CARBOHYDRATE DERIVATIVES IN ANTIBIOTICS RESEARCH

by

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In this study, we investigated the potential applications of carbohydrates in the development of new antibiotics. To tackle the problem of multidrug-resistant variants of \textit{M. tuberculosis} (MDR-TB), we investigated the biosynthesis pathways of trehalose, which has contributed to significant drug resistance. Some new methods were developed for the synthesis of potential inhibitors (6-azido-trehalose and 6,6’-diazido-trehalose) that have been designed to imitate the intermediate molecule (trehalose 6-phosphate, TPP) of \textit{OtsA-OtsB} pathway. At the same time, some new antibacterial agents based on trehalose have been synthesized.

Members of the 1,2,3-triazole family have shown interesting biological properties. Steroid derivatives have been developed in antibiotics area. Therefore click reaction was utilized to build a 1,2,3-triazole ring and combine cholesterol with different carbohydrate moieties. A series of new compounds has been synthesized and their bioactivities have been tested. (122 pages)
ACKNOWLEDGMENTS

I would like to thank Dr. Tom Chang for his help and supervising during my graduate study period. I would especially like to thank my committee members, Dr. Alvan Hengge and Dr. Philip Silva, for their support and assistance throughout the entire process. Also, I would like to thank all the people in the chemistry department of Utah State University for their help in my research and study.

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ABBREVIATIONS

NBS: N-Bromosuccinimide
TIBSCI: 2,4,6-Triisopropyl bezenesulfonyl chloride
DMAP: 4-Dimethylaminopyridine
TrCl: Triphenylmethyl chloride
BnBr: Benzyl bromide
TBAI: Tetrabutyl Ammonium Iodide
TsOH: p-Toluenesulfonate acid
BOC: t-Butoxycarbonyl
EDC: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
HOBt: Hydroxybenzotriazole
TFA: Trifluoroacetic acid
TsCl: 4-Toluenesulfonyl chloride
Tf₂O: Triflic acid anhydride
DMSO: Dimethyl sulfoxide
Ac₂O: Acetic anhydride
CHAPTER 1

INTRODUCTION

One of the successful themes in human therapeutics from the 20th century was the development of antibiotics and antibacterial agents for the treatment of bacterial infections. Antibiotics helped drop the death rate from infectious disease from 797 per hundred thousand in 1900 to 36 per hundred thousand in 1980.\textsuperscript{1} Two basic routes of chemical investigation were proved as well-suited in antibiotics research: the isolation from natural products with antibiotic activities and the synthesis of antibacterial agents. Table 1 shows the first route is most common.

1. Resistance for antibiotics

In the 1950s, when antibiotics were first introduced for the treatment of microbial infection, the bacterial geneticists believed by mistake that the development of antibiotic resistance during therapy was unlikely. For instance, most early research of antibiotic resistance involved lab experiments with Enterobacteriaceae such as \textit{Escherichia coli} or \textit{Salmonella typhimurium}. For these procaryotes a mutation frequency of $10^{-9}$ or less per bacterial generation was found. Hence at that time, the development of antibiotic-resistant strains was not supposed to be a serious problem. However, today a wide variety of resistance mechanisms have been known. For example, genes encoding resistance to antibiotics would be carried by autonomously replicating transferable elements. Table 2 identifies some mechanisms.
### Table 1. History of antibiotic classes in clinical use

<table>
<thead>
<tr>
<th>Year</th>
<th>Antibiotic</th>
<th>Class</th>
<th>Nature product</th>
<th>Synthetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1929 (activity)</td>
<td>penicillin</td>
<td>β-lactam</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1940 (purification)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1932</td>
<td>sufapyridine</td>
<td>sulfonamide</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>1944</td>
<td>streptomycin</td>
<td>aminoglycoside</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1945</td>
<td>cephalosporin</td>
<td>β-lactam</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1947</td>
<td>chloramphenicol</td>
<td>phenypropanoid</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1948</td>
<td>chlortetracycline</td>
<td>tetracycline</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1950</td>
<td>erythromycin</td>
<td>macrolide</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>vancomycin</td>
<td>glycopeptides</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>virginiamycin</td>
<td>streptogramin</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>amphotericin</td>
<td>polyene(antifungal)</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1955</td>
<td>lincomycin</td>
<td>lincosamide</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1959</td>
<td>rifamycin</td>
<td>ansamycin</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>1962</td>
<td>nalidixic acid</td>
<td>quinolone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>fosfomycin</td>
<td>phosphonate</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>linezolid</td>
<td>oxazolidinone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>daptomycin</td>
<td>lipopeptide</td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Transferable antibiotic resistance in bacteria. For the thorough overview of biochemical mechanisms of drug resistance, see Hayes and Wolf.4

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced uptake into cell</td>
<td>Chloamphenicol</td>
</tr>
<tr>
<td>Active efflux from cell</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Modification of target to eliminate or reduce binding of antibiotic</td>
<td>β-lactams</td>
</tr>
<tr>
<td>Inactivation of antibiotic by enzyme modification</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Hydrolysis</td>
<td>β-lactams</td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Derivatization</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td></td>
<td>Chloamphenicol</td>
</tr>
<tr>
<td></td>
<td>Fosfomycin</td>
</tr>
<tr>
<td>Sequestration of antibiotic by protein binding</td>
<td>Fusidic acid</td>
</tr>
<tr>
<td>Metabolic bypass of inhibited reaction</td>
<td>Sulfonamides</td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
</tr>
<tr>
<td>Binding of specific immunity protein to antibiotic</td>
<td>Bleomycin</td>
</tr>
<tr>
<td>Overproduction of antibiotic target</td>
<td>Sulfonamide</td>
</tr>
<tr>
<td></td>
<td>Tremethoprim</td>
</tr>
</tbody>
</table>

For those, there are three major mechanisms (Figure 1) of antibiotic resistance revealing a few common themes underlying principle about bacterial defense against antibiotics: A, the antibiotic is pumped out by the bacteria efflux pump on the cell wall. B, the antibiotic’s interaction with the target is weakened through modification by bacterial enzymes. C, the antibiotic target is modified in the bacterial genome.
Figure 1. Mechanisms of bacterial drug resistance

The emergence of multi-drug-resistant microorganisms leads to stringent public health problems.\textsuperscript{5-9} The annual cost of treating life-threatening antibiotic resistant infections in the United States has been estimated to be as high as $30 billion.\textsuperscript{10, 11} This situation promotes new drug development worldwide.

To date, even vancomycin, the last resort of antibiotics active against \textit{Staphylococcus aureus} and some other Gram-positive bacteria, has encountered resistance,\textsuperscript{12} and this new public health crisis has renewed the interest in antibacterial development.\textsuperscript{13}

Currently, the common strategies for resolving the problem of antibiotic resistance involve chemical modification of existing antibiotics to resist those mechanisms or development of new types of antibiotics. Our interest in this area is to develop new antibiotics by modification of carbohydrates and their derivatives.
2. Carbohydrate in new antibiotics

2.1 New fields in carbohydrate research

Although the development of vaccines based on carbohydrates has a long history, there are some new emerging fields of interest in the chemistry and biology of carbohydrate. Carbohydrate modifications of proteins and lipids are very important processes which can modulate the structures and functions of these biomolecules and affect intercellular recognition in infection, cancer, and immune response. The most recent efforts in this field are to develop new tools for use to understand the molecular-level carbohydrate recognition and to enable the carbohydrate-based drug discovery process.

Figure 2. The cell walls of bacteria\textsuperscript{14}

For example, both Gram-positive and Gram-negative bacteria have substantial differences in the structures surrounding the cells which form a strong, protective peptidoglycan layer (Figure 2). Many Gram-positive bacteria modify their peptidoglycan layers with anionic wall teichoic acid (WTA) polymers (Figure 3) via
a highly conserved disaccharide bridge. Inhibition of the biosynthesis of this bridge is a potential antibiotic strategy. Ideally, compounds that specifically target the biosynthesis of these anionic polymers could be developed as new antibiotics. The first chemical and enzymatic synthesis of key substrates for the first two committed steps in WTA biosynthesis are reported along with characterization of the two enzymes, TagA and TagB, which carry out this chemistry.\textsuperscript{14}

\textbf{Figure 3.} Teichoic acid polymer linked to peptidoglycan\textsuperscript{14}

Unlike those of Gram-negative bacteria, the peptidoglycan layers of Gram-positive bacteria are often modified by teichoic acids. Penicillin inhibits the biosynthesis of the peptidoglycan layer of many Gram-positive bacteria and some Gram-negative bacteria, but resistance to this and related antibiotics make inhibition of alternate biosynthetic targets very attractive.

TagA and TagB catalyze the biosynthesis of the highly conserved bridge between peptidoglycan and teichoic acid polymers. One potential antibiotic strategy that can be explored is to inhibit the reactions catalyzed by TagA and TagB.
2.2 Carbohydrates as future anti-adhesion drugs for infectious diseases

To effectively colonize a host animal and cause disease, many bacterial pathogens have evolved means for attachment or adhesion to the host cells and tissues. Adhesion is required so that the organisms are not swept away by the natural cleansing mechanisms of the host. The most common means of adhesion, expressed by numerous bacteria, are surface lectins that combine with complementary carbohydrates present on the host cell surfaces. Blocking or inhibition of these lectins by suitable carbohydrates or their analogs for the prevention and treatment of microbial diseases is the aim of anti-adhesion therapy of such diseases.\textsuperscript{15}

From a medical point of view, molecular intervention of this kind may be considered mild and gentle, and more sound ecologically, as well as safer, compared with present chemotherapy approaches. Saccharides are ideal for this purpose as they are unlikely to be toxic or immunogenic.

Although only a small number of the bacterial surface lectins have been well-characterized, the existence of many has been inferred from, and their specificity investigated by inhibition experiments, in which the effect of different carbohydrates is examined on adhesion of the bacteria to relevant animal cells or on the agglutination by the bacteria of erythrocytes\textsuperscript{16} or other kinds of cell, such as yeasts.\textsuperscript{17} Until the early 1980s, bacteria specific for mannose were identified, namely type 1 fimbriated strain \textit{E. coli}. Since then, \textit{E. coli} strains with diverse specificities were discovered (\textbf{Table 3}). (Glc:glucose; Gal:galactose; Fuc:fucose; Man:mannose; NeuAc:N-acetylneuraminic acid).
Table 3. Carbohydrates as attachment sites for bacterial pathogens on animal tissues.

\(^a\)Unless otherwise noted, see Ref. 18. \(^b\)Predominant form in tissue: GP, glycoproteins; GSL, glycolipids. \(^c\)Ref. 19. \(^d\)Ref. 20. \(^e\)Ref. 21. \(^f\)Ref. 22.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Target tissue</th>
<th>Carbohydrate</th>
<th>Form(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni(^c)</td>
<td>Intestinal</td>
<td>Fuca2GalβGlcNAc</td>
<td>GP</td>
</tr>
<tr>
<td>E. coli Type 1</td>
<td>Urinary</td>
<td>Manα3Manα6Man</td>
<td>GP</td>
</tr>
<tr>
<td>P</td>
<td>Urinary</td>
<td>Galα4Gal</td>
<td>GSL</td>
</tr>
<tr>
<td>S</td>
<td>Neural</td>
<td>NeuAc (α2–3)Galβ3GalNAc</td>
<td>GSL</td>
</tr>
<tr>
<td>CFA/1</td>
<td>Intestinal</td>
<td>NeuAc (α2–8)</td>
<td>GP</td>
</tr>
<tr>
<td>F1C(^d)</td>
<td>Urinary</td>
<td>GalNAcβ4Galβ</td>
<td>GSL</td>
</tr>
<tr>
<td>F17(^e)</td>
<td>Urinary</td>
<td>GlcNAc</td>
<td>GP</td>
</tr>
<tr>
<td>K1</td>
<td>Endothelial</td>
<td>GlcNAcβ4GlcNAc</td>
<td>GP</td>
</tr>
<tr>
<td>K99</td>
<td>Intestinal</td>
<td>NeuAc(α2–3)Galβ4Glc</td>
<td>GSL</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>Respiratory</td>
<td>[NeuAc(α2–3)]0,1 Galβ4GlcNAc</td>
<td>GSL</td>
</tr>
<tr>
<td>H. pylori</td>
<td>Stomach</td>
<td>NeuAc(α2–3)Galβ4GlcNAc</td>
<td>GP</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>Respiratory</td>
<td>Man</td>
<td>GP</td>
</tr>
<tr>
<td>N. gonorrhoea</td>
<td>Genital</td>
<td>Galβ4Glc(NAc)</td>
<td>GSL</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>Respiratory</td>
<td>[NeuAc(α2–3)]0,1 Galβ4GlcNAc</td>
<td>GSL</td>
</tr>
<tr>
<td>P. aeruginosa(^f)</td>
<td>Respiratory</td>
<td>L-Fuc</td>
<td>GP</td>
</tr>
<tr>
<td></td>
<td>Respiratory</td>
<td>Galβ3Glc(NAc)β3Galβ4Glc</td>
<td>GSL</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>Intestinal</td>
<td>Man</td>
<td>GP</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>Respiratory</td>
<td>[NeuAc(α2–3)]0,1</td>
<td>GSL</td>
</tr>
<tr>
<td>S. suis</td>
<td>Respiratory</td>
<td>Galα4Galβ4Glc</td>
<td>GSL</td>
</tr>
</tbody>
</table>
The presence of multiple lectins with distinct sugar specificities that are encoded by DNA often located on the same chromosome of the pathogen is most likely the major impediment for the use of sugars as anti-adhesion drugs. To overcome this problem, it is necessary to learn more about the lectins of the bacterial pathogens and the factors affecting their expression in the course of natural infection. This will allow the preparation of suitable cocktails of inhibitory sugars for the treatment of bacterial infections, instead of the single sugars in use until now. The low affinity of free saccharides to the bacterial lectins is another stumbling block, which may be overcome by their attachment to polymeric carriers or presentation as dendrimers. Production of the oligosaccharides is still extremely costly, and to act effectively, large doses are required. However, new technologies, in particular the use of engineered bacteria, promise to lower this cost markedly. Another possibility is to develop suitable carbohydrate analogs (glycomimetics) that are more potent inhibitors.

