Synthesis of Two Bromotyrosine Derived Natural Alkaloids and Analogues, Total Synthesis of Mutisianthol and Enzyme-catalysed Resolution of Fine Chemicals and Intermediates in the Presence of Phosphorus Ionic Liquids

A THESIS

SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)

TO THE

UNIVERSITY OF PUNE

BY

SULAKE ROHIDAS SHIVAJI

UNDER THE GUIDANCE OF

Dr. BHANU M. CHANDA

DIVISION OF ORGANIC CHEMISTRY
NATIONAL CHEMICAL LABORATORY
PUNE - 411 008
INDIA

February 2009

DEDICATED
TO MY BELOVED
PARENTS

D E C L A R A T I O N

The research work presented in this thesis entitled **"Synthesis of Two Bromotyrosine Derived Natural Alkaloids and Analogues, Total Synthesis of Mutisianthol and Enzyme-catalysed Resolution of Fine Chemicals and Intermediates in the Presence of Phosphorus Ionic Liquids"** and being submitted for the degree of Doctor of Philosophy in Chemistry to the University of Pune, India has been carried out under the supervision of **Dr. Bhanu M. Chanda** at Division of Organic Chemistry, National Chemical Laboratory, Pune- 411 008. The work presented here is original and has not been submitted in part or in full by me for any other degree or diploma of this or any other university.

Date: February 2009

Sulake Rohidas Shivaji
Division of Organic Chemistry,
National Chemical Laboratory.
Pune - 411 008.

NATIONAL CHEMICAL LABORATORY

Dr. Homi Bhabha Road, PUNE - 411 008(INDIA).

Dr.(Mrs.) Bhanu M. Chanda
Ex-Scientist G

Telephone(Res): + 91-20-25898567
E-mail: bhanuchanda@yahoo.com

C E R T I F I C A T E

This is to certify that the work presented in this thesis entitled "**Synthesis of Two Bromotyrosine Derived Natural Alkaloids and Analogues, Total Synthesis of Mutisianthol and Enzyme-catalysed Resolution of Fine Chemicals and Intermediates in the Presence of Phosphorus Ionic Liquids**" and being submitted to the University of Pune by **Mr. Sulake Rohidas S**. has been carried out by the candidate at National Chemical Laboratory, Pune, under my supervision. The work presented is original and has not been submitted for any other degree or diploma of this or any other University. Whenever references have been made to previous works of others it has been clearly indicated as such and included in the bibliography.

Pune - 411 008
Date:

Dr. Bhanu M. Chanda
(Research Guide)

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A c k n o w l e d g e m e n t s

*It gives me immense pleasure to express my deep sense of gratitude to my teacher and research guide **Dr. Bhanu M. Chanda**, who has helped me a lot to learn and think more about chemistry. I thank her for an excellent and inspiring guidance, constant encouragement, sincere advice and unstinted support during all the times of my Ph.D. work. Working with her was a great pleasure and learning experience.*

I wholeheartedly appreciate the support and help extended by Drs H. B. Borate, D. D. Sawaikar, Hotha Srinivas, S. P. Chavan, U. R. Kalkote, R. A. Joshi, and M. N. Deshmukh and Mr. I. Shivakumar, throughout my stay at NCL.

I could interact effectively with a large scientific workforce at NCL and Dept. of Chemistry, University of Pune. Although it is not possible to name each of them, these interactions have undoubtedly augmented my professional and personal life in one or the other way. I wholeheartedly thank all of them for their help and time. I extend my thanks to the technical and office staff of NCL for their co-operation. Help from spectroscopy, comprising IR, NMR, mass and related groups are gratefully acknowledged.

The warm and friendly attitude of my colleagues at NCL Pune helped to have a cheerful atmosphere in the laboratory. I extend my deep sense of gratitude towards them.

Whatever I am and whatever I will be in future is because of the goodwill, unstinted support that I have received from my parents, brothers, and sister with whom I can share my delights and worries. Thank you for your enormous support in materializing this work into a reality. Their kind cooperation helped me in pursuing my PhD work and no words are enough to acknowledge them. Their constant encouragement, sacrifice and support made me achieve the goal.

*I am grateful to my wife **Bharati** for her patience, understanding and being extremely encouraging during these years. I would like to thank her for taking care of our daughter **Pratibha** who is also the source of inspiration in my endeavors.*

Thanks are also expressed to Dr. Al Robertson of CYTEC, Canada and Dr. A. Ramani for gift of ionic liquids and useful discussions.

My sincere thanks to Dr. M. K. Gurjar Ex. Head, and Dr. G. Pandey, Head, Division of Organic Chemistry for their cooperation and support. Finally I thank Director, NCL, Pune for providing infrastructural facilities and CSIR, New Delhi for the financial assistance in the form of a fellowship.

Sulake Rohidas Shivaji

ABBREVIATIONS

Ac	-	Acetyl
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
BINAP	-	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
Bn	-	Benzyl
BnBr	-	Benzyl bromide
Boc	-	*tert*-Butoxy carbonyl
$(Boc)_2O$	-	Di-*tert*-butyl dicarbonate
CSA	-	Camphorsulfonic acid
DCC	-	Dicyclohexylcarbodiimide
DIBAL-H	-	Diisobutylaluminium hydride
DIPEA	-	Diisopropylethylamine
DMA	-	*N*,*N*-Dimethylacetamide
DMAP	-	*N*,*N*-Dimethylaminopyridine
DMF	-	*N*,*N*-Dimethylformamide
DMS	-	Dimethyl sulfate
DMSO	-	Dimethyl sulfoxide
EDCI	-	1-(3-Dimethylaminopropyl)-3-ethylcarbo-diimide hydrochloride
Et	-	Ethyl
EtOAc	-	Ethyl acetate
Et_3N	-	Triethyl amine
Et_2O	-	Diethyl ether
g	-	gram
h	-	hours
HMPA	-	Hexamethylphosphoramide
HOBt	-	1-Hydroxybenzotriazole hydrate
K_2CO_3	-	Potassium carbonate
LAH	-	Lithium aluminium hydride
MCA	-	Mycothiol *S*-conjugate amidase

mCPBA	-	*meta*-Chloroperoxybenzoic acid
MEK	-	Methyl ethyl ketone
MeOH	-	Methanol
mL	-	millilitre
mmol	-	millimole
MsCl	-	Methanesulfonyl chloride
Me	-	Methyl
MOM	-	Methoxymethyl
$NaIO_4$	-	Sodium metaperiodate
NBS	-	*N*-Bromosuccinimide
NMO	-	*N*-Methylmorpholine-*N*-Oxide
NMR	-	Nuclear Magnetic Resonance
Pd/C	-	Palladium on Carbon
Ph	-	Phenyl
PIDA	-	Phenyliodine(III) diacetate
PIFA	-	Phenyliodine(III) bis(trifluoroacetate)
Py	-	Pyridine
p-TSA	-	*para*-Toluenesulfonic acid
PMB	-	*para*-Methoxybenzyl
rt	-	room temperature
TBAF	-	Tetra-*n*-butylammonium fluoride
TEA	-	Triethylamine
TFA	-	Trifluoroacetic acid
TFAA	-	Trifluoroacetic anhydride
THF	-	Tetrahydrofuran
TMOF	-	Trimethylorthoformate
TPP	-	Triphenyl phosphine
Ts	-	Tosyl
TsCl	-	*para*-Toluenesulphonyl chloride
TTN	-	Thallium trinitrate

General Remarks

- Melting points were recorded on Buchi 535 melting point apparatus and are uncorrected.
- Optical rotations were measured with a JASCO DIP 370 digital polarimeter.
- Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and are measured in cm^{-1}.
- ^{1}H Nuclear Magnetic Resonance spectra were recorded on Varian FT-200 MHz (Gemini), AC-200 MHz, MSL-300 MHz, AV-400 MHz and Bruker-500 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C Nuclear Magnetic Resonance spectra were recorded on AC-50 MHz, MSL-75 MHz, AV-100 MHz and Bruker-125 MHz spectrometer.
- Mass spectra were recorded on a CEC-21-110B, AP-1 QSTAR PULSAR, Finnigan Mat 1210 or MICRO MASS 7070 spectrometer at 70 eV using a direct inlet system.
- All reactions were monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV, I_2 and anisaldehyde reagent in ethanol as development reagents.
- All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 50 °C.
- All solvents and reagents were purified and dried according to procedures given in Vogel's Text Book of Practical Organic Chemistry.
- Silica gel (60-120 & 230-400 mesh) used for column chromatography was purchased from Spectrochem Company, Mumbai, India.
- Molecular weights of the compounds and *m/z* values in the mass spectra are corrected to nearest integers.

ABSTRACT

The thesis entitled **"Synthesis of Two Bromotyrosine Derived Natural Alkaloids and Analogues, Total Synthesis of Mutisianthol and Enzyme-catalysed Resolution of Fine Chemicals and Intermediates in the Presence of Phosphorus Ionic Liquids"** consists of three chapters. Chapter 1 describes approaches towards the total synthesis of a spiroisoxazoline derived natural alkaloid. It includes the synthesis of three units of the target molecule and attempted coupling of these parts to realise the target molecule. Chapter 2 is divided into two sections; Section I describes an expeditious convergent synthesis of a dibromotyrosine alkaloid inhibitor of mycothiol-*S*-conjugate amidase whereas Section II deals with preparation of analogues of dibromotyrosine alkaloids. Chapter 3 is divided into two sections; Section I describes total synthesis of Mutisianthol, whereas Section II describes enzyme-catalysed resolution of 1-phenylethanol *via* transesterification in the presence of phosphorus ionic liquids.

Chapter 1: Approaches towards the total synthesis of a spiroisoxazoline derived natural alkaloid

In 2001 Bewley *et al* reported the isolation of novel dibromotyrosine alkaloids from an Australian non-verongid sponge of the *Oceanpia* species. In recent years, *Mycobacterium tuberculosis* has re-emerged as a leading cause of death and the appearance of drug resistant strains continues to rise. Related mycobacterial species, such as *Mycobacterium avium* and *M. avium* complex, that are otherwise non-deleterious to human, pose serious threats to immuno compromised people. Consequently, there is a continuing need for the discovery of new antituberculars with novel modes of action.

In an effort to identify new classes of antimycobacterials, a variety of marine extracts were screened for their ability to inhibit a novel mycobacterial enzyme, mycothiol-*S*-conjugate amidase (MCA) by Bewley *et al*. Compound **1** was isolated from an Australian non-verongid sponge of the *Oceanapia* species and is the most effective inhibitor of MCA. It was therefore found desirable and appropriate to synthesise this compound.

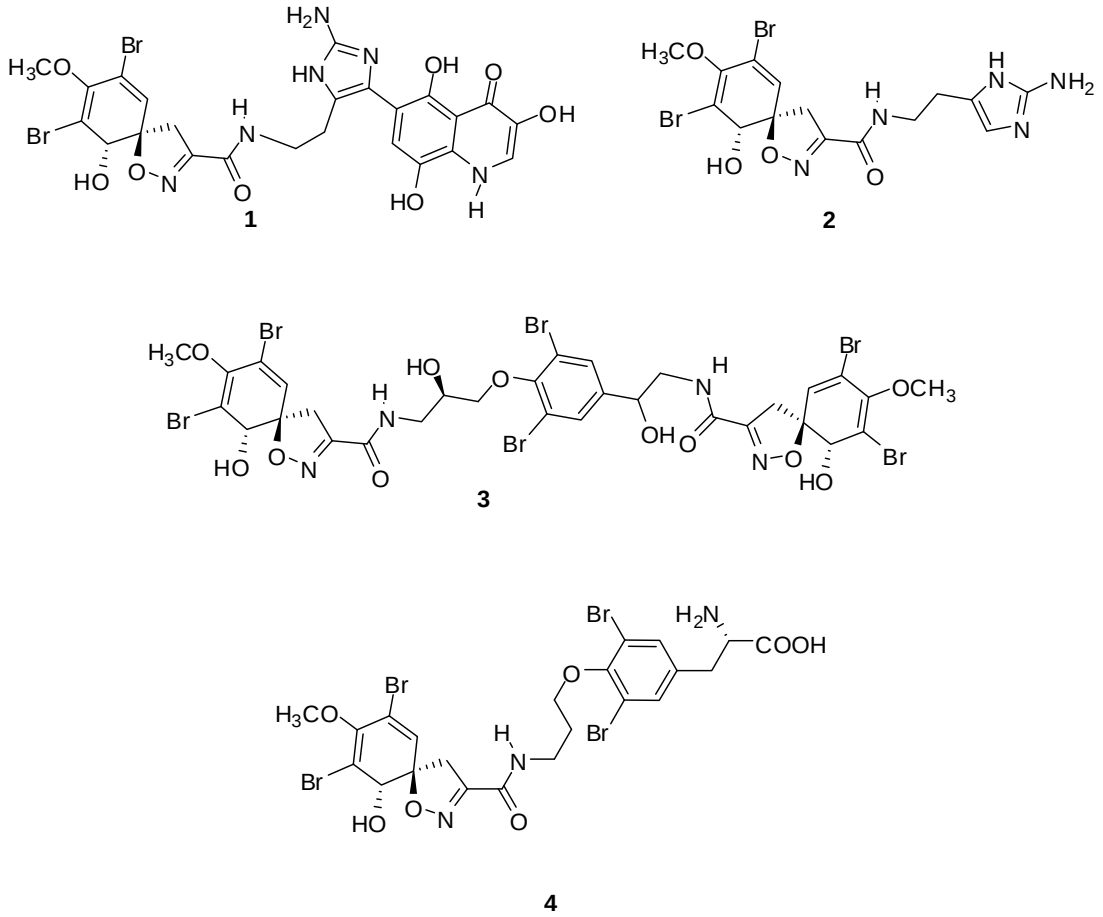
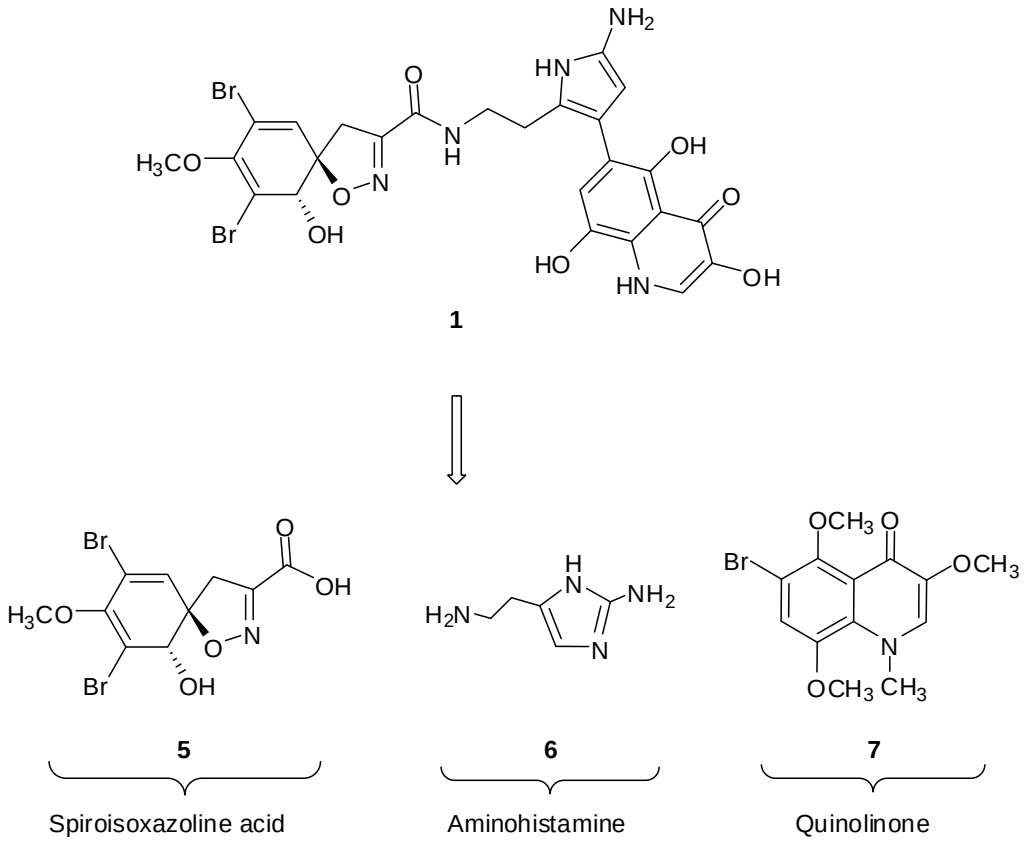


Figure1: Structures of spiroisoxazoline derived alkaloids

Palladium catalyzed coupling reactions (Stille coupling, Negishi coupling, Heck coupling, Suzuki coupling) are widely used for carbon-carbon bond formation in the synthesis of complex molecules since they are usually performed under mild conditions and provide excellent selectivities. In the present work, design of retrosynthesis was based on palladium catalyzed coupling reactions. According to retrosynthetic analysis shown in **figure 2**, molecule **1** can be derived from three fragments: left side spiroisoxazoline acid **5**, a middle aminohistamine spacer **6** and a right side quinolinone **7**. Cleavage of bond between spiroisoxazoline acid and aminohistamine and bond between aminohistamine and quinolinone provides three fragments. In the synthetic sense fragments **5** and **6** would be united by acid-amine coupling and fragments **6** and **7** would be united by C-C coupling (Heck, Suzuki).

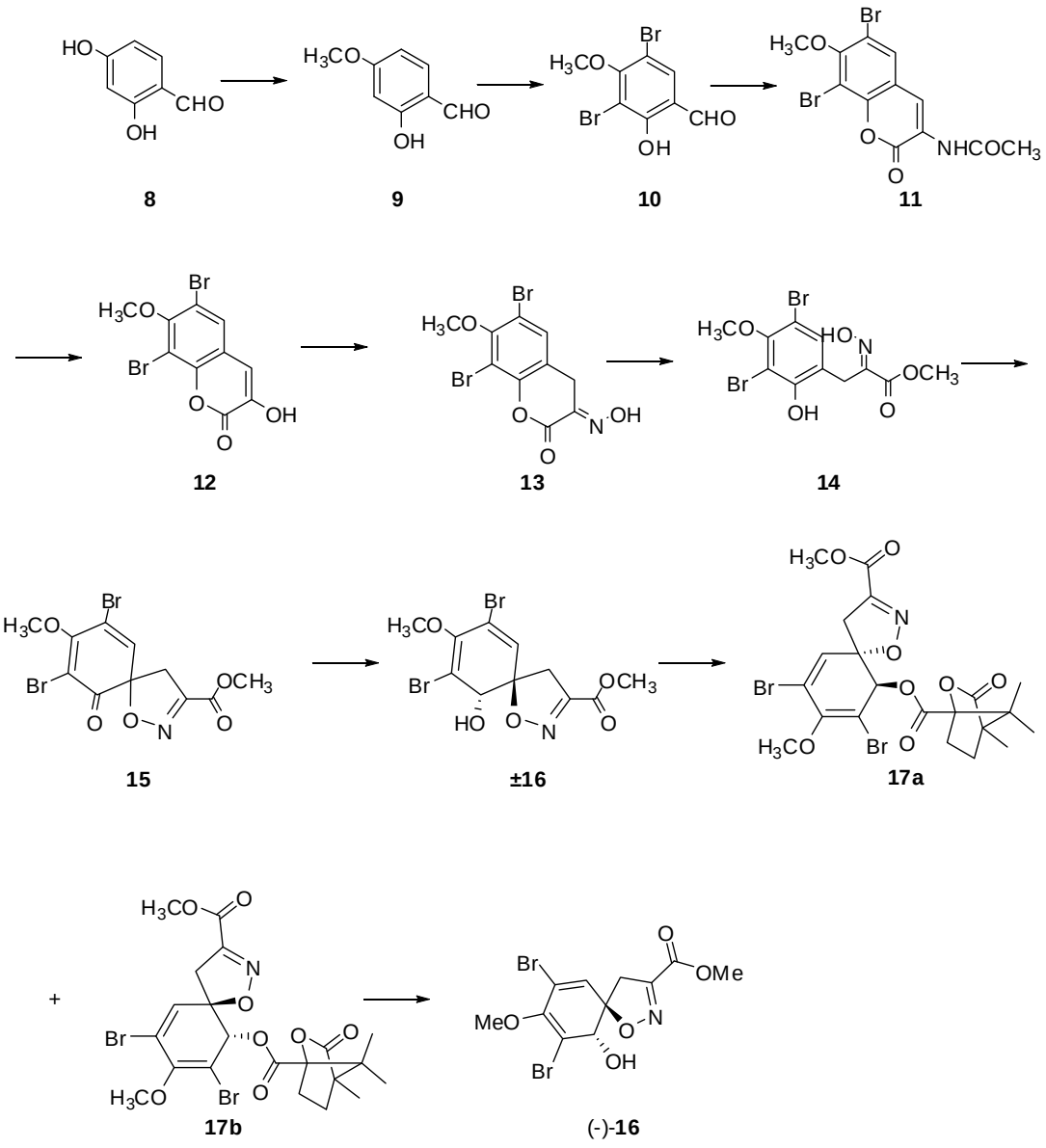


Scheme 1: Retrosynthetic analysis

Synthesis of spiroisoxazoline

The spiroisoxazoline methyl ester was synthesized according to Hoshino's approach. Commercially available 2,4-dihydroxybenzaldehyde **8** was selectively methylated and then brominated to dibromosalicylaldehyde **10**. During azlactone preparation under Erlenmeyer conditions from benzaldehyde **10**, acetamido coumarin **11** was obtained which on hydrolysis followed by oximation furnished oximino coumarin **13**. Opening of coumarin **13** with sodium methoxide followed by oxidation with NBS in DMF, (diacetoxyiodo)benzene or with polymer supported (diacetoxyiodo)benzene (PSDIB) reagent in acetonitrile proceeded smoothly to afford spiroisoxazoline methyl ester **15.** Spiroisoxazoline methyl ester **15** was reduced with $Zn(BH_4)_2$ (Yamamura's method) to give cyclohexadienylisoxazoline **16**. Synthesis of the optically active spiroisoxazoline unit was achieved by coupling reaction of ester **16** with (-)-camphanic chloride which gave the chromatographically separable diastereomeric mixture of **17a** and **17b**. The chiral intermediate formed was

deprotected by hydrolysis with K_2CO_3 in MeOH to furnish pure spiroisoxazoline methyl ester (-)-**16.**

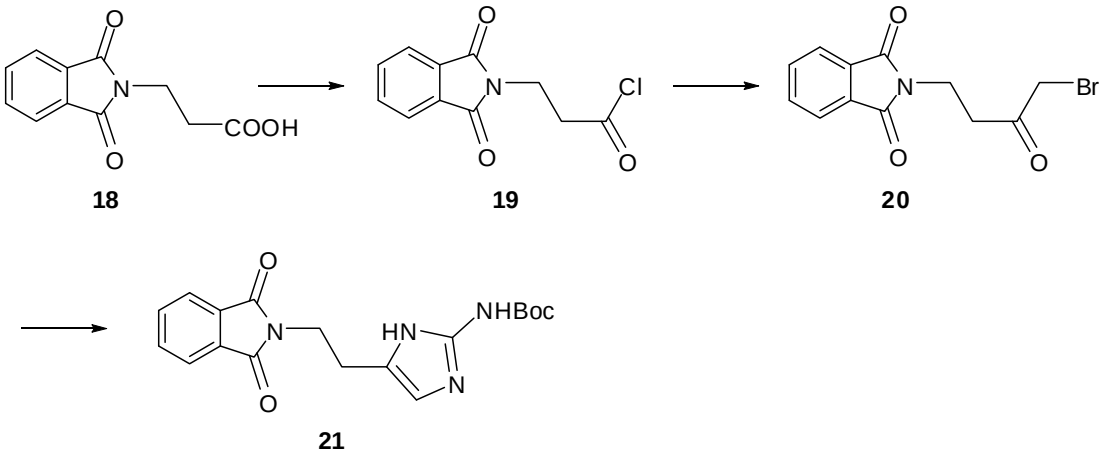


Scheme 2: Synthesis of spiroisoxazoline part

Synthesis of aminohistamine

Aminohistamine part **21** was synthesised starting from commercially available β-alanine. Amino group of β-alanine was protected with phthalic anhydride to give protected acid **18** which was converted to acyl chloride **19** with thionyl chloride. The acyl chloride **19** was treated with diazomethane and subsequent quenching by HBr

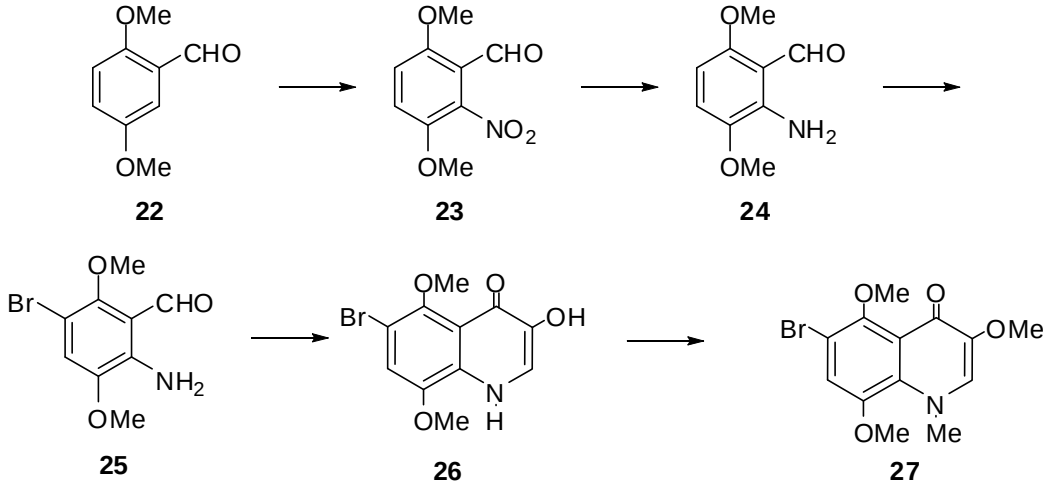
gave α-bromo keto compound **20**. This keto compound was condensed with Boc-protected guanidine to furnish Boc-protected aminohistamine **21** (Scheme 3).



Scheme 3: Synthesis of aminohistamine part

Synthesis of Quinolinone part

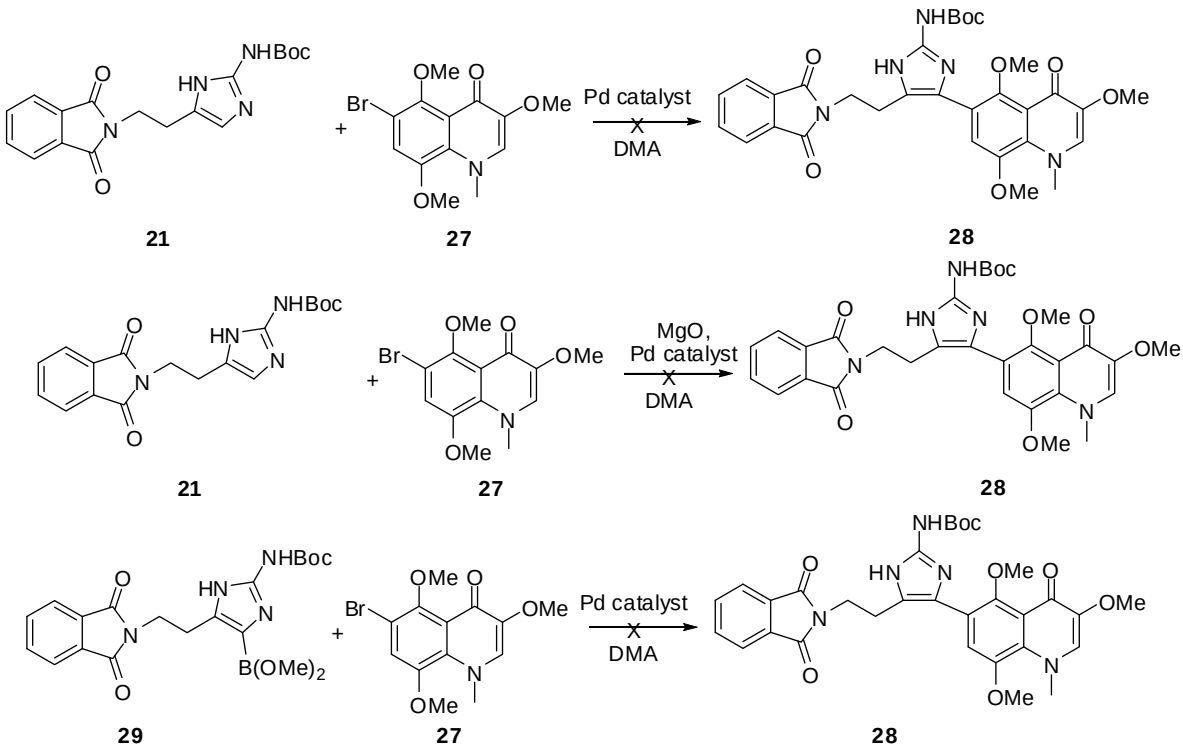
Preparation of the third quinolinone fragment **27** (Scheme 4) was effected from commercially available 2,5-dimethoxybenzaldehyde **22**. The latter was nitrated; subsequent reduction of nitro group afforded substituted aniline **24**. Bromination followed by benzoin condensation of *ortho*-aminobenzaldehyde **25** with glyoxalbisulfite furnished the quinolinone **26** which upon methylation yielded the required fragment **27.**



Scheme 4: Synthesis of quinolinone part

Coupling of fragments

Coupling of fragment **21** with **27** in the presence of tetrakistriphenylphosphine palladium, $Pd(OAc)_2$, $PdCl_2$ or Pd (0) as catalysts did not yield the expected product **28**; changes in either solvent or protection of amino group did not afford the desired product. In order to increase the nucleophilicity of carbon to prevent the reactivity of amino group, magnesium salt of histamine **21** was prepared and the reaction carried out. However, the desired coupling reaction did not take place under these conditions also and the starting materials were recovered. It was then decided to apply Suzuki reaction for coupling, for which compound **21** was brominated and corresponding boronic ester **29** was prepared. However, a highly polar product, practically insoluble in all common organic solvents was isolated which could not be characterised. Hence, this approach was abandoned.



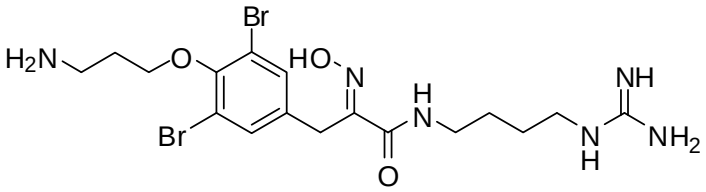
Scheme 5

In conclusion, during the total synthesis of the unusual dibromotyrosine based marine natural product **1**, the three important and interesting segments in their protected form have been successfully synthesised. Efforts are underway to achieve the coupling of these fragments to yield the target compound.

Chapter 2

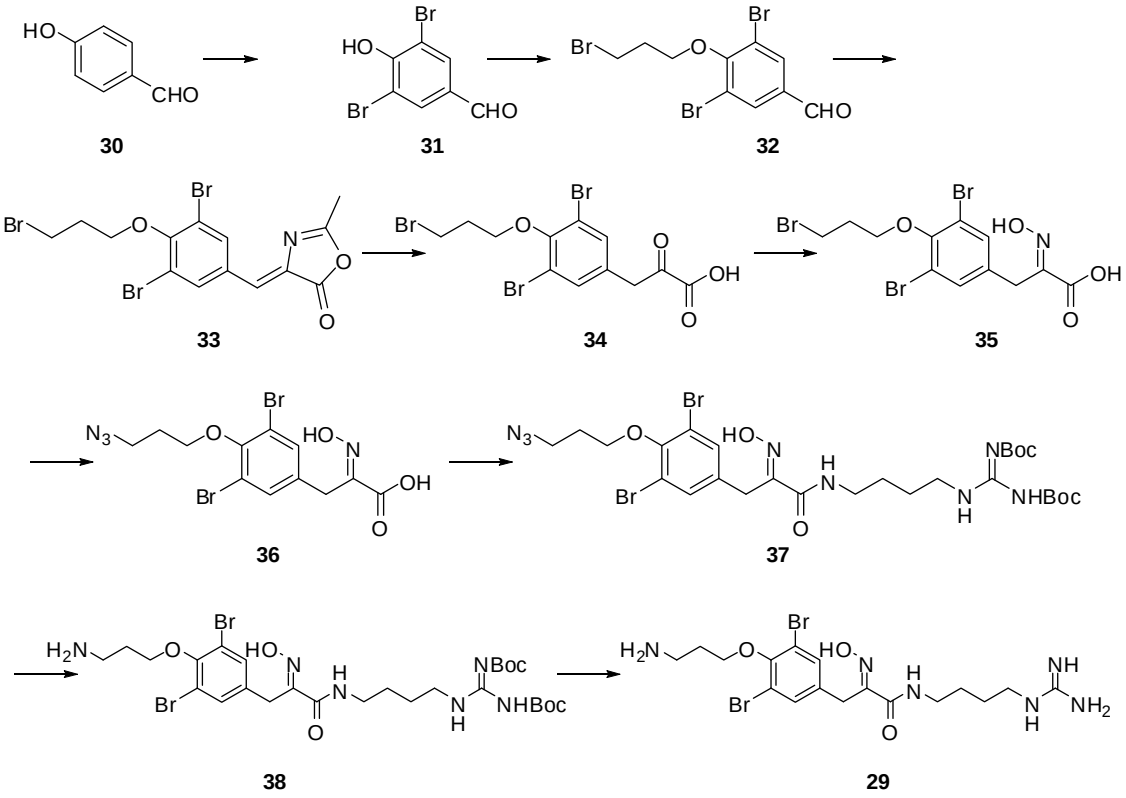
Section I: An expeditious convergent synthesis of a dibromotyrosine alkaloid inhibitor of mycothiol-*S*-conjugate amidase

Four novel alkaloids isolated from an Australian non-verongid sponge of the *Oceanapia* species exhibited significant inhibitory activity against mycothiol-*S*-conjugate amidase (MCA). Due to the crucial role played by MCA, such alkaloids are marked as potentially useful therapeutic agents against *Mycobacterium tuberculosis* and related pathogens. Of the various structures studied, kinetics experiments established that the bromotyrosine-derived alkaloid, such as **29**, is the most active inhibitor of the group and is also competitive inhibitor of the *M. tuberculosis* detoxification enzyme MCA. Motivated by these findings, the synthesis of **29** was undertaken and reported here.



29

Synthesis of **29** was initiated from commercially available 4-hydroxybenzaldehyde **30**. Erlenmeyer approach was used for the synthesis of azlactone **33** from dibromobenzaldehyde **32**. Hydrolysis of azlactone **33** yielded the pyruvic acid **34** which was subjected to oximation to afford oxime **35**. Azidation of bromo derivative **35** gave the corresponding azide **36**. Amide **37** was obtained by EDCI-HOBt mediated coupling of acid **36** with di-Boc protected agmatine. Azide **37** was reduced to amine **38** under Staudinger conditions. Finally, complete deprotection of Boc-groups in **38** was achieved with TFA in DCM to produce the target **29** as its bis(trifluoroacetate) salt in good yield. The spectral data of bis(trifluoroacetate) salt of **1** were in agreement with the reported data. In conclusion, the total synthesis of **1,** which has been successfully accomplished, provided a methodology amenable to scale up. This synthetic approach also offers methodology for related analogues with equal/better activity as inhibitors of MCA



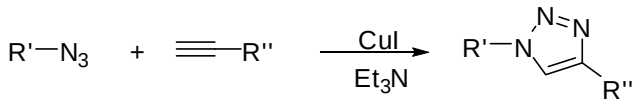
Scheme 6

Section II: Synthetic analogues of dibromotyrosine derived natural alkaloids

Modern drug discovery often entails the synthesis and biological testing of molecule collections, referred to as libraries, which arise from the combinations of different building blocks by the same chemical strategy. Over the last decade, combinatorial library synthesis has become a very important field both in academic and industrial research. Combinatorial techniques on the solid and solution-phase have significantly increased the efficiency of the drug discovery process. Importantly, combinatorial chemistry allows for high throughput synthesis of drug candidates with broad diversity and/or complexity.

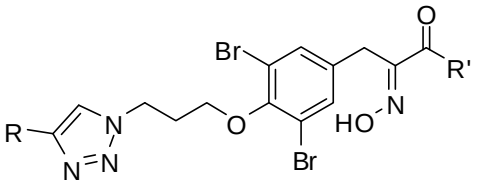
1,3-Dipolar cycloaddition of azides and alkynes (**Scheme 7**) is an effective way to make connections between structures that bear a wide variety of functional groups. The process is strongly favored in thermodynamic terms, as both azide and alkyne units are highly selective in their reactivity; however they are inert to most other chemical functionalities like esters, acids etc and are also stable in wide ranges of solvents, temperatures and pH values. The discovery of copper (I) catalysis of this

process has opened a myriad of applications in bioconjugation, organic synthesis, materials and surface science and combinatorial chemistry.



Scheme 7: Triazole preparation

Dibromotyrosine alkaloid **29** consists of three segments: left segment is the propylamino block, middle segment is the bromophenyl oxime-acid building block and the right segment is the agmatine building block. By using various left and right segments, a library of compounds can be created. In the synthetic route (**Scheme 6**) of compound **29,** there is an azide intermediate **36**, so it was decided to modify this azide group for preparation of different analogues. Coupling of this azide with different acetylenic compounds furnished triazoles as the left segment. For the right segment, different amine compounds were used and coupling of these amines with acids afforded the desired compounds. All the coupling reactions were carried out by the same protocol. The three amines chosen for the library synthesis were agmatine **39**, 3-amino guanidinyl propane **40** and histamine **41**.



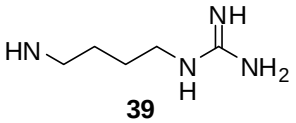
R = Ph

C_6H_{13}

C_5H_{11}

C_5H_7Cl

R' =



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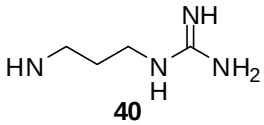

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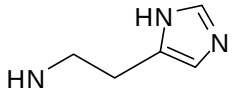

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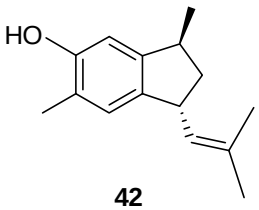
Thus, all the twelve analogues synthesised comprising a library have been submitted for screening and the results are awaited.

Chapter 3

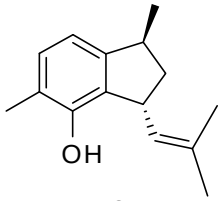
Section I: Total synthesis of mutisianthol

Structurally different indanes have been isolated along the years which exhibit potent biological activities. The phenolic sesquiterpene mutisianthol **42** is also a member of this class of compounds. It was isolated by Bohlmann and co-workers in 1979, from the roots of *Mutisia homoeantha* along with a related indane jungianol **43**. Mutisianthol in its racemic form is plausibly derived in nature from an α-curcumene type precursor by cyclisation to form the indane nucleus. Recently, mutisianthol came under structural revision in five membered ring by its first total synthesis. The most intriguing aspect of these phenolic terpene molecules is the *trans* relationship assigned for the two side chains in the five member rings of mutisianthol. This feature, suggesting a congested folding of the precursor for cyclisation, attracted our attention to investigate the synthesis of these molecules. In the present work, synthetic approaches to mutisianthol have been elaborated.



42

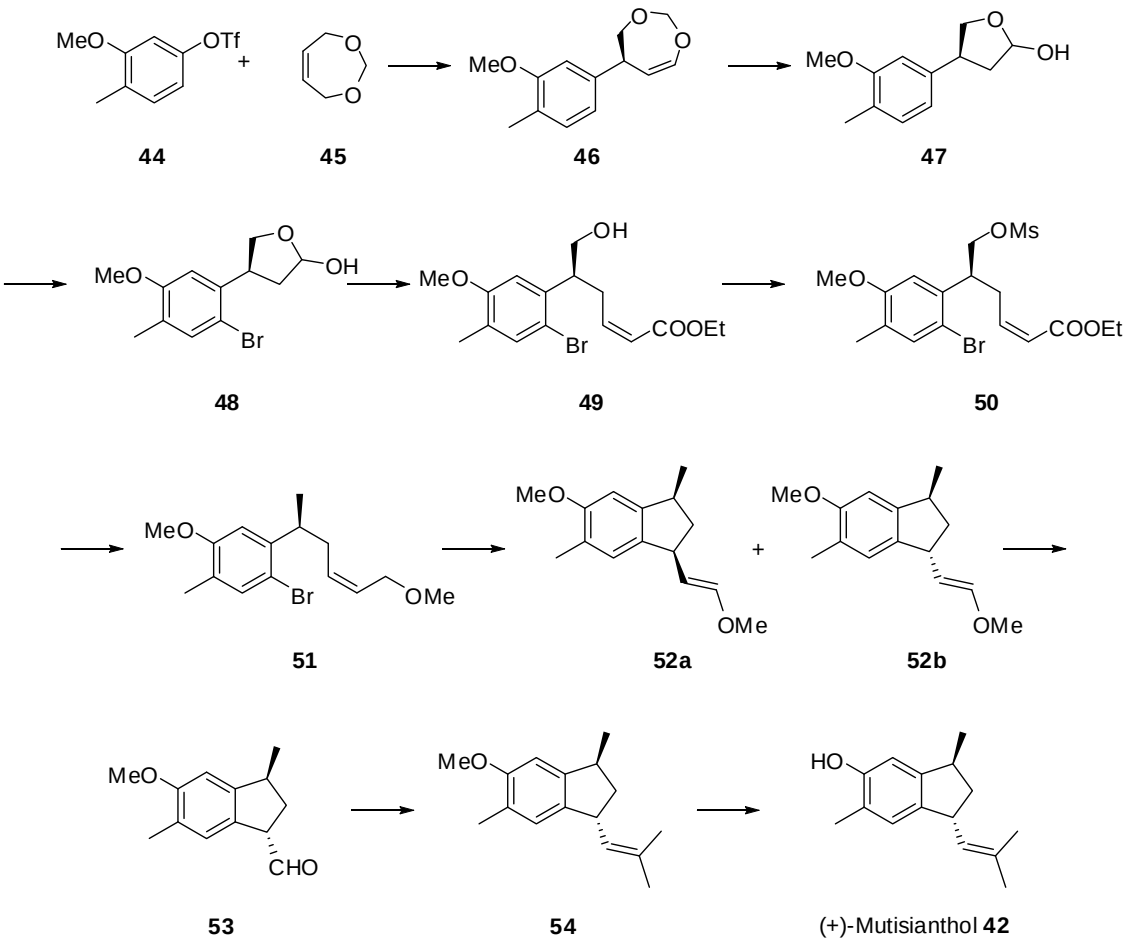
Mutisianthol



43

Jungianol

Synthesis of mutisianthol was initiated by asymmetric Heck reaction of triflate **44** with dioxepin **45**. Deprotection of acetal, bromination and Wittig homologation yielded the unsaturated ester **49**. Deoxygenation was achieved *via* mesylation and reduction of ester followed by protection of alcohol as methylether gave the crucial intermediate **51**. Asymmetric Heck reaction for cyclisation was successfully used to generate a chiral center at benzylic position, followed by a series of reactions involving dihydroxylation, reduction, $NaIO_4$ cleavage of the diol formed etc. Wittig reaction and demethylation completed the synthetic sequence for mutisianthol **42**.



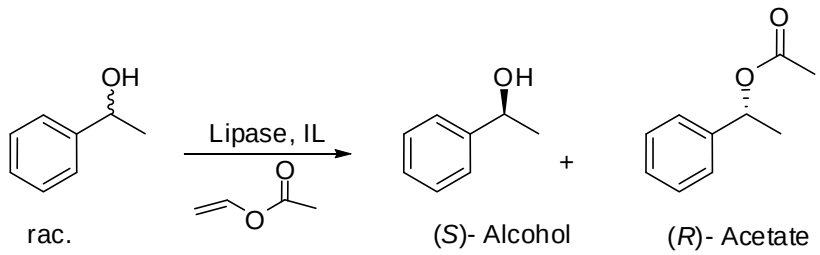
Scheme 8

In conclusion, the first total synthesis of (+)-mutisianthol has been achieved. The key steps in the synthesis involved asymmetric Heck reaction, both intermolecular and intramolecular. The methodology developed in the synthesis can be applied to several other related molecules.

Section II: Enzyme-catalysed resolution of 1-phenylethanol *via* transesterification in the presence of phosphorus ionic liquids

Recently, ionic liquids (ILs) have gained increasing attention for performing all types of reactions with sometimes remarkable results. By modification of the cation and anion, their properties can be tuned in many ways. For all catalytic processes, there are basically three modes of operations: use of the ionic liquid as a co-solvent, as a pure solvent or in biphasic systems. Phosphorous based ionic liquids were used in the present study for the resolution of racemic 1-phenylethanol. 1-

Phenylethanol is a fine chemical and is an important intermediate in many drugs and natural products.



Scheme 9: Transesterification of (R, *S*)-1-phenylethanol with vinyl acetate

The synthetic utilities of lipases were investigated by carrying out transesterification reaction. An activated ester, vinyl acetate was used as an acyl donor in this acyl transfer reaction. Racemic (*R*,*S*)-1-phenylethanol was resolved by acylation with vinyl acetate to produce (*R*)-1-phenylethyl acetate, vinyl alcohol and unreacted (*S*)-1-phenylethanol. All the enzymes tried were found to be highly selective towards the (*R*)-enantiomer of the racemic alcohol.

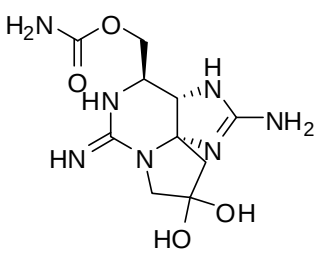
In conclusion, the efficiency of ILs belonging to the phosphonium salts in lipase catalysed kinetic resolution of 1-phenylethanol has been demonstrated in this work. Several variations in ILs to improve the enantioselectivity are currently being worked out which could match the work of the use of enzymes in pure organic solvents.

Chapter 1

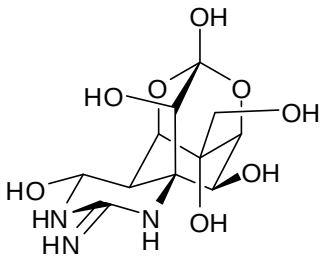
Approaches towards the total synthesis of a spiroisoxazoline derived natural alkaloid

Introduction

Natural products are secondary metabolites that are not directly involved in primary metabolic processes. They play important biological roles in the interactions between organisms. The relationship between biological activity and toxicity of natural products isolated from marine organisms has long been a question of study due to phenomena like the red tides and human seafood poisonings. For example, saxitoxin and tetrodotoxin (Figure 1), which are important tools used to investigate cell biochemistry, are responsible for severe seafood poisoning.



Saxitoxin



Tetrodotoxin

Figure 1

Marine natural products

Many marine derived secondary metabolites possess interesting biological activities. Some of the most important, structurally and biologically interesting marine natural products are listed (Figure 3).

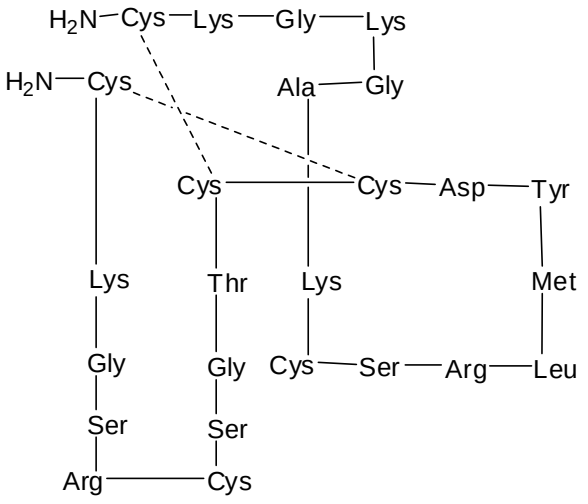
Their limited availability from natural sources, however, limits further investigation about their activities making them attractive targets for studies toward their total synthesis. Beyond the investigation of selected marine natural products exhibiting important biological activities, basic research must take the opportunity to identify and explore the underlying principles of their biological functions.

An exciting ‘‘marine pipeline’’ of new anticancer clinical and preclinical agents has emerged from intense efforts over the past decade to more effectively explore the rich chemical diversity offered by marine life. It is not truly known how many species inhabit the world’s oceans; however, it is becoming increasingly clear that the number of microbial species is many times larger than previously estimated, and the total marine species may approach 1 to 2 million. Whereas the oceans are vast and constitute 70% of the world’s surface, the major diversity of this species is found

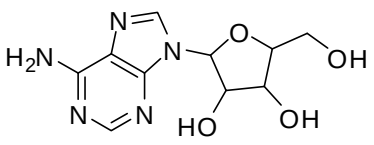
in the ocean fringe. This slender land-sea interface with its high concentration of species is among the most biodiverse and productive environments on the planet. Deep ocean thermal vent communities represent another highly biodiverse and productive habitat, albeit one of limited extent.

Marine derived secondary metabolites are structurally complex with unique functionalities and possess pronounced biological activity. This is due in part to an extreme and harsh living environment, with high ionic concentrations, high pressure, variable temperatures, and lack of light as well as low nutrient availability. Because conditions in the ocean are so markedly distinct, the chemistry produced by its inhabitants is also quite varied. While terrestrial sources of pharmaceuticals and biochemicals have been considerably explored, less than one percent of marine species have been examined for production of novel chemistry. Thus, the oceans represent a rich and still largely untapped resource for biologically active compounds. In addition, completely unknown biochemical pathways in pathogens or disease may be discovered and targeted by such unique chemotypes, leading to the development of novel therapeutics.

Till now, two marine derived natural products have advanced to the pharmaceutical drug market including the new analgesic ziconotide (trade name "Prialt") isolated from the venom of fish hunting cone snails that recently entered the market in the US and an unusual sponge derived nucleosides that served as model compounds for anti viral drugs such as Vidarabin (Figure 2).



Zinconotide



Vidarabin

Figure 2

Bioactive compounds of marine origin

A number of promising compounds have been identified from marine sources that are already at advanced stages of clinical trials, mostly for the treatment of cancer, or have been selected as promising candidates for extended preclinical evaluation. The majority of marine natural products currently in clinical trials or under preclinical evaluation are produced by invertebrates such as sponges, soft corals, sea squirts, and bryozoans. The types of compounds characterized from marine organisms are diverse and represent many different structural classes (Figure 3), including polyethers, terpenoids, alkaloids, macrolides, and polypeptides.

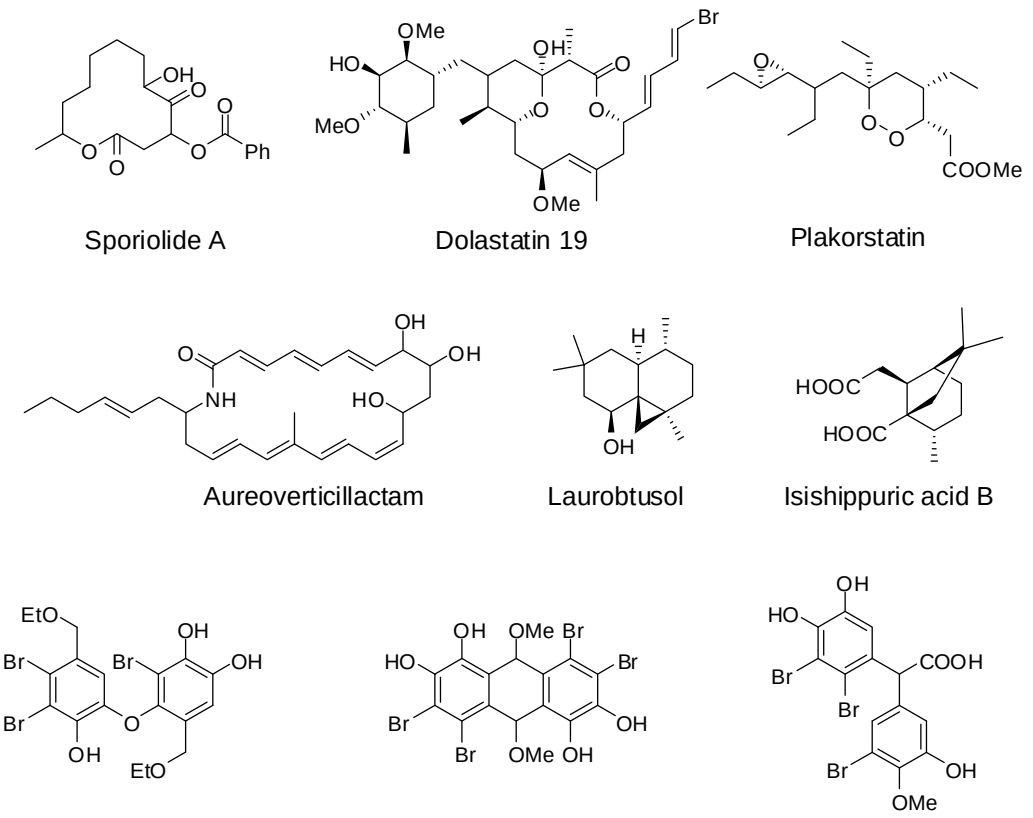


Figure 3

Among the bioactive compounds isolated from marine organisms, those currently under intense investigation as potential anticancer agents include ecteinascidin 743 and bryostatin (Figure 4).

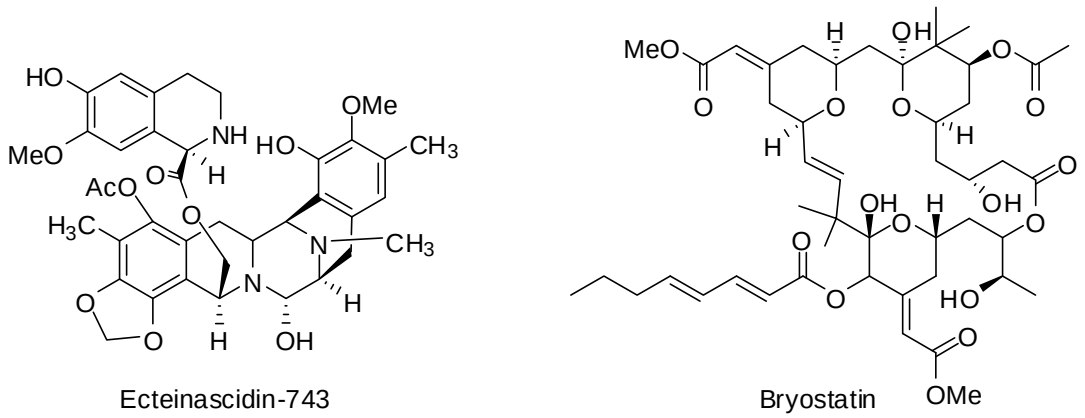
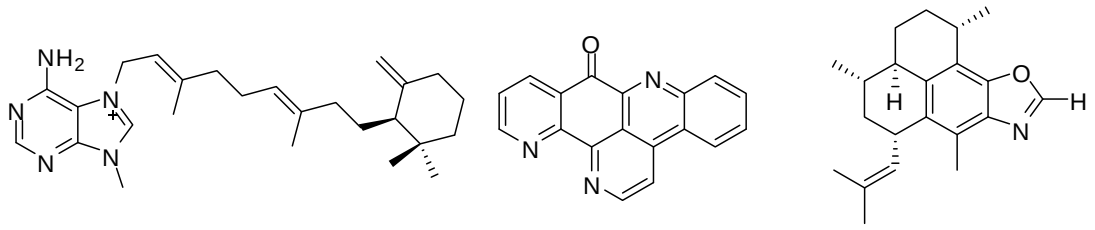


Figure 4

Marine natural products with antitubercular activity

In recent years, *Mycobacterium tuberculosis* has re-emerged as a leading cause of death and the appearance of drug resistant strains continues to rise. Related mycobacterial species, such as *Mycobacterium avium* and *M. avium* complex, that are otherwise non-deleterious to human beings, pose serious threats to immuno compromised people. Consequently, there is a continuing need for the discovery of new antituberculars with novel modes of action. In an effort to identify new classes of antimycobacterials, Bewley and co-workers have screened a variety of marine extracts for their ability to inhibit a novel mycobacterial enzyme, mycothiol-*S*-conjugate amidase (MCA). Mycothiol (MSH)[1] is a low molecular weight thiol that replaces glutathione in actinomycetes.[2] In conjunction with MCA, MSH plays a central role in protecting actinomycetes against alkylating agents and other toxins.[2,3] Recently a second highly homologous amidase from *M. tuberculosis* that is involved in the biosynthesis of MSH has been described.[4] Because these mycothiol-dependent pathways are not found in eukaryotes, the enzymes involved represent potentially useful new antimycobacterial targets since inhibition of both enzymes would permit blocking of MSH-dependent detoxification at two distinct levels, namely, biosynthesis and detoxification. Some of the most important anti-tubercular compounds isolated from marine sources are agelasine, ascididemin and pseudopteroxazole (Figure 5).



