SYNTHESIS OF CMI-546 AND OLIGOSACCHARIDES OF MOTIFS D AND E OF *M. TUBERCULOSIS*

Submitted by

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To

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For

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(In Chemistry)

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Dedicated to My Beloved Grandmother

DECLARATION

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

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CERTIFICATE

The research work presented in this thesis entitled **"Synthesis of CMI-546 and Oligosaccharides of Motifs D and E of *M. tuberculosis*"** has been carried out under my supervision and is bonafide work of **Mr. L. Krishnakanth Reddy.** This work is original and has not been submitted for any other degree or diploma of this or any other University.

Pune-8

Date:

(Dr. M. K. Gurjar)
(Research Guide)

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It gives me immense pleasure to express my deep sense of gratitude to my teacher and research guide Dr. M. K. Gurjar FNASc, Deputy Director and Head of Organic Chemistry: Technology Division, National Chemical Laboratory, Pune for his inspiring guidance and constant encouragement. It would have been impossible to accomplish this work, without his able guidance and unabated perseverance in the quest for success.

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L. Krishnakanth Reddy

General Remarks

- Melting points were recorded on Buchi 535 melting point apparatus and are uncorrected.
- Optical rotations were measured with a JASCO DIP 370 digital polarimeter.
- Infrared spectra were scanned on Shimadzu IR 470 and Perkin-Elmer 683 or 1310 spectrometers with sodium chloride optics and are measured in cm^{-1}.
- Proton magnetic resonance spectra were recorded on Varian FT-200 MHZ (Gemini), AC-200 MHz, MSL-300 MHz and Bruker-500 MHz spectrometer using tetra methyl silane (TMS) as an internal standard. Chemical shifts have been expressed in ppm units downfield from TMS.
- ^{13}C Nuclear magnetic spectra were recorded on AC-50 MHz, MSL-75 MHz and Bruker-125 MHz spectrometer.
- Mass spectra were recorded on a CEC-21-110B, Finnigan Mat 1210 or MICRO MASS 7070 spectrometer at 70 eV using a direct inlet system. FABMS were recorded on a VG auto spec mass spectrometer at 70 eV using a direct inlet system.
- All reactions are monitored by Thin Layer chromatography (TLC) carried out on 0.25 mm E-Merck silica gel plates (60F-254) with UV, I_2 and anisaldehyde reagent in ethanol as development reagents.
- All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 50 °C.
- All solvents and reagents were purified and dried by according to procedures given in Vogel's Text Book of Practical Organic Chemistry.
- Silica gel (60-120) used for column chromatography was purchased from ACME Chemical Company, Bombay, India.

Abbreviations

Ac	- Acetyl
AcOH	- Acetic acid
Ac_2O	- Acetic anhydride
Ara	-Arabinose
Ara*f*	-Arabinofuranosyl
BF_3:OEt_2	- Borontrifluoride diethyletherate
Bn	- Benzyl
BnBr	- Benzyl bromide
BnCl	- Benzyl chloride
Boc	- *tert*-Butoxy carbonyl
$(Boc)_2O$	- Di-*tert*-butyl dicarbonate
Bu_2SnO	- Di *n*-butyltin oxide
Bu_3SnOEt	- Tri *n*-butyltin ethoxide
CAN	- Ammonium cerium(IV) nitrate
DBU	- 1,8-Diazabicyclo [5.4.0]undec-7-ene
DIBAL-H	- Diisobutylaluminium hydride
DMAP	- *N*, *N'*-Dimethylaminopyridine
DMF	- *N*, *N'*-Dimethylformamide
DMP	- 2,2-Dimethoxypropane
DMSO	- Dimethyl sulfoxide
Et	- Ethyl
EtMgBr	- Ethylmagnesiumbromide
EtOAc	- Ethyl acetate
EtOH	- Ethanol
EtSH	- Ethanethiol
Gal	- Galactose
Gal*f*	- Galactofuranosyl
IBX	- 2-Iodoxybenzoic acid
Im	- Imidazole
Man*p*	- Mannopyranosyl
*m*CPBA	- *meta*-Chloroperbenzoic acid
MeOH	- Methanol
NaOMe	- Sodium methoxide
NBS	- *N*-Bromosuccinimide
NIS	- *N*-Iodosuccinimide
NPhth	- Phthalimide
Pd/C	- Palladium on carbon
$Pd(OH)_2$/C	- Palladium hydroxide on carbon
PMB	- *para*-Methoxy benzyl

pTSA - *para*-Toluenesulfonic acid

Py - Pyridine

Rham*p* -Rhamnopyranosyl

TBAB - Tetrabutylammonium bromide

TBAF - Tetrabutylammonium fluoride

TBAI - Tetrabutylammonium iodide

TBDMS-Cl - *tert*-Butyldimethylchlorosilane

TBDPS-Cl - *tert*-Butyldiphenylchlorosilane

TEA - Triethyl amine

TFA - Trifluoroacetic acid

TfOH - Trifluoromethanesulfonic acid

THF - Tetrahydrofuran

TMSBr - Bromotrimethylsilane

Contents

ABSTRACT

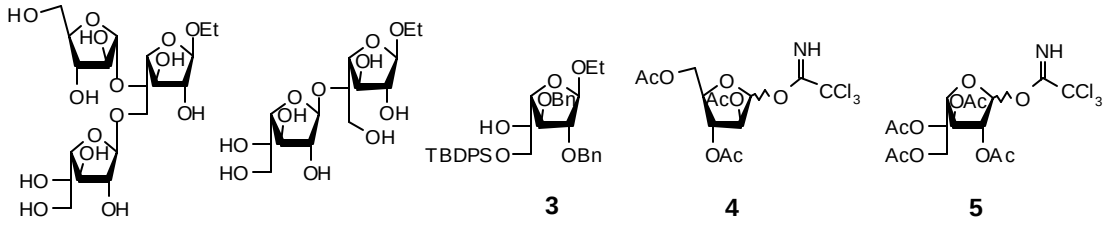
Abstract

The thesis entitled **" Synthesis of CMI-546 and oligosaccharides of motifs D and E of *M. tuberculosis*"** is divided into three chapters. The first chapter highlights the synthesis of oligosaccharides of motifs D and E of *M. tuberculosis*. The second chapter deals with the synthesis of CMI-546 (Section I) and new and efficient method for (±)-*trans*-2,5-diaryl tetrahydrofurans (Section II). The third chapter describes the synthesis of 9-*epi*-manzacidin B.

Chapter I: Synthesis of olig osaccharides of motifs D and E of *M. tuberculosis*

M. tuberculosis, which causes tuberculosis, contains two major polysaccharides, lipoarabinomannan (LAM) and arabinogalactan (AG) on its cell wall in which both the arabinose and galactose residues are found in the furanose form. The five major motifs of AG namely motifs A-E have been isolated and characterized. The oligosaccharide structure of motif E is sterically much complicated because both 5- and 6- positions of the reducing galactofuranose component are linked with arabino- and galactofuranosyl residues respectively. Motif D mainly consists ß-D-gal*f* (1→5)-gal*f* repeating units. This chapter describes the synthesis of trisaccharide (**1**) and disaccharide (**2**) present in motifs E and D respectively. We have envisioned that the synthesis of these oligosaccharides requires three principal building blocks, *viz*, aglycone **3** and glycosyl donors **4** and **5** (figure 1).

Figure 1

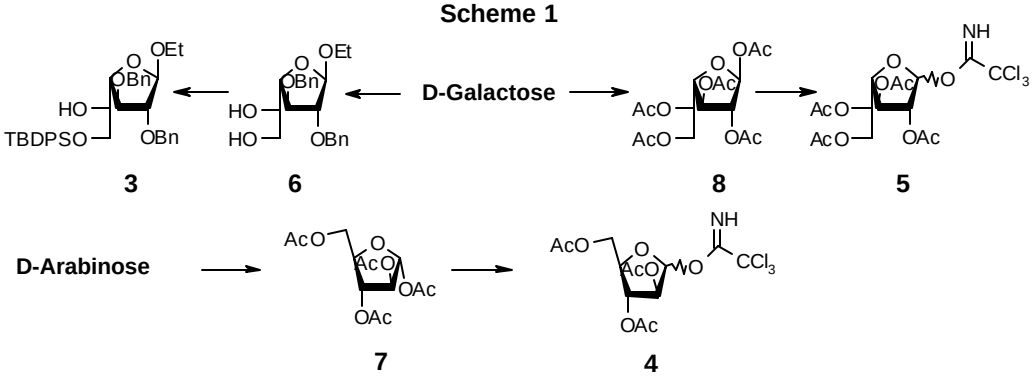


Trisaccharide (1) **Disaccharide (2)**

Our first concern was the preparation of **3,** which was obtained from known diol (**6**) by the treatment of TBDPS-Cl. Glycosyl donor **4** was obtained by selective deprotection of 1-*O*-acetyl group of known tetra acetate (**7**) with Bu_3SnOEt and

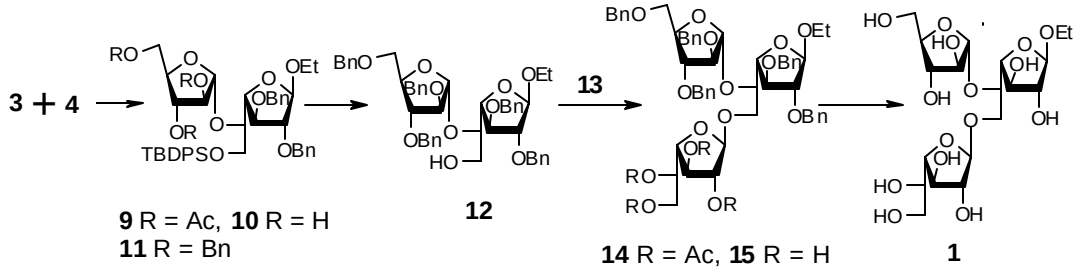
subsequent exposure to CCl_3CN-DBU. Compound **5** was ynthesized from known penta acetate (**8**) (scheme 1).

Scheme 1



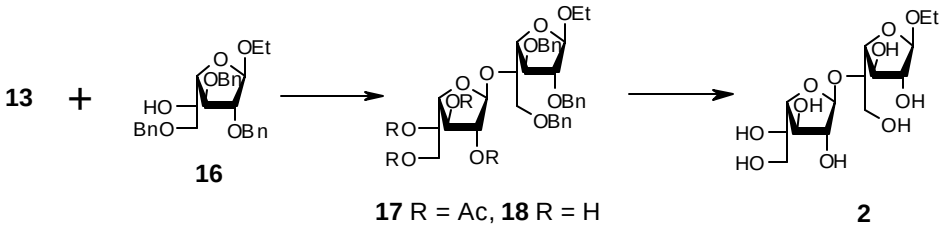
The coupling reaction between **3** and **4** was carried out with BF_3:OEt_2 as an activator to give **9**, which was isolated and characterized after Zemplen deacetylation. The ^1H, ^{13}C NMR and FAB MS spectral data clearly confirmed the structure of **10**. Compound **10** was transformed into penta-*O*-benzyl derivative (**11**) followed by cleavage of TBDPS ether linkage gave **12**. The glyco- sylation reaction of **12**, with galactofuranosyl derivatives, turned out to be a difficult proposition because trichloroacetimidate methodology, SEt mediated glycosidation and Helferich reaction failed. Finally, Fraser-Reid's n-pentenyl glycosidation technique was successful. Thus, pentaacetate (**8**) was converted to pentenyl glycoside (**13**) in the presence of 4-pentene-1-ol and BF_3:OEt_2. The coupling reaction between **12** and **13** in the presence of *N*-iodosuccnimide and triflic acid gave **14**. Zemplen deacetylation of **14** provided **15**, which was analyzed by ^1H and ^{13}C NMR spectral data. Finally compound **15** was debenzylated over $Pd(OH)_2$ to the target molecule (**1**) (scheme 2).

Scheme 2



Synthesis of disaccharide (2): Having both the aglycone (**3**) and glycosyl donor (**13**) in hand, the coupling reaction was attempted. But the reaction was not successful. Thus, the known diol (**6**) was converted to the tri benzyl derivative **16** with Bu_2SnO/BnBr. The coupling reaction between **13** and **16** mediated by NIS and TfOH gave the disaccharide (**17**), which was then deacetylated under Zemplen conditions. The ^1H and ^{13}C NMR spectra of **18** were in complete agreement with the assigned structure. Finally, compound **18** was debenzylated by hydrogenolysis over $Pd(OH)_2$ to give the target disaccharide (**2**) (scheme 3).

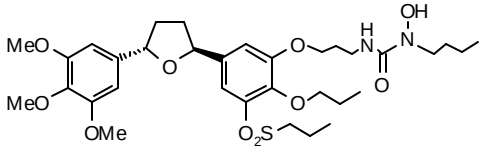
Scheme 3



Chapter II

Section I: Synthesis of CMI-546 (1): A potent PAF receptor antagonist

Lignans of 2,5-diaryl-tetrahydrofuran 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 CMI-546 (**1**) has identified and developed as a potent 5-LO inhibitor and PAF receptor antagonists there by adjusting as the curator for chronic asthma. This section describes the synthesis of CMI-546 (**1**).

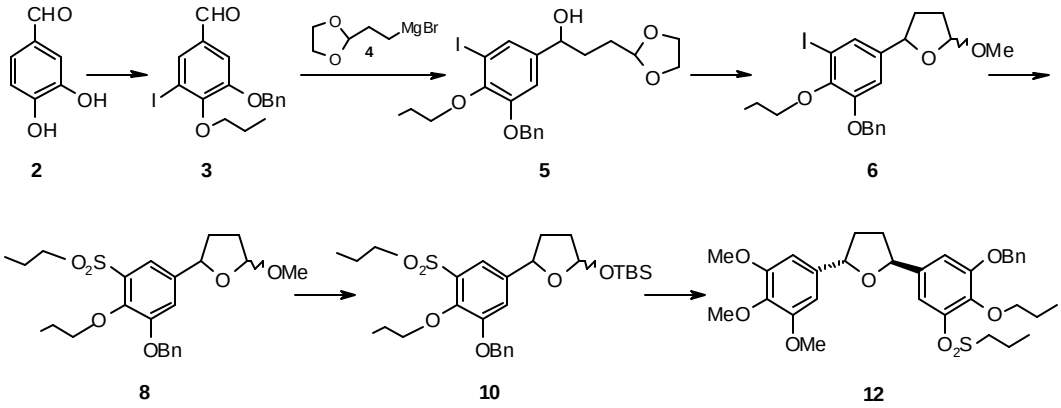


CMI-546(1)

We began the synthesis from 3,4-dihydroxybenzaldehyde (**2**), which was converted to compound **3** following the sequence of reactions (i) monobenzylation (ii) iodination and (iii) *O*-alkylation. Compound **3** on treatment with grignard reagent (**4**) afforded the alcohol**5**, which was cyclized to **6** in the presence of *p*TSA. The ^1H NMR and FAB MS spectral data confirmed the structure of **6**. Conversion of iodide in compound **6** to propyl

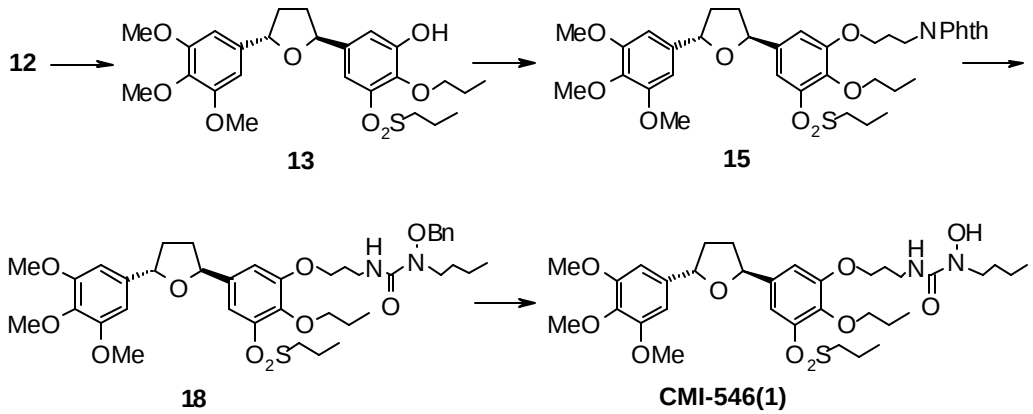
sulfide **7** and oxidation of sulfide **7** gave sulfone **8**. The silyl acetal **10** was obtained by hydrolysis of **8** to lactol followed by protection with TBS-Cl. The structure of **10** was confirmed by ^{1}H NMR and FAB MS spectral data. Exposure of **10** to TMSBr at $-78\ ^{o}$C followed by the addition of 3,4,5-trimethoxyphenylmagnesiumbromide (**11**) provided the (±)- *trans*-**12** (scheme 1) whose structure was confirmed by ^{1}H NMR, FAB MS and HRMS spectral data.

Scheme 1



Unmasking of *O*-Bn group from **12** followed by *O*-alkylation with *N*-(3-bromopropyl) phthalimide (**14**) gave **15**. The phthalimide group in **15** was deprotected with hydrazine hydrate to provide an amine (**16**), which on treatment with triphosgene and *N*-(*O*-benzyl) butyl amine (**17**) furnished the compound **18**. Finally, CMI-546 (**1**) was obtained by the deprotection of benzyl group from compound **18** (scheme 2). The ^{1}H NMR, FAB MS and HRMS spectral data were in agreement with the assigned structure.

Scheme 2

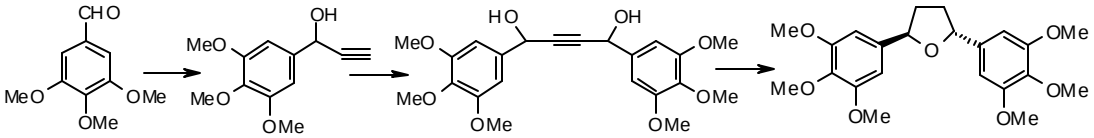


Section II: New and efficient method for (±)- *trans*-2, 5-diaryltetrahydrofurans

Recently a number of trans-2,5-diaryltetrahydrofurans have been identified, synthesized and their anti-PAF activities evaluated. All these syntheses have involved a number of synthetic steps, frequently proceed in low overall yield which prompted us to develop a new and efficient method for the synthesis of (±)- *trans*-2, 5-diaryltetrahydrofurans. This section describes the synthesis of (±)- *trans*-2, 5-diaryltetrahydrofurans (**19** & **20**).

Aldehyde **21** on exposure to acetylenemagnesiumbromide provided the alkyne **22**, which on treatment with ethylmagnesiumbromide at 60 ^{o}C followed by the addition of **21** gave **23**. Compound **23** upon hydrogenation gave the diol **24**, which was cyclized in the presence of TFA to afford (±)- *trans*-**19** (scheme 3). Similarly, (±)-*trans*-2- (3,4,5-Trimethoxyphenyl)-5- (3-methoxy-4-propoxyphenyl) tetrahydrofuran **(20)** was also obtained starting from 3-methoxy-4-propoxybenzaldehyde.

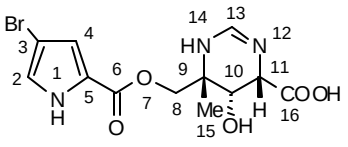
Scheme 3



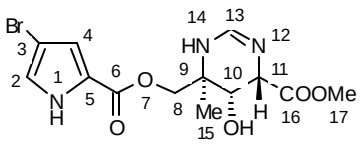
21 22 23 19

Chapter III: Synthesis of 9-*epi*-manzacidin B

Recently a novel class of alkaloids manzacidin A-C (**1-3**) have been isolated, which possess a unique structure consisting of an ester-linked bromopyrrole carboxylic acid and a 3,4,5,6-tetrahydropyrimidine ring in which one of the amino group is attached to the C-9 quaternary carbon center. Although manzacidins exhibit similar biological activities to those of other bromopyrrole alkaloids, only recently tests have been carried out, owing to the extremely small amount of samples available from marine sources. In this context, we intended to synthesize both manzacidin B (**2**) and 9-*epi*-manzacidin B. The present chapter describes the highly stereoselective synthesis of 9-*epi*-manzacidin B as its methyl ester derivative (**4**).



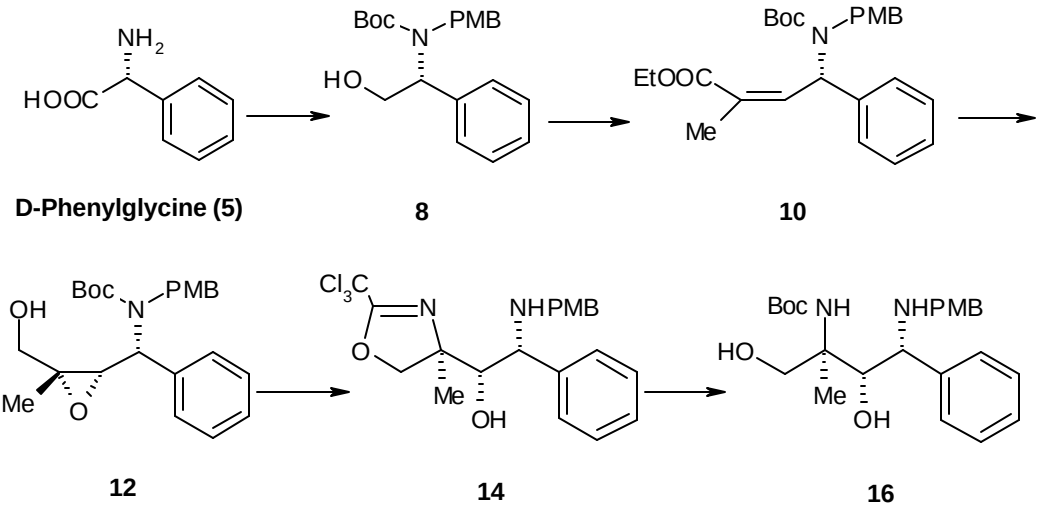
manzacidin B (2)



9-*epi*-manzacidin B methyl ester (4)

We began the synthesis of **4** from D-phenyl glycine (**5**) which was reduced with $NaBH_4/I_2$ followed by the protection of the amine functionality with PMB and Boc groups gave **8.** Alcohol **8** was oxidized and the resulting aldehyde then subjected to modified Horner-Emmons olefination conditions using Still's reagent, bis(trifluoroethyl)-2-phosophonopropionate ethyl ester to give the (Z)-olefin **10**. Compound **10** was then reduced with DibaI-H at −78 °C to *cis*-γ-amino allylic alcohol **11**. The *m*-CPBA epoxidation of **11** at −10 °C afforded the *syn* epoxide **12**. Our next aim was to introduce the amine group regioselectivity at C-2, for which Hatakeyama's method was employed. Thus, treatment of **12** with CCl_3CN/DBU followed by intramolecular epoxide-opening reaction catalyzed by BF_3:OEt_2 at −25 °C afforded the oxazoline **14**. Oxazoline **14** was then converted in to alcohol **16** by acid hydrolysis followed by *tert*-butoxy carbonylation (Scheme 1).

Scheme 1



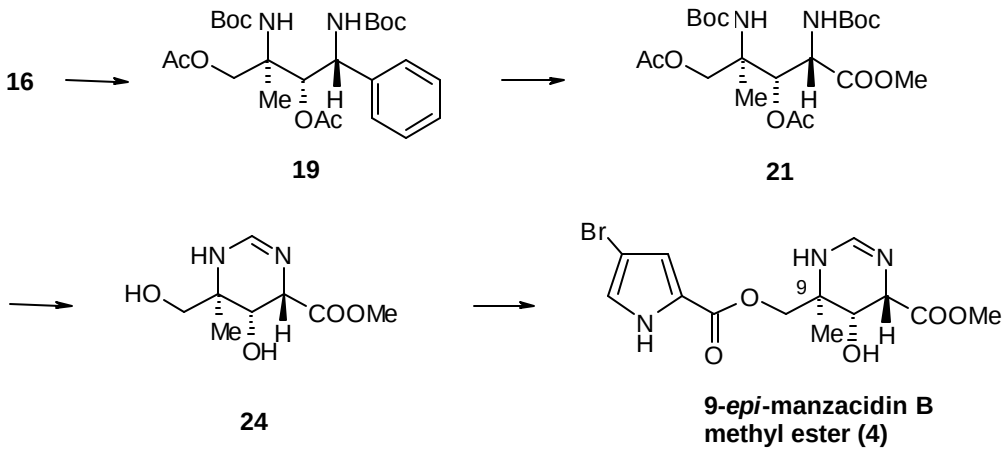
D-Phenylglycine (5) **8** **10**

12 **14** **16**

Our next concern was the oxidation of phenyl group in **16** to acid. For this purpose, the PMB group was first deprotected and the resulting free amine was then protected with $(Boc)_2$ to give **18**. The two hydroxyl groups in compound **18** were protected with Ac_2O to give **19**. Compound **19** on oxidation with $RuCl_3/NaIO_4$ followed by esterification with CH_2N_2 afforded **21**. The deprotection of acetate functionalities from compound **21** with K_2CO_3/MeOH resulted in the formation of the expected lactone **22**. Construction of the tetrahydropyrimidine ring was performed by successive treatment of **22** with (i) TFA and (ii) methyl orthoformate to give the acid which was purified after the esterification with

CH_2N_2. Completion of the synthesis now required esterification of the bromopyrrole carboxylate with **24** . Thus, treatment of **24** with NaH/trichloroacetylbromopyrrole(**25**) in DMF at room temperature to give the required compound **4** (Scheme 2).

Scheme 2



CHAPTER I

Synthesis of Oligosaccharides of Motifs D and E of *M. tuberculosis*

Introduction

The social and economical burden due to mycobacterial diseases such as leprosy and tuberculosis are of unprecedented nature particularly as it relates to developing countries.[1] In spite of enormous efforts to develop anti-infective drug molecules, tuberculosis still constitutes the leading killer disease.[2] Seven million new cases and three million deaths occur every year due to tuberculosis, and with AIDS becoming epidemic in many developing countries; this mortality figure is bound to increase at an alarming rate.[3] After antibiotics to treat tuberculosis became widely available in the 1950s, it was believed that the disease would eventually be eliminated. But *M. tuberculosis,* the organism that causes the disease, has proven to be very resilient. The specter of a worldwide resurgence of tuberculosis (TB) and its drug resistant forms has generated an intense effort to develop new and more effective therapeutic agents. Following lines highlight a brief view to this growing public health threat in a chemist's perspective with little insight into biology.

Introduction to *Mycobacteria*

The genus mycobacterium contains three important bacterial pathogens *Mycobacterium* (*M.*) *tuberculosis*, *M. leprae*, *M. avium*, and an important fast growing non-pathogenic research species *M. smegmatis.* Mycobacteria, although strictly speaking gram-positive, are readily distinguished from other bacteria by their unique cell wall, which confirms neither to the classical gram-positive nor gram-negative cell wall but includes features of both.[4] *M. tuberculosis* has a very slow growth cycle (dividing every 24 hours, compared with every 20 minutes for *Escherichia coli*), a complex cell envelope, the ability to colonize macrophages and the ability to remain quiescent and then reactivate decades later.[5]

M. tuberculosis is transmitted almost exclusively by air-borne route and " infectious unit" is a small bacillus-containing particle called a droplet nucleus. When a droplet nucleus containing one or two viable bacilli is inhaled by an immuno compromised person, it is deposited in the alveolar surface where the bacilli begin to multiply. Initially, the infecting organism meets only limited resistance from the host, as phagocytosis by alveolar macrophages has little effect on the bacilli, which continue to multiply intracellularly in the human host. After several weeks of infection, the number of leukocytes in the area decreases and the mononuclear cells predominate; these crowd together and contain pale, foamy, cytoplasmic material which is rich in lipid. The resulting unit is called a tubercle, the fundamental lesion of tuberculosis.[4]

Tuberculosis research highlights

The first milestone in tuberculosis research came way back in 1882 from a German microbiologist Robert Koch who announced that the disease TB is caused by a rod shaped bacteria when he managed to make the bacteria visible only through a staining procedure that was complicated to perform.[6] Since then, tuberculosis has claimed at least 200 million lives while scientists have been struggling to explain why *M. tuberculosis* is such a successful pathogen. Also, Koch attributed the complication to the "covering of the bacterium" and the likelihood that "the tubercle bacillus is surrounded with a special wall of unusual properties". After about 90 years, a group led by Patrick J. Brennan in a seminal work showed[7] the importance of the unique cell wall of *M. tuberculosis* and unraveled the complete fine structure of its cell wall.

The primary structure of the cell wall

The basic cell wall structure of *M. tuberculosis* does not differ from that of other nonpathogenic mycobacteria. It consists of three interconnected "macromolecules".[8] The

outermost of these are mycolic acids, unique 70-90 carbon branched fatty acids, which form outer lipid layer similar to, but differing from, the classical outer membrane of gram-negative bacteria. The mycolic acids are esterified to the middle component, arabinogalactan (AG), a polymer composed primarily of D-galactofuranosyl and D-arabinofuranosyl residues. AG is connected *via* a linker disaccharide phosphate to the 6-position of a muramic acid residue of the peptidoglycan. The peptidoglycan is the inner most of the three cell wall core macromolecules. The major polysaccharide components are arabinoglactan (AG) and a lipoarabinomannan (LAM) in which all of the galactose and arabinose residues are present in the furanose form.

Structural features of Lipoarabinomannan (LAM)

The LAM, which is an antigenic polymer, contains about 120 sugar residues 71 of which are arabinoses and 49 of which are mannoses. Distinct features are:

1. Within LAM, all *ara* are in furanose form and *man* are in pyranosyl form
2. The terminal end is a branched hexaarabinofuranoside with the structure [β-D-ara*f*-(1→2)-α-D-ara*f*)]$_2$-3,5-α-D-ara*f*- (1→5)-α-D-ara*f*, similar to that in AG
3. A linear β-D-ara*f*- (1→2)-α-D-ara*f*- (1→5)-α-D-ara*f*
4. *Ara* termini are extensively capped with man*p* residues
5. Mycolic acids are not present in LAM

Structural features of Arabinogalactan (AG)

Partial depolymerisation of the per-*O*-alkylated polysaccharide and analysis of the generated oligomers by GC-MS and FAB MS has established[7a] the fine structure of Arabinogalactan as depicted in figure 1. The AG, which is a structural polymer, contains approximately 100 sugar residues, 69 of which are arabinofuranoses and 31 of which are galactofuranoses. Salient features are:

1. Within AG, all *ara* and *gal* are in the furanose form[9]

2. The non-reducing termini of arabinan consist of a branched pentaarabinofuranosyl structure [β-D-ara*f*- (1→2)-α-D-ara*f*] $_2$-3,5-α-D-ara*f*- (1→5)-

3. The majority of the arabinan consists of 5-linked α-D-ara*f* residues with branching introduced by 3,5-α-D-ara*f* residues replaced at both branched positions with 5-α-D-ara*f*

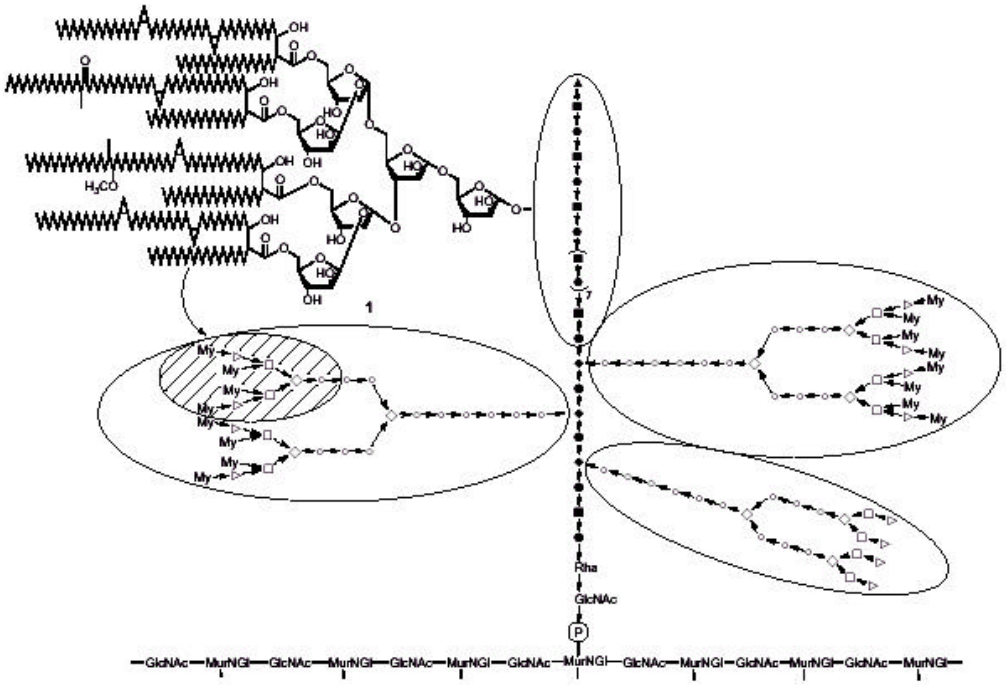


Fig. 1 Schematic diagram of the proposed illustration of the macro structural motifs of the cell wall arabinogalactan. My, Mycolic acid; (▼) t-β-D-Ara*f*; (□) 2-α-D-Ara*f*; (◇) 3, 5-α-D-Ara*f*; (▲) t-β-D-Gal*f*; (■) 6-β-D-Gal*f*; (●) 5-β-D-Gal*f*; (◆) 5,6-β-D-Gal*f*; GlcNAc, *N*-acetylglucosamine; Rha, rhamnose; MurNGl, *N*-glycolylmuramic acid.

4. The arabinan chains are attached to the galactan core through the G-5 of some of the 6-linked alternating 5- and 6- linked β-D-gal*f* moieties.

5. The galactan of AG is linked to the C-6 of some muramyl residues of peptidoglycan *via* the glycophosphoryl bridge L-Rha*p*- (1→3)-D-GlcNAc- (1→*p*)

6. The mycolic acids are located in clusters of four on the terminal pentaarabinofuranosyl units.

The major *ara*- containing degradation products were the hexaarabinofuranoside and linear disaccharide, α-D-ara*f*-(1→5)-D-ara*f*. Oligosaccharide fragments containing upto 23 *ara* residues were obtained by gentle acid hydrolysis of the per-*O*-methylated AG and all the major structural motifs of AG, namely motifs A-E were as represented in figure 2.

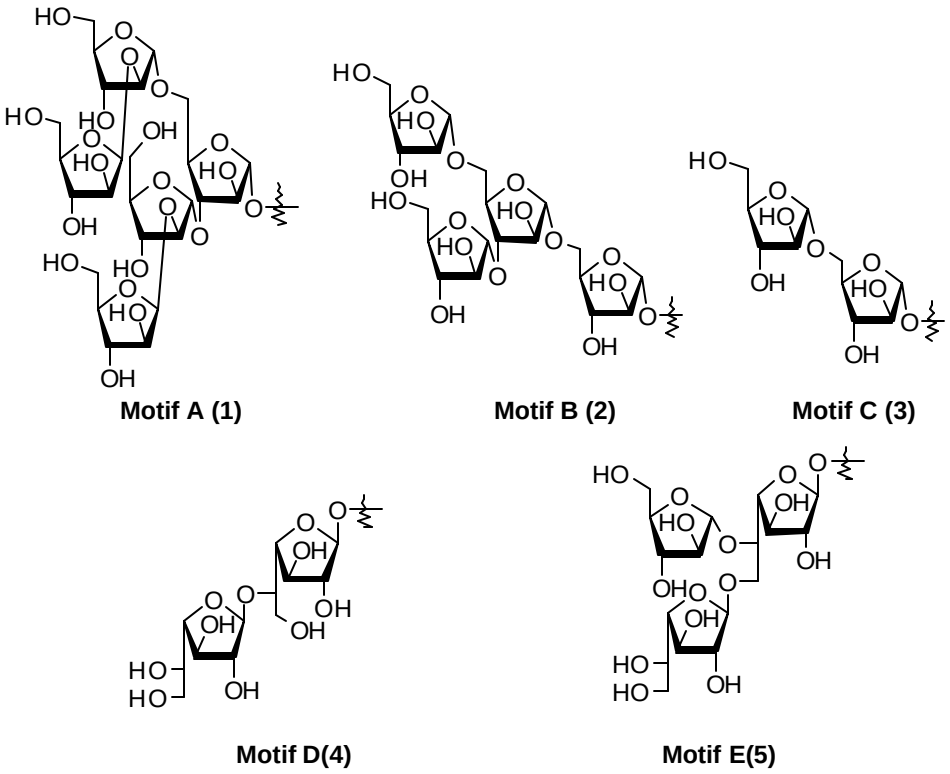


Figure 2: Five major Structural motifs A-E of AG.

Synthesis of these motifs provides tremendous opportunities to understand their role in the survival and pathogenicity of these organisms. The structural analysis revealed that motifs A, B, and C are composed of arabinofuranose units having subtle differences between them with respective *O*-glycosidic linkage. Structural motif A is significant due to the presence of two 1→2 *cis* linkages[7d], whereas structural motifs B and C account for the bulk of internal portions of the arabinan segments of the arabinogalactan and structural

motif D, composed of alternating 6-linked and 5-linked galactofuranosyl residues, is supported by the presence of the disaccharide, 6-*O*-β-D-galactofuranosyl-D-galactose, among the products of the degradation of AG. Motif E is unique in that it contains both arabinofuranose and galactofuranose residues and both 5- and 6- positions of the galactofuranose component are linked with arabinofuranosyl and galactofuranosyl residues, respectively.

A major impetus for the study of the cell wall core molecule AG arises from the need for new drugs against *M. tuberculosis* and *M. avium*.[10] AG of *M. tuberculosis* has special interest for two fundamental reasons, 1) it appears to be essential for viability[11] and 2) three out of the four sugars of which it is composed, D-Ara*f*, D-Gal*f* and L-Rha*p* are not found in humans. Thus any of a score or more of enzymes involved in the formation of sugar donors and their polymerization are potential drug targets. The isolation and expression of the genes for these enzymes is a high research priority. Inhibitors of the resultant enzymes can be obtained by using "high through put" screens and by enzyme characterization (ultimately X-ray analysis) and the subsequent design of "rational" inhibitors.

The terminal ends of both AG and LAM are capped with a pentaarabinofuranosyl motif A, which is linked to the remainder of the polymer *via* a α-(1→5)-linked linear chain of arabinofuranosyl residues. This motif A serves as an attachment site for other functionalities present in the cell wall. These groups are located at the periphery of the cell wall complex and are therefore interface between the microorganisms and the environment.[2a] In LAM, the primary hydroxyl groups in motif A are often substituted with mannopyranosyl oligosaccharides, which have been implicated in the initial stages of infection through their interaction with human mannose binding proteins.[12] In the AG, the

same hydroxyl groups are esterified with mycolic acids, branched, long chain fatty acids.[8] Through the tight packing of the alkyl chains, the mycolic acids form a protective hydrophobic façade that in some cases is nearly crystalline.[13]

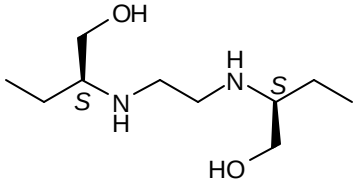
The peptidoglycan-bound arabinogalactan of a virulent strain of *M. tuberculosis* was per-*O*-methylated, partially hydrolyzed with acid and the resulting oligosaccharides were separated by high pressure liquid chromatography and the structures of all those 43 constituent oligosaccharide fragments were identified by exhaustive NMR studies.[7d] Based on availability of sugars and number of glycosyl linkages, the fine structure of AG polymer has been characterized.[7a] It has been proven that arabinosyl residues are responsible for the antigenicity of AG, and that serological activity resides largely in fraction containing 2-linked arabinosyl residues.[14] Thus it is logical to speculate that part, or all, of structural motif A is the major humoral immunological epitope of arabinogalactan and, consequently, of whole mycobacteria.[15] Monoclonal antibodies raised against lipoarabinomannan also react with purified cell walls,[16] suggesting an arabinose-containing epitope common to lipoarabinomannan and arabinogalactan.

As the distal ends of both polymers (AG and LAM) are terminated with motif A, this motif is believed to play critical role in both infection by and survival of the organism in the human host.[8,13b] Ethambutol (**6**), one of the drugs currently used to treat tuberculosis, has recently been shown to be an arabinosyltransferase inhibitor.[17] Thus, new compounds that act, as does Ethambutol (**6**), in preventing complete arabinan biosynthesis are likely to be potent antimycobacterial agents. Furanosyl oligo- and polysaccharides are not found in mammalian glycoconjugates and therefore inhibitors of the biosynthetic pathways leading to their formation are particularly attractive drug candidates.

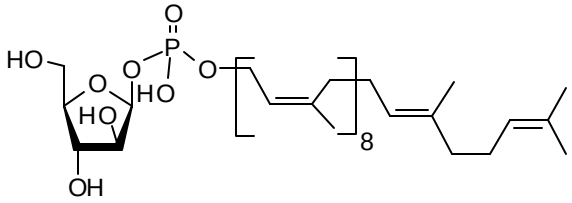
Attention is now being focused on understanding mycobacterial cell wall biosynthesis[18] but there is still much to be learned concerning the details of this process,

especially the assembly of the arabinan component. Decaprenol arabinofuranosyl phosphate (**7**) has been identified as the source of the arabinose in mycobacteria[7b,19] and there is presumably an array of glycosyl transferases that use **7** and various oligosaccharide acceptors to produce the glycan. None of these putative arabinosyltransferases have yet been isolated or purified;[18] however, an assay for their activity using mycobacterial membrane preparations as the enzyme source has been developed.[17a]

The transfer of arabinose from **7** to an arabinofuranosyl dimer and trimer has been evaluated using this assay; the effect of the aglyconic group (*e.g.* methyl vs. octyl) was also investigated.[20] However, a major limiting factor in these studies is the lack of availability of discrete oligosaccharide structures that can be used for unraveling the



Ethambutol (6)



b**-D-Arabinofuranosyl-1-mono phosphoryldecaprenol (7)**

biosynthetic pathways, including the isolation and purification of the enzymes and the development of individual assays for their activity. Such compounds are most easily obtained *via* chemical synthesis but synthetic studies are rare. Thus the current endeavor stands as a pivotal point in this direction.

