METHODOLOGICAL STUDIES OF CHIRAL AUXILIARY ASSISTED GALACTOSYLATION AND THE SYNTHESIS OF THE HEXASACCHARIDE UNIT OF *CLOSTRIDIUM DIFFICILE*

by

WEI HUANG

(Under the Direction of Geert Jan Boons)

ABSTRACT

The rapid assembly of the complex carbohydrates is the one of the major challenges in the development of glycoscience. Herein, we established a set of strategies which combined the selected orthogonal protecting groups, glycosyl donors modified by a (*S*)-phenylthiomethylbenzyl ether at C-2 and fluorous tag-assisted solution phase synthesis into one synthetic procedure. Through this strategy, the rapid preparation of the complex branched carbohydrates with biological importance is possible. The C-2 auxiliaries controlling the 1,2-*cis* galactosylation were investigated and the technique guaranteed the desired glycosylic linkages in the complex molecule. 2-Naphthylmethyl ether (Nap) and levulinic ester (Lev) were installed as the orthogonal protecting groups to generate glycosyl acceptors and served as the branch spots. Once the glycosylation is completed, the C-2 auxiliary can be selectively removed under acidic condition, but the conditions kept the Lev and Nap orthogonal protecting groups, which made the immediate installation of the 1,2-*cis* linkage possible. The light fluorous tag simplified

the purification process into a simple filtration procedure by using fluorocarbons modified silica gel. The synthesis of the hexasaccharide moiety of GPI anchor of *Trypanosoma brucei* was accomplished, and it could be a potential target for the development of carbohydrate conjugate vaccine against sleeping sickness in humans and similar diseases in domestic animals.

Clostridium difficile can cause severe nosocomial infections which further cause high mortality worldwide. The traditional antibiotics failed to stop such infection, and the patients suffer from the reoccurrence of the *C.difficile*. Thus, there is an urgent need to development a vaccine which could stop the infection. The *C. difficile* surface glycan PS-II now is one of the high potential targets for the vaccine development. Herein, we investigated five possible synthetic routes for the assembly of the hexasaccharide from PS-II. By using phenyldiselenide as the anomeric protection group, the aglycon transfer was prevented. A new method to remove the trichloroacetyl amine protecting group by using cesium carbonate was established in the synthesis. Moreover, a reliable route to synthesize the hexasaccharide was confirmed.

INDEX WORDS: Auxiliary;Fluorous Tag;Stereoselective Glycosylations;Sulfonium Ion; Carbohydrate Assembly

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by

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

Ac.....Acetyl
AD.....Alzheimer's Disease
Ac_2O..... Acetic Anhydride
AcOH..... Acetic Acid
Alloc..... Allyloxycarbonyl
BACE..... β-site APP-Cleaving Enzyme
BCR..... B-Cell Receptor
BF_3-Et_2O..... Boron Trifluoride Diethyl Etherate
Bn..... Benzyl
BnBr..... Benzyl Bromide
BSA.....Bovine Serum Albumin
Bz..... Benzoyl
cBSA..... cationized Bovine Serum Albumin
CIP..... Contact Ion Pair
CMD..... Congenital Muscular Dystrophy
COSY..... Correlation Spectroscopy
CS ..... Chondroitin Sulfate
CSA ..... Camphorsulfonic Acid
CSPG..... Chondroitin Sulfate Proteoglycan
DBU..... 1,8-Diazabicycloundec-7-ene
DCC..... N,N'-Dicyclohexylcarbodiimide
DCE..... 1,2-Dichloroethane
DCM.....Dichloromethane
DDQ..... 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DTT ..... Dithiothreitol
DFT ..... Density Functional Theory
DHB ..... 2,5-Dihydroxybenzoic Acid
DIC ..... N,N'-Diisopropylcarbodiimide
DIPEA..... Diisopropylethylamine
DMAP..... 4-N,N'-Dimethylaminopyridine
DMDO..... Dimethyldioxirane
DMF.....N,N'-Dimethylformamide
DTBMP..... 2,6-Di-tert-Butyl-4-Methyl-Pyridine
ECM..... Extracellular Matrix
ER..... Endoplasmic Reticulum
F6P..... Fructose 6-Phosphate
FBP..... Fructose 1,6-bisphosphate
Fmoc 9-Fluorenylmethyloxycarbonyl
FmocCl 9-Fluorenylmethylchloroformate
FUT..... Fucosyltransferase

GAG ..... Glycosaminoglycan
Gal …..... Galactose
Glc .....Glucose
HA..... Hemagglutinin
HIV.....Human Immunodeficiency Virus
HMBC..... Heteronuclear Multiple Bond Correlation Spectroscopy
HS..... Heparin Sulfate
HSPG..... Heparin Sulfate Proteoglycan
HSQC..... Heteronuclear Single Quantum Coherence Spectroscopy
KDO ..... 3-Deoxy-D-Manno-Octulosonic Acid
KHMDS..... Potassium bis(trimethylsilyl)amide
Lev…..... Levulinyl
LG..... Leaving group
LGMD..... Limb-Girdle muscular dystrophy
LPS…..... Lipopolysaccharide
LSD..... Liposome storage disease
MAB..... Monoclonal Antibody
MALDI-TOF ..... Matrix Assisted Laser Desorption Ionization Time-Of-Flight
Man ..... Mannose
mCPBA ..... meta-Chloroperbenzoic Acid
Me ..... Methyl
MHC ..... Major Histocompatibility Complex
MS ..... Molecular Sieves
NADPH ..... Nicotinamide Adenine Dinucleotide Phosphate
NA ..... Neuraminidase
Nap 2-Methylnaphthyl
NBO ..... Natural Bond Order
Neu5Ac ..... N-Acetylneuraminic Acid
NIS ..... N-Iodosuccinimide
NMR ..... Nuclear Magnetic Resonance
NPC ..... Niemann–Pick disease type C
OMe ..... Methoxy
OGA ..... O-GlcNAcase
OGT ..... O-GlcNActransferase
PBS ..... Phosphate Buffered Saline
PFK ..... Phosphofructokinase
POMT ..... Protein O-Mannosyl Transferase
PTM .....Post-Translational Modification
Ph ..... Phenyl
Pse ..... Pseudaminic acid
PTP ..... Protein tyrosine phosphatases
Py ..... Pyridine
R5P Ribose-5-Phosphate
Sia ..... Sialic acid
SSIP ..... Solvent Separated Ion Pair
TACA ..... Tumor-Associated Carbohydrate Antigen

TBAF ..... Tetrabutyl Ammonium Fluoride
TDS ..... dimethyl (1,1,2-trimethylpropyl) silyl
TCA ..... Trichloroacetonitrile
TCEP ..... Tris(2-carboxyethyl)phosphine hydrochloride
TEA ..... Triethylamine
Tf_2O .....Trifluoromethanesulfonic Anhydride
TFA ..... Trifluoroacetic Acid
TfOH ..... Trifluoromethanesulfonic Acid
THF ..... Tetrahydrofuran
TLC ..... Thin Layer Chromatography
TLR ..... Toll-Like Receptor
TMEDA ..... N,N,N',N'-Tetramethylethylenediamine
TMSOTf ..... Trimethylsilyl Trifluoromethanesulfonate
TMP 2,4,6-Tri-Methoxylphenyl
TOCSY ..... Total Correlation Spectroscopy
Troc 2,2,2-Trichloroethyloxycarbonyl
TS ..... Transition State
SSEA ..... Stage-Specific Embryonic Antigen

CHAPTER 1:

INTRODUCTION AND LITERATURE OVERVIEW

Though carbohydates had been previously thought of energy sources, it is now well established that they play critical roles in most of the biological activities.[1] However, due to the complexity and heterogeneity nature of carbohydrates, the understanding of the molecular basis of glycan functions is still under development.[2] Unlike proteins and nucleic acids that are made by template driven biosynthesis, carbohydrates act like an exotic language between cells. Such a language has certain rules and regulations. However, there are not any uniform ways to express a particular meaning. For example, N-linked glycans all start with the pentasaccharide core containing mannoses and GlcNacs (N-acetylglucosamine) moieties; however the extensions of the GlcNacs could be varied from cell to cell.[3] Glycosylation site, monosaccharide composition, and glycan length can all be different between cells and even within a single cell[4]. Such complexity makes the understanding of such language particularly difficult. However, this difficulty offers scientists opportunities to explore this tremendous and mysterious area that had been previously underestimated. In this chapter, the current research progress of carbohydrates will be present in the following order: 1) The importance of biological functions of carbohydrates; 2) The methods used in the carbohydrate analysis; 3) Carbohydrate conjugate vaccines; 4) The method utilized in the carbohydrate synthesis and purification.

The biological functions of carbohydrates in mammals

The heterogeneous structure and complexity of glycans on cell surface enable them to play a significant role in signaling, recognition, and adhesion[5,6]. Based on those functions, cell surface glycans take part in many bio-physiology functions including normal embryonic development, cell signaling, host–pathogen interaction during infection, disease development, metastasis and localization, the rate of degradation and membrane rigidity[7-9]. Because of their heterogeneity and complexity, little has been explored in living systems, although the physical and chemical properties of simple carbohydrates have been well studied. Studies focusing on glycobiology have been increasing at high speed in the past years[10,11].

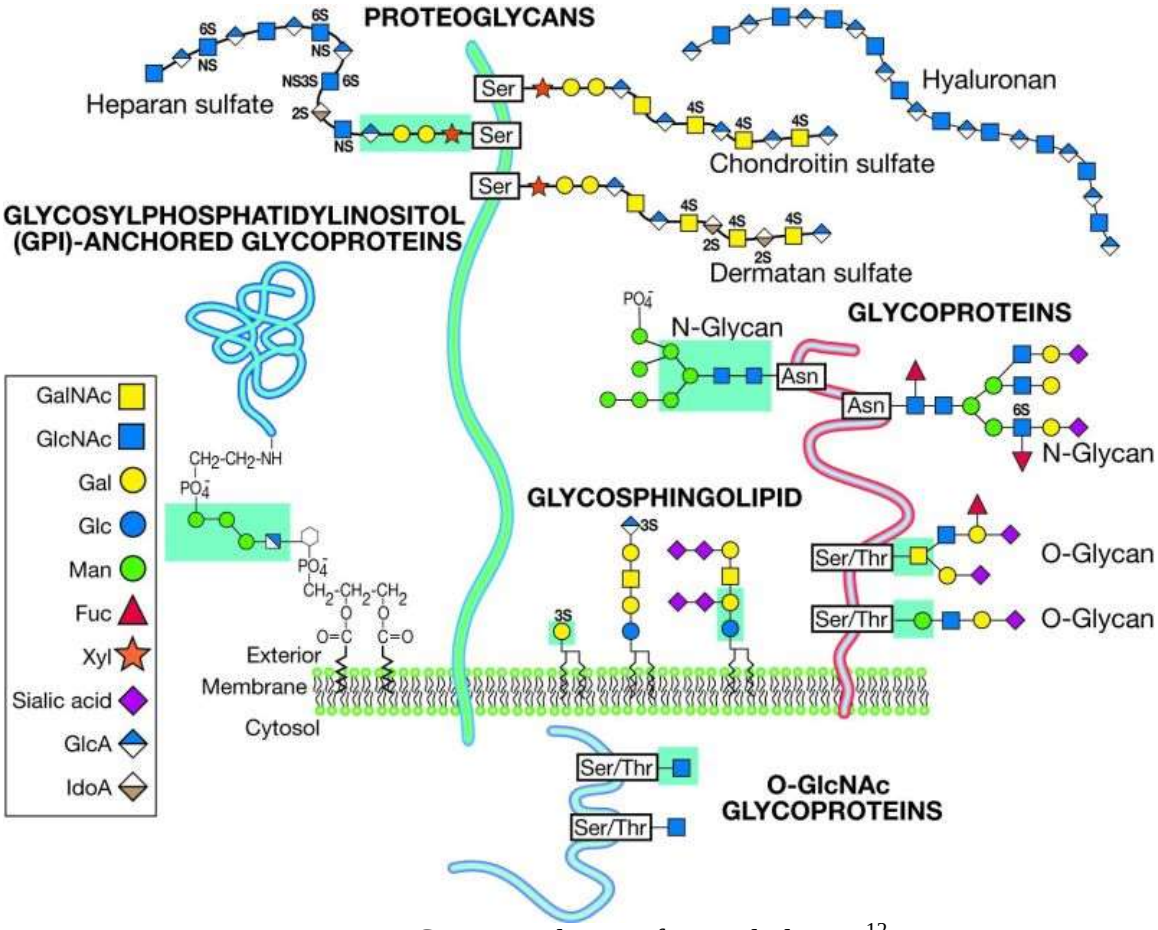


Figure 1.1: Common classes of animal glycans[12]

Several types of glycans exist in mammals. For example, N-glycans are attached to the N-terminal of an asparagine residue of a protein carrier; similarly, O-glycans are appended to the O terminal of a serine or a threonine residue[13]; and glycosaminoglycans (GAGs) are attached to serine residues of proteoglycan moieties. Also, carbohydrates attached to sphingolipids are named as glycosphingolipids and carbohydrates that connected to the protein and lipid containing an inositol moiety are named glycophosphatidylinositol (GPI) anchors. Hyaluronans often act as free glycan chains.

Oligosaccharides can attach to proteins, thus forming a glycosylic bond by two kinds of linkages. The first one is called N-glycan and is a glycan linked to asparagine

residues in the sequence of Asn-X-Ser (Thr), where X can be any amino acid except proline. The second type involves a glycan linked to the hydroxyl of serine or threonine, which is called an O-linked glycan[14].

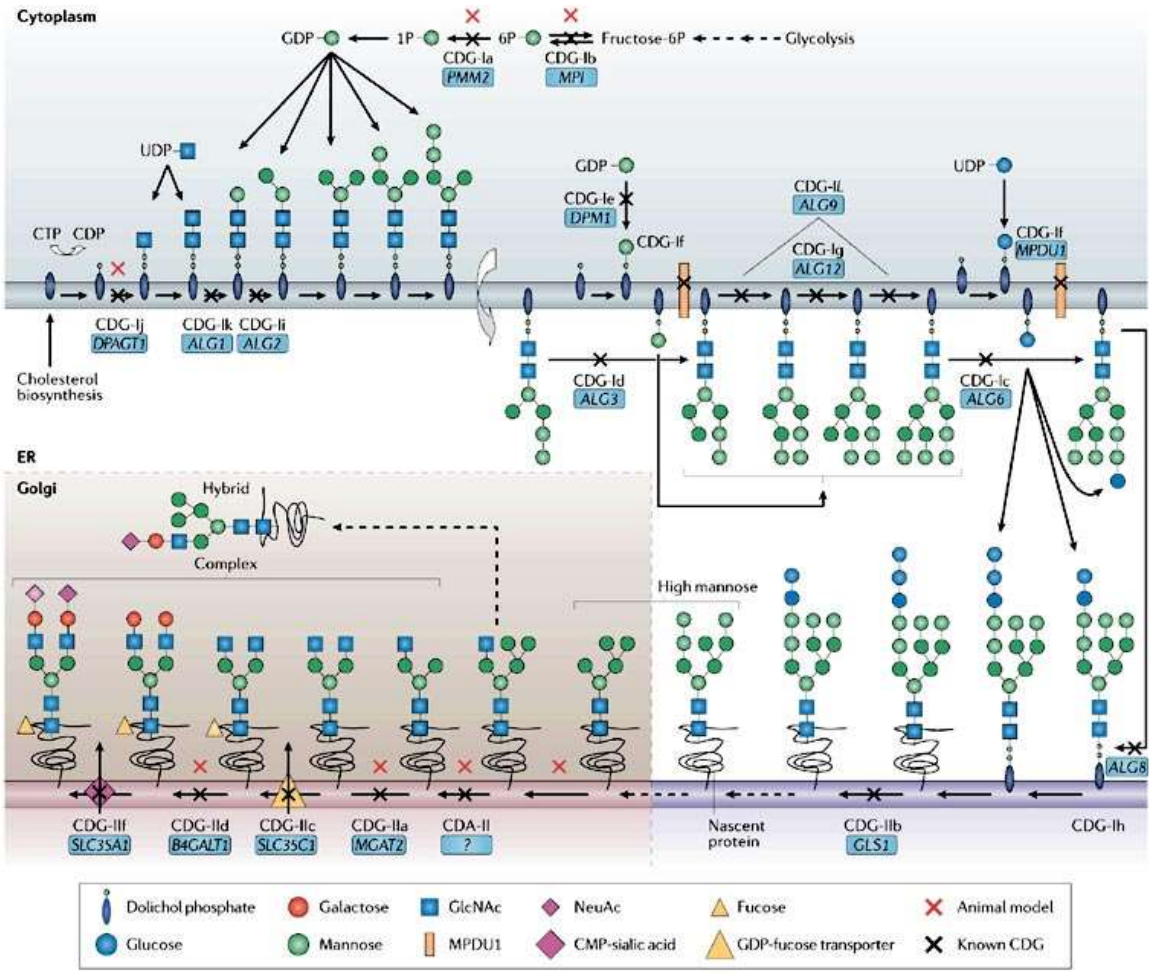


Figure 1.2: N-glycan biosynthesis[15]

N-glycans in eukaryotes start from the attachment of N-acetylglucosamine to a dolichol lipid chain. After several glycosylic additions by glycosyltransferases, a 14 carbohydrate unit oligosaccharide precursor is formed. Moreover, the precursor is

transferred to the asparagine of the target protein chain. During this step, the glycan plays a critical role in quality control of protein folding; only the properly folded protein could proceed to the next stage and the misfolded protein will enter the lysosome for recycling. In the next step, glucosidases remove three glucose moieties from the N-glycan which attaches to correctly folded protein and then the proteins are transferred from ER to the Golgi apparatus. In Golgi, more mannose moieties will be deleted and other monosaccharides units will be attached to extend the oligosaccharides chain. The oligosaccharide will differentiate into three major classes of N-glycan: 1) High-mannose; 2) Complex; 3) And, hybrid. Such a synthetic pathway in the Golgi apparatus complicates the structures of N-glycans. Moreover, all three major classes of N-glycans are attached to different portions of protein further increasing the complexity of glycoproteins.

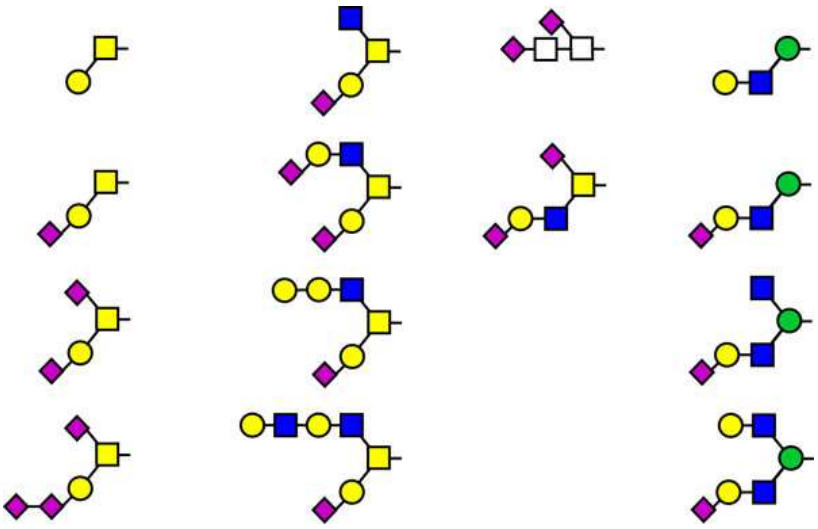


Figure 1.3: Different types of O-linked glycans

O-glycan modification of proteins is believed to have most likely happened in the Golgi apparatus[16]. In the case of O-glycans, the anomeric center of N-acetylgalactosamine is covalently linked to the hydroxyl group of a serine or threonine of the target protein[17]. Once the attachment is completed, the extension of the O-glycans then proceeds by glycosylation of other monosaccharide moieties such as galactose, N-acetylglucosamine, fucose and sialic acid. Several different types of O-glycan have been found, for example, O-mannose, O-fucose, O-glucose and O-N-Acetylglucosamine and even more novel linkages are being identified[18]. It has been shown that the dynamic modification of proteins by the O-β-N-acetylglucosamine plays a paramount role in the modulation of protein biological functions. In this case, the modulation is through several types of mechanisms: 1) Modification of protein via phosphorylation; 2) Protein and protein interactions regulation; 3) Protein degradation; 4) Protein localization; 5) And, transcription regulation[19]. For example, it has been found that in many site-mapping studies, O-phosphate and O-GlcNAc modify the same protein residue. The data implicates that those two modifications modulate the protein functions by competing to attach to the same residues on serine or threonine. Therefore, we can conclude that the O-GlcNAc regulates protein function by changing the phosphorylation patterns[9].

Figure 1.4: Cancer-associated glycans

In cancer, changed glycosylation patterns are treated as the hallmark of the tumor. Meezan et al. found this phenomenon in 1969, that healthy fibroblasts had smaller glycoproteins than tumor cells[20]. This result further proves that the lectins had different binding affinities to healthy tissue compared with tumor tissue[21]. With the development of monoclonal antibodies and mass spectrometry[22], cancer-associated cell surface glycans could be directly identified, which further proved the previous finding[23].

Those changes include under or overexpression of natural glycans and also with a neo expression of glycans that is usually restricted to embryonic tissues. The reason for changes in the expression levels is the increasing expression of glycosyltransferases in the Golgi apparatus of cancer cells. Such difference in glycosyltransferase expression could cause the modification of the essential structure of N- or O- glycans. Among those changes, one of most prominent changes is the bigger size and more branching of N-glycans. The increased branching comes from the increased activity of the glycosyltransferase of N-acetylglucosamine (GlcNAc-TV)[24]. The increased branching generates more sites for the sialic acid modification at terminal branches. Together with upregulated sialyltransferases, those changes eventually lead to an increase of sialylation of the whole body[25]. Furthermore, the changes that happened to the terminal structures of glycans could also relate to malignancy. For example, sialyltransferases and fucosyltransferases will modify the terminal residues of glycans and there is evidence of overexpression in the malignant tissue. The increasing activity of these glycosyltransferases causes the overexpression of certain glycan moieties. There are examples of terminal glycan modifications that are commonly discovered on the tumorous cell surface, such as sialyl Lewis X (sLe x), sialyl Tn (sTn), Globo H, Lewis y (Le y) and polysialic acid. Many of those biomarkers have appeared in cancer tissues from brain, breast, colon, and prostate[26-28].

Besides the glycan modifications, certain glycoproteins and glycolipids could be another common markers of cancer, which are also overexpressed[29]. As with the epithelial tumors, the mucin proteins that are heavily decorated with O-linked glycans are usually overexpressed. This type of protein has been recognized as a marker for cancer

diagnosis. The mucin proteins could also be the frame for other cancer-related epitopes. Researchers also found that the overexpression of gangliosides is another marker for cancer. For example, GD2, GD3, and fucosyl GM1 have been found with an increased amount of expression in small-cell lung carcinomas, neuroblastomas, and melanomas[30]. However, even though it is easy to assess the global glycosylation changes in cancer patients, it is impossible to determine if the single change would lead to a malignant cell. It could only be determined by a different combination of changes in glycan expression[31,32].

The research methods in glycan analysis

To investigate the functions of carbohydrates in biological activities, numerous tools have been invented and utilized in the research field. In this section, several different tools used in the carbohydrate analysis will be discussed.

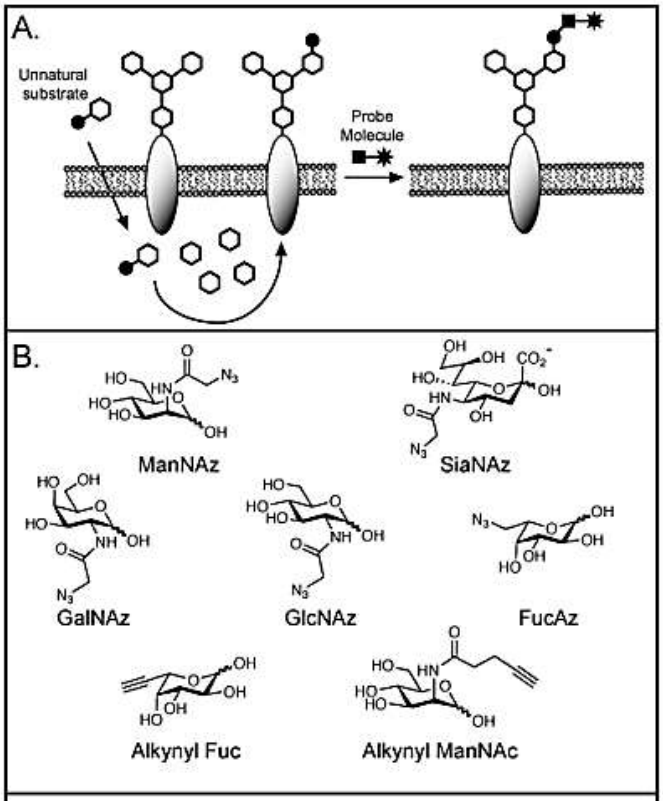


Figure 1.5 The bioorthogonal chemical reporter strategy for profiling and visualizing glycans.

(A) Metabolic labeling of cell-surface glycans. (B) Azidosugars and alkynyl sugars.

The physiological changes related to glycosylation need a tool to visualize target biopolymers in a physiologically similar background. By modification of target proteins with fluorescent protein fusion, the target protein would be tagged and tracked, and the information such as location and function will be obtained. The method of tagging and tracking of proteins had been well established for decades. However, the method to track and tag carbohydrates is still under development. With an aim to explore the abundance, distribution and dynamics of carbohydrates in the cell, many methods have been

developed. The bioorthogonal chemical reporter is just one of such methods to address the issue of glycan imaging. The very first method is copper-catalyzed azide and alkyne reaction. Through this technique, researchers can tag certain sialic acid-containing carbohydrate polymers with azide-modified mannosamine or galactosamine in cells since azide modification is not present in nature so the selectivity is guaranteed. Before fixing the cell with formaldehyde[33,34], a probe that contains both alkyne functionality and a fluorescent moiety is cultured with the cell under copper (I) catalysis and a click reaction will happen. By such means, the probe will selectively react with azide modified carbohydrates and accumulate on the cell surfaces. Thus, the particular type of glycan can be visualized and imaged.

