SYNTHETIC STUDIES TOWARD CMI-977, SCYPHOSTATIN, (*R*)-(-)-PHENYLEPHRINE AND HERBICIDIN

SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
(IN CHEMISTRY)

TO
OSMANIA UNIVERSITY

BY
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DEDICATED

TO

MY PARENTS

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CERTIFICATE

The research work presented in thesis entitled " **Synthetic studies toward CMI-977, Scyphostatin, (*R*)-(-)-Phenylephrine and Herbicidin**" has been carried out under my supervision and is bonafide work of Mr. L. Murali Krishna. This work is original and has not been submitted for any other degree or diploma of this or any other University.

Pune-8 (**M. K. Gurjar**)

6th November, 2000 Research Guide

DECLARATION

The research work embodied in this thesis has been carried out at Indian Institute of Chemical Technology, Hyderabad and National Chemical Laboratory, Pune under the supervision of **Dr. M. K. Gurjar**, Deputy director and Head, Division of organic chemistry: Technology, National Chemical Laboratory, Pune-411008. This work is original and has not been submitted part or full, for any degree or diploma of this or any other University.

Pune-8 (L. Murali Krishna)
6th, November, 2000

ABBREVIATIONS

Ac	-	Acetyl
AcOH	-	Acetic acid
Ac_2O	-	Acetic anhydride
BnBr	-	Benzyl bromide
$(Boc)_2O$	-	Di-*tert*-butyldicarbonate
CuCN	-	Cuprous cyanide
DBU	-	1,8-Diazobicyclo[5.4.0]undec-7-ene
DCC	-	Dicyclohexyl carbodiimide
DCM	-	Dichloromethane
DEAD	-	Diethyl azodicarboxylate
DIPEA	-	Di-isopropylethylamine
DMAP	-	*N,N'*-Dimethylaminoformamid
DMSO	-	Dimethyl sulfoxide
EtOH	-	Ethanol
EtOAc	-	Ethyl acetate
HMDS	-	Hexamethyldisilazane
IBX	-	Iodoxybenzoic acid
Im	-	Imidazole
MeOH	-	Methanol
MeI	-	Methyl iodid
MEM-Cl	-	Methoxyethoxymethyl chloride
NaH	-	Sodium hydride
Py	-	Pyridine
$PhSO_2H$	-	Benzenesulpinic acid
TEA	-	Triethyl Amine
TBDMS-Cl	-	*tert*-Butyldimethyl Chlorosilane
TPP	-	Triphenyl Phosphine
pTSA	-	*para*-Toluenesulfonic acid
TrCl	-	Trityl Chloride
TsCl	-	*p*-Toluene Sulfonyl chloride
$ZnBr_2$	-	Zinc bromide

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}

- Proton magnetic resonance spectra were recorded on Varian FT-200 MHZ (Gemini), AC-200 MHz, MSL-300 MHz, Bruker-500 MHz and Varian Unity-400 MHz spectrometer using tetramethyl silane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.

- ^{13} C Nuclear magnetic spectra were recorded on Varian FT-50 MHz (Gemini), AC-50 MHz, MSL-75 MHz spectrometer.

- Mass spectra were recorded on a CEC-21-110B, Finnigan Mat 1210 or MICRO MASS 7070 spectrometer at 70 eV using a direct inlet system. FABMS were recorded on a VG autospec mass spectrometer at 70 eV using a direct inlet system.

- All reactions are monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV, I_2 and Molisch's reagent or Anisaldehyde reagent in ethanol as development reagents.

- All evaporation were carried out under reduced pressure on Buchi rotary evaporator below 50 °C.

- All solvents and reagents were purified and dried by according to procedures given in Vogel's Text Book of Practical Organic Chemistry.

- Silica gel (60-120) used for column chromatography was purchased from ACME Chemical Company, Bombay, India.

CONTENTS

ACKNOWLEDGEMENT

I thank the galaxy of friends, who came across my research career and left permanent impression through their good words and support.

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Last but not the least, I thank my parents and brother who shaped me to this status with their bluntless vision and selfless agenda.

L.Murali Krishna

ABSTRACT

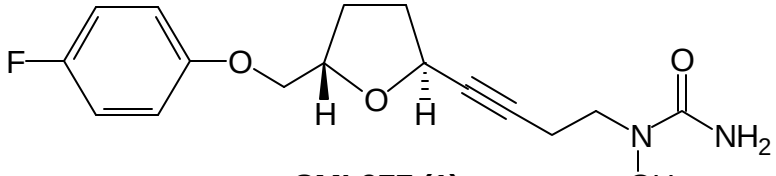
ABSTRACT

The thesis entitled "Synthetic studies toward CMI-977, Scyphostatin, (*R*)-(-)-Phenylephrine and Herbicidin" is divided into three chapters. The first chapter concerns the syntheses of some novel anti-asthmatic agents. The first chapter is further divided into three sections. The first section deals with the synthesis of a new potent anti-asthmatic agent (CMI-977). The second and third sections, comprises the synthesis of six and seven (*cis* and *trans* isomers) membered analogues of CMI-977 respectively. The synthetic efforts toward the advanced template of scyphostatin has been described in chapter 2. Finally the third chapter describes a practical synthesis of (R)-phenylephrine hydrochloride using hydrolytic kinetic resolution of a styrene oxide derivative (Section I) and furan precursor of herbicidin (Section II).

CHAPTER –1

Section-I: Synthesis of (2S,5S)-5-(4-fluorophenoxymethyl)-2-(1-*N*-hydroxyureidyl-3-butyn-4-yl)tetrahydrofuran (CMI-977). A new potent anti-asthmatic agent.

The role of leukotrienes in inflammatory and allergic responses including arthritis, asthma, psoriasis, and thrombotic disease has been well recognized. The alarming rise of asthma constitutes the biggest mystery in modern health care at the beginning of this century and the exact reasons still evade the researchers despite the advances in molecular biology and asthma chemotherapy. This urge to develop antagonists or inhibitors of leukotriene biosynthesis to prevent inflammatory responses. CMI-977 (**1**) has been reported with lipoxygenase inhibitory activity and is being currently developed by Millenium Pharmaceuticals, USA, as a promising candidate for chronic asthma.

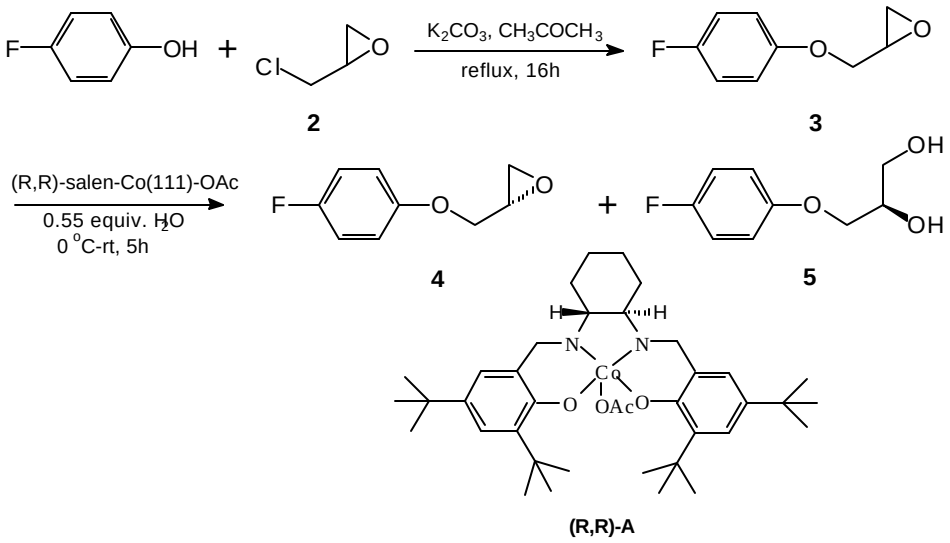


CMI-977 (1)

The compound CMI-977 (**1**) has a interesting diversely substituted tetrahydrofuran ring with trans juxtaposition of functionalities. The inadequacies of the flexible and practical routes for the synthesis of these type of compounds prompted us to evaluate alternate, novel methodologies involving the olefin metathesis of vinyl ethers and a stereoselective alkylation

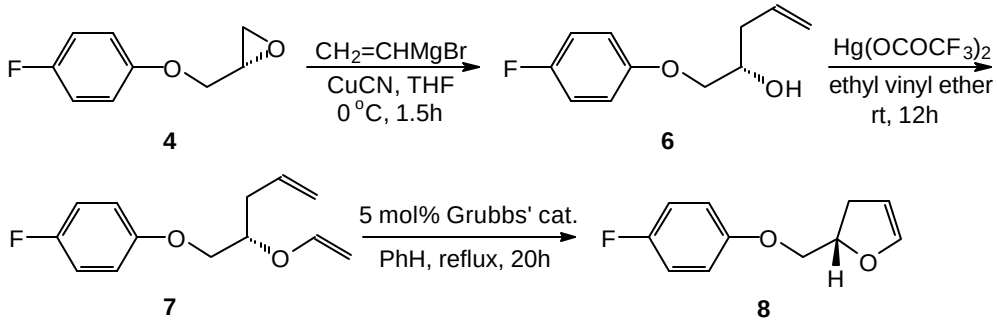
of 2-benzenesulphoyl derivative. Additionally, it was expected that the method would be amenable to higher analogues of CMI-977.

Scheme 1



Our synthetic endeavour began with application of Jacobsen's hydrolytic kinetic resolution of racemic 4-fluorophenyl glycidyl ether. O-alkylation of 4-fluorophenol with racemic epichlorohydrin (**2**) in anhydrous acetone in the presence of K_2CO_3 provided racemic 4-fluorophenyl glycidyl ether (**3**) as a colourless liquid in 98% yield, which was subjected to hydrolytic kinetic resolution with 0.5 mol % of (R, R)-(salen)Co(III)OAc catalyst (**A**) and 0.55 equivalent of distilled water afforded the requisite (S)-4-fluorophenyl glycidyl ether (**4**) in 45% yield with 92% ee along with the (R)-1-(4-fluorophenyl)glycerol (**5**) in 49% yield with 97 % ee (Scheme 1).

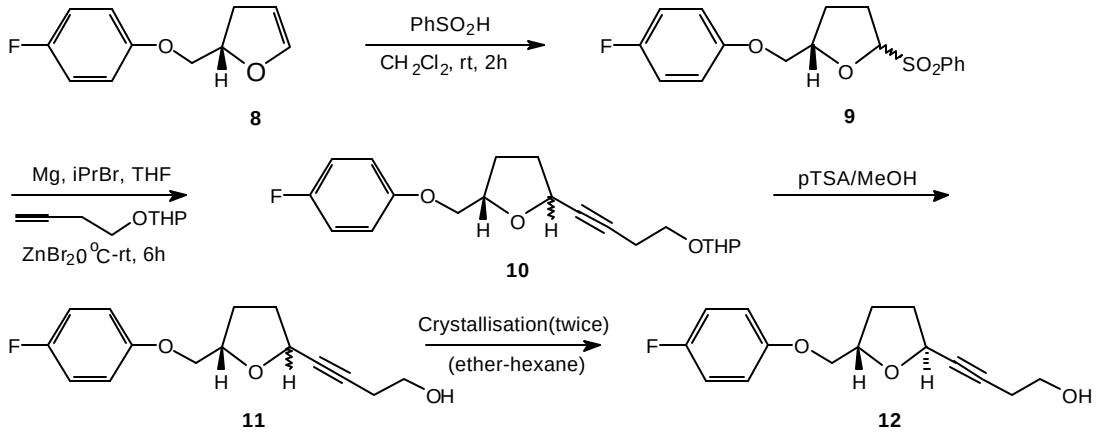
Scheme 2



Subseqent reaction of **4** with vinylmagnesium bromide in presence of CuCN in dry THF provided compound **6** in 78% yield. The conversion of free OH group into the vinyl

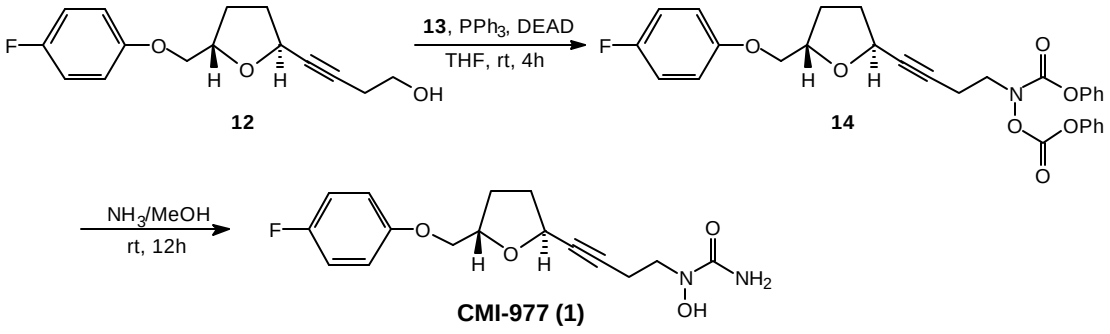
ether **7** was accomplished by treatment of **6** with ethyl vinyl ether and $Hg(OCOCF_3)_2$. The ring-closing metathesis of **7** in presence of 5 mol% of Grubbs' catalyst in refluxing benzene for 20 h gave the dihydrofuran derivative **8** in 52% yield (Scheme 2).

Scheme 3



The approach to incorporate 4-*N*-hydroxyureidyl-1-butynyl side chain of CMI-977 was based on Ley's alkylation of 2-benzenesulphonyltetrahydrofuran. For this endeavour, compound **8** was treated with benzenesulphinic acid in CH_2Cl_2 to give sulphone derivative **9** in 81% yield. Subsequent C-C bond formation at C-2 was carried out by treating compound **9** with dialkyl zinc reagent derived from BrMg-C≡C-CH_2CH_2OTHP and $ZnBr_2$ followed by deprotection with pTSA in methanol to give a 7:3 mixture of (2S, 5S) and (2R, 5S) diastereomers (Scheme 3). The required trans (2S,5S)-5-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydrofuran (**12**) was isolated in 50% yield with high enantiomeric purity after crystallization from ether-light petroleum.

Scheme 4

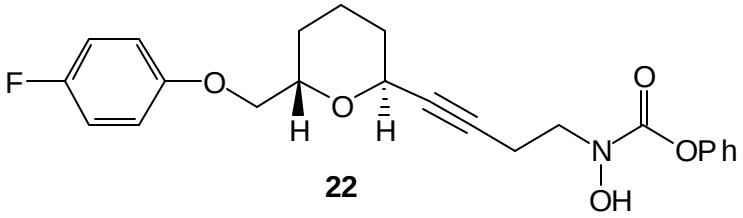


The introduction of *N*-hydroxyurea derivative was carried out by the Mitsunobu reaction of **12** with *N,O*-bis(phenoxycarbonyl)hydroxylamine (**13**) to give (2S, 5S)-5-(4-

fluorophenoxylmethyl)-2-(1-*N*,*O*-bis-(phenoxycarbonyl)hydroxylamino-3-butyn-4-yl)tetrahydrofuran (**14**) (Scheme 4). Treatment of compound **10** with methanolic ammonia simultaneously cleared the benzoate ester and converted urethane into urea thus affording the target molecule CMI-977 (**1**).

Section-II: Synthesis of (2S,6S)-6-(4-fluorophenoxymethyl)-2-(1-N-hydroxyureidyl-3-butyn-4-yl)tetrahydropyran (22).

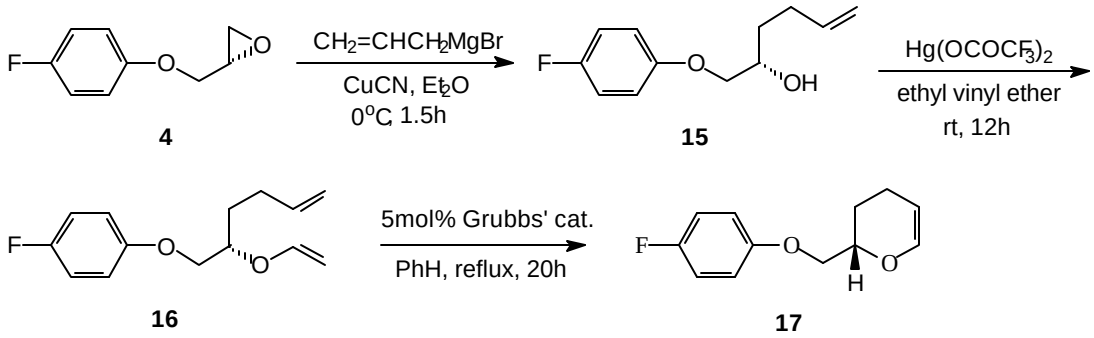
Inspired by an efficient approach to 2,5-disubstituted tetrahydrofuran as described in Section-I, we sought to explore the versatility of this approach in the preparation of so far unknown six membered 2,6-disubstituted tetrahydropyran analogue **22** of CMI-997 (**1**). Availability of **22** and its biological profile is useful to evaluate the influence of ring size on the biological activity of these anti-asthmatic compounds.



22

Our synthetic endeavour began from (S)-4-fluorophenyl glycidyl ether (**4**), which was converted into the diene derivative **16** by first reacting with allylmagnesiumbromide in the presence of CuCN to open the expoxide group followed by O-vinylation with ethyl vinyl ether/$Hg(OCOCF_3)_2$. The ring closing metathesis with Grubbs' catalyst (Scheme 5) gave the dihydropyran derivative **17**, which was subsequently converted into 2-benzenesulphonyl tetrahydropyran derivative **18**.

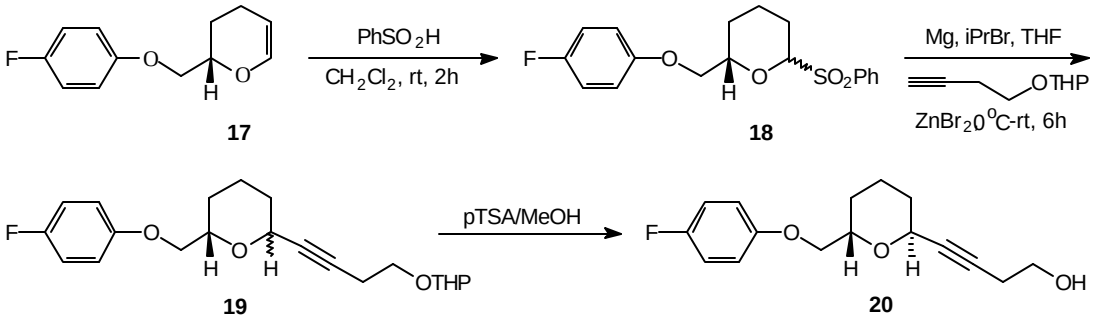
Scheme 5



The nucleophilic displacement of the sulfone **18** with 4-tetrahydropyranyloxy-1-butynylmagnesium bromide in the presence of anhydrous $ZnBr_2$ in dry THF provided the GC

coupled derivative followed by deprotection of THP group gave trans (2S,6S)-6-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydropyran (**20**) as a single diastereomeric product (Scheme 6). The formation of a single isomer during the C-C bond formation with tetrahydropyransulphonyl derivative **18** compared to the tetrahydrofuran precursor **9** (with 7:3 selectivity) was indeed gratifying and this observation is attributed to the anomeric effect forming the axial bond formation. The all trans stereochemical assignment was proved by the extensive NOE studies carried out on this intermediate.

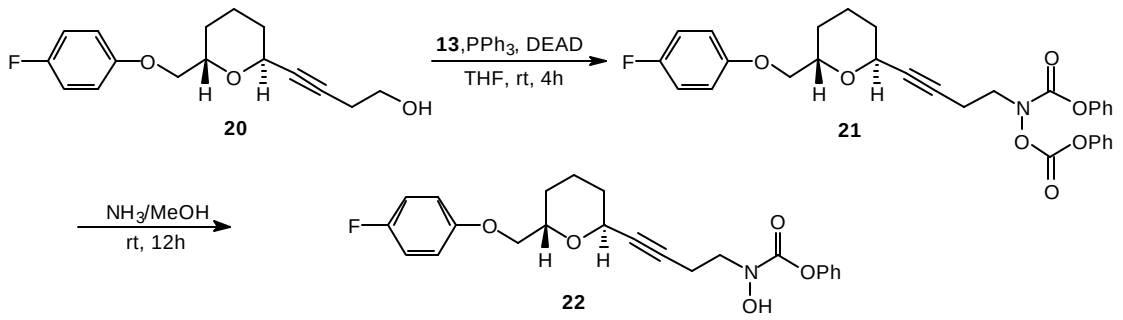
Scheme 6



17 **18** **19** **20**

The introduction of N-hydroxy urea was essentially carried out by the approach reported above for CMI-977 to give the target molecule (2S, 6S)-6-(4-fluorophenoxymethyl)-2-(1-N-hydroxyureidyl-3-butyn-4-yl)tetrahydropyran (**20**) as shown in (scheme 7).

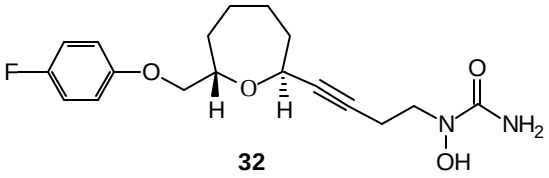
Scheme 7



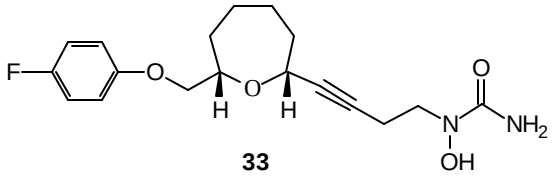
20 **21** **22**

Section-III: Synthesis of (2S,7S)-7-(4-fluorophenoxyl)-2-(1-N-hydroxyureidyl-3-butyn-4-yl)oxepane (32).

In continuation of our earlier enantioselective construction of CMI-977 and its six membered analogue, we were further interested to build a library of compounds that will extend to lead discovery and structure activity relationship. Hence, our next agenda in this series is to develop stereoselective synthesis of seven membered analogue of CMI-977 (**1**).



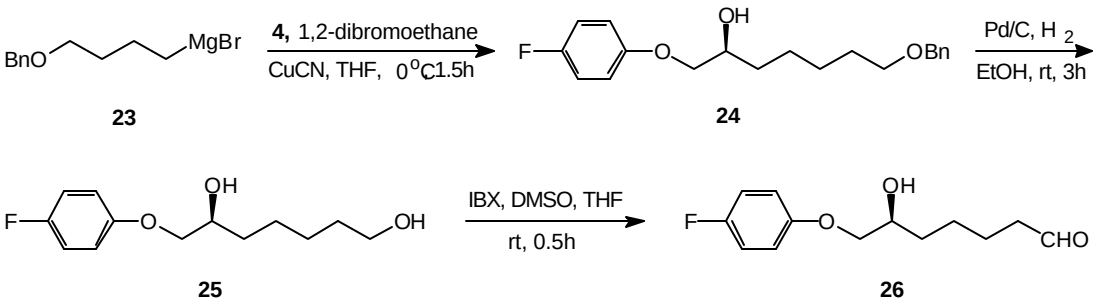
32



33

Our synthetic endeavour began from (S)-4-fluorophenyl glycidyl ether **4** which was converted into the diol derivative **25** by first reacting with CuCN-coordinated opening of **4** with (4-benzyloxy)butyl magnesiumbromide (**23**) followed by hydrogenolysis of benzyl group. Selective oxidation of primary hydroxy group is a challenging task and the methods to accomplish this is very rare. We observed that this could be easily performed with IBX in DMSO to provide (6*S*)-7-(4-fluorophenoxy)-6-hydroxyheptan-1-al (**26**) in 62 % yield (Scheme 8). The presence of aldehyde proton in its ^{1}H NMR spectrum revealed that **26** existed in open chain form.

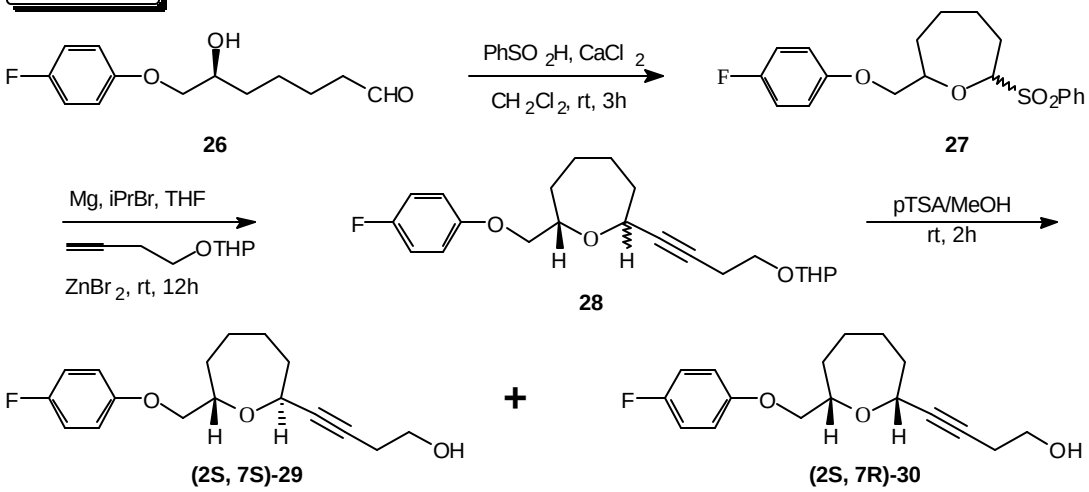
Scheme 8



23 **24** **25** **26**

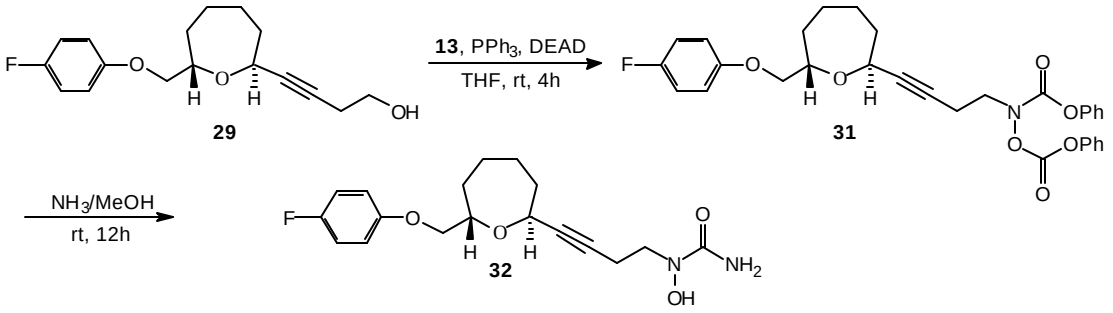
Our next concern is to obtain cyclic 2-benzenesulfonyloxepane derivative (**27**). We believed that during sulphonylation the open chain form of **26** will collapse to cyclic form **27**. The driving force for the reaction will be the stability of benzene sulfonyloxy function. Accordingly, when compound **26** was treated with benzenesulfinic acid in dry CH_2Cl_2 provided (2*RS*,7*S*)-2-(benzenesulfonyl)-7-(4-fluorophenoxymethyl)oxepane (**27**) in 81% yield (Scheme 9). Subsequent C-C bond formation at C-2 was carried out by treating compound (**27**) with dialkyl zinc reagent derived from BrMg-C≡C-CH_2CH_2OTHP and $ZnBr_2$ followed by deprotection of THP group with PTSA in methanol to give a 8:2 mixture of (2S, 7S) and (2R, 7S) diastereomers, which were separated by silica gel column chromatography. (Scheme 9). The major compound **29** was confirmed as *trans* by NOE studies in which irradiation of H-2 proton did not show the enhancement of H-7 proton and vice versa. Whereas in the case of minor product **30** the irradiation of H-2 proton shown the enhancement

Scheme 9



in H-7 and vice versa, indicating the presence of spacial interaction which confirms the *cis* isomer. The synthesis of the target molecule was then accomplished on similar lines as discussed for synthesis of CMI-977 and shown in (scheme 10).

Scheme 10

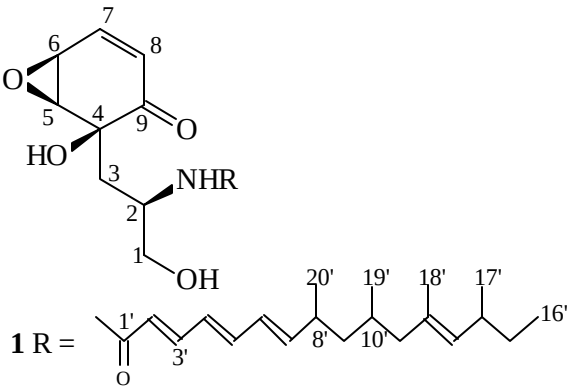


CHAPTER 2

Synthetic studies towards advanced template 2 of Scyphostatin (1).

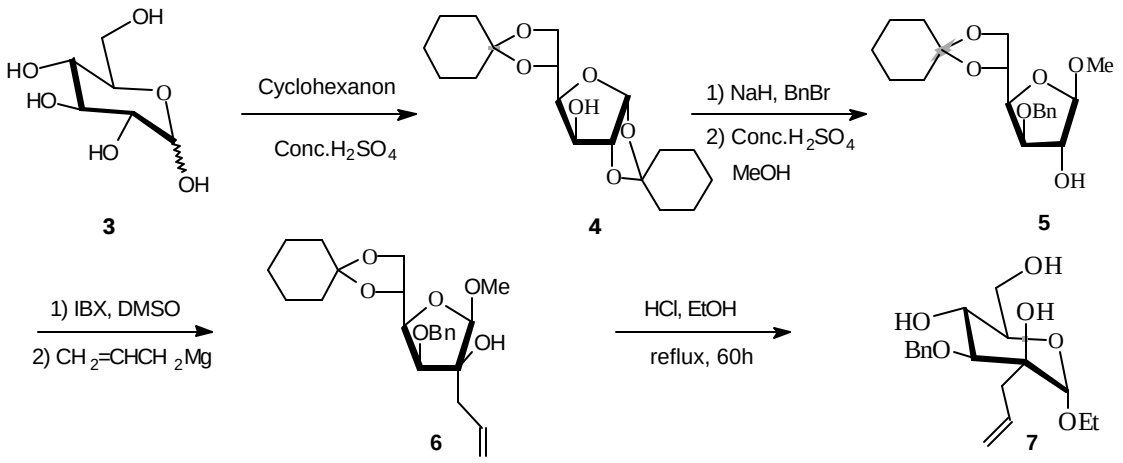
Scyphostatin (**1**) is a novel neutral sphingomyelinase inhibitor isolated during the search for N-smase inhibitors from the fermentation broth of *Dascysphus mollissimus* SANK –13892. N-Smase inhibitors are useful in the regulation of ceramide level and thus are of immense need in the therapy of autoimmune diseases and inflammation. This natural product is endower with unprecedented structural features specially, a cyclohexenone epoxide moiety in which the tertiary chiral center at C_4 is linked to an n-propyl amino alcohol under lipophilic side chain. The synthetic chemistry was undertaken in lieu of its potent biological activity and novel structure, which lead to realise the advanced congenor of this target. Under the thematic

strategy carbohydrate to carbocycle, we envisioned to arrive at the target with installation of the stereochemical features at the appropriate positions through the string of manipulations.



D-Glucose (**3**) was chosen as starting material for the current exploration, which was trapped in furanose form as 1,2;5,6-di-*O*-cyclohexylidene-α-D-glucofuranoside (**4**) by known procedure. The free hydroxyl group at C-3 of compound **4** was protected as benzyl ether derivative, which on treating with $MeOH/H_2SO_4$ at -50°C for 3 h was resulted in α, β (1:2) mixture of methyl-5,6-*O*-cyclohexylidene-α-D-glucofuranoside. Separation of anomers and

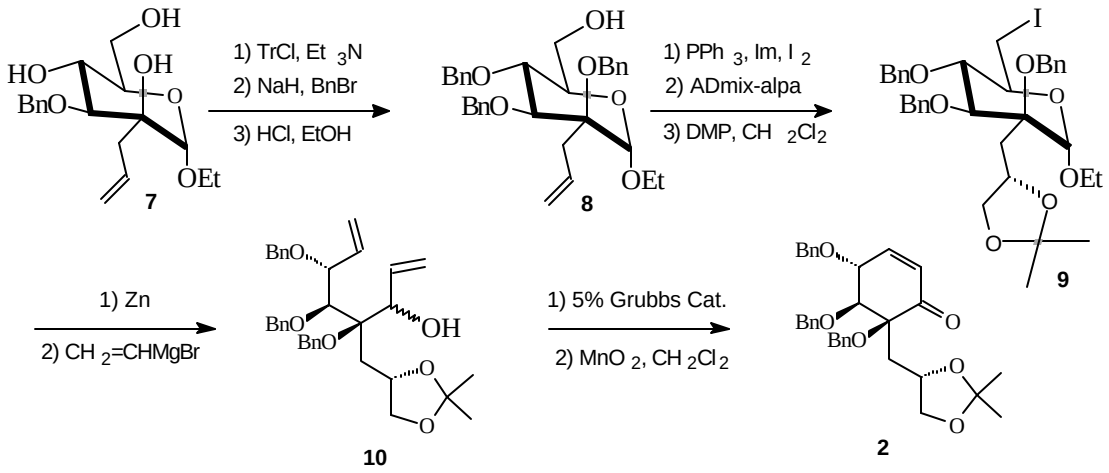
Scheme 1



subsequent oxidation of β-isomer **5** with iodoxy benzoic acid gave 2-ulose derivative. The diastereoselective addition of allyl Grignard reagent generated the product with required tertiary center **6** (Scheme 1), which was confirmed by extensive NOE studies. Compound **6** on kinetic equilibration with refluxing ethanolic HCl provided the pyranose derivative **7**. Selective primary tritylation, benzylation of 2,4-diol, followed by detritylation under acidic condition provided compound **8**. Conversions of alcohol to iodo group with Ph_3P-I_2-Imidazole

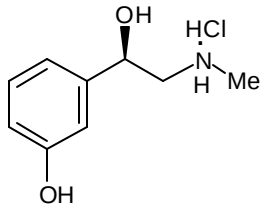
in reflux toluene, sharpless dihydroxylation with Admix-α followed by isopropylidene protection of resulted diol affording compound **9**. Exposure of compound **9** to activated Zn in iPrOH : H_2O (9:1) at 80 °C for 30 min provided the alkenal derivative, which on subjected to vinyl Grignard reaction, afforded the diene **10** (Scheme 2). which was submitted to ring closing metathesis with 5 mol % of Grubbs' catalyst followed by allylic oxidation with MnO_2 imparted the cyclohexenone **2**, which carries the thumb impression of the target molecule **1**. Further studies are underway to achieve the total synthesis of scyphostatin.

Scheme 2



CHAPTER–III

Section-I: Hydrolytic kinetic resolution of a styrene oxide derivative: A practical synthesis of optically pure (R)-(-)phenylephrine hydrochloride

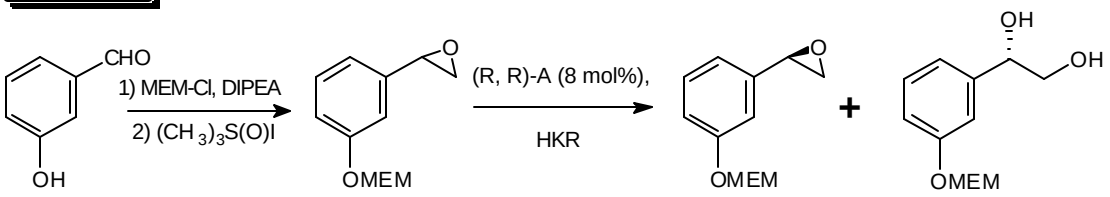


(R)-1

Recently, Jacobsen *et al* reported hydrolytic kinetic resolution of terminal epoxides using readily accessible chiral (R,R)-cobalt salen complex (A). This process is advantageous in many aspects, like no solvent requirement and low loading of a recyclable catalyst. It

affords highly valuable terminal epoxides and 1,2-diols in good yield and high enantiomeric enrichment.

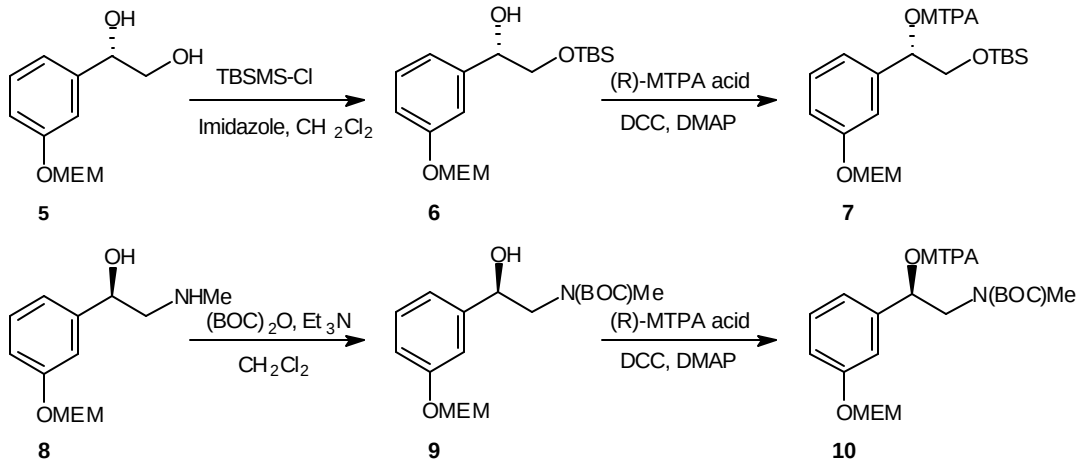
Scheme 1



2 **3** **4** **5**

The growing awareness of chirality in the context of biological activity has led to the discovery of many new asymmetric reactions in order to produce drugs and drug intermediates in enantiomerically pure forms. Catalytic asymmetric reactions have distinct advantages over stoichometric versions for economic and environmental reasons. Due to growing concern about chiral drugs being sold as racemates, many pharmaceutical industries are switching over to producing enantiomeric pure forms of the chiral drug. Since its discovery, phenylephrine hydrochloride, a potent adrenergic agent and β-receptor sympathomimetic drug has been marketed in the optical (*R*)-form. This chapter describes a practical synthesis of (*R*)-phenylephrine hydrochloride (**1**) using hydrolytic kinetic resolution of a styrene oxide derivative.

Scheme 2



5 **6** **7**

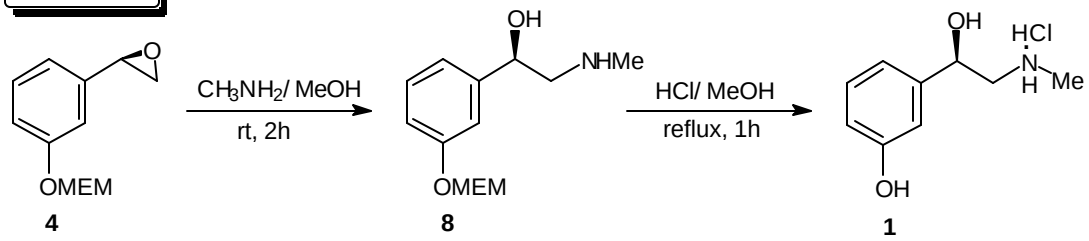
8 **9** **10**

Our synthetic endeavour initiated from m-hydroxybenzaldehyde (**2**), which was protected as the methoxyethoxymethyl ether derivative followed by treatment with trimethyl sulfoxonium iodide in the presence of NaH/DMSO at ambient temperature for 30 min. yielded the racemic epoxide **3** (Scheme 1). The epoxide **3** was resolved by hydrolytic kinetic

resolution (salen)CO(III)OAc (A) (0.8 mol%) and water (0.55 equiv) at room temperature for 60 h to afford (*R*)-styrene oxide (**4**) in 45% yield and (S)-diol **5** (yield 45%, 95% ee).

The 95% enantiomeric excess of the diol **5** was determined by ^{1}H and ^{19}F NMR spectral studies of the corresponding (*R*)-Mosher ester derivative **7** (scheme 2). The (*R*)-epoxide **4** was treated with methylamine in methanol to provide the N-methylamino alcohol derivative **8** (yield 90%, 97% ee). The enantiomeric excess of **8** was determined by converting into optically pure (*R,R*)-N-BOC-MTPA ester **10** in two steps (scheme 2). Similarly (±)–**7** was transformed into (*RS, R*)-N-BOC-MTPA ester **10**. Comparison of ^{1}H and ^{11}F-NMR spectra of (*R, R*)-**10** and (*RS, R*)-**10** conclusively confirmed 97% ee for the parent compound (*R*)-**8**. Removal of the methoxyethoxymethyl (MEM) group with concomitant hydrochloride formation occurred in one step when Compound **8** was heated under reflux in methanolic HCl for 1 h to give **1**. The ^{1}H-NMR spectrum in D_2O and the optical rotation data were comparable to the literature values.

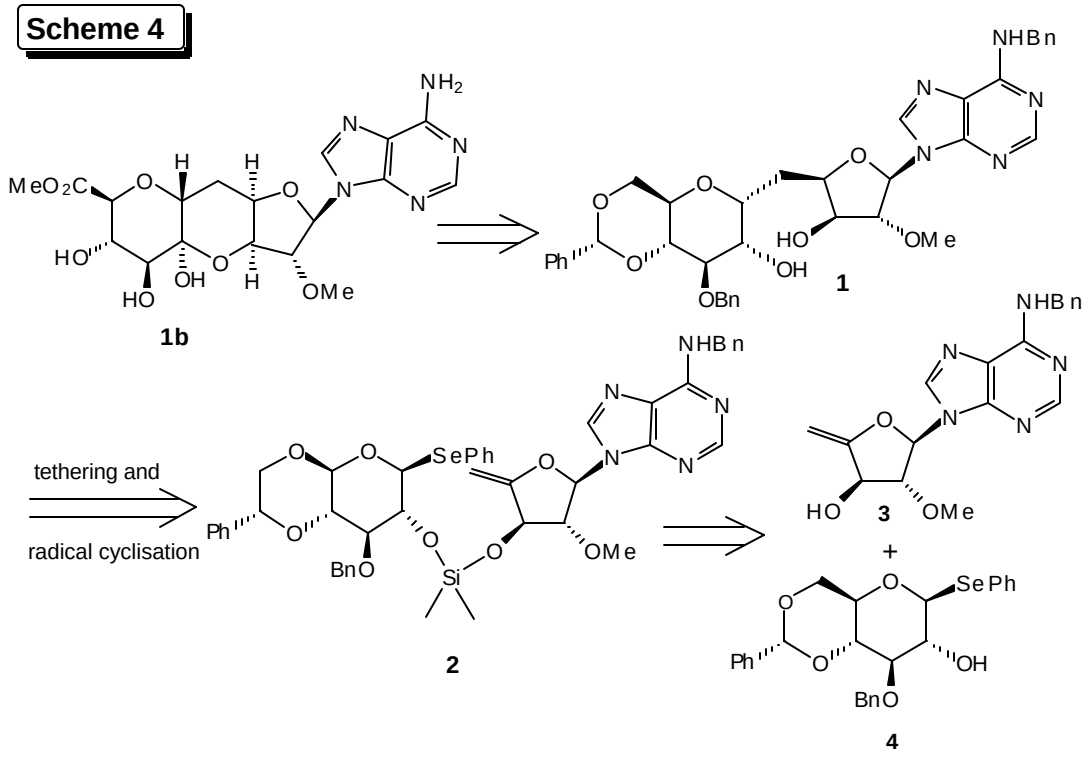
Scheme 3



Section-II: Synthesis of furan precursor 3 of Herbicidin.

