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SYNTHETIC STUDIES TOWARDS THE NATURAL PRODUCT LEOPOLIC ACID A

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ATUL ARUN DHAVAN

TUTORE

PROF.SSA SABRINA DALLAVALLE

COORDINATORE

PROF.DANIELE DAFFONCHIO

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Dedicated to

Sudhir and Narayan

CERTIFICATE

This is to certify that the work incorporated in the thesis entitled **"Synthetic studies towards the natural product Leopolic acid A"** which is being submitted to the **University of Milan** for the award of **Doctor of Philosophy** in Chemistry, Biochemistry and Ecology of pesticides by **Atul Arun Dhavan** was carried out by him under my supervision at **University of Milan, Milan**. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other University.

Prof. Sabrina Dallavalle

(Research Supervisor)

Candidate's Declaration

I hereby declare that the thesis entitled **"Synthetic studies towards the natural product Leopolic acid A"** submitted for the award of degree of **Doctor of Philosophy** in Chemistry, Biochemistry and Ecology of pesticides to the **University of Milan, Milan**. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other University. This work was carried out by me at the **DeFENS, Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy**.

Atul Arun Dhavan December 02, 2015

Department of Food, Environmental and Nutritional Sciences

Division of Chemistry and Molecular Biology,

University of Milan

Milan, Italy.

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Atul Arun Dhavan

CONTENTS

Abbreviations

Ac	-	Acetyl
ACN	-	Acetonitrile
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
Ar	-	Aryl
Bn	-	Benzyl
BnBr	-	Benzyl bromide
Brs	-	Broad singlet
Boc	-	*tert*-Butoxy carbonyl
$(Boc)_2O$	-	Di-*tert*-butyl dicarbonate
t-Bu	-	*tert*-Butyl
BuLi	-	Butyl Lithium
CAN	-	Ceric ammonium nitrate
Cat.	-	Catalytic/Catalyst
$CDCl_3$	-	Deuterated chloroform
COSY	-	Correlation spectroscopy
DBE	-	Double bond equivalent
DCM	-	Dichloromethane

d	-	Doublet
dd	-	Doublet of doublet
DCC	-	Dicyclohexylcarbodiimide
DDQ	-	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL-H	-	Diisobutylaluminium hydride
DIPEA	-	Diisopropylethylamine
DMAP	-	*N, N'*-Dimethylaminopyridine
DMF	-	*N, N'*-Dimethylformamide
DMF.DMA	-	*N*,*N*-Dimethylformamide dimethyl acetal
DMSO	-	Dimethyl sulfoxide
Et	-	Ethyl
Et_3N	-	Trimethyl amine
EtOAc	-	Ethyl acetate
Et_2O	-	Diethylether
EtOH	-	Ethanol
g	-	grams
h	-	hours
HMBC	-	Heteronuclear Multiple Bond Correlation
HMPA	-	Hexamethylphosphoramide

HPLC	-	High performance liquid chromatography
HREI MS	-	High-resolution electrospray ionisation mass spectrum
Hz	-	Hertz
IBX	-	2-Iodoxybenzoic acid
LAH	-	Lithium aluminium hydride
LDA	-	Lithiumdiisopropylamide
LiHMDS	-	Lithium bis(trimethylsilyl)amide
m	-	Multiplate
Me	-	Methyl
mg	-	Miligram
MIC	-	Minimum inhibitory concentration
min	-	Minutes
MeOH	-	Methanol
mmol	-	mmol
m.p	-	Melting point
MRSA	-	Methicillin-resistant *Staphylococcus aureus*
NaOEt	-	Sodium ethoxide
NBS	-	N-Bromosuccinimide
NIS	-	N-Iodosuccinimide

NMR	-	Nuclear magnetic resonance
NOESY	-	Nuclear overhauser effect spectroscopy
NMM	-	N- methyl morpholine
PCC	-	Pyridiniumclorochromate
PDC	-	Pyridiniumdichromate
PFP	-	Pentaflourophenol
PMB	-	Paramethoxybenzyl
Ph	-	Phenyl
Pd/C	-	Palladium on carbon
ppm	-	Parts per million
*p*TSA	-	*para*-Toluenesulfonic acid
Py	-	Pyridine
rt	-	Room temperature
s	-	Singlet
TBAF	-	Tetrabutylammonium fluoride
TBS	-	*tert*-Butyldimethylsilyl
TBSOTf	-	*tert*-Butyldimethylsilyl triflate
TEA	-	Triethyl amine
THF	-	Tetrahydrofuran

TFA	-	Trifluoroacetic acid
TLC	-	Thin layer chromatography
TMSCl	-	Trimethylsilyl chloride
Val	-	Val

CHAPTER 1

General Introduction

1.1 Natural products in history

Natural products (secondary metabolites) have been the most successful source of potential drug leads and impressive number of modern drugs finds their origin in natural products[1-5]. Natural products can also be prepared by chemical synthesis (including both semi synthetic and total synthesis) and have played a vital role in the development of the field organic chemistry by providing challenging synthetic targets. Natural product chemistry has experienced vast and diversified growth, making natural products the subject of much interest and promise in the present day research directed towards drug design and discovery. The natural products are source of new compounds with diverse structural arrangements possessing interesting and useful biological activities. Historically pharmaceutical companies have utilised plant extract to produce relatively crude therapeutic formulation, but with advancement of antibiotic drug formulation of fairly purified compounds have become more typical.

Natural products can come from anywhere. People most commonly think of plants first when talking about natural products but trees and shrubs also provide good source of natural compounds that can be used as basis of new therapeutic agents. Animals too, whether highly developed or poorly developed, whether they live on land, sea, or in the air can be excellent sources of natural products. Bacteria, smuts, rusts, yeasts, molds, fungi, and many other forms of what we consider to be primitive life can provide compounds or leads to compounds that can potentially be very useful

therapeutic agents. Thus natural products have played and continue to play invaluable role in drug discovery.

1.2 Medicinal plants in Folklore

The natural products including plants, animals and minerals have been the basis of treatment of human diseases and ancient wisdom has been used in modern medicinal chemistry and will remain an important source in future medicine. The use of natural products has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. Healing with medicinal plants is an old concept for mankind. The nexus between man and his search for drugs in nature dates from the far past, of which there is number of evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. In ancient time people looked for drugs for rescue of their diseases. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. Modern science has acknowledged their active action, mode of action, effectiveness and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The dominant source of knowledge of natural products uses from medicinal plants is a result of man experiencing by trial and error for hundred of centuries[6-7]. One example involves the plant genus *Salvia* which grows throughout the south-western region of the United States as well as north-western Mexico and which was used by Indian tribes of southern California as an aid in childbirth. Male newborn babies were "cooked" in the hot *Salvia* ashes as it was believed that these babies consistently grew to be the strongest and healthiest members of their respective tribes and are claimed to have been immune from all respiratory ailments for life. The plant, *Alhagi maurorum* Medik (Camels thorn) secretes a sweet, gummy material from the stems and leaves during hot days. This gummy sap is called "*manna*" and consists mostly of melezitose, sucrose and invert sugar and it has been documented and claimed by the

Ayurvedic people that the plant aids in the treatment of anorexia, constipation, dermatosis, epistaxis, fever, leprosy, and obesity[8]. It was also used by the Israelis who boiled the roots and drank the extract as it stopped bloody diarrhea. The Konkani people smoked the plant for the treatment of asthma, whilst the Romans used the plant for nasal polyps[8]. The plant *Ligusticum scoticum* Linnaeus found in Northern Europe and Eastern North America was eaten raw first thing in the morning and was believed to protect a person from daily infection[9]; the root was a cure for flatulence[10-12], an aphrodisiac and was used as a sedative in the Faeroe Islands[10, 13]. *Atropa belladonna* Linnaeus (deadly nightshade) is found in central and Southern Europe, Western Asia, North Africa, North America and New Zealand. Its notoriously poisonous nature (three berries are sufficient to kill a child) firmly excluded it from the folk medicine compilation and seemed to have been accepted as dangerous to handle or to experiment with[14].

1.3 Types of Natural products

Natural products broadly divided into four parts

1.3.1 Natural products from microorganisms

1.3.2Natural products from plant sources

1.3.3 Natural products from marine sources

1.3.4 Natural products from animal sources

1.3.1 Natural products from microorganisms

Microorganisms are capable of producing natural products with widely divergent structures. Greatest attention in the past has been paid to natural products that have antibiotic properties. Microorganisms have wide variety of potentially active substances so a large number of terrestrial and marine microorganisms have been taken great tool of antibiotics.

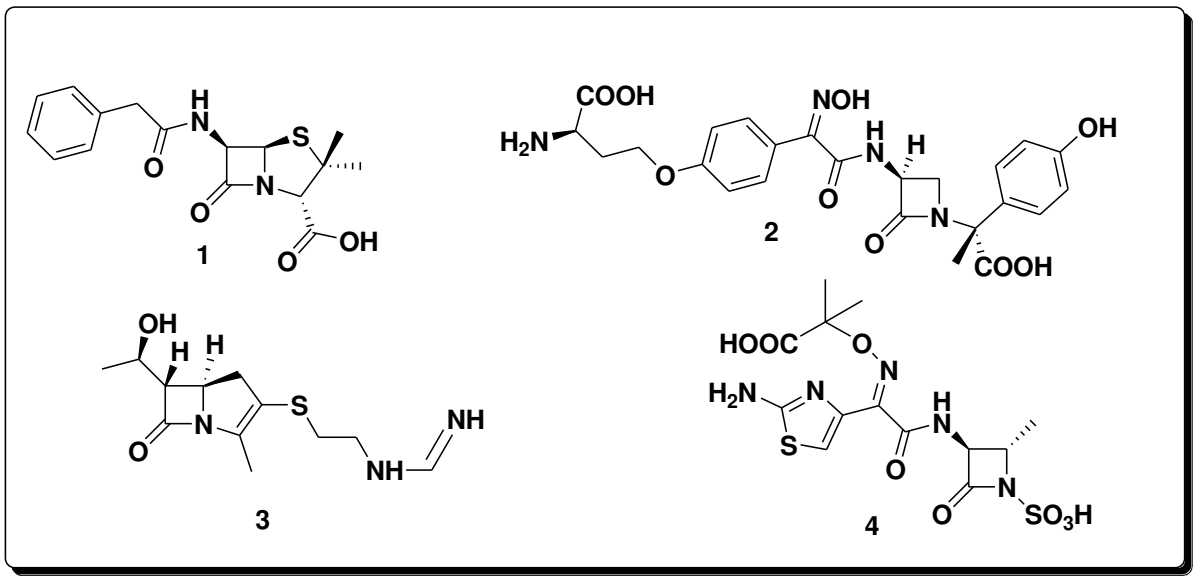
One of the most famous natural product discoveries derived from a fungus (microorganism) is that of penicillin (**1**) from the fungus, *Penicillium notatum* discovered by Fleming in 1929[15]. A countercurrent extractive separation technique which produced **1** in high yields was required for the *in vivo* experimentation that ultimately saved countless lives and won Chain and Florey (together with Fleming) the 1945 Nobel prize in Physiology and Medicine[16]. This discovery led to the re-isolation and clinical studies by Chain, Florey and co-workers in the early 1940s and commercialization of synthetic penicillins, which ultimately revolutionized drug discovery research[17-20].

There was huge worldwide research to discover new antibiotics from microorganisms and bioactive natural products after preliminary research on penicillin[20-21]. Up until 1968, old methods for detecting β-lactams were still being utilized and it was concluded that all natural β-lactams had been discovered. Nevertheless, this was not the case as with the introduction, in the 1970s, of new screening methods, the production of bacterial

strains supersensitive to β-lactams, tests for the inhibition of β-lactamases and specificity for sulphur- containing metabolites resulted in the discovery of novel antibiotic structural classes (nocardicins, carbapenems and monobactams) including the isolation of the antibiotics, nocardicin (**2**), imipenem (**3**) and aztreonam (**4**), respectively (Figure 1)[21-23].A number of β-lactams (cephalosporins, carbapenems and penems) have reached the clinical practice, along with the novel class of broad spectrum antibiotics called the glycylcyclines[23].

Figure 1

Structure of Penicillin (1), Nocardicin (**2**), Imipenem (**3**) and Aztreonam (**4**),

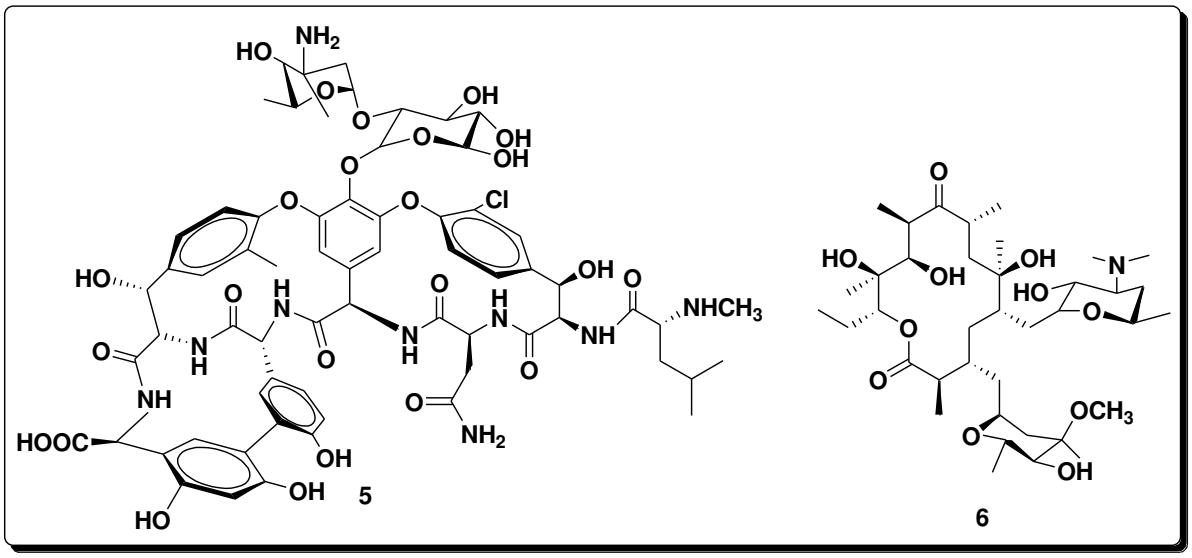


Vancomycin (**5**) a glycopeptide antibiotic produced in cultures of *Amycolatopsis orientalis* which is active against a wide range of gram-positive organisms such as *Staphylococci* and *Streptococci* and against gram-negative bacteria, mycobacteria and fungi, was approved by the FDA in 1958 (Figure 2). It is used for the treatment of severe infection and against susceptible organisms in patients hypersensitive to penicillin. The 14-membered macrocycle erythromycin (**6**) from *Saccharopolyspora erythraea* is an antibacterial drug, which contains propionate units (Figure 2). Erythromycin has broad spectrum activities

against gram-positive *cocci* and *bacilli* and is used for mild to moderate, upper and lower respiratory tract infections. Currently there are three semisynthetic ketolide derivatives of erythromycin, cethromycin (ABT-773, Restanza™), EP-420 (by Enanta Pharmaceuticals) and BAL-19403 (by Basilea) which are in clinical development.

Figure 2

Structure of Vancomycin (**5**), Erythromycin (**6**)



1.3.2 Natural products from plant

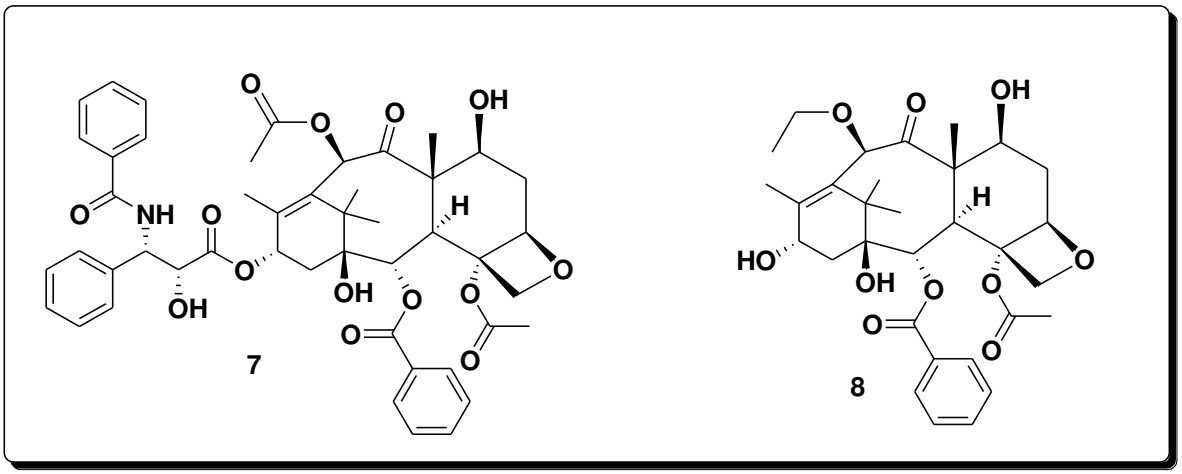
Plants have been part of our lives since beginning of time. We get numerous products from plants. The use of plants to heal or combat illness is probably as old as humankind. For centuries native people of various cultures have used plants as medicine for all sorts of healing. The ethnopharmacological properties have been used as a primary source of medicines for early drug discovery[24-25]. According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care[26] and 80% of 122 plant derived drugs were related to their original

ethnopharmacological purpose[27]. The knowledge associated with traditional medicine (complementary or alternative herbal products) has promoted further investigations of medicinal plants as potential medicines and has led to the isolation of many natural products that have become well known pharmaceuticals.

The most commonly used breast cancer drug is paclitaxel (Taxol®) (**7**), isolated from bark of *Taxus brevifolia* (Pacific Yew). In 1962 the United States Department of Agriculture (USDA) first collected the bark as part of their exploratory plant screening program at the National Cancer Institute (NCI) (Figure 3)[28]. The bark from about three mature 100 year old trees is required to provide 1 gram of **7** given that a course of treatment may need 2 grams of the drug. Current demand for **7** is in the region of 100–200 kg per annum (*i.e.*, 50,000 treatments/year) and is now produced synthetically. The first of several FDA approvals for various uses for Taxol® was announced in 1992 [29]. Taxol® (**7**) is present in limited quantities in natural sources, but its synthesis (though challenging and expensive) has been achieved[30]. Baccatin III (**8**) present in much higher quantities and readily available from the needles of *T. brevifolia* and associated derivatives is an example of a structural analogue that can be efficiently transformed into **7** (Figure 3).

Figure 3

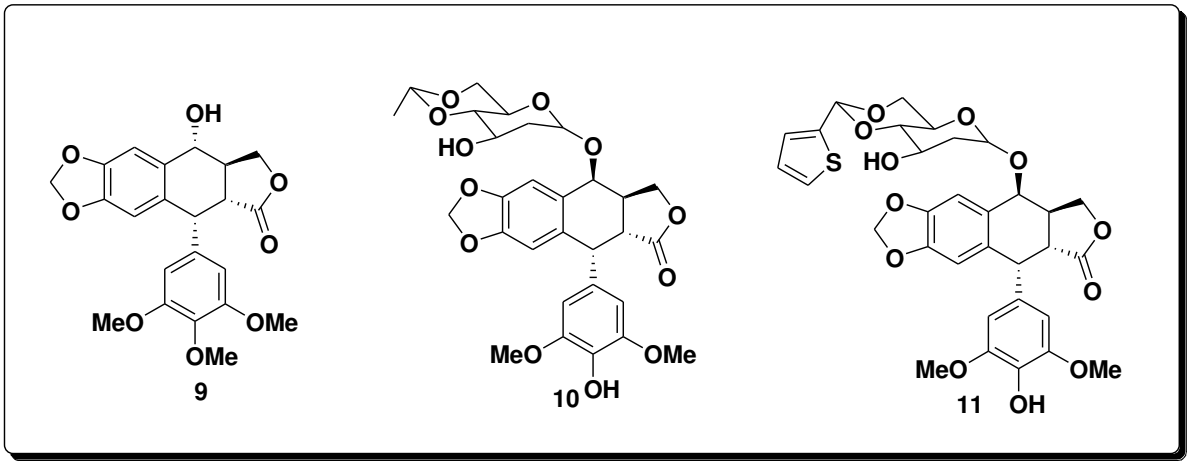
Structure of paclitaxel (Taxol®) (**7**) and Baccatin III (**8**)



Most of drugs available are not selective to cancer cells and affects normal cells as well, thus leading to several side effects. The aim of cancer research drug development is to find new drugs administrated such that they act only on target cells and not the regular targeting cells. One of early compounds isolated as anticancer drug was Podophyllotoxin (**9**), a compound obtained from *Podophyllum peltatum* in 1944[31].It was initially used therapeutically as a purgative and in the treatment of venereal warts[32]. Later it was found that it acts as an anticancer agent by binding irreversibly to tubulin[33]. Etoposide (**10**) and teniposide (**11**), two modified analogs of podophyllotoxin, however, cause cell death by inhibition of topoisomerase II, thus preventing the cleavage of the enzyme- DNA complex and arresting the cell growth[34].Both these analogs are used in the treatment of various cancers[35].

Figure 4

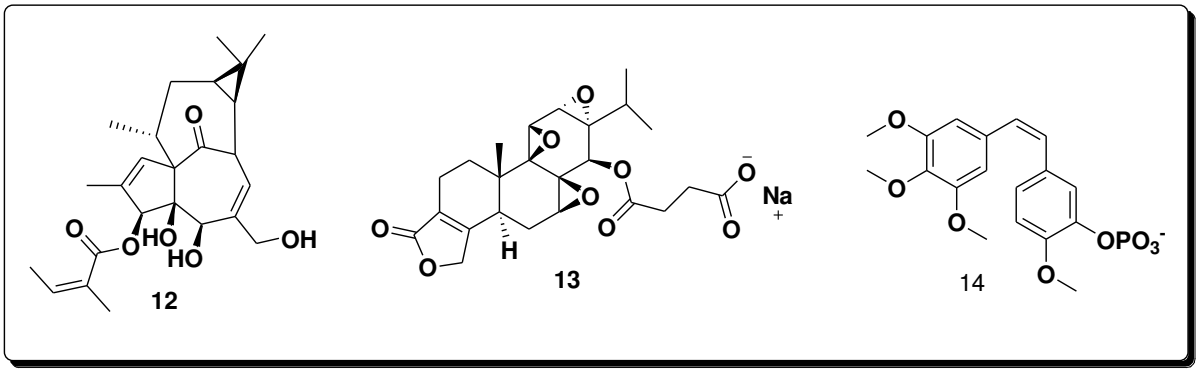
Structure of Podophyllotoxin(**9**), Etoposide (**10**) and Teniposide (**11**).



Other examples of antitumor compounds currently in clinical trials include ingenol 3-*O*-angelate (**12**) a derivative of the polyhydroxyditerpenoid ingenol isolated from the sap of *Euphorbia peplus* (known as "*petty spurge*" in England or "*radium weed*" in Australia) which is a potential chemotherapeutic agent for skin cancer currently under clinical development by Peplin Biotech for the topical treatment of certain skin cancers[36,37](Figure 5). PG490-88 (**13**) (14-succinyl triptolide sodium salt), a semisynthetic analogue of triptolide is diterpene-diepoxide isolated from *Tripterygium wilfordii* which is used for autoimmune and inflammatory diseases in the People's Republic of China[38-39]. Combretastatin A-4 phosphate (**14**) a stilbene derivative from the South African Bush Willow, *Combretum caffrum* acts as an anti-angiogenic agent causing vascular shutdowns in tumors (necrosis) and is currently in Phase II clinical trials[40- 41]

Figure 5

Ingenol 3-*O*-angelate (**12**), PG490-88 (**13**), Combretastatin A-4 phosphate (**14**)



1.3.3 Natural products from marine sources

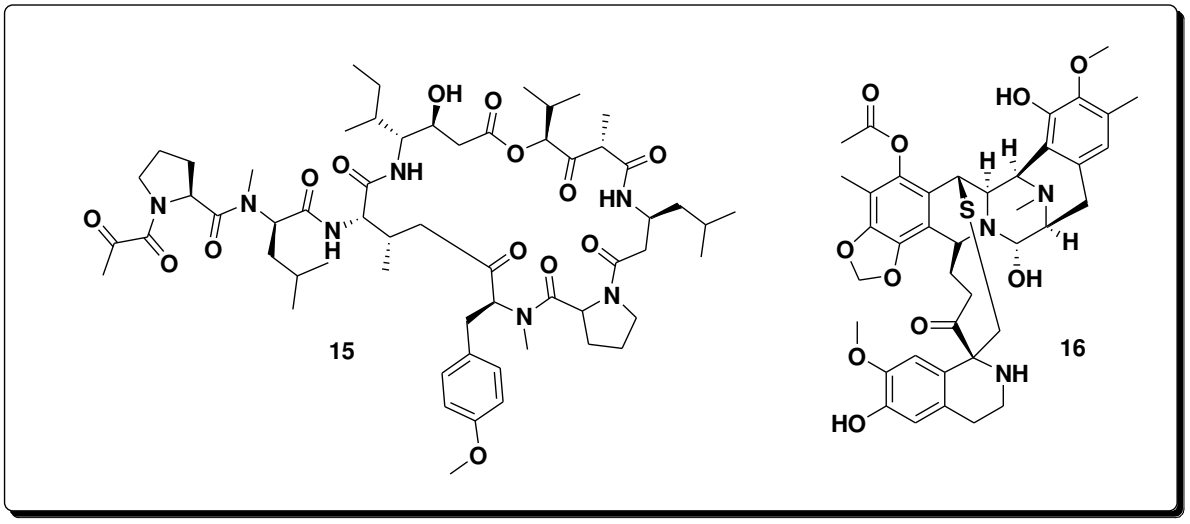
More than 70% of planet's surface is covered by ocean and life on earth has its origin in the sea. In certain marine ecosystems, such as coral reefs or the deep sea floor, experts estimate that the biological diversity is higher than in tropical rain forests. Many marine organisms are soft bodied and have a sedentary life style necessitating chemical means of defence. Therefore, they have evolved the ability to synthesize toxic compounds or to obtain them from marine microorganisms. These compounds help them deter predators, keep competitors at bay or paralyze their prey. The overwhelming biological diversity of marine microbes has so far only been explored to a very limited extent. This diversity is believed to give rise to an equally high diversity of secondary metabolites synthesized by the marine microfauna and microflora[42]. Immense biological diversity in the sea as a whole, it is increasingly recognized that a huge number of natural products and novel chemical entities exist in the oceans, with biological activities that may be useful in the quest for finding drugs with greater efficacy and specificity for the treatment of many human diseases[43-44].So

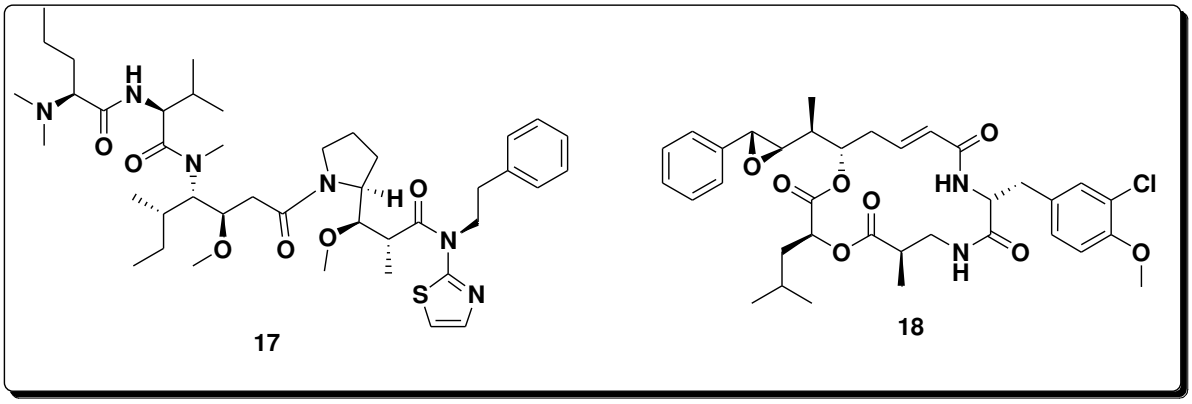
pharmaceutical companies began to realise that ocean would possess unique biodiversity and may be a possible source of potential drug candidates.

Plitidepsin (**15**) (also known as dehydrodidemnin B, marked by PharmaMar under the trade name of Aplidin) depsipeptide was isolated from the Mediterranean tunicate *Aplidium albicans.*[45-46] Plitidepsin is effective in treating various cancers, including melanoma, small cell and non-small cell lung, bladder as well as non-Hodgkin lymphoma and acute lymphoblastic leukemia and is currently in Phase II clinical trials [47](Figure 6). Ecteinascidin 743 (ET743; Yondelis™) (**16**) was isolated in very low yields from the ascidian *Ecteinascidia turbinate*[48-49]. The quantities of ET743 required for advanced pre-clinical and clinical studies were achieved by adopting very large-scale aquaculture of *E. turbinata* in open ponds; however the semisynthesis of ET743 has been well established[50-51]. In October 2007, Trabectedin also known as Ecteinascidin 743 or ET-743) (Yondelis, PharmaMar) became the first marine anticancer drug to be approved in the European Union. Trabectedin (**16**) has been approved by the European Agency for the Evaluation of Medicinal Products (EMEA) and is completing key Phase III studies in the US for approval. Spisulosine (**17**), isolated from the marine clam *Spisula polynyma*, exhibited substantial selective activity against tumor cells compared to normal cells. It advanced to Phase I clinical trials against solid tumors but was withdrawn in late 2006. Cryptophycin (**18**) was selected for clinical trials in the mid 1990s then advanced to phase II trials but was terminated in 2002 due to toxicity and lack of efficacy.[52]

Figure 6

Structure of Plitidepsin (**15**), Ecteinascidin 743(**16**), Spisulosine (**17**), Cryptophycin (**18**)





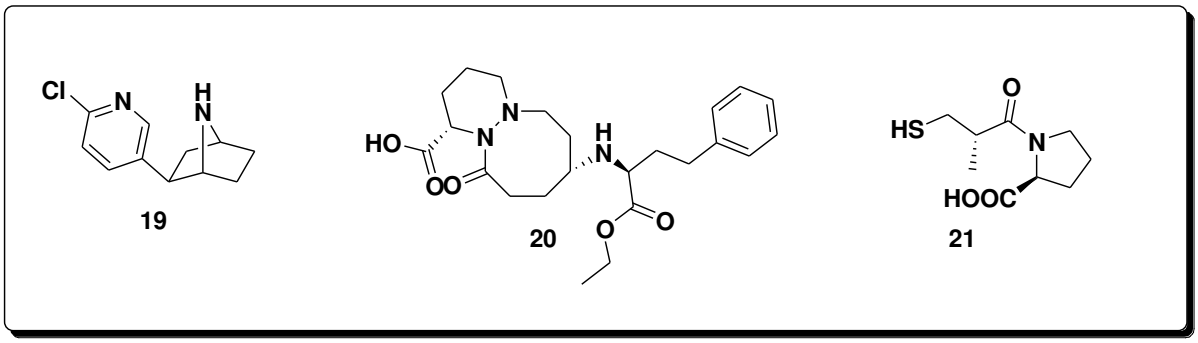
1.3.4 Natural products from animals

The terrestrial and marine ecosystems remain nature's most efficient synthetic laboratories, as they biosynthesize natural products for the survival and healing of mankind and generate renewable industrial and medical chemicals for the wealth of the nations. Animal products like their plant counterparts have been exploited throughout history to treat

human ailments and develop medical tools. Their usage phenomenon is marked by a broad geographical distribution across all continents**.** Animals have been source of some of interesting compounds that can used as drugs. Epibatidine(**19**) (Figure 7) a potent non-opioid analgesic has been isolated from skins of the Ecuadoran poison frog, *Epipedobates tricolor*[53].Venom and toxins plays an important role in designing a multitude of cures for several diseases. Cilazapril (**20**) and Captopril (**21**), extracted from Brazilian viper are effective against hypertension.

Figure 7

Structure of Epibatidine(**19**) ,Cilazapril (**20**) and Captopril (**21**)



1.4 The Success of Natural products in drug discovery

An integrative approach by combining the various discovery tools and the new discipline of integrative biology will surely provide the key for success in natural product drug discovery and development. Natural products can be predicted to remain an essential component in the search and development for new, safe and economical medicaments. Pharmaceutical industry must awaken in this case to change its mindset and reorient its resources towards the natural product-based drug discovery programs.

According to Lutz[54], natural products not only complement synthetic molecules, they also exhibit drug- relevant features unsurpassable by any synthetic compound. One key feature of natural products is their enormous structural and chemical diversity. In fact, about 40% of the chemical scaffolds found in natural products are absent in today's medicinal chemistry, and therefore complementary to synthetically produced molecules. Most possibly this is one of the reasons for their historical success in drug discovery, with 45% of today's best-selling drugs originating from natural products or their derivatives.

Another important advantage of natural products is that they have a biological history. Biosynthesis of natural products involves repeated interaction with modulating enzymes, and the actual biological function of many natural products comprises binding to other proteins. Thus, the ability of natural products to interact with other molecules, an indispensable prerequisite to making an effective drug, might be considered as biologically validated. It is an unsurprising, but often overlooked, fact that many natural products exhibit advanced binding characteristics compared with synthetics. Most probably, the sterically more complex structure of natural products contributes to this. Furthermore, natural products have higher molecular weights; incorporate fewer nitrogen, halogen, or sulfur atoms but more oxygen atoms; and are sterically more complex, with more bridgehead tetrahedral carbon atoms, rings, and chiral centers.

The success of natural products is related to the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, and pharmacology to exploit the vast diversity of chemical structures and biological activities of these products. Moreover, the exploration of structural chemical data-bases comprising a wide variety of chemotypes, in conjunction with databases on target genes and proteins, will facilitate the creation of new chemical entities through computational molecular modeling for pharmacological evaluation[55]

In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine, plants, and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities[56].

There is no doubt that natural products, containing inherently large-scale structural diversity than synthetic compounds, have been the principal resources of biologically active agents and will continually act as protagonists for the discovery of new drugs. In fact, the value of natural products in the treatment and prevention of human diseases can be assessed, according to Chin *et al* [57]. using three criteria: 1) the rate of introduction of new chemical entities of a wide structural diversity, including their application as templates for semi synthetic and total synthetic modification; 2) the number of diseases treated or prevented by these substances; and 3) their frequency of use in the treatment of diseases. Natural products are important sources for new drugs and are also good lead compounds suitable for further modification during drug development. The large proportion of natural products in drug discovery has stemmed from the diverse structures and the intricate carbon skeletons of natural products. Since secondary metabolites from natural sources have been elaborated within living systems, they are often perceived as showing more "drug-likeness and biological friendliness than totally synthetic molecules", making them good candidates for further drug development.

A potential explanation beyond the success of natural products as drugs is the classification of natural compounds as so-called privileged structures. This concept is based on the fact that chemical agents produced by living organisms (particularly the secondary metabolites) have evolved throughout millenniums under the evolutionary pressure, and is therefore more likely to have a specific biological activity than "randomly" assembled,

manmade synthetic chemicals. Despite the enormous potential, only a minor part of globe's living species has ever been tested for any bioactivity. For instance, approximately only 10% of all existing plant species has been assayed, and in the case of microbes the value is even lower.

In medicine, biotechnology and pharmacology, drug discovery is the process by which drugs are discovered and/or designed. In the past most drugs have been discovered either by identifying the active ingredient from traditional remedies or by serendipitous discovery. A new approach has been to understand how disease and infection are controlled at the molecular and physiological level and to target specific entities based on this knowledge. The process of drug discovery so long involves the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Once a compound has shown its value in these tests, it will begin the process of drug development prior to clinical trials.

1.4.1 Screening and Design

There is vast increase in molecular targets due to increased research in molecular mechanism, cellular biology and genomics. So this helped to decrease drug discovery timeline. Major pharmaceutical industry started natural product discovery (NPD) programs which focused on antibacterial, antifungal as well as infectious diseases. Natural product and synthetic organic chemistry placed an important role and driving force for drug discovery. Natural product has shown potent biological activities and provided lead compound for treatment of cancer, microbial infection inflammation, hypercholesteremia, and tissue rejection in organ transplantation.

However molecular target based drug discovery has replaced classical drug based discovery. Classical drug based discovery involves extraction, assay-based functional fractionation, isolation, characterization, and target validation. The advent of High Throughput Screening (HTS) which increased momentum in biological testing and combinatorial chemistry began to prompted as better approach to creating drug like compounds for HTS. The classical drug based discovery were not able to generate the numbers of target which were required to keep pace with the HTS requirements.

The organisation that conduct Natural product discovery (NPD) programs used actinomycetes, fungi, bacteria, plants and medicinal herbs as screening sources. Some apply invertebrates and microalgae to screening. Insects are rarely exploited. But some seek microbes from unusual environments. Since initial natural products are rarely launched unaltered as a drug, unreasonable expectations on financial investment will prevent many countries from being involved in the drug discovery process. The process of finding a new drug against a chosen target for a particular disease usually involves HTS, where large libraries of chemicals are tested for their ability to modify the target. One of important role of HTS is to show the selectivity of compounds for chosen target. The ideal condition is to find a molecule which should show selectivity towards only the chosen target, but not other, related targets. To this end, other screening runs will be made to see whether the "hits" against the chosen target will interfere with other related targets-this is the process of cross-screening. Cross-screening is important, because the more unrelated targets a compound hits, the more likely that off-target toxicity will occur with that compound once it reaches the body. Though HTS is a common method used for novel drug discovery, other methods such as virtual high throughput screening in which screening is done using computer-generated models and attempting to dock virtual libraries to a target, are often used. Drug design is also an important method for drug discovery, where the biological and physical properties of the

target are studied and predication is made of the sort of chemicals that might fit into an active site. There is vast revolution in natural product research due to recent advancement in combinatorial biosynthesis, microbial genomics and screening process. Also Liquid Chromatography-Mass Spectrometry, Liquid Chromatography-Nuclear Magnetic Resonance have placed drastic role by reducing the time and cost involved in natural product research by using dereplication process.

1.4.2 Dereplication

A drug discovery program always search for novel bioactive natural products which possess some form of potent biological activity. Natural products (NPs) sources are well known to produce chemical metabolites with unique features, highly complex structures and properties for human health care and well-being, exhibiting a wide range of applications that have inspired a number of industrial arenas. There is no doubt that NPs are the most consistently successful source of drug leads. The dereplication is a process to indentify known compounds responsible for activity of extract prior to bioassay–guided isolation. At present there are numerous modern methods and protocols that can discriminate novel entities from natural compounds at early stage drug discovery programs or natural product isolation strategy. The isolation of novel natural products was far more frequent during 1970s and it is steadily declining[58]. Dereplication strategies generally involve combination of bioassay, separation science, spectroscopic methods and database searching and can be regarded as chemical or biological screening process. There are number of ways in which natural product program approach dereplication, which is based upon availability of screening methods/instrumentation, time and cost to identify possible biological leads or novel compounds from crude extract.

1.4.3 Chemical Diversity of Natural Products

Natural products remain the best sources of drugs and drug leads, and this remains true today despite the fact that many pharmaceutical companies have deemphasized natural products research in favour of HTP screening of combinatorial libraries during the past 2 decades**.** Natural products possess enormous structural and chemical diversity that is unsurpassed by any synthetic libraries. About 40% of the chemical scaffolds found in natural products are absent in today's medicinal chemistry repertoire. Based on various chemical properties, combinatorial compounds occupy a much smaller area in molecular space than natural products. Although combinatorial compounds occupy a well-defined area, natural products and drugs occupy all of this space as well as additional volumes. Most importantly, natural products are evolutionarily optimized as drug-like molecules. This is evident upon realization that natural products and drugs occupy approximately the same molecular space. Chemical diversity in nature is based on biological and geographical diversity, so researchers travel around the world obtaining samples to analyze and evaluate in drug discovery screens or bioassays. This effort to search for natural products is known as bioprospecting. On the other hand, some medicines are developed from a lead compound originally obtained from a natural source. This means that the lead compound can be produced by total synthesis, or can be a starting point (precursor) for a semi-synthetic compound, or can act as a template for a structurally different total synthetic compound.

This is because most biologically active natural product compounds are secondary metabolites with very complex structures. This has an advantage in that they are extremely novel compounds but this complexity also makes many lead compounds' synthesis difficult and the compound usually has to be extracted from its natural source - a slow, expensive and inefficient process. As a result, there is usually an advantage in designing simpler analogues. The natural product structures have high chemical diversity, biochemical specificity and other

molecular properties which helped make them as lead structures for drug discovery. So it is helped to discriminate from libraries of synthetic and combinatorial compounds in terms of ratio of aromatic ring atoms to total heavy atoms, number of solvated hydrogen bond donors and acceptors and molecular rigidity. Natural-product libraries also have a broader distribution of molecular properties such as molecular mass, octanol-water partition coefficient and diversity of ring systems compared with synthetic and combinatorial counterparts.