Because of the toxicity of copper (I) catalyst, the tracking and tagging of living cells is impossible to accomplish with this type of method. The next important step is the development of an imaging tool that will avoid the usage of copper (I) catalyst. Staudinger ligation is a clever invention to prevent the usage of the catalyst. A molecular probe containing phosphine and ester-containing dyes is cultured with living cells. After the phosphine reduces the azide into an amine, an intramolecular ester exchange will occur and the azide-modified carbohydrates will be tagged with fluorescence. It has been proven that this method has good selectivity with azides and also low background staining during cell surface glycan labeling[35].

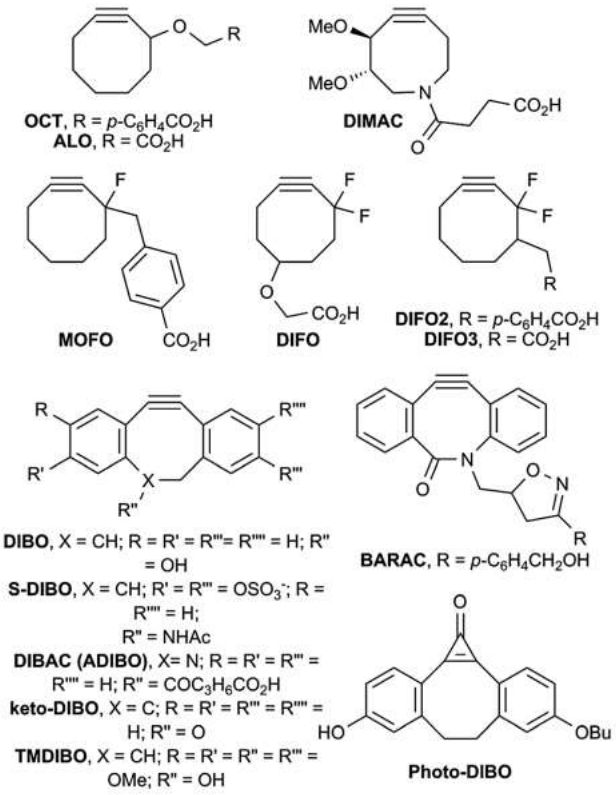


Figure 1.6: Cyclooctyne reagents for Copper-free click chemistry[36]

However, the Staudinger ligation suffers from low kinetic rates. In an aim to address these issues, the Bertozzi group developed a difluorinated cyclooctyne reagent named DIFO, which can react with azides rapidly without any toxicity in animals[37,38]. The DIFO-azide reaction that has a high kinetic rate was employed to observe the dynamic cellular glycosylation of zebrafish embryo development. In this research, the zebrafish embryo was labeled with azide-modified galactosamine and reacted with fluorescent DIFO reagents to identify the total O-glycan variation in the fish[39]. This method was also extended to monitor dynamic glycosylation through quenching of the unreacted azides with TCEP. After feeding the embryos with another azido modified galactosamine and labeling again, the blue-shifted DIFO product was probed. The areas

that are involved in rapid O-glycan biosynthesis are the fins, jaw, and olfactory organs. Moreover, these areas show more labeling intensity with the blue shifted conjugate. The quench-label-tag method exhibits high reactivity of DIFO to monitor the concentration changes of glycans, which are below the limit of Staudinger conjugation. Furthermore, compared with lectin or antibody labeling, this method is easier for the reagents to approach the tissue because of the nature of the small molecule. Most importantly, the zebrafish continued its normal development, which further proved its low cytotoxicity and in vivo compatibility. To further improve this technology, Boons group also developed DIBO (dibenzo cyclooctyne) which is even faster and easier to synthesize and the kinetics of the click reaction is much higher than conventional methods.

It is not hard to imagine in the near future that this technology can extend to the human systems and will help the tumor identification and microbial infection imaging.

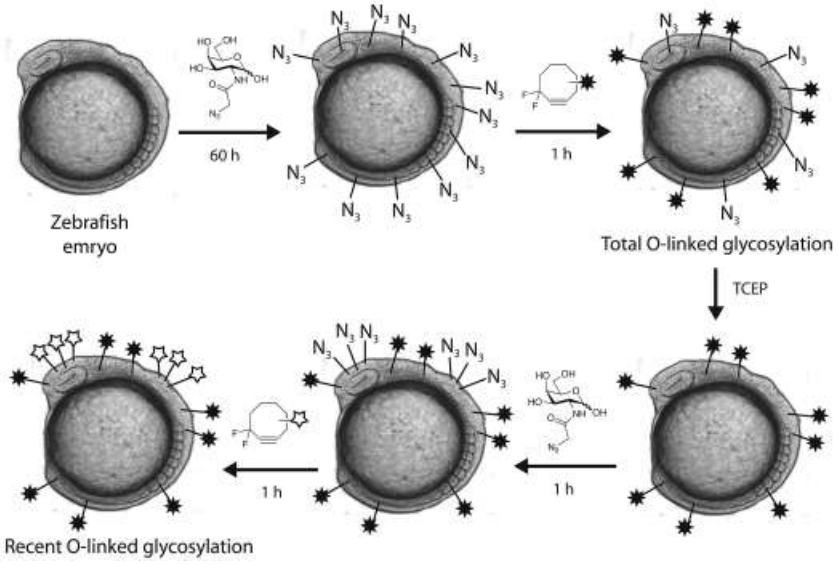


Figure 1.7: The quench-label-tag sequence for visualizing dynamic glycosylation

The bioorthogonal chemical reporter strategy also has been used in the dynamic glycoconjugates purification and inventory. In this procedure, the azide-modified monosaccharides will attach to the glycans by the cell's metabolic machinery[40]. With the incubation time and concentration changes, the newest synthesized glycans will utilize modified monosaccharides and present the azide containing sugar to the cell surface. In such case, the carbohydrate will be labeled. Subsequently, reagents bearing a biotin tag for purification will specifically react with azide functionalities on the cell surface. By affinity chromatography, the targeted glycans can be captured and enriched.

Even though metabolic labeling is a powerful tool for visualizing and enriching glycans and glycoproteins, the detailed carbohydrate and protein structure informations are impossible to obtain by this method. In this case, mass spectrometry could extend the search to another dimension.

MS, particularly tandem MS, have further improved the analysis of both glycomics and glycoproteomics. Collision-induced dissociation (CID) has been extensively employed in the detailed protein or glycoprotein analyses. Another technique such as electron-transfer dissociation (ETD) has even better potential for characterization of glycopeptides and glycoproteins. The process is initiated by a radical, which benefits glycosylated peptides due to the glycans staying intact during the fragmentation process, so the ETD can research the site of glycan modification and obtain the information on glycans yields on each site[41,42]. Another cutting edge tandem MS is the higher energy collisional dissociation (HCD) which is utilized on the octopole of orbitrap instruments[43]. One of the most important features is the generation of Y1-fragment (peptide with GlcNAc), which can be selectively isolated and subsequently dissociated generating the

amino acid sequence[44]. Moreover, this method can selectively trigger ETD if oxonium ion is detected.

Mass spectrometry with all kinds of ionization methods and mass analyzer modes can provide an ideal and the most sensitive tools to analyze the expected detailed structure of N- or O- linked glycans. However, due to the isomerism of complex carbohydrates and its heterogeneous nature, MS cannot identify a specific isomer unless it has been isolated or selectively derivatized. Capillary liquid chromatography and electrophoresis, luckily, can separate such different isomer structures. By combining with MS, it can be a powerful and efficient analytical method for glycan mapping.

Before the application of all those advanced MS techniques, one of the most important steps is that the interested glycans must be released quantitatively from the target glycoprotein. A quantitative and repeatable releasing method has great significance for the current need for high sensitivity measurements. Conventionally, hydrazine was used as the chemical way to release N-linked glycans, which usally results in side reactions and imcomplete reactions. Now, this method has been replaced by a more advanced enzymatic approach using N-glycanases (N-glycosidase F and A), which are easy to handle and result in reliable reaction products. However, for the O-glycans that attach to threonine or serine, β-elimination is still the most efficient procedure due to the shortage of the O-glycanases. In general, N-glycans are easier to research than O-glycans. One of the most important goals for derivatization is reducing reaction time without compromising its digestion efficiency. To achieve such goal, ultra-high pressure cycling[45] or microwave assisted heating[46] with immobilized reactors were involved[47]. In the research of O-linked glycans, the alternative chemical cleavage approaches other than

β-elimination were actively under development to recover O-glycans, such as dimethylamine combined with microwave radiation[48]; the use of pronase to achieve a complete digestion[49]; as well as the combination of solid-phase permethylation and β-elimination.

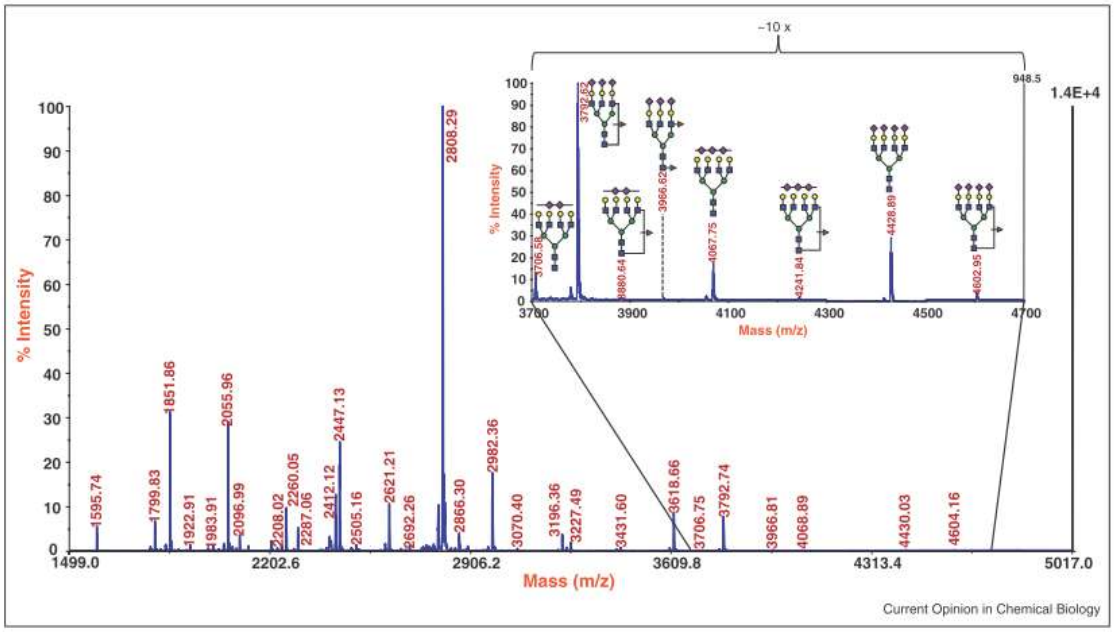


Figure 1.8 A MALDI-TOF profile of permethylated glycans of a blood serum sample originated from an ovarian cancer patient[49]

After release, the profiles of the glycans can be established through MALDI-MS or ESI- MS quantitatively. ESI-MS is often combined with liquid chromatography (LC) to achieve better profiling. Both methods have features to address specific issues across different areas. The greatest advantage of MALDI based techniques is high sensitivity and relatively being able to tolerate impurities. ESI has the disadvantage of generating a set of ion peaks for a single molecule, which is due to multiple ions being attached to one molecule. Since many glycosylic linkages and decorations (sialic acid and fucose; sulfate

and phosphate decorations) are presented, the derivatization before MS analysis is recommended. Permethylation is currently a most popular method for chemical modification. The reasons for the chemical derivatization are because it will stabilize the glycan analytes and both neutral and acidic glycans can be presented in one spectrum profile. At last, this will largely enhance the sensitivity and generate more individual data for MS fragmentation studies. Permethylation can also be a useful tool for the reverse phase LC-MS system, which converts the glycan from hydrophilic to hydrophobic[50]. Isotopic labeling combined with permethylation even further extends the scope of this derivatization method for comparative profiling measurement[51].

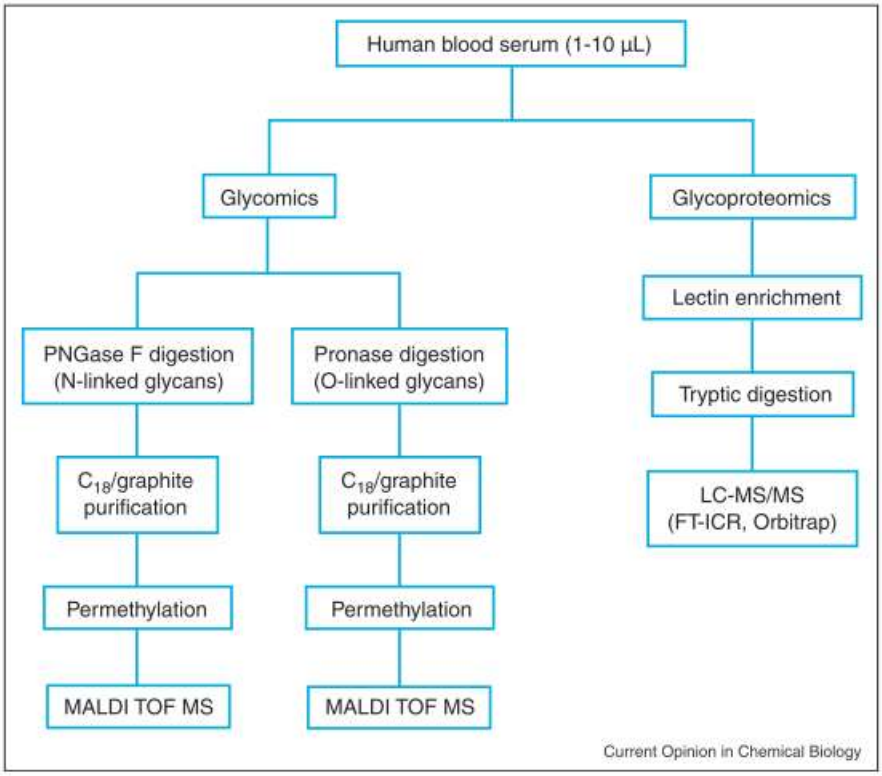


Figure 1.9: Workflow for high-sensitivity glycoprotein analysis

Glycan profiling can also be enhanced by the combination of separation techniques such as HPLC, capillary LC, and capillary electrophoresis (CE). In these cases, fluorescent derivatization is necessary for the profiling process because the UV detector is usually used. There are numerous successful examples of the discovery of disease biomarkers by applying HPLC and fluorescent derivatization[52,53]. Hydrophilic interaction chromatography (HILIC) is another powerful tool featuring separation of different glycan isomers with high resolution[54]. For the same reason, a special chromatographic material, porous graphitized carbon (PGC) is drawing increasing attention[55]. PGC columns takes a part in analyzing the minuscule amount of biological molecules by attaching with MS [56].

The utilization of microfabricated devices (microchips) is becoming increasingly popular for its ability in various glycan separations. One of the reasons for its popularity is due to the fact that separating channels can be tightly loaded with chromatographic material and give it high efficiency. On the other hand, it can also integrate with different parts such as various trapping column, microreactors, and switching valves. Such a combination largely reduces the time of manual operation and sample transfer. Furthermore, the microchip can be linked with a different "MS inlet" and further improve this technology. The microchip, which contains the LC system and packing with different chromatographic materials, has been used in separating permethylated blood serum glycans and analyzed by ESI-MS system. By using graphitic carbon as padding material, both serum and breast milk glycans were carefully identified by Lebrilla group[57,58]. Another technique used in separation is CE, which has been employed to separate various glycan mixtures labeled with a particular fluorophore moiety for detection.

Unfortunately, CE has difficulties to connect to MS. The current shortage of characterized glycan standards is the most serious hindrance to CE analysis.

In conclusion, mass spectrometries combined with different separating tools can be a powerful strategy for providing glycan information such as carbohydrate composition, linkage pattern, and glycosylation sites. However, detailed structure information such as α or β configuration is still under development. In this case, NMR currently is the only reliable analytical tool to address the glycan configuration issues. However, it is nearly impossible to isolate enough amounts of certain glycans from patients or animals in relatively high purity. So, the synthesis of bioactive complex carbohydrates as the standard is the only solution.

Carbohydrate conjugate vaccines

Carbohydrates often are expressed on the surface of pathogenic bacteria. Most of those carbohydrates are structurally unique and could be the potential target for vaccination and diagnostic development. Vaccines that are based on capsular polysaccharides (CPS) have existed for decades and have been widely utilized in the pharmaceutical industries against different pathogenic bacteria[59,60]. Those carbohydrate materials are extracted from target bacterial cultures[61]. However, it is common that the impurities, such as cell-wall polysaccharides, are co-isolated with the desired polysaccharide. Moreover, such impurities may cause hyporesponsiveness and other side effects[62]. Because the polysaccharides purified from bacteria culture are heterogeneous, repeating purification and quality control steps are necessary for the carbohydrate antigens to be utilized in the vaccine products. The synthetic oligosaccharides derived

from the repeating unit of CPS is the other option to obtain a glycoconjugate vaccine without contamination and with predictable clinical results[63]. However, it is not straightforward and it is scientifically challenging to designing a vaccine based on synthetic oligosaccharides. Moreover, there is a major bottleneck about the identification of an effective epitope.

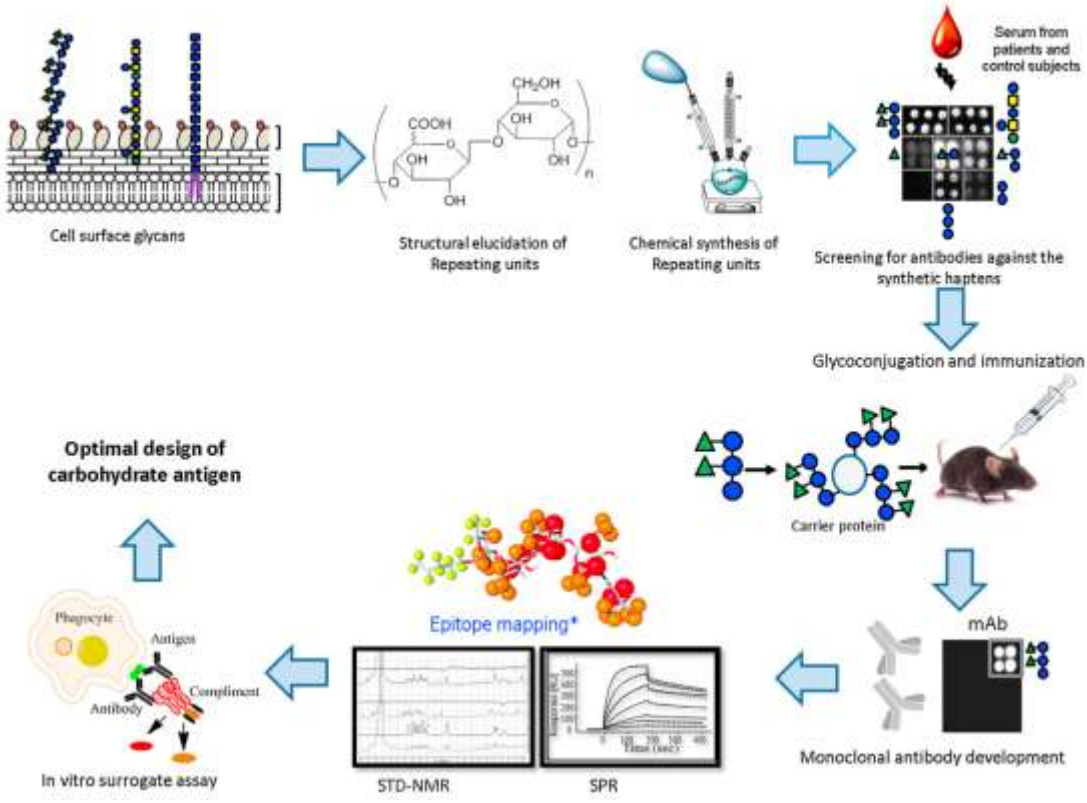


Figure 1.10: Major Steps Involved in Rational Design of Synthetic Carbohydrate Vaccines[4]

The effective epitopes are the segments of antigenic polysaccharides which could be recognized by membrane-bound immunoglobulin (Ig) molecules on B cell, and those epitopes are called glycan B cell epitopes[64]. A short-term and IgM dependent immune

response can be induced by the polysaccharides, which are T cell-independent antigens. However, this type of antigen fails to generate an efficient immunological memory. It could be converted to T cell dependent antigens by conjugating to a carrier protein that will eventually induce a long-lasting memory response. Such a long-lasting memory response will accompany the switch of antigen recognizing B cells to a plasma cell. Moreover, either reinfection from a pathogen or a boosting from a vaccine will induce plasma cells' rapid proliferation and the secretion of a vast number of antibodies.

It has been shown that carbohydrate conjugate vaccines have succeeded to prevent infectious diseases that are caused by *Neisseria meningitides*, *Haemophilus influenza,* and *Streptococcus pneumoniae*[65]. However, the vaccine manufacturer faces certain bottlenecks despite the success of CPS-based vaccines. Moreover, one of the major disadvantages is the isolation and purification of pure capsular polysaccharides coming from pathogenic bacteria. The large scale bacterial culture is not applicable to all bacteria strains[66]. Growth conditions need carefully optimization with an aim to produce polysaccharides[67]. Additionally, certain CPSs are not stable and will be decomposed under isolation conditions or formulation processed[68]. The further purification of polysaccharides must be carefully checked on different levels that are specified by guidelines coming from the regulatory authorities. For example, the contaminants such as proteins, nucleic acid, and especially other cellular polysaccharides must be eliminated. The structure of the desired polysaccharides must be evaluated by chemical and instrumental analysis, for example, nuclear magnetic resonance (NMR) spectroscopy[69]. After careful depolymerization and chemical activation, the polysaccharides are then conjugated to the carrier protein and the quality control is

necessary during glycoconjugate manufacture, which further raises the cost of the final vaccine. Furthermore, after chemical derivatization of polysaccharides, artificial and non-protective epitopes are common in the vaccine formulation that further compromises vaccine efficiency. Identification of the correct antigenic epitope is one of the most important steps toward an effective vaccine that can protect the host from the pathogen. However, it is almost impossible to purify and obtain a homogeneous polysaccharide to improve vaccine efficiency[70].

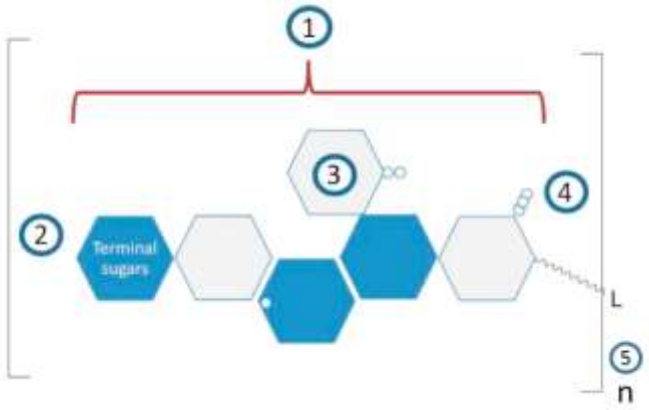


Figure 1.11: The Immunogenic Determinants of a Synthetic Carbohydrate Antigen Size/span of the epitope.

(1), terminal glycan residues (2), the presence of branching points (3), side chain functional groups (4), and some repeating units (5), preinstalled linker for conjugation[71].

There is an alternative way to obtain the structurally uniform vaccine without any impurities, which utilizes synthetic oligosaccharides replacing the repeating unit of polysaccharides. Because synthetic oligosaccharides could be structurally well-characterized, the production of glycoconjugate vaccines is reproducible. On the other

hand, a more efficient formulation could be achieved with a structurally defined antigen and the cost of vaccine development will further decrease.

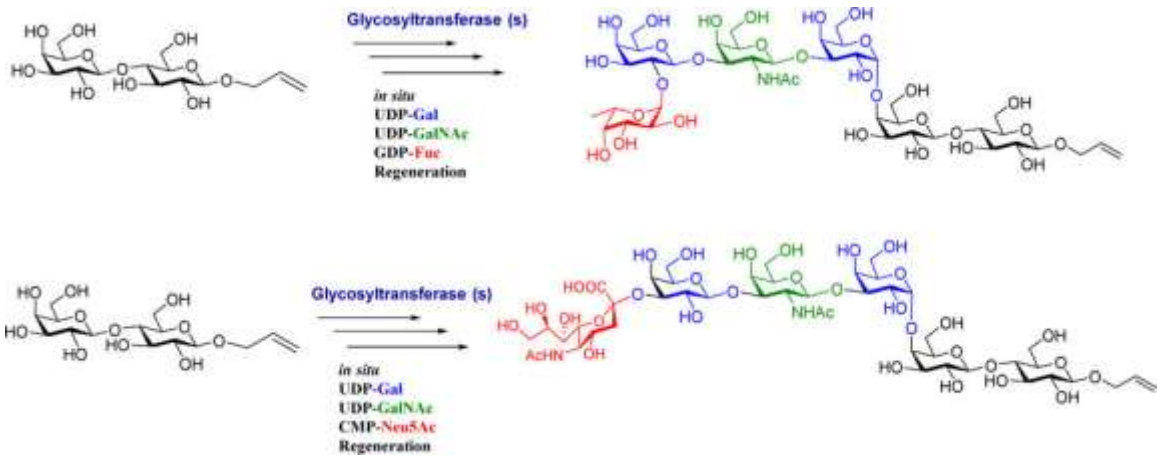


Figure 1.12: Effective Sugar Nucleotide Regeneration for the Large-Scale Enzymatic Synthesis of Globo H and SSEA4[72]

Recently, extensive research on the oligosaccharide syntheses have been done, which led to highly antigenic compounds[73]. The potent synthetic oligosaccharides vaccine that offers a protective effect on *Haemophilus influenzae* type b type b (Hib) has been commercialized in Cuba[74]. Another example is tumor associated antigens Globo H and SSEA-4 hexasaccharide. Both are attractive targets for the development of anti-tumor vaccines[72]. The Globo H development combined the basic biochemical and clinical result and further improved an entirely synthetic antigen. Moreover, carbohydrate conjugate vaccines based on the synthetic antigen have reached the early clinical trials[75].

Carbohydrate Synthesis and Purification Methods

Although glycobiology and carbohydrate-related therapeutic methods have made significant progress in recent decades, one of the major bottlenecks in the further development of glycoscience is the shortage of well-defined carbohydrate structures and their conjugates in high purity. The heterogeneity of carbohydrates in nature creates a problem isolating pure compounds in appropriate quantities. It is almost impossible to isolate one particularly desired glycan in the proper amount to obtain detailed structure characterization. In most of the cases, the only source for obtaining structurally well-defined oligosaccharides is through chemical or enzymatic synthesis.