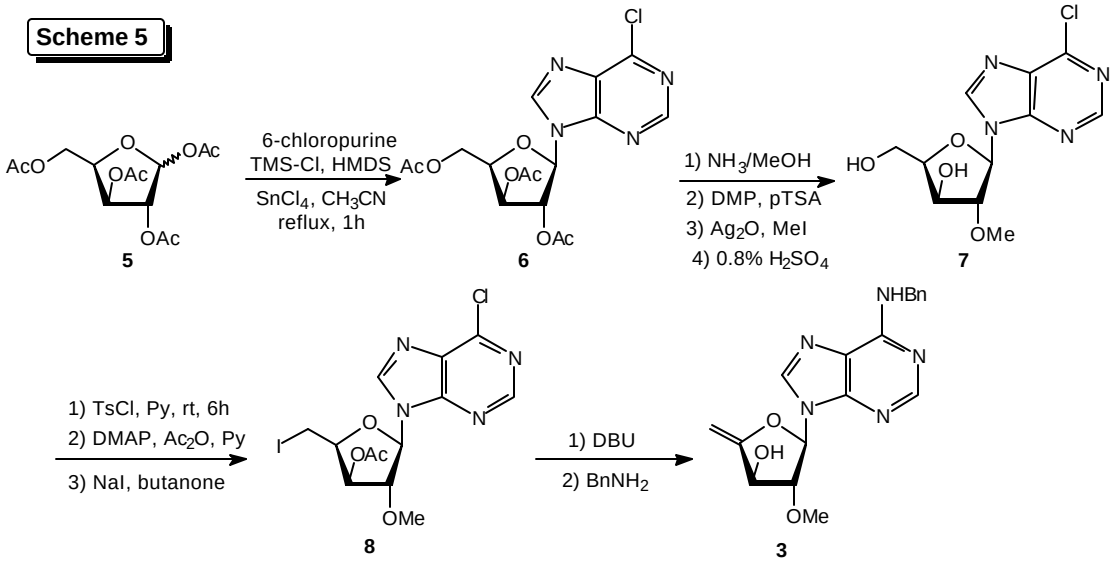
Herbicidins and aureoneucleomycines, a group of adenine nucleoside antibiotics, isolated from strains of Streptomyces Saganonesis are replete with potential herbicidal activity. They are environmentally friendly i. e, non-toxic to animals and inhibit the growth of Xanthomonas Oryzae, the causatory for rice leaf blight, and also acts selectively against dicotyledonous plants. These compounds explicit the novel structures with rich stereochemical functionality, the core skeleton being tricyclic furanpyranopyran structure of undecose. We herein report the synthesis of furan precursor **3** of herbicidin as part of the ongoing Indo-French collaborative program for pesticide control in our laboratories. It is candid that the THF precursor is one of the synthons derived out of antithetic analysis involving the disconnection of glycosidic bond in Herbicidin **B** considering the equilibration of **1** with **2**.

Scheme 4



Accordingly, tetra-*O*-acetyl-D-xylofuranose **5** was chosen as our starting material, which was subjected to diastereoselective nucleophilic substitution of 6-chloropurine at the anomeric position via Lewis acid promoted formation of acetoxonium ion to give 6-Chloro–9-(2', 3', 5'-tri-*O*-acetyl-β-D-xylofuranosyl)purine (**6**). Hydrolysis of acetate provided the triol with concomitant replacement of chloro group of purine by methoxy group under

Scheme 5



Zemplen's conditions and also K_2CO_3/ MeOH. Success was met only when exposed to methanolic ammonia at 0^0C where the required product carried the 'chloro' group intact in purine. Protection of 3', 5'-dihydroxy as its isopropylidene derivative with DMP and catalytic *p*TSA, methylation of 2'-hydroxyl group with MeI and Ag_2O followed by deblocking of acetonide provided compound **7** (Scheme 5). Selective conversion of primary hydroxy group of **7** into the corresponding bromide was achieved using TPP and CBr_4 in neat pyridine, at 50 ^{o}C albeit in moderate yield (40%). Acetylation of 3'-hydroxy group under routine conditions gave the acetate. The poor yield in direct bromination prompted us to deploy the two step procedure, i.e., selective primary tosylation with tosyl chloride in pyridine, protection of 3'-hydroxy group as its acetate, followed by refluxing with sodium iodide in 2-butanone to give the iodo product **8**. Compound **8** was then subjected to elimination of iodide using DBU at 80 ^{o}C in DMF and subsequent nucleophilic substitution of chloro group of purine, along with concurrent hydrolysis of acetate, culminated in the formal synthesis of furan precursor **3** of Herbicidine.

CHAPTER-1

SYNTHETIC STUDIES TOWARD CMI-977 AND ITS ANALOGUES

SECTION–I

STEREOSELECTIVE SYNTHESIS OF (2*S*,5*S*)-5-(4-FLUORO PHENOXYMETHYL)-2-(1-*N*-HYDROXYUREIDYL-3-BUTYN-4-YL)TETRAHYDROFURAN (CMI-977)

INTRODUCTION

Asthma is an obstructive lung disorder, it occurs when the bronchial tubes swell up and go into a spasm, blocking the passage of air in and out of lungs, which is characterized by wheezing, breathlessness, chest tightness and cough. Asthma can develop at any age, but occurs most-commonly in children. Although no country is immune, it occurs predominantly in industrialised western countries. The severity of asthma often worsens in spring and early summer. Although there is no cure for asthma, it is a disease that can be managed, enabling most people to lead active and productive life. This preliminary discussion will brief the current status of the biological and medicinal aspects of asthma (asthma chemotherapy) that would have eventually formed the basic tenet of our interest to develop the synthesis of anti-asthmatic compounds.

According to the estimate of World Health Organization, asthma affects 150 million people worldwide, and the number of patients has doubled over the decade.[1] Asthma has now emerged as one of the major health threats in this century. The rapid rise of asthma constitutes the biggest mystery in modern medicine and the exact reasons for the increase still evade the researchers.[2] Although several factors were put forward, like diesel fuel exhaust, allergies, diet, smoking, viral infections, cold air, and physical exercise, it is now concluded that a combination of genetic and environmental factors is responsible for the onset of asthma.[3] While some people are genetically predisposed while others suffer from the early-life allergen exposure, especially air-pollution, damp housing, poor ventilation, dusty carpets, furry pets, cockroaches, and indoor chemicals. A combination of cold air and physical exercise leads to asthma in athletes, esp., cross-country skiers, swimmers, and track-and field runners since they pump, thousands of cubic meters of cold air, during the race. On average, 10% of family budget goes meeting the treatment of asthma. Even passive or second hand smoking by parents especially mothers increases the risk of asthma in children. Infants born of mothers

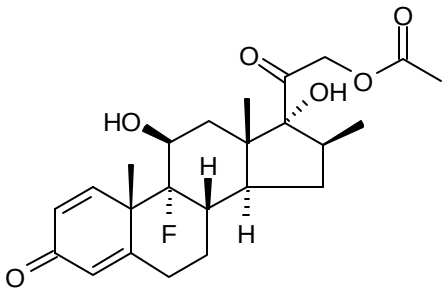
who smoke have higher risk of developing asthma. The good news is that 95% of asthma is controllable, given proper and continuous medication.

The advances in molecular biology indicate that allergy and asthma are not inherited as single-gene disorders and do not show a simple pattern of inheritance. Environmental and genetic factors interact in a complex fashion to produce disease susceptibility and expression. A genetic predisposition to asthma and atopy is influenced by several factors. An increased risk of atopy exists when the mother herself has a history of allergy. Environmental factor such as infection in early life (by tuberculosis, hepatitis A, measles, and other unidentified pathogens for which these conditions are markers of) might reduce the risk of developing allergy. Other factors might increase this risk such as exposure to certain allergens, respiratory viral and helminth infection and can cause increased immunoglobulin E (IgE) serum levels. Once asthma is established, attacks can be precipitated or exacerbated by cigarette smoke, aeroallergens, respiratory viral infections, and air pollution.

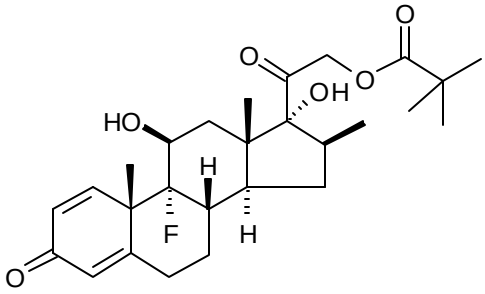
Glucocorticosteroides, â$_2$-adrenoreceptor agonists and theophylline:

Inhaled â$_2$-adrenoreceptor agonists are the most effective bronchodilators, currently prescribed for symptomatic relief in asthma.[4] The mechanism of action, i.e., causing smooth muscle relaxation involves camp-dependent and independent pathways. Inhaled glucocorticosteroides, e.g., betamethasone acetate (**1**), dexamethasone pivalate (**2**), fluticasone dipropionate (**3**), and cortisone (**4**) are mainstay therapy for reducing airway inflammation in asthma. The effects of steroids are mediated largely via changes in gene transcription: steroid binds to a cytosotic glucocorticoid receptor (GR) and the resulting dimer translocates to the nucleus where it interacts with a glucocorticoid response element (GRE) to increase or decrease gene transcription (*trans*-activation and *trans*-repression respectively). Activated GR can also interact directly with cytoplasmic transcription factors such as activator protein 1

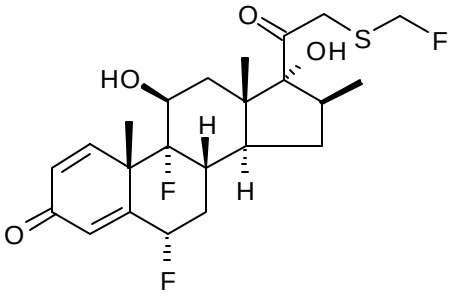
(AP-1), and nuclear factor êB (NF-êB), which alter gene transcription in response to inflammatory stimuli.



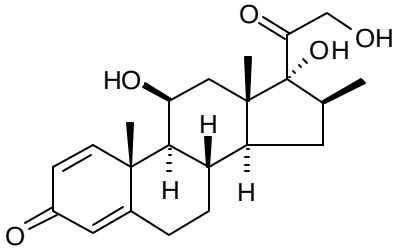
Betamethasone acetate (**1**)



Dexamethasone.pivalate (**2**)

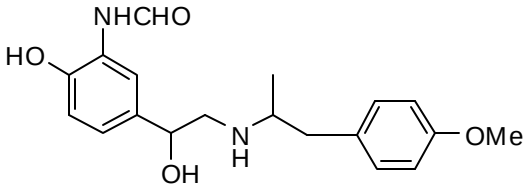


Fluticasone.dipropionate (**3**)

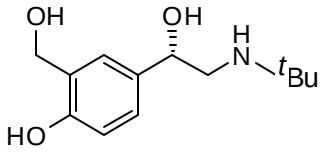


Cortisone (**4**)

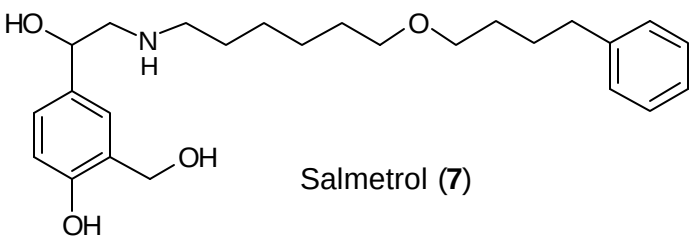
Salbutamol (**5**) is a potent â$_2$-adrenergeric receptor antagonist. â$_2$-adrenergeric receptors are found on the smooth muscle lining airways of the lungs. The binding of salbutamol to â$_2$-adrenoceptor causes the conformational change in that G-protein. A GDP (Guanosine 5'-diphosphate) group associated with the G-protein becomes dissociated and is then replaced with a GTP group. This is in turn causes alpha sub unit to dissociate from the G-complex. The dissociated alpha sub unit is then free to move in the membrane and has a binding site for the enzyme adenylyl cyclase. It binds to this enzyme, which catalyses the conversion of ATP (adenosine 5'-triphophate) to cAMP. The latter activates protein kinase A that transfers the terminal phosphate group of an ATP to several target proteins which leads to muscle relaxation in the airways of lung. Long action of â$_2$-adrenoreceptor agonists can be achieved by exosite binding (e.g., salmetrol)[5] (**7**) and by alterations in pharmacokinetics



Formoterol (**6**)



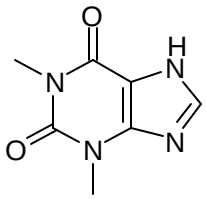
(R)-Salbutamol (**5**)



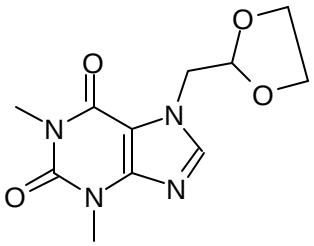
Salmetrol (**7**)

(e.g., formoterol) (**6**).[6] Although highly lipophilic, the extended duration of action of salmeterol appears due to anchoring in the vicinity of the â$_2$-adrenoreceptor via a second binding interaction (the exosite) near the cytoplasmic face of the fourth trans membrane domain. In addition, new steroids are being developed with the aim of maximizing topical anti-inflammatory effects and minimizing adverse systematic effects, as exemplified by RU-24858. Clinical studies have recently demonstrated the benefit of combining long-acting â$_2$-adrenoreceptor agonists with inhaled steroids, e.g., seretide (salmetrol and fluticasone) combined in a single formulation.

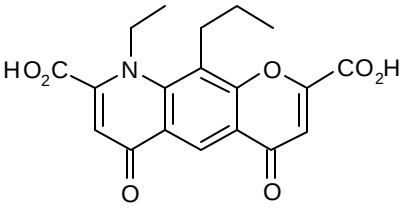
Theophylline (**8**) has a long historic background through its ability to bronchodilate asthmatic subjects. Although limited by side-effects profile, theophylline is effective in reducing the symptoms and improving lung function in patients with mild chronic asthma. Theophylline is believed to inhibit the enzyme PDE-4 of specifically cyclic nucleotide phosphodiesterase, an enzyme that catalyses the hydrolysis of intracellular second messengers cAMP and cGMP. Nedocromil (**10**) and cromolyn are the drugs work in the inflammatory cells to prevent the release of histamine and other chemicals involved in airway inflammation. They also help in the treatment of exercise-induced asthma.



Theophylline (**8**)



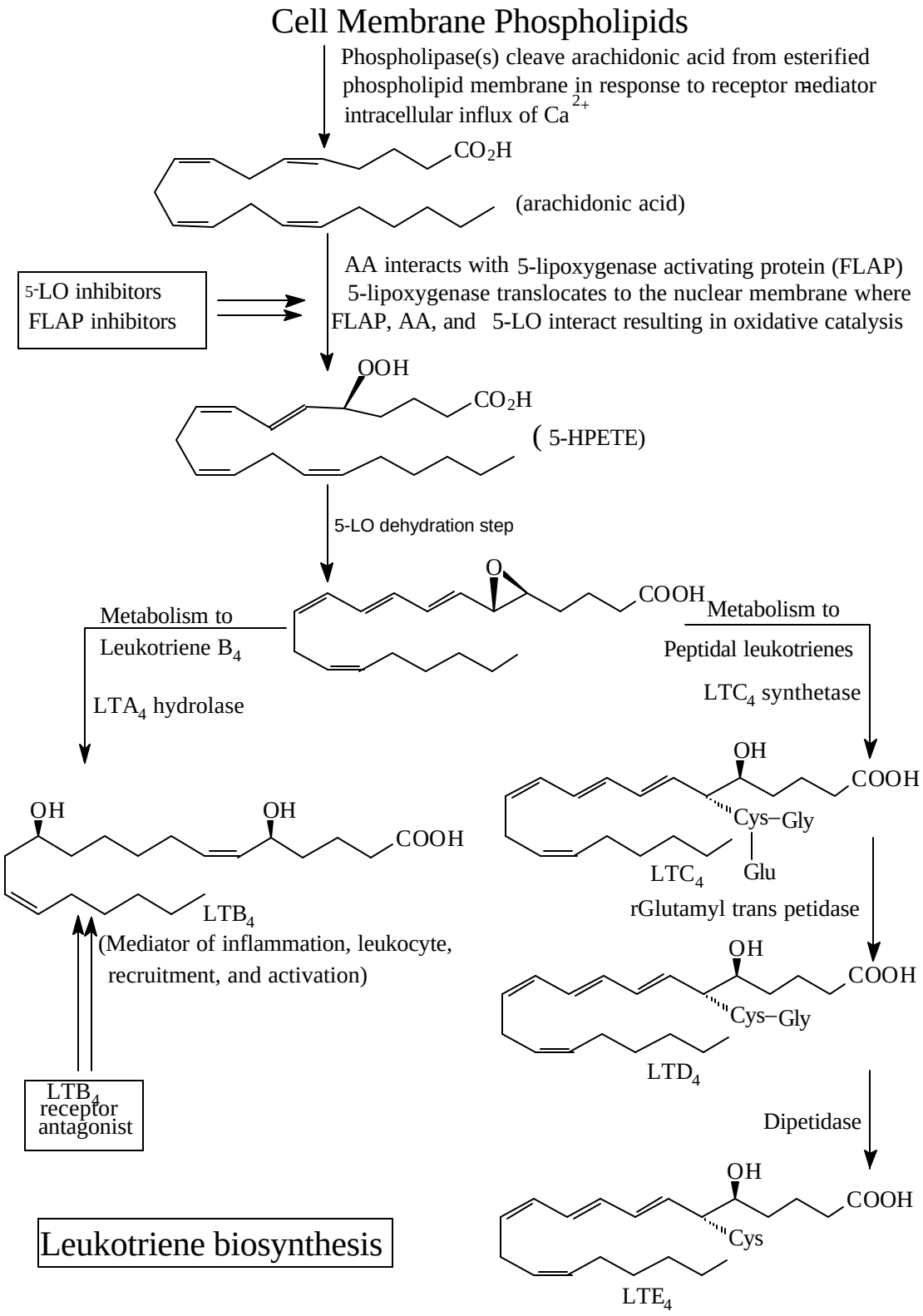
Doxophylline (**9**)



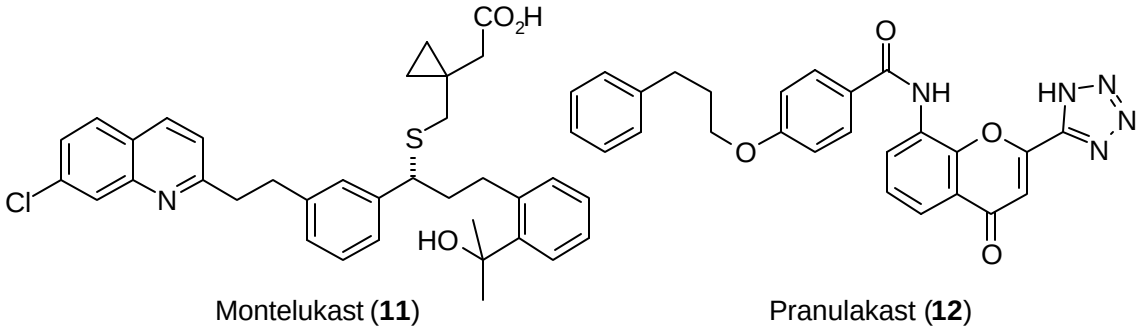
Nedocromil (**10**)

Inflammatory mediator receptor antagonists:

Small molecule receptor antagonists for a number of inflammatory mediators have been developed. Trailing for so long in the wake of other putative mediators, the cysteinyl leukotrienes have assumed a central role in asthma and in drug development with *Cys*T_1 receptor antagonists such as zafirlukast (**19**) and pranlukast (**12**), being the first new treatment for asthma in 25 years.[7] The cysteinyl LTs C_4, D_4, and E_4 produced by resident mast cells and by infiltrating eosinophils and basophils are implicated in bronchial constriction and submucosal odema of airways in asthmatics. The biosynthesis of LTs is initiated by activation signals of Ca^{2+} influx which then activates and translocates cytosolic phopholipase A_2 to the nuclear membrane, where it catalyses the release of arachidonic acid from phospholipids.[8] Arachidonic acid is subsequently presented by an 18 kDA integral perinuclear protein and 5-lipoxygenase (5-LO)-activating protein (FLAP) to 5-LO which is also translocated to the nuclear membrane. 5-LO catalysed the sequential formation of 5-HPETE and LTA_4. LTA_4 is then conjugated with reduced glutathione by LTC_4 synthase, the only enzyme committed to the biosynthesis of LTC_4. Human LTC_4 synthase has been cloned and mapped on human chromosome 5 (5q35) distal to genes relevant to allergic diseases such as IL-4 and IL-5. Cell activation by cytokines modulates the dynamics of arachidonic acid pools causing the redistribution of these fatty acids


Cell Membrane Phospholipids
Phospholipase(s) cleave arachidonic acid from esterified phospholipid membrane in response to receptor mediator intracellular influx of Ca2+
CO2H
(arachidonic acid)
5-LO inhibitors
FLAP inhibitors
AA interacts with 5-lipoxygenase activating protein (FLAP)
5-lipoxygenase translocates to the nuclear membrane where FLAP, AA, and 5-LO interact resulting in oxidative catalysis
OOH
CO2H
(5-HPETE)
5-LO dehydration step
O
COOH
Metabolism to
Leukotriene B4
LTA4 hydrolase
Metabolism to
Peptidal leukotrienes
LTC4 synthetase
OH
OH
COOH
LTB4
(Mediator of inflammation, leukocyte, recruitment, and activation)
OH
COOH
Cys–Gly
LTC4
Glu
rGlutamyl trans petidase
OH
COOH
Cys–Gly
LTD4
Dipetidase
OH
COOH
Cys
LTE4
LTB4 receptor antagonist
Leukotriene biosynthesis

Which in turn, is largely responsible for the amount and type of eicosanides immunologically produced by inflammatory cells. The removal of glutamate from LTC_4 by the enzyme γ-glutamyltranspeptidase (GTP) gives the corresponding cysteinylglycinyl-5-hydroxy (7E, 9E, 11Z, 14Z)-eicosatetraenoic acid, leukotriene D_4 (LTD_4) which on further peptido hydrolysis by the enzyme dipetidase gives leukotriene E_4 (LTE_4).



Montelukast (**11**) Pranulakast (**12**)

TYPES OF LIPOXYGENASE INHIBITORS:

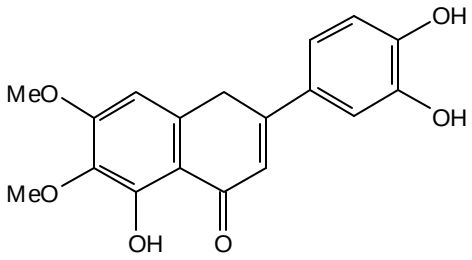
Both natural and synthetic compounds have been reported as lipoxygenase inhibitors. The former category encompasses compounds isolated from both animate and inanimate sources while the latter class is comprised of lipoxygenase substrate and product analogues synthetically modified natural products and novel structures obtained by total synthesis.

1. Natural product inhibitors:

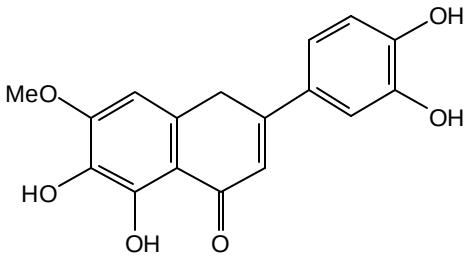
This is the large and the most thoroughly studied class of lipoxygenase inhibitors because of the wide distribution of these compounds in nature and thus the availability of many structural analogues.

i) Flavonoids:

S. Yamamoto and coworkers[9] have studied the effect of various flavanoids on 5-lipoxygenase from rat basophilic leukemia cells (RBL) and guinea pig PMNs. Cirsitiol (**13**) and pedalitin (**14**) were found to be the most potent.



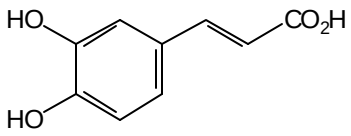
Crisitiol (**13**)



Pedalitin (**14**)

ii) Caffeic acid:

Caffeic acid (**15**) is the most potent of the naturally occurring compounds with an IC_{50} in the micromolar range.[10] It is interesting to note that conversion of one of the phenols of caffeic acid to a methyl ether to yield ferulic acid, substantially reduces the ability to inhibit lipoxygenase. This indicates the possible role of catechol for lipoxygenase inhibition.



Caffeic acid (**15**)

2. Synthetic inhibitors:

Three principle types of lipoxygenease inhibitors will be discussed in this section. These are: (1) Synthetic analogues of the natural substrate, arachidonic acid, or the products of the enzyme, (2) Synthetically modified natural products and (3) Novel synthetic compounds.

Much of the efforts with synthetic inhibitors have been directed towards the 5-lipoxygenase pathway with a lesser amount of work aimed at 12-lipoxygenase pathway. This is principally due to the biological prominence of the products of the 5-lipoxygenase pathway. Synthetic inhibitors span wide range of potential uses from possible therapeutic agents, from pharmacological tools, to mechanistic probes of lipoxygenase enzyme. Synthetically modified natural products and novel synthetic compounds are invariable attempts to find a therapeutic

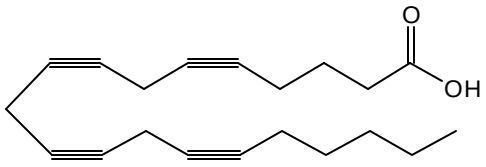
agent, while substrate and product analogues are mainly pharmacological tools and mechanistic probes. Naturally, compounds which fail as therapeutic agents often find use as pharmacological tools.

Substrate and product analogues:

This class of inhibitors comprise two types (i) those based on arachidonic acid and (ii) those based on lipoxygenase products which are to inhibit the lipoxygenase enzyme.

Arachidonic acid related inhibitors:

One of the first compounds reported to inhibit the synthesis of leukotrienes was 5,8,11,14 eicosatetraynoic acid (**16**) (ETYA). In this compound, double bonds of arachidonic acid were replaced by triple bonds. Also other fatty acids such as 5,6-dihydro arachidonic acid, (5,6-DHA) are time dependent irreversible inhibitors that are believed to act as suicide substrates for 5-LO. Number of other analogues of arachidonic acid have been reported as inhibitor of 5-LO. e.g. compounds such as **17** and **18** which lack a 7-pro (5)-hydrogen are 5-LO inhibitors. These inhibitors inactive the enzyme through covalent coupling. They mimic the shape of the arachidonic acid and instead of arachidonic acid, enzymes react with them and thus the natural biosynthesis is inhibited.



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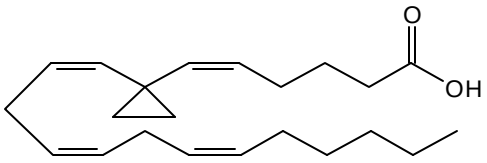
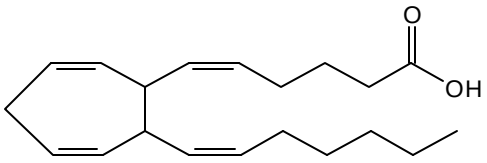
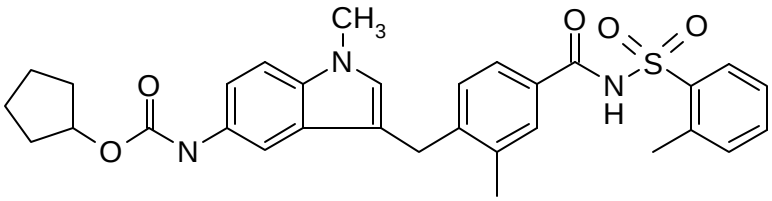


17



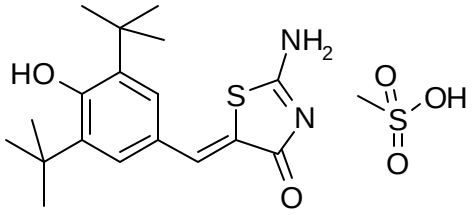
18

Zafirlukast (**19**) is a potent leukotriene receptor antagonist, which improves the symptom, and pulmonary function, reduces the use of rescue bronchodilation medication and reduces the likelihood of asthma exacerbation.



Zafirlukast (**19**)

CI-1004 (**20**) (PD-136095) is a dual inhibitor of lipoxygenase and cyclooxygenase-2 (COX-2) inhibitor that is currently under development by Parke-Davis as a potential treatment for asthma.[11] It is recognized that an inducible form of cycloxygenase (COX-2) is upregulated in inflammatory processes, significant efforts are ongoing to identify highly selective COX-2 inhibitors with the aim to separate the beneficial actions from the side effects of non-steroidal anti-inflammatory drugs (NSAIDs). On the other hand, leukotrienes, produced through the 5-lipoxygenase (5-LO) enzyme pathway also contribute to NSAID-induced side effects. When evaluated against the formation of $PGF_{2\alpha}$ (a product of COX formation) and LTB_4 (a product of 5-LO), it was found to inhibit potentially both 5-LO (IC_{50} = 0.77 μM) and COX (IC_{50} = 0.39 μM)

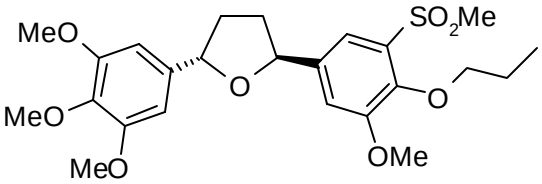


CI-1004 (**20**)

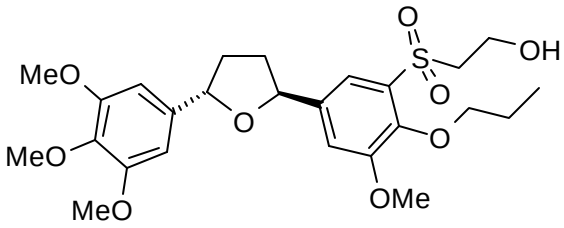
PAF antagonists:

Platelet activating factor is a phospholipid, exhibiting potent pro-inflammatory effects. It is produced by a number of cells, including eosinophils, basophils, neutrophils,

macrophages, and endothelial cells. 2,5-disubstitutedtetrahydrofurans have been investigated for their role as PAF antagonists. In general, *trans*-isomers have been found to be more potent than cis isomers. Further structure activity studies indicated that more potent PAF antagonists contained an electron-withdrawing group on one but not both aromatic rings. These features are incorporated in L-659, 989 (**21**) in which a metabolically stable methylsulphone serves as the electron withdrawing functional unit and a trimethoxy aryl ring is appended at C_5. In order to achieve increased metabolic activity and pharmacokinetic profile, polar head group modifications were investigated from which the (2S, 5S)-*trans*-isomer of MK-287 (**22**) emerged as a potent, specific and orally active PAF receptor antagonist and chosen for clinical trail.[12]



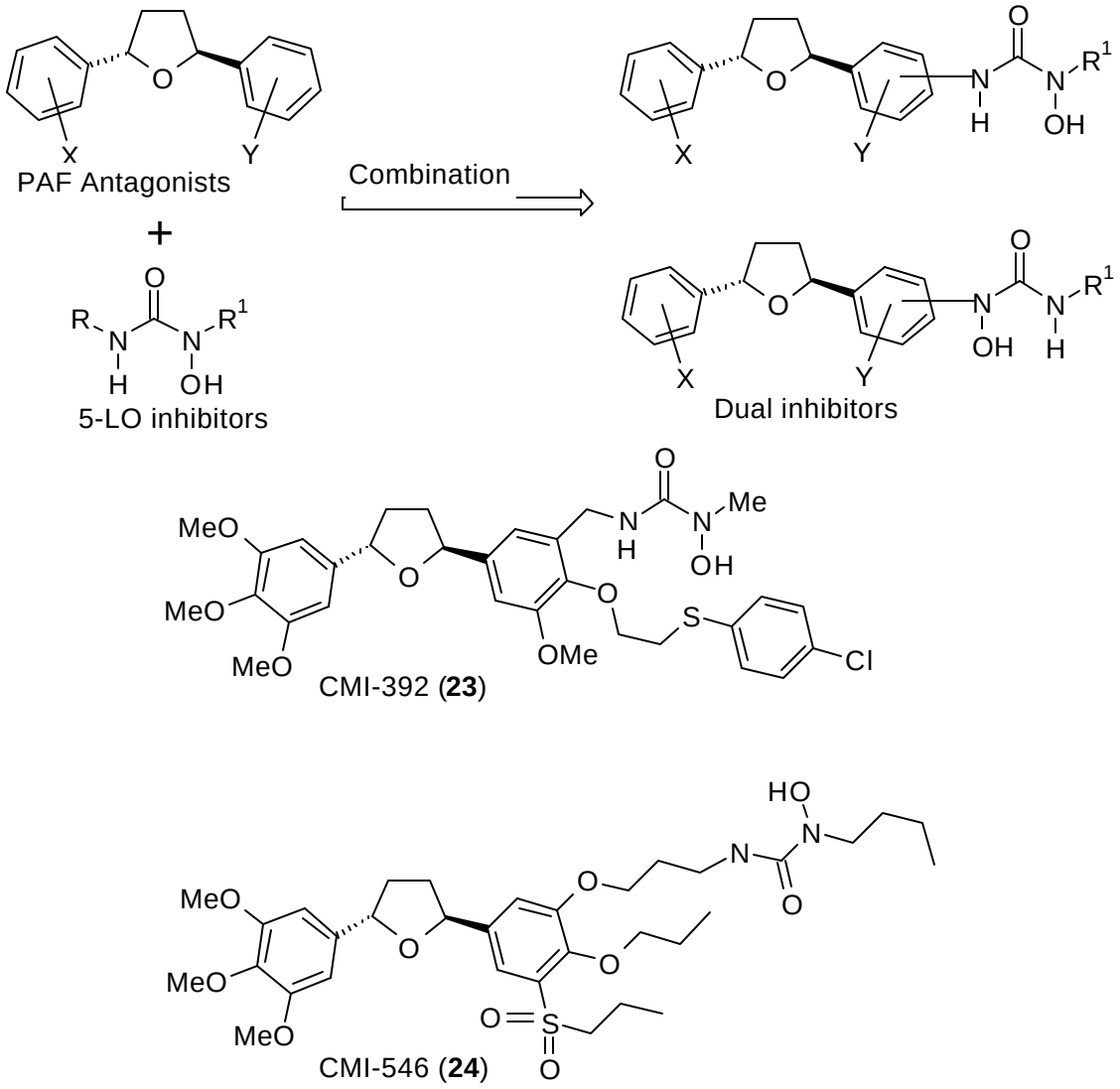
L-659,989 (**21**)



MK-287 (**22**)

Since both PAF and leukotriene are released simultaneously from leukocytes and upon cellular activation, act synergistically in many biological models, a single compound which inhibits the actions of both PAF and leukotrienes may offer certain therapeutic advantages in terms of efficacy and pharmcokinetics over reagents which inhibits either mediator alone. The basic knowledge that 2,5-diaryltetrahydrofuran class of compounds are PAF inhibitors while hydroxy ureas are potent 5-LO inhibitors, the introduction of hydroxy urea functionality onto certain scaffolds carrying THF skeleton should provide the candidates with dual inhibition.

The inhibiting activity of hydroxy urea derivative is probably due to chelation of Fe^{3+} required for oxidative catalysis in leukotriene biosynthesis. Recently, Cytomed Inc. has reported CMI-392 (**23**) and CMI-546 (**24**) as a potent dual 5-LO and PAF inhibitor, which

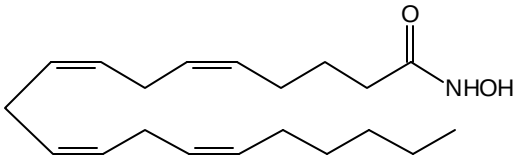


is currently being evaluated in human clinical trials as a novel-inflammatory agent. CMI-392 (**23**) showed very potent and balanced activities against both 5-LO and PAF and more potency than zileuton in 5-LO inhibition and equally potent as MK-287 as PAF antagonist.[13]

Hydroxamic acid and N-hydroxyurea type inhibitors:

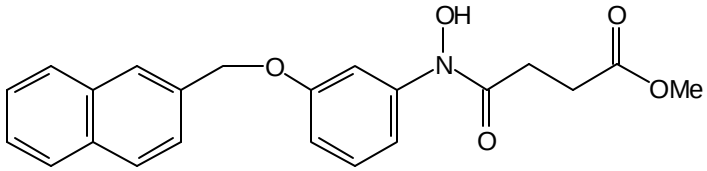
5-Lipoxygenase enzymes contains an iron atom, therefore several substrate analogues have been prepared that contain an iron-chelating moiety. Hydroxamic acids are excellent ligands for ferric ion with high association constant. Corey was the first to report that this property could be used to prepare compounds, which might bind tightly to iron and produce

competitive LO inhibitor. He reported that hydroxamic acid derivative of arachidonic acid, **25** was a potent inhibitor of rat basophilic leukocytes (RBL-1), 5-LO.[14]



25

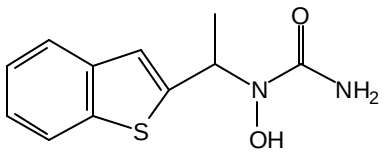
It was suggested that a hydroxamic acid function would more closely approximate the C-5 carbinol of the 5-HETE and LTD_4 and it would also have potential to chelate iron in the active site of 5-lipoxygenase/cyclooxygenase.[15] Therefore Wy-45911, 17 was prepared and it inhibited 5-lipoxygenase enzyme (IC_{50} = 1.4 μM, rat PMN) and antagonised LTD_4 receptor.[16] Wy-45911 was the first hydroxamic acid possessing 5-lipoxygenase inhibitory activity which was investigated *in vivo*. Compound **26** showed variable activity, it not only inhibited action of 5-LO but also the production of PGE_2 and TXB_2.



26

Zileuton (**27**), an iron chelating substance was proved to be effective during clinical trials of rhematoid arthritis, [17,18] asthma[19] and airway function diseases. Zileuton has been approved in the US for prophylaxis and chronic treatment of asthma in adults. Clinical studies of Zileuton have demonstrated modest increase in pulmonary function, improvement in symptom scores and a reduced requirement for escape bronchodilator medication. The significance of hepatic enzyme elevation seen in larger clinical trials remains to be established

but at present hepatic enzyme monitoring is recommended. This profile suggests that there is an opportunity to develop a 5-LO inhibitor, which could have an improved risk/benefit ratio.

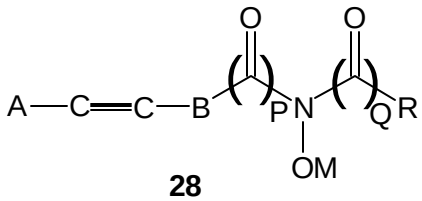


Zileuton (**27**)

A series of potent selective, orally active inhibitors of the enzyme 5-lipoxygenase has been investigated which contain the N-hydroxy urea as necessary component for biological activity. [20]

Acetylene derivatives having lipoxygenase inhibitory activity: [21]

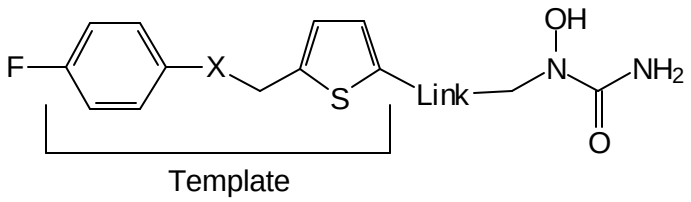
Compounds of the structure **28** shown below where P and Q are zero or one, but not both the same, M represents hydrogen, a pharmaceutically acceptable cation or a metabolically cleavable group, B is a valence bond or straight or branched alkylene group, R is alkyl, cycloalkyl or $-NR^1R^2$ where R^1 and R^2 are hydrogen, alkyl, cycloalkyl or alkanoyl, and A is optionally substituted carbocyclic aryl, furyl, benzo [b] furyl, theieneyl, or benzo [b] thienyl are potent inhibitors of lipoxygenase enzymes and thus inhibit the biosynthesis of leukotrienes.



28

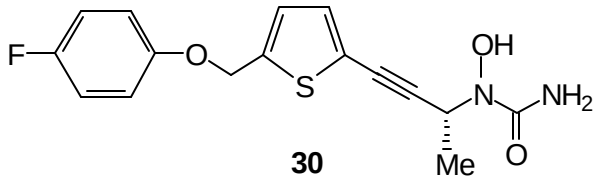
When **P** is one and **Q** zero, the compounds belong to a class of hydroxamic acids. When **P** is zero and **Q** one, the compounds comprise a class of *N*-hydroxy amide and urea compounds.