1.4.4 Isolation and Purification

Due to incomparable structural diversity and drug like properties, natural products are expected to play an important role as one of the major sources of new drugs so isolation of natural products from higher plants, marine organisms and microorganisms is therefore absolutely necessary, calling for state-of-the-art methodologies for separation and isolation procedures.The active compound has to be isolated from mixture of compounds present in natural sources. This isolation and purification depends on structure, stability, volatility and quantity of the natural compound. Often this isolation and purification is long and tedious which involves combination of various separation techniques.

1.4.5 Structure-Activity Relationship (SAR) of Natural compounds

The analysis of SAR enables the determination of the chemical groups responsible for evoking a target biological effect in the organism. One of important characteristic of natural product is their frequent occurrence as complexes of structurally related analogues. A structure activity relationship relates features of a chemical structure to a property, effect, or biological activity associated with that chemical. In so doing there can be both qualitative and quantitative considerations. The fundamental premise is that the structure of a chemical implicitly determines its physical and chemical properties and reactivity, which, in interaction

with a biological system, determine its biological/toxicological properties. The process of developing a SAR is one of attempting to understand and reveal how properties relevant to activity are encoded within and determined by the chemical structure.

1.5 Development of New Technologies and Modern Method in Drug Discovery

The drug derived from natural product provided driving force for screening for novel, biologically active metabolite from this product. The technologies such as combinatorial chemistry, high-throughput screening, bioinformatics, proteomics and genomic play a vital role in the field of modern drug discovery. This technologies have enormous potential to make use of the chemical diversity of natural products. Other techniques have only been developed in the last years and continue to evolve rapidly. These include molecular diversity, compound library design, protein 3D-structures, NMR- based screening, 3D QSAR in modern drug design. This modern methods helped chemist to improve and accelerate the drug discovery research and development process. This modern method enables to screen 100,000 test substances per week against several different targets. In keeping with these trends, natural products acquisition programs are now scoped to collect large numbers of diverse tissue samples, but only to collect small wet weights. This format allows collectors to provide large numbers of samples for screening, and they rely on recollection for follow-on studies. This means that discovery programs actively testing natural products must track a greater number of samples. In addition, in- formation on known natural products chemistry must be considered in an effort to contain the costs of follow-on studies[59].

1.5.1Combinatorial Chemistry

Combinatorial chemistry is a laboratory technique in which millions of molecular constructions can be synthesized and tested for biological activity. It has generated massive numbers of targeted molecules for testing and the developing techniques of high throughput screening has automated the screening process so larger numbers of biological assays can be done. As compared to Natural product, libraries generated by Combinatorial chemistry are

relatively simple planner molecules. Combinatorial chemistry helped to reduce the time and the cost associated with producing effective and competitive new drugs and also can be used to generate new leads for a specific target as well as powerful alternative method to optimise the initial lead. The principle of Combinatorial chemistry depends on synthesis of directly large number of compounds through preparing many single compounds in parallel of many compound simultaneously in mixture. Increasing pressure to identify, optimize, develop, and commercialize novel drugs more rapidly and more cost- effectively has led to an urgent demand for technologies that can reduce the time to marke for new products. Molecular diversity, of both natural and synthetic materials, provides a valuable source of compounds for identifying and optimizing new drug leads. Through the rapidly evolving technology of combinatorial chemistry, it is now possible to produce libraries of small molecules to screen for novel bioactivities. This powerful new technology has begun to help pharmaceutical companies to find new drug candidates quickly, save significant dollars in preclinical development costs, and ultimately change their fundamental approach to drug discovery.

1.5.2 High-Throughput-Screening

High-throughput screening (HTS) is the process of testing a large number of diverse chemical structures against disease targets to identify 'hits'. As compared to traditional screening methods HTS is a simple, rapid, low cost and highly efficient process. HTS involves an automated operation-platform, highly sensitive testing system, specific screening model (in vitro), an abundant components library, and a data acquisition and processing system. Using this robotics researchers are able to conduct thousands of biochemical, genetic or pharmacological tests. Due to HTS it is easy to identify active compounds, antibodies or genes which modulate a particular biomolecular pathway. The results of these experiments

provide starting points for drug design and for understanding the interaction or role of a particular biochemical process in biology. Automation is an important part in HTS usefulness. A specialized robot is often responsible for much of the process over the lifetime of a single assay plate, from creation through final analysis. An HTS robot can usually prepare and analyze many plates simultaneously, further speeding the data-collection process. HTS robots currently exist which can test up to 100,000 compounds per day. HTS is relatively recent innovation which includes effective technologies to support the acquisition and inventory of the natural products sources. Information handling is key part of this process. Information handling requirements at every stage of this process will grow, and computer technology is available to meet the demand.

1.5.3 Bioinformatics, Proteomics and Genomics

Resistance to current medical treatment plays an important role in human and animal health, so better resistance-breaking drugs are urgently needed. Recently, there has been a change in the way that medicines are being developed due to our increased understanding of molecular biology. In the past, new synthetic organic molecules were tested in animals or in whole organ preparations. This has been replaced with a molecular target approach in which in-vitro screening of compounds against purified, recombinant proteins or genetically modified cell lines is carried out with a high throughput. This change has come about as a consequence of better and ever improving knowledge of the molecular basis of disease. The major advances in genomics and sequencing means that finding an attractive target is no longer a problem but finding the targets that are most likely to succeed has become the challenge. The focus of bioinformatics in the drug discovery process has therefore shifted from target identification to target validation. Numerous factors must be taken into account to

select a candidate target from a multitude of heterogeneous resources. The types of information that one needs to gather about potential targets include nucleotide and protein sequencing information, homologues, mapping information, function prediction, pathway information, disease associations, variants, structural information, gene and protein expression data and species/taxonomic distribution among others. Different bioinformatics tools can be used to gather this information. The accumulation of this information into databases about potential targets means that the pharmaceutical companies can save themselves much time, effort and expense exerting bench efforts on targets that will ultimately fail. The information that is gathered helps to characterise the different targets into families and subfamilies. It also classifies the behaviour of the different molecules in a biochemical and cellular context. Decisions about which families provide the best potential targets is guided by a number of criteria. It is important that the potential target has a suitable structure for interacting with drug molecules. Structural genomics helps to prioritise the families in terms of their 3D structures.

The use of proteomics technology is protein mapping (separating, distinguishing, and quantifying the protein present in individual sample) and also involves the technique for indentifying specific protein and characterising their complete structure and functional role. The main protein mapping technology in use today is two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), which can resolve up to 2000 proteins on a single gel significantly better than other separation techniques. Though new technology likes HTS and combinatorial placed an important role, natural products still offers unprecedented structural diversity. The human genome has pro- vided the pharmaceutical industry with innumerable starting points for new drug discovery approaches. And yet, the expected flood of new chemical entities has failed to materialize.

1.6 Status of Natural products in Drug Discovery today

One of most important breakthroughs in drug discovery was the use of mechanism-based screening for bioassay-guided fractionation and continuous improvement of screening formats, reagent production, robotics, and data management, mechanism-based screening has since become the mainstay of high-throughput screening (HTS).Though there is high competition from other drug discovery methods, NPs are still providing their fair share of new clinical candidates and drugs. This was demonstrated recently by Newman and Cragg who analyzed the number of NP-derived drugs present in the total drug launches from 1981 to 2010[60]. They concluded that NPs were still a significant source of new drugs, especially in the anticancer and antihypertensive therapeutic areas[61]. Drug discovery is a complex, interdisciplinary pursuit of chemistry, pharmacology, and clinical sciences, which has benefited humankind immensely over the last 100 years. Although drug discovery has been traditionally a difficult and expensive process, the amount of money currently being invested in R&D and clinical development has skyrocketed, while the output of newly launched drugs has fallen. Given that NPs have historically provided many novel drugs leads, one would assume that NPs would still play a pivotal role in the drug discovery strategy of Big Pharma. However, most Big Pharma companies have terminated or significantly scaled down their NP operations in the last 10 years. To a certain extent the downsizing or termination of these NP research programs has been offset by biotech companies offering NP-related services such as pure NP libraries and more traditional extract based screening services. To better understand why Big Pharma has scaled down its NP research programs, it is prudent to examine the differences between the pharmaceutical industry of today and that of 10-20 years ago.

1.7 Natural product based drugs approved during 2005-2010

A total of 19 NP based drugs were approved for marketing worldwide in between the year 2005 to 2010 among of which 7 are classified as NPs, 10 as semi-synthetic NPs, and 2 as NP derived drugs. Sativex (GW Pharmaceuticals) is the world's first pharmaceutical prescription medicine derived from the cannabis plant[62]. Sativex, a mixture of dronabinol and cannabidol , was launched in Canadian April 2005 for neuropathic pain relief in multiple sclerosis[63] and it was also approved by Health Canada in August 2007 as an adjunctive analgesic for severe pain in advanced cancer patients, reducing the need for the opioid medications. Sativex efficiently reduces pain in patients with advanced cancer and has been recommended by the FDA for direct entry into Phase III trials. In November 2009, GW Pharmaceuticals disclosed that recruitment for a Phase II/III cancer pain trial of Sativex had been completed. In March 2010, GW Pharmaceuticals provided an update on the progress of regulatory submission for Sativex oromucosal spray for the treatment of the symptoms of spasticity due to multiple sclerosis.

Fumagillin (Flisint, Sanofi-Aventis), an antimicrobial lead capable of inhibiting the proliferation of endothelial cells, was isolated from *Aspergillus fumigatus*[64]. In September 2005, France approved Fumagillin against intestinal microsporidiosis, a disease caused by the sporeforming unicellular parasite *Enterocytozoon bieneusi*, causing chronic diarrhea in immunocompromised patients[65]. There are presently nine β -lactams (two cephalosporins, six carbapenems and one penem) in clinical trials or undergoing drug registration. Among carbapenem-type β -lactams, doripenem and thienamycin are the ultra-broad spectrum injectable antibiotics. Doripenem (Finibax, Doribax) exhibiting a broad antibacterial spectrum was launched in Japan by Shionogi & Co. in 2005. In October 2007, Johnson &Johnson (J&J) obtained formal FDA approval for use of Doripenem in intra-abdominal and urinary tract infections.

Tigecycline (Tygacil) is among a new generation of antibiotics called glycylcyclines, and is structurally similar to tetracycline. It contains a centralized four-ring carbocyclic skeleton substituted at the D-9 position conferring broad spectrum activity. Tigecycline effectively binds to the 30S subunit of bacterial ribosome and blocks the entry of amino-acyl tRNA molecules into the A site of the ribosome, thus inhibiting protein translation[66]. Tigecycline was developed by Wyeth and in June 2005 the FDA approved it for treatment of complicated skin and skin structure infections (cSSSIs) and intra-abdominal infections. Tigecycline was approved in Europe in May 2006, and a supplemental NDA for community acquired pneumonia (CAP) was submitted to the FDA in October 2007.

Ziconotide (Prialt) is a synthetic form of the peptide ω-conotoxin, which was isolated from the toxinof *Conus magus*, is an N-type voltage sensitive calcium channel blocker and is proposed to regulate neurotransmission by inhibiting pro-nociceptive neurochemical releases in the brain and spinal cord, thus causing pain relief[67]. Zotarolimus, a derivative of sirolimus, is the active principle of the Endeavor™ stent that inhibits cell proliferation, preventing scar tissue formation and minimizing restenosis in angioplasty patients[68]. Anidulafungin (Eraxis™ in the US, Ecalta™ in Europe), is a semisynthetic derivative of the fungal metabolite echinocandin B. Anidulafungin was originally developed for use against invasive and oesophageal candidiasis and candidemia by Eli Lilly and was licensed to Vicuron Pharmaceuticals, which was purchased by Pfizer in June 2005. Exenatide (Byetta®), is a 39 amino acid peptide, structurally similar to glucagon-like peptide-1 (GLP-1) and was isolated from the oral secretions of the poisonous lizard *Heloderma suspectum* (Gila monster). Among the incretin (human hormone) mimetics, Exenatide can mimic the antidiabetic or glucose-lower in properties of incretins. Lisdexamfetamine (Vyvanse™, NRP104) consisting of dextroamphetamine coupled with the essential amino acid L-lysine was designed by New River Pharmaceuticals to help Attention-Deficit Hyperactivity Disorder (ADHD).

Pleuromutilin is a metabolite of fungal origin that binds to the peptidyltransferase and exhibits antibacterial activity by inhibiting protein synthesis in bacteria[69]. Retapamulin (SB-275833) a semi-synthetic derivative of Pleuromutilin, is the first among pleuromutilin antibiotics developed by GlaxoSmithKline for topical use in impetigo caused by Gram-positive *Staphylococcus aureus* or *Streptococcus pyogenes*. GlaxoSmithKline gained FDA approval for 17 in April 2007. Temsirolimus (Torisel, CCI-779) is a sirolimus derivative and is an intravenous drug developed by Wyeth[70].It was approved by the FDA in late May 2007 and by the EMEA in November 2007 for use against renal cell carcinoma (RCC)[71]. Temsirolimus is a semisynthetic derivative of sirolimus and is the first mTOR inhibitor developed by Wyeth Pharmaceuticals. Trabectedin (Yondelis®, ecteinascidin-743, ET-743), a tetrahydroisoquinoline alkaloid produced by *Ecteinascidia turbinate* is sold by Zeltia and Johnson & Johnson for use in the treatment of advanced soft tissue sarcoma[72]. Trabectedin binds to the minor groove of DNA and inhibits cell proliferation by disrupting the cell cycle. It was approved by the EMEA in September 2007 for use against soft tissue sarcomas and ovarian cancer. Ixabepilone (Ixempra™, BMS-247550) is a semi-synthetic derivative of epothilone B produced by *Sorangium cellulosum*. Bristol-Myers Squibb (BMS) developed Ixabepilone as an anticancer drug that binds directly to β-tubulin subunits on microtubules, leading to the suppression of microtubule dynamics, blocking of cells in the mitotic phase, ultimately leading to cell death.

Methylnaltrexone (MOA-728, Relistor® by Wyeth), an N methyl derivative of naltrexone, contains a charged tetravalent nitrogen atom and is unable to cross the blood-brain barrier, and so has antagonist effects throughout the body. Methylnaltrexone blocks peripheral opioid receptors activated by opioids administered for pain relief and is thus useful in management of alcohol and opioid dependence[73]. Everolimus (Luveniq™ or LX211), a mTOR inhibiting derivative is marketed as an immunosuppressant by Novartis under the

trade Afinitor® for use in advanced renal carcinoma. In March 2009 the FDA has approved Everolimus against advanced renal cell carcinoma after failure of Sutent (sunitinib) or Nexavar (sorafenib)[74].Telavancin (Vibativ™,TD-6424), a semi-synthetic analog of vancomycin inhibits bacterial growth through binding to the D-Ala-D-Ala terminus of the bacterial peptidoglycan precursors[75]. Romidepsin (depsipeptide, FK228, FR901228,Istodax®) a naturally occurring histone deacetylase inhibitor obtained from the bacteria *Chromobacterium violaceum*, was developed and evaluated by Gloucester Pharmaceuticals in various Phase I/II trials sponsored by the National Cancer Institute (NCI) for use against cutaneous and peripheral T-cell lymphoma (TCL)[76].

Capsaicin an active component of chili peppers belonging to the genus *Capsicum*, was first isolated in pure and crystalline form by John Clough Thresh in 1876. Capsaicin produces a burning sensation when it comes in contact with tissues though binding to the ion channel receptor vanilloid receptor subtype (VR 1). In November 2009, NeurogesX gained FDA approval for Qutenza® (a transdermal 8% patch of Capsaicin) against neuropathic pain combined with post-therapeutic neuralgia. In April 2010, NeurogesX launched Qutenza® in US and is planning to market it in Europe by Astellas Pharma Europe Ltd.

Aztreonam lysine (Cayston™), an inhaled lysine salt formulation, wherein the β-lactam ring is alone and not fused o another ring, has been evaluated by Gilead in various Phase III trials against cystic fibrosis (CF) in patients having a pulmonary infection of the Gram-negative bacteria *Pseudomonas aeruginosa* .

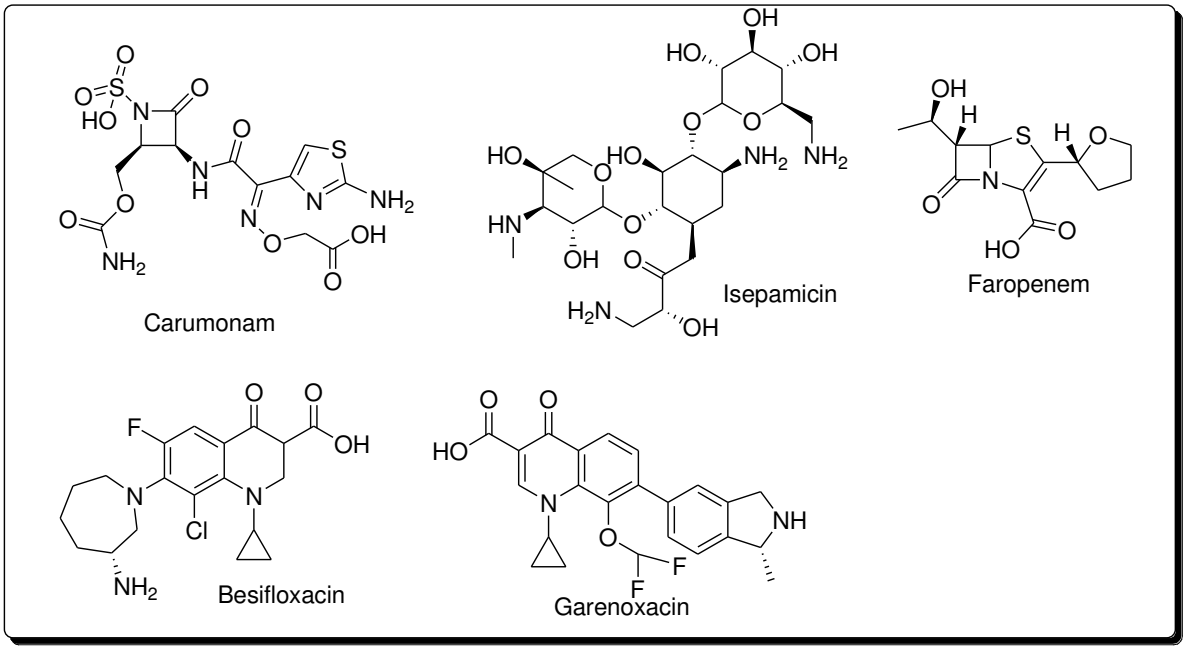
NP-Derived drugs launched since 2005, lead compound, classification, therapeutic areas

Year	Generic name(trade name)	Lead compound	classification	Diseases area
2005	Dronabinol//Cannabidol	Dronabinol	NP	Pain
2005	Fumagillin	Fumagillin	NP	Antiparasitic
2005	Doripenem	Thienamycin	NP-Derived	Antibacterial
2005	Tigecycline	Tetracycline	Semi-synthetic NP	Antibacterial
2005	Ziconotide	Ziconotide	NP	Pain
2005	Zotarolimus	Sirolimus	Semi-synthetic NP	Cardiovascular surgery
2006	Anidulafungin	Echinocandin	Semi-synthetic NP	Antifungal
2006	Exenatide	Exenatide-4	NP	Diabetes
2007	Lisdexamfetamine	Amphetamine	NP-Derived	ADHD
2007	Retapamulin	Pleuromutilin	Semi-synthetic NP	Antibacterial
2007	Temsirolimus	Sirolimus	Semi-synthetic NP	Oncology
2007	Trabectedin	Trabectedin	NP	Oncology
2007	Ixabepilone	Epothilone B	Semi-synthetic NP	Oncology
2008	Methylnaltrexone	Naltrexone	Semi-synthetic NP	Pain
2009	Everolimus	Sirolimus	Semi-synthetic NP	Oncology
2009	Telavancin	Vancomyci	Semi-synthetic NP	Antibacterial
2009	Romidepsin	Romidepsin	NP	Oncology
2009	Capsaicin	Capsaicin	NP	Pain
2010	Monobactam aztreonam	Aztreonam	Semi-synthetic NP	Antibacterial

Antibacterial drugs from 1981 to 2010 by generic name within source

Generic name	Trade name	Year introduced	Source
Carumonam	Amasulin	1988	N
Deptomycin	Cubicin	2003	N
Insepamicin	Isepacin	1988	N
Miokamycin	Bactroban	1985	N
Apalcillin sodium	Lumota	1982	ND
Biapenem	Omegacin	2002	ND
Cefcapene pivoxil	Flomox	1997	ND
Doripenem	Finibax	2005	ND
Fropenam	Farom	1997	ND
Tigecycline	Tygacil	2005	ND
Balafloxacin	Q-Roxin	2002	S
Besifloxacin	Besivance	2009	S
Garenoxacin	Geninax	2007	S
Grepafloxacin	Vexor	1997	S
MCV-4	Menactra	2005	V
MenACWY-CRM	Menveo	2010	V
PsA	MenAfriVac	2010	V

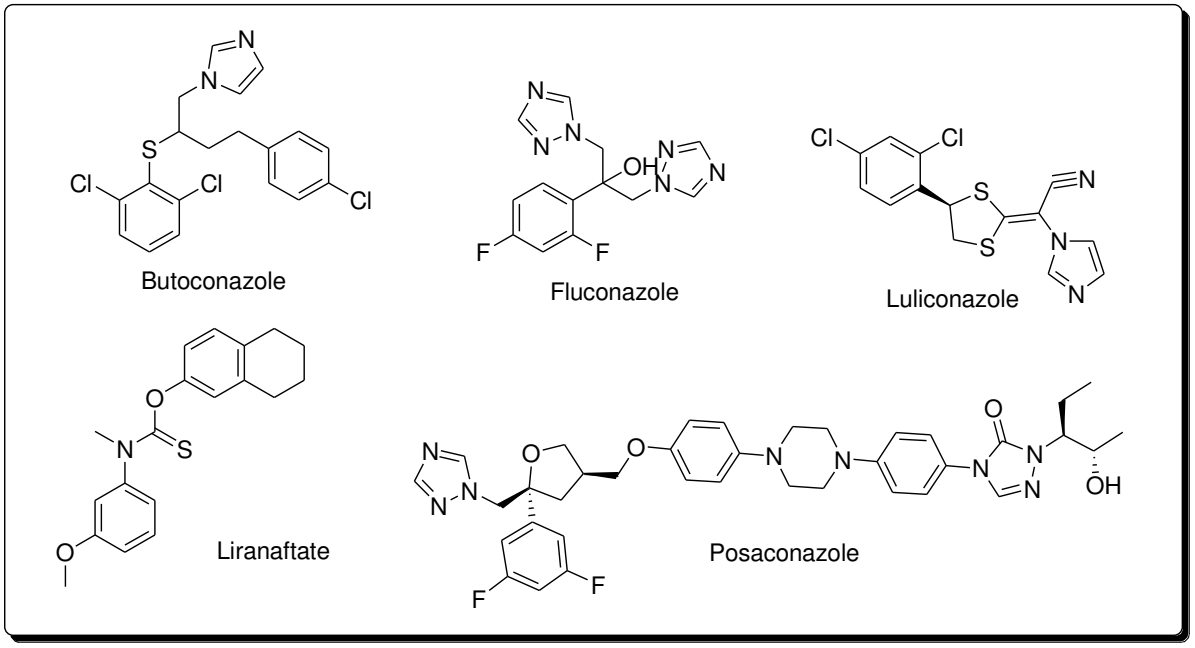
N-Natural product, ND-Derived from natural product, S-Totally synthetic drug, V-Vaccine



Antifungal drugs from 1981 to 2010 by generic name within source

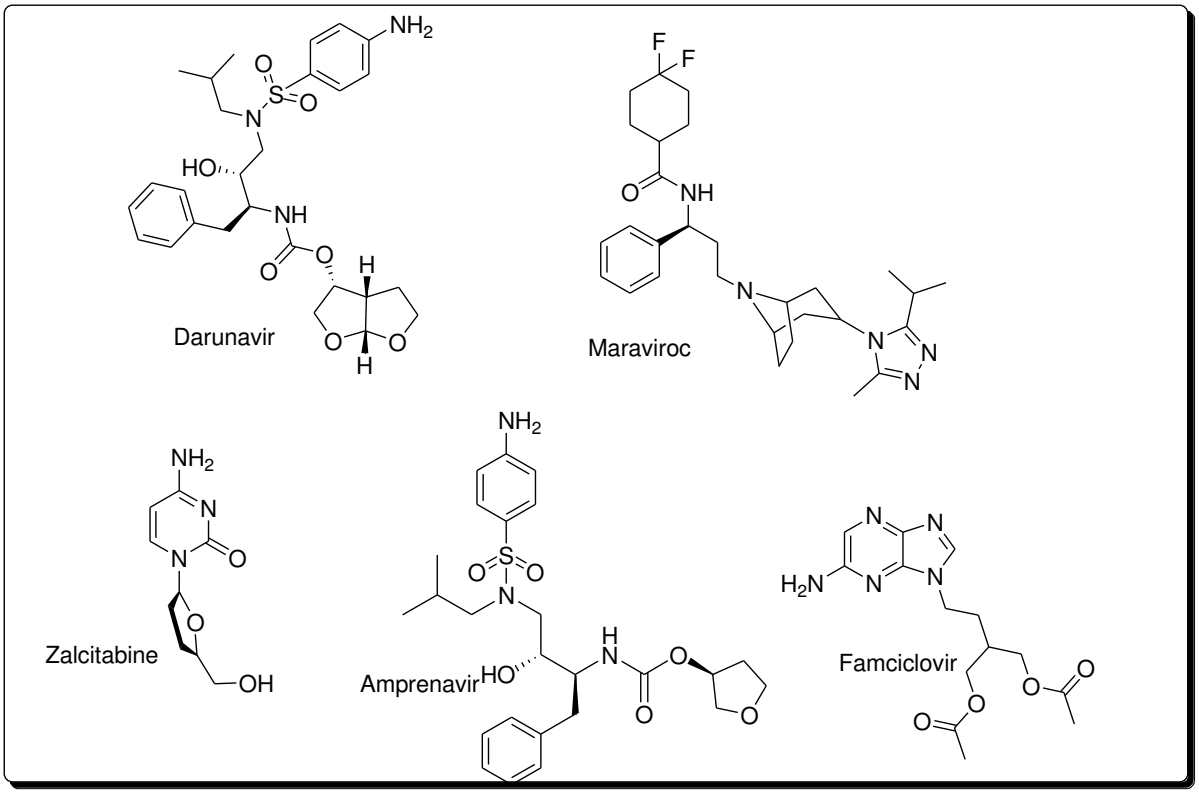
Generic name	Trade name	Year introduced	Source
Interferon γ-n1	OGamma100	1996	B
Anidulafungin	Eraxis	2006	ND
Caspofungin acetate	Cancidas	2001	ND
Micafungin sodium	Fungard	2002	ND
Butoconazole	Femstat	1986	S
Cloconazole HCI	Pilzcin	1986	S
Eberconazole	Ebernet	2005	S
Fluconazole	Diflucan	1988	S
Itraconazole	Sporanox	1988	S
Luliconazole	Lulicon	2005	S
Posaconazole	Noxafil	2005	S
Sertaconazole nitrate	Dermofix	1992	S
Sulconazole nitrate	Exelderm	1985	S
Soriconazole	Vfend	2002	S
Terbinafine hydrochloride	Lamisil	1991	S/NM
Liranaftate	Zefnart	2000	S/NM

NM-Natural product mimic, B-Biological



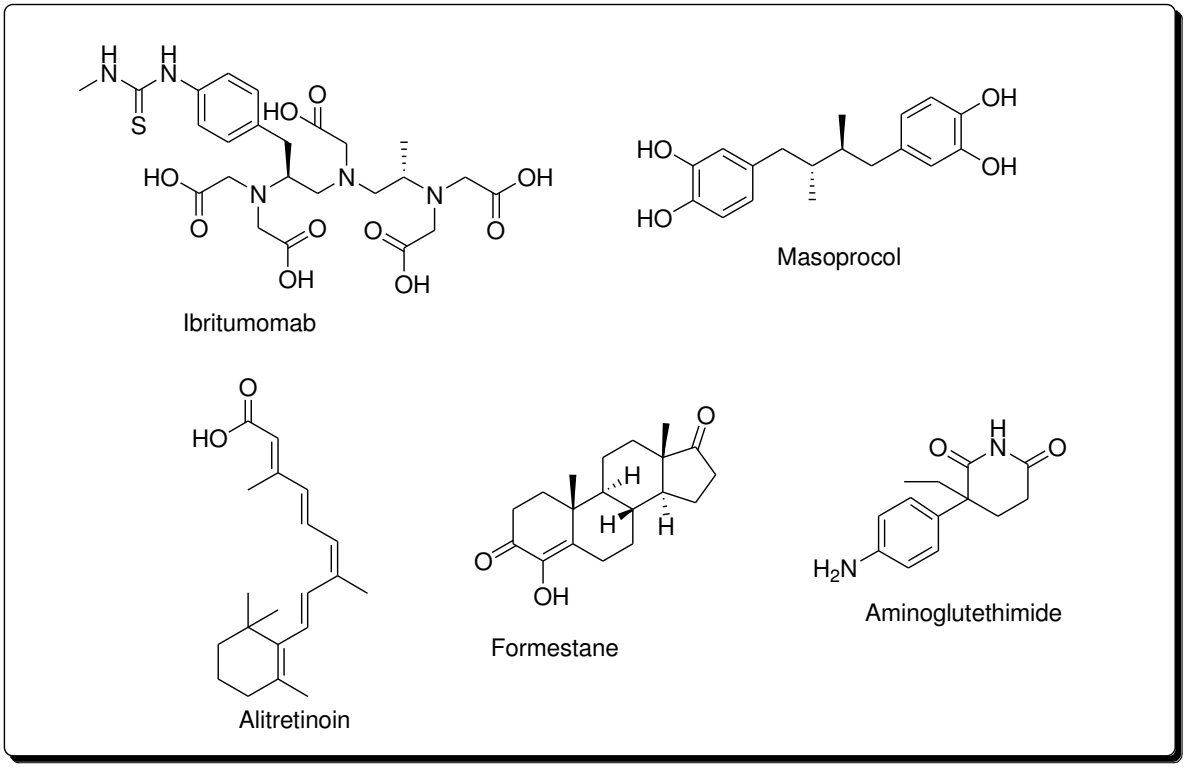
Antiviral drugs from 1981 to 2010 by generic name within source

Generic name	Trade name	Year introduced	Source
Interferon α	Alfaferone	1987	B
Interferon α -n3	Alferon N	1990	B
Interferon ß	Frone	1985	B
Immunoglobulin intravenous	Gammagard Liquid	2005	B
Interferon alfacon-1	Infergen	1997	B
IGIV-HB	Niuliva	2009	B
Peginterferon a-2a	Pegasys	2001	B
Peginterferon a-2b	Pegintron	2000	B
Resp syncytial virus IG	RespiGam	1996	B
Palivizumab	Synagis	1998	B
Enfuvirtide	Fuzeon	2003	ND
Maraviroc	Celsentri	2007	S
Darunavir	Prezista	2006	S
Zalcitabine	Hivid	1992	S
Amprenavir	Agenerase	1999	NM
Famciclovir	Famvir	1994	S



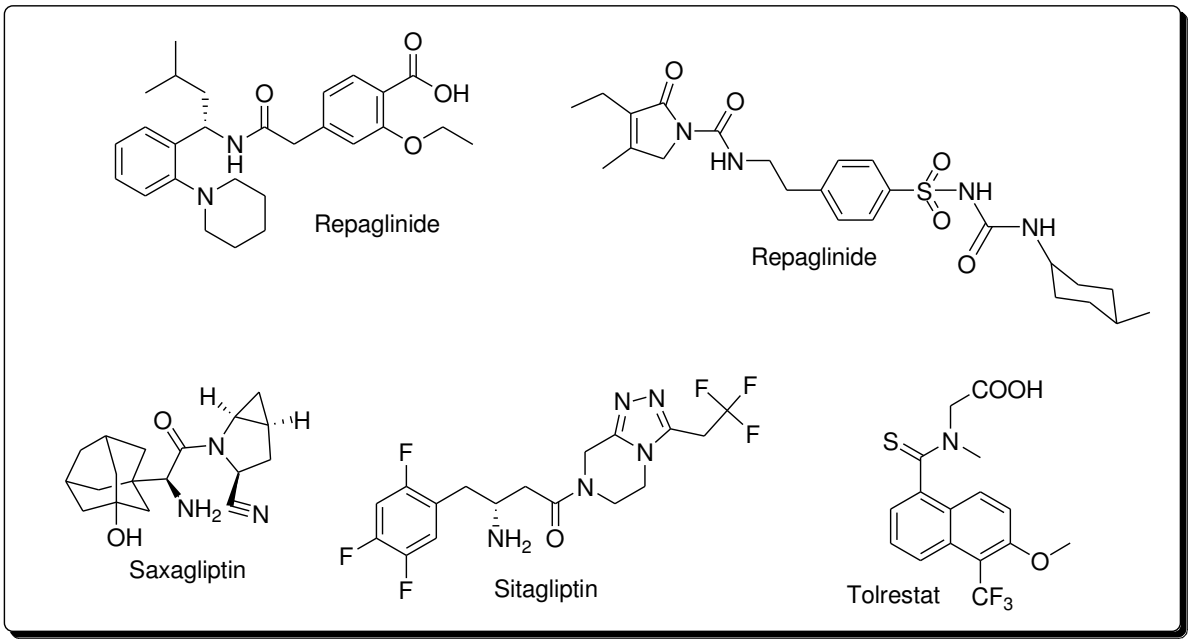
Anticancer drugs from 1981 to 2010 by generic name within source

Generic name	Trade name	Year introduced	Source
Bevacizumab	Avastin	2004	B
Catumaxomab	Removab	2009	B
Celmoleukin	Celeuk	1992	B
Ibritumomab	Zevalin	2002	B
Aclarubicin	Aclacin	1981	N
Masoprocol	Actinex	1992	N
Pentostatin	Nipent	1992	N
Alitretinoin	Panretin	1999	ND
Eribulin	Halaven	2010	ND
Formestane	Lentaron	1993	ND
Mifamurtide	Junovan	2010	ND
Aminoglutethimide	Cytadren	1981	S
Fotemustine	Muphoran	1989	S
Lobaplatin	Lobaplatin	1998	S
Oxaliplatin	Eloxatin	1996	S
Ranimustine	Cymerine	1987	S



Antidiabetic drugs from 1981 to 2010 by generic name within source

Generic name	Trade name	Year introduced	Source
Biphasic porcine insulin	Pork Mixtard 30	1982	B
Hu neutral insulin	Insuman	1992	B
Insulin aspart	NovoRapid	1999	B
Insulin glargine	Lantus	2000	B
Insulin glulisine	Apidra	2005	B
Acarbose	Glucobay	1990	ND
Extenatide	Byetta	2005	ND
Liraglutide	Victoza	2009	ND
Mitiglinide calcium hydrate	Glufast	2004	S
Repaglinide	Prandin	1998	S
Epalrestat	Kinedak	1992	S
Glimepiride	Amaryl	1995	ND
Saxagliptin	Onglyza	2009	S
Sitagliptin	Januvia	2006	S
Tolrestat	Alredase	1989	S
Nateglinide	Starsis	1999	S



1.8 Challenges faced by Natural product synthesis

The natural products have made major contribution in biomedical science due to their broad structural diversity and interesting biological activity. So natural products have made major driving force for drug discovery programs in pharmaceutical industry. But major pharmaceutical industry have declined their focus from natural product chemistry. This decline has attributed to a number of factors: first, the development of combinatorial chemistry and introduction of high-throughput screening (HTS) against defined molecular targets, which prompted many companies to shift away from natural products extract libraries; second, the challenges associated with isolation and purification of active principles from complex natural product extracts; third, the lack of novel entities in natural products; and last, the challenges with compound supply and the lack of adequate structural diversification strategies for preclinical and clinical studies. However, the modest success of combinatorial chemistry and HTS, the considerable advances in automation of chromatographic and spectroscopic techniques, and the advent of genome mining, novel heterologous expression systems, and metabolic engineering have rekindled interest in natural products as valuable resources for drug discovery. At the same time, to address the challenges of material supply and lack of adequate structural diversification strategies, the organic chemistry community has been introducing new, exciting developments to natural product synthesis. As a consequence, natural product synthesis has become increasingly sophisticated.

1.9 Summary

Natural products offer a “privileged” starting point in the search for highly specific and potent modulators of biomolecular function. The natural product synthesis has been the main driving force behind the crucial contributions made by natural products to drug discovery and chemical biology. With the introduction of novel concepts and strategies inspired by the remarkable efficiency of biosynthesis, natural product synthesis in the 21st century is well poised to meet the challenges and complexities in natural product chemistry such as difficulties in material supply and lack of strategies for structural modifications. Therefore, by choosing the right target and using both efficient and innovative synthetic technology, there is no doubt that natural product synthesis will remain not only relevant, but also essential to progress in drug discovery and chemical biology.

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CHAPTER 2

SCOPE OF THESIS

Feeding the world is an increasing challenge. On the other hand the size of arable land is limited and cannot be expanded without further inroads into vital natural habitats like the rain forests. Population growth combined with a shift to a more protein-rich diet containing beef and pork will increase the demand on food production in the future. As more people need more food per head from less land, the yields need to be increased by a combination of methods which is known as ‘‘Integrated Crop Management’’. This practice includes the selection of optimized seeds and the right locations, optimized sowing and harvesting techniques, crop rotation, fertilization and mechanical, biological or chemical crop protection. Chemical crop protection products with activity against weeds, plant diseases, insects and mites help the farmer to assure or increase yields. In the most drastic cases crop protection products can prevent the total loss of a crop. Furthermore the quality of food has improved and the costs of production are cheaper. Finally, the consumer can also benefit from having access to high quality food. In modern days, we have become habitual to a wide variety of cheap, nutritious food which is one of the basic needs of living organisms, while plants are the major source of food. Rapidly growing world population put tremendous pressure to increase and preserve the food supply, Additionally, in recent years public pressure to reduce the use of synthetic pesticides in agriculture has increased. Concerns have been raised about both the environmental impact and the potential health risk related to the use of these pesticides. There are already abundant of crop protection products on the market and the standards of technology are high. The new agrochemicals should be target orienting and should have desired biological spectrum which can fulfil farmer’s needs and the

company's product portfolio. Various types of pests, such as insects, fungi, weeds, bacteria, rodents and other biological organisms, have bothered humans or threatened human health.

Nature is a rich source of compounds exhibiting biological activity against weeds, plants diseases, insects and mites. A large number of volumes and reviews have been written about the use of natural products as pesticides, each having strengths and weakness when considering the vast array of compounds that have found use or are currently being used in crop protection.

Natural products, with their tremendous structural diversity, are an important source of new toxophores. Many of these natural products have complex structures, insufficient biological activity and low persistence under field condition. Thus the share of natural products being used as active ingredients per se in today's crop protection market is relatively small. In some cases the natural products have been further modified to provide semi-synthetic derivatives with improved biological properties. More importantly, natural products served as lead structures inspiring chemists to prepare new synthetic analogues with often improved biological activity, simplified structure, increased safety towards human and the environment an optimized persistence[1].

All the methods and technologies discussed in Chapter 1 regarding the preparation of new drugs can be, and are, applied for the development of new active compounds of agrochemical interest.

The need to develop new fungicides has been a major driving force for applied phytopathological and mycological research efforts in the past, and this is likely to remain the case in future as fungal plant pathogens continue to develop resistance against existing fungicides at great speed, and also because new disease situations continually arise. One increasingly stringent requirement for new fungicides is their biodegradability in natural

situations. This is likely to be met most readily by substances derived from natural products, as has already been shown for the strobilurin-derived fungicides, e.g. kresoxim-methyl.

A second important requirement is the selectivity of their mode of action. Ideally, a fungicide should prevent or cure infections by fungal plant pathogens without hitting neutral or benign species such as saprotrophic soil fungi or mycorrhizal associates, and of course without toxicity against non-fungal organisms. Non-target effects can be reduced by screening for fungicides which do not inhibit vegetative hyphal growth but specifically interfere with developmental events involved in pathogenesis, such as spore germination, formation of penetration structures, or sporulation. Fungi themselves are a rich source of secondary metabolites which can be used as 'lead molecules' for the development of new fungicides if their mode of action is sufficiently selective.