Introduction to *O*-glycosylation methods

In nature carbohydrates are present as *C*-glycosides, *O*-glycosides, and *N*-glycosides. Of all the three *O*-glycosides are the most important class of compounds. *O*-glycosides are formed from the condensation of the anomeric hydroxyl group of sugar

with the hydroxyl group of another molecule. The latter can be a simple alcohol, a hydroxylated amino acid, another sugar or a more complex molecule.

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

Stereoselective formation of *O*-glycosidic bond is one of the most important problems in carbohydrate chemistry.[21] Recently, much effort has been devoted to the stereoselective synthesis of glycopyranosides and several efficient methods have been developed by the appropriate combination of sugar donors and activators (figure 3).[22]

Despite significant advances in the synthesis of complex oligosaccharides, construction of *O*-furanosidic linkages remains a challenging task.[23] To address at the stereoselectivities, it is advantageous to clarify glycosylation methods as 1,2-*cis*- and 1,2-*trans*- furanosides instead of a- and ß- glycosides as the latter would be confusing. The

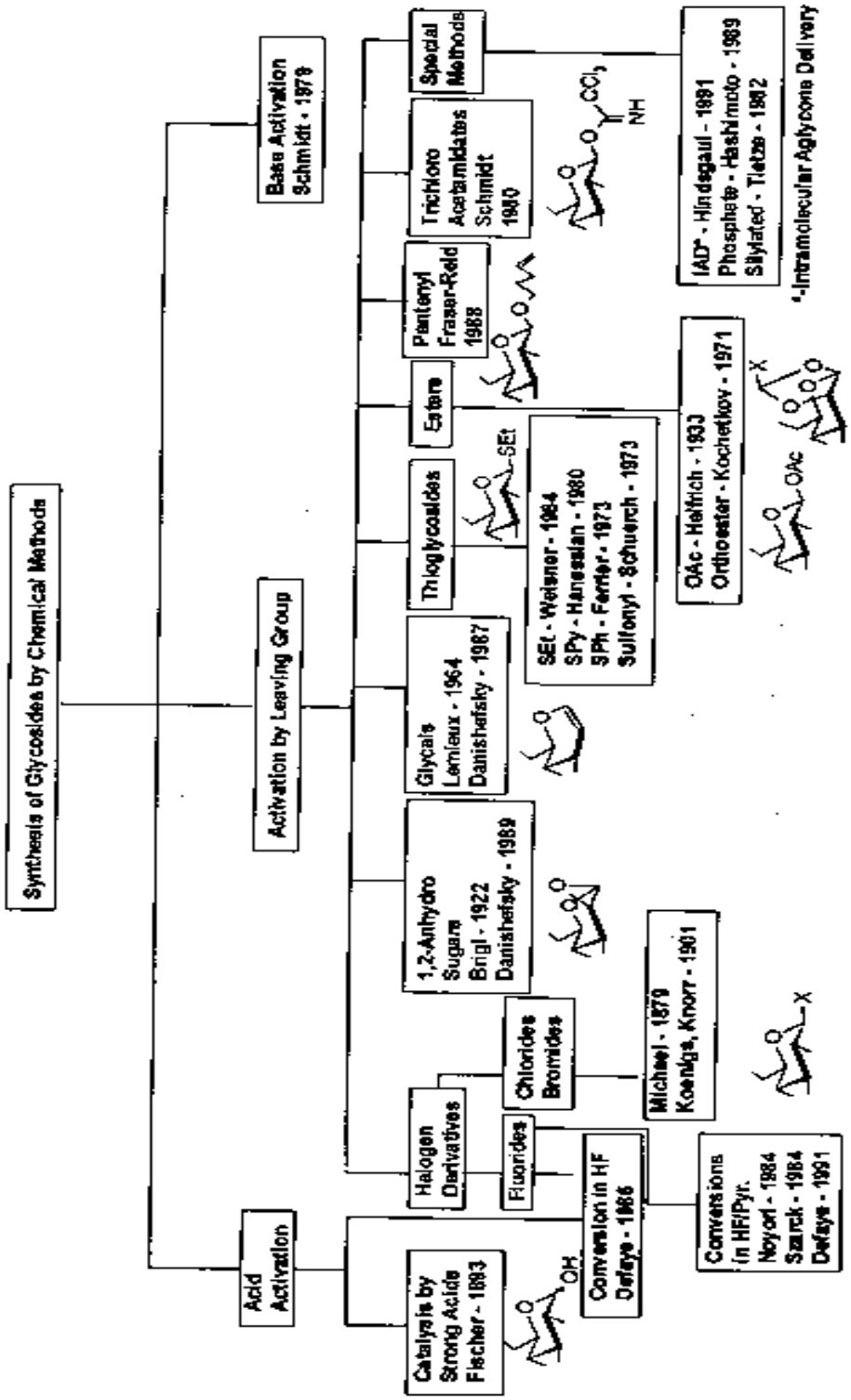


Figure 3: Development of Pyranoside Glycosylation Methods

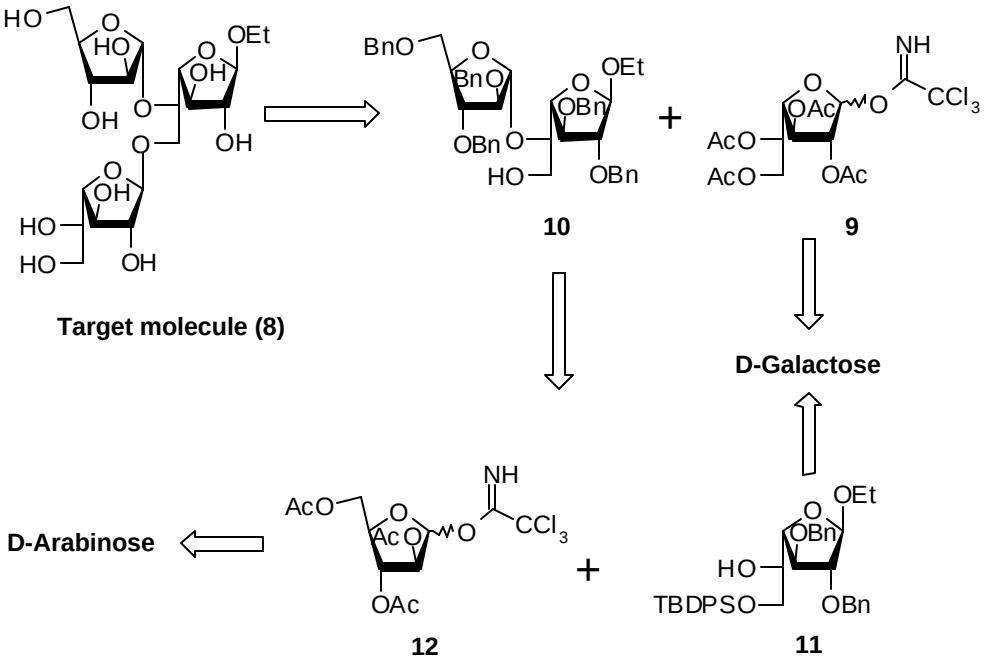
chemical synthesis of oligosaccharides containing furanose residues has been relatively unexplored until recently.[24]

Synthesis of the sugar components of AG of *M. tuberculosis* has been a topic of immense activity[25-27]. The first major contribution appeared from our laboratory and reported the synthesis of the pentaarabinofuranoside of motif A.[25] Subsequently Lowary *et al*[26] reported a series of publications on motifs A, B, and C and studied their ring conformation by NMR techniques. Very recently Prandi *et al*[27] also published the synthesis of the pentaarabinofuranosyl structure of motif A.

Arabinogalactan (AG) is one of the major polysaccharide segments of *M. tuberculosis* cell wall. Its usefulness in developing new therapeutic agents against multi-drug resistant strains and also a single dose drug for dreadful tuberculosis has been well understood. Given the structural complexity in AG, involving mycolic acids, arabinose, galactose and mannose, many glycosyl transferases must be involved in its formation. Synthesis of all the possible glycosyl motifs provides tremendous opportunities to understand their role in the survival and pathogenecity of these organisms.[28] Synthesis of oligosaccharide fragments from *Mycobacterium* species is a topic of interest.[25,29] This chapter deals with our synthetic endeavor that resulted in the first synthesis of oligosaccharides of motifs D and E of arabinogalactan present on the *M. tuberculosis* cell wall.

Synthesis of Ethyl 5-*O*-(a-D-arabinofuranosyl)-6-*O*-(ß-D-galactofuranosyl)-ß-D-galactofuranoside (8) Present in Motif E

Motif E is novel and unique because of the presence of both arabinofuranose and galactofuranose residues in its structural frame work. In addition, the oligosaccharide structure of motif E is sterically more demanding because both the 5- and 6- positions of the reducing galactofuranose component are linked with arabinofuranosyl and galactofuranosyl residues respectively. The synthetic strategy utilized for the preparation of ethyl 5-*O*-(a-D-arabinofuranosyl)-6-*O*-(ß-D-galactofuranosyl)-ß-D-galactofuranoside (**8**) belonging to motif E is based on the stepwise assembly of three sugar components as shown in the retrosynthetic analysis (scheme 1).

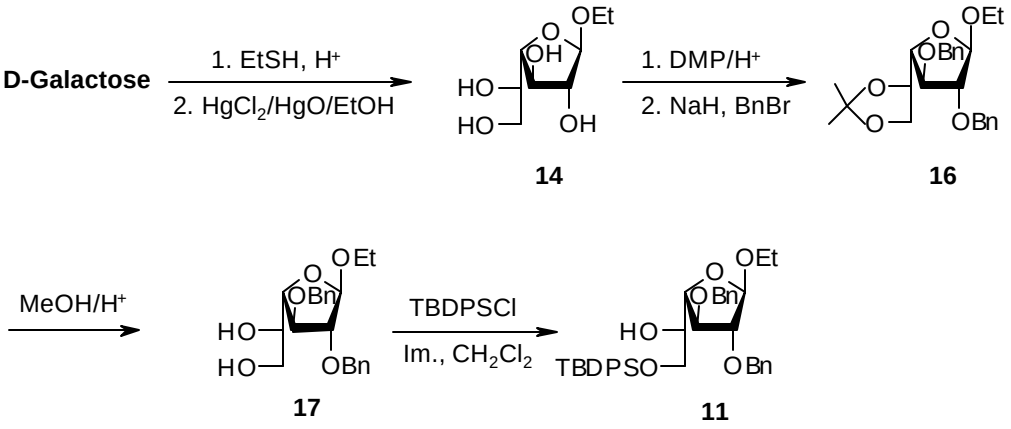


Scheme 1: Retro Synthetic Analysis

Our first concern was the preparation of the aglycone (**11**) in which both 2- and 3-positions were blocked with permanent protecting benzyl ethers while the 6-position was blocked with a temporary protecting TBDPS ether. This preparation was accomplished by a protocol reported by Thiem *et al.*[30] Thus, D-galactose was treated with ethane thiol in the presence of conc. HCl to give the diethyl dithioacetal derivative of galactose (**13**), which on the exposure to $HgCl_2$/HgO in ethanol gave the ethyl ß-D-galactofuranoside (**14**) in 70% yield. Compound **14**, on treatment with 2,2-dimethoxy propane in acetone in the presence of cat. *p*TSA followed by benzylation using NaH/BnBr in DMF afforded compound **16**. In the ^{1}H NMR spectrum of **16**, the characteristic singlets at 1.35 and 1.43 ppm due to isopropylidene group whereas a multiplet at 4.4-4.65 ppm due to benzylic protons were observed. A singlet at 5.05 ppm was attributed to the anomeric proton. Rest of the spectrum was in complete agreement with the assigned structure. Cleavage of 5,6-isopropylidene group in compound **16** with *p*TSA in methanol afforded the diol **17** whose

structure was confirmed by the ^1H NMR spectrum in which singlets due to isopropylidene were absent. Selective protection of the OH group at C-6 in compound **17** was carried out with TBDPS-Cl/imidazole in CH_2Cl_2 to provide the 6-*O*-TBDPS derivative **11** in 90% yield (scheme 2).

Scheme 2

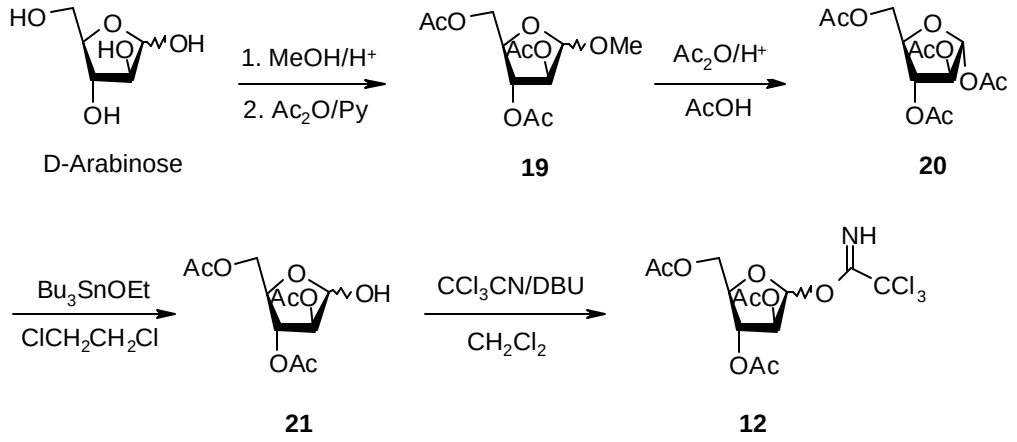


The singlet at 1.05 ppm due to *tert*-butyl group of TBDPS was identified in the ^1H NMR spectrum of **11** while the rest of the spectrum was in complete agreement with the assigned structure. Further confirmation of **11** came from its ^{13}C NMR and DEPT spectral data. For example, in the ^{13}C NMR spectrum, characteristic carbons of TBDPS group were identified at 19.1 and 26.8 ppm while ß-anomeric carbon was located at 106 ppm. In addition, FAB MS (Na) analysis of **11** indicated single highest mass peak at m/z: 649 accounting for M^++23 while elemental analysis was satisfactory (Calcd. for $C_{38}H_{46}O_6Si$: C, 72.84; H, 7.34. Found: C, 73.16; H, 7.55).

The glycosylating agent **12** was prepared as follows. Methyl D-arabinofuranoside (**18**) was obtained from D-arabinose with methanolic HCl at room temperature. Compound **18** upon treatment with Ac_2O, pyridine afforded methyl 2,3,5-tri-*O*-acetyl-D-arabinofuranoside (**19**). Subsequently, compound **19** was acetolysed using Ac_2O/AcOH/

H_2SO_4 to afford per-*O*-acetyl D-arabinose (**20**).[31] Selective deprotection of 1-*O*-acetyl group of **20** was achieved using Bu_3SnOEt in refluxing $ClCH_2CH_2Cl$. The resulting lactol **21** was then exposed to CCl_3CN-DBU-CH_2Cl_2 at 0 ^oC to afford the corresponding arabinosyl trichloroacetimidate derivative **12** as the glycosyl donor (scheme 3).

Scheme 3



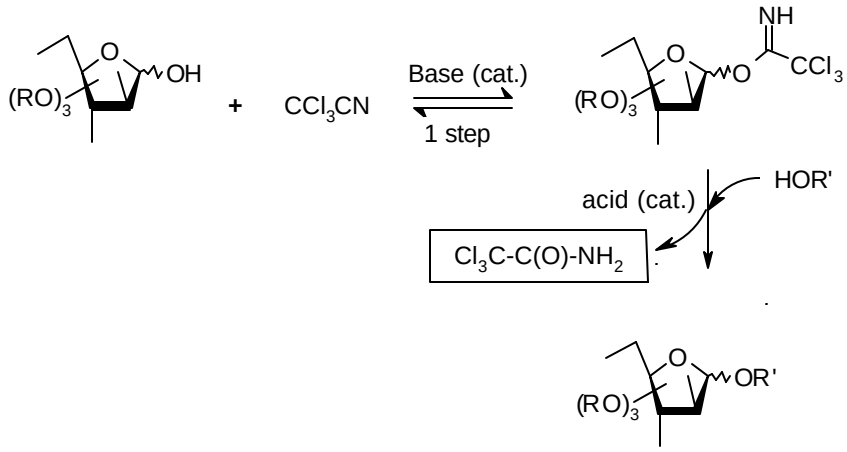
A Short Account of *O*- Glycosyl Trichloroacetimidate Donors

Glycosyl imidate esters, particularly the trichloroacetimidates introduced by Schmidt[32] have proved to be more versatile glycosylating agents because of their higher reactivity, and applicability toward different acceptors under mild conditions.

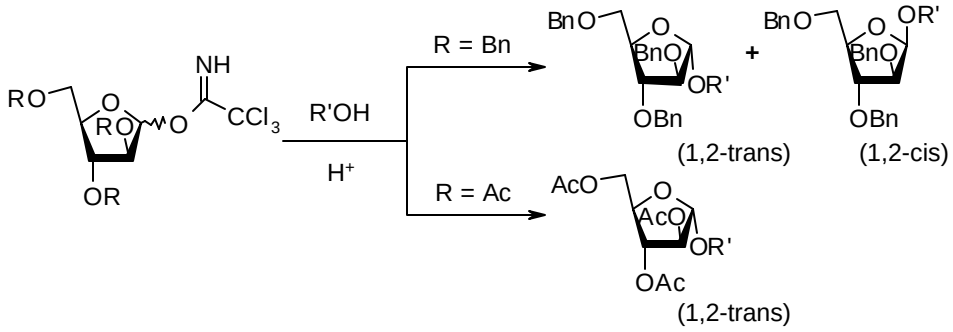
The base- catalyzed generation of O- glycosyl trichloroacetimidates and ensuing acid- catalyzed glycosylation have become a very competitive alternative to direct, often uncontrolled acid- catalyzed transformation of sugars into glycosides (Fischer- Helferich method) or to glycosyl halide formation for the activation step, which requires at least equimolar amounts of promoter system for the glycosylation step (Koenigs- Knorr method or variations). In addition, the trichloroacetimidate method may be readily adapted for large- scale preparations. Therefore, after base- promoted trapping of anomeric O-

glycosyl trichloroacetimidates (first step), mild acid treatment in the presence of acceptors, leading to formation of glycosides in an irreversible manner (second step) constitutes the simple means of catalysis desired for the efficient glycosylation method (scheme 4).

Scheme 4



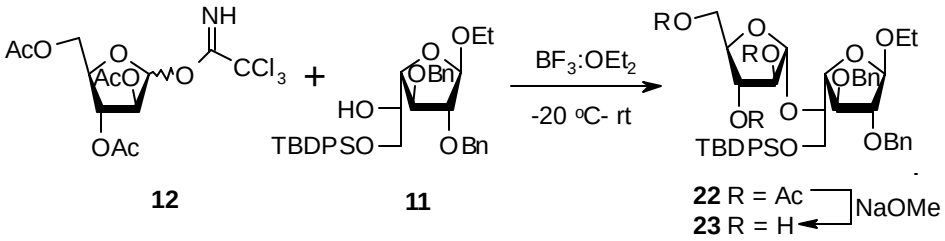
Glycosyl trichloroacetimidates undergo nucleophilic displacement reactions with predictable stereochemical outcome under mild conditions. Those with non- participating groups at G-2 react at low temperatures in the presence of BF_3:OEt_2 catalyst with inversion at C-1. In this way 1,2 -cis compounds may be prepared from sugars. Sugar trichloroacetimidates with participating groups at C-2 would yield 1,2 -trans compounds as expected (scheme 5).



Scheme 5: Effect of the Neighbouring Group in Glycosidation

Thus the *O*- glycosylation of aglycone **11** with **12** was carried out[32] in the presence of BF_3:OEt_2 and 4A° MS powder in CH_2Cl_2 at −20 °C followed by stirring at room temperature for 2 h to afford the disaccharide **22** (contaminated with **11** as a minor impurity), which was isolated and characterized after Zémplen deacetylation (scheme 6).[33]

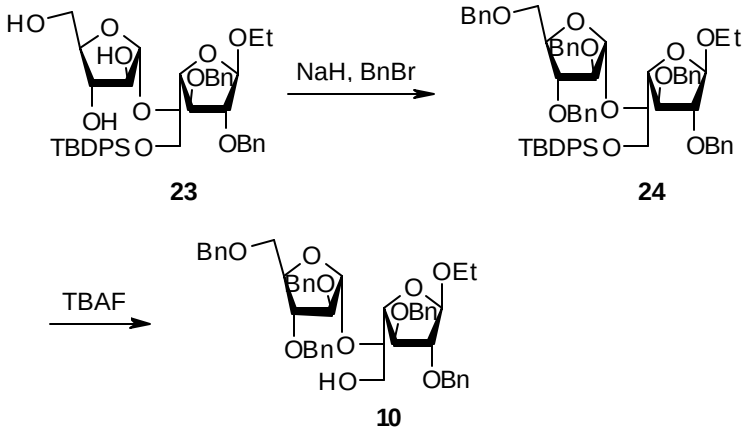
Scheme 6



The 1,2-trans linkage of disaccharide of **23** was evidenced from its ^1H and ^{13}C NMR spectral studies. For example, in the ^1H NMR spectrum of **23**, the 1,2-trans linked protons were observed at 5.01 and 5.21 ppm as characteristic singlets. The location of the signal due to C-1' at 104.5 ppm in the ^{13}C NMR spectrum of **23** confirmed the a-linkage at the newly formed glycosidic bond.[34] In addition, FAB MS (Na) with highest mass peak at m/z: 781 (M^++23) and satisfactory elemental analysis supported the structure of **23**.

The disaccharide **23** was *O*-benzylated using NaH, BnBr in DMF at room temperature for 3 h to afford the penta *O*-benzyl derivative **24**. The structure of **24** was thoroughly characterized by high-resolution ^1H, ^{13}C NMR and DEPT spectral data. Further, FAB MS (Na) [(M^++23) at 1051] and elemental analysis (Calcd. for $C_{64}H_{72}O_{10}Si$: C, 74.7; H, 7.0. Found: C, 74.4; H, 7.14) supported the structure of *O*-benzylated product. Cleavage of TBDPS ether in compound **24** was affected with TBAF (1M solution in THF) to afford the aglycone (**10**) containing the 6-hydroxyl free in 92% yield (scheme 7). In the ^1H NMR spectrum of **10** absence of the peaks due to TBDPS group were clearly noted. In addition, FAB MS and elemental analysis also supported the structure of **10**.

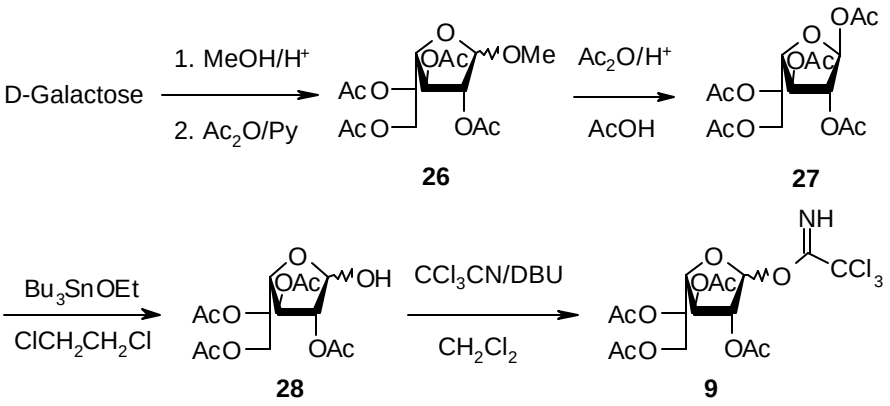
Scheme 7



Synthesis of Target Trisaccharide (8)

Our next endeavor was the crucial *O*-glycosylation of aglycone **10** with galactofuranosyl residue in order to complete the synthesis of the trisaccharide (**8**). For this purpose the trichloroacetimidate of galactofuranose (**9**) was choosen as the glycosyl donor, which was obtained from D-galactose (scheme 8).[35]

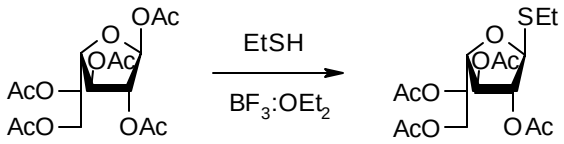
Scheme 8



The coupling reaction between aglycone **10** and glycosyl donor **9** was attempted in the presence of 4A^{o} MS powder with BF_3:OEt_2 as an activator in CH_2Cl_2 at $-20\ ^{o}C$. The product isolated from the reaction mixture was the starting material **10** and the hydrolyzed product **28**. No other product with appreciable yield resulted. When the reaction was

conducted at 0 ^{o}C and later at room temperature, no new product was formed as observed in TLC. This indicated that the glycosyl donor **9** was not sufficiently reactive to couple with sterically crowded aglycone **10**. This prompted us to resort to other *O*-glycosylating protocols. The one choosen for the investigation was S-Et mediated glycosylation. Thus, S-ethyl derivative (**29**) was synthesized from **27** in one step (scheme 9).[36]

Scheme 9

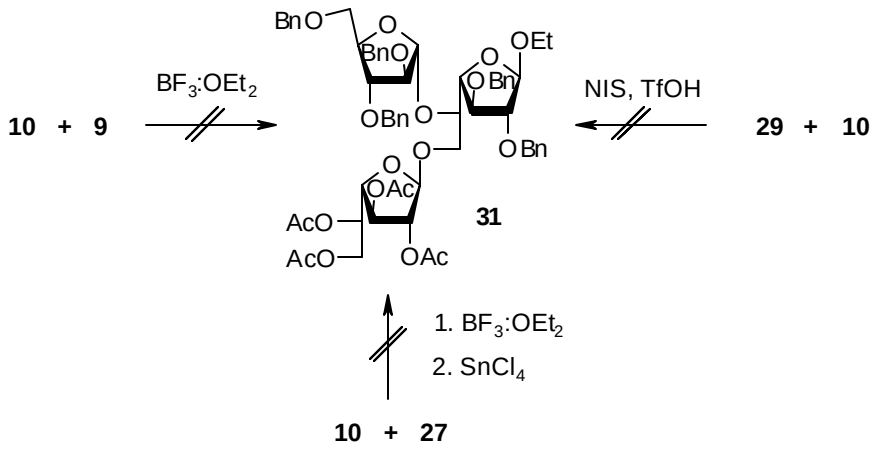


27 **29**

The aglycone **10** was treated with **29** in the presence of *N*-iodo succinimide (NIS) and trifluoromethanesulfonic acid (TfOH).[37] Unfortunately this reaction also failed perhaps due to insufficient reactivity of **29**. In addition, we also attempted the glycosylation under classical Helferich conditions[38], but this attempt also met with failure.

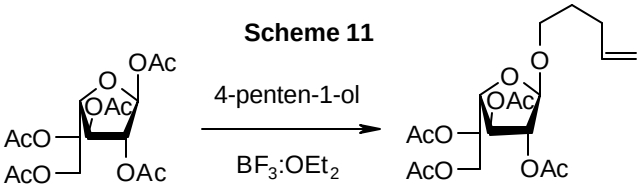
Thus, the synthesis of trisaccharide turned out to be a Herculean task as all our efforts to install the third galactofuranose residue at C-6 of **10** *via* Schmidt's trichloroacetimidate method, SEt mediated glycosidation, and classical Helferich reaction failed to materialise (scheme 10).

Scheme 10



Finally the chemistry that worked for the trisaccharide synthesis was Fraser-Reid's *n*-pentenyl mediated *O*-glycosidation approach.[39] The synthesis of pent-4-enyl-2,3,5,6-tetra-*O*-acetyl- ß-D-galactofuranose **30** was accomplished in one step by treating penta-*O*-acetyl- ß-D-galactofuranose (**27**)[35] and 4-penten-1-ol with a catalytic amount of BF_3:OEt_2 and 4A° MS powder in CH_2Cl_2 at room temperature (scheme 11).The structure of compound **30** was confirmed by the ^1H and ^{13}C NMR spectral analysis.

Scheme 11

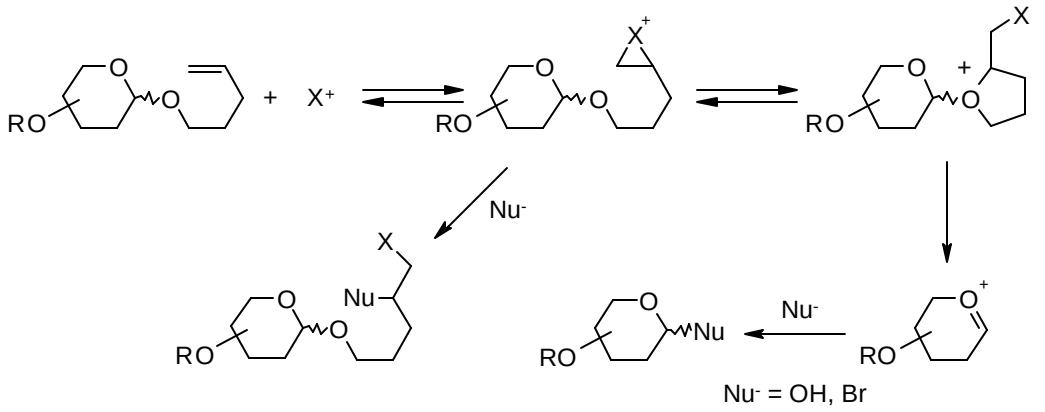


27 **30**

Before we discuss the approach it is pertinent to mention the concepts of *n*-pentenyl mediated *O*-glycosylation reaction.

Salient Features of Fraser-Reid's Glycosylation Method

The serendipitous discovery of n-pentenyl leaving group strategy by Fraser-Reid's group[39] opened a new era in the art of oligosaccharide synthesis. n-Pentenyl glycosides (NPGs) are readily obtained by standard glycosidations including Fischer's direct method and although they are stable to a wide range of reagents, they are easily activated by treatment with a halonium ion (scheme 12). The effect of some of the commonly used protecting groups upon glycoside reactivity has been probed with these substrates, and the "armed/disarmed" strategy for oligosaccharide assembly emanated directly from these investigations. Thus esters disarm electronically, while benzylidene and isopropylidene groups disarm by torsional strain.

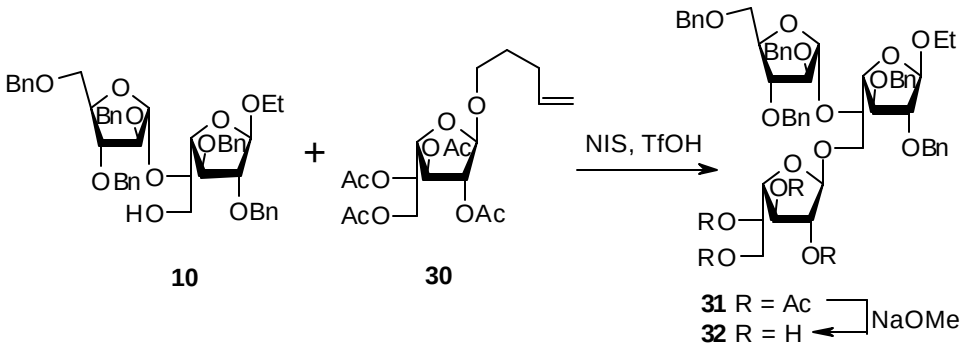


Scheme 12: n-Pentenyl mediated Glycosylation method

Most commonly used activators for NPGs are NBS, IDCP (Iodonium dicollidine per chlorate). However, IDCP is not commercially available, a circumstance which compromised its attractiveness. Later on alternative promoters were sought that would, among other things not require laboratory preparation. A non-nucleophilic counter anion was essential, and trifluoro methanesulfonate (triflate) was preferred and it was found that NIS reacted with TfOH to generate a ready source of iodonium ion solved the problem, as both NIS and TfOH are commercially available. A general drawback of this protocol is the use of strong acid and thus care should be taken while planning and executing the glycosylation reaction.

The coupling reaction[39] between **10** and **30** in the presence of NIS, TfOH (cat.) and4A^{o} MS powder in CH_2Cl_2 at room temperature gave the crude trisaccharide **31** (contaminated with minor quantity of aglycone **10**), which after Zémplen deacetylation provided the pure penta-*O*-benzylated derivative **32** (scheme 13).

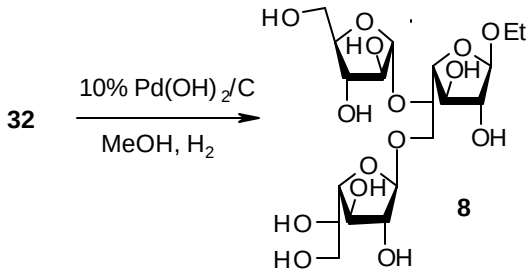
Scheme 13



The high-resolution ^{1}H NMR spectrum of **32** showed three anomeric protons at 4.84, 4.96 and 5.17 ppm as distinguishable singlets. As we have already fixed stereochemistry at anomeric positions in the disaccharide as 1,2-trans linkages, the remaining singlet indicates the newly formed galactofuranosyl linkage as 1,2-trans (β-linkage) configuration. This was further confirmed by the ^{13}C NMR and DEPT spectral studies wherein three anomeric carbons were identified[40] at 105.3, 106.8 and 107.7 ppm. In addition, FAB MS (Na) [(M^{+}+23) at 952] and elemental analysis (Calcd. for $C_{54}H_{64}O_{15}$: C, 68.06, H, 6.72. Found: C, 68.35, H, 6.95) supported the structure of **32**.

Finally compound **32** was subjected to exhaustive hydrogenolysis over 10% $Pd(OH)_2/C$ in methanol at normal temperature and pressure (ntp) to provide the target trisaccharide (**8**) (scheme 14).

Scheme 14

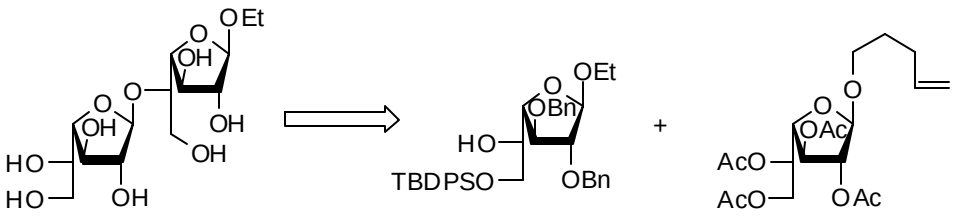


The high-resolution ^{1}H, ^{13}C NMR and DEPT spectral data established the structure of **8**. For example, in the ^{1}H NMR spectrum of **8**, resonances typical of anomeric protons (1,2-trans) were identified at 4.88, 4.92 and 5.10 ppm as three singlets while rest of the spectrum was in complete agreement with the assigned structure. The chemical shifts of anomeric carbons were observed at 107.6, 108.6 and 109.6 ppm in its ^{13}C NMR spectrum. Further, FAB MS (Na) analysis of **8** showed the mass m/z at: 525 accounting for M^{+}+23 while elemental analysis (Calcd. for $C_{19}H_{34}O_{15}$: C, 45.42, H, 6.77. Found: C, 45.13, H, 7.0) was satisfactory. Compound **8** constitutes the oligosaccharide segment of motif E of arabinogalactan present in *M. tuberculosis*.

Synthesis of Ethyl 5-*O*-(ß-D-galactofuranosyl)-ß-D-galactofuranoside (33) Present in Motif D

After successfully completing the synthesis of the trisaccharide segment (**8**) belonging to motif E, we undertook the synthesis of ethyl 5-*O*-(ß-D-galactofuranosyl)-ß-D-galactofuranoside (**33**) representing motif D of *M. tuberculosis* (scheme 15). This disaccharide serves as an anchor that couples arabinan and galactan chains of arabinogalactan.

Scheme 15

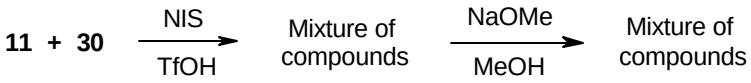


Target Molecule (33) **11** **30**

Having both the aglycone **11** and glycosyl donor **30** in hand, the coupling reaction was carried out in the presence of NIS, TfOH (cat.) and 4A° MS powder in CH_2Cl_2 at

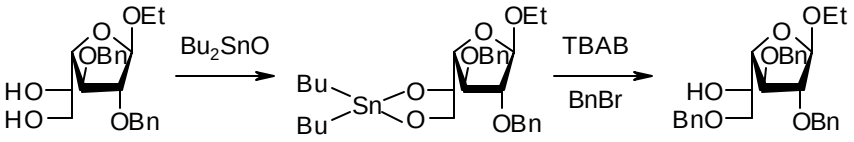
room temperature. Unfortunately the reaction showed on TLC a large number of compounds whose separations into individual product was not amenable. We attributed the formation of mixture of compounds to the presence of TBDPS group which might have cleaved during the reaction. The free OH group will undergo further *O*-glycosidation reaction (scheme 16).

Scheme 16



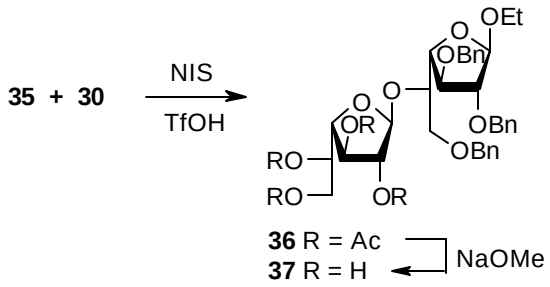
Thus, protection of 6-OH group with stable benzyl ether was considered. In order to selectively block the primary OH group in the presence of secondary OH group, the alkylation of stannylacetal strategy was adapted.[41] Accordingly, compound **17** and Bu_2SnO were heated under reflux in toluene for 6 h to give the dibutyltinacetal derivative (**34**) to which was subsequently added TBAB and BnBr. After 24 h of refluxing and workup the tri-*O*-benzyl derivative **35** (scheme 17) was isolated and whose structure was supported by the ^{1}H and ^{13}C NMR spectroscopic data.

Scheme 17



The coupling reaction between **35** and **30** mediated by NIS and TfOH (cat.) in CH_2Cl_2 at room temperature gave the disaccharide (**36**) which was isolated after Zémplen deacetylation reaction to give **37** (scheme 18).

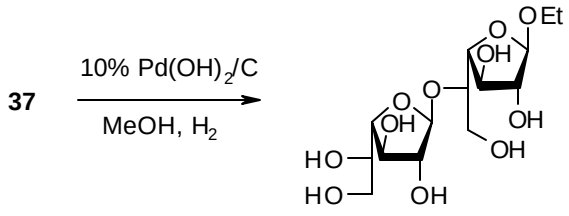
Scheme 18



The ^{1}H and ^{13}C NMR spectroscopic studies of **37** revealed the assigned structure. For example, in the ^{1}H NMR spectrum of compound **37**, signals due to two anomeric protons were observed at 5.0 and 5.34 ppm as singlets. The ^{13}C NMR spectrum of **37** showed anomeric carbons at 104.9 and 106.1 ppm, thus confirming the ß-linkage (1,2-trans) at the newly formed glycosidic bond.

Finally, compound **37** was exhaustively debenzylated by hydrogenolysis over 10% $Pd(OH)_2/C$ in methanol at normal temperature and pressure to afford the target disaccharide **33** (scheme 19). The structure of compound **33** was fully analyzed by high-resolution ^{1}H and ^{13}C NMR spectroscopic data coupled with satisfactory elemental analysis.

Scheme 19



33

Conclusion

For the first time, syntheses of oligosaccharides of motifs D and E of arabinogalactan complex present in *M. tuberculosis* cell wall have been achieved.[42] The arabinogalactan caused profound interest for two fundamental reasons, (1) it is essential for viability and (2) three out of four sugars, namely *araf*, *galf* and *rhamp*- are not found in human beings. Only recently it has been reported that (S, S)-Ethambutol, the drug of choice for TB since 35 years, is involved in the inhibition of biosynthetic pathway of arabinan, thus establishing it to be a valuable target for the discovery of new drugs.[17] The present synthesis opens a new vista in this direction.

Ethyl ß-D-Galactofuranoside (14)

To a solution of D-galactose (25.0 g, 138.9 mmol) in conc. HCl (20 ml) was added ethanethiol (25.6 ml, 347.2 mmol). The mixture was stirred vigorously at room temperature. After 30 min. a heavy crystalline mass was formed, cooled in ice-bath, filtered, washed with water and dried to afford **13** (25 g, 63 %). The above product (**13**) (20 g, 69.9 mmol), $HgCl_2$ (37.9 g, 139.8 mmol) and HgO (30.2 g, 139.8 mmol) in ethanol (400 ml) were stirred at room temperature for 2 h. The reaction mixture was filtered, and pyridine (10 ml) added to the filtrate. The precipitate was filtered, concentrated and recrystallized from 2-propanol to give **14** (10.2 g, 70 %).

m.p.: 87-88 ^{o}C; lit[30] m.p.: 86 ^{o}C;

[a] $_{D}$ = – 98 ^{o} (c 1, H_2O), lit[30] **[a]** $_{D}$ = – 97.5 ^{o} (c 1, H_2O**)**.