As a conceptual working hypothesis, the *N*-hydroxy function was conserved as the pharmacophore for inhibitory activity, and we defined two variable components, the template and the link, for structure-activity optimisation (compound of type **29**) as shown below. The



29

template was envisioned as the entity providing lipophilic binding interactions. The link group via proximity to the pharmacophore could modulate inhibition and metabolism phenomena. The link group also influenced the spatial orientation of the lipophilic template with respect to the pharmacophore and thus could have a dramatic effect on pharmacological properties. Optimization of the lipophilic template lead to the discovery of R (+)-*N* [3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1methyl-2-propynyl]-*N*-hydroxyurea (**30**) with more effective and prolonged inhibition of leukotriene biosynthesis in monkey and men.[22] The optimized 5-lipoxygenase inhibitor 21 was selected for development as an investigational drug for leukotriene-mediated disorders.



30

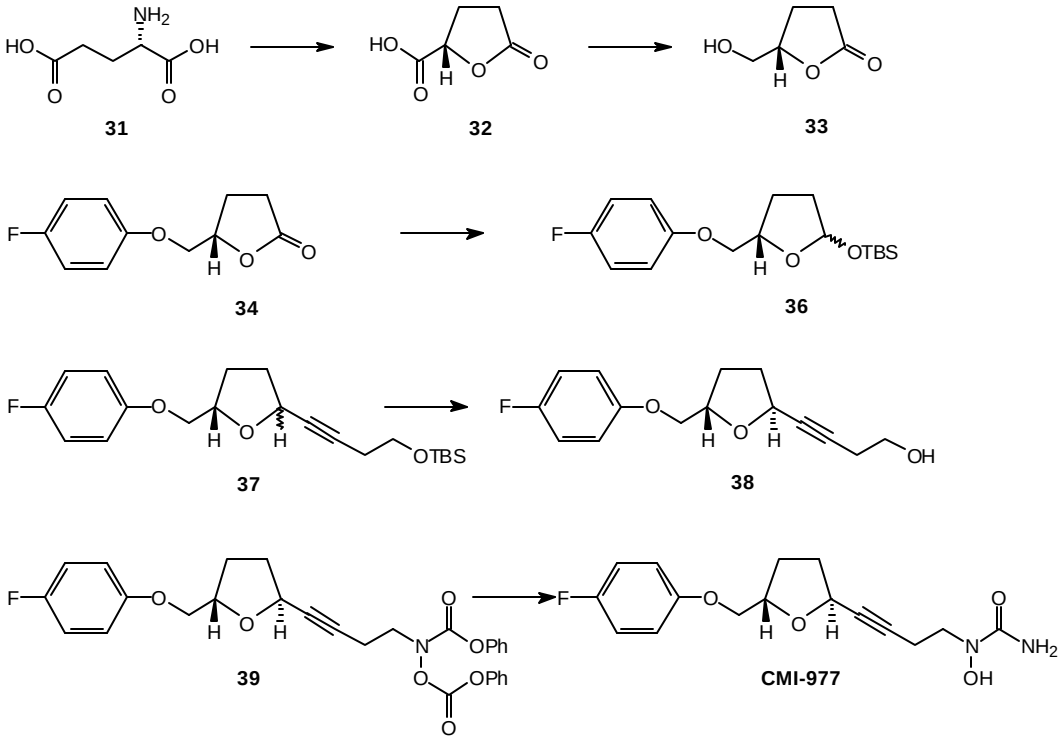
The foregoing discussion indicates the importance of lipoxygenase inhibitor for controlling various respiratory diseases specifically asthma.[23] Thus a new agent has been designed conserving the *N*-hydroxyurea function as the pharmacophore for 5-lipoxygenase inhibitory activity.

Recently Cytomed Inc., USA, has investigated (2S, 5S)-*trans*-5-(4-fluorophenoxy methyl)-2-(1-*N*-hydroxyureidyl-3-butyn-4-yl) tetrahydrofuran (CMI-977) for the synthetic studies owing to its potential anti-asthmatic properties. It is an enantiomerically pure, potent, selective and orally active inhibitor of 5-LO. In *vitro*, CMI-977 is a potent blocker of LTB_4 production from human whole blood (IC_{50} = 100 nM) and human bronchiolar constriction (IC_{50}=10 nM). In animal model, oral administration of CMI-977 effectively blocks ovalbumin-induced bronchoconstriction, airway eosinophil accumulation and plasma extravasation in guinea pigs. In addition, oral dosing of CMI-977 blocks ovalbumin-induced airway hyper responsiveness in mice. In sheep asthma model, oral dosing with CMI-977 blocks both early and late airway responses as well as blocking hyper responsiveness. Pharmacokinetic and pharmacodynamic studies carried out in monkeys following a single oral dose of CMI-977 suggest that it will be efficacious in human beings.

The first synthesis, coincidentally, the discovery route,[24] was developed by choosing (*S*)-(+)-hydroxymethyl-γ-butyrolactone (**33**) as core chiral synthon. Accordingly, L-glutamic acid (**31**) was converted to this intermediate (**33**) in two-step procedure: Initial diazotisation to give (*S*)-(+)-γ-butyrolactone carboxylic acid (**32**) followed by reaction with BH_3.DMS complex. Mitsunobu etherification with 4-fluorophenol/TPP/DIAD gave the corresponding lactone (**34**). Reductive chemistry with DIBAL-H afforded the lactol (**35**), which was subsequently protected as its silyl ether **36** with TBDMS-Cl. The introduction of homopropargylic unit at C-2 position was accomplished by initial activation of anomeric position on exchange with TMS-Br (Oxenium ion type intermediate), and subsequent exposure to the carbonion of 1-*tert*-butyldimethylsiloxy-3-butyne. This reaction resulted in a mixture of diastereomers **37** (*cis/trans*) in 1:1 ratio. Deprotection of silyl group gave the corresponding homopropargyl alcohol derivative **38**. It is interesting to note that the diastereomers were easily separated at this stage by simple recrystallisation taking advantage

of the different state of the isomers (*trans*-isomer-solid, cis-isomer-liquid). The introduction of N-hydroxyurea derivative was effected on Mitsunobu reaction of **38** with N,O-bis(phenoxycarbonyl)hydroxylamine /TPP/ DIAD, followed by ammonolysis of the resultant urethane derivative **39**.

Scheme 1

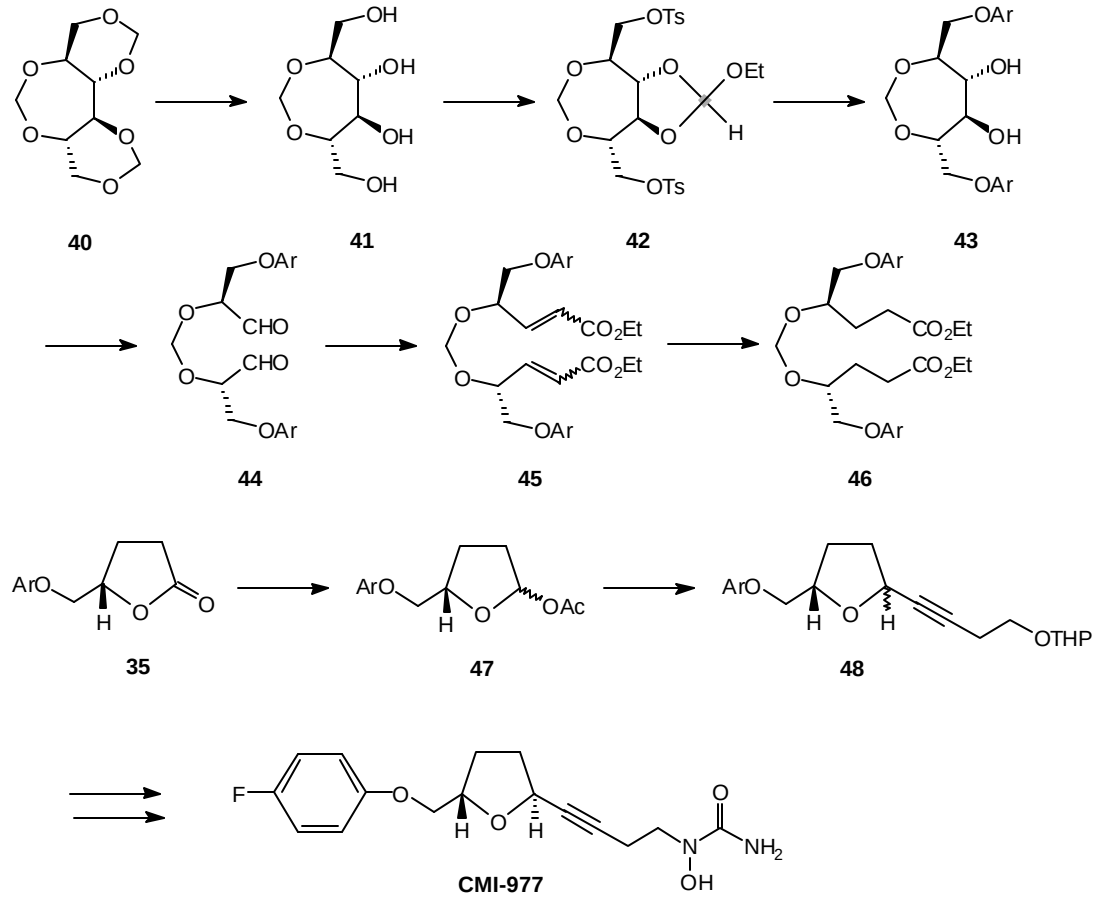


The inadequacy of the discovery route for large scale synthesis challenged our group (Gurjar *et al*) to undertake route selection efforts aimed at defining cost effective syntheses.[25] The carbohydrate based route presented herein demonstrate the versatility of D-mannitol as an inexpensive commodity chemical and synthon available in high enantiomeric purity endowed with C-2 axis of symmetry.

The conversion of D-mannitol into 1,3;2,5;4,6-tri-*O*-methylene-D-mannitol (**40**) using formalin and conc. HCl proceeded in high yield. Subsequently **40** was subjected to acid

catalyzed acetylation with Ac_2O, AcOH, conc. H_2SO_4 followed by Zamplen deacetylation condition to give the tetrol derivative **41** as a white crystalline solid. Selective protection of hydroxyl group at C-1 and C-6 of **41** with tosyl chloride and pyridine at room temperature followed by 3,4-diol was elaborated to ethoxymethylidene ether by using triethylorthoformate and pTSA as catalyst to provide **42**. Nucleophilic displacement of tosyl group with 4-fluorophenol in the presence of aqueous KOH in acetonitrile followed by orthoester deprotection with 1% aqueous HCl in THF provided 3,4-diol derivative **43**. Oxidative cleavage 3,4-diol with KIO_4 in MeOH-water (8:2) followed by stable two carbon Wittig

Scheme 2



reaction gave the α, β-unsaturated ester **45**. The reduction in the presence of Pd/C in ethanol followed by acid catalysed cylization resulted in to two equivalent frame works of desired

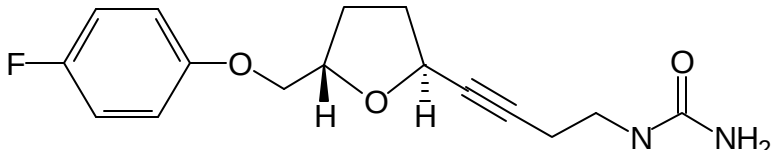
lactone **35**. Lactone **35** was further reduced with DIBAL-H at -78° C followed by acetylation to give **47** in quantitative yield. Subsequent treatment of **47** with TMS-Br at -78° C provided rather unstable bromide which was consequently treated with lithium salt of homopropargyl alcohol THP ether at same temperature followed by deprotection of THP group resulted into 65:35 mixture of diastereomer which was crystallised in 50% yield of all required trans isomer **38**. In order to introduce 1-hydroxyuriedyl moiety **38** was subjected to mitsunobu reaction condition with N,O-bis-(carbophenoxy) hydroxyla mmine, TPP, DEAD followed by amminolysis to provide CMI-977.

This discovery route was plagued with several problems that mitigated against efficient scale up and cost effective production of the target molecule (Scheme 1). Many reactions necessitated cryogenic conditions; silyl protecting groups were used in numerous instances. The atom economy in the protection-deprotection sequence was not in the desired direction. The initial Mitsunobu coupling and follow-up steps generated lot of hazardous waste that was difficult to dispose. Above all, the preponderant issue was that the C-C bond formation at the anomeric centre was in totally non-stereodirective fashion, resulting in 1:1 mixture of *cis*/*trans* isomers. Hence, the undesired formation of *cis*-isomer by half after several steps was a major concern. The carbohyrate route developed by colleague in our laboratory was efficient to synthesis the lactone **35** in multy gram scale, has only one draw back of being multistep synthesis.

The problems encountered hitherto prompted us to undertake route-selection efforts to devise novel and a versatile approach, which can be used to synthesize analogues of CMI-977 (like aza, thia, six, seven etc.,). Availability of higher analogues and their biological profile is useful to evaluate structure-activity relationship.

PRESENT WORK

Asthma is a chronic inflammatory disease complicated by periodic acute inflammatory changes. The relationship between inflammatory conditions and prostaglandins, thromboxanes and leukotrienes has been established for a long time. Leukotrienes (LT) are metabolites of arachidonic acid and are produced by the activity of 5-lipoxygenase (5-LO) enzyme.[26] This activity leads to the formation of the unstable intermediate LTA_4 which is converted to the chemotaxin LTB_4 by LTA_4 hydrolase and to the cysteinyl leukotriene LTC_4 by LTC_4 synthatase. LTC_4 is further converted to LTD_4 and ultimately to LTE_4 by enzymes, which are ubiquitous in both tissue and in circulation. Both LTB_4 and the cysteinyl lekotrienes (LTC_4, LTD_4 and LTE_4) are known to play significant role in the induction of inflammation in a variety of conditions including asthma. These compounds can be found in a number of biological fluids including bronchoalveolar lavage fluid (BALE) from asthmatic patients. The physiological effects of these leukortrienes include augmentation of eosinophil and neutrophil migration. The overall effect of the leukotrienes mimics the recognized pathophysiological features of asthma, in particular, bronchial constriction, increased airways responsiveness, increased microvascular permeability and hypersecretion of mucus. Blocking the synthesis or function of leukotrienes has been shown to be of therapeutic benefit in asthmatic patients.

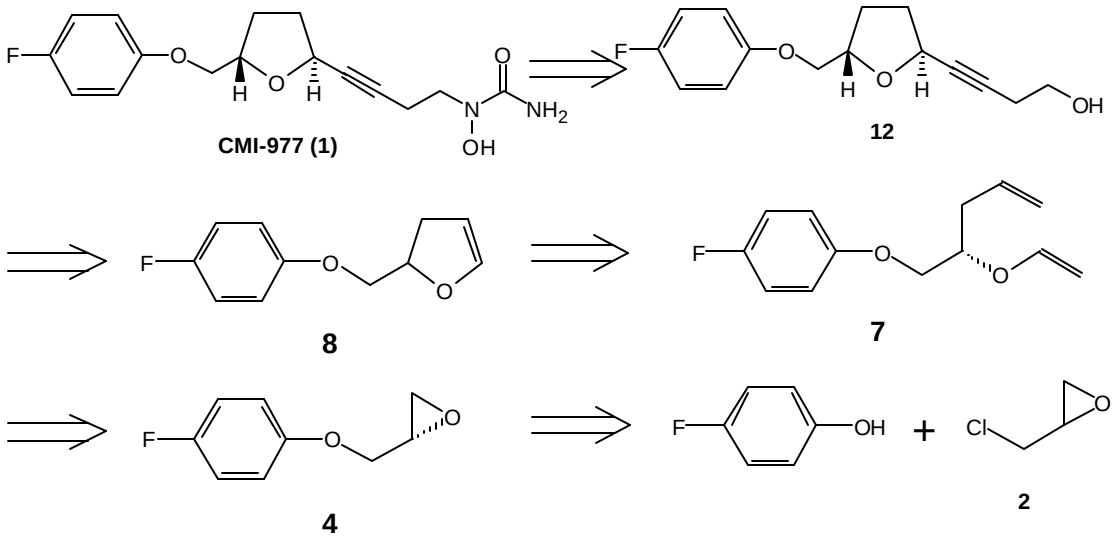


CMI-977 (1)

CMI-977 (**1**) is an anti-asthmatic lead drug candidate, which is undergoing human clinical trials.[27] The attractive feature is that the molecule has shown excellent pharmacological profile with lesser side effects compared to the existing drugs for the treatment of asthma. Chemically, it has an interesting diversely substituted tetrahydrofuran

ring with *trans* juxtaposition of functionalities. The inadequacies of the flexible and practical routes for the synthesis of these type of compounds and the striking structural feature, adjuvenated by the good activity profile prompted us to evaluate new synthectic method. This method will also address as a general synthectic protocol for the synthesis of higher (6, 7 membered) and hetero (aza., thio.) analogues.

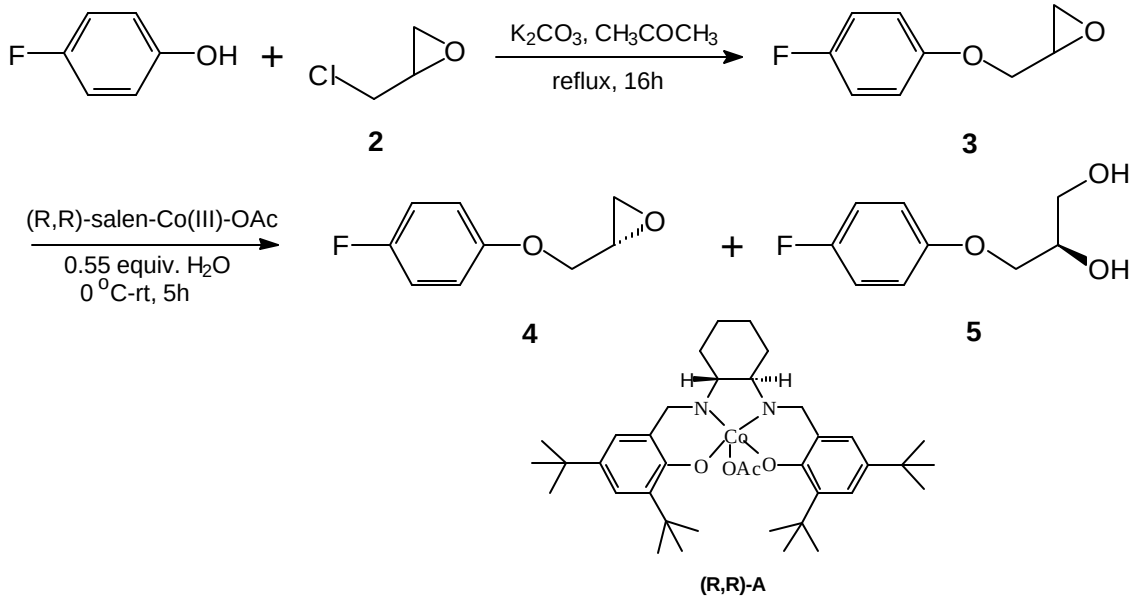
Scheme 1



The retrosynthetic analysis of CMI-977 (**1)** reveals that the key features of this approach will be: (1) the ring closing olefin metathesis of vinyl ether, (2) a stereoselective alkylation of 2-benzenesulphonyl derivative, and (3) the introduction of the hydroxyureidyl moiety (Scheme 1). The very first strategic disconnection should disembark N-hydroxyurea moiety to provide the precursor **12**. It was envisaged that the homopropargyl part should arise from the addition to the 5-membered oxenium ion where the diastereoinduction should be made possible through the inherent chirality of the substrate. Accordingly, the potential electrophilic counterpart that undergoes addition at C_2 position is substituted dihydrofuran (**8**). The next conceivable operation is the *retro*-RCM to generate the diene (**7**), which in turn could be obtained by few-steps procedure from 4-fluorophenyl glycidyl ether.

The synthetic endeavour begins with the synthesis of (***S***)-4-fluorophenylglycidyl ether (**4**). *O*-Alkylation of 4-fluorophenol with (*R*)-epichlorohydrin in anhydrous acetone in the presence of K_2CO_3 as a base provided (*S*)-4-fluorophenyl glycidyl ether (**4**) in 98 % yield. In spite of the high yield, the cost of chiral (***R***)-epichlorohydrin was not amicable for the present studies. Therefore alternatively, Jacobsen hydrolytic kinetic resolution (HKR) of terminal epoxide would be the proper strategy. In 1997 Jacobsen group reported a powerful tool to resolve the terminal epoxide by describing HRK process.[28] Accordingly, (*R,R*)-salen Co(IIIOAC (**A**) complex with 0.55 eq. of H_2O effect the resolution of racemic terminal epoxide proving optically pure epoxide and corresponding optically active diol in good chemical and optical purity.

Scheme 2



(R,R)-A

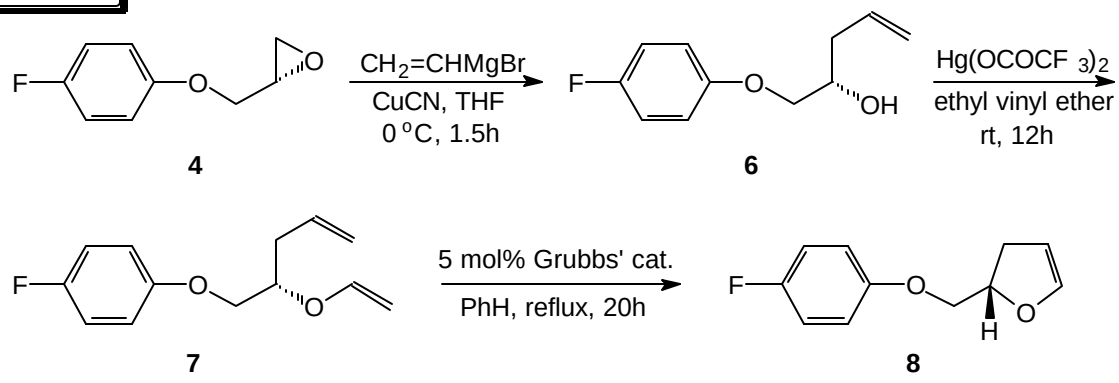
The catalyst (*R,R*)-salen Co(III)OAc (**A**) used in the synthesis was prepared in the laboratory in good yields and optical purity. Thus the relevant starting material (*S*)-4-flurophenyl glycidyl ether **4** was made available by a two-step procedure first *O*-alkylation of 4-fluorophenol with racemic epichlorohydrin (**2**) gave (±)-4-fluorophenyl glycidyl ether (**3**) in

98% yield as a colourless liquid. The compound **3** was subjected to hydrolytic kinetic[29] resolution according to Jacobsen's protocol with 0.5 mol % of (*R,R*)-salen Co(III)-OAc (**A**) and 0.55 equivalent of distilled water at 0 °C. It was stirred at room temperature for 5 h to afford the requisite (*S*)-4-fluorophenyl glycidyl ether (**4**) in 45% yield with 92% ee along with (*R*)-1-*O*-(4-fluorophenyl)glycerol (**5**) in 49% yield with 97% ee (Scheme 2). The ^{1}H NMR spectra of the epoxide (**4**) and diol (**5**) were in agreement with the assigned structures.

The EI mass spectrum of **4** showed the molecular ion peak at *m/z* 168 (M^+). The next step involving the regioselective epoxide[30] opening of **4** with vinylmagnesium bromide in the presence of 0.1 equiv. of cuprous cyanide in dry THF provided (2*S*)-1-(4-fluorophenoxy)-pent-4-en-2-ol (**6**) in 78% yield. The structure **6** was elucidated by its ^{1}H NMR spectrum in which the conspicuous absence of peaks due to epoxy group were noted and the appearance of olefinic protons in the downfield region at 5.14 ppm (m, 1H) due to terminal protons and δ 5.84 (m, 1H) corresponding to internal olefinic proton, whereas allylic methylene resonated at δ 2.35. The molecular ion peak (M^+) was observed at *m/z* 196 in EI mass spectrum and which was further confirmed by elemental analysis (Calcd. for $C_{11}H_{13}FO_2$: C, 67.35; H, 6.63. Found C, 67.45; H, 6.73). The conversion of the alcohol **6** into the corresponding vinyl ether (**7**) was accomplished by treating with freshly distilled ethyl vinyl ether and 0.1 equiv. of $Hg(OCOCF_3)_2$,[31] followed by stirring at room temperature for 12 h (Scheme 3). In its ^{1}H NMR spectrum, the characteristic signals due to *O*-vinyl group were distinctly visible at δ 4.04 (dd, 1H, *J* = 1.9, 6.8 Hz) [the olefinic proton *cis* to internal vinylic proton], 4.34 (dd, 1H, *J* = 1.9, 14.2 Hz) [the olefinic proton *trans* to internal vinylic proton], 6.38 (dd, 1H, *J* = 6.8, 14.2 Hz) [the internal olefinic proton of *O*-vinyl group] and resonances due to rest of the protons were in confirmity with the assigned structure. In addition, the molecular ion peak (M^+) was observed at *m/z* 222 in its mass spectrum. Our next endeavour was the ring closing metathesis of **7** which was performed in the presence of 5 mol % of Grubbs' catalyst in

refluxing benzene for 20 h to give the dihydrofuran derivative **8** in 52% yield.[32] The ^{1}H NMR spectrum of **8** displayed the characteristic dihydrofuran vinylic signals at δ 4.90 (m, 1H) and 6.29 as doublet respectively and also two allylic methylene protons at C_3 were located at δ 2.48 and 2.82. The peaks correspond to the rest of the protons were appeared at their expected chemical shifts. In addition, the structure of **8** was confirmed by the ^{13}C NMR spectrum in which the characteristic olefinic carbon signals were located at δ 145.24 and 98.85.

Scheme 3

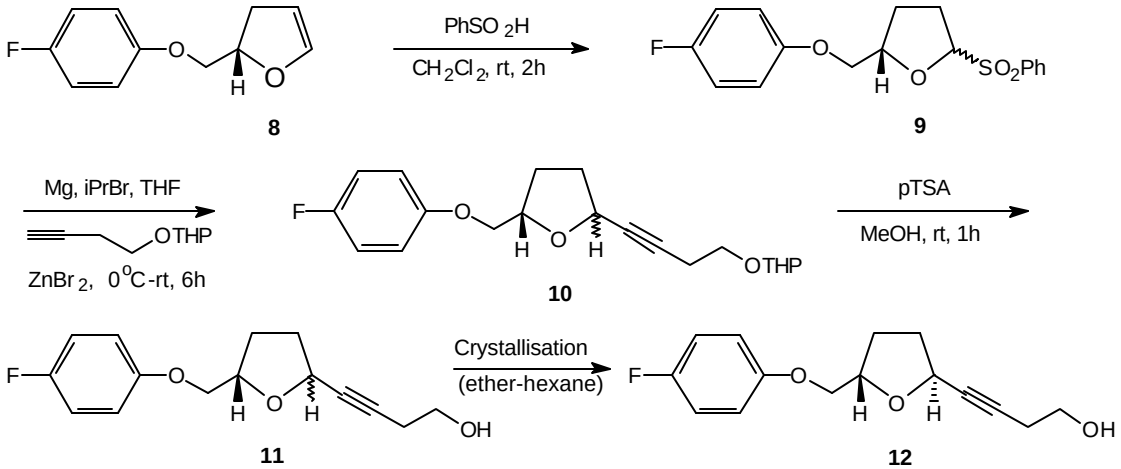


4 **6** **7** **8**

The approach to incorporate 4-*N*-hydroxyureidyl-1-butynyl moiety, the side chain of CMI-977, was based on Ley's alkylation[33] of 2-benzenesulphonyltetrahydrofuran. For this endeavour, compound **8** was treated with benzenesulphinic acid in dry CH_2Cl_2 at room temperature for 2 h followed by work up provided the crystalline sulfone derivative **9** in 81% yield. In the ^{1}H NMR spectrum of **9**, the peaks correspond to ring proton H-2 of THF ring was localised as two sets of multiplets at δ 4.89 and 4.94 due to the presence of diastereomer mixture, whereas aromatic ring protons of sulphone group appeared as two sets of multiplets at δ 7.58 and 7.88. The signals correspond to rest of the protons were appeared at their expected chemical shifts. In addition, the structure was confirmed by the high resolution mass spectrum in which the molecular ion peak (M^+) was observed at *m/z* 336.0823 (Calcd. for $C_{17}H_{17}FO_4S$: 336.0831). Treatment of the sulfone **9** with dialkyl zinc reagent, derived from BrMg-C≡C-CH_2CH_2OTHP and $ZnBr_2$, in anhydrous THF at 0 °C to room temperature for 6 h provided

(2*RS*,5*S*)-5-(4-fluorophenoxymethyl)-2-(1-tetrahydropyranyloxy-3-butyn-4-yl)tetra hydrofuran (**10**). Subsequently **10** was treated with catalytic amount of pTSA in methanol at room temperature for 1 h to deprotect the THP group affording the alcohol **11** in an overall 88% yield. From the ^{1}H NMR spectrum, it was revealed that **11** was a mixture of diastereomers. The HPLC analysis on ODS column with methanol and water (6:4) as an eluting solvent system showed 7:3 ratio of diastereomers. The ^{1}H NMR spectrum of **11** revealed the presence of acetylenic side chain and the tetrahydrofuran moiety. However, due to diastereomeric mixture, a complex ^{1}H NMR pattern was observed. Crystallization of crude product twice from ether-hexane yielded the pure *trans* (2*S*,5*S*)-5-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydrofuran (**12**) in 50% yield with HPLC purity above 96 % ee. The ^{1}H NMR spectrum of enantiomerically pure **12** was amiable to first order splitting. For example, the methylene protons were observed in the region of δ 1.88-2.24 ppm, whereas the H-2 and H-5 protons of tetrahydrofuran ring resonated at δ 4.73 and 4.45 respectively (Scheme 4). NOE studies on compound **12** showed that the relative stereochemistry of the ring protons H-2 and H-5 was *trans*, which was further confirmed by the single crystal X-ray crystallography. The

Scheme 4



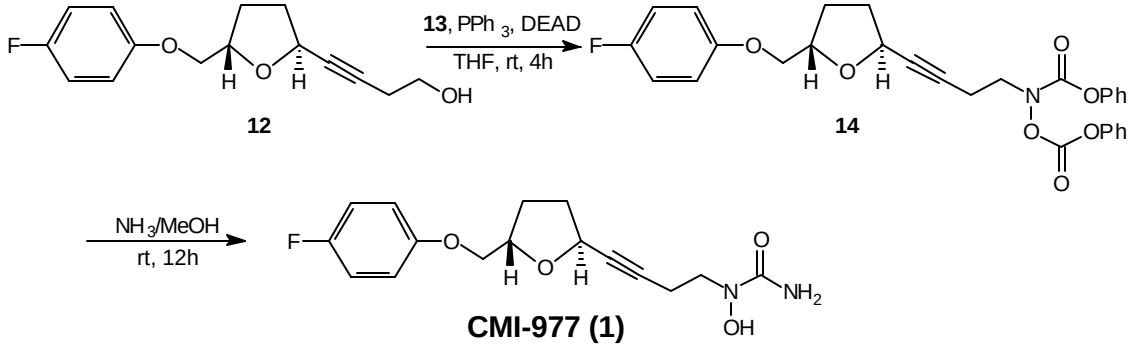
optical rotation $[\alpha]_D$ -34.2 (c 1.3, $CHCl_3$) was comparable to the authentic product provided by M/s. Steroids, Chicago, USA {lit.,[24] $[\alpha]_D$-34 (c 1, $CHCl_3$)}.

The next step of the synthetic planning was to introduce the *N*-hydroxyurea group at the end of the acetylene side chain with which the total synthesis of CMI-977 would complete. Synthesis of *N*,*O*-bis(carbophenoxy)hydroxylamine **13** was achieved by the patented procedure using $NH_4OH.HCl$ and 2 equivalents of phenyl chloroformate.[34] The Mistsunobu[35] reaction of *N*,*O*-bis(carbophenoxy)hydroxylamine (**13**) with the compound **12** in the presence of TPP and diethylazodicarboxylate (DEAD) in anhydrous THF, at room temperature for 4 h furnished the corresponding(2*S*,5*S*)-5-(4-fluorophenoxymethyl)-2-(1-*N*,*O*-bis(phenoxycarbonyl)hydroxylamino-3-butyn-4-yl)tetrahydrofuran (**14**) in 90% yield. In the ^1H NMR spectrum, the signals correspond to 10 protons of phenoxy group (2 × Ph) appeared in the region of δ 7.05-7.45 as multiplet and all other protons resonated at expected chemical shift values, which was further confirmed by HRMS analysis which showed molecular ion peak (M^+ + 1) at 520.1770 (Calcd. for $C_{29}H_{27}FNO_7$ 520.1771).

The final step involved the conversion of urethane group (NCOOPh) into the urea (N-CO-NH_2) with concomitant ammonolysis of acyl group (OCOPh) to hydroxy group. Treatment of **14** with saturated methanolic ammonia at room temperature for 12 h followed by workup to provide the final product of CMI-977 (**1**) in 64 % yield as a white crystalline solid. Scanning electron microscopy was employed to observe the morphology and partical size. CMI-977 was seen as plate like very agglomerated crystals with a smooth surface texture. The size of the particles ranged from 5-10 μm with a rounded shape. Some particles were seen to have a more angular shape. The substance is strongly cohesive in nature, X-ray powder diffraction of CMI-977 showed that the material is highly crystalline with very intense narrow diffraction line and no amorphous background. The structure of **1** was also

confirmed by comparing with the ^{1}H NMR, ^{13}C NMR, mass spectrum, optical rotation and melting point of the authentic sample.[24]

Scheme 5



In conclusion, we have demonstrated a short and steroselective synthesis of CMI-977 using (±)-4-fluorophenyl glycidyl ether (**3**) as a raw material. The tetrahydrofuran ring was effectively constructed involving the ring closing metathesis of vinyl ether while stereoslective introduction of 1-*N*-hydroxyureidyl-3-butyn-4-yl side chain was achieved by C-alkylation of 2-benzenesulphonyl derivative.

The CMI-977 (**1**) was tested for Leukotriene B_4 (LTB_4) inhibition in the human whole blood assay. Heparinized human whole blood was pre-incubated with selected concentrations of test compound for 15 minutes at 37 ^{o}C and stimulated with 50 μM calcium ionophor for 30 minutes at 37 ^{o}C. The reaction was stopped by placing samples on ice and cold centrifugation at 4 ^{o}C for 10 minutes at 1100 xg. Test sample plasma was diluted in buffer and assayed for LTB_4 content. Test compound activity was determined as per Cayman LTD enzyme immunoassay (EIA) and evaluated as IC_{50} [nM].

CMI-977 (**1**) is proven to inibit 5-LO activity in human whole blood with an IC_{50} of 120 nM and to block anti-IgE-induced contractions of human airways tissue with an IC_{50} of 100 nM, being 5-10 times more potent than zileution. CMI-977 provides to be a valueable addition to the therapeutic armamentarium available for the management of asthma.

EXPERIMENTAL SECTION

(±)-4-Fluorophenyl glycidyl ether (3).

To a stirred solution of 4-fluorophenol (20.0 g, 178.4 mmol) and (±)-epichlorohydrin (**2**) (66.0 g, 713.6 mmol) in anhydrous acetone (400 mL) was added potassium carbonate (96.0 g, 713.6 mmol). The reaction mixture was heated under reflux for 16 h until complete consumption of 4-fluorophenol. The reaction mixture was filtered off and the filtrate concentrated *in vacuo* to afford a pale yellow oil. Excess of epichlorohydrin was distilled off. The residue was distilled under reduced pressure (100 °C at 4 mm/Hg) to give **3** (29.4 g, 98 %) as a colorless liquid.

(*S*)-4-Fluorophenyl glycidyl ether (4) and (*R*)-1-*O*-(4-fluorophenyl)glycerol (5).

To an oven dried 100 mL round bottom flask equipped with a spinbar, racemic 4-fluorophenyl glycidyl ether (**3**) (20 g, 119 mmol), (*R,R*)-salenCo(III)OAc (**A**) catalyst (0.396 g, 0.595 mmol) were added. The reaction mixture was cooled to 0 °C. Distilled water (1.2 mL, 65.47 mmol) was added dropwise over a period of 1 h. The reaction mixture was stirred for 5 h, and diluted with hexane. The precipitated solid was filtered off and recrystallised from a mixture of $CHCl_3$ and hexane to afford **5** (10.85 g, 49 %) as a solid. The filterate was concentrated and purified by distillation under reduced pressure (100 ^{o}C at 4 mm/Hg) to give (*S*)-4-fluorophenyl glycidyl ether (**4**) (9 g, 45 %) as a liquid.

Compound **4**

$[\alpha]_D$ +5.27° (c 1.46, $CHCl_3$), ee: 92%.

IR (neat): 2892, 1493, 1215, 831 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.73 (m, 1H, H-3), 2.88 (t, *J* = 4.5 Hz, 1H, H-3'), 3.30 (m, 1H, H-2), 3.92 (dd, *J* = 6.7, 15.7 Hz, 1H, H-1), 4.16 (dd, J = 4.5, 15.7 Hz, 1H, H-1'), 6.78-7.09 (m, 4H, Ar).

EIMS *m/z* (rel. intensity): 168 (M^+, 54), 125 (20), 112 (98), 95 (31), 83 (80), 75 (23), 57 (100).

HRMS (EI): Calcd. for ($C_9H_9FO_2$, M^+): 168.0586. Found: 168.0580.

Compound **5**

M.P.: 58-59 ^oC.

$[\alpha]_D$ -9.6° (c 1.6, EtOH), ee: 97%.

IR (neat) 3200 (br), 3020, 1493, 1046, 846 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 2.08 (br s, 1H, OH), 2.77 (br s, 1H, OH), 3.64-3.90 (m, 2H, CH_2O), 3.93-4.16 (m, 3H, CH_2O, OCH), 6.73-7.03 (m, 4H, Ar).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 63.6 (CH_2OH), 69.7 (CHOH), 70.4 (OCH_2), 115.4 (CH, Ar), 115.6 (2xCH, Ar), 116.1 (CH, Ar), 154.4 (F-C, Ar), 159.8 (O-C, Ar).

EIMS *m/z* (rel. intensity): 186 (M^+, 9), 112 (100), 95 (21), 83 (34), 57 (34), 43 (21).

HRMS (EI): Calcd. for ($C_9H_{11}FO_3$, M^+): 186.0692. Found: 186.0693.

(2*S*)-1-(4-Fluorophenoxy)pent-4-en-2-ol (**6**).

To a suspension of magnesium (1.63 g, 67.9 mmol) in dry THF (20 mL) at 0 ^0C was added a solution of vinyl bromide (3.6 g, 33.9 mmol) in dry THF (15 mL). After 0.5 h, CuCN (60 mg, 0.68 mmol) and (*S*)-4-flurophenyl glycidyl ether (**4**) (4.0 g, 23.8 mmol) were sucessively added. After stirring for 1 h at rt., the reaction mixture was quenched with saturated NH_4Cl solution, concentrated and the residue partitioned between ethyl acetate and water. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:4) to give **6** (3.64 g, 78%) as a colourless liquid.

$[\alpha]_D$ +15.0° (c 2.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.35 (dt, *J* = 1.4, 7.3 Hz, 2H, C<u>H</u>$_2$CH=CH$_2$), 2.51 (br s, 1H, OH), 3.84 (dd, *J* = 5.8, 9.3 Hz, 1H, CH$_2$O), 3.92 (dd, *J* = 4.4, 9.3 Hz, 1H, CH$_2$O), 4.0 (m, 1H, C<u>H</u>OH), 5.14 (m, 2H, =CH$_2$), 5.84 (m, 1H, CH=), 6.82 (m, 2H, Ar), 6.95 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 37.54 (CH_2), 69.06 (CH_2O), 71.80 (CHOH), 115.28 (1 x CH, Ar), 115.34 (2 x CH, Ar), 115.52 (1 x CH, Ar), 117.60 (=CH_2), 133.61 (CH=), 154.46 (F-C, Ar), 157.99 (O-C, Ar).

EI (MS) *m/z* (rel. intensity): 196 (M^+, 46), 137 (20), 112 (100), 95 (26).

Anal. Calcd. for ($C_{11}H_{13}FO_2$): C, 67.35; H, 6.63. Found: C, 67.45; H, 6.73.

(2*S*)-2-(1-Ethenoxy)-1-(4-fluorophenoxy)-4-pentene (**7**).

A mixture of Compound **6** (3.6 g, 18.4 mmol), ethyl vinyl ether (350 mL) and $Hg(OCOCF_3)_2$ (0.8 g, 1.8 mmol) was stirred for 12 h at rt. The reaction was neutralized by addition of saturated $NaHCO_3$ solution and concentrated. The residue layer was extracted with ether, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:50) to give **7** (2.85 g, 70%) as a syrup.

$[\alpha]_D$ +3.7° (c 2.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.49 (dt, *J* = 1.5, 7.3 Hz, 2H, C<u>H</u>$_2$CH=CH$_2$), 3.97 (m, 2H, CH$_2$O), 4.04 (dd, *J* = 1.9, 6.8 Hz, 1H, OCH=C<u>H</u>$_2$), 4.14 (m, 1H, OCH), 4.34 (dd, *J* = 1.9, 14.2 Hz, 1H, OCH=C<u>H</u>$_2$), 5.15 (m, 2H, =CH$_2$), 5.84 (m, 1H, CH=), 6.38 (dd, *J* = 6.8, 14.2 Hz, 1H, OC<u>H</u>=CH$_2$), 6.84 (m, 2H, Ar), 6.95 (m, 2H, Ar).

EIMS *m/z* (rel.intensity): 222 (M^+, 12), 194 (66), 125 (100), 112 (26), 95 (40).

(2*S*)-2-(4-Fluorophenoxymethyl)-2,3-dihydro-2H-furan (**8**).

A solution of **7** (2.8 g, 12.6 mmol) and Grubbs' catalyst (0.52 g, 0.63 mmol) in benzene (750 mL) was heated under reflux for 20 h, evaporated and the residue was chromatographed on silica gel using ethyl acetate-light petroleum (1:50) to give **8** (1.27 g, 52 %) as a syrup.

$[\alpha]_D$ +95° (c 2.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.48 (m, 1H, H-3), 2.82 (m, 1H, H-3'), 3.89 (dd, *J* = 6.6, 9.9 Hz, 1H, CH_2O), 4.03 (dd, *J* = 4.2, 9.9 Hz, 1H, CH_2O), 4.90 (m, 2H, H-2, H-4), 6.29 (d, *J* = 2.2 Hz, 1H, H-5), 6.81 (m, 2H, Ar), 6.95 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 31.77 (CH_2), 70.68 (CH_2O), 78.77 (C-5), 98.85 (C-3), 115.58 (CH, Ar), 115.79 (CH, Ar), 115.88 (2 x CH, Ar), 145.24 (C-2), 155.01 (F-C, Ar), 159.10 (O-C, Ar).