A survey of recent literature supplies lists of novel bioactive compounds, which have been reported very recently and show promising biological activity.

Among a number of possible candidates we have selected two natural products, Leopolic acid A and 3-O-Methylfunicone as synthetic targets, as they have been reported to be endowed with fungitoxic activity.

Thus, the aim of our thesis is to synthesize these natural products, their analogues in order to study the SAR and evaluation of their biological activity.

In chapter 3, we discuss natural compound Leopolic acid A^2 with respect to isolation, structure elucidation, antifungal activity and study towards total synthesis of molecule. The unusual structure of Leopolic acid A, together with the scarcity of compounds with the 2,3-pyrrolidinedione core in the literature, attracted our attention, so that a total synthesis of this compound was planned. Also we developed the idea of making analogues exploiting the

sequence to make SAR studies and to increase the activity.We also discuss the approach toward total synthesis of a positional isomer of Leopolic acid A.

In chapter 4 we discuss the synthesis of various compounds containing the 2,3-pyrrolidinedione core present in Leopolic acid.

In chapter 5, we discuss the antifungal and antimicrobial activity of various intermediate containing the 2,3-pyrrolidinedione skeleton, considering a combination of substituents at different positions to have a large variety of structurally diverse molecules. The compounds were tested on *S. mutans*, *Cladosporium cladosporioides* and *C. albicans* and preliminary structure-activity relationship (SAR) have emerged.

In chapter 6, we discuss the efforts towards the total synthesis of 3-O-Methylfunicone. We also discuss the reported synthesis of 3-O-Methylfunicone based on Stille coupling reaction[3,4].

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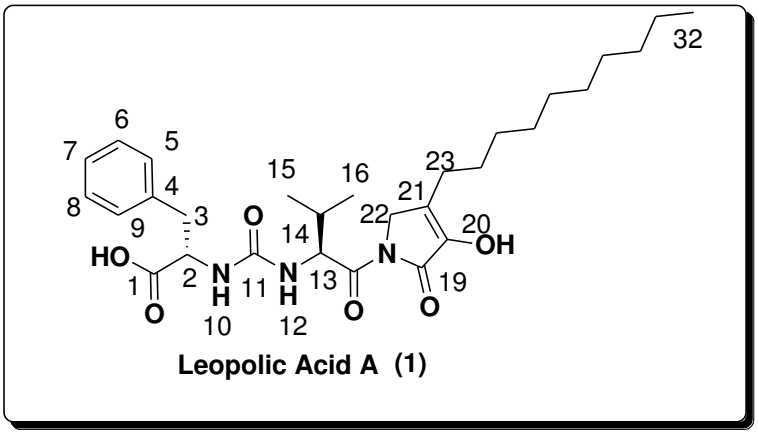
CHAPTER 3

Part A: Synthetic studies towards the total Synthesis of Leopolic Acid A

3.1 Introduction



Chemical analysis of a terrestrial-derived *Streptomyces sp*. isolated from the rhizosphere of the plant *Juniperus* excels collected from the Crimean Mountains (Ukraine) yielded a new acid, leopolic acid A (**1**)[1]. HRESI(+)MS analysis of **1** revealed a pseudomolecular ion ($[M+H]^+$) indicative of a molecular formula ($C_{29}H_{43}N_3O_6$) requiring 10 double bond equivalents. The ^{13}C NMR (DMSO-d_6) data revealed 4 ester/amide carbonyls (δ_C 157.8, 166.5, 172.5, and 174.2) and a further 8 sp^2 resonances (δ_C 126.6 to 141.7), accounting for 8 DBE and requiring 2 rings. Consideration of the 1D and 2D NMR data revealed a mono-substituted benzene ring [COSY correlations from H-5 to H-9 extended by HMBC correlations to the methylene C-3 (δ_C 37.9) which further extended to a deshielded methine C-2 (δ_C 54.3) and the amide NH (δ_H 6.35). and methine H-2 (δ_H 4.29) to the ester/amide carbonyl C-1 (δ_C 174.2)]. This led to the construction of the amino acid residue phenylalanine (C1–C10). Further examination of the ^1H and COSY NMR data documented

an isolated spin system, indicative of two secondary methyls H_3- 15 and H_3-16 (δ_H's 0.91 and 0.75) extending through the deshielded methine H-13 (δ_H 5.36) to the amide NH (δ_H 6.47). HMBC correlations were observed from the amide NH to the ester/amide carbonyl C-17 (δ_C 172.5). The generation of the fragment C-13–C-17 was reminiscent of the amino acid residue, valine. HMBC correlations from both the a protons H-2 (δ_H 4.29) and H-13 (δ_H 5.36) to the amide carbonyl C-11 (δ_C 157.8) linked the two amino acid residues together generating subunit A. The remaining structure fragment of **1**, subunit B(cyclised part with long chain) consisted of an isolated spin system, indicative of a primary methyl H_3-32 (δ_H 0.85) linked through a set of methylenes generating the aliphatic chain C-23–C-32. A deshielded methylene H_2-22 (δ_H 4.00, 4.09) (δ_C 46.5) suggested its connectivity to a nitrogen, which in turn with the terminal methylene H_2-23 (δ_H 2.30) showed HMBC correlations to one another and to the quaternary carbons C-20 (δ_C 141.7) and C-21 (δ_C 129.2). The downfield chemical of C-20 suggested its attachment to a heteroatom, in this case hydroxyl functionality (δ_H 9.45). Also observed were correlations from H_2-22 to an amide carbonyl C-19 (δ_C 166.5). A large coupling (J = 18.6 Hz) for the methylene H_2-22 and to accommodate for the remaining DBE led to the construction of the 5-dihydro-3-hydroxy-pyrrole-2-one ring (C-19–C- 22). Finally a HMBC correlation from the methylene H_2-22 to C-17 of subunit A (Ureido moiety) led to the formulation of the planar structure of **1**. Marfey's analysis of **1** confirmed the presence of L-Phe and L-Val residues, thereby assigning a 2S, 13S configuration.

Leopolic acid A (**1**) was identified to possess a rare ureido dipeptide, Phe-CO-Val, attached to a 5-dihydro-3-hydroxy-pyrrole-2-one ring. Ureido-peptides with varying number of amino acids have been previously isolated from actinomycetes. Noteworthy examples of ureido peptides include the microbial alkaline protease inhibitors, (MAPI) - α and β,[2] GE20372 factor A and B, as HIV-1 protease inhibitors,[3] and the pacidamycins,[4] exhibiting *anti-Pseudomonas aeruginosa* activity. Closest resemblance of the substituted

pyrrole-2- one ring is found in the tetramic acid incorporating metabolites which include the nocamycins, isolated from a terrestrial *Nocardiopsis sp*.[5] Lydicamycin, isolated from *Streptomyces* showing selective Gram +ve antibacterial activity,[6] and TPU-0037-B, isolated from the marine-derived *Streptomyces sp*. exhibiting antimicrobial activity against MRSA.[7] The examples were not limited to microbes but spanned to cyanobacteria, isomalyngamide A isolated from *Lyngbya majuscule*.[8]

Leopolic acid (**1**), has unprecedented structural features consisting of an aliphatic side chain attached to the novel 3-hydroxy-dihydropyrrole-2-one residue connected to the ureido dipeptide L-Phe-L-Val.

3.1.1 Biological Activity

Leopolic acid A (**1**) was screened against a panel of Gram positive and negative bacterial strains, but it did not show significant activity. The cytotoxicity of leopolic acid A against HCT-116 (human colon carcinoma) cells was in the range of 20 µg/mL. However, it did show antifungal and antibacterial activity against *Mucor hiemalis* and *Staphylococcus aureus* with a MIC of 32 and 16 µg/mL, respectively.

MIC and IC_{50} (µg/mL) values of leopolic acid A (**1**)

Test organism	**MIC (µg/mL)**
Candida albicans	> 64
Pichia anomala	> 64
Mucor hiemalis	32
Pseudomonas aeruginosa PA14	> 64
Escherichia coli DH5a	> 64
Escherichia coli TolC	> 64
Micrococcus luteus	32
Staphylococcus aureus Newman	16
Bacillus subtilis	32
Mycobacterium diernhoferi	32
	IC50 (µg/mL)
Human colon carcinoma cells HCT-116	ca. 20

3.2 Importance of Ureido containing compounds

Ureides are compounds, which essentially incorporate urea as a substructural component either in open or cyclic form. Ureido derivatives are one of the oldest classes of bioactives, widely used as antiinfective agents. Several of these compounds, including aminoquinuride, aminocarbalide, imidurea, cloflucarban, nitrofurazone, urosulfan, viomycin are used in clinical situations. The ureides are one of the most simple and commonly used antibacterial agents. The aryl ureas are one of the simplest of all the chemicals to be used in clinics. In studies reported in the middle of the last century, Beaver et al described the synthesis and antibacterial activity of several diarylureas[9]. A number of these diaryl ureas displayed significant antibacterial activity and triclocarban **(2)** was developed for the clinical use. In present times, triclocarban is compulsorily used in the cleansing and disinfecting solutions in hospitals, households, cosmetics, toys, textiles and plastics. It is active against gram positive bacteria and certain fungi, but less so against gram negative bacteria. It is now known that triclocarban disables the activity of ENR (enoyl-acyl carrier-protein reductase), an enzyme vital for building the cell wall of the bacteria and fungus[10]. Another analogous urea derivative the chloflucarban **(3)** has been reported to have similar antibacterial properties and commercial utility. TAN-1057A(**4**) isolated from *Flexibacter spp*. and TAN-1057A has been found to exhibit potent activity against several MRSA strains and was found to be very similar to vancomycin. An array of ureide including several peptidomimetics has been reported to display potent antiviral activity including anti-HIV activity. The anti-HIV agents comprises of the nucleosidic reverse transcriptase inhibitors, the HIV-protease inhibitors and the NNRTIs (non-nucleosidic reverse transcriptase inhibitors). Guieffier and coworkers designed the synthesis of ureidoimidazo[1,2-α]pyridine derivatives **(5)** for anti-HIV activity[11]. Rodgers and Cocuzza from DuPont filed a patent for 1,3-benzodiazepin-2-ones and 1,3-benzoxazepin-2-ones **(6)** useful as HIV reverse transcriptase inhibitors[12]. They claimed

the treatment of HIV infection by coadministration of these compounds with at least one of the HIV-reverse transcriptase inhibitor and/or HIV protease inhibitor.

Figure 1

Examples of Ureido containing compound as antiinfectives

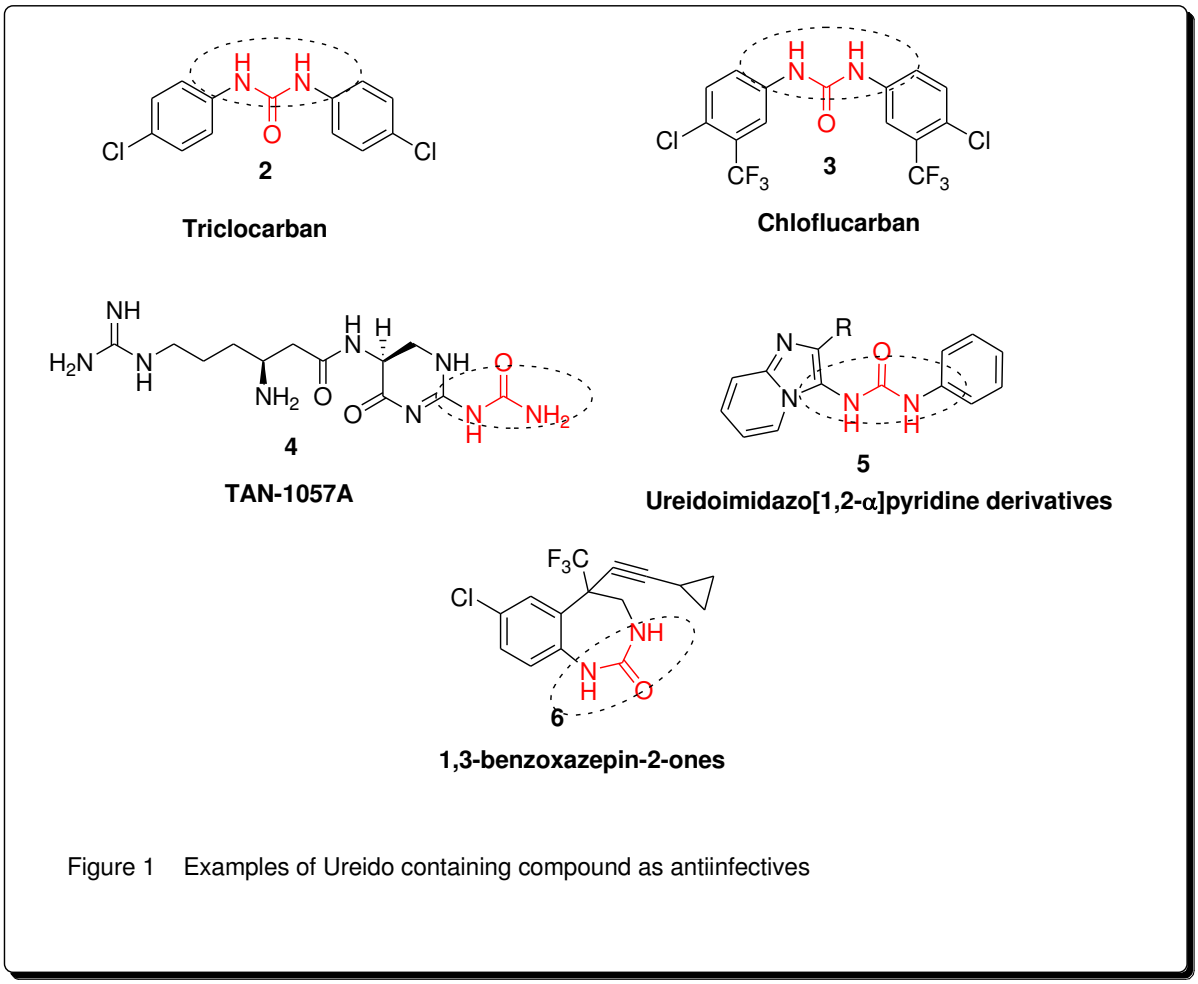


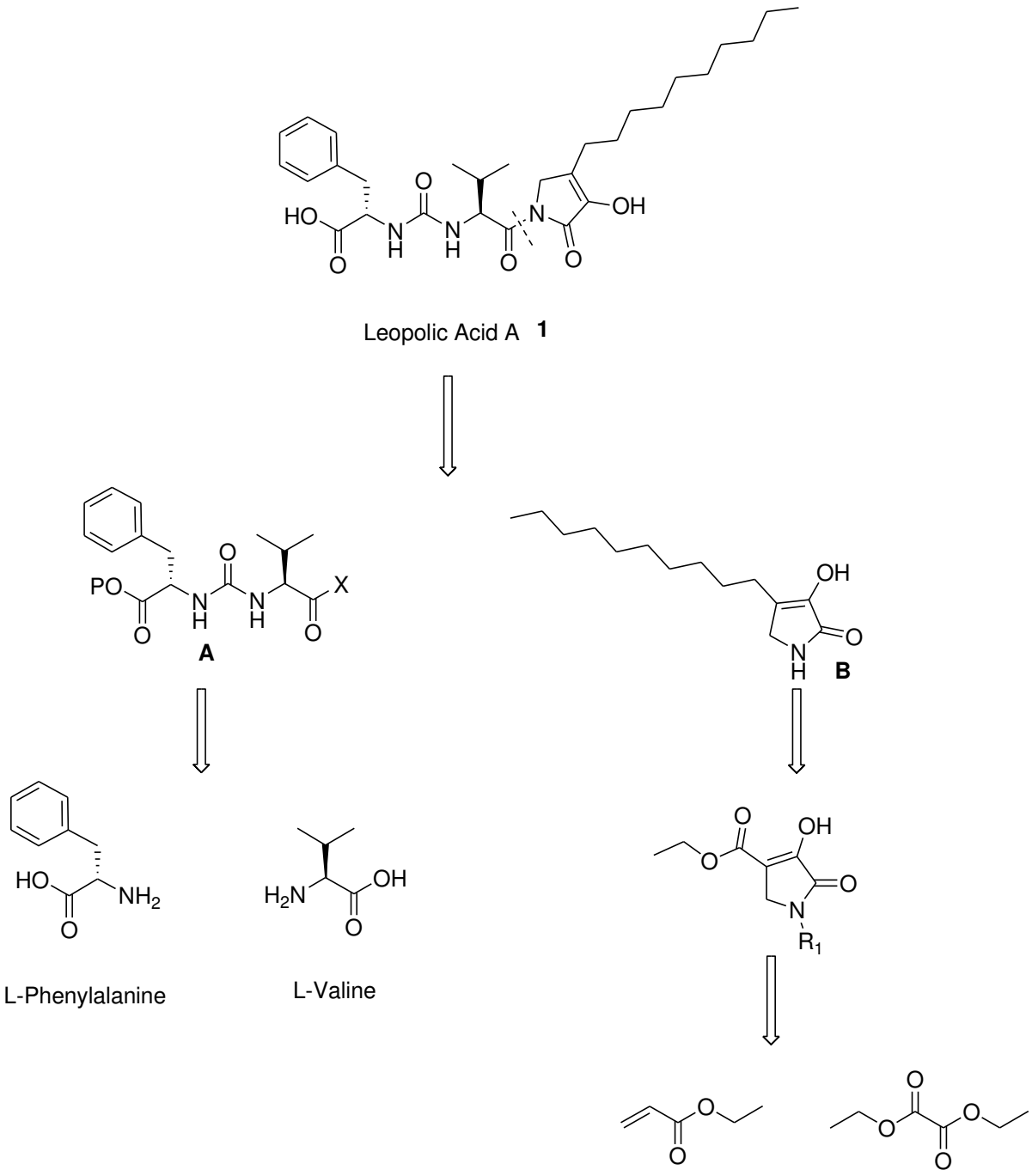
Figure 1 Examples of Ureido containing compound as antiinfectives

3.3 Synthesis of Leopolic Acid A

3.3.1 Retrosynthetic Approach

Our initial retrosynthetic analysis is outlined in scheme 3.1. In the beginning we envisioned that Leopolic Acid A could be constructed by a convergent synthetic strategy. Our first strategy for the synthesis of Leopolic acid A was based on the coupling of two domains: a protected ureidodipeptide **A** and 4-decyl-3-hydroxy-1,5-dihydroxypyrrol-2-one ring **B**. The ureido dipeptide **A** could be constructed from commercially available L-Phenylalanine and L-Valine and 4-decyl-3-hydroxy-1,5-dihydroxypyrrol-2-one ring **B** could be constructed from ethyl acrylate and diethyl oxalate.

Scheme 3.1



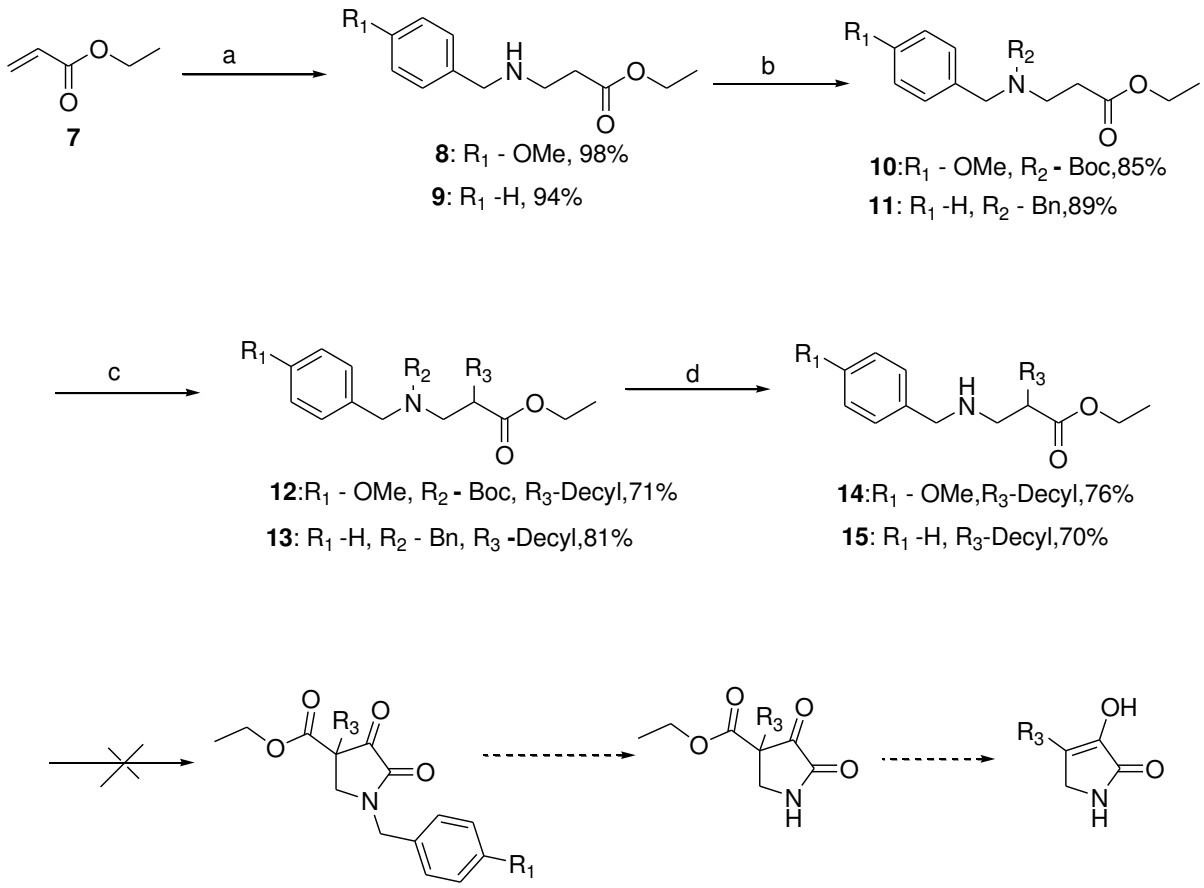
3.3.2 Attempts towards the synthesis of subunit B

The attempts towards the synthesis of subunit **B** are shown in scheme 3.2, scheme 3.3, scheme 3.4 and scheme 3.5.

The Michael addition of p-methoxybenzylamine and benzylamine on ethyl acrylate **7** gave respectively amine **8** and **9**[13].The Boc and benzyl protection of the respective amines furnished compounds **10** and **11** with 85% and 89% yield. The LDA- and HMPA-induced α alkylation of these amines gave **12** and **13**[14].The selective deprotection of Boc and benzyl groups of the amines was carried out with TFA and ceric ammonium nitrate respectively. Several attempts for Dieckmann cyclisation of compounds **14** and **15** with diethyl oxalate and different bases failed. The steric hindrance of the α–decyl chain might be the reason for the failure of Dieckmann cyclisation.

Then we turned our attention to cyclise a compound before the incorporation of the decyl chain. Dieckmann cyclisation of Michael products **8** and **9** with diethyl oxalate, NaOEt furnished the 2,3-pyrrolidinones **16** and **17**. As compounds with this skeleton are known to exist in the enolic 3-hydroxy-1,5-dihydro-pyrrol-2-one form, the enolic OH was first protected with a TBS group, by reacting it with TBSCl and imidazole to give compounds **18** and **19**[15]. Reduction of protected enols **18** and **19** with DIBAL-H was successful and gave alcohol **20** and **21** in72% and 90% yield. Several oxidation conditions were carried out on the alcohol, but resulted into decomposition of compounds. We tried different oxidising agents like PCC, PDC, Swern, MnO_2 and Dess-Martin.

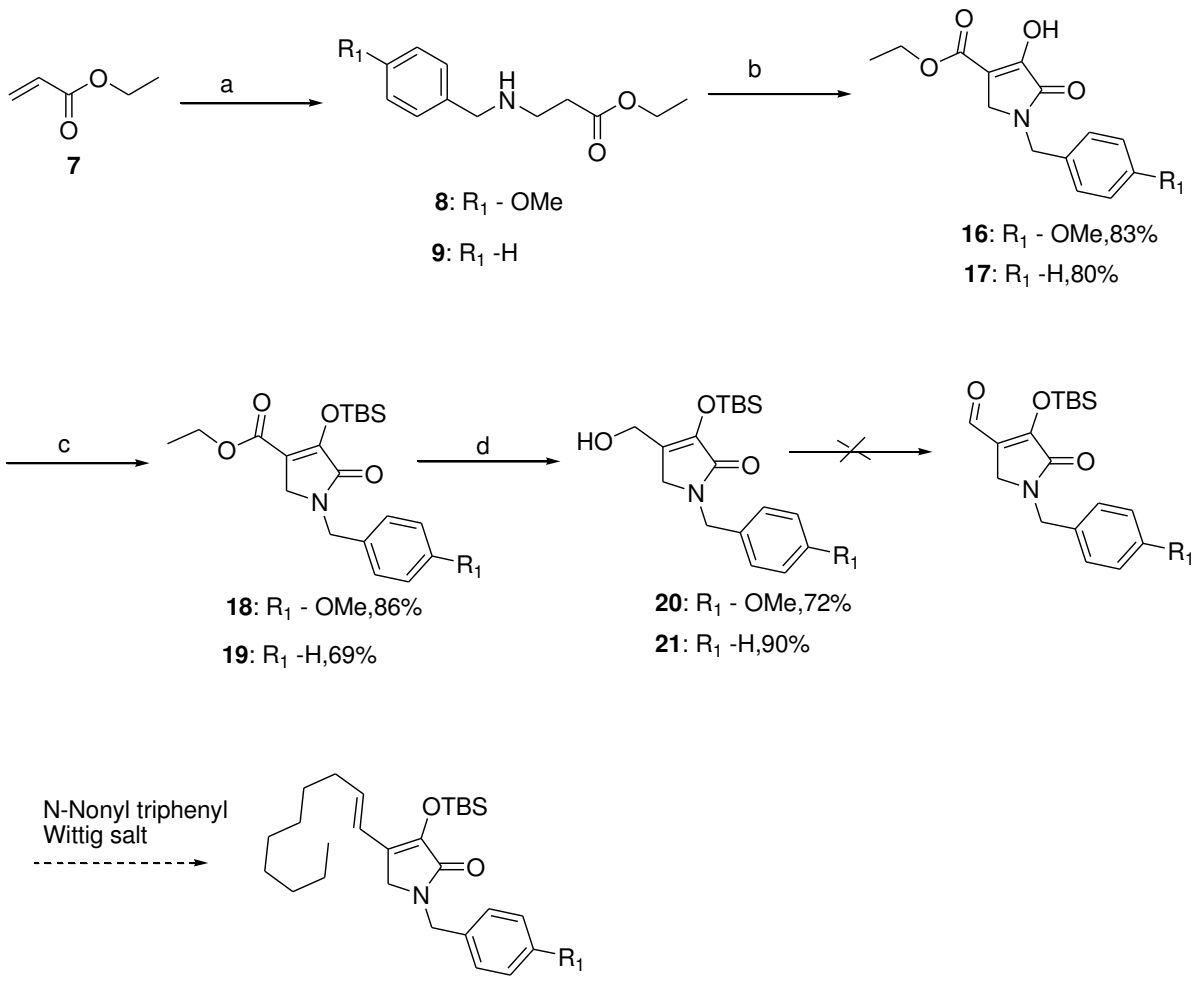
Scheme 3.2



Reagents and Conditions:

a) Benzylamine or p-methoxybenzylamine, EtOH, RT,12h b) BnBr, K_2CO_3,DMF,RT,12h and $(Boc)_2O$,TEA, DCM, RT, 2h c) LDA, HMPA, Decyl iodide, THF, -78^oC to RT, 12h d)TFA, DCM,RT, 12h and CAN, ACN:Water, RT, 4h

Scheme 3.3



7

8: R_1 - OMe

9: R_1 -H

16: R_1 - OMe,83%

17: R_1 -H,80%

18: R_1 - OMe,86%

19: R_1 -H,69%

20: R_1 - OMe,72%

21: R_1 -H,90%

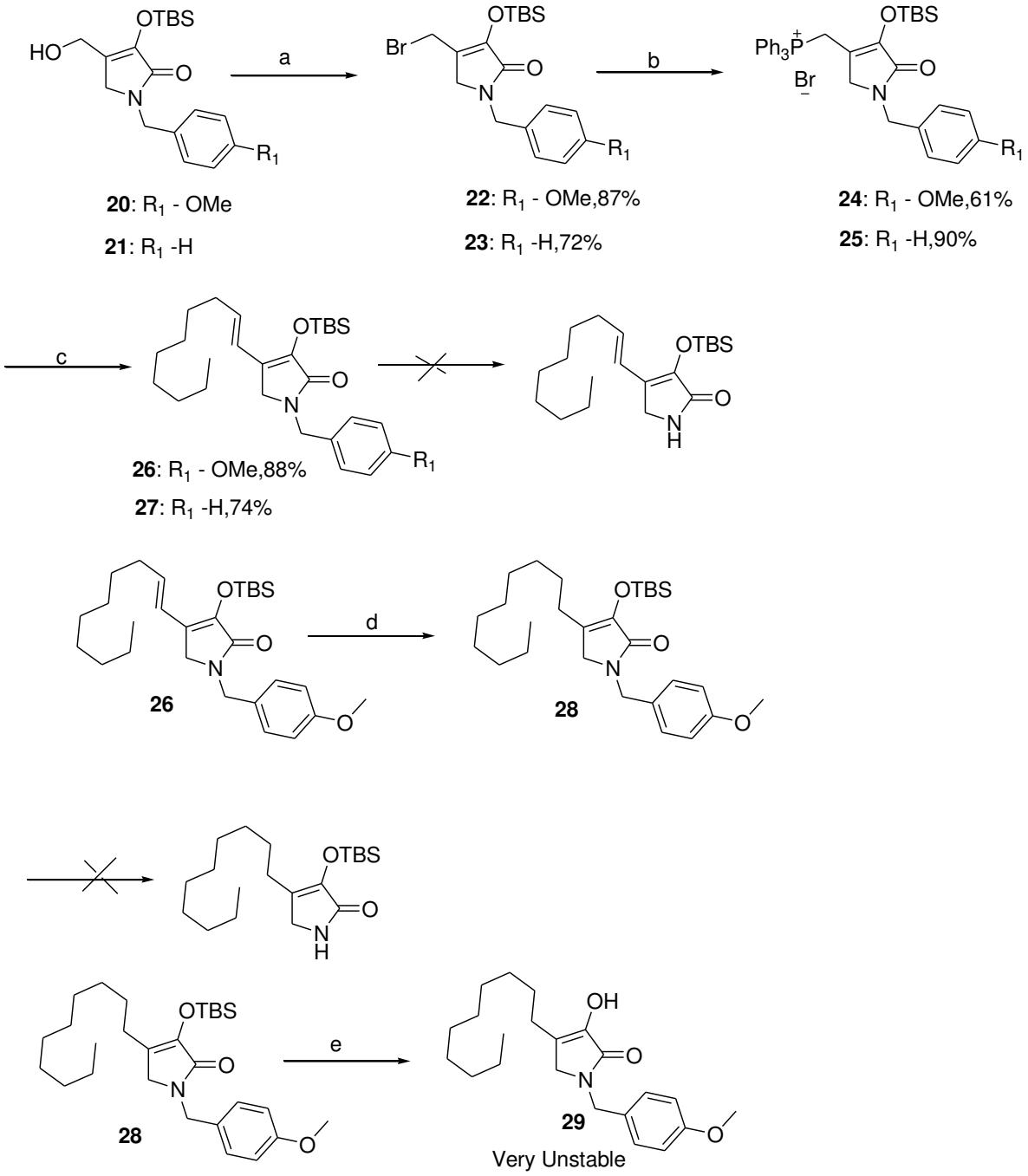
Reagents and Conditions:

a) Benzylamine or p-methoxybenzylamine, EtOH, RT,12h b) Diethyl oxalate, NaOEt, EtOH, Reflux, 4h c)TBSCl, Imidazole, DCM, 0^o to RT, 3h d) DiBAL-H,DCM, -78^oC, 2h

The alcohols **20** and **21** were then converted into the corresponding bromides **22** and **23** by Appel reaction with PPh_3 and CBr_4[16].This is shown in scheme 3.4. The bromide was reacted with PPh_3 to give the bromonium salts **24** and **25** which were, then, subjected to Wittig condensation with a long chain aldehyde, nonanal, to obtain compounds **26** and **27**[17]. Several attempts were carried out for deprotection of PMB protecting group but always resulted into decomposition of products. Deprotection was tried with the help of CAN, DDQ, TFA and hydrogenation. This decomposition may be attributed to the free 3-hydroxypyrrolidinone nucleus.

The double bond of compound **26** was, then, reduced by catalytic hydrogenation to give compound **28**. Then we tried to remove PMB group with different reagents but resulted into decomposition. Also deprotection of enolic TBS group of compound **28** with TBAF resulted into a very unstable and temperature sensitive compound which got decomposed during water work up.

Scheme 3.4

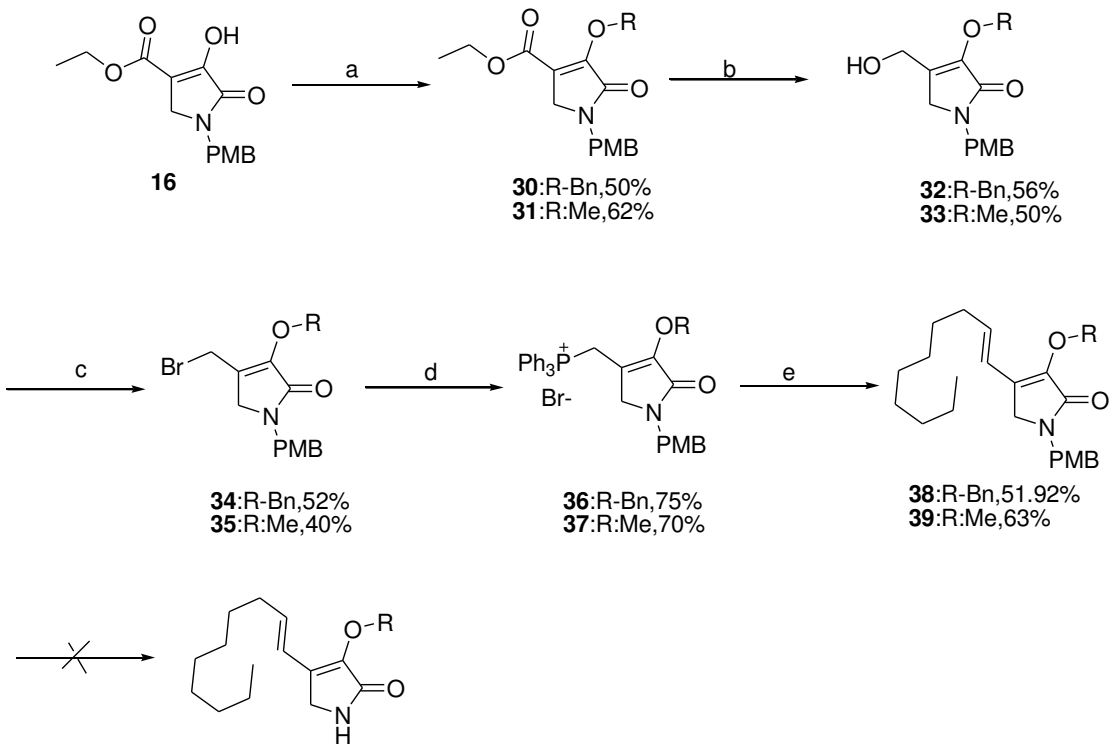


Reagents and Conditions:

a) PPh_3, CBr_4, DCM, 12h b) PPh_3, Toluene, reflux, 5h c) n-Nonanal, LiHMDS, THF, -78^oC to RT, 5h d) H_2, 10%Pd/C, RT, 1h, 89% e) TBAF, THF, O^oC, 1h.

In order to check the stability of compounds we changed the enolic protecting group and used benzyl and methyl protecting groups for enol protection. This is shown in scheme 3.5. The enol of compound **16** was protected with benzyl and methyl by using benzyl bromide and methyl iodide in DMF. The DIBAL-H reduction of esters **30** and **31** furnished alcohols **32** and **33**. The Appel reaction followed by bromination gave bromo compounds **34** and **35**.The long chain was incorporated by Wittig olefination reaction to gave **38** and **39**. Again we carried out different attempts for removal of PMB protecting group but resulted into decomposition. Isolation and purification for such compounds were very difficult. We also attempted the coupling of crude amide with ureido fragments **44**, **45** and **46** in different coupling conditions, but it resulted into no further reaction.

Scheme 3.5



Reagents and Conditions:

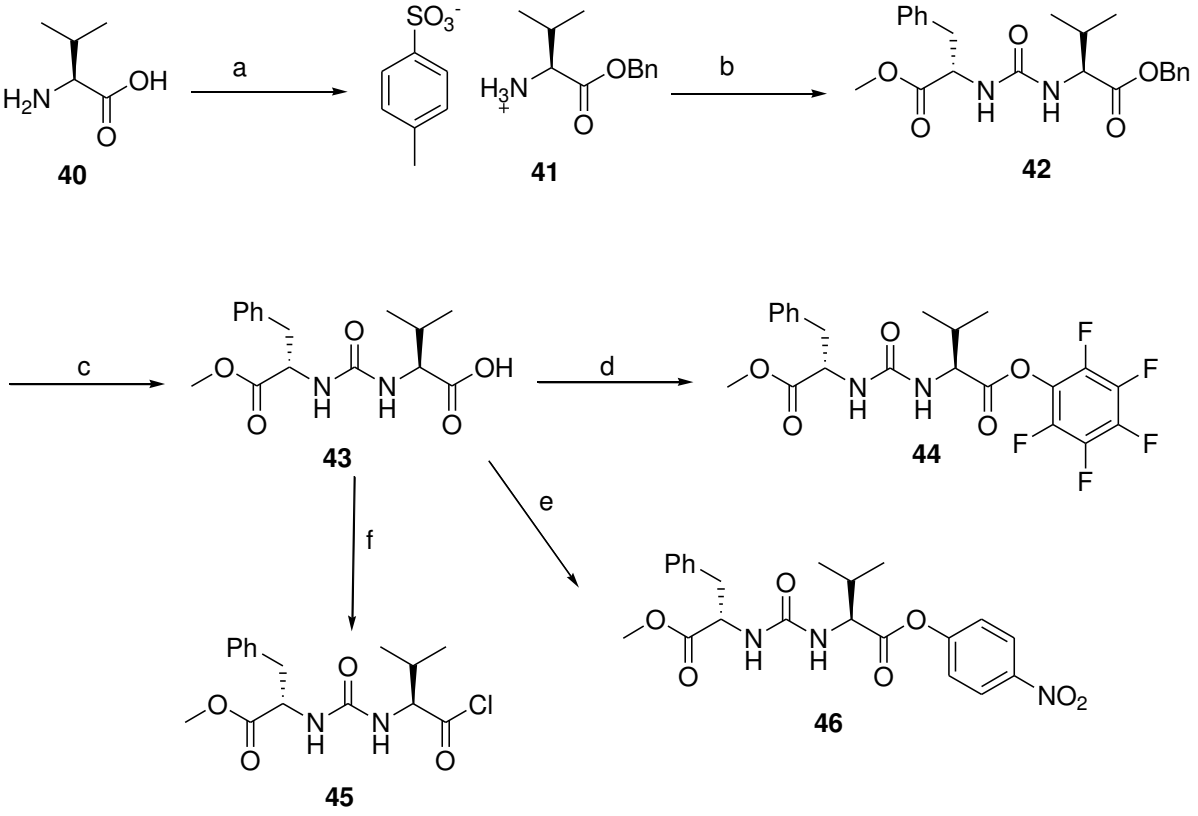
a) BnBr, K_2CO_3,DMF, 0^oC to RT, 3h and MeI, K_2CO_3,DMF, 0^oC to RT, 3h b) DIBAL-H, DCM, -78^oC, 2h c) PPh_3, CBr_4, DCM, 3h d) PPh_3, Toluene, reflux, 5h d) N-Nonanal, LiH, MDS, THF, -78^oC to RT, 5h.

3.3.3 Synthesis of subunit A

The linear synthesis of activated ureido fragment was achieved in 4 steps. This is shown in scheme 3.6 The benzyl protection of L-valine was carried out with PTSA and benzyl alcohol in toluene in 91% yield[18]. An unsymmetrical urea was synthesised between **41** and L-phenylalanine methyl ester hydrochloride with the help of triphosgene at room temperature in 85% yield[19].The catalytic hydrogenation of compound **42** furnished the desired acid **43**.The

acid **43** was activated by using different activating group like pentafluorophenol, p-nitrophenol and acid chloride.