Ethyl 2,3-Di-*O*-benzyl 5,6-*O*-isopropylidene -ß-D-galactofuranoside (16)

A solution of ethyl ß-D-galactofuranoside (**14**) (10 g, 48.1 mmol), 2,2-dimethoxy-propane (11.8 ml, 96.2 mmol) and *p*TSA in acetone (100 ml) was stirred at room temperature for 12 h, neutralized with Et_3N and concentrated. The residual syrup was dissolved in $CHCl_3$, washed with brine, dried (Na_2SO_4), concentrated and then the residue purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to afford **15** (8.6 g, 72 %).

To compound **15** (8 g, 32.2 mmol) in dry DMF (20 ml) at 0 ^{o}C, was added NaH (3.87 g, 161.3 mmol, 60% dispersion in oil). After 30 min. at room temperature, TBAI (0.1 g) and BnBr (8.42 ml, 70.9 mmol) were introduced and stirred for 2 h. Excess NaH was destroyed by adding methanol (10 ml). The reaction mixture was partitioned between diethyl ether and water. The ethereal layer was washed with brine, dried (Na_2SO_4),

concentrated and the residue purified by silica gel chromatography using ethyl acetate-light petroleum (1:10) as eluent to give **16** (10.2 g, 74 %).

[a] $_{D}$ = – 62.5 ^{o} (c 2, $CHCl_3$), lit[30] **[a]** $_{D}$ = – 58.5 ^{o} (c 1.85, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.25 (t, 3 H, *J* = 7.11 Hz), 1.35 (s, 3 H), 1.43 (s, 3 H), 3.75-3.85 (m, 4 H), 3.97-4.05 (m, 2 H), 4.12-4.32 (m, 2 H), 4.4-4.65 (m, 4 H), 5.05 (s, 1 H), 7.25-7.38 (m, 10 H).

Ethyl 2,3-Di-*O*-benzyl-ß-D-galactofuranoside (17)

A solution of ethyl 2,3-di-*O*-benzyl-5,6-*O*-isopropylidene-ß-D-galactofuranoside (**16**) (10 g, 23.3 mmol) in methanol (100 ml) was stirred at room temperature in the presence of *p*TSA (cat.). After 3 h, the reaction mixture was neutralized with Et_3N and solvent removed. The residue was taken in ethyl acetate (150 ml), washed with brine, dried (Na_2SO_4), concentrated and purified by silica gel column chromatography with ethyl acetate-light petroleum (1:2) as eluent to give **17** (6.16 g, 68 %).

[a] $_{D}$ = – 74.2 ^{o} (c 1.5, $CHCl_3$), lit[30] **[a]** $_{D}$ = – 71.7 ^{o} (c 1.27, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.2 (t, 3 H, *J* = 7.51 Hz), 3.4-3.52 (m, 1 H), 3.63-3.72 (m, 4 H), 3.95-4.05 (m, 3 H), 4.45-4.62 (m, 4 H), 5.0 (s, 1 H), 7.23-7.35 (m, 10 H).

Ethyl 2,3-Di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-ß-D-galactofuranoside (11)

A solution of **17** (3 g, 7.75 mmol), imidazole (1.1 g, 16.28 mmol), *tert*-butyldiphenylsilyl chloride (2.13 g, 7.75 mmol) in CH_2Cl_2 (50 ml) was stirred at room temperature for 3 h. Then the reaction mixture was diluted with CH_2Cl_2, washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel column with light petroleum-EtOAc (10:1) as eluent to give **11** (4.36 g, 90 %).

[a] $_{D}$ = - 53.2 ^{o} (c 1, $CHCl_3$);

^{1}H NMR d (300 MHz, $CDCl_3$): 1.05 (s, 9 H), 1.2 (t, 3 H, *J* = 7.1 Hz), 3.4-3.48 (m, 1 H), 3.65-3.79 (m, 4 H), 3.96 (dd, 1 H, *J* = 3.0, 1.3 Hz), 4.03-4.1 (m, 1 H), 4.21 (dd, 1 H, *J* = 6.65, 2.7 Hz), 4.44-4.58 (m, 4 H), 5.0 (s, 1 H), 7.2-7.4 (m, 15 H), 7.6-7.65 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 15.0, 19.1, 26.8, 62.9, 64.9, 71.2, 71.8, 72.2, 80.7, 83.4, 87.8, 106.0, 127.5-129.7, 133.0, 134.6-138.0;

FAB MS (Na): 649 (M + 23);

Anal. Calcd. for $C_{38}H_{46}O_6Si$: C, 72.84, H, 7.34. Found: C, 73.16, H, 7.55.

1,2,3,5-Tetra-*O*-acetyl-a-D-arabinofuranose (20)

D-arabinose (10.0 g, 66.7 mmol) and 2% methanolic HCl (100 ml) were stirred at room temperature for 4 h and neutralized with solid $BaCO_3$. The solid was filtered and the filtrate concentrated to give a syrup which was treated with Ac_2O (30 ml) and pyridine (20 ml) in the presence of DMAP (cat.) in CH_2Cl_2 (100 ml) at room temperature 8 h. The reaction mixture was successively washed with dil. HCl, water, brine, dried (Na_2SO_4) and concentrated. The resulting product, AcOH (100 ml), Ac_2O (20 ml) and conc. H_2SO_4 (5 ml) were stirred at room temperature for 12 h and poured over crushed ice. After 2 h, the solution was extracted with $CHCl_3$, washed with aq. $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated to give a syrup which was purified on silica gel with ethyl acetate-light petroleum (1:3) as eluent to afford **20** (9.15 g, 43 %).

[a] $_D$ = + 50.8 ^{o} (c 2.8, $CHCl_3$), lit[31] **[a] $_D$ =** + 51.9 ^{o} (c 3, $CHCl_3$);

^{1}H NMR d (200 MHz, $CDCl_3$): 2.0-2.12 (4 s, 12 H), 4.07-4.23 (m, 2 H), 5.0-5.05 (m, 1 H), 5.2 (s, 1 H), 5.3-5.34 (m, 1 H), 6.17 (s, 1 H).

2,3,5-Tri-*O*-acetyl-a, ß-D-arabinofuranosyl trichloroacetimidate (12)

A solution of per-*O*-acetyl-a-D-arabinofuranose (**20**) (4.0 g, 12.57 mmol), tri n-butyltin ethoxide (8.4 g, 25.15 mmol) in $ClCH_2CH_2Cl$ (40 ml) was heated under reflux for 3 h and concentrated. The residue was purified on silica gel with light petroleum-ethyl

acetate (EtOAc) (3:2) as eluent to give **21** (2.43 g, 70 %). The resulting product (**21**) was immediately treated with CCl_3CN (6.34 g, 44.02 mmol) in the presence of DBU (1.47 g) in CH_2Cl_2 (50 ml) at 0 °C for 2 h. Then solvent was removed and the residue purified by flash chromatography on silica gel with light petroleum-EtOAc (3:1) as eluent to give **12** (2.35 g, 65 %), which was used without delay.

Ethyl 5-*O*-(a-D-Arabinofuranosyl)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-ß-D-galactofuranoside (23)

To a stirred solution of **11** (3.0 g, 4.8 mmol), **12** (2.1 g, 5 mmol) and 4A° molecular sieves (MS) powder (0.5 g) in CH_2Cl_2 (50 ml) under nitrogen at –20 °C, BF_3:OEt_2 (0.2 ml) was added. After 2 h at room temperature, solid $NaHCO_3$ (0.2 g) was added and then filtered. The filtrate was washed with brine, dried (Na_2SO_4) and concentrated. The residue was passed through a short column of silica gel with light petroleum-EtOAc (9:1) as eluent to obtain the disaccharide **22** (2.96 g) (contaminated with **11** as a minor impurity) and treated with 0.05 M NaOMe in methanol (15 ml) at room temperature for 2 h. The reaction mixture was de-ionized by the addition of Amberlite IR 120 (H^+) resin (pH 6), filtered and concentrated. The residue was purified by silica gel column chromatography with light petroleum-EtOAc (1:1) as eluent to obtain **23** (2.03 g, 56 %).

[a]$_D$ = - 22.4 ^{o} (c 1, $CHCl_3$);

^{1}H NMR d (200 MHz, $CDCl_3$): 1.07 (s, 9 H), 1.2 (t, 3 H, *J* = 7.5 Hz), 3.6-3.7 (m, 5 H), 3.9-4.02 (m, 4 H), 4.06-4.17 (m, 4 H), 4.3-4.58 (m, 4 H), 5.01 (s, 1 H), 5.21 (s, 1 H), 7.2-7.45 (m, 15 H), 7.6-7.75 (m, 5 H);

^{13}C NMR d (125 MHz, $CDCl_3$): 15.0, 18.0, 25.6, 60.2, 61.7, 62.1, 71.0, 76.8, 77.1, 77.3, 77.4, 79.1, 82.9, 85.8, 86.9, 104.5, 106.5, 126.1-128.4, 134.0, 136.0;

FAB MS (Na): 781 (M + 23);

Anal. Calcd. for $C_{43}H_{54}O_{10}Si$: C, 68.07, H, 7.12. Found: C, 68.34, H, 6.93.

Ethyl 5-*O*-(2′,3′,5′-Tri-*O*-benzyl-a-D-arabinofuranosyl)-2,3-di-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-ß-D-galactofuranoside (24)

To a stirred solution of **23** (1.5 g, 1.98 mmol) and NaH (60 % dispersion in paraffin, 0.35 g, 14.8 mmol) in dry DMF (10 ml) under nitrogen was added benzyl bromide (1.11 g, 6.53 mmol). After 3 h at room temperature, the reaction was quenched with methanol (2 ml) and partitioned between water and diethyl ether. The ether layer was washed with brine, dried (Na_2SO_4) and concentrated to give a residue which was purified on silica gel with light petroleum-EtOAc (10:1) as eluent to give **24** (1.77 g, 87 %).

[a] $_D$ = - 18.4 ^{o} (c 1.5, $CHCl_3$);

^{1}H NMR d (500 MHz, $CDCl_3$): 1.08 (s, 9 H), 1.2 (t, 3 H, *J* = 6.25 Hz), 3.45-3.5 (m, 3 H), 3.74-3.8 (m, 2 H), 3.9 (d, 2 H, *J* = 5.2 Hz), 3.92-4.0 (m, 3 H), 4.05-4.1 (m, 3 H), 4.28-4.6 (m, 10 H), 5.09 (s, 1 H), 5.20 (s, 1 H), 7.18-7.37 (m, 30 H), 7.68-7.72 (m, 5 H);

^{13}C NMR d (125 MHz, $CDCl_3$): 14.8, 18.9, 26.5, 62.5, 63.0, 68.4, 71.4, 71.6, 71.7, 73.0, 76.4, 76.6, 76.9, 79.5, 79.6, 82.8, 83.4, 88.3, 105.1, 106.6, 127.2-129.2, 133.2, 135.3, 137.6;

FAB MS (Na): 1051 (M + 23);

Anal. Calcd. for $C_{64}H_{72}O_{10}Si$: C, 74.7, H, 7.0. Found: C, 74.4, H, 7.14.

Ethyl 5-*O*-(2′,3′,5′-Tri-*O*-benzyl-a-D-arabinofuranosyl)-2,3-di-*O*-benzyl-ß-D-galacto-furanoside (10)

Compound **24** (1.5 g, 1.46 mmol) and 1 M solution of TBAF (3 ml) in THF (10 ml) were stirred for 30 min at room temperature and then concentrated. The residue was purified on short silica gel column with light petroleum-EtOAc (3:1) to provide **10** (1.06 g, 92 %).

[a] $_D$= - 20.6 ^{o} (c 2, $CHCl_3$);

^{1}H NMR d (300 MHz, $CDCl_3$): 1.2 (t, 3 H, *J* = 7.5 Hz), 3.52-3.65 (m, 6 H), 3.85-4.1 (m, 7 H), 4.35-4.58 (m, 10 H), 5.02 (s, 1 H), 5.3 (s, 1 H), 7.05-7.35 (m, 25 H);

FAB MS (Na): 813 (M + 23)

Anal. Calcd. for $C_{48}H_{54}O_{10}$: C, 72.91, H, 6.83. Found: C, 73.24, H, 7.15.

1,2,3,5,6-Penta-*O*-acetyl-ß-D-galactofuranose (27)

To a solution of D-galactose (5 g, 27.8 mmol) in anhydrous MeOH (100 ml), conc. H_2SO_4 (1 ml) was added drop wise at 0 °C. The reaction mixture was allowed to proceed at room temperature for 4 h. Then the reaction mixture was neutralized with $BaCO_3$ and the insoluble inorganic materials were filtered through Celite and washed with methanol (50 ml). The filtrate was concentrated to give a syrup, which was treated with Ac_2O (20 ml) and pyridine (25 ml) for 4 h at 0 °C and then at room temperature for 2 days. The usual work-up procedure yielded a syrup which, was dissolved in AcOH (35 ml), Ac_2O (7.5 ml) and treated with conc. H_2SO_4 (1.5 ml) at 0 °C. After 15 h at room temperature, the brown solution was worked up, and the residual syrup was crystallized from isopropanol (10 ml) at 0 °C. Recrystallisation from the same solvent gave **27** (3.8 g, 35 %).

m.p.: 103-105 °C; lit[35] mp: 102-103 °C;

[a]$_D$ = – 41° (c 3.1, CH_2Cl_2); lit[35] **[a]$_D$** = – 41.5 ° (c 3, CH_2Cl_2);

^{1}H NMR d (200 MHz, $CDCl_3$): 2.0-2.1 (5 s, 15 H), 4.1-4.25 (m, 2 H), 5.05-5.1 (m, 1 H), 5.15 (d, 1 H, *J* = 2.0 Hz), 5.28-5.36 (m, 2 H), 6.17 (s, 1 H).

Pent-4-enyl 2,3,5,6-tetra-*O*-acetyl-ß-D-galactofuranoside (30)

To a stirred solution of per-*O*-acetyl ß-D-galactofuranose (**27**) (2 g, 5.13 mmol), 4-pentene-1-ol (1.1 ml, 10.25 mmol) and 4A° MS powder (0.5 g) in CH_2Cl_2 (20 ml) under nitrogen, at 0 °C was added BF_3:OEt_2 (0.1 ml). After 2 h, solid $NaHCO_3$ (0.2 g) was added and filtered over Celite. The filtrate was washed with brine, dried (Na_2SO_4), and

concentrated. The residual syrup was purified by silica gel column chromatography by eluting with light petroleum-EtOAc (4:1) to give **30** (1.81 g, 85 %).

[a] $_D$ = - 50.2 ^{o} (c 2, $CHCl_3$);

^{1}H NMR d (200 MHz, $CDCl_3$): 1.64-1.76 (m, 4 H), 2.06-2.14 (4 s, 12 H), 3.4-3.51 (m, 1 H), 3.62-3.74 (m, 1 H), 4.16-4.38 (m, 3 H), 4.96-5.08 (m, 5 H), 5.35-5.42 (m, 1 H), 5.71-5.92 (m, 1 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 20.3, 28.2, 29.7, 62.2, 66.5, 68.9, 76.2, 79.5, 81.0, 105.1, 114.6, 137.6, 169.6;

Anal: Calcd. for $C_{19}H_{28}O_{10}$: C, 54.8, H, 6.73. Found: C, 54.65, H, 6.52.

Ethyl 5-*O*-(2¢,3¢,5¢-Tri-*O*-benzyl-a-D-arabinofuranosyl)-6-*O*-(ß-D-galactofuranosyl)-2,3-di-*O*-benzyl-ß-D-galactofuranoside (32)

To a stirred solution of **10** (1 g, 1.26 mmol), **30** (0.63 g, 1.52 mmol) and 4A ° MS powder (0.5 g) in dry CH_2Cl_2 (20 ml) was added NIS (0.7 g, 3.16 mmol) followed by TfOH (0.1 ml in two intervals of 30 min). The reaction was stirred under dark at room temperature for 48 h, filtered through Celite bed. The filtrate was washed with saturated solutions of $NaHSO_3$, $NaHCO_3$ followed by brine solution. The organic layer was dried (Na_2SO_4) and concentrated. The residue was subjected to column chromatography on silica gel with light petroleum-EtOAc (3:1) as eluent to obtain the crude trisaccharide derivative **31** (0.92 g). The crude trisaccharide (**31**) was treated with 0.05 M NaOMe in methanol (5 ml) at room temperature for 2 h. After usual work up as indicated above for Zémplen reaction, gave the residue, which was chromatographed on silica gel with light petroleum-EtOAc (2:3) as eluent to give **32** (0.56 g, 47 %).

[a] $_D$ = - 22.6 ^{o} (c 0.6, $CHCl_3$);

^{1}H NMR d (500 MHz, $CDCl_3$): 1.1 (t, 3 H, *J* = 7.5 Hz), 3.36-3.65 (m, 8 H), 3.76-3.97 (m, 10 H), 4.0-4.05 (m, 1 H), 4.27-4.51 (m, 10 H), 4.84 (s, 1 H), 4.96 (s, 1 H), 5.17 (s, 1 H), 7.12-7.25 (m, 25 H);

^{13}C NMR d (125 MHz, $CDCl_3$): 14.8, 62.8, 63.7, 66.6, 69.5, 70.7, 71.5, 71.6, 71.9, 72.0, 73.2, 76.2, 78.3, 78.6, 80.0, 80.6, 83.5, 83.6, 86.9, 87.8, 87.9, 105.3, 106.8, 107.7, 127.4-128.2, 137.1-137.6;

FAB MS (Na): 952 (M + 23);

Anal. Calcd. for $C_{54}H_{64}O_{15}$: C, 68.06, H, 6.72. Found: C, 68.35, H, 6.95.

Ethyl 5-*O*-(a-D-Arabinofuranosyl)-6-*O*-(ß-D-galactofuranosyl)-ß-D-galactofuranoside (8)

Compound **32** (0.3 g, 0.31 mmol) and 10 % $Pd(OH)_2$/C (50 mg) in methanol (10 ml) were stirred under hydrogen atmosphere at normal temperature and pressure (ntp) for 8 h. The catalyst was filtered off and the filtrate concentrated to give **8** (0.14 g, 90 %).

[a]$_D$ = -26.8 ^{o} (c 1.1, MeOH);

^{1}H NMR d (500 MHz, D_2O): 1.1 (t, 3 H, *J* = 7.0 Hz), 3.48-3.72 (m, 8 H), 3.78-4.05 (m, 11 H), 4.88 (s, 1 H), 4.92 (s, 1 H), 5.1 (s, 1 H);

^{13}C NMR d (125 MHz, D_2O): 15.2, 62.1, 63.8, 65.3, 68.2, 71.8, 77.2, 77.3, 77.4, 77.8, 81.9, 82.0, 82.2, 82.9, 83.9, 84.8, 107.6, 108.6, 109.6;

FAB MS (Na): 525 (M + 23);

Anal: Calcd. for $C_{19}H_{34}O_{15}$: C, 45.42, H, 6.77. Found: C, 45.13, H, 7.0.

Ethyl 2,3,6-Tri-*O*-benzyl-ß-D-galactofuranoside (35)

Compound **17** (1 g, 2.1 mmol) and dibutyltin oxide (0.78 g, 3.13 mmol) in dry toluene (20 ml) were heated under reflux for 6 h with azeotropic removal of water, then TBAB (0.1 g) and benzyl bromide (0.35 g, 2.1 mmol) were introduced. The reaction was heated under reflux for 24 h, solvent evaporated, and the residue taken up in ethyl acetate

(20 ml). The organic layer was successively washed with 10 % aq. KF solution, brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography with light petroleum-EtOAc (9:1) as eluent to give **35** (0.87 g, 71 %).

[a] $_D$ = - 48.2 ^{o} (c 1.1, $CHCl_3$);

^{1}H NMR d (200 MHz, $CDCl_3$): 1.24 (t, 3 H, *J* = 7.3 Hz), 3.43-3.56 (m, 3 H), 3.72-3.8 (m, 1 H), 4.08-4.14 (m, 4 H), 4.54-4.6 (m, 6 H), 5.05 (s, 1 H), 7.28-7.33 (m, 15 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 14.8, 62.6, 69.6, 71.3, 71.6, 71.9, 72.9, 80.9, 83.0, 87.6, 105.6, 127.2-128.0, 137.1-137.8;

Anal. Calcd. for $C_{29}H_{34}O_6$: C, 72.8, H, 7.11. Found: C, 72.42, H, 7.01.

Ethyl 5-*O*-(ß-D-Galactofuranosyl)-2,3,6-tri-*O*-benzyl-ß-D-galactofuranoside (37)

To a stirred solution of **35** (0.5 g 1.04 mmol), **30** (0.52 g, 1.25 mmol), 4A ° MS powder (0.5 g) and NIS (0.58 g, 2.61 mmol) in CH_2Cl_2 (20 ml) under nitrogen and in dark was addded TfOH (0.05 ml). The reaction mixture was stirred for 24 h at room temperature, filtered through Celite, the filtrate washed with saturated solutions of $NaHSO_3$ and $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated. The residual syrup was chromatographed on silica gel with light petroleum-EtOAc (4:1) as eluent to give the crude disaccharide **36** (0.57 g), which was treated with 0.05 M NaOMe in methanol (5 ml) at room temperature. After 2 h, and usual work up as given above for Zémplen reaction, the residue was purified on silica gel with light petroleum-EtOAc (1:4) as eluent to give **37** (0.32 g, 48 %).

[a] $_D$ = - 84.1 ^{o} (c 1.2, $CHCl_3$);

^{1}H NMR d (200 MHz, $CDCl_3$): 1.18 (t, 3 H, *J* = 7.1 Hz), 3.37-3.72 (m, 6 H), 3.85-3.9(m, 2 H), 3.93-4.1 (m, 6 H), 4.33-4.61 (m, 6 H), 5.0 (s, 1 H), 5.34 (s, 1 H), 7.27-7.35 (m, 15 H);

^{13}C NMR d (75 MHz, $CDCl_3$): 14.5, 62.5, 63.5, 69.2, 70.7, 71.5, 71.6, 72.8, 73.0, 78.3, 78.6, 80.2, 83.9, 86.5, 87.0, 104.9, 106.1, 127.1-128.0, 136.6-137.3;

FAB MS (Na): 663 (M + 23);

Anal. Calcd. for $C_{35}H_{44}O_{11}$: C, 65.62, H, 6.87. Found: C, 65.32, H, 6.95.

Ethyl 5-*O*-(ß-D-Galactofuranosyl)-ß-D-galactofuranoside (33)

A solution of **37** (0.2 g, 0.31 mmol), 10 % $Pd(OH)_2/C$ (50 mg) in methanol (5 ml) was stirred under hydrogen atmosphere at normal temperature and pressure. After 8 h, the catalyst was filtered off and the filtrate concentrated to give **33** (0.1 g, 88 %).

[a] $_D$ = - 105.2 ^{o} (c 1.1, MeOH);

^{1}H NMR d (500 MHz, D_2O): 0.92 (t, 3 H, *J* = 6.4 Hz), 3.29-3.32 (m, 1 H), 3.4-3.44 (m, 2 H), 3.46-3.55 (m, 4 H), 3.64 (br s, 1 H), 3.73-3.77 (m, 4 H), 3.81-3.85 (m, 2 H), 4.92 (s, 2 H);

^{13}C NMR d (125 MHz, D_2O): 15.1, 61.9, 63.6, 65.1, 71.4, 76.7, 77.0, 77.3, 81.7, 82.1, 82.2, 83.5, 107.5, 107.9;

FAB MS (Na): 393 (M + 23);

Anal. Calcd. for $C_{14}H_{26}O_{11}$: C, 45.4, H, 7.02. Found: C, 45.75, H, 7.25.

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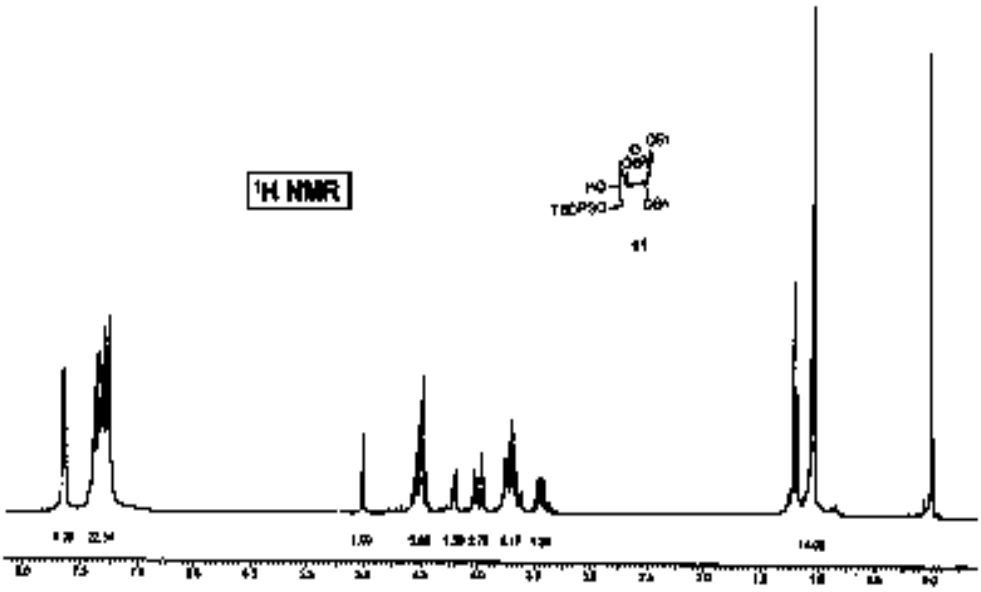
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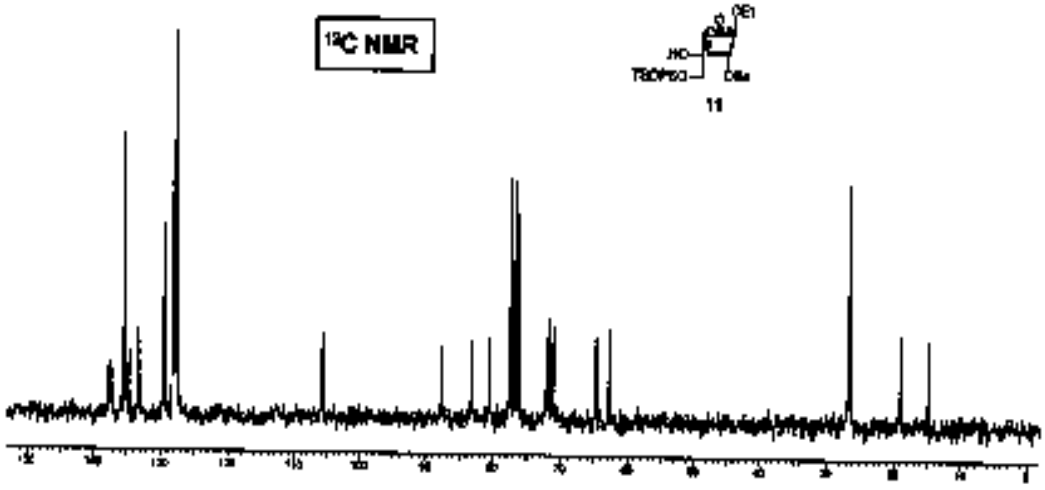
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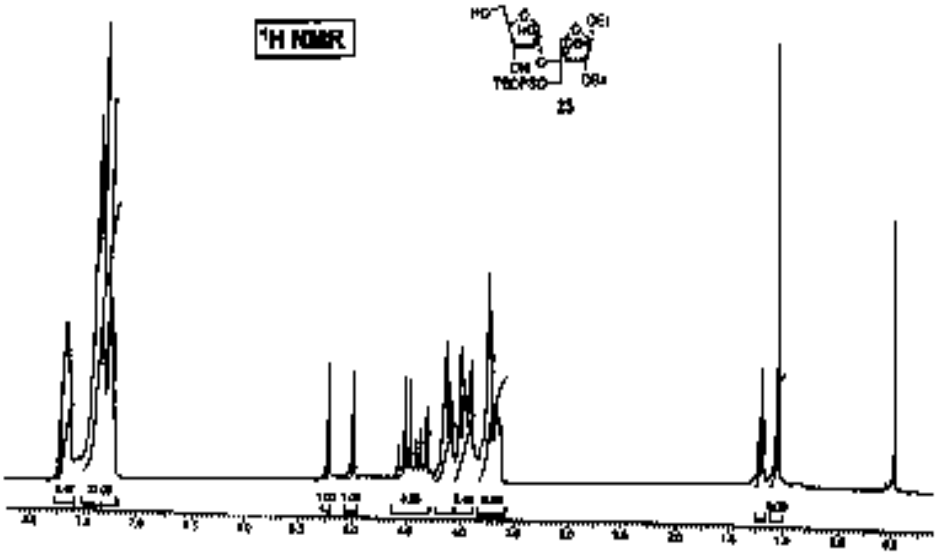
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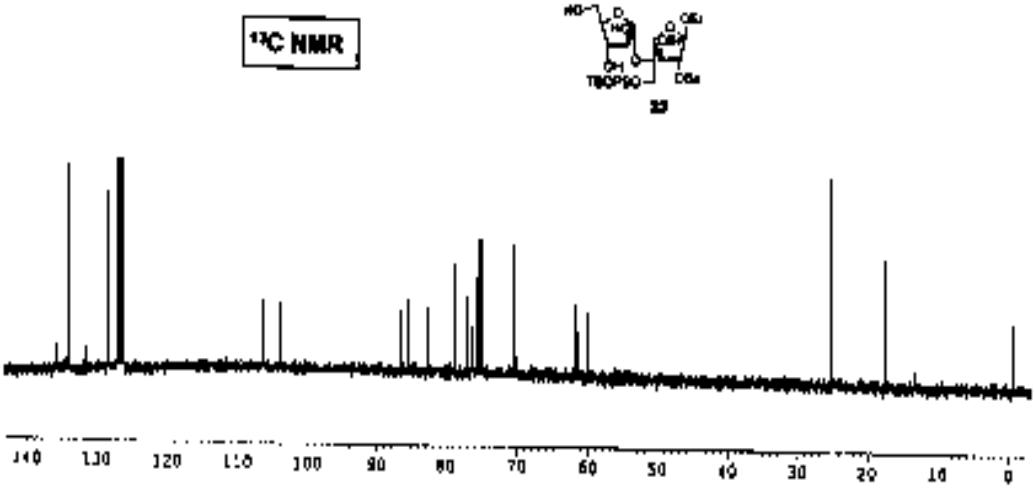
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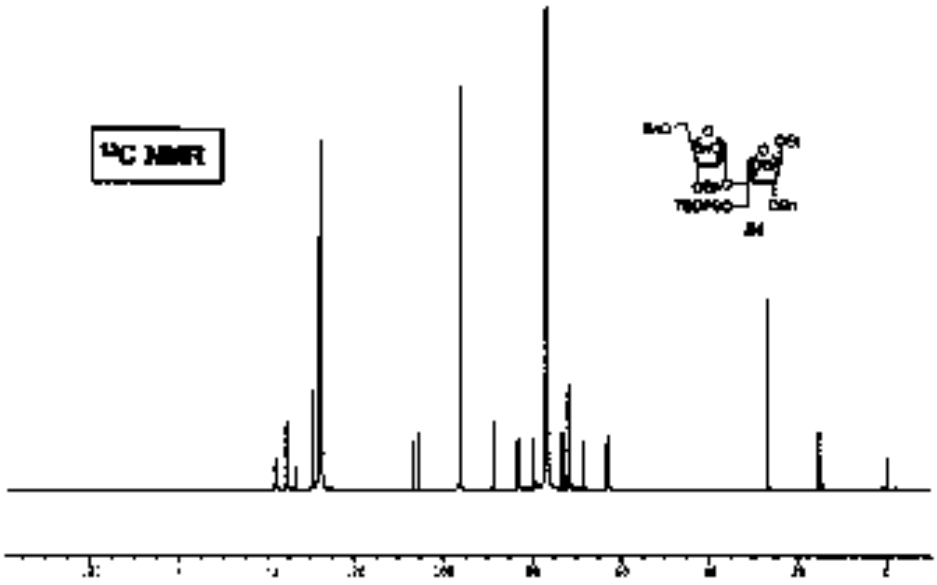
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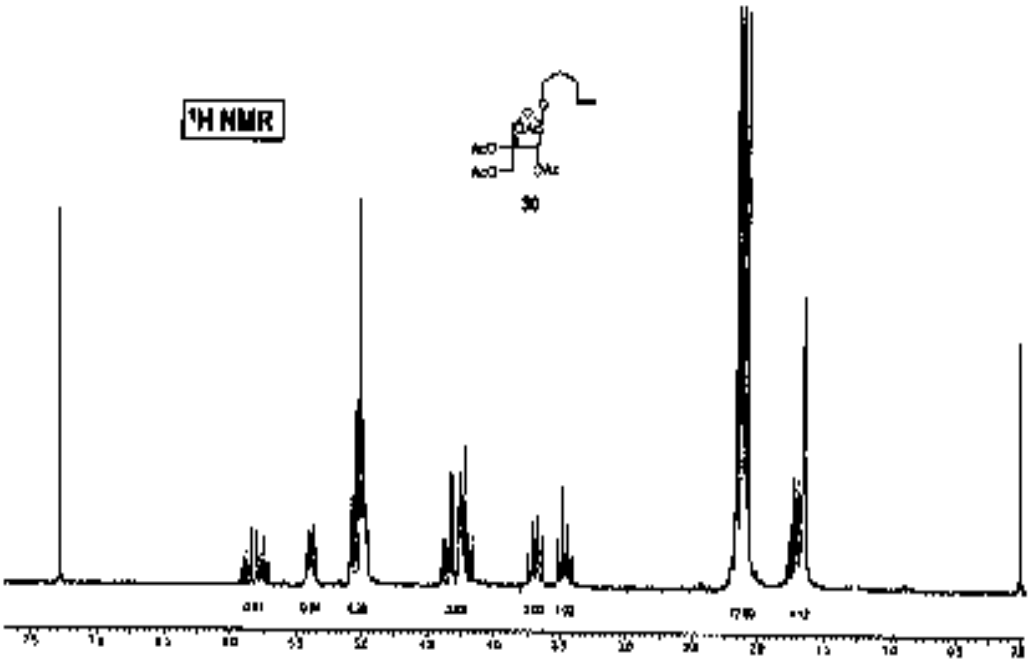
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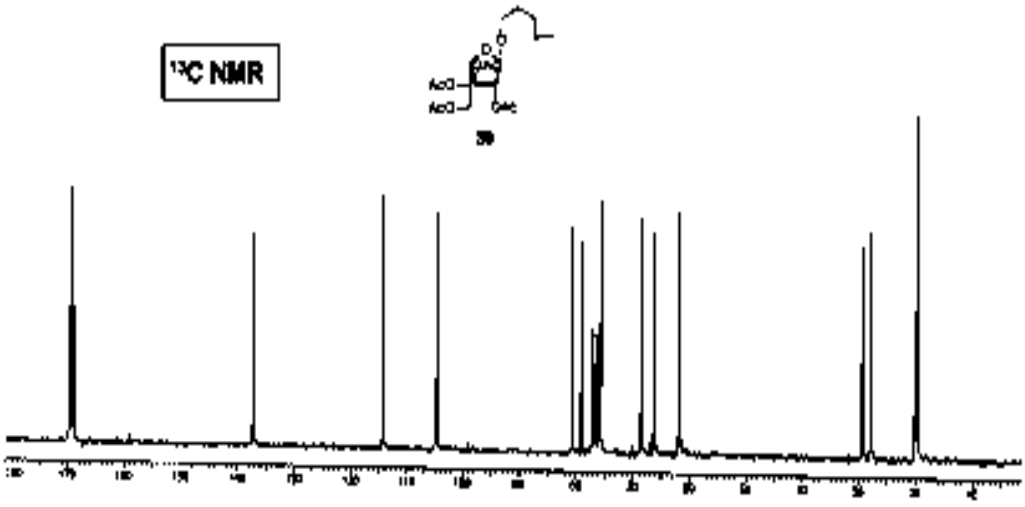
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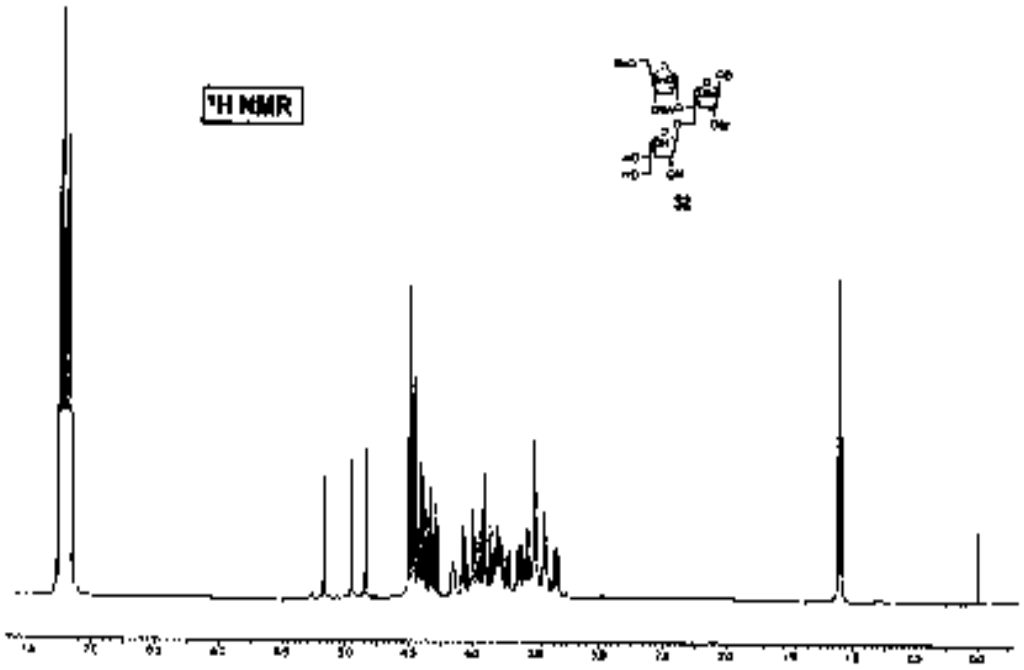
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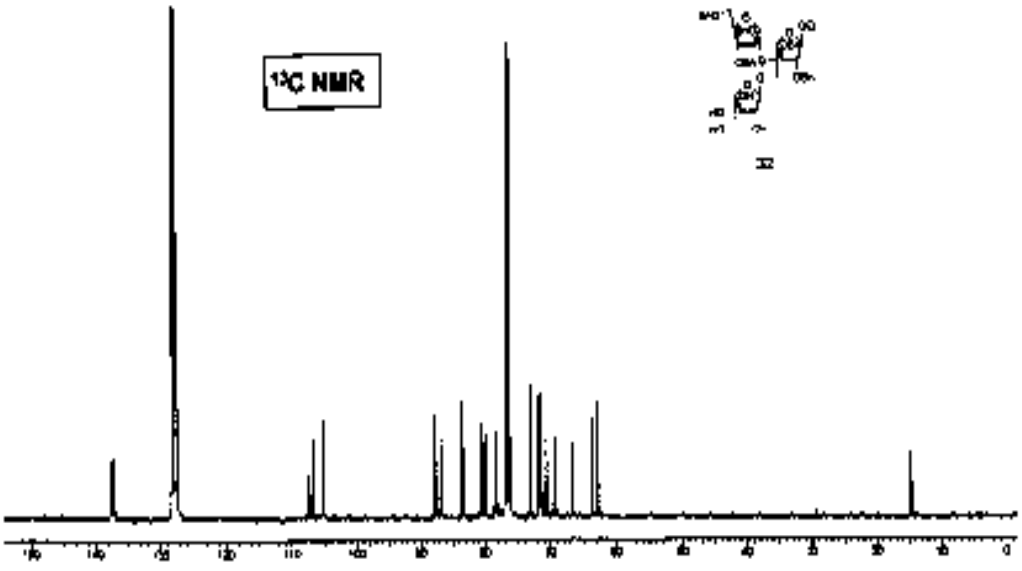
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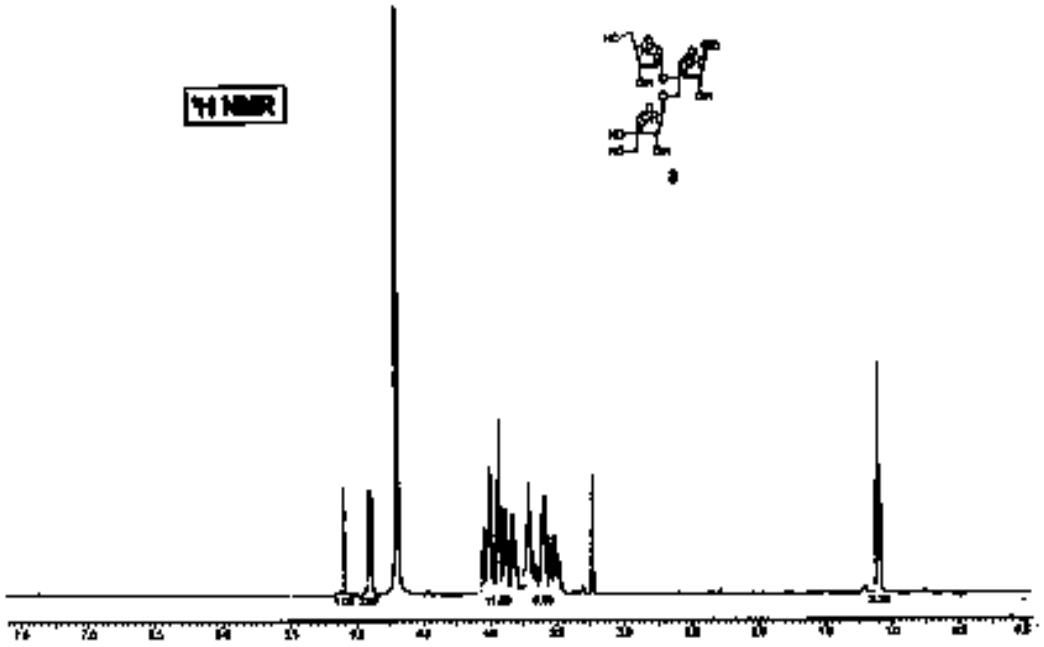
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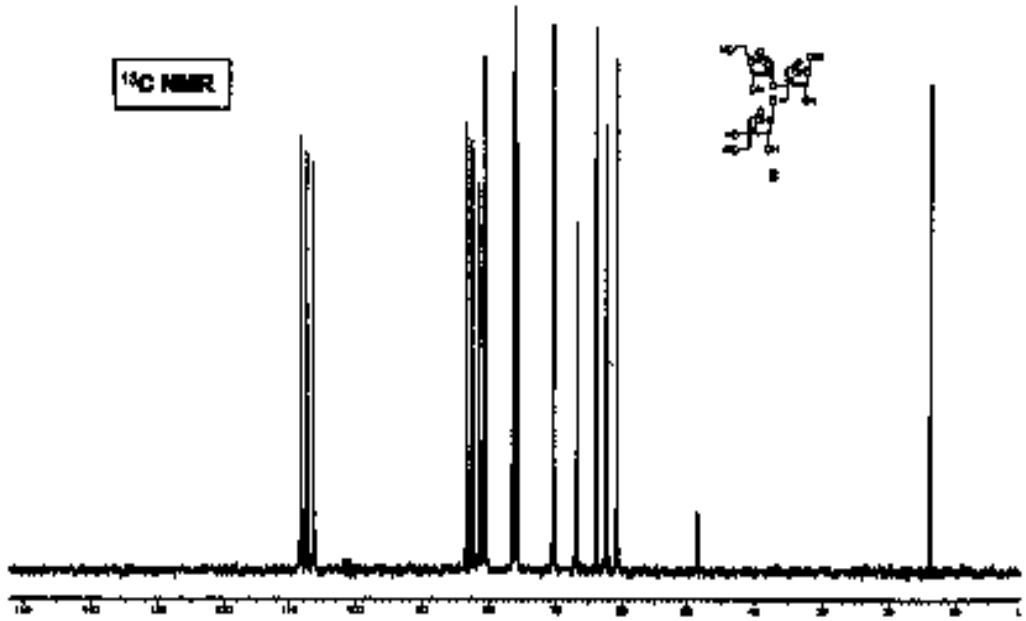
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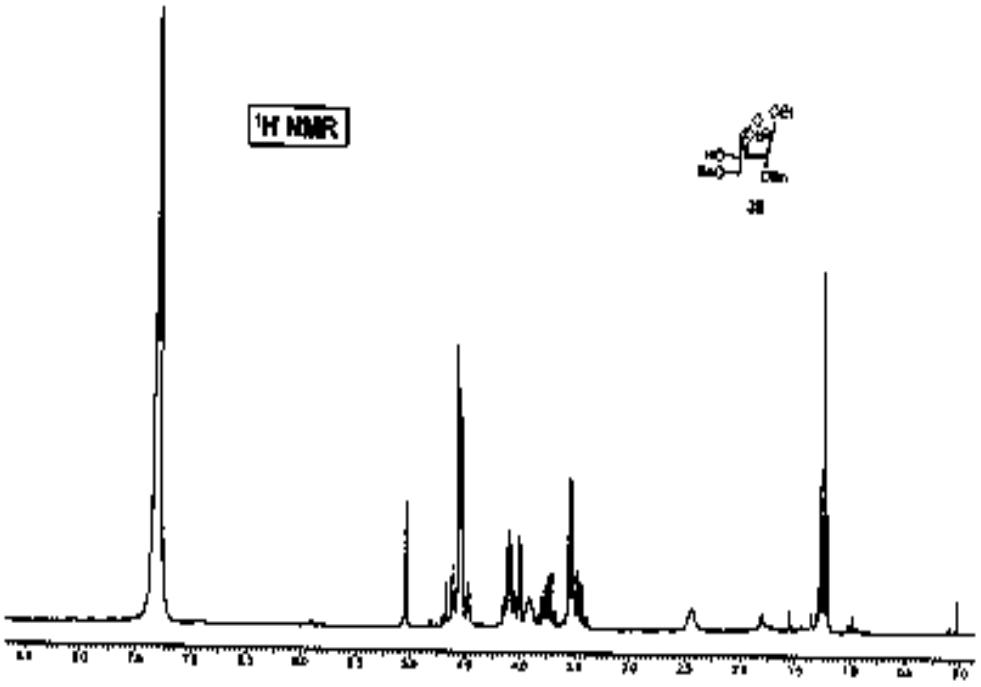
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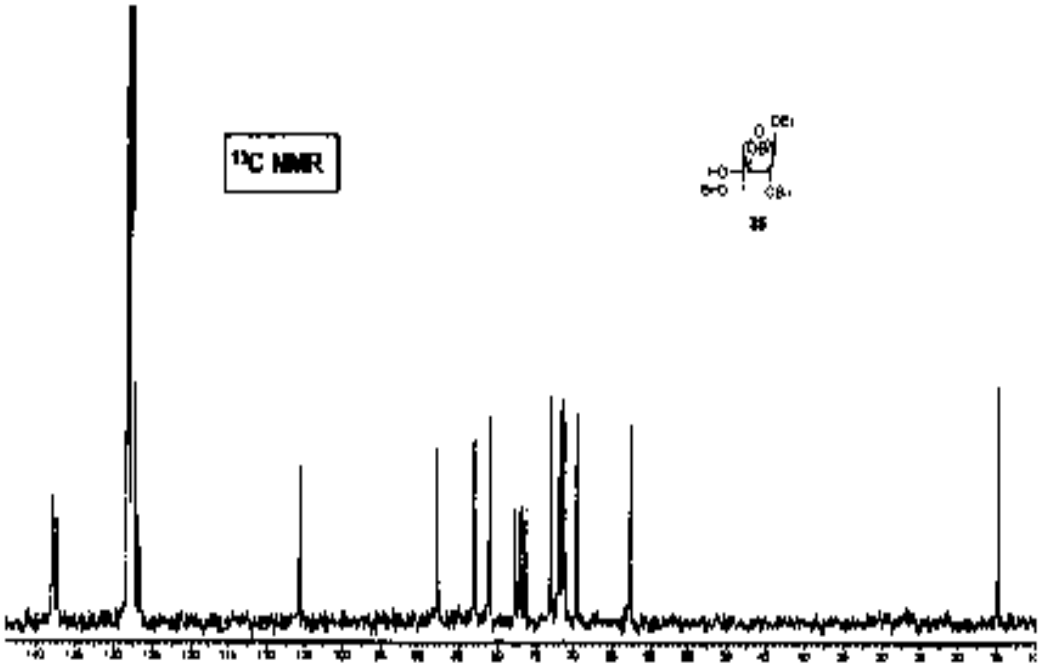
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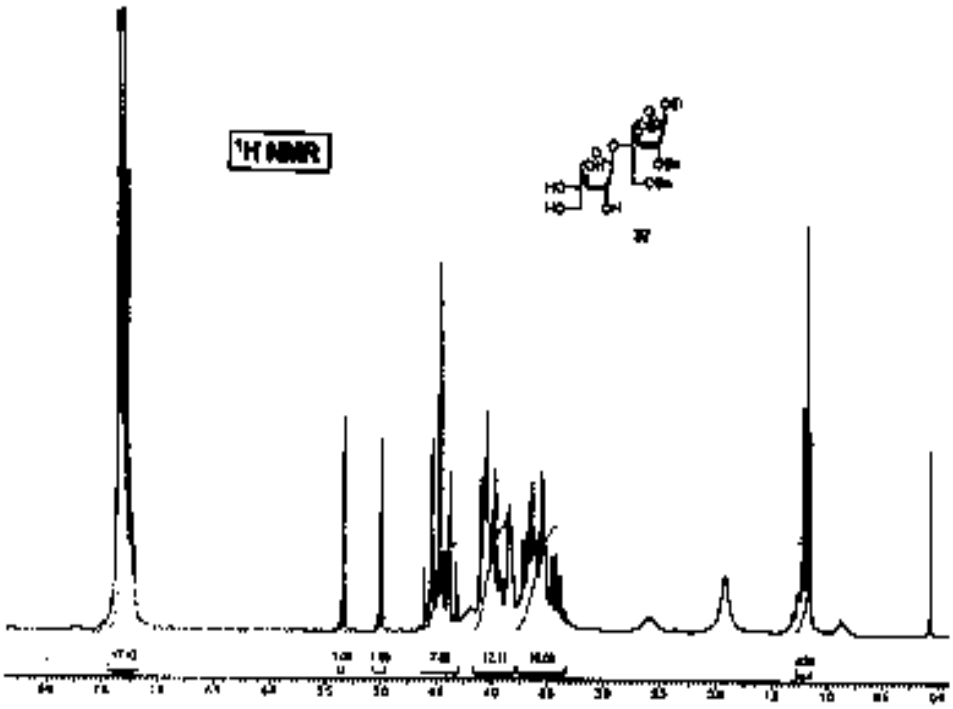
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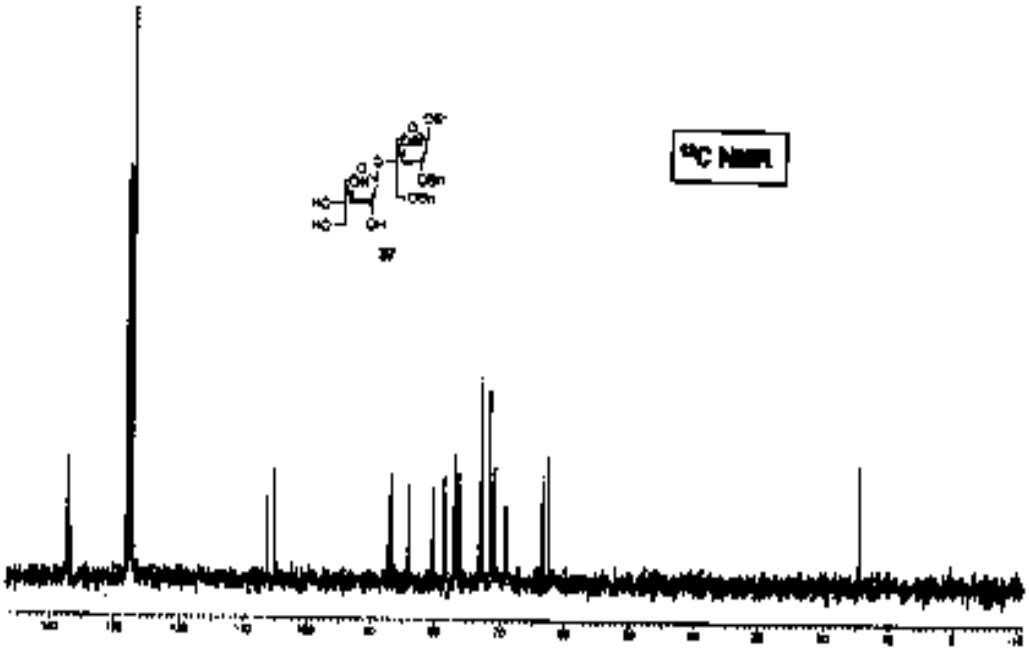
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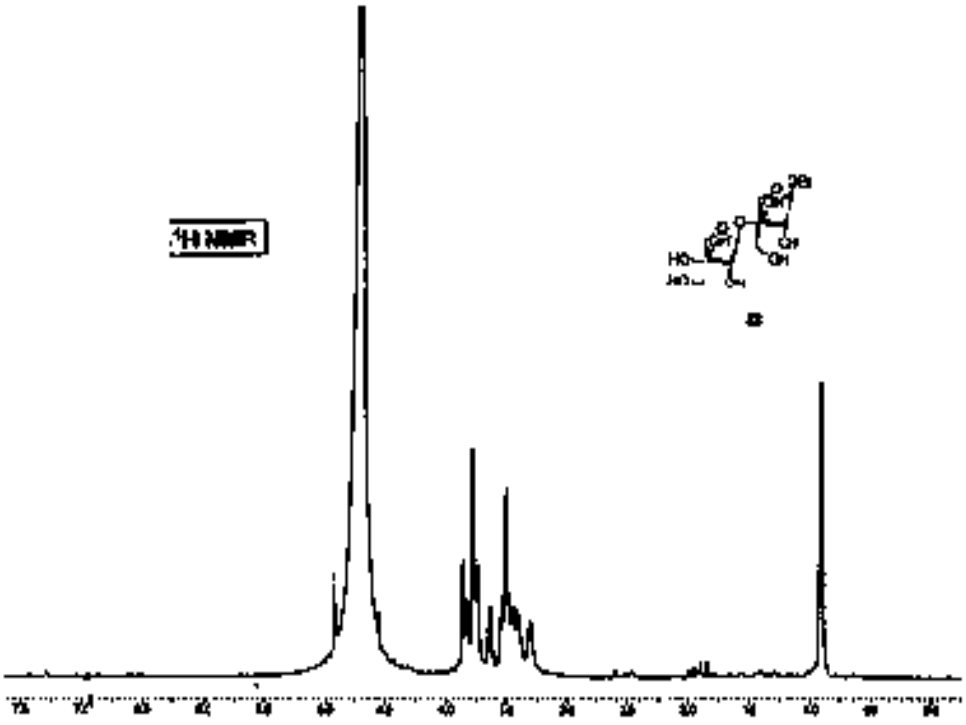
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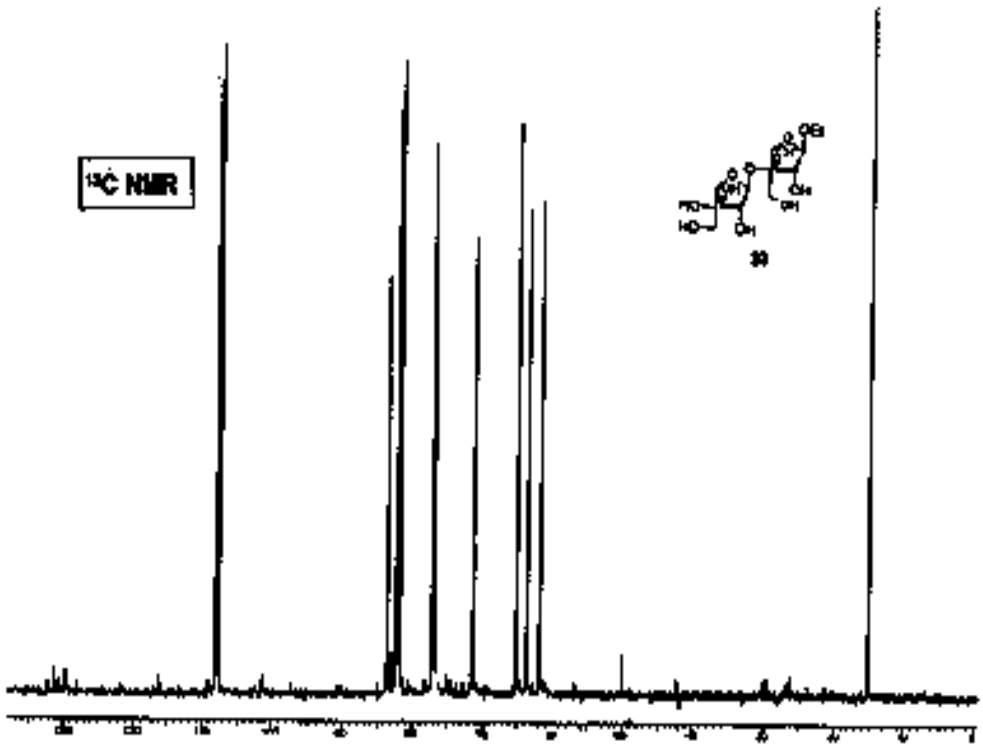
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CHAPTER II