The bio-organic synthesis of peptides and nucleotides has been streamlined and utilized efficiently for decades, due to the limited available substrate reaction sites and well developed automated solid phase synthetic methods. An advancement in biological development resulted through the ease of obtaining peptides and nucleotides conveniently through simple and efficient chemistry. However, due to the scientific challenges of heterogeneity and complexity of carbohydrates, the field of automating carbohydrate synthesis is still under development.

The development of an automated chemical carbohydrate synthesis is hindered by the diversity and complexity of the carbohydrate itself. There are two aspects that cause complexity of carbohydrate automated synthesis in comparison to other biological polymers. Monosaccharides have a diverse range of linkages, and oligosaccharides can be highly branched molecules containing a vast array of monosaccharides. Since protein and nucleic acids can only be attached to each other through one-dimensional linkages (amide bonds for proteins and [3'-5']-phosphodiester bonds for nucleic acids), it is much

easier to control the outcome of each coupling reaction. However, in the case of carbohydrates, the synthesis can be multi-dimensional which provides numerous variations in their structures, making it scientifically challenging to develop specific linkages without the use of laborious synthetic and enzymatic techniques. In addition to variations in linkage placement, carbohydrates contain an anomeric center that can be linked through an acetal functionality, or glycosidic bond. The coupling reaction can result in two configuration isomers α and β which further complicates the whole structure.

The anomeric stereochemistry is usually defined by the relative position of the C-2 hydroxyl group as either 1, 2- cis or 1, 2-trans or the relationship to the last chiral substituent on the six-member ring, either an α or a β linkage. Moreover, to attach a monosaccharide unit to another carbohydrate substrate, protecting group manipulation is necessary for preventing mis-coupling products and other side reactions. Normally an oligosaccharide synthesis process will undergo three stages. First, the protecting groups need to be installed on the monosaccharide building blocks, and orthogonal protection is necessary since one particular hydroxyl group needs to be exposed as an acceptor/donor before each glycosylation reaction. The next step is performing the glycosylation reactions that assemble monosaccharide building blocks together. Once the target oligosaccharide assembly is completed, a global deprotection reaction is performed to yield the desired oligosaccharide product.

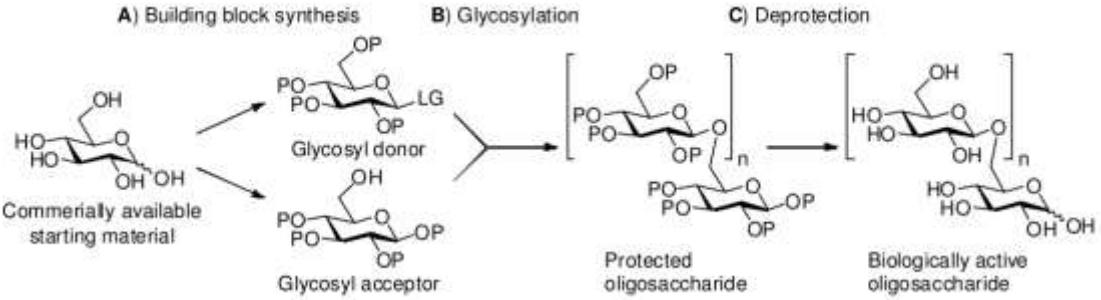


Figure 1.13: An overview of a typical oligosaccharide synthesis

As mentioned before, the first challenge in carbohydrate chemistry is the intensive labor costing carbohydrate assembly. Much work has been done to simplify the whole process[76,77]. For example, one-pot glycosylation has been developed to avoid time-consuming intermediate purification and shorten the assembly time[78,79]. Solid phase oligosaccharide synthesis (SPOS) was also developed for the much easier reaction product purification[80,81]. If the whole SPOS procedure can be executed in an automated fashion, the technique further reduces the laboratory labor requirement[82]. There is an example that average skilled personnel can perform the oligosaccharide synthesis by automated SPOS within 24 hours.

Recently, the discovery of one-pot introduction of multiple protecting groups can speed-up the monosaccharide building block synthesis, which further reduces the time and labor of the oligosaccharide synthesis[83,84].

However, all those advances do not address the issue of the stereo-control of a glycosylation reaction. If different isomers are generated during a glycosylation, a time-consuming and tedious separation is required. The control of stereoselectivity becomes

another major challenge that hinders the development of carbohydrate chemistry. In the next section, the development of stereoselective glycosylation reactions will be discussed.

Emil Fisher discovered the first chemical glycosylation method in 1893[85]. Under the acid-catalyzed reaction conditions, an anomeric lactol can be coupled with one hydroxyl group on the sugar ring yielding a glycoside product. The method was improved by Koenigs and Knorr in 1902 by using glycosyl halides and a silver salt to catalyze the coupling reaction[86].

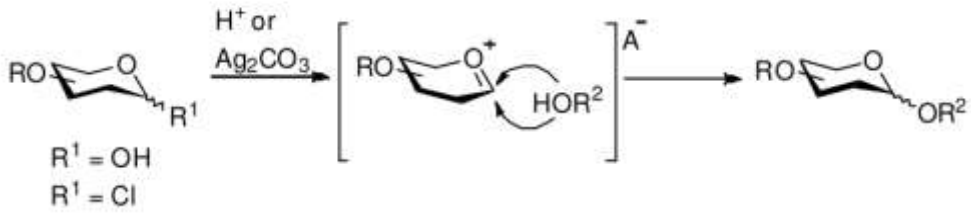


Figure 1.14 Fisher glycosylation and Koenigs and Knorr glycosylation

In a glycosylation reaction, the glycosyl donor requires a leaving group attached to the anomeric center, and the acceptor contains a nucleophilic functionality (OH or SH) to couple with the donor. Under proper promoting conditions, the leaving group will detach and form an oxocarbenium ion. Then, the acceptor will attack from either top face or the bottom face resulting in either an α- or β-glycoside respectively. In the cases in which neighboring group participation is not a factor, the glycosylation outcome is determined by the so-called "anomeric effect."

Generally, in a six member ring system, all substituents prefer an equatorial position that is energetically favored due to steric hindrance of the 1, 3-diaxial interactions. Thus, the β-direction should be preferred in glycosylation.

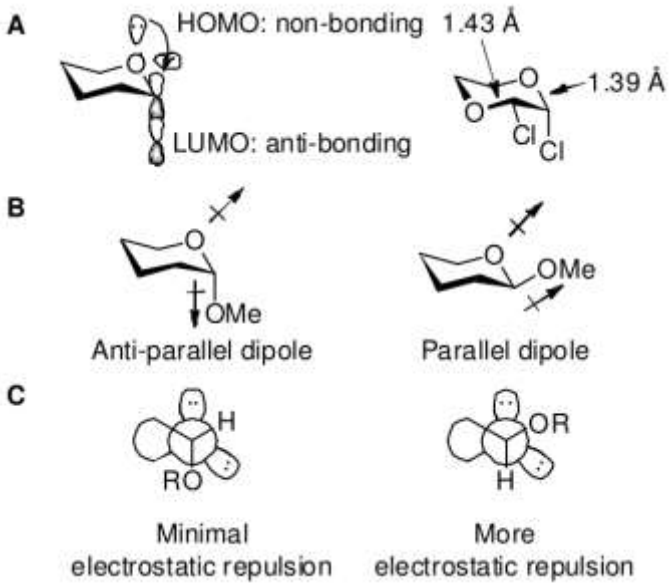


Figure 1.15: The anomeric effect

However, when an electron withdrawing functionality is linked to the anomeric center, the axial outcome is preferred. Edward first observed this phenomenon, and Lemieux further defined it as the "anomeric effect"[87].

Among several explanations of the anomeric effect, the most widely accepted is the antiperiplanar lone pair hypothesis (ALPH) in which in an α -configuration, the HOMO of the endocyclic oxygen donates electrons to the LUMO antibonding orbital of C-1 and O1 bond. The distribution of electron density favors the stabilization of the α-isomer and the bond length of endo-O-C1 is reduced. In the model study of 2, 3-dichloro-

1, 4-dioxane, the equatorial chloride endo-O-C1 bond is much shorter than the bond length of axil O-C3.

Another explanation for the anomeric effect suggests that the dipole direction of the α- and β- isomers plays a role in isomer formation. The first dipole aligns on the C-1-O1 bond. The second is in a bisected position among the two pair electrons of sugar ring oxygen. When the OMe group points the equatorial direction, the angle between two dipoles is too small to result in favorable dipole-dipole interaction. However, when the OMe points in the axial direction, the dipole-dipole interaction is favored because of its anti-parallel position. The anti-parallel dipole overcomes the unfavored energy barrier created by 1, 3-diaxial interactions.

Besides the anomeric effect, other factors also play important roles in the anomeric stereoselectivity and the yield of glycosylation. Reaction conditions such as solvents and temperature are crucial. However, neither anomeric effect, nor reaction conditions are strong enough to control the glycosylation outcomes effectively. The protecting groups and well-designed chiral auxiliaries have been invented to address such an issue. The protecting groups, in general, can stabilize the intermediate oxocarbenium ion through neighboring group participation (NGP), controlling the stereo-outcome of a glycosylation reaction. In most of the cases, the donor will determine the stereochemical result of glycosylation. In the next section, different C-2 functionalities which can generate different glycosylic linkages will be discussed.

The nature of the protecting group of C-2 of a glycosyl donor will determine the anomeric selectivity. After the activation of the leaving group at the anomeric center, a 2-O-actyl group will participate to yield 1-2 *trans* glycosylic linkage in the reaction below.

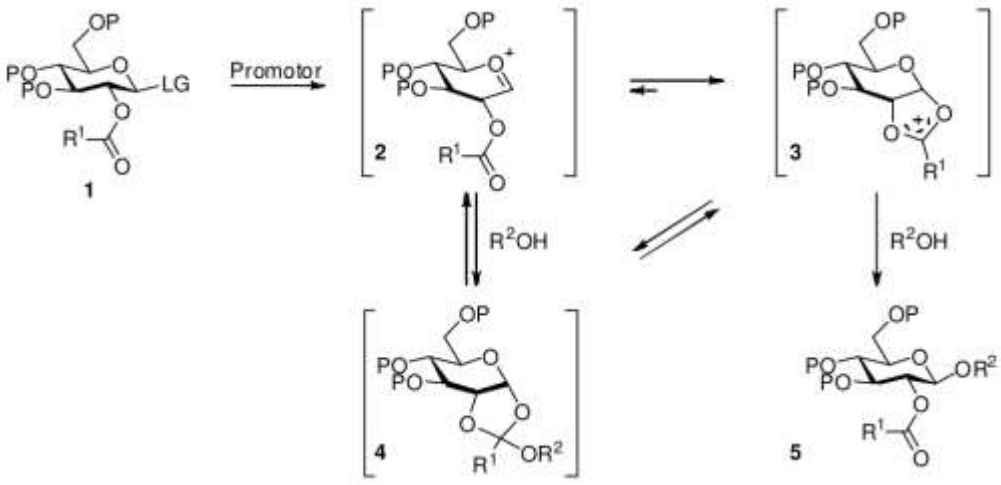


Figure 1.16: Neighboring group participation of a 2-O-acyl functionality [88].

Once the intermediate oxocarbenium ion **2** is formed, the C-2 ester group will attack the anomeric center to form a five-membered dioxaleniumion **3**. The five-membered ring only prefers a 1,2- *cis* fused ring system as confirmed by NMR spectroscopy. Also, the intermediate **3** has been proven by the isolation of crystal dioxalenium ions[89]. In the following step, a glycosyl acceptor or a substrate attacks the anomeric center that can only be approached from the β-face to yield the formation of 1,2-*trans* glycoside **5**. However, the dioxalenium ion can also be attacked by the acceptor molecule that leads to the formation of orthoester **4** as the side product. Fortunately, the orthoester **4** is unstable under acidic condition and can rearrange to the stable 1,2-*trans* glycosylic linkage eventually if the proper amount of acid is added. In most of the cases,

the use of 2-O-ester as the method to yield 1,2-*trans* glycosylic linkage has been a part of routine work in carbohydrate synthesis, which is reliable and highly stereoselective. In the case of glucose as the donor substrate, a β-linkage will be formed, whereas mannose will give α products. Several ester protecting groups have been developed, either to prevent the formation of the orthoester or to utilize an orthogonal protecting group that can be selectively removed in the presence of other ester functionalities, revealing the hydroxyl group.

Remote neighboring group participation is another method to control stereoselectivity. Instead of C-2 protecting group participation, the non-vicinal hydroxyl groups are used during glycosylation reactions[90-92]. In this case, the intermediate dioxalenium ion is formed by the involvement of a non-vicinal ester, and there is evidence that this exists beyond the stereochemical argument[93,94]. For example, a recent study to trap the intermediate dioxalenium ion through a *tert*-butoxycarbonyl (Boc) group was reported.

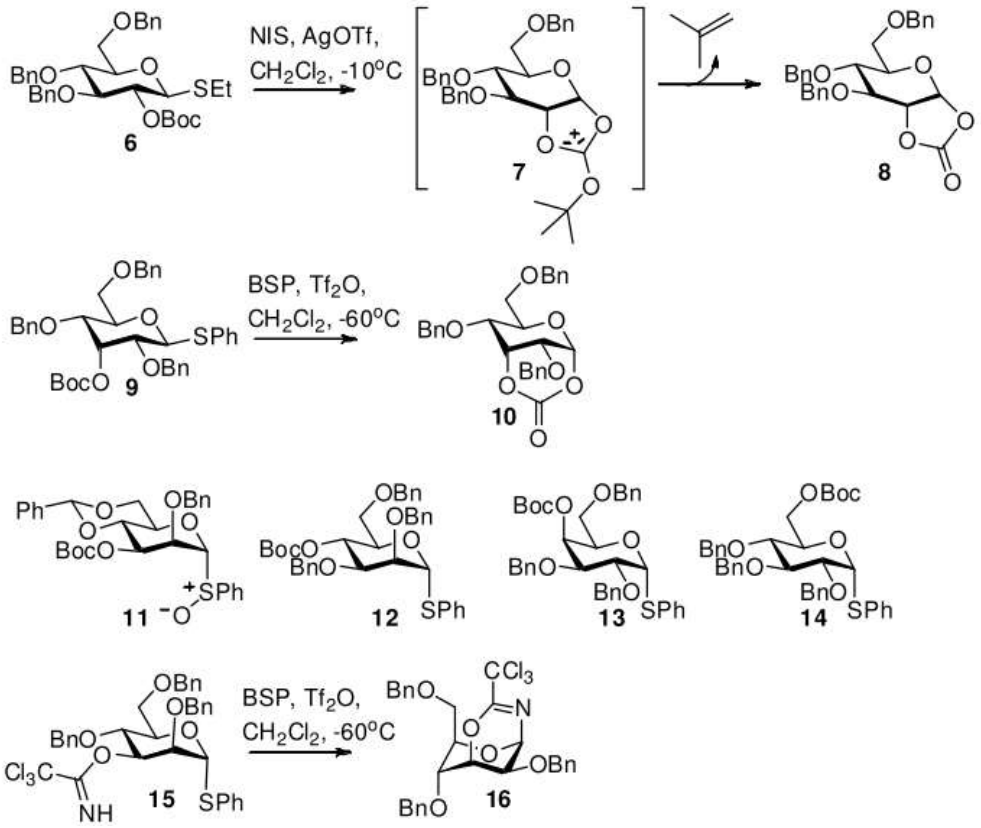


Figure 1.17: Nucleophilic traps to investigate remote neighboring group participation[89,95]

Recently, efforts to trap the intermediate dioxolenium ion using a *tert*-butoxycarbonyl (Boc) group have been reported (Scheme 1.5)[95]. The mechanism of trapping the intermediate is upon the participation of the Boc, the dioxolenium ion **7**, will form the stable ring carbonate **8** and lose isobutylene spontaneously. Based on such a method, thioglycoside **6** was reacted with NIS/AgOTf, and the formation of carbonate **8** was observed. Subsequently, some donors with a Boc group at different positions were tested. However, only allose donor **9** was observed in the corresponding carbonate **10**, which proves the occurrence of remote participation. In the other cases, thioglycosides **11** and **12** failed to form bicyclic carbonate and formed hydrolyzed donor or glycosylated products. Similar results were observed with thioglycosides **13** and **14**. Based on all the

observations, it was concluded that the remote participation can only happen in particular positions. However, later research showed that the remote participation on a C-3 equatorial position is possible[96]. In this research, trichloroacetimidate was employed as the intermediate trap. When the thioglycoside **15** was activated by benzenesulfonyl piperidine (BSP) and trifluoromethanesulfonic anhydride (Tf_2O), the trichlorooxazine product **16** was detected. However, the same results did not apply to the C-4 and C-6 trichloroacetimidoyl group. In summary, remote participation indeed plays a significant role in glycosylation. However, such a phenomenon is highly depended on the nature of the sugar, such as configuration and the electronic and steric properties of the participating group.

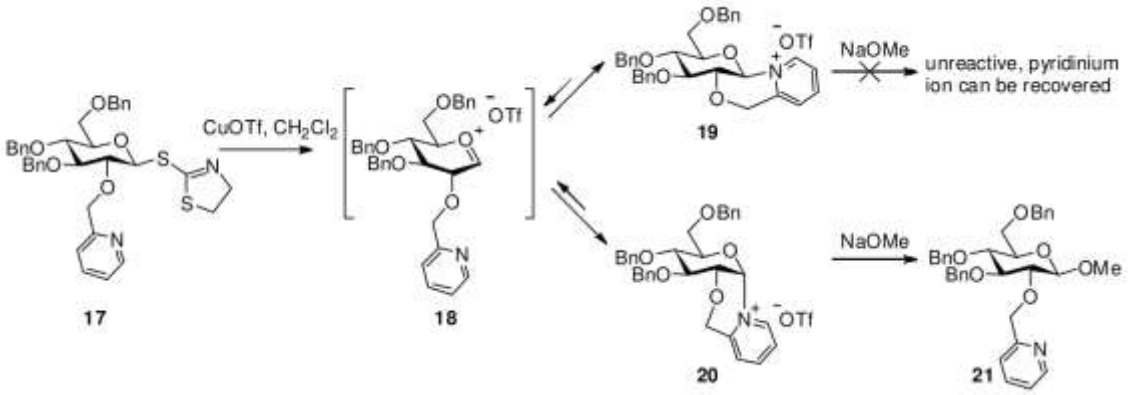


Figure 1.18: the picolinyl ether for the stereoselective synthesis[97]

There are other neighboring participation groups in addition to ester type and remote participation. It was reported by the Demchenko group that modified ethers can be NPG, and control the stereoselectivity and obtain the glycosylation product in high yield. In this report, the picolinyl ether was used as the participating functionality and

generates the 1, 2-*trans*-glycosylic linkage. Theoretically, the 2-picolinyl mediated glycosylation needs heat to drive the reaction to complete since it deactivates the oxocarbenium ion[97]. However, it was found that the reaction can occur at low temperatures. The reaction of donor **17** which was activated by $Cu(OTf)_2$, can complete in one hour. An NMR study confirmed that the two pyridinium ions **19** and **20** existed, and the latter one was formed predominantly. The pyridinium ions are remarkably stable and even tolerant to silica gel chromatography conditions. The purified pyridinium ion **20** then reacted with nucleophiles, resulting in 1,2-*trans*-glycoside **21**. However, pyridinium ion **19** was too inert to be activated.

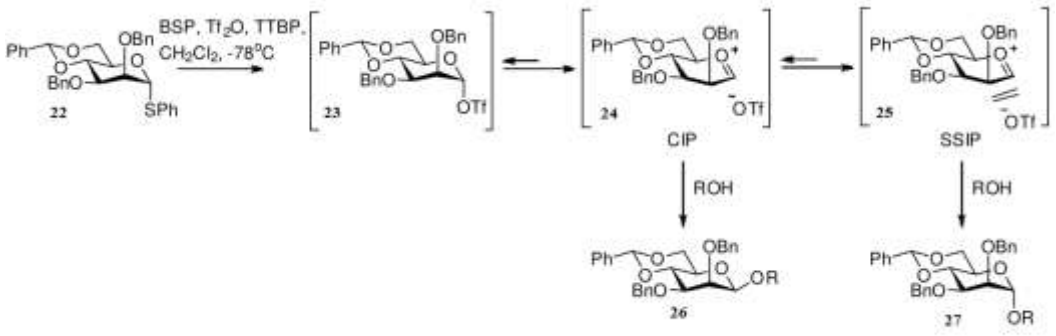


Figure 1.19: Using the 4,6-O-benzylidene acetal, to generate an α-triflate intermediate.

Anomeric stereochemistry can be controlled by electronic and steric effects through a new development in stereoselectivity control. The Crich group found that by using pre-activated 4,6-O-benzylidene protected mannosyl sulfoxide donors at -78 ℃ and subsequent addition of acceptor, the formation of β-mannosides is favored. The pre-activation and low temperature were proven to be the keys for a good β-selectivity. The reason is that an intermediate formed under such conditions will react with an acceptor in

a stereoselective way to form a β-mannoside. In addition, the 4,6-O-benzylidene acetal is necessary for the high β-selectivity due to its torsional disarming effect.

It has been proven that during the glycosylation, the oxocarbenium ion interacts with a triflate anion and forms a stable α-triflate **23**. An S_N-2 like a nucleophilic attack by the acceptor displaces the α-triflate to yield the desired β-mannoside product **26**. To further confirm the mechanism, ^{1}H, ^{13}C and ^{19}F NMR experiments of the mannosyl donor were carried out at low temperature.[98] These experiments confirmed the existence of α-glycosyl triflates but failed to establish the reaction mechanism. To further investigate this phenomenon, kinetic isotope effects (KIE) were employed[99]. A 50% deuterated mannosyl donor was synthesized, H-1 for KIE and H-7 as an internal standard. The resulting KIE value was around 1.12, which is consistent with the transition state of an oxocarbenium ion. Moreover, it was further hypothesized that an α-triflate contact ion pair (CIP) **24** was formed, in which the triflate anion blocked the α-face and induced the formation of β-mannosides, or the nucleophile associated with the oxocarbenium ion upon the departure of the triflate in an opened transition state. Additionally, the product **27** was explained as the solvent separated ion pair (SSIP) [100] where the selectivity lost.

As mentioned before, the neighboring group participation has been widely used in the control of stereo outcomes of glycosylation reactions. The C-2 acetyl group is so powerful that it can mediate most of the reactions and generate 1,2 *trans*-glycosides. However, there is still no effective way to control the formation of 1,2 *cis*-glycosides. Inspired by the C-2 ester mediated glycosylation, the chiral auxiliary was invented and developed by the Boons group to solve the scientific challenges in the formation of 1,2-*cis*-glycosylation.

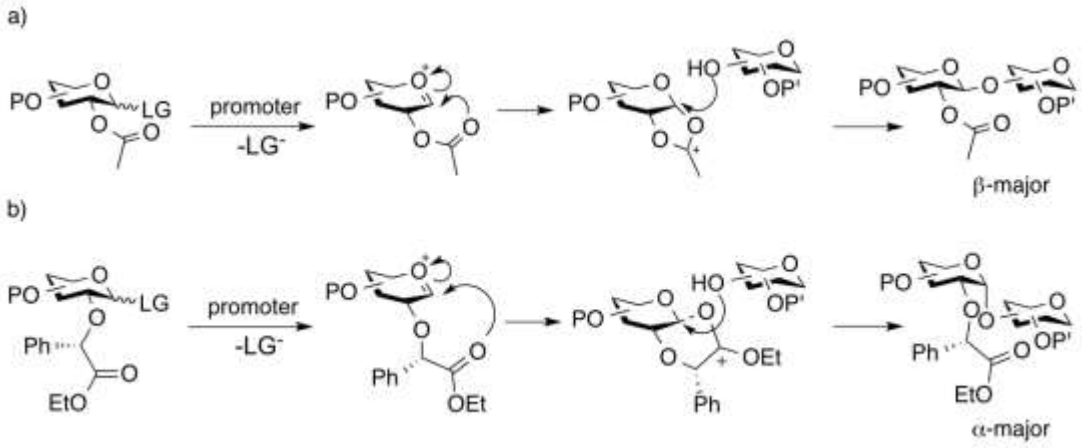


Figure 1.20: Neighboring group participation in the synthesis of *cis*- and *trans*-glycosides.

Using commercially available ethyl mandelates as the source of the chirality, the optically pure ethyl mandelate was installed at a C-2 position to mimic an ester. Upon the formation of the oxocarbenium ion, the nucleophilic moiety of the auxiliary would participate with oxocarbenium ion to generate a putative *trans*- or *cis*-decalin system. The *trans*- or *cis*- decalin is determined by the chiral source from the mandelate. The (S)-mandelate preferred the formation of the *trans*-decalin, because the phenyl moiety, in this case, would be in an axial position and unstabilize the whole structure. In the next step, the O- nucleophile was expected to attack from α face of the *trans*-decalin acyl oxonium ion and yield excellent stereoselectivity. Some acceptors were tested with the *trans*-decalin system and excellent stereoselectivity was accomplished. The hypothesized mechanism was further confirmed with the *cis*-decalin system, and the chirality of products was reversed.