2.3 Vaccines based on the carbohydrates

In recent decades, the gradual increase of the resistance to antibiotics leads to a serious threat for treatment of bacterial infection. Establishing the structure of carbohydrate immuno-determinants in conjunction with improvements in carbohydrate synthesis has rendered it feasible to develop new generations of carbohydrate-based vaccines.24

With a long history from 1923, polysaccharides have been used as vaccines, providing immunity. And also the glycoproteins have an even larger immune response
for the same antigens. The role of carbohydrate catabolism in the pathogenesis of invasive streptococci has been investigated (Figure 4).²⁵

In Figure 4, proteins involved in deglycosylation of host glycoproteins such as NanA, StrH, BgaA and EndoS, StrH and BgaA are cell-surface exoglycosidases studied in S. pneumoniae. EndoS is a secreted endoglycosidase studied in group A Streptococcus. Pathways for nutrient acquisition are shown in Figure 4. SusX and MalE are cell-surface lipoprotein components of ATP-binding cassette transport systems. Bacterial cell-wall-linked proteins involved in binding to eukaryotic cell surface carbohydrate residues are PuA and BgaA. PuA is a pullulanase mainly studied in group A Streptococcus. BgaA is a β-galactosidase studied in S. pneumoniae.

Figure 4 Summary of mechanisms by which carbohydrate utilization proteins contribute to pathogenesis in S. pneumoniae and group A Streptococcus.²⁶

For several types of bacterial infections, such as Streptococcus pneumoniae,
Neisseria meningitides, Haemophilus influenzae, and Salmonella typhi, glycoconjugate vaccines can be based on fragments of capsular polysaccharides. Various oligosaccharide-conjugate vaccines based on the capsular or O-specific polysaccharides exhibit good immunogenic properties. The synthesis of clearly defined molecular entities will render possible the study of the influences of these parameters as well as of the characterization of the effects of different carrier molecules.

Anti-cancer vaccines are intended to treat cancers (therapeutic vaccines) or to delay or preferably prevent a relapse of cancer (prophylactic vaccines) after any other form of (radical) therapy. Cancer cell antigens may be unique to individual tumors, shared by several tumor types, or expressed by the normal tissue from which the tumor grows. Some of these antigens are carbohydrates and several trials are being carried out to explore their possibilities to be used for the development of vaccines. For example, on breast cancer cells Mucin-1 (MUC-1), NER-2/neu, carcino-embryonic antigen (CEA), Sialyl Tn (STn) and Globo H have been found. The MUC-1 STn epitope: Neu5Aca2-6GalNAca1-O conjugated to KLH (Biomira Inc. Edmonton, Alta., Canada), given in combination with the adjuvant DETOX-B is currently under investigation.27, 28

Since the large-scale synthesis of carbohydrates is no longer a limiting factor, the preparation of carbohydrate-based and structurally well-defined vaccines has become feasible.29 We now screening for the optimal length of the carbohydrate chain and the most suitable spacer and most effective (protein). The vaccines based on the
carbohydrates will lead to wide clinical applications.

2.4 Aminoglycoside in antibiotics

Aminoglycoside antibiotics (Figure 5) are the drug of choice in the anti-infective armamentarium. Despite the potential for renal toxicity, ototoxicity, and bacterial resistance, several members of this class of antibiotics have enjoyed several decades of clinical use.\textsuperscript{30} Unfortunately, years of continuous use and misuse have also caused the bacteria to acquire different types of resistance which has seriously compromised the clinical usage of aminoglycosides.

The aminoglycosides are active against both Gram-positive and Gram-negative bacteria. They exert their antibacterial activities by first entering the cell irreversibly through active transportation and then binding to the particular regions or sites of RNA molecules in the bacterial ribosome, thus effectively interfering with the fidelity of mRNA translation. This interference of the translation process results in malformed proteins. A few of these miscoded proteins become part of the cell wall causing the disruption of the integrity of bacterial membrane.\textsuperscript{31,32} The damaged membrane subsequently allows more antibiotic molecules to enter the cell. Structurally related aminoglycosides bind to this region of rRNA and interfere with the protein synthesis by inducing codon misreading (Figure 6).
Figure 5. Structures of aminoglycosides

Figure 6. Ribosomal protein synthesis and the 16S rRNA site for aminoglycoside binding
3. Click reaction and 1,2,3-triazole in antibiotic

1,2,3-triazole is one of a pair of isomeric chemical compounds with molecular formula $C_2H_3N_3$, which have a five-membered ring of two carbon atoms and three nitrogen atoms, a basic aromatic heterocycle, which can be used in research as a building block for more complex chemical compounds, such as pharmaceutical drugs like tazobactam. 34 1,2,3-Triazole and substituted 1,2,3-triazoles can be produced using the Azide Alkyne Huisgen Cycloaddition 35 (Figure 7) in which an azide and an alkyne undergo a 1,3-dipolar cycloaddition reaction. But under the heat condition, the reaction always gives a mixture of 1,2,3-triazole and 1,2,4-triazole which means the regioselectivity is poor.

Several members of the 1,2,3-triazole family have indeed shown interesting biological properties, such as anti-allergic, 36 anti-bacterial, 37 and anti-HIV activity. 38 Additionally, 1,2,3-triazoles are found in herbicides, fungicides, and dyes.

![Figure 7. Azide Alkyne Huisgen Cycloaddition](image)

In 2001, K. Barry Sharpless’s group introduced “click chemistry” as a new chemical methodology which could generate substances quickly and reliably by joining small units together. “Click Chemistry” refers to a 1,3-cyclo-addition of azides with terminal acetylenes using a $Cu^+$ catalyst (Figure 8) at room temperature discovered concurrently and independently by the groups of K. Barry Sharpless and Morten...
The cyclozation reactions of Sharpless’s research gave a very good yield and regioselectivity under very mild reaction conditions. The catalyst was Cu(I), which was formed by Cu(II) salt and the reductive reagent sodium ascorbate. “Click chemistry” has emerged as a fast and efficient approach to synthesize novel compounds with desired function by using selected “near perfect” reactions.

In 2002, the mechanism of this kind click reaction was first proposed (Figure 9). The reaction began with formation of copper (I) acetylide I, then the concerted [2+3] cycloaddition (B-directed) and pointed to a stepwise, annealing sequence.
(B-1_B-2_B-3, hence the term “ligation”, which proceeded via the intriguing sixmembered copper-containing intermediate III. In 2007, Bernd F. Straub’s group isolated a Cu(I) triazolide, the click intermediate. They developed a straightforward synthesis to Cu(I) phenyl acetylide complexes with a saturated NHC (N-heterocyclic carbene-based) ligand (Scheme 1). The NHC Cu(I) halide complexes have recently been reported as highly active catalysts for triazole click reaction.

Scheme 1. Preparation of a copper(I) triazolide complex

In carbohydrate chemistry, many methods were available in the current literature to prepare homo and hetero dimers of oligosaccharides and glycoconjugates. Some of the important methods that gave access to these interesting compounds were (i) olefin metathesis of alkenyl glycosides, (ii) intermolecular enyne metathesis of alkynyl and alkenyl glycosides; (iii) native chemical ligation of oligosaccharides to peptides; (iv)
glycosylation of diols;\textsuperscript{44} (v) the cycloaddition of azide and alkyne under modified Huisgen (“click”) conditions;\textsuperscript{45} and (vi) coupling of alkynyl glycosides.\textsuperscript{46} Since click reactions were found, they have been quickly applied in the protocol of carbohydrate substrates. Regarding the biological significance of the products, continued interests (in the application of click chemistry to carbohydrate substrates) prompted us to develop an efficient procedure, which was for the conjugation of oligosaccharides to oligosaccharides/amino acids. For instance, in 2008, a carbohydrate sensing “click-fluor” was reported which displayed a nontypical binding preference with sample saccharides. A fluorescent sensor was generated as a result of click reaction which formed a triazole ring (Figure 10).\textsuperscript{47}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure10.png}
\caption{Modular fluorescent saccharide sensors based on a 1,2,3-triazole ring}
\end{figure}

Most of our group’s works were based on carbohydrate chemistry such as modifications on different carbohydrate derivatives, in order to improve the antibiotic activities. For example, the trehalose was modified, which was very important in antitubercular drug development. Recently in our approach, 1,2,3-triazole ring was introduced via click reaction between different saccharide part and other bioactive moieties like cholesterol. Some synthetic modifications were carried out by utilizing the click reaction between azido sugar and cholesterol alkyne moiety to get
sugar-cholesterol conjugant containing 1,2,3-triazole ring. Some new techniques were used to assist our synthetic procedure, such as sonication. In recent years, the application of sonication in synthetic methodologies has gained considerable interest as it can enhance the rate, yield and selectivity of such reactions. It can also facilitate reactions at ambient conditions eliminating requirement of drastic conditions such as temperature, pressure and concentration.
CHAPTER 2
DESIGN AND SYNTHESIS OF TREHALOSE BASED INHIBITORS AND ANTIBIOTICS

1. Rationale for the design of trehalose

Tuberculosis (TB) is still the leading cause of death from a single infectious agent.⁴⁹,⁵⁰ Once curable, M. tuberculosi(s MDR-TB) has shown multi-drug-resistant variants which challenged normal therapeutic practices and increased mortality rates worldwide.⁵¹ The high lipophilicity of the bacterial cell wall of Mycobacterium, which lowers the permeability of antitubercular agents and contributes to significant drug resistance, imposes great challenges in developing new drugs against TB.

Recent advances in the studies of the lipid envelope of TB have uncovered a unique structural component, 6,6’-dimycolyltrehalose (TDM), which forms the outmost layer of the bacterial cell wall of TB (Figure 11).

Figure 11. The lipid cell wall of Mycobacterium tuberculosis and the TDM structure.
Three homologous proteins (AG 85 A, B, C), which possess mycolyltransferase activity, are responsible for the biosynthesis of TDM. Damage to the cell wall, due to the inhibition of ag 85, has been shown to increase the efficacy of various antibiotics. Also, the biosynthesis pathways of trehalose have been discovered (Figure 12). So we designed some trehalose derivatives to imitate the trehalose and those derivatives would inhibit the biosynthesis pathway of trehalose (Figure 13). In our group’s previous work, an analog molecule containing a trehalose core with mono- or di-substituted hydrocarbon side chain had been synthesized. As the linkages for attaching these hydrocarbon chains, amide and urea linkages were introduced (Figure 14), which were commonly found in the designs of inhibitors against proteases, esterases, and peptidases.

Figure 12. Biosynthesis pathways of trehalose
Figure 13. Potential inhibitors for trehalose biosynthesis pathway

Figure 14. Structures of the second generation inhibitors.
After completion of the synthesis, the trehalose derivatives were assayed against *M. smegmatis* (ATCC 14468), using isoniazid (INH) as the positive control. Some synthesized compounds showed positive result in this test (Table 4). Especially for compound YH23 and YH37, they almost had the same result as INH (Figure 15).

**Table 4. Inhibition zones of trehalose derivatives against *M. smegmatis***

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
<th>Compound</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH (Isoniazid)</td>
<td>18</td>
<td>YH008</td>
<td>6</td>
<td>YH023</td>
<td>18</td>
<td>YH031</td>
<td>8</td>
</tr>
<tr>
<td>YH001</td>
<td>Inactive</td>
<td>YH011</td>
<td>Inactive</td>
<td>YH024</td>
<td>5</td>
<td>YH032</td>
<td>11</td>
</tr>
<tr>
<td>YH002</td>
<td>Inactive</td>
<td>YH012</td>
<td>10</td>
<td>YH025</td>
<td>10</td>
<td>YH033</td>
<td>6</td>
</tr>
<tr>
<td>YH003</td>
<td>Inactive</td>
<td>YH014</td>
<td>7</td>
<td>YH026</td>
<td>8</td>
<td>YH034</td>
<td>Inactive</td>
</tr>
<tr>
<td>YH004</td>
<td>Inactive</td>
<td>YH017</td>
<td>6</td>
<td>YH027</td>
<td>Inactive</td>
<td>YH035</td>
<td>Inactive</td>
</tr>
<tr>
<td>YH005</td>
<td>Inactive</td>
<td>YH018</td>
<td>5</td>
<td>YH028</td>
<td>Inactive</td>
<td>YH036</td>
<td>12</td>
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<tr>
<td>YH006</td>
<td>Inactive</td>
<td>YH019</td>
<td>5</td>
<td>YH029</td>
<td>Inactive</td>
<td>YH037</td>
<td>19</td>
</tr>
<tr>
<td>YH007</td>
<td>Inactive</td>
<td>YH020</td>
<td>Inactive</td>
<td>YH030</td>
<td>Inactive</td>
<td>YH041</td>
<td>Inactive</td>
</tr>
</tbody>
</table>

**Figure 15.** Result of YH23 assayed against *M. smegmatis*
2. Synthesis of potential inhibitors

Based on previous work, the potential inhibitors 6-azido-trehalose and 6,6’-diazido-trehalose (Figure 16) were chosen as the target compounds.