SECTION I
Synthesis of CMI-546: A potent PAF receptor antagonist

Introduction

According to the estimate of World Health Organization, asthma affects 150 million people worldwide, and the number of patients has doubled over the decade.[1] Especially, the situation is alarming in industrialized countries, where the rapid surge in air pollution has become the primary cause of this lung disease. Although there is no cure for asthma, it is a disease that can be managed, enabling most people to lead active and productive life. This preliminary discussion will brief the current status of the biological and medicinal aspects of asthma that would have eventually formed the basic tenet of our interest to develop the synthesis of anti-asthmatic compounds.

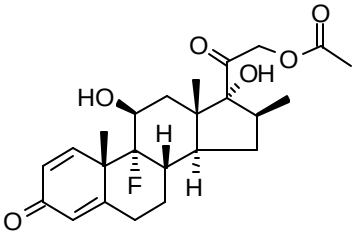
The rapid rise of asthma constitutes the biggest mystery in modern medicine and the exact reasons for the increase still evade the researchers.[2] Although several factors were put forward, it is now concluded that a combination of genetic and environmental factors are responsible for the onset of asthma.[3] The good news is that 95% of asthma is controllable, given proper and continuous medication. The advances in molecular biology indicate that allergy and asthma are not inherited as single-gene disorders and do not show a simple pattern of inheritance. Environmental and genetic factors interact in a complex fashion to produce disease susceptibility and expression.

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 at least 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, are more i.e., histamine, 5-lipoxygenase (5-LO) and platelet activating factor (PAF) have been developed over the years.

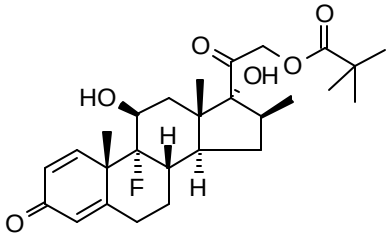
The present anti-asthmatic therapy is largely based on corticosteroides (inhaled and systemic) and symptomatic treatment, and to the lesser extent, immunotherapy which revolve around the inhibition of various inflammatory mediators that enter the various stages of asthmatic process e.g., cytokines, chemokines, adhesion molecules, proteinases and growth factors as discussed below.

Glucocorticosteroids, b_2-adrenoreceptor agonists and theophylline

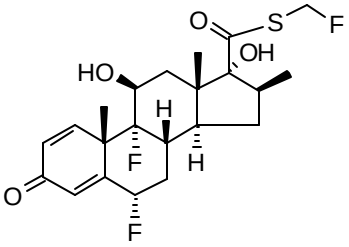
Inhaled β_2-adrenoreceptor agonists are the most effective bronchodilators, currently predescribed for symptomatic relief in asthma.[4] The mechanism of action, i.e., causing smooth muscle relaxation involves camp-dependent and independent pathways. Inhaled glucocorticosteroids, e.g., betamethasone (**1**), dexamethasone (**2**), fluticasone (**3**), and cortisone (**4**) are mainstay therapy for reducing airway inflammation in asthma.



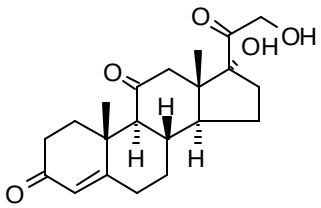
Betamethasone (**1**)



Dexamethasone (**2**)

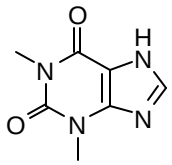


Fluticasone (**3**)

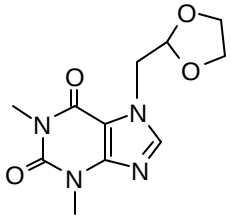


Cortisone (**4**)

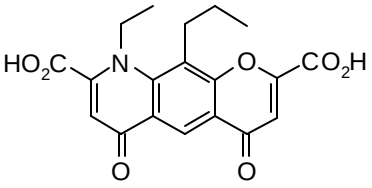
Theophylline (**5**) has a long historic background through its ability to bronchodilate asthmatic subjects. Although limited by side-effects profile, theophylline is effective in reducing the symptoms and improving lung function in patients with mild chronic asthma. Doxofylline (**6**) and nedocromil (**7**) are the drugs work in the inflammatory cells to prevent the release of histamine and other chemicals involved in airway inflammation.



Theophylline (**5**)

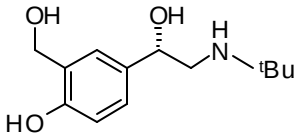


Doxofylline (**6**)

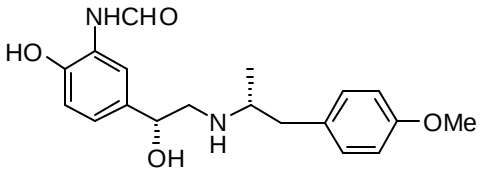


Nedocromil (**7**)

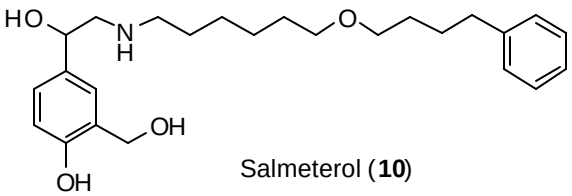
Salbutamol (**8**) is a potent β_2-adrenoreceptor antagonist. β_2-Adrenergeric receptors are found on the smooth muscle lining airways of the lungs. Long action of β_2-adrenoreceptor agonists can be achieved by alterations in pharmacokinetics [e.g., formoterol (**9**)][5] and by exosite binding [e.g., salmeterol (**10**)].[6] Also, new steroids are being developed with the aim of maximizing topical anti-inflammatory effects and minimizing adverse systemic effects, as exemplified by RU-24858. Clinical studies have recently demonstrated the benefit of combining long-acting β_2-adrenoreceptor agonists



(R)-Salbutamol (**8**)



Formoterol (**9**)

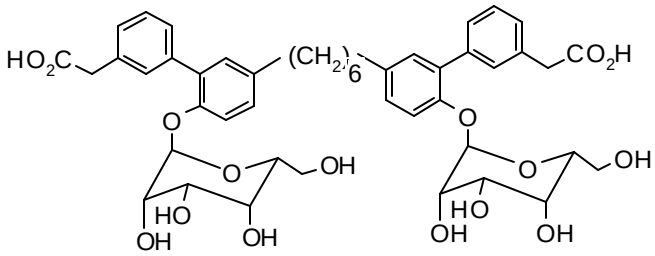


Salmeterol (**10**)

with inhaled steroids, e.g., seretide (salmeterol and fluticasone) combined in a single formulation.

Adhesion molecules

Suppression of eosinophil adhesion with consequent inhibition of influx into the lung is a strategy to suppress the asthmatic airway inflammation. The selectin family of adhesion molecules, which are expressed on activated endothelial cells (E- and P- selectin), activated platelets (P- selectin), and peripheral blood leukocytes (L- selectin) are involved in tethering and rolling of leukocytes in the microcirculation, leading to leukocyte tissue infiltration. Interruption of leukocyte-endothelium interaction is a current strategy to target asthma. TBC-1269 (**11**) is the lead compound of a series of orally active, low molecular weight E-, P-, L- selectin antagonists for the potential treatment of asthma and psoriasis.[7]



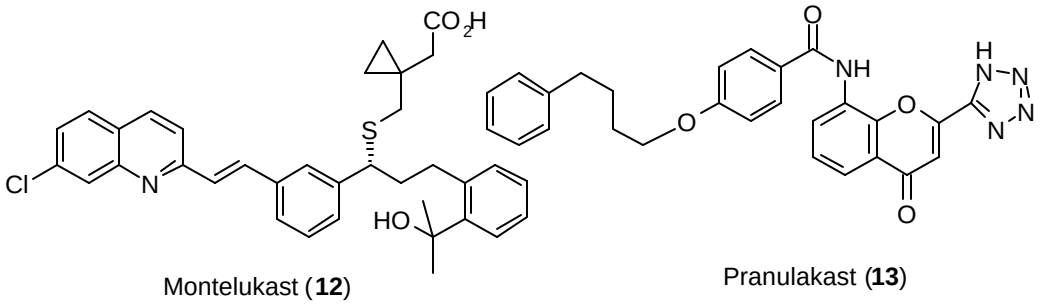
TBC-1269 (**11**)

Cytokines

Cytokines play a key role in the chronic inflammation of asthma and appear to orchestrate, amplify, and perpetuate the inflammatory process.[8] The chemotactic cytokines (CC chemokines) act by attracting leukocytes to sites of inflammation. The advancement in understanding the intracellular signaling pathways and inflammatory gene transcription of key pro- and anti-inflammatory cytokines is laying the foundation for a new era in anti- inflammatory drug discovery. SP 650003 and SP 100030 are the small molecule inhibitors, which are great promise in the treatment of asthma.

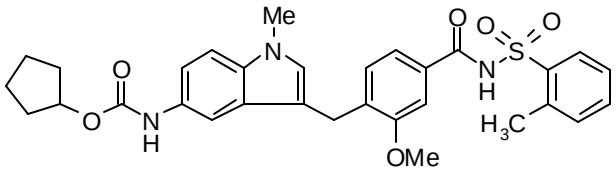
Inflammatory mediator receptor antagonists

Small molecule receptor antagonists for a number of inflammatory mediators have been developed. The cysteinyl leukotrienes have assumed a central role in asthma and in drug development with $CysT_1$ receptor antagonists such as montelukast (**12**), pranulakast (**13**), and zafirlukast (**14**) being the first new treatment for asthma in 25 years.[9]



Montelukast (**12**)

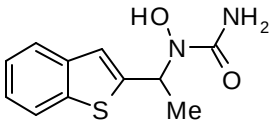
Pranulakast (**13**)



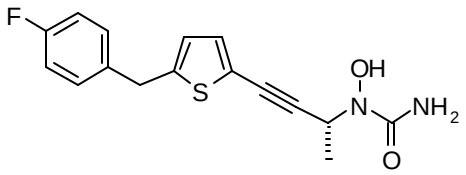
Zafirlukast (**14**)

In a biological system for the regulation of various cell functions arachidonic acid (**15**) 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. Studies on the metabolism of arachidonic acid in poly morphonuclear leukocytes (PMLS) led to the recognition of a new class of pro- inflammatory products called leukotrienes. The enzyme 5-LO catalyzes the conversion of arachidonic acid to leukotrienes.[10]

Much evidence has accumulated implicating LTs in disease states having inflammatory components, including psoriasis, asthma, and allergy. In the hope of finding anti-inflammatory drugs with reduced side-effects (or) greater efficacy, a major effort has been mounted by the pharmaceutical industry over the past decade to identify either selective inhibitors of 5-LO (or) dual inhibitors of CO and 5-LO. A wide variety of agents have been reported as 5-LO inhibitors, through the untiring efforts of medicinal chemists.[11] 5-LO inhibitor, 5-LO-activating protein antagonist, and CysL-T receptor antagonists are three classes of LT modulators, and subsequently as drug targets now in clinical practice. Zileuton (**16**) is a selective orally active inhibitor of 5-LO proven to exert anti-inflammatory and anti-allergic effects in animal models and humans.[12] Another lead discovery, ABT-761 (**17**) is undergoing final clinical trials with potent 5- LO inhibiting activity and minimal side effects.[13] CI-1004 (**18**) is a dual inhibitor of lipoxygenase and cyclooxygenase-2 (COX-2) that is currently under development as a potential treatment for asthma.[14]



Zileuton (**16**)

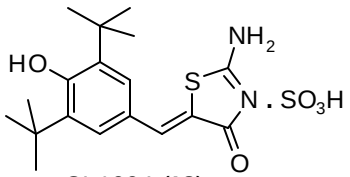


ABT-761 (**17**)

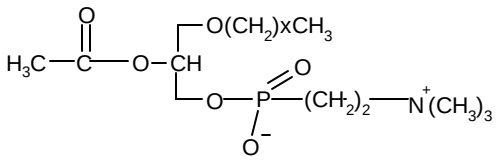
Platelet activating factor

Platelet activating factor (PAF),[15] chemically identified as 1-*O*-alkyl-2-*O*-acetyl-*Sn*-glycero-3-phosphocoline (**19**) has been implicated as a mediator of pathophysio-logical reactions in various animal models as well as in human disease.[16] PAF is produced by a variety of inflammatory cells such as basophils, platelets, and macrophages. PAF exerts a wide variety of biological actions including degrannulation, platelet aggregation and

cardiac dysfunction. 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.



CI-1004 (**18**)



Structural formula of platelet activating factor (**19**)

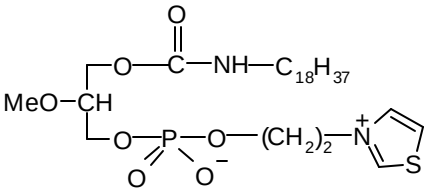
Binding of PAF to its specific receptor is thought to be the first step necessary to display its biological functions both *in vitro* and *in vivo*. The affinity and number of binding sites appear to correlate with tissue and species specificity of the biological effects of PAF.[17] It has become increasingly likely that PAF has multiple effects, which may be relevant in many human diseases, and recent activity in studying these effects has been as anticipated consequence.

PAF antagonists

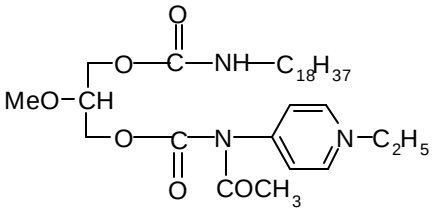
Specific and potent PAF receptor antagonists are valuable tools in the elucidation of the patho physiological roles of PAF and are expected to be of clinical importance. A variety of structurally diverse antagonists of the binding of PAF to its receptors have been reported. These compounds have been obtained by three different chemical approaches.[16]

Non-constrained backbone: In this series, the antagonists derive directly from the PAF framework. The first compound described in this family is CV 3988 (**20**), which is an orally active, potent antagonist and is specifically inhibited PAF induced platelet aggregation. A new CV analogue, CV 6209 (**21**) is developed that is about 80 times more potent than CV 3988. Replacement of phosphoryl ethyl thiazolium moiety of CV 3988 with a hepta methylene thiazolium on C_3 yielded another group of antagonists among

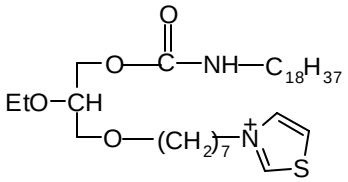
which ONO-6240 (**22**) is the most potent one. The potent antagonists were obtained by replacing the phosphoryl group by an ester linkage i.e., RU 45703 (**23**).



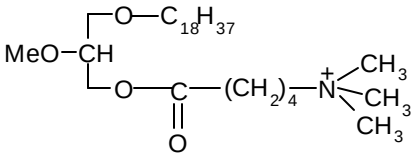
CV 3988 (**20**)



CV 6209 (**21**)

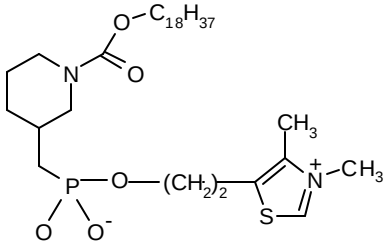


ONO - 6240 (**22**)

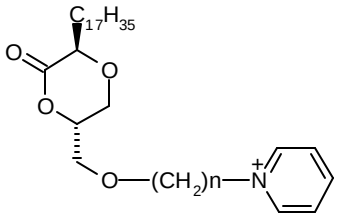


RU 45703 (**23**)

Constrained backbone: Moderately active PAF antagonists were produced from cyclisation of the PAF framework such as SRI 63-073 (**24**) and dioxanone type compound (**25**). SRI 63-073 inhibits PAF induced human and guinea pig platelet aggregation.

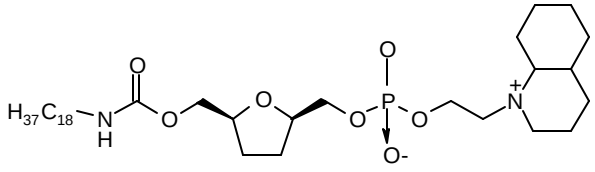


SRI 63-073 (**24**)



Dioxanone (**25**)

Tetrahydrofuran derivatives: The most potent one in this series, which is having PAF

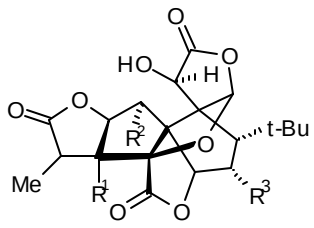
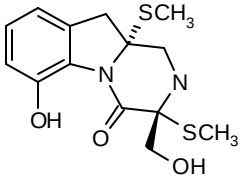
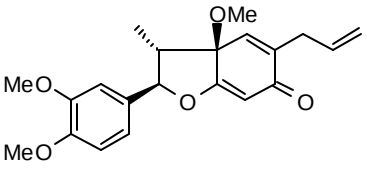


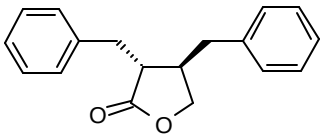
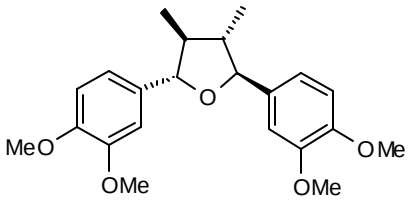
SRI 63-441 (**26**)

framework is, SRI 63-441 (**26**) that is a specific inhibitor of PAF-induced human platelet aggregation.

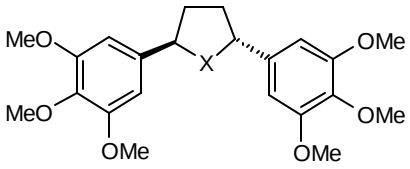
Natural products: The most promising chemical series of PAF inhibitors include natural products isolated from various bacterial strains (Table -I).

Table -I

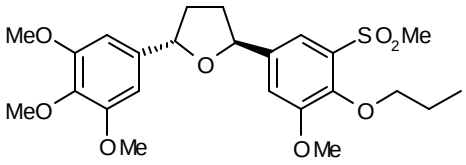
Natural Product Class	**Example**
1. Terpenes	 Ginkgolides (**27**)
2. Gliotoxin and related compounds	 Gliotoxine (**28**)
3. Lignans	
a. Benzofuranoid neolignans	 Kadsurenone (**29**)

b. Butanolide derived lignans	 Dibenzyl butyrolactone (**30**)
c. Substituted furanoid lignans	 Galbelgin (**31**)

Synthetic compounds: Systematic synthetic study from the natural tetrahydrofuran framework led to the synthesis of dinor type 'C' tetrahydrofuran lignan L-652, 731 (**32**) and thio analogue L- 653, 150 (**33**).[15]



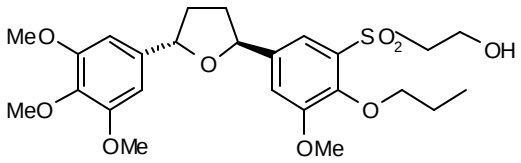
X = O; L - 652, 731 (**32**)
X = S; L - 653,150 (**33**)



L - 659, 989 (**34**)

In the type C tetrahydrofuran series, 2,5-diaryl tetrahydrofurans have been investigated for their role as PAF antagonists. In general, *trans*- isomers have been found to be more potent than *cis*- isomers. Further, structure activity studies indicated that more potent PAF antagonists contained an electron-withdrawing group on one but not both aromatic rings. These features are incorporated in L-659, 989 (**34**)[18] in which a metabolically stable methyl sulfone serves as the electron withdrawing functional unit and

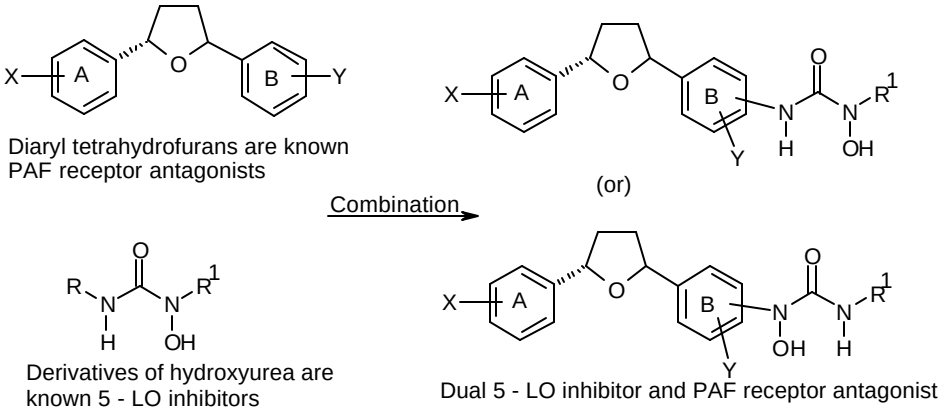
a trimethoxy aryl ring is appended at C_5. In order to achieve increased metabolic activity and pharmacokinetic profile, polar head group modifications were investigated from which the (-)- (2S, 5S)-*trans*-isomer of MK-287 (**35**)[19] emerged as a potent, specific and orally active PAF receptor antagonist and chosen for clinical trial for asthma.



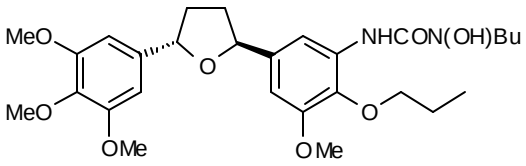
MK 287 (**35**)

Since both PAF and leukotriene are released simultaneously from leukocytes 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 therapeutic advantages in terms of efficacy and pharmacodyanamics over agents which inhibits either mediator alone.

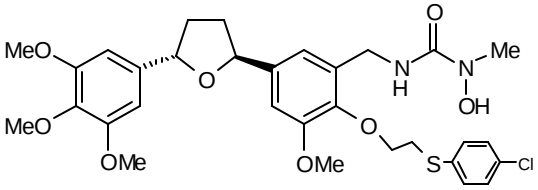
The basic knowledge that 2,5-diaryl tetrahydrofurans are PAF inhibitors while hydroxy ureas are potent 5-LO inhibitors, the introduction of hydroxy urea functionality onto certain scaffolds carrying THF skeleton should provide the candidates with dual inhibition. The inhibiting activity of hydroxy urea derivatives is probably due to chelation of Fe^{+3} required for oxidative catalysis in leukotriene biosynthesis.



Recently, Cytomed Inc. has reported CMI-206 (**36**)[20] and CMI-392 (**37**)[21] as potent dual 5-LO and PAF inhibitors, which are currently being evaluated in human clinical trials as novel inflammatory agents. CMI-392 showed very potent and balanced activities against both 5-LO and PAF and more potent than Zileuton (**16**) in 5-LO inhibition and equally potent as MK-287 (**35**) as PAF antagonist.



CMI - 206 (**36**)

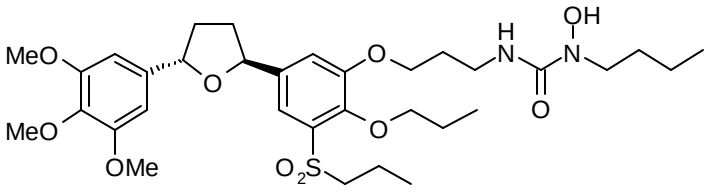


CMI - 392 (**37**)

In conclusion, the old drugs, which are now used as mainstay therapy, mainly, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroides are flawed with their limited efficacy and inadequate safety profiles. Several gene targets that control cell influx and activation, inflammatory mediator release and activity, and tissue proliferation and degradation have been identified. Since multiple gene products have been identified at the site of inflammation, there has been a surge of interest in identifying intracellular signaling targets, including transcription factors that control inflammatory gene expression, and are amenable to drug discovery. The recent advances in the pathophysiology of asthma, together with advances in drug development bodes well for the introduction of rational therapy in 21st century where leukotriene antagonists boom as promising drugs in the days ahead.[22]

Present Work

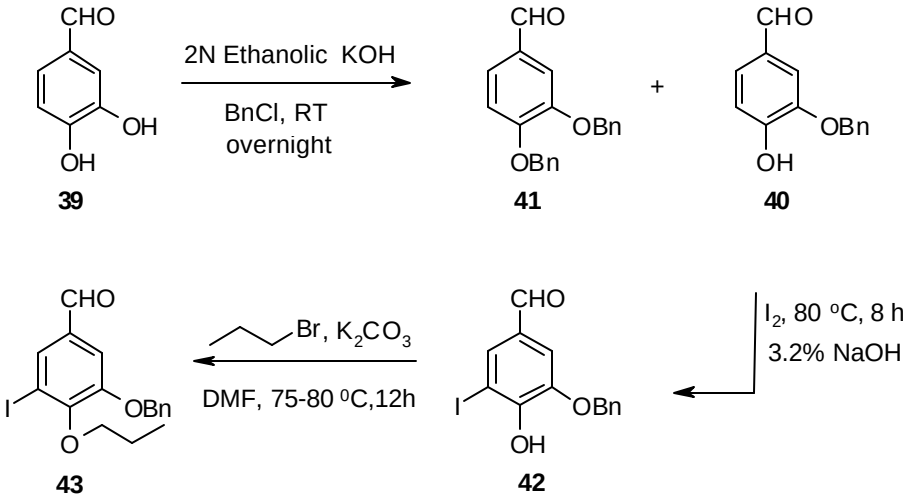
Today, at the start of 21st century, the search for new anti asthmatic agents remains unabated. The reason is that there is currently no complete cure for asthma; presently treatment of condition depends primarily upon inhaled glucocorticoides to reduce inflammation and inhaled bronchodilators to reduce symptoms. Such treatments are far from idea and significant effort is being directed in both academic and commercial laboratories to the development of more efficacious and safer drugs, especially those that are orally active. The path-breaking advances in understanding the pathology of asthma and subsequent discovery of new drug targets, together with tremendous burst of innovation in drug development, have propelled the pharmaceutical majors for the introduction of safer and efficacious drugs. In this scenario, Cytomed Inc. USA has recently announced the development of CMI-546, (±)-*trans*–2-{3-[3-(N^1-butyl-N^1-hydroxyureidyl) propoxy]-4-propoxy-5-(propylsulfonyl)phenyl}-5-(3,4,5-trimethoxyphenyl) tetrahydrofuran (**38**) as a potent dual 5-LO inhibitor and PAF receptor antagonist there by adjucating as the curator for chronic asthma.[23] The molecule having spectacular pharmacological and pharmacodyanamic properties and enriched with rich 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 (**38**).



