SYNTHESIS OF α-*C*-D-GLUCOSPHINGOSINE, CALLIPELTOSIDE AND CMI-546

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

TO

OSMANIA UNIVERSITY

BY

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ORGANIC CHEMISTRY: TECHNOLOGY
NATIONAL CHEMICAL LABORATORY
PUNE 411 008

AUGUST 2000

DEDICATED

TO

MY BELOVED PARENTS

CERTIFICATE

The research work presented in this thesis entitled "**Studies Directed toward the Syntheses of α-*C*-D-Glucosphingosine, Callipeltose and CMI-546**" has been carried out under my supervision and is a bonafide work of **Mr. Ranga Reddy**. This work is original and has not been submitted for any other degree or diploma of this or any other University.

Pune 8
5 August 2000

(M. K. GURJAR)
Research Guide

DECLARATION

The research work embodied in this thesis has been carried out at Indian Institute of Chemical Technology, Hyderabad, under supervision of **Dr. M. 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 part or full, for any degree or diploma of this or any other University.

Pune 8 (RANGA REDDY)
5 August 200

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RANGA REDDY

ABBREVIATIONS/ACRONYMS

AcOH	:	Acetic acid
Ac	:	Acetyl
Ac_2O	:	Acetic anhydride
AIBN	:	Azobis isobutyronitrile
Bn	:	Butyloxy carboxyl
BOC	:	Benzyl
DCM	:	Dichloromethane
DIBAL-H	:	Diisobutylaluminium hydride
DIPT	:	Diisopropyl tartarate
DMSO	:	Dimethyl sulfoxide
DMAP	:	4-(Dimethylamino pyridine
DMF	:	Dimethylformamide
EtOH	:	Ethanol
Et	:	Ethyl
EtOAc	:	Ethyl acetate
IDCP	:	Iodonium di-(S-collidine) perchlorate
LAH	:	Lithium alunimium hydride
MeOH	:	Methanol
mCPBA	:	meta-chloroperbenzoic acid
NMO	:	4-Methylmorpholine N-oxide
Ph	:	Phenyl
Py	:	Pyridine
PTSA	:	para-toluenesulfonic acid
TBS	:	tert-butyldimethyl silyl
TBDPS	:	tert-butyldiphenylsilyl
TBHP	:	tert-butylhydroperoxide
TMS-Br	:	Trimethylsilyl bromide
TBAF	:	Tetrabutylammonium fluoride

THF	:	Tetrahydro furan
TFA	:	Trifluoroacetic acid
TFAA	:	Trifluoroacetic anhydride
TPP	:	Triphenylphosphine
Ts	:	Tosyl

GENERAL REMARKS

1. NMR spectra were recorded on Varian Gemini 200 instrument in ppm. IR spectra were recorded on Schimadazu IR-470 and Perkin-Elmer 283b instruments measured in cm^{-1}. Mass spectra were recorded on Finnigan Mat 1210 spectrometer under Electron Impact (EI) or Chemical Ionisation (CI) condition. FABMS were recorded on VG Autospec (M series). Melting points determined on Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured on TASCO DIP-300 instrument.

2. All reactions were monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) with UV light, I_2 and α-napthal (or) anisaldehyde-heat as developing agents. Acme, India silica gel (60-120 mesh) was used for column chromatography.

3. All reactions were carried out under nitrogen atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Organic extracts were dried over sodium sulfate unless otherwise mentioned.

4. All solvents and reagents were dried utilizing standard techniques. All evaporations were done on Buchi rotary evaporator under reduced pressure below 40°C.

CONTENTS

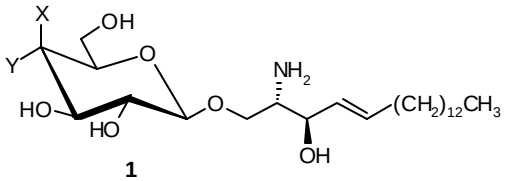
ABSTRACT

The thesis entitled "Studies directed toward the syntheses of á-*C*-D-Glucosphingosine, callipeltose and CMI-546" is divided into three chapters. The first chapter deals with the synthesis of á-*C*-D-glucosphingosine, where as second chapter describes the synthesis of callipeltose and third chapter deals with the synthesis of CMI-546.

CHAPTER –I

NEW SYNTHESIS OF α-*C*-D-GLUCOSPHINGOSINE DERIVATIVE

Glycosphingosine (**1**) and their dihydroderivatives (**2**) are of immense importance as they are present on mammalian tissues and show ability to be involved numerous physiological functions, most importantly in transmembrane signalling. α-Galactosyl ceramide structures namely agelaspins (**3**) from marine sponge agelas–*mauritianus* are recognized as potential antitumor and immunostimulant agents. Surprisingly, β anomers of agelaspins are inactive.



1

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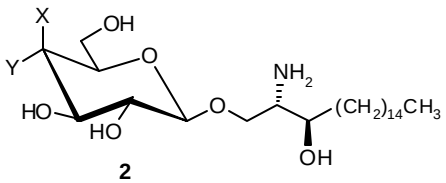
Y = H, X = OH



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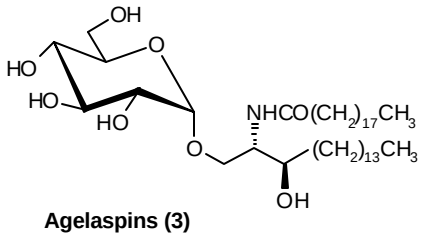
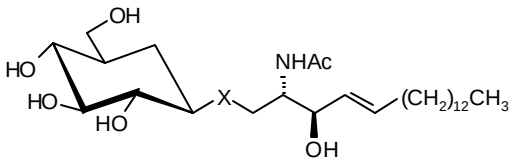


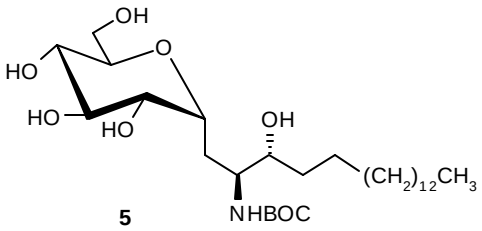
Agelaspins (3)



4 X = O, NH, S

In order to understand the mode of action of glycosphingolipids, many N- and S-glycosyl analogues and also of carbasugars (**4**) were synthesized and tested. However, to our

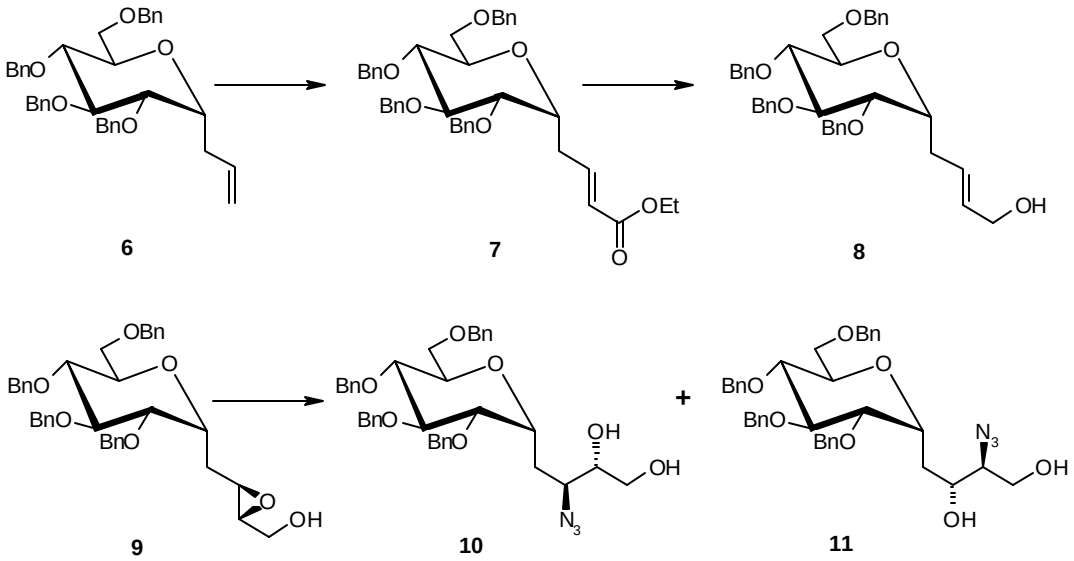
knowledge, no attempt has yet been made to prepare *C*-glycospingosine such as **5** having *C*-glycoside residue. These analogues possessing non-hydrolysable (metabolically stable) *C*-glycoside unit will be interesting and useful products since many *C*-glycosides are recognized as glycosidase inhibitors. In this background we put forward for a stereocontrolled synthesis of á-*C*-D-glucosphingosine (**5**) as a general strategy for these novel compounds.



5

The synthesis of **5** was started with 3-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-1-propene (**6**) which was obtained from glucosepentaacetate by the procedure known in literature. Subsequent oxidative cleavage of the double bond using OsO_4-NMO-$Pb(OAc)_4$ furnished the aldehyde which was treated with $Ph_3P{=}CHCO_2Et$ in CH_2Cl_2 to afford (E)-α, β-unsaturated ester (**7**). Reduction of ester **7** with DIBAL-H at -78 °C followed by Sharpless asymmetric epoxidation using (-)-DIPT as a chiral ligand afforded the epoxide **9**.

Scheme-1



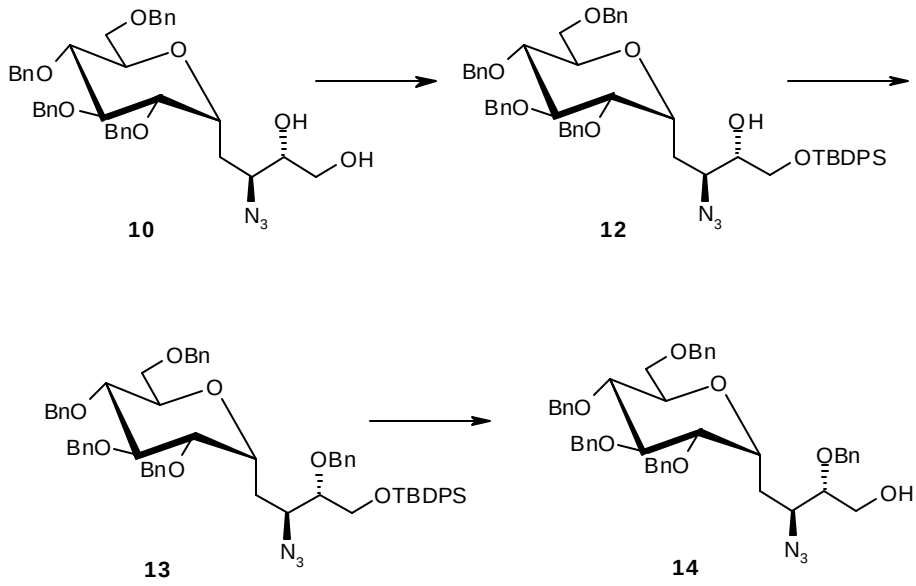
6 **7** **8**

9 **10** **11**

Our next endeavour was to regioselectively open the epoxide ring with azide. The epoxide was treated with NaN_3 and NH_4Cl to give 1,2-diol (**10**) and the corresponding 1,3-diol (**11**) (Scheme-1).

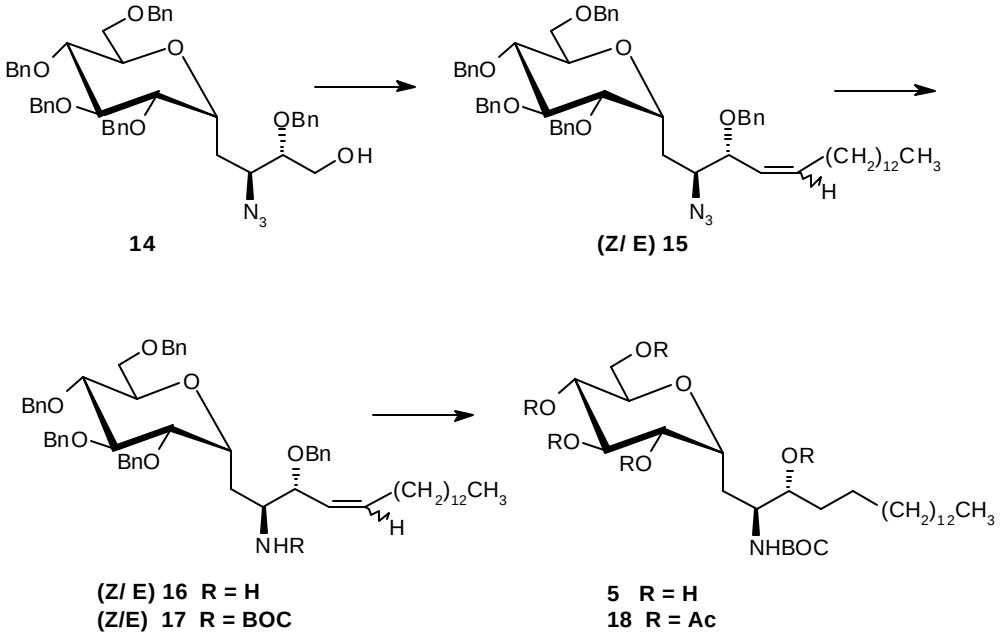
Protection of primary hydroxyl group in **10** as its TBS ether and adjacent secondary hydroxyl group as benzyl ether followed by removal of TBS group with TBAF gave the compound **14** (Scheme-2).

Scheme-2



Swern oxidation of **14** followed by Wittig reaction with ylide generated in situ from $Ph_3P^+(CH_2)_{12}CH_3Br^-$ and n-BuLi at −78 °C gave a mixture of E,Z-olefins (**15**), the predominant being the Z-olefin. Selective reduction of azido group with Ph_3P-H_2O gave the amine **16** which was then converted into the N-BOC derivative **17**. Catalytic reduction of **17** over 10 % Pd-C at room temperature and baloon pressure reduced the olefin and concurrently cleaved the benzyl groups to give **5**. The structure of target molecule (**5**), was unambiguously established, by converting as penta-acetyl derivative (**18**) (Scheme-3).

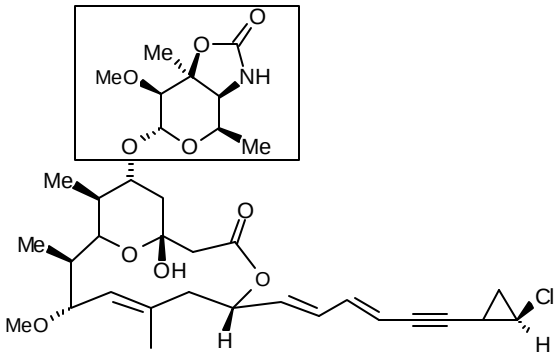
Scheme-3



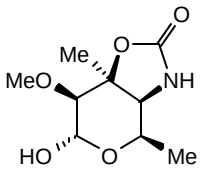
CHAPTER-II

SYNTHESIS OF CALLIPELTOSE: AN UNUSUAL AMINODEOXY SUGAR COMPONENT OF CYTOTOXIC MACROLIDE CALLIPELTOSIDE-A

Callipeltoside A (**1**) is a glycosidic macrolide isolated from the shallow water lithisted sponge, *callipelta Sp*. It is found to inhibit *in vitro* proliferation of KB and P 388 cells as to protect from infection by HIV virus. Undoubtedly the unusual structural parameters coupled with cytotoxic activity made callipeltoside A, a meaningful target for synthesis. As a part of our studies on the total synthesis of callipeltoside A, we now describe the synthesis of callipeltose (**2**), as its methyl glycoside derivative **20**, which is present on the main structural backbone of callipeltoside A (**1**).



Callipeltoside A (1)



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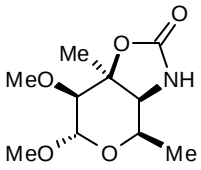
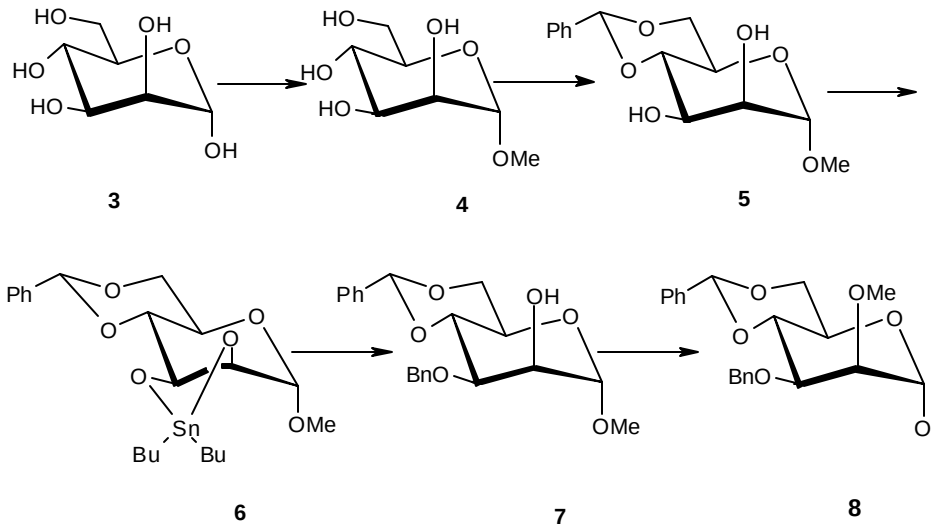


20

We began synthesis of methyl callipeltoside (**20**) starting from D-mannose. D-Mannose was converted to 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranoside derivative (**8**) as shown in scheme-1.

Scheme-1



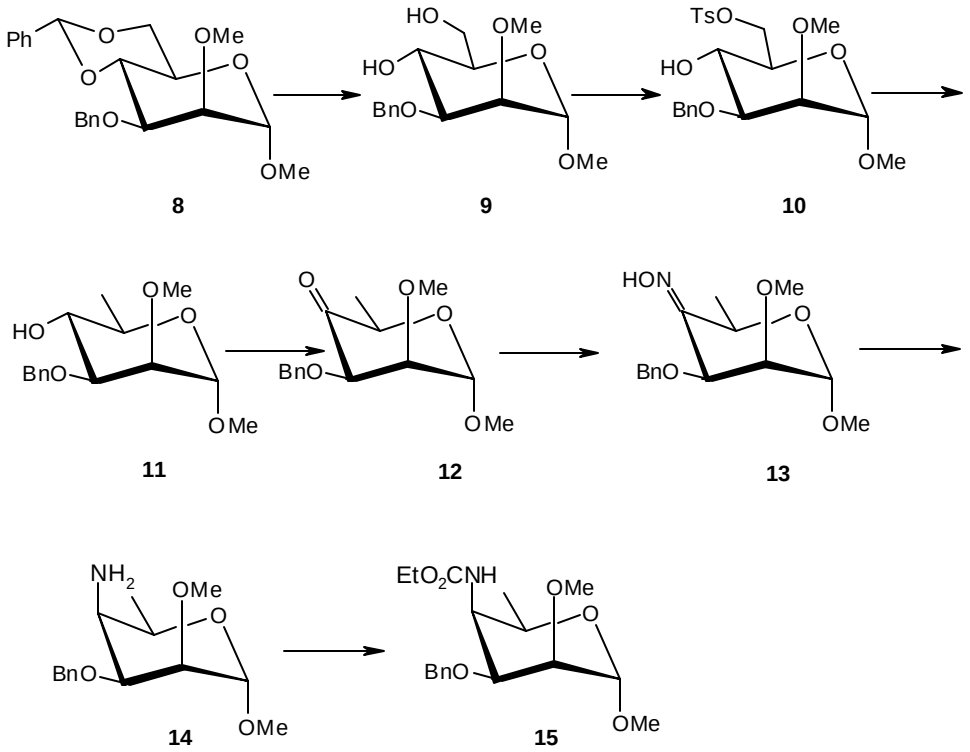
3 **4** **5**

6 **7** **8**

Cleavage of benzylidene group of compound **8** in the presence of 80 % acetic acid followed by selective tosylation of primary hydroxyl group of **9** with TsCl-Py gave the 6-*O*-tosyl derivative **10**. Reduction of **10** with $LiAlH_4$-THF under reflux provided 6-deoxy product **11**. In order to introduce the aminogroup at C-4, compound **11** was oxidized with $(CF_3CO)_2O$-DMSO and Et_3N at −78 °C to give 4-ulose derivative **12**. Compound **12** was immediately

treated with excess of NH_2OH in methanol at room temperature for 18 h to afford the oxime **13**. Stereoselective reduction **13** with $LiAlH_4$ in THF followed by protection of amino group with ethyl chloroformate furnished the carbamate derivative **15**. The D-talo configuration of **15** was assigned by the ^1H NMR spectrum in which the characteristic coupling constant $J_{3,4} = J_{4,5}$ = 3.3 Hz was observed. The G-4 isomeric D-mannose derivative could not be detected in the ^{1}HNMR spectrum (Scheme-2).

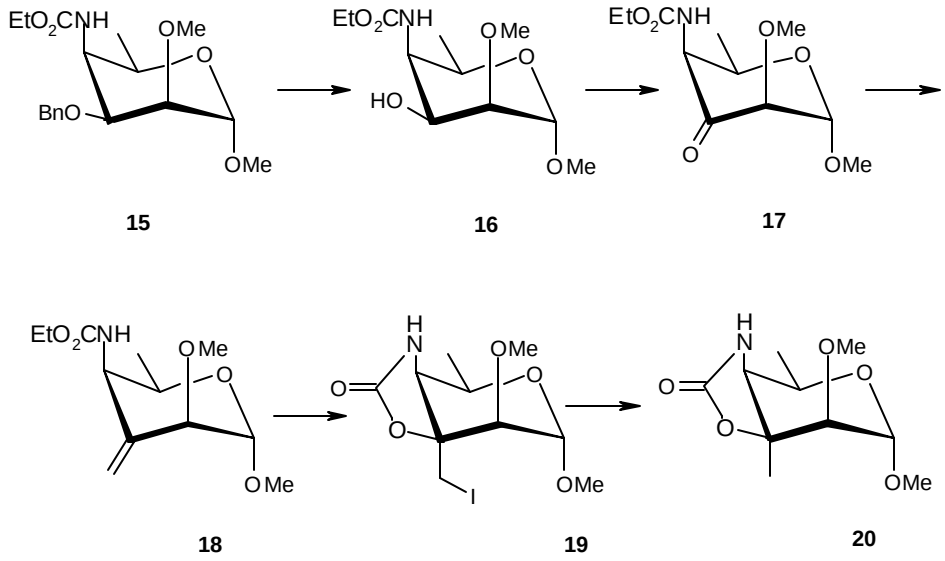
Scheme-2



Our next aim was to introduce an exo-methylene group at C-3 position. The benzyl group in compound **15** was first removed by hydrogenolysis over 10 % Pd-C to afford **16**. Subsequent oxidation of **16** to keto derivative (**17**) followed by reaction with $Ph_3P^+CH_3I^-$-n-BuLi in THF provided **18**. The iodolactonisation of **18** was performed in the presence of iodonium dicollidine perchlorate in $CHCl_3$ to give the iododerivative **19**. Reductive deiodination of **19** with Bu_3SnH-AIBN in refluxing toluene furnished the methyl glycoside of

callipeltose **20** whose structure was supported by the ^1H NMR and high resolution mass spectral data [m/z. 232.1174 (M^++1)] (Scheme-3).

Scheme – 3



15 **16** **17** **18** **19** **20**

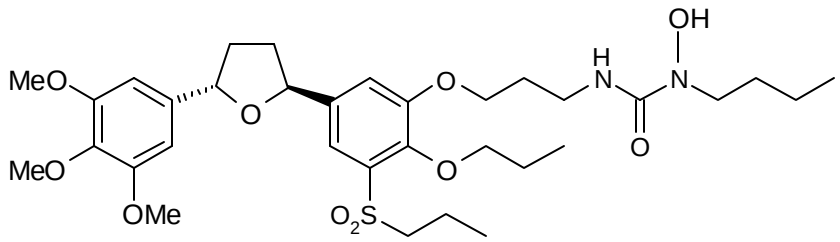
CHAPTER – III

SYNTHESIS OF CMI-546: A POTENT PAF-RECEPTOR ANTAGONIST

Platelet activating factor is a potent inflammatory phospholipid mediator with a wide variety of biological activities. It also appears to play a role in pathological immune and inflammatory responses. Many published studies have provided evidence for the involvement of PAF in human diseases, including arthritis, acute inflammation, asthma, endotoxic, shock, pain psoriasis, opthamalic inflammation, ischemia gastrointestinal, ulceration, mycocordial infarction, inflammatory bowel diseases, and acute respiratory distress syndrome. Lignans of the 2,5-diaryltetrahydrofuran series were identified as competitive PAF receptor antagonists. The compound endowed with 2,5-*trans* junction diaryl stereochemical relationship, exhibited excellent activity profile than that of *cis*-isomer.

Recently, the *trans*-2-[3-(3-(N'-butyl-N'-hydroxyuridyl)propoxy)-4-propoxy-5-propylsulfonylphenyl]-5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (**CMI-546**) was identified

and developed as a potent 5-LO inhibitor and PAF receptor antagonist thereby adjudicating as the curator of chronic asthma. The compound is now undergoing human clinical trials as anti-inflammatory agent.



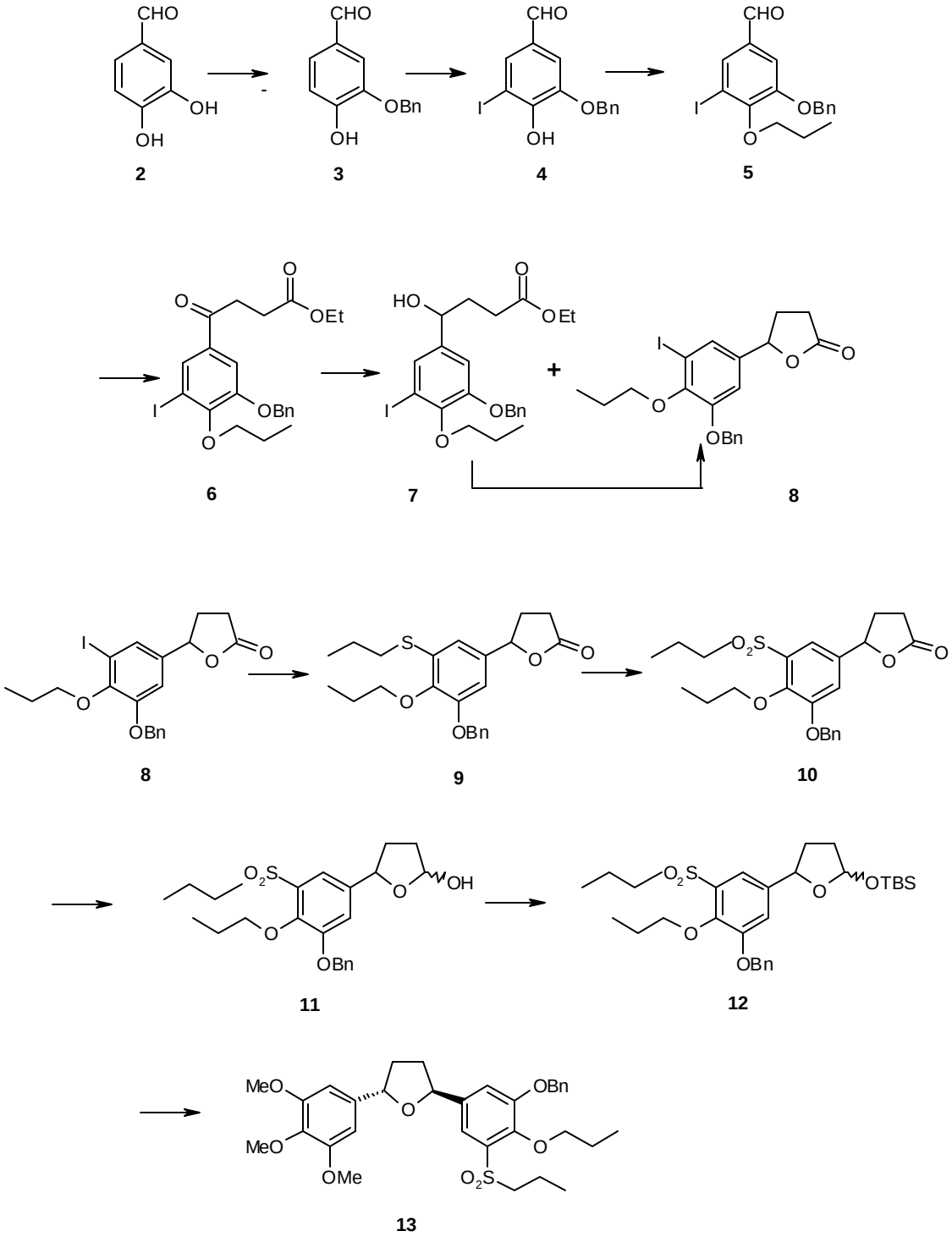
CMI-546 (1)

The molecule having spectacular pharmacological and pharmacodynamic properties and enriched with rich functionality and complexity poses copious attention to every synthetic chemist and hence our synthetic endavour to capitulate the total synthesis in economically viable fashion.

This chapter precisely describes the synthesis of CMI-546 (**1**) starting from 3,4-dihydroxybenzaldehyde. The compound **5** was obtained from 3,4-dihydroxy benzaldehyde as given in scheme-1. The compound **5** was converted into γ-ketoester (**6)** by using conditions described by Stettere *et al*. The ketoester was reduced with sodium borohydride in ethanol to afford a mixture of γ-hydroxyester (**7)** and iodolactone (**8)**. This mixture was converted into iodolactone **8** exclusively by treating with PTSA in DCM.

Conversion of iodide in compound **8** to propyl sulfide **9** and oxidation of sulfide to sulfone gave the compound **10**. The silyl acetal **12** was obtained by reduction of lactone **10** to lactal with DIBAL-H followed by protection with TBDMS-Cl. Exposure of the silyl acetal with TMSBr in DCM at −78 °C followed by the addition of 3, 4, 5-trimethoxyphenyl magnesium bromide produced (±) *trans*-2,5 disubstituted- tetrahydrofuran derivative **13** (Scheme-1).

