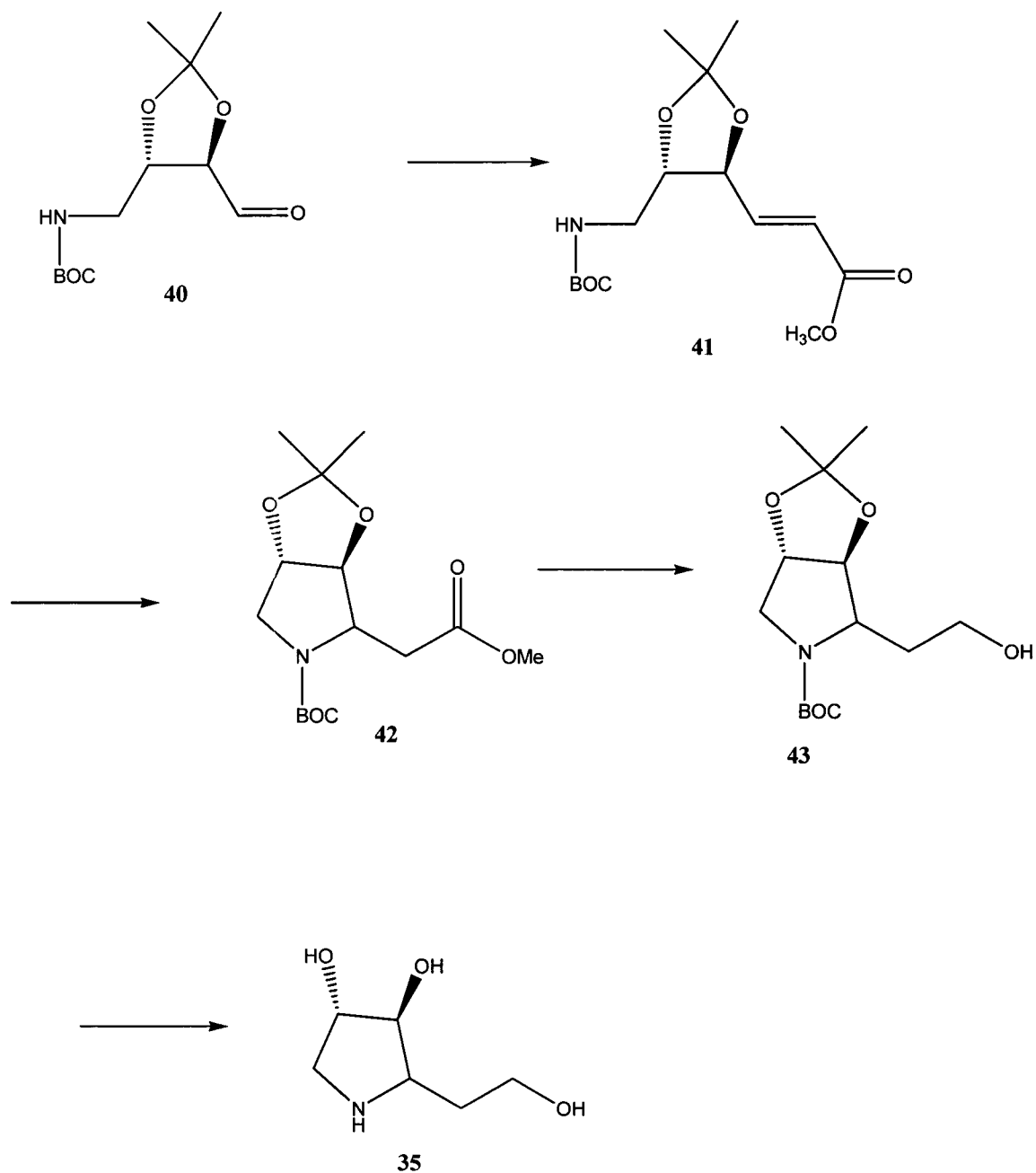
The second approach commenced with alcohol **(28)** which was taken from the first approach and it was treated with methane sulfonyl chloride and triethyl amine in DCM at RT to afford compound **(36)** in good yield **(88%)**. Compound **(36)** was then treated with sodium azide to give compound (37). Catalytic hydrogenation of azide **(37)** with 10% palladium on charcoal in ethyl acetate with Boc anhydride furnished Boc-

protected amine **(38)** in a single step. Subsequent removal of the TBS group with TBAF

in dry THF at RT provided alcohol **(39)** in 82% yield, thus releasing the hydroxy function

to be used for the final chain elongation. Swern oxidation of **(39)** in the presence of

oxalyl chloride–DMSO in dry CH_2Cl_2 at -72 °C, followed by treatment with

triethylamine, gave aldehyde **(40)** (76%) which was immediately homologated with the

lithium salt of trimethoxy phosphonoacetate in THF at -72 °C to provide **(41)** in 72%

yield. Then I have tried with different reagents under different reaction conditions [1.

NaH, 0 °C-RT, THF; 2. KH, 0 °C-RT, THF; 3) HCl, MeOH] to afford the desired

cyclized product. I couldn't get the cyclised product. We believe that stereochemistry at

the carbons 4 and 5 is playing a key role in preventing the cyclization.

According to Baldwin, in 5-membered ring closure systems, favoured bond angle

between the attacking nucleophile and the electrophilic carbon must be around 105°±5;

this is called the Dunitz bond angle. 5-exo-trig is favoured for cyclisations.

In the molecule **(41)** theoretically the cyclization is 5-exo-trig Fig. 2. 3. the

molecule is favoured for ring cyclisation. But the stereochemistry at 4th and 5th carbon

(from ester) makes the bond angle and bond distance between the attacking nucleophile

and electrophilic carbon more severe, so that approach at the Dunitz bond angle is not

possible.
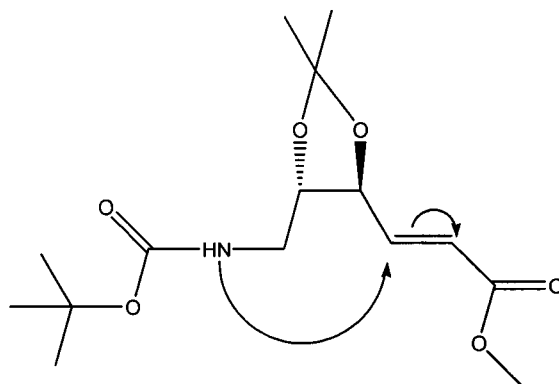
41

Fig. 2. 3.

Ideally, the desire was to design and synthesize different pyrrolidine derivatives Fig. 2. 4 to test their efficacy and potency as glycosidase inhibitors (which are involved in the biosynthesis of bacterial cell walls).

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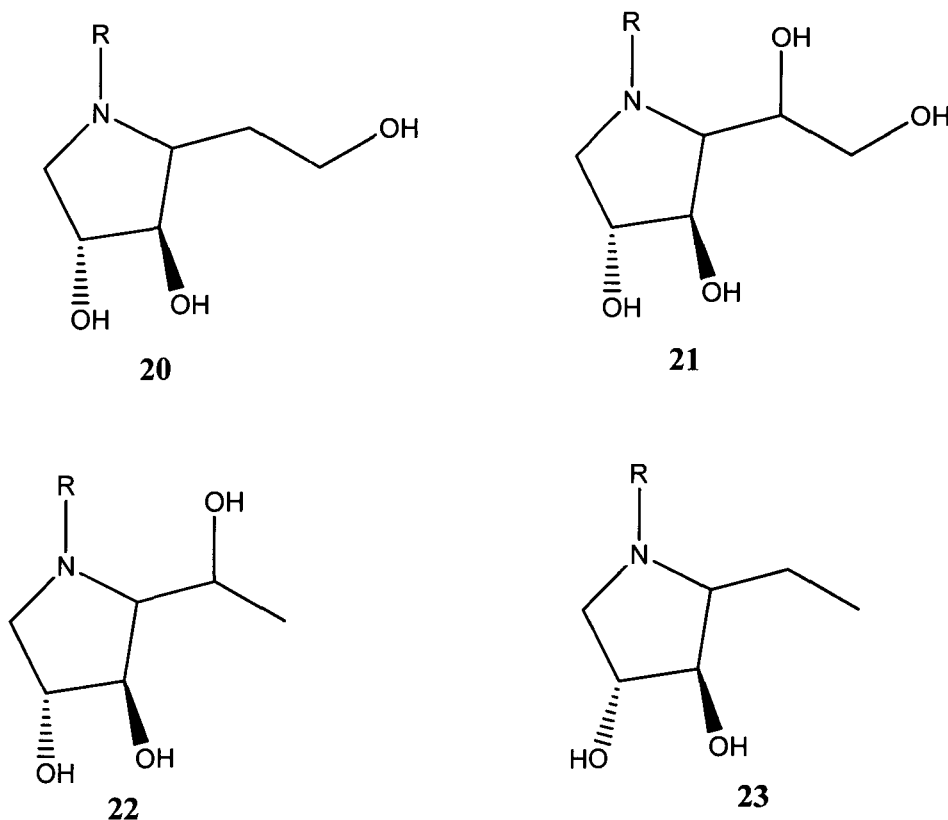
Fig. 2. 4.

R = H, alkyl

This in conjunction with, we wanted to isolate and synthesize different flavonoid compounds described above in a test strain completely devoid of MDR, would potentially improve the chances of discovering a wide variety of antimicrobials and anti-cancer agents. Once identified with the aid of MDR mutants, new antimicrobials could be deployed effectively either in combination with MDR inhibitors or at a concentration

sufficiently high to overcome the pump. Another possibility is chemical modification of these compounds (i.e. newly discovered drugs) to increase the potency and to decrease their affinity to MDR or both.

CHAPTER 3 Synthesis of a

Plantensimycin Analogue:

3.1. Introduction:

Though several antibiotics are available, bacterial infection remains a serious threat to human lives because of MDR to existing antibiotics. The discovery of new chemotypes with antibacterial activity is a primary research goal making this venture less successful but few innovative methods were developed in drug discovery laboratories, it results minimal success.[49] Recently Jun Wang and his colleagues reported the discovery of platensimycin, a previously unknown class of antibiotics produced by *Streptomyces platensis*. Platensimycin shows strong and broad spectrum Gram-positive antibacterial activity by selectively inhibiting cellular lipid biosynthesis. It selectively targets the β-ketoacyl-(acyl-carrier-protein (ACP)) synthase I/II (FABF/B) in the synthetic pathway of fatty acids[50]. Fatty acid synthesis is a vital process in cell physiology. The initiation condensing enzyme, Fab H, and elongation condensing enzyme, FabF/B, are essential components of fatty acid biosynthesis. There are two types of FAS architectures. FAS I is found in mammals and consists of a single gene that produces a polypeptide, which contains all of the reaction centers required to produce fatty acids.[51]

FAS II is found in bacteria, plants and parasites. In this system each component is encoded by a separate gene that produces a unique protein which catalyses a single step in the pathway.[51] Type II fatty acid synthetic pathway is the principal route for the production of phospholipid acyl chains in bacteria and plants. Type II fatty acid synthesis is a target for antibacterial drug discovery.[52] So far no drug is used clinically targeting

condensing enzymes, but cerulenin[53] and thiolactomycin[54] selectively inhibit the condensation enzymes FabF/B and Fab H. Platensimycin interacts specifically with the acyl enzyme intermediate of the target protein. Treatment with platensimycin eradicates Staphylococcus aureus infection in mice. Platensimycin shows no cross resistance to other antibiotic resistant stains. Platensimycin is the most potent inhibitor for the FABF/B condensing enzymes and it does not show any toxic effect.[50]

After screening 250,000 natural product extracts, with the use of a combination of target based whole cell and biochemical assays, Jun Wang et al found a potent and selective small molecule from a strain of *Streptomyces platensis*, which was recovered form a soil sample collected in South Africa. Platensimycin consists of two distinct structural elements [with the molecular weight of 441.47 ($C_{24}H_{27}NO_7$)] connected by an amide bond. In the whole cell labelling experiments, Platensimycin showed selective inhibition of lipid biosynthesis in *S. aureus* with an IC_{50} of 0.1 µg. mL^{-1}. Similar results were obtained with *S.pneumoniae*, in which the IC_{50} for inhibition of cellular lipid biosynthesis was 0.8 µg. mL^{-1}. Platensimycin selectively inhibits lipid biosynthesis, but it did not inhibit DNA, RNA, protein and cell wall biosynthesis even at concentrations up to 500 µg. mL^{-1}. Platensimycin kills bacteria entirely through the inhibition of fatty acid biosynthesis.

Based on the above encouraging reports we attempted the synthesis of platensimycin analogues starting with resorcinol.
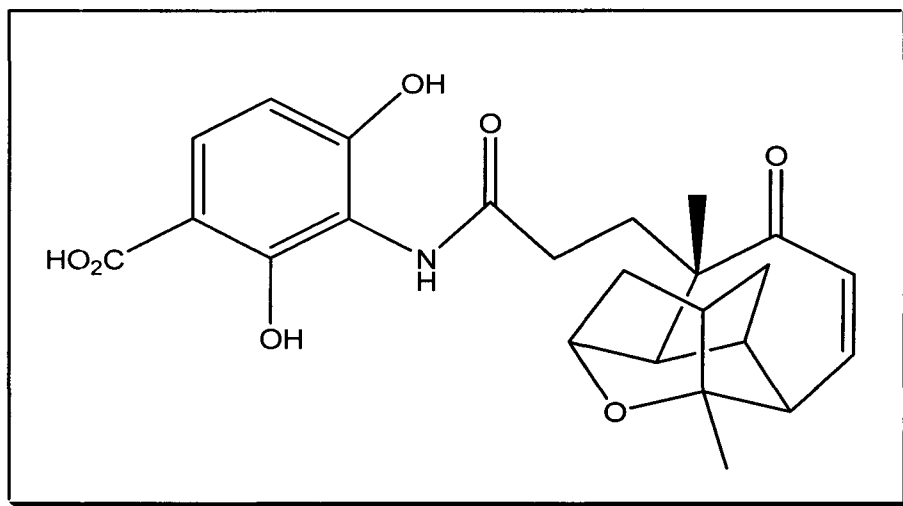
18