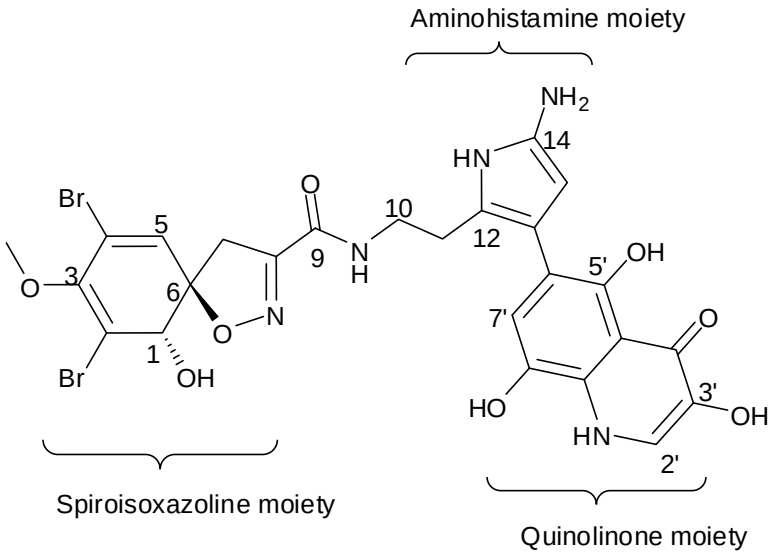
Agelasine E Ascididemin Pseudopteroxazole

Figure 5

Bromotyrosines from marine sponges

Although seawater contains a much higher concentration of chloride (559 mM) than bromide (0.86 mM) or iodide (0.45 μM), brominated secondary metabolites predominate in marine organisms. Bromide is more easily and readily oxidized than chloride and the resulting bromonium ions undergo electrophilic addition to alkenes and aromatic systems. Haloperoxidases are the enzymes which are able to oxidise chloride, bromide and iodide in the marine environment. Consequently, various marine organisms effectively used the available bromonium ions in the biosynthesis of defensive and other necessary constituents. Out of nearly 3200 known natural organohalogen compounds, more than 1600 contain bromine more than 90% of the latter are from marine sources. Illustrative are the bromotyrosine-derived secondary metabolites of marine sponges and certain tunicates.

Over the past 30 years an ever increasing number of tyrosine derived secondary metabolites have been isolated from marine sponges. Bromotyrosine-derived spiroisoxazoline **1**, a marine natural product isolated[5] in 2001 from an Australian non-verongid sponge of the *Oceanpia* species is one of the hundreds of dibromotyrosine-derived natural products.[6] Its structure, shown in **Figure 6** consists of a central amino histamine unit which acts as a spacer and is attached on the right to a quinolinone unit and to the left through an amide bond with dibromotyrosine spiroisoxazoline unit. The absolute configuration of the naturally occurring enantiomer is established as *S* and *R* respectively at C1 and C6 centers. Compound **1** contains a rare example of an amino-imidazole coupled to another aromatic substituent.



1

Figure 6: Structure of bromotyrosine-derived spiroisoxazoline

A preliminary screening of the extract of a specimen of *Oceanapia* sp. showed strong activity against MCA as determined by the absence of the MCA/MSH cleavage product. Compound **1** is the first example of natural product that inhibits an enzyme central to a mycothioldependent detoxification pathway found in mycobacteria. Till now there is no report of synthesis of compound **1**. Its interesting biological activity coupled with novelty in the structure (spiroisoxazole and quinolinone) with the spacer, prompted its total synthesis. Attempted synthesis of dibromotyrosine derived alkaloid **1** is described in the following section.

Several bromotyrosine alkaloids have been isolated in recent years. A few examples related to **1** are given in **Figure 7.** The bromotyrosine-derived spiroisoxazolines are a structurally diverse class of physiologically active natural products. The family contains a number of structural types, including both monomeric and dimeric compounds derived from brominated tyrosine precursors.

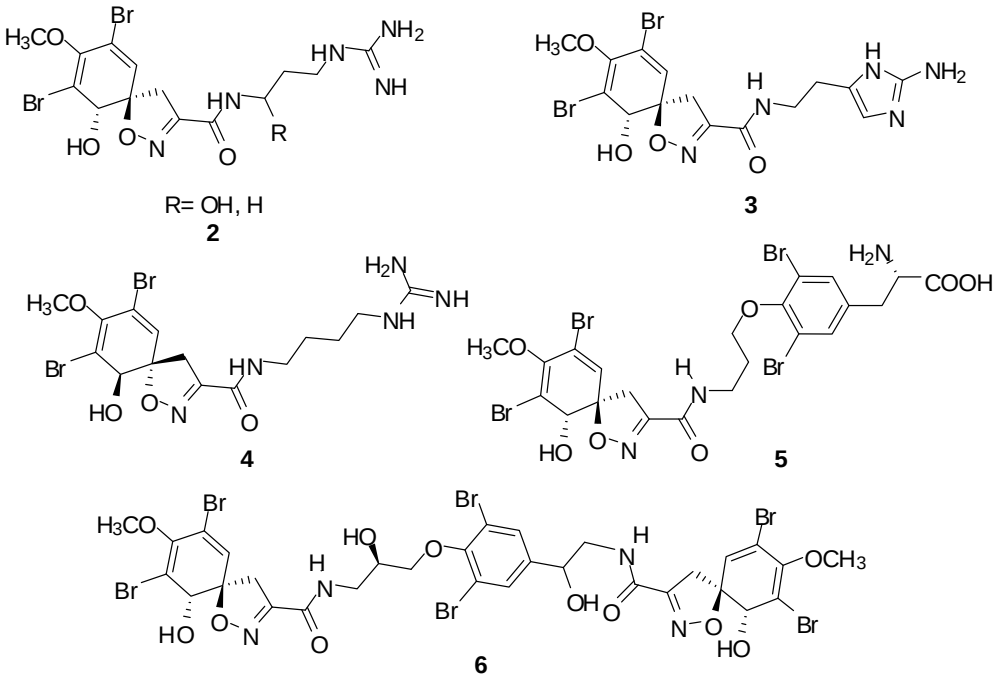


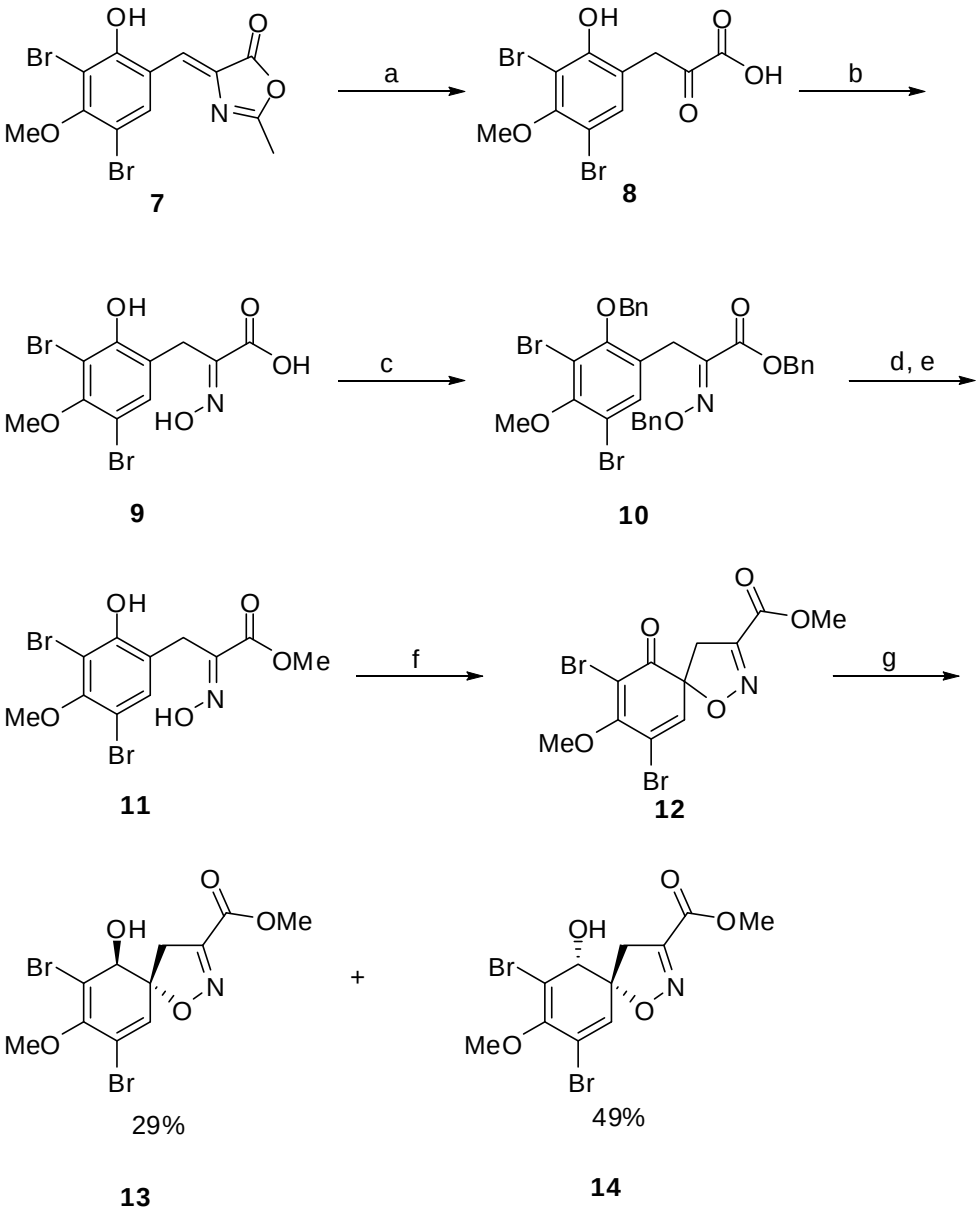
Figure 7: Bromotyrosine-derived metabolites of marine sponges

Various reported synthesis of spiroisoxazole ring

A central feature of **1** and its relatives is the dibromotyrosine-derived spiroisoxazoline ring. The spiroisoxazoline and related molecules have been synthesized in several different ways. These approaches are reported in the following pages.

Yamamura's approach (1985)[7]

Yamamura first reported the synthesis of ester **13** (Scheme 1). When readily accessible azlactone[8] **7** was subjected to alkaline hydrolysis in 10% KOH followed by oximation and benzylation, the tribenzyl derivative **10** was obtained in 35% yield. Transesterification of **10** in methanol containing K_2CO_3 afforded the corresponding methyl ester, which on hydrogenolysis led to the desired dihydroxy methyl ester **11** in 74% yield. On treatment with thallium (III) trifluoroacetate in CF_3COOH, methyl ester **11** was converted to spiroisoxazoline **12** in 27% yield. Reduction of the ketone **12** was successfully carried out with excess $Zn(BH_4)_2$ to give the corresponding *trans* and *cis* isomers (**13** and **14**) in 29% and 40% yields respectively. The stereostructure of **13** and **14** was established on the basis of comparison of ^1H NMR spectroscopy with those of similar compounds.[9]



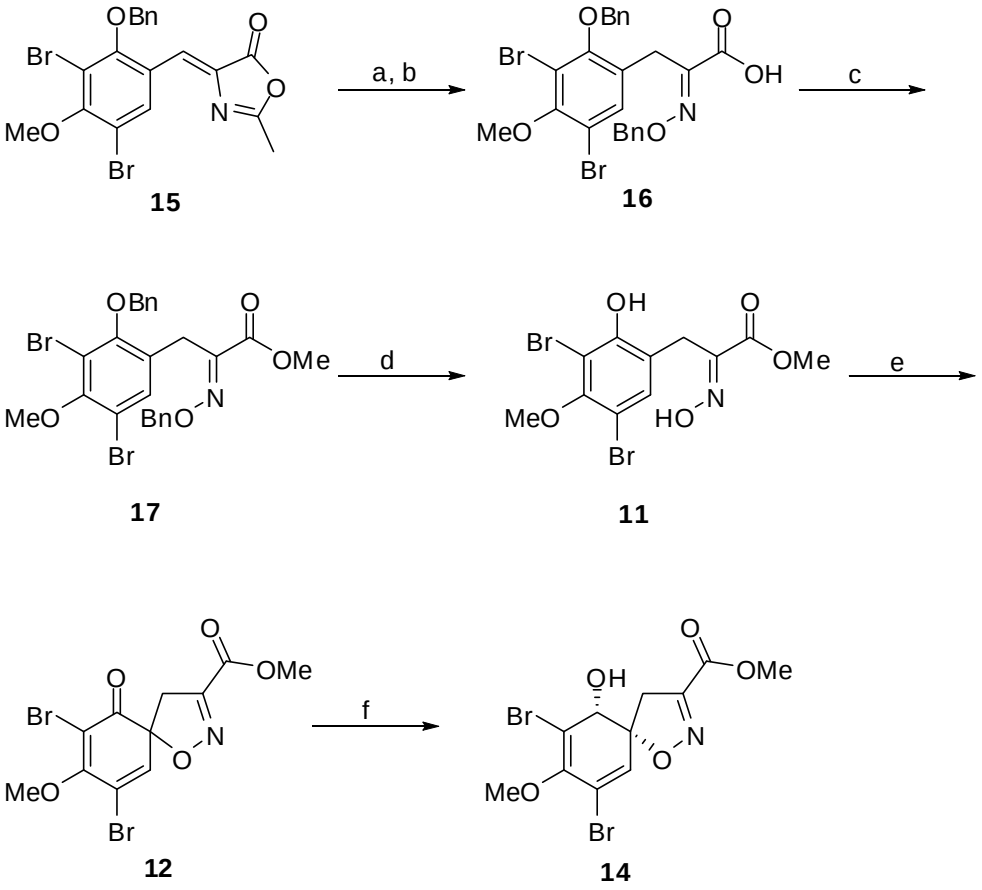
Scheme 1: Synthesis of spiroisoxazole methyl ester

Reagents and conditions: (a) KOH, H_2O; (b) $NH_2OH.HCl$; (c) BnCl, K_2CO_3, 35%; (d) MeOH, K_2CO_3; (e) H_2, Pd/C, 74%; (f) $Tl(CF_3COO)_3$, 27%; (g) $Zn(BH_4)_2$, ether.

Hoshino's approach (1996)[10]

Starting with the similar azlactone **15**, Hoshino reported the synthesis of spiroisoxazoline **12** in much higher yield (Scheme 2). The azlactone **15** was hydrolyzed with $Ba(OH)_2$ in the presence of *O*-benzylhydroxylamine in aqueous dioxane to give oxime **16** in 75% yield. Methylation of acid **16** with CH_2N_2 followed by hydrogenolysis over Pd-black afforded dihydroxy methyl ester **11** in 66% yield. The reaction of dihydroxy methyl ester **11** with phenyliodonium diacetate in

acetonitrile at 0 °C proceeded smoothly to afford spiroisoxazoline **12** in 76% yield. Reduction of the ketone **12** was successfully carried out with $NaBH_4$ to give the dienol **14** in 46% yield.



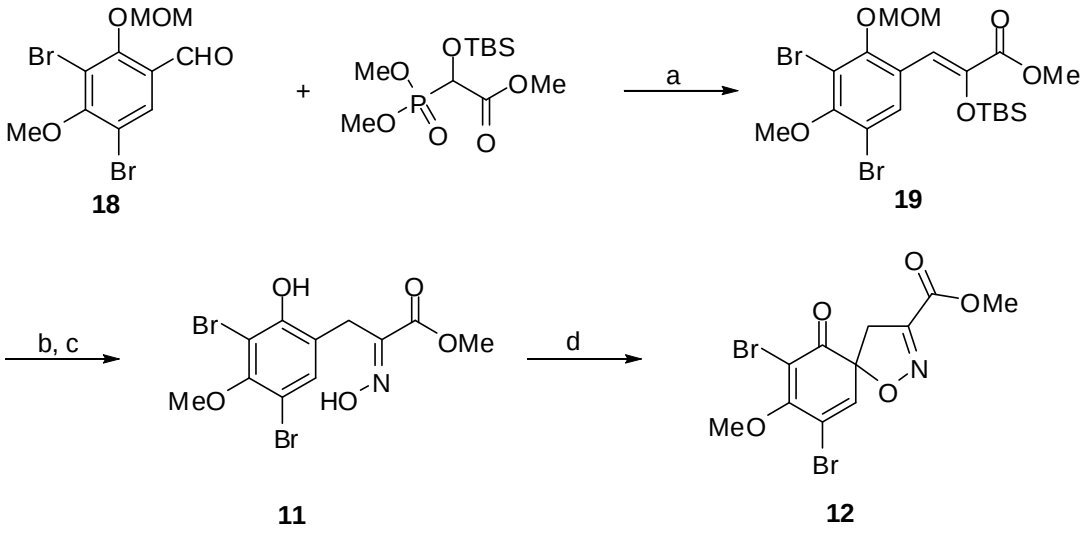
Scheme 2: Synthesis of spiroisoxazole

Reagents and conditions: (a) $Ba(OH)_2$; (b) $NH_2OBn.HCl$, 75%; (c) CH_2N_2; (d) H_2, Pd-black, 66%; (e) $PhI(OAc)_2$, 76%; (f) $NaBH_4$, MeOH, 46%.

Spilling's approach (2001)[11]

Spilling synthesized spiroisoxazoline **12** in a different way (Scheme 3). Reaction of the MOM-protected aldehyde **18** with methyl 2-(*tert*-butyldimethylsilyloxy)-2-(dimethylphosphono) acetate gave the silyl enol ether **19** in 92% yield. Deprotection of TBS group in **19** with $Et_3N{\cdot}HF$ in MeOH followed by addition of $NH_2OH{\cdot}HCl$ and Et_3N yielded dihydroxy oxime methyl ester **11** in 90% yield. The methyl ester **11** was cyclised with NBS in DMF to give the spiroisoxazoline **12** in 93% yield. Manganese tris(acetylacetonate)[12] and 2,4,4,6-

tetrabromocyclohexa-2,5-dienone (TBCO)[13] were also reported to oxidatively cyclise the phenolic oxime ester **11**.

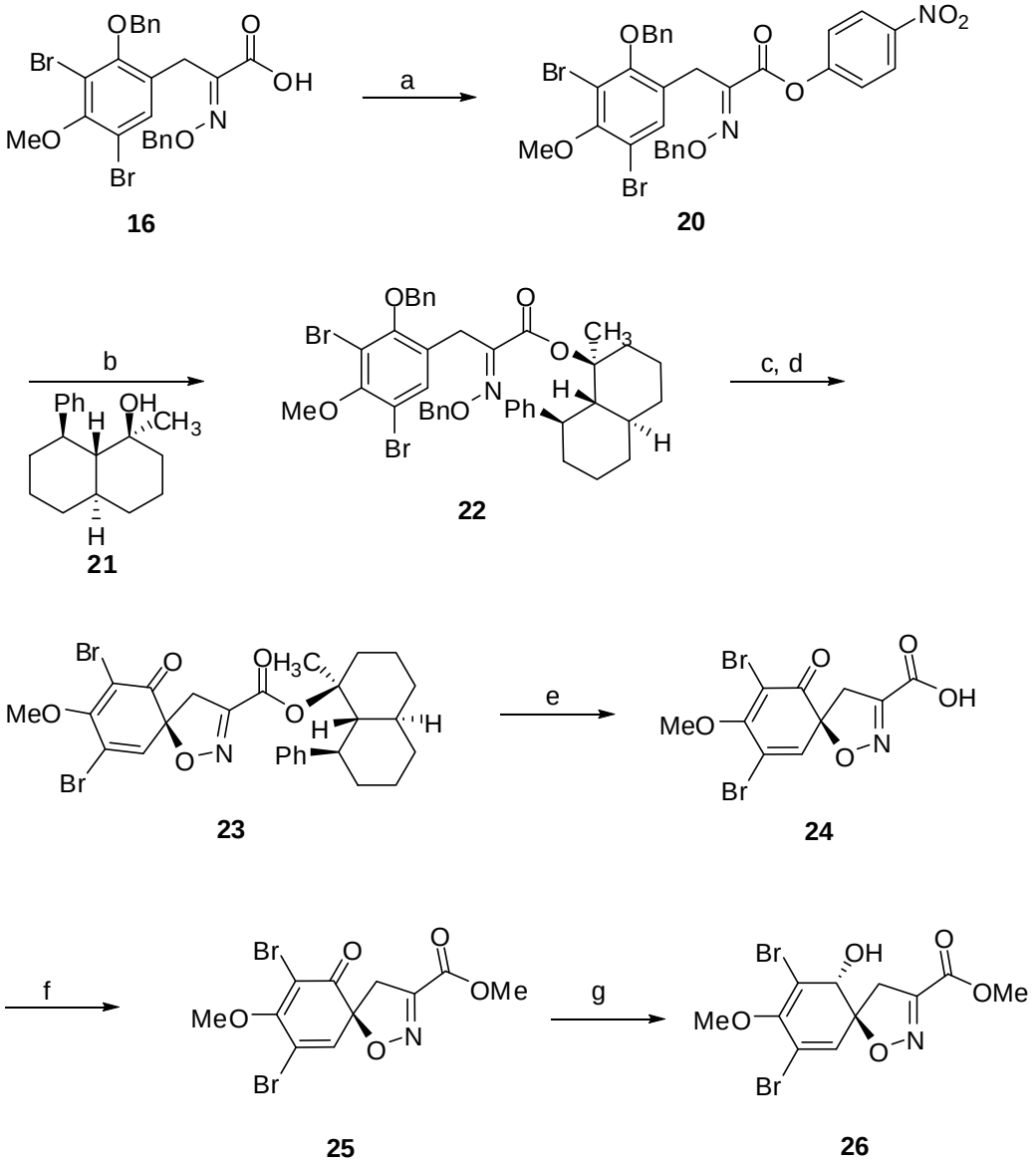


Scheme 3: Synthesis of spiroisoxazole

Reagents and conditions: (a) LDA, THF, 92%; (b) HF.TEA, MeOH; (c) $NH_2OH.HCl$, MeOH, TEA; (d) NBS, DMF, 93%;

Asymmetric synthesis of a spiroisoxazoline (1997[14] and 2006[15])

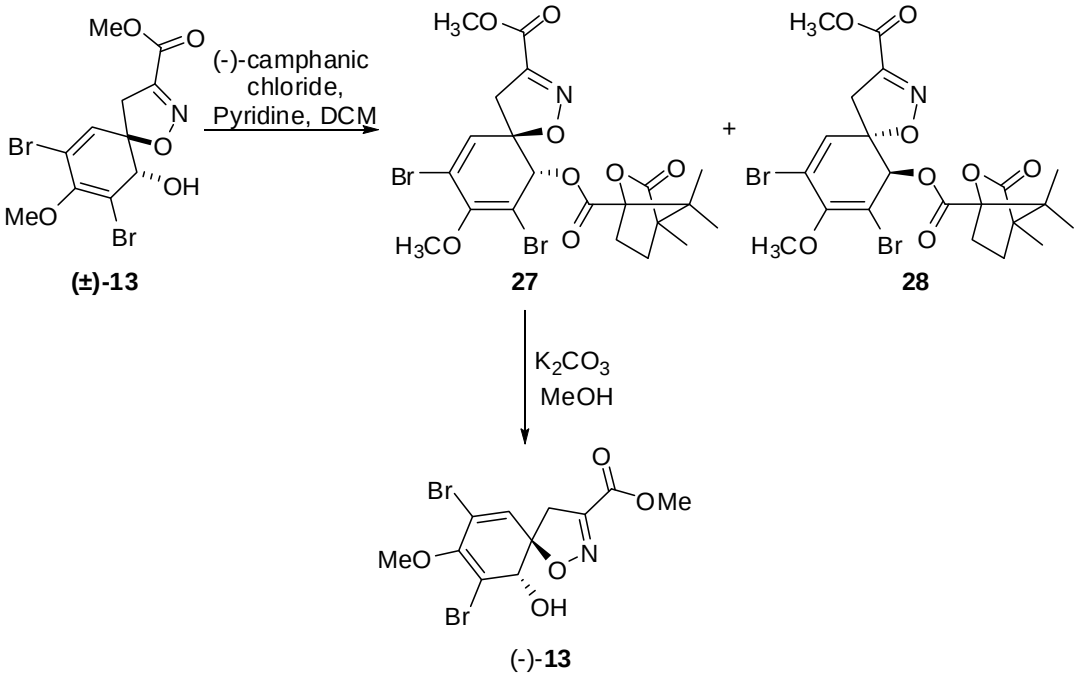
The first asymmetric synthesis of a spiroisoxazoline ester was achieved by Hoshino[13] and coworkers (Scheme 4). Treatment of dibenzyloxime **16** with *p*-nitrophenol afforded the *p*-nitrophenyl ester **20** in 91% yield, and transesterification of ester **20** with lithiated chiral alcohol **21** produced ester **22** in 85% yield. Hydrogenolysis and oxidation of oxime ester **22** with PhIO in the presence of CSA at –78 °C afforded spiroisoxazoline **23** in 83% yield and 70-80% de. The 'de' was estimated on the basis of the resolved methylene proton signals of the isoxazoline ring. Removal of the chiral auxiliary from spiroisoxazoline **23** followed by methylation by DCC and MeOH gave (*S*)-methyl ester **25** in 71% yield and in 74% ee. The enantiomeric excess (ee) was determined by chiral HPLC. Finally the spiroisoxazoline methyl ester **25** was reduced to give the *trans* alcohol **26** with 84% ee according to Yamamura method.[7]



Scheme 4: Asymmetric synthesis of spiroisoxazoline

Reagents and conditions: (a) DCC, *p*-nitrophenol, DCM; (b) MeLi, HMPA-toluene; (c) H_2, Pd-black, dioxane; (d) PhIO-CSA, DCM; (e) TFA; (f) DCC, MeOH, DMAP; (g) $Zn(BH_4)_2$, ether.

In 2006, synthesis of chirally pure spiroisoxazoline unit without contamination of its diastereomer was achieved by Nishiyama[15] and coworkers. The coupling reaction of ester **13** with (-)-camphanic chloride gave the chromatographically separable diastereomeric mixture of **27** and **28**. The chiral reagent was deprotected with K_2CO_3 in MeOH to give pure (-)-**13**.



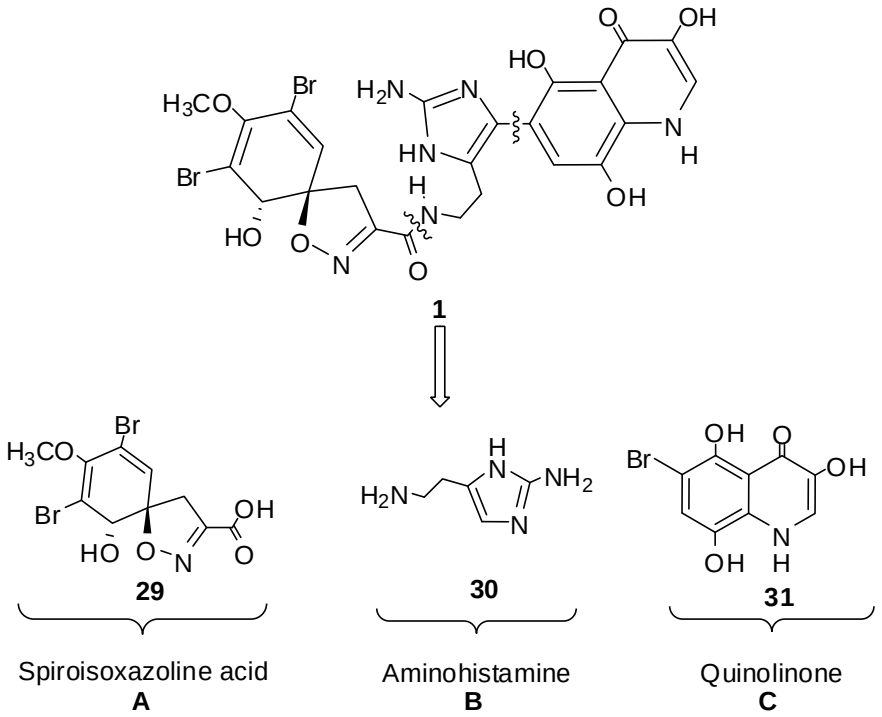
Scheme 5: Asymmetric synthesis of spiroisoxazoline

Present Work

Many marine natural products that appear to be biogenetically derived from a bromotyrosine precursor have been described earlier. Mycothiol (MSH) is the major low molecular weight thiol limited to actinomycetes, which include mycobacteria. MSH plays a major role in detoxification and maintenance of a reductive intracellular environment. Since enzyme mycothiol-*S*-conjugate amidase appears to play a critical role in protecting mycobacteria against alkylating agents and antibiotics, there is continuing need to isolate compounds showing significant inhibitory activity against these enzymes. Studies of enzymes involved in mycothiol biosynthesis and mycothiol-dependent detoxification, as well as those of inhibitors to these enzymes, are rapidly increasing in number. In this connection Bewley et al. described a series of marine natural product inhibitors of MCA, some of which are lethal to *M. smegmatis*. Of those structures studied, kinetics experiments established that the bromotyrosine-derived alkaloid, such as **1**, is active inhibitor of the group and is also competitive inhibitor of the *M. tuberculosis* detoxification enzyme MCA.

Retrosynthetic analysis of 1

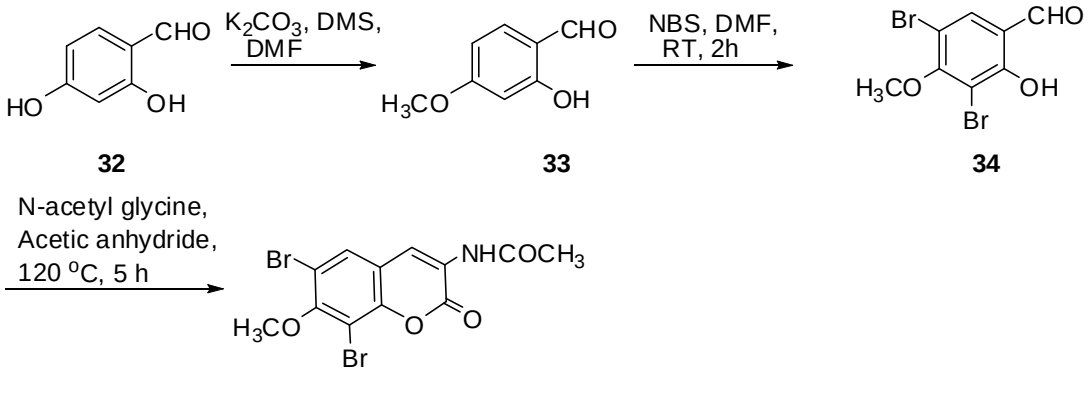
Palladium catalyzed coupling reactions (Stille coupling, Negishi coupling, Heck coupling, Suzuki coupling) are widely used for carbon-carbon bond formation in the synthesis of complex molecules since they are usually performed under mild conditions and provide excellent selectivities. A retrosynthetic scheme of **1** was designed based on palladium catalyzed coupling reactions. According to retrosynthetic analysis shown in **Scheme 5**, molecule **1** can be derived from three fragments: left side spiroisoxazoline acid **29**, a middle aminohistamine spacer **30** and a right side quinolinone **31**. Cleavage of bonds between spiroisoxazoline acid and aminohistamine and between aminohistamine and quinolinone provides three fragments. In the synthetic sense fragments **29** and **30** would be united by acid-amine (amide formation) coupling, and fragments **30** and **31** would be united by C-C coupling (Heck, Suzuki).



Scheme 5: Retrosynthetic analysis

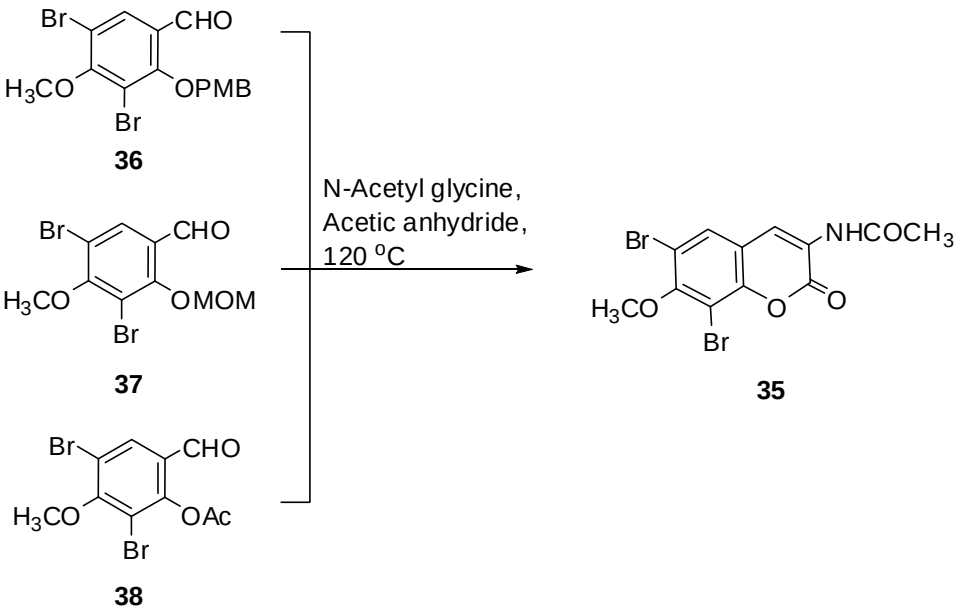
Synthesis of spiroisoxazoline part A

Spiroisoxazoline methyl ester **13** was synthesized according to Hoshino's approach[10]. Commercially available 2,4-dihydroxybenzaldehyde **32** was selectively methylated to give 2-hydroxy-4-methoxybenzaldehyde **33**, which was treated with NBS in DMF at room temperature to provide the dibromosalicylaldehyde **34** in 98% yield. By heating dibromosalicylaldehyde **34** with *N*-acetyl glycine[16] in acetic anhydride, the acetamido coumarin **35** was obtained in yields ranging from 70 to 80%, wherein the intermediate azlactone formed underwent cyclisation with phenol to form lactone (Scheme 6).



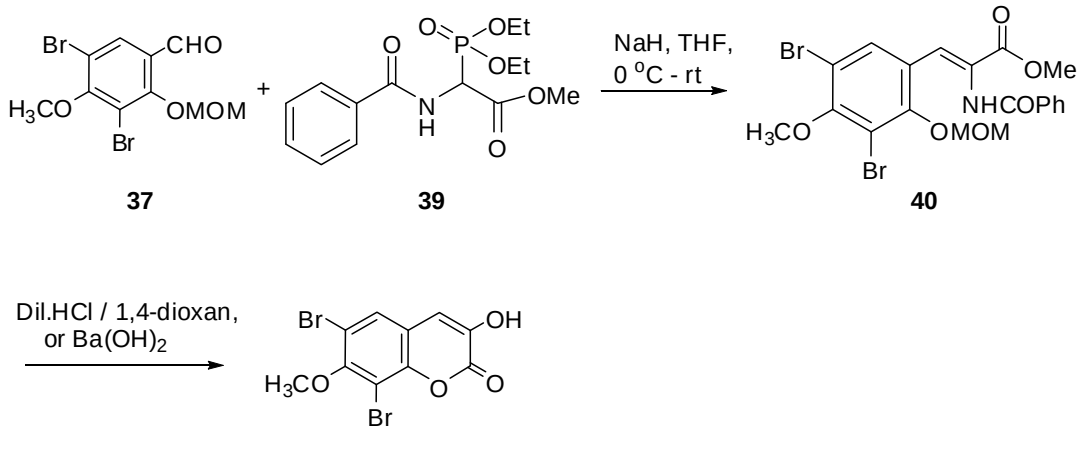
Scheme 6

Free phenolic group is necessary for the synthesis of spiroisoxazoline unit; hence, for the preparation of azlactone with free phenolic group, benzaldeydes **36**-**38** with different protecting groups (OAc, OMOM, and OPMB) were used for reaction. However, during heating with *N*-acetyl glycine in acetic anhydride, both deprotection and lactonisation took place and the acetamido coumarin **35** was the exclusive product observed in yields ranging from 70-80% (Scheme 7).



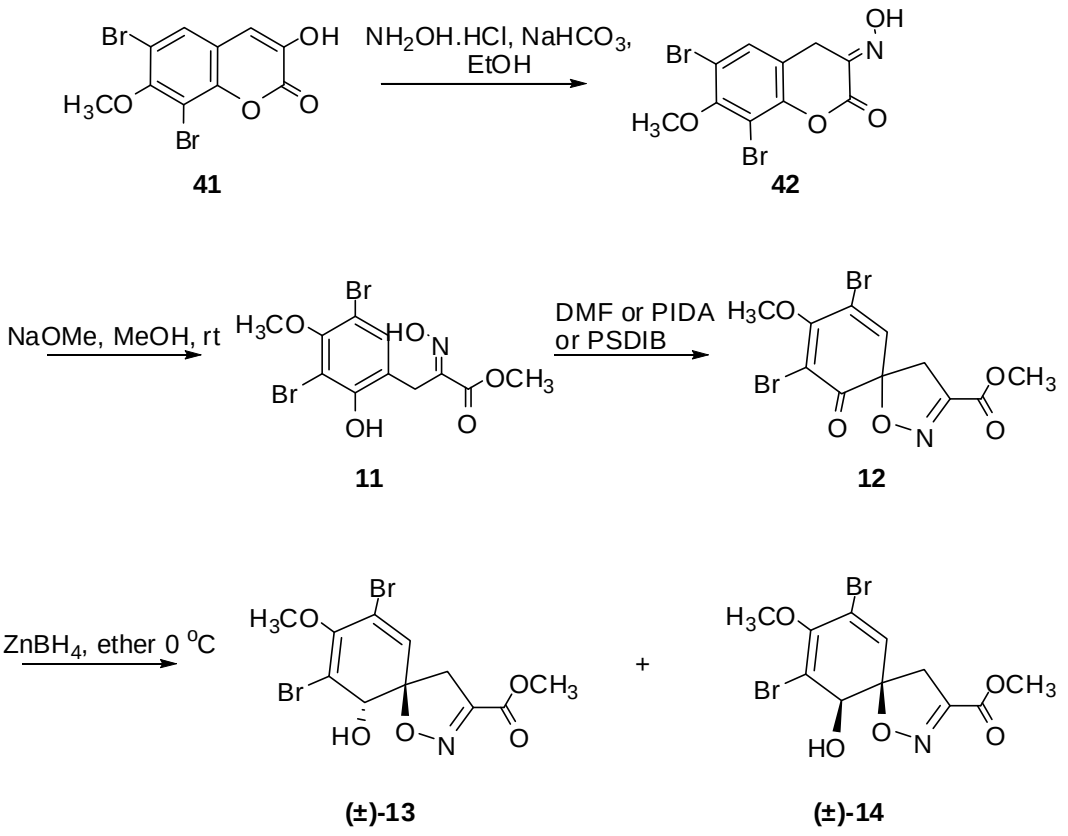
Scheme 7

Wittig olefination of benzaldehyde **37** with phosphonate **39** provided the cinnamic ester **40**, hydrolysis of the latter with 1M HCl or with $Ba(OH)_2$ gave the 3-hydroxycoumarin derivative **41** (Scheme 8).



Scheme 8

Reaction of the coumarin **41** with hydroxylamine under reflux in 80% EtOH for 1-2 h yielded the oximino coumarin **42**. Prolonged reaction of enol coumarin with an excess of hydroxylamine led to formation of the corresponding hydroxamic acid. In the ^{1}H NMR and ^{13}C NMR spectra of **42** signals due to benzylic methylene group were observed at δ 3.65 (s, 2H) and δ 24.7 respectively. The coumarin derivative **42** was treated with sodium methoxide in methanol to give oxime ester **11**. Reaction of *ortho*-phenolic oxime-ester **11** with NBS in DMF, (diacetoxyiodo)benzene or with polymer supported (diacetoxyiodo)benzene (PSDIB) reagent in acetonitrile proceeded smoothly to afford spiroisoxazoline methyl ester **12.** While this synthesis was in progress, D. Spilling *et al*. published[17] the synthesis of spiroisoxazoline **12** following an identical route (Scheme 9).



Scheme 9

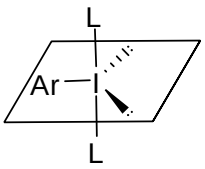
Spiroisoxazoline methyl ester **12** was not stable and was reduced with $Zn(BH_4)_2$ (Yamamura's method) to give cyclohexadienylisoxazoline **13** along with *cis* isomer **14** which was separated by column chromatography. In the ^{1}H NMR spectrum of **13**, signals at δ 4.21 (d, J = 8.0 Hz, 1H) and 5.40 (d, J = 8.0 Hz, 1H) were

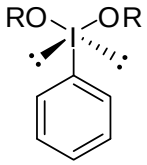
observed which were assigned to the alcohol and methine protons attached to the carbon bearing alcoholic group. Signals for the methylene protons in the ^{1}H NMR spectrum of **14**, observed at δ 3.39 and 3.46, while for **13** at δ 3.21 and 3.85. Optically active spiroisoxazoline compound (-)-**13** was synthesised following literature procedure.[15]

As the synthesis described above apply reagents involving hypervalent iodine chemistry, it becomes relevant to discuss at length these reagents. Following pages furnish details with mechanism.

Hypervalent Iodine Chemistry

Iodine is a large-sized halogen element, easily polarizable and low in electronegativity. It forms hypervalent iodine compound beyond the octet rule by readily extending its valence. The central iodine atom forms a plane with two lone pairs and one σ-bond and furthermore, this iodine atom coordinate two ligands with larger electronegativity in an apical position orthogonal to the plane resulting in the formation of a linear three center-four electron bond. The I-L bond in the apical position is longer than the covalent bond.





R = $COCF_3$, PIFA
R = $COCH_3$, PIDA

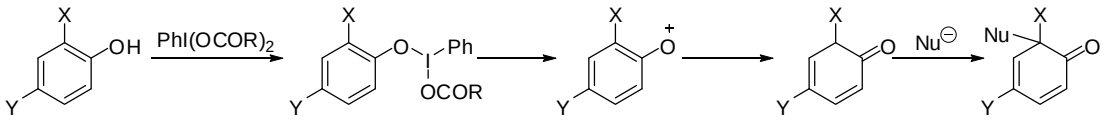
Weaker and longer than covalent linkages, hypervalent bonds are the result of a linear three-center, four-electron (3c-4e) electronic distribution (hypervalent model). The great advantages of hypervalent iodine compounds, for example, their low toxicity compared with heavy-metal reagents, mild reaction conditions, fast accessibility of a large variety of reagents, and easy handling have led to their increased use in synthesis.

PIFA and PIDA reagents

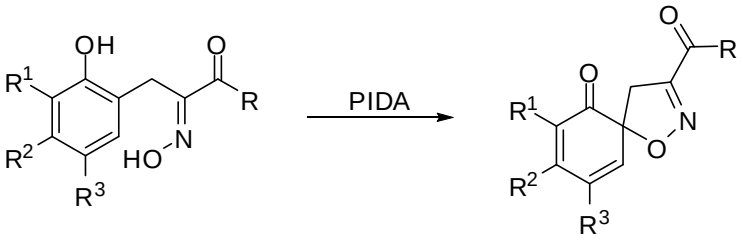
PIFA [phenyliodine bis(trifluoroacetate)] can serve as an oxidant similarly to Tl(III), Hg(II) and Pb(IV), while being less toxic than these heavy metal containing reagents.

Facile oxidation of alcohols to the corresponding carbonyl compounds is one of the prominent features of hypervalent iodine compounds appreciated by many synthetic chemists.[18] Reactions leading to the formation of carbon–carbon bonds are important applications of hypervalent iodine reagents. These reactions proceed either through reactive intermediates such as radicals or carbocations, or they are ligand-coupling reactions mediated by trivalent iodine derivatives. The oxidative coupling of appropriately substituted phenol derivatives has been developed into a powerful tool for the synthesis of polycyclic compounds.

Mechanism of phenolic oxidation by $PhI(OCOR)_2$



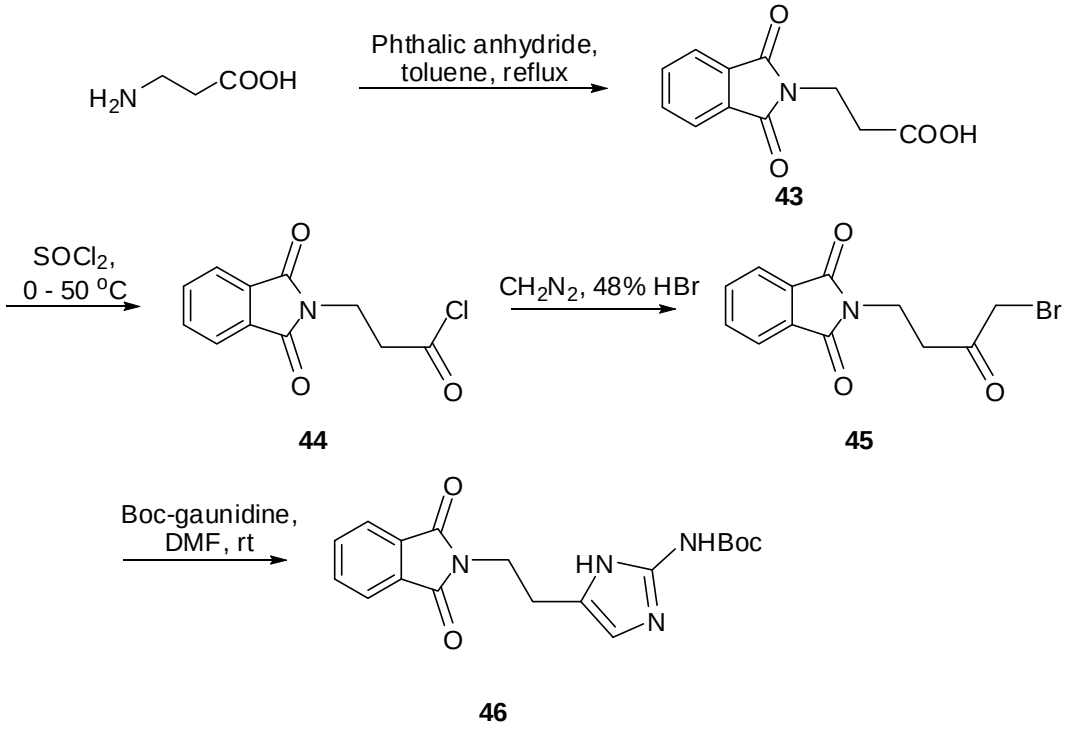
Cyclisations involving phenoxenium ions are known in which the attacking nucleophile is a hydroxy group.[19] The formation of lactones by cyclisation of carboxylic acids was early demonstrated and more recently[10] spiroisoxazolines have been synthesised from phenolic oxime acid derivatives. The reaction of o-phenolic oxime-ester with PIDA in acetonitrile at 0 °C proceeded smoothly to afford spiroisoxazoline in good yield.



Synthesis of aminohistamine part B of 1

Aminohistamine **46**, with protecting groups, was synthesized according to a known method with slight modification in procedures.[20,21] Amino group of commercially available β-alanine was protected as phthalimido group by heating it with phthalic anhydride in toluene with catalytic PTSA; water formed was removed azeotropically (Dean Stark apparatus) to afford β-phthalimidopropionic acid **43**. The

acid **43** was mixed with thionyl chloride and allowed to stand for four hours. The mixture was then warmed and the resulting clear solution was evaporated under vacuum to remove the excess thionyl chloride. The crystalline residue was taken up in dry benzene, and this solution was diluted with petroleum ether and filtered to afford acid chloride **44**. The acyl chloride **44** was treated with diazomethane and subsequent quenching by HBr furnished 1-bromo-4-phthalimido-2-butanone **45**. In the ^{1}H NMR spectrum of **45**, signal for the newly added methylene group which is in between bromo and keto group was observed at δ 3.93 (s, 2H). This α-bromoketo compound **45** was condensed with Boc-protected guanidine in DMF to furnish Boc-protected aminohistamine **46**. Product obtained was fully confirmed by spectroscopic analysis.



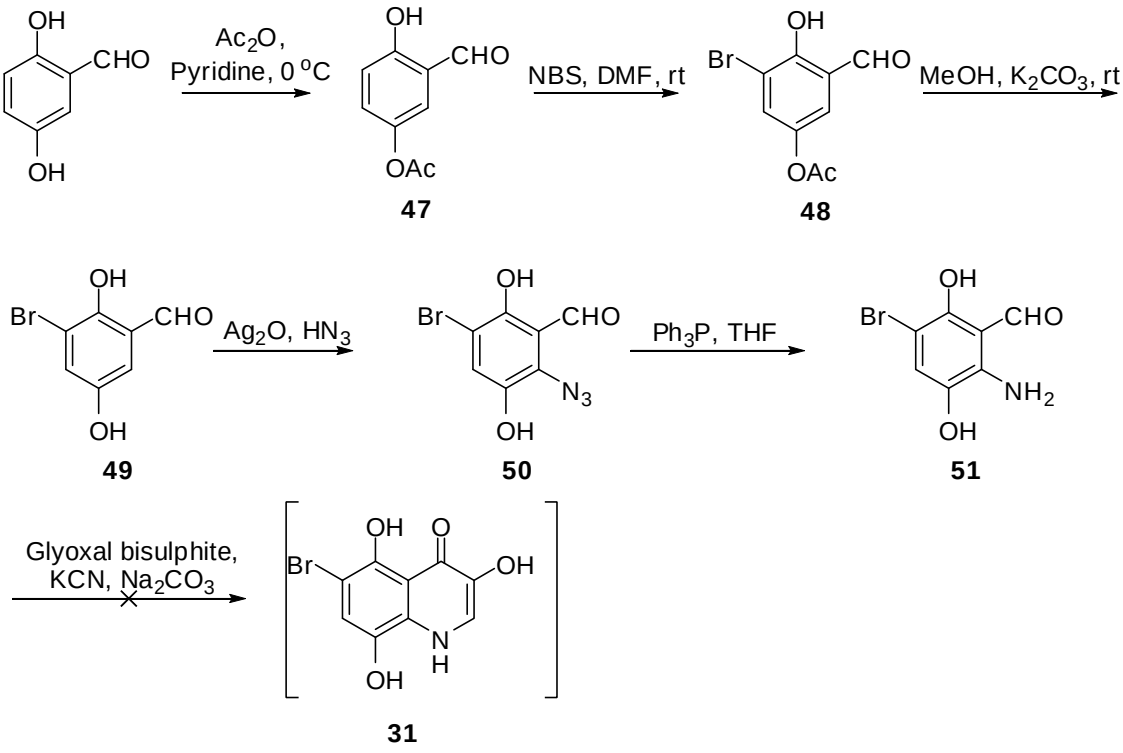
Scheme 10: Synthesis of aminohistamine part

Synthesis of Quinolinone part C of 1

After successful synthesis of two fragments the next target was to synthesise quinolinone part. Commercially available 2,5-dihydroxybenzaldehyde was selected as the starting material.

Protection of C-5 phenolic group in 2,5-dihydroxybenzaldehyde was necessary to achieve regioselective bromination to furnish benzaldehyde **49** because direct bromination of 2,5-dihydroxybenzaldehyde gave the isomeric 2-bromo-3,6-dihydroxybenzaldehyde. Thus regioselective protection[22] was achieved with acetic

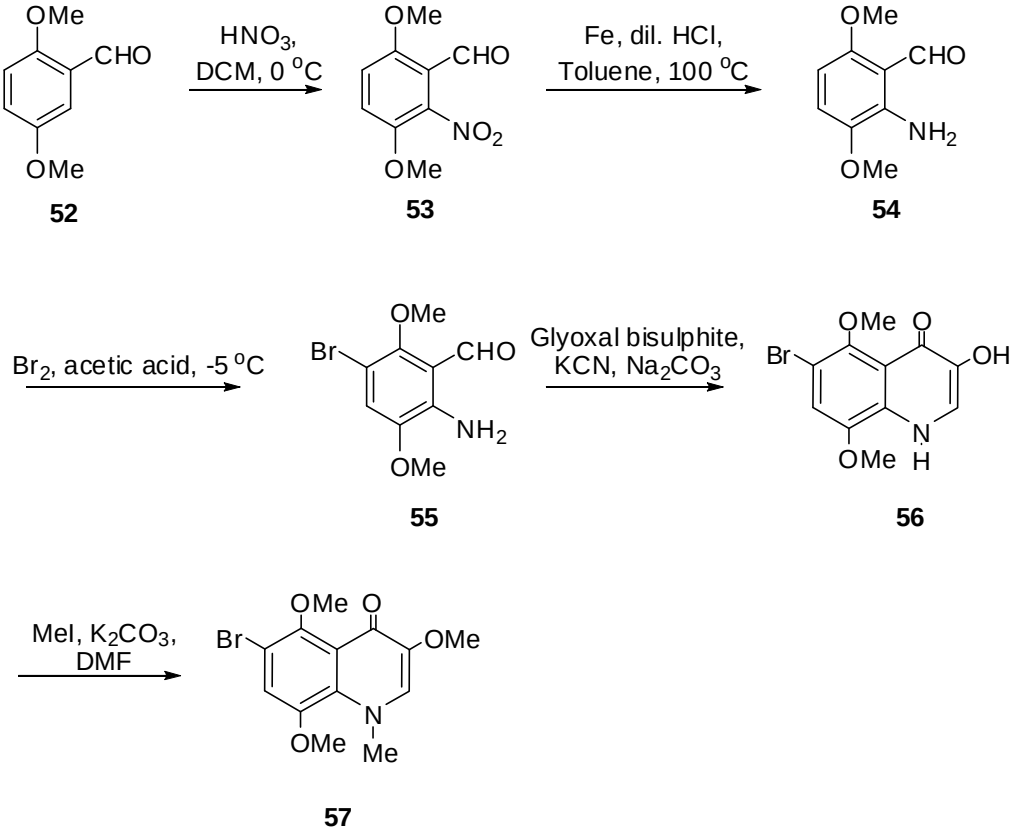
anhydride in pyridine to afford benzaldehyde **47**. Benzaldeyde **47** was brominated with NBS in DMF followed by deprotection of acetyl group by K_2CO_3 in MeOH gave 3-bromo-2,5-dihydroxybenzaldehyde **49**. Azido group was introduced *ortho* to benzaldehyde following literature procedure.[23] The unstable quinone was generated[24] from 3-bromo-2,5-dihydroxybenzaldehyde with silver (I) oxide[25] in benzene and the resulting orange solution was added to a solution of hydrazoic acid in benzene-diethylether (4:l) at 5-10 °C. Under these conditions the expected addition product **50** was isolated in 71% yield. Azido group was reduced with TPP in THF to give *ortho*-aminobenzaldehyde **51** in the 67% yield. Attempts to synthesise the quinolinone **31** by reacting **51** with glyoxal bisulphite and KCN were not successful as a black sticky polymeric material insoluble in most organic solvent was obtained. Probably the phenolic compound **31** was readily oxidised to the corresponding quinone (Scheme 11).



Scheme 11: Attempted synthesis of quinolinone part

As a free phenolic group was readily oxidized to quinone, it was decided to protect phenolic groups in benzaldehyde **51** as the methyl ether. Following this protocol, reactions as per **Scheme 12** were carried out. For the preparation of dimethyl *ortho*-aminobenzaldehyde **55**; commercially available 2,5-dimethoxybenzaldehyde **52** was nitrated[26] in DCM at room temperature to provide

3,6-dimethoxy-2-nitrobenzaldehyde **53**, which upon reduction with iron powder[27] in ethanol, acetic acid and water (2:2:1) afforded substituted aniline **54**. This aniline derivative which was unstable in air was brominated[28] immediately with bromine in acetic acid to give 2-amino-5-bromo-3,6-dimethoxybenzaldehyde **55**. Benzoin condensation of *ortho*-aminobenzaldehyde **55** with glyoxal bisulfite and KCN in 1M sodium carbonate solution furnished the quinolinone **56**. As in literature reports are there that free phenolic and aniline protons take part in Heck reaction, it was decided to protect these groups with methyl iodide. Methylation of quinolinone **56** was achieved with CH_3I and K_2CO_3 in DMF to furnish quinolinone **57** in 91% yield (Scheme 12).



