SYNTHESIS OF AZA ANALOGUE OF CMI-977, TERMINAL DISACCHARIDE UNIT OF *K. PNEUMONIAE* AND SOME USEFUL ORGANIC TRANSFORMATIONS.

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

To

University of Pune

By

ARINDAM TALUKDAR

Division of Organic Chemistry: Technology
National Chemical Laboratory
Pune – 411 008
INDIA
December 2002

AN ADORE TO MY PARENTS

DECLARATION

The research work embodied in this thesis submitted for Ph. D. degree to the University of Pune has been carried out at Indian Institute of Chemical Technology, Hyderabad and National Chemical Laboratory, Pune under the supervision of **Dr. Mukund. K. Gurjar,** Deputy director and Head, Division of Organic Chemistry: Technology, National Chemical Laboratory, Pune – 411 008. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other University.

Date: **(ARINDAM TALUKDAR)**

Organic Chemistry: Technology
National Chemical Laboratory
Pune - 411 008.

NATIONAL CHEMICAL LABORATORY

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

Dr. M. K. Gurjar
Deputy Director & Head
Division of Organic Chemistry: Technology

Telephone and Fax: + 91-20-5893614
+ 91-20-5882456
E-mail: gurjar@dalton.ncl.res.in
Website: http://www.ncl-india.org

CERTIFICATE

The research work presented in this thesis entitled **"Synthesis of aza analogue of CMI-977, terminal disaccharide unit o*f K. pneumoni*ae and some useful organic transformations"** has been carried out under my supervision and is bonafide work of **Mr. Arindam Talukdar.** This work is original and has not been submitted for any other degree or diploma of this or any other University.

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

Date: (Research Guide)

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CHAPTER: IV

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Arindam

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 and Bruker-500 MHz spectrometer using tetra methyl 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 AC-50 MHz, MSL-75 MHz and Bruker-125 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 auto spec 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 anisaldehyde reagent in ethanol as development reagents.

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

- All solvents and reagents were purified and dried 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.

Ac	- Acetyl
AcOH	- Acetic acid
Ac_2O	- Acetic anhydride
BF_3:OEt_2	- Borontrifluoride diethyletherate
Bn	- Benzyl
BnBr	- Benzyl bromide
BnCl	- Benzyl chloride
Boc	- *tert*-Butoxy carbonyl
$(Boc)_2O$	- Di-*tert*-butyl dicarbonate
CAN	- Ammonium cerium(IV) nitrate
DBU	- 1,8-Diazabicyclo [5.4.0]undec-7-ene
DIBAL-H	- Diisobutylaluminium hydride
DMAP	- *N, N'*-Dimethylaminopyridine
DMF	- *N, N'*-Dimethylformamide
DMP	- 2,2-Dimethoxypropane
DMSO	- Dimethyl sulfoxide
Et	- Ethyl
EtOAc	- Ethyl acetate
EtOH	- Ethanol
IBX	- 2-Iodoxybenzoic acid
Im	- Imidazole
Man*p*	- Mannopyranosyl
*m*CPBA	- *meta*-Chloroperbenzoic acid
MeOH	- Methanol
NaOMe	- Sodium methoxide
NIS	- *N*-Iodosuccinimide
NPhth	- Phthalimide
Pd/C	- Palladium on carbon
$Pd(OH)_2/C$	- Palladium hydroxide on carbon
PMB/MPM	- *para*-Methoxy benzyl

*p*TSA	- *para*-Toluenesulfonic acid
Py	- Pyridine
TBAB	- Tetrabutylammonium bromide
TBAF	- Tetrabutylammonium fluoride
TBAI	- Tetrabutylammonium iodide
TBDMS-Cl	- *tert*-Butyldimethylchlorosilane
Et_3N	- Triethyl amine
TFA	- Trifluoroacetic acid
THF	- Tetrahydrofuran

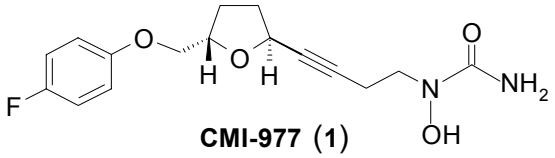
The thesis entitled **"Synthesis of aza analogue of CMI-977, terminal disaccharide unit o*f K. pneumoni*ae and some useful organic transformatio**ns" is divided into four chapters. The first chapter provides the synthesis of aza analogue of CMI-977 (Section I), a new synthesis for potent anti-asthmatic lead candidate and (*S*)-Metoprolol (Section II), β-blocker. The second chapter describes the synthesis of 9-*epi*-manzacidin B methyl ester, an α-adrenoceptor blocker, antagonists of serotonergic receptor etc. The third chapter deals with the synthesis of terminal disaccharide unit of *K. pneumoniae*. A facile methodology for the synthesis of cinnamyl glycine derivatives (Section I) *via* Heck reaction and an unprecedented reaction of Grubbs' catalyst for the conversion of sugar oximes to sugar nitriles (Section II) have been described in the fourth chapter.

CHAPTER – I

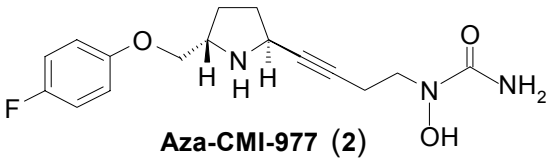
Section – I: Stereoselective synthesis of Aza CMI-977.

Bronchial asthma is characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper responsiveness to various stimuli. The relationship between inflammatory conditions and prostaglandins, thromboxanes and leukotrines has been established for a long time. Researchers are now striving to develop leukotrienes inhibitors as a new class of asthma drugs that will strike at the root cause of the disease.

CMI-977 (**1**) is being currently developed by Millenium Pharmaceuticals, USA as a potential lead candidate for chronic asthma and presently at clinical trial stage. In order to understand the structure activity relationship, we have been engaged in the synthesis of compounds related to **1** in which variation in ring size, introduction of heteroatom, change in side chain etc. This chapter deals with the synthesise of aza analogue (**2**) of CMI-977, where in furan ring has been replaced by pyrrolidine ring.



CMI-977 (1)

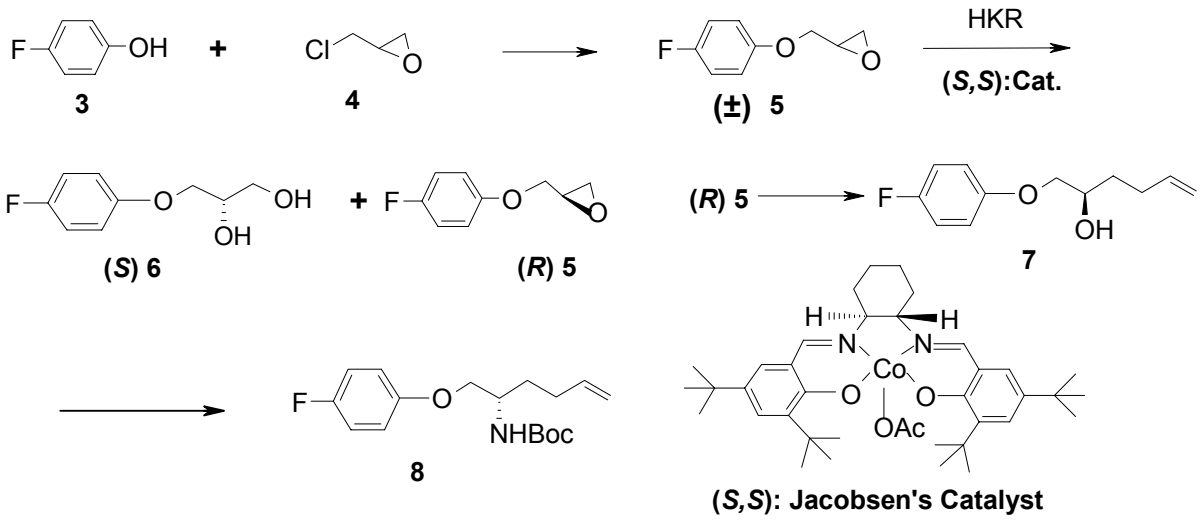


Aza-CMI-977 (2)

Accordingly, reaction of 4-flurophenol (**3**) with epichlorohydrine (**4**)/K_2CO_3 gave racemic (±) 4-flurophenyl glycidyl ether (**5**) (Scheme: **1**), which was subjected to Jacobsen's Hydrolytic Kinetic Resolution (HKR) conditions using (*R*, *R*)-Cobalt salen complex to provide (*S*)-epoxide **5**

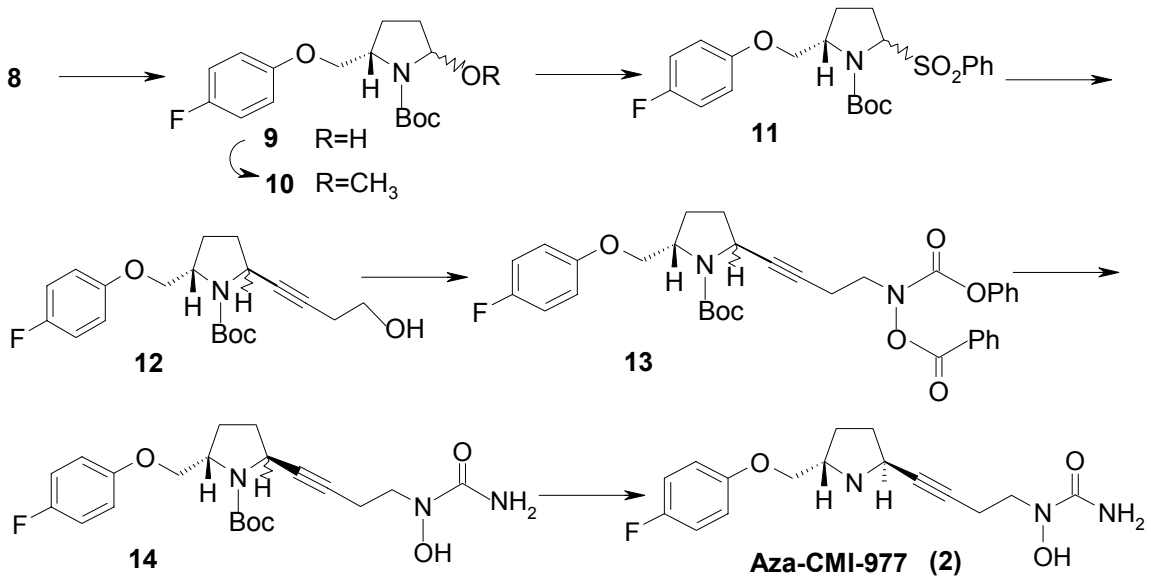
and (*R*)-diol **6**. In order to generate the correct stereochemistry at C-5 of **2**, our synthesis started with (*R*)-epoxide, which was obtained by converting (*R*)-diol into (*R*)-epoxide **5** with TPP and DEAD. The CuCN-coordinated opening of (*R*)-**5** with allylmagnesium bromide, and subsequent treatment of **7** under Mitsunobu condition with pthalimide, DEAD and TPP gave pthalimide derivative, which was hydrolysed using $NH_2NH_2.H_2O$ to give free amine. The amine was immediately protected with $(BoC)_2O$ to give **8**.

Scheme: **1**



Ozonolysis of **8** provided (Scheme: **2**) the corresponding 2-hydroxypyrrolidine **10** as an anomeric mixture, which upon exposure to PTSA in MeOH gave **10**. Compound **10** on treatment with $PhSO_2H$ in DCM in the presence of $CaCl_2$ gave 2-benzenesulphonyl pyrrolidine **11**. Homopropargyl alcohol derivative **12** was obtained by nucleophilic displacement of the sulfone **11** with 4-tetrahydropyranyloxy-1-butynylmagnesium bromide in presence of anhydrous $ZnBr_2$ and subsequent removal of THP protecting group with catalytic PPTS in MeOH provided the C-C coupled derivative (2S/2R; 87:13, by analytical HPLC). Introduction of *N*-hydroxyurea derivative was achieved by carrying out Mitsunobu reaction of **12** with *N*, *O*-bis(phenoxycarbonyl) hydroxylamine / PPh_3 / DEAD to give **13**. Subsequent ammonolysis and Boc deprotection of **14** with TFA culminated in the total synthesis of target compound (**2**), which was confirmed by ^1H NMR, ^{13}C NMR, DEPT and FABMS studies. It was interesting that chiral HPLC indicated only one isomer, whose stereochemistry was established as *trans* by NOESY studies.

Scheme: **2**

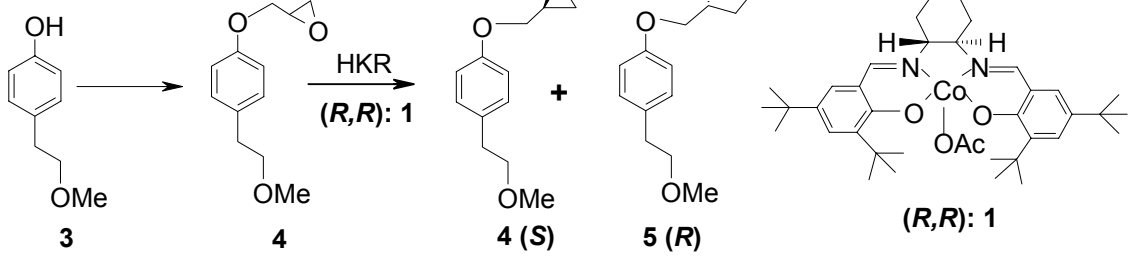


Section- II: Application of Hydrolytic Kinetic Resolution (HKR) Towards the Synthesis of Optically Pure β-blocker *S*-Metoprolol.

Jacobsen *et*. *al*. reported hydrolytic kinetic resolution (HKR) of terminal epoxides using readily accessible chiral (R, R)-Cobalt Salen complex (**1**). (HKR) reaction is highly enantioselective and uses water as the only reagent. It affords terminal epoxides and 1,2-diols with high enantiomeric enrichment. Metoprolol (**2**) has been shown to be an effective agent against hypertension, myocardial infraction and angina pectoris. It's '*S*'-isomer possesses most of the β-blocking activity.

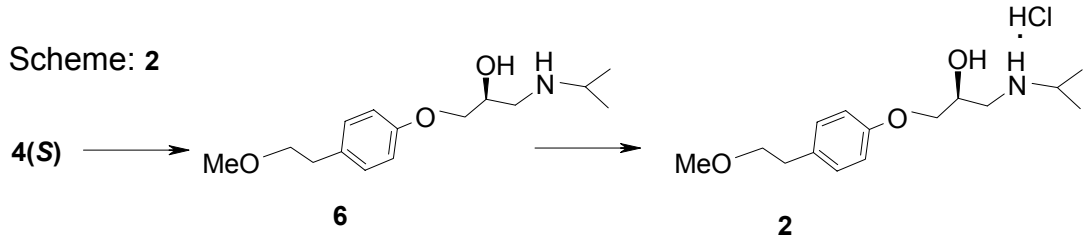
The known compound **3** (Scheme: **1**) on treatment with epichlorohydrine/K_2CO_3 afforded racemic (±) aryl glycidyl ether (**4**). Compound **4** was subjected to HKR using Salen complex **1** (5 mol %) and water (0.55 eq) provided aryl glycidyl ether **4** (*S*) in 93 % *ee* and diol **5** (*R*) in 97 % *ee*.

Scheme: **1**



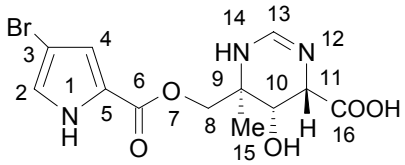
Treatment of **4(*S*)** (Scheme: **2**) with isopropylamine, followed by salt formation with methanolic HCl provided the desired (*S*)-metoprolol hydrochloride (**2**) whose optical rotation and NMR data are in accordance with reported values.

Scheme: **2**



Chapter II: Synthesis of 9-*epi*-manzacidin B methyl ester

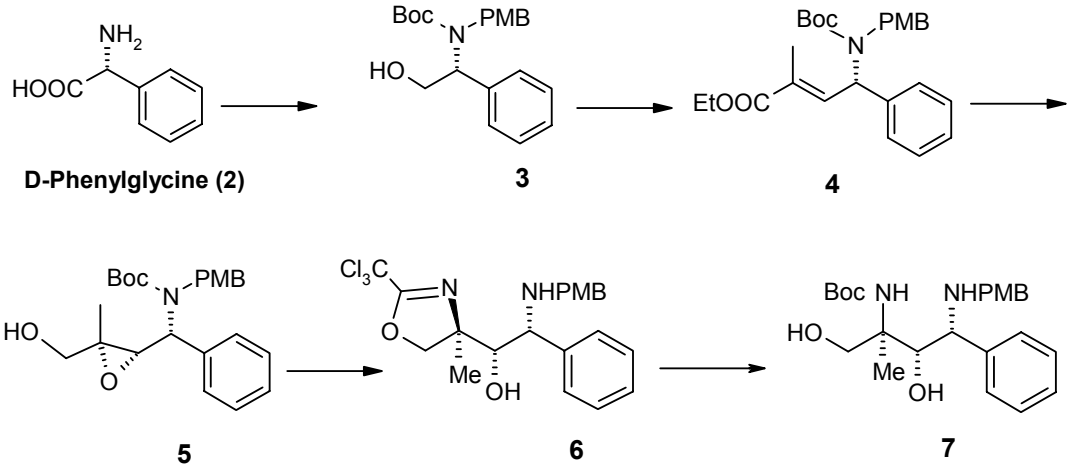
Recently a novel class of alkaloids manzacidin A-C have been isolated from *Hymeniacidon* sp., which possess a unique structure consisting of an ester-linked bromopyrrole carboxylic acid and a 3,4,5,6-tetrahydropyrimidine ring in which one of the amino group is attached to the C-9 quaternary carbon center. The present chapter describes the highly stereoselective synthesis of 9-*epi*-manzacidin B as its methyl ester derivative (**1**). The perpose of synthesis of **1** was to develop a synthetic route amenable to prepare the natural product as well as stereomeric analogues useful to study structure-activity relationship.



9-*epi*-manzacidin B (1) methyl ester

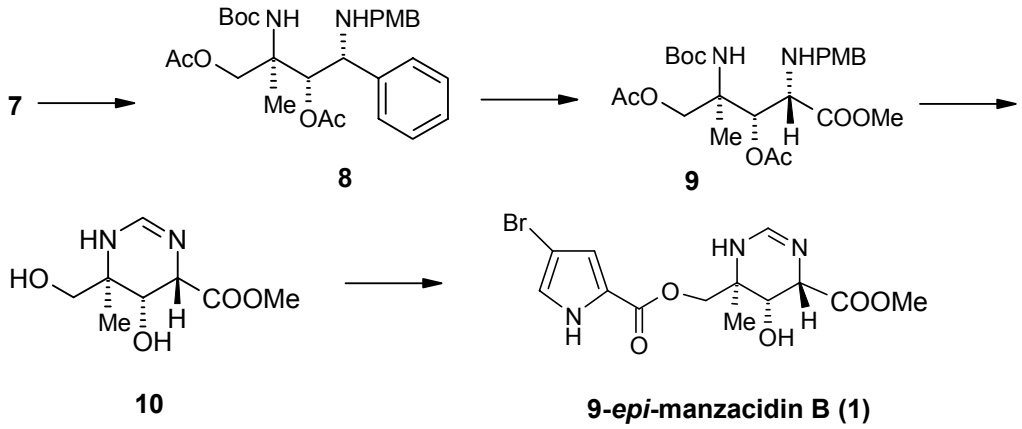
We began the synthesis from D-phenyl glycine (**2**), which was reduced with $NaBH_4/I_2$ followed by the protection of the amine functionality with PMB and Boc groups to give **3.** Alcohol **3** was oxidized and the resulting aldehyde then subjected to Wittig-Horner olefination conditions to give the (E)-olefin **4**. Olefin **4** when reduced with Dibal-H at –78 ^oC gave *trans*-γ-amino allylic alcohol. The Sharlpless epoxidation of allylic alcohol with $Ti(OPr\text{-}i)_4$, (+)-DIPT and TBHP at –20 ^oC afforded the *syn* epoxide **5**. Our next aim was to introduce the amine group regioselectivity at C-2, for which Hatakeyama's method was employed. Thus, treatment of **5** with CCl_3CN/DBU followed by intramolecular epoxide-opening reaction catalyzed by BF_3:OEt_2 at –25 ^oC afforded the oxazoline **6**. Oxazoline **6** was then converted in to alcohol **7** by acid hydrolysis followed by *tert*-butoxy carbonylation (Scheme **1**).

Scheme **1**



Our next concern was the oxidation of phenyl group in **7** to acid. For this purpose, the PMB group was first deprotected and the resulting free amine was then protected with $(Boc)_2$ and subsequently two hydroxyl groups were protected with Ac_2O to give **8**. Compound **8** on oxidation with $RuCl_3/NaIO_4$ followed by esterification with CH_2N_2 afforded **9**. The deprotection of acetate functionalities from compound **9** with K_2CO_3/MeOH resulted in the formation of the expected lactone. The lactone on successive treatment with (i) TFA and (ii) methyl orthoformate gave the acid which was purified after the esterification with CH_2N_2 **10**. Completion of the synthesis now required esterification of the bromopyrrole carboxylate with **10**. Thus, treatment of **10** with NaH/trichloroacetylbromopyrrole in DMF at room temperature gave the required compound **1** (Scheme **2**).

Scheme 2



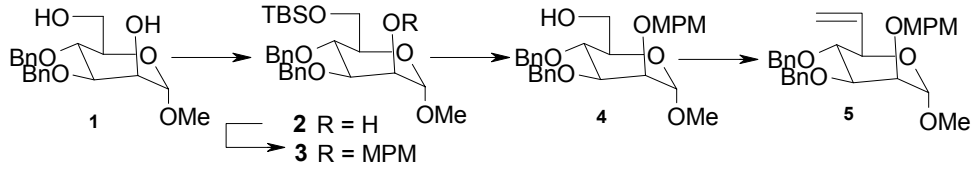
CHAPTER- III: Synthesis of terminal disaccharide unit of *K. pneumoniae* strain R20

Klebsiella pneumoniae is an important Gram-negative pathogenic bacterium associated with nosocomial infections. *Klebsiella* infections are encountered more often probably due to the bacterium's resistance towards antibiotics. Herein, we report the synthesis of a terminal disaccaride unit of the heptoglycan of α linkage present in *K. pneumoniae* ssp. *pneumoniae* strain R20.

Approach A

Our synthesis started with methyl α -D-mannopyranoside, which was (Scheme **1**) converted to diol **1** by following the reported procedure. Protection of primary hydroxyl as its silyl derivative **2** followed by the protection of 2-OH with MPM-Br (**3**) and subsequent removal of silyl group using *n*-Bu_4NF in THF afforded **4.** Swern oxidation of **4** yielded the corresponding aldehyde,

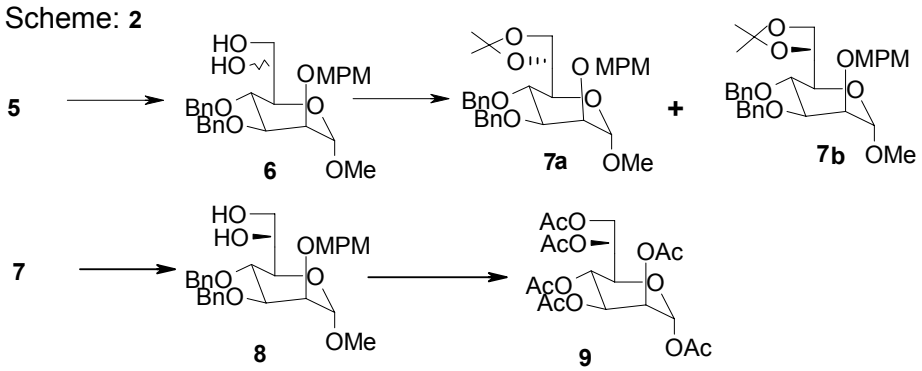
Scheme :**1**



which upon treatment with $Ph_3P{=}CH_2$ gave **5**.

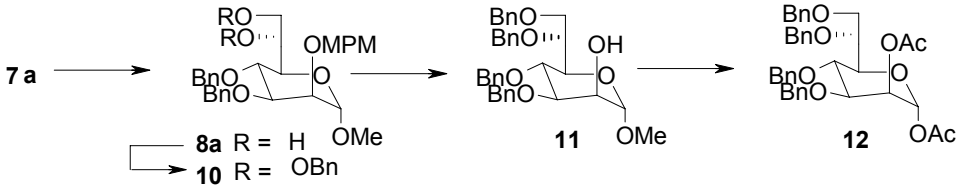
Sharpless asymmetric dihydroxylation of **5** (Scheme: **2**) with $(DHQ)_2PYR$ ligand gave inseparable 9:1 mixture of diastereomers **6**. The corresponding acetonide derivative (**7a** and **7b**) was found to easily separable, with **7a** being the major and required product. The stereochemistry at C-6 was confirmed by comparing the optical rotation of **9** $[\alpha]_D$ + 28.1 (c 0.85, $CHCl_3$) with that of the reported value $[\alpha]_D$ + 27 (c 1, $CHCl_3$). Compound **9** was synthesized from **8b** by deprotection of the benzyl and MPM group by hydrogenation and then subjected to acetolysis condition.

Scheme: **2**



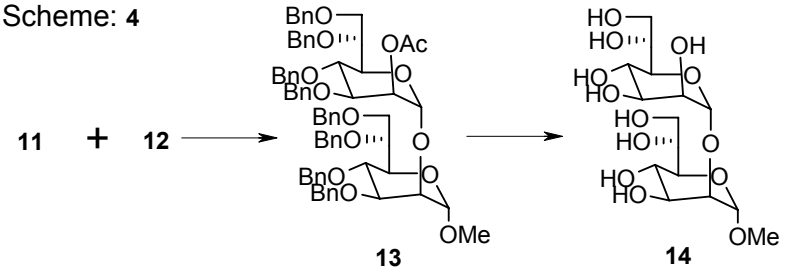
The acetonide group in **7a** was subsequently deprotected (Scheme: **3**) with PPTS and the diol **8a** thus obtained was further benzylated using NaH/BnBr to afford compound **10**. The MPM group at C-2 was then deprotected with DDQ to give **11** and subsequently under acetolysis conditions was transformed to glycosyl donor **12** in 75 % yield.

Scheme: **3**



Glycosidation was done with **10** and **11** (Scheme: **4**) in presence of catalytic BF_3:OEt_2, 4 Å molecular sieves in dry CH_2Cl_2 to obtain **13**, which finally on deacetylation with MeONa and exhaustive debenzylation using 10% $Pd(OH)_2$ in MeOH gave the required terminal disaccharide unit **14** . The spectroscopic data are comparable with the similar reported compounds.

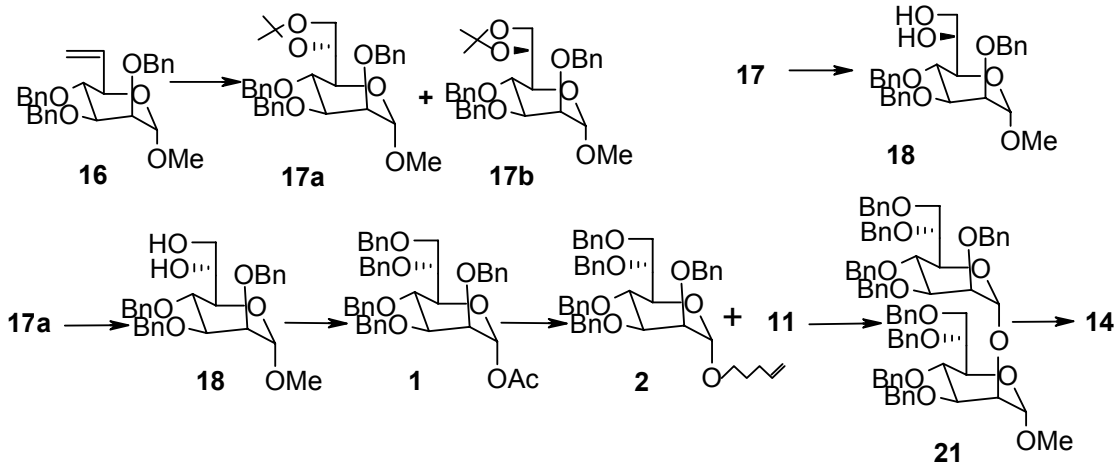
Scheme: **4**



Approach B

In an alternative method (Scheme: **5**), methyl α-D-mannopyranoside was converted to **16**, which was subjected to the same reaction sequences as in Scheme **1** and **2** to obtain compound **17a** and **17b**. The stereochemistry at C-6 was confirmed by comparing the optical rotation of **18b**, $[\alpha]_D$ + 23.8 (c 0.90 , $CHCl_3$), with that of the reported value $[\alpha]_D$ + 23 (c 1, $CHCl_3$) . Deprotection of acetonide followed by benzylation of **18a** and subsequent acetolysis provided compound **19**, which on treatment with pentenyl alcohol in presence of BF_3:OEt_2, 4 Å molecular sieves in dry CH_2Cl_2 afforded pentenyl glycoside **20**. Glycosidation was carried out adopting Fraser-Reid's pentenyl glycosidation strategy with **20** and glycosyl acceptor **11** (from Scheme **3**), in presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH) and 4 Å molecular sieves. Final deprotection of benzyl group using 10 % $Pd(OH)_2$ in MeOH gave the terminal disaccharide unit **14** with α–glycosidic linkage, which was proved by comparison of analytical and spectroscopic data with the sample prepared earlier (Scheme **4**).

Scheme: **5**

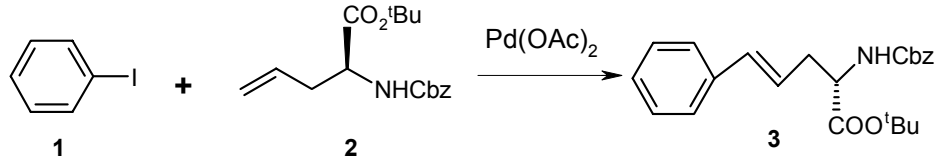


CHAPTER: IV

Section – I: Heck Reaction of Allyl Glycine with Aromatic Halides

The allyl glycines belong to the class of an unnatural and non-proteogenic α -amino acid. They also act as building blocks, exemplified by the synthesis of homophenylalanine derivatives, the latter being used in the synthesis of drugs with angiotensin converting enzyme inhibitory activity. The strategies utilised for the synthesis of allyl glycines include Wittig reaction, Pd-catalysed allylation of glycine or glycine equivalent, etc. The Heck reaction between allyl glycine and aryl halides, which would provide a direct access to aryl allyl glycines, has not been reported to the best of our knowledge.

Scheme: **1**

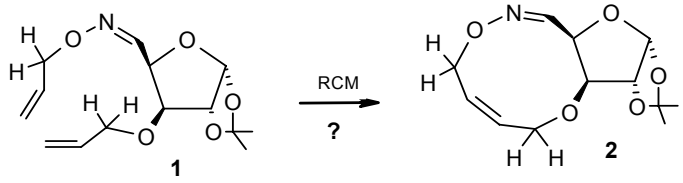


When *N*-Cbz-allylglycine *tert*-butyl ester (**2**) (Scheme: **1**) was reacted with iodobenzene (**1**) in presence of $Pd(OAc)_2$ in acetonitrile at 70 °C afforded the 5-phenyl-allylglycine derivative (**3**) in 70 % yield with high isomeric purity. The ^{1}H and ^{13}C NMR, mass spectral data coupled with elemental analysis of **3** was in conformity with the assigned structure. In conclusion, the above strategy founded on the Heck reaction between allyl glycine and various aromatic halides is indeed a simple and efficient method to prepare cinnamyl glycine derivative with high isomeric purity.

Section- II: Unusual Conversion of Sugar-Oximes to Sugar-Nitriles with Ruthenium Catalysts.

The use of olefin metatheses in synthesis has fully emerged as a powerful method of C-C bond formation. peptidomimetics. Keeping this in mind we plan to synthesize new heterocyclic compounds (**2**) with a carbohydrate backbone by applying RCM (Scheme: **1**).

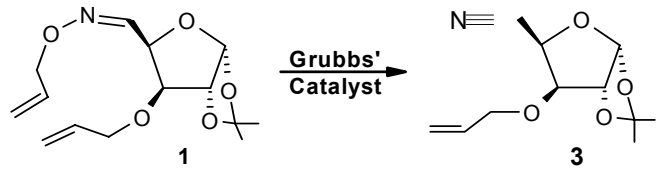
Scheme: **1**



1 **2**

Treatment of **1** with 3 mol % of Grubbs' catalyst $Cl_2(PCy_3)_2Ru=CHPh$ in benzene (Scheme: **2**) at 60 °C gave a single major product whose structure was not in conformity with expected product (**2**). It was observed that the unprotected oxime also underwent transformation with ruthenium salts to provide the same nitrile derivative **3**, thus, indicating that the protecting group is not essentiaThe scanty reports on the synthesis of sugar substituted nitriles may be

Scheme: **2**



1 **3**

attributed to the use of acidic dehydrating reagents in the existing methods, which cleave acid labile isopropylidene groups leading to a mixture of products. The conversion of oxime to nitrile was studied in wide range of substrates, by changing the substituent at C-3 of glucofuranose, in glucopyranose, ribose etc.

Arindam Talukdar
(Candidate)

Dr. M. K. Gurjar
(Research Supervisor)

CHAPTER- 1

Section-I

Stereoselective Synthesis of Aza CMI-977

INTRODUCTION

The famous Greek physician, Hippocrates, first used the word Asthma. Physicians during the seventeenth and eighteenth centuries realized that asthma was due to constrictions of the Bronchi (the airways into the lungs). They called asthma as "Epilepsy of the lungs" reflecting the sudden and unpredictable nature of asthma "attacks". A Spanish doctor, Moses Maimonides, wrote the first book specifically about asthma in AD 1190. It was not until the 1960's that physicians discovered that asthma is an inflammatory disease. This discovery started a revolution in the treatment of asthmatics - instead of just treating the constriction of the airways; doctors now treat the underlying inflammation as well. According to WHO, asthma affects 150 million people worldwide, and the number of patients has doubled over the decade. With more than one-half of the sufferers' children and mortality rates inexplicably on the rise, researchers are now striving to develop a new class of asthma drugs that will strike at the root cause of the disease.[1]

WHAT IS ASTHMA?

Asthma is a chronic reaction disorder involved episodic reversible airway obstruction because of bronchospasms, increased mucus secretion and mucosal cell edema.[1] This makes breathing out more difficult than breathing in, causing stale air to be retained with each new breath, leading to suffocation. Asthma may occur due to an allergy (atopic/extrinisic)[2] or because of a respiratory infection (non-atopic/intrinsic). [3] Smooth muscles then undergo spasmodic contractions, swelling in membranes that line the bronchial tubes, and increased mucus secretion occurs. It is characterized clinically by wheezing, dyspnea, and cough.

CAUSE OF ASTHMA

There are multiple triggering stimuli that will increase symptoms. Allergic asthma is the most common form. Other precipitating factors include infection,[4] exercise,[5] drugs,[6] air pollution,[7] industrial exposures[8] commonly causes asthma symptoms. The

common factor seems to be an irritation of the respiratory mucous membrane. The advances in molecular biology indicated that allergy and asthma are not inherited as single-gene disorder and do not show a simple pattern of inheritance. Environmental and genetic factors interact in a complex fashion to produce disease susceptibility and expression.[9] An increased risk of atopy exists when the mother herself has a history of allergy. Even passive or second hand smoking by parents especially mothers' increases the risk of asthma in children.

During an attack, asthma triggers causes Immunoglobulin E (IgE) to destabilize Mast Cells located in the airway. When Mast Cell membranes destabilize, they release a chemical soup made up of substances such as prostaglandin D2, histamines, and cysteinyl leukotrienes, which causes tracheo- bronchial hyper-reactivity leading to paroxysmal airway narrowing.[10]

MEDIATORS OF ASTHMA[11]

The discovery of slow reacting substance (SRS) began in 1938, when Feldberg and Kellaway observed a substance released from perfuse guinea pig lung when injected with cobra venom. They introduced the term SRS to represent a substance which caused contraction of the pig ileum but differ from histamine in that the contraction was delayed and prolonged. It was not until 1979, Merphy, Hammerstrom and Samuelsson characterized SRS as a cystine containing metabolites of arachidonic acid via the lipoxygenase pathway. Subsequently Samuelsson and coworkers made a rapid progress in the elucidation of different SRS from metabolism of arachidonic acid and renamed SRS as leukotrienes (LTs). In 1982, Samuelsson was awarded Nobel Prize in medicine and physiology for his explanation of this extraordinarily interesting class of natural products and for an account of the conversion of arachidonic acid into different leukotrines LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4 as well as their implication in physiological disorders.

ARACHIDONIC ACID METABOLISM[12]

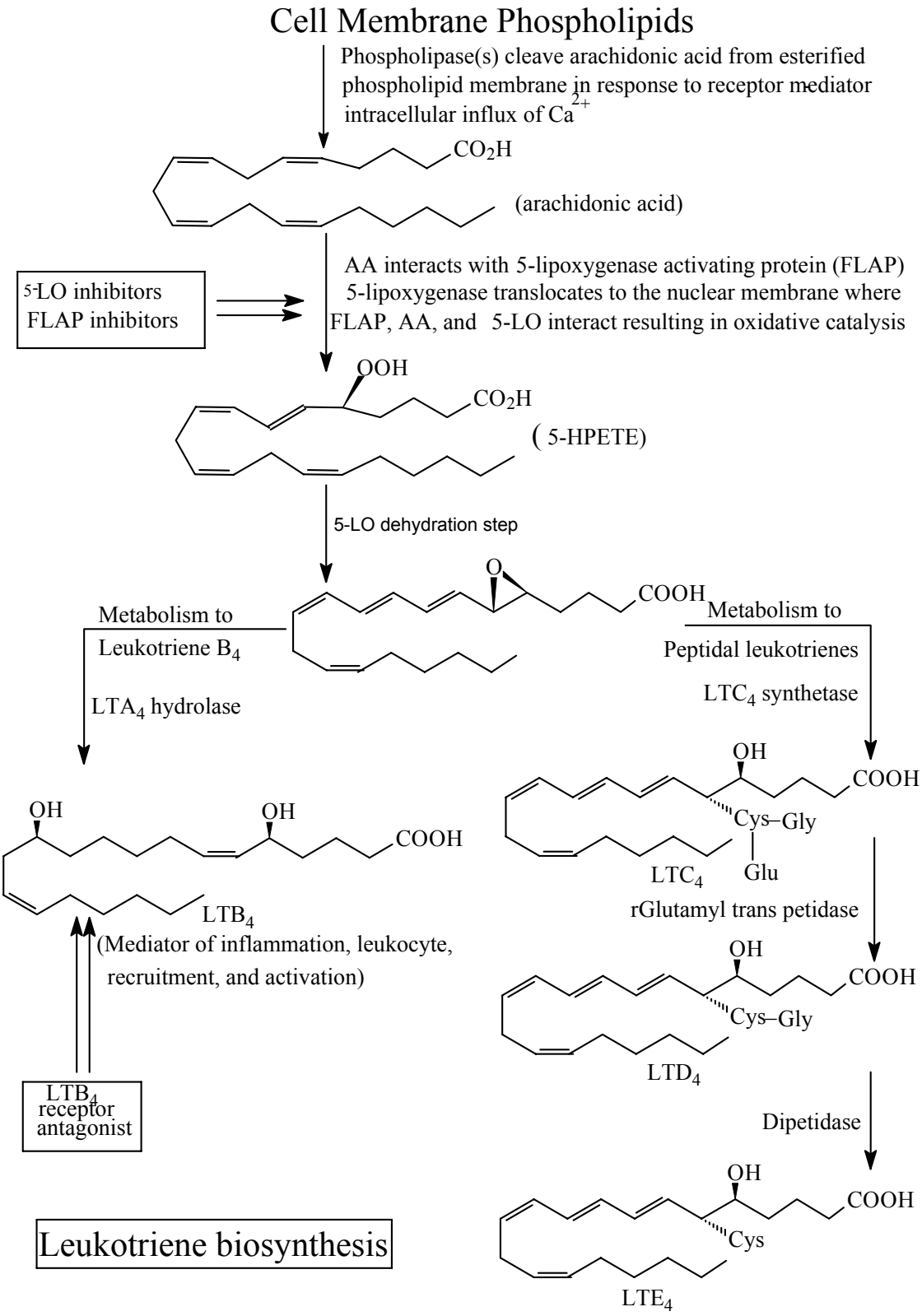
Arachidonic acid, an unsaturated C_{20} fatty acid has attracted the researchers over the past few decades. It forms an essential acyl component of the phospholipids in all

animal cells. Arachidonic acid is enzymatically formed from linolenic acids by the action of phospholipase enzyme. Once released from phospholipids, two types of enzymes, Cyclooxygenase and lipoxygenase metabolize it.

Cyclooxygenase catalyze the incorporation of molecular oxygen into arachidonic acid leading to peroxidation. The product of this reaction is cyclic endoperoxide[13] prostaglandin G2 (PGG_2) that is reduced to chemically unstable prostaglandin H_2 (PGH_2) and is isomerised into different products. The major products of endoperoxide[13] metabolism are the stable prostaglandins PGD_2, PGE_2, PGF_2. The PGH_2 is also metabolized into highly biologically active compounds. Thromboxane A_2 (TXA_2), formed by enzyme, thromboxane synthetase. TXA_2 further breaks down non-enzymatically into stable thromboxane B_2 (TXB_2).

LEUKOTRINES

Simply stated, when allergies or viral infections occur, inflammation begins with the release of a molecule from airway cell membranes called arachidonic acid. Arachidonic acid is acted on by two different enzyme pathways and becomes either leukotrienes or other molecules called prostaglandins. Leukotrienes and prostaglandins can both trigger asthma. Leukotrienes[14] interact with their own special receptor molecules in the lung to increase mucus production, airway irritability and swelling, which lead to coughing, wheezing, shortness of breath or chest tightness. Most importantly, leukotrienes attract other cells to the tissue, and over a period of hours, these new cells along with other, potent chemical “mediators” in the cells, cause the tissue inflammation. The inflammation must be controlled, or the lungs will become “twitchy” and, if left uncontrolled, will result in an attack. Leukotrienes are of particular interest because of their potential as bronchoconstrictors. They are 1000 times more potent bronchoconstrictors than histamines and prostaglandins. Leukotrienes are a byproduct of arachidonic acid found in the phospholipid by-layer in the outer membranes of Mast Cells. During asthma attack a chemical process eventually breaks arachidonic acid into five leukotrienes (LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4).