Fig. 3. 1. **Platensimycin**

3.2. Results and Discussion

SCHEME 3.1.
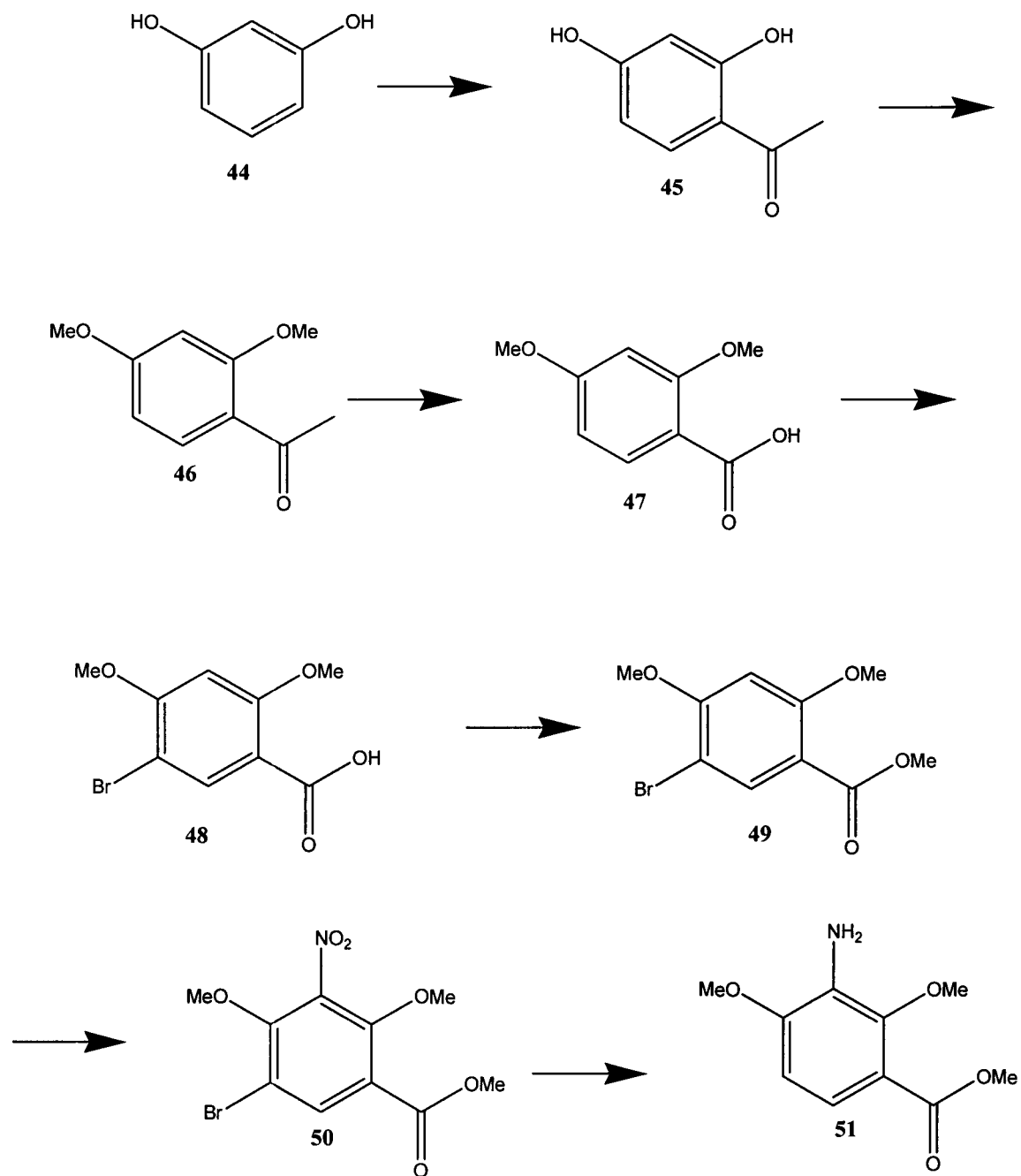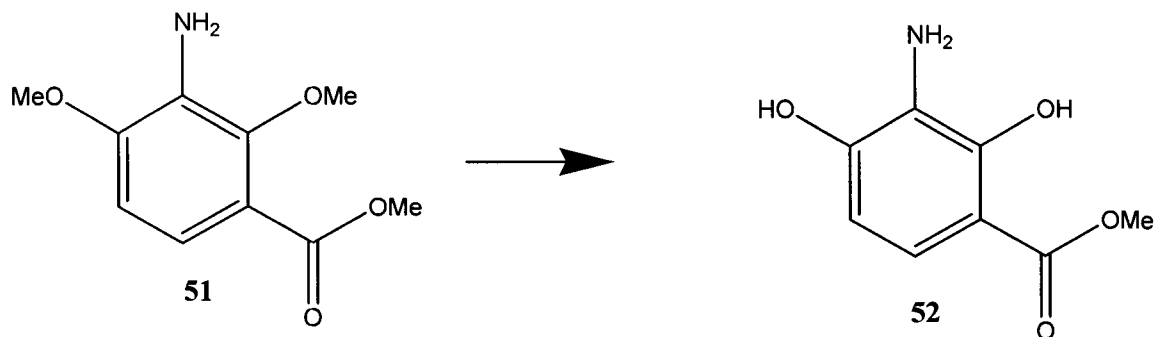
In our synthesis, the approach commenced with commercially available resorcinol (44), which was converted into the corresponding 2, 4-dihydroxyacetophenone (45) with acetic anhydride and zinc chloride at 200 °C under microwave irradiation through Fries rearrangement. 2, 4-dihydroxyacetophenone (45) was then treated with dimethylsulfate (DMS) in the presence of K_2CO_3 in acetonitrile to yield the corresponding dimethoxyacetophenone (46)[55] in an excellent yield (90%). Subsequent oxidation of 2, 4-dimethoxyacetophenone (46) with KOH in DMF at 65 °C gave 2, 4-dimethoxybenzoic acid (47)[56], which was treated with oxone and KBr at RT to afford the 5-bromo-2, 4-dimethoxybenzoic acid (48)[57]. The aryl bromo compound (48) was converted to the corresponding methyl ester (49) which was then treated with different nitrating agents under different reaction conditions, Table. 3.1 to synthesize the desired nitro derivative (50). Moreover the ipso product was observed as the major product 40% instead of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid (50).

Table. 3.1.

Reagents and reaction conditions	Product	% of yield
$Ca(NO_3)_2$, AcOH, MWI, 7min, 100 °C	Starting Material (49)	100
$NaNO_3$, $AlCl_3$, DCM, TMSCl, 0 °C-RT & 50 °C	Ipso nitration (58)	15
TBAB, HNO_3, EDC	Ipso nitration (58)	25
HNO_3, H_2SO_4, 0 °C	Ipso nitration (58)	30
$ZnCl_2$, HNO_3, 65 °C	Ipso nitration (58)	27
$(AcO)_2$, HNO_3, -70 °C- RT	Ipso nitration (58)	33
I_2, $AgNO_2$, CH_3CN, RT-reflux	Starting material (49)	100
Cerium (IV) Ammonium nitrate, AcOH	Starting material (49)	100
Ac_2O, fuming HNO_3, 0 °C	Ipso nitration (58)	25

However, in most cases ipso-nitration of the aryl bromide occurred giving the 5- nitro compound (**58**) in which the bromine had been substituted by nitro through electrophilic aromatic substitution. Theoretically two electron donating methoxy substituents should favour nitration in the ortho, para direction i.e. C-3 and/or C-6, while the bromo and ester groups should direct substituents towards C-3. We believe that steric factors have precluded substitution at C-3, giving the ipso product instead. The proposed mechanism is given below.
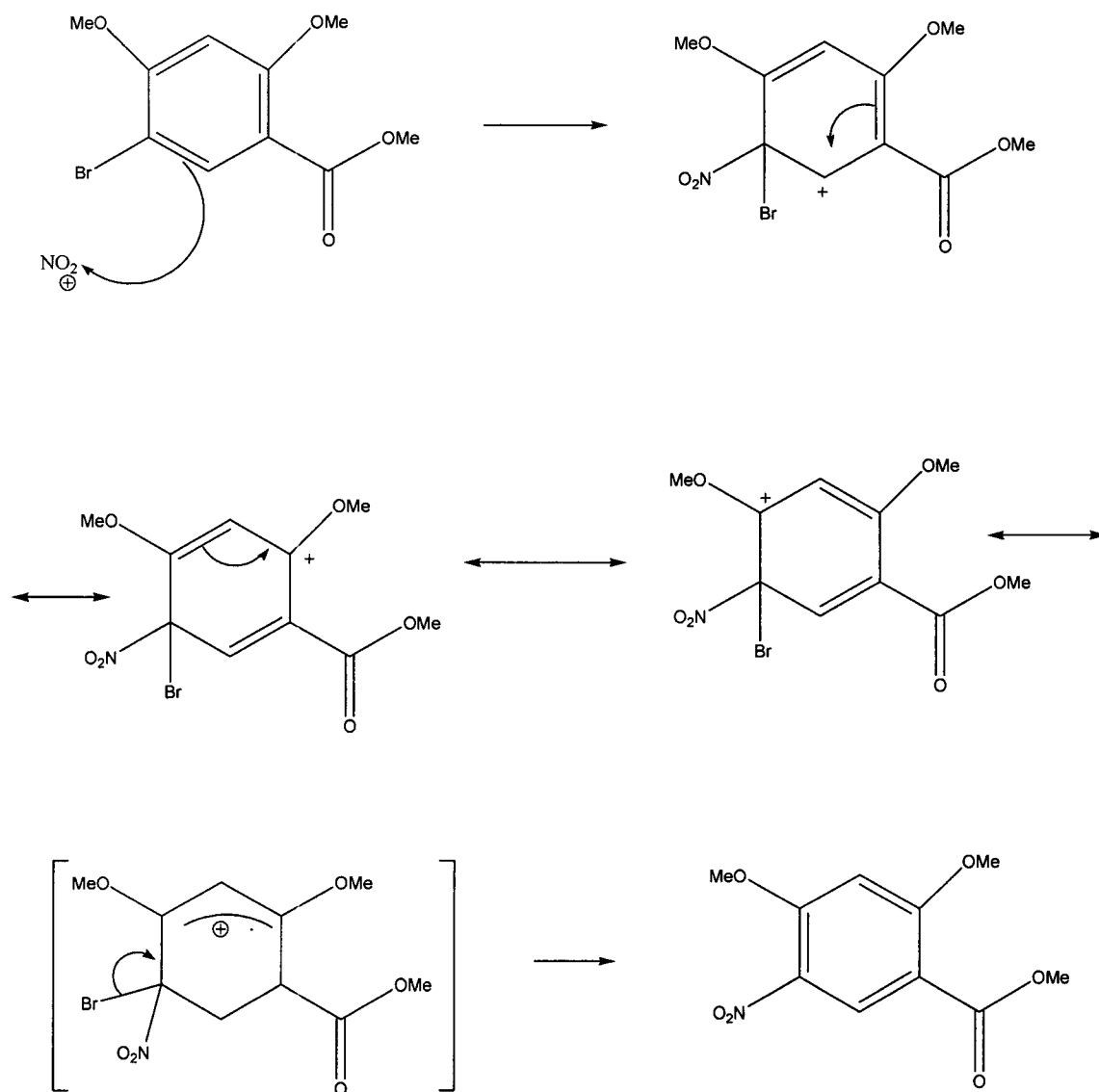
Fig. 3. 2.

3.3. Conclusions

A large amount of chemical and biochemical knowledge has been accumulated with respect to the understanding of resistance to anti-tumour drugs and especially in MDR modulation. Potent and safe MDR modulators are still needed for clinical use and some of the P-gp inhibitors that are in the advanced clinical evaluation stage may be expected

finally to enter therapeutical use in the near future. On the other hand, since new inhibitors of MDR transporters are continuously being described and some structural features of P-gp modulators are already well recognised, the discovery of relevant new lead compounds should be expected. However, the involvement of multiple mechanisms of multidrug resistance makes the development of efficient therapies in this area a very difficult and ongoing task.

In summary, we are trying to develop a simple and concise synthesis of trihydroxylated pyrrolidine alkaloids as well as new antibiotics based on the aniline core of Plantensimycin. 1, 4-cyclization to the pyrrolidine could not be achieved even with Boc-protected amine substituents. This is most probably because of steric reasons due to the near-planar conformation imposed by the 2, 3-acetonide ring and resulting distance between the substituent at positions 1 and 4 preventing cyclization. Theoretically in 5, 5-trans bicyclic systems cyclization is difficult due to ring strain. The use of open-chain 2, 3-protected derivatives may alleviate this problem. A synthetic approach to the aniline core of platensimycin was investigated. We identified ipso-nitration as a major side reaction on attempted nitration of an advanced aryl bromide intermediate during the reaction (Electrophilic substitution). An alternate synthesis of the aryl core using Pd-mediated carbonylation chemistry may prove to be more feasible in the future.

Experimental:

The heartwood of *Prunus avium* was obtained from a felled tree and air-dried chips (10.73 g) crushed and macerated with $CHCl_3$ for 24 h after which the solvent was removed under reduced pressure and the residue (1.2 g) dissolved in ethyl acetate (4.5 mL). Crude extract (1.2 g) was subjected to column (40 cm x 2.5 cm) chromatography on silica gel by gradient elution with *n*-hexane, *n*-hexane-EtOAc, EtOAc. Ten fractions were generated in this manner. Similar fractions were combined after TLC indicated that they contained same flavonoids. Tectochrysin and sakuranetin were obtained from the 3rd and 8th fractions respectively. Fraction 3 was rechromatographed by column (25 cm x 1 cm) chromatography on silica gel using and *n*-hexane-EtOAc step gradient which gave a mixture of compounds. This mixture was further purified by gel filtration on Sephadex LH-20 in chloroform and methanol, from which tectochrysin (2 mg) was obtained. Similarly, Fraction 8 yielded sakuranetin (5 mg) by gel filtration on Sephadex LH-20. The residue obtained after chloroform filtration was macerated with MeOH (75 mL) for 24 h after which solvent was removed under reduced pressure and the residue (2.03 g) dissolved in EtOAc (8 mL). Crude extract after TLC was subjected to column (40 cm x 2.5 cm) chromatography on silical gel by gradient elution with *n*-hexane-EtOAc, EtOAc, EtOAc-MeOH and finally MeOH. Fifteen fractions were generated in this manner. Similar fractions were combined after TLC [*n*-hexane-EtOAc 1:1] indicated that they contained same flavonoids, and subjected to column chromatography on silica gel by gradient elution with *n*-hexane-EtOAc, EtOAc, EtOAc-MeOH, finally MeOH, followed by gel filtration on Sephadex LH-20 and prep. TLC (*n*-hexane and EtOAc, 80:20 run thrice) afforded different flavonoids: catechin (10 mg), naringenin (2 mg),

dihydrowogonin (3 mg), dihydrokaempfeol (3 mg).
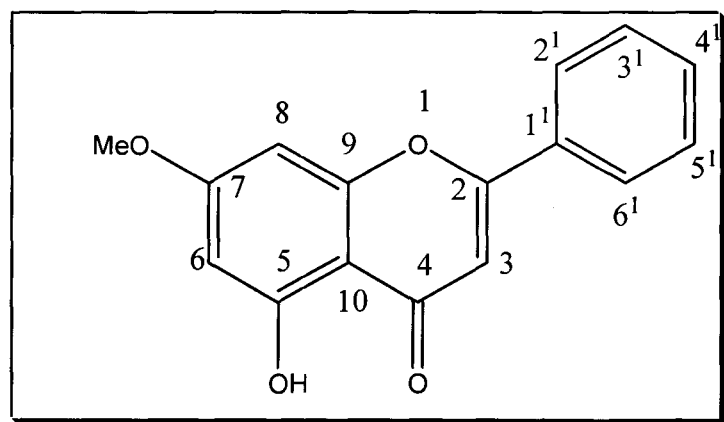
5-hydroxy-7-methoxy flavone

1

Tectochrysin[20]

^{1}H-NMR: δ (500 MHz, CDCl$_3$) 3.89 (3H, s, OCH$_3$), 6.38 (1H-8, d, J 2.2), 6.51 (1H-6, d,

J 2.2), 6.67 (H-3, s), 7.53 (2^1, 6^1-H, d, J 8.0), 7.54 (H-4^1, t, J 1.3, 8.0), 7.89 (H-3^1 & 5^1,

dd, J 1.1, 8.0 Hz).

