Fall 12-17-2011

Synthesis and Antifungal Evaluation of Spirostane Saponins

Sunil Upadhyay
University of New Orleans

Follow this and additional works at: https://scholarworks.uno.edu/td

Part of the Chemistry Commons

Recommended Citation
https://scholarworks.uno.edu/td/1414

This Dissertation is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UNO. It has been accepted for inclusion in University of New Orleans Theses and Dissertations by an authorized administrator of ScholarWorks@UNO. The author is solely responsible for ensuring compliance with copyright. For more information, please contact scholarworks@uno.edu.
Synthesis and Antifungal Evaluation of Spirostane Saponins

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Chemistry

By

Sunil Kumar Upadhyay

B.S. Ramjas College, Delhi University, India, 2002
M.S. Indian Institute of Technology Madras, Chennai, 2004

December 2011
Dedicated to
My Parents
and
My wife Rajni Upadhyay
ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor, Professor Branko S. Jursic, for his unwavering support and guidance. Without his encouragement and constant inspiration it would have not been possible to achieve this stage in my life. I would also like to thank the members of my committee, Dr. Mark L. Trudell, Dr. Richard B. Cole, Dr. Lee Roy Morgan and Dr. Ananthakrishnan Sankaranarayanan for all of their help and guidance throughout this process.

I would also like to thank to Dr Donna M. Neumann for allowing me to work in her lab and guiding me through doing antifungal assays and helping me gain a better understanding of the biological aspect of my project.

Additionally, I would like to recognize all the former of Dr. Jursic’s group – Dr. Katherine L. Bowdy, for helping me with getting started on my project and Dr. Sarada Sagiraju, for being very supportive during my early days in the group. I would also like to thank all current members of Dr. Jursic’s group- Ravi Kiran Pingali, Rajesh Komati and Monika Madhav for their support and help.

I would like to thank my parents for their tremendous emotional support. My special thank to my lovely wife Rajni Upadhyay for constantly motivating me and being much more than supportive through my entire journey. At the end I would like to thank God for giving me this opportunity and being so kind to me.
TABLE OF CONTENTS

List of Figures ........................................................................................................ v
List of Tables .......................................................................................................... ix
List of Scheme ....................................................................................................... x
Abbreviations ........................................................................................................ xii
Abstract ................................................................................................................ xiii

Chapter 1: Introduction .......................................................................................... 1
1.1 Pharmacological need for Novel Antifungal compounds ................................. 4
1.2 Classes of Antifungal compounds ................................................................... 6
1.3 Antifungal Agents: Mode of Action .............................................................. 13
1.4 Mechanism of resistance to Azols ................................................................. 23
1.5 Fungal Strains for Antifungal Assay ............................................................... 26
1.6 Specific Aims for the synthesis and biological assay of steroidal antifungals .... 32
1.7 References for Chapter 1 .............................................................................. 33

Chapter 2: Synthesis of Cholestan and Androstane derivatives .......................... 40
2.1 Retro-analysis for 2α, 3β- Cholestane derivative .......................................... 44
2.2 Synthesis of Cholestane derivatives ................................................................ 45
2.3 Synthesis of Androstane derivatives .............................................................. 53
2.4 Antifungal assay of Cholestane and Androstane derivatives ....................... 58
2.5 Conclusions for Chapter 2 .......................................................................... 60
2.6 Experimental for Chapter 2 ........................................................................... 61
2.7 References for Chapter 2 .............................................................................. 71

Chapter 3: Synthesis and Antifungal evaluation of functionalized 2,3-spirostanes.... 75
3.1 Retro-analysis for 2α, 3β-spirostane ............................................................... 81
3.2 Synthesis of Spirostane derivatives ............................................................... 82
3.3 First attempt for synthesis of \(2\alpha, 3\beta\)-spirostane isomer ...........................................83
3.4 Second attempt for synthesis of \(2\alpha, 3\beta\)-spirostane isomer ...........................................86
3.5 Third attempt for synthesis of \(2\alpha, 3\beta\)-spirostane isomer ...........................................88
3.6 Antifungal assay of spirostane derivatives .................................................................92
3.7 Conclusions for Chapter 3 .........................................................................................95
3.8 Experimental for chapter 3 .........................................................................................96
3.9 References –Chapter 3 .............................................................................................107

Chapter 4: Synthesis and Antifungal assay of steroidal saponins.................................111
4.1 Synthesis of Glycosides and saccharides .................................................................112
4.2 Anomeric Effect ..........................................................................................................115
4.3 Glycosilation method for oligosaccharides ...............................................................116
4.4 Synthesis of tetra-O-benzoyl-\(\alpha\)-D-glucopuranosyl trichloroacetimidate ..........119
4.5 Retroanalysis of saccharide moiety of saponin CAY-1 ..............................................120
4.6 Synthesis of \((1\rightarrow3)\)-\(\beta\) linked disaccharide trichloroacetimidate ..................121
4.7 Synthesis of various disaccharide trichloroacetimidates .........................................121
4.8 General methodology for Glycosylation ................................................................122
4.9 Monosaccharide derivatives of steroids ....................................................................123
4.10 Disaccharide derivatives of steroids .........................................................................124
4.11 Synthesis of branched oligosaccharides ..................................................................125
4.12 Synthesis of Fatty acid derivatives of steroids .......................................................129
4.13 Antifungal assay of steroidal saponins ....................................................................131
4.14 Conclusions-Chapter 4 ..........................................................................................136
4.15 Experimental-Chapter 4 .........................................................................................137
4.16 references- Chapter 4 .............................................................................................171

Chapter 5: Tag molecule based approach for synthesis of branched oligosaccharides .175
5.1 Synthesis of tag molecules .......................................................................................179
5.1.1 Synthesis of Phthalimides and Naphthalimides ...................................................179
5.1.2 Synthesis of Succinimides ................................................................. 182
5.1.3 Synthesis of Maleimides .................................................................. 184
5.2 Microwave-assisted NBS bromination of \( p \)-iminotoluenes .................. 187
  5.2.1 Microwave-assisted NBS mono-bromination of \( p \)-iminotoluenes .... 189
  5.2.2 Microwave-assisted NBS di-bromination of \( p \)-iminotoluenes ......... 191
5.3 Synthesis of aldehyde based tag molecules ......................................... 193
5.4 Tagged glucose for synthesis of branched oligosaccharides .................. 194
5.5 Conclusions- Chapter 5 ...................................................................... 201
5.6 Experimental- Chapter 5 ..................................................................... 202
5.7 References- Chapter 5 ........................................................................ 217
LIST OF FIGURES

Figure 1.1 Timelines for systemic antifungals .......................................................... 2
Figure 1.2 Antifungal Polynes ..................................................................................... 6
Figure 1.3 Antifungal Azoles ...................................................................................... 7
Figure 1.4 Antifungal Sordarines ............................................................................... 8
Figure 1.5 Antifungal echinocandins ....................................................................... 9
Figure 1.6 Classes of Saponins .................................................................................. 10
Figure 1.7 Antifungal Saponins ............................................................................... 11
Figure 1.8 Structure of Antifungal Saponin CAY-1 .................................................. 12
Figure 1.9 Schematic representation of Polyenes with fungal cell membrane .......... 14
Figure 1.10 Mode of action of Antifungals ................................................................. 14
Figure 1.11 Ergosterol bio-synthetic pathway ............................................................. 18
Figure 1.12 The Transmembrane complex of two proteins Fks1p ans Fks2p ............ 19
Figure 1.13 The effect of CAY-1 compared to DMSO controls on fungal cells .......... 21
Figure 1.14 Proposed model for membrane disruption by saponins ......................... 22
Figure 1.15 Mechanism by which microbial cells might develop resistance .......... 24
Figure 1.16 Candida albicans .................................................................................... 27
Figure 1.17 Cryptococcus neoformans ..................................................................... 28
Figure 1.18 Candida glabrata .................................................................................... 30
Figure 1.19 Aspergillus fumigatus ............................................................................. 31
Figure 2.1 Oxygentaed Cholesterol derivatives for antimicrobial activity ............... 40
Figure 2.1 Active fatty acid analogues of cholesterol ................................................. 42
Figure 2.3 2α, 3β- Cholestan and Spirostane derivatives ........................................... 43
Figure 2.4 1H-NMR for enolization and epoxidation ............................................... 49
Figure 2.5 1H-NMR for regeioselective acylation and carbonyl reduction ................ 52
Figure 2.6 2α, 3β- Cholestane, Androstane and Spirostane derivatives ................. 53
Figure 2.7 Androstane derivatives with antifungal activity ........................................54
Figure 2.8 $^1$H-NMR for Androstane derivatives ..........................................................56
Figure 3.1 Portion $^1$H-NMR for 3α-hydroxyspirostan-2β-yl acetate .......................85
Figure 3.2 ORTEP drawing of 3α-hydroxyspirostan-2β-yl acetate ..........................86
Figure 3.3 Portion $^1$H-NMR for 2,3- spirostane isomers .........................................90
Figure 4.1 Anomeric effect .........................................................................................115
Figure 4.2 Explanation for Anomeric effect ...............................................................116
Figure 4.3 Monosaccharide derivatives of steroids ..................................................124
Figure 4.4 Disaccharide and trisaccharide derivatives of steroids .............................126
Figure 5.1 Tagged glucose as a synthon for preparation of branched oligosaccharides.177
Figure 5.2 New alcohol, thiol, and amino protection groups ....................................187
Figure 5.3 Possible attachment sites for tag molecule .............................................195
Figure 5.4 $^1$H-NMR for tagged monosaccharide key intermediate .......................198
LIST OF TABLES

Table 1.1 Fungal diseases, symptoms and causing agents ......................................................... 26
Table 2.1 Antifungal assay of Cholestane and Androstane derivatives .................................. 59
Table 3.1 Antifungal assay of spirostane derivatives .................................................................. 94
Table 4.1 Antifungal activity of novel steroid monosaccharide analogues ............................. 133
Table 4.2 Antifungal activity of novel steroid disaccharide analogues ............................... 134
Table 4.3 Antifungal activity of novel steroid trisaccharide and fatty acid analogues ... 135
Table 5.1 Preparation of phthalimides ....................................................................................... 180
Table 5.2 Preparation of naphthalimides ................................................................................. 182
Table 5.3 Preparation of succinimides ..................................................................................... 183
Table 5.4 Preparation of maleimides ......................................................................................... 185
Table 5.5 Preparation of benzyl bromides ............................................................................... 190
Table 5.6 Preparation of benzylidene dibromides ................................................................. 192
LIST OF SCHEMES

Scheme 2.1 5,6-fused steroidal oxazoles in cholestane series by Shamusuzzaman et al......41
Scheme 2.2 Synthesis of hydrazone derivatives of cholesterol by C. Loncle et al.............41
Scheme 2.3 Synthesis of cholestane derivatives by G.A.G. Santos et al..........................44
Scheme 2.4 Retro-analysis of 2α, 3β-choelstane derivatives ........................................45
Scheme 2.5 Synthesis of cholestan-3-one by Min Serk et al............................................46
Scheme 2.6 Synthesis of 2α-acetoxy-lupan-3-one................................................................47
Scheme 2.7 Regoeoselective enolization of ketone ................................................................47
Scheme 2.8 Synthesis of enol acetate using mantmorillonite KSF clay ..............................48
Scheme 2.9 Mechanism-stereoselective epoxidation..........................................................48
Scheme 2.10 Probable mechanism for epoxide ring opening by acetic acid ..........................50
Scheme 2.11 Synthesis of 2α, 3β-choelstane derivatives ......................................................51
Scheme 2.12 Synthesis of 2α, 3β-Androstane derivatives....................................................55
Scheme 3.1 Synthesis of 2α, 3β-aglycone moiety by Sondheimer et al............................78
Scheme 3.2 Synthesis of 2α, 3β-aglycone moiety by Holzapfel et al ................................79
Scheme 3.3 Synthesis of 2α, 3β-aglycone moiety by Yu et al ............................................79
Scheme 3.4 Synthesis of 2,3 spirostane derivatives...............................................................81
Scheme 3.5 Retro-analysis of 2α, 3β-spirostane derivatives .................................................82
Scheme 3.6 First attempt synthesis of of 2α, 3β-spirostane derivatives .............................84
Scheme 3.7 Stereoselective mechanism for epoxidation ......................................................84
Scheme 3.8 Epoxide ring opening by acetic acid.................................................................85
Scheme 3.9 Platinum catalyzed reduction of diosgenin.......................................................87
Scheme 3.10 Final Synthesis of of 2α, 3β-spirostane derivatives .........................................89
Scheme 4.1 Synthesis of Glycosides and Saccharides .........................................................113
Scheme 4.2 Schmidt’s trichloroimidate method .................................................................114
Scheme 4.3 Generalized mechanism for glycosylation reaction ........................................117
Scheme 4.4 Glycosylation of sapogenins reported by Yu et al...........................................118
Scheme 4.5 Synthesis of tetra-benzoyl-α-D-glucopyranosyl trichloroacetimidate ..........119
Scheme 4.6 Retro-analysis of CAY-1 pentasaccharide moiety...............................120
Scheme 4.7 Synthesis of (1-3)-β- linked disaccharide trichloroacetimidate..................121
Scheme 4.8 Synthesis of various disaccharide trichloroacetimidates..........................122
Scheme 4.9 Glycosylation for synthesis of steroidal saponins-general scheme ............123
Scheme 4.10 Synthesis of 2,3- branched oligosaccharides ....................................128
Scheme 4.11 fatty acid derivatives of steroids ..........................................................130
Scheme 5.1 Monophasic and Biphasic reaction .........................................................178
Scheme 5.2 Synthesis of aldehyde based tag molecules ............................................193
Scheme 5.3 Synthesis of dimethoxyacetal based tag molecules ...............................193
Scheme 5.4 Synthesis of thiol based tag molecule .....................................................196
Scheme 5.5 Synthesis of activated and tagged glucose ............................................198
Scheme 5.6 Glycosylation, Oligosacchride deprotection and tag molecule removal ......199
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>Bz</td>
<td>Benzoyl</td>
</tr>
<tr>
<td>CSA</td>
<td>Camphorsulfonic acid</td>
</tr>
<tr>
<td>DME</td>
<td>Ethylene glycol dimethyl ether</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-Dimethylforamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>HMDS</td>
<td>1,1,1,3,3,3-hexamethyldisilazane</td>
</tr>
<tr>
<td>$m$-CPBA</td>
<td>$m$-Chloroperoxybenzoic acid</td>
</tr>
<tr>
<td>Ms</td>
<td>Mesylate</td>
</tr>
<tr>
<td>9-BBN</td>
<td>9-Borabicyclo[3.3.1]nonane</td>
</tr>
<tr>
<td>NIS</td>
<td>$N$-Iodosuccinimide</td>
</tr>
<tr>
<td>NBS</td>
<td>$N$-Bromosuccinimide</td>
</tr>
<tr>
<td>PE</td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl triflate</td>
</tr>
<tr>
<td>PTSA</td>
<td>$p$-Toluenesulfonic acid</td>
</tr>
</tbody>
</table>
ABSTRACT

Methods for the preparation of novel antifungal saponins have been investigated in order to further explore their medicinal utility and provide the opportunity to synthesize their derivatives.

Through this work, several partially protected stereoisomers of Cholestane, Androstane and Spirostan have been prepared which could be used for the synthesis of various saponin derivatives in order to discover novel saponin based antifungal agent. Various mono and disaccharide derivatives of these steroids have been synthesized and evaluated for their antifungal activity against four pathogenic fungal strains. Among the various derivatives maltose derivatives were found to have the best antifungal activity. However there is a need for more extensive SAR studies to discover compounds with better potency.

Additionally, the branched oligosaccharide synthesis was explored in two parts. First, these results demonstrated that the central 2,3-branched portion can be synthesized efficiently from a partially protected glucopyranosyl acceptor since the C-2 and C-3 alcohols differ in their reactivity in glycosylation reactions. Second, a tagged sugar based strategy for synthesis of branched oligosaccharides was developed, and found to be effective for general synthesis of branched oligosaccharides.

Microwave assisted synthesis of cyclic imides have been explored this was a key precursor for the synthesis of our tag molecules which were required for synthesis of branched oligosaccharides. A comparison of microwave versus conventional methods for synthesis of cyclic imides has been studied. The synthesis of tagged sugars and their selective deprotection to remove tag molecules were successfully explored in order to have proof of concept for its applicability towards synthesis of branched
oligosaccharides.

Benzylic mono and dibromination was achieved in very high yields using microwave conditions using environmentally friendly solvent in order to avoid use of carcinogenic carbon tetrachloride as solvent for this type of reactions. In addition reaction time was reduced to 30 minutes to 3 hours compared to convention methods, which needed more than 15 hours for the benzylic bromination reaction.

CHAPTER-1: INTRODUCTION

The clinical needs for novel antifungal agents have increased steadily with the rise and fall of AIDS-related mycoses, and the increased spectrum of fatal fungal infections that has accompanied changes in immunosuppressive therapies. The search for new molecular targets for antifungals has generated considerable research using modern genomic approaches, so far without generating new agents for clinical use. Invasive fungal infections have transitioned from a rare curiosity to an everyday problem for the practicing physician. Invasive candidiasis is the third to fourth most common bloodstream infection in surveys in the United States. Similar trends have been reported in several regions throughout the world\textsuperscript{1-3}. Rates of invasive aspergillosis and mucormycosis are increasing parallel to the growth of immunocompromised patient populations\textsuperscript{4-8}. Invasive fungal infections are associated with substantial morbidity and mortality, despite optimal antifungal therapy, and carry a high financial burden. Improved diagnostics, recent epidemiological studies and the availability of variety of new antifungals have advanced the field of medical mycology in the past few decades.

Although systemic antifungals have been available since the 1950s, compounds characterized the early antifungal pipeline had limited efficacy and high toxicity, such as the polyenes nystatin and amphotericin B\textsuperscript{9}. Triazoles were developed in the 1980s, which revolutionized medical mycology due to their availability in both intravenous and oral formulations. They were effective against fungal pathogens that were refractory to the polyenes. Triazoles were further developed and amphotericin B reformulations with lipid compounds were reported in 1990s (Fig.1.1). Lipid-based formulations were much less toxic and allowed the true efficacy of polyenes to be explored, expanding the pharmacokinetic and pharmacodynamic limits of these agents\textsuperscript{9}. The twenty first century brought the echinocandins, a new class of antifungals.
to the market. They inhibit the synthesis of (1,3)-β-glucan (a key component of fungal cell walls) and represent the first class of antifungal agents that act against a specific component of the fungal cells and not mammalian cells\textsuperscript{10-12}.

![Timeline of systemic antifungals](image)

**Figure 1.1** Timeline of systemic antifungals.\textsuperscript{13}

Antifungal research and development is challenging because apart from the fungal cell wall, fungal cells are metabolically similar to mammalian cells and offer few pathogen-specific targets. Clinical development is further hampered by challenges of timely and definitive diagnoses of the more resistant fungi.

Over the past two decades, fungi have emerged as important causes of infectious morbidity and mortality in immunocompromised patients. The most significant risk factors include profound and prolonged granulocytopenia, immunosuppression with corticosteroids, acquired deficiencies in the number and/or function of T-helper cells, and severe illness requiring
multiple invasive medical procedures, such as the use of intravascular devices and extensive abdominal surgery\textsuperscript{14}. While the species \textit{Aspergillus fumigatus} and \textit{Candida albicans} traditionally account for the majority of invasive opportunistic infections, more recent epidemiological trends indicate a shift toward infections by non-fumigatus \textit{Aspergillus spp.}, non-albicans \textit{Candida spp.}, and previously uncommon fungi that often display resistance to current antifungal agents \textit{in vitro} and \textit{in vivo}\textsuperscript{14}. Human immunodeficiency virus (HIV)-infected patients with advanced immune dysfunction are particularly susceptible to cryptococcal meningitis, disseminated histoplasmosis, coccidioidomycosis, and penicillosis, and recent outbreaks highlight that endemic fungi can become a significant public health concern beyond their baseline prevalence\textsuperscript{15}.

Improved blood culture, antigen, and nucleic acid detection techniques, the advent of high-resolution two-dimensional imaging, and an increased awareness among physicians of the fungal pandemic have all had considerable impact on improving the early clinical diagnosis of invasive fungal infections, and major advances have been achieved in disease definitions, in defining paradigm for antifungal interventions, and in designing and implementing clinical trials\textsuperscript{16-17}. Finally, standardized methods for testing the \textit{in vitro} susceptibility of fungi have become available and are continuously refined, and concentration-effect relationships \textit{in vitro} and \textit{in vivo} are increasingly explored. Nevertheless, despite these advances, invasive fungal infections remain difficult to diagnose and to manage, and there is a continuing need for improved antifungal therapy.
1.1 Pharmacological Need for Novel Antifungal Compounds

Members of the genera *Candida* and *Aspergillus* most commonly cause invasive fungal infections. For example, the incidences of invasive *Candida* and *Aspergillus* infections in bone marrow transplant recipients are 10-15% and 3-7% respectively, and the mortality rate is 60-95%\(^{18a}\). Overall, *Candida* infections are the fourth most common type of nosocomial bloodstream infection in the United States and have a higher mortality rate (33-47%) than any other bloodstream infection\(^{18b}\). Furthermore, there has been a 14-fold increase in the frequency of opportunistic invasive fungal infections from 1980-1992 in the United States and Europe\(^{19}\).

This increase in incidence of invasive fungal infections in immunocompromised individuals is largely attributed to medical advancements in the treatment of these patients, especially in the management of bacterial infections. It has been estimated that the development of antibiotics has increased the life expectancy of humans by ten years\(^{20}\). Although physicians have had success in increasing the life expectancy of immunocompromised patients by treating bacterial infections, they are now struggling to treat the resulting opportunistic invasive fungal infections. Just as antibiotic resistant bacteria appeared soon after the introduction of the first antibiotics, the problem of antifungal resistance has been emerging as the use of existing antifungal agents has increased in response to increasing incidences of life-threatening fungal infections.

In order to avert the problem of antimicrobial resistance, the successful management of fungal infections requires a large number of antifungal agents belonging to a wide variety of classes with different mechanisms of action. Additionally, the patients most at risk for invasive fungal infections are typically taking several medications to treat the underlying diseases causing their neutropenia (a hematological disorder characterized by abnormally low number of
neutrophils, the most important type of white blood cell). Therefore, it is also important that physicians have access to a large number of antifungal medications to improve their chances of finding one that is both effective for treating the infection and will not interact with other medications the patient may be taking. Unfortunately, due to increased resistance and the emergence of new opportunistic infections, there is still a dire need for the development of new antifungal compounds. As of 2000, there were only ten medications approved by the U.S. Food and Drug Administration (FDA) for use in the treatment of invasive fungal infections and these drugs belong to only three classes: polyenes, pyrimidines, and azoles.
1.2 Classes of antifungal compounds:

1.2.1 Polyenes: The broad-spectrum polyene antifungal amphotericin B (Fig.2) was an early mainstay for the treatment of various types of invasive antifungal infections. Amphotericin B binds strongly to ergosterol, the principal sterol in fungal cell membranes. The preferential binding of Amphotericin B to ergosterol results in the formation of a membrane pore, which leads to leakage of ions from the internal of the cell. This ultimately results in the disruption of the osmotic integrity of the fungal cell and leads to cell death. The polyene, nystatin (fig.1.2) was originally isolated from Streptomyces noursei in 1951 is a conjugated tetraene, it was first clinically useful polyene antifungal. Natamycin, another polyene, was isolated from cultures of Streptomyces natalensis intended for the treatment of fungal conjunctivitis, blepharitis and keratitis.

![Amphotericin B and Nystatin](image.png)

**Figure 1.2** Antifungal Polyenes.

1.2.2 Azoles: The azoles are the largest class of synthetic antimycotics and account for approximately 20 antifungal agents on the market today. The class itself can be broken down into two subdivisions, the imidazoles and the triazoles. The first members of the class were highly substituted imidazoles (clotrimazole, miconazole). Imidazole antifungals were not absorbed orally and were primarily formulated for topical use, usually because of toxicity or bioavailability problems that limited their potential as systemic agents. However the imidazole
Ketoconazole was introduced in 1984 and was the first effective oral therapy in the class of imidazoles for treatment of *Candida spp*. Subsequently, the triazoles Itraconazole and Fluconazole (Fig.3) were developed as more potent azoles that are more potent azoles that are less toxic and provide effective oral therapy for many systemic fungal infections\(^\text{23}\). Since then, triazoles remain the mainstay of treatment for antifungal infections and their development is currently ongoing. Posaconazole and Voriconazole (Fig. 1.3) represent specific advances in the understanding of the SAR for the antifungal azole class, as evident by their progression over past 30 years. Albaconazole (UR-9825) is currently under development by Stiefel under licence from its discoverer, Uriach \(^\text{24}\). Isavuconazole (BAL-4815) is undergoing development in the form of orally active, Isavuconazole is currently in phase-III clinical trials \(^\text{25}\).

![Antifungal Azoles](image)

**Figure 1.3 Antifungal Azoles.**

**1.2.3 Sordarins:** Sordarins are semi-synthetic natural products shown to have antifungal activity in early 1970s \(^\text{26}\). Sordarin R-135853 (Fig.4) has shown *in vivo* efficacy in various experimental
models of candidiasis\textsuperscript{27}. FR290581 is a sordarin derivative that in under development has shown excellent antifungal activity against \textit{Candida albicans}\textsuperscript{28}. (Fig. 1.4) To date, neither compounds has made it through FDA, and are currently not available as a drug.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure1.4.png}
\caption{Antifungal Sordarines.}
\end{figure}

\textbf{1.2.4 Echinocandins:} The echinocandins are fungal secondary metabolites comprising a cyclic hexapeptide core with a lipid side-chain responsible for antifungal activity. Random screening in the 1970s discovered antifungal activity in the prototypes, echinocandin B and aculeacin A. A modified form of echinocandin B, cilofungin, was developed to the point of phase 2 trials, but was abandoned when its formulation showed toxicity to patients\textsuperscript{29}. In the late 1990s, three echinocandin-class compounds, anidulafungin, caspofungin and micafungin, all entered clinical development. The three-dimensional configuration of all three molecules is similar. A central, common core bears a long, ‘gun-barrel’-like side chain known to be a determinant of the spectrum of susceptible species (Fig.1.5), and a hydroxylated side chain that appears opposite the ‘gun barrel’ in flat structural representations but is adjacent in energy-minimized 3-D structures.
The FDA approved caspofungin in 2001 for therapy of aspergillosis. The echinocandin spectrum excludes some pathogens such as *Cryptococcus neoformans*, *Fusarium spp.* and *Scedosporium spp.* However, they are active against the opportunistic pathogen *Pneumocystis jiroveci*. All of the three new echinocandins can only be administered by intravenous injection, which also restricts their use.

**1.2.5 Saponins:** The saponin is a family of natural product compounds that shows promise in the identification of novel antifungal compounds. They are divided based on the structure of their aglycone moiety into three classes: the steroid class, the triterpene class, and the steroid alkaloid class (Fig. 1.6). In addition to aglycone moiety, saponins also have an oligosaccharide moiety of up to five sugar molecules usually attached to the C-3 position of the aglycone and some saponins also have a C-26 or C-28 sugar moiety. The oligosaccharide usually consists of a
combination of glucose, arabinose, glucuronic acid, rhamnose, or xylose\textsuperscript{31}.

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{saponin_classes}
\caption{Classes of saponin.}
\end{figure}
\end{center}

Saponins are named after one of their most notable characteristics, their amphiphilicity due to the lipophilic steroidal moiety and the hydrophilic oligosaccharide moiety\textsuperscript{32}. Many plants produce saponins as secondary metabolites that primarily serve as antimicrobial agents and traditionally it was believed that plants were the only natural source of saponins. More recently, however, biologists have discovered that saponins are also abundant in many marine animals, most notably in echinoderms, but they also appear occasionally in higher animals such as fish\textsuperscript{33}.

Saponins have a wide range of biological activity that goes beyond their antimicrobial roles to include hypocholesterolemic, anti-inflammatory, anti-diabetic, anti-allergenic, immunomodulatory, anti-hepatotoxic, and anti-cancer capabilities\textsuperscript{34}. It has been shown that both the aglycone and oligosaccharide moieties of saponins are important for their biological activity.\textsuperscript{35-39} While the effectiveness of saponins has been recognized for centuries in herbal medicine, they were not typically viewed as potential drug targets in the past due to their structural complexity. One exception is digoxin (Fig.1.7), a cardiac glycoside that has been isolated from the leaves of the foxglove plant \textit{Digitalis lanata} and marketed for over 200 years to treat congestive heart failure. Digoxin binds to Na\(^+/\)K\(^+\) ATPase pumps in cardiac cell membranes. This alters the membrane potential by changing the concentrations of ions and ultimately leads to an increase in the strength of the contraction of the cardiac muscles.\textsuperscript{34}
Unfortunately, digoxin is an unusual saponin. Its availability is only by isolation from natural sources in sufficient quantities for distribution. Even so, recent advances in the synthesis of glycoconjugates allowed further exploration of additional medicinal uses for saponins.

![Digoxin](image1)

**Figure 1.7** Antifungal saponins.

In fact, saponins have drawn much attention in recent years owing in large part to OSW-1 (Fig.7); a steroidal saponin isolated from bulbs of the lily plant *Ornithogalum saundersiae*. OSW-1 shows *in vitro* anti-tumor activity levels that are 10-100 times more effective than many well-known anticancer compounds including taxol and cisplatin. Furthermore, this saponin has the potential to be effective against cancers that are resistant to currently available chemotherapy because it has an unusual mechanism of action. Upon entering a cancer cell, OSW-1 causes damage to the mitochondrial membranes, which in turn disrupts the calcium levels in the cytoplasm of the cell and ultimately causes cell death by apoptosis. Like OSW-1 many other saponins have also been well documented to possess potent *in vitro* antifungal properties. For example, a derivative of the triterpene medicagenic acid (Fig.1.7), which was isolated from alfalfa root extracts. Initial testing revealed that it was effective against fungal skin infections in
guinea pigs and did not show dermal toxicity in rhesus monkeys. It also showed promise toward the treatment of invasive fungal infections by successfully treating *Cryptococcus neoformans* infections in mice with a minimal inhibitory concentration of 4 µg/mL. This saponin was also effective against amphotericin B-resistant strains of *Candida tropicalis*\(^{41}\). It was synthesized in three steps from medicagenic acid and acetobromomaltose in 49% overall yield.\(^{41}\)

With respect to the scope of this work, CAY-1 is an important newly identified saponin that has the potential to be effective in the treatment of invasive fungal infections (Fig.1.8). CAY-1 was isolated in 0.1 % yield from the fruit of the cayenne pepper plant, *Capsicum frutescens*. Sufficient quantities of CAY-1 were available by this isolation from cayenne pepper plant in order to perform preliminary testing for antifungal activity and cytotoxicity toward mammalian cells\(^{42}\). Following these preliminary studies- CAY-1 was shown to be an effective antifungal agent against sixteen fungal strains of *Candida* species, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Neurospora crassa*. Further, each of these strains are human pathogens known to cause invasive fungal infections in immunocompromised patients. The most promising result was the activity of CAY-1 against *C. neoformans* (IC\(_{90} < 1\)µg/mL), a fungal species responsible for cryptococcal meningitis in HIV/AIDS patients, a fatal disease if left untreated.\(^{43}\)

![Figure 1.8](image-url)  
*Figure 1.8 Structure of antifungal saponin CAY-1.*
CAY-1 was tested in vitro for cytotoxicity against 55 mammalian cell lines. Nevertheless given these preliminary results, we opted to use CAY-1 as a model saponin for the development of the project.

1.3 Antifungal Agents: Mode of Action:

Of the given antifungals on the market today, there are a number of mechanism of action, all of which have selective toxicity for the fungal cell over the mammalian cell. In terms of number of classes of antifungal agents that can be used to treat life-threatening mycoses, the targets are highly focused, directly or indirectly, on the cell envelope (wall and plasma membrane), and particularly on the fungal membrane sterol, ergosterol, and its biosynthesis.

1.3.1 Polyene antifungal agents

Amphotericin B has for many years been the only antifungal polyene that can be administered systemically to treat a visceral infection. Its mode of action is atypical for an antimicrobial molecule: instead of inhibiting an enzyme, it binds to ergosterol, the principal sterol in fungal membranes (Fig.1.9), thereby perturbing membrane function to the point of causing leakage of cellular contents. The precise way in which this fungicidal effect occurs still remains unclear. The ergosterol molecule of fungi has a cylindrical three-dimensional structure, unlike cholesterol, the major sterol in mammalian membranes, which has a sigmoid shape11. This conformational difference is probably sufficient to explain the greater binding affinity of amphotericin B for ergosterol over cholesterol.
Figure 1.9 Schematic representation of the interaction between amphotericin B and ergosterol in fungal cell membrane.\textsuperscript{53}

Figure 1.10 Mode of action of antifungals. a) Echinocandins and nikkomycin Z inhibit the formation of the fungal cell wall. b) Sordarins interfere with protein assembly. c) Azoles, polyenes and terbinafine disrupt the fungal cell membrane. d) Flucytosine interferes with DNA synthesis. Antibodies and vaccines prevent fungal infection or block and/or destroy the fungal cells.\textsuperscript{13}

This shape difference and the higher ratio of ergosterol in fungal cells are likely the basis for the antifungal selectivity of amphotericin B. However, the selectivity of amphotericin B is
low and suggests that amphotericin B can be toxic to mammalian cells. Binding to a sterol leaves the amphoteric amphotericin B molecule with its hydrophilic edge unbalanced relative to the larger hydrophobic portion of the complex. Almost certainly, such a complex when formed in a membrane will migrate to align the hydrophilic faces within aggregations of complexes, thus creating areas of local tension within the membrane. The mechanism of action correctly predicts that amphotericin B will act on a broad spectrum of fungal species. As predicted on the basis of its mechanism of action, amphotericin B is toxic to mammalian cells, particularly causing nephrotoxicity\textsuperscript{46}. This was observed from the earliest days of clinical use of the drug. To overcome amphotericin B toxicity, a variety of reformulated versions of the agent have been introduced. These reformulations reduce nephrotoxicity by slowing the rate at which amphotericin B is delivered to the kidneys.

The most successful, clinically proven versions of novel formulations are based on lipid combinations with amphotericin B, encapsulated in liposomes or in ribbon-like and disc-like lipid complexes. Other formulations are under investigation, including an amphotericin B – cochleate preparation and an arabinogalactan complex. The antifungal polyene nystatin is also being developed in a liposomal formulation for systemic use.

1.3.2 Antifungal azoles

Imidazoles and triazoles (‘azoles’) are the largest class of antifungal agents in clinical use. Their main effect is to inhibit 14α-demethylation of lanosterol in the ergosterol biosynthetic pathway\textsuperscript{47}, but in some fungal species, they can also inhibit the subsequent Δ22-desaturase step (Fig.1.11)\textsuperscript{48}. With ergosterol depleted and replaced with unusual sterols, the normal permeability and fluidity of the fungal membrane is altered, with secondary consequences for membrane-bound enzymes, such as those involved in cell wall synthesis\textsuperscript{49}. Antifungal azoles bind to the
iron atom via a nitrogen atom in the imidazole or triazole ring. The remainder of the azole molecule binds to the apoprotein in a manner dependent on the individual azole’s structure. The exact conformation of the active site differs between fungal species and amongst the many mammalian P450 mono-oxygenases (P450 mono-oxygenases are heme protein-dependent mixed function oxidase systems that utilize NADPH and/or NADH to reductively cleave atmospheric dioxygen to produce a functionalized organic substrate and a molecule of water). The precise nature of the interaction between each azole molecule and each kind of P450 therefore determines the extent of the azole’s inhibitory effect in different fungal species. A triazole nucleus has replaced imidazole in the active pharmacophore, to enhance the specificity of binding to fungal P450. For molecules derived from the fluconazole prototype, the inclusion of an α-O-methyl group confers activity against *Aspergillus* species and many other filamentous fungi. For molecules derived from the ketoconazole prototype, extension of the side chain enhances binding of the azole to the P450 apoprotein. The principal molecular target of azole antifungals is a cytochrome P450 – Erg11p or Cyp51p, according to different gene-based nomenclatures, which catalyzes the oxidative removal of the 14α-methyl group of lanosterol and/or eburicol in fungi by a typical P450 mono-oxygenase activity\(^5\). This protein contains an iron protoporphyrin moiety located at the active site, and the structural differences might seem small, but they dictate antifungal potency and spectrum, bioavailability and drug interaction and toxic potential – very important considerations for compounds that bind to heme groups in P450s. Voriconazole, which is the first approved and so far the most fully characterised of the three new triazoles, enjoys a very broad spectrum of target fungal species and, like itraconazole, is even fungicidal against some isolates of filamentous species. Furthermore, it shows activity against the hard-to-treat *Fusarium* and *Scedosporium* infections.
However, there is a long list of other drugs with which voriconazole interacts (similar to the list for itraconazole), including some immunosuppressants, benzodiazepines, prednisolone, digoxin and other drugs in common use, which can cause problems for physicians treating seriously ill patients who receive multiple medications. Posaconazole also acts against a broad spectrum of susceptible fungi, and shows interestingly promising efficacy against coccidioidomycosis in preclinical studies. Ravuconazole, with an identical pharmacophore but a longer side-chain than voriconazole, stands out for its unusually long plasma half-life in humans.
Figure 1.11 Ergosterol bio-synthetic pathway. Steps at which various antifungal agents exert their inhibitory activities are shown. FLU, fluconazole; ITRA, itraconazole; VOR, voriconazole.55

1.3.3 Echinocandins

The target for the echinocandins is the complex of proteins responsible for synthesis of cell wall β-1,3 glucan polysaccharides (Fig. 1.12). Mechanistic details of glucan synthesis and its inhibition by echinocandins still remain unclear, largely because a membrane-associated protein
complex is involved. There is no doubt that the component to which echinocandins bind is \( Fks1p \), but their non-competitive inhibitory effects on glucan synthesis do not necessarily imply that \( Fks1p \) itself is the catalytic subunit, nor is it clear whether the echinocandin-binding site on \( Fks1p \) is external or internal to the cell membrane.

**Figure 1.12** The transmembrane complex of two proteins, Fks1p and Fks2p, involved in synthesis of \( \beta \)-1:3 glucan in the cell walls of Saccharomyces cerevisiae.\(^\text{11}\)

The rationale is similar to that used to treat tuberculosis and HIV infection, in which effects on two or more disparate targets are a better guarantee of clinical efficacy than effects on a single target. So far, resistance to caspofungin has only appeared by mutation of the \( FKS1 \) gene in \( C.\ albicans\)\(^\text{29}\). The known fungal efflux pumps do not export the drug.