Scheme 3.6

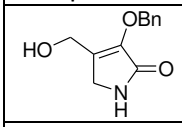
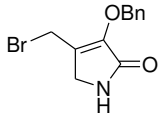
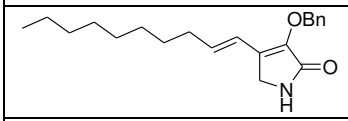
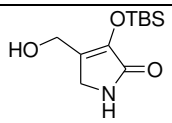
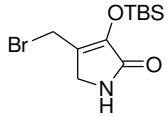
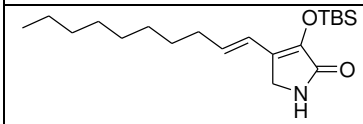
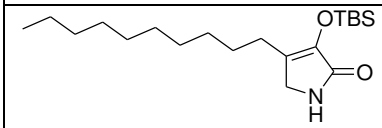
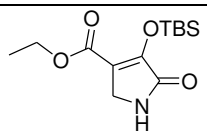
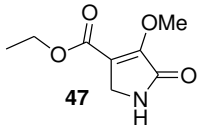
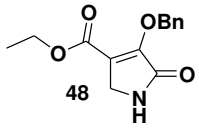


Reagents and Conditions:

a) PTSA.H_2O, Benzyl alcohol, Toluene, Reflux, 5h, 91% b)L-Phenylalanine methyl ester hydrochloride, Triphosgene, DIEA, DCM, RT, 2h,85% c) H_2,Pd/C,EtOAC,RT,2h,80% d) Pentaflourophenol,DCC,EtOAc,RT,12h,66% e) P-nitrophenol, DCC, EtOAc, 12h, RT,90% f) Oxalyl chloride, DCM, DMF, 0^oC to RT, 1h.

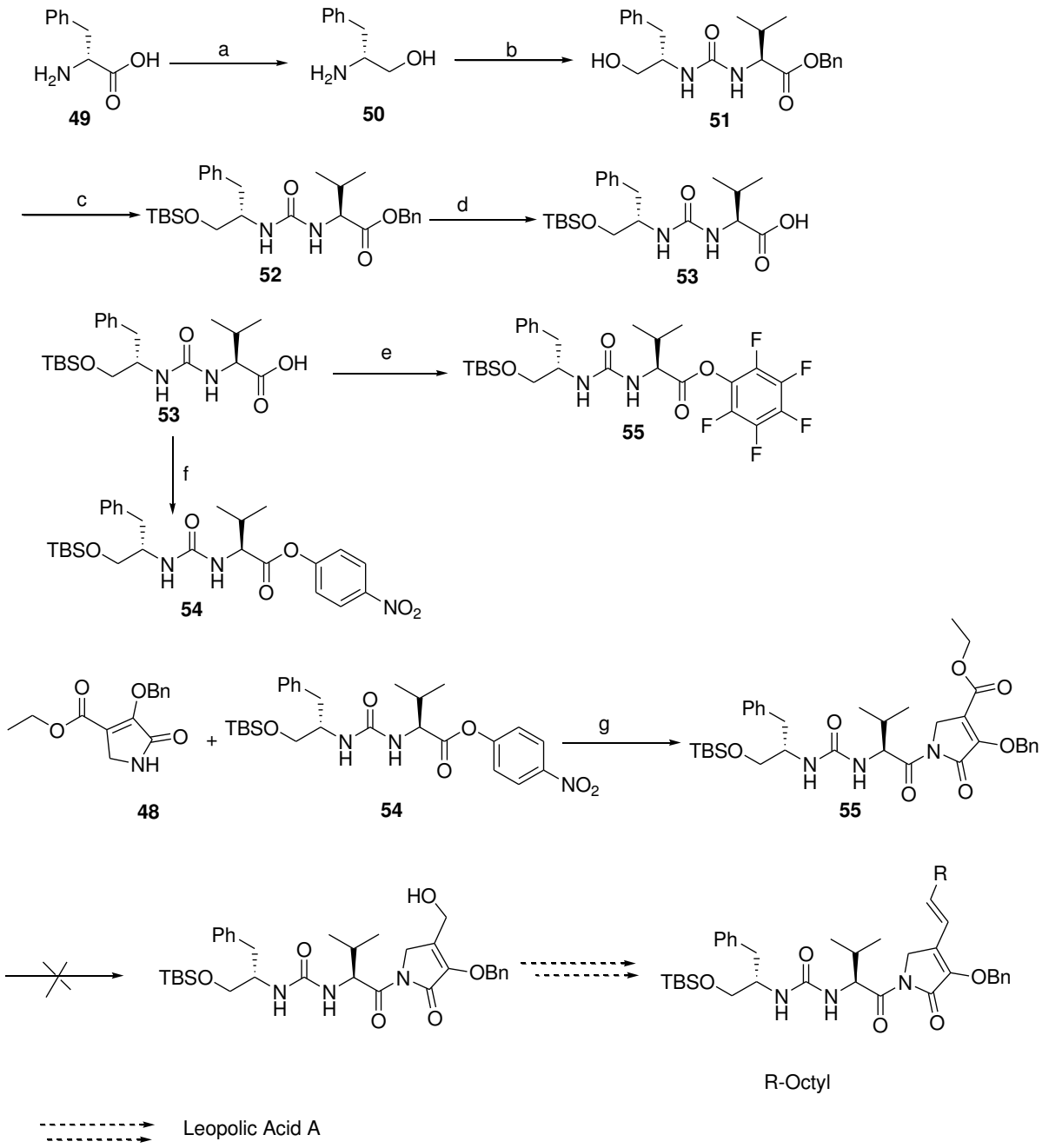
3.3.4 Attempts to remove the PMB group

The fragment A was synthesised in good yield following a coupling protocol from commercially available amino acids, L-phenylalanine and L-valine. Conversely the synthesis of fragment B was found to be quite cumbersome. Several attempts were made for removal of PMB protecting group on different intermediats, but resulted into decomposition. Finally we succeeded to remove the PMB group from compounds **30** and **31** with the help of CAN[20].

compounds	Result
	Unstable
	Unstable
	Unstable
	Unstable
	Unstable
	Unstable
	Unstable
	Complex mixture of compounds
 **47**	Stable but low yield
 **48**	Stable with good yield

Once having succeeded to deprotect PMB group from compound **30**, we planned to couple the protected ureido fragment **54**. This is shown in scheme 3.7. The reduction of amino acid **49** to amino alcohol **50**, using $LiBH_4$/TMSCl, proceeded cleanly and in high yield[21]. The ureido fragment between amino alcohol **50** and amino acid **24** was synthesised by using triphosgene, DIEA at RT. The alcoholic group of compound **51** was first protected as a TBS group with TBSCl and imidazole to give the corresponding ether **52** which was subjected to catalytic hydrogenation to give acid **53**. Intial attempts of synthesis of pentafluoro-activated ester of **55** resulted into unstable compound. Later we succeeded to synthesise the para-nitrophenol ester **54**. The coupling between amide **48** and the activated ester **54** was carried out by using NaH in THF. Several attempts of reduction of this ester failed.

Scheme 3.7



Reagents and Conditions:

a) $LiBH_4$,TMSCl, THF,RT,16h,94% b) **24**, DIEA, Triphosgene, DCM, RT,4 h,74% c) TBSCl, Imidazole, DCM, RT, 2h,84% d) H_2, 10% Pd/C, EtOAc,RT,1h,92% e) PFP,DCC, EtOAc,RT,16 h f) p-Nitrophenol, DCC, EtOAc, RT, 16h,77% g) NaH,THF,RT, 30 min, 20%

We were particularly surprised by the failure of the reduction of the ester group with DIBAL-H, a reaction which had been successful in other cases, e.g. on compound **30**. Therefore we attempted to repeat the reaction on the analogue **58** of compound **55**, where only the valine residue was attached to the pyrrolone ring, instead of both aminoacids (scheme 3.8). The NMR data shows that DIBAL-H reduction of compound **58** had reduced not only the ester, but also the CO group of the valine moiety, leading to compound **59**. Further Swern oxidation of compound **59** proceeded only on primary alcohol, CHOH group of the valine residue remaining unoxidised. We decided anyway to proceed until the end of the sequence, to verify the feasibility of the synthetic approach. When the second aminoacid residue was coupled using triphosgene, this reagent also reacted with the hydroxyl and amino groups of compounds **62/63** to give the oxazolone **65/66**. Subsequent catalytic hydrogenation in EtOAc of **65/66** afforded **67** in 50% yield. The core structure of compound **67** is new and a survey of the literature showed that compounds with this scaffold have never been isolated or synthesized before. Studies to evaluate its biological activity are underway. The NMR spectra of derivative **67** are reported in the next pages (Figures 2, 3, 4).

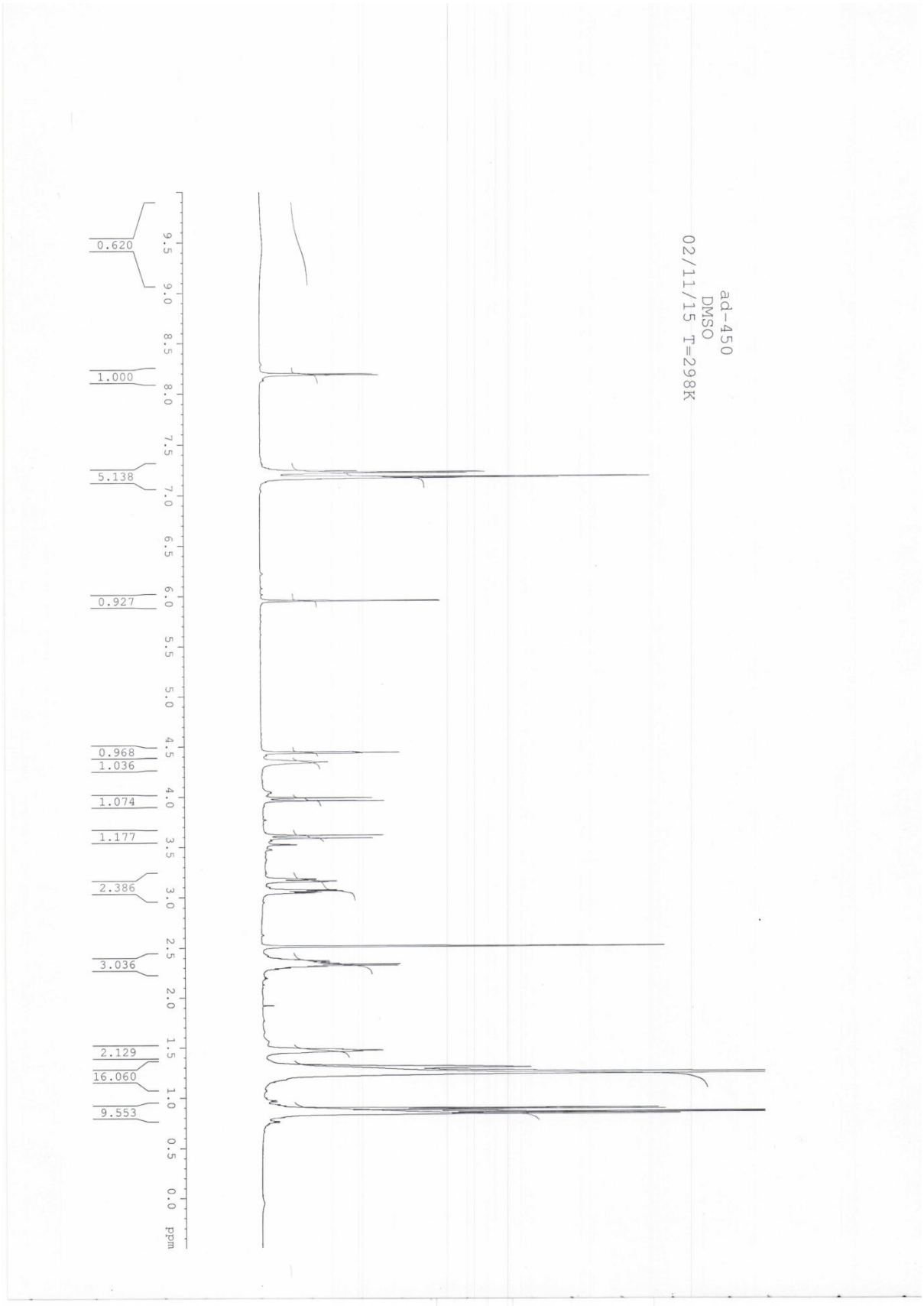


Figure 2. ^{1}H NMR of compound **67**

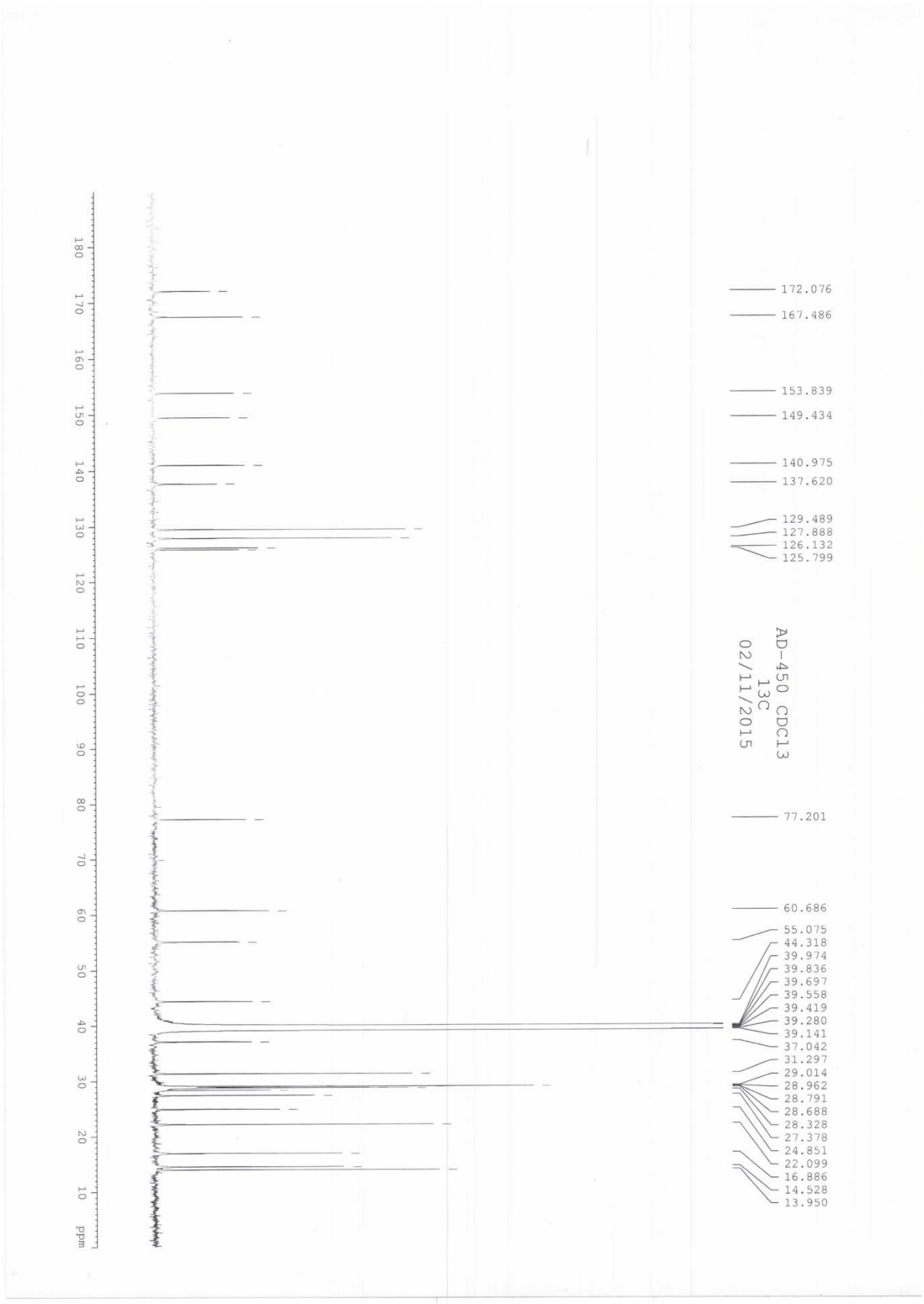


Figure 3 ^{13}C NMR of compound **67**

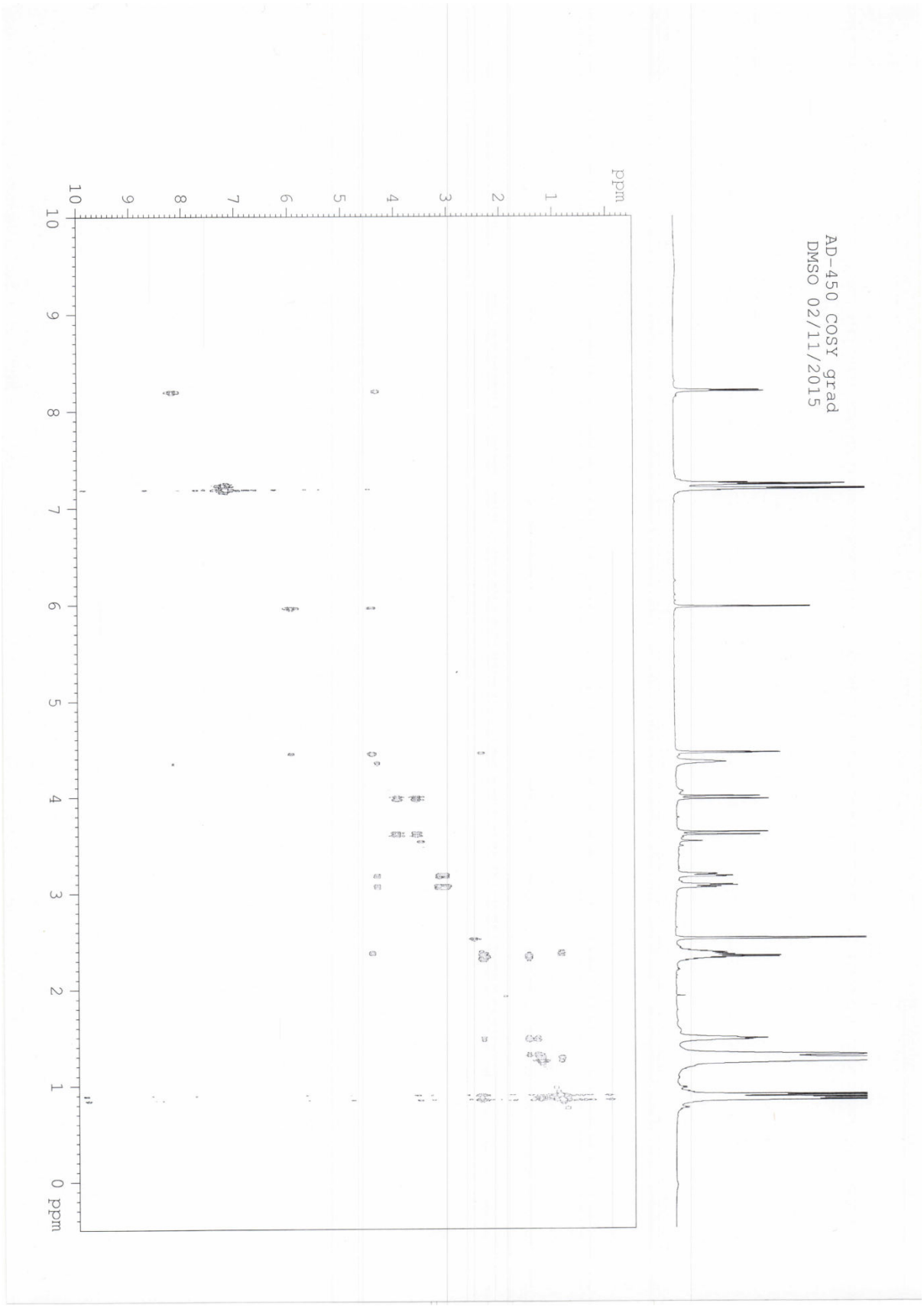
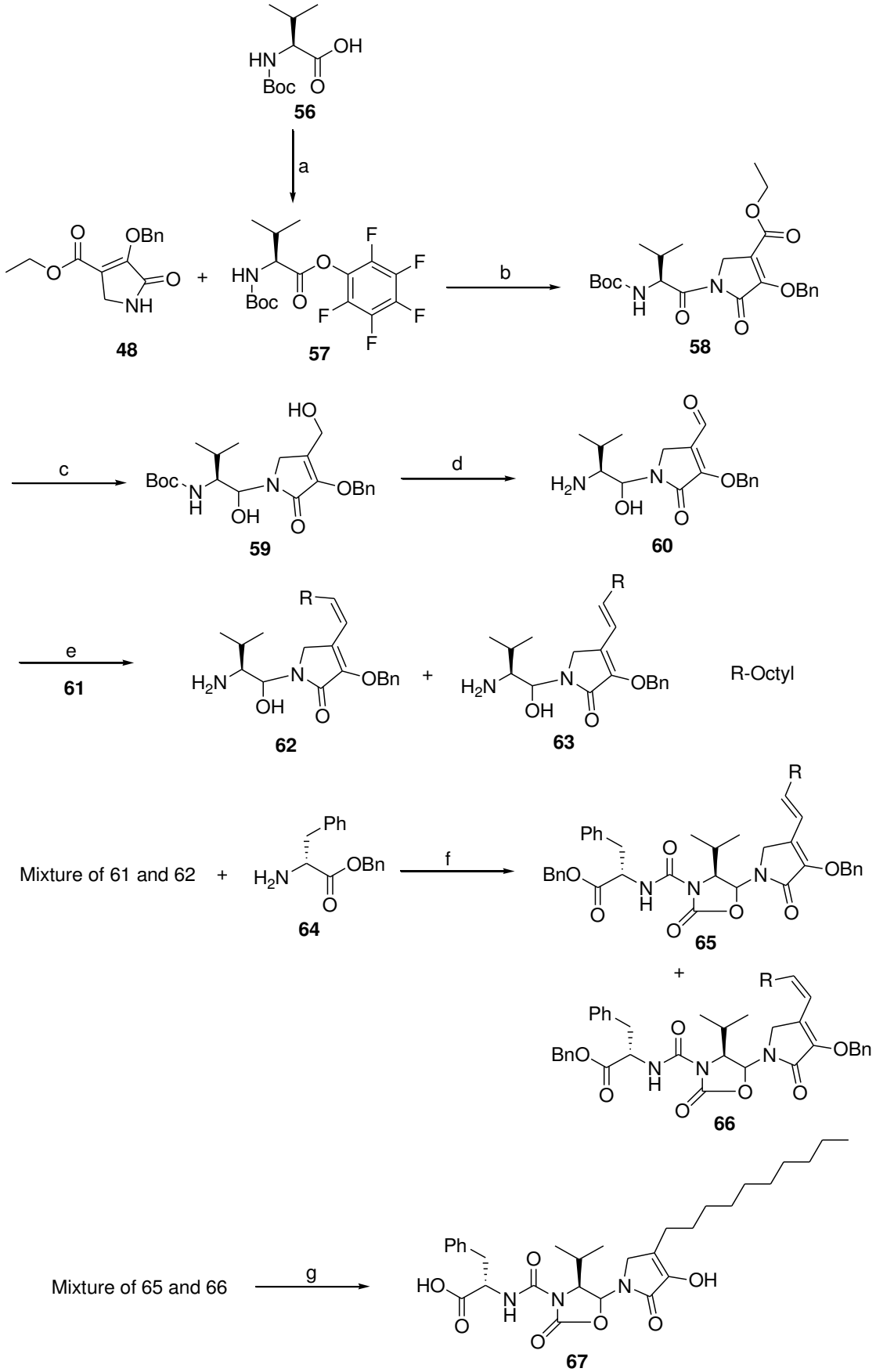


Figure 4. COSY spectrum of compound **67**

To obtain Leopolic acid we turned back to compound **59**. Both primary and secondary alcohols of compound **59** were oxidised with the help PCC to obtain aldehyde **88**. The long chain was incorporated on **88** by Wittig olefination reaction with n-nonyl triphenylphoshonium bromide and BuLi at -78^{o}C. The ureido fragment between amino acid **64** and compound **90** was synthesised by using triphosgene at rt. This crude compound was subjected to catalytic hydrogenation affording final desired Leopolic acid A.

Scheme3.8


HN
Boc
O
OH
56
a
O
OBn
O
O
NH
48
+
HN
Boc
O
O
F
F
F
F
F
57
b
Boc
N
H
O
N
O
O
OBn
O
58
c
HO
Boc
N
H
OH
N
O
OBn
59
d
O
H2N
OH
N
O
OBn
60
e
61
R
H2N
OH
N
O
OBn
62
+
R
H2N
OH
N
O
OBn
63
R-Octyl
Mixture of 61 and 62
+
Ph
H2N
O
OBn
64
f
Ph
BnO
O
O
N
H
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O
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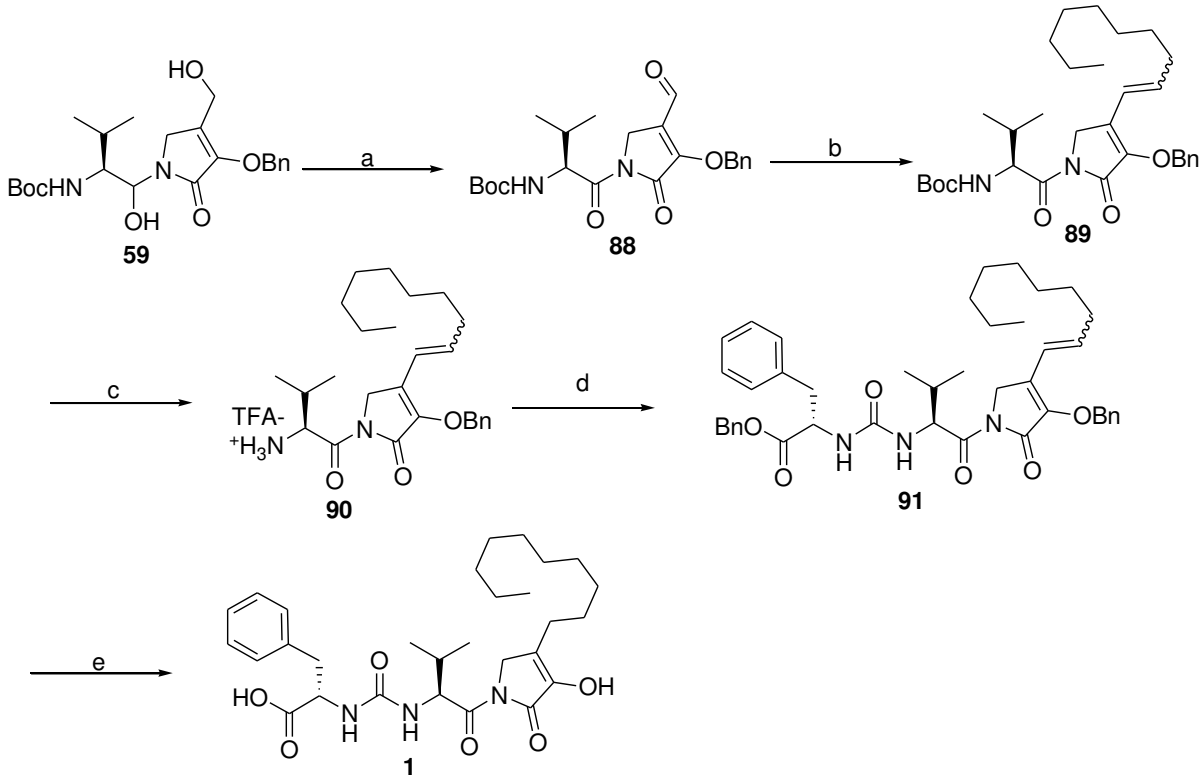
N
O
OBn
65
+
R
Ph
BnO
O
O
N
H
O
N
O
O
N
O
OBn
66
Mixture of 65 and 66
g
Ph
HO
O
O
N
H
O
N
O
O
N
O
OH
67

Reagents and Conditions:

a) PFP, DCC, EtOAc, 0°C to RT, 1h, 91 % b) BuLi, THF, -78°C,1h,86% c) DIBAL-H, DCM, -78°C, 2h, 30% d) Oxalyl chloride, DMSO,THF, TEA, -78°C to 0°C, 2h, 60% e) N-Nonyl triphenylphoshonium bromide (**61**),THF,-78°C to 0°C, 2h, 73.80% f) DIEA, Triphosgene, DCM, RT,72h, 30% g) H_2, Pd/C, EtOAc, RT, 2h, 50%.

Scheme 3.9



Reagents and Conditions:

a) PCC, DCM, 0°C to RT, 48h, 46% ; b) N-Nonyl triphenylphoshonium bromide (**61**), THF,-78°C to 0°C, 2h, 36% c)TFA, DCM, 0°C to RT d) **64**, DIEA, Triphosgene, DCM, RT, 72h, 50% e) H_2,Pd/C, EtOAc, RT, 2h, 70%.

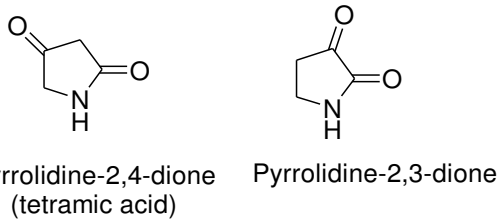
Part B

Approach towards the synthesis of a positional isomer of leopolic acid A

The interesting structure of Leopolic acid A, with the ureido fragment linked to the pyrrolone core, and the antibacterial activity of some ureido compounds (see 3.2) induced us in the meantime to undergo the synthesis of a positional isomer of Leopolic acid A, i.e. a compound with the same side chains, but with a tetramic acid (instead of a 2,3-pyrrolidinedione) core, with the hope of increasing the antibacterial activity of tetramic acids (Figure 5).

Figure 5

General structure of 2,3 and 2,4-pyrrolidinediones



Pyrrolidine-2,4-dione (tetramic acid)

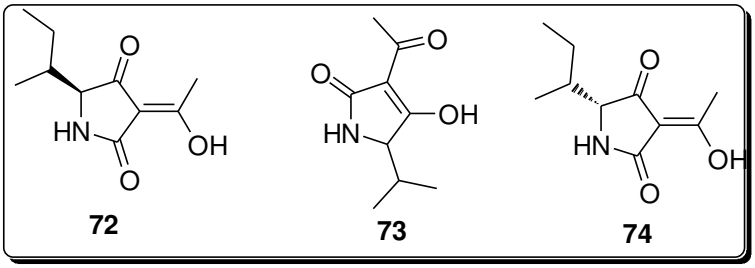
Pyrrolidine-2,3-dione

3.4 Importance of Tetramic acid products

Natural products containing the tetramic acid structure (pyrrolidine-2,4-dione) are isolated from various terrestrial and marine organism; often they are metabolites originating from bacteria, fungi and assorted sponges. These structures can be characterised as simple heterocycles or more complex systems possibly containing long-chain fused polycyclic skeletons. This types of natural products often display wide ranging and potent biological activities including antibacterial, antiviral and antitumoral activities. Due to the intriguing structure and biological activity, tetramic acid containing products are attracting increasingly significant attention. Therefore this tetramic acid core is a key structure both for the synthesis of natural products as well as their unnatural analogues.

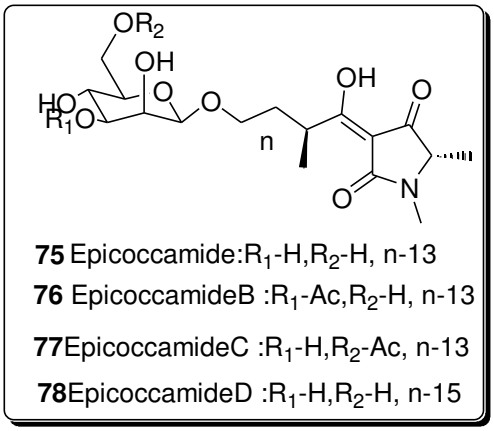
3.5 Natural compounds containing Tetramic acid core

3.5.1 L-tenuazonic acid



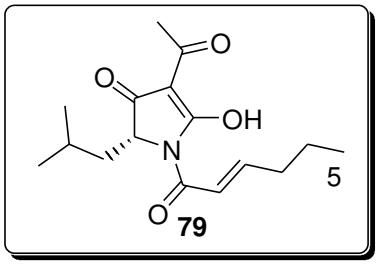
The phytotoxin L-tenuazonic acid (**72**) was first isolated from the phytopathogenic fungus *Alternaria alternate*, the causative agent behind brown leaf spot of *Eupatorium adenophorum*[22]. The related analogue 3-acetyl-5-isopropyltetramic acid (3-AIPTA, **73**) was isolated from the same strain by supplementing the culture with precursor L-valine[23]. In 2004, **72** and **73** along with D-allo-tenuazonic acid (**74**) were re-discovered from two fungal strains *A. brassicicola* and *A. raphani* originating from pollen collected from beehives, which showed inhibitory activity against the causative agent of American foulbrood *Paenibacillus larvae*[24]. Further bioassays revealed that **74** accounts for the activity against *P. larvae* (MIC 32 μg/mL), and displays activity comparable to the antibiotic oxytetracycline currently used to control American foulbrood[25]. Compounds **72** and **73** were also found to inhibit root and shoot growth rates and **73** displayed phytotoxicity against a wide range of plants.

3.5.2 Epicoccamide



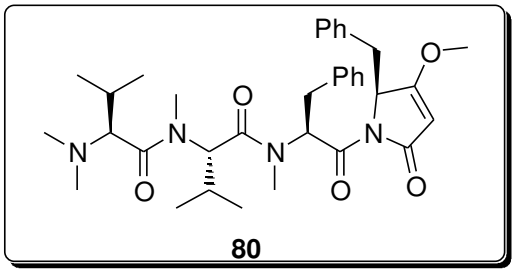
Epicoccamide (**75**) was first isolated from cultures of the fungus *Epicoccum purpurascens* originating from the inner tissue of the jellyfish *Aurelia aurita* collected in the North Sea in 2001[26]. The compound was re-isolated as the major metabolite along with epicoccamides B–D from the endofungal *Epicoccum sp*. associated with the tree fungus *Pholiota squarrosa* in 2008[27]. Epicoccamides consist of three biosynthetically distinct subunits: a β -D-mannose, an aliphatic chain, and a tetramic acid moiety, which differ in their mannose substitution patterns and the length of their aliphatic chains. Epicoccamide (**75**) has thus far failed to show antimicrobial or cytotoxity activity although epicoccamides B–D (**76-78**) have demonstrated antiproliferative activity against cell lines L-929, K-562 and HeLa cell[26-27]. Epicoccamide D is the most active against HeLa (CC_{50}=17.0 μM), L-929 (GI_{50}= 50.5 μM) and K-562 (GI_{50} = 33.3 μM). Furthermore, epicoccamide D induces morphogenesis and pigment formation in surface cultures of the fungus *Phoma destructia* at a concentration of 1.7 μM.

3.5.3 Reutericyclin



Reutericyclin (**79**), another tetramic acid was found to possess N-1 substitution with an α,β unsaturated fatty acid, and was the first low molecular weight antibiotic isolated from the broth of lactic acid bacteria *Lactobacillus reuteri* LTH2584 originating from an industrial sourdough SER[28]. Reutericyclin has shown potent activity against a broad range of Gram-positive bacteria and drug-resistant strains with the values of MICs in the range 0.08 to 6.25 mg mL^{-1}. However, thus far, reutericyclin has failed to inhibit the growth of yeast, fungi and Gram-negative bacteria. Differences in the activity against *L. sanfranciscensis* between natural reutericyclin (MIC=0.10 mg mL^{-1}) and synthetic racemic reutericyclin (MIC=0.75 mg mL^{-1}) indicated that stereochemistry is vital to the compounds bioactivity[29].

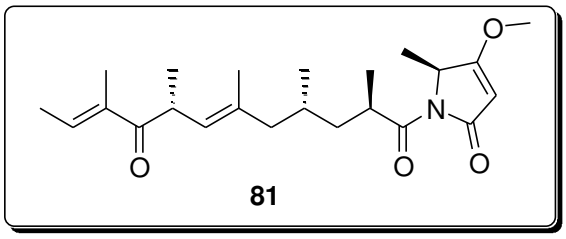
3.5.4 BelamideA



80

Belamide A (**80**), a highly methylated structural analogue of dolastatin was isolated from the Panamanian marine cyanobacterium *Symploca Sp*[30]. Compound **80** was found to be cytotoxic to MCF7 and HCT-116 cell lines with IC_{50} values of 1.6 μM and 0.74 μM, respectively. In addition, **80** showed classic microtubule depolymerizing effects toward A-10 cells at 20 μM[30].

3.5.5 Eliamid



81

Eliamid (**81**) was isolated from two strains: ambruticins producer *Sorangium cellulosum* So ce439 (DSMZ 11529) and soraphen producer *S. cellulosum* So ce241[31]. Eliamid was found to exert potent activity against a panel of transformed cell lines with the IC50 values in the range 0.5–30.0 ng ML^{-1}. In addition, **81** showed cytotoxic activity against brine shrimp *Artemia salina* at 0.2–0.3 μg/mL, lethal activity against soil nematodes (*Panagrellus spec*.) at 5 μg/mL, and moderate inhibitory activity against fungi and yeast in agar diffusion assays (ranging from 15–22 mm zone at 20 μg per disk). The mode of action was determined by examining the effects of **81** on the mitochondrial respiratory chain. Compound **81** was found

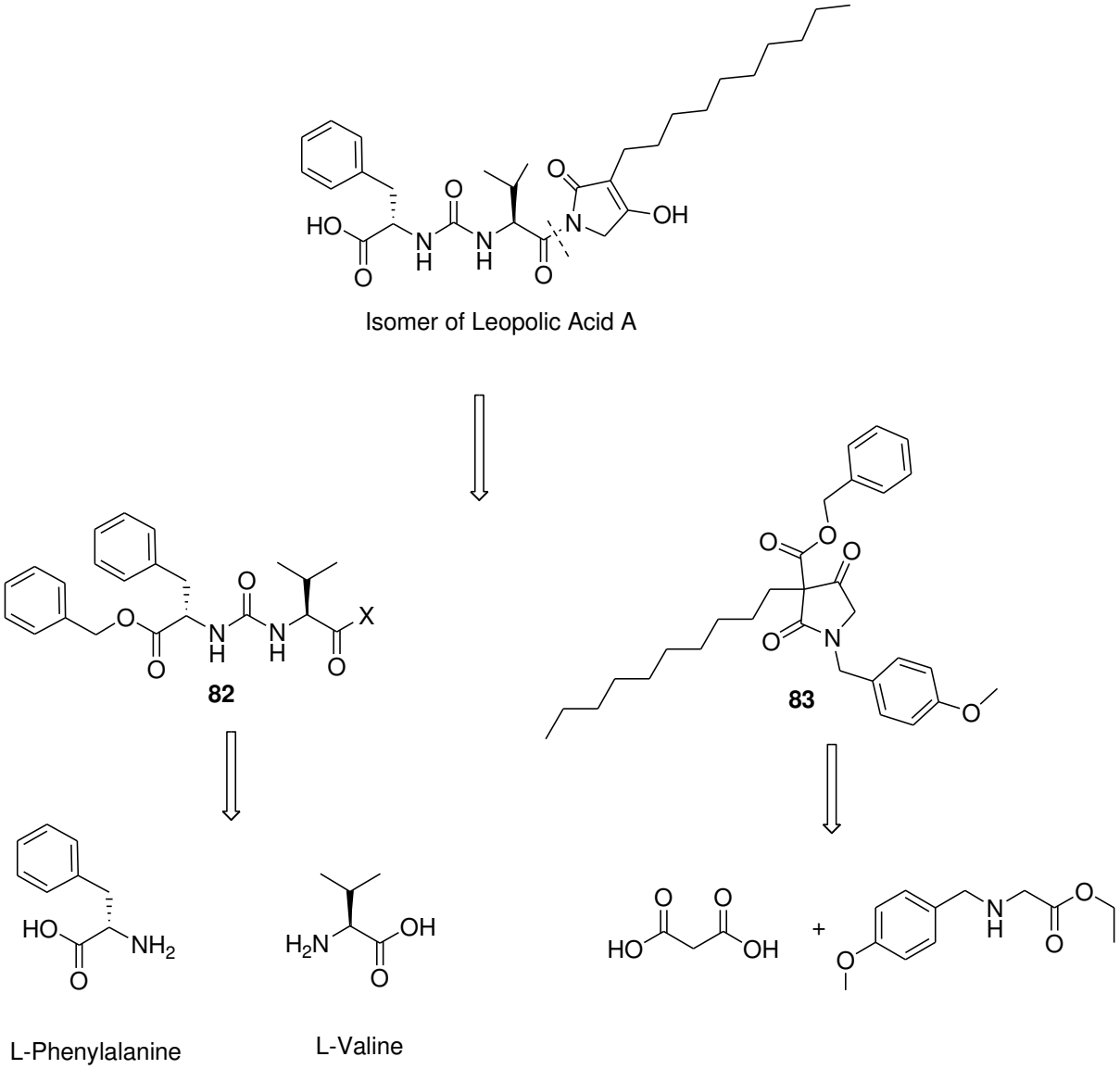
to strongly inhibit NADH oxidation in beef heart submitochondrial particles (SMP) with an IC_{50} of 8 ng/mL and differential spectroscopy experiments with beef SMP indicated that **81** is a potent inhibitor of complex I (NADH-ubiquinone oxidoreductase) within the eukaryotic respiratory chain.