Cell Membrane Phospholipids
Phospholipase(s) cleave arachidonic acid from esterified phospholipid membrane in response to receptor mediator intracellular influx of Ca^{2+}
CO_2H
(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
CO_2H
(5-HPETE)
5-LO dehydration step
O
COOH
Metabolism to
Leukotriene B_4
LTA_4 hydrolase
Metabolism to
Peptidal leukotrienes
LTC_4 synthetase
OH
OH
COOH
LTB_4
(Mediator of inflammation, leukocyte, recruitment, and activation)
OH
COOH
Cys–Gly
Glu
LTC_4
rGlutamyl trans petidase
OH
COOH
Cys–Gly
LTD_4
Dipetidase
OH
COOH
Cys
LTE_4
LTB_4 receptor antagonist
Leukotriene biosynthesis

It's within this chemical process where Leukotriene modifiers[15] (inhibitors) do their work. Of the five leukotrienes produced during an attack, the cysteinyl leukotrienes[16] (LTC_4, LTD_4, and LTE_4) are the most potent bronchoconstrictors. It will be rational to divide leukotrines effects into two groups

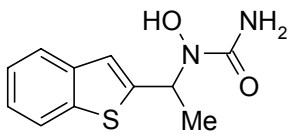
i) LTB4 and its congeners

ii) LTC4 and its metabolites

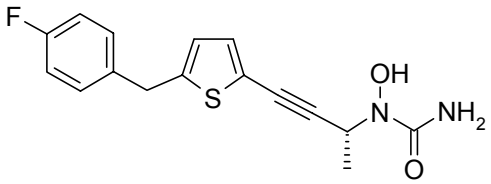
LTB_4 is a powerful chemotactic signal for polymorphonuclear leukocytes, causing diapedesis and accumulation of thesis cells in the extravascular space. LTB_4 also causes activation of leukocytes leading to release of cytotoxic substances presumably superoxide generation. On the other hand cystenyl-containing LTC_4, LTD_4, and LTE_4 lack capacity to activate polymorphonuclear leukocytes. They are important mediators of cardinal manifestations of immediate-type bronchial asthma.

LEUKOTRINES INHIBITORS

Much evidence has accumulated implicating LTs in disease states having inflamm atory components, including psoriasis, asthma, and allergy. In the hope of finding anti-inflammatory drugs with reduced side-effects (or) greater efficacy, a major effort has been mounted by the pharmaceutical industry over the past decade to identify either selective inhibitors of 5-LO (or) dual inhibitors of CO and 5-LO. A wide variety of agents have been reported as 5-LO inhibitors, through the untiring efforts of medicinal chemists. 5-LO inhibitor, 5-LO-activating protein antagonist, and CysL-T receptor antagonists are three classes of LT modulators, and subsequently as drug targets now in clinical practice.



Zileuton (**16**)

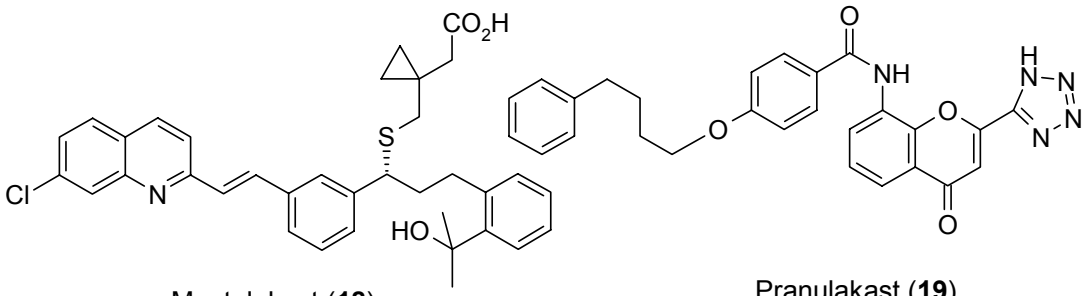


ABT-761 (**17**)

Zileuton[17] (**16**) is a selective orally active inhibitor of 5-LO proven to exert anti-inflammatory and anti-allergic effects in animal models and humans. Another lead discovery, ABT-761[18] (**17**) is undergoing final clinical trials with potent 5- LO inhibiting

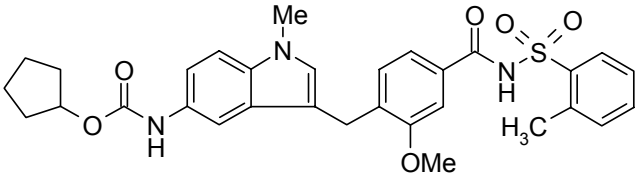
activity and minimal side effects. CI-1004 is a dual inhibitor of lipoxygenase and cyclooxygenase-2 (COX-2) that is currently under development as a potential treatment for asthma.[19]

Small molecule receptor antagonists for a number of inflammatory mediators have been developed. The cysteinyl leukotrienes have assumed a central role in asthma and in drug development with $CysT_1$ receptor antagonists such as montelukast (**18**)[20], pranulakast[21] (**19**), and zafirlukast[22] (**20**) being the first new treatment for asthma in 25 years.



Montelukast (**18**)

Pranulakast (**19**)



Zafirlukast (**20**)

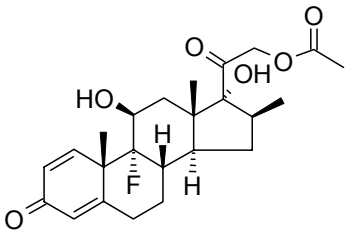
PAF antagonists

Specific and potent PAF receptor antagonists are valuable tools in the elucidation of the patho physiological roles of PAF and are expected to be of clinical importance. A variety of structurally diverse antagonists of the binding of PAF to its receptors have been reported. These compounds have been obtained by three different chemical approaches.[23]

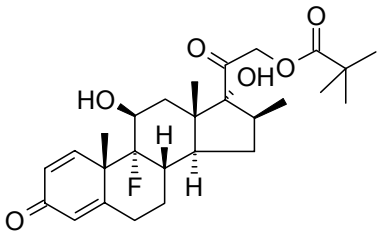
Glucocorticosteroids, β_2-adrenoreceptor agonists and theophylline:

Inhaled β_2-adrenoreceptor agonists are the most effective bronchodilators, currently prescribed for symptomatic relief in asthma.[24] The mechanism of action, i.e., causing smooth muscle relaxation involves camp-dependent and independent pathways.

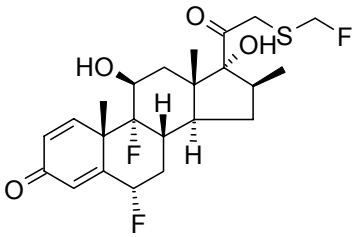
Inhaled glucocorticosteroids, e.g., betamethasone acetate (2**1**), dexamethasone pivalate (**22**), fluticasone (**23**), and cortisone (**24**) are mainstay therapy for reducing airway inflammation in asthma.



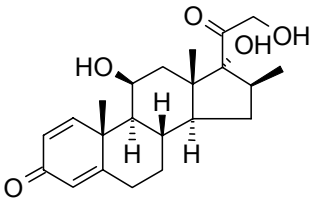
Betamethasone acetate (**21**)



Dexamethasone.pivalate (**22**)

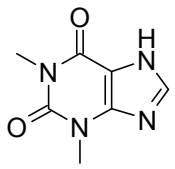


Fluticasone.dipropionate (**23**)

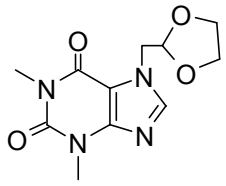


Cortisone (**24**)

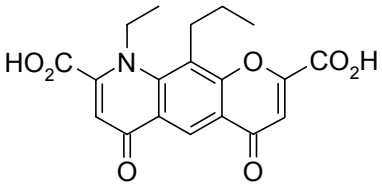
Theophylline (**25**) has a long historic background through its ability to bronchodilate asthmatic subjects. Although limited by side-effects profile, theophylline is effective in reducing the symptoms and improving lung function in patients with mild chronic asthma. Doxophylline (**26**) and nedocromil (**27**) are the drugs work in the inflammatory cells to prevent the release of histamine and other chemicals involved in



Theophylline (**25**)



Doxophylline (**26**)

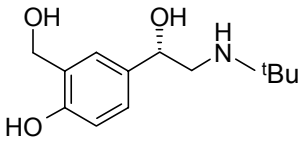


Nedocromil (**27**)

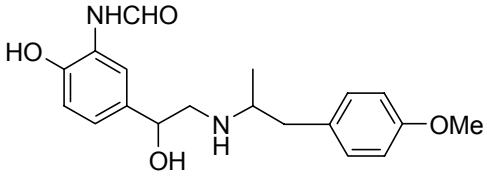
airway inflammation.

Salbutamol (**28**) is a potent β_2-adrenoreceptor antagonist. β_2-Adrenergeric receptors are found on the smooth muscle lining airways of the lungs. Long action of β_2-adrenoreceptor agonists can be achieved by alterations in pharmacokinetics [e.g., formoterol (**29**)] [25] and by exosite binding [e.g., salmeterol (**30**)] [26]. Also, new steroids are

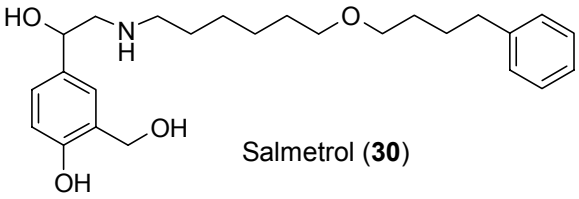
being developed with the aim of maximizing topical anti-inflammatory effects and minimizing adverse systemic effects, as exemplified by RU-24858. Clinical studies have



(*R*)-Salbutamol (**28**)



Formoterol (**29**)

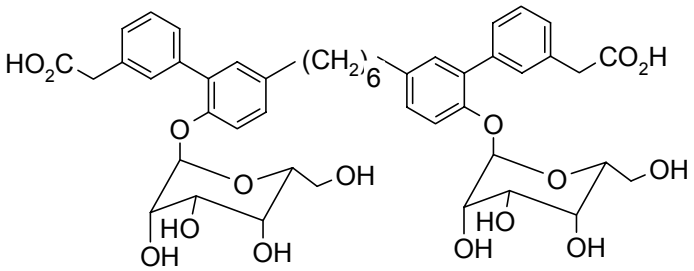


Salmetrol (**30**)

recently demonstrated the benefit of combining long-acting β_2-adrenoreceptor agonists with inhaled steroids, e.g., seretide (salmeterol and fluticasone) combined in a single formulation.

Adhesion molecules:

Suppression of eosinophil adhesion with consequent inhibition of influx into the lung is a strategy to suppress the asthmatic airway inflammation. The selectin family of adhesion molecules, which are expressed on activated endothelial cells (E- and P-selectin), activated platelets (P- selectin), and peripheral blood leukocytes (L- selectin) are involved in tethering and rolling of leukocytes in the microcirculation, leading to leukocyte tissue infiltration. Interruption of leukocyte-endothelium interaction is a current strategy to target asthma. TBC-1269 (**31**) is the lead compound of a series of orally active, low molecular weight E-, P-, L- selectin antagonists for the potential treatment of asthma and psoriasis.[27]



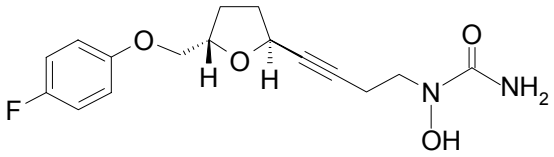
TBC-1269 (**31**)

Cytokines:

Cytokines play a key role in the chronic inflammation of asthma and appear to orchestrate, amplify, and perpetuate the inflammatory process.[28] The chemotactic cytokines (CC chemokines) act by attracting leukocytes to sites of inflammation. The advancement in understanding the intracellular signaling pathways and inflammatory gene transcription of key pro- and anti-inflammatory cytokines is laying the foundation for a new era in anti- inflammatory drug discovery. SP 650003 and SP 100030 are the small molecule inhibitors, which are great promise in the treatment of asthma.

Related Work

Recently, Cytomed Inc. has announced the development of CMI-977 (**32**) for final clinical trails for asthma.[29] It acts primarily by inhibiting the 5-LO pathways and



CMI-977 (32)

thus blocking the production of inflammatory mediator, leukotrines. CMI-977 has successfully been evaluated in animal models. In guinea pigs, oral administration of CMI-977 effectively blocks ovalbumin-induced bronchoconstriction, airway eosinophil accumulation, and plasma extravasation. CMI-977 blocked LTB4 production with IC50 of 117 nm and 10mg/Kg inhibited eosinophil influx by 63%. Data from phase IIa trial out of one randomized, double blind, placebo-controlled analysis to evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of a single dose of CMI-977 in normal subjects, showed that PK/PD profile is comparable with a single dose of zileuton, i.e., it may administered orally once or twice a day. Overall, CMI-977 has shown a high degree of potency, excellent oral bioavailability and exceptionally favourable safety profile.[30]

CMI-977 belongs to lignan family of 2,5-disubstituted tetrahydrofurans, featured with diverse substitution and *trans*-juxtapositioned ring and is chirally homogeneous (all

other three stereoisomers have shown poor pharmacological profile). The unique structural ensemble augmented with eutomer-dependent attractive therapeutic index should invite the proposal to undertake a 'single enantiomer synthesis' that would deliver the target molecule with relevant stereochemical information and the functionalities at their respective positions.

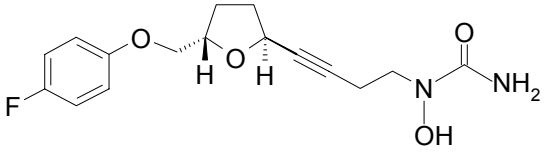
Except the inaugural medicinal chemistry route by Cytomed Inc.(now Millenium Pharmaceuticals), much of the synthetic chemistry for CMI-977 has been explored in our laboratory.[31] The original discovery route[32] was plagued with several problems that mitigated against efficient scale up and cost effective production of the target molecule. 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.

In the aftermath of completion of enantioselective synthesis of anti-asthmatic lead candidate, CMI-977, it was planned in these laboratories to synthesise a library of similar compounds that will differ in ring size, heteroatom, side chain length/homologation etc., without disturbing the main 'pharmacological core' responsible for activity that will lead to systematic investigation of structure activity relationship.

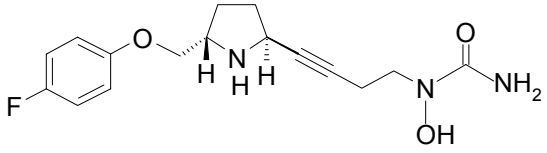
PRESENT WORK

Asthma is a chronic condition in the airways of lungs, and it has two main components- constrictions, the tightening of the muscles surrounding the airways, and inflammation, which releases either leukotrienes or other molecules called prostaglandins causing asthma. Leukotrienes are of particular interest because of their potential as bronchoconstrictors.[14] They are 1000 times more potent bronchoconstrictors than histamines and prostaglandins. Inhibitors of leukotrienes represent a large family of structurally unrelated compounds including cys-LT receptor antagonists, LTB_4 receptor antagonists and LT synthesis inhibitors. Researchers are now striving to develop a new class of asthma drugs that will strike at the root cause of the disease. The leukotriene inhibitors are the first new class of anti-inflammatory asthma medication introduced in the last 10 years.

CMI-977,[29] (2*S*,5*S*)-*trans*-5-(4-fluorophenoxy)methyl-2-(4-*N*-hydroxyureidyl-1-butynyl) tetrahydrofuran (**32**) is being currently developed by Cytomed Inc., USA, as a potential lead candidate for chronic asthma. It acts primarily by inhibiting the 5-lipoxygenase pathway[33] (5-LO), blocking the production of leukotriene B4. CMI-977 has shown a high degree of potency, excellent oral bioavailability and favorable safety profile. It belongs to lignan family of 2,5-disubstituted tetrahydrofurans, featured with diverse substitution and *trans*-juxtaposition ring. After successfully completing the asymmetric synthesis of CMI-977 in our laboratory,[31] we intend to synthesize compounds that will vary in ring size, heteroatom, side chain length/homologation etc., without disturbing the main 'pharmacophore' responsible for activity that will lead to a systematic structure activity relationship study.



CMI-977 (32)



Aza-CMI-977(33)

One of our interests was to synthesize the aza analogue (**33**) of CMI-977. The rationale for preparing **33** was that nitrogen is an indispensable part of many natural compounds including alkaloids, amino acids, azacarbohydrates and macromolecules (including proteins, DNA, RNA etc.). These compounds play major role starting from life-making process, chemical communication in mammals to ageing process and photosynthesis.

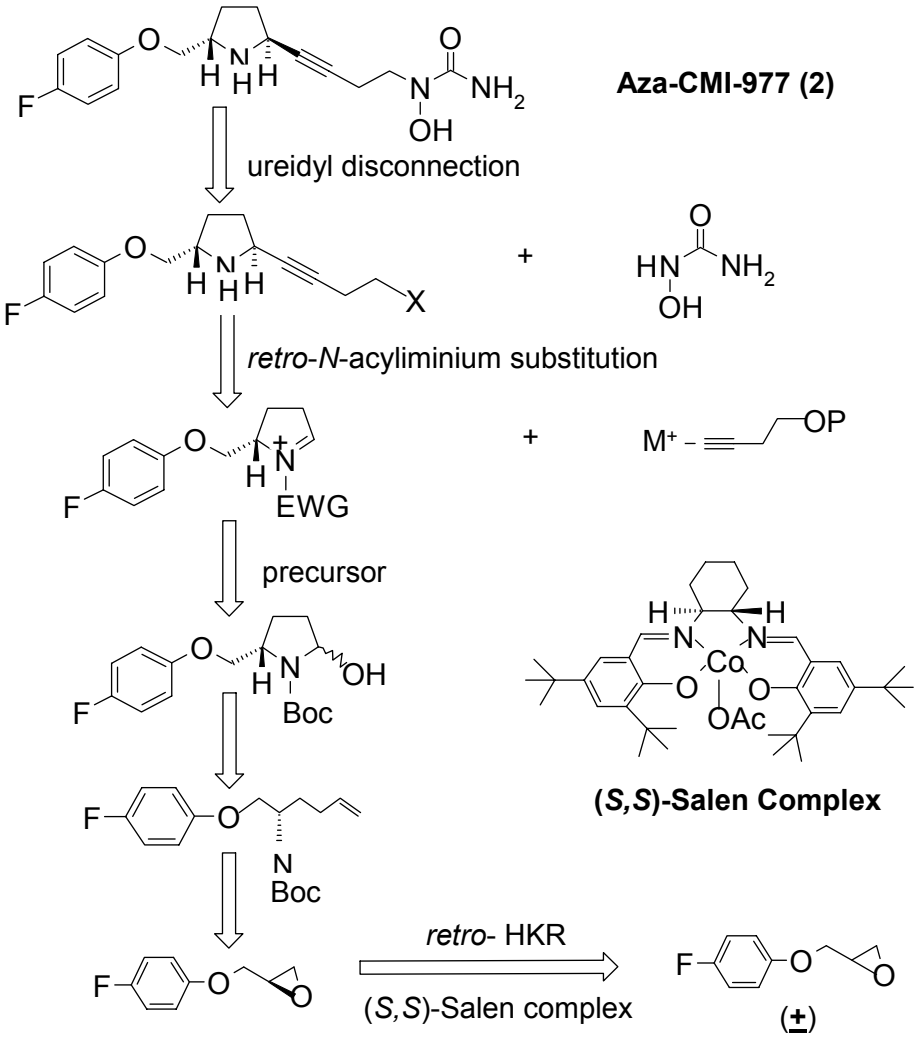
An ideal synthesis should project the ease of reaction conditions, ready accessibility of raw materials and convincing yields in synthetic sequences. The key features of this strategy will be (i) the construction of the *trans* fused pyrrolidine ring, (ii) C-C bond formation and (iii) introduction of the hydroxyureidyl moiety. The overall chiron strategy is described in Scheme **1**. The pivotal step in this strategy is the C-C bond formation to affix the side chain at C-2 position of lactam with control of stereochemistry *via* Lewis acid mediated nucleophilic addition to *N*-acyliminium ion.

Though hemiaminal compounds have been reported to undergo nucleophilic substitution in the presence of various Lewis acids, especially BF_3:OEt_2, $TiCl_4$, $SnCl_4$, etc., this method has several drawbacks like, only countable strength of nucleophiles like allyl trimethylsilane, TMSCN, $TMSN_3$, etc., have to used with variable degree of stereoselectivity.[34] A modified version pioneered by Ley, has scored popularity in recent years.[35] This method replaces the less stable iminal with stable electrophilic counterpart 2-arenesulphonylpyrrolidine. The attractive features of this methodology include the stability and crystalline nature of most sulphone derivatives facilitating easy purification by recrystallisation, applicable to broad range of nucleophiles, efficient, good stereoselectivity and no requirement for external Lewis acid.

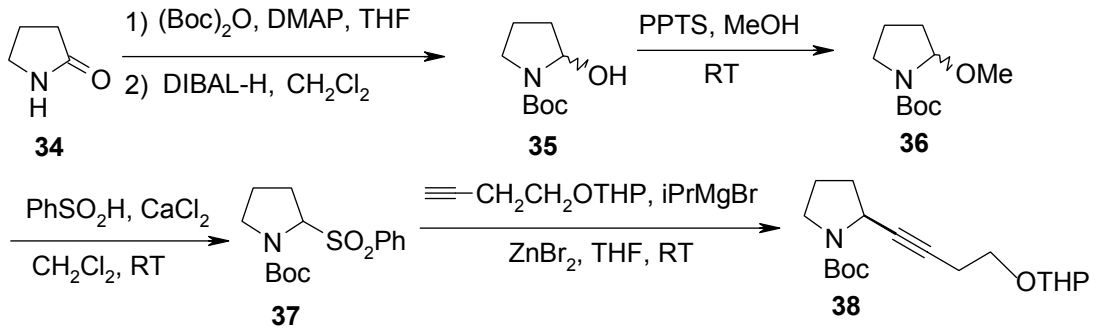
Before starting the synthesis, as planned in the preceding lines, a model study was conducted to probe the conceivability of the strategy, the C-C bond formation in the nucleophilic addition of *N*-acyliminium ion. Accordingly, 2-pyrrolidinone (**34**) (Scheme **2**) was chosen that is reminiscent of the compound used to access the target molecule. It was converted into the lactol derivative (**35**) through protection as the carbamate derivative followed by reduction with DIBAL-H. Conversion of **35** into 2-

methoxypyrrolidine (**36**) was effected by reacting with methanol and PPTS. The structure of **36** was confirmed by the ^{1}H NMR spectrum.

Scheme **1:** Retrosynthetic analysis



Scheme **2**

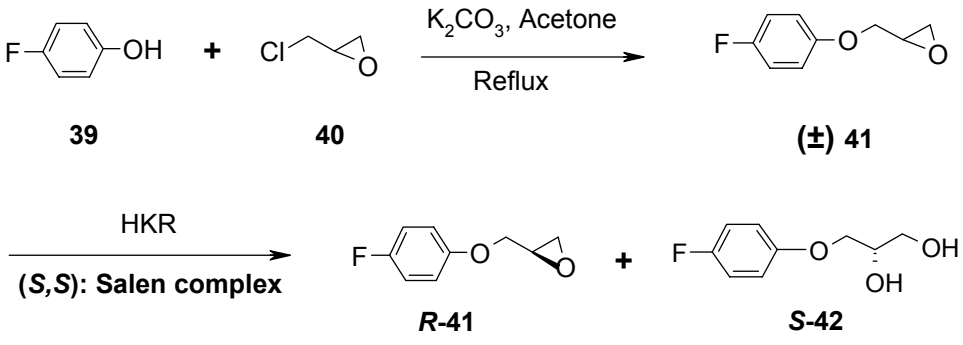


34 **35** **36** **37** **38**

Replacement of methoxy group with benzenesulphonyl group was easily accomplished on treatment with freshly prepared $PhSO_2H$ in CH_2Cl_2. The dialkyl zinc reagent, prepared *in situ* from $ZnBr_2$ and 4-tetrahydropyranyloxy-1-butynylmagnesium bromide smoothly reacted with the substrate to provide homopropargylpyrrolidine derivative (**38**). The ^{1}H NMR spectrum of **38** highlighted the major structural features evidently. Synthesis of **7** was gratifying to us because it paved a way singularly to adapt the C-C bond strategy directly on our target molecule leading to aza-CMI-977 (**33**).

Our synthetic endeavor began with the application of Jacobsen's hydrolytic kinetic resolution (HKR)[36] of racemic (±)-4-fluorophenyl glycidyl ether (**41**). Accordingly, *O*-alkylation of 4-fluorophenol (**39**) (Scheme **3**) with (±)-epichlorohydrine (**40**) in anhydrous acetone in the presence of K_2CO_3 afforded (±)-4-fluorophenyl glycidyl ether (**41**) in 98 % yield. Resolution of the racemic glycidyl ether using Jacobsen's HKR protocol with 0.5 mol % of (*S,S*)-Cobalt Salen complex and 0.55 equivalent of distilled water at 0 °C followed by stirring at room temperature for 5 h provided the diol (***S***)-**42** in 49 % yield with 97 % ee along with 45 % of (*R*)-4-fluorophenyl glycidyl ether (***R***)-**41** with 92 % ee.

Scheme **3**

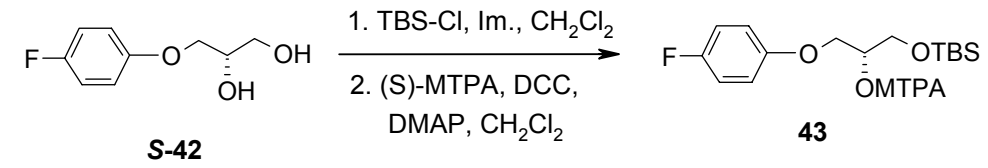


39 **40** **(±) 41** ***R*-41** ***S*-42**

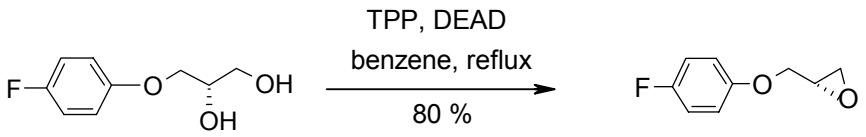
The enantiomeric excess of the (*R*)-epoxide **(*R*)-41** was confirmed by converting (Scheme **4**) enantiomerically pure (*S*)-diol **(*S*)-42** into (S)-epoxide, under Mitsunobu reaction condition[38] to provide **(*S*)-41** {$[\alpha]_D$ + 4.5 (c 1.4, $CHCl_3$)}. It conclusively confirmed the 92 % ee of **(*R*)-41** {$[\alpha]_D$ - 4.9 (c 1.46, $CHCl_3$)}.

The enantiomeric excess of diol (***S***)-**42** was also confirmed by converting (Scheme **4**) into its Mosher ester derivative. The primary hydroxy group in (***S***)-**42** was first protected with TBSCl and subsequently was subjected to esterification using (*S*)-(α, α') methoxytrifluromethyl phenyl acetic acid [(*S*)-MTPA acid] to get compound **43**. Similarly racemic Mosher ester derivative of diol **(±)-42** was also prepared. The comparision of the racimic Mosher ester derivative and chiral derivative (**43**) ^{19}F NMR spectra confirmed the 97 % enantimeric excess of the diol **(*S*)-42**.

Scheme **4**



***S*-42** **43**

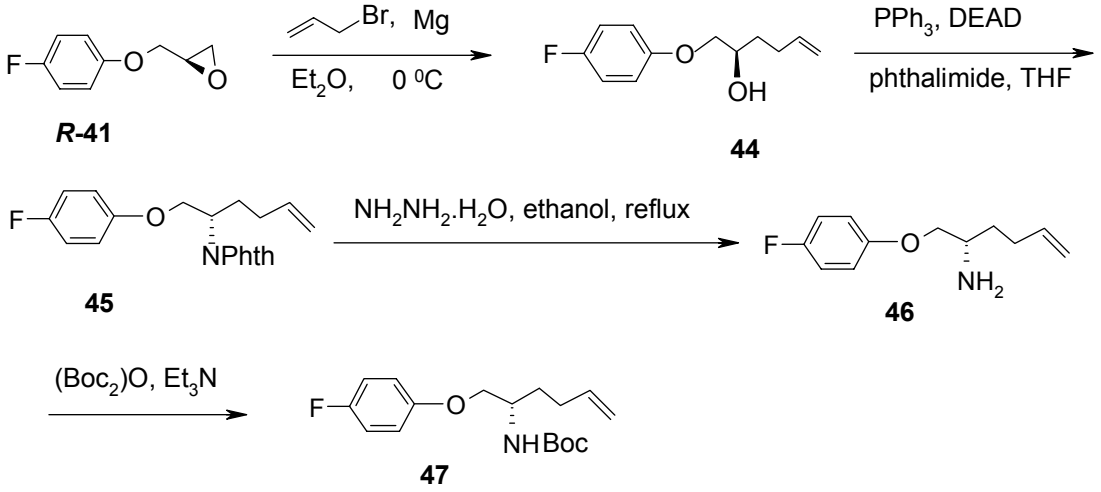


***S*-42** ***S*-41**

The epoxide (***R***)-**41** (Scheme **5**) was treated with allylmagnesium bromide-CuCN[37] to give compound **44**. In the ^1H-NMR spectrum of **44**, the characteristic resonances typical of terminal olefin were observed at 5.06 and 5.84 ppm as multiplets. Treatment of **44** with phthalimide, diethyl azodicarboxylate and triphenyl phosphine in THF under Mitsunobu conditions[38] gave **45**. Hydrolysis of phthalimide derivative was

done with hydrazine hydrate in refluxing ethanol to get free amine **46**, which was protected with Boc anhydride/triethyl amine to afford **47**. In the ^{1}H NMR spectrum of **47,** characteristic singlet due to Boc group was seen at 1.4 ppm and a broad singlet at 4.65 was assigned to NH proton. Further confirmation was obtained by MS, which revealed highest mass peak at *m/z* 310 (M+1).

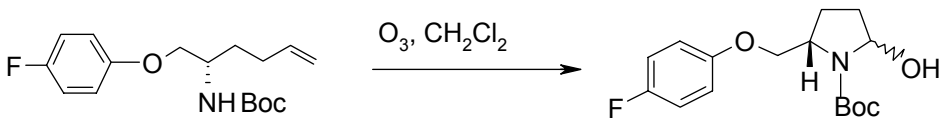
Scheme **5**



***R*-41** **44** **45** **46** **47**

Ozonolysis of **47** in CH_2Cl_2 (Scheme **6**) for 2 h gave the cyclized 2-hydroxy pyrrolidine derivative **48** as a diastereomeric mixture. The peaks accounted and the environment for protons was in complete agreement with the expected product. The HRMS analysis [Calcd for ($C_{16}H_{22}FNO_4$): 311.1532. Found: 311.1545] further confirmed the structure of **48**.

Scheme **6**

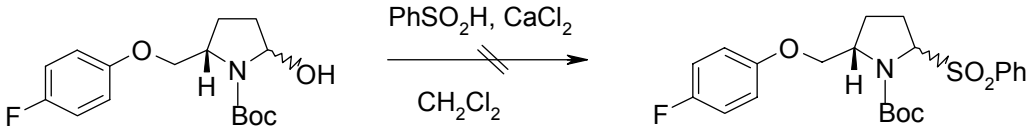


47 **48**

Our next concern was to affix the side chain at C-2 position *via* the Lewis acid mediated nucleophilic addition to *N*-acyliminium ion in diastereoselective way as

described earlier. The hemiaminal **48** was treated with to $PhSO_2H$ in CH_2Cl_2 (Scheme **7**) at room temperature as per the literature precedent; no product was imminent from the reaction. When the reaction was conducted with refluxing $CHCl_3$, no change was observed in TLC. Raising the temperature of the reaction medium further (110 ^oC) with refluxing toluene resulted in the deterioration of starting material. The addition of H_2SO_4/*p*TSA that will facilitate the formation of putative intermediate *N*-acyliminium ion because of their superior acidity to $PhSO_2H$ didn't cause any improvement.

Scheme **7**

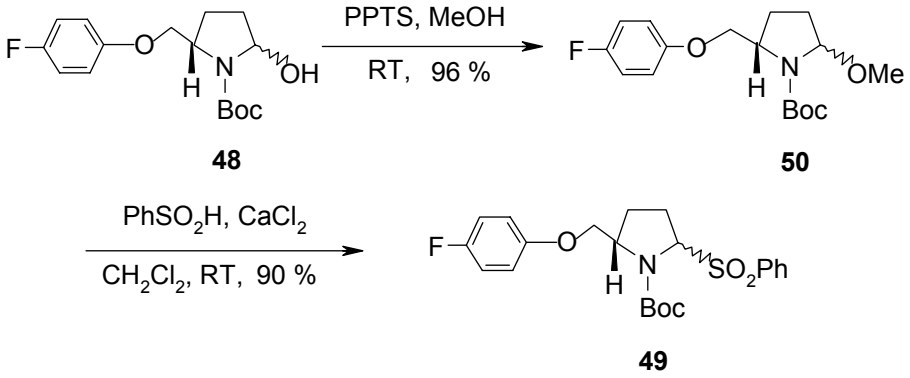


48 **49**

In accordance with the model studies, we converted 2-hydroxypyrrolidine derivative (**48**) to the 2-methoxypyrrolidine derivative (**50**) (Scheme **8**) on treatment with PPTS and MeOH. The product was found to be a diastereomeric mixture, as it was evident from ^1H NMR spectrum, which showed two distinct signals for OMe group at 3.29 and 3.53 ppm, two broad multiplets were also observed at 5.10 and 5.57 ppm for C-1 proton and two distinct singlets for *t*-butyl group at 1.49 and 1.55 ppm. Exposure of compound **19** with $PhSO_2H$ in CH_2Cl_2 in the presence of $CaCl_2$ at ambient temperature afforded the desired 2-benzenesulphonylpyrrolidine derivative (**49**).

In the ^1H NMR spectrum of **49**, characteristic multiplets were observed due to SO_2Ph in the aromatic region at 7.63 and 7.91 ppm. The FABMS: (*m/z*) 436 (M+1) and HRMS analysis [Calcd for $(C_{22}H_{27}FNO_4S)$: 436.1593. Found: 436.1624] confirmed the structure to be 2-benzenesulphonylpyrrolidine derivative.

Scheme **8**



48 **50**

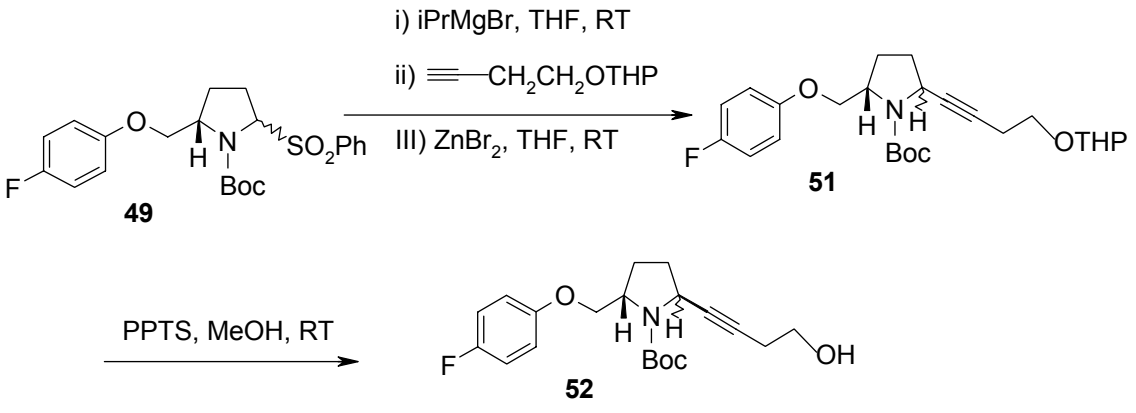
49

The feasibility of this reaction at the minuscule difference at the reaction site, i.e., from hemiaminal to aminal invites some mechanistic explanation since the same reaction has been reported[39] to proceed at equal ease in the case of glycal and its methylated derivative glycoside. The probable explanation that will account for this stereoelectronic bias relies on the better leaving nature of OMe group as MeOH compared to the OH group as H_2O, after initial protonation with $PhSO_2H$.

The sulphone derivative (**49**) when treated with dialkyl zinc reagent (Scheme **9**), prepared *in situ* from $ZnBr_2$ and 4-tetrahydropyranyloxy-1-butynylmagnesium bromide in THF for 10 h, smoothly reacted with the substrate to provide homopropargyl pyrrolidine derivative (**51**). The THP group was subsequently removed by exposure to catalytic amount of PPTS in MeOH to retrieve the homopropargyl alcohol derivative **52** in 80% yield (2 steps).

In the ^{1}H NMR spectrum of **52**, the resonance due to two CH_2 groups of homopropargylic part appeared as two sets of triplet at 2.44 and 3.70 ppm. The proposed structure **52** was also supported by ^{13}C NMR, DEPT, FABMS (*m/z*) [364 (M^++1)] and HRMS (Calcd. for [$C_{20}H_{26}FNO_4$]: 364.1924. Found: 364.1917) analysis. The diastereomeric excess information could not be quantified from the ^{1}H NMR spectrum, probably; because of the rotameric distortion of signals.[40] The diastereomers were not separable by flash chromatography either. However, the mixture was found to be in 87:13 ratio through analytical HPLC (ODS column, mobile phase: 40 % acetonitrile + 20 % water + 40 % methanol; retention times: 20.5 and 24.2). We have earlier in our lab synthesized CMI-977, thio analogue, six membered and seven membered analogue of CMI-977, and in all instance we obtained the required *trans* isomer in > 85 %, with *cis* isomer being the minor product. Based on the above data the *trans* isomer in **52** was

Scheme **9**

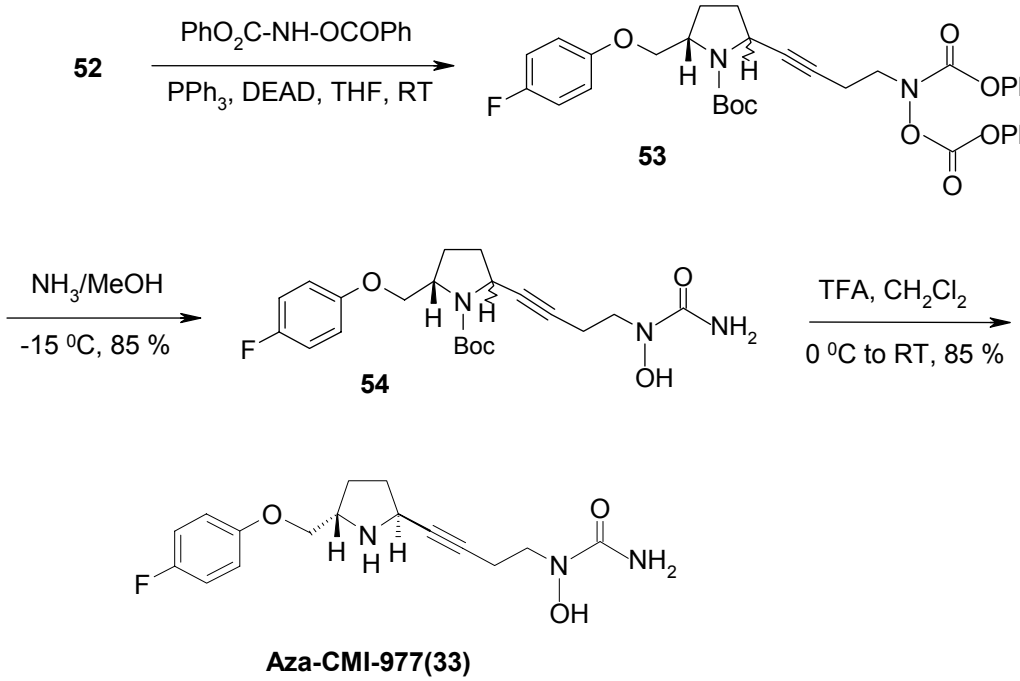


thought to be the predominant one.

Our next concern was to introduce hydroxyurea functionality at the acetylenic side chain. The Mitsunobu reaction[38] of propargyl alcohol derivative (Scheme **10**) with *N,O*-bis (phenoxycarbonyl) hydroxylamine in presence of PPh_3 and DEAD provided the fully protected urethane derivative **53**. The surge in the integration values in the aromatic region (2 x Ph = 10 H) and the downfield shifted resonance of CH_2NR_2 at 4.11 ppm (compared to CH_2OH) pinpointed the conversion, duly supported by FABMS [(M+1) at (*m*/*z*) 604].

Compound **53** on exposure to methanolic solution of NH_3 simultaneously cleaved benzoate ester and converted urethane into urea, thus affording *N*-hydroxy urea derivative **54** in 80 % yield. The structure was characterized on the basis of information from ^1H NMR, ^{13}C NMR, DEPT, IR and FAB MS spectral analysis. Finally, the deprotection of Boc group with trifluoroacetic acid in CH_2Cl_2 at room temperature culminated in the total synthesis of target compound Aza-CMI-977 (**33**),[41] which was confirmed by ^1H NMR, ^{13}C NMR, DEPT and FABMS studies.

Scheme **10**



It was interesting to note that the HPLC analysis of the final compound showed only one peak with both reverse phase and chiral column, indicating that the minor *cis* isomer might have been removed from the mixture during purification.

There was no NOE enhancement observed during the irradiation of protons at C-2 and C-5, which suggested that these protons are not in close proximity, thus establishing the *trans* stereochemistry.

In conclusion, we have synthesized Aza CMI-977 (**33**) in a concise, efficient and stereocontrolled manner with the application of Jacobsen's Hydrolytic Kinetic Resolution. The compound has been submitted for evaluating it's pharmacological profile (PK/PD) in the treatment of asthma, especially for 5-lipoxygenase inhibition.