Scheme 12: Synthesis of quinolinone part

Having successfully completed the synthesis of all the three required fragments, focus was directed towards coupling of these fragments one by one. Linking of aminohistamine fragment **46** and quinolinone fragment **57** first followed by condensation with spiroisoxazoline part **13** was considered to be a convenient and logical approach. Especially, the methods including carbon-carbon coupling are of high value. Heck, Suzuki, Stille, Sonogashira, Negishi are the most useful C-C bond

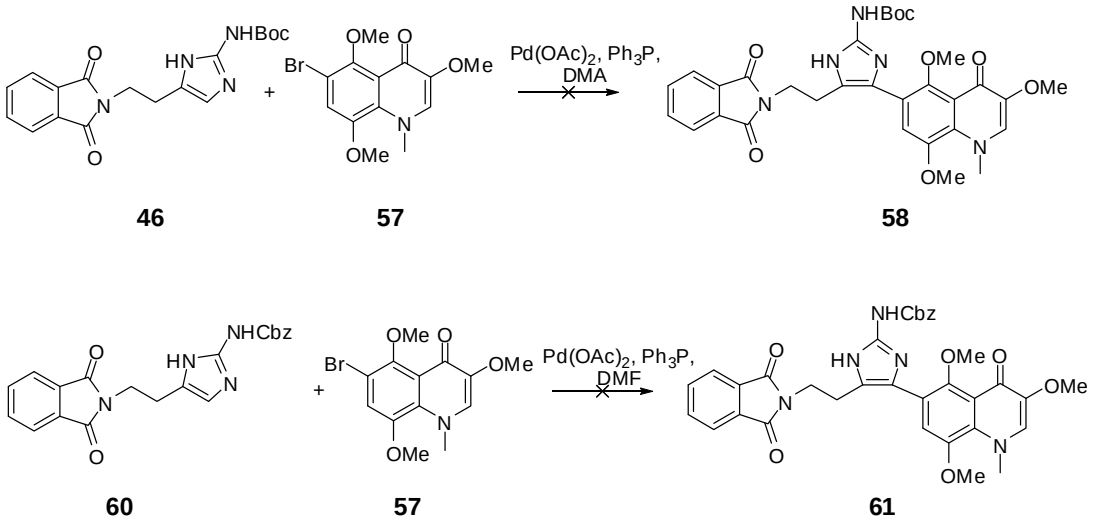
formation reactions. Palladium is a soft metal and hence according to HSAB (Hard Soft Acid Base) theory it reacts preferably with soft bases like aryls, alkenes and alkynes. In the quinolinone fragment**,** there is an aromatic bromo group; hence the strategy was to follow well known C-C bond formation reaction like Heck – coupling, Suzuki-coupling etc.

Attempted coupling *via* Heck reaction of 46 and 57

Heck reaction is a carbon-carbon bond forming reaction in which coupling of two sp^2-hybridised species in the presence of palladium catalyst is affected. The inter and intramolecular versions of Heck reaction have been widely applied for the total synthesis of myriad of bioactive organic compounds. By applying Heck reaction it is possible to form polyene, to couple fragments and to form cyclic frameworks. Numerous reviews covering the utility of the Heck reaction attest to it being one of the most widely utilized methodologies for the formation of C-C bonds. A traditional Heck reaction requires one electrophilic partner and one nucleophilic partner. Aryl/benzyl/vinyl halides as well as aryl/benzyl/vinyl triflates can act as the electrophilic partner and alkenes as the nucleophilic partner. However, the rate of reaction is high for olefins containing electron-withdrawing groups. The most widely used halide partners (electrophilic) for the coupling reactions are aryl halides. Reactivity of aryl halides toward the coupling reaction depends on the bond dissociation energy of the C-X bond. In the halogen family of the periodic table, the bond dissociation energy decreases from top to bottom and hence the reactivity order of these aryl halides increases in the same order. Various catalytic systems have been developed for Pd catalyzed Heck reactions including homogenous as well as heterogeneous catalysts, ligands as well as ligand free system, stable colloids, nanoparticles and polymer supported catalysts. For the Heck reactions catalyzed by Pd the turnover number (TON) is good. Nitrogen and phosphorous compounds are commonly used ligands in transition metal chemistry. Palladium complexes with various phosphines as ligands have been most commonly used as catalysts for the Mizoroki-Heck reaction. The ligand free catalytic systems have also been reported.

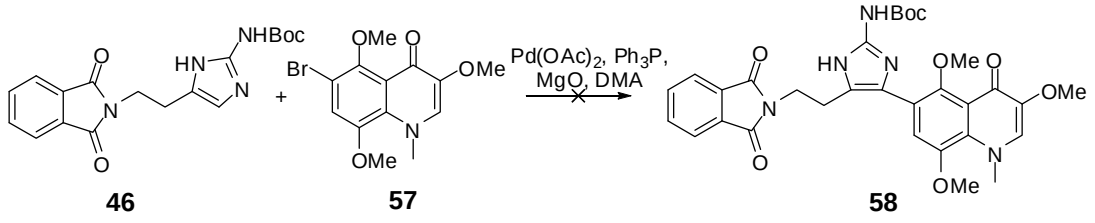
Coupling of fragments **46** with **57** in the presence of tetrakistriphenylphosphine palladium, $Pd(OAc)_2$, $PdCl_2$ or Pd(0) as catalysts did not yield the expected product **58**. However**,** in some reactions, formation of Boc deprotected aminohistamine fragment and debrominated quinolinone **59** was

observed. Changes in either solvent or protection of amino group from Boc to Cbz **60** did not afford the desired product (Scheme 13).



Scheme 13: Attempted coupling of quinolinone and aminohistamine fragments

In order to increase the nucleophilicity of carbon and to prevent the reactivity of amino group of imidazole in Heck reaction, magnesium salt of aminohistamine **46** was prepared and the reaction was carried out. However, the desired coupling reaction did not take place under these conditions also and the starting materials were recovered (Scheme 14).

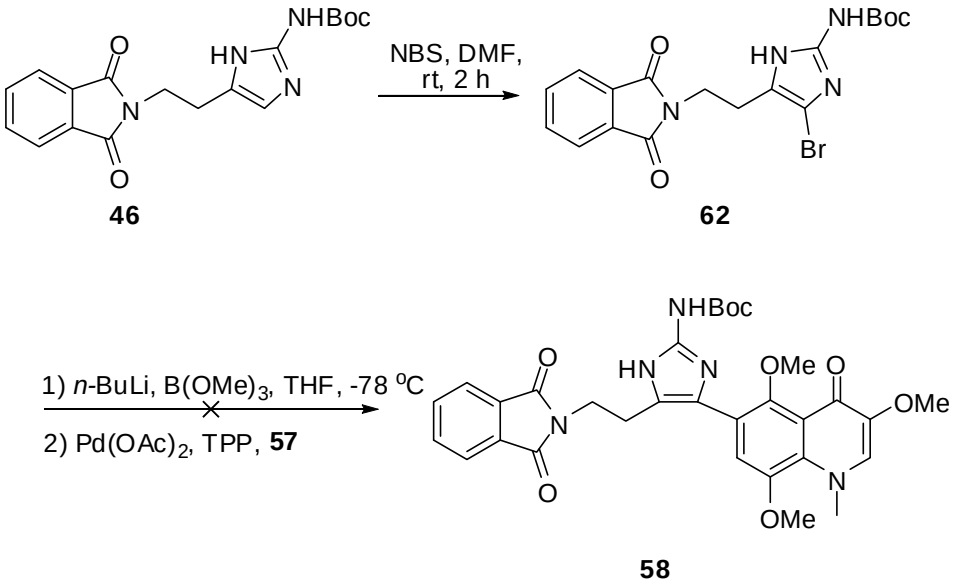


Scheme 14: Attempted coupling of quinolinone and aminohistamine fragments

Attempted coupling *via* Suzuki reaction of 62 and 57

Having met with failures to couple two fragments by Heck reaction, it was then decided to apply Suzuki reaction for coupling. According to the redesigned synthetic strategy focused on Suzuki coupling, requisite coupling partner has to be modified. As there is an aromatic bromo group in quinolinone part, the required boronic acid was to be prepared from aminohistamine part. In order to carry out the

desired coupling compound **46** was brominated with NBS in DMF to afford **62** and then it was converted to the corresponding boronate *via* metalation with *n*-BuLi and then reaction with trimethoxy borane in THF at -78 °C. This boronate was subjected to Suzuki coupling with quinolinone **57**; however, a highly polar product, practically insoluble in all common organic solvents was isolated which could not be characterised. Hence, this approach was abandoned (Scheme 15).



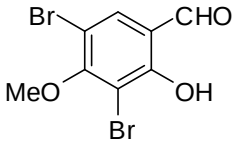
Scheme 15: Attempted coupling of quinolinone and aminohistamine fragments

Conclusion:

It is concluded that, during the total synthesis of the unusual dibromotyrosine based marine natural product **1**, the three important and interesting segments in their protected form have been successfully synthesised. Various coupling reactions of aminohistamine moiety with quinolinone part were carried out. However, the expected reactions did not proceed to yield the required product. It was also observed that hindered aryl bromide ring with electron donating groups is unreactive in C-C bond formation reactions. Further experiments are underway to achieve the total synthesis of the target molecule.

EXPERIMENTAL

3,5-Dibromo-2-hydroxy-4-methoxybenzaldehyde (34)



A solution of NBS (25.74 g, 144.60 mmol) in DMF (20 mL) was added to a solution of 2-hydroxy-4-methoxybenzaldehyde (10.00 g, 65.73 mmol) in DMF (50 mL) over 15 min at 0-5 °C. After the addition was complete, the reaction mixture was stirred for 1 h at rt. It was diluted with water (300 mL) and extracted with EtOAc (2 x 200 mL). The organic layer was washed with water, saturated aq. $Na_2S_2O_3$ solution and dried over Na_2SO_4. The solid was filtered off and the solvent was removed under vacuum to yield of 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (20.10 g, 98.7%) as a pale yellow solid; mp 100 ^oC.

Mol. Formula : $C_8H_6Br_2O_3$

Mol. Weight : 310

IR ($CHCl_3$) ν : 3020, 1658, 1607, 1467, 1417, 1289, 1060 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.96 (s, 3H), 7.73 (s, 1H), 9.75 (s, 1H), 11.72 (s, 1H)

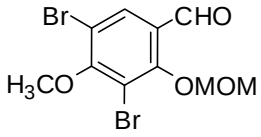
^{13}C NMR (50 MHz, $CDCl_3$) : δ 60.8, 107.8, 108.0, 118.7, 136.2, 159.6, 161.1, 193.9

ESI-MS *m/z* : 310.8/312.8 $[M+H]^+$

Elemental Analysis : Calcd: C, 31.00; H, 1.95; Br, 51.56%

Found: C, 30.87; H, 2.06; Br, 51.42%

3,5-Dibromo-4-methoxy-2-(methoxymethoxy)benzaldehyde (37)



A mixture of 3,5-dibromo-2-hydroxy-4-methoxybenzaldehyde (5.00 g, 16.13 mmol), Et_3N (2.45 g, 24.20 mmol) and methoxymethylchloride (1.43 g, 17.75 mmol) in THF (50 mL) was stirred under N_2 at rt for 2 h. The reaction mixture was poured in water and extracted with EtOAc (2 x 50 mL). The combined extract was washed with water, dried over Na_2SO_4, filtered and the solvent was removed under vacuum. The crude product obtained was purified by flash column chromatography (silica gel, hexane/EtOAc 5:1) to afford 3,5-dibromo-4-methoxy-2-(methoxymethoxy) benzaldehyde as a viscous oil (5.30 g, 93%).

Mol. Formula : $C_{10}H_{10}Br_2O_4$

Mol. Weight : 354

IR ($CHCl_3$) ν : 1643, 1615, 1485, 1080 cm^{-1}

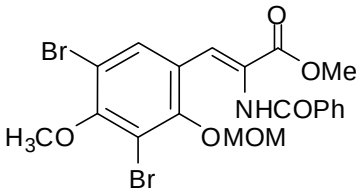
^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.60 (s, 3H), 3.95 (s, 3H), 5.20 (s, 2H), 8.05 (s, 1H), 10.20 (s, 1H)

ESI-MS *m/z* : 355.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 33.93; H, 2.85; Br, 45.14%

Found: C, 33.75; H, 2.89; Br, 44.95%

Methyl 2-benzamido-3-(3,5-dibromo-4-methoxy-2-(methoxymethoxy)phenyl) acrylate (40)



To a suspension of NaH (0.62 g, 15.54 mmol, 60% emulsion in oil) in THF (25 mL), a solution containing methyl 2-benzamido-2-(diethoxyphosphoryl)acetate **39** (5.58 g, 16.95 mmol) in THF (10 mL) was added over 10 min at rt. The reaction

mixture was stirred for an additional 30 min and then a solution of the aldehyde **37** (5.00 g, 14.12 mmol) in THF (10 mL) was added. The resultant mixture was stirred at rt until the reaction was complete as indicated by TLC. The solution was quenched with saturated NH_4Cl (20 mL) and extracted with EtOAc (3 X 50 mL). The combined EtOAc layer was washed with water (2 X 50 mL), dried over Na_2SO_4, and filtered. Evaporation of the solvent under vacuum yielded **40** as yellow viscous oil (6.45 g, 86%).

Mol. Formula : $C_{20}H_{19}Br_2NO_6$

Mol. Weight : 529

IR ($CHCl_3$) ν : 3316, 1727, 1654, 1580, 1279, 1160, 927 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.55-3.90 (m, 9H), 5.05-5.16 (m, 2H), 7.35-7.60 (m, 5H), 7.80-8.44 (m, 3H)

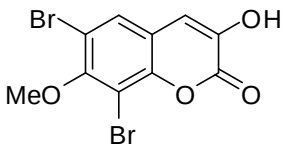
^{13}C NMR (50 MHz, $CDCl_3$) : δ 52.7, 52.9, 58.1, 58.4, 60.6, 99.8, 100.5, 112.2, 114.1, 123.2, 127.3, 128.8, 131.8, 132.2, 133.4, 152.6, 154.3, 155.3, 165.0, 165.2, 165.7

ESI-MS *m/z* : 310.8/312.8 $[M+H]^+$

Elemental Analysis : Calcd: C, 31.00; H, 1.95; Br, 51.56%

Found: C, 30.87; H, 2.06; Br, 51.42%

6,8-Dibromo-3-hydroxy-7-methoxy-coumarin (41)

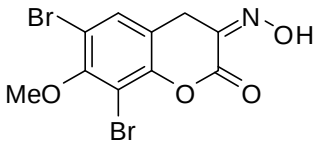


A solution of **40** (5.00 g, 10.39 mmol) in EtOH (50 mL) and HCl (6N, 50 mL) was heated under reflux for 12 h. The reaction mixture was concentrated in vacuum to give a yellow solid which was collected by filtration. The filtrate was washed successively with copious amounts of water, hexane and the product obtained was

dried in vacuum to give 6,8-dibromo-3-hydroxy-7-methoxy-coumarin **41** as a pale-yellow crystalline solid (3.35 g, 92%); mp 178-179 °C.

Mol. Formula	**:**	$C_{10}H_6Br_2O_4$
Mol. Weight	**:**	350
IR ($CHCl_3$) ν	**:**	3360, 1711, 1650, 1590, 1473, 1309, 1052, 886 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 3.91 (s, 3H), 6.87 (s, 1H), 7.56 (s, 1H), 10.11 (br s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 60.1, 105.5, 112.3, 112.6, 118.8, 127.4, 141.3, 145.5, 152.5, 157.1
ESI-MS *m/z*	**:**	350.9 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 34.32; H, 1.73; Br, 45.66% Found: C, 34.14; H, 1.81; Br, 45.47%

6,8-Dibromo-7-methoxy-3-hydroximino-3,4-dihydrocoumarin (42)



A solution of 6,8-dibromo-3-hydroxy-7-methoxycoumarin (3.00 g, 8.57 mmol), hydroxylamine hydrochloride (2.98 g, 42.86 mmol) and NaOAc (5.63 g, 68.58 mmol) in aqueous EtOH (80%, 30 mL) was heated at reflux until starting material consumed (monitored by TLC). The reaction mixture was concentrated in vacuum and the pale-yellow solid obtained was collected by filtration. It was then washed with copious amounts of water and dried in vacuum. Further purification by column chromatography (silica gel, hexanes: EtOAc 1:1) afforded 6,8-dibromo-7-methoxy-3-hydroximino-3,4-dihydrocoumarin **42** as a pale yellow solid (2.75 g, 88%); mp 193.5-195 °C.

Mol. Formula : $C_{10}H_7Br_2NO_4$

Mol. Weight : 365

IR ($CHCl_3$) ν : 3476, 2925, 1651, 1621, 1463, 1377, 1050, 964 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.65 (s, 2H), 3.67 (s, 3H), 7.40 (s, 1H), 10.27 (br s, 1H)

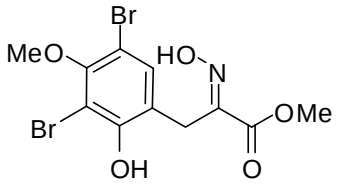
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) : δ 24.7, 59.9, 106.1, 107.7, 120.3, 133.7, 148.8, 152.7, 153.3, 162.2

ESI-MS *m/z* : 365.9 $[M+H]^+$

Elemental Analysis : Calcd: C, 32.91; H, 1.93; Br, 43.79; N, 3.84%

Found: C, 32.86; H, 1.97; Br, 43.65; N, 3.73%

Methyl 3-(3,5-dibromo-2-hydroxy-4-methoxy-phenyl)-2(E)-(hydroximino) propanoate (11)



A solution of 6,8-dibromo-7-methoxy-3-hydroximino-3,4-dihydrocoumarin **42** (1.00 g, 2.74 mmol) and NaOMe (162 mg, 162.82 mmol) in MeOH (10 mL) was stirred until the starting material was fully consumed. The solvent was removed under vacuum and residue obtained was dissolved in EtOAc (50 mL). The organic layer washed with dilute HCl (0.1M, 50 mL), brine (50 mL) and dried over Na_2SO_4 concentration of the solvent under vacuum to give **11** as colorless solid (0.98 g, 90%); mp 146-147 °C.

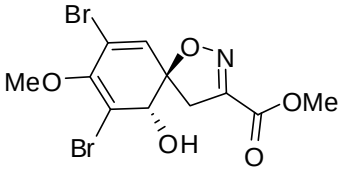
Mol. Formula : $C_{11}H_{11}Br_2NO_5$

Mol. Weight : 397

IR ($CHCl_3$) ν : 3465, 3020, 2956, 1730, 1547, 1215, 668 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 3.86 (s, 3H), 3.90 (s, 3H), 3.93 (s, 2H), 7.40 (s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 25.5, 53.6, 60.6, 107.7, 119.3, 133.4, 149.8, 151.4, 153.6, 164.4
ESI-MS *m/z*	**:**	398.1 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 33.28; H, 2.79; Br, 40.25; N, 3.53%
		Found: C, 33.16; H, 2.83; Br, 40.06; N, 3.43%

(5*R*,10*S*)-Methyl 7,9-dibromo-10-hydroxy-8-methoxy-1-oxaspiro[4.5]deca-2,6,8-triene-3-carboxylate (13)



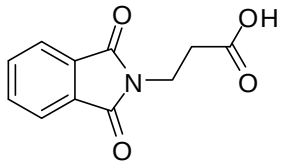
A mixture of NBS (0.54 g, 3.02 mmol) and oxime ester **11** (0.80 g, 2.02 mmol) in DMF (10 mL) was stirred at rt for 4 h. Ethyl acetate (20 mL) was added to the reaction mixture, the resultant solution was then washed with water followed by aq. $Na_2S_2O_3$ solution, dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to provide crude spiroisoxazoline **12.** To a solution of spiroisoxazoline **12** in CH_2Cl_2 (5 mL) was added $Zn(BH_4)_2$ (ethereal solution, 0.5 M, 4 mL) at rt under an argon atmosphere; the resultant mixture was then stirred for 10 min. After the addition of water (0.4 mL), the mixture was stirred for another 20 min, dried over Na_2SO_4. The resulting mixture was filtered and the filtrate was concentrated under vacuum. Further purification by column chromatography (silica gel, hexanes/EtOAc 3:1) furnished compound **13** (0.27 g, 34%).

The thick oil **13** resolved by dissolving in DCM (10 mL) to which pyridine (0.29 g, 3.68 mmol) was added. It was cooled to 0 °C and camphanic chloride (0.21 g, 1.00 mmol) was added with stirring. The reaction mixture was stirred for 1 h, poured into water and extracted with DCM (20 mL). The organic layer was washed with water, concentrated and the product obtained was purified by column chromatography (silica gel, hexanes/EtOAc 9:1) to afford the two resolved isomers as camphonic esters. The required isomer ester was dissolved in MeOH (10 mL), to which K_2CO_3

was added and the reaction mixture was stirred for 4 h at rt. Methanol was removed under vacuum and the residue was diluted with water (20 mL), then acidified with dil HCl to pH 6 followed by extraction with chloroform. Organic layer was washed with water, brine and concentrated to afford the pure isomer (- **13**) (0. 11 g, 16%).

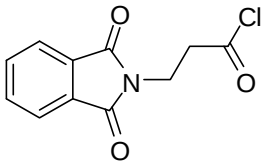
Mol. Formula	:	$C_{11}H_{11}Br_2NO_5$
Mol. Weight	:	397
$[\alpha]_D^{25}$	:	-206° (*c* 1.0, benzene) [lit. -210 (*c* 1.0, benzene]
IR ($CHCl_3$) ν	:	3430, 2931, 1721, 1581, 1216, 670 cm^{-1}
^{1}H NMR (200 MHz, acetone-d_6)	:	δ 3.21 (d, *J* = 18.0 Hz, 1H), 3.73 (s, 3H), 3.83 (s, 3H), 3.85 (d, *J* = 18.0 Hz, 1H), 4.21(d, *J* = 8.0 Hz, 1H), 5.40 (d, *J* = 8.0 Hz, 1H), 6.51 (s, 1H)
^{13}C NMR (50 MHz, acetone-d_6)		δ 39.9, 52.8, 60.3, 75.2, 92.4, 113.9, 122.2, 132.2, 148.9, 152.4, 161.1
ESI-MS *m/z*	:	398.2 $[M+H]^+$
Elemental Analysis	:	Calcd: C, 33.28; H, 2.79; Br, 40.25; N, 3.53 %
		Found: C, 33.05; H, 2.86; Br, 40.13; N, 3.49 %

3-Phthalimidopropionic acid (43)



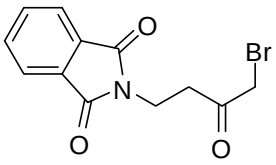
A mixture of phthalic anhydride (14.80 g, 100 mmol), β-alanine (8.91 g, 100 mmol) and *p*-toluenesulfonic acid (10 mg) was refluxed together for 8 h in toluene (100 mL); with continuous removal of water (Dean-Stark). The mixture was then washed with water and the solvent toluene concentrated to get the desired product (21.30 g, 97%) as pure white crystals; mp 150-151.5 ^oC, (Lit.[20] 151 °C).

3-Phthalimidopropionyl chloride (44)



3-Phthalimidopropionic acid (5.00 g, 22.81 mmol) was mixed with thionyl chloride (20 mL) and stirred for 4 h at rt. The mixture was then warmed to 50 ^{o}C for 1 h, and the resulting clear solution was distilled out to remove excess thionyl chloride. The crystalline residue was taken up in hot dry benzene (30 mL) and this solution was diluted with petroleum ether (30 mL). The white solid obtained was filtered out and washed with petroleum ether (20 mL). The yield of white crystalline acid chloride was 5.30 g (98%); mp 105.0-106.5 ^{o}C (Lit.[21] 107-108 °C).

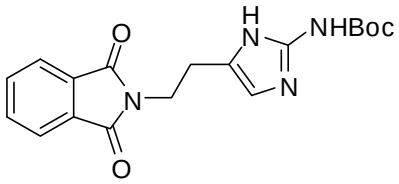
1-Bromo-4-phthalimido-butan-2-one (45)



A solution of diazomethane in diethyl ether (100 mL) was prepared from 18.00 g of nitrosomethyl urea was dried for 2 h over potassium hydroxide pellets. This solution was placed in a flask provided with a stirrer, a dropping funnel, thermometer and cooled to 0 ^{o}C. 3-Phthalimidopropionyl chloride (5.00 g, 21.04 mmol) in benzene (20 mL) was added to this solution from a dropping funnel during 1 h. The resulting solution was stirred at 0 ^{o}C for 2 h and then allowed to warm up to 20 ^{o}C. The solution was again cooled to 0 ^{o}C and 48% hydrobromic acid (10 mL) was added drop wise with stirring over a period of fifteen minutes. After stirring for 1 h, the solution was placed in a separating funnel and shaken with excess aqueous sodium carbonate solution. It was then dried over Na_2SO_4 and concentrated in vacuum giving a white crystalline residue. The crude product obtained was purified by column chromatography (silica gel, hexane/EtOAc 7:3) to yield 1-bromo-4-phthalimido-2-butanone (5.60 g, 90%); mp 118-119 ^{o}C (Lit.[21] 119-120 °C).

Mol. Formula	:	$C_{12}H_{10}BrNO_3$
Mol. Weight	:	296
IR ($CHCl_3$) ν	:	3022, 2967, 1848, 1771, 1708, 1463, 1437, 1375, 1081, 876 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	:	δ 3.12 (t, *J* = 7.1 Hz, 2H), 3.93 (s, 2H), 4.01 (t, *J* = 7.1 Hz, 2H), 7.65-7.80 (m, 2H), 7.80-7.90 (m, 2H)
^{13}C NMR (50 MHz, $CDCl_3$)	:	δ 33.0, 33.8, 37.94, 123.3, 131.9, 134.1, 167.9, 168, 199.6
ESI-MS *m/z*	:	296.2/298.2 $[M+H]^+$
Elemental Analysis	:	Calcd: C, 48.67; H, 3.40; Br, 26.98; N, 4.73%
		Found: C, 48.48; H, 3.53; Br, 26.77; N, 4.63%

***tert*-Butyl 5-(2-(1,3-dioxoisoindolin-2-yl)ethyl)-1*H*-imidazol-2-ylcarbamate (46)**



α-Bromo ketone **45** (3.00 g, 10.13 mmol) was added to a stirred solution of mono-Boc-guanidine (3.23 g, 20.26 mmol) in 20 mL of dry DMF. After 48 h, the reaction mixture was poured into water and mixture was extracted with EtOAc (2 x 20 mL). The combined extracts was washed with water and dried over Na_2SO_4. Concentration of the solvent the crude product which was further purified by column chromatography (silica gel, hexane/EtOAc 7:3) to afford *tert*-butyl 5-(2-(1,3-dioxoisoindolin-2-yl)ethyl)-1*H*-imidazol-2-ylcarbamate (3.10 g, 86%).

Mol. Formula : $C_{18}H_{20}N_4O_4$

Mol. Weight : 356

IR ($CHCl_3$) ν : 3393, 3020, 1772, 1738, 1715, 1621, 1437, 1129, 1004, 847 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.56 (s, 9H), 2.76 (t, *J* = 7.2 Hz, 2H), 3.94 (t, *J* = 7.2 Hz, 2H), 5.66 (s, 1H), 6.61 (s, 1H), 7.65-7.75 (m, 2H), 7.75-7.90 (m, 2H)

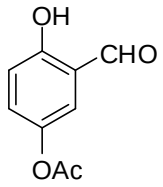
^{13}C NMR (50 MHz, $CDCl_3$) : δ 27.1, 27.9, 37.2, 84.7, 107.6, 123.2, 132.2, 133.8, 135.0, 150.2, 168.2

ESI-MS *m/z* : 357.3 $[M+H]^+$

Elemental Analysis : Calcd: C, 60.66; H, 5.66; N, 15.72%

Found: C, 60.44; H, 5.71; N, 15.56%

5-Acetoxy-2-hydroxybenzaldehyde (47)



2,5-Dihydroxybenzaldehyde (5.00 g, 36.2 mmol) dissolved in 15 mL of dry pyridine, was cooled to -10 ^oC and acetic anhydride (3.70 g, 36.2 mmol) was added over a period of 10 minutes, maintaining the temperature -5 ^oC . The mixture was allowed to stand in an ice-bath for 1 h and then warmed to rt. The product was poured into ice-water, filtered and the crude solid dissolved in hot ethanol and then refrigerated. The crystalline material obtained was filtered off to yield 5-acetoxy-2-hydroxybenzaldehyde (4.60 g, 71%); mp 80-81 ^oC (Lit.[22] 80-81 °C).

Mol. Formula : $C_9H_8O_4$

Mol. Weight : 180

IR ($CHCl_3$) ν : 3023, 2852, 1760, 1661, 1589, 1482, 1215, 1014 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.32 (s, 3H), 7.00 (d, *J* = 8.8 Hz, 1H), 7.26 (dd, *J* = 2.8 8.8 Hz, 1H), 7.33 (d, *J* = 2.8 Hz, 1H), 9.86 (s, 1H), 10.92 (s, 1H)

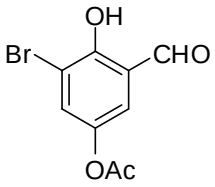
^{13}C NMR (50 MHz, $CDCl_3$) : δ 20.9, 118.7, 120.1, 125.3, 130.7, 142.9, 159.2, 169.5

ESI-MS *m/z* : 181.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 60.00; H, 4.48%

Found: C, 59.87; H, 4.53%

3-Bromo-5-acetoxy-2-hydroxybenzaldehyde (48)



A solution containing NBS (3.26 g, 18.32 mmol) in dry DMF (5 mL) was added slowly over 15 min to a solution of 5-acetoxy-2-hydroxybenzaldehyde (3.00 g, 16.65 mmol) in dry DMF (15 mL) and the resultant mixture was stirred at rt for 5 h. It was then poured into water and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic layer was washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield 3-bromo-5-acetoxy-2-hydroxybenzaldehyde as yellow thick oil (4.12 g, 95%).

Mol. Formula : $C_9H_7BrO_4$

Mol. Weight : 259

IR ($CHCl_3$) ν : 3685, 2856, 1769, 1666, 1443, 1373, 1123, 980, 669 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.32 (s, 3H), 7.35 (d, *J* = 2.7 Hz, 1H), 7.57 (d, *J* = 2.7 Hz, 1H), 9.82 (s, 1H), 11.49 (s, 1H)

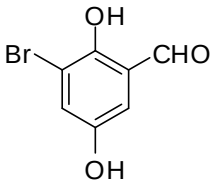
^{13}C NMR (50 MHz, $CDCl_3$) : δ 20.9, 111.3, 120.2, 124.9, 133.6, 142.9, 156.0, 169.2, 195.2

ESI-MS *m/z* : 259.1/261.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 41.73; H, 2.72; Br, 30.84%

Found: C, 41.55; H, 2.76; Br, 30.71%

3-Bromo-2,5-dihydroxybenzaldehyde (49)



To a stirred solution of acetate **48** (4.00 g, 15.44 mmol) in MeOH (20 mL) was added K_2CO_3 (2.77 g, 20.07 mmol) in one portion at rt. The reaction mixture was stirred for 2 h and methanol was removed under vacuum. The residue obtained was dissolved in water, acidified with dil. HCl and was extracted with EtOAc (2 x 30 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated. Purification by flash column chromatography (silica gel, hexane/EtOAc 4:1) afforded the 3-bromo-2,5-dihydroxybenzaldehyde as thick oil (3.15 g, 94%).

Mol. Formula : $C_7H_5BrO_3$

Mol. Weight : 217

IR ($CHCl_3$) ν : 3778, 3020, 1774, 1718, 1469, 1373, 1019 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) : δ 7.04 (d, *J* = 2.8 Hz, 1H), 7.38 (d, *J* = 2.8 Hz, 1H), 9.15 (s, 1H), 9.78 (s, 1H), 11.00 (s, 1H)

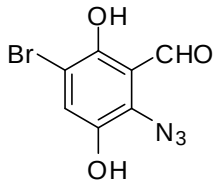
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) : δ 110.5, 118.0, 120.5, 128.1, 150.4, 151.0, 195.8

ESI-MS *m/z* : 217.1/219.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 38.74; H, 2.32; Br, 36.82%

Found: C, 38.58; H, 2.39; Br, 36.64%

2-Azido-5-bromo-3,6-dihydroxybenzaldehyde (50)



3-Bromo-2,5-dihydroxybenzaldehyde (2.00 g, 9.22 mmol), silver oxide (6.41 g, 27.65) and anhydrous Na_2SO_4 (5.00 g) were added simultaneously to dry benzene (100 mL) contained in RB flask. The mixture was stirred at rt for 1 h and then filtered. This filtrate was added slowly to the vigorously stirred solution of HN_3 in diethyl ether at 5-10 ^oC. Stirring was continued for 30 min at rt, the mixture was then concentrated under reduced pressure and the residue obtained was purified by flash column chromatography (silica gel, hexane/EtOAc 4:1) to afford the 2-azido-5-bromo-3,6-dihydroxybenzaldehyde as yellow solid (1.85 g, 78%).

Mol. Formula : $C_7H_4BrN_3O_3$

Mol. Weight : 258

IR ($CHCl_3$) ν : 3465, 2942, 2087, 1889, 1741, 1571, 1447 1047, 848 cm^{-1}

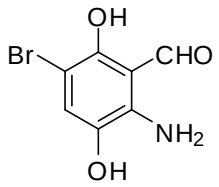
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) : δ 7.36 (s, 1H), 9.70 (s, 1H), 10.24 (s, 1H), 11.82 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) : δ 104.3, 112.5, 127.8, 128.2, 144.3, 151.9, 193.8

ESI-MS *m/z* : 258.1/260.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 32.58; H, 1.56; Br, 30.97; N, 16.29%
Found: C, 32.43; H, 1.67; Br, 30.73; N, 16.02%

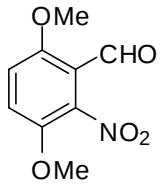
2-Amino-5-bromo-3,6-dihydroxybenzaldehyde (51)



To a solution of azide **50** (2.00 g, 7.75 mmol) and triphenylphosphine (2.24 g, 8.53 mmol) in THF (20 mL), water (1 mL) was added and reaction mixture was stirred for 6 h at rt and then concentrated under vacuum. The residue obtained was extracted with EtOAc (2 x 30 mL) and the combined EtOAc layer was dried over Na_2SO_4. After filtration and concentration under vacuum the crude product was obtained yellowish oil. Further purification by flash column chromatography (silica gel, hexane/EtOAc 8:2) yielded the aniline derivative **51** (1.45 g, 81%).

Mol. Formula	**:**	$C_7H_6BrNO_3$
Mol. Weight	**:**	232
IR ($CHCl_3$) ν	**:**	3460, 3330, 1652, 1560, 1396, 1095, 920, 667 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 6.60 (br s, 2H), 7.05 (s, 1H), 9.80 (s, 1H), 10.28 (s, 1H), 11.23 (s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 98.4, 113.2, 118.5, 142.4, 143.6, 152.6, 191.5
ESI-MS *m/z*	**:**	232.0/234.0 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 36.23; H, 2.61; Br, 34.44; N, 6.04% Found: C, 35.98; H, 2.74; Br, 34.25; N, 5.94%

3,6-Dimethoxy-2-nitrobenzaldehyde (53)



To a stirred solution of 2,5-dimethoxybenzaldehyde (10.00 g, 60.18 mmol) in DCM (100 mL) at 0 °C was added a solution of 80% HNO_3 (5.69 g, 72.21 mmol). The resultant solution was stirred at rt for 2 h, diluted with water (100 mL) and extracted with chloroform (3 x 50 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated. The residue obtained was purified by flash column chromatography (silica gel, hexane/EtOAc 7:3) to give 3,6-dimethoxy-2-nitrobenzaldehyde as yellow solid (9.50 g, 75%); mp 162 ^oC (Lit.[26] 163-165 °C).

Mol. Formula : $C_9H_9NO_5$

Mol. Weight : 211

IR ($CHCl_3$) ν : 3102, 2892, 1684, 1528, 946, 816 cm^{-1}

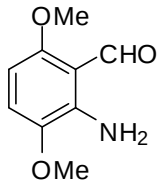
^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.87 (s, 3H), 3.95 (s, 3H), 7.11 (d, *J* = 9.4 Hz, 1H), 7.29 (d, *J* = 9.4 Hz, 1H), 10.36 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 56.7, 57.2, 114.1, 116.0, 116.7, 120.0, 144.4, 155.2, 186.1

ESI-MS *m/z* : 212.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 51.19; H, 4.30; N, 6.63%
Found: C, 50.93; H, 4.38; N, 6.47%

2-Amino-3,6-dimethoxybenzaldehyde (54)



3,6-Dimethoxy-2-nitrobenzaldehyde (5.00 g, 23.68 mmol) and iron powder (10.00 g) were added to a mixture containing ethanol (40 mL), acetic acid (40 mL), water (20 mL) and 35% hydrochloric acid (1 drop). The resultant suspension was refluxed while stirring vigorously for 30 min. It was then cooled and filtered through celite. The filtrate was diluted with water (300 mL) and extracted with chloroform (3 x 50 mL). The organic layers were combined, sequentially washed with 9% aq. sodium bicarbonate (100 mL) and water (2 x 100 mL), dried over Na_2SO_4 and evaporated to give 2-amino-3,6-dimethoxybenzaldehyde as yellow solid (4.10 g, 96%); mp 68 ^oC (Lit.[26] 67-68 °C).

Mol. Formula : $C_9H_{11}NO_3$

Mol. Weight : 181

IR ($CHCl_3$) ν : 3436, 1651, 1558, 1481, 1448, 1266, 1095, 1019, 666 cm^{-1}

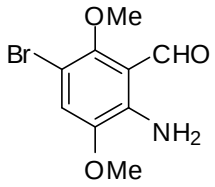
^{1}H NMR (200 MHz, $CDCl_3$) : δ 3.81 (s, 3H), 5.98 (d, *J* = 8.6 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 1H), 10.40 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 55.1, 55.6, 94.2, 108.0, 114.4, 140.4, 142.1, 156.3, 191.3

ESI-MS *m/z* : 182.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 59.66; H, 6.12; N, 7.73%
Found: C, 59.45; H, 6.19; N, 7.53%

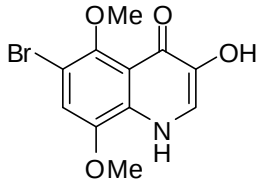
2-Amino-5-bromo-3,6-dimethoxybenzaldehyde (55)



To a stirred solution of 2-amino-3,6-dimethoxybenzaldehyde (3.00 g, 16.56 mmol) in acetic acid (10 mL) at 10-15 °C was added a solution of bromine (0.40 g, 2.5 mmol) in acetic acid (5 mL). The resultant mixture was stirred at rt for 1 h, diluted with water (50 mL) and extracted with chloroform (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4 and evaporated. The residue was purified by rapid column chromatography (silica gel (230-400), hexane/EtOAc 7:3) to give of 2-amino-5-bromo-3,6-dimethoxybenzaldehyde (2.75 g, 67%) as yellow oil.

Mol. Formula	**:**	$C_9H_{10}BrNO_3$
Mol. Weight	**:**	260
IR ($CHCl_3$) ν	**:**	3408, 3018, 1748, 1653, 1492, 1475, 1051 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 3.85 (s, 3H), 3.88 (s, 3H), 6.67 (br s, 2H), 6.93 (s, 1H), 10.25 (s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$)	**:**	δ 55.8, 62.7, 98.5, 111.7, 117.6, 141.7, 143.4, 153.4, 191.4
ESI-MS *m/z*	**:**	260.1/262.1 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 41.56; H, 3.88; Br, 30.72; N, 5.39% Found: C, 41.33; H, 3.95; Br, 30.58; N, 5.26%

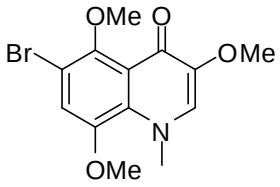
6-Bromo-3-hydroxy-5,8-dimethoxyquinolin-4(1*H*)-one (56)



Potassium cyanide (4.01 g, 61.52 mmol) was added to a stirred 1 M solution of sodium carbonate (50 mL) under argon atmosphere. Glyoxal bisulphite (10.25 g, 46.14 mmol) followed by 2-amino-5-bromo-3,6-dimethoxybenzaldehyde (4.00 g, 15.38 mmol) were added to this reaction mixture. Resultant mixture was then stirred for 5 h at 60 ^{o}C, cooled to rt and acidified with acetic acid to pH 6. The mixture was stirred for further 5 h and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum to afford crude product as thick oil. Purification by flash column chromatography (silica gel, MeOH/EtOAc 1:9) yielded 6-bromo-3-hydroxy-5,8-dimethoxyquinolin-4(1*H*)-one as yellowish thick oil (1.60 g, 35%).

Mol. Formula	**:**	$C_{11}H_{10}BrNO_4$
Mol. Weight	**:**	300
IR ($CHCl_3$) ν	**:**	3778, 3019, 1650, 1571, 1518, 1424, 1078, 929 cm^{-1}
^{1}H NMR (500 MHz, DMSO-d_6)	**:**	δ 3.81 (s, 3H), 3.99 (s, 3H), 7.15 (s, 1H), 7.52 (s, 1H), 8.12 (s, 1H), 11.13 (br s, 1H)
^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 54.7, 59.4, 106.2, 110.6, 116.1, 117.0, 128.8, 141.1, 143.2, 146.4, 166.9
ESI-MS *m/z*	**:**	300.1/302.1 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 44.02; H, 3.36; Br, 26.63; N, 4.67% Found: C, 43.94; H, 3.41; Br, 26.53; N, 4.58%

6-Bromo-3,5,8-trimethoxy-1-methylquinolin-4(1*H*)-one (57)



A mixture of 6-bromo-3-hydroxy-5,8-dimethoxyquinolin-4(1*H*)-one (1.00 g, 3.33 mmol), K_2CO_3 (1.15 g, 8.33 mmol) and methyl iodide (1.04 g, 7.33 mmol) in DMF (10 mL) was stirred at rt for 2 h. The reaction mixture was poured in water and extracted with EtOAc (2 X 20 mL). The combined extract was washed with water, dried over Na_2SO_4, filtered and the solvent was removed under vacuum. The crude product was purified by column chromatography (silica gel, EtOAc /MeOH 9:1) to afford 6-bromo-3,5,8-trimethoxy-1-methylquinolin-4(1*H*)-one as a brown solid (0.99 g, 91%).

Mol. Formula	**:**	$C_{13}H_{14}BrNO_4$
Mol. Weight	**:**	328
IR ($CHCl_3$) ν	**:**	3020, 2932, 1713, 1637, 1476, 1434, 1112, 1037, 929 cm^{-1}
^{1}H NMR (500 MHz, DMSO-d_6)	**:**	δ 3.69 (s, 3H), 3.71 (s, 3H), 3.88 (s, 3H), 3.99 (s, 3H), 7.36 (s, 1H), 7.70 (s, 1H)
^{13}C NMR (50 MHz, DMSO)	**:**	δ 46.2, 57.1, 57.2, 61.0, 109.6, 116.6, 122.6, 131.7, 132.8, 143.1, 146.9, 149.3, 168.6
ESI-MS *m/z*	**:**	328.1/330.1 $[M+H]^+$
Elemental Analysis	**:**	Calcd: C, 47.58; H, 4.30; Br, 24.35; N, 4.27% Found: C, 47.06; H, 4.38; Br, 24.19; N, 4.06%

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Chapter 2

Section I

An expeditious convergent synthesis of a dibromotyrosine alkaloid inhibitor of mycothiol-*S*-conjugate amidase

Introduction

Marine Natural Products

Nature continues to be one of the most important sources of pharmacologically active compounds in the quest for drugs against life threatening diseases such as microbial infections, diseases of the heart and the circulatory system, cancer and others. Almost 40% of the top selling pharmaceutical drugs are natural products or natural product derivatives.

The first active compounds to be isolated from marine species were spongouridine and spongothymidine (Figure 1) from the Carribean sponge *Cryptotheca crypta* in the 1950s. These compounds are nucleotides and show great potential as anticancer and antiviral agents. Their discovery led to an extensive research to identify novel drug candidates from marine sources. About 70% of the earth's surface is covered by the oceans, providing significant biodiversity for exploration for drug sources. Many marine organisms have a sedentary lifestyle and thereby synthesize many complex and extremely potent chemicals as their means of defense from predators.[1] These chemicals can serve as possible remedies for various ailments, especially cancer. One such example is discodermolide, isolated from the marine sponge, *Discodermia dissoluta*.

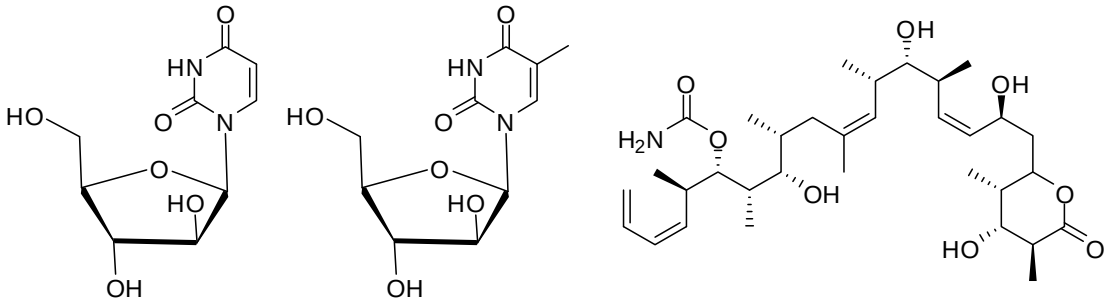


Figure 1

While chemical investigations of terrestrial plants and insects started in the beginning of the 19th century, the first reports of marine natural products chemistry did not appear until early in the 20th century, with the isolation of sterols from marine sponges by Henze[2] and Doree[3] followed by the isolation and characterization of the ancient Egyptian dye tyrian purple from molluscs.[4] In 1943, Bergmann isolated

gorgosterol from a gorgonian,[5] but only in 1969, when Weinheimer and Spraggins discovered prostaglandins in the octocoral Plexaura homomalla,[6] did the pharmaceutical industry become interested in the discovery of new natural products from marine origin. The retarded development of marine compared to "terrestrial" natural product chemistry was mainly due to the difficulties involved in the collection of marine organisms. Only with the development of the SCUBA diving technology in the 1960 were scientists finally able to explore the seas to look for new compounds with interesting biological and pharmacological activities. Over the past 30 years, marine natural products have been isolated from many types of marine organisms, including algae, invertebrates and micro-organisms (Figure 2).[7]

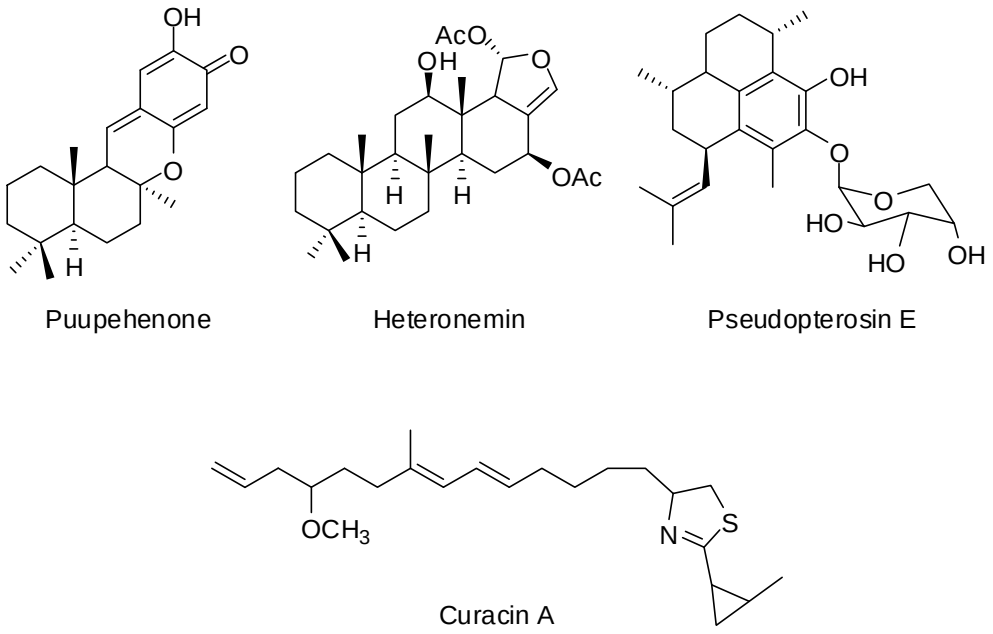


Figure 2: marine natural products

Role of Marine Natural Products in the Treatment of infectious Diseases

Tuberculosis

Tuberculosis (abbreviated as **TB**) is a common and often deadly infectious disease caused by mycobacteria, mainly *Mycobacterium tuberculosis*.[8] Tuberculosis usually attacks the lungs (as pulmonary TB) but can also affect the central nervous system and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti* and *Mycobacterium microti* also cause tuberculosis, but these species are less common.

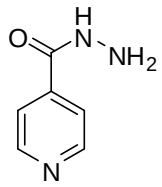
Transmission of tuberculosis

Tuberculosis is spread through the air, when people who have the disease cough, sneeze or spit. One third of the world's current population has been infected with *M. tuberculosis* and new infections occur at a rate of one per second.

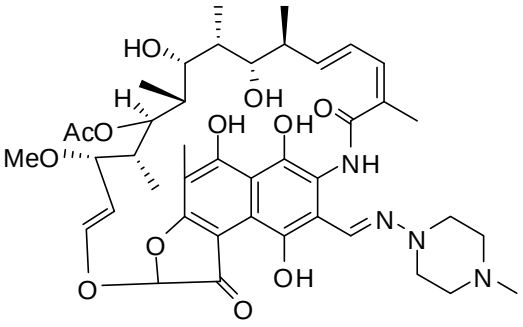
Diagnosis of tuberculosis

Tuberculosis is diagnosed definitively by identifying the causative organism (*Mycobacterium tuberculosis*) in a clinical sample (for example, sputum or pus). When this is not possible, a probable diagnosis may be made using imaging (X-rays or scans) and/or a tuberculin skin test. The main problem with tuberculosis diagnosis is the difficulty in culturing this slow-growing organism in the laboratory. A complete medical evaluation for TB must include a medical history, a physical examination, a chest X-ray and microbiological cultures. It may also include a tuberculin skin test, a serological test. Currently, latent infection is diagnosed in a non-immunized person by a tuberculin skin test, which yields a delayed hypersensitivity type response to an extract made from *M. tuberculosis*.

Isoniazid **1** (INH) is one of the most common drugs used for TB. It is inexpensive, effective and easy to take; it can prevent most cases of TB and, when used in conjunction with other drugs, cure most TB.



Isoniazid **1**



Rifampin **2**

Figure 3: Drugs used for TB

Isoniazid **1** and rifampin **2** (Figure 3) are the keystones of treatment; however due to increasing resistance to them, pyrazinamide and either streptomycin sulfate or ethambutol hydrochloride are added to regimens. Drug resistance may be either

primary or acquired. Primary resistance occurs in patients who have had no previous antimycobacterial treatment. Acquired resistance occurs in patients who have been treated in the past and it is usually a result of non-adherence to the recommended regimen or incorrect prescribing. It has been estimated that one in seven cases of tuberculosis is resistant to drugs that previously cured the disease. Resistance arises when patients fail to complete their drug therapy, lasting six months or longer. The hardiest TB bacteria are allowed to survive and as they multiply, they spread their genes to a new generation of bacteria and to new victims. The drug-resistant forms of TB that do not respond to the usual drug therapy might be treatable by other, sometimes more toxic drugs.

Mycothiol in treatment for Tuberculosis

Mycothiol biosynthesis[9] and mycothiol-dependent enzymes such as mycothiol-dependent formaldehyde dehydrogenase and mycothione reductase have been proposed to be good drug targets for treatments of tuberculosis. Mycothiol (MSH) **3** (Figure 4) is the major low molecular weight thiol limited to actinomycetes, which include mycobacteria. Analogous to glutathione in gram negative bacteria and eukaryotes, MSH plays a major role in detoxification and maintenance of a reductive intracellular environment.[10] Owing to the continuing need for new classes of antibiotics effective against *Mycobacterium tuberculosis*,[11] studies of enzymes involved in mycothiol biosynthesis and mycothiol-dependent detoxification, as well as those of inhibitors to these enzymes, are rapidly increasing in number.

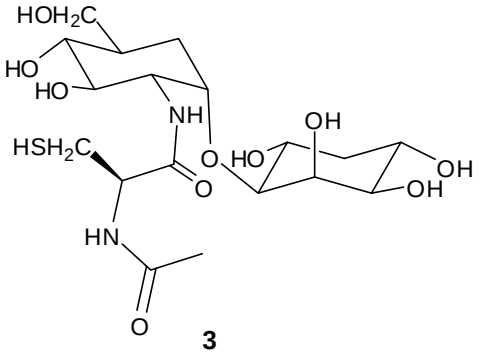


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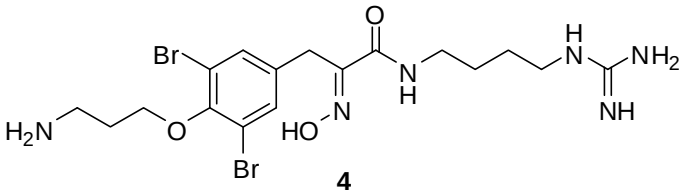
Figure 4: Mycothiol

The rise of mycobacterial resistance to common antituberculars such as isoniazid and rifampin, along with the high prevalence of tuberculosis and *Mycobacterium avium* complex in AIDS patients, has led to a renewed interest in the

discovery of antimycobacterial agents with new modes of action. In an effort to identify new classes of antimycobacterials, Bewley and coworkers have screened a variety of marine extracts for their ability to inhibit a novel mycobacterial enzyme, mycothiol-*S*-conjugate amidase (MCA)[12] and report the isolation[13] and structure elucidation of new alkaloids that inhibit MCA. Inhibitors are chemical compounds that can bind to enzymes and form stable complexes, thus, decreasing their activity. Of those structures studied, kinetics experiments established that the bromotyrosine-derived series of alkaloids, such as **4** (Figure 5), is the most active inhibitors of the group and is also competitive inhibitors of the *M. tuberculosis* detoxification enzyme MCA. Kinetics, NMR and modeling experiments suggest that this class of compounds presumably acts by chelation of a metal cation in the active site *via* the oxime moiety.[14]



4

Figure 5

Bromotyrosines from marine sponges

Many bromotyrosine alkaloids have been isolated in recent years and a few examples are given in **Figure 6**. Pseudoceratinines A **5** and pseudoceratinines B **6** have been isolated[15] from two specimens of the sponge *Pseudoceratina verrucosa*. Aplysamine **7** and purpuramine I **8** and purpuramine J **9** were isolated[16] from the Fijian marine sponge *Druinella* sp. These two compounds were found to have moderate cytotoxic activity. Purealidin A **10** was isolated[17] from an Okinawan marine sponge, which was a precursor of purealin. Lipopurealins A **11**, B **12** and C **13** were isolated[18] from *psammaplysilla purea*. Compared to purealin, lipopurealins A, B and C belong to a new type of bromotyrosine derived metabolites, which have side chain fatty acids rather than spiroisoxazoline rings.

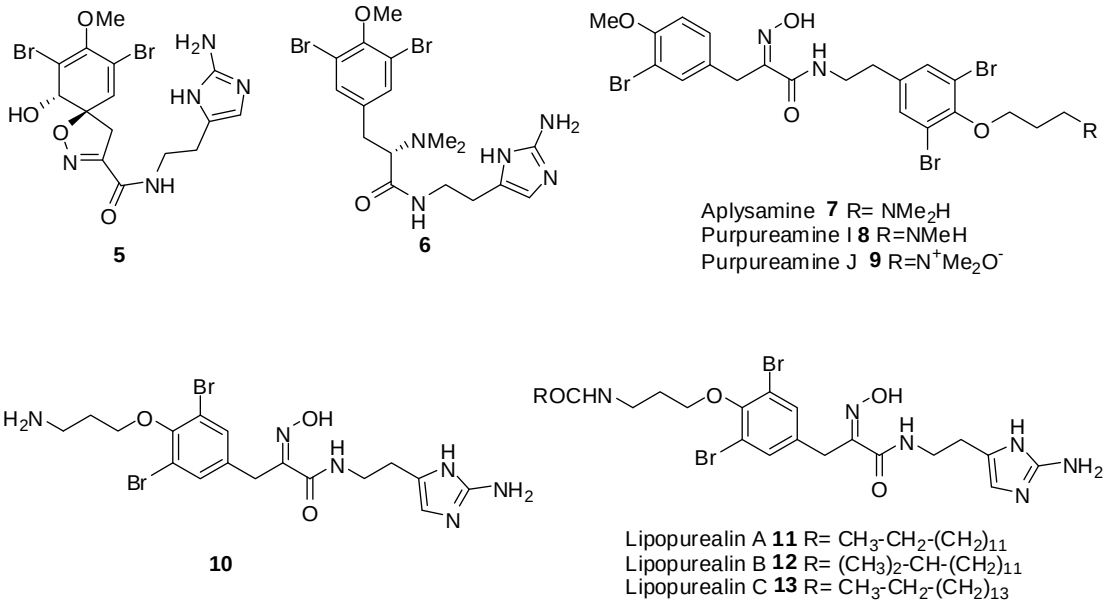


Figure 6: Structures of bromotyrosine derived natural alkaloids

Several marine natural products that appear to be biogenetically derived from a bromotyrosine precursor have been synthesised during the past three decades. Owing to the variety of interesting biological activities exhibited by members of this class of compounds, a number of syntheses have also been completed.