(*2RS*,5*S*)-2-(Benzenesulfonyl)-5-(4-fluorophenoxymethyl)tetrahydrofuran (9).

To an ice cold solution of freshly prepared benzenesulfinic acid (1.1 g, 7.4 mmol) in dry CH_2Cl_2 (10 mL), was added a solution of **8** (1.2 g, 6.2 mmol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred for 2 h, filtered through celite and washed with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:7) to give **9** (1.68 g, 81%) as white solid.

M.P.: 112-113 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.90-2.95 (m, 4H, 2 x CH_2), 3.86-4.28(m, 2H, CH_2O), 4.56 (m, 1/3 H, H-5), 4.73 (m, 2/3 H, H-5), 4.89 (m, 1/3 H, H-2), 4.94 (m, 2/3 H, H-2), 6.7-7.05 (m, 4H, Ar), 7.58 (m, 3H, Ph), 7.88 (m, 2H, Ph).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.72 (CH_2), 26.90 (CH_2), 69.95 (OCH_2), 80.50 (OCH, C-5), 94.65 (OCH, C-2), 115.51 (2 x CH, Ar), 115.66 (CH, Ar), 115.97 (CH, Ar), 129.00 (2 x CH, Ph), 129.18 (2 x CH, Ph), 129.27 (C, Ph), 133.84 (C, Ph), 155.01 (O-C, Ar), 159.76 (F-C, Ar).

FABMS *m/z* (rel. intensity): 336 (M^+, 6), 195 (82), 167 (10), 151 (100), 125 (24).

HRMS (FAB): Calcd. for ($C_{17}H_{17}FO_4S$, M^+): 336.0831. Found: 336.0823.

(2*S*,5*S*)-5-(4-Fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydrofuran(12).

To a solution of isopropylmagnesiumbromide [prepared from 0.35 g (14.7 mmol) of Mg and 1.2 g (9.8 mmol) of isopropyl bromide in THF] was added 4-tetrahydropyranyloxy-1-butyne (1.5 g, 9.8 mmol) in anhydrous THF (5 mL). After 30 min, freshly prepared 1 M solution of $ZnBr_2$ (5.9 mL, 5.9 mmol) in THF was introduced followed by the addition of compound **9** (1.65 g, 4.9 mmol) in THF after 45 min. The reaction was stirred for 6 h and then quenched with saturated NH_4Cl solution. THF was removed under reduced pressure and the residue partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried (Na_2SO_4) and concentrated to give **10**. The crude product was stirred with *p*TSA (0.02 g) in MeOH (10 mL) for 1 h, neutralized with Et_3N and concentrated to provide **11**. The HPLC analysis on ODS column with MeOH and H_2O solvent system (60:40) ratio, showed 70:30 ratio of diastereomers (RT: 6.3: 5.5 min.). The residue was crystallized twice from ether-light petroleum to yield the pure *trans*-alcohol (**12**) (0.65 g, 50 %) with 96 % HPLC purity.

M.P.: 76 °C; [lit.[24] M.p.: 77-79 °C].

$[\alpha]_D$ -34.2 ^{o} (c 1.3, $CHCl_3$). {lit., $[\alpha]_D$ -34 ^{o} (c 1, $CHCl_3$)}

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.88 (m, 1H, CH_2), 2.05 (m, 1H, CH_2), 2.24 (m, 2H, CH_2), 2.48 (t, *J* = 6.2 Hz, 2H, ≡-CH_2), 3.69 (t, *J* = 6.2 Hz, 2H, C$\underline{H}_2$OH), 3.91 (d, *J* = 4.7 Hz, 2H, OCH_2), 4.45 (m, 1H, H-5), 4.73 (m, 1H, H-2), 6.85 (m, 2H, Ar), 6.94 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.97 (CH_2), 27.68 (CH_2), 33.32 (≡-CH_2), 60.74 (CH_2OH), 68.89 (OCH_2), 70.64 (C-5), 76.76 (C-2), 81.13, 82.13 (C≡C), 115.35 (CH, Ar), 115.55 (CH, Ar), 115.80 (2 x CH, Ar), 154.80 (F-C, Ar), 159.55 (O-C, Ar).

FABMS *m/z* (rel.intensity): 264 (M^+, 37), 153 (22), 132 (100), 121 (56).

HRMS (FAB): Calcd. for ($C_{15}H_{17}FO_3$, M^+): 264.1161. Found: 264.1152.

Anal. Cacld. for ($C_{15}H_{17}FO_3$): C, 68.18; H, 6.44. Found: C, 68.51; H, 6.35.

***N,O*-Bis(phenoxycarbonyl)hydroxylamine (13).**

To a stirred solution of sodium bicarbonate (21.5 g, 256.0 mmol) in water (150 mL) at 0 °C was added hydroxylamine hydrochloride (8.80 g, 127.0 mmol) and the reaction mixture was stirred for 30 min at 0 °C. Phenylchloroformate (60.0 g, 383 mmol) was introduced directly into the vigorously stirred mixture. Sodium bicarbonate (32.3 g, 385.0 mmol) in water (270 mL) was added to the mixture, followed by an additional quantity of water (30 mL) to wash the remaining sodium bicarbonate. The mixture was stirred for 30 min. at 0 °C and then for 2 h at room temperature. The resulted suspension was filtered and the filter cake washed with water. The filter cake was collected, suspended in hexane, filtered and again washed with hexane. The solid was then dissolved in warm ether and hexane. The solution was cooled to room temperature to precipitate the product. The solid was collected by filtration to give **13** (23.5 g, 68 %) as a white solid.

^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26 (m, 5H, OCO_2Ph), 7.42 (m, 5H, CO_2Ph), 8.54 (bs, 1H, NH).

EIMS *m/z*: 273 (M^+).

(2*S*,5*S*)-5-(4-Fluorophenoxymethyl)-2-(1-*N,O*-bis(phenoxycarbonyl)hydroxylamino-3-butyn-4-yl)tetrahydrofuran (14).

To a stirred ice cooled solution of *trans* alcohol **12** (0.62 g, 2.3 mmol) in THF (10 mL) were added triphenylphosphine (0.74g, 2.8 mmol) and *N,O*-bis(phenoxycarbonyl)-hydroxylamine (**13**) (0.64 g, 2.8 mmol). After 10 minutes, diethylazodicarboxylate (DEAD) (0.49 g, 2.8 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature, stirred for 4 h and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4) and concentrated. The product was purified on silica gel using ethyl acetate-light petroleum (1:6) to give **14** (1.1 g, 90%) as a solid.

M.P.: 85-86 °C.

$[\alpha]_D$ −18.4° (c 0.9, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.76 (m, 1H, CH_2), 1.95 (m, 1H, CH_2), 2.13 (m, 2H, CH_2), 2.64 (t, *J* = 6.8 Hz, 2H, ≡-CH_2), 3.79 (dd, *J* = 2.0, 4.5 Hz, 2H, OCH_2), 3.95 (t, *J* = 6.8 Hz, 2H, CH_2N), 4.33 (m, 1H, H-5), 4.63 (m, 1H, H-2), 6.70-6.95 (m, 4H, Ar), 7.05-7.45 (m, 10H, 2x Ph).

HRMS (FAB): Calcd for ($C_{29}H_{27}FNO_7$, M^++1): 520.1771. Found: 520.1770.

(2*S*,5*S*)-5-(4-Fluorophenoxymethyl)-2-(1-*N*-hydroxyureidyl-3-butyn-4-yl)tetrahydro-furan (CMI-977) (1).

To a stirred solution of **14** (1.0 g, 1.9 mmol) in methanol (5 mL) was added saturated methanolic ammonia solution (10 mL) at room temperature, the reaction mixture was stirred was continued for 12 h and concentrated. The residue was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:1) to give **1** (0.39 g, 64%) as a white solid.

M.P.: 107-108 °C.

$[\alpha]_D$ -47° (c 1.1, CH_3OH); lit.,[24] $[\alpha]_D$ -47.8 ° (c 0.3, CD_3OD).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.82 (m, 1H, CH_2), 2.01 (m, 1H, CH_2), 2.22 (m, 2H, CH_2), 2.54 (t, *J* = 7.9 Hz, 2H, ≡-CH_2), 3.68 (t, *J* = 7.9 Hz, 2H, CH_2N), 3.91 (m, 2H, OCH_2), 4.46 (m, 1H, H-5), 4.73 (m, 1H, H-2), 5.68 (br s, 2H, NH_2), 6.78-7.02 (m, 4H, Ar), 8.95 (s, 1H, N-OH).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.13 (CH_2), 27.66 (CH_2), 33.28 (≡-CH_2), 48.62 (CH_2N), 69.08 (OCH_2), 70.36 (C-5), 76.72 (C-2), 80.72, 82.80 (C≡C), 115.50 (2 x CH, Ar), 115.63 (CH, Ar), 115.97 (CH, Ar), 154.98 (F-C, Ar), 159.70 (O-C, Ar), 161.84 ($NCONH_2$).

FABMS *m/z* (rel.intensity): 323 (M^++1, 100), 280 (11), 154 (54), 137 (42).

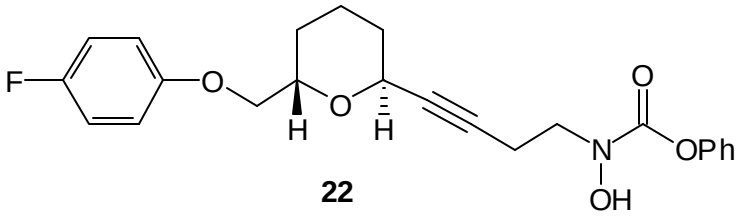
HRMS (FAB): Calcd. for ($C_{16}H_{19}FN_2O_4$, M^++1): 323.1407, Found: 323.1424.

SECTION–II

STEREOSELECTIVE SYNTHESIS OF (2*S*,6*S*)-6-(4-FLUOROPHENOXYMETHYL)-2-(1-*N*-HYDROXYUREIDYL-3-BUTYN-4-YL)TETRAHYDROPYRAN (SIX MEMBERED ANALOGUE)

PRESENT WORK

Inspired by an efficient approach to 2,5-disubstituted tetrahydrofuran CMI-977 (**1**) as described in the preceding section, we sought to explore the versatility of this approach in the preparation of the so far unknown six membered 2,6-disubstituted tetrahydropyran analogue **22** of CMI-977 (**1**). We envisage that availability of **22** and its bilogical profile is useful to evaluate the influence of ring size on the biological activity of these anti-asthmatic compounds.



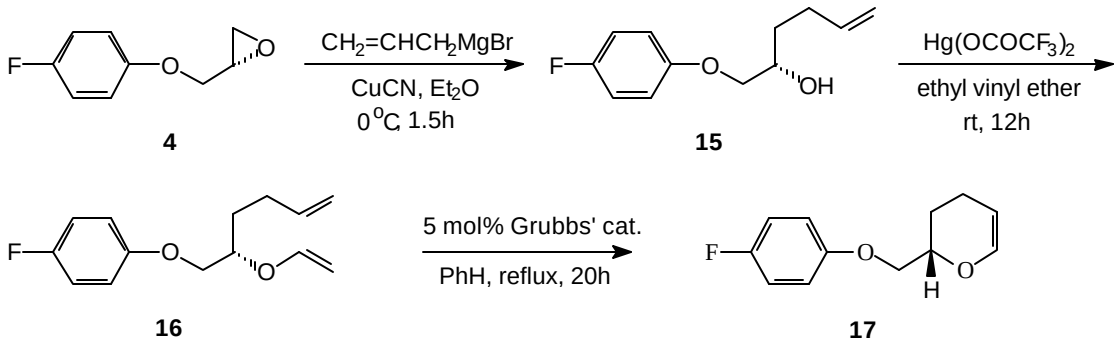
22

The six membered analogue **22** of CMI-977 was prepared starting from (S)-4-fluorophenyl glycidyl ether (**4**), which was obtained from hydrolytic kinetic resolution of racemic 4-fluorophenyl glycidyl ether **3** as dicussed in (scheme 2).

The regioselective opening of epoxide **4** with allylmagnesium bromide was achieved in the presence of cuprous cyanide in dry ether to provide (2S)-1-(4-fluorophenoxy)hex-5-en-2-ol (**15**) in 80 % yield. The conversion of free hydroxy group in **15** to the vinyl ether **16** was accomplished by treatment with ethyl vinyl ether and catalytic amount of mercuric trifluoroacetate for 12 h in 70 % yield. The structure was elucidated by its ^{1}H NMR and EIMS spectra in which the molecular ion peak M^+ was observed at *m/z* 236. The ring closing metathesis of **16** in presence of 5 mol % of Grubbs' catalyst in refluxing benzene for 20 h gave the dihydro-2H-pyran derivative **17** in 55 % yield (Scheme 6). In its ^{1}H NMR spectrum the characteristic dihydropyran signals were observed at δ 4.71 (m, 1H), 6.39 (d, 1H) ppm while all other protons resonated at expected chemical shifts. Formation of product **17** was

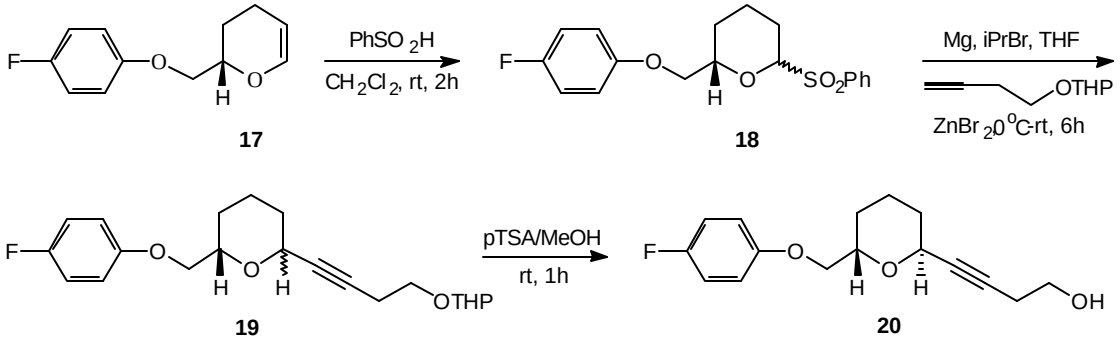
further confirmed by ^{13}C NMR as well as EIMS spectra in which the molecular ion peak M^+ was observed at *m/z* 208.

Scheme 6



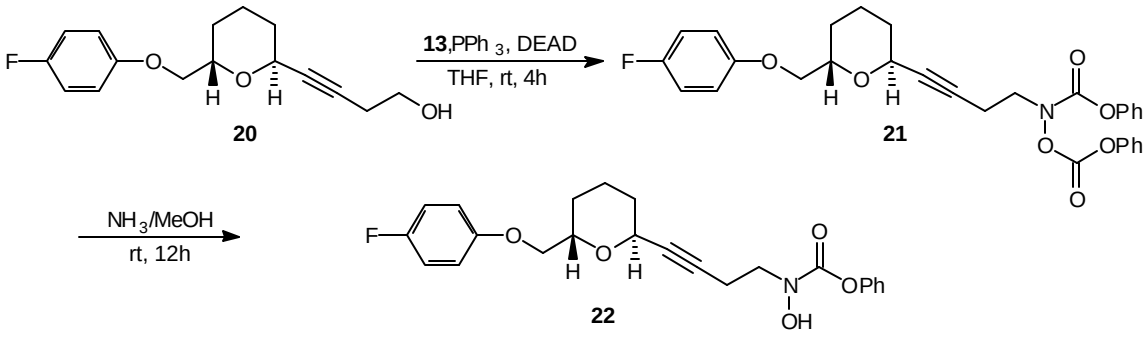
The compound **17** was converted into 2-benzenesulphonyltetrahydropyran derivative **18** with benzenesulphinic acid in dry CH_2Cl_2 at room temperature for 2 h in 82 % yield. Subsequent C-C bond formation at C-2 was carried out by treating sulphone derivative **18** with 4-tetrahydropyranyloxy-1-butynylmagnesium bromide in the presence of anhydrous zinc bromide in dry THF at 0 °C to room temperature for 3 h followed by deprotection of THP with pTSA in methanol gave *trans*-(2S,6S)-6-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydropyran (**20**) as a single diastereomeric product in 84 % overall yield after two steps. The formation of a single isomer during the C-C bond formation with tetrahydropyransulphonyl derivative **18** compared to the tetrahydrofuran precursor **8** (7:3 selectivity) was indeed gratifying and this observation is attributed to the anomeric effect forming the axial bond formation.[33a] In the ^1H NMR spectrum, the resonance due to acetylenic side chain and the tetrahydropyran moiety were observed. In addition, the structure was confirmed by ^{13}C-NMR, EIMS and elemental analysis (Calcd. for $C_{16}H_{19}FO_3$: C, 69.06; H, 6.83. Found C, 68.98; H, 7.05). The all *trans* stereochemical assignment was provided by the extensive NOE studies carried out on this intermediate.

Scheme 7



The next step was to introduce *N*-hydroxy urea group at the end of acetylenic side chain, which was described in section-I of this chapter. The Mitsunobu reaction of *N*,*O*-bis(phenoxycarbonyl)hydroxylamine with alcohol **20** in presence of TPP and DEAD in anhydrous THF provided the adduct **21** in 88 % yield. Treatment of **21** with saturated methanolic ammonia solution, simultaneously cleaved benzoate ester and converted urethane into urea affording the target molecule **22** in 65 % yield (Scheme 8). The structure **22** was fully assigned by ^{1}H NMR and ^{13}C NMR spectral analysis along with further confirmation by combustion data (Calcd. for $C_{17}H_{21}FN_2O_4$: C, 60.71; H, 6.25; N, 8.33. Found C, 60.60; H; 6.42, N; 8.25).

Scheme 8



In conclusion, we have reported a short and stereoselective approach to 6-membered anologue of CMI-977 (**22**) involving the ring closing metathesis of vinyl ethyl derivative **17**.

EXPERIMENTAL SECTION

(2*S*)-1-(4-Flourophenoxy)hex-5-en-2-ol (15).

To a solution of allylmagnesium bromide [prepared from 1.23 g (51.4 mmol) of magnesium and 3.1 g (25.7 mmol) of allyl bromide in dry ether (10 mL)], were successively added CuCN (45 mg) and (S)-4-fluorophenylglycidyl ether (**4**) (3.0 g, 18.0 mmol) at 0 ^{o}C. The reaction mixture was stirred for 15 min at rt., quenched with saturated NH_4Cl solution and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel using ethyl acetate-light petroleum (1:4) to give **15** (3.0 g, 80%) as viscous liquid.

$[\alpha]_D$ +21.4^{o} (c 2.1, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.65 (m, 2H, CH_2), 2.18 (m, 2H, C$\underline{H}_2$CH=CH_2), 2.65 (br s, 1H, OH)), 3.85 (m, 2H, OCH_2), 4.0 (m, 1H, C$\underline{H}$OH), 5.02 (m, 2H, =CH_2), 5.83 (m, 1H, CH=), 6.85 (m, 2H, Ar), 6.95 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 29.55 (CH_2), 32.11 ($\underline{C}H_2CH=CH_2$), 69.40 (CH_2O), 72.75 (CHOH), 115.01 (CH, Ar), 115.42 (CH, Ar), 115.50 (CH, Ar), 115.57 (CH, Ar), 115.96 (=CH_2), 137.91 (CH=), 154.64 (F-C, Ar), 159.69 (O-C, Ar).

EIMS *m/z* (rel.intensity): 210 (M^+, 17), 126 (19), 112 (100), 95 (18).

Anal. Calcd. for ($C_{12}H_{15}FO_2$): C, 68.57; H, 7.14. Found: C, 68.35; H, 7.23.

(2*S*)-2-(1-Ethenoxy)-1-(4-fluorophenoxy)-5-hexene (16).

A mixture of compound **15** (2.95 g, 14.0 mmol), ethyl vinyl ether (250 mL) and $Hg(OOCCF_3)$ (0.6 g, 1.4 mmol) were stirred for 12 h. The reaction was worked up as described earlier for the preparation of compound **8**. The residue was purified on silica gel using ethyl acetate-light petroleum (1:50) to give **16** (2.32 g, 70%) as a colourless liquid.

$[\alpha]_D$ –6.1^{o} (c 2.8, $CHCl_3$).

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.82 (m, 2H, CH_2), 2.23 (m, 2H, $CH_2CH=CH_2$), 3.90-4.20 (m, 4H, H-5, OCH_2, $OCH=CH_2$), 4.34 (dd, *J* = 1.4, 14.2 Hz, 1H, $OCH=CH_2$), 5.05 (m, 2H, $=CH_2$), 5.83 (m, 1H CH=), 6.39 (dd, *J* = 6.4, 14.2 Hz, 1H, $OCH=CH_2$), 6.85 (m, 2H, Ar), 6.95 (m, 2H, Ar).

EIMS *m/z* (rel.intensity): 236 (M^+, 2), 208 (57), 125 (81), 112 (37), 95 (38), 81 (100).

(2*S*)-2-(4-Fluorophenoxymethyl)-3,4-dihydro-2H-pyran (17).

A mixture of **16** (2.3 g, 9.7 mmol) and Grubbs' catalyst (0.4 g, 0.48 mmol) in benzene (600 mL) was heated under reflux for 20 h and processed as described earlier for the preparation of compound **8**, to give **17** (1.1 g, 55%) as a syrup.

$[\alpha]_D$ +47.3° (c 1.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.65-2.40 (m, 4H, 2 x CH_2), 3.83 (dd, *J* =5, 9.3 Hz, 1H, OCH_2), 3.95 (dd, *J* = 6.1, 9.3 Hz, 1H, OCH_2), 4.16 (m, 1H, H-2), 4.71 (m, 1H, H-5), 6.39 (d, *J* = 5 Hz, 1H, H-6), 6.80-7.00 (m, 4H, Ar).

EIMS *m/z* (rel.intensity): 208 (M^+, 40), 125 (19), 112 (100), 95 (27), 83 (45).

Anal. Cacld. for ($C_{12}H_{13}FO_2$): C, 69.23; H, 6.25. Found C, 68.97; H, 6.42.

(2*RS*,6*S*)-2-(benzenesulfonyl)-6-(4-fluorophenoxymethyl)tetrahydropyran (18).

A solution of **17** (1.1 g, 5.3 mmol) and freshly prepared benzenesulphinic acid (0.9 g, 6.3 mmol) in CH_2Cl_2 (20 mL) was stirred for 2 h at rt. and processed as described earlier for the preparation of compound **9**, to provide **18** (1.52 g, 82%) as white solid.

M.P.: 124-126 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.55 (m, 2H, CH_2), 1.66 (m, 1H, CH_2), 1.91 (m, 1H, CH_2), 2.30 (m, 1H, CH_2), 2.70 (m, 1H, CH_2), 3.82 (m, 2H, OCH_2), 4.72 (d, *J* = 5.0 Hz, 1H, H-2), 4.92 (m, 1H, H-7), 6.75 (m, 2H, Ar), 6.95 (m, 2H, Ar), 7.60 (m, 3H, Ph), 7.95 (m, 2H, Ph).

(2*S*,6*S*)-6-(4-Fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)tetrahydropyran (20).

The compound **18** (1.5 g, 4.28 mmol) was converted to (2*RS*,6*S*)-6-(4-fluorophenoxy methyl)-2-(1-tetrahydropyranyloxy-3-butyn-4-yl)tetrahydropyran (**19**) using 4-tetrahydropyr anyloxy-1-butynylmagnesium bromide (prepared from 1.32 g/ 8.6 mmol of 4-tetrahydro pyranyloxy-1-butyne, 1.05 g/8.57 mmol of isopropyl bromide and 0.3 g of Mg), and anhydrous $ZnBr_2$ solution (1 M in THF, 5.14 mL, 5.14 mmol) in THF by using the same procedure described earlier for the preparation of **10**. The crude product was immediately treated with pTSA (25 mg) in MeOH (10 mL), neutralized with Et_3N and concentrated. The residue was chromatographed on silica gel using EtOAc-light petroleum (1:15) to obtain **20** (0.83 g, 84%) as a solid.

$[\alpha]_D$ -32° (c 1.1, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.5-2.1 (m, 6H, 3 x CH_2), 2.55 (t, *J* = 6.3 Hz, 2H, ≡-CH_2), 3.73 (t, *J* = 6.3 Hz, 2H, CH_2OH), 3.82 (dd, *J* = 6.4, 9.7 Hz, 1H, OCH_2), 3.98 (dd, *J* = 4.7, 9.7 Hz, 1H, OCH_2), 4.22 (m, 1H, H-6), 4.8 (s, 1H, H-2), 6.83 (m, 2H, Ar), 6.93 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 125 MHz): δ18.88 (CH_2), 23.18 (CH_2), 27.04 (CH_2), 30.38 (≡-CH_2), 61.11 (CH_2OH), 65.52 (OCH_2), 69.96 (C-6), 71.92 (C-2), 80.08, 84.02 (C≡C), 115.12 (CH, Ar), 115.63 (CH, Ar), 115.72 (CH, Ar), 115.79 (CH, Ar), 154.97 (F-C, Ar), 158.28 (O-C, Ar).

EIMS *m/z* (rel.intensity): 278 (M^+, 18), 153 (28), 125 (37), 112 (100), 95 (75), 79 (73).

Anal. Calcd. for ($C_{16}H_{19}FO_3$): C, 69.06; H, 6.83. Found C, 68.98; H, 7.05.

(2*S*,6*S*)-6-(4-Fluorophenoxymethyl)-2-(1-*N*,*O*-bis(phenoxycarbonyl)hydroxylamino-3-butyn-4-yl)tetrahydropyran (21).

The compound 21 was prepared from *trans*-alcohol **20** (0.81 g, 2.9 mmol), PPh_3 (0.92 g, 3.49mmol), *N*,*O*-bis(phenoxycarbonyl)hydroxylamine (0.95 g, 3.49mmol) and DEAD (0.6 g, 3.49 mmol) in THF (15 mL) by using the same procedure described earlier for the preparation of compound **14**, to provide **21** (1.39 g, 88%) as a solid.

M.P.: 115-116 ^{o}C.

$[\alpha]_D$ -18.4^{o} (c 1.32, $CHCl_3$) .

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.45-1.80 (m, 6H, 3 x CH_2), 2.75 (t, *J* = 6.8 Hz, 2H, ≡-CH_2), 8.83 (m, 2H, OCH_2), 4.05 (t, *J* = 7.32 Hz, 2H, CH_2N), 4.23 (m, 1H, H-6), 4.8 (s, 1H, H-2), 6.70-6.95 (m, 4H, Ar), 7.1-7.45 (m, 10, 2 x Ph).

(2*S*,6*S*)-6-(4-fluorophenoxymethyl)-2-(1-*N*-hydroxyureidyl-3-butyn-4-yl)tetrahydropyran (22).

The compound 21 (1.3 g, 2.44 mmol) was converted to 22 using methanolic ammonia solution (10 mL) by using the same procedure described earlier for the preparation of compound 1, to provide 22 (0.53 g, 65%) as a solid.

$[\alpha]_D$ -28.6^{o} (c 1.2, $CHCl_3$).

M.P.: 98-99 ^{o}C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.5-2.0 (m, 6H, 3 x CH_2), 2.52 (t, *J* = 7.3 Hz, 2H, ≡-CH_2), 3.65 (t, *J* = 7.3 Hz, 2H, CH_2N), 3.83 (m, 2H, OCH_2), 4.2 (m, 1H, H-6), 4.75 (s, 1H, H-2), 5.77 (br s, 2H, NH_2), 6.75-7.0 (m, 4H, Ar), 9.0 (br s, 1H, N-O<u>H</u>).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.15 (CH_2), 18.71 (CH_2), 27.80 (CH_2), 30.40 (≡-CH_2), 48.95 (CH_2N), 65.43 (OCH_2), 69.98 (C-6), 72.24 (C-2), 79.53, 84.54 (C≡C), 115.52 (CH, Ar), 115.82 (CH, Ar), 115.94 (CH, Ar), 116.03 (CH, Ar), 155.01 (F-C, Ar), 159.01 (O-C, Ar), 161.84 ($NCONH_2$).

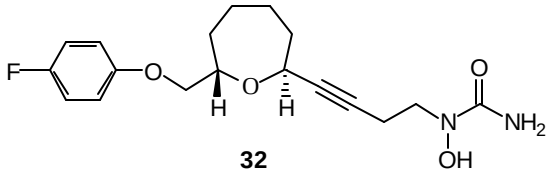
Anal. Cacld. for ($C_{17}H_{21}FN_2O_4$): C, 60.71; H, 6.25, N, 8.33. Found: C, 60.60; H, 6.42; N, 8.25.

SECTION–III

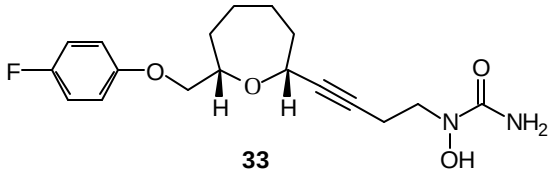
STEREOSELECTIVE SYNTHESIS OF (2*S*,7*S*)-7-(4-FLUOROPHENOXYMETHYL)-2-(1-*N*-HYDROXYUREIDYL-3-BUTYN-4-YL)OXEPANE (SEVEN MEMBERED ANALOGUE)

PRESENT WORK

In continuation of our earlier entantioselective construction of CMI-977 and its six membered analogue, we were further interested to build a library of compounds that will extend to lead discovery and optimisation from the medicinal chemist point of view. Hence, our next agenda in this series was to develop a single enantiomer synthesis of oxepane derivative i.e. the seven membered analogue of CMI-977. In this chapter, we have disclose the synthesis of seven membered analogues (*cis* and *trans*) of CMI-977 with two carbon increase in the ring size i.e., compound **32** and compound **33**.



32

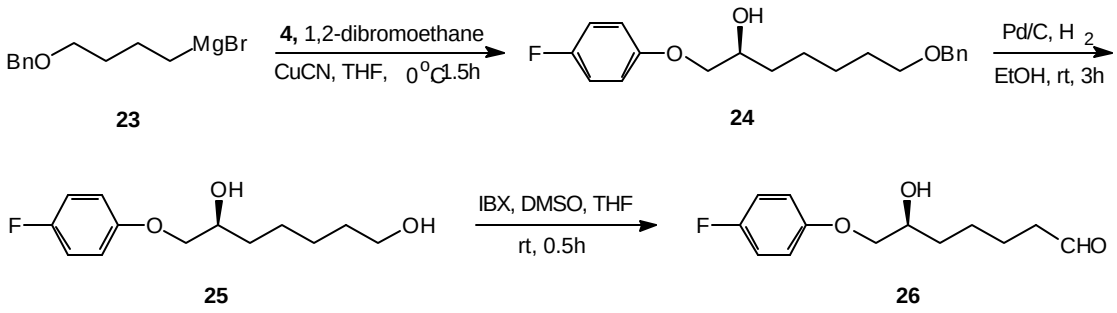


33

Accordingly, we initiated starting from (S)-4-flulorophenyl glycidyl ether (**4**), which was obtained from hydrolytic kinetic resolution of (±)-4-fluorophenyl glycidyl ether (**3**) as described in the scheme **2**. The regioselective opening of epoxide **4** with (4-benzyloxy)butylmagnesium bromide (**23**) in the presence of cuprous cyanide in dry THF gave the (2S)-7-benzyloxy-(4-fluorophenoxy)heptan-2-ol (**24**) in 73 % yield. In its ^{1}H NMR spectrum, the characteristic signals due to benzyl as well as *p*-fluorophenyl groups were localised in the region of 6.77-7.35 ppm (m, 9H). In addition, the structure was confirmed by high resolution mass spectrum in which the molecular ion peak (M^+) was observed at *m/z* 322.1803 (Calcd. for $C_{20}H_{25}FO_3$: 322.1787). Our next endeavour was to obtain dihydroxy derivative **25** which was achieved on hydrogenolysis of benzyl group of **24** on treating with 10% Pd/C in ethanol under hydrogen atmosphere for 3 h to provide **25** in 93 % yield. In the ^{1}H NMR spectrum, the conspicuous absence of peaks due to benzyl group was noted. The molecular formula of the structure was further confirmed by HRMS spectrum in which the

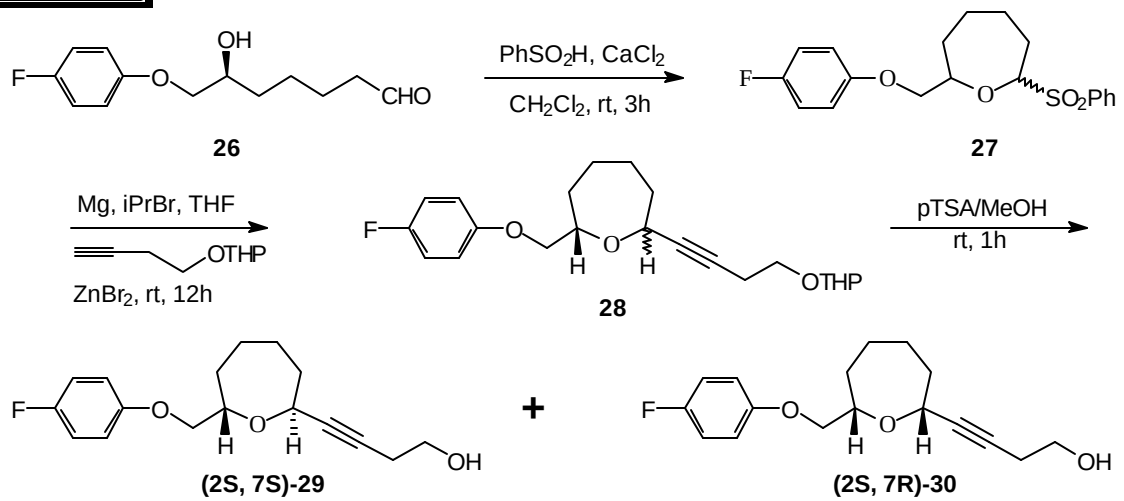
molecular ion peak (M^+) was found at *m/z* 242.1319 (Calcd. for $C_{13}H_{19}FO_3$: 242.1318). Selective oxidation of primary hydroxy group is a challenging task and the method to accomplish this is very rare. We observed that this could be easily performed with IBX in DMSO. Thus, The primary hydroxy group was selectively oxidised with slow addition of 2-iodoxybenzoic acid[36] in dry DMSO to the compound **25** in dry THF for the period of 15min. at the room temperature to provide (6*S*)-7-(4-fluorophenoxy)-6-hydroxyheptan-1-al (**26**) in 62 % yield. The presence of aldehyde proton revealed that **26** existed in open chain form. The structure was in accordance with the ^{1}H NMR spectrum in which a singlet at the down field region δ 9.80 due to the aldehyde proton appeared along the resonances of methylene group in CH_2CHO at 2.49 ppm, further confirmation by HRMS analysis which showed the molecular ion peak at *m/z* 240.1164 (Calcd. for $C_{13}H_{17}FO_3$: 240.1161).

Scheme 9



Our next concern is to obtain cyclic 2-benzenesulfonyloxepane derivative (**27**). We believed that during sulphonylation the open chain form of **26** will collapse to cyclic form **27**.The driving force for the reaction will be the stability of benzene sulfonyloxy function. The compound **26** was treated with benzenesulfinic acid in dry CH_2Cl_2 at 0 °C to room temperature for 3 h to provide (2*RS*,7*S*)-2-(benzenesulfonyl)-7-(4-fluorophenoxymethyl) oxepane (**27**) in 81% yield. In the ^{1}H NMR spectrum, the signal due to H-2 and H-7 protons appeared at δ 4.72 (dd) and 4.45 (m) as expected for cyclic structure.

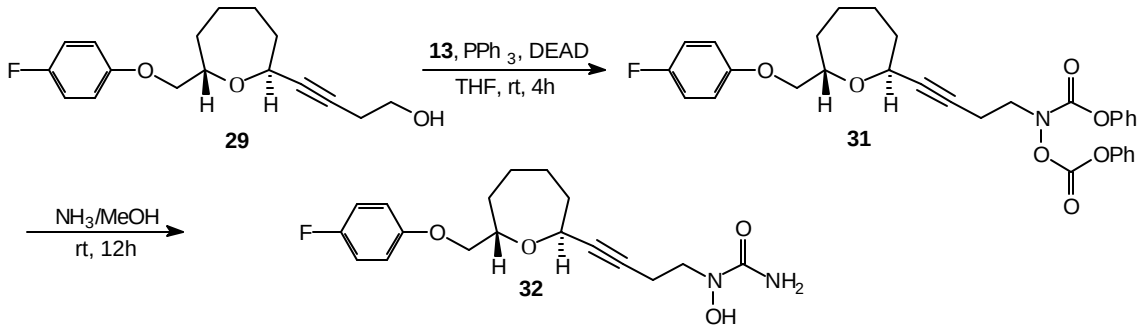
Scheme 10



Our next concern was the elaboration of sulphone **27** into the target molecule **32**. Accordingly nucleophilic displacement of the sulphone **27** with 4-tetrahyropyranyloxy-1-butynylmagnesium bromide in the presence of anhydrous zinc bromide in dry THF at 0 °C to room temperature for 30 h provided the C-C coupled derivative **28** which on THP deprotection furnished a mixture of (2*S*,7*S*) and (2*R*,7*S*) diastereomers in ratio of (8:2). This diastereomer were separated by silica gel column chromatography using ethyl acetate-hexane (1:8) to give (2*S*,7*S*)-7-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)oxepane (**29)** in 70% yield (*trans*) and the corresponding *cis* (2*R*,7*S*) isomer **30** in 17.5 yield (Scheme 10). The major compound **29** was confirmed as *trans* by NOE studies in which irradiation of H-2 proton did not show the enhancement of H-7 proton and vice versa, whereas in the case of minor product **30** the irradiation of H-2 proton shown the enhancement in H-7 and vice versa, indicating the presence of spacial interaction which confirms the *cis* isomer. The ^{1}H NMR spectrum of enantiomerically pure **29** (*trans*) was amiable to first order splitting. For example, the peaks corresponding eight methylene protons were observed in the region of δ 1.40-2.12, whereas the oxepane ring protons H-2, H-7 were localised at δ 4.56 and 4.16 respectively. The molecular

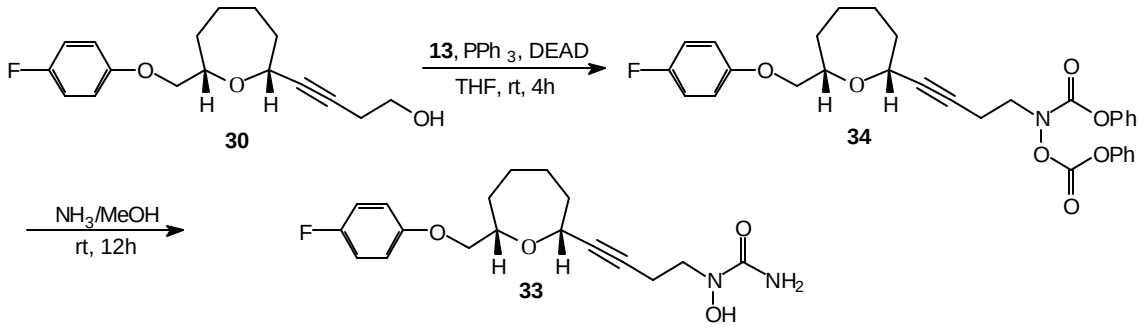
formula was further confirmed by HRMS analysis with the observed molecular ion peak (M^+) at 292.1474 (Calcd. for $C_{17}H_{21}FO_3$: 292.1477).

Scheme 11



The next step of the synthetic planning was to introduce *N*-hydroxyurea group at the end of the acetylene side chain of **29** (Scheme 11). Thus, the Mistunobu reaction of **29** with *N,O*-bis(phenoxycarbonyl)hydroxylamine (**13**) using TPP and DEAD in THF at room temperature gave the compound **31** in 92 % yield (Scheme 11). Subsequent treatment of **31** with ammonia in methanol effected the two reactions. In the first place the urethane group (NCOOPh) was converted into urea (N-$CONH_2$) whereas benzoate group (OCOPh) was ammonolysed to hydroxy, thus providing the requisite product **32** (7-membered *trans*-analogue of CMI 977) as a crystalline solid with 98 % purity by HPLC. The structure **32** was fully assigned by ^{1}H NMR, ^{13}C-NMR and HRMS spectral analysis.

Scheme 12



Similarly, *cis*-isomer **30** was also converted into final target **33** as depicted in scheme 12, which was fully characterised by spectral data.

In summary, the stereoselective syntheses of the 7-membered analogues of CMI-977 (**1**) have been achieved efficiently. The highlights of the synthetic strategy include regio-selective oxidation of hydroxy group and the adventurous ring closure of open chain aldehyde to 2-benzenesulfonyl oxepane derivative with $PhSO_2H$.

The seven membered analogues of CMI-977(**1**), namely *trans*-isomer (**32**) and *cis*-isomer (**33**) were obtained as a colorless crystalline solid after multi-step synthesis. This compounds were then tested for Leukotriene B_4 inhibition in the human whole blood assay. Test compound activity was determined as per Cayman LTD enzyme immunoassay (EIA) and evaluated as IC_{50} [nM]. The cis isomer **33** had an IC_{50} of 518 nM where as its trans isomer **32** with an 1339 nM. It is really surprising that *cis*-isomer shows better activity than the *trans*-isomer, thereby establishing the new paradigm in the pathology of asthma, where in generally, it has been proven that *trans* substituted compounds (mostly 5-membered) are the most effective compounds with inhibitory activity.

EXPERMENTAL SECTION

(2*S*)-7-Benzyloxy-1-(4-fluorophenoxy)heptan-2-ol (24).

To a suspension of magnesium (1.4 g, 57.6 mmol) in dry THF (25 mL) was added 1,2-dibromoethane (0.2 mL) dropwise followed by a solution of 4-benzyloxy-1-bromo butane (7 g, 28.8 mmol) in dry THF (25 mL) slowly at room temperature. The reaction mixture was stirred for 1 h, cooled in an ice-salt bath and then CuCN (50.0 mg, 0.57 mmol) was added followed by a solution of (S)-4-fluorophenyl glycidyl ether (**4**) (2.9 g, 17.3 mmol) in dry THF (30 ml). The reaction was stirred for 15 min and quenched with saturated aqueous ammonium chloride solution at 0 ^{o}C. THF was removed under vacuum and the residue partitioned between EtOAc and water. The organic layer was successively washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified on silica gel column chromatography using EtOAc-hexane (1:6) as eluent to give **24** (5.8 g, 73%) as colourless liquid.