^{13}C-NMR: δ (125 MHz, CDCl$_3$) 55.9 (OCH$_3$), 92.2 (C-8), 97.7 (C-6), 105.2 (C-10),

105.4 (C-3), 125.8 (C-3^1, 5^1), 128.6 (C-2^1, 6^1), 130.8 (C-2), 131.3 (C-4^1), 157.3 (C-9),

161.7 (C-5), 163.5 (C-1^1), 165.1 (C-7), 182.0 (C-4).

HRMS: m/z (rel.int.) EI: Mass: 268.0706, Calc. Mass for C$_{16}$H$_{14}$O$_4$: 268.0736, 268 (100),

239 (40), 225 (10), 138 (7), 95 (7).

IR ν_{max} (film)/cm^{-1}: 1160, 1653, 2854.

R$_f$ = 0.62 (n-hexane-EtOAc; 80:20).
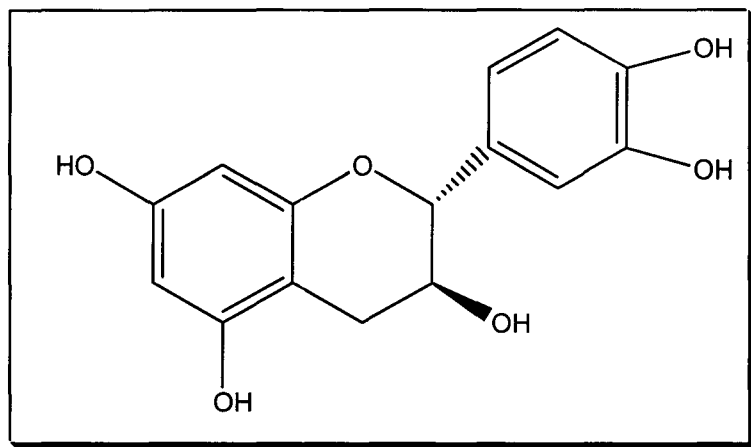
2. (+)-Catechin[21]

^{1}H-NMR : δ (600 MHz, CDCl$_3$) 2.51 (1H, dd, J 13.0, 13.0, H-4), 2.84 (1H, dd, J 9.0, 9.0, H-4), 3.97 (1H, m, H-3), 4.56 (1H, d, J 12.0, H-2), 5.85 (1H, d, J 4.0, H-8), 5.93(1H, d, J 4.0, H-6), 6.71-6.83 (3H, m, Ar).

^{13}C-NMR: δ (150 MHz, CD$_3$OD) 28.5 (C-4), 68.8 (C-3), 82.8 (C-2), 95.5 (C-8), 96.2 (C-6), 100.8 (C-10), 115.2 (C-2^1), 116.0 (C-5^1), 120.0 (C-6^1), 132.2 (C-1^1), 146.2 (C-3^1,4^1), 156.9 (C-5), 157.5 (C-7), 157.8 (C-9).

HRMS: m/z (rel. int.) EI: Mass: 290.0780, Calc. Mass for C$_{15}$H$_{14}$O$_6$: 290.0790, 290 (5), 256 (20), 213 (15), 185 (20), 129 (40).

IR ν_{max} (film)/cm^{-1}: 1520, 1618, 3350.

[α] $_D^{25}$: +11.7° (c 0.028, MeOH), lit. [α]$_D^{22}$: +20° (c 0.1, MeOH).

R$_f$ = 0.20 (n-hexane-EtOAc; 70:30).
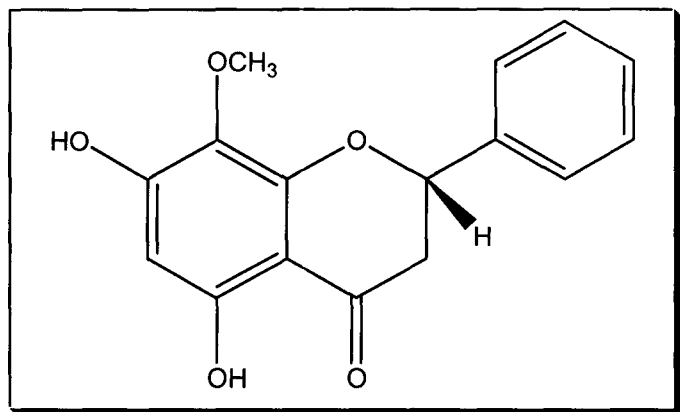
3. Dihydrowogonin[20]

^{1}H-NMR: δ (500 MHz, CD$_3$OD) 2.85 (1H, dd, J 16.9, 3.0), 3.08 (1H, dd, J 16.9, 13.2), 3.88 (3H, s, OCH$_3$), 5.49 (1H, dd, J 13.0, 3.0), 6.15 (1H, s, H-6), 7.39-7.48 (5H, m, Ar).

^{13}C-NMR : δ (125 MHz, CD$_3$OD) 43.6 (C-3), 61.7 (OCH3), 79.7 (C-2), 96.1 (C-6), 103.1 (C-10), 126.1 (C-2^1, C-6^1), 127.7 (C-80), 129.0 (C-3^1, C-4^1, C-5^1), 138.4 (C-1^1), 152.9 (C-9), 158.0 (C-5), 160.0 (C-7), 195.7 (C-4).

HRMS: m/z (rel. int.): CI: Mass: 287.0915, Calc. Mass for C$_{16}$H$_{14}$O$_5$: 287.0919, 287 (30), 286 (28), 257 (5), 209 (5), 182 (15), 143 (10), 48 (100).

IR ν_{max} (film)/cm^{-1}: 1574, 1638, 3400.

$[\alpha]_D^{25}$: -27.42° (c 0.07, MeOH).

R$_f$ = 0.24 (n-hexane-EtOAc; 70:30 run twice in the same solvent)
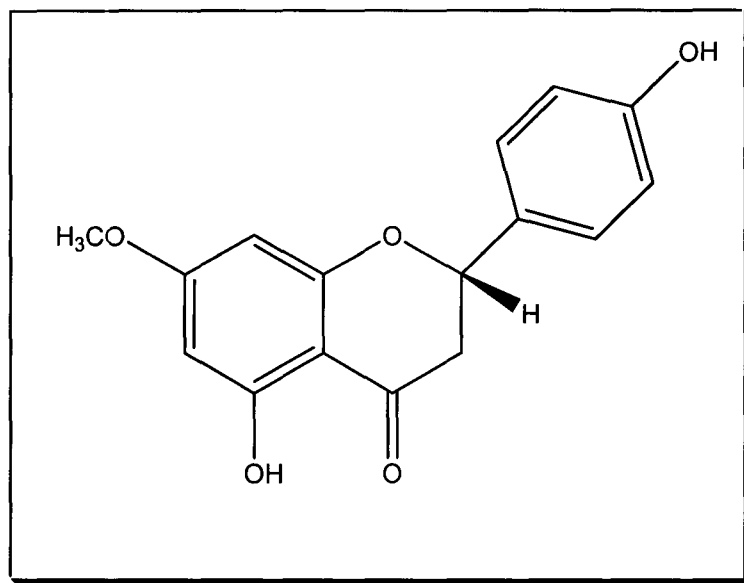
4. Sakuranetin[20]

^{1}H-NMR: δ (600 MHz, CD$_3$OD) 2.78 (1H, dd, J 17.4, 3.0), 3.09 (1H, dd, J 17.3, 13.2),

5.35 (1H, dd, J 13.2, 3.0), 6.05 (2H, d, J 2.4), 6.88 (2H, d, J 8.4, Ar-H), 7.32(2H, d, J 8.4,

Ar-H).

^{13}C-NMR: δ (150 MHz, CD$_3$OD) 43.1 (C-3), 55.8 (OCH$_3$), 79.1 (C-2), 94.4 (C-8), 95.2

(C-6), 103.2 (C-10), 115.8 (C-3^1, 5^1), 128.0 (C-2^1, 6^1), 130.5 (C-1^1), 156.4 (C-9), 159.1

(C-4^1), 163 (C-5), 168.1 (C-7), 196.0 (C-4).

HRMS: m/z (rel. int.): CI: Mass: 287.0891, Calc. Mass for C$_{16}$H$_{14}$O$_5$: 287.0919, 287 (15),

286 (93), 285 (63), 269 (6), 193 (35), 180 (40), 167 (100), 166 (30), 138 (22), 120 (33).

IR ν_{max} (film)/cm^{-1}: 1641, 2925, 3343.

[α]$_D^{25}$: -18° (c 0.02, CHCl$_3$), lit. [α]$_D^{25}$: -27° (c 0.1, MeOH).

R$_f$ = 0.22 (n-hexane-EtOAc; 80:20).
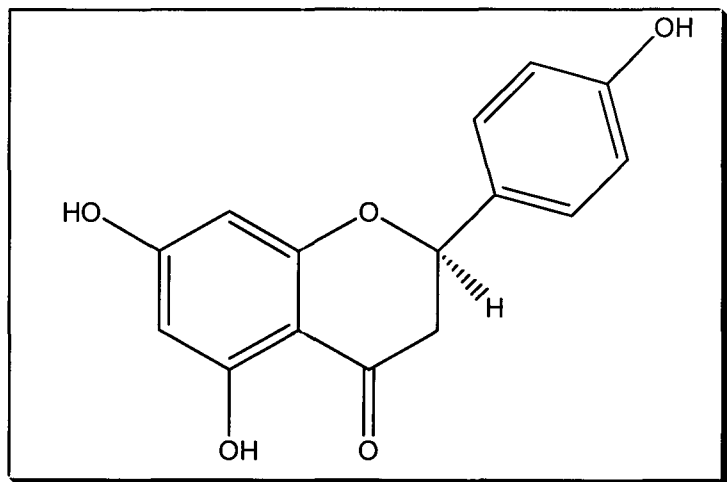
5. Naringenin[20]

^{1}H-NMR: δ (600 MHz, CD$_3$OD) 2.70 (1H, dd, J 3.0, -17.1, H-3e), 3.11 (1H, dd, J 12.9, -17.1, 3a), 5.34 (1H, dd, J 3.0, 13.0, H-2), 5.88 (1H, d, 2.2, H-6), 5.89 (1H, d, 2.2, H-8), 6.82 (2H, d, J 8.6, H-3^1, 5^1), 7.31 (2H, d, J 8.6, H-2^1, 6^1).

^{13}C-NMR: δ (150 MHz, CD$_3$OD) 44.0 (C-3), 80.4 (C-2), 96.1 (C-8), 97.0 (C-6), 103.3 (C-10), 116.3 (3^1, 5^1), 129.0 (C-2^1, 6^1), 131.1 (C-1^1), 150.0 (4^1), 164.8 (C-9), 165.4 (C-5), 168.4 (C-7), 197.7 (C-4).

HRMS: m/z (rel. int.): CI: Mass: 273.0753, Calc. Mass for C$_{15}$H$_{12}$O$_5$: 273.0763, 273 (100), 272 (45), 179 (20), 153 (32).

IR v_{max} (film)/cm^{-1}: 1250, 1620, 3010.

$[\alpha]_D^{25}$: +12.8° (c 0.06, MeOH), lit. $[\alpha]_D^{22}$: +15.8° (c 0.3, EtOH)[20b].

R$_f$ = 0.31 (n-hexane-EtOAc; 70:30).
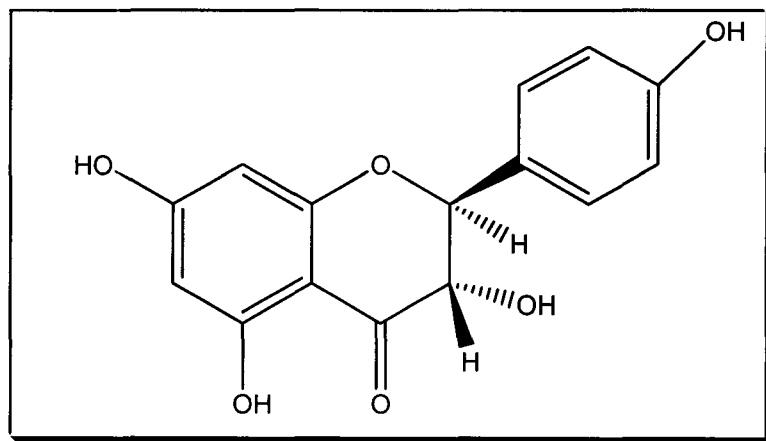
6. dihyrokaempferol[22]

^{1}H-NMR: δ (500 MHz, CD$_3$OD) 4.55 (1H, d, J 11.6, H-3), 4.97 (1H, d, J 11.6, H-2), 5.88 (1H, d, J 2.0), 5.92 (1H, d, J 2.1), 6.83 (2H, d, J 8.5), 7.34 (2H, d, J 8.5).

^{13}C-NMR: δ (125 MHz, CD$_3$OD) 73.6 (C-3), 84.9 (C-2), 96.3 (C-8), 97.3 (C-7), 101.8 (C-10), 116.1 (C-2^1, 6^1), 129.3 (C-1^1), 130.3 (C-3^1, 5^1), 159.2 (C-4^1), 164.5 (C-9), 165.3 (C-5), 168.8 (C-6), 198.4 (C-4).

HRMS: m/z (rel. int.): EI: Mass: 288.0626, Calc. Mass for C$_{15}$H$_{12}$O$_6$: 288.0634, 289 (3), 288 (7), 270 (10), 237 (100), 153 (45), 136 (60).

IR v_{max} (film)/cm^{-1}: 1520, 1640, 3300.

$[\alpha]_D^{25}$: +8.8° (c 0.31, MeOH), lit. $[\alpha]_D^{27}$: +13° (c 4, EtOH)[22, 23].

R_f = 0.23 (n-hexane-EtOAc; 35:65 run twice in the solvent).

Synthesis of dimethyl 2, 3-(*O*)-isopropylidenedioxy-L-tartrate (26):
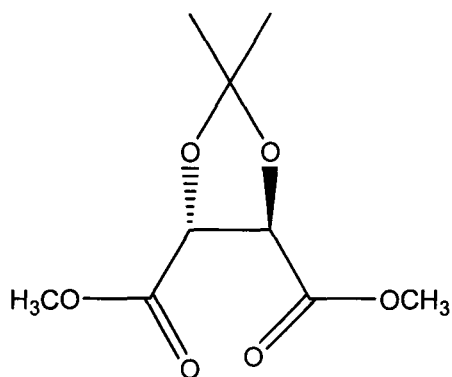
To a flame-dried round-bottom flask containing 10 g (6.6 mmol) of L-tartaric acid, 15.6 g

2, 2-dimethoxypropane (27.5 mL, 0.22 mmol) 12 mL of dry methanol and 40 mg of *p*-

toluenesulphonic acid monohydrate were added and the mixture was warmed gently with

constant stirring for 1.5h under reflux condenser. Then, 9.2 mL of 2, 2-dimethoxypropane

and 45 mL of cyclohexane were added. The resulting two-layer solution was refluxed

with stirring while the acetone-cyclohexane and methanol-cyclohexane mixtures were

slowly removed with a Dean-Stark condenser. After 48 hrs another 1 mL of 2, 2-

dimethoxypropane was added again followed by a 20 min reflux, lowering the

temperature to below 40°C. Anhydrous K_2CO_3 (100mg) was added to neutralize the

catalyst. The solvent and unreacted 2, 2-dimethoxypropane were removed under reduced

pressure giving a yellow liquid which was distilled under high vacuum to give (**26**) as a

colourless oil, 70%.