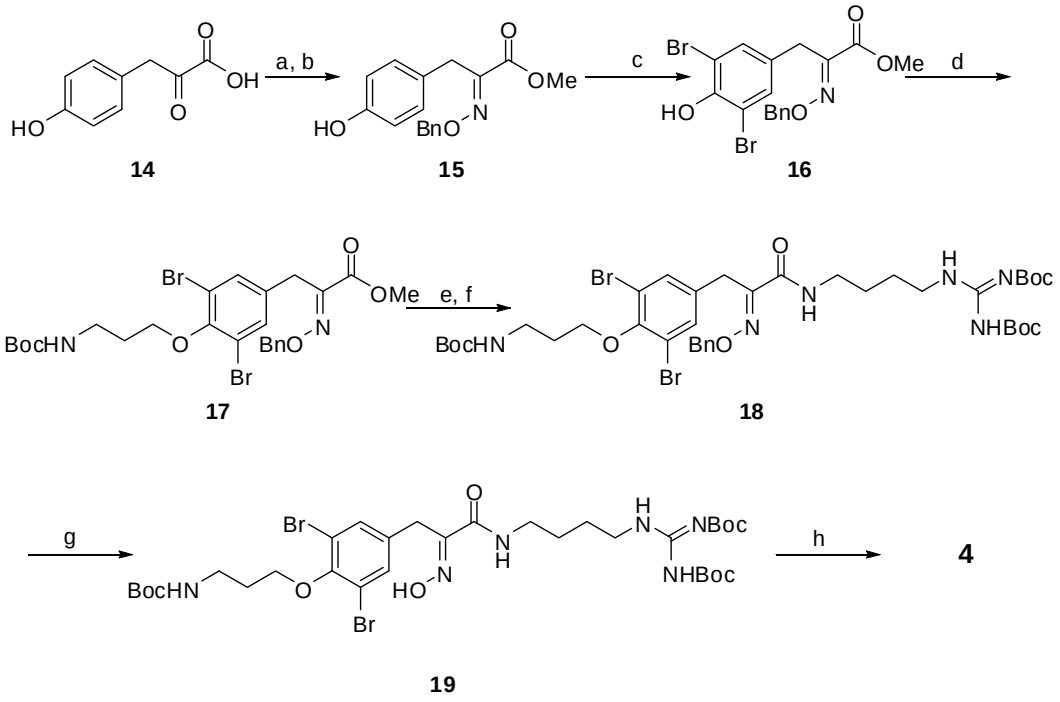
Reported Synthetic Approaches for 4

Synthesis of **4** in 2003 was undertaken for the first time when no other synthesis was reported. While the work was in progress Kende *et al*. completed the first total synthesis of **4** in 2004 from two complementary schemes starting with 4-hydroxyphenylpyruvic acid. Second report for the synthesis of **4** came from Bewley *et al*. also in 2004 who used commercially available 3,5-dibromotyrosine as the starting material. Both these approaches are described below in detail.

(i) Kende's approach (2004)[19]

Kende's synthetic route (Scheme 1) proceeded from 4-hydroxyphenylpyruvic acid **14**, which was converted to the *O*-benzyloxime and then methylated with diazomethane to give the oxime methyl ester **15**. Dibromination of **15** with 2.0 eq. of NBS in THF at room temperature gave the dibromo derivative **16**. Direct *O*-alkylation of the phenolic hydroxyl in **16** with $BocNH(CH_2)_3Br$ introduced the protected propylamine tail to give **17**. Saponification of the ester followed by coupling of the

resulting acid with a Boc-protected agmatine established the Eastern chain to yield the amide **18**. The delicate chemoselective catalytic debenzylation of **18** required use of the 1:1 dioxane–acetic acid solvent system and led to the free oxime **7** in only 56% yield. Deprotection of all Boc groups in **19** was achieved in 30% CF_3CO_2H in dichloromethane to produce the target **4** as its bis(trifluoroacetate) salt in good yield.

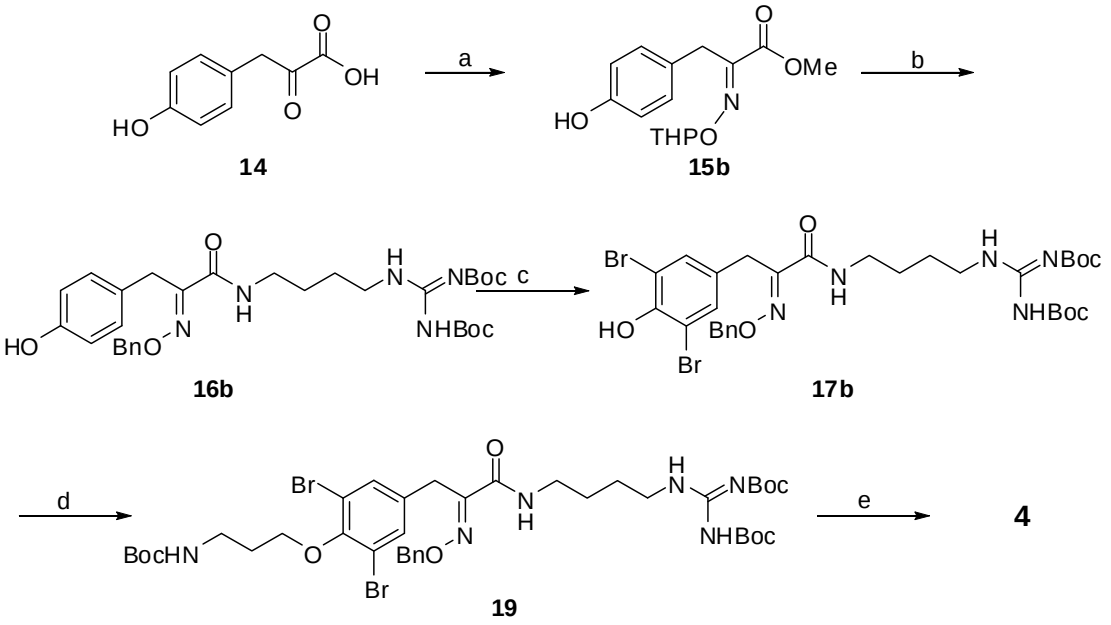


14 **15** **16** **17** **18** **19**

Scheme 1

Reagents and conditions: (a) $BnONH_2$, (b) CH_2N_2, 91%; (c) NBS, THF, 87%; (d) K_2CO_3, $BocNHC_3H_6Br$, 78%; (e) LiOH, CH_3OH/THF; (f) EDCI, HOBT, DiBoc-agmatine, 86%; (g) Pd/C, H_2, Dioxane-AcOH(1:1), 56%; (h) 30%, TFA/DCM, 82%.

In the second synthetic route (Scheme 2), 4-hydroxyphenylpyruvic acid **14** was converted to the THP-oxime acid **15b**, which was coupled with the protected 4-aminobutylguanidine to install the Eastern chain. Then **16b** was dibrominated to the dibromophenol amide **17b**. At this point, Mitsunobu coupling of the phenolic hydroxyl with 3-(*t*-butoxycarbonylamino)propanol produced the fully elaborated system **19**. Removal of both the THP and all Boc groups by 30% CF_3CO_2H in dichloromethane led smoothly to the target aminoguanidine **4** as its bis trifluoroacetate salt.

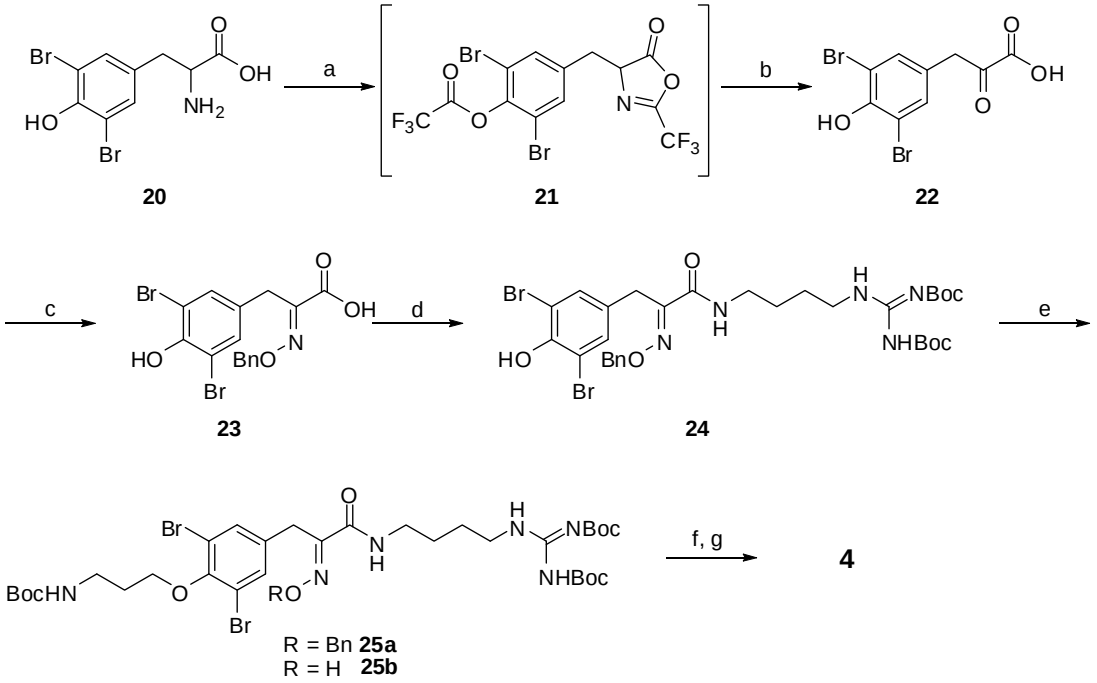


Scheme 2

Reagents and conditions: (a) $THPONH_2$, (b) EDCI, HOBT, DiBoc-agmatine, 87% (c) NBS, THF, 89%; (d) DEAD, Ph_3P, $BocNHC_3H_6OH$, 82%; (e) 30%, TFA/DCM, 82%.

(ii) Bewley's approach (2004)[20]

Total synthesis of **4** was completed by applying Hoshino's approach in 38% overall yield starting from the 3,5-dibromotyrosine. Commercially available 3,5-dibromotyrosine **20** was treated with trifluoroacetic anhydride (TFAA) at 80 °C for 18 h to generate the unstable trifluoromethyloxazolone intermediate **21**. Oxazolone **21** was dissolved in 70% aq trifluoroacetic acid (TFA) and allowed to stand overnight to yield the solid α-keto acid **22** as a white solid in 91% yield over two steps. Treatment of ketone **22** with O-benzyl hydroxylamine in ethanol provided the protected oxime **23** in 87% yield. Coupling of N-Boc-protected agmatine with intermediate **23** was affected with 1-Ethyl-3-(3-Dimethylaminopropyl)carbodiimide (EDCI) and N-hydroxylsuccinimide giving intermediate **24** in 63% yield. In the final step of the synthesis, alkylation of **24** with *tert*-butyl 3-bromopropylcarbamate under basic conditions provided **25** in 86% yield. Hydrogenation of **25** using palladium black with equal amounts of acetic acid and 1,4-dioxane provided the deprotected oxime **25b** in 89% yield with a minimal amount of the corresponding fully reduced amine. Cleavage of all the three *N*-*tert*-butyl carbamate protecting groups was effected with 20% TFA in dichloromethane completed the synthesis of **4** (Scheme 3) in 38% overall yield starting from the 3,5-dibromotyrosine.



20 21 22 23 24

Scheme 3

Reagents and conditions: (a) $(CF_3CO)_2O$, TFA, 80 °C; (b) 70% TFA, 91%; (c) NH_2OBn, EtOH, 87%; (d) *N*-Hydroxylsuccinimide, EDCI, dioxane, then DiBoc-agmatine, Et_3N, 63%; (e) K_2CO_3, $BocNHC_3H_6Br$, 86%; (f) Pd-black, dioxane:AcOH, 89%; (g) 20% TFA in DCM, 92%.

The synthesis initiated in 2003 was carried out with cheaper, commercially available 4-hydroxybenzaldehyde. The scheme is efficient and yields are good-excellent. Details of this synthesis constitute the contents of present work.

Present Work

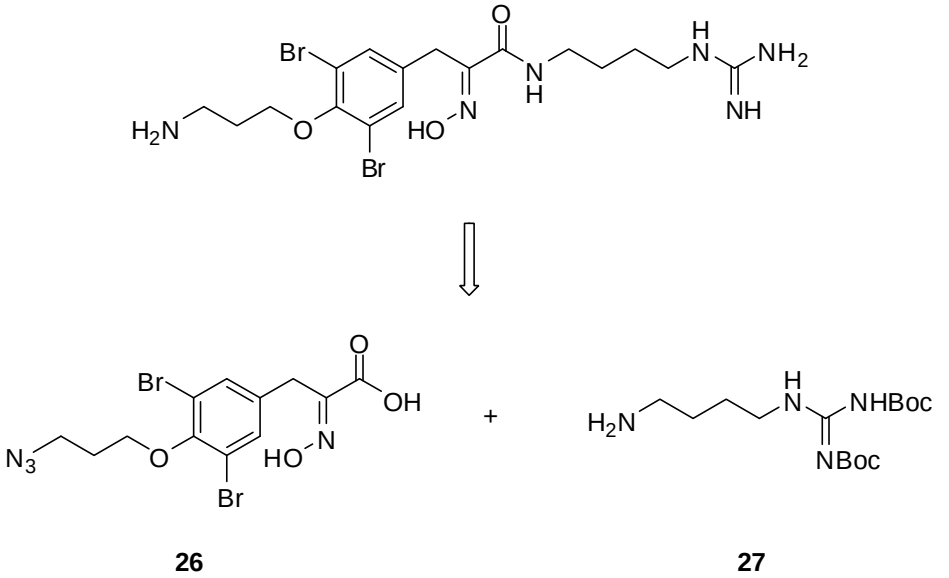
Many marine natural products that appear to be biogenetically derived from a bromotyrosine precursor have been described previously. Mycothiol (MSH) is the major low molecular weight thiol limited to actinomycetes, which include mycobacteria. MSH plays a major role in detoxification and maintenance of a reductive intracellular environment. Since enzyme mycothiol-*S*-conjugate amidase appears to play a critical role in protecting mycobacteria against alkylating agents and antibiotics, there is continuing need to isolate compounds showing significant inhibitory activity against these enzymes and studies of enzymes involved in mycothiol biosynthesis and mycothiol-dependent detoxification, as well as those of inhibitors to these enzymes, are rapidly increasing in number. For this purpose Bewley *et al*. described a series of marine natural product inhibitors of MCA, some of which are lethal to *M. smegmatis*. Of those structures studied, kinetics experiments established that the bromotyrosine-derived alkaloid, such as **4**, is the most active inhibitor of the group and is also competitive inhibitor of the *M. tuberculosis* detoxification enzyme MCA.

Bromotyrosine derived alkaloid **4** was isolated from an Australian non-verongid sponge of the *Oceanapia* species. Chemical structure of **4** was elucidated on the basis of its spectral data. Motivated by these findings, the synthesis of **4** was undertaken and the results are reported here.

While our synthesis was in progress, two reports appeared on the synthesis of **4**. The first total synthesis of **4** by Kende *et al*. utilized 4-hydroxyphenylpyruvic acid as the starting material and involved *O*-benzylated hydroxylamine and *O*-THP protected hydroxylamine for oximation. In the former case, a careful chemoselective catalytic debenzylation was achieved in only 56% yield. In the second synthetic approach, Bewley *et al*. utilized the expensive dibromotyrosine as the starting material and, via an unstable trifluoromethyl oxazolone intermediate, completed the synthesis. Debenzylation was not smooth and gave rise to the amine (instead of the oxime) as the major product. Finally, palladium black was used for this purpose, which also gave the amine as a minor product in addition to the required oxime as the major product.

Retrosynthetic analysis:

According to retrosynthetic analysis shown in **Scheme 4**, synthesis of alkaloid can be derived from two fragments: a left side oxime acid **26** and right side Boc protected agmatine **27**. The two fragments could be attached to each other through amide bond. The coupling of these fragments, reduction of azide to amine and deprotection will afford the natural product. It was decided to introduce the amine group of left side at the end after coupling of oxime acid and protected agmatine.

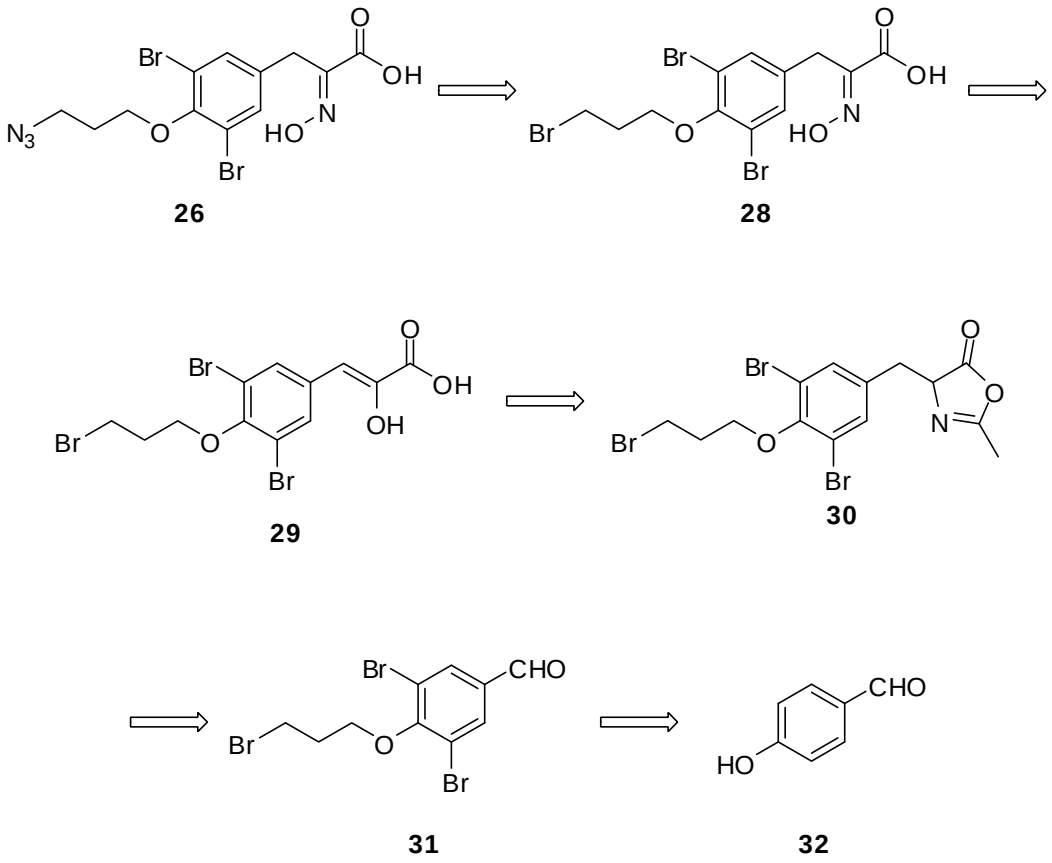


26 **27**

Scheme 4: Retrosynthetic analysis

Oxime acid **26** was planned to be synthesized from inexpensive and commercially available 4-hydroxybenzaldehyde using a tactical combination of transformations, as outlined in **Scheme 5**.

Azide group could be introduced by azidation of bromide **28.** Oximation of enol acid **29** will give oxime **28**. Synthesis of azlactone **30** could be achieved by using Hoshino approach from benzaldehyde **31**. Bromination of 4-hydroxybenzaldehyde followed by protection of phenol with 1,3-dibromopropane could give benzaldehyde **31**. Agmatine part could be obtained by treating 1,4-diaminobutane with *N*,*N*'-Bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea.



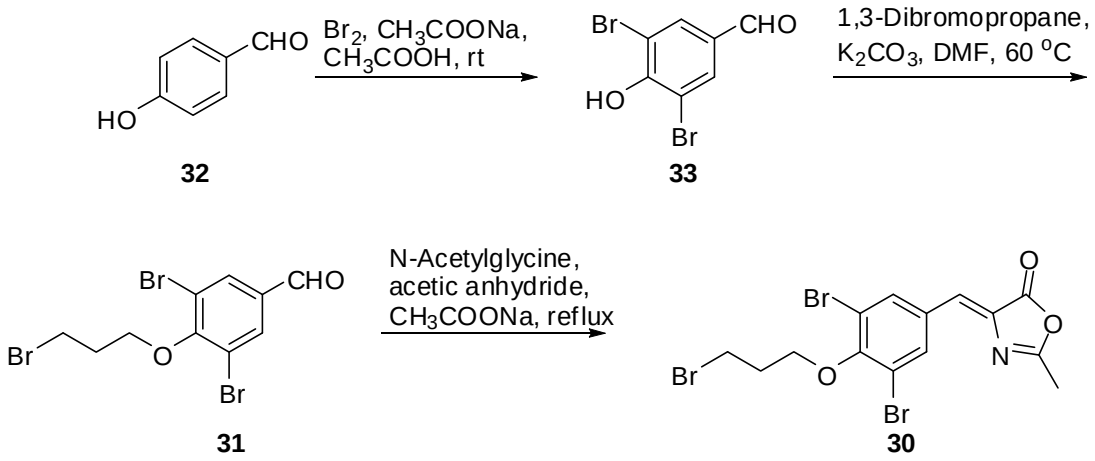
Scheme 5: Retrosynthetic analysis for oximeacid part

Synthesis of oxime acid fragment

Retrosynthetic analysis outlined in (Scheme 5) identified compound **30** as one of the potential synthetic intermediates and the construction of **30**, would mark the first synthetic objective in the construction of fragment **26**. Therefore, the synthesis of azlactone **30** began with commercially available 4-hydroxybenzaldehyde as shown in **Scheme 6**.

Commercially available 4-hydroxybenzaldehyde **32** was treated with bromine in acetic acid[21] in the presence of sodium acetate to provide the dibromobenzaldehyde **33** in 95% yields. The phenolic group of dibromobenzaldehyde **33** was converted to 3-bromopropyl ether **31** by reacting with 1,3-dibromopropane in the presence of K_2CO_3 as base and DMF as solvent at 80 °C. As the phenolic group is sterically hindered due to the presence of two bulky bromo groups *ortho* to it, this transformation[22] required higher temperature and prolonged time (80 °C, 8 h).

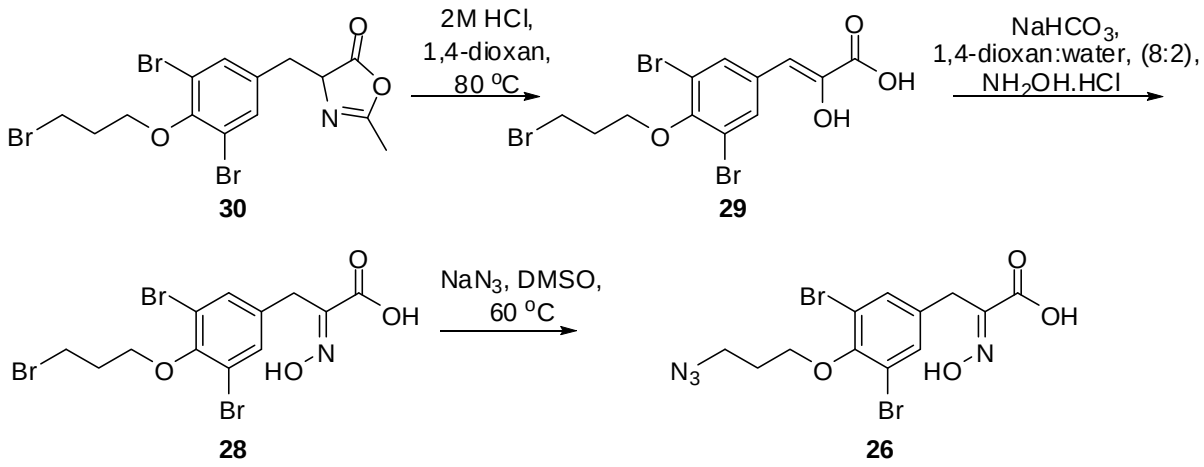
Dimerisation was minimized by using excess (3 eq.) of the alkylating reagent. Azlactone **30** was prepared by condensation of benzaldehyde **31** and N-acetyl glycine in acetic anhydride in the presence of sodium acetate for 5 h (Scheme 6). The yellow solid obtained was quite stable and the spectral data were in full agreement with azlactone **30**. The ^{1}H NMR revealed absence of that aldehyde proton at δ 9.86 in **31** and appearance of a singlet due to methyl group at δ 2.43 was observed thus confirming azlactone formation.



Scheme 6: Synthesis of azlactone

Complete hydrolysis of azlactone is known to give α-keto acid. Hydrolysis of azlactone can be achieved either under acidic or either basic conditions and for both the processes reaction temperature required is 80-100 °C. In the present case, hydrolysis under acidic conditions was resorted as the azlactone **30** has a base sensitive alkyl halide group. Thus, the required hydrolysis was achieved with 2M HCl in 1,4-dioxane. The acid **29** was obtained in 81% yield.

This α-keto acid **29** remains in enol form rather than keto form in 9:1 ratio as confirmed by integration values in ^{1}H NMR spectrum. Enol-acid **29** was converted to corresponding oxime **28** with hydroxylamine hydrochloride and $NaHCO_3$ in aq. 1,4-dioxan. The structure of **28** for the oxime was confirmed by spectral and analytical data. In the ^{1}H NMR spectrum, peak due to benzylic methylene at δ 3.70 observed which was also confirmed by the DEPT (Distortionless enhancement by polarisation transfer) spectrum with appearance of a peak at a 28.8. Bromo group of **28** was converted to azide; the later on reduction would give the amino group. This conversion was therefore achieved by heating oxime acid **28** with sodium azide in DMSO at 60 °C (Scheme 7). The IR spectrum of **26** revealed absorption at 2098 cm^{-1}, characteristic of azido group.

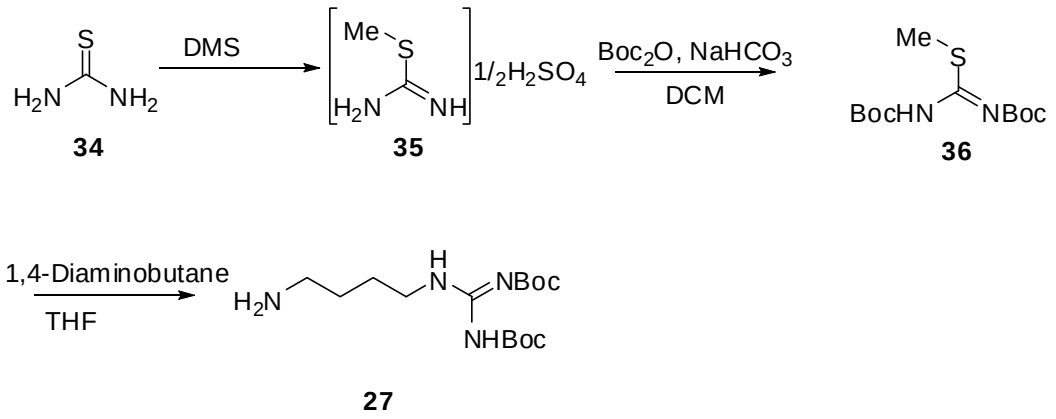


Scheme 7: Synthesis of acid part

Synthesis of agmatine part

Agmatine, an amine is an endogenous ligand at α-2-adrenergic and imidazoline receptors, to which it binds with high affinity. In addition, agmatine has properties of an endogenous neurotransmitter. Thus, agmatine is locally synthesized in brain by a specific enzyme, arginine decarboxylase. Agmatine is stored in a large number of neurons with selective distribution in the central nervous system (CNS). Agmatine can be enzymatically degraded by agmatinase in synaptosomes. Agmatine meets most criteria to establish it as a novel neurotransmitter/neuromodulator in the CNS. However, agmatine differs from forms of clonidine displacing system with respect to distribution, bioactivity and capacity to interact with antibodies raised to imidazoline-like drugs. Thus, there are multiple endogenous ligands of the imidazoline receptors, one of which is agmatine.

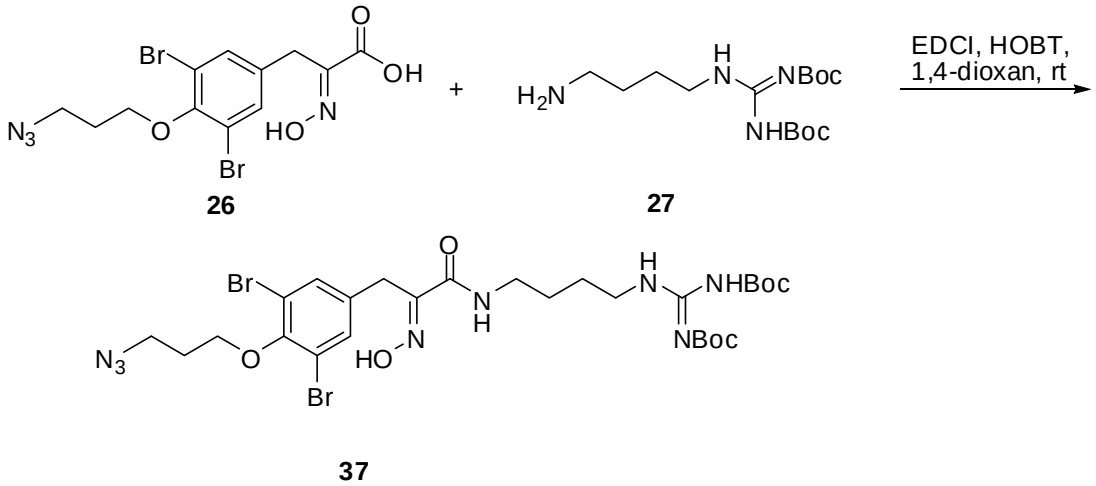
Synthesis of Boc-protected agmatine was accomplished following literature method.[23] Thus, methylation of commercially available thiourea **34** with dimethylsulphate gave the sulphate salt **35** which was protected with di-*tert*-butyl dicarbonate in DCM and water in the presence of $NaHCO_3$ to give *N*,*N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea **36**. Substitution of the thiomethyl group of **36** was achieved by replacement with 1,4-diaminobutane in THF to give Boc-protected agmatine **27** (Scheme 8). Spectral and analytical data of **27** were in full agreement with the reported values.



Scheme 8: Synthesis of agmatine part

Coupling of two fragments

Having the acid **26** in hand, the coupling reaction of **26** with Boc-protected agmatine **27** was the next target. The coupling reaction between amine **27** and acid **26** with EDCI and HOBt as the coupling reagents and 1,4-dioxan as the solvent proceeded smoothly and the amide **37** (Scheme 9) was obtained in 86% yield after purification with flash chromatography. Amide **37** was fully characterized by IR, ^{1}H NMR and ^{13}C NMR spectroscopic analysis.

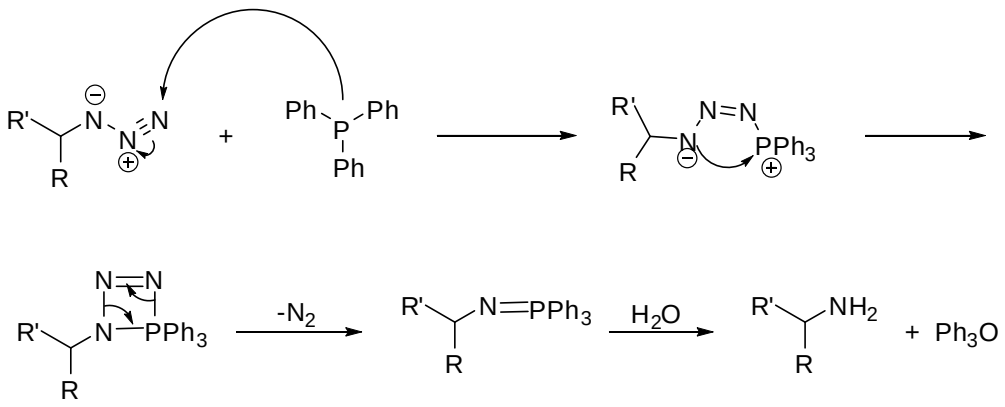


Scheme 9: Coupling of acid and agmatine fragments

After coupling of two fragments, the next task was to reduce the azide group and deprotection of Boc-groups to get the target molecule. Organic azides are easily reduced to the corresponding amines by hydrogenation using Pd or Pt as catalyst. In the amide compound **37**, alkenyl halide (bromo) as well as double bond (C=N) are present, hence catalytic hydrogenation is not the recommended option. It was decided to use Staudinger reaction for reduction of azide group to corresponding amine.

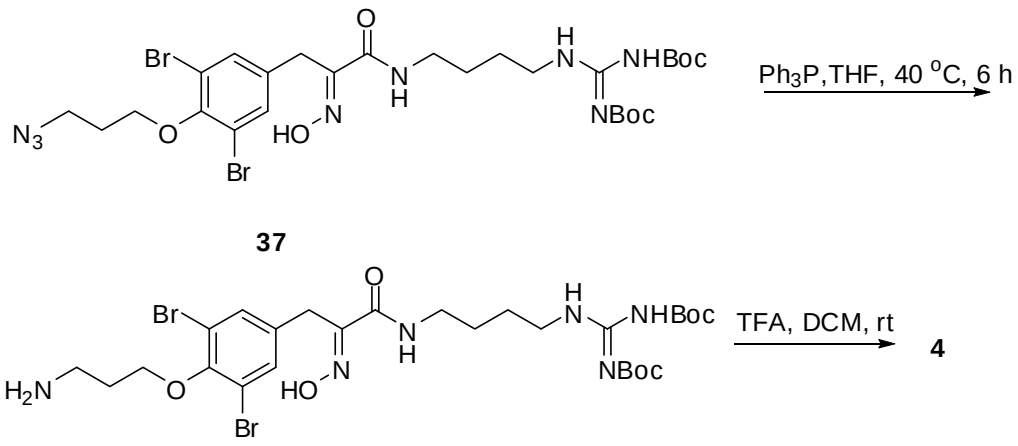
Staudinger reaction

Staudinger reduction is a mild method for reducing azide to amine in which the combination of an azide with a phosphine or phosphite produces an iminophosphorane intermediate which on hydrolysis produce a phosphine oxide and an amine (Scheme 10).



Scheme 10: Reaction mechanism of Staudinger reaction

Following the Staudinger conditions reduction of azide **37** was achieved with triphenylphosphine in THF at 40 °C for 6 h to furnish the amine **38** in 86% yield (Scheme 11). The IR spectrum of **38** revealed the absence of absorption at 2100 cm^{-1} pertaining to azide functionality, thereby indicating that the reduction has occurred. The amine thus formed was fully characterised spectroscopically.



37

38

Scheme 11

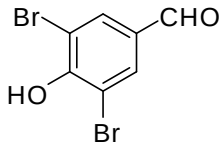
Complete deprotection of Boc groups in **38** was achieved with TFA in DCM to produce the target **4** as its bis(trifluoroacetate) salt in good yield. The ^{1}H NMR and ^{13}C NMR spectral data of bis(trifluoroacetate) salt of **4** were in agreement with the reported data.[17, 18]

Conclusion:

The total synthesis of **4,** which has been successfully accomplished, provides a methodology amenable to scale up. The starting material is cheap and easily accessible as well as all the reactions lead to good to excellent yields of the products. This synthetic approach[24] also offers a methodology for design and development of analogues with equal/better activity as inhibitors of MCA. This aspect of the work constitutes Section II of this chapter.

EXPERIMENTAL

3,5-Dibromo-4-hydroxybenzaldehyde (33)



Bromine (27.48 g, 172.00 mmol) in AcOH (50 mL) was added to a mixture of 4-hydroxybenzaldehyde (10.00 g, 90.00 mmol) and sodium acetate (22.90 g, 181.90 mmol) in AcOH (200 mL) at rt over 30 min and then stirred for 2 h at rt. A solid precipitated after addition of water (200 mL) which was filtered, washed with water and dried in a vacuum desiccator overnight to afford **33** as a pale-yellow solid (21.80 g, 95%) mp 181-183 °C (Lit. 181-185 °C).

Mol. Formula : $C_7H_4Br_2O_2$

Mol. Weight : 280

IR ($CHCl_3$) ν : 2954, 1672, 1577, 1544, 1473, 1194, 739 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 6.38 (br s, 1H), 8.01 (s, 2H), 9.80 (s, 1H)

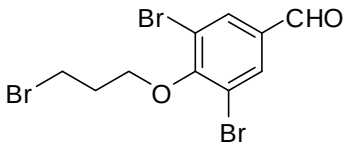
^{13}C NMR (50 MHz, $CDCl_3$) : δ 110.3, 128.7, 132.0, 154.7, 187.1

ESI-MS *m/z* : 280.8 $[M+H]^+$

Elemental Analysis : Found: C, 29.84; H, 1.52; Br, 56.83%

Calcd: C, 30.04; H, 1.44; Br, 57.09%

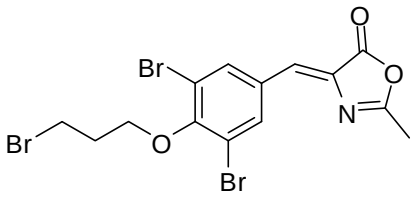
3,5-Dibromo-4-(3-bromopropoxy)benzaldehyde (31)



A mixture of 3,5-dibromo-4-hydroxybenzaldehyde (20.00 g, 71.70 mmol), K_2CO_3 (11.89 g, 86.10 mmol) and 1,3-dibromopropane (43.40 g, 215.10 mmol) in DMF (100 mL) was stirred at 80 °C for 4 h. The reaction mixture was cooled, poured in water and extracted with EtOAc (3 x 100 mL). The combined organic extract was washed with water, saturated brine and then dried over Na_2SO_4. After filtration the solvent was removed under vacuum. The crude product obtained was purified by column chromatography (silica gel, hexane/EtOAc 5:1) to afford **31** as a colorless solid (22.70 g, 79%); mp 83 °C.

Mol. Formula	**:**	$C_{10}H_9Br_3O_2$
Mol. Weight	**:**	401
IR ($CHCl_3$) ν	**:**	3018, 1701, 1547, 1215, 668 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 2.44 (quintet, *J* = 6.2 Hz, 2H), 3.77 (t, *J* = 6.4 Hz, 2H), 4.23 (t, *J* = 5.7 Hz, 2H), 8.04 (s, 2H), 9.86 (s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$)		δ 29.6, 33.2, 71.0, 119.3, 133.8, 134.1, 157.7, 188.3
ESI-MS *m/z*	**:**	400.9/402.9 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 29.78; H, 2.35; Br, 59.71%
		Calcd: C, 29.96; H, 2.26; Br, 59.80%

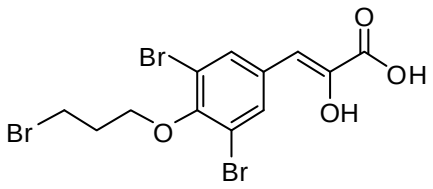
4-[3,5-Dibromo-4-(3-bromopropoxy)benzylidene]-2-methyloxazol-5(4*H*)-one (30)



A mixture of aldehyde **31** (10.00 g, 24.90 mmol), sodium acetate (3.10 g, 37.40 mmol) and *N*-acetylglycine (2.90 g, 24.90 mmol) in Ac_2O (50 mL) was stirred at 120 °C for 5 h. During the course of the reaction the solution turned dark brown and solidified upon cooling to rt. The yellowish brown solid obtained was then suspended in ice water, filtered off, washed with ice-cold water and then dried in a vacuum desiccator overnight to afford **30** as a yellowish solid (9.75 g, 81%); mp 125-127 °C.

Mol. Formula	**:**	$C_{14}H_{12}Br_3NO_3$
Mol. Weight	**:**	482
IR ($CHCl_3$) ν	**:**	3019, 1809, 1661, 1606, 1455, 1215, 669 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 2.43 (s, 3H), 2.44 (quintet, *J* = 5.9 Hz, 2H), 3.72 (t, *J* = 6.3 Hz, 2H), 4.18 (t, *J* = 5.8 Hz, 2H), 6.89 (s, 1H), 8.24 (s, 2H)
^{13}C NMR (50 MHz, $CDCl_3$)		δ 15.5, 29.4, 33.2, 70.9, 118.4, 126.6, 131.7, 133.6, 135.6, 154.4, 166.6, 167.2
ESI-MS *m/z*	**:**	481.93/483.93 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 34.66; H, 2.47; Br, 49.56; N, 2.83%
		Calcd: C, 34.89; H, 2.51; Br, 49.74; N, 2.91%

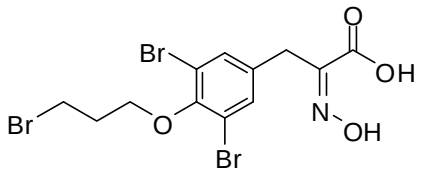
3-[3,5-Dibromo-4-(3-bromopropoxy)phenyl]-2-hydroxyacrylic acid (29)



A mixture of azlactone **30** (5.00 g, 10.37 mmol) and 2M dil. HCl (10 mL) in 1,4-dioxane (40 mL) was stirred at 80 °C for 17 h. The reaction mixture was cooled to rt and 1,4-dioxane was removed under vacuum. The residue was extracted with EtOAc (2 X 50 mL), the combined extract was washed with water, saturated brine and then dried over Na_2SO_4. The crude product obtained after removal of EtOAc under vacuum was purified by column chromatography (silica gel, EtOAc/MeOH 10:1) to afford 3-[3,5-dibromo-4-(3-bromopropoxy) phenyl]-2-hydroxyacrylic acid (3.85 g, 81%) as a pale yellow solid; mp 147-149 °C.

Mol. Formula	**:**	$C_{12}H_{11}Br_3O_4$
Mol. Weight	**:**	459
IR ($CHCl_3$) ν	**:**	2925, 1699, 1456, 1027, 769 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 2.15-2.25 (m, 2H), 3.63-3.85 (m, 2H), 4.00-4.25 (m, 2H), 6.29 (s, 1H), 7.33 (br s, 1H), 7.85 (s, 2H)
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 28.7, 31.8, 68.6, 105.1, 116.3, 131.8, 133.1, 141.8, 149.8, 164.9
ESI-MS *m/z*	**:**	459.1/461.1 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 31.23; H, 2.46; Br, 51.97% Calcd: C, 31.41; H, 2.42; Br, 52.23%

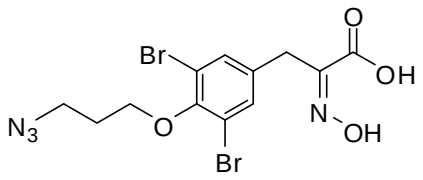
3-[3,5-Dibromo-4-(3-bromopropoxy)phenyl]-2-(hydroxyimino)propanoic acid (28)



A solution of 3-(3, 5-dibromo-4-(3-bromopropoxy) phenyl)-2-hydroxyacrylic acid (3.00 g, 6.54 mmol), hydroxylamine hydrochloride (2.27 g, 32.69 mmol) and $NaHCO_3$ (3.29 g, 39.22 mmol) in aqueous 1,4-dioxane (80%, 50 mL) was stirred till starting material disappeared (usually 5 h, thin-layer chromatography). Reaction mixture was concentrated in vacuum, residue obtained was dissolved in water (50 mL) and dil. HCl (2M) was added till it became acidic to give pale-yellow solid which was collected by filtration. The solid was washed with copious amounts of water and dried in vacuum. Further purification by column chromatography (silica gel, EtOAc/MeOH 10:1) afforded 3-[3, 5-dibromo-4-(3-bromopropoxy) phenyl]-2-(hydroxyimino) propanoic acid as a pale yellow solid (2.37 g, 76%); mp 170-172 °C.

Mol. Formula	**:**	$C_{12}H_{12}Br_3NO_4$
Mol. Weight	**:**	474
IR ($CHCl_3$) ν	**:**	2925, 1699, 1456, 1027, 769 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 2.23 (quintet, *J* = 6.5 Hz, 2H), 3.71 (t, *J* = 6.4 Hz, 2H), 3.82 (s, 3H), 4.04 (t, *J* = 6.0 Hz, 2H), 7.43 (s, 2H)
^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6)	**:**	δ 28.8, 29.5, 32.9, 69.4, 117.4, 133.1, 135.4, 148.2, 150.9, 166.3
ESI-MS *m/z*	**:**	474.2/476.2 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 30.14; H, 2.51; Br, 50.37% Calcd: C, 30.41; H, 2.55; Br, 50.58%

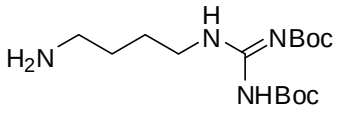
3-[4-(3-Azidopropoxy)-3,5-dibromophenyl]-2-(hydroxyimino)propanoic acid (26)



3-[3,5-Dibromo-4-(3-bromopropoxy)phenyl]-2-(hydroxyimino)propanoic acid (5.00 g, 10.55 mmol) was added into the solution of sodium azide (3.43 g, 52.75 mmol) in DMSO (30 mL). The reaction temperature was maintained at 60 ^{o}C and stirring was continued overnight (16 h). The reaction mixture was allowed to cool and then the contents were poured into 100 ml of ice-cold water. A white precipitate formed immediately, it was stirred for 25 min and then the white solid was collected by filtration and then dried in vacuum to yield 3-[4-(3-azidopropoxy)-3, 5-dibromophenyl]-2- (hydroxyimino) propanoic acid (4.20 g, 91%).

Mol. Formula	**:**	$C_{12}H_{12}Br_2N_4O_4$
Mol. Weight	**:**	436
IR ($CHCl_3$) ν	**:**	2927, 2098, 1700, 1456, 1215, 1029 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 1.95 (quintet, *J* = 6.5 Hz, 2H), 3.51 (t, *J* = 6.7 Hz, 2H), 3.70 (s, 2H), 3.90 (t, *J* = 5.9 Hz, 2H), 7.34 (s, 2H)
^{13}C NMR (50 MHz, $CDCl_3$)	**:**	δ 27.9, 28.3, 47.0, 68.8, 116.5, 132.1, 134.8, 148.2, 150.0, 164.0
ESI-MS *m/z*	**:**	437.1 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 32.87; H, 2.84; Br, 36.53; N, 12.62%
		Calcd: C, 33.05; H, 2.77; Br, 36.65; N, 12.85%

***tert*-Butyl (4-aminobutylamino)(*tert*-butoxycarbonylamino) methylenecarbamate (27)**



To a solution of 1,4-diaminobutane (5.00 g, 56.72 mmol) in THF (50 mL), *N*,*N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (8.24 g, 28.36 mmol) was added and the reaction mixture was stirred at rt for 24 h. The solvent was removed under vacuum; the residue obtained was taken in EtOAc (40 mL), washed twice with water (20 mL) and then dried over Na_2SO_4. After evaporation of the solvent, the residue obtained was purified by silica gel column chromatography with EtOAc to afford the Boc-protected guanidine **27** (8.40 g, 90%) as colorless oil.

Mol. Formula : $C_{15}H_{30}N_4O_4$

Mol. Weight : 330

IR ($CHCl_3$) ν : 3324, 2982, 1717, 1638, 1334, 1132 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.49 (s, 9H), 1.50 (s, 9H), 1.55-1.68 (m, 4H), 2.73 (t, *J* = 6.3 Hz, 2H), 3.43 (dt, *J* = 5.3, 7.0 Hz, 2H), 8.33 (br s, 1H), 11.49 (br s, 1H)

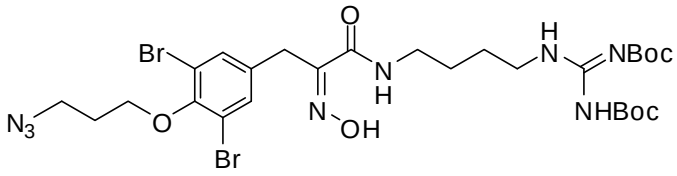
^{13}C NMR (50 MHz, $CDCl_3$) : δ 26.0, 27.7, 28.0, 31.0, 40.3, 41.0, 78.9, 82.8, 153.0, 155.9, 163.2

ESI-MS *m/z* : 331.2 $[M+H]^+$

Elemental Analysis : Found: C, 54.23; H, 9.27; N, 16.78%

Calcd: C, 54.52; H, 9.15; N, 16.96%

***tert*-Butyl 15-[4-(3-azidopropoxy)-3,5-dibromophenyl]-14-(hydroxyimino)-2,2-dimethyl-4,13-dioxo-3-oxa-5,7,12-triazapentadecan-6-ylidenecarbamate (37)**



To a stirred solution of the 3-[4-(3-azidopropoxy)-3, 5-dibromophenyl]-2-(hydroxyimino)propanoic acid (2.00 g, 4.59 mmol) in 1,4-dioxane (20 mL) were added 1-ethyl-3-(3′-dimaminopropyl)carbodiimide (EDCI) (0.88 g, 4.59 mmol) and N-hydroxybenzotriazole (HOBt) (0.62 g, 4.59 mmol) at rt and allowed to stir for 15 min, followed by slow addition of Boc-protected agmatine (1.52 g, 4.59 mmol) over 5 min. Stirring was continued for 2 h at rt and then the reaction mixture was quenched by addition of water and extracted with EtOAc (3 x 30 mL). The combined EtOAc layer was washed with saturated brine solution, dried over Na_2SO_4 and the solvent from filtrate was evaporated to give the crude product. The later was purified by column chromatography (silica gel, hexane/EtOAc 6:4) to afford **37** as colorless oil (2.96 g, 86%).

Mol. Formula : $C_{27}H_{40}Br_2N_8O_7$

Mol. Weight : 748

IR ($CHCl_3$) ν : 3419, 3330, 3017, 2099, 1719, 1671, 1637 cm^{-1}

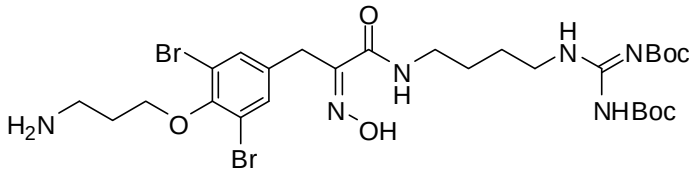
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.45 (s, 9H), 1.48 (s, 9H), 1.52-1.63 (m, 4H), 2.02 (quintet, *J* = 6.7 Hz, 2H), 3.25-3.40 (m, 4H), 3.79 (s, 2H), 4.00 (t, *J* = 6.9 Hz, 2H), 4.66 (s, 1H), 6.78 (t, *J* = 4.3 Hz, 1H), 7.40 (s, 2H), 8.29 (s, 1H), 11.5 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 26.5, 26.7, 28.0, 28.6, 29.5, 39.0, 40.4, 48.2, 69.7, 79.1, 83.0, 117.7, 133.5, 134.9, 151.4, 153.2, 156.0, 162.0

ESI-MS *m/z* : 749.2 $[M+H]^+$

Elemental Analysis : Found: C, 43.17; H, 5.42; Br, 21.14; N, 14.97%
Calcd: C, 43.33; H, 5.39; Br, 21.35; N, 14.97%

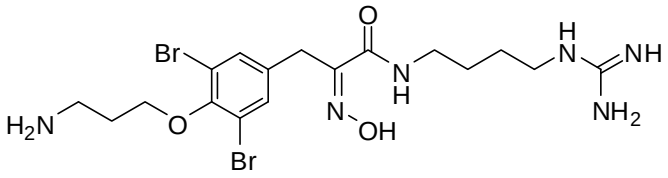
***tert*-Butyl 15-[4-(3-aminopropoxy)-3,5-dibromophenyl]-14-(hydroxyimino)-2,2-dimethyl-4,13-dioxo-3-oxa-5,7,12-triazapentadecan-6-ylidenecarbamate (38)**



To a solution of azide **37** (1.00 g, 1.34 mmol) in THF (10 mL) and water (0.5 mL), was added triphenylphosphine (0.42 g, 1.60 mmol) was added and the reaction mixture was stirred for 6 h at 40 ^{o}C. Volatiles of the reaction mixture were removed; the residue obtained was diluted with water and then extracted with EtOAc (2 x 30 mL). The combined organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated under vacuum to afford the crude product as thick oil. The later was purified by flash column chromatography (silica gel, EtOAc/MeOH 9:1) to yield pure amine **38** as yellowish oil (0.82 g, 84%).

Mol. Formula	**:**	$C_{27}H_{42}Br_2N_6O_7$
Mol. Weight	**:**	722
IR ($CHCl_3$) ν	**:**	3673, 3419, 3330, 3018, 1720, 1672, 1633 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 1.48 (s, 18H), 1.50-1.68 (m, 4H), 2.00 (br s, 2H), 2.10-2.18 (m, 2H), 3.00-3.42 (m, 6H), 3.80 (s, 2H), 4.02 (t, *J* = 5.3 Hz, 2H), 6.78 (t, *J* = 3.9 Hz, 1H), 7.41 (s, 1H), 11.47 (s, 1H)
^{13}C NMR (50 MHz, $CDCl_3$)	**:**	δ 26.1, 26.3, 27.5, 27.8, 28.2, 29.0, 29.1, 38.6, 39.9, 43.5, 69.3, 78.1, 82.6, 117.3, 134.4, 150.8, 150.9, 152.8, 155.6, 161.6
ESI-MS *m/z*	**:**	723.1 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 44.73; H, 5.91; Br, 21.97; N, 11.58% Calcd: C, 44.89; H, 5.86; Br, 22.12; N, 11.63%

3-[4-(3-Aminopropoxy)-3,5-dibromophenyl]-N-(4-guanidinobutyl)-2-(hydroxyimino)propanamide (4)



TFA (1.5 mL) was added to an ice-cold solution of the Boc-protected compound **38** (0.50 g, 0.69 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was warmed to rt and then stirring was continued for 2 h. The volatiles were evaporated and the residue obtained was dried under high vacuum. The bistriflate salt of **4** was obtained in quantitative yield (0.49 g).

Mol. Formula	**:**	$C_{17}H_{26}Br_2N_6O_3$
Mol. Weight	**:**	522
IR ($CHCl_3$) ν	**:**	3363, 2930, 1670, 1574, 1122 cm^{-1}
^{1}H NMR (200 MHz, MeOH)	**:**	δ 1.50-1.65 (m, 4H), 2.10-2.25 (m, 2H), 3.20-3.33 (m, 6H), 3.84 (s, 2H), 4.10 (t, *J* = 5.6 Hz, 2H), 7.46 (s, 2H)
^{13}C NMR (50 MHz, DMSO-d_6)	**:**	δ 25.8, 26.1, 27.5, 28.3, 36.5, 38.3, 40.4, 69.9, 117.0, 132.9, 136.5, 149.8, 151.0, 156.7, 162.8
ESI-MS *m/z*	**:**	523.1 $[M+H]^+$

Chapter 2

Section II

Synthetic analogues of dibromotyrosine derived natural alkaloids

Introduction

Discovery and development of novel drugs is a complex process. Historically, the main source of biologically active compounds used in drug discovery programs has been natural products, isolated from plants, animals or fermentation sources. In medicine, biotechnology and pharmacology, drug discovery is the process by which drugs are discovered and/or designed, most drugs have been discovered either by identifying the active ingredients from traditional remedies or by serendipitous discovery. A new approach has been to understand how the mechanism of diseases and infections control at the molecular and physiological level and to target specific entities based on this knowledge. The process of drug discovery 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. Despite advances in technology and understanding of biological systems, drug discovery is still an expensive and long process with low rate of new therapeutic discovery.

Modern drug discovery often entails the synthesis and biological testing of collected molecules both natural and synthetic, referred to as libraries, which arise from the combinations of different building blocks by the same chemical strategy. Over the last decade; combinatorial library synthesis has become a very important field both in academic and industrial research[25] in which the combinatorial techniques on the solid and solution-phase have significantly increased the efficiency of the drug discovery process. Importantly, combinatorial chemistry allows for the high throughput synthesis (HTS) of drug candidates with broad diversity and/or complexity.[26]

Combinatorial chemistry

Combinatorial chemistry is one of the important new methodologies developed by researchers in the pharmaceutical industry to reduce the time and costs associated with producing effective and competitive new drugs. Through acceleration of the process of chemical synthesis, this method has generated profound effect on all branches of chemistry, especially on drug discovery. Through the rapidly evolving technology of combi-chemistry, it is now possible to produce compound libraries to screen for novel bioactivities, this powerful new technology has therefore begun to

assist pharmaceutical companies to generate new drug candidates fast, save significant money in preclinical development costs and ultimately change the fundamental approach to drug discovery.

Combinatorial library methods were first applied to peptides and oligonucleotides. Since then, the field has been expanded to include proteins, synthetic oligomers, small molecules and oligosaccharides. The method of library preparation is dependent on the type of library desired, however all combinatorial library methods involve three main steps: preparation of the library, screening of the library components and determination of the chemical structures of active compounds.