### 1.3.4 Sordarins

Sordarins inhibit protein synthesis by blocking the function of fungal translation Elongation Factor 2. Interest in sordarins was increased as a result of a prospective screen for inhibitors of \( C.\ albicans\) protein synthesis *in vitro*, which pinpointed the nature of the sordarin antifungal effect. When refined research work revealed EF2 as the specific target of the
sordarins, the result engendered surprise because *C. albicans* EF2 displays more than 85% amino acid sequence identity to the human equivalent, and EF2 would never have emerged as a potential target from genomics-based screening\(^5\). Different sordarin derivatives have different spectra of susceptible species, for reasons that are not yet clear. This might reflect problems of penetration of these agents into target fungi. Nevertheless, their high specificity for the fungal target and the relative ease with which new sordarin variants can be synthesized holds promise for positive future developments with this series.

### 1.3.5 Saponins

Saponins are known to alter the permeability of fungal cell membranes, which allows intracellular components to leak out of the cell and ultimately causes cell death\(^{31a}\). This was demonstrated with CAY-1 by treating *N. crassa* spores with \(^{14}\text{C}\)-alpha-amino-isobutyric acid. The radioactivity remaining in the cells after treatment with 25 \(\mu\text{g/mL}\) of CAY-1 was measured using a liquid scintillation counter and compared to controls that were dosed with dimethylsulfoxide (DMSO) (Fig.1.13). The results show that the radioactivity in the treated cells decreased significantly after five minutes, suggesting that the integrity of the cellular membranes had been compromised.\(^{31a}\)
However the exact mechanism by which saponins alter the permeability of fungal cell membranes is not clear. The tendency of saponins to bind to steroids suggests that antifungal saponins, which are not toxic toward mammalian cells may bind preferentially to ergosterol, the major sterol component in fungal cell membranes, rather than cholesterol, the major sterol component in mammalian cell membranes (Fig. 1.14). Once bound, the sterol-saponin complexes may aggregate to create pores in the membrane or to extract the sterol components from the membrane. Evidence to support the formation of pores in fungal membranes upon exposure to saponins has been provided by electron microscopic analysis.

The mechanism of saponin resistance may vary. Whereas saponin resistance can be conferred by non-degradative mechanism, a number of pathogenic fungi degrade the saponins of their respective host plants, usually by hydrolysis of sugar molecule from the sugar chain attached at C-3 of the saponin backbone.
Figure 1.14 Proposed mechanism for membrane disruption by saponins.\textsuperscript{31b}
1.4 Mechanisms of resistance to azoles

As noted above, there are as yet no reports of modification of azole antimicrobials as a mechanism of resistance. Resistant strains therefore either exhibit a modification in the quality or quantity of target enzyme, reduced access to the target, or some combination of these mechanisms. These mechanisms are discussed in detail below and are summarized in Fig.15. Several lines of evidence implicate a modification in the quantity or quality of 14α-demethylase in the expression of resistance toazole antifungalagents. A recent study examined the biochemical mechanisms for resistance to fluconazole by comparing sterol composition, fluconazole accumulation, and inhibition of 14α-demethylase by fluconazole in two clinical C. krusei strains (expressing intrinsic resistance to fluconazole) and a susceptible C. albicans isolate. No significant differences in the sterol content of C. krusei and C. albicans were detected (ergosterol was the major sterol in both species). Studies performed on cell extracts indicated that the concentration of fluconazole required to inhibit the synthesis of ergosterol by 50% was approximately 24 to 46-fold higher in C. krusei than in C. albicans, suggesting that affinity of the target enzyme is different in the two species. A comparison of fluconazole accumulation by C. albicans and C. krusei indicated that fluconazole accumulation in the first 60 min was similar in all study strains. However, analysis after 90 min of incubation revealed that C. krusei accumulated 60% less fluconazole than did C. albicans, implicating active efflux in the fluconazole resistance expressed by these C. krusei strains. The potential coexistence of two resistance mechanisms precludes a precise calculation of the level of resistance contributed by the low-affinity 14α-demethylase. Over expression of 14α-demethylase has also been implicated as a mechanism of resistance toazole antifungals. Vanden Bosse et al. characterized as azole-resistant C. glabrata strain and showed that its ergosterol content was increased compared with
that of the permanent isolate. This increase was accompanied by a decrease in susceptibility to both azoles and amphotericin B. 

Figure 1.15 Mechanisms by which microbial cell might develop resistance. 1, The target enzyme is overproduced, so that the drug does not inhibit the biochemical reaction completely. 2, The drug target is altered so that the drug cannot bind to the target. 3, The drug is pumped out by an efflux pump. 4, The entry of the drug is prevented at the cell membrane/cell wall level. 5, The cell has a bypass pathway that compensates for the loss-of-function inhibition due to the drug activity. 6, Some fungal “enzymes” that convert an inactive drug to its active form are inhibited. 7, The cell secretes some enzymes to the extracellular medium, which degrade the drug. 

Prevention And Control of Antifungal Resistance

Strategies to avoid and suppress the emergence of antifungal resistance have not been defined. However, approaches analogous to those recommended for antibacterials could be suggested. These measures include

(i) Prudent use of antifungals.

(ii) Appropriate dosing with emphasis on avoiding treatment with low antifungal dosage.

(iii) Therapy with combinations of existing agents.
(iv) Treatment with the appropriate antifungal.

(v) Use of surveillance studies to determine the true frequency of antifungal resistance.

It should be emphasized that data supporting the use of the suggested measures is largely lacking, and ongoing studies may provide some specific guidelines in the near future. Additionally, advances in rapid diagnosis of fungi may be helpful in reducing the use of inappropriate antifungals to treat organisms that are resistant to a particular agent \(^{56}\).
1.5 Fungal Strains for antifungal assay

The kingdom fungi include yeasts, molds, rusts and mushrooms. Fungi, like animals, are heterotrophic, that is, they obtain nutrients from environment, not from endogenous sources (like plants with photosynthesis). Most fungi are beneficial and are involved in bio-degradation; however, a few can cause cutaneous infections if they are introduced into the skin through wounds, or into the lings and nasal passages if inhaled. To enter the alveolar spaces of the lungs and establish pulmonary infection, an organism must produce viable forms smaller than 4μm in diameter. Organisms that cause opportunistic infections will not gain a foothold in healthy individuals, but in immunocompromised they can cause serious, sometimes life-threatening infections.

Table-1.1: Fungal diseases, symptoms and causing agents.

<table>
<thead>
<tr>
<th>Opportunistic Infections</th>
<th>Causative agent</th>
<th>Target Organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candiaisis, Thrush, Vulvovaginitis</td>
<td><em>Candida albicans</em></td>
<td>GI tract and vagina</td>
</tr>
<tr>
<td>Cryptococcal meningitis</td>
<td><em>Cryptococcus neoformans</em></td>
<td>Lung infection, Mainly affects CNS</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td><em>Aspergillus sp.</em></td>
<td>Lung, brain, sinuses and other organs</td>
</tr>
<tr>
<td>Mucormycosis</td>
<td><em>Murcor sp.</em></td>
<td>Sinuses, eyes, blood and brain</td>
</tr>
<tr>
<td>Penumocystis carinii pneumonia</td>
<td><em>Penumocystis carinii</em></td>
<td>Lungs (especially in HIV patients)</td>
</tr>
<tr>
<td>Candiaisis, Thrush, Vulvovaginitis</td>
<td><em>Candida glabrata</em></td>
<td>GI tract and vagina</td>
</tr>
</tbody>
</table>

Patients susceptible to these infections include individuals with leukemia and other blood diseases, cancer, HIV and other immunodeficiencies, and diabetes. These organisms can be found throughout U.S.
Taking these facts into consideration we decided to do antifungal assay on four different fungal strains that are central cause for most of the immunocompromised fungal infection. Fungal species for our study are following:

1.5.1 *Candida albicans*: *Candida albicans* is a diploid fungus (a form of yeast) and a causal agent of opportunistic oral and genital infections in humans (Fig.1.16). Systemic fungal infections (fungemias) have emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). *C. albicans* biofilms readily form on the surface of implantable medical devices. In addition, hospital-acquired infections in patients not previously considered at risk (e.g., patients in an intensive care unit) have become a cause of major health concern. *C. albicans* is commensal and is found among the gut flora, the many organisms that live in the human mouth and gastrointestinal tract. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis. Candidiasis is often observed in immunocompromised individuals such as HIV-positive patients. Candidiasis also may occur in the blood and in the genital tract. Candidiasis, also known as "thrush", is a common condition, usually easily cured in people who are not immunocompromised. To infect host tissue, the usual unicellular yeast-like form of *C. albicans* reacts to environmental cues and switches into an invasive, multicellular filamentous form.

![Figure 1.16 Candida albicans.](image-url)
1.5.2 Cryptococcus neoformans: Cryptococcus neoformans usually grows as yeast (unicellular) and replicates by budding. Under certain conditions, both in nature and in the laboratory, C. neoformans can grow as a filamentous fungus. When grown as yeast, C. neoformans has a prominent capsule composed mostly of polysaccharides. Microscopically, the India ink stain is used for easy visualization of the capsule. The particles of ink pigment do not enter the capsule that surrounds the spherical yeast cell, resulting in a zone of clearance or "halo" around the cells. This allows for quick and easy identification of C. neoformans. They are encapsulated fungal organism that can cause disease in apparently immunocompetent, as well as immunocompromised, hosts. Most susceptible to infection are patients with T-cell deficiencies. Organism enters the host by the respiratory route in the form of dehydrated haploid yeast or as basidiospores. After sometime in lungs, the organism hematogenously spreads to extrapulmonary tissues; since it has a predilection for the brain. Infected persons usually contract meningoencephalitis. If untreated, cryptococcal meningoencephalitis is 100% fatal, and even if treated with most effective antifungal drugs, cryptococcal infections can be fatal if the host doest not have adequate T-cell-dependent immune function.

![Image of Cryptococcus neoformans](image)

**Figure 1.17** Proposed means of infection by Candida neoformans.

In high glucose and salt conditions that may be found in the nature Cryptococcus neoformans becomes un-capsulated. The small size is necessary for the organism to get into the
alveolar spaces in the lungs. Once in the lungs the organism can become rehydrated and acquire the thick polysaccharide capsule (fig.1.17)\textsuperscript{58}.

1.5.3 *Candida glabrata:*

*Candida glabrata* is haploid yeast of the genus *Candida* previously known as *Torulopsis glabrata*. This species of yeast is non-dimorphic and no mating activity has been observed. Until recently, *C. glabrata* was thought to be a primarily non-pathogenic organism\textsuperscript{59}. However, with the ever-increasing population of immunocompromised individuals, trends have shown *C. glabrata* to be a highly opportunistic pathogen of the urogenital tract, and of the bloodstream (*Candidemia*). Historically, *Candida glabrata* has been considered a relatively non-pathogenic saprophyte of the normal flora of healthy individuals, rarely causing serious infection in humans. However, following the widespread and increased use of immunosuppressive therapy together with broad-spectrum antimycotic therapy, the frequency of mucosal and systemic infections caused by *C. glabrata* has increased significantly. In fact, depending on the site of infection, *C. glabrata* is often the second or third most common cause of candidiasis after *C. albicans*. *C. glabrata* infections can be mucosal or systemic and are common in abnormal hosts (e.g., immunocompromised persons or those with diabetes mellitus). In contrast to other Candida species, *C. glabrata* is not dimorphic; consequently, it is found as blastoconidia both as a commensal and as a pathogen (Fig.1.18). *C. glabrata* infections are difficult to treat and are often resistant to many azole antifungal agents, especially fluconazole. Consequently, *C. glabrata* infections have a high mortality rate in compromised, at-risk hospitalized patients.
**C. glabrata**, together with other Candida species, belongs to the class Fungi Imperfecti, the order Moniliales, and the family Cryptococcaceae. *C. glabrata* is a nondimorphic yeast that exists as small blastoconidia under all environmental conditions as a pathogen.

### 1.5.4 Aspergillus fumigatus

*Aspergillus fumigatus* is a fungus of the genus *Aspergillus*, and is one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency disease. *A. fumigatus*, a saprotroph widespread in nature, is typically found in soil and decaying organic matter, such as compost heaps, where it plays an essential role in carbon and nitrogen recycling. Colonies of the fungus produce from conidiophores thousands of minute grey-green conidia (2–3 µm) that readily become airborne (Fig.1.19). For many years, *A. fumigatus* was thought to only reproduce asexually, as neither mating nor meiosis had ever been observed. In 2008, however, *A. fumigatus* was shown to possess a fully functional sexual reproductive cycle, 145 years after its original description by Fresenius.

The fungus is capable of growth at 37°C/99°F (normal human body temperature), and can grow at temperatures up to 50°C/122°F, with conidia surviving at 70°C/158°F—conditions it regularly encounters in self-heating compost heaps. Its spores are ubiquitous in the atmosphere, and it is estimated that everybody inhales several hundred spores each day; typically these are...
quickly eliminated by the immune system in healthy individuals. In immunocompromised individuals, such as organ transplant recipients and people with AIDS or leukemia, the fungus is more likely to become pathogenic, over-running the host's weakened defenses and causing a range of diseases generally termed aspergillosis.

Figure 1.19 *Aspergillus fumigatus*. 61
1.6 Specific Aims for the synthesis and biological assay of steroidal antifungals:

The rationale for the research towards the synthesis of novel steroidal antifungal compounds is threefold. First, due to the difficulty in characterizing the structure of saponins isolated from nature, the synthesis of these compounds will provide verification for the precise structure and stereochemistry of the complex glycoconjugates.

Second, the development of a practical synthesis of steroidal saponins will potentially provide a sample in sufficient quantity and purity to further explore its biological activity and medicinal utility as well as its mechanism of action. While the isolated yield of most of the naturally occurring saponins from the plant is exceptionally low, the laborious isolation process has limited their availability in sufficient quantity and purity for additional biological testing.

Finally, a practical synthetic strategy would also allow for the opportunity to synthesize various derivatives of saponins, which could be investigated in the future in structure-activity relationship studies. Since CAY-1 is only one of many biologically active saponins contained in the epithelial cells of the Capsicum frutescens plant, it is likely that the synthesis of structurally similar saponins will yield other biologically active compounds. Simple oligosaccharide derivatives of aglycone moiety of CAY-1 will also be explored for antifungal activity in search of possibly more active compounds, which might have a much simpler structure.

Perform antifungal assay for various steroidal derivatives synthesized against four pathogenic fungal strains Candida albicans, Aspergillus fumigates, Candida glabrata and Cryptococcus neoformans. Structure activity relationship studies will be done in order to get novel and highly active antifungal steroid based compounds. Cell line assay for active compounds will also be done for their toxicity in vitro. Preliminary effect of most active compounds on animal models like mice will be done depending on their toxicity results in vitro.
1.7 References:


CHAPTER-2: SYNTHESIS OF CHOLESTANE AND ANDROSTANE DERIVATIVES

As opportunistic fungal infections continue to represent continuous obstacles to individuals with compromised immune systems, the development of novel and efficient antifungal therapeutic agents that have the capability of broad-spectrum activity becomes inevitable. Synthetic derivatives of natural steroids have become a promising approach for discovery of new antifungal agents. According to J. M. Brunel et al., the best antifungal in vitro activity for oxygenated cholesterol was observed for hydroxy ketones 1, 3 and 4 and diketone 6 exhibiting activity against *S. cerevisiae* and *Candida albicans* (Fig.2.1).

![Figure 2.1 Oxygenated cholesterol derivatives reported for antimicrobial activity by J. M. Brunel et al.](image)

Shamsuzzaman et al. have synthesized 6,5-fused steroidal oxazoles in cholestane series and tested for their inhibitory action against five different fungal strains and minimum inhibitory concentration (MIC) of all compounds were determined. (Scheme-2.1) All compounds 13, 14 and 15 synthesized were active against all the five strains of fungi (*Candida albicans, Candida*...
glabrata, Penicillium spp., Fusarium oxyporium and Aspergillus niger) and both types of bacteria (Gram-positive and Gram-negative).\textsuperscript{5}

C. Loncle \textit{et al.} have also explored a series of cholesterol-hydrazone derivatives for antifungal activity against human pathogens. The best results were reported for tosylhydrazone cholesterol derivatives 16 and 17 exhibiting activities against \textit{Candida albicans} at a concentration of 1.5µg/ml (Scheme 2.2).\textsuperscript{6}

### Scheme 2.1
6,5-fused steroidal oxazoles in cholestane series by Shamusuzzaman \textit{et al.}\textsuperscript{5}

### Scheme 2.2
Synthesis of hydrazone derivatives of cholesterol by C. Loncle \textit{et al.}\textsuperscript{6}

In addition, a number of fatty-acid derivatives have been characterized as antimicrobials and
antifungals yet fatty acid esters of cholesterol have not been explored exhaustively as potent therapeutic antifungal agents\textsuperscript{7-8}. M. R. Banday \textit{et al.} have reported synthesis and antifungal activity of eight different fatty acid derivatives of cholesterol and found that two of these compounds 18 and 19 were having excellent antifungal activity. (Fig. 2.2)\textsuperscript{8}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Active fatty acid analoges of cholesterol reported by M. R. Banday \textit{et al.} \textsuperscript{8}}
\end{figure}

Finally, 2,3-functionalized steroids are well known compounds with potentially interesting biological properties\textsuperscript{9-12}. Furthermore, proper functionalization of 2,3-steroids can be an important step in the development of building blocks for novel fatty-acid esters of cholesterol and other oxygenated and hydrazone-cholesterol derivatives. A novel approach to the synthesis of 2,3-functionalized cholesterol and androstane derivatives needs to be developed which will be further explored and optimized for synthesis of tigogenin derivatives. As we had previously tried syntheses of tigogenin derivatives, after several attempts we were able to synthesize desired product in eight steps and the final step leads to two isomers, which was then purified. We figured out that there was an intensive need for development of simple and efficient synthetic procedure to prepare the desired derivatives in a stereo selective way.

Since the starting material for this synthesis was hecogenin acetate which is a very expensive starting material we decided to develop synthetic methodology with a fairly inexpensive model
steroid molecule and then apply the optimized synthesis for tigogenin. We selected for cholesterol as our model compound to establish synthetic scheme, as it have the similar skeleton as our target saponin except the aliphatic chain in place of furan ring system in tigogenin. We also hypothesized that the spiral structure of the spirostandiol is not a pharmacophore and therefore can be totally removed, as in the case of androstane derivatives, or replaced by a hydrocarbon chain, as in the case of cholestane derivatives, to yield novel 2,3-functionalized targets that maintain the potential to elicit antifungal activity (Fig. 2.3)

![Figure 2.3 2α, 3β- Cholestane and Spirostane derivatives](image.png)

Over the last few decades, many naturally occurring products have been deemed effective antimicrobial agents, including saponins, typically isolated from plant and marine species\(^{13-15}\). Synthetic modifications of cholesterol and other traditional steroid molecules have become a promising area for the exploration and development of novel antifungal agents, especially with respect to the development of fatty-acid esters of steroids. In addition, 2,3-functionalized steroids have potentially interesting biological properties and proper functionalization of 2,3-steroids can lead to the development of efficient syntheses of building blocks for novel fatty-acid esters of steroids. An efficient approach for the synthesis of 2,3-functionalized cholestane and androstane derivatives would be developed and further optimized for the synthesis of similar tigogenin derivatives.
G. A. G. Santos et al. have reported on synthesis of cholestane derivatives starting from commercially available 3β-hydroxy-5α-cholestane in four steps to get compound 24. Although it gives us an idea about synthesis of cholestane derivative but we are not looking for the same isomer (2β, 3α) they synthesized instead our goal was to develop synthesis of 2α, 3β-isomer, more over we would like our end product to be selectively protected 2α, 3β-isomer in order to attach oligosaccharide moiety at C-3 position for the purpose of exploration of SAR in search of lead candidate for antifungal activity.

![Scheme 2.3](image)

**Scheme 2.3** i) p-TsCl, pyridine; ii) LiCO₃, LiBr, DMF; iii) m-CPBA, H₂O-CH₂Cl₂; iv) H₂SO₄, THF.

2.1 Retro-analysis for 2α, 3β- Cholestane derivative:

The retro-analysis of 2α, 3β- Cholestane derivative indicates that we could get it by reduction of carbonyl group of compound 37, which can be obtained in two different ways, Firstly by enolization and protecting enol group with TMS or acetyl group followed by epoxidation to form epoxide ring which can be opened in presence of acetic anhydride/acetic acid to get compound 37(Scheme 2.4). Secondly leadtetraacetate in presence of acetic acid and acetic anhydride reflux
will stereoselectively form 37 from compound 26. Cholestane 26 can be obtained from cholesterol in two steps by reduction of double bond followed by oxidation of alcohol group at 2-position.

Scheme 2.4 Retro-analysis of 2α, 3β-Cholestane derivatives

2.2 Synthesis of Cholestane derivatives:

Min Serk Kwon at el. have reported oxidation of alcohol to keto group by air oxidation in presence of a new aluminum hydroxide-supported palladium catalyst made by one-pot synthesis through nanoparticle generation and gelation shows dual activity for olefinic hydrogenation and aerobic alcohol oxidation. They were able to get cholestan-3-one from cholesterol very effectively (Scheme-2.5). It gave us an idea that we should be able to reduce the not easily accessible double bond in cholesterol, but conditions have to be found and optimized. However, we were not interested in using nanoparticle generation and gelation techniques in our synthesis for the sake of simplicity and expertise required to handle and characterize those materials.
Scheme 2.5 Synthesis of Chlestan-3-one by Min Serk et al.17a

Our synthesis of the cholestane derivatives began with cholesterol (25, Scheme-2.11), which is very inexpensive and commercially available in bulk quantities. The double bond of 25 was then reduced by platinum dioxide catalyzed hydrogenation, which led to the formation of intermediate alcohol 20.17b The intermediate alcohol 20 was later oxidized by chromic acid (Jone’s oxidation) by following standard procedures.23 From this point, we then branched the synthetic scheme into two different paths; the first involved the direct introduction of an acetyl group into the 2α-position with lead tetraacetate to generate 2α-acetoxycholestan-3-one (37, Scheme-2.11). This approach was inspired by a literature report for synthesis of 2α-acetoxy-3-ketone starting from lupan-3-one by A. D. Boul et al., they further explored the rearrangement of 2α-acetoxy-3-ketone into 3β-acetoxy-2-ketone under various conditions including treatment with alkaline alumina. (Scheme-2.6) The position of the acetoxy groups follows from the NMR results, also the equatorial conformations for these groups were suggested by high C=O stretching frequency in IR spectra.24,25
Scheme 2.6 Synthesis of 2α-acetoxy-lupan-3-one and its rearrangement to 3β-acetoxy-lupan-2-one

The second approach generated the same compound 37 using a three-step procedure (Scheme 2.11, steps iv–vi). Initially we tried enolization by lithium bis(trimethylsilyl)amide and trimethyl silyl chloride at -78°C in dry tetrahydrofurane to give compound OTMS protected enol in an isolated yield of 60% (Note- Purification by column chromatography should be done fast). Regioselective enolization 3-keto steroids have been reported by M. Sobukawa et al. and have very good selectivity of 9:1 for the desired regio-isomer 27 (Scheme 2.7). 19

Scheme 2.7 Regioselective enolization of ketone 19

TMS group was found to be cleaving while running column chromatography for purification; shortening the time for column purification helped to minimize this problem to certain extent. That was one of the reasons for low to moderate yield for this step of the synthesis. Since we already had issues with same strategy in case of spirostane derivatives synthesis and were hoping for some improvement, which didn’t happen in this case, so we decided to go for acetyl-enol protection instead of TMS to simplify and in hope to find a better way to achieving synthesis of desired cholestane isomers.
The first step of the alternative preparation of 37 is the synthesis of vinyl acetate 35 (Scheme 2.11, step iv) using montmorillonite KSF clay was inspired by acid catalyzed enolization reported by Biswajit kalita et al. for the preparation of enol acetates from ketones using montmorillonite KSF clay at room temperature in excellent yields\textsuperscript{20} (Scheme-2.8). They also tried these reactions in microwave irradiation to dramatically reduce the reaction time. Very simple and mild conditions attracted our attention towards this method.

![Scheme 2.8 Synthesis of enol acetates using montmorillonite KSF clay.\textsuperscript{20}](image)

Stereoselective epoxidation of 35 with \textit{m}-CPBA was done to obtain corresponding epoxide product 36 (Scheme 2.12, step v). Epoxidation of enolic double bond happens only from behind the plane in cholestane-based molecule as C-19 methyl group sterically hinders the top face of the steroid so epoxidation occurs from $\alpha$-face nearly exclusively. (Scheme-2.9) This result is reasonable since epoxidations with \textit{m}-CPBA generally occur from the least hindered side of the alkene.\textsuperscript{21,22}

![Scheme 2.9 Mechanism-stereo selective epoxidation](image)
Vinyl acetate 35 have peak for H-2 proton at 5.23 ppm whereas after epoxidation H-2 peak moves to 3.38 ppm. Compound 35 have H-2 proton at 5.23 ppm which is up field compares to H-2 proton in case of TMS-enol at 4.78 ppm as would be expected by having ester protection of enol instead of alcoholic TMS protection. (Fig. 2.4)

Figure 2.4 $^1$H-NMR for enolization and epoxidation

Finally, epoxide ring opening was done by treatment with acetic acid and acetic anhydride to yield the desired compound 37. We have proposed Probable mechanism for epoxide ring opening by acetic acid and acetic anhydride involving esterification at the last step by acetic anhydride seems to be the reason for retained steroselectivity at C-2 position by acetyl group (Scheme-2.10).
The carbonyl group of acetyl gets protonated in first step followed by nucleophilic attack by acetate ion on carbonyl carbon would give the acetal intermediate, which rearrange to give 2α-hydroxy cholest-3-one. Acid catalyzed esterification by acetic anhydride would give 2α-acetoxy-cholestan-3-one (37). Stereochemistry of compound 37 was confirmed by $^1$H-NMR showing a doublet of double at 5.25ppm which will be otherwise be a nice triplet for the 2β-acetoxy-cholestan-3-one due to coupling with two prochiral protons (Fig. 2.5).

![Scheme 2.10 Probable mechanism for epoxide ring opening by acetic acid and acetic anhydride](image-url)

Although longer, the alternative route through the preparation of vinyl ester 35 and epoxide 36 has an added advantage with respect to the purification of the product, and this method gave us comparable yields of 37 (Scheme-2.11). Moreover use of lead tetraacetate was avoided without much compromise of yield. Finally, to generate the 2,3-functionalized steroids, we followed a straightforward Luche’s reduction NaBH$_4$/CeCl$_3$ reduction of the carbonyl group at C-3 position gave desired 2α, 3β-Cholestane derivative, 38 in very good yield. $^{26, 27}$
Scheme 2.11 i) PtO₂/CH₂Cl₂·CH₃CO₂H; ii) CrO₃/H₂SO₄; iii) (CH₃CO)₂O/Montmorillonite clay; iv) m-chloroperbenzoic acid CH₂Cl₂; v) (CH₃CO)₂/N(C₂H₅)₃; vi) Pb(O₂CCH₃)₄/(CH₃CO)₂O·CH₃CO₂H; vii) CeCl₃·7H₂O/CH₃OH/tetrahydrofuran/NaBH₄; viii) NaOH/CH₂Cl₂/CH₃OH; ix) succinic anhydride/pyridine.

The acetate ester hydrolysis of 38 gave hydrolyzed product 42 which was clearly evident from ¹H-NMR, multiplet for H-2 proton have shifted from 4.8 to 3.6 ppm and singlet for acetyl group at 2.23 ppm have vanished completely in the compound 42 (Fig. 2.5). Succinic acid derivatives were prepared from the corresponding alcohols by a well-reported classical alcohol esterification by succinic acid anhydride.²⁸,³⁰
We were able to develop a very efficient and short synthetic methodology for synthesis of various 2α, 3β-cholestane derivatives. Succinic acid mono and di-derivative of 2α, 3β-cholestane were also synthesized and were tested for antifungal activity. Stereochemistry for various isomers were well compared and confirmed by $^1$H-NMR studies and reported literature evidences for these kinds of compounds.
2.3 Synthesis of Androstane derivatives:

Since synthesis was well developed and optimized for cholestane based derivatives, we decided to use the same strategy for synthesis of androstane derivatives. The reason we wanted to synthesize these derivatives in order to complete the SAR study of antifungal assay and see if our hypothesis of not having spirostane ring for antifungal activity is actually true or not. We wanted to have comparison among three steroid derivatives bearing siprostane ring system (tigogenin), aliphatic chain (in cholestane) and no substitution on the basic four-ring skeleton (Fig. 2.6).

![Figure 2.6 2α, 3β- Cholestane, Androstane and Spirostane derivatives](image)

This will give us a fair idea about if the entire ring system in spirostane is needed for better antifungal activity. Adrosterone derivatives have been reported to have antifungal activity by N. R. Moamed et al. against *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus flavus*.²⁹ Four compounds were found to be active antifungal agents with activity close to Nystatin by disc-diffusion method for the antifungal assay. (Fig. 2.7)
The synthesis of androstane derivatives began with 5-androsten-17-one-3β-acetate (49, Scheme 2.12) (Sigma–Aldrich). A Wolff–Kishner reduction of the carbonyl group of dehydroisoandrosterone-3β-acetate (49, Scheme 2.12) followed by the hydrolysis of the acetate group was performed in one step by applying Reyes and coworkers’ elegant steroid carbonyl reduction procedure. This method was used to prepare 5-androsten-3β-ol (50, Scheme 2.12) in large quantities with very high yields. The double bond of 50 was then reduced by platinum dioxide catalyzed hydrogenation, which led to the formation of intermediate alcohol 51. The intermediate alcohol 51 was later oxidized by chromic acid by following standard procedures. From this point, we then branched the synthetic scheme into two different paths; the first involved the direct introduction of an acetyl group at 2α-position of 52 with lead tetraacetate to generate 55 (Scheme 2.12, step vii).
The second approach generated compound 55 using a three-step procedure (Scheme 2.12, steps iv–vi). The first step of the alternative preparation of compound 55 is the synthesis of vinyl acetate (53, Scheme 2.12, step iv), followed by epoxidation of 53 with mCPBA to yield epoxide 54 (Scheme 2.12, step v). Vinyl acetate, 53 have peak for H-2 proton at 5.23ppm whereas after epoxidation H-2 peak moves to 3.38 ppm. Finally, epoxide ring opening occurs following treatment with acetic acid to yield the desired compound 55. Although longer, the alternative route through the preparation of vinyl ester and epoxide has an added advantage with respect to the purification of the product, and this method gave us comparable yields of compound 55 (Scheme-2.12).
Stereochemistry of 2α-acetoxy-androstane-3-one was confirmed by $^1$H-NMR showing a doublet of doublet at 5.25 ppm which will be otherwise be a nice triplet for the 2β-acetoxy-androstan-3-one due to coupling with two prochiral protons.

Finally, to generate the 2,3-functionalized steroids, we followed a straightforward NaBH₄/CeCl₃ reduction of the carbonyl group of 2α-acetoxy-androstane-3-one, followed by the acetate ester hydrolysis to produce desired 2α, 3β-androstane, 56 and 2α, 3β-androstandiol, 57 derivative respectively. The acetate ester hydrolysis of 2α, 3β-androstan-3-one gave corresponding diol
which was clearly evident from $^1$H-NMR, multiplet for H-2 proton have shifted from 4.8 to 3.6ppm and singlet for acetyl group at 2.23 ppm have vanished completely (Fig. 2.8).
2.4 Antifungal assay of Cholestane And Androstane derivatives

The antifungal activity of the synthesized stereoisomers of the 2,3-functionalized steroids evaluated in vitro. The susceptibility studies and minimal inhibitory concentrations (MIC) values for *Candida albicans* (ATCC no. 10231), *Cryptococcus neoformans* (ATCC no 36556), *Candida glabrata* (ATCC no 48435), and the filamentous fungus *Aspergillus fumigates* (ATCC no. 16424) were determined by the broth dilution technique in accordance with NCCLS reference documents M27-A. Dilution panels ranged from 0.01 to 128 μg/mL. Master stock concentrations of drugs (soluble in DMSO) were prepared to ensure that the maximum final concentration of DMSO in tested antifungal solutions was 1% or less. Subsequent 2-fold serial dilutions were made using RPMI 1640 broth or sterile water to a final concentration of 1280-0.1 μg/ml. A final 10-fold dilution of each drug was made by aliquotting 0.1 mL of each dilution to 0.9 mL of inoculating media, giving final drug concentrations tested in the range of 128-0.01 μg/mL. Antifungal drug controls used for these studies were Amphotericin B and Itraconazole (Sigma-Aldrich). All controls used were diluted to 1.0 μg/mL concentrations, according to the manufacturer’s instructions and run in parallel to each in vitro screening of the 2,3-functionalized steroid analogs. The results of these screenings are summarized in Table 2.1. Several of the derivatives from the androstane and cholestane groups showed reasonable antifungal activity (as measured spectroscopically by a >25% reduction in fungal growth compared to control wells) against at least one species of fungus.
Table 2.1 Antifungal activity of cholestanone and androstane derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MIC&lt;sub&gt;25&lt;/sub&gt; (µg/mL)&lt;sup&gt;a,f&lt;/sup&gt;</th>
<th>C. albicans&lt;sup&gt;b&lt;/sup&gt;</th>
<th>C. glabrata&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C. neoformans&lt;sup&gt;d&lt;/sup&gt;</th>
<th>A. fumigates&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>54</td>
<td></td>
<td>4.0</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>55</td>
<td></td>
<td>0.125</td>
<td>NC*</td>
<td>NC*</td>
<td>1.0</td>
<td>NC*</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>57</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>128</td>
<td>NC*</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>128</td>
<td>NC*</td>
<td>NC*</td>
<td>8.0</td>
<td>NC*</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>4.0</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td>44</td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>8.0</td>
<td>2.0</td>
<td>NC*</td>
</tr>
<tr>
<td>43</td>
<td></td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
<td>8.0</td>
<td>NC*</td>
</tr>
</tbody>
</table>

<sup>a</sup> MIC values are reported only for compounds displaying (1) a prominent decrease in turbidity by visual comparison to the control wells containing no antifungal and (2) a >25% reduction in fungal growth compared to controls containing no antifungal, as measured spectroscopically by absorption at 530 nm.

<sup>b</sup> ATCC no. 10231.

<sup>c</sup> ATCC no. 48435.

<sup>d</sup> ATCC no. 36556.

<sup>e</sup> ATCC no. 16424.

<sup>f</sup> All values were determined after incubation at 30–35 °C for 48 h.

<sup>g</sup> Compounds that had (1) a slight reduction in turbidity to no change and (2) had less than a 10% reduction in growth compared to controls, as measured spectroscopically by absorption at 530 nm.
2.5 Conclusions

We have successfully developed and optimized a very efficient and elegant four to six step synthesis for $2\alpha$, $3\beta$- isomers of cholestane and androstane. Large quantities of these derivatives have been synthesized and preliminary antifungal evaluation was done in vitro. Antifungal assay protocols were optimized and reproduced several times in order to check reproducibility of susceptibility assay experiments. The susceptibility studies and minimal inhibitory concentrations (MIC) values for *Candida albicans*, *Cryptococcus neoformans*, *Candida glabrata*, and the filamentous fungus *Aspergillus fumigates* were determined by the broth dilution technique in accordance with NCCLS reference documents M27-A.\(^1\) Several of the derivatives from the androstane and cholestane groups showed reasonable antifungal activity (as measured spectroscopically by a $>25\%$ reduction in fungal growth compared to control wells) against at least one species of fungus. Most of these derivatives were showing good inhibition of fungal growth in case of filamentous fungi *Aspergillus fumigates*. Further, the 2,3-functionalized derivatives can further be used as building blocks for the synthesis of novel fatty-acid esters of steroids with potential antimicrobial activities. Although we found some good antifungal activity with some cholestane and androstane derivatives, they were not potent enough to get a probable lead compound, this gave an indication that we need to have spirostane ring system for better activity. The next step at this stage of the project was to optimize synthesis of $2\alpha$, $3\beta$-spirostane derivatives and test them for antifungal activity to complete a SAR study towards finding a good antifungal agent in the saponin class. We also decided to synthesize some glycosidic derivatives of active compounds from cholestane and androstane series and test them for antifungal assay. The details of glycosides derivatives of cholestane and androstane will be discussed in chapter 4 along with their antifungal activity and comparison them with similar derivatives from the spirostane class.
2.6 Experimental

2.6.1 General Experimental

All reagents and solvents were purchased from Sigma-Aldrich and were analytical grade. Thin-layer chromatographic analysis (TLC) was performed using silica gel on glass plates and was detected under ultraviolet (UV) light and using PMA solution. Column chromatography was performed using silica gel porosity 60 Å, partial size 40-75 mm from Sorbent Technologies. The $^1$H and $^{13}$C NMR spectra were run on Varian 400 MHz Unity in CDCl$_3$ or DMSO-d$_6$ as solvents and internal standards.

**Preparation of 5α-cholestan-3β-ol (20):** A suspension of cholesterol (25) (1.0 g; 2.6 mmol) and platinum dioxide (100 mg) in dichloromethane (20 ml) and acetic acid (20 ml) was shaken under hydrogen pressure (60 psi) for 16 hours. The catalyst was separated by filtration and the filtrate was neutralized with 10% potassium carbonate. The resulting mixture was extracted with dichloromethane (3x50 ml). The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated to give 0.98 g (98%) white solid as pure product. Selected $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 3.61 (1H, m, CH$_3$-3), 1.12 (3H, s,CH$_3$-19), 0.85, 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), and 0.67 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR $\delta$: 74.4, 56.1, 56.0, 53.7, 47.8, 44.8, 43.5, 42.5, 39.7, 39.4, 37.1, 36.1, 35.7, 34.6, 31.5, 28.3, 28.2, 27.9, 24.1, 23.7, 22.8, 22.5, 21.6, 20.7, 18.6, 12.7 and 12.0 ppm.

**Preparation of 5α-cholestan-3-one (26):** Into a stirring acetone (200 ml) solution of 5α-cholestan-3β-ol (20) (0.95 g; 2.45 mmol), chromic acid made from chromium trioxide (1 g) and 40% sulfuric acid (4 ml) was added drop-wise at 30°C. The reaction mixture was stirred at 30°C
for an additional 2 hours then diluted with water (50 ml) and extracted with dichloromethane (3x50 ml). Combined dichloromethane extracts were dried over anhydrous magnesium sulfate and evaporated to dryness to give pure product (0.9 g; 95%). Selected $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.12 (3H, s, CH$_3$-19), 0.85, 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), and 0.67 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR: 160.2, 74.4, 56.1, 56.0, 53.7, 47.8, 44.8, 43.5, 42.5, 39.7, 39.4, 37.1, 36.1, 35.7, 34.6, 31.5, 28.3, 28.2, 27.9, 24.1, 23.7, 22.8, 22.5, 21.6, 20.7, 18.6, 12.7 and 12.0 ppm.