Synthesis of 2, 3-(*O*)-isopropylidenedioxy-butane-1, 4-diol (27):
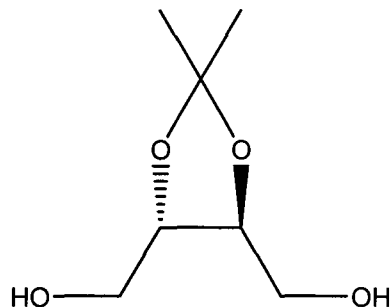
Lithium aluminium hydride (LAH) (0.8 g, 2 mmol) was slurried in dry ether (10 mL) and refluxed for 30 min, after which dimethyl 2, 3-(*O*)-isopropylidene-L-tartrate (**26**) (3.0 g, 1.37 mmol) dissolved in dry ether (13 mL) was slowly added at room temperature to the slurry which was left to stir overnight. After cooling with ice, ethyl acetate (4 mL) was carefully added dropwise, followed by water (2 mL), 4 M NaOH (2 mL) and, further 7 mL of water. The suspension was stirred until gas release ceased, the conspicuous ivory precipitate which had settled down was filtered off and washed with abundant ether and the pooled ethereal extracts dried over $MgSO_4$, filtered and concentrated under reduced pressure. Distillation of the residue afforded the threitol (**27**) in the form of pale yellow dense oil.

Synthesis of 4-ᵗButyldimethylsilyloxy- 2, 3-(*O*)-isopropylidenedioxy-butan-1-ol (28):
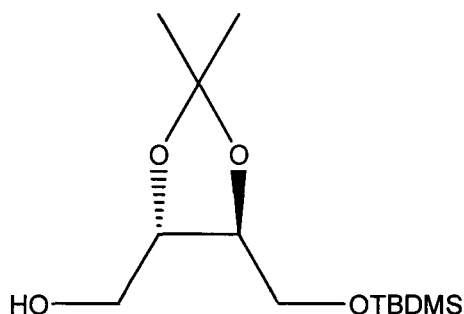
Sodium hydride (0.135 g, 5.6 mmol: as 60% dispersion in mineral oil) was added portion wise to a stirred solution of L-threitol acetonide (27) (0.82 g, 5.56 mmol) in anhydrous THF (30 mL) and the solution cooled to 0 °C under argon. When effervescence ceased, a solution of *tert-* butyldimethylsilylchloride (1 g, 6.63 mmol) in dry THF (5 mL) was slowly added to the slurry at RT and the mixture was stirred vigorously overnight under argon. The milky solution was poured into water (30 mL), and the yellow clear organic layer separated and collected in a beaker. The aqueous phase was extracted with EA (3 x 10 mL). The pooled organic phase was dried over $MgSO_4$ and solvent removed under reduced pressure to yield a yellow residue which was chromatographed on silica gel (hexane: EA, 10:90) to afford the desired monosilylated alcohol (28) (50%) as a lemon-yellow oil.

Synthesis of 4-^{t}Butyldimethylsilyloxy- 2, 3-(*O*)-isopropylidenedioxy-butan-1-al (29):
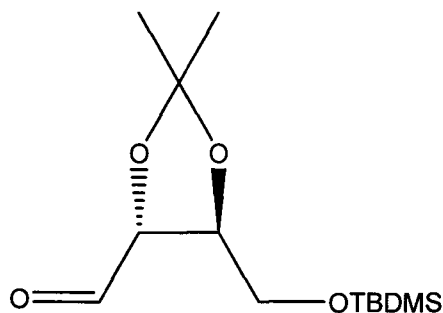
A solution of DMSO (56 μL, 0.79 mmol) in anhydrous CH_2Cl_2 (0.1 mL) was carefully added dropwise under Ar flow over a 3 min period to a stirred solution of oxalyl chloride (34 μL, 0.79 mmol), in the same solvent (0.4 mL), cooled at -76 °C. The mixture was allowed to react for 30 min at the same temperature, until a milky turbid appearance was observed. Thereafter, alcohol (28) (100 mg, 0.36 mmol) was dissolved in dry CH_2Cl_2 (0.5 mL) and slowly added at -72 °C to the white mixture over a 15 min period. After stirring for 1h at -65 °C under Ar flow, anhydrous triethylamine (0.2 mL, 1.79 mmol) was added

dropwise at -61 °C. The reaction mixture was stirred for 15 min at the same temperature,

left to warm to RT over 2 h and poured into 3 mL of water. The whitish clear bottom

phase was separated from the aqueous layer, which was extracted with CH_2Cl_2 (3 × 5

mL); the organic phases were combined, washed with 1 M HCl (3 mL), saturated

$NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, filtered and evaporated under

reduced pressure to give the crude aldehyde (29), in the form of lemon yellow thick oil

(76%), which was used for the next Wittig reaction without further purification, except

for repeated treatments with anhydrous toluene followed by rotary evaporation.

Synthesis of 6-^{t}Butyldimethylsilyloxy-4, 5-(O)-isopropylidenedioxy-hex-2-enoic acid

(E)-methyl ester (30):
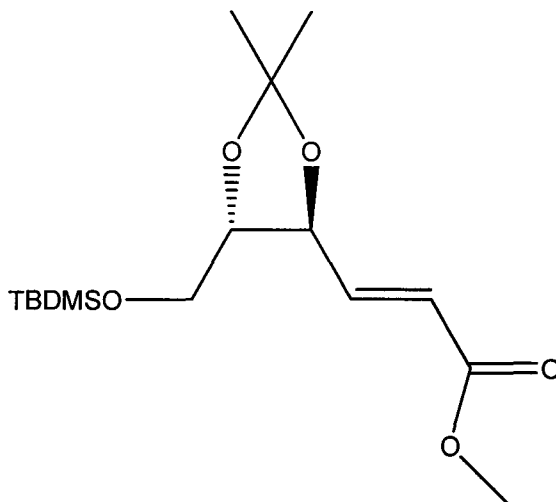
Trimethyl phosphono acetate (60 μL, 0.25 mmol) was slurried in 2 mL freshly distilled

anhydrous THF. The white suspension was cooled to -78 °C and LiHMDS (0.25 mL of a

2.0 M solution in THF, 0.25 mmol) was added over 5 min under Ar flow and magnetic

stirring. The bright yellow mixture was vigorously stirred and allowed to warm to rt for 1

h 40 min; gas bubbles were observed to develop and a white solid to settle. The crude

aldehyde (70 mg, 0.25 mmol) was dissolved in dry THF (1 mL) and added dropwise over

a 5 min period to the yellow slurry at -78 °C and stirred for 30 min at the same temperature. The reaction mixture was allowed warm to RT and stirred continuously over night. 7 mL of EA was added to the mixture and the organic layer was collected and washed with water (3 x 4 mL), dried over MgSO$_4$ and filtered. Concentration *in vacuo* provided a crude residue which was purified by silica gel chromatography (hexane: EA, 70: 30 and finally 60: 40) to afford the ester (30) (90%).

Synthesis of Methyl-6-hydroxy-4, 5-(O)-isopropylidinedioxy hex-2-enoate (31):
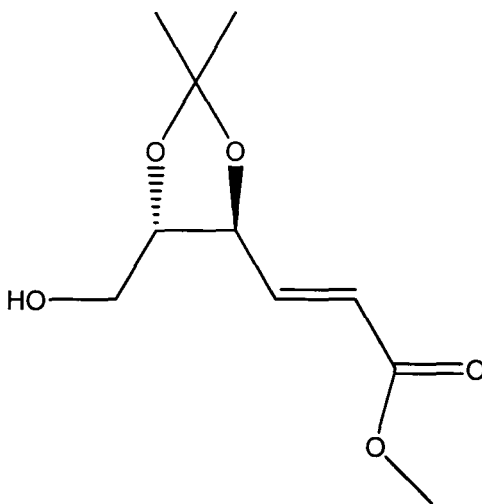
Into flame-dried round bottom flask containing dry THF 2 mL and α, β-unsaturated ester (80 mg, 0.24 mmol) was added and Tetra butyl ammonium fluoride (0.27 mL; 0.27 mmol) was injected via syringe under Argon. The reaction mixture was stirred at RT and left over night under argon. The solvent was evaporated under reduced pressure, the yellowish residue dissolved in ethyl acetate (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EA (5 mL x 3) and pooled organic phases were dried over MgSO$_4$ and filtered. Concentration invacuo followed by chromatography on silica gel (hexane: EA 60: 40) yielded free alcohol (31) as a pale yellow oil in 85%.

**Synthesis of Methyl-6-methanesulfonyl-4, 5-(*O*)-isopropylidinedioxy hex-2-enoate**

(32):
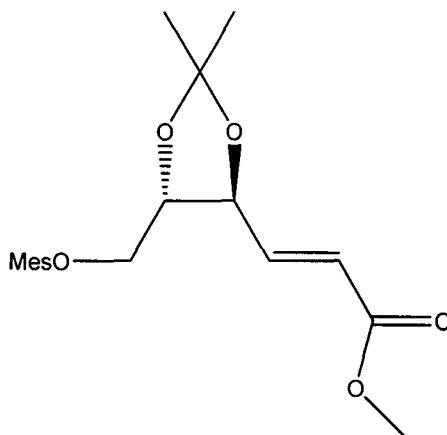
Into a flame-dried round-bottom flask containing dry DCM 10 mL and monosilylated alcohol (40 mg, 0.18 mmol) was added TEA (51.5 μL, 0.37 mmol) and methane sulfonyl chloride (16.3 μL, 23 mg, 0.2 mmol) slowly injected via syringe (under argon). The reaction mixture was stirred at room temperature for over night under argon. The solvent was evaporated under reduced pressure 5 mL DCM was added and the mixture washed with water (5 mL). The aqueous phase was extracted with DCM (8 mL x 3). The pooled organic phases were dried over MgSO$_4$ and concentrated under reduced pressure. The residue was chromatographed over silica gel (hexane: EA 10:90) and afforded the desired product (32) as yellow oil (85%).

Synthesis of 4-ᵗButyldimethylsilyloxy-2, 3-(*O*)-isopropylidenedioxy-1-

Methanesulfonate (36):
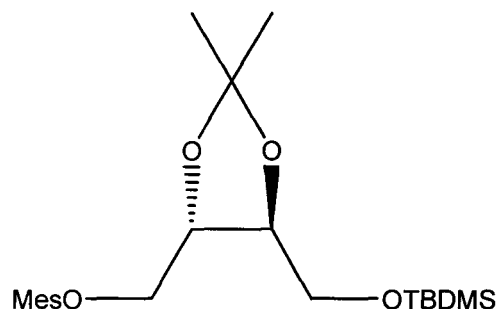
Into a flame-dried round-bottom flask containing dry DCM 10 mL and monosilylated alcohol (215 mg, 0.78 mmol) was added TEA (225 µL, 1.6 mmol) and methane sulfonyl chloride (66 µL, 91.6 mg, 0.8 mmol) slowly injected via syringe under argon. The reaction mixture was stirred at room temperature for over night under argon. Total solvent was evaporated under reduced pressure and DCM (10 mL) was added and washed with water (10 mL). The aqueous phase was extracted with DCM (8 mL x 3). The pooled organic phases were dried over MgSO$_4$ and concentrated under reduced pressure which was chromatographed over silica gel (hexane: EA 10:90) and afforded the desired product (**36**) as yellow oil (85%).

^{1}H-NMR: δ (500 MHz, CDCl$_3$) 0.06 (6H, s), 0.88 (9H, s), 1.40 (3H, s), 1.41 (3H, s), 3.06 (3H, s), 3.68 (1H, dd, *J* 11.8, 4.2Hz), 3.82 (2H, m), 4.23 (2H, m), 4.43 (1H, dd, *J* 2.3, 2.3Hz).

^{13}C-NMR: δ (125 MHz, CDCl$_3$) -5.0, 18.1, 25.7, 26.8, 37.3, 63.1, 69.4, 78.2, 79.0, 109.8.

MS: *m/z* (rel.int.): 355 (5), 339 (8), 297 (5), 239 (15), 152 (100).

IR v$_{max}$ (film)/cm^{-1}: 2930, 1087, 777.

Synthesis of 1-Azido-4-t-butyldimethylsilyloxy 2, 3-(*O*)-isopropylidene-butanol (37):
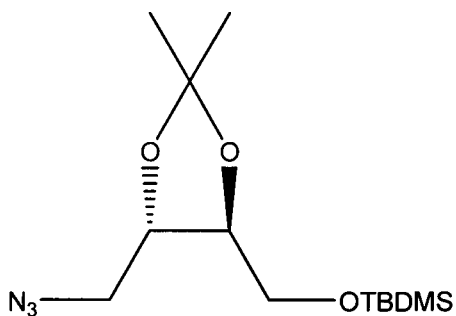
Into a flame-dried round–bottom flask containing DMF (5 mL), was added mesylate (1.16 g, 3.3 mmol) and sodium azide (1.29 g, 19.8 mmol). The reaction mixture was stirred overnight at 100 °C. The reaction was then cooled to RT and 10 mL water added and the mixture extracted with EA (15 mL x 3). Pooled organic phase was dried over MgsO₄, filtered and concentrated under reduce pressure to yield yellow residue, which was chromatographed over silica gel (hexane) and afforded the desired product (37) in the form of a pale yellow oil 65%.

¹H-NMR: δ (500 MHz, CDCl₃) 0.049 (6H, s), 0.87 (9H, s), 1.38 (3H, s), 1.42 (3H, s), 3.33 (1H, dd, *J* 5.9, 5.9Hz), 3.84 (2H, m), 3.60 (2H, m), 4.04 (1H, m).

¹³C-NMR: δ (125 MHz, CDCl₃) -5.0, 25.0, 32.6, 33.7, 59.2, 70.3, 73.6, 81.4, 116.5.

MS: *m/z* (rel. int.): 316 (20), 274 (20), 244 (12), 216 (13), 158 (30).

IR ν_max (film)/cm⁻¹: 2930, 2098, 1253.

Synthesis of 4-ᵗButyldimethylsilyloxy-2, 3-(O)-isopropylidenedioxy-1-

ᵗButyloxycarbo- amino butane (38):

Into a flame-dried round bottom flask containing ethyl acetate (1 mL) was added silylated

azide (70 mg; 0.23 mmol) and 10% Pd/C (25 mg; 0.04 mmol) and Boc-anhydride (75

mg; 0.35 mmol). The reaction mixture was stirred under a hydrogen atmosphere pressure

overnight. The reaction mixture was filtered and evaporated the solvent and residue was

subjected to column chromatography over silica gel (hexane: ethyl acetate 95:5), which

afforded as a colourless oil (**38**) in 40% yield.

^{1}H-NMR: δ (500 MHz, CDCl$_3$) 0.065 (6H, s), 0.88 (9H, s), 1.35 (3H, s), 1.37 (3H, s),

1.41 (9H, s), 3.95 (3H, s), 3.34 (2H, t, 4.2Hz), 3.72-4.0 (3H, m), 4.10 (1H, m), 5.14 (1H,

s).

^{13}C-NMR: δ (125 MHz, CDCl$_3$) -5.0, 25.2, 32.3, 33.6, 35.1, 49.0, 60.0, 69.1, 70.0, 73.9,

115.7, 162.6.

MS: *m/z* (rel. int.): 376 (5), 320 (10), 288 (100), 276 (15), 218 (22).