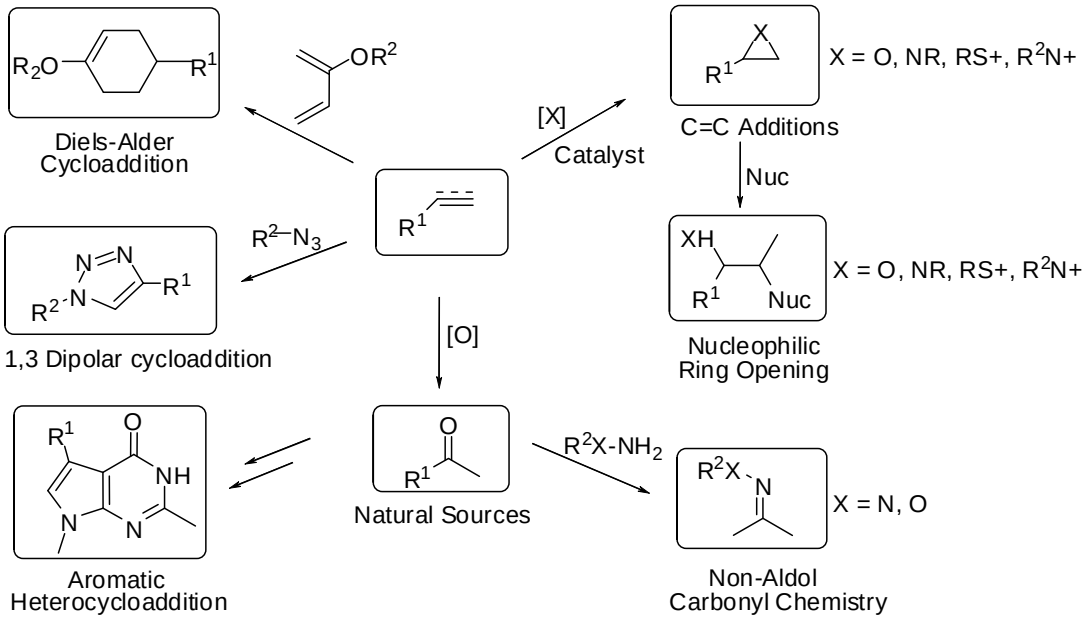
Click chemistry

Click chemistry is a chemical philosophy introduced by Sharpless in 2001 and describes chemistry tailored to generate substances quickly and reliably by joining small units together. This is inspired by the fact that nature also generates substances by joining small modular units. Click chemistry (CC) can be summarized neatly in one sentence: "*all searches must be restricted to molecules that are easy to make"*. A set of stringent criteria that a process must meet to be useful in the context of CC has been defined by Sharpless *et al*, as reactions that: "*are modular, wide in scope, high yielding, create only inoffensive by-products (that can be removed without chromatography), are stereospecific, simple to perform and that require benign or easily removable solvent"*. Although meeting the requirements of a click reaction is a tall order, several processes have been identified which step up to the mark (Scheme 12) *viz*. nucleophilic ring opening reactions, non-aldol carbonyl chemistry, additions to carbon-carbon multiple bonds and cycloaddition reactions.

Chemical transformations which are called as click reactions

- Cycloadditions of unsaturated species, especially 1,3-dipolar cycloaddition reactions, but also the Diels-Alder family of transformations.
- Nucleophilic substitution chemistry, particularly ring-opening reactions of strained heterocyclic electrophiles such as epoxides, aziridines, aziridiniumions and episulfoniumions.

- Carbonyl chemistry of the "non-aldol" type, such as formation of ureas, thioureas, aromatic heterocycles, oxime ethers, hydrazones and amides.
- Additions to carbon-carbon multiple bonds, especially oxidative cases such as epoxidation, dihydroxylation, aziridination and sulfenyl halide addition, but also Michael additions of Nu-H reactants.



Scheme 12 : Click Chemistry Reaction Types

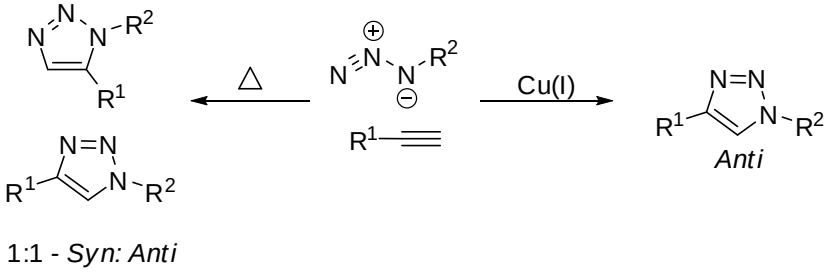
Click chemistry and drug discovery

Click chemistry is being used increasingly in biomedical research, ranging from lead discovery and optimization, to tagging of biological systems, such as proteins, nucleotides and whole organisms.

Azide-alkyne Cycloaddition Reaction

Among these carefully selected reactions, CuI-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes to afford 1,2,3-triazoles[27] (Scheme 13) become the gold standard of click chemistry due to its reliability, specificity, biocompatibility and this reaction has also been termed the "cream of the crop" of click reactions. Further interest in this reaction stems from the interesting biological activity of 1,2,3-triazoles. These heterocycles function as rigid linking units that can mimic the atom placement and electronic properties of a peptide bond

without the same susceptibility to hydrolytic cleavage.[28] Since the foundations of click reactions were laid, there has been an explosive growth in publications describing a wealth of applications of this practical and sensible chemical approach. Its applications are separated into the three most relevant categories: (i) bioconjugation[29] (ii) polymer and materials science[30] (iii) drug discovery.[31]



Scheme 13: Topological and electronic similarities of amides and 1,2,3-triazoles

Thermal 1,3-dipolar cycloaddition of alkynes to azides is not a regiospecific reaction, it always gives 1:1 mixture of *syn* and *anti* regioisomers. The analogous copper (I)-catalyzed reaction gives only one regioisomer, the 1,4-substituted [1,2,3] triazole. Some structural differences between triazoles and amide bonds of course exist; most notably, the extra atom in the triazole backbone leads to a calculated increase in R^1–R^2 distance of 1.1 Å over the typical amide bond (Scheme 12). Triazoles also possess a much stronger dipole moment than an amide bond, but this may actually enhance peptide bond mimicry by increasing the hydrogen bond donor and acceptor properties of the triazole. In addition to the possibility of both the N(2) and N(3) triazole atoms acting as hydrogen bond acceptors, the strong dipole may polarize the C(5) proton to such a degree that it can function as a hydrogen-bond donor, like the amide proton. Perhaps due in part to their ability to mimic certain aspects of a peptide bond, many known 1,2,3-triazoles possess varied biological activity, including *anti*-HIV activity,[32] selective β_3 adrenergic receptor inhibition,[33] *anti*-bacterial activity,[34] potent *anti*-histamine activity,[35] and more.

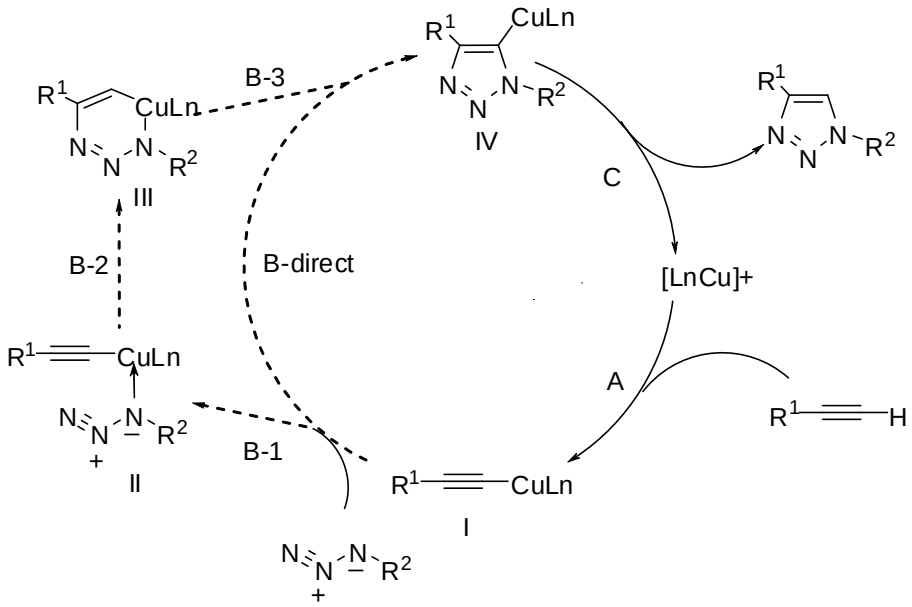
Role of Copper (I) in Alkyne-Azide Coupling Reaction

The catalytic mechanism has not been investigated, but it is known that copper (I) readily inserts into terminal alkynes in the presence of base, e.g. the Sonogashira coupling.[36] The polarization of the terminal triple bond by the covalently bound

copper (I) catalyzes the cycloaddition, which probably changes from a concerted reaction into a stepwise addition. Experiments conducted so far showed that the 1,3-dipolar cycloaddition was catalyzed (0.01 eq. was the lowest stoichiometry) only by copper (I) chloride, copper (I) bromide-dimethyl sulfide complex and copper (I) iodide but not by copper (II) salts. However, the copper (I) catalysis does not work on internal alkynes where only the starting material was recovered. This suggested a reaction intermediate in which copper (I) was terminally bound to the alkyne, since copper (I) does not catalyze reactions with internal triple bonds. It may therefore be concluded that the 1,3-dipolar cycloaddition of terminal alkynes to azides is catalyzed by copper (I) salts through a preformed copper-acetylide complex followed by a stepwise or concerted addition to an azide.

Mechanism of CuI-Catalyzed Alkyne-Azide Coupling

The mechanism for CuI-catalyzed alkyne-azide coupling tolerates most organic functional groups and shows a wide scope with respect to both alkyne and azide reactants. The reaction proceeds in a variety of solvents, tolerates a wide range of pH values and performs well over a broad temperature range. Although the thermal dipolar cycloaddition of azides and alkynes occurs through a concerted mechanism, DFT calculations on monomeric copper acetylide complexes indicate that the concerted mechanism is strongly disfavored relative to a stepwise mechanism (Scheme 14).



Scheme 14: Proposed catalytic cycle for the Cu (I)-catalysed ligation

Structure-activity relationship (**SAR**)

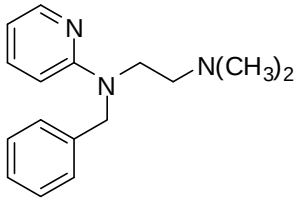
The basic assumption for all molecule based hypotheses is that similar molecules have similar activities. This principle is also called **S**tructure-**A**ctivity **R**elationship (SAR). The underlying problem is therefore how to define a *small* difference on a molecular level, since each kind of activity, e.g. reaction ability, biotransformation ability, solubility, target activity and so on, might depend on another kinds of differences.

Structure-activity relationships are the traditional practices of medicinal chemistry which try to modify the effect or the potency (i.e. activity) of bioactive chemical compounds by modifying their chemical structure. Medical chemists use the chemical techniques of synthesis to insert new chemical groups into the biomedical compound and test the modifications in their biological effect.

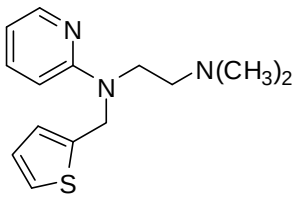
Bioisosterism

In 1919, Langmuir first developed the concept of chemical isosterism to describe the similarities in physical properties among atoms, functional groups, radicals and molecules. The similarities among atoms described by Langmuir primarily resulted from the fact that these atoms contained the same number of valence electrons and came from the same columns within the periodic table.

Bioisosterism can be defined as: "Bioisosteres are (functional) groups or molecules that have chemical and physical similarities producing broadly similar biological properties."



Tripelennamine

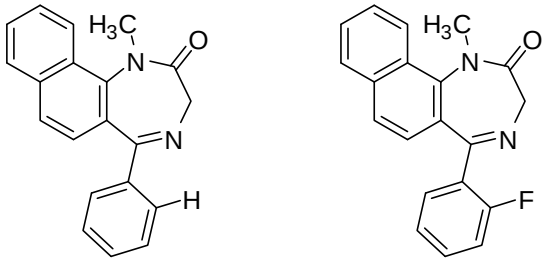


Methapheniliene

Isostric substitution of thiophene for benzene

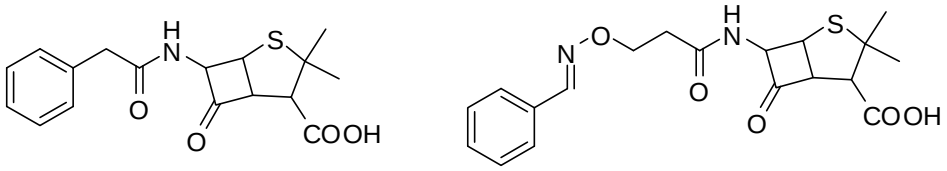
Bioisosteres have been classified as either classical or nonclassical

- Classical bioisosteres are those which have similar steric and electronic features and have the same number of atoms as the substituent moiety for which they are used as a replacement.



Fluorine vs Hydrogen Replacements

- Nonclassical bioisosteres do not obey the strict steric and electronic definition of classical isosteres and they do not have the same number of atoms as the substituent moiety for which they are used as a replacement. These isosteres are capable of maintaining similar biological activity by mimicking the spatial arrangement, electronic properties, or some other physicochemical property of the molecule or functional group that is critical for the retention of biological activity.



Replacement of phenyl group of penicillin G with a β-aminooxypropionyl group

Bioisosterism is an effective way to design bioactive compounds. In medicinal chemistry, bioisosteres are substituents or groups with similar physical or chemical properties that impart similar biological properties to a chemical compound. In drug design, the purpose of exchanging one bioisostere for another is to enhance the desired biological or physical properties of a compound without making significant changes in chemical structure. Bioisosterism is a strategy of Medicinal Chemistry for the rational design of new drugs, applied with a lead compound (LC) as a special process of molecular modification.

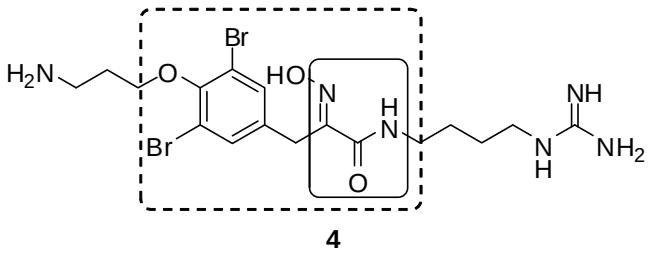
A structure activity relationship relates features of a chemical structure to a property, effect, or biological activity associated with that chemical. In so doing there can be both qualitative and quantitative considerations. The fundamental premise is that the structure of a chemical implicitly determines its physical and chemical properties and reactivities, 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.

In the pharmaceutical and chemical industries, SARs have long been used to design chemicals with commercially desirable properties. This has been particularly the case in the area of drug design where chemicals with desired pharmacologic and therapeutic activities are sought. In the environmental health protection field, SAR is being used to predict ecological and human health effects, with applications varying widely. It is even being used to help industry design safer chemicals for commercial use as a part of their desirable properties.

The importance of click chemistry and combinatorial synthesis in drug discovery as well as the great importance of triazole in these activities has been discussed. Based on these information, it was decided to undertake synthesis and characterisation of a library of compounds with dibromotyrosine as the core unit and with 1,2,3-triazoles and different amines as the modifications. The approaches and the key results obtained are described in the following pages under "present work".

Present Work

Four novel alkaloids isolated from an Australian non-verongid sponge of the *Oceanapia* species exhibited significant inhibitory activity against mycothiol-*S*-conjugate amidase (MCA). Due to the crucial role played by MCA, such alkaloids are marked as potentially useful therapeutic agents against *Mycobacterium tuberculosis* and related pathogens. Of the various structures studied, kinetics experiments established that the bromotyrosine-derived alkaloid, such as **1**, is the most active inhibitor of the group and is also competitive inhibitor of the *M. tuberculosis* detoxification enzyme MCA. Motivated by these findings, the synthesis of **4** (Figure 7) was completed and it was decided to synthesize analogues of natural product by modifying its left segment to triazole and using different amino compounds including of agmatine.



4

Figure 7: Bromotyrosine derived natural alkaloid

The importance of bromotyrosine linked amide-oxime as the core unit (for biological activity)

Screening of approximately 1500 organic extracts, 1200 of which have been derived from marine plants and invertebrates and 300 from terrestrial fungal cultures, were carried out in a fluorescence-detected assay that measures the extent of cleavage of the substrate mycothiol bimane, by MCA. This initial screening effort yielded about 20 active extracts that inhibited MCA at concentrations less than 50 micrograms per milliliter. On the basis of potency and availability of sufficient material the chemistry of these extracts were undertaken. The active compounds of each mixture were isolated by bioassay-guided fractionation.

Inhibition curves were measured for each of the pure compounds using natural MCA isolated from *Mycobacterium smegmatis* and recombinant *Mycobacterium*

tuberculosis MCA, which have shown cleaves mycothiol bimane (MSmB) with rates identical to those of natural *M. smegmatis* MCA. To further investigate the mode of MCA inhibition of these active compounds, competition assays on different compounds which include the spiroisoxaline and linear oximinoamide containing bromotyrosine-derived compounds, the glycosylated sphingolipid and the tricyclic piperazine-containing toxins was carried out. Results of these experiments show that the compounds containing a centrally located oximino-amide linkage (Figure 7) compete directly with the substrate MSmB for MCA. The studies strongly suggest that MCA contains a metal ion in its active site that may be coordinated by the oximinoamides moieties, thereby disrupting MCA activity. These compounds most likely mimic the amide bond connecting cysteine and glucosamine in mycothiol, which is cleaved by MCA. In support of this notion, bromotyrosine-containing natural products lacking an amide group, such as molokaiamine, oceanapiside and gliotoxin are found to be non-competitive inhibitors. It was observed that compound **4** exhibits anti-mycobacterial activity having IC_{50} value 3 μM and shows potent activity it was decided to synthesise analogues of this compound. Additionally, the results show the central oximino-amide linkage is essential for the activity, hence change in side chains was considered to be a logical approach in the synthesis of analogues.

Natural product **4** consists of two segments: left segment is the 3-aminopropyloxy bromophenyl oxime-acid building block; right segment is the agmatine building block. By using four different left segments and three different right segments keeping middle (core) segment constant, a 12-compound-library can be designed and synthesised. Here, the first step would be the preparation of different left segments followed by coupling reaction with different right segments to afford different analogues. In the synthetic route (described in the Section I) for the compound **4**, the azide intermediate **26** was formed which need to be modified for yielding the different analogues. Coupling of this azide with different acetylenic compounds would give triazoles as the left segment and for the right segment, different amine compounds would be used and coupling of these amines with acids would afford the desired analogues.

Click chemistry and bioconjugation

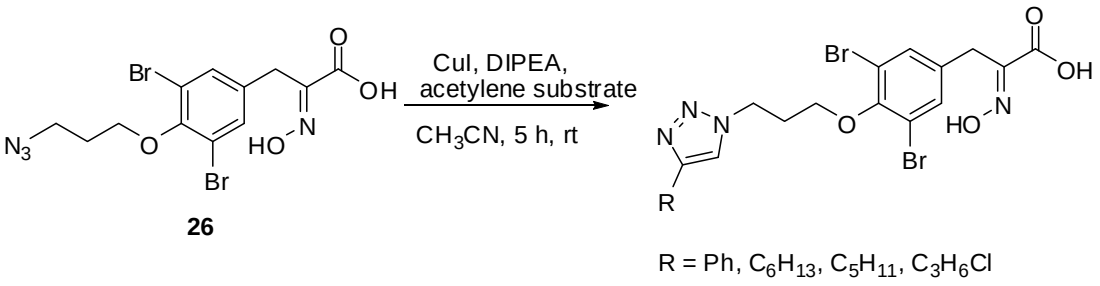
In vivo and *in vitro* bioconjugation applications benefit from the unprecedented reliability of the copper-catalyzed azide–acetylene union, the inertness of the reactants under physiological conditions and the mild reaction conditions.

The copper-(I)-catalyzed formation of 1,2,3-triazoles has recently been used to prepare functionalized resins for the solid phase synthesis of a library of dopaminergic arylcarbamides.[37] In another resin-based approach, Yli-Kauhaluoma *et al.* prepared 1,2,3-triazoles via thermal 1,3-dipolar cycloaddition of polymer-bound azides to alkynes, followed by cleavage from the solid support with TFA.[38]

Synthesis of oxime-acid segment

1,2,3-triazole was chosen as the building block for the left segment. Synthesis of azide-oximeacid **26** has already been reported in Section I (Scheme 5). It was therefore decided to modify azide to different triazoles using Huisgen cycloaddition reaction of azide with terminal acetylenic compounds. The crucial 1,3-dipolar cycloaddition reaction in acetonitrile was successfully carried out between azide **26** and acetylenic compounds (phenyl acetylene, 1-octyne, 1-heptyne, 5-chloro-1-pentyne) using CuI in the presence of *N*,*N*-diisopropylethylamine (Scheme 15).

To a solution of acetylene compound (1 mmol) and azide **26** (1 mmol) in 30 mL of anhydrous acetonitrile, were added CuI (2 mmol) and *N*,*N*-diisopropylethylamine (3 mmol) and the reaction mixture was stirred for 5 h at room temperature. It was then filtered through celite and the filtrate was diluted with 50 mL of water and 20 mL of NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic layer was washed with water followed by brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to obtain a crude residue that was purified by column chromatography to give the desired triazole as yellow solid.



26

R = Ph, C_6H_{13}, C_5H_{11}, C_3H_6Cl

Scheme 15 : Synthesis of a library of triazole

It is interesting to note that the Huisgen reaction was found to be highly regioselective, yielding 1,4-disubstituted 1,2,3-triazoles **39, 40, 41, 42** (Figure 8) with high yields. In the ^{1}H NMR spectrum of triazoles the olefinic proton associated with the 1,2,3-triazole moiety was identified as a singlet at δ 7.45-7.50 ppm along with other resonances in accordance with the assigned structure. The ^{13}C NMR spectrum of compounds confirmed the presence of triazole product as the olefinic carbons were noticed around δ 122 and 148 ppm which is the characteristic of triazole system. Furthermore, the IR spectra of compounds revealed absence of the azido group which usually has a sharp peak at ~ 2100 cm^{-1}.

Table 1: Synthesis of triazoles from the dibromo derivative **26** using different acetylenes and catalysed by CuI

S. No	Acetylenic compound	Reaction conditions	Product	Yield
1	Phenyl acetylene	CuI, DIPEA, CH_3CN, 5 h, rt	**39**	85%
2	1-Octyne	CuI, DIPEA, CH_3CN, 10 h, rt	**40**	72%
3	1-Heptyne	CuI, DIPEA, CH_3CN, 8 h, rt	**41**	80%
4	5-Chloro-1-pentyne	CuI, DIPEA, CH_3CN, 10 h, rt	**42**	83%

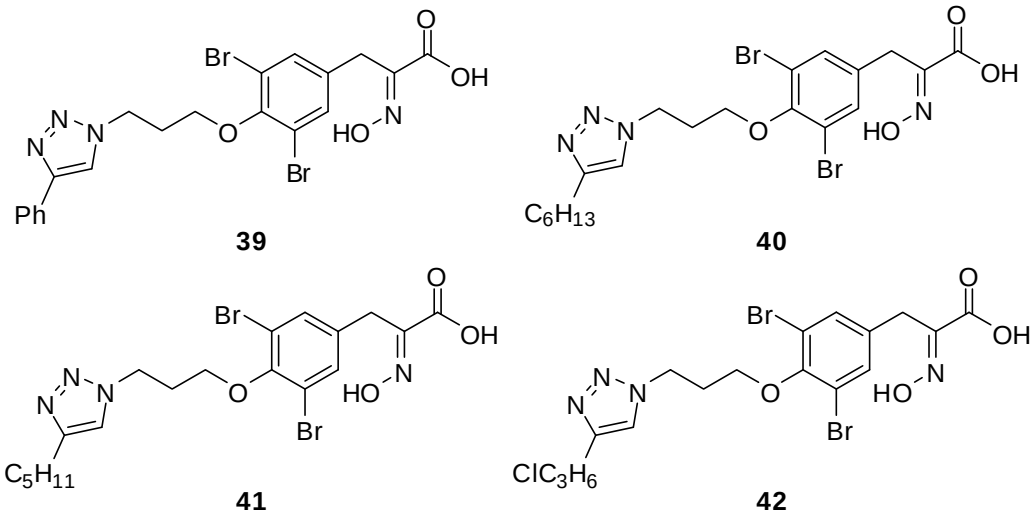


Figure 8: Structures of triazoles synthesised

Coupling of triazoles with different amino compounds

Three different amino compounds (Figure 9) were chosen as the building blocks for right segment. These are Boc-protected agmatine **27**, histamine **43** and Boc-protected amine **44**. Synthesis of Boc-protected agmatine was discussed in Section I (Scheme 6). 1,3-Diaminopropane was used in the displacement of thiomethyl group for the preparation of amine **44**. Histamine used was procured from commercially sources.

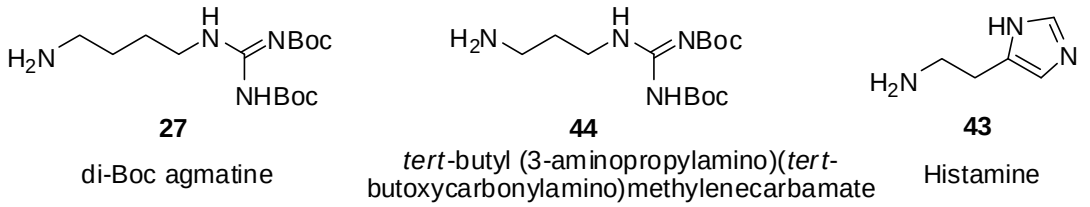
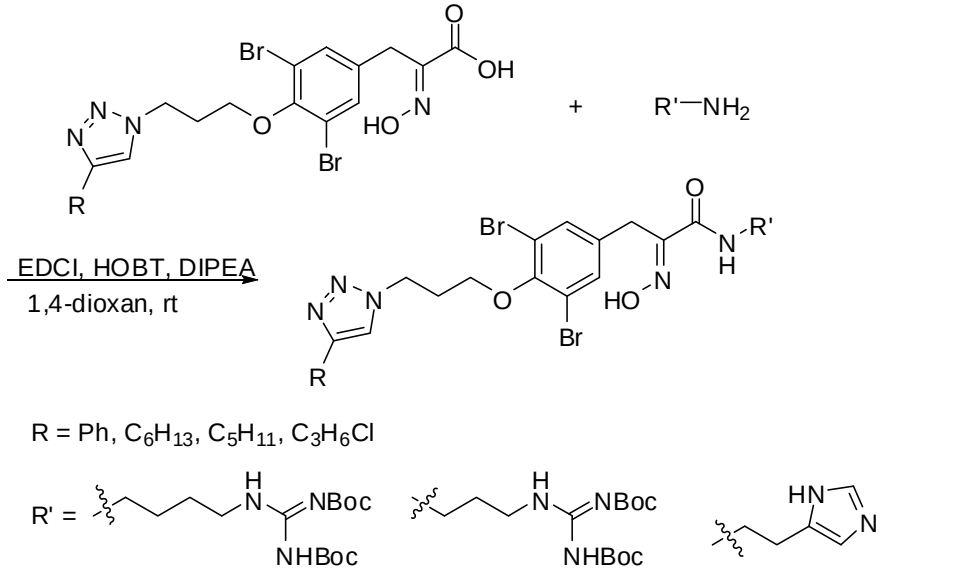


Figure 9: Different amines used for synthesis of analogues

All the 12 coupling reactions were carried out using the same protocol as described below (Scheme 16, Table 2). In a typical procedure a mixture of 1 eq. acid, 1 eq. amine, 1 eq. EDCI and 2 eq. DIPEA was stirred in 1,4-dioxan at room temperature for 6-10 h. After the reaction was completed, it was quenched by water and extracted with EtOAc. The combined EtOAc layer was washed with water followed by brine and dried over Na_2SO_4. The solvent was removed under vacuum to give crude product, which was purified by column chromatography (silica gel, hexane/EtOAc 3:2) to afford amide compound.



Scheme 16: Analogues synthesised with di-Boc-agmatine and 3-aminoguanidine part and histamine

Deprotection of the Boc group of the amides was carried out according to a standard procedure by using TFA in DCM at 0 °C to rt. Excess TFA from the reaction mixture was removed under vacuum. The residue obtained was taken up in 10% $NaHCO_3$ solution and then extracted with DCM to furnish required compound (Figure 10).

Table 2: Reaction conditions utilized for the synthesis of analogues

S. No	Substrate 1	Substrate 2	Reaction conditions	Product	Yield (%)
1	**39**	**27**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**45**	56
2	**40**	**27**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**46**	62
3	**41**	**27**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**47**	42
4	**42**	**27**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**48**	55
5	**39**	**44**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**49**	61
6	**40**	**44**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**50**	53
7	**41**	**44**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**51**	52
8	**42**	**44**	1) EDCI, HOBT, DIPEA, 1,4-dioxan 2) TFA, DCM, rt	**52**	48
9	**39**	**43**	EDCI, HOBT, DIPEA, 1,4-dioxan	**53**	74
10	**40**	**43**	EDCI, HOBT, DIPEA, 1,4-dioxan 2	**54**	80
11	**41**	**43**	EDCI, HOBT, DIPEA, 1,4-dioxan	**55**	77
12	**42**	**43**	EDCI, HOBT, DIPEA, 1,4-dioxan 2	**56**	70

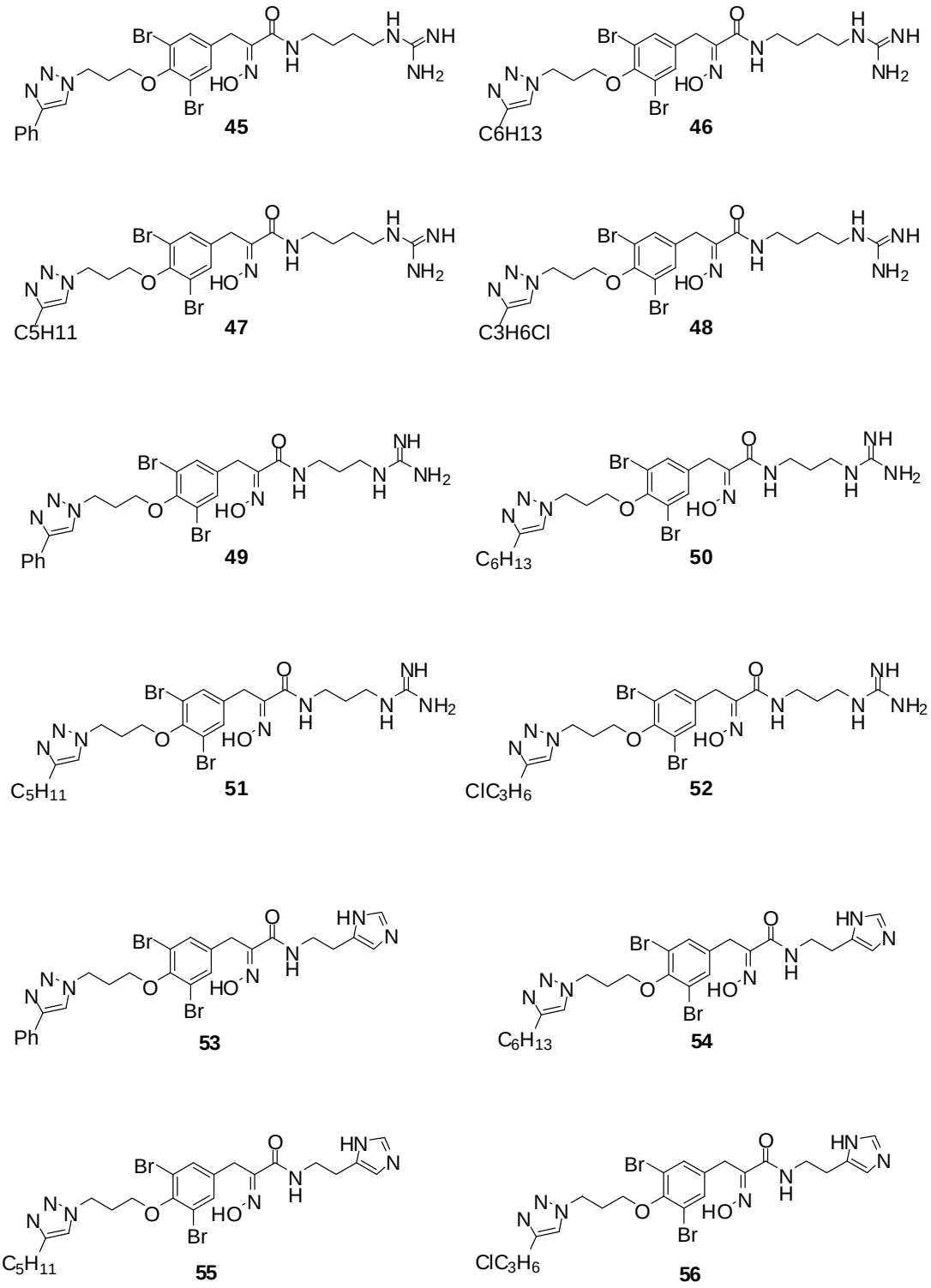


Figure 10: Structures of analogues synthesised

Each library member was synthesised in about 50-100 mg scale and was characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the twelve analogues of the library have been submitted for screening and activity testing and the results are awaited.

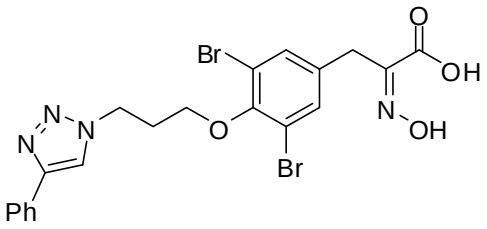
Conclusion:

- For the first time analogues of dibrotyrosine derived alkaloids having anti-tuberculosis activity were synthesised.
- Huisgen reaction was successfully used for the synthesis of 1,2,3-triazoles from oxime-acid intermediate.
- A library of 12 compounds of dibromotyrosine derived alkaloids containing triazole unit has been synthesized with good to excellent yields.
- These synthesised compounds were submitted for screening.

General Procedure for synthesis of 1,2,3-triazoles

To a solution of acetylenic compound (1 mmol) and azide **26** (1 mmol) in CH_3CN (30 mL), CuI (2 mmol) and *N*,*N*-diisopropylethylamine (3 mmol) were added and then the reaction mixture was stirred for 5 h at rt. It was then filtered through celite and the filtrate was diluted with 50 mL of water and 20 mL of NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 50 mL), the combined organic layer was washed with water followed by brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to give a crude residue that was further purified by column chromatography (silica gel, hexane/EtOAc 2:3) to afford the desired triazole as yellow solid.

3-{3,5-Dibromo-4-[3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanoic acid (39)



Mol. Formula : $C_{20}H_{18}Br_2N_4O_4$

Mol. Weight : 538

IR ($CHCl_3$) ν : 3325, 2960, 1701, 1632, 1286, 1215 cm^{-1}

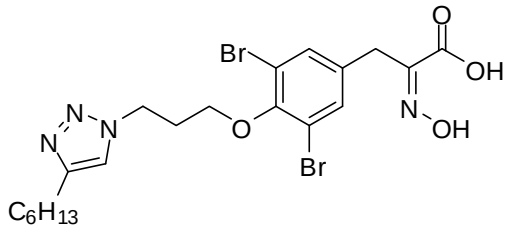
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) : δ 2.46 (quintet, *J* = 6.2 Hz, 2H), 3.78 (s, 3H), 4.06 (t, *J* = 5.8 Hz, 2H), 4.47 (t, *J* = 6.5 Hz, 2H), 7.30-7.45 (m, 3H), 7.46 (s, 2H), 7.75-7.90 (m, 2H), 8.51 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) : δ 28.3, 28.6, 50.3, 70.9, 117.6, 120.7, 125.3, 127.4, 128.6, 131.4, 133.2, 136.4, 146.7, 150.8, 151.3, 164.8

ESI-MS *m/z* **:** 538.9 $[M+H]^+$

Elemental Analysis **:** Found: C, 46.88; H, 3.83; Br, 29.69; N, 10.21%
Calcd: C, 47.04; H, 3.76; Br, 29.80; N, 10.45%

3-{3,5-Dibromo-4-[3-(4-hexyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanoic acid (40)



Mol. Formula **:** $C_{20}H_{26}Br_2N_4O_4$

Mol. Weight **:** 546

IR ($CHCl_3$) ν **:** 3319, 2936, 1699, 1654 cm^{-1}

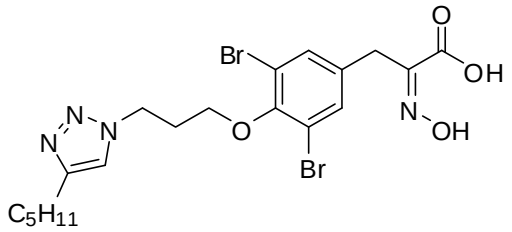
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) **:** δ 0.89 (t, *J* = 6.8 Hz, 3H), 1.30-1.45 (m, 6H), 1.60-1.75 (m, 2H), 2.50 (quintet, *J* = 6.7 Hz, 2H), 2.67 (t, *J* = 6.3 Hz, 2H), 3.78 (s, 2H), 4.07 (t, *J* = 5.8 Hz, 2H), 4.48 (t, *J* = 6.4 Hz, 2H), 7.45 (s, 2H), 8.41 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) **:** δ 14.4, 22.7, 25.8, 28.6, 29.3, 29.5, 29.6, 31.7, 50.4, 70.3, 117.7, 122.6, 133.2, 136.5, 148.5, 150.4, 151.5, 164.8

ESI-MS *m/z* **:** 547.2 $[M+H]^+$

Elemental Analysis **:** Found: C, 43.76; H, 4.87; Br, 29.21; N, 10.18%
Calcd: C, 43.97; H, 4.80; Br, 29.26; N, 10.26%

3-{3,5-Dibromo-4-[3-(4-pentyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanoic acid (41)



Mol. Formula : $C_{19}H_{24}Br_2N_4O_4$

Mol. Weight : 532

IR ($CHCl_3$) ν : 3360, 2925, 1698, 1656 cm^{-1}

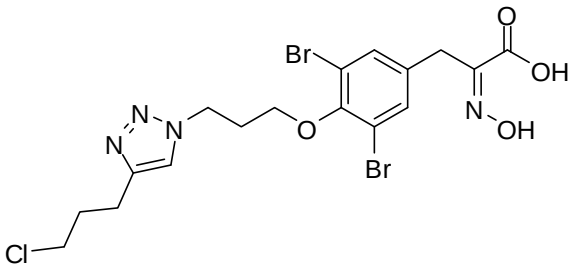
^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) : δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.23-1.41 (m, 4H), 1.65-1.80 (m, 2H), 2.46 (quintet, *J* = 6.4 Hz, 2H), 2.64 (t, *J* = 6.6 Hz, 2H), 3.76 (s, 2H), 4.04 (t, *J* = 5.8 Hz, 2H), 4.48 (t, *J* = 6.4 Hz, 2H), 7.46 (s, 2H), 8.46 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 14.4, 22.8, 25.6, 28.4, 29.6, 29.8, 31.7, 50.1, 70.3, 117.9, 122.3, 133.3, 136.5, 148.6, 150.4, 151.1, 163.9

ESI-MS *m/z* : 533.2 $[M+H]^+$

Elemental Analysis : Found: C, 42.65; H, 4.63; Br, 29.95; N, 10.46 %
Calcd: C, 42.88; H, 4.55; Br, 30.03; N, 10.53%

3-(3,5-Dibromo-4-(3-(4-(3-chloropropyl)-1*H*-1,2,3-triazol-1-yl)propoxy)phenyl)-2-(hydroxyimino)propanoic acid (42)



Mol. Formula	**:**	$C_{17}H_{19}Br_2ClN_4O_4$
Mol. Weight	**:**	538.5
IR ($CHCl_3$) ν	**:**	3352, 2930, 1702, 1658, 1648, 1215 cm^{-1}
^{1}H NMR (200 MHz, DMSO-d_6)	**:**	δ 1.90 (quintet, *J* = 6.7 Hz, 2H), 2.48 (quintet, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 6.3 Hz, 2H), 3.64 (t, *J* = 6.3 Hz, 2H), 3.77 (s, 2H), 4.06 (t, *J* = 5.8 Hz, 2H), 4.46 (t, *J* = 6.4 Hz, 2H), 7.48 (s, 2H), 8.42 (s, 1H)
^{13}C NMR (50 MHz, DMSO-d_6)	**:**	δ 20.6, 28.3, 29.5, 35.2, 44.3, 50.1, 70.0, 117.7, 122.4, 133.3, 136.2, 148.5, 150.1, 151.4, 164.7
ESI-MS *m/z*	**:**	539.6 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 37.68; H, 3.69; N, 10.21% Calcd: C, 37.91; H, 3.56; Br, 29.67; Cl, 6.58; N, 10.40%

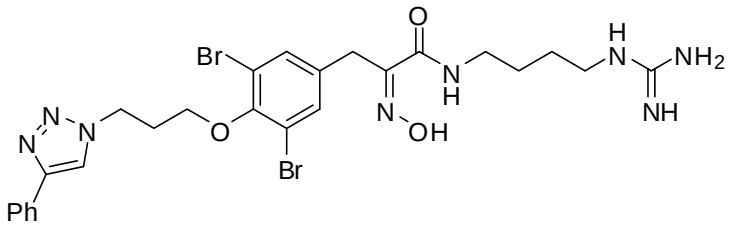
General procedure for the synthesis of amides (by using Boc-agmatine and aminopropyl guanidine)

To a stirred solution of the triazole acid compound (1 mmol) in 1,4-dioxane (10 mL) 1-ethyl-3-(3′-dimaminopropyl)carbodiimide hydrochloride (EDCI) (1 mmol) and *N*-hydroxybenzotriazole (HOBt) (1 mmol) were added at rt and the resultant mixture was allowed to stir for 30 min at rt. Amine compound (1 mmol) and DIPEA (2 mmol) were added and stirring of the reaction mixture was continued for 2 h at rt. The reaction mixture was then quenched by addition of water (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layer was washed with water followed by brine and dried over Na_2SO_4. The solvent was removed under vacuum to give crude product, which was further purified by column chromatography (silica gel, hexane/EtOAc 3:2) to afford amide compound.

A solution of the amide thus obtained and TFA (0.5 mL) in anhydrous CH_2Cl_2 (20 mL) was stirred at rt under argon atmosphere for 12 h. A solution of 10% $NaHCO_3$ in water was added drop wise until the pH was adjusted to ~7-8. Reaction mixture was extracted with CH_2Cl_2 (3 x 30 mL), organic layer was dried over Na_2SO_4,

filtered and concentrated under reduced pressure to afford amide compound as thick oil.

3-{3,5-Dibromo-4-[3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(4-guanidinobutyl)-2-(hydroxyimino)propanamide (45)



Mol. Formula : $C_{25}H_{30}Br_2N_8O_3$

Mol. Weight : 650

IR ($CHCl_3$) ν : 3360, 3256, 2969, 1669, 1202 cm^{-1}

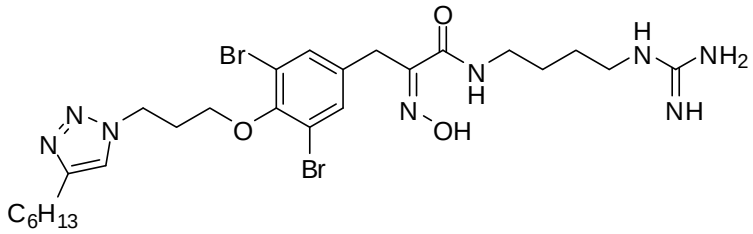
^{1}H NMR (200 MHz, DMSO-d_6) : δ 1.25-150 (m, 4H), 2.50 (quintet, *J* = 6.4 Hz, 2H), 3.01-3.15 (m, 4H), 3.79 (s, 2H), 4.10 (t, *J* = 5.8 Hz, 2H), 4.50 (t, *J* = 6.4 Hz, 2H), 7.25-7.50 (m, 3H), 7.49 (s, 2H), 7.62 (t, *J* = 5.6 Hz, 1H), 7.75-7.95 (m, 2H), 8.28 (t, *J* = 5.5 Hz, 1H), 8.45 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 25.8, 26.3, 28.3, 29.5, 38.0, 40.5, 50.1, 70.0, 117.2, 120.6, 125.3, 127.6, 128.7, 131.6, 132.9, 136.5, 146.8, 150.5, 151.1, 156.7, 162.6

ESI-MS *m/z* : 651.1 $[M+H]^+$

Elemental Analysis : Found: C, 45.94; H, 4.71; Br, 24.36; N, 17.15%
Calcd: C, 46.17; H, 4.65; Br, 24.57; N, 17.23%

3-{3,5-Dibromo-4-[3-(4-hexyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(4-guanidinobutyl)-2-(hydroxyimino)propanamide (46)



Mol. Formula : $C_{25}H_{38}Br_2N_8O_3$

Mol. Weight : 658

IR ($CHCl_3$) ν : 3210, 2926, 1667, 1256, 856 cm^{-1}

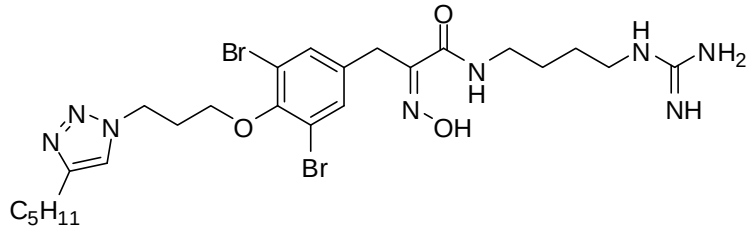
^{1}H NMR (200 MHz, DMSO-d_6) : δ 0.89 (t, *J* = 6.8 Hz, 3H), 1.20-1.48 (m, 10H), 1.60-1.75 (m, 2H), 2.50 (quintet, *J* = 6.4 Hz, 2H), 2.76 (t, *J* = 6.3 Hz, 2H), 3.01-3.15 (m, 4H), 3.78 (s, 2H), 4.07 (t, *J* = 5.9 Hz, 2H), 4.48 (t, *J* = 6.6 Hz, 2H), 7.45 (s, 2H), 7.65 (t, *J* = 5.6 Hz, 1H), 8.41 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 14.2, 22.8, 25.7, 25.9, 26.3, 28.3, 29.1, 29.5, 29.52, 31.8, 38.0, 40.4, 50.0, 69.8, 117.2, 122.3, 132.8, 136.4, 148.5, 150.5, 151.1, 156.7, 163.0

ESI-MS *m/z* : 659.1 $[M+H]^+$

Elemental Analysis : Found: C, 45.46; H, 5.89; Br, 24.13; N, 16.93%
Calcd: C, 45.60; H, 5.82; Br, 24.27; N, 17.02%

3-{3,5-Dibromo-4-[3-(4-pentyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(4-guanidinobutyl)-2-(hydroxyimino)propanamide (47)



Mol. Formula : $C_{24}H_{36}Br_2N_8O_3$

Mol. Weight : 644

IR ($CHCl_3$) ν : 3353, 2915, 1663, 1215, 805 cm^{-1}

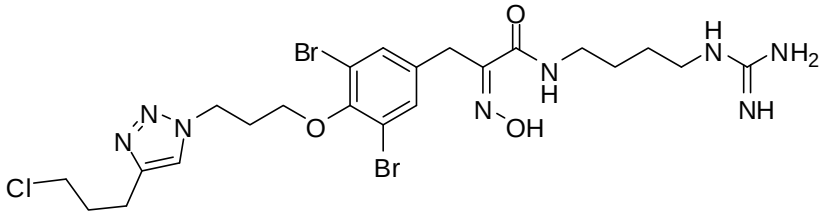
^{1}H NMR (200 MHz, DMSO-d_6) : δ 0.80 (t, *J* = 6.8 Hz, 3H), 1.24-1.40 (m, 4H), 1.65-1.75 (m, 2H), 2.51 (quintet, *J* = 6.5 Hz, 2H), 2.72 (t, *J* = 6.4 Hz, 2H), 3.00-3.16 (m, 4H), 3.80 (s, 2H), 4.10 (t, *J* = 5.8 Hz, 2H), 4.51 (t, *J* = 6.8 Hz, 2H), 7.25 (t, *J* = 5.6 Hz, 1H), 7.48 (s, 2H), 8.47 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 14.3, 22.8, 25.6, 25.8, 26.4, 28.3, 29.4, 29.6, 31.8, 38.1, 40.3, 50.1, 70.0, 117.0, 122.2, 132.5, 136.2, 148.3, 150.4, 151.4, 156.7, 163.1

ESI-MS *m/z* : 645.1 $[M+H]^+$

Elemental Analysis : Found: C, 44.52; H, 5.72; Br, 24.66; N, 17.26%
Calcd: C, 44.73; H, 5.63; Br, 24.80; N, 17.39%

3-{3,5-Dibromo-4-{3-[4-(3-chloropropyl)-1*H*-1,2,3-triazol-1-yl]propoxy}phenyl}-N-(4-guanidinobutyl)-2-(hydroxyimino)propanamide (48)



Mol. Formula : $C_{22}H_{31}Br_2ClN_8O_3$

Mol. Weight : 650.5

IR ($CHCl_3$) ν : 3504, 2935, 1669, 1459, 1235, 809 cm^{-1}

^{1}H NMR (200 MHz, DMSO- : δ 1.36-1.45 (m, 4H), 1.91 (quintet, *J* = 6.7 Hz, 2H), 2.47 (quintet, *J* = 6.3 Hz, 2H), 2.95 (t, *J* = 6.3 Hz, 2H), 3.10-

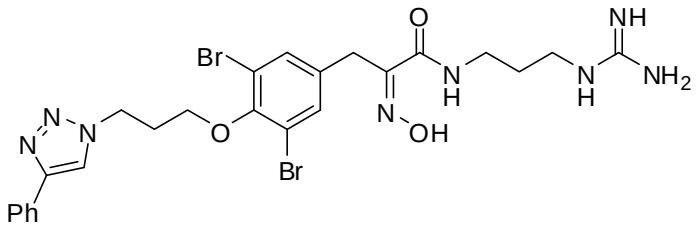
d_6) 3.40 (m, 4H), 3.68 (t, *J* = 6.4 Hz, 2H), 3.78 (s, 2H), 4.05 (t, *J* = 5.8 Hz, 2H), 4.46 (t, *J* = 6.7 Hz, 2H), 7.42 (s, 2H), 7.53 (t, *J* = 5.6 Hz, 1H), 8.51 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) **:** δ 20.3, 25.6, 26.5, 28.1, 29.7, 34.9, 38.3, 40.4, 44.2, 50.0, 70.1, 117.6, 122.6, 132.8, 136.5, 148.3, 150.3, 151.2, 156.4, 162.8

ESI-MS *m/z* **:** 651.7 $[M+H]^+$

Elemental Analysis **:** Found: C, 40.48; H, 4.86; Br, 24.39; N, 17.15%
Calcd: C, 40.60; H, 4.80; Br, 24.56; Cl, 5.45; N, 17.22%

3-{3,5-Dibromo-4-[3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(3-guanidinopropyl)-2-(hydroxyimino)propanamide (49)



Mol. Formula **:** $C_{24}H_{28}Br_2N_8O_3$

Mol. Weight **:** 636

IR ($CHCl_3$) ν **:** 3456, 1669, 1635, 1134 cm^{-1}

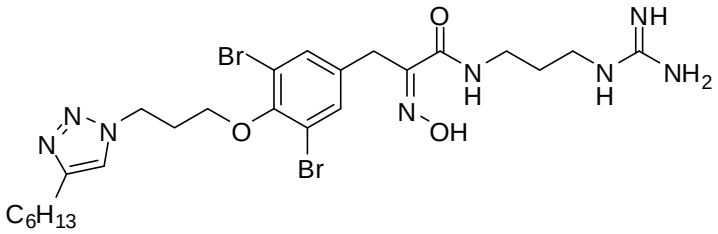
^{1}H NMR (200 MHz, DMSO-d_6) **:** δ 1.60-1.75 (m, 2H), 2.52 (quintet, *J* = 6.4 Hz, 2H), 2.70-2.80 (m, 2H), 3.00-3.20 (m, 2H), 3.80 (s, 2H), 4.04 (t, *J* = 5.8 Hz, 2H), 4.52 (t, *J* = 6.3 Hz, 2H), 7.32-7.49 (m, 3H), 7.48 (s, 2H), 7.85-7.95 (m, 2H), 8.16 (t, *J* = 5.6 Hz, 1H), 8.41(s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) **:** δ 26.5, 28.2, 29.6, 35.9, 38.1, 50.2, 70.4, 117.5, 120.6, 125.4, 127.5, 128.4, 131.5, 133.1, 136.4, 146.8, 150.4, 151.2, 156.8, 162.5

ESI-MS *m/z* **:** 637.1 $[M+H]^+$

Elemental Analysis **:** Found: C, 45.13; H, 4.51; Br, 25.08; N, 17.43%
Calcd: C, 45.30; H, 4.44; Br, 25.11; N, 17.61%

3-{3,5-Dibromo-4-[3-(4-hexyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(3-guanidinopropyl)-2-(hydroxyimino)propanamide (50)



Mol. Formula **:** $C_{24}H_{36}Br_2N_8O_3$

Mol. Weight **:** 644

IR ($CHCl_3$) ν **:** 3370, 1660, 1139, 870, 665 cm^{-1}

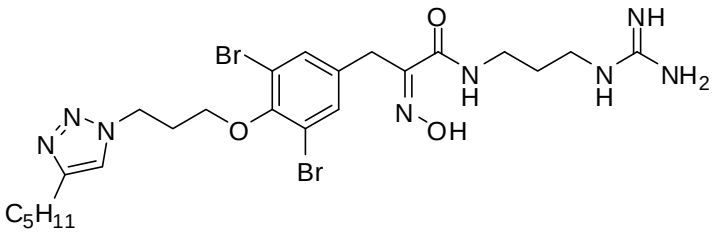
^{1}H NMR (200 MHz, DMSO-d_6) **:** δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.25-1.45 (m, 6H), 1.60-1.81 (m, 4H), 2.54 (quintet, *J* = 6.5 Hz, 2H), 2.78 (m, 4H), 3.10-3.30 (m, 2H), 3.82 (s, 2H), 4.03 (t, *J* = 5.9 Hz, 2H), 4.48 (t, *J* = 6.4 Hz, 2H), 7.42 (s, 2H), 7.63 (t, *J* = 5.6 Hz, 1H), 8.40 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) **:** δ 14.4, 22.9, 25.8, 26.6, 28.3, 29.2, 29.5, 29.7, 31.9, 35.8, 38.2, 50.4, 70.2, 117.8, 122.4, 133.1, 136.3, 148.6, 150.2, 151.4, 156.7, 163.1

ESI-MS *m/z* **:** 645.1 $[M+H]^+$

Elemental Analysis **:** Calcd: C, 44.73; H, 5.63; Br, 24.80; N, 17.39%
Found: C, 44.62; H, 5.69; Br, 24.68; N, 17.26%

3-{3,5-Dibromo-4-[3-(4-pentyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-N-(3-guanidinopropyl)-2-(hydroxyimino)propanamide (51)



Mol. Formula : $C_{23}H_{34}Br_2N_8O_3$

Mol. Weight : 630

IR ($CHCl_3$) ν : 3290, 2936, 1666, 1138, 947 cm^{-1}

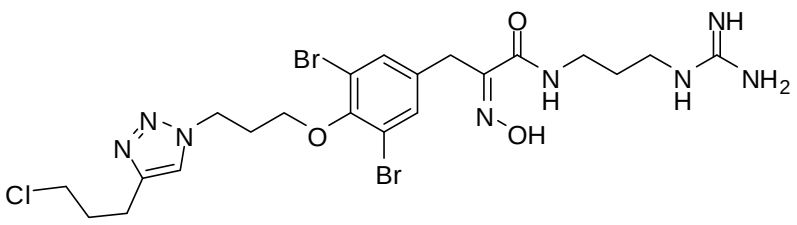
^{1}H NMR (200 MHz, DMSO-d_6) : δ 0.85 (t, *J* = 6.8 Hz, 3H), 1.25-1.40 (m, 4H), 1.65-1.75 (m, 4H), 2.53 (quintet, *J* = 6.6 Hz, 2H), 2.80-2.90 (m, 2H), 3.05-3.20 (m, 2H), 3.78 (s, 2H), 4.06 (t, *J* = 5.9 Hz, 2H), 4.46 (t, *J* = 6.6 Hz, 2H), 7.46 (s, 2H), 7.56 (t, *J* = 5.6 Hz, 1H), 8.51 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 14.2, 22.6, 25.8, 26.5, 28.4, 29.7, 29.8, 31.7, 35.6, 38.4, 50.1, 69.8, 117.1, 122.0, 133.0, 136.4, 148.2, 150.5, 151.6, 156.2, 162.7

ESI-MS *m/z* : 631.1 $[M+H]^+$

Elemental Analysis : Calcd: C, 43.82; H, 5.44; Br, 25.35; N, 17.78%
Found: C, 43.67; H, 5.52; Br, 25.26; N, 17.58%

3-{3,5-Dibromo-4-{3-[4-(3-chloropropyl)-1*H*-1,2,3-triazol-1-yl]propoxy}phenyl}-N-(3-guanidinopropyl)-2-(hydroxyimino)propanamide (52)

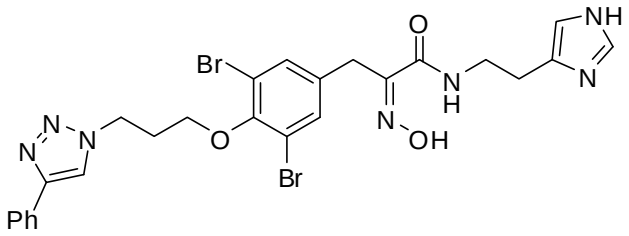


Mol. Formula	:	$C_{21}H_{29}Br_2ClN_8O_3$
Mol. Weight	:	636.5
IR ($CHCl_3$) ν	:	3320, 1658, 1640 cm^{-1}
^{1}H NMR (200 MHz, DMSO-d_6)	:	δ 1.60-1.79 (m, 2H), 1.91 (quintet, *J* = 6.6 Hz, 2H), 2.46 (quintet, *J* = 6.4 Hz, 2H), 2.75-3.20 (m, 6H), 3.65 (t, *J* = 6.3 Hz, 2H), 3.77 (s, 2H), 4.04 (t, *J* = 5.8 Hz, 2H), 4.47 (t, *J* = 6.7 Hz, 2H), 7.46 (s, 2H), 7.62 (t, *J* = 5.6 Hz, 1H), 8.46 (s, 1H)
^{13}C NMR (50 MHz, DMSO-d_6)	:	δ 20.2, 26.4, 28.4, 29.6, 34.9, 35.7, 38.1, 44.2, 50.2, 70.1, 117.6, 122.1, 133.2, 136.1, 148.3, 150.4, 151.6, 156.7, 163.5
ESI-MS *m/z*	:	637.6 $[M+H]^+$
Elemental Analysis	:	Found: C, 39.46; H, 4.63; Br, 24.98; N, 17.48% Calcd: C, 39.61; H, 4.59; Br, 25.10; Cl, 5.57; N, 17.60%

General procedure for the synthesis of amides (by using histamine)

To a stirred solution of triazole acid compound (1 mmol) in 1,4-dioxane (10 mL) 1-ethyl-3-(3′-dimaminopropyl)carbodiimide hydrochloride (EDCI) (1 mmol) and *N*-hydroxybenzotriazole (HOBt) (1 mmol) were added at rt and allowed to stir for 30 min. Histamine (1 mmol) and DIPEA (2 mmol) were then added and stirring of the reaction mixture was continued for 6 h at rt. The reaction mixture was then quenched by addition of water and extracted with EtOAc (3 X 30 mL). The combined EtOAc layer was washed with water followed by brine and dried over Na_2SO_4. The solvent was removed under vacuum to give crude product, which was further purified by column chromatography (silica gel, hexane/EtOAc 3:2) to afford the required amide compound.