**Preparation of 3-acetoxy-5α-cholest-2-ene (35):** Acetic anhydride (10 ml) suspension of 5α-cholestan-3-one (26) (1 g; 2.6 mmol) and monomorillonite clay (500 mg) was stirred at room temperature overnight. The catalyst was separated by filtration and diluted by diethyl ether (100 ml). The ether solution was washed with saturated sodium bicarbonate (3x20 ml), dried on anhydrous magnesium sulfate, and evaporated to give pure product (1.0 g; 90%). Selected $^1$H- and $^{13}$C-NMR spectra. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 5.20 (d, 1H), 2.15 (3H, s, CH$_3$CO), 1.12 (3H, s, CH$_3$-19), 0.85, 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), and 0.67 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): $\delta$: 170.1, 74.4, 56.1, 56.0, 53.7, 47.8, 44.8, 43.5, 42.5, 39.7, 39.4, 37.1, 36.1, 35.7, 34.6, 31.5, 28.3, 28.2, 27.9, 24.1, 23.7, 22.8, 22.5, 21.6, 20.7, 18.6, 12.7 and 12.0 ppm.

**Preparation of 3β-acetoxy-2α, 3α-epoxy-5α-cholestan (36):** Dichloromethane (15 ml) solution of 3-aceto-5α-cholest-2-ene (35) (1 g; 2.16 mmol) and m-chloroperbenzoic acid (0.443 g; 2.56 mmol) was stirred at 10ºC for 1 hour. The reaction mixture was mixed with 10% sodium sulfite (15 ml) stirred at 10ºC for additional 20 minutes. Into this reaction mixture ethyl ether (100 ml) was added. The organic layer was separated from water, washed with saturated sodium
bicarbonate (3x20 ml), brine (3x20 ml), dried over anhydrous magnesium sulfate and evaporated to a solid residue. Isolated yield of white powder is 0.380 mg (85%). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 3.21 (d,1H), 2.15 (3H, s, CH$_3$CO), 1.12 (3H, s,CH$_3$-19), 0.85, 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), and 0.67 ppm (3H, s, CH$_3$-18). $^{13}$C NMR (CDCl$_3$) $\delta$: 170.1, 74.4, 56.1, 56.0, 53.7, 47.8, 44.8, 43.5, 42.5, 39.7, 39.4, 37.1, 36.1, 35.7, 34.6, 31.5, 28.3, 28.2, 27.9, 24.1, 23.7, 22.8, 22.5, 21.6, 20.7, 18.6, 12.7 and 12.0 ppm.

**Preparation of 2α-acetoxy-5α-cholestan-3-one (37): Method I:** Acetic anhydride (20 ml) solution of 3β-acetoxy-2α,3α-epoxy-5α-cholestan (36) (700 mg; 1.57 mmol) and triethylamine (0.25 ml) was refluxed for 2 hours. Reaction mixture was cooled to room temperature and extracted with diethyl ether (3x20 ml). Combined ether extracts were dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure to give 0.62 mg (89%) product as white solid. **Method II:** A mixture of acetic acid (40 ml) and acetic anhydride (5 ml) was refluxed for 10 minutes. After reaction mixture was cooled to room temperature 5α-cholestan-3-one (1.0 g; 2.57 mmol) and lead tetraacetate (1.6 g; 4.36 mmol) were added reaction mixture was refluxed for 24 hours. The reaction mixture was diluted with diethyl ether (100 ml) and extracted with 5% hydrochloric acid (3x15 ml), saturated sodium bicarbonate (3x15 ml), brine (3x15 ml). Solvent was evaporated and the oily residue was purified by silica gel column chromatography (20% ethyl acetate in hexane). The isolated yield was 80% of pure product. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 5.29 (1H, dd ), 2.15 (3H, s, CH$_3$CO), 1.12 (3H, s,CH$_3$-19), 0.85 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), and 0.67 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$) $\delta$: 204.3, 170.1, 74.4, 56.1, 56.0, 53.7, 47.8, 44.8, 43.5, 42.5, 39.7, 39.4, 37.1, 36.1, 35.7, 34.6, 31.5, 28.3, 28.2, 27.9, 24.1, 23.7, 22.8, 22.5, 21.6, 20.7, 18.6, 12.7 and 12.0 ppm.
Preparation of 2\(\alpha\)-acetoxy-5\(\alpha\)-cholestan-3\(\beta\)-ol (38): Tetrahydrofuran (20 ml) solution of methanol (10 ml), 2\(\alpha\)-acetoxy-5\(\alpha\)-cholestan-3-one 37 (0.35 g; 0.8 mmol), and CeCl\(_3\)·7H\(_2\)O (373 mg; 1 mmol) was stirred at room temperature for 10 minutes. Into this solution sodium borohydride was added (60 mg; 1.6 mmol) and continued to stir at room temperature for additional 30 minutes. The excesses of the borohydride were quenched with 5% hydrochloric acid. The resulting reaction mixture was poured into water (100 ml) and the water mixture was extracted by ether (3x50 ml). Combined ether extracts were dried over anhydrous magnesium sulfate and evaporated to dryness yielding 0.32 g (90%) of pure. Selected \(^1\)H- and \(^{13}\)C-NMR spectra. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 4.82 \) (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH\(_3\)CO), 0.84-0.90 (12H, s, CH\(_3\)-19, CH\(_3\)-21, CH\(_3\)-26, CH\(_3\)-27), and 0.64 ppm (3H, CH\(_3\)-18). \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta: 171.5, 76.4, 73.5, 56.2, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.4, 37.2, 36.1, 35.8, 35.7, 34.7, 31.7, 28.1, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

Preparation of 5\(\alpha\)-cholestan-2\(\alpha\),3\(\beta\)-diol (42): A methanol (2 ml) and dichloromethane (10 ml) solution of 2\(\alpha\)-Acetoxy-5\(\alpha\)-cholestan-3\(\beta\)-ol (38) (0.5 g; 1.12 mmol) and sodium hydroxide (0.12 g; 3 mmol) was stirred at room temperature overnight. Into the reaction mixture acidic resin-dowex (1g) was added and this suspension was stirred at room temperature for 5 minutes. The resin was separated by filtration and solvent was removed on rotavapour to get pure product in 98% (0.44 g) isolated yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 3.59 \) (1H, ddd, JZ4.7, 9.0, 11.5 Hz, H-2b), 3.40(1H, ddd, JZ5.1, 8.9, 10.9 Hz, H-3a), 0.84–0.91 (12H, CH\(_3\)-19, CH\(_3\)-21, CH\(_3\)-26, CH\(_3\)-27), and 0.64 ppm (3H, CH\(_3\)-18). \(^{13}\)C-NMR (CDCl\(_3\)) \(\delta: 73.1, 72.3, 56.3, 56.2, 54.2, 45.0, 44.8, 39.9, 39.5, 38.1, 37.5, 36.1, 35.8, 35.6, 34.7, 31.9, 28.2, 28.0, 27.9, 24.1, 23.8, 22.8, 22.5, 21.3, 18.6, 13.5 and 12.0 ppm.
Preparation 4-(2α-acetoxy-5α-cholestan-3β-yloxy)-4-oxobutanoic acid (44): Pyridine (5 ml) solution of 2α-acetoxy-5α-cholestan-3β-ol (38) (150 mg, 0.32mmols) and succinic anhydride (96mg, 0.96mmols) was refluxed for 4 hours. The reaction mixture was cooled to room temperature and added to crushed ice (~100 g). Resulted mixture was extracted with ethyl acetate (100 ml). Ethyl acetate extract was washed with water (3x30 ml), dried over anhydrous magnesium sulfate and evaporated to dryness. Selected $^1$H-NMR (400 MHz, CDCl$_3$): δ 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.60(m, 2H), 2.08 (3H, s, CH$_3$CO), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C NMR (CDCl$_3$) δ:180.2, 175.3, 171.5, 76.4, 73.5, 56.3, 56.2, 54.2, 44.4, 42.5,42.1, 41.0, 40.9, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.3, 18.6, 13.0 and 12.0 ppm.

Preparation of 4,4'-/(5α-cholestan-2α,3β-diyloxy)bis(4-oxobutanoic acid) (43): Pyridine solution (5 ml) of 5α-cholestan-2α,3β-diol (42) (100 mg, 0.25mmols) and succinic anhydride (63.3mg, 0.63mmols) was refluxed for 16 hours. Room temperature cooled reaction mixture was poured over crushed ice (~100 g) and extract with ethyl acetate (3x50 ml). Combined organic layers were washed with water (3x20 ml), dried over anhydrous magnesium sulfate and evaporate to dryness to yield pure product. $^1$H NMR (400 MHz, CDCl$_3$): δ 5.15 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 2.60 (m, 2H), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C NMR (CDCl$_3$) δ:180.2, 178.2, 175.3,174.6, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 41.0, 40.9, 39.8, 39.4, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.3, 18.6, 13.0 and 12.0 ppm.
Preparation of 5-androsten-3β-ol (50): A mixture of 5-androsten-17-one-3β-yl acetate (49) (1g, 2.12 mmol), hydrazine hydrate (1ml, 19.98mmol) and 2-ethoxyethanol (10ml) was stirred at room temperature and under nitrogen atmosphere for 20 minutes followed by 1 hour refluxing. After cooling to room temperature potassium hydroxide (1g, 17.82 mmol) was added and the resulting mixture was heated at 136°C for an additional three hours. After cooling to room temperature, the reaction mixture was neutralized with concentrated hydrochloric acid and the formed white solid was separated by filtration, washed with water (3x20 ml) and dried in the air. The isolated yield of the product was 0.863 mg (98%). Selected 1H NMR (400 MHz, CDCl3): δ 5.41(d, 1H), 3.47(m, 1H), 0.90 (3H, d, J=6.5 Hz, CH3-21), 0.87 (6H, two d, J = 6.5 Hz, CH3-26 and CH3-27), and 0.82 ppm (3H, s, CH3-19). 13C NMR δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.8, 18.6, 12.5, and 12.0 ppm.

Preparation of 3α-androstan-3β-ol (51): A suspension of 5-androstene-3β-ol (50) (1.0 g; 3.64 mmol) and platinum dioxide (200 mg) in dichloromethane (20 mL) and acetic acid (20 mL) under hydrogen pressure (60 psi) was shaken in a Parr hydrogenator for 16 hours. The solid catalyst was separated by filtration. The filtrate was neutralized with aqueous (10%) potassium hydroxide and extracted with dichloromethane (3x50 mL). Dichloromethane extracts were dried over anhydrous magnesium sulfate and evaporated to yield a solid residue that corresponded to pure product (0.987g; 98%). Selected 1H NMR (400 MHz, CDCl3): δ 3.47(m, 1H), 0.90 (3H, d, J = 6.5 Hz, CH3-21), 0.87 (6H, two d, J = 6.5 Hz, CH3-26 and CH3-27), and 0.82 ppm (3H, s, CH3-19). 13C NMR δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.9, 18.6, 12.5 and 12.0 ppm.
Preparation of 5α-androstan-3-one (52): Cromic acid (4 mL solution made from equal amount of CrO₃ and 40% sulfuric acid) was slowly added into an acetone (200 ml) solution of 51 (0.95 g; 3.44 mmol) by keeping the temperature of the reaction mixture below 30°C. The resulting reaction mixture was stirred at 30°C for an additional 2 hours. The reaction mixture was diluted with water (50 mL) and extracted with dichloromethane (3x30 mL). The combined dichloromethane extracts were dried over anhydrous magnesium sulfate and evaporated to a solid residue. Isolated yield of the product is 0.93 g (95%). Selected ¹H NMR (400 MHz, CDCl₃): δ 0.90 (3H, d, J = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and and 0.82 (3H, s, CH₃-19). ¹³C NMR δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.3, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.9, 18.6, 12.5 and 12.0 ppm.

Preparation of 3-acetoxy-5α-androst-3-ene (53): Acetic anhydride (10 ml) suspension of 3-androstanone (52) (1g; 3.64 mmol) and montomorillonite clay (500mg) was stirred at room temperature overnight. The reaction was monitor by TLC (15% ethyl acetate in hexane). Upon reaction completion (~ 12 hours) the clay catalyst was separated by filtration, the filtrate was diluted by ether (150 ml) and washed with saturated sodium bicarbonate (5x 20 ml). The ether layer was dried over magnesium sulfate and evaporated to solid residue to give pure product (1.04g; 90% yield. Selected ¹H NMR (400 MHz, CDCl₃): δ 5.20 (d, 1H), 2.12 (3H, s, CH₃CO), 0.90 (3H, d, JZ = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19). ¹³C NMR δ: 171.5, 120.2, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.2, 36.7, 36.1, 35.7, 34.71, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.8, 18.6, 12.5 and 12.0 ppm.
**Preparation of 3β-acetoxy-2α,3α-epoxy-5α-androstane (54):** To a dichloromethane (15 ml) solution of 53 (1g, 2.16 mmol) *m*-chloroperbenzoic acid (0.44g, 2.56 mmol) was added at 10°C. The resulting reaction mixture was stirred at room temperature for 1 hour. Into the reaction mixture 10% sodium sulfite (10 ml) was added and resulting mixture was stirred at room temperature for additional 20 minutes. The reaction mixture was diluted with ether (100 ml). The aqueous layer was discarded and the organic layer was washed with saturated sodium bicarbonate (3×20 ml), dried over anhydrous magnesium sulfate and evaporated to a white powdery residue, which was the pure product. The isolated yield 85%. Selected 1H NMR (400 MHz, CDCl₃): δ 3.20 (d, 1H), 2.12 (3H, s,CH₃CO), 0.90 (3H, d, J = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19) ppm. 13C NMR δ: 171.5, 73.03, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.4, 39.2, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.8, 18.6, 12.5 and 12.0 ppm.

**Preparation of 2α-acetoxy-5α-androstan-3-one (55) from epoxide (54):** The acetic anhydride (20 ml) solution of epoxide 54 (700 mg; 2.1 mmol) and triethylamine (0.250 ml) was refluxed for 2 hours. The reaction mixture was extracted with diethyl ether (3×15 ml) and the combined ether extracts were evaporated and the solid white residue was dried under reduced pressure yielding pure product (620 mg, 89%). 4 1H NMR (400 MHz, CDCl₃): δ 5.29 (dd, 1H), 2.12 (3H, s,CH₃CO), 0.90 (3H, d, J = 6.5 Hz, CH3-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19) ppm. 13C NMR δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.2, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.8, 18.6, 12.5 and 12.0 ppm.
Preparation of 2α-acetoxy-5α-androstan-3-one (55) from ketone (52): A mixture of acetic acid (40 ml) and acetic anhydride (5 ml) was refluxed for 10 minutes. This mixture was cooled to room temperature and the compound 2 (1g; 2.57 mol) was added followed by the addition of lead tetraacetate (1.6 g; 4.36 mmol). The reaction mixture was refluxed with stirring for 24 hours. After cooling, the reaction mixture was diluted by ether (100 mL) and washed 5% hydrochloric acid (3x20 ml), saturated sodium bicarbonate (3x20 ml), and brine (3x20 ml). The solvent was evaporated and the oily residue was purified by column chromatography on silica gel (15% ethyl acetate in hexane). The isolated yield of the product was 0.855 g (80%). ¹H NMR (400 MHz, CDCl₃): δ 5.29 (dd, 1H), 2.12 (3H, s, CH₃CO), 0.90 (3H, d, J = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19) ppm. ¹³C NMR δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.2, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.8, 18.6, 12.5 and 12.0 ppm.

Preparation of 3β-hydroxy-5α-androstan-2α-yl acetate (56): A methanol (10 ml) and tetrahydrofuran (20 ml) solution of ketone 55 (270 mg; 0.8 mmol) 2:1 and CeCl₃.7H₂O (373mg,1.0 mmol) was stirred at room temperature for 10 minutes. The reaction mixture was stirred for additional 30 minutes after NaBH₄ (60 mg; 1.6 mmol) was added. After sodium borohydride was quenched with 5% hydrochloric acid (~0.5 ml) the reaction mixture was diluted with water and extracted with ether (3x20 ml). Combined ether extracts were dried over anhydrous magnesium sulfate and evaporated to dryness yielding to 245 mg (90%) of pure product. Selected ¹H NMR (400 MHz, CDCl₃): δ 4.82 (1H, ddd, J = 4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, J = 4.7, 9.0, 11.5 Hz, H-2b), 2.12 (3H, s, CH₃CO), 0.90 (3H, d, J = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19) ppm. ¹³C NMR
δ: 171.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.5, 39.3, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.9, 18.6, 12.5 and 12.0 ppm.

**Preparation of 2α,3β-dihydroxy-5α-androstane (57):** A methanol-dichloromethane (10 ml 1:1) solution of potassium hydroxide (120 mg; 2 mmol) and ester 56 (170 mg; 0.5 mol) was stirred at room temperature for five hours. The solvent was evaporated, the solid residue was partitioned between dichloromethane (20 ml) and water (20 ml). The organic layer was separated, washed with water (3×10 ml) and dried over anhydrous magnesium sulfate. Solvent was evaporated yielding 165 mg (98%) of pure diol 9. Selected ¹H NMR (400 MHz, CDCl₃): δ 3.59 (1H, ddd, J = 4.7, 9.0, 11.5 Hz, H-2b), 3.40 (1H, ddd, J = 5.1, 8.9, 10.9 Hz, H-3a), 0.90 (3H, d, J = 6.5 Hz, CH₃-21), 0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 ppm (3H, s, CH₃-19). ¹³C NMR (CDCl₃) δ: 82.2, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.4, 39.3, 36.7, 36.1, 35.7, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.9, 18.6, 12.5, and 12.0 ppm.
2.7 References:


11. Marston, A.; Potterat, O.; Hostettmann, K. J. “Isolation of biologically active plant constituents


CHAPTER-3. SYNTHESIS ANTIFUNGAL EVALUATION OF FUNCTIONALIZED 2,3-SPIROSTANES

Several saponins have been described as having potent antimicrobial properties. Furthermore, a number of saponins with either a spirostanol or a 2,3-spirostadiol moiety linked to a glycoside exhibit broad-spectrum antifungal activity against multiple fungal species, where the mechanism of action is probably through the formation of a complex with sterols in fungal membranes causing a loss of membrane integrity or by inhibiting 1,3-β glucan synthase activity. Therefore, it is reasonable to investigate the development of novel 2,3-spirostane based glycosides as potential antifungal agents. Nonetheless, while there are reports showing successful syntheses of spirostanols, a major obstacle remains in the limited synthetic methodology applicable to large-scale preparations of functionalized 2,3-spirostanes. Developing this synthetic methodology would not only allow the preliminary investigation of functionalized 2,3-spirostanes as potential antifungal agents but would also provide a library of functionalized 2,3- spirostane derivatives that could be used as building blocks for the development of novel synthetic spirostane-linked glycosides with potential antimicrobial properties. Functionalized steroids are well known compounds with potentially interesting biological properties\(^1\)-\(^3\). Many of these compounds are only available in milligram quantities, as they are isolated from natural sources. This limited availability hampers both chemical and biological exploration of these compounds and their analogs. Therefore, there is an urgency to develop well-defined synthetic procedures for the preparation of these valuable compounds in large quantities starting with inexpensive and commercially available steroid precursors.

Our overarching goal was to develop an efficient synthetic procedure for the synthesis and isolation of functionalized 2,3-spirostanes that could be further used to develop novel saponin-like analogs with potential therapeutic value. We explored a number of different synthetic pathways in
the preparation of functionalized 2,3-spirostanes. Our main goal was to develop most efficient routes, each of which will be superior to other alternative synthetic routes in simplicity, isolated yields, and applicability to the preparation of other substituted steroids in large quantities. In designing the synthetic methodology to prepare functionalized 2,3-spirostane stereoisomers, we identified two major obstacles that required resolution prior to the development of a practical synthetic approach. The first was (a) how to selectively introduce hydroxyl groups into the C-2 position; and the second was (b) how to differentiate one of the two-hydroxyl groups for hydroxyl group protection in order to modify the other synthetically. We have already developed a synthesis of similar isomers of cholestane and androstane, which will be explored and optimized for spirostane derivatives to achieve the desired goal for the synthesis.

The non-protected 2α, 3β-aglycone (26, R = H) is a known compound derived from the hydrolysis of the saponin gitonin (1). This saponin is one of the minor saponin components obtained during the isolation of the saponin digitonin (2) from *Digitalis purpurea.* Digitonin is a toxic saponin, which precipitates cholesterol and is therefore useful in the permeabilization of cellular and nuclear membranes. While this saponin mixture is commercially available from a number of sources, it is impractical as a starting material for the preparation of 2α, 3β-aglycone.

This is due to the fact that gitonin is only a minor component of the commercially available digitonin mixture. Consequently, obtaining 26 from commercial sources would require separation of a minor saponin component from a saponin mixture followed by hydrolysis of the saponin to obtain non-protected aglycone component 26. Furthermore, selective protection and isolation of one of two neighboring equatorial secondary alcohols would need to be accomplished before a glycosylation with an oligosaccharide moiety could be performed.
A strategy for the synthesis of aglycone 25 from more abundant sterols was investigated. A literature search revealed a synthesis by Sondheimer et al. of the 2α, 3β-aglycone in 5 steps from diosgenin (Scheme 3.1). In this synthesis, diosgenin (3) was oxidized to α,β-unsaturated ketone 4 by an Oppenauer oxidation which was then subjected to an allylic bromination to give 5. The key step in this approach, involving an acetolysis of 5 to form α-acetoxy ketone 6, proceeded in only 25% yield, severely limiting the usefulness of this strategy in a more complicated synthesis. To complete the synthesis, hydrogenation of the alkenyl functionality of 6 was followed by reduction of both the C-2 acetate protecting group and the C-3 ketone of 7 by LiAlH₄ to give unprotected trans-1,2 diol 8 in 5% overall yield from diosgenin. (Scheme-1) There were two concerns about the utility of this synthesis for the preparation of 2α, 3β-aglycone . The first concern was the low yield since aglycone 26 would be an intermediate in the saponin synthesis. More importantly, the lack of a protecting group on either the C-2 or C-3 alcohol in 7 renders it unusable in a glycoconjugate preparation. Since both of these secondary alcohols are equatorial, it would not be feasible to selectively protect one over the other. The development of a new synthetic strategy was therefore considered necessary in the hopes of discovering a practical synthesis of 2α, 3β-aglycone moiety.
Holzapfel et al. reported the synthesis of a glycoconjugate containing the same steroid moiety as 2α, 3β- aglycone (Scheme 3.2). Their synthesis began with diosgenin (3), which provided tigogenin (9) by catalytic hydrogenation. Tigogenin was oxidized using chromic acid to spirostan-3-one (23), which was then converted to trimethylsilyl enol ether 19. Enol ether 19 was oxidized with dimethyldioxirane (DMDO) and the resulting α-hydroxy ketone was protected as allyl carbonate 19a. To this point, each step in this literature report was accomplished in high yields of no less than 83%. Unfortunately, the last step, requiring a stereoselective reduction of the C-3 ketone of 19a to the β-alcohol using sodium borohydride, provided the desired stereoisomer 19b in only 63% yield.

Successful syntheses of trans-1,2 diols from trimethylsilyl enol ethers using hydroboration-oxidation has been reported for substituted cyclohexenol derivatives. Therefore, a new retrosynthesis (Scheme 3.2) for the steroid moiety of 2α, 3β-aglycone was designed using the trimethylsilyl enol ether intermediate 19 described in the synthesis by Holzapfel et al. Although the reported yields for the hydroboration-oxidation of trimethylsilyl enol ethers are low (~60%), this method would still provide the opportunity for a higher yielding synthesis since it would require fewer steps. In this proposed synthesis, the aglycone would be prepared by the
hydroboration-oxidation of trimethylsilyl enol ether 23 to give selectively protected diol 19b. This silyl enol ether 19 would be synthesized from spirostan-3-one 23, which is available from the oxidation of tigogenin (9).

Scheme 3.2 i) H₂, Catalytic; ii) H₂CrO₄; iii) HMDS, TMSCl; iv) DMDO, ClCO₂Allyl; v) NaBH₄. Literature reported synthesis of 2α, 3β-agnlecone by Holzapfel et al.⁷ Even so, it is worth highlighting the fact that these authors choose to invert the axial C-3 alcohol of 17a by oxidizing it to ketone 17b and then reducing it to the corresponding equatorial alcohol 17c. Yu et al.⁸ employed a Luche reduction and made no improvement on the yield of this step.

Scheme 3.3 Reagents and conditions: (i) H₂, Pd/C, CH₂Cl₂–MeOH, r.t.; (ii) TsCl, pyridine, 0°C then r.t.; (iii) silica gel, EtOAc–PE, r.t.; (iv) OsO₄, NMO, t-BuOH–THF–H₂O (10:8:1), r.t.; (v) Bu₃SnO, toluene, reflux, 6 h; then BnBr, Bu₃N+Br–, reflux, 2 h; (vi) Dess–Martin periodinane, CH₂Cl₂, r.t.; (vii) NaBH₄, CeCl₃·7H₂O, MeOH, –40 °C, 55% (two steps for 17c), 28% (two steps for 17). Literature reported synthesis of the 2α, 3β-agnlecone by Yu et al.³
Synthesis of 2β, 3α-spirostan and 2β, 3β-spirostan isomers have been previously developed in our group previously starting from hecogenin acetate. Tigogenin 9 was easily obtained in quantitative yield by Wolff-Kishner reduction of hecogenin acetate. It is well documented that the α-steroid face is less sterically hindered and therefore is the more reactive face of the steroid. This selective reactivity was used to prepare the axial trans dihydroxy derivatives 12 and 13 (Scheme-3.4). The synthesis started with a modified Chugaiev water elimination9 from tigogenin 9. As in many other cases, epoxidation of 2-spirostene (10, Scheme-3.4) occurred from the α-face10, 11 and generated epoxide 11. The acetic acid epoxide ring opening resulted in the axial hydroxyl acetate 12 and 13. The structure of 13 was also confirmed by X-ray crystallography.

In addition to produce functionalized isomers 12 and 13, cis-diols from compound 10 were also generated synthetically. This was accomplished by using an established procedure involving OsO₄ oxidation8 of unsaturated steroids, such as 10. Using this method, 5α-spirostan-2α, 3α-diol (17) was prepared in 83% isolated yield. Of the isomers prepared, the more demanding was 5α-spirostan-2β, 3β-diol. Considering that the preparation of acetyl 13 was a straightforward process, it was decided to use this compound as a starting point to explore its C-3 stereochemical inversion. Inversion through acetyl group rearrangement is a well-known reaction12. This approach was used to explore the effect of acetyl neighboring group participation on the hydrolysis of mesylate 14 (Scheme 3.4). Hydrolysis of mesylate in compound 14 gave major product 15 and minor product 16 by refluxing with pyridine/water, which were purified and characterized by 1H and 13C-NMR (Fig. 3.3).
Scheme 3.4 (i) NaH/CS₂/propargyl bromide/THF; (ii) collidinium trifluoromethanesulphonate (0.1 equiv)/toluene/reflux; (iii) m-chloroperbenzoic acid/CH₂Cl₂; (iv) acetic acid/reflux; (v) MsCl/Et₃N/CH₂Cl₂; (vi) Pyridine/water/reflux; (vii) OsO₄/N-methylmorpholine-N-oxide.

3.1 Retro-analysis for 2α, 3β-spirostane:

The retro-analysis of 2α, 3β-spirostane derivative indicated that we can get it by reduction of 2α-acetoxy-spirostan-3-one (7), which can be obtained in two different ways. Firstly by enolization and protecting enol group with TMS or acetyl group followed by epoxidation to form epoxide ring which can be opened in presence of acetic anhydride and acetic acid to get. Secondly leadtetraacetate in presence of acetic acid and acetic anhydride reflux will stereoselectively form 7. Spirostan-3-one (23) could be obtained from Diosgenin in two steps by reduction of double bond followed by oxidation of alcohol group at 2-position alternatively from commercially available hecogenin acetate in two steps by classical Wolff-Kishner reduction followed by oxidation of hydroxyl group to keto. (Scheme 3.5)
Scheme 3.5 Retro-synthetic analysis of 2α, 3β-spirostane isomers

3.2 Synthesis of Spirostane derivatives:

Efficient and simple synthesis of 2α, 3β-spirostane isomers was not successfully explored so far as having both group equatorial at C-2 and C-3 is naturally not favoured due to sterically hindered attack to form 2α functionality. We have developed a method for synthesis of 2α, 3β-cholestane and 2α, 3β-androstane derivatives which was discussed in chapter-2 in details, the developed synthesis needs to be explored to optimize reaction conditions for the synthesis of 2α, 3β-spirostane isomer. Our first attempt towards this synthesis started well before we actually have developed synthesis of 2α, 3β-cholestane and 2α, 3β-androstane derivatives, there were constant problems involved in the synthesis which led us to look for another strategy and to reduce the cost of synthesis we decided to develop synthesis with some starting material which is much cheaper
compared to hecogenin acetate. Thus we decided to use cholesterol as our model starting material to explore the synthesis and apply the developed strategy for the synthesis of 2α, 3β-spirostane isomer.

3.3 First attempt for synthesis of 2α, 3β-spirostane isomer.

Our first attempt towards synthesis of 2α, 3β-spirostane derivative started with hecogenin acetate as starting material, with the one-pot simultaneous ester group hydrolysis and Wolff-Kishner keto group reduction to get tigogenin13. This procedure was adapted from a well-developed method for the preparation of tigogenin from hecogenin acetate14. Using this approach, we produced a large quantity of tigogenin in excess of 98% yield, which was then used for further chemical modification. Jones oxidation of tigogenin gave keto compound 23 in 93 % of isolated yield15. Enolization was done by lithium bis-(trimethylsilyl)amide and trimethylsilyl chloride at -78°C in dry tetrahydrofurane to give compound 19 in an isolated yield of 60% (Note- Purification by column chromatography should be done fast)7. TMS group was found to be cleaving while doing column chromatography for purification; shortening the time for column purification did improved the yield but not with very high success towards minimizing this problem. That was one of the reasons for low to moderate yield for this step of the synthesis. Regioselectivity for this type of enolization was not an issue as it was well documented by M. Sobukawa et al. and has very good selectivity of 9:1 for the desired regio-isomer in case of cholestan-3-one16. (Scheme-2.5)

Epoxidation of O-TMS enol 19 was achieve by m-CPBA after several attempts to optimize the reaction to get epoxide compound 21 in 70% of isolated yield after purification. Epoxidation occurs selectively from α-face of the spirostane molecule due to steric hindrance by methyl groups on the β-face. (Scheme 3.7)
Most notably, the chemical shift for the C-19 methyl group for 11a was 0.75 ppm as compared to 1.04 ppm for 11b. The downfield shift for this signal in the case of 11b is due to the fact that the methyl group is on the same side of the molecule as the epoxide in this isomer. This result is supported by the fact that in ring systems, nucleophilic attack on epoxides generally occurs from an axial approach.\(^{17}\)
The epoxide ring opening of 11a and 11b was explored previously in our group. In both cases the acetic acid nucleophile approaches each epoxide axially to give the diaxial isomers 12 and 13 in 84% and 71% yield respectively (Scheme 3.8).

Additionally, the appearance of H-2 and H-3 as broad singlets in the $^1$H-NMR of 3α-hydroxy-spirostan-2β-yl acetate (13) confirms their equatorial orientation (Figure 3.1). Axial protons, as in H-3 of tigogenin (9) which is also shown in Figure 1 for comparison, appear as multiplets. This is due to the difference in the coupling constants between equatorial and axial protons. The stereochemistry of 13 was further confirmed by X-ray crystallography (fig. 3.2). Therefore, further attempts to synthesize 11b in good yield were abandoned considering the fact that either epoxide leads to a diaxial trans-1,2 diol derivative upon reaction with acetic acid.²

![Scheme 3.8 Epoxide ring opening with acetic acid.](image)

![Figure 3.1 Portion of $^1$H-NMR of 3α-hydroxy-spirostan-2β-yl acetate (13) and tigogenin (9) showing C-2 and C-3 stereochemistry.](image)
Epoxide ring was successfully opened by acetic acid and acetic anhydride mixture on reflux to give compound 7 in 65% of isolated yield after column purification. TMS group present at C-3 tends to favor formation of 2α-acetyl groups and the mechanism of reaction is assumed to be similar as in case of cholestane and androstane epoxide ring opening step.

A sodium borohydride reduction of compound 8 gave us a mixture of two isomers 8 and 24. Although our major product was the desired isomer but were not able to purify more than 90% by column chromatography, there was a 10% mixture of two were confirmed by several trials of purification. The minor isomer 24 was purified successfully and confirmed by 1H-NMR in 10-25% yield. At this point of synthesis we decided to change our strategy as there were four synthetic steps involving column purification with moderate yield of 60-70%, more over gives two isomers at the last step, which were practically hard to purify by column chromatography.

3.4 Second attempt for synthesis of 2α, 3β-Spirostane isomer.

The problem associated with previous approach forced us to look for alternate synthetic strategy. Hecogenin acetate being very expensive made the whole process of exploration very expensive; we were constantly looking for alternate starting material, which is cheaper. Since we always wanted to design a viable and economic synthetic pathway, we decided to use Diosgenin as our starting material, which is at least five times cheaper than Hecogenin acetate. Structural features
of Diosgenin were attractive for us as it have similar double bond as cholesterol and synthesis for which was well developed and reported by us previously. Reduction of double bond was performed with platinum dioxide in dichloromethane and acetic acid (1:1) mixture as solvent at high-pressure hydrogen parr apparatus for 12 hours (Scheme 3.9)). Initially we thought that the reaction is going well and we have very nice product, monitored reaction by $^1$H-NMR. The doublet proton peak at 5.2 for double bond proton was completely vanished. We continued next step, which was oxidation of the double bond by chromic acid solution (40% sulfuric acid) in dry acetone.

After completion of oxidation reaction and purification of product analysis was done by $^1$H-NMR. It was to our surprise that the nice peaks for proton at C-17 have changed their pattern to nice quartet instead of doublet of doublet signature peak at 3.4 ppm. We were surprised but wanted to make sure that every thing was done in desired way and various parameters, which could lead to opening of the oxygen containing spirostane ring. When we did a series of repeated trials to see what is going wrong we realized that the spirostane rings were opening up in presence of acetic acid under high pressure of oxygen.

Very low percentage of acetic acid and lesser amount of platinum oxide catalyst was also tried for this reduction reaction, which indeed hampers the reduction reaction.

![Scheme 3.9 Platinum catalyzed reduction of diosgenin.](image-url)
3.5 Third attempt for synthesis of 2α, 3β-spirostan isomer.

Since there was not much success with optimization of double bond reduction reaction we decided to go with second strategy, which was to use previous starting material hecogenin acetate. The preparation of our functionalized 2,3-spirostanes started with the one-pot simultaneous ester group hydrolysis and Wolff-Kishner keto group reduction of hecogenin acetate\textsuperscript{13}. This procedure was adapted from a well-developed method for the preparation of tigogenin from hecogenin acetate\textsuperscript{14}. Using this approach, we produced a large quantity of tigogenin in excess of 98% yield, which was then used for further chemical modification to produce alternative stereoisomers. In designing the synthetic methodology to prepare functionalized 2,3-spirostanne stereoisomers, we identified two major obstacles that required resolution prior to the development of a practical synthetic approach. The first was (a) how to selectively introduce hydroxyl groups into the C-2 position; and the second was b) how to differentiate one of the two-hydroxyl groups for hydroxyl group protection in order to modify the other synthetically. In the first step, a Jones oxidation\textsuperscript{15} of the OH group in the C-3 position of tigogenin was used to prepare ketone (23, Scheme 3.7). This oxidation was necessary because it allows for an acetoxy group to be introduced into the α-position to the carbonyl\textsuperscript{18}. Typically, this reaction produces several acetoxy isomers, but in the case of steroid substitution, the reaction is both highly site and stereospecific. The acetoxy group is introduced in the C-2 position rather than in C-4 position due to steric hindrance of C-4 steroids. Further, the reaction occurs from the α-face of steroid, due to β-face steric hindrance, and therefore the only isolated product is the keto ester 7 (Scheme 3.10). The preference for α-face substitutions in steroid reactions has been well documented. Finally, to generate the 2α, 3β-spirostanne (8) we followed a straightforward NaBH\textsubscript{4}/CeCl\textsubscript{3} reduction of the carbonyl group of 7 in 90% isolated yield.\textsuperscript{19} Finally, to complete our synthetic route toward the preparation of 5α-spirostan-2α, 3β-diol
(Scheme 3.7) the ester group of was hydrolyzed in NaOH/methanol + dichloromethane to yield compound 25.

Alternatively, we also developed a highly efficient alternative synthetic approach for the preparation of keto acetate 7, avoiding the use of lead tetraacetate, due to pharmacological controversy surrounding this reagent. In our alternative approach, we replaced the lead tetraacetate step (Scheme 3.10, step iv) with three very simple and efficient synthetic steps: (a) the transfer of ketone 23 into its vinyl acetate 20 (step iii-Scheme 3.10) (b) the mCPBA epoxidation of vinyl acetate into epoxide 22 (step v-Scheme 10), and finally; (c) the acetic acid epoxide 22 ring opening (step vii-Scheme 3.10). This three step combination that replaced lead tetraacetate with less controversial reagents did ultimately give a slightly lower overall yield (75%) for compound 7 than what is isolated from a direct reaction with lead tetraacetate (80%), however it can still be used as a safe alternative for the preparation of intermediate 5 in our synthesis of functionalized 2,3-spirostanes.

Scheme 3.10  i) Hydrazine hydrate, 2-ethoxyethanol, KOH, 136°C; ii) CrO₃/H₂SO₄/dry Acetone; iii) (CH₃CO)₂O/Montmorillonite clay; iv) Pb(O₂CCH₃)₄/(CH₃CO)₂O-CH₃CO₂H; v) m-chloroperbezoic acid/CH₂Cl₂; vi) CeCl₃x7H₂O/CH₃OH/tetrahydrofuran/NaBH₄; vii) (CH₃CO)₂/N(C₂H₅)₃; viii) NaOH/CH₂Cl₂/CH₃OH.
Stereoselective 2α- acetylation of ketone 23 was clearly evident from 1H-NMR spectra a doublet of doublet at 5.25 ppm indicates that acetyl group is on α-face of the molecule, which would have been a nice triplet if the acetyl group has been on β-face. Epoxidation of the vinyl acetate occurred from the α-face was indicated by similar peak at 3.68 ppm for H-2 proton as was in case of cholestane and androstane derivative.