(±)-4-Fluorophenyl glycidyl ether (41)

A stirred solution of 4-fluorophenol (40.0 g, 0.3 mol), K_2CO_3 (148.0 g, 1.0 mol) and (±) epichlorohydrine (98.0 g, 1.0 mol) in dry acetone was refluxed for 15 h, filtered and concentrated. The residue was distilled under vaccum (100 ^oC/ 4mm) to give **41** (52.0 g, 85 %) as colorless oil.

(*R*)-Glycidyl-4-fluorophenyl ether [(R)-41]

To a mixture of (±) 4-fluorophenyl glycidyl ether (**41**) (52.0 g, 0.3 mol) and (*S,S*) Co-Salen(III)OAc catalyst (1.03 g, 1.5 mol) at 0 ^oC was added distilled water (3.06 mL, 0.2 mmol) over a period of 1 h and monitored by HPLC. The reaction mixture was diluted with hexane, the solid was filtered to afford (S)-1-*O*-(4-fluorophenyl)glycerol **(S)-42** (26.4 g) in 49 % yield. The filtrate obtained was concentrated. The residue was distilled under vaccum (100°C/mm) to give pure **(R)-41** (23.5 g, 46 %).

$[\alpha]_D$ = – 4.9° (c 1.46, $CHCl_3$);

IR (neat, cm^{-1}): 2890, 1493, 1215, 831;

^{1}H NMR ($CDCl_3$, 200MHz): δ 2.68 (dd, 1 H, *J* = 2.2, 4.5 Hz), 2.85 (t, 1 H, *J* = 4.6 Hz), 3.27 (m, 1 H), 3.89 (dd, 1 H, *J* = 6.7, 15.7 Hz), 4.11 (dd, 1 H, *J* = 4.5, 15.7 Hz), 6.88 (m, 4 H);

EIMS *m/z*: 168 (M^+);

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

(2*R*)-1-(4-Fluorophenoxy)-hex-5-ene-2-ol (44)

To a suspension of ally magnesium bromide (prepared from 8.5 g of Mg and 9.1 mL of allyl bromide) and CuCN, a solution of **R-41** (12.0 g, 70.0 mmol) in anhydrous ether was added. After 20 min, saturated aq. NH_4Cl was added and the reaction mixture extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), concentrated and the

residue purified on silica gel using ethyl acetate- light petroleum ether (3:17) as eluent to afford **44** (13.5 g, 90 %).

$[\alpha]_D$ = – 17.2 (c 1.6, $CHCl_3$);

^{1}H NMR ($CDCl_3$, 200MHz): δ 1.67 (m, 2 H), 2.30 (q, 2 H, *J* = 6.8 Hz), 2.65 (brs, 1 H), 3.92 (m, 3 H), 4.0 (m, 1 H), 5.06 (m, 2 H), 5.84 (m, 1 H), 6.90 (m, 4 H);

^{13}C NMR ($CDCl_3$, 50 MHz): δ 29.6, 32.1, 69.4, 72.8, 115.0, 115.4, 115.5, 115.6, 116.0, 138.0, 154.6, 159.7;

EIMS *m/z*: 210 (M^+);

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

(2*S*)-1-(4-Fluorophenoxy)-2-phthalimido-hex-5-ene (45)

To a solution of **44** (8.4 g, 40.0 mmol) in dry THF (25 mL) at 0 ^oC under nitrogen was added phthalimide (7.0 g, 80.0 mmol) and triphenylphosphine (12.6 g, 0.1 mmol). After 10 min, DEAD (7.6 mL, 80.0 mmol) was added and the reaction stirred for 5 h. Solvent was removed and the residue extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over Na_2SO_4, concentrated and the residue purified by silica gel chromatography using ethyl acetate- light petroleum ether (1:9) as eluent to give **45** (9.6 g) in 70 % yield.

$[\alpha]_D$ = + 11.3 (c 2.1, $CHCl_3$);

^{1}H NMR ($CDCl_3$, 200MHz): δ 2.07 (m, 4 H), 4.17 (dd, 1 H, *J* = 4.5, 13.6 Hz), 4.49 (t, 1 H, *J* = 9 Hz,), 4.66 (m, 1 H), 5.01 (m, 2 H), 5.76 (m, 1 H), 6.87 (m, 4 H), 7.80 (m, 4 H);

EIMS *m/z*: 340 (M^++1);

Anal. Calcd. for ($C_{20}H_{18}FNO_3$): C, 70.78; H, 5.35; N, 4.13. Found: C, 70.71; H, 5.54; N, 3.65.

(2*S*)-1-(4-fluorophenoxy)-2-*N*-*tert*-butyloxycarbonyl-hex-5-ene (47)

A solution of **45** (7.2 g, 20.0 mmol), hydrazine hydrate (1.5 mL, 0.03 mmol) and EtOH (100.0 mL) was refluxed for 4 h. Ethanol was removed and the residue extracted with ethyl acetate, washed with brine, dried (Na_2SO_4) and concentrated to afford free amine **46** (3.8 g), which was treated with triethylamine (1.2 mL) and $(Boc)_2O$ (2.1 mL, 13.0 mmol) and stirred for 4 h. Solvent was evaporated and the residue extracted with

ethyl acetate, washed with water, dried (Na_2SO_4), concentrated and the residue purified on silica gel using ethyl acetate- light petroleum ether (1:9) as eluent to give **47** (4.6 g, 70 %).

$[\alpha]_D$ = + 19.7 (c 1.6, $CHCl_3$);

^{1}H NMR ($CDCl_3$, 200MHz): δ 1.40 (s, 9 H), 1.74 (m, 2 H), 2.21 (q, 2 H, *J* = 4.5 Hz), 3.90 (s, 3 H), 4.65 (br s, 1 H), 4.96 (m, 2 H), 5.80 (m, 1 H), 6.89 (m, 4 H);

EIMS *m/z*: 310 (M^++1);

Anal. Calcd. for ($C_{16}H_{20}FN_3O_3$): C, 66.00; H, 7.82; N, 4.53. Found: C, 65.97; H, 7.51; N, 3.99.

(2*RS*, 5*S*)-*N*-*tert*-Butyloxycarbonyl-5-(4-fluorophenoxy)-methyl-2-hydroxy-pyrrolidine (48)

Ozone gas was passed through a solution of compound **47** (3.0 g) in dry CH_2Cl_2 (50.0 mL) at –78 ^oC. After 2 h, dimethyl sulfide was added and concentrated. The residue was purified on silica gel using ethyl acetate- light petroleum ether (3:17) as eluent to afford **48** (1.6 g) in 54 % yield.

^{1}H NMR (200MHz, $CDCl_3$): δ 1.52 (s, 9 H), 2.11 (m, 4 H), 3.93 (m, 3 H), 5.46 (m, 1 H), 7.04 (m, 4 H);

^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 26.9, 27.6, 70.1, 80.7, 82.4, 89.8, 115.5, 115.8, 116.1, 154.9, 159.7, 160.2;

EIMS *m/z*: 311 (M^+);

HRMS: Calcd for $C_{16}H_{22}FNO_4$: 311.1532 (M^+). Found: 311.1545;

(2*RS*, 5*S*)-*N*-*tert*-Butyloxycarbonyl-5-(4-fluorophenoxy)-methyl-2-methoxy-pyrrolidine (50)

A mixture of compound **48** (10.0 g, 32.1 mmol) and PPTS (0.8 g, 3.2 mmol) in methanol (100.0 mL) was stirred for 8 h at RT. The reaction mixture was neutralized with triethylamine, concentrated and the residue purified on silica gel using ethyl acetate- light petroleum ether (1:9) as eluent to afford **50** (10 g, 96 %).

IR (neat, cm^{-1}): 2976, 2944, 1696, 1504, 1392, 1208, 1186, 1078, 824;

^{1}H NMR (200MHz, $CDCl_3$): δ 1.49 and 1.55 (2 s, 9 H), 2.0 (m, 4 H), 3.29 and 3.53 (2 s, 3 H), 4.04 (m, 3 H), 4.2 (m, 2 H), 5.10 and 5.57 (m, 1 H), 6.89 (m, 4 H);

Anal. Calcd. for ($C_{17}H_{24}FNO_4$): C, 62.75; H, 7.38; N, 4.30. Found: C, 63.05; H, 7.06; N, 4.36.

(2*RS*, 5*S*)-2-Benzenesufonyl-1-*tert*-butyloxycarbonyl-5-(4-fluorophenoxy)-methyl pyrrolidine (49)

A solution of **50** (9.8 g, 30.1 mmol), powdered $CaCl_2$ (5.0 g) and benzenesulfinic acid (4.3 g, 30.1 mmol) in CH_2Cl_2 (100.0 mL) was stirred for 2 h at ambient temperature. Saturated solution of $NaHCO_3$ was added and stirring continued for 1 h. The suspension was filtered and filtrate washed with water and brine, dried over anhydrous Na_2SO_4, concentrated and the residue purified on silica gel chromatography using ethyl acetate-light petroleum ether (1:19) as eluent to afford **49** (11.8 g, 90 %) as a colorless solid.

Melting point: 113-114 ^oC;

IR (neat, cm^{-1}): 2976, 1752, 1504, 1488, 1456, 1368, 1312, 1216, 1152, 1088, 1040, 832, 768, 688, 560;

^1H NMR (200MHz, $CDCl_3$): δ 1.20 (s, 9 H), 2.34 (m, 3 H), 2.77 (m, 1 H), 4.30 (m, 3 H), 5.18 (m, 1 H), 6.97 (m, 4 H), 7.63 (m, 3 H), 7.91 (m, 2 H);

^{13}C NMR ($CDCl_3$, 50 MHz): δ 25.7, 26.9, 27.6, 70.1, 80.7, 82.4, 94.8, 115.5, 115.8, 116.1, 129.1, 129.3, 129.4, 133.2, 154.9, 159.7, 160.3;

FABMS (m/z): 436 (M^++1);

HRMS: Calcd. for $C_{22}H_{27}FNO_5S$: 436.1594 (M^+). Found 436. 1625;

Anal. Calcd. for ($C_{22}H_{26}FNO_5S$): C, 60.55; H, 6.02; N, 3.22. Found: C, 60.79; H, 6.29; N, 3.01.

(2*RS*, 5*S*)-*N*-*tert*-Butyloxycarbonyl-5-(4-fluorophenoxy)-methyl-2-(4-hydroxy-1-butynyl)-pyrrolidine (52)

A solution of 4-tetrahydropyranyloxy-1-butynylmagnesium bromide {prepared *in situ* by the addition of isopropylmagnesium bromide (1.1 g, 7.1 mmol) to 2-(3-butynyl-1-oxy)-tetrahydropyran (1.08 g, 7.0 mmol)} and zinc bromide in THF (0.78 g, 3.5 mmol, 2 M solution) was stirred for 30 min. Compound **49** (1.5 g, 3.5 mmol) in THF (12.0 mL) was added and stirring continued for 10 h. The reaction was quenched with saturated solution of NH_4Cl, diluted with ether, washed with brine, dried (Na_2SO_4) and concentrated to afford the crude product **51**, which was dissolved in methanol (15.0 mL) containing *p*-TSA (66.0 mg, 0.3 mmol) and stirred at room temperature for 10 h. Solvent was concentrated and the residue purified on silica gel (30 % ethyl acetate in hexane) to afford **52** (1.0 g, 86%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (s, 9 H), 1.66 (br s, 1 H), 2.11 (m, 4 H), 2.44 (t, 2 H, *J* = 6.74 Hz), 3.70 (t, 2 H, *J* = 4.0 Hz), 3.74 (t, 0.5 H, *J* = 8.0 Hz) and 3.86 (t, 0.5 H, *J* = 8.0 Hz), 4.02 (d, 0.5 H, *J* = 8.0 Hz) and 4.09 (d, 0.5 H, *J* = 8.0 Hz), 4.18 (m, 1 H), 4.49 (m, 1 H), 6.9 (m, 4 H);
^{13}C NMR ($CDCl_3$, 50 MHz): δ 22.6, 26.3, 28.2, 31.3, 49.2, 55.5, 60.7, 69.8, 79.8, 82.5, 115.2, 115.7, 153.8, 153.9, 154.6, 159.5;
FABMS(m/z): 364 (M^++1);
HRMS: Calcd. for ($C_{20}H_{27}FNO_4$): 364.1924. Found: 364.1917;
Anal. Calcd. for ($C_{20}H_{26}FNO_4$): C, 66.10; H, 7.21; N, 3.85. Found: C, 66.49; H, 7.20; N, 3.66.

(2*RS*, 5*S*)-*N*-*tert*-Butyloxycarbonyl-2-(4-fluorophenoxy)-methyl-5-(4-*N*,*O*-bis-phenoxy carbonylhydroxyamino-1-butynyl)-pyrrolidine (53)

To a solution of **52** (0.82 g, 2.3 mmol), triphenyphosphine (0.71 g, 2.7 mmol) and *N*,*O*-bis-phenoxycarbonyl hydroxylamine (0.70 g, 2.7 mmol) in dry THF (10.0 mL) was added DEAD (0.47 g, 2.7 mmol). The reaction mixture was stirred for 6 h at RT and then concentrated. The residue was subjected to silica gel chromatography using ethyl acetate-light petroleum ether (1:3) as eluent to afford **53** (1.12 g, 80 %).

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.52 (s, 9 H), 1.94 (t, 1 H, *J* = 5.6 Hz), 2.21 (m, 3 H), 2.70 (t, 2 H, *J* = 7.0 Hz), 3.81 (m, 1 H), 4.11 (m, 4 H), 4.52 (m, 1 H), 6.93 (m, 4 H), 7.41 (m, 10 H)

FABMS (m/z): 619 (M^++1);

(2*S*,5*S*)-*N*-*tert*-Butyloxycarbonyl-5-(2-hydroxyethyl)-ethynyl-2-(4-fluorophenoxy)-methyl -5-(4-hydroxyureidyl-1-butynyl)-pyrrolidine (54)

Ammonia gas was purged into a solution of **53** (1.4 g, 2.3 mmol) in methanol (30.0 mL) and THF (10.0 mL) at 0 ^oC for 15 min. After overnight stirring at RT, solvent was evaporated and the residue purified on silica gel using ethyl acetate- light petroleum ether (3:7) as eluent to give **54** (0.81 g, 85 %).

IR (neat, cm^{-1}): 3504, 3450-3000 (br.), 2960, 1688, 1512, 1392, 1208, 1160, 760;

^{1}H NMR (200MHz, $CDCl_3$): δ 1.45 (s, 9 H), 2.31 (m, 6 H), 3.59 (m, 1 H), 3.81 (m, 2 H), 4.03 (dd, 1 H, *J* = 3.8, 8.8, Hz), 4.14 (m, 1 H), 4.45 (m, 1 H), 5.23 (br s, 2 H), 6.87 (m, 4 H), 8.45 (s, 1 H);

FABMS (m/z): 422 (M^+ +1);

Anal. Calcd. for ($C_{21}H_{28}FN_3O_5$): C, 59.85; H, 6.65; N, 9.97. Found: C, 59.95; H, 6.38; N, 9.65.

(2*S*, 5*S*)-2-(4-Fluorophenoxy)-methyl-5-(4-hydroxyureidyl-1-butynyl)-pyrrolidine (Aza-CMI-977, 33)

Trifluoroacetic acid (0.5 mL, 5.5 mmol) and **54** (0.8 g, 1.8 mmol) in CH_2Cl_2 (10.0 mL) were stirred at rt for 3 h and then neutralized with saturated solution of $NaHCO_3$. The organic layer was separated, washed with brine, dried (Na_2SO_4) and concentrated to afford the brown residue, which was purified by column chromatography on silica gel (10 % methanol in ethyl acetate) to afford **33** (0.55 g, 86 %). The compound was rendered acidic with dilute HCl and the corresponding hydrochloride salt of **33** was isolated by extraction with ethyl acetate and concentration of solvent gave foam.

HPLC: Peak- (retention time: 1.9, area: 99.2 %); stationary phase-chiracel OJ; Mobile phase- 10 % isopropanol in hexane; flow rate- 1 mL/min; UV detection- 254 nm.

^{1}H NMR (500 MHz, D_2O) : δ 1.81 (m, 1 H), 2.11 (m, 1 H), 2.33 (m, 2 H), 2.52 (dt, 2 H, *J* = 2.0, 6.0 Hz), 3.58 (t, 2 H, *J* = 6.36 Hz), 4.01 (dd, 1 H, *J* = 3.17, 10.7 Hz), 4.13 (dq, 1 H, *J* = 3.18, 7.55 Hz), 4.20 (dd, 1 H, *J* = 3.18, 10.73 Hz), 4.41 (dt, 1 H, *J* = 2.0, 6.0 Hz), 6.90 (m, 2 H), 7.01 (t, 2 H, *J* = 8.75);

^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.2, 25.1, 31.4, 46.9, 50.2, 57.9, 66.7, 74.4, 86.2, 115.7, 115.8, 153.4, 156.4, 158.3, 162.3;

FABMS *m/z*: 321[M^+];

Anal. Calcd. for ($C_{16}H_{20}FN_3O_3$): C, 59.80; H, 6.23; N, 13.07. Found: C, 59.67; H, 6.13; N, 12.71.

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CHAPTER- 1

Section- II

Application of Hydrolytic Kinetic Resolution (HKR) Towards the Synthesis of Optically Pure β-blocker *S*-Metoprolol

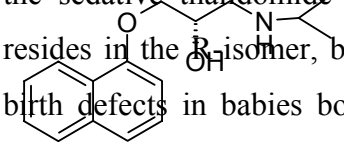
INTRODUCTION

The importance of chirality in the context of the biological function has been fully appreciated and therefore chirality has emerged as a major theme in drug design, discovery and development[1–5] as stereoisomer distinction is a significant component in many pharmacological events. The advances in chiral technology and the ability to produce enantiomerically pure compounds have an important impact on drug design, research and development and on the strategies and policies of the pharmaceutical industry.[6-8] 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.[9]

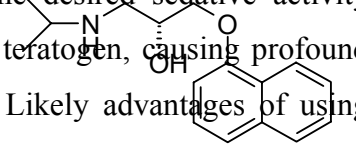
Synthetic chemists have ever since taken it as a challenge to induce chirality in a molecule. Seldom has there been an area of chemistry where the scientific goals are so challenging, the economic benefits so obvious, and the ethical reason for doing the research so compelling.

Until recently, it was a common practice for pharmaceutical company to market a chiral drug as the racemate.[10] One enantiomer acts as a very effective therapeutic drug, whereas the other is highly toxic. This approach in effect meant that each dose of drug was contaminated with an equal weight of an isomer, which usually had no therapeutic value, but had the potential to cause unsuspected deleterious side effects. For example, the sedative thalidomide was marketed as a racemate. The desired sedative activity resides in the R-isomer, but the contaminant S-isomer is a teratogen, causing profound birth defects in babies born to mothers using this drug.[11] Likely advantages of using stereochemically pure drugs are that: (1) the total dose could be reduced, (2) the dose–response relationship would be simpler, (3) a source of inter[illegible]ld have been removed and (4) toxicity from the inactive stereoisomer would be minimized. These pharmacodynamic and pharmacokinetic factors have led to an increasing preference for single enantiomers. Indeed, single enantiomer drugs continue to take an increasing share of the market with worldwide sales of these drugs surging by 21% between 1996 and 19[illegible]st $90 billion.[5] There are two principal sce[illegible] drug development: the first is the *de novo* development of an enantiomerically pure chiral drug; the second is a switch from an existing racemic drug to the single enantiomer(s) of that drug.[1] Chiral switches[4] are chiral drugs that are already approved as racemates but th[illegible]veloped and launched as single enantiomers.[5,7]

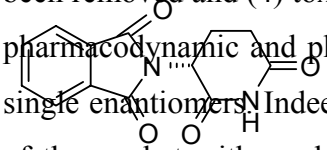
Table 1: Drugs that have different activities for different isomers



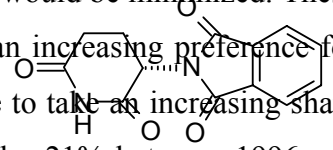
***S*)-Propranolol**
β blocker



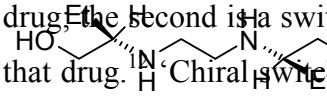
***R*)-Propranolol**
aginal contraceptive



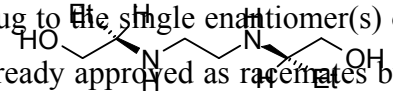
***R*)-Thalidomide**
Sedative



***S*)-Thalidomide**
Teratogen



***S,S*)-Ethambutol**
Tuberculastic



(*R,R*)-Ethambutol
Causes blindness

There are several methods to obtain enantiomerically pure materials, which include classical optical resolution via diastereomers, chromatographic separation of enantiomers, enzymatic resolution, chemical kinetic resolution and asymmetric synthesis. Recently, enantioselective catalysis using metal complexes[13] has advanced to the point where it can often provide a viable alternative to biocatalyst.

The strength of metal catalyst tend to complement those of enzymes:

1) Metals can promote reaction not known to occur in nature.
2) The chirality of the catalyst is easily modified by appropriate changes in the ligand.
3) One can use substrates not accepted by enzymes.
4) Separation and recoveries of products are relatively easy (Enzymes most often work in aqueous or near aqueous environments).
5) Organometallic reagents are generally less capricious than enzymes, which are often susceptible to degradation caused by heat, oxidation and pH.

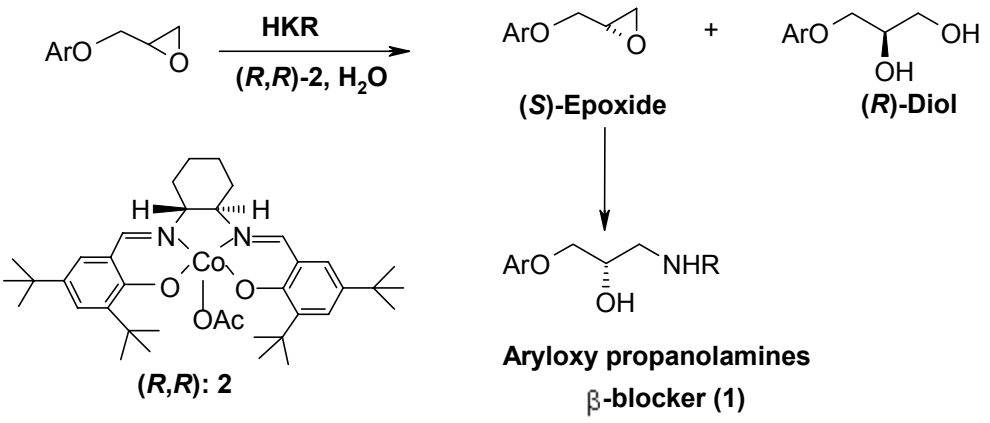
The numbers of metal-catalyzed asymmetric processes that are commercially applicable for the synthesis of various drugs are steadily increasing. Some typical examples are listed in Table 2.

Table 2: Synthesis of various drugs via metal-catalyzed asymmetric process

Reaction Type	Metal	drug developed
Hydrogenation	Rh	(L)-DOPA
Cyclopropanation	Cu	Cilastatin
Hydrogenation	Rh	(L)-Phenylalanine
Epoxidation	Ti	Disparlure
Rearrangement	Rh	(L)-Menthol
Carbonyl Reduction	B	MK-0417
C-C bond formation	Ni	(*S*)-Naproxen

In our research work programme on enantioselective catalysis, we focused our attention on hydrolytic kinetic resolution (HKR) of terminal epoxide using chiral cobalt salen complexes **2** (Scheme **1**). The amazing methodology pioneered by Jacobsen[14] prompted us to investigate the HKR of aryl glycidyl ethers, because the resulting chiral arylglycidyl ether would become useful in optically active β-blocker synthesis. The features of the HKR include the following: the use of water as the nucleophile for epoxide ring opening; the high accessibility of racemic terminal epoxides; the low loadings and recyclability of the commercially available catalyst and the ease of product separation from unreacted epoxide due to large boiling point and polarity differences. Epihalohydrins and glycidol derivatives are particularly attractive substrates for HKR because the racemates are available inexpensively and on a large scale and the chiral three-carbon (C-3) building blocks derived from these compounds are extremely versatile synthetic intermediates with numerous applications for β-blockers, MAO inhibitors, alkyl glycerophospolipids and other pharmaceuticals as well as organic synthesis.[15]

Scheme 1



β-Adrenergic blocking agents (aryloxypropanolamino series) have received major attention because of their utility in the management of cardiovascular disorders, including hypertension, angina pectoris and cardiac arrhythmias.

Pharmacology of β-blockers[16]

Ahlquist has suggested the convenient designation of α and β-receptors to distinguish major differences in responses elicited in various organ systems. The α receptor plays an important role in vasopressor action, intestinal relaxation, and contraction of vasdeferens.[17]

The β-receptors are generally concerned with myocardial infaraction.[17] Propranolol was the first β-adrenergic antagonist to come into wide clinical use. It is a highly potent, nonselective β-adrenergic blocking agent with no intrinsic sympathomimatic activity. However, because of its ability to block β-receptors in bronchial smooth muscle and skeletal muscle, Propranolol interferes with bronchodilation produced by epinephrine and other sympathomimatic amines and with glycogenolysis, which ordinarily occurs during hypoglycemia. Thus, the drug is usually not used in individuals with bronchial asthma and must be used cautiously in diabetics who are receiving insulin or oral hypoglycemic agents.[18] As a consequence, there has been a search for β-adrenergic blocking agents that are cardioselective, and a number of drugs have now been developed that exhibit some degree of specificity for β_1-adrenergic receptors. Among the various selective β_1-adrenergic blocking agents that are introduced into therapy, metoprolol is used worldwide.

Metoprolol is an effective antihypertensive agent, and its efficacy appears to be comparable to that of propranolol in the management of mild or moderate disease. Metoprolol is also effective in the control of anginal attacks. It reduces the incidence of recurrent myocardial infarctions and mortality in patients who receive the drug after an infarction.[19]

Proper substitution at the alkylamine side chain results in cardioselectivity (β_1) or vascular selectivity (β_2). (*S*)-Atenolol[20] a β-blocker specific for the β_1-adrenoceptors and hence a very potent cardio selective[21] β-adrenergic blocker, used as an antihypertensive and antianginal agent.

Most of the β-blockers being sold today as racemates and are nonspecific for β_1 or β_2-receptor activity. Most of them are structural variants of 3-aryloxypropanolamine.

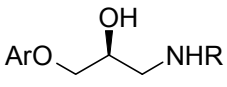
Stereochemistry of aryloxypropanolamines (1)

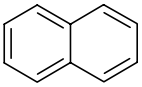
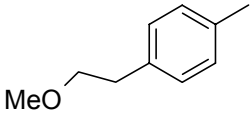
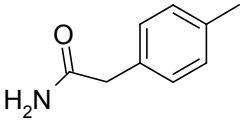
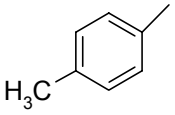
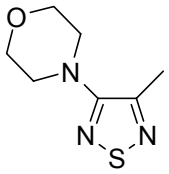
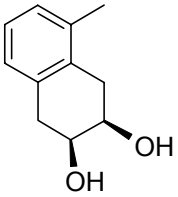
It is apparent that aryloxypropanolamines have one chiral center (Nadolol as an exception which contain three centers). The chirality of a drug and its biological profile are interrelated. For instance, in the case of Propranolol,[22] (*S*)-isomer has β-blocker activity, while (*R*)-isomer possesses vaginal contraceptive activity. Similarly, (*S*)-Metoprolol[23, 24] is the active β-blocker and not (*R*)-isomer. Important representations of this class of β-blockers are listed in Table **3**.

Past Work: Synthesis of (*S*)-Metoprolol

This chapter describes the practical synthesis of (*S*)-metoprolol via HKR reaction. It will be appropriate to discuss about the related work, [25,26,33] before describing the present work. Almost all the work reported on metoprolol is patented.

Table 3: Aryloxypropanolamine class of β-blocker



Generic Name	Ar	R
Propranolol		$CH(CH_3)_2$
Metoprolol		$CH(CH_3)_2$
Atenolol		$CH(CH_3)_2$
Toliprolol		$CH(CH_3)_2$
Timolol		$C(CH_3)_3$
Nadolol		$C(CH_3)_3$

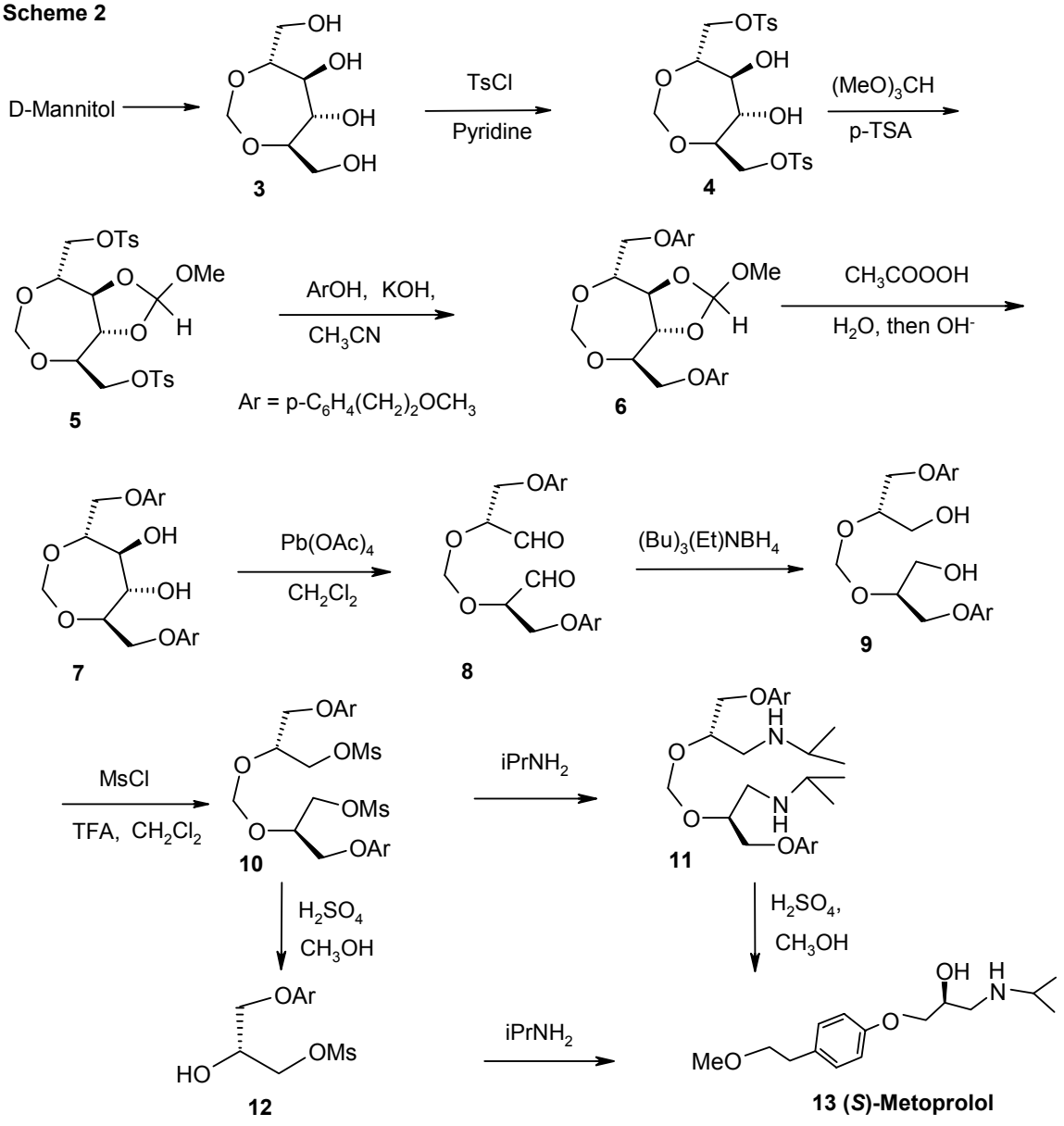
Lamm's Approach[25]

2,5-*O*-Methylene-D-mannitol (**3**), obtained from D-mannitol, was converted into the di-O-tosyl derivative **4**, and consequently free 3,4-hydroxyl groups were protected using trimethyl orthoformate to provide compound **5**. Treatment of compound **5** with properly substituted aryl alcohol/KOH provides derivative **6**. Removal of diol protection with peracetic acid followed by $Pb(OAc)_4$ treatment afforded dialdehyde **8**. Reduction of

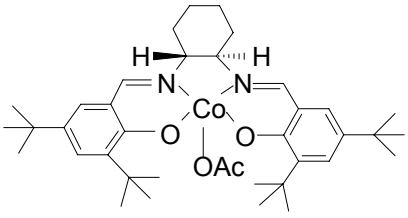
aldehyde (**8**) using Bu_3EtNBH_4 furnished diol **9**, which was dimesylated to afford dimesylate derivative **10** (Scheme **2**).

Conversion of compound **10** into (*S*)-metoprolol (**13**) was accomplished in two ways. 1) Replacement of mesylated moiety with propylamine and then removal of methylene bridge using H_2SO_4 provided (*S*)-metoprolol (**13**) in good yield.

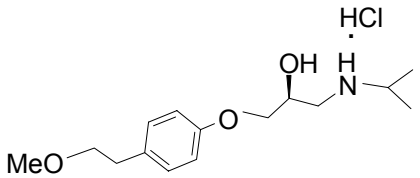
Scheme 2



The importance of epoxides in organic synthesis arises partly from the occurrence of the strained three-membered ring unit in a number of interesting natural products[27] but more so because the ring opening of epoxides allows straightforward elaboration to useful new functionality, often with generation of new carbon-carbon bonds. The stereospecific manner in which epoxides generally react renders these compounds attractive chiral building blocks for asymmetric synthesis.[28] As a consequence; the preparation of enantioenriched epoxides has long stood as a most significant target for asymmetric synthesis. Chiral glycidyl deridatives are perhaps the most versatile C_3-chiral synthons with numerous applications for β-blockers, MAO inhibitors, alkyl glycerophospholipids and other pharmaceuticals as well as in organic synthesis.[29] Among various available methods for the preparation of enantioenriched epoxides, Hydrolytic Kinetic Resolution (HKR) of terminal epoxides using SalenCo(III)OAc complex **1** appeared to hold considerable promise.[14] HKR provides a practical method for obtaining enantiopure epoxides and 1,2- diols with > 99 % ee. It uses water as the sole solvent; along with low loading of recyclable catalyst (< 0.5 %) made this new method appeared to hold considerable promise.



(R,R): 1

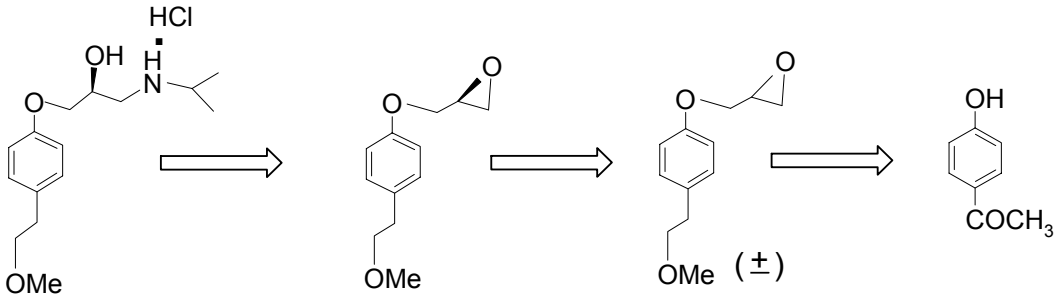


2

Metoprolol (**2**) has been shown to be an effective agent against hypertension, myocardial infraction and angina pectoris. Metoprolol is cardioselective. It blocks selectively the β-$_1$ receptors found predominantly in the heart and do not block the β-$_2$ receptors (found predominantly in the bronchi and peripheral blood vessels). Although most of the β-blockers are sold as racemates, only the (*S*)-isomer is associated with β-blocking activity. Since (*R*)-isomer does not have any detrimental activity, the racemates are considered reasonably safe for consumer use. However, there is a growing concern all

over the world as to why unwanted (*R*)-isomer should be given to the patient.[30] This has prompted organic chemists to investigate various optically active synthetic strategies for (*S*)-β-blocking agents. This section describes a practical synthesis of (*S*)-Metoprolol, belonging to aryloxypropanolamine class, as a representative example using HKR as shown in retrosynthetic analysis (Scheme **1**).

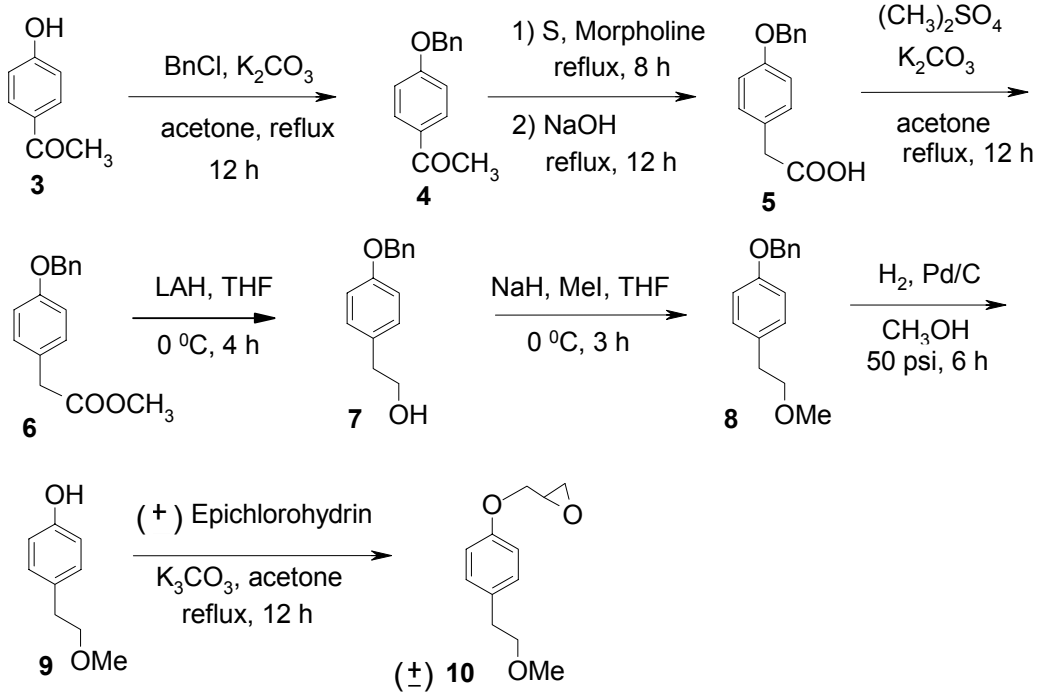
Scheme **1** (Retrosynthetic analysis)



Our first concern was the preparation of racemic aryl glycidyl ether, [31] that was achieved starting with 4-hydroxy acetophenone (**3**) as shown in Scheme **2**. Treatment of compound **3** with $BnCl/K_2CO_3$ under reflux condition in acetone provided benzylated compound **4**. ^1H NMR spectrum of **4** clearly indicated the presence of benzyl group. Compound **4** was refluxed for 6 h with sulfur in morpholine to provide thiomorpholine derivative, which was immediately heated under reflux with NaOH solution to afford aryl acetic acid derivative **5**. The acid derivative **5** was converted to the alcohol derivative **7**,[32] *via* the formation of its methyl ester derivative **6** in presence of $(CH_3)_2SO_4$ and K_2CO_3 in refluxing acetone, and subsequent reduction of ester derivative **6** with $LiAlH_4$. The characteristic signals in ^1H NMR spectrum due to $PhCH_2$ and CH_2OH were observed at 2.76 and 3.76 ppm respectively and the rest of the protons appeared at their expected regions. Methylation of alcohol **7** with NaH/MeI in THF, followed by hydrogenolysis of **8** using Pd/C in MeOH at 50 psi hydrogen pressure afforded compound **9**.[33] In the ^1H NMR spectrum, the absence of signal due to OCH_2Ph at 5.05 and 7.38 ppm along with presence of characteristic signal due to phenolic hydroxy at 5.88 ppm, confirms the structure of compound **9**. Compound **9** on treatment with (±) epichlorohydrin and K_2CO_3

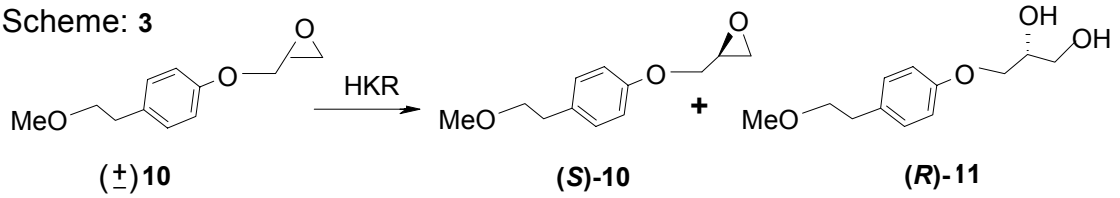
in acetone under refluxing condition for 12 h was converted to racemic arylglycidyl ether **10**. The structure of the compound **10** was confirmed by in ^{1}H NMR spectral analysis.[33b]

Scheme: **2**



The racemic arylglycidyl ether **10** was resolved by hydrolytic kinetic resolution technique.[14] For this purpose, (R,R)-Salen Co(III)OAc complex (**1**) was prepared according to the literature procedure.[14] Compound **10** when subjected to HKR (Scheme **3**) using complex **1** (5 mol %) and water (0.55 eq.) for 18 h (monitored by HPLC) provided (*S*)-[4-(2-methoxyethyl)] phenylglycidylether (**10**) in 45 % yield with 93 % ee; $[\alpha]_D$ +4.9° (c 1.27, $CHCl_3$). Enantiomeric excess of (***S***)-**10** was confirmed at later stage. The ^{1}H NMR spectral analysis conclusively confirmed the structure of the compound (***S***)-**10**. Further conformation came from HRMS spectral analysis, which shows M^+ peak at 208.1108 (Calcd. for $C_{12}H_{16}O_3$: 208.1099; Found: 208.1108).