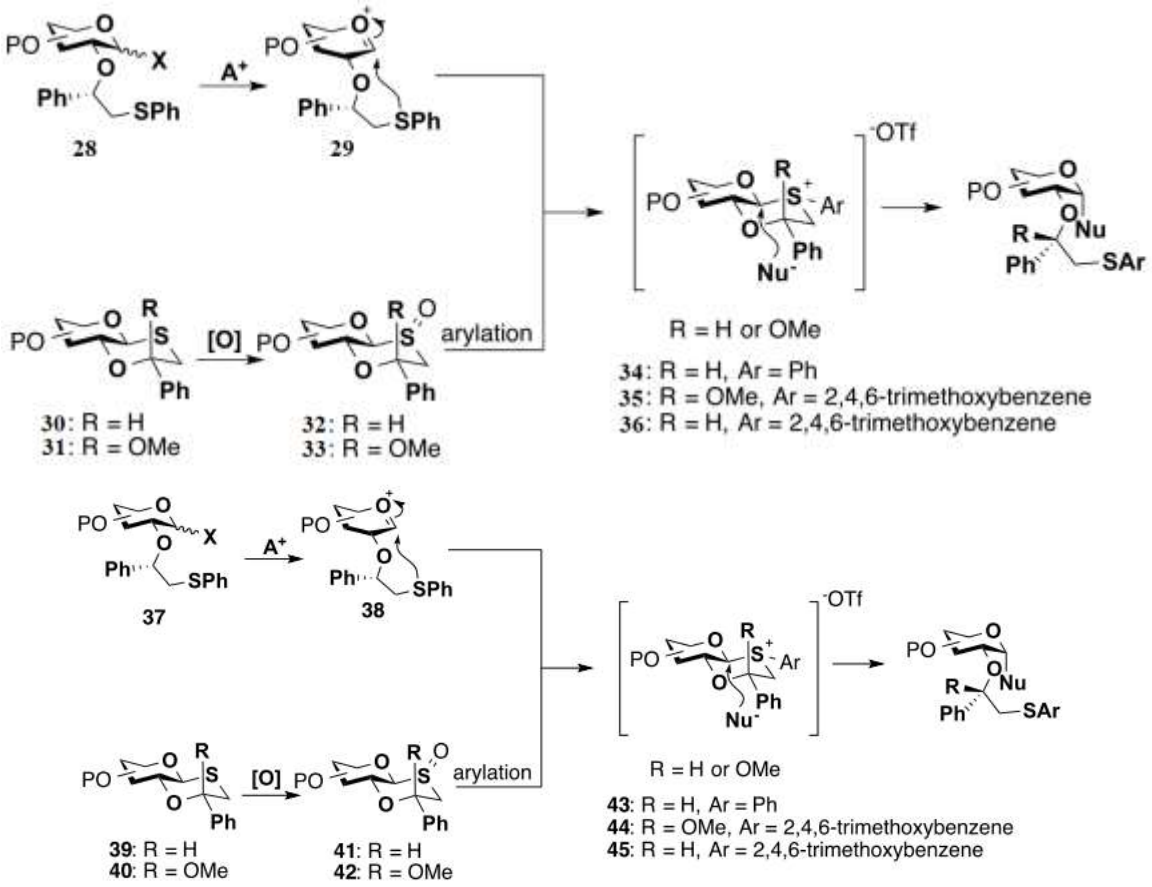


Figure 1.21: α-Selective glycosylations by chiral auxiliaries from different precursors

With the success of the first generation of the chiral auxiliary, the effort was paid to improve further the auxiliary system. Instead of using oxygen as the participation moiety, sulfur was used in the second generation to improve the stereoselectivity. Compared with oxygen, sulfur is more attractive since it has a higher nucleophilicity and larger atomic radius. With an aim to introduce sulfur into auxiliary, acetic acid (1S)-phenyl-2-(phenyl sulfonyl) ethyl ester was used as a chiral source, and it was installed onto the C-2 hydroxyl group by BF_3-OEt_2. The reaction was driven by the loss of the acetate, and an active episulfonium ion was formed subsequently. Then the C-2 hydroxyl group of sugar would nucleophilically attack the benzylic position of the episulfonium

ion and results in the reversion of the configuration. The donor **28** was activated without the presence of acceptor by TMSOTf, and low-temperature NMR study showed the presence of *trans*-decalin **34**. The structure was further identified by HMBC indicating the coupling between C1 and C18. Moreover, the NOE experiment confirmed the formation of the *trans*-decalin[101]. The various acceptors were reacted with the β-sulfonium of *trans*-decalin, and different α-glycosides were obtained with high selectivity. This method was further applied to the solid phase synthesis of an α-glucan that was essential for host innate immune response[102].

Since the chiral auxiliary is acid labile and the installation of the auxiliary is synthetically challenging, the further development was focused on those two aspects. The Turnbull group cleverly used the stereo- and regioselective acetal formation to install oxathiane acetal **31**[103]. The oxathiane acetal has the conformation of *trans*-decalin. By treating with Tf_2O, the sulfoxide **33** attacked trimethoxylbenzene. Due to the electron donating nature of trimethoxylbenzene, the generated sulfonium ions **35** were more stable than the sulfonium ion **34,** and the higher temperature was needed for driving the reaction to completion. However, with the presence of OMe moiety in the chiral auxiliary, the auxiliary was even more acid liable, and this limited the application of the auxiliary chemistry. To address this issue, the Boons group development a method that reduced the oxathiane acetal to the ether **30,** which is more acid-toleratant. Different types of acceptors were tested to explore the oxathiane donors' capability. A wide range of acceptors, such as primary and secondary hydroxyl acceptors, thioglycosides, and protected amino acid all showed high α selectivity.

Based on the excellent result in the methodology study, other oxathiane donors with orthogonal protecting groups were synthesized and utilized for the synthesis of oligosaccharide from *P. boydii*[104]. However, we still didn't know the mechanism of sulfonium ion mediated glycosylation and the explanation for the high stereoselectivity[105,106].

In previous research, we have synthesized different donors bearing two stereoisomers of chiral auxiliary and also the auxiliary without any chirality, allowing the nature of the mechanism of auxiliary to be revealed. Interestingly, when the (*R*)-isomer was used during a glycosylation instead of the desired (*S*)-isomer, a significant increase of β linkage was observed. It is easy to rationalize that the bulky phenyl substitution destabilizes the *trans*-decalin system by the unfavorable spatial hindrance. In the case of the non-chiral auxiliary, the *trans*- and *cis*- decalins will both form during the glycosylation due to the absence of the configuration orienting functionality. The glycosylation outcome (α/β=8/1) further proves the anticipation. In the following research, Woerpel and White field attempt to isolate the effect of stereo-directing from the neighboring group participation. In their experiments, a simplified facial-selective glycosylation system was employed. They found out that the sulfonium ion derivated from 4-sulfur substituted acetal was a resting state in the simplified tetrahydropyran system. In the nucleophilic addition, the sugar preferred oxocarbenium ion state rather than S_N-2 like mechanism[107]. However, only poor nucleophiles were tested, and the most common O-nucleophiles were not included. In another set of experiments, the Whitefield group discovered that the stereoselectivity of glycosylation can be controlled by a non-participating protecting group that is chiral and attach to the C-2 of glucose[108]. Their

computational studies showed the rotation barrier along the C2-O2 δ-bond that orients the C-2 protecting group in a syn-faction, blocking the β-face[109]. However, they also explained that there was not a single factor that dominated the glycosylation outcome; the selectivity was the result of multiple factors.

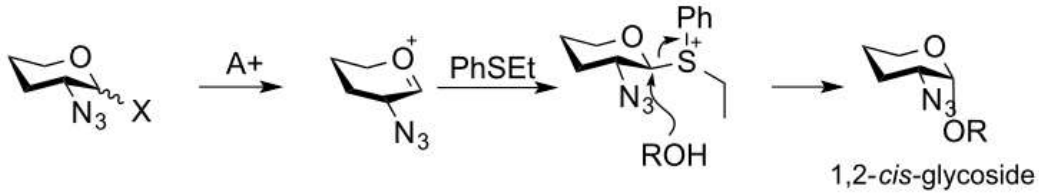


Figure 1.22: α-Selective glycosylation with C-2 deoxy donor by adding sulfur-containing reagent

Stereoselective glycosylation with C-2 deoxy sugars is another challenge in the carbohydrate synthesis field. However, it offers the sulfonium ion mediated glycosylation an opportunity to address such issue. However, of course, the conventional chiral auxiliary will not work in this case. The using of ethyl phenyl sulfide or thiophenol has accomplished excellent selectivity in 2-deoxy-glycosides[110]. Similar to the idea of chiral auxiliary-mediated glycosylation, the sulfur-containing reagents can attach to oxocarbenium ion during the preactivation procedure to form chiral sulfonium ions. In this case, a reversed anomeric effect causes the sulfonium ion to adopt the equatorial orientation and an S_N-2 like nucleophilic attacks the anomeric center to result in the desired α isomer.

The development of stereoselective glycosylation is not only a method by which the glycosylation outcomes are under control, but the method offers the opportunity for a

much easier purification due to fewer impurities and byproducts. Moreover, the method also expands the application scope of solid phase oligosaccharides synthesis and fluorous tag supported oligosaccharides synthesis. This is because both purification methods only purify the compound at the very last step after detachment from either tag or resin, and there is no intermediates purification during the whole process. In this case, if any configuration isomers are generated in one reaction step, the purification of the final compound will be extremely painful and time-consuming, and the yield will be largely compromised. In many even worse cases, if more than one glycosylation loses stereo-control, the number of different stereoisomers will increase exponentially, and such a mixture is almost impossible to be purified. However, with the combination of C-2 ester and chiral auxiliary technologies, a clean and high yield glycosylation will be guaranteed in the most of the cases. The additional combination of stereoselective glycosylations with solid phase synthesis or fluorous tag synthesis will largely improve the efficiency of the total oligosaccharide synthesis.

By attaching any chemical substrate to a resin particle, solid-phase synthesis can remove the excess reagent easily by washing the resin. As previously mentioned, the purification of the desired compound at the very end of synthesis limits the chromatographic steps. The solid phase synthesis of peptides and oligonucleotides has been well established, and the automation has been commercially available for decades. However, due to the extremely different chemical nature of carbohydrates and scientifical difficulties of glycosylation, the solid phase synthesis of oligosaccharides is still under development. Several issues are still trapping the development of polymer supported oligosaccharides synthesis. Glycosylation yield is often compromised in the solid phase

synthesis due to the significant reduction of the reaction site of the substrate activity after coupling to the polymer resin. To boost the yield of glycosylation reactions, excess donor will be consumed during coupling reaction. Moreover, the monitoring of the reaction is painful in the solid phase synthesis of carbohydrates. Either measuring the weight changes after reactions or detaching a small amount of reaction crude from resin, both methods will end up with uncertain results or loss of desire products.

In an aim to overcome the drawbacks of solid phase synthesis of oligosaccharides, fluorous tag supported glycosylation was invented. Compared with solid phase synthesis, the fluorous tag synthesis just avoids all issues that happened in the solid phase synthesis. First, the fluorous tagged reactions are performed in organic solvents and usually in one phase reactions. In such cases, it is not necessary to utilize the a large excess amount of donors. Moreover, since the fluorous tag is compatible with mass spectrometry, NMR, and other analytical methods, there is no need to detach the compound from the fluorous tag, that avoids the unnecessary loss of product. In addition, all detections are as accurate and easy as normal organic reactions.

It was found that a fluorous solvent such as perfluorohexane can not dissolve in most of the organic solvents and water. However, if a compound is highly fluorinated, it could be easily extracted from organic solvents by simple fluorous-organic solvent partition [111]. Fluorous Solid Phase Extraction (F-SPE) was invented as an improved fluorous tag technology. By using commercially available fluorous silica gel, the F-SPE and HPLC could be performed. The reaction crude is loaded onto the F-SPE cartridge with a minimal amount of solvent. Moreover, then the cartridge is firstly eluted with a fluorophobic solvent, for example, 80:20 MeOH/H_2O to remove non-fluorous tagged

compounds. Then, a more fluorophilic solvent (MeOH, acetonitrile or THF) was used for elution of the fluorous tagged compounds.

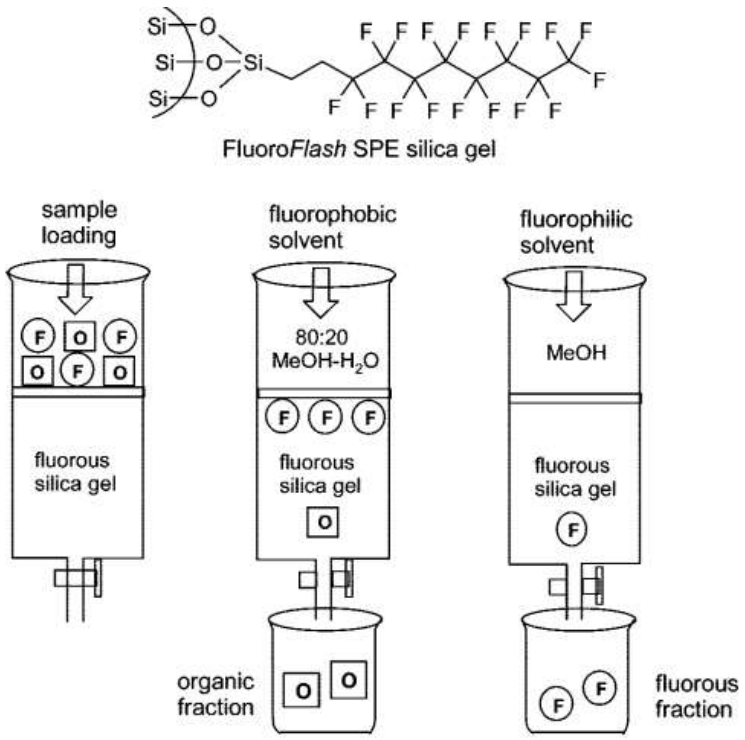


Figure 1.23 Fluorous Solid Phase Extraction (F-SPE)[112]

Several key points have to be well designed before the development of fluorous tag supported oligosaccharides synthesis. First, it has been determined whether the synthesis starts from the reducing end of carbohydrates or begins with the nonreducing end and which end attaches to the fluorous tag. The fluorous tag linker has to be inert to all chemical conditions in the whole synthetic procedure, and it could be easily cleaved from the substrate. The selection of orthogonal protecting groups has to be compatible with the linker of the fluorous tag.

Reference

(1) Yoshida-Moriguchi, T.; Yu, L.; Stalnaker, S. H.; Davis, S.; Kunz, S.; Oldstone, M. B. A.; Schachter, H.; Wells, L.; Campbell, K. P. *Science (New York, N.Y.)* **2010**, *327*, 88.

(2) Ghazarian, H.; Idoni, B.; Oppenheimer, S. B. *Acta Histochem* **2011**, *113*, 236.

(3) Varki A, C. R., Esko JD *Essentials of Glycobiology. 2nd edition*; Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, 2009.

(4) Oberli, M. A.; Tamborrini, M.; Tsai, Y. H.; Werz, D. B.; Horlacher, T.; Adibekian, A.; Gauss, D.; Moller, H. M.; Pluschke, G.; Seeberger, P. H. *Journal of the American Chemical Society* **2010**, *132*, 10239.

(5) (a)Sharon, N.; Lis, H. *Science* **1989**, *246*, 227; (b)Bucior, I.; Burger, M. M. *Current Opinion in Structural Biology* **2004**, *14*, 631.

(6) (a)Akiyama, S. K.; Yamada, S. S.; Yamada, K. M. *Journal of Biological Chemistry* **1989**, *264*, 18011; (b)Chammas, R.; Veiga, S. S.; Line, S.; Potocnjak, P.; Brentani, R. R. *Journal of Biological Chemistry* **1991**, *266*, 3349; (c)Zachara, N. E.; Hart, G. W. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids* **2006**, *1761*, 599.

(7) (a)Wormald, M. R.; Sharon, N. *Current Opinion in Structural Biology* **2004**, *14*, 591; (b)Rek, A.; Krenn, E.; Kungl, A. J. *British Journal of Pharmacology* **2009**, *157*, 686.

(8) Fuster, M. M.; Esko, J. D. *Nat Rev Cancer* **2005**, *5*, 526.

(9) Wang, P.-H. *Taiwanese Journal of Obstetrics and Gynecology* **2004**, *43*, 53.

(10) Gorelik, E.; Galili, U.; Raz, A. *Prostate Cancer: New Horizons in Research and Treatment* **2002**, 109.

(11) Song, X.; Lasanajak, Y.; Xia, B.; Heimburg-Molinaro, J.; Rhea, J. M.; Ju, H.; Zhao, C.; Molinaro, R. J.; Cummings, R. D.; Smith, D. F. *Nat Meth* **2011**, *8*, 85.

(12) Rottger, S.; White, J.; Wandall, H. H.; Olivo, J. C.; Stark, A.; Bennett, E. P.; Whitehouse, C.; Berger, E. G.; Clausen, H.; Nilsson, T. *Journal of Cell Science* **1998**, *111*, 45.

(13) Patsos, G.; Hebbe-Viton, V.; Robbe-Masselot, C.; Masselot, D.; Martin, R. S.; Greenwood, R.; Paraskeva, C.; Klein, A.; Graessmann, M.; Michalski, J. C.; Gallagher, T.; Corfield, A. *Glycobiology* **2009**, *19*, 382.

(14) Miyake, M.; Taki, T.; Hitomi, S.; Hakomori, S. *New England Journal of Medicine* **1992**, *327*, 14.

(15) Hart, G. W.; Housley, M. P.; Slawson, C. *Nature* **2007**, *446*, 1017.

(16) Meezan, E.; Wu, H. C.; Black, P. H.; Robbins, P. W. *Biochemistry* **1969**, *8*, 2518.

(17) Turner, G. A. *Clinica Chimica Acta* **1992**, *208*, 149.

(18) Shriver, Z.; Raguram, S.; Sasisekharan, R. *Nature Reviews Drug Discovery* **2004**, *3*, 863.

(19) Matsushita, Y.; Cleary, K. R.; Ota, D. M.; Hoff, S. D.; Irimura, T. *Laboratory Investigation* **1990**, *63*, 780.

(20) Dennis, J. W.; Laferte, S.; Waghorne, C.; Breitman, M. L.; Kerbel, R. S. *Science* **1987**, *236*, 582.

(21) Kim, Y. J.; Varki, A. *Glycoconjugate Journal* **1997**, *14*, 569.

(22) (a)Gabius, H. J. *Angewandte Chemie-International Edition in English* **1988**, *27*, 1267; (b)Hakomori, S.; Zhang, Y. M. *Chemistry & Biology* **1997**, *4*, 97; (c)Orntoft, T. F.; Vestergaard, E. M. *Electrophoresis* **1999**, *20*, 362.

(23) Hollingsworth, M. A.; Swanson, B. J. *Nature Reviews Cancer* **2004**, *4*, 45.

(24) Hakomori, S. *Glycoconjugate Journal* **2000**, *17*, 627.

(25) (a)Zhang, S. L.; CordonCardo, C.; Zhang, H. S.; Reuter, V. E.; Adluri, S.; Hamilton, W. B.; Lloyd, K. O.; Livingston, P. O. *International Journal of Cancer* **1997**, *73*, 42; (b)Zhang, S. L.; Zhang, H. S.; CordonCardo, C.; Reuter, V. E.; Singhal, A. K.; Lloyd, K. O.; Livingston, P. O. *International Journal of Cancer* **1997**, *73*, 50.

(26) (a)Prescher, J. A.; Dube, D. H.; Bertozzi, C. R. *Nature* **2004**, *430*, 873; (b)Dube, D. H.; Prescher, J. A.; Quang, C. N.; Bertozzi, C. R. *Proceedings of the National Academy of Sciences of the United States of America* **2006**, *103*, 4819.

(27) Hsu, T. L.; Hanson, S. R.; Kishikawa, K.; Wang, S. K.; Sawa, M.; Wong, C. H. *Proceedings of the National Academy of Sciences of the United States of America* **2007**, *104*, 2614.

(28) Chang, P. V.; Bertozzi, C. R. *Chemical Communications* **2012**, *48*, 8864.

(29) (a)Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proceedings of the National Academy of Sciences of the United States of America* **2007**, *104*, 16793; (b)Codelli, J.

A.; Baskin, J. M.; Agard, N. J.; Berozzi, C. R. *Journal of the American Chemical Society* **2008**, *130*, 11486.

(30) Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664.

(31) Prescher, J. A.; Bertozzi, C. R. *Nature Chemical Biology* **2005**, *1*, 13.

(32) (a)Alley, W. R.; Mechref, Y.; Novotny, M. V. *Rapid Communications in Mass Spectrometry* **2009**, *23*, 161; (b)Wu, S. L.; Huehmer, A. F. R.; Hao, Z. Q.; Karger, B. L. *Journal of Proteome Research* **2007**, *6*, 4230.

(33) Olsen, J. V.; Macek, B.; Lange, O.; Makarov, A.; Horning, S.; Mann, M. *Nature Methods* **2007**, *4*, 709.

(34) Segu, Z. M.; Mechref, Y. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 1217.

(35) Szabo, Z.; Guttman, A.; Karger, B. L. *Analytical Chemistry* **2010**, *82*, 2588.

(36) Tzeng, Y. K.; Chang, C. C.; Huang, C. N.; Wu, C. C.; Han, C. C.; Chang, H. C. *Analytical Chemistry* **2008**, *80*, 6809.

(37) Jmeian, Y.; Hammad, L. A.; Mechref, Y. *Analytical Chemistry* **2012**, *84*, 8790.

(38) Maniatis, S.; Zhou, H.; Reinhold, V. *Analytical Chemistry* **2010**, *82*, 2421.

(39) Goetz, J. A.; Novotny, M. V.; Mechref, Y. *Analytical Chemistry* **2009**, *81*, 9546.

(40) Alley, W. R.; Madera, M.; Mechref, Y.; Novotny, M. V. *Analytical Chemistry* **2010**, *82*, 5095.

(41) Alvarez-Manilla, G.; Warren, N. L.; Abney, T.; Atwood, J.; Azadi, P.; York, W. S.; Pierce, M.; Orlando, R. *Glycobiology* **2007**, *17*, 677.

(42) (a)Arnold, J. N.; Saldova, R.; Galligan, M. C.; Murphy, T. B.; Mimura-Kimura, Y.; Telford, J. E.; Godwin, A. K.; Rudd, P. M. *Journal of Proteome Research* **2011**, *10*, 1755; (b)Marino, K.; Bones, J.; Kattla, J. J.; Rudd, P. M. *Nature Chemical Biology* **2010**, *6*, 713.

(43) Gilar, M.; Yu, Y. Q.; Ahn, J.; Xie, H. W.; Han, H. H.; Ying, W. T.; Qian, X. H. *Analytical Biochemistry* **2011**, *417*, 80.

(44) Davies, M. J.; Smith, K. D.; Carruthers, R. A.; Chai, W.; Lawson, A. M.; Hounsell, E. F. *Journal of Chromatography* **1993**, *646*, 317.

(45) Wilson, N. L.; Schulz, B. L.; Karlsson, N. G.; Packer, N. H. *Journal of Proteome Research* **2002**, *1*, 521.

(46) (a)Aldredge, D.; An, H. J.; Tang, N.; Waddell, K.; Lebrilla, C. B. *Journal of Proteome Research* **2012**, *11*, 1958; (b)Wu, S. A.; Tao, N. N.; German, J. B.; Grimm, R.; Lebrilla, C. B. *Journal of Proteome Research* **2010**, *9*, 4138.

(47) (a)Ada, G.; Isaacs, D. *Clinical Microbiology and Infection* **2003**, *9*, 79; (b)Adamo, R.; Romano, M. R.; Berti, F.; Leuzzi, R.; Tontini, M.; Danieli, E.; Cappelletti, E.; Cakici, O. S.; Swennen, E.; Pinto, V.; Brogioni, B.; Proietti, D.; Galeotti, C. L.; Lay, L.; Monteiro, M. A.; Scarselli, M.; Costantino, P. *Acs Chemical Biology* **2012**, *7*, 1420.

(48) Evans, S. V.; Sigurskjold, B. W.; Jennings, H. J.; Brisson, J. R.; To, R.; Tse, W. C.; Altman, E.; Frosch, M.; Weisgerber, C.; Kratzin, H. D.; Klebert, S.; Vaesen,

M.; Bittersuermann, D.; Rose, D. R.; Young, N. M.; Bundle, D. R. *Biochemistry* **1995**, *34*, 6737.

(49) Esposito, S.; Tansey, S.; Thompson, A.; Razmpour, A.; Liang, J.; Jones, T. R.; Ferrera, G.; Maida, A.; Bona, G.; Sabatini, C.; Pugni, L.; Emini, E. A.; Gruber, W. C.; Scott, D. A.; Principi, N. *Clinical and Vaccine Immunology* **2010**, *17*, 1017.

(50) Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046.

(51) Mazmanian, S. K.; Kasper, D. L. *Nature Reviews Immunology* **2006**, *6*, 849.

(52) Garegg, P. J.; Maron, L. *Acta Chemica Scandinavica Series B-Organic Chemistry and Biochemistry* **1979**, *33*, 39.

(53) Rappuoli, R.; Telford, J.; Rosini, R.; Moschioni, M.; Sakharkar, K. R.; Sakharkar, M. K.; Chandra, R. *Vaccine Discovery and Translation of New Vaccine Technology*; River Publishers: Aalborg, 2015; Vol. 5.

(54) Cimini, D.; Restaino, O. F.; Catapano, A.; De Rosa, M.; Schiraldi, C. *Applied Microbiology and Biotechnology* **2010**, *85*, 1779.

(55) Pujar, N. S.; Huang, N. F.; Daniels, C. L.; Dieter, L.; Dayton, M. G.; Lee, A. L. *Biopolymers* **2004**, *75*, 71.

(56) Johnson, H. L.; Deloria-Knoll, M.; Levine, O. S.; Stoszek, S. K.; Hance, L. F.; Reithinger, R.; Muenz, L. R.; O'Brien, K. L. *Plos Medicine* **2010**, *7*, 13.

(57) Wessels, M. R.; Paoletti, L. C.; Guttormsen, H. K.; Michon, F.; D'Ambra, A. J.; Kasper, D. L. *Infection and Immunity* **1998**, *66*, 2186.

(58) Anish, C.; Schumann, B.; Pereira, C. L.; Seeberger, P. H. *Chemistry & Biology* **2014**, *21*, 38.

(59) Tsai, T. I.; Lee, H. Y.; Chang, S. H.; Wang, C. H.; Tu, Y. C.; Lin, Y. C.; Hwang, D. R.; Wu, C. Y.; Wong, C. H. *Journal of the American Chemical Society* **2013**, *135*, 14831.

(60) Berkin, A.; Coxon, B.; Pozsgay, V. *Chemistry-a European Journal* **2002**, *8*, 4424.

(61) Verez-Bencomo, V.; Fernandez-Santana, V.; Hardy, E.; Toledo, M. E.; Rodriguez, M. C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdes, Y.; Cosme, K.; Deler, M. L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Torano, G.; Sosa, I.; Hernandez, I.; Martinez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. *Science* **2004**, *305*, 522.

(62) Gilewski, T.; Ragupathi, G.; Bhuta, S.; Williams, L. J.; Musselli, C.; Zhang, X. F.; Bencsath, K. P.; Panageas, K. S.; Chin, J.; Hudis, C. A.; Norton, L.; Houghton, A. N.; Livingston, P. O.; Danishefsky, S. J. *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98*, 3270.