![Figure 3.3 Portion of 1H-NMR spectra of 2,3-spirostan isomers showing C-2 and C-3 stereochemistry.](image)

The stereochemistry of 2α, 3β-spirostane derivative was confirmed by 1H-NMR spectra comparison with the previously reported isomers as well as literature. There is nice multiplet at 4.80 ppm for H-2 proton and a multiplet at 3.61 ppm for H-3 proton due to differential coupling with the proximal prochiral and chiral protons in case of compound 8. Whereas in case of 2β, 3α -spirostane 13 peaks for H-2 at 4.81 ppm and for H-3 at 3.80 ppm are broad singlet (figure 3.3). Compound 15
shows nice multiplet at 3.65 ppm for H-3 proton, which a 3β-isomer hence have similar splitting as compound 8. Similar is the case with compound 16, which is a 3β-isomer, have nice multiplet splitting for H-3 proton whereas H-2 proton peak is broad singlet as in case of compound 13. Switching of acetyl group from C-2 in compound 15 to C-3 in compound 16 can be easily seen by H-3 proton peak going up field from 3.65 ppm to 4.81 ppm. The $^1$H-NMR spectrum was also found to be matching with literature report of reported $^1$H-NMR of 2α, 3β-spirostan by Sondheimer et al. Moreover the splitting pattern for H-2 and H-3 proton for similar isomers from cholestane and androstan were well matching.
3.6 Antifungal assay of spirostane derivatives

The antifungal activity of the synthesized functionalized 2,3-spirostanes were evaluated in vitro using four species of fungi, including Candida albicans, Cryptococcus neoformans, Candida glabrata, and the filamentous fungus Aspergillus fumigatus. The results of these screenings are summarized in Table 3.1. Of the nine analogs tested, 2α-acetoxy-5α-spirostan-3β-ol (65) was the only compound that showed significant antifungal activity (>50% inhibition at the minimal concentration) against all four species tested, with C. glabrata being the most sensitive at the lowest concentration (6.25 µg/mL) and the filamentous fungi A. fumigatus being the least sensitive (where 50% inhibition was only observed in the highest dose tested- 128µg/mL). Hydrolysis of the acetoxy group of 8 (yielding compound 25) resulted in the total loss of antifungal activity against all species tested, as did inversion of the stereocenter on the C-3 (to yield compound 15) as well as on the C-2 and C-3 positions (to yield compound 13) of isomer 8 (Table 3.1). The combination of the change in stereochemistry of both the chiral C-2 and C-3 centers and the acetyl group migration from C-2 to C-3 of 8 resulted in the total loss of antifungal activity (see 12-Table 3.1). Finally, the inversion of only one of the chiral centers of 8 (C-2 inversion to yield 16-Table 3.1) resulted in the total loss of antifungal activity.

In addition to analyzing in vitro the antifungal properties of all acetate ester and free 2,3-spirostane isomers, we also analyzed three intermediate compounds with structural similarities to compound 8. These analogs included compounds 9 (tigogenin), 23 and 7 (Table 3.1). Interestingly, the analog tigogenin (9), which contains a β-face OH group in the C-3 position, similar to 8, displayed significantly increased activity against C. glabrata and A. fumigatus (>62 µg/mL).
µg/mL, respectively) compared to 8 (Table 3.1). One can speculate this might be due to the orientation of the binding sites to which these compounds may be binding. Further studies with analogs that contain various sugar and hydrocarbon substitutions to both the α- and β-faces of the spirostane molecule were tested (see chapter-4). Nonetheless, the preliminary antifungal evaluation of 2,3-functionalized spirostanes identified several of the derivatives that showed moderate antifungal activity (as measured spectroscopically by a >50% reduction in fungal growth compared to control wells), against at least one species of fungus.

These 2,3-functionalized derivatives were be used as building blocks for the synthesis of novel spirostane-linked glycosides (chapter-4). At this stage of the project we knew that some of these derivatives were very active and have potential to be taken further and SAR of these active compound could give us a molecule which might be much more active than the naturally occurring saponin CAY-1. There are several reports of glycosides linked spirostane molecules having decent antifungal activity\textsuperscript{23, 24}. We decided to synthesize branched and linear oligosaccharide and attach them to various spirostane derivatives. Detailed synthetic strategy for oligosaccharide synthesis will be discussed in details in chapter-4 various approaches for glycosidation with steroid moiety were explored. We have synthesized more than thirty compounds with glycosides attached to various steroid derivatives with a variation of mono, di and trisaccharides. Similar compounds originated from all the three different steroid derivatives we synthesized so far were synthesized and tested \textit{in vitro} for their antifungal activity details of which will be discussed in chapter-5.
Table 3.1 Antifungal activities of novel spirostane analogs (MIC in µg/mL).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MIC$_{50}$ (µg/mL)$^{a, f}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. albicans$^b$</td>
</tr>
<tr>
<td>9</td>
<td><img src="image1" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>23</td>
<td><img src="image2" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>7</td>
<td><img src="image3" alt="Structure" /></td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td><img src="image4" alt="Structure" /></td>
<td>16</td>
</tr>
<tr>
<td>25</td>
<td><img src="image5" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>13</td>
<td><img src="image6" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>22</td>
<td><img src="image7" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>15</td>
<td><img src="image8" alt="Structure" /></td>
<td>NC*</td>
</tr>
<tr>
<td>16</td>
<td><img src="image9" alt="Structure" /></td>
<td>NC*</td>
</tr>
</tbody>
</table>

$^a$MIC values are reported only for compounds displaying 1) a prominent decrease in turbidity by visual comparison to the control wells containing no antifungal and 2) a >50% reduction in fungal growth compared to controls containing no antifungal, as measured spectratically by absorption at 530 nm.

$^b$ATCC no. 10231; $^c$ATCC no. 48435; $^d$ATCC no. 36556; $^e$ATCC no. 16424

$^f$All values were determined after incubation at 35°C for 48 h.

* denotes compounds that had 1) a slight reduction in turbidity to no change and 2) had less than a 25% reduction in growth compared to controls, as measured spectratically by absorption at 530 nm.
3.7 Conclusions

The syntheses discussed above by Sondheimer et al. and by Holzapfel et al., Yu et al. published a 7-step synthesis of the 2α, 3β-aglycone from diosgenin in 28% yield. Although their report is more recent than that of Holzapfel et al., it is less efficient in that it is both lower yielding and requires more steps. Even so, it is worth highlighting the fact that these authors chose to invert the axial C-3 alcohol of 17a by oxidizing it to ketone 17b and then reducing it to the corresponding equatorial alcohol 17c. Yu et al. employed a Luche reduction and made no improvement on the yield of this step. Synthesis for 2α, 3β-spirostane derivatives were successfully achieved and after a lot of hurdles we finally have a method to synthesize these derivatives in large quantities in just four to six steps with very good yield (60-56% overall yield for synthesis). Comparison of $^1$H-NMR spectrum of synthesized isomer was done with previously synthesized isomers and literature reported $^1$H-NMR of similar isomers has proved to be good evidence for the stereochemistry at C-2 and C-3 positions. Antifungal susceptibility tests for various derivatives of spirostane against four different fungal strains have shown some very promising activity. At this stage of the project we knew that some of these derivatives were very active and have potential to be taken further and SAR of these active compound could give us a molecule which might be much more active than the naturally occurring saponin CAY-1. We decided to take these active compounds further and make glycosides linked steroidal saponins in hopes that antifungal activity would improve and we might find our lead compound in nanomolar range of activity.
3.8 Experimental

3.8.1 General Remarks—All necessary starting materials for the preparation of targeted molecules were purchased from Aldrich and were used without further purification. Thin-layer chromatographic analysis (TLC) was performed using silica gel on glass plates and was detected under ultraviolet (UV) light and using PMA solution. Column chromatography was performed using silica gel porosity 60 Å, partial size 40-75 mm from Sorbent Technologies. The final products of the steroid derivatives were purified by silica gel chromatography and were more than 96% pure, as determined by HPLC chromatography (Agilent Technologies, 1200 series). Structures of all intermediates and final products were determined by $^1$H NMR, $^{13}$C NMR spectroscopy (Varian Unity 400 MHz). In one instance, the structure was also determined by X-ray analysis (Steroid 11) using a Bruker SMART 1KCCD automated diffractometer. Crystals of compound were obtained by slow crystallization from a diluted dichloromethane solution. Computing x-ray data collection and the cell refinement was performed by Bruker SMART. Computing x-ray data reduction was performed by Bruker Saint. Computing structure solution and structure refinement from x-ray data were performed by SHELXL-97.

3.8.2 Biological Evaluation: Antifungal susceptibility studies and minimal inhibitory concentrations (MIC) values for Candida albicans (ATCC no. 10231), Cryptococcus neoformans (ATCC no 36556), Candida glabrata (48435), and the filamentous fungus Aspergillus fumigatus (ATCC no. 16424) were determined by the broth microdilution technique in accordance with NCCLS reference documents M27-A. Microdilution panels ranged from 0.01 to 128 µg/mL. All organisms were subcultured on Sabouraud or potato dextrose agar and passaged to ensure purity and viability. The initiating inocula were prepared by picking 5 isolated colonies ~1mm in diameter from freshly prepared plates. Colonies were resuspended in sterile water, vortexed and
the cell density was adjusted spectrophotometrically to the transmittance of a 0.5 McFarland standard at 530 nm wavelength, to yield a stock suspension of $1 \times 10^6 - 5 \times 10^6$ cells/mL. A working suspension was then made by diluting the stock 1:100 with RPMI 1640 broth media. The working stock was used for all drug studies. The plates were incubated at 30-35°C for 48 h in a humid atmosphere and scored visually with the aid of a reading mirror and spectrophotometrically with an automatic plate reader set at 530 nm. MIC values are defined as the lowest concentration of agent that prevents any discernible growth, ~50% reduction of growth, as compared with drug-free control wells. Control drugs used were Amphotericin B, and itraconazole, all diluted to 1.0 µg/mL concentrations (Sigma-Aldrich). Controls were run parallel to each in vitro screening of the functionalized 2,3-steroid analogs.

### 3.8.3 Preparation of spirostanes:

**Preparation of 5α-spirostan-3β-ol (tigogenin) (9):** A 2-Ethoxyethanol (10 mL) solution of hecogenin acetate (18) (1 g; 2.12 mmol) and hydrazine hydrate (1 mL; 20 mmol) was stirred under nitrogen atmosphere for 20 minutes and refluxed for one hour. Into the room temperature reaction mixture potassium hydroxide (1g; 17.8 mmol) was added and the reaction mixture was refluxed for an additional 3 hours. After cooling to room temperature, the reaction mixture was acidified by concentrated hydrochloric acid. A white crystalline material precipitated and was separated by filtration, washed with water, and dried under reduced pressure to give 863 mg (98%) of pure product that has $^1$H NMR and $^{13}$C NMR consistent with literature data 31. Selected $^1$H NMR (400 MHz, CDCl$_3$): δ 4.38 (q, $J = 11.2$ Hz, 1H), 3.58 (m, 1H), 3.47 (m, 1H), 3.37 (t, $J = 10.8$ Hz, 1H), 0.96 (d, $J = 3.4$ Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J = 3.2$ Hz, 3H), 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): δ 109.5, 81.0, 71.5, 67.0, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 39.6 ppm.
Preparation of 5α-spirostan-3-one (23): Tigogenin (9) (1 g, 2.40 mmol) was dissolved in acetone (50 mL) at 30°C. 8N chromic acid in 40% H₂SO₄ (2.5 mL) was added and the mixture was stirred for 30 minutes. Water (100 mL) was added and the resulting solid was filtered and washed with water and purified by flash column chromatography (1:2 ethyl acetate: hexanes) to give (3) as a white solid (930 mg, 93 % yield) which was used immediately in the next reaction. Selected ¹H NMR (400 MHz, CDCl₃): δ 4.39 (q, J = 11.2 Hz, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 1.02 (s, 3H), 0.96 (d, J = 3.6 Hz, 3H), 0.78 (s, 3H), 0.78 (d, J = 2.0 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 212.1, 109.4, 80.9, 67.0, 62.4, 56.3, 54.0, 46.8, 44.8, 41.8, 40.7, 40.1, 38.7, 38.3, 35.9, 35.2, 32.0, 31.9, 31.5, 30.4, 29.0, 29.0, 21.4, 17.3, 16.6, 14.7 and 11.7 ppm.

Preparation of 3-acetoxy-5α-spiro-2-ene (20): An acetic acid (100 mL) suspension of 5α-spirostan-3-one (23) (1g; 2.4 mmol) and montomorillonite clay (500 mg) was stirred at room temperature overnight. The clay catalyst was separated by filtration and the filtrate was extracted with ether (3x30 mL). The combined ether extracts were washed with saturated sodium bicarbonate (3x20 mL), brine (3x20 mL) and dried over anhydrous magnesium sulfate. The ether was evaporated under reduced pressure to give 1.0 g (90%) of white crystalline product (20). Selected ¹H NMR (400 MHZ, CDCl₃): δ 5.21 (d, 1H), 4.38 (q, J = 11.2 Hz, 1H), 3.47 (m,1H), 3.37 (t, J = 10.8 Hz, 1H), 2.08 (s, 3H), 0.96 (d, J = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, J = 3.2 Hz,3H), and 0.76 (s, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 165.2, 81.0, 71.5,
Preparation 2α-acetoxy-5α-spirostan-3-one (7): Method A: A mixture of acetic acid (30 mL) and acetic anhydride (5 mL) was refluxed for ten minutes. Into a room temperature cooled reaction mixture, 5α-spirostan-3-one (23) (830 mg; 2 mmol) and lead tetraacetate (1.1 g; 3 mmol) was added and the resulting mixture was refluxed for 24 hours. Into the room temperature cooled reaction mixture diethyl ether (150 mL) was added and the resulting mixture was washed with 5% hydrochloric acid (3x20 mL), saturated bicarbonate (3x30 mL) and brine (3x20 mL). The ether layer was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give (80%) of pure product (7). Method B: An acetic anhydride solution (20 mL) of 2α-acetoxy-2β,3α-epoxy-5α-spirostane (22) (950 mg; 2 mmol) and a catalytic amount of triethylamine (0.15 mL) was refluxed for 2 hours. After cooling to room temperature, the reaction mixture was extracted with ether (3x20 mL). The ether was evaporated giving pure product in 98% isolated yield. Selected data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.29 (dd, 1H), 4.38 (q, $J$ = 11.2 Hz, 1H), 3.47 (m,1H), 3.37 (t, $J$ = 10.8 Hz, 1H), 2.08 (s, 3H), 0.96 (d, $J$ = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J$ = 3.2 Hz,3H), and 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2,162.5, 81.0, 71.5, 67.0, 62.3, 56.5,54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7, 12.5 ppm. [Physical properties of our compound are in agreement with ones previously published $^{27}$].
Preparation 2α-acetoxy-2β,3α-epoxy-5α-spirostane (22): A dichloromethane (20 mL) solution of 3-acetoxy-5α-spirost-2-ene (4) (910 mg; 2 mmol) and m-chloroperbenzoic acid (520 mg; 3 mmol) was stirred at room temperature for 1 hour. Into this reaction mixture 10% sodium sulfite (20 mL) was added and reaction mixture was stirred for additional 20 minutes. The reaction mixture was extracted with ether (3x30 mL). The combined ether extracts were washed with water (3x20 mL) dried over anhydrous magnesium sulfate and evaporated to give 800 mg (85%) pure epoxide 22. Selected data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.38 (q, $J = 11.2$ Hz, 1H), 3.47 (m, 1H), 3.37 (t, $J = 10.8$ Hz, 1H), 3.25 (d, 1H), 2.08 (s, 3H), 3.25 (d, 1H), 0.96 (d, $J = 3.4$ Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J = 3.2$ Hz, 3H), and 0.76 ppm (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2, 81.0, 71.5, 67.0, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7, 12.5 ppm.

Preparation of 2α-acetoxy-5α-spirostan-3β-ol (8): A tetrahydrofuran (10 mL) and methanol (5 mL) solution of 2α-acetoxy-5α-spirostan-3-one (23) (350 mg; 0.8 mmol) CeCl$_3$·7H$_2$O (373, 1 mmol) was stirred at room temperature for 10 minutes prior addition of sodium borohydride (60 mg; 1.6 mmol). The reaction mixture was stirred for additional 30 minutes following sodium borohydride addition. The reaction mixture was acidified with 5% hydrochloric acid and extracted with ether (3x20 mL). Combined ether extracts were dried over anhydrous magnesium sulfate and evaporated to give pure product in 90% isolated yield (8). Selected data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, $J = 4.8$, 9.4, 11.6 Hz, H-2b), 4.38 (q, $J = 11.2$ Hz, 1H), 3.59 (1H, ddd, $J = 4.7$, 9.0, 11.5 Hz, 1H), 3.47 (m, 1H), 3.37 (t, $J = 10.8$ Hz, 1H), 2.08 (s, 3H), 0.96 (d, $J = 3.4$ Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J = 3.2$ Hz, 3H), 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2, 162.5, 81.0, 71.5, 67.0, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7, 12.5 ppm.
35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7, 12.5 ppm. [Physical properties of our compound are in agreement with literature 28-31].

**Preparation of 5α-spirostan-2α, 3β-diol (25):** A methanol (2 mL) and dichloromethane (10 mL) solution of 2α-acetoxy-5α-spirostan-3β-ol (8) (475 mg; 1 mmol) and sodium hydroxide (0.12 g; 3 mmol) was stirred at room temperature overnight. Into the reaction mixture the acidic resin-dowex (1g) was added and this suspension was stirred at room temperature for 5 minutes. The resin was separated by filtration and the filtrate was evaporated to result in 368mg (85%) of pure product (25). Selected data: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.38 (q, $J$ = 11.2 Hz, 1H), 3.59 (1H, ddd, $J$ =4.7, 9.0, 11.5 Hz, 1H), 3.47 (m,1H), 3.40(1H, ddd, $J$ = 5.1, 8.9, 10.9 Hz, 1H) 3.37 (t, $J$ = 10.8 Hz, 1H), 0.96 (d, $J$ = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J$ = 3.2 Hz,3H), and 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 81.0, 72.5, 72.0, 71.5, 67.0, 62.3, 56.5,54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

**5α-Spirost-2-ene (10):** NaH (5.8 mg, 0.24 mmol) was stirred in tetrahydrofuran (10 mL). Tigogenin (9) (100 mg, 0.24 mmol) was added and stirred at room temperature for 30 minutes. Carbon disulfide (130 mg, 1.7 mmol) was added and stirred another 2 hours. 3-Bromopropyne (140 mg, 1.2 mmol) was added and the mixture was stirred an additional 3 hours at room temperature. The reaction was quenched with saturated NH$_4$Cl (40 mL), extracted with dichloromethane (3x50 mL), and the organic layers were combined and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (1:1 ethyl acetate: hexanes) to give the propargyl 5α-spirostan-3β-yl
xanthate, which was used immediately in the following reaction. Selected $^1$H NMR of the xanthate (500 MHz, CDCl$_3$): $\delta$ 5.50 (m, 1H), 4.40 (q, $J$ = 11.3 Hz, 1H), 3.85 (s, 1H), 3.82 (s, 1H), 3.48 (m, 1H), 3.38 (t, $J$ = 11.0 Hz, 1H), 0.97 (d, $J$ = 3.5 Hz, 3H), 0.88 (s, 3H), 0.80 (d, $J$ = 3.0 Hz, 3H), and 0.77 ppm (s, 3H). Trifluoromethanesulfonic acid (1.0 mL, 11 mmol) and 2,4,6-trimethylpyridine (1.5 mL, 11 mL) were stirred at 0°C in diethyl ether (5 mL). A portion of the resulting sulfonate salt (7.0 mg, 0.02 mmol) was transferred to a solution of propargyl 5α-spirostan-3β-yl xanthate (0.24 mmol) in toluene (10 mL). The reaction was refluxed 30 hours, cooled to room temperature, and the solvent was removed under reduced pressure. The resulting solid was purified by flash column chromatography (1:10 ethyl acetate: hexanes) to give 9 as a white solid (88 mg, 91 % yield over 2 steps from 2). Selected $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.56 (m, 2 H), 4.37 (q, $J$ = 11.2 Hz, 1H), 3.45 (m, 1H), 3.35 (t, $J$ = 10.4 Hz, 1H), 0.94 (d, $J$ = 3.8 Hz, 3H), 0.78 (s, 3H), 0.75 (s, 3H), and 0.75 (d, $J$ = 3.2 Hz, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 126.0, 109.4, 81.0, 68.0, 67.0, 62.4, 56.5, 54.2, 41.8, 41.6, 40.6, 40.2, 39.9, 35.4, 34.9, 32.2, 32.1, 31.9, 31.6, 30.5, 29.9, 29.0, 28.8, 20.9, 17.3, 16.6, 14.7 and 11.9 ppm. Alternatively, 10 was also prepared from 9 via methyl 5α-spirostan-3β-yl xanthate. Sodium hydride (10 mg, 0.42 mmol) was suspended in tetrahydrofuran (10 mL). Tigogenin (9) (100 mg, 0.24 mmol) was added and stirred at room temperature 30 minutes. Carbon disulfide (130 mg, 1.7 mmol) was added and stirred another 2 hours. Methyl iodide (170 mg, 1.2 mmol) was added and the mixture was stirred an additional 3 hours at room temperature. The reaction was quenched with saturated NH$_4$Cl (40 mL), extracted with dichloromethane (3x50 mL), and the organic layers were combined and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (1:15 ethyl acetate: hexanes) to give methyl 5α-spirostan-3β-yl xanthate, which was used immediately in the following reaction.
Selected $^1$H NMR of methyl 5α-spirostan-3β-yl xanthate (400 MHz, CDCl$_3$): δ 5.47 (m, 1H), 4.37 (q, $J = 11.4$ Hz, 1H), 3.45 (m, 1H), 3.35 (t, $J = 10.8$ Hz, 1H), 2.52 (s, 3H), 0.94 (d, $J = 3.6$ Hz, 3H), 0.85 (s, 3H), 0.77 (d, $J = 3.2$ Hz, 3H), 0.74 (s, 3H) ppm. Methyl 5α-spirostan-3β-yl xanthate was heated at 200°C for 2 hours under reduced pressure. The resulting solid was purified by flash column chromatography (1:10 ethyl acetate: hexanes) to give (10) as a white solid (90 mg, 94 % yield). [Preparation was performed by modification of general procedures for preparation of olefins from alcohols $^{32}$].

**Preparation of 2α,3α-epoxy-5α-spirostone (11):** A dichloromethane solution of 5α-spirost-2-ene (10) (4.44 g; 11.15 mmol) and $m$-chloroperbenzoic acid (2.12 g; 12.3 mmol) was stirred at room temperature for 30 minutes. Aqueous (5%) sodium sulfite (100 mL) was added and the mixture was stirred for an additional 15 minutes. The organic layer was separated and the aqueous phase was extracted with dichloromethane (3x100 mL). Combined organic layers were washed with saturated sodium bicarbonate (3x30 mL), followed by brine (3x30 mL), and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the resulting solid was purified by flash column chromatography in hexane-ethyl acetate (5:1) to give 4.20 g (91 %) of white crystalline product. Selected $^1$H NMR (400 MHz, CDCl$_3$): δ 4.36 (q, $J = 11.2$ Hz, 1H), 3.45 (m, 1H), 3.35 (t, $J = 10.8$ Hz, 1H), 3.13 (br s, 1H), 3.09 (m, 1H), 0.94 (d, $J = 3.6$ Hz, 3H), 0.77 (d, $J = 3.4$ Hz, 3H), 0.75 (s, 3H), 0.73 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): δ 109.4, 80.9, 67.0, 62.3, 56.3, 53.8, 52.5, 51.1, 41.8, 40.5, 40.1, 38.4, 36.4, 35.4, 33.9, 32.0, 31.9, 31.5, 30.4, 29.2, 29.0, 28.5, 20.8, 17.3, 16.5, 14.7 and 13.1 ppm.
Preparation of 3α-hydroxy-5α-spirostan-2β-yl acetate (12) and 2β-hydroxy-5α-spirostan-3α-yl acetate (13): An acetic acid (10 mL) solution of epoxide 11 (100 mg; 0.24 mmol) was refluxed for 2 hours. The solution was co-evaporated with toluene (3x5 mL) and the resulting solid was purified by gravity column chromatography in hexane-ethyl acetate (4:1) to give 12 (70 mg; 64%) and 13 (19 mg; 17%). Selected 1H NMR for 12 (400 MHz, CDCl3): δ 4.87 (br s, 1H), 4.38 (q, J = 11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), and 0.75 ppm (s, 3H). 13C NMR (100 MHz, CDCl3): δ 170.5, 109.5, 81.0, 73.3, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 21.0, 17.6, 16.8, 14.7, and 13.9 ppm. Selected 1H NMR for 13 (400 MHz, CDCl3): δ 4.78 (m, 1H), 4.39 (q, J = 11.2 Hz, 1H), 4.10 (br s, 1H), 3.66 (m, 1H), 3.38 (t, J = 10.8 Hz, 1H), 2.09 (s, 3H), 1.06 (s, 3H), 0.96 (d, J = 3.4 Hz, 3H), 0.79 (d, J = 3.0 Hz, 3H), 0.77 (s, 3H) ppm. 13CNMRof 12 (100 MHz, CDCl3): δ 170.3, 109.4, 81.0, 75.7, 68.8, 67.0, 62.3, 56.3, 55.3, 45.6, 43.1, 41.7, 40.8, 40.1, 35.6, 34.6, 32.2, 31.9, 31.5, 30.4, 28.9, 28.7, 28.2, 21.5, 21.2, 17.3, 16.7, 14.7 and 14.6 ppm.

Preparation of 2β-acetoxy-5α-spirostan-3α-yl methylsulfonate (14): A dichloromethane (15 mL) solution of acetate 12 (474 mg; 1 mmol), methanesulfonyl chloride (0.15 mL; 1.5 mmol), and triethylamine (0.25 mL; 1.75 mmol) were stirred at 0°C under nitrogen atmosphere for 2 hours. Ice cold hydrochloric acid (1M, 50 mL) was added. The organic layer was separated and the aqueous layer was extracted with dichloromethane. Combined organic layers were washed with water (3x20 mL), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give 458 mg (83%) of 14 that was used in next reaction without further purification. Selected 1H NMR (400 MHz, CDCl3): δ 4.99 (br s, 1H), 4.68 (br s, 1H), 4.40 (q, J = 11.6 Hz,
1H), 3.48 (m, 1H), 3.37 (t, J = 11.1 Hz, 1H), 3.08 (s, 3H), 0.96 (d, J = 3.5 Hz, 3H), 0.94 (s, 3H), 0.79 (d, J = 3.2 Hz, 3H), and 0.76 (s, 3H) ppm.

Preparation of 3β-hydroxy-5α-spirostan-2β-yl acetate (15) and 2β-hydroxy-5α-spirostan-3β-yl acetate (16): A pyridine solution (20 mL) of sulfonate 14 (50 mg; 0.09 mmol) and water (5 mL) was refluxed for 7 hours. The solvent was removed under reduced pressure and the product was co-evaporated with toluene (3x5 mL) and purified by silica gel flash column chromatography in hexane-ethyl acetate (4:1 → 1:1) to give 15 (87%) and 16 (10%) as white solids. Selected 1H NMR of 15 (400 MHz, CDCl3): δ 5.14 (br s, 1H), 4.39 (q, J = 11.4 Hz, 1H), 3.69 (m, 1H), 3.47 (m, 1H), 3.37 (t, J = 10.8 Hz, 1H), 2.10 (s, 3H), 0.96 (d, J = 3.4 Hz, 3H), 0.93 (s, 3H), 0.79 (d, J = 3.0 Hz, 3H), 0.76 (s, 3H) ppm. Selected 1H NMR of 16 (400 MHz, CDCl3): δ 4.78 (m, 1H), 4.39 (q, J = 11.2 Hz, 1H), 4.10 (br s, 1H), 3.66 (m, 1H), 3.38 (t, J = 10.8 Hz, 1H), 2.09 (s, 3H), 1.06 (s, 3H), 0.96 (d, J = 3.4 Hz, 3H), 0.79 (d, J = 3.0 Hz, 3H), 0.77 (s, 3H) ppm.

Preparation of diluted compounds: None of the compounds tested over the course of these studies were soluble in water. Therefore, DMSO was used to dilute each compound tested. Master stock concentrations of drugs were prepared to ensure that the maximum final concentration of DMSO in tested antifungal solutions was 1% or less. Subsequent 2-fold serial dilutions were made using RPMI 1640 broth or sterile water to a final concentration of 1280-0.1 μg/mL. A final 10-fold dilution of each drug was made by aliquotting 0.1 mL of each dilution to 0.9 mL of inoculating media (see preparation below), giving final drug concentrations tested in
the range of 128-0.01 ug/mL. All drug controls used were diluted to 1.0 ug/mL concentrations, according to the manufacturer’s instructions.
3.9 References:


26. Johns, W. Patent 2875201, **1959**.


CHAPTER-4: SYNTHESIS AND ANTIFUNGAL EVALUATION OF SPIROSTANE SAPONINS

Spirostane saponins are an important class of natural products, which probably best known as starting materials for synthesis of various steroidal hormones. Spirostane saponins are composed of an aglycone moiety (also referred to as steroidal sapogenin) and a sugar chain of one or more monosaccharides. There are at least 90 plant families containing such compounds, particularly in monocotyledons\(^1\). The antifungal activity of spirostane saponins, particularly against agricultural pathogens has been known for long time\(^2-4\), while other reported activities of this class of compounds include antitumour, hypoglycemic, immunoregulatory, and cardiovascular disease prevention and treatment\(^1\). Saponins exist in the plants in such a complicated manner that one plant species normally contains more than several dozen structurally similar saponins. Isolation of a single pure saponin from plants, especially in considerable amounts, is extremely difficult. Consequently, many saponin extracts from herbs have been directly used to treat various human diseases without evaluation of the pharmacological activities of each component. Chemical synthesis would provide a realistic way to obtain homogeneous saponins and therefore opportunities for understanding the actions of a saponin on the human body.

Our specific aim towards finding high potency antifungal saponin focused on synthesis of various saccharide derivatives of the most active steroid derivatives identified so far. In order to explore Structure Activity Relationship (SAR) study it was decided to synthesize mono-, di- and tri-saccharide saponins with a variety of sugar linkages. It was planned to synthesize linear as well as branched oligosaccharide in order to find out ideal linkage between sugar units for better
antifungal activity. Developed synthesis for glycoside derivatives of steroid could be used for

total synthesis of various naturally occurring antifungal saponins including CAY-1.

4.1 Synthesis of glycosides and saccharides:

Glycoside syntheses in past years were initially focused mainly on the improvements of
the well-known Koenigs-Knorr method, introduced in 1901, which requires an exchange of the
anomeric hydroxyl group by bromine or chlorine as the first step (generation of glycosyl-group
donor). The second step involves glycosyl group transfer to the glycosyl acceptor in the presence
of a heavy metal ion promoter (Scheme-4.1, path B). Several inherent disadvantages associated
with this method makes it often experimentally demanding and certainly unsuitable for large–
scale synthesis. For example it requires at least equimolar amounts of the heavy metal salt
promoter, often incorrectly termed as “catalyst,” is a limiting factor\(^5\)-\(^7\). Hence, alternative
methods are of interest in order to overcome these shortcomings.

The Fischer-Helferich method, as a direct anomeric-oxygen replacement reaction
(Scheme-4.1, method A), has been very successfully applied for the synthesis of simple alkyl
glycosides. However, because of its reversibility, it has not gained general importance in the
synthesis of complex oligosaccharides and glycoconjugates\(^6\)-\(^7\).

The requirements for glycoside syntheses, high chemical and stereochemical yield, and
applicability to large-scale preparations were not effectively met by any of the methods just
described. However, the general strategy for glycoside synthesis seemed to be reasonable. Only
simple means of meeting these requirements will lead to a generally acceptable and useful
methodology. Therefore, besides acid activation (Scheme-4.1, A and B), the simplest form of
activation would be base activation generating first as anomeric alkoxide structure of pyranose or
furanose (Scheme-4.1, paths C and D). This method was very interesting as nature has a similar
approach for generating glycosyl donors, namely glycosyl phosphate formation\(^8\,^9\).

In beginning, direct anomeric \(O\)-alkylation seemed very unlikely to fulfill all the
requirements for glycoside and saccharide synthesis. Although all remaining functional groups
are protected, the ring-chain tautomerism between the anomeric forms and open side chain form
(Schem-4.1, path C) already gives three possible sites for attack of the alkylating agent. Base
catalyzed elimination in the open-chain form of the sugar could be a destructive side reaction.
Therefore, the yield, regioselectivity and stereoselectivity of such direct anomeric \(O\)-alkylation
would not be expected to be very good.
The desired formation for the stable anomeric $O$-activated inetermediates via base catalysis requires system for reactivity in the subsequent glycosylation step. Therefore, after base-promoted trapping of anomeric $O$-activated inetermediates, mild acid treatment in the presence of acceptors, leads to formation of glycosides in an irreversible manner (Scheme-4.1, Path D) $^5,9$. Electron-deficient nitriles, such as trichloroacetonitrile and trifluoroactonitrile are known to undergo direct and reversible, base catalyzed addition of alcohols providing $O$-alkyl trichloroacetimidates $^{10}$. This imidate synthesis has the advantage that the free imidates can be isolated as stable adducts, which are less sensitive to hydrolysis than corresponding salts.

Scheme 4.2 Schmidt’s trichloroacetimidate method.$^{11}$

A detailed study of addition of trichloroacetonitrile to 2,3,4,6-tetra-$O$-benzyl-D-glucose revealed that from equatorial 1-oxide ion, the $\beta$-trichloroacetimidate is generated preferentially or even exclusively in a very rapid and reversible addition reaction (Schem-4.2) $^5,7$. However, this product anomerizes in a slow, base catalyzed reaction (via retroreaction, anomerization of 1-oxide ion, and renewed trichloroacetonitrile addition) to the $\alpha$-trichloroacetimidate having electron withdrawing 1-substituent in an axial position as favored by the thermodynamically operating anomeric effect$^{11}$. Thus, with different bases for example $K_2CO_3$, $Cs_2CO_3$, $NaH$ and 1,8-diazobicyclo[5,4,0]undec-7-ene(DBU) both $O$-activated anomers may be isolated in pure
form and high yields via kinetic and thermodynamic reaction-control. Both anomers are commonly thermally stable and may be easily stored. Therefore if we wish to form thermodynamic product the base catalyzed reaction must go for longer period of time in order to anomerize kinetic product.

4.2 **Anomeric effect:** Anomeric effect is defined as the preference of an electronegative substituent at the anomeric position of a carbohydrate to be axially rather than equatorially oriented. There are two most accepted explanations for anomeric effect being used over the years.

![Figure 4.1. The anomeric effect](image)

One explanation for the anomeric effect is that there is less lone pair interaction between the substituent and the ring oxygen atom for gauche conformations (Figure 4.1).

A second explanation is that the dipoles generated by the substituent and the ring oxygen atom is in opposing directions in the axial anomer and they are in similar directions in the equatorial anomer\textsuperscript{11,12}. Finally, the most widely accepted explanation for the anomeric effect is the stabilizing effect created by hyperconjugation between the axial lone pair on the ring oxygen atom and the $\sigma^*$ orbital of an axial heteroatom substituent on the anomeric carbon (Fig. 4.2). This type of stabilization by hyperconjugation is not possible when the substituent is equatorial.
4.3 Glycosylation method for oligosaccharides:

Many well-developed glycosylation methods for oligosaccharide synthesis follow the same general mechanism (Scheme 4.1), in the presence of a promoter; the leaving group of the glycosyl donor is freed from the molecule\textsuperscript{5,7, \textsuperscript{13-14}. The structure of the oxocarbenium intermediate formed depends on the nature of the C-2 substituent of the glycosyl donor. Participating groups, such as alkyl or aryl esters and amides, can help stabilize the intermediate and tend to favor the formation of 1,2-\textit{trans}-glycosides (Scheme 4.3). It is important to note that in this case a side reaction occurs to form an orthoester. Even though orthoester formation is reversible, it can still have a deleterious effect on the glycosylation depending on the nature of the glycosyl donor and acceptor and the reaction conditions.

Glycosyl donors with non-participating groups at C-2, such as ethers, called armed donors, react faster than those with participating groups at C-2 (disarmed donors). This is due to the fact that the C-2 substituent of an armed donor is less electron withdrawing than that of a disarmed donor and therefore the oxocarbenium intermediate forms faster (Scheme 4.3). In this case, the stereoselectivity of the glycosylation is largely solvent dependent.
The trichloroacetimidate glycosylation method was selected due to the fact that it is generally high yielding and highly stereoselective even in complex oligosaccharide syntheses\(^{15-18}\). The trichloroacetimidate donors are formed by the reaction of the anomeric alcohol with trichloroacetonitrile under basic conditions. Strong bases, such as sodium hydride, and longer reaction times favor the formation of the thermodynamically more stable \(\alpha\)-trichloroacetimidate.

The stereoselectivity of the donor is important for S\(\text{N}_2\) type glycosylations, which can be favored for the trichloroacetimidate method by using weaker promoters, such as BF\(_3\)·OEt\(_2\)\(^8\). When starting trichloroacetimidate is a mixture of the two anomers, the stereochemical outcome of the reaction depends on the nature of neighboring group participation. Not surprisingly, donors with neighboring participating group give only corresponding 1,2-\textit{trans} products, whilst
donors without a neighbouring group give mixture of the α- and β-anomeric glycosides.

Yu et al. have reported synthesis of trifluoroacetimidate and demonstrated to be effective glycosyl donors in the presence of TMSOTf\(^{20}\). Their method utilized 2,3,4,6-tetra-O-benzoyl-α-D-glucopuranosyl trichloroacetimidate as glycosyl donor and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.05equivalent) as a promoter that produces the corresponding saponin quantitatively. (Scheme 4.4)

No benzoyl groups transfer products or ortho-esters were detected. The newly formed glycosidic bonds were proved to be exclusively 1,2-trans on the basis of their \(^1\)H NMR spectra\(^{19}\). They have also reported condensation of glycosyl trichloacetimidate with cholesterol in high yield and selectivity. The same methodology was applied to the synthesis of a trisaccharide saponin, dioscin.\(^{20}\)

Scheme 4.4. Glycosylation of sapogenins reported by Yu et al.\(^{20}\)
4.4 Synthesis of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate:

Our synthesis of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate started with α-D-glucose, all hydroxyl groups were protected with benzoyl group with well-known esterification by benzoyl chloride in pyridine to get compound 7. The anomeric benzoyl group was selectively deprotected by mild base ethanolamine in ethylacetate and dimethylsulfoxide to get anomeric free hydroxyl group in compound 8 (Scheme 4.5) [21]. Base catalysed activation of anomeric oxygen in presence of trichloroacetonitrile in dichloromethane gave the desired compound in almost quantitative yield [22, 23]. The last step of reaction took 48hrs as we desired to make α-trichloroacetimidate which is the thermodynamic product, as was discussed previously that the kinetic product of this reaction β-trichloroacetimidate formed preferentially. However, this product anomerizes in a slow, base catalyzed reaction to the α-trichloroacetimidate having electron withdrawing 1-substituent in an axial position as favored by the thermodynamically operating anomeric effect. (Scheme 4.2)

![Scheme 4.5](image)

**Scheme 4.5** Synthesis of 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate: i) Pyridine, benzoyl chloride, reflux; ii) Ethanolamine, DMSO, EtOAc; iii) Trichloroacetonitrile, K₂CO₃, DCM, RT, 48hrs.