Scheme-1

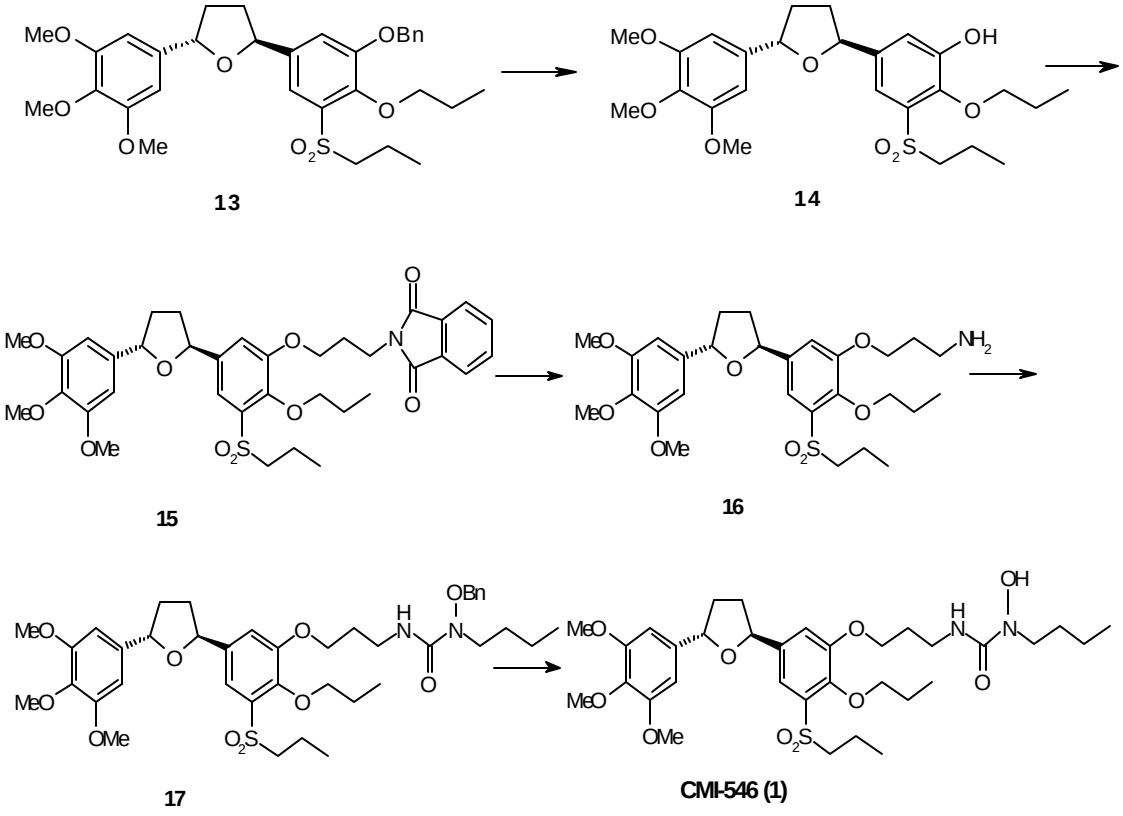


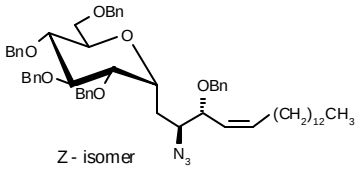
2 **3** **4** **5** **6** **7** **8** **8** **9** **10** **11** **12** **13**

The removal of benzyl group from **13** followed by *O*-alkylation with N-(-3-bromopropyl)phthalimide gave compound **15**. The phthalimide group in **15** was removed with

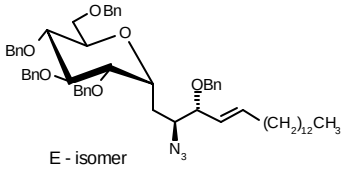
hydrazine monohydrate to produce an amine **16** which on treatment with triphosgene and *N*-(*O*-benzyl) butylamine furnished the compound **17**. The CMI-546 (**1**) was obtained by removal of benzyl group from compound **17** (Scheme-2).

Scheme-2

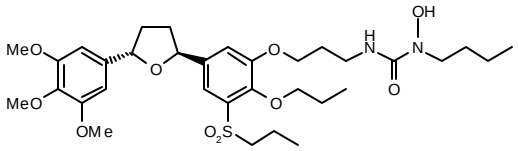




Z - isomer



E - isomer



INTRODUCTION:

In this introductory section, the chemistry and biology of glycosphingolipids will be deliberated incorporating the structural features, biological functions, physiological disorders and structural amplifications in detail. Glycosphingolipids are a class of naturally occurring bioactive compounds usually embedded in the membrane of all animal cells and in some plant cells. Glycosphingolipids[1] consist of three characteristic building block components : one of sphingosine (or one of its derivatives) and one molecule of a fatty acid with carbohydrate residues of varying complexity as their polar head groups. Sphingosine itself is a nonglycosylated longchain amino alcohol (Figure-1).

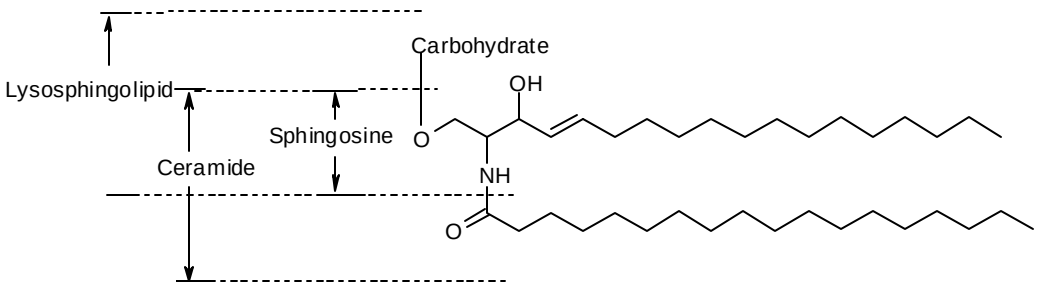


Figure-1: Generic structure of Glycosphingolipid

The amino group of the sphingoid base is attached to a long chain fatty acyl group (that can be saturated (or) monounsaturated depending on different factors, such as, cell type, diet, or age) to form ceramide. While the ceramide moiety is located in the membrane lipid bilayer, the saccharide head group extends out from the plasma membrane surface to the extracellular space. The sugar residue in glycosidic linkage (always β) to the ceramide is either glucose (or) galactose, with glucose the more prevalent. The most complex sphingolipids are the glycosphingolipids, which include neutral lipids containing from one (cerebrosides) to 20 (or) more glucose units and acidic glycosphingolipids contianing one (or) more sialic acid residues (gangliosides) or sulfate estes (sulfatides).

Several classes of glycosphingolipids with different carbohydrate core structures have been characterized. Substitution of these core structures gives rise to glycosphingoplipids with a high degree of variation in the carbohydrate moiety. Glycosphingolipids are classified according to their carbohydate components. These complex lipids can be divided into two major groups: neutral and acidic. Cerebrosides are the simplest glycosphingolipids and consist of sphingosine, a fatty acid residue, and a single monosaccharide polar head group.

More complex neutral glycosphingolipids have unbranched (or) branched oligosaccharide head groups containing upto 20 sugar residues. Acidic glycosphingolipids are sulfatides (or) gangliosides. Gangliosides have a similar basic structure to the neutral glycosphingolipids but are differentiated from each other by the fatty acid residue they contain and the sialylated oligosaccharides which comprise the polar head group. Over 100 gangliosides have been identified sofar. Gangliosides are found in all vertibrate cells but are highly enriched in nervous tissues. They have considerable physiological and medical significance.

BIOFUNCTIONAL ROLE OF GSLs:

Since the monoclonal antibody (mAb) approach was introduced in tumor immunology and developmental biology, a surprisingly large number of tumor associated (or) developmentally regulated antigens have been identified as glycosphingolipids[2]. GSLs may serve as receptors for viral and bacterial toxins, and microbial infections may be mediated by interaction of cellular GSLs with microbial membrane lectins often providing the internal domain of the CHO chain of GSL as primary binding epitope. During the past decade studies on two other categories of GSL function have evolved: (i) modulators of transmembrane signal transducers, resulting in regulation of cell proliferation (ii) mediators for cell- cell (or) cell substratum recognition.

The concept that membrane gangliosides may regulate cell growth has developed slowly during the past two decades, based initially on changes in GSL synthesis associated with oncogenic transformation, cell cycle, and "contact inhibition of" cell growth. GSLs have the amphipathic property that they are capable of being incorporated into cellular membrane when oxogenously added; thus the cells display new antigenicity and growth behaviour. This approach has extensively been used in order to identify the function of specific GSLs.

GLYCOSPHINGOLIPIDS AS RECEPTORS FOR CELLULAR INTERACTION:

The hypothesis that gangliosides act as receptors for bacterial toxins and hormones was proposed based on observed interactions between gangliosides and these bioactive factors. Specificities of the interactions between bioactive factors and GSLs were obscure, and the physiological significance of these interactions remains unknown. Even in the case of cholera toxin, whose binding specificity to G_{M1}, has been best characterized, there is no strong evidence that G_{M1}, really plays a role in the pathogenesis of cholera. Intestinal epithelial cells are the initial targets of cholera toxin, but G_{M1}, is absent in these cells. A clear picture has emerged of the interactions of GSLs with microorganisms (particularly a large variety of bacteria), strengthening the possibility that GSLs may act as receptors in microbial infections. A number of microorganisms may have specific lectins that recognize specific sugar residues in host cell GSLs. In many cases recognition is directed toward an internal sugar chain moiety rather than terminal structure.

The *a priori* fact that dramatic changes in GSL composition are closely correlated with changes in cell recognition during ontogenesis and oncogenesis suggests that GSLs function as receptor sites in cell-cell recognition. In support of this concept, there is evidence that GSLs (or) corresponding oligosaccharide sequences, particularly multivalent synthetic compounds, block cell-cell recognition, an essential step during embryogenesis and morphogenesis. Naturally, cell surface proteins interacting with specific CHO sequences of GSLs have

received great attention, i.e. glycosyltransferases, hydrolases, and lectins. Particular interest was aroused, some time ago, by the possible presence of cell surface glycosyltransferase as cell recognition site. However, in the majority of cells, localization of glycosyltransferases appears to be restricted to the golgi membrane as evidenced by application of monoclonal antibodies (mAb) to glycosyl- transferases. Lopez and Shur, however, provided evidence that β-Gal.transferase is located in the sperm cell surface and possibly has some function in cell recognition during fertilization.

Similarly, surface expression of endogenous lectins in the majority of animal cell is limited, except in the cases of the hepatocyte receptor that recognizes serum asialoglycoprotein (Ashwell receptor), the galactose binding lectin highly expressed at melanoma and other tumor cell surfaces and the recently discovered leukocyte adhesion receptor. The sequence homologies with animal lectins have been found in a few receptors (known as LECCAM (or) selection family) involved in leukocyte adhesion to endothelial cells (or) matrices[2c].

GLYCOSPHINGOLIPID STORAGE DISORDERS:

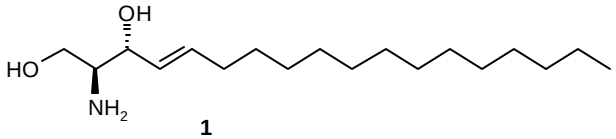
Glycosphingolipids are known to involve in various lipid storage disorders[1b,3].These results from the inheritance of defects in the genes encoding catabolic enzymes (which are then impaired) required for the sequential breakdown of the glycolipids within lysosomes. The therapeutic options for the treatment of these disorders are very limited. Currently, only the nonneuronopathic form of Gaucher's disease, a condition characterized by glucocerebrolidase deficiency, which occurs at high frequency in Ashkenazi Jews, is being successfully treated using enzyme-replacement therapy. However, the use of specific inhibitors glycosphingolipid biosynthesis to reduce their levels in cells, is one approach which may be generally applicable to treating storage disorders. This might then allow the impaired enzymes to catabolize fully the reduced levels of glycosphingolipids thus preventing their accumulation. One example of this has recently been shown using NB-DNJ which in addition to being an α-glucosidase I and

II inhibitor, is also a potent inhibitor of glycosphingolipid biosynthesis. This compound inhibits the initial monosaccharide attachment and so this approach may be useful in all glycosphingolipid storage disorders. Interestingly, the glactose analogue N-butyl deoxygalactonojinimycin (NB-DGJ) was found to inhibit glycosphingolipid biosynthesis. This is a more selective compound in that unlike NB-DNJ it does not inhibit the α-galactosidases.

Clearly the ability to manipulate glycoprotein and glycolipid biosynthetic pathways using specific glycosyltransferases and glycosidase inhibitor has enormous potential. In this way, the roles of glycolipids in areas such as neuronal and embryonic development can also be investigated as well as their roles in adhesion processes and tumor metasis.

SPHINGOSINES AND THEIR BIOLOGICAL EFFECTS:

Sphingosine **1** was first isolated in 1884 by Thudichum as a waxy unstable substance obtained from the hydrolysate of a lipid fraction of brain tissue[4]. Sphingosines are long chain amino alcohols found in the hydrophobic moiety of glycosphingolipids and sphingomyelins.



1

Sphingosines were found to inhibit a number of cellular functions[2b]. In human platelets, sphingosine inhibited secondary aggregation and secretion in response to various platelet activators, but did not effect the initial response (or) shape change. Inhibition of platelet function was accompanied by inhibition of phorbol dibutyrate binding and by inhibition of protein kinase C activation by endogenous diacylglycerols. In neutrophils, sphingosine inhibited phorbol ester binding, the induction of superoxide generation, and the secretion of specific but not azurophilic granules. Sphingosine also inhibited the synthesis of platelet activating factor and leukotriene LTB_4. Likewise sphingosine inhibited the differentiation of HL-60 cells in response to phorbol 12-myristate 13-acetate, inhibited neurite

outgrowth in PC-12 cells in response to nerve growth factor, and inhibited insulin-stimulated uptake of 2-deoxyglycose in 3T3-L1 fibroblasts. In A431 cells, sphingosine inhibited the phosphorylation and affinity changes of the epidermal growth factor (EGF) receptor in response to phorobol esters. In addition, sphingosine led to selective enhancement of phosphorylation of the EGF receptor at sites known not to be direct substrates for protein kinase C. In S49 lymphoma cells, sphingosine inhibited the translocation of protein kinase C in response to phorbol myristate acetate.

In vitro studies of the structure activity relation ship of sphingosine that would ideally influence inhibition of protein kinase C requires the hydrophobic character and the positively charged amine. Sphingosine, N-methyl sphingosine, 3-ketosphingosine, and stearylamine all inhibit, whereas ceramide, N-acetyl sphingosine, short chain sphingosines with fewer than 11 carbons are inactive as inhibitors. Similar structure activity relations were observed in intact cells. Sphingosine, but not N-acetyl sphingosine, inhibited protein kinase C activity and phorbol binding in human platelets, HL-60 cells, and neutrophils. Studies with sphingosine analogs having variable chain lengths showed that the 18-carbon molecule had optimal cellular and *in vitro* activity and that both shorter and longer chain analoges showed progressively less activity.

Since sphingosine, lysosphingolipids and structural analogs are potentially selective inhibitors of protein kinase C, they may serve as important pharmacological tools to dissect the role and function of protein kinase C in different cell systems and to develop agents that can selectively prevent the effects of tumor promotors by inhibiting their effects on protein kinase C. The sphingosine, structural unit common to almost all sphingolipids in eukaryotic cells, is an amino alcohol D-*erythro* sphingosine [(2S, 3R, 4E)-2-amino-3-hydroxyoctadec-4-ene-1-ol] with two asymmetric carbons and can exist in four stereoisomers. Comparison of the four

stereoisomers (Figure-2) shows that all have approximately the same potency as the inhibitors of PKC *in vitro*, with slight preference for L-*threo*-sphingosine (2S, 3S). A yet another isomer

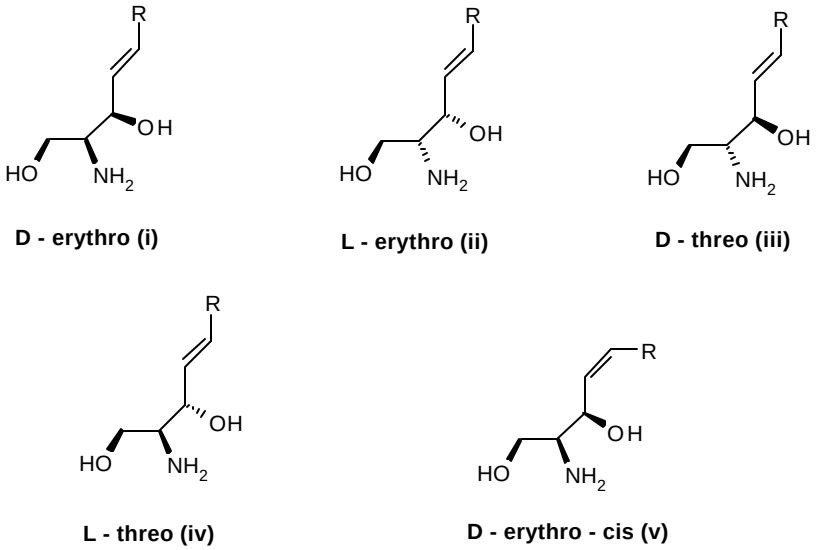


Figure-2

of the D-*erythro*-sphingosine with a cis-double bond instead of the trans-double bond between C_4-C_5 was also found to be half as inhibitory[5].

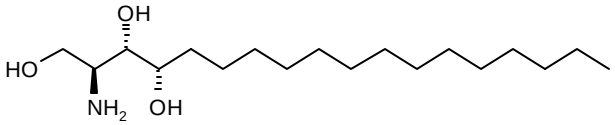
CHART

Effect of long-chain bases on PKC activity *in vitro*

Stereoisomer of sphingosines	Structure	Cu for 50% inhibition *in vitro* in mol%
D-erythro	(i)	2.8
L-Erythro	(ii)	3.3
D-Threo	(iii)	2.8
L-Threo	(iv)	2.2
D-Erythro-cis	(v)	7.0

In addition to D-*erythro*-sphingosine there are over 60 other sphingoid base structures found elsewhere in nature[6]. Phytosphingosines **2** constitute the major base component of

higher plants, protozoa, yeast and fungi, and have also been found in human kidney cerebrosides, and in some cancer cell types[7].

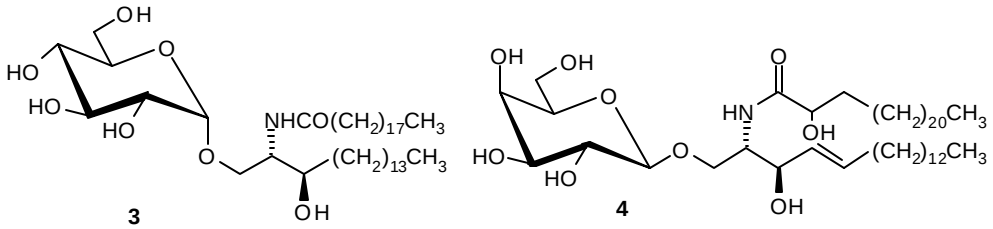


2

Because of the multifarious role exerted by sphingosines and their derivatives glycosphingolipids, ceramides and cerebrosides, there is an increasing demand for these compounds for biological studies and pharmaceutical applications. Synthetic chemists have turned up with equal tenor to execute the synthesis of these compounds of type over the five dacades, us ing elegant tactics and strategies with up-to-dated chemistries[8].

NATURAL GLYCOSPHINGOLIPIDS:

The first recognition of GSLs in variety of marine invertebrates came in early 1970's. Several types of â-Galcers have been isolated from marine organisms and organ tissues. Galcer **3** was identified as essential components of neural receptor for type 1 human immunodeficiency (HIV) surface glycoprotein[9]. á-Galactosyl ceramide structures namely agelaspins **4** from marine sponge *agelasmauritianus* are recognised as potential antitumor and

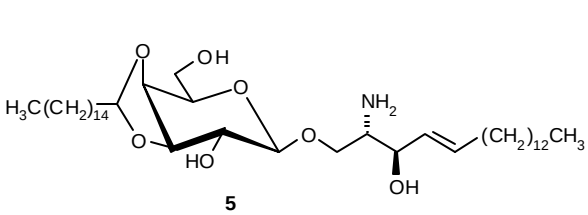


3 **4**

immunostimulant agents[10]. Surprisingly, β-anomers of agelaspins are inactive. Various GSLs have been isolated from different types of sponges such as *Agelas dispar, clathrods, agelas confera* and *A.longissima*[11].

Two novel lysosphingolipids plasmalopsychosines **5** and **6** possessing inhibitory protein kinase C activity were found in human brain. Plasmalopsychosines **5** and **6** were

structurally identified as conjugates of "plasmals" (long chain aliphatic aldehydes) with galactose residue of psychosine **7** through 3,4 and 4,6 cyclic acetal linkages respectively[12].



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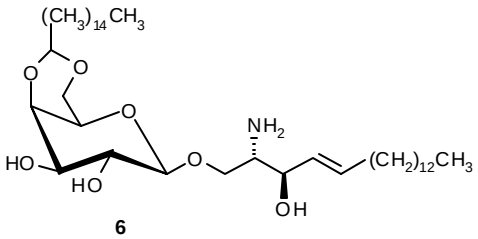

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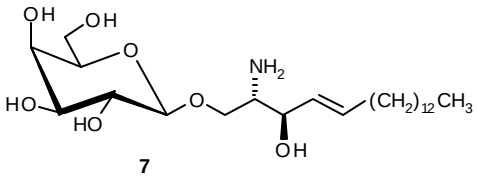
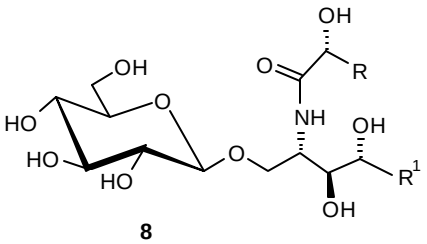


7

The new cerebrosides, asterocerebrosides **8** have been isolated from starfish *Asterina pectinifera*[13].



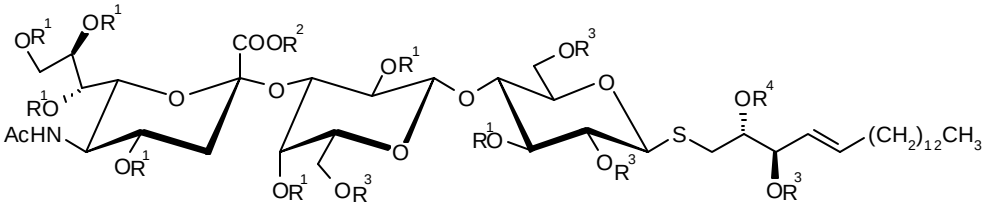
8

Inspite of the wide range of biological functions exhibited by glycosphingolipids, they are actually relatively scare and difficult to obtain in homogeneous form from biological sources. Because of this scarcity and difficulty of isolation, the synthesis of isomerically pure glycosphingolipids[14] and their analogues is necessary inorder to explore the functions of these compounds in biological systems.

ANALOGUES OF GSLs:

The extensive biological role, played by GSLs, usurped the medicinal community to prepare several analogues to understand the mechanisms of GSL-related disorders. In this direction several classes of glycosphingolipid analogues were prepared, mainly, aza, thia and

carba variant of *O*-glycosides. For example, Hasegawa et al[15]. have synthesized several ganglioside analogues containing â-thioglycosidically linked ceramide **9** since several ganglioside analogues containing â-thioglycosides of sialic acid are potent inhibitors of sialidase activities of different types influenza viruses. Thomas Bar and R.R.Schmidt[16] have reported the synthesis of â-Lactosyl 1-thioceramide **10**.



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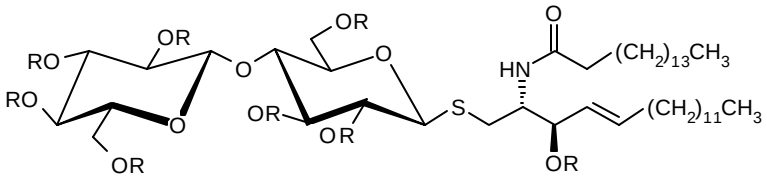
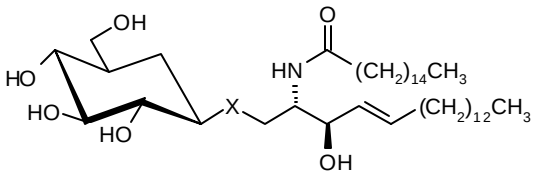
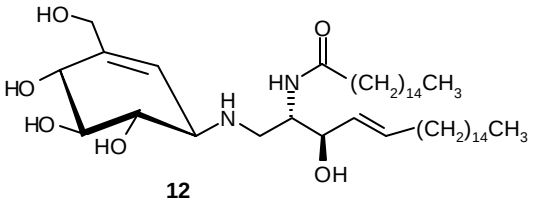


10 R = H, Ac

Analogues with carbocycles instead of carbohydrates, carrying N- and S- glycosyl acceptors were also synthesized and tested which were shown to be the potent and specific â-D-glucocerebrosidase inhibitors[17].



11 X = O, NH, S

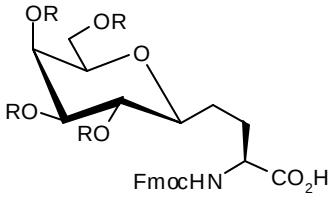


12

_C_-GLYCOSIDES:

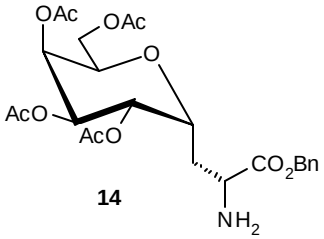
The potential of peptide *O*-glycoconjugates as therapeutic agents has attracted great attention by virtue of the improved activity and bioavailability of peptidyl drugs. To combine

these features with an increased metabolic stability, it was planned to replace oxygen atom of glycosidic linkage with methylene group. These isosters are precious tools for the studies at molecular level of the role that carbohydrates play in numerous biological processes and for the explorative work in drug discovery[18]. The problem has been addressed by the development of synthetically useful strategies for the synthesis of *C*-glycosylated α-aminoacid subunit to be incorporated into pharmacologically active peptides. Thus, for example β-Gal-Ser (**13)** was synthesized by Bednarski.

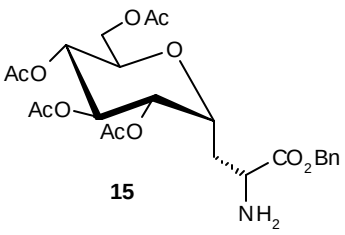


13

Aimed at increasing the metabolic stability of the gonadodropin-releasing hormone against Buserelin, a nonapeptide developed by Hoechst, Kessler and his colleagues prepared *C*-α-D-galctosylated alanine derivative **14**. The *C*-glycosylated GnRH agonist featured with increased water solubility and proved to be more active than the natural GnRH. The corresponding sugar analogue 1-*C*-(α-D-glucopyranosyl)-D-alanine **15** was prepared by Gurjar through a three carbon stereoselective homologation of peracetyl glucose at anomeric centre.



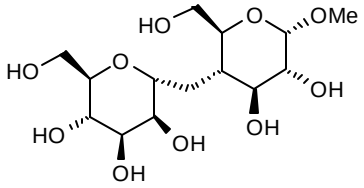
14



15

Carbon oligosaccharides are a class of non-natural analogues of oligosaccharides wherein the interglycosidic oxygen bridge is replaced by a methylene linker. In a broader context this subclass also includes examples in which the monosaccharide units are connected

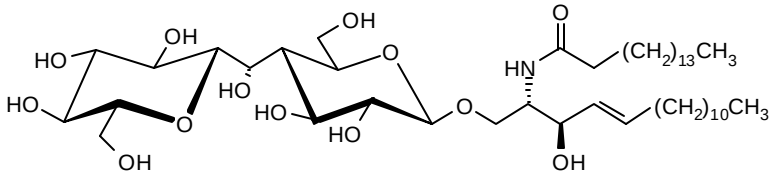
either directly (or) by way of more extended carbon linkers. This novel class of pseudocarbohydrates the first example **16** was reported in 1983 by Sinay is of considerable interest and has attracted wide attention as result of its biological and structural significance.



16

Among the various approaches culminating in the syntheses of well-defined *C*-oligosaccharides, strategies based on stereocontrolled connection of suitably preformed monosaccharides constitute the avenue of choice for a number of research groups.

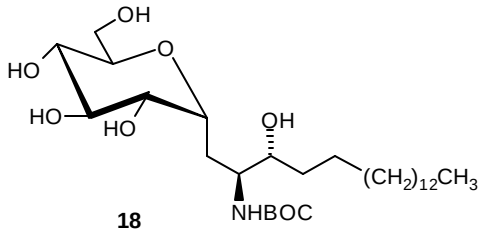
R. R. Schmidt and co-workers[19] reported the *C*-Galactosyl ceramide **17** which shows carbon-carbon bond between the two monosaccharide moities while the natural *O*-glycosidic linkage still holds the ceramide.



17

PRESENT WORK:

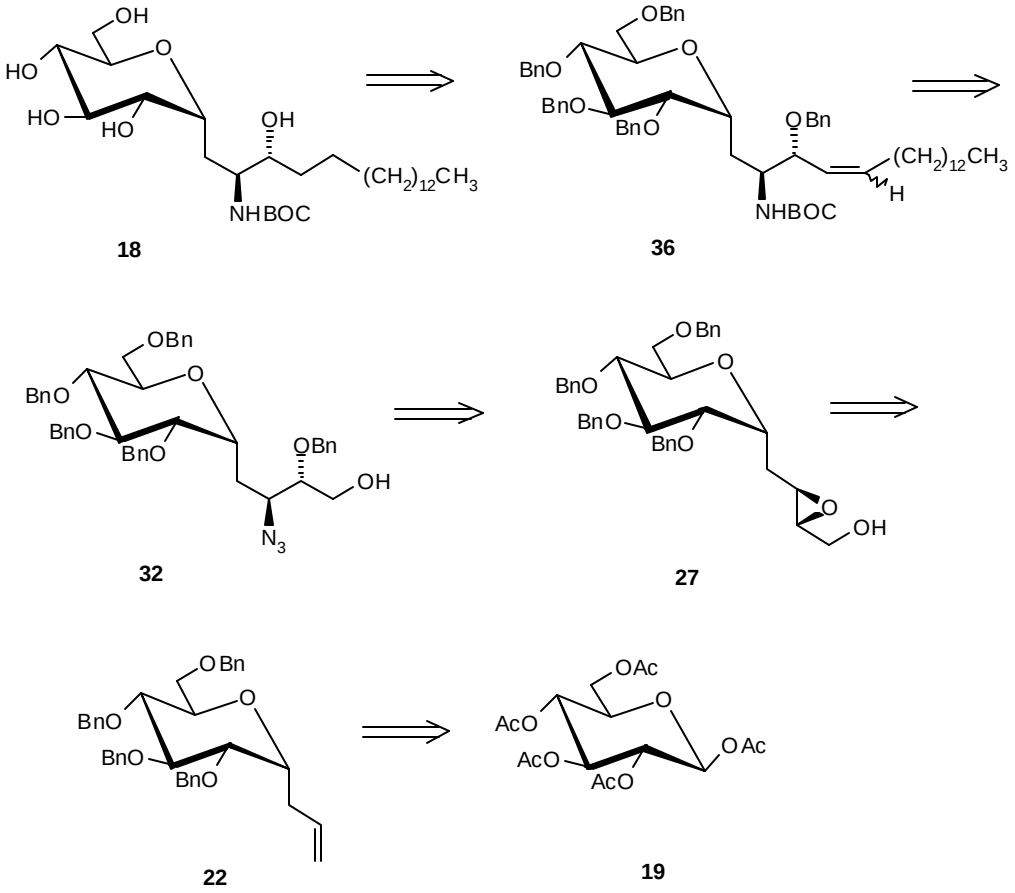
From the preceeding lines, it is apparent that glycosphingosines are biologically active compounds. One of the limitations of these compounds is the presence of *O*-glycosidic bond which hydrolyses under physiological conditions. This characteristic problem is associated with oligosaccharides, *O*-glycopeptides etc. In order to overcome such difficulty, scientists have initiated preparing *C*-glycosides (C_1-*O* bond is replaced with C_1-*C*). The presence of *C*-*C* bond at the anomeric carbon makes these molecules resistant to hydrolysis. Many *C*-glycopeptides have been synthesized and evaluated as glycosidase inhibitors. Although many syntheses of sphingosines are reported, no attempt has yet been made to prepare *C*-sphingosine derivatives. It is believed that á-*C*-D-glucosphingosine such as **18** will be useful as bioactive materials because of the presence of nonhydrolysable *C*-*C* bond.



18

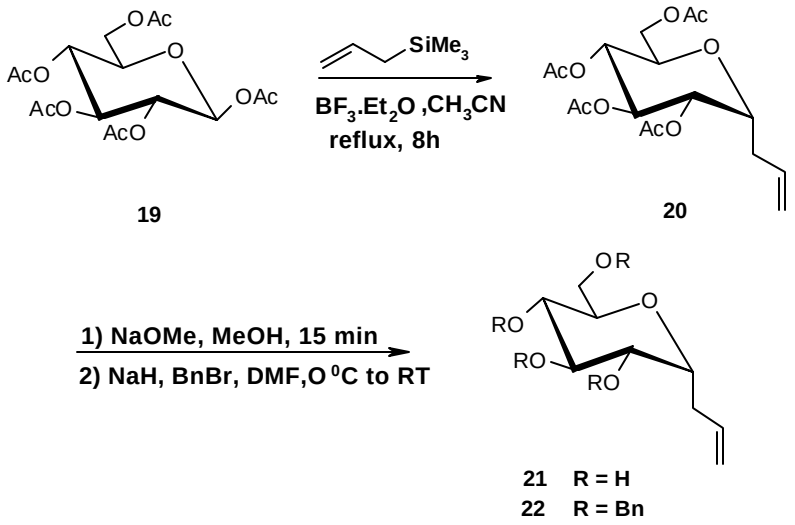
The retrosynthetic analysis of **18** (Scheme-1) clearly demonstrates that the synthesis of side-chain containing two chiral centers is most critical phenomenon. *C*-Allyl glycoside **22** is a known and obtainable in high yields. The two carbon homologation, Sharpless epoxidation should provide **27** which in turn could be elaborated to **18** by regioselective epoxide (**27**) opening with azide and elongation of side chain through Wittig reaction.