***N*-[2-(1*H*-Imidazol-4-yl)ethyl]-3-{3,5-dibromo-4-[3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanamide (53)**



Mol. Formula : $C_{25}H_{25}Br_2N_7O_3$

Mol. Weight : 631

IR ($CHCl_3$) ν : 3412, 1665, 1138, 804, 770 cm^{-1}

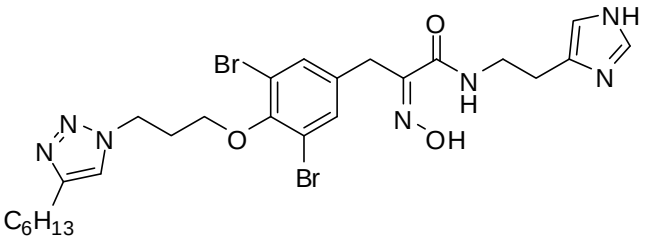
^{1}H NMR (200 MHz, DMSO-d_6) : δ 2.50 (quintet, *J* = 6.3 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 3.39 (dt, *J* = 5.7, 7.1 Hz, 2H), 3.76 (s, 2H), 4.04 (t, *J* = 5.9 Hz, 2H), 4.47 (t, *J* = 6.4 Hz, 2H), 6.84 (s, 1H), 7.30-7.48 (m, 3H), 7.47 (s, 2H), 7.80-7.95 (m, 2H), 8.52(s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 27.9, 28.5, 29.6, 40.7, 50.2, 70.2, 117.2, 117.8, 120.4, 125.2, 127.4, 128.5, 131.4, 132.1, 133.2, 134.1, 136.3, 146.4, 150.4, 151.5, 162.9

ESI-MS *m/z* : 632.0 $[M+H]^+$

Elemental Analysis : Found: C, 47.32; H, 4.07; Br, 25.17; N, 15.37%

***N*-[2-(1*H*-Imidazol-4-yl)ethyl]-3-{3,5-dibromo-4-[3-(4-hexyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanamide (54)**



Mol. Formula : $C_{25}H_{33}Br_2N_7O_3$

Mol. Weight : 639

IR ($CHCl_3$) ν : 3430, 2926, 1651, 1205, 668 cm^{-1}

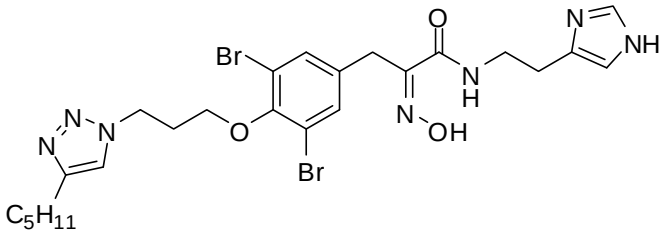
^{1}H NMR (200 MHz, DMSO-d_6) : δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.23-1.46 (m, 6H), 1.60-1.75 (m, 2H), 2.25-2.50 (m, 4H), 2.62 (t, *J* = 6.9 Hz, 4H), 3.40 (t, *J* = 7.2 Hz, 2H), 3.78 (s, 2H), 4.06 (t, *J* = 5.8 Hz, 2H), 4.50 (t, *J* = 6.7 Hz, 2H), 6.82 (s, 1H), 7.45 (s, 2H), 8.45 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) : δ 14.3, 22.8, 25.6, 27.6, 28.2, 29.3, 29.5, 29.6, 31.8, 40.4, 50.2, 70.1, 117.4, 117.6, 122.6, 132.2, 133.2, 134.2, 136.5, 148.5, 150.4, 151.5, 162.8

ESI-MS *m/z* : 640.1 $[M+H]^+$

Elemental Analysis : Found: C, 46.79; H, 5.28; Br, 24.72; N, 15.18%
Calcd: C, 46.96; H, 5.20; Br, 24.99; N, 15.33%

***N*-[2-(1*H*-Imidazol-4-yl)ethyl]-3-{3,5-dibromo-4-[3-(4-pentyl-1*H*-1,2,3-triazol-1-yl)propoxy]phenyl}-2-(hydroxyimino)propanamide (55)**



Mol. Formula : $C_{24}H_{31}Br_2N_7O_3$

Mol. Weight : 625

IR ($CHCl_3$) ν : 3458, 2928, 1660, 1358 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6) : δ 0.86 (t, *J* = 6.8 Hz, 3H), 1.25-1.45 (m, 4H), 1.65-1.75 (m, 2H), 2.45-2.63 (m, 4H), 2.68 (t, *J* = 6.5 Hz, 2H), 3.42 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 2H), 4.04 (t, *J* = 5.8 Hz, 2H), 4.48 (t, *J* = 6.4 Hz, 2H), 7.48 (s, 2H), 8.47 (s, 1H)

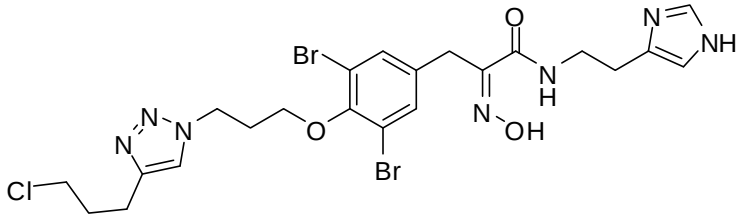
^{13}C NMR (50 MHz, DMSO- : δ 14.4, 22.7, 25.7, 27.6, 28.5, 29.6, 29.8, 31.8, 40.3, 50.2,

d_6) 70.3, 117.6, 117.8, 122.3, 132.3, 133.1, 134.3, 136.5, 148.6, 150.4, 151.5, 162.8

ESI-MS *m/z* **:** 626.1 $[M+H]^+$

Elemental Analysis **:** Found: C, 45.96; H, 5.08; Br, 25.38; N, 15.52%
Calcd: C, 46.09; H, 5.00; Br, 25.55; N, 15.68%

N-[2-(1*H*-Imidazol-4-yl)ethyl]-3-{3,5-dibromo-4-{3-[4-(3-chloropropyl)-1*H*-1,2,3-triazol-1-yl]propoxy}phenyl}-2-(hydroxyimino)propanamide (56)



Mol. Formula **:** $C_{22}H_{26}Br_2ClN_7O_3$

Mol. Weight **:** 631.5

IR ($CHCl_3$) ν **:** 3473, 2915, 1654, 1289 cm^{-1}

^{1}H NMR (200 MHz, DMSO-d_6) **:** δ 1.90 (quintet, *J* = 6.5 Hz, 2H), 2.32-2.63 (m, 4H), 2.91 (t, *J* = 6.3 Hz, 2H), 3.40 (t, *J* = 7.3 Hz, 2H), 3.65 (t, *J* = 6.4 Hz, 2H), 3.76 (s, 2H), 4.02 (t, *J* = 5.9 Hz, 2H), 4.47 (t, *J* = 6.7 Hz, 2H), 6.84 (s, 1H), 7.46 (s, 2H), 8.47 (s, 1H)

^{13}C NMR (50 MHz, DMSO-d_6) **:** δ 20.4, 27.5, 28.4, 29.4, 35.1, 40.7, 44.2, 50.4, 70.2, 117.6, 117.8, 122.2, 132.4, 133.2, 134.5, 136.4, 148.6, 150.2, 151.3, 162.7

ESI-MS *m/z* **:** 632.6 $[M+H]^+$

Elemental Analysis **:** Found: C, 41.65; H, 4.23; Br, 25.19; N, 15.47%
Calcd: C, 41.83; H, 4.15; Br, 25.30; Cl, 5.61; N, 15.52%

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Chapter 3

Section I

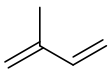
Total synthesis of mutisianthol

Introduction

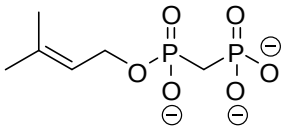
Terpenes

Terpenes are a large and varied class of hydrocarbons, produced primarily by a wide variety of plants, particularly conifers, though also by some insects such as swallowtail butterflies, which emit terpenes from their osmeterium. They are also the major components of resin and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine". In addition to their roles as end-products in many organisms, terpenes are major biosynthetic building blocks within nearly every living creature.

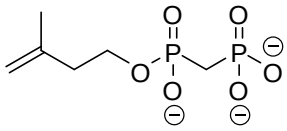
Terpenes are derived biosynthetically from units of isoprene, which has the molecular formula C_5H_8. The basic molecular formulae of terpenes are multiples of that, $(C_5H_8)_n$ where n is the number of linked isoprene units. This is called the *isoprene rule* or the *C_5 rule*. The isoprene units may be linked together "head to tail" or “tail to tail” to form linear chains or they may be arranged to form rings. Isoprene itself does not undergo the building process, but rather activated forms, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), are the components in the biosynthetic pathway.



Isoprene



Dimethylallyl pyrophosphate



Isopentenyl pyrophosphate

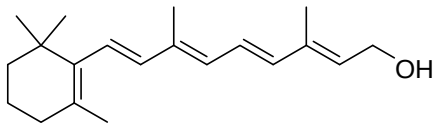
Classification of terpenes

Terpenes are classified by the number of terpene units present in the molecule.

- **Hemiterpenes** consist of *a single isoprene* unit. eg. Isoprene
- **Monoterpenes** consist of *two isoprene* units and have the molecular formula $C_{10}H_{16}$. eg. geraniol, limonene and terpineol.
- **Sesquiterpenes** consist of *three isoprene* units and have the molecular formula $C_{15}H_{24}$. eg. farnesol.
- **Diterpenes** are composed for *four isoprene* units and have the molecular formula $C_{20}H_{32}$. eg. cafestol, kahweol, cembrene and taxadiene.

- **Sesterterpenes**, terpenes having 25 carbons and *five isoprene* units, are rare relative to the other sizes. eg. geranylfarnesol.
- **Triterpenes** consist of *six isoprene* units and have the molecular formula $C_{30}H_{48}$. eg. Squalene.
- **Tetraterpenes** contain *eight isoprene* units and have the molecular formula $C_{40}H_{64}$. eg. lycopene, γ-carotene, α and β -carotenes.
- **Polyterpenes** consist of long chains of *many isoprene* units. eg. Natural rubber.

When terpenes are modified by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids or isoprenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Retinol (Vitamin A) is an example of a terpene.



Retinol (Vitamin A)

Sesquiterpenes

Sesquiterpenes are a diverse group of terpenoid compounds containing a 15-carbon skeleton. Their structure diversification and pharmacological activity make this group of exceptional interest. The phenolic sesquiterpenes of the bisabolane family have been isolated from many different natural sources.[1] These are the olfactorally active components of a large number of essential oils and they show a wide range of biological activities. Curcuphenol **1,** curcuquinone **2** and curcuhydroquinone **3** (Figure 1) were isolated from the Caribbean gorgonian *Pseudopterogorgia rigida*[2] and show antibacterial properties against *Staphylococcus aureus* and *Vibrio anguillarum*. The closely related xanthorrhizol **4** was extracted

from *Curcuma xanthorrhixa* and *Iostephane heterophylla* which are plants of different geographic origins, used in traditional medicine.

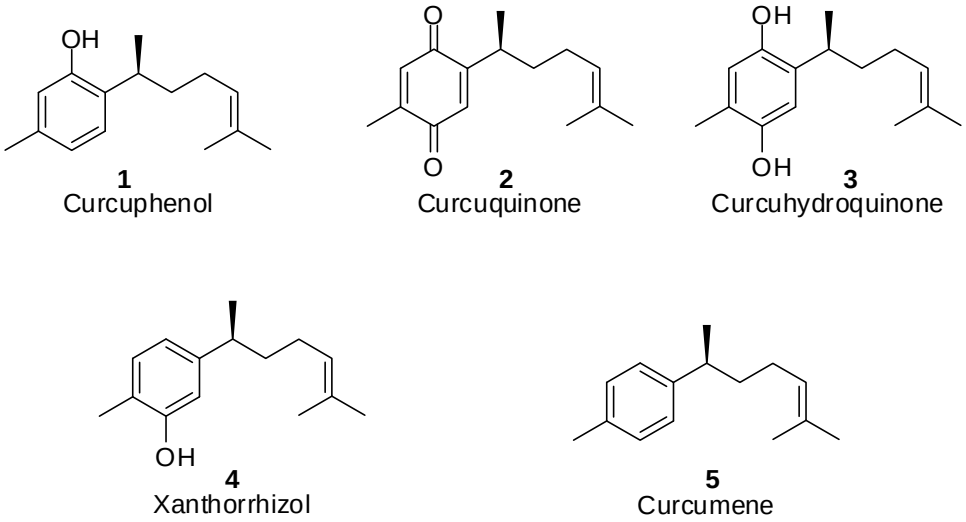
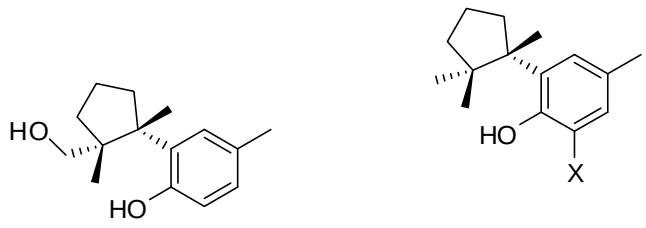


Figure 1: Important naturally occuring sesquiterpenes

Out of these phenolic sesquiterpenes, α-curcumene **5** is the most important sesquiterpene having antibacterial activity and was isolated from the rhizomes of *Curcuma aromatica* Salisb.[3] The monocyclic aromatic sesquiterpenes of the bisabolane family are constituents of a large number of essential oils. Most of these compounds possess a benzylic asymmetric centre.

Cuparanes and herbertanes belong to an expanding family of sesquiterpenes that possess two vicinal quaternary carbon centers, some of which exhibit interesting biological activities. Herbertanes are considered as chemical markers for the liverworts belonging to the genus Herbertus.[4] Asakawa and co-workers have recently reported[5] the isolation of (+)-1,14-herbertenediol **6**, along with other new members of the herbertane group and the dimeric herbertanes mastigophorenes, from the Japanese liverwort *Herberta sakuraii*. Isolation of (-)-α-herbertenol **7** and (-)-herbertenediol **8**, (Figure 2) along with other herbertenes, from the liverwort *Herberta adunca* was reported earlier by Matsuo and co-workers.[5]



6

(+)-1,14-Herbetenediol

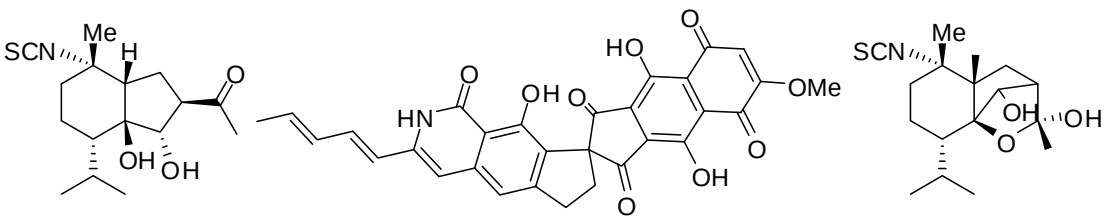
X = H (-)-α-Herbertenol **7**

X = OH (-)-Herbertenediol **8**

Figure 2

Biologically active naturally occuring indanes

Structurally diverse indanes have continuously been isolated[6] along the years, yielding new challenges to organic synthesis. Recent examples are fredericamycin, isolated[7] from *Streptomyces griseus* in 1981, which exhibits a potent antitumor activity against several tumor models (*in vivo*) such as P388 leukemia, B16 melanoma and CD8F mammary carcinoma and does not show mutagenicity in the Ames test. Also the architecturally complex alkaloid ribasine isolated[8] from fumariaceae plants in 1983, is the parent compound of a class of alkaloids that contain an indanobenzazepine in their skeleton and are biogenetically related to the isoquinoline alkaloid family. Axiplyns D **9** and E **10** (Figure 3), isothiocyanate sesquiterpenes from the sponge *Axinyssa aplysinoides* are potent brine shrimp toxins with LD50 values between 1.5 and 1.8 μg/mL.[9]



Axiplyn D

9

Fredericamycin

Axiplyn E

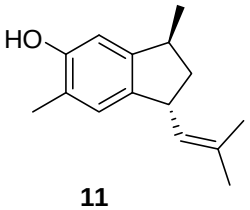
10

Figure 3: Structures of indane sesquiterpenes

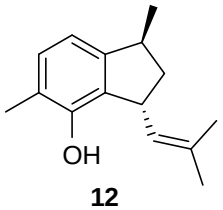
Mutisianthol and its synthesis

The phenolic sesquiterpene mutisianthol **11** is one of the important members of this class of indane compounds. This molecule was isolated[10] by Bohlmann and co-workers in 1979, from the roots of *Mutisia homoeantha*. Mutisianthol is plausibly

derived in nature from an α-curcumene type precursor by cyclisation to form the indane nucleus. Interestingly, an isomer of mutisianthol, jungianol **12** (Figure 4), was found in *Jungia malvaefolia*[11] and apparently arises from intramolecular *ortho*-alkylation of the same biogenetic precursor. The most intriguing aspect of these terpene molecules is the *trans*-relationship assigned for the two side chains in the five-membered ring of mutisianthol and of jungianol based on the spectroscopic data. After nearly two decades of the work of Bohlmann, Ho and his group reported[12] the total synthesis of mutisianthol. In the first route, the authors obtained the acetylated *cis*-indan, whose structure is very similar to **11**. However, comparing the NMR spectra of this intermediate with those of the natural compound, the authors considered that the relative configuration of mutisianthol could be *trans*. Ho *et al*. then modified their synthetic route and prepared the *trans* diastereoisomer, which showed spectroscopic data identical to natural mutisianthol.



11
Mutisianthol



12
Jungianol

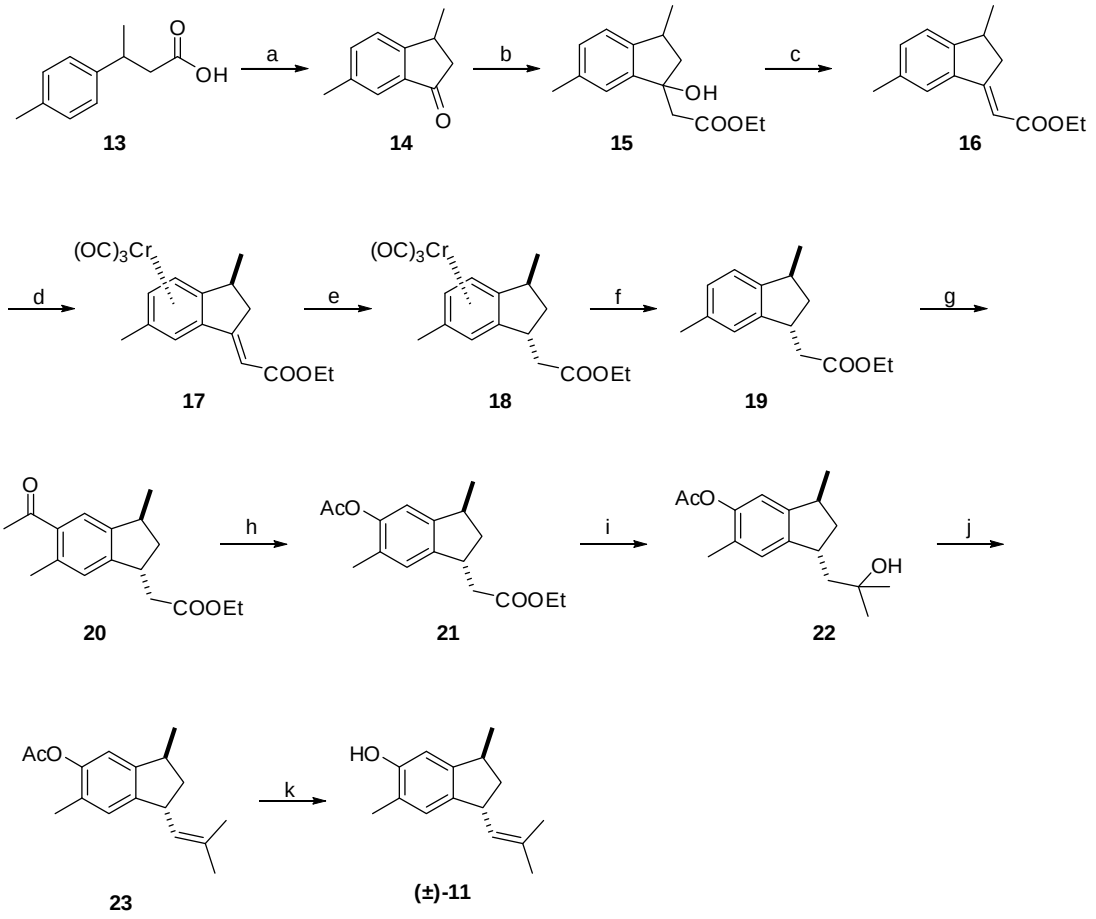
Figure 4

Synthesis of sesquiterpenes possessing a fused bicyclic system has been an important theme in organic chemistry for decades. *Trans*-relationship assigned for the two side chains in the five-membered ring of mutisianthol prompted several synthetic chemists to develop an efficient total synthesis. Till the time the present work has been undertaken, one stereoselective synthesis[12] of (±)-mutisianthol and a diastereoselective total synthesis[14] of (±)-mutisianthol have been reported.

Ho's approach (1997)[12]

The synthetic plan adopted by Ho *et al*. for the mutisianthol relied on template effect[13] of a tricarbonylchromium complex. Friedel-Craft acylation of Rupe's acid provided indalone **14**. Applying Reformatsky reaction, the indanone was converted into a tertiary alcohol **15** which on dehydration produced unsaturated ester **16**. Template effect of tricarbonylchromium complex was used for the stereoselective reduction of alkene **17** to give *trans*-disubstituted groups in the five membered ring of

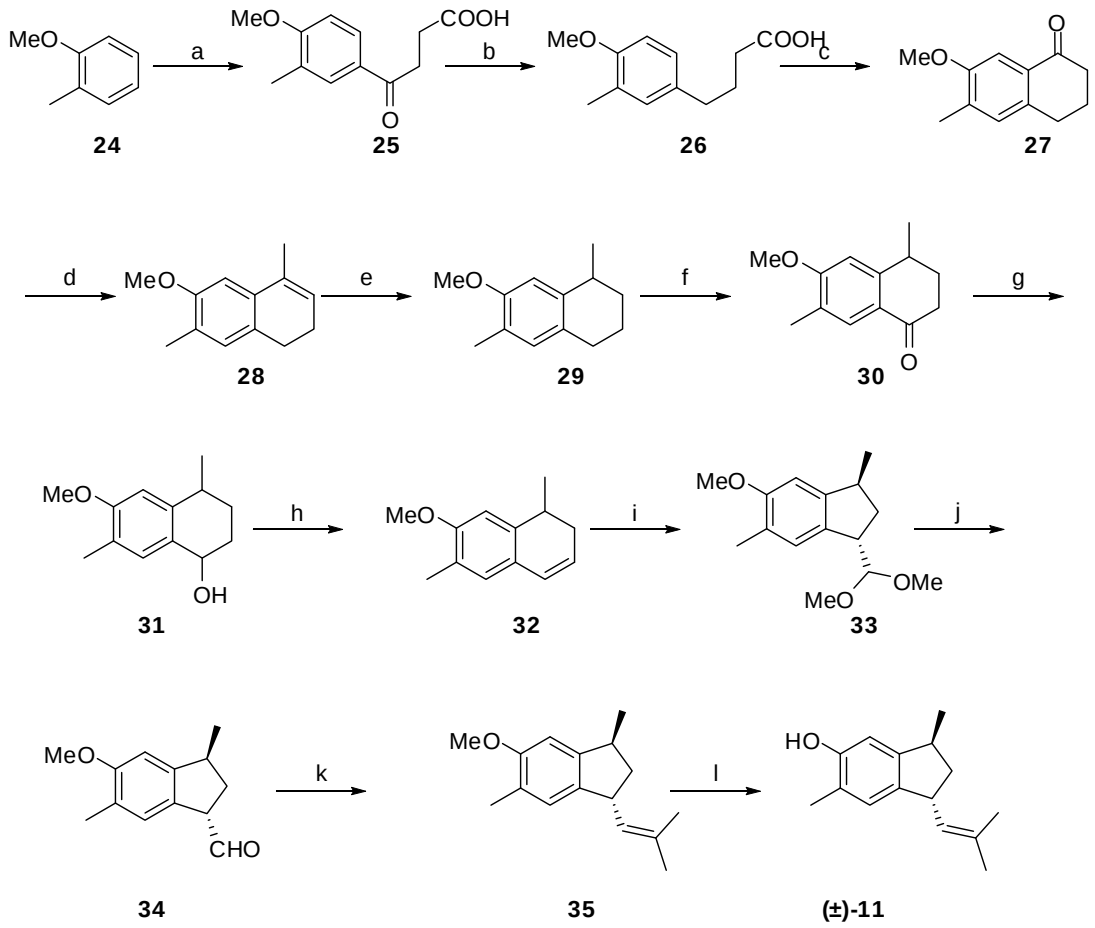
indane **18**. On treatment of **18** with iodine the desired *trans*-ester **19** was obtained. The latter ester compound **19** was acetylated to give the 6-acetyl derivative **20**. Baeyer-Villiger oxidation of the ketone **20** with *m*-chloroperbenzoic acid led to partial hydrolysis to give a mixture of phenol and acetate; this mixture was reacetylated to afford **21**. Methyllithium attacked both the ester functions of the acetate **21** and product obtained as mixture was reacetylated to yield acetate **22**. Dehydration of **22** by refluxing with catalytic amount of *p*-toluenesulfonic acid in toluene under a Dean-Stark trap led to **23**. Final deacylation was achieved with LAH in ether to furnish mutisianthol as a racemic mixture (Scheme 1).



Scheme 1

Reagents and conditions: (a) P_2O_5, CH_3SO_3H, rt, 75%; (b) Zn, $BrCH_2COOEt$, < 30 °C, 80%; (c) TsOH, toluene, 110 °C, 70%; (d) $Cr(CO)_6$, dioxane, 52%; (e) Mg, MeOH, 71%; (f) I_2, THF, 80%; (g) AcCl, $AlCl_3$, DCM, 82%; (h) MCPBA, Ac_2O, Py, 56%; (i) MeLi, Ac_2O, Py, DMAP, 67%; (j) TsOH, PhH, 80 °C, 85%; (k) LAH, Et_2O, 92%.

Ferraz Helena's approach (2003)[14]



Scheme 2

Reagents and conditions: (a) Succinic anhydride, $AlCl_3$, 80%; (b) Zn(Hg), HCl 68%; (c) TFAA, TFA, 97%; (d) i) CH_3MgI, ii) HCl, rt, 90%; (e) H_2, Pd/C, 92%; (f) CrO_3, 55%; (g) $NaBH_4$, MeOH:THF, 0 °C; (h) TsOH, PhH, rt, 74% (two steps); (i) TTN, TMOF, 0 °C, 91%; (j) AcOH, 70-80 °C, 86%; (k) $Ph_3PCH(CH_3)_2Br$, nBuLi, 10 °C, THF, 63%; (l) NaSEt, DMF, 150 °C, 89%.

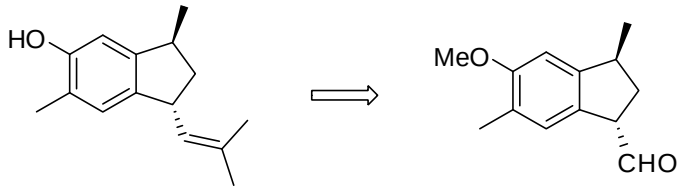
The first distereoselective synthesis of mutisianthol was accomplished in 12 steps (Scheme 2) from the readily available 2-methylanisole by Ferraz *et al*. 2-Methylanisole on Friedel-Craft acylation with succinic anhydride afforded keto-acid **25**. The reduction of the keto-acid **25** under Clemmensen's conditions was carried out with zinc amalgam. Cyclisation was achieved with TFA to furnish tetralone **27**. Grignard reaction of **27** with methyl magnesium iodide, followed by work-up with 10% aqueous solution of HCl afforded the corresponding alkene **28**. Hydrogenation of

alkene followed by oxidation with chromium trioxide yielded tetralone **30**. Reduction of the latter **30** with $NaBH_4$ in a mixture of MeOH and THF gave the tetralol intermediate which was converted to the required 1,2-dihydronaphthalene **32** with TsOH in benzene. Later, this intermediate was treated with Thallium trinitrate (TTN), providing the *trans*-1,3-disubstituted indan **33** in excellent yield. Hydrolysis of the latter **33** with acetic acid furnished the desired aldehyde **34** in good yield. Wittig reaction of the aldehyde **34** with ylide prepared from isopropyl(methyl)diphenylphosphonium iodide introduced the required isopropenyl moiety leading to **35**. Finally, deprotection of the methyl group with sodium ethanethiolate gave mutisianthol.

Both the synthetic routes described in literature for mutisianthol are reported for the racemic product only. They employ toxic and hazardous chemicals like TTN, $Cr(CO)_6$ etc. for the routes; therefore, it was necessary to develop a new synthetic route for mutisianthol which would include routes to both racemic and chiral compounds. With this in view, the present work was undertaken. Successful implementation was possible *via* Heck reaction and asymmetric Heck reaction for both racemic and chiral mutisianthol respectively. Details are given in the following pages.

Present Work

The salient feature in the structure of mutisianthol **11** is the presence of *trans* bisubstituted five membered ring attached to phenyl ring. The basic strategy for the synthesis of mutisianthol **11** is delineated in the retrosynthetic analysis (Scheme 3). An appealing strategy for the linear synthesis of mutisianthol can be envisaged by the stereo controlled synthesis of two side chains in the five membered ring *via* asymmetric Heck reaction or asymmetric alkylation on aromatic ring. Synthesis of mutisianthol from aldehyde **34** was earlier reported[14] by Ferraz *et al*. Thus aldehyde **34** was also considered to be the starting material in the present synthesis (Scheme 3).



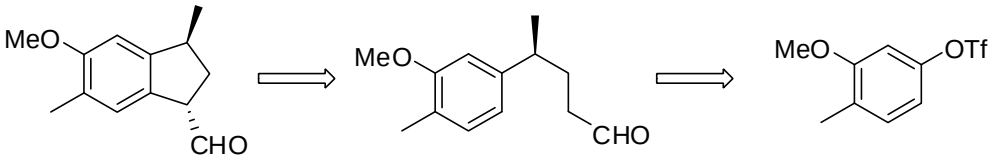
11 **34**

Scheme 3: Retrosynthetic analysis

Retrosynthetic Analysis:

Aldehyde **34** was planned to be synthesised *via* two different approaches as outlined below (Scheme 4 and Scheme 5).

Aldehyde **34** could be obtained through hypervalent iodine oxidation of enamine prepared from aldehyde **36**. Heck reaction of triflate **37** with methyl crotonate followed by reduction and one-carbon Wittig homologation would provide aldehyde **36** (Scheme 4).

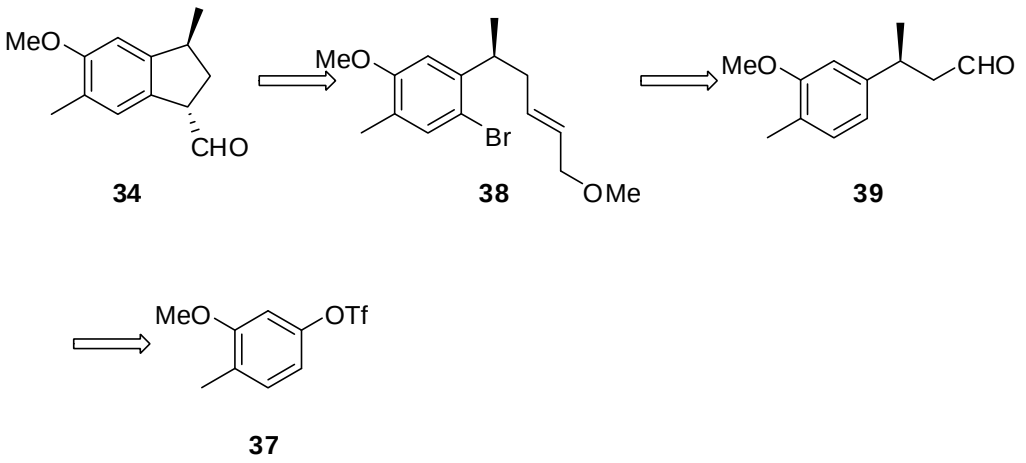


34 **36** **37**

Scheme 4: Retrosynthetic analysis

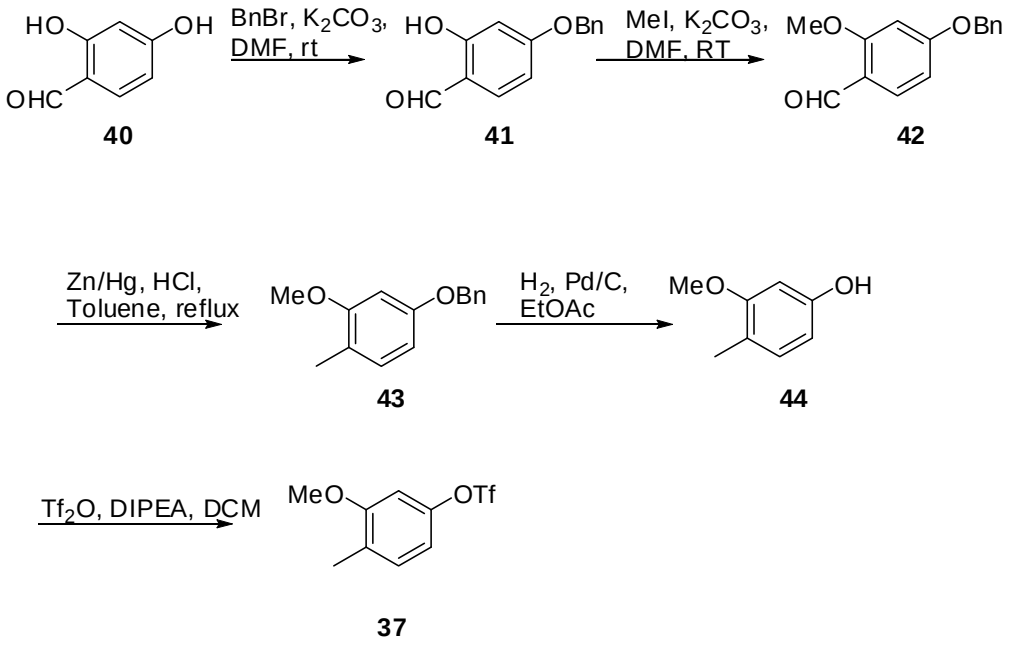
Asymmetric intramolecular Heck reaction with (*R*)-BINAP as chiral ligand of methyl ether **38** followed by dihydroxylation and cleavage of diol is expected to produce aldehyde **34**. Two-carbon Wittig homologation of aldehyde **39** followed by DIBAL-H reduction of ester and methylation of allyl alcohol would furnish

intermediate **38**. Aldehyde **39** can be prepared by intermolecular asymmetric Heck reaction of triflate **37** with 4,7-dihydro-1,3-dioxepin *via* asymmetric intermolecular Heck reaction with (*S*)-BINAP as chiral ligand (Scheme 5).



Scheme 5: Retrosynthetic analysis

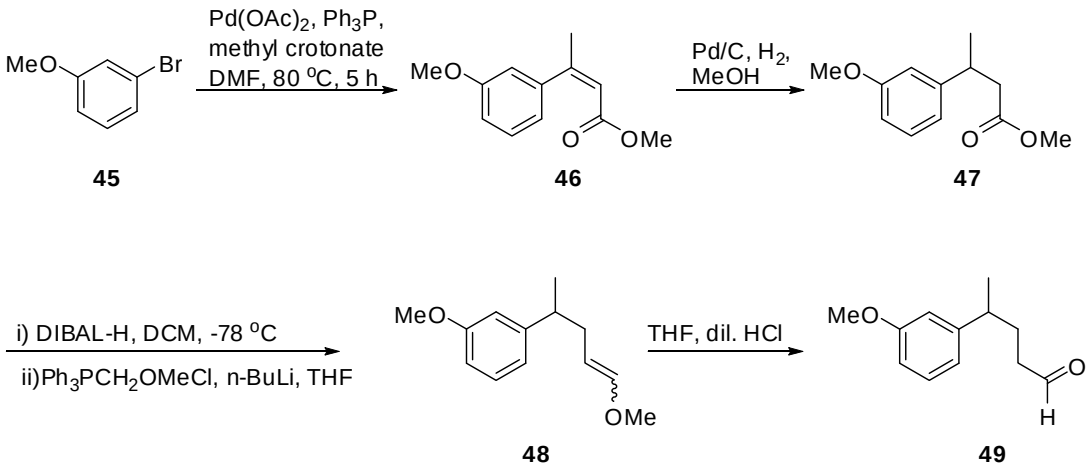
The retrosynthetic analysis outlined in **Scheme 4** and **Scheme 5** identified compound **37** as a potential synthetic intermediate and its synthesis would be the first milestone of the synthetic objective in the total synthesis of mutisianthol. For that task 2,4-dihydroxybenzaldehyde **40** was chosen as an appropriate starting material. Regioselective benzylation of the *para* hydroxy group of 2,4-dihydroxybenzaldehyde using benzyl bromide and K_2CO_3 in DMF at room temperature afforded 2-hydroxy-4-benzyloxybenzaldehyde **41**. Protection of the *ortho*-hydroxy group was carried out with methyl iodide and K_2CO_3 in DMF at room temperature to give 2-methoxy-4-benzyloxybenzaldehyde **42**. Clemmensen reduction of benzaldehyde **42** with zinc amalgam in toluene followed by deprotection of benzyl group by hydrogenation (Pd/C) furnished the required anisole **44**. For the synthesis of triflate **37,** triflic anhydride in the presence of DIPEA as a base was employed (Scheme 6).



Scheme 6

Synthesis of triflate **37** required more steps and chemicals required are expensive. Model study was conducted to test the feasibility of the route and to standardise the reaction conditions for constructing the requisite aldehyde **34**. For this model study, 3-methoxybromobenzene was chosen to be starting material, as it is commercially available and has similarity in structure with respect to natural product.

Thus Heck reaction of 3-methoxybromobenzene **45** with methyl crotonate with catalytic $Pd(OAc)_2$ and triphenylphosphine in DMF gave the unsaturated ester **46**. Reduction of double bond was carried out by hydrogenation with Pd/C as catalyst in methanol to furnish the ester **47**. Homologation was achieved with a two step sequence of reactions. Thus, controlled reduction of ester group to aldehyde by DIBAL-H was achieved followed by Wittig reaction of aldehyde formed with ylide generated from triphenyl salt of MOM chloride and *n*-BuLi. Deprotection of enol methyl ether **48** carried out with dil. HCl in THF at room temperature to furnish the aldehyde **49** (Scheme 7).



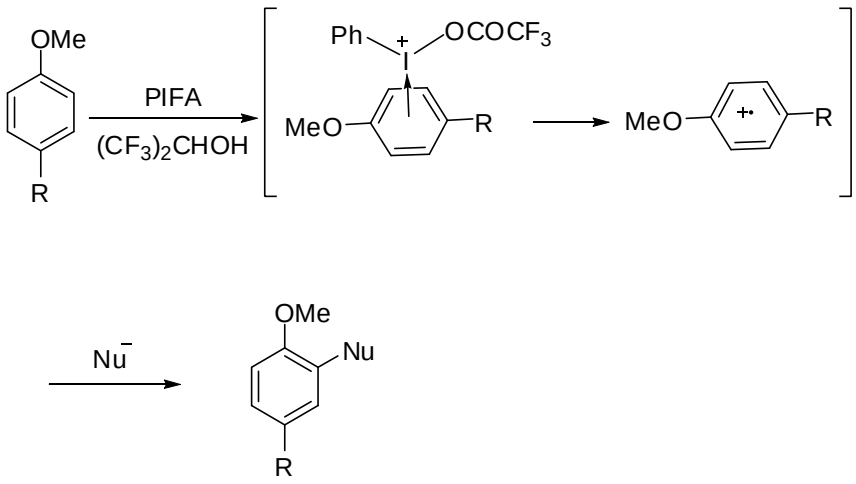
Scheme 7

As hypervalent iodine oxidation with phenyliodinebistrifluoroacetate (PIFA) has been increasingly applied in the present case for the alkylation on aromatic ring, it becomes relevant to discuss at length these reagents. Following pages furnish details with mechanism.

Hypervalent iodine oxidation

The organic chemistry of polyvalent iodine compounds has experienced an unprecedented, explosive development during the last decade. This surging interest in iodine compounds is mainly due to the very useful oxidizing properties of polyvalent organic iodine reagents combined with their benign environmental character and commercial availability. Several areas of organic polyvalent iodine chemistry have recently attracted especially active interest and research activity. These areas include the synthetic applications of the Dess-Martin periodinane and similar oxidizing reagents based on iodine (V), the use of iodosylbenzene in the transition-metal-catalyzed biomimetic oxygenations, catalytic imidations with iodonium imides, azidations with azidoiodanes, the chemistry of benziodoxoles and benziodazoles and synthetic and mechanistic studies of alkynyl and alkenyl iodonium salts.

Yasuydu Kita *et al*. reported[16] the nucleophilic substitution of *para* substituted phenol ethers to proceed *via* cation radicals as reactive intermediates by SET from the complex of phenol ethers with PIFA. Proposed mechanism for nucleophilic substitution is as follows (Scheme 8).



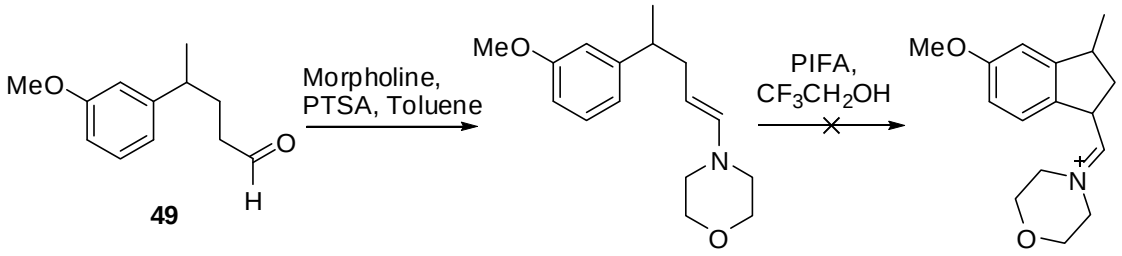
Scheme 8

In the hypervalent iodine oxidation reaction, *para* position with respect to methoxy group is most electron deficient. So the nucleophilic attack prefers *para* position if it is unsubstituted.

α-Alkylations of carbonyl compounds are important C-C bond forming reactions in organic synthesis. Asymmetric variants mostly rely on the use of chiral auxiliaries. Although two catalytic asymmetric α-alkylation strategies based on chiral phase transfer catalysts and chiral oligoamines are reported, these methods are most commonly applied in the synthesis of R-amino acids *via* alkylation of glycine derivatives and are by no means general. In 2004 List B. *et al*. reported[17] an organocatalytic approach for catalytic asymmetric intramolecular α-alkylation of aldehyde. This reaction shows the potential of general applicability and represents the first example of enamine catalysis where a nucleophilic substitution reaction takes place.

In the present work, instead of asymmetric cyclisation using chiral ligands, it was decided to cyclise aldehyde **49** by converting it to the respective enamine followed by α-alkylation of enamine with electron deficient aromatic system prepared by treating with PIFA (Scheme 9). For this purpose enamine prepared from morpholine was used. By heating aldehyde **49** and morpholine with the catalytic amount of PTSA in toluene and subsequent removal of water by Dean Stark apparatus gave the intermediate enamine. Toluene from the reaction mixture was removed and then residue obtained was taken in trifluoroethanol. Hypervalent oxidation and *in situ* cyclisation was tried with PIFA but reaction did not proceed even after heating

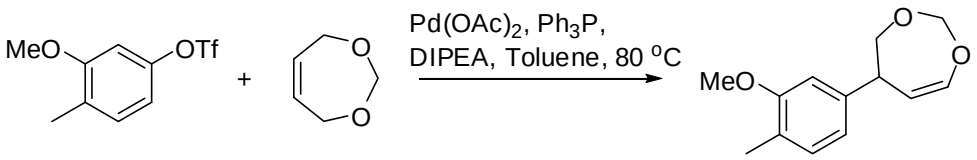
reaction mixture at refluxing temperature. Variation of amine from morpholine to pyrolidine, piperidine and proline also did not succeed. Change of solvent from trifluoroethanol to acetonitrile, 1,4-dioxan and THF failed to give product. When reaction mixture was refluxed for longer time and worked up only demethylation of aldehyde **49** was observed.



Scheme 9

After several unsuccessful attempts for the cyclisation of aldehyde **49**, it was decided to proceed with the second route as shown in retrosynthetic analysis (Scheme 5). It is well known in literature[18] that the product of Heck reaction between 4,7-dihydro-1,3-dioxepin **50** with different aryl halide gives the enolethers that are easily converted to chiral β-aryl-γ-butyrolactones which are useful synthetic intermediates.[19] Despite this interesting characteristic, there are only few reports that study this substrate.[18,20]

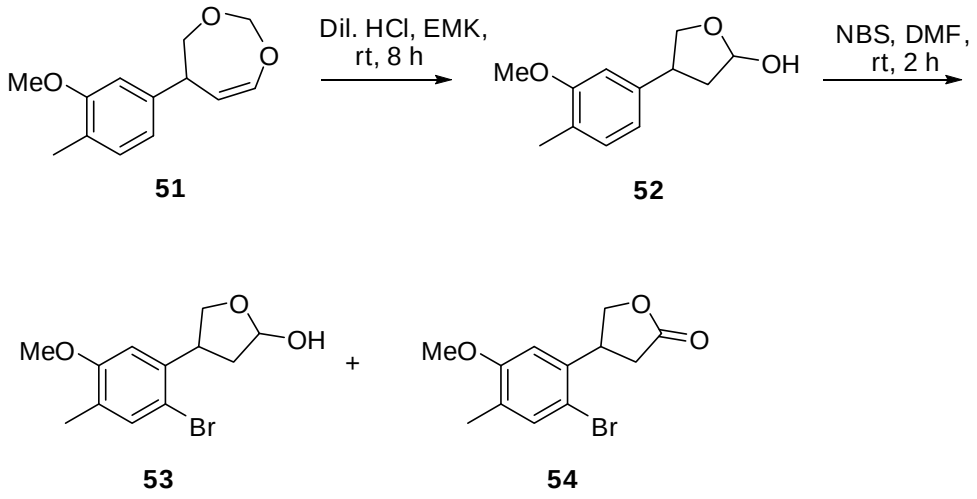
4,7-Dihydro-1,3-dioxepin **50** was prepared by condensation of *cis*-butenediol with formaldehyde in toluene, water formed during the reaction was removed by Dean Stark apparatus and product was purified by distillation. In the first set of experiments, optimal reaction conditions were determined by conducting a series of experiments in which variation in the solvent, temperature and base was carried out. In all cases, the formation of the desired product **51** was observed with the variation in the yield. In the end the optimum trade-off between selectivities and reaction rates was achieved with toluene as solvent, a temperature of 80 °C and diisopropylethylamine as a base, $Pd(OAc)_2$ as catalyst and triphenylphosphine as ligand. Heck reaction of triflate **37** with dioxepin **50** adopting optimized condition gave enolether **51** in 87% yield (Scheme 10). Product obtained was confirmed by spectral and analytical data.



37 **50** **51**

Scheme 10

Racemic enolether **51** was reacted to check the feasibility of the route. Deprotection of acetal of enolether **51** was achieved with dil. HCl in ethylmethylketone to afford the lactol **52**. After deprotection, enol formed tautomerised to aldehyde and then cyclised to form lactol **52**. Formation of lactol was confirmed by spectroscopic data. The ^{1}H NMR spectrum of lactol displayed the signal at δ 5.71 (d, J = 4.6 Hz, 1H) which is the characteristic value for the chemical shift for anomeric proton and absence of carbonyl carbon in ^{13}C NMR spectrum further cemented the conclusion. Bromination of lactol **52** was carried out in DMF with stoichiometric quantity of NBS. Excess NBS and prolonged stirring oxidises the lactol **53** to give lactone **54** (Scheme 11).

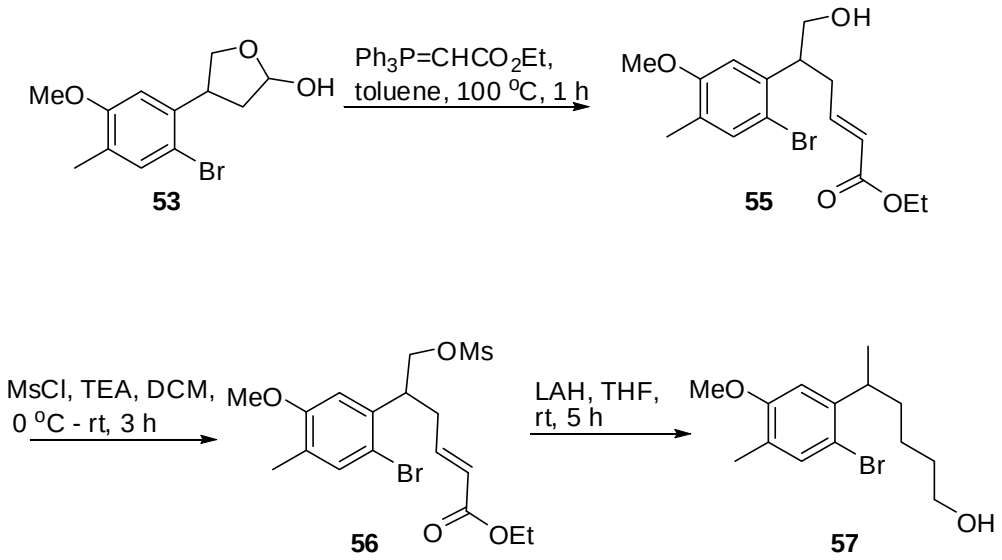


51 **52** **53** **54**

Scheme 11

After achieving the synthesis of lactol **53**, next target was to obtain the alkene **38** which is the important intermediate required for the synthesis of indane system. Two-carbon Wittig homologation of lactol **53** by treatment with $Ph_3P{=}CHCO_2Et$ in toluene at 100 °C furnished the α,β-unsaturated ester **55**. In the ^{1}H NMR spectrum of

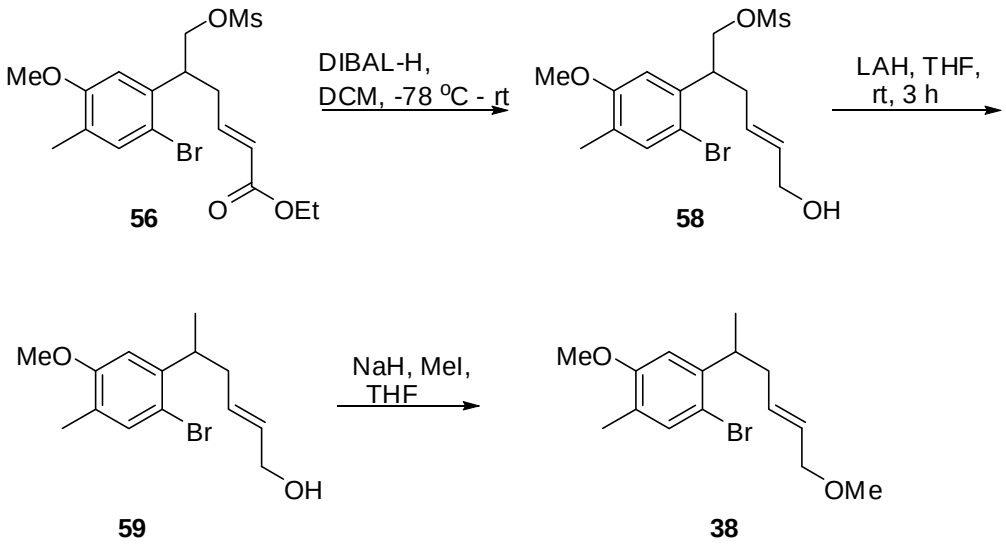
55 olefinic protons appeared at δ 5.87 and δ 6.84 with coupling constant 15.7 Hz, which was due to *trans* geometry of double bond.



Scheme 12

Deoxygenation of alcohol in **55** could give benzylic methyl group. For this purpose alcohol group of α,β-unsaturated ester **55** was converted to its mesylate derivative with mesyl chloride and TEA in DCM. Simultaneous demesylation and reduction of ester with LAH to corresponding allyl alcohol did not proceed as there was formation of saturated alcohol **57** as the only product (Scheme 12). So reduction of α,β-unsaturated ester to allyl alcohol followed by demesylation was carried out.