Synthesis of monosaccharide trichloroacetimidate was achieved in almost quantitative yield and our next task was to use developed synthesis for synthesis of various disaccharide trichloroacetimidates.
4.5 Retroanalysis of saccharide moiety of saponin CAY-1:

Synthesis of (1-3)-β linked disaccharide trichloroacetimidate was our first priority as CAY-1 oligosaccharide moiety has a (1-3)-β linked disaccharide attached to the central glucose. Retroanalysis of pentasaccharide moiety of CAY-1 was done in order to emphasize importance of having synthetic methodology for (1-3)-β linked disaccharide trichloroacetimidate, which could be attached directly to central sugar unit in its convergent synthesis (Scheme 4.6). The developed synthesis will give an edge over for the convergent synthetic approach for synthesis of branched oligosaccharides with similar skeleton as CAY-1 oligosaccharide moiety.

Scheme 4.6 Retro-analysis of CAY-1 Pentasaccharide Moiety
4.6 Synthesis of (1→3)-β linked disaccharide trichloroacetimidate:

Synthesis of (1→3)-β linked disaccharide trichloroacetimidate utilizes glycosylation of 2,3,4,6-tetra-O-benzoyl-α-D-glucopuranosyl trichloroacetimidate with diacetone-D-glucose, 9 in presence of acid catalyst BF$_3$.OEt$_2$ to give the coupled product 10. In presence of trifluoroacetic acid and water the diacetone-protecting group was cleaved followed by rearrangement to give partially benzoyl protected disaccharide compound 11 (Scheme 4.7, step ii). Benzoyl protection of 11 was achieved by pyridine and benzoyl chloride under reflux conditions to get octa-benzoylated disaccharide compound 12. Anomeric benzoyl group was cleaved by mild base ethanolamine in ethyl acetate and DMSO to get compound 13 in 85% of isolated yield after column purification. Anomeric oxygen of 13 was then activated by base catalyzed reaction with trichloroacetonitrile to give the disaccharide trichloroacetimidate compound 14 in quantitative yield. (Scheme 4.7)

Scheme 4.7 Synthesis of (1-3)-β linked disaccharide trichloroacetimidate: i) BF$_3$.OEt$_2$, DCM; ii) TFA, THF/H$_2$O; iii) pyridine, benzoyl chloride, reflux; iv) ethanolamine, DMSO, EtOAc; v) trichloroacetonitrile, K$_2$CO$_3$, DCM, room temperature, 48hrs.
4.7 Synthesis of various disaccharide trichloroacetimidates:

After accomplishing the synthesis of mono and disaccharide trichloroacetimidate we utilized this methodology to synthesize various other disaccharide trichloroacetimidate starting from commercially available simple disaccharide molecules like maltose, lactose, cellobiose, and melibiose (Scheme 4.8). The idea behind synthesis of various disaccharide units and attach them to steroidal part in order to extensively explore SAR study and see what kind of linkage between two sugars would be needed to achieve best antifungal activity. Synthesis of a variety of disaccharide trichloroacetimidate will also provides us an opportunity to synthesis a wide variety of branched oligosaccharide which might be needed for antifungal activity as in case of antifungal saponin CAY-1, which was not evident at this stage of project.

Scheme 4.8 Synthesis of various disaccharide trichloroacetimidates: i) Pyridine, benzoyl chloride, reflux; ii) Ethanolamine, DMSO, EtOAc; iii) Trichloroacetonitrile, K₂CO₃, DCM, RT, 48hrs.
4.8 General methodology for Glycosylation:

Glycosylation of trichloroacetimidates with various steroid derivatives synthesized previously was the next objective in our mind. Since we had no idea how many sugar units were optimum to achieve best antifungal activity, it was decided to start attaching one sugar unit first then two or three sugar units depending on the assay results. In order to have a fare SAR study and comparison between three different steroidal derivatives, it was decided that similar glycoside derivatives will be synthesized for all three steroids in order to comparative study of their antifungal activity.

Glycosylation was achieved in general by TMSOTf catalyzed reaction in dichloromethane of activated sugar trichloroactimidate and steroidal derivative with free hydroxyl group at either C-2 or C-3 positions (Scheme 4.9). The benzoyl protection of sugar was removed after glycosylation reaction by sodium methoxide in methanol and dichloromethane mixture to get the desired saponin product after column purification.

Scheme 4.9 Glycosylation for synthesis of Steroidal saponin: a general scheme.
4.9 Monosaccharide derivatives of steroids:

Antifungal evaluation of glycosides linked steroid derivatives was initially done with monosaccharide derivatives and a significant increase in antifungal activity was observed compared to corresponding steroid derivatives. Monosaccharide derivatives of spirostane were far more active than corresponding cholestane and androstane derivatives. Of the eleven monosaccharide derivatives of various steroids tested, 21, 24, 27 and 28 were not significantly active against all four species tested, all other monosaccharide derivatives were active against at least two of the fungal strains. The C-2 substituted monosaccharide derivative 29 (with same steroidal unit as compound 28) was found to be active against C. albicans, C. glabrata and C. neoformans. (Table 4.1)

![Figure 4.3 Monosaccharide derivatives of steroids](image-url)
Best activity among monosaccharide derivatives was observed with spirostane-based compound 22, 25, 26 and 30 ranging from 2 to 16 µg/mL (around 50% inhibition). Cholestane and androstane based monosaccharide derivatives were had lesser activity (32µg/mL and above-25 to 50% inhibition) compared to corresponding spirostane derivatives (Table 4.1). Motivated by better activity with monosaccharide derivatives of steroids we decided to explore the effect of increased sugar chain length on efficacy of these series of compounds. The next step of our project at this moment was to synthesize various disaccharide derivatives of steroids and test them for antifungal activity against all the four pathogenic fungal strains.

**4.10 Disaccharide derivatives of steroids:**

Disaccharide derivatives of various steroid isomers were synthesized and evaluated against all four pathogenic fungal strains. Most of the disaccharide derivatives of steroids were more active than corresponding monosaccharide derivatives with exception of lactose based derivatives of steroids, which were having moderate activity and not much improvement from corresponding monosaccharide derivatives (Table 4.2). Probably the β- (1→4) linkage between galactose and glucose units in lactose didn’t provide the required orientation for interaction with fungal cell wall. Out of five different disaccharide derivatives synthesized most of them were active against at least two of the fungal strains but maltose linked spirostane derivatives seemed to be the most potent molecules so far (Fig. 4.3). These sets of assay results did emphasized on the kind of linkage between sugar units needed for better antifungal activity.

Maltose having α- (1→4) linkage between two glucose units seemed to be the best fit for antifungal activity amongst all other disaccharide derivatives synthesized and tested so far. Since we already had a bunch of very active antifungal maltose derivatives of various steroids, it was obvious to go a step further and see if attaching another sugar unit to maltose with α- (1→4) linkage
will increase the potency any further. In fact we were going in the right direction and it was amazing to find out that the four different derivatives of maltotriose synthesized (45, 46, 47 and 48) showed significant increase in antifungal activity (Table 4.3). There was a clear indication that the α- (1→4) linkage in these derivatives of steroids provided the desired orientation needed for interaction with fungal cell wall.

Figure 4.4. Disaccharide and trisaccharide derivatives of steroids
After having the promising results from linear disaccharide and trisaccharide derivatives of various steroid isomers we were also aimed to explore possibility of exploring branched oligosacchride derivatives which would be quite similar to the branched oligosacchride in various naturally isolated saponins like CAY-1. That would give us an idea about how having linear versus branched oligosaccharide unit attached to steroids affects the antifungal activity in order to explore a critical part of SAR studies towards finding potent antifungal saponins.

4.11 Synthesis of branched oligosacchride:

Synthesis of branched oligosaccharides started from penta-\textit{O}-Acetyl glucopyranose, which can be prepared from D-glucose in quantitative yield by acetic anhydride and pyridine reflux. Thioisopropyl group was introduced at anomeric position by acid catalyzed glycosylation with thioisopropanol. Acetyl group protection was cleaved by sodium hydroxide in methanol to yield compound 51 (step ii, Scheme 4.10). Dimethoxymethylbenzene under acidic conditions with camphor sulfonic acid in DMF leads to 4,6-diol protection to give the key intermediate 52 in good yield\textsuperscript{25,26}. At temperature below 0\degree C glycosylation of 52 with trichloroacetimidate activated monosaccharide sugar 1 with TMSOTf gave C-3 glycosylated disaccharide 53, which was glycosylated further using another mole of 1 at room temperature to get branched trisaccharide 54 (step iv, Scheme 4.10). Glycosylated 4, 6-\textit{O}-benzylidene-1-thio-\textit{\beta}-D-glucopyranoside (54) could be coupled with another sugar\textsuperscript{27} or steroid\textsuperscript{28} having acceptor unit by N-iodosuccinimide (2.5eq) promoter and acid catalyst TMSOTf (0.1-0.2eq) in dichloromethane. These literatures reported coupling reaction could be used to get complex branched oligosaccharides and their derivatives with various steroids\textsuperscript{27,28}.

There were some major issues with the glycosylation reaction (step iv, Scheme 4.10), which was the cause for low yield of reaction. The reaction was highly moisture sensitive, even
after using molecular sieves and dry solvent for the reaction trichloroacetimidate activated sugar (1) was found to be hydrolyzing back to compound 8 in presence of trace of moisture. All three spots on TLC were isolated and confirmed by $^1$H-NMR to prove the observation. Use of freshly prepared trichloroacetimidate activated sugar and freshly purchased TMSOTf did improve the yield of reaction to certain extent but not much for this methodology to be effective for large-scale synthesis of branched oligosaccharides.

Scheme 4.10 Synthesis of 2,3-branched trisaccharide: (i) propane-2-thiol, BF$_3$·OEt$_2$, DCM, (ii) NaOH, MeOH/DCM, (iii) dimethoxymethylbenzene, CSA, DMF, 51% (3 steps), (iv) 1, TMSOTf, DCM, 23% (2 steps).
In order to simplify the synthesis of branched oligosaccharides and develop methodology, which can be useful for their large-scale synthesis it was decided to changed our strategy. We propose to design TAG molecules which would be attached to sugar and hence change the physical property of sugars in order to simply handling of intermediate and easier monitoring of reaction. Tag molecules are large aromatic molecules, which on attachment to sugars would make them less soluble in common organic solvents hence easy to re-crystallize and purify. The detailed of synthesis of tag molecules and their attachment of them to sugar will be discussed in details in chapter-5.

4.12 Synthesis of fatty acid derivatives derivatives of steroids:

We also synthesized some fatty acid derivatives of active molecules from steroid series in order to see the effect of having long chain aliphatic ring attached at C-3 position of the steroids. This was inspired by the fact that there are some literature reports showing antifungal activity with the fatty acid derivatives of steroids. Synthesis of fatty acid derivatives of various steroid isomers was achieved by two methods, Method-A involved coupling to corresponding acid chloride of a fatty acid to the steroid unit while Method-B involved direct coupling of fatty acids by in situ activation of carboxylic acid group with DCC and base DMAP to steroid isomers (Scheme 4.11). Two compounds 55 and 58 were synthesized by Method-A in almost quantitative yield whereas four other fatty acid derivatives were synthesized using Method-B. Most of these compounds were re-crystallized by polar solvents like ethanol and acetonitrile.

It was not very surprising that most of the cholestane based fatty acid derivatives had solubility issue with our common solvent for micro-dilution, dimethylsulfoxide. The solubility issue was solved using a mixture of isopropyl alcohol and dimethylsulfoxide mixture as solvent
system for microdilution and compared with same solvent control. Only three out of six fatty acid derivative showed some antifungal activity, which was not very impressive. Compound 55 and 59 were having 25% inhibition at 62µg/mL concentration for *C. glabrata* whereas compound 60 had 40% inhibition at 32µg/mL for the same pathogenic fungus. Overall there is a need for more fatty acid derivatives to be explored in order to achieve better antifungal activity of these series of compounds. Synthesis and antifungal evaluation of fatty acid derivatives of steroids will give a nice comparison with glycosidic derivatives, therefore hydrophilic versus hydrophobic substitution on steroids and their impact on antifungal activity could be studied in detailed for a complete SAR study.

**Scheme 4.11** Fatty acid derivatives of steroids
4.13 Antifungal assay of steroidal saponins

The antifungal activity of the synthesized glycoside linked 2,3-spirostanes were evaluated in vitro using four species of fungi, including Candida albicans, Cryptococcus neoformans, Candida glabrata, and the filamentous fungus Aspergillus fumigatus. The results of these screenings are summarized in Table 4.1, 4.2 and 4.3. Of the eleven monosaccharide derivatives of various steroids tested, 21, 24, 27 and 28 were the compounds that showed no significant antifungal activity against all four species tested, other all other five monosaccharide derivatives were active against at least two of the fungal strains. The C-2 substituted monosaccharide derivative with same steroid 29 was found to be active against C. albicans, C. glabrata and C. neoformans (where more than 25% inhibition was observed in the highest dose tested 125µg/mL –Table 4). Best activity among monosaccharide derivatives was observed with spirostane-based compounds 22, 25, 26 and 30 ranging from 2 to 16 µg/mL (around 50% inhibition). Cholestane and androstane based monosaccharide derivatives had lesser activity (32µg/mL about-25 to 50% inhibition) compared to corresponding spirostane derivatives (Table-4.1).

Disaccharide derivatives of steroids with lactose did not improve the antifungal activity for almost all of the steroids, which forced us to look for some other disaccharide units to be attached to the steroids to improve their activity more than the monosaccharide units. We have synthesized other disaccharide-like maltose, cellobiose, melibiose- based derivatives of active series of steroids. Among these disaccharide derivatives maltose seemed to gave the best antifungal activity, for example compound 34 have an activity 8µg/mL for C. glabrata and 16µg/mL for C. albicans (100% inhibition). Among maltose derivatives of steroids spirostane based compounds are more active than corresponding cholestane and androstane compounds, for instance cholestane compound
33 have 25% inhibition at 125µg/mL with *C. albicans* and at 16µg/mL with *C. glabrata* (Table 4.2). Androstane based maltose derivative, 31 is only active with *C. albicans* at 62µg/mL (25% inhibition) and had no activity with *C. glabrata*. It was very interesting to find out that maltose derivative of commercially available diosgenin (39) also had moderate activity at 16µg/mL (25% inhibition) with *C. albicans* and 32µg/mL (50% inhibition) with *C. glabrata*.

Among the fatty acid derivatives of steroids three out of six had moderate activity, compound 55 and 59 had 25% inhibition at 62µg/mL for *Candida glabrata*. Fatty acid derivative of cholestane compound 60 was having 40% inhibition at 32µg/mL for *C. galbrata* (Table 4.3). The low to moderate activity of fatty acid derivatives might be due to their solubility issues, so there is need for more fatty acid derivatives to be synthesized and explored against pathogenic fungal strains in order to get a potent antifungal agent in this series of steroid derivatives.
**Table 4.1** Antifungal activities of novel steroid monosaccharide analogs (MIC in \(\mu g/mL\))

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC(_{50}) (µg/mL)(^a)</th>
<th>C. albicans(^b)</th>
<th>C. glabrata(^c)</th>
<th>C. neoformans(^d)</th>
<th>A. fumigatus(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Compound 1" /></td>
<td>NC*</td>
<td>16</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td><img src="image2.png" alt="Compound 2" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td><img src="image3.png" alt="Compound 3" /></td>
<td>2</td>
<td>2</td>
<td>0.125</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><img src="image4.png" alt="Compound 4" /></td>
<td>16</td>
<td>NC*</td>
<td>16</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image5.png" alt="Compound 5" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image6.png" alt="Compound 6" /></td>
<td>32</td>
<td>62</td>
<td>32</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td><img src="image7.png" alt="Compound 7" /></td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><img src="image8.png" alt="Compound 8" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image9.png" alt="Compound 9" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image10.png" alt="Compound 10" /></td>
<td>125</td>
<td>125</td>
<td>125</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td><img src="image11.png" alt="Compound 11" /></td>
<td>8</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)MIC values are reported only for compounds displaying 1) a prominent decrease in turbidity by visual comparison to the control wells containing no antifungal and 2) a >50% reduction in fungal growth compared to controls containing no antifungal, as measured spectroscopically by absorption at 530 nm.

\(^b\)ATCC no. 10231;\(^c\)ATCC no. 48435;\(^d\)ATCC no. 36556;\(^e\)ATCC no. 16424

\(^f\)All values were determined after incubation at 35°C for 48 h.

\(^\star\)denotes compounds that had 1) a slight reduction in turbidity to no change and 2) had less than a 25% reduction in growth compared to controls, as measured spectroscopically by absorption at 530 nm.
Table 4.2 Antifungal activities of novel steroids disaccharide analogs (MIC in µg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C. albicans&lt;sup&gt;b&lt;/sup&gt;</th>
<th>C. glabrata&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C. neoformans&lt;sup&gt;d&lt;/sup&gt;</th>
<th>A. fumigatus&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>62</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>32</td>
<td>16</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>8</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>16</td>
<td>125</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>&gt;125</td>
<td>&gt;125</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>32</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>16</td>
<td>125</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
<td>NC*</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>32</td>
<td>16</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>64</td>
<td>8</td>
<td>NC*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NC*</td>
<td>&gt;125</td>
<td>32</td>
<td>64</td>
</tr>
</tbody>
</table>
Table 4.3 Antifungal activities of steroid trisaccharide and fatty acid analogs (MIC in µg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C. albicans&lt;sup&gt;b&lt;/sup&gt;</th>
<th>C. glabrata&lt;sup&gt;c&lt;/sup&gt;</th>
<th>C. neoformans&lt;sup&gt;d&lt;/sup&gt;</th>
<th>A. fumigatus&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Compound" /></td>
<td>NC*</td>
<td>16</td>
<td>8</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image2.png" alt="Compound" /></td>
<td>NC*</td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image3.png" alt="Compound" /></td>
<td>NC*</td>
<td>&gt;125</td>
<td>&gt;125</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image4.png" alt="Compound" /></td>
<td>NC*</td>
<td>NC*</td>
<td>32</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><img src="image5.png" alt="Compound" /></td>
<td>NC*</td>
<td>62</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image6.png" alt="Compound" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image7.png" alt="Compound" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image8.png" alt="Compound" /></td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image9.png" alt="Compound" /></td>
<td>NC*</td>
<td>62</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
<tr>
<td><img src="image10.png" alt="Compound" /></td>
<td>NC*</td>
<td>32</td>
<td>NC*</td>
<td>NC*</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>MIC values are reported only for compounds displaying 1) a prominent decrease in turbidity by visual comparison to the control wells containing no antifungal and 2) a >50% reduction in fungal growth compared to controls containing no antifungal, as measured spectroscopically by absorption at 530 nm.

<sup>b</sup>ATCC no. 10231;<sup>c</sup>ATCC no. 48435;<sup>d</sup>ATCC no. 36556;<sup>e</sup>ATCC no. 16424

<sup>f</sup>All values were determined after incubation at 35°C for 48 h.

<sup>*</sup>denotes compounds that had 1) a slight reduction in turbidity to no change and 2) had less than a 25% reduction in growth compared to controls, as measured spectroscopically by absorption at 530 nm.
4.14 Conclusions

We have developed a synthesis for glycoside linked steroid derivatives from mono, di and trisaccharides. Antifungal assay of all synthesized derivatives against four pathogenic fungal strains was performed. Most of the monosaccharide derivatives were found to have better activity than corresponding un-substituted steroid units with the exception of compound 28, 20 which were not active against any of the tested fungal strains. Compound 22 among cholestane and 26 among spirostane monosaccharide derivatives were found to be most potent.

Disaccharide based saponins were found to have better antifungal activity than corresponding monosaccharide derivatives with an exception of lactose based derivatives (36,37 and 38), which were not active against any of the four fungal strains. Among the active disaccharide saponins the maltose derivatives (31-35 and 39) were found to be most potent compounds in this series so far for all the four fungal strains tested. Maltose derivative of 2,3-spirostanol had best activity across the board and with 0.5µg/mL activity for Cryptococcus neoformans.

Maltotriose derivatives showed a loss in activity over the corresponding disaccharide derivative (Maltose). Further studies are required in order to find the optimum number of sugar units needed in the saponin for the best antifungal activity. Synthesis and coupling of branched oligosaccharides with steroid isomers need to be explored in future works, to see the effect of branching in oligosaccharide unit on the antifungal activity of various saponins.

Fatty acid derivatives of active steroid isomers were also synthesized and assayed for antifungal activity. Out of six derivatives synthesized only three (55, 59 and 60) had mild antifungal activity. There is a need for a more extensive study of other possible fatty acid derivatives in order to find a potent antifungal molecule in this series of compounds.
4.15 Experimental

4.15.1 Synthesis of monosaccharide and disaccharide trichloroacetimidate: Synthesis of monosaccharide trichloroacetimidate was achieved starting from commercially available D-glucose in three steps in 75 to 80% of isolated yield. Our synthesis of 2,3,4,6-tetra-O-benzoyl-α-D-glucopuranosyl trichloroacetimidate started with α-D-glucose, all hydroxyl groups were protected with benzoyl group with well-known esterification by benzoyl chloride in pyridine to get compound 7. The anomic benzoyl group was selectively deprotected by mild base ethanolamine in ethylacetate and dimethylsulfoxide to get anomic free hydroxyl group in compound 8. Base catalysed activation of anomic oxygen in presence of trichloroacetonitrile in dichloromethane gave the desired monosaccharide trichloroacetimidate compound 1 in almost quantitative yield.

Penta-O-benzoyl-β-D-glucopyranose (7): D-glucose 6 (5.0 g, 28 mmol) was refluxed in pyridine (100 mL) for 1 hour. The solution was then added to benzoyl chloride (20 mL) which had been heated to 65°C. After the mixture was cooled to room temperature, water (400 mL) was added and stirred until the product solidified, approximately 30 minutes. The solid was filtered and washed with water and recrystallized from ethyl acetate to give 7 as a white solid (11 g, 56 % yield). $^1$H NMR (400 MHz, CDCl₃): $\delta$ 8.01 (d, $J = 4.0$ Hz, 4H), 7.89 (d, $J = 3.8$ Hz, 4H), 7.84 (d, $J = 3.9$ Hz, 2H), 7.53 – 7.21 (m, 15 H), 6.28 (d, $J=3.9$ Hz, 1H), 6.02 (t, $J = 9.3$ Hz, 1H), 5.83 (q, $J=12.9$ Hz, 2H), 4.64 (dd, $JI= 6.0$ Hz, $J2= 1.4$ Hz, 1H), 4.49 (dd, $JI = 8.1$ Hz, $J2 = 2.1$ Hz, 1H), 4.394 (m, 1H). $^{13}$C NMR (400 MHz, CDCl₃): $\delta$ 166.3, 165.8, 165.30, 165.28, 164.8, 134.0, 133.7, 133.6, 133.5, 133.3, 130.4, 130.0, 129.99, 129.96, 129.7, 128.9, 128.84, 128.82, 128.7, 128.62, 128.59, 128.5, 92.9, 73.3, 73.0, 71.0, 69.2, 62.8.
**2,3,4,6-Tetra-**O-**benzoyl-D-glucopyranose (8):** Pentabenzoyl-δ-D-glucopyranose 7 (10 g, 14 mmol) and freshly distilled 2-aminoethanol (2.0 g, 36 mmol) were stirred at room temperature in ethyl acetate (150 mL) and DMSO (2 mL) overnight, during which time the initial suspension formed a solution. The resulting solution was washed with water (3 x 150 mL) and the organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography (CH$_2$Cl$_2$ followed by Et$_2$O) to give 8 as a white solid (6.0 g, 70 % yield). 100 mg of the product was recrystallized from diethyl ether/hexanes for $^1$H and $^{13}$C NMR analysis.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J=9.9$ Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H). $^{13}$C NMR of 3 (CDCl$_3$): $\delta$ 166.6, 166.1, 166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.2, 129.6, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 90.6, 72.5 and 70.4 ppm.

**O–(Tetra-**O-**benzoyl-α-D-glucopyranosyl)-trichloroacetimidate (1):** Tetrabenzoyl- D-glucopyranose 8 (2.0 g, 3.3 mmol), K$_2$CO$_3$ (1.1 g, 8.2 mmol), and trichloroacetonitrile (5 mL) were stirred at room temperature in dichloromethane (20 mL) for 48 hours. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH$_2$Cl$_2$ and Et$_2$O (150 mL). The filtrate was concentrated to give 1 as a pale yellow solid (2.0 g, 83 % yield). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.62 (s, 1H) 8.02 (d, $J=3.9$ Hz, 2H), 7.93 (br d, $J=5.7$ Hz, 4H), 7.85 (d, $J=4.2$ Hz, 2H), 7.56 – 7.25 (m, 12H), 6.82 (d, $J=2.0$ Hz, 1H), 6.25 (t, $J=9.9$ Hz, 1H), 5.79 (t, $J$=9.9 Hz, 1H), 5.60 (dd, $J_1$ = 5.1 Hz, $J_2$ = 1.8 Hz, 1H), 4.62(brd,$J$=5.1Hz,2H),4.46(dd,$J_1$ =6.5Hz,$J_2$ =3.0Hz,1H). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ 166.2, 165.8, 165.6, 165.4, 160.7, 133.7, 133.5,
Tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-1,2,5,6-di-O-isopropylidene-α-D-glucofuranose (5): Trichloroacetimidate 1 (2.0 g, 2.7 mmol) and diacetone D-glucose 9 (650 mg, 2.5 mmol) were stirred under an atmosphere of nitrogen in anhydrous dichloromethane (20 mL) with 3Å molecular sieves for 1 hour. The solution was cooled in an ice bath for 30 minutes before BF$_3$·OEt$_2$ (2 mL) was added and stirred for 1 hour in the ice bath followed by 1 hour at room temperature. The solution was added to an ice-cold saturated aqueous sodium bicarbonate solution (100 mL) with vigorous stirring and then extracted with ether (2 x 75 mL). The organic layers were combined and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (CH$_2$Cl$_2$:THF 9:1 to 0:1) to give 10 as a white solid (1.1 g, 53 % yield). 1H NMR (400 MHz, CDCl$_3$): $\delta$ 8.03 (d, $J$ = 4.4 Hz, 2H), 7.91 (t, $J$ = 9.8 Hz, 4H), 7.80 (d, $J$ = 4.4 Hz, 2H), 7.57 – 7.26 (m, 12H), 5.89 (m, 2H), 5.66 (t, $J$=8.0Hz,1H),5.51(t,$J$=8.8Hz,1H),4.90(d,$J$=3.8Hz,1H),4.73(dd,$Jl$ =6.2Hz, $J2$ = 1.4 Hz, 1H), 4.42 (m, 2H), 4.28 (d, $J$ = 1.4 Hz, 1H), 4.16 (m, 1H), 4.07 (m, 2H), 4.01 (dd, $Jl$= 3.4 Hz, $J2$= 1.2 Hz, 1H), 3.84 (q, $J$ = 9.2 Hz, 1H), 1.36 (s, 6H), 1.26 (s, 6H). 13C NMR (400 MHZ, CDCl$_3$): $\delta$ 166.5, 166.0, 165.6, 165.4, 133.8, 133.7, 133.6, 130.1, 130.0, 129.6, 129.2, 128.9, 128.7, 128.6, 112.0, 105.3, 102.0, 85.2, 79.7, 75.8, 72.84, 72.77, 72.5, 72.2, 69.5, 69.4, 62.8, 27.0 and 26.4 ppm.

Tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-D-glucopyranose (11): Disaccharide 10 (3.2 g, 3.8 mmol) was stirred at 40°C in THF (10 mL) and a 1:1 mixture of trifluoroacetic acid and water (40 mL) for 24 hours. The solution was then added slowly to water (400 mL) with stirring.
The resulting solid was filtered and washed with water to give 11 as a white solid (2.5 g, 86 % yield). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.99 – 7.86 (m, 6H), 7.80 (d, $J$ = 3.8 Hz, 2H), 7.47 – 7.21 (m, 12H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.71 (t, $J$ = 9.6 Hz, 1H), 5.52 (t, $J$ = 9.2 Hz, 1H), 5.05 (d, $J$ = 1.6 Hz, 1H), 4.94 (d, $J$ = 4.0 Hz, 1H), 4.69 (t, $J$ = 12.8 Hz, 1H), 4.41 (m, 1H), 4.14 (m, 2H), 3.94 (m, 1H), 3.80 (m, 1H), 3.69 (t, $J$ = 9.6 Hz, 1H), 3.41 (m, 1H), 3.31 (m, 1H). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ 166.6, 166.0, 165.7, 165.3, 133.6, 133.5, 133.4, 130.0, 129.9, 129.5, 129.2, 129.2, 129.0, 128.9, 128.6, 128.5, 128.4, 128.4, 102.0, 101.9, 96.6, 92.5, 77.4, 76.4, 75.2, 74.7, 73.6, 73.1, 72.3, 72.2, 72.1, 70.8, 70.6, 70.4, 70.1, 69.9, 69.7 and 63.1 ppm.

**Tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-tetra-O-benzoyl-D-glucopyranose (12):**

Disaccharide 11 (2.5 g, 3.3 mmol) was refluxed in pyridine (50 mL) for 30 minutes. The solution was added to benzoyl chloride (10 mL) that had been heated to 65°C. After the mixture was cooled to room temperature, water (200 mL) was added. The resulting syrup was separated from the aqueous layer and dissolved in CH$_2$Cl$_2$ (50 mL). The solution was washed with water (3 x 50 mL), dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was crystallized from CH$_2$Cl$_2$/hexanes to give 12 as a white solid (3.0 g, 78 % yield). $^1$H NMR of 12 (400 MHz, CDCl$_3$): $\delta$ 8.12 – 7.77 (m, 32H), 7.62 – 7.15 (m, 48H), 6.69 (d, $J$ = 1.8 Hz, 1H), 6.19 (t, $J$ = 10.0 Hz, 1H), 6.14 (d, $J$ = 4.2 Hz, 1H), 5.92 (m, 2H), 5.71 – 5.41 (m, 7H), 5.08 (d, $J$ = 3.8 Hz, 1H), 5.00 (d, $J$ = 4.0 Hz, 1H), 4.58 (dd, $J_1$ = 6.0 Hz, $J_2$ = 1.6 Hz, 1H), 4.51 – 4.37 (m, 5H), 4.23 (m, 1H), 4.18 – 3.99 (m, 5H), 3.94 (dd, $J_1$ = 6.2 Hz, $J_2$ = 3.0 Hz, 1H), 3.86 (dd, $J_1$ = 6.4 Hz, $J_2$ = 3.0 Hz, 1H). $^{13}$C NMR of 7 (400 MHz, CDCl$_3$): $\delta$ 172.0, 166.3, 166.0, 165.8, 165.4, 164.9, 134.0, 133.6, 133.5, 133.4, 133.3, 130.4, 130.22, 130.16, 130.1, 130.0, 129.8, 129.5, 129.4, 129.0, 128.9, 128.8, 128.7, 128.6, 128.51, 128.48, 128.4, 101.1, 100.5, 92.9, 90.1, 75.4,
Tetra-\(O\)-benzoyl-\(\beta\)-D-glucopyranosyl-(1→3)-2,4,6-tri-\(O\)-benzoyl-D-glucopyranose (13):

Disaccharide 12 (3.4 g, 2.9 mmol) was stirred with freshly distilled 2-aminoethanol (500 mg, 8.277 mmol) and DMSO (0.5 mL) in ethyl acetate (50 mL) for 24 hours at room temperature. The mixture was washed with water (3 x 50 mL), dried over sodium sulfate, and concentrated. The resulting syrup was purified by flash column chromatography (CH\(_2\)Cl\(_2\) followed by Et\(_2\)O) to give 13 as a white solid (1.2 g, 40 % yield). \(^1\)H NMR of 13 (400 MHz, CDCl\(_3\)): \(\delta\) 8.08 – 7.77 (m, 14 H), 7.53 – 7.26 (m, 21 H), 6.11 (t, \(J = 10.0\) Hz, 1H), 5.90 (t, \(J = 9.6\) Hz, 1H), 5.69 (t, \(J = 9.6\) Hz, 1H), 5.49 (t, \(J = 10.0\) Hz, 1H), 5.39 (d, \(J = 1.8\) Hz, 1H), 5.28 (t, \(J = 10.4\) Hz, 1H), 5.10 (d, \(J = 1.8\) Hz, 2H), 4.95 (d, \(J = 3.8\) Hz, 1H), 4.71 (dd, \(J_1 = 6.2\) Hz, \(J_2 = 1.4\) Hz, 1H), 4.62 (m, 1H), 4.49 (m, 1H), 4.39 (dd, \(J_1 = 6.0\) Hz, \(J_2 = 2.2\) Hz, 1H), 4.13 (m, 2H). \(^{13}\)C NMR of 13 (400 MHz, CDCl\(_3\)): \(\delta\) 166.5, 166.0, 165.9, 165.7, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.1, 130.0, 130.0, 129.8, 129.7, 129.6, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 102.5, 90.4, 77.4, 72.8, 72.6, 72.4, 72.3, 70.2, 69.9, 69.5, 69.0 and 63.0 ppm.

\(O\)-(Tetra-\(O\)-benzoyl-\(\beta\)-D-glucopyranosyl-(1→3)-tri-\(O\)-benzoyl-\(\alpha\)-D-glucopyranosyl)-trichloroacetimdate (14): Disaccharide 13 (1.2 g, 1.2 mmol), potassium carbonate (0.42 g, 3.0 mmol), and trichloroacetonitrile (4 mL) were stirred 48 hours in CH\(_2\)Cl\(_2\) (20 mL) at room temperature. The resulting suspension was filtered through silica gel using a 1:1 mixture of CH\(_2\)Cl\(_2\) and Et\(_2\)O (150 mL). The filtrate was concentrated to give 14 as a pale yellow solid (1.3 g, 94 % yield) which was used immediately in the next reaction without further purification. \(^1\)H NMR of 14 (400 MHz, CDCl\(_3\)): 8.36 (s, 1H), 7.98 – 7.73 (m, 14H), 7.51 – 7.26 (m, 21H), 6.64
(d, J = 2.0 Hz, 1H), 6.14 (t, J = 9.8 Hz, 1H), 5.88 (t, J = 10.1 Hz, 1H), 5.63 – 5.32 (m, 4H), 4.98 (d, J = 4.1 Hz, 1H), 4.40 – 4.61 (m, 3H), 4.13 (m, 2H), 3.84 (m, 1H). $^{13}$C NMR of 14 (400 MHz, CDCl$_3$): δ 166.5, 166.0, 165.9, 165.7, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.1, 130.0, 130.0, 129.8, 129.7, 129.6, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 102.5, 90.4, 77.4, 72.8, 72.6, 72.4, 72.3, 70.2, 69.9, 69.5, 69.0 and 63.0 ppm.

**General procedure for glycosylation:**

Glycosylation was achieved in general by TMSOTf catalyzed reaction in dichloromethane of activated sugar trichloroactimidate and steroidal derivative with free hydroxyl group at either C-2 or C-3 positions (Scheme-below). The benzoyl protection of sugar was removed after glycosylation reaction by sodium methoxide in methanol and dichloromethane mixture to get the desired saponin product after column purification.

**4.15.2 General Procedure for monosaccharide derivatives:**

Synthesis of Tigogenyl-3β-glucose (25): Trichloroacetimidate glucosyl donor 1 (200 mg, 0.27 mmol) and steroidal acceptor tigogenin (100 mg, 0.27 mmol) were stirred under an atmosphere of nitrogen in anhydrous CH$_2$Cl$_2$ (10 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (15 µL, 0.1 mmol) was added. The reaction was stirred for 3 hours at 0°C to room temperature, quenched with triethylamine, and extracted with dichloromethane. The solvents were removed under reduced pressure and the resulting syrup was purified by gravity column chromatography (15% ethyl acetate: hexane) to give 175mg of benzoylated saccharide-coupled tigogenin(65% isolated yield). $^1$H NMR (400 MHz, CDCl$_3$): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, J=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H),
3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J = 10.8$ Hz, 1H), 3.12 (d, $J = 1.3$ Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J = 3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J = 3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Benzoylated monosaccharide coupled tigogenin (175mg,0.18mmols) was dissolved in Methanol and dichloromethane mix (1:1, 10ml) and freshly prepared sodium methoxide solution in methanol was added dropwise until pH 9-11 was achieved. Reaction mixture was stirred at room temperature for one hour. On completion of reaction the reaction mixture was neutralized by acidic Dowex resin to neutral PH and filtered off resin. Solvent was removed under reduced pressure and crude product was purified by column chromatography (Methanol:Dichloromethane:: 1:6) to get the 91mg of desired product 25 (90% yield). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J=10.8$ Hz, 1H), 3.12 (d, $J=1.3$ Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J=3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J=3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$119.6, 109.8, 82.9, 81.5, 81.0, 78.7, 76.8, 74.1, 71.5, 67.0, 66.3, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.9, 35.5, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

**Synthesis of Androstanyl-2α-hydroxy-3β-glucose (20):** Benzoyl protected 20: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J=9.9$ Hz, 1H), 5.72 (m, 2H), 4.82 (1H, ddd, $J = 4.8, 9.4, 11.6$ Hz, H-2b), 3.59 (1H, ddd, $J = 4.7, 9.0, 11.5$ Hz, H-2b), 2.12 (3H,
s, CH$_3$CO), 0.90 (3H, d, J = 6.5 Hz, CH$_3$-21), 0.87 (6H, two d, J = 6.5 Hz, CH$_3$-26 and CH$_3$-27), and 0.82 (3H, s, CH$_3$-19) ppm. $^{13}$C NMR δ: 171.6, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 109.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.4, 20.9, 18.6, 12.5 and 12.0 ppm.

**Androstanyl-2α-hydroxy-3β-glucose (20):** $^1$H NMR (400 MHz, CDCl$_3$): δ 5.03, 4.82 (1H, ddd, J = 4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, J = 4.7, 9.0, 11.5 Hz, H-2b), 0.90 (3H, d, J = 6.5 Hz, CH$_3$-21), 0.87 (6H, two d, J = 6.5 Hz, CH$_3$-26 and CH$_3$-27), and 0.82 (3H, s, CH$_3$-19) ppm. $^{13}$C NMR δ: 109.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.4, 20.9, 18.6, 12.5 and 12.0 ppm.

**Synthesis of Androstanyl-3β-glucose (21):** Benzoyl protected 21: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.19–7.82 (m, 8H), 7.57–7.19 (m, 12H), 6.23 (t, J=9.9 Hz, 1H), 5.72 (m, 2H), 4.82 (1H, ddd, J = 4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, J = 4.7, 9.0, 11.5 Hz, H-2b), 0.90 (3H, d, J = 6.5 Hz, CH$_3$-21), 0.87 (6H, two d, J = 6.5 Hz, CH$_3$-26 and CH$_3$-27), and 0.82 (3H, s, CH$_3$-19) ppm. $^{13}$C NMR δ: 171.6, 166.6, 166.1, 166.1, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.5, 128.4, 109.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.9, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.3, 20.9, 12.5 and 12.0 ppm.