$[\alpha]_D$ +12 (c 2.2, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 Hz): δ 1.35-1.69 (m, 8H, 4 x CH_2), 3.45 (t, *J* = 6.25 Hz, 2H, C$\underline{H}_2$OBn), 3.71-3.95 (m, 3H, OC$\underline{H}_2$, C$\underline{H}$OH), 4.48 (s, 2H, OC$\underline{H}_2$Ph), 6.82 (m, 2H, Ar), 6.95 (m, 2H, Ar), 7.27-7.35 (m, 5H, Ph).

FABMS *m/z* (rel.intensity): 332 (M^+, 32), 261 (14), 207 (43), 181 (90), 125 (61), 112 (78), 107 (100).

HRMS (FAB): Calcd. for ($C_{20}H_{25}FO_3$, M^+): 332.1787. Found 332.1803.

(6*S*)-7-(4-Fluorophenoxy)heptane-1,6-diol (25).

A solution of **24** (5.8 g, 17.5 mmol) in ethanol (30 mL), containing10% of Pd/C (100 mg) was stirred under H_2 atmosphere for 3 h. The reaction mixture was filtered through celite, washed with ethanol and concentrated. The residue was purified by silica gel column chromatography using EtOAc-hexane (1:1) to afford **25** (3.92 g, 93%) as viscous liquid.

$[\alpha]_D$ +12.5° (c 3.1, $CHCl_3$).

^1H NMR (200 MHz, $CDCl_3$): δ 1.29-1.69 (m, 8H, 4 x CH_2), 3.65 (t, *J* = 6.8 Hz, 2H, C$\underline{H}_2$OH), 3.82-4.02 (m, 3H, OCH_2, CHOH), 6.82 (m, 2H, Ar), 6.94 (m, 2H, Ar).

EIMS *m/z* (rel.intensity): 242 (M^+, 9), 126 (13), 112 (100), 95 (31), 43 (78).

HRMS (EI): Calcd. for ($C_{13}H_{19}FO_3$, M^+): 242.1318. Found 242.1319.

(6*S*)-7-(4-Fluorophenoxy)-6-hydroxyheptanal (26).

To a solution of **25** (3.6 g, 14.8 mmol) in dry THF (60 mL) was added dropwise a solution of IBX (5 g, 17.8 mmol) in dry DMSO (4 mL) over a period of 15 min. at room temperature. After 15 min. the reaction mixture was diluted with ice water, filtered through celite and concentrated. The residue was extracted with diethyl ether, washed with brine, dried over Na_2SO_4 and the organic solvent removed under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc-hexane (1:9) to give **26** (2.2 g, 62 %) as viscous liquid.

$[\alpha]_D$ +12.0 (c 3.77, $CHCl_3$).

IR (neat): 3694-3200 (br), 2941, 1684, 1498, 1208, 824 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 1.4-1.8 (m, 6H, 3 x CH_2), 2.49 (t, *J* = 5.47 Hz, 2H, C$\underline{H}_2$CHO), 3.71-4.05 (m, 4H, OC$\underline{H}_2$, C$\underline{H}$O$\underline{H}$), 6.85 (m, 2H, Ar), 6.94 (m, 2H, Ar), 9.8 (s, 1H, CHO);

FABMS *m/z* (rel.intensity): 240 (M^+, 37), 223 (80), 205 (18), 154 (37), 136 (65), 125 (58), 109 (100).

HRMS (FAB): Calcd. for ($C_{13}H_{17}FO_3$, M^+): 240.1161. Found 240.1164.

(2*RS*, 7*S*)-2-(Benzenesulfonyl)-7-(4-fluorophenoxymethyl)oxepane (27).

To an ice-cold mixture of benzenesulfinic acid (1.8 g, 12.4 mmol) and $CaCl_2$ (1.4 g, 12.5 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise a solution of **26** (2 g, 8.3 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 3 h and filtered through celite and washed with CH_2Cl_2. The combined organic layer was washed with saturated aqueous

Na_2CO_3, water, brine and dried over Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using EtOAc-hexane (1:6) as eluent to give **27** (2.45 g, 80.8%) as white solid.

M.P. 126- 128 ^oC.

^1H NMR ($CDCl_3$, 200 MHz): δ 1.43-2.20 (m, 7H, 3xCH_2, ½CH_2), 2.5 (m, 1H, ½CH_2), 3.62 (dd, *J* = 5.84, 10.75 Hz, 1H, OCH_2), 3.82 (dd, *J* = 4.49, 10.75 Hz, 1H, OCH_2), 4.45 (m, 1H, H-7), 4.72 (dd, J= 6.6, 10.75 Hz, 1H, H-2), 6.75 (m, 2H, Ar), 6.95 (m, 2H, Ar), 7.49 (m, 3H, Ph), 7.91 (m, 2H, Ph).

(2*S*,7*S*)-7-(4-Fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)oxepane (29) and (2*R*,7*S*) -7-(4-fluorophenoxymethyl)-2-(1-hydroxy-3-butyn-4-yl)oxepane (30).

Compound **27** (2.2 g, 6.0 mmol) was converted to (2*RS*,7*S*)-7-(4-fluorophenoxy methyl)-2-(1-tetrahydropyranyloxy-3-butyn-4-yl)tetrahydropyran (**28**) using 4-tetrahydro pyranyloxy-1-butynylmagnesium bromide (prepared from 1.86 g/ 12.0 mmol of 4-tetrahydro pyranyloxy-1-butyne, 1.85 g/15.0 mmol of isopropyl bromide and 0.58 g of Mg) and anhydrous $ZnBr_2$ solution (1 M in THF, 7.25 mL, 7.2 mmol) in THF by using the same procedure described earlier for the preparation of **10**. The crude product was treated with pTSA (25 mg) in MeOH (10 mL), neutralized with Et_3N and concentrated. The residue was purified by silica gel chromotography using EtOAc-hexane (1:8) to elute first-low polar *cis* isomer (**30**) (0.26g, 15%) as a solid and then high-polar *trans* isomer (**29**) (1.06g, 60%) as a solid.

Compound **30** (*tras* isomer)

$[\alpha]_D$ -74^o (c 3.63, $CHCl_3$).

IR (neat): 3634-3200 (br), 2909,1498, 1208, 824 cm^{-1}.

^1H NMR ($CDCl_3$, 400 MHz): δ 1.50 (m, 2H, CH_2), 1.76 (m, 2H, CH_2), 1.89 (m, 2H, CH_2), 2.12 (m, 2H, CH_2), 2.48 (dt, *J* = 7.17, 1.4 Hz, 2H, ≡-CH_2), 3.68 (t, *J* = 7.17 Hz, C$\underline{H}_2$OH), 3.81

(dd, *J* = 6.04, 9.32 Hz, 1H, OCH_2), 3.92 (dd, *J* = 5.11, 9.32 Hz, 1H, OCH_2), 4.16 (m, 1H, H-7), 4.56 (m, 1H, H-2), 6.84 (m, 2H, Ar), 6.95 (m, 2H, Ar);

EIMS *m/z* (rel.intensity): 292 (M^+, 32), 123 (85), 112 (88), 95 (70), 41 (100).

HRMS (EI): Calcd. for ($C_{17}H_{21}FO_3$, M^+): 292.1477. Found 292.1474.

Compound **29** (*cis* isomer)

$[\alpha]_D$ + 26.9 (c 2.2, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.50-2.05 (m, 8H, 4xCH_2), 2.48 (t, *J* = 7.2 Hz, 2H, ≡-CH_2), 3.68 (t, *J* = 7.2 Hz, 2H, C$\underline{H}_2$OH), 3.77 (dd, *J* = 7.2, 10.1 Hz, 1H, OCH_2), 3.88 (m, 1H, H-7), 3.98 (dd, *J* = 6.48, 10.1 Hz, 1H, OCH_2), 4.36 (m, 1H, H-2), 6.83 (m, 2H, Ar), 6.94 (m, 2H, Ar).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 23.13 (CH_2), 25.01 (2 x CH_2), 32.24 (CH_2), 37.86 (≡-CH_2), 60.86 (CH_2OH), 70.26 (OCH_2), 71.66 (C-7), 77.49 (C-2), 81.58, 82.26 (C≡C), 115.45 (CH, Ar), 115.66 (2 x CH, Ar), 115.82 (CH, Ar), 115.91 (CH, Ar), 154.90 (F-C, Ar), 159.65 (O-C, Ar).

(2*S*,7*S*)-7-(4-Fluorophenoxymethyl)-2-[1-*N*,*O*-bis(phenoxycarbonyl)-3-butyn-4-yl]oxepane (31).

The compound **31** was prepared from *trans*-alcohol **29** (0.81 g, 2.9 mmol), PPh_3 (0.92 g, 3.49mmol), *N*,*O*-bis(phenoxycarbonyl)hydroxylamine (0.95 g, 3.49 mmol) and DEAD (0.6 g, 3.49 mmol) in THF (15 mL) by using the same procedure described earlier for the preparation of compound **14**. The product was purified by silica gel column chromatography using EtOAc-hexane (1:9) to give (1.55 g, 92%) as a solid.

$[\alpha]_D$ -46.0° (c 2.4, $CHCl_3$).

IR (neat) 3600-3200 (br), 2941, 1678, 1502, 1215, 824 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.39-2.18 (m, 8H, 4 x CH_2), 2.70 (t, J= 6.9Hz, 2H, ≡-CH_2), 3.70-4.05 (m, 4H, C$\underline{H}_2$OH, OC$\underline{H}_2$), 4.13 (m, 1H, H-7), 4.51 (m, 1H, H-2), 6.76-6.96 (m, 4H,

Ar), 7.13-7.44 (m, 10H, 2 x Ph).

FABMS *m/z* (rel.intensity): 547 (M^+, 23), 410 (5), 300 (10), 206 (19), 151 (19), 95 (42), 77 (100).

(2*R*,7*S*)-7-(4-Fluorophenoxymethyl)-2-[1-*N*,*O*-bis(phenoxycarbonyl)-3-butyn-4-yl] oxepane (34).

The compound **30** was converted to **34** as described above

$[\alpha]_D$ + 11 (c 4.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.47-2.03 (m, 8H, 4 x CH_2), 3.74 (dt, *J* = 1.4, 7.0 Hz, 2H, ≡-CH_2), 3.68-4.08 (m, 5H, H-7, OCH_2, CH_2O), 4.34 (m, 1H, H-2), 6.74-6.99 (m, 4H, Ar), 7.12-7.47 (m, 10H, 2 x Ph).

(2*S*,7*S*)-2-(4-fluorophenoxymethyl)-7-(1-*N*-hydroxyureidyl-3-butyn-4-yl)oxepane (32).

The compound **31** (1.3 g, 2.44 mmol) was converted to **32** using methanolic ammonia solution (10 mL) by using the same procedure described earlier for the preparation of compound **1**, to provide **32** (0.55 g, 65 %) as a colourless solid.

$[\alpha]_D$ -56.0° (c 2.15, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.41-2.18 (m, 8H, 4 x CH_2), 2.51 (dt, *J* = 1.1, 7.1 Hz, 2H, ≡-CH_2), 3.69 (t, J = 7.1 Hz, 2H, CH_2N), 3.81 (dd, *J* = 9.52, 4.76 Hz, 1H, OCH_2), 3.9 (dd, *J* = 5.71, 9.52 Hz, 1H, OCH_2), 4.13 (m, 1H, H-7), 4.51 (m, 1H, H-2), 5.25 (s, 2H, $CONH_2$), 7.02 (m, 4H, Ar), 7.69 (s, 1H, N-OH).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 17.19 (CH_2), 24.59 (CH_2), 27.45 (CH_2), 32.0 (CH_2),37.13 (≡-CH_2), 48.89 (CH_2N), 67.11 (OCH_2), 72.03 (C-7), 72.31 (C-2), 81.57, 82.41 (C≡C), 115.51 (2 x CH, Ar), 115.66 (CH, Ar), 115.81 (CH, Ar), 115.97 (CH, Ar), 154.90 (F-C, Ar), 159.66 (O-C, Ar), 161.78 ($NCONH_2$).

FABMS *m/z* (rel.intensity): 351 (M^++1, 100), 308 (8), 291 (6), 154 (27), 111 (6), 95 (17).

HRMS(FAB): Calcd. for ($C_{18}H_{24}FN_2O_4, M^+ + 1$): 351.1720. Found 351.1736.

(2*R*,7*S*)-7-(4-Fluorophenoxymethyl)-2-(1-*N*-hydroxyureidyl-3-butyn-4-yl)oxepane (33).

The compound **34** was converted to **33** as described above

$[\alpha]_D$ + 32.0° (c 0.5, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.40-1.98 (m, 8H, 4 x CH_2), 2.50 (t, *J* = 7.1 Hz, 2H, ≡-CH_2), 3.57 (t, J = 7.1Hz, 2H, CH_2N), 3.69-3.99 (m, 3H, OCH_2, H-7), 4.33 (m, 1H, H-2), 5.48 (s, 2H, $CONH_2$), 6.74-6.99 (m, 4H, Ar), 8.6 (s, 1H, N-OH).

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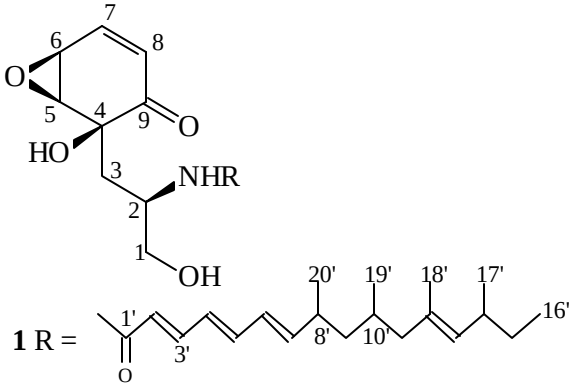
CHAPTER-2

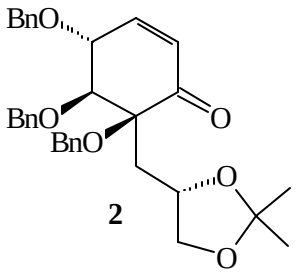
SYNTHETIC STUDIES TOWARD

SCYPHOSTATIN

INTRODUCTION

Scyphostatin (**1**) is a novel neutral sphingomyelinase inhibitor isolated during the search for N-smase inhibitors from the fermentation broth of *Dasyscyphus millissimus* SANK – 13892 by Ogita *et al* in 1997.[1] N-Smase inhibitors are useful in the regulation of ceramide level and thus are of immense need in the therapy of autoimmune diseases and inflammation.[2] This compound is the first low molecular weight inhibitor of the enzyme, either natural sources or of synthetic origin. This natural product is endowed with unprecedented structural features specially a cyclohexenone epoxide moiety in which the tetiary chiral centre at C_4 is linked to an n-propyl amino alcohol under a lipophilic side chain. The initial report[1] did not confirm the absolute configuration of the lipophilic side chain. Later satio *et al*[3] has confirmed the absolute and relative configuration by the degradation of scyphostatin. Recently, Hoye *et al*[4] reported have synthesis of lipophilic side chain, where the synthesis of polar cyclohexanone moiety was largely neglected.





Evidence suggests that tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) employ the sphingomyelin pathway to effect signal transduction by their receptors. This pathway is initiated by hydrolysis of plasma membrane sphingomyelin to ceramide by the action of a sphingomyelinase. Ceramide serves as a second messenger, stimulating a serine/threonine ceramide-activated protein kinase to transduce the cytokine signal, in part

through mitogenactivated protein (MAP) kinase and transcription factors such as NF-$_K$B. The extent to which this signaling system is used in inflammmation, immune responses, and apoptosis is not known, but accumulating evidence suggests that it is a commonly employed pathway that could be exploited therapeutically.

Sphingomyelin is preferentially concentrated in the outer leaflet of the plasma membrane of most mammalian cells, it is comprised of a long chain sphingoid base backbone (predominantly sphingoshine), a fatty acid, and a phosphocholine head group (Figure 1). The fatty acid in amide linkage at the second position of the sphingoid base constitutes ceramide. Hydrolysis of the phosphodiester bond by a sphingomyelinase to yield ceramide and phosphocholine is the only clearly defined mechanism for sphingomyelin degradation in mammalian cells.[5]

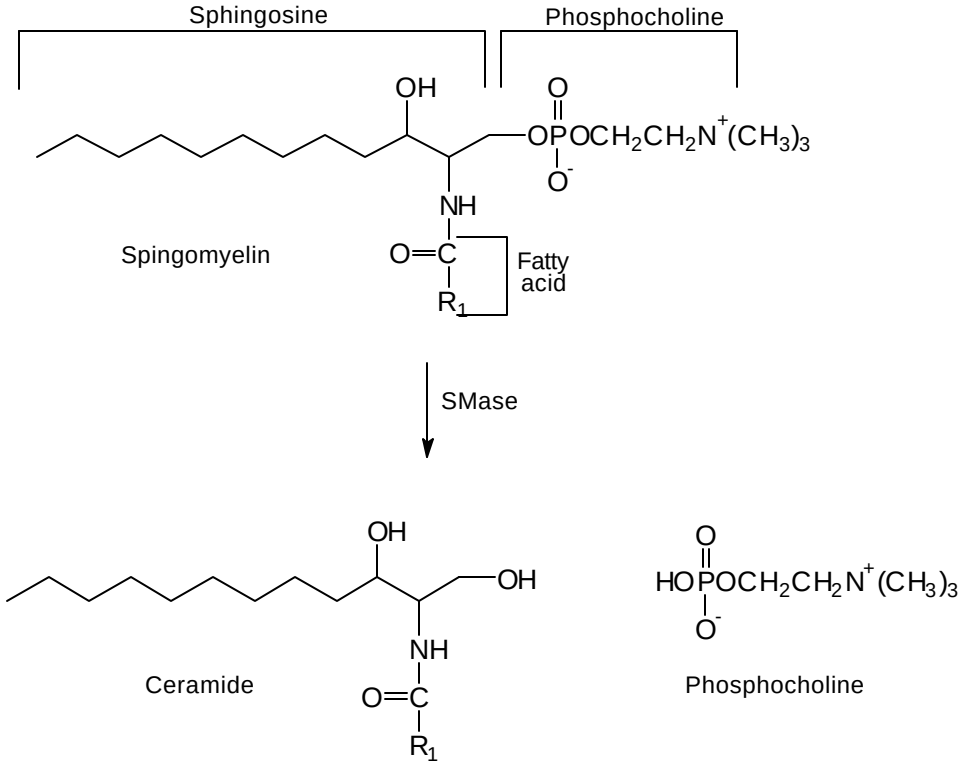


Figure 1. Spingomyelin Hydrolsis to Ceramide mediated by Sphingomyelinase

Sphingomyelin was considered only a structral element of the plasma membrane. However, 1,2-diacylglycerol (DG), a physiologic activator of protein kinase C, stimulated rapid sphingomyelin degradation to ceramide in GH_3 rat pituitary cells. Little of the generated ceramide was deacylated to sphingoid bases, potential inhibitors of protein kinase C,[6] prompting a search for additional derivatives of ceramide. Seveal investigations establisehd the existence of a specific metabolic pathway from sphingomyelin to ceramide 1-phosphate.[7]

The sphingomyelin metabolic pathway is similar to the phosphoinositide signal transduction pathway. The central lipids in these pathways, ceramide and DG, both serve as substrates for the same bacterial DG kinase, implying they posses structural similarity. Their phosphorylated forms, ceramide 1-phosphate and phosphatidic acid were therefore, also structurally similar. Further, neutral sphingomyelinase, the enzyme that initiates the sphingomyelin pathway, is a phospholipase C concentrated in the plasma membrane (Kolesnick, 1991), like the enzyme initiating the phosphoinositide pathway. Ceramide also appears to be an ideal candidate second messenger and it readily redistributes across a membrane bilayer.[8] Since DG utilized a specific kinase, protein kinase C, for signaling, it was considered that ceramide might stimulate a kinase. Ceramide does not activate protein kinase C.

TNFα and interferon-γ, agents that induce monocytic differentiation of HL-60 cells, also stimulated sphingomyelin degradation after 15 min. of stimulation.[9] Evidence was provided that ceramide mediated TNF-induced down-regulation of c-*myc*, an event that may be important for cessation of proliferation during terminal differentiation. Although these studies suggested that ceramide could play a prominent role in TNF-induced monocyte differentiation, the extended time courses implied that these events, even if critical for differentiation were downstream of the initial signaling steps.

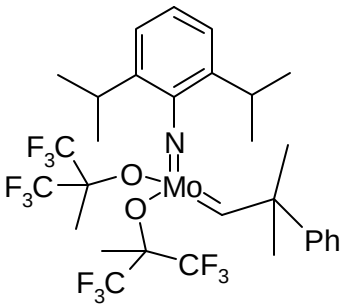
Since TNF induced a physiologic elevation in ceramide levels, its effect on ceramide-activated protein kinases was assessed. HL-60 cells contained an activity indistinguishable

from that defined in A431 cells that increased within minutes of TNF stimulation. Other differentiating agents did not enhance kinase activity. Subsequent investigations showed sphingomyelin degradation to ceramide and stimulation of the kinase within the first minutes of TNF stimulation. Therefore, the cascade of sphingomyelin degradation to ceramide and stimulation of the ceramide-activated protein kinase is initiated early in TNF signaling.

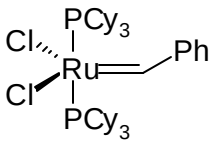
The sphingomyelin pathway appears to have sufficient selectivity for NF-$_K$B translocation to the nucleus, HIV-1 replication, IL-2 transcription, and apoptotic DNA damage to conclude that it is a major signaling mechanism through which TNF and IL-1 induce these events. The specificity for these events is inherent in the structure of ceramide and is manifest even in the most proximal events in this pathway, stimulation of ceramide activated protein kinease and phosphatase. Thus, the sphingomyelin pathway appears to be bonafide-signaling system analogous to the more well defined cAMP and phosphoinositide pathways. This system contains as many as three targets, neutral sphingomyelinase, ceramide activated protein kinase, and perhaps ceramide activated protein phosphatase, for pharmacologic intervention in the processes of host defense, inflammation, and nooplasia.

The synthesis of advanced congenor **2** of Scyphostatin (**1**) was undertaken in lieu of its potent biological activity and novel structure, under the thematic strategy carbohydrate to carbocycle involving ring closing metathesis.

Ring closing metathesis (RCM) has emerged as an attractive tool among synthetic chemists for C=C bond formation.[10] The field has reached to the matured level because of the steadfast advances in recent years. The exotic reasons attributable to this are: i) well defined, stable and highly active catalysts ii) very high TON in the catalytic reaction iii) efficacy in medium to macro-ring cyclisation iv) its superiority over other cyclisations like Diels-Alder, macrolactonisation, etc., because of favourable thermodynamic profile v) adaptability for both



Schrock catalyst (**A**)



Grubbs' catalyst (**B**)

solution phase and solid phase reactions vi) availability of polymer bound catalysts vii) water soluble catalysts enabling the metathesis in water and methanol viii) applicability to broad scope of substrates like ene-yne and yne-yne metathesis, in addition to tri- and tetra-substituted systems ix) ecosafety profile including viability in solvents like $ScCO_2$ and x) compatibility to various functional groups xi) combinatorial RCM libraries. The pioneering efforts of Schrock and Grubbs led to the introduction of their respective catalysts (**A**) and (**B**)

Scheme **1**

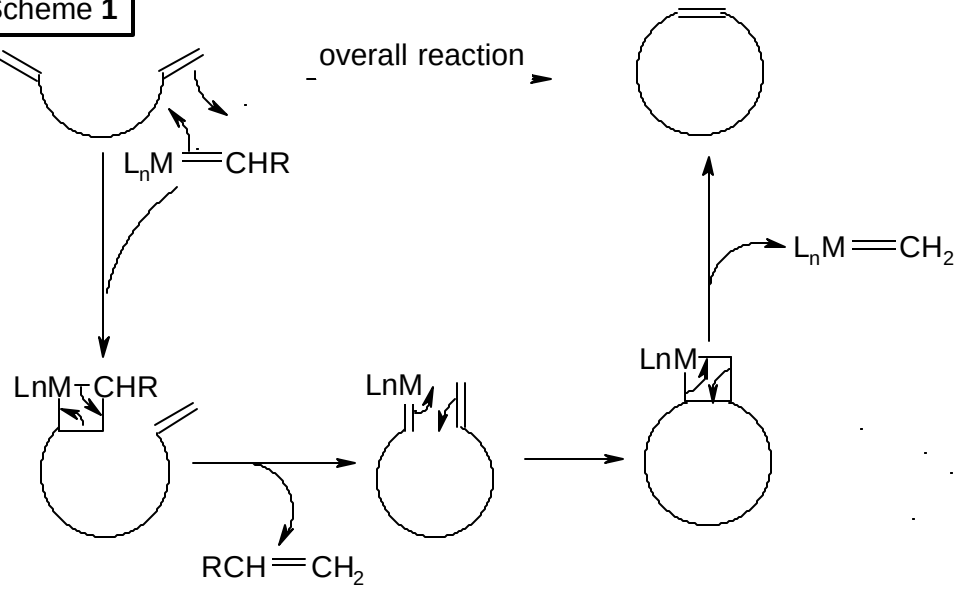


Fig. Ring Closing Metathesis Mechanism

which find widespread use now-a-days, although the discovery and development of new and

Robust catalysts is presently a hot-pursuit.[11] This reaction has changed the strategy of synthetic chemists and it is very common to find RCM as key transformation in the recent total syntheses of natural products, esp., for ring construction.

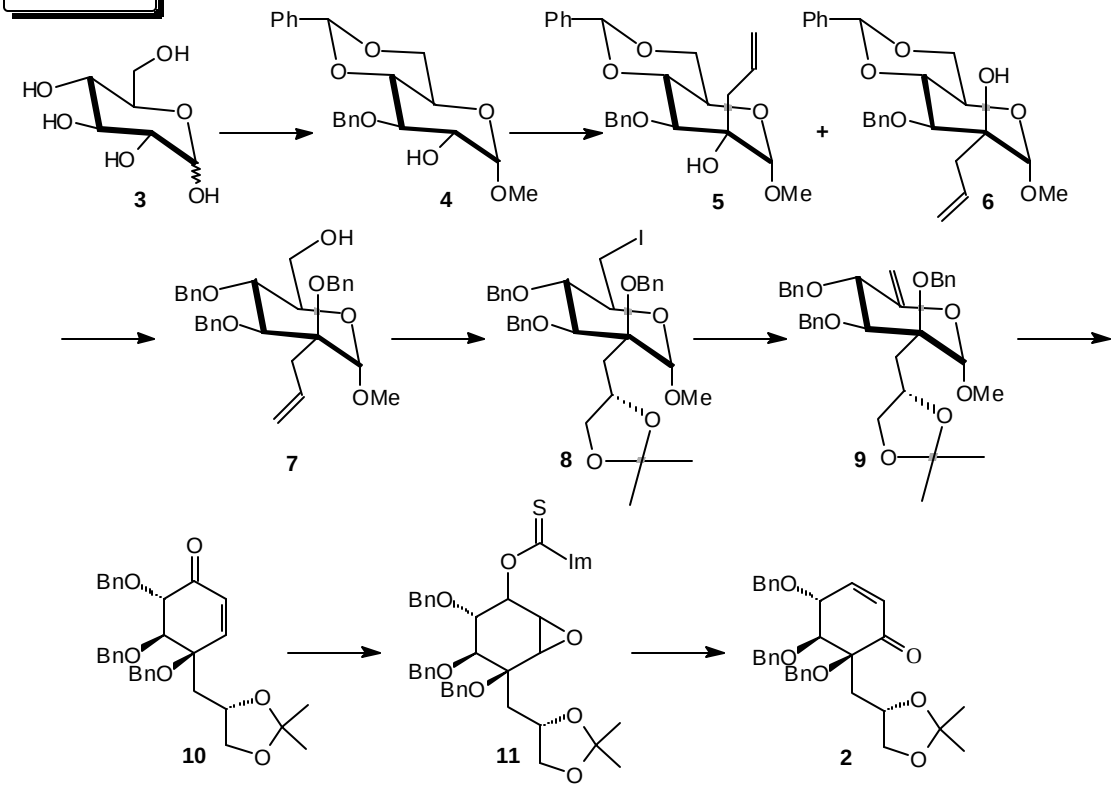
The postulated mechanism involves an iterative process of [2+2] cycloaddition and cycloreversion between the olefins, metal alkylidene and metallocyclobutane species (Scheme 1). The initial retro-type intermolecular [2+2] cycloaddition between the catalyst and one of the olefins of diene leads to the incorporation of the metal alkylidene in the substrate. The second cycloaddition takes place in a facile intramolecular fashion and ring opening of resulting metallocyclobutane leads to the cycloalkene and regeneration of the catalyst. In the first turn of the cycle, the volatile nature of the alkene by-product (the gaseous ethene in most cases) tends the reaction to proceed forward thermodynamically.

PAST WORK

Earlier our group (Gurjar *et al*) reported first synthectic strategy,[12] involving ferrier rearrangment as key step, which is discussed below.

D-Glucose (**3**) was converted into methyl 3-*O*-benzyl-4,6-benzylidene-α-D-glucopyranoside (**4**) whose oxidation at C-2 under Swern conditions, followed by *C*-allylation with allylmagnesium bromide in anhydrous Et_2O/CH_2Cl_2 at -78° C gave a 9:1 diastereomeric mixture of *tertiary* alcohols (**5**) and (**6**) which was separated by silica gel column chromatography. The major product was subjected to NOE studies, which unequivocally proved the structure as **6**. The tertiary hydroxyl group protected as *O*-benzyl derivative followed by hydrogenolysed in the presence of $LiAlH_4$-$AlCl_3$ to give rise to the 4-*O*-benzyl derivative **7**. Compound **8** was obtained in a sequence of three steps. The Ferrier rearrangement of **8** in the presence of $Hg(OAc)_2$ in aqueous acetone gave the carbocyclic derivative **9** whose dehydration in the presence of MsCl-Et_3N-CH_2Cl_2 provided the α, β-

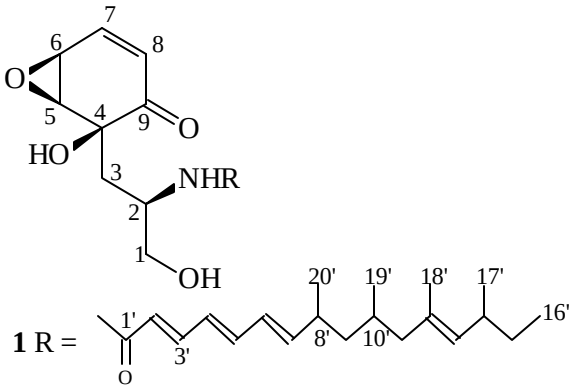
Scheme 2

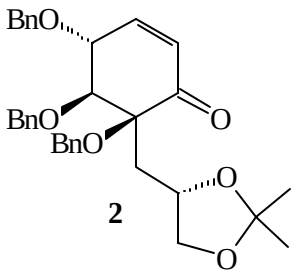


unsaturated derivative **10**. Compound **11** was obtained by epoxidation with 30 % H_2O_2-K_2CO_3, reduction with $NaBH_4$ and protection of the resultant hydroxy group as its thiocarbonate derivative. Treatment of **11** with Bu_3SnH followed by MnO_2 oxidation afforded cyclohexenone segment **2** of Scyphostatin (**1**).

PRESENT WORK

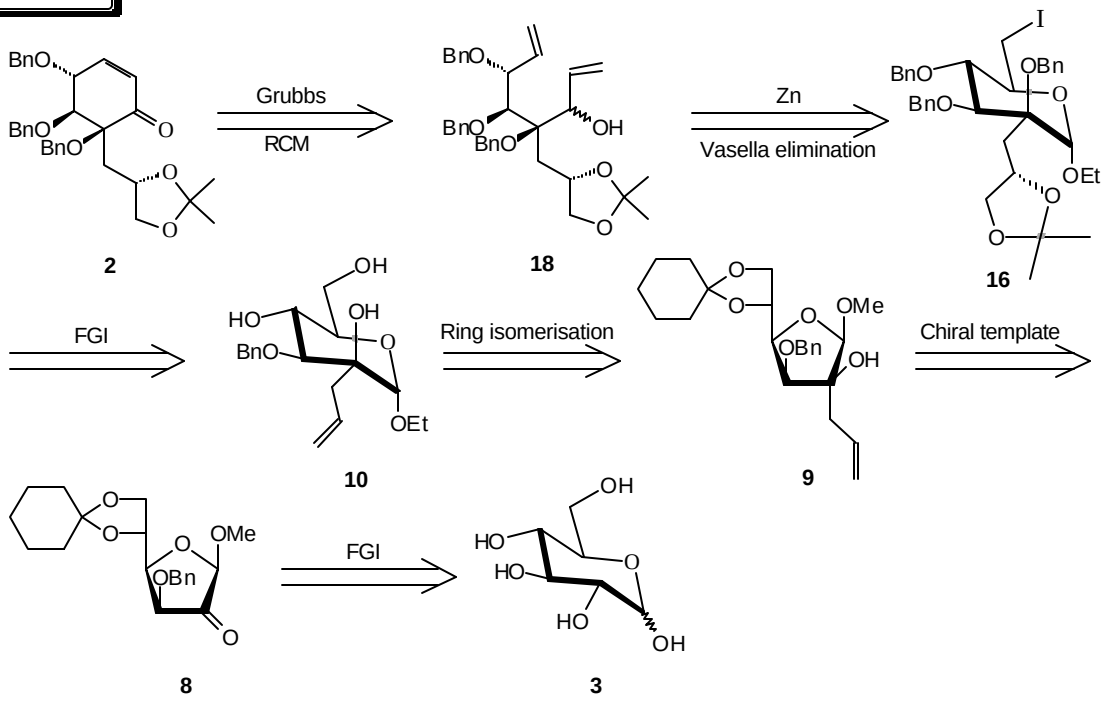
Scyphostatin (**1**) is a novel neutral sphingomyelinase inhibitor isolated during the search for N-smase inhibitors from the fermentation broth of *Dasyscyphus millissimus* SANK –13892. N-Smase inhibitors are useful in the regulation of ceramide level and thus are of immense need in the therapy of autoimmune diseases and inflammation. This natural product is endowed with unprecedented structural features specially a cyclohexenone epoxide moiety in which the tertiary chiral centre at C_4 is linked to an n-propyl amino alcohol under a lipophilic side chain. The synthesis was undertaken in lieu of its potent biological activity and novel structure, which led to realise the advanced congenor of this target, under the thematic strategy carbohydrate to carbocycle, we envisioned to arrive at the target to install the stereochemical features at the appropriate positions through the string of manipulations.





The cyclohexenone derivative (**2**) was chosen as a target because of appropriate functionality related to **1**. The retrosynthetic plan is given in the Scheme 1. The basic design based on ring closing metathesis of diene (**18**). The derivatization of diene by a series of reactions are given in Scheme 1. The particularly interesting aspect is the derivatization of chiral tertiary center following by its manipulation of chiral diol unit. The requisite ketone after a simple FGI should be matched with D-glucose in all respects.

Scheme 1

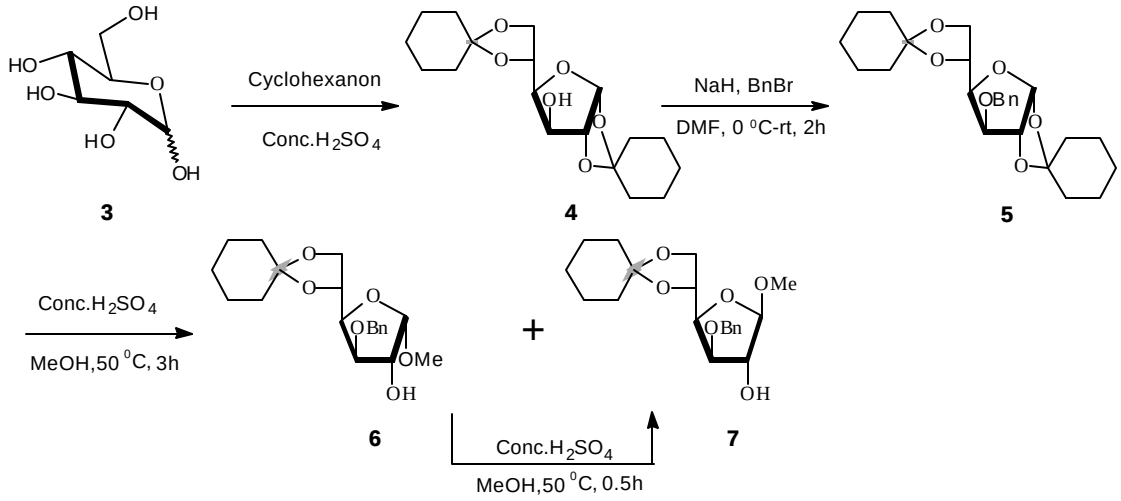


There is a great deal of appreciation for carbohydrates as suitable chiral building blocks in the synthesis of optically active natural products. In carbohydrates, stereochemically well defined carbons are present. In them, we have molecules with different chain lengths and varied functional group distribution, unmatched with any other source of carbon compounds. Due to rigid ring structure, the reaction with carbohydrate precursors occur with conformational and stereochemical control.

D-Glucose (**3**) was chosen as starting material for the current exploration, which was trapped in furanose form as 1,2;5,6-di-*O*-cyclohexylidene-α-D-glucofuranoside (**4**) by known procedure. The free hydroxyl group at C-3 of compound **4** was protected as benzyl ether **5** by conventional method with sodium hydride and benzyl bromide in dry DMF, whose ^{1}H NMR spectrum was comparable with literature source.[13] The cyclohexylidene group at C_1-C_2 was selectively cleaved over C_5-C_6[14] on treating with few drops of conc. H_2SO_4 in MeOH at 50 °C for 3 h to provide methyl 3-*O*-benzyl-5,6-*O*-cyclohexylidene-D-glucofuranoside as a (1:2)

mixture of α and β-anomers, which were separated by silica gel chromatography. The α isomer **6** was converted into the required β isomer **7** on sequential anomeric equilibration as per the above procedure, followed by separation (Scheme 2). The presence of OMe group was clearly indicated by the appearance of singlet at δ 3.36. The anomeric β-configuration was established by its ^{1}H NMR spectrum in which H-1 proton was found as singlet at δ 4.75 and further confirmed by ^{13}C NMR, where the C-1 appeared at δ 109.63.

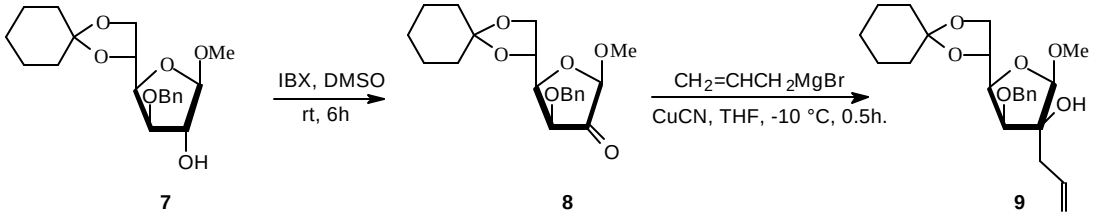
Scheme 2



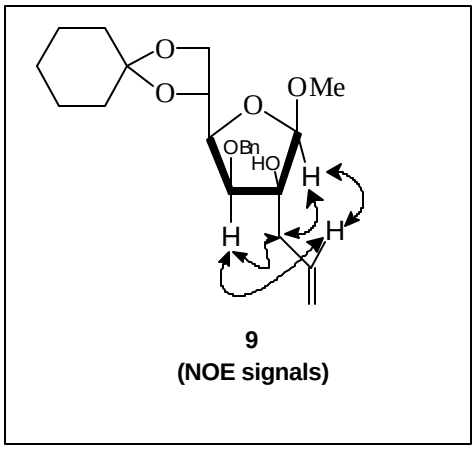
Our next concern was to convert **7** into manno derivative **9** by introducing propenyl group at C-2 carbon. For this endeavour, the hydroxy group at C-2 of **7** was oxidised with IBX in dry DMSO at room temperature for 6 h to provide the 2-ulose derivative (**8**).[15] The Grignard reaction of carbonyl compounds derived from carbohydrates, normally occurs with high degree of stereoselectivity through steric control exerted by adjacent chiral centres.[16] The carbonyl derivative (**8**) was treated with freshly prepared allylmagnesium bromide at -10 °C to provide the single diasteromeric product **9** in overall 78% yield for the two steps (Scheme 3). In contrast, a mixture of 1:1 diastereomers was observed in the case allylation of the corresponding α-anomer of the 2-ulose derivative. The all β-configuration 2-ulose derivative

(**8**) would have directed the allyl nucleophile for exclusive α-facial selectivity, whereas the absence of selectivity in α-anomer can be accounted for mixed configuration, thereby allowing equal facial exposure. The structure of **9** was substantiated by ^{1}H NMR spectrum in

Scheme 3



7 **8** **9**



9
(NOE signals)

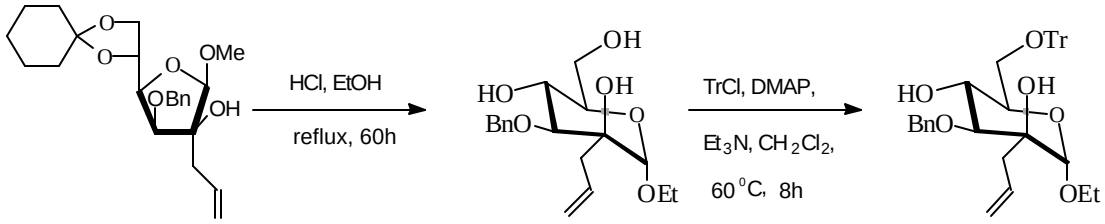
which the characteristic signals were observed due to the two terminal olefin protons at δ 5.15 (m) and internal olefinic proton as multiplet at δ 5.92 (m), whereas two allylic protons at δ 2.27 and 2.37 as double doublet. The rest of the protons resonated at their expected chemical shift values. The manno configuration of **9** was confirmed by extensive NOE and NOESY studies (Scheme 3).