![Inhibitors for trehalose biosynthetic OtsA-OtsB Pathway](image)

**Figure 16.** Inhibitors for trehalose biosynthetic OtsA-OtsB Pathway

2.1 Synthesis of 6, 6’-diazido-trehalose

Firstly, the trehalose was tosylated. The solubility of trehalose was very low in most organic solvents because of its polarity, so pyridine was used as the reaction solvent and DMAP as the catalyst. Then without further purification, the crude product was directly used to do azido substitution. TLC showed the reaction complete, but the product was too polar to be purified by column chromatography. In order to lower the polarity, acetylation reaction was run. And then the mixture was purified by a gradient column chromatography. After hydrolysis, the target compound 6,6’-diazido-trehalose was obtained with a low yield (only 17%).

In order to improve the yield, the procedure was modified by switching the order of acetylation and azido substitution. The modified route gave a much better result with 64.7% yield in four steps (Scheme 2).
2.2 Synthesis of 6-azido-trehalose

However, for another compound, 6-azido-trehalose, the synthesis was more difficult compared with 6,6'-diaza-dio-trehalose. Treatment of trehalose with 1 equiv. TsCl in pyridine (Scheme 3) only gave di-substituted product instead of desired mono-substituted product. The reason for this result might be that the solubility of trehalose was not good in pyridine. Once it was tosylated, the reduced polarity made it more soluble than trehalose which meant the mono-substituted trehalose was more reactive. The mono-substituted one then continued to react with TsCl which made the final product disubstituted.

Scheme 3. Route 1 for synthesis of monosubstituted trehalose

Therefore the second route (Scheme 4) was tried. 2,4,6-Triisopropylbenzenesulfonyl chloride was chosen because it was a more bulky leaving group, slowing down the substitution rate. The reaction was very slow and would not go without heating or sonication.
Scheme 4. Synthesis route of monosubstituted trehalose by TIBSCl

The reaction worked when NaI was added and was assisted by sonicating for half hour. After acetylation and purification, the yield of desired monosubstituted compound was only 22%. To improve the yield, another modification was made (Scheme 5). First, the trehalose was brominated by using NBS and PPh₃ in DMF, which created a mixture of mono- and di-substituted products. After acetylation and purification, the desired compound, the mono-substituted one, was obtained with 52% yield with two steps. Then after the azido substitution and hydrolysis reactions, 6-azido-trehalose was obtained.

3. Trehalose-based antitubercular agents

Our group has synthesized a library of antitubercular compounds, which were based on trehalose core. In order to do further research work on this project, another molecule was designed to prepare (Figure 17), which was very similar to YH23 and YH37.

Trehalose reacted with TrCl in DMF to form compound 11. The reaction was catalyzed by triethylamine. Then compound 11 reacted with BnBr and NaH in DMF,
catalyzed by TBAI. After the deprotection for -Tr groups, compound 13 was obtained (Scheme 6). These three steps had a total yield 40% and can be run in a big scale.

Scheme 5. Synthesis route of 6-azido-trehalose
Figure 17. New molecule for antitubercular assay

Scheme 6. Synthesis route of trehalose diol

The side chain part was synthesized by n-heptylamine and BOC-glycine via peptide coupling condition (Scheme 7). The next step was to introduce a leaving group to the trehalose diol, then run the nucleophilic substitution by amine side chain. Several different methods were tried, but the results were not good (Scheme 8).

\[
\text{C}_7\text{H}_{15}\text{NH}_2 + \text{Boc-NHCH}_2\text{COOH} \xrightarrow{\text{EDC, HOBT, Et}_3\text{N, DMF}} \text{BocNHCH}_2\text{CONHC}_7\text{H}_{15}
\]

\[
\text{NH}_2\text{CH}_2\text{CONHC}_7\text{H}_{15}
\]

Scheme 7. Synthesis route of compound 15.
Scheme 8. Synthesis approaches for compound 10

So, slight modification was carried on this trehalose based molecule (Figure 18). An amino acid moiety was the first choice to be introduced between trehalose and aliphatic chain to form two amides. Amino acids were chosen because of their biological activity, and because they are abundant and easily available. So, a new library of trehalose analogues would be synthesized by using different commercial amino acids. The synthetic route for this molecule is shown as Scheme 9.

Figure 18. Modified trehalose analogue molecules with amino acid moiety (in red).
Scheme 9. Synthesis route of compound 19

Primarily, the trehalose diol was treated by tosylation followed by azido substitution. The azido compound was reduced by Staudinger reaction into amine. This amine coupled with BOC-glycine. The BOC group was removed by trifluoro acetic acid. Then another peptide coupling reaction with heptanoic acid was run. After hydrogenation, the desired compound was obtained.

However, the solubility of this product in water was low. If a chemical was able to be applied in drug, it was required to have some solubility in water. Otherwise it was hard to be absorbed by human being. So in order to improve this kind compound’s solubility, compound 25 was designed (Figure 19). Amine was introduced to replace amide as the functional groups which could increase the hydropphilicity.
Based on this idea, the first approach was to directly reduce the amide by LiAlH$_4$, under reflux (**Scheme 10**). Even in a high boiling point solvent (pyridine) and long reaction time (2 days), TLC result showed there was no reaction, which meant the amide was hard to be reduced in this condition. The reason might be a large steric hindrance resulting from the long side chain which made the reduction reaction difficult to occur. Then an effort was made to reduce the amide after deprotection of BOC group (**Scheme 11**). There was still no reaction.

**Figure 19. Structure of Compound 25**

**Scheme 10.** Approach 1 for compound 25
It seemed that the direct reduction of amide connected at 6 and 6’ positions would not work. An alternative approach was tried. After nucleophilic substitution, a reaction was run to reduce the amide which was connected to the side chain instead of trehalose. The result showed that this substitution reaction still did not work (Scheme 12).

Scheme 11. Approach 2 for amide reduction

Scheme 12. Approach 3 for compound 26
The fourth approach was reductive amination. Swern oxidation was used to convert alcohol into aldehyde. The aldehyde reacted with primary amine and reductive reagent, generally NaBH₃CN. During the reaction, the aldehyde was reduced and coupled with the amine on the carbon in carbonyl group at the same time. A model reaction was run. A reductive amination reaction was utilized to reduce the dialdehyde which was formed by oxidation of trehalose diol. The reaction worked well (Scheme 13).

Scheme 13. Model reaction for reductive amination

Then another amine was tried, N-hexylethlenediamine, to run the same reduction (Scheme 14). The reductive reaction happened since the aldehyde disappeared on TLC plate. After purification, some aldehyde was reduced into diol, but it was difficult to afford our desired compound.

Scheme 14. Approach 4 for compound 25
Conclusion

For this project, two trehalose based inhibitors (6,6’-diazido-trehalose and 6-azido-trehalose) have been successfully synthesized. The reaction conditions have been optimized. The best route for 6-azido-trehalose’s synthesis was bromination of trehalose with NBS followed by acetylation (see Scheme 5). Then the monobrominated trehalose was separated with an acceptable yield. This route could be used in synthesis of other mono-substituted trehalose analogues. And a new trehalose analogue was designed and synthesized for antitubercular assay. Comparing with other core trehalose reagents, these analogues contained different amino acid units between trehalose and the side aliphatic chain. These analogues could serve as the leading compounds for further synthetic modification and pave the way to the development of a new generation of antibiotic. We are currently working on increasing the water solubility of this analogue. Further work is required for the modification on the aliphatic chain.
CHAPTER 3

DESIGN AND SYNTHESIS OF CHOLESTEROL DERIVATIVES

1. Background

Steroid is a terpenoid lipid characterized by a carbon skeleton with four fused rings, generally arranged in a 6-6-6-5 fashion. Steroids vary by the functional groups attached to these rings and the oxidation state of the rings. Hundreds of distinct steroids are found in plants, animals, and fungi. All steroids are made in cells either from the sterol lanosterol (Figure 20) (animals and fungi) or the sterol cycloartenol (plants). Both sterols are derived from the cyclization of the triterpene squalene.

Figure 20. Steroid skeleton of lanosterol and its synthesis pathway
It is possible to classify steroids based upon their chemical composition. **Table 5** shows examples.

**Table 5** Classification of steroids

<table>
<thead>
<tr>
<th>Class</th>
<th>Examples</th>
<th>Number of carbon atoms</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholstanes</td>
<td>cholesterol</td>
<td>27</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>Cholanes</td>
<td>cholic acid</td>
<td>22</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>Pregnanes</td>
<td>progesterone</td>
<td>21</td>
<td><img src="image3" alt="Structure" /></td>
</tr>
<tr>
<td>Androstanes</td>
<td>testosterone</td>
<td>19</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>Estranes</td>
<td>estradiol</td>
<td>18</td>
<td><img src="image5" alt="Structure" /></td>
</tr>
</tbody>
</table>

Many steroids have bioactivities, such as anabolic-androgenic steroids (AAS), a class of steroid hormones which are related to the hormone testosterone. AAS increase protein synthesis within cells, resulting in the buildup of cellular tissue (anabolism), especially in muscles. Anabolic steroids also have androgenic and virilizing properties, including the development and maintenance of masculine characteristics such as the growth of the vocal cords and body hair.
Cholesterol is a lipid found in the cell membranes of all animal tissues, and it is transported in the blood plasma of all animals. Cholesterol is also a sterol (a combination steroid and alcohol). Because cholesterol is synthesized by all eukaryotes, trace amounts of cholesterol are also found in membranes of plants and fungi. Most of the cholesterol in the body is synthesized by the body and some has dietary origin. Cholesterol is more abundant in tissues which either synthesize more or have more abundant densely-packed membranes, for example, the liver, spinal cord and brain. It plays a central role in many biochemical processes, such as the composition of cell membranes and the synthesis of steroid hormones.

Cholesterol is required in the membrane of mammalian cells for normal cellular function, and is either synthesized in the endoplasmic reticulum, or derived from the diet, in which case it is delivered by the bloodstream in low-density lipoproteins. These are taken into the cell by LDL receptor-mediated endocytosis in clathrin-coated pits, and then hydrolyzed in lysosomes. Cholesterol is primarily synthesized from acetyl CoA through the HMG-CoA reductase pathway (Figure 21) in many cells and tissues. About 20–25% of total daily production (~1 g/day) occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs.
Recently, steroid derivatives have been studied in antibiotic assays such as antiinflammatory and antitumor.\textsuperscript{53-55} Many of them have high efficacy and low toxicity. These compounds can be isolated from natural source or synthesized in lab. For example, new cationic steroid antibiotics (CSA) were developed to imitate the antibacterial behavior of some endogenous peptide antibiotics.\textsuperscript{56} This behavior includes selective association of the antibiotics with and disruption of bacterial membranes.\textsuperscript{57,58}
This activity results in rapid bactericidal activity with a minimal potential for causing the emergence of resistance.

These antibiotics, in general, adopt cationic, facially amphiphilic conformations (Figure 22), which appears to be the key requirement for antibacterial activity, and membrane selectivity is primarily derived from ionic recognition of negatively charged bacterial membranes.

![Figure 22. Structure of CSA (1) and a schematic representation of a CSA-peptide conjugate (2).](image)

It is reported that some steroidal oligoglycosides isolated from the Antarctic starfish *Acodontaster conspicuous* have very good antimicrobial abilities. Those compounds are dominated by glycosides composed of steroidal aglycons having the hydroxyl groups typically disposed on one side of the tetracyclic nucleus, i.e., 3β, 4β, 6α, 8, 15β-, with some having a sulfate at C-6, and differing in the side chains and/or in the disaccharide moieties that are usually attached at C-26, with some at C-28 and C-29 (Figure 23).
Figure 23. The steroid oligoglycosides isolated from the Antarctic starfish *Acodontaster conspicuous*
2. Design and synthesis

Our project was based on cholesterol in which the sugar moiety was introduced by using click reaction and formed a 1,2,3-triazole ring (Figure 24). First, the azido sugar part was synthesized as Scheme 15.