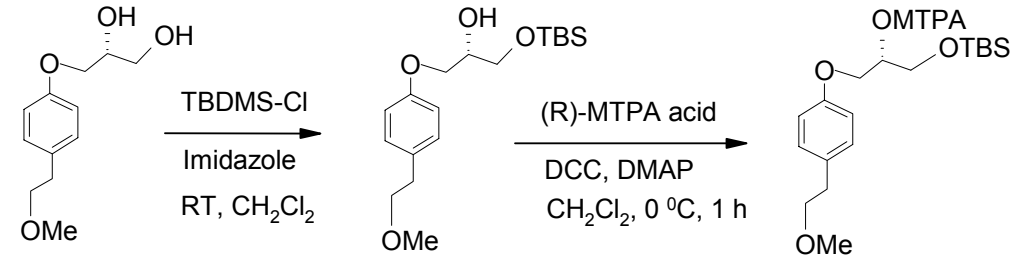
Scheme: **3**



(±)**10** (***S***)-**10** (***R***)-**11**

The HKR reaction also provided (*R*)-1-[4-(2-methoxyethyl)] phenylglycerol **11** in 49 % yield with 97 % ee; $[\alpha]_D$ –9.0° (c 2.28, MeOH) (ee confirmed at later stage). The ^{1}H NMR spectrum along with HRMS analysis (Calcd. for $C_{12}H_{18}O_4$: 226.1205; Found: 226.1208) confirmed the structure of compound **11**.

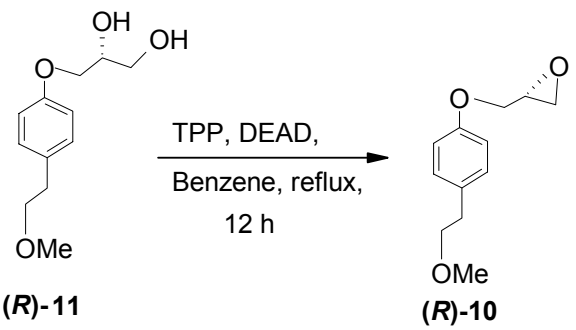
Scheme: **4**



The enantiomeric excess of the (*R*)-diol (**11**) was confirmed by converting into its Mosher ester derivative (Scheme **4**). The primary hydroxy group in (*R*)-diol (**11**) was first protected with TBSCl to give **12**, which was subjected to esterification using (*R*)-(α, α') methoxytrifluromethylphenyl acetic acid [(*R*)-MTPA acid] / DCC in CH_2Cl_2 to give (*R*,*R*)-**13.** Similarly racemic-**12** was also prepared. The ^{19}F NMR spectral analysis of (*R*,*R*)-**13** and (±) **13** confirmed the 97 % enantimeric excess of the diol **(*R*)-11**.

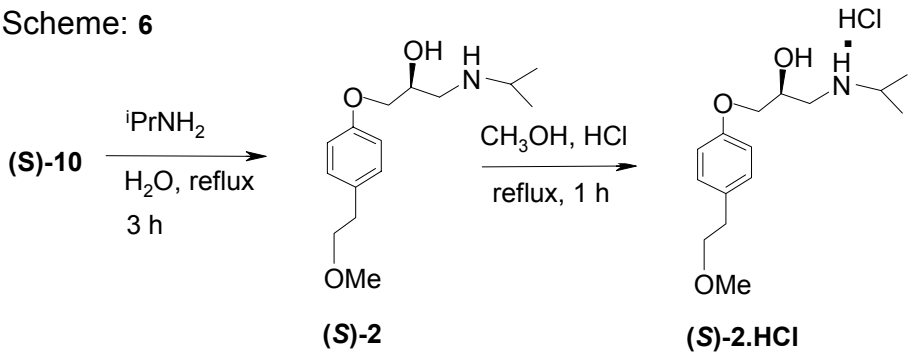
The enantiomeric excess of (*S*)-epoxide **10** was confirmed by converting (*R*)-diol (97 % ee) **11** into (*R*)-epoxide (97 % ee) by Mitsunobu[34] reaction (Scheme: **5**). Accordingly, (*R*)-diol was treated with TPP and DEAD and the mixture heated under reflux in benzene for 12 h to provide (*R*)-epoxide (***R*-10**) with $[\alpha]_D$ –5.2° (c 1.2, $CHCl_3$). It conclusively confirmed the 93 % enantiomeric excess of (***S*)-10** with $[\alpha]_D$ +4.9° (c 1.27, $CHCl_3$).

Scheme: **5**



(*S*)-epoxide **10** was then finally converted to (*S*)-metoprolol as shown in Scheme: **6**. The (*S*)-epoxide **10** on treatment with isopropylamine / H_2O under reflux condition for 3 h provided metoprolol (**2**). The characteristic resonance due to isopropyl group and hydroxyl groups were distinctly visible. The ^1H NMR spectroscopic data of **2** was in full agreement with the reported data. Further confirmation came from HRMS analysis [Calcd. for $C_{14}H_{22}NO_3$ ($M\text{-}Me^+$): 252.1599; Found: 252.1602]. The enantiomeric excess of (***S***)-**2** was determined by converting it into its HCl salt (***S***)-**2.**HCl. (***S***)-**2** was refluxed with HCl-methanol gave (***S***)-**2.**HCl, which on recrystallization in diethyl ether provided crystalline solid $[\alpha]_D$ –21.0° (c 1.26, MeOH), lit.[25, 26] $[\alpha]_D$ –21.5° (c 1.0, MeOH), Mp 92 °C; lit.[25, 26] MP 92-94 °C. Based on the optical rotation, enantiomeric excess of (***S***)-**2.**HCl was found to be 96 %.

Scheme: **6**



In conclusion, a practical synthesis of (*S*)-metoprolol was achieved[33] by the hydrolytic kinetic resolution of arylglycidyl ether using chiral Salen Co(III)OAc complex.

EXPERIMENTAL

1-(4-Benzyloxy-phenyl)-ethanone (4)

A solution of 4-hydroxy acetophenone (**3**) (25.0 g, 183.8 mmol), K_2CO_3 (38.0 g, 275.7 mmol) and BnCl (30.0 mL, 220.6 mmol) in acetone (100.0 mL) was reflux for 12 h. The reaction was filtered and the residue purified on silica gel using ethyl acetate- light petroleum (1:9) as eluent to afford compound **4** (35.0 g, 84 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.51 (s, 3 H), 5.11 (s, 2 H), 6.95 (d, 2 H, *J* = 9.3 Hz), 7.35 (m, 5 H), 7.88 (d, 2 H, *J* = 9.3 Hz).

(4-Benzyloxy-phenyl)-acetic acid (5)

A solution of **4** (35.0 g, 154.8 mmol), morpholine (16.85 mL, 193.6 mmol) and sulphur (7.5 g, 232.3 mmol) was reflux for 5 h and poured into water. The yellow solid formed was filtered to give thioacetomorpholide (48.8 g, 96 %), which was added to a solution of 10 % NaOH (300.0 mL) in ethanol, refluxed for 10 h and concentrated. The residue was diluted with water, acidified with HCl, extracted with ether, dried (Na_2SO_4) and concentrated to afford **5** (28.0g, 78 %).

(4-O-Benzyloxy-phenyl)-acetic acid methyl ester (6)

A solution of **5** (28.0 g, 115.7 mmol), K_2CO_3 (24.0 g, 173.6 mmol) and dimethyl sulfate (14.3 ml, 150.4 mmol) in dry acetone (200.0 mL) was reflux for 10 h. The reaction was filtered, concentrated and the residue purified on silica gel using ethyl acetate-light petroleum (1:9) as eluent to give **6** (26.4 g, 89 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.71 (s, 3 H), 4.12 (s, 2 H), 5.08 (s, 2 H), 6.91 (d, 2 H, *J* = 9.3 Hz), 7.12 (d, 2 H, *J* = 9.3 Hz), 7.35 (m, 5 H).

2-(4-Benzyloxy-phenyl)-ethanol (7)

A solution of **6** (26.4 g, 103.0 mmol) and LAH (7.8 g, 206.0 mmol) in dry THF (100.0 mL) was stirred for 6 h. Excess of LAH was destroyed by the addition of ethyl acetate (20.0 mL) and the reaction filtered, extracted with ethyl acetate, dried (Na_2SO_4), concentrated and the residue purified on silica gel using ethyl acetate – light petroleum (1:2) as eluent to give **7** (21.2 g, 90 %).[32]

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.76 (t, 2 H, *J* = 6.8 Hz), 3.76 (t, 2 H, *J* = 6.8 Hz), 5.01 (s, 2 H), 6.86 (d, 2 H, *J* = 9.3 Hz), 7.09 (d, 2 H, *J* = 9.3 Hz), 7.31 (m, 5 H).

EIMS *m/z*: 228 (M^+);

1-benzyloxy-4-(2-methoxy-ethyl)-benzene (8)

A solution of **7** (20.0 g, 87.7) and NaH (8.8 g of 60 % dispersion in oil, 219.3 mmol) in dry THF was stirred for 45 min and subsequently MeI (13.8 mL, 219.3 mmol) was added. After 6 h, excess sodium hydride was decomposed with methanol (15.0 mL) and concentrated. The residue was dissolved in ethyl acetate (100.0 mL), washed with water, brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate – light petroleum (1:4) as eluent to yield **8** (19.1 g, 91 %). [33a]

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.82 (t, 2 H, *J* = 6.8 Hz), 3.34 (s, 3 H), 3.55 (t, 2 H, *J* = 6.8 Hz), 5.05 (s, 2 H), 6.86 (d, 2 H, *J* = 9.1 Hz), 7.11 (d, 2 H, *J* = 9.1 Hz), 7.37 (m, 5 H).

EIMS *m/z*: 242 (M^+);

4-(2-methoxy-ethyl)-phenol (9)

A suspension of **8** (19.0 g, 78.5 mmol) and 10 % Pd-C (200.0 mg) in methanol (70.0 mL) were stirred under hydrogen pressure at 50 psi for 6 h. The reaction was filtered, concentrated and the residue purified on silica gel using ethyl acetate – light petroleum (1:2) as eluent to afford **9** (10.0 g, 84 %). [33b]

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.82 (t, 2 H, *J* = 6.8 Hz), 3.39 (s, 3 H), 3.59 (t, 2 H, *J* = 6.8 Hz), 5.88 (s, 1 H), 6.71 (d, 2 H, *J* = 9.1 Hz), 7.02 (d, 2 H, *J* = 9.1 Hz).

EIMS *m/z*: 152 (M^+);

(±)-1-[4-(2-Methoxy-ethyl)]-phenylglycidyl ether (10)

To a suspension of **9** (10.0 g, 65.8 mmol) and K_2CO_3 (18.2 g, 131.6 mmol) in dry acetone (100.0 mL), (±)-epichlorohydrin (7.7 mL, 98.7 mmol) was added and refluxed for 12 h. The reaction was filtered, concentrated and the residue purified on silica gel using ethyl acetate – light petroleum (1:9) as eluent to afford **10** (12.2 g, 89 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.80 (m, 4 H), 3.34 (m, 4 H), 3.55 (t, 2 H, *J* = 6.8 Hz), 3.98 (dd, 1 H, *J* = 5.4, 12.0 Hz), 4.18 (dd, 1 H, *J* = 4.2, 12.0 Hz), 6.82 (d, 2 H, *J* = 8.4 Hz), 7.10 (d, 2 H, *J* = 8.4 Hz).

EIMS *m/z*: 208 (M^+);

(*S*)-1-[4-(2-Methoxy-ethyl)]-phenylglycidyl ether [(*S*)-10]

(*R*)-1-[4-(2-Methoxy-ethyl)]-phenyl glycerol [(*R*)-11]

To a suspension of (±) aryl glycidyl ether **10** (10.0 g, 48.1 mmol) and (*R*, *R*)-Salen Co (III) OAc complex (**1**) (0.16 g, 0.2 mmol), water (0.48 mL, 26.4 mmol) was added over a period of 1 h. The reaction was stirred at room temperature and monitored by HPLC (ODS column, UV: 225 nm, 60 % CH_3CN in H_2O). The reaction was diluted with ethyl acetate, dried (Na_2SO_4), concentrated and the residue purified on silica gel. The first fraction to be eluted with 1:9 ethyl acetate – light petroleum gave the **(*S*)-10** (4.5 g, 45 %).

$[\alpha]_D$ + 4.9° (c 1.27, $CHCl_3$);

HRMS Calcd. for $C_{12}H_{16}O_3$: 208.1099 (M^+); Found 208.1108.

Further elution with 1:1 ethyl acetate – light petroleum gave (***R*)-11** (5.3 g, 49 %).

$[\alpha]_D$ –9.0° (c 2.28, $CHCl_3$);

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2 (brs, 1 H), 2.6 (brs, 1 H), 2.8 (t, 2 H, *J* = 6.9 Hz), 3.34 (s, 3 H), 3.55 (t, 2 H, *J* = 6.9 Hz), 3.70 (m, 2 H), 4.07 (m, 3 H), 6.83 (d, 2 H, *J* = 8.5 Hz), 7.12 (d, 2 H, *J* = 8.5 Hz).

HRMS Calcd. for $C_{12}H_{18}O_4$: 226.1205; Found: 226.1208.

(R)-1-[4-(2-Methoxy ethyl)]-phenyl-[3'(t-butyl dimethylsilyloxy)]-glycerol (12)

To a solution of **11** (0.2 g, 0.9 mmol) and imidazole (0.18 g, 2.6 mmol) in dry CH_2Cl_2, TBS-Cl (0.14 g, 0.9 mmol) was added and stirred for 1 h. The organic layer was

washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate – light petroleum (1:3) as eluent to afford **12** (0.28 g, 91 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.10, 0.20 (2s, 6 H), 0.94 (s, 9 H), 2.44 (d, 1 H, *J* = 4.5 Hz), 2.82 (t, 2 H, *J* = 6.7 Hz), 3.36 (s, 3 H), 3.56 (t, 2 H, *J* = 6.7 Hz), 3.77 (d, 2 H, *J*= 4.5 Hz), 3.98 (m, 3 H), 6.83 (d, 2 H, *J* = 9.0 Hz), 7.12 (d, 2 H, *J* = 9.0 Hz).

(R)-1-[4-(2-Methoxy ethyl)]phenyl-[2'-(R)-(α, α')-(methoxytrifloromethylphenyl acetate)-3'-(t-butyldimethoxy silyloxy)]glycerol (13)

A solution of **12** (50.0 mg, 0.15 mmol), DCC (45 mg, 0.2 mmol), DMAP (catalytic) and (R)-(α, α')-(methoxytrifloromethylphenyl acetic acid (52 mg, 0.2 mmol) in dry CH_2Cl_2 (10.0 mL) was stirred for 1 h. The organic layer was washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate – light petroleum (1:9) as eluent to afford **13** (0.77 mg, 94 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.00, 0.01 (2s, 6 H), 0.84 (s, 9 H), 2.81 (t, 2 H, *J* = 6.8 Hz), 3.34 (s, 3 H), 3.54 (t, 2 H, *J* = 6.8 Hz), 3.58 (s, 3 H), 3.85 (m, 2 H), 4.21 (m, 2 H), 5.41 (m, 1 H), 6.82 (d, 2 H, *J* = 9.1 Hz), 7.14 (d, 2 H, *J* = 9.1 Hz), 7.46 (m, 5 H).

(R)-1-[4-(2-Methoxyethyl)]-phenyl glycidyl ether [(R)-10]

A solution of **(*R*)-11** (2.0 g, 8.8 mmol), Ph_3P (3.5 g, 13.4 mmol) and DEAD (2.1 mL, 13.4 mmol) in benzene (25.0 mL) was reflux for 20 h and concentrated. The residue was purified on silica gel using ethyl acetate – light petroleum (1:9) as eluent to afford **(*R*)-10** (1.49 g, 81 %).

$[\alpha]_D$ –5.2° (c 1.2, $CHCl_3$).

(S)-Metoprolol hydrochloride [(*S*)-2-HCl]

A solution of **(*S*)-10** (1.6 g, 7.7 mmol), isopropylamine (6.6 mL, 77.0 mmol) and water (0.15 mL) was heated under reflux for 6 h. The reaction mixture was concentrated to dryness to give metoprolol **(*S*)-2** (1.85 g, 90 %).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.10 (d, 6 H, *J* = 6.4 Hz), 2.17 (brs, 1 H), 2.80 (m, 5 H), 3.38 (s, 3 H), 3.59 (t, 2 H, *J* = 7.4 Hz), 3.98 (m, 3 H), 6.86 (d, 2 H, *J* = 8.5 Hz), 7.14 (d, 2 H, *J* = 8.5 Hz).

HRMS Calcd. for $C_{14}H_{22}NO_3$: 252.1599 ($M\text{-}Me^+$); Found 252.1602.

To a solution of **(*S*)-2** (1.85 g, 6.9 mmol) in methanol (15.0 mL), 11 N HCl (2.0 mL) was added and reflux for 1 h. The reaction was concentrated and the residue recrystallized from diethyl ether to afford metoprolol **(*S*)-2-HCl** as a solid (1.9 g, 90 %).

M.P. 92 °C; lit. [25,26] 92-94 °C;

$[\alpha]_D$ –21.0° (c 1.26, CH_3OH); lit.[25, 26] $[\alpha]_D$ –21.8° (c 1.0, CH_3OH).

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CHAPTER- 2

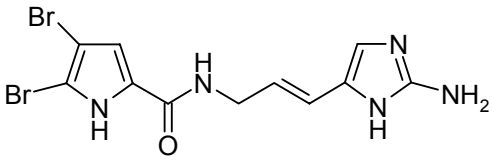
Synthesis of 9-*epi*-Manzacidin B Methyl Ester

INTRODUCTION

Bromopyrrole alkaloids comprise a typical class of marine natural products, frequently encountered as secondary metabolites of marine sponges of various species.[1] Bromopyrrole alkaloids are very interesting metabolities because of their structural variety and pharmacological activities. Among these species *Agelasiidae*, *Axinellidae* and *Hymeniacidonidae* families constitute the bromopyrrole alkaloids.

In the last 25 years, sponges of the genus *Agelas* have been extensively investigated yielding a prodigious harvest of new natural compounds, going from α-glycosphingolipids to derivatized terpenoids and bromopyrrole alkaloids. Oroidin[2] (**1**) is a major metabolite of several species of marine sponges of the genus *Agelas* and is the basis of the oroidin group of alkaloids. It was first isolated in 1971 from *Agelas oroides* but its structure was established in 1973[3] and proven by total synthesis in 1986.[4] Subsequently, many other species of *Agelas, Axinella, Acanthella, Hymeniacidon, Phakellia* and *Pseudaxinyssa* have been reported to contain high levels of oroidin or cyclic analogues. It is thus clear that oroidin alkaloids are useful chemotaxonomic markers for axinellid sponges that were once allied with the Agelasida. In 1981, Faulkner and co-workers[5] isolated sceptrin (**2**) from *Agelas sceptrum.* Formally, sceptrin is related to debromooroidin by a head-to-head [2 + 2]-cycloaddition that must be photochemically allowed. As oroidin is achiral, if this were a photochemical reaction, sceptrin ought to have been isolated as a racemic mixture. In contrast, sceptrin is chiral ($[\alpha]_D$ - 7.4 ^{o}) suggesting that it is formed by an enzyme catalysed reaction. If this is the case, it will be the first example of a biological [2 + 2] cycloaddition or, indeed, any pericyclic reaction. Slight variations on the structure of sceptrin [eg dibromosceptrin, debromosceptrin, oxysceptrin (**3**) and nakamuric acid (**4**)] have been isolated in subsequent work from *Agelas* cf *nemoechinata*, [6] *A. conifer*[7] and *A. nakamurai*.[8] These compounds are also of pharmaceutical interest as many have shown α-adrenoceptor blocking activity that does not interfere with the action of potassium chloride or seratonin.[9] Specifically, sceptrin (**2**),

and its analogues have potent antibacterial/antifungal activities,[10] anti-muscarinic activity,[11] anti-histaminic activity[12] and oxysceptrin is also a potent actomyosin ATPase



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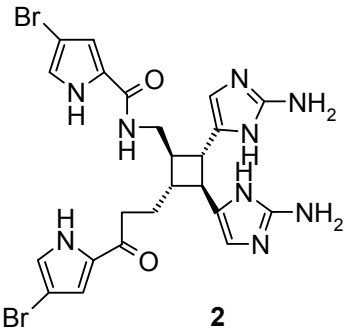

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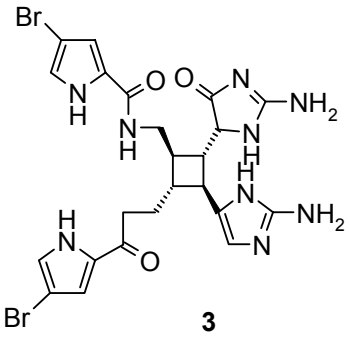

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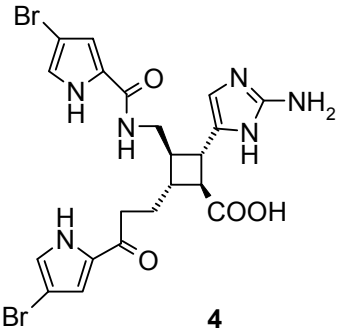
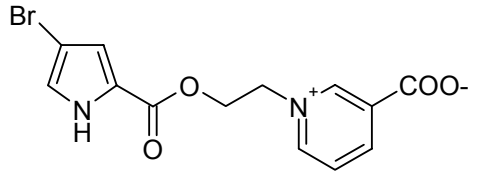


4

activator.[6] As a first step toward isolation of enzymes involved in the biosynthesis of sceptrin, we need to find a local sponge that contains this compound.

A series of structurally related alkaloids were obtained from four Carabbean species, namely A. clatherodes, A. conifera, A. dispar, and A. longissima. They are agelongine[13] (**5**, a pyridinium alkaloid that has selective antiserotonergic activity), clathramides[14] (**6**), and dispacamides A[15] (**7**), B[15] (**8**), C[16] (**9**) and D[16] (**10**).



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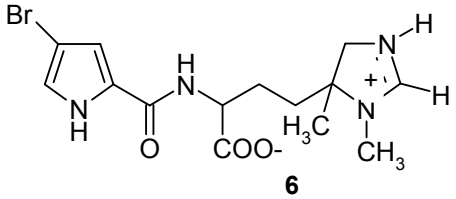

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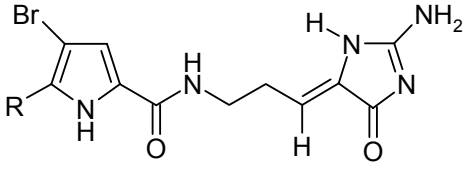
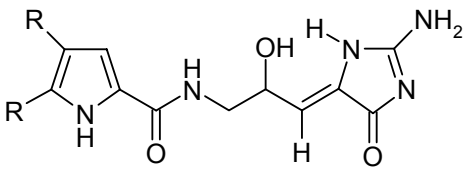
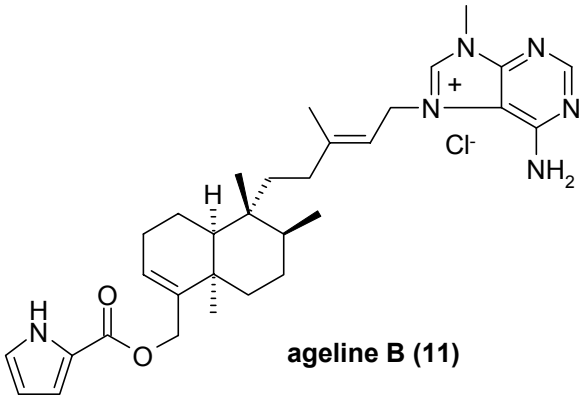


7 R = Br **8** R = H



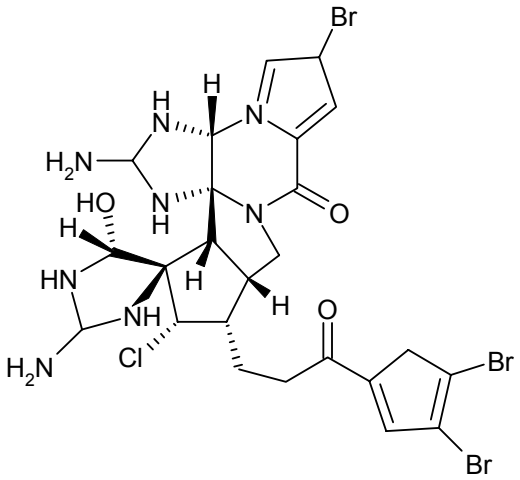
9 R = Br **10** R = H

Ageline B (**11**) is an example of a pyrrole-2-carboxylic acid derivative attached to the terpenoid through an ester bond.[17] Natural products containing a tetrahydropyrimidine ring are rare, and these apparently are the first examples from marine sources.[18]



ageline B (11)

Kobayashi *et al* during the studies on bioactive substances from Okinawan marine sponges examined the extracts of numerous marine sponges, and isolated a new fused-hexacyclic alkaloid possessing two bromopyrrole carbonyl groups and two guanidine units named konbu'acidin A[19] (**12**), with cdk4 inhibitory activity from an Okinawan marine sponges *Hymeniacidon* sp. There are some other brommopyrrole alkoloids obtained from Hymeniacidonidae family and they are hymenialdisine[20] (**13**), hymenine (**14**), etc.



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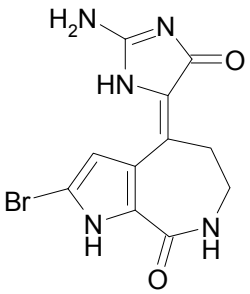
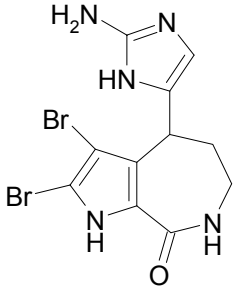
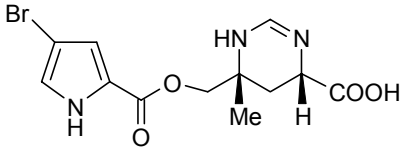


hymenialdisine (13)

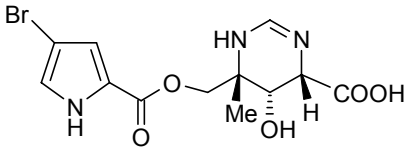


hymenine (14)

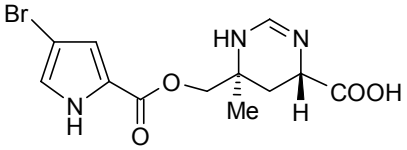
During further studies on bioactive substances from marine organisms, Kobayashi *et al*[21] have examined the extracts of numerous marine sponges and isolated several bromopyrrole alkaloids, which were found to be pharmacologically useful as α-adrenoceptor blockers,[22] antagonists of serotonergic receptor,[23] actomyosin ATPase



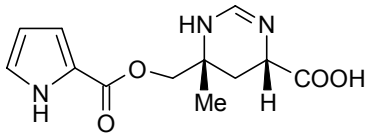
manzacidin A (15)



manzacidin B (16)



manzacidin C (17)



manzacidin D (18)

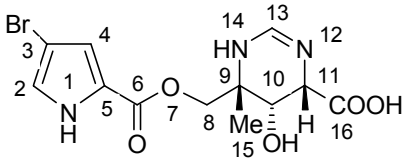
activators.[24]

Recently they have investigated bio active constituents of *Hymeniacidon* sp.[25] and isolated three novel compounds, named manzacidins A-C (**15-17**) belonging to an unprecedented class of bromopyrrole alkaloids with an unusual 3,4,5,6- tetrahydro pyrimidine ring. Also very recently, manzacidin D (**18**) structurally related manzacidin A

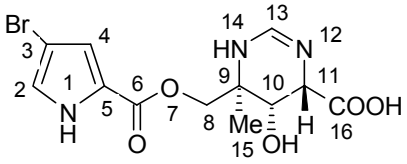
(**15**) has been isolated.[26] Manzacidins A-C are the first bromopyrrole alkaloids with a tetrahydropyrimidine ring attached through an ester linkage.

PRESENT WORK

Recently a novel class of alkaloids manzacidin A-C (**15-17**) have been isolated,[23, 24] which possess a unique structure consisting of an ester-linked bromopyrrole carboxylic acid and a 3,4,5,6-tetrahydro pyrimidine ring in which one of the amino group is attached to the C-9 quaternary carbon center. Although manzacidins exhibit similar biological activities to those of other bromopyrrole alkaloids, only recently tests have been carried out, owing to the extremely small amount of samples available from marine sources. Ohfune *et al* reported the synthesis of manzacidin A (**15**).[28] The difference between manzacidin A (**15**) and manzacidin B (**16**) is an additional hydroxy group at C-10. No synthesis of manzacidin B (**16**) or its derivatives has yet been attempted. The importance of synthesis of manzacidin B (**16**) can be visualized in two ways. First, the total synthesis of manzacidin B (**16**) would provide unambiguous proof for its stereochemical structure but more importantly it will pave a way to prepare analogues of manazacidin B (**16**) useful for structure activity relationship. This chapter deals with synthetic approaches toward manzacidin B (**16**), particularly 9-*epi*-manzacidin B (**19**). The perpose of synthesis of **19** was to develop a synthetic route amenable to prepare the natural product as well as stereomeric analogues useful to study structure-activity relationship. It is pertinent to mention that based on the investigation reported in this chapter, the total synthesis of naturally occurring manzacidin B (**16**) has been recently undertaken in this



manzacidin B (16)

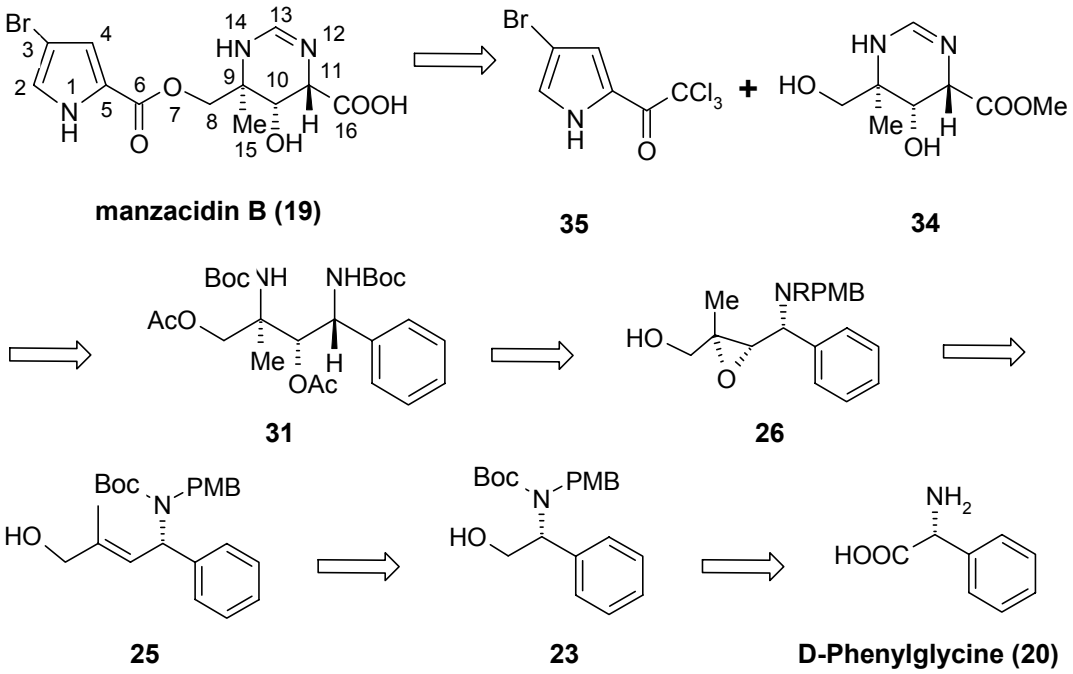


9-*epi*-manzacidin B (19)

laboratory.

Compound **19** can be visualized by the esterification of the alcohol (**34**) with bromopyrrole (**35**), the former being envisaged from the diamine (**31**) by cyclisation. The formation of the diamine (**31**) by regioselective opening of the epoxide (**26**) with nitrogen nucleophile was a straightforward exercise. The epoxide (**26**) can be obtained using *m*-CPBA protocol constitutes of diastereoselective *syn* epoxidation of *trans*-γ-amino allylic alcohol (**25**).

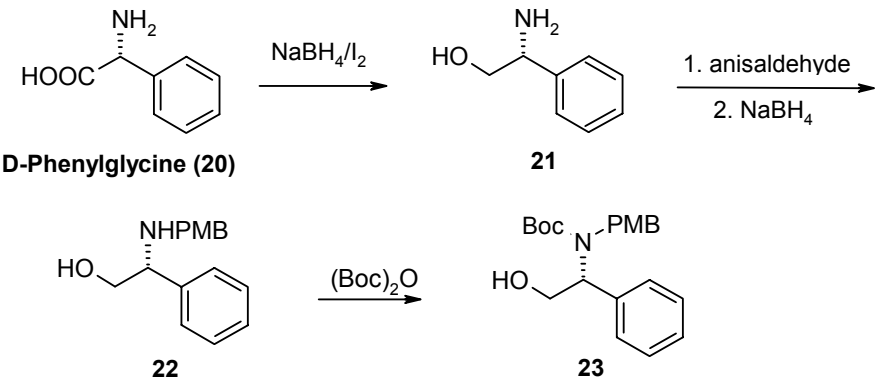
Retrosynthetic analysis



Synthetic approach

We began the synthesis from D-phenylglycine (**20**), which was reduced[29] with $NaBH_4/I_2$ followed by protection of the amino functionality with PMB and Boc groups to give **23** (Scheme **1**) whose structure was confirmed by the ^1H and ^{13}C NMR spectral data.

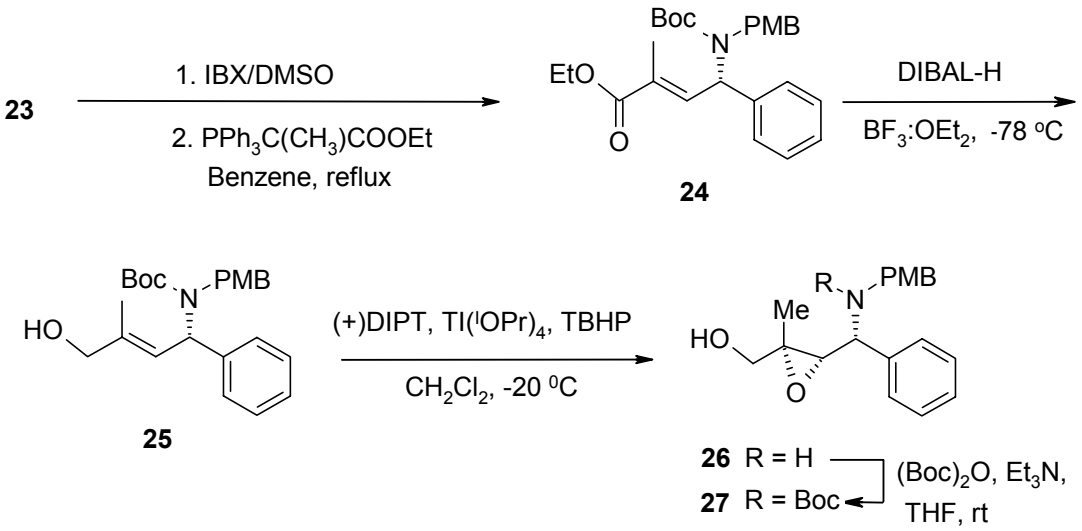
Scheme 1



The alcohol (**23**) was oxidized[30] with IBX and the resulting aldehyde subjected to Wittig olefination with $Ph_3PC(Me)COOEt$ in refluxing benzene to afford the *trans*-olefin **24** in 65 % yield. The reduction of **24** with DIBAL-H in the presence of BF_3:OEt_2 at –78 °C gave the *trans*-γ-amino allylic alcohol (**25**) in 87 % yield (Scheme **2**). The structure of **25** derived from the ^1H NMR spectrum showed the presence of the olefinic proton at 6.77 ppm. The ^{13}C NMR spectrum and elemental analysis further supported the structure of **25**.

The Sharpless asymmetric epoxidation[31] reaction of **25** with (+)-DIPT as a chiral auxilary at –20 °C gave the epoxide (**26**) (9:1 as judged by chiral HPLC). The structural features of **26** were established by the ^1H and ^{13}C NMR spectral data. It was interesting to note that Boc protecting group cleaved during the Sharpless asymmetric epoxidation. Subsequently, the Boc group was again attached in presence of $(Boc)_2O$ and Et_3N in THF to give the epoxide **27**.The stereochemistry of **26**, although confirmed at a later stage was given as indicated based on Sharpless empirical rules.

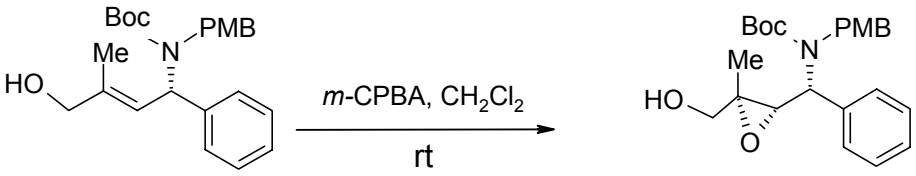
Scheme 2



To further prove the stereochemistry of the epoxide (**26**), we carried out epoxidation of **25** by *m*-CPBA (Scheme **3**) at room temperature in CH_2Cl_2. Literature survey[32] revealed that *m*-CPBA epoxidation of *trans*-amino allylic alcohols afforded exclusive diastereoselective *syn* epoxides. Accordingly, *m*-CPBA epoxidation gave

compound **27** exclusively whose spectral data was super imposable with the epoxide obtained *via* Sharpless asymmetric epoxidation reaction, thus confirming the stereochemistry of the epoxide **26**. The highly diastereoselective epoxidation of **25** was explained in terms of co-operative effect reported by Kishi.[32c] Namely, it was postulated that the hydroxy group of the allylic alcohol and the carbonyl oxygen form hydrogen bonds with the peracid in the transition state.

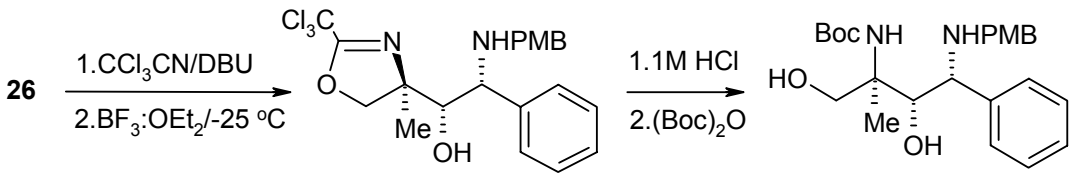
Scheme 3



25 **27**

Our next aim was to introduce the amino group regioselectivity at C-2, for which Hatakeyama's method[33] was employed. Thus, treatment of **26** with CCl_3CN/DBU in CH_2Cl_2 at 0 ^oC (Scheme **4**) was followed by BF_3:OEt_2 catalyzed intramolecular epoxide-opening reaction at –25 ^oC to afford the oxazoline **28** in 62 % yield over two steps. In addition, 20 % yield of the hydrolysis product was also isolated. The structure of **28** was confirmed by the ^1H and ^{13}C NMR spectroscopic data. The oxazoline derivative (**28**) was then converted to the alcohol **29** by acid hydrolysis followed by *tert*-butoxy carbonylation in 82 % yield (Scheme **4**). In the ^1H NMR spectrum of **29**, the presence of a singlet at 1.47 ppm due to Boc protecting group was noted. The ^{13}C NMR spectrum and elemental analysis were compatible with the assigned structure.

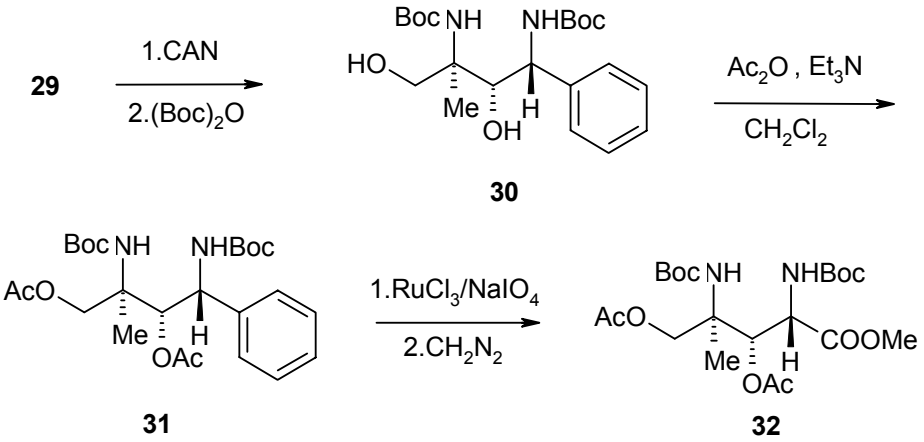
Scheme 4



28 **29**

Our next concern was the oxidation of phenyl group present in compound **29**. In order to accomplish the oxidation, the PMB protecting group was first deprotected using CAN at –5 °C and the free amine was then converted into the Boc derivative (**30**) in 73 % yield. The two-hydroxyl groups in compound **27** were transformed into the diacetate (**31**) whose ^{1}H, ^{13}C NMR spectroscopic data and elemental analysis established the assigned structure. For example, the two singlets at 1.34 and 1.47 ppm were attributed to Boc groups while other two singlets at 2.06 and 2.15 ppm were from acetyl methyl groups.

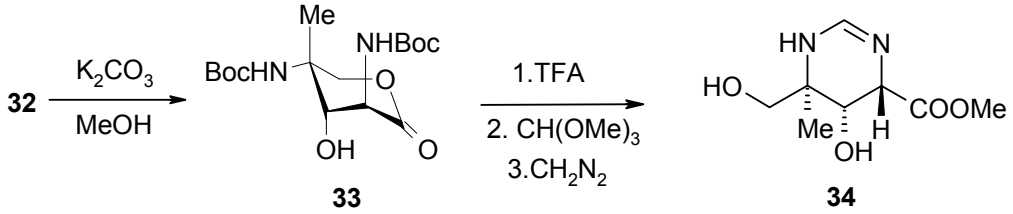
Scheme 5



The $RuCl_3/NaIO_4$ oxidation[34] of **31** followed by esterification with CH_2N_2 afforded the ester **32** in 52 % yield (Scheme **5**). The singlet at 3.85 ppm was assigned to methyl ester while rest of the spectrum was in complete agreement with the assigned structure. In addition, the ^{13}C NMR spectrum also supported the structure **32**.