**Androstanyl-3β-glucose (21):** $^1$H NMR (400 MHz, CDCl$_3$): δ 5.03, 4.82 (1H, ddd, J = 4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, J = 4.7, 9.0, 11.5 Hz, H-2b), 0.90 (3H, d, J = 6.5 Hz, CH$_3$-21),
0.87 (6H, two d, J = 6.5 Hz, CH₃-26 and CH₃-27), and 0.82 (3H, s, CH₃-19) ppm. $^{13}$C NMR δ: 109.5, 73.0, 68.1, 56.3, 56.2, 44.1, 42.5, 41.7, 39.8, 39.4, 39.3, 36.7, 36.1, 35.8, 34.7, 32.2, 31.7, 28.2, 27.9, 27.4, 24.1, 23.8, 22.8, 22.5, 21.4, 20.9, 18.6, 12.5 and 12.0 ppm.

**Synthesis of Cholestanyl-2α-hydroxy-3β-glucose (22):** Synthesis of benzyolated 22: $^1$H NMR (400 MHz, CDCl₃): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.82 (1H, ddd, $J$=4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, $J$=5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH₃CO), 0.84-0.90 (12H, CH₃-19, CH₃-21, CH₃-26, CH₃-27), and 0.64 ppm (3H, s, CH₃-18). $^{13}$C-NMR (CDCl₃): δ 171.6, 166.6, 166.1, 166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.4, 73.5, 56.2, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.4, 37.2, 36.1, 35.9, 35.7, 34.7, 31.7, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

**Cholestanyl-2α-hydroxy-3β-glucose (22):** $^1$H NMR (400 MHz, CDCl₃): δ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.82 (1H, ddd, $JZ4.8$, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, $JZ5.4$, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH₃-19, CH₃-21, CH₃-26, CH₃-27), and 0.64 ppm (3H, s, CH₃-18). $^{13}$C-NMR (CDCl₃): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.3, 18.6, 13.1 and 12.0 ppm.

**Synthesis of Cholestanyl-3β-glucose (23):** Synthesis of benzoylated 23: $^1$H NMR (400 MHz, CDCl₃): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.82 (1H, ddd, $JZ4.8$, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd,
JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): δ171.59, 166.6, 166.1, 166.1, 165.5, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.2, 54.1, 44.4, 42.5, 42.1, 39.8, 39.4, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

**Cholestanyl-3β-glucose (23):** $^1$H NMR (400 MHz, CDCl$_3$): δ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.3, 18.6, 13.0 and 12.0 ppm.

**Synthesis of Cholesteryl-3β-glucose (24):** Synthesis of benzoylated 24: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.38(d, 1H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): δ171.6, 166.6, 166.1, 166.1, 165.5, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.
**Cholesteryl-3β-glucose (24):** ¹H NMR (400 MHz, CDCl₃): δ 5.72 (m, 2H), 5.38(d, 1H), 5.30 (dd, J₁=6.45 Hz, J₂=1.2 Hz, 1H), 4.66 (m, 2H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH₃-19, CH₃-21, CH₃-26, CH₃-27), and 0.64 ppm (3H, s, CH₃-18). ¹³C-NMR (CDCl₃): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

**Synthesis of Diosgenyl-3β-glucose (26):** Synthesis of benzoylated 26: ¹H NMR (400 MHz, CDCl₃): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, J=9.9 Hz, 1H), 5.72 (m, 2H), 5.38(d, 1H), 5.30 (dd, J₁=6.45 Hz, J₂=1.2 Hz, 1H), 4.87 (br, s,1H), 4.46 (dd, J₁=8.6 Hz, J₂=2.4 Hz, 1H), 4.38(q, J=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.12 (d, J=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). ¹³C NMR of (CDCl₃): δ 170.5, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.5, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7, 13.9.

**Diosgenyl-3β-glucose (26):** ¹H NMR (400 MHz, CDCl₃): δ 5.72 (m, 2H), 5.38 (d, 1H) 5.30 (dd, J₁=6.45 Hz, J₂=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, J₁=8.6 Hz, J₂=2.4 Hz, 1H), 4.38(q, J=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.12 (d, J=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). ¹³C NMR of (CDCl₃): δ119.6, 109.8, 82.9, 81.5, 81.0, 78.7, 76.8, 74.1, 71.5, 67.0, 66.3, 62.3, 56.5, 54.5, 45.0,
Synthesis of Tigogenyl-2α--hydroxy-3β-glucose (27): Synthesis of benzoylated 27: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.12(3H, s, CH$_3$CO), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.1, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7, 13.9.

Tigogenyl-2α--hydroxy-3β-glucose (27): $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$119.6, 109.8, 82.9, 81.5, 81.0, 78.7, 76.8, 74.1, 71.5, 67.0, 66.3, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.9, 35.5, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

Synthesis of Tigogenyl-2β--hydroxy-3α-glucose (28): Synthesis of benzoylated 28: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.12(3H, s, CH$_3$CO), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.1, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7, 13.9.
Tigogenyl-2β--hydroxy-3α-glucose (28): \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 5.72 (m, 2H), 5.30 (dd, \(J_1=6.45\) Hz, \(J_2=1.2\) Hz, 1H), 4.66 (m, 2H), 4.46 (dd, \(J_1=8.6\) Hz, \(J_2=2.4\) Hz, 1H), 4.38(q, \(J=11.2,1\)H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, \(J=10.8\) Hz, 1H), 3.12 (d, \(J=1.3\) Hz, 1H), 2.04 (s, 3H), 0.95 (d, \(J=3.2\) Hz, 3H), 0.75 (s, 3H). \(^{13}\)C NMR of (CDCl\(_3\)): \(\delta\) 170.5, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Synthesis of Tigogenyl-3β--hydroxy-2α-glucose (29): Synthesis of benzoylated 29: \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, \(J=9.9\) Hz, 1H), 5.72 (m, 2H), 5.30 (dd, \(J_1=6.45\) Hz, \(J_2=1.2\) Hz, 1H), 4.87 (br s, 1H), 4.46 (dd, \(J_1=8.6\) Hz, \(J_2=2.4\) Hz, 1H), 4.38(q, \(J=11.2,1\)H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, \(J=10.8\) Hz, 1H), 3.12 (d, \(J=1.3\) Hz, 1H), 2.04 (s, 3H), 0.95 (d, \(J=3.2\) Hz, 3H), 0.75 (s, 3H). \(^{13}\)C NMR of (CDCl\(_3\)): \(\delta\) 170.5, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.
Tigogenyl-3β--hydroxy-2α-glucose (29): $^1$H NMR (400 MHz, CDCl$_3$): δ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38 (q, $J$=11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$= 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$= 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$= 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): δ 119.6, 109.8, 82.9, 81.5, 81.0, 78.7, 76.8, 74.1, 71.5, 67.0, 66.3, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.9, 35.5, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Synthesis of Tigogenyl-3β--hydroxy-2β-glucose (30): Synthesis of benzyloylated 30: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.19- 7.82 (m, 8H), 7.57-7.19 (m, 12H), 6.23 (t, $J$=9.9 Hz, 1H), 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.87 (br, s, 1H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38 (q, $J$=11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$= 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.12 (3H, s, CH$_3$CO), 2.04 (s, 3H), 0.95 (d, $J$= 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$= 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): δ 170.5, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 109.5, 90.6, 81.0, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Tigogenyl-3β--hydroxy-2β-glucose (30): $^1$H NMR (400 MHz, CDCl$_3$): δ 5.72 (m, 2H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38 (q,
\[ J = 11.2, 1H, 3.84 \text{ (br s, 1H)}, 3.46 \text{ (m, 1H)}, 3.36 \text{ (t, } J = 10.8 \text{ Hz, 1H)}, 3.12 \text{ (d, } J = 1.3 \text{ Hz, 1H)}, 2.04 \text{ (s, 3H)}, 0.95 \text{ (d, } J = 3.4 \text{ Hz, 3H)}, 0.92 \text{ (s, 3H)}, 0.78 \text{ (d, } J = 3.2 \text{ Hz, 3H)}, 0.75 \text{ (s, 3H)}. \]

\[ ^{13}C \text{ NMR of (CDCl}_3\text{): } \delta 119.6, 109.8, 82.9, 81.5, 81.0, 78.7, 76.8, 74.1, 71.5, 67.0, 66.3, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.9, 35.5, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.6, 20.8, 17.3, 16.7, 14.7 \text{ and 13.9 ppm.} \]

4.15.3 General Procedure for disaccharide derivatives: Synthesis of Compound Tigogenyl-3\(\beta\)-maltose (35) Trichloroacetimidate glucosyl donor (476mg, 0.39 mmol) and steroidal acceptor tigogenin (163mg, 0.39 mmol) were stirred under an atmosphere of nitrogen in anhydrous CH\(_2\)Cl\(_2\) (10 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (15 µL, 0.1 mmol) was added. The reaction was stirred for 3 hours at 0°C to room temperature, quenched with triethylamine, and extracted with dicholomethane. The solvents were removed under reduced pressure and the resulting syrup was purified by gravity column chromatography (15% ethyl acetate: hexane) to give 385mg of benzoylated disaccharide-coupled tigogenin 35 (67% isolated yield).

Tigogenyl-3\(\beta\)-maltose (35): Benzoylated disaccharide coupled tigogenin (375mg, 0.25mmols) was dissolved in Methanol and dichloromethane mix (1:1, 10ml) and freshly prepared sodium methoxide solution in methanol was added dropwise until pH 9-11 was achieved. Reaction mixture was stirred at room temperature for one hour. On completion of reaction the reaction mixture was neutralized by acidic dowex resin to neutral PH and filtered off resin. Solvent was removed under reduced pressure and crude product was purified by column chromatography (Methanol:Dichloromethane:: 1:6) to get the 160mg of desired product 35 (85%-yield).
Synthesis of Androstanyl-2α-hydroxy-3β-maltose (31): Synthesis of benzoylated 31: \(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, \(J=9.9\) Hz, 1H), 5.90 (t, \(J = 9.6\) Hz, 1H), 5.72 (m, 2H), 5.69 (t, \(J = 9.6\) Hz, 1H), 5.49 (t, \(J = 10.0\) Hz, 1H), 5.39 (d, \(J = 1.8\) Hz, 1H), 5.30 (dd, \(J_1=6.45\) Hz, \(J_2=1.2\) Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH\(_3\)CO), and 0.64 ppm (3H, s, CH\(_3\)-18). 

\(^{13}\)C-NMR (CDCl\(_3\)): δ 171.6, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 22.5, 21.4, 13.0 and 12.0 ppm.

Cholestanyl-2α-hydroxy-3β-maltose (32): \(^1\)H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (t, \(J = 9.6\) Hz, 1H), 5.49 (t, \(J = 10.0\) Hz, 1H), 5.39 (d, \(J = 1.8\) Hz, 1H), 5.28(t,J=10.4Hz,1H),5.10(dd,\(J_1=5.2\)Hz,\(J_2=1.8\)Hz, 1H), 4.95(d,J = 3.8 Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH\(_3\)-19, CH\(_3\)-21, CH\(_3\)-26, CH\(_3\)-27), and 0.64 ppm (3H, s, CH\(_3\)-18). \(^{13}\)C-NMR (DMSO): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.8, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

Synthesis of Cholestanyl-2α-hydroxy-3β-maltose (32): Synthesis of benzoylated 32: \(^1\)H NMR (400 MHz, CDCl\(_3\)): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, \(J=9.9\) Hz, 1H), 5.90 (t, \(J = 9.6\) Hz, 1H), 5.72 (m, 2H), 5.69 (t, \(J = 9.6\) Hz, 1H), 5.49 (t, \(J = 10.0\) Hz, 1H), 5.39 (d, \(J = 1.8\)Hz,1H), 5.30 (dd, \(J_1=6.45\) Hz, \(J_2=1.2\) Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH\(_3\)CO), 0.84-0.90 (12H, CH\(_3\)-19, CH\(_3\)-21, CH\(_3\)-26, CH\(_3\)-27), and 0.64 ppm (3H, s, CH\(_3\)-18). \(^{13}\)C-NMR (CDCl\(_3\)): δ171.6, 166.6, 166.1,
166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.8, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

**Cholestanyl-2α-hydroxy-3β-maltose (32):** $^1$H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J = 5.2$ Hz, $J = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (DMSO): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

**Synthesis of Cholestanyl-3β-maltose (33):** Synthesis of benzoylated 33: $^1$H NMR (400 MHz, CDCl$_3$): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J = 9.9$ Hz, 1H), 5.90 (t, $J = 9.6$ Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.30 (dd, $J = 6.4$ Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): δ171.6, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.5, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.
Cholestanyl-3β-maltose (33): $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J_1 = 5.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.82 (2H, s), 3.59 (1H, ddd, $J = 5.4$, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C NMR (DMSO): $\delta$ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

Synthesis of Tigogenyl-2α-hydroxy-3β-maltose (34): Synthesis of benzoylated 34: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08-7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J = 9.9$ Hz, 1H), 5.90 (t, $J = 9.6$ Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J_1 = 5.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J_1 = 6.2$ Hz, $J_2 = 1.4$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$ Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J = 10.8$ Hz, 1H), 3.12 (d, $J = 1.3$ Hz, 1H), 2.12 (3H, s, CH$_3$CO), 2.04 (s, 3H), 0.95 (d, $J = 3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J = 3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.13, 166.10, 165.9, 165.7, 165.5, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.9, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Tigogenyl-2α-hydroxy-3β-maltose (34): $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J_1 = 5.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J_1 = 6.2$ Hz, $J_2 = 1.4$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$ Hz, 1H),
3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.12 (d, J = 1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). 13C NMR of (DMSO): δ 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Synthesis of Tigogenyl-3β-maltose (35):** Benzoyl protected 35: 1H NMR (400 MHz, CDCl3): δ 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, J = 9.9 Hz, 1H), 5.90 (t, J = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, J = 9.6 Hz, 1H), 5.49 (t, J = 10.0 Hz, 1H), 5.39 (d, J = 1.8 Hz, 1H), 5.28 (t, J = 10.4 Hz, 1H), 5.10 (dd, J = 5.2 Hz, J = 1.8 Hz, 1H), 4.95 (d, J = 3.8 Hz, 1H), 4.71 (dd, J = 6.2 Hz, J = 1.4 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 4.38 (q, J = 11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.12 (d, J = 1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). 13C NMR of (CDCl3): δ 166.6, 166.13, 166.10, 165.9, 165.7, 165.5, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Tigogenyl-3β-maltose (35):** 1H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (s, 2H), 5.49 (t, J = 10.0 Hz, 1H), 5.39 (d, J = 1.8 Hz, 1H), 5.28 (t, J = 10.4 Hz, 1H), 5.10 (dd, J = 5.2 Hz, J = 1.8 Hz, 1H), 4.95 (d, J = 3.8 Hz, 1H), 4.71 (dd, J = 6.2 Hz, J = 1.4 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, J = 8.6 Hz, J = 2.4 Hz, 1H), 4.38 (q, J = 11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, J = 10.8 Hz, 1H), 3.12 (d, J = 1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, J = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, J = 3.2 Hz, 3H), 0.75 (s, 3H). 13C NMR of (DMSO): δ 119.1, 102.5, 90.6, 81.0,
Synthesis of Tigogenyl-3β-cellobiose (36): Synthesis of benzoylated Compound 36: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H),5.28(t,$J$=10.4Hz,1H),5.10(dd,$J_l$=5.2Hz,$J_2$ =1.8Hz,1H), 4.95(d,$J$ = 3.8 Hz, 1H), 4.71 (dd, $J_l$ = 6.2 Hz, $J_2$ = 1.4 Hz, 1H),4.66 (m, 2H), 4.46 (dd, $J_l$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.1, 166.1, 165.9, 165.7, 165.5, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Tigogenyl-3β-cellobiose (36): $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H),5.28(t,$J$=10.4Hz,1H),5.10(dd,$J_l$=5.2Hz,$J_2$ =1.8Hz,1H), 4.95(d,$J$ = 3.8 Hz, 1H), 4.71 (dd, $J_l$ = 6.2 Hz, $J_2$ = 1.4 Hz, 1H),4.66 (m, 2H), 4.46 (dd, $J_l$=8.6 Hz, $J_2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (DMSO): $\delta$ 119.1,102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.
Synthesis of Cholestanyl-3β-cellobiose (37): Synthesis of benzyolated 37: $^1$H NMR (400 MHz, CDCl$_3$): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): δ171.6, 166.6, 166.2, 166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

Cholestanyl-3β-cellobiose (37): $^1$H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H), 5.28(t,$J$=10.4Hz,1H),5.10(dd,$J_1$=5.2Hz,$J_2$=1.8Hz,1H), 4.95(d,J = 3.8 Hz, 1H), 4.82 (2H,s), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (DMSO): δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

Synthesis of Cholestanyl-2α-hydroxy-3β-maltose (38): Synthesis of benzyolated Compound 38: $^1$H NMR (400 MHz, CDCl$_3$): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H), 5.30 (dd, $J_1$=6.45 Hz, $J_2$=1.2 Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH$_3$CO), 0.84-0.90 (12H,
CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): $\delta$171.6, 166.6, 166.2, 166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

**Cholestanyl-2$\alpha$-hydroxy-3$\beta$-maltose (38):** $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H), 5.28(t,$J$=10.4Hz,1H),5.10(dd,$J$=5.2Hz,$J$=1.8Hz,1H), 4.95(d,$J$ = 3.8 Hz, 1H), 4.82 (2H,s), 3.59 (1H, ddd, J=5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (DMSO): $\delta$ 76.4, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

**Synthesis of Diosgenyl-3$\beta$-maltose (39):** Synthesis of benzoylated Compound 39: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.39 (d, $J$ = 1.8Hz,1H),5.28(t,$J$=10.4Hz,1H),5.10(dd,$J$=5.2Hz,$J$=1.8Hz,1H), 4.95(d,$J$ = 3.8 Hz, 1H), 4.71 (dd, $JI$ = 6.2 Hz, $J2$ = 1.4 Hz, 1H),4.66 (m, 2H), 4.46 (dd, $JI$=8.6 Hz, $J2$=2.4 Hz, 1H), 4.38(q, $J$=11.2,1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$ = 10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$ = 3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$ = 3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (CDCl$_3$): $\delta$ 166.6, 166.1, 166.1, 165.9,165.7,165.5,165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4,130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5,
Diosgenyl-3β-maltose (39): $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J = 5.2$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J = 6.2$ Hz, $J = 1.4$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J = 8.6$ Hz, $J = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J = 10.8$ Hz, 1H), 3.12 (d, $J = 1.3$ Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J = 3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J = 3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (DMSO): $\delta$ 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Synthesis of Tigogenyl-3β-cellobiose (40): Synthesis of benzoylated 40: $^1$H NMR (400 MHz, CDCl$_3$): 8.08-7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J = 9.9$ Hz, 1H), 5.90 (t, $J = 9.6$ Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.30 (dd, $J = 6.45$ Hz, $J = 1.2$ Hz, 1H), 4.82 (1H, ddd, $J = 4.8$, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, $J = 5.4$, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$): $\delta$ 171.6, 166.6, 166.1, 166.1, 165.5, 133.6, 133.5, 133.3, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

Tigogenyl-3β-cellobiose (40): $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J = 5.2$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J = 6.2$ Hz, $J = 1.4$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J = 8.6$ Hz, $J = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J = 10.8$ Hz, 1H), 3.12 (d, $J = 1.3$ Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J = 3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J = 3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (DMSO): $\delta$ 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.
=5.2Hz, J2 =1.8Hz, 1H), 4.95(d, J =3.8 Hz, 1H), 4.82 (2H, s), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH3-19, CH3-21, CH3-26, CH3-27), and 0.64 ppm (3H, s, CH3-18).  

**13C-NMR (DMSO):** δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

**Synthesis of Tigogenyl-3β-laminaribiose (41):** Synthesis of benzoylated 41: 1H NMR (400 MHz, CDCl3): 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, J=9.9 Hz, 1H), 5.90 (t, J = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, J = 9.6 Hz, 1H), 5.49 (t, J = 10.0 Hz, 1H), 5.39 (d, J = 1.8Hz,1H), 5.30 (dd, J1=6.45 Hz, J2=1.2 Hz, 1H), 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH3-19, CH3-21, CH3-26, CH3-27), and 0.64 ppm (3H, s, CH3-18).  

**13C-NMR (CDCl3):** δ171.6, 166.6, 166.13, 166.10, 165.5, 133.6, 133.5, 133.1, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 76.5, 73.5, 56.3, 56.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.

**Tigogenyl-3β-laminaribiose (41):** 1H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (t, J = 9.6 Hz, 1H), 5.49 (t, J = 10.0 Hz, 1H), 5.39 (d, J = 1.8Hz,1H), 5.28(t,J=10.4Hz,1H),5.10(dd,J1 =5.2Hz,J2 =1.8Hz,1H), 4.95(d,J =3.8 Hz, 1H), 4.82 (2H, s), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 0.84-0.90 (12H, CH3-19, CH3-21, CH3-26, CH3-27), and 0.64 ppm (3H, s, CH3-18).  

**13C-NMR (DMSO):** δ 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.
Synthesis of Cholestanyl-3β-cellobiose (42): Synthesis of benzoylated 42: $^1$H NMR (400 MHz, CDCl3): δ 8.08-7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.28 (t, $J$=10.4 Hz, 1H), 5.10 (dd, $J$ = 5.2 Hz, $J$ = 1.8 Hz, 1H), 4.95 (d, $J$ = 3.8 Hz, 1H), 4.71 (dd, $J$ = 6.2 Hz, $J$ = 1.8 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J$=8.6 Hz, $J$=2.4 Hz, 1H), 4.38 (q, $J$=11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$=10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$=3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$=3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR (CDCl3): δ 166.6, 166.1, 166.10, 166.5, 165.7, 165.5, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Cholestanyl-3β-cellobiose (42): $^1$H NMR (400 MHz, DMSO): δ 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.28 (t, $J$=10.4 Hz, 1H), 5.10 (dd, $J$ = 5.2 Hz, $J$ = 1.8 Hz, 1H), 4.95 (d, $J$ = 3.8 Hz, 1H), 4.71 (dd, $J$ = 6.2 Hz, $J$ = 1.4 Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J$=8.6 Hz, $J$=2.4 Hz, 1H), 4.38 (q, $J$=11.2 Hz, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J$=10.8 Hz, 1H), 3.12 (d, $J$=1.3 Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J$=3.4 Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J$=3.2 Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR (DMSO): δ 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

Synthesis of Cholestanyl-3β-melibiose (43): Synthesis of benzoylated 43: $^1$H NMR (400 MHz, CDCl3): δ 8.08-7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, $J$=9.9 Hz, 1H), 5.90 (t, $J$ = 9.6 Hz, 1H), 5.72 (m, 2H), 5.69 (t, $J$ = 9.6 Hz, 1H), 5.49 (t, $J$ = 10.0 Hz, 1H), 5.28 (t, $J$=10.4 Hz, 1H), 5.10 (dd, $J$ = 5.2 Hz, $J$ = 1.8 Hz, 1H), 4.95 (d, $J$ = 3.8 Hz, 1H), 4.71 (dd, $J$ =
6.2 Hz, \( J_2 = 1.4 \text{ Hz}, 1\text{H}\), 4.66 (m, 2H), 4.46 (dd, \( J_1 = 8.6 \text{ Hz}, J_2 = 2.4 \text{ Hz}, 1\text{H}\)), 4.38 (q, \( J = 11.2, 1\text{H}\)), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, \( J = 10.8 \text{ Hz}, 1\text{H}\)), 3.12 (d, \( J = 1.3 \text{ Hz}, 1\text{H}\)), 2.04 (s, 3H), 0.95 (d, \( J = 3.4 \text{ Hz}, 3\text{H}\)), 0.92 (s, 3H), 0.78 (d, \( J = 3.2 \text{ Hz}, 3\text{H}\)), 0.75 (s, 3H). 13C NMR of (CDCl3): \( \delta \) 166.6, 166.13, 166.10, 165.9, 165.7, 165.5, 165.4, 163.9, 163.7, 163.6, 163.5, 163.4, 163.3, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Cholestanyl-3β-melibiose (43):** \(^1\)H NMR (400 MHz, DMSO): \( \delta \) 5.72 (m, 2H), 5.69 (t, \( J = 9.6 \text{ Hz}, 1\text{H}\)), 5.49 (t, \( J = 10.0 \text{ Hz}, 1\text{H}\)), 5.28(t,\( J=10.4\text{Hz},1\text{H}\)),5.10(dd,\( JI =5.2\text{Hz},J2 =1.8\text{Hz},1\text{H}\)), 4.95(d,\( J = 3.8 \text{ Hz}, 1\text{H}\)), 4.71 (dd, \( J_1 = 6.2 \text{ Hz}, J_2 = 1.4 \text{ Hz}, 1\text{H}\)), 4.66 (m, 2H), 4.46 (dd, \( J_1 = 8.6 \text{ Hz}, J_2 = 2.4 \text{ Hz}, 1\text{H}\)), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, \( J = 10.8 \text{ Hz}, 1\text{H}\)), 3.12 (d, \( J = 1.3 \text{ Hz}, 1\text{H}\)), 2.04 (s, 3H), 0.95 (d, \( J = 3.4 \text{ Hz}, 3\text{H}\)), 0.92 (s, 3H), 0.78 (d, \( J = 3.2 \text{ Hz}, 3\text{H}\)), 0.75 (s, 3H). 13C NMR of (DMSO): \( \delta \) 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Synthesis of Tigogenyl-3β-melibiose (44):** Synthesis of benzyolated 44: \(^1\)H NMR (400 MHz, CDCl3): \( \delta \) 8.08- 7.77 (m, 14H), 7.57-7.26 (m, 21H), 6.13 (t, \( J=9.9 \text{ Hz}, 1\text{H}\)), 5.90 (t, \( J = 9.6 \text{ Hz}, 1\text{H}\)), 5.72 (m, 2H), 5.69 (t, \( J = 9.6 \text{ Hz}, 1\text{H}\)), 5.49 (t, \( J = 10.0 \text{ Hz}, 1\text{H}\)), 5.39 (d, \( J = 1.8 \text{Hz}, 1\text{H}\)), 5.28(t,\( J=10.4\text{Hz},1\text{H}\)),5.10(dd,\( JI =5.2\text{Hz},J2 =1.8\text{Hz},1\text{H}\)), 4.95(d,\( J = 3.8 \text{ Hz}, 1\text{H}\)), 4.71 (dd, \( J_1 = 6.2 \text{ Hz}, J_2 = 1.4 \text{ Hz}, 1\text{H}\)), 4.66 (m, 2H), 4.46 (dd, \( J_1 = 8.6 \text{ Hz}, J_2 = 2.4 \text{ Hz}, 1\text{H}\)), 4.38(q, \( J=11.2,1\text{H}\)), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, \( J = 10.8 \text{ Hz}, 1\text{H}\)), 3.12 (d, \( J = 1.3 \text{ Hz}, 1\text{H}\)), 2.04 (s, 3H), 0.95 (d, \( J = 3.4 \text{ Hz}, 3\text{H}\)), 0.92 (s, 3H), 0.78 (d, \( J = 3.2 \text{ Hz}, 3\text{H}\)), 0.75 (s, 3H). 13C NMR of (CDCl3): \( \delta \) 166.6, 166.13, 166.10, 165.9, 165.7, 165.5, 165.4, 138.9, 133.7, 133.6, 133.5, 133.4, 133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

162
133.3, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.46, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Tigogenyl-3β-melibiose (44):** $^1$H NMR (400 MHz, DMSO): $\delta$ 5.72 (m, 2H), 5.69 (t, $J = 9.6$ Hz, 1H), 5.49 (t, $J = 10.0$ Hz, 1H), 5.39 (d, $J = 1.8$ Hz, 1H), 5.28 (t, $J = 10.4$ Hz, 1H), 5.10 (dd, $J_1 = 5.2$ Hz, $J_2 = 1.8$ Hz, 1H), 4.95 (d, $J = 3.8$ Hz, 1H), 4.71 (dd, $J_1 = 6.2$ Hz, $J_2 = 1.4$ Hz, 1H), 4.66 (m, 2H), 4.46 (dd, $J_1 = 8.6$ Hz, $J_2 = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$, 1H), 3.84 (br s, 1H), 3.46 (m, 1H), 3.36 (t, $J = 10.8$ Hz, 1H), 3.12 (d, $J = 1.3$ Hz, 1H), 2.04 (s, 3H), 0.95 (d, $J = 3.4$ Hz, 3H), 0.92 (s, 3H), 0.78 (d, $J = 3.2$ Hz, 3H), 0.75 (s, 3H). $^{13}$C NMR of (DMSO): $\delta$ 119.1, 102.5, 90.6, 81.0, 77.4, 73.3, 72.5, 70.4, 67.7, 67.0, 62.3, 56.4, 55.1, 41.8, 40.7, 40.2, 38.7, 37.3, 35.9, 34.8, 32.2, 32.0, 31.9, 31.5, 30.5, 29.0, 28.2, 21.6, 20.8, 17.3, 16.7, 14.7 and 13.9 ppm.

**Cholestan-3β-deca-benzoyl-maltotriose:** $^1$H NMR of benzoylated 45 (400 MHz, CDCl$_3$): $\delta$ 8.04 (d, $J = 3.6$ Hz, 2H), 7.92 – 7.74 (m, 12H), 7.52 – 7.23 (m, 21H), 5.92 (t, $J = 9.6$ Hz, 1H), 5.78 (t, $J = 9.6$ Hz, 1H), 5.66 (t, $J = 9.6$ Hz, 1H), 5.53 (dd, $J_1 = 4.9$ Hz, $J_2 = 4.2$ Hz, 1H), 5.39 (m, 2H), 5.28 (m, 2H), 4.65 (d, $J = 4.0$ Hz, 1H), 4.64 (d, $J = 4.8$ Hz, 1H), 4.47 (dd, $J_1 = 6.1$ Hz, $J_2 = 2.4$ Hz, 1H), 4.17 (m, 1H), 4.04 – 3.92 (m, 4H), 3.84 – 3.80 (m, 3H), 3.63 – 3.56 (m, 2H), 1.12 (3H, s, CH$_3$-19), 0.85, 0.91 (9H, CH$_3$-21, CH$_3$-26 and CH$_3$-27), 0.67 (3H, s, CH$_3$-18) ppm $^{13}$C NMR of 45 (400 MHz, CDCl$_3$): $\delta$ 166.8, 166.5, 165.9, 165.7, 165.5, 165.4, 133.8, 133.7, 133.5, 130.1, 130.0, 129.9, 129.7, 129.4, 128.9, 128.7, 128.6, 128.5, 101.6, 101.2, 92.9, 77.4, 75.1, 74.7, 74.4, 73.4, 72.6, 72.5, 72.0, 71.9, 70.4, 70.2, 69.9, 69.8, 69.1, 67.9, 63.2, 56.1, 56.0, 53.8, 47.8, 44.8, 43.6, 42.5, 39.7, 39.5, 37.2, 36.1, 35.7, 34.7, 31.6, 28.4, 28.2, 27.9, 24.1, 23.8, 22.8, 22.5, 21.6, 20.8, 18.6, 12.7 and 12.0 ppm.
Cholesta-2α-hydroxy-3β-deca-benzoyl-maltotriose:  \(^1\)H NMR of benzoylated 46 (400 MHz, CDCl\(_3\)):  δ 8.04 (d,  \(J = 3.6\) Hz, 2H), 7.92–7.74 (m, 12H), 7.52–7.23 (m, 21H), 5.92 (t,  \(J = 9.6\) Hz, 1H), 5.78 (t,  \(J = 9.6\) Hz, 1H), 5.66 (t,  \(J = 9.6\) Hz, 1H), 5.53 (dd,  \(J_1 = 4.9\) Hz,  \(J_2 = 4.2\) Hz, 1H), 5.39 (m, 2H), 5.28 (m, 2H), 4.65 (d,  \(J = 4.0\) Hz, 1H), 4.64 (d,  \(J = 4.8\) Hz, 1H), 4.47 (dd,  \(J_1 = 6.1\) Hz,  \(J_2 = 2.4\) Hz, 1H), 4.17 (m, 1H), 4.04–3.92 (m, 4H), 3.84–3.80 (m, 3H), 3.63 (m, 1H), 3.41 (m, 1H), 1.12 (3H, s, CH\(_3\)-19), 0.85, 0.91 (9H, CH\(_3\)-21, CH\(_3\)-26 and CH\(_3\)-27), 0.67 (3H, s, CH\(_3\)-18) ppm.

\(^{13}\)C NMR of Benzoylated 46 (400 MHz, CDCl\(_3\)):  δ 166.8, 166.5, 165.9, 165.7, 165.5, 165.4, 133.8, 133.7, 133.5, 130.1, 130.0, 129.9, 129.7, 129.4, 128.9, 128.7, 128.6, 128.5, 128.5, 101.6, 101.2, 92.9, 77.4, 75.1, 74.7, 74.5, 73.4, 72.6, 72.5, 72.0, 71.9, 70.4, 70.2, 69.9, 69.8, 69.1, 67.9, 63.2, 56.1, 56.0, 53.8, 47.8, 44.8, 43.6, 42.5, 39.7, 39.4, 37.2, 36.1, 35.7, 34.6, 31.5, 28.4, 28.2, 27.9, 24.1, 23.8, 22.8, 22.5, 21.6, 20.8, 18.6, 12.7 and 12.0 ppm.

Tigogenyl-3β-deca-benzoyl-maltotriose:  \(^1\)H NMR of benzoylated 47 (400 MHz, CDCl\(_3\)):  δ 8.04 (d,  \(J = 3.6\) Hz, 2H), 7.92–7.74 (m, 12H), 7.52–7.23 (m, 21H), 5.92 (t,  \(J = 9.6\) Hz, 1H), 5.78 (t,  \(J = 9.6\) Hz, 1H), 5.66 (t,  \(J = 9.6\) Hz, 1H), 5.53 (dd,  \(J_1 = 4.9\) Hz,  \(J_2 = 4.2\) Hz, 1H), 5.39 (m, 2H), 5.28 (m, 2H), 4.65 (d,  \(J = 4.0\) Hz, 1H), 4.64 (d,  \(J = 4.8\) Hz, 1H), 4.47 (dd,  \(J_1 = 6.1\) Hz,  \(J_2 = 2.4\) Hz, 1H), 4.38 (q,  \(J = 11.2\) Hz, 1H), 4.17 (m, 1H), 4.04–3.92 (m, 4H), 3.84–3.80 (m, 3H), 3.63–3.56 (m, 2H), 3.47 (m, 1H), 3.37 (m, 1H), 0.96 (d,  \(J = 3.4\) Hz, 3H), 0.82 (s, 3H), 0.76 (s, 3H) ppm.  \(^{13}\)C NMR of benzoylated 47 (400 MHz, CDCl\(_3\)):  δ 166.8, 166.5, 165.9, 165.7, 165.5, 165.4, 133.8, 133.7, 133.5, 130.1, 130.0, 129.9, 129.7, 129.4, 128.9, 128.7, 128.6, 128.5, 109.5, 101.6, 101.2, 92.9, 81.0, 77.4, 75.1, 74.7, 74.5, 73.4, 72.6, 72.5, 72.0, 71.9, 71.5, 70.4, 70.2, 69.9, 69.8, 69.1, 67.9, 67.0, 63.2, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.
**Tigogenyl-2α-hydroxy-3β-deca-benzoyl-maltotriose:** $^1$H NMR of benzoylated 48 (400 MHz, CDCl$_3$): $\delta$ 8.04 (d, $J = 3.6$ Hz, 2H), 7.92 – 7.74 (m, 12H), 7.52 – 7.23 (m, 21H), 5.92 (t, $J = 9.6$Hz,1H), 5.78(t,$J=9.6$Hz,1H), 5.66(t,$J=9.6$Hz,1H), 5.53(dd,$J_1 =4.9$Hz,$J_2 =4.2$Hz, 1H), 5.39 (m, 2H), 5.28 (m, 2H), 4.65 (d, $J = 4.0$ Hz, 1H), 4.64 (d, $J = 4.8$ Hz, 1H), 4.47 (dd, $J_1 = 6.1$ Hz, $J_2 = 2.4$ Hz, 1H), 4.38 (q, $J = 11.2$ Hz, 1H), 4.17 (m, 1H), 4.04 – 3.92 (m, 4H), 3.84 – 3.80 (m, 3H), 3.63 – 3.56 (m, 2H), 3.47(m, 1H), 3.37(m, 1H), 0.96(d, $J = 3.4$Hz, 3H), 0.82 (s, 3H), 0.76(s, 3H) ppm. $^{13}$C NMR of benzoylated 48 (400 MHz, CDCl$_3$): $\delta$ 166.8, 166.5, 165.9, 165.7, 165.5, 165.4, 133.8, 133.7, 133.5, 130.1, 130.0, 129.9, 129.7, 129.4, 128.9, 128.7, 128.6, 128.5, 109.5, 101.6, 101.2, 92.9, 81.0, 77.4, 75.1, 74.7, 73.4, 72.6, 72.5, 72.0, 71.9, 71.5, 70.4, 70.2, 69.9, 69.8, 69.1, 67.9, 67.0, 63.2, 62.3, 56.5, 54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

**Penta-O-acetyl-D-glucopyranose (49):** D-glucose (20 g, 110 mmol) was refluxed in pyridine (150 mL) for 1 hour. Acetic anhydride (100 mL) was added and the mixture was refluxed an additional 2 hours. The resulting solution was cooled to room temperature, poured over ice (300 mL) and stirred until the product solidified, about 2 hours. The solid was filtered, washed with water, and recrystallized from ethyl acetate/hexanes to give 49 as a white solid (36 g, 83 % yield). $^1$H NMR of 49 (400 MHZ, CDCl$_3$): $\delta$ 5.72 (d, $J = 4.0$ Hz, 1H), 5.26 (t, $J = 9.2$ Hz, 1H), 5.14(t,$J=8.8$Hz,1H), 5.13(t,$J=10.0$Hz,1H), 4.30(dd,$J_1 =6.2$Hz,$J_2 =2.2$Hz,1H), 4.12 (dd, $J_1 = 6.3$ Hz, $J_2 = 1.0$ Hz, 1H), 3.84 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H). $^{13}$C NMR of 49 (400 MHz, CDCl$_3$): $\delta$ 170.7, 170.3, 169.8, 169.5, 168.9, 89.1, 69.9, 69.2, 67.9, 61.5, 20.9, 20.8, 20.7, 20.6 and 20.5ppm.

**Isopropyl tetra-O-acetyl-1-thio-β-D-glucopyranoside (50):** Penta-O-acetyl-β-D-glucopyranose
Isopropyl 1-thio-β-D-glucopyranoside (51): Thioglucopyranoside 50 was dissolved in 2:1 MeOH: CH₂Cl₂ (45 mL). Sodium hydroxide (1N) was added to a pH ~ 9-10. The solution was stirred at room temperature 6 hours, neutralized with acidic Dowex resin, and filtered. The solvents were removed under reduced pressure and the resulting solid was co-evaporated with toluene (3 x 10 mL) and used immediately in the following reaction without further purification. 