Scheme-1



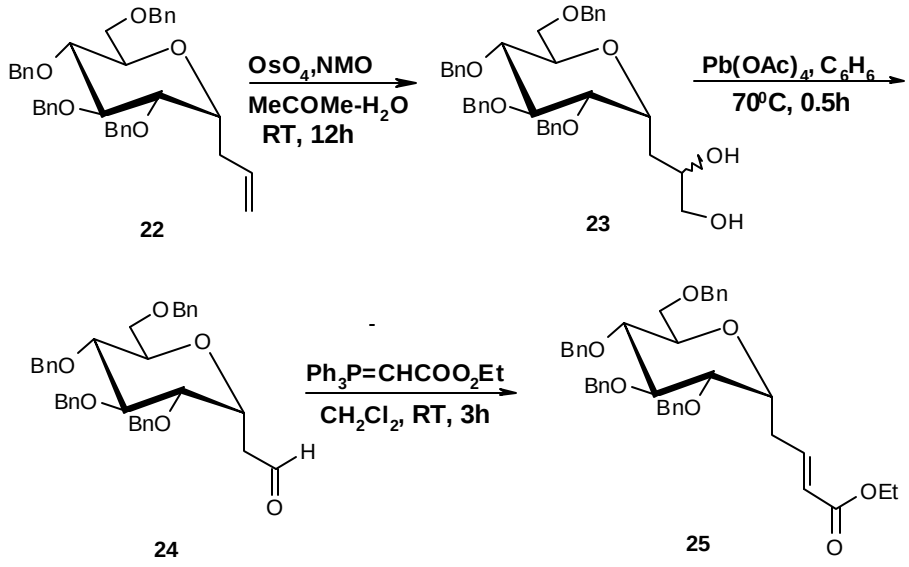
The glucose-pentaacetate (**19**) was treated with allyltrimethylsilane and $BF_3.Et_2O$ in dry CH_3CN under reflux to give 3-(2',3',4',6'-tetra-*O*-acetyl-α-*C*-D-glucospyranosyl)-1-propene (**20**) in 43 % yield[20]. Zemplen deacetylation of **20** in the presence of methanolic sodium methoxide gave the tetrol **21** which was subjected to benzylation using excess of both sodium hydride and benzyl bromide to give the tetrabenzylate **22** in 95 % yield (Scheme-2). The structure of **22** was confirmed by comparision of ^{1}H-NMR, optical rotation and mp data with reported values[21].

Scheme-2



Subsequent cleavage of double bond in **22** in two steps by using OsO_4-NMO-$Pb(OAc)_4$ furnished the aldehyde **24** which was treated with $Ph_3P{=}CHCO_2Et$ in CH_2Cl_2 to afford the (E) α,β-unsaturated ester **25** (Scheme-3). The E-stereochemistry of **25** was ensured by its ^{1}H-NMRspecturm in which characteristic coupling constant (J = 15 Hz) was observed for olefinic

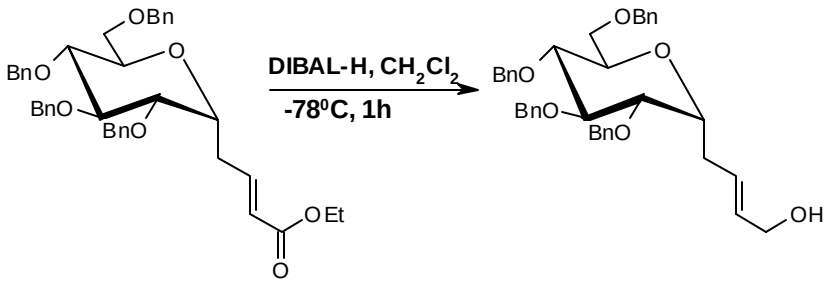
Scheme-3



protons. The ^{1}H-NMR of **25** showed a doublet (J = 15 Hz) at 5.87 ppm and a doublet of triplet (J = 7.5 Hz, 15 Hz) at 6.90 ppm due to olefinic protons. The methyl group of $COOCH_2\underline{CH_3}$ appeared as a triplet at 1.27 ppm while methylene group of ($\underline{CH_2}$-C=) was observed as a triplet at 2.62 ppm. The rest of the protons appeared at their expected chemical shifts.

The α,β-unsaturated ester **25** was reduced with DIBAL-H at −78 °C to afford the allyl alcohol **26** in 56 % yield whose ^{1}H NMR spectrum revealed the doublet at 3.95 ppm due to methylene protons.The two olefinic protons appeared as a multiplet at 5.65 ppm.The rest of the peaks were consistent with the assigned structure (Scheme-4).

Scheme-4

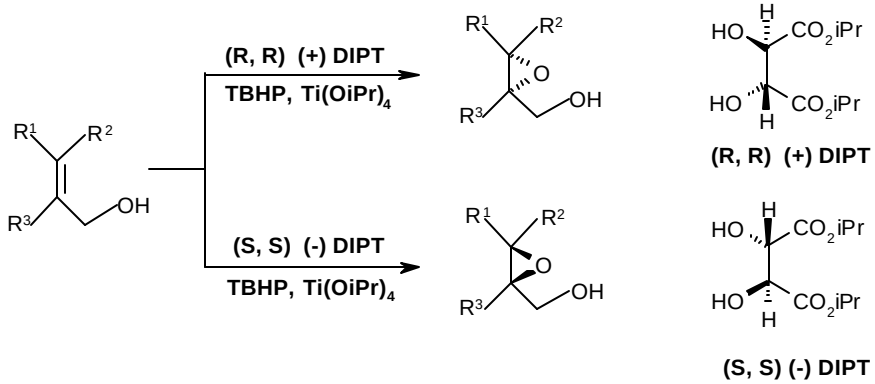


25 **26**

Sharpless et al.[22] discovered a metal catalysed asymmetric expoxidation process of allyl alcohol which is more selective than any of the previously known methods. The simplicity of this new method is one of its more attractive aspects, the necessary components are all commercially available at low cost. This new chiral epoxidation system possesses two especially striking features. First, it gives uniformly high asymmetric inductions throughout a range of substitution patterns in the allylic alcohol substrate. Second upon use of a given tartarate enantiomer, the system seems obliged to deliver the epoxide oxygen from the same enantioface of the olefin regardless of substitution pattern. This latter characteristic is highlighted in fig. When the olefinic unit is in the plane of the paper, with the hydroxymethyl substituent at the lower right side the use of (+) DET (or) (+) DIPT leads to addition of the

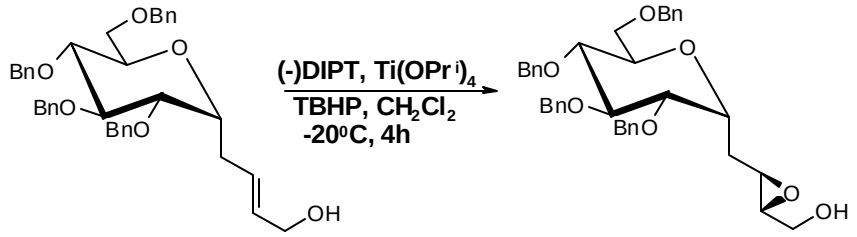
epoxide oxygen from the bottom as shown in the fig 3. Similarly, when (-) DET (or) (-) DIPT is employed the epoxide oxygen is added from the top (Figure-3).

Figure-3



In accordance with the Sharpless reaction, the allylic alcohol **26** was converted into the epoxy alcohol **27** by using (-) DIPT, $Ti(OiPr)_4$, TBHP and molecular seives 4A in CH_2Cl_2 at –20 °C in 82 % yield (Scheme -5).

Scheme-5



26 **27**

The structure of the epoxide **27** was supported by its ^1H NMR, ^{13}C NMR and mass spectral data while the configuration was deduced by the empirical rules reported by Sharpless. The ^1H-NMR spectrum of **27** showed a multiplet at 2.9 ppm and a doublet of a triplet (J = 2.3 Hz, 5.6 Hz) for the two epoxy protons. In addition, the structure of **27** was further confirmed by ^{13}C-NMR as well as by the FABMS [m/z: 611 (M^++1)]. The diastereomeric excess of epoxy alcohol **27** determined by HPLC and was found to be above 99%.

Our next endeavour was to regioseletively open the epoxide ring in **27** with azide. The ring opening reactions of 2,3-epoxy alcohols under non-isomerizing conditions were investigated by Beherens and Sharpless[23]. There is an inherent tendency for ring opening at the C-3 position of 2,3-epoxy alcohols, due to an electronic effect of hydroxyl group at C-1. Since the hydroxyl group is relatively weak, inductively electron withdrawing group, ring opening reactions at C-3 is observed only with certain simple 2,3-epoxy alcohols. (Figure-4).

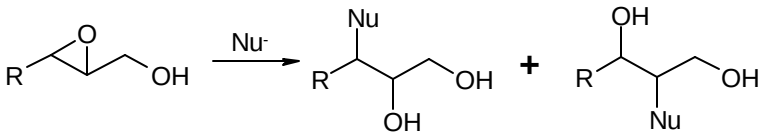
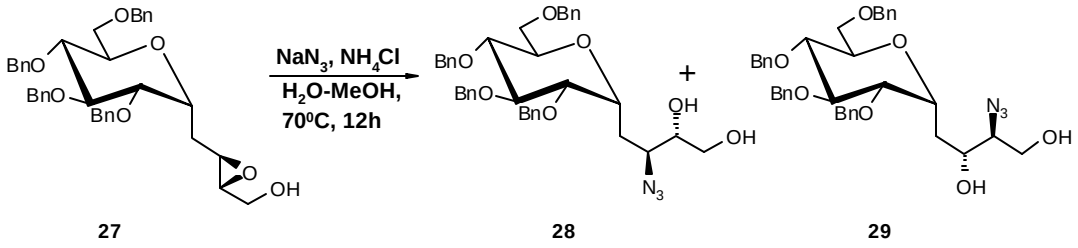


Figure-4

The epoxy alcohol **27** was treated with NaN_3 and NH_4Cl in refluxing aqueous methanol to afford 1,2-diol **28** and corresponding 1,3-diol **29** in the ratio of 5.7:1. The mixture was chromatographically separated on silica gel to furnish the pure 1,2-diol **28**. The structure of **28** was supported by the ^{1}H-NMR and IR spectra. The IR spectrum of **28** showed a characteristic absorption due to N_3 group at 2100 cm^{-1}. The chemical degradation of **28** with $NaIO_4$ was observed whereas **29** resisted such degradation as expected (Scheme-6).

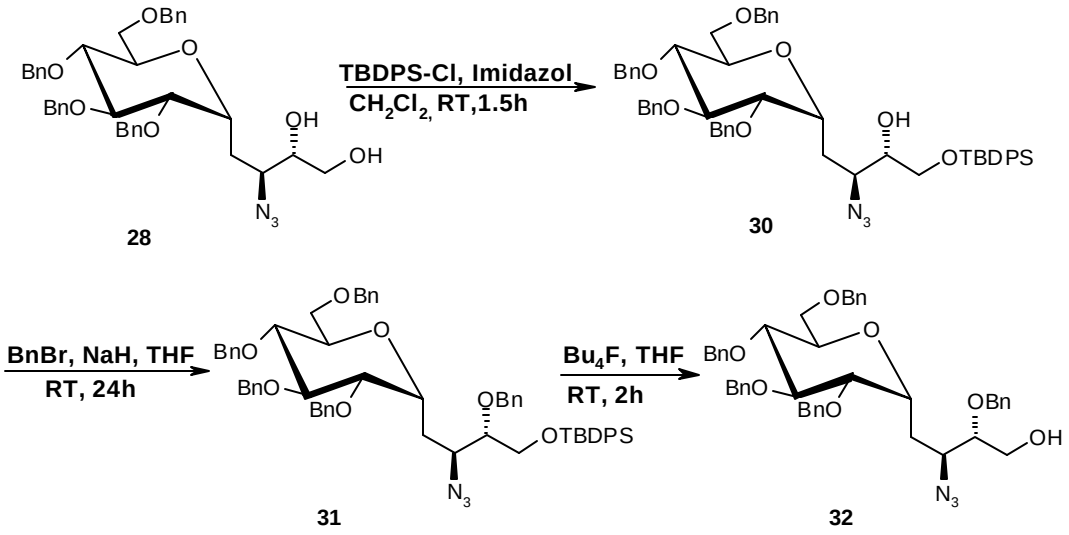
Scheme-6



To achieve the regeoselective oxidation of CH_2OH group, the hydroxyl at C-2 has to be protected. The primary hydroxyl group of compound **28** was protected with TBDPS-Cl and imidazole to give compound **30** in 78 % yield whose structure was confirmed by the presence of t-butyl and phenyl groups in the ^{1}H-NMR spectrum. The free OH group in **30** was

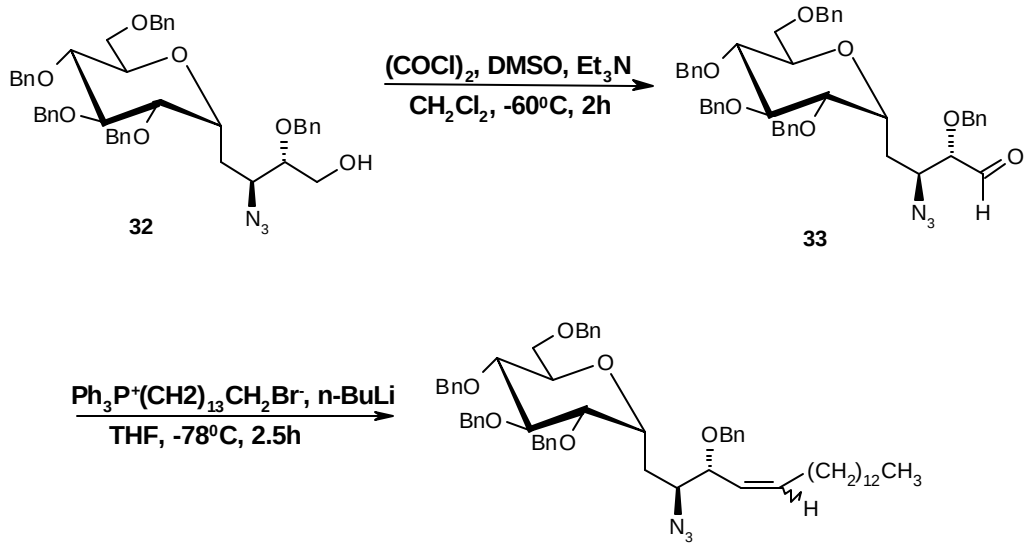
converted into the benzyl ether (**31**) using NaH-BnBr in dry THF in 82 % yield.The structrure of **31** was confirmed by ^{1}H-NMR spectrum. Compound **31** was treated with tetrabutylammonium fluoride in THF to cleave the TBDPS group and afford **32** in 87 % yield. The ^{1}H-NMR spectrum of **32** revealed the absence of characteristic resonances due to TBDPS group (Scheme-7).

Scheme-7



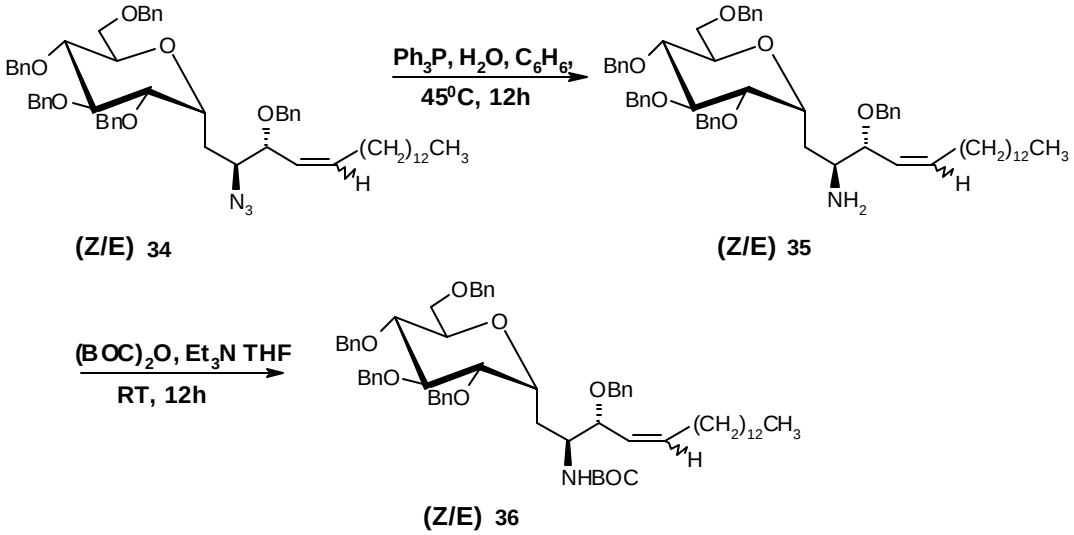
The primary hydroxyl group in **32** was oxidized to the corresponding aldehyde **33** using $(COCl)_2$, DMSO and Et_3N (Swern oxidation condition) at −78 °C in CH_2Cl_2. The resulting aldehyde **33** was subjected to Wittig reaction with tetradecanylphosphonium bromide and n-butyllithium at −78 °C to give the (E,Z)-olefin **34** in which Z-isomer predominated ($\delta_{H\text{-}4}$ 5.74 dt, J = 8.0, 11.0 Hz) ($\delta_{H\text{-}5}$ 5.38, t, J = 11.0 Hz). The ^{1}H-NMR spectrum of **34** showed a triplet (J = 7.2 Hz) at 0.88 ppm due to methyl group and broad singlet at 1.36 ppm due to methylene groups. The rest of the chemical shifts were consistent with the assigned structure **34** (Scheme-8).

Scheme-8



The conversion of azido compound **34** into the corresponding amine **35** was accomplished by reducing the azido group with Ph_3PO-H_2O in benzene[24]. The resulting crude amine **35** was converted into N-BOC derivative **36** with $(BOC)_2O$ and Et_3N in 67% yield (Scheme-9). The ^{1}H-NMR spectrum of **36** showed a singlet due to N-BOC at 1.35 ppm. The remaining protons appeared at their expected values. In the IR spectrum of **36** the characteristic absorption due to N_3 group at 2100cm^{-1} had disappeared. The IR spectrum of **36** showed absorptions at 1650 cm^{-1} and 3400cm^{-1}. The structure of **36** was further confirmed by FABMS m/z: 896 (M^++1- BOC). Catalytic reduction of **36** over 10 % Pd-C at room temperature and balloon pressure reduced the olefin and cleaved the benzyl groups to give **18**. In order to confirm the structure of **18** it was derivatised into the penta-acetate **37** using Ac_2O-Py in 95 % yield (Scheme-10).

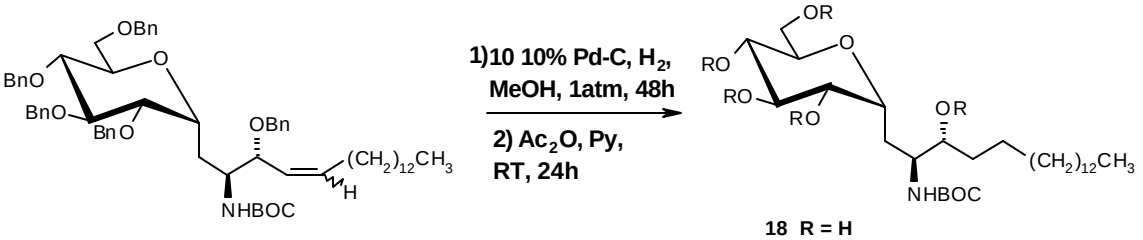
Scheme-9



(Z/E) 34 **(Z/E) 35** **(Z/E) 36**

The ^{1}H-NMR spectrum showed five distinctive signals due to acetyl groups. In addition the sugar ring protons H-1', H-2', H-3' and H-4' were clearly located at 4.05 (m), 5.06 (dd, J = 5.6, 8.4 Hz), 5.19 (t, J = 8.4 Hz), and 4.92 (t, J = 8.4 Hz) respectively. In addition the structure was further confirmed by FABMS m/z 658 (M^{+}+1).

Scheme-10



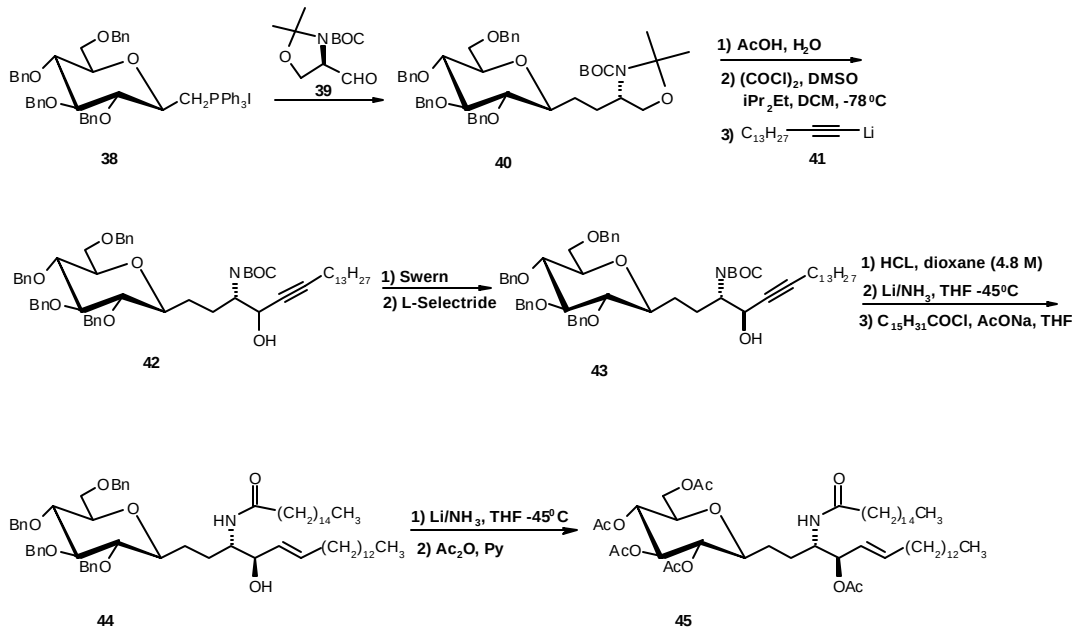
(Z/E) 36

18 R = H
37 R = Ac

POST PUBLICATION:

Dondoni et al[25]. have recently reported the synthesis of carbon-linked isostere of natural â-D-galactosyl ceramide starting from sugar phosphorane **38** and aldehyde **39** obtained from D-serine. Thus, coupling of these reagents and diimide reduction of double bond afforded **40**. The removal of the acetonide protective group under standared conditions (AcOH-H_2O, 4:1) transformed **40** into the N-BOC amino alcohol which in turn was converted into the aldehyde by Swern oxidation and the aldehyde was treated

Scheme-11



with excess of lithium1-pentadecyne (**41**) to afford **42** as a mixture of S (syn adduct) and R (anti adduct) isomers in 70:30 ratio. To obtain the desired ***anti*-43**, alcohol **43** was oxidized and resulting ynone was reduced with lithium tri-*sec*-butylborohydride (L-selectride). Stereoselective hydrogenation of triple bond and the replacement of N-BOC with N-palmitoyl group afforded **44** whose on debenzylation and exhaustive acetylation produced **45** in 4 % overall yield (Scheme-11).

In conclusion we have developed an efficient method for the synthesis of á-C-D-glucosphingosine from cheap and easily available starting material. This strategy provides opportunities to prepare several anomeric and diastereomeric analogues of *C*-glucosphingosine. These analogues possessing non-hydrolysable (metabolically stable) *C*-glycoside unit will be interesting and useful products since many *C*-glycosides are recognized as glycosidase inhibitors.

EXPERIMENTAL:

b-D-Glucopyranose pentaacetate (19):

A suspension of anhydrous sodium acetate (40 g) in acetic anhydride (560 ml) in a 1L round bottomed flask was heated over a flame to the boiling point in an efficient fume hood. D-glucose (80 g) was added in portion (4-5 g) and the flask without shaking was heated carefully at the point nearest the sugar laying on the bottom. Initiation of the reaction was indicated by continued boiling after removal of the flame, the flask was placed on a cork ring and the flame was extinguished. The remainder of the sugar was then added in small portions at a rate which maintains the boiling temperature of the mixture. The flask was shaken occasionally to prevent an accumulation of solid sugar on the bottom of the flask. After the addition of all the sugar and after the reaction had subsided, the solution was brought to a full boil. It was then cooled and poured with stirring onto cracked ice. After standing 2 h with occasional stirring, the material was filtered with suction and washed with cold water. The crude solid was crystalised with hot 500 ml ethanol to afford â-D-glucose pentaacetate **19** (63.5g) in 73 % yield as a solid.

mp: 131 °C

3-(2',3',4',6'-Tetra-*O*-acetyl-a-*C*-D-glucopyranosyl)-1-propene (20):

To a solution of **19** (10 g, 0.025 mol) in dry acetonitrile were added allyltrimethylsilane (40.8 ml, 0.025 mol) and BF_3: Et_2O (32.2 ml) under N_2. The reaction mixture was refluxed for 8 h, cooled to room temperature, solvent was evaporated, and poured into a saturated solution of $NaHCO_3$ and extracted with ethyl acetate, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:5) as eluent to afford **20** (4.10 g) in 43 %yield.

$[\alpha]_D$ +71 ° (c 1, $CHCl_3$) lit[20] $[\alpha]_D$ +72 ° (c 1, $CHCl_3$)

mp: 106 °C lit[20] mp: 108 °C

^{1}H NMR ($CDCl_3$, 200 MHz): ä 2.03, 2.04, 2.05 and 2.07 (4s, 12H, 4×CH_3CO), 2.30 (m, 1H, H-3a), 2.50 (m, 1H, H-3b), 3.82 (m, 1H, H-5'), 4.00 (dd, 1H, J = 2.4, 11.6 Hz, 6'a), 4.10-4.30 (m, 2H, H-1', H-6'b), 4.90 (t, 1H, J = 9.8 Hz, H-4'), 5.00-5.20 (m, 4H, H-1a, H-1b, H-2'), 5.27 (t, 1H, J = 9.8 Hz, H-3'), 5.70 (m, 1H, H-2).

3-(2',3',4',6'-Tetra-*O*-benzyl-a-*C*-D-glucopyranosyl)-1-propene (22):

A solution of compound **20** (4.00 g, 0.01 mol) in dry MeOH (10 ml) was added 0.1 N NaOMe (15 ml) in MeOH and stirred for 15 min, then the mixture was neutralised with ion exchange resin (Amberlite IR-120, H^+), filtered and the filtrate was concentrated to give tetrol (**21**). The tetrol **21** was used for the next step without further purification.

To a suspension of sodium hydride (2.25 g, 60 % dispersion in oil) in dry DMF was added a solution of **21** (2.30 g, 0.01 mol) in dry DMF at 0 °C under nitrogen atmosphere and stirred for 2 h. Then benzyl bromide (5.39 ml, 0.045 mol) was added and stirring was continued for 18 h. The mixture was poured into water and extracted with diethyl ether. The ether layer was washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:20) as eluent to afford **22** (5.82 g) as a syrup in 96 % yield.

$[\alpha]_D$ +28 ° (c 1.8, $CHCl_3$) lit[21] $[\alpha]_D$ +29 ° (c 1.8, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.45 (m, 2H), 3.50-3.81 (m, 6H), 4.12 (m, 1H, H-1'), 4.40-4.92 (m, 8H, 4×$PhCH_2$), 5.10 (m, 2H, -CH=CH_2), 5.83 (m, 1H, -CH=CH_2), 7.10-7.40 (m, 20H, 4×Ph).

3-(2',3',4',6'-Tetra-*O*-benzyl-a-*C*-D-glucopyranosyl)-1,2-propanediol (23):

To a stirred solution of **22** (5.80 g, 10.28 mmol) in a mixture of acetone and water (9:1) were added OsO_4 (cat. 0.1 ml) and NMO (2.76 ml, 20.42 mmol). The resulting reaction mixture was stirred for 12 h at room temperature. The OsO_4 was quenched with sodium

metabilsulfate, and acetone was evaporated, water was added and extracted with ethyl acetate, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using methanol-chloroform (1:99) as eluent to give **23** (5.64 g) as a solid in 92 % yield.

mp: 114-115 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.70-2.10 (m, 2H, CH_2-3). 3.30-3.90 (m, 9H), 4.22 (m, 1H, H-1'), 4.40-4.93 (m, 8 H, 4 x $PhCH_2$), 7.12-7.40 (m, 20H, 4 x Ph).

(2E)-4-(2',3',4',6'-Tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-2-butenoate (25):

To compound **23** (5.60 g, 9.36 mmol) 30 ml of benzene was added and the suspension was just brought to the boil while stirring moderately. Then lead tetraacetate (6.22 g, 14.04 mmol) was added in equal portions (1 g) at intervals of 1 min. This mixture without further heating and the stirring was continued for an additional 15 min at 70 °C. Excess of lead tetraacetate was reduced with few drops of ethylene glycol and after cooling to room temperature the precipitated lead diacetate was filtered off and washed with benzene. The combined filtrates were extracted with ethyl acetate, dried over Na_2SO_4 and concentrated to furnish an aldehyde **24** (5.25 g) which was used for the next reaction without further purification.

To an ice cooled (0 C) solution of **24** (5.25 g, 9.27 mmol) in dry CH_2Cl_2 (30 ml) was added Ph_3P=CH-COOEt (3.22 g, 9.27 mmol) and stirred at room temperature for 3 h. Then the dichloromethane was evaporated and the residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:9) as eluent to afford **25** (5.08 g) as a syrup in 86 % overall yield.

$[\alpha]_D$ +43 ° (c 1.2, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.27 (t, 3H, J = 7.5 Hz, $COOCH_2CH_3$), 2.62 (t, 2H, J = 7.5 Hz, CH_2-4), 3.45-3.80 (m, 6H), 4.15 (m, 3H, $COOCH_2CH_3$ and H-1'), 4.30-5.00 (m, 8H, 4 x $PhCH_2$), 5.87 (d, 1H, J = 15.0 Hz, H-2), 6.90 (dt, 1H, J = 7.5, 15.0 Hz, H-3), 7.32 (m, 20H, 4 x Ph).

(2E)-4-(2',3',4',6'-Tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-but-2-ene-1-ol (26):

To a solution of **25** (5.08 g, 7.9 mmol) in dry CH_2Cl_2 (30 ml) at –78 °C was added DIBAL-H (12.10 ml, 15.97 mmol of a 1.4 M solution in toluene). After stirring for 1 h at –78 C, the reaction was decomposed with sodium potassium tartarate, and the white precipitate was filtered through a pad of Celite bed and washed with dichloromethane. The filtrate was extracted with dichloromethane, washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:4) to give **26** (2.80 g) in 56 % yield.

$[\alpha]_D$ +46 ° (c 1, $CHCl_3$), lit[26] $[\alpha]_D$ +44 ° (c 1.56, $CHCl_3$),

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.40 (m, 2H, CH_2-4), 3.30-3.80 (m, 6H), 3.95 (d, 2H, J = 5.00 Hz, 1-CH_2), 4.02 (m, 1H, H-1'), 4.32-4.90 (m, 8H, 4 x Ph CH_2), 5.56 (m, 2H), 7.32 (m, 20H, 4 x Ph).