3.6 Approach towards the synthesis of a positional isomer of Leopolic Acid A

3.6.1 Retrosynthetic Approach

Our initial retrosynthetic analysis is outlined in scheme 3.9. We envisioned that an isomer of Leopolic acid A containing a tetramic acid core could be constructed by a convergent synthetic strategy. Our strategy for the synthesis of the isomer of Leopolic acid A was based on the coupling of two domains: Ureido fragment **82** and Tetramic core **83**.

Scheme 3.9

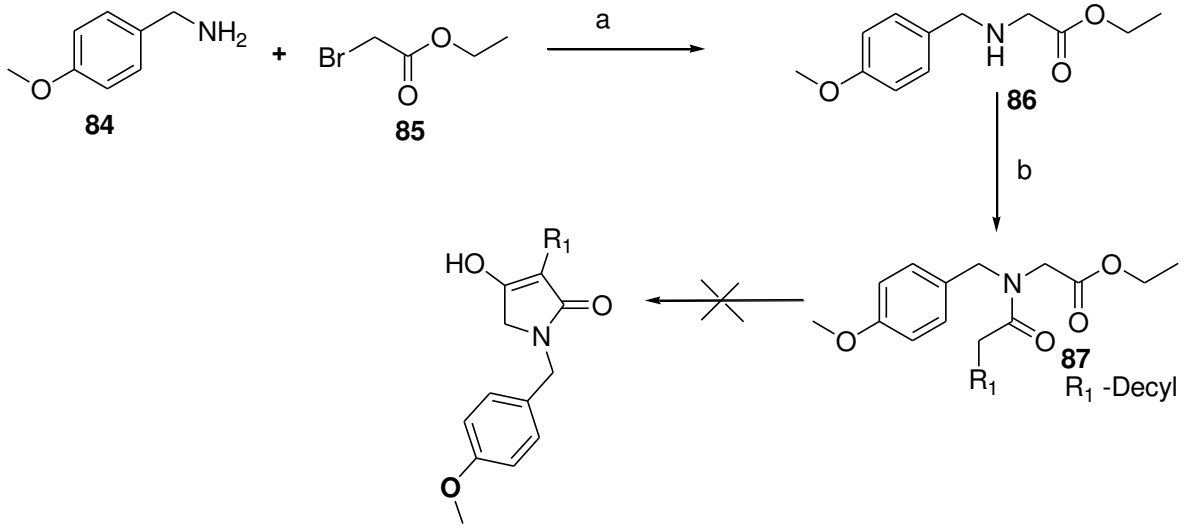


3.6.2 Synthesis of Tetramic core 83

N-(4-methoxybenzyl)glycine ethyl ester **86** was synthesized from ethyl bromoacetate and p-methoxybenzylamine[32]. The amine **86** was converted into N-dodecanoyl-(4-methoxybenzylamino)acetate **87** with the help of $CHCl_3$ and TEA[33]. The Dieckmann

condensation of **87** was carried out in different conditions but resulted into no reaction. The synthesis of Tetramic core **83** is shown in scheme 3.11. N-(4-methoxybenzyl)glycine ethyl ester **86** was converted into amide **88** by condensation with benzyl malonyl chloride with the help of DCC , DMAP in 83% yield. Tetrabutylammonium fluoride in ether at room temperature induced cyclisation and formation of an enolate which was subsequently treated with 1-iododecane to afford **89**[34]. PMB deprotection of **89** with ceric ammonium nitrate afforded amide **90**. The coupling between amide **91** and pentafluoro-activated ureido fragment **44** by using BuLi in dry THF afforded compound **91**. Now we are trying to remove the benzyl ester with hydrogenolysis and subsequentally decarboxylation of the acid .

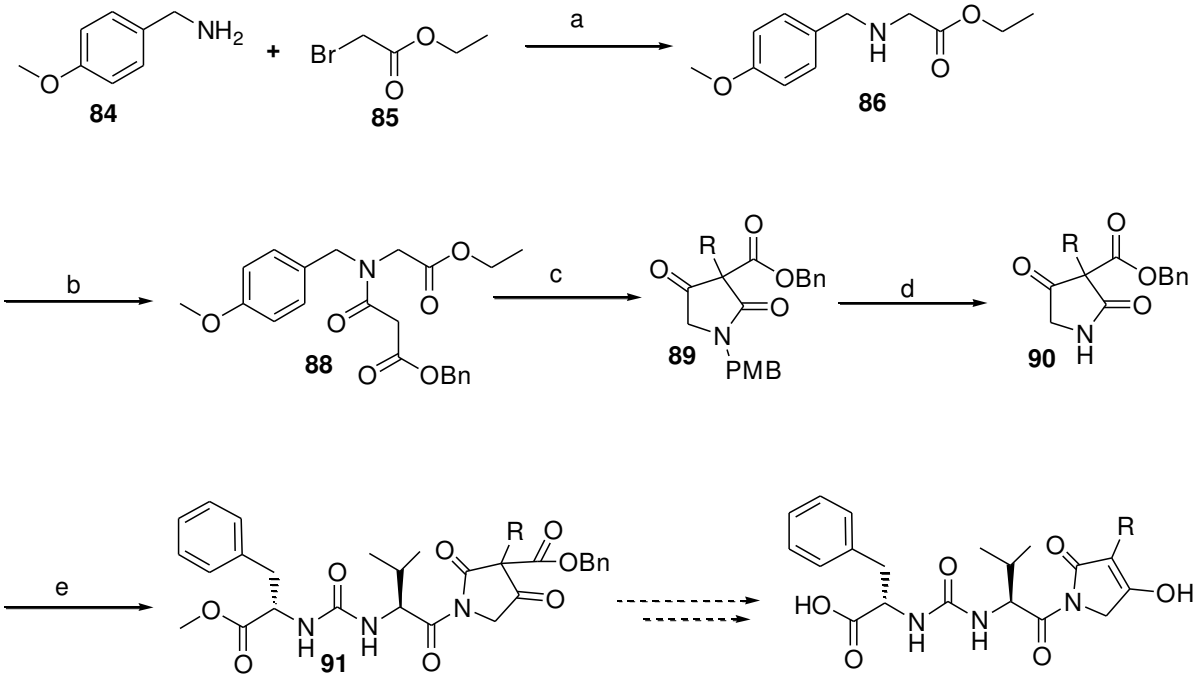
Scheme 3.10



Reagent and Conditions:

a) TEA, THF, 0^{o}C to RT, 2.5h, 87%; b) Dodecanoyl chloride, TEA, $CHCl_3$, 83%

Scheme 3.11



Reagent and Conditions:

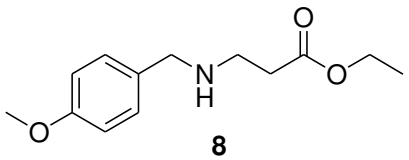
a) TEA, THF, 0^{o}C to RT, 2.5h, 87%; b) DCC, DMAP, DCM, 0^{o}C to RT, 24h, 83% c) TBAF,Et_2O,THF,1-Iododecane, RT, 24h 30% d) CAN, ACN:Water (3:1), 0^{o}C to RT, 1h, 66%.

3.7 Experimental section

General Experimental methods

All reagents and solvents were reagent grade or were purified by standard methods before use. Melting points were determined in open capillaries and are uncorrected. NMR spectra were recorded in $CDCl_3$ (where not otherwise stated) at 300 MHz. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were obtained by distillation from sodium-benzophenone ketyl; dry methylene chloride was obtained by distillation from phosphorus pentoxide. All reactions requiring anhydrous conditions were performed under a positive nitrogen flow, and all glassware were oven dried and/or flame dried. Isolation and purification of the compounds were performed by flash column chromatography on silica gel 60 (230-400 mesh). Analytical thin-layer chromatography (TLC) was conducted on Fluka TLC plates (silica gel 60 F_{254}, aluminum foil).

Ethyl 3-(4-methoxybenzylamino)propanoate



8

A mixture of 4-methoxybenzylamine (10 g, 72.90 mmol) in ethanol (100 mL) and ethyl acrylate (6.27 g, 72.90 mmol) was stirred at room temperature for 12 h. After completion of reaction, the mixture was concentrated in vacuo to give product **8** (16.88 g, 98%)

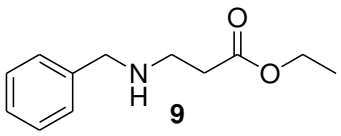
Physical appearance- Pale yellow liquid

R_f 0.5 (EtOAc/ Hexane 6:2)

^{1}H-NMR ($CDCl_3$) δ: 7.23 (2H, d, J = 8.5 Hz); 6.85 (2H, d, J = 8.5 Hz); 4.13 (2H, q, J = 7.2 Hz); 3.80 (3H, s); 3.74 (2H, s); 2.88 (2H, t, J = 6.6 Hz); 2.52 (2H, t, J = 6.6 Hz); 1.25 (3H, t; J = 7.2 Hz).

Anal. calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: 66.01; H, 8.03; N, 5.92.

Ethyl 3-(benzylamino)propanoate



9

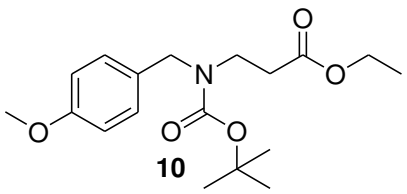
A mixture of benzylamine (10 g, 93.35 mmol) in ethanol (100 mL) and ethyl acrylate (6.27 g, 93.35 mmol) was stirred at room temperature for 12 h. After completion of reaction, the mixture was concentrated in vacuo to give product **8** (18 g, 94%)

Physical appearance- White viscous liquid

R_f 0.5 (EtOAc/ Hexane 6:2)

^{1}H-NMR ($CDCl_3$) δ: 7.40-7.32 (3H, m); 7.38-7.25 (2H, m); 2.16 (2H, t, J = 7.3 Hz); 3.83 (2H, s); 2.95 (2H, t, J = 7.2 Hz); 2.53 (2H, t, J = 7.2 Hz); 1.23 (3H, t, J = 7.3 Hz).

Tert-butyl 2-(ethoxycarbonyl)ethyl-4-methoxybenzylcarbamate



10

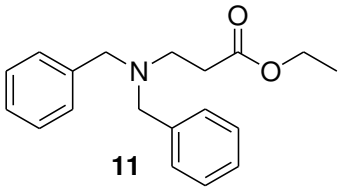
To the stirred solution of compound **8** (2.0g, 8.4mmol) in dry DCM were added TEA (2.2 g, 10.11mmol) and $(Boc)_2O$ (1.8g, 1.8 mmol) at 0^oC, then the solution was allowed to stir for 5h at room temperature. After the completion of reaction, it was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **10** (2.4 g, 85%)

Physical appearance- White viscous liquid

R_f 0.7 (EtOAc/ Hexane 2:8)

^{1}H-NMR ($CDCl_3$) δ: 7.16 (2H, m); 6.85 (2H, d, J = 8.5 Hz); 4.38 (2H, s); 4.10 (2H, q, J = 7.0 Hz); 3.80 (3H, s); 3.46 (2H, m); 2.48 (2H, m); 1.44 (9H, s); 1.24 (3H, t, J = 7.0 Hz).

Ethyl 3-(dibenzylamino)propanoate



11

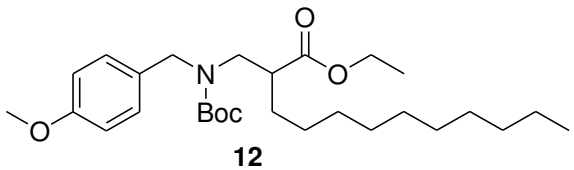
To the stirred solution of compound **9** (2.0g, 9.6mmol) in dry DMF (40mL) was added K_2CO_3 (8.0 g, 57.93 mmol) at 0^oC and the solution was stirred for 10 min, then BnBr (4.95g, 28.96 mmol) was added. Then reaction was allowed to stir for 24h at room temperature. After the completion, the reaction mixture was concentrated in vacuo. The crude product was purified by flash column chromatography (04% EtOAc/Hexane) to afford **11** (2.5 g, 89%)

Physical appearance- White viscous liquid

R_f 0.5 (EtOAc/ Hexane 1:9)

^{1}H-NMR ($CDCl_3$) δ: 7.42-7.15 (10H, m); 4.08 (2H, q, J = 7.3 Hz); 3.58 (4H, s); 2.81 (2H, t, J = 7.2 Hz); 2.49 (2H, t, J = 7.2 Hz); 1.20 (3H, t, J = 7.3 Hz).

Tert-butyl 2-(ethoxycarbonyl)dodecyl- 4-methoxybenzylcarbamate



12

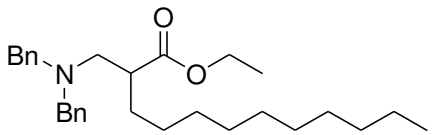
To the stirred solution of compound **10** (1.0g, 2.9 mmol) in dry THF (20mL) was added a 1.8M solution of LDA (4.1mL, 7.4 mmol) at -78^{o}C. After 45 min HMPA (3.1g, 17.78 mmol) was added and the solution was stirred for 20 min at same temperature. After addition of neat 1-iododecane (3.9 g, 14.81 mmol) at - 78^{o}C, the solution was allowed to raise the temperature and stirred for 24 at RT. After the completion of reaction, the mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (6% EtOAc/Hexane) to afford **12** (1.0 g, 71%)

Physical appearance- Colorless viscous liquid

R_f 0.5 (EtOAc/ Hexane 1:9)

^{1}H-NMR ($CDCl_3$) δ: 7.16 (2H, m); 6.86 (2H, d, J = 8.2 Hz); 4.14 (2H, q, J = 6.7 Hz); 3.81 (3H, s); 3.30 (2H, m); 2.78 (1H, m); 1.65-1.20 (30H, m); 0.89 (3H, t, J = 6.7 Hz).

Ethyl 2-((dibenzylamino)methyl)dodecanoate



13

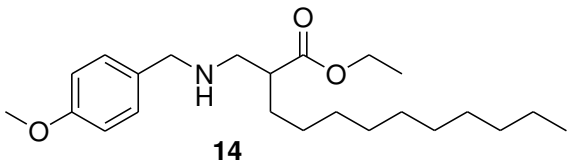
To the stirred solution of compound **11** (1.0g, 3.36mmol) in dry THF (20 mL) was added a 1.8M solution of LDA (15.15mL, 8.4 mmol) at - 78^{o}C. After 45 min HMPA (3.6 g, 20.20 mmol) was added and the solution was stirred for 20 min at same temperature. After addition of neat 1-iododecane (4.5 g, 16.83 mmol) at - 78^{o}C, the solution was allowed to raise the temperature and stirred for 24 at RT. After the completion of reaction, the mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (4% EtOAc/Hexane) to afford **13** (1.2 g, 81%)

Physical appearance-White solid

R_f 0.5 (EtOAc/ Hexane 1:9)

^{1}H-NMR ($CDCl_3$) δ: 7.37-7.07 (10H, m); 4.23 (2H, m); 3.68 (2H, m); 3.44-3.39 (2H, m); 2.65 (1H, m); 2.48-2.30 (4H, m); 1.60-1.10 (21H, m); 0.88 (3H, t, J = 6.7 Hz).

Ethyl 2-((4-methoxybenzylamino)methyl)dodecanoate



14

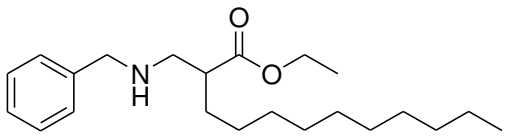
To the stirred solution of compound **12** (0.5g, 1.0 mmol) in DCM (10 mL) was added TFA (0.5 mL) at 0^{o}C and then the solution was allowed to stir for 2h at RT. After the completion of reaction, the mixture was evaporated under reduced pressure. The crude was basified with sat. NaHCO3, extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (50% EtOAc/Hexane) to afford **14** (0.3g, 76%)

Physical appearance- Colorless viscous liquid.

R_f 0.5 (EtOAc/ Hexane 1:9)

^{1}H-NMR ($CDCl_3$) δ: 7.26 (2H, d, J = 8.5 Hz); 6.87 (2H, d, J = 8.5 Hz); 4.17 (2H, q, J = 6.7 Hz); 3.88 (3H, s); 3.80-3.70 (2H, m); 2.89 (1H, m); 2.80-2.61 (12H, m); 2.01 (1H, brs); 1.65-1.18 (21H, m); 0.89 (3H, t, J = 6.7 Hz).

Ethyl 2-((benzylamino)methyl)dodecanoate



15

To the stirred solution of **13** (0.2 g, 0.4 mmol) in ACN:Water (3:1) was added cerium ammonium nitrate (0.6 g, 1.1mmol) at 0°C, then the mixture was allowed to stir for 1h at room temperature. After the completion of reaction, the mixture was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (50% EtOAc/Hexane) to afford **14** (0.11 g, 70%)

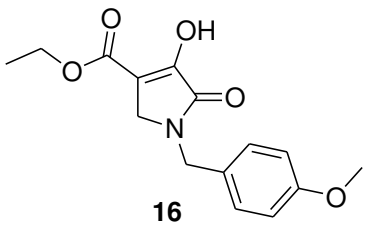
Physical appearance Yellowish viscous liquid.

R_f 0.3 (EtOAc/Hexane 5:5)

MP 160°C

^{1}H NMR ($CDCl_3$) δ: 7.41-7.14 (5H, m); 4.15 (2H, q, J = 7.2 Hz); 3.85-3.72 (2H, m); 2.87 (1H, dd, J = 8.9, 11.7 Hz); 2.69 (1H, dd, J = 4.7, 11.7 Hz); 2.58 (1H, m); 1.75 (2H, m); 1.65-1.20 (19H, m); 0.87 (3H, t, J = 7.2 Hz).

Ethyl 1-(4-methoxybenzyl)-2,5-dihydro-4-hydroxy-5-oxo-1H-pyrrole-3-carboxylate



16

A mixture of ethyl 3-(4-methoxybenzylamino)propanoate **8** (15 g, 63.21 mmol), NaOEt (2.1 g, 94.21 mmol) and diethyl oxalate (11.08 g,75.85 mmol) was refluxed for 3h. After removal of all the volatiles under vacuum the solid residue obtained was dissolved in boiling water and filtered while hot. Neutralization of the filtrate with 2 N HCl gave a precipitate, which was filtered, washed with water and dried under vacuum to give the desired product **16** (18 g, 83%)

Physical appearance- white solid

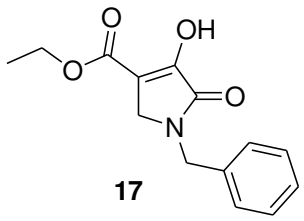
R_f 0.5(MeOH/DCM 1:9)

MP 133°C

^{1}H NMR ($CDCl_3$) δ: 7.18 (2H, d, J = 8.7 Hz); 6.86 (2H, d, J = 8.7 Hz); 4.60 (2H, s); 4.26 (2H, s); 3.83 (2H, s); 3.79 (3H, s); 1.29 (3H, t, J = 7.0 Hz).

Anal. calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.60; H, 5.90; N, 4.82.

Ethyl 1-benzyl-2,5-dihydro-4-hydroxy-5-oxo-1H-pyrrole-3-carboxylate



17

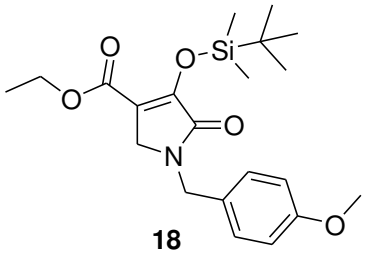
A mixture of Ethyl 3-(benzylamino)propanoate **9** (2.0 g, 9.6 mmol), NaOEt (0.98 g, 14.48 mmol) and diethyl oxalate (1.6 g, 11.58 mmol) was refluxed for 3h. Removed all the volatiles under vacuum the solid residue obtained was dissolved in boiling water and filtered while hot. Neutralization of the filtrate with 2 N HCl gave precipitate, which was filtered, washed well with water and dried in vacuum to give desired product **17** (2.0 g, 80%)

Physical appearance- white solid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.42-7.15 (5H, s); 4.67 (2H, s); 4.26 (2H, q, J = 7.2 Hz); 3.86 (2H, s); 1.29 (3H, t, J = 7.2 Hz).

4-(tert-Butyl-dimethyl-silanyloxy)-1-(4-methoxy-benzyl)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester



18

To the solution of **16** (5 g, 51.49 mmol) in dry DCM (50 mL), imidazole (2.33 g, 34.32 mmol) was added portionwise at 0°C and mixture was stirred for 10 min. TBSCl (5.1 g, 34.32 mmol) was added at 0°C and resulting reaction mixture was stirred 6h at RT. The organic solvent was removed under reduced pressure .The residue was dissolved in EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (12% EtOAc/Hexane) to afford **18** (6 g, 86%)

Physical appearance- White solid

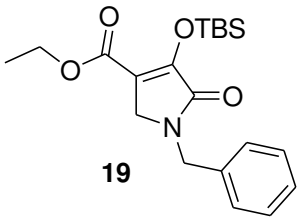
R_f 0.5(EtOAc/Hexane 2:8)

MP 85°C

^{1}H NMR ($CDCl_3$) δ: 7.15 (2H, d, J = 8.7 Hz); 6.81 (2H, d, J = 8.7 Hz); 4.52 (2H, s); 4.22 (2H, q, J = 7.0 Hz); 4.11 (2H, q, J = 7.0 Hz, 2nd rotamer); 3.83 (2H, s); 3.78 (3H, s); 1.28 (3H, t, J = 7.0 Hz); 1.24 (3H, t, J = 7.0 Hz, 2nd rotamer); 0.99 (9H, s); 0.35 (6H, s).

Anal. calcd for $C_{21}H_{31}NO_5Si$: C, 62.19; H, 7.70; N, 3.45. Found: C, 62.41; H, 7.66; N, 3.46.

1-Benzyl-4-(tert-butyl-dimethyl-silanyloxy)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester



19

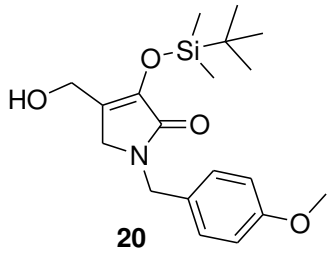
To the solution of **17** (5 g, 19.29 mmol) in dry DCM (50 mL) , imidazole (2.6 g, 38.59 mmol) was added portionwise at 0°C and mixture was stirred for 10 min. TBSCl (5.7 g, 38.59 mmol) was added at 0°C and resulting reaction mixture was 6h at RT. The organic solvent was removed under reduced pressure .The residue was dissolved in EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated .The crude product was purified by flash column chromatography (12% EtOAc/Hexane) to afford **19** (5.0g, 69%)

Physical appearance White solid

R_f 0.5(EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.42-7.12 (5H, m); 4.62 (2H, s); 4.23 (2H, q, J = 7.2 Hz); 3.87 (2H, s); 1.29 (3H, t, J = 7.2 Hz); 1.01 (9H, s); 0.37 (6H, s).

3-(tert-Butyl-dimethyl-silanyloxy)-4-hydroxymethyl-1-(4-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one



20

To the solution of **18** (4 g, 9.8mmol) in dry DCM (40 mL) was added diisobutylaluminium hydride (1.0 M in DCM, 19.72 mL, 19.72 mmol) at -78^{o}C and the solution was stirred for 1h at the same temperature. After completion of reaction, the mixture was quenched with sat. Rochelle salt and EtOAc were added, and stirring was continued for 10 min. The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **20** (2.5 g, 72%)

Physical appearance Off white solid

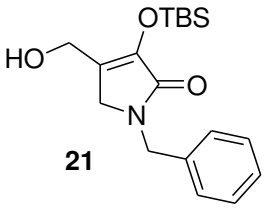
R_f 0.5(EtOAc/Hexane 4:6)

MP 148°C

^{1}H NMR ($CDCl_3$) δ: 7.16 (2H, d, J = 8.5 Hz); 6.84 (2H, d, J = 8.5 Hz); 4.52 (2H, s); 4.41 (2H, s); 3.79 (3H, s); 3.70 (2H, s); 0.96 (9H, s); 0.29 (6H, s).

Anal. calcd for $C_{19}H_{29}NO_4Si$: C, 62.78; H, 8.04; N, 3.85. Found: C, 62.55; H, 8.06; N, 3.86.

1-Benzyl-3-(tert-butyl-dimethyl-silanyloxy)-4-hydroxymethyl-1,5-dihydro-pyrrol-2-one



21

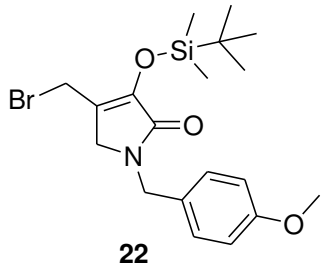
To the solution of **19** (2.5 g, 6.6 mmol) in dry DCM (40mL) was added Diisobutylaluminium hydride (1.0 M in DCM, 13.3 mL, 13.32 mmol) at -78^{o}C and the solution was stirred for 1h at same temperature. After completion of reaction, the mixture was quenched with sat. Rochelle salt and EtOAc was added, then stirring was continued for 10 min. The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **21** (2.0g, 90%)

Physical appearance Off white solid

R_f 0.5 (EtOAc/Hexane 4:6)

^{1}H NMR ($CDCl_3$) δ: 7.46-7.13 (5H, m); 4.61 (2H, s); 4.44 (2H, s); 3.74 (2H, s); 0.99 (9H, s); 0.30 (6H, s).

4-Bromomethyl-3-(tert-butyl-dimethyl-silanyloxy)-1-(4-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one



22

To the solution of **20** (2 g, 5.5 mmol) in dry DCM (40mL) were added PPh_3 (2.8 g, 11mmol) and CBr_4 (3.6 g, 11 mmol) at 0°C. Then reaction was allowed to stir for 3 h at RT. After completion of reaction, the mixture was evaporated in vacuo. The crude product was purified by flash column chromatography (8% EtOAc/Hexane) to afford **22** (2 g, 87%)

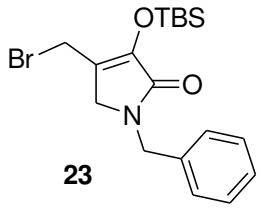
Physical appearance Colorless viscous liquid.

R_f 0.7 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.16 (2H, d, J = 8.4 Hz); 6.86 (2H, d, J = 8.4 Hz); 4.53 (2H, s); 4.17 (2H, s); 3.79 (3H, s); 3.75 (2H, s); 0.99 (9H, s); 0.32 (6H, s).

Anal. calcd for $C_{19}H_{28}BrNO_3Si$: C, 53.69; H, 6.58; N, 3.28. Found: C, 53.52; H, 6.62; N, 3.27.

1-Benzyl-4-bromomethyl-3-(tert-butyl-dimethyl-silanyloxy)-1,5-dihydro-pyrrol-2-one



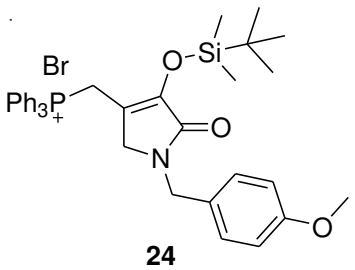
23

To the solution of **21** (1.0g, 2.9 mmol) in dry DCM (40mL) were added PPh_3 (1.5g, 5.9 mmol) and CBr_4 (1.9g, 5.9 mmol) at 0°C, then the reaction was allowed to stir for 3 h at RT. After completion of reaction, the mixture was evaporated in vacuo. The crude product was purified by flash column chromatography (8% EtOAc/Hexane) to afford **23** (0.8g, 72%)

Physical appearance Yellowish Viscous liquid.

R_f 0.7 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.43-7.14 (5H, m); 4.60 (2H, s); 4.19 (2H, s); 3.78 (2H, s); 1.00 (9H, s); 0.33 (6H, s).



24

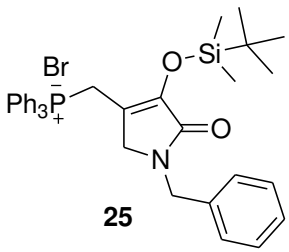
To the solution of **22** (2 g, 4.7 mmol) in dry toluene (40mL) was added PPh_3 (1.84 g, 7mmol) and the mixture was refluxed for 6 h. After cooling toluene was decanted, the solid was triturated with diethyl ether to give the desired phosphonium bromide **24** (2 g, 61%)

Physical appearance White solid

MP 220°C

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.86-7.38 (15H, m); 6.97 (2H, d, J = 7.9 Hz); 6.76 (2H, d, J = 7.9 Hz); 5.27 (2H, d, J = 15.9 Hz); 4.37 (2H, s); 3.81 (3H, s); 3.53 (2H, s); 0.76 (9H, s); 0.22 (6H, s).



25

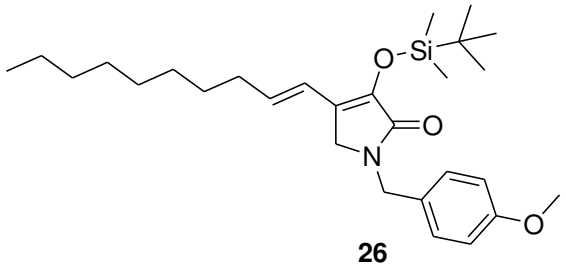
To the solution of **23** (1.0 g, 2.5 mmol) in dry toluene (10 mL) was added PPh_3 (0.9 g, 3.7 mmol) and the mixture was refluxed for 6 h. After cooling toluene was decanted, the solid was triturated with diethyl ether to give the desired phosphonium bromide **25** (1.4g, 90%)

Physical appearance White solid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.86-7.65 (12H, m); 7.64-7.51 (8H, m); 5.40 (2H, d, J = 15.3 Hz); 4.45 (2H, s); 3.37 (2H, s); 0.76 (9H, s); 0.21 (6H, s).

3-(tert-Butyl-dimethyl-silanyloxy)-4-dec-1-enyl-1-(4-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one



26

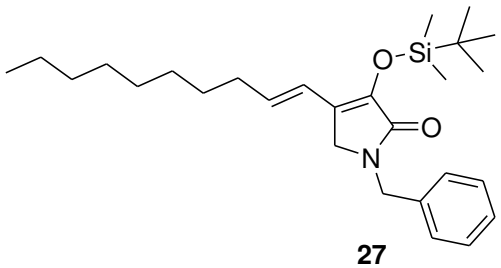
To the solution of **24** (1.5 g, 2.1 mmol) was added LiHMDS (1 M in THF, 3.2 mL, 3.2mmol) at -78^{o}C dropwise under nitrogen atmosphere. After stirring for 30 min, it was added a solution of n-nonanal in THF. Then reaction was allowed to warm up and stirred further for 5 h at RT. After the completion of reaction, mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **26** (0.9 g, 88%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.19 (2H, d, J = 8.2 Hz); 6.88 (2H, d, J = 8.2 Hz); 6.28 (1H, d, J = 11.9 Hz); 5.49 (1H, m); 4.58 (2H, s); 3.93 (2H, s); 3.81 (3H, s); 2.07 (2H, m); 1.46-1.18 (12H, m); 1.00 (9H, s); 0.89 (3H, t, J = 7.0 Hz); 0.22 (6H, s).

1-Benzyl-3-(tert-butyl-dimethyl-silanyloxy)-4-dec-1-enyl-1,5-dihydro-pyrrol-2-one



27

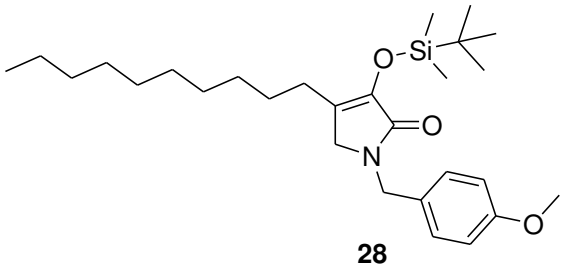
To the solution of **25** (1.0 g, 1.5 mmol) was added LiHMDS (1 M in THF, 1.5 mL, 1.5mmol) at -78^{o}C dropwise under nitrogen atmosphere. After stirring for 30 min, it was added a solution of n-nonanal (0.129 g, 0.9 mmol) in THF. Then reaction was allowed to warm up and stirred further for 5 h at RT. After the completion of reaction, the mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **27** (0.5g, 74%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.41-7.16 (5H, m); 6.27 (1H, d, J = 11.3 Hz); 5.49 (1H, m); 4.62 (2H, s); 3.93 (2H, s); 2.05 (2H, m); 1.56-1.10 (12H, m); 1.03 (9H, s); 0.87 (3H, t, J = 6.9 Hz); 0.30 (6H, s).

3-(tert-Butyl-dimethyl-silanyloxy)-4-decyl-1-(4-methoxy-benzyl)-1,5-dihydro-pyrrol-2-one



28

To the solution of **26** (0.5 g, 1mmol) in EtOAc (20mL) was added a catalytic amount of 10%Pd/C. The reaction mixture was stirred for 1h at RT under hydrogen atmosphere. After the completion of reaction, the mixture was filtered through a celite bed and it was washed with EtOAc three times. EtOAc was concentrated under vacuo. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **28** (0.45 g, 89%)

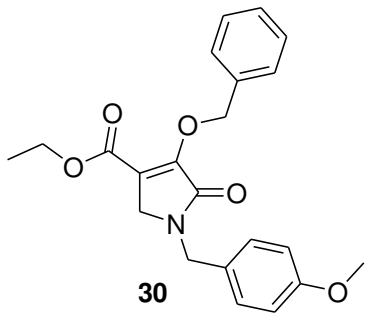
Physical appearance White viscous liquid

R_f 0.5(EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.16 (2H, d, J = 8.5 Hz); 6.86 (2H, d, J = 8.5 Hz); 4.53 (2H, s); 3.81 (3H, s); 3.54 (2H, s); 2.27 (2H, t, J = 8.2 Hz); 1.56-1.12 (16H, m); 0.99 (9H, s); 0.89 (3H, t, J = 7.0 Hz); 0.22 (6H, s).

Anal. calcd for $C_{28}H_{47}NO_3Si$: C, 70.98; H, 10.00; N, 2.96. Found: 701.20; H, 10.03; N, 2.95.

Ethyl 1-(4-methoxybenzyl)-4-(benzyloxy)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate



30

To the stirred solution of **16** (15 g, 51 mmol) in dry DMF (150mL) was added anhydrous K_2CO_3 (21 g, 154 mmol) at 0°C and the solution was stirred for 15 min, then was added BnBr (9.6 g, 56 mmol). The reaction mixture was allowed to warm up and stirred further for 1h. After completion of reaction, the mixture was diluted with EtOAc and washed with cold brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (25% EtOAc/Hexane) to afford **30** (10 g, 50%)

Physical appearance White solid

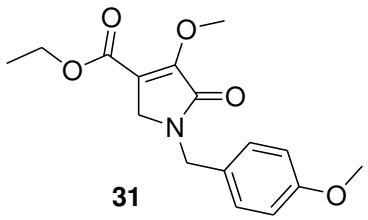
R_f 0.5 (EtOAc/Hexane 3:7)

MP 72°C

^{1}H NMR ($CDCl_3$) δ: 7.54-7.45 (2H, m); 7.42-7.31 (3H, m); 7.18 (2H, d, J = 8.5 Hz); 6.88 (2H, d, J = 8.5 Hz); 5.82 (2H, s); 4.57 (2H, s); 4.24 (2H, q, J = 7.0 Hz); 3.86 (2H, s); 3.82 (3H, s); 1.30 (3H, t, J = 7.0 Hz).

Anal. calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 69.05; H, 6.10; N, 3.66.

Ethyl 1-(4-methoxybenzyl)-2,5-dihydro-4-methoxy-5-oxo-1H-pyrrole-3-carboxylate



31

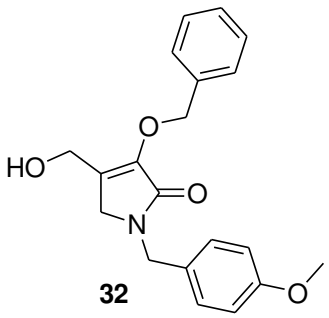
To the stirred solution of **16** (1.0 g, 3.4 mmol) in dry DMF (20 mL) was added anhydrous K_2CO_3 (1.4 g, 10 mmol) at 0°C and the resulting solution was stirred for 15 min, then was added BnBr (0.58 g, 4.1 mmol) . The reaction mixture was allowed to warm up and stirred further for 1h. After completion of reaction, the mixture was diluted with EtOAc and washed with cold brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/Hexane) to afford **31** (0.65 g, 62%)

Physical appearance White solid

R_f 0.5 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.20 (2H, d, J = 8.5 Hz); 6.88 (2H, d, J = 8.5 Hz); 4.58 (2H, s); 4.34 (3H, s); 4.22 (2H, q, J = 7.0 Hz); 3.86 (2H, s); 3.81 (3H, s); 1.29 (3H, t, J = 7.0 Hz). Anal. calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.70; H, 6.26; N, 4.60.

1-(4-methoxybenzyl)-3-(benzyloxy)-4-(hydroxymethyl)-1H-pyrrol-2(5H)-one



32

To the solution of **30** (5 g, 13 mmol) in dry DCM (50mL) was added diisobutylaluminium hydride (1.0 M in DCM, 26 mL, 26 mmol) at -78^{o}C and the solution was stirred for 1h at the same temperature. After completion of reaction, the mixture was quenched with sat. Rochellesalt and EtOAc was added, stirred for 10 min. The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **32** (2.5 g, 56%)

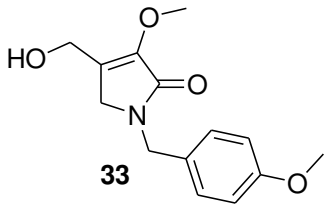
Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 4:6)

^{1}H NMR ($CDCl_3$) δ: 7.45-7.33 (5H, m); 7.18 (2H, d, J = 8.5 Hz); 6.88 (2H, d, J = 8.5 Hz); 5.41 (2H, s); 4.57 (2H, s); 3.82 (3H, s); 3.62 (2H, s).

Anal. calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 71.00; H, 6.23; N, 4.12.

1-(4-methoxybenzyl)-4-(hydroxymethyl)-3-methoxy-1H-pyrrol-2(5H)-one



33

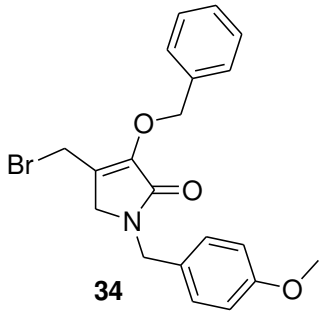
To the stirred solution of **31** (0.5 g, 1.6 mmol) in EtOH was added $NaBH_4$ (0.18 g, 4.9 mmol) at 0^oC and the solution was allowed to stir for 5h at RT. After the completion of reaction, the mixture was evaporated by rotavapor and quenched with sat.NH_4Cl, extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **33** (0.2 g, 50%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 6:4)

^{1}H NMR ($CDCl_3$) δ: 7.18 (2H, d, J = 8.8 Hz); 6.86 (2H, d, J = 8.8 Hz); 4.53 (2H, s); 4.46 (2H, s); 4.03 (3H, s); 3.80 (3H, s); 3.72 (2H, s).