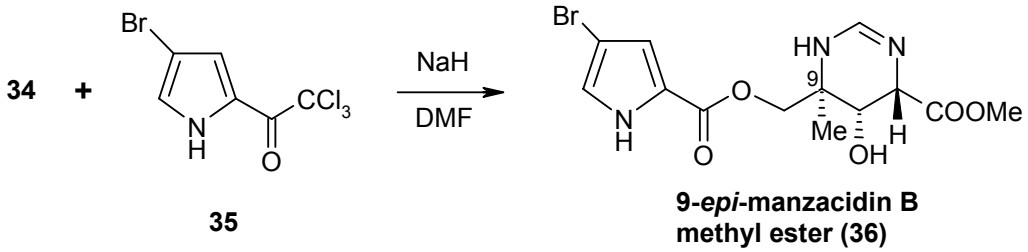
The deprotection of acetate functionalities from **32** with K_2CO_3/MeOH resulted in the formation of the expected lactone **33** in 71 % yield. The ^{1}H and ^{13}C NMR spectroscopic studies of **33** revealed the assigned structure. Construction of the tetrahydro pyrimidine ring[35] was performed by successive treatment of **33** with (i) TFA and (ii) methyl orthoformate to give the acid, which was purified after the esterification with CH_2N_2 to provide **34** in 64 % yield (Scheme **6**). In the ^{1}H NMR spectrum, a singlet at 7.97 was assigned to the imine proton while other two singlets at 1.37 and 3.80 were due to methyl and ester group. In addition ^{13}C NMR spectroscopic data and elemental analysis established the assigned structure **34**.

Scheme 6



Completion of the synthesis now required esterification of the bromopyrrole carboxylate with **34**. Thus, treatment of **34** with NaH/trichloroacetylbromopyrrole (**35**) [36] in DMF at room temperature gave the manzacidin B methyl ester (**36**) in 45 % yield (Scheme **7**).

Scheme 7



The structure of compound **36** was fully analyzed by ^{1}H and ^{13}C NMR spectroscopic data coupled with satisfactory elemental analysis. For example, in its ^{1}H NMR spectrum the two *meta* coupled protons of the bromopyrrole moiety were appeared as two doublets at 6.79 (J = 1.92 Hz) and 7.01 ppm (J = 1.92 Hz). The singlet at 8.07 ppm was attributed to H-13. The two singlets at 3.83 and 1.27 ppm were due to ester and methyl groups respectively, while rest of the spectrum was in complete agreement with the assigned structure.

In order to convert **36** into the natural product (**14**), selective hydrolysis of **35** was attended under various basic conditions. This reaction was not successful in our hand due to the presence of two susceptible groups prone to basic hydrolysis. Our next concern to achive the total synthesis of manzacidin B (**16**) is in progress in our laboratory.

Conclusion

In conclusion, synthesis of the 9-*epi*-manzacidin B methyl ester derivative (**36**) has been achieved *via* Sharpless asymmetric epoxidation reaction and the regioselective opening of the epoxide with nitrogen nucleophile. The present synthetic route enables the synthesis of diastereomers of manzacidin B (**16**) that permits further pharmacological studies on these alkaloids.

(2*R*)-2-[(*tert*-Butoxycarbonyl) (*p*-methoxybenzyl)-amino]-2-phenylethanol (23)

A solution of $NaBH_4$ (12.9 g, 331.1 mmol), D-phenylglycine (**20**) (20.0 g, 132.4 mmol) and iodine (33.6 g, 132.4 mmol) in THF (200.0 mL) was refluxed for 18 h, diluted with methanol, concentrated. The residue was dissolved in aq. 20 % KOH solution (200.0 mL), stirred for 4 h and extracted with CH_2Cl_2. The organic layer extract was washed with brine, dried (Na_2SO_4), concentrated to give **21** (17.0 g), which was dissolved in methanol (100.0 mL) and *p*-methoxybenzaldehyde (16.9 g, 124.0 mmol) was added. After 1 h $NaBH_4$ (5.4g, 136.5 mmol) was introduced and stirred further for 2 h. The reaction was quenched with dilute acetic acid and concentrated. The residue was dissolved in ethyl acetate, washed with brine (Na_2SO_4), dried and concentrated. The residue was suspended in 1:1 mixture of THF-H_2O (100.0 mL) and $(Boc)_2O$ (29.7 g, 136.4 mmol) added. After 2 h, solvent was concentrated, and washed with brine, dried (Na_2SO_4), concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **23** (39.2 g, 83 %).

$[\alpha]_D$ = -37.2° (c 2.2, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.46 (s, 9 H), 3.79 (s, 3 H), 3.96 (d, 2 H, *J* = 5.4 Hz), 4.20 (br s, 2 H), 5.08 (t, 1 H, *J* = 6.8 Hz), 6.82 (d, 2 H, *J* = 8.8 Hz), 7.25 (m, 7 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 28.1, 48.2, 54.8, 61.5, 62.6, 80.1, 113.6, 127.2-128.2, 131.0, 138.1, 156.5, 158.4;

Anal: Calcd. for $C_{21}H_{27}NO_4$: C, 70.58; H, 7.56; N, 3.92. Found: C, 70.15; H, 7.22; N, 3.61.

Ethyl (2*E*, 4*S*)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2-methyl-4-phenyl but-2-enoate (24)

A solution of **23** (10.0 g, 28.0 mmol) and IBX (8.62 g, 30.8 mmol) in DMSO (30.0 mL) was stirred at room temperature for 1 h, diluted with water and filtered. To the

filtrate, was added, ethyl acetate, washed with brine, dried (Na_2SO_4) and concentrated. The resulting product (8.7 g) and PPh_3=C(Me)COOEt (5.6 g, 15.5 mmol) in benzene (100.0 mL) were refluxed for 3 h. Benzene was removed and the residue purified on silica gel using ethyl acetate-light petroleum (1:10) as eluent to give **24** (4.12 g, 65 %).

[α] $_D$ = - 18.2 (c 0.59, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.24 (t, 3 H, *J* = 6.1 Hz), 1.39 (s, 9 H), 1.79 (s, 3 H), 3.76 (s, 3 H), 4.15 (m, 4 H), 4.55 (d, 1 H, *J* = 14.5 Hz), 6.77 (d, 2 H, *J* = 8.5 Hz), 7.06 (d, 2 H, *J* = 8.5 Hz), 7.27 (m, 6 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 12.3, 13.7, 27.8, 47.9, 54.6, 56.8, 60.1, 79.8, 113.2, 126.7, 127.0, 127.8, 128.1, 128.3, 130.1, 130.8, 137.7, 138.9, 155.3, 158.3, 167.0;

Anal: Calcd. for $C_{26}H_{33}NO_5$: C, 71.07; H, 7.51; N, 3.18. Found: C, 70.75; H, 7.32; N, 3.51.

(2*E*, 4*S*)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2-methyl-4-phenylbut-2-en-1-ol (25)

To a solution of **24** (4.0 g, 8.8 mmol) in CH_2Cl_2 (40.0 mL) at –78 ^{o}C was added a solution of DIBAL-H (11.0 mL, 22.0 mmol, 2.5 M in toluene). After 1 h, it was quenched with aq. sodium potassium tartarate, extracted with CH_2Cl_2. The combined CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **25** (3.16 g, 87 %).

[α] $_D$ = - 2.8 (c 0.84, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.33 (s, 9 H), 1.58 (s, 3 H), 3.72 (s, 4 H), 3.88 (s, 2 H), 3.97 (d, 1 H, *J* = 15.0 Hz), 4.62 (brd, 1 H, *J* = 16.2), 5.62 (d, 1 H, *J* = 16.2), 6.79 (d, 2 H, *J* = 8.5 Hz), 7.09 (d, 2 H, *J* = 8.5 Hz), 7.29 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 13.8, 28.1, 47.8, 54.9, 56.3, 67.4, 79.8, 113.2, 122.3, 126.7, 128.1, 128.3, 131.7, 139.2, 140.8, 155.8, 158.2;

Anal: Calcd. for $C_{24}H_{31}NO_4$: C, 72.54; H, 7.80; N, 3.52. Found: C, 72.95; H, 7.42; N, 3.81.

(2*S*, 3*S*, 4*R*)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2,3-epoxy-2-methyl-4-phenylbutan-1-ol (26)

Molecular sieves powder 4 A° (0.1 g), $Ti(^{i}OPr)_4$ (1.5 mL, 4.6 mmol) and (+)-DIPT (1.35 mL, 6.2 mmol) in CH_2Cl_2 were cooled to –20 °C. After 10 min, a solution of **25** (1.6 g, 3.9 mmol) in CH_2Cl_2 (10.0 mL) was added. After stirring for 15 min, TBHP (1.3 mL, 11.6 mmol) was added and stirred for 8 hr. To the reaction mixture, water and 30 % NaOH in saturated brine were added, organic layer separated, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to give **26** (0.8 g, 66 %).

$[\alpha]_D$ = - 11.2 (c 0.6, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 0.98 (s, 3 H), 3.35 (d, 1 H, *J* = 11.9 Hz), 3.54 (d, 1 H, *J* = 14.9 Hz), 3.66 (d, 1 H, *J* = 11.9 Hz), 3.81 (s, 3 H), 4.36 (d, 1 H, *J* = 4.9 Hz), 4.55 (d, 1 H, *J* = 4.9 Hz), 4.85 (d, 1 H, *J* = 14.9 Hz), 6.87 (d, 2 H, *J* = 8.6 Hz), 7.09 (d, 2 H, *J* = 8.6 Hz), 7.35 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 17.4, 45.2, 55.2, 58.8, 66.1, 73.0, 82.0, 114.0, 127.0, 127.3, 128.6, 129.2, 130.0, 138.8, 157.6, 159.3;

Anal: Calcd for $C_{19}H_{23}NO_3$: C, 72.84; H, 7.35; N, 4.47. Found: C, 72.61; H, 7.64; N, 4.21.

(2*S*, 3*S*, 4*R*)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2,3-epoxy-2-methyl-4-phenylbutan-1-ol (27)

A solution of **25** (4.5 g, 11.3 mmol) and *m*-CPBA (3.34 g, 13.6 mmol, 70 %) in CH_2Cl_2 (50.0 mL) at -10 ^oC was stirred 12 h. 5 % K_2CO_3 solution was added, the organic layer separated and washed with brine, dried (Na_2SO_4), concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **27** (3.13 g, 67 %).

$[\alpha]_D$ = -24.4 (c 0.62, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 0.49 (s, 3 H), 1.06 (s, 9 H), 2.79 (d, 1 H, *J* = 9.3 Hz), 3.26 (d, 1 H, *J* = 9.3 Hz), 3.57 (d, 1 H, *J* = 13.1 Hz), 3.73 (s, 3 H), 4.56 (q, 2 H, *J* = 6.2 Hz), 4.80 (d, 1 H, *J* = 13.1 Hz), 6.77 (d, 2 H, *J* = 8.8 Hz), 6.98 (d, 2 H, *J* = 8.8 Hz), 7.18 (m, 2 H), 7.1-7.3 (m, 3 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 14.1, 28.1, 48.8, 54.9, 58.6, 59.1, 62.0, 65.3, 80.0, 113.6, 126.6-131.0, 138.3, 155.8, 158.6;

Anal: Calcd. for $C_{24}H_{31}NO_5$: C, 69.73; H, 7.50; N, 3.38. Found: C, 70.15; H, 7.12; N, 3.81.

(1′*S*, 2′*R*, 4*R*)-4-[2′-(*p*-Methoxybenzyl)-amino-2′-phenyl-1′-hydroxyethyl]-4-methyl-2-(trichloromethyl)-2-oxazoline (28)

A solution of **26** (1.5 g, 4.8 mmol), CCl_3CN (0.55 mL, 5.3 mmol) and DBU (0.07 mL, 0.5 mmol) in CH_2Cl_2 (15.0 mL) was stirred at 0 °C for 2 h. The solvent was removed and the residue purified on silica gel with ethyl acetate-light petroleum (1:5) as eluent to give the trichloroacetimidate derivative (1.7 g), which was exposed to BF_3:OEt_2 (0.25 mL, 1.9 mmol) in CH_2Cl_2 (15.0 mL) at –25 ^oC for 45 min. The reaction was washed with saturated $NaHCO_3$ solution, brine, dried (Na_2SO_4) and concentrated. The resulting residue was purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **28** (1.37 g, 62 %).

[α] $_D$ = + 7.3 (c 1.4, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.21 (s, 3 H), 3.46 (d, 1 H, *J* = 14.8 Hz), 3.77 (s, 3 H), 3.94 (d, 1 H, *J* = 8.3 Hz), 4.25 (d, 1 H, *J* = 8.3 Hz), 4.47 (d, 1 H, *J* = 5.9 Hz), 4.67 (d, 1 H, *J* = 5.9 Hz), 4.80 (d, 1 H, *J* = 14.8 Hz), 6.83 (d, 2 H, *J* = 8.6 Hz), 7.06 (d, 2 H, *J* = 8.6 Hz), 7.36 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 17.3, 45.2, 55.2, 59.4, 75.8, 82.4, 84.7, 103.0, 114.1, 116.0, 127.1, 127.4, 128.9, 129.2, 130.0, 138.3, 156.8, 159.4;

Anal: Calcd. for $C_{21}H_{23}Cl_3N_2O_3$: C, 55.14; H, 5.03; N, 6.12. Found: C, 55.97; H, 5.82; N, 6.51.

(2*R*, 3*S*, 4*R*)-2-(*tert*-Butoxycarbonylamino)-2-methyl-4-(*p*-methoxybenzylamino)-4-phenyl butan-1,3-diol (29)

A solution of **28** (2.12 g, 4.6 mmol) and 1M HCl (10.0 mL) in THF (25.0 mL) was stirred for 4 h and basified with $NaHCO_3$. $(Boc)_2O$ (3.04 g, 13.9 mmol) was added and after 24 h, the reaction mixture extracted with ethyl acetate, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to afford **29** (1.63 g, 82 %).

[α] $_D$ = -16 (c 0.65, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.06 (s, 3 H), 1.41 (s, 9 H), 2.57 (brs, 1 H), 3.54 (d, 1 H, *J* = 14.4 Hz), 3.79 (s, 3 H), 4.00 (q, 2 H, *J* = 11.3 Hz), 4.30 (d, 1 H, *J* = 4.9 Hz), 4.51 (d, 1 H, *J* = 4.9 Hz), 4.84 (d, 1 H, *J* = 14.4 Hz), 6.84 (d, 2 H, *J* = 8.5 Hz), 7.08 (d, 2 H, *J* = 8.5 Hz), 7.1-7.3 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 18.5, 27.3, 45.0, 54.9, 58.5, 70.0, 71.8, 82.2, 113.8, 126.9, 127.2, 128.5, 128.9, 129.7, 138.4, 152.8, 157.1, 159.1;

Anal: Calcd. for $C_{24}H_{34}N_2O_5$: C, 66.97; H, 7.90; N, 6.51. Found: C, 66.75; H, 7.52; N, 6.85.

(2*R*, 3*S*, 4*R*)-2,4-Bis-(*tert*-butoxycarbonyl amino)-2-methyl-4-phenylbutan-1,3-diol (30)

A solution of **29** (1.63 g, 3.8 mmol) and CAN (6.23 g, 11.4 mmol) in CH_3CN-H_2O (3:1) (40.0 mL) at –5 ^oC was stirred for 10 h, diluted with water, extracted with EtOAc. The organic extract was washed with aq. 5 % $NaHCO_3$, aq. 10 % Na_2SO_3, brine, dried (Na_2SO_4), concentrated to give free amine (1.2 g) which was treated with $(Boc)_2O$ (1.52 g, 7.0 mmol) in the presence of Et_3N (1.0 mL, 7.0 mmol) in THF (15.0 mL). Solvent was removed and the residue diluted with ethyl acetate, washed with brine, dried (Na_2SO_4), concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to give **30** (1.14 g, 73 %).

$[\alpha]_D$ = -17.1 (c 0.4, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 0.72 (s, 3 H), 1.27 (s, 9 H), 1.50 (s, 9 H), 3.86 (q, 2 H, *J* = 11.72 Hz), 4.77 (d, 1 H, *J* = 6.8 Hz), 5.35 (d, 1 H, *J* = 6.8 Hz), 7.35 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 19.3, 27.4, 27.5, 61.6, 71.0, 71.3, 77.7, 82.5, 83.2, 127.9, 128.3, 128.6, 136.1, 148.2, 151.8, 153.2;

Anal: Calcd. for $C_{21}H_{34}N_2O_6$: C, 61.46; H, 8.29; N, 6.82. Found: C, 61.95; H, 7.87; N, 6.41.

(2*R*, 3*S*, 4*R*)-1,3-Di-*O*-acetyl-2-methyl-2,4-bis(*tert*-butoxycarbonylamino)-4-phenyl butane-1,3-diol (31)

A solution of **30** (1.0 g, 2.4 mmol), Ac_2O (0.9 mL, 9.7 mmol), Et_3N (2.0 mL, 14.6 mmol) and DMAP (50.0 mg) in CH_2Cl_2 (10.0 mL) was stirred at room temperature for 8

h. The reaction was diluted with CH_2Cl_2, washed with water, brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to give **31** (0.97 g, 80 %).

[α] $_D$ = -31.7 (c 0.88, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.02 (s, 3 H), 1.26 (s, 9 H), 1.49 (s, 9 H), 1.96 (s, 3 H), 2.15 (s, 3 H), 4.23 (d, 1 H, *J* = 11.8 Hz), 4.47 (d, 1 H, *J* = 11.8 Hz), 5.07 (d, 1 H, *J* = 5.6 Hz), 5.38 (d, 1 H, *J* = 5.6 Hz), 7.39 (m, 5 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 11.1, 19.2, 28.3, 31.3, 47.9, 59.7, 70.7, 72.9, 82.0, 84.6, 126.7, 129.1, 129.7, 141.0, 152.6, 153.8, 168.8, 171.0;

Anal: Calcd. for $C_{25}H_{38}N_2O_8$: C, 60.72; H, 7.69; N, 5.66. Found: C, 60.56; H, 7.52; N, 5.41.

Methyl (2*R*, 3*S*, 4*S*)-2,4-Bis-(*tert*-butoxycarbonylamino)-3,5-bis(acetoxy)-4-methyl-penta-noate (32)

A mixture of **31** (0.95 g, 1.9 mmol), $NaIO_4$ (4.13 g, 19.2 mmol) and $RuCl_3.H_2O$ (8.0 mg, 0.04 mmol) in CH_3CN (10.0 mL), CCl_4 (ethanol free) (10.0 mL) and H_2O (15.0 mL) was stirred at room temperature for 12 h, diluted with ether and the organic phase separated. The aqueous phase was extracted with ether, washed with brine, dried (Na_2SO_4) and concentrated. The residue was treated with CH_2N_2 (30.0 mL in ether) at 0 ^oC for 30 min, concentrated and purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to give **32** (0.48 g, 52 %).

[α] $_D$ = -27.7 (c 0.58, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.35, (s, 9 H), 1.49 (s, 9 H), 1.66 (s, 3 H), 2.11 (s, 3 H), 2.17 (s, 3 H), 3.84 (s, 3 H), 4.21 (d, 1 H, *J* = 12.5 Hz), 4.47 (d, 1 H, *J* = 12.5 Hz), 5.11 (d, 1 H, *J* = 6.1 Hz), 5.38 (d, 1 H, *J* = 6.1 Hz);

^{13}C NMR δ (50 MHz, $CDCl_3$): 12.2, 20.6, 28.1, 32.2, 47.3, 51.9, 56.1, 66.9, 72.0, 82.1, 85.5, 155.5, 158.6, 166.4, 168.0, 170.6;

Anal: Calcd. for $C_{21}H_{36}N_2O_{10}$: C, 52.94; H, 7.56; N, 5.88. Found: C, 52.66; H, 7.42; N, 5.61.

***N*-[(3*R*, 4*S*, 5*S*)-5-(*tert*-Butoxycarbonylamino)-4-hydroxy-3-methyl-6-oxo-(2H-3,4,5-dihydropyran-3-yl)](*tert*-butoxy)carboxamide (33)**

A solution of **32** (0.46 g, 1.0 mmol) and K_2CO_3 (0.53 g, 3.9 mmol) in methanol (10.0 mL) was stirred for 10 h. The residue was de-ionized by the addition of Amberlite IR 120 (H^+) resin (pH 6), filtered, concentrated and purified on silica gel with light petroleum-EtOAc (1:3) as eluent to give **33** (0.25 g, 71 %).

[α] $_D$ = -39.2 (c 0.55, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.37 (s, 3 H), 1.43 (s, 9 H), 1.62 (s, 9 H), 2.67 (brs, 1 H), 4.08 (q, 2 H, *J* = 8.8 Hz), 4.51(d, 1 H, *J* = 4.7 Hz), 5.08 (d, 1 H, *J* = 4.7 Hz);

^{13}C NMR δ (50 MHz, $CDCl_3$): 13.7, 27.8, 31.7, 47.8, 54.5, 60.0, 69.9, 79.7, 83.5, 155.2, 158.3, 170.5;

Anal: Calcd. for $C_{16}H_{28}N_2O_7$: C, 53.33; H, 7.77; N, 7.78. Found: C, 53.66; H, 7.42; N, 7.61.

Methyl (4*S*, 5*S*, 6*R*)-5-Hydroxy-6-(hydroxymethyl)-6-methyl-1,4,5,6-tetrahydro-pyrimidine-4-carboxylate (34)

A solution of **33** (0.25 g, 0.7 mmol) and TFA (5.0 mL) in CH_2Cl_2 (10.0 mL) was stirred for 2 h and then $CH(OMe)_3$ (30.0 mL) and conc. HCl (0.5 mL) were added. The reaction was heated under reflux for 4 h, concentrated and the residue diluted with ethyl acetate, extracted with 2 N HCl. The residue obtained after concentration of aqueous layer was stirred with CH_2N_2 (20.0 mL in ether) at 0 ^oC for 30 min, concentrated and purified on silica gel using ethyl acetate-light petroleum (3:1) as eluent to afford **34** (89.0 mg, 64 %).

[α] $_D$ = -49.8 (c 0.82, $CHCl_3$);

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.37 (s, 3 H), 3.80 (s, 3 H), 4.22 (d, 1 H, *J* = 10.6 Hz), 4.36 (d, 1 H, *J* = 10.6 Hz), 4.64 (d, 1 H, *J* = 2.4 Hz), 4.83 (d, 1 H, *J* = 2.4 Hz), 7.97 (s, 1 H);

^{13}C NMR δ (50 MHz, $CDCl_3$): 19.8, 46.3, 54.9, 60.7, 65.0, 69.5, 158.1, 170.7;

Anal: Calcd. for $C_8H_{14}N_2O_4$: C, 47.52; H, 6.93; N, 13.86. Found: C, 47.84; H, 7.32; N, 13.57.

9-*epi*-manzacidin B methyl ester (36)

A solution of **34** (0.08 g, 0.4 mmol) and NaH (52.0 mg, 1.4 mmol, 60 % w/w dispersion in oil) in DMF (2.0 mL) was stirred for 15 min. Compound **35** (0.13 g, 0.4 mmol) was added, stirred for 4 h and quenched with 2 N HCl. The reaction mixture was concentrated and purified on silica gel using ethyl acetate-methanol (5:1) as eluent to give manzacidin B methyl ester (**36**) (67.0 mg, 45 %).

[α] $_{D}$ = -66.2 (c 0.55, MeOH);

^{1}H NMR δ (200 MHz, Acetone-d$_6$): 1.45 (s, 3 H), 3.91 (s, 3 H), 4.41 (q, 1 H, *J* = 10.6 Hz), 4.68 (d, 1 H, *J* = 2.2 Hz), 4.89 (d, 1 H, *J* = 2.2 Hz), 6.94 (d, 1 H, *J* = 1.6 Hz), 7.13 (d, 1 H, *J* = 1.6 Hz), 8.13 (s, 1 H);

^{13}C NMR δ (50 MHz, Acetone-d$_6$): 23.4, 48.8, 55.5, 58.2, 65.3, 66.8, 98.2, 118.5, 123.5, 125.3, 151.6, 160.8, 170.2;

Anal: Calcd. for $C_{13}H_{16}BrN_3O_5$: C, 41.71; H, 4.27; N, 11.23. Found: C, 41.94; H, 4.62; N, 11.54.

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INTRODUCTION

We all have millions of bacteria in our gastrointestinal tracts, primarily in the colon (or "large" bowel). These bacteria are important for normal bowel health and function. *Klebsiella* is the genus name for one of these bacteria. When *Klebsiella* bacteria get outside of the gut, however, serious infection can occur. As a general rule, *Klebsiella* infections tend to occur in people with a weakened immune system. *Klebsiella pneumoniae* is among the most common gram-negative bacteria encountered by physicians worldwide. It is a common hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumonia, and intra abdominal infections.[1, 2] *K. pneumoniae* is also a potential community-acquired pathogen. *Klebsiella'*s pathogenicity can be attributed to its production of a heat-stable enterotoxin.[3] In fact; *K. pneumoniae* is second only to E. coli as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now than in the past. This is probably due to the bacterium's antibiotic resistance properties.[4] *Klebsiella* bacteria are generally resistant to many antibiotics, such as penicillin. Often, two or more powerful antibiotics are used to help eliminate a Klebsiella infection. *Klebsiella* species may contain resistant plasmids (R-plasmids), which confer resistance to antibiotics such as ampicillin and carbenicillin. To make matters worse, the R-plasmids can be transferred to other enteric bacteria not necessarily of the same species.

First, *K. pneumoniae* has been a recognized pulmonary pathogen since its discovery >100 years ago. The classic clinical presentation is dramatic: toxic presentation with sudden onset, high fever, and hemoptysis (currant jelly sputum). Chest radiographic abnormalities such as bulging interlobar fissure and cavitary abscesses are prominent.[5] At times; surgery may be needed to "rescue" a lung that is trapped in irregular pockets of pus and scar tissue. *Klebsiella* can also cause less serious respiratory infections, such as bronchitis, which is usually a hospital-acquired infection.[6] *K. pneumonia* tends to affect people with underlying diseases, such as alcoholism, diabetes and chronic lung disease.[7] Classically, *Klebsiella* causes a severe, rapid-onset illness that often causes areas of destruction in the lung.

Studies from the 1920s to 1960s, *K. pneumoniae* was considered as an important causative organism for community-acquired pneumonia,[5a] however, it was noticed that in the last decade *K. pneumoniae* accounted for <1% of cases of pneumonia requiring hospitalization in North America.[8-10] Second, a striking clinical finding concerning a new manifestation of community-acquired *K. pneumoniae* infections has been documented. An unusual invasive presentation of *K. pneumoniae* infection, primary bacteremic liver abscess, has been described by numerous investigators in Asia, >900 patients with Klebsiella liver abscess have been reported from Taiwan in the last 10 years.[11] In addition, case reports and small series from Korea, Singapore, Japan, India, and Thailand have been published.[12] The Taiwanese patients with *K. pneumoniae* liver abscess have no history of hepatobiliary disease. Seventy percent of such patients have diabetes mellitus,[7] 11% to 12% of the reported patients with Klebsiella liver abscess have other septic metastatic lesions, including pulmonary emboli or abscess, brain abscess, pyogenic meningitis, endophthalmitis, prostatic abscess, osteomyelitis, septic arthritis, or psoas abscess.[7] The third striking clinical observation is the preponderance of *K. pneumoniae* as a cause of community-acquired bacterial meningitis in adults in Taiwan, even in the absence of liver abscess or other sites of infection. The proportion of cases of culture-proven bacterial meningitis due to *K. pneumoniae* in one Taiwanese hospital increased from 8% during 1981 to 1986 and 18% during 1987 to 1995.[13] In contrast, in a recent large review only 3 (1.2%) of 253 cases of community-acquired bacterial meningitis from the Massachusetts General Hospital were due to *K. pneumoniae*.[6]

Diagnosis

The clinical picture depends on the site of infection; diagnosis relies on culturing the organism and on biochemical and/or serologic identification. A variety of phenotypic (i.e., biotyping, serotyping, antibiograms, bacteriocin and phage typing) and genotypic (i.e., plasmid analysis, RFLP, ribotyping, and PCR) methods are used for epidemiological investigations.

Control

The most effective way to reduce transmission of nosocomial organisms is for all hospital personnel to wash hands meticulously after attending to each patient. Vaccines and hyperimmune sera are not currently available. Various antibiotics are the backbone of treatment; drug resistance (often multiple) due to conjugative plasmids is a major problem.

Given these empiric observations, an international collaboration of researchers was established from each of the world's populated continents. These investigators worked in large tertiary-care hospitals or hospitals serving veterans. The aim was to delineate in a single time period, with a consistent set of definitions, global differences in the clinical manifestations of serious *K. pneumoniae* infections; also the influence of prior antibiotic use on these differences in *K. pneumoniae* infections was examined.

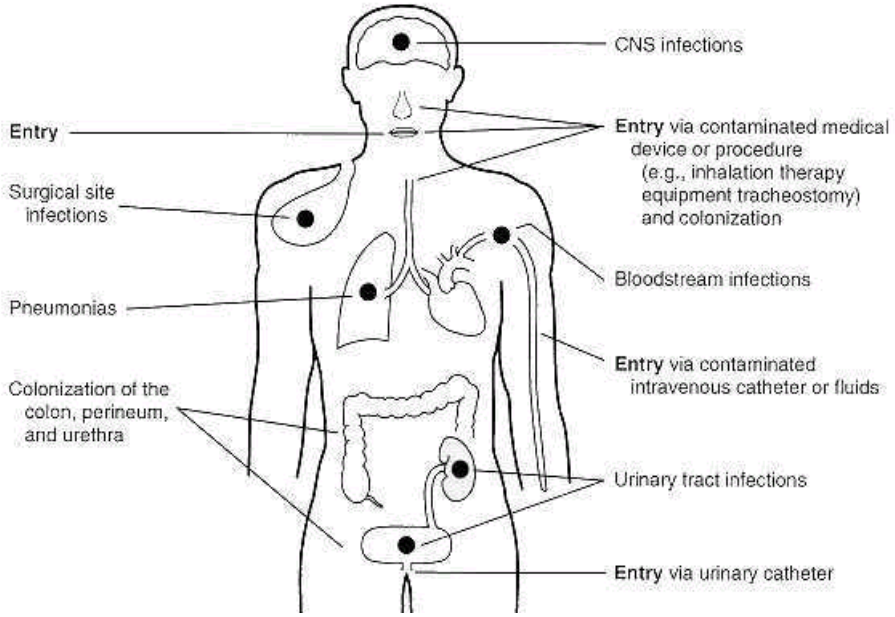


Figure 1: Sites of colonization and extraintestinal disease production by the K. pneumoniae

Role of *K. pneumoniae* in nitrogen fixation

Klebsiella pneumoniae is a member of the Enterobacteriaceae but unlike related organisms such as *Escherichia coli* it has the ability to fix nitrogen i.e., to convert atmospheric nitrogen gas to ammonium.[14] This process, which is only found in bacteria, is of major global importance in the provision of fixed nitrogen for plant growth.

Klebsiella is naturally found as a free-living soil bacterium and unlike other nitrogen fixers such as *Rhizobium* it does not participate in symbiotic interactions with leguminous plants. This ability to fix nitrogen in the free-living state has made it the organism of choice for studies of both the biochemistry and the genetics that underlie the nitrogen fixation process.

Nitrogen fixing (diazotrophic) organisms synthesise an enzyme called *nitrogenase* which is a complex oxygen-sensitive molybdoprotein containing a number of novel metal centres that are required for the binding of molecular nitrogen and its subsequent reduction to ammonia.[15] Nitrogen fixation is in energy intensive process, requiring some 16 molecules of ATP for the reduction of one molecule of nitrogen to ammonia. Given this requirement and the oxygen-sensitivity of the process *Klebsiella pneumoniae*, which is a facultative anaerobe, only fixes nitrogen when it is under anaerobic or micro aerobic conditions and is nutritionally starved of other sources of fixed nitrogen.

Lipopolysaccharides (LPS)

Lipopolysaccharides (LPSs; endotoxins) are biologically active components of gram-negative bacteria and are important virulent factors of *K. pneumoniae*.[17] The virulence of *Klebsiella* is not well understood, but it is thought that capsular polysaccharides and lipopolysaccharides (LPS) contribute to its virulence. The O-polysaccharide and core oligosaccharide moieties of LPS are immunogenic, giving rise to antibodies having specific serological properties that may be protective and that can also be of diagnostic importance. The structures of the former and O-antigens from LPS have been investigated extensively. In strains of *K. pneumoniae* serotypes O1:K1 and O1:K2, a toxic complex is released from the cell surface during growth.[18, 19] The complex consists of capsular polysaccharide, LPS, and proteins and is responsible for characteristic lung tissue damage caused by *K. pneumoniae* infections. It is known that LPS is the critical component of the complex, although the toxicity of purified LPS is lower than that of the complex.[19] These toxic effects abrogated antibodies against LPS.[18] However, LPS within the extracellular complex is also implicated in generating a transient reduction in the response of the reticuloendothelial system, allowing proliferation of the bacterium in the early stages of infection.[20] Consideration of conserved core structures has stimulated

interest in their potential use in creation of a vaccine, which might be cross protective for pathogenic *Enterobacteriaceae* and perhaps other bacteria. This is not possible with O-specific polysaccharide-based vaccines because of the enormous structural, variation in this portion of the LPS molecule. Passive immunization with antibodies directed against this conserved region of LPS might provide an alternative approach to chemotherapy for infections such as those resulting from *K. pneumoniae*. Such applications require a detailed knowledge of the molecular structure of the targeted LPS molecules and the diversity of structures in related bacteria. Early chemical analyses on the LPSs of *Klebsiella* spp. showed similar composition of cores from different serotypes.[21] More recently, a crossreactive monoclonal antibody (MAb) (V-9.5) was used to demonstrate a conserved epitope in the lipid A-core fraction of LPS from various Klebsiella O serotypes.[22]

A structural investigation of LPS core region began only recently. In a preliminary investigation[23] of LPS from the rough mutant *K. pneumoniae* ssp. *pneumoniae* R20 ($O1^-$: $K20^-$),[24] a major fraction of the carbohydrate backbone was isolated and its structure was characterized. It possessed a terminal *threo*-hex-4-enuronic acid residue, which resulted from β-elimination under the alkaline conditions used, indicating a further substituent at position O-4 of the second Gal*p*A is D-Glc*p*N. In another investigation,[25] the structure of the core region of LPS from serotype O8 was found to be similar to that of LPS from serotype O1. In this case the eliminated substituent was α-D-Glc*p* linked at O-4 of the second GalA residue. Both core region lack phosphate residues, which is so far unique in enterobacterial LPS. LPS is substituted at O-6 by three or four residue of D-*glycero*-D-*manno*-heptopyranose (D,D-Hep*p*).

Several attempts have been made to develop anti-LPS anti bodies as therapeutic agents in septic patients. All three regions of LPS, i.e. the O-specific polysaccharide, the core region, and the lipid A, can act as immunogen; however, the O-antigen expresses high structural variability, even within one species, and lipid A antigenicity is cryptic in LPS and exposed as a neoantigen only after removal of the lipid A-distal saccharide moiety.[26] The core region has been identified as a suitable target for the induction of antibodies with broad cross-reactivity among all *E. coli* strains.

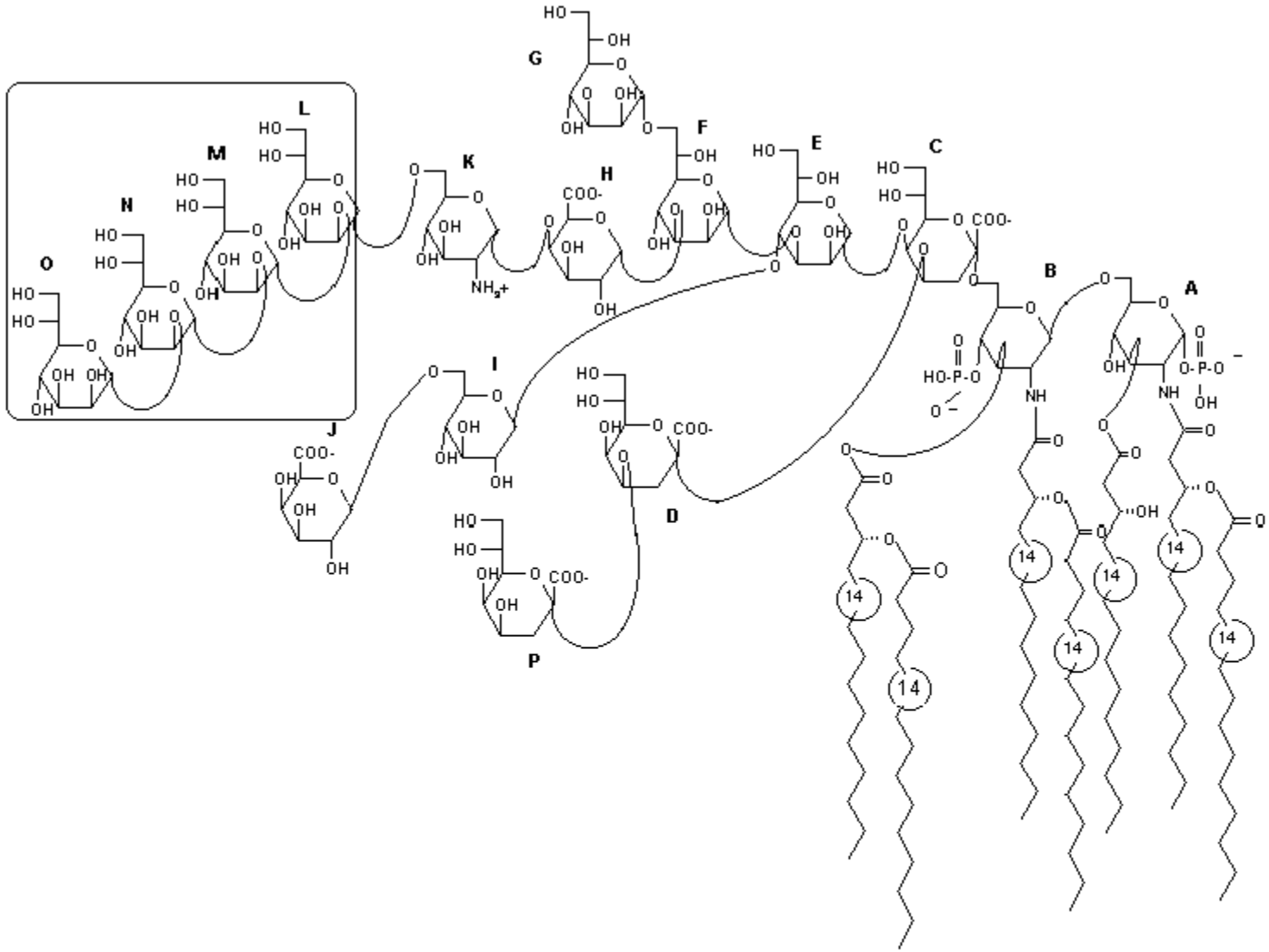


Figure 2. The structure of LPS from *K. pneumoniae* ssp. *pneumoniae* strain R20.

Introduction to *O*-glycosylation methods

In nature carbohydrates are present as *C*-glycosides, *O*-glycosides, and *N*-glycosides. Of all the three *O*-glycosides is the most important class of compounds. *O*-glycosides are formed from the condensation of the anomeric hydroxyl group of sugar with the hydroxyl group of another molecule. The latter can be a simple alcohol, a hydroxylated amino acid, another sugar or a more complex molecule.

The importance of cell surface carbohydrates in biological processes ranging from antibody-antigen interaction to cell-cell recognition and development has led to a great deal of activity at the carbohydrate frontier. An integral part of this has involved the development of new chemical methods of oligosaccharide synthesis requiring fewer manipulations and/or resulting in higher yields, increased stereoselectivity and selective activation. Although many advances have been made in the synthesis of oligosaccharides, each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic research and a good deal of know how. There are no universal reaction conditions for oligosaccharide synthesis. In an oligosaccharide synthesis, two poly functional sugar units must be coupled. Regioselectivity in such coupling reactions is generally achieved when the glycosylating agent (glycosyl donor) possesses selectively protected hydroxyl groups and an activating group at the anomeric carbon atom and when the sugar component with free hydroxyl group (glycosyl acceptor) possesses protecting groups at all other hydroxyl functions. Thus, complicated protecting strategies and suitable procedures for activation at the anomeric carbon atom are required.