(63) (a)Zhu, X. M.; Schmidt, R. R. *Angewandte Chemie-International Edition* **2009**, *48*, 1900; (b)Boons, G. J. *Carbohydrate Chemistry* **1998**, 175.

(64) (a)Tanaka, H.; Yamada, H.; Takahashi, T. *Trends in Glycoscience and Glycotechnology* **2007**, *19*, 183; (b)Wang, Z.; Gilbert, M.; Eguchi, H.; Yu, H.; Cheng, J. S.; Muthana, S.; Zhou, L. Y.; Wang, P. G.; Chen, X.; Huang, X. F. *Advanced Synthesis & Catalysis* **2008**, *350*, 1717.

(65) (a)Weishaupt, M.; Eller, S.; Seeberger, P. H. In *Methods in Enzymology, Vol 478: Glycomics*; Fukuda, M., Ed.; Elsevier Academic Press Inc: San Diego, 2010;

Vol. 478, p 463; (b)Zhu, T.; Boons, G. J. *Journal of the American Chemical Society* **2000**, *122*, 10222.

(66) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523.

(67) (a)Wang, C. C.; Lee, J. C.; Luo, S. Y.; Kulkarni, S. S.; Huang, Y. W.; Lee, C. C.; Chang, K. L.; Hung, S. C. *Nature* **2007**, *446*, 896; (b)Jones, R. A.; Davidson, R.; Tran, A. T.; Smith, N.; Galan, M. C. *Carbohydrate Research* **2010**, *345*, 1842.

(68) Fischer, E. *Berichte der deutschen chemischen Gesellschaft* **1893**, *26*, 2400.

(69) Koenigs, W.; Knorr, E. *Berichte der deutschen chemischen Gesellschaft* **1901**, *34*, 957.

(70) Lemieux, R. U.; Kullnig, R. K.; Bernstein, H. J.; Schneider, W. G. *Journal of the American Chemical Society* **1958**, *80*, 6098.

(71) Echegoyen, L. *Journal of Physical Organic Chemistry* **2011**, *24*, 743.

(72) Crich, D.; Li, M. *Organic Letters* **2007**, *9*, 4115.

(73) (a)Demchenko, A. V.; Rousson, E.; Boons, G.-J. *Tetrahedron letters* **1999**, *40*, 6523; (b)Mukaiyama, T.; Suenaga, M.; Chiba, H.; Jona, H. *Chemistry Letters* **2002**, 56; (c)Cheng, Y.-P.; Chen, H.-T.; Lin, C.-C. *Tetrahedron letters* **2002**, *43*, 7721.

(74) (a)Van Boeckel, C.; Beetz, T.; Van Aelst, S. *Tetrahedron* **1984**, *40*, 4097; (b)Smid, P.; De Ruiter, G.; Van der Marel, G.; Rombouts, F.; Van Boom, J. **1991**.

(75) Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *Journal of the American Chemical Society* **2009**, *131*, 17705.

(76) Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *Journal of the American Chemical Society* **2010**, *132*, 7229.

(77) Smoot, J. T.; Pornsuriyasak, P.; Demchenko, A. V. *Angewandte Chemie* **2005**, *117*, 7285.

(78) Crich, D.; Sun, S. *Journal of the American Chemical Society* **1997**, *119*, 11217.

(79) Crich, D.; Chandrasekera, N. S. *Angewandte Chemie* **2004**, *116*, 5500.

(80) Zhang, Y.; Gaekwad, J.; Wolfert, M. A.; Boons, G.-J. *Journal of the American Chemical Society* **2007**, *129*, 5200.

(81) Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *Journal of the American Chemical Society* **2005**, *127*, 12090.

(82) Nigudkar, S. S.; Demchenko, A. V. *Chemical Science* **2015**, *6*, 2687.

(83) Fascione, M. A.; Webb, N. J.; Kilner, C. A.; Warriner, S. L.; Turnbull, W. B. *Carbohydrate research* **2012**, *348*, 6.

(84) Raj, D. A.; Biju, S.; Reddy, M. *Dalton Transactions* **2009**, 7519.

(85) (a)Fang, T.; Mo, K.-F.; Boons, G.-J. *Journal of the American Chemical Society* **2012**, *134*, 7545; (b)Fascione, M. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chemistry-A European Journal* **2012**, *18*, 321.

(86) Beaver, M. G.; Billings, S. B.; Woerpel, K. *Journal of the American Chemical Society* **2008**, *130*, 2082.

(87) Moumé-Pymbock, M.; Crich, D. *The Journal of organic chemistry* **2012**, *77*, 8905.

(88) Whitfield, D. M.; Nukada, T. *Carbohydrate research* **2007**, *342*, 1291.

(89) Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J. *Organic letters* **2007**, *9*, 1959.

(90) Horváth, I. T.; Rábai, J. *Science* **1994**, *266*, 72.

(91) Zhang, W. *Chemical reviews* **2009**, *109*, 749.

(92) (a)Cosgrove, D. J. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 850; (b)Huskens, J. *Curr. Opin. Chem. Biol.* **2006**, *10*, 537.

(93) (a)Dube, D. H.; Bertozzi, C. R. *Nature reviews* **2005**, *4*, 477; (b)Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855; (c)Dalziel, M.; Crispin, M.; Scanlan, C. N.; Zitzmann, N.; Dwek, R. A. *Science (New York, N.Y* **2014**, *343*, 1235681; (d)Hudak, J. E.; Bertozzi, C. R. *Chem. Biol.* **2014**, *21*, 16.

(94) Cummings, R. D. *Mol. BioSyst.* **2009**, *5*, 1087.

(95) (a)Hart, G. W.; Copeland, R. J. *Cell* **2010**, *143*, 672; (b)Cummings, R. D.; Pierce, J. M. *Chem. Biol.* **2014**, *21*, 1.

(96) (a)Boltje, T. J.; Buskas, T.; Boons, G. J. *Nat. Chem.* **2009**, *1*, 611; (b)Yasomanee, J. P.; Demchenko, A. V. *Trends Glycosci. Glyc.* **2013**, *25*, 13.

(97) (a)Codee, J. D. C.; Litjens, R.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chemical Society reviews* **2005**, *34*, 769; (b)Kaeothip, S.; Demchenko, A. V. *Carbohydr. Res.* **2011**, *346*, 1371; (c)Yang, L.; Qin, Q.; Ye, X. S. *Asian J. Org. Chem.* **2013**, *2*, 30.

(98) (a)Seeberger, P. H. *Chem Commun* **2003**, 1115; (b)Hsu, C. H.; Hung, S. C.; Wu, C. Y.; Wong, C. H. *Angew. Chem. Int. Ed.* **2011**, *50*, 11872.

(99) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837.

(100) Tang, S. L.; Pohl, N. L. *Org. Lett.* **2015**, *17*, 2642.

(101) (a)Jaipuri, F. A.; Pohl, N. L. *Org Biomol Chem* **2008**, *6*, 2686; (b)Ko, K. S.; Park, G.; Yu, Y.; Pohl, N. L. *Org. Lett.* **2008**, *10*, 5381; (c)Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162; (d)Zhang, F.; Zhang, W.; Zhang, Y.; Curran, D. P.; Liu, G. *J. Org. Chem.* **2009**, *74*, 2594; (e)Liu, L.; Pohl, N. L. *Org. Lett.* **2011**, *13*, 1824; (f)Tanaka, H.; Tanimoto, Y.; Kawai, T.; Takahashi, T. *Tetrahedron* **2011**, *67*, 10011; (g)Zong, C.; Venot, A.; Dhamale, O.; Boons, G. J. *Org. Lett.* **2013**, *15*, 342; (h)Cai, C.; Dickinson, D. M.; Li, L. Y.; Masuko, S.; Suflita, M.; Schultz, V.; Nelson, S. D.; Bhaskar, U.; Liu, J.; Linhardt, R. J. *Org. Lett.* **2014**, *16*, 2240; (i)Hwang, J.; Yu, H.; Malekan, H.; Sugiarto, G.; Li, Y. H.; Qu, J. Y.; Nguyen, V.; Wu, D. Y.; Chen, X. *Chem Commun* **2014**, *50*, 3159; (j)Macchione, G.; de Paz, J. L.; Nieto, P. M. *Carbohydr. Res.* **2014**, *394*, 17; (k)Roychoudhury, R.; Pohl, N. L. B. *Org. Lett.* **2014**, *16*, 1156.

(102) Demchenko, A. V. *Synlett* **2003**, 1225.

(103) (a)Kim, J. H.; Yang, H.; Park, J.; Boons, G. J. *J. Am. Chem. Soc.* **2005**, *127*, 12090; (b)Park, J.; Boltje, T. J.; Boons, G. J. *Org. Lett.* **2008**, *10*, 4367; (c)Boltje, T. J.; Kim, J. H.; Park, J.; Boons, G. J. *Org. Lett.* **2011**, *13*, 284.

(104) Ferguson, M. A.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science (New York, N.Y* **1988**, *239*, 753.

(105) Nagamune, K.; Nozaki, T.; Maeda, Y.; Ohishi, K.; Fukuma, T.; Hara, T.; Schwarz, R. T.; Sutterlin, C.; Brun, R.; Riezman, H.; Kinoshita, T. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 10336.

(106) (a)Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95; (b)Khiar, N.; Martinlomas, M. *J. Org. Chem.* **1995**, *60*, 7017; (c)Baeschlin,

D. K.; Chaperon, A. R.; Green, L. G.; Hahn, M. G.; Ince, S. J.; Ley, S. V. *Chem. Eur. J.* **2000**, *6*, 172; (d)Ziegler, T.; Dettmann, R.; Duszenko, M. *Carbohydr. Res.* **2000**, *327*, 367; (e)Dettmann, R.; Ziegler, T. *Carbohydr. Res.* **2011**, *346*, 2348.

(107) van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *17*, 4875.

(108) (a)Gaunt, M. J.; Yu, J. Q.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172; (b)Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169.

(109) (a)Fang, T.; Mo, K. F.; Boons, G. J. *J. Am. Chem. Soc.* **2012**, *134*, 7545; (b)Fascione, M. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem.-Eur. J.* **2012**, *18*, 321.

(110) Fascione, M. A.; Turnbull, W. B. *Beilstein J. Org. Chem.* **2010**, *6*, 19.

(111) Zhu, X. M.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900.

(112) Fascione, M. A.; Adshead, S. J.; Stalford, S. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem Commun* **2009**, 5841.

(113) Yu, B.; Tao, H. C. *Tetrahedron Lett.* **2001**, *42*, 2405.

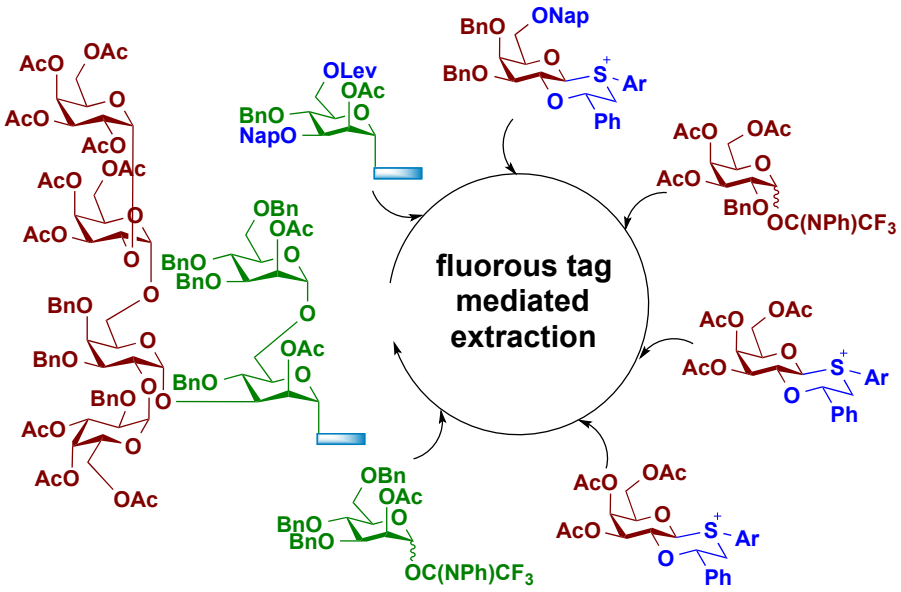
(114) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.

(115) Boltje, T. J.; Zhong, W.; Park, J.; Wolfert, M. A.; Chen, W.; Boons, G. J. *J. Am. Chem. Soc.* **2012**, *134*, 14255.

(116) Crich, D.; Vinogradova, O. *J. Org. Chem.* **2007**, *72*, 3581.

CHAPTER2:

ASSEMBLY OF A COMPLEX BRANCHED OLIGOSACCHARIDE BY COMBINING FLUOROUS SUPPORTED SYNTHESIS AND STEREOSELECTIVE GLYCOSYLATIONS USING ANOMERIC SULFONIUM IONS



Introduction

It is now well established that a dense layer of complex carbohydrates covers the surface of all prokaryotic and eukaryotic cells. These carbohydrates have been implicated in a wide range of biological processes such as protein folding, fertilization, embryogenesis, host-guest interactions, and cell differentiation and mobility.[113,114] In addition, overwhelming data supports the relevance of glycosylation in pathogen recognition, inflammation, innate immunity and the development of autoimmunity and cancer.[115-118] Although the importance of cell surface carbohydrates in health and disease is widely appreciated, advances in glycoscience have been slow due to the staggering complexity of the glycome.[119] This complexity makes it difficult to define glycan structures expressed by a given cell type and complicates the identification of specific glycan recognition determinants of glycan-binding proteins.[120,121] Libraries of well-defined glycans will make it possible to address these difficulties.

The need for diverse collections of complex glycans has stimulated the development of fast and convenient methods for their synthesis.[122,123] For example, several synthetic strategies make it possible to assembly complex oligosaccharides from carefully selected monosaccharide building blocks using a minimal number of chemical steps.[124-126] Among these strategies, one-pot multi-step glycosylations, in which several glycosyl donors are sequentially reacted in the same flask, are particularly attractive and can furnish target oligosaccharides without the need for protecting group manipulations

and isolation and purification of synthetic intermediates.[126] Within the past few years, automated solid-phase oligosaccharide synthesis has also substantially advanced.[127,128] A host of glycosylating agents, new linker systems, different solid supports and a variety of protecting groups have been carefully evaluated and these efforts have resulted in the first commercially available glycan synthesizer.

Soluble light fluorous tags offer another attractive means to simplify the process of oligosaccharide synthesis. In this case, tagged carbohydrates can easily be separated from nonfluorous-tagged side products by solid phase extraction using silica gel modified by fluorocarbons.[129] This generic procedure, which more closely resembles filtration than chromatography, depends primarily on the presence or absence of a fluorous tag and not on the polarity or other molecular features of the compound. Unlike solid phase supported synthesis, light fluorous technology does not require large excesses of reagents to drive the reactions to completion. Fluorous-tagged compounds can easily be analyzed by standard spectroscopic methods, thereby providing control over the synthesis. Furthermore, efforts are underway to develop a liquid handler to automate fluorous supported oligosaccharide synthesis.[130] Several fluorous versions of protecting groups have been developed for a variety of functional groups, and thus tags can easily be installed.[131-141] Additionally, it is possible to array fluorous-tagged glycans, thereby eliminating the necessity to install reactive functional groups for glycan immobilization.[142]

Despite the promise of fluorous supported oligosaccharide synthesis, it has mainly been employed for the preparation of relatively simple linear compounds.[131-141] This limited application is most likely due to the difficulties of controlling anomeric

selectivities in glycosylations and challenges to install branching points in high yield.[122,124,142] In this respect, many complex oligosaccharides are branched and due to steric crowding, the corresponding glycosylations are often low yielding. Furthermore, 1,2-*trans*-glycosides, such as β-glucosides and β-galactosides, can reliably be introduced by neighboring group participation of an ester-protecting group at C-2 of a glycosyl donor (Scheme 1A). On the other hand, the installation of 1,2-*cis* glycosidic linkages, such as α-glucosides and α-galactosides, requires glycosyl donors that have a non-assisting functionality at C-2, and often these coupling reactions result in mixtures of anomers.[122,142] Low yielding glycosylations and the formation of anomers defeat the purpose of fluorous support synthesis that relies on simple filtration protocols for purification.

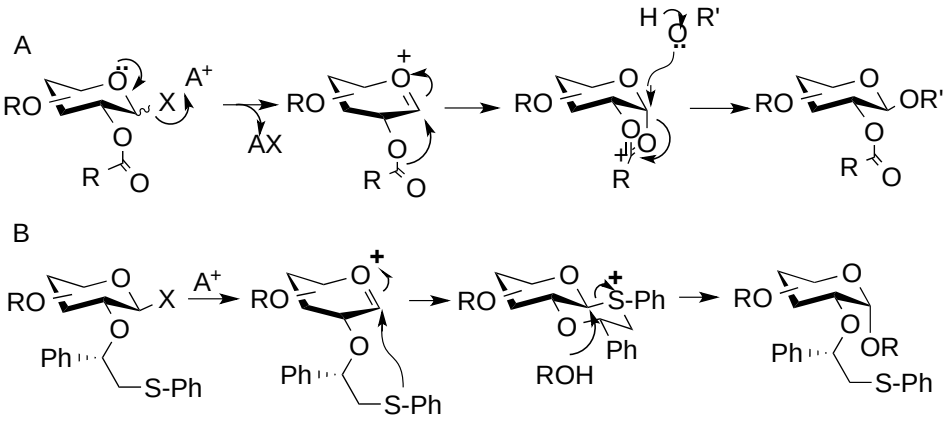


Figure 2. 1**.** Control of anomeric selectivity in glycosylations.

A) Neighboring group by C-2 esters to give a five membered ring oxocarbenium ion intermediate to form selectively 1,2-*trans* glycosides. B) Neighboring group participation by chiral auxiliary to give a *trans*-decalin anomeric sulfonium ion intermediate to provide 1,2-*cis* glycosides.

Recently, we introduced a stereoselective glycosylation approach based on neighboring group participation by a (*S*)-phenylthiomethylbenzyl moiety at C-2 of a glycosyl donor, which can readily provide 1,2-*cis*-glycosides (Scheme 1B).[143-145] Upon activation of the donor and formation of an oxacarbenium ion, the thiophenyl moiety of the C-2 auxiliary participates resulting in the formation of an intermediate sulfonium ion having a *trans*-decalin configuration. This stereoisomer is strongly favored because of the absence of unfavorable gauche interactions. Furthermore, the alternative *cis*-decalin system places the phenyl-substituent in an axial position thereby inducing unfavorable steric interactions. Displacement of the anomeric sulfonium ion by a sugar alcohol then results in the formation of a 1,2-*cis*-glycoside.

We describe here that the use of glycosyl donors modified by a C-2 (*S*)-phenylthiomethylbenzyl ether or ester-protecting group to stereoselectively introduce 1,2-*cis* or 1,2-*trans* glycosides, respectively and glycosyl acceptors modified by a fluorous tag can readily provide highly complex branched oligosaccharides of biological importance. The strategy was applied to the preparation of the carbohydrate moiety of the GPI anchor of *Trypanosoma brucei* (Figure 1), which is the parasite causing sleeping sickness in humans and similar diseases in domestic animals.[146] The oligosaccharide is composed of a branched tri-mannoside core, which is a structurally conserved motif of GPI anchors of many different organisms. It is further elongated by α-galactosides that are unique to *T. brucei*. It is expected that synthetic carbohydrates of different compositions will aid in the development of therapeutics and diagnostic for infections

caused by this pathogen.[147] Previous attempts to prepare such oligosaccharides entailed low yielding galactosylations and provided mixtures anomers.[148-152]

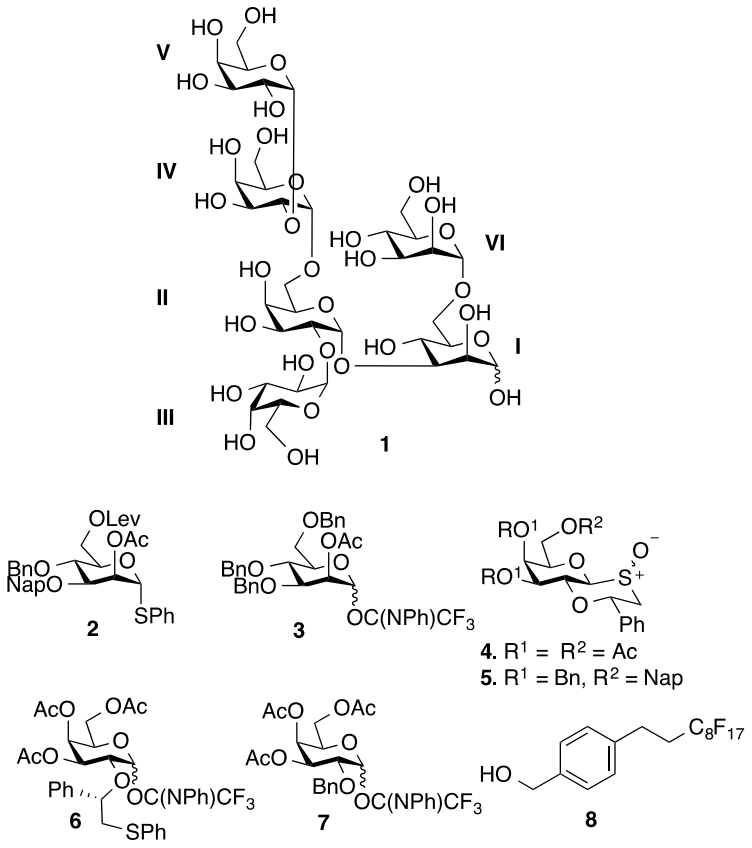


Figure 2.2**.** The structure of hexasaccharide **1** of the GPI anchor of *T. Brucei* and monosaccharide building blocks for its assembly.

Results and discussion

The synthesis of building blocks: We envisaged that building blocks **2-7** and fluorous tag modified benzyl alcohol **8** (Figure 2) would make it possible to assemble target compound **1**. Levulinic ester (Lev)[153] and 2-Naphthylmethyl ether (Nap)[154,155] were employed as a convenient set of orthogonal protecting groups for glycosyl acceptor

formation and branching point installation. The donors **2** and **3**, having participating esters at C-2, were used to install the mannosyl moieties. Furthermore, it was anticipated that galactosyl donors **4**-**6**, having a chiral auxiliary at C-2, could be employed for the stereoselective introduction of the challenging α-galactosides.

First, attention was focused on the preparation of galactosyl donors **4**-**6** (Scheme 2). It was expected that activation of a trifluoro-*N*-phenyl imidate of **6** would result in the formation of an oxacarbenium ion which will undergo neighboring group participation by the (*S*)-(phenylthiomethyl)benzyl ether leading to a 1,2-*trans* anomeric sulfonium ion. Nucleophilic displacement of the anomeric sulfonium ion by a sugar alcohol will then provide an α-galactoside.[143] Alternatively, arylation of the 1,2-oxathiane of compounds such as **4** and **5** will also provide anomeric sulfonium ions and such a transformation can easily be accomplished by activation the sulfoxide with triflic anhydride followed by reaction with 1,3,5-trimethoxybenzene.[156,157] An attractive feature of the 2-oxathianes is that they can be converted into compounds such as **7** by treatment with benzyne which leads to a derivative having a (*S*)-(phenylthiomethyl)benzyl ether at C-2 and an acetate at the anomeric center.[158] Standard procedures can then be employed to install an anomeric imidate for glycosylations.[159] Thus, it was anticipated that 2-oxathiane **11** would be an appropriate precursor for the synthesis of glycosyl donors **4**-**6**.

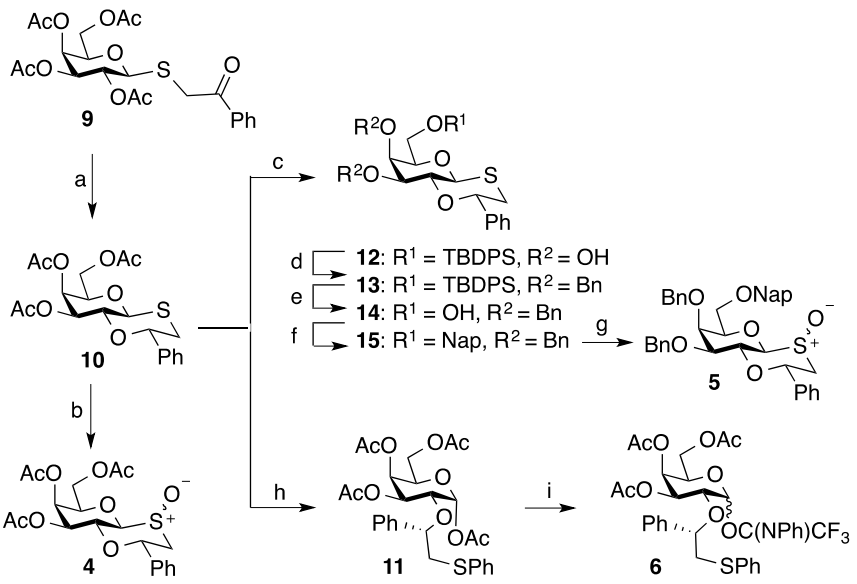


Figure 2.3 Preparation of building blocks for the GPI anchor carbohydrate moiety of *T. brucei.*

Reagents and conditions: a) MeONa, MeOH, rt, 1 h, then *p*-TSA, MeOH, rt, 18 h; then acetic anhydride, pyridine, rt, 3 h, 73% (for 3 steps) then $TiCl_4$, Et_3SiH, DCM, 0 ℃, 8 h, 83%; b) *m*-CPBA, DCM, -15 ℃, 30 min, 96%; c) NaOMe, MeOH, rt, 1 h, then TBDPSCl, Imidazole, DMF, 0 ℃, 2 h, 98%; d) BnBr, NaH, DMF, 0 ℃, 1 h, 75%; e) HF-pyridine in pyridine, rt, 18 h, 61%; f) NaH, NapBr, DMF, 0 ℃, 5 h, 95%; g) *m*-CP*B*A, DCM, -15 ℃, 30 min, 72%; h) $Pb(AcO)_4$, 1-aminobenzotriazole, DCM, -78 ℃, 1 h, 95%; i)NH_2NH_2-AcOH, DMF, 50 ℃, 4 h; then 2,2,2-trifluoro-*N*-phenyl-acetimidoyl chloride, DBU, DCM, rt, 1 h, 71%.