(2S,3S)-2,3-Epoxy-4-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-1-butanol (27):

To a stirred solution of titanium tetra-isopropoxide (0.069 ml, 0.23 mmol), molecular sieves (4A°) powder in dry CH_2Cl_2 at –20 °C was added (-) diisopropyl tartarate (0.06 mol, 0.28 mmol) and stirred at that temperature for 20 min. To this solution was added compound **26** (2.80 g, 4.7 mmol) in dry CH_2Cl_2 (15 ml) and after stirring for 20 min *tert*-butyl hydroperoxide (1.44 ml, 10.83 mmol of a 7.5 N in isooctane) was added and stirred for 4 h at –20 °C. The reaction mixture was filtered through celite. The filtrate was cooled to 0 °C, distilled water was added and stirred for 20 min. Then 30 % NaOH solution saturated with

NaCl (1.2 ml) was added and stirred for 30 min. The emulsion was filtered through a pad of Celite and extracted with dichloromethane, washed with brine dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether as eluent to afford **27** (2.35 g) as a solid in 82 % yield.

$[\alpha]_D$ 46 ° (c 1, $CHCl_3$)

%de = 99,

mp: 112 °C

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.82-2.20 (m. 2H, CH_2-4) 2.93 (m, 1H, H-2), 3.00 (dt, 1H, J = 2.3, 5.6 Hz, H-3) 3.43 (t, 1H, J = 7.9 Hz), 3.50-3.74 (m, 7H), 4.25 (m, 1H, H-1) 4.47 (1/2 AB_q, 1H, J = 11.2 H, PhCH), 4.52 (AB_q, 2H, J = 11.2 Hz, $PhCH_2$), 4.65 (AB_q, 2H, J = 11.2 Hz, $PhCH_2$), 4.76 (1/2 AB_q, 1H, J = 11.2 Hz, PhCH), 4.79 (1/2 AB_q, 1H. J = 11.2 Hz, PhCH), 4.88 (1/2 AB_q, 1H, J = 11.2 Hz, Ph-CH), 7.30 (m, 20H, 4 x Ph).

^{13}C-NMR ($CDCl_3$, 50 MHz): δ 27.66, 53.81, 58.86, 61.90, 69.29, 71.66, 71.81, 73.11, 73.55, 75.01, 75.36, 78.05, 79.34, 82.00, 127.62, 127.77, 127.84, 127.95, 128.38, 137.69, 132.93, 138.50.

HRMS (FAB): calcd for $C_{38}H_{42}O_7$ (M^+) 611.3008, found 611.3058.

(2S,3R)-3-Azido-4-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-1-2-butane diol (28)

A mixture of **27** (2.3 g, 3.7 mmol), sodium azide (1.22 g, 18.7 mmol) and ammonium chloride (0.44 g, 8.2 mmol) in MeOH : H_2O (8:1) (27 ml) was heated under reflux for 7 h. The methanol was removed under reduced pressure, water was added and extracted with ethyl acetate, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1: 4) as eluent to furnish **28** (1.87 g) and **29** (0.32 g) in 5.7: 1 ratio (87 % yield).

$[\alpha]_D$ +38 ° (c 1, $CHCl_3$),

mp: 74-76 °C

compound **28:** ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.05-2.35 (m, 2 H, CH_2-4), 3.12-3.84 (m, 9H), 3.85 (t, 1H, J = 9.0 Hz, H-4'), 4.10 (m, 1H, H-1'), 4.30-4.95 (m, 8H, 4 x $PhCH_2$), 7.10-7.40 (m, 20H, 4 x Ph) IR (KBr) 3400 (OH), 3050 , 2910, 2850, 2910, 2100 (N_3), 1450, 1090, 1060 cm^{-1}.

compound **29:** ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.60-2.00 (m, 2H), 3.25-3.42 (m, 2H), 3.48-3.85 (m, 8H), 4.22 (m, 1H, H-1'), 4.30-4.90 (m, 8H), 7.00-7.300 (m, 20H, 5 x Ph).

(2S,3R)-3-Azido-1-*tert*-butyldiphenylsilyoxy-4-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-butane-2-ol (30):

To a solution of compound **28** (1.21 g, 1.85 mmol) in dry CH_2Cl_2 (10 ml) were added TBDPS-Cl (0.45 ml), imidazole (0.25 g, 3.67 mmol) and the reaction mixture was stirred for 90 min at room temperature. Water was added and extracted with dichlromethane, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:6) as eluent to afford **30** (1.10 g) as a syrup in 78 % yield.

$[\alpha]_D$ +28 ° (c 1, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.05 (s, 9H ^{t}-Bu), 2.06 (m, 2H, CH_2-4), 3.20-3.80 (m, 10H), 4.25 (m, 1H, H-1'), 4.92 (m, 8H, 4 x $PhCH_2$) 7.20-7.40 (m, 25H, 5 x Ph), 7.65 (m, 5H, Ph).

(2S,3R)-3-Azido-2-benzyloxy-1-*tert*butyldiphenylsilyloxy-4-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-butane (31):

A solution of compound **30** (0.42 g, 0.52 mmol) in dry THF (8 ml) was added to a suspension of sodium hydride (24 mg) in THF at 0 °C under nitrogen atmosphere and after stirring for 30 min, benzyl bromide (0.075 ml, 0.62 mmol) was added. The reaction mixture was stirred at room temperature for 6 h. The excess sodium hydride was quenched with methanol and solvents were evaporated, water was added and extracted with ethyl acetate,

dried over Na_2SO_4 and concentrated. Purification of residue by column chromatography on silica gel using ethyl acetate: petroleum ether (1:9) gave **31** (0.37 g) as a syrup in 82 % yield.

$[\alpha]_D$ +23 ° (c 1.03, $CHCl_3$)

^{1}HNMR ($CDCl_3$, 200 MHz): δ 1.09 (s, 9H, *t*-Bu), 1.80-2.20 (m, 2H, CH_2-4), 3.50-3.90 (m, 10H), 4.22 (m, 1H, H-1'), 4.40-4.95 (m, 10H, 5 x Ph CH_2), 7.10-7.42 (m, 30H, 6 x Ph), 7.72 (m, 5H, Ph).

(2S,3R)-3-Azido-2-benzyloxy-4-(2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranosyl)-1-butanol (32):

Tetrabutylammonium fluoride (0.21 g, 0.82 mmol) was added to a stirred solution of compound **31** (0.37 g, 0.41 mmol) in dry THF (5 ml) and stirred at room temperature for 2 h, water was added and extracted with diethyl ether, dried over Na_2SO_4. Upon concentration and purification of the residue over silica gel column using ethyl acetate: petroleum ether (1:3) as eluent afforded the alcohol **32** (0.26g) as a syrup in 87 % yield.

$[\alpha]_D$ +59 ° (c 1.7, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.95 (m, 2H, CH_2-4), 3.40-3.85 (m, 10H) 4.12 (m, 1H,H-1'), 4.40-5.00 (m, 10H, 5 Ph CH_2, 7.35 (m, 25H, 5 x Ph),

^{13}C NMR ($CDCl_3$, 50 MHz): δ 29.61, 60.52, 60.86, 68.90, 71.85, 72.23, 72.39, 73.36, 73.39, 74.49, 74.93, 77.96, 79.8o, 81.32, 82.10, 127.58, 127.64, 127.82, 128.31, 128.42, 137.65, 137.75, 137.92, 138.05, 138.52,

IR (KBr): 2092 cm^{-1} (N_3), 3450 (OH) cm^{-1}.

FABMS: m/z 716 (M^++1-N_2). HRMS (FAB) calcd for $C_{45}H_{49}NO_7$ (M^++1-N_2) 716.3587, found 716.3635.

(2S,3R)-[(2-Azido-3-benzyloxy)-(Z/E)-octadec-4-enyl]-2',3',4',6'-tetra-*O*-benzyl-α-*C*-D-glucopyranoside (34):

To a stirred solution of oxalyl chloride (0.054 ml, 0.62 mmol) in dry CH_2Cl_2 at −78 C was added dimethyl sulfoxide (0.09 ml, 01.2 mmol) drop wise under N_2 atmosphere, stirred for 30 min at that temperature and a solution of alcohol **32** (0.23 g, 0.3 mmol) in dry CH_2Cl_2 was added. The resulting reaction mixture was stirred for 1 h at −78 °C and triethyl amine (0.26 ml, 1.86 m mol) was added. The reaction mixture was warmed to room temperature, water was added and extracted with dichloromethane, washed with brine, dried over Na_2SO_4, filtered and evaporation of the solvent under reduced pressure gave aldehyde **33** (0.23 g) which was utilised for the next reaction without further purification.

To a stirred, cold (−78 C) solution of tetradecanyltriphenylphosphonium bromide (0.67 g, 1.26 mmol) in dry THF (10 ml) was added n-BuLi (0.8 ml, 1.1 mmol of a 1.4 M solution in hexane) under N_2, stirred for 30 min at −78 °C, then the above prepared aldehyde **33** (0.23 g, 0.31 mmol) in dry THF (5 ml) was added and stirring was continued for 2 h. Then saturated ammonium chloride solution was added and extracted with diethyl ether, washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:20) as eluent to give the title compound **34** (0.17 g) as an oil in 62 % yield.

Z-isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.88 (t, 3H, J = 7.2 Hz, 18-CH_3), 1.36 (brs, 22H, 11 x CH_2), 1.60-2.35 (m, 4H, 2 x CH_2), 3.45-3.80 (m, 6H), 4.00-4.90 (m, 13H, 5 x $PhCH_2$, H-1', H-2 and H-3), 5.38 (t, 1H, J = 11.0 Hz, H-4), 5.74 (dt, 1H, J = 8.0 Hz, J = 11.0 Hz, H-5), 7.30 (m, 25H, 25 x Ph).

E-isomer: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.82 (t, 3H, J = 7.2 Hz, 18-CH_3), 1.25 (brs, 22H, 11 x CH_2), 1.50-2.50 (m, 4H, 2 x CH_2), 3.42-3.80 (m, 6H), 4.03-4.85 (m, 13H, 5 x $PhCH_2$, H-1',

H-2 and H-3), 5.34 (t, 1H, J = 10.4 Hz, H-4), 5.70 (dt, 1H, J = 6.8 Hz, J = 10.4 Hz, H-5), 7.30 (m, 25H, 25 x Ph).

FABMS: m/z 894 (M^++1-N_2).

(2S,3R)-[2-N-(*tert*-Butyloxycarbonylamino)-3-benzyloxy-(Z/E)-octadec-4-enyl]-2',3',4',6'-tetra-*O*-benzyl-a -*C*-D-glucopyranoside (36):

To a stirred solution of **34** (83 mg, 0.09 mmol) in benzene (4 ml) at 45 °C was added triphenylphosphine (51 mg, 0.19 mmol). After 30 min. water (0.015 ml, 9.7 mmol) was added and stirring was continued at 45 °C for 11 h. The mixture was cooled to room temperature, water was added and extracted with ethyl acetate, washed with saturated ammonium chloride solution (NH_4Cl), dried over Na_2SO_4, filtered and concentrated to give amine product **35** (72 mg) which was used in the next step without further purification.

To a solution of crude amine **35** (72 mg, 0.080 mmol) in dry THF (4 ml) were added Et_3N (0.033 ml, 0.24 mmol) and $(BOC)_2O$ (26 mg, 0.12 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h. The solvent was evaporated in *vacuo* and the residue was purified by silica gel chromatography using ethyl acetate: pertroleum ether (1:15) as eluent to give **36** (60 mg) in 67 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.85 (t, 3H, J = 7.2 Hz, 18-CH_3), 1.20 (s, 22H, -$(CH_2)_{11}$-), 1.35 (s, 9H, NHCOO^t-Bu), 1.40 (m, 2H, 1-CH_2), 2.04 (m, 2H, 6-CH_2), 3.50-3.70 (m, 7H), 4.10-4.90 (m, 12H), 5.32 (t, 1H, J = 11.0 Hz, 4-H), 5.60 (m, 1H, 5-H), 7.00-7.32 (m, 25H, 5×ph).

IR (KBR): 3400, 2960, 2880, 1740 cm^{-1}. FABMS: m/z 896 (M^++1-BOC).

(2S,3R)-[2-N-(*tert*-Butyloxycarbonylamino)-3-*O*-acetyl-octadecanyl]-2',3',4',6'-tetra-*O*-acetyl-a -*C*-D-glucopyranoside (37):

To a solution of compound **36** (50 mg, 0.05 mmol) in MeOH was added 10% Pd-C (20 mg) and mixture was stirred for 48 h under baloon pressure and room temperature. The

catalyst was filtered through pad of Celite and the filtrate was concentrated to give **18**. A solution of **18** in pyridine (0.5 ml) and acetic anhydride (0.25 ml) was stirred for 24 h at room temperature. The solvent was evaporated in *vacuo* and the residue was purified by column chromatography on a silica gel using ethyl acetate: petroleum ether (1:3) as eluent to afford **37** (36 mg) in 95 % yield.

$[\alpha]_D$ +37 (c 0.3, $CHCl_3$)

^1H NMR ($CDCl_3$, 200 MHz): δ 0.79 (t, J = 7.1 Hz, 3H, 18-CH_3), 1.20 (bs, 28H, 14 x CH_2), 1.35 (s, 9H, NHCOOt-Bu), 1.62-1.81 (m, 2H, 1-CH_2), 1.96 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.99 (s, 3H), CH_3CO), 2.00 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 3.84 (m, 2H, H-2 and H-5'), 4.05 (m, 1H, H-6'a), 4.21 (m, 2H, H-1' and H-6'b), 4.63 (d, 1H, J = 9.4 Hz, N-H), 4.80 (dt, 1H, J = 4.0, 8.6 Hz, H-3), 4.92 (t, 1H, J = 8.6 Hz, H-4'), 5.06 (dd, 1H, J = 5.6, 8.6 Hz, H-2'), 5.19 (t, 1H, J = 8.6 Hz, H-3').

FABMS: m/z 658 (M^++1-BOC)

HRMS (FAB): calcd for $C_{34}H_{58}NO_{11}$ (M^++1-BOC) 658.4166, found 658.4146.

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INTRODUCTION :

It seems two and only two diseases in the world that have been considered "most formidable" in the threshold of 21st century. They are century-old cancer and recent AIDS. Although the every nook and corner of scientific research directed to find plausible answers to remedy these diseases, the target still seems to be far from sight. Cancer is not one disease, but a group of diseases affecting different organs and systems of the body. It develops due to the abnormal and uncontrolled cell division, frequently at a rate greater than that of the most normal body cells. In general the multiplication of cells is carefully regulated and responsive to specific needs of the body. In a young animal, cell multiplication exceeds cell death, so the animal increases in size; in an adult, the process of cell birth and death are balanced to produce a steady state. Very occasionally, the exquisite controls that regulate cell multiplication breaks down and although the body has no need for further cells of its type, a cell begins to grow and divide. When such a cell has descendents that inherit the propensity to grow without responding to regulation, the result is a clone of cells able to expand indefinitely. Ultimately, a mass called a tumor may be formed by this clone of unwanted cells. Tumors arise with great frequency, especially in older animals and humans, but most pore little risk to their host because they are localized. Such a tumor is called benign; an example is warts. Tumor become life threatening if they spreadout throughout the body. Such tumors are called malignant and are the cause of cancers[1].

The incidence of bronchogenic carcinoma has reached epidemic proportions in the developed world. The disease is the most common malignancy and leading cause of death from cancer among men in many countries. Cigarette smoking is the single major contributing factor.

The efforts to provide preventive measures to cancers have not been very effective, as variety of chemicals and environmental factors can cause cancer. Thus once the cancer has

developed one has to resort to its treatment. There are four major modalities for the treatment of the cancer 1) surgery, 2) radiation therapy, 3) immunotherapy, 4) chemotherapy and 5) genetic therapy. Surgery cannot be applied when the disease is spread throughout the body and radiation therapy damages not only the cancerous cells but also the normal cells. Thus, in this situation, the only treatment for the disseminated cancer is chemotherapy, although immunotherapy, the manipulation of immune response holds encouraging promise, but it is still in its infancy.

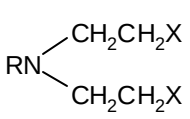
Chemotherapy is today providing increasing cure rates in many forms of human cancer[2]. Because of our day by day progress in understanding the effects of drugs on cells, both normal and cancerous, there has been a continued improvement in this mode of cancer treatment. However, it cannot be denied that for continued advances more effective drugs still need to be found.

The mechanism by which the anticancer drugs selectively kill cancer cells has not been clearly established, but evidence points out these drugs might interfere with the synthesis (or) function of nucleic acid or with the mitotic process itself. The advances in quantitative structure-activity relationship (QSAR) have evidenced the scope of rationalizing of drug design and finding the mechanism of drug actions. QSARs have proven their worth in the interpretation of mechanism of inhibition of a number of enzyme systems and in elucidating the modes of actions of local anesthetics and a variety of drugs acting at the central nervous system.

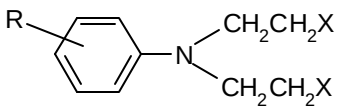
CLASSIFICATION OF ANTICANCER DRUGS:

Anticancer drugs belong to different categories of chemicals and follow different modes of action. On the basis of their modality of action they are classified as chemically reactive drugs having nonspecific action, Mitotic inhibitors, Cellular respiration inhibitors, Hypoxia -selective and radiosensitizing agents, agents binding to DNA , antimetabolites.

A variety of chemicals elicit their anticancer activity by alkylating nucleic acids. Alkylating agents can combine covalently with nucleophilic center and thus can attack non-selectively at any nucleophilic center available *in vivo*. These centers can be nitrogen, sulfur, or oxygen atoms of biologically important functional groups, such as amino groups, thiolate anions of proteins and ring nitrogen atoms and phosphate anions of nucleic acids DNA is the critical target of biological alkylating agents and that the 7 position guanine is the primary site of attack. Aliphatic and aromatic nitrogen mustards having simple general structures as **1** and **2** respectively, form an important class of alkylating agents – drugs that have non-specific action. Among the variety of alkylating agents, nitrogen mustards are widely studied and better exploited for chemotheraphy[3].



1

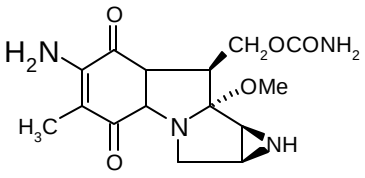


2

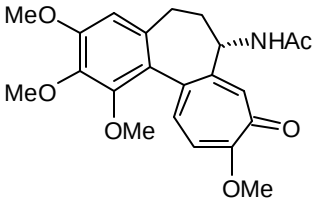
Mitomycin C(**3**) is one of the most useful antitumor antibiotics which have been clinically used in cancer chemotheraphy, but its use is limited by side effects, severe bone marrow suppression (or) gastrointestinal damage. Mitomycin C (**3**) has shown antitumor activity against four different human tumor cell lines, WiDr colon, 2780 ovarian, MCF-7 breast cancer, and P_{388} leukemia in culture[4].

Colchicine (**4**) is a potent mitotic inhibitor. Its antitumor property long since has been recognized, but its use in the treatment of neoplasms is still limited. Its activity is though to be due to its ability to bind to cysteine residue in tubulin polypeptide chain, thus preventing the cell division[5].

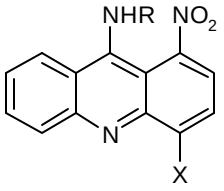
Recently, a variety of compounds were studied for their hypoxia-selective antitumor activity[6]. These compounds were substituted nitracrines (**5**), nitroaniline (**6**) mustards and nitroquinolines (**7**).



Mitomicine C (3)



Cholchicine (4)



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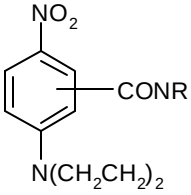

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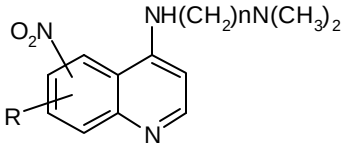
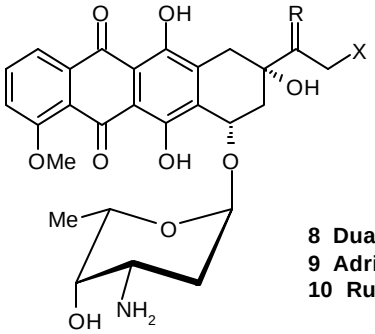


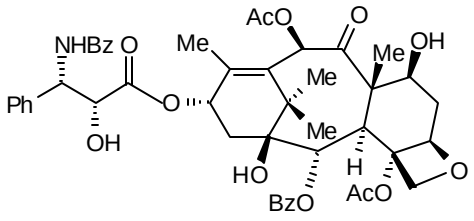
7

DNA-binding agents constitute one of the most important classes of anticancer drugs in clinical use today whose antineoplastic effectiveness depends upon the mode and intensity of binding. A great number of DNA-binding agents such as 9-anilinoacridines 9-amino acridines, anthracyclines and their derivatives were prepared and tested for their antitumor activities. For example, duanomycin (**8**) and adriamycin (**9**), which exhibit impressive activity against a wide range of experimental and human tumors, have become important for cancer chemotherapy[7] and their usefulness has generated considerable interest in developing analogues with improved properties. Among these analogues, rubidazone (**10**) has received considerable attention, people were encouraged by its decreased cytotoxicity relative to adriamycine.

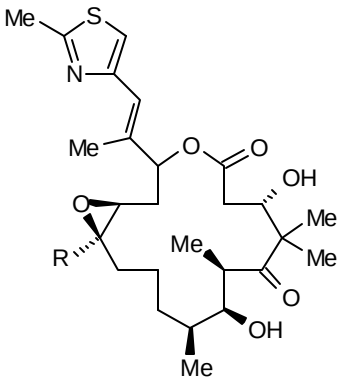


8 Duanomycin R = O, X = OH
9 Adriamycine R = O, X = H
10 Rubidazone R = NNHCOC$_6$H$_5$, X = H

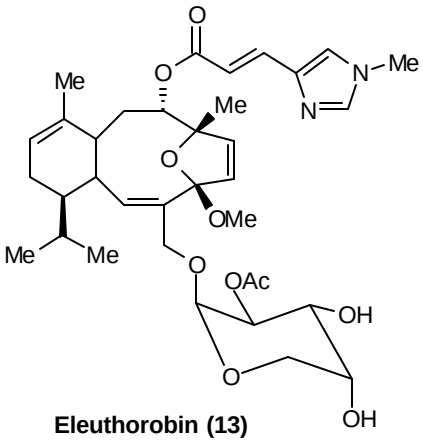
In the ongoing search for new effective chemotherapeutic agents, a wide variety of cytotoxic compounds such as taxol (**11**), epothilone (**12**),eleutherobin (**13**) and sarcodictyin (**14**) etc have emerged in very recent period. Therefore, there has been considerable interest in the synthesis of this class of compounds in recent years[8].



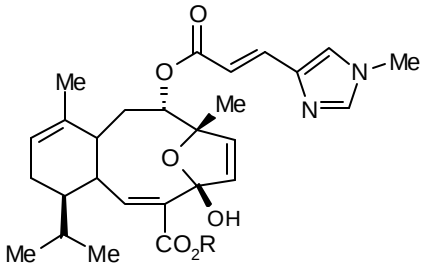
Taxol (11)



(12) R = H : Epothilone A
R = Me : Epothilone B



Eleuthorobin (13)

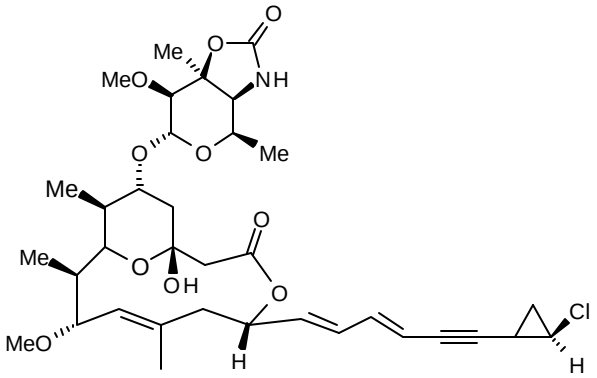


(14) R = Me : Sarcodictyin A
R = Et : Sarcodictyin B

In search of new antitumor agents from different natural sources, every year thousands of natural products are isolated and screened by natural products chemists and phytochemists. And this way some new anticancer compounds with promising therapeutic value have been discovered. Sponges of the order *lithistida* continue to be exceptionally rich sources of structurally unique and biologically active natural products[9]. Examples include potent cytotoxic macrolides[10] such as swinholides, misakinolide, theonezolides, sphinxolides, superstolides, discodermolide and callipeltoside A[11].

Due to their recognized importance as antitumor agents, these molecules have attracted considerable attention from the isolation and synthetic point of view. Inspite of the biological activities exhibited by these natural compounds, they are actually relatively scare and difficult to obtain in homogeneous form from natural sources. Because of this scarcity and the difficulty of isolation in sufficient quantity, the synthesis of isomerically pure compounds is a challenging goal for organic chemists.

The much attention has not been focused on callipeltoside A (**15**) since its isolation from the shallow lithisted sponge, *callipetta sp*. The structure of callipeltoside A was elucidated by NMR, NOE experiments and other spectral studies. Only the relative configuration of callipeltoside A has been established. Callipeltoside A, a cytotoxic glycoside macrolide, is the first member of unprecedented class of marine natural products with unusual structural features such as previously unknown 4-amino-4,6-dideoxy-2,*O*,3-*C*-dimethyl-α-talopyranosyl-3,4-urethane (callipeltose), linked through an α-glycoside linkage to an-hemiketal oxane ring, which is part of a 14-membered macrocycle lactone with dienyne cyclopropane side chain.



Callipeltoside A (15)

Callipeltoside A exhibited remarkable activity in cytotoxic assays against KB and P_{388} cells and in the anti-HIV tests. Callipltoside A has a moderate activity with IC_{50} values against NSCLC-N_6 human bromchopulmonary non-small-cell-lung carcinoma and P_{388} of 11.26 and

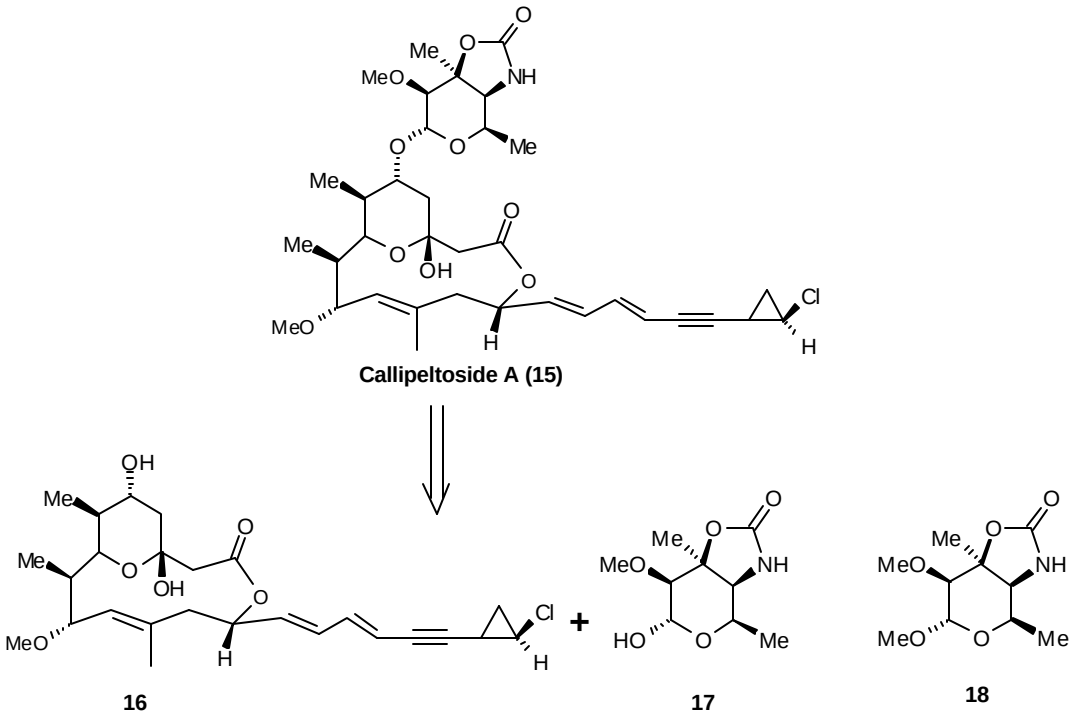
15.26 μg/mL respectively. Further cell cycle analysis by flow cytometry assays of the NSCLC-N_6 cell line treated with callipeltoside A revealed a cell cycle dependent effect, involving a dependent GI blockage. These results are indicative of the NSCLC-N_6 cell proliferation *in vitro* at the level of GI phase or by enzyme inhibition or inducing terminal cell differentiation.

Barring a few reports concerning the synthetic studies directed towards this molecule, no full-breight total synthesis has been achieved sofar. Undoubtedly, the unique structural parameters present a meaningful challenge to every synthetic chemist. In continuation of ongoing research towards the synthesis of biologically-active compounds, we planned to construct the glycon frame work of callipeltoside A.

PRESENT WORK:

As part of total synthesis of natural product callipeltoside A (**15**), we simultaneously initiated studies on both the aglycone segment (**16**) and sugar component callipeltose (**17**). This chapter describes the total synthesis of sugar component (**17**) as its methyl glycoside derivative (**18**). The structural parameters of **18** are quite unique as it contains a oxazolidinone fused on the 6-deoxy-pyranose ring having D-talose configuration. The presence of tertiary C-methyl group at C-3 makes **18** as a branched-chain carbohydrate derivative (Scheme-1).

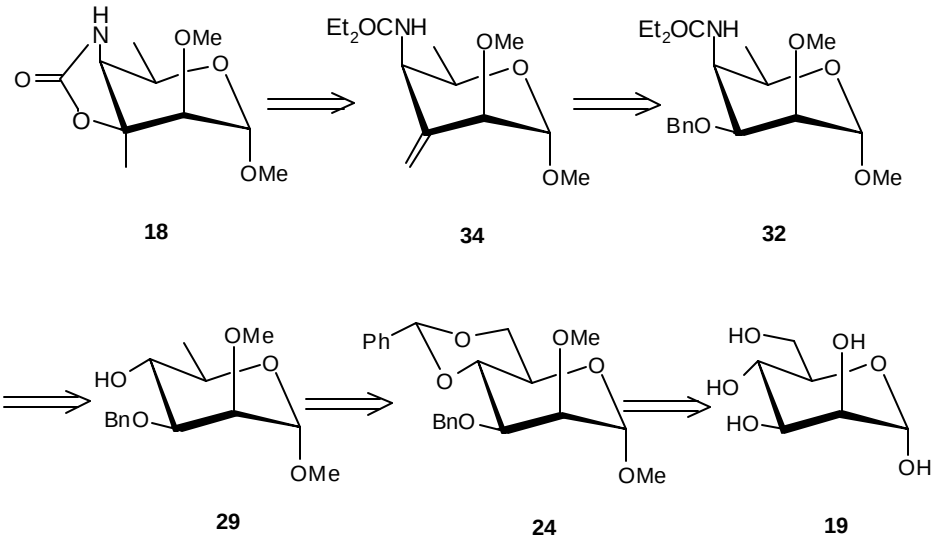
Scheme-1



Retrosynthetic analysis of methyl callipeltoside (**18**).

The basic premise by which the tertiary chiral centre at C-3 present in the callipeltose **18** could be generated related to the stereocontrolled iodolactonisation reaction. Accordingly the target molecule **18** was envisioned to have arisen from the offspring synthon **34**, obtainable by the introduction of ethoxycarbonylamine at C-4 with desired stereochemistry and exo-methylene group at C-3 (Scheme-2).