Stereoselective formation of *O*-glycosidic bond is one of the most important problems in carbohydrate chemistry.[27] Recently, much effort has been devoted to the stereoselective synthesis of glycopyranosides and several efficient methods have been developed by the appropriate combination of sugar donors and activators (figure 3).[28]

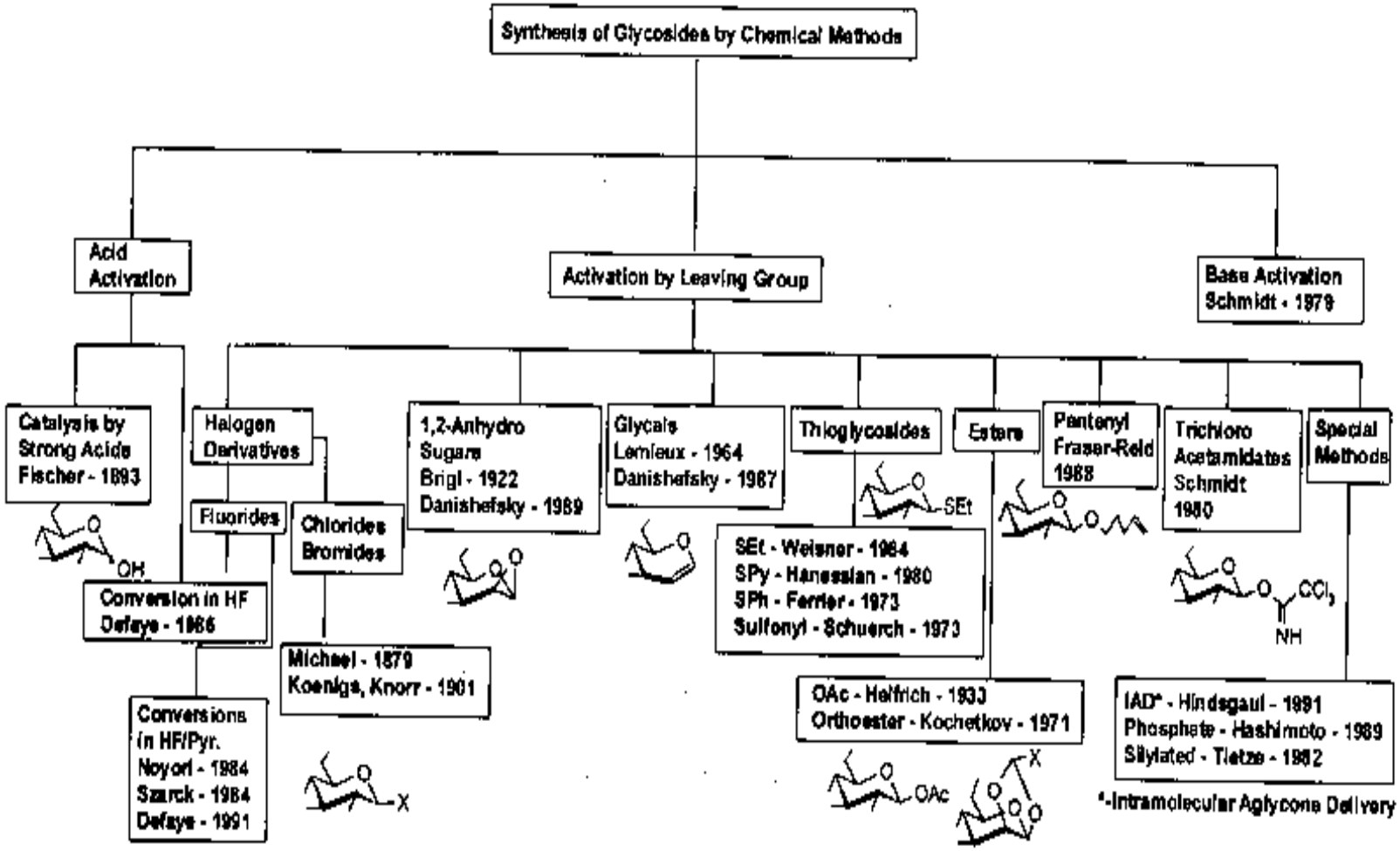


Figure 3: Development of Pyranoside Glycosylation Methods

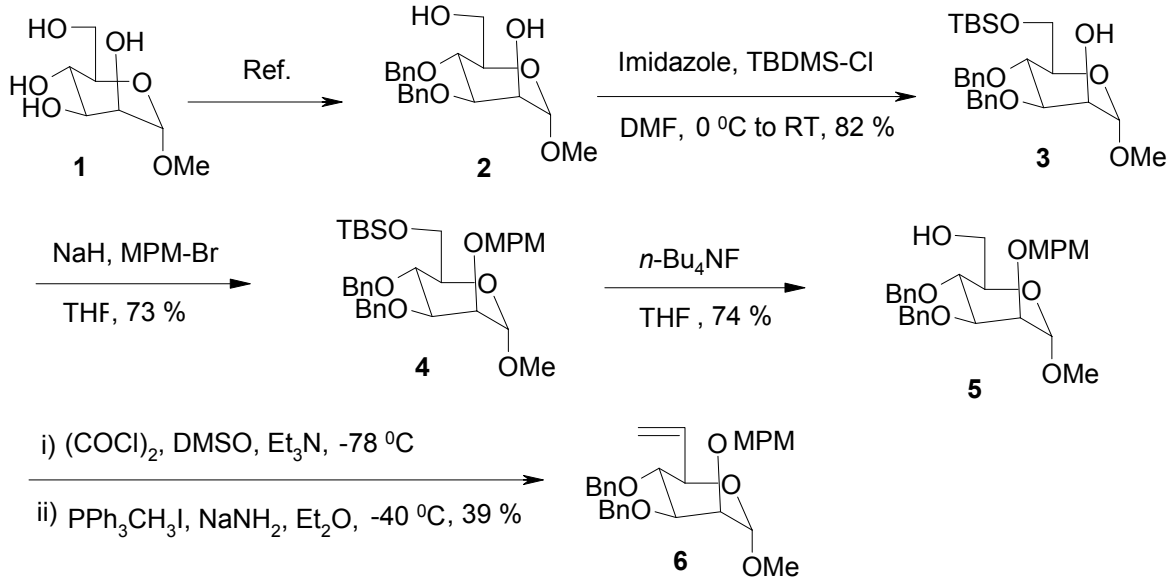
Klebsiella pneumoniae is an important Gram-negative pathogenic bacterium associated with nosocomial infections.[1,2] *Klebsiella's* pathogenicity can be attributed to its production of a heat-stable enterotoxin. Although *Klebsiella pneumoniae* can cause a severe pneumonia, it is most commonly the cause of hospital-acquired urinary tract infections or burn wound infections. In fact, *K. pneumoniae* is second only to *E. coli* as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now probably due to the bacterium's resistance towards antibiotics. The virulence of *Klebsiella* is not well understood, but it is thought that capsular polysaccharides and lipopolysaccharides (LPS) contribute to its virulence.[17] A structural investigation of the LPS core region from *K. pneumoniae* ssp. *pneumoniae* strain R20[24] was shown to have substitution at O-6 of the D-GlcpN by three or four residue of D-glycero-D-manno-heptopyranose (D,D-Hepp), where all hexoses possess the D-configuration. The structure (Figure **2**) is unique with regard to the presence of a novel heptoglycan of α 1 → 2 linkage and does not contain phosphate substituent in the core region.

Approach A

Herein, we report the synthesis of a terminal disaccaride unit of the heptoglycan of α 1 → 2 linkage present in *K. pneumoniae* ssp. *pneumoniae* strain R20, starting from methyl α-D-mannopyranoside (**1**). Compound **1** (Scheme 1) was converted into the dibenzyl derivative **2** by following the reported procedure.[29] The primary hydroxy group of **2** was first protected as its TBDMS ether on treatment with TBDMS-Cl and imidazole in DMF and subsequent exposure to NaH and MPM-Br in THF afforded MPM protected derivative **4**. In the ^{1}H NMR spectrum of **4** the characteristic resonances due to TBDMS group and MPM group were distinctly visible. Removal of TBDMS group by *n*-Bu_4NF in THF afforded compound **5**, whose structure was confirmed by the absence of signals due to TBDMS group in the up field region of its ^{1}H NMR spectrum. Compound **5** was subjected to Swern oxidation reaction[30] to yield the corresponding aldehyde, which on treatment with PPh_3CH_3I and sodamide in anhydrous ether gave **6**.[31] In the ^{1}H NMR

spectrum the characteristic resonances due to terminal olefin were observed at 5.34 and 5.96 ppm.

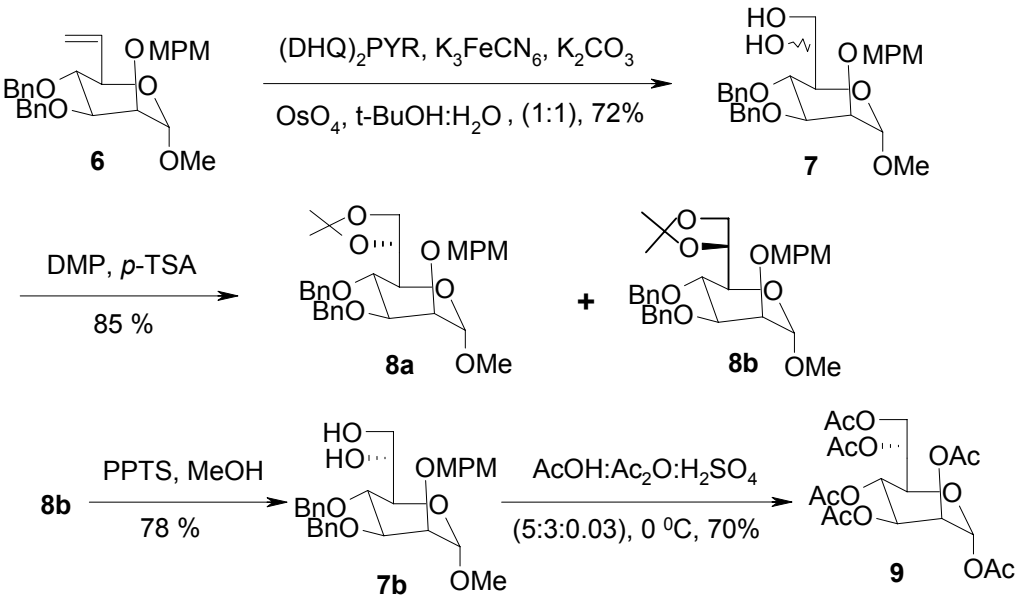
Scheme: 1



Sharpless asymmetric dihydroxylation[32] of **6** (Scheme: **2**) with $(DHQ)_2PYR$ ligand, $K_3Fe(CN)_6$, K_2CO_3 and OsO_4 in *tert*-BuOH:H_2O (1:1 v/v) gave inseparable (9:1) mixture of diastereomers **7**. However, the corresponding acetonide derivatives (**8a** and **8b**) prepared by treating **7** with DMP and PPTS were easily separated by chromatography. The stereochemistry at C-6 of **8b** was confirmed by converting it into the known compound **9** {$[\alpha]_D$ + 28.1 (c 0.85, $CHCl_3$), lit.[33] $[\alpha]_D$ + 27 (c 1, $CHCl_3$)} by hydrogenolysis using Pd/C in MeOH and acetolysis.[34] The confirmation of structure of **8b**, indirectly suggested the structure of **8a** as shown.

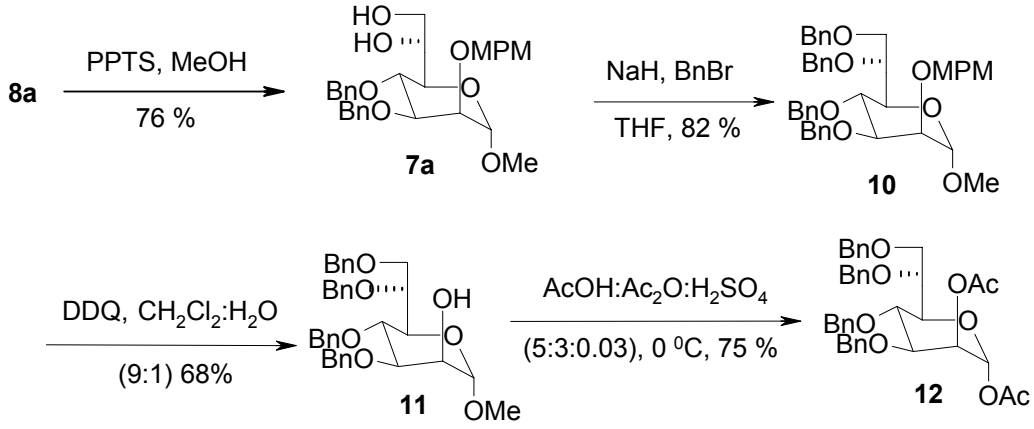
The diol (**7a**) on treatment with BnBr and NaH in THF provided the benzylated product (**10**). The ^1H NMR, ^{13}C NMR spectra and elemental analysis were in conformity with the proposed structure. The MPM group at C-2 was then deprotected with DDQ[35] to give **11**, which was destined as the glycosyl acceptor. In the ^1H NMR spectrum of 11, the absence of a singlet at 3.77 ppm (OMe) and A_2B_2 pattern in the aromatic region suggested that the reaction had occurred. In addition ^{13}C NMR spectrum and elemental analysis also confirm the structure of **11**.

Scheme: 2



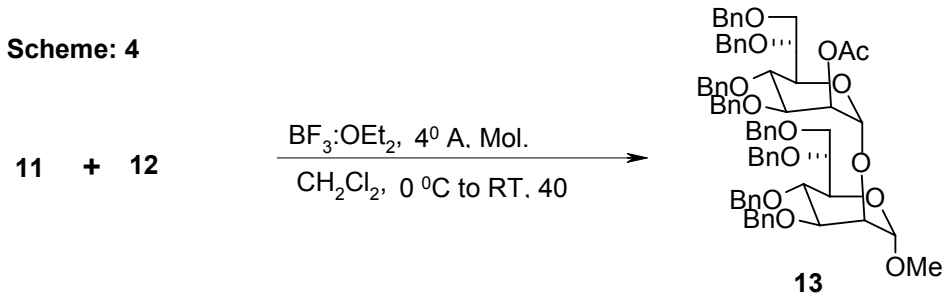
Compound **11** was also converted into glycosyl donor (**12**) under acetolysis condition.[34] Thus 11 was treated with acetic acid: acetic anhydride: sulphuric acid (25: 5: 1) to give **12** in 75 % yield. The ^{1}H NMR spectrum of **12** showed two characteristic singlets at 2.05 and 2.11 ppm and a distinct down field chemical shift for H-1 and H-2 protons observed at 6.05 ppm.

Scheme: 3



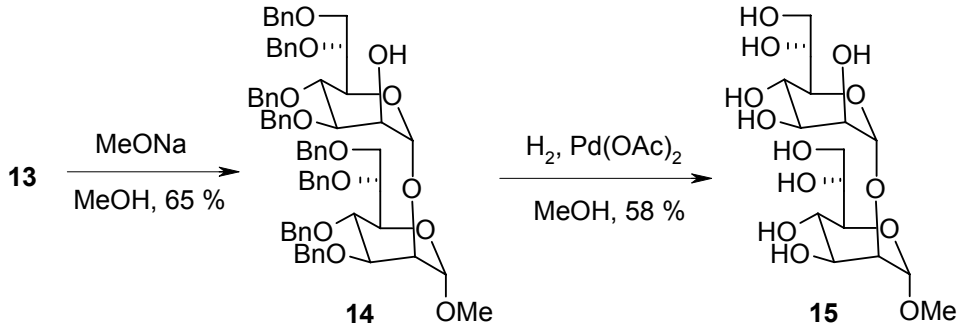
Glycosidation reaction between **11** and **12** was mediated [36] (Scheme: **4**) through catalytic BF_3:OEt_2, 4ºA molecular sieves in dry CH_2Cl_2 to give the disaccharide **13**. The ^{1}H NMR spectrum of **13** showed resonances due to acetate protons and methoxy group attached to anomeric carbon were observed as two singlets at 2.09 and 3.29 ppm. A broad doublet at 5.22 ppm was assigned to H-2 proton. In the ^{13}C NMR spectrum, two anomeric carbon signals were visible at 99.5 and 99.8 ppm. The chemical shifts of anomeric carbon being less than 100 ppm clearly indicated α-configuration at both the anomeric centers.

Scheme: 4



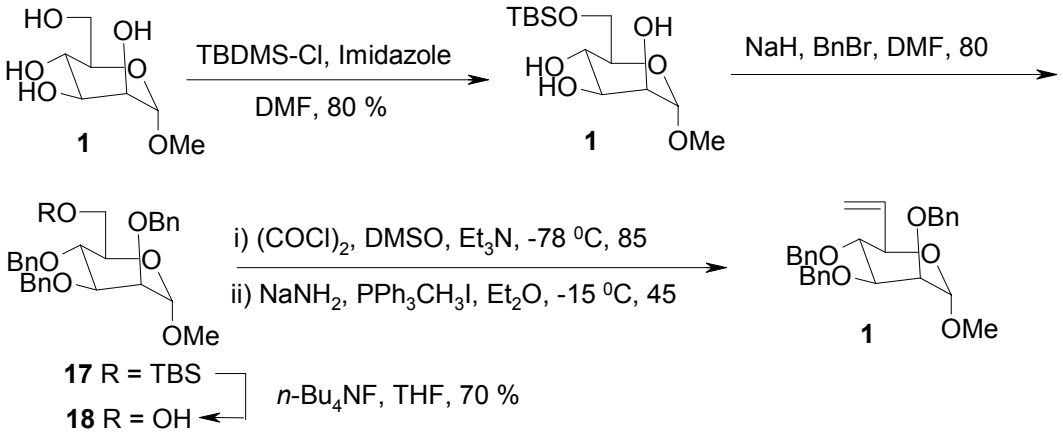
Compound **13** on deacetylation (Scheme **5**) under Zemplen[37] condition gave **14**. Hydrogenolysis[38] of **14** using 10 % $Pd(OH)_2$ in MeOH gave the required terminal disaccharide (**15**). In the ^{1}H NMR spectrum of **15**, signals due to two anomeric protons were observed at 4.77 and 4.9 ppm as doublet with J = 1.7 Hz. The characteristic coupling constants observed, revealed α-configuration at C-1 as well as C-1'.

Scheme: 5



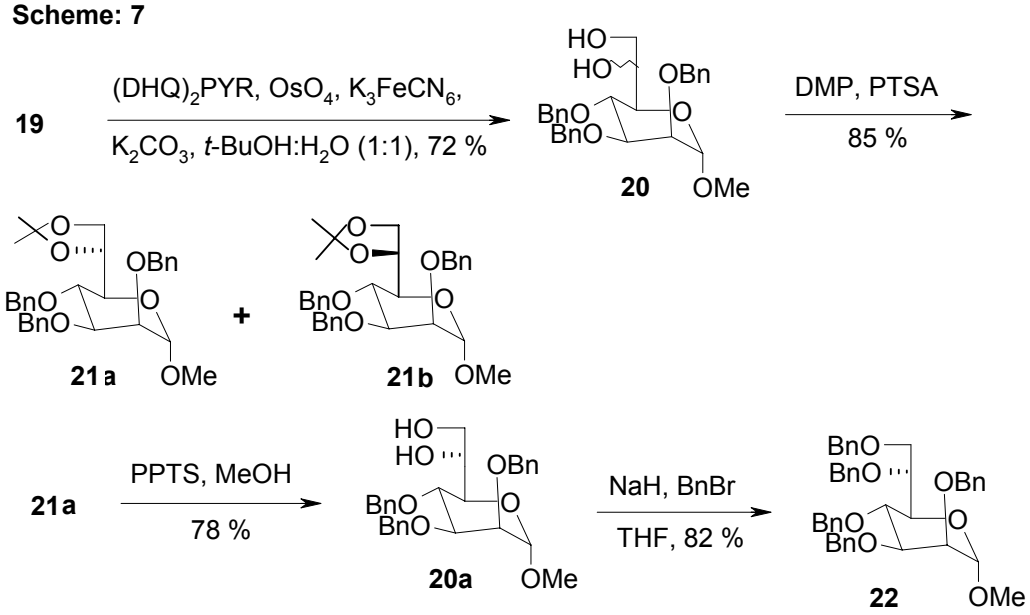
Approach B

In an alternative approach towards the terminal disaccharide (**15**), methyl α-D-mannopyranoside (**1**) was converted into 6-O-silyl derivative **16** using TBS-Cl and imidazole in DMF. The three secondary hydroxyl groups were protected as benzyl ether (**17**) with NaH and BnBr in DMF. Deprotection of TBS group with *n*-Bu_4NF in THF afforded **18**, whose structure was confirmed by spectroscopic data and comparision of optical rotation with the reported value.[39] Swern oxidation[30] of **18** yielded the corresponding aldehyde, which was immediately treated with $PPh_3{=}CH_2$ (prepared from PPh_3CH_3I and sodamide[31] in anhydrous ether) to afford **19**.[40a] In the ^1H NMR spectrum the characteristic resonances due to terminal olefin were observed (Scheme **6**).



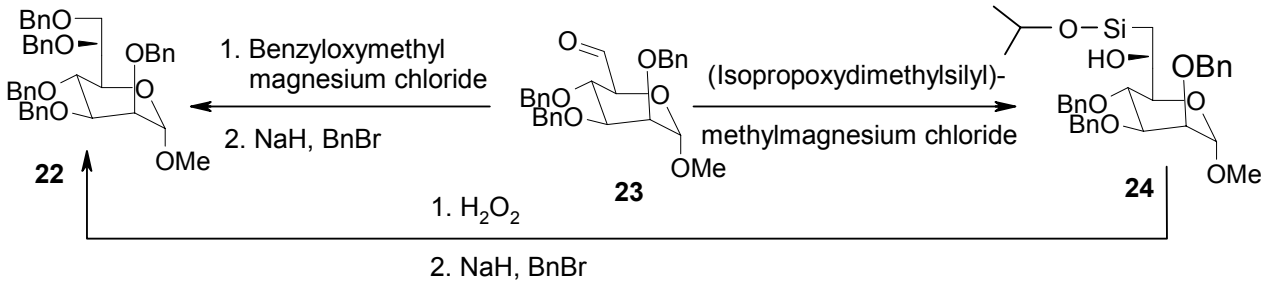
Transformation of **19** into **22** was performed as described earlier in Scheme **1** and **2**. All the intermediates described in Scheme **7** were fully charecterised by spectroscopic studies (see experimental).

Scheme: 7



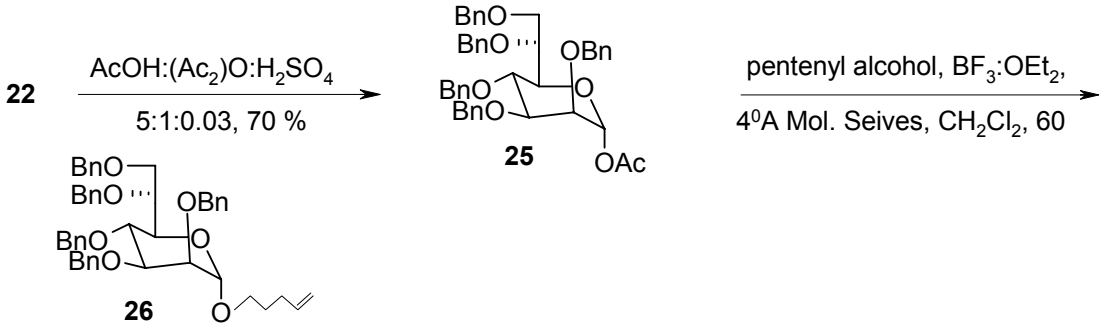
The stereochemical structural identity of **20b** was confirmed by comparing its optical rotation $[\alpha]_D$ + 22.2 (c 0.98, $CHCl_3$) with that of the literature value[32] $[\alpha]_D$ + 23 (c 1, $CHCl_3$). The literature procedure to introduce a new center at C-6 involved the reaction of α-D-manno-hexodialdo-1,5-pyranoside and the Grignard complex of isopropoxydimethylsilyl- methyl chloride, followed by oxidative cleavage of the carbon-silicon bond (Scheme **8**). [40]

Scheme **8**



Acetolysis[34] (Scheme: **9**) of **22** afforded compound **25**, whose ^{1}H NMR spectrum showed presence of a characteristic singlet at 2.0 ppm due to acetyl group. A distinct down field shift of 1.5 ppm was observed for H-1 proton.

Scheme: 9



Treatment of **25** with pentenyl alcohol[37] in presence of BF_3:OEt_2 and 4 Å molecular sieves in dry CH_2Cl_2 gave the pentenyl glycoside **26**. The characteristic resonances due to pentenyl olefinic protons were located at 5.66 ppm and methylene protons at 1.56 and 2.0 ppm of the ^{1}H NMR spectrum proved the structure of **26**. The ^{13}C NMR spectrum was consistent with the assigned structure.

A short account on Fraser-Reid's Glycosylation Method

The serendipitous observations that led to the discovery of n-pentenyl glycosides (NPGs) by Fraser-Reid have provided an avenue in the art of oligosaccharide synthesis. NPGs are readily prepared by standard glycoside forming procedures, including Fischer's direct method, and although they are stable to a wide range of reagents, they are readily activated by treatment with a halonium ion. The effect of some of the commonly used protecting groups upon glycoside reactivity has been probed with these substrate, and the "armed/disarmed" stategy for oligosaccharide assembly emanated directly from these investigations. Thus, esters disarm electronically, while benzylidene and isopropylidene groups disarm by torsional strain.

Most commonly used activators for activating NPGs are NBS, IDCP (iodonium dicollidine perchlorate). However, IDPC is not commercially available, a circumstance which compromised its attractiveness. Later on an alternative promoter were therefore sought that would, among other things not required laboratory preparation.

A non-nucleophilic counter anion was essential, and trifluoromethanesulfonate (triflate) was preferred and it was found that N-halosuccinimide (NIS) reacted with trifluoromethanesulfonic acid (TfOH) to generate a ready source of iodonium ion[41]

solved the problem as both NIS and TfOH are commercially available. A general drawback of this protocol is the use of strong acid and thus a judicious planning and care should be taken while planning and executing the glycosylation reaction.

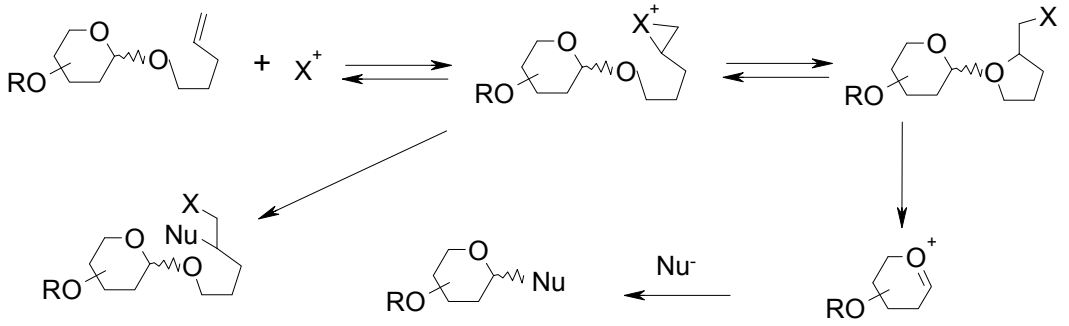
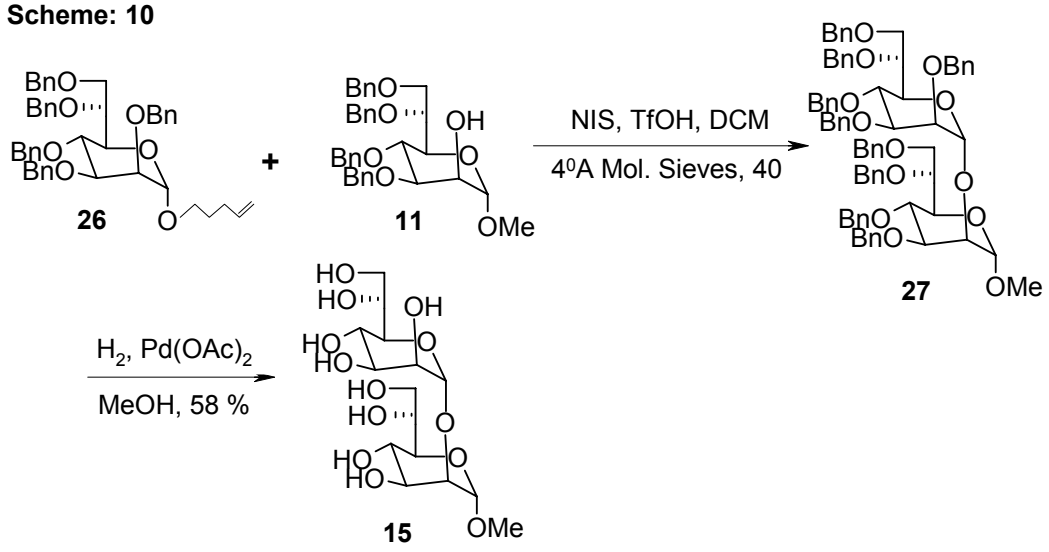


Figure : Fraser Reid's Glycosidation Method

The coupling reaction between **11** and **26** (Scheme: **10**) was conducted in presence of NIS, TfOH [42, 43] and 4ºA molecular sieves to give the disaccharide **27**. In the ^{13}C NMR spectrum, two β-anomeric carbon signals were visible at 99.9 and 100.3 ppm. Finally deprotection of benzyl group of **27** using $Pd(OH)_2$ in MeOH gave the terminal disaccharide unit **15**.

Scheme: 10



The structure of **15** was proved by comparison of analytical and spectroscopic data with the sample prepared earlier (Scheme **5**).

Methyl 3,4-di-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-D-mannopyranoside (3)

A solution of **2** (15.0 g, 40.0 mmol), imidazole (8.2 g, 120.0 mmol) and TBS-Cl (6.0 g, 40.0 mmol) in CH_2Cl_2 was stirred for 1 h. The reaction was concentrated and the residue purified on silica gel using EtOAc and light petroleum ether (1:9) to afford **3** (16.0 g, 82 %).

$[\alpha]_D$ = + 33.9 (c 0.49, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 0.0 (s, 6 H), 0.80 (s, 9 H), 2.27 (br s, 1 H), 3.33 (s, 3 H), 3.7 (m, 6 H), 4.66 (m, 5 H), 7.29 (m, 10 H);

Anal. Calcd for $C_{27}H_{40}O_6Si$: C, 66.39; H, 8.19. Found: C, 66.67; H, 8.26.

Methyl 3,4-di-*O*-benzyl-6-*O*-(*tert*-butyldimethyl-silyl)-2-*O*-(*p*-methoxy-benzyl)-α-D-mannopyranoside (4)

A solution of **3** (11.0 g, 22.0 mmol) and NaH (1.8 g, 45.0 mmol, 60 % dispersion in oil) in THF was stirred for 30 min and subsequently MPM-Br (5.0 g, 24.0 mmol) was added. After 4 h, the reaction was quenched with ice and concentrated. The residue was extracted with EtOAc, washed with water, brine, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (0.5:9.5) to give **4** (10.0 g, 73 %).

$[\alpha]_D$ = + 30.2 (c 0.9, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 0.0 (s, 6 H), 0.82 (s, 9 H), 3.2 (s, 3 H), 3.4 (m, 1 H), 3.96 (s, 3 H), 3.74 (m, 5 H), 4.5 (m, 6 H), 4.82 (d, 1 H, J = 10.8 Hz), 6.76 (d, 2 H, J = 8.8 Hz), 7.21 (m, 12 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ –5.4, 18.1, 25.7, 54.1, 54.6, 62.6, 71.8, 71.9, 72.9, 74.4, 74.7, 76.7, 80.0, 98.6, 113.4, 127.2, 127.3, 127.6, 128.0, 129.0, 130.4, 138.5, 138.7;

Anal. Calcd for $C_{35}H_{48}O_7Si$: C, 69.05; H, 7.95. Found: C, 68.91; H, 7.65.

Methyl 3,4-di-*O*-benzyl-2-*O*-(*p*-methoxy-benzyl)-α-D-mannopyranoside (5)

A solution of **4** (10.0 g, 16.0 mmol) and 1 *n*-Bu_4NF (33.0 mL, 33.0 mmol, 1 M) in THF (40.0 mL) was stirred for 1 h and concentrated. The residue was dissolved in

EtOAc, washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (1:4) to give **5** (6.0 g, 74 %).

$[\alpha]_D$ = + 28.6 (c 0.7, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.05 (brs, 1 H), 3.21 (s, 3 H), 3.55 (m, 1 H), 3.77 (s, 3 H), 3.8 (m, 5 H), 4.57 (m, 7 H), 6.76 (d, 2 H, *J* = 8.7 Hz), 7.23 (m, 12 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 55.1, 62.4, 72.2, 72.6, 74.4, 74.9, 75.1, 80.2, 99.5, 113.8, 127.5, 128.0, 128.3, 129.4, 130.3, 138.6;

Anal. Calcd for $C_{29}H_{34}O_7$: C, 70.44; H, 6.88. Found: C, 70.59; H, 7.11.

Methyl 3,4-di-*O*-benzyl-6-eno-2-*O*-(*p*-methoxy-benzyl)-α-D-manno-heptopyranoside (6)

A solution of DMSO (2.8 mL, 32.4 mmol) and oxalyl chloride (1.4 mL, 16.2 mmol) in CH_2Cl_2 at – 78 ºC was stirred for 30 min and then **5** (4.0 g, 8.1 mmol) was added. After 45 min, Et_3N (6.8 mL, 48.6 mmol) was added and the reaction slowly brought to RT. The CH_2Cl_2 layer was washed with water, dried (Na_2SO_4) and concentrated to obtain the aldehyde (3.4 g, 85 %), which was dissolved in dry ether, cooled to – 40 ºC and treated with $Ph_3P{=}CH_2$ {generated from PPh_3CH_3I (11.2 g, 276.8 mmol) and sodamide (1.0 g, 256.0 mmol)}. After 30 min, ether was removed and the residue purified on silica gel using EtOAc and light petroleum ether (0.7:0.93) to give **6** (1.3 g, 39 %).

$[\alpha]_D$= + 24.4 (c 1, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.29 (s, 3 H), 3.71 (m, 3 H), 3.78 (s, 3 H), 3.91 (t, 1 H, *J* = 6.9 Hz), 4.64 (m, 7 H), 5.25 (d, 1 H, *J* = 8.8 Hz), 5.43 (d, 1 H, *J* = 14.4 Hz), 5.96 (m, 1 H), 6.8 (d, 2 H, *J* = 8.4 Hz), 7.28 (m, 12 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 55.2, 72.5, 72.5, 72.9, 74.7, 75.1, 79.0, 80.1, 99.4, 113.9, 117.7, 127.6, 128.0, 128.3, 128.4, 129.5, 130.6, 135.8.

Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.46; H, 6.94. Found: C, 73.68; H, 6.92.

Methyl 3,4-di-*O*-benzyl-6,7-*O*-isopropylidine-2-*O*-(*p*-methoxy-benzyl)-D-glycero-α-D-mannopyranoside (8a)

Methyl 3,4-di-*O*-benzyl-6,7-*O*-isopropylidine-2-*O*-(*p*-methoxy-benzyl)-L-glycero-α-D-mannopyranoside (8b)

A solution of K_2CO_3 (1.1 g, 8.0 mmol), $K_3Fe(CN)_6$ (2.6 g, 8.0 mmol), OsO_4 (27.0 mg, 0.1 mmol) and $(DHQ)_2$ PYR (23.0 mg, 0.026 mmol) in *t*-BuOH : H_2O (16.0 mL, 1:1) was added to **6** (1.3 g, 2.7 mmol). After 6 h at 0 ºC, the reaction was quenched with sodium sulfite and extracted with EtOAc, dried (Na_2SO_4) and concentrated to give **7a / 7b** (1.0 g), which was treated with DMP (5.0 mL) and PPTS (0.53 g, 2.1 mmol). After 4 h, the reaction was neutralized with Et_3N, concentrated and the mixture of diastereomers were separated by column chromatography on silica gel using EtOAc and light petroleum ether (1: 9) to give **8a** (0.71 g, 66 %) and further elution gave **8b** (78.0 mg, 7.2 %) in 9:1 ratio.

8a: $[\alpha]_D$ = + 30.8 (c 0.8, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 1.2 and 1.3 (2 s, 6 H), 3.18 (s, 3 H), 3.56 (m, 5 H), 3.65 (s, 3 H), 3.85 (t, 1 H, *J* = 7.9 Hz), 4.18 (m, 1 H), 4.47 (m, 6 H), 4.83 (d, 1 H, *J* = 11.0), 6.67 (d, 2 H, *J* = 8.7 Hz), 7.17 (m, 12 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 27.2, 54.9, 55.6, 65.1, 69.5, 72.3, 72.6, 73.0, 74.5, 74.8, 75.2, 80.2, 99.7, 127.6, 127.7, 127.8, 128.0, 128.4, 128.6, 129.8, 138.5;

Anal. Calcd for $C_{33}H_{40}O_8$: C, 70.2; H, 7.0. Found: C, 69.92; H, 7.02.

8b: $[\alpha]_D$ = + 19.4 (c 1.05, $CHCl_3$).

^{1}H NMR (200MHz, $CDCl_3$): δ 1.38 and 1.44 (2 s, 6 H), 3.32 (s, 3 H), 3.8 (s, 3 H), 3.83 (m, 3 H), 4.0 (m, 3 H), 4.4 (dd, 1 H, *J* = 2.3, 6.1 Hz), 4.62 (m, 6 H), 4.98 (d, 1 H, *J* = 8.3 Hz), 6.83 (d, 2 H, *J* = 7.2 Hz), 7.29 (m, 12 H);

Anal. Calcd for $C_{33}H_{40}O_8$: C, 70.21; H, 7.09. Found: C, 70.04; H, 7.26.

Methyl 3,4,6,7-tetra-*O*-benzyl-2-*O*-(*p*-methoxy-benzyl)-D-glycero-α-D-manno-hepto-pyranoside (10)

A solution of **8a** (0.68 g, 1.2 mmol) and PPTS (0.33 g, 1.3 mmol) in MeOH was stirred for 6 h, neutralized by Et_3N and concentrated to obtain diol **7a** (0.5 g, 1.0 mmol),

which was dissolved in dry THF (7.0 mL) and NaH (150.0 mg, 3.8 mmol, 60 % dispersion in oil) was added. After 30 min, BnBr (0.3 mL, 2.4 mmol) was introduced, stirred for another 12 h, quenched with ice and concentrated. The residue dissolved in EtOAc, washed with water, dried (Na_2SO_4), evaporated and purified on silica gel using EtOAc and light petroleum ether (1:10) to give **10** (0.55 g, 82 %).

$[\alpha]_D$ = +29.2 (c 1.07, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.3 (s, 3 H), 3.7 (m, 5 H), 3.77 (m, 3 H), 3.96 (s, 2 H), 4.65 (m, 11 H), 6.76 (d, 2 H, *J* = 7.3 Hz), 7.26 (m, 22 H);

Anal. Calcd. for $C_{44}H_{48}O_8$: C, 75.00; H, 6.82. Found: C, 74.69; H, 7.07.

Methyl 3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-manno-heptopyranoside (**11**)

A solution of **10** (1.3 g, 1.8 mmol) and DDQ (0.46 g, 2.0 mmol) in CH_2Cl_2:H_2O (9:1) was stirred for 3 h, concentrated and the residue purified on silica gel using EtOAc and light petroleum ether (1:4) to give **11** (0.76 g, 70 %).

$[\alpha]_D$ = + 17.3 (c 0.935, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.35 (s, 3 H), 3.85 (m, 7 H), 4.65 (m, 9 H), 7.3 (m, 20 H).

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.8, 68.2, 70.8, 72.0, 72.6, 73.3, 74.4, 78.3, 80.8, 100.2, 127.5, 127.6, 127.7, 127.9, 128.3, 128.5, 138.0, 138.6;

Anal. Calcd. for $C_{36}H_{40}O_7$: C, 73.97; H, 6.85. Found: C, 73.91; H, 6.90.

1, 2-di-*O*-acetyl-3,4, 6,7-tetra-*O*-benzyl-D-glycero-α-D-manno-heptopyranoside (**12**):

A mixture of acetic acid, acetic anhydride and sulfuric acid (3.0 mL; 25:5:1) was added to **11** (0.3 g, 0.5 mmol) and stirred for 1 h at 0 ºC. The reaction was neutralized by $NaHCO_3$, diluted with water, extracted with EtOAc, dried (Na_2SO_4), evaporated and purified on silica gel using EtOAc and light petroleum ether (1:9) to give **12** (0.2 g, 60 %).

$[\alpha]_D$ = + 22.5 (c 0.9, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.05, 2.11 (2 s, 6 H), 3.94 (m, 4 H), 4.24 (m, 2 H), 4.64 (m, 8 H), 5.28 (br.d, 1 H, *J* = 5.6 Hz), 6.05 (d, 1 H, *J* = 2.0 Hz), 7.32 (m, 20 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 20.4, 64.3, 71.8, 72.1, 72.3, 73.6, 74.4, 74.8, 79.1, 91.5, 127.1, 127.3, 127.5, 127.7, 127.9, 128.1, 128.7, 137.7, 137.8, 138.0, 138.2, 168.2, 169.8;

Anal. Calcd for $C_{39}H_{42}O_9$: C, 71.56; H, 6.42. Found: C, 71.41; H, 6.26.

Methyl 3,4,6,7-tetra-*O*-benzyl- D-glycero-α-D-manno-heptopyranosyl-(2→1)-2-*O*-acetyl –3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-manno-heptopyranoside (13)

To a mixture of **11** (0.14 g, 0.2 mmol), **12** (0.2 g, 0.3 mmol) and activated 4 ºA molecular sieves in dry CH_2Cl_2 (10.0 mL), BF_3:OEt_2 (0.05 mL) was added and stirred for 12 h. The reaction was neutralized with Et_3N, filtered, concentrated and purified on silica gel using EtOAc and light petroleum ether (1:10) to give the disaccharide **13** (72.0 mg, 26 %).

$[\alpha]_D$ = + 28.9 (c 1.01, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.09 (s, 3 H), 3.29 (s, 3 H); 3.76 (m, 13 H), 4.59 (m, 18 H), 5.22 (brd, 1 H, *J* = 5.4 Hz), 7.2 (m, 40 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 21.0, 54.7, 68.8, 72.0, 72.3, 72.4, 75.2, 75.3, 78.5, 78.6, 79.5, 99.5, 99.8, 127.5, 127.7, 128.0, 128.4, 135.1, 135.6, 138.5, 169.8;

Anal. Calcd for $C_{73}H_{78}O_{14}$: C, 74.36; H, 6.62. Found: C, 74.51; H, 7.02.

Methyl 3,4,6,7-tetra-*O*-benzyl- D-glycero-α-D-manno-heptopyranosyl-(2→1)–3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-manno-heptopyranoside (14)

A solution of **13** (72.0 mg, 0.06 mmol) and MeONa (3.0 mg, 0.7 mmol) in MeOH (5.0 mL) was stirred for 10 min, quenched by adding small pieces of dry ice and concentrated. The residue extracted with EtOAc, washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (1:9) to give **14** (50.0 mg, 72 %).