Thus, thioglycoside **9** was prepared by sequential treatment of per-*O*-acetyl-galactose with thiourea and 2-bromoacetophenone. The acetyl esters of **9** were cleavage with sodium methoxide in methanol and the resulting tetraol was treated with methanol in

the presence of camphorsulfonic acid (CSA) to form a 1,2-oxathiane ketal. Due to the poor solubility of the latter compound, it was not purified and immediately treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) or BF_3OEt_2 in the presence Et_3SiH to reduce the ketal to a 1,2-oxathiane ether. Although the latter reaction proceeded smoothly for glucose derivatives,[156,160] in the case of galactose no reaction occurred. Fortunately, the use of $TiCl_4$ as the Lewis acid in the presence of Et_3SiH gave, after *O*-acetylation with acetic anhydride in pyridine, the target compound **10** in a yield of 83%. Oxidation of compound **10** using meta-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane (DCM) at -15 ^oC gave the galactosyl donor **4**. Compound **11** was readily prepared by treatment of **10** with 1-aminobenzotriazole and $Pb(OAc)_4$ to generate benzyne for arylation of the 1,2-oxathiane. The latter compound was treated with hydrazine acetate to remove the anomeric acetate and the resulting lactol was converted into an *N*-phenyl trifluoroacetimidate (**6**) using 2,2,2,-trifluoro-*N*-phenylacetimidoyl chloride in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[161]

The selectively protected galactosyl donor **5** was synthesized by removal of the acetyl esters of **10** followed by selective silylation of the primary hydroxyl using tert-butyl(chloro)diphenylsilane (TBDPSCl) in the presence of imidazole in DMF to give **12**. The latter compound was benzylated under standard conditions (→**13**) followed by removal of the TBDPS ether using HF-pyridine to give **14**, which was converted into Nap ether **15** by alkylation with NapBr in the presence of sodium hydride in dimethylformamide (DMF). Prior to glycosylation, the 1,2-oxathiane **15** was oxidized to the corresponding sulfoxide **5** using *m*-CPBA. The mannosyl donors **2** and **3** were

prepared by standard protecting group manipulations as detailed in the supporting information.

Assembly of the carbohydrate moiety of the GPI anchor of *T. brucei*

First, target compound **1** was prepared by a conventional purification protocol using silica gel or size exclusion column chromatography (Scheme 3). In this case, each intermediate was carefully characterized by two-dimensional NMR spectroscopy and mass spectrometry. After establishing an appropriate synthetic protocol, the target compound was resynthesized in a rapid manner by employing fluorous solid phase extraction and in this case only the fully assembled oligosaccharide was characterized. The attraction of this approach is that a streamlined synthetic protocol for **1** can easily be adapted for the preparation of many analogs.

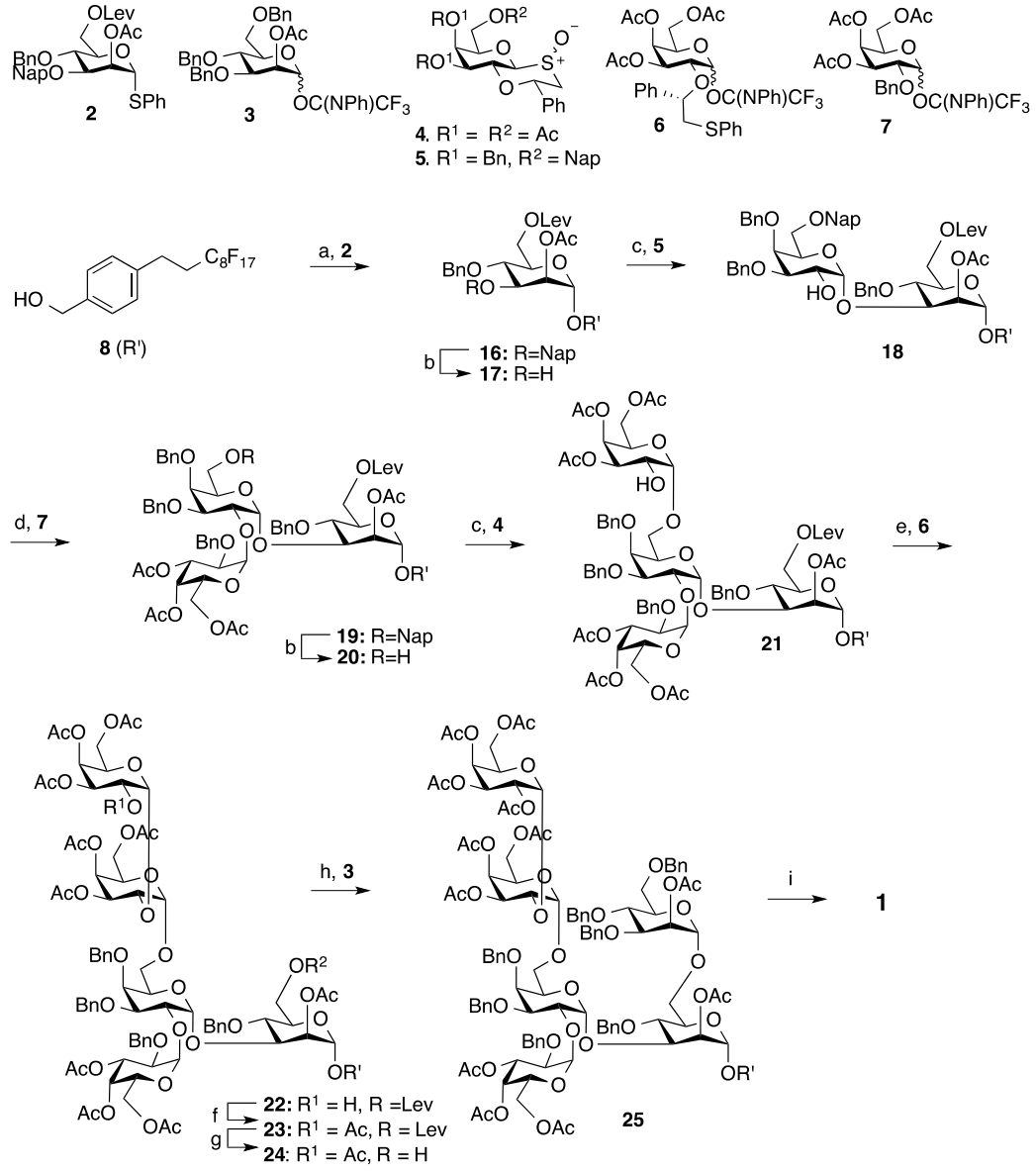


Figure 2.4**.** The assembly of the GPI anchor moiety of *T. brucei*.

Reagents and conditions: a) NIS, TfOH, DCM, -25 ℃, 30 min, 89%; b) DDQ, DCM: H_2O = 10: 1, rt, 2 h, **17**: 82%; **20**: 77%; c) Tf_2O, TMB, DTBMP -40 ℃ to rt then 10% TFA in DCM, rt, 1 h; (**18**: 87%, α only; **21**: 67%, α-only); d) TfOH, DCM, -25 ℃ to rt, 3 h, 71%, α,β; e) TfOH, DTBMP, DCM, -60 ℃ to rt, 18 h, then 10% TFA in DCM, rt, 1 h, 76%, α-only; f) Ac_2O, pyridine, DMAP, rt, 4 h; g) NH_2NH_2-AcOH, pyridine, rt, 1 h; h)

TMSOTf, DCM, -25 ℃ to rt, 1 h, 51% over three steps; i) H_2, Pd/C, AcOH, MeOH, rt, 24 h, then MeONa, MeOH, rt, 1 h, 65%.

Thus, glycosyl donor **2** was coupled with 4-(1*H*,1*H*,2*H*,2*H*-perfluorodecyl)benzyl alcohol (**8**) using *N*-Iodosuccinimide (NIS) and triflic acid (TfOH) as the activator[162] at -25 ℃ to give, after a reaction time of 30 min, fluorous tagged mannoside **16** in high yield. As expected, only the α-anomer was formed due to neighboring group participation of the acetyl ester of **2**. Next, the Nap ether of **16** was removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in the mixture of DCM and water to give glycosyl acceptor **17**, which was coupled with glycosyl donor **5** to provide, after acid mediated removal of the C-2 auxiliary, disaccharide **18**. In this glycosylation, **5** was arylated by treatment with a stoichiometric amount of triflic anhydride (Tf_2O) and 1,3,5-trimethoxybenzene (TMB) in the presence of 2,6-di-tert-butyl-4-methylpyridine (DTBMP) in DCM at -40 ℃ to form a sulfonium ion intermediate. Next, glycosyl acceptor **17** was added and the reaction mixture was allowed to warm to room temperature and after a reaction time of 11 h and purification by silica gel column chromatography, a glycoside product was obtained having a (trimethoxyphenylthiomethyl)benzyl ether moiety at C-2. The latter functionality was cleaved by treatment with 10% trifluoroacetic acid (TFA) in DCM to give glycosyl acceptor **18**. Careful analysis by ^{1}H NMR spectroscopy confirmed that only the expected α-anomer had formed.

The installation of the α(1,2)-linked galactoside of **1** proved challenging. Preactivation of **4** followed by the addition of acceptor **18** did not lead to glycoside formation. A TMSOTf mediated coupling of **6** with **18** gave only a trace amount of

product as shown by MALDI-TOF mass spectrometry. The use of 5 equivalents of **6** provided the corresponding trisaccharide in a disappointing yield of 25%. We reasoned that the failures of these glycosylations was due to the rather low reactivity of C-2 hydroxyl of **18** and the bulky nature of the C-2 auxiliary of glycosyl donors **4** and **6**.[163] Therefore, a smaller and more reactive glycosyl donor was required for this glycosylation. Indeed, a triflic acid mediated coupling of **7** with **18** led to the formation of trisaccharide **19** in an isolated yield of 71% and fortunately only a trace amount of the unwanted β-anomer was detected. Removal of Nap ether of **19** to give glycosyl acceptor **20** was accomplished by oxidation with DDQ in a mixture of DCM and water. In this reaction, care had to be taken to avoid oxidative removal of one of the benzyl ethers and in particular the use of only a small excess of recrystallized DDQ was critical to avoid overoxidation.[164] α-Galactosylation of **20** was easily accomplished by preactivation of **4** using Tf_2O and TMB in the presence of DTBMP in DCM at -40 ℃ followed by the addition of glycosyl acceptor **20**. The remnant of the auxiliary of the resulting tetrasaccharide was cleaved by treatment with 10% trifluoroacetic acid (TFA) in DCM to give glycosyl acceptor **21** in an overall yield of 67% as only the α-anomer. Surprisingly, a glycosylation of **21** with **4** gave a pentasaccharide a disappointing yield of 20%. Fortunately, a TMSOTf mediated glycosylation of **21** with **6** in DCM gave, after cleavage of the auxiliary, pentasaccharide **22** in an overall yield of 76% as only the α-anomer. The HSQC data of **22** showed that all H^1-C^1 coupling constants were in the range of 171 to 176 confirming the α-configurations of the glycosidic linkages.

The hydroxyl of **22** was acetylation and the Lev ester of the resulting compound (**23**) was removed using hydrazine acetate to give glycosyl acceptor **24**, which was coupled with mannosyl donor **3** using TMSOTf as the catalyst to provide hexasaccharide **24** in an excellent overall yield of 51% (three steps). In this case, only the α-anomeric product was formed due to neighboring group participation of the acetyl ester at C-2 of the glycosyl donor. The overall yield of the assembly of the hexasaccharide, starting from the monomeric building blocks, was 9%. Finally, hexasaccharide **24** was converted into target compound **1** by hydrogenation over Pd/C followed by removal of the acetyl esters using sodium methoxide in methanol.

Fluorous assisted target glycan assembly

Having established a robust synthetic approach for the preparation of **1**, the synthesis of this compound was performed using a purification protocol based fluorous solid phase extraction (Scheme 4). In this case, each glycosylation was performed twice to ensure completion of these critical reactions. Thus, the Nap ether of **16** was oxidatively removed with DDQ and the resulting acceptor **17** was isolated by fluorous solid phase extraction (F-SPE) using 20% water in methanol as the eluant to remove untagged compounds and the desired compound was isolated by elution with acetone. Next, acceptor **17** was coupled with **5** using the standard preactivation protocol and, as expected, aqueous workup and solid phase extraction resulted in the removal of hydrolyzed donor and other non-fluorous by-products. The glycosylation was repeated and the remnant of the auxiliary was removed using 10% TFA in DCM to give, after standard fluorous solid phase extraction, disaccharide **18**. The latter compound was

coupled twice with donor **4** using triflic acid as the promoter to provide trisaccharide **19**, which was subjected to DDQ oxidation to remove the NAP ether to provide acceptor **20**. Next, the α(1-6)-galactoside was installed by preactivation of **4** using Tf_2O, TMB and DTBMP followed by glycosylation with **20** and, after repeating the coupling protocol, the remnant of the auxiliary was removed by treatment with 10% TFA in DCM to give tetrasaccharide acceptor **21**. This compound was coupled twice with donor **6** using a standard preactivation protocol to give, after removal of the C-2 auxiliary and passing the material through a F-SPE cartridge, pentasaccharide **22**. The hydroxyl of **22** was acetylated and the resulting compound was treated with hydrazine acetate to remove the Lev ester to give an acceptor which was subjected to a double coupling with mannosyl donor **2**. After each step, the product was isolated by solid phase extraction and immediately used in the next reaction step. Homogeneous hexasaccharide **25** was obtained after purification by silica gel and LH-20 size exclusion column chromatography. This compound was obtained in an overall yield of 16.7%, which corresponds to an 85% yield per reaction step. The assembly of the hexasaccharide could be completed within 6 days. Standard deprotection of **25** gave target compound **1**, the analytical data of which were identical to the compound prepared by the conventional approach described above.

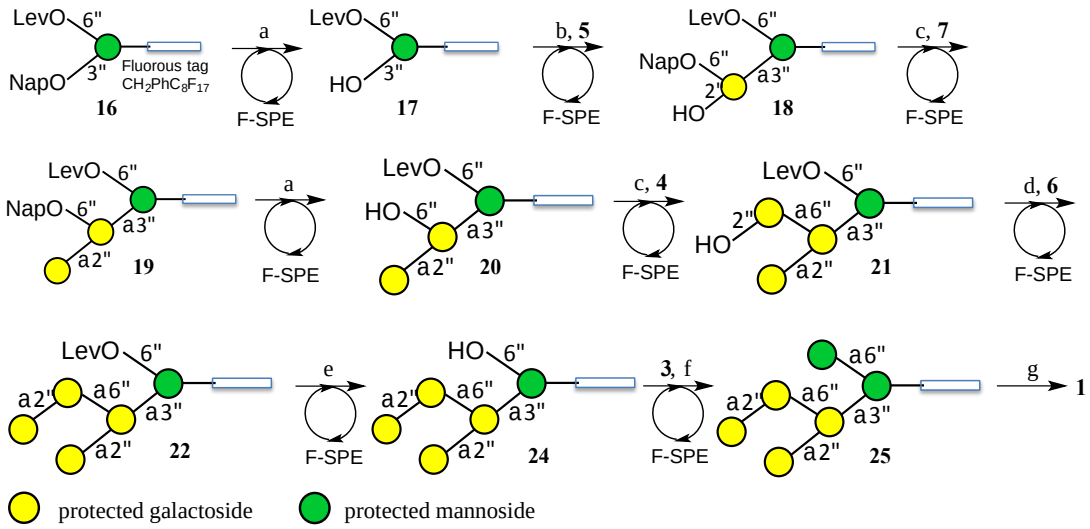


Figure 2.5 The assembly of the GPI anchor moiety of *T. brucei* by fluorous solid phase extraction.

Reagents and conditions: a) DDQ, DCM:H_2O = 10:1, 2 h, b) Tf2O, TMB, DTBMP -40 ℃ to rt then 10% TFA in DCM, 1 h; c) TfOH, DCM, -25 ℃ to rt, 3 h; d) TfOH, DTBMP, DCM, -60 ℃ to rt, 18 h, then 10% TFA in DCM, 1 h; e) Ac_2O, pyridine, DMAP, 4 h, then NH_2NH_2-AcOH, pyridine, 1 h; f) TMSOTf, DCM, -25 ℃ to rt, 1 h; g) H_2, Pd/C, AcOH, MeOH, 24 h, then NaOMe, MeOH, 1 h.

After establishing a protocol for the efficient fluorous supported synthesis of **1**, it could easily be adapted to the preparation of structurally related compounds and for example, a pentasaccharide was assembled by appropriate protecting group manipulations and sequential coupling of **2** with **8** to give a product that was further extended with **5**, **4**, **4** and **3**, respectively. The preparation of this compound was completed within 5 days.

Conclusion

We demonstrate here that a set of strategically selected orthogonal protecting groups, glycosyl donors modified by a chiral auxiliary and glycosyl acceptors containing a fluorous tag, make it possible to prepare rapidly complex branched oligosaccharides of biological importance. After the glycosylations, the chiral auxiliary could be removed using moderately strong acidic conditions, which were compatible with the presence of the orthogonal protecting groups Lev and Nap, thereby allowing efficient installation of 1,2-*cis*-linked glycosides. Previously, the auxiliary-mediated methodology was employed for the installation of α-glucosides,[143,145,156,157] and it is shown here that it can easily be extended to other monosaccharides such as galactosides. An exploratory study was required to identify potential synthetic problems. For example, due to the bulky nature of the auxiliary, a glycosylation of a sterically hindered acceptor site was challenging and in this case, a conventional donor had to be used. The attraction of the fluorous supported methodology is that after establishing a successful synthetic approach, target compounds can rapidly be resynthesized by routine procedures. Also, it allows for fast preparation of structural analogs and for example the approach for fluorous supported synthesis of **1** could easily be adapted to the preparation of structurally related compounds. Efforts are underway to develop a liquid handling system to automate fluorous supported synthesis,[130] which will make it possible to further speed up the process of oligosaccharide assembly.

Experimental Section

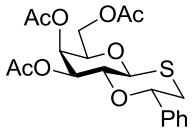
General procedure for the preparation of sulfoxide donors 4 and 5 from their corresponding oxathianes 10 and 15: *m*-CPBA (≤ 77%, l.05 eq) was dissolved in DCM and the resulting solution was slowly added to a cooled (−78 °C) solution of oxathiane in DCM. The reaction mixture was stirred at −78 °C for 30 min, diluted with DCM (20 mL) and then poured into 10% $Na_2S_2O_3$ aqueous solution. The organic layer was washed with aq. saturated $NaHCO_3$, dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography.

General glycosylation procedure for oxathiane donors with various acceptors: Oxathiane donor (1.2 eq), 1,3,5 trimethoxybenzene (2.5 eq) and 2,6-di-tert-butyl-4-methylpyridine (3.0 eq) were dissolved in DCM. Molecular sieves (4 Å) were added and the resulting suspension was cooled to −15 °C. Trifluoromethanesulfonic anhydride (1.2 eq) was added dropwise to the solution and stirring was continued for 10 min. The reaction mixture was further cooled to −40 °C and a solution of acceptor (1.0 eq) in DCM, which was dried over molecular sieves (4 Å) was added dropwise. After a reaction time of 30 min, the reaction was quenched with aq. saturated $NaHCO_3$ (30 mL). The organic phase was washed with brine (30 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography or Sephadex LH-20 size exclusion chromatography (DCM/MeOH = 1:1, 0.2 mL/min).

General procedure for the removal of a C-2 auxiliary: Trifluoroacetic acid was added dropwise to a solution of the glycosylation product in DCM at 0 ℃ adjusting the final concentration to 10% (v/v). The reaction mixture was stirred for 3 h until TLC indicated complete consumption of starting material. The reaction mixture was diluted

with DCM and poured into saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography or sephadex LH-20 size exclusion chromatography (DCM/MeOH = 1:1, 0.2 mL/min).

General fluorous supported purification protocol: F-SPE cartridges (FluoroFlash® SPE Cartridges, 10 grams, 20 cc tube) were purchased from Fluorous Technologies. Inc. The fluorous tagged compound with (200 mg compound per 1 g resin) was loaded using a minimum amount of mixture of water and DMF (9:1, v:v). The order of elution was 20% water and methanol (3×20 mL), hexane (3×20 mL). The desired fluorous-tagged compound was obtained by elution with acetone (3×20 mL). The formation of the desired compound was determined by TCL and MALDI-TOF. The product containing fractions were concentrated *in vacuo*.

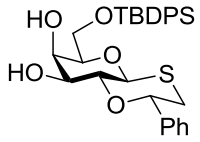


10

2-(*S*)-Phenyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-galactopyranoso)[1,2-*e*]-1,4-oxathiane (10).

2-Methoxy-2-(*S*)-phenyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-galactopyranoso)[1,2-*e*]-1,4-oxathiane[1] (2.80 g, 6.17 mmol) was dissolved in DCM (50 mL). After cooling the mixture to 0 °C, titanium tetrachloride (0.68 mL, 2.08 mmol) was added and stirring was continued for 10 min. Triethylsilane (0.89 mL, 10.54 mmol) was added dropwise and the resulting reaction was allowed to warm to room temperature and the stirring was continued for 18 h. The reaction mixture was quenched with methanol

(10 mL) and triethylamine (5 mL) after which MALDI-TOF indicated completion of the reaction. The resulting mixture was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **10** (2.26 g, 83%). R_f = 0.21 (EtOAc/Hexane, 20%, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 7.15 (m, 5H, *H*Ph), 5.48 (d, *J* = 3.5 Hz, 1H, H-1), 5.12 (dd, *J* = 10.2, 3.4 Hz, 1H, H-2), 4.85 – 4.67 (m, 1H, PhC*H*CH_2 -Aux), 4.54 (d, *J* = 9.0 Hz, 1H, H-4), 4.23 – 3.90 (m, 4H, H-$6_{a,b}$, H-3, H-5), 3.15 – 2.72 (m, 2H, PhCHCH_2Aux), 2.23 – 1.84 (s, 9H, 3×Ac). ^{13}C NMR (75 MHz, $CDCl_3$) δ 125.10, 127.34, 125.93, 68.38, 71.04, 71.05, 80.17, 80.05, 77.09, 76.77, 61.91, 61.85, 76.54, 77.74, 78.15, 36.29, 36.23, 36.26, 36.29, 36.23, 21.00, 21.00, 21.04, 21.03; HR MALDI-TOF MS: m/z: calcd for $C_{20}H_{24}O_8S$ $[M+Na]^+$: 424.1192; found: 424.1211.

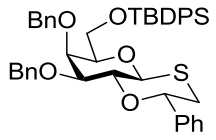


12

2-(*S*)-Phenyl-[6-*O*-(*t*-butyldiphenylsilyl)-1,2-dideoxy-β-D–galactopyranoso][1,2-*e*]-,4-oxathiane (12).

Compound **10** (2.10 g, 4.95 mmol) was dissolved in MeOH (100 mL) and fresh prepared sodium methoxide (100 mg, 1.8 mmol) was added. The reaction mixture was stirred for 1 h after which TLC analysis showed the absence of starting material. The reaction mixture was concentrated *in vacuo*. The resulting white solid was re-dissolved in DMF (30 mL) and the resulting solution was placed under an atmosphere of argon. Imidazole (0.67 g 9.9 mmol) and TBDPSCl (1.93 mL, 6.64 mmol) were subsequently

added and the reaction mixture was stirred for 3 h at room temperature. It was then diluted with DCM (100 mL), washed with aq. saturated $NaHCO_3$ (20 mL) and brine (20 mL). The organic phase was dried ($MgSO_{4)}$, filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 20%, v/v) to give compound **12** (2.60 g, 98%). R_f = 0.64 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 – 6.93 (m, 15H, 3× Ph), 4.70 (dd, J = 10.7, 2.0 Hz, 1H, CH, Aux), 4.37 (d, J = 8.8 Hz, 1H, H-1), 4.22 (dt, J = 3.2, 1.3 Hz, 1H, H-4), 4.01 – 3.81 (m, 3H, H-2, H-$6_{a,b}$), 3.74 – 3.55 (m, 2H, H-3, H-5), 3.05 (dd, J = 14.1, 10.7 Hz, 1H, CH_{2a}, Aux), 2.93 – 2.77 (m, 2H, OH×2), 2.73 (dd, J = 13.9, 2.0 Hz, 1H, CH_{2b}, Aux), 1.17 (s, 9H, TBDPS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.03, 138.00, 134.98, 127.92, 127.66, 127.65, 126.27, 80.42, 80.44, 76.14, 69.66, 61.49, 63.79, 63.72, 63.77, 81.21, 81.24, 72.57, 79.04, 35.81, 35.76, 35.83, 35.82, 35.81, 27.19; HR MALDI-TOF MS: m/z: calcd for $C_{30}H_{36}O_5SSi$ $[M+Na]^+$: 536.2053; found: 536.2061.



13

2-(*S*)-Phenyl-[3,4-*O*-benzyl-6-*O*-(*t*-butyldiphenylsilyl)-1,2-dideoxy-β-D–galactopyranoso] [1, 2-*e*]-1,4-oxathiane (13).

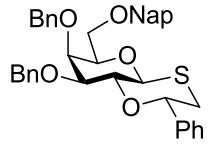
Compound **12** (2.60 g, 4.85 mmol) was dissolved in DMF (30 mL) after which benzyl bromide (1.40 mL, 5.68 mmol) was added, followed by 60% sodium hydride (0.58 g, 14.55 mmol). The reaction was stirred at 0 °C for 4 h. The reaction mixture was diluted with DCM (50 mL) and quenched with water (10 mL) after which TLC analysis

showed the completion of the reaction. The organic phase was washed with brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 5%, v/v) to give compound **13** (2.62 g, 75%). R_f = 0.73 (EtOAc/Hexane, 10%, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 – 7.07 (m, 25H, Ph), 5.11 – 4.61 (m, 5H, 2×CH_2, CH, Aux), 4.34 (d, J = 8.6 Hz, 1H, H-1), 4.21 (t, J = 9.0 Hz, 1H, H-2), 4.05 (s, 1H, H-4), 3.91 – 3.73 (m, 2H, H-$6_{a,b}$), 3.66 – 3.51 (m, 2H, H-3, H-5), 3.01 (dd, J = 13.7, 10.9 Hz, 1H, CH_{2a}, Aux), 2.76 (d, J = 13.9 Hz, 1H, CH_{2b}, Aux), 1.05 (s, J = 1.3 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 120.04, 127.99, 128.42, 129.56, 128.39, 128.39, 128.36, 128.07, 128.08, 128.03, 127.82, 127.82, 127.98, 128.08, 128.15, 128.08, 128.09, 127.77, 128.22, 128.63, 124.38, 74.40, 73.16, 73.28, 73.28, 76.40, 74.14, 80.08, 73.36, 74.59, 74.34, 74.23, 73.57, 73.94, 82.13, 84.98, 68.96, 76.81, 81.79, 74.75, 75.91, 80.20, 80.55, 27.13, 27.17; HR MALDI-TOF MS: m/z: calcd for $C_{44}H_{48}O_5SSi$ $[M+Na]^+$: 716.2992; found: 716.2984.