$^1$H NMR (400 MHz, DMSO- D₆): $\delta$ 4.31 (d, $J = 4.8$ Hz, 1H), 3.65 (dd, $J_1 = 5.9$ Hz, $J_2 = 0.6$ Hz, 1H), 3.40 (dd, $J_1 = 5.9$ Hz, $J_2 = 2.9$ Hz, 1H), 3.18 – 2.93 (m, 5H), 1.22 (dd, $J_1 = 3.4$ Hz, $J_2 = 2.1$ Hz, 6H). $^{13}$C NMR (400 MHz, DMSO-D₆): $\delta$ 84.5, 80.9, 78.3, 73.3, 70.1, 61.3, 33.4, 23.9 and 23.8 ppm.
**Isopropyl (R)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (52):** Thioglucopyranoside 51 and dimethoxymethylbenzene (1.7 g, 11 mmol) were dissolved in DMF (15 mL). The solution was cooled to 0°C and CSA was added to a pH ~ 2-3. After the mixture was stirred at room temperature overnight, it was neutralized with triethylamine. Ethyl acetate (100 mL) was added and the resulting solution was washed with brine (2 x 100 mL), dried over sodium sulfate. The solvents were removed under reduced pressure and the product was purified by flash column chromatography (1:1 ethyl acetate: petroleum ether) to give 52 as a white solid (1.6 g, 51 % yield in 3 steps from 49). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.55 – 7.36 (m, 5H), 5.55 (s, 2H), 4.54 (d, $J$ = 5.0 Hz, 1H), 4.35 (dd, $J_1$ = 5.2 Hz, $J_2$ = 2.3 Hz, 1H), 3.88 – 3.75 (m, 2H), 3.62 – 3.45 (m, 3H), 3.24 (m, 1H), 1.36 (d, $J$ = 3.3 Hz, 6H). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ 129.4, 128.5, 126.6, 126.5, 102.1, 86.5, 81.2, 72.3, 72.3, 68.9, 63.6, 36.5, 24.4 and 23.8 ppm.

**Isopropyl tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-(tetra-O-benzoyl-β-D-glucopyranosyl-(1→2)]-(R)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (54):** Trichloroacetimidate donor 1 (3.7 g, 5.0 mmol) and thioglucopyranoside 52 (1.5 g, 4.5 mmol) were co-evaporated with toluene (3 x 10 mL), dried under reduced pressure overnight, and then stirred under an atmosphere of nitrogen in anhydrous CH$_2$Cl$_2$ (50 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (91 μL, 0.5 mmol) was added. The reaction was stirred at 0°C for 5 hours, neutralized with triethylamine (0.3 mL), and filtered. The solvents were removed under reduced pressure and the product was purified by gravity column chromatography (2:3 ethyl acetate: petroleum ether) to give isopropyl tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-(R)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (53), which was used immediately in the following reaction. $^1$H NMR of 53 (400 MHz, CDCl$_3$): $\delta$ 8.14 – 7.82 (m,
10H), 7.23 – 7.57 (m, 15 H), 5.92 (t, J = 9.2 Hz, 1H), 5.71 (t, J = 9.2 Hz, 1H), 5.57 (s, 2H), 5.56 (dd, J1 = 3.6 Hz, J2 = 1.0 Hz, 1H), 5.22 (d, J = 4.0 Hz, 1H), 4.49 (dd, J1 = 5.9 Hz, J2 = 1.8 Hz, 1H), 4.44 (d, J = 5.0 Hz, 1H), 4.33 (t, J = 12.0 Hz, 1H), 4.32 (t, J = 12.4 Hz, 1H), 3.94 (m, 1H), 3.90 (t, J = 8.4 Hz, 1H), 3.77 (t, J = 10.8 Hz, 1H), 3.71 (t, J = 9.6 Hz, 1H), 3.49 – 3.44 (m, 2H), 3.14 (m, 1H), 1.24 (d, J = 2.9 Hz, 6H). Disaccharide 53 and trichloroacetimidate donor 1 (3.0 g, 4.0 mmol) were co-evaporated with toluene (3 x 10 mL), dried under reduced pressure overnight, and then stirred under an atmosphere of nitrogen in anhydrous CH2Cl2 (50 mL), with 3Å molecular sieves for 1 hour. The mixture was cooled to 0°C before TMSOTf (91 μL, 0.5 mmol) was added. The reaction was stirred at 0°C for 3 hours, neutralized with triethylamine (0.5 mL), and filtered. The solvents were removed under reduced pressure and the product was purified by gravity column chromatography (2:3 ethyl acetate: petroleum ether) and then recrystallized from methanol to give 54 (1.6 g, 23% yield in 2 steps from 52). 1H NMR (400 MHz, CDCl3): δ 8.29 – 7.21 (m, 45H), 5.83 (t, J = 9.2 Hz, 1H), 5.79 (t, J = 9.6 Hz, 1H), 5.61 – 5.52 (m, 3H), 5.51 (s, 2H), 5.43 (t, J = 10.0 Hz, 1H), 4.97 (d, J = 3.8 Hz, 1H), 4.80 (d, J = 4.2 Hz, 1H), 4.49 (d, J = 4.8 Hz, 1H), 4.37 (dd, J1 = 5.9 Hz, J2 = 1.6 Hz, 1H), 4.28 – 4.18 (m, 4H), 4.00 (t, J = 8.8 Hz, 1H), 3.90 (t, J = 9.6 Hz, 1H), 3.69 (t, J = 10.8 Hz, 1H), 3.62 (t, J = 9.2 Hz, 1H), 3.30 (m, 1H), 3.07 (m, 1H), 2.79 (m, 1H), 2.67 (m, 1H), 1.22 (d, J = 3.2 Hz, 3H), 1.09, (d, J = 3.4 Hz, 3H). 13C NMR (400 MHz, CDCl3): δ 166.5, 166.1, 166.0, 165.5, 165.4, 165.3, 163.9, 133.8, 133.7, 133.6, 133.5, 133.3, 130.3, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.7, 129.6, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.9, 128.7, 128.6, 128.5, 128.5, 128.3, 128.3, 126.2, 101.4, 100.0, 99.6, 96.1, 92.1, 84.0, 80.9, 78.6, 77.4, 76.7, 73.1, 72.5, 71.5, 71.3, 70.8, 69.2, 68.7, 63.6, 62.2, 34.3, 23.8 and 23.6 ppm.
Synthesis of **Diosgenyl-3β-hexanoate (55):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, $J$ =4.8, 9.4, 11.6 Hz, H-2b), 4.38 (q, $J$ = 11.2 Hz, 1H), 3.59 (1H, ddd, $J$ = 4.7, 9.0, 11.5 Hz, 1H), 3.47 (m, 1H), 3.37 (t, $J$ = 10.8 Hz, 1H), 2.08 (s, 3H), 0.96 (d, $J$ = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J$ = 3.2 Hz, 3H), 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2,162.5, 81.0, 71.5, 67.0, 62.3, 56.5,54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

Synthesis of **Tigogenyl-2α-acetoxy-3β-tetradecanoate (56):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, $J$ =4.8, 9.4, 11.6 Hz, H-2b), 4.38 (q, $J$ = 11.2 Hz, 1H), 3.59 (1H, ddd, $J$ = 4.7, 9.0, 11.5 Hz, 1H), 3.47 (m, 1H), 3.37 (t, $J$ = 10.8 Hz, 1H), 2.08 (s, 3H), 0.96 (d, $J$ = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J$ = 3.2 Hz, 3H), 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2,162.5, 81.0, 71.5, 67.0, 62.3, 56.5,54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.

Synthesis of **Tigogenyl-2α-acetoxy-3β-hexadecanoate (57):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, $J$ =4.8, 9.4, 11.6 Hz, H-2b), 4.38 (q, $J$ = 11.2 Hz, 1H), 3.59 (1H, ddd, $J$ = 4.7, 9.0, 11.5 Hz, 1H), 3.47 (m,1H), 3.37 (t, $J$ = 10.8 Hz, 1H), 2.08 (s, 3H), 0.96 (d, $J$ = 3.4 Hz, 3H), 0.82 (s, 3H), 0.79 (d, $J$ = 3.2 Hz, 3H), 0.76 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 165.2,162.5, 81.0, 71.5, 67.0, 62.3, 56.5,54.5, 45.0, 41.8, 40.7, 40.2, 38.4, 38.2, 37.1, 35.7, 35.3, 32.4, 31.9, 31.7, 31.5, 30.5, 29.0, 28.8, 21.2, 17.3, 16.7, 14.7 and 12.5 ppm.
Synthesis of **Cholestanyl-2α-acetoxy-3β-hexanoate (58):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH$_3$CO), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$) $\delta$: 171.6, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

Synthesis of **Cholestanyl-2α-acetoxy-3β-tetradecanoate (59):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH$_3$CO), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$) $\delta$: 171.6, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.5, 37.3, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.0 and 12.0 ppm.

Synthesis of **Cholestanyl-2α-acetoxy-3β-hexadecanoate (60):**

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.82 (1H, ddd, JZ4.8, 9.4, 11.6 Hz, H-2b), 3.59 (1H, ddd, JZ5.4, 9.5, 11.1 Hz, H-3a), 2.08 (3H, s, CH$_3$CO), 0.84-0.90 (12H, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27), and 0.64 ppm (3H, s, CH$_3$-18). $^{13}$C-NMR (CDCl$_3$) $\delta$: 171.6, 76.5, 73.5, 56.3, 56.1, 54.1, 44.4, 42.5, 42.1, 39.8, 39.4, 37.2, 36.1, 35.9, 35.7, 34.7, 31.8, 28.2, 27.9, 27.7, 24.1, 23.7, 22.8, 22.5, 21.4, 18.6, 13.1 and 12.0 ppm.
4.16 References:


14. Lindhorst, T. K. *Essentials of Carbohydrate Chemistry and Biochemistry, 2nd Ed.* Wiley-VCH: Germany, **2003**.


27. Calinaud, P.; Gelas, J. *Preparative Carbohydrate Chemistry*, Dekker: New York, **1997**.


Glycoproteins, glycolipids, and glycophospholipids are major components of membranes. The oligosaccharide residues are responsible for intercellular recognition and interaction and they also act as receptors for proteins, hormones, and viruses and regulate immune reactions. These significant activities have attracted significant research interest in oligosaccharides and glycoconjugates. Unlike peptides and nucleotides, in which the information content is determined solely by the number and sequence of different monomer units, the information content of oligosaccharides is also determined by the site of coupling, the configuration of glycosidic linkage (α or β), and by the occurrence of branching. Thus, polymers made by carbohydrates can carry considerably more information per building block than proteins and nucleic acids. Therefore, the chemical synthesis of oligosaccharides is more complicated than that of other biopolymers. A major source of branched oligosaccharides is still isolated from natural resources. Unfortunately, the amplification methods—such as the polymerase chain reaction (PCR) for nucleic acids or bacterial expression systems for protein production—do not exist for glycoconjugates. Therefore, the overall progress in glycobiology has suffered from a lack of tools that would quickly provide a diverse library of oligosaccharides in a similar fashion to ones that are currently available for nucleic acids and amino acids. Classically all solution-phase carbohydrate synthetic methodologies are relatively complicated and time-consuming, therefore oligosaccharide synthesis requires more expertise and has been mostly performed in specialized laboratories. There have been some recent advances in the development of modern sequencing methods such as the development of protocols toward solution-phase and solid-phase oligosaccharide synthesis. However, more advances in the oligosaccharide synthesis are
necessary to drive the development of diagnostic tests, vaccines and carbohydrate therapeutics\textsuperscript{7}. The synthesis of carbohydrates has been pursued for more than a century, and many oligosaccharides can now be synthesized, albeit with considerable effort\textsuperscript{8}. This tremendously hampers the exploration of oligosaccharide biological studies. One can argue that a fully automated oligosaccharide synthesis process must be developed.\textsuperscript{9} However for an efficient automated synthesis there is a need for a large number of oligosaccharide building blocks (monomers) and the methodology to connect them to the oligomers. Applying polymer-supported syntheses in the preparation of oligosaccharides has its drawbacks. For oligosaccharide synthesis, the main problem is associated with use of an excess of the expensive carbohydrate building blocks that are required to drive the heterogeneous reaction to completion, and it is very difficult to monitor the sugar–sugar coupling process by normal characterization methods such as TLC, NMR and mass spectrometry. Furthermore, the heterogeneous nature of the insoluble polymers and restricted reaction conditions often result in non-linear reaction kinetics, unequal distribution, difficult access to the reaction sites, improper solvation and inefficient coupling rates to name a few.\textsuperscript{10}

In order to overcome some of these problems, new synthetic methodology with TAG molecules\textsuperscript{11} was proposed that would take into account the advantages of both polymer supported reactions (such as easy purification) and all solution chemistry (such as easy reaction monitoring). Since the preparation of branched oligosaccharides is a difficult task, we have employed the use of a tagged glucose that can be used as a key building block for various branched oligosaccharides (Fig. 1). Once we synthesize the oligosaccharide building block, other saccharide units can be added in positions 1, 2, and 3 selectively.\textsuperscript{12} (Fig. 5.1)
Figure 5.1 Proposed tagged glucose as a synthone for preparation of branched oligosaccharide.

The tag attachment should give the tagged saccharide desirable physical properties that saccharide by itself does not have, such as low moisture sensitivity, solubility in low polarity organic solvents, easy purification, and easy reaction monitoring and structure elucidation. In addition the tag attachment should be stable under reaction conditions required to prepare oligosaccharides, and at the same time easily removed once the final reaction is completed and the product is purified.

In general, a tag is a group of atoms or a molecular fragment that is attached to a molecule for identification\textsuperscript{13}. However in some instances a tag is used to give a particular nature to a molecule. Several types of tags, such as affinity tags and fluorescent tags have been widely used in field of chemistry and biochemistry. A tag that facilitates phase separation is called a phase tag. A phase tag is a functional group attached to a molecule to control the favorable phase of the parent molecule in phase separation. In other words a phase tag changes the natural phase affinity of the molecule, which makes it easy to separate tagged molecule, that have strong interaction to a phase determined by the tag, from other untagged molecule which have natural phase affinity.
Generally, a monophasic system is advantageous for reactions although a biphasic (or multiphasic) system is essential for phase separation (Scheme 5.1). Heterogeneous reactions usually proceed less effectively than the homogeneous counterparts. The most popular way of solving this problem is the use of a solvent that dissolves both tagged compound and untagged compounds. After reaction is finished, the solvents are changed to form a biphasic system. Sometimes temperature-dependent miscibilities or solubilities are used to switch from monophasic to biphasic system.\textsuperscript{14-16}

Substrate tags should meet the following requirements\textsuperscript{17}:

- It should be easily introduced to a starting molecule (tagging).
- It should be stable under the conditions of desired chemical transformations.
- It should not interfere with the desired chemical transformations.
- It should effectively change the phase affinity of the molecule to ensure effective phase separation.
- After the transformation is completed, a tag should be removable from the product without affecting other functional groups in the molecule (detagging).
5.1: Synthesis of TAG molecules:

5.1.1 Synthesis of phthalimides and naphthalimides: Microwave-assisted preparation of several cyclic imides was performed with four different cyclic anhydrides. All the reactions are significantly faster and the isolated yields are significantly higher compared to conventionally heated reactions. Cyclic imides\(^\text{18}\) play an important role in organic syntheses and in medicinal chemistry. For instance, cyclic imides, particularly phthalimides, have been widely used as amino acid protection groups\(^\text{19}\) and have attracted considerable attention in medicinal chemistry.\(^\text{20}\) Maleimides are important constituents of peptide-conjugate haptens, antibody–antibody conjugates, immune conjugates, and enzyme inhibitors.\(^\text{21}\) Recently, a solvent-free procedure using TaCl\(_5\)-silica gel as a catalyst was described for the preparation of imides under microwave irradiation.\(^\text{22}\) Sandhu and co-workers advocated the use of a more eco-friendly solvent-free system, involving the reaction of equal amounts of anhydride and amines or amino acids in the absence of a solvent in a domestic oven without any catalyst.\(^\text{23}\) In the case of the reaction of the anhydride with amino acids, a reaction between two solids was involved, for example between phthalic anhydride and glycine. Unfortunately, no temperature measurements were reported.\(^\text{23}\)

In order to address these limitations we have developed microwave-assisted preparation of cyclic imides in polar solvents such as DMF and pyridine from cyclic anhydrides and corresponding amines. In the course of our microwave-assisted reaction studies, we explored the preparation of phthalimides from phthalic anhydride and the corresponding amine in DMF as a solvent. The magnetron power for microwave was adjusted for gentle solvent refluxing. After the reaction was completed, the product was isolated by ice-water precipitation from the hot reaction mixture. The reaction time and isolated yields are presented in Table 5.1. Using conventional reaction methods, time taken for transformation into the corresponding imides ranged from 12 to
16 h (Table 5.1). However, utilizing the new microwave-assisted method of preparation gave quantitative yields of product in no more than 1 h (Table 5.1).

Table 5.1 Preparation of phthalimides

```
\[ \text{phthalic anhydride} + \text{H}_2\text{N-R} \rightarrow \text{N-R} \]

1: \( R = 4\text{-CH}_3\text{C}_6\text{H}_4 \), 2: \( R = \text{C}_6\text{H}_5 \)
3: \( R = 3\text{-BrC}_6\text{H}_4 \), 4: \( R = 2\text{-C}_6\text{H}_5\text{-C}_6\text{H}_4 \)
5: \( R = \text{CH}_3(\text{CH}_2)_4\text{CH}_2 \), 6: \( R = \text{CH}_3(\text{CH}_2)_5\text{CH}_2 \)
```

<table>
<thead>
<tr>
<th>Compound</th>
<th>Imide</th>
<th>Time (min)</th>
<th>Power (W)</th>
<th>Yield (%)</th>
<th>Time(^a) (hr)</th>
<th>Yield(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Imidazole 1]</td>
<td>60</td>
<td>300</td>
<td>94</td>
<td>16</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>![Imidazole 2]</td>
<td>60</td>
<td>300</td>
<td>95</td>
<td>16</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>![Imidazole 3]</td>
<td>30</td>
<td>300</td>
<td>97</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>![Imidazole 4]</td>
<td>60</td>
<td>450</td>
<td>85</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>![Imidazole 5]</td>
<td>40</td>
<td>450</td>
<td>95</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>![Imidazole 6]</td>
<td>40</td>
<td>450</td>
<td>90</td>
<td>16</td>
<td>63</td>
</tr>
</tbody>
</table>

\(^a\) Conventional heating in toluene as a solvent catalytic amount of TEA
For conventional preparation of 1 toluene is the superior solvent in comparison to DMF in regards to both the reaction time and isolated yield. For instance, after refluxing the DMF solution for two days the isolated yield of 1 was only 45%. This is not an unusual finding because the preparation of aromatic and aliphatic phthalimides under conventional heating usually requires prolonged time in solvents such as toluene, acetic acid.\textsuperscript{26} There are also reports of microwave-assisted synthesis without solvent.\textsuperscript{26} However, the no-solvent approach can only be successful if one of the reactants absorbs microwave irradiation and has relatively low melting point. On the other hand, our procedure is applicable to broad range of amines regardless of their physical state or microwave radiation absorption because our solvent (DMF) is an excellent microwave reaction media, as it was demonstrated on the examples of aromatic amines (1-4) and aliphatic amines (5 and 6, Table 5.1) and microwave-assisted reaction is superior to conventional reaction.

In general, five-membered cyclic anhydrides are more reactive than six-membered cyclic anhydrides. Therefore, it does not come as a surprise that microwave-assisted reaction conditions used for the preparation of cyclic imides with 1,8-naphthalic anhydride are more demanding than those with phthalic anhydride, where the reaction time is more than quadruples. However by replacing DMF as the solvent with pyridine, the isolated yield was higher and the reaction time was shortened (Table 5.2). The isolation of the product requires quenching the reaction with water or with aqueous hydrochloric acid followed by crystallization of the product. As demonstrated in Table 5.2, the isolated yields are higher and the reaction times are shortened by 10–20 times in the case of microwave-assisted preparation.
Table 5.2 Synthesis of 1,8-naphthylimides\textsuperscript{26}

![Image of 1,8-naphthylimides](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Imide</th>
<th>Time (hr)</th>
<th>Power (W)</th>
<th>Yield (%)</th>
<th>Time\textsuperscript{a} (hr)</th>
<th>Yield\textsuperscript{a} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>4</td>
<td>300</td>
<td>70</td>
<td>48</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>2</td>
<td>450</td>
<td>90</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4</td>
<td>300</td>
<td>65</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>2</td>
<td>450</td>
<td>95</td>
<td>24</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>1</td>
<td>450</td>
<td>93</td>
<td>24</td>
<td>50</td>
</tr>
</tbody>
</table>

Note: different power for DMF and pyridine is required for reflux due to difference in their polarity.

\textsuperscript{a} With conventional heating

5.1.2 Synthesis of Succinimides:

We were not able to prepare succinimide by simple microwave heating in either DMF or pyridine solution of succinic anhydride and the corresponding amine. The major product was the corresponding open amide acid that, upon continued microwave heating, decomposed. Even when the DMF reaction mixture was microwaved for 8 hrs, only traces of the product were detected with substantial amount of decomposition material. However, if the DMF reaction mixture was first microwaved for a short time and then a water-removing reagent, such as DCC was added followed by short microwave irradiation, then the corresponding succinimide was successfully prepared.

To simplify the isolation and purification of the product, acetic anhydride was used as a dehydration reagent instead of DCC. The reaction was practically completed in 20 min. To eliminate the excess of the acetic anhydride, water was added and the reaction mixture was microwaved for an additional 20 min (Table 5.3). Considering the nature of the reaction
media (DMF + Ac₂O + H₂O), the product can be isolated by ice-water precipitation and purified by simple washing with water or aqueous hydrochloride. If necessary, further purification can be accomplished by column chromatography. The reaction conditions and isolated yields for some of the prepared succinimides are presented in Table 5.3.

**Table 5.3 Synthesis of succinimides**

\[
\text{Imide} + \text{H}_2\text{N}-\text{R} \xrightarrow{\text{Microwave}} \text{N}=\text{R}
\]

10: R = Benzyl; 11: R = (CH₂)₆; 12: R = 4-methylphenyl; 13: R = 4, 4'- methanedi phenyl; 14: R = 1-naphthyl

<table>
<thead>
<tr>
<th>Entry</th>
<th>Imide</th>
<th>Yield(^a) (%)</th>
<th>Time(^b) (hr)</th>
<th>Yield(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Imide1" /></td>
<td>91</td>
<td>24</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Imide2" /></td>
<td>84</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Imide3" /></td>
<td>85</td>
<td>16</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Imide4" /></td>
<td>92</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Imide5" /></td>
<td>88</td>
<td>24</td>
<td>35</td>
</tr>
</tbody>
</table>

\(^a\) All microwave reactions were performed with a power of 300W and reaction time 30+30+20 minutes.

\(^b\) For conventional method only heating with acetic anhydride was reported.
5.1.3 Synthesis of Maleimides:

The described procedure that was so successful for the preparation of both aliphatic and aromatic succinimides cannot be accepted as a general procedure for the preparation of maleimides. In many instances, using this preparation approach generated a complex mixture with majority (more than 60%) of the maleimide as a product. Unfortunately, the formed byproducts make the isolation and purification of the maleimide difficult. However this approach is still applicable for the preparation of simple aromatic maleimides such as phenyl and naphthyl maleimides (Table 5.4). In the case of aliphatic maleimides, the conversion of the amine into maleimide was around 60% therefore a new microwave-assisted preparation strategy was required.

Maleic anhydride followed by the ring closure in acetic anhydride with a catalytic amount of acetic acid. Isolated yields of the maleimides range around 50%. In our microwave-assisted preparation approach we also applied the two-step synthesis (Method B Table 10). In the first step, tetrahydrofuran (THF) solutions of the corresponding amine and maleic anhydride were mixed at room temperature. The reaction was completed after a few minutes. The formed precipitate was mixed with acetic anhydride and a catalytic amount of sodium acetate and then microwaved at 450 W. The reaction conditions and isolated yields are presented in Table 5.4.
Table 5.4 Synthesis of Maleimides²⁶

\[ \text{Imide} + \text{H}_2\text{N}-\text{R} \xrightarrow{\text{Microwave A or B}} \text{Imide} \]

Method A: DMF, Ac₂O, H₂O  
Method B: a) THF b) Ac₂O-AcONa

<table>
<thead>
<tr>
<th>Entry</th>
<th>Method</th>
<th>Imide</th>
<th>Time (min)</th>
<th>Power (W)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>![Imide1]</td>
<td>70</td>
<td>450</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>![Imide2]</td>
<td>70</td>
<td>450</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>![Imide3]</td>
<td>30</td>
<td>450</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>![Imide4]</td>
<td>30</td>
<td>450</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>![Imide5]</td>
<td>30</td>
<td>450</td>
<td>93</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>![Imide6]</td>
<td>30</td>
<td>450</td>
<td>85</td>
</tr>
</tbody>
</table>

15: R = Phenyl;  16: R = 1-naphthyl  
17: R = 4-methylphenyl;  18: R = bis(4-phenylene)methane
We have explored the effects of microwave irradiation on the preparation of cyclic imides. Given the possibility to control the power of microwave radiation, it was possible to use microwave irradiation for the preparation of cyclic imides that require microwave radiation in the range of a few minutes to a few hours. This approach is superior to conventional synthetic approach in both isolated yield of the product and required reaction time.
5.2 Microwave-assisted NBS bromination of \( p \)-iminotoluenes:

A simple, efficient, and rapid microwave-assisted method for the preparation of protected \( p \)-bromomethyl and \( p \)-dibromomethylanilines was developed as new alcohol, thiol, and amine protection groups. The procedure involves microwave-assisted NBS radical bromination of readily available \( N \)-protected \( p \)-toluidine. The microwave-assisted radical bromination was found to be superior to the conventional NBS radical bromination.

We selected cyclic imides of \( p \)-aminobenzyl and \( p \)-aminobenzylidene bromides as ideal protection groups for the purposes of our natural product syntheses. Cyclic imides are compounds that crystallize well and are relatively stable in acid, neutral, and dry basic reaction media. When these groups are part of the protected compound either in the ether, thioether, or amine form, they are relatively stable in the above mentioned reaction conditions. The major advantage of these benzyl based protection groups over others that their acid sensitivity can be significantly increased by removal of imides protection using hydrazine or a base. The resulting \( p \)-amino benzyl ethers, thioethers, or amines are easily cleaved with moderate acids. For alcohols, the corresponding phenylmethyl bromide \( \text{A} \) is an ideal protection group, while for 1, 2- and 1, 3-diols the phenylmethylene dibromide \( \text{B} \) is the ideal protection group and finally, for thiols and amines, the triphenylmethyl bromide \( \text{C} \) is the ideal protection group (Figure 5.2). 26-28

![Diagram](image)

**Figure 5.2** New alcohol, thiol, and amino protection groups
One of the requirements for broad use of these protection groups in natural product synthesis is that they be available in large amounts either commercially or through simple preparation procedures. The classical approach to prepare benzyl bromides and benzyldene dibromides is through the direct bromination of the benzylic methyl, or through deoxybromination of benzylic alcohol or benzaldehyde derivatives. The latter route implies a multistep synthesis, which is often time consuming. Alternatively, the two-step bromination method, via the generation of the methyl anion developed by Fraser, appears attractive. However, this method is incompatible with the presence of nucleophile sensitive functional groups on the molecule, such as an ester group.

Radical mediated bromination is frequently used to achieve selective activation of the benzyl and allyl positions of an organic molecule. Using N-bromosuccinimide (NBS) in tetrachloromethane with various radical initiators normally carries out the reaction. However, tetrachloromethane presents a relatively high toxicity and carcinogenicity, which restricts its use in general synthesis. Other solvents, such as methyl acetate, in light and some microwave-assisted benzylic brominations, replaced tetrachloromethane but to avoid using it, Golding and coworkers used (trifluoromethyl) benzene as a solvent in the benzylic bromination with NBS. Subsequently, Ulrich and coworkers found that dichloromethane and benzene are better solvents for radical bromination than carbontetrachloride.
5.2.1 Microwave-assisted NBS mono-bromination of \( p \)-iminotoluenes:

Our microwave-assisted NBS bromination was performed in benzene as solvent instead of tetrachloromethane with only one equivalent of NBS. The reaction is basically over in less than two hours and the isolated yields are higher than 80% (Table 11). By comparison, if the reaction is performed under conventional methods (refluxing of benzene) then the reaction requires a reaction time 3-10 times longer. Longer reaction times generate more byproducts that hamper the isolation and purification of the final product, resulting in lower isolated yields. In addition, for conventional NBS bromination, more than one equivalent (1.3-1.5 eq.) of NBS is required for full conversion (disappearance of starting material).

On the other hand if carbon tetrachloride is used as a solvent, then the required reaction time is more than doubled in comparison with benzene as a solvent. For instance, seventeen hours is required for the preparation of 19 in benzene (Table 5.5). For example, to shorten reaction times to two hours for compound 19 (Table 5.5) using more conventional reaction conditions, a combination of both carbon tetrachloride refluxing and ultraviolet radiation was used.\(^{40}\) These examples only emphasize superiority of microwave-assisted NBS benzyl bromination in benzene as solvent (Table 5.5).
19: $R^1$-$R^2$ = 1,2-$C_6H_4$; 20: $R^1$-$R^2$ = $CH_2CH_2$
21: $R^1$-$R^2$ = 1,8-$C_{10}H_8$; 22: $R^1$ = $C_6H_5$, $R^2$ = $CH_3$
23: ortho isomer of 19

Table 5.5 Preparation of benzyl bromides$^{24}$

<table>
<thead>
<tr>
<th>Product</th>
<th>Microwave</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (min.)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>30</td>
<td>85</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>40</td>
<td>83</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>60</td>
<td>87</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td><img src="image.png" alt="image" /></td>
<td>90</td>
<td>82</td>
</tr>
</tbody>
</table>
5.2.2 Microwave-assisted NBS di-bromination of \( p \)-iminotoluenes:

Microwave-assisted NBS dibromination is as effective as monobromination (Table 12) and this method again is superior, considering that the usual isolated yields for dibromination are between 5-40%\(^{38}\). Furthermore, using conventional methods, the reaction mixture always contains mixture of dibromo and monobromo products and require more than one day to complete and at least three equivalents of NBS. In the case of the conventional heating, the benzene solution was refluxed for at least 12 hours and a small amount of monobrominated product was still present. The isolated yields are somewhere between 60-70%\(^{39}\).

Microwave-assisted reaction requires only a few hours with more than 95% conversion into dibromo product and the isolated yields are between 80 and 90% (Table 5.6). This is the cleanest, simplest, shortest, and the highest yielding procedure for preparation of dibromomethylarene derivatives from methylarenes.

Finally, benzyl NBS bromination can be performed in environmentally friendly solvents such as ethyl acetate and diethyl carbonate\(^{25}\). For instance, if NBS radical bromination was performed in ethyl acetate instead of benzene for 24 (Table 5.6), then the required reaction time was slightly longer (one hour) and the isolated yield moderately lower (75%). However, if the NBS bromination was performed in DMF as a reaction media, then the reaction was completed in 10 minutes but isolated yield is only 60%.
Table 5.6. Isolated yields of benzylidene bromides$^{24}$

<table>
<thead>
<tr>
<th>Product</th>
<th>Microwave</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (hrs)</td>
<td>Yield (%)</td>
</tr>
<tr>
<td><img src="image1" alt="Chemical Structure 1" /></td>
<td>1</td>
<td>88</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical Structure 2" /></td>
<td>3</td>
<td>83</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure 3" /></td>
<td>3</td>
<td>92</td>
</tr>
</tbody>
</table>

In conclusion, it can be stated that with using this new NBS radical benzyl bromination method, desirable bromomethyl and dibromomethylarenes can be prepared from readily available methylarenes in high quantities and short reaction times coupled with almost quantitative isolated yields. This synthetic approach was proven to be superior to conventional NBS bromination.
5.3 Synthesis of aldehyde based tag molecules:

Dibromomethylarenes were also key precursor for synthesis other tag molecules which could be used for 1,3-diol protection, for instance corresponding aldehyde and dimethyl acetals. They were hydrolyzed by pyridine/water to corresponding aldehyde in quantitative yield (Scheme 5.2).

![Scheme 5.2 Synthesis of aldehyde based tag molecules](image)

Aldehyde based tag molecules were also transformed into corresponding methyl acetal in presence of cobalt chloride, which could be used as potential tag molecule for sugars as 4,6-acetal protection (Scheme 3). It's well known that 4,6-bezylacetal protection of sugars are done by dimethoxylbenzaldehyde in presence of camphor sulfonic acid. We wanted to use similar strategy to attach tag molecules to sugar hence synthesizing dimethoxy acetals seemed obvious.

![Scheme 5.3 Synthesis of dimethoxyactal tag molecules](image)
5.4 Tagged glucose for the synthesis of branched oligosaccharides

The tag attachment to the sugar our next step which should give the tagged saccharide desirable physical properties that saccharide by itself does not have, such as solubility in low polarity organic solvents, easy purification, and easy reaction monitoring and structure elucidation. In addition the tag attachment should be stable under reaction conditions required to prepare oligosaccharides, and at the same time easily removable once the final reaction is completed and the product is purified.

Several variations of these types of tag molecules were explored and phthalamide derivatives of p-toluidine (Fig. 5.3) have proven to be the best in compliance with these required conditions. These compounds are crystalline and are easily purified by crystallization; they are stable in acidic media, which is commonly required for glycosylation\(^\text{38}\), but not stable in a base and can be removed from newly formed oligosaccharide at the same time when ester groups (OH protection) of oligosaccharides are hydrolyzed. Additionally, the remaining part of the tag (p-aminobenzyl group) can easily be removed upon neutralization of the reaction mixture. The thiophenyl group in position 1 is also a protection group that upon oxidation becomes a good leaving group and the whole molecule is now a good glycoside donor.\(^\text{39}\)

There are two very promising sites to attach tag molecule to sugar molecule, First being the anomeric position which the most reactive center and second is 4,6-diol protection utilizing selectivity towards reaction to primary alcohol group (Fig.5.3). We need to have 1, 4 and 6-hydroxy groups protected in order to have possibility to do glycosylation at 2 and 3 position for the synthesis of branched oligosaccharides.
Since we have developed synthesis of 1-thioisopropanol-glucopyranose in our initial attempt for synthesis of branched oligosaccharides it was decided that 4,6-diol protections by our tag molecule could be very easy way to attach it to sugar. However our initial attempts to attach tag molecule to sugar using acid catalyzed reaction by camphor sulfonic acid by $27-28$ and $30-31$ were not successful. In case of coupling of $27-28$ no attachment with sugar was observed even after 18hrs of reaction while in case of $30-31$ the hyrdolysis of tag molecule to corresponding aldehyde to give $27-28$ was observed. Our failure with acid catalyzed attachment of tag molecules to sugar had forced us to look for alternative strategy and we decided to go with based catalyzed coupling using dibromomethylarenes itself. There is literature report of such kind of reaction with not very impressive yield. We were able to attach tag molecule to sugar by refluxing with pyridine but with not more than 40% of isolated yield, the major product of the reaction was the hydrolyzed tag to corresponding aldehyde (Note- this is one of the way to make aldehyde based tag molecules). It was speculated that moisture content in pyridine was the cause behind the major side reaction hence we decided to use freshly distilled pyridine for the reaction. It did help to improve the yield a little bit but not good enough for synthesizing these tagged sugars in large-scale. The reaction yield was down to 20% in case of naphthyl based tag.
molecules compared to 40% in phthalyl based tag molecules, probably because of poor reactivity of former towards base catalyzed coupling to the sugar.

Our focus changed after several failure attempts towards other point of attachment which is the anomeric position. Thiol based tag molecule was synthesized by reacting chlorosulfonic acid and phosphorus pentachloride to cyclic imide 2 to get corresponding para-substituted sulfonyl chlorides (32), which could be reduce to corresponding thiols 33 by zinc metal catalyzed acidic reduction (Scheme 5.4).

Scheme 5.4 Synthesis of thiol based tag molecule

Thiol based tag molecule 33 could be to attached to the anomeric position of sugar in a similar fashion as thioisopropyl group was introduced (Scheme 4.10) by Lewis acid catalyst BF$_3$OEt$_2$ in dry dichloromethane. However, we were not successful in attaching our tag molecule to the sugar may be because it was not reactive enough to attack the nucleophilic anomeric carbon, as there was no reaction even after 8 hrs of stirring at room temperature by BF$_3$OEt$_2$ in dry dichloromethane.

Starting with D-glucose (34) all hydroxyl groups were protected with an acetyl group.$^{41}$ The anomeric acetyl group of 35 was substituted by thiophenol in methylene dichloride using borontrifloride acidic catalyst to get compound 36 in 96% yield.$^{42}$ Acetyl protection groups were removed in almost quantitative yields with sodium methoxide in dry methanol to give compound 37.$^{43}$ Same compound was also prepared from D-glucose and thiophenyl in aqueous media by using 2-chloro-1,3-dimethylimidazolinium chloride as catalyst.$^{44}$

196
There are several classic methods for benzaldehyde protection of monosaccharide.\textsuperscript{43} It is now common to use dimethyl acetals of benzaldehyde derivatives in DMF and camphorsulfonic acid as the catalyst.\textsuperscript{45} A similar procedure to introduce our tag molecules with dimethyl acetals of aldehyde 27 was not successful. The main product of the reaction was the free aldehyde 27. To avoid this problem, a study for preparation of tagged monosaccharide 38 from 37 and benzyl dibromides 24 in pyridine as reaction media was used. Although there is some literature evidence that supports this synthetic approach\textsuperscript{46} in our case isolated yields were around 40\% at best. Therefore, this approach was not applicable to large-scale preparation of tagged monosaccharides. Considering that in the process of tagging 37 with dimethylacetal of 27, the corresponding aldehyde 27 was detected, we developed a method of monosaccharide tagging with the aldehyde 27 (Scheme 5.5). To simplify and make the reaction more economic, p-toluenesulfonic acid monohydrate was used as an acid catalyst with a minimal amount of DMF to make the benzene solution homogeneous. The reaction was carried with benzene reflux and a Soxhlet extraction apparatus filled with anhydrous calcium chloride for the removal of water from azeotropically distilled benzene–water mixture. The reaction was completed after 3–4 h with isolated yield of tagged monosaccharide between 80\% and 90\%. The prepared tagged glucose corresponded to the properties that were initially sought after.
Scheme 5.5 Synthesis of activated and tagged glucose. Reagents and conditions: (i) (CH$_3$CO)$_2$O, Pyridine; (ii) C$_6$H$_5$SH/CH$_2$Cl$_2$/BF$_3$.OEt$_2$; (iii) CH$_3$ONa/CH$_3$OH; (iv) TAG-38,39,40/p-CH$_3$C$_6$H$_4$SO$_3$H/DMF-benzene; (v) C$_6$H$_5$SH+2-chloro-1,3-dimethylimidacolinium chloride in (C$_2$H$_5$)$_3$N/H$_2$O/CH$_3$CN.