Scheme-2

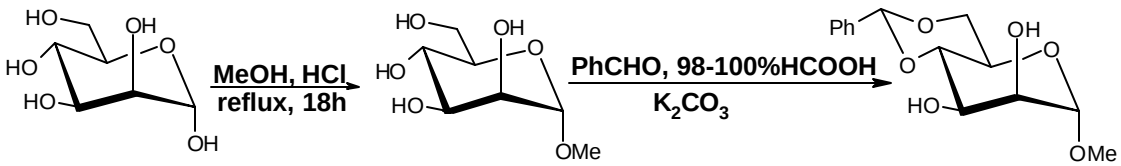


18 **34** **32**

29 **24** **19**

Synthesis of olefin intermediate **34** was initiated from D-mannose (**19**). D-Mannose was converted into methyl α-D-mannopyranoside (**20**) by using methanol and HCl (g) under reflux for 18 h. Subsequently, **20** on treatement with benzaldehyde 98-100 % formic acid gave methyl 4,6-*O*-benzylidene α-D-mannopyranoside (**21**) in 35 % yield (Scheme-3)[12].

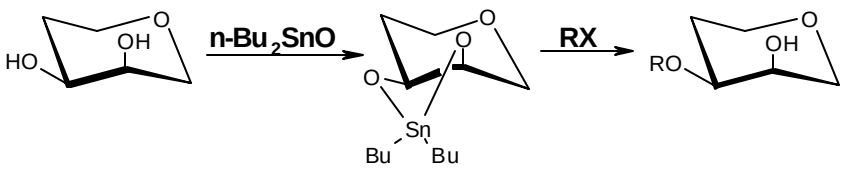
Scheme-3



19 **20** **21**

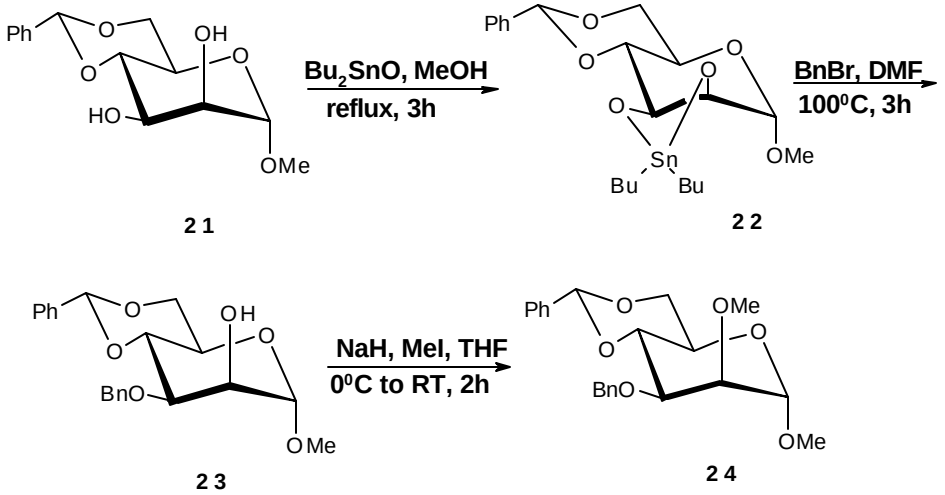
In order to selectively block the equatorial OH group at C-3 of **21**, the alkylation of stannylacetal strategy was adopted (Scheme-4)[13].

Scheme-4



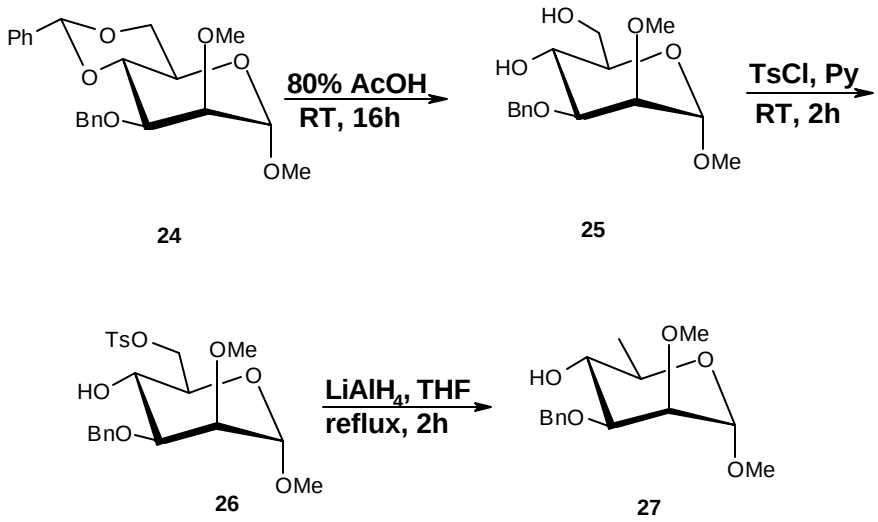
Thus compound **21** and n-Bu_2SnO were heated under reflux in methanol for 1 h to give dibutyltin acetal derivative (**22**) after removal of MeOH. The crude product **22** in DMF and benzyl bromide were heated at 100 C to give the 3 -*O*-benzylated derivative (**23**). The characteristic peaks due to benzyl group were clearly observed in the ^{1}H NMR spectrum of **23**. The hydroxy group at C-2 of **23** was protected as its methyl ether using sodium hydride and methyl iodide in THF to afford **24** (Scheme-5). The ^{1}H-NMR spectrum of **24** showed two showed two singlets at 3.36 and 3.56 ppm due to two methoxyl groups and the benzylic signals

Scheme-5



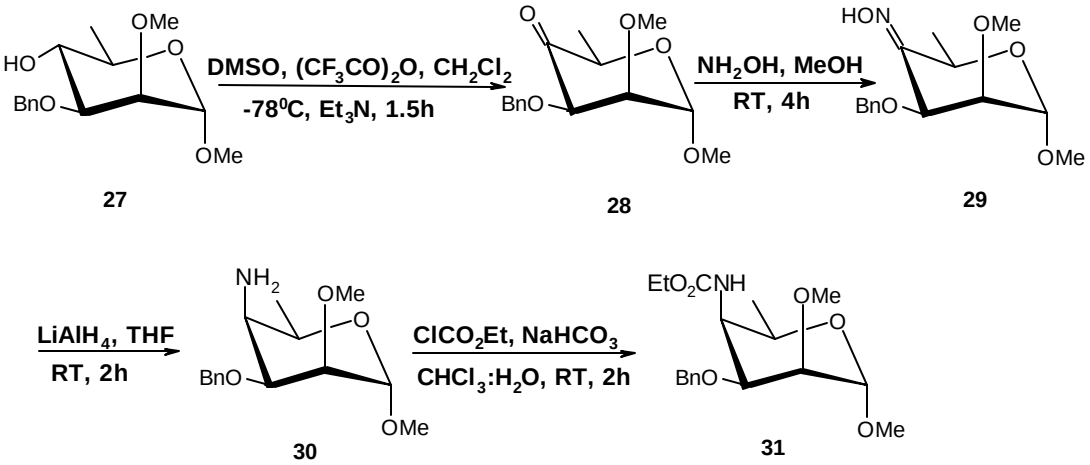
at doublets at 4.68 and 4.90 ppm. The anomeric proton and acetal proton appeared at 4.70 and 5.60 ppm as singlets. The remaining signals were in complete agreement with the assigned structure **24**. The benzylidene group in compound **24** was cleaved with 80 % acetic acid to afford the diol **25**, which was subjected to selective tosylation at O-6 with TsCl and pyridine to give the 6-*O*-tosylate derivative **26** in 61 % yield. The ^{1}H-NMR spectrum of **26** showed a singlet at 2.44 ppm due to tosyl methyl group. The remaining protons resonated at their expected chemical shifts. Reduction of **26** with LAH in THF under reflux provided the 6-deoxy product **27** (Scheme-6). In the ^{1}H-NMR spectrum of **27** the characteristic doublet (J = 6.5 Hz) due to H-6, 6', 6" was located at 1.28 ppm.

Scheme-6



In order to introduce an amino group at C-4, coumpound **27** was oxidized with oxidizing agent $(CF_3CO)_2O$-DMSO and Et_3N at −78 °C to furnish the 4-ulose derivative **28.** The ^{1}H-NMR spectrum of **28** showed a doublet at 3.87 ppm due to H-3 proton and H-5 appeared as a quartet at 4.12 ppm. While H-2 appeared as a doublet (J = 4.5 Hz) at 4.36 ppm due to coupling between H-3 and H-2 only. The 4-ulose derivative (**28**) was immediately treated with NH_2OH in methanol at room temperature for 18 h to afford the oxime **29**. Stereoselective reduction[14] of oxime **29** with $LiAlH_4$ in THF followed by protection of the

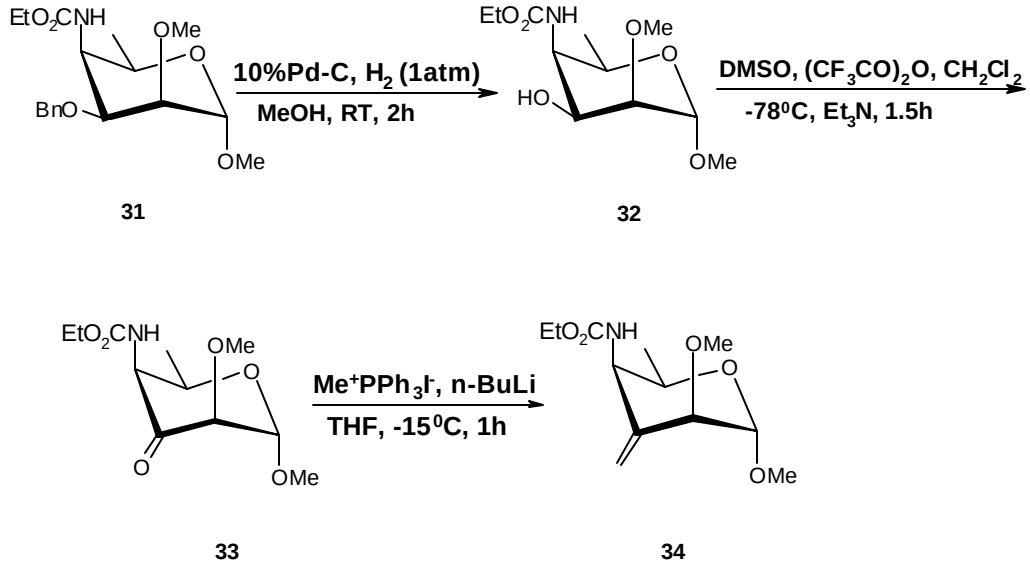
Scheme-7



amine group of **30** with ethyl chloroformate furnished the carbamate derivative **31** (Scheme-7). The D-talo configuration of **31** was assigned by the ^{1}H NMR spectrum in which the characteristic coupling constant $J_{3,4} = J_{4,5} = 3.5$ Hz was observed. The epimeric D-mannose configuration could not be detected in the ^{1}H-NMR spectrum.

Our next aim was to introduce an exo-methylene group at C-3 position. The benzyl group in compound **31** was cleaved by hydrogenolysis over 10 % Pd-C under baloon pressure and room temperature to afford **32** in 95 % yield. The signals due to benzyl group had disappeared in ^{1}H NMR spectrum of **32**. The structure of **32** was further confirmed by CIMS m/z: 264 (M^++1). Subsequent oxidation of **32** with $(CF_3CO)_2O$-DMSO and Et_3N at –78 °C gave the 3-keto derivative **33** which was subjected to Wittig olefination with $Ph_3P^+CH_3I^-$ and n-BuLi in THF to provide 3-exo-methylene derivative **34** in 56 % yield (Scheme-8).

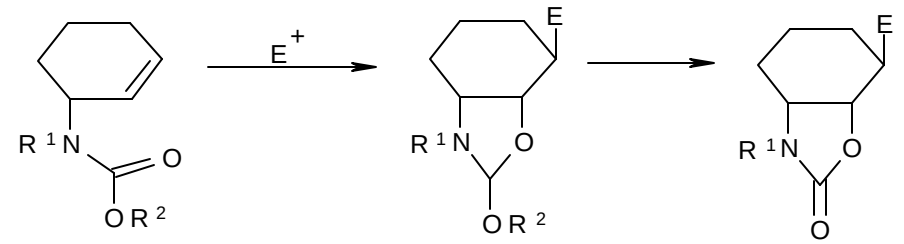
Scheme-8



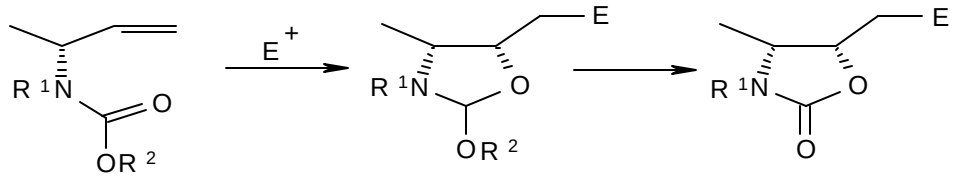
The ^{1}H NMR spectrum of **34** showed two singlets at 5.12 and at 5.40 ppm due to methylene protons and H-2 proton appeared as a singlet at 3.48 ppm. The remaining signals resonated at their expected chemical shifts. The structure of **34** was further confirmed by HRMS (CI): calcd. for $C_{12}H_{21}NO_5$(M^++1) 260.1497, found 260.1503.

Halocyclocarbamation is an interesting reaction which efficiently introduces functional groups such as aminoalcohols. Fraser-Reid *et al*. have made a significant advance towards the synthesis of aminosugars by using this reaction for cyclic systems. In fact the halocyclofunctionalization of double bonds in acyclic system is a simple strategy that proceeds under high regio and stereo control, as shown in the following cyclic system (Scheme-9)[15].

Scheme-9



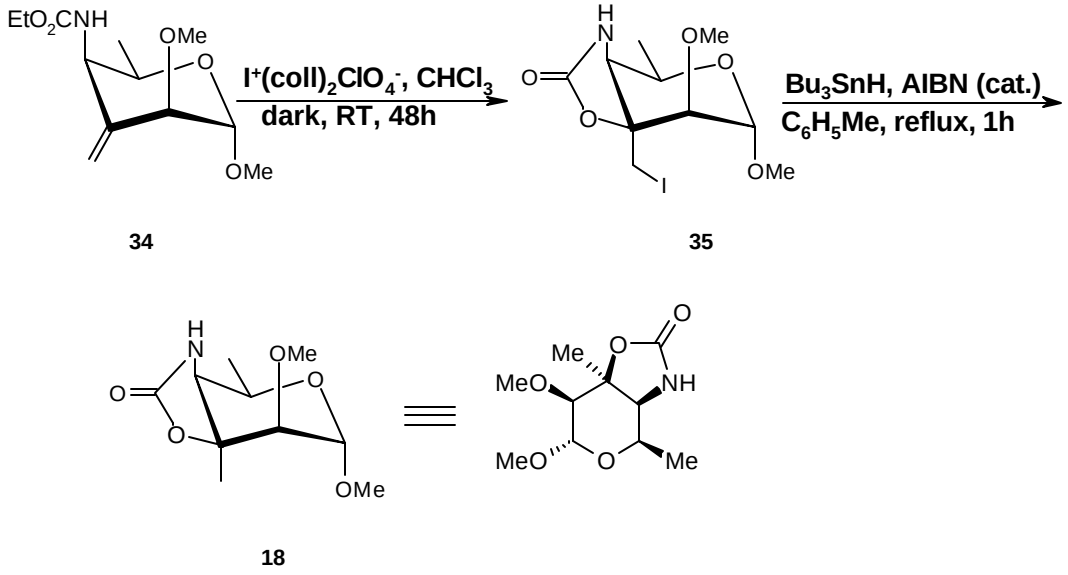
R 1 = H, Alkyl
R 2 = Alkyl



R 1 = H, Alkyl
R 2 = Alkyl

Compound **34** was subjected to iodolactonisation with iodoniumdicollidine perchlorate in $CHCl_3$ at room temperature to afford **35** in 50 % yield. The structure of **35** was confirmed by ^{1}H-NMR and mass spectral analysis. The ^{1}H-NMR spectrum of **35** showed a doublet at 3.36 ppm due to one CH_2I protons, a singlet at 3.77 ppm due to H-4 and a multiplet at 3.85 ppm for H-2, H-5 and one of CH_2I protons. The anomeric proton appeared as a doublet (J = 4.5 Hz) at 4.61 ppm and NH proton was located at 7.0 ppm as a broad singlet. The rest of the protons appeared at their expected values. The structure of **35** was further confirmed by CIMS: m/z 358 (M^++1).

Scheme-10



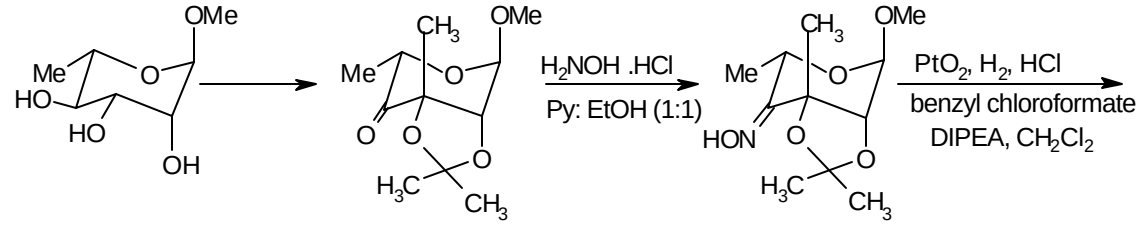
Reductive deiodination of **35** with tributyltin hydride and cat. AIBN in refluxing toluene furnished the methyl glycoside of callipetose **18** in 95 % yield (Scheme-10). The structure of **18** was supported by the ^{1}H NMR and high resolution mass spectral data. The ^{1}H NMR spectrum of **18** revealed a singlet due to C_4-Me, a doublet ($J_{1,2}$ = 4.5 Hz) at 3.18 ppm due to H-2 and H-4 proton appeared as a broad singlet at 3.39 ppm. The anomeric proton appeared at 4.69 ppm as a doublet ($J_{1,2}$ = 4.5 Hz) while the NH proton was located at 7.45 ppm as a broad singlet. Remaining signals were in complete agreement with the assigned structure. The structure of **18** was further confirmed by the high resolution mass spectrum (HRMS) in which the highest mass peak (M^++1) was observed at m/z. 232.1174 calcd. for $C_{10}H_{17}NO_5$ (M^++1) 232.1184.

In conclusion an efficient synthesis of methyl callipeltosside (**18**) has been developed. Studies related to the macrocyclic structural frame work of callipeltoside A (**15**) to which the above sugar is bound , are under investigation.

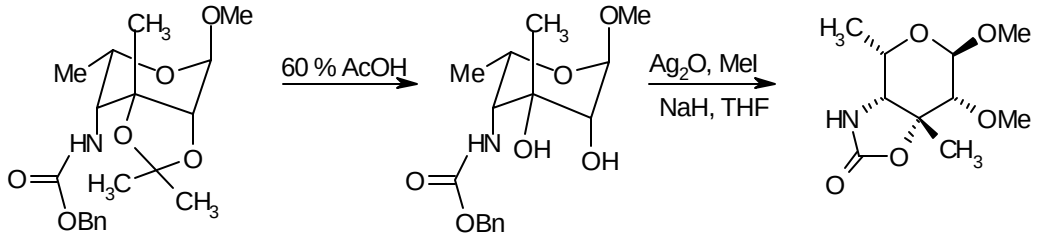
POST PUBLICATION:

Synthesis of methyl α-L-callipeltoside **41**, since the only relative configuration of sugar fragment in callipeltoside A has been established, was reported by Giuliano *et al*[16] almost simultaneously with our report. Methyl α-L-rhamnopyranoside (**36**) was converted to the 3*C* -methyl branched sugar **37** by a procedure known in literature. The compound **37** was treated with hydroxylamine hydrochloride to give an oxime **38**. Reduction of oxime **38** with reduced platinum oxide followed by protection of resulting amine with benzyl chloroformate gave benzyl carbamate derivative **39**. Treatement of **39** with 60 % acetic acid resulted in complete removal of isopropylidene group to give **40**. Finally the methyl α-L-callipeltoside (**41**) was obtained by the selective methylation of **40** using Ag_2O-CH_2I_2 in DMF followed by cyclisation in the presence of sodium hydride (Scheme-11).

Scheme-11



36 **37** **38**



39 **40** **41**

EXPERIMENTAL:

Methyl 4,6-*O*-Benzylidene-α-D-mannopyranoside (21):

D-Mannose (**19**) (75 g, 416.66 mmol) was dissolved in methanolic HCl (p^H = 2, 350 ml) and the reaction mixture was heated under reflux for 18 h, cooled and neutralised with lead carbonate. The solid was filtered and the methanol was removed at diminished pressure to give **20** (66.50 g). Finely powdered methyl α-D-mannoside (**20**) (66.40 g, 0.342 mol) was dissolved as rapidly as possible in 98-100 % formic acid (332 mol) and distilled benzaldehyde (332 ml) was immediately added to the solution. After 5min the mixture was poured with stirring into light petroleum (bp 60-80°, 2650 ml) and water (2650 ml) containing potassium carbonate (913 g anhydrous) inorganic material separated from the aqueous layer which was discarded. The upper layer was filtered and concentrated, the residue washed with light petroleum ether and crystallised from chloroform: benzene to give **21** (33.80 g) in 35 % yield.

mp: 140-142 °C lit[12] mp: 140-143 °C

$[\alpha]_D$ +61 ° (c 1.85, $CHCl_3$) lit[12] $[\alpha]_D$ +61 ° (c 1.84, $CHCl_3$).

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (23):

Compound **21** (33.80 g, 119 mmol) and di-n-butyltin oxide (35.80 g, 143 mmol) in dry MeOH (180 ml) was heated under reflux for 3 h. The solution was concentrated to give **22** and the residue **22** was dissolved in dry DMF (120 ml) and benzyl bromide (14.25 ml, 119 mmol) was added. The mixture was heated at 100 °C for 3 h, cooled to room temperature, partitioned between ether and water. The ether layer was washed with water, brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:5) as eluent to afford **23** (28.46 g) in 63 % yield.

$[\alpha]_D$ +37 (c, 1 EtOH) lit[13] $[\alpha]_D$ +38 (c, 1 EtOH)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.78 (bs, 1H, OH), 3.38 (s, 3H, OMe), 3.75-3.95 (m, 3H, H-5, H-6', 6"), 4.00 (brs, 1H, H-2), 4.10 (t, 1H, J = 8.6 Hz, H-4), 4.25 (d, 1H, J = 6.9 Hz, H.3), 4.70 (m, 2H, $\underline{CH_2}$Ph, H-1), 4.88 (d, 1H, J = 13.0 Hz, $\underline{CH_2}$Ph), 5.6 (s, 1H, O$\underline{CH}$Ph), 7.25-7.55 (m, 10H, 2xPh).

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-methyl-α-D-mannopyranoside (24):

To an ice cooled suspension of sodium hydride (3.61 g, 90.25 mmol, 60 % dispersion oil) in dry THF (120 ml) was added a solution of **23** (28 g, 0.075 mol) in THF over 15 min. After stirring at room temperature for 30 min, the reaction mixture was cooled to 0 °C and then methyl iodide (12.82 g, 0.09 mol) was added. The reaction mixture was stirred for 2 h at room temperature, then excess of sodium hydride was quenched with methanol and concentrated. The residue was extracted with ethyl acetate, washed with water, brine and dried over Na_2SO_4. Upon concentration and chromatographic purification on silica gel with ethyl acetate: petroleum ether (1:9) afforded **24** (26.32 g) in 90 % yield as a syrup.

$[\alpha]_D$ +65 (c 1.3, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 200 MHz): δ 3.36 (s, 3H, OMe), 3.56 (s, 4H, OMe, H-2), 3.78-3.96 (m, 3H, H-6', 6", H-5), 4.12 (t, 1H, J = 8.6 Hz, H-4), 4.25 (dd, 1H, $J_{2,3}$ = 4.3 Hz, $J_{3,4}$ = 8.6 Hz, H-3), 4.70 (s, 1H, H-1), 4.68, 4.90 (2d, 2H, J = 13.0 Hz, $\underline{CH_2}$Ph), 5.60 (s, 1H, $\underline{CH}$ Ph), 7.22-7.54 (m, 10H, 2 x Ph).

Methyl 3-*O*-benzyl-2-*O*-methyl α-D-manopyranoside (25):

Compound **24** (26.20 g, 0.067 mol) in 80 % acetic acid (70 ml) was stirred overnight at room temperature. Then acetic acid was neutralized with sodium carbonate, water was added and extracted with ethyl acetate, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (3:2) as eluent to afford **25** (13.80 g) in 68 % yield as a syrup.

$\alpha]_D$ +26 (c 2.05, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.32 (s, 3H, OMe), 3.40 (s, 3H, OMe), 3.45 – 3.54 (m, 2H, H-6', 6"), 3.64 (d, 1H, J = 4.6 Hz, H-2), 3.80 (m, 2H, H-3, H-5), 3.90 (t, 1H, J = 8 .6 Hz, H-4), 4.80-4.85 (m, 3H, H-1, CH_2Ph), 7.30 (m, 5H, Ph).

Methyl 3-*O*-benzyl-2-*O*-methyl-6-*O*-tosyl-α-D-manopyranoside (26):

To a cooled (0 C) solution of compound **25** (13.80 g, 0.046 mol) in dry pyridine (50 ml) was added tosyl chloride (8.35 g, 0.043 mol) and the mixture was stirred for 2 h at room temperature. Then pyridine was removed and extracted with diethyl ether, washed with water, brine and dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:4) as eluent to afford **26** (12.92 g) in 61 % yield as a syrup.

$[\alpha]^{25}_D$ +24 (c 1.08, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.30 (s, 1H, OH), 2.44 (s, 3H, Ph-$\underline{CH_3}$), 3.36 and 3.42 (2s, 6H, 2×OMe), 3.44 (s, 1H, H-2), 3.60 - 3.80 (m, 3H, H-5, H-6, H-6"), 4.16 (m, 1H, H-4), 4.32 (d, 1H, J = 8.3 Hz, H-3), 4.56 (d, 1H, J = 13.2 Hz, $\underline{CH_2}$Ph), 4.70 (s, 2H, H-1, CH_2Ph), 7.32 (m, 7H, Ph, Ts), 7.80 (d, 2H, J = 3.8 Hz, Ts)

Methyl-3-*O*-benzyl-6-deoxy-2-*O*-methyl-α-D-mannopyranoside (27):

A solution of compound **26** (16.21 g, 35.84 mol) in dry THF (30 ml) was added dropwise to a stirred suspension of LAH (3.75 g, 101.35 mmol) in THF (50 ml). After addition was complete, the reaction mixture was heated under reflux for 2 h, then the reaction mixture was cooled to room temperature and the excess hydride was decomposed with aqueous solution of saturated Na_2SO_4. The white precipitate was filtered. The filtrate was extracted with diethyl ether, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography using ethyl acetate: petroleum ether (1:5) as eluent to give **27** (8.20 g) as a syrup in 87 % yield.

$[\alpha]_D$ +9 ° (c 1.12, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.28 (d, 3H, J = 6.5 Hz, H-6, 6',6"), 3.36 and 3.45 (2s, 6H, 2xOMe), 3.51 (s, 1H, H-2), 3.58 (m, 3H, H-3, H-4, H-5), 4.55 (d, 1H, J = 13.5 Hz, $PhCH_2$), 4.70 (m, 2H, $PhCH_2$ and H-1), 7.30 (m, 5H, Ph).

Methyl 3-*O*-benzyl-6-deoxy-2-*O*-methyl-α-D-lyxo-hexopyranosid-4-ulose (28):

A solution of DMSO (1.88 ml, 26.51 mol) in dry CH_2Cl_2 (15 ml) was cooled to -78 °C under N_2 and to this was added $(CF_3CO)_2O$ (2.48 ml, 17.71 mmol) slowly and the mixture was stirred at that temperature for 30 min. A solution of compound **27** (5 g, 17.73 mmol) in dry CH_2Cl_2 (10 ml) was added slowly at −78 °C. After addition the reaction mixture was stirred for 1 h at −78 °C and Et_3N (4.97 ml, 35.42 mmol) was added slowly. The reaction mixture was then warmed to room temperature, water was added and extracted with dichloromethane, dried over Na_2SO_4 and solvent was evaporated. The residue was purified by silica gel column using ethyl acetate: petroleum ether (1:9) as eluent to give the compound **28** (4.61 g) in 92 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.30 (d, 3H, J = 6.8 Hz, H-6, 6', 6"), 3.44 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3), 3.87 (t, 1H, J = 1.3 Hz, H-2), 4.12 (q, 1H, J = 6.8 Hz, H-5), 4.36 (d, 1H, J = 4.5 Hz, H-3), 4.52, 4.92 (ABq, 2H, J = 12.7 Hz, CH_2Ph), 4.78 (d, 1H, J = 1.3 Hz, H-1), 7.28 (m, 5H, Ph).

Methyl-4-amino-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-talopyranoside (30):

Hydroxylamine hydrochloride (4.50 g, 65.71 mmol) was added to a stirred solution of NaOMe (prepared by dissolving Na (1.50 g) in MeOH (40 ml) and stirred for 20 min. Then the compound **28** (4.62 g, 16.47 mol) in MeOH (20 ml) was added to the above solution. The reaction mixture was stirred at room temperature for 4 h. The solid was filtered and filtrate

was concentrated, then water was added and extracted with ethyl acetate, dried over Na_2SO_4, filtered and concentrated to afford **29** and which was used in the next reaction without further purification.

The above prepared compound **29** (3.54 g, 11.86 mmol) in dry THF (20 ml) was added to a suspension of LAH (0.87 g, 23.70 mmol) in dry THF (15 ml) at 0 °C. The mixture was stirred at room temperature for 20 h. The excess LAH was decomposed with saturated Na_2SO_4 solution, white precipitate was filtered through pad of Celite bed and washed with ethyl acetate. The combined filtrates were concentrated and extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, concentrated to give amine **30** (2.31 g).

Methyl 3-*O*-benzyl-4,6-dideoxy-4-(N-ethoxycarbonylamino)-2-*O*-methyl-α-D-talopyranoside (31):

To a solution of compound **30** (2.30 g, 8.20 mmol) in 1:1 mixture of $CHCl_3$ (5 ml) and H_2O (5 ml) was added sodium bicarbonate (0.50 g) at 0 °C and ethyl chloroformate. The reaction mixture was stirred for 2 h at room temperature. The two layers were separated and the aqueous layer was extracted with chloroform, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:20) as eluent to afford **31** (1.47 g, 23 % overall yield after 3 steps) as a syrup.

$[\alpha]_D$ +125 ° (c 1.92, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (m, 6H, H-6, 6', 6" and $CO_2CH_2CH_3$), 3.30, 3.50 (2s, 6H, 2 x OMe), 3.40 (s, 1H, H-2), 3.70 (t, 1H, $J_{3,4}$ =, $J_{4,5}$ = 3.3 Hz, H-4), 3.80 (m, 1H, H-5), 4.08 (m, 3H, H-3 and $CO_2CH_2CH_3$), 4.43, 4.73 (2d, 2H, J = 13.3 Hz, $PhCH_2$), 4.70 (s, 1H, H-1), 5.90 (d, 1H, J = 8.0 Hz, NH), 7.32 (m, 5H, Ph).

CIMS: m/z 353 (M^+)

Methyl 4,6-dideoxy-4-(N-ethoxycarbonylamino)-2-*O*-methyl-α-D-talopyranoside (32):

A solution of compound **31** (1.40 g, 3.96 mmol) and 10 % Pd-C (300 mg) in dry MeOH was stirred at room temperature and balloon pressure for 15 h. The catalyst was filtered through pad of Celite, washed with methanol and the filtrate was concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:4) as eluent to give **32** (0.83 g) in 80 % yield.