CMI - 546 (**38**)

Our synthetic endeavor started from the commercially available 3,4-dihydroxy benzaldehyde (**39**). It was reacted with benzyl chloride and 2N ethanolic KOH solution to give a mixture of monobenzyl ether and dibenzylether derivatives (**40** and **41**), separated by crystallization.[24] The monobenzyl ether derivative (**40**) was treated with iodine in 3.2 % aq. NaOH solution at 80 ^{o}C to afford the iodo derivative (**42**) in 77% yield. The structure of **42** 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 **42** was alkylated with *n*-propyl bromide in presence of K_2CO_3 in DMF at 75-80 ^{o}C affording the propyl ether derivative (**43**) in 82 % yield (scheme 1). The ^{1}H NMR spectrum of **43** showed characteristic peaks at 1.03 (triplet), 1.82 (sextet) and 4.05 (triplet) ppm corresponding to the propyl group. The remaining chemical shifts were in complete agreement with the assigned structure.

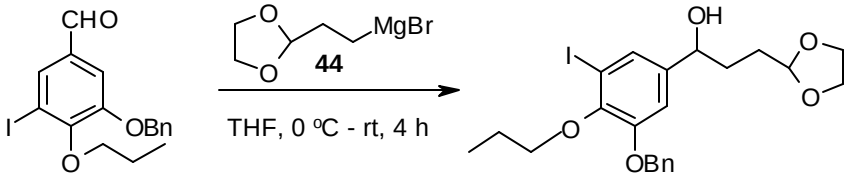
Scheme 1



The aldehyde (**43**) was treated with the Grignard reagent **44**[25] [obtained from 2-(2-bromoethyl)-1,3-dioxolane and magnesium in THF] to afford the alcohol **45** in 78% yield (scheme 2). The structure of **45** was confirmed by its ^{1}H NMR spectrum that showed a

triplet at 0.98, two sets of multiplets between 1.66-2.0 and 3.75-4.0 ppm. The rest of the spectrum was in agreement with the assigned structure. The FAB MS spectrum showed highest mass peak at m/z 498 which corresponded to molecular ion peak of **45**.

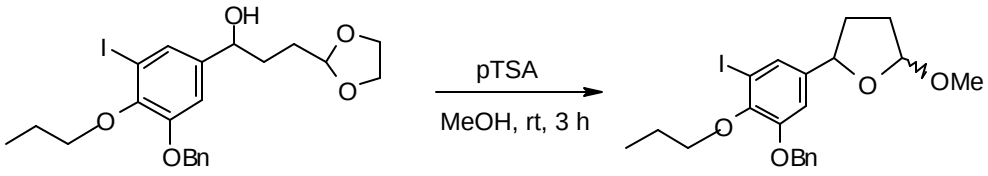
Scheme 2



43 **45**

In order to cleave the acetal group present in compound **45**, it was treated with *p*TSA in methanol to give **46** in 85% yield (scheme 3). The hydrolysis with concomitant formation of methyl acetal was observed to give **46** whose structure was supported by spectroscopic data.

Scheme 3

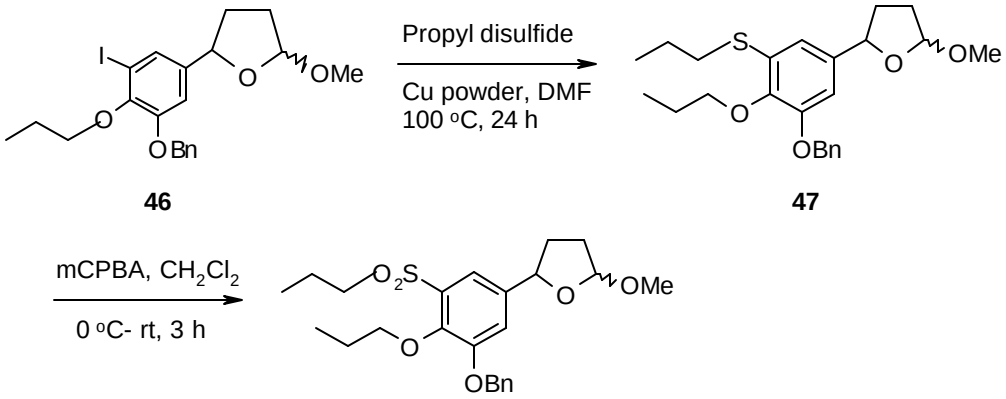


45 **46**

Displacement of iodine with sulfide has been performed by making use of Ullmann conditions.[26] Thus, **46** was treated with propyl sulfide and copper powder in DMF at 100 °C to afford **47** in 65% yield. The ^{1}H NMR spectrum of **47** showed a characteristic triplet at 2.86 ppm corresponding to S-CH_2 group. The structure of **47** was further confirmed by mass spectrum in which highest mass peak was found at m/z 416 (M^+). The sulfide **47** was oxidized with *m*CPBA in CH_2Cl_2 to give the sulfone **48** as a solid in 80% yield (scheme

4). In the ^{1}H NMR spectrum of **48** the methylene protons adjacent to SO_2 group showed a downfield shift and appeared at 3.40 ppm. The aromatic protons also showed downfield trend due to SO_2 group. The structure of **48** was further supported by its mass spectrum which showed molecular ion peak at m/z 448.

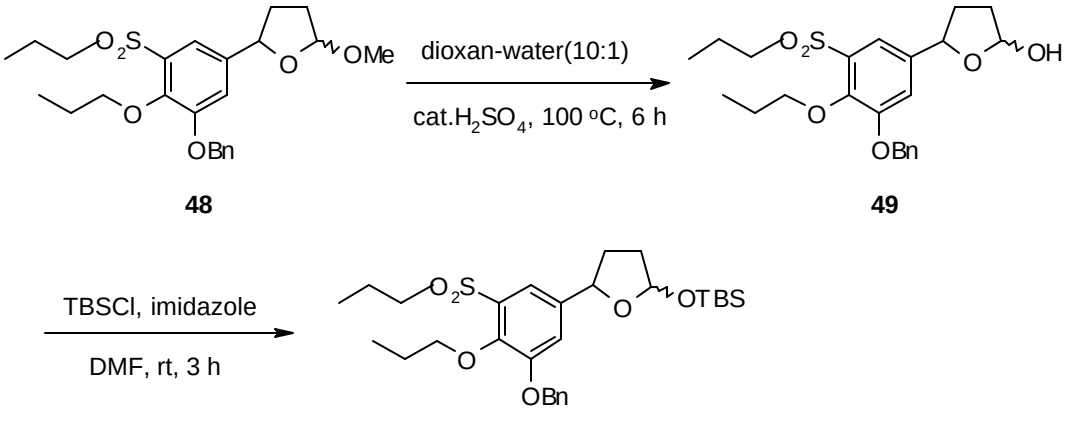
Scheme 4



46 **47** **48**

Hydrolysis of methyl acetal group of compound **48** was accomplished in presence of H_2SO_4 in a mixture of dioxan-water under reflux. The formed lactol derivative **49** was scrutinized by ^{1}H NMR spectrum which clearly showed absence of methoxy group. The FAB MS spectrum gave molecular ion peak at m/z: 434 (M^+). The free hydroxyl group in

Scheme 5



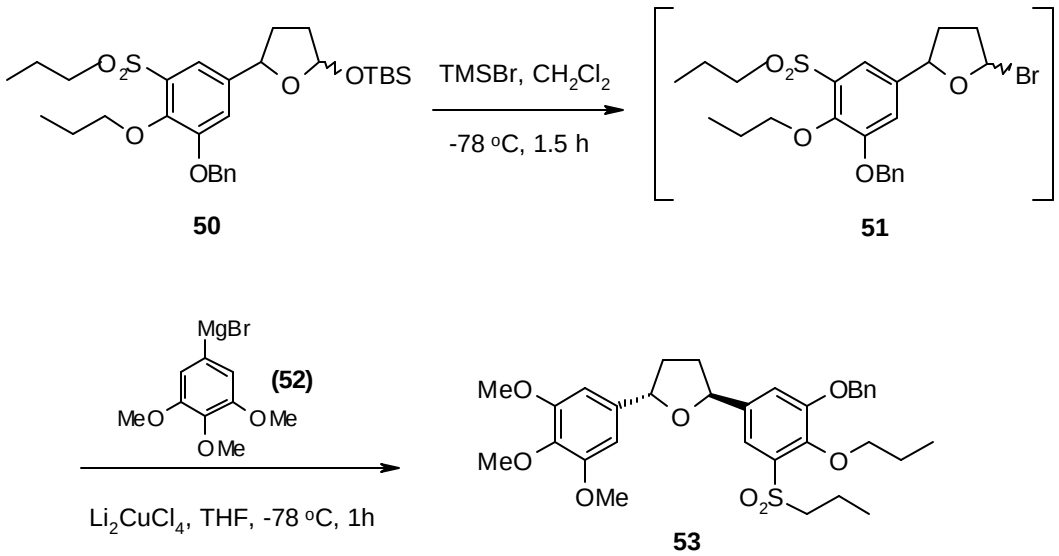
48 **49** **50**

49 was protected as its TBS ether derivative with TBDMS-Cl and imidazole in DMF at 25 ^{o}C to afford **50** as a 2:1 mixture of diastereomers in 95% yield (scheme 5). The ^{1}H NMR spectrum of **50** revealed the typical singlets due to TBS group at 0.13 and 0.90 ppm. The anomeric proton appeared at 5.68 ppm as a broad doublet. The FAB MS spectrum of **50** revealed highest mass peak at m/z: 491 corresponding to the loss of *tert.* butyl group.

Installation of the Trimethoxyphenyl Ring

Our next aim was to introduce the trimethoxyphenyl segment at C-5 of tetrahydrofuran ring with a view to complete the synthesis of CMI-546 (**38**). Although several approaches[26-33] have been found in the literature for this endeavor, we believe that the most promising in terms of controlling relative and absolute stereochemistry was the Corey's approach.[34] Accordingly, silyl acetal **50** was treated with TMSBr at –78 ^{o}C to afford the corresponding bromo derivative (**51**), which being rather unstable was used immediately. To the above reaction vessel, at – 78 ^{o}C a mixture of 3,4,5-trimethoxyphenylmagnesiumbromide (**52**)[19] and Li_2CuCl_4 in THF was added. This led to

Scheme 6

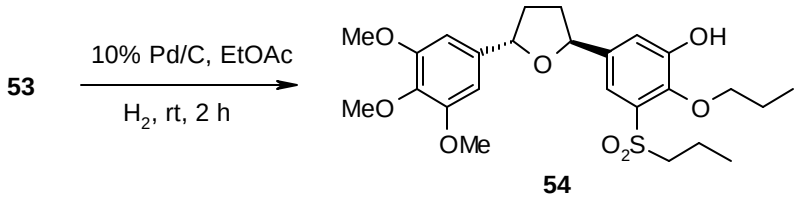


50 **51** **53**

the C-C bond formation at C-5 giving rise the product **53** in 65 % yield (scheme 6). The structure of **53** was derived from the spectroscopic data. In the ^{1}H NMR spectrum of **53** three methoxyl groups appeared at 3.83 (singlet, 3H) and 3.88 (singlet, 6H) ppm. The protons at C-2 and C-5 were observed as a multiplet between 5.08–5.27 ppm. The structure of **53** was also supported by its FAB MS data [585 (M^++1)] and HRMS (Calcd. for $C_{32}H_{40}O_8S$: 585.2536. Found: 585.2522).

Unmasking of *O*-Bn group in **53** was accomplished by hydrogenolysis in presence of 10 % Pd/C in ethyl acetate to give **54** as a solid material in 63 % yield (scheme 7). The conspicuous absence of signals due to benzyl group was noticed in the ^{1}H NMR spectrum of **54**. The FAB MS spectrum showed the highest mass peak at m/z: 495 corresponding to M^++1.

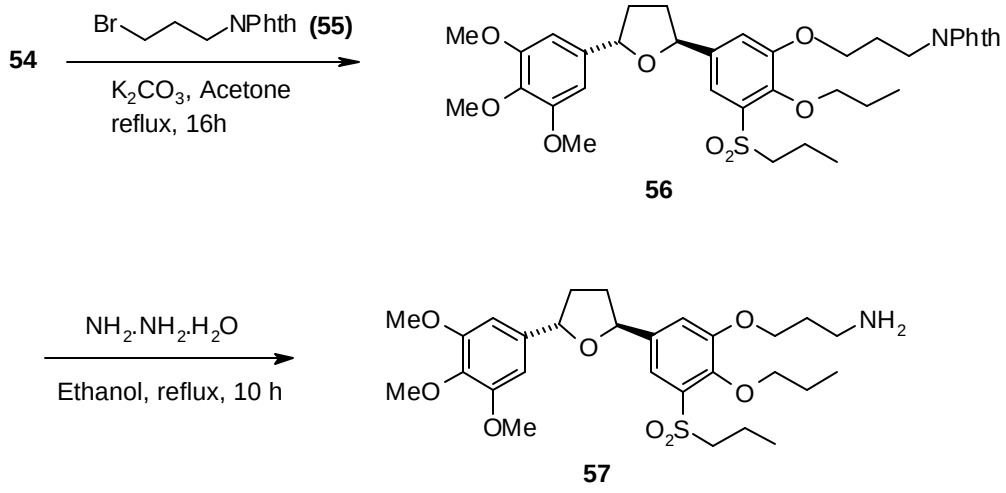
Scheme 7



Our next concern was to introduce *N*-hydroxyurea segment at the free phenolic group of **54**. For this endeavor, first the *O*-alkylation with 3-phthalimido-1-bromopropane (**55**) in presence of K_2CO_3 in acetone under reflux was performed to afford the phthalimido derivative **56** in 92 % yield. The structure of the product **56** was confirmed by the ^{1}H NMR spectrum which showed two multiplets between 7.69-7.75 and 7.80-7.88 ppm typical of the phthalimide group. In the IR spectrum characteristic absorptions at 1775 and 1715 cm^{-1} due to phthalimido carbonyls were observed. The FAB MS spectrum gave a highest mass peak at m/z: 681 attributed to M^++1. The HRMS

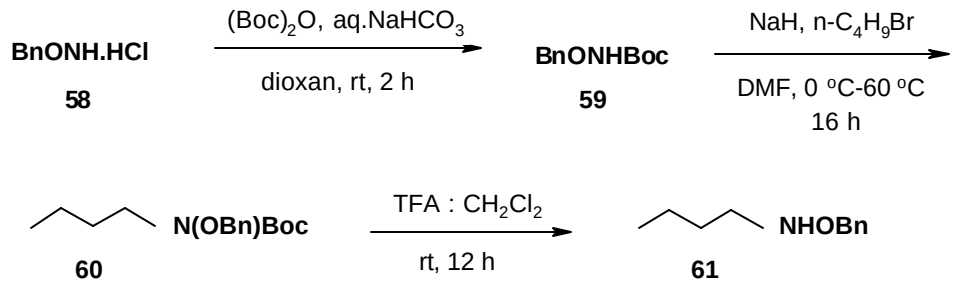
(Calcd. for $C_{36}H_{43}NO_{10}S$: 681.2607. Found: 681.2650) was consistent with **56**. The deprotection of phthalimide group present in **56** using hydrazine monohydrate in refluxing ethanol provided the amine **57** (scheme 8).

Scheme 8



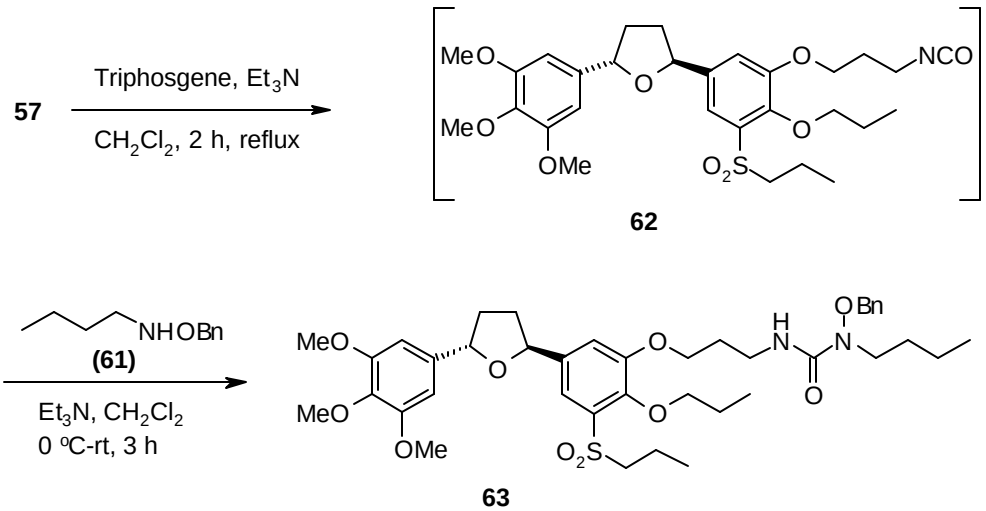
It is pertinent to mention that many hydroxamic acids and hydroxy ureas act as potent 5-LO inhibitors. In order to optimize dual 5-LO inhibition and PAF receptor antagonist activities in a single molecule, the introduction of butyl *N*-ureidyl functionality was sought. Both *cis* and *trans* isomers containing a medium size alkyl group are the most active in both rat basophilic leukemia (RBL) cell extracts and PAF assays. Thus, butyl *N*-(*O*-benzyl)amine (**61**) was prepared as shown in scheme 9. The *O*-benzyl hydroxylamine hydrochloride (**58**) in aqueous $NaHCO_3$ was treated with $(Boc)_2O$ to afford the carbamate **59** whose structure was confirmed by the ^1H NMR spectrum. The carbamate **59** was alkylated with NaH and butyl bromide in DMF to give **60**, the acidolysis of which with trifluoroacetic acid in CH_2Cl_2 provided butyl *N*-(*O*-benzyl)amine (**61**). The ^1H NMR spectrum of **61** showed a triplet at 0.92 (CH_3), a multiplet at 1.27-1.57 ($2xCH_2$) and a triplet at 2.89 ppm (CH_2N). The benzylic protons appeared as a singlet at 4.65 ppm.

Scheme 9



The final endeavor was appending of *N*-hydroxyureidyl moiety to **57** which was effectively accomplished with triphosgene in the presence of triethylamine in CH_2Cl_2 to produce an isocyanate intermediate (**62**) not isolated for being unstable. The isocyanate (**62**) was immediately treated with butyl *N*-(*O*-benzyl)amine (**61**) and triethylamine to afford **63** in 82% yield (scheme 10). The structure of **63** was unambiguously derived from the relevant chemical shifts in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **63** showed singlets at 4.72 (benzylic methylene) and 7.30 ppm (phenyl) corresponding to N^1-OBn group. The triplet due to NH appeared at 5.74 ppm while the CH_2N showed another triplet at 3.48 ppm. The IR spectrum of **63** showed the characteristic absorptions at 3440

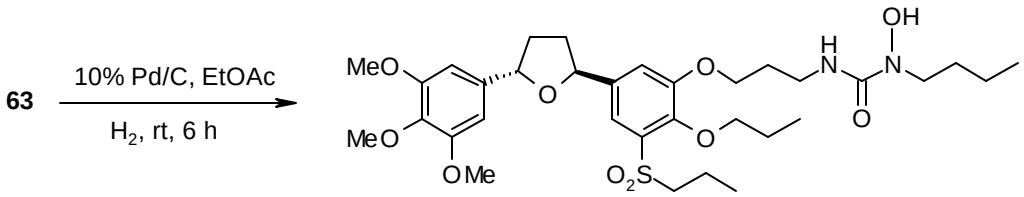
Scheme 10



and 1685 cm^{-1} corresponding to NH and C=O stretchings respectively. The FAB MS spectrum with the highest mass peak at m/z: 757(M^++1) and HRMS data (Calcd. for $C_{40}H_{56}N_2O_{10}S$ (M^++1): 757.3733. Found: 757.3721) were also in favour of the structure.

Hydrogenation of **63** in the presence of 10% Pd/C in ethyl acetate at normal temperature and pressure afforded the target molecule CMI-546 (**38**) in 72% yield (scheme 11). The structural verification was performed with ^1H NMR, IR and mass spectral analyses.

Scheme 11



CMI-546 (38)

The ^1H NMR spectrum of CMI-546 (**38**) revealed characteristic singlets due to methoxy groups. The signals due to C-2 and C-5 ring junction protons appeared as a multiplet between 5.1-5.22 ppm. In the aromatic protons chemical shift region, the two protons of trimethoxy phenyl group appeared as a singlet at 6.54 ppm. While those from the substituted phenyl sulphone ring appeared at 7.20 (d, J = 1.4 Hz) and 7.42 (d, J = 1.4 Hz) ppm. The IR spectrum of CMI-546 (**38**) showed the amide carbonyl absorption at 1650 cm^{-1} whereas the OH and NH groups were observed at 3410 and 3230 cm^{-1} respectively. The structure of CMI-546 (**38**) was further confirmed by FAB MS [m/z: 667 (M^++1)] and HRMS [Calcd. for $C_{33}H_{50}N_2O_{10}S$(M^++1): 667.3264. Found: 667.3287] spectral data.

The above synthetic endeavor provided diastereomeric mixture of (R, R) and (S, S)-CMI-546. In order to obtain both the diastereomers in enantiomerically pure form,

chiral HPLC separation was attempted. Thus (R, R)-CMI-546 and (S, S)-CMI-546 were easily separated on chiracel OD HPLC column using 40% isopropanol in hexane as mobile phase. The diastereomers were analyzed by ^{1}H NMR spectra, which were identical for both while optical rotations had opposite sign.

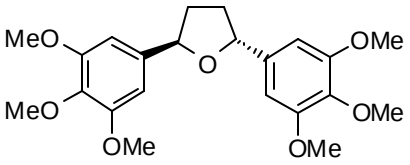
Conclusion

In summary, a concise and expedient synthesis of CMI-546 (**38**) has been achieved. The compound has been submitted to Cytomed Inc. USA for evaluating pharmacological profile (PK/PD) in the treatment of asthma, especially for 5-LO inhibition. The future will envision that such small molecule inhibitors and potential analogues hammer out asthma and enhance the human life for new world order.

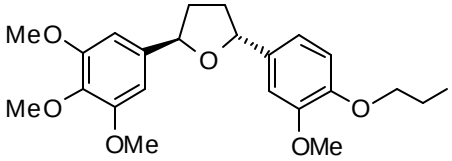
SECTION II

New and efficient Method for (±)-*trans*-2,5-Diaryltetrahydrofurans

Recent efforts in identifying and developing effective agents to treat inflammatory and immune disorders have led to the synthesis of a family of important compounds, *trans*-2, 5-diaryl tetrahydrofurans.[16] These compounds have been found to be particularly useful in treating psoriasis and atopic dermatitis, both chronic inflammatory disorders affecting millions of people. A number of pharmaceutical compositions containing these drugs have been prepared.[23] However, there remains a need for an improved synthetic route to prepare these valuable agents. This part describes the synthesis of (±)-*trans*-2,5-diaryltetrahydrofurans (**32** & **64**). It is more appropriate to discuss about the related work before describing the present work.



32



64

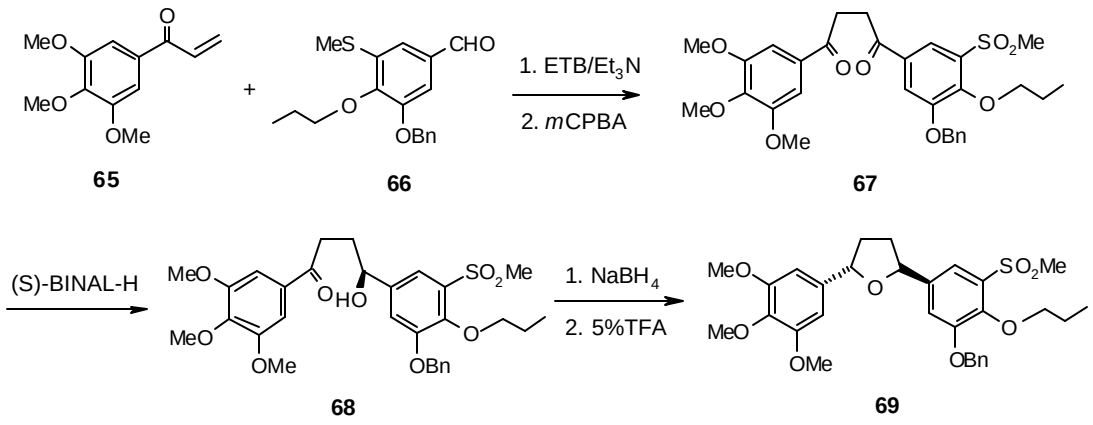
Approaches Toward *trans*-2,5-Diaryltetrahydrofurans

(i) Merck's approach[26]

Regio- and enantioselective reduction of 1,4-diketone **67** and subsequent cyclisation in the presence of acid are the key steps in Merck's approach. Condensation of vinyl ketone **65** with aldehyde **66** in the presence of thiazolium catalyst and Et_3N gave 1,4-diketone (**67**). Regio- and enantioselective reduction of diketone **67** to (1S)–isomer (**68**) was accomplished with (S)-BINAL-H as the reducing agent. Reduction of **68** with $NaBH_4$

followed by cyclisation with 5 % TFA provided the optically pure *trans*-2,5-diaryl tetrahydrofuran **69** (55 %) and its *cis*- isomer (20 %) (scheme 12).

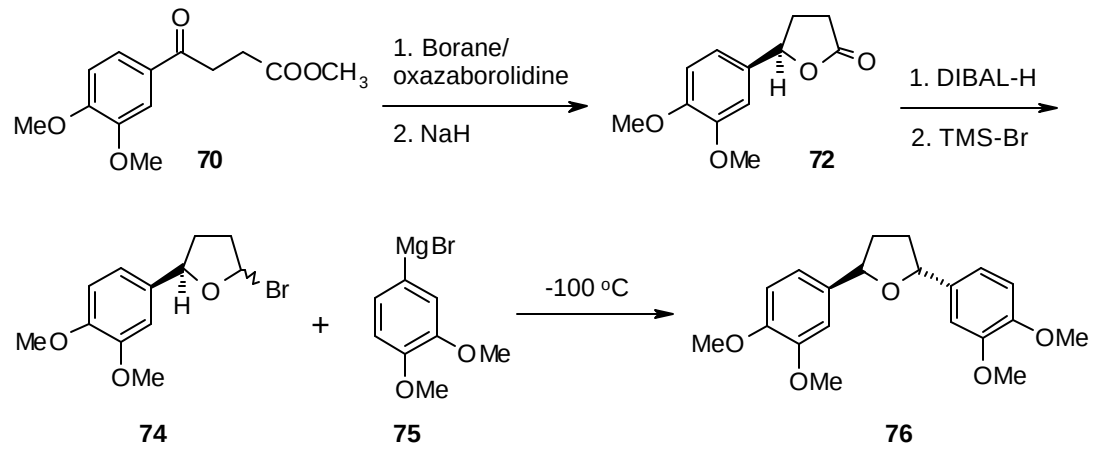
Scheme 12



(ii) Corey's approach[34]

Enantioselective reduction of γ-keto ester **70** with oxazaborolidine catalyst and coupling of bromo ether **74** with aryl magnesiumbromide are the key steps in Corey's approach. Accordingly, reduction of ketone **70** with borane in the presence of oxazaborolidine catalyst gave the corresponding alcohol (**71**) in high enantiomeric excess, which upon treatment with NaH furnished the lactone (**72**). Reduction of lactone **72** with DIBAL-H afforded the lactol (**73**), which was converted to the corresponding a-

Scheme 13

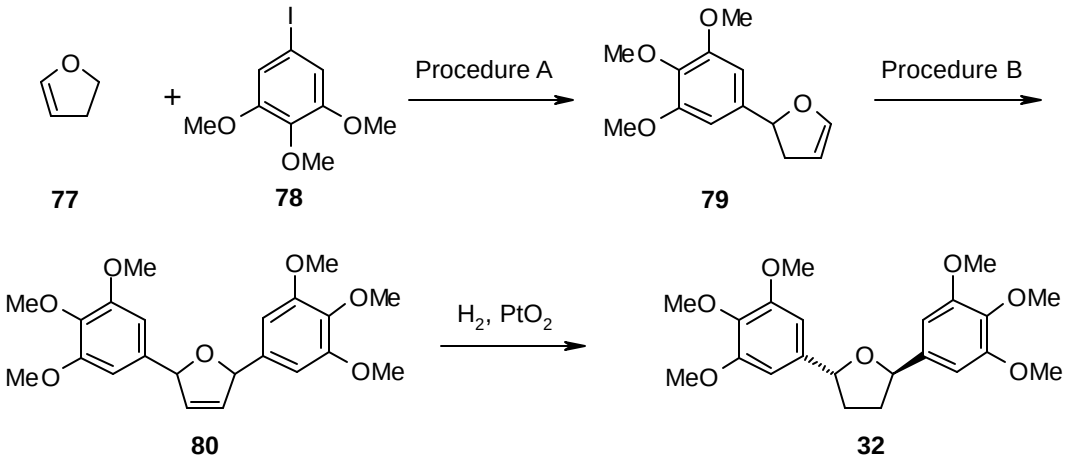


bromoether (**74**) using TMS-Br at –78 °C. Coupling of this brome ether **74** with 3,4-dimethoxyphenylmagnesiumbromide (**75**) at – 100 °C afforded the *trans* product **76** selectively (*cis*-*trans*: 1:10) (scheme 13).

(iii) Larock's approach[33]

Regio- and stereoselective palladium catalyzed diarylation of 2,3-dihydrofuran and subsequent hydrogenation are the key steps in Larock's approach. Accordingly, diarylation of 2,3-dihydrofuran (**77**) using $Pd(OAc)_2$, Ph_3P, *n*-Bu_4Cl, KOAc, and aryl iodide (**78**) in DMF at room temperature (procedure A) followed by second arylation using $Pd(OAc)_2$, Ph_3P, Ag_2CO_3, and aryl iodide (**78**) in CH_3CN at 80 °C (procedure B) and subsequent hydrogenation over PtO_2 catalyst afforded the desired *trans*-2,5-diaryl tetrahydrofuran (**32**) as the only product (scheme 14).

Scheme 14



77 **78** **79** **80** **32**

Procedure A: 2.5% $Pd(OAc)_2$, 2.5% Ph_3P, n-Bu_4NCl, KOAc, DMF, rt.

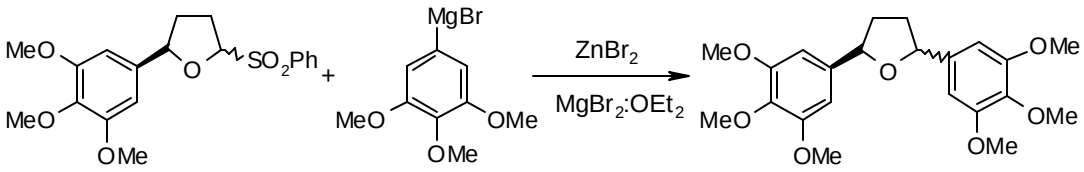
Procedure B: 3-4% $Pd(OAc)_2$, 9% Ph_3P, Ag_2CO_3, **78**, CH_3CN, 80 °C.

(iv) Ley's approach[31]

Diastereoselective C-C bond formation *via* nucleophilic substitution of 2-benzenesulphonyl tetrahydrofuran is key step in Ley's approach. Accordingly, various

sulphones were prepared and direct substitution with organozinc reagents gave 2,5-diaryl tetrahydrofurans (scheme 15).

Scheme 15

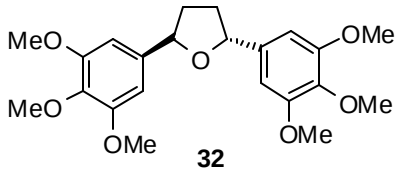


81 **52** **32**

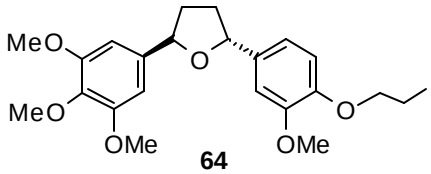
cis :trans-15:85

All these syntheses have involved a number of synthetic steps, frequently proceeded in low overall yield, which prompted us to undertake rapid and efficient synthesis of these compounds.

In the preceding section we have demonstrated a novel approach to synthesize CMI-546 (**38**). Propelled by the success, we also undertook the synthesis of bioactive compounds **32** and **64** using a new and efficient method.



32



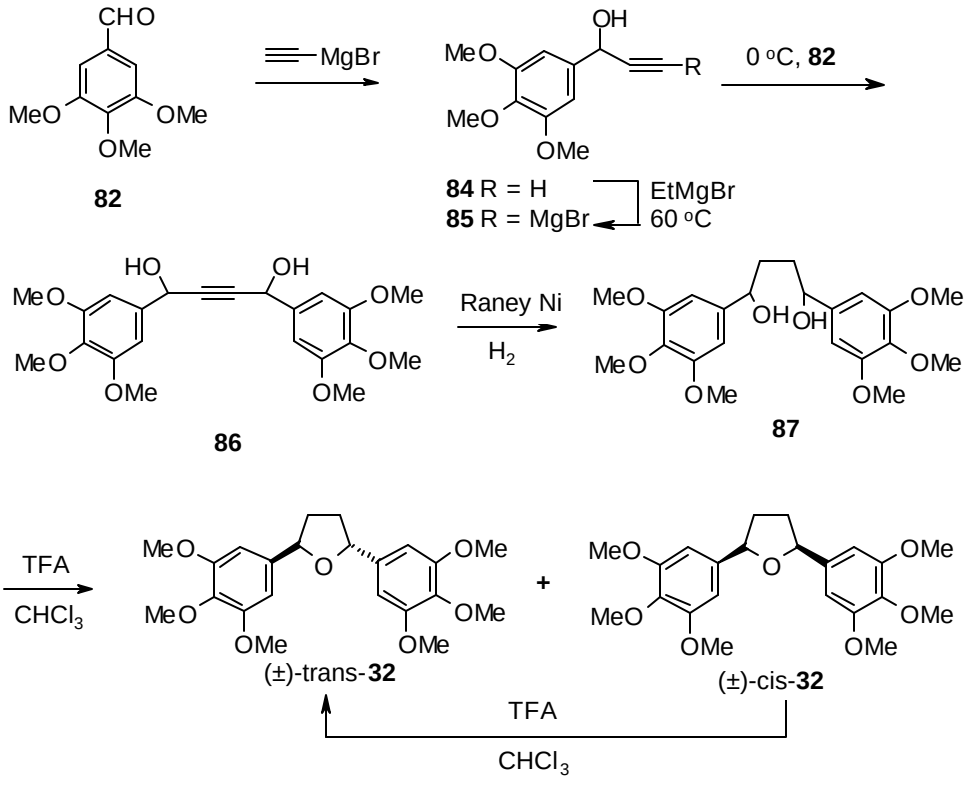
64

Synthesis of (±)-*trans*-2, 5-Bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (32)

We began the synthesis of **32** from commercially available 3,4,5-trimethoxybenzaldehyde (**82**) which on treatment with acetylene magnesiumbromide (**83**) (generated *in situ* by acetylene gas and ethyl magnesiumbromide in THF) at room temperature gave the alkyne derivative **84** in 62 % yield. The structure of **84** was confirmed by the ^{1}H NMR spectrum in which the acetylene proton appeared as a singlet at 2.65 ppm. Our next aim was to install the second trimethoxyphenyl carbinol segment at the free acetylenic carbon. For this endeavor, **84** was treated with ethyl magnesium bromide at 60 ^{o}C which led to the generation of acetylenic Grignard. Subsequent reaction with **82** gave the diol **86**. Compound **86**, being a C_2-symmetrical molecule, showed in its ^{1}H NMR spectrum resonances corresponding to half molecule only. For example, resonances due to benzylic methine proton appeared at 5.50, methoxyl groups at 3.85, and aromatic protons at 6.75 ppm, all as singlets. Its structure was further supported by mass [m/z: 418 (M^+)] spectroscopic data.

Compound **86** was hydrogenated in the presence of Raney Ni to reduce the triple bond. In the ^{1}H NMR spectrum of the resulting product **87** presence of a multiplet between 1.75-1.85 ppm due to methylene protons clearly confirmed the reduction step. The diol **87** was cyclized in presence of 5 % TFA in $CHCl_3$ to provide a mixture of (±)-*cis*- and (±)-*trans*-2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (**32**). The mixture was not separated but subjected to prolong treatment of TFA. This treatment converted *cis* isomer into *trans*. Thus the reaction mixture contained predominantly the *trans*-isomer along with minor quantity of *cis*-isomer (**32**) (scheme 16). Upon work-up and crystallization, pure *trans*-isomer of **32** was obtained. The ^{1}H NMR and mass spectral data and melting point of compound **32** were identical with reported values.[31b]

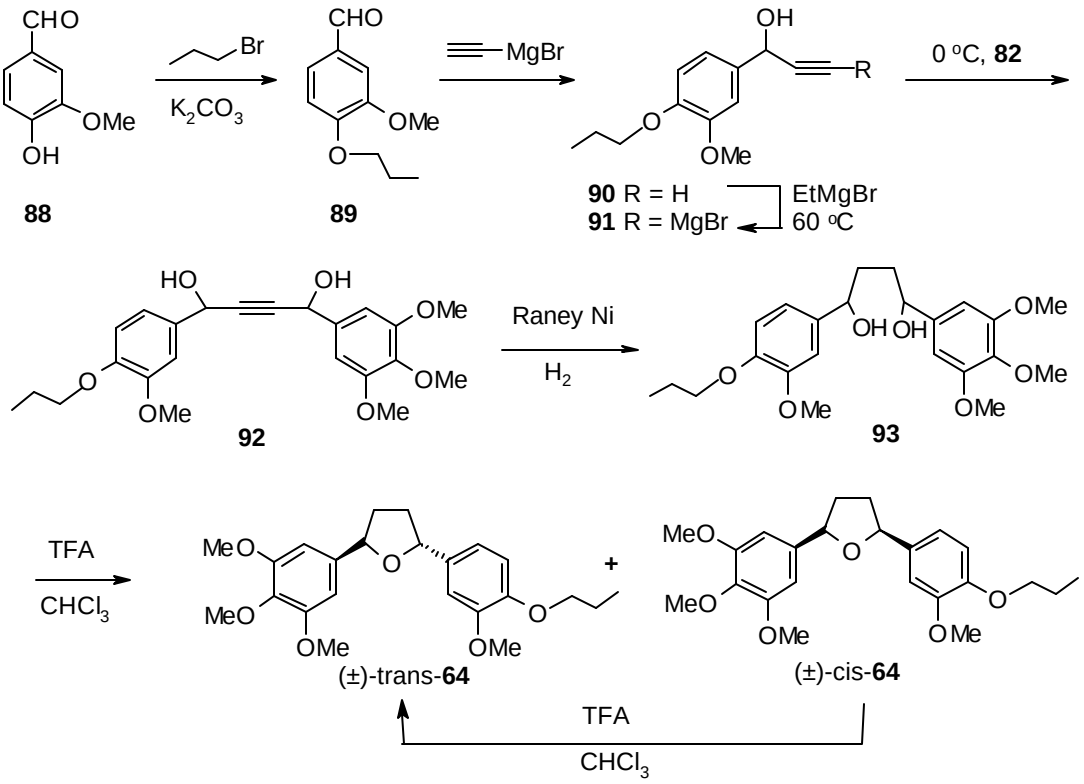
Scheme 16



Synthesis of (±)-*trans*-2-(3,4,5-Trimethoxyphenyl)-5-(3-methoxy-4-propoxyphenyl) tetrahydrofuran (64)

Synthesis of **64** was initiated from commercially available vanillin (**88**) which was *O*-alkylated with 1-bromopropane in the presence of K_2CO_3 to give the propyl ether derivative **89**. The compound on treatment with acetylenemagnesiumbromide gave the alkyne **90** whose structure was confirmed by the ^1H NMR and mass spectroscopic data. The Grignard reagent (**91**) (generated *in situ* by compound **90** and ethyl magnesiumbromide in THF at 60 ^oC) was treated with **82** to give the diol **92**. The ^1H NMR spectrum of **92** showed singlets due to methoxyl groups at 3.73 (6H), 3.77 (3H), and 3.80 (3H) ppm while rest of the spectrum was in complete agreement with the assigned structure. Reduction with Raney Ni followed by cyclisation in the presence of TFA in $CHCl_3$ provided the crystalline (±)-*trans*-**64** (scheme 17). The ^1H NMR and EI mass spectral data clearly confirmed the structure of **64**.