Figure 5.4 $^1$H-NMR for Tagged monosaccharide key intermediate
To demonstrate the efficiency of our tag approach in the preparation of oligosaccharides, the simplicity of the reaction conditions, and the recyclability of tag aldehydes, we have demonstrated as example preparations, the synthesis of compounds 42, 43, and 44 (Scheme 5.6). All reactions were performed in a common organic solvent (dichloromethane).

![Scheme 5.6 Glycosylation, Oligosaccharide deprotection, and tag molecule removal. Reagents and conditions: (i) (CH₃)₃SiOSO₂CF₃/ CH₂Cl₂/(0°C to room temperature in 2hrs) 75%; (ii) NaOCH₂CH₂OH/CH₂Cl₂ (room temperature), yield 93%; (iii) CF₃COOH/CH₂Cl₂ (room temperature), yield 95%.](image)

The glycosylation was selectively performed with 41 in the C-3 position of the tagged monosaccharide 40 and our isolated yield was 75% using standard glycosylation procedures. Product 42 was purified by filtration of the reaction mixture through a short silica gel column using dichloromethane–ethyl acetate (3/1) as an elutent. Selective benzoate hydrolysis was started at 0 °C with sodium methoxide in a dichloromethane–methanol solution. The solution was allowed to stir at room temperature for 4 h, was evaporated and mixed with
dichloromethane–ethyl acetate and filtered through short column of silica gel. The tag molecule was removed with trifluoroacetic acid in dichloromethane. The formed precipitate was an oligosaccharide solution containing the tag aldehyde 27.
5.5 Conclusions

In conclusion, we have developed synthetic procedures for the preparation of tag reagents based on phthalimide of p-toluidine. Syntheses for cyclic imides were explored for a broad range of substrates including aliphatic and aromatic derivatives. The developed methods have potential to be used in large-scale industrial synthesis of cyclic imides very efficiently in a short period of time using microwave conditions.

Benzylic mono and di-bromination was successfully achieved under microwave condition using benzene as solvent which is a much better option compared to carbon tetrachloride, which is carcinogenic. The possibility to use environment friendly organic solvents like ethyl acetate and diethylcarbonate were also explored and found to be very effective for benzylic bromination by N-bromosuccinimide. All procedures are simple and materials can be obtained in large quantities.

Tagging monosaccharides with aldehyde based tag molecules is simple, and requires refluxing the DMF/benzene solution of the corresponding tag aldehyde and monosaccharide under Soxhlet extraction apparatus. Tagged monosaccharides can be glycosylated first in position 3, then 2, and finally 1 with the same or different saccharide building blocks. In the last step of the synthesis, the tag molecule can be selectively deprotected, isolated as an aldehyde, and reused in new oligosaccharide syntheses. Recyclibility of aldehyde tag molecules gives an advantage over other tag molecules we explored so far and seems to be best one among various other tag molecules synthesized. Model reactions to prove the applicability of tag strategy is amazing and we could selectively remove protecting groups on the tagged sugar depending on the next step in the synthesis. Over all we have developed very nice synthesis of tag molecules, their coupling to sugar (tagging) and selective removal (detagging).
5.6 Experimental

5.6.1 General Consideration. Chemicals were purchased from Sigma-Aldrich, Acros, and Merck Co. The $^1$H and $^{13}$C NMR spectra were run on Varian 400 MHz Unity in CDCl$_3$ or DMSO-d$_6$, as solvent and internal standards. Our laboratory microwave reactor was made by using magnetron (700W) from domestic microwave. The microwave cavity was reduced to 21.6 cm in height, 17.30 cm wide, and 25.4 cm deep with two 2.54 cm. The bottom of the microwave cavity was replaced with brass and magnetic stirrer was installed. On the top of the microwave two openings of one inch in diameter were made on onsite cavity and on the outside cover of the microwave reactor. The test performed shows the there is no microwave radiation above the openings. The power of the microwave radiation was controlled by direct wiring of variable autotransformer for control of the microwave magnetron. The power was monitored with ECM meter. The composition of the reaction mixture influence change of the ECM power. When reaction is completed the power measured with ECM meter become constant. Detail electronic, and mechanic schemes of the microwave reactor will be available in our new patent.

5.6.2 General procedure for synthesis of phthalimides (1-6):

In round bottom flask (100 ml) phthalic anhydride (1.5 g; 0.01 mol), corresponding amine (0.01) and N,N-dimethyformamide (20 ml) are mixed together. The round bottom flask with reaction mixture was put in our microwave reactor equiped with condense and magnetic stirrer. The reaction mixture stirred under microwave radiation with mild solvent refluxing (magnetron power between 300 and 450 W). After reaction was completed reaction mixture was poured onto crushed ice (~200 g). Formed white precipitate was separated by filtration, washed with water and dried under reduced pressure. According to the NMR spectra compounds are more than 96% pure. IF higher purity is required further purification can be performed by column
chromatography. The $^1$H-NMR and $^{13}$C-NMR spectra were recorded in CDCl$_3$ or in DMSO-d$_6$ and they are identical to authentic samples.

**4-methylphenylphthalimide (1):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.98 (d, 2H), 7.80 (d, 2H), 7.35 (s, 4H), 2.41 (s, 3H) ppm. $^{13}$C NMR $\delta$: 168.0, 138.2, 134.5, 132.0, 130.0, 129.5, 127.1, 124.0, and 21.5 ppm.

**Phenylphthalimide (2):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.98 (d, 2H), 7.80 (d, 2H), 7.35-7.60 (m, 5H) ppm. $^{13}$C NMR $\delta$: 168.0, 136.1, 134.5, 132.0, 129.5, 128.1, 126.5, and 124.0 ppm.

**3-Bromophenylphthalimide (3):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.98 (d, 2H), 7.80 (d, 2H), 7.62 (s, 1H), 7.55 (d, 1H), 7.40 (dd, 2H) ppm. $^{13}$C NMR $\delta$: 168.0, 136.1, 134.0, 132.2, 132.0, 131.5, 131.0, 127.1, 124.0, and 122.0 ppm.

**2-Phenylphenylphthalimide (4):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.82 (dd, 2H), 7.70 (dd, 2H), 7.5-7.6 (m, 2H), 7.40-7.52 (m, 2H), 7.21-7.38 (m, 2H), 6.85 (t, 1H), 6.80 (d, 2H) ppm. $^{13}$C NMR $\delta$: 168.0, 140.1, 134.0, 132.1, 130.8, 130.5, 128.8, 128.5, 128.0, 127.1, 124.0, 118.5 and 116.0 ppm.

**Hexylphthalimide (5):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.85 (dd, 2H), 7.65 (dd, 2H), 3.62 (t, 2H), 1.62 (m, 2H), 1.20-1.24 (s, 6H), 0.85 (t, 3H) ppm. $^{13}$C NMR $\delta$: 168.0, 134.0, 132.1, 123.5, 38.0, 32.0, 28.5, 26.1, 22.2 and 14.0 ppm.
**Hexadecylphthalimide (6):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.82 (dd, 2H), 7.65 (dd, 2H), 3.62(t, 2H), 1.62(m, 2H), 1.20-1.35(s, 11H), 0.82 (t, 3H) ppm. $^{13}$C NMR $\delta$: 168.0, 134.1, 132.1, 123.5, 38.0, 32.1, 30.0, 30.0, 29.9, 29.8, 29.7, 29.5, 29.0, 28.5, 28.0, 27.0, 23.1 and 14.0 ppm.

**5.6.3 General Procedure for synthesis of 1,8-naphthalimides (7, 8 and 9):**

Pyridine (100 ml) solution of anhydride (5mmol for 1,8-naphthalic anhydride and 2.5 mmol for dianhydride) and corresponding amine (8mmol) were microwave heated at magnetron power of 450W with gentle solvent refluxing. Cold reaction mixture was poured over crushed ice and formed white precipitate was separated by filtration and washed with water (3x20ml). Solid material was dissolved in dichloromethane (100 ml), this solution was washed with 10% hydrochloric acid (3x50 ml), water (3x50 ml), and dried over anhydrous sodium sulfate. After evaporated of dichloromethane 96% pure product was obtained.

**Phenylnaphthalimide (7):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.65 (d, 2H), 8.24 (d, 2H), 7.80 (t, 2H), 7.42-7.60(m, 3H), 7.35(d, 2H) ppm. $^{13}$C NMR $\delta$: 164.1, 136.1, 134.5, 132.0, 130.1, 129.2, 128.2, 128.1, 128.0, 127.5 and 123.5 ppm.

**4-Methlyphenylnaphthalimide (8):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.65 (d, 2H), 8.24 (d, 2H), 7.80(t, 2H), 7.20(d, 2H), 2.42(s, 3H) ppm. $^{13}$C NMR $\delta$: 164.1, 128.1, 125.5, 125.0, 124.0, 123.1, 122.0, 121.1, 119.5, 118.0, 116.1 and 31.1 ppm.
2,7-dip-tolylbenzo[3,8]phenanthroline-1,3,6,8-tetraone (9): $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.82 (s, 4H), 7.38(d, 4H), 7.20(d, 4H), 2.42(s, 6H) ppm. $^{13}$C NMR $\delta$: 164.1, 140.2, 132.1, 131.8, 130.4, 128.2, 128.0, 127.2 and 22.1 ppm.

5.6.4 General method for synthesis of succinimidess (10-14)

DMF (20 ml) solution of succinic anhydride (2 g; 0.02 mol) and corresponding amine (0.02 mol for 11 and 13; 0.01 mol for 10, 12 and 14) was heated in microwave reactor with slow solvent refluxing for 30 minutes. Reaction mixture was cooled to room temperature and acetic anhydride (5.7 ml; 6.13 g; 0.06 mol) and gently refluxed in microwave oven for 20 minutes. Into room temperature cooled reaction mixture water (5 ml) was added and reaction mixture was gently refluxed in microwave oven for additional 20 minutes. Resulting reaction mixture was deluted with dichloromethane (100 ml). Resulting dichloromethane solution was washed with saturated water solution of sodium bicarbonate (3x50 ml), water (3x50ml) and dried over anhydrous sodium sulfate. After solvent was evaporated crude product was purified by column chromatography with ethylacetate-hexane (5:1) as an eluent.

**Benzylsuccinimide (10):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.21-7.40 (m, 5H), 2.61(s, 4H) ppm. $^{13}$C NMR $\delta$: 176.5, 135.05, 128.7, 128.5, 127.8, 44.2 and 28.1 ppm.

**Hexyl-1,6-disuccinimide (11):** $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.21-7.40 (m, 5H), 2.61(s, 4H) ppm. $^{13}$C NMR $\delta$: 176.5, 135.05, 128.7, 128.5, 127.8, 44.2 and 28.1 ppm.
4-methylphenylsuccinimide (12): \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.30\) (d, 2H), 7.15(d, 2H), 2.91(s, 4H), 2.38 (s, 3H) ppm. \(^{13}\)C NMR \(\delta: 176.9, 138.1, 130.2, 129.1, 126.0, 28.5\) and 21.3 ppm.

4, 4'- Methanediphenyldisuccinimide (13): \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 7.36\) (d, 4H), 7.21(d, 4H), 4.02 (s, 2H), 2.75(s, 8H) ppm. \(^{13}\)C NMR \(\delta: 177.5, 141.0, 130.8, 129.1, 127.1, 40.2, 39.9, 39.7, 39.5, 39.3, 39.1\) and 28.4 ppm.

1-Naphthylsuccinimide (14): \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta 8.01-8.06\) (t, 2H), 7.81( d, 1H), 7.51-7.65( m, 3H), 7.42 (d, 1H), 2.80- 3.08(m, 4H) ppm. \(^{13}\)C NMR \(\delta: 177.5, 137.7, 129.7, 129.6, 129.2, 128.2, 126.8, 126.6, 126.5, 125.6, 122.87, 39.7, 39.5, 39.2\) and 28.9 ppm.

5.6.5 General Procedure for synthesis of maleimides (15-18). Thetrahydrofuran (20 ml) solution of maleic anhydride (1g; 0.01 mol) and tetrahydrofuran solution (20 ml) of corresponding amine (1.1 equivalent for monoamine and 0.55 equivalent for diamine) was mixed together at room temperature. Immediately precipitate was formed. Reaction mixture was stirred at room temperature for 15 minutes, solvent was decanted and solid dried under nitrogen flow. Acetic anhydride (21 ml) and sodium acetate (1g) were added to the solid material. Reaction mixture was heated for 30 minutes in microwave oven with magnetron power of 450W. Cooled to room temperature, reaction mixture was in water (300 ml). Resulting mixture was extracted with ether (3x50 ml). Ether was dried over anhydrous sodium sulfate and evaporated to solid residue. Product was purified by column chromatography with 5% ethylacetate in dichloromethane.
Phenylmaleimide (15): $^1$H NMR (400 MHz, CDCl$_3$): δ 7.30-7.51 (m, 5H), 6.85(s, 2H) ppm. $^{13}$C NMR δ: 170.1, 132.5, 131.1, 130.0, 128.1 and 126.0 ppm.

1-Naphthylmaleimide (16): $^1$H NMR (400 MHz, CDCl$_3$): δ 7.81-8.02 (m, 2H), 7.45-7.60(m, 4H), 7.41(d, 1H), 6.98(s, 2H) ppm. $^{13}$C NMR δ: 170.15, 134.5, 130.5, 130.10, 129.0, 127.8, 127.1, 126.6, 126.1, 125.0 and 122.5 ppm.

4-MethlyPhenylmaleimide (17): $^1$H NMR (400 MHz, CDCl$_3$): δ 7.30(d, 2H), 7.21(d, 2H), 6.85(s, 2H), 2.42(s, 3H) ppm. $^{13}$C NMR δ: 169.9, 138.3, 134.4, 130.04,128.7, 126.2 and 21.3 ppm.

4, 4'- Methanediphenyldimaleimide (18): $^1$H NMR (400 MHz, CDCl$_3$): δ 7.20-7.32(m, 8H), 6.85(s, 4H), 4.02(s, 2H)ppm. $^{13}$C NMR δ: 169.9, 140.1, 134.2, 130.2, 129.8, 126.2 and 40.8 ppm.

5.6.6 Typical procedure for preparation of monobromoarene (19-23): Benzene (10 ml) solution of methyl benzene derivative (5 mmol), N-bromosuccinamide (0.89 g; 5 mmol), and benzyolperoxide (0.12 g; 0.5 mmol) was refluxed under microwave heating (magnetron power 600 W). After reaction was completed (See Table 1) solvent was evaporated; solid residue was dissolved in dichloromethane (100 ml) and washed with saturated water solution of sodium bicarbonate (3x15 ml) and water (3x15 ml). Dichloromethane was evaporated and the solid residue was purified by silica gel column chromatography with hexane-dichloromethane as an eluent.
4-(Bromomethyl)phenylphthalimide (19): Isolated yield (85%). $^1$H-NMR (CDCl$_3$): 7.98 (2H, m), 7.80 (2H, m), 7.55 (2H, d, J = 8Hz), 7.45 (2H, d, J = 8Hz), and 4.55 (2H, s) ppm. $^{13}$C-NMR (CDCl$_3$): 167.1, 140.0, 138.2, 135.1, 132.0, 130.2, 127.5, 124.3 and 33.0 ppm. C$_{15}$H$_{10}$BrNO$_2$ (MW 316.15). MS (m/z) 317.99 (10%), 314.99 (100%), and 316.99 (95%).

4-(Bromomethyl)phenylsuccimide (20): Isolated yield 83%. $^1$H-NMR (CDCl$_3$): 7.51 (2H, d, J = 7.6 Hz), 7.32 (2H, d, J = 7.6Hz), 4.48 (2H, s) ppm. $^{13}$C-NMR (CDCl$_3$): 176.0, 138.2, 130.2, 127.5, 126.1, 33.0 and 28.1 ppm. C$_{11}$H$_{10}$BrNO$_2$ (MW 268.11). MS (m/z) 269.99 (8%), 268.99 (95%), 266.99 (100%).

4-(Bromomethyl)phenylnapthalimide (21): Isolated yield (87%). $^1$H-NMR (CDCl$_3$): 8.65 (2H, d, J = 7.2 Hz), 8.27 (2H, d, J = 8.4 Hz), 7.80 (2H, t, J = 7.2 Hz), 7.58 (2H, d, J = 8.4 Hz), 7.30 (2H, d, J = 8.4 Hz), and 4.57 (2H, s) ppm. $^{13}$C-NMR (CDCl$_3$) 164.1 , 138.2, 135.0, 134.3, 131.2, 130.2, 129.5, 127.1, 123.0, and 33.0 ppm. C$_{19}$H$_{12}$BrNO$_2$ (MW 366.21) m/z: 368.01 (15%), 367.00 (100%), 365.01 (100.0%), 366.01 (10%)

4-(Bromomethyl)-N-benzoyl-N-methylaniline (22): Isolated yield (80%). $^1$H-NMR (CDCl$_3$): 7.28 (2H, d, J = 7.8Hz), 7.25 (7H, m), 7.02 (2H, d, J = 7.8Hz), 4.40 (2H, s), and 3.47 (3H, s) ppm. $^{13}$C-NMR (CDCl$_3$): 171.0, 145.2, 136.1, 135.5, 130.2, 129.1, 128.0, 127.6, 39.4, and 33.0 ppm. C$_{15}$H$_{14}$BrNO (MW 304.18). MS (m/z) 306.03 (10%) 305.02 (90%), 303.03 (100).

2-(Bromomethyl)phenylphthalimide (23): Isolated yield (82%). $^1$H-NMR (CDCl$_3$): 7.98 (2H, m), 7.80 (2H, m), 7.30 (4H, m), and 4.46 (2H) ppm. $^{13}$CNMR (CDCl$_3$): 167.0, 136.1, 135.2,
Typical procedure for preparation of dibromoarene 24-26: Benzene (10 ml) mixture of the methylbenzene derivative (3.3 mmol), N-bromsucinimide (1.8 g; 10 mmol), and benzoylperoxide (75 mg; 0.31 mmol) was refluxed for three hours under microwave radiation (magnetron power 600W). The solvent was evaporated under reduced pressure and the solid residue was dissolved in dichloromethane (100 ml). Dichloromethane solution was washed with saturated sodium bicarbonate (3x15 ml), water (3x20 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated and residue purified by filtration through short silica gel column with hexane-dichloromethane as solvent.

4,4’-(dibromomethyl)phenylphthalimide (24): Isolated yield (88%). $^1$H-NMR (CDCl$_3$): 7.98 (2H, m), 7.82 (2H, m), 7.72 (2H, d, $J$ = 8.0 Hz), 7.51 (2H, d, $J$ = 8.0 Hz), and 6.68 (1H, s) ppm. $^{13}$C-NMR (CDCl$_3$): 167.1, 141.0, 135.3, 133.2, 131.0, 128.5, 127.1, 124.0 and 40.2 ppm. C$_{15}$H$_9$Br$_2$NO$_2$ (MW 395.05). MS (m/z) 396.90 (50%), 395.90 (10%), 394.90 (100%), 392.90 (50%).

4,4’-(dibromomethyl)phenylsuccinimide (25): Isolated yield (83%). $^1$H-NMR (CDCl$_3$): 7.68 (2H, d, $J$ = 7.4 Hz), 7.32 (2H, d, $J$ = 7.4 Hz), 6.64 (1H, s), and 2.99 (4H, s) ppm. $^{13}$C-NMR (CDCl$_3$): 178.1, 133.0, 128.5, 127.2, 126.3, 40.0 and 28.1 ppm. C$_{11}$H$_9$Br$_2$NO$_2$ (MW 347.00) m/z 348.90 (50%), 347.90 (10%), 346.90 (100%), 344.90 (50%).
4,4’-(dibromomethyl)phenylnaphthalimide (26): Isolated yield (92%). $^1$H-NMR (CDCl$_3$): 8.82 (2H, t, J = 8 Hz), 8.48 (2H, d, J = 8 Hz), 7.83 (2H, t, J = 8 Hz), 7.75 (2H, d, J = 7.8 Hz), 7.36 (2H, d, J = 7.8 Hz), and 6.72 (1H, s) ppm. $^{13}$C-NMR (CDCl$_3$, 400 MHz) 164.2, 142.0, 137.5, 135.1, 132.0, 129.3, 128.1, 127.0, 123.2 and 40.0 ppm. C$_{19}$H$_{11}$Br$_2$NO$_2$ (MW 445.10). MS (m/z) 447.91 (10%), 446.9 (50%) 445.9 (20%), 444.9 (100%), 443.9 (10%). 442.9 (50%).

5.6.8 Penta-O-acetyl-D-glucopyranose (35): D-glucose (20 g, 110 mmol) was refluxed in pyridine (150 mL) for 1 hour. Acetic anhydride (100 mL) was added and the mixture was refluxed an additional 2 hours. The resulting solution was cooled to room temperature, poured over ice (300 mL) and stirred until the product solidified, about 2 hours. The solid was filtered, washed with water, and recrystallized from ethyl acetate/hexanes to give 35 as a white solid (36 g, 83 % yield).: $^1$H NMR of 35 (400 MHZ, CDCl$_3$): δ 5.72 (d, J = 4.0 Hz, 1H), 5.26 (t, J = 9.2 Hz, 1H), 5.14(t,J=8.8Hz,1H),5.13(t,J=10.0Hz,1H),4.30(dd,J1 =6.2Hz,J2 =2.2Hz,1H), 4.12 (dd, J1 = 6.3 Hz, J2 = 1.0 Hz, 1H), 3.84 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H). $^{13}$C NMR of 35 (400 MHz, CDCl$_3$): δ 170.7, 170.3, 169.8, 169.5, 168.9, 89.1, 69.9, 69.2, 67.9, 61.5, 20.9, 20.8, 20.7, 20.6 and 20.5 ppm.

5.6.9 Phenyl tetra-O-acetyl-1-thio-β-D-glucopyranoside (36): Penta-O-acetyl-β-D-glucopyranose 35 (3.7 g, 9.4 mmol) and thiophenol (1.0 mL, 13 mmol) were stirred at room temperature in CH$_2$Cl$_2$ with 3 Å molecular sieves for 30 minutes under an atmosphere of nitrogen. BF$_3$·OEt$_2$ (3.5 mL) was added and the mixture was stirred an additional 2 hours. The molecular sieves were filtered and the filtrate was added to 5 % NaHCO$_3$ (50 mL) with vigorous stirring. The organic layer was separated, washed with 5 % NaHCO$_3$ (3 x 50 mL), and dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting solid was
used immediately in the next reaction without further purification. 1H NMR (400 MHz, CDCl₃):

δ  7.48-7.32 (m, 5H), 5.11 (t, J = 9.6 Hz, 1H), 4.94(t, J=9.6Hz,1H),4.86(t,J=9.6Hz,1H),4.49(d,J=5.2Hz,1H),4.11(dd,J1 = 6.2Hz,J2 =2.8Hz,1H),4.00(dd,J1 =6.0Hz,J2 =1.2Hz,1H),3.61(m,1H),3.05(m, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H). 13C NMR (400 MHz, CDCl₃): δ 170.6, 170.2, 169.4, 169.3, 120.1, 118.3, 115.0, 105.2, 83.3, 75.6, 73.9, 70.1, 68.4, 62.2, 24.0, 23.7, 20.7 and 20.7 ppm.

5.6.10 Phenyl-1-thio-β-D-glucopyranoside (37): Thioglucopyranoside 36 was dissolved in 2:1 MeOH: CH₂Cl₂ (45 mL). Sodium methoxide was added to a pH ~ 9-10. The solution was stirred at room temperature 6 hours, neutralized with acidic Dowex resin, and filtered. The solvents were removed under reduced pressure and the resulting solid was co-evaporated with toluene (3 x 10 mL) and used immediately in the following reaction without further purification. 1H NMR (300 MHz, DMSO- D6): δ 7.48-7.32 (m, 5H), 4.31 (d, J = 4.8 Hz, 1H), 3.65 (dd, J1 = 6.0 Hz, 1H), 3.40 (dd, J1 = 5.9 Hz, J2 = 2.9 Hz, 1H), 1.94-2.93 (m, 5H). 13C NMR (400 MHz, DMSO-D6): δ 120.1, 118.3, 115.0, 105.2, 84.5, 80.9, 78.3, 73.3, 70.1 and 61.3 ppm.

5.6.11 Synthesis of naphthylimide-4-benzaldehyde (28): Dibromo tag molecule 25 (1gm) was refluxed in pyridine (15ml) for two hours and then poured over crushed ice and stirred for 30 minutes. Filtered off the white precipitate (840mg) as our product. 1H NMR of 28 (400 MHZ, CDCl₃): δ 10.1 (s, 1H), 8.1 (d, 2H), 8.0 (dd,2H), 7.8 (dd, 2H),7.65 (d, 2H). 13C NMR of 28 (400 MHz, CDCl₃): δ 169.0, 162.4, 138.2, 134.5, 132.1, 130.2, 127.1 and 124.0 ppm.

5.6.12 Synthesis of 2-[4-(7,8-Dihydroxy-6-phenylsulfanyl-hexahydro-pyranol[3,2-d][1,3]dioxin-2-yl)-phenyl]-isoindole-1,3-dione (38) : Dissolved 4-(1,3-Dioxo-1,3-dihydro-
isoindol-2-yl)-benzaldehyde (400mg, 1.59mmols) and 2-Hydroxymethyl-6-phenylsulfanyl-tetrahydro-pyran-3,4,5-triol (450mg, 1.65mmols) in dry N,N-dimethyl formamide (8ml) and added benzene (25ml) to the mixture. Added p-Toluene sulfonic acid (15mg) to the reaction mixture. Stirred the reaction at 50°C under vacuum and condenser with chilled water condenser which is attached through Din-Stark’s apparatus (half filled with calcium chloride anhydrous) for 3hrs and 30minutes. On completion of reaction diluted reaction mixture with dichloromethane and washed twice with saturated sodium bicarbonate solution (30ml X 2). Concentrated organic portion on rotavapour to get crude product which was then purified by column chromatography on silica-gel (20% EtOAc: DCM elution concentration to get out product from column) to get 685mg white solid as desired product. Product was confirmed by ¹H and ¹³C –NMR in CDCl₃. (isolated yield 85%) ¹H NMR of 38 (400 MHZ, CDCl₃): δ 7.98 (dd, 2H), 7.8 (dd, 2H), 7.6 (d, 2H), 7.48 (m, 2H), 7.42 (d, 2H), 7.27 (m, 3H), 5.6 (s, 1H), 4.62(d, 1H), 4.4 (dd, 1H), 3.8(m, 1H), 3.58-3.40( m, 2H) ¹³C NMR of 38 (400 MHz, CDCl₃): δ 167.2, 141.1, 135.0, 133.2, 131.3, 128.0, 127.1, 124.0, 8120.1, 118.3, 115.0, 105.2, 84.5, 80.9, 78.3, 73.3, 70.1, 61.3 and 40.0 ppm.

5.6.13 Synthesis of 2-[4-(7,8-Dihydroxy-6-phenylsulfanyl-hexahydro-pyran[3,2-d][1,3]dioxin-2-yl)-phenyl]-benzo[f]isoindole-1,3-dione (39) : Dissolved 4-(1,3-Dioxo-1,3-dihydro-benzo[f]isoindol-2-yl)-benzaldehyde (222mg, 1.59mmols) and 2-Hydroxymethyl-6-phenylsulfanyl-tetrahydro-pyran-3,4,5-triol (200mg, 1.65mmols) in dry N,N-dimethyl formamide (8ml) and added benzene (25ml) to the mixture. p-Toluene sulfonic acid (15mg) was added to the reaction mixture. Stirred the reaction at 60°C under vacuum and condenser with chilled water condenser which is attached through Din-Stark’s apparatus (half filled with calcium chloride anhydrous) for 3hrs and 30minutes. On completion of reaction diluted reaction mixture with dichloromethane and washed twice with saturated sodium bicarbonate solution
Concentrated organic portion on rotavapour to get crude product which was then purified by column chromatography on silica-gel (20% EtOAc: DCM elution concentration to get out product from column) to get 335mg white solid as desired product. Product was confirmed by $^1$H and $^{13}$C –NMR in CDCl$_3$. (isolated yield 82%) $^1$H NMR of 39: 8.52 (s, 2H), 8.15 (m, 2H), 7.68 (m, 4H), 7.62(dd, 2H), 7.5-7.45( m, 4H), 7.28( m, 3H) 5.6 (s, 1H), 4.62(d, 1H), 4.4 (dd, 1H), 3.8(m, 1H), 3.58-3.40( m, 2H) $^{13}$C NMR of 39: δ 168.2, 138.0, 136.1, 134.2, 131.0, 129.6, 129.5, 129.4, 129.0, 128.1, 126.5, 126.0, 102.0, 89.5, 80.9, 75.3, 72.5, 71.1 and 68.2 ppm.

5.6.14 Synthesis of 2-[4-(7,8-Dihydroxy-6-phenylsulfanyl-hexahydro-pyran[3,2-d][1,3]dioxin-2-yl)-phenyl]-benzo[de]isoquinoline-1,3-dione (40) : Dissolved 4-(1,3-Dioxo-1H,3H-benzo[de]isoquinolin-2-yl)-benzaldehyde (100mg, 0.33mmols) and 2-Hydroxymethyl-6-phenylsulfanyl-tetrahydro-pyran-3,4,5-triol (90mg, 0.33mmols) in dry N,N-dimethyl formamide (5ml) and added benzene (25ml) to the mixture. p-Toluene sulfonic acid (10mg) was added to the reaction mixture. Stirred the reaction at 70$^0$C under vacuum and condenser with chilled water condenser which is attached through Din-Stark apparatus (half filled with calcium chloride anhydrous) for 4hrs and 30minutes. On completion of reaction diluted reaction mixture with dichloromethane and washed twice with saturated sodium bicarbonate solution (30ml X 2). Concentrated organic portion on rotavapour to get crude product which was then purified by column chromatography on silica-gel (30% EtOAc: DCM elution concentration to get out product from column) to get 67mg white solid as desired product. Product was confirmed by $^1$H and $^{13}$C –NMR in CDCl$_3$. (isolated yield 80%) $^1$H NMR of 40: 8.82 (2H, t, J = 8 Hz), 8.48 (2H, d, J = 8 Hz), 7.83 (2H, t, J = 8 Hz), 7.75 (2H, d, J = 7.8 Hz), 7.36 (2H, d, J = 7.8 Hz), and 6.72 (1H, s), 5.6 (s, 1H), 4.62(d, 1H), 4.4 (dd, 1H), 3.8(m, 1H), 3.58-3.40( m, 2H) $^{13}$C NMR of 40 (400 MHz, CDCl$_3$): δ...
MHz, CDCl₃): δ 164, 142, 137, 135, 132, 129, 128, 127, 123, 120.1, 118.3, 115.0, 105.2, 84.5, 80.9, 78.3, 73.3, 70.1, 61.3 and 40.0.

5.6.15 Synthesis of naphthylimide-4-benzaldehyde dimethyl acetal (30-31): Compound 28 (500mg) and trimethylorthoformate (0.4ml) dissolved in methanol (4ml) and to was added amberlite IR-120 H⁺, mixture was stirred at 60°C for 15hrs. On completion, reaction mixture was cooled to room temperature and diluted with 10ml of methylenechloride. Filtered off mixture to remove amberlite IR-120 and concentrated to get the desired product in 85% yield. 1H NMR of 10 (400 MHZ, CDCl₃): δ 7.9 (dd, 2H), 7.8 (dd, 2H), 7.6 (d, 2H), 7.45 (d, 2H), 5.42(s, 1H), 3.38 (s, 6H) 13C NMR of 31 (400 MHz, CDCl₃): δ 162.4, 138.2, 134.5, 132.1, 130.2, 127.1, 124.0, 40.0 and 34.0 ppm.

Typical procedure for glycosidation at C-3 of tagged sugar 37: Trichloroacetimidate donor 41 was prepared according to well known literature procedure. Freshly prepared Trichloroacetimidate donor 41 (370 mg, 0.5 mmol) and tagged thioglucopyranoside 37 (150mg, 0.45 mmol) were dissolved in CH₂Cl₂ (10 mL). The mixture was cooled to 0°C before TMSOTf (10 µL, 0.05 mmol) was added. The reaction was stirred at 0°C to room temperature for 2 hours, neutralized with triethylamine (0.3 mL). The solvents were removed under reduced pressure and the product was purified by gravity column chromatography (1:3 ethyl acetate: CH₂Cl₂) to give desired product 10 in 75% isolated yield. ¹H NMR of 42 (400 MHz, CDCl₃): δ 8.52 (s, 2H), 8.15 (m, 2H), 8.14 – 7.82 (m, 10H), 7.68 (m, 4H), 7.23 – 7.57 (m, 19 H), 5.92 (t, 1H), 5.71 (t, 1H), 5.57 (s, 1H), 5.56(dd, 1H), 5.22(d, 1H), 4.49(dd, 1H), 4.44 (d,1H), 4.33 (t, 1H), 4.32 (t, 1H), 3.94 (m, 1H), 3.90 (t,1H), 3.77 (t, 1H), 3.71, (t, 1H), 3.49 – 3.44 (m, 2H) ppm. ¹³C NMR δ 167.1, 166.2, 165.9, 133.9, 133.0, 132.9, 132.0, 131.8, 130.0, 129.9, 129.8, 129.7, 129.3, 128.9,
128.6, 128.5, 127.6, 125.1, 123.7, 123.5, 109.2, 106.7, 93.2, 82.4, 81.9, 79.3, 74.8, 72.6, 70.9,
70.2, 69.2, 68.1 and 64.2 ppm.

**Typical procedure for deprotection:**

a) deprotection of tag molecule from sugar. Substrate for deprotection compound 42 (100mg) was dissolved in CH$_2$Cl$_2$ (5mL) and trifluoroacetic acid (2mL) was added with vigorous stirring at room temperature. Reaction was continued at room temperature till completion of reaction (10-12hrs) monitored by 1H-NMR. On completion of reaction evaporated solvent and purified by flash column chromatography to get 71mg of desired product 44. $^1$H NMR of 44 (400 MHz, CDCl$_3$): $\delta$ 8.14 – 7.82 (m, 10H), 7.68 (m, 4H), 7.23 – 7.57 (m, 16 H), 5.92 (t, 1H), 5.71 (t, 1H), 5.56(dd, 1H), 5.22(d, 1H), 4.49(dd, 1H), 4.44 (d,1H), 4.33 (t, 1H), 4.32 (t, 1H), 3.94 (m, 1H), 3.90 (t,1H), 3.77 (t, 1H), 3.71, (t, 1H), 3.49 – 3.44 (m, 2H). $^{13}$C-NMR $\delta$ 167.1, 133.9, 132.9, 132.3, 132.2, 132.0, 131.8, 129.3, 128.5, 127.6, 125.1, 110.4, 109.2, 93.2, 82.7, 81.9, 81.5, 79.3, 76.8, 74.1,72.6, 71.5, 68.1 and 62.2 ppm.

b) Benzoyl deprotection of tagged sugar molecule 42. Substrate for deprotection compound 42 (50mg) was dissolved in CH$_2$Cl$_2$ (5mL) and methanol (5mL). Sodium methoxide was added at room temperature with stirring till obtain a PH of 9 for reaction mixture. Reaction was continued at room temperature till completion of reaction (4-5hrs) monitored by 1H-NMR. On completion of reaction neutralized by acidic dowex resin and filtered off to remove resin, evaporated solvent and purified by flash column chromatography to get 28mg of desired product 43. $^1$H NMR of 43 (400 MHz, DMSO): $\delta$ 8.45 (s, 2H), 8.13 (m, 2H), 7.68 (m, 4H), 7.23 – 7.57 (m, 5H), 5.92 (t, 1H), 5.71 (t, 1H), 5.60 (s, 1H), 5.56(dd, 1H), 5.22(d, 1H), 4.49(dd, 1H), 4.44 (d,1H), 4.33 (t, 1H),
4.32 (t, 1H), 3.94 (m, 1H), 3.90 (t, 1H), 3.77 (t, 1H), 3.71, (t, 1H), 3.49 – 3.44 (m, 2H). $^{13}$C-NMR δ 165.9, 165.9, 165.8, 165.6, 133.9, 133.0, 130.1, 129.9, 129.8, 129.7, 129.3, 128.9, 128.6, 106.7, 93.2, 86.7, 84.6, 74.8, 72.3, 70.9, 70.2, 69.2, 68.9, 64.2 and 61.5 ppm.
5.7 Reference:


18. (a) Filho, V. C.; de Campos, F.; Correa, R.; Yunes, R. A.; Nunes, R. J. “Chemical
241; (b) Benjamin, E.; Hijji, Y. “The Synthesis of Unsubstituted Cyclic Imides Using
Hydroxylamine under Microwave Irradiation.” Molecules 2008, 13, 157–169; (c) Abdel-
Aziz, A. A.-M. “Novel and Versatile Methodology for Synthesis of Cyclic Imides and
Evaluation of Their Cytotoxic, DNA Binding, Apoptotic Inducing Activities and
Ahrens, M. J.; Scheidt, K. A.; Wasielewski, M. R. “Copper-Promoted N-Arylations of
Cyclic Imides within Six-Membered Rings: A Facile Route to Arylene-Based Organic
“Microondas doméstico na síntese de derivados ftalimídicos.” Revista de Ciencias

19. (a) Osby, J. O.; Martin, M. G.; Ganem, B. “An exceptionally mild deprotection of
phthalimides.” Tetrahedron Lett. 1984, 25, 2093; (b) Wuts, P. G. M.; Greene, T. W.

20. For instance see: (a) Sondhi, S. M.; Rani, R.; Roy, P.; Agrawal, S. K.; Saxena, A. K.
“Microwave-Assisted Synthesis of N-Substituted Cyclic Imides and Their Evaluation for
1538; (b) Yunes, J. A.; Cardoso, A. A.; Yunes, R. A.; Correa, R.; de Campos-Buzzi, F.;
Cechinel, F. V. “Antiproliferative effects of a series of cyclic imides on primary
endothelial cells and a leukemia cell line.” Z. Naturforsch 2008, 63c, 675–680; (c)
Stewart, S. G.; Polomska, M. E.; Lim, R. W. “A concise synthesis of maleic anhydride


VITA

Sunil Kumar Upadhyay was born in Bihar, India. He received his B.S. degree from Ramjas College, Delhi University, India in 2002. He did his M.S. degree from Indian Institute of Technology Madras, Chennai, India in 2004. He worked in Aurigene Discovery Technology Ltd Bangalore, India as Research Associate from 2004 to 2006. He joined University of New Orleans to pursue his Ph.D. in Organic Chemistry in fall 2006. In December of same year he joined Dr. Branko S. Jursic’s group to pursue research in the field of Organic Chemistry for the Ph.D. candidacy.