Figure 24. Target compound of steroid-carbohydrate conjugates

Scheme 15. Synthesis route of azido sugar moieties
In the synthesis of those sugar moieties, the general method was applied in most of sugars except for two aminosugars, glucosamine and galactosamine. Those two sugars were hard to get their chlorine or bromine substituted intermediates, which made it impossible to run azido substitution. So another route was used to synthesize those two azido sugars. Sugar was put in dry acetic chloride and kept stirring for 2 days. Then the reaction afforded 1-chloride sugar and some peracetylated products.

The cholesterol part was synthesized by using cholesterol to react with propargyl bromide. Then it reacted with azido sugar via click reaction forming 1,2,3-triazole ring as expected. (Scheme 16).

Scheme 16. Synthesis route of cholesterol sugar conjugated compound

After a series of this kind of compounds was synthesized, some antibiotic tests were applied to them such as against *Mycobacterium smegmatis*. Unfortunately those
compounds did not show any obvious bioactivity.

Conclusion

In summary, we have synthesized a new class of cholesterol derivative compounds, in which carbohydrate moiety and cholesterol were linked together by click reaction. 1,2,3-Triazole was introduced in this kind of compounds. The reaction conditions for the synthesis route have been optimized. Although there is no obvious bioactivity found in antibiotic tests, it is still possible to apply this method in other similar projects.
CHAPTER 4

EXPERIMENTAL SECTION AND DATA

Proton magnetic resonance spectra were recorded using a Jeol 300 MHz or Bruker
400 MHz spectrometers. Chemical shifts were reported as parts per million (ppm)
downfield from tetramethylsilane in unit, and coupling constants were given in cycles
per second (Hz). Splitting patterns were designed as s, singlet; d, doublet; t, triplet; q,
quartet; m, multiplet. 13C spectra were obtained using the Jeol 270 spectrometer at 68
MHz or Bruker 400 spectrometers at 100 MHz. Routine 13C NMR spectra were fully
decoupled by broad-band waltz decoupling. All NMR spectra were recorded at ambient
temperature unless otherwise noted. Low-resolution fast-atom bombardment (LRFAB)
and High-resolution fast-atom bombardment (HRFAB) or High-resolution MALDI
were provided by the Mass Spectrometry Facilities, University of California, Riverside.

Chemical reagents and starting materials were purchased from Aldrich Chemical
Co. or Acros Chemical Co. and were used without purification unless otherwise noted.
Dichloromethane was distilled over CaH2. Other solvents were used without
purification.

6, 6’-Diazido-α-D-trehalose (1)

2.4 equiv. TsCl (6.7 g, 35.1 mmol) and DMAP (0.02 g) were added to a solution
containing 5 g trehalose (14.6 mmol) in pyridine (50 mL). The reaction was stirred for
12 hours. After the completion of the reaction, the crude product was directly used for
the next step. 9 equiv. Ac2O (6.2mL, 0.13 mol) was added to the reaction solution. The
reaction was stirred for 4-5 hours and monitored by TLC. Then it was quenched with saturated NaHCO₃ (aq, 10 mL) and diluted by ethyl acetate (30 mL). The organic solution was washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄ (10 g). After removal of the organic solvent, the crude product was dissolved in DMF (40 mL). 4 equiv. NaN₃ (2 g) was added. The reaction was stirred for 12 hours under 80°C. Then it was cooled down to room temperature. After removal of most of DMF, the solution was diluted by ethyl acetate (50 mL). The organic solution was washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄. After removal of the solvent, the mixture was purified with a gradient column chromatography (hexanes:EtOAc = 100:0 to 50:50), the product was obtained as clear oil (4.3 g, 65%). Then 1 g product was dissolved in methanol and 2 drops MeOH/NaOMe solution was added as catalyst. The reaction was monitored by TLC. Two hours later it was quenched by H⁺ resin (2 g) and filtered through celite. After removal of the solvent; compound 1 was obtained as clear oil. (0.5 g, 100%). (1). ¹H NMR (CD₃OD, 400 MHz): δ 5.12 (d, J = 3.7 Hz, 1H), 4.03 (m, 1H), 3.75 (t, J = 9.4 Hz, 1H), 3.52 (t, J = 3.9 Hz, 2H), 3.49 (d, J = 3.2 Hz, 2H), 3.40 (dd, J₁ = 13.2 Hz, J₂ = 3.8 Hz, 2H), 3.31 (m, 2H). ¹³C NMR (CD₃OD, 100 MHz): δ 94.4 (2C), 73.2 (2C), 71.9 (2C), 71.8 (2C), 71.4 (2C), 51.4 (2C).
6-O-2,4,6-triisopropylbenzenesulfonyl-2,2',3,3',4,4',6'-hepta-acetyl-α-D-trehalose (3):

Trehalose (0.3 g, 0.88 mmol), TIBSCI (0.32 g, 2.2 mmol) and 1.2 equiv. NaI (0.16 g, 1.07 mmol) were dissolved in pyridine (20 mL). The reaction was sonicated for 20 min. After acetylation (refer to the acetylation in preparation of compound 1), the mixture was purified by a gradient column chromatography (hexane:EtOAc = 100:0 to 50:50), compound 3 was obtained as yellow oil (0.08 g, 22%). 1H NMR (CDCl₃, 400 MHz): δ 7.19 (s, 2H), 5.50 (m, 2H), 5.25 (d, J = 3.8 Hz, 1H), 5.24 (d, J = 2.5 Hz, 1H), 5.10 (dd, J₁ = 10.3 Hz, J₂ = 3.8 Hz, 1H), 5.06 (dd, J₁ = 10.1, J₂ = 9.4, 1H), 4.95 (m, 2H), 4.25 (m, 1H), 4.22 (m, 1H), 4.15-3.95 (m, 6H), 2.90 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.07 (s, 6H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.28 (s, 3H), 1.26 (s, 6H), 1.25 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H).

6,6′-Dibromo-2,2′,3,3′,4,4′-hexa-acetyl-α-D-trehalose (6) and 6-bromo-2,2′,3,3′,4,4′,6′-hepta-acetyl-α-D-trehalose (7)

Trehalose (2 g, 5.8 mmol), NBS (2.6 g, 14.5 mmol) and PPh₃ (3.8 g, 14.5 mmol) were dissolved in pyridine (50 mL). The reaction was stirred for 3 days. After acetylation (refer to the acetylation in preparation of compound 1) and removal of the solvent, the mixture was purified with a gradient column chromatography (hexane:EtOAc = 100:0 to 50:50), compound 6 (0.3 g, 22%) and compound 7 (2.6 g, 44%) were obtained.

6-Azido-α-D-trehalose (9)

Compound 7 (0.5 g, 0.7 mmol) and 4 equiv. NaN₃ (0.2 g, 2.8 mmol) were dissolved in DMF (10 mL). The reaction was stirred for 12 hours under 80°C. Then it was cooled
down to room temperature. After the removal of most DMF by compressed air, it was
diluted by ethyl acetate and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20
mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by
anhydrous Na₂SO₄ (5 g). After purification with a gradient column chromatography
(hexane:EtOAc = 100:0 to 50:50), compound 8 was obtained as clear oil (0.33 g, 52%).
Then 0.1 g compound 8 was dissolved in methanol (10 mL) and 2 drops
MeOH/NaOMe solution were added as catalyst. After the reaction was stirred for 2
hours, it was quenched by H⁺ resin and filtered through celite. Removal of the solvent
afforded compound 9 as clear oil (0.05 g, 100%). ¹H NMR (CD₃OD, 400 MHz): δ 5.14
(d, J = 3.7 Hz, 1H), 5.12 (d, J = 3.7 Hz, 1H), 4.02 (m, 1H), 3.85 (m, 1H), 3.79 (m, 3H),
3.67 (m, 1H), 3.5 (m, 3H), 3.39 (dd, J₁ = 13.2 Hz, J₂ = 5.8 Hz, 1H), 3.32 (m, 2H). ¹³C
NMR (CD₃OD, 100 MHz): δ 94.5 (1C), 94.0 (1C), 73.5 (1C), 73.2 (1C), 72.7 (1C), 72.0
(1C), 71.6 (1C), 71.4 (1C), 70.8 (1C), 61.5 (1C), 60.3 (1C), 51.5 (1C).

2,2’,3,3’,4,4’-Hexa-benzyl-α-D-trehalose (13)

Trehalose (10 g, 0.03 mol), TrCl (20 g, 0.072 mol) and Et₃N (12 mL, 0.072 mL)
were dissolved in DMF (40 mL). The reaction was stirred for 12 hours. Then it was
quenched by 2 mL MeOH. After removal of most of DMF, the crude product of
compound 11, BnBr (32 mL, 0.27 mol), 9 equiv. NaH (10.6 g, 0.27 mol) and TBAI (0.1
g, catalyst) were dissolved in anhydrous THF (60 mL). The reaction was stirred for 12
hours. Then it was quenched by 5 mL MeOH. The reaction solution was poured into a
beaker containing 300 g ice. The mixture was stirred for half an hour and diluted by 200
mL ethyl acetate. The organic solution was washed twice by saturated NaHCO₃ (aq, 20
mL), brine (20 mL) and H2O (20 mL) respectively. The organic layer was collected and dried by anhydrous Na2SO4 (20 g). After removal of the solvent, it afforded the crude product 12. The crude compound 12 and 3 equiv. TsOH (17 g) were dissolved in CH2Cl2 (250 mL) and MeOH (50 mL). The reaction was stirred for 12 hours. Then it was quenched by saturated NaHCO3 (aq, 20 mL). The solution was washed twice by saturated NaHCO3 (aq, 20 mL), brine (20 mL) and H2O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na2SO4 (20 g). After removal of the solvent, the mixture was purified with a gradient column chromatography (hexanes/EtOAc 100:0 to 50:50). Compound 13 was obtained as yellow oil (32 g, 68%).

**Aminoacetic heptylamide (15)**

BOC-glycine (0.76 g, 4.3 mmol), n-heptyl amine (0.64 mL, 4.3 mmol), 1.5 equiv. EDC (1.25 g, 6.5 mmol), 1.5 equiv. HOBr (0.88 g, 6.5 mmol) and 2 equiv. Et3N (1.22 mL, 8.6 mmol) were dissolved in DMF (20 mL). The reaction was stirred for 2 days. After removal of the solvent, crude compound 14 was obtained. Compound 14 and TFA (3 mL) were dissolved in CH2Cl2 (25 mL). One hour later, after removal of the solvent, crude compound 15 was obtained as yellow oil.

**6,6’-Di-p-toluenesulfonyl-2,2’,3,3’,4,4’-hexa-benzyl-α-D-trehalose (16)**

Compound 13 (0.6 g, 0.68 mmol), TsCl (0.54 g, 2.7 mmol), Et3N (0.48 mL, 0.68 mmol) and DMAP (0.1 g) were dissolved in CH2Cl2 (10 mL). The reaction was stirred for 12 hours. After removal of the solvent, crude compound 16 was obtained.
6,6'-Ditrifluoromethanesulfonyl-2,2',3,3',4,4'-hexa-benzyl-α-D-trehalose (17)

Compound 13 (0.1 g, 0.11 mmol), Tf2O (0.1 mL, 0.3 mmol) and pyridine (0.48 g, 0.35 mmol) were dissolved in CH2Cl2 (10 mL) under 0°C. The reaction was stirred for half hour. After removal of the solvent, crude compound 17 was obtained.

6,6'-Dimethanesulfonyl-2,2',3,3',4,4'-hexa-benzyl-α-D-trehalose (18)

Compound 13 (0.1 g, 0.11 mmol), MsCl (0.1 mL, 0.44 mmol) and Et3N (0.148 mL, 0.11 mmol) were dissolved in CH2Cl2 (10 mL). The reaction was stirred for half hour. Then the solution was washed by brine (10 mL). The organic layer was collected and dried by anhydrous Na2SO4 (2 g). After removal of the solvent, crude compound 18 was obtained.

6,6’-Dideoxy-6,6’-di(monohexanoylaminoacetyl)amino-α-D-trehalose (19)

The crude compound 16 (from 4.6 g compound 13 as the procedure above) and 4 equiv. NaN3 (3.3 g, 20.8 mmol) were dissolved in DMF (50 mL). The reaction was stirred for 12 hours under 80°C. After the reaction was complete, most DMF was removed by compressed air. The solution was diluted by EtOAc (50 mL) and washed it twice by saturated NaHCO3 (aq, 20 mL), brine (20 mL) and H2O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na2SO4 (10 g). After removal of the solvent followed by purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 75:25), it afforded compound 20 as yellow oil (4.11 g, 89%). Compound 20 (0.46 g, 0.49 mmol) was dissolved with PMe3 (0.12 mL, 1.18 mmol) in THF (5 mL), One drop water was added. The reaction was heated to 50°C. After 2.5
hours it was cooled down to room temperature. After removal of the solvent, it was purified by a gradient column chromatography (hexane:EtOAc = 100:0 to 25:75), which afforded compound 21 as yellow oil (0.31 g, 81%). Compound 21 (0.31 g, 0.35 mmol), BOC-glycine solid (0.15 g, 0.84 mmol), EDC (0.1 g, 0.53 mmol), HOBt (0.07 g, 0.53 mmol) and Et₃N (0.2 mL, 0.7 mmol) were dissolved in DMF (10 mL). The reaction was stirred for 12 hours. After removal of most DMF, the solution was diluted by EtOAc (20 mL) and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄ (5 g). After removal of the solvent, the mixture was purified by a gradient column chromatography (hexane:EtOAc = 100:0 to 25:75), compound 22 was obtained as yellow oil (0.27 g, 79%). ¹H NMR (CDCl₃, 400 MHz): δ 7.37-7.26 (m, 15H), 5.95 (s, 1H), 5.10 (d, J = 3.4 Hz, 1H), 5.02-4.61 (m, 6H), 4.1 (m, 2H), 3.78 (m, 1H), 3.64 (d, J = 4.0 Hz, 2H), 3.52 (dd, J₁ = 9.7 Hz, J₂ = 3.5 Hz, 1H), 3.32 (t, J = 9.3 Hz, 1H), 3.06 (m, 1H), 1.44 (s, 9H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.5 (1C), 138.8 (1C), 138.2 (1C), 138.1 (1C), 128.7-127.5 (15C), 94.0 (1C), 81.8 (1C), 80.4 (1C), 79.6 (1C), 78.5 (1C), 75.8 (1C), 75.5 (1C), 73.3 (1C), 69.7 (1C), 44.5 (1C), 39.4 (1C), 28.5 (3C).