Scheme 17



In conclusion, a short and efficient method for the synthesis of (±)-*trans*-2,5-diaryl tetrahydrofurans has been developed. One of our colleague successfully synthesized CMI-392 (**37**) using this methodology.[35] Further, enantioselective synthesis of *trans*-2,5-diaryl tetrahydrofurans is under progress.

3-Benzyloxy-4-hydroxybenzaldehyde (40)

Benzyl chloride (41.7 ml, 362.3 mmol) was slowly added to a solution of 3,4-dihydroxybenzaldehyde (**39**) (50.0 g, 362.3 mmol) in 2N ethanolic potassium hydroxide (400 ml). The reaction mixture was stirred overnight under nitrogen, ethanol was concentrated and then diluted with ice water. The solution was extracted with diethyl ether to remove the dibenzyl ether derivative (**41**). The aqueous layer was acidified with concentrated hydrochloric acid and extracted with CH_2Cl_2, dried (Na_2SO_4) and concentrated to a volume of 200 ml and light petroleum (50 ml) introduced. After overnight stirring the starting material (**39**)(18.2 g) was filtered, while the mother liquor concentrated to afford **40** (25.6 g, 31 %).

m.p.: 109-111 °C; lit[24] m.p.: 110-113 °C.

^{1}H NMR d **(200 MHz, $CDCl_3$):** 5.06 (s, 2 H), 6.39 (s, 1 H), 6.95 (d, 1 H, *J* = 8.0 Hz), 7.12-7.4 (m, 7 H) and 9.57 (s, 1 H).

3-Benzyloxy-4-propoxy-5-iodobenzaldehyde (43)

To a solution of **40** (20.0 g, 87.7 mmol) in aq. 3.2 % NaOH solution (200 ml), iodine (22.3 g, 87.7 mmol) was added and the mixture heated at 80 °C for 8 h. The reaction mixture was cooled to room temperature and concentrated hydrochloric acid added and the precipitated solid filtered. It was recrystallized with isopropanol to give **42** (23.9 g, 77 %).

m.p.: 136-138 °C.

A mixture of **42** (20.0 g, 56.5 mmol), potassium carbonate (10.1 g, 73.4 mmol) and 1-bromopropane (7.7 ml, 84.7 mmol) in DMF (50 ml) was stirred at 75-80 °C for 12 h. The reaction mixture was diluted with water and extracted with diethyl ether which was

dried (Na_2SO_4), and concentrated. The crude product was purified on silica gel using ethyl acetate: light petroleum (1:9) as eluent to give **43** (18.3 g, 82 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.03 (t, 3 H, *J* = 7.2 Hz), 1.82 (sextet, 2 H), 4.05 (t, 2 H, *J* = 6.6 Hz), 5.15 (s, 2 H), 7.3-7.45 (m, 6 H), 7.83 (d, 1 H, *J* = 1.5 Hz), 9.79 (s, 1 H).

1-(1,3-Dioxolan-2-yl)-3-hydroxy-3-(3-benzyloxy-4-propoxy-5-iodophenyl)propane (45)

The solution of Grignard reagent (**44**)[25] [prepared from 1.21 g of magnesium and 6.85 g of 2-(2-bromoethyl)-1,3-dioxolane in THF (50 ml)] was cannulated to a solution of **43** (10.0 g, 25.2 mmol) in THF (100 ml) at 0 ^oC. The reaction mixture was allowed to stir at room temperature for 4 h, quenched with saturated aq. NH_4Cl solution and extracted with ethyl acetate. The organic extract was washed with brine solution, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to afford **45** (9.8 g, 78 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.98 (t, 3 H, *J* = 7.0 Hz), 1.66-2.0 (m, 6 H), 2.65 (br s, 1 H), 3.75-4.0 (m, 6 H), 4.56 (t, 1 H, *J* = 5.1 Hz), 4.83 (t, 1H, *J* = 3.9 Hz), 5.03 (s, 2 H), 6.90 (d, 1 H, *J* = 1.6 Hz), 7.25-7.42 (m, 6 H).

IR (NEAT): 3480 cm^{-1}.

FAB MS (Na): 498 (M^+).

2-Methoxy-5-[3-benzyloxy-4-propoxy-5-(propylthio)phenyl]tetrahydrofuran (47)

A solution of **45** (9.4 g, 18.8 mmol) and *p*TSA (0.1 g) in methanol (100 ml) was stirred at room temperature for 3 h, neutralized with triethylamine and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:9) as eluent to afford **46** (7.5 g, 85%).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.05 (t, 3 H, *J* = 7.0 Hz), 1.73-2.3 (m, 6 H), 3.4 (s, 3 H), 3.95 (t, 2 H, *J* = 6.8 Hz), 4.82-5.2 (m, 4 H), 6.97 (d, 1 H, *J* = 1.4 Hz), 7.28-7.48 (m, 6 H). **FAB MS:** 468(M^+).

Compound **46** (5.0 g, 10.7 mmol), copper powder (2.71 g, 42.7 mmol) and propyl disulfide (4.2 ml, 26.7 mmol) in DMF (30 ml) were heated to 100 ^oC for 24 h and filtered through Celite. To the filtrate ethyl acetate and NH_4Cl: NH_4OH (9:1) solutions were added and the layers separated. The organic layer was washed with brine solution, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel using ethyl acetate-light petroleum (1:9) as eluent to afford **47** (2.9 g, 65 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.05 (q, 6 H, *J* = 6.81 Hz), 1.6-2.3 (m, 8 H), 2.86 (t, 2 H, *J* = 7.26 Hz), 3.4 (s, 3 H), 3.98 (t, 2 H, *J* = 6.12 Hz), 4.88-4.96 (m, 1 H), 5.05-5.1 (m, 3 H), 6.82 and 6.85 (2 s, 2 H), 7.3-7.45 (m, 5 H).

FAB MS: 416 (M^+).

2-Hydroxy-5-[3-benzyloxy-4-propoxy-5-(propylsulfonyl)phenyl]tetrahydrofuran (49)

A solution of **47** (2.8 g, 6.73 mmol) and *m*-chloroperoxybenzoic acid (3.13 g, 18.2 mmol) in dry CH_2Cl_2 (20 ml) was stirred at room temperature for 3 h, quenched with the addition of saturated aq.sodium thiosulfate followed by aq. $NaHCO_3$ solution. The solid was filtered through Celite, washed with CH_2Cl_2. The organic layer was separated, washed with brine solution, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to give **48** (2.41 g, 80 %).

m.p.: 168-170 ^oC; **^{1}H NMR** d **(200 MHz, $CDCl_3$):** 1.03 (q, 6 H, *J* = 6.8 Hz), 1.63-2.5 (m, 8 H), 3.3-3.42 (m, 5 H), 4.13 (t, 2 H, *J* = 6.75 Hz), 4.96-5.23 (m, 4 H), 7.2 (d, 1 H, *J* = 1.4 Hz), 7.3-7.48 (m, 6 H); **FAB MS (Na):** 448 (M^+).

Compound **48** (2.15 g, 4.79 mmol) and conc. H_2SO_4 (0.3 ml) in dioxan-water (10:1) was heated under reflux for 6 h, neutralized with $NaHCO_3$ and evaporated. The

residue was extracted with ethyl acetate, washed with brine solution, dried (Na_2SO_4) and concentrated to provide a residue which was purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **49** (1.25 g, 60 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.86-0.97 (m, 6 H), 1.5-2.5 (m, 8 H), 2.7 and 2.9 (broad singlets, 1 H), 3.28-3.38 (m, 2 H), 4.08 (t, 2 H, *J* = 6.3 Hz), 4.95 (t, 0.5 H, *J* = 7.2 Hz,), 5.05-5.2 (m, 2.5 H), 5.55 (br s, 0.5 H), 5.7 (d, 0.5 H, *J* = 2.3 Hz), 7.15 (d, 1 H, *J* = 1.4 Hz), 7.3-7.45 (m, 6 H).

FAB MS: 434 (M^+).

2-(*O-tert*-Butyldimethylsilyl)-5-[3-benzyloxy-4-propoxy-5-(propylsulfonyl)phenyl] tetrahydrofuran (50)

A solution of **49** (1.0 g, 2.3 mmol), imidazole (0.33 g, 4.83 mmol) and *t*-butyldimethylsilyl chloride (0.38 g, 2.76 mmol) in dry DMF (5 ml) was stirred for 3 h, diluted with water and extracted with diethyl ether. The ether layer was washed with brine solution, dried (Na_2SO_4), and concentrated. The crude product was purified on silica gel using ethyl acetate: light petroleum (1:15) as eluent to give **50** (1.2 g, 95 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.13 (2 s, 6 H), 0.9 (s, 9 H), 1.0 (q, 6 H, *J* = 6.5 Hz), 1.6-2.1 (m, 7 H), 2.4- 2.58 (m, 1 H), 3.3-3.4 (m, 2 H), 4.13 (t, 2 H, *J* = 5.9 Hz), 5.1-5.18 (m, 3 H), 5.68 (br d, 1 H), 7.16 (d, 1 H, *J* = 1.3 Hz), 7.32-7.46 (m, 6 H).

FAB MS: 491 (M - ^{t}bu).

(3,4,5-Trimethoxyphenyl) magnesium bromide (52)

To a suspension of Magnesium (0.2 g, 8.54 mmol) in THF (10 ml) under nitrogen, dibromoethane (0.1 ml) and 3,4,5-trimethoxy-bromobenzene (0.1 g, 0.4 mmol) were added. After 15 min., the reaction was initiated at which time remaining phenyl bromide derivative (2.17 g, 8.8 mmol) in THF (20 ml) was introduced. The reaction mixture was

stirred for 18 h. The generated Grignard reagent was cooled in ice-water bath and then a solution of dilithium tetrachlorocuprate (0.5 M in THF, 0.2 ml, 0.1 mmol) was added. The reaction was stirred at 0 °C for 15 min and used for the next coupling reaction.

(±)-*trans*-2-[3-Benzyloxy-4-propoxy-5-(propylsulfonyl)phenyl]-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (53)

To a solution of **50** (2.1 g, 3.83 mmol) in CH_2Cl_2 (20 ml) at –78 °C under N_2 was added TMSBr (0.55 ml, 4.21 mmol). After 1.5 h, the above prepared Grignard reagent (**52**) was cannulated. The mixture was stirred for 1 h at –78 °C and quenched with 10:1 saturated NH_4Cl/NH_4OH solution (15 ml). The reaction mixture was partitioned between water-CH_2Cl_2. Then organic layer was separated, washed with brine solution, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate: light petroleum (1:5) as eluent to give **53** (1.45 g, 65 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.03 (q, 6 H, *J* = 6.9 Hz), 1.67-2.05 (m, 6 H), 2.38-2.52 (m, 2 H), 3.3-3.4 (m, 2 H), 3.83 (s, 3 H), 3.88 (s, 6 H), 4.14 (t, 2 H, *J* = 7.4 Hz), 5.08-5.27 (m, 4 H), 6.57 (s, 2 H), 7.3-7.48 (m, 7 H).

FAB MS: 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-(propylsulfonyl)phenyl]–5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (54)

A mixture of **53** (1.4 g, 2.39 mmol) and Pd/C (0.2 g) in ethyl acetate (10 ml) was stirred at room temperature under hydrogen atmosphere (balloon pressure) for 2 h. The catalyst was filtered through Celite and washed with ethyl acetate. The filtrate was concentrated and the crude product purified on silica gel using ethyl acetate: light petroleum (1:3) as eluent to give **54** (0.74 g, 63 %).

m.p.: 116-118 °C.

^{1}H NMR δ (200 MHz, $CDCl_3$): 0.95 (t, 3 H, *J* = 7.7 Hz), 1.0 (t, 3 H, *J* = 6.8 Hz), 1.6-1.95 (m, 6 H), 2.3-2.42 (m, 2 H), 3.2-3.3 (m, 2 H), 3.75 (s, 3 H), 3.8 (s, 6 H), 4.03 (t, 2 H, *J* = 6.1 Hz), 5.0-5.13 (m, 2 H), 6.28 (s, 1 H), 6.5 (s, 2 H), 7.18 (d, 1 H, *J* = 1.5 Hz), 7.34 (d, 1 H, *J* = 1.5 Hz).

FAB MS: 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 (56)

A mixture of **54** (0.7 g, 1.42 mmol), potassium carbonate (0.25 g, 1.84 mmol) and *N*-(3-bromopropyl) phthalimide **(55)** (0.57 g, 2.12 mmol) in acetone (20 ml) was refluxed for 16 h. The reaction mixture was concentrated and partitioned between water and ethyl acetate. The organic layer was washed with brine solution, dried (Na_2SO_4), concentrated and the crude product purified on silica gel using ethyl acetate: light petroleum (2:5) as eluent to give **56** (0.88 g, 92 %).

m.p.: 195-196 °C.

^{1}H NMR δ (200 MHz, $CDCl_3$): 1.05 (t, 3 H, *J* = 7.1 Hz), 1.08 (t, 3 H, *J* = 6.6 Hz), 1.68-2.06 (m, 6 H), 2.2-2.3 (m, 2 H), 2.4-2.55 (m, 2 H), 3.3-3.38 (m, 2 H), 3.83 (s, 3 H), 3.9 (s, 6 H), 3.95 (t, 2 H, *J* = 6.6 Hz), 4.15 (t, 4 H, *J* = 6.1 Hz), 5.12-5.26 (m, 2 H), 6.58 (s, 2 H), 7.23 (d, 1 H, *J* = 1.5 Hz), 7.45 (d, 1 H, *J* = 1.5 Hz), 7.69-7.75 (m, 2 H), 7.8-7.88 (m, 2 H).

IR (NEAT): 1775, 1715 cm^{-1}.

FAB MS: 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-(propylsulfonyl)phenyl]-5-(3,4,5-tri-methoxyphenyl)tetrahydrofuran (57)

A solution of **56** (0.8 g, 1.17 mmol) and hydrazine monohydrate (0.2 g, 4.11 mmol) in ethanol (20 ml) was heated under reflux for 10 h and concentrated. The residue was partitioned between water and ethyl acetate. The organic layer was washed with brine solution, dried (Na_2SO_4) and concentrated to afford **57** (0.71 g) which was used in the next step without further purification.

Butyl *N*-(*O*-benzyl) amine (61)

To a solution of *O*-benzyl hydroxylamine hydrochloride **58** (5.0 g, 31.3 mmol) in dioxan (30 ml) were added aq.1M $NaHCO_3$ solution (50 ml) and di-*tert*-butyl dicarbonate (6.83 g, 31.3 mmol). The resulting milky solution was stirred for 2 h at room temperature and concentrated to remove dioxan. The P^H was adjusted to 4 with the addition of citric acid and then extracted with CH_2Cl_2. The organic layer was washed with brine solution, dried (Na_2SO_4) and evaporated to afford **59** (6.85 g, 98 %).

m.p.: 44-45 ^oC; lit[36] m.p.: 45-46 ^oC

^{1}H NMR d **(200MHz, $CDCl_3$):** 1.45 (s, 6 H), 1.52 (s, 3 H), 4.85 (s, 2 H), 7.05 (br s, 1 H), 7.35-7.42 (m, 5 H).

To a solution of **59** (6.6 g, 29.6 mmol) and sodium hydride (1.77 g, 74 mmol, 60 % dispersion in oil) in DMF (30 ml) under nitrogen was added 1-bromobutane (3.81 ml, 35.5 mmol). The reaction mixture was heated to 60 ^oC for 16 h, diluted with water and extracted with diethyl ether. The ether extract was washed with brine solution, dried (Na_2SO_4) and evaporated. The residue was purified by silica gel using ethyl acetate: light petroleum (1:10) as eluent to provide **60** (7.93 g, 96 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.93 (t, 3 H, J = 7.0 Hz), 1.22-1.4 (m, 2 H), 1.45 (s, 3 H), 1.5 (s, 6 H), 1.52-1.62 (m, 2 H), 3.38 (t, 2 H, J = 6.8 Hz), 4.8 (s, 2 H), 7.3-7.4 (m, 5 H).

A solution of **60** (7.85 g, 28.1 mmol) and trifluoroacetic acid (4.3 ml, 56.3 mmol) in CH_2Cl_2 (20 ml) was stirred at room temperature for 12 h and concentrated. The residue was taken into ethyl acetate, washed with saturated $NaHCO_3$ and brine solutions, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate: light petroleum (1:5) as eluent to give **61** (4.53 g, 90 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.92 (t, 3 H, *J* = 4.2 Hz), 1.27-1.57 (m, 4 H), 2.89 (t, 2 H, *J* = 5.8 Hz), 4.65 (s, 2 H), 7.22-7.34 (m, 5 H).

(±)-*trans*-2-{3-[3-(*N*^{1}-Butyl-*N*^{1}-benzyloxyureidyl)propoxy]-4-propoxy-5-(propylsulfonyl)phenyl}-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (63)

To a solution of **57** (0.7 g, 1.27 mmol) and triphosgene (0.19 g, 0.63 mmol) in CH_2Cl_2 (20 ml), triethylamine (0.35 ml, 2.54 mmol) was added. The reaction mixture was refluxed for 2 h and cooled at 0 ^oC, and then compound **61** (0.57 g, 3.17 mmol) and triethylamine (0.7 ml, 5.08 mmol) were added. The reaction mixture was stirred at room temperature for 3 h, quenched with water and extracted with chloroform. The organic layer was washed with brine solution, dried (Na_2SO_4) and concentrated. The crude product was purified on silica gel using ethyl acetate: light petroleum (1:3) as eluent to give **63** (0.72 g, 82 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.83-1.08 (m, 9 H), 1.2-1.4 (m, 2 H), 1.5-2.05 (m, 10 H), 2.38-2.5 (m, 2 H), 3.25-3.4 (m, 4 H), 3.48 (t, 2 H, *J* = 6.8 Hz), 3.8 (s, 3 H), 3.85 (s, 6 H), 3.94 (t, 2 H, *J* = 6.1 Hz), 4.08 (t, 2 H, *J* = 6.3 Hz), 4.72 (s, 2 H), 5.13-5.25 (m, 2 H), 5.74 (t, 1 H, *J* = 4.5 Hz), 6.58 (s, 2 H), 7.2 (d, 1 H, *J* = 1.3 Hz), 7.3 (s, 5 H), 7.48 (d, 1 H, *J* = 1.3 Hz).

IR (NEAT): 3440, 1685 cm^{-1}.

FAB MS: 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-trimethoxyphenyl)tetrahydrofuran (CMI-546) (38)

A solution of **63** (0.5 g, 0.66 mmol and 10 % Pd/C (50 mg) in ethyl acetate (5 ml) was stirred at room temperature under hydrogen atmosphere (balloon pressure) for 6 h. The catalyst was filtered through Celite, washed with ethyl acetate and concentrated. The crude product was purified on silica gel using ethyl acetate: light petroleum (3:2) as eluent to afford **CMI-546** (**38**) (0.32 g, 72 %).

m.p.: 102-104 °C.

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.83 (t, 3 H, *J* = 6.9 Hz), 0.95 (q, 6 H, *J* = 6.9 Hz), 1.22 (sextet, 2 H), 1.4-1.53 (m, 2 H), 1.6-2.05 (m, 8 H), 2.38-2.48 (m, 2 H), 3.28-3.4 (m, 6 H), 3.79 (s, 3 H), 3.83 (s, 6 H), 4.08 (t, 4 H, *J* = 6.0 Hz), 5.1-5.22 (m, 2 H), 6.03 (t, 1 H, *J* = 5.1 Hz), 6.54 (s, 2 H), 7.0 (broad peak, 1 H), 7.2 (d, 1 H, *J* = 1.4 Hz), 7.42 (d, 1 H, *J* = 1.4 Hz);

IR (NEAT): 3410, 3230, 1650 cm^{-1}.

FAB MS: 667 (M + 1).

HRMS (FAB): Calcd for $C_{33}H_{50}N_2O_{10}S$ (M + 1): 667.3264. Found, 667.3287.

Chiral Separation of CMI-546 (38) with chiral HPLC

Column: Chiracel – OD

Mobile phase: 40 % isopropanol in *n*-hexane

Flow rate: 2 ml/min

UV: 225 nm

Retention time

(a) (+) diastereomer: 15.0 min, $[\alpha]_D$ +28^o (c 1, $CHCl_3$)

(b) (-) diastereomer: 21.0 min, $[\alpha]_D$ -31^o (c 1, $CHCl_3$)

1-(3,4,5-Trimethoxyphenyl)-2-propyne-1-ol (84)

To a suspension of magnesium (1.58 g, 66.1 mmol) in dry THF (20 ml) ethyl bromide (4.9 ml, 66.1 mmol) was added. After 15 min, acetylene gas was bubbled into the above prepared solution of ethyl magnesiumbromide for 20 min at 0 ^{o}C and stirred 30 min. A solution of **82** (3.25 g, 16.5 mmol) in THF (20 ml) was introduced and after 30 min, quenched with saturated aq. NH_4Cl solution. It was partitioned between water and diethyl ether. The ether layer was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **84** (2.28 g, 62 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 2.65 (br s, 1 H), 3.82 (s, 3 H), 3.86 (s, 6 H), 5.37 (br s, 1 H), 6.75 (s, 2 H);

EI MS: 222 (M^+).

1,4-Bis-(3,4,5-trimethoxyphenyl)-2-butyne-1,4-diol (86)

A solution of ethyl magnesiumbromide (prepared from 1.38 g of magnesium and 4.3 ml ethyl bromide in THF) and **84** (3.2 g, 14.4 mmol) in THF (40 ml) was heated at 60 ^{o}C for 90 min. It was cooled to 0 ^{o}C, compound **82** (2.83 g, 14.4 mmol) in THF (15 ml) was added. After 2 h, saturated aq. NH_4Cl solution was introduced and the mixture partitioned between water and diethyl ether. The ether layer was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel with ethyl acetate-light petroleum (3:2) as eluent to afford **86** (3.91 g, 65 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 3.85 (s, 9 H), 5.5 (br s, 1 H), 6.75 (s, 2 H).

EI MS: 418 (M^+).

1,4-Bis-(3,4,5-trimethoxyphenyl)butane-1,4-diol (87)

A mixture of **86** (2 g, 4.78 mmol) and Raney Ni (0.5 g) in ethanol (10 ml) was stirred under hydrogen atmosphere at normal temperature and pressure for 6 h. The catalyst was filtered through Celite and washed with ethanol. The filtrate was concentrated

and the crude product purified on silica gel using ethyl acetate-light petroleum (4:1) as eluent to give **87** (1.61 g, 80 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.75-1.85 (m, 2 H), 3.85 (s, 3 H), 3.9 (s, 6 H), 4.60-4.70 (m, 1 H), 6.5 (s, 2 H).

EI MS: 422 (M^+).

(±)-*trans*-2,5-Bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (32)

A solution of **87** (1 g, 2.36 mmol) and TFA (0.4 ml) in chloroform (5 ml) was stirred for 24 h, partitioned between $CHCl_3$ and water. The organic layer was separated and washed with aq. NaOH solution, brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to give **32** (0.52 g, 55 %). The (±)-*trans*-isomer was recrystallized with EtOAc: hexane (1:2).

m.p.: 138-139 ^oC, lit[31b] m.p.: 139-140 ^oC.

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.92-2.07 (m, 2 H), 2.38-2.54 (m, 2 H), 3.83 (s, 12 H), 3.88 (s, 6 H), 5.01 (apparent t, 0.8 H), 5.18 (apparent t, 1.2 H), 6.61 (s, 2.5 H), 6.63 (s, 1.5 H).

EI MS: 404 (M^+).

3-Methoxy-4-propoxybenzaldehyde (89)

A mixture of vanillin (**88**) (5 g, 32.9 mmol), 1-bromopropane (4.5 ml, 49.3 mmol) and K_2CO_3 (5.9 g, 42.7 mmol) in acetone (30 ml) was refluxed for 6 h. The reaction mixture concentrated and partitioned between water and ethyl acetate. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified on silica gel using ethyl acetate-light petroleum (1:5) as eluent to provide **89** (5.56 g, 87 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.1 (t, 3 H, J = 6.52 Hz), 1.9 (sextet, 2 H, J = 6.52 Hz), 3.92 (s, 3 H), 4.05 (t, 2 H, J = 6.52 Hz), 6.92 (d, 1 H, J = 7.4 Hz), 7.35-7.42 (m, 2 H), 9.81 (s, 1 H).

1-(3-Methoxy-4-propoxyphenyl)-2-propyne-1-ol (90)

Compound **90** was prepared from **89** as described previously. A solution of acetylene magnesiumbromide (generated *in situ* from 2.47 g of Magnesium and 7.7 ml of ethyl bromide and acetylene gas in THF) and compound **89** (5 g, 25.8 mmol) in THF (50 ml) was stirred for 30 min at room temperature, after the usual workup, the crude product was purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **90** (4.02 g, 71 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.06 (t, 3 H, *J* = 6.97 Hz), 1.87 (sextet, 2 H, *J* = 6.97 Hz), 2.0 (br s, 1 H), 2.6 (s, 1 H), 3.88 (s, 3 H), 3.95 (t, 2 H, *J* = 6.51 Hz), 5.36 (br s, 1 H), 6.8 (d, 1 H, *J* = 8.37 Hz), 6.98-7.08 (m, 2 H).

EI MS: 220 (M^+).

1-(3,4,5-Trimethoxyphenyl)-4-(3-methoxy-4-propoxyphenyl)-2-butyne-1,4-diol (92)

Compound **92** was obtained from **90** as described previously. A solution of ethyl magnesiumbromide (prepared from 1.3 g of Magnesium and 4.1 ml of ethyl bromide in THF) and compound **90** (3 g, 13.6 mmol) in THF (30 ml) was heated at 60 ^oC for 90 min, then cooled to 0 ^oC and compound **82** (2.67 g, 13.6 mmol) in THF (15 ml) was added. After 2 h, it was worked up and the crude product was purified on silica gel using ethyl acetate-light petroleum (1:1) as eluent to afford **92** (3.8 g, 67 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.03 (t, 3 H, *J* = 7.27 Hz), 1.83 (sextet, 2 H, *J* = 7.27 Hz), 3.73 (s, 6 H), 3.77 (s, 3 H), 3.8 (s, 3 H), 3.9 (t, 2 H, *J* = 6.94 Hz), 5.35 (br s, 2 H), 6.68 (s, 2 H), 6.75 (d, 1 H, *J* = 7.95 Hz), 6.9-7.0 (m, 2 H);

EI MS: 424 (M^+).

1-(3,4,5-Trimethoxyphenyl)-4-(3-methoxy-4-propoxyphenyl)butane-1,4-diol (93)

A mixture of **92** (3 g, 7.21 mmol) and Raney Ni (1 g) in ethanol (20 ml) was stirred under hydrogen atmosphere at normal temperature and pressure. After 6 h, the catalyst

was filtered through Celite and washed with ethanol. The filtrate was concentrated and the crude product was purified on silica gel using ethyl acetate-light petroleum (3:2) as eluent to give **93** (2.57 g, 85 %).

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.07 (t, 3 H, *J* = 7.27 Hz), 1.75-1.95 (m, 6 H), 3.8 (s, 3 H), 3.85 (s, 9 H), 3.93 (t, 2 H, *J* = 7.27 Hz), 4.61 (apparent t, 2 H), 6.5 (s, 2 H), 6.78 (s, 2 H), 6.85 (s, 1 H).

EI MS: 402 (M^+-H_2O).

(±)-*trans*-2-(3,4,5-Trimethoxyphenyl)-5-(3-methoxy-4-propoxyphenyl)tetrahydro-furan (64)

A solution of **93** (1 g, 2.38 mmoles) and TFA (0.4 ml) in $CHCl_3$ (5 ml) was stirred for 24 h, partitioned between $CHCl_3$ and water. The organic layer was separated and washed with aq. NaOH solution, brine, dried (Na_2SO_4), concentrated and purified on silica gel with ethyl acetate-light petroleum (1:5) as eluent to afford **64** (0.6 g, 63 %). The (±)-*trans*-isomer was recrystallized with EtOAc: hexane (1:2).

m.p.: 155-157 ^oC.

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.05 (t, 3 H, *J* = 7.65 Hz), 1.8-2.08 (m, 4 H), 2.37-2.54 (m, 2 H), 3.86 (s, 9 H), 3.9 (s, 3 H), 4.0 (t, 2 H, *J* = 7.44 Hz), 5.04 (apparent t, 1.2 H), 5.16-5.27 (m, 0.8 H), 6.64-6.69 (m, 2 H), 6.84-7.02 (m, 3 H);

EI MS: 402 (M^+).