1-(4-methoxybenzyl)-3-(benzyloxy)-4-(bromomethyl)-1H-pyrrol-2(5H)-one



34

To the solution of **32** (2 g, 5.8 mmol) were added PPh_3 (3.1 g, 11.79 mmol) and CBr_4 (3.9 g, 11.79 mmol) at 0°C. The reaction was allowed to stir for 3h at RT. After completion of reaction, the mixture was evaporated in vacuo. The crude product was purified by flash column chromatography (22% EtOAc/Hexane) to afford **34** (1.2 g, 52%)

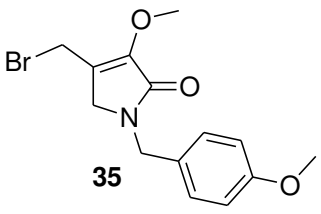
Physical appearance Yellowish liquid

R_f 0.6 (EtOAc/Hexane 3:7)

^{1}H NMR ($CDCl_3$) δ: 7.48-7.30 (5H, m); 7.18 (2H, d, J = 8.5 Hz); 6.88 (2H, d, J = 8.5 Hz); 5.53 (2H, s); 4.56 (2H, s); 4.11 (2H, s); 3.82 (3H, s); 3.76 (2H, s).

Anal. calcd for $C_{20}H_{20}BrNO_3$: C, 59.71; H, 5.01; N, 3.48. Found: C, 59.90; H, 5.00; N, 3.49.

1-(4-methoxybenzyl)-4-(bromomethyl)-3-methoxy-1H-pyrrol-2(5H)-one



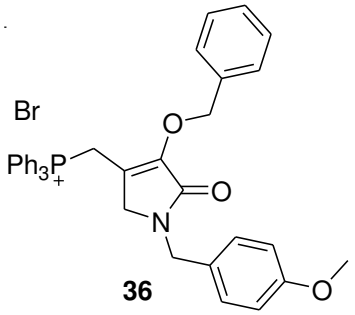
35

To the solution of **33** (0.3g, 1.1mmol) were added PPh_3 (0.57g, 2.2mmol) and CBr_4 (0.75g, 2.2mmol) at 0°C.The reaction was allowed to stir for 3 h at RT. After completion of reaction, the mixture was evaporated in vacuo. The crude product was purified by flash column chromatography (22% EtOAc/Hexane) to afford **35** (0.15g, 40%)

Physical appearance White viscous liquid.

R_f 0.6 (EtOAc/Hexane 3:7)

^{1}H NMR ($CDCl_3$) δ: 7.19 (2H, d, J = 8.8 Hz); 6.88 (2H, d, J = 8.8 Hz); 4.55 (2H, s); 4.22 (2H, s); 4.16 (3H, s); 3.81 (3H, s); 3.77 (2H, s).



36

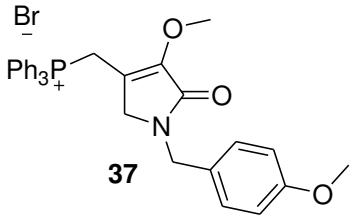
To the solution of **34** (1 g, 2.4 mmol) in dry toluene (10 mL) was added PPh_3 (1 g, 3.7 mmol) and the mixture was refluxed for 6 h. After cooling toluene was decanted, the solid was triturated to give the desired phosphonium bromide **36** (1.2 g, 75%)

Physical appearance White solid

MP 167°C

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.85–7.61 (8H, m); 7.57-7.42 (6H, m); 7.41-7.37 (3H, m); 7.25-7.14 (3H, m); 7.06 (2H, m, J = 8.5 Hz); 6.28 (2H, d, J = 8.5 hz); 5.33 (2H, s); 5.21 (2H, d, J = 15.3 Hz); 4.37 (2H, s); 3.84 (3H, s); 3.58 (2H, d, J = 4.0 Hz).



37

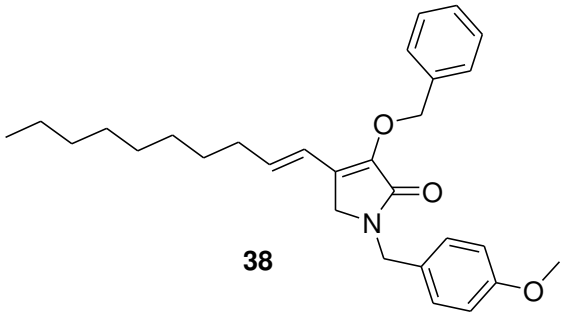
To the solution of **35** (1g, 3.0 mmol) in dry toluene (10 mL) was added PPh_3 (2.4g, 9.1 mmol) the mixture was refluxed for 6 h. After cooling toluene was decanted, the solid was triturated to give the desired phosphonium bromide **37** (1.26 g, 70%)

Physical appearance White solid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.87-7.67 (9H, m); 7.65-7.52 (6H, m); 7.02 (2H, d, J = 8.5 Hz); 6.79 (2H, d, J = 8.5 Hz); 5.24 (2H, d, J = 15.4 Hz); 4.35 (2H, s); 3.81 (3H, s); 3.80 (3H, s); 3.56 (2H, d, J = 3.4 Hz).

1-(4-methoxybenzyl)-3-(benzyloxy)-4-((E)-dec-1-enyl)-1H-pyrrol-2(5H)-one



38

To the solution of **36** (1 g, 1.5mmol) was added LiHMDS (1 M in THF, 1.5 mL, 1.5 mmol) at -78^{o}C dropwise under nitrogen atmosphere. After stirring for 30 min, added solution of n-nonanal (0.25 g, 1.8 mmol) in THF. Then reaction was allowed to warm up and stirred further for 5 h at RT. After the completion of reaction, mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **38** (0.35 g, 52%)

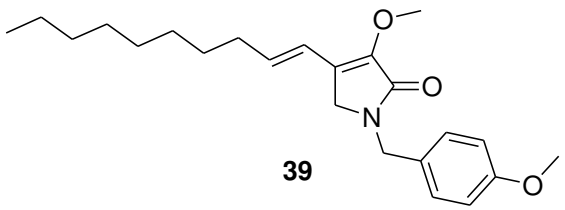
Physical appearance White viscous liquid

R_f 0.5(EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.51-7.42 (2H, m); 7.41-7.31 (3H, m); 7.18 (2H, d, J = 8.2 Hz); 6.88 (2H, d, J = 8.2 Hz); 6.23 (1H, d, J = 11.6 Hz); 5.58-5.44 (3H, m); 4.58 (2H, s); 3.90 (2H, s); 3.82 (3H, s); 2.14-1.97 (2H, m); 1.75-1.14 (12H, m); 0.89 (3H, t, J = 7.3 Hz).

Anal. calcd for $C_{29}H_{37}NO_3$: C, 77.82; H, 8.33; N, 3.13. Found: C, 78.10; H, 8.31; N, 3.14.

1-(4-methoxybenzyl)-4-(dec-1-enyl)-3-methoxy-1H-pyrrol-2(5H)-one



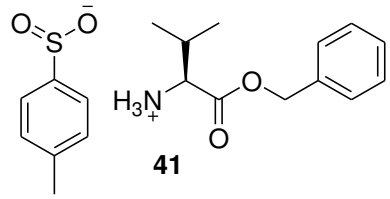
39

To the solution of **37** (0.1g**,** 0.16 mmol) was added LiHMDS (1 M in THF, 0.16 mL, 0.16 mmol) at -78^{o}C dropwise under nitrogen atmosphere. After stirring for 30 min, it was added a solution of n-nonanal (0.028g, 0.2 mmol) in THF. Then reaction was allowed to warm up and stirred further for 5 h at RT. After the completion of reaction, the mixture was quenched with sat.NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **39** (0.040 g, 63%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) (mixture E/Z 7:3) δ: 7.14 (2H, d, J = 8.4 Hz, E+Z); 6.89 (2H, d, J = 8.4 Hz, E+Z); 6.42 (1H, d, J = 16.2 Hz, E); 6.23 (1H, d, J = 11.5 Hz, Z); 5-76-5.50 (1H, m, E+Z); 4.58 (2H, s, Z); 4.55 (2H, s, E); 4.08 (3H, s, Z); 4.07 (3H, s, E); 3.88 (2H, s, Z), 3.85 (3H, s, E+Z); 3.81 (2H, s, E); 2.17-1.99 (2H, m, E+Z); 1.44-1.16 (12H, m, E+Z); 0.89 (3H, t, J = 7.3 Hz, E+Z).



41

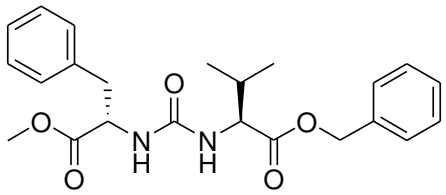
A mixture of L-valine **40** (5.0 g, 43 mmol), benzyl alcohol (18.5 g, 171 mmol) and *p*-toluene sulfonic acid monohydrate (8.9 g, 47 mmol) in toluene (71 mL) was heated at reflux for 5 h, with the removal of water *via* the aid of a Dean-Stark apparatus. The resulting reaction mixture was allowed to cool to room temperature, resulting in the formation of white crystals. The mixture was diluted with toluene (38 mL), cooled on an ice bath and the resulting precipitate collected *via* filtration, then washed with cold toluene (2 × 38 mL) and dried under reduced pressure to desired compound **41** (11.61 g, 91 %)

Physical appearance White fluffy solid

MP 155-156^{o}C

^{1}H NMR δ: 8.22 (3H, brs); 7.74 (2H, d, J = 8.4 Hz); 7.32-7.25 (5H, m); 7.10 (2H, d, J = 8.4 Hz); 5.13 (1H, d, J = 12.2 Hz, AB); 5.01 (1H, d, J = 12.2 Hz, AB); 3.90 (1H, m); 2.32 (3H, s); 2.20 (1H, m); 0.90 (3H, d, J = 6.7 Hz); 0.88 (3H, d, J = 6.7 Hz).

2-[3-(1-Methoxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid benzyl ester



42

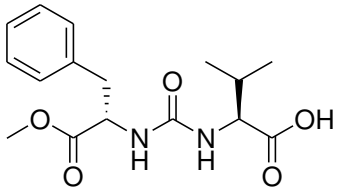
A mixture of L-valine benzyl ester *p*-toluenesulfonate **41** (2 g, 5.2 mmol) and diisopropylethylamine (1.49 g, 11 mmol) in dry DCM (20 mL) was slowly added to the stirred solution of triphosgene (0.57 g, 1.9 mmol) dropwise at RT. After a further 5 min stirring, a solution of L-phenylalanine methyl ester hydrochloride (1.13 g, 5.2 mmol) and DIEA (1.4 g, 11 mmol) was added in one portion. The reaction mixture was stirred for 10 min at rt, evaporated to dryness, diluted with ethyl acetate, washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **42** (1.8 g, 85%)

Physical appearance- White viscous liquid

R_f 0.5 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.40-7.02 (10H, m); 5.30-4.98 (4H, m); 4.80 (1H, brs); 4.50 (1H, brs); 3.70 (3H, s); 3.20-2.98 (2H, m); 2.09 (1H, m); 0.90 (3H, d, J = 7.2 Hz); 0.79 (3H, d, J = 7.2 Hz).

2-[3-(1-Methoxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid



43

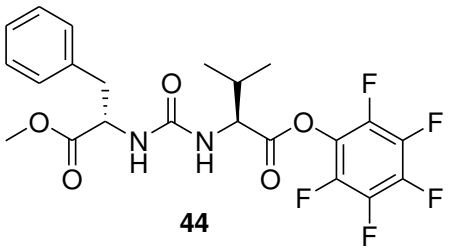
To the solution of **42** (1.5g, 3.6 mmol) in EtOAc (20 mL) was added a catalytic amount of 10% Pd/C. Then reaction mixture was stirred for 1h at RT under hydrogen atmosphere. After the completion of reaction, the mixture was filtered through a celite bed and bed was washed with EtOAc three times. EtOAc was concentrated under vacuo. The crude product was purified by flash column chromatography (5% MeOH/DCM) to afford **43** (0.937 g, 80%)

Physical appearance- white viscous liquid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.30-7.15 (3H, m); 7.11-7.01 (2H, m); 5.68-5.55 (2H, m); 4.80 (1H, m); 4.36 (1H, m); 3.65 (3H, s); 3.13-3.04 (2H, m); 2.16 (1H, m); 0.95 (3H, d, J = 6.7 Hz); 0.87 (3H, d, J = 6.7 Hz).

2-[3-(1-Methoxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyricacid pentafluorophenyl ester



44

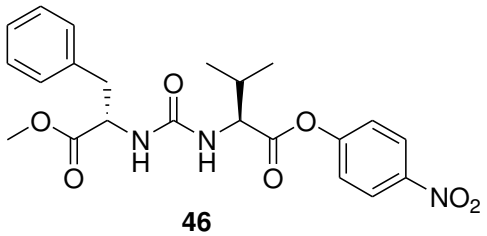
A solution of acid **43** (0.5 g, 1.5 mmol), PFP (0.31 g, 1.7 mmol), DCC (0.35g, 1.7 mmol) in EtOAc (10 mL) was stirred at 0^{o}C for 1h and then stirred for 3h at RT. The resultant mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (25% EtOAc/Hexane) to afford **44** (0.5g, 66%)

Physical appearance- white viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.30-7.18 (3H, m); 7.11-7.04 (2H, m); 5.38-5.26 (2H, m); 4.87-4.74 (2H, m); 3.72 (3H, s); 3.09-3.02 (2H, m); 2.30 (1H, m); 1.05 (3H, d, J = 6.7 Hz); 0.96 (3H, d, J = 6.7 Hz).

2-[3-(1-Methoxycarbonyl-2-phenyl-ethyl)-ureido]-3-methyl-butyric acid 4-nitro-phenyl ester



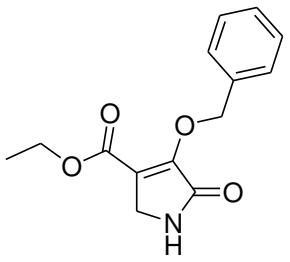
46

A solution of acid **43** (0.5 g, 1.5 mmol), p-nitrophenol (0.48 g, 3.4 mmol), DCC (0.48 g, 2.3 mmol) in EtOAc (10mL) was stirred at 0^{o}C for 1h and then stirred for 3h at RT. The resultant mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product (0.612 g, 90%) was used for the next step without further purification.

Physical appearance White solid

R_f 0.5 (EtOAc/Hexane 2:8)

Ethyl 4-(benzyloxy)-2,5-dihydro-5-oxo-1H-pyrrole-3-carboxylate



48

To a stirred solution of **30** (5 g, 13 mmol) in ACN:Water (3:1) was added cerium ammonium nitrate (28 g, 52 mmol) at 0^oC, then the solution was allowed to stir for 1h at room temperature. After the completion of reaction, the mixture was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (50% EtOAc/Hexane) to afford **48** (2.5 g, 73%)

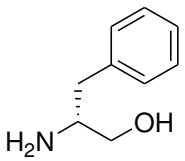
Physical appearance White solid

R_f 0.3 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.51-7.42 (2H, m); 7.41-7.30 (3H, m); 6.71 (1H, brs); 5.77 (2H, s); 4.29 (2H, q, J = 7.0 Hz); 4.06 (2H, s); 1.34 (3H, t, J = 7.0 Hz).

Anal. calcd for $C_{14}H_{15}NO_4$: C, 64.36; H, 5.79; N, 5.36. Found: C, 64.60; H, 5.77; N, 5.35.

(R)-2-amino-3-phenylpropan-1-ol



50

To a cold solution of lithium borohydride (5.2 g, 24.19 mmol) in anhydrous THF (30mL) was added trimethylsilyl chloride (5.26 g , 48.39 mmol). The ice/water bath was removed and the mixture was allowed to stir at room temperature for 15 min. The mixture was recooled to 0 °C, and L-phenylalanine (2.0 g, 12.09 mmol) was added. The ice/water bath was removed, and the reaction mixture was stirred overnight. The mixture was again cooled to 0°C, and methanol (45 mL) was added dropwise, followed by 2.5 M aqueous sodium hydroxide (25mL) This mixture was evaporated in vacuo, and the residue extracted with chloroform. The combined extracts were dried (Na_2SO_4), filtered, and evaporated in vacuo to give **50** (1.7 g, 94.4%)

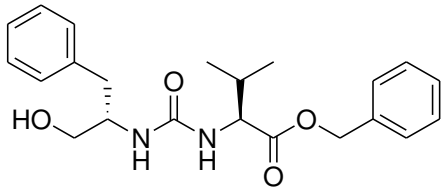
Physical appearance White crystalline solid

R_f 0.2 (MeOH/DCM 1:9)

MP 90^{o}C

^{1}H NMR ($CDCl_3$) δ: 7.39-7.05 (5H, m); 3.64 (1H, dd, J = 3.7, 10.7 Hz); 3.38 (1H, dd, J = 7.0, 10.7 Hz); 3.12 (1H, m); 2.79 (1H, dd, J = 5.3, 13.6 Hz); 2.53 (1H, dd, J = 13.6, 8.5 Hz).

2-[3-(1-Benzyl-2-hydroxy-ethyl)-ureido]-3-methyl-butyric acid benzyl ester



51

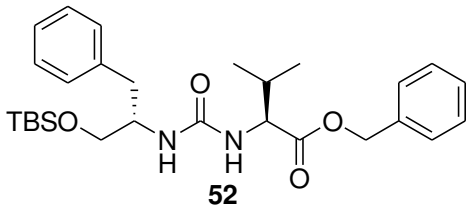
A mixture of L-valine benzyl ester *p*-toluenesulfonate **41** (1 g, 2.6 mmol) and diisopropylethylamine (0.749 g, 5.7 mmol) in dry DCM (20 mL) was slowly added to the stirred solution of triphosgene (0.289 g, 0.97 mmol) dropwise at RT. After a further 5 min stirring, a solution of L-phenylalaninol **50** (0.398g, 2.6 mmol) and DIEA (0.748 g, 5.7 mmol) was added in one portion. The reaction mixture was stirred for 10 min at RT, evaporated to dryness, diluted with ethyl acetate, washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (60% EtOAc/Hexane) to afford **51** (0.750 g, 74%)

Physical appearance- Yellowish viscous liquid

R_f 0.4 (EtOAc/Hexane 6:4)

^{1}H NMR ($CDCl_3$) δ: 7.42-7.15 (5H, m); 5.21-5.11 (2H, m); 4.50 (1H, t, J = 8.2 Hz); 4.39 (1H, d, J = 4.6 Hz); 4.23-4.05 (2H, m); 3.93 (1H, m); 3.73 (1H, dd, J = 2.7, 11.0 Hz); 3.61 (1H, dd, J = 6.4, 11.0 Hz); 2.97-2.69 (2H, m); 2.28-2.08 (1H, m); 0.92 (3H, d, J = 6.7 Hz); 0.84 (3H, d, J = 6.7 Hz).

2-{3-[1-Benzyl-2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-ureido}-3-methyl-butyric acid benzyl ester



52

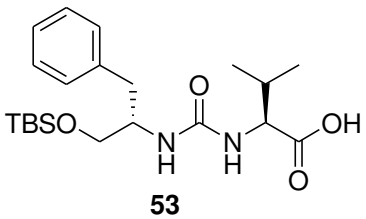
To the solution of **51** (0.5 g, 1.3 mmol) in dry DCM (5 mL), imidazole (0.17 g, 2.6 mmol) was added portionwise at 0°C and the mixture was stirred for 10 min. TBSCl (0.39g, 2.6 mmol) was added at 0°C and resulting reaction mixture was 4h at RT. The organic solvent was removed under reduced pressure .The residue was dissolved in EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/Hexane) to afford **52** (0.55 g, 85%)

Physical appearance- Yellowish solid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.46-7.14 (5H, m); 5.22 (1H, d, J = 12.5 Hz, AB); 5.14 (1H, d, J = 12.5 Hz, AB); 4.81 (2H, brs); 4.48 (1H, brs); 3.97 (1H, m); 3.56 (1H, dd, J = 4.3, 10.0 Hz); 3.50 (1H, dd, J = 3.0, 10.0 Hz); 2.90 (1H, dd, J = 5.8, 13.1 Hz); 2.81 (1H, dd, J = 4.9, 13.1 Hz); 2.15 (1H, m); 0.94 (9H, s); 0.93 (3H, d, J = 7.0 Hz); 0.84 (3H, d, J = 7.0 Hz); 0.06 (6H, s).

2-{3-[1-Benzyl-2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-ureido}-3-methyl-butyric acid



53

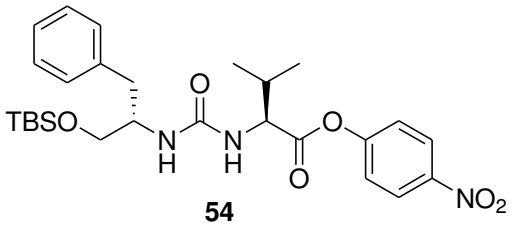
To the solution of **52** (0.5 g, 1.0 mmol) in EtOAc (10 mL) was added a catalytic amount of 10% Pd/C. Then reaction mixture was stirred for 1h at RT under hydrogen atmosphere. After the completion of reaction, the mixture was filtered through a celite bed and bed was washed with EtOAc three times. EtOAc was concentrated under vacuo. The crude product was purified by flash column chromatography (5% MeOH/DCM) to afford **53** (0.38 g, 92%)

Physical appearance White viscous liquid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.36-7.11 (5H, m); 5.63 (1H, brs); 5.25 (1H, brs)4.15 (1H, m); 3.95 (1H, m); 3.52-3.45 (2H, m); 2.85-2.75 (2H, m); 2.12 (1H, m); 1.10-0.80 (15H, m); 0.04 (6H, s).

2-{3-[1-Benzyl-2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-ureido}-3-methyl-butyric acid 4-nitro-phenyl ester



54

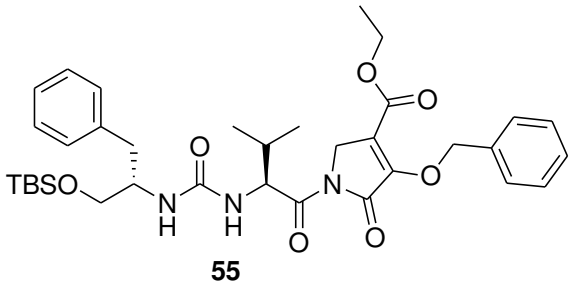
A solution of acid **53** (0.5 g, 1.2mmol), p-nitrophenol (0.187 g, 1.3 mmol), DCC (0.268 g, 1.3 mmol) in EtOAc (10mL) was stirred at 0°C for 1h and then stirred for 3h at RT. The resultant mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash column chromatography (15%EtOAc/Hexane) to afford **54** (0.5 g, 77%)

Physical appearance Yellowish viscous liquid

R_f 0.4 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 8.30 (2H, d, J = 9.2 Hz); 7.35-7.17 (7H, m); 4.81 (1H, brs); 4.62 (1H, brs); 4.13 (1H, m); 4.00 (1H, m); 3.67-3.47 (2H, m); 2.93-2.82 (2H, m); 2.29 (1H, m); 1.10 (3H, d, J = 6.7 Hz); 1.04 (3H, d, J = 6.7 Hz); 0.95 (9H, s); 0.07 (6H, s).

1-(2-{3-[1-Benzyl-2-(tert-butyl-dimethyl-silanyloxy)-ethyl]-ureido}-3-methyl-butyryl)-4-benzyloxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester



55

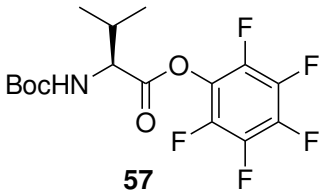
A stirred solution of NaH (0.02 g, 0.4 mmol) in dry THF (2 mL) was added to a solution of amide **48** (0.1 g, 0.3 mmol) in THF (2 mL) at 0°C, The mixture was allowed to stir for 10 min and then added with a solution of the activated ester **54** (0.2 g, 0.3 mmol) in THF (2 mL). Then reaction was allowed to stir for 30 min at 0°C. After the completion of reaction, the mixture was quenched with sat NH_4Cl, extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/Hexane) to afford **55** (0.05g, 20%)

Physical appearance White viscous oil.

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.51-7.14 (5H, m); 5.69 (1H, AB); 5.62 (1H, AB); 5.53 (1H, brs); 5.14 (1H, brs), 4.92 (1H, m); 4.40-4.20 (4H, m); 3.98 (1H, m); 3.58 (1H, dd, J = 4.3, 10.1 Hz); 3.51 (1H, dd, J = 3.4, 10.1 Hz); 2.93-2.78 (2H, m); 2.12 (1H, s); 1.34 (3H, t, J = 7.0 Hz); 1.07 (3H, d, J = 7.0 Hz); 0.94 (9H, s); 0.84 (3H, d, J = 7.0 Hz); 0.07 (6H, s).

Tert-butyl (S)-1-((pentafluorophenoxy)carbonyl)-2-methylpropylcarbamate



57

N-Boc-L-Valine (2g, 9.2mmol), C_6F_5OH (1.86g, 10.12mmol) and DCC (2g, 10.12mmol) were added to EtOAc (30mL) at 0°C. The mixture was stirred at 0°C for 1 h, then at room temperature for 3 h. After filtration, the solvent was removed and the residue was purified by column chromatography on silica gel (6% EtOAc /Hexane) to give activated ester **57** (3.2g, 91.42%)

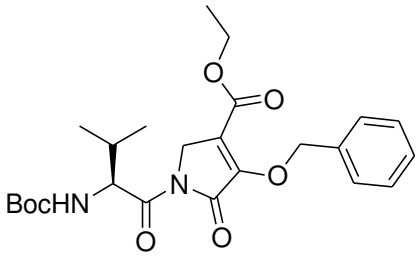
Physical appearance White solid

R_f 0.6 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 5.04 (1H, m); 4.60 (1H, m); 2.35 (1H, m); 1.49 (9H, s); 1.11 (3H, d, J = 7.0 Hz), 1.05 (3H, d, J = 7.0 Hz).

Anal. calcd for $C_{16}H_{18}F_5NO_4$: C, 50.13; H, 4.73; N, 3.65. Found: C, 49.94; H, 4.72; N, 3.66.

4-Benzyloxy-1-(2-tert-butoxycarbonylamino-3-methyl-butyryl)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester



58

To a stirred solution of **48** (1.0g, 3.8 mmol) in anhydrous THF (30mL) was added n-BuLi (1.6M in hexane, 2.6mL, 4.2mmol) at –78 °C over 10 min. Active ester **57** (1.6g, 4.2mmol) in THF (2mL) was then added, and the mixture was stirred for 30 min at the same temperature. The reaction was quenched with sat. NH_4Cl and extracted with EtOAc (3 ×15mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (12% EtOAc/Hexane) to afford **58** (6 g, 86%)

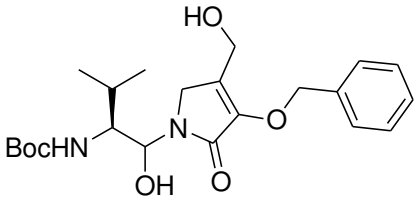
Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.50-7.31 (5H, m); 5.67 (2H, s); 5.41 (1H, m); 4.35 (1H, AB); 4.31 (2H, q, J = 7.0 Hz); 4.30 (1H, AB); 4.14 (2H, q, J = 7.0 Hz, 2nd rotamer); 2.15 (1H, m); 1.46 (9H, s); 1.34 (3H, t, J = 7.0 Hz); 1.28 (3H, t, J = 7.0 Hz, 2nd rotamer); 1.11 (3H, d, J = 7.0 Hz); 0.84 (3H, d, J = 7.0 Hz, 2nd rotamer).

Anal. calcd for $C_{24}H_{32}N_2O_7$: C, 62.59; H. 7.00; N, 6.08. Found: C, 62.80; H. 7.02; N, 6.09.

{1-[(3-Benzyloxy-4-hydroxymethyl-2-oxo-2,5-dihydro-pyrrol-1-yl)-hydroxy-methyl]-2-methyl-propyl}-carbamic acid tert-butyl ester



59

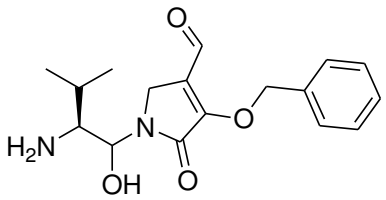
To the solution of **58** (1 g, 2.1 mmol) in dry DCM (20mL) was added diisobutylaluminium hydride (1.0 M in DCM, 5.4mL, 5.4mmol) at -78^{o}C and stirred for 2.5h at the same temperature. After completion of reaction, the mixture was quenched with sat. Rochelle salt and EtOAc was added, stirring for 10 min. The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **59** (0.27 g, 30%)

Physical appearance Yellowish solid.

R_f 0.3 (EtOAc/Hexane 4:6)

^{1}H NMR ($CDCl_3$) δ: 756-7.30 (5H, m); 5.47 (1H, d, J = 9.4 Hz); 5.29 (1H, d, J = 11.9 Hz, AB); 5.16 (1H, d, J = 11.9 Hz, AB); 4.63 (1H, d, J = 11.0 Hz); 4.35-4.02 (4H, m); 3.80 (1H, m); 2.23 (1H, m); 1.44 (9H, s); 0.99 (3H, d, J = 7.3 Hz); 0.94 (3H, d, J = 7.3 Hz).

1-(2-Amino-1-hydroxy-3-methyl-butyl)-4-benzyloxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carbaldehyde



60

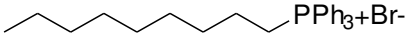
To a stirred solution of oxalyl chloride (0.37 mL, 4. 3 mmol) in dry DCM (3 mL), was added DMSO (0.4 mL, 5.7 mmol) in dry DCM (3 mL) dropwise at -78 °C under N_2 atmosphere. Stirring was continued for 15 min, then **59** (0.6 g, 1.43 mmol) in dry DCM (10 mL) was added dropwise. After complete addition, the reaction mixture was stirred at -78 °C for 1 h, then NEt_3 (1.98 mL, 14.33mmol) was added dropwise. The reaction mixture was gradually warmed to 0°C and stirred at this temperature till complete conversion was observed. The mixture was diluted with diethyl ether and poured in cold sat. $NaHCO_3$. The organic layer was separated; and the aqueous layer was extracted with diethyl ether (3×150 mL). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (20% EtOAc/Hexane) to afford **60** (0.3 g, 60%)

Physical appearance Yellowish Solid.

R_f 0.5 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 10.11 (1H, s); 7.49-7.36 (5H, m); 6.18 (1H, d, J = 3.9 Hz); 6.12 (1H, brs); 5.72 (2H, s); 4.19-4.02 (2H, m); 3.49 (1H, dd, J = 3.9, 6.7 Hz); 1.90 (1H, m); 1.01 (3H, d, J = 6.7 Hz); 0.99 (3H, d, J = 6.7 Hz).

n-Nonyltriphenylphosphonium bromide



61

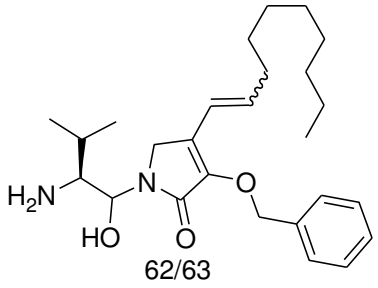
A solution of 1-bromononane (1 g, 4.8 mmol) and triphenylphosphine (1.5 g, 5.7 mmol) in 20 mL of toluene was refluxed overnight. The reaction mixture was then cooled to room temperature and poured into diethyl ether. The resulting oil was washed three times with ether, dissolved in CH_2Cl_2 and then concentrated in vacuo. The oil was left standing in diethyl ether at 0°C overnight and then concentrated in vacuo to give the desired phosphonium bromide **61** (1.8 g, 94%)

Physical appearance Yellowish sticky solid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.98-7.60 (15H, m); 3.90-3.62 (2H, m); 190-1.10 (14H, m); 0.89 (3H, m).

1-(2-Amino-1-hydroxy-3-methyl-butyl)-3-benzyloxy-4-dec-1-enyl-1,5-dihydro-pyrrol-2-one



62/63

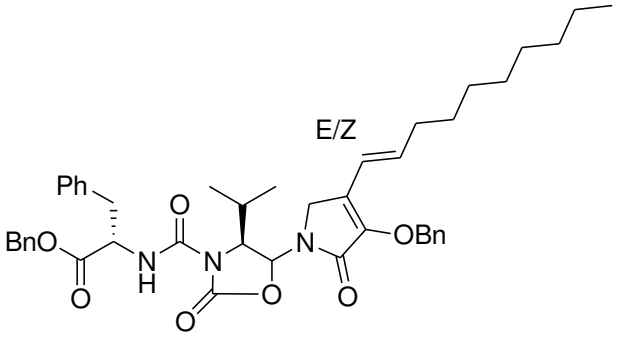
To an ice cold solution of (1-Nonyl)triphenylphosphonium bromide **61** (0.623 g, 1.3 mmol) in 10mL of dry THF under nitrogen was added dropwisely (0.790 mL,1.6 M,1.2 mmol) of n-BuLi in Hexane and then allowed stir for 45 min at same temperature. Then orange solution was cooled to -78°C and added aldehyde **60** (0.2g, 0.63mmol) in dry THF was added dropwise. After addition was completed, the reaction was stirred for 1 h at -78°C,warmed to 0°C and quenched with added Sat. NH_4Cl. . The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (40% EtOAc/Hexane) to afford **62/63** (0.2 g, 73.8%)

Physical appearance White viscous liquid

R_f 0.6 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.44-7.28 (5H, m); 6.27-6.15 (2H, m); 5.94 (1H, brs); 5.63 (1H, m); 5.45-5.30 (2H, m, AB); 4.16 (1H, d, J = 16.8 Hz, AB); 4.04 (1H, d, J = 16.8 Hz, AB); 3.51 (1H, m); 2.18-2.06 (2H, m); 1.78-1.64 (4H, m); 1.50-1.15 (12H, m); 1.00 (3H, d, J = 6.7 Hz); 0.97 (3H, d, J = 6.7 Hz).

2-{[5-(3-Benzyloxy-4-dec-1-enyl-2-oxo-2,5-dihydro-pyrrol-1-yl)-4-isopropyl-2-oxo-oxazolidine-3-carbonyl]-amino}-3-phenyl-propionic acid benzyl ester



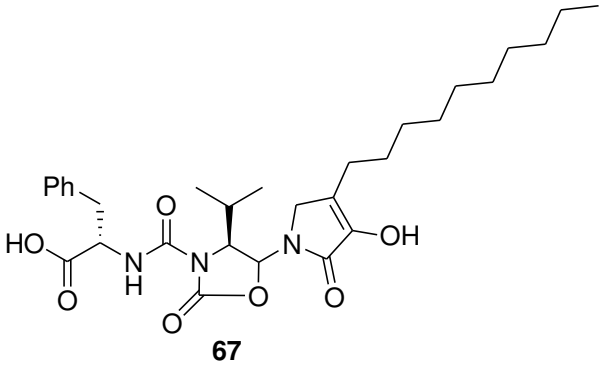
65/66

A mixture of L-phenylalanine benzyl ester **64** (0.059 g, 0.2 mmol) and diisopropylethylamide (0.06 g, 0.5 mmol) in dry DCM (2mL) was slowly added to the stirred solution of triphosgene (0.025 g, 0.086 mmol) dropwise at RT. After a further 15 min stirring, a solution of **62/63** (0.1 g, 0.2 mmol) and DIEA (0.06 g, 0.5 mmol) in dry DCM (2 mL) was added in one portion. The reaction mixture was stirred for 3 days at RT, evaporated to dryness, diluted with ethyl acetate, washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered and concentrated. The crude product **65/66** (0.05g, 30%) was used for the next step without further purification.

Physical appearance- White viscous liquid

R_f 0.5 (EtOAc/Hexane 5:5)

2-{[5-(4-Decyl-3-hydroxy-2-oxo-2,5-dihydro-pyrrol-1-yl)-4-isopropyl-2-oxo-oxazolidine-3-carbonyl]-amino}-3-phenyl-propionic acid



67

To the solution of **65/66** (0.04 g, 0.05 mmol) in EtOAc (10mL) was added a catalytic amount of 10%Pd/C. Then reaction mixture was stirred for 2h at RT under hydrogen atmosphere. After the completion of reaction, the mixture was filtered through a celite bed and the bed was washed with EtOAc three times. EtOAc was concentrated under vacuo. The crude product was purified by flash column chromatography (05%MeOH/DCM) to afford **67** (0.015 g, 50%)

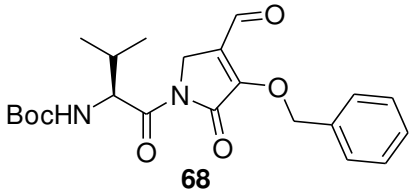
Physical appearance White viscous liquid

R_f 0.5 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 8.19 (1H, d, J = 7.9 Hz); 7.36-7.21 (5H, m); 6.03 (1H, d, J = 2.4 Hz); 4.77 (1H, m); 4.23 (1H, dd, J = 2.4, 4.0 Hz); 3.74-3.59 (2H, m, AB); 3.32 (1H, dd, J = 15.1, 14.1 Hz); 3.11 (1H, dd, J = 14.1, 8.5 Hz); 2.49-2.30 (3H, m); 1.60-1.45 (2H, m); 1.40-1.20 (14H, m); 0.94 (3H, d, J = 7.3 Hz); 0.93 (3H, d, J = 7.3 Hz); 0.88 (3H, t, J = 7.2 Hz).

^{13}C NMR (DMSO) δ: 172.1, 167.5, 153.8, 149.4, 141.0, 137.6, 129.5, 127.9, 126.1, 125.8, 77.2, 60.7, 55.1, 44.3, 37.0, 31.3, 29.0, 28.96, 28.8, 28.7, 28.3, 27.4, 24.8, 22.1, 16.9, 14.5, 13.9.

[1-(3-Benzyloxy-4-formyl-2-oxo-2,5-dihydro-pyrrole-1-carbonyl)-2-methyl-propyl]-carbamic acid tert-butyl ester



68

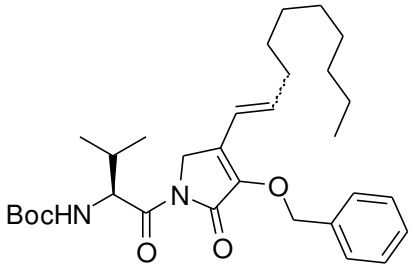
To the stirred solution of compound **59** (0.22 g, 0.5 mmol) in DCM (5 mL) was added PCC (1.12 g, 5.23 mmol) at 0^{o}C, then the solution was allowed to stir for 48 h at RT. After completion of reaction, the mixture was filtered through a celite bed and washed with DCM. The DCM was concentrated under vacuo. The crude product was purified by flash column chromatography (20% EtOAc/Hexane) to afford **68** (0.087 g, 40%)

Physical appearance- White viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 10.14 (1H, s); 7.51-7.31 (5H, m); 5.72-5.59 (2H, m, AB); 5.41 (1H, dd, J = 3.4, 9.5 Hz); 5.18 (1H, d, J = 9.5 Hz); 4.40 (1H, d, J = 18.8 Hz, AB); 4.24 (1H, d, 18.8 Hz, AB); 2.14 (1H, m); 1.44 (9H, s); 1.09 (3H, d, J = 7.1 Hz); 0.84 (3H, d, J = 7.1 Hz).

[1-(3-Benzyloxy-4-dec-1-enyl-2-oxo-2,5-dihydro-pyrrole-1-carbonyl)-2-methyl-propyl]-carbamic acid tert-butyl ester



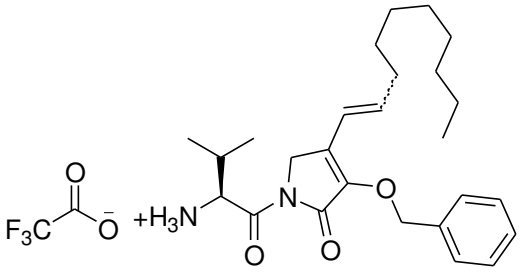
69

To an ice cold solution of (1-nonyl)triphenylphosphonium bromide **61** (0.067 g, 0.144 mmol) in 3 mL of dry THF under nitrogen was added dropwise (0.18 mL,1.6 M, 0.18 mmol) n-BuLi in Hexane and then the mixture was allowed stir for 45 min at the same temperature. Then orange solution was cooled to -78°C and aldehyde **68** (0.030 g, 0.072 mmol) in dry THF was added dropwise. After addition was completed, the reaction was stirred for 1 h at -78°C, warmed to 0°C and quenched with added Sat. NH_4Cl. The aqueous phase was extracted three times with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. To give crude **69** (0.013 g, 36 %) which was used for the next step without further purification.