The BOC groups of compound 22 were deprotected as the procedure in preparation of compound 15. Compound 23 was obtained. Without further purification, it was directly used to run peptide coupling reaction with heptoic acid following the same procedure for compound 22. After purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 25:75), compound 24 was obtained as yellow oil (0.14 g, 68%). ¹H NMR (CDCl₃, 400 MHz): δ 7.41-7.23 (m, 15H), 6.77 (s, 1H), 6.42 (s, 1H),
5.10 (d, J = 3.4 Hz, 1H), 4.99-4.59 (m, 6H), 4.08 (m, 2H), 3.82 (d, J = 4.9 Hz, 2H), 3.63 (m, 1H), 3.53 (dd, J₁ = 9.6 Hz, J₂ = 3.4 Hz, 1H), 3.31 (t, J = 9.3 Hz, 1H), 3.20 (m, 1H), 2.19 (t, J = 7.4 Hz, 2H), 1.56 (m, 2H), 1.24 (m, 6H), 0.87 (t, J = 6.6 Hz, 3H).

Compound 24 (0.14 g, 0.12 mmol) was dissolved in degassed MeOH (5 mL). Pd/C (0.1 g, 10%) was added as catalyst. The reaction was stirred for 12 hours under N₂ atmosphere. The reaction mixture was filtered through celite-silica gel. After removal of the solvent, compound 19 was obtained as white crystal (0.06 g, 93%). 

\(^1^H\) NMR (CD₃OD, 400 MHz): δ 5.05 (d, J = 2.9 Hz, 1H), 3.90 (t, J = 6.8 Hz, 1H), 3.85 (s, 2H), 3.76 (t, J = 9.2 Hz, 1H), 3.62 (d, J = 13.2 Hz, 1H), 3.53 (dd, J₁ = 9.4 Hz, J₂ = 2.8 Hz, 1H), 3.35 (m, 2H), 3.16 (t, J = 9.2 Hz, 1H), 2.80 (m, 2H), 2.28 (t, J = 7.1 Hz, 2H), 1.96 (m, 2H), 1.63 (m, 2H), 1.33 (m, 6H), 0.91 (t, J = 6.0 Hz, 3H). 

\(^1^3^C\) NMR (CD₃OD, 100 MHz): δ 175.8 (1C), 170.9 (1C), 94.7 (1C), 72.9 (1C), 72.1 (1C), 72.0 (1C), 70.9 (1C), 42.5 (1C), 40.3 (1C), 35.7 (1C), 31.5 (1C), 28.9 (1C), 25.6 (1C), 22.4 (1C), 13.2 (1C).

**Approaches for 6,6’-Dideoxy-6,6’-di(monohexaminoethylene)amino-α-D-trehalose (25)**

Compound 24 (0.06g) and LiAlH₄ (0.3 g) were dissolved in anhydrous THF (10 mL). The reaction was refluxed for 12 hours. The solution was cooled down to room temperature. The reaction was quenched by pouring the solution into ice-water.

Compound 23 was prepared from 0.12 g compound 22. The crude compound 23 was dissolved in anhydrous THF (10 mL) with LiAlH₄ (0.3 g). The reaction was refluxed for 2 days.
6,6'-Dideoxy-6,6'-di(monoheptyl)amino-α-D-trehalose (28)

Dry CH₂Cl₂ was cooled by acetone-dry ice to -78°C. (COCl)₂ (0.18 mL, 1 mmol) and DMSO (0.22 mL, 1.5 mmol) were added. Then compound 13 (0.22 g, 0.25 mmol) and Et₃N (0.6 mL, 1 mmol) were added at -50°C. The reaction was under N₂ atmosphere. Two hours later, the solution was washed by HCl (3 M, 10 mL) three times and diluted by CH₂Cl₂ (20 mL). The organic layer was dried by anhydrous Na₂SO₄ (5 g). After removal of the solvent, it afforded crude product compound 27. Crude compound 27, heptyl amine (0.19 mL, 0.55 mmol), 3 equiv. NaCNBH₃ (0.06 g, 0.75 mmol) and 10 drops acetic acid were dissolved in MeOH (10 mL). The reaction was stirred for 12 hours. It was quenched by saturated NaHCO₃ (aq, 5 mL). The solution was extracted by CH₂Cl₂ (20 mL). After removal of the solvent, the mixture was purified with a gradient column chromatography (hexane:EtOAc = 100:0 to 35:65), it afforded compound 28 as yellow oil (0.11 g, 51%).

2,3,4,6-O-tetraacetyl-1-azido-α-D-glucofuranose (47)

Glucose (1.0 g, 5.6 mmol) and 8 equiv. Et₃N (12 mL, 44 mmol) were dissolved in Ac₂O (25 mL). The reaction was cooled to 0°C by ice. Two hours later, the reaction mixture was diluted by ethyl acetate (40 mL) and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄ (5 g). After removal of the solvent, the crude compound 29 was obtained. Compound 29 (0.5 g, 0.74 mmol) and HBr (33% in HOAc, 3 mL) were dissolved in CH₂Cl₂ (10 mL). Two hours later, the solution was diluted by
ethyl acetate (20 mL) and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL) respectively. After removal of the solvent, the crude compound 38 was obtained. The crude compound 38 and NaN₃ (0.15 g, 1.5 mmol) were dissolved in DMF (20 mL). The reaction was stirred under 80 °C for 12 hours. Then most of DMF was removed by compressed air. The solution was diluted by ethyl acetate (20 mL) and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL) and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄ (5 g). After removal the solvent, the mixture was purified with a gradient column chromatography (hexane:EtOAc = 100:0 to 35:65), compound 47 was obtained as white crystal (0.4g, 82%).

**Synthesis of compound 48-55:**

Refer the procedure for synthesis of compound 47.

**2-Acetamido1-azido-3,4,6-triacetyl-α-D-glucopyranose (56)**

N-acetate glucosamine (2.0 g, 4.6 mmol) was dissolved in dry AcCl (20 mL). The reaction was stirred for 2 days. After the reaction was complete, it was quenched by pouring the solution into ice/water mixture. The reaction solution was extracted by ethyl acetate (3 × 15 mL). The organic layer was collected and dried by anhydrous Na₂SO₄. After removal of the solvent, the mixture was dissolved in CH₂Cl₂ (40 mL). Saturated NaHCO₃ (aq, 20 mL), NaN₃ (1 g, 18 mmol) and phase transfer catalyst n-Bu₄NHSO₄ (1.2 g) were added. The reaction was stirred for 24 hours. The organic layer was collected and washed twice by saturated NaHCO₃ (aq, 20 mL), brine (20 mL)
and H₂O (20 mL), respectively. The organic layer was collected and dried by anhydrous Na₂SO₄ (5 g). After removal of the solvent, the mixture was purified with a gradient column chromatography (hexane:EtOAc = 100:0 to 25:75), compound 56 was obtained as white crystal (1.6 g, 40%).

2-Acetamido1-azido-3,4,6-triacetyl-α-D-galactopyranose (57)

Refer the procedure for synthesis of compound 56.

O-propargyl-cholesterol (58)

Cholesterol (2.0 g, 5.17 mmol), NaH (0.3 g, 15.5 mmol), propargyl bromide (1.7 mL, 15.5 mmol) and catalyst TBAI (0.1 g) were dissolved in anhydrous THF (50 mL). The reaction was sonicated for 35 min. After the reaction was complete, the solution was diluted by CH₂Cl₂ (39 mL) and filtered through celite. After purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 0:100), compound 58 was obtained as a white crystal (1.6 g, 77%).

1-(2,3,4,6-Tetraacetyl-D-glucosyl)-4-cholesteryl-1,2,3-triazole (59)

Compound 58 (0.05 g, 0.12 mmol), 1 equiv. compound 47 (0.05 g, 0.12 mmol), Cu(OAc)₂ (0.01 g) and Na-ascorbate (0.02 g) were dissolved in a mixed solvent (MeOH:THF:H₂O = 4 mL:1.1 mL:0.7 mL). The reaction was sonicated for 28 min. Then the solution was filtered through celite. After removal of the solvent, the mixture was purified with a gradient column chromatography (hexane:EtOAc = 100:0 to 50:50), compound 59 was obtained as yellow oil (0.06 g, 73%). ¹H NMR (CDCl₃, 400 MHz): δ 7.78 (s, 1H), 5.89 (d, J = 9.0 Hz, 1H), 5.37 (m, 2H), 5.23 (m, 1H), 5.13 (m, 1H), 4.67 (s,
2H), 4.27 (dd, $J_1 = 12.7$ Hz, $J_2 = 5.1$ Hz, 1H), 4.18 (dd, $J_1 = 14.6$ Hz, $J_2 = 2.3$ Hz, 1H), 4.00 (m, 1H), 3.30(m, 1H), 2.40 (m, 1H), 2.25 (m, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 2.15-1.78 (m, 5H), 2.04 (s, 3H), 1.88 (s, 3H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.91 (d, $J = 6.5$ Hz, 3H), 0.85 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.7$ Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 170.7 (1C), 170.1 (1C), 169.6 (1C), 169.1 (1C), 147.1 (1C), 140.9 (1C), 122.0 (1C), 120.9 (1C), 85.9 (1C), 79.3 (1C), 75.3 (1C), 73.0 (1C), 70.4 (1C), 67.9 (1C), 61.8 (1C), 61.6 (1C), 57.0 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 40.0 (1C), 39.7 (1C), 39.2 (1C), 37.4 (1C), 37.0 (1C), 36.4 (1C), 36.0 (1C), 32.2 (1C), 32.1 (1C), 32.0 (1C), 29.9 (1C), 28.4 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 20.9 (1C), 20.7 (1C), 20.4 (1C), 19.6 (1C), 18.9 (1C), 12.1 (1C).

Synthesis of compound 60-69:

Refer the procedure for synthesis of compound 59.

60. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.83 (s, 1H), 5.84 (d, $J = 9.3$ Hz, 1H), 5.55 (m, 2H), 5.35 (d, $J = 5.7$ Hz, 1H), 5.22 (dd, $J_1 = 9.6$ Hz, $J_2 = 3.5$ Hz, 1H), 4.67 (s, 2H), 4.12 (m, 2H), 3.29 (m, 1H), 2.40 (m, 1H), 2.25 (m, 1H), 2.20 (s, 3H), 2.05-1.78 (m, 5H), 2.03 (s, 3H), 2.0 (s, 3H), 1.89 (s, 3H).160-1.14 (m, 23H), 1.01 (s, 3H), 0.96 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.5$ Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz) : $\delta$ 170.6 (1C), 170.2 (1C), 170.0 (1C), 169.3 (1C), 146.9 (1C), 140.9 (1C), 122.0 (1C), 121.0 (1C), 86.4 (1C), 79.2 (1C), 74.2 (1C), 71.1 (1C), 68.0 (1C), 67.1 (1C), 61.7 (1C), 61.4 (1C), 57.0 (1C), 56.4 (1C), 50.4 (1C), 42.5 (1C), 39.9 (1C), 39.7 (1C), 39.2 (1C), 37.4 (1C), 37.1 (1C), 36.4 (1C), 36.0 (1C), 32.2 (1C), 32.1 (1C), 28.4 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 20.9 (1C), 20.7 (1C), 20.5 (1C), 19.6 (1C), 18.9 (1C),
12.1 (1C).

61. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.83 (s, 1H), 5.79 (d, $J$ = 9.3 Hz, 1H), 5.55 (t, $J$ = 10.0 Hz, 1H), 5.38 (dd, $J_1$ = 13.3 Hz, $J_2$ = 2.8 Hz, 1H), 5.23 (dd, $J_1$ = 10.2 Hz, $J_2$ = 3.3 Hz, 1H), 4.67 (q, $J_1$ = 14.7 Hz, $J_2$ = 12.4 Hz, 2H), 4.12 (m, 1H), 3.29(m, 1H), 2.40 (m, 1H), 2.25 (s, 3H), 2.00 (s, 3H), 2.15-1.78 (m, 5H), 1.89 (s, 3H), 1.60-1.14 (m, 23H), 1.26 (d, $J$ = 6.4 Hz, 3H), 1.01 (s, 3H), 0.92 (d, $J$ = 6.5 Hz, 3H), 0.86 (dd, $J_1$ = 6.6 Hz, $J_2$ = 1.8 Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 170.6 (1C), 170.1 (1C), 169.4 (1C), 146.8 (1C), 140.9 (1C), 122.0 (1C), 120.9 (1C), 86.5 (1C), 78.9 (1C), 72.8 (1C), 71.5 (1C), 70.1 (1C), 68.1 (1C), 61.6 (1C), 57.0 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 40.0 (1C), 39.7 (1C), 39.1 (1C), 37.4 (1C), 37.1 (1C), 36.4 (1C), 36.0 (1C), 35.8 (1C), 32.2 (1C), 32.1 (1C), 28.5 (1C), 28.4 (1C), 28.2 (1C), 24.9 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 20.9 (1C), 20.8 (1C), 20.5 (1C), 19.6 (1C), 18.9 (1C), 16.3 (1C), 12.1 (1C).

62. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.79 (s, 1H), 6.09 (s, 1H), 5.70 (s, 1H), 5.35(d, $J$ = 5.7 Hz, 1H), 5.22 (m, 2H), 4.67 (s, 2H), 3.80 (m, 1H), 3.29 (m, 1H), 2.40 (m, 1H), 2.25 (m, 1H), 2.10 (s, 3H), 2.15-1.78 (m, 5H), 1.98 (s, 3H), 1.57 (s, 3H), 1.35 (d, $J$ = 5.9 Hz, 3H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.96 (d, $J$ = 6.5 Hz, 3H), 0.86 (d, $J$ = 6.5 Hz, 6H), 0.7 (s, 3H).

63. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.76 (s, 1H), 6.11 (s, 1H), 5.71 (d, $J$ = 2.0 Hz, 1H), 5.32(m, 3H), 4.67 (s, 2H), 4.30 (dd, $J_1$ = 12.4 Hz, $J_2$ = 6.2 Hz, 1H), 4.20 (dd, $J_1$ = 12.0 Hz, $J_2$ = 2.1 Hz, 1H), 3.94 (m, 1H), 3.28 (m, 1H), 2.40 (m, 1H), 2.25 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.15-1.78 (m, 5H), 1.99 (s, 3H), 1.55 (s, 3H), 1.60-1.14 (m, 23H),
0.99 (s, 3H), 0.88 (d, J = 6.5 Hz, 3H), 0.85 (dd, J₁ = 6.8 Hz, J₂ = 1.0 Hz, 6H), 0.7 (s, 3H).

$^1$H NMR (CDCl₃, 400 MHz): δ 7.74 (s, 1H), 5.79 (d, J = 9.0 Hz, 1H), 5.40 (m, 3H), 5.20 (m, 1H), 4.67 (s, 2H), 4.30 (dd, J₁ = 11.7 Hz, J₂ = 5.7 Hz, 1H), 3.59 (t, J = 10.5 Hz, 1H), 3.27 (m, 1H), 2.38 (m, 1H), 2.25 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 2.15-1.78 (m, 5H), 1.89 (s, 3H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.86 (dd, J₁ = 6.6 Hz, J₂ = 1.7 Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl₃, 100 MHz): δ 170.1 (1C), 170.0 (1C), 169.2 (1C), 140.9 (1C), 127.1 (1C), 122.0 (1C), 117.3 (1C), 86.5 (1C), 79.1 (1C), 72.3 (1C), 70.6 (1C), 68.6 (1C), 65.8 (1C), 61.6 (1C), 57.0 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 40.0 (1C), 39.7 (1C), 39.2 (1C), 37.4 (1C), 37.0 (1C), 36.4 (1C), 36.0 (1C), 32.1 (1C), 32.0 (1C), 29.9 (1C), 28.4 (2C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 20.9 (1C), 20.4 (1C), 19.6 (1C), 18.9 (1C), 12.1 (1C).

64. $^1$H NMR (CDCl₃, 400 MHz): δ 7.71 (s, 1H), 5.83 (d, J = 9.0 Hz, 1H), 5.46-5.35 (m, 3H), 5.17 (t, J = 9.3 Hz, 1H), 5.09 (t, J = 9.7 Hz, 1H), 4.97 (dd, J₁ = 9.1 Hz, J₂ = 8.0 Hz, 1H), 4.68 (q, J₁ = 14.0 Hz, J₂ = 12.5 Hz, 2H), 4.55 (d, J = 7.9 Hz, 1H), 4.51 (dd, J₁ = 12.3 Hz, J₂ = 1.5 Hz, 1H), 4.39 (dd, J₁ = 12.6 Hz, J₂ = 4.4 Hz, 1H), 4.15 (dd, J₁ = 12.3 Hz, J₂ = 2.3 Hz, 1H), 3.95 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.70 (dd, J₁ = 13.0 Hz, J₂ = 1.5 Hz, 1H), 3.60 (dd, J₁ = 13.0 Hz, J₂ = 1.5 Hz, 1H), 3.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H).

65. $^1$H NMR (CDCl₃, 400 MHz): δ 7.71 (s, 1H), 5.83 (d, J = 9.0 Hz, 1H), 5.46-5.35 (m, 3H), 5.17 (t, J = 9.3 Hz, 1H), 5.09 (t, J = 9.7 Hz, 1H), 4.97 (dd, J₁ = 9.1 Hz, J₂ = 8.0 Hz, 1H), 4.68 (q, J₁ = 14.0 Hz, J₂ = 12.5 Hz, 2H), 4.55 (d, J = 7.9 Hz, 1H), 4.51 (dd, J₁ = 12.3 Hz, J₂ = 1.5 Hz, 1H), 4.39 (dd, J₁ = 12.6 Hz, J₂ = 4.4 Hz, 1H), 4.15 (dd, J₁ = 12.3 Hz, J₂ = 2.3 Hz, 1H), 3.95 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.70 (dd, J₁ = 13.0 Hz, J₂ = 1.5 Hz, 1H), 3.60 (dd, J₁ = 13.0 Hz, J₂ = 1.5 Hz, 1H), 3.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 3.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 2.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 1.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.80 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.60 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.40 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.20 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H), 0.00 (dd, J₁ = 12.6 Hz, J₂ = 1.5 Hz, 1H).
$J_2 = 5.0$ Hz, $J_1 = 12.5$ Hz, $J_2 = 2.2$ Hz, 1H), 3.91 (m, 2H), 3.69 (m, 1H), 3.30 (m, 1H), 2.39 (m, 1H), 2.25 (m, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.052 (s, 3H), 2.051 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.87 (s, 3H), 1.60-1.14 (m, 33H), 1.04 (s, 3H), 0.91 (d, $J = 6.4$ Hz, 3H), 0.86 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 170.7 (1C), 170.5 (1C), 170.4 (1C), 169.7 (1C), 169.5 (1C), 169.3 (1C), 169.2 (1C), 146.9 (1C), 140.8 (1C), 122.1 (1C), 120.9 (1C), 101.1 (1C), 85.7 (1C), 79.3 (1C), 76.1 (1C), 73.0 (1C), 72.6 (1C), 72.3 (1C), 71.8 (1C), 70.5 (1C), 67.9 (1C), 61.9 (1C), 61.7 (1C), 56.9 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 39.9 (1C), 39.7 (1C), 39.2 (1C), 37.3 (1C), 37.0 (1C), 36.4 (1C), 36.0 (1C), 32.1 (1C), 32.0 (1C), 28.4 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 21.0 (1C), 20.9 (1C), 20.8 (1C), 20.7 (1C), 20.5 (1C), 19.6 (1C), 18.9 (1C), 12.0 (1C).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.71 (s, 1H), 5.90 (d, $J = 9.1$ Hz, 1H), 5.49-5.27 (m, 10H), 5.09 (t, $J = 9.9$ Hz, 1H), 4.78 (dd, $J_1 = 10.5$ Hz, $J_2 = 4.0$ Hz, 1H), 4.79 (dd, $J_1 = 10.3$ Hz, $J_2 = 4.0$ Hz, 1H), 4.68 (t, $J = 12.9$ Hz, 2H), 4.48 (m, 3H), 4.34-4.18 (m, 3H), 4.11-4.08 (m, 2H), 4.03-3.94 (m, 6H), 3.30 (m, 1H), 2.39 (m, 1H), 2.25 (m, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.023 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.85 (s, 3H), 1.60-1.14 (m, 33H), 1.01 (s, 3H), 0.89 (d, $J = 4.3$ Hz, 3H), 0.86 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.7$ Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 170.9 (1C), 170.8 (1C), 170.7 (1C), 170.6 (1C), 170.5 (1C), 170.2 (1C), 170.1 (1C), 169.9 (1C), 169.7 (1C), 169.5 (1C), 146.9 (1C), 140.8 (1C), 122.1 (1C), 120.9 (1C), 96.3 (1C), 95.9 (1C), 87.6 (1C), 85.4 (1C), 79.3 (1C), 75.4 (1C), 75.3 (1C), 73.7 (1C), 72.7 (1C), 71.8 (1C), 71.1 (1C), 70.6 (1C), 70.3 (1C), 69.6 (1C), 69.4 (1C), 68.7
67. $^{1}H$ NMR (CDCl$_3$, 400 MHz): $\delta$ 7.70 (s, 1H), 5.79 (d, $J = 8.9$ Hz, 1H), 5.46-5.33 (m, 4H), 5.12 (dd, $J_1 = 10.4$ Hz, $J_2 = 7.9$ Hz, 1H), 4.96 (dd, $J_1 = 10.4$ Hz, $J_2 = 3.4$ Hz, 1H), 4.67 (t, $J = 12.9$ Hz, 2H), 4.51 (d, $J = 7.9$ Hz, 1H), 4.45 (dd, $J_1 = 12.4$ Hz, $J_2 = 1.5$ Hz, 1H), 4.11 (m, 4H), 3.93 (m, 3H), 3.28 (m, 1H), 2.40 (m, 1H), 2.23 (m, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.96 (s, 3H), 1.85 (s, 3H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.89 (d, $J = 4.3$ Hz, 3H), 0.86 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.7$ Hz, 6H), 0.7 (s, 3H). $^{13}C$ NMR (CDCl$_3$, 100 MHz): $\delta$ 170.6 (1C), 170.4 (1C), 170.3 (1C), 170.2 (1C), 169.7 (1C), 169.4 (1C), 169.3 (1C), 146.9 (1C), 140.8 (1C), 122.1 (1C), 120.9 (1C), 101.3 (1C), 85.6 (1C), 79.3 (1C), 76.0 (1C), 75.9 (1C), 72.9 (1C), 71.1 (1C), 71.0 (1C), 70.6 (1C), 69.2 (1C), 66.8 (1C), 62.0 (1C), 61.7 (1C), 61.0 (1C), 56.9 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 39.9 (1C), 39.7 (1C), 39.2 (1C), 37.3 (1C), 37.0 (1C), 36.4 (1C), 35.9 (1C), 32.1 (1C), 32.0 (1C), 29.9 (1C), 28.4 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 21.0 (1C), 20.9 (1C), 20.85 (1C), 20.83 (1C), 20.7 (1C), 20.4 (1C), 19.6 (1C), 18.9 (1C), 12.0 (1C).