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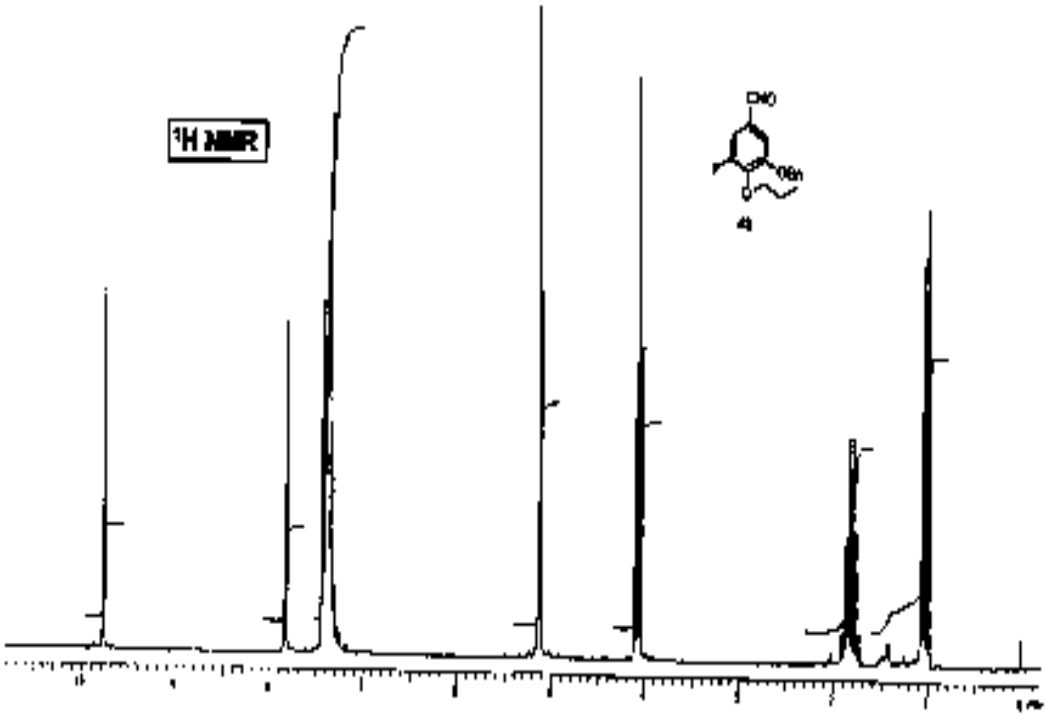
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OBn
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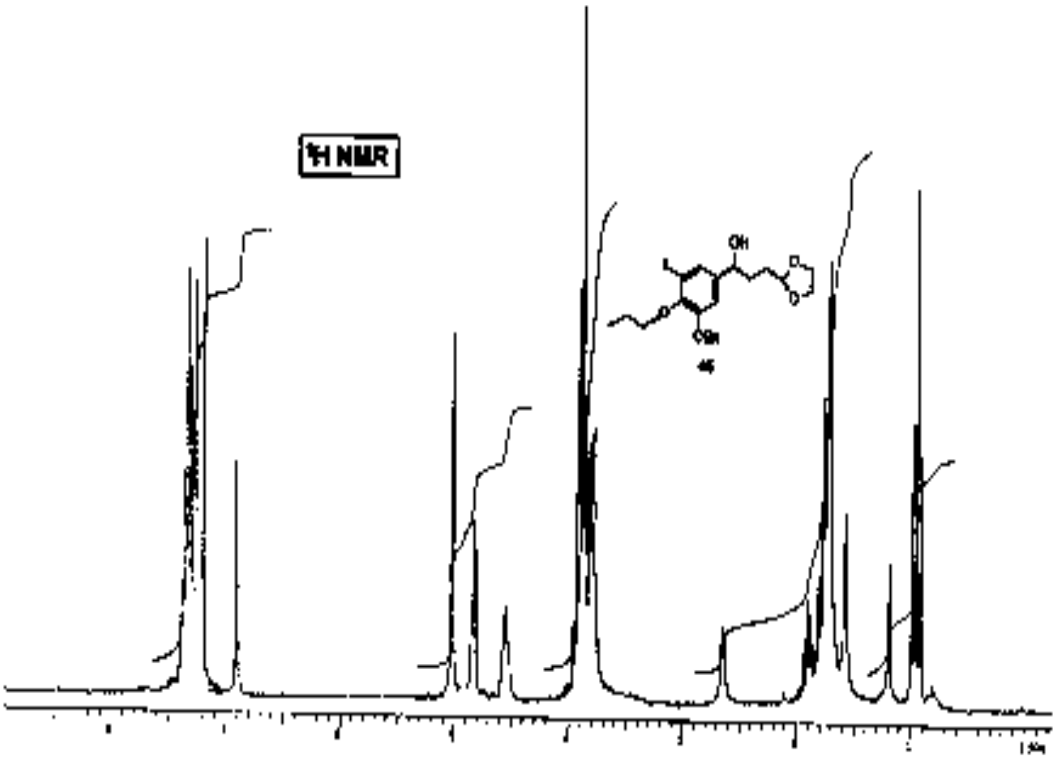
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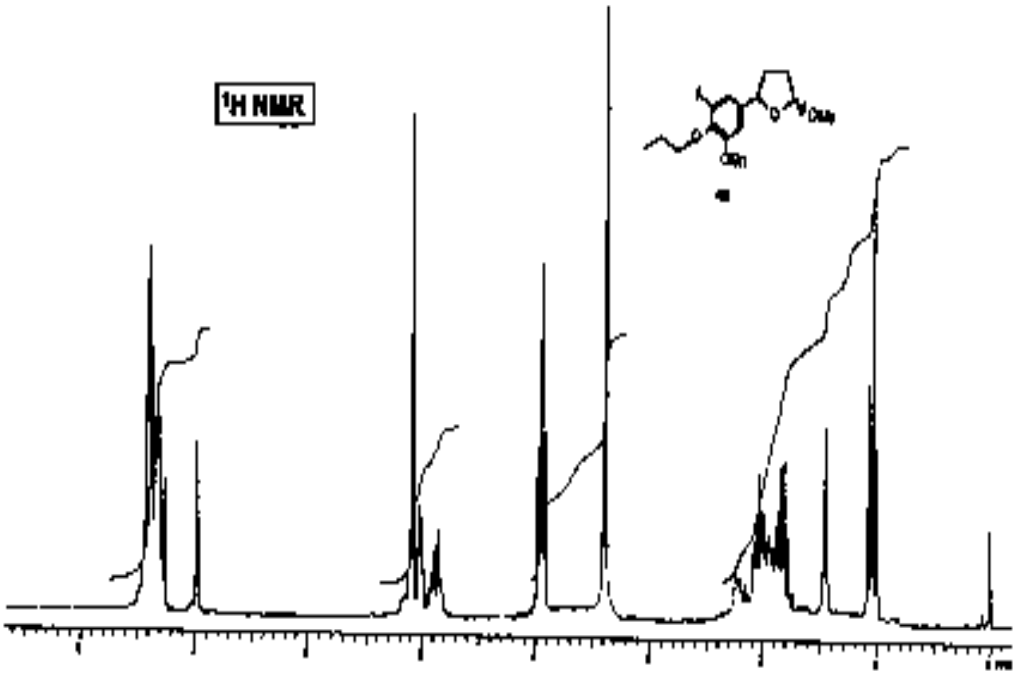
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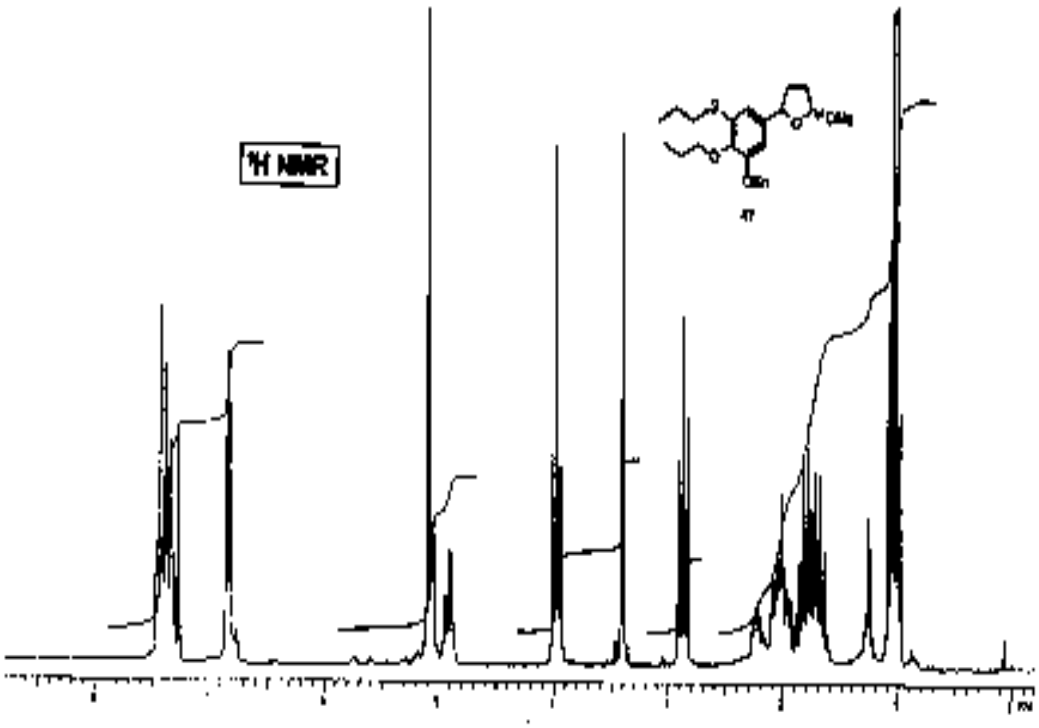
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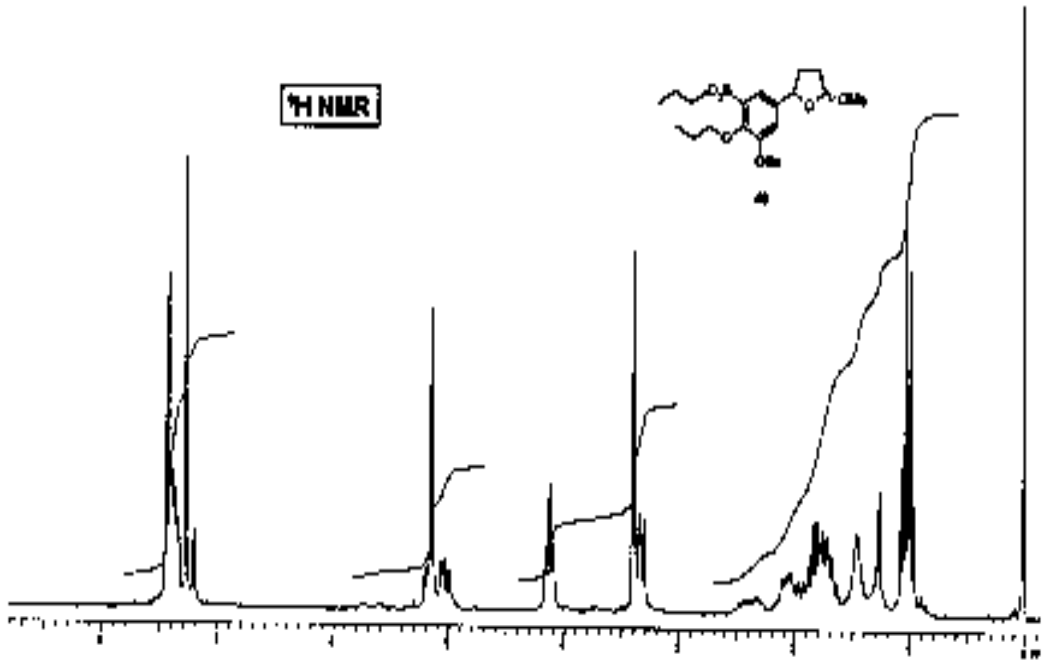
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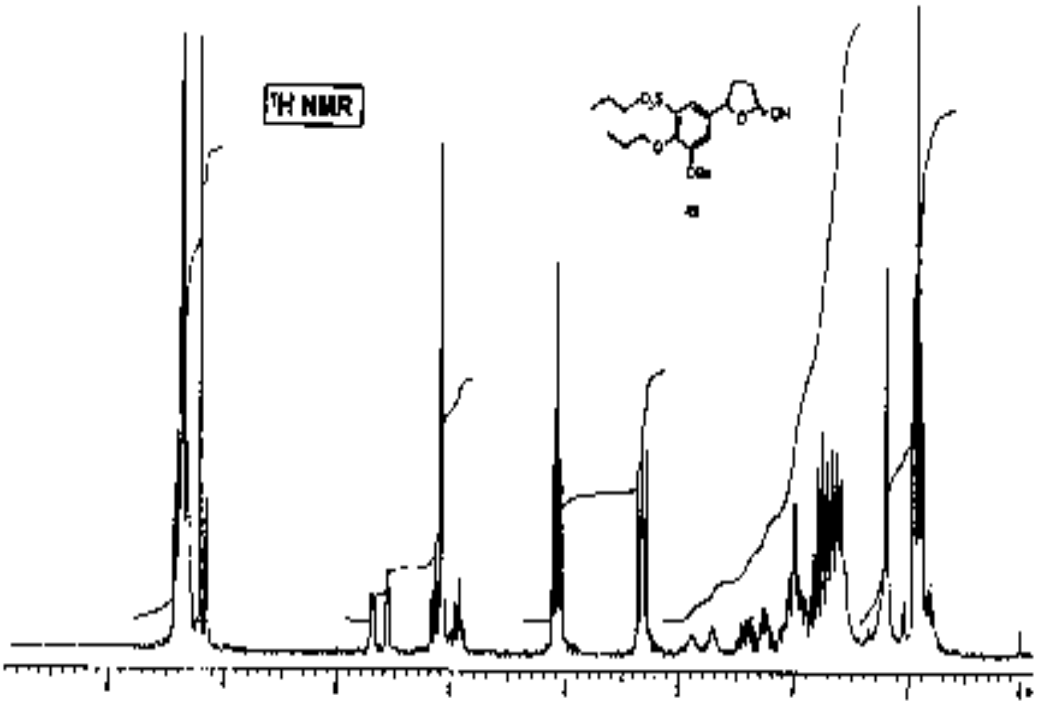
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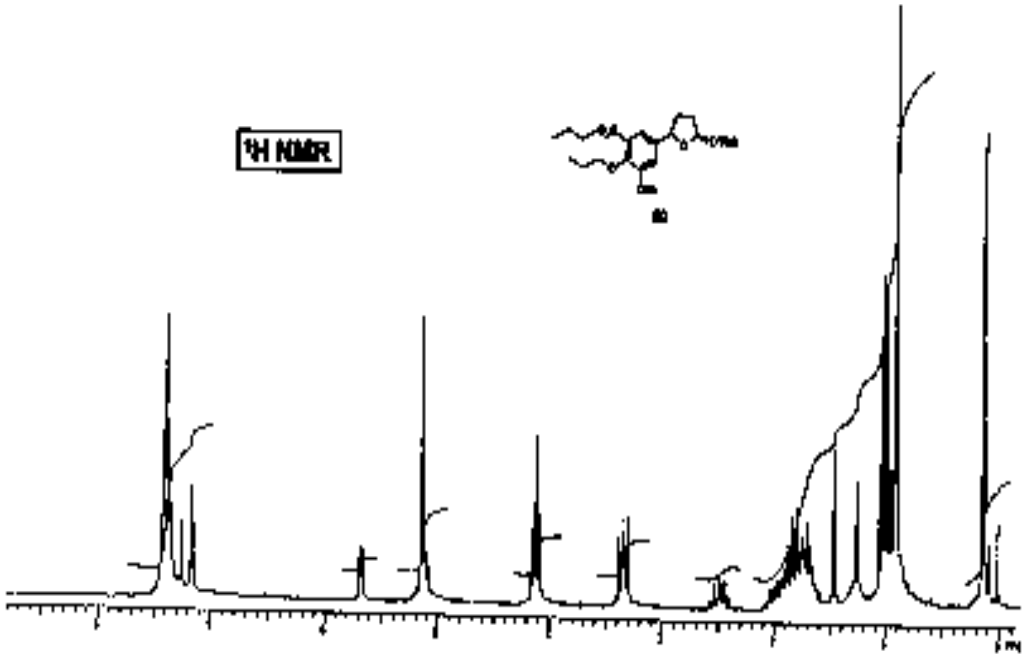
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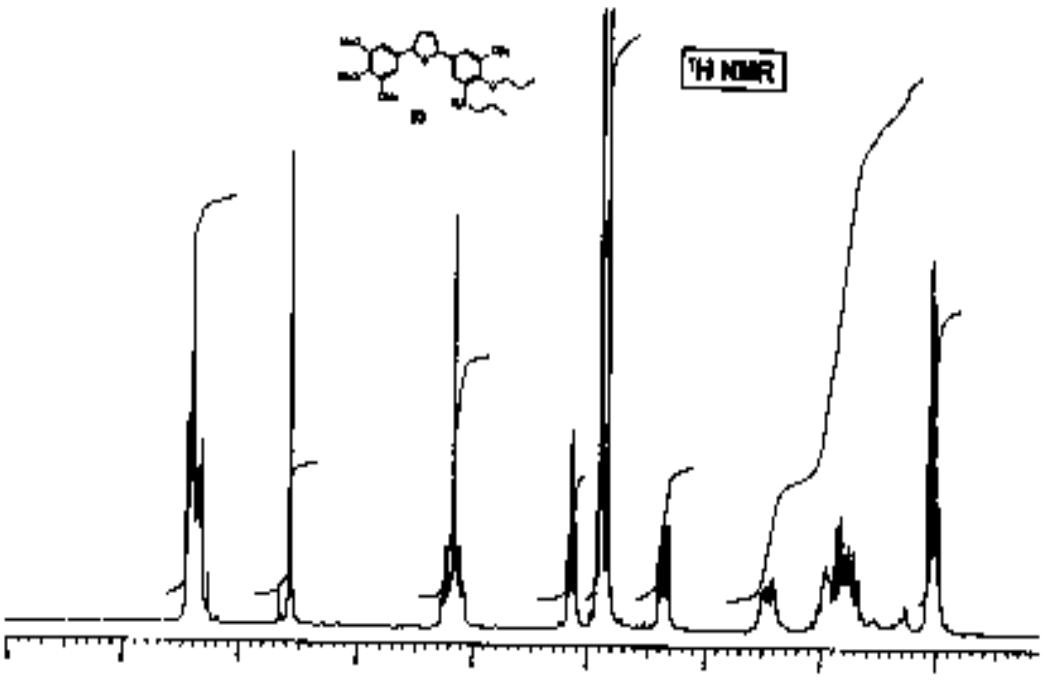
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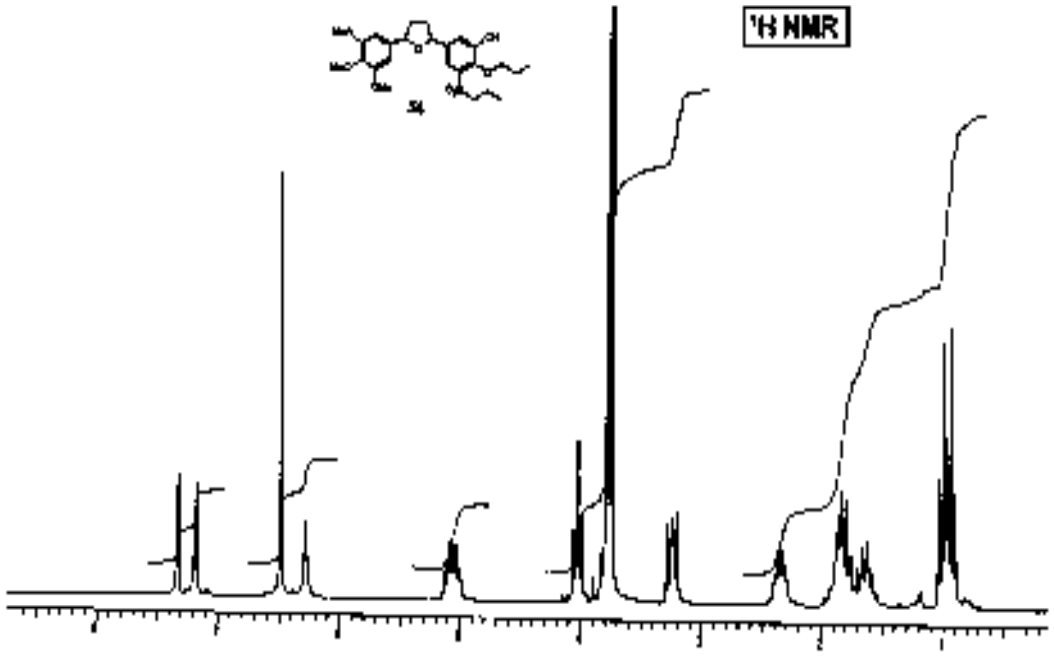
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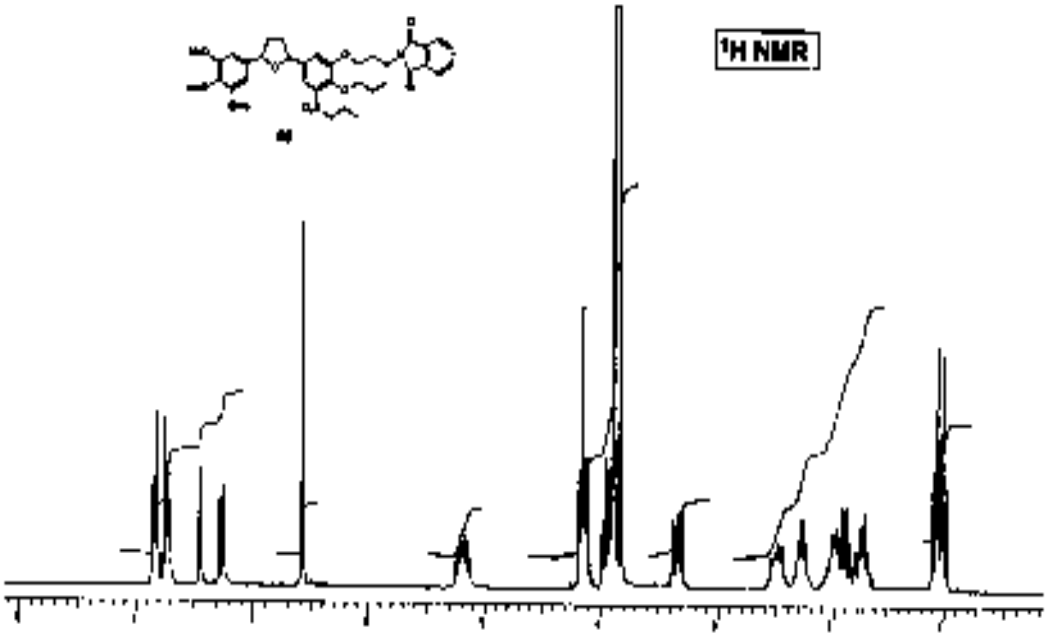
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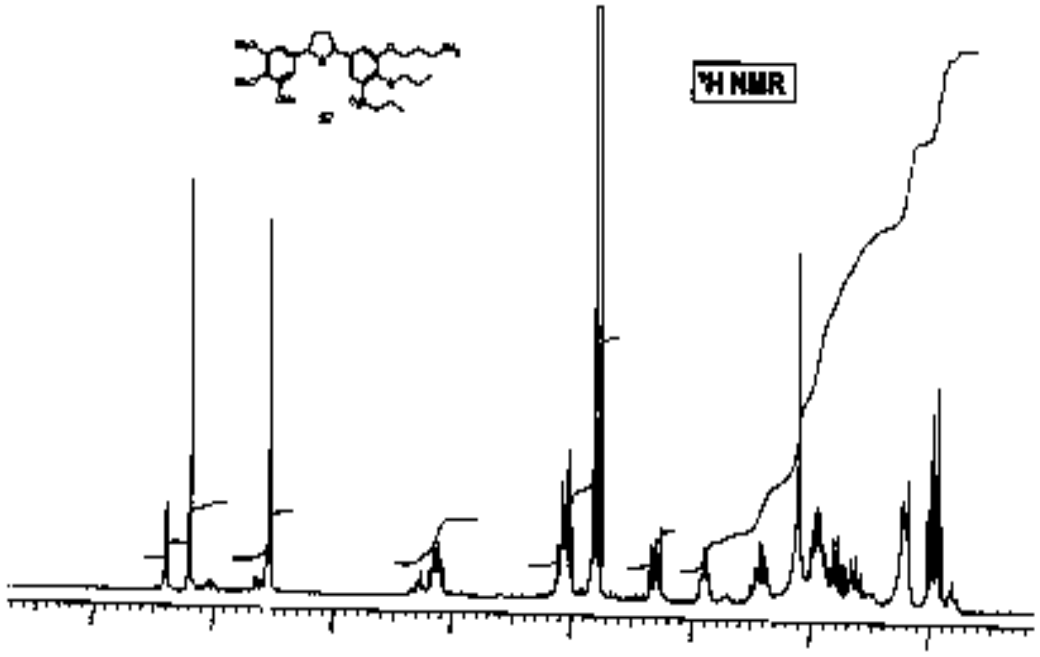
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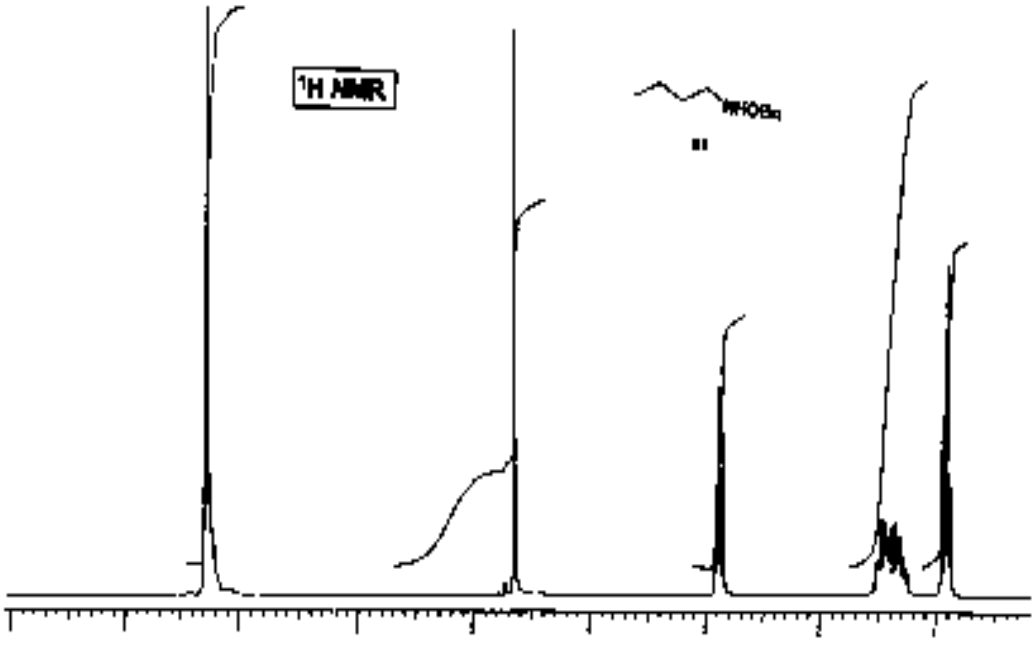
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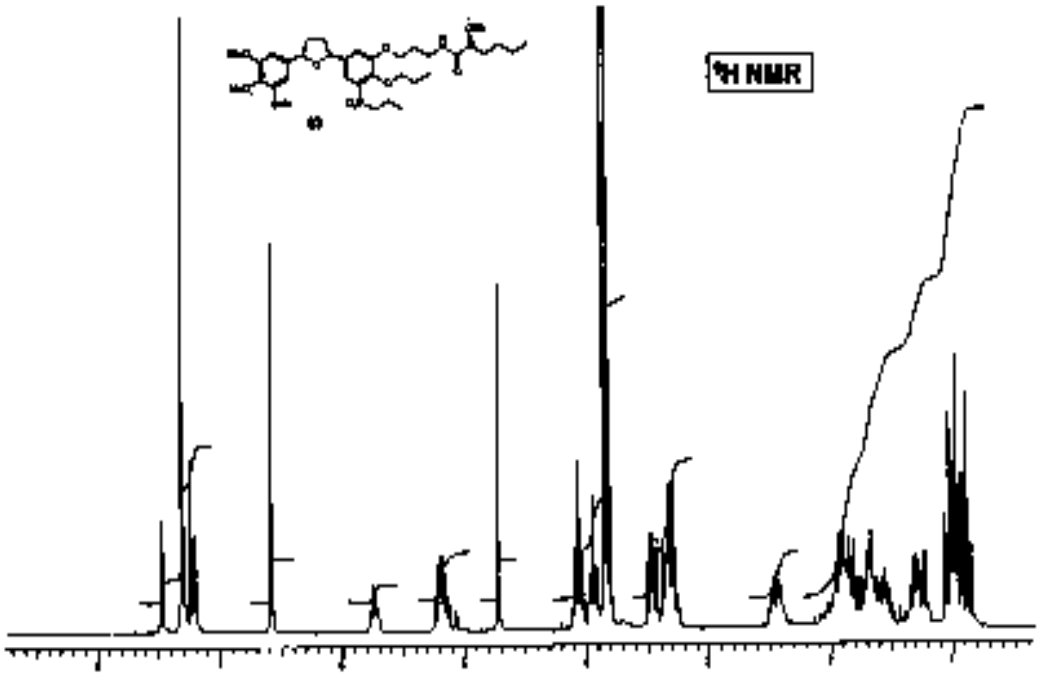
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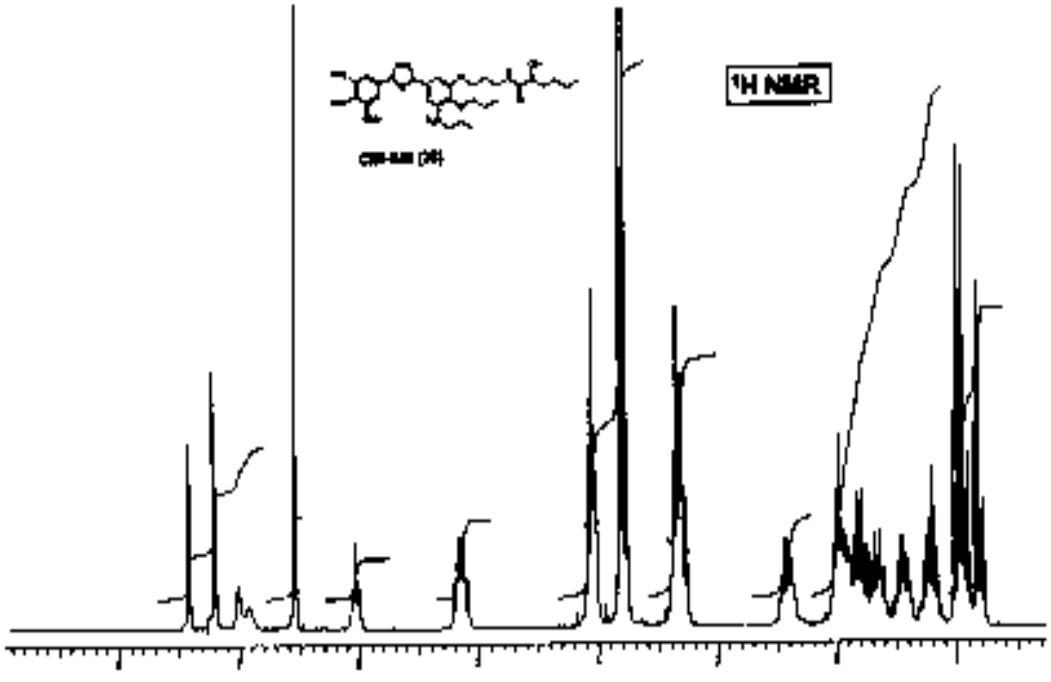
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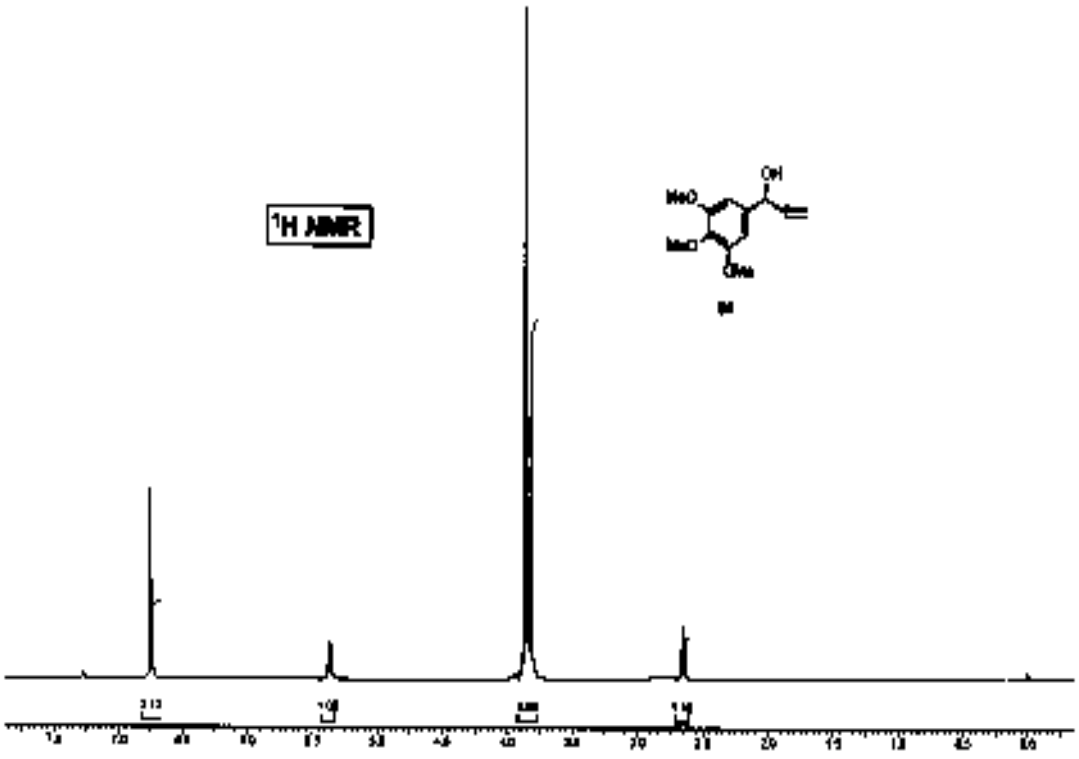
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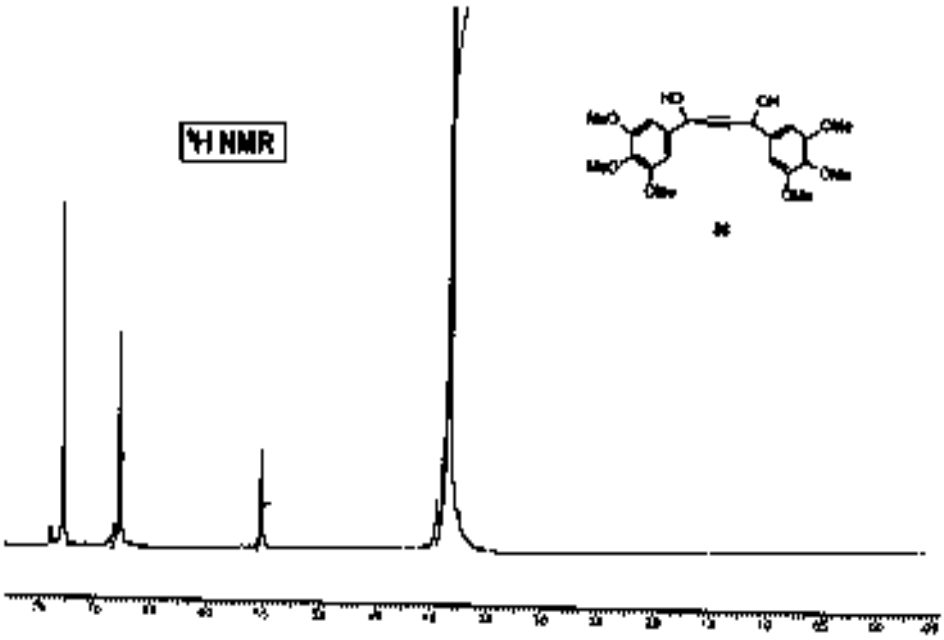
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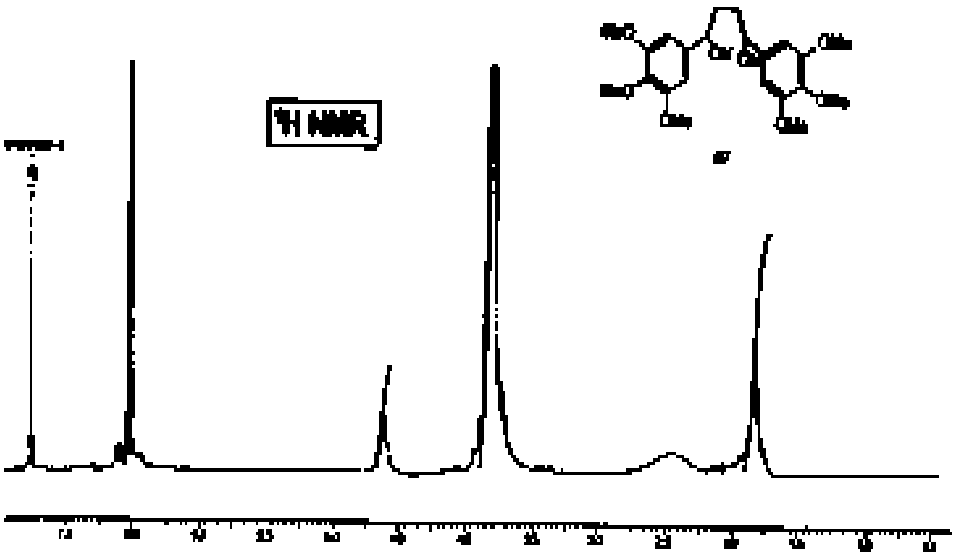
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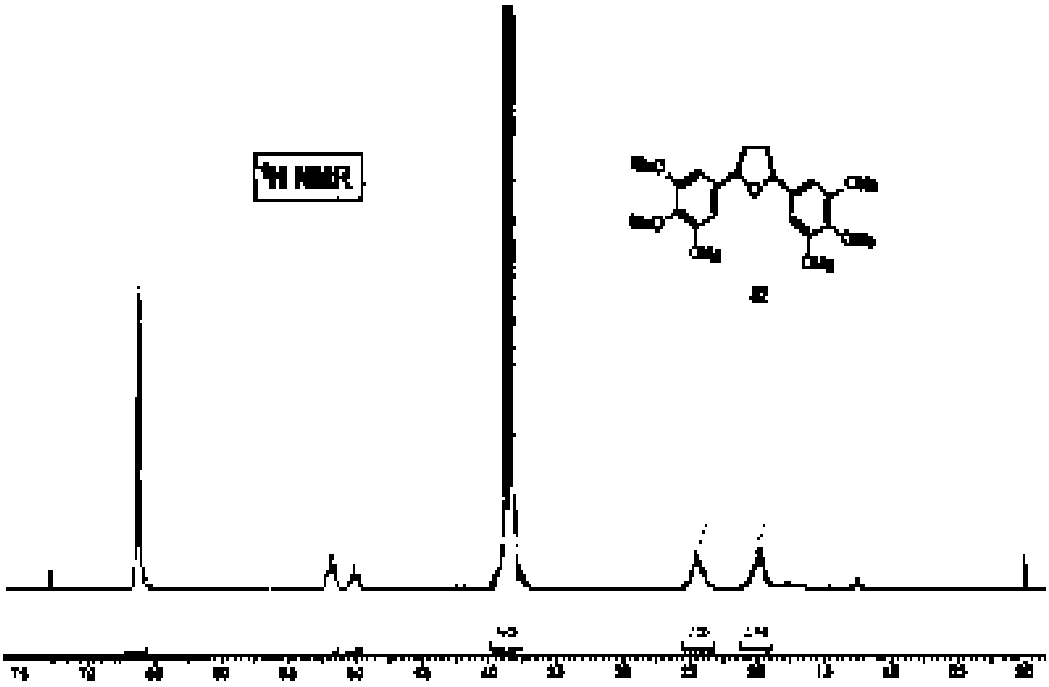
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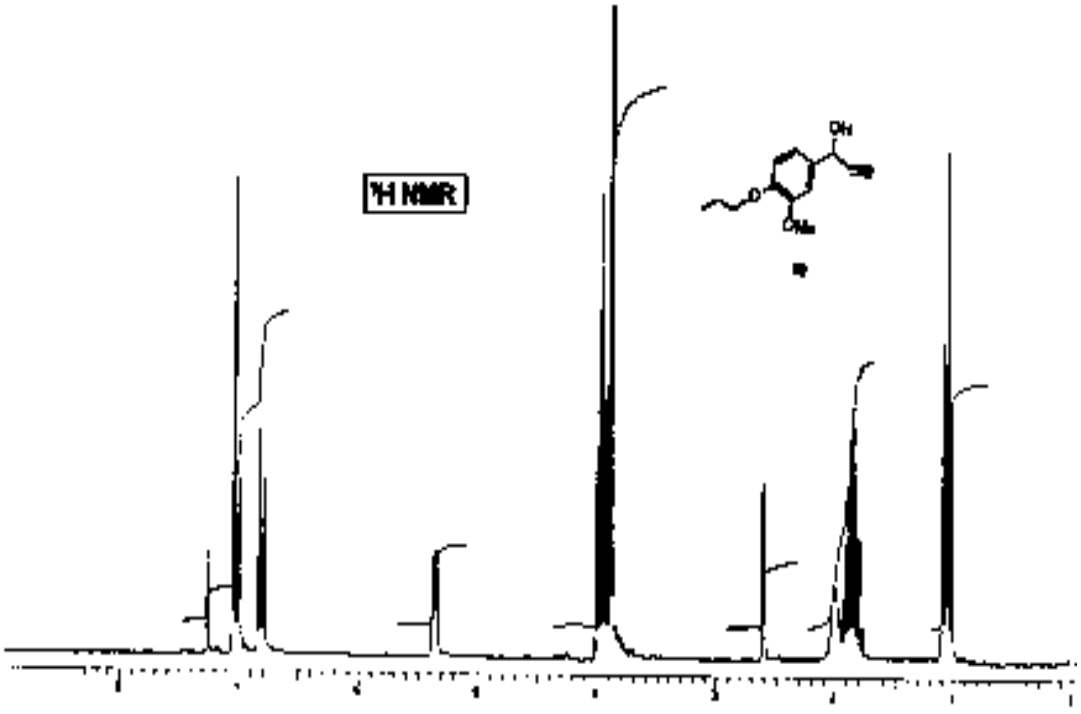
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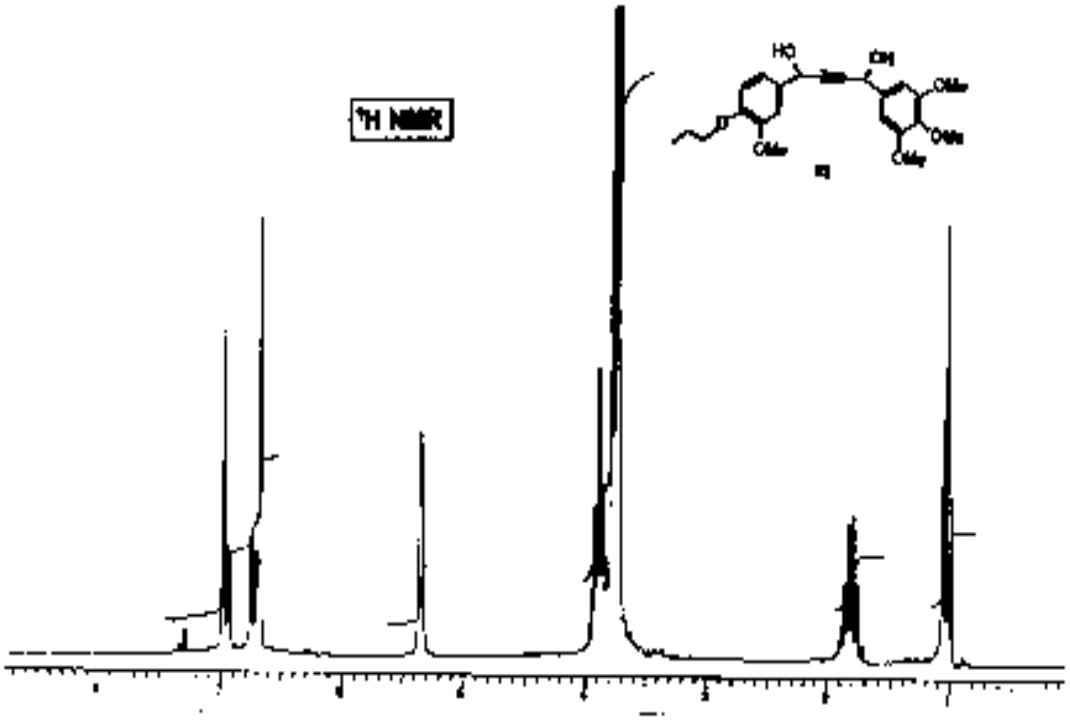
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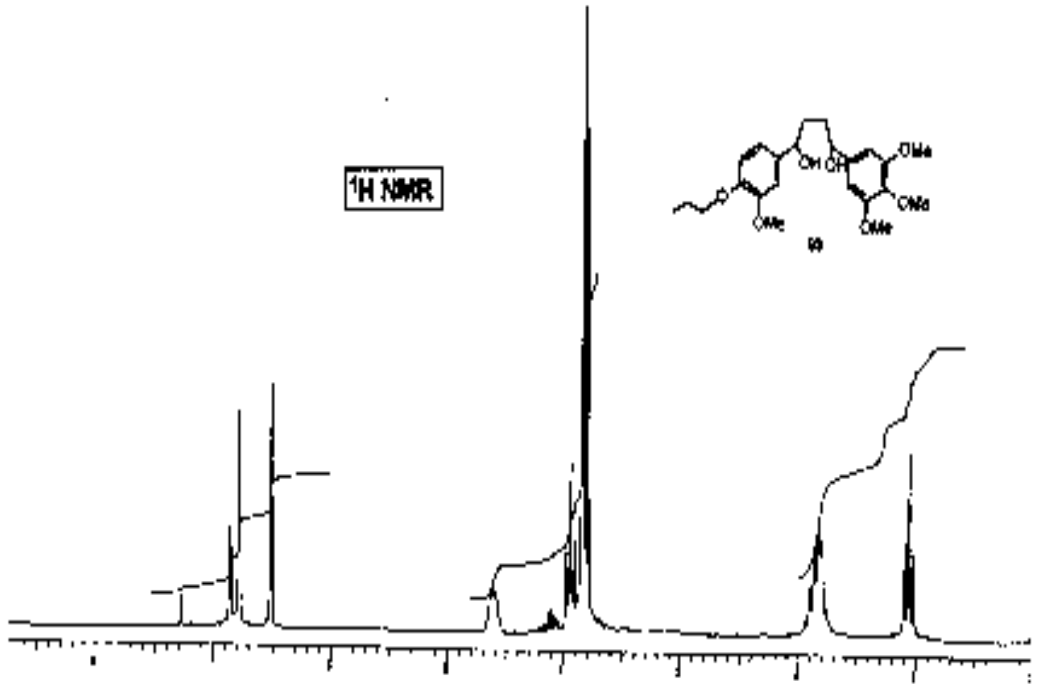
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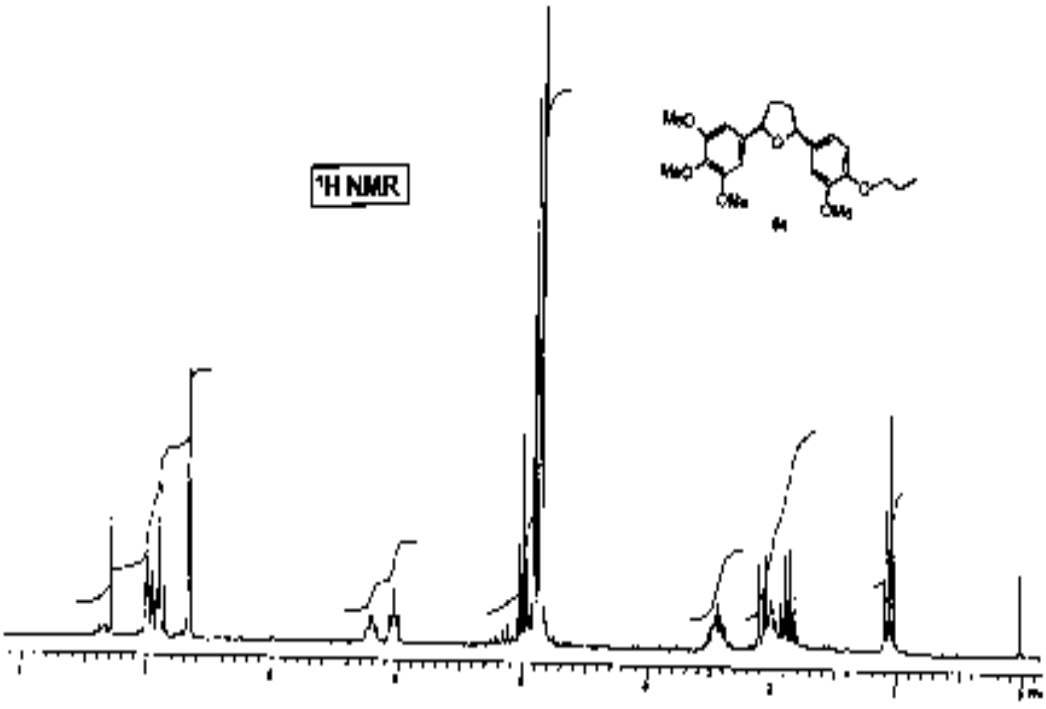
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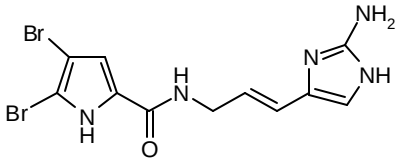
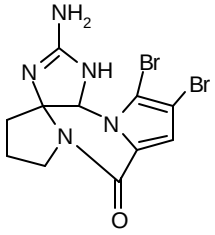
CHAPTER III
Synthesis of 9-*epi*-Manzacidin B

Bromopyrrole alkaloids comprise a typical class of marine natural products, frequently encountered as secondary metabolites of marine sponges of various species. Among these species *Agelasiidae*, *Axinellidae* and *Hymeniacidonidae* families constitute the bromopyrrole alkaloids.