$[\alpha]_D$ +91 (c 1.92, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (d, 3H, J = 6.5 Hz, H-6, 6', 6"), 1.3 (t, 3H J = 4.3 Hz, , $CH_2\underline{CH_3}$), 2.70 (d, 1H, J = 5.6 Hz, OH), 3.38 (m, 4H, H-2, OMe), 3.5 (s, 3H, OMe), 3.92 (m, 3H, H-3, H-4, H-5), 4.15 (m, 2H, $\underline{CH_2}CH_3$), 4.8 (s, 1H, H-1), 5.28 (d, 1H, J = 6.1 Hz, NH).

CIMS: 264 (M^++1).

Methyl 3,4,6-trideoxy-4-[(N-ethoxycarbonyl)amino]-3-*C*-methylene-2-*O*-methyl-α-D-talopyranoside (34) :

To a cold (-78 °C), stirred solution of DMSO (0.63 ml, 8.95 mmol) in dry CH_2Cl_2 (10 ml) was added $(CF_3CO)_2O$ (0.836 ml, 5.97 mmol) in CH_2Cl_2 (2 ml). This mixture was stirred for 20 min at –78 °C under N_2 atmosphere. The compound **32** (0.78 g, 2.98 mmol) in CH_2Cl_2 (10 ml) was added slowly and the reaction mixture was stirred for 45 minutes, at –78 °C dry Et_2O (1.67 ml, 11.95 mmol) was added, the reaction mixture warmed to room temperature, water was added and extracted with dichloromethane, dried over Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:5) as eluent to give **33** (0.52 g) in 67 % yield.

To an ice-salt cooled solution of methyltriphenylphosphonium iodide (3.74 g, 9.25 mmol) in dry THF (20 ml) was added n-butyl lithium (2.52 ml of 2.2 M solution, 5.55 mmol) slowly. After stirring for 1 h, a solution of keto compound **33** (0.48 g, 1.85 mmol) in THF

(8 ml) was cannulated. The mixture was stirred for 2 h. at room temperature, then saturated aqueous ammonium chloride solution was added. The THF was evaporated under reduced pressure, extracted with ethyl acetate washed with water, brine, dried over Na_2So_4. Upon concentration and chromatographic purification of the residue on silica gel using ethyl acetate: petroleum ether (1:15) as eluent gave the compound **34** (207 mg) in 43 % yield as a syrup.

$[\alpha]_D$ +112 ° (c 2.04, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.10 (m, 6H, H-6, 6', 6" and $CO_2CH_2\underline{CH_3}$), 3.32, 3.40 (2s, 6H, 2 x OMe), 3.48 (s, 1H, H-2), 3.96 (m, 1H, H-5), 4.12 (m, 3H, H-4 and $CO_2\underline{CH_2}CH_3$), 4.72 (s, 1H, H-1), 5.12 (s, 1H, one of $=\underline{CH_2}$), 5.40 (s, 1H, one of $=\underline{CH_2}$), 5.60 (d, 1H, J = 8.0 Hz, NH).

CIMS: m/z 260 (M^++1).

HRMS (CI) calcd for $C_{12}H_{21}NO_5$ (M^++1) 260.1497, found 260.1503.

Methyl 4-amino-3,4-*N,O*-carbonyl-4,6-didexy-3-iodomethyl-2-*O*-methyl-α-D-talopyranoside (35):

Iodoniumdicollidine perchlorate (592 mg, 1.26 mmol) was added to a solution of compound **34** (0.164 g, 0.63 mmol) in $CHCl_3$ (5 ml) and the solution was stirred in dark for 2 days at room temperature. Then chloroform was evaporated and the residue was dissolved in diethyl ether, then the solid was filtered and the filtrate was washed with dilute HCl (0.5 N), sodium thiosulfate, dried over Na_2SO_4 and the residue was purified by column chromatography on silica gel using ethyl acetate: petroleum ether (1:3) as eluent to afford **35** (113 mg) in 50 % yield.

$[\alpha]_D$ +95 ° (c 0.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.22 (d, 3H, J = 6.5 Hz, H-6, 6', 6"), 3.36 (d, 1H, J = 9.0 Hz, one of CH_2I), 3.61, 3.79 (2s, 6H, 2 x OMe), 3.77 (s, 1H, H-4), 3.85 (m, 3H, H-2, H-4 and one of CH_2I), 4.61 (d, 1H, J = 4.5 Hz, H-1), 7.00 (s, 1H, NH).

CIMS: m/z 358 (M^++1).

Methyl 4-amino-3,4-*N*,*O*-carbonyl-4,6-didexy-2-*O*-3-*C*-dimethyl-α-D-talopyranoside (18):

To a solution of compound **35** (32 mg, 0.089 mmol) in toluene were added AIBN (cat) and tributyltin hydride (78 mg, 0.20 mmol) at room tempeature. The reaction mixture was stirred at 100 °C for 1 h and cooled to room temperature. The solvent was evaporated and the residue was dissolved in acetonitrile (20 ml) and washed thrice with petroleum ether (3 x 10 ml), and evaporated. The crude product was purified by silica gel column chromatography using ethyl acetate: petroleum ether (3:2) as eluent to afford **18** (19 mg) in 95 % yield.

$[\alpha]_D$ +76 ° (c 1.0, $CHCl_3$)

^1H NMR ($CDCl_3$, 200 MHz): δ 1.16 (d, 3H, J = 6.5 Hz, H-6, 6', 6"), 1.40 (s, 3H, C_3-methyl), 3.18 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-2), 3.39 (bs, 1H, H-4), 3.43, 3.55 (2s, 6H, 2 x OMe), 3.86 (m, 1H, H-5), 4.59 (d, 1H, $J_{1,2}$ = 4.5 Hz, H-1), 7.45 (bs, 1H, NH).

CIMS: m/z 232 (M^++1)

HRMS (CI): calcd for $C_{10}H_{17}NO_5$ (M^++1) 232.1184 found 232.1174.

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INTRODUCTION:

Asthma has now emerged as one of the major health threats in this century. Especially, the situation is alarming in industrialized countries, where the rapid surge in air pollution has become the primary cause of this lung disease. This present action is a platform of discussion around biochemical and medicinal aspects related to asthma. Asthma is a chronic inflammatory disease of the conducting airways. The asthmatic inflammation is highly-complexd process and driven by a vast array of mediators and cytokines produced by mix of inflammatory cells, principally macrophages, lymphocytes and eosinophils[1]. Pro-inflammatory mediators such as histamine, leukotrienes and platelet activating factor (PAF) released from pulmonary tissues (or) migrating inflammatory cells possess specific vasoactive, bronchoactive and oedemagenic properties that are thought to be pivotal in the initiation and propagation of the asthmatic response[2]. However, the relative contributions of these mediators to pathophysiology of asthma are not fully understood. Given the complexity of chronic asthma it is unlikely that a single inflammatory mediator could account for all the pathology. Consequently, it can be speculated that only drugs which can simultaneously interfere with atleast two mediators are likely to have any profound effect on the inflammation. Keeping this in mind, new original molecules which could simultaneously interfere with any two or more of histamine, 5-lipoxygenase (5-LO) and PAF have been developed over the years.

HISTORICAL BACK GROUND:

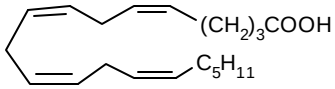
The first report of a substance which possesses the sort of biological activity now associated with leukotrienes came in 1938 when Feldberg[3] and Kellaway injected Cobra venom into guinea pig perfuse lungs and observed the release of a substance which caused contraction of the pig ileum which they called “slow reacting substances”.

Two years later Kellaway and Trethewic[4] demonstrated that a similar substance was produced in the effluent of guinea pig perfuse lung following challenge with appropriate

antigen. This immunologically produced mediator was later termed SRS-A, i.e., slow reacting substances of Anaphylaxis, by Brockelburst. In 1963, Berstrom[5] and Samuelsson[6] showed that prostaglandins were, in fact, a family of compounds of which two prostaglandins (PGS) were isolated in crystalline form and their structures were elucidated. Later the structure of thromboxane A_2 (TXA_2) and the degradation product of TXA_2 viz., thromborane B_2 (TXB_2) were elucidated.

In early 1980s., Samuelsson[6,8] and Corey[9] made a rapid progress in the elucidation of different products i.e. slow reaction substances (SRS) from metabolism of arachidonic acid, derived from membrane phospholipids initiated by enzyme 5-lipoxygenase and renamed SRS as leukotrienes (LTS) because of their initial discovery in leukocytes and their conjugated triene structures.

LEUKOTRIENES:



Arachidonicacid (1)

In a biological system for the regulation of various cell functions arachidonic acid plays a unique role as a precursor molecule which is transformed into a number of potent mediators with wide ranging effects. Oxidation of arachidonic acid in mammalian cells gives rise to a number of metabolites, collectively called eicosanoids, which are intimately involved in inflammation and the physiological and pathological processes. Prostaglandins were the first members of this family to be identified and characterized and are involved in inflammatory and pain responses. The enzyme cyclo-oxygenase (CO) converts arachidonic acid to prostaglandins. Studies on the metabolism of arachidonic acid in polymorphonuclear leukocytes (PMLS) led to the recognition of a new class of proinflammatory products called leukotrienes. The enzyme 5-lipoxygenase catalyzes the conversion of arachidonic acid to leukotrienes.

LIPOXYGENASE PATHWAY OF ARACHIDONIC ACID METABOLISM:

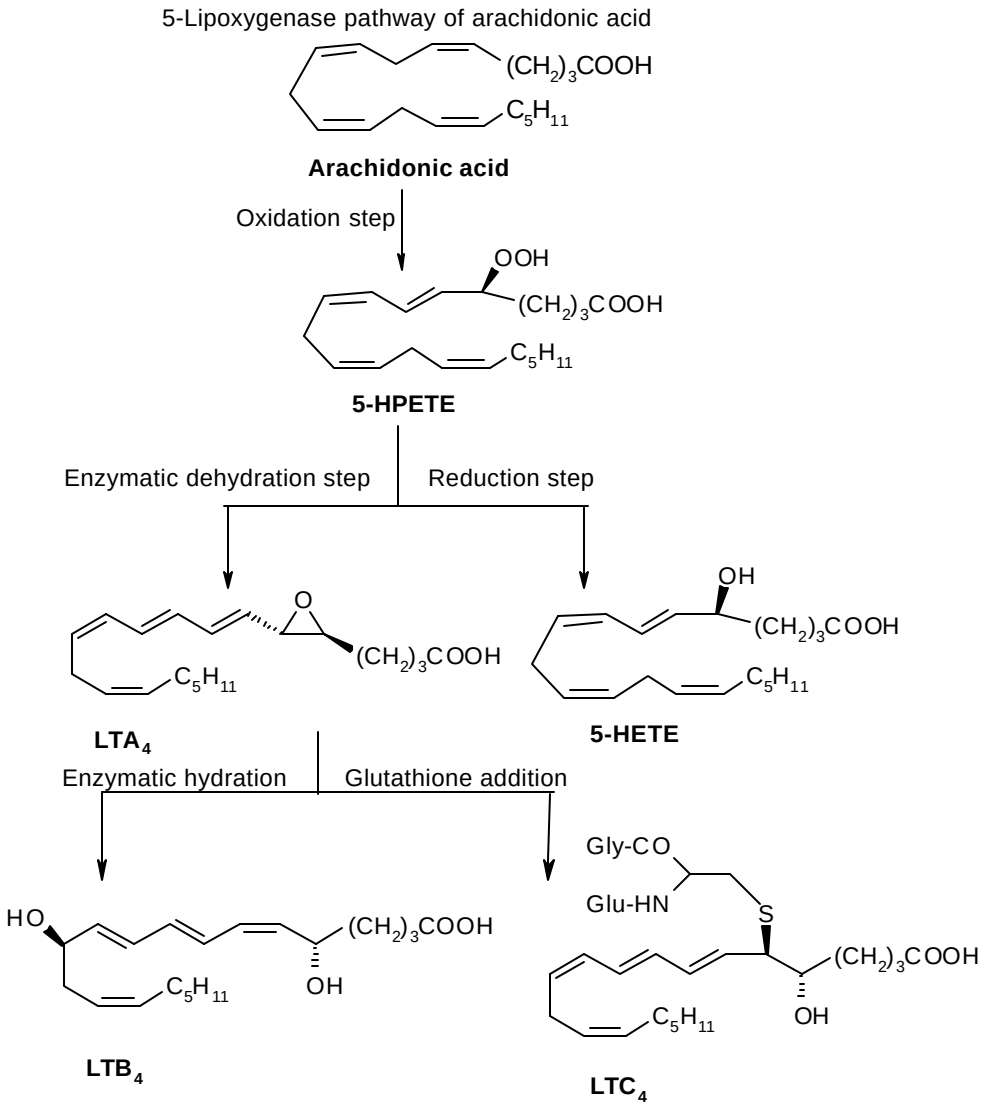
Arachidonic acid, an unsaturated C_{20} fatty acid has attracted interest over the last 15 years far beyond the bounds of nutritional research. It forms an essential acyl component of the phospholipids in all animal cells. Arachidonic acid is enzymatically formed from linoleic and linolenic acids by the action of phospholipase. It may also be metabolized by a group of enzymes known as lipoxygenases (Scheme-1). Lipoxygenases are the dioxygenases enzymes, which contain nonheme iron and stereospecifically catalyze the insertion of molecular oxygen into arachidonic acid. In 1976, Borgeat, Haniberg and Samuelsson[10,11] described the transformation of arachidonic acid to 5(S)-hydroxeroxy (6E, 8Z, 11Z, 14Z) eicosatetranoic acid (5-HPETE) by rabit polymorphonuclear leukocytes thus providing the first evidence for the existence of the 5-lipoxygenase enzyme. 5-Lipoxygenase is one of the most physiologically important enzymes and the subject of the greater interest in recent years.

The 5-lipoxygenase is the first dedicated enzyme in the pathway leading to the biosynthesis of leukotrienes. This important enzyme has a rather restricted distribution, being found predominantly in leukocytes and mast cells of most mammals. Normally 5-lipoxygenase is present in the cell in an inactive form, however, when leukocytes respond to external stimuli, intracellular 5-lipoxygenase can be rapidly activated. This enzyme catalyzes the addition of molecular oxygen to fatty acids with cis, cis-1,4-pentadiene structures, converting them to 1-hydroperoxy trans, cis-2,4-pentadienes. Arachidonic acid substrate which leads to leukotriene products, is found in very low concentrations in mammalian cells and must first be hydrolysed from membrane phsopholipids through the actions of phospholipases in response to extracellular stimuli.

5-LO catalyses the stereospecific conversion of arachidonic acid (**1**) to a 5-hydroxyperoxy eicosatetraenoic acid (5-HPETE)[12] (Scheme-1). Since the active site of the

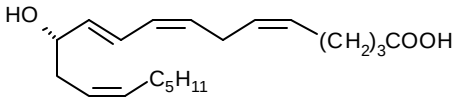
enzyme is non-heme iron, the mechanism of the transformation probably involves an organoiron intermediate (or) a dienyl radical which is trapped by molecular

Scheme-1



oxygen to produce 5-HPETE. Reduction of 5-HPETE generates 5-HETE, while enzymatic dehydration produces the unstable epoxyleukotriene A_4 (LTA_4). LTB_4 arises from the enzymatic hydration of LTA_4, while addition of glutathione generates LTC_4. The latter compound, along with its proteolytic metabolites LTD_4 and LTE_4, constitutes slow reacting substances of anaphylaxis (SRS-A).

Additional lipoxygenases are known which oxygenate different positions on the arachidonic acid chain. 12-LO, resulting in the formation of 12-HETE (**2**), is best known in platelets, while the 15-LO from soyabean has been studied in detail for many years. 15-HETE (**3**) is also produced by mammalian cells; the enzymes from neutrophils and particularly rabbit reticulocytes are the best characterized.



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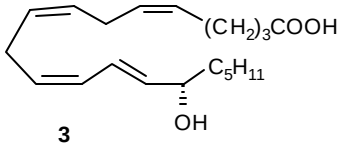


3

LTB_4, C_4, D_4 and E_4 play major roles in inflammatory and allergic responses. For example LTB_4, a potent chemotactic agent for neutrophils and eosinophils, is an important mediator of inflammation. LTC_4, D_4 and E_4 are potent bronchoconstrictors, as well as the slow-reacting substance of anaphylaxis.

Much evidence has accumulated implicating LTs in diseases states having inflammatory components, including arthritis, asthma and allergy, psoriasis and other inflammatory skin diseases, inflammatory bowel diseases such as ulcerative colitis and Crohn's disease and more recently in circulatory diseases such as shock and myocardial ischaemia. 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 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 effects of medicinal chemists.

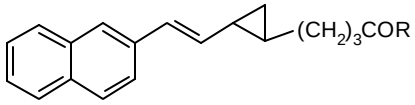
TYPES OF LIPOXYGENASE INHIBITORS[13]:

Both natural products and synthetic compounds have been reported as lipoxygenase inhibitors. The former category encompasses compounds isolated from both animate and inanimate sources while the latter class is comprised of lipoxygenase substrate and product

analogs. Synthetically modified natural products and novel structures have been obtained by total synthesis.

Substrate and product analogues:

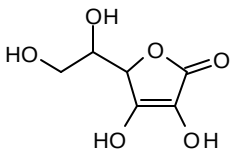
The LTB_4 analogues TE_1-8005 (**4**) and TEI-1338 (**5**), which inhibited LT production by ionophore-stimulated human blood at micromolar concentrations, also showed oral activity in CPE and topical activity in endotoxin induced endophthalimitis in rabbit eyes.



4 R = OH
5 R = NHC_6H_4(2-COOMe)

Phenolic compounds:

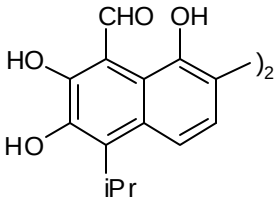
Beginning as early as 1970, a vast array of phenolic compounds have been explored as inhibitors of lipoxygenases. Since phenols are well-known as reducing agents activity against an oxidase would not be expected. All active compounds of this class contain at least one free aromatic hydroxyl group, or a phenolic ester which presumably acts as a prodrug form of the phenol. Lipophilic character is a common feature of most of these inhibitors, as might be expected for activity against an enzyme whose normal substrate is a fatty acid. (Surprisingly, however, incorporation of a carboxyl group into many of these molecules destroys activity). The requirement for lipophilicity in a reducing agent is demonstrated by the inactivity of ascorbic acid (**6**), a water soluble physiological antioxidant, in rat-ISN below millimolar concentrations. However, a polmitate ester of (**6**) is a potent inhibitor (1.5 μm) of LTB_4 synthesis in human ISN and in cell hanogenates.



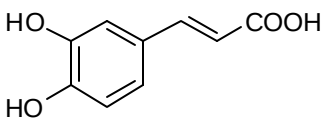
6

Catecols and *ortho* aminophenols:

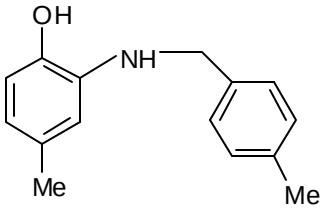
A large number of catechol containing compounds have been reported to inhibit a variety of 5-LOs, most commonly in cRBL and ISN. Many of these are natural products (or) synthetic analogues, such as gossypol (**7**), caffeic acid (**8**) and derivatives. Caffeic acid is the most potent of the naturally occurring compounds with IC_{50} in the micromolar range. It is interesting to note that conversion of one of the phenols of caffeic acid to methyl ether to yield ferulic acid, substantially reduces the ability to inhibit lipoxygenase. This indicates the possible role of catechol for lipoxygenase inhibition.



Gossypol (7)



Caffeic acid (8)

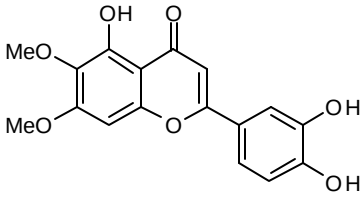


9

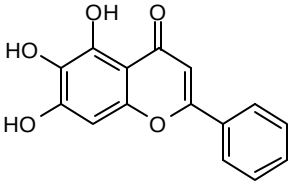
The *ortho*-aminophenols, which are stereoelectronic with catechols, inhibit the 5-LO. The N-aryl methyl derivative (**9**) was a potent inhibitor of cRBL (0.029 μm) and inhibited the release of SRS-A in rat passive peritoneal anaphylaxis. Variation of the 4-substituent as well as the *para*-substituent on the benzyl group, indicated that overall lipophilicity was important for good potency, electronic effects in the benzyl group had a much smaller effect.

Flavonoids:

S. Yamamoto and co-workers have studied the effect of various flavonoids on 5-lipoxygenase from rat basophilic leukemia cells (RBL) and guinea pig PMNS. Cirsiliol (**10**) was the most potent flavonoid against cRBL (0.1μm). Baicalein (**11**) is another example of flavonoid inhibiting the 5-LOs from rat and human neutrophils and lymphocytes.



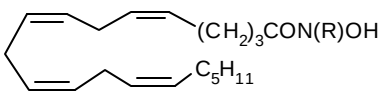
Cirsiliol (10)



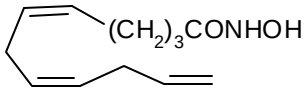
Baicalein (11)

Hydroxamic acids and related compounds:

In 1984, Corey and co-workers reported that N-hydroxyarachidonamides (**12**) were potent reversible inhibitors of cRBL (0.03 – 0.2 μm). Alkylation on nitrogen increased the inhibitory potency significantly and truncation to (**13**) still gave activity (1.9 μm). An alternative approach at Abott placed the hydroxamic acid moiety in the 5-position, giving analogues of 5-HPETE such as (**14**) which also inhibited cRBL.Although the simple hydroxamate analogues of arachidonic acid showed no anti-inflammatory activity the concept prompted a number of research groups to explore hydroxamic acids with more stable lipophilic residues. This approach has yielded some of the most interesting anti-inflammatory 5-LO inhibitors reported to date.



12 R = H, Me, nBu



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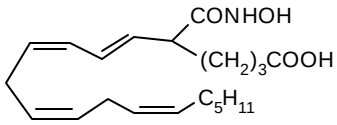


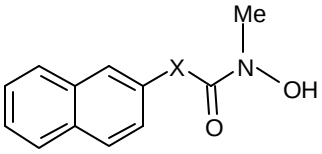
14

A series of aralkyl hydroxamic acids from Bristol-Myers is represented by 9-phenylnonanohydroxamic acid (BMY 30094). This compound inhibited the production of 5-HETE by human ISN (5.8 μm), with about one third that potency against CO and 12-LO in platelets. Alkylene chains shorter than 6 (or) longer than 11 carbons gave inactive compounds

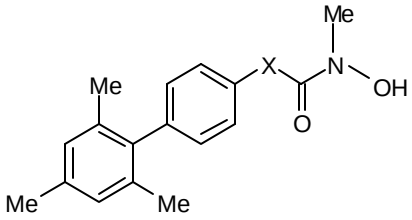
(>100 μm). Alkylation on nitrogen greately improved potency (<1μm). Small substituents on the phenyl ring (methyl, methoxy, chloro) had little effect on potency, but larger substituents (butyloxy) led to greately decreased activity. 9-Phenylnonanohydroxamic acid (BMY 30094) showed topical anti-inflammatory activity in AAE and phorbol ester ear oedemia.

Hydroxamic acids have been extensively investigated at Abott, where a hypothetical binding site hypothesis was based on examination of many simple ω-aralkyl hydroxamic acids. Several series of conjugated hydroxamic acids were explored based on this hypothesis, yielding potent 5-LO inhibitors exemplified by **15-18**.

The hydroxamic acids in earlier examples (**15-18**) have the large lipophilic group attached to the carbonyl and small alkyl group on nitrogen. Reversal of this pattern (dubbed

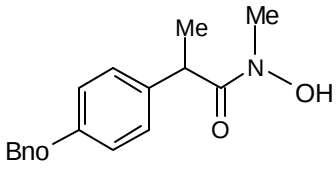


15 X = bond
16 X = CH=CH

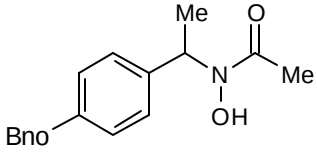


17 X = bond
18 X = CH=CH

'type A' by the Abbot workers) to yield N-substituted acetohydroxamic acids (type B') afforded surprising increases in oral potency without affecting *in vitro* activity. Abbott's A63162 (**20**) was 2 to 5 fold more potent in rat peritoneal anaphylaxis than the corresponding arylacetohydroxamic acid (**19**).

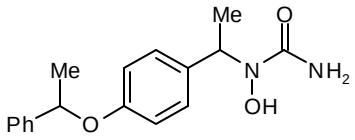


Type A (19)



Type B (20)

Conversion of the type B-hydroxamic acids to hydroxyureas led to further enhancements of oral bioavailability and *in vivo* potency. Abbott's **21** and BW-B70C (**22**) from Burroughs welcome are the examples.



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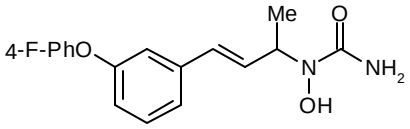
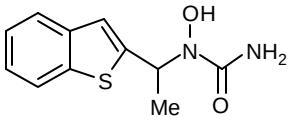


22

The benzothiphene analogue zileuton (**23**) is one of the more interesting 5-LO inhibitors studied to date which has been recently approved by FDA for the treatment of asthma. Zileuton inhibited cRBL (0.5 μm) and human ISN (0.7 μm) was specific for 5-LO, and was orally active in rat peritoneal anaphylaxis. Promising activities have been reported in a number of diseases such as antigen induced nasal congestion, overtsymptoms as well as LTB_4 and 5-HETE levels in nasal secretions were reduced, the response of asthamatic patients to cold air bronchoconstriction decreased LTB_4 levels in rectal dialysis fluid, preliminary indications of efficacy in rheumatoid arthritis.

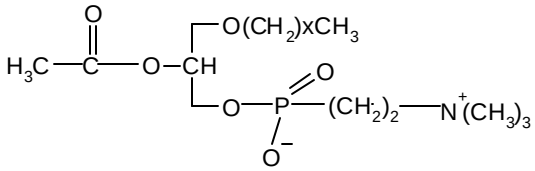


Zileuton (23)

PLATELET ACTIVATING FACTOR:

Platelet activating factor (PAF)[14], chemically identified as 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocoline (**25**) has been implicated as a mediator of pathophysio-logical reactions in various animal models as well as in human disease[15]. PAF is produced by a variety of inflammatory cells such as basophils, neutrophils, platelets, macrophages, endothelial cells, and IgE-sensitized bone marrow mast cells. PAF exerts a wide variety of biological actions including *interalia* smooth muscle contraction, neutrophil, degranulation,

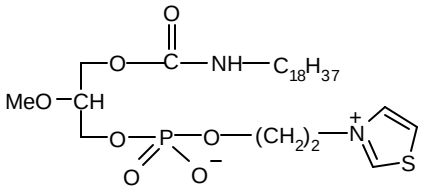
platelet aggregation and cardiac, renal and gastrointestinal dysfunction. In various animal models, PAF induces bronchocostriction, systemic hypotension, neutrophenia, increased vascular permeability, and elevated plasma lysosomal hydrolase levels. PAF plays a major role in asthma, especially in the pathogenesis of late asthmatic responses, and is the only mediator able to elicit a selective recruitment of eosinophils in allergic subjects.



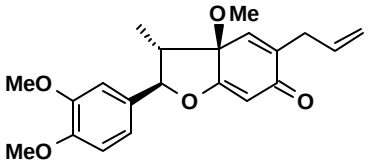
Structural formula of plateactivating factor (25)

Binding of PAF to its specific receptor is thought to be the first step necessary to display its biological functions both *in vitro* and *in vivo.* The existence of high affinity PAF-specific binding sites in rabit platelet and guinea pig smooth muscle membranes, human platelets, human polymorphonuclear leukocytes and human lung tissues has been demonstrated by experiments using [^{3}H] PAF. The affinity and number of these binding sites appear to correlate with tissue and species specificity of the biological effects of PAF[16]. It has become increasingly likely that PAF has multiple effects, which may be relevant in many human diseases, and enormous recent activity in studying these effects has been an anticipated consequence.

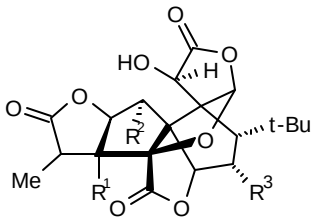
Specific and potent PAF receptor antagonists are valuable tools in the elucidation of the pathophysiological roles of PAF and are expected to be of clinical importance. A variety of structurally diverse antagonists of the binding of PAF to its receptors have been reported, including structural analogs of PAF such as CV-3988 (**26**) complex natural products isolated from medicinal plants such as Kadsurenone (**27**) and ginkgolide(**28**) as well as the totally synthetic substance such as L-652,731 (**29**) etc.



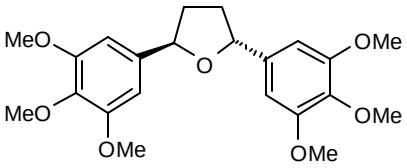
CV 3988 (26)



Kadsurenone (27)



Ginkgolides (28)

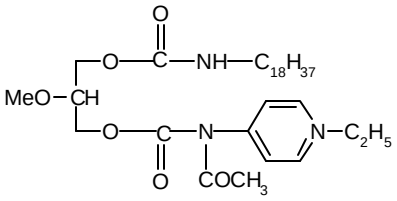


L - 652, 731 (29)

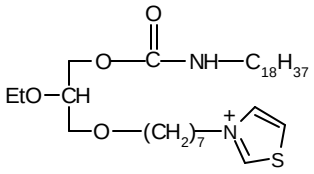
PAF – RELATED ANTAGONISTS:

These compounds have been obtained by three different chemical approaches[15].

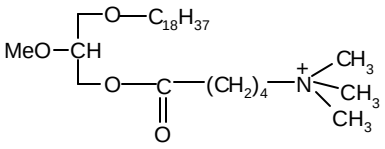
Nonconstrained backbone: In this series, the antagonists derive directly from the PAF frame work. The first compound described in this family is CV3988 (**26**). CV3988 is an orally active and potent antagonist. The first reports by Terashita *et al*. claimed that CV3988 specifically inhibited PAF – induced platelate aggregation. The inhibition of PAF – induced platelate aggregation by CV3988 is accompanied by an inhibition of the membrane production of phosphoinositides. CV3988 inhibits PAF induced hypotension, thrombopenia, and hemoconcentration. CV3988 also counteracts the decrease in blood pressure induced by unclipping of the renal artery in the one-kidney one clip hypertensive rat. A new CV analog CV6209 (**30**) has a N-acetylcarbamoylmethylpyridinium side-chain in place of the phosphoryl choline group. It is about 80 times more potent than CV 3988 in vitro and in vivo replacement of phsophoryl ethyl thiazolium moiety of CV3988 with a heptamethylene thiazolium on C_3 yielded another group of antagonists among which ONO-6240 (**31**) is the most potent one. Thr potent antagonists were obtained by replacing the phosphory group by an ester linkage i.e. RU 45703 (**32**).



CV 6209 (30)

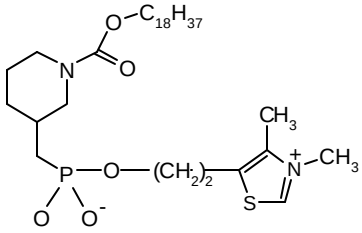


ONO - 6240 (31)

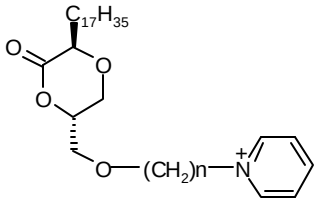


RU 45703 (32)

Constrained backbone: Moderately active PAF antagonists were produced from cyclization of the PAF framework such as the Sandoz piperidine derived SRI 63-073 (**33**) (or) the dioxanone-related (**33**) Hoffmann La Roche series (**34**). SRI 63-073 synthesis was designed by combination of thiamine phosphate (which displays a modest PAF inhibitory effect *in vivo* with a modified PAF fram work. SRI 63-073 inhibits PAF induced human and guinea pig platelet aggregation.