Physical appearance- Colorless liquid

R_f 0.4 (EtOAc/Hexane 1:9)

1-(3-Benzyloxy-4-dec-1-enyl-2-oxo-2,5-dihydro-pyrrole-1-carbonyl)-2-methyl-propyl-ammonium; trifluoro-acetate



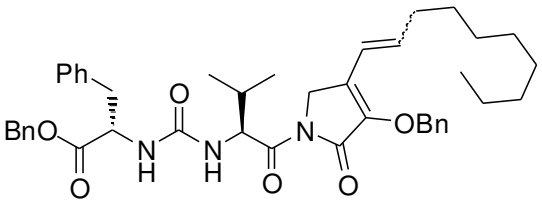
70

To a stirred solution of compound **69** (0.02 g, 0.037 mmol) in dry DCM (2 mL) was added TFA (0.05 mL) at 0^{o}C. The reaction mixture was allowed to stir at room temperature for 4h. After completion of the reaction, the mixture was concentrated under vacuo. The residue was triturated with diethyl ether and dried under vacuum, to afford **70** (0.015g, 73%). The crude product was used for the next step without further purification.

Physical appearance- White sticky solid.

R_f 0.2(MeOH/DCM 1:9)

2-{3-[1-(3-Benzyloxy-4-dec-1-enyl-2-oxo-2,5-dihydro-pyrrole-1-carbonyl)-2-methyl-propyl]-ureido}-3-phenyl-propionic acid benzyl ester



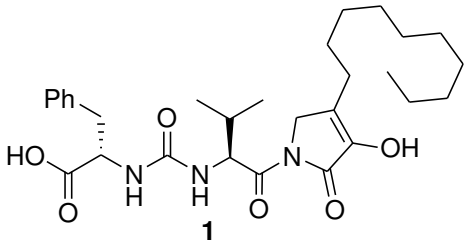
71

A mixture of L-phenylalanine benzyl ester **64** (0.016 g, 0.036 mmol) and diisopropylethylamide (0.010 g, 0.081 mmol) in dry DCM (2 mL) was slowly added to the stirred solution of triphosgene (0.004 g, 0.013 mmol) dropwise at RT. After a further 15 min stirring, a solution of **70** (0.020 g, 0.036 mmol) and DIEA (0.010 g, 0.081 mmol) in dry DCM (2 mL) was added in one portion. The reaction mixture was stirred for 24h at RT, evaporated to dryness, diluted with ethyl acetate, washed with 10% aqueous $KHSO_4$, 5% aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was used for the next step without further purification.

Physical appearance-White viscous liquid.

R_f 0.4 (EtOAc/Hexane 3:7)

2-{3-[1-(4-Decyl-3-hydroxy-2-oxo-2,5-dihydro-pyrrole-1-carbonyl)-2-methyl-propyl]-ureido}-3-phenyl-propionic acid



1

To the solution of **71** (0.02 g, 0.025 mmol) in EtOAc (5 mL) was added a catalytic amount of 10% Pd/C. Then reaction mixture was stirred for 2h at RT under hydrogen atmosphere. After the completion of reaction, the mixture was filtered through a celite bed and the bed was washed with EtOAc three times. EtOAc was concentrated under vacuo. The crude product was purified by flash column chromatography (0.5% MeOH/DCM) to afford **1** (0.01 g, 80 %)

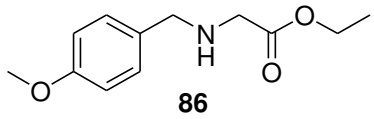
Physical appearance- Clear oil.

R_f 0.4 (MeOH/DCM 1:9)

^{1}H NMR (DMSO) δ: 9.46 (1H, s); 7.28 (2H, dd, J = 7.6, 7.3 Hz); 7.19 (2H, d, J = 7.3 Hz); 7.21 (1H, dd, J = 7.6, 7.7 Hz) ; 6.47 (1H, d, J = 9.3 Hz) ; 6.35 (1H, d, J = 8.0 Hz) ; 5.36 (1H, dd, J = 9.3, 3.7 Hz) ; 4.29 (1H, m); 4.09 (1H, d, J = 18.6 Hz); 4.00(1H, d, J=18.6 Hz) ; 2.99(1H, dd, J = 14.3, 5.2 Hz); 2.84 (1H, dd, J = 14.3, 5.3 Hz); 2.00 (1H, m); 2.30 (2H, m); 1.22-1.44 (16H, m); 0.91(1H, d, J = 7.2 Hz); 0.75 (1H, d J = 7.2 Hz); 0.87 (1H, t, J = 6.7 Hz)

^{13}C NMR (DMSO) δ:174.2, 172.5, 166.5, 157.8, 141.7, 137.9, 129.6, 129.2, 128.5,126.6, 56.9, 54.3, 46.5, 37.9,31.7, 30.2, 29.2, 29.6, 29.6, 29.6, 29.6, 27.2, 25.2, 22.3, 19.9, 16.6, 14.3

Ethyl 2-(4-methoxybenzylamino)acetate



86

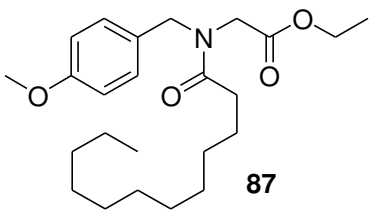
Ethyl bromoacetate (1.0 g, 7.2 mmol) in THF (20 mL) was added dropwise to a cooled solution of p-methoxybenzylamine(1.27 g,7.6 mmol) . After stirring 2.5 h, reaction mixture was filtered and the filtrate was concentrated under vacuo. The crude product was purified by flash column chromatography (80% Ether/Hexane) to afford **86** (1.4g, 87%)

Physical appearance White viscous liquid

R_f 0.5 (Ether/Hexane 9:1)

^{1}H NMR ($CDCl_3$) δ: 7.26 (2H, d, J = 8.2 Hz); 6.88 (2H, d, J = 8.2 Hz); 4.20 (2H, q, J = 7.3 Hz); 3.81 (3H, s); 3.76 (2H, s); 3.41 (2H, s); 1.29 (3H, t, J = 7.3 Hz).

Ethyl 2-(N-(4-methoxybenzyl)dodecanamido)acetate



87

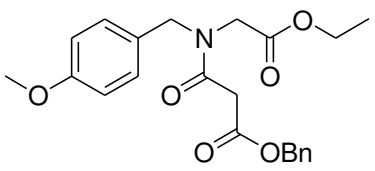
Dodecanoyl chloride (1.1g, 8.8 mmol) was added dropwise to the solution of compound **86** (1.0 g, 4.4 mmol) and TEA (0.9 g, 8.9mmol) in $CHCl_3$ at 0°C over 10 min. After completion of reaction the mixture was diluted with $CHCl_3$ and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated . The crude product was purified by flash column chromatography (60% Ether/Hexane) to afford **87** (1.5g, 83%)

Physical appearance White viscous liquid

R_f 0.5(EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.13 (2H, d, J = 8.8 Hz); 6.90 (2H, d, J = 8.8 Hz); 4.58 (2H, s); 4.18 (2H, q, J = 7.3 Hz); 4.02 (2H, s); 3.82 (3H, s); 2.46 (2H, t, J = 7.3 Hz); 1.75-1.58 (2H, m); 1.41-1.117 (16H, m); 0.89 (3H, t, J = 7.3 Hz).

N-Ethoxycarbonylmethyl-N-(4-methoxy-benzyl)-malonamic acid benzyl ester



84

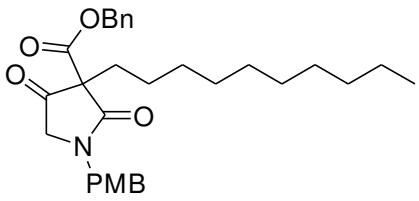
To the solution of **87** (2.4 g, 10.75 mmol) in DCM was added benzyl hydrogen malonate (4.1g, 21.51 mmol) at 0°C, followed by a solution of DCC (3.32 g, 16.13 mmol) and DMAP (0.065 g, 0.5 mmol) in DCM. The solution was allowed to stir at RT for 12h. After the completion of reaction, the mixture was filtered and the filtrate was washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **88** (3.0g, 83%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$ δ: 7.42-7.28 (5H, m); 7.21-7.03 (2H, m); 6.93-6.76 (2H, m); 5.18 (2H, s); 4.55 (2H, s); 4.16 (2H, q, J = 7.3 Hz); 4.05 (2H, s); 3.80 (3H, s); 3.63 (2H, s); 1.23 (3H, t, J = 7.3 Hz).

Benzyl 1-(4-methoxybenzyl)-3-decyl-2,4-dioxopyrrolidine-3-carboxylate



89

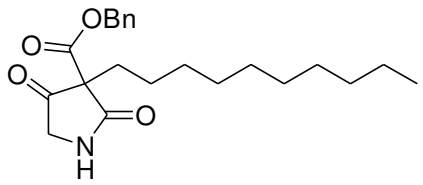
Tetrabutylammonium fluoride (9.5 mL,9.5 mmol) was added to a solution of diester **88** (1.9 g, 4.7 mmol) and diethyl ether; the mixture was stirred for under nitrogen at room temperature for 2h , and the colourless solid was removed by filtration and washed with diethyl ether. The solid was suspended in THF at room temperature, and decyl iodide (2.5 g, 9.5 mmol) was added. The mixture was stirred for twenty four hours, and evaporated to dryness. The crude was purified by column chromatography to afford **89** (0.69 g, 30%)

Physical appearance White viscous liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$ δ: 7.46-7.28 (3H, m); 7.28-7.15 (2H, m); 7.14-6.99 (2H, m); 6.81-6.63 (2H, m); 5.15 (2H, s); 4.79 (1H, d, J = 14.6 Hz, AB); 4.44 (1H, d, J = 14.6 Hz, AB); 3.82 (1H, d, J = 17.8 Hz, AB); 3.77 (3H, s); 3.57 (1H, d, J = 17.8 Hz, AB); 2.18 (2H, m); 1.48-1-1.12 (16H, m); 0.88 (3H, t, J = 7.2 Hz).

Benzyl 3-decyl-2,4-dioxopyrrolidine-3-carboxylate



90

To the stirred solution of **89** (0.1 g, 0.2 mmol) in ACN:Water (3:1) was added cerium ammonium nitrate (0.3g, 0.6mmol) at 0^{o}C. The solution was then allowed to stir for 1h at room temperature. After the completion of reaction, the mixture was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/Hexane) to afford **90** (0.05 g, 66%)

Physical appearance Colorless liquid

R_f 0.5 (EtOAc/Hexane 5:5)

^{1}H NMR ($CDCl_3$) δ: 7.43-7.17 (5H, m); 6.95 (1H, brs); 5.16 (2H, s); 4.05 (2H, d, J = 18.1 Hz, AB); 3.81 (2H, d, J = 18.1 Hz, AB); 2.16 (2H, m); 1.46-0.96 (16H, m); 0.88 (3H, t, J = 7.3 Hz).

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CHAPTER 4

Synthesis of 2,3-pyrrolidinedione derivatives

4.1 Introduction

Leopolic Acid possesses a 2,3-pyrrolidinedione core (**2**). This is a very attractive skeleton as natural compounds with the isomeric 2,4-pyrrolidinedione ring (**1**, tetramic acids) show remarkable activities, ranging from antibacterial and antiviral to antifungal as well as anticancer, as discussed in Chapter 3.

On the contrary, compounds with a 2,3-pyrrolidinedione skeleton have been much less investigated. To the best of our knowledge, only leopolic acid[1] and a few synthetic compounds[2-4] have been reported in the literature so far. Therefore, this skeleton can be considered a very attractive target for biological evaluation, offering the possibility of preparing products with several points of diversity, similarly to what occurred for tetramic acid. Thus, using leopolic acid as a starting point, we planned to develop a chemical routes to the substituted 2,3-pyrrolidinedione core, with the aim of preparing derivatives with a wide range of diversity. In the perspective of a potential evaluation of their antifungal and antimicrobial activity, a number of analogues with various substituents on the 2,3 pyrrolidinedione core were synthesized.

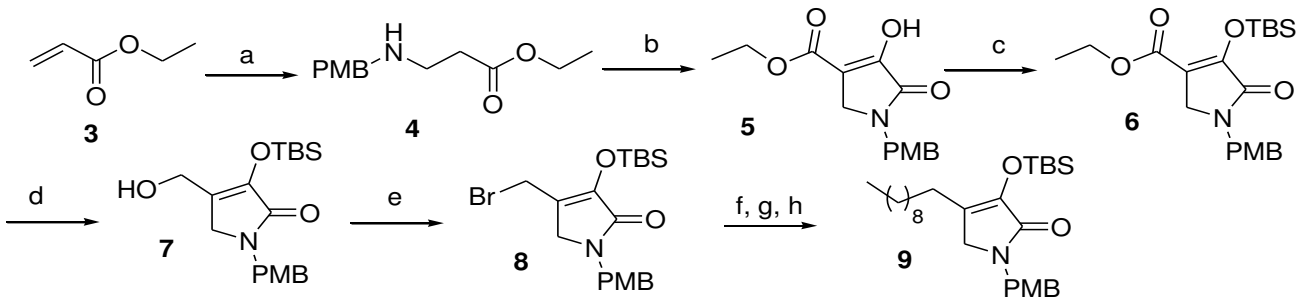
4.2 Synthesis

We envisaged that the most straightforward route to the synthesis of the 2,3-pyrrolidinedione system could be the Michael addition of a suitably protected amine to ethyl acrylate, followed by a Dieckmann cyclization with diethyl oxalate.[5] We opted for the *p*-methoxybenzyl (PMB) protecting group, which could be easily removed by cerium ammonium nitrate (CAN) or 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ).

Thus, ethyl acrylate **3** was reacted with *p*-methoxybenzylamine to obtain compound **4**, which was then treated with diethyl oxalate to give 2,3-pyrrolidinedione **5**. (Scheme 4.1). NMR observations showed that the compound exists as an enol tautomer (see SI). Indeed, it has been reported[6-8] that apparently all 4-monosubstituted 2,3-dioxopyrrolidines are highly enolized, regardless of the nature of the substituent in position 4.

With compound **5** in hands, we planned to prepare a series of analogues to obtain a range of diversity around the heterocyclic core. Initially, it was decided to compare different functionalities at position 4. The enolic OH was first protected with a tertbutylsilyl (TBS) group, by treatment of **5** with TBSCl, to obtain compound **6**.

Scheme 4.1. Synthesis of compounds **5-9**.



(a) *p*methoxybenzylamine, EtOH, rt, 12h, 98%; (b) diethyloxalate, NaOEt, EtOH, reflux, 3h, 83%; (c) TBSCl, imidazole, DCM, rt, 6h, 86%; (d) DIBAL-H, DCM, -78°C to rt, 1h, 72%; (e) PPH_3, CBr_4, DCM, rt, 12h, 87%; (f) PPh_3, toluene, reflux, 6h, 61%; (g) n-nonanal, LiHMDS, THF, -78°C to rt, 5h, 88%; (h) H_2/Pd/C, EtOAc, rt, 1h, 89%

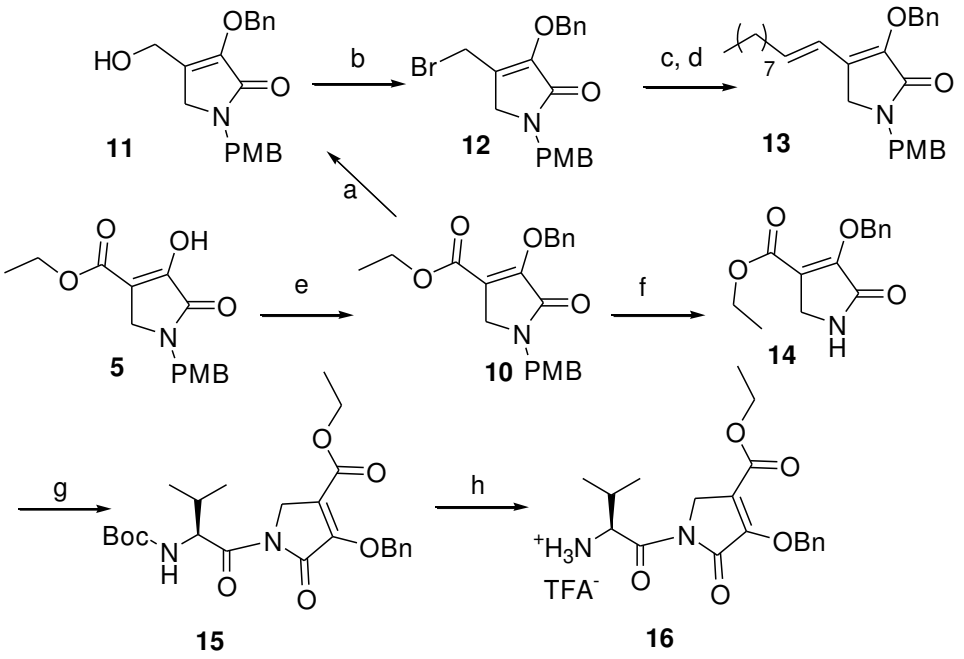
Reduction of **6** with diisobutylaluminium hydride (DIBALH) successfully gave alcohol **7** in 72% yield. Inspired by Leopolic acid we planned the synthesis of a 4-alkyl substituted derivative. Thus, alcohol **7** was converted into the corresponding bromide by Appel reaction with PPh_3 and CBr_4. The bromide was reacted with PPh_3 to give a bromonium salt which was then subjected to Wittig reaction with a nine-carbons aldehyde. Reduction of the double bond by catalytic hydrogenation gave compound **9** in 89% yield.

Unfortunately, attempts to remove the PMB group from compounds **6**-**9** (CAN in ACN /water or DDQ, CH_2Cl_2) resulted into unstable compounds which decomposed during purification.

Assuming that this instability could be due to the enol protecting group, we decided to use a benzyl group as an alternative. Thus, compound **5** was treated with benzyl bromide to obtain compound **10** (Scheme 4.2). To compare compounds with the benzyl group to analogues with the TBS group on the enolic OH, we repeated on compound **10** (Scheme 2) the same sequence reported above for compound **6** (see Scheme 1). Reduction with DIBALH, followed by Appel bromination and Wittig reaction, allowed the introduction in position 4 of the aliphatic chain (compound **14**).

Deprotection of PMB group from compound **10** was successful and gave **14**. Having the compound with a free NH in hands, we explored the effect of a polar group linked to nitrogen, to compare with compound **10**, carrying a lipophilic PMB group. Thus, **14** was acylated with activated N-protected valine, already present in Leopolic acid to obtain **15**. Removal of the Boc protecting group afforded **16**, containing the free aminoacidic residue.

Scheme 4.2. Synthesis of compounds **10-16**.



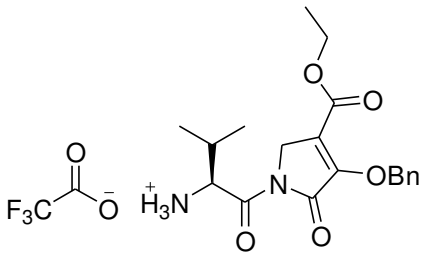
(a) DIBAL-H, DCM, -78°C to rt, 1h, 56%; (b) PPh_3, CBr_4, DCM, rt, 3h, 52%; (c) PPh_3, toluenene, reflux, 6h, 75%; (d) n-nonanal, LiHMDS, THF, -78°C, 5h, 52%; (e) BnBr, K_2CO_3, DMF, rt, 1h, 50%; (f) CAN, ACN: water, rt, 1h, 73%; (g) 2-*tert*-butoxycarbonylamino-3-methyl-butyric acid pentafluorophenyl ester, BuLi, THF, -78°C to rt, 0.5h, 86%; (h) TFA, DCM, 0°C, 0.5h, 90%.

4.3 Experimental section

For general experimental methods, see Chapter 3

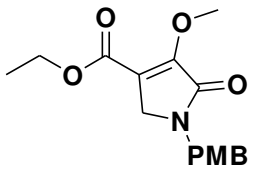
The preparation of compounds **3-15** is described in Chapter 3

(S)-1-(3-(benzyloxy)-4-(ethoxycarbonyl)-2-oxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-1-oxobutan-2-aminium 2,2,2-trifluoroacetate (16)



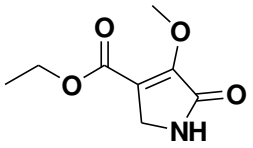
To a stirred solution of compound **15** (0.10 g, 0.21 mmol) in dry DCM (3 mL) was added TFA (1 mL) at 0^{o}C. The reaction mixture was allowed to stir at room temperature for 1h. After completion of the reaction, the mixture was concentrated under vacuo. The residue was triturated with diethyl ether and dried under vacuum, to afford **16** (0.09 g, 90%) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 7.46-7.26 (5H, m); 5.64-5.57 (2H, m); 5.07 (1H, m); 4.48 (1H, d, J = 19.8 Hz, AB); 4.28 (1H, d, J = 19.8 Hz, AB); 4.27 (2H, q, J = 7.3 Hz); 2.33 (1H, m); 1.32 (3H, t, J = 7.3 Hz); 1.24 (3H, d, J = 7.0 Hz); 1.01 (3H, d, J = 7.0 Hz). Anal. calcd for $C_{21}H_{25}F_3N_2O_7$: C, 53.16; H, 5.31; N, 5.90. Found: C, 53.33; H, 5.30; N, 5.91.

4-Methoxy-1-(4-methoxybenzyl)-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester (19)



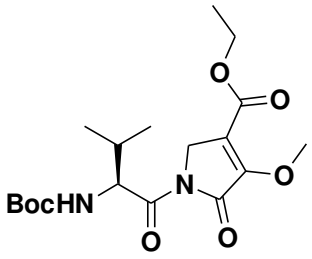
To a stirred solution of **5** (0.50 g, 1.71 mmol) in dry DMF(10 mL) was added anhydrous K_2CO_3 (0.71 g, 5.14 mmol) at 0°C. The solution was stirred for 15 min, then MeI (0.29 g, 2.05 mmol) was added. The reaction mixture was allowed to warm up and stirred for further 1h. After completion of reaction, the mixture was diluted with EtOAc and washed with cold brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (30% EtOAc/Hexane) to afford **19** (0.32 g, 61%) as a white sticky solid. R_f 0.4 (EtOAc/Hexane 1:1). ^{1}H NMR ($CDCl_3$) δ:: 7.20 (2H, d, J = 8.5 Hz); 6.88 (2H, d, J = 8.5 Hz); 4.58 (2H, s); 4.34 (3H, s); 4.22 (2H, q, J = 7.0 Hz); 3.86 (2H, s); 3.81 (3H, s); 1.29 (3H, t, J = 7.0 Hz). Anal. calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.70; H, 6.26; N, 4.60.

4-Methoxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester (20)



To a stirred solution of **19** (0.25 g, 0.81 mmol) in ACN:Water (3:1) (8 mL) was added cerium ammonium nitrate (1.79 g, 3.27 mmol) at 0 °C. The mixture was stirred for 1h at room temperature, then it was diluted with EtOAc and washed with water and brine solution. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (50% EtOAc/Hexane) to afford **20** (0.18 g, 71%) as a colorless viscous oil. R_f 0.5 (EtOAc/Hexane 6:4). ^{1}H NMR ($CDCl_3$) δ:: 4.37-4.23 (6H, m); 4.07 (2H, s); 1.34 (3H, t, J = 7.0 Hz). Anal. calcd for $C_8H_{11}NO_4$: C, 51.89; H, 5.99; N, 7.56. Found: C, 52.06; H, 6.01; N, 7.59.

1-(2-tert-butoxycarbonylamino-3-methylbutyryl)-4-methoxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylic acid ethyl ester (17)



To a stirred solution of **20** (0.12 g, 0.64 mmol) in anhydrous THF (2mL) was added n-BuLi (1.6 M in hexane, 0.40 mL, 0.64 mmol) at –78°C over 10 min. The active ester **18** (0.27 g, 0.71 mmol) in THF (2 mL) was then added, and the mixture was stirred for 30 min at the same temperature. The reaction was quenched with sat. NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (16% EtOAc/Hexane) to afford **17** (0.19 g, 80%) as a colorless viscous oil. R_f 0.5 (EtOAc/Hexane 2:8). ^1H NMR ($CDCl_3$) δ (mixture of rotamers): 5.39 (1H, d, J = 3.6 Hz); 5.36 (1H, d, J = 3.6 Hz, 2nd rotamer); 4.44 (1H, d, J = 19.2 Hz, AB); 4.30 (2H, q, J = 7.0 Hz); 4.29 (1H, d, J = 19.2 Hz, AB); 4.27 (3H, s); 4.13 (2H, q, J = 7.0 Hz, 2nd rotamer); 1.45 (9H, s); 1.34 (3H, t, J = 7.0 Hz); 1.27 (3H, t, J = 7.0 Hz, 2nd rotamer); 1.09 (3H, d, J = 6.7 Hz); 0.85 (3H, d, J = 6.7 Hz). Anal. calcd for $C_{18}H_{28}N_2O_7$: C, 56.24; H, 7.34; N, 7.29. Found: C, 56.03; H, 7.36; N, 7.28.

4.4 References and notes

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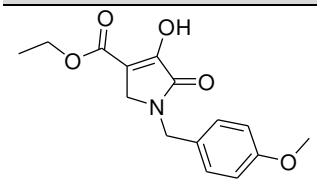
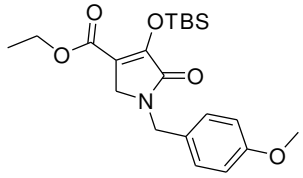
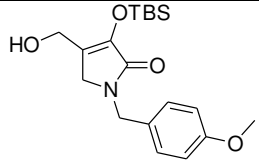
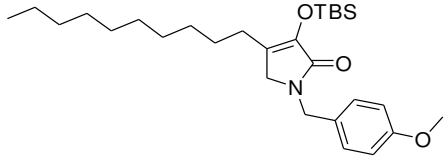
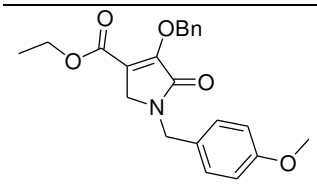
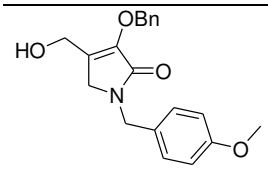
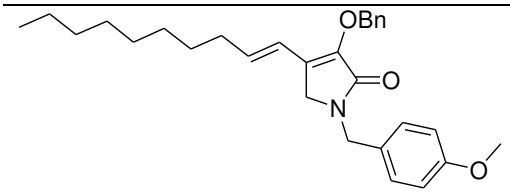
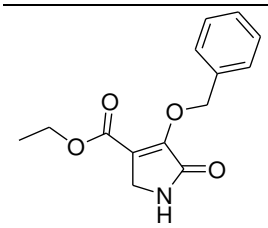
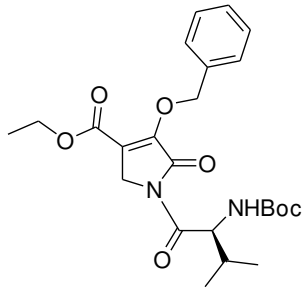
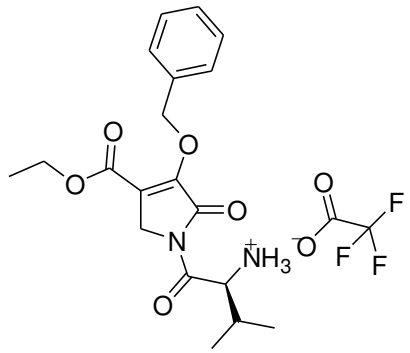
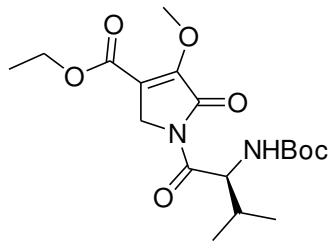
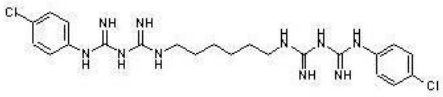
CHAPTER 5

Antimicrobial and antifungal activity evaluation of 2,3-pyrrolidinedione derivatives

The prepared compounds containing the 2,3-pyrrolidinedione core (see Chapter 4) were investigated for their antimicrobial and antifungal activity on *S. mutans, C. cladosporioides* and *C. albicans.* From the analysis of the results obtained preliminary structure-activity relationships (SAR) have emerged.

Table 1. Compounds tested

Compound	N°	MW	Solubility
	**5**	291.30	DCM EtOAc
	**6**	405.56	DCM EtOAc
	**7**	363.52	DCM EtOAc
	**9**	473.33	DCM EtOAc
	**10**	381.16	DCM EtOAc
	**11**	339.39	DCM EtOAc
	**13**	471.3	DCM EtOAc

	**14**		
	**15**	460.52	DCM EtOAc
	**16**	474.43	DCM EtOAc
	**17**	384.42	DCM EtOAc
	**CHX**[1]		

[2]chlorhexidine (positive control).

5.1 Antimicrobial activity against *Streptococcus mutans* and *Candida albicans*

The results of antibacterial activity are shown in Figure 1 and Table 2.

Compound **5**, containing a free enolic OH, showed a mean OD of 0.394. Introduction on the OH of a TBS group did not cause a significant change in activity (**6**, OD = 0.407), whereas a benzyl group in the same position (**10**), showed a two-fold decrease in activity (OD = 0.770, not significantly different from the negative control). Significance was assessed by one-way analysis of variance (ANOVA) and Tukey-Kramer *post hoc* test (significant differences between groups were considered at $p \leq 0.05$) (see SI).

Reduction of the ester group to introduce a polar alcohol moiety at position 4 of both TBS and benzyl substituted compounds gave derivatives **7** and **11** (OD = 0.647 and OD = 0.776, respectively). Compound **11** showed a similar activity to the corresponding ester **10**, whereas **7** showed a decreased activity compared to the ester **6**.

Having not gained potency by introducing a polar group, we replaced the ester group with an apolar aliphatic chain to obtain **9** and **13**. Comparing the activities of **6** with **9**, we observed a decrease of activity due to the introduction of the aliphatic chain (OD = 0.762 in **9** vs OD = 0.407 in **6**). Interestingly, the introduction of the apolar chain on the benzyl substituted derivative **13** resulted in the highest antibacterial activity recorded (OD = 0.054) against *S. mutans* biofilm, not significantly different from CHX when diluted up to 0,2% w/v (p=1.0000).

Successively, the effect of substituents on the nitrogen atom was investigated. Removal of the PMB group from compound **10** did not cause any change in activity (**14**: OD = 0.761). An

unexpected gain in antibacterial activity was observed with the introduction of the protected aminoacidic moiety (compound **15**) (OD = 0.084).

Indeed, the derivative **15** showed a significant decrease in the *S. mutans* viable biomass when diluted up to 0.008% w/v (p<0.0001). In the highest concentration tested (1% w/v), it showed an antibacterial activity not significantly different from the positive control (p=1.0000).

Removal of the Boc protecting group (compound **16**) resulted in a 5-fold lower activity (OD = 0.422).

Finally, considering the significant activity showed by compound **15**, we synthesized the corresponding derivative with a methyl in place of the benzyl group (**17).**. This replacement caused a drop in activity (OD = 0.539), suggesting that the presence of a lipophilic benzyl group in position 3 was productive in terms of antibacterial activity.

Figure 2 and Table 2 report the results on antifungal activity of derivatives. Almost every compound possessed fungicidal activity. In particular, derivatives **10**, **11**, **13**, **14**, **15** and **17** exhibited a concentration-dependent activity on *C. albicans* biofilm.

The TBS-substituted compounds (**6**, **7**, **9**) were less effective than the corresponding benzyl-substituted compounds, which, in turn, showed a significant activity comparable to their antimicrobial activity on *Streptococcus mutans.* Again, compounds **13** and **15** were the most effective antifungal agents. In fact, at the highest concentration tested, they showed an activity comparable to 0.2% CHX.

Table 2. Antimicrobial activity of pyrrolidinedione derivatives on *Streptococcus mutans* and *Candida albicans*.

Compound	Viable biomass (OD)[1]	
	S. mutans	*C. albicans*
5	0.394	0.160
6	0.407	0.224
7	0.647	0.231
9	0.762	0.201
10	0.771	0.198
11	0.776	0.187
13	0.054	0.115
14	0.761	0.167
15	0.084	0.144
16	0.422	0.186
17	0.539	0.203
CHX[2]	0.051	0.147

[1]Residual viable biomass of either a *Streptococcus mutans* or a *Candida albicans* 24h-grown monospecific biofilm after 1h exposure to the most active dilution of the derivative, as assessed by MTT assay (data shown as OD values, higher OD meaning a higher amount of adherent, viable microbial biomass after the exposure); [2]Chlorhexidine (positive control).

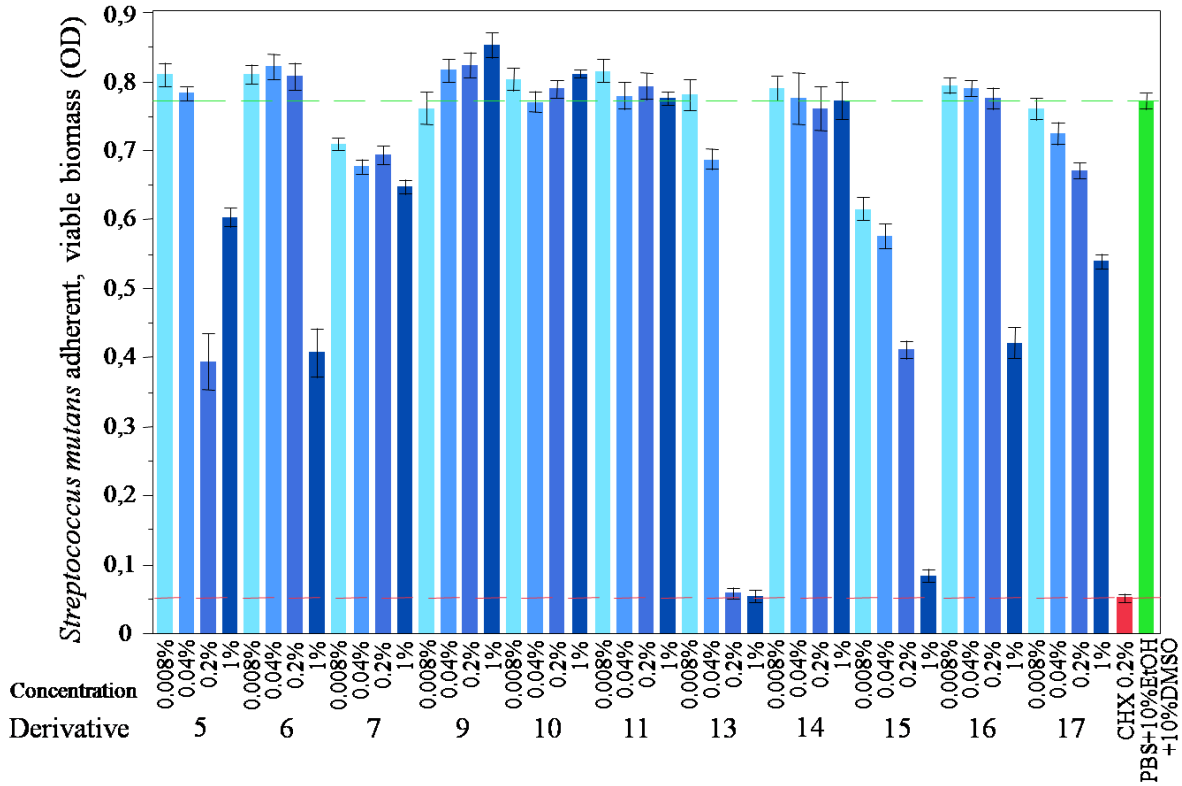


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Figure 1. Residual *Streptococcus mutans* viable biomass after 1h exposure to different concentrations of the tested derivatives. The negative control (the solution used to dilute the derivatives) is shown as a green bar and green dashed line while the positive control (a 0.2% solution of Chlorhexidine) is highlighted in red. Data are presented as OD values proportional to the amount of viable bacterial cells ± 1 standard error

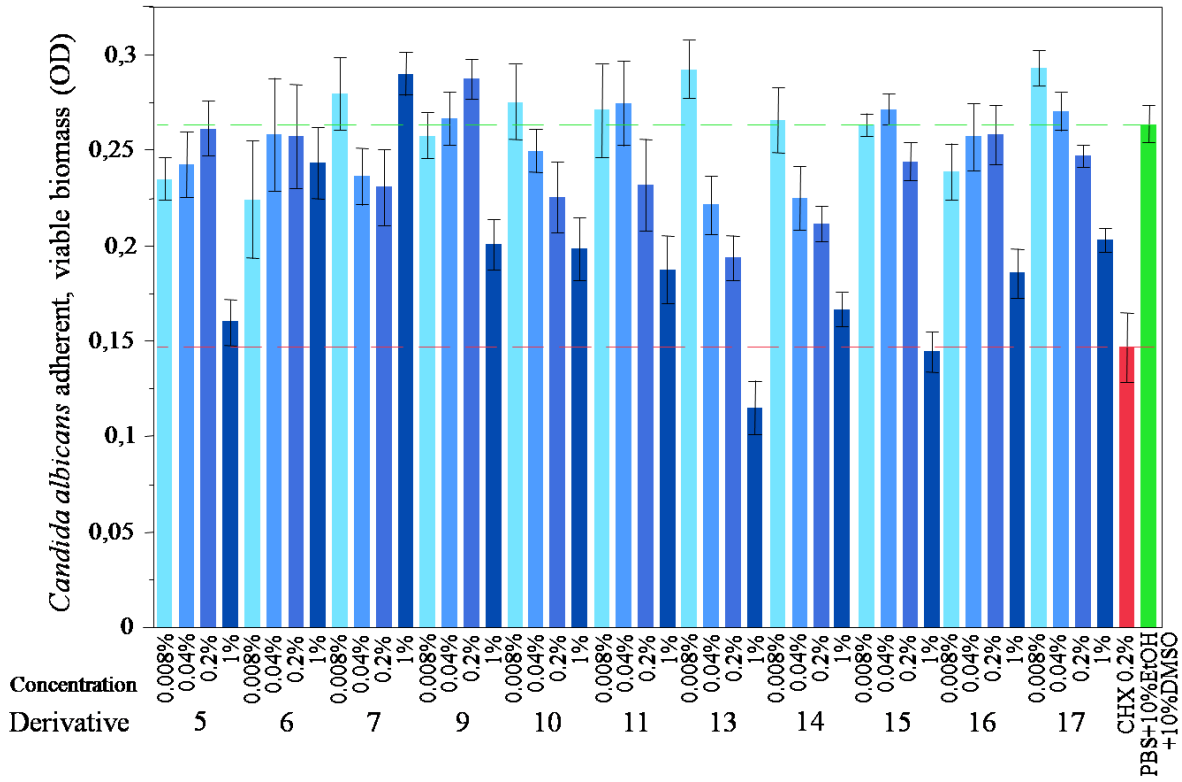


Figure 2. Residual *Candida albicans* viable biomass after 1h exposure to different concentrations of the tested derivatives. The negative control (the solution used to dilute the derivatives) is shown as a green bar and green dashed line while the positive control (a 0.2% solution of Chlorhexidine) is highlighted in red. Data are presented as OD values proportional to the amount of viable fungal cells ± 1 standard error.

5.2 Antifungal activity against *Cladosporium cladosporioides*

We also decided to assess the activity of substituted 2,3-pyrrolidinedione-containing compounds against *Cladosporium cladosporioides,* a fungal plant pathogen that affects wheat. For the antifungal assay 10.0 µL of solution corresponding to 50.0, 40.0, 30.0, 20.0, 10.0, 5.0, 1.0, 0.5, 0.1 µg were applied to precoated Si gel TLC plates. The chromatograms were sprayed with spore suspension of *Cladosporium cladosporioides* in CZAPEK broth and incubated for 72h in darkness in a moistened chamber at 25°C. None of compound found significant activity. Prochloraz was used as control.

The results obtained suggest that compounds possessing a 2,3-pyrrolidinone skeleton can be considered promising candidates in the development of new antibacterial and antifungal compounds. In particular, compound **13** showed a remarkable activity both on *Streptococcus mutans* and *Candida albicans.*

5.3 Experimental

5.3.1 Antimicrobial activity

5.3.1.1 Microorganisms

All the culture media were obtained from Becton-Dickinson (BD Diagnostics-Difco, Franklin Lakes, NJ, U.SA). A pure suspension of *Streptococcus mutans* (ATCC 35668) in brain-heart infusion (BHI) broth was obtained after 24h incubation at 37 °C in a 5% CO_2 supplemented environment. Cells were harvested by centrifugation (2200 rpm at 19°C for 5 min), washed twice with sterile phosphate-buffered saline (PBS) and resuspended in the same buffer. The cell suspension was subsequently subjected to low intensity ultrasonic energy (Sonifier model B-150; Branson, Danbury, CT, USA; operating at 7W energy output for 30 s) in order to disperse bacterial chains. Then, the suspension was adjusted to a turbidity equivalent to that of a 1.0 McFarland standard.