$[\alpha]_D$ = + 32.7 (c 1.12, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.41(s, 3 H), 3.86 (m, 14 H), 4.72 (m, 18 H), 7.26 (m, 40 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.6, 65.5, 68.8, 72.6, 73.0, 73.5, 75.4, 78.1, 78.4, 79.8, 98.7, 98.9, 127.4, 127.7, 127.8, 128.0, 128.2, 128.4, 135.2, 135.8, 138.0;

Anal. Calcd for $C_{71}H_{76}O_{12}$: C, 75.00; H, 6.69. Found: C, 75.18; H, 6.99.

Methyl-D-glycero-α-D-manno-heptopyranosyl-(2→1)–D-glycero-α-D-manno-hepto-pyranoside (15)

A suspension of 10 % Pd/$(OH)_2$-C (10.0 mg) and **14** (50.0 mg, 0.04 mmol) in MeOH (5.0 mL) was hydrogenolyzed at RT for 40 h. Filtration of the reaction mixture, followed by concentration gave **15** (9.0 mg, 50 %).

$[\alpha]_D$ = + 65 (c 0.81, H_2O);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.41 (s, 3 H), 3.79 (m, 14 H), 4.77 (d, 1 H, *J* = 1.7 Hz), 4.9 (d, 1 H, *J* = 1.7 Hz);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.3, 62.1, 62.2, 67.6, 67.8, 69.9, 70.1, 71.0, 72.2, 72.4, 74.2, 74.2;

Anal. Calcd for $C_{15}H_{28}O_{13}$: C, 43.27; H, 6.73. Found: C, 43.41; H, 6.93.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-α-D-manno-pyranoside (17)

A solution of **1** (15.0 g, 77.3 mmol), imidazole (8.2 g, 231.0 mmol) and TBS-Cl (6.0 g, 40.0 mmol) in dry DMF (20.0 mL) was stirred for 1 h, diluted with water, extracted with ether, dried (Na_2SO_4) and concentrated to afford **16** (16.0 g, 82 %), which was dissolved in 5.0 mL of dry DMF and NaH (8.3 g, 207.8 mmol, 60 % dispersion in oil) added. After 30 min BnBr (25.0 mL, 234.0 mmol) was added and stirred for 12 h. The reaction was quenched with ice, extracted with ether, washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (1:10) to give **17** (0.55 g, 82 %).

$[\alpha]_D$ = + 24.7 (c 0.92, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 0.0 (s, 6 H), 0.84 (s, 9 H), 3.24 (s, 3 H), 3.44 (m, 1 H), 3.76 (m, 5 H), 4.65 (m, 6 H), 4.85 (d, 1 H, *J* = 11.0 Hz), 7.25 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ –5.7, 18.4, 26.0, 54.4, 55.2, 62.9, 72.1, 72.2, 73.2, 74.7, 75.0, 80.3, 98.9, 113.7, 127.5, 127.6, 127.9, 128.3, 130.7, 138.8, 138.9;

Anal. Calcd. for $C_{34}H_{46}SiO_6$: C, 70.59; H, 7.96. Found: C, 70.49; H, 7.71.

Methyl 2,3,4-tri-*O*-benzyl-D-manno-pyranoside (18)

A solution of **17** (10.0 g, 17.3 mmol) and *n*-Bu_4NF (23.0 mL, 22.5 mmol, 1 M) in THF (40.0 mL) was stirred for 1 h. The reaction was concentrated, dissolved in EtOAc,

washed with water, dried (Na_2SO_4), concentrated and purified on silica gel using EtOAc and light petroleum ether (1:4) to give **18** (6.0 g, 74 %).

$[\alpha]_D$ = + 24.1 (c 1.08, $CHCl_3$); lit.[39] **$[\alpha]_D$** = + 24.8 (c 1.7, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.14 (brs, 1 H), 3.31 (s, 3 H), 3.62 (m, 1 H), 3.87 (m, 5 H), 4.63 (m, 6 H), 4.91 (d, 1 H, *J* = 10.9 Hz), 7.29 (m, 15 H);

Anal. Calcd. for $C_{28}H_{32}O_6$: C, 72.41; H, 6.89. Found: C, 72.18; H, 6.57.

Methyl 2,3,4-tri-*O*-benzyl-6-eno-D-manno-heptopyranoside (19)

A solution of DMSO (2.9 mL, 34.5 mmol) and oxalyl chloride (1.5 mL, 17.2 mmol) in CH_2Cl_2 (60.0 mL) was stirred for 30 min at –78 ºC, and **5** (4.0 g, 8.6 mmol) were added. After 45 min, Et_3N (7.2 mL, 51.6 mmol) was added and the reaction slowly brought to RT. The CH_2Cl_2 layer was washed with water, dried (Na_2SO_4) and concentrated to obtain aldehyde (3.4 g, 85 %), which was dissolved in dry ether (40.0 mL), and the ylide generated from PPh_3CH_3I (11.2 g, 276.8 mmol) and sodamide (1.0 g, 256.0 mmol) in dry ether (70.0 mL) was added and stirred for 30 min. Ether was removed and the residue purified on silica gel using EtOAc and light petroleum ether to give **19** (1.3 g, 39 %).

$[\alpha]_D$ = + 23.9 (c 0.9, $CHCl_3$); lit. [40] **$[\alpha]_D$** = + 24.6 (c 1, $CHCl_3$).

^{1}H NMR (200MHz, $CDCl_3$): δ 3.33 (s, 3 H), 3.81 (m, 2 H), 3.92 (dd, 1 H, *J* = 3.4, 8.4 Hz), 4.03 (m, 1 H), 4.76 (m, 7 H), 5.32 (d, 1 H, *J* = 10.2 Hz), 5.53 (d, 1 H, *J* = 15.5 Hz), 5.93 (m, 1 H), 7.35 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 55.2, 72.5, 72.6, 72.9, 74.7, 75.1, 79.0, 80.1, 99.4, 113.9, 117.7, 127.5, 127.6, 128.0, 128.3, 128.4, 129.5, 130.6, 135.9;

Anal. Calcd. for $C_{29}H_{32}O_5$: C, 75.65; H,6.95. Found: C, 75.64; H, 7.23.

Methyl 2,3,4-tri-*O*-benzyl-6,7-*O*-isopropylidine-D-glycero-α-D-manno-heptopyranoside (21a)

Methyl 2,3,4-tri-*O*-benzyl-6,7-*O*-isopropylidine-L-glycero-α-D-manno-heptopyranoside (21b)

A solution of K_2CO_3 (1.2 g, 8.5 mmol), $K_3Fe(CN)_6$ (2.8 g, 8.5 mmol), OsO_4 (29.0 mg, 0.1 mmol) and $(DHQ)_2$ PYR (25.0 mg, 0.03 mmol) in *t*-BuOH : H_2O (16.0 mL, 1:1)

was added to **19** (1.3 g, 2.8 mmol). After stirring for 6 h at 0 ºC, the reaction was quenched with sodium sulfite, extracted with EtOAc, washed with water, dried (Na_2SO_4) and concentrated to give **20a / 20b** (1.0 g), which was treated with DMP (5.0 mL) and PPTS (0.56 g, 2.2 mmol). After 4 h, the reaction was neutralized by Et_3N, concentrated and mixture of diastereomers were separated by flash chromatography on silica gel using EtOAc and light petroleum ether (1:9) to give **21a** (0.68 g, 63 %) and further elution gave **21b** (70.0 mg, 7 %) in 9:1 ratio.

21a: $[\alpha]_D$ = + 21.4 (c 0.33, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 1.37 and 1.48 (2s, 6 H), 3.37 (s, 3 H), 3.55 (m, 1 H), 3.75 (m, 5 H), 4.06 (m, 1 H), 4.40 (m, 1 H), 4.65 (m, 6 H), 4.86 (d, 1 H, *J* = 11.1 Hz), 7.35 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 27.4, 54.9, 65.1, 69.6, 72.3, 72.6, 73.1, 74.5, 74.9, 75.2, 80.2, 99.7, 127.6, 127.8, 127.9, 128.4, 129.8, 138.5;

Anal. Calcd for $C_{32}H_{38}O_7$: C, 71.91; H, 7.12. Found: C, 71.79; H, 7.11.

21b: $[\alpha]_D$ = + 14.4 (c 1.05, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 1.43 and 1.50 (2s, 6 H), 3.34 (s, 3 H), 3.51 (m, 1 H), 4.00 (m, 5 H), 4.37 (q, 1 H, *J* = 5.7 Hz), 4.70 (m, 6 H), 4.92 (d, 1 H, *J* = 11.2 Hz), 7.31 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 29.7, 54.9, 65.0, 69.6, 72.3, 72.6, 73.1, 74.5, 75.0, 75.2, 80.2, 99.8, 127.6, 127.8, 127.9, 128.4, 129.8, 138.5;

Anal. Calcd for $C_{32}H_{38}O_7$: C, 71.90; H, 7.12. Found: C, 71.98; H, 7.31.

Methyl 2,3,4-tri-*O*-benzyl--D-glycero-α-D-manno-heptopyranoside (20b)

A solution of **21b** (0.68 g, 1.2 mmol) and PPTS (0.33 g, 1.3 mmol) in MeOH (5.0 mL) was stirred for 6 h, neutralized by Et_3N and concentrated to afford diol **20b**.

$[\alpha]_D$ = + 22.2 (c 0.98, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.42 (brs, 1 H), 3.36 (s, 3 H), 3.74 (m, 4 H), 3.94 (m, 3 H), 4.71 (m, 6 H), 5.02 (d, 1 H, *J* = 11.5 Hz), 7.38 (m, 15 H);

Anal. Calcd. for $C_{29}H_{34}O_7$: C, 70.44; H, 6.88. Found C, 70.56; H, 7.11.

Methyl 2,3,4,6,7-penta-*O*-benzyl--D-glycero-α-D-manno-heptopyranoside (22)

A solution of **20** (0.5 g, 1.0 mmol) and NaH (160.0 mg, 3.8 mmol, 60 % dispersion in oil) in dry THF (10.0 mL), was stirred for 30 min and BnBr (0.3 mL, 2.5 mmol) was added to the reaction. After 12 h, the reaction was quenched with ice, and the residue dissolved in EtOAc, washed with water, dried (Na_2SO_4), evaporated and purified on silica gel using EtOAc and light petroleum ether (1:10) to give **22** (0.55 g, 82 %).

$[\alpha]_D$ = + 16.3 (c 1.18, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.26 (s, 3 H), 3.81 (m, 7 H), 4.55 (m, 11 H), 7.25 (m, 25 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 54.7, 70.9, 72.1, 72.4, 72.6, 73.2, 74.8, 75.0, 75.2, 78.5, 80.5, 98.8, 127.2, 127.3, 127.5, 127.6, 128.2, 138.5, 138.6;

Anal. Calcd. for $C_{43}H_{46}O_7$: C, 76.56; H, 6.82. Found: C, 76.30; H, 6.77.

Acetyl 2,3,4,6,7-penta-*O*-benzyl--D-glycero-α-D-manno-heptopyranoside (25)

A mixture of acetic acid, acetic anhydride and sulfuric acid (3.0 mL, 25:5:1) was added to **22** (0.3 g, 0.4 mmol) and stirred for 1 h at 0 ºC. The reaction was neutralized by $NaHCO_3$, diluted with water, extracted with EtOAc, dried (Na_2SO_4), evaporated and purified on silica gel using EtOAc and light petroleum ether (1:9) to give **23** (0.2 g, 60 %).

$[\alpha]_D$ = + 10.8 (c 0.55, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 2.00 (s, 3 H), 3.66 (m, 3 H), 3.79 (dd, 1H, *J* = 3.4, 10.6 Hz), 3.91 (m, 2 H), 4.15 (m, 1 H), 4.42 (s, 2 H), 4.51 (s, 2 H), 4.66 (m, 6 H), 6.13 (d, 1 H, *J* = 1.9 Hz), 7.24 (m, 25 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 21.0, 71.0, 72.3, 72.7, 72.8, 73.3, 74.0, 74.3, 74.7, 75.3, 78.8, 79.5, 92.0, 127.3, 127.4, 127.6, 127.8, 127.9, 128.3, 128.4, 138.1, 138.3, 138.5, 138.6, 139.0, 168.7;

Anal. Calcd. for $C_{44}H_{46}O_8$: C, 75.21; H, 6.55. Found: C, 74.90; H, 6.81.

Pentenyl 2,3,4,6,7-penta-*O*-benzyl--D-glycero-α-D-manno-heptopyranoside (26)

A mixture of **23** (150.0 mg, 0.2 mmol), 4-pentenol (0.05 mL, 0.5 mmol) and activated 4 ºA molecular sieves in CH_2Cl_2 (5.0 mL) were stirred under argon for 30 min

and subsequently BF_3:OEt_2 (0.02 mL) was added. After 8 h, reaction was neutralized, filtered, concentrated and the residue purified on silica gel using EtOAc and light petroleum ether (1:5) to give **24** (110.0 mg, 71 %).

$[\alpha]_D$ = + 15.6 (c 1.08, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.56 (m, 2 H), 2.0 (m, 2 H), 3.7 (m, 9 H), 4.56 (m, 13 H), 5.66 (m, 1 H), 7.2 (m, 20 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 29.7, 30.3, 67.0, 71.1, 72.5, 72.63, 73.3, 74.7, 78.6, 80.6, 97.8, 114.8, 127.4, 127.6, 127.7, 127.9, 128.2, 128.3, 138.1, 138.7, 139.0;

Anal. Calcd for $C_{47}H_{52}O_7$: C, 77.47; H, 7.14. Found: C, 77.40; H, 7.32.

Methyl 3,4,6,7-tetra-*O*-benzyl-D-glycero-α-D-manno-heptopyranosyl-(2→1)-2-3,4, 6, 7-penta-*O*-benzyl-D-glycero-α-D-manno-heptopyranoside (**27**)

A suspension of **24** (110.0 mg, 0. 15 mmol), alcohol **12** (70.0 mg, 0.14 mmol), NIS (67.0 mg, 0.3 mmol) and freshly activated 4 ºA molecular sieves in anhydrous CH_2Cl_2 (5.0 mL) were stirred under argon for 20 min. A portion (2 drops) of solution (ca. 0.14 M) of TfOH in CH_2Cl_2 was added and stirred for 2 h, diluted with CH_2Cl_2 and filtered. The filtrate was washed with 10 % aq. $Na_2S_2O_3$ and saturated solution of $NaHCO_3$, dried (Na_2SO_4), concentrated and the residue purified on silica gel using EtOAc and light petroleum ether (1:9) to give disaccaride **25** (48.0 mg, 33 %).

$[\alpha]_D$ = + 25.2 (c 0.84, $CHCl_3$);

^{1}H NMR (200MHz, $CDCl_3$): δ 3.37 (s, 3 H); 3.86 (s, 14 H); 4.65 (m, 20 H); 7.4 (m, 45 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 55.1, 69.5, 72.4, 72.8, 73.3, 75.6, 79.0, 79.2, 99.9, 100.3, 127.9, 128.7, 135.75, 136.1, 139.0;

Anal. Calcd for $C_{78}H_{82}O_{13}$: C, 76.34; H, 6.68. Found: C, 75.9; H, 7.23.

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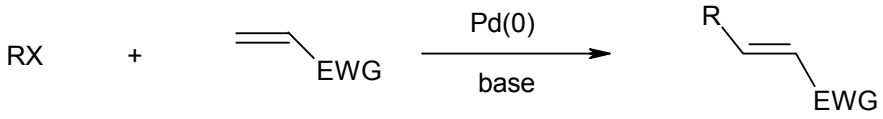
INTRODUCTION

Palladium catalysis has achieved the status of an indispensable tool for both common and state-of-art organic synthesis. Among basic types of palladium-catalyzed transformations, the Heck reaction and related chemistry occupy a special place. The arylation or vinylation of alkenes under the influence of palladium catalyst, generally reffered to as the Heck reaction,[1-7] has been known to synthetic chemists since the late 1960s.[8] Despite displaying many of the advantages usually associated with Pd-mediated reactions (particularly ease of scale up and tolerance of water and/or other functional groups) interest in the reaction has been sporadic, largely due to problems of regiocontrol in the case of unsymmetrical alkene substrates and to an incomplete understanding of the reaction mechanism. In recent years, however the attention paid to the reaction has increased dramatically,[9] and perhaps the most significant development to date has been the advent of an enantioselective variant.[10, 11]

A traditional Heck coupling was based on an aryl iodide as the electrophilic partner and a terminal alkene as the nucleophilic partner [(Scheme **1**), **R** = aryl, vinyl and **X** = I, Br]. The initial promise of a convenient method for carbon-carbon bond formation was only realized satisfactorily for terminal alkenes possessing an electron-withdrawing group (for example, EWG = COOR, CN, Ph).

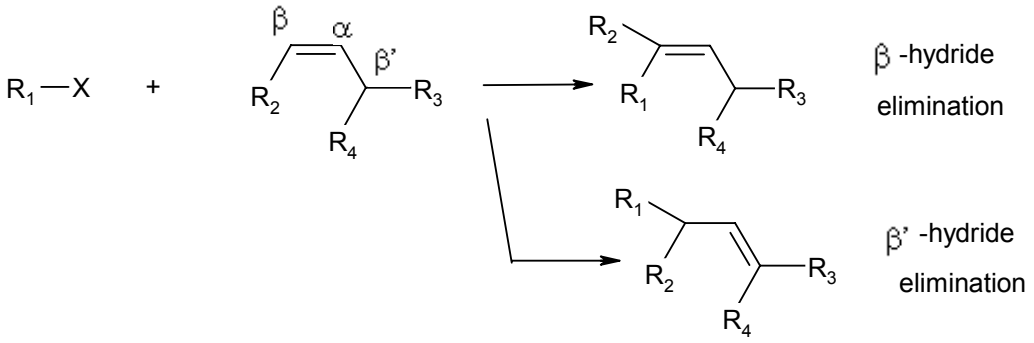
Scheme 1



The regiochemistry found for such transformations was consistent with carbon-carbon bond formation at the least hindered terminus of the alkene, much like in a Michael addition.[9] The stereochemistry of addition usually delivered a *trans* disubstituted alkene. The formation of regioisomers arose as a major problem for electronically neutral alkenes or those substituted with an electron-donating group (such as OR or NR_2). After considerable experimentation with a variety of ligands, palladium sources, solvents and additives many of the disadvantages associated with the traditional Heck conditions have been alleviated and we now have a convenient methodology for the construction of complex, multifunctional

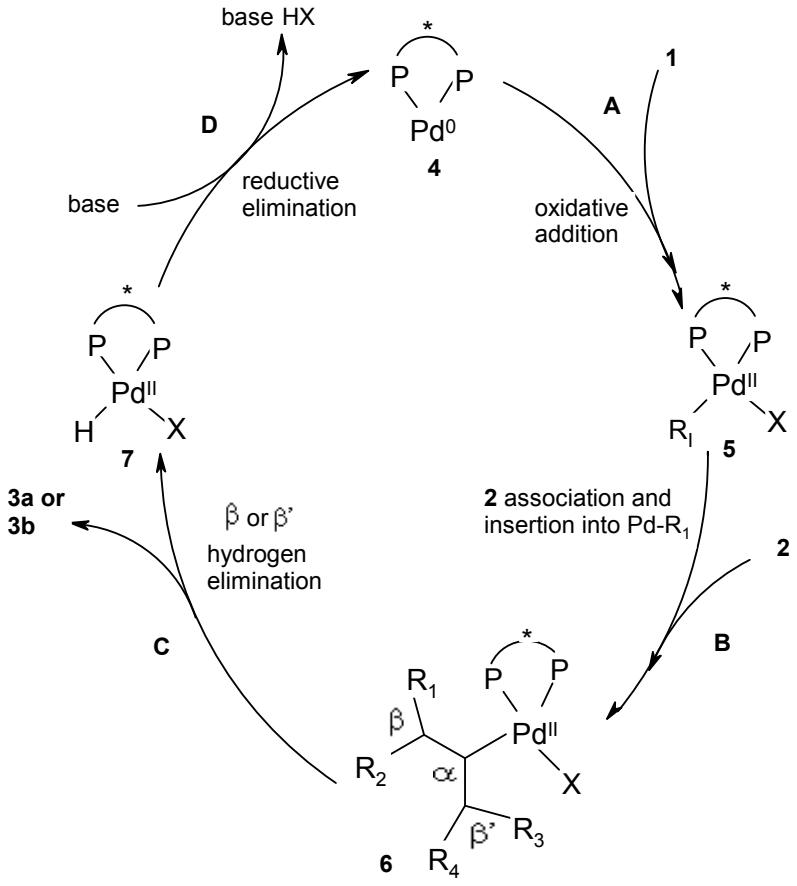
molecules. The key to using the Heck reaction as a crucial step in synthetic endeavors is to identify the class of Heck reaction in terms of both the type of alkene (whether electron donating or electron withdrawing) and the electrophile (whether a halide or a trifluoromethane sulfonate is the leaving group) and then select the most appropriate conditions in order to maximize the conversion. Various aspects of the Heck reaction have been reviewed in recent years including an extensive compilation of recent applications to the synthesis of complex natural products and cascade processes,[9] the use of the intramolecular Heck reaction for the construction of quaternary centers,[11, 12] the intramolecular asymmetric Heck reaction[6] and a detailed summary of mechanistic details for the Heck reaction under a variety of conditions.[4] In view of plethora of excellent reviews we want to concentrate on the factors, which impart the regio- and enantio control necessary for a successful AHR.

1) Factors Governing Regioselectivity



Scheme 1 Heck reaction with disubstituted alkenes bearing β and β' hydrogen

The mechanism of the Heck reaction (Scheme **1**) with bidented phosphene ligands is generally thought to follow the four-step catalytic cycle shown in Scheme **2**, with individual steps being: A) oxidative addition of **1** to the bidented phosphine ligand bearing Pd^0 catalytic species **4** to give the Pd^{II} species **5**, B) co-ordination and then syn-insertion of alkene substrate **2** into the **5** Pd-R_1 bond to give **6**, C) β- or β'-hydride elimination from **6** to give either **3a** or **3b**, and finally D) regeneration of **4** by reductive elimination of HX from **7**.



Scheme 2. Catalytic cycle for Heck reaction

The three major factor imparting regioselectivety are:

i) The regioselectivity of the insertion into Pd-R_1 is heavily dependent upon the nature of the steric and electronic environment provided by R_2, R_3 and R_4 for unsymmetrical alkenes, which has tended to limit the scope of the reaction somewhat.

ii) The problem of competing β- and β'-hydride elimination from 6 further completes the regioselectivity issue, to the extent that the majority of reported heck reactions simply avoid the problem by using simple acrylate ester substrates (R_2 = COOR, monosubstituted alkenes), which through their highly unsymmetrical steric and electronic environment also avoid any problems with regioselectivity in step B. Although this constitutes a mild and powerful method for the synthesis of aryl

acrylates, by eliminating the possibility of β'-hydride elimination an opportunity to form a tertiart chiral center is lost.

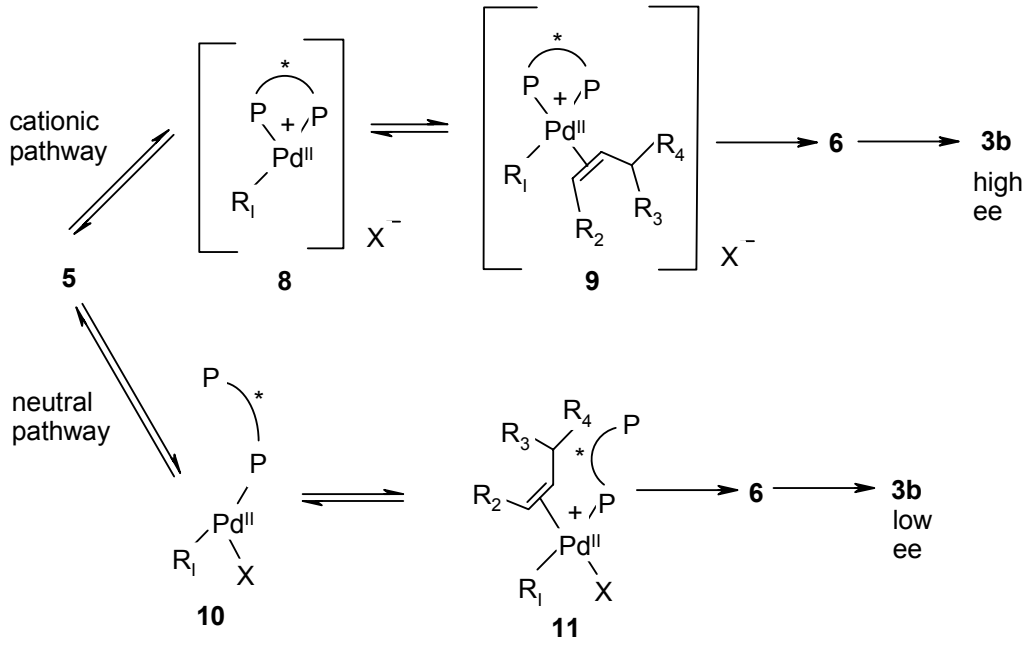
iii) Even if the regioselectivity of step C can be controlled a further problem lies in its reversibility, which can result in reinsertion of the **3b** alkene into the Pd-H bond in **7** either to regenerate **6** or to form a regioisomer of it with the Pd atom attached to the same carbon atom as R_3 and R_4. If either of these substituents contains a suitably positioned hydrogen atom then the possibility exist of isomerisation of the α and β'-alkene into a β', χ position. Fortunately methods has been developed to suppress this by adding thalium[13] or silver salts[14, 15] to the reaction mixture-latter are usually preferred owing to their lower toxicity and fortuitous double role as enhancer of enantioselectivity.

2) Factors Governing Enantioselectivity

The key step in the catalytic cycle with regard to enantioselectivity is clearly the association of alkene **2** and insertion of it into the Pd-R_1 bond. As with the Heck reaction itself the mechanism for this process remains a matter for conjecture, with the overall rationale currently in favour having been proposed in 1991 by Ozawa and Hayashi[16] and independently by Cabri[17] (although the cationic pathway via **8** and **9** had been proposed as early as 1990).[18] The latter author has recently reviewed its development and subsequent evolution.[4]

Two possible routes are proposed (Scheme **2**), the former (cationic) pathway being with the dissociation of X from **5** to generate the tri-coordinate 14e cationic complex **8** with accompanying X counterion. Complexation of **2** into vacant site then gives the **16e** species **9**, and insertion of **2** into the pd-R1 bond followed by reformation of the Pd-X bond gives **6** as desired, with the chiral bidented ligand having remained fully chelated throughout and so having maximized the asymmetric induction. The alternative (neutral) pathway starts with dissociation of one arm of the bidented ligand giving the neutral species **10**; association and complexation into the vacant site of **2** gives the neutral species **11**, which by alkene insertion into Pd-R_1 and re-complexation of the previously displaced phosphine moiety also gives **6**. The partial dissociation of the chiral ligand during the neutral process would seem to make it

less suited to asymmetric induction, however, and the evidence of most of the AHRs reported so far seems to indicate that conditions, which favour the cationic route, also give the best enantiomeric excesses. The nature of X in **1** is clearly an important factor, unless the reaction conditions are modified aryl and vinyl triflates are generally assumed to follow the cationic pathway[19] with either route being available to reactions using aryl/vinyl halides. In practice it is possible to influence which pathway will be followed in a Heck process, either by adding silver salts to the reaction of an aryl/vinyl halide, or by adding excess of halide anions to reactions using triflates.[20] The nature of the alkene substrate is also important; with electron-rich olefins favoring the cationic pathway while the neutral pathway makes for faster reaction with electron-poor substrates.[17]



Scheme 3 Cationic and neutral pathways for the AHR mechanism

There are no certainties in synthesis yet in view of the plethora of excellent published data concentrating on the Heck reaction and detailed mechanistic information, chemists can now propose a series of protocols which can be used to perform a successful Heck coupling. For *intermolecular* couplings involving reactive electrophiles (aryl or vinyl iodides) and alkenes containing an electron withdrawing group a traditional catalyst systems such as $Pd(OAc)_2$ alone or with 2–4 equivalents of L or PdL_2Cl_2 or PdL_4 [L = PPh_3 or P(*o*-tolyl)$_3$][9] with an

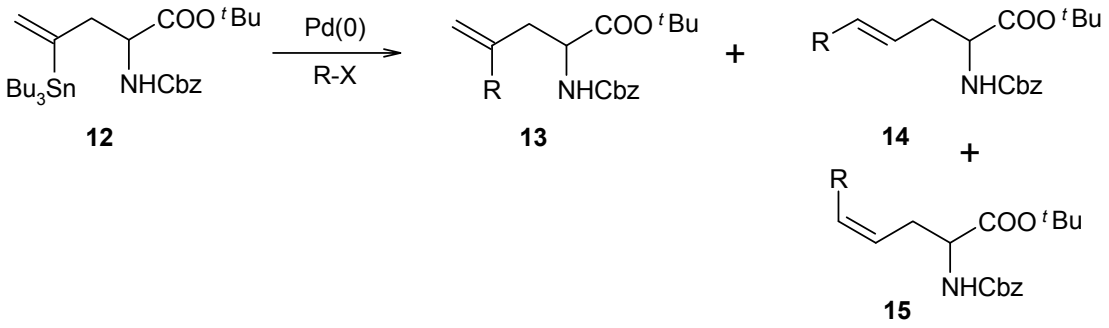
organic or inorganic base will usually suffice. Such systems will usually require temperatures in the range 50–100 °C. In order to lower the temperature the most effective protocol is to add phase-transfer catalyst[21] R_4NX (X = Cl, Br) and use an aqueous solvent with K_2CO_3 as the base. For electrophiles that undergo oxidative addition more slowly (aryl bromides with electron donating groups or aryl chlorides) high temperatures (above 120 °C) are usually required along with a ligand, which will not decompose for a long-lived catalyst (L = PPh_3 will not be suitable). For aryl or vinyl triflates with alkenes containing an electron withdrawing group a traditional catalyst system can also be used. For alkenes which do not contain an electron withdrawing group then halide free conditions, achieved by either using aryl or vinyl triflates as the electrophile or adding a halide sequestering agent (Ag^+) for aryl or vinyl halides, will be advantageous. For *intramolecular* Heck cyclisations the reaction conditions appear to vary depending on whether a tertiary or quaternary center is being formed, on the ring size and the stereochemistry of the alkene.[6, 22] The presence of halides does not appear to impede the cyclisation at elevated temperatures and can be beneficial for high ee's in asymmetric Heck couplings. The use of halide free conditions can produce rapid Heck couplings but variable ee's for asymmetric cyclisations.

Unnatural α-amino acids

Unnatural and non-proteinogenic α-amino acids are important as enzyme inhibitors, therapeutic agents and chiral synthons.[23] An important class of non-proteinogenic α-amino acids is that based on allylglycines, many of these compounds have been reported to act as irreversible, mechanism-based, inhibitors of pyridoxal phosphate dependent enzymes.[24] They also act as building blocks exemplified by the synthesis of homophenylalanine derivatives, the latter being used in the synthesis of drugs with angiotensin converting enzyme inhibitory activity. Allylglycine derivatives have been prepared previously by routes such as allyl electrophiles reacting with glycine anions,[25] allyl nucleophiles reacting with glycine cations[26] and Wittig reactions of L-aspartic acid semialdehyde derivatives.[27] Stille coupling methodology was also exploited for the elaboration of the side-chain of α-amino acids since the reactions take place under mild conditions and are tolerant of a wide variety of functionality.[28] Palladium-catalysis has been used previously for the synthesis of allylglycine

derivatives, where tri-n-butylstannylallylglycine derivatives (**12**) undergo coupling with organic electrophiles (R-X). Though the reaction (Scheme **4**) proceeds with over all high yields, it produced significant amount of *cine*-substituted products (**14** and **15**). The reaction was repeated using a number of different conditions and in all instances the three isomeric products were obtained. Also, the metal impurity present in the reaction mixture poses problem in separating the products in its pure form. Hence, it was thought to apply Heck coupling as a better alternative process to obtain allylglycine derivatives with high isomeric purity.[29]

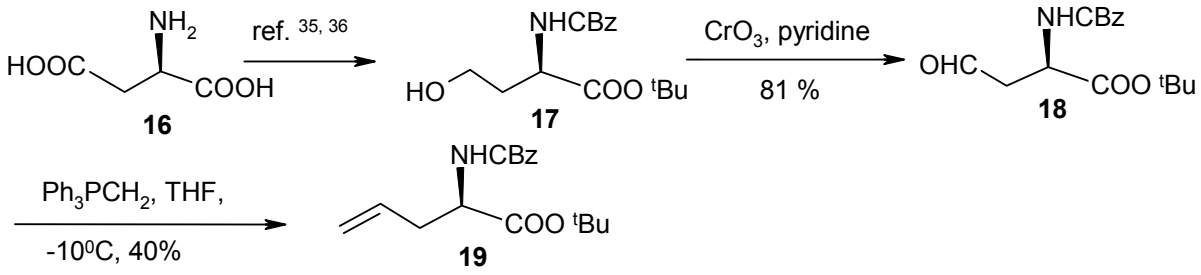
Scheme 4



Unnatural and non-proteinogenic α-amino acids[30] are important as enzyme inhibitors, theraputic agents and chiral synthons. An important class of non-proteinogenic α-amino acids is that based on allyl glycines, many of these compounds have been reported to act as irreversible, mechanism-based, inhibitors of pyridoxal phosphate dependent enzymes. They also act as building blocks exemplified by the synthesis of homophenylalanine derivatives,[31] the latter being used in the synthesis of drugs with angiotensin converting enzyme inhibitory activity.[32] Palladium-catalysis has been used previously for the synthesis of allylglycine derivatives including allyl acetate coupling with glycine anions,[25] vinyl electrophiles coupling with an organozinc derivative of β-iodoalanine,[33] rearrangements of imino acid allyl ester derivatives[34] and a Stille and Suzuki coupling of a bromoallylglycine derivative.[28] These reactions are marred by poor selectivity of products and difficulty in purification due to the presence of metal impurity. Surprisingly, the Heck reaction between allyl glycine derivatives and aryl halide, which would provide a direct access to aryl allyl glycines, has not been reported to the best of our knowledge.

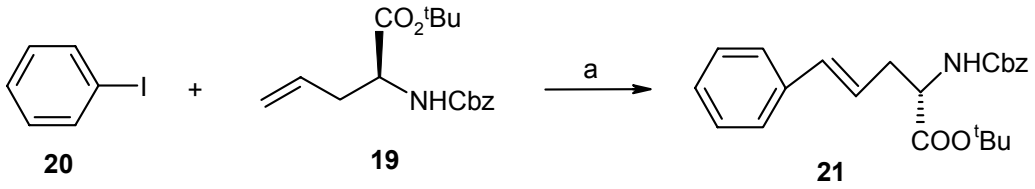
Synthetic chemists have extensively exploited the arylation and alkenylation of alkenes under the influence of a palladium catalyst, commonly referred to as the Heck reaction,[1-7] since its debut in the late 1960's. Herein, we report palladium-catalysed elaboration of allyl glycine *via* Heck reaction for the synthesis of allyl glycine derivatives with high isomeric purity. The stereochemistry of addition usually delivered a *trans* disubstituted alkenes.

Synthesis of (*S*)-*N*-Cbz-allylglycine *tert*-butyl ester (**19**) (Scheme **5**) was achieved starting with aspartic acid (**16**) by following the reported procedure.[35, 36] The alcohol (**17**) was oxidized with PCC in presence of 4 Å molecular sieves and NaOAc in CH_2Cl_2 to get aspartic acid β-semialdehyde (**18**).[27] The aldehyde function of **18** provides a useful handle for manipulation to more complex structures.[37] Wittig reaction of the aldehyde (**18**) with methylenetriphenylphosphorane in THF at –10 °C provided (*S*)-N-Cbz-allylglycine *tert*-butyl ester (**19**) in 40 % yield. In the ^{1}H- NMR spectrum **19**, resonances typical of olefin were observed at 5.66 and 5.09 ppm as multiplets.



The allylglycine derivative (**19**) (Scheme **6**) when reacted with iodobenzene (**20**) in presence of $Pd(OAc)_2$ in acetonitrile at 70 °C, gave the 5-phenyl-allylglycine derivative (**21**) in 70 % yield. The ^1H and ^{13}C NMR spectra, mass spectral data coupled with elemental analysis of **6** was in conformity with the assigned structure. For example, in the ^1H NMR spectrum of (**21**), the olefinic protons appeared at δ 6.09 (double-triplet, J = 7.3, 15.6 Hz) and at δ 6.46 (doublet, J = 15.6 Hz), which indicated that the *E*-isomer was predominantly formed. Table-**1** demonstrated versatility of the Heck reaction of allyl glycine with various substituted aryl halides.

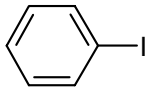
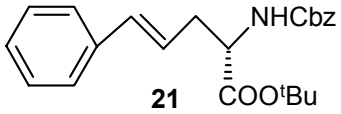
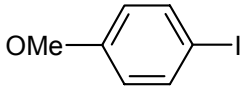
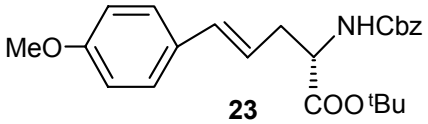
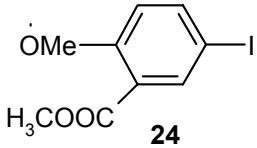
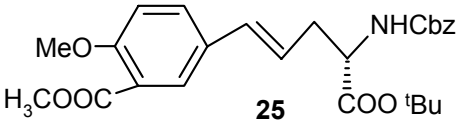
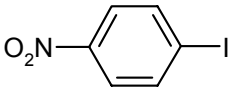
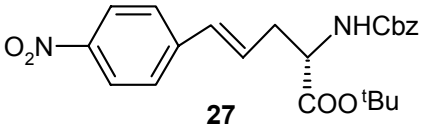
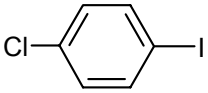
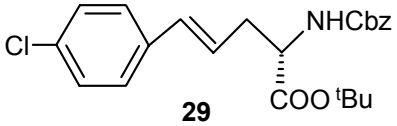
Scheme 6



a) $NaHCO_3$, Bu_4NBr, $Pd(OAc)_2$, CH_3CN, 70 °C .

In conclusion, the above strategy founded on the Heck reaction between allyl glycine and aromatic halide is indeed a simple and efficient method to prepare aryl allyl glycine derivative with high isomeric purity.

Table 1: Heck coupling between aryl halide and (S)-*N*-(benzyloxycarbonyl) allylglycine *tert*-butyl ester.

Entry	Substrate	Yield (%)	Product	E : Z [a]
1	 **20**	70	 **21**	96 : 4
2	 **22**	69	 **23**	100 : 0
3	 **24**	68	 **25**	98 : 2
4	 **26**	62	 **27**	96 : 4
5	 **28**	60	 **29**	100 : 0

a) determined on the basis of relative areas of the C(δ)H signal.

[*S*-(*E*)]-2-[(Benzyloxycarbonyl)amino]-5-phenyl-pent-4-enoic acid tert-butyl ester (21)

A mixture of iodobenzene (**20**) (0.10 g, 0.5 mmol), allylglycine derivative (**19**) (0.16 g, 0.5 mmol), $NaHCO_3$ (0.12 g, 1.4 mmol) and Bu_4NBr (0.17 g, 0.5 mmol) in CH_3CN (6.0 mL) was degassed. $Pd(OAc)_2$ (10.0 mg) was added to it and the reaction mixture was heated at 70 °C for 6 h. The solvent was removed and residue was purified on silica gel with light petroleum-ethyl acetate (10:1) as eluent to give **21** (0.13 g, 70 %), as syrup.

$[\alpha]_D$ + 39 (*c* 0.85, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (s, 9 H), 2.57 (m, 2 H), 4.42 (m, 1 H), 5.12 (s, 2 H), 5.42 (d, 1 H, *J* = 7.0 Hz), 6.09 (dt, 1 H, *J* = 7.3, 15.6 Hz), 6.46 (d, 1 H, *J* = 15.6 Hz), 7.3 (m, 10 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 28.0, 36.3, 54.0, 66.8, 82.2, 123.7, 126.2, 128.0, 128.4, 133.8, 136.3, 136.9, 155.6, 170.7;

EIMS *m/z*: 381 (M^+);

Anal. Calc for $C_{23}H_{27}NO_4$: C, 72.44; H, 7.08; N, 3.67. Found: C, 72.26; H, 7.12; N, 3.62.

[*S*-(*E*)]-2-[(Benzyloxycarbonyl)amino]-5-(4-methoxyphenyl)-pent-4-enoic acid tert-butyl ester (23)

A mixture of 1-iodo-4-methoxybenzene (**22**) (0.10 g, 0.4 mmol), allylglycine derivative (**19**) (0.14 g, 0.5 mmol), $NaHCO_3$ (0.11 g, 1.2 mmol) and Bu_4NBr (0.15 g, 0.5 mmol) in CH_3CN (6.0 mL) was degassed. $Pd(OAc)_2$ (9.0 mg) was added to it and the reaction mixture was heated at 70 °C for 6 h. The solvent was removed and residue was purified on silica gel with light petroleum-ethyl acetate (11:1) as eluent to give **23** (0.12 g, 69 %), as syrup.

$[\alpha]_D$ + 32 (*c* 1.4, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (s, 9 H), 2.66 (m, 2 H), 3.80 (s, 3 H), 4.38 (m, 1 H), 5.09 (ABq, 2 H, *J* = 10.0 Hz), 5.29 (d, 1 H, *J* = 8.1 Hz), 5.83 (dt, 1 H, *J* = 7.1, 15.3 Hz), 6.31 (d, 1 H, *J* = 15.3 Hz), 6.83 (d, 2 H, *J* = 7.3 Hz), 7.22 (d, 2 H, *J* = 7.3 Hz), 7.3 (s, 5 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 28.1, 36.5, 54.3, 55.3, 66.9, 82.1, 114.1, 121.5, 127.4, 128.1, 128.5, 129.9, 133.4, 155.6, 170.7;

EIMS *m/z*: 411 (M^+);

Anal. Calc for $C_{24}H_{29}NO_5$: C, 70.07; H, 7.05; N, 3.4. Found: C, 70.10; H, 7.22; N, 3.31.