68. $^{1}H$ NMR (CDCl$_3$, 400 MHz): $\delta$ 7.83 (s, 1H), 6.00 (d, $J = 9.9$ Hz, 1H), 5.62 (d, $J = 9.2$ Hz, 1H), 5.45 (t, $J = 9.4$ Hz, 1H), 5.37 (m, 1H), 5.24 (t, $J = 9.83$ Hz, 1H), 4.65 (s, 2H), 4.55 (m, 1H), 4.28 (dd, $J_1 = 12.6$ Hz, $J_2 = 5.1$ Hz, 1H), 4.14 (dd, $J_1 = 12.7$ Hz, $J_2 = 9.4$ Hz, 1H), 4.10 (dd, $J_1 = 12.5$ Hz, $J_2 = 9.0$ Hz, 1H), 4.06 (dd, $J_1 = 12.3$ Hz, $J_2 = 8.7$ Hz, 1H), 4.03 (dd, $J_1 = 12.1$ Hz, $J_2 = 8.5$ Hz, 1H), 3.99 (dd, $J_1 = 11.9$ Hz, $J_2 = 8.3$ Hz, 1H), 3.96 (dd, $J_1 = 11.7$ Hz, $J_2 = 8.1$ Hz, 1H), 3.93 (dd, $J_1 = 11.5$ Hz, $J_2 = 7.9$ Hz, 1H), 3.90 (dd, $J_1 = 11.3$ Hz, $J_2 = 7.7$ Hz, 1H), 3.87 (dd, $J_1 = 11.1$ Hz, $J_2 = 7.5$ Hz, 1H), 3.84 (dd, $J_1 = 10.9$ Hz, $J_2 = 7.3$ Hz, 1H), 3.81 (dd, $J_1 = 10.7$ Hz, $J_2 = 7.1$ Hz, 1H), 3.78 (dd, $J_1 = 10.5$ Hz, $J_2 = 6.9$ Hz, 1H), 3.75 (dd, $J_1 = 10.3$ Hz, $J_2 = 6.7$ Hz, 1H), 3.72 (dd, $J_1 = 10.1$ Hz, $J_2 = 6.5$ Hz, 1H), 3.69 (dd, $J_1 = 9.9$ Hz, $J_2 = 6.3$ Hz, 1H), 3.66 (dd, $J_1 = 9.7$ Hz, $J_2 = 6.1$ Hz, 1H), 3.63 (dd, $J_1 = 9.5$ Hz, $J_2 = 5.9$ Hz, 1H).
2.0 Hz, 1H), 3.97 (m, 1H), 3.32 (m, 1H), 2.42 (m, 1H), 2.28 (m, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.78 (s, 3H), 2.05-1.82 (m, 8H), 1.58-1.06 (m, 17H), 1.04 (s, 3H), 0.91 (d, \( J = 6.4 \) Hz, 4H), 0.86 (dd, \( J_1 = 6.6 \) Hz, \( J_2 = 1.8 \) Hz, 6H), 0.7 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta 170.7 \) (1C), 170.5 (1C), 170.4 (1C), 169.5 (1C), 140.8 (1C), 122.1 (1C), 120.9 (1C), 85.7 (1C), 79.3 (1C), 75.2 (1C), 72.4 (1C), 68.0(1C), 61.9 (1C), 61.7 (1C), 57.0 (1C), 56.3 (1C), 53.9 (1C), 50.4 (1C), 42.5 (1C), 39.9 (1C), 39.7 (1C), 39.2 (1C), 37.4 (1C), 37.1 (1C), 36.4 (1C), 36.0 (1C), 32.2 (1C), 32.1 (1C), 29.9 (1C), 28.5 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.2 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 20.9 (1C), 20.8 (1C), 19.6 (1C), 18.9 (1C), 12.0 (1C).

69. \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta 7.83 \) (s, 1H), 6.00 (d, \( J = 9.9 \) Hz, 1H), 5.84 (d, \( J = 8.9 \) Hz, 1H ), 5.45 (t, \( J = 10.3 \) Hz, 1H), 5.35 (m, 1H), 5.22 (t, \( J = 9.6 \) Hz, 1H), 4.65 (s, 2H), 4.55 (m, 1H), 4.28 (dd, \( J_1 = 12.4 \) Hz, \( J_2 = 4.8 \) Hz, 1H), 4.14 (d, \( J_1 = 12.3 \) Hz, 1H), 3.98 (m, 1H), 3.32 (m, 1H), 2.42 (m, 1H), 2.28 (m, 1H), 2.07 (s, 3H), 2.06 (s, 6H), 1.78 (s, 3H), 2.05-1.82 (m, 8H), 1.58-1.06 (m, 17H), 1.04 (s, 3H), 0.91 (d, \( J = 6.4 \) Hz, 4H), 0.86 (d, \( J_1 = 6.6 \) Hz, 6H), 0.67 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \( \delta 171.0 \) (1C), 170.8 (1C), 170.6 (1C), 169.5 (1C), 140.9 (1C), 128.9 (1C), 127.1 (1C), 122.1 (1C), 86.0 (1C), 79.3 (1C), 75.2 (1C), 72.5 (1C), 68.1(1C), 61.9 (1C), 61.5 (1C), 57.0 (1C), 56.3 (1C), 53.9 (1C), 50.4 (1C), 42.5 (1C), 40.0 (1C), 39.7 (1C), 39.1 (1C), 37.4 (1C), 37.1 (1C), 36.4 (1C), 36.0 (1C), 32.2 (1C), 32.1 (1C), 32.0 (1C), 29.9 (1C), 28.5 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.2 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 20.9 (1C), 20.8 (1C), 19.6 (1C), 18.9 (1C), 12.0 (1C).
1-D-glucosyl-4-cholesteryl-1,2,3-triazole (70):

Compound 59 (0.06g, 0.08 mmol) was dissolved in MeOH (5 mL). Two drops NaOMe/MeOH solution were added as catalyst. The reaction was stirred for 1 hour. Then it was quenched by H\(^+\) resin (1 g) and filtered through celite. After removal of the solvent, compound 70 was obtained as white solid (0.04 g, 99\%). \(^1\)H NMR (CD\(_3\)OD, 300 MHz): \(\delta\) 8.15 (s, 1H), 5.61 (d, \(J = 9.1\) Hz, 1H), 5.37 (d, \(J = 5.2\) Hz, 1H), 4.67 (s, 2H), 4.34 (m, 1H), 4.28 (m, 1H), 3.89 (m, 1H), 3.69 (dd, \(J_1 = 12.3\) Hz, \(J_2 = 5.4\) Hz, 1H), 3.58 (m, 1H), 3.54, (dd, \(J_1 = 16.6\) Hz, \(J_2 = 9.3\) Hz, 1H), 2.40 (m, 1H), 2.20 (m, 1H), 2.05-1.78 (m, 5H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.96 (d, \(J = 6.5\) Hz, 3H), 0.86 (dd, \(J_1 = 6.5\) Hz, 6H), 0.73 (s, 3H). \(^13\)C NMR (CD\(_3\)OD, 100 MHz): \(\delta\) 145.4 (1C), 140.6 (1C), 123.0 (1C), 121.7 (1C), 88.4 (1C), 79.9 (1C), 78.9 (1C), 77.3 (1C), 72.8 (1C), 69.7 (1C), 61.2 (1C), 60.6 (1C), 57.0 (1C), 56.4 (1C), 50.5 (1C), 42.3 (1C), 40.0 (1C), 39.5 (1C), 38.8 (1C), 37.2 (1C), 36.7 (1C), 36.2 (1C), 35.9 (1C),32.0 (1C), 31.9 (1C), 29.6 (1C), 28.1 (1C), 28.0 (1C), 24.1 (1C), 23.7 (1C), 22.0 (1C), 21.7 (1C), 21.0 (1C), 18.6 (1C), 18.0 (1C), 11.1 (1C). ESI/APCI Calcd for C\(_{36}\)H\(_{59}\)N\(_3\)O\(_6\)Na ([M+Na\(^+\)]\(^+)\) m/e 652.4295; measured m/e 652.4296.

Synthesis of compound 71-80:

Refer the procedure for synthesis of compound 70.

71. \(^1\)H NMR (CD\(_3\)OD, 300 MHz): \(\delta\) 8.18 (s, 1H), 5.56 (d, \(J = 9.3\) Hz, 1H), 5.35 (d, \(J = 5.7\) Hz, 1H), 4.67 (s, 2H), 4.12 (t, \(J = 9.6\) Hz, 1H), 3.96 (d, \(J = 3.1\) Hz, 1H), 3.80 (q, \(J_1 = 11.7\) Hz, \(J_2 = 5.5\) Hz, 1H), 3.73 (t, \(J = 5.5\) Hz, 1H), 3.65 (dd, \(J_1 = 9.6\) Hz, \(J_2 = 3.5\) Hz,
1H), 2.40 (m, 1H), 2.20 (m, 1H), 2.05-1.78 (m, 5H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.86 (dd, J = 6.5 Hz, 6H), 0.7 (s, 3H). 13C NMR (CD3OD, 100 MHz) : δ 145.6 (1C), 140.6 (1C), 122.4 (1C), 121.7 (1C), 89.0 (1C), 78.8 (1C), 74.1 (1C), 70.3 (1C), 69.2 (1C), 61.2 (1C), 60.6 (1C), 60.0 (1C), 56.4 (1C), 50.5 (1C), 42.3 (1C), 39.9 (1C), 39.5 (1C), 38.8 (1C), 37.2 (1C), 36.7 (1C), 36.2 (1C), 35.9 (1C), 32.0 (1C), 31.8 (1C), 28.1 (1C), 27.9 (1C), 24.1 (1C), 23.7 (1C), 22.0 (1C), 21.7 (1C), 21.0 (1C), 18.6 (1C), 18.0 (1C), 11.1 (1C). ESI/APCI Calcd for C36H59N3O6Na ([M+Na]^+) m/e 652.4295; measured m/e 652.4298.

72. 1H NMR (CD3OD, 300 MHz): δ 8.16 (s, 1H), 5.51 (d, J = 9.3 Hz, 1H), 5.35 (d, J = 5.2 Hz, 1H), 4.64 (s, 2H), 4.05 (t, 1H, J = 9.3 Hz), 3.93 (dd, J1 = 12.7, 6.5 Hz, 1H), 3.73 (d, J = 3.5 Hz, 2H), 3.66 (dd, J1 = 9.6 Hz, J2 = 3.4 Hz, 1H), 2.38 (m, 1H), 2.19 (m, 1H), 1.96 (m, 2H), 2.01-1.86 (m, 6H), 1.60-1.35 (m, 10H), 1.28 (d, J = 6.2 Hz, 3H), 1.22-1.05 (m, 5H), 1.01 (s, 3H), 0.92 (d, J = 6.5 Hz, 3H), 0.86 (d, J = 5.8, 6H), 0.7 (s, 3H). 13C NMR (CD3OD, 100 MHz) : δ 145.6 (1C), 140.6 (1C), 122.3 (1C), 121.7 (1C), 89.0 (1C), 79.0 (1C), 74.3 (1C), 74.1 (1C), 71.8 (1C), 70.0 (1C), 60.7 (1C), 57.0 (1C), 56.4 (1C), 50.5 (1C), 42.3 (1C), 39.9 (1C), 39.5 (1C), 38.8 (1C), 37.2 (1C), 36.7 (1C), 36.2 (1C), 35.9 (1C), 32.0 (1C), 31.9 (1C), 28.1 (1C), 28.0 (1C), 24.1 (1C), 23.8 (1C), 22.0 (1C), 21.8 (1C), 21.0 (1C), 18.6 (1C), 18.1 (1C), 15.6 (1C), 11.1 (1C). ESI/APCI Calcd for C36H59N3O6Na ([M+Na]^+) m/e 652.4295; measured m/e 652.4305.

73. 1H NMR (CD3OD, 300 MHz): δ 8.1 (s, 1H), 5.95 (s, 1H), 5.36 (d, J = 4.8 Hz, 1H), 4.63 (s, 2H), 4.07 (d, J = 2.8 Hz, 1H), 3.65 (dd, J1 = 8.9 Hz, J2 = 3.1 Hz, 1H), 3.58-3.43 (m, 2H), 2.39 (m, 1H), 2.2 (m, 1H), 2.05-1.78 (m, 5H), 1.65-1.40 (m, 10H),
1.35 (d, J = 5.9 Hz, 3H), 1.27-1.04 (m, 6H), 1.01 (s, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.5 Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CD$_3$OD, 100 MHz) : δ 145.6 (1C), 140.6 (1C), 123.2 (1C), 121.5 (1C), 86.9 (1C), 78.9 (1C), 75.6 (1C), 73.4 (1C), 71.8 (1C), 71.1 (1C), 60.5 (1C), 56.8 (1C), 56.2 (1C), 48.5 (1C), 42.2 (1C), 39.8 (1C), 39.4 (1C), 38.7 (1C), 37.0 (1C), 36.6 (1C), 36.0 (1C), 35.8 (1C), 31.9 (1C), 31.7 (1C), 28.0 (1C), 27.8 (1C), 24.0 (1C), 23.6 (1C), 21.9 (1C), 21.6 (1C), 20.8 (1C), 18.5 (1C), 17.9 (1C), 16.7 (1C), 11.0 (1C). ESI/APCI Calcd for C$_{42}$H$_{69}$N$_3$O$_{11}$Na ([M+Na]$^+$) m/e 814.4830; measured m/e 814.4834.

74. $^1$H NMR (CD$_3$OD, 400 MHz): δ 8.28 (s, 1H), 6.03 (d, J = 1.1 Hz, 1H), 5.37 (d, J = 5.1 Hz, 1H), 4.67 (s, 2H), 4.12 (s, 1H), 3.92 (dd, $J_1$ = 12.2 Hz, $J_2$ = 2.2 Hz, 1H), 3.80-3.70 (m, 2H), 3.54 (m, 1H), 2.39 (m, 1H), 2.2 (m, 1H), 2.05-1.78 (m, 5H), 1.60-1.14 (m, 23H), 1.01 (s, 3H), 0.96 (d, J = 6.5 Hz, 3H), 0.86 (dd, $J_1$ = 6.6 Hz, $J_2$ = 1.4 Hz, 6H), 0.7 (s, 3H). $^{13}$C NMR (CD$_3$OD, 100 MHz) : δ 145.6 (1C), 140.6 (1C), 123.4 (1C), 121.7 (1C), 87.3 (1C), 80.4 (1C), 79.0 (1C), 73.7 (1C), 71.1 (1C), 66.5 (1C), 61.4 (1C), 60.5 (1C), 56.9 (1C), 56.4 (1C), 50.5 (1C), 42.3 (1C), 39.9 (1C), 39.5 (1C), 38.8 (1C), 37.2 (1C), 36.7 (1C), 3620 (1C), 35.9 (1C), 32.0 (1C), 31.8 (1C), 28.1 (1C), 27.9 (1C), 24.1 (1C), 23.7 (1C), 22.0 (1C), 21.7 (1C), 21.0 (1C), 18.6 (1C), 18.0 (1C), 11.1 (1C). ESI/APCI Calcd for C$_{36}$H$_{59}$N$_3$O$_5$ (M)$^+$ m/e 614.4488; measured m/e 614.4524.