Synthesis of 4-^{t}Butyloxycarboamino-2, 3-(O)-isopropylidenedioxy-butan-1-ol (39):
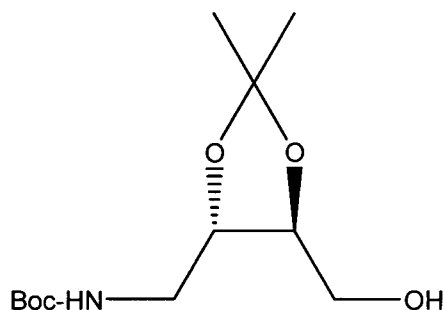
Into flame-dried round bottom flask containing dry THF (2 mL) and Boc-amine (**38**) (40 mg, 0.106 mmol) was added t-butyl ammonium fluoride (0.1 mL; 0.11 mmol) via syringe under argon. The reaction mixture was stirred at RT for 1 hr and the solvent removed under reduced pressure, the yellowish residue dissolved in ethyl acetate (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EA (5 mL x 3), the pooled organic phase dried over MgSO$_4$ filtered and concentrated under reduced pressure to yield a gum which on chromatography on silica gel (hexane: EA 60: 40) to yield alcohol (**39**) as a pale yellow oil in 85%.

^{1}H-NMR: δ (500 MHz, CDCl$_3$) 1.28 (3H, s, CH$_3$), 1.36 (3H, s, CH$_3$), 1.42 (9H, s, 3-x-CH$_3$), 3.35 (2H, m), 3.6-3.9 (3H, m), 4.02 (1H, m), 5.08 (1H, s).

^{13}C-NMR: δ (125 MHz, CDCl$_3$) 33.8, 33.1, 48.4, 68.7, 73.8, 84.9, 86.6, 115.8, 166.3.

MS: m/z (rel. int.): 262 (3), 230 (2), 206 (100), 162 (40).

IR ν_{max} (film)/cm^{-1}: 3350, 2930, 1692, 1367.

Synthesis of 4-^{t}Butoxycarbo amino-2, 3-(O)-isopropylidenedioxy-butan-1-al (40):
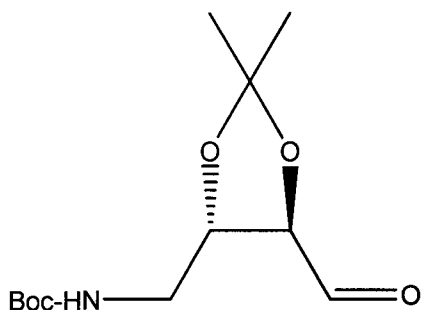
A solution of DMSO (15 mL, 0.2 mmol) in anhydrous CH_2Cl_2 (0.4 mL) was carefully added drop wise under Ar flow over a 3 min period to a stirred DCM solution of oxalyl chloride (13 μL, 0.099 mmol) cooled at -78 °C. The mixture was allowed to react at the same temperature fro 30 min, at which point milky turbid appearance was observed. Thereafter, alcohol (**39**) (26 mg, 0.1 mmol) was dissolved in dry CH_2Cl_2 (0.3 mL) and slowly added at -72 °C to the white mixture over a 15 min period. After stirring for 1h at -65 °C under Ar flow, anhydrous triethylamine (70 μL, 0.5 mmol) was added drop wise and the reaction mixture stirred for 15 min at the same temperature then left to warm to RT over 2 hr and poured into 3 mL of water. The whitish clear bottom phase was separated from the aqueous layer, which was further extracted with CH_2Cl_2 (3 × 5 mL); the organic phases were combined, washed with 1 M HCl (3 mL), followed by saturated $NaHCO_3$ (5 mL) and brine (5 mL), dried (Na_2SO_4), filtered and evaporated under reduced pressure to give the crude aldehyde (**40**), in the form of lemon yellow thick oil (76%) which was used for the next Wittig reaction without further purification, except for repeated treatments with anhydrous toluene followed by rotary evaporation.

Synthesis of Methyl-6-ᵗButyloxycarbo amino-4, 5-(*O*)-isopropylidinedioxy hex-2-enoate (41):
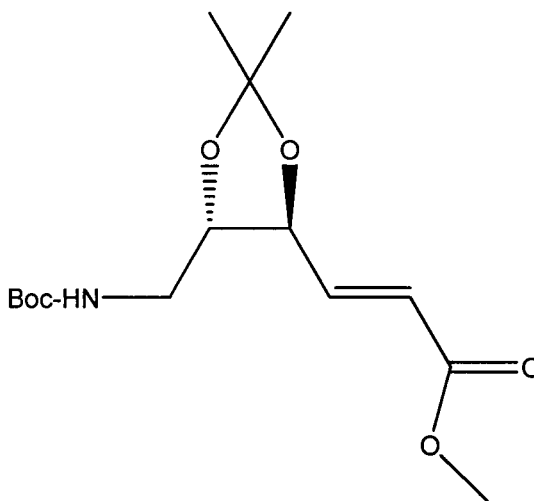
Trimethylphosphono acetate (18.28 μL, 0.1 mmol) was slurried in 1mL freshly distilled anhydrous THF and the stirred white suspension cooled to -78 °C and LiHMDS (18 μL of a 2.0 M solution in THF) added dropwise over 2 min under Ar flow and magnetic stirring. The bright yellow mixture was stirred at -78 °C fro 1 hr when the crude aldehyde (25 mg, 0.1 mmol) dissolved in dry THF (1 mL) was added dropwise over a 3 min period and the reaction mixture allowed to warm to RT for over night. 7 mL of EA was added to the mixture and washed with water (3 x 4 mL), dried over MgSO₄ and filtered. Concentrated in vacuo provided a crude residue which was purified on silica gel (hexane: EA, 70: 30 and finally 60: 40) to afford the ester (**41**) (90%).

¹H-NMR: δ (500 MHz, CDCl₃) 1.39 (3H, s), 1.40 (3H, s), 1.44 (9H, s), 3.75 (3H, s), 3.81(1H, m), 4.89 (1H, s), 3.41 (2H, m), 6.15 (1H, dd, *J* 2.0, 2.0Hz), 6.88 (1H, dd, *J* 9.0, 9.0Hz), 4.28 (1H, m).

¹³C-NMR: δ (125 MHz, CDCl₃) 26.7, 27.0, 28.4, 40.7, 51.8, 62.1, 78.3, 79.7, 109.5, 122.7, 143.8, 156.1, 166.4.

MS: *m/z* (rel. int.): 316(25), 260 (64), 216(100), 202.08(93).

IR v_{max} (film)/cm^{-1}: 3230, 2924, 1738, 1268.

Experimental: Synthesis of Aniline core:

Synthesis of 2, 4-dihydroxyacetophenone (45):
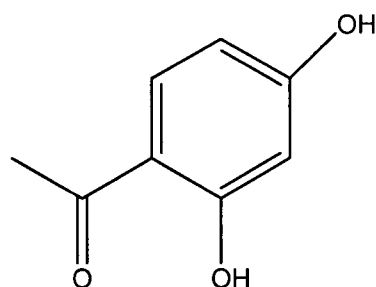
To a solution of resorcinol 100 mg (0.909 mmol) and acetic acid 2 mL, 1equivalent of zinc chloride (1.0 M in ethyl ether solution) was added. This reaction mixture was irradiated in a microwave at 200 °C for 7 min and transferred into a round bottom flask and concentrated under reduced to pressure to remove solvent. The crude residue was then extracted with ethyl acetate (10 mL x 3). Organic layers were pooled together and dried over magnesium sulphate. The solvent was concentrated under reduced pressure using rotary evaporator. The crude material was subjected to column chromatography over silica gel. Finally a pure compound (45) was obtained in 80% yield. The structure was determined by NMR and melting point (mp). 137 °C (lit.141-144°).

Synthesis of 2, 4-Dimethoxyacetophenone (46):
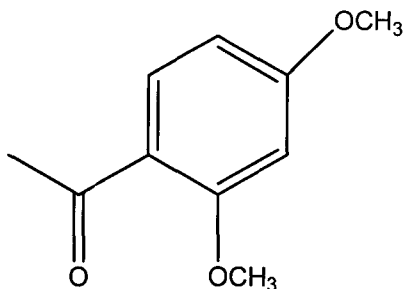
To a flame dried flask 2, 4-Dihydroxy acetophenone (100 mg, 0.65 mmol) and K_2CO_3 (180 mg, 1.315 mmol) were dissolved in 6 mL acetonitrile cooled to 0 °C. To this solution, dimethyl sulphate (166 µL, 1.315 mmol) was added at the same temperature. The reaction mixture was allowed to warm to room temperature and continued overnight. Reaction mixture was filtered and the solution was concentrated under reduced pressure. The remaining crude was extracted with ethyl acetate solution (10 mL x 3) and pooled organic layers were washed with saturated Ammonium chloride solution (10 mL). The organic layer was dried over magnesium sulphate. The solvent was removed under reduced pressure and a pure compound (90 %) was isolated by column chromatography using hexane and ethyl acetate as eluent. The structure of the compound was determined by using NMR and compared with the literature data.

Synthesis of 2, 4-Dimethoxy Benzoic Acid (47):
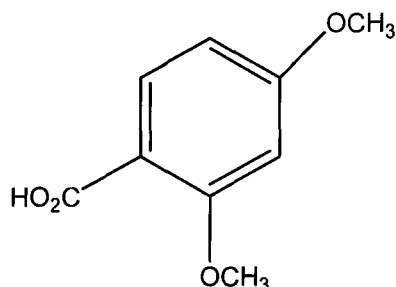
To a flame dried flask Ketone (90 mg, 0.5 mmol) was added to a suspension of finely ground KOH (150 mg, 2.7 mmol) in DMF (12 mL) and gradually heated to 65 °C. After 3 h an additional amount of KOH (130 mg, 2.3 mmol) was added and heated for overnight. The reaction mixture was cooled to RT, diluted with water (50 mL) and extracted with DCM (3 x 20 mL). The aqueous layer was acidified with Conc.HCl to P^H1 and extracted with DCM to yield acid. Crude product was subjected to column chromatography and the final product was obtained as white solid in 68% yield.

Synthesis of 5-bromo-2, 4-dimethoxy benzoic acid (48):
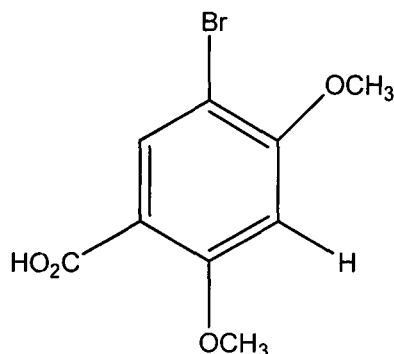
To a flame dried flask KBr (78.5 mg, 0.66 mmol) was added to a solution of 2, 4-dimethoxy benzoic acid (100 mg, 0.549 mmol) in methanol. The reaction mixture was

stirred for 15 min at RT. Oxone (405 mg, 0.66 mmol) was added. After TLC indicated

the reaction to be completed in 5 h. The reaction mixture was filtered to remove

precipitated salts and solvent removed under reduced pressure to give a gum which was

subjected to column chromatography and the pure product (48) obtained by using hexane

and ethyl acetate as eluents. The pure product was obtained in 83% yield. The structure

of the compound was determined by using NMR and mass spectroscopic techniques.

^{1}H-NMR: δ (200 MHz, CDCl$_3$) 3.950 (3H, s, OCH$_3$), 4.119 (3H, s, OCH$_3$), 6.574 (1H, s,

H-3), 8.397 (1H, s, 6H).

^{13}C-NMR: δ (125 MHz, CDCl$_3$) 56.8 (C-2 OCH$_3$), 57.0 (C-4 OCH$_3$), 97.2 (C-3), 102.2

(C-5), 106.0 (C-1), 138.2 (C-6), 158.6 (C-2), 160.2 (C-4), 162.5 (COOH).

MS: m/z (rel. int.): 262 (10), 261 (100), 259 (100), 230 (50), 186 (45).

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
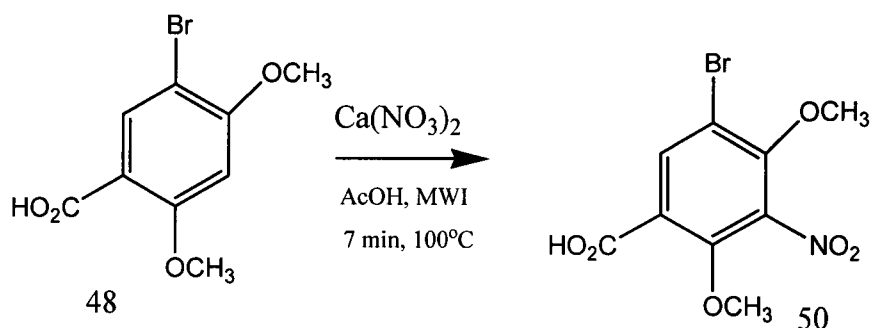
To a solution of 5-bromo2, 4-dimethoxy benzoic acid (13 mg, 0.050 mmol) in AcOH (0.3

mL, 0.1 mmol) calcium nitrate was added at RT. The reaction carried out under MWI for

7 min at 100 °C. The solvent was evaporated under reduced pressure, the residue

dissolved in ethyl acetate (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EA (5 mL x 3) and pooled organic phases were dried over MgSO$_4$ and filtered. Concentration invacuo followed by chromatography on silica gel (hexane: EA 80: 20) yielded starting material in 100%.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
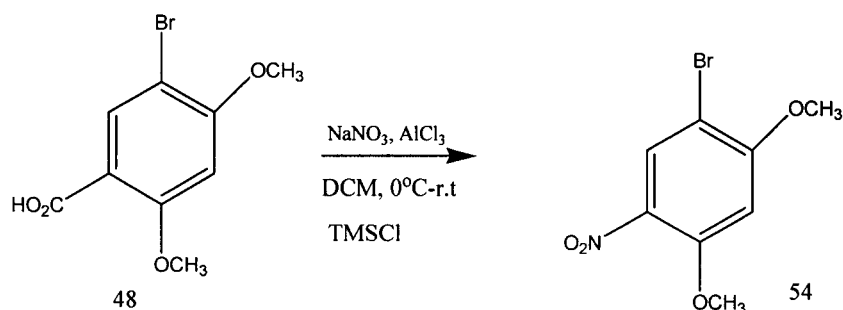
Chloro trimetheylsilane (60 μL, 0.477 mmol) was added dropwise to a stirred heterogeneous mixture comprising NaNO$_3$, bromo acid to be nitrated (50 mg, 0.194 mmol) and powdered AlCl$_3$ (83.4 mg, 0.627 mmol) stirred in anhydrous DCM (2.5 mL) at 0 °C for 2 h. The stirring was continued further at RT and left overnight. No reaction was observed by TLC. So the reaction mixture was refluxed overnight. The solvent was removed under reduced pressure and the residue was extracted with Et$_2$O. The organic layers were mixed together and washed with ice cold sodium bicarbonate until the washings were neutral. The organic layer was further washed with brine and dried over magnesium sulphate.