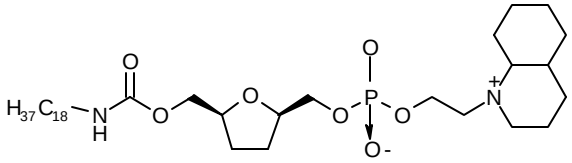


SRI 63-073 (33)



Dioxanone (34)

Tetrahydrofuran derivatives: Sandoz has synthesized a series of tetrahydrofuran related PAF antagonists related to the PAF framework. The most potent one, SRI 63-441 (**35**) is a specific inhibitor of PAF– induced human platelet aggregation.



SRI 63-441 (35)

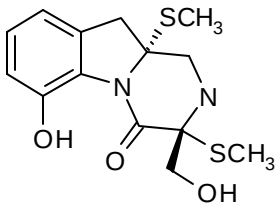
NATURAL PRODUCTS:

The most promising chemical series of PAF inhibitors include natural compounds isolated from Chinese (or) Brazilian plants (terpenes and lignans) and from various bacterial strains (gliotoxins).

Terpenes: A family of potent PAF antagonists is formed by ginkgolides (**28**) and terpenes isolated from Chinese tree Ginkgo biloba L Ginkgolides are unique cage molecules which are C_{20} compounds, incorporating a *tert*-Bu group and six 5-membered rings including a spiro [4.4.0]nonane system, a tetrahydrofuran cycle and three lactonic groups.

Gliotoxin and related compounds:

PAF antagonists are also produced by fermentaion of different fungi and microorganisms. These products derive from bisdethiobis(methylthio)gliotoxin which was first isolated from wood fungus G. delquescens. Most of these products possess the dialkylthiopiperazinedione skeleton. The most potent antagonists is gliotoxine (**36**). This product inhibits PAF-induced rabbit platelet aggregation, but a slight inhibition of cellagen induced aggregation was recorded.



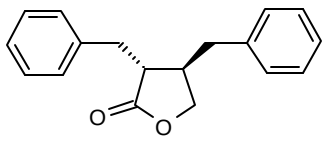
Gliotoxine (36)

Lignans: Lignans and neoliganans are parts of an immense chemical family formed biogenetically by oxidative dimerization of hydroxyalkoxyphenylpropane. The term lignan is traditionally reserved for compounds in which the precursors are linked by β-carbons of each lateral chain, whereas the term neolignan is applied to products in which dimerization intervenes on carbons other than β.

Benzofuranoid neolignans: A neolignan isolated by the Merck group from *piper futokadsurae* (haifenteng, piperaceae), a plant used in Southern China as antirheumatic and anti-allergic was the first natural product discovered as a potent inhibitor of the binding of [^{3}H] PAF to a rabbit platelet membrane preparation. It was named Kadsurenone (**27**) and was shown to be a specific and potent inhibitor of PAF induced platelet aggregation.The chemical specificity of Kadsurenone was demonstrated by the weak activity of several related compounds isolated from the same plant (e.g. Kadsurin A, Kadsurin B and piperenone) and several synthetic analogues with altered stereochemistry (or) ring substituents. Only the dihydroderivative, obtained by hydrogenation of the allyl side chain, retained full activity. The inversion of the configuration at either the 2-or 3a position results in a significant decrease of the receptor inhibitory activity, since the related methoxyimino or alcohol analogues are weak inhibitors. The neolignan is active in blocking PAF-induced cutaneous permeability in the guinea pig.

Dibenzyl butyrolactone (butanolide) derived lignans:

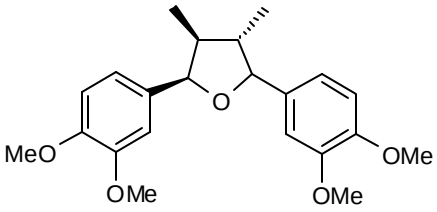
Butanolide lignans (**37**) are isosters of type A furanoid lignans. In this series, prestganes A and B and various methoxylated matariresinols isolated from *stegantaenia araliacea* are moderate antagonists. Lignans of this series dibenzyl butyrolactone (Butanolides) were discovered in animal urines (including human). They are characaterized by the presence of one meta OH-group in each aryl ring, the main lignan identified in mammalian rings being enterolactone.



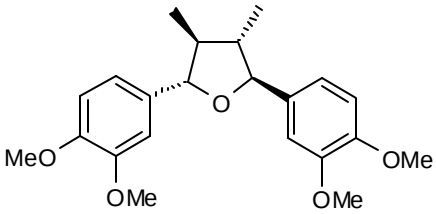
37

Substituted furanoid lignans:

Several structures in lignan series are potent PAF antagonists. This is especially the case with tetrahydrofuran derived compounds, type C (2,3,4,5 tetrasubstituted). Veraguensin (**38**) , Galbelgin (**39**) and Galgranin (**40**) respectively isolated from *Magnolia accuminata* (USA) and *Himantadrabelgravena* (New Guinea), are relatively potent and specific inhibitors of PAF-induced rabbit platelet aggregation..

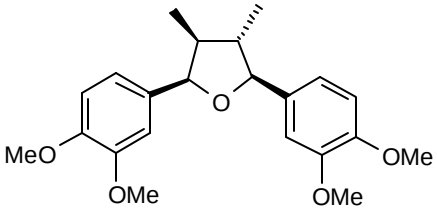


Veraguensine (38)

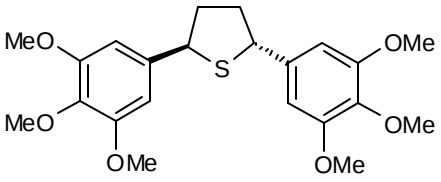


Galbelgin (39)

Systematic synthetic study from the natural tetrahydrofuran fram work led to the synthesis of a dinor type C tetrahydrofuran lignan L-652, 731 (**29**) and the thioester of L-652,731 *trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrothiophene (L-653, 150) (**41**).

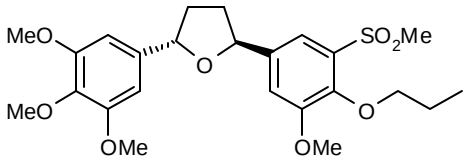


Galgravin (40)



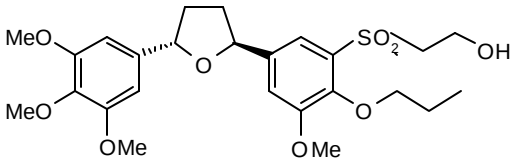
L - 653, 150 (41)

In the type C tetrahydrofuran series, the trans isomers are generally more potent than the corresponding cis isomers. Further structure activity studies indicated that more potent PAF antagonists contained an electron-withdrawing group on one but not both aromatic rings. These features are incorporated in L-659,989 (**42**)[17] in which a metabolically stable methylsulfone serves as the electron withdrawing functional unit and a trimethoxy aryl ring is appended at C_5.

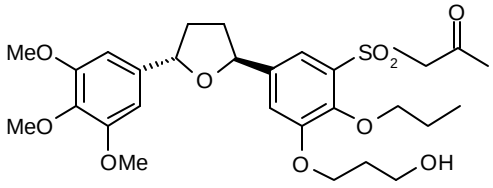


L - 659, 989 (42)

In order to achieve improved metabolic stability and pharmacokinetic profile, polar head group modifications were investigated from which the (-)-(2S, 5S) *trans* isomer of MK 287 (**43**)[18] emerged as a potent, specific and orally active PAF receptor antagonist and chosen for clinical trial for asthma. The development of (-)-*trans*(2S,5S)-2-[3-(2-oxopropyl) sulfonyl]-4-*n*-propoxy-5-(3-hydroxypropoxy)phenyl-5-(3,4,5trimethoxyphenyl) tetrahydrofuran (**44**)[19] with further improvement of *in vivo* potency and drug characteristics has been described. Since both PAF and leukotrienes are released simultaneously from le ukocytes and upon cellular activation, act synergistically in many biological models, a single compound which effectively inhibits the actions of both PAF and leukotrienes may offer certain therapeutic advantages in



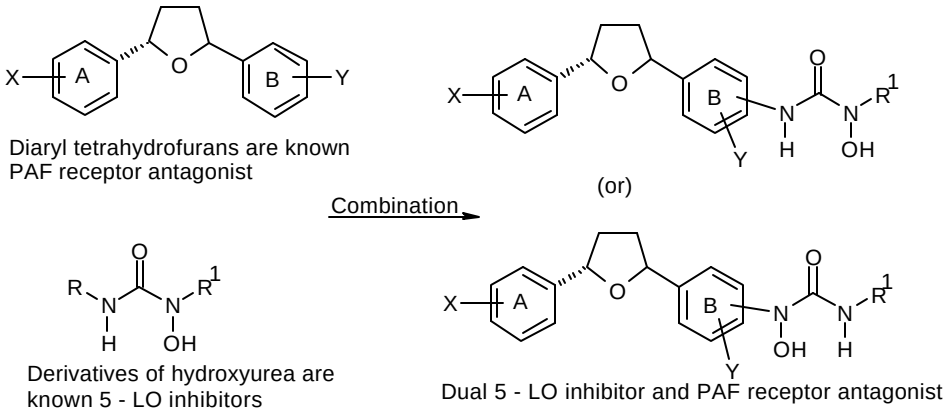
MK 287 (43)



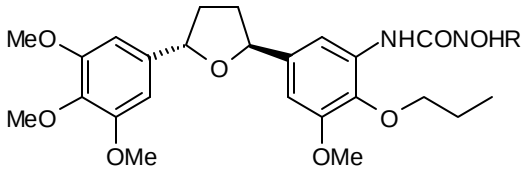
44

terms of effic acy and Pharmacodynamics over agents which inhibits either mediator alone. The 2,5 diaryltetrahydrofuran class of compounds are extensively studied PAF-receptor antagonists. On the other hand, hydroxamic acids and hydroxy ureas are the most potent 5-lipoxygenase inhibitor known. A number of monofunctional and dual functional 5-LO inhibitors and PAF receptor antagonists have been reported. The introduction of hydroxyurea functionality onto certain scaffolds has been shown to confer 5-LO inhibitory activity, possibly involving chelation of Fe^{3+} required for catalysis. To optimize dual 5-LO inhibition and PAF receptor antagonist activities in a single compound, the N-hydroxyl urea functionality was

incorporated by Cytomed Inc. into a well-characterized family of PAF receptor antagonists, 2,5-diaryl tetrahydrofurans.



A number of hydroxy ureidyl derivatives of diaryl tetrahydrofurans have been synthesized, which show dual 5-LO inhibitory and PAF receptor antagonistic activities. Md. Sajjat Hussion et al.[20] have replaced the sulfonyl group in MK 287 (**43**), potent PAF-antagonist, with hydroxy ureidyl groups to introduce dual activity in this type of molecule. For anti-PAF activity the *trans* isomer was always more potent, however, isomer preference has not been determined for 5-LO inhibition. Further introduction of hydroxy urea might change the conformation of the molecule to give anti-PAF activity irrespective of the geometry of the isomer. So both the *cis* and *trans* isomers of the diaryl tetrahydrofuran type of compounds

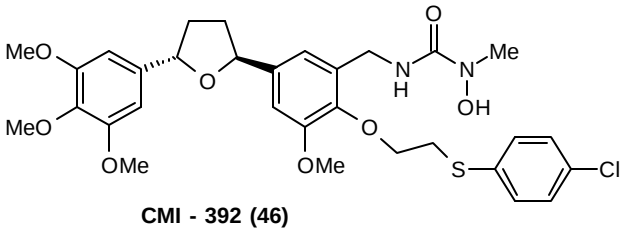


45 R = H, Alkyl

were synthesized (**45**). *In vitro* dual activities were determined by a PAF receptor binding assay and a 5-LO inhibition assay using a rat basophilic leukemiae (RBL) cell extracts. There was practically no difference between *cis* and trans *isomers* in antagonizing PAF. Compounds

(both *cis* and *trans* isomers) containing a medium size alkyl R are the most active in both RBL and PAF assays.

Xiang Cal *et al*[21]. have reported (±) trans-2-[3-methoxy-4-(4-chlorophenylthioethoxy)-5-(N-methyl-N-hydroxyureidyl)methylphenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (CMI-392) (**46**) as a potent dual 5-LO inhibitor and PAF receptor antagonist which is currently being evaluated in human clinical trials as a novel anti-inflammatory agent. CMI-392 showed

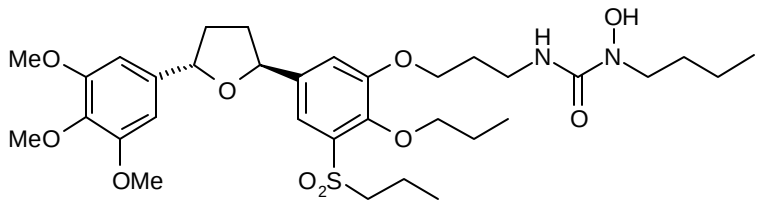


CMI - 392 (46)

very potent and balanced activities against both 5-LO and PAF compared with reference compounds, CMI-392 (**46**) is more potent than zileuton (**23**) in 5-LO inhibitory activity and is almost equally potent as MK-287 (**43**) in PAF receptor antagonist activity.

PRESENT WORK:

The (±)-*trans*-2-[3-3(N'-butyl-N'-ureidyl]propoxy)-4-propoxy)-5-propyl sulfonyl phenyl] -5-(3,4,5-trimethoxyphenyl)-tetrahydrofuran (CMI-546) **47** was identified and developed as a potent 5-LO inhibitor and PAF receptor antagonist thereby adjudicating as the curator of chronic asthma[22]. The compound is now undergoing human clinical trials as anti-inflammatory agent. The molecule having spectacular pharmacological and pharmacodynamic properties and enriched with rich functionality and functionality posses copious attention to every synthetic chemist and hence our synthetic endeavor to capitulate the total synthesis in economically viable fashion. This chapter deals with the synthesis of CMI-546 (**47**).



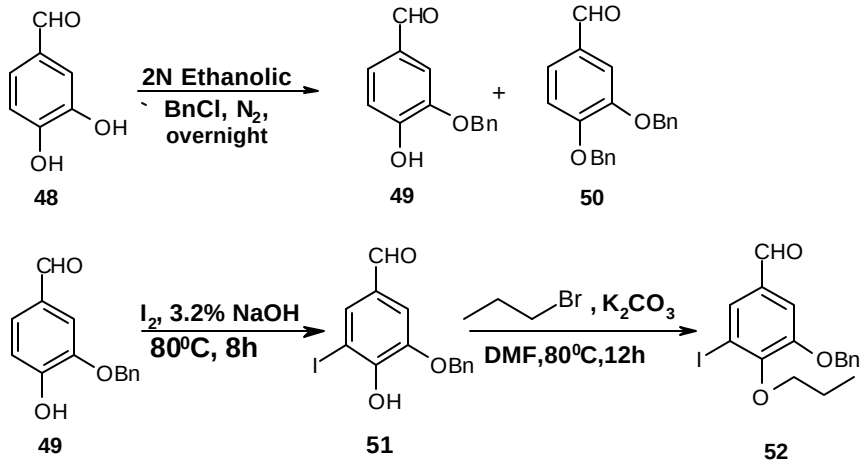
CMI - 546 (47)

We began the synthesis of CMI-546 (**47**) from commercially available 3,4-dihydroxy-benzaldehyde (**48**). The compound **48** was treated with benzyl chloride and 2N ethanolic potassium hydroxide to give a mixture of monobenzyl ether (**49**) and dibenzyl ether (**50**) which could be separated by column chromatography[23].

The pure monobenzyl ether (**49**) was heated with iodine and 3.2 % NaOH at 80 °C to afford the iodo derivative (**51**) in 79 % yield. The structure of **51** was confirmed by the ^{1}H NMR spectrum in which two meta-coupled aromatic protons were located at 7.45 and 7.85 ppm. The iodo derivative **51** was alkylated with n-propyl bromide and potassium carbonate in dimethylformamide at 75-80 °C, affording the propyl ether (**52**) in 82% yield (Scheme-1). The ^{1}H-NMR spectrum of **52** showed characteristic peaks such as a triplet at 1.03, a multiplet at

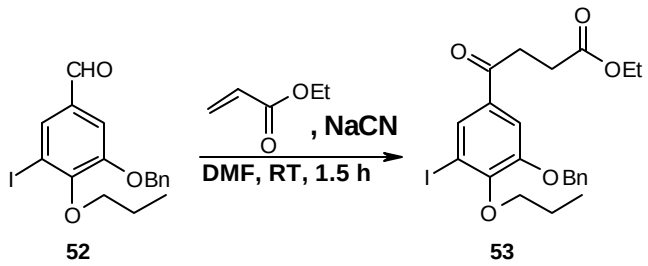
1.75 and a triplet at 4.05 ppm corresponding to the propyl group. The remaining chemical shifts were in agreement with the assigned structure.

Scheme-1



The γ-keto ester (**53**), containing the carbon backbone of tetrahydrofuran ring was obtained from compound **52** using conditions described below. Thus, the aldehyde **52** was reacted with 25 mol % sodium cyanide and ethyl acrylate to afford the γ-keto ester (**53**) in 74 % yield (Scheme-2)[24]. The structure of **53** was confirmed by its ^{1}H-NMR spectrum which showed the triplet at 1.30, a quartet at 4.18 ppm due to ethyl ester.Two triplets at 2.70 and 3.20

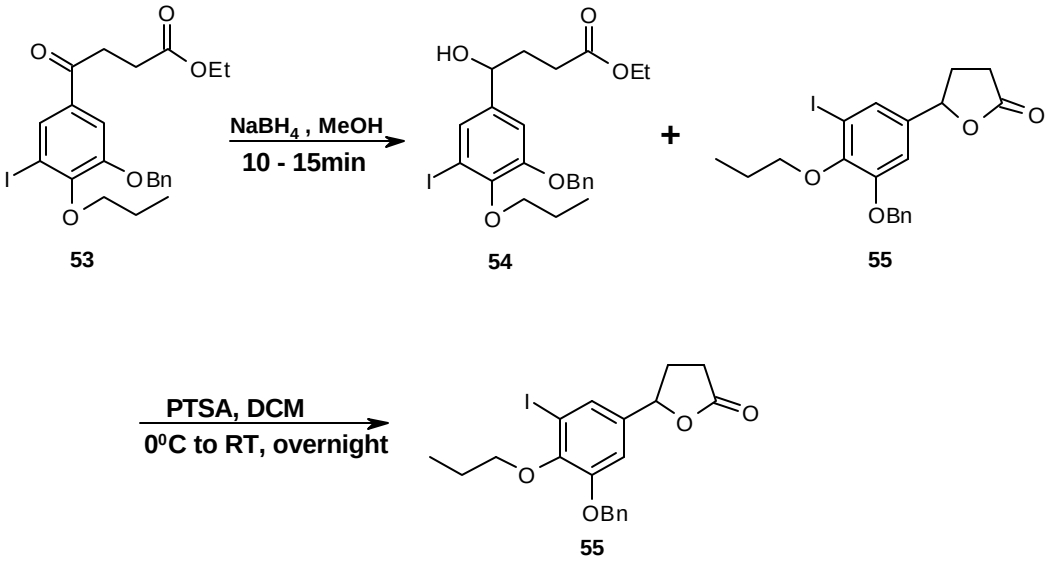
Scheme-2



ppm corresponding to (CH_2-CH_2) group. The rest of the protons appeared at their expected values. The structure of **53** was further supported by the IR spectrum in which characteristic absorptions due to C=O and COOEt were observed at 1690 and1735 cm^{-1}. The mass spectrum showed highest mass peak at m/z 496 which represented molecular weight of **53**.

The keto group in **53** was reduced with $NaBH_4$ in EtOH to afford a mixture of γ-hydroxy ester (**54**) and γ-lactone (**55**). The mixture of **54** and **55** was treated with p-toluenesulfonic acid in CH_2Cl_2 to convert the above mixture into **55** in 79 % yield (Scheme-3). The structure of **55** was confirmed by its ^{1}H-NMR, IR and mass spectral analysis. The ^{1}H-NMR spectrum of **55** showed multiplets between 2.0-2.65ppm due to methylene protons at C-3

Scheme-3

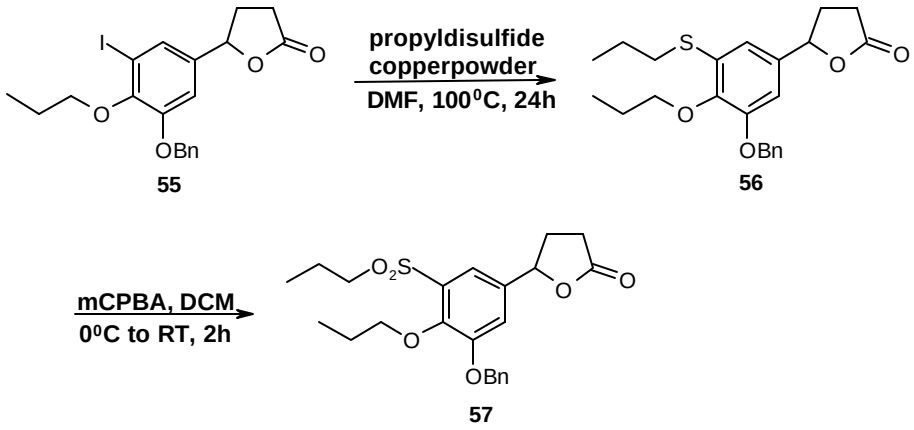


and C-4 while the methine proton at G5 was appeared as a triplet at 5.35 ppm.. The two meta coupled aromatic protons appeared at 6.92 and 7.28 ppm as two doublets. In the IR spectrum of **55**, the characteristics C=O streching absorption due to lactone group was observed at 1760 cm^{-1}. The mass spectrum of **55** showed molecular ion peak at m/z 452 (M^+).

The iodide present in **55** was displaced with propyl sulfide under Ulmann type cross-coupling reaction by using propyl disulfide and copper powder in DMF to afford **56** in 86 % yield. The ^{1}H-NMR spectrum of **56** showed a triplet at 2.85 ppm corresponding to S-CH_2 group. The aromatic proton (H-6`) was shifted upfield in sulfide product (**56**) by 0.7 ppm as compared to the iodide **55**. The structure was further supported by mass spectrum in which

highest mass peak was found at m/z 400 (M^+). The sulfide (**56**) was cleanly oxidized with m-chloroperbenzoic acid in CH_2Cl_2 to give the sulfone (**57**) as a solid in 73 % yield (Scheme-4).

Scheme-4

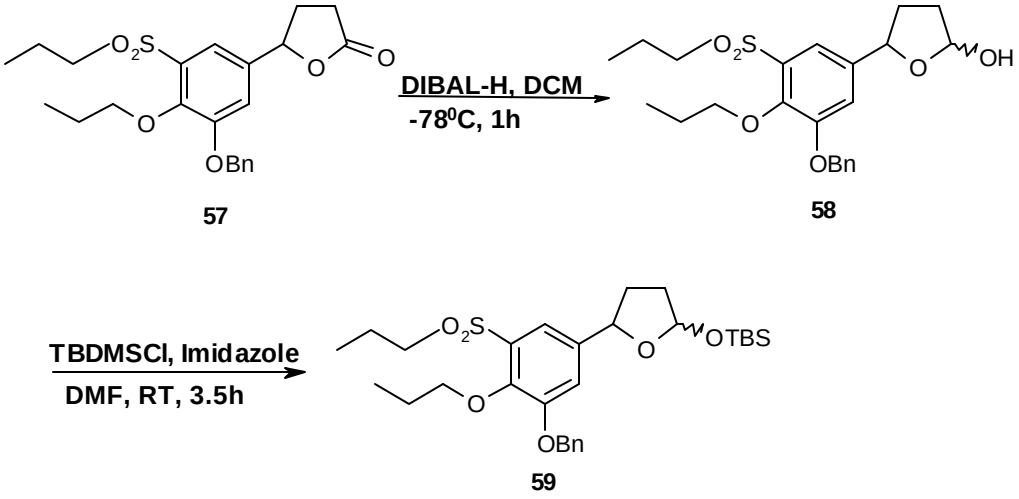


In the ^{1}H-NMR spectrum of **57** the CH_2 protons adjacent to SO_2 group was located in the downfield region at 3.35 ppm. The aromatic protons also showed downfield trend in their chemical shifts due to SO_2 group. The structure of **57** was further confirmed by its mass spectrum showing molecular ion peak at m/z. 432 (M^+) while IR spectrum showed absorptions at 1765 (C=O), 1600, 1490 and 1460 cm^{-1} in agreement with the structure of **57**.

Partial reduction of the lactone (**57**) to the lactol (**58**) was accomplished with DIBAL-H in CH_2Cl_2 at −78 °C. Compound **58** was a 1:1 mixture of *trans* and *cis* isomers. The structure of the lactol (**58**) was confirmed by ^{1}H-NMR and IR spectral analysis. The IR spectrum of **58** showed the absence of lactone carbonyl (C=O) absorption at 1765 cm^{-1}. The structure of **58** was confirmed by FABMS which gave molecular ion peak at m/z. 434 (M^+). The hydroxyl group of **58** was protected as its TBS ether with TBS-Cl and imidazole in DMF at room temperature to afford **59** as a 2:1 mixture of anomers 97 % yield (Scheme-5). The structure of **59** was confirmed by the ^{1}H-NMR spectrum which showed singlets due to Me_2Si group at 0.13 ppm (6H) and t-BuSi group at 0.90 ppm (9H). The anomeric proton appeared at 5.68 ppm as a

broad doublet. The FABMS supported the structure of **59** as highest mass peak was observed at m/z 491 (M^+-^{t}Bu).

Scheme-5

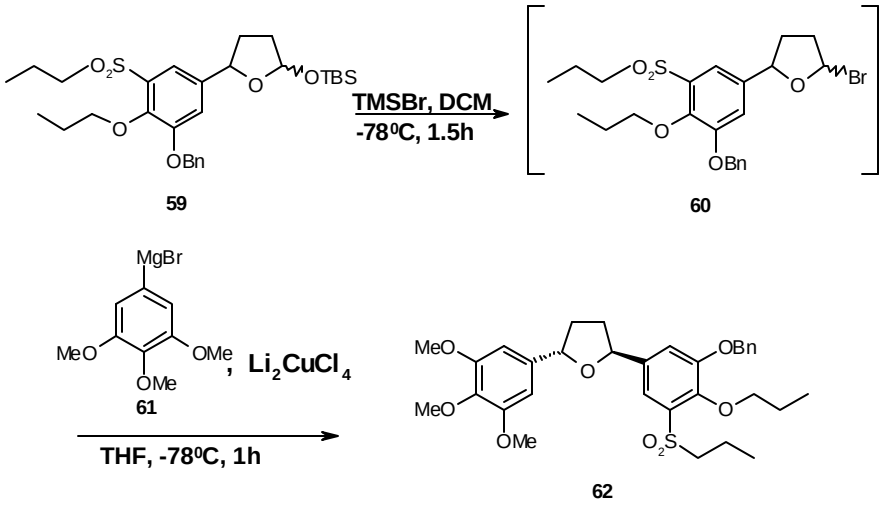


57 **58** **59**

Our next aim was to introduce the second trimethoxyaryl segment. Although many approaches for this endeavour have been forwarded, we believe that the most promising in terms of controlling relative and absolute stereochemistry was the Corey's approach[25].

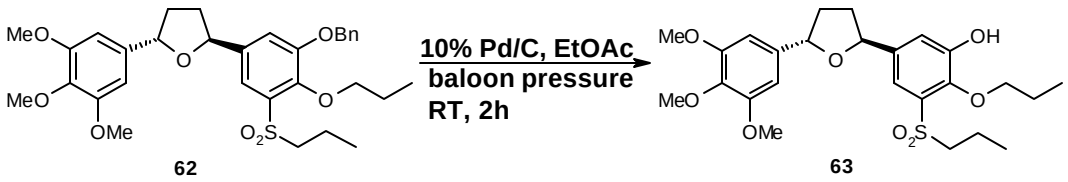
The reaction of silylacetal **59** at −78 °C with TMSBr gave the corresponding bromide derivative (**60**) which being unstable was used immediately without isolation for the next reaction. Thus, to the above reaction, a mixture the 3,4,5-trimethoxyphenylmagnesium bromide (**61**) and Li_2CuCl_4 in THF was added to afford **62** in 71 % yield (Scheme-6). The ^{1}H-NMR spectrum of **62** revealed two singlets due to methoxy groups at 3.83 (3H), 3.88 (6H). The H-2 and H-5 protons appeared as a multiplet between 5.08 – 5.27 ppm. The rest of the protons resonated at their expected shifts. The structure of **62** was further confirmed by its mass spectrum [FABMS m/z: 585 (M^+ +1)] and HRMS: found 585.2522. calculated for $C_{32}H_{40}O_8S$ 585.2536.

Scheme-6



The hydrogenolysis of **62** in the presence of 10 %Pd/C in ethyl acetate gave **63** as a solid in 61 % yield (Scheme-7). The structure of **63** was confirmed by its ^{1}H-NMR spectrum which showed the absence benzylic resonances while rest of the spectrum was in complete agreement with the structure. In FABMS the molecular ion peak at m/z 495 (M^+) was observed.

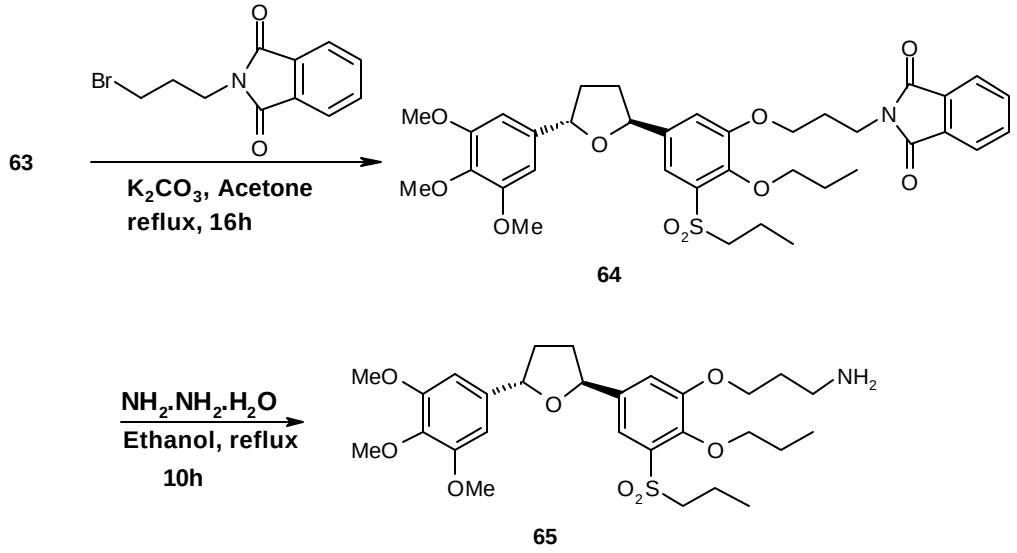
Scheme-7



The O-alkylation of **63** with 3-phthalimido-1-bromopropane in the presence of K_2CO_3 - acetone under reflux temperature afforded the phthalimido derivative (**64**) in 94 % yield. The structure of the corresponding N-phthalimido derivative (**64**) was supported by the IR spectrum which revealed characteristic absorptions at 1775 and 1715 cm^{-1} whereas the ^{1}H-NMR spectrum of **64** revealed the two typical multiplets at 7.70 and 7.85 ppm due to phthalimido protons. The structure of **64** was further confirmed by its mass spectrum: FABMS m/z: 682 (M^+ +1) and HRMS cald for $C_{36}H_{43}NO_{10}S$: 681.2607, found 681.2650. The

removal of phthalimide group from **64** using hydrazine monohydrate in ethanol at reflux temperature provided **65** (Scheme-8). The structure **65** was confirmed by ^{1}H-NMR spectrum in which typical resonances due to phthalimodo group were absent. The methylene protons (CH_2NH_2) appeared as a triplet at 2.90 ppm.