[*S*-(*E*)]-5-[4-(Benzyloxycarbonyl)amino-4-tert-butoxycarbonyl-but-1-enyl]-2-methoxy-benzoic acid methyl ester (25)

A mixture of 5-iodo-2-methoxy-benzoic acid methylester (**24**) (0.10 g, 0.3 mmol), allylglycine derivative (**19**) (0.11 g, 0.4 mmol), $NaHCO_3$ (0.09 g, 1.0 mmol) and Bu_4NBr (0.12 g, 0.4 mmol) in CH_3CN (6.0 mL) was degassed. $Pd(OAc)_2$ (8.0 mg) was added to it and the reaction mixture was heated at 70 °C for 6 h. The solvent was removed and residue was purified on silica gel with light petroleum-ethyl acetate (12:1) as eluent to give **25** (0.11 g, 68 %), as syrup.

$[\alpha]_D$ + 35 (*c* 0.8, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (s, 9 H), 2.60 (m, 2 H), 3.84 (s, 6 H), 4.35 (m, 1 H), 5.08 (s, 2 H), 5.38 (d, 1 H, *J* = 7.6 Hz), 5.96 (dt, 1 H, *J* = 7.3, 15.8 Hz), 6.34 (d, 1 H, *J* = 15.8 Hz), 6.84 (d, 1 H, *J* = 7.5 Hz), 7.3 (m, 6 H), 7.71 (d, 1 H, *J* = 1.5 Hz);

^{13}C NMR (50 MHz, $CDCl_3$): δ 28.1, 36.5, 51.9, 54.2, 56.1, 66.9, 82.1, 112.4, 120.6, 123.1, 128.1`, 128.5, 129.6, 130.8, 132.4, 136.5, 155.5, 158.6, 166.4, 170.6;

EIMS *m/z*: 469 (M^+);

Anal. Calc for $C_{26}H_{31}NO_7$: C, 66.52; H, 6.61; N, 2.98. Found; C, 66.55; H, 6.49; N, 3.03.

[*S*-(*E*)]-2-[(Benzyloxycarbonyl)amino]-5-(4-nitrophenyl)-pent-4-enoic acid tert-butyl ester (27)

A mixture of 1-iodo-4-nitrobenzene (**26**) (0.10 g, 0.4 mmol), allylglycine derivative (**19**) (0.14 g, 0.5 mmol), $NaHCO_3$ (0.10 g, 1.2 mmol) and Bu_4NBr (0.14 g, 0.5 mmol) in CH_3CN (6.0 mL) was degassed. $Pd(OAc)_2$ (9.0 mg) was added to it and the reaction mixture was heated at 70 °C for 6 h. The solvent was removed and residue was purified on silica gel with light petroleum-ethyl acetate (9:2) as eluent to give **27** (0.11 g, 62 %), as syrup.

$[\alpha]_D$ + 34 (*c* 0.7, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.45 (s, 9 H), 2.74 (m, 2 H), 4.39 (m, 1 H), 5.09 (ABq, 2 H, *J* = 10.0 Hz), 5.49 (d, 1 H, *J* = 7.3 Hz), 6.30 (dt, 1 H, *J* = 8.0, 16.0 Hz), 6.50 (d, 1 H, *J* = 16.0 Hz), 7.32 (s, 5 H), 7.42 (d, 2 H, *J* = 8.0 Hz), 8.15 (d, 2 H, *J* = 8.0);

^{13}C NMR (50 MHz, $CDCl_3$): δ 28.0, 36.7, 53.8, 66.9, 82.5, 124.0, 126.6, 128.0, 128.5, 129.4, 131.7, 136.4, 143.2, 146.9, 155.5, 170.3;

EIMS *m/z*: 369 (M^+- ^{t}butyl);

Anal. Calc for $C_{23}H_{26}N_2O_6$: C, 64.78; H, 6.1; N, 6.57. Found: C, 64.90; H, 6.35; N, 6.47.

[*S*-(*E*)]-2-[(benzyloxycarbonyl)amino]-5-(4-chlorophenyl)-pent-4-enoic acid tert-butyl ester (29)

A mixture of 1-chloro-4-iodobenzene (**28**) (0.10 g, 0.4 mmol), allylglycine derivative (**19**) (0.14 g, 0.5 mmol), $NaHCO_3$ (0.1 g, 1.3 mmol) and Bu_4NBr (0.15 g, 0.5 mmol) in CH_3CN (6.0 mL) was degassed. $Pd(OAc)_2$ (9.0 mg) was added to it and the reaction mixture was heated at 70 °C for 6 h. The solvent was removed and residue was purified on silica gel with light petroleum-ethyl acetate (12:1) to give **29** (0.11 mg, 60 %), as syrup.

$[\alpha]_D$ +39 (*c* 0.7, $CHCl_3$);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (s, 9 H), 2.67 (m, 2 H), 4.39 (m, 1 H), 5.09 (ABq, 2 H, *J* = 10.0 Hz), 5.40 (d, 1 H, *J* = 8.0 Hz), 6.04 (dt, 1 H, *J* = 8.0, 16.0 Hz), 6.37 (d, 1 H, *J* = 16.0 Hz), 7.3 (m, 9 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 28.1, 36.5, 54.0, 66.9, 82.3, 124.6, 127.4, 128.1, 128.5, 128.7, 132.6, 133.2, 135.4, 136.3, 155.6, 170.5;

EIMS *m/z*: 415 (M^+);

Anal. Calc for $C_{23}H_{26}ClNO_4$: C, 66.42; H, 6.25; N, 3.36. Found: C, 66.66; H, 6.37; N, 3.48.

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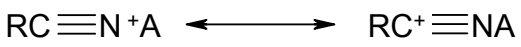
INTRODUCTION

Nitrile is a key constituent in numerous natural products, and it also serves as an important synthetic intermediate for pharmaceuticals, agricultural chemicals, dyes, and material sciences.[1] Nitriles can be converted to amides, carboxylic acids, amines, ketones and esters.[2] The reactivity of nitriles is fundamentally due to the polarization of the C≡N triple bond, which arise from the greater electronegativity of nitrogen compared to carbon, RC≡N $\leftrightarrow$ $RC^+ = N^-$. Neucleophilic reagents will attack at the electrophilic carbon atom while the nitrogen atom is weakly basic site. Interaction with an acid A^+ enhances the polarization and gives a species having increased susceptibility to nucleophilic attack.[3]



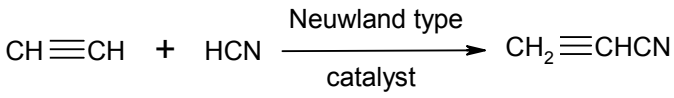
GENERAL METHODS FOR SYNTHESIS OF NITRILES

The synthetic methods for preparation of nitriles can be related to four reaction types: addition, substitution, elimination and conversion of other nitriles.

A) Preparation of nitriles by addition of HCN

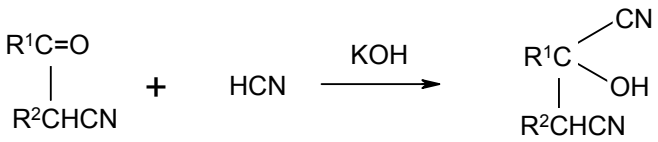
Acetylenes readily add hydrogen cyanides (Scheme **1**). The reaction requires a Neuwland-type catalyst, an aqueous solution of cuprous chloride, ammonium chloride and hydrogen chloride. Thus a yield of 80 % acrylonitrile is obtained at 80 °C from acetylene and hydrogen cyanide.[4]

Scheme 1



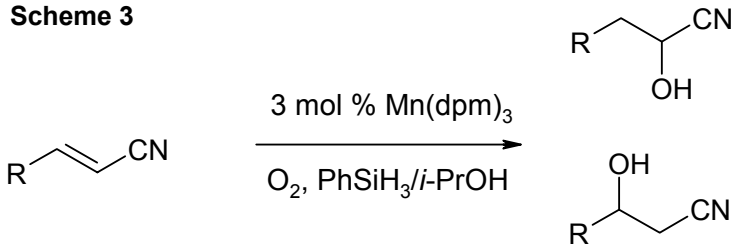
The reaction of the carbonyl group with hydrogen cyanide yields cyanohydrins. The first synthesis of aromatic cyanohydrins was reported in 1832,[5] that of an aliphatic one in 1867.[6] Several methods have been developed for the synthesis of cyanohydrins.

Scheme 2



α,β-Unsaturated nitriles can be converted to cyanohydrins by using tris(dipivaloylmethanato) manganese(III) [abbreviated to $Mn(dpm)_3$],[7] phenylsilane and oxygen (Scheme **3**). Approximately 1:1 mixtures of the *E*- and *Z*-isomers were obtained.

Scheme 3

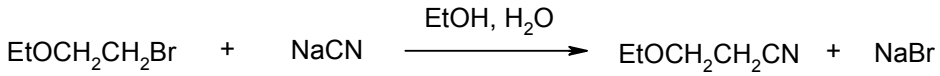


B) Preparation of nitriles by substitution

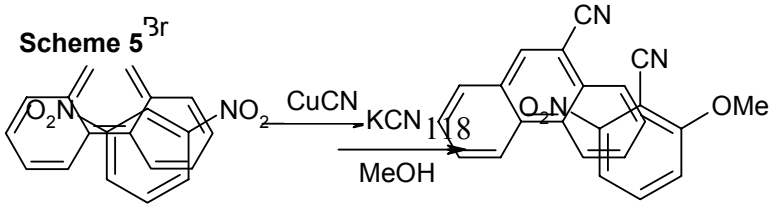
The reaction between organic halogen compounds and metal cyanides is a frequently used nitrile synthesis (Scheme **4**). [8] Often a certain amount of isonitrile is formed along with the nitrile. Alkali cyanides predominantly yield nitriles, whereas heavy metal cyanides such as copper, silver and mercury cyanide give increasing amount of isonitriles. At reaction temperature above 150 °C the yield of nitrile increases, since at about that temperature, considerable isomerization of isonitriles to nitriles already takes place.

The reaction of alkali cyanides with polynitrobenzenes and substituted phenols has found limited application because of the complexity of the products formed. A relative simple

Scheme 4



Scheme 5



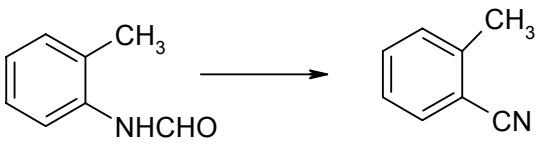
example is the substitution of a hydrogen atom by cyanide in *m*-dinitrobenzene (Scheme **5**). [9]

In 1884 Sandmeyer[10] discovered a nitrile synthesis, which consists of the reaction of an aromatic diazonium salt with an aqueous solution of cuprous cyanide and potassium cyanide (Scheme **6**). With some modifications this is still a very important method for synthesis of aromatic nitriles.

C) Preparation of nitriles by conversion of other nitriles

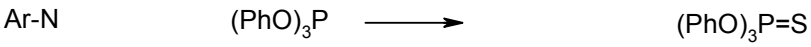
Isonitriles start to rearrange to nitriles at temperature around 150 °C. Correspondingly, reactions that give isonitriles in the first step will produce nitriles if performed at a sufficiently high temperature. Thus pyrolysis of *N*-formyl amines at temperatures around 500 °C in presence of a silica gel catalyst yields nitriles (Scheme **7**). [11]

Scheme 7



Aryl isothiocyanates can be desulphurized by treatment with triphenyl phosphite, the resulting isonitriles subsequently rearranging to nitriles (Scheme **8**). [12]

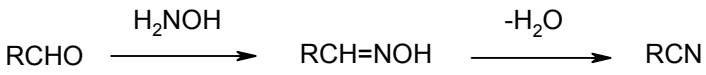
Scheme 8



D) Preparation of nitriles by elimination

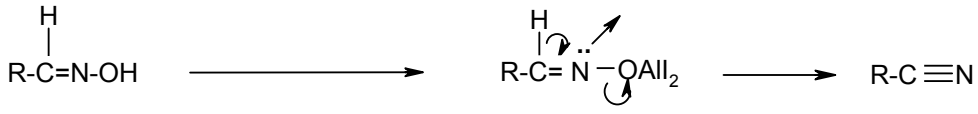
The most frequently used method to convert aldehydes to nitriles is the dehydration of the corresponding oximes (Scheme **9**).

Scheme 9



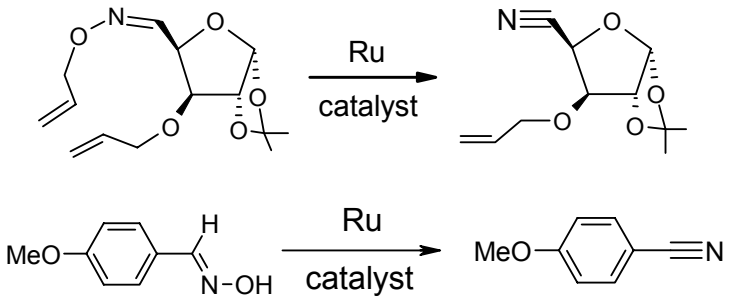
This may be affected by a number of metal/nonmetal reagents.[13] However, nonmetal reagents suffer from some disadvantages such as acidic reagents[14] or give rise to acidic byproducts, which is detrimental to acid sensitive substrates, inconvenient preparation of the reagents[15], limited substrate scope[16], incompatibility of sensitive groups to the reaction conditions.[17] Recently, there have been several reports describing dehydration methods of aldoximes with the use of stoichiometric amounts of certain main or transition metal complexes. [18]

Scheme 10



Despite the recent progress, there is still a strong need for a preparative method of highly efficient and catalytic conversion of aldoximes to nitriles under neutral conditions. In the present chapter described herein is a realization of this goal with Ru catalyst. [19]

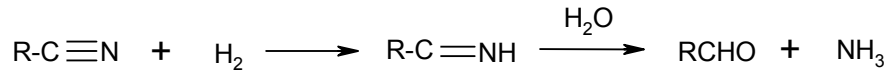
Scheme 11



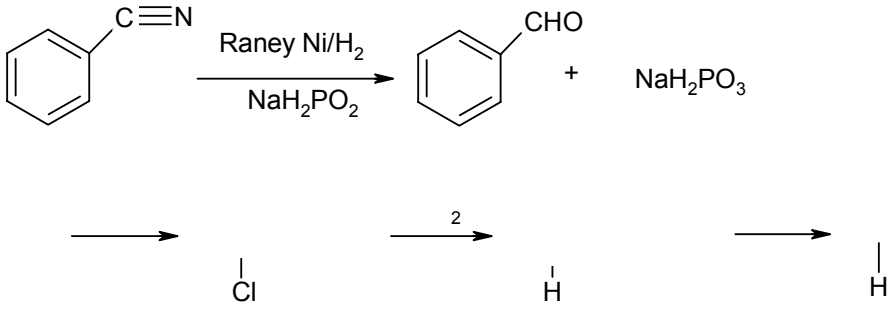
REACTION OF NITRILES

Reduction

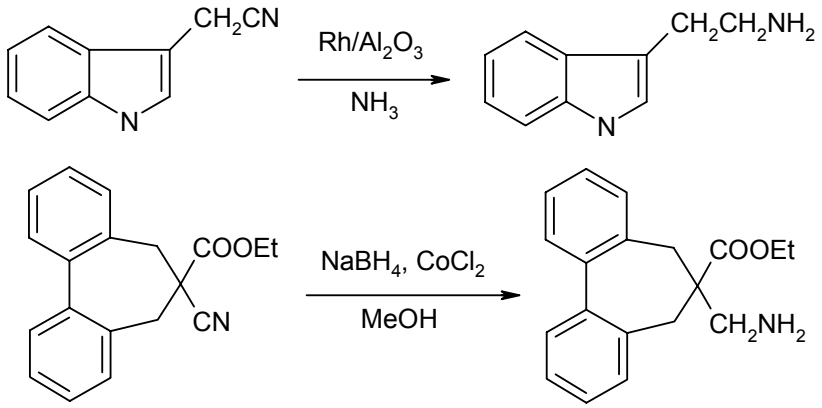
The cyano group of the nitriles is unsaturated. As a consequence it enter into reaction with a large variety of reagents, resulting in partial or complete loss of unsaturation. Important among these reactions is reduction, in which hydrogen is added to the triple bond. The most important reduction products are aldehydes and amines, which can be utilized, in many organic reactions. The conversion of nitriles to aldehyde proceeds via an aldimine intermediate, which is further hydrolysed to the aldehyde.[20]



Reduction of nitrile with Raney nickel in the presence of sodium hypophosphite gave corresponding aldehyde in excellent yields.[21] In the Stephen method, dry hydrogen chloride in ether reacts with nitrile and subsequent reduction by $SnCl_2$ gave aldehyde.[22]

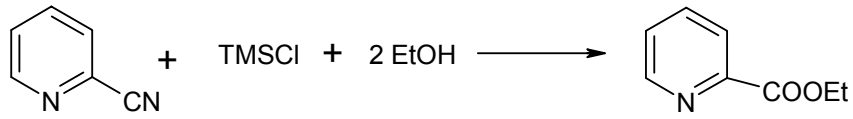


Amines are the usual final reduction products of nitriles. Various methods are described in the literature about the reduction of nitriles to amines. They are mainly catalytic hydrogenation [23] and hydride reduction.[24]



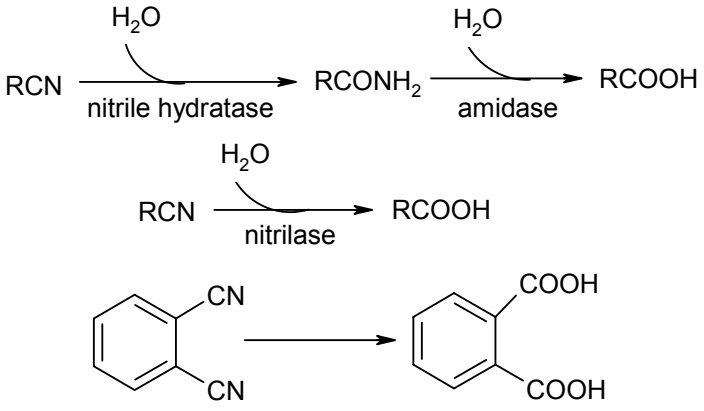
Transformation of nitriles into ester

There is plethora of reports in the literature to bring about the same transformation. Most of the methods reported involve using gaseous HCl or strong acid at higher temperature.[25] Treatment of nitriles with alcohol and TMSCl at 50 °C could give ester in good yields.[26]



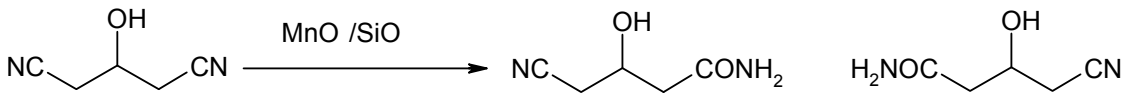
Hydrolysis of Nitriles to Acid

The ability of enzymes to hydrolyse nitriles is well known,[27] selective hydrolysis has been demonstrated in some instances[28] and the mechanism of some of these enzymes has been extensively studied. [29] Two distinct pathways have been recognised,[27b] namely the step wise conversion of nitriles to amides (*via* a hydratase) followed by hydrolysis of the amide to a carboxylic acid (via an amidase), or the direct conversion of nitriles to carboxylic acids (via a nitrilase).[30]



Dehydartion of Nitriles to Amide

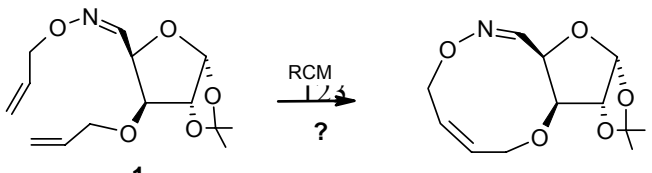
The classical methods of dehydration of nitriles to amides are usually involving the use of either concentrated acids or hydrogen peroxide in alkaline medium. β-hydroxy-nitriles can be converted into corresponding amides by treating with manganese dioxide, deposited onto silica gel for a few day at room temperature.[31]



PRESENT WORK

Synthesis of novel heterocyclic derivatives fused to sugar backbone is of current interest.[32] We have identified[33] ring closing metathesis (RCM) [34] reaction as a tool to fabricate some novel heterocyclic molecules. Synthesis of cyclic molecules of the type **2** from sugar dienes (**1**) by using RCM method was undertaken (Scheme **1**) in the present work.

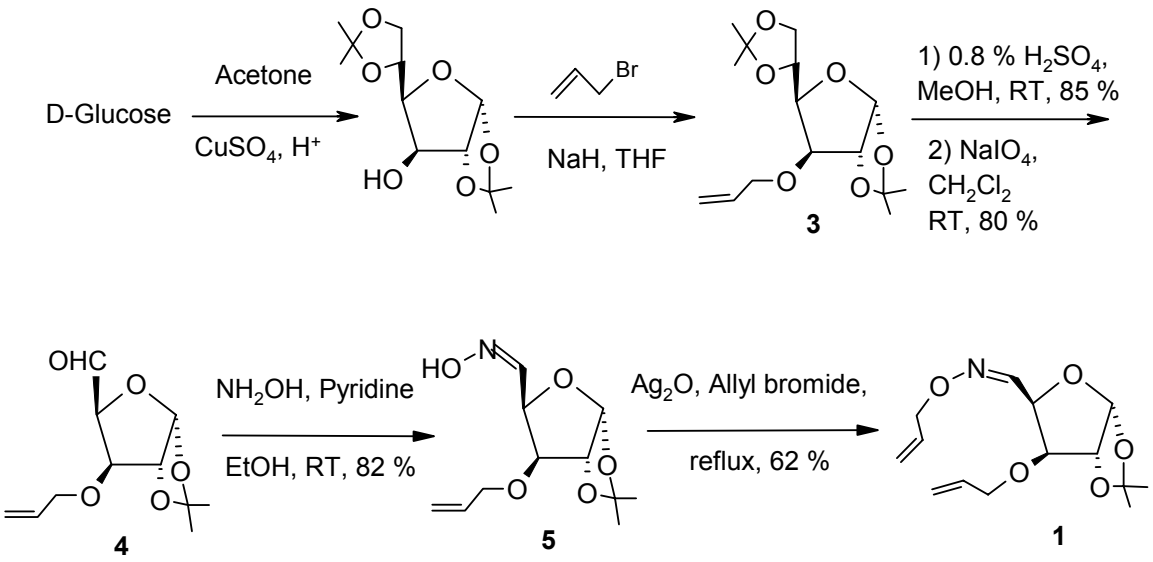
Scheme 1



1 **2**

Preparation of **1** was accomplished from 3-*O*-allyl-1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose (**3**) in 4 steps (Scheme **2**).[35] The acetonide group in compound **3** was deprotected with 0.8 % H_2SO_4 in methanol and the diol that formed was treated with $NaIO_4$ to give aldehyde **4**, which was subsequently treated with hydroxylamine hydrochloride and pyridine in ethanol to provide oxime **5**.

Scheme 2



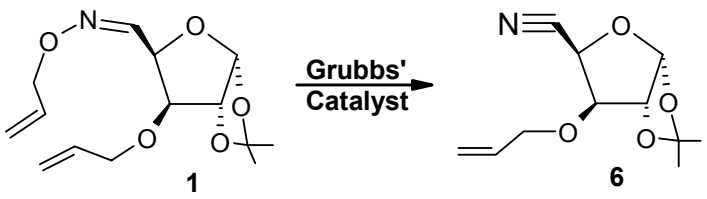
In the ^{1}H NMR spectrum of **5**, characteristic signal due to oxime proton was observed as a set of broad doublet at 8.12 and 8.53 ppm. Treatment of **1** with 3 mol % of Grubbs' catalyst $Cl_2(PCy_3)_2Ru$=CHPh in benzene at 60 °C gave a single major product whose structure was not in conformity with expected product (**2**). Based on spectral data and elemental analysis, structure **6** was proposed for the new product. For example, in the ^{1}H NMR spectrum of **6**, the resonances due to H-4 was observed at 4.81 ppm as a doublet ($J_{3,4}$ = 3.5 Hz) while rest of the spectra was in complete agreement with the assigned structure **6**. The ^{13}C NMR ($\delta_{C\equiv N}$ = 115.5 ppm) and IR ($\gamma_{C\equiv N}$ = 2360 cm^{-1}, weak) spectra of compound **6** also supported the assigned structure (Scheme **3**).

The cyanide functionality is useful in many chemical transformations.[1] Although several methods are reported for its preparation, conversion of oxime to nitrile using a dehydrating reagent is a widely used methodology.[13] The scanty reports on the synthesis of sugar substituted nitriles[36] may be attributed to the use of acidic reagents in the existing

methods,[14] which may cleave acid labile groups such as isopropylidene group, leading to a mixture of products. The method reported herein involves mild conditions and hence the acid labile groups are tolerated.[19]

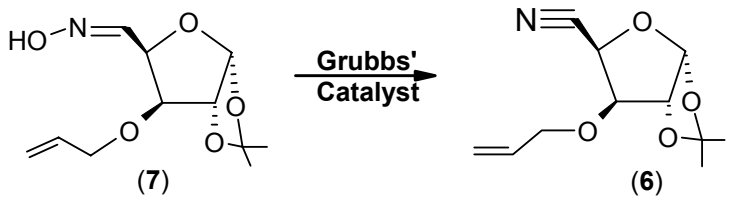
In order to probe the unexpected nitrile formation, we performed several experiments to address some critical issues. For instance, unprotected oxime (**7**) also underwent transformation (Scheme **4**) with catalytic amount of $Cl_2(PCy_3)_2Ru=CHPh$ to obtain the same nitrile derivative (**6**), thus, indicating that protecting groups are not needed (Table **1**). The 3-oxime derivative (**8**) as well as acetophenone oxime (**9**), without the α-hydrogen, were inert to the treatment of $Cl_2(PCy_3)_2Ru=CHPh$ in benzene at 60 °C, suggesting the essentiality of α-proton.

Scheme 3

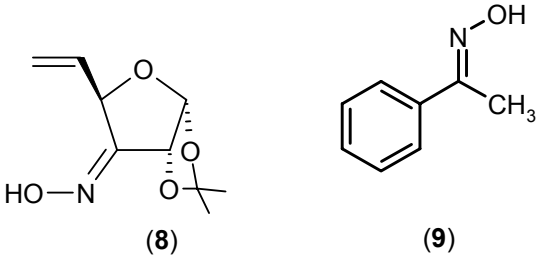


1 **6**

Scheme 3



(**7**) (**6**)

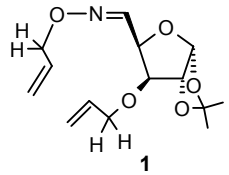
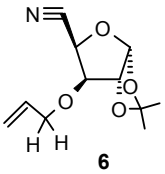
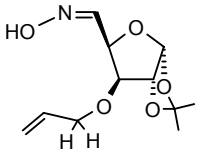
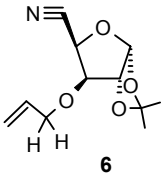
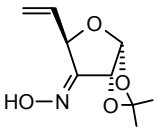
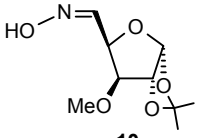
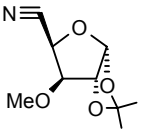
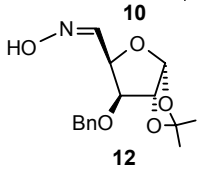
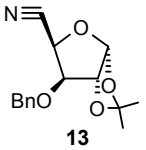
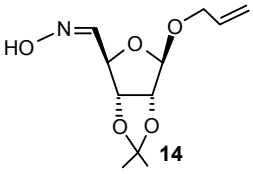
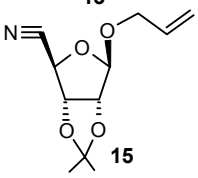
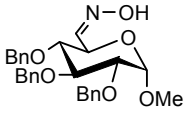
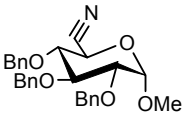
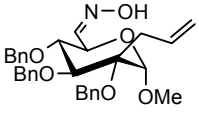
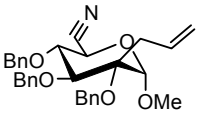
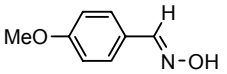
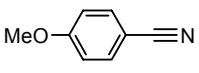


(**8**) (**9**)

Conversion of **1** and **7** into **6** also occurred with 3 mol % of $Ru(PPh_3)_3Cl_2$ in benzene at 60 °C. However, with 3 mol % of $RuCl_3$, transformation of **1** → **6** was sluggish. With addition of increased quantity of $RuCl_3$ (10 mol %), complete conversion was observed in refluxing benzene after 16 h. With PPh_3 alone in refluxing benzene, compound **1** and **7** were recovered unchanged. The versatility of this method was evident by several examples as shown in Table **1**.

In conclusion, we have developed a new method of conversion of sugar-oximes into sugar-nitriles with Grubbs' catalyst as well as other ruthenium salts. The reaction is high yielding, with easy workup procedure and involves mild conditions, which enables acid labile isopropylidene groups to remain intact. This method is also applicable to aryl-oxime as depicted in entry **9** (Table **1**).

Table 1: Conversion of Aldoximes to Nitriles

Entry	Oxime	Reagent [a]	Time in hrs [b]	Nitrile	Yield (%) [c]
1.	 **1**	$Cl_2(PCy_3)_2Ru=CHPh$	10.5	 **6**	81
2.	 **5**	$Cl_2(PCy_3)_2Ru=CHPh$ $(PPh_3)_3RuCl_2$ $RuCl_3$	10 10 16	 **6**	85 81 81
3.	 **8**	$Cl_2(PCy_3)_2Ru=CHP$	24	No Product	-
4.	 **10**	$Cl_2(PCy_3)_2Ru=CHPh$ $(PPh_3)_3RuCl_2$ $RuCl_3$	6 6.5 15	 **11**	88 83 80
5.	 **12**	$Cl_2(PCy_3)_2Ru=CHPh$	9	 **13**	78
6.	 **14**	$Cl_2(PCy_3)_2Ru=CHPh$	1	 **15**	90
7.	 **16**	$Cl_2(PCy_3)_2Ru=CHPh$	8	 **17**	76
8.	 **18**	$Cl_2(PCy_3)_2Ru=CHPh$ $(PPh_3)_3RuCl_2$ $RuCl_3$	9 9 16	 **19**	79 76 70
9.	 **20**	$Cl_2(PCy_3)_2Ru=CHPh$	12	 **21**	75

(a) $Cl_2(PCy_3)_2Ru=CHPh$ (3 mol %), $(PPh_3)_3RuCl_2$ (3 mol %) , $RuCl_3$ (10 mol %); (b) reaction were carried out in benzene at 60°C (c) isolated

General Procedure

Oxime (0.5 g, 2.05 mmol) in dry benzene (10 mL) was heated at 60 °C in the presence of ruthenium salts (3 mol % of $Cl_2(PCy_3)_2Ru=CHPh$ / $Cl_2Ru(PPh_3)$ or 10 mol % of $RuCl_3$) under nitrogen atmosphere. After completion of the reaction solvent was removed and the residue purified on silica gel (60-120 mesh) with EtOAc and light petroleum ether as eluent to give respective nitrile products.

3-*O*-Allyl-4-*C*-[(*O*-allyl)-oxime]-4-deoxy-1,2-*O*-isopropylidine-α-D-glucofuranose (1)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.31, 1.52 (2s, 6 H), 4.07 (m, 3 H), 4.63 (m, 4 H), 5.27 (m, 4 H), 5.85 (m, 2 H), 5.92 (d, 1 H, *J* = 3.6 Hz), 6.85 (d, 0.25 H, *J* = 2.4 Hz), 7.44 (d, 0.75 H, *J* = 7.4 Hz);

^{13}C NMR (50 MHz, $CDCl_3$): δ 26.3, 26.6, 71.4, 74.3, 74.8, 75.9, 78.0, 82.1, 82.3, 82.5, 83.6, 104.7, 105.2, 111.6, 111.7, 117.7, 133.4, 133.8, 146.7, 148.4.

3-*O*-Allyl-4-*C*-oxime-4-deoxy-1,2-*O*-isopropylidine-α-D-glucofuranose (5)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35, 1.51 (2s, 6 H), 4.08 (m, 3 H), 4.65 (m, 2 H), 5.24 (m, 2 H), 5.78 (m, 1 H), 5.95 (d, 1 H, *J* = 3.2 Hz), 6.91 (d, 0.35 H, *J* = 1.4 Hz), 7.46 (d, 0.65 H, *J* = 7.1 Hz), 8.12 (brs, 0.35 H), 8.48 (brs, 0.65 H).

3-*O*-Allyl-4-*C*-cyano-4-deoxy-1,2-*O*-isopropylidine-α-D-glucofuranose (6)

IR: $\gamma_{C\equiv N}$ 2360 cm^{-1} (weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.24, 1.40 (2s, 6 H), 4.12 (d, 1 H, *J* = 3.5 Hz), 4.25 (m, 2 H), 4.54 (d, 1 H, *J* = 3.5 Hz), 4.81 (d, 1 H, *J* = 3.5 Hz), 5.33 (m, 2 H), 5.90 (m, 1 H), 5.94 (d, 1 H, *J* = 3.5 Hz);

^{13}C NMR (50 MHz, $CDCl_3$): δ 26.4, 27.6, 70.2, 82.3, 82.9, 105.9, 113.6, 115.5, 119.3, 134.0;

Anal. calcd. for $C_{11}H_{15}NO_4$: C, 58.6; H, 6.6; N, 6.2. Found: C, 58.5; H, 6.8; N, 6.2.

5-Deoxy-1,2-*O*-isopropylidine-5-eno-3-*C*-oxime-α-D-glucofuranoside (8)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.34, 1.52 (2s, 6 H), 4.59 (t, 1 H, *J* = 3.6 Hz), 5.26 (m, 3 H), 5.81 (m, 1 H), 5.94 (d, 1 H, *J* = 3.7 Hz), 8.11 (brs, 0.6 H), 8.51 (brs, 0.4 H).

4-*C*-Oxime-4-deoxy-1,2-*O*-isopropylidine-3-*O*-methyl-α-D-glucofuranoside (10)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.33, 1.51 (2s, 6 H), 3.40 (d, 3 H, *J* = 2.9 Hz), 3.80 (d, 0.5 H, *J* = 2.9 Hz), 4.13 (d, 0.5 H, *J* = 2.9 Hz), 4.60 (t, 1 H, *J* = 4.1 Hz), 4.71 (dd, 0.5 H, *J* = 3.4, 7.3 Hz), 5.18 (t, 0.5 H, *J* = 3.4 Hz), 5.94 (d, 1 H, *J* = 3.9 Hz), 6.88 (d, 0.5 H, *J* = 3.9 Hz), 7.43 (0.5 H, *J* = 7.8 Hz), 8.39 (brs, 0.5 H), 8.79 (brs, 0.5 H).

4-*C*-cyano-4-deoxy-1,2-*O*-isopropylidine-3-*O*-methyl-α-D-glucofuranoside (11)

IR: $\gamma_{C\equiv N}$ 2365 cm^{-1} (weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25, 1.41 (2s, 6 H), 3.49 (s, 3 H), 3.92 (d, 1 H, *J* = 3.4), 4.55 (d, 1 H, *J* = 3.9 Hz), 4.79 (d, 1 H, *J* = 3.4), 5.90 (d, 1 H, *J* = 3.4);

^{13}C NMR (50 MHz, $CDCl_3$): δ 26.1, 26.9, 58.7, 69.1, 81.5, 84.3, 105.5, 112.9, 114.5;

Anal. calcd. for $C_9H_{13}NO_4$: C 54.26, H 6.58, N 7.03. Found: C 54 48, H 6.75, N 6.79.

3-O-benzyl-4-*C*-oxime-4-deoxy-1,2-*O*-isopropylidine-3-*O*-α-D-glucofuranoside (12)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.32, 1.50 (2s, 6 H), 4.36 (d, 1 H, *J* = 2.9 Hz), 4.57 (m, 3 H), 5.21 (t, 1 H, *J* = 3.6 Hz), 5.98 (d, 1 H, *J* = 3.9 Hz), 6.97 (d, 0.9 H, *J* = 3.9 Hz), 7.28 (m, 5 H), 8.15 (brs, 0.1 H), 8.30 (brs, 0.9 H).

3-O-benzyl-4-*C*-cyano-4-deoxy-1,2-*O*-isopropylidine-3-*O*-α-D-glucofuranoside (13)

IR: $\gamma_{C\equiv N}$ 2250 cm^{-1} (weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (s, 3 H), 1.38 (s, 3 H), 4.15 (d, 1 H, *J* = 3.1 Hz), 4.58 (d, 1 H, *J* = 3.1 Hz) 4.77 (s, 2 H), 4.86 (d, 1 H, *J* = 3.1 Hz), 6.00 (d, 1 H, *J* = 3.1 Hz), 7.3 (m, 5 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 25.9, 27.2, 69.2, 72.6, 81.9, 105.2, 112.4, 114.1, 127.6, 128.0, 128.4.

Anal. calcd. for $C_{15}H_{17}NO_4$: C, 65.45; H, 6.18; N, 5.09. Found: C, 65.60; H, 6.36; N, 5.05.

Allyl-4-*C*-oxime-4-deoxy-2,3-*O*-isopropylidine-3-*O*-α-D-ribofuranoside (14)

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.31, 1.44 (2s, 6 H), 4.11 (m, 2 H), 4.75 (m, 3 H), 5.24 (m, 3 H), 5.86 (m, 1 H), 6.77 (d, 0.35 H, *J* = 4.4 Hz), 7.35 (d, 0.65 H, *J* = 7.0 Hz), 8.38 (brs, 0.65 H), 8.93 (brs, 0.35 H).

Allyl-4-*C*-cyano-4-deoxy-2,3-*O*-isopropylidine-3-*O*-α-D-ribofuranoside (15)

IR: $\gamma_{C\equiv N}$ 2295 cm^{-1} (Weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 1.29, 1.38 (2s, 6 H), 4.00 (dd, 1 H, *J* = 5.9, 11.7 Hz) 4.29 (dd, 1 H, *J* = 4.4, 11.7 Hz), 4.76 (m, 2 H), 5.25 (m, 4 H), 5.94 (m, 1 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 24.9, 26.0, 67.6, 70.9, 82.3, 83.4, 106.9, 113.4, 115.6, 117.2, 132.5;

Anal calcd. for $C_{11}H_{15}NO_4$: C, 58.6; H, 6.6; N, 6.2. Found: C, 58.8; H, 6.7; N, 6.2.

Methyl-2,3,4-tri-*O*-benzyl-5-*C*-oxime-5-deoxy-α-D-glucopyranoside (16)

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.37 (m, 5 H), 4.0 (t, 1 H, *J* = 9.3 Hz), 4.28 (dd, 1 H, *J* = 4.2, 6.3 Hz), 4.78 (m, 7 H), 7.32 (m, 16 H), 8.03 (brs, 0.85 H).

Methyl-2,3,4-tri-*O*-benzyl-5-*C*-cyano-5-deoxy--α-D-glucopyranoside (17)

IR: $\gamma_{C\equiv N}$ 2240 cm^{-1} (Weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.40 (s, 3 H), 3.57 (m, 3 H), 4.62 (m, 8 H), 7.32 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): 56.1, 60.1, 73.5, 75.7, 75.8, 78.9, 79.4, 80.4, 98.7, 117.1, 127.7, 127.8, 127.9, 128.0, 128.3, 136.9, 137.7, 138.2;

Anal. calcd. for $C_{28}H_{29}NO_5$: C 73.18, H 6.36, N 3.05. Found: C 73 29, H 6.59, N 3.17.

Methyl-2-*C*-allyl-2,3,4-tri-*O*-benzyl-5-C-oxime-5-deoxy--α-D-glucopyranoside (18)

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.48 (dd, 1 H, *J* = 8.3, 13.1 Hz), 2.92 (dd, 1 H, *J* = 5.4, 13.2 Hz), 3,41 (s, 3 H), 5.65 (t, 1 H, *J* = 9.4 Hz), 4.26 (m, 2 H), 4.75 (m, 7 H), 5.17 (m, 2 H), 6.07 (m, 1 H), 7.28 (m, 16 H), 7.62 (brs, 0.9 H).

Methyl-2-*C*-allyl-2,3,4-tri-*O*-benzyl-5-*C*-cyano-5-deoxy--α-D-glucopyranoside (19)

IR: $\gamma_{C\equiv N}$ 2335 cm^{-1} (Weak);

^{1}H NMR (200 MHz, $CDCl_3$): δ 2.52 (dd, 1 H, *J* = 7.7, 12.9 Hz), 2.96 (dd, 1 H, *J* = 5.8, 12.9 Hz), 3.42 (s, 3 H), 3.93 (t, 1 H, *J* = 9.6 Hz), 4.12 (d, 1 H, *J* = 9.6 Hz), 4.51 (d, 1 H, *J* = 9.6 Hz), 4.61 (d, 1 H, *J* = 12.9 Hz), 4.84 (m, 6 H), 5.22 (m, 2 H), 6.1 (m, 1 H), 7.32 (m, 15 H);

^{13}C NMR (50 MHz, $CDCl_3$): δ 35.7, 56.3, 62.6, 65.4, 75.1, 75.7, 79.1, 79.6, 81.4, 102.0, 117.4, 118.5, 126.7-128.2, 132.8, 136.8, 138.0, 138.5;

Anal. calcd. for $C_{31}H_{33}NO_5$: C, 74.5; H, 6.6; N, 2.8. Found: C, 74.3; H, 6.7; N, 2.9.

4-Methoxy-benzyloxime (20)

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.83 (s, 3 H), 6.94 (d, 2 H, *J* = 10.9 Hz), 7.49 (d, 1 H, *J* = 10.9 Hz), 8.12 (s, 1 H), 9.49 (brs, 1 H).

4- methoxy-benzonitrile (21)

IR: $\gamma_{C\equiv N}$ 2349 cm^{-1};

^{1}H NMR (200 MHz, $CDCl_3$): δ 3.92 (s, 3 H), 6.97 (d, 2 H, *J* = 10.2 Hz), 7.63 (d, 2 H, *J* = 10.2 Hz);

^{13}C NMR (50 MHz, $CDCl_3$): δ 55.3, 104.6, 113.7, 118.6, 133.8;

Anal. calcd. for C_8H_7NO: C 72.16, H 5.30, N 10.52. Found: C 72.33, H 5.13, N 10.26.

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