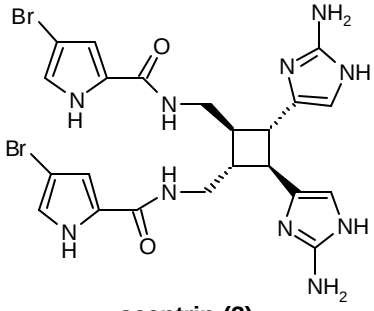
(i) *Agelasiidae* family



oroidin (1)

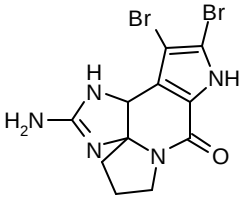


dibromophakellin (2)

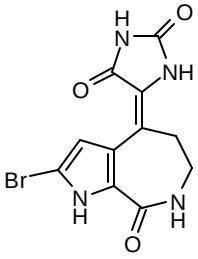


sceptrin (3)

(ii) *Axinellidae* family

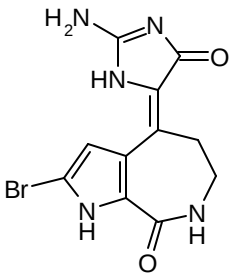


dibromo cantharelline (4)

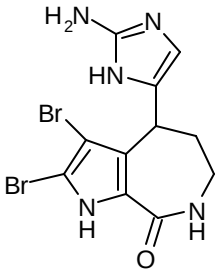


axinohydantoin (5)

(iii) *Hymeniacidonidae* family



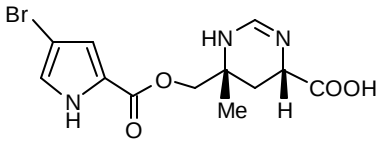
hymenialdisine (6)



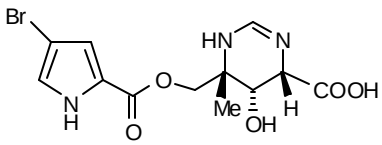
hymenine (7)

During the studies on bioactive substances from marine organisms, Kobayashi *et al*[2] have examined the extracts of numerous marine sponges and isolated several bromopyrrole alkaloids, which were found to be pharmacologically useful as α-adrenoceptor blockers,[3] antagonists of serotonergic receptor,[4] actomyosin ATPase activators.[5]

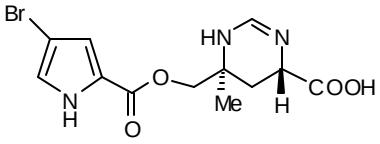
Recently they have investigated bio active constituents of *Hymeniacidon* sp.[7] and isolated three novel compounds, named manzacidins A-C (**9-11**) belonging to an unprecedented class of bromopyrrole alkaloids with an unusual 3,4,5,6- tetrahydro pyrimidine ring. Also very recently, manzacidin D (**12**) structurally related manzacidin A (**9**) has been isolated.[8]



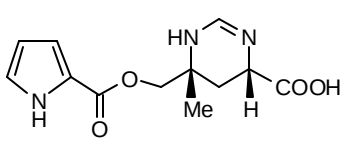
manzacidin A (9)



manzacidin B (10)

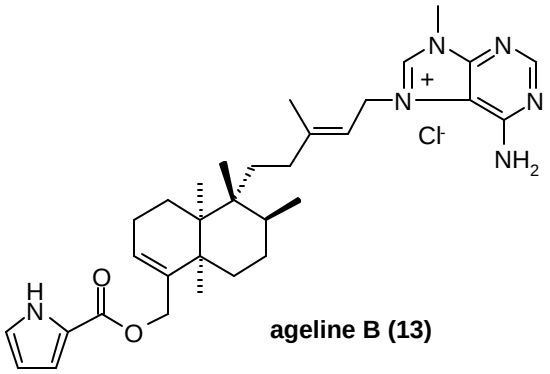


manzacidin C (11)



manzacidin D (12)

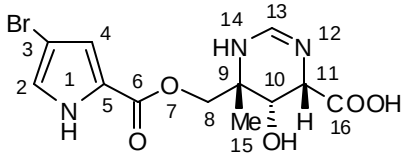
Manzacidins A-C (**9-11**) are the first bromopyrrole alkaloids with a tetrahydropyrimidine ring attached through an ester linkage. Most of the pyrrole-2-carboxylic acid derivatives obtained from marine sources possess amide bonds.[1,3-5] Ageline B (**13**) is an example of a pyrrole-2-carboxylic acid derivative attached to the terpenoid through an ester bond.[9] Natural products containing a tetrahydropyrimidine ring are rare, and these apparently are the first examples from marine sources.[10]



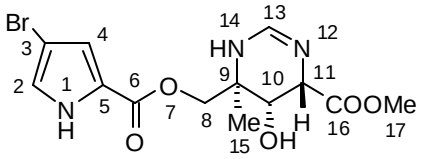
ageline B (13)

Present Work

A number of bromopyrrole alkaloids have been found from marine sponges which are known to exhibit pharmacologically useful activities such as α-adrenoceptor blockers, antagonists of serotonergic receptor, actomyosin ATPase activators, etc.[11] Recently a novel class of alkaloids manzacidin A-C (**9-11**) have been isolated,[6] which possess a unique structure consisting of an ester-linked bromopyrrole carboxylic acid and a 3,4,5,6-tetrahydro pyrimidine ring in which one of the amino group is attached to the C-9 quaternary carbon center. Although manzacidins exhibit similar biological activities to those of other bromopyrrole alkaloids, only recently tests have been carried out, owing to the extremely small amount of samples available from marine sources. The synthesis of manzacidin A (**9**) was recently reported by Ohfune *et al.*[12] The difference between manzacidin A (**9**) and manzacidin B (**10**) is an additional hydroxy group at C-10. No synthesis of manzacidin B (**10**) has yet been attempted. The importance of synthesis of manzacidin B (**10**) can be visualized in two ways. First, total synthesis of manzacidin B (**10**) would provide unambiguous proof for its stereochemical structure but more importantly it will pave a way to prepare analogues of manazacidin B (**10**) useful for structure activity relationship. In this chapter we have designed a synthetic scheme which will provide 9-*epi*-manzacidin B (**14**). We believe that extension of this protocol coupled with requisite modifications in our strategy we should be able to prepare the natural



manzacidin B (10)



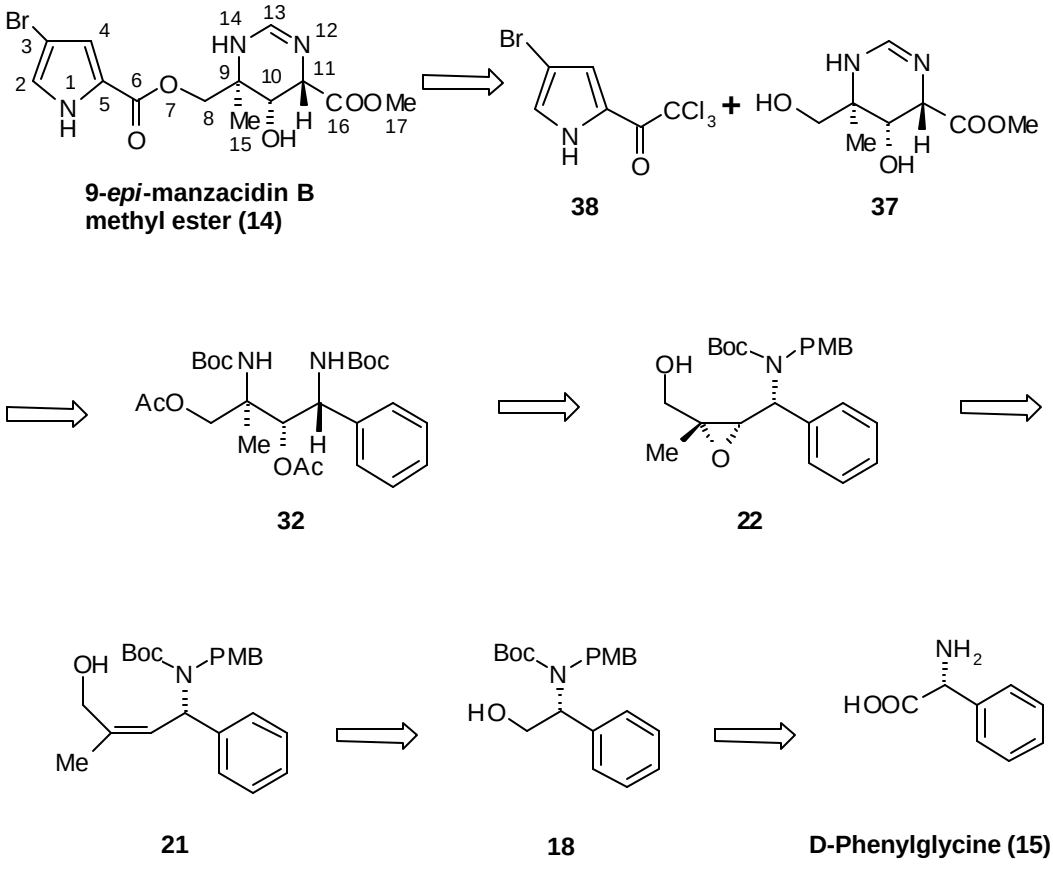
9-*epi*-manzacidin B methyl ester (14)

product manzacidin B (**10**) at a later date. This chapter deals with the synthesis of 9*epi*-manzacidin B as its methyl ester derivative (**14**).

Retrosynthetic analysis

9-*epi*-Manzacidin B methyl ester (**14**) can be obtained by the esterification of alcohol (**37**) with bromopyrrole (**38**), the former being envisaged from the diamine (**32**) by catalytic cyclisation. The formation of diamine (**32**) by regioselective opening of the epoxide (**22**) with nitrogen nucleophile was a straight forward exercise. The obtaintion of **22** using *m*-CPBA protocol constitutes of diastereoselective *syn* epoxidation of *cis*-γ-amino allylic alcohol (**21**) was planned.

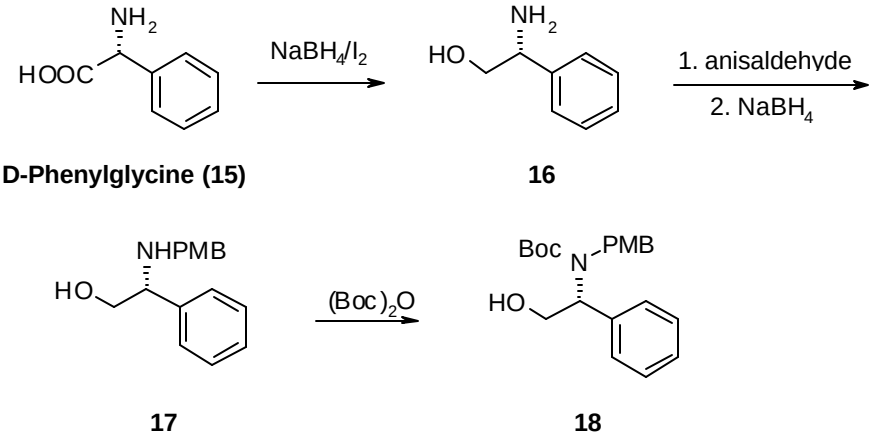
Retrosynthetic analysis



Synthetic approach

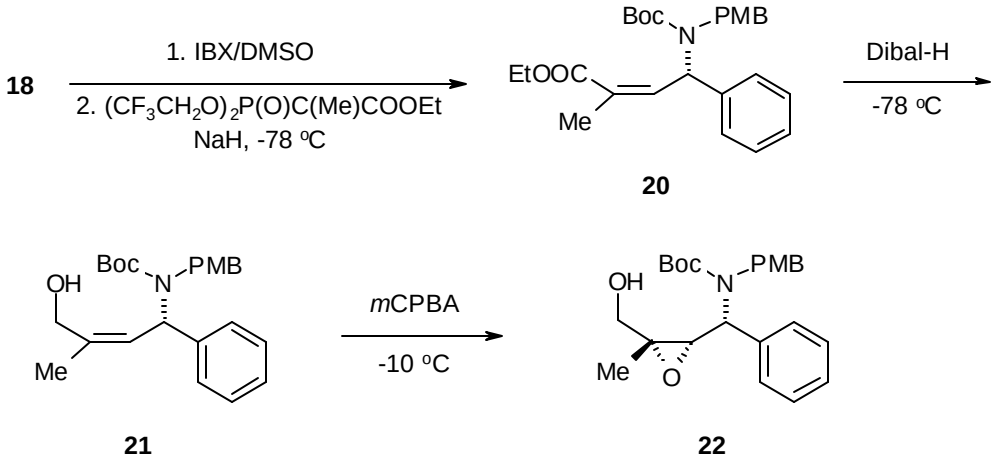
We began the synthesis of **14** from D-phenylglycine (**15**) which was reduced[13] with $NaBH_4/I_2$ followed by protection of the amine functionality with PMB and Boc groups to give **18** (Scheme 1) whose structure was confirmed by the ^1H and ^{13}C NMR spectral data.

Scheme 1



The alcohol (**18**) was oxidized with IBX and the resulting aldehyde then subjected to modified Horner-Emmons olefination conditions using Still's reagent,[14] $(CF_3CH_2O)_2P(O)C(Me)COOEt$ and NaH at −78 °C to give the (Z)-olefin **20** in 56 % yield over two steps. The *cis*-γ-amino-α,β-unsaturated ester **20** was then reduced with Dibal-H at −78 °C to *cis*-γ-amino allylic alcohol **21** in 87 % yield. The structure of **21** derived from the ^1H NMR spectrum showed the presence of the olefinic proton at 5.65 ppm. The ^{13}C NMR spectrum and elemental analysis further supported the structure of **21**. The next step required diastereoselective *syn* epoxidation[15] of **21**. Thus, the *m*-CPBA epoxidation of **21** at −10 °C in CH_2Cl_2 afforded the epoxide **22** in 67 % yield (Scheme 2). The structural features of **22** were established by the ^1H and ^{13}C NMR spectral data. The stereochemistry of **22**, although confirmed at a later stage was given as indicated based on hypothesis.

Scheme 2



The highly diastereoselective epoxidation of **21** was explained in terms of co-operative effect reported by Kishi.[16] Namely, it was postulated that the hydroxy group of the allylic alcohol and the carbonyl oxygen form hydrogen bonds with the peracid in the transition state as shown in figure 1.

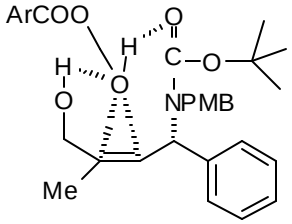
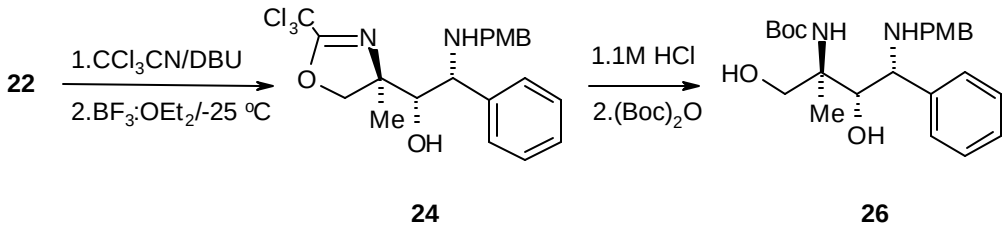


Figure 1

Our next aim was to introduce the amino group regioselectivity at C-2, for which Hatakeyama's method was employed.[17] Thus, treatment of **22** with CCl_3CN/DBU in CH_2Cl_2 at 0 °C was followed by intramolecular epoxide-opening reaction catalyzed by BF_3:OEt_2 at −25 °C afforded the oxazoline **24** in 64 % yield over two steps. In addition, 20 % yield of the hydrolysis product was also isolated. It was pertinent to mention that the BF_3:OEt_2 catalyzed epoxide opening also resulted in the deprotection of the Boc group. The structure of oxazoline deivative (**24**) was confirmed by the ^1H and ^{13}C NMR spectral

data. The oxazoline derivative (**24**) was then converted in to the alcohol **26** by acid hydrolysis followed by *tert*-butoxy carbonylation in 82 % yield (Scheme 3). In the ^{1}H NMR spectrum of **26** presence of a singlet at 1.47 ppm due to Boc protecting group confirmed its structure. In addition, the ^{13}C NMR spectrum and satisfactory elemental analysis supported the structure of **26**.

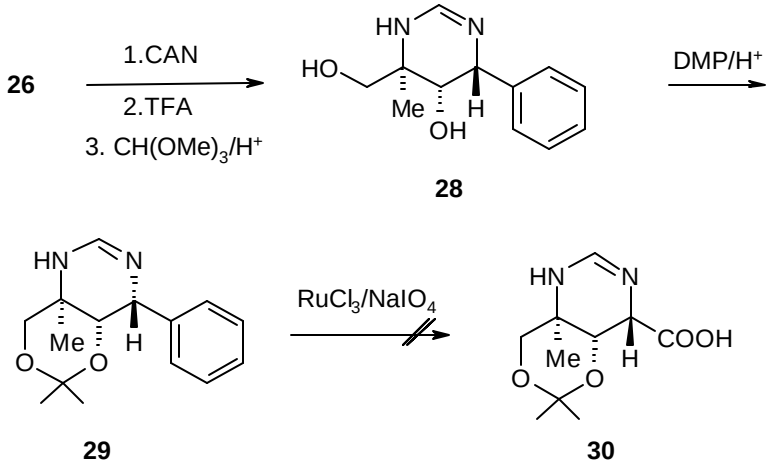
Scheme 3



24 **26**

Our next concern was the construction of tetrahydropyrimidine ring which necessitated the deprotection of PMB group from compound **26** which was affected with CAN. The resulting free amine, on successive treatment[12] with (i) TFA and (ii) methyl orthoformate afforded the tetrahydropyrimidine derivative **28** in 62 % yield. The structure of **28** was confirmed by the ^{1}H and ^{13}C NMR spectral data. For example, the presence of a singlet at 8.10 ppm due to imino group identified in the ^{1}H NMR spectrum of **28** while the rest of the spectrum was in complete agreement with the assigned structure. Completion of

Scheme 4



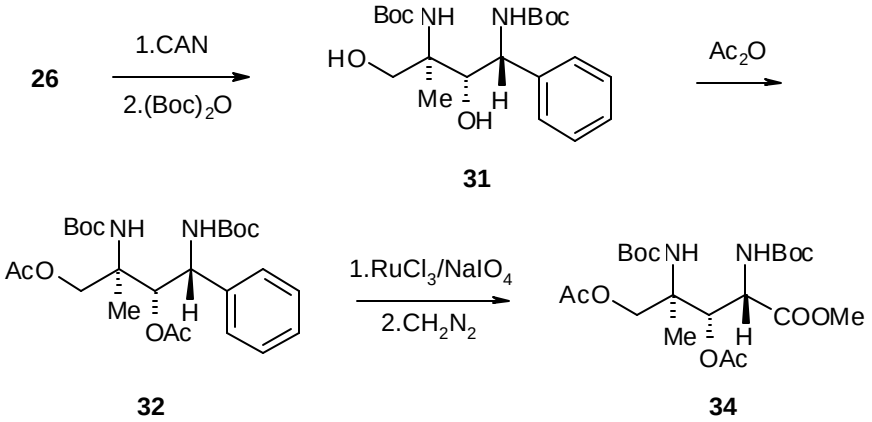
28

29 **30**

the synthesis now required the oxidation of phenyl group to acid. For this endeavor the diol was first protected as isopropylidene derivative (**29**), then the $RuCl_3/NaIO_4$ oxidation[18] was attempted, but the reaction resulted in the decomposition of **29** (Scheme 4).

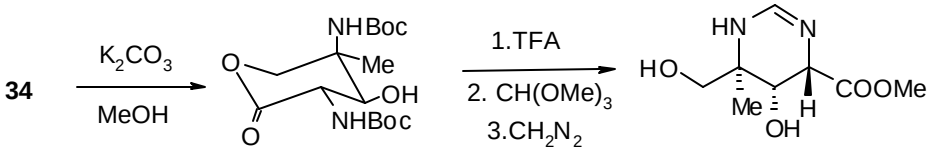
Hence the oxidation of phenyl group in compound **26** was first sought before the cyclisation. In order to accomplish the oxidation, the PMB protecting group was first deprotected using CAN and the free amine was then protected with $(Boc)_2O$ to give compound **31** in 85 % yield. The two hydroxyl groups in compound **31** were protected as their acetate derivatives. The ^1H, ^{13}C NMR spectral data and elemental analysis established the structure of **32**. For example the two singlets at 1.25 and 1.47 ppm due to Boc groups and another two singlets at 1.93 and 2.05 ppm typical of acetate groups, were identified in the ^1H NMR spectrum of **32**. The $RuCl_3/NaIO_4$ oxidation of **32** followed by esterification with CH_2N_2 afforded the ester **34** in 63 % yield (Scheme 5). The singlet at 3.85 ppm due to methyl ester was identified in the ^1H NMR spectrum of **34** while rest of the spectrum was in complete agreement with the assigned structure. In addition, the ^{13}C NMR spectrum and elemental analysis confirmed the structure of **34**.

Scheme 5



The next reaction, deprotection of acetate functionalities from compound **34** with K_2CO_3/MeOH resulted in the formation of the expected lactone **35** in 78 % yield. The ^1H and ^{13}C NMR spectroscopic studies of **35** revealed the assigned structure. Construction of the tetrahydro pyrimidine ring was performed by successive treatment of **35** with (i) TFA and (ii) methyl orthoformate to give the acid which was purified after the esterification with CH_2N_2 (Scheme 6). The structure of **37** was thoroughly characterized by ^1H, ^{13}C NMR spectral data.

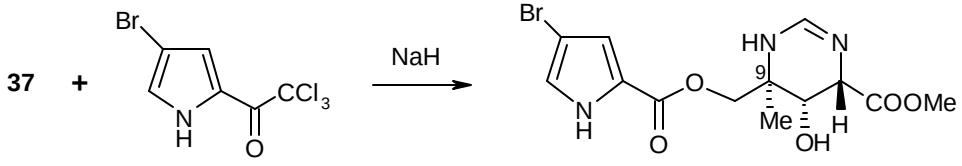
Scheme 6



35 **37**

Completion of the synthesis now required esterification of the bromopyrrole carboxylate with 37 . Thus, treatment of 37 with NaH/trichloroacetylbromopyrrole(38)[19] in DMF at room temperature gave the required compound 14 in 58 % yield (Scheme 7).

Scheme 7



38

9-*epi*-manzacidin B methyl ester (14)

The structure of compound **14** was fully analyzed by ^1H and ^{13}C NMR spectroscopic data coupled with satisfactory elemental analysis. For example, in its ^1H NMR spectrum the two meta coupled protons of the bromopyrrole moiety were appeared as two doublets at 6.79 (J = 1.92 Hz) and 7.01 ppm (J = 1.92 Hz). The singlet at 8.07 ppm

was attributed to H-13. The two singlets at 3.83 and 1.27 ppm were due to ester and methyl groups respectively, while rest of the spectrum was in complete agreement with the assigned structure.

Conclusion

In conclusion, synthesis of the 9-*epi*-manzacidin B methyl ester derivative (**14**) has been achieved *via* the stereoselective *syn* epoxidation and the regioselective opening of the epoxide with nitrogen nucleophile. The present synthetic route enables the synthesis of diastereomers of manzacidin B (**10**) which permits further pharmacological studies on these alkaloids.

(2R)-2-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2-phenylethanol (18)

To a solution of $NaBH_4$ (12.9 g, 331.1 mmol) and D-phenylglycine (**15**) (20.0 g, 132.4 mmol) in THF (200 ml) was added a solution of iodine (33.6 g, 132.4 mmol) in THF (100 ml) drop wise at 0 ^oC. The reaction mixture was refluxed for 18 h, cooled to 0 ^oC and quenched with methanol, solvent concentrated and the residue dissolved in aq. 20 % KOH solution (200 ml) and stirred for 4 h. The mixture was then extracted with CH_2Cl_2, the combined organic extract was washed with brine, dried (Na_2SO_4), concentrated to give **16** (17 g).

A solution of **16** (17 g, 124 mmol) and *p*-methoxy benzaldehyde (16.9 g, 124 mmol) in methanol was stirred at room temperature for 1 h, cooled to 0 ^oC and $NaBH_4$ (5.4g, 136.5 mmol) added. After 2 h, the reaction mixture was quenched with dil. AcOH, solvent concentrated and the residue taken in ethyl acetate, washed with brine, dried (Na_2SO_4) and concentrated to give a residue which was suspended in 1:1mixture of THF-H_2O (100 ml) and $(Boc)_2O$ (29.7 g, 136.4 mmol) introduced into the reaction mixture. After 2 h, solvent was concentrated and extracted with ethyl acetate, the combined organic extract was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **18** (39.2 g, 83 %).

[a] $_D$ = -37.2° (c 2.2, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.46 (s, 9 H), 3.79 (s, 3 H), 3.96 (d, 2 H, *J* = 5.37 Hz), 4.20 (br s, 2 H), 5.08 (t, 1 H, *J* = 6.84 Hz), 6.82 (d, 2 H, *J* = 8.79 Hz), 7.11-7.37 (m, 7 H);

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

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

Ethyl(2Z,4S)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2-methyl-4-phenyl-but-2-enoate (20)

A solution of compound **18** (10 g, 28 mmol) and IBX (8.62 g, 30.8 mmol) in DMSO (30 ml) was stirred at room temperature for 1 h, quenched with water and filtered. The filtrate was diluted with ethyl acetate, washed with brine, dried (Na_2SO_4) and concentrated to afford the aldehyde (8.7 g) which was used as such for the next reaction.

A suspension of NaH (1.27 g, 31.8 mmol) and bis(trifluoroethyl)-2-phosophonopropionate ethyl ester (9.32 g, 26.9 mmol) in THF (75 ml) was stirred at 0 ^oC for 30 min. and then cooled to –78 ^oC. To the mixture were added successively, a solution of 18-crown-6 (32.3 g, 122.5 mmol) in THF (100 ml) and a solution of aldehyde (8.7 g, 24.5 mmol) in THF (60 ml). After 2 h at –78 ^oC, the mixture was quenched with sat. aq. NH_4Cl, extracted with ethyl acetate, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:10) as eluent to give **20** (6.88 g, 56 %).

[a] $_D$ = -14.7° (c 2.1, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.26 (t, 3 H, *J* = 7.32 Hz), 1.40 (s, 9 H), 1.79 (s, 3 H), 3.77 (s, 3 H), 4.06-4.24 (m, 4 H), 4.55 (d, 1 H, *J* = 7.81 Hz), 6.25 (d, 1 H, *J* = 7.33 Hz), 6.77 (d, 2 H, *J* = 8.79 Hz), 7.07 (d, 2 H, *J* = 8.30 Hz) 7.16-7.36 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 12.3, 13.7, 27.9, 47.9, 54.5, 56.7, 60.0, 79.7, 113.2, 126.7-128.3, 130.0, 130.8, 137.6, 139.0, 155.2, 158.3, 166.7;

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

(2Z,4S)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2-methyl-4-phenylbut-2-en-1-ol (21)

To a solution of **20** (6 g, 13.6 mmol) in CH_2Cl_2 (75 ml) at −78 ^oC was added a solution of DIBAL-H (17 ml, 34.2 mmol, 2.0 M in toluene). After 1 h, it was quenched with aq. sodium potassium tartarate, extracted with CH_2Cl_2. The combined CH_2Cl_2 layer was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **21** (4.72 g, 87 %).

[a] $_D$ = -27.6° (c 1.5, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.42 (s, 9 H), 1.65 (s, 3 H), 3.78 (s, 4 H), 3.91 (s, 2 H), 4.0 (br d, 1 H, *J* = 15.62 Hz), 4.64 (d, 1 H, *J* = 7.88 Hz), 5.65 (d, 1 H, *J* = 7.48 Hz), 6.79 (d, 2 H, *J* = 8.79 Hz), 7.08 (d, 2 H, *J* = 8.30 Hz), 7.20-7.36 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 14.5, 28.3, 48.5, 56.4, 61.0, 67.8, 80.3, 115.0, 122.6, 124.7-128.9, 139.6, 140.0, 141.0, 154.1, 156.0;

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

(2R,3S,4R)-4-[(*tert*-Butoxycarbonyl)(*p*-methoxybenzyl)amino]-2,3-epoxy-2-methyl-4-phenylbutan-1-ol (22)

To a solution of **21** (4.5 g, 11.3 mmol) in CH_2Cl_2 (50 ml) was added *m*-CPBA (3.34 g, 13.6 mmol, 70 %) at -10 ^oC. After 15 h at -10 ^oC, the reaction mixture was quenched with aq. 5 % K_2CO_3 solution and extracted with CH_2Cl_2. The combined organic extract was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **22** (3.13 g, 67 %).

[a] $_D$ = -12.4° (c 1, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.28 (s, 3 H), 1.38 (s, 9 H), 3.45 (d, 1 H, *J* = 3.91 Hz), 3.69 (br s, 2 H), 3.79 (s, 3 H), 4.14 (br d, 2 H), 4.68 (d, 1 H, *J* = 7.36 Hz), 6.82 (d, 2 H, *J* = 8.79 Hz), 7.10-7.38 (m, 7 H);

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

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

(1¢S,2¢R,4R)-4-[2¢-(*p*-Methoxybenzyl)amino-2¢-phenyl-1¢-hydroxyethyl]-4-methyl-2-(trichloromethyl)-2-oxazoline (24)

A solution of **22** (3 g, 7.26 mmol) in CH_2Cl_2 (25 ml) was treated with CCl_3CN (0.8 ml, 7.99 mmol) in the presence of DBU (0.1 ml, 0.72 mmol) at 0 °C. After 2 h, solvent was removed and the residue purified on silica gel with ethyl acetate-light petroleum (1:5) as eluent to give trichloroacetimidate (3.4 g) which was immediately dissolved in CH_2Cl_2 (25 ml) and treated with BF_3:OEt_2 (0.3 ml, 2.18 mmol) at –25 °C for 45 min.. The reaction mixture was diluted with CH_2Cl_2, washed with aq. $NaHCO_3$, brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:3) as eluent to give **24** (2.12 g, 64 %).

[a]$_D$ = -5.6° (c 1.5, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.84 (s, 3 H), 3.58 (br d, 1 H, *J* = 14.65 Hz), 3.81 (s, 3 H), 3.88 (d, 1 H, *J* = 8.79 Hz), 4.15 (d, 1 H, *J* = 3.41 Hz), 4.46 (d, 1 H, *J* = 7.33 Hz), 4.65 (d, 1 H, *J* = 7.33 Hz), 4.85 (d, 1 H, *J* = 7. 81 Hz), 6.83 (d, 2 H, *J* = 8.79 Hz), 7.04 (d, 2 H, *J* = 8.79 Hz), 7.17 (br s, 2 H), 7.40-7.43 (m, 3 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 13.3, 45.6, 49.1, 54.9, 59.4, 61.8, 74.2, 101.4, 114.6, 127.3-128.8, 134.9, 137.6, 156.8, 173.0;

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

(2R,3S,4R)-2-(*tert*-Butoxycarbonylamino)-4-(*p*-methoxybenzylamino)-4-phenyl butane-1,3-diol (26)

A solution of **24** (2.0 g, 4.37 mmol) in THF (25 ml) was treated with 1M HCl (10 ml) at room temperature. After 2 h, it was carefully basified with $NaHCO_3$, $(Boc)_2O$ (2.86 g, 13.1 mmol) added and stirred for 24 h. The reaction mixture was saturated with NaCl, extracted with ethyl acetate, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to afford **26** (1.54 g, 82 %).

[a] $_D$ = +6.7° (c 1.5, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.77 (s, 3 H), 1.47 (s, 9 H), 3.73 (d, 1 H, *J* = 11.23 Hz), 3.81 (s, 3 H), 3.90 (d, 1 H, *J* = 11.23 Hz), 4.33 (d, 1 H, *J* = 3.87 Hz) 4.61 (ABq, 2 H, *J* = 7.82 Hz), 4.84 (d, 1 H, *J* = 8.65 Hz), 6.83 (d, 2 H, *J* = 8.31 Hz), 7.03 (d, 2 H, *J* = 8.79 Hz), 7.20-7.45 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 20.1, 27.6, 45.1, 50.1, 55.0, 60.9, 71.1, 71.6, 82.4, 114.0, 127.6-129.6, 134.0, 139.0, 157.5, 159.3;

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

(4R,5S,6R)-6-(Hydroxymethyl)-6-methyl-4-phenyl-1,4,5,6-tetrahydropyrimidine-5-ol (28)

A solution of **26** (0.3 g, 0.69 mmol) in 3:1 mixture of CH_3CN-H_2O (10 ml) was stirred in the presence of CAN (1.14g, 2.09 mmol) at -5 °C for 1 h, diluted with water, extracted with EtOAc. The combined organic extract was washed with aq. 5 % $NaHCO_3$, aq. 10 % Na_2SO_3, brine, dried (Na_2SO_4), concentrated to give amine (0.2 g) which was treated with TFA (4 ml) in CH_2Cl_2 (10 ml) at room temperature for 2 h. To the reaction

mixture was added $CH(OMe)_3$ (20 ml) and conc. HCl (0.5 ml) and refluxed for 4 h, then cooled to 0 ^oC, neutralized with $NaHCO_3$, extracted with CH_2Cl_2. The combined organic extract was washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate - light petroleum (1:1) as eluent to afford **28** (95 mg, 62 %).

[a] $_D$ = +16.4° (c 1.3, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.77 (s, 3 H), 3.95 (ABq, 2 H, *J* = 7.82 Hz), 4.35 (d, 1 H, *J* = 3.90 Hz), 4.77 (d, 1 H, *J* = 7.85 Hz), 7.20-7.45 (m, 5 H), 8.1 (s, 1 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 19.8, 47.8, 53.5, 62.5, 68.1, 128.6, 129.0, 134.0, 160.2;

Anal: Calcd. for $C_{12}H_{16}N_2O_2$: C, 65.45, H, 7.27, N, 12.72. Found: C, 65.85, H, 7.52, N, 12.41.

(4aR,8R,8aS)-2,2,4a-Trimethyl-8-phenyl-4a,5,8,8a-tetrahydro-4H-[1,3]dioxino[5,4-d] pyrimidine (29)

A solution of **28** (80 mg, 0.36 mmol), 2,2-dimethoxypropane (0.12 ml, 0.90 mmol) and *p*TSA (cat.) in acetone (5 ml) was stirred at room temperature for 12 h, neutralized with Et_3N and concentrated. The residual syrup was dissolved in $CHCl_3$, washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate - light petroleum (1:4) as eluent to afford **29** (80 mg, 85 %).

[a] $_D$ = +17.6° (c 1.1, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.85 (s, 3 H), 1.43 (s, 3 H), 1.55 (s, 3 H), 3.85 (ABq, 2 H, *J* = 7. 81 Hz), 4.45 (d, 1 H, *J* = 3.85 Hz), 4.67 (d, 1 H, *J* = 7. 74 Hz), 7.11-7.45 (m, 5 H), 8.20 (s, 1 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 20.6, 26.8, 27.0, 48.3, 52.9, 63.0, 72.0, 109.3, 128.7, 129.1, 134.3, 158.4;

Anal: Calcd. for $C_{15}H_{20}N_2O_2$: C, 69.23, H, 7.69, N, 10.76. Found: C, 69.55, H, 7.52, N, 10.41.

(2R,3S,4R)-2,4-Bis(*tert*-butoxycarbonylamino)-2-methyl-4-phenylbutane-1,3-diol (31)

A solution of **26** (1.5 g, 3.48 mmol) in 3:1 mixture of CH_3CN-H_2O (40 ml) was stirred in the presence of CAN (5. 73g, 10.46 mmol) at $-5\ ^{o}C$ for 1 h, then diluted with water, extracted with EtOAc. The combined organic extract was washed with aq. 5 % $NaHCO_3$, aq. 10 % Na_2SO_3, brine, dried (Na_2SO_4), concentrated to give amine (1.1g) which was treated with $(Boc)_2O$ (1.52 g, 6.97 mmol) in the presence of Et_3N (1 ml, 6.97 mmol) in THF (15 ml) at room temperature for 12 h. Solvent was concentrated and the residue taken in ethyl acetate, washed with brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to give **31** (1.21 g, 85 %).

[a] $_{D}$ = +8.9° (c 1.4, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.72 (s, 3 H), 1.28 (s, 9 H), 1.50 (s, 9 H), 3.88 (q, 2 H, *J* = 11.72 Hz), 4.75 (d, 1 H, *J* = 3.42 Hz), 5.08 (d, 1 H, *J* = 7.32 Hz), 7.30-7.38 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 20.1, 27.6, 30.1, 45.0, 55.0, 60.9, 71.6, 85.7, 86.0, 127.6-129.6, 134.0, 157.5, 159.2;

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

(2R,3S,4R)-1,3-Di-*O*-acetyl-2-methyl-2,4-bis(*tert*-butoxycarbonylamino)-4-phenyl butane-1,3-diol (32)

A solution of **31** (1.0 g, 2.43 mmol), Ac_2O (0.9 ml, 9.75 mmol) and Et_3N (2.0 ml, 14.63 mmol) in CH_2Cl_2 (10 ml) was stirred in the presence of DMAP (50 mg) at room temperature for 8 h. The reaction mixture was diluted with CH_2Cl_2, washed with water, brine, dried (Na_2SO_4), concentrated and purified on silica gel using ethyl acetate-light petroleum (1:4) as eluent to give **32** (1.07 g, 89 %).

[a] $_{D}$ = +19.2° (c 1.7, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.02 (s, 3 H), 1.25 (s, 9 H), 1.47 (s, 9 H), 1.93 (s, 3 H), 2.05 (s, 3 H), 4.20 (d, 1 H, *J* = 11.72 Hz), 4.45 (d, 1 H, *J* = 12.21 Hz), 5.08 (d, 1 H, *J* = 7.83 Hz), 5.33 (d, 1 H, *J* = 3.91 Hz), 7.20-7.40 (m, 5 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 13.0, 20.0, 28.9, 31.5, 48.0, 56.1, 62.9, 66.5, 79.9, 80.8,126.7-129.0, 137.7, 153.1, 153.9, 169.1, 170.0;

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

Methyl (2S,3S,4R)-2,4-Bis(*tert*-butoxycarbonylamino)-3,5-bis(acetoxy)-4-methyl-pentanoate (34)

A mixture of **32** (1.0g, 2.02 mmol), $NaIO_4$ (4.34 g, 20.2 mmol) and $RuCl_3.H_2O$ (10 mg, 0.04 mmol) in CH_3CN (10 ml), CCl_4 (10 ml) and H_2O (15 ml) was stirred at room temperature for 12 h. The reaction mixture was then cooled to 0 ^oC, diluted with ether and the organic phase separated. The aqueous phase was extracted with ether and the combined ether layer washed with brine, dried (Na_2SO_4), concentrated to give a residue which was treated with excess CH_2N_2 in ether (30 ml) at 0 ^oC for 30 min. then concentrated and purified on silica gel using ethyl acetate-light petroleum (1:2) as eluent to give **34** (0.6 g, 63 %).

[a]$_D$ = +23.5° (c 1.1, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.05 (s, 3 H), 1.27 (s, 9 H), 1.49 (s, 9 H), 1.91 (s, 3 H), 2.05 (s, 3 H), 3.85 (s, 3 H), 4.23 (d, 1 H, *J* = 11.76 Hz), 4.46 (d, 1 H, *J* = 12.32 Hz), 4.78 (d, 1 H, *J* = 7.85 Hz), 5.32 (d, 1 H, *J* = 3.86 Hz);

^{13}C NMR d (50 MHz, $CDCl_3$): 13.8, 20.3, 28.8, 32.9, 46.0, 50.9, 62.2, 66.5, 68.9, 79.6, 81.0, 153.8, 154.5, 169.2, 169.6, 170.2;

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

***N*-[(3R,4S,5S)-5-(*tert*-Butoxycarbonylamino)-4-hydroxy-3-methyl-6-oxo-(2H-3,4,5-dihydropyran-3-yl)](*tert*-butoxy)carboxamide (35)**

A solution of **34** (0.5 g, 1.05 mmol) in methanol (10 ml) was treated with K_2CO_3 (0.58 g, 4.20 mmol) at room temperature for 8 h. The reaction mixture was de-ionized by the addition of Amberlite IR 120 (H^+) resin (pH 6), filtered, concentrated and purified on silica gel with light petroleum-EtOAc (1:3) as eluent to give **35** (0.29 g, 78%).

[a] $_D$ = +32.1° (c 1.2, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 0.72 (s, 3 H), 1.26 (s, 9 H), 1.50 (s, 9 H), 2.33 (br s, 1 H), 3.87 (q, 2 H, *J* = 11.72 Hz), 4.39 (d, 1 H, *J* = 7.23 Hz), 4.76 (d, 1 H, *J* = 3.41 Hz);

^{13}C NMR d (50 MHz, $CDCl_3$): 15.1, 27.3, 30.1, 45.5, 53.4, 62.9, 66.4, 79.9, 81.1, 154.6, 155.4, 172.2;

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

Methyl(4S,5S,6R)-5-Hydroxy-6-(hydroxymethyl)-6-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylate (37)

A solution of **35** (0.25 g, 0.69 mmol) and TFA (5 ml) in CH_2Cl_2 (10 ml) was stirred at room temperature for 2 h. To the reaction mixture was added $CH(OMe)_3$ (30 ml) and conc. HCl (0.5 ml) and heated to reflux. After 4 h, the reaction mixture was concentrated and the residue diluted with ethyl acetate and extracted with 2 N HCl. The combined aqueous layer was concentrated to afford a residue which was treated with excess CH_2N_2 in ether (20 ml) at 0 ^oC. After 30 min., it was concentrated and purified on silica gel using ethyl acetate-light petroleum (3:1) as eluent to afford **37** (0.1 g, 77 %).

[a] $_D$ = +44.1° (c 1.6, $CHCl_3$);

^{1}H NMR d **(200 MHz, $CDCl_3$):** 1.08 (s, 3 H), 3.78 (s, 3 H), 4.22 (d, 1 H, *J* = 11.71 Hz), 4.46 (d, 1 H, *J* = 12.21 Hz), 4.58 (d, 1 H, *J* = 3.43 Hz), 4.80 (d, 1 H, *J* = 7.81 Hz), 8.08 (s, 1 H);

^{13}C NMR d (50 MHz, $CDCl_3$): 19.9, 48.1, 53.6, 57.5, 62.7, 68.4, 160.1, 168.2;

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

9-*epi*-Manzacidin B methyl ester (14)

To a solution of **37** (0.1 g, 0.49 mmol) in DMF (2 ml) was added NaH (65 mg, 1.63 mmol, 60% dispersion in oil) at room temperature and stirred for 15 min. To the mixture was added 1-(4-bromopyrrol-2-yl)-2,2,2-trichloroethan-1-one (**38**) (0.16 g, 0.54 mmol) and continued the stirring for 4 h, quenched with 2 N HCl, concentrated and purified on silica gel using ethyl acetate-methanol (5:1) as eluent to give **14** (0.1 g, 58 %).

[a] $_D$ = +56.5° (c 0.92, MeOH);

^{1}H NMR d **(200 MHz, acetone-d_6):** 1.27 (s, 3 H), 3.83 (s, 3 H), 4.17 (d, 1 H, *J* = 11.72 Hz), 4.39 (d, 1 H, *J* = 12.23 Hz), 4.58 (d, 1 H, *J* = 3.41 Hz), 4.81 (d, 1 H, *J* = 7.83 Hz), 6.79 (d, 1 H, *J* = 1.92 Hz), 7.01 (d, 1 H, *J* = 1.92 Hz), 8.07 (s, 1 H);

^{13}C NMR d (50 MHz, acetone-d_6): 19.8, 46.6, 52.0, 56.4, 62.7, 66.9, 98.7, 117.5, 122.1, 126.0, 156.1, 161.9, 171.7;

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

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PUBLICATIONS

List of Publications

1. Method for synthesizing diaryl substituted heterocyclic compounds including tetrahydrofurans, A V Rama Rao, M S Chorgade, Aminul Islam, V V K Rao, A S Prasad, B V Rao, Ranga Reddy, **L Krishnakanth Reddy**, K Shailaja, M K Gurjar S V Mhaskar, WO 2000 023409A1.

2. Synthesis of Ethyl 5-*O*-(α-D-arabinofuranosyl)-6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside Present in Motif E of *Mycobacterium tuberculosis* Cell Wall, M K Gurjar, **L Krishnakanth Reddy**, Srinivas Hotha, *Organic Lett*., **2001**, *3*, 321-323.

3. Synthesis of Oligosaccharides of Motifs D and E of arabinogalactan present in *Mycobacterium tuberculosis*, M K Gurjar, **L Krishnakanth Reddy**, Srinivas Hotha, *J. Org. Chem.*, **2001**, *66*, 4657-4660.