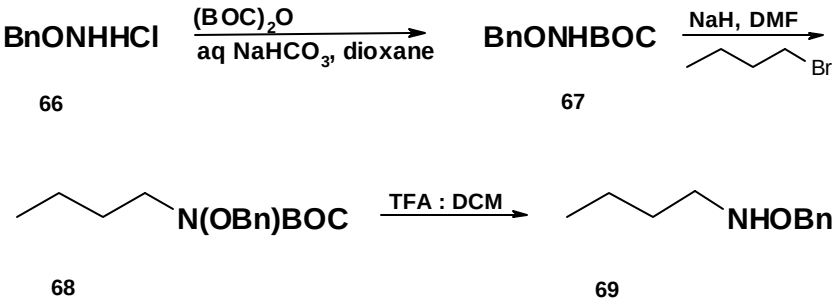
Scheme-8



We next focused our efforts to introduce N-hydroxyurea functionality into the molecule since many hydroxamic acids and hydroxyureas have shown to be the most potent 5-Lipoxygenase inhibitors. The introduction of hydroxyurea functionality onto the molecule has shown to confer 5-LO inhibitory activity, possibly involving chelation of Fe^{+3} required for catalysis. In order to optimize dual 5-LO inhibition and PAF receptor antagonist activities in a single compound we intended to introduce butyl N-uriedyl functionality since both *cis* and *trans* isomers containing a medium size alkyl group are the most active in both rat basophilic leukemia (RBL) cell extracts and PAF assays. The butyl N-(*O*-benzyl)amine was prepared as outlined in scheme-9 . The *O*-benzyl hydroxyl amine hydrochloride (**66**) in aqueous base was treated with di-t-butyl dicarbonate to afford the carbamate **67**. The structure of **67** was confirmed by the ^{1}HNMR spectrum. The carbamate (**67**) was subjected to alkylation with

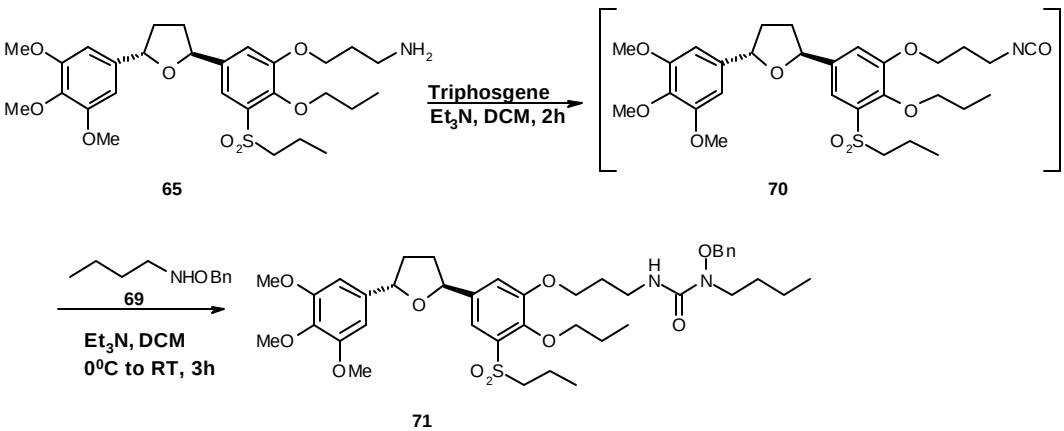
sodium hydride and butyl bromide in dimethylformamide to afford **68** followed by acidolysis with trifluroacetic acid in CH_2Cl_2 provided butyl N-(*O*-benzyl)amine (**69**) (Scheme-9). The ^{1}H-NMR of spectrum **69** showed a triplet at 0.92 (CH_3), a multiplet at 1.27-1.57 ($2\times CH_2$) and a triplet at 2.88 (CH_2N) ppm due to butyl group and a singlet of benzyl group at 4.65, a multiplet at 7.25 ppm corresponding to phenyl group.

Schenme-9



The amine **65** was treated with triphosgene and triethyl amine in dichloromethane at room temperature to produce an isocyanate (**70**) which being unstable was not isolated. The isocyanate (**70**) was reacted with butyl N-(*O*-benzyl) amine (**69**) and triethylamine to afford **71** in 80 % yield (Scheme-10).

Scheme-10

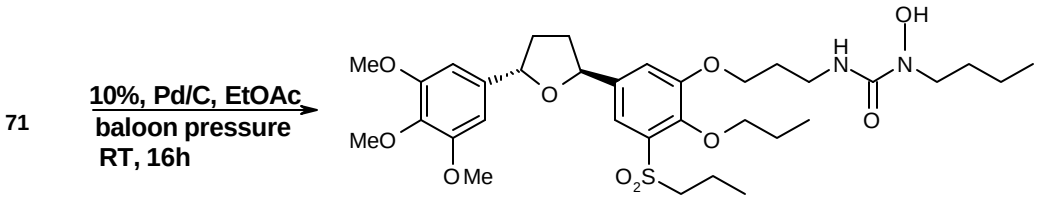


The structure of **71** was confirmed by ^{1}H-NMR, IR and mass spectral analysis. The ^{1}H-NMR spectrum of **71** showed two singlets at 4.72 (benzylic) and 7.30 ppm aromatic of N'-*O*-

Bn group. The NH proton appeared as a triplet at 5.77 ppm while the CH_2N appeared as a triplet at 3.48 ppm. The remaining signals were in complete agreement with the assigned structure **71**. The IR spectrum of **71** showed the characteristic absorption bands at 3440 and 1680 cm^{-1} due to NH and C=O stretching respectively. The structure of **71** was also confirmed by FABMS: m/z 757 (M^+) and high resolution mass spectrum (HRMS) in which the highest mass peak (M^+) was observed at m/z 757.3721 calculated for $C_{40}H_{56}N_2O_{10}S$: 757.3733.

Removal of benzyl group in **71** in presence of 10 % Pd/C in ethyl acetate under baloon pressure and room temperature gave CMI-546 (**47**) in 75 % yield (Shceme-11). The structure of CMI-546 was confirmed by ^{1}H-NMR, IR, and mass spectral analysis.

Shceme-11



CMI-546 (47)

The ^{1}H-NMR spectrum of CMI-546 showed a triplet at 0.83 ppm (3H), a quartet at 0.95 ppm (6H), a sextet at 1.22 ppm (2H) and two multiplets at 1.40-2.05 (10H) and 2.40 ppm (2H). A multiplet was observed at 3.35 ppm (6H) corresponding to 2 x CH_2N and CH_2 protons adjacent to sulfonyl group. The two singlets at 3.79 ppm (3H), 2.83 ppm (6H), and a triplet at 4.08 ppm (J = 6.0 Hz) were appeared due to three methoxyl groups and 2 x OCH_2 groups respectively. It also revealed a quartet at 5.17 ppm (2H) due to C_2-H and C_5-H protons and the aromatic protons resonated at 6.54 ppm (2H) as a singlet and two doublets at 7.20 ppm (J = 1.4 Hz) and 7.42 ppm (J = 1.4 Hz). The NH proton was located as a triplet at 6.03 ppm.

The IR spectrum of CMI-546 showed amide carbonyl absorption at 1650 cm^{-1} and the signals for hydroxyl and NH groups were observed at 3410 and 3230 cm^{-1} respectively.The

structure of CMI-546 (**47**) was further confirmed by FABMS: m/z 66 (M^+ +1) and high resolution mass spectrum of (HRMS) in which the highest mass (M^++1) peak was observed at m/z: 667.3287, calcd for $C_{53}H_{50}N_2O_{10}S$: 667.3264.

The above synthetic sequence provided diastereomeric mixture of (R, R) and (S, S)-CMI-546. In order to obtain both the diastereomers in enantiomerically pure form, chiral HPLC seperation was attempted. It was observed that on HPLC column chiracel OD with isopropane-hexane as mobile phase, (R, R)-CMI-546 and (S, S)-CMI-546 were easily separated. They were analysed by ^{1}H-NMR which were identical for both while optical rotations had opposite sign.

EXPERIMENTAL:

3-Benzyloxy-4-Hydroxybenzaldehyde (49):

To a mixture of **48** (40.80 g, 0.29 mol) and 2N ethanolic potassium hydroxide (320 ml), benzyl chloride (37.54 g, 0.29 mol) was added slowly at room temperature. The reaction mixture was stirred overnight under nitrogen. The ethanol was removed on rotavapour and remaining solution treated with ice water. For removal of dibenzyl ether alkaline solution was extracted with diethyl ether (500 ml). Then the aqueous layer was acidified with concentrated hydrochloric acid and extracted thrice with ethyl acetate (800 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:9) as eluent to give **49** (19.80 g) in 29 % yield.

mp: 109-111 °C. lit[23] mp : 110-113 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.06 (s, 2H), 6.39 (s, 1H), 6.95 (d, J = 8.0 Hz, 1H), 7.12-7.40(m, 7H) and 9.57 (s, 1H).

3-Benzyloxy-4-hydroxy-5-Iodobenzaldehyde (51):

To a solution of **49** (15.00 g, 0.065 mol) in 3.2 % NaOH solution (150 ml), iodine (17.54 g, 0.13 mol) was added and the mixture was heated at 80 °C for 8 h. The reaction mixture was cooled to room temperature and concentrated hydrochloric acid was added and the solid was filtered. Then the compound was recrystalized with isopropanol. The solid was filtered and dried to give **51** (18.40 g) in 79 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.20 (s, 2H), 7.23-7.45 (m, 6H), 7.80 (d, J =1.4 Hz, 1H), 9.73 (s, 1H).

3-Benzyloxy-4-propoxy-5-Iodo benzaldehyde (52):

To a mixture of **52** (23.10 g, 0.065 mol) and potassium carbonate (11.70 g, 0.084 mol) in DMF (55 ml) was added 1-bromo propane (12.03 g, 0.09 mol). The mixture was stirred at 75-80 °C for 12 h. The reaction mixture was cooled to room temperature, diluted with water

and extracted with diethyl ether (480 ml), dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:9) as eluent to give **52** (21.42 g) in 83 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (t, J = 7.2 Hz, 3H), 1.75 (m, 2H), 4.05 (t, J = 6.6 Hz, 2H), 5.15 (s, 2H), 7.30-7.45 (m, 6H), 7.83 (d, J = 1.5 Hz, 1H), 9.79 (s, 1H).

Ethyl-4-(3-benzyloxy 4-propoxy-5-Iodophenyl)-4-oxo-1-butanoate (53):

To a solution of **52** (25.50 g, 0.064 mol) in DMF (155 ml) was added sodium cyanide (0.78 g, 0.016 mol) and stirred at room temperature for 45 min under nitrogen atmosphere. Ethyl acrylate (5.21 g, 0.057 mol) in DMF (30 ml) was added slowly and stirred at room temperature for 45 min. Ethyl acetate (185 ml) and 15 % NaCl solution were added to the reaction mixture and the two layers were separated. The aqueous phase was extracted with ethyl acetate (250 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (125 ml) followed by 5 % aqueous NaCl (180 ml). The ethyl acetate layer was dried over Na_2SO_4, filtered and concentratred. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:12) as eluent to give **53** (23.80 g) in 74 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.05 (t, J = 7.4 Hz, 3H), 1.30 (t, J = 6.9 Hz, 3H) 1.78 (m, 2H), 2.70 (t, J = 6.5 Hz, 2H), 3.20 (t, J = 6.5 Hz, 2H), 4.06 (t, J = 5.6 Hz, 2H), 4.18 (q, J = 7.1Hz, 2H), 5.12 (s, 2H), 7.33-7.43 (m, 5H), 7.58 (d, J =1.6 Hz, 1H), 7.97 (d, J = 1.6 Hz, 1H).

IR (NEAT): 2970, 2930, 1735, 1690, 1585, 1550 cm^{-1}

4-(3-Benzyloxy-4-propoxy-5-Iodophenyl) butyrolactone (55):

To a stirred solution of **53** (15.80 g, 0.031 ml) in EtOH (80 ml) at 0 °C was added sodium borohydride (0.90 g, 0.023 mol) slowly. The reaction mixture stirred for 10-15 min at 0 °C, then ethanol was removed on rotavapor, water was added and extracted with ethyl acetate (300 ml), dried over Na_2SO_4, filtered and concentrated to afford a mixture of **54** and **55**. A solution of mixture of **54** and **55** in CH_2Cl_2 was stirred overnight with catalytic amount of

PTSA at room temperature and the solvent was removed on rotavapor. The residue was purified on silica gel column using ethyl acetate: petroleum ether (1:5) to give **55** (11.40 g) in 79 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (t, J = 7.9 Hz, 3H), 1.75-1.90 (m, 2H), 2.00-2.30 (m, 1H), 2.45-2.65 (m, 3H), 3.96 (t, J = 6.0 Hz, 2H), 5.08 (s, 2H), 5.35 (t, J = 6.9 Hz, 1H), 6.88 (d, J = 1.6 Hz, 1H), 7.28 (d, J = 1.6 Hz, 1H), 7.30-7.40 (m, 5H).

IR (NEAT): 3070, 2960, 2895, 1760, 1600, 1585 cm^{-1}.

Mass (EI): 452 (M^+).

4-(3-Benzyloxy-4-propoxy-5-propylthiophenyl)butyrolactone (56):

To compound **55** (11.40 g, 0.025 mol) in DMF (60 ml) were added propyl disulfide (10.23 g, 0.068 mol) and copper powder (6.40 g, 0.10 mol) and the mixture was stirred at 100 °C for 24 h. The reaction mixture was cooled to room temperature, the solid was filtered through pad of Celite, washed with ethyl acetate (80 ml) and NH_4Cl: NH_4OH (9:1) solution (50 ml) was added and extracted with ethyl acetate (180 ml), dried over Na_2SO_4, filtered concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:4) to afford **56** as syrup (8.75 g) in 86 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.05 (q, J = 6.8 Hz, 6H), 1.60- 1.90 (m, 4H), 2.04-2.20 (m,1H), 2.52-2.65 (m, 3H), 2.85 (t, J = 6.5 Hz, 2H), 3.97 (t, J = 6.8 Hz, 2H), 5.08 (s, 2H), 5.40 (t, J = 6.8 Hz, 1H), 6.70 (s, 1H), 6.75 (s, 1H), 7.30-7.45 (m, 5H).

IR (NEAT): 2975, 2945, 2880, 1745, 1590 cm^{-1}.

Mass (EI): 400 (M^+).

4-(3-Benzyloxy-4-propoxy-5-propylsulfonylphenyl) butyrolactone (57):

To a cooled (0 C) solution of **56** (8.75 g, 0.021 mol) in dry CH_2Cl_2 (80 ml) was added m-chloroperbenzoic acid (9.43 g, 0.054 mol) slowly. Then the reaction mixture was stirred at room temperature for 2 h. Then the solid was filtered through pad of Celite and washed with

dichloromethane (100 ml). The organic layer was washed with saturated sodium bicarbonate solution followed by brine, dried over Na_2SO_4, filtered and concentrated. The crude compound was purified on silica gel column using ethyl acetate: petroleum ether (2:3) as eluent to give **57** as solid (6.90 g) in 73 % yield.

mp: 95-96 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.00 (q, J = 6.4 Hz, 6H), 1.62-1.90 (m, 4H), 2.05-2.30 (m, 1H), 2.45-2.75 (m, 3H), 3.35 (t, J = 6.0 Hz, 2H), 4.14 (t, J = 6.4 Hz, 2H), 5.13 (s, 2H), 5.45 (m, 1H), 7.27 (d, J = 1.4 Hz, 1H), 7.33-7.45 (m, 6H).

IR (NEAT): 3010, 2930, 1765, 1600, 1490, 1460 cm^{-1}.

Mass (EI): 432 (M^+).

2-Hydroxy-5-(3-benzyloxy-4-propoxy-5-propylsulfonylphenyl)tetrahydrofuran (58):

To a solution of **57** (6.90 g, 0.015 mol) in dry toluene (65 ml) at −78 °C was added DIBAL-H (3.40g, 26.61ml of 0.9 M toluene solution) drop wise at −78 °C and stirred for 1 h. Upon completion, the reaction was quenched by adding methanol (7 ml) at −78 °C. The mixture was warmed to −20 °C followed by the addition of saturated sodium potassium tartarate solution while maintaining the temperature between −10 °C and 0 °C. The mixture was stirred at 0 °C for 1 h. Then the two phases were separated, the aqueous layer was extracted with ethyl acetate and the combined organic layer was washed with water followed by brine, dried over Na_2SO_4, filtered and concentrated to afford **58** (6.86 g, 99 % yield) which was used for the next step without further purification.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (m, 6H), 1.65-2.15 (m, 7H), 2.30-2.60 (m, 1H), 3.35 (m, 2H), 4.15 (t, J = 6.4 Hz, 2H), 4.98 (apparent t, J = 6.7 Hz, 0.5H), 5.18 (t, J = 6.5 Hz, 0.5H), 5.15 (2s, 2H), 5.60, 5.75 (brs, 1H), 7.10-7.22 (m, 1H), 7.30-7.48 (m, 6H).

FABMS: m/z 434 (M^+).

2-(O-*t*-Butyldimethylsilyl)-5-(3-benzyloxy-4-propoxy-5-propylsulfonylphenyl) tetrahydrofuran (59):

To a solution of **58** (6.86 g, 0.015 mol) in dry DMF (30 ml) at 25 °C under nitrogen was added imidazole (2.36 g, 0.034 mol) followed by t-butyldimethylsilyl chloride (2.62 g, 0.017 mol). The mixture was stirred at 25 °C under nitrogen for 3.5 h. After completion of the reaction, ethyl acetate and water were added, extracted with ethyl acetate (120 ml). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude products were purified on silica gel column using ethyl acetate: petroleum ether (1:15) as eluent to give the 2:1 mixture of **59** (8.45 g) in 97 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.13 (m, 6H), 0.90 (s, 9H), 1.00 (q, J = 6.5 Hz, 6H), 1.60-2.10 (m, 7H), 2.50 (m, 1H), 3.35 (m, 2H), 4.13 (t, J = 5.9 Hz, 2H), 5.15 (m, 3H), 5.68 (bd, 1H), 7.16 (d, J = 1.3 Hz, 1H), 7.32-7.46 (m, 6H).

FABMS: 491 (M^+-^{t}bu).

(3,4,5-Trimethoxyphenyl) magnesium bromide (61):

Magnesium (0.82 g, 33.74 mmol) was taken in flame dried 100 ml two necked flask and dry THF (20 ml) was added. Then the dibromoethane (0.1 ml) and trimethoxy bromobenzene (0.30 g, 1.20 mmol) were added at room temperature and stirred for 20 min. The reaction initiates as indicated by temperature rise. The remaining bromide/THF (8.8 g, 35.48 mmol) in THF (20 ml) was added over 15 min. After the addition was completed reaction mixture was stirred at 25 °C under nitrogen for 18 h. To the grignard reagent at 0 °C was added a solution of dilithium tetrachlorocuprate (0.5 M, .76 ml, 0.38 mmol). The reaction was stirred at 0 °C for 15 min and was used immediately for the coupling reaction.

(±)-*trans*-2-(3-Benzyloxy-4-propoxy-5-propylsulfonylphenyl)-5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (62):

To a solution of **59** (8.45 g, 15.41 mmol) in CH_2Cl_2 (80 ml) was added TMSBr (2.59 g, 2.20 ml, 16.96 mmol) at −78 °C under N_2. The mixture was stirred at −78 °C for 1.5 h. The above prepared grignard **61** /Li_2CuCl_4 mixture was transferred via cannula over 10 min to the reaction vessel containing bromoether **60**. The mixture was stirred for 1 h at −78 °C and quenched with 10:1 saturated NH_4Cl/NH_4OH (50 ml) and water was added to dissolve the salts. The mixture was stirred for 30 min without external cooling. The organic layer was removed and aqueous phase was extracted with ethyl acetate (150 ml). The combined organic layer was washed with brine (100 ml), dried over Na_2SO_4, filtered and concetrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:5) as eluent to give **62** (6.48 g, 71 %). The product contains some colour impurity at the same R_f which was removed in the next step.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.03 (q, J = 6.9 Hz, 6H), 1.67-2.05 (m, 6H), 2.38-2.52 (m, 2H), 3.35 (t, J = 7.9 Hz, 2H), 3.83 (s, 3H), 3.88 (s, 6H), 4.14 (t, J = 7.4 Hz, 2H), 5.08-5.27 (m, 4H), 6.57 (s, 2H), 7.30-7.48 (m, 7H).

IR (NEAT): 2975, 2920, 2865, 1600, 1445 cm^{-1}.

FABMS: 585 (M^++1), HRMS (FAB): calcd for $C_{32}H_{40}O_8S$ (M^++1) 585.2536, found. 585.2522.

(±)-*trans*-2-(3-Hydroxy-4-propoxy-5-propylsulfonylphenyl)–5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (63):

To a solution of (6.35 g, 10.87 mol) in ethyl acetate (50 ml) was added 10 % Pd/C (0.80 g). The reaction mixture was stirred at room temperature under baloon pressure for 2 h. The catalyst was filtered through pad of Celite and washed with ethyl acetate (80 ml). The filtrate was concentrated and the crude product was purified through silica gel column using ethyl acetate: petroleum ether (1:3) to give **63** as a solid (3.30 g) in 61 % yield.

mp:115-117 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.95 (t, J = 7.7 Hz, 3H), 1.00 (t, J = 6.8 Hz, 3H), 1.60-1.95 (m, 6H), 2.30-2.42 (m, 2H), 3.25 (m, 2H), 3.75 (s, 3H), 3.80 (s, 6H), 4.03 (t, J = 6.1 Hz, 2H), 5.00-5.13 (m, 2H), 6.28 (s, 1H), 6.5 (s, 2H), 7.18 (d, J = 1.5 Hz, 1H), 7.34 (d, J = 1.5 Hz, 1H).

IR (NEAT): 3400, 2975, 2945, 2860, 1725, 1540, 1490, 1440 cm^{-1}.

FABMS: m/z 495 (M^++1), HRMS ((FAB) calcd for $C_{25}H_{34}O_8S$ (M^++1) 495.2052, found. 495.2072.

(±)-*trans*-2-[3-(3-Phthalimidopropoxy)-4-propoxy-5-propylsulfonyl phenyl]-5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (64):

A mixture of **63** (2.80 g, 5.66 mol), potasssium carbonate (1.01 g, 7.36 mol) and N-(3-bromopropyl)phthalimide (2.06 g, 8.50 mmol) in acetone (40 ml) was heated at reflux temperature for 16 h. The reaction mixture was cooled to room temperature and acetone was removed, water was added and extracted with ethyl acetate (120 ml), dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (2:5) to give **64** as solid (3.71 g) in 94 % yield.

mp: 195-196 °C.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.05 (t, J = 7.1 Hz, 3H), 1.08 (t, J = 6.6 Hz, 3H), 1.68-2.06 (m, 6H), 2.20-2.30 (m, 2H), 2.40-2.55 (m, 2H), 3.35 (m, 2H), 3.83 (s, 3H), 3.9 (s, 6H), 3.95 (t, J = 6.6 Hz, 2H), 4.15 (t, J = 6.1 Hz, 4H), 5.20 (m, 2H), 6.58 (s, 2H), 7.23 (d, J = 1.5 Hz, 1H), 7.45 (d, J = 1.5 Hz, 1H), 7.72 (m, 2H), 7.85 (m, 2H).

IR (NEAT): 3010, 2975, 1775, 1715, 1600, 1490, 1380 cm^{-1}.

FABMS: m/z 681(M^+), HRMS (FAB) calcd for $C_{36}H_{43}NO_{10}S$ (M^+) 681.2607, found. 681.2650.

(±)-*trans*-2-[3-(3-Aminopropoxy)-4-propoxy-5-propylsulfonylphenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (65):

To a solution of **64** (3.71 g, 5.44 mmol) in EtOH (60 ml) was added hydrazine monohydrate (0.95 g, 19.06 mmol). The reaction mixture was heated under reflux for 10 h, then mixture was cooled to room temperature. The ethanol was removed, water was added and extracted with chloroform (120 ml). Organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated to afford an amine **65**. The crude amine **65** was used in the next step without further purification (3.43 g).

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.95-1.02 (m, 6H), 1.62-2.00 (m, 8H), 2.35-2.50 (m, 2H), 2.90 (t, J = 6.8 Hz, 2H), 3.30 (m, 2H), 3.78 (s, 3H), 3.82 (s, 6H), 4.08 (m, 4H), 5.05-5.20 (m, 2H), 6.55 (s, 2H), 7.20 (d, J = 1.3 Hz, 1H), 7.40 (d, J = 1.3 Hz, 1H).

***tert*-Butyl N-benzyloxy carbamate (67):**

To a stirred solution of di-*tert*-butyl dicarbonate (6.90 g, 31.34 mmol) in dioxane (30 ml) was added O-benzylhydroxylamine hydrochloride **(66)** (5.00 g, 31.34 mmol) and then sodium bicarbonate solution (1M, 50 ml). The resulting milky solution was stirred 2 h at room temperature and then partially evaporated in *vacuo* to remove dioxane. The residue was cooled and adjusted with citric acid to P^H 4. The mixture was extracted with dichloromethane, dried over $NaSO_4$, filtered and evaporated to afford **67** (6.70 g) in 96 % yield.

^{1}H NMR($CDCl_3$, 200MHz): δ 1.45(s, 6H), 1.52(s, 3H), 4.85(s, 2H), 7.05(bs, 1H), 7.35(m, 5H).

***tert*-Butyl N-butyl-N-benzyloxycarbamate (68):**

Sodium hydride (1.80 g, 60 %dispersion in oil) was added to a solution of compound **67** (6.70 g, 30.04 mmol) in DMF (50 ml) at room temperature and stirred for 30 min, then 1-bromobutane (3.87 ml, 36.00 mmol) was added and the reaction mixture was heated to 60 °C for 16 h. The reaction mixture was cooled, and poured into water and extracted with hexane, dried over Na_2SO_4, filtered and evaporated. The residue was purified by column

chromatography on silica gel using ethyl acetate: petroleum ether (1:10) to provide **68** (7.96 g) in 97 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.93 (t, J = 7.0 Hz, 3H), 1.22-1.40 (m, 2H), 1.45 (s, 3H), 1.50 (s, 6H), 1.50-1.62 (m, 2H), 3.38 (t, J = 6.8 Hz, 2H), 4.80 (s, 2H), 7.30-7.40 (m, 5H).

Butyl N (*O*-benzyl) amine (69):

To a solution of compound **68** (7.90 g, 28.31 mmol) in CH_2Cl_2 was added trifluoroacetic acid (4.34 ml, 56.63 mmol) in CH_2Cl_2 and stirred for 12 h. Then trifluoroacetic acid was removed, and saturated $NaHCO_3$ solution was added and extracted with ethyl acetate, dried over Na_2SO_4, filtered and evaporated. The residue was chromatographed on silica gel using ethyl acetate: petroleum ether to give **69** (4.90 g) in 97 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.92 (t, J = 4.2 Hz, 3H), 1.27-1.57 (m, 4H), 2.89 (t, J = 5.8 Hz, 2H), 4.65 (s, 2H), 7.25 (m, 5H).

(±)-*trans*-2-[3-(3-(N^1-Butyl-N^1-benzyloxyureidyl)propoxy)-4-propoxy-5-propyl sulfonylphenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (71):

To a solution of amine **65** (3.43 g, 6.22 mmol) in CH_2Cl_2 (30 ml) was added triphosgene (0.92 g, 3.11 mol) and triethyl amine (1.73 ml, 12.44 ml) at room temperature. The reaction mixture was refluxed for 2 h and then cooled with an ice bath, to this cold solution was added butyl N-(*O*-benzyl)amine **69** (2.78 g, 15.56 mmol) and triethyl amine (3.45 ml, 24.84 mmol). The reaction mixture was stirred at room temperature for 3 h and then quenched with water and extracted with chloroform (120 ml). Organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (1:3) to give **71** as syrup (3.32 g) in 80 % yield.

^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.83-1.08 (m, 9H), 1.20-1.40 (m, 2H), 1.50-2.05 (m,10H), 2.23-2.50 (m, 2H), 3.25-3.40 (m, 4H), 3.48 (t, J = 6.8 Hz, 2H), 3.8 (s, 3H), 3.85 (s, 6H), 3.94 (t, J = 6.1 Hz, 2H), 4.08 (t, J = 6.3 Hz, 2H), 4.72 (s, 2H), 5.20 (m, 2H), 5.74 (t, J = 4.5 Hz, 1H),

6.58 (s, 2H), 7.20 (d, J = 1.3 Hz, 1H), 7.30 (s, 5H), 7.48 (d, J = 1.3 Hz, 1H). IR (NEAT): 3440, 2960, 2880, 1685, 1660, 1500, 1472 cm^{-1}.

FABMS: m/z 757 (M^++1), HRMS (FAB): calcd for $C_{40}H_{56}N_2O_{10}S$ (M^++1) 757.3733, found. 757.3721.

(±)-*trans*-2-[3-(3-(N^1-Butyl-N^1-hydroxyureidyl)propoxy)-4-propoxy-5-propylsulfonyl phenyl]- 5-(3,4,5-trimethoxy phenyl) tetrahydrofuran (CMI-546) (47):

To a solution of **71** (3.10 g, 4.10 mmol) in ethyl acetate (15 ml) was added 10 % Pd/C (465 mg). The reaction mixture was stirred at room temperature under baloon pressure for 6 h. Then catalyst was filtered through pad of Celite, washed with ethyl acetate (80 ml) and the filtrate was concentrated. The crude product was purified on silica gel column using ethyl acetate: petroleum ether (3:2) to afford **CMI-546 (47)** as solid (2.07 g) in 75 % yield.

mp: 102-104 °C.

^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.83 (t, J = 6.9 Hz, 3H), 0.95 (q, J = 6.9 Hz, 6H), 1.22 (sextet, 2H), 1.40-1.53 (m, 2H), 1.60-2.05 (m, 8H), 2.40 (m, 2H), 3.28-3.40 (m, 6H), 3.79 (s, 3H), 3.83 (s, 6H), 4.08 (t, J = 6.0 Hz, 4H), 5.17 (m, 2H), 6.03 (t, J = 5.1 Hz, 1H), 6.54 (s, 2H), 7.00 (broad peak, 1H), 7.20 (d, J = 1.4 Hz,1H), 7.42 (d, J = 1.4 Hz, 1H).

IR (NEAT): 3410, 3230, 2945, 2830, 1650, 1585, 1520, 1460 cm^{-1}.

FABMS: m/z 667 (M^++1), HRMS (FAB): calcd for $C_{33}H_{50}N_2O_{10}S$ (M^++1) 667.3264, found. 667.3264.

HPLC: Optical purity of the compound **47** determined by HPLC was 98.25 %. conditions [column: ODS, mobile phase: 70 % methanol in water, flow rate 1.0 ml/min, UV detection at 225 nm]. The two enantiomers (R, R)- and (S, S) were separated on chiral HPLC column. HPLC conditions [CHIRACEL (OD), mobile phase: 40 % isopropanol in n-hexane, flow rate: 2.0 ml/min UV detection at 225 nm, t_R = 15 min, $[\alpha]_D$ 24 (c 1, $CHCl_3$), t_R = 21 min, $[\alpha]_D$ -31 (c 1, $CHCl_3$).

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List of publications:

1. New synthesis of α-*C*-D-Glucosphingosine Derivative
   Gurjar, M. K.; **Reddy, R**. Carbohydr.Lett. 1997, 2, 293.

2. Synthesis of Callipeltose: An unusual aminodeoxy sugar component of cytotoxic macrolide callipeltoside-A.
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3. Rao, A.V.R.; Chorghade, M.S.; Rao, B. V.; **Reddy, R**.; Reddy, L.K.; Gurjar, M.K.; Mhaskar, S.V.; Islam, A.; Rao, V. V. K.; Prasad, A.S.; Shailaja, K. US Patent. Application No. 09/418, 637, Oct, 1999.

4. Unusual formation of 2-butyl-5-alkyloxymethylfuran
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