10 % of the ipso-nitration product was observed and 90 % starting material was recovered after purification.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
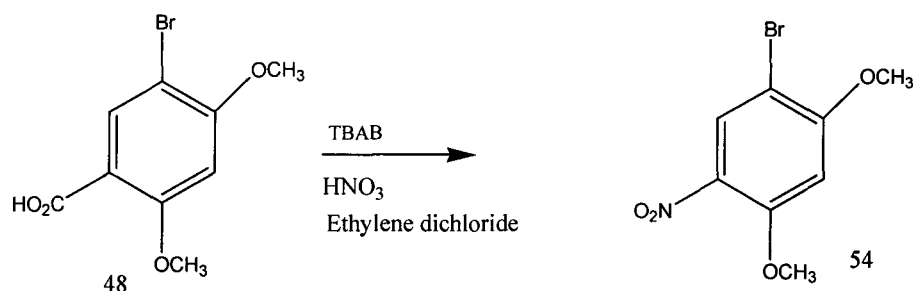
To a solution of 5-bromo-2, 4-dimethoxy benzoic acid (50 mg, 0.191 mmol) and TBAB,

10 mol % of substrate in EDC (5 mL), nitric acid (0.38 mmol) was added in r.b. flask and

stirred at RT for overnight. Solvent was removed under reduced pressure extracted with

DCM, washed with water and finally dried over magnesium sulphate. The residue was

subjected to column chromatography and purified the product in 10% yield.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
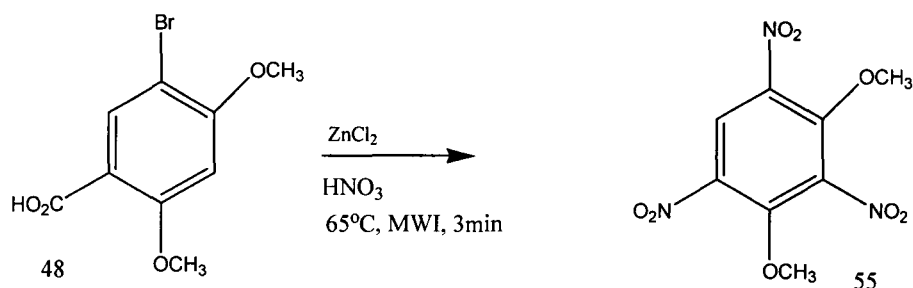
To a solution of 5-bromo-2, 4-dimethoxy benzoic acid (16 mg, 0.0597 mmol) and $ZnCl_2$

in 1.0 M ethyl ether solution (70 μL), nitric acid (6 μL) was added . The reaction mixture

was irradiated in microwave at 65 °C for 3 min. The reaction mixture was extracted with EA and washed with water. Organic layers were mixed together and dried over magnesium sulphate. Crude product was subjected to column chromatography. Trinitro compounds were obtained as 40% yield.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
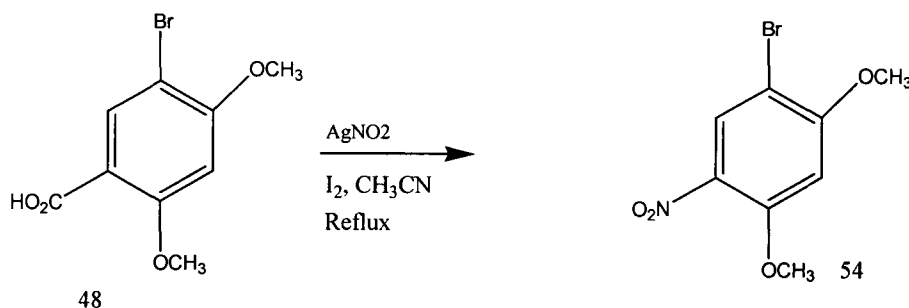
To a solution of bromo derivative to be nitrated (30 mg, 0.114 mmol) and I_2 in (20 mL) acetonitrile, silver nitrite (67 mg) was added.

At room temperature no reaction was observed and then after refluxed at 80 °C overnight and cooled to RT and concentrated the solvent under reduced pressure, EA (7 mL) was added and washed with water. Pooled organic layers were dried over magnesium sulphate and the crude product was subjected to column chromatography 1-bromo-2, 4-dimethoxy 5-nitro benzene was obtained in 10 % yield and 90 % starting material was retained.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
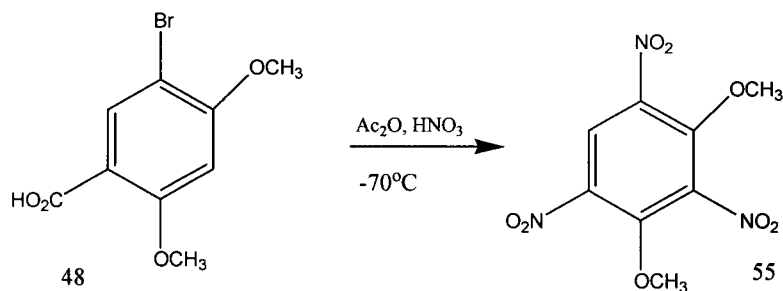
Acylnitrate was generated by the dropwise addition of nitric acid (12 µL, 0.174 mmol) to cooled (0 °C) acetic anhydride (0.2 mL) followed by standing at room temperature for 10 min and then was used immediately.

To a stirred solution of bromo compound to be nitrated (0.0574 mmol) in acetic anhydride (0.3 mL) at -70 °C, a solution of performed acetyl nitrate was added dropwise via an addition funnel over 30 min. The mixture was continued at the same temperature for 4 hours and left over night at room temperature. The solvent was removed under reduced pressure and washed with water, sodium bicarbonate (5%) and extracted with EA. Pooled organic layers were dried over magnesium sulphate and the crude product was subjected to column chromatography and tri nitro products were obtained in 20% yield and 80% starting material.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
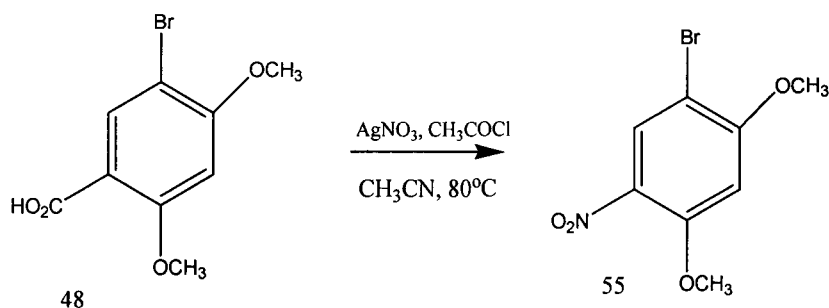
Acetyl chloride (2.198 mg, 0.028 mmol) in acetonitrile (50 µL) was added drop wise to a rapidly stirred solution of silver nitrate (4.76 mg, 0.028 mmol) in acetonitrile (50 µL), containing bromo derivative to be nitrated at reflux. Silver chloride precipitated immediately. The reaction was continued for another 48 h. Filtered the silver nitrate precipitate and washed with water and extracted with EA. Pooled organic layers were dried over magnesium sulphate and the crude product was subjected to column chromatography. 85% of the starting material was recovered from the column.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy benzoic acid:
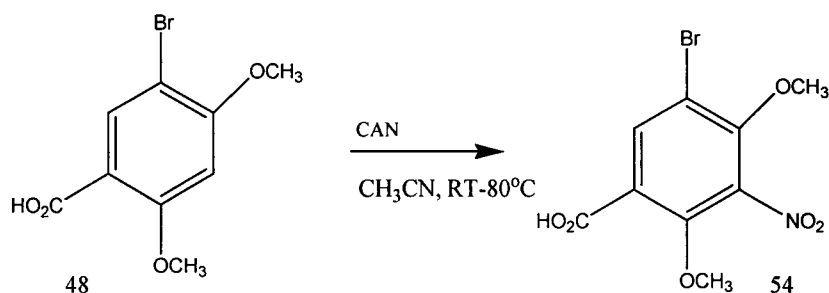
Bromo derivative to be nitrated (20 mg, 0.076 mmol) was dissolved in acetonitrile (1 mL). Cerium (IV) ammonium nitrate (42 mg) was dissolved in acetonitrile (1.2 mL) was added drop wise to the above mixture fro about 25 min. At RT no reaction was observed and continued to heat at 80 °C for over night. Workup with EA and dried over magnesium sulphate. The crude product was subjected to column chromatography. 100% starting material was isolated.

Synthesis of 5-bromo-2, 4-dimethoxy acetophenone (56):
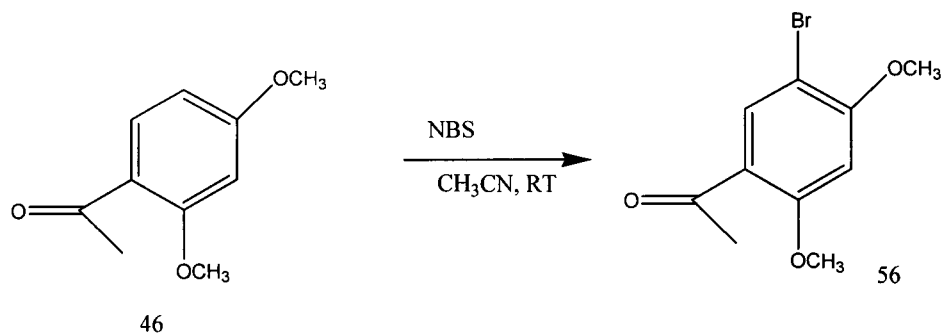
To a solution of 2, 4-dimethoxy acetophenone (83 mg, 0.461 mmol) in 25 mL, NBS (82 mg, 0.461 mmol) was added. The reaction mixture was stirred for over night at room temperature. Then filtered the solvent and concentrated under reduced pressure and extracted with EA and dried over magnesium sulphate and subjected to column chromatography and the desired bromo derivative was obtained in 85% yield.

Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy acetophenone (57):
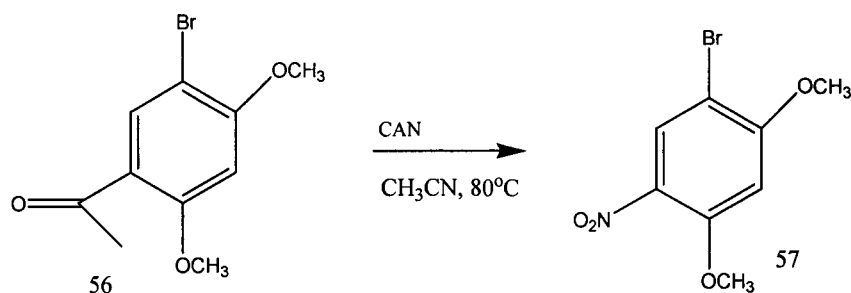
To a solution of 5-bromo-2, 4-dimethoxy acetophenone (20 mg, 0.0772mmol) in acetonitrile (1.2 ml), CAN (42 mg, 0.0772 mmol) in 1 mL acetonitrile was added drop wise for about 30 min. The reaction was heated at 80 °C. The solvent was evaporated under reduced pressure, the yellowish residue dissolved in ethyl acetate (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EA (5 mL x 3) and pooled organic phases were dried over $MgSO_4$ and filtered. Concentration invacuo

followed by chromatography on silica gel (hexane: EA 60: 40) yielded ipso product as a major product.
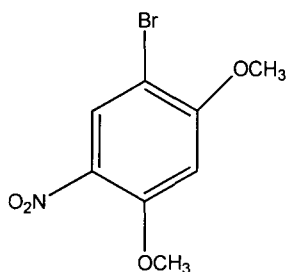
^{1}H-NMR: δ (200 MHz, CDCl$_3$) 4.014 (6H, s, 2 x OMe), 6.524 (1H, s, 3-H), 8.287 (1H, s, 6H).

^{13}C-NMR: δ (65 MHz, CDCl$_3$) 56.98 (2 x OCH$_3$), 96.93 (C-3), 101.83 (C-1), 131.14 (C-6), 132.75 (C-5), 155.31 (C-4), 160.95 (C-2).
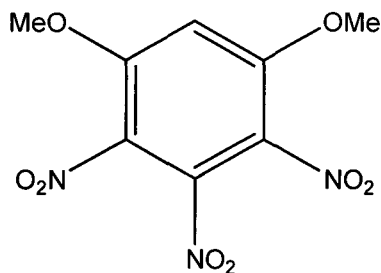
MS: *m/z* (rel. int.): 263 (22), 262 (65), 215 (100).
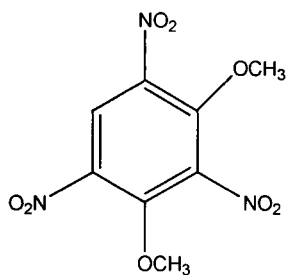
^{1}H-NMR: δ (200 MHz, CDCl$_3$) 4.143 (6H, s, 2 x OMe), 8.498 (1H, s, 6-H).

^{13}C-NMR: δ (65 MHz, CDCl$_3$) 63.33 (2 x OCH$_3$), 118.83 (C-3), 121.93 (C-6), 140.05 (C-1, 5), 157.83 (C-2, 4).

MS: *m/z* (rel. int.): 273 (7), 243 (5), 183 (4).

^{1}H-NMR: δ (200 MHz, CDCl$_3$) 4.108 (6H, s, 2 x OMe), 8.761 (1H, s, 6-H).

^{13}C-NMR: δ (65 MHz, CDCl$_3$) 64.59 (2 x OMe), 124.35 (C-6), 136.83 (C-3), 150.55 (C-2, 4).

Synthesis of 5-bromo-2, 4-dimethoxy methyl benzoate (49):
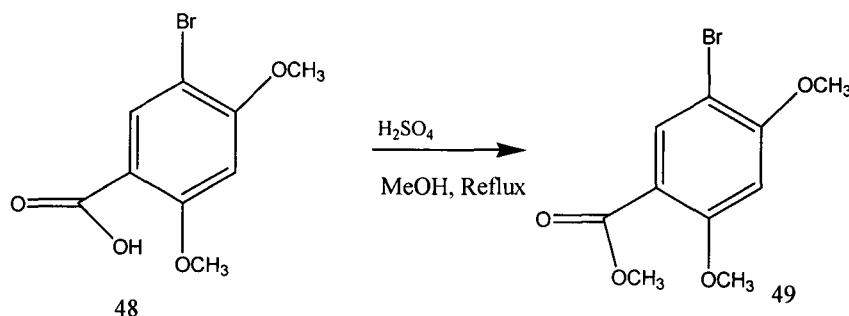
In a flame dried flask 2, 4-dimethoxy-5-bromo benzoic acid (50 mg, 0.195 mmol) was treated with a solution of Conc.H$_2$SO$_4$ (20 μL) in methanol (1.2 mL) and refluxed for 3 h. The solution was cooled to room temperature and concentrated under reduced pressure. EA was added and the organic layer was washed with 5% sodium bicarbonate and brine solution, dried over magnesium sulphate. Desired ester was obtained in 85% yield.

^{1}H-NMR: δ (200 MHz, CDCl$_3$) 3.839 (3H, s, COOMe), 3.923 (6H, d, 2 x OMe), 6.520 (1H, s, 3-H), 8.234 (1H, s, 6-H).

^{13}C-NMR: δ (65 MHz, CDCl$_3$) 51.90 (COOCH3), 56.37 (2 x OCH$_3$), 96.57 (C-3), 101.64 (C-5), 115 (C-1), 136.30 (C-6), 159.96 (C-2), 160.88 (C-4), 165 (C=O).

MS: *m/z* (rel. int.): 275 (48), 273 (53), 242 (100).