A pure suspension of *Candida albicans* (ATCC 90028) in BHI broth was obtained after a 24h incubation at 37 °C in a 5% CO_2 supplemented environment. Cells were harvested by centrifugation (2200 rpm at 19 °C for 5 min), washed twice with sterile PBS and resuspended in the same buffer and, then, the suspension was adjusted to a value of 1.0 on McFarland scale. The concentration for both the tested strains was confirmed by plate-count on Mitis salivarius agar (MSA) and on Sabouraud dextrose agar (SDA) for *S. mutans* and *C. albicans* suspensions, respectively.

5.3.1.2 Solutions of derivatives

All reagents, including the multi-well plates used in this study were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA), unless otherwise specified. Solutions of the tested compounds were prepared for each derivative using 10% (v/v) DMSO and 10% (v/v)

EtOH in sterile PBS. The following dilutions were prepared for each derivative: 1%, 0.2%, 0.04%, 0.008%, w/v. A solution consisting in10% (v/v) DMSO + 10% (v/v) EtOH in sterile PBS was used as negative control, whereas another solution of 0.2% chlorhexidine (CHX) in 10% (v/v) DMSO + 10% (v/v) EtOH in sterile PBS was used as positive control. Finally, all solutions were filter-sterilized (Millipore filter, 0.2µm). The researchers who assessed the microbiological activity of the solutions were blinded regarding the derivatives and the dilutions of the tested solutions.

5.3.1.4 Biofilm formation

Two independent experimental runs were performed in two different months in order to exclude day-to-day variability, and data from the two runs were averaged.

In each well of 96 well-plates, 200 µL of sterile TSB broth additioned with 1% D-glucose (Klein et al., 2014) and 20 µL of cell suspension were inoculated. The plates were then incubated at 37°C and 5% CO_2 supplemented atmosphere for 24 h to allow the development of a multilayer biofilm on the polystyrene surfaces. After that, the supernatant broth solution was gently removed and each well was gently rinsed with sterile PBS in order to remove non-adherent cells. Then, the rinsing solution was discarded and 25 µL of each dilution of the tested derivative solutions were added to each well. A total of 14 replicate wells were inoculated in tandem for each solution and strain. After 1h, the test solutions were discarded and the wells were gently rinsed with sterile PBS. The residual viable biomass on the polystyrene surfaces was evaluated for both tested strains by the MTT assay.

5.3.1.5 MTT assay

The MTT assay was conducted as follows: a tetrazolium salt (MTT) stock solution was prepared by dissolving 5 mg mL^{-1} 3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide in sterile PBS; a phenazinium salt (PMS) stock solution was prepared by dissolving 0.3 mg mL^{-1} of *N*-methylphenazinium methyl sulphate in sterile PBS. The solutions were stored at 4°C in light-proof vials until the day of the experiment, when a fresh measurement solution (FMS) was prepared by mixing 1 mL of MTT stock solution, 1 mL of PMS stock solution and 8 mL of sterile PBS for each 96-well plate. A lysing solution (LS) was also prepared by dissolving 10% v/v of sodium dodecyl sulphate (SDS) and 50% v/v of dimethylformamide (DMF) in distilled water.

At the end of the incubation period, the adherent, viable and metabolically active biomass was measured by MTT assay as follows. At first, 100 µL of FMS were added in each well and the plates were incubated at 37°C in light-proof conditions, for 30 min (*S. mutans*) or 3h (*C. albicans*). During incubation, electron transport across the microbial plasma membrane and, to a lesser extent, microbial redox systems converted the yellow MTT salt to insoluble purple formazan. The conversion was facilitated by the intermediate electron acceptor (PMS). The unreacted FMS was gently removed from the wells by aspiration and the formazan crystals were dissolved by adding 100 µL of LS to each well; plates were then incubated for 1h at room temperature in light-proof conditions. From each well, 80 µL of the suspension were collected and optical density (OD) at 550 nm was measured with a spectrophotometer (Genesys 10-S, Thermo Spectronic, Rochester, NY, USA). OD units are proportional to the number of adherent, viable cells in each well.

5.3.2 Statistical analysis

Statistical analyses were carried out using JMP 10.0 software (SAS Institute, Cary, NC, U.S.A.). Homogeneity of variance was preliminarily checked and verified using Bartlett's test. One-way analysis of variance (ANOVA) and Tukey-Kramer *post hoc* test were used to assess significant differences between groups at $p \leq 0.05$.

CHAPTER 6

Approach towards synthesis of 3-O-Methylfunicone

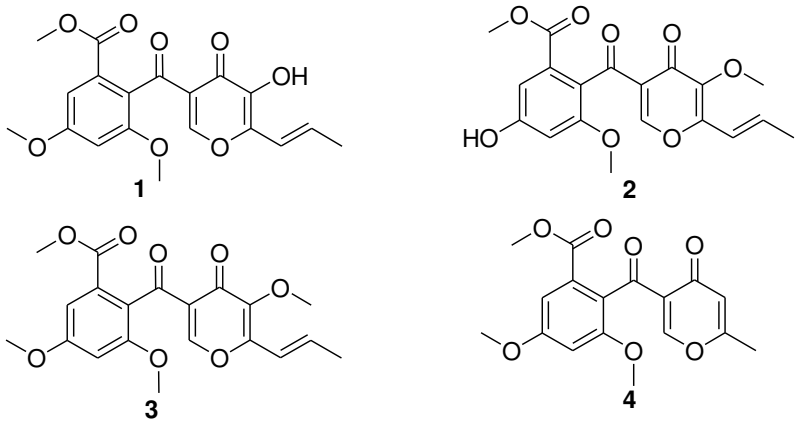
6.1 Introduction

The structures of funicone-related compounds are based on a γ-pyrone ring which is linked through a keto function to a β-resorcylic acid nucleus whose carboxylic group is esterified by methanol; depending on the specific metabolite, one or more hydroxyl groups may be methylated, while several substitutions occur at the carbon atoms of the γ -pyrone nucleus. The family embraces a number of derivatives showing promising biomedical potential as fungicides, antiviral and antitumor candidates. Funicone (**1**) was first isolated from *Penicillium funiculosum*[1] but structural analogs have been reported from many other terrestrial fungi. Some funicone-related products differ by just one or few substitutions on this fundamental molecular frame. This is the case of isofunicone (**2**), a structural isomer produced by an unidentified *Penicillium* strain[2], in which a methoxyl group on the aryl moiety is interchanged with the hydroxyl group on the γ -pyrone nucleus.

6.2 Isolation and Biological activity of 3-OMF

3-OMF(**3**), produced by soil strain of *P. Pinophilum*[3] shows consistent fungitoxic properties that suppressed *in vitro* mycelial growth of a number of plant pathogenic fungi, such as *R. solani, A. alternata, Cylindrocladium scoparium* and *F. solani*, at a concentration of 100 μg/mL[4]. The same concentration also inhibited dermatophytic species, such as *Trichophyton rubrum* and *Microsporum canis*, while, as already pointed out for other compounds of the series, it was not effective against *C. albicans*[5]. Antiproliferative properties by 3-*O*-methylfunicone have later resulted against human tumor cell lines. In fact, cytostatic effects and the induction of programmed death were observed on HEp-2 cells (derived from larynx carcinoma) at a concentration of 60 μg/ml[6].The anti-proliferative effects of 3-OMF were

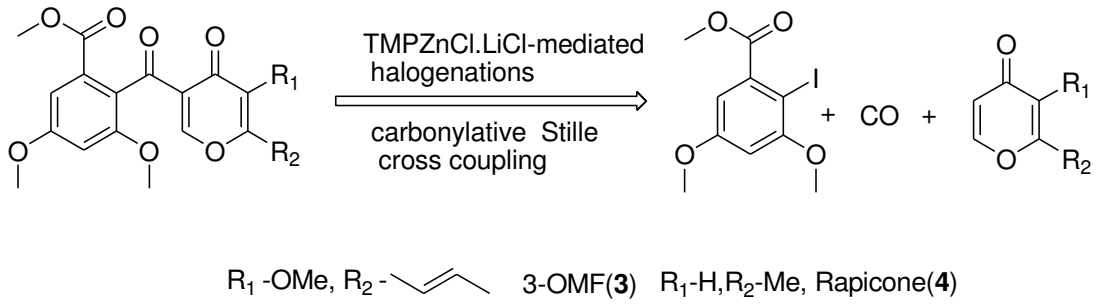
investigated on human breast cancer MCF-7 cells selected as mammospheres derived from MCF-7s.



6.3 Reported Syntheses of 3-OMF

The total synthesis of the natural product 3-OMF(**3**), a member of the funicone class of compounds, and its derivative is reported[7]. The key reaction in synthesis was TMPZnCl. LiCl-mediated halogenations and carbonylative Stille cross coupling .

Scheme 6.1 Reported Retrosynthesis of 3-OMF

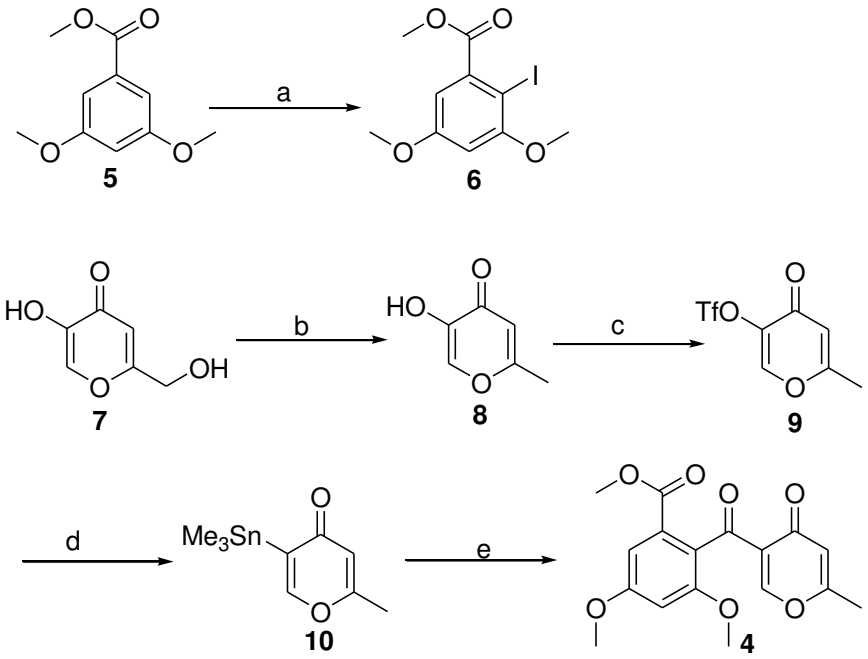


The synthesis of Rapicone is shown in scheme 6.2.

Rapicone was synthesised from commercially available methyl 3,5-dimethoxybenzoate **5** and kojic acid **7**. Methyl 2-iodo-3, 5-dimethoxybenzoate **6** was synthesised from **5** by using NIS in ACN with 82%. Kojic acid was converted into *allo* maltol **8** in two steps with 50% yield. To convert this synthon into a tin–organyl, the hydroxy group was triflated to provide **9**. Subsequent direct stannylation with hexamethylditin afforded the cross-coupling partner **10.** The carbonylative Stille cross-coupling reaction with **6** using the $Pd(OAc)_2$/X-Phos system yielded the final product rapicone **4.**

For the synthesis of 3-OMF, they have started with maltol (**11**), cheap commercially available compound (scheme 6.3). The methoxy group was introduced by methylation with iodomethane to afford **12** in 95% yield. Regioselective monobromination at allylic position was achieved by using NBS and AIBN to provide bromide **13**.The bromide was substituted in aqueous dioxane to afford alcohol **14**, which was subjected to DMP oxidation to yield aldehyde **15**. The aldehyde **15** was then converted to alcohol **16** by using diethyzinc and (-) MIB in 50% yield. The alcohol was protected with TBS group giving **17**. The protected pyranone was regioselectively iodinated at α position with TMPZnCl.LiCl in 99% yield. The stannylation of iodide with Sn_2Me_6, LiCl, $Pd(PPh_3)_4$, in THF afforded stannane **19**. The carbonylative Stille coupling followed by deprotection and dedydration afforded 3-OMF.

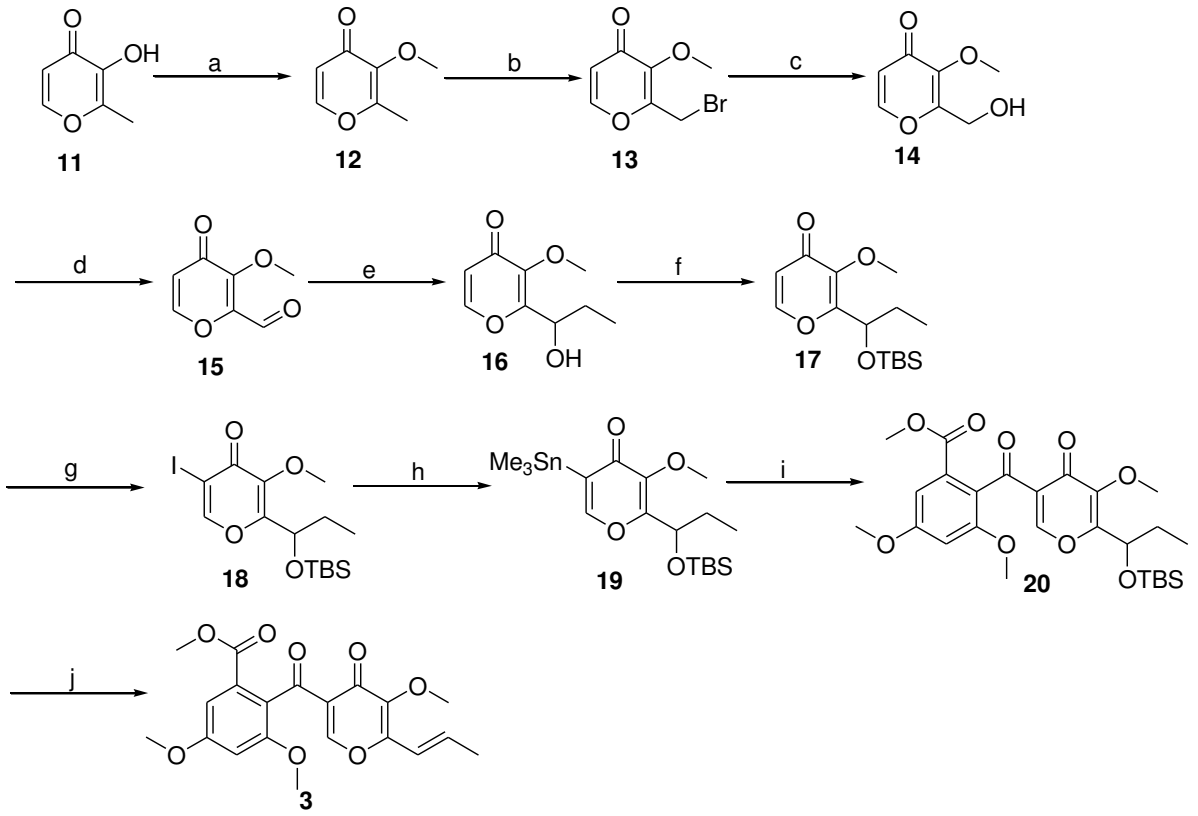
Scheme 6.2



Reagents and Conditions

a) NIS, ACN, 82°C, 59% b) i) $SOCl_2$, rt ii) Zn, HCl, H_2O,75°C 50% over 2 steps c) Tf_2O, Py, 0°C to rt, 90% d) Sn_2Me_6,LiCl, $Pd(OAc)_2$/X-Phos,DMF, rt, 81% e) Pd(OAc)2/X-Phos, CsF, CO(5bar), dioxane, 95°C, 50%

Scheme 6.3



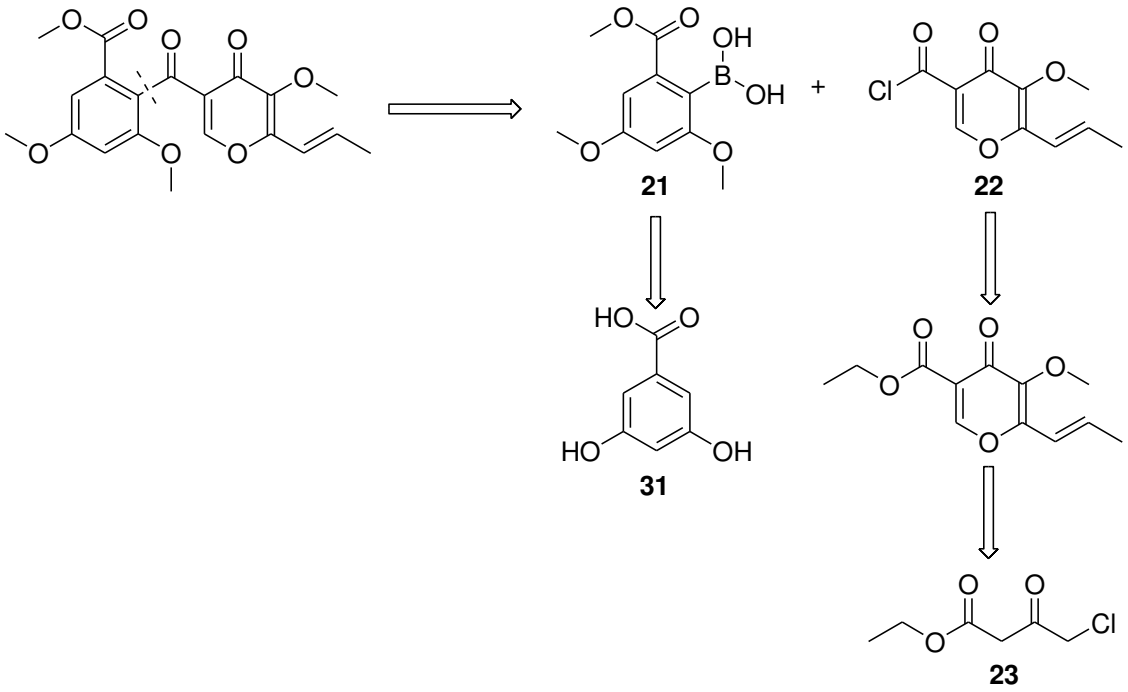
Reagents and Conditions

a) MeI, K_2CO_3, Acetone, 56^oC, 95% b) NBS, AIBN, CCl_4, 77^oC, 85% c) $CaCO_3$, H_2O/dioxane, 100^oC, 94% d) DMP, DCM, rt, 98% e) (-)MIB, Et_2Zn,THF,-78 ^oC to rt, 50% f) TBSOTf, 2,6-lutidine, DCM,0 ^oC,82% g) TMPZnCl.LiCl, I_2, THF, rt, 99% h) Sn_2Me_6,LiCl, $Pd(PPh_3)_4$,THF,65^oC,83% I) $Pd(OAc)_2$/X-Phos, CsF, CO(5bar), dioxane, 95^oC,67% j) PTSA, toluene, 110^oC, 50%

6.4 Retrosynthesis of 3-OMF

Our initial retrosynthetic analysis is outlined in scheme 6.4. In the beginning we envisioned that 3-OMF could be constructed by a convergent synthetic strategy. The subunit **21** could be constructed from 3,5dihydroxybenzoic acid **31** and subunit **22** could be constructed from ethyl 4-chloroacetoacetate **23**.

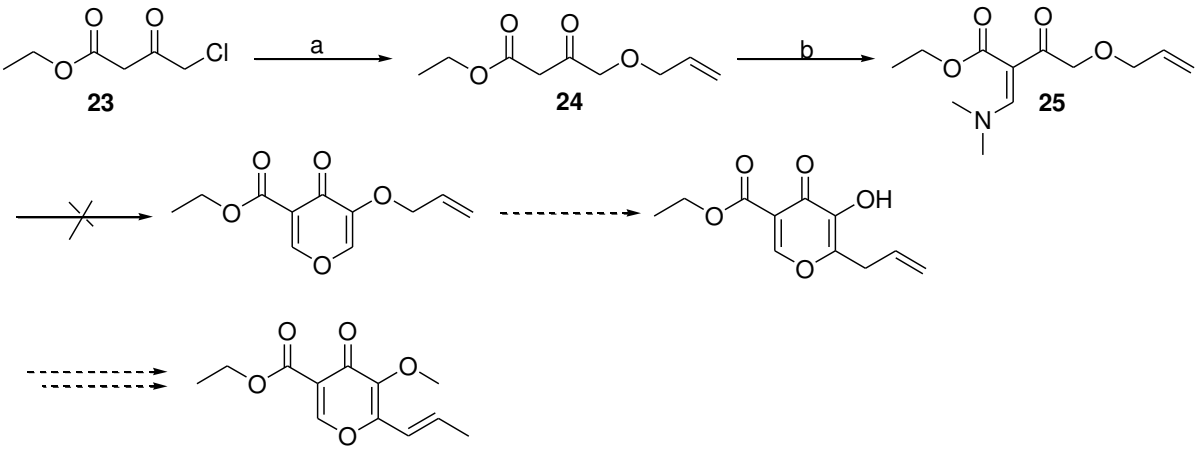
Scheme 6.4



6.5 Approach towards 3-OMF based on Suzuki coupling

In order to construct the γ-pyrone, we started from commercially available ethyl 4-chloroacetoacetate. Ethyl 4-(allyloxy)-3-oxobutanoate **24** was obtained in 78% yield[8] from **23** by using allyl alcohol and NaH. The enaminone **25** was synthesised in good yield by using DMF-DMA at RT[9]. Further cyclisation of enaminone **25** to γ-pyrone was failed.

Scheme 6.5

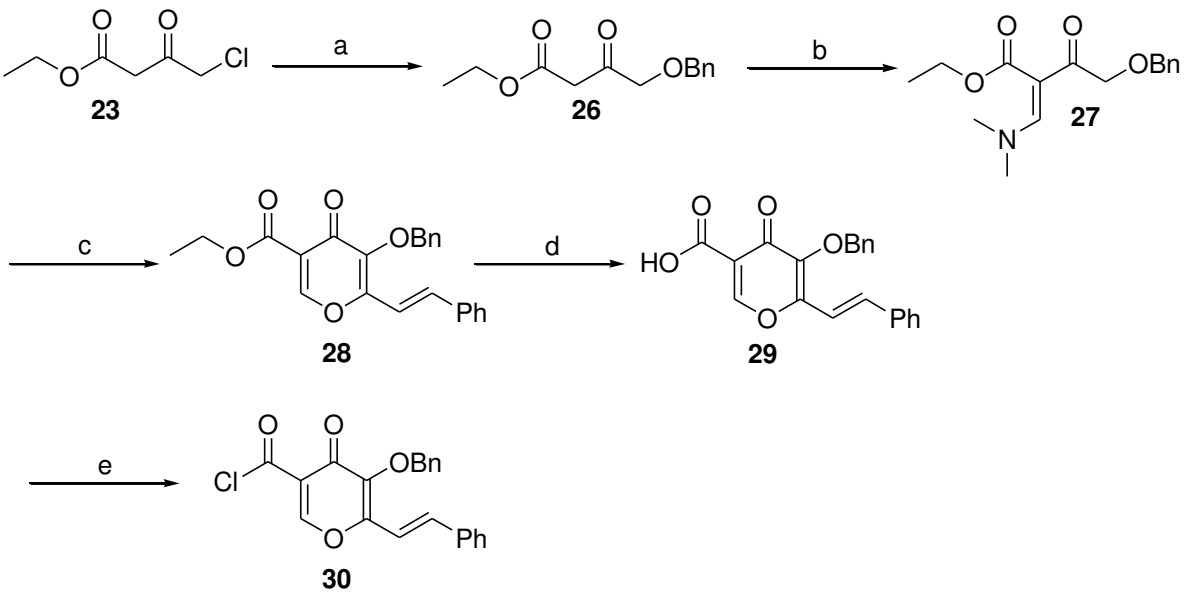


Reagents and conditions:

a) Allyl alcohol, NaH, THF, 0°C to RT, 16h, 78% b) DMF.DMA, RT,16h, 90%

Ethyl 4-(benzyloxy)-3-oxobutanoate **26** was synthesised from **23** by using BnOH and NaH in 70% yield[10]. Subsequently, **26** was reacted with DMF-DMA to obtain enaminone **27** in 91% yield. Compound **27** was cyclised by using cinnamoyl chloride and LiHMDS at -78^{o}C to give γ-pyrone moiety **28**. The ester of **28** was hydrolysed in basic condition in EtOH:water to afford acid **29**. Further acid chloride **30** was synthesised from acid **29** by using oxalyl chloride and cat.DMF at 0^{o}C in 63%.

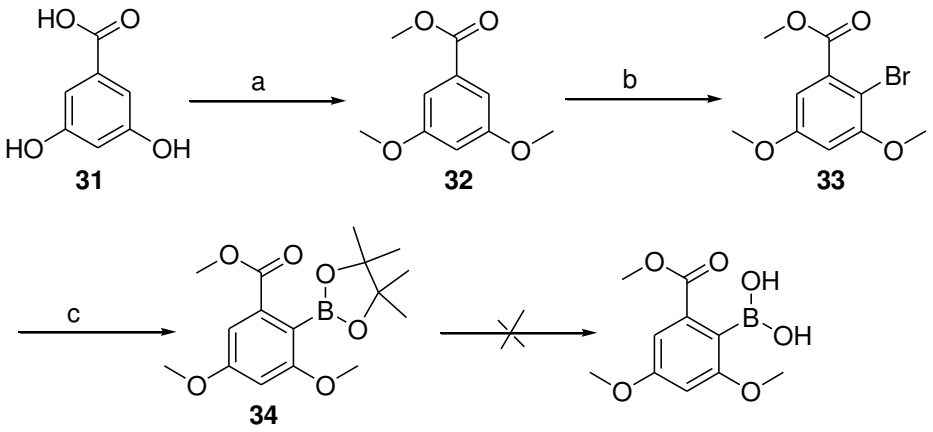
Scheme 6.6



Reagents and conditions:

a) BnOH, NaH, THF, 0°C to RT, 16h, 70% b) DMF.DMA, RT,16h, 91% c) Cinnamoyl chloride, LiHMDS, -78°C to RT , 1h, 60% d) NaOH, EtOH:Water, 0°C to RT,72% d) Oxalyl chloride, DMF, DCM, 0°C to RT,63%

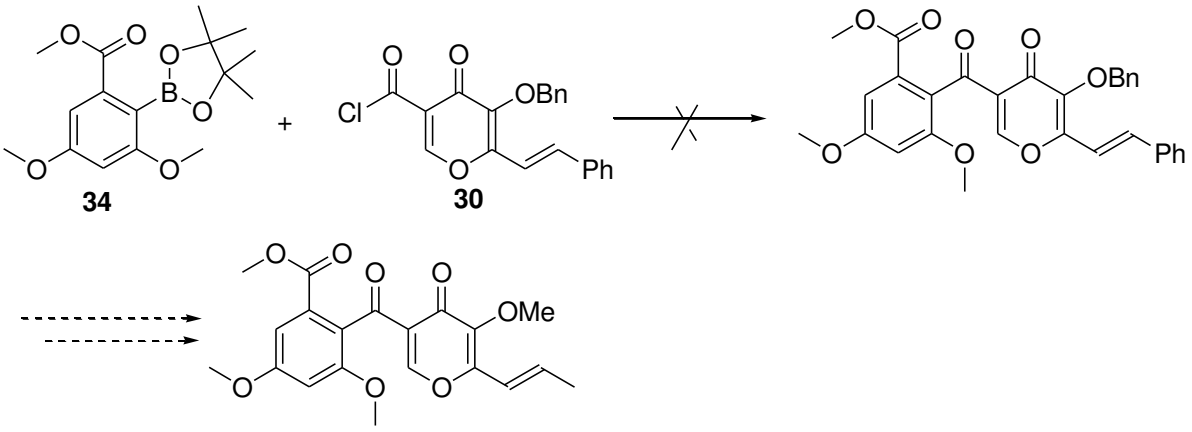
After developing a successful procedure for acid chloride of γ-pyrone moiety, we have synthesised the aromatic boronate ester. Methyl 3,5-dimethoxybenzoate **32** was prepared from 3,5-dihydroxybenzoic acid **31** by using dimethyl sulphate and K_2CO_3 in 98% yield[11].The regioselective bromination of **31** was carried out by using NBS in ACN in 75%[12]. Aryl boronate ester **34** was synthesised by Miyaura boraylation reaction in 67%[13]. The hydrolysis of boronate ester to boronic acid under different conditions failed.



Reagents and conditions:

a) Dimethyl sulphate, K_2CO_3, acetone, reflux, 4h, 98% b) NBS, ACN, 0°C to RT, 24h, 75%

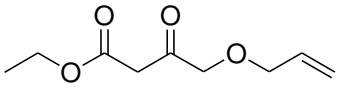
c) Bis(pinacolato)diborane, K_3PO_4, $(dppf)_2PdCl_2$.dcm, dioxane, reflux, 12h, 67%.



A few attempts to couple boronate **34** and acid chloride **30** failed as well. Now work is in progress to find the conditions for Suzuki coupling of the two key fragments obtained.

6.6 Experimental

Ethyl 4-(allyloxy)-3-oxobutanoate



24

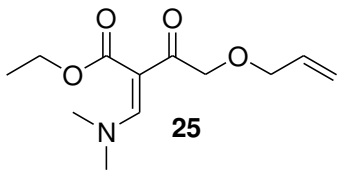
A solution of allyl alcohol (3.3 mL, 47 mmol) in THF (17 mL) was added to a stirred suspension of sodium hydride (60% dispersion in oil; 3.8 g, 94 mmol) in THF (33 mL) at 0°C, and the resulting mixture was stirred at 0°C for 1 h, then at reflux for 1 h, then cooled to 0°C. A solution of ethyl 4-chloroacetoacetate (4.94 mL, 36.7 mmol) in THF (17 mL) was added dropwise over 2 min, and the resulting mixture was allowed to warm to room temperature with stirring over 16 h. The mixture was acidified to pH 2 by dropwise addition of hydrochloric acid (1 M). Water (50 mL) and ethyl acetate (50 mL) were added. The aqueous phase was further extracted with ethyl acetate (3 × 50 mL) and the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The crude product was purified by flash column chromatography (10% EtOAc/Hexane) to afford **24** (5.32 g, 78%)

Physical appearance Pale yellow liquid

R_f 0.5(EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 5.88 (1H, m); 5.28 (2H, m); 4.30-4.00 (6H, m); 3.65 (2H, m); 1.27 (3H, t, J = 7.2 Hz).

(Z)-Ethyl 4-(allyloxy)-2-((dimethylamino)methylene)-3-oxobutanoate



25

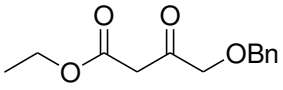
A mixture of N, N-Dimethylformamide dimethyl acetal (DMF-DMA) (0.153 g, 1.288 mmol) and ethyl 4-(allyloxy)-3-oxobutanoate **24** (0.2 g, 1.073 mmol) was allowed to stir for 24h at rt. After the completion of reaction, crude was purified by column chromatography (90% EtOAc/Hexane) afforded **25** (0.207 g, 90%)

Physical appearance Yellow viscous liquid

R_f 0.5(EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.71 (1H, s); 5.92 (1H, m); 5.40-5.12 (2H, m); 4.42 (2H, s); 4.19 (2H, q, J = 7.3 Hz); 4.04 (2H, d, J = 5.5 Hz); 3.25 (3H, brs); 2.90 (3H, brs); 1.30 (3H, t, J = 7.3 Hz).

Ethyl 4-(benzyloxy)-3-oxobutanoate



26

Benzyl alcohol (0.722 g, 6.683 mmol) was added dropwise to a stirred suspension of 60% sodium hydride (0.65 g, 13.36 mmol) in THF (20 mL); occasional cooling was required with an ice bath to maintain ambient temperature. After hydrogen evolution ceased, the thick slurry was allowed to stir for 2 h. Ethyl 4-chloroacetoacetate (1.00 g, 6.075 mmol) was then added dropwise within 3 h, and the reaction mixture was stirred for 16 h. The reaction mixture was carefully added into 5% HCl solution (200 mL) at 5 °C and extracted with EtOAc (100 mL × 3). The organic layers were washed with saturated $NaHCO_3$ (60 mL x 2) and then saturated NaCl (60 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (10% EtOAc/Hexane)

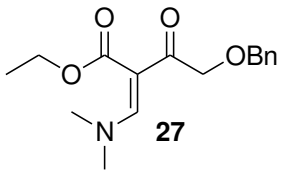
to give **26** (1.02 g, 70%)

Physical appearance Yellow liquid

R_f 0.4 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 3.54 (2H, s), 4.14 (2H, s), 4.17 (2H, q, J=7.2 Hz), 4.59 (2H, s), 7.28-7.40 (5H, m).

(Z)-Ethyl 4-(benzyloxy)-2-((dimethylamino)methylene)-3-oxobutanoate



27

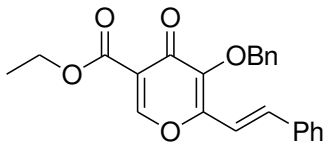
A mixture of N, N-Dimethylformamide dimethyl acetal (DMF-DMA) (0.32 g, 2.53 mmol) and ethyl 4-(benzyloxy)-3-oxobutanoate **26** (0.50 g, 2.11 mmol) was allowed to stir for 24h at rt. After the completion of reaction, the crude was purified by column chromatography (90% EtOAc/Hexane) to give **27** (0.53 g, 91%)

Physical appearance Yellow liquid

R_f 0.5 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.74 (1H, s); 7.42-7.24 (5H, m); 4.60 (2H, s); 4.47 (2H, s); 4.16 (2H, q, J = 7.2 Hz); 3.25 (3H, brs); 2.92 (3H, brs); 1.26 (3H, t, J = 7.2 Hz).

Ethyl 5-(benzyloxy)-4-oxo-6-styryl-4H-pyran-3-carboxylate



28

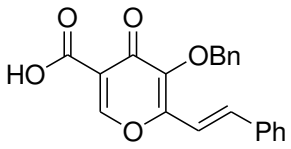
A 1N LiHMDS in THF (0.858 mL, 0.85mmol) solution was cooled to -78^{o}C and a solution of compound **27** (0.10 g, 0.343 mmol) and cinnamoyl chloride (0.068 g, 0.411mmol) in THF was added dropwise over 10 min while same temperature was retained. The reaction mixture was stirred for 45 min at -78^{o}C and then quenched with 2N HCl at 0^{o}C, stirred for 15min, extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography (18% EtOAc/Hexane) to afford **28** (0.077 g, 60 %)

Physical appearance White solid

R_f 0.5 (EtOAc/Hexane 3:7)

^{1}H NMR ($CDCl_3$) δ: 8.44 (1H, s); 7.60-6.90 (6H, m); 6.45 (1H, d, J = 16.2 Hz); 5.27 (2H, s); 4.38 (2H, q, J = 7.2 Hz); 1.38 (3H, t, J = 7.2 Hz).

5-(Benzyloxy)-4-oxo-6-styryl-4H-pyran-3-carboxylic acid



29

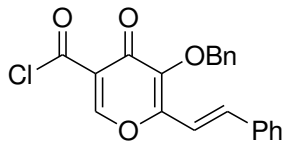
To the stirred solution of compound **28** (0.75 g, 1.99 mmol) in EtOH:Water(3:1) was added NaOH (0.12 g, 2.98 mmol) at 0^{o}C, then the solution was allowed stir for 4h at rt. After the completion of reaction, the mixture was concentrated in vacuo. The crude was acidified with 2N HCl at 0^{o}C, extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give acid **29** (0.5 g, 72%)

Physical appearance Yellow solid

R_f 0.2 (MeOH/DCM 1:9)

^{1}H NMR ($CDCl_3$) δ: 9.78 (1H, s); 7.81-6.80 (7H, m); 5.02 (2H, s).

5-(Benzyloxy)-4-oxo-6-styryl-4H-pyran-3-carbonyl chloride



30

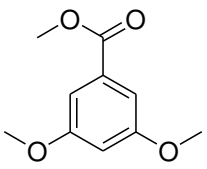
To the stirred solution of **29** (0.06 g, 0.17mmol) in dry DCM (3 mL) was added oxalyl chloride (0.043 g, 0.344 mmol) and cat. DMF (1drop) at 0^{o}C, then the reaction mixture was allowed to stir for 5h at rt. After the completion of reaction, the mixture was concentrated in vacuo. The crude product was purified by silica gel chromatography (10% EtOAc/Hexane) to give **26** (0.04 g, 63%)

Physical appearance Yellow viscous liquid

R_f 0.5 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.54-7.32 (7H, m); 6.80 (1H, d, J = 15.8 Hz); 5.00 (2H, s).

Methyl 3,5-dimethoxybenzoate



32

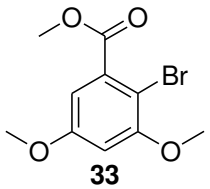
To 3,5-dihydroxybenzoic acid **31** (15.4 g, 0.1 mol) dissolved in acetone (150 mL) was added potassium carbonate (42.8 g, 0.3 mol, 3.1 equiv.) followed by dimethyl sulphate (19 mL, 2 equiv.). The mixture was then heated to reflux for 4 h with vigorous stirring. Upon cooling to room temperature, water was added so as to completely dissolve all the potassium carbonate and the mixture extracted with diethyl ether (3 x 30 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford **32** (16.58 g, 98%)

Physical appearance White solid

R_f 0.5 (EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 7.17 (2H, d, J = 2.4 Hz,), 6.63 (1H, t, J = 2.4 Hz,), 3.88 (3H, s), 3.81 (6H, s)

Methyl 2-bromo-3,5-dimethoxybenzoate



33

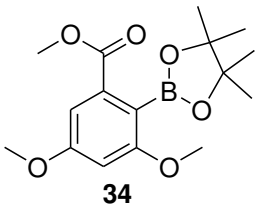
Methyl 3,5-dimethoxybenzoate **32** (0.115 g, 0.586 mmol) in acetonitrile (3 mL) was treated with N-bromosuccinimide (0.125 g, 0.703 mmol) at 0°C and the solution was stirred at room temperature for 24h. After removal of the solvent, the residue was purified by column chromatography (PE/Et_2O, 2:1), yielding the desired compound **33** (0.12 g, 75%)

Physical appearance White solid

R_f 0.3(EtOAc/Hexane 1:9)

^{1}H NMR ($CDCl_3$) δ: 6.82 (1H, d, J = 2.7 Hz); 6.60 (1H, d, J = 2.7 Hz); 3.95 (3H, s); 3.91 (3H, s); 3.84 (3H, s).

Methyl 3,5-dimethoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate



34

The aromatic bromide (0.1 g, 0.363 mmol) was dissolved in dioxane (5 mL) and cooled to -78°C and vacuum degassed and back filled with nitrogen. To this degassed reaction mixture were added bis(pinacolato)diborane (0.12 g, 0.472 mmol), K_3PO_4 (0.23 g, 1.090 mmol) and $(dppf)_2PdCl2$. DCM (0.015 g, 0.0181 mmol). The reaction mixture was refluxed overnight under nitrogen. After the completion of reaction, the mixture was filtered through a celite bed and washed with EtOAc. The organic layer was washed water and brine solution. The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography (20% EtOAc/Hexane) to give **34** (0.09 g, 67%)

Physical appearance White solid

R_f 0.6 (EtOAc/Hexane 2:8)

^{1}H NMR ($CDCl_3$) δ: 7.10 (1H, d, J = 1.8 Hz); 6.58 (1H, d, J = 1.8 Hz); 3.91 (3H, s); 3.84 (3H, s); 3.79 (3H, s); 1.43 (12H, s).

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CHAPTER 7

Conclusions

1. The first total synthesis of the natural compound Leopolic acid A was designed and carried out. The synthesis of Leopolic Acid A has been achieved starting from commercially available amino acids L-Valine and L-Phenyl alanine in 11 steps.

2. During the synthesis of the natural compound Leopolic acid A an oxazolidone analogue of Leopolic acid A was also synthesized. This core structure is new and a survey of the literature showed that compounds with this scaffold have never been isolated or synthesized before. Studies to evaluate its biological activity are underway.

3. A series of compounds containing the 2, 3-pyrrolidinedione skeleton were prepared, considering a combination of substituents at different positions to have a large variety of structurally diverse molecules. The antifungal and antimicrobial activity of these compounds were evaluated and preliminary structure-activity relationship (SAR) have emerged.

4. An approach towards total synthesis of the positional isomer of natural compound Leopolic acid A was developed.

5. Efforts were directed towards the total synthesis of natural compound 3-O-Methylfunicone.