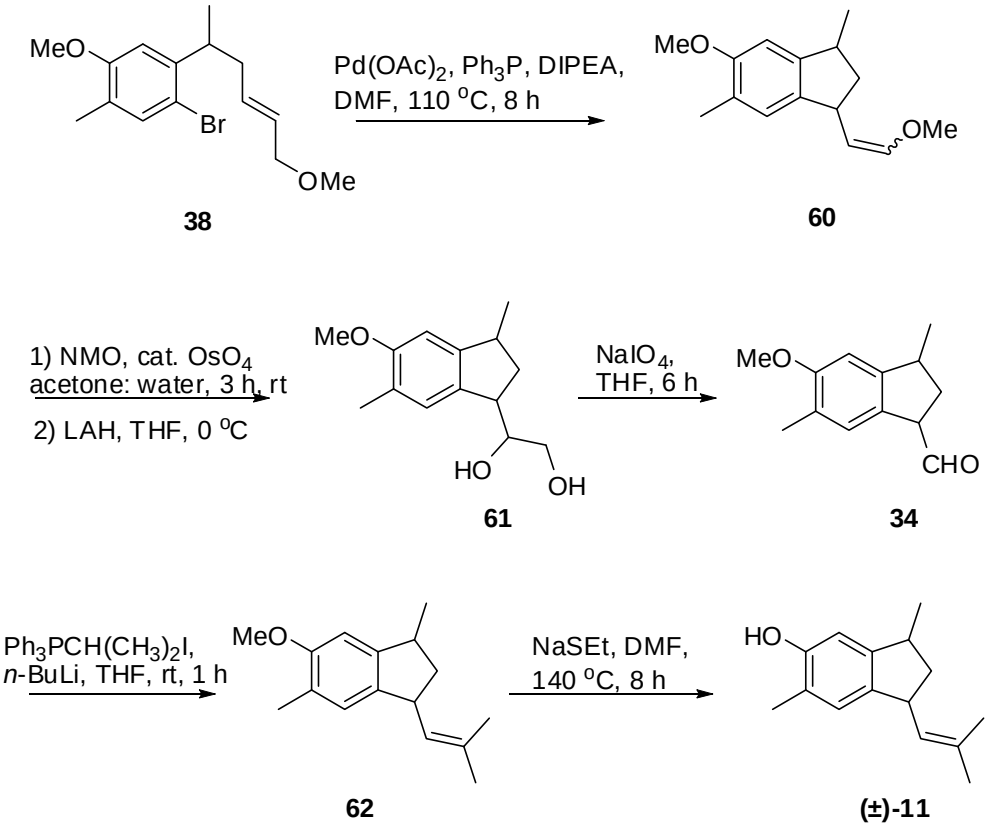
Reduction of α,β-unsaturated ester **56** was carried out by DIBAL-H at –78 °C to room temperature to afford allylic alcohol **58** in good yield. The ^{1}H and ^{13}C NMR spectral data and elemental analysis of **58** were in agreement with the assigned structure. Demesylation was achieved by nucleophilic displacement of mesyl group in **58** with LAH in THF at room temperature. In the ^{1}H NMR spectrum of **59,** methyl group resonated as a doublet at δ 1.24 (J = 6.8 Hz). The allyl alcohol was protected as methyl ether by treating with NaH and MeI in THF at 0 °C to furnish the desired product **38** (Scheme 13).



56 **58** **59** **38**

Scheme 13

Having the desired allyl methyl ether **38** in hand, attempts for developing indane system by intramolecular Heck reaction was considered to be right pathway.



38 **60** **61** **34** **62** **(±)-11**

Scheme 14

Intramolecular Heck cyclisation of **38** was achieved with $Pd(OAc)_2$, Ph_3P and DIPEA as a base in DMF at 110 °C to afford bicyclic system **60**. Methyl enol ether **60** was subjected to dihydroxylation by using catalytic OsO_4 along with co-oxidant NMO in acetone:water (8:2) and subsequent reduction of aldehyde with LAH yielded the diol **61**. Oxidative cleavage of the diol **61** with $NaIO_4$ in THF afforded the aldehyde **34**, which was immediately used for the Wittig reaction[21] with $PPh_3CH(CH_3)_2I$[22] and *n*-BuLi in THF to introduce the isopropenyl moiety of the target molecule leading to **62** in good yield. Finally, deprotection of the methyl group with sodium ethanethiolate[23] gave racemic mutisianthol **11** in 82% yield (Scheme 14). The spectroscopic data of **11** was in full conformity with those reported earlier.[12,14]

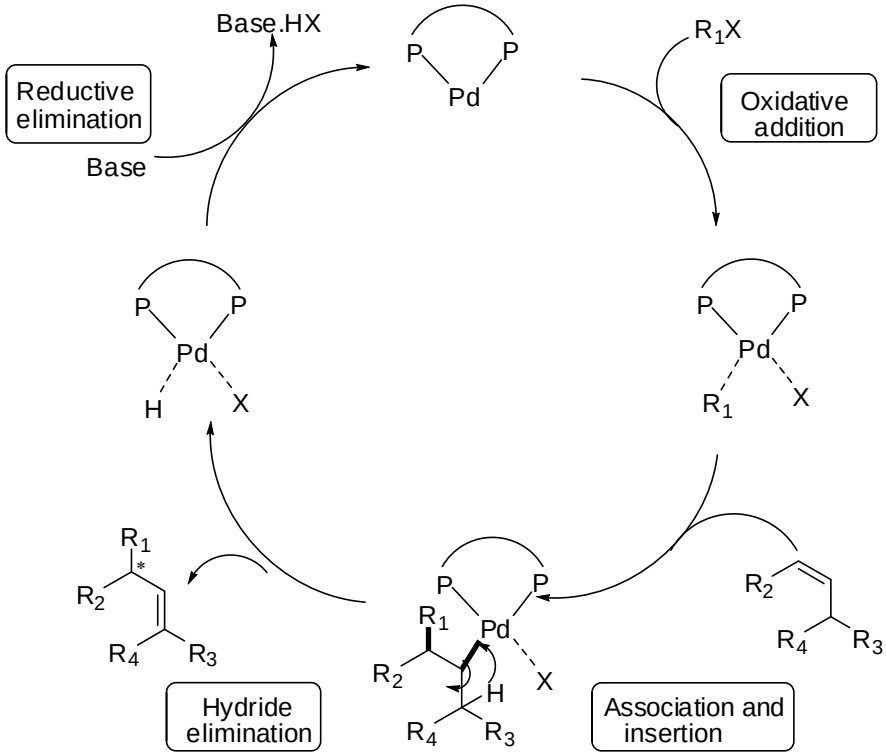
Following the successful completion of the scheme for racemic mutisianthol, next target was to synthesize (+) mutisianthol. Intermolecular asymmetric Heck reaction between triflate **37** and dioxepin **50** is the key step for generation of chiral benzylic methyl center.

A Short Account on Intermolecular and Intramolecular Asymmetric Heck Reactions

Asymmetric Heck reaction is a powerful method for the synthesis of both tertiary and quaternary chiral carbon centers, with an enantiomeric excess often greater than 80% and in some cases much higher (up to 99% ee). A variety of carbocyclic and heterocyclic systems can be constructed, including spirocyclic systems. The scope of the reaction with respect to the product alkene isomerisation is somewhat limited by problems of regioselectivity, however, these problems are surmountable and a new generation of ligands that dissociate more rapidly from the products, might improve both enantio- and regiocontrol. A variety of chiral compounds prepared by the asymmetric Heck reaction were successfully utilized in the enantioselective syntheses of complex natural products.

The mechanism of the Heck reaction (Scheme 15) with bidentate phosphine ligands is generally considered to follow the four-step catalytic cycle shown in Scheme 15, with the following steps: (A) oxidative addition of alkenyl halide to the Pd^0 species, (B) coordination and *syn*-insertion of the alkene substrate into the Pd

complex, (C) β-hydride elimination and finally (D) regeneration of Pd^0 by reductive elimination of HX.



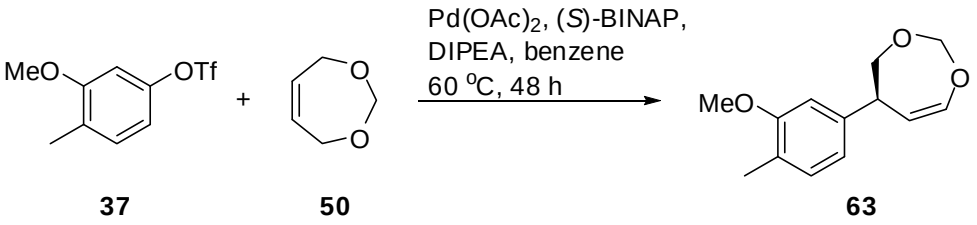
Scheme 15: Mechanism of Heck reaction

(*S*)-BINAP (*R*)-BINAP

Figure 5: Structures of BINAP ligands

Different ligands have been reported[24] for the asymmetric Heck reaction of dioxepin **50** with various triflate compounds. Except (*S*)-BINAP (Figure 5) all the ligands give the (*R*) isomer as major product. It was therefore decided to use (*S*)-BINAP as ligand. A set of asymmetric Heck reactions of the triflate **37** with dioxepin **50** using $Pd(OAc)_2$ as catalyst, (*S*)-BINAP as ligand and DIPEA as base in different solvents (toluene, acetonitrile, chloroform, DMF, benzene) were carried out. The enantioselectivity of **63** was determined using chiral HPLC column: Kromasil 5-Cellucoat (250X4.6 mm, 5 μ). Retention times for the two isomers were 11.5 and 12.5

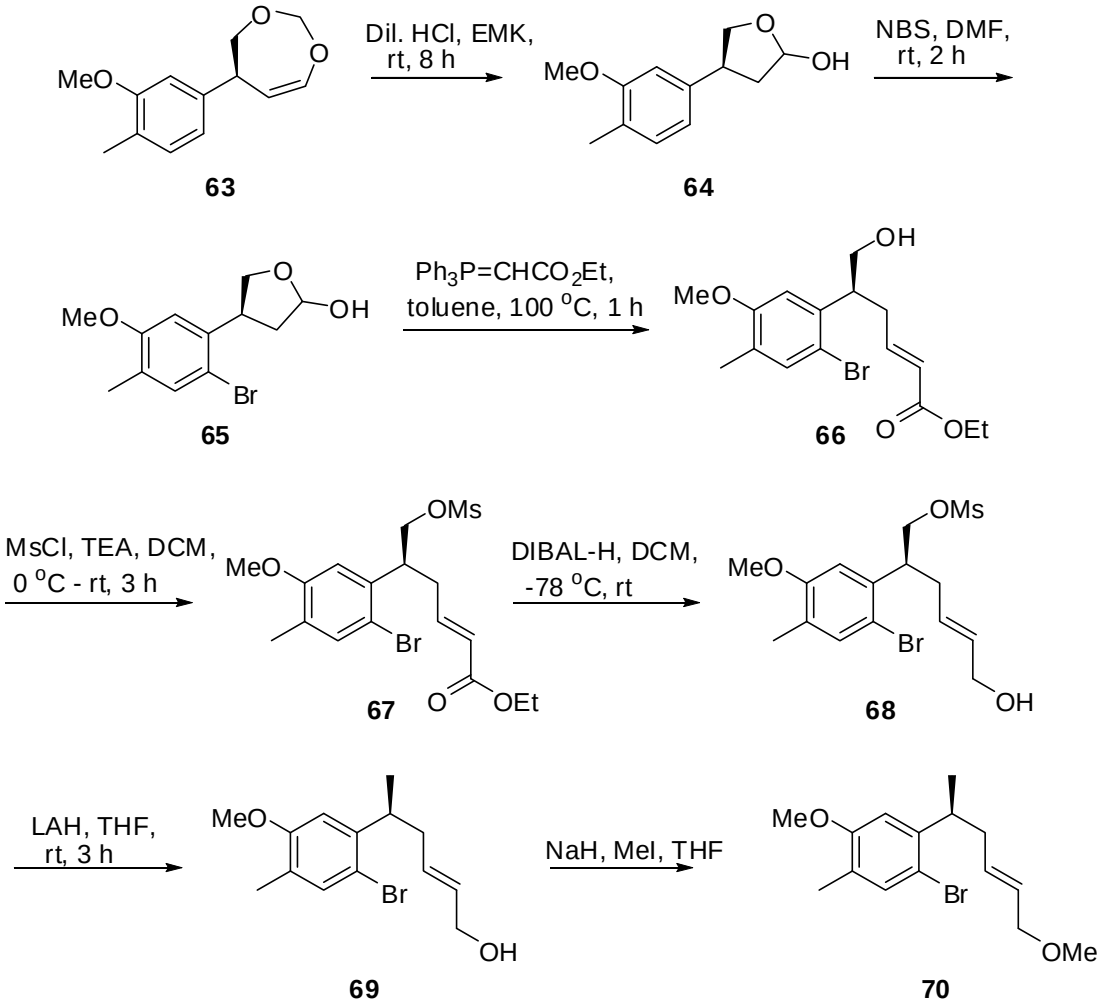
min. Maximum enantioselectivity (85%) was observed with benzene as solvent (Scheme 16).



37 **50** **63**

Scheme 16

The reaction conditions as described before for racemic compound were employed for the synthesis of allyl methyl ether **70** from enol ether **63** (Scheme 17).

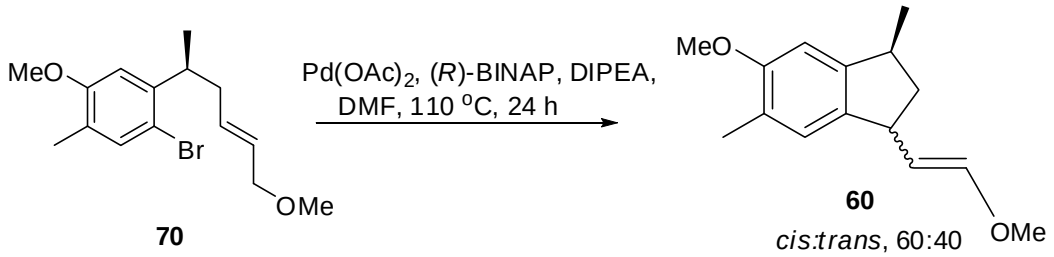


63 **64** **65** **66** **67** **68** **69** **70**

Scheme 17

After the synthesis of allyl methyl ether **70**, for the synthesis of disubstituted five membered ring, intramolecular asymmetric Heck reaction was considered to be

one of the best method for obtaining the bicyclic skeleton. To obtain cyclic product, methyl ether **70** was subjected to intramolecular asymmetric Heck reaction with (*R*)-BINAP as chiral ligand to furnish indane derivative **60** (Scheme 18) as a mixture of *trans* and *cis* in the ratio 40:60 (HPLC).

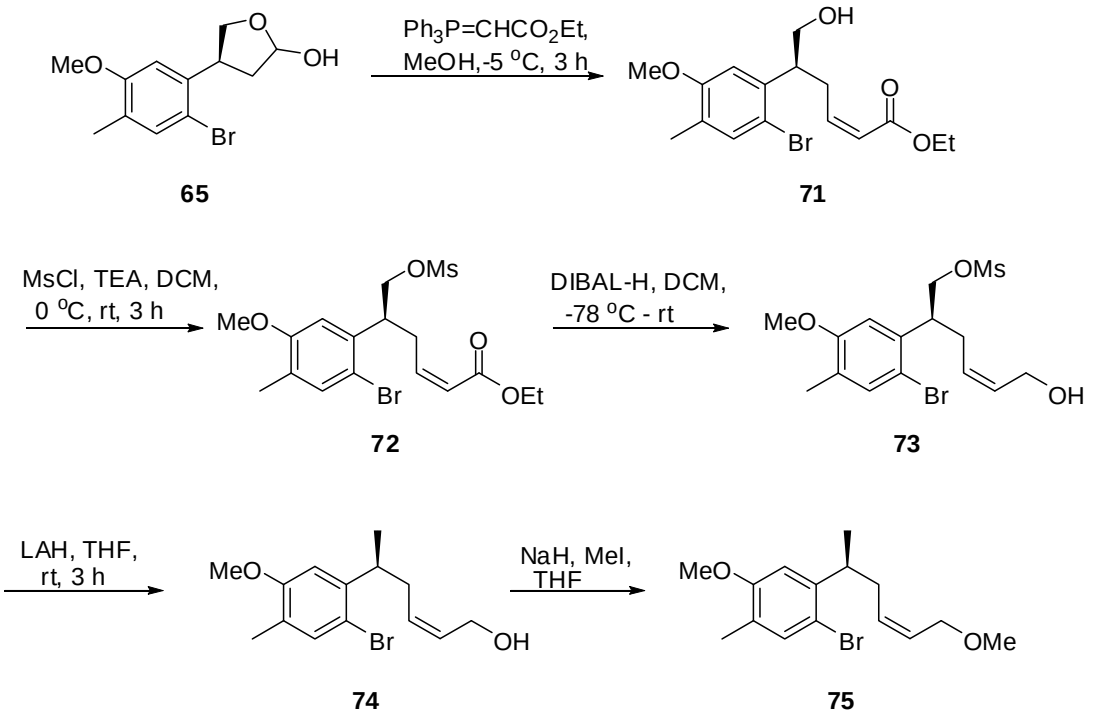


70 **60**

cis:*trans*, 60:40

Scheme 18

As *trans*-alkene **70** produced mixture of products, it was considered appropriate to synthesise *cis*-alkene for asymmetric Heck cyclisation. Thus the lactol **65** was subjected to Wittig reaction with $Ph_3P{=}CHCO_2Et$ in methanol[25] at -5 °C to give mixture of *cis* and *trans* isomers in 65:35 ratio which were separated by flash column chromatography. The structures of *cis* and *trans* isomers were confirmed by coupling constants of alkene protons in the ^1H NMR spectrum. The (Z)-α,β-unsaturated ester **71** was converted to allyl methyl ether **75** using the same reaction conditions as described for *trans* isomer (Scheme 19).



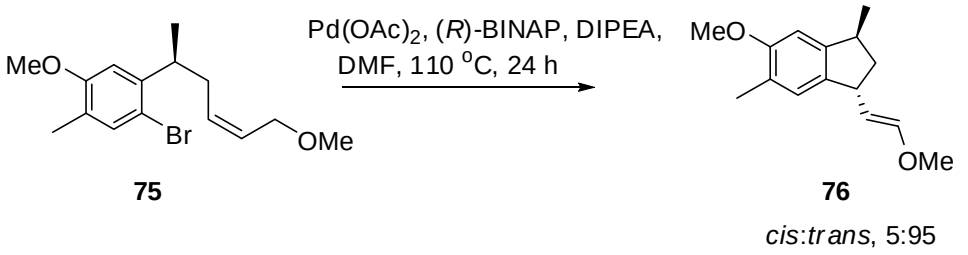
65 **71**

72 **73**

74 **75**

Scheme 19

Heck reaction of **75** with (*R*)-BINAP as chiral ligand and $Pd(OAc)_2$ as catalyst in DMF at 110 °C furnished an inseparable *trans*:*cis* mixture of the disubstituted indane system in 95:5 ratio as confirmed by HPLC (Scheme 20).

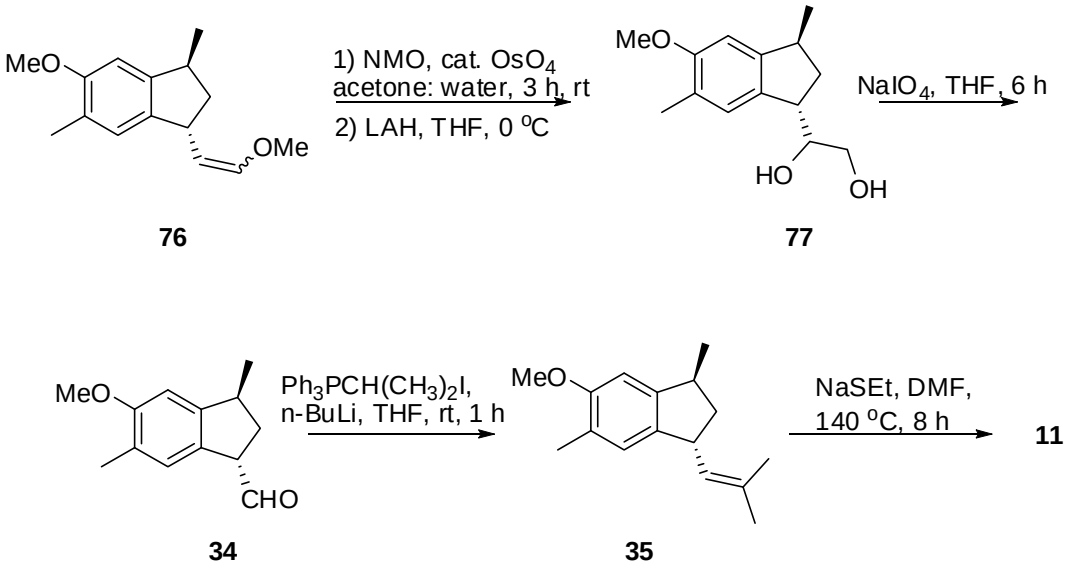


75 **76**

cis:*trans*, 5:95

Scheme 20

After successful synthesis of requisite *trans* disubstituted five membered ring, total synthesis of (+) mutisianthol was achieved *via* the same reaction conditions as described for racemic mutisianthol and which is elaborated below(Scheme 21).



76 **77**

34 **35**

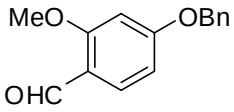
Scheme 21

Conclusion:

The first total synthesis of (+)-mutisianthol has been achieved. The key steps in the synthesis involved asymmetric Heck reaction, both intermolecular and intramolecular. Chiral induction of 85% and 95% *trans* selectivity respectively have been achieved. The methodology developed in the synthesis could also be applicable to several other related molecules.

EXPERIMENTAL

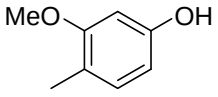
4-Benzyloxy-2-methoxybenzaldehyde (42)



To a stirred solution of 2,4-dihydroxybenzaldehyde (10.00 g, 72.40 mmol) and K_2CO_3 (10.00 g, 72.40 mmol) in DMF (100 mL) under N_2 atmosphere, benzyl bromide (12.31 g, 72.40 mmol) was added over 30 min and reaction mixture was stirred for 1 h at rt. It was then poured into water and extracted with EtOAc (2 X 100 mL). EtOAc layer was washed with water, brine and concentrated under vacuum to give crude product. The crude product obtained was purified by column chromatography (silica gel, hexane/EtOAc 5:1) to afford **41** as a white solid (14.20 g, 86%).

A mixture of **41**(14.00 g, 61.34 mmol), K_2CO_3 (8.48 g, 61.34 mmol) and methyl iodide (8.71 g, 61.34 mmol) in DMF (100 mL) was stirred at rt for 2 h. The reaction mixture was poured into water and extracted with EtOAc (3 x 100 mL). The combined organic layer was washed with water, dried over Na_2SO_4, filtered and the solvent was removed under vacuum to afford **42** as a colorless solid (14.3 g, 96%) mp: 98-99 °C [Lit. 98-100 °C].

3-Methoxy-4-methylphenol (44)



A mixture of 4-benzyloxy-2-methoxybenzaldehyde (14.00 g, 57.79 mmol), 1M HCl (1 mL) and Zn (Hg) (10.00 g) in toluene was refluxed for 3 h. The reaction mixture was cooled, filtered, washed with water and then concentrated to afford 4-(benzyloxy)-2-methoxy-1-methylbenzene (which was utilised in the next step without purification). The crude product obtained was dissolved in EtOH (50 mL), Pd/C (1.00 g) was added and reaction mixture was stirred for 3 h under H_2

atmosphere. Pd/C was filtered, washed with EtOAc and the organic layer was concentrated under vacuum to afford 3-methoxy-4-methylphenol as colorless oil (6.30 g, 78%).

Mol. Formula : $C_8H_{10}O_2$

Mol. Weight : 138

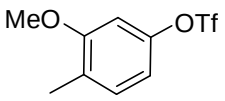
IR ($CHCl_3$) ν : 3354, 1645, 739 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.12 (s, 1H), 3.77 (s, 3H), 4.60 (br s, 1H), 6.31 (d, *J* = 8.3 Hz, 1H), 6.37 (s, 1H), 8.01 (s, 2H), 6.94 (d, *J* = 8.3 Hz, 1H)

Elemental Analysis : Found: C, 69.43; H, 7.35%

Calcd: C, 69.54; H, 7.30%

3-Methoxy-4-methylphenyl trifluoromethanesulfonate (37)



3-Methoxy-4-methylphenol (10.00 g, 72.32 mmol) and triethylamine (10.99 g, 108.57 mmol) were dissolved in dry DCM (100 mL). Trifluoromethanesulfonic anhydride (22.48 g, 79.62 mmol) was added drop wise at 0 ^oC and the solution was stirred for 1 h under nitrogen atmosphere. White precipitate formed was removed by filtration, the solid was washed with DCM (20 mL) and the combined filtrate was washed with water, dried over Na_2SO_4 and then concentrated under vacuum to give yellow oil. The crude product obtained was purified by column chromatography (silica gel, hexane/EtOAc 5:1) to yield 3-methoxy-4-methylphenyl trifluoromethanesulfonate (15.50 g, 79%) as yellowish oil.

Mol. Formula : $C_9H_9F_3O_4S$

Mol. Weight : 270

IR ($CHCl_3$) ν : 3019, 1731, 1649, 1216, 929 cm^{-1}

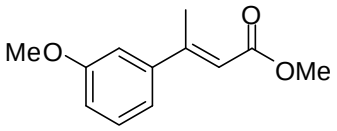
^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.22 (s, 3H), 3.85 (s, 3H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.78 (dd, *J* = 2.4, 7.6 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.8, 55.6, 103.6, 112.4, 127.2, 130.9, 148.2, 158.5

ESI-MS *m/z* : 271.2 $[M+H]^+$

Elemental Analysis : Found: C, 39.87; H, 3.48%
Calcd: C, 40.00; H, 3.36; F, 21.09; S, 11.87%

(*E*)-Methyl 3-(3-methoxyphenyl)but-2-enoate (46)



To a mixture of $Pd(OAc)_2$ (0.60 g, 2.76 mmol) and triphenyl phosphine (2.80 g, 10.69 mmol) in DMF (30 mL), which had been pre-stirred at room temperature for 10 min, 1-bromo-3-methoxybenzene (5.00 g, 26.73 mmol) was added. Then methyl crotonate (3.21 g, 32.08 mmol) and K_2CO_3 (5.54 g, 40.10 mmol) were successively added and the resultant solution was stirred at 100 ^oC for 6 h. The mixture was cooled, filtered through a 2 cm layer of silica gel and eluted with EtOAc (50 mL). Combined organic layer was washed with water, brine and concentrated to obtain crude product. The crude product obtained was further purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford **46** (4.60 g, 89%) as colorless oil.

Mol. Formula : $C_{12}H_{14}O_3$

Mol. Weight : 206

IR ($CHCl_3$) ν : 1708, 1649, 1612, 1576, 1048 cm^{-1}

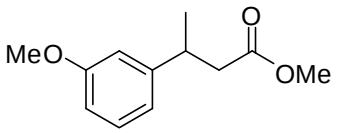
^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.45 (s, 3H), 3.65 (s, 3H), 3.82 (s, 3H), 6.02 (s, 1H), 6.80-7.05 (m, 3H), 7.15-7.25 (m, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 18.0, 51.1, 55.5, 113.2, 115.2, 116.9, 119.0, 129.5, 143.7, 155.6, 158.9, 167.1

ESI-MS *m/z* : 207.1 $[M+H]^+$

Elemental Analysis : Found: C, 69.63; H, 6.92 %
Calcd: C, 69.88; H, 6.84 %

Methyl 3-(3-methoxyphenyl)butanoate (47)



A solution containing (*E*)-methyl 3-(3-methoxyphenyl)but-2-enoate (4.00 g, 19.40mmol) in MeOH (30 mL), was subjected to reduction with the Pd/C (0.5 g), resultant mixture was hydrogenated (Parr reactor). The catalyst was filtered off and the filtrate was concentrated under vacuum to afford methyl 3-(3-methoxyphenyl)butanoate as colorless oil (3.98 g, 98%).

Mol. Formula : $C_{12}H_{16}O_3$

Mol. Weight : 208

IR ($CHCl_3$) ν : 1745, 1640, 1612, 1563, 1048 cm^{-1}

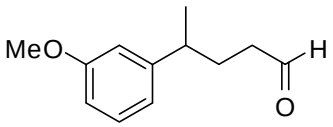
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.25 (d, *J* = 6.8 Hz, 3H), 2.50-2.65 (m, 2H), 3.10-3.25 (m, 1H), 3.63 (s, 3H), 3.83 (s, 3H), 6.60-6.70 (m, 3H), 7.05-7.15 (m, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) **:** δ 21.7, 36.3, 42.6, 51.7, 55.4, 113.5, 113.8, 118.8, 129.7, 147.4, 155.9, 173.4

ESI-MS *m/z* **:** 209.1 $[M+H]^+$

Elemental Analysis **:** Found: C, 69.01; H, 7.85 %
Calcd: C, 69.21; H, 7.74 %

4-(3-Methoxyphenyl)pentanal (49)



To a cooled (-78 °C) and stirred solution of ester **47** (3.00 g, 14.41 mmol) in CH_2Cl_2 (30 mL) DIBAL-H (1.6 M solution in toluene, 9.90 mL, 15.85 mmol) was added. After stirring at -78 °C for 5 h, a saturated solution (25 mL) of tartaric acid sodium potassium salt was added. The resultant mixture was stirred for 1 h at rt and then was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum to afford 3-(3-methoxyphenyl)butanal (2.15 g, 77%) as colorless oil.

To a stirred suspension of methoxymethyltriphenylphosphonium chloride (7.41 g, 21.62 mmol) in anhydrous THF (50 mL) under argon atmosphere at 0 ^oC, n-BuLi (1.6 M in hexane, 13.51 mL, 21.61 mmol) was added drop wise. The resulting red solution was stirred for 30 min at rt and a solution containing the 3-(3-methoxyphenyl)butanal (2.25 g, 12.06 mmol) in anhydrous THF (10 mL) was added drop wise. The mixture was stirred for 1 h at rt, quenched with dil. HCl and then extracted with EtOAc (2 X 20 mL). The organic layer was washed with water, brine and dried over Na_2SO_4. The organic solvent was concentrated under reduced pressure and the resulting crude residue obtained was purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford 4-(3-methoxyphenyl) pentanal (0.45 g, 79%) as a colorless oil.

Mol. Formula : $C_{12}H_{16}O_2$

Mol. Weight : 192

IR ($CHCl_3$) ν : 3060, 2965, 1726, 1607 cm^{-1}

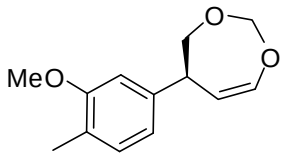
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.26 (d, *J* = 6.9 Hz, 3H), 1.80-2.00 (m, 2H), 2.25-2.40 (m, 2H), 2.60-2.75 (m, 1H), 3.82 (s, 3H), 6.60-6.70 (m, 3H), 7.05-7.15 (m, 1H), 9.65 (d, *J* = 1.5 Hz, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 22.1, 30.4, 39.3, 42.1, 55.4, 113.4, 113.9, 119.3, 129.8, 147.5, 156.1, 202.1

ESI-MS *m/z* : 193.1 $[M+H]^+$

Elemental Analysis : Found: C, 74.73; H, 8.45%
Calcd: C, 74.97; H, 8.39%

(*S*,*Z*)-5-(3-Methoxy-4-methylphenyl)-4,5-dihydro-1,3-dioxepine (63)



A mixture containing $Pd(OAc)_2$ (0.42 g, 1.85 mmol) and (*S*)-BINAP (2.30 g, 3.70 mmol) in argon saturated benzene (50 mL), which had been pre-stirred at room temperature for 10 min, was reacted with the aryl triflate **37** (5.00 g, 18.50 mmol). Then 4,5-dihydro-1,3-dioxepine (3.70 g, 37.01 mmol) and ethyldiisopropylamine (4.78 g, 37.01 mmol) were successively added and the resultant mixture was stirred at 60 ^oC for 48 h. Concentration yielded an orange suspension which was filtered through a 2 cm layer of silica gel and eluted with EtOAc. The concentration of the combined filtrate gave a yellow oil, which was purified by column chromatography (silica gel, hexane/EtOAc 5:1) to afford **63** (3.12 g, 76%) as a colorless oil.

Mol. Formula : $C_{13}H_{16}O_3$

Mol. Weight : 220

$[\alpha]_D^{25}$: -46.1° (*c* 2.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3448, 2926, 1731, 1649, 1612, 1583, 1262, 1041 cm^{-1}

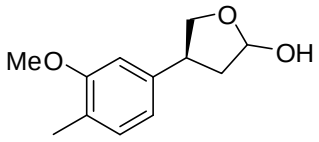
^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.20 (s, 3H), 3.45 (dd, *J* = 8.7, 11.4 Hz, 1H), 3.70-3.80 (m, 1H), 3.84 (s, 3H), 3.95 (dd, *J* = 4.4, 11.4 Hz, 1H), 4.85 (d, *J* = 7.1 Hz, 1H), 4.96 (dd, *J* = 3.5, 7.3 Hz, 1H), 5.22 (d, *J* = 7.0 Hz, 1H), 6.46 (dd, *J* = 2.4, 7.3 Hz, 1H), 6.70-6.80 (m, 2H), 7.08 (d, *J* = 7.3 Hz, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.7, 48.3, 55.1, 76.7, 98.0, 109.6, 112.3, 119.6, 125.2, 130.6, 139.6, 145.8, 157.7

ESI-MS *m/z* : 221.2 $[M+H]^+$

Elemental Analysis : Found: C 70.67; H, 7.36%
Calcd: C, 70.89; H, 7.32%

(4*S*)-4-(3-Methoxy-4-methylphenyl)tetrahydrofuran-2-ol (64)



5-(3-Methoxy-4-methylphenyl)-4, 5-dihydro-1, 3-dioxepine (5.00 g, 22.7 mmol) was dissolved in 50 mL of ethyl methyl ketone, then 1M dil. HCl (2 mL) added and the resultant mixture was stirred for 8 h at rt. Ethyl methyl ketone was removed under vacuum to give an oily residue which was dissolved in EtOAc (50 mL). The EtOAc layer was washed with water dried and concentrated to give a colorless residue which was purified by column chromatography (silica gel, hexane/EtOAc 8:2) to afford 4-(3-methoxy-4-methylphenyl) tetrahydrofuran-2-ol as a light coloured oil (4.25 g, 89%).

Mol. Formula : $C_{12}H_{16}O_3$

Mol. Weight : 208

$[\alpha]_D^{25}$: +2.4° (*c* 1.0, $CHCl_3$)

IR ($CHCl_3$) ν : 3437, 3020, 1643, 1216, 1018 cm^{-1}

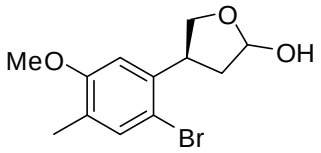
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.95-2.20 (m, 1H), 2.20 (s, 3H), 2.30-2.65 (m, 1H), 3.30-3.70 (m, 1H), 3.80-3.90 (m, 1H), 3.83 (s, 3H), 4.20-4.50 (m, 1H), 5.71 (d, *J* = 4.6 Hz, 1H), 6.72 (d, *J* = 3.7 Hz, 1H), 6.82 (dd, *J* = 3.7, 7.8 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.7, 41.6, 41.8, 42.3, 44.6, 55.1, 73.0, 74.0, 98.7, 99.0, 108.9, 109.1, 118.5, 119.2, 124.8, 130.5, 139.4, 140.5, 157.9

ESI-MS *m/z* : 209.2 $[M+H]^+$

Elemental Analysis : Found: C, 68.96; H, 7.83%
Calcd: C, 69.21; H, 7.74%

(4*S*)-4-(2-Bromo-5-methoxy-4-methylphenyl)tetrahydrofuran-2-ol (65)



4-(3-Methoxy-4-methylphenyl) tetrahydrofuran-2-ol (4.00 g, 19.21 mmol) was dissolved in DMF (30 mL). To this solution was added NBS (3.42 g, 19.21 mmol) in three portions at intervals of 15 min. When the reaction was complete as determined by TLC analysis, the mixture was poured into crushed ice and then it was extracted with EtOAc (2 X 30 mL). The organic layers were mixed and washed with water (60 mL), saturated brine and concentrated to give crude product, which was purified by column chromatography (silica gel, hexane/EtOAc 8:2) to afford 4-(2-bromo-5-methoxy-4-methylphenyl) tetrahydrofuran-2-ol as a thick yellowish oil(4.85 g, 87%).

Mol. Formula : $C_{12}H_{15}BrO_3$

Mol. Weight : 287

$[\alpha]_D^{25}$: -14.4° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3444, 2926, 1716, 1645, 1036 cm^{-1}

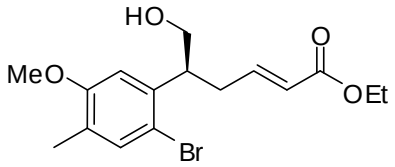
^{1}H NMR (500 MHz, $CDCl_3$) : δ 2.00-2.20 (m, 1H), 2.20 (s, 3H), 2.31-2.65 (m, 1H), 3.35-3.74 (m, 1H), 3.80-3.92 (m, 1H), 3.83 (s, 3H), 4.15-4.50 (m, 1H), 5.71 (d, *J* = 4.6 Hz, 1H), 6.67 (s, 1H), 7.33 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.6, 41.5, 41.9, 42.5, 44.7, 55.3, 73.2, 74.3, 98.6, 99.1, 108.1, 109.2, 113.7, 114.1, 128.4, 134.9, 136.8, 157.4, 176.4

ESI-MS *m/z* : 287.1/289.1 $[M+H]^+$

Elemental Analysis : Found: C, 49.93; H, 5.34; Br, 27.76%
Calcd: C, 50.19; H, 5.27; Br, 27.83%

(*S*,*E*)-Ethyl 5-(2-bromo-5-methoxy-4-methylphenyl)-6-hydroxyhex-2-enoate (66)



A mixture containing bromolactol **65** (3.00 g, 10.45 mmol) and ethyl (triphenyl phosphoranylidene) acetate (3.64 g, 10.45 mmol) was heated at 100 °C in toluene (30 mL) under nitrogen atmosphere for 1 h. Then the mixture was cooled and diluted with ether (50 mL) and the resultant precipitate of triphenylphosphine oxide was removed by filtration. The organic filtrate on concentration and column chromatographic purification (silica gel, hexane/EtOAc 8:2) yielded **66** (3.45 g, 92%) as colorless oil.

Mol. Formula : $C_{16}H_{21}BrO_4$

Mol. Weight : 357

$[\alpha]_D^{25}$: +12.1° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3448, 2931, 1708, 1654, 1492, 1370, 1044, 878 cm^{-1}

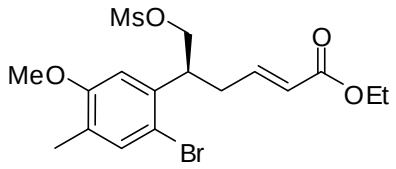
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.27 (t, *J* = 7.2 Hz, 3H), 2.16 (s, 3H), 2.50-2.75 (m, 2H), 3.45-3.60 (m, 1H), 3.70-3.85 (m, 2H), 3.81 (s, 3H), 4.16 (q, *J* = 7.2 Hz, 2H), 5.85 (d, *J* = 15.7 Hz, 1H), 6.67 (s, 1H), 6.80-7.00 (m, 1H), 7.33 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 14.2, 15.6, 34.1, 45.3, 55.5, 60.3, 65.2, 109.6, 115.2, 123.2, 127.6, 134.5, 137.8, 146.1, 157.3, 166.3

ESI-MS *m/z* : 357.1/359.1 $[M+H]^+$

Elemental Analysis : Found: C, 53.66; H, 5.98; Br, 22.23%
Calcd: C, 53.79; H, 5.93; Br, 22.37%

(*S*,*E*)-Ethyl 5-(2-bromo-5-methoxy-4-methylphenyl)-6-(methylsulfonyloxy)hex-2-enoate (67)



Methanesulfonyl chloride (1.15 g, 10.08 mmol) and Et_3N (1.27 g, 12.60 mmol) were added successively to a stirred solution of **66** (3.00 g, 8.40 mmol) in CH_2C1_2 (20 ml) at 0 °C under N_2 atmosphere. Stirring was continued at room temperature for 3 h, water (10 mL) was then added and the mixture was extracted with CH_2Cl_2. The combined organic extract was washed with brine, dried over Na_2SO_4 and concentrated to afford the mesylate **67** (3.20 g, 87%) as colorless viscous oil.

Mol. Formula : $C_{17}H_{23}BrO_6S$

Mol. Weight : 435

$[\alpha]_D^{25}$: -44.2° (*c* 1.15, $CHCl_3$)

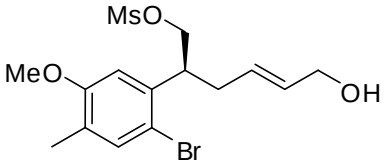
IR ($CHCl_3$) ν : 3318, 1708, 1658, 1360, 1177 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.27 (t, *J* = 7.2 Hz, 3H), 2.17 (s, 3H), 2.62-2.83 (m, 2H), 2.91 (s, 3H), 3.70-3.80 (m, 1H), 3.81 (s, 3H), 4.16 (q, *J* = 7.2 Hz, 2H), 4.31 (dd, *J* = 6.3, 10.0 Hz, 1H), 4.40 (dd, *J* = 5.8, 10.0 Hz, 1H), 5.87 (dt, *J* = 1.4, 15.5 Hz, 1H), 6.64 (s, 1H), 6.84 (dt, *J* = 7.3, 15.5 Hz, 1H), 7.33 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 14.1, 15.5, 33.6, 37.2, 42.3, 55.5, 60.3, 70.7, 109.6, 114.5, 123.7, 128.2, 134.5, 135.5, 144.4, 157.3, 166.0

ESI-MS *m/z* : 435.1/437.1 $[M+H]^+$

(*S*,*E*)-2-(2-Bromo-5-methoxy-4-methylphenyl)-6-hydroxyhex-4-enyl methanesulfonate (68)



A cooled (-78 °C) and stirred solution containing the ester **67** (3.00 g, 6.89 mmol) in CH_2Cl_2 (30 mL) under argon atmosphere was reacted with DIBAL-H (1.6 M solution in toluene, 10.77 mL, 17.23 mmol) with slow addition. Stirring was continued at -78 °C for 30 min, the mixture was then warmed to room temperature and a saturated solution (25 mL) of tartaric acid sodium potassium salt was added. The reaction mixture was again stirred for 12 h at rt and then was extracted with CH_2Cl_2 (2 X 20 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue obtained was purified by column chromatography (silica gel, hexane/EtOAc 7:3) to afford **68** (2.35 g, 87%) as colorless oil.

Mol. Formula : $C_{15}H_{21}BrO_5S$

Mol. Weight : 393

$[\alpha]_D^{25}$: -12.6° (*c* 0.5, $CHCl_3$)

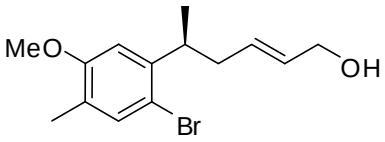
IR ($CHCl_3$) ν : 3450, 1648, 1364, 1245, 1178 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 2.17 (s, 3H), 2.45-2.65 (m, 2H), 2.89 (s, 3H), 3.60-3.65 (m, 1H), 3.82 (s, 3H), 4.08 (d, *J* = 5.1 Hz, 2H), 4.34 (dd, *J* = 6.1, 10.0 Hz, 1H), 4.42 (dd, *J* = 5.9, 10.0 Hz, 1H), 5.55-5.85 (m, 2H), 6.68 (s, 1H), 7.33 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.6, 34.0, 37.3, 43.0, 55.6, 63.2, 71.3, 109.8, 127.9, 128.2, 128.7, 132.2, 134.3, 136.6, 157.2

ESI-MS *m/z* : 393.1/395.1 $[M+H]^+$

(*S*,*E*)-5-(2-Bromo-5-methoxy-4-methylphenyl)hex-2-en-1-ol (69)



A solution containing mesylate **68** (2.00 g, 5.09 mmol) in THF (30 mL) was stirred at 0 °C. LAH (386 mg, 10.17 mmol) was added to it and stirring was continued at 0 °C for 30 min, the mixture was then stirred for 3 h at rt. It was then quenched with water and the stirring was continued for another 5 h. The reaction mixture was then extracted with CH_2Cl_2 (2 X 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residue obtained was purified by column chromatography (silica gel, hexane/EtOAc 7:3) to afford **69** (1.25 g, 85%) as a colorless thick oil.

Mol. Formula : $C_{14}H_{19}BrO_2$

Mol. Weight : 299

$[\alpha]_D^{25}$: -42.2° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3418, 3019, 1645, 1404, 1216, 1020, 669 cm^{-1}

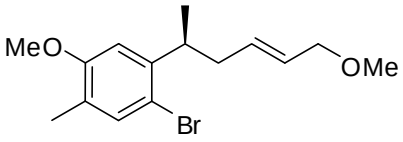
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.24 (d, *J* = 6.8 Hz, 3H), 2.16 (s, 3H), 2.20-2.46 (m, 2H), 3.20-3.35 (m, 1H), 3.82 (s, 3H), 4.07 (d, *J* = 5.0 Hz, 2H), 5.56-5.75 (m, 2H), 6.66 (s, 1H), 7.28 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.8, 21.7, 39.9, 41.0, 55.4, 63.6, 108.7, 118.5, 126.9, 128.3, 130.8, 134.0, 143.5, 157.2

ESI-MS *m/z* : 299.2/301.2 $[M+H]^+$

Elemental Analysis : Found: C, 55.96; H, 6.45; Br, 26.57 %
Calcd: C, 56.20; H, 6.40; Br, 26.71 %

(*S*,*E*)-1-Bromo-4-methoxy-2-(6-methoxyhex-4-en-2-yl)-5-methylbenzene (70)



To a suspension of sodium hydride (176 mg, 4.41 mmol, 60% in mineral oil) in THF (10 mL) methyl iodide (683 mg, 4.81 mmol) was added. Allylic alcohol **69** (1.20 g, 4.01 mmol) was then slowly added at 25 °C to this stirred suspension. The mixture was stirred for 2 h at this temperature, then hydrolysed at 0 °C with a saturated NH_4Cl solution and extracted with ether (2 X 30 mL). The combined organic extract was washed with water, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue obtained was purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford **70** (1.15 g, 91%) as colorless oil.

Mol. Formula : $C_{15}H_{21}BrO_2$

Mol. Weight : 313

$[\alpha]_D^{25}$: -29.6° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3433, 1637, 1404, 1018, 670 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.23 (d, *J* = 6.8 Hz, 3H), 2.15 (s, 3H), 2.20-2.50 (m, 2H), 3.26 (m, 1H), 3.27 (s, 3H), 3.82 (s, 3H), 3.83-3.90 (m, 2H), 5.50-5.75 (m, 2H), 6.66 (s, 1H), 7.28 (s, 1H)

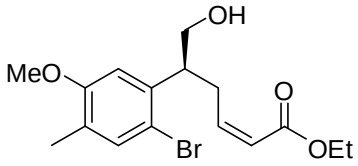
^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.8, 21.7, 39.9, 41.2, 55.2, 57.5, 73.1, 108.9, 118.5, 124.1, 127.7, 130.4, 133.0, 146.0, 157.6

ESI-MS *m/z* : 313.1/315.1 $[M+H]^+$

Elemental Analysis : Found: C, 57.14; H, 6.85; Br, 25.13%

Calcd: C, 57.52; H, 6.76; Br, 25.51%

(*S*,*Z*)-Ethyl 5-(2-bromo-5-methoxy-4-methylphenyl)-6-hydroxyhex-2-enoate (71)



A mixture of bromolactol **65** (2.50 g, 8.71 mmol) and ethyl (triphenylphosphoranylidene) acetate (3.03 g, 8.71 mmol) in MeOH (30 mL) was stirred at –5 °C under nitrogen atmosphere for 3 h. The reaction mixture was then concentrated under vacuum and the resultant crude residue was purified by flash column chromatography (silica gel, hexane/EtOAc 8:2) to afford **71** (1.86 g, 60%) as colorless viscous oil.

Mol. Formula : $C_{16}H_{21}BrO_4$

Mol. Weight : 357

$[\alpha]_D^{25}$: +23.8° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 3440, 2931, 1710, 1656, 1493, 1370, 1044, 878 cm^{-1}

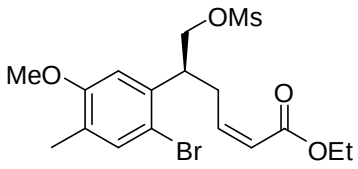
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.29 (t, *J* = 7.1 Hz, 3H), 2.17 (s, 3H), 2.85-3.05 (m, 2H), 3.15-3.30 (m, 1H), 3.38-3.40 (m, 1H), 3.70-3.81 (m, 2H), 3.77 (s, 3H), 4.18 (q, *J* = 7.1 Hz, 2H), 5.85 (d, *J* = 11.5 Hz, 1H), 6.17 (ddd, *J* = 3.6, 7.9, 11.5 Hz, 1H), 6.68 (s, 1H), 7.34 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 14.0, 15.8, 34.3, 45.4, 55.6, 60.5, 65.6, 109.6, 115.2, 123.2, 126.8, 134.5, 137.8, 145.4, 157.5, 166.6

ESI-MS *m/z* : 357.12/359.12 $[M+H]^+$

Elemental Analysis : Found: C, 53.66; H, 5.98; Br, 22.13%
Calcd: C, 53.79; H, 5.93; Br, 22.37%

(*S*,*Z*)-Ethyl 5-(2-bromo-5-methoxy-4-methylphenyl)-6-(methylsulfonyloxy)hex-2-enoate (72)



Methanesulfonyl chloride (1.15 g, 10.08 mmol) and Et_3N (1.27 g, 12.60 mmol) were added successively to a stirred solution of **71** (3.00 g, 8.40 mmol) in CH_2C1_2 (2 mL) at 0 °C. The stirring was continued at rt for a further 3 h, water was then added and the mixture was extracted with CH_2C1_2 (2 X 20 mL). The combined organic extract was washed with water, brine, dried over Na_2SO_4 and concentrated to afford the mesylate **72** (3.20 g, 87%) as colorless thick oil.

Mol. Formula : $C_{17}H_{23}BrO_6S$

Mol. Weight : 435

$[\alpha]_D^{25}$: -13.6° (*c* 1.25, $CHCl_3$)

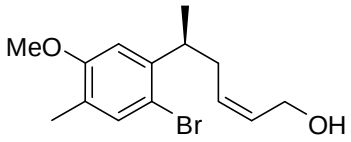
IR ($CHCl_3$) ν : 3336, 1711, 1661, 1364, 1172 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.29 (t, *J* = 7.2 Hz, 3H), 2.18 (s, 3H), 2.92 (s, 3H), 3.10-3.25 (m, 2H), 3.76-3.85 (m, 1H), 3.79 (s, 3H), 4.18 (q, *J* = 7.2 Hz, 2H), 4.30 (dd, *J* = 6.9, 10.0 Hz, 1H), 4.40 (dd, *J* = 6.3, 10.0 Hz, 1H), 5.80 (d, *J* = 11.3 Hz, 1H), 6.05-6.20 (m, 1H), 6.65 (s, 1H), 7.34 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 13.9, 15.5, 33.8, 37.3, 42.6, 55.5, 60.4, 70.8, 109.6, 114.5, 123.4, 127.5, 134.6, 135.8, 143.8, 157.3, 166.0

ESI-MS *m/z* : 435.1/437.1 $[M+H]^+$

(*S*,*Z*)-5-(2-Bromo-5-methoxy-4-methylphenyl)hex-2-en-1-ol (74)



To a cooled (-78 °C) and stirred solution of ester **72** (2.30 g, 5.28 mmol) in CH_2Cl_2 (30 mL) under argon atmosphere, DIBAL-H (1.6 M solution in toluene, 8.26 mL, 13.21 mmol) was added maintaining the temperature at -78 °C. Stirring was continued for another 30 min and the mixture was warmed to rt. A saturated solution (20 mL) of tartaric acid sodium potassium salt was added and the resultant mixture was stirred for 1 h. Extraction was carried out with CH_2Cl_2 (2 x 20 mL). The combined organic layer was dried and concentrated in vacuum. The residue which was a thick oil used as such for further reaction.

A stirred solution of mesylate (2.00 g, 5.09 mmol) in THF (30 mL) was reduced with LAH (386 mg, 10.17 mmol). Stirring was continued at 0 °C for 30 min, the mixture was then stirred for 3 h at rt and was quenched with water. The

resultant mixture was stirred for another 5 h, it was then extracted with CH_2Cl_2 (2 X 20 mL). The combined organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated under vacuum. The residue obtained was purified by column chromatography (silica gel, hexane/EtOAc 7:3) to afford **74** (1.15 g, 76%) as colorless viscous oil.

Mol. Formula : $C_{14}H_{19}BrO_2$

Mol. Weight : 299

$[\alpha]_D^{25}$: -42.2° (*c* 0.5, $CHCl_3$)

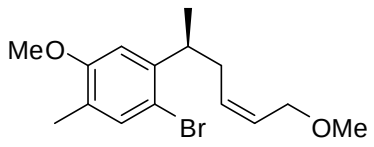
IR ($CHCl_3$) ν : 3418, 3019, 1645, 1404, 1216, 1020, 669 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.24 (d, *J* = 6.7 Hz, 3H), 2.17 (s, 3H), 2.30-2.45 (m, 2H), 3.16-3.38 (m, 4H), 3.80 (s, 3H), 4.00-4.25 (m, 2H), 5.20-5.46 (m, 1H), 5.80-6.05 (m, 1H), 6.65 (s, 1H), 7.31 (s, 1H)

ESI-MS *m/z* : 299.2/301.2 $[M+H]^+$

Elemental Analysis : Found: C, 55.96; H, 6.45; Br, 26.57 %
Calcd: C, 56.20; H, 6.40; Br, 26.71 %

(*S*,*Z*)-1-Bromo-4-methoxy-2-(6-methoxyhex-4-en-2-yl)-5-methylbenzene (75)



Methyl iodide (683 mg, 4.81 mmol) was added to a suspension of sodium hydride (176 mg, 4.41 mmol, 60% in mineral oil) in THF (10 mL) at 0 °C. Allylic alcohol **74** (1.20 g, 4.01 mmol) was then slowly added at 25 ^oC to this stirred suspension. The resultant mixture was stirred for 2 h at this temperature, then hydrolysed at 0 ^oC with a saturated NH_4Cl solution and extracted with ether (2 X 30 mL). The combined extract was washed with water, brine and dried over Na_2SO_4. It was then filtered and concentrated under vacuum to give crude product. The crude

product obtained was further purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford **75** (1.15 g, 91%) as colorless viscous oil.

Mol. Formula : $C_{15}H_{21}BrO_2$

Mol. Weight : 313

$[\alpha]_D^{25}$: -35.7° (*c* 0.5, $CHCl_3$)

IR ($CHCl_3$) ν : 1642, 1467, 1089, 776 cm^{-1}

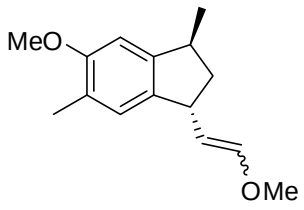
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.23 (d, *J* = 6.8 Hz, 3H), 2.15 (s, 3H), 2.20-2.50 (m, 2H), 3.26 (m, 1H), 3.27 (s, 3H), 3.82 (s, 3H), 4.05-4.20 (m, 2H), 5.20-5.40 (m, 1H), 5.80-5.95 (m, 1H), 6.68 (s, 1H), 7.28 (s, 1H)

ESI-MS *m/z* : 313.1/315.1 $[M+H]^+$

Elemental Analysis : Found: C, 57.23; H, 6.87; Br, 25.24%

Calcd: C, 57.52; H, 6.76; Br, 25.51%

(1*R*,3*S*)-5-Methoxy-1-(2-methoxyvinyl)-3,6-dimethyl-2,3-dihydro-1*H*-indene (76)



Mixture containing $Pd(OAc)_2$ (72 mg, 0.32 mmol) and (*R*)-BINAP (0.79 g, 0.13 mmol) in argon saturated DMF (10 mL) was stirred at rt for 10 min and then the methyl ether derivative **75** (1.00 g, 3.19 mmol) in DMF (1 mL) and ethyldiisopropylamine (0.83 g, 6.39 mmol) were added successively. The resultant reaction mixture was stirred at 110 ^oC for 8 h, then it was cooled to rt and filtered through a 2 cm layer of silica gel. Filtrate was diluted with EtOAc (20 mL), washed with water and dried over Na_2SO_4. It was filtered and concentrated under vacuum to

give yellow oil, which was further purified by column chromatography (silica gel, hexane/EtOAc 5:1) to afford **76** (0.45 g, 61%) as thick oil.