## Synthesis of 5-bromo-3-nitro-2, 4-dimethoxy methyl benzoate (58):
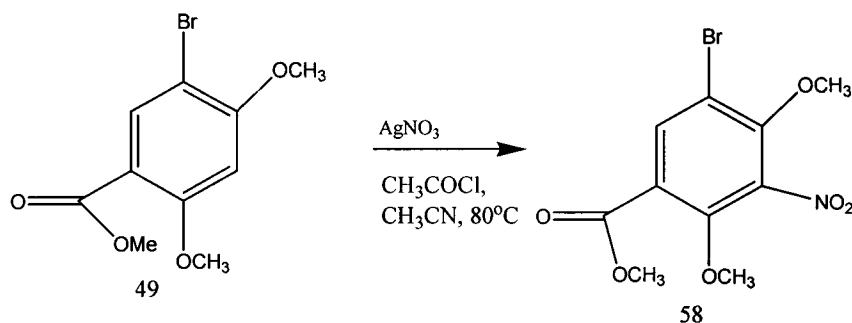
No Reaction observed after over night heating. 100% starting material obtained after workup. The solvent was evaporated under reduced pressure, the pale yellowish residue dissolved in ethyl acetate (5 mL) and washed with water (5 mL). The aqueous layer was extracted with EA (5 mL x 3) and pooled organic phases were dried over MgSO$_4$ and

filtered. Concentration invacuo followed by chromatography on silica gel (hexane: EA 60: 40) yielded ipso product as major product in 33%.
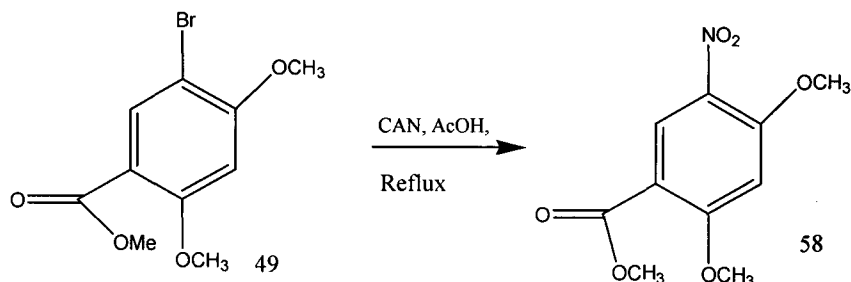
To a solution of 5-bromo-2, 4-dimethoxy benzoate (10.3 mg, 0.0363 mmol) in AcOH (0.5 mL), CAN (22 mg, 0.040 mmol) was added and heated to 95 °C. After over night reaction mixture was cooled to room temperature and concentrated under reduced pressure and EA was added, washed with H_2O. Pooled organic layers were dried over magnesium sulphate. The crude product was subjected to column chromatography. A mixture of two products was obtained. I could not separate these two compounds by using column chromatography because two compounds are very close to each other.

^{1}H-NMR: δ (200 MHz, CDCl$_3$) 3.880 (3H, s, COOMe), 3.967 (6H, d, 2 x OMe), 6.656 (1H, s, 3-H), 8.634 (1H, s, 6-H).
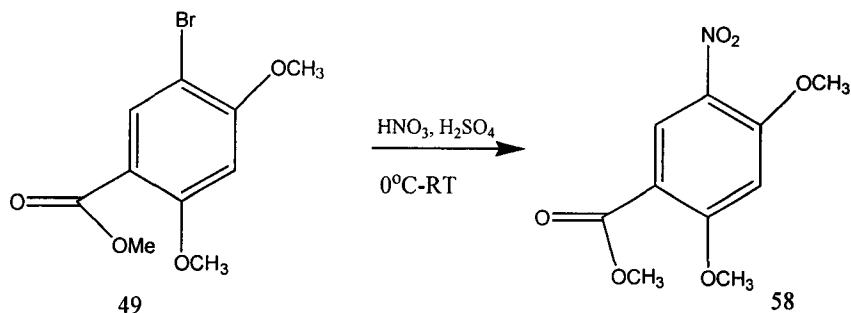
5-bromo-2, 4-dimethoxybeanzoate (15 mg) was added to Conc.H_2SO_4 (15 µL) cooled to 0 °C. Nitration mixture prepared by mixing equal amounts of sulphuric acid and nitric acid and cooled to 0 °C. 10 µL of cooled nitration mixture was added to the above reaction mixture drop by drop for about 25 min. Reaction continued at the same temperature for another 2 h. Then the reaction mixture was allowed to warm to room temperature. And water (1 mL) added to the reaction mixture and extracted with EA and washed with 5% sodium bicarbonate and brine solution. Pooled organic layers were dried over magnesium sulphate and subjected to column chromatography.

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Appendix: NMR, Mass, IR spectra of

SAKURANETIN.

Sample: F-307 AV-700 Reference Spectrum
1H NMR in CD3OD (15 Jan. 2006)
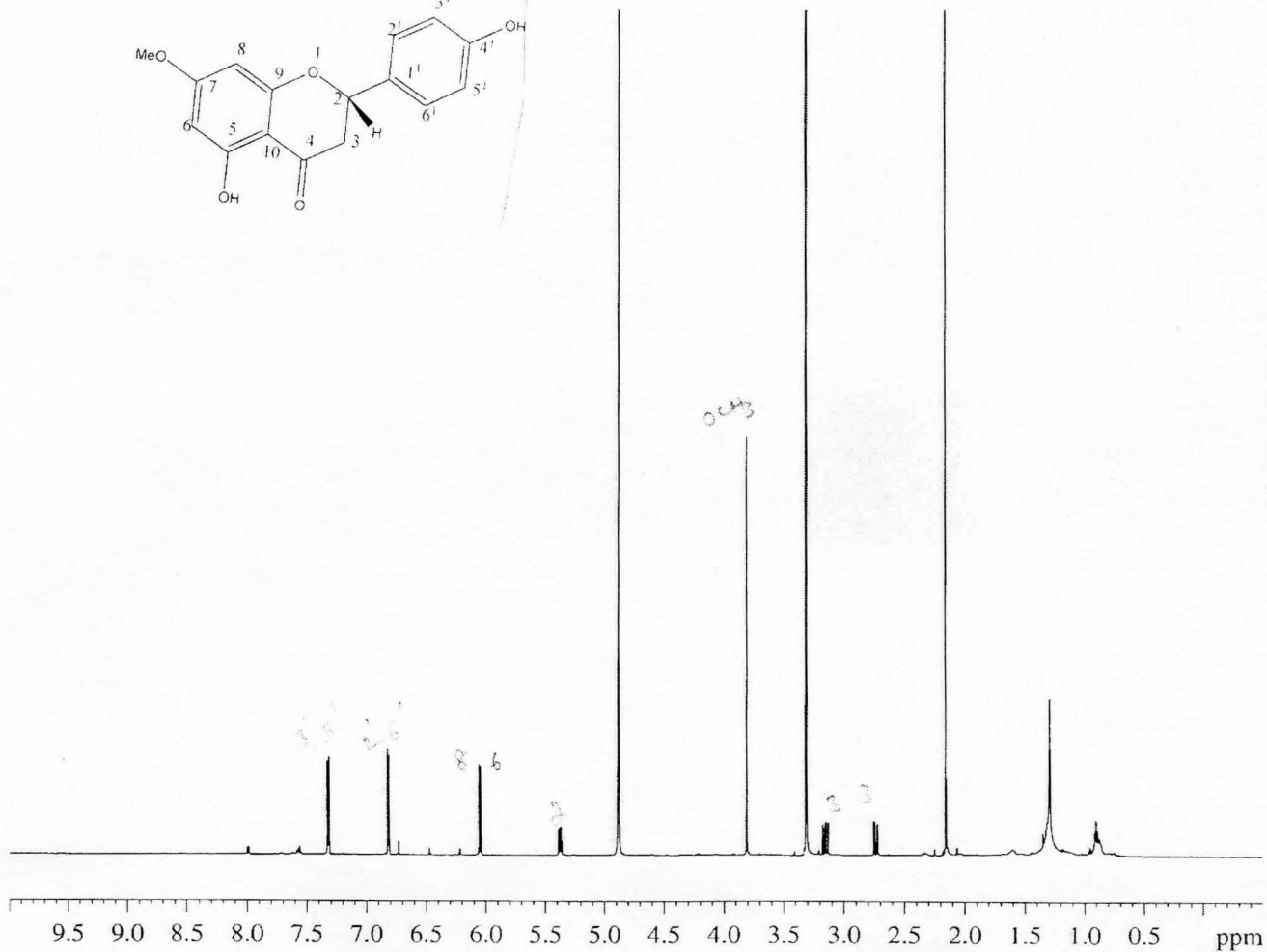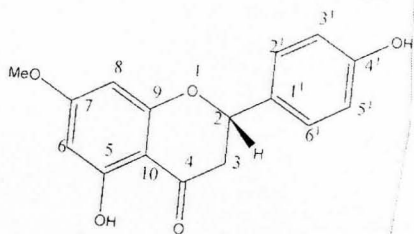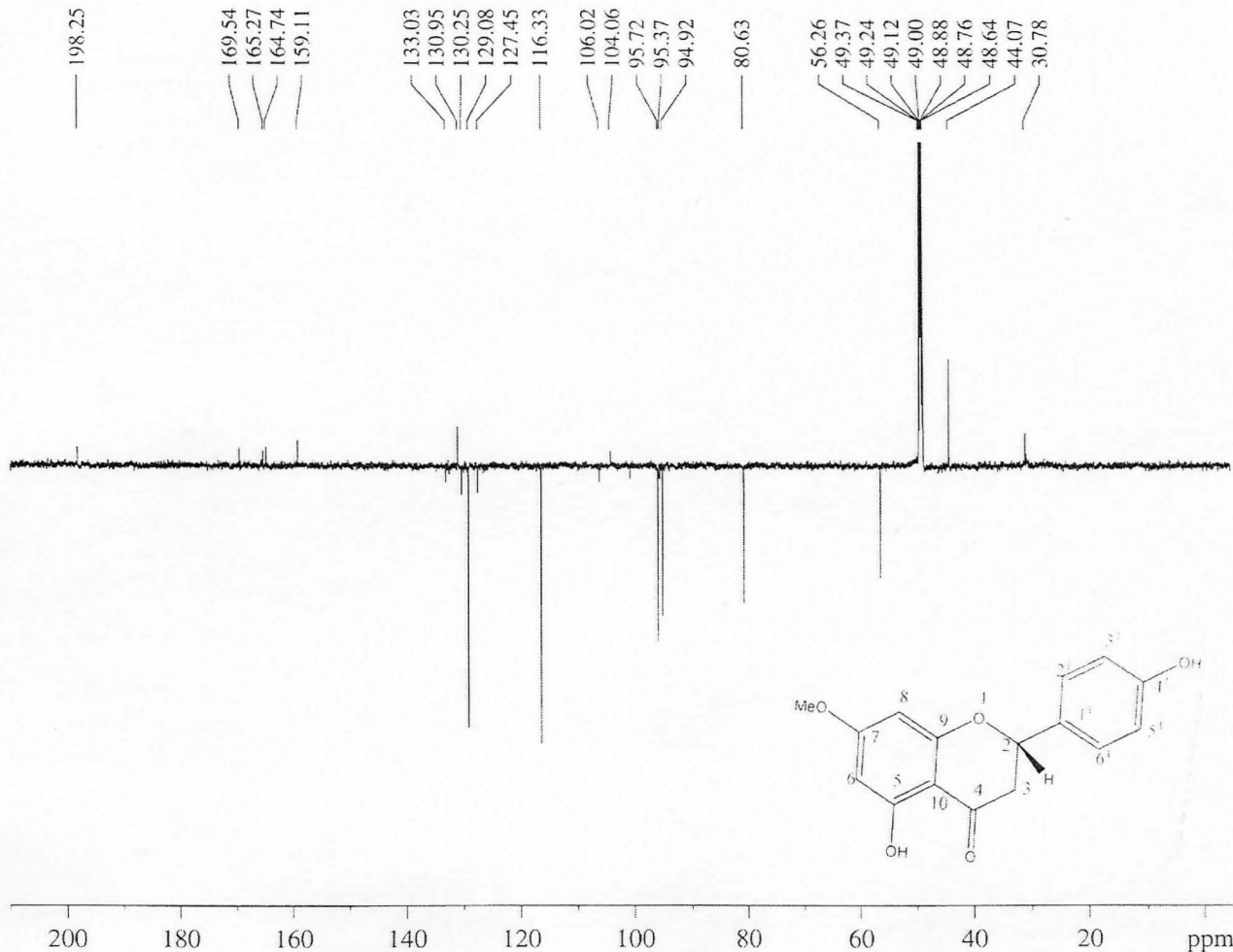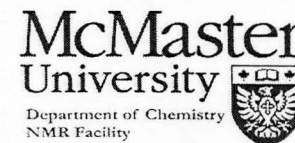
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EXPNO   35
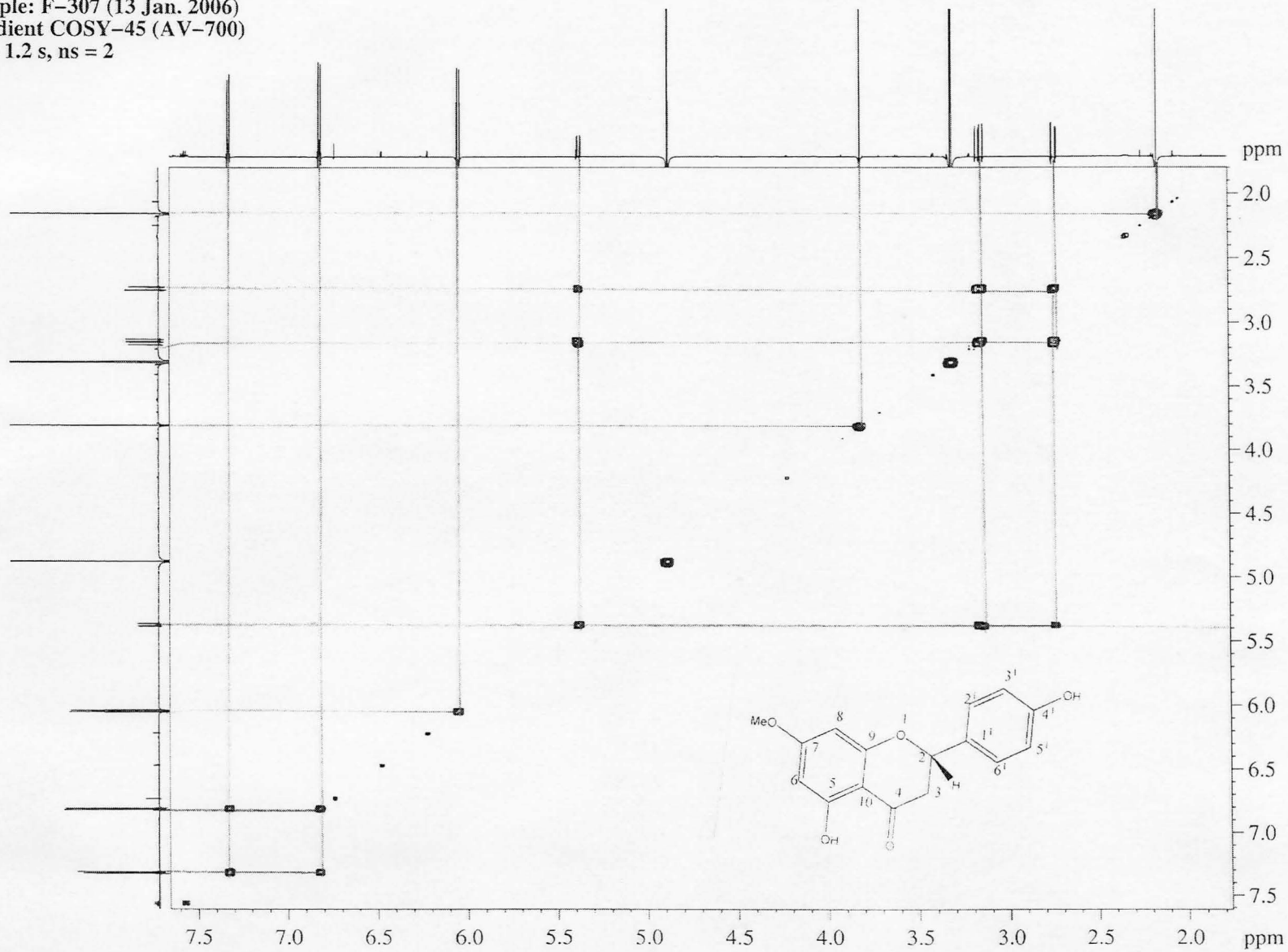PROCNO   1