2-(*S*)-Phenyl-(3,4-*O*-benzyl-1,2-dideoxy-β-D–galactopyranoso) [1, 2-*e*]-1,4-oxathiane (14).

Compound **13** (2.60 g, 3.63 mmol) was dissolved in pyridine (20 mL), cooled to 0 °C and then HF/pyridine (10 mL) was added dropwise to the solution. The reaction mixture was stirred for 1 h after which it was diluted with DCM (30 mL) and quenched with aq. saturated $NaHCO_3$ (20 mL). The organic phase was washed with aq. saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography

over silica gel (EtOAc/ Hexane, 0% to 20%, v/v) to give compound **14** (1.4 g, 61%). R_f = 0.24 (EtOAc/Hexane, 20%, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 – 7.10 (m, 15H, Ph), 5.16 – 4.60 (m, 5H, 2×CH_2, CH, Aux), 4.41 (d, *J* = 8.7 Hz, 1H, H-1), 4.35 – 4.12 (m, 1H, H-2), 3.87 (dt, *J* = 12.9, 6.5 Hz, 1H, H-4), 3.80 (dd, *J* = 10.9, 6.5 Hz, 1H, H-6_a), 3.68 – 3.40 (m, 3H, H-3, H-5, H-6_b), 3.05 (dd, *J* = 13.9, 10.6 Hz, 1H, 1H, CH_{2a}, Aux), 2.79 (dd, *J* = 13.9, 2.1 Hz, 1H, 1H, CH_{2b}, Aux), 1.57 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 123.03, 124.37, 128.49, 128.54, 128.56, 128.56, 128.49, 128.54, 128.05, 128.56, 128.54, 128.54, 128.54, 128.56, 124.08, 128.18, 128.18, 128.23, 74.73, 80.18, 73.63, 80.10, 73.56, 77.07, 77.12, 81.93, 74.33, 62.04, 80.49, 62.30, 36.17, 36.21; HR MALDI-TOF MS: m/z: calcd for $C_{28}H_{30}O_5S$ $[M+Na]^+$: 478.1814; found: 478.1826.

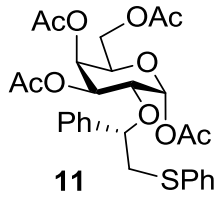


15

2-(*S*)-Phenyl-[3,4-*O*-di-benzyl-6-*O*-(2-methylnaphthyl)-1,2-dideoxy-β-D–galactopyranoso) [1, 2-*e*]-1,4-oxathiane (15).

Compound **14** (1.40 g, 2.29 mmol) was dissolved in DMF (40 mL) and the resulting solution was placed under an atmosphere of argon. 1-(Bromomethyl)naphthalene (0.77g, 3.5 mmol) and 60% NaH (0.11g, 4.38 mmol) were subsequently added and the reaction mixture was stirred for 5 h. The reaction mixture was quenched with H_2O (5 mL) and DCM (20 mL). The organic phase was washed with aq. saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash

chromatography over silica gel (EtOAc/ Hexane, 0% to 10%, v/v) to give compound **15** (1.81 g, 95%). R_f = 0.61 (EtOAc/Hexane, 20%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 – 6.61 (m, 22H), 4.61 – 3.88 (m, 8H, H_4, 3×CH_2, CH, Aux), 3.87 – 3.72 (m, 1H, H-3), 3.58 – 3.46 (d, 1H, H-1), 3.36 – 3.07 (m, 4H, H-2, H-5, H-$6_{a,b}$), 2.64 – 2.50 (m, 1H, CH_{2a}, Aux), 2.32 (d, J = 14.1 Hz, 1H, CH_{2b}, Aux); ^{13}C NMR (126 MHz, $CDCl_3$) δ 124.89, 123.89, 123.02, 122.87, 122.93, 123.82, 125.02, 125.09, 124.99, 71.93, 71.92, 70.15, 76.99, 76.98, 70.00, 70.00, 70.11, 70.11, 70.97, 71.82, 70.78, 70.78, 70.62, 70.65, 74.00, 73.95, 78.66, 78.70, 78.75, 71.78, 71.84, 76.03, 65.72, 65.72, 77.01, 77.07, 33.26, 33.21, 33.18, 33.27, 33.17; HR MALDI-TOF MS: m/z: calcd for $C_{39}H_{38}O_5S$ $[M+Na]^+$: 618.2240; found: 618.2219.

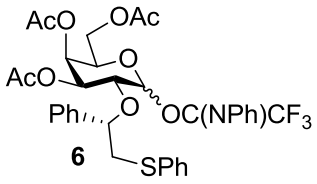


11

1,3,4,6-Tetra-*O*-acetyl-2-*O*-[1-(*S*)-phenyl-2-(phenylsulfanyl)-ethyl]-α-D-galactopyranose (11).

Compound **10** (500 mg, 1.18 mmol) was dissolved in DCM (20 mL) and the resulting solution was cooled to -78 °C. 1-aminobenzotriazole (577 mg, 1.30 mmol) and lead (IV) acetate (174 mg, 1.30 mmol) were added. The reaction mixture was stirred for 1 h and then quenched with water (10 mL). The organic phase was washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **11** (627 mg, 95%). R_f

= 0.32 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 – 7.05 (m, 10H), 6.51 (d, *J* = 3.7 Hz, 1H, H-1), 5.45 – 5.33 (m, 1H, H-4), 5.31 – 5.18 (m, 1H, H-3), 4.52 (dd, *J* = 8.4, 4.6 Hz, 1H, CH, Aux), 4.33 – 4.19 (m, 1H, H-5), 4.12 – 3.92 (m, 2H, H-6), 3.90 – 3.79 (m, 1H, H-2), 3.35 – 2.92 (m, 2H, CH_2, Aux), 2.25 -1.69 (m, 12H, 4 ×CH_3, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.41, 125.98, 127.47, 126.86, 90.13, 88.49, 68.08, 67.94, 69.07, 69.13, 81.59, 68.62, 61.45, 61.46, 71.82, 71.82, 41.80, 41.79, 41.80, 21.01, 20.81, 20.53, 20.32, 20.38; HR MALDI-TOF MS: m/z: calcd for $C_{28}H_{32}O_{10}S$ $[M+Na]^+$: 560.1716; found: 560.1711.

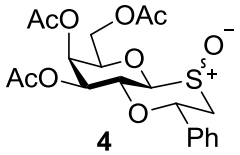


6

3,4,6-*O*-Acetyl-2-*O*-[(*S*)-2-(phenylthiomethyl)benzyl]-D-α/β–galactopyranosyl-2,2,2-trifluoro-*N*-phenyl-acetimidate (6).

Compound **11** (627 mg, 1.17 mmol) was dissolved in DMF (10 mL) and hydrazine acetate (215 mg, 2.34 mmol) was added to the resulting solution. The reaction mixture was heated to 50 °C for 5 h. The reaction mixture was concentrated *in vacuo*. The resulting yellow oil was re-dissolved in DCM (20 mL) and 2,2,2-trifluoro-*N*-phenyl-acetimidoyl chloride (0.3 mL, 1.12 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.5 mL, 3.23 mmol) were added. The reaction mixture was concentrated *in vacuo* after a reaction time of 2 h. The resulting dark yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **6** (572 mg, 71%). R_f = 0.54 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ

7.43 – 7.09 (m, 15H, 3×Ph), 6.70 (d, J = 3.5 Hz, 1H, H-1), 5.49 – 5.39 (m, 1H, H-4), 5.28 (dd, J = 10.5, 3.3 Hz, 1H, H-3), 4.63 – 4.48 (m, 1H, CH, Aux), 4.39 (t, J = 6.6 Hz, 1H, H-5), 4.16 – 3.99 (m, 2H, H-$6_{a,b}$), 3.98 – 3.85 (dd, 1H, H-2), 3.30 – 3.08 (m, 2H, CH_2, Aux), 2.09 – 1.56 (m, 9H, 3×Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 129.22, 126.70, 129.27, 129.50, 126.78, 127.13, 129.81, 129.10, 125.39, 125.25, 129.87, 127.05, 93.46, 67.87, 69.19, 69.20, 83.65, 81.19, 68.96, 68.94, 68.96, 61.63, 61.69, 61.64, 61.67, 61.66, 72.48, 72.49, 42.76, 42.76, 42.77, 42.76, 42.76, 42.76, 42.75, 21.96, 21.88, 21.86; HR MALDI-TOF MS: m/z: calcd for $C_{34}H_{34}F_3NO_9S$ $[M+Na]^+$: 689.1906; found: 689.1917.

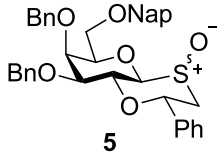


4

2-(*S*)-phenyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-galactopyranoso)[1,2-*e*]-1,4-oxathiane (*R*,*S*)-*S*-oxide (4).

Compound **10** (1.0 g, 2.36 mmol) was dissolved in DCM (20 mL) and cooled to -20 °C. Meta-chloroperoxybenzoic acid (0.58 g, 77%, 2.60 mmol) was added. The reaction was quenched with saturated $NaHCO_3$ (20 mL) after a reaction time of 30 min. The organic phase was washed with brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 50%, v/v) to give compound **4** (1.0 g, 96%, the ratio of the two isomer = 1:0.7). R_f = 0.24 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$, the R/S configuration of two isomers was not determined) δ 7.51 – 7.18 (m, 5H, Ph), 5.56 – 5.46 (m, 1.6H, H-$4^{R/S}$), 5.41 (d, J = 9.5 Hz, 0.7H,

CH^1,Aux), 5.35 – 5.21 (m, 1.5H, H-$3^{R/S}$), 4.88 – 4.78 (m, 0.6H, H-2^R), 4.74 (d, *J* = 10.7 Hz, 1H, CH^R, Aux), 4.37 (d, *J* = 10.0 Hz, 1H, H^1-1), 4.29 –4.03 (m, 5H, H^S-1, $H^{R/S}$-5, $H^{R/S}$-6), 3.87 (t, *J* = 9.9 Hz, 1H, H^1-1), 3.66 (dd, *J* = 12.7, 1.3 Hz, 1H, CH^1_2, Aux), 3.22 (dd, *J* = 14.5, 1.5 Hz, 1H, CH^2_2, Aux), 3.12 (dd, *J* = 12.6, 12.0 Hz, 1H, CH^1_2, Aux), 2.89 – 2.71 (m, 1H, CH^R_2, Aux), 2.23 – 1.93 (m, 12H, 4× CH_3, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.74, 128.74, 129.14, 125.64, 123.62, 67.39, 67.34, 70.40, 68.73, 71.02, 65.37, 75.54, 95.56, 61.28, 86.53, 75.76, 57.83, 39.53, 52.96, 57.70, 52.88, 20.70, 20.74, 20.74; HR MALDI-TOF MS: m/z: calcd for $C_{20}H_{24}O_9S$ $[M+Na]^+$: 440.1141; found: 440.1157.

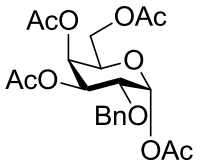


5

2-(*S*)-Phenyl-[3,4-*O*-benzyl-6-*O*-(2-methylnaphthyl)-1,2-dideoxy-β-D–galactopyranoso)[1, 2-*e*]-1,4-oxathiane(*R,S*)-*S*-oxide (5).

Compound **15** (1.81 g, 2.92 mmol) was dissolved in DCM (20 mL) and cooled to -20 °C. Meta-chloroperoxybenzoic acid (0.71 g, 77%, 3.21 mmol) was added. The reaction mixture was quenched with aq. saturated $NaHCO_3$ (20 mL) after a reaction time of 30 min. The organic phase was washed with brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 40%, v/v) to give compound **5** (1.3 g, 72%, ratio of two isomer =1:0.4); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.17 (m, 22H), 4.99 – 4.40 (m, 7H, 3×CH_2, CH, Aux), 4.22 (dt, *J* = 12.2, 4.8 Hz, 1H, H-1), 4.10 –

4.00 (m, 2H, H-2, H-4), 3.79 – 3.68 (m, 4H, H-5, H-$6_{a,b}$), 3.61 (dt, *J* = 6.4, 3.4 Hz, 1H, CH_{2a}), 3.12 – 3.03 (m, 1H, CH_{2b}); ^{13}C NMR (126 MHz, $CDCl_3$) δ 127.85, 127.16, 127.55, 126.05, 128.60, 127.78, 127.97, 77.35, 74.94, 75.84, 73.09, 73.93, 73.71, 90.45, 95.76, 74.45, 74.04, 79.00, 67.71, 79.96, 57.64, 57.69, 57.72; HR MALDI-TOF MS: m/z: calcd for $C_{39}H_{38}O_6S$ $[M+Na]^+$: 634.2389; found: 634.2377.

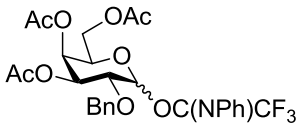


31

1,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α-D-galactopyrannose (31).

Compound **30** (1.30 g, 3.70 mmol) was dissolved in diethyl ether (20 mL) and the resulting solution was cooled to 0 °C. Benzyl 2,2,2-trichloroacetimidate (1.4 mL, 4.06 mmol) and 4 Å molecular sieve were subsequently added to the resulting solution. Trifluoromethanesulfonic acid (1.65 mL, 6.47 mmol) was added dropwise. The reaction was quenched with pyridine (2 mL) after a reaction time of 2 h and the resulting solution was concentrated *in vacuo*. The resulting yellow oil was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **31** (1.20 g, 73%). R_f = 0.54 (EtOAc/Hexane, 40%, v/v); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48 – 7.17 (m, 5H, Ph), 6.43 (dd, *J* = 8.3, 3.8 Hz, 1H, H-1), 5.62 – 5.39 (m, 1H, H-4), 5.29 (dt, *J* = 12.2, 8.2 Hz, 1H, H-3), 4.78 – 4.45 (m, 2H, CH_2, Bn), 4.32 (t, *J* = 6.7 Hz, 1H, H-5), 4.23 – 4.03 (m, 2H, H-$6_{a,b}$), 4.01 – 3.87 (m, 1H, H-2), 2.22 – 1.89 (m, 12H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.19, 127.93, 90.11, 67.86, 69.36, 69.26, 73.39, 73.32,

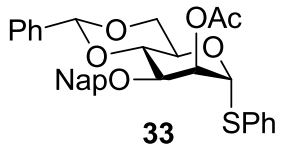
73.30, 73.23, 68.57, 61.34, 61.45, 72.43, 21.12, 20.78, 20.81, 20.87; HR MALDI-TOF MS: m/z: calcd for $C_{21}H_{26}O_{10}$ [M+Na]$^+$: 438.1526; found: 438.1575.



7

3,4,6-tri-*O*-Acetyl-2-*O*-benzyl-D-α/β–galactopyranosyl-2,2,2-trifluoro-*N*-phenyl-acetimidate (7).

Compound **30** (1.20 g, 2.71 mmol) was dissolved in the mixture of methanol and THF (7:3, v/v). Ammonia gas was then bubbled through the resulting solution. The reaction mixture was concentrated *in vacuo* after a reaction time of 3 h and the residue re-dissolved in DCM (20 mL). 2,2,2-Trifluoro-N-phenyl-acetimidoyl chloride (0.44 mL) and DBU (0.56 mL) were added to the resulting solution. The reaction mixture was concentrated *in vacuo* after a reaction time of 2 h. The resulting dark yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **7** (1.09 g, 71%). R_f = 0.82 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 – 6.61 (m, 10H, Ph), 5.55 (d, *J* = 1.7 Hz, 1H, H-1), 5.48 – 5.30 (m, 2H, H-3, H-4), 4.92 – 4.66 (m, 2H, CH_2, Bn), 4.39 (dd, *J* = 22.5, 15.5 Hz, 1H, H-5), 4.22 – 4.09 (m, 2H, H-$6_{a,b}$), 4.07 – 3.88 (m, 1H, H-2), 2.33 – 1.99 (m, 9H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.10, 128.63, 128.53, 124.46, 124.41, 119.16, 119.17, 119.36, 67.82, 67.24, 69.19, 69.27, 72.26, 75.19, 75.22, 73.54, 74.88, 68.98, 61.11, 61.42, 72.78, 72.77, 75.35, 20.73, 20.69, 20.76, 20.78, 20.72, 20.72; HR MALDI-TOF MS: m/z: calcd for $C_{27}H_{28}F_3NO_9$ [M+Na]$^+$: 567.1716; found: 567.1701.

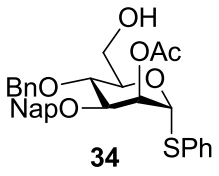


33

Phenyl-4,6-O-benzylidene-3-*O*-(2-naphthyl)-2-*O*-acetyl-1-thio-α-D-mannopyranoside (33).

Phenyl-4,6-*O*-benzylidene-1-thio-α-D-glucopyranoside **(32)** (5.0 g, 13.9 mmol) was dissolved in toluene (100 mL). Tert-dibutyltin(IV) oxide (3.6 g, 15.3 mmol) was added to the resulting solution. The solution was then heated to 85 °C and refluxed for 4 h and concentrated *in vacuo*. The resulting reaction mixture was re-dissolved in DMF (200 mL). NapBr (4.6 g, 20.9 mmol) and caesium fluoride (4.2 g, 28.0 mmol) were then added to the resulting solution. The reaction mixture was then diluted with DCM (100 mL) after a reaction time of 18 h. The organic phase was washed with aq. saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting colorless oil was then re-dissolved in a mixture of pyridine (20 mL) and acetic anhydride (10 mL). The reaction mixture was concentrated *in vacuo* after a reaction time of 4 h. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 20%, v/v) to give compound **33** (5.90 g, 78%). R_f = 0.31 (EtOAc/Hexane, 20%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 – 7.24 (m, 17H), 5.73 (dt, *J* = 15.9, 5.6 Hz, 2H, H-1, H-2), 5.53 (d, *J* = 0.9 Hz, 1H, CH), 5.02 – 4.85 (m, 2H, CH_2), 4.48 – 4.36 (m, 1H, H-5), 4.28 (dt, *J* = 11.1, 5.6 Hz, 1H, H-6_a), 4.23 (t, *J* = 9.7 Hz, 1H, H-4), 4.17 (dd, *J* = 8.8, 5.4 Hz, 1H, H-3), 3.92 (t, *J* = 10.3 Hz, 1H, H-6_b), 2.27 (s, 3H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 126.51, 127.94, 128.07, 128.01, 126.35, 125.82, 132.10, 132.12, 128.63, 128.70, 71.36, 101.79, 87.23, 72.26,

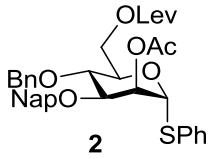
72.27, 72.27, 72.28, 65.28, 68.48, 78.55, 78.54, 78.58, 74.17, 74.17, 68.48, 68.50, 68.48, 21.10; HR MALDI-TOF MS: m/z: calcd for $C_{32}H_{30}O_6S$ $[M+Na]^+$: 542.1763; found: 542.1761.



34

Phenyl-2-*O*-acetyl-3-*O*-(2-naphthyl)-4-*O*-benzyl-1-thio-α-D-mannopyranoside (34).

Compound **33** (1.0 g, 1.85 mmol) was dissolved in DCM (30 mL) and triethylsilane (1.5 mL, 1.77 mmol) was then added. The resulting solution was cooled to -78 °C and dichlorophenylborane (0.24 mL, 1.23 mmol) was added dropwise. The reaction was quenched by a mixture of MeOH (5 mL) and triethylamine (2 mL) after a reaction time of 2 h. The mixture was concentrated *in vacuo* and the resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **22** (0.70 g, 68%). R_f = 0.21 (EtOAc/Hexane, 20%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.26 (m, 17H), 5.71 (dt, *J* = 10.7, 5.3 Hz, 1H, H-2), 5.51 (d, *J* = 1.1 Hz, 1H, H-1), 5.09 – 4.66 (m, 4H, CH_2), 4.24 (dt, *J* = 9.7, 3.4 Hz, 1H, H-5), 4.10 – 4.05 (m, 1H, H-3), 3.97 (t, *J* = 9.6 Hz, 1H, H-4), 3.91 – 3.82 (m, 2H, H-$6_{a,b}$), 2.19 (s, 4H, Ac), 2.05 (s, 1H, OH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.14, 127.32, 127.89, 126.15, 132.22, 128.16, 128.53, 70.34, 86.40, 75.39, 75.40, 72.03, 72.03, 72.03, 72.03, 75.39, 75.39, 73.15, 78.36, 78.35, 74.14, 74.30, 74.35, 62.02, 21.12; HR MALDI-TOF MS: m/z: calcd for $C_{32}H_{32}O_6S$ $[M+Na]^+$: 544.1920; found: 544.1938.

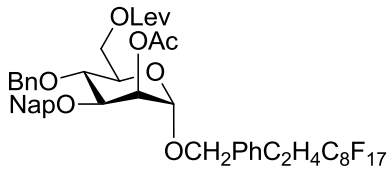


2

Phenyl-2-*O*-acetyl-3-*O*-(2-naphthyl)-4-*O*-benzyl-6-levulinoyl-1-thio-α-D-mannopyranoside (2).

Compound **34** (0.68 g, 1.23 mmol) and levulinic acid (0.28 g, 2.47 mmol) were dissolved in DCM (30 mL). The resulting solution was cooled to 0 °C. And 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.38 g, 2.47 mmol) and 4-dimethylaminopyridine (14 mg, 0.12 mmol) were added. The reaction mixture was stirred under an atmosphere of argon. The reaction was quenched with water (10 mL) after a reaction time of 2 h. The organic phase was washed with aq. saturated $NaHCO_3$ (30 mL), brine (30 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 20%, v/v) to give compound **2** (0.61g, 77%). R_f = 0.51(EtOAc/Hexane, 20%, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89-7.18 (m, 17H, Ar*H*), 5.66 (dd, *J* = 3.2, 1.6 Hz, 1H, H-2), 5.49 (d, *J* = 1.6 Hz, 1H, H-1), 4.93 (m, 4H, 2× CH_2Ph), 4.47 – 4.26 (m, 3H, H-5, H-$6_{a,b}$), 4.02 (dd, *J* = 9.2, 3.2 Hz, 1H, H-3), 3.82 (t, *J* = 9.4 Hz, 1H, H-4), 2.76 – 2.63 (m, 2H, C*H*$_2$CH$_2$, Lev), 2.55 (td, *J* = 6.5, 1.0 Hz, 2H, CH$_2$C*H*$_2$, Lev), 2.16 (s, 3H, CH_3, Lev), 2.15 (s, 3H, CH_3, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 128.04, 128.25, 127.84, 126.41, 132.20, 126.29, 132.13, 132.14, 128.42, 128.36, 128.53, 70.31, 70.37, 86.39, 86.39, 75.67, 75.60, 75.55, 72.31, 72.31, 72.23, 72.34, 72.25, 72.33, 72.25, 75.64, 75.64, 75.63, 71.14, 63.68, 71.00, 63.59, 63.76, 63.65, 78.61, 78.63, 78.65, 74.59, 38.19, 28.20, 38.04, 38.14, 28.24, 28.25,

28.13, 21.17, 21.33, 30.02, 30.21; HR MALDI-TOF MS: m/z: calcd for $C_{37}H_{38}O_8S$ $[M+Na]^+$: 665.2185; found: 666.2181.

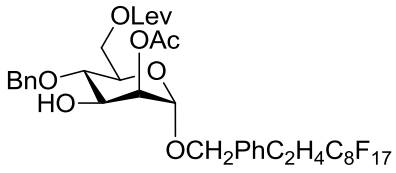


16

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-2-*O*-acetyl-3-*O*-(2-naphthyl)-4-*O*-benzyl-6-levulinoyl-1-*O*-α-D-mannopyranoside (16).

Compound **2** (0.58 g, 0.90 mmol) and compound **8** (0.50 g, 0.90 mmol) were dissolved in DCM (30 mL), and flame dried molecular sieve (4Å) was added. The reaction mixture was placed under an atmosphere of argon and cooled to -25 °C in dark. After stirring for 10 min subsequently NIS (0.24 g, 1.08 mmol) and TfOH (16 μl) were added. The reaction was allowed to reach room temperature over 40 min. The reaction mixture was diluted with DCM (20 mL), and then quenched with aq. saturated NaS_2O_3 solution (20 mL). The organic phase was washed with aq. saturated $NaHCO_3$ (30 mL), brine (30 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **16** (0.89g, 89%). R_f= 0.45 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 – 7.08 (m, 16H, Ar*H*), 5.49 (dd, *J* = 3.5, 1.8 Hz, 1H, H-2), 5.13 -4.47 (m, 7H, 3× CH_2Ar, H-1), 4.46 – 4.40 (m, 1H, H-6_a), 4.40 – 4.32 (m, 1H, H-6_b), 4.12 (dd, *J* = 9.3, 3.4 Hz, 1H, H-3), 3.94 (ddd, *J* = 10.2, 5.0, 2.1 Hz, 1H,H-5), 3.82 (t, *J* = 9.6 Hz, 1H, H-4), 3.04 – 2.87 (m, 2H, C*H*$_2$CH$_2$C$_8$F$_{17}$,), 2.86 – 2.73

(m, 2H, CH_2CH_2, Lev), 2.70 – 2.64 (m, 2H, CH_2CH_2, Lev), 2.47 – 2.32 (m, 5H, CH_3, Lev, $CH_2CH_2C_8F_{17}$), 2.21 (s, 3H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 127.87, 128.10, 127.12, 127.83, 127.01, 126.05, 125.97, 128.26, 128.47, 128.50, 128.31, 128.50, 68.66, 66.01, 75.36, 75.37, 97.00, 71.91, 71.93, 71.89, 71.59, 69.19, 69.12, 75.37, 75.36, 69.05, 69.05, 63.54, 63.53, 63.54, 26.22, 37.92, 27.89, 33.08, 21.51, 32.96, 21.04; HR MALDI-TOF MS: m/z: calcd for $C_{48}H_{43}F_{17}O_9$ $[M+Na]^+$: 1109.2533; found: 1109.2539.