75. $^1$H NMR (CD$_3$OD, 400 MHz): δ 8.13 (s, 1H), 5.50 (d, J = 9.1 Hz, 1H), 5.38 (m, 1H), 4.67 (s, 2H), 4.02 (dd, $J_1$ = 11.3 Hz, $J_2$ = 5.4 Hz, 1H), 3.92 (t, $J_1$ = 9.0 Hz, 1H), 3.78-3.67 (m, 2H), 3.52-3.45 (m, 3H), 2.40 (m, 1H), 2.25 (m, 1H), 2.07-1.86 (m, 5H), 1.67-0.87 (m, 22H), 1.01 (s, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.89 (dd, $J_1$ = 6.6 Hz, $J_2$ = 1.7 Hz, 3H), 0.86 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 6.5 Hz, 3H).
Hz, 6H), 0.73 (s, 3H). 13C NMR (CD3OD, 75 MHz): δ 145.4 (1C), 140.6 (1C), 122.9 (1C), 121.7 (1C), 89.0 (1C), 79.0 (1C), 77.4 (1C), 72.7 (1C), 69.5 (1C), 68.6 (1C), 60.6 (1C), 57.0 (1C), 56.4 (1C), 50.5 (1C), 42.3 (1C), 40.0 (1C), 39.5 (1C), 38.8 (1C), 37.2 (1C), 36.7 (1C), 36.2 (1C), 35.9 (1C), 32.0 (1C), 31.9 (1C), 28.1 (2C), 28.0 (1C), 24.1 (1C), 23.7 (1C), 22.0 (1C), 21.7 (1C), 21.0 (1C), 18.6 (1C), 18.0 (1C), 11.1 (1C).

ESI/APCI Calcd for C36H59N3O5Na ([M+Na]+) m/e 636.4352; measured m/e 636.4352.

76. 1H NMR (DMSO, 400 MHz): δ 8.21 (s, 1H), 5.57 (d, J = 9.3 Hz, 1H), 5.31 (m, 1H), 4.99 (m, 1H), 4.79 (m, 1H), 4.53 (s, 2H), 4.30 (d, J = 7.9 Hz, 1H), 3.97-2.96 (m, 11H), 2.35 (m, 1H), 2.07 (m, 1H), 1.96-0.96 (m, 32H), 1.04 (s, 3H), 0.86 (d, J = 6.4 Hz, 3H), 0.82 (dd, J 1 = 6.6 Hz, J 2 = 1.8 Hz, 6H), 0.62 (s, 3H). 13C NMR (DMSO, 100 MHz): δ 145.2 (1C), 141.0 (1C), 123.6 (1C), 121.9 (1C), 103.7 (1C), 87.6 (1C), 79.9 (1C), 78.4 (1C), 78.3 (1C), 77.4 (1C), 77.0 (1C), 75.8 (1C), 73.9 (1C), 72.4 (1C), 70.7 (1C), 70.3 (1C), 61.6 (1C), 60.9 (1C), 60.5 (1C), 56.8 (1C), 56.2 (1C), 50.2 (1C), 42.5 (1C), 37.3 (1C), 36.9 (1C), 36.3 (1C), 35.8 (1C), 32.1 (1C), 28.5 (1C), 28.4 (1C), 28.0 (1C), 26.7 (1C), 24.5 (1C), 23.8 (1C), 23.7 (1C), 23.3 (1C), 23.0 (1C), 21.2 (1C), 19.8 (1C), 19.7 (1C), 19.2 (1C), 12.3 (1C). ESI/APCI Calcd for C35H57N3O5Na ([M+Na]+) m/e 622.4196; measured m/e 622.4196.

77. 1H NMR (CD3OD, 400 MHz): δ 8.2 (s, 1H), 5.64 (d.), 5.39 (m, 1H), 5.26 (d, J = 3.7 Hz, 1H), 5.17 (m, 1H), 4.67 (s, 2H), 4.48 (m, 3H), 3.98-3.20 (m, 31H), 2.40 (m, 1H), 2.22 (m, 1H), 2.07-1.83 (m, 6H), 1.63-1.01 (m, 27H), 1.03 (s, 3H), 0.93 (d, J = 6.4 Hz, 3H), 0.86 (dd, J 1 = 6.6 Hz, J 2 = 1.3 Hz, 6H), 0.7 (s, 3H). 13C NMR (CD3OD, 100 MHz): δ 170.9 (1C), 170.8 (1C), 170.7 (1C), 170.6 (1C), 170.5 (1C), 170.2 (1C), 170.1 (1C),
169.9 (1C), 169.7 (1C), 169.5 (1C), 146.9 (1C), 140.8 (1C), 122.1 (1C), 120.9 (1C), 96.3 (1C), 95.9 (1C), 87.6 (1C), 85.4 (1C), 79.3 (1C), 75.4 (1C), 75.3 (1C), 73.7 (1C), 72.7 (1C), 71.8 (1C), 71.1 (1C), 70.6 (1C), 70.3 (1C), 69.6 (1C), 69.4 (1C), 68.7 (1C), 68.1 (1C), 62.9 (1C), 62.4 (1C), 61.7 (1C), 61.6 (1C), 56.9 (1C), 56.3 (1C), 50.4 (1C), 42.5 (1C), 39.9 (1C), 39.7 (1C), 39.1 (1C), 37.3 (1C), 37.0 (1C), 36.4 (1C), 35.9 (1C), 32.1 (1C), 32.0 (1C), 29.9 (1C), 28.4 (1C), 28.2 (1C), 24.5 (1C), 24.0 (1C), 23.0 (1C), 22.8 (1C), 21.3 (1C), 21.0 (1C), 20.9 (1C), 20.85 (1C), 20.83 (1C), 20.7 (1C), 20.4 (1C), 19.6 (1C), 18.9 (1C), 12.0 (1C).

78. $^1$H NMR (DMSO, 400 MHz): $\delta$ 8.25 (s, 1H), 5.60 (d, $J = 9.3$ Hz, 1H), 5.55 (d, $J = 5.9$ Hz, 1H), 5.33 (m, 1H), 5.12 (d, $J = 4.2$ Hz, 1H), 4.89 (m, 1H), 4.82 (m, 1H), 4.68 (m, 2H), 4.54 (m, 2H), 4.24 (d, $J = 7.0$ Hz, 1H), 3.87-3.74 (m, 2H), 3.62-3.24 (m, 5H), 2.36 (m, 1H), 2.10 (m, 1H), 1.96-0.96 (m, 33H), 1.04 (s, 3H), 0.86 (d, $J = 6.4$ Hz, 4H), 0.82 (dd, $J_1 = 6.6$ Hz, $J_2 = 1.8$ Hz, 6H), 0.62 (s, 3H). $^{13}$C NMR (DMSO, 75 MHz): $\delta$ 145.1 (1C), 141.0 (1C), 123.5 (1C), 121.8 (1C), 104.3 (1C), 87.5 (1C), 80.3 (1C), 78.3 (2C), 76.1 (1C), 75.7 (1C), 73.8 (1C), 72.3 (1C), 71.1 (1C), 70.2 (2C), 68.7 (1C), 61.0 (1C), 60.9 (1C), 60.5 (1C), 56.7 (1C), 56.1 (1C), 50.1 (1C), 42.4 (1C), 37.2 (1C), 36.8 (1C), 36.2 (1C), 35.7 (1C), 32.0 (2C), 28.5 (1C), 28.4 (1C), 27.9 (1C), 26.6 (1C), 24.4 (1C), 23.7 (1C), 23.2 (1C), 22.9 (1C), 21.2 (1C), 19.6 (1C), 19.7 (1C), 19.1 (1C), 12.2 (1C).

79. $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 8.13 (s, 1H), 5.80 (d, $J = 9.8$ Hz, 1H), 5.38 (m, 1H), 4.63 (s, 2H), 4.22 (m, 1H), 3.91 (m, 1H), 3.78-3.67 (m, 2H), 3.61-3.24 (m, 3H), 2.40 (m, 1H), 2.25 (m, 1H), 2.07-1.86 (m, 6H), 1.67-0.87 (m, 24H), 1.01 (s, 3H), 0.91 (d,
$J = 6.5 \text{ Hz, 3H}$, 0.89 (dd, $J_1 = 6.6 \text{ Hz, } J_2 = 1.7 \text{ Hz, 6H}$), 0.73 (s, 3H). $^{13}$C NMR (CD$_3$OD, 75 MHz): $\delta$ 145.1 (1C), 140.5 (1C), 122.4 (1C), 121.6 (1C), 86.9 (1C), 80.1 (1C), 78.8 (1C), 74.5 (1C), 70.1 (1C), 61.1 (1C), 60.5 (1C), 56.9 (1C), 56.4 (1C), 55.5 (1C), 50.5 (1C), 42.3 (1C), 39.9 (1C), 39.5 (1C), 38.8 (1C), 37.1 (1C), 36.7 (1C), 36.1 (1C), 35.9 (1C), 32.0 (1C), 31.8 (1C), 29.5 (1C), 28.1 (1C), 28.0 (1C), 26.3 (1C), 24.1 (1C), 23.7 (1C), 22.0 (1C), 21.7 (1C), 21.4 (1C), 21.0 (1C), 18.6 (1C), 18.1 (1C), 11.1 (1C).

80. $^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 8.13 (s, 1H), 5.80 (d, $J = 9.8 \text{ Hz, 1H}$), 5.38 (m, 1H), 4.63 (s, 2H), 4.22 (t, $J = 10.0 \text{ Hz, 1H}$), 3.90 (m, 1H), 3.78-3.32 (m, 5H), 2.38 (m, 1H), 2.21 (m, 1H), 2.07-1.11 (m, 28H), 0.96 (d, $J = 6.5 \text{ Hz, 4H}$), 0.89 (dd, $J_1 = 6.6 \text{ Hz, } J_2 = 1.7 \text{ Hz, 6H}$), 0.73 (s, 3H). $^{13}$C NMR (CD$_3$OD, 100 MHz): $\delta$ 143.1 (1C), 140.7 (1C), 122.0 (1C), 121.7 (1C), 86.9 (1C), 80.2 (1C), 74.6 (1C), 70.5 (1C), 70.1 (1C), 61.1 (1C), 57.1 (1C), 56.4 (1C), 55.5 (1C), 50.5 (1C), 42.8 (1C), 39.1 (1C), 38.8 (1C), 37.2 (1C), 36.2 (1C), 35.9 (1C), 32.0 (1C), 31.9 (1C), 29.6 (1C), 28.1 (1C), 28.0 (1C), 26.4 (1C), 23.7 (1C), 23.6 (1C), 22.0 (1C), 21.7 (1C), 21.4 (1C), 19.5 (1C), 18.6 (1C), 18.0 (1C), 12.7 (1C), 11.1 (1C). ESI/APCI Calcd for C$_{48}$H$_{79}$N$_3$O$_{16}$Na ([M+Na]$^+$) m/e 976.5358; measured m/e: (m/z) 976.5346.
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CURRICULUM VITAE

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SUMMARY
Excellent synthetic, purifying and characterizing skills
Familiar with organic total synthesis process
Familiar with the recent developments of glycosylation and click reactions
Familiar with NMR, IR, GC and MS
Strong ability to work in a team
Knowledge of hardware and software: MS office, Chemoffice, Photoshop and CorelDraw

WORKING EXPERIENCE
Position: Technical Engineer
Responsibility: QC analysis of new refrigerants which can be used in automotive air conditioner. I am Familiar with GC analysis, including TCD and FID detectors. Maintain instruments, organize data and draft monthly report to manager.

2005.08—present Chemistry Department of Utah State University
Position: Teaching Assistant
Responsibility: Instruct undergraduate chemistry lab, both organic and general labs. Maintain chemical instruments, including GC, IR, MS, and NMR. Clean the lab.

2005.08—present Chemistry Department of Utah State University
Position: Research Assistant
Responsibility: Develop some new antibiotic compounds by glycosylation and click reactions. Purify them by column chromatography. Analyze them by MS and NMR.

EDUCATION
2005.8—present Department of chemistry and biochemistry, Utah State University
Major: Organic Chemistry Expected degree: M.S.
2000.9 — 2003.6 Department of Chemistry, Wuhan University
Major: Organic Chemistry Academic Degree: M.S.

1996.9 — 2000.6 Department of Chemistry, Wuhan University
Major: Organic Chemistry Academic Degree: B.S.

RESEARCH EXPERIENCE:
1. Click Reaction in Carbohydrate Derivatives Synthesis: 1-glycopyranosyloxylhexyl - 4- methylenethiol -1,2,3- triazoles carbohydrate chips synthesis.

2. Synthesis of some inhibitors and potential targets for the development of new antitubercular drugs based on the trehalose.

3. Potential anticancer drugs: cholesterol derivatives