F2 – Acquisition Parameters
Date_   20060115
Time   13.09
INSTRUM   av700
PROBHD 5 mm CPTCI 1H–
PULPROG   zg30
TD   65536
SOLVENT   MeOD
NS   8
DS   2
SWH   9124.088 Hz
FIDRES   0.139223 Hz
AQ   3.5914776 sec
RG   35.9
DW   54.800 usec
DE   6.00 usec
TE   300.0 K
D1   1.50000000 sec
MCREST   0.00000000 sec
MCWRK   0.01500000 sec

======== CHANNEL f1 ========
NUC1   1H
P1   8.00 usec
PL1   2.80 dB
SFO1   700.2338573 MHz

F2 – Processing parameters
SI   65536
SF   700.2300135 MHz
WDW   EM
SSB   0
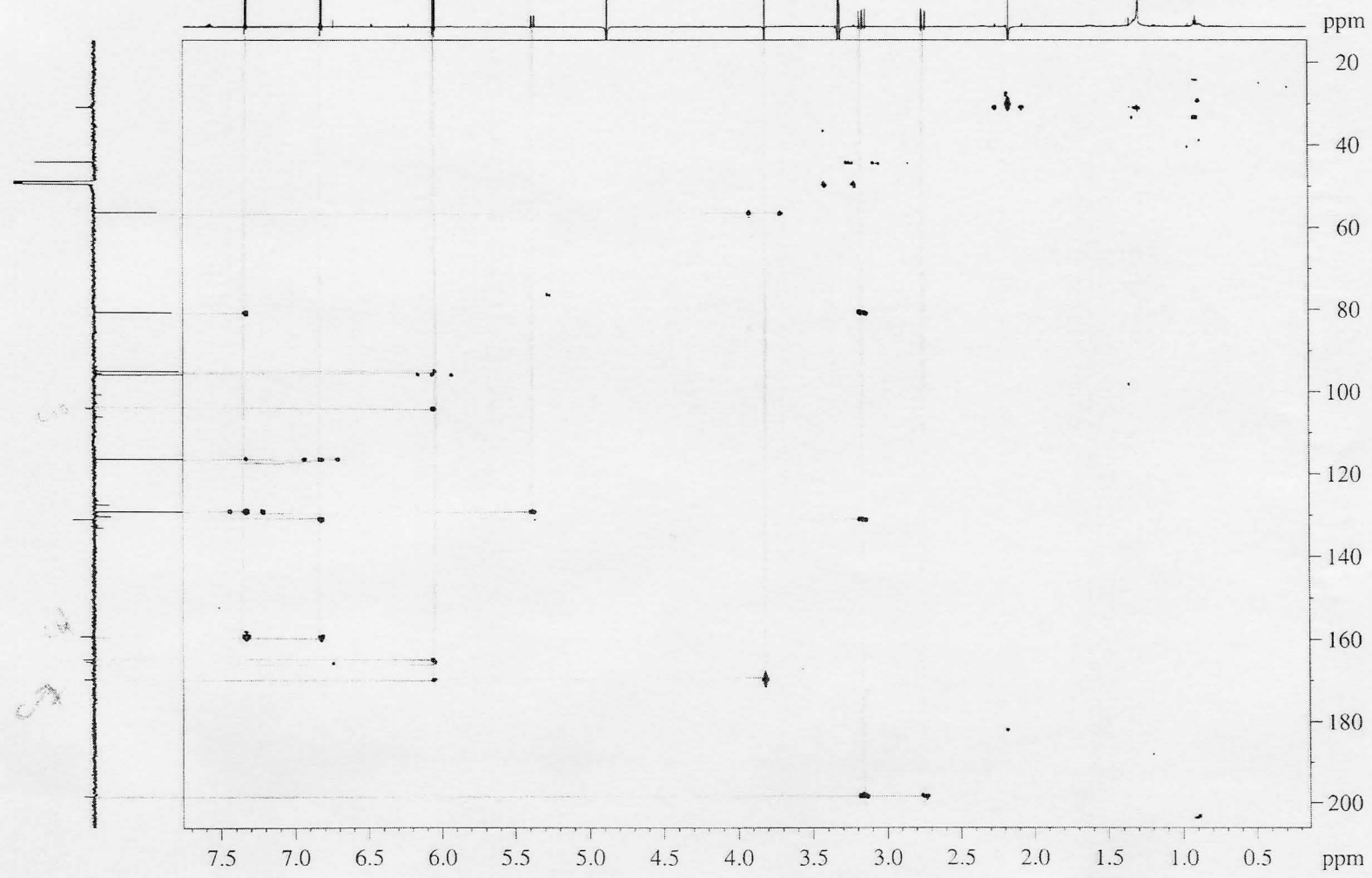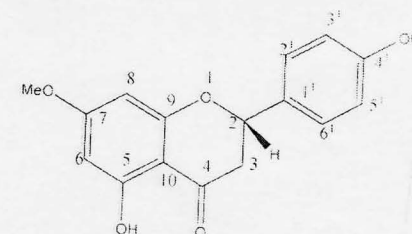LB   0.20 Hz
GB   0
PC   1.40

Sample: F-307 AV-700 (13 Jan. 2006)
13C Jmod in CD3OD
CH and CH3 −ve Phase; C and CH2 +ve Phase

198.25

169.54
165.27
164.74
159.11

133.03
130.95
130.25
129.08
127.45
116.33

106.02
104.06
95.72
95.37
94.92

80.63

56.26
49.37
49.24
49.12
49.00
48.88
48.76
48.64
44.07
30.78

200   180   160   140   120   100   80   60   40   20   ppm

Sample: F-307 AV-700 (13 Jan. 2006)
1H-13C Gradient HMBC
d1 = 1.2 s, ns = 2

100

180.0288

%

287.0520

288.2486

167.0179

193.0396

285.0540

166.0215

254.0504

120.0533

255.0605

65.0400   91.0550

289.0975

147.0473   226.0639   301.1021

369.3457 379.4193   460.3445 479.4963

0

40   60   80   100   120   140   160   180   200   220   240   260   280   300   320   340   360   380   400   420   440   460   480   500   m/z

Bio-Rad Win-IR

530
574
616
739
807
833
1031
1074
1090
1157
1191
1205
1264
1305
1383
1450
1519
1615
1641
1743
2284
2349
2854
2925
3343

View Mode

1/31/06 7:12 PM   Res=8cm-1

1000

2000

3000

48

46

44

42
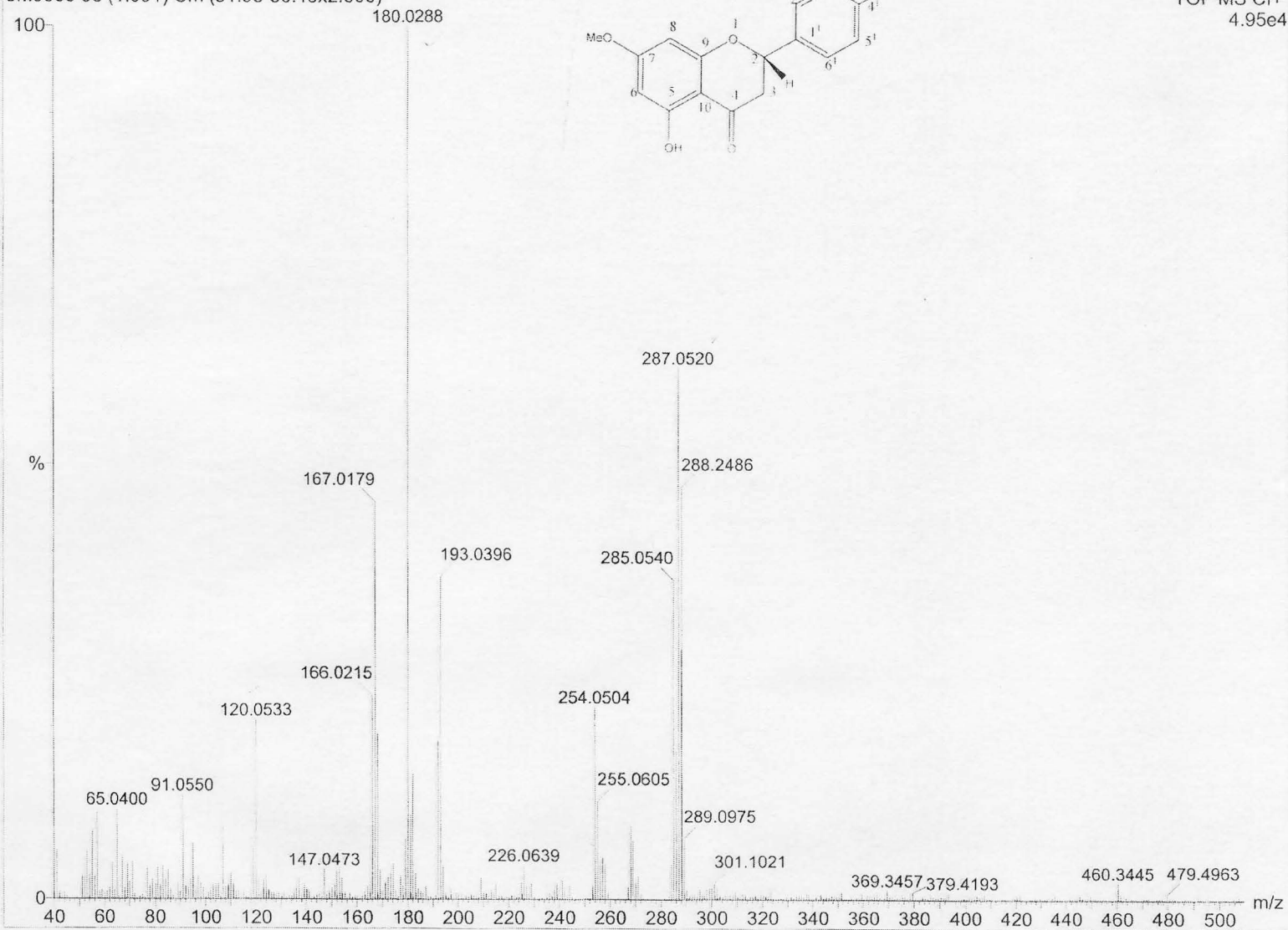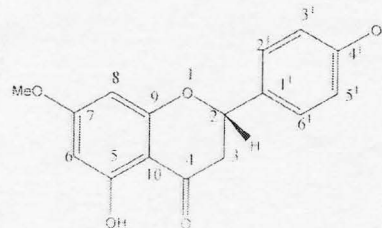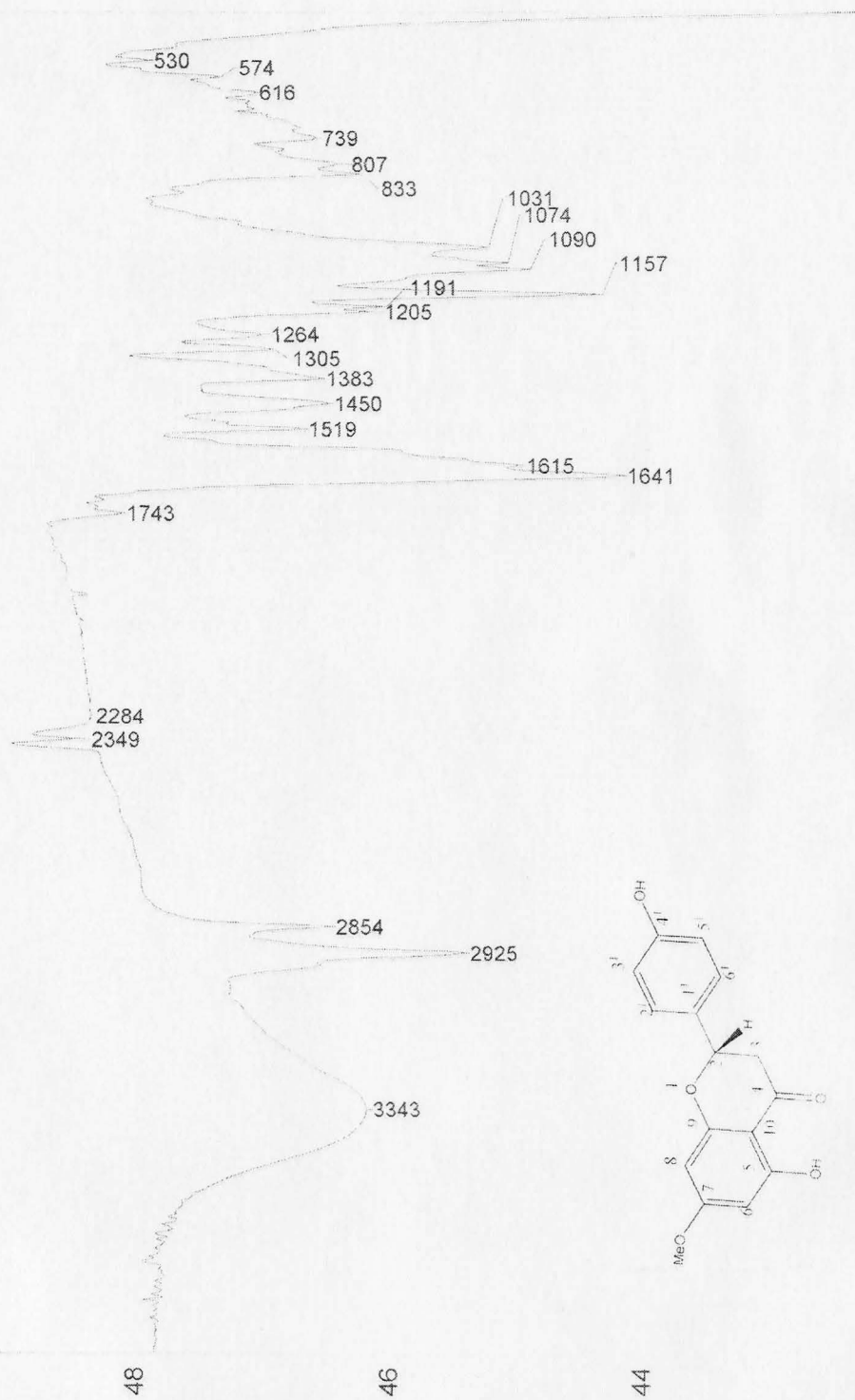
File # 3 : BREDDYSAKURANETIN

Number of Scans: 16

Comment: nacl film