Having established the absolute stereochemistry at C-2 center, our next effort was to convert mannofuranose derivative to pyranose derivative. Accordingly, compound **9** was refluxed with saturated methanolic HCl for prolonged duration (60 h),[17] no ring isomerisation was observed but only the anomeric equilibration to provide the anomeric mixture of

furanoside with concomitant deprotection of cyclohexylidene group. This furanose form chemically confirmed as it succumbed to sodiumperiodate oxidation condition implying the presence of *vic*-diol.

The desired transformation was finally met by changing the solvent from methanol to ethanol i.e. compound **9** on kinetic equilibration with refluxing ethanolic HCl for 60 h provided the mannopyranoside derivative (**10**) as a single product in 75 % yield. The structure of **10** was elucidated by its ^{1}H NMR spectrum in which the anomeric proton resonated as a singlet at δ 4.42 along with the methyl group in the upfield region as a triplet at δ 1.55 of anomeric ethoxy group. The resonances due to the rest of the protons were in conformity with the assigned structure. To further confirm the pyranose structure, compound **10** was treated with silica gel supported sodium periodate in CH_2Cl_2, no reaction was observed,

Scheme 4

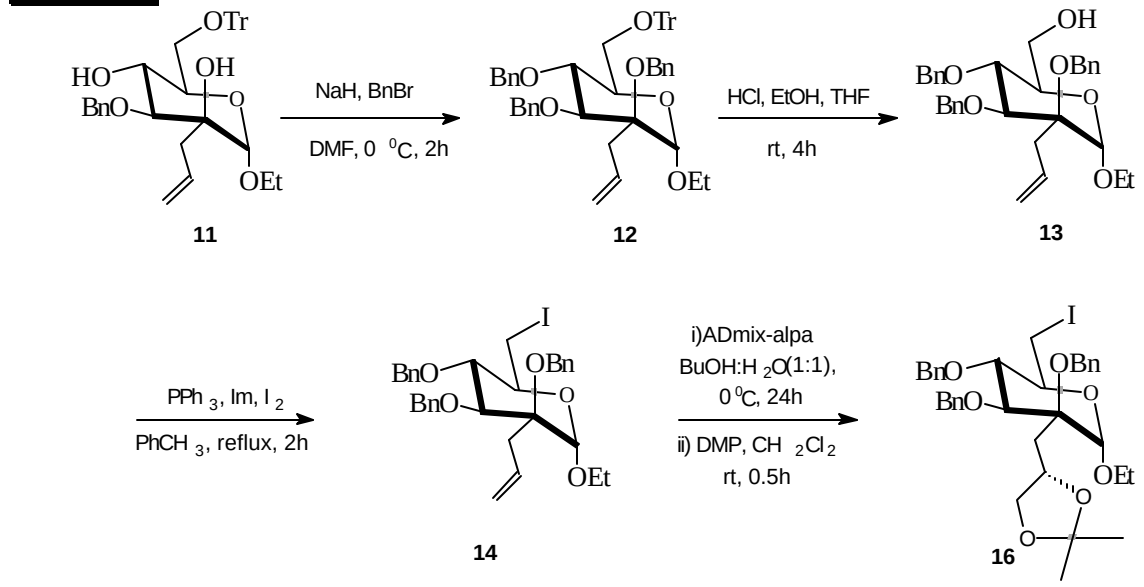


9 **10** **11**

indicating the absence of vic-diol and presence of pyranose form. In addition, structure was confirmed as α-anomer by its ^{13}C NMR, in which the characteristic anomeric carbon resonated at δ 100.35. The rest of the carbons resonated at their expected positions. The molecular formula of the compound was further confirmed by elemental analysis (Calcd. for $C_{18}H_{26}O_6$: C, 63.90; H, 7.69. Found: C, 63.60; H, 8.21). The selective primary tritylation[18] was achieved by treatment of compound **10** with tritylchloride, triethylamine, DMAP (cat.) in dry CH_2Cl_2 followed by stirring at 60 °C for 8 h provided the trityl derivative **11** in 94% yield

(Scheme 4). In the ^{1}H NMR spectrum of **11**, the peaks corresponding to aromatic protons due to trityl and benzyl group were observed in the region of δ 7.12-7.41.

Scheme 5



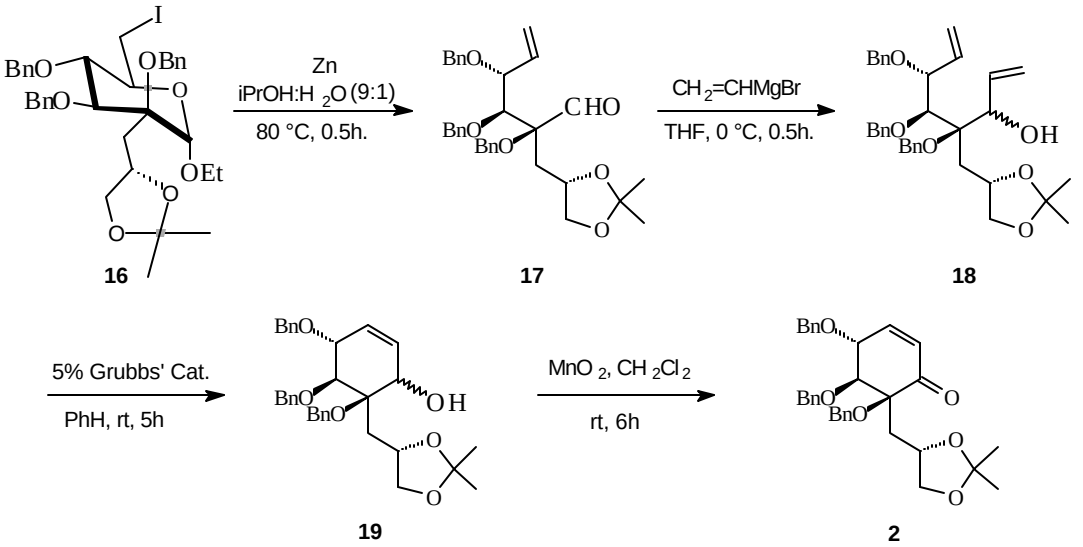
Dibenzylation of the hydroxyl groups (C-2 and C-4) in **11** gave the fully protected derivative **12** using sodium hydride and benzyl bromide in dry DMF for 2 h in 90% yield. The structure was confirmed by its ^{1}H NMR and ^{13}C-NMR spectral studies. The detritylation was achieved by treating **12** with 1M HCl in mixture of ethanol/THF (1:1) for 4 h to provide 6-hydroxy derivative **13** (Scheme 5). In the ^{1}H NMR spectrum the conspicuous absence of peaks due to trityl group were noted. The primary hydroxyl group in compound **13** was converted into its iodo derivative **14** with TPP-I_2-Imidazole in toluene at reflux for 2 h in 95 % yield.[19] The structure was elucidated by its ^{1}H NMR and ^{13}C NMR spectrum. For example, signal due to C-6 carbon was observed in upfield region at 7.42 ppm in ^{13}C-NMR spectrum. Further elemental analysis accounted for the molecular formula of structure (Calcd. for $C_{32}H_{37}IO_5$: C, 61.14; H, 5.89; I, 19.16. Found: C, 60.95; H, 6.10; I, 18.75).

Catalytic asymmetric dihydroxylation of **14** in *tert*-butanol and water in the presence of AD-mix-α gave a single product **15**, whose stereochemical assignment was based on

hypothesis reported by Sharpless *et al*.[20] The diol **15** was protected as its acetonide derivative **16** with catalytic amount of pTSA in DMP and CH_2Cl_2. The ^1H NMR spectrum of **16** revealed the presence of two singlets at δ 1.29, 1.38 ppm due to isoproplyidene group. In addition, molecular formula of the structure was confirmed by elemental analysis (Calcd. for $C_{35}H_{43}IO_7$: C, 59.82; H, 6.12; I, 18.09. Found: C, 60.10; H, 6.32; I, 17.95).

It is pertinent to mention that after this stage the further work was carried out along with my colleague Shri Hotha Srinivas and it would also form a part of his thesis. The details of the work will be presented there. The iodo derivative **16** on exposure to activated Zn in iPrOH : H_2O (9:1) at 80°C for 30 min. provided the alkenal derivative **17**.[21] The presence of aldehyde proton was clearly indicated by the appearance of a singlet at δ 9.85 in its ^1H NMR spectrum. The resonances due to the rest of the protons were in conformity with the assigned structure. The reaction of aldehyde with vinylmagnesium bromide afforded the di-ene derivative **18**.

Scheme 6



In the ^1H NMR spectrum of **18**, signals due to terminal olefins were clearly observed in the region of 5-6 ppm. The ring closing metathesis reaction of **18** with Grubbs' catalyst[22]

followed by allylic oxidation[23] with MnO_2 imparted in the cyclohexenone **2** which carries the thumb impression of the target molecule **1** (Scheme 6). The ^1H NMR spectrum displayed the characteristic α, β unsaturated olefinic protons at δ 5.96 (dd) and 6.85 (dd). The resonance due to the rest of protons were in conformity with the assigned structure. In addition, structure of **2** was confirmed by ^{13}C NMR spectrum in which the characteristic olefinic carbons along with carbonyl carbon signals were located at δ 83.19, 146.99 and 193.98 respectively. Further elemental analysis accounted for the molecular formula of structure (Calcd. for $C_{33}H_{36}O_6$: C, 75.0; H, 6.82. Found: C, 74.91, H, 7.10). Further studies are underway to achieve the total synthesis.

In summary, the realm of chiron approach has elegantly been implemented to realise the advanced template **2** for scyphostatin **1**. The construction of diene derivative through inheritance of natural chirality from D-glucose and Vasella elimination laid the ground for subsequent carbocycle under the repertoire of RCM. The future work should relay on this foundation to reach the original ensemble through appropriate exploitation.

EXPERIMENTAL SECTION

Methyl (R) 3-*O*-benzyl-5,6-*O*-cyclohexylidene-â-D-glucofuranoside (7).

Compound **5** (6.6 g, 16.3 mmol) was taken in saturated methanolic HCl (50 mL) and gently heated for 3 h at 50 ^{o}C. The reaction mixture was quenched with triethyl ammine and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried ($NaSO_4$) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:6) to give **7** (4.14 g, 60 %) as viscous liquid.

$[\alpha]_D$ -41.1 ^{o} (c 1.6, $CHCl_3$).

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.60 (m, 10H, 5 x CH_2), 2.65 (br s, 1H, OH), 3.36 (s, 3H, OCH_3), 3.88 (dd, *J* = 1.95, 4.88 Hz, 1H, H-4), 4.03 (m, 2H, CH_2-6), 4.16 (s, 1H, H-2), 4.34 (m, 2H, H-3, H-5), 4.64 (d, *J* = 2.4 Hz, 2H, C$\underline{H}_2$Ph), 4.75 (s, 1H, H-1), 7.32 (m, 5H, Ph).

EIMS *m/z* (rel. intensity): 364 (M^+, 92), 321 (25), 289 (59), 248 (57), 217 (59), 199 (92), 91 (100).

^{13}C-NMR ($CDCl_3$, 75 MHz):δ 23.71 (CH_2, Cy), 23.90 (CH_2, Cy), 25.06 (CH_2, Cy), 34.79 (CH_2, Cy), 36.20 (CH_2, Cy), 55.58 (OCH_3), 66.56 (OCH_2, C-6), 72.09 (O$\underline{C}H_2$Ph), 73.37 (OCH), 78.71 (OCH), 82.22 (OCH), 82.74 (OCH), 109.44 (OCO, Cy), 109.69 (OCHO, C-1), 127.54 (3 x CH, Ph), 128.21 (2 x CH, Ph), 137.86 (C, Ph).

Anal. Calcd. for $C_{20}H_{28}O_6$: C,65.93; H, 7.69. Found: C, 65.58; H, 7.98.

Methyl (*R*) 3-*O*-benzyl-5,6-*O*-cyclohexyledene-2-*C*-propenyl-â-D-mannofuranoside (9).

A mixture of **7** (7.8 g, 21.43 mmol), IBX (7.19 g, 25.71 mmol) in dry DMSO (50 mL) was stirred at r.t. for 6 h. The reaction mixture was then poured into water (100 mL) and filtered through celite, washed with diethyl ether (100 mL).The aqueous layer extracted with diethyl ether, the combined organic extracts dried ($NaSO_4$) and concentrated. The crude keto compound **8** was taken in dry THF (100 mL) and cooled to –10 ^{o}C to which Allyl magnesium

bromide [prepared from1.0g (42.85 mmol) of magnesium and 3.88 g (32.2 mmol) of allyl bromide in dry ether (60 mL)] was added dropwise. The reaction mixture was stirred for 30 min., quenched with saturated NH_4Cl solution and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried ($NaSO_4$) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:19) to give **9** (6.75 g, 78 %) as a syrupy liquid.

$[\alpha]_D$ -27.6^o (c 2.56, $CHCl_3$).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.65 (m, 10H, 5xCH_2), 2.27 (dd, *J* = 7.3, 14.3 Hz, 1H, CH_2-$CH{=}CH_2$), 2.37 (dd, *J* = 5.3, 14.3 Hz, 1H, CH_2-$CH{=}CH_2$), 3.38 (s, 1H, OH), 3.40 (s, 3H, OCH_3), 3.70 (d, *J* = 5.4 Hz, 1H, H-3), 4.06 (m, 2H, H-6), 4.14 (m, 1H, H-4), 4.34 (q, *J* = 7.03, 13.26 Hz, 1H, H-5), 4.43 (s, 1H, H-1), 4.73 (q, *J* = 11.64, 16.77, 2H, OCH_2Ph), 5.15 (m, 2H, $=CH_2$), 5.92 (m, 1H, CH=), 7.29- 7.40 (m, 5H, Ph).

^{13}C-NMR ($CDCl_3$, 50 MHz):δ 23.72 (CH_2, Cy), 23.99 (CH_2, Cy), 25.09 (CH_2, Cy), 34.69 (CH_2, Cy), 36.34 (CH_2, Cy), 40.20 (CH_2-$CH{=}CH_2$), 55.45 (OCH_3), 66.43 (OCH_2, C-6), 73.83 (OCH), 74.50 (OCH_2Ph), 79.61 (C, C-2), 81.50 (OCH), 105.37 (OCHO, C-1), 109.24 (OCO, Cy), 118.60 ($=CH_2$), 27.60 (CH, Ph), 127.96 (2 x CH, Ph), 128.18 (2 x CH, Ph), 132.42 (CH=), 137.81 (C, Ph).

EIMS *m/z* (rel. intensity): 404 (M^+, 12), 257 (5), 239 (18), 203 (26), 167 (18), 156 (47), 141 (100), 91 (87).

Anal. Calcd. for $C_{18}H_{26}O_6$: C, 68.31; H, 7.92. Found: C, 67.73; H, 8.16.

Ethyl (*R*) 3-*O*-benzyl-2-*C*-propenyl-a -D-mannopyranoside (10).

Compound **9** (6.6 g, 16.3 mmol) was taken in saturated ethanolic HCl (50 ml) and vigorously refluxed for 60 h. The reaction mixture was quenched with triethyl amine and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4)

and concentrated. The crude product was purified on silica gel using ethyl acetate-light petroleum (1:1) to give **10** (4.14 g, 75 %) as a colourless liquid.

$[\alpha]_D$ +51.4 ^{o} (c 2.0, $CHCl_3$).

^{1}H-NMR ($CDCl_3$, 200 MHz):δ 1.15 (t, *J* = 6.83 Hz, 3H, $OCH_2C\underline{H}_3$), 2.20 (dd, *J* = 9.3, 13.7 Hz, 1H, $C\underline{H}_2CH=CH_2$), 2.44 (dd, *J* = 4.89, 13.7 Hz, 1H, $C\underline{H}_2CH=CH_2$), 3.23-4.07 (m, 10H, OCH_2, H-3, H-4, H-5, CH_2-6, 3 x OH), 4.42 (s, 1H, H-1), 4.79 (dd, *J* = 11.23 Hz, 2H, $OC\underline{H}_2Ph$), 4.97 (m, 2H, $CH=C\underline{H}_2$), 5.73 (m, 1H, $C\underline{H}=CH_2$), 7.23- 7.41 (m, 5H, Ph).

^{13}C-NMR ($CDCl_3$, 50 MHz):δ 14.95 ($OCH_2\underline{C}H_3$), 39.71 ($\underline{C}H_2CH=CH_2$), 61.25 (CH_2OH), 63.24 (OCH_2CH_3), 67.66 (CHOH, C-4), 71.96 (CHOH, C-5), 75.78 (C-OH, C-2), 76.42 ($O\underline{C}H_2Ph$), 83.53 (CHOBn, C-3), 100.35 (OCHO, C-1), 118.17 ($CH=\underline{C}H_2$), 127.94 (CH, Ph), 128.24 (2 x CH, Ph), 128.43 (2 x CH, Ph), 132.85 ($\underline{C}H=CH_2$), 138.10 (C, Ph).

EIMS *m/z* (rel. intensity): 338 (M^+, 6), 247 (6), 203 (38), 184 (28), 161 (11), 131 (14), 91 (100).

Anal. Calcd. for $C_{18}H_{26}O_6$: C,63.90; H, 7.69. Found: C, 63.60; H, 8.21.

Ethyl (*R*) 3-*O*-benzyl-2-*C*-propenyl-6-*O*- trityl- a -D-mannopyranoside (11).

A mixture of compound **10** (4.0 g, 11.8 mmol), trityl-chloride (3.95 g, 14.2 mmol), DMAP (143 mg, 1.2 mmol) and triethylamine (2.5 mL, 17.8 mmol) in CH_2Cl_2 (50 mL) were stirred for 8 h at 60 ^{o}C. TLC showed complete consumption of starting material. The reaction mixture was then poured into water, extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:19) to give **11** (6.45 g, 94 %) as viscous liquid.

$[\alpha]_D$ +14.8 ^{o} (c 2.05, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.15 (t, 3H, *J*=6.96 Hz, $OCH_2\underline{C}H_3$), 2.29 (dd, *J* = 8.8, 13.9 Hz, 1H, $\underline{C}H_2CH=CH_2$), 2.51 (dd, *J* = 5.5, 13.9, 1H, $\underline{C}H_2CH=CH_2$), 3.34 (m, 3H, 2 x H-6, H-5),

3.43 (d, *J* = 8.79 Hz, 1H, H-3), 3.66 (m, 2H, $OC\underline{H}_2CH_3$), 3.83 (t, *J* = 9.2 Hz, 1H, H-4), 4.46 (s,1H, H-1), 4.74 (dd, *J* = 11.7, 12.83 Hz, 2H, $OC\underline{H}_2Ph$),5.03 (d, 2H, *J*=12.8 Hz, $CH=C\underline{H}_2$), 5.84 (m, 1H, $C\underline{H}=CH_2$), 7.12- 7.41 (m, 20H, 4xPh).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.97 ($OCH_2\underline{C}H_3$), 39.77 ($\underline{C}H_2CH=CH_2$), 63.19 ($O\underline{C}H_2CH_3$), 64.98 ($\underline{C}H_2OTr$, C-6), 69.92 (CHOH, C-4), 71.57 (OCH, C-5), 75.08 (C-OH, C-2), 75.84 ($O\underline{C}H_2Ph$), 82.78 ($\underline{C}HOBn$, C-3), 87.16 ($O\underline{C}(Ph)_3$), 100.16 (OCHO, C-1), 118.23 ($CH=\underline{C}H_2$), 127.02 (3 x CH, Ph), 127.81 (6 x CH, Ph), 127.90 (3 x CH, Ph), 128.35 (2 x CH, Ph),128.54 (6 x CH, Ph), 132.91 ($\underline{C}H=CH_2$), 138.36 (C, Ph), 143.60 (3 x C, Tr).

EIMS *m/z* (rel. intensity): 580 (M^+, 3), 503 (6), 489 (9), 337 (57), 291 (100), 259 (61), 243 (74), 165 (37), 91 (23).

Anal. Calcd. for $C_{37}H_{40}O_6$: C,76.55; H, 6.89. Found: C, 76.32; H, 6.99.

Ethyl (*R*) 2, 3, 4-tri-*O*-benzyl-2-*C*-propenyl-6-*O*-trityl- a -D-mannopyranoside (12)

Sodium hydride (650 mg, 60 % dispersion in mineral oil, 27.15 mmol) was added portion wise to an ice-cooled solution of compound **11** (6.3 g, 10.86 mmol) in dry DMF (25 mL), the resulting mixture was left at rt. for 1 h and benzyl bromide (2.8 ml, 23.9 mmol) was added dropwise at O ^oC. The reaction mixture was then poured into water (50ml) and extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:50) to give **12** (7.42 g, 90%) as colourless liquid.

$[\alpha]_D$ −14.4 ^{o} (c 2.5, $CHCl_3$).

^1H-NMR ($CDCl_3$, 300 MHz):δ 1.22 (t, 3H, *J*=6.96 Hz, $OCH_2C\underline{H}_3$), 2.40 (dd, *J* = 8.8, 14.7 Hz, 1H, $C\underline{H}_2CH=CH_2$), 3.10 (dd, *J* = 5.5, 14.7 Hz, 1H, $C\underline{H}_2CH=CH_2$), 3.26 (dd, *J* = 4.4, 9.9 Hz, 1H, H-5), 3.51 (m, 2H, 2 x H-6), 3.80 (m, 3H), 4.16 (t, *J* = 9.51 Hz, 1H), 4.27 (d, *J* = 10.3 Hz, 1H), 4.56 (d, *J* = 10.3 Hz, 1H), 4.66 (d, *J* = 11.7 Hz, 1H), 4.79 (s, 1H, H-1), 4.81 (d, *J*=12.5

Hz, 1H), 4.92 (d, *J* = 11.7 Hz, 1H), 5.12 (m, 2H, CH=C$\underline{H}_2$), 5.90 (m, 1H, C$\underline{H}$=CH$_2$), 7.08-7.58 (m, 30H, 6 x Ph).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 15.14 (OCH$_2\underline{C}$H$_3$), 33.92 ($\underline{C}$H$_2$CH=CH$_2$), 62.92 (O$\underline{C}$H$_2$CH$_3$), 66.05 ($\underline{C}$H$_2$OTr), 72.15 (OCH, C-5), 74.94 (O$\underline{C}$H$_2$Ph), 76.37 (O$\underline{C}$H$_2$Ph), 77.0 ($\underline{C}$HOBn, C-4), 80.12 ($\underline{C}$-OBn, C-2), 84.94 ($\underline{C}$HOBn, C-3), 86.26 (O$\underline{C}$(PhP)$_3$), 100.05 (OCHO, C-1), 117.98 (CH=$\underline{C}$H$_2$), [126.73, 127.10, 127.43,127.69,128.20] (CH, 6 x Ph), 133.05 ($\underline{C}$H=CH$_2$), 137.98 ($\underline{C}$, Ph), 138.90 (C, Ph), 139.74 (C, Ph), 144.15 (3 x C, Tr).

Anal. Cacld. for $C_{51}H_{52}O_6$: C, 80.52; H, 6.84. Found: C, 79.42; H, 7.29.

Ethyl (R) 2, 3, 4-tri-*O*-benzyl-2-*C*-propenyl- a -D-mannopyranoside (13).

Compound **12** (7.3 g, 9.6 mmol) was dissolved in mixture of THF and EtOH (50 mL, 1:1) and 5 ml of 1M HCl was stirred at r.t., for 4 h, . The reaction mixture was quenched with triethyl amine and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate -light petroleum (1:9) to give **13** (4.16 g, 86 %).

$[\alpha]_D$ −3.36 ^{o} (c 3.15, $CHCl_3$).

^{1}H NMR ($CDCl_3$, MHz): δ 1.19 (t, *J* = 6.84 Hz, 3H, OCH$_2\underline{C}$H$_3$), 2.32 (dd, *J* = 8.8, 15.2 Hz, 1H, C$\underline{H}_2$CH=CH$_2$), 3.11 (dd, *J* = 5.4, 15.2 Hz, 1H, C$\underline{H}_2$CH=CH$_2$), 3.40 (m, 1H), 3.73 (m, 5H), 4.03 (t, *J* = 9.3 Hz, 1H), 4.56-5.18 (m, 9H), 5.82 (m, 1H, C$\underline{H}$=CH$_2$), 7.18- 7.38 (m, 15H, 3 x Ph).

^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.92 (OCH$_2\underline{C}$H$_3$), 33.26 ($\underline{C}$H$_2$CH=CH$_2$), 61.75 (CH$_2$OH, C-6), 63.07 (O$\underline{C}$H$_2$CH$_3$), 66.30 (O$\underline{C}$H$_2$Ph), 72.81 (OCH, C-5), 75.02 (O$\underline{C}$H$_2$Ph), 76.23 ($\underline{C}$HOBn, C-4), 76.30 (O$\underline{C}$H$_2$Ph), 80.01 ($\underline{C}$-OBn, C-2), 84.54 ($\underline{C}$HOBn, C-3), 100.12 (OCHO, C-1), 118.13 (CH=$\underline{C}$H$_2$), [127.06, 127.32, 127.51,127.95,128.24] (15 x CH, 3 x Ph), 132.0 ($\underline{C}$H=CH$_2$), 138.02 (C, Ph), 138.61 (C, Ph), 139.19 (C, Ph).

EIMS *m/z* (rel. intensity): 518 (M^+, 5), 427 (17), 381 (20), 337 (7), 293 (48), 231 (64), 181 (100), 91 (57).

Anal. Calcd. for $C_{32}H_{38}O_6$: C, 74.13; H, 7.34. Found: C, 73.65; H, 7.45.

Ethyl (*R*) 2,3,4-tri-*O*-benzyl-2-*C*-propenyl-6-deoxy-6-iodo-a -D-mannopyranoside (14).

A mixture of alcohol **13** (4.0 g, 7.7 mmol), Ph_3P (6.1 g, 23.2 mmol), imadazole (1.6 g, 23.2 mmol) and Iodine (3.9 g, 15.4 mmol) in dry toluene (100 ml) was stirred at reflux for 2 h. The reaction was quenched with saturated $NaHCO_3$ solution and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with NaS_2O_3 solution to remove excess of iodine, dried (Na_2SO_4) and concentrated. The crude product was purifie d on silica gel column chromatography using ethyl acetate-light petroleum (1:50) to give **14** (4.6 g, 95%) as viscous liquid.

$[\alpha]_D$ +13.2 ^{o} (c 2.1, $CHCl_3$).

^{1}H-NMR ($CDCl_3$, MHz): δ 1.21 (t, 3H, *J* = 6.84 Hz, $OCH_2\underline{C}H_3$), 2.33 (dd, *J* = 8.8, 15.2 Hz, 1H $C\underline{H}_2CH=CH_2$), 3.08 (dd, *J* = 5.4, 15.2 Hz, 1H, $C\underline{H}_2CH=CH_2$), 3.26-3.55 (m, 4H), 3.72-3.85 (m, 3H), 4.60-5.17 (m, 9H), 5.82 (m, 1H, $C\underline{H}=CH_2$), 7.18-7.44 (m, 15H, 3 x Ph).

^{13}C-NMR ($CDCl_3$, 50 MHz):δ 7.42 (CH_2I), 14.92 ($OCH_2\underline{C}H_3$), 33.63 ($\underline{C}H_2CH=CH_2$), 63.22($O\underline{C}H_2CH_3$), 66.05 ($O\underline{C}H_2Ph$), 71.67 (OCH, C-5), 75.16 ($O\underline{C}H_2Ph$), 76.30 ($O\underline{C}H_2Ph$), 80.05 ($\underline{C}HOBn$, C-4), 80.38 80.12 ($\underline{C}$-OBn, C-2), 84.57 ($\underline{C}HOBn$, C-3), 100.08 (OCHO, C-1), 118.02 ($CH=\underline{C}H_2$), [126.92, 127.14, 127.39, 127.8,127.98,128.24] (15 x CH, 3 x Ph), 132.54 ($\underline{C}H=CH_2$), 137.94 (C, Ph), 138. 42 (C, Ph), 139.34 (C, Ph).

FABMS *m/z* (rel. intensity): 628 (M^+, 2), 537 (10), 491 (12), 429 (43), 389 (100), 293 (65), 231 (40), 181 (90), 91 (45)

Anal. calcd. for $C_{32}H_{37}IO_5$: C,61.14; H, 5.89, I, 19.16. Found: C, 60.95, H; 6.10, I, 18.75.

Ethyl (*R*) 2,3,4-tri-*O*-benzyl-2-(2,2-dimethoxy-1,3-dioxalanmethyl)-6-deoxy-6-iodo-α-D-mannopyranoside (14).

A mixture of alkene **14** (4.5 g, 7.11 mmol), AD-mix-α (8.25 g) was taken in mixture of ^{t}BuOH & H_2O (70 ml, 1:1). The reaction mixture was stirred vigorously for 24 h at 0 ^{o}C, quenched with $NaHSO_3$ solution and concentrated. The residue was extracted with ethyl acetate, dried ($NaSO_4$) and concentrated to give required diol **15**. The crude compound **15** was dissolved in CH_2Cl_2 (25 ml), DMP (5 ml) and catalytic amount of p-TSA. The reaction mixture was stirred for 30 min. at rt., quenched with triethyl ammine and concentrated. The residue was dissolved in ethyl acetate, washed with water, dried ($NaSO_4$) and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:20) to give **16** (4.14 g, 75 %).

$[\alpha]_D$ +21.5 ^{o} (c 1.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz):δ 1.23 (t, *J*=6.84 Hz, 3H, $OCH_2C\underline{H}_3$), 1.29, 1.38 (2s, 6H, $(C\underline{H}_3)_2C$), 1.76 (dd, *J* = 7.8, 15.2 Hz, 1H), 2.79 (dd, *J* = 3.9, 15.2 Hz, 1H), 3.25-3.51 (m, 5H), 3.72-4.0 (m, 4H), 4.21 (m, 1H), 4.52-5.11 (m, 7H), 7.14- 7.46 (m, 15H).

^{13}C-NMR ($CDCl_3$, 50 MHz):δ 7.31 (CH_2I), 14.92 ($OCH_2\underline{C}H_3$), 25.65, 26.75 (2 x CH_3, $(CH_3)_2C$), 32.45 (CH_2, C-7), 62.92 ($O\underline{C}H_2CH_3$), 66.38 ($O\underline{C}H_2Ph$), 70.46 (CH_2O, C-9), 71.34 (OCH, C-8), 71.56 (OCH, C-5), 75.38 (OCH_2Ph), 76.38 (OCH_2Ph), 79.39 (CHOBn, C-4), 80.12 (C-OBn, C-2), 84.46 (CHOBn, C-3), 100.19 ($O\underline{C}HO$, C-1), 108.02 (OCO), [127.10, 127.54, 127.76, 128.02, 128.35] (15 x CH, 3xPh), 137.76.54 (C, Ph), 138.28 (C, Ph), 139.23 (C, Ph).

FABMS *m/z* (rel. intensity): 702 (M^+, 5), 611 (8), 565 (12), 507 (9), 401 (30), 367 (78), 318 (93), 305 (100), 247 (65), 127 (90), 91 (65).

Anal. Calcd. for ($C_{35}H_{43}IO_7$): C, 59.82; H, 6.12, I, 18.09. Found: C, 60.10, H, 6.32, I, 17.95.

(2S,4S,5S,6*R*)-4,5,6-tri-Benzyloxy-1,2-isopropylidene-4-(1-formyl)-oct-8-en-1,2-diol (17).

The iodo derivative **16** (4 g, 5.69 mmol) was heated to reflux with the activated Zinc (3.6 g, 56.9 mmol) in mixture of 2-propanol and water (9:1) for 0.5 h. The zinc was removed by filteration, washed with diethylether and the combine organic layers were washed with water, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column chromatography using ethyl acetate-light petroleum (1:20) to give **17** (2.56 g, 85 %) as viscous liquid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.24, 1.30 (2s, 6H, $(C\underline{H}_3)_2C$), 1.59 (dd, *J* = 8.03, 17.4 Hz, 1H, H-3), 2.05 (m, 1H, H-3'), 3.25 (t, *J* = 8.20 Hz, 1H, H-1), 3.90 (t, *J* = 7.50 Hz, 1H, H-1'), 4.01 (d, *J* = 3.5 Hz, 1H), 4.10-4.20 (m, 2H), 4.25, 4.56 (Abq, *J* = 11.80 Hz, 2H, $OC\underline{H}_2Ph$), 4.42 (br s, 2H, $OC\underline{H}_2Ph$), 4.73, 4.92 (Abq, *J* = 11.32 Hz, 2H, $OC\underline{H}_2Ph$), 5.30 (m, 2H, $=CH_2$), 5.88 (m, 1H, CH=), 7.22-7.44 (m, 15H), 9.85 (s, 1H, CHO).

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CHAPTER-3

SECTION–I

HYDROLYTIC KINETIC RESOLUTION

OF A STYRENE OXIDE DERIVATIVE: A PRACTICAL

SYNTHESIS OF OPTICALLY PURE (*R*)-(-)-PHENYLEPHRINE

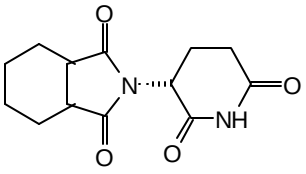
HYDROCHLORIDE

INTRODUCTION

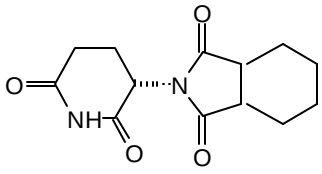
In most cases biologic al systems, recognize the members of a pair of enantiomers as different substances, and the two enantiomers will elicit different responses.[1] The importance of chirality in the context of the biological function has been fully appreciated and therefore efforts toward the development of technology for the synthesis of chiral compounds with commercial viability have been one of the major objectives of many researchers. The *vista* of asymmetric synthesis dates back to 1890, with Emil Fischer's remarkable piece of chemical research on the cyanohydrin reaction in sugar units[2]. Synthetic chemists have ever since taken it as a challenge to induce chirality in a molecule. Among various methods, enantioselective catalysis has brought a revolution in asymmetric synthesis. Seldom has there been an area of chemistry where the scientific goals are so challenging, the economic benefits so obvious, and the ethical reason for doing the research so compelling.

Until recently, it was common practice for a pharmaceutical company to market a chiral drug as the racemate[3]. In many cases the desired activity resides solely in one of the enantiomer[4]. The mirror image enantiomer is either totally inactive or carries some undesirable properties that have been tolerated as an unavoidable necessity.[5] This approach in effect meant that each dose of a drug was contaminated with an equal weight of other isomer, which usually had no therapeutic value, but had the potential to cause unsuspected deleterious side effects. Activities of enantiomers of some drugs are depicted in Table-I. Probably the most well known and tragic example of a drug where the isomers causes serious effects is thalidomide, which was sold in the market in 1960s as a sedative and administered as a racemate. Unfortunately, it was not known during that period the (S)-enantiomer is highly teratogenic and causing profound birth defects in babies born to mother using the drug.[5] In the case of ethambutol, it is the (S,S)-isomer that is an active turberculostatic whereas its (R,R)-enantiomer causes optical neuritis that can result in blindness. Similarly, racemic

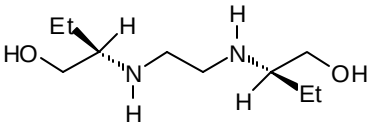
Table 1: Drugs that have different activities for different isomer



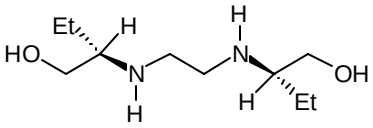
(R)-Thalidomide
Sedative



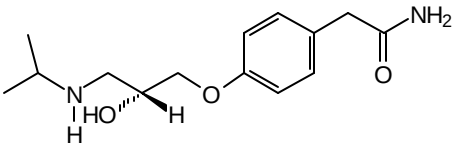
(S)-Thalidomide
Teratogen



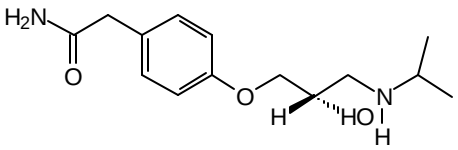
(S,S)-Ethambutol
Tuberculastic



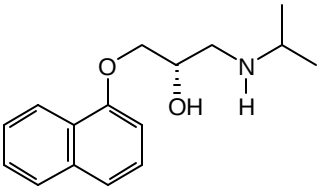
(R,R)-Ethambutol
causes blindness



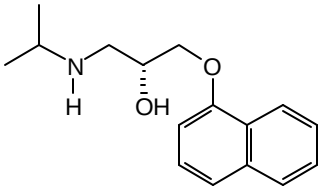
(R)-Atenolol
Hypertension, Angina



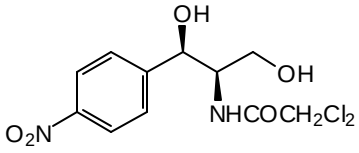
(S)-Atenolol
Lower heart rate



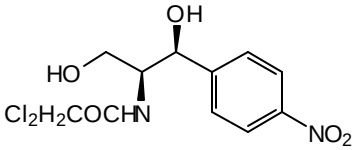
(S)-Propranolol
Beta-Blocker



(R)-Propranolol
Vaginal contraceptive



(R,R)-Chloramphenicol
Antibacterial Typhoid



(S,S)-Chloramphenicol
Inactive

atenolol is presently being marketed for the treatment of hypertension, angina and has shown promise in the treatment of post myocardial infarction where as the (S) isomer has recently been found to present the occasional side effect of a lowered heart rate sometimes encountered with the racemate.

The enantiomer of most drugs are metabolized by different biochemical paths and at different rates. Therefore, it is virtually impossible to follow the biochemical fate and actions of the individual isomers when the dose is a racemic mixture. There are also differences in how individuals react to the R and S forms of a drug.[6]

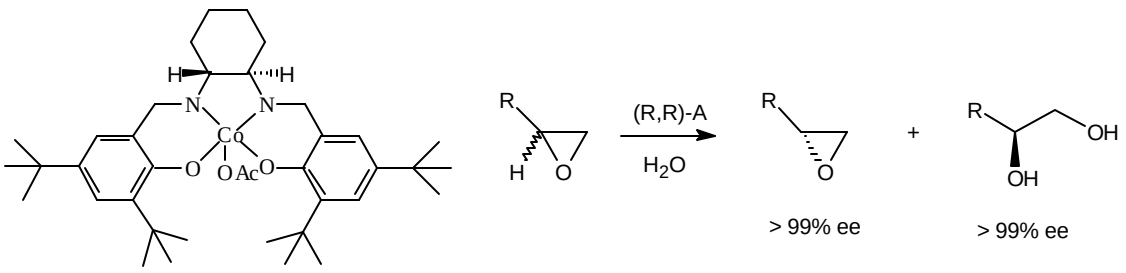
Introduction of new regime of drug substances in consumer market has become increasingly difficult since the implementation of new regulations by Food and Drug Administration, USA.[7] The number of single enantiomer drugs are steadily increasing because of better safety and efficacy of single enantiomer over its racemate. Approval for racemic mixture is almost impossible unless it is proven beyond doubt that the racemate displays no undesirable side effects and is safe for human consumption. Although enantiomers are chemically indistinguishable, the chiral nature of the biological milieu including plasma protein binding sites, enzymes and receptors recognizes them as discrete species leading to pharmacological differences. Examples in table-I clearly demonstrate the chiral recognition.[8]

It is pertinent to mention the different methods to obtain enantiomerically pure compounds, which include classical optical resolution via diastereromers, chromatographic separation of enantiomers, enzymatic resolution, chemical kinetic resolution and asymmetric synthesis.

The enatioseletive technologies, which utilize chemo and bio-catalysts for asymmetric induction can be successfully transferred to the industrial applications, provided they are (i) efficient by route selection (ii) economically viable and (iii) environmentally benign. Catalyst efficiency is also the crucial factor in terms of (i) catalyst selectivity (ee) (ii) catalyst productivity (turn over number) (iii) catalyst activity (turn over factor) and (iv) availabity and cost.There is a great deal of importance attached to the preparation of chiral building blocks as they are versatile starting materials for the preparation of complex bioactive molecules. In

this domain, epoxides due to their ease of formation and ready reactivity towards nucleophiles, are important starting materials and intermediates in organic synthesis. Optically active epoxides have attracted much attention as versatile intermediates[9] for the synthesis of a wide variety of chiral compounds, for example, biologically active compounds[10] such as prococene II derivatives as selective cytotoxic agent of insects[11] or medicinal compounds for the remedy of hypertension and asthma[12] and functional organic materials such as ferroelectric liquids etc.[13] However, terminal epoxides are arguably the most important subclass of these compounds and no general and practical method exists for their production in enantiomerically pure form. Terminal epoxides are available very inexpensively as racemic mixture, and kinetic resolution is an attractive strategy for the production of optically active epoxies, with an economical and operationally simple method. Jacobsen described,[14] a practical route to enantiomerically enriched terminal epoxides following a hydrolytic kinetic resolution using simple chiral cobalt based Salen complex.

Scheme 1



(R,R)-A

This process uses water as the only reagent, no added solvent, and low loadings of a recyclable catalyst (<0.5%). It affords highly valuable enantiomeric enrichment (Scheme 1), which are valuable chiral building blocks of demonstrated utility for organic synthesis.[15] As water is effecting the kinetic resolution, this resolution is named as hydrolytic kinetic resolution (HKR).