Mol. Formula : $C_{15}H_{12}O_2$

Mol. Weight : 232

IR ($CHCl_3$) ν : 2928, 2851, 1652, 1458, 1210, 1130, 1106 cm^{-1}

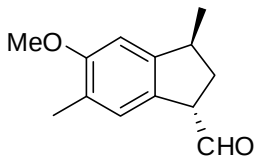
^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.25 (d, *J* = 6.8 Hz, 3H), 1.60-2.00 (m, 2H), 2.20 (s, 3H), 3.10-3.30 (m, 1H), 3.50 (s, 3H), 3.84 (s, 3H), 4.00-4.10 (m, 1H), 5.00-5.25 (m, 1H), 5.70-5.90 (m, 1H), 6.69 (s, 1H), 6.95 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 16.4, 21.0, 38.5, 38.7, 39.1, 42.6, 55.2, 55.4, 55.8, 102.4, 105.7, 106.1, 125.1, 126.9, 138.0, 146.3, 146.9, 147.5, 155.1

ESI-MS *m/z* : 233.2 $[M+H]^+$

Elemental Analysis : Found: C, 77.36; H, 8.75%
Calcd: C, 77.55; H, 8.68%

(1*S*,3*S*)-5-Methoxy-3,6-dimethyl-2,3-dihydro-1*H*-indene-1-carbaldehyde (34)



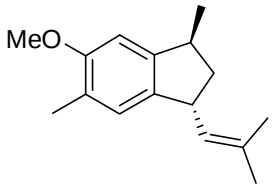
N-Methylmorpholine-*N*-oxide (0.64 g, 4.73 mmol) was dissolved in a solution of **76** (1.00 g, 4.30 mmol) in acetone/water 8:2 (20 mL) and then osmium tetraoxide (50 mg, 196 μmol) was added at rt. The reaction mixture was stirred for 3 h and then quenched with aq. 10% Na_2SO_3. Stirring was continued for additional 15 min and resultant mixture was extracted with EtOAc (2 X 15 mL). The organic layer was separated, dried over Na_2SO_4 and concentrated under vacuum to give colorless residue which was used foe next step without any purification.

LAH (82 mg, 2.15 mmol) was added to a stirred solution of residue obtained above in THF (20 mL) 0 °C. Reaction mixture was stirred at 0 °C for 30 min and then quenched with water. Stirring was continued for 5 h and then it was extracted with CH_2Cl_2 (2 X 20 mL). The combined organic layer was dried over Na_2SO_4 and concentrated under vacuum to give diol **61**.

$NaIO_4$ (0.92 g, 4.30 mmol) was added to a solution containing diol **61** in THF (15 mL) and water (5 mL) at rt and stirring was continued for 6 h. The mixture was filtered though celite and concentrated under vacuum. The residue obtained was diluted with EtOAc (20 mL), washed with water, dried and concentrated under vacuum to give crude product which was further purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford aldehyde **34** (0.51 g, 58%) as colorless thick oil.

Mol. Formula	**:**	$C_{13}H_{16}O_2$
Mol. Weight	**:**	204
$[\alpha]_D^{25}$	**:**	-52.1° (*c* 0.5, $CHCl_3$)
IR ($CHCl_3$) ν	**:**	2918, 1787, 1770, 1497, 1255, 1163, 879 cm^{-1}
^{1}H NMR (200 MHz, $CDCl_3$)	**:**	δ 1.29 (d, *J* = 7.3 Hz, 3H), 1.86 (ddd, *J* = 7.3, 8.5, 13.2 Hz, 1H), 2.20 (s, 3H), 2.66 (ddd, *J* = 3.8, 7.8, 13.2 Hz, 1H), 3.25-3.44 (m, 1H), 3.80-3.89 (m, 1H), 3.83 (s, 1H), 6.71 (s, 1H), 7.04 (s, 1H), 9.58 (d, *J* = 2.9 Hz, 1H)
^{13}C NMR (50 MHz, $CDCl_3$)	**:**	δ 16.3, 20.9, 35.1, 38.7, 55.5, 56.2, 105.8, 125.5, 126.8, 129.0, 148.3, 158.3, 200.6
ESI-MS *m/z*	**:**	205.1 $[M+H]^+$
Elemental Analysis	**:**	Found: C, 76.26; H, 8.05% Calcd: C, 76.44; H, 7.90%

(1*R*,3*S*)-5-Methoxy-3,6-dimethyl-1-(2-methylprop-1-enyl)-2,3-dihydro-1*H*-indene (35)



Under argon atmosphere n-BuLi (1.6 M in hexane, 1.84 mL, 2.94 mmol) was added drop wise to a stirred suspension of isopropyltriphenylphosphonium iodide (1.27 g, 3.18 mmol) in anhydrous THF (10 mL) at 0 ^{o}C. The resulting red solution was stirred for 30 min at rt and then the solution of aldehyde **34** (0.50 g, 2.45 mmol) in anhydrous THF (5 mL) was added drop wise into the reaction mixture. The mixture was stirred for 1 h at rt, quenched with saturated aq. NH_4Cl solution and then extracted with EtOAc (2 X 20 mL). The combined organic layer was washed with water, brine and dried over Na_2SO_4. The organic solvent was removed under reduced pressure and the resulting residue was further purified by column chromatography (silica gel, hexane/EtOAc 9:1) to afford **35** (0.45 g, 79%) as colorless oil..

Mol. Formula : $C_{16}H_{22}O$

Mol. Weight : 230

IR ($CHCl_3$) ν : 3460, 1620, 1245, 848 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.23 (d, *J* = 7.0 Hz, 3H), 1.74 (d, *J* = 1.3 Hz, 3H), 1.78 (d, *J* = 1.3 Hz, 3H), 1.87-2.02 (m, 2H), 2.18 (s, 3H), 3.15-3.30 (m, 1H), 3.81 (s, 3H), 3.95-4.05 (m, 1H), 5.11-5.23 (m, 1H), 6.67 (s, 1H), 6.83 (s, 1H)

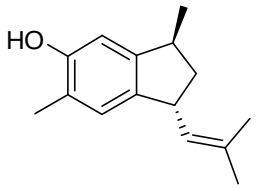
^{13}C NMR (50 MHz, $CDCl_3$) : δ 16.3, 18.2, 21.0, 25.9, 38.5, 41.7, 42.5, 55.5, 105.7, 125.0, 126.2, 128.8, 131.1, 138.0, 147.2, 157.3

ESI-MS *m/z* : 231.1 $[M+H]^+$

Elemental Analysis : Found: C, 83.29; H, 9.73%

Calcd: C, 83.43; H, 9.63%

(1*R*,3*S*)-3,6-Dimethyl-1-(2-methylprop-1-enyl)-2,3-dihydro-1H-inden-5-ol (11)



EtSH (1.08 g, 17.37 mmol) in DMF (3 mL) was added to a suspension of NaH (0.70 g, 17.37 mmol, 60% in mineral oil) in dry DMF (5 mL) at 0 ^{o}C and resulting solution was stirred for 1 h at rt under argon atmosphere. A solution of **35** (0.40 g, 1.74 mmol) in DMF (2 mL) was added and resulting mixture was heated for 8 h at 140 ^{o}C. The mixture was cooled to room temperature and a saturated solution of NH_4Cl (20 mL) was added. The mixture was extracted with EtOAc (2 X 20 mL), organic layer was washed with water followed by brine and dried over Na_2SO_4. The organic solvent was removed under reduced pressure and the resulting brown oil was purified by flash column chromatography (silica gel, hexane/EtOAc 7:3) to afford **11** as a white solid: mp 82 ^{o}C, [Lit.[12] 82 ^{o}C].

Mol. Formula : $C_{15}H_{20}O$

Mol. Weight : 216

$[\alpha]_D^{25}$: +79.8° (*c* 1, $CHCl_3$)

IR ($CHCl_3$) ν : 3370, 1620, 1245, 820 cm^{-1}

^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.20 (d, *J* = 7.5 Hz, 3H), 1.74 (d, *J* = 1.3 Hz, 3H), 1.77 (d, *J* = 1.3 Hz, 3H), 1.90-2.00 (m, 2H), 2.21 (s, 3H), 3.15-3.25 (m, 1H), 3.95 (q, *J* = 9.0 Hz 1H), 5.05-5.20 (m, 1H), 6.60 (s, 1H), 6.80 (s, 1H)

^{13}C NMR (50 MHz, $CDCl_3$) : δ 15.8, 18.1, 20.9, 25.8, 38.0, 41.5, 42.3, 110.1, 121.7, 126.3, 128.6, 131.2, 138.6, 147.8, 152.7

ESI-MS *m/z* : 217.1 $[M+H]^+$

Elemental Analysis : Found: C, 83.03; H, 9.42%

Calcd: C, 83.28; H, 9.32%

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Chapter 3

Section II

Enzyme-catalysed resolution of 1-phenylethanol *via* transesterification in the presence of phosphorus ionic liquids

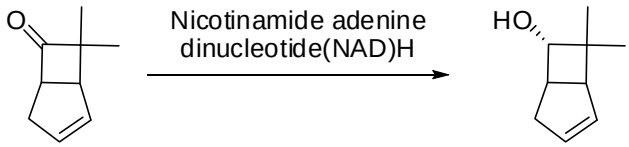
Introduction

Biotransformation in organic synthesis

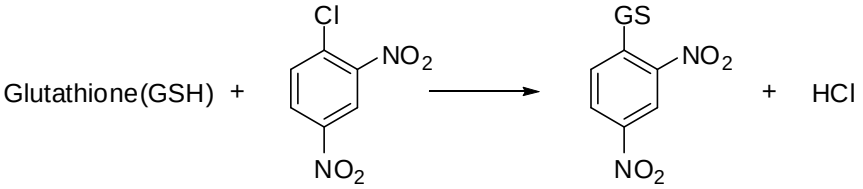
Biotransformation is the alteration of chemical structure of a compound into a new compound by enzyme. It is a specific reaction because a compound is modified chemically into a defined final product *via* selective transformation. The majority of useful biotransformations carried out in organic synthesis are by the hydrolase class of enzyme. The oxidoreductases are a mediocre second and the remaining classes are of low, but increasing, utility.

Enzyme-catalyzed reactions can be divided into six main groups according to the International Union of Biochemistry. These groups are:

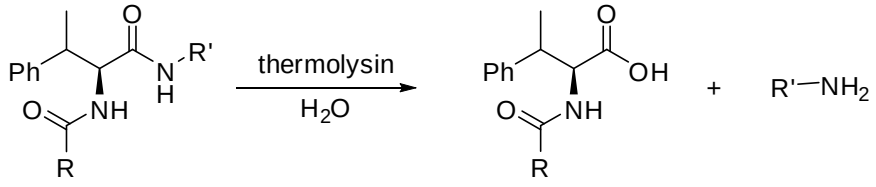
1. Oxidoreductases: Oxidation - reduction: oxygenation of C-H, C-C and C=C bonds, removal of hydrogen atom equivalents.



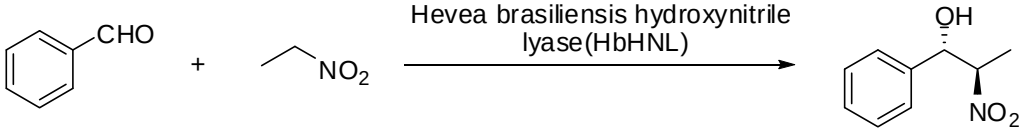
2. Transferases: Transfer of groups such as acyl, sugar, phosphoryl, aldehydic, and ketonic.



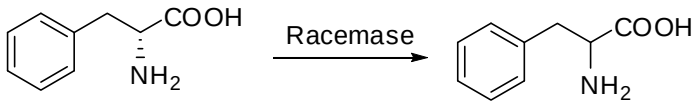
3. Hydrolases: Hydrolysis of glycosides, anhydrides, esters, amides, peptides and other C-N containing functions.



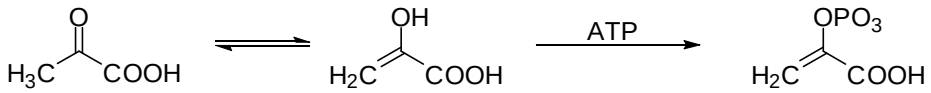
4. Lyases: Reactions such as the addition of HX to double bonds as in C=C, C=N and C=O and the reverse process.



5. Isomerases: Isomerizations such as C=C bond migration, *cis-trans* isomerization and racemization.



6. Ligases: Catalyze the joining of two large molecules by forming a new chemical bond.



Chirality and its significance

Chirality is an important feature of nature and it plays a key role in chemo and biodiversity of the planet earth. Chirality means a compound having one or more centers of asymmetry (usually carbon). The asymmetry center permits the compound to exist in two or more stereospecific configurations or stereoisomers. The word chiral is derived from the Greek word chiros meaning "hand", the term chiral suggests handedness and is applied to molecules having structures that are mirror image of each other analogous to left and right hands. The significance of using chirally pure compounds is being increasingly realized in pharmaceutical industry and also to some extent for other specialty chemicals, viz., fragrance & flavor, agrochemicals and health care products. Biological systems, in most cases, recognize the members of a pair of enantiomers as different substances and the two enantiomers will elicit different responses. Thus, one enantiomer may act as a very effective therapeutic drug, whereas the other enantiomer is highly toxic as seen from Table 1.

Table 1. Contrasting biological activity of some enantiomers:

Sr.No	**Drug/chemical**	***S*-form**	***R*-form**
1	Chloramphenicol	Inactive	antibacterial
2	Ethambutol	Tuberculostatic	Causes blindness
3	Carvone	Caraway flavour	Spearmint flavour
4	Asparagine	Bitter taste	Sweet taste
5	Propranolol	b-blocking agent	100 times less active
6	Fluazifop butyl	inactive	herbicide
7	Paclobutrazol	Plant growth regulator	fungicide

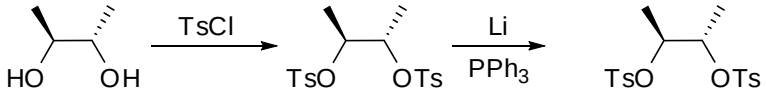
Strategies for synthesis of chiral molecules

To obtain a pure enantiomer, two approaches are followed:

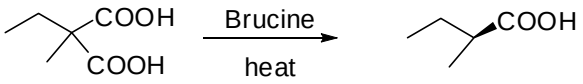
- To design asymmetric synthesis for desired stereoisomer
- To synthesize the molecule in its racemic form and then resolve it

There are three main approaches for asymmetric synthesis:

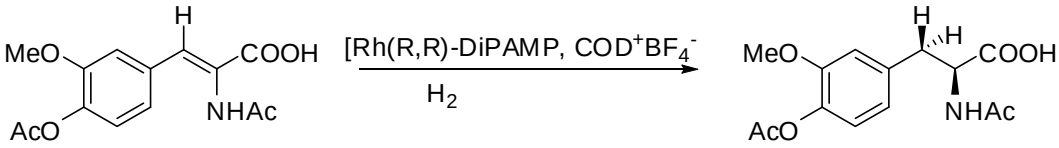
- **Chiral pool synthesis**: It is the easiest approach in which starting material is manipulated through successive reactions using achiral reagents which retain its chirality to obtain the desired target molecule.



- **Chiral auxiliaries**: Chiral auxiliary forms adduct with the starting materials and physically blocks the other trajectory for attack, leaving only the desired trajectory open.



- **Asymmetric catalysis**: Small amounts of chiral, enantiomerically pure (or enriched) catalysts promote reactions and lead to the formation of large amounts of enantiomerically pure or enriched products. Mostly, the three different kinds of chiral catalysts employed are metal ligand complexes derived from chiral ligands, chiral organocatalysts and biocatalysts.

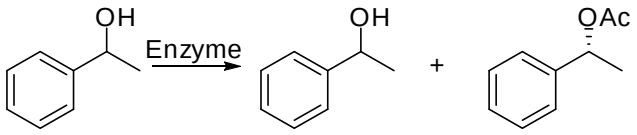


Kinetic resolution

In kinetic resolution, one enantiomer will undergo chemical transformation at a rate higher than the other enantiomer under optimal conditions. The difference in rate arises from the difference in activation energy required to reach the transition state of reaction.

This approach is often more economical than asymmetric synthesis. Furthermore, kinetic resolution can be performed at high substrate concentration.

However, asymmetric synthesis often requires high dilution in order to achieve acceptable enantioselectivity. As a consequence, the kinetic resolution of racemate is considered as the best enzymatic approach to produce enantioenriched or enantiopure compounds. A chiral reagent, a chiral solvent or a chiral physical force must be introduced in order to carry out kinetic resolution. However, the reagent used is not required in stoichiometric amount.



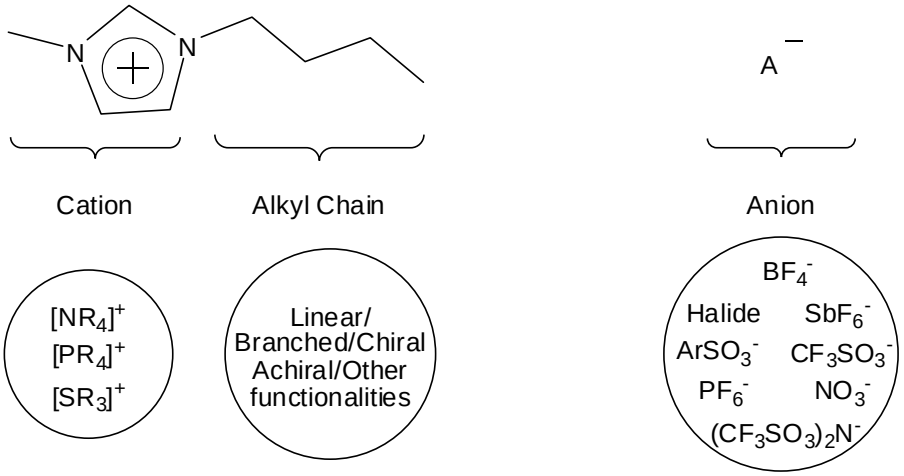
Role of ionic liquids (ILs) as solvents in enzyme catalysed reactions

Ionic liquids offer new possibilities for the application of solvent to biocatalytic reactions. Although in many cases ionic liquids have simply been used to replace organic solvents, they have often led to improved process performance. Unlike conventional organic solvents, ionic liquids possess no vapor pressure, are able to dissolve many compounds and can be used to form two-phase systems with many solvents. To date, reactions involving lipases have benefited most from the use of ionic liquids and the use of ionic liquids with other enzymes and in whole-cell processes have also been described. In some cases, remarkable results with respect to yield, (enantio) selectivity or enzyme stability were observed. More detailed are discussed in the later pages.

In the following pages a brief history of ionic liquids and some recent developments in this area are described.

Brief history of ionic liquids

Generally ILs refers to molten salts, which contain ions. Only those liquids, which are non-corrosive and have low viscosity, are chosen to be called as Ionic Liquids. Ionic liquids are broadly defined as *low melting's salts*. Ionic liquids consist of an organic cation and an anion as shown in **Figure 1**.



Figire 1: Diagrammattic representation of various components of ionic liquids

The early history of ionic liquids began in 1914 when the first report of a room temperature molten salt was reported by Walden.[1] He reported the physical properties of ethyl ammonium nitrate($[C_2H_5NH_3]NO_3$), which has a melting point of 12 ºC, formed by the reaction of ethylamine with concentrated nitric acid. This salt is liquid at room temperature but usually contains a small amount of water (200-600 ppm).

The first ionic liquid with chloroaluminate ions were developed in 1948 for applications in aluminium electroplating by Hurley and Weir[2] by mixing and warming 1-ethylpyridinium chloride with aluminum chloride. As early as 1967, a publication by Swain *et al*. described the use of tetra-n-hexylammonium benzoate as a solvent for kinetic and electrochemical investigation.[3] Room temperature ionic liquids only really reached a more general audience with the reopening of development in this area by the groups of Osteryoung *et al*.[4], Hussey *et al*.[5] and Seddon *et al*.[5b,5c] In 1980s, they carried out extensive research on organic chloride-aluminium chloride ambient temperature ionic liquids and the first major review of room temperature ionic liquids was written by Hussey.[6] The ionic liquids based on $AlCl_3$ can be regarded as the first generation of ionic liquids.

Classification of Ionic Liquids

ILs are classified into two categories.

1. Binary ionic liquids – salts where equilibrium is involved.
2. Simple salts – made of single anion and cation.

In the first category, the first generation ILs contains a mixture of metal halide and dialkylimidazolium chloride. These contain several ionic species and their melting point and other properties depend on the mole fractions of the individual components. The second class, generally termed as second generation ILs, consists of simple cation and anion e.g. ethyl ammonium nitrate ($[EtNH_3]^+[NO_3]^-$), dialkylimidazolium ILs bmim]Br. The third generation ILs consists of chiral ILs made from either chiral cations or anions, mono-alkyl imidazolium ILs and task specific ILs. ILs generally are composed of relatively large organic cations and inorganic or organic anions and have a melting range of –96 °C to 100 °C. Cations are mainly alkyl quaternary ammonium or phosphonium moiety which may be a part of a heterocyclic ring.

Recent developments in cations and anions in ionic liquids

The cations are generally organic components with low symmetry and bulk in size. Those described until now are based on ammonium **1**, sulfonium **2**, phosphonium **3, i**midazolium **4**, pyridinium **5**, pyrrolidinium **6**, thiazolium **7**, triazolium **8**, oxazolium **9** and pyrazolium **10** cations (Figure 2).

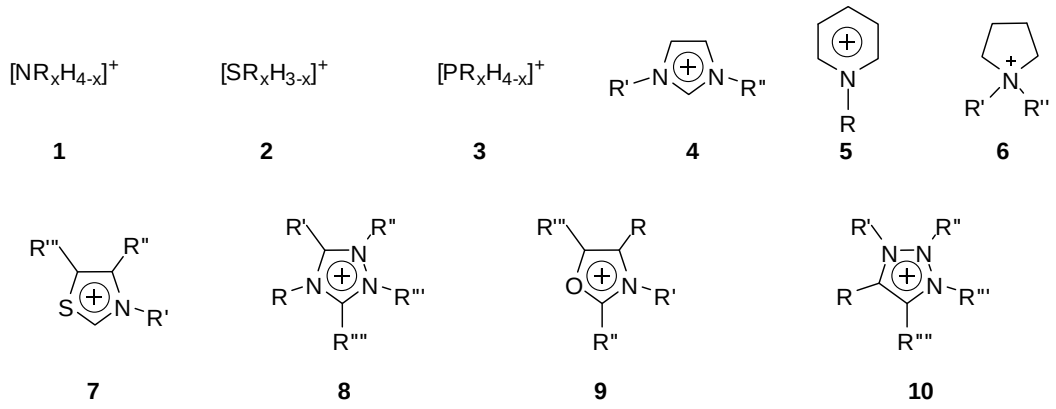


Figure 2: Examples of cations described in ionic liquids

Concerning the anions, they can be classified in two categories, those which give polynuclear anions, e.g. Al_2Cl_7, Al_3Cl_{10}, Fe_2Cl_7, Sb_2F_{11} and mononuclear anions which gives neutral stoichiometric ionic liquids like Cl, Br, ClO_4, CF_3SO_3, CH_3SO_3, CF_3CO_2, etc. The series of zwitterionic type ionic liquids (Figure 3) consisting of imidazolium cations containing covalently bound counter anionic sites, such as sulfonate or a sulfonamide group have been prepared.

Figure 3: Examples of zwitterionic salts

Chiral Ionic Liquids

Even though a limited number of chiral ILs have been designed and synthesized in an attempt to influence the outcome of asymmetric organic reactions,[7] there are only a few chiral ionic liquids that can effectively influence the outcome of asymmetric reactions.[8] These new chiral solvents should play a central role in enantioselective organic synthesis and hopefully expand the scope of chiral solvents. Chiral ILs can be particularly attractive if one considers their potential applications to chiral discrimination, including asymmetric syntheses and optical resolution of racemates.

A thorough literature review reveals that the design of existing chiral ILs is based on modifications of the ammonium,[9] pyridinium,[10] oxazolinium,[10] or thiazolium cations.[11] There are several chiral ILs in which the chiral moiety is contained in the anion **11** and **12** (Figure 4), but the modification of imidazolium cation-derived ILs **13-16** offers extreme promise in the design of chiral ILs due to their facile preparation, low melting points, and relatively favorable viscosity.

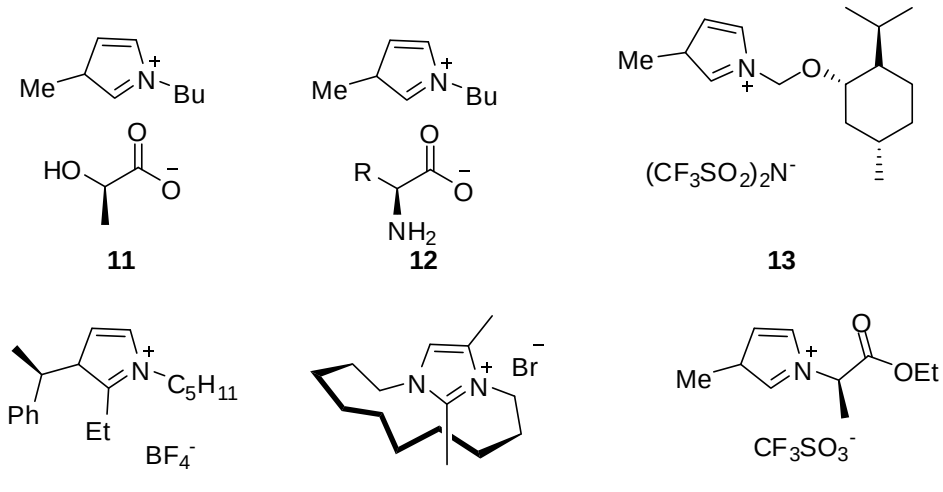


Figure 4: Chiral ionic liquids

Task Specific Ionic Liquids [TSILs]

The covalent tethering of a functional group to one or both of the ions of an otherwise ordinary ionic liquid can inculcate the resulting salt with a capacity to interact with dissolved substrates in specific ways generates what are called as ‘‘task-specific’’ ionic liquids (TSILs). These low melting salts are finding an increasing number of applications in synthesis, separations, catalysis, and electrochemistry.

The ionic liquids are defined as TSILs when they have the following features:

- Ionic liquids in which a functional group is covalently tethered to the cation or anion (or both) of the imidazolium salts, which behave not only as a reaction medium but also as a reagent or catalyst.
- A conventional ionic liquid solution of a functionalized imidazolium salt, which is not a liquid form at ambient temperature, could also be defined as a TSIL since the functionalized imidazolium salt become integral elements of the overall ionic liquid solution and can introduce a functional group into the liquid.

Applications of ionic liquids

Large volume of literature is available which shows the importance of ILs which is covered in a number of excellent books,[12] recent general reviews[13] as well as those covering specific topics such as catalysis,[14] synthesis of organometallic complexes[15] in ionic liquids, biphasic systems and supported ionic liquids,[16] analytical applications of ionic liquids,[17] electrochemistry[18] in ionic liquids. In addition, a number of special issues have appeared covering a range of topics including ionic liquids as green solvents, physical and thermodynamic data and organometallic chemistry in ionic liquid.

The first publications in which ionic liquids were described as new reaction media and catalyst for organic synthesis appeared in 1986. Acidic ionic liquids with chloroaluminate ions proved to be effective Friedal-Crafts catalysts.[19] Phosphonium halide melts were used successfully in nucleophilic aromatic substitution reactions.[20] The use of ionic liquids as solvents for homogeneous transition metal catalysts was described for the first time in 1990 by Chauvin *et al*. and by Wilkes *et al*. Chauvin’s group dissolved nickel catalyst in weakly acidic chloroaluminate melts and investigated the resulting ionic catalysed solutions for the dimerization of

propene.[21] Also Wilkes *et al*. used weekly acidic chloroaluminate melts and studied therein in the ethylene polymerization with Ziegler-Natta catalyst.[22]

Phosphorus containing ionic liquids

Phosphorus containing ionic liquids can be classified in to two groups; a) ionic liquids where phosphorus is an **anion** (dialkylimidazolium hexafluorophosphate) and b) where phosphorus acts as a **cation** (quaternary phosphonium salts).

The first group of ionic liquids (phosphorus containing imidazolium based ionic liquids) was introduced[23] and thoroughly studied and lot of literature is available regarding properties, synthesis and applications. A review article by Keglevich[24] explains various green chemistry aspects of both phosphorus based ionic liquids along with their applications. Various applications of 1-alkyl-3-methylimidazolium hexafluorophosphates as alternative reaction media are found in the literature.[25] Dzyuba *et al*.[26] reported the synthesis and properties of 1,3-dialkylimidazolium hexafluorophosphates (Figure 5). All these 1,3-dialkylimidazolium hexafluorophosphates with dibutyl, dioctyl, dinonyl, and didecyl substitutents have melting points less than 100 °C.

Imidazolium phosphine type ionic liquids (Figure 5) were introduced for reactions involving metal complexes as catalyst.[27]

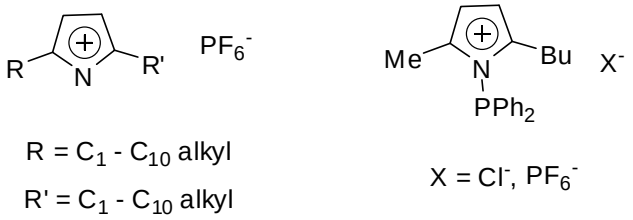


Figure 5

Phosphonium-based ionic liquids (PILs)

Recently, a new class of ionic liquids has been introduced in organic reactions. They are phosphonium based ionic liquids, which differ from the well known imidazolium ILs.

The other group of phosphorus containing ionic liquid is where phosphorus acts as a cation (quaternary phosphonium salts), are popularly known as phosphonium ionic liquids (PILs). Following are some anions (Figure 6) that can be paired with phosphorus cation.

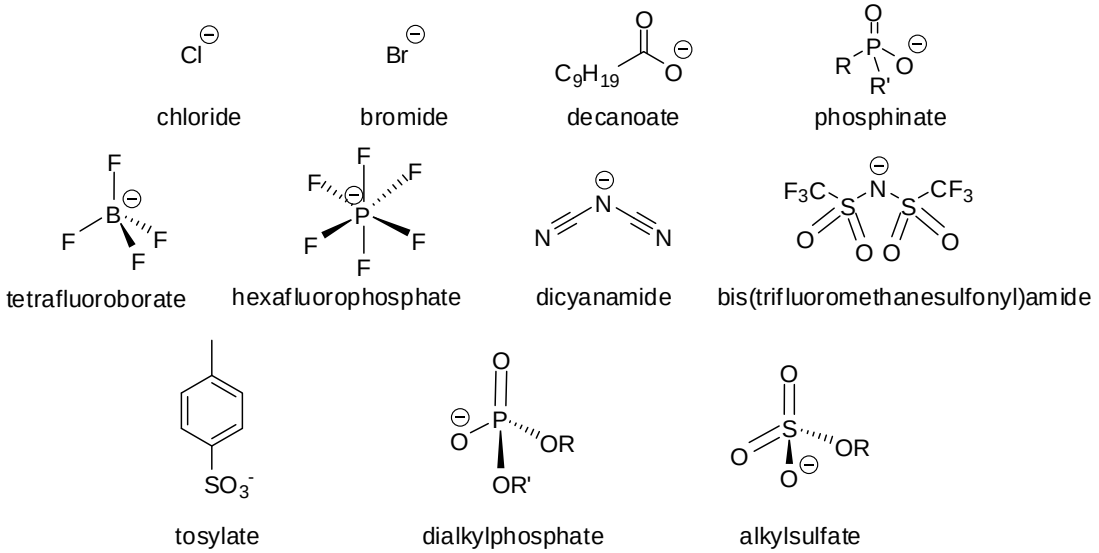


Figure 6: Anions in phosphonium based ionic liquids

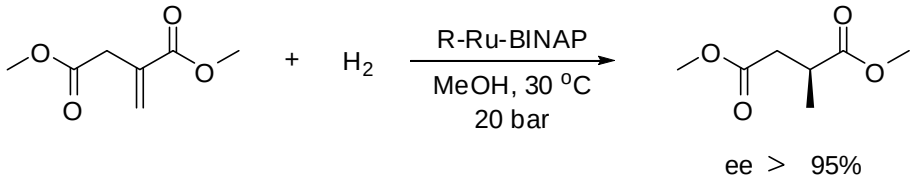
Features and advantages of phosphonium ionic liquids

- Phosphorus has larger radius and more polarizable lone pair electron that make them more nucleophilic than other amine based ionic liquids.
- Phosphonium salts are generally thermally more stable than ammonium salts.
- Phosphonium based ionic liquids tend to have viscosities somewhat higher than their ammonium counterparts, especially at or near room temperature.
- Important difference between imidazolium and phosphonium salts are the acidic protons present in the former. Relative to phosphonium cations, imidazolium cations are not entirely inert and can interact with solutes either through hydrogen bonding interactions or through the aromatic nature of the ring system.
- Unlike their ammonium counterparts, which can undergo facile Hoffmann or β-elimination in the presence of base, phosphonium salts decompose to yield a tertiary phosphine oxide and alkane under alkaline conditions.

Applications of phosphonium ionic liquids

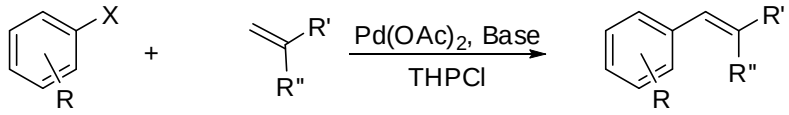
Phosphonium ionic liquids have been used as reaction medium for various chemical transformations. Trihexyl(tetradecyl)phosphonium chloride (THPCl), has been used for the enhancement of the enantioselectivity and the stability of Ru-BINAP during hydrogenation[28] of dimethyl itaconate to (*S*)-dimethyl methylsuccinate. The catalyst and ionic liquid were efficiently separated by

nanofiltration from the product, followed by simultaneous recycling of the catalyst and ionic liquid. Palladium catalyzed Suzuki cross coupling of aryl boronic acids with aryl halides under mild conditions was demonstrated in same ionic liquid with 76-100% yield.[29] It was also demonstrated by Wong *et al.*[30] for 4-bromo acetophenone in three different PILs. Catalyst and ionic liquid were efficiently separated by nanofiltration from the product for further recylization.



Buchwald–Hartwig amination of aryl halides using palladium as catalyst was studied[31] in phosphonium ionic liquids containing trihexyl(tetradecyl)phosphonium cation with a range of anions. Trihexyl(tetradecyl)phosphonium bis(trifluoromethylsulfonyl)imide was found to be better among used ionic liquids.

The Heck cross-coupling of aryl iodides and bromides with methyl acrylate was reported[32] in trihexyl(tetradecyl)phosphonium chloride and trihexyl(tetradecyl) phosphonium decanoate in 78% and 75% yield respectively. Trihexyl(tetradecyl)phosphonium chloride was easier to separate during the purification stage. Addition of hexane and brine to the reaction mixture gave three distinct phases with the palladium remaining in the middle ionic liquid layer, the coupled product in the organic layer and salts in the aqueous layer. Tetrabutylphosphonium bromide was used[33] for thiolyzation of epoxides with aryl disulfides catalyzed by cerium (III) chloride heptahydrate.



Comyns *et al.*[34] demonstrated hydrogen transfer to acetophenone using ethyltrioctylphosphonium tosylate and Ru as catalyst. The product was separated by simply decantation as PIL is solid at ambient temperature. Similarly selective hydrogenation of 1,3-butadiene to 1-butene[35] and the hydroformylation of 1-hexene to C-7- aldehyde are described[36] in phosphonium ionic liquids.

The conversion of methanol to acetic acid was reported[37] in presence of phosphonium ionic liquids with tetrafluoroborate as anion and Ru and Co as catalyst.

Highly regioselective O-alkylation of β-naphthol with benzyl bromide was demonstrated[38] in tetraalkylphosphonium halides.

Design of novel phosphonium ionic liquids that are compatible with Grignard reagents have been investigated.[39] It has been established that even basic aliphatic Grignard reagent-mediated reactions are possible when methoxyethyl tri(n-butyl) phosphonium bis(trifluoromethanesulfonyl)imide is used as the solvent. Ramani *et al*.[40] reported in 2005 that Grignard reagents are persistent in tetradecyl(trihexyl)phosphonium chloride and may be more suitable for reactions involving strong bases. They further suggested[41] that Grignard reagents in phosphonium ionic liquids possessing *O*-donor anions are excellent reaction media for electron transfer processes and transmetallation reactions.

Itoh *et al*.[39] in 2007 further demonstrated that Grignard reagent-mediated reactions are possible when methoxyethyl (tri-*n*-butyl) phosphonium bis (trifluoromethanesulfonyl) imide is used as the solvent. Reactions of saturated chlorides with hydrogen fluoride in PILs were also reported.[42] This method may be suitable for the preparation of industrially useful fluorine derivatives such as freons.

Phosphonium tosylates were used in Diels-Alder reactions of isoprene with methyl acrylate, but-2-en-2-one and acrylonitrile; demonstrating a high regioselectivity (>99%) of 1,4-isomer with oxygen-containing dienophiles even without Lewis acids as catalysts.[43]

Forbes *et al*.[44] demonstrated esterification of acetic acid using ethanol and phosphonium ionic liquid bearing sulfopropyl moiety which act as an acid catalyst for esterification reaction.

Friedel-Crafts acylation of substituted benzene with benzoyl chloride was also reported by Earle *et al*.[45-48] in presence of $(C_6H_{13})_3P^+C_{14}H_{29}\ X^-$.

Biotransformations in ionic liquids

Ionic liquids are used with three different methods in the enzyme systems, namely; 1) as a co-solvent in aqueous phase, 2) as a pure solvent and 3) as a two-phase system together with other non-aqueous solvents. The use of ionic liquids in biocatalytic transformations has solved some of the problems encountered in their applications in aqueous and organic solvents.

Their use in lipase-catalyzed reactions has increased the solubility of the substrate by 3-fold, and in the N-acetyl-lactosamine synthesis using β-galactosidase,

the yield has been doubled by minimizing the side reactions.[49] Enhanced enantioselectivity in dynamic, kinetic and distillative work-up is possible for lipases in ionic liquids and, additionally, the physical properties of the ionic liquids can be varied very widely by using different cation and anion combinations.[50] This makes it possible to dissolve enzymes directly in certain ionic liquids and to dissolve substrates (e.g. carbohydrates) that would not normally dissolve adequately in organic solvents.[51] Different enzymes such as hydrolases (proteases and lipases) and oxidoreductases (peroxidases and dehydrogenases) retain their activity when suspended in ionic liquids, which present a very promising 'green' alternative to organic solvents for enzyme-catalysed reactions. Cull *et al*. have published[52] first results on the use of an ionic liquid as a reaction medium for whole-cell biotransformations. They reported the use of a [BMIm][PF_6] ionic liquid in a two-phase system for the hydration of 1,3-cyanobenzene catalysed by nitrile hydratase from Rhodococcus 312 to give 3-cyanobenzamide and 3-cyanobenzoic acid. Recently Udo Kragl *et al*. reported[53] the lipase catalysed kinetic resolution of 1-phenylethanol in imidazolium based ionic liquids.

Thus, above literature indicates utility of phosphonium-based ionic liquids for various catalytic reactions along with nucleophilic and electrophilic reactions. As all reactions dealing with ionic liquids and biotransformation reactions in or in absence of ionic liquids are classified as green reactions, it is considered necessary to discuss green chemistry and its principles here.

Green Chemistry

It is widely acknowledged that there is a growing need for more environmentally acceptable processes in the chemical industry. This trend towards what has become known as 'Green Chemistry' or 'Sustainable Technology' necessitates a paradigm shift from traditional concepts of process efficiency, that focus largely on chemical yield, to one that assigns economic value to eliminating waste at source and avoiding the use of toxic and/or hazardous substances.

A reasonable working definition of green chemistry can be formulated as follows: *green chemistry efficiently utilizes (preferably renewable) raw materials, eliminates waste and avoids the use of toxic and/or hazardous reagents and solvents in the manufacture and application of chemical products.*

Twelve Principles of Green Chemistry

Since its inception in 1991, green chemistry has grown into a significant, internationally engaged focus area within chemistry. The twelve principles of green chemistry are viewed as the guidelines or directions that have been set by some pioneering scientists who have laid the groundwork for future are prevent waste, design chemical products should be fully effective, use substance with low or no toxicity, renewable feedstocks, catalytic reactions, avoid using blocking or protecting groups, design syntheses so that the final product contains the maximum proportion of the starting materials, avoid using solvents, separation agents, run chemical reactions at ambient temperature and pressure, design chemical products to break down to innocuous substances, minimize or eliminate the formation of by products and minimize the potential for chemical accidents including explosions and fires.

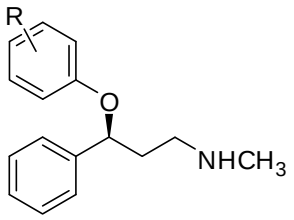
A green solvent must ideally have negligible vapour pressure, high boiling point, be nontoxic, have capacity to dissolve wide range of organic, inorganic and organometallic compounds, it should be chemically and physically stable, recyclable, reusable, inexpensive and eventually easy to handle. In addition to these, solvents that allow more selective and rapid transformations will have a significant impact. Therefore, many attempts have been made to substitute conventional organic solvents with novel alternative reaction media which include:

- Supercritical fluids
- Poly (ethylene glycol) – PEG
- Perfluorinated (fluorous) solvents
- Water
- Ionic liquid

Present Work

Generally only one of the enantiomer of chiral compounds has the desired biological activity. The biologically inactive enantiomer is at best useless compound, but it may also cause unwanted side-effects. Among the various possibilities to produce single isomers, catalytic kinetic resolution is used extensively because of its suitability for large scale production. The catalytic chiral resolution based on the principle that optical isomers react at different rates and therefore a homochiral derivate can be separated from the reaction products.

A few methods have been developed for the enantioselective synthesis of chiral alcohols but still enzymatic resolution of racemic alcohols is a convenient alternative for preparing enantiomerically pure alcohols. Chiral alcohols are important synthetic intermediates and are structural elements in biologically active compounds (Figure 7) and natural products.[54]



Fluoxetine R = 4-CF_3

Tomoxetine R = 2-CH_3

Nisoxetine R = 2-OCH_3

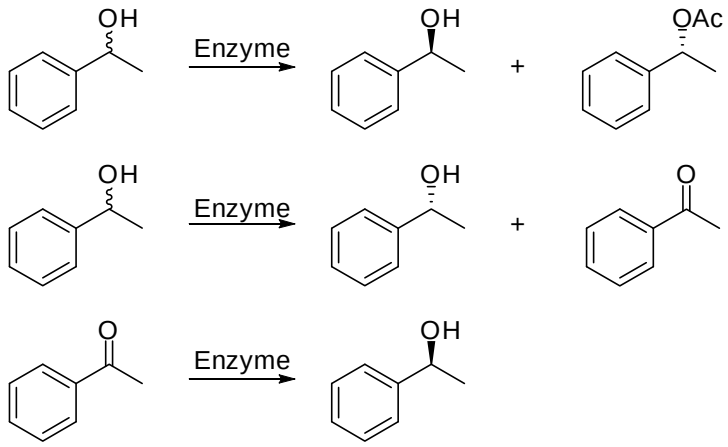
Figure 7

Importance of chiral 1-phenylethanol

The optically active 1-phenylethanol is used as chiral building block and synthetic intermediate in fine chemical, pharmaceutical and agrochemical industries.[54] In pharmaceutical industry, 1-phenylethanol is used as ophthalmic preservative. This chiral compound may also inhibit cholesterol intestinal adsorption and thus decrease high cholesterol level. Since (*R*)-1-phenylethanol contains mild floral odour, it is used as hyacinth like fragrance in cosmetic industries. It is also used as perfumery ingredient. Moreover, (*R*)-1-phenylethanol can be used in solvatochromic dye and present in many natural products and in its chirally pure form is important intermediate in large number of total synthesis of biologically active natural products. Both the isomers of 1-phenylethanol were used as chiral auxiliary in the total synthesis of compactin and dihydromevinolin.[54d]

Kinetic Resolution of 1-Phenylethanol

The chemical method for the preparation of optically active 1-phenylethanol requires heavy metal catalyst, namely ruthenium (II) complexes and lithium aluminum hydride complexes in the asymmetric reduction of acetophenone.[55] In addition to the negative impact on the environment, this method is also unable to produce chiral 1-phenylethanol with sufficient optical purity (48% ee) compared to the enzymatic approach. The asymmetric reduction of prochiral ketones and the enantioselective oxidation of single enantiomer are another two possible microbial methods of chiral alcohol preparations. The yeast-mediated reduction required the regeneration of coenzyme NAD(P)H and hence energy sources must be added to the system. Furthermore, only about 10% of acetophenone was converted to 1-phenylethanol as catalyzed by yeast cells. On the other hand, the enantioselective oxidation required an oxygen source for the reaction. The reaction could produce (*R*)-1-phenylethanol with sufficient optical purity (> 90%) only after 80 hours of continuous production. Lipases are still considered as the most suitable enzyme for preparing enantiomerically pure 1-phenylethanol. Lipases, especially from the genera of *Pseudomonas* can produce 1-phenylethanol with high enantiomeric excess, >99 %.[56] Lipase-catalyzed reactions could be carried out in a wide variety of reaction conditions. They require no cofactor and are readily available at low cost. Different enzymatic pathways to produce chiral 1-phenylethanol are depicted in **Scheme 1**.



Scheme 1: Different enzymatic paths to produce chiral phenyl ethanol

Acyl donor in enzymatic resolutions

An acyl donor can be divided into two moieties, namely acyl part and its leaving group. The acyl moiety of acyl donor is covalently bound to the active site of enzyme. The length and the shape of acyl part indeed influence the enantioselectivity of reaction. However, the leaving group affects the reversibility of reaction in organic solvent. It is either a poor nucleophile or easy to vaporize in order to drive the reaction towards desired direction. The common acyl donors for the enzymatic resolution of racemic alcohols are carboxylic acids, anhydrides, vinyl ester, diketene and ester. Each class of acyl donors produces different types of by-products. The effects of the by-products on the reactions are the main consideration in the acyl donor selection process. Out of all acyl donor reagents vinyl acetate was used in majority of reactions involved in the resolution by transesterification of alcohol (Table 2).

Table 2: Selected reported studies on enzymatic (*R*,*S*)-1-phenylethanol resolution

Enzyme	**Acyl donor**	**Ref.**
Lipid-coated lipase from *Pseudomonas fragi 22-39B*	Lauric acid	57
Lipid-coated lipase from *Pseudomonas fragi 22-39B*	Lauric acid	58
Lipid-coated lipase from *Pseudomonas fragi 22-39B*	Lauric acid	59
Microemulsion-based gel lipase (Genzyme) from: i) *Chromobacterium viscosum* ii) *Pseudomonas sp.*	Hexanoic acid & lauric acid	60
i) *Fusarium oxysporum* ii) *Ovadendron sulphureoochraceum* lipase	Vinyl acetate	61
Lipase SAM-II (Amano) from *Pseudomonas sp.*	Vinyl acetate	62
i). Dry powder lipase B from *Pseudomonas fragi,* ii). Lipase AS from *Pseudomonas nitroreducens* iii). Lipase P from *Pseudomonas fluorescens*	Vinyl acetate	63
Celite immobilized lipase from *Pseudomonas fluorescens*	Vinyl acetate	64

Results and discussion

In this study, the efficiency of lipases in the resolution of 1-phenylethanol in phosphorus based ionic liquids was determined by kinetic analysis. Kinetic studies were carried out using the data obtained by optical rotation of acetate which was transesterified in the reaction. The objective of this research was to study an enzymatic resolution of (*R,S*)-1-phenylethanol *via* enantioselective esterification with vinyl acetate catalyzed by lipases in the phosphorus based ionic liquids.

As maintained in earlier pages, imidazole based ionic liquids have been reported for lipase catalysed kinetic resolution of 1-phenyletanol. Except two lipases, *Candida antarctica* lipase and *Alcaligenes sp.* lipase conversion (%) of alcohol is below 5% in imidazole based ionic liquids. While comparing the results, it clearly indicates that conversion (%) of alcohol and ee (%) of corresponding acetate are higher in phosphonium ionic liquids.

(*R,S*)-1-Phenylethanol and vinyl acetate

High purity grade of substrates such as (*R,S*)-1-phenylethanol and vinyl acetate were purchased from Fluka. The physical properties of the substrates are summarized in **Table 3**.

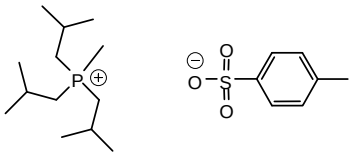
Table 3: Physical properties of substrates

Substrate	***(R,S)*-1-phenylethanol**	**Vinyl acetate**
Formula	$C_6H_5CH(CH_3)OH$	$C_4H_6O_2$
Molecular weight	122.17	86.04
Purity (%)	~ 96	~ 99
Melting point °C	19 - 21	-100
Boiling point °C	94 - 96	72
Density (g/ml)	1.009	0.93
Appearance	Colorless liquid	Colorless liquid
Odor	Mild floral	Sweet-to-sharp
Hazardness	Peroxidizable chemical	Flammable in presence of open flames and sparks

Ionic liquids used in the present study

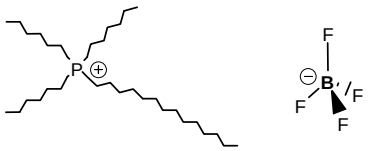
The following ionic liquids are used.

Triisobutyl (methyl) phosphonium tosylate (IL-1), trihexyl (tetradecyl)phosphonium tetrafluoroborate(IL-2), trihexyl(tetradecyl) phosphoniumhexa fluorophosphate (IL-3), trihexyl(tetradecyl)phosphonium dicyanamide (IL-4), trihexyl (tetradecyl) phosphonium bis (2,4,4 -trimethylpentyl) phosphinate (IL-5), trihexyl (tetradecyl) phosphonium bis(trifluoromethane) sulphonimide(IL-6).



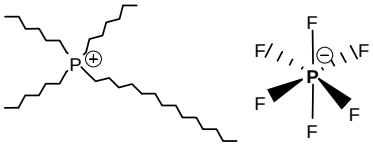
IL-1

Triisobutyl (methyl)
phosphonium tosylate



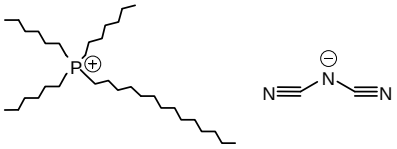
IL-2

Trihexyl (tetradecyl)
phosphonium tetrafluoroborate



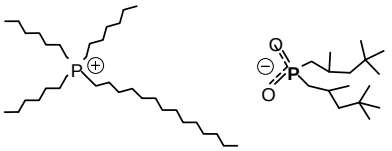
IL-3

Trihexyl (tetradecyl)
phosphoniumhexafluoro phosphate



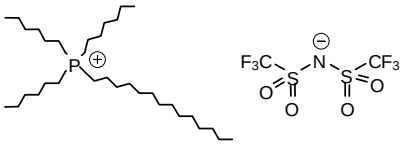
IL-4

Trihexyl (tetradecyl)
phosphonium dicyanamide



IL-5

Trihexyl (tetradecyl) phosphonium
bis (2,4,4 -trimethylpentyl) phosphinate



IL-6

Trihexyl (tetradecyl) phosphonium
bis(trifluoromethane)sulphonylamide

Figure 8: Ionic liquids used for reaction

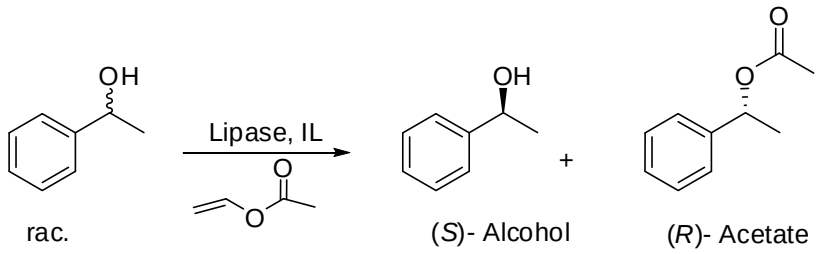
Enzymes

The commercial immobilized lipases used for the reaction are tabulated below (Table 4). They are microorganism lipases from yeast.

Table 4: Commercial immobilized lipases supplied by M/s Aldrich chemicals, USA

Microorganism	**Size (mm)**	**Appearance**
Candida antarctica	> 0.5	Brown bead
Pseudomonas cepacia	0.01 –0.03	Brown powder
Porcine pancreas	> 0.1	Brown powder
Candida rugosa	0.01 –0.03	Yellow powder
Candida cylindraecea	> 0.1	White powder

The synthetic utilities of lipases were investigated by carrying out transesterification reaction. An activated ester, vinyl acetate was used as acyl donor in this acyl transfer reaction. The racemic (*R*,*S*)-1-phenylethanol was enantiomerically acylated by vinyl acetate to produce (*R*)-1-phenylethyl acetate, vinyl alcohol and unreacted (*S*)-1-phenylethanol. The enzymes are highly selective toward the (*R*)-enantiomer of the racemic alcohol. The by-product, vinyl alcohol is reactive and unstable, so it tautomerizes into volatile acetaldehyde rapidly. The release of acetaldehyde promotes the reaction toward synthesis direction based on the principle of Le Chatelier. The schematic diagram of the reaction is presented in **Scheme 2**.



Scheme 2: Transesterification of (*R*,*S*)-1-phenylethanol with vinyl acetate

The data obtained are summarized in **Table 4**. Under the conditions employed for the transformation, *Candida rugosa* lipase showed no activity even after 72 h at 35 °C (All reactions were carried out at 35 °C with time varying from 15 to 72 h). Both *Candida antartica* lipase and *Porcine pancreas* lipase gave the best results in several of the ionic liquids. Enantioselectivity of the product improved significantly with *Porcine pancreas* lipase compared to the reaction in MTBE. In *Candida antartica* lipase, the resolution took an average of 20 h whereas with *Porcine pancreas* lipase, the time taken varied from 48 to 72 h. Although there is no single ionic liquid which

could be termed best, both IL-2 and IL-3 were found to be good for the reaction under consideration.

Table 4: Screening of lipases in various ionic liquids. Yield (%) of (*R*)-acetate and ee's (%) of (*R*)-acetate (in brackets) are given. Enantiomeric excess (ee) was estimated by comparison of optical rotation of (*R*)–acetate with authentic value. The last values in each column denote the time of the reaction in hours.

Solvents	**Enzyme**				
	L-1	**L-2**	**L-3**	**L-4**	**L-5**
MTBE	46, (100), 24	38, (92.3), 72	12, (90.6), 72	NR, 72	28, (100), 72
IL-1	8, (44.2), 20	8, (5.2), 72	12, (100), 48	NR, 72	21, (<5), 72
IL-2	22, (97.8), 20	10.9, (97), 48	32, (100), 72	NR, 72	17.5, (66), 72
IL-3	24, (100), 15	20, (98), 24	14, (87), 60	NR, 72	14, (68), 72
IL-4	23, (92), 20	19, (95), 48	27, (96), 72	NR, 72	19, (66), 72
IL-5	25, (14.2), 20	15.2, (6), 48	28, (56), 72	NR, 72	12, (37), 72
IL-6	29, (100), 20	9.4, (79), 72	43, (100), 72	NR, 72	13.6, (71), 72

L-1 *Candida antartica* lipase. **L-2** *Pseudomonas sp*. lipase. **L-3** *Porcine pancreas* lipase. **L-4** *Candida rugosa* lipase. **L-*5*** *Candida cylindraecea* lipase. **MTBE** Methyl *tert*-butyl ether. **NR** No reaction.

All the enzymes except *Candida rugosa* lipase were efficient in catalysing the reaction. They showed a preference for the (*R*)-configuration. The (*R*)-enantiomer of 1-phenylethanol was converted into (*R*)-ester while the (*S*)-enantiomer remained unchanged.

From the results[65] of enzyme screening, the enzymes that showed the highest performance in the chiral resolution were *Candida antartica* lipase, *Pseudomonas sp*. lipase and *Porcine pancreas* lipase. The reaction has to be terminated at an appropriate time when maximum yield with satisfactory enantiomeric excess value of the desired compound is obtained.

The product could be isolated by distillation or by aqueous work-up and extraction. In the former case, the lipase could be used efficiently up to two cycles after addition of the fresh substrate, without appreciable change in chemical yield and enantioselectivity.

Experimental procedure:

In a typical experiment, the lipase (5% w/w of substrate) was added to racemic 1-phenylethanol (0.5 g, 4.10 mmol) and vinyl acetate (1.4 g, 16.27 mmol) in ionic liquid (~ 3-4 mL). The resulting suspension was stirred at 35 °C for time ranging from 15 h to 72 h depending on the ionic liquids and lipases used. The reaction mixture was then extracted with hexane (3 X 10 mL) and the combined extract was washed with brine and dried (Na_2SO_4). Removal of solvent gave a crude product, which was purified by silica gel column chromatography to give enantiorich (*S*)-alcohol and (*R*)-acetate.

Conclusion:

The results obtained clearly demonstrate the potential of ionic liquids for enzymatic biotransformations. The efficiency of ILs belonging to the phosphonium salts in lipase catalysed kinetic resolution of 1-phenylethanol has been demonstrated in this work. The racemic alcohol was resolved *via* enantioselective esterification using vinyl acetate as acyl donor. Several commercial immobilized lipases were screened for the resolution. The combination of the lipases with phosphorus based ionic liquids can be used for kinetic resolution of 1-phenylethanol and other alcohols for the development of environmentally friendly methodologies for industrial production of enantiomerically pure or enriched compounds.

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