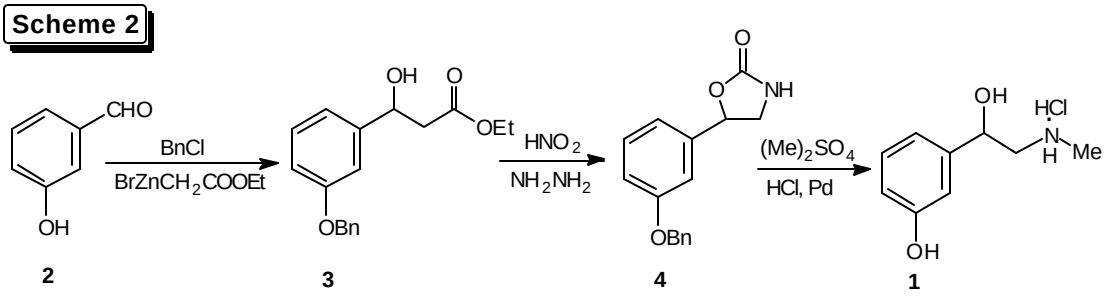
Among drugs, sympathomimetic agents are of potential interest to synthetic organic chemists because they have actions similar to those, which follow stimulation of postaganglionic sympathetic or adrenegic nerves. Most of the phenylethylamine drugs such as dopamine, adrenaline, phenylephrine etc. are having sympathomimetic activity and show effect on cardiac, metabolic as well as central nerves system stimulation.[16]

In the family of sympathomimetic agent, (*R*)-phenylephrine, an adrenergic agent is an important drug with strong α-agonistic activity as a vasoconstrictors and a decongestant. (*R*)-phenylephrine is known to produce mydriacin without cyclopleyia in the eye. It has also been used to maintain blood pressure during anesthesia.[17] Although many nonchiral syntheses of phenylephrine hydrochloride have been reported, the asymmetric synthesis has been largely neglected. This chapter describes the practical synthesis of (R)-phenylephrine hydrochloride, using hydrolytic kinetic resolution of a styrene oxide derivative. It is appropriate to discuss about the related work before describing the present work.

Ernst's approach:

Ernst *et al*[18] synthesised *dl*-phenylephrine using Schroeter's reaction involving isomerisation of *m*-hydroxy acid azides (**3**) to oxazolidones (**4**), which can be manipulated to get dl-phenylephrine. They have synthesised phenylephrine hydrochloride (**1**) starting from m-hydroxy benzaldehyde (**2**) as shown in the Scheme 2.

Scheme 2

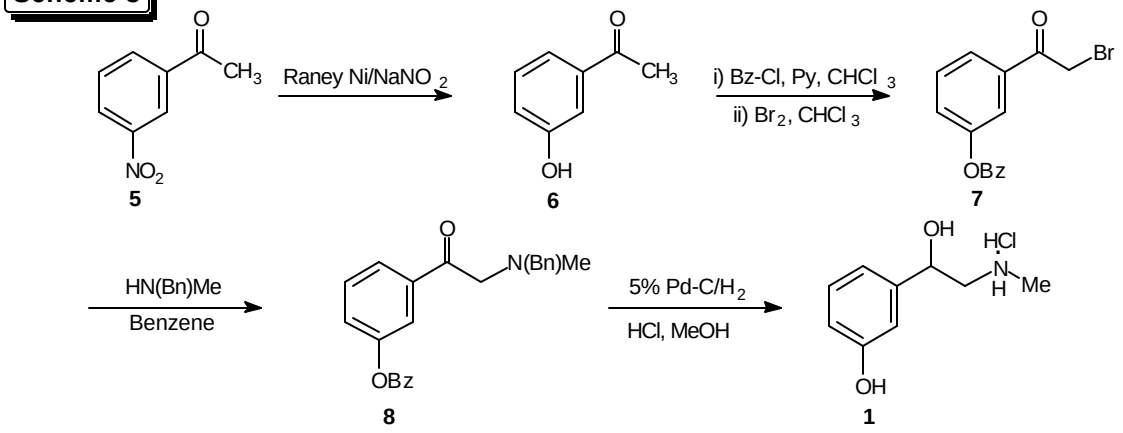


2 **3** **4** **1**

Ravdel's approach:

Ravdel *et al*[19] reported synthesis of dl-phenylephrine hydrochloride (**1**) from *m*-nitroacetophenone (**5**). Compound **5** was converted to *m*-hydroxyacetophenone which in turn was converted to *dl*-phenylephrine as depicted in the Scheme 3.

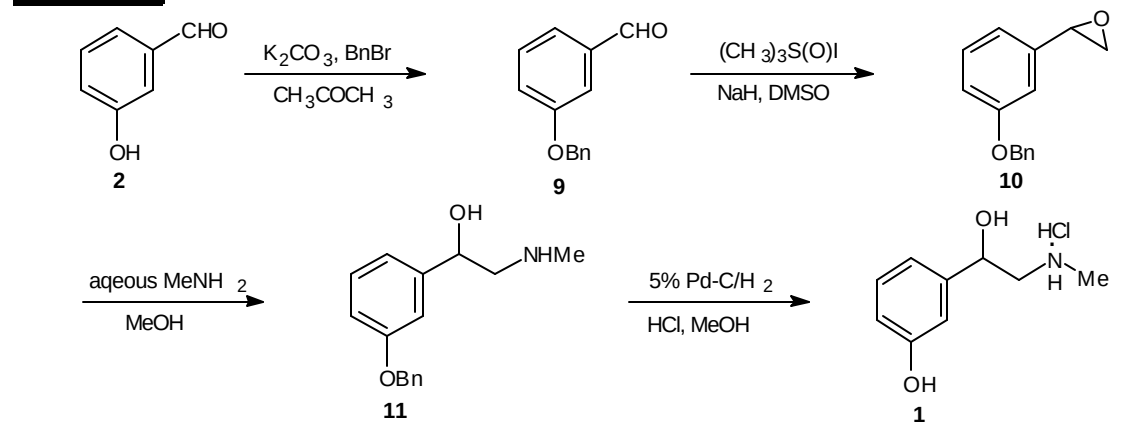
Scheme 3



Britten's approach:

Britten *et al*[20] synthesised the styrene oxide (**10**) from *m*-benzyloxybenzaldehyde (**9**) by the treatment of sulphonium methylide. The epoxide (**10**) was treated with methylamine followed by debenzylation to give phenylephrine hydrochloride (**1**) as demonstrated in Scheme 4.

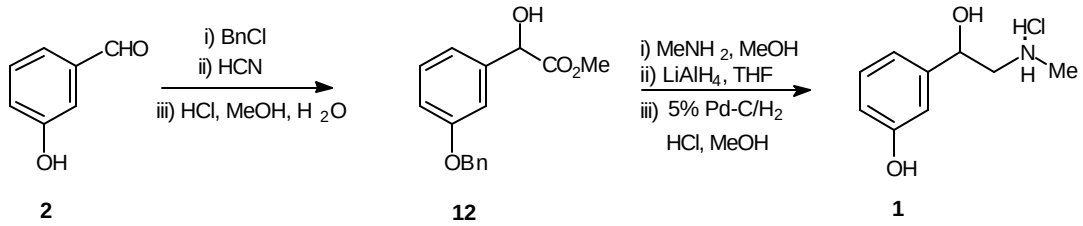
Scheme 4



Peter's approach:

Peter *et al*[21] synthesised dl-phenylephrine starting from *m*-hydroxy benzaldehyde (**2**) by using of cyanohydrin reaction. Compound **2** on treatment with HCN gave cyanohydrin, which on reaction with HCl/MeOH-H_2O gave the ester **12**. The ester on treatment with methylamine and subsequent reaction with LAH followed by reduction with pd/C in methanolic HCl gave phenylephrine hydrochloride (**1**) (Scheme **5**).

Scheme 5

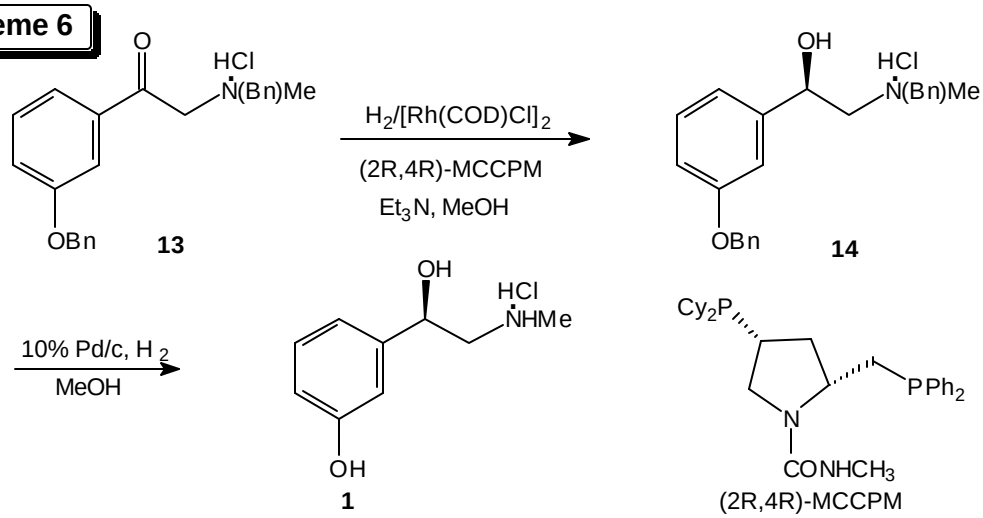


2 **12** **1**

Takeda's approach:

Takeda *et al*[22] reported the asymmetric hydrogenation of 3-benzyloxy-2-(N-benzyl-*N*-methyl) aminoacetophenone hydrochloride (**13**) with neutral (2R, 4R)-MCCPM-rhodium catalyst to give **14** which on reduction with Pd-C under hydrogen atmosphere to give (R)-(-)-phenylephrine hydrochloride (**1**) in 85% ee.

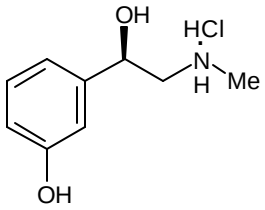
Scheme 6



13 **14** **1** (2R,4R)-MCCPM

PRESENT WORK

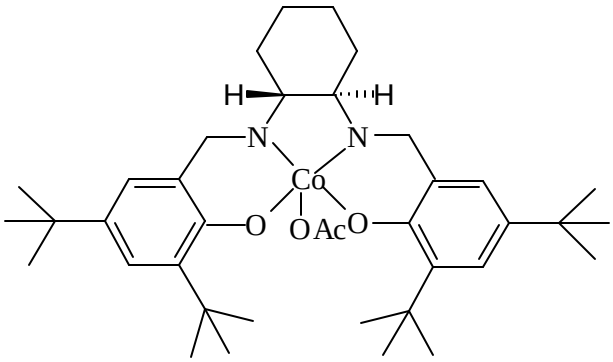
The growing awareness of chirality in the context of biological activity has led to the discovery of many new asymmetric reactions in order to produce drugs and drug intermediates in enantiomerically pure forms. Catalytic asymmetric reactions have distinct advantages over stoichiometric versions for economic and environmental reasons. Due to growing concern about chiral drugs being sold as racemates, many pharmaceutical industries are switching over to produce enantiomerically pure form of the corresponding chiral drug. Since its discovery, phenylephirine hydrochloride, a potent adrenergic agent and β-receptor sympathomimetic drug has been marketed as (*R*)-enantiomer. This chapter describes a practical synthesis of (*R*)-phenylephrine hydrochloride (1) using hydrolytic kinetic resolution of a styrene oxide derivative as a key step.



(R)-1

Recently, Jacobsen *et al*[23] reported hydrolytic kinetic resolution (HKR) of terminal epoxides using readily accessible chiral (*R,R*)-cobalt salen complex (A). This process is prospective in many directions such as no solvent is required; higher catalytic turn over of the catalyst; the recyclisation of the catalyst. It affords highly valuable terminal epoxides and 1,2-diols in good yield and high enantiomeric enrichment.

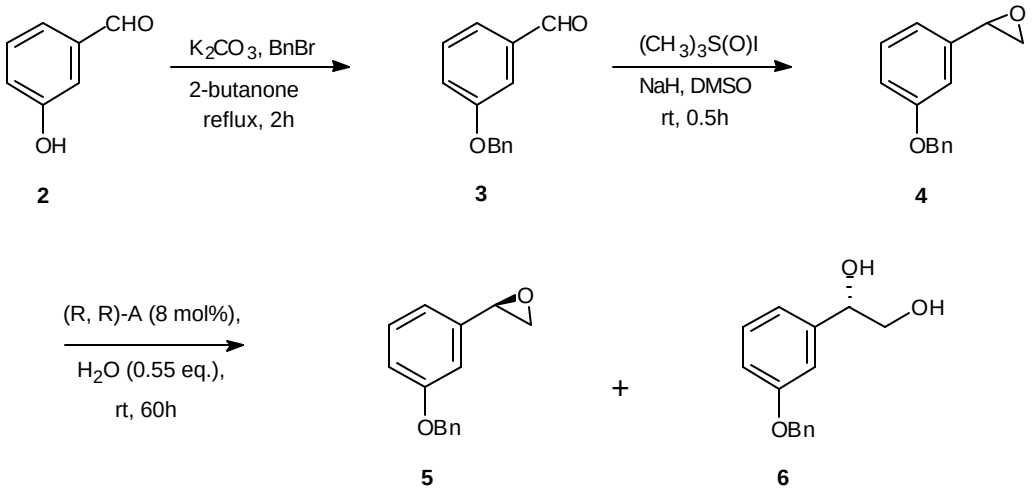
Synthesis of (*R*)-(-) phenylephrine hydrochloride (**1**) was initiated from *m*-hydroxy benzaldehyde (**2**), a commercially available inexpensive starting material. The phenolic hydroxy group was protected as its benzyl ether (**3**) with benzyl bromide and K_2CO_3 in refluxing 2-butanone. The formation of benzyl ether (**3**) was confirmed by its ^{1}H NMR



(R,R)-A

spectrum, which showed peak corresponding to benzylic protons at δ 4.70. Our next concern was to generate the epoxide from the corresponding aldehyde through Corey's protocol, ahead of the chiral resolution. Accordingly, reaction of **3** with trimethylsulfoxonium iodide[24] in the presence of NaH/DMSO at ambient temperature for 30 min. yielded the racemic epoxide (**4**) in 77% yield (Scheme 1). In the ^{1}H NMR spectrum of **4** the characteristic signals due to epoxy protons were observed as three doublets of doublet at δ 2.73, 3.12, 3.81. The rest of protons resonated at expected chemical shift values. This sets the stage for HKR. For this puropose (*R,R*)-(-)-[*N,N*'-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamino]cobalt(III) acetate complex (**A**) was prepared according to literature procedure.[25] The epoxide (±)-**4** was then resolved as per Jacobsen's procedure,[14] using (*R,R*)-salenCo(III)OAc complex (A) (0.8 mol%) and water (0.55 equivalent) at 0 °C with vigorous stirring. The reaction was monitored by HPLC with an ODS column (flow rate 1.0 ml/min, uv, 225nm) using 60% acetonitrile in water as a mobile phase. Unfortunately, the reaction was extremely slow and did not reach the theoretical completion 50:50 ratio of starting material and product, even after several days (Scheme 1). Since simple styrene oxides have been reported to undergo HKR efficiently within shorter reaction time, only the innocuous benzyl group should be blamed for its probable aromatic stagnation and steric-bulky nature to block the access of the steric-sensitive cobalt complex to coordinate the oxirane to facilitate hydrolytic opening.

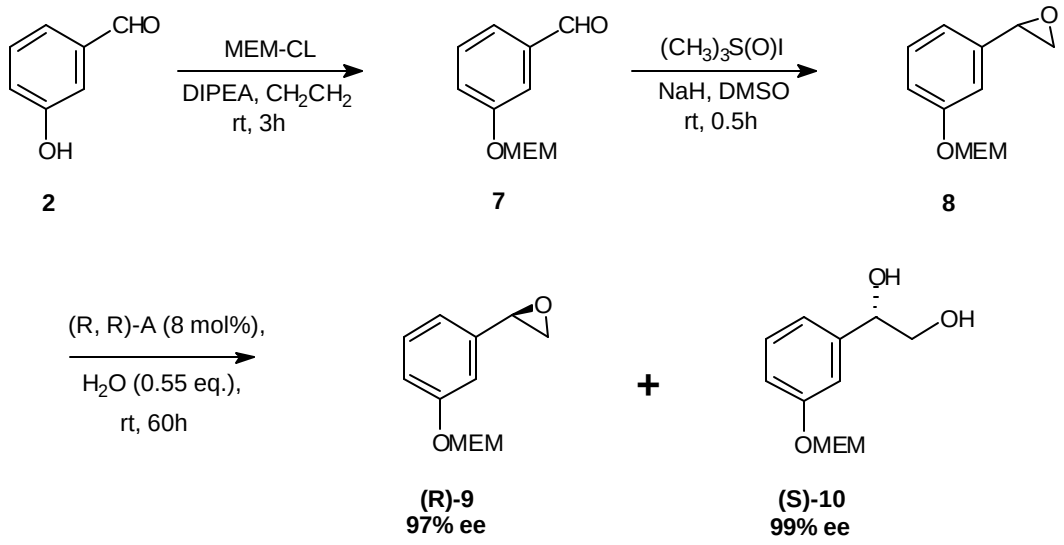
Scheme 1



2 **3** **4** **5** **6**

To circumvent this problem, the benzyl group was replaced by sterically less-congestive methoxyethoxymethyl group because of its linearity. Compound **2** was treated with *N,N*-diisopropylethylamine and MEM-Cl in CH_2Cl_2 for 3 h to provide 3-methoxyethoxymethyloxy benzaldehyde (**7**). The ^{1}H NMR spectrum of **7** clearly indicated the presence of MEM group with characteristic pattern of resonance. The benzaldehyde **7** was then converted into its corresponding styrene oxide **8** using trimethylsulfoxonium iodide as described above (*vide infra*) in 75% yield. In the ^{1}H NMR spectrum, the corresponding epoxy protons were observed in the region δ 2.72-3.78. The rest of the protons resonated at their expected chemical shifts. Compound **8** was subjected to hydroytic kinetic resolution using 0.8 mol% of catalyst **A** (0.8 mol%) and water (0.55 equiv). This solvent free reaction was stirred at room temperature and monitored by HPLC with an ODS column (flow rate 1.0 ml/min, UV, 225 nm) using 60% acetonitrile in water as a mobile phase. To the much of our satisfaction, the change of protecting group worked well and the reaction was complete in 60 h. The HRK reaction provided (*R*)-1-(3-methoxyethoxymethyloxy)phenylethylene oxide (**9**) (45 %, 97 % ee). Enantiomeric excess of (*R*)-**9** was determined at later stage. The ^{1}H NMR spectral analysis conclusively confirmed the structure of compound **9**. Further, the HKR reac-

Scheme 2



2 **7** **8**

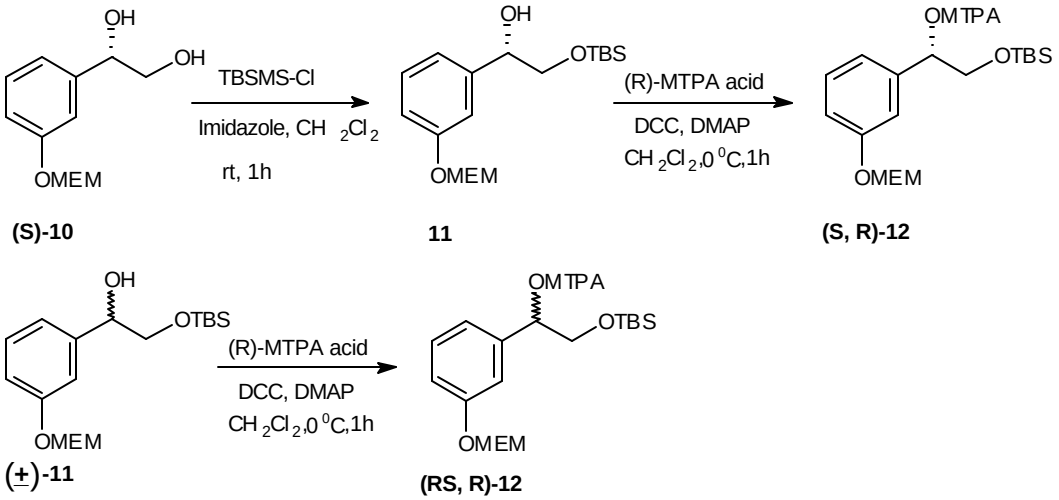
(R)-9
97% ee

(S)-10
99% ee

tion provided (*S*)-1-(3-methoxyethoxymethyl- oxy)phenyl-1,2-ethane diol (**10**) (48% yield, 95 % ee). In its ^{1}H NMR spectrum of diol (**10**), the characteristic signals due to two hydroxy groups were distinctly visible as broad singlets at δ 2.38, 2.90 respectively. The H-2,2' and H-1 protons were located in the region δ 3.60-3.77 (m) and 4.77 (dd) ppm respectively. Peaks corresponding to rest of the protons appeared at expected chemical shift values. The structure was further confirmed by HRMS analysis, which showed the highest mass peak at *m/z* 243.1243 (M^{+}+1) (Calcd. for $C_{12}H_{19}O_5$: 243.1232).

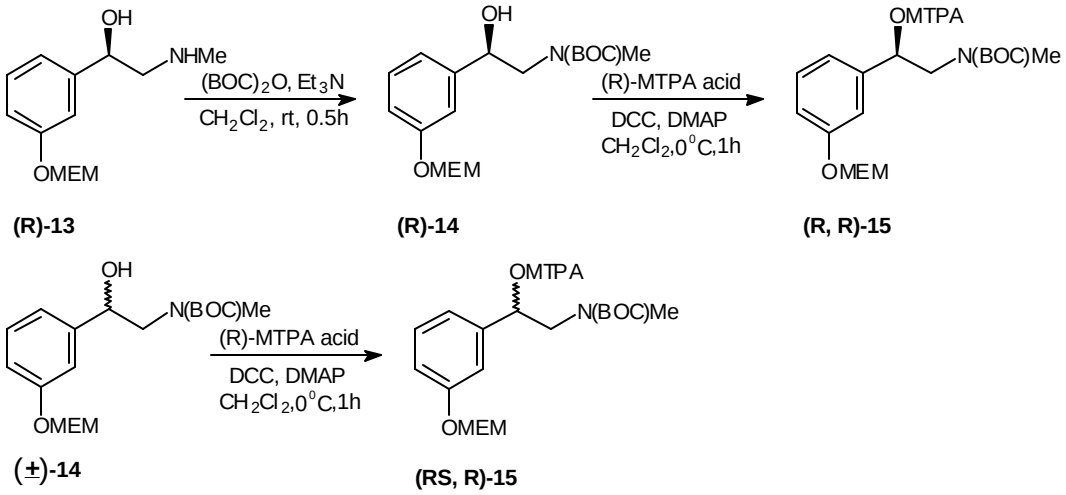
The enantiomeric excess of the diol **10** was determined by converting into Mosher ester derivative as shown in the scheme 3. Accordingly, the (*S*)-diol **10** was treated with 1 equiv. of TBDMS-Cl to provide **11**, which was subjected to esterification using (*R*)-MTPA acid [(*R*)-(α,α′)-methoxytrifluoromethylphenylacetic acid], DCC and DMAP (5 mg) in CH_2Cl_2 to give corresponding ester derivative (*S,R*)-**12**. Similarly, racemic (*RS, R*)-**12** was also prepared by treating (±)-**11** with (*R*)-MTPA acid. The ^{1}H NMR and ^{19}F NMR spectral analysis of (*S,R*)-**12** and (*RS, R*)-**12** confirmed the 95 % enantiomeric excess of the *S*-diol **10**.

Scheme 3



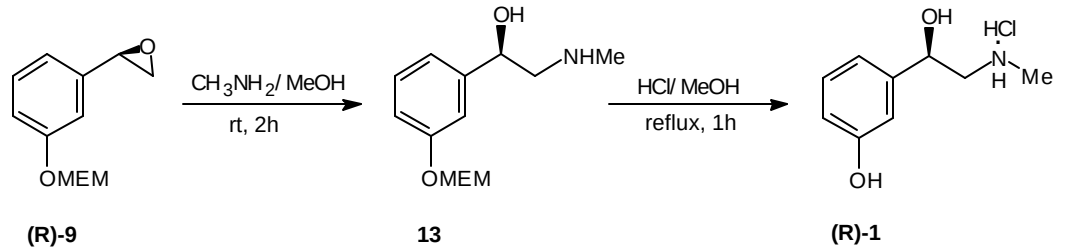
The (R)-epoxide **9** was treated with methylamine in methanol to provide the *N*-methylamino alcohol derivative **13** (90 % yield, 97 % ee). The presence of *N*-methyl group was clearly indicated by the appearance of a doublet at δ 2.53 along with upfield shift in H-2a and H-2b protons carrying *N* at δ 2.76 and 2.88 ppm in its ^{1}H NMR spectrum. All other protons resonated at their expected chemical shift values. In addition, the molecular formula **13** was further confirmed by HRMS analysis, which shows the molecular ion peak at m/z 255.1478 (M^{+}) (Calcd. for $C_{13}H_{21}NO_4$: 255.1470). The enantiomeric excess of (*R*)-**13** was confirmed by converting into its Mosher ester derivative. Accordingly, the compound **13** was treated 1 equiv. of $(Boc)_2O$ to provide *R*-**14**, which was then converted into optically pure (*R,R*)-*N*-Boc-MTPA ester following the conventional method. Similarly racemic (±)-**13** was transformed into (*RS, R*)-*N*-Boc-MTPA ester (**15**). Comparison of the ^{1}H NMR and ^{19}F NMR spectral analysis of (*R, R*)-**15** and (*RS, R*)-**15** conclusively confirmed 97% ee for the parent compound (*R*)-**13**. Removal of the methoxyethoxymethyl (MEM) group with concomitant hydrochloride formation occurred in one step when compound **13** was heated under reflux in

Scheme 4



methanolic HCl for 1 h to provide **1** in 90 % yield (Scheme 5). After recrystallization in 2-propanal, **1** was obtained as a white crystalline solid. The ^{1}H NMR spectrum in D_2O and the optical rotation data were comparable to the literature values.[26] [M.P. 141 °C; lit.[22] mp. 141-144 °C; $[\alpha]_D$-21.0 °C (C 1.26, MeOH), lit. $[\alpha]_D$-21.5 °C (C 1.0, MeOH)]. Based on optical rotation, enantiomeric excess of (R)-(**1**) was determined as 97.6% ee.

Scheme 5



Conclusion

In summary, an efficient and practically viable method for the synthesis of (*R*)-(-)-phenylephrine hydrocloride (**1**) involving hydrolytic kinetic resolution technique has been described.

EXPERIMENTAL SECTION

(3-Methoxyethoxymethyloxy)benzaldehyde (7).

To a stirred solution of *m*-hydroxybenzaldehyde (**2**) (20.0 g, 163.9 mmol) in dry CH_2Cl_2 (200 mL) at 0 °C, were added diisopropylethylamine (42.4 mL, 327.8 mmol) and MEM-Cl (22.4 mL, 196.6 mmol). After 3 h, the reaction mixture was washed with water (100mL). The organic layer was dried (Na_2SO_4), concentrated and the residue purified on silica gel column chromatography by eluting with EtOAc-light petroleum (1:9) to furnish compound **7** (31.0 g, 90% yield) as a colourless liquid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.27 (s, 3H, OCH_3), 3.47 (t, *J* = 4.5 Hz, 2H, $OC\underline{H}_2CH_2OCH_3$), 3.75 (t, *J* = 4.5 Hz, 2H, $OCH_2C\underline{H}_2OCH_3$), 5.25 (s, 2H, OCH_2O), 7.20-7.50 (m, 4H, Ar), 9.89 (s, 1H, CHO).

1-(3-Methoxyethoxymethyloxy)phenylethylene oxide (8).

To a stirred suspension of NaH (60% dispersion in oil, 5.7 g, 142.8 mmol) in dry DMSO (40 mL) at 0 °C, was added trimethylsulfoxonium iodide (31.4 g, 142.8 mmol). After 15 min, compound **7** (25.0 g, 119.0 mmol) in DMSO (50 mL) was introduced. The reaction was stirred for 30 min. at room temperature, diluted with water (100 mL), and extracted with diethyl ether (2 x 200 mL). The organic layer was washed with brine (100 mL), dried (Na_2SO_4), concentrated, and the residue eluted through a pad of silica gel with EtOAc-light petroleum (1:9) to give **8** (20.0 g, 75%) as a colourless liquid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.72 (dd, *J* = 2.1, 6.4 Hz, 1H, H-2a), 3.09 (dd, *J* = 4.3, 6.4 Hz, 1H, H-2b), 3.36 (s, 3H, OCH_3), 3.53 (t, *J* = 4.2 Hz, 2H, $OCH_2C\underline{H}_2O$), 3.78 (m, 3H, H-1 and $OC\underline{H}_2CH_2O$), 5.23 (s, 2H, OCH_2O), 6.86-7.27 (m, 4H, Ar).

(*R*)-1-(3-Methoxyethoxymethyloxy)phenylethylene oxide (9) and

(*S*)-1-(3-Methoxyethoxymethyloxy)phenyl-1,2-ethanediol (10).

A stirred solution of the epoxide (±)-**8** (18.0 g, 80.3 mmol) and (*R*,*R*)-salenCo(III)OAc complex (**A**) (0.43 g, 0.64 mmol) was cooled to 0 °C. Water (0.8 mL, 44.2 mmol) was added dropwise over a period of 1 h. The reaction mixture was then stirred at room temperature for 60 h, diluted with ethyl acetate (100 mL), dried (Na_2SO_4), and concentrated. [TLC: ethyl acetate-light petroleum (3: 2), R_f = 0.8 for compound **9** and R_f = 0.2 for compound **10**]. The brownish residue was chromatographed on silica gel using ethyl acetate-light petroleum. The (R)-epoxide **9** (8.1 g, 45 %, 97 % ee) was eluted with (1:9) mixture of ethyl acetate-light petroleum as a colourless liquid followed by (S)-diol **10** (9.3 g, 48 %, 95 % ee) with (1:1) mixture of ethyl acetate-light petroleum as a syrup.

Compound 9

$[\alpha]_D$ -13.14° (c 1.34, $CHCl_3$).

Compound 10

$[\alpha]_D$ +34.44° (c 1.49, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.38 (br s, 1H, OH), 2.90 (br s, 1H, OH), 3.36 (s, 3H, OCH_3), 3.55 (t, *J* = 4.5 Hz, 2H, $OCH_2C\underline{H}_2OCH_3$), 3.60-3.77 (m, 2H, CH_2-2), 3.81 (t, *J* = 4.5 Hz, 2H, $OC\underline{H}_2CH_2OCH_3$), 4.77 (dd, *J* = 3.4, 6.8 Hz, 1H, H-1), 5.27 (s, 2H, OCH_2O), 6.93-7.30 (m, 4H, Ar).

EIMS *m/z* (rel. intensity): 243 (M^++1, 6), 212 (M^+-30, 13), 136 (6), 89 (87), 59 (100), 45 (15).

HRMS (EI): Calcd. for ($C_{12}H_{19}O_5$, M^++1): 243.1232. Found 243.1243.

(S)-2-(*tert*-Butyldimethylsilyloxy)-1-(3-Methoxyethoxymethyloxy)phenyl-1-ethanol (11).

To an ice cooled mixture of compound **10** (0.2 g, 0.83 mmol) and imidazole (0.11g, 1.65 mmol) in dry CH_2Cl_2 (10 mL), was added TBDMS-Cl (0.12 g, 0.83 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was washed with water, dried over Na_2SO_4, concentrated and purified by silica gel column chromatography using ethyl acetate-hexane (1:3) as eluent to afford **11** (0.27 g, 92%) as a viscous liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.076 (s, 6H, $(CH_3)_2Si$), 0.91 (s, 9H, $(CH_3)_3CSi$), 2.96 (s, 1H, OH), 2.96 (s, 1H, OH), 3.39 (s, 3H, OCH_3), 3.50-3.62 (m, 3H, H-2a, $OCH_2C\underline{H}_2O$), 3.78 (dd, *J* = 3.1, 12.3 Hz, 1H, H-2b), 3.83 (t, *J* = 4.5 Hz, 2H, $OC\underline{H}_2CH_2O$), 4.37 (dd, *J* = 3.5, 6.7 Hz, 1H, H-1), 5.27 (s, 2H, OCH_2O), 6.94-7.27 (m, 4H, Ar).

(S,R')-2-(*tert*-Butyldimethylsilyloxy)-1-(3-methoxyethoxymethyloxy)phenyl ethyl(2-methoxy-2-phenyl)trifluoroacetate (12).

To an ice cooled mixture of compound **11** (100 mg, 0.28 mmol), DCC (68 mg, 0.33 mmol) and DMAP (5 mg) in dry CH_2Cl_2 (5 mL) was added (*R*)-MTPA acid [(R)-(α, α')-methoxytrifluoromethylphenyl acetic acid] (78 mg, 0.33 mmol). The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, it was diluted with CH_2Cl_2, washed with water, dried over Na_2SO_4, concentrated and purified by silica gel column chromatography using ethyl acetate-hexane (1:9) as eluent to afford **12** (152 mg, 95 %) as a colourless liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ -0.05 (2s, 6H, $(CH_3)_2Si$), 0.83 (s, 9H, $(CH_3)_3CSi$), 3.36 (s, 3H, $OCH_2CH_2OC\underline{H}_3$), 3.49 (s, 3H, OCH_3), 3.53 (t, *J* = 4.5 Hz, 2H, $OCH_2C\underline{H}_2O$), 3.74-392 (m, 4H, $C\underline{H}_2$OTBS, $OC\underline{H}_2CH_2O$), 5.25 (s, 2H, OCH_2O), 6.02 (m, 1H, C<u>H</u>OMTPA), 7.02 (m, 3H, Ar), 7.26 (m, 1H, Ar), 7.35 (m, 3H, Ph), 7.50 (m, 2H, Ph).

(*R*)-1-(3-Methoxyethoxymethyloxy)phenyl-2-(N-methyl)amino-1-ethanol (13).

Compound **9** (8.0 g, 35.7 mmol) was treated with dry methanol (60 mL) saturated with methylamine gas and stirred at room temperature for 2 h. The reaction mixture was concentrated to give **13** (8.1 g, 90%) as a viscous liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.53 (d, *J* = 4.2 Hz, 3H, NCH_3), 2.76 (m, 1H, H-2a), 2.88 (m, 1H, H-2b), 3.36 (s, 3H, OCH_3), 3.58 (t, *J* = 4.5 Hz, 2H, $OCH_2C\underline{H}_2OCH_3$), 3.80 (t, *J* = 4.5 Hz, 2H, $OC\underline{H}_2CH_2OCH_3$), 4.79 (m, 1H, H-1), 5.29 (s, 2H, OCH_2O), 6.95-7.23 (m, 4H, Ar).

EIMS *m/z* (rel. intensity): 255 (M^+, 3), 237 (12), 148 (19), 89 (52), 59 (91), 44 (100).

HRMS (EI): Calcd. for ($C_{13}H_{21}NO_4$, M^+): 255.1470. Found 255.1478.

(R)-2-(N-*tert*-Butyloxycarbonyl-N-methyl)amino-1-(3-methoxyethoxymethyloxy)phenyl ethanol (14).

The compound **13** (0.2 g, 0.78 mmol) and $(Boc)_2O$ (0.21 g, 0.94 mmol) in a solvent mixture of THF and H_20 (4 mL, 1:1 ratio) were stirred for 2 h at the room temperature. The solvent was then evaporated and the residue taken in ethyl acetate and washed with water, dried over Na_2SO_4 and concentrated. The crude product purified by silica gel column chromatography using ethyl acetate-hexane (1:9) as eluent to afford **14** (0.26 g, 94%) as a syrupy liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H, $(CH_3)_3CCO$), 2.82 (s, 2H, CH_2N), 3.38 (s, 3H, OCH_3), 3.58 (t, *J* = 4.6 Hz, 2H, $OCH_2C\underline{H}_2O$), 3.82 (t, *J* = 4.6 Hz, 2H, $OC\underline{H}_2CH_2O$), 4.89 (br s, 1H, H-1), 5.28 (s, 2H, OCH_2O), 6.92-7.29 (m, 4H, Ar).

(R,R')-[2-(N-t-Butyloxycarbonyl-N-methyl)amino-1-(3-Methoxyethoxymethyloxy)phenyl] ethyl (2-methoxy-2-phenyl)trifluoroacetate (15).

To an ice cooled mixture of compound **14** (100 mg, 0.28 mmol), DCC (69 mg, 0.33 mmol) and DMAP (5 mg) in dry CH_2Cl_2 (5 mL) was added (*R*)-MTPA acid (79 mg, 0.33 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was washed with water, dried over Na_2SO_4, concentrated and purified by silica gel column chromatography using ethyl acetate-hexane (2:8) as eluent to afford **15** (149 mg, 93%) as a viscous liquid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.43 (s, 9H, $(CH_3)_3CCO$), 2.79 (s, 2H, CH_2N), 3.33 (s, 3H, $OCH_2CH_2OC\underline{H}_3$), 3.53 (m, 5H, $OCH_2C\underline{H}_2O$, $OC\underline{H}_3$), 3.79 (t, *J* = 4.5 Hz, 2H, $OC\underline{H}_2CH_2O$), 5.20 (s, 2H, OCH_2O), 6.08 (br s, 1H, H-1), 6.90 (m, 3H, Ar), 7.22 (m, 1H, Ar), 7.35 (m, 5H, Ph).

(*R*)-Phenylephrine Hydrochloride (1).

To a solution of **13** (8.0 g, 31.4 mmol) in methanol (80 mL), was added concentrated HCl (2 mL). The reaction mixture was heated under reflux for 1 h, concentrated and the residue crystallized from 2-propanol to afford **1** (5.7 g, 90% yield) as a solid.

M.P.: 141 °C, (lit.[26] M.P.: 141-145° C).

$[\alpha]_D$ -44° (c 2.16, H_2O), lit.[22] $[\alpha]_D$ -45.2° (c 2.0, H_2O).

^{1}H NMR (D_2O, 400 MHz): δ 2.78 (s, 3H, NCH_3), 3.28 (m, 2H, H-2), 5.03 (m, 1H, H-1), 6.92-7.34 (m, 4H, Ar).

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SECTION–II

SYNTHESIS OF FURAN PRECURSOR OF

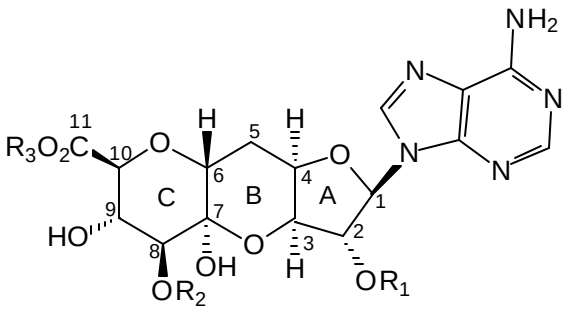
HERBICIDIN

INTRODUCTION

Nucleoside antibiotics are fascinating compounds that show a variety of biological activities.[1] Their biological activity is also wide ranging, including herbicidal,[2] antibacterial, antifungal, antitrypanosomal, antitumor, antiviral insecticidal, immunostimulating and often immunosuppressive properties. These are also found in diverse groups of secondary metabolites of microbial origin and include a variety of structural modifications leading to intricate molecules. Nucleoside antibiotics exhibit such diverse biological activities, because nucleosides and nucleotides play pleiotric roles in most fundamental cellular metabolic pathways such as metabolite carriers, energy donors, secondary messengers and cofactors for various enzymes. Not only nucleic acid synthesis but also protein, glycan and glycoprotein synthesis leads to nucleoside antibiotics.[3] The protein kinases which are key enzymes for cell proliferation and differentiation, require nucle otides as phosphate donors. As from the above biological evidences, it shows nucleoside antibiotics are potential candidates for the regulation of all aspects of cell growth and differentiation. Because of its important biological activities as well as our investigation directed toward the discovery of new antibiotics with herbicidal activity, we took up some adenine nucleoside antibiotics.

Herbicidins (**1a-f**),[4] aureonucleomycin (**2**),[5] and S12245 (**3**)[6] all belongs to a class of adenine nucleoside antibiotics compounds with same backbone structure. Herbicidines were isolated from strains of *Streptomyces saganonesis*.

The herbicidins exhibit herbicidal and antialgal activity, and herbicidin **A** (1a) and **B** (1b) as well as aureonuclemycin (**2**), are efficient inhibitors of *Xanthomonas oryzae*, a bacterium that causes leaf blight infection in the rice crops. Inhibitory effects on seed germination and on algal growth were also observed, while the groups are markedly non-toxic to animals.



	R_1	R_2	R_3
Herbicidin A (1a)	Me	$CH_3CH=C(CH_2OH)CO$	Me
Herbicidin B (1b)	Me	H	Me
Herbicidin C (1c)	H	H	Me
Herbicidin C (1d)	Me	$(CH_3)_2CHCHO$	Me
Herbicidin C (1e)	Me	$CH_3CH=C(CH_3)CO$	Me
Herbicidin C (1f)	H	$CH_3CH=C(CH_3)CO$	H
Aureonuclemycin (2)	Me	CH_3CO	Me
S12245 (3)	H	H	H

Fifure1: Structures of herbicidins

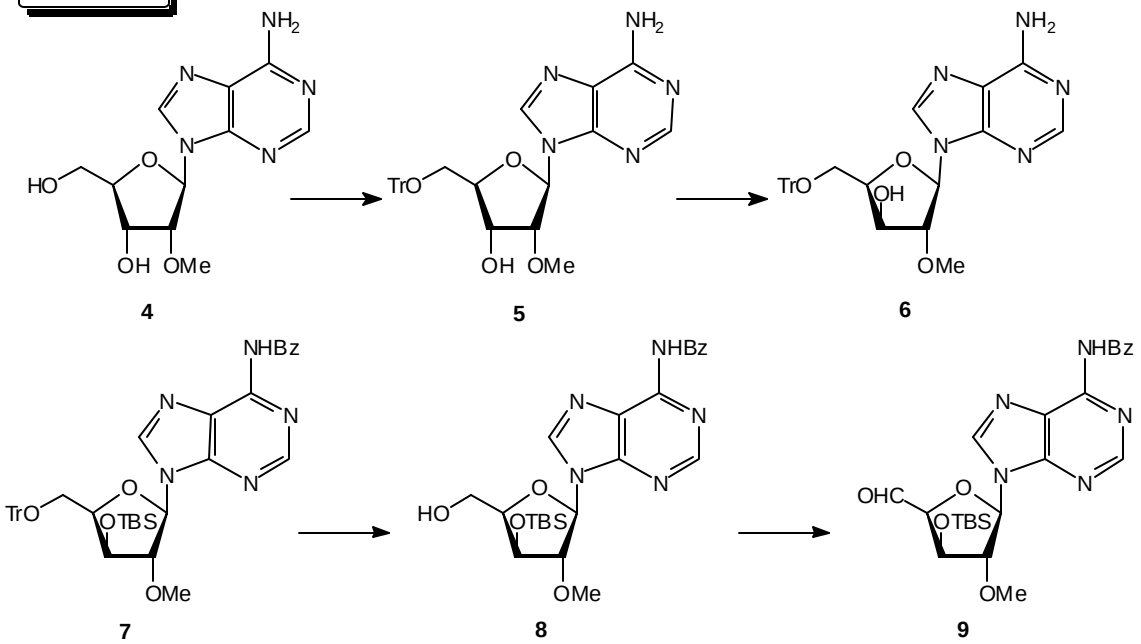
The herbicidins encompass a number of interesting structural features.

1. Adenine is glycosylated at the 1-β-position of an unusual sugar, undecose, which has a tricyclic furano-pyrano-pyran strucure.
2. The B/C ring junction is held as an internal hemiacetal (at C-7) with a C-glycosyl linkage between (C-5 and C-6).
3. The C-ring substituents all occupy an axial orientation due to the tricyclic structure of the undecose.

Till to date very few synthesis of this type molecule have been reported, which involved mainly the model studies and attempts toward the synthesis of tricyclic core of herbicidin without adenine moiety.[7] The recent report by Akira Mastuda *et al.*[8] constitutes as the first and only total synthesis of Herbicidine **B**, which was discussed below.

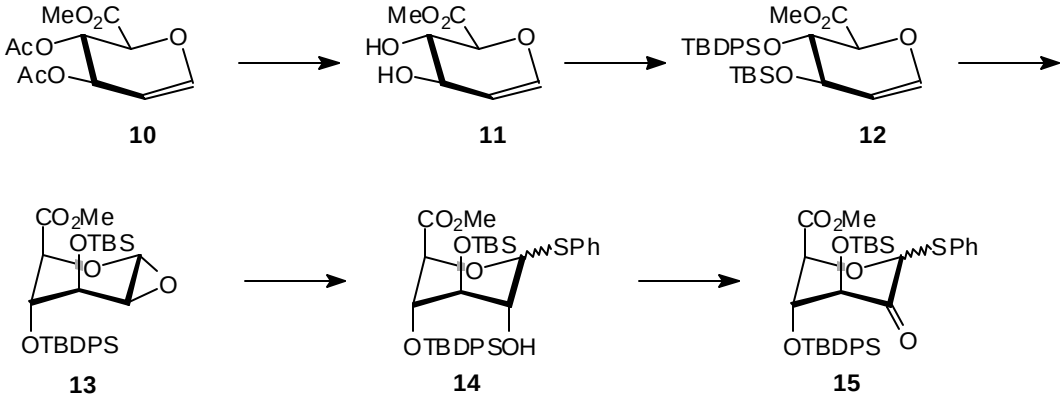
The 1-β-D-Xylosyladenine 5'-Aldehyde Unit **9** was prepared from 2' -*O*-Methyl adenosine (**4**) as shown in the Scheme 1. The 5'-primary hydroxyl group of **4** was protected by a triphenylmethyl (Tr) group, followed by inversion of 3'-hydroxyl group by successive oxidation-reduction with CrO_3/Ac_2O/pyridine and $NaBH_4$/AcOH afforded xylonucleoside **6**. The free 3'-hydroxy and 6-amino groups of **6** were protected with TBS and benzoyl groups respectively to give **7**. Removal of Tr group of **7** under acidic conditions followed by oxidation with Dess-Martin periodinane provided **9** which was later on used as an acceptor of the aldol reaction (Scheme 1).

Scheme 1



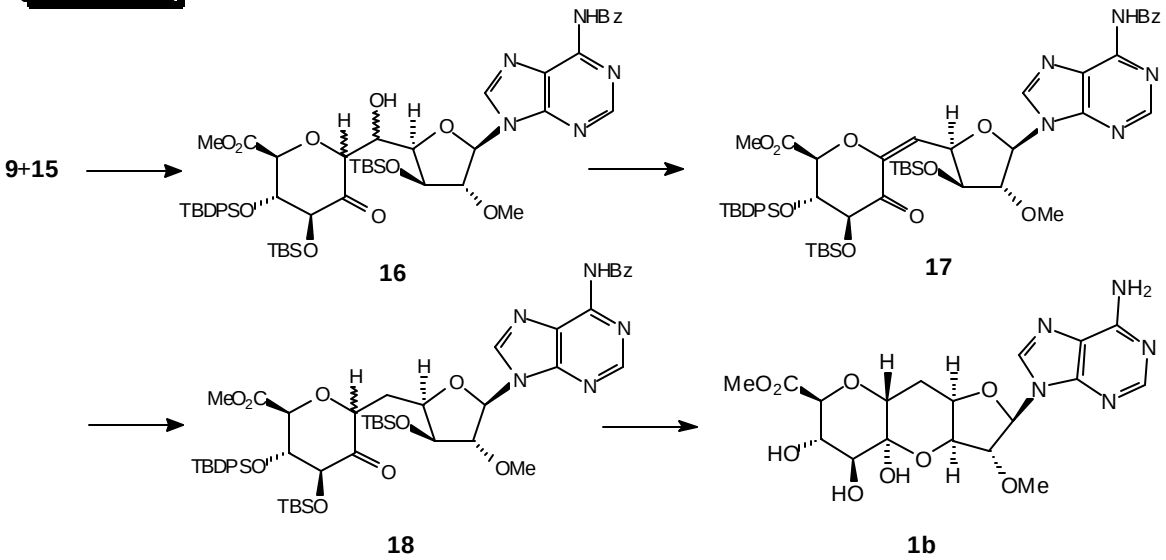
The preparation of 1-phenylthio-2-uloses (**15**) was summarized in Scheme 2. Glycal **10** derived from D-glucurono-3,6-lactone, was treated with NaOMe in MeOH to give **11**, which was on successive treatment with TBDMS-Cl and TBDPS-Cl in the presence of imidazole in DMF followed by epoxidation with dimethyl-dioxirane gave the glyco type of epoxide **13**. Compound **13** was treated with PhSH in the presence of catalytic amount of BF_3-OEt_2 afforded **14** as an anomeric mixture (Scheme 2). Compound **14** when subjected to Dess-Martin periodinane gave assisted oxidation provided 1-phenyl thio-2-ulose derivative **15**.

Scheme 2



When **15** was treated with SmI_2 in THF regioselectively gave the corresponding 1-enolate, which was readily trapped with 5'-aldehyde derivative **9** to afford the product as an anomeric mixture **16**. Dehydration of the 5'-hydroxyl of **16** using Burgress's inner salt gave the enone **17**, which was subsequently hydrogenated to give undeculofuranuronyl adenine derivative **18** (Scheme 3). Deprotection of silyl groups of **18** lead to an internal ketal linkage between the 3'-and 7'-positions, which spontaneously gave herbicidin B (**1b**).

Scheme 3

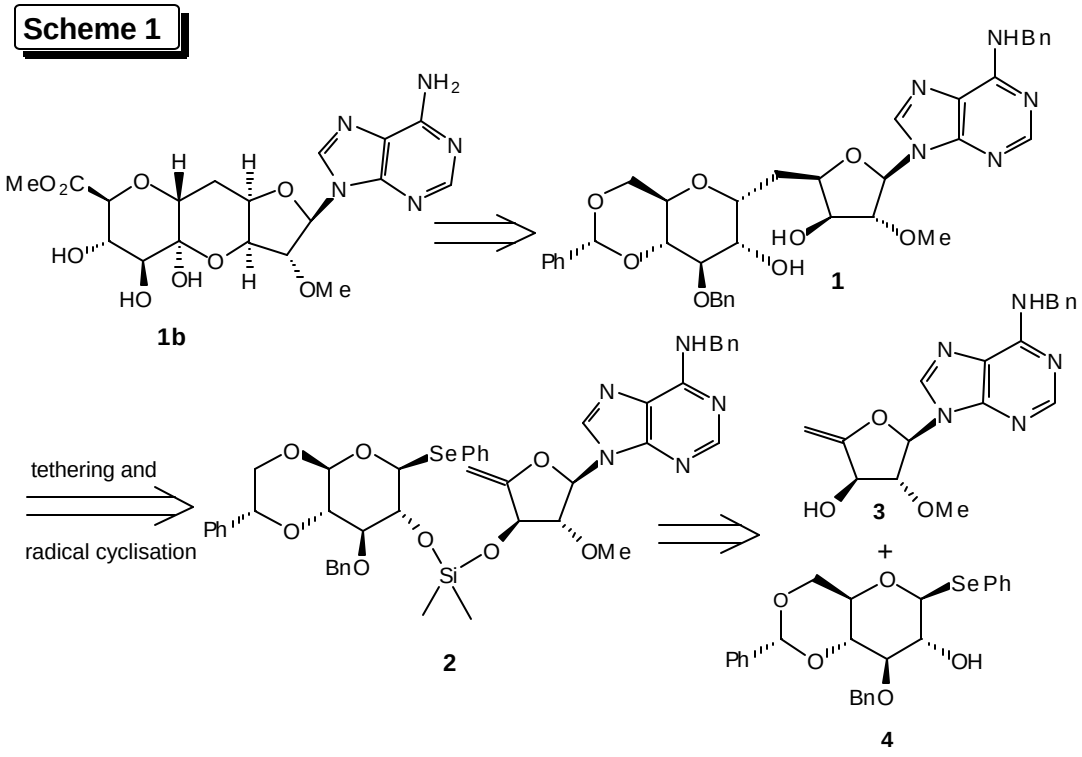


PRESENT WORK

Herbicidins adorned with novel structures with dense functionalities are attractive targets for synthetic chemists. The synthesis of herbicidin was a collaborative program between our group and Prof. Pierre sinay's laboratory at Ecole normale superieure, Paris, France. We herein report the synthesis of furan precursor **3** of herbicidin as part of the ongoing Indo-French collaborative program.

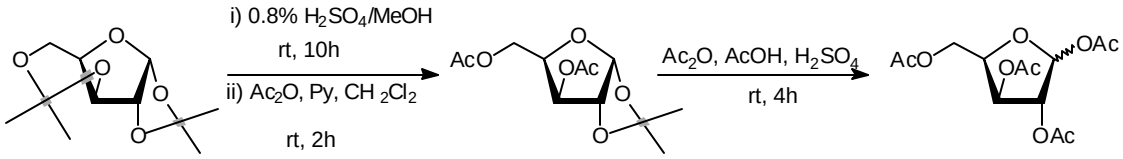
Retrosynthectic analysis of herbicidin (**1b**) involved initial disconnection of the central cyclic hemiketal to produce the diol **1** (Scheme 1). Close inspection of this material reveals a logical disconnection of the C5-C6 interglycosidic bond which is applied by the presence of the two free hydroxyl groups. These hydroxyl groups suggest that a molecular tethering and radical cyclization approach result the C-disaccharide. The further disconnection lead us to the exomethylene nucleoside **3** and the selenoglucopyranoside **4**. It was envisaged that temporary linking of these two materials, *via* a silaketal ether, followed by an 8-endo trig radical cyclization could provide a route to the herbicidin carbon skeleton. The work assigned to our laboratory involved the synthesis of unsaturated nucleoside **3**. Whose synthesis is described below.

Scheme 1



D-Xylose (**5**) was converted into 1,2,3,5-tetra-*O*-acetyl-D-xylofuranose (**8**) by a sequence involving four high yielding steps. On stirring a mixture of D-xylose (**5**), anhydrous $CuSO_4$ and dry acetone in the presence of catalytic conc. H_2SO_4, 1,2,3,5-di-*O*-isopropylidene-α-D-xylofuranose (**6**) was obtained in 84% yield. The structure of **6** was confirmed by comparing of the analytical data reported in the literature.[9] Selective hydrolysis of the 3,5-*O*-isopropylidene groups with 0.8% H_2SO_4 in MeOH followed by conventional acetylation provided the diacetate **7**, whose ^{1}H NMR spectrum showed two distinct acetyl methyl singlets at δ 2.06 and 2.09. The downfield shift of signals due to H-5,5' and H-3 observed at δ 4.14 (dd), 4.27 (dd) and 5.22 (d) respectively indicated the presence of acetate groups at these posititions.

Scheme 2



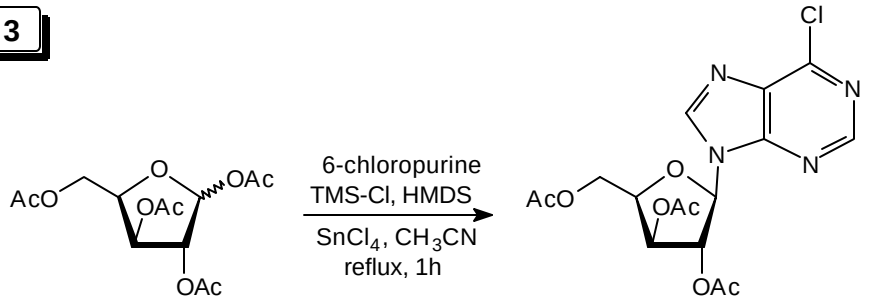
6 **7** **8**

Subsequent hydrolysis of the 1,2-*O*-isopropylidene group and concomitant acetylation was carried out by Reist-Goodmann[10] method in the presence of $Ac_2O/AcOH/H_2SO_4$ to yield 1,2,3,5-tetra-*O*-acetyl-D-Xylofurnaose (**8**) as a 1:1 mixture of α and β anomers (Scheme 2). In the ^{1}H NMR spectrum **8**, the anomeric protons due to α and β anomers resonated at δ 6.38 (d, J = 6.1 Hz) and 6.07 (s) respectively.

The Vorbruggen[11] reaction was utilized for the condensation of the sugar moiety with the purine base. Consequently, the compound tetra-***O***-acetyl-α-D-xylopfuranose (**8**) was subjected to diastereoselective nucleophilic substitution of 6-chloropurine (**9**) at the anomeric centre in the presence of HMDS, TMS-Cl and $SnCl_4$ in CH_3CN at 60 °C to provide the β-

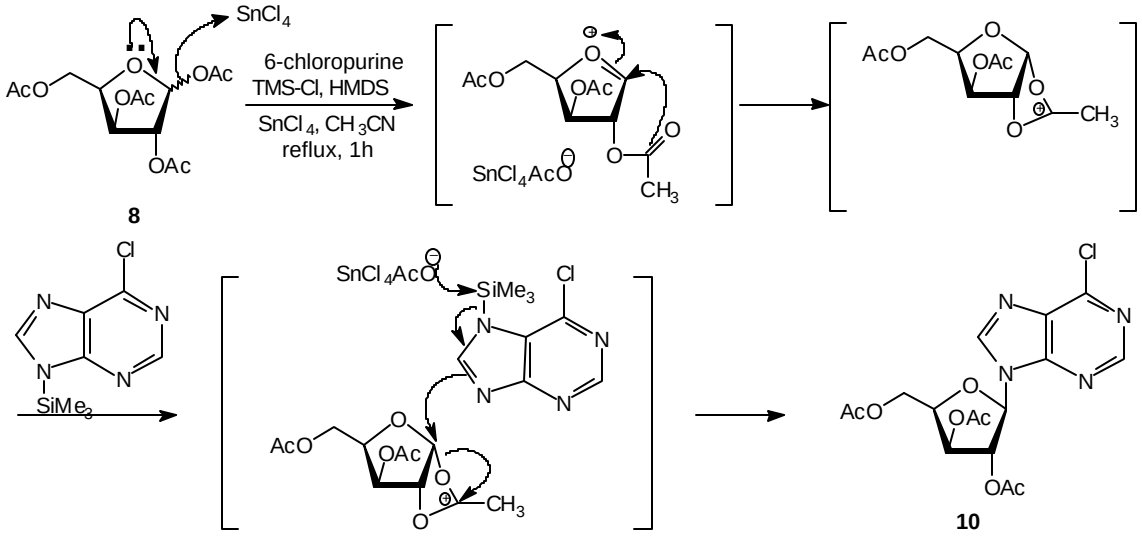
nucleoside **10** in 72 % yield. The exclusive formation of β-isomer can be explained by the Lewis acid promoted formation of acetoxonium ion by the participation of the neighbouring acetyl group at 2-position. The mechanism of the coupling reaction is visualised in the scheme 3.

Scheme 3



8 **10**

Mechanism of the b-nucleoside formation (anchimeric assistance).

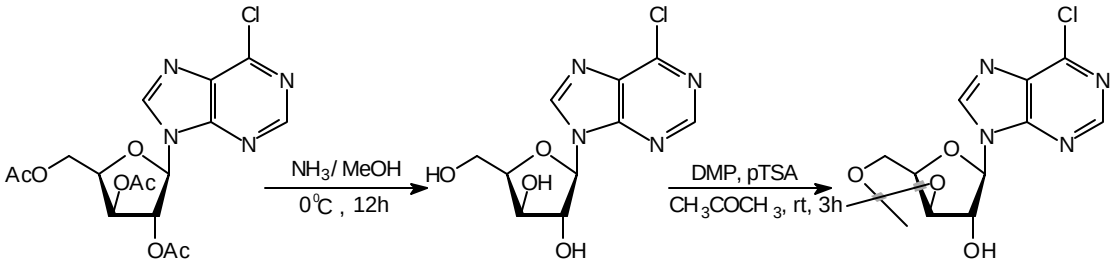


8 **10**

Compound **10** was in agreement with assigned structure due to comparable spectral and analytical data with those reported in the literature. For example, in the ^{1}H NMR spectrum, singlets at δ 8.33 and 8.69 (integration for one proton each) were assigned to H-2 and H-8 of the 6-chloropurine moiety. The anomeric proton appeared at δ 6.2 as a doublet with coupling constant $J_{H\text{-}1',\ H\text{-}2'}$ = 2.4 Hz. The small coupling constant suggested β-configuration at the

anomeric carbon. The resonances due to the rest of protons were in conformity with the assigned structure. Hydrolysis of the acetate in **10** provided the triol with concomitant replacement of chloro group of purine by methoxy group under Zemplen's conditions. Same reaction also found to occur with K_2CO_3/MeOH. Success was met only when **10** was exposed to methanolic ammonia at 0 °C where the required product **11** with chloro group intact was formed.[12] Compound **11** was subsequently protected as its 3', 5'-isopropylidene derivative **12** with dimethoxy propane and catalytic PTSA in acetone for 3 h in 84 % yield. Since hydroxyl groups at C-2 and C-3 are trans, the isopropylidene derivative was expected to form between C-3 and C-5 hydroxyls only. In the ^{1}H NMR spectrum of **12**, the characteristic signals of isopropylidene protons were observed at δ 1.30, 1.48 as two singlets of three proton each. The remaining signals were in complete agreement with the assigned structure, which was further confirmed by HRMS analysis, which showed the highest mass peak at *m/z* 325.0704 (M^+-1) (Calcd. for $C_{13}H_{14}ClN_4O_4$: 325.0703).

Scheme 4

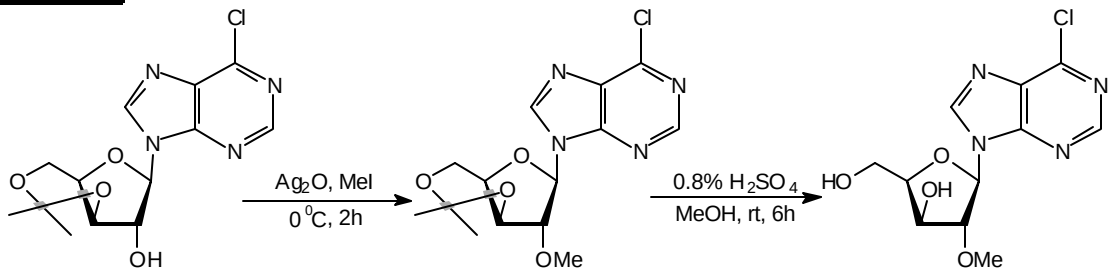


10 **11** **12**

The hydroxy group at C-2 of **12** protected as its methyl derivative **13** with MeI and Ag_2O for 2 h in 86 % yield.[13] The presence of O-methyl group clearly indicated by the appearance of a singlet at δ 3.55 in its ^{1}H NMR spectrum. In addition, the molecular formula of the structure was confirmed by HRMS analysis in which highest mass peak (M^++1) was observed at m/z 341.1025 (Calcd. for $C_{14}H_{17}ClN_4O_4$: 341.1016). Deblocking of acetonide with 0.8% of H_2SO_4 in MeOH gave the diol **14** in 90% yield. In the ^{1}H NMR spectrum of **14**,

the conspicuous absence of peaks due to isopropylidene group was noted. The structure was further confirmed HRMS mass spectrum, in which the highest mass peak (M^++1) was observed at *m/z* 301.0708 (Calcd. for $C_{11}H_{14}ClN_4O_4$: 301.0703).

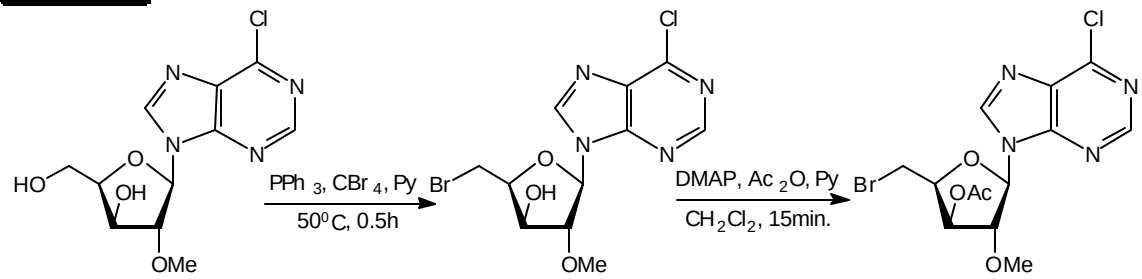
Scheme 5



12 **13** **14**

The selective conversion of primary hydroxy group into the corresponding bromide **15** was achieved using TPP and CBr_4 in neat pyridine at 50 °C albeit in moderate yield.[14] Acetylation of 3'-hydroxy group under routine condition gave the acetate derivative **16**. The ^{1}H NMR spectrum of **16** revealed the presence of a singlet at δ 2.05 due to acetyl group along with the 1 ppm downfield shift of methine proton carrying the *O*-Ac group was observed as the doublet due to H-3 located at δ 5.37. The high resolution mass spectral analysis of **16** showed the molecular ion peak at *m/z* 406.0035 (M^++1) (Calcd. for $C_{13}H_{14}ClN_4O_4$: 406.0043).

Scheme 6

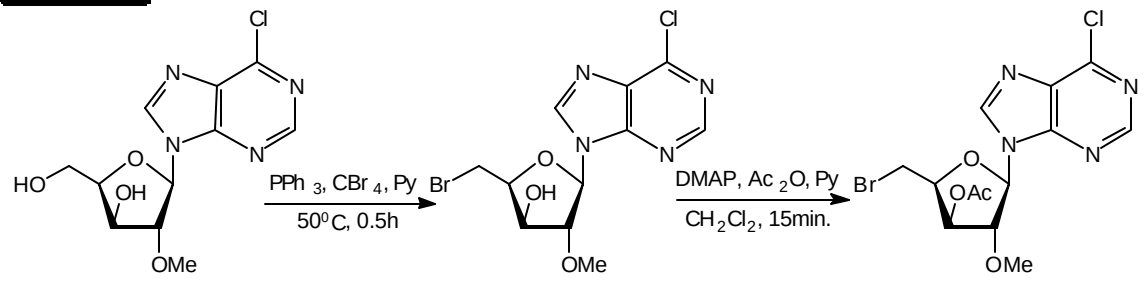


14 **15** **16**

The poor yield in direct bromination of C-5' hydroxyl group prompted us to deploy the two step procedure, i.e. selective primary tosylation of **14** with tosyl chloride in pyridine for 6 h in

85 % followed by protection of 3'-hydroxy group as its acetate derivative **17**. In the ^{1}H NMR spectrum of **17**, the characteristic singlet due to acetyl group was observed at δ 1.98 and tosyl

Scheme 6

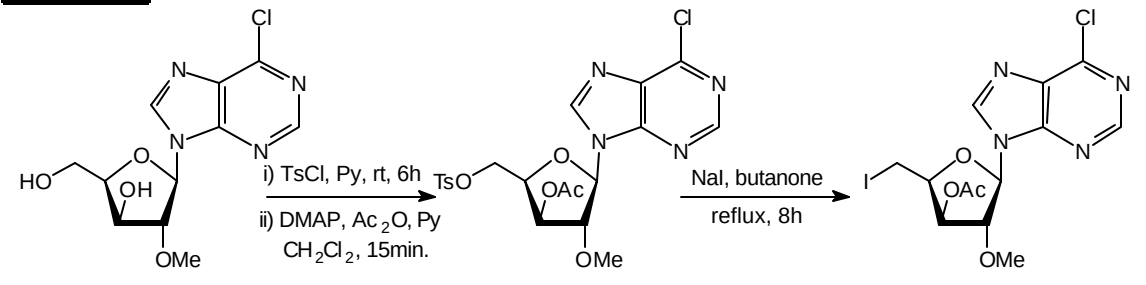


14 **15** **16**

group was indicated by the signals at δ 2.45 (Me), 7.39 and 7.75 (A_2B_2). The rest of the protons had expected chemical shift values. The molecular formula of the structure was further confirmed by HRMS analysis, which showed the molecular ion peak at *m/z* 498.0969 (M^++1) (Calcd. for $C_{20}H_{23}ClO_7S$: 498.0975).

The replacement of tosyl by iodo group was achieved by refluxing **17** with sodium iodide in acetone for 8 h to provide the iodo derivative **18** in 75 % yield. In the ^{1}H NMR spectrum of **18**, the conspicuous absence of peak due to tosyl group was indicated. In addition, the molecular formula of structure was confirmed by HRMS analysis, which showed the molecular ion peak at *m/z* 454.9907 (M^++2) (Calcd. for $C_{13}H_{16}ClIN_4O_4$: 454.9904).

Scheme 7

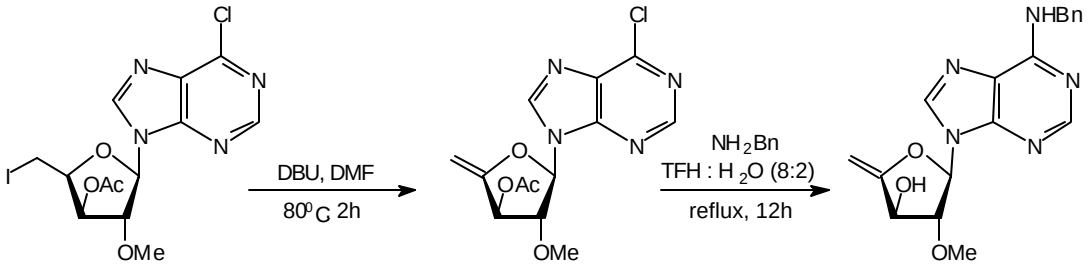


14 **17** **18**

Compound **18** was then subjected to elimination of halogenide using DBU at 80 ^{0}C in dry DMF to provide 4, 5-ene derivative **19** in 84 % yield. In the ^{1}H NMR spectrum, the

characteristic signal due to two exocyclic protons were distinctly visible as two doublets at δ 4.60, 4.88, the structure was further confirmed by its HRMS in which the highest molecular ion peak (M^++1) was observed at *m/z* 325.0711 (Calcd. for $C_{13}H_{14}ClN_4O_4$: 325.0703). Our final concern was nucleophilic substitution of chloro group of purine **19** which was achieved with excess of benzylamine in the mixture of THF: H_2O (8: 2) gave the furan precursor **3** of Herbicidin B (**1**) with the concomitant hydrolysis of acetate in 78 % yield.

Scheme 8



18 **19** **3**

The presence of NHBn group was clearly indicated by a appearance of broad singlet at δ 4.86 for benzylic group and multiplet in the region of 7.22-7.30 for aromatic protons of *N*-benzyl group. The remaining signals were in complete agreement with the assigned structure and while HRMS analysis showed the molecular ion peak at *m/z* 354.1575 (Calcd. for $C_{18}H_{20}N_5O_3$: 354.1566)

This intermediate was (**3**) provided to Prof. P. Sinay who is now working into the total synthesis of herbicidin by the strategy described earlier (scheme 1).[7b]

EXPERIMENTAL SECTION

6-Chloro-9-(2',3',5'-tri-*O*-acetyl-b-D-xylofuranosyl)purine (10).

To a stirred suspension of 6-chloropurine **9** (7.28 g, 47.16 mmol) in anhydrous acetonitrile (300 mL) were successively added hexamethyldisilazane (7.8 mL, 37.73 mmol), trimethylsilyl chloride (4.7 mL, 37.73 mmol) and stannic chloride (6.66 mL, 56.59 mmol). The temperature was raised to 60 ^{o}C. To the resulting clear solution was added tetra-acetylated xylose **8** (15 g, 47.16 mmol) in anhydrous acetonitrile (100 mL) over a period of 15 min. The mixture was heated under reflux for 1 h, cooled to room temperature, concentrated under reduced pressure, diluted with dichloromethane (500 mL) and poured into a cold sat. $NaHCO_3$ solution (500 mL) with vigorous stirring. The emulsion was filtered through celite layer, the organic phase separated and the aqueous phase extracted with dichloromethane. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4 and evaporated. Subsequent silica gel column chromatography using ethyl acetate-light petroleum (3:7) of the residue afforded **10** (13.3 g, 72%) as a viscous liquid.

$[\alpha]_D$ +62.6^{o} (c 1.2, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03, 1.07, 1.13 (3s, 3H each, OAc), 4.32 (m, 2H, CH_2-5'), 4.6 (m, 1H, H-4'), 5.45 (m, 2H, H-2', H-3'), 6.2 (d, *J* = 2.38, 1H, H-1'), 8.33 (s, 1H, H-2), 8.69 (s, 1H, H-8).

6-Chloro-9-(3',5'-*O*-isopropylidene-b-D-xylofuranosyl)purine (12).

To a stirred solution of **10** (13 g, 33.4 mmol) in methanol (100 mL) was added saturated methanolic ammonia (5 mL) at 0 ^{o}C. The reaction flask was left refrigerated for 12 h. The reaction mixture was concentrated to give the crude product **11** (7.45 g, 83.8 %) as a white solid, which was dissolved in acetone (100 mL), followed by the addition of 2,2-dimethoxypropane (50 mL) and a pinch of *p*TSA. The reaction mixture was stirred for 3 h,

quenched with triethylamine and concentrated. The crude product was purified on silica gel column chromatography using ethyl acetate-light petroleum (3:7) to give **12** (7.53 g, 84 %) as a colourless solid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.30, 1.48 (2s, 3H each, $(CH_3)_2C$), 4.27 (s, 2H, CH_2-5'), 4.35 (s, 1H), 4.42 (s, 1H), 4.48 (s, 1H), 5.5 (br s, 1H, OH), 6.21 (s, 1H, H-1'), 8.71 (s, 1H, H-2), 8.88 (s, 1H, H-8).

FABMS m/z (rel. intensity): 325 (M^+-1, 10), 213 (15), 185 (20), 59 (30), 43 (100).

HRMS (FAB): Calcd. for ($C_{13}H_{14}ClN_4O_4$, M^++1): 325.0703. Found 325.0704.

6-Chloro-9-(3',5'-*O*-isopropylidene-2'-*O*-methyl-b-D-xylofuranosyl)purine (13).

To a stirred solution of **12** (7.4 g, 22.7 mmol) in methyl iodide (100 mL) was added silver oxide (15.7 g, 68.0 mmol) portion wise at 0 °C. The reaction mixture was stirred for 2 h, filtered through celite layer and the filtrate concentrated in vaccuo. The residue was partitioned between $CHCl_3$ and water, organic layer has separated, dried over anhydrous Na_2SO_4 and concentrated to give **13** (7.74 g, 86%) as a viscous liquid.

$[\alpha]_D$ +52.6^o (c 0.81, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.27, 1.39 (2s, 3H each, $(CH_3)_2C$), 3.55 (s, 3H, OCH_3), 3.85 (s, 1H), 4.07 (s, 1H), 4.15 (s, 2H, CH_2-5'), 4.30 (s, 1H), 6.17 (s,1H, H-1'), 8.61 (s, 1H, H-2), 8.67 (s, 1H, H-8).

FABMS m/z (rel. intensity): 341 (M^++1, 100), 325 (10), 187 (13), 155 (18), 109 (15).

HRMS (FAB): Cacld. for ($C_{14}H_{18}ClN_4O_4$, M^++1): 341.1016. Found 341.1025.

6-Chloro-9-(2'-*O*-methyl-b-D-xylofuranosyl)purine (14).

To the residue of **13** (6.5 g, 19.0 mmol) in methanol (100 mL) was added dropwise 0.8 % of H_2SO_4 (25 mL). The reaction mixture was stirred for 6 h, neutralized with $BaCO_3$,

filtered through celite and concentrated. The residue was purified over a pad of silica gel ($CHCl_3$-MeOH = 98:2) to afford **14** (5.2 g, 90%) as a solid.

^{1}H NMR ($CDCl_3$+DMSO-d_6, 200 MHz): δ 3.52 (s, 3H, OCH_3), 3.90 (m, 2H, CH_2-5'), 4.01 (s, 1H, H-2'), 4.26 (m, 1H, H-4'), 4.69 (t, *J* = 7.69 Hz, 1H, H-3'), 5.47 (d, *J* = 5.12 Hz, 1H, OH), 6.21 (s, 1H, H-1'), 8.71 (s, 2H, H-2, H-8).

FABMS m/z (rel. intensity): 301 (M^++1, 100), 211 (5), 155 (74), 136 (39), 107 (15).

HRMS (FAB): Cacld. for ($C_{11}H_{14}ClN_4O_4$, M^++1): 301.0703. Found 301.0708.

6-Chloro-9-(5'-bromo-2'-*O*-methyl-b-D-xylofuranosyl)purine (15).

To a stirred solution of **14** (5 g, 16.6 mmol) in dry pyridine (100 mL) were successively added TPP and CBr_4 at 0 °C. The temperature was raised to 50 °C. After 1 h, methanol was added after being stirred for 10 minutes. The solvent was evaporated under vacuuo and the residue purified on silica gel column chromatography using ethyl acetate-light petroleum (1:3) to give **15** (2.4 g, 40 %) as a solid.

M.P.: 176 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.46-3.75 (m, 5H, CH_2-5', OCH_3), 4.25 (s, 1H, H-2'), 4.35 (m, 2H, H-3', H-4'), 5.80 (d, 1H, *J* = 10.0 Hz, OH), 5.90 (s, 1H, H-1'), 8.26 (s, 1H, H-2), 8.77 (s, 1H, H-8).

6-Chloro-9-(3'-*O*-acetyl-5'-bromo-2'-*O*-methyl-b-D-xylofuranosyl)purine (16).

To the mixture of compound **15** (2.4 g, 6.6 mmol), DMAP (20 mg) and pyridine (3 mL) in CH_2Cl_2 (20 mL), was added acetic anhydride (2 mL) at 0 °C. The reaction mixture was stirred for 15 min. and the solvent evaporated under reduced pressure. Subsequent silica gel column chromatography of the residue using ethyl acetate-light petroleum (1:4) gave **16** (2.54 g, 95 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.05 (s, 3H, OAc), 3.58-3.68 (m, 5H, CH_2-5', OCH_3), 4.11 (s, 1H, H-2'), 4.68 (m, 1H, H-4'), 5.37 (d, *J* = 2.5 Hz, 1H, H-3'), 6.27 (s, 1H, H-1'), 8.28 (s, 1H, H-2), 8.72 (s, 1H, H-8).

FABMS m/z (rel. intensity): 406 (M^++2, 100), 404 (67), 360 (10), 325 (9), 250 (57), 191 (54), 155 (67).

HRMS (FAB): Calcd. for ($C_{13}H_{14}BrClN_4O_4$, M^++1): 406.0043. Found: 406.0035.

6-Chloro-9-(3'-*O*-acetyl-2'-*O*-methyl-5'-*O*-tosyl-b-D-xylofuranosyl)purine (17).

To a stirred solution of **14** (4 g, 11.7 mmol) in dry pyridine (75 mL), TsCl (3.4 g, 17.6 mmol) was added at 0 ºC. The reaction mixture was stirred at room temperature for 6 h. The solvent was evaporated under vacuo and the residue passed through silica gel column chromatography using ethyl acetate- light petroleum (3:7) to give 5'-O-tosyl derivative (4.6 g, 88 %) as a solid.

M.P.: 192 ºC.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.43 (s, 3H, $PhCH_3$), 3.48 (s, 3H, OCH_3), 4.17-4.54 (m, 5H, H-2', H-3', H-4', CH_2-5'), 5.77 (d, *J* = 10.12 Hz, 1H, OH), 5.89 (s, 1H, H-1'), 7.26 (A_2B_2, *J* =7.59 Hz, 2H, Ph), 7.78 (A_2B_2, *J* = 7.59 Hz, 2H, Ph), 8.27 (s, 1H, H-2), 8.72 (s, 1H, H-8).

FABMS m/z (rel. intensity): 455 (M^++1, 100), 421 (19), 155 (47), 111 (36).

HRMS (FAB): calcd.for ($C_{18}H_{20}ClN_4O_6S$, M^++1): 455.0792. Found 455.0780.

To a mixture of 5'-O-tosyl derivative (4.5 g, 9.9 mmol), DMAP (20 mg) and pyridine (3 mL) in CH_2Cl_2 (50 mL), acetic anhydride (2 mL) was added at 0 ºC. The reaction mixture was stirred for 15 min. and evaporated under reduced pressure. Subsequent passage of the residue through silica gel using ethyl acetate-light petroleum (1:4) afforded **17** (4.6 g, 94%) viscous liquid.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.98 (s, 3H, OAc), 2.45 (s, 3H, PhC$\underline{H}_3$), 3.58 (s, 3H, OCH_3), 4.13(s, 1H, H-2'), 4.33 (m, 2H, CH_2-5'), 4.60 (m, 1H, H-4'), 5.33 (d, *J* = 3.79 Hz, 1H, H-3'), 6.18 (s, 1H, H-1'), 7.33 (A_2B_2, *J* = 7.59 Hz, 2H, Ph), 7.79 (A_2B_2, *J* = 7.59 Hz, 2H, Ph), 8.19 (s, 1H, H-2), 8.70 (s, 1H, H-8).

FABMS m/z (rel. intensity): 498 (M^++2, 17), 497 (M^++1, 74), 343 (100), 261 (25), 227 (34), 175 (80), 129 (85).

HRMS (FAB): Calcd. for ($C_{20}H_{23}ClN_4O_7S$, M^++1): 498.0975. Found 498.0969.

6-chloro-9-(3'-*O*-acetyl-5'-Iodo-2'-*O*-methyl-b-D-xylofuranosyl)purine (18).

A mixture of **17** (4.5 g, 9 mmol) and sodium iodide (6.7 g, 45.3 mmol) in 2-butanone (100 mL), was refluxed for 8 h and concentrated. The residue was treated with saturated sodium thiosulphate solution, extracted with ethyl acetate and evaporated under reduced pressure. Subsequent silica gel chromatography using ethyl acetate-light petroleum (1:4) of the residue gave **18** (3.1 g, 75 %).

$[\alpha]_D$ +68.52^o (c 1.52, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.04 (s, 3H, OAc), 3.41 (m, 2H, CH_2-5'), 3.65 (s, 3H, OCH_3), 4.12 (s, 1H, H-2'), 4.71 (m, 1H, H-4'), 5.4 (d, *J* = 2.5 Hz, 1H, H-3'), 6.30 (s, 1H, H-1'), 8.28 (s, 1H, H-2), 8.73 (s, 1H, H-8).

FABMS m/z (rel. intensity): 454 (M^++2, 16), 453 (M^++1, 100), 299 (39), 176 (5), 155 (37), 127 (14).

HRMS (FAB): Calcd. for ($C_{13}H_{16}ClIN_4O_4$, M^++2): 454.9904. Found 454.9907.

6-Chloro-9-(3'-*O*-acetyl-2'-*O*-methyl-4'-eno-b-D-xylofuranosyl)purine (19).

To a stirred solution of **18** (2.5 g, 6.15 mmol) in dry DMF (25 mL) was added dropwise DBU (1.1 mL, 7.4 mmol) at 0 °C. The temperature of the reaction mixture was raised to 80 °C. The reaction mixture was stirred for 2 h at the same temperature, cooled to

rt. and partitioned between ether and water. The ether layer was evaporated and the residue on purification by silica gel column chromatography using ethyl acetate-light petroleum (2:8) provided **19** (1.99 g, 84 %) as a solid.

M.P.: 184-185 ^{o}C.

$[\alpha]_D$ +84.63^{o} (c 1.23, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.01(s, 3H, OAc), 3.61 (s, 3H, OCH_3), 4.18 (s, 1H, H-2'), 4.60 (d, *J* = 2.5 Hz, 1H, H-5'), 4.88 (d, *J* = 2.5 Hz, 1H, H-5''), 5.62 (s, 1H, H-3'), 6.47 (s, 1H, H-1'), 8.25 (s, 1H, H-2), 8.75 (s, 1H, H-8).

CIMS m/z (rel. intensity): 325 (M^++1, 100), 172 (16), 118 (96), 101 (32), 59 (92), 43 (82).

HRMS (CI): Calcd. for ($C_{13}H_{14}ClN_4O_4$, M^++1): 325.0703. Found 325.0711.

6-Benzylamino-9-(2'-*O*-methyl-4'-eno-b-D-xylofuranosyl)purine (3).

To a stirred solution of **19** (1.6 g, 4.9 mmol) in the mixture of THF: H_2O (8:2 ratio, 10 mL) was added dropwise benzylamine (2 mL). The reaction mixture was refluxed for 12 h. After evaporation of the solvent, the residue was purified on silica gel using methanol-chloroform (2:98) to give **3** (1.35 g, 78 %) as a solid.

M.P.: 210-212 ^{o}C.

$[\alpha]_D$ +90.10^{o} (c 0.93, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.48 (s, 3H, OCH_3), 4.36 (s, 1H, H-2'), 4.43 (s, 1H, H-5'), 4.51(s, 1H, H-5''), 4.55 (s, 1H, H-3'), 4.86 (br d 2H, NCH_2Ph), 6.01 (s, 1H, H-1'), 6.35 (br t, 1H, NH), 7.22-7.30 (m, 5H, Ph), 7.82 (s, 1H, H-2), 8.35 (s, 1H, H-8).

FABMS m/z (rel. intensity): 354 (M^++1, 56), 322 (15), 282 (52), 226 (100), 186 (27), 159 (14).

HRMS (FAB): Calcd. for ($C_{18}H_{20}N_5O_3$, M^++1): 354.1566. Found 354.1575.

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