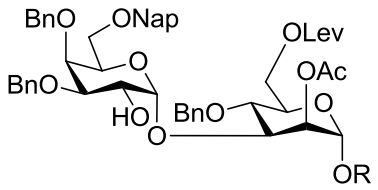


17

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-2-*O*-acetyl-4-*O*-benzyl-6-levulinoyl-1-α-D-mannopyranoside (17).

Compound **16** (0.89 g, 0.82 mmol) was dissolved in a mixture of DCM (10 mL) and water (0.5 mL). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.28 g, 1.23 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. It was diluted with DCM (20 mL) and the organic phase was washed with saturated $NaHCO_3$ (30 mL×2), brine (30 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 50%, v/v) to give compound **17** (0.64 g, 82%). R_f = 0.24 (EtOAc/Hexane, 40%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.17 (m, 9H, Ar*H*), 5.17 (dd, *J* = 3.6, 1.7 Hz, 1H, H-2), 4.93 (d, *J* = 1.9 Hz, 1H, H-1), 4.90 – 4.47 (m, 4H, 2× CH_2Ph), 4.45 – 4.30 (m, 2H, H-$6_{a,b}$), 4.23 (dd, *J* = 9.4, 3.5 Hz, 1H, H-3), 3.93 – 3.86 (m, 1H, H-5), 3.69 (t, *J* = 9.6 Hz, 1H, H-4), 2.98 – 2.88 (m, 2H, $CH_2CH_2C_8F_{17}$), 2.86 – 2.75

(m, 2H, CH_2CH$_2$, Lev), 2.70 – 2.64 (m, 2H, CH$_2$CH_2, Lev), 2.48 – 2.30 (m, 3H, CH$_2$CH_2C$_8$F$_{17,}$ O*H*), 2.21 (s, 3H, CH$_3$, Lev), 2.18 (s, 3H,Ac); ^{13}C NMR (126 MHz, CDCl$_3$) δ 125.99, 128.31, 128.54, 128.63, 125.41, 125.36, 128.55, 128.65, 72.44, 96.78, 75.08, 75.06, 69.14, 75.08, 69.13, 75.07, 69.10, 69.09, 63.38, 63.36, 63.35, 70.53, 70.51, 69.60, 75.72, 75.77, 75.74, 26.03, 37.63, 27.70, 32.87, 32.83, 29.83, 20.92; HR MALDI-TOF MS: m/z: calcd for $C_{37}H_{35}F_{17}O_9$ $[M+Na]^+$: 969.1907; found: 969.1918.



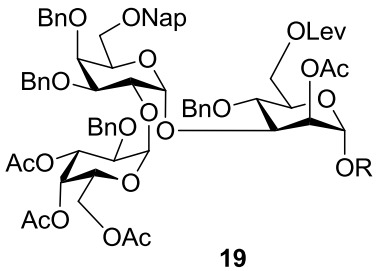
18

R = $CH_2PhC_2H_4C_8F_{17}$

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(3,4-di-benzyl-6-2-*O*-acetyl-4-*O*-benzyl-6-(2-naphthyl)-α-D-galactopyranosyl)-(1→3)-2-acetyl-4-benzyl-6-levulinoyl-1-α-*D*-mannopyranoside (18).

Compound **5** (0.2 g, 0.32 mmol) and compound **17** (0.2 g, 0.21 mmol) were coupled using general glycosylation procedure. The purification was achieved through sephadex LH20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **18** (0.14 g, 87%, only); R_f = 0.21 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.13 – 6.91 (m, 26H, ArH), 5.31 (d, J = 4.3 Hz, 2H, H-2, H^I-1), 5.05 – 4.81 (m, 3H, CH_2Ar, H-1), 4.78 – 4.33 (m, 9H, 4×CH_2Ar, H-6$_a$), 4.33 – 4.26 (m, 2H, H-6$_b$, H^I-2), 4.24 – 4.20 (m, 1H, H-3), 4.16 (t, J = 6.8 Hz, 1H, H^I-5), 4.06 (s, 1H, H^I-4), 3.91 – 3.82 (m, 2H, H-5, H^I-3), 3.74 – 3.67 (m, 1H, H-4), 3.64 (dd, J = 9.5, 7.0 Hz, 1H, H^I-6$_a$), 3.57 (dd, J = 9.6, 5.7 Hz, 1H, H^I-6$_b$), 2.90 – 2.81 (m, 2H ,CH_2CH$_2$C$_8$F$_{17}$), 2.80

– 2.70 (m, 2H, C*H*$_2$CH$_2$, Lev), 2.73 – 2.58 (m, 2H, CH$_2$C*H*$_2$, Lev), 2.40 – 2.24 (m, 3H, CH$_2$C*H*$_2$C$_8$F$_{17,}$ O*H*), 2.21 (s, 3H, CH$_3$, Lev), 2.14 (s, 3H, Ac). ^{13}C NMR (126 MHz, CDCl$_3$) δ 127.80, 127.87, 126.66, 126.02, 125.86, 125.79, 128.28, 128.28, 128.08, 128.06, 128.23, 128.29, 128.19, 72.11, 100.54, 75.10, 75.13, 74.63, 96.76, 74.66, 72.06, 72.05, 73.35, 73.41, 72.09, 73.19, 73.44, 69.21, 74.38, 69.21, 74.44, 74.98, 74.96, 63.27, 69.18, 69.17, 63.23, 76.32, 63.29, 68.94, 70.52, 73.62, 74.70, 70.02, 74.72, 79.09, 79.09, 68.83, 68.88, 26.13, 37.91, 27.87, 32.97, 29.89, 21.14; HR MALDI-TOF MS: m/z: calcd for C$_{68}$H$_{65}$F$_{17}$O$_{14}$ [M+Na]$^+$: 1451.4001; found: 1451.4009.



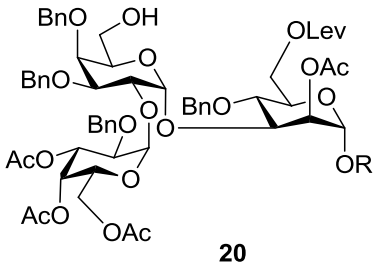
19

R = CH$_2$PhC$_2$H$_4$C$_8$F$_{17}$

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(3,4-di-benzyl-6-2-*O*-acetyl-4-*O*-benzyl-6-(2-naphthyl)-α-D-galactopyranosyl)-(1→3)-2-acetyl-4-benzyl-6-levulinoyl-1-α-D-mannopyranoside (19).

Compound **7** (0.11g, 0.194 mmol) and **18** (0.19 g, 0.097 mmol) were dissolved in DCM (15 mL) and then flame dried molecular sieve (4Å) was added. The mixture was placed under an atmosphere of argon at room temperature, followed by cooling to -25 °C.

TMSOTf (4 L, 0.02 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature over a period of 3 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (10 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by LH-20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **19** (0.12 g, 71%, = 15:1). R_f = = 0.21 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 – 6.94 (m,31H, ArH), 5.38 (dd, J = 10.6, 3.5 Hz, 1H, H^{II}-3), 5.29 – 5.25 (m, 1H, H-2), 5.22 (q, J = 3.9 Hz, 1H, H^{I}-1), 5.17 (d, J = 3.6 Hz, 1H, H^{II}-4), 5.15 – 5.10 (d, 1H, CH_2Ar), 4.95 – 4.90 (m, 2H, H-1, H^{II}-1), 4.88 (d, J = 10.9, 3.8 Hz, 1H, CH_2Ar), 4.73 – 4.41 (m, 11H, 5×CH_2Ar, H^{II}-5), 4.35 – 4.31 (m, 1H, H-3), 4.28 – 4.21 (m, 2H, H^{I}-2, H-6_a), 4.18 – 4.10 (m, 2H, H-6_b, H^{I}-5), 4.09 – 4.04 (m, 1H, H^{I}-4), 4.04 – 3.99 (m, 1H, H^{I}-3), 3.89 – 3.83 (m, 1H, H-5), 3.82 – 3.70 (m, 4H, H^{II}-$6_{a,b}$, H-4, H^{II}-2), 3.67 – 3.63 (m, 1H, H^{I}-6_a), 3.44 – 3.38 (m, 1H, H^{I}-6_b), 2.87 – 2.77 (m, 2H ,CH_2CH_2C$_8$F$_{17}$), 2.74 – 2.63 (m, 2H, CH_2CH_2, Lev), 2.59 – 2.45 (m, 2H, CH_2CH_2, Lev), 2.35 (s, 3H, Lev), 2.33 – 2.20 (m, 2H, CH_2CH_2, Lev), 2.11 – 2.06 (s, 3H, Ac), 2.05 – 2.00 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.73 (s, 3H, Ac). ^{13}C NMR (151 MHz, $CDCl_3$) δ 127.88, 126.71, 126.04, 127.65, 127.85, 128.06, 128.05, 127.94, 131.80, 128.28, 69.58, 69.55, 71.68, 97.66, 68.55, 74.36, 74.32, 96.20, 96.50, 74.88, 74.91, 72.70, 72.75, 72.83, 73.43, 69.20, 73.56, 69.16, 73.63, 74.19, 72.88, 69.35, 66.09, 69.30, 71.85, 77.49, 73.35, 73.30, 63.52, 63.50, 63.66, 70.48, 74.56, 77.05, 77.09, 69.71, 74.09, 74.04, 61.43, 73.95, 61.52, 73.15, 73.11, 68.33, 68.36, 26.11, 26.08, 37.83, 27.75, 27.77, 21.49, 32.87, 32.78, 29.88, 21.01, 20.67, 20.77, 20.51. HR MALDI-TOF MS: m/z: calcd for $C_{87}H_{87}F_{17}O_{22}$ $[M+Na]^+$: 1829.5315; found: 1829.5324.



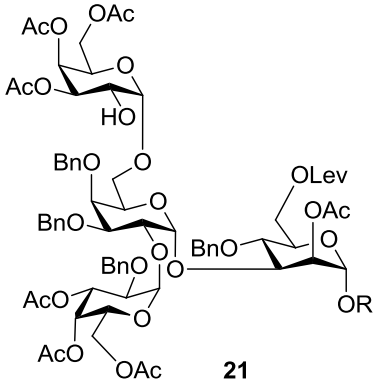
20

R = $CH_2PhC_2H_4C_8F_{17}$

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(3,4-di-benzyl-6-2-*O*-acetyl-4-*O*-benzyl-6-α-D-galactopyranosyl)-(1→3)-2-acetyl-4-benzyl-6-levulinoyl-1-α-D-mannopyranoside (20).

Compound **19** (57 mg, 0.031 mmol) was dissolved in a mixutre of DCM (0.5 mL) and water (0.01 mL). DDQ (8 mg, 0.034 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with DCM (20 mL) and the organic phase was washed with aq. saturated $NaHCO_3$ (20 mL), brine (20mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 50%, v/v) to give compound **20** (40 mg, 77%). R_f = 0.21 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.08 (m, 24H, Ar*H*), 5.45 – 5.36 (dd, 1H, H^{II}-3), 5.32 – 5.27 (d, 1H, H^{I}-1), 5.27 – 5.22 (t, 1H, H-2), 5.20 – 5.17 (t, 1H, H^{II}-4), 5.10 (d, J = 11.7, 4.3 Hz, 1H, CH_2Ar), 4.99 – 4.86 (m, 3H, H^{II}-1, H-1, CH_2Ar), 4.77 – 4.42 (m, 8H, 4×C*H*$_2$Ar), 4.41 – 4.31 (m, 2H, H^{I}-4, H^{II}-5), 4.29 – 4.19 (m, 2H, H^{I}-2, H-6_a), 4.18 – 4.07 (m, 2H, H-3, H-6_b), 4.02 – 3.92 (m,2H, H-4, H^{I}-5), 3.89 (dt, J = 4.8, 3.8 Hz, 1H, H^{I}-3), 3.88 –

3.70 (m, 5H, H^{II}-$6_{a,b}$, H-5, H-3, H^{II}-2), 3.65 (ddd, J = 12.3, 6.7, 3.6 Hz, 1H, H^{I}-6_a), 3.58 – 3.46 (m, 1H, H^{I}-6_b), 2.97 – 2.24 (m, 8H, CH_2CH_2 Lev, CH_2CH_2 Linker), 2.24 – 1.54 (m, 15H, 5×CH_3, Ac, Lev). ^{13}C NMR (151 MHz, $CDCl_3$) δ 127.99, 127.89, 132.09, 128.34, 131.81, 127.90, 128.24, 127.90, 131.74, 128.16, 128.27, 69.57, 69.56, 97.78, 71.42, 68.59, 100.04, 74.33, 74.28, 97.01, 74.56, 74.51, 74.44, 96.26, 96.74, 73.25, 73.32, 73.14, 72.46, 69.26, 69.16, 74.43, 69.18, 74.45, 72.92, 74.28, 69.18, 74.24, 69.16, 66.41, 62.92, 76.80, 73.83, 63.11, 73.84, 63.09, 75.95, 63.49, 76.14, 68.68, 63.48, 68.64, 63.48, 60.86, 77.38, 73.64, 77.46, 74.50, 71.87, 77.45, 70.09, 74.55, 74.53, 74.29, 74.21, 61.62, 73.31, 73.25, 79.22, 62.16, 79.16, 62.16, 26.15, 26.12, 37.82, 37.83, 27.74, 32.88, 29.88, 29.87, 21.18, 20.76, 20.71, 20.74, 20.55; HR MALDI-TOF MS: m/z: calcd for $C_{76}H_{79}F_{17}O_{22}$ $[M+Na]^+$: 1689.4689; found:1689.4695.



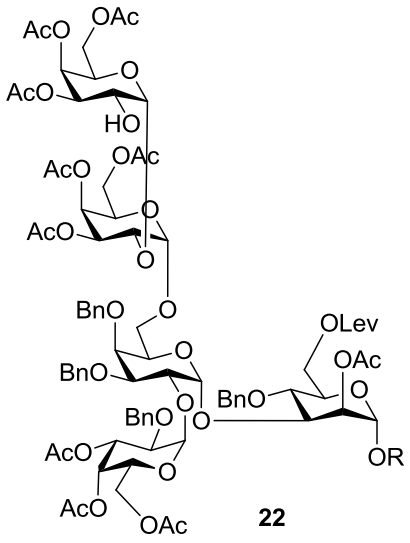
21

R = $CH_2PhC_2H_4C_8F_{17}$

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl- -D-galactopyranosyl)-(1→2)(3,4,6-tri-*O*- -D-galactopyranosyl)-(1→6)-(3,4-di-benzyl-

2-*O*-acetyl-4-*O*-benzyl-6- -D-galactopyranosyl)-(1→3)-2-acetyl-4-benzyl-6-levulinoyl-1- -D-mannopyranoside (21).

Compound **4** (13 mg, 0.031 mmol) and **20** (40 mg, 0.024 mmol) were coupled by the general glycosylation method. The purification was achieved through LH-20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **21** (31 mg, 67%, 2 steps, only); R_f = 0.35 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 – 7.11 (m, 24H, HAr), 5.41 – 5.34 (m, 2H, H^{II}-3, H^{I}-1), 5.29 (t, J = 3.9 Hz, 1H, H^{III}-4), 5.24 – 5.19 (dd, 1H, H-2), 5.19 – 5.11 (m, 2H, H^{III}-4, H^{II}-4), 5.08 – 5.02 (m, 2H, H^{II}-1, CH_2Ar), 4.94 – 4.90 (m, 1H, H-1, CH_2Ar), 4.81 (t, J = 5.1 Hz, 1H, H^{III}-1), 4.78 – 4.46 (m, 8H, 4×CH_2Ar), 4.40 (t, J = 6.6 Hz, 1H, H^{III}-5), 4.35 (dd, J = 9.4, 3.1 Hz, 1H, H-3), 4.27 (dd, J = 10.3, 3.2 Hz, 1H, H^{I}-2), 4.20 (dt, J = 5.6, 2.9 Hz, 1H, H^{II}-6_a), 4.16 (q, J = 6.7 Hz, 1H, H^{I}-4), 4.10 (ddd, J = 17.3, 9.4, 4.8 Hz, 2H, H^{II}-5, H^{II}-6_b), 4.04 – 3.99 (m, 2H, H^{I}-3, H-5), 3.94-3.76 (m, 8H, H^{III}-2, H-4, H-$6_{a,b}$, H^{III}-$6_{a,b}$, H^{I}-6_a), 3.76 – 3.71 (m, 1H, H^{II}-2), 3.29 (dd, J = 10.1, 3.9 Hz, 1H, H^{I}-6_b), 2.90 – 2.84 (m, 2H ,CH_2CH_2C$_8$F$_{17}$), 2.70 – 2.64 (m, 2H, CH_2CH_2, Lev), 2.55 – 2.43 (m, 4H, CH_2CH_2, Lev), 2.38 – 2.28 (m, 5H, CH_2CH_2C$_8$F$_{17}$, CH_3 Ac), 2.16 – 1.74 (m, 21H, 7×CH_3, Ac); ^{13}C NMR (151 MHz, $CDCl_3$) δ 128.08, 128.41, 128.02, 127.81, 128.40, 69.41, 69.41, 97.75, 68.34, 72.32, 68.53, 70.48, 70.52, 73.19, 73.16, 96.24, 74.64, 74.64, 95.32, 98.91, 68.36, 68.37, 74.61, 74.60, 68.27, 72.74, 68.20, 72.67, 73.00, 66.27, 75.12, 75.28, 72.93, 63.41, 63.49, 66.56, 63.27, 77.13, 61.89, 77.24, 69.96, 61.97, 67.17, 67.89, 62.07, 75.21, 74.22, 61.50, 74.26, 73.32, 73.22, 67.96, 26.18, 37.75, 27.62, 21.48, 32.87, 29.85, 20.68, 21.07, 20.68, 20.82, 20.70, 20.65, 20.53, 20.67, 20.51; HR MALDI-TOF MS: m/z: calcd for $C_{88}H_{95}F_{17}O_{30}$ $[M+Na]^+$: 1954.5636; found:1954.5641.



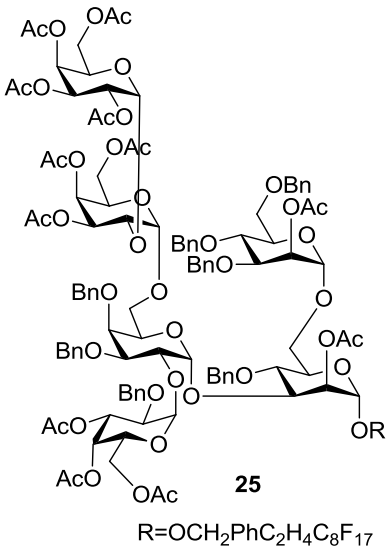
22

R = $CH_2PhC_2H_4C_8F_{17}$

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl)-(1→2)(3,4,6-tri-*O*-α-D-galactopyranosyl)-(1→2)-(3,4,6-tri-*O*-α-D-galactopyranosyl)-(1→6)-(3,4-di-benzyl-2-*O*-acetyl-4-*O*-benzyl-6-α-D-galactopyranosyl)-(1→3)-2-acetyl-4-benzyl-6-levulinoyl-1-α-D-mannopyranoside (22).

Compound **6** (15 mg, 0.023 mmol) was dissolved in DCM (2 mL) and activated molecular sieve (4 Å) was added. The reaction mixture was placed under an atmosphere of argon and cooled to -78 ℃ for 30 min. TfOH (2 µl, 0.02mml) was added. The mixture of compound **21** (15 mg, 0.0076 mmol) and DTBMP (7 mg, 0.03 mmol) with activated molecular sieve (4Å) were added to the reaction mixture dropwise at -35 ℃. The reaction mixture was allowed to rise to room temperature for 18 h. 15% TFA in DCM (10 mL) was added to the reaction mixture after TLC analysis showed the absence of the starting material and stirred for 1.5 h. The reaction was quenched with aq. saturated $NaHCO_3$

solution (10 mL) and the organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by LH-20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **22** (12mg, 76%, 2steps, only), R_f = 0.28 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 – 7.08 (m, 24H, HAr), 5.40 – 5.33 (m, 3H, H^{II}-3, H^{III}-4, H^{IV}-4), 5.27 (dt, *J* = 7.3, 4.4 Hz, 3H, H^{III}-3, H^{I}-1, H-2), 5.21 – 5.16 (t, 1H, H^{II}-4), 5.11 – 5.06 (d, 1H, CH_2Ar), 5.04 – 4.93 (m, 4H, CH*H*Ar, H^{II}-1, H-1, H^{IV}-3), 4.86 (dd, *J* = 7.0, 3.8 Hz, 2H, H^{III}-1, H^{IV}-1), 4.80 – 4.40 (m, 9H, 4×CH_2Ar, H^{IV}-5), 4.34 – 4.29 (m, 1H, H-3), 4.26 (dt, *J* = 11.4, 5.6 Hz, 1H, H^{I}-2), 4.22 – 4.15 (m, 3H, H^{IV}-6_a, H^{III}-5, H^{I}-4), 4.12 – 4.02 (m, 5H, H-$6_{a,b}$, H^{IV}-6, H^{II}-5, H^{II}-3), 4.01 – 3.95 (m, 3H, H^{III}-6_a, H^{I}-5, H-5), 3.91 – 3.87 (m, 2H, H^{III}-6_a, H^{IV}-2), 3.85 – 3.73 (m, 4H, H^{I}-3, H-4, H^{II}-$6_{a,b}$), 3.72 – 3.65 (m, 2H, H^{II}-2, H^{I}-6_a), 3.61 (dd, *J* = 10.4, 6.3 Hz, 1H, H^{I}-6_b), 2.91 – 2.84 (m, 2H, C*H*$_2$$CH_2C_8F_{17}$), 2.61 (d, *J* = 11.4 Hz, 2H, C*H*$_2$$CH_2$, Lev), 2.56 – 2.42 (m, 3H, CH_2C*H*$_2$, Lev, OH), 2.31 (dq, *J* = 19.4, 10.6 Hz, 2H, CH_2C*H*$_2$$C_8F_{17}$), 2.20 – 1.70 (m, 33H, 11×CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 127.98, 128.15, 127.78, 128.17, 68.19, 71.74, 68.45, 98.50, 68.48, 74.01, 73.85, 70.62, 74.46, 74.46, 96.32, 96.07, 72.46, 72.64, 68.89, 68.88, 74.42, 74.45, 68.86, 68.76, 72.74, 66.21, 77.24, 72.93, 66.78, 63.26, 70.30, 74.54, 62.11, 76.73, 70.38, 61.81, 61.83, 66.51, 69.58, 74.06, 61.50, 73.26, 67.67, 67.89, 26.12, 37.82, 27.72, 32.81, 29.87, 20.68, 21.00, 20.68, 20.78, 20.66, 20.76, 20.54; HR MALDI-TOF MS: m/z: calcd for $C_{100}H_{111}F_{17}O_{38}$ $[M+Na]^+$: 2243.6515; found: 2243.6507.

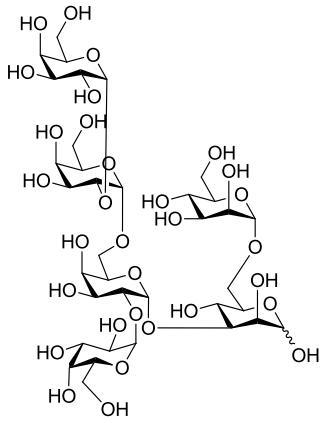


4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(2,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl- -D-galactopyranosyl)-(1→2)(3,4,6-tri-*O*-α-D-galactopyranosyl)-(1→2)-(2-*O*-benzyl-3,4,6-tri-*O*-α-D-galactopyranosyl)-(1→6)-(3,4-di-benzyl-2-*O*-acetyl-4-*O*-benzyl-6-α-D-galactopyranosyl)-(1→3)(4,5,6-tri-benzyl-2,acetyl-α-D-mannopyranosyl)-(1→6)-2-acetyl-4-benzyl-6-1-α-D-mannopyranoside (25).

Compound **22** (12 mg, 0.0056 mmol) was dissolved in pyridine (3 mL). Acetic anhydride (0.05 mL) and 4-dimethylaminopyridine (1 mg) were added. The reaction was then quenched with aq. saturated $NaHCO_3$ solution (10 mL) after which MALDI–TOF analysis showed completion of the reaction. The organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was re-dissolved in the mixture of pyridine (0.3 mL) and acetic acid (0.1 mL). Hydrazine acetate (3 mg, 0.032 mmol) was added to the resulting solution and stirred for 2 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (10 mL) and the organic phase was washed with washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate

was concentrated *in vacuo*. Compound **24** (9.5 mg, 0.016 mmol) and the residue were re-dissolved in DCM (2 mL) and cooled to -25 ℃. TMSOTf (2 L) was added and stirred for 3 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (10 mL) and the organic phase was washed with washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by LH-20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **25** (7.6mg, 51% 3 steps), R_f = 0.22 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (800 MHz, $CDCl_3$) δ 7.56 – 6.95 (m, 39H, *H*Ar), 5.42 (tt, *J* = 10.4, 4.2 Hz, 3H, H-2, H^{III}-4, H^{IV}-4), 5.34 (dd, *J* = 10.5, 3.5 Hz, 1H, H^{II}-3), 5.32 – 5.26 (m, 2H, H^{III}-3, H^{IV}-3), 5.25 – 5.20 (m, 4H, H^{I}-1, H^{IV}-1,H^{V}-2, C*H*HPh), 5.10 – 5.06 (m, 2H, H^{II}-4, H^{IV}-2), 4.99 (d, *J* = 11.3 Hz, 1H, CH*H*Ph), 4.90 (dd, *J* = 5.0, 3.1 Hz, 3H, H-1, H^{II}-1, H^{III}-1), 4.82 (ddd, *J* = 16.4, 11.8, 7.0 Hz, 3H, CH_2Ph), 4.79 (d, *J* = 1.9 Hz, 1H, H^{V}-1), 4.75 (d, *J* = 11.3 Hz, 1H, CH_2Ph), 4.69–4.39 (m, 10H, 5×CH_2Ph), 4.32 – 4.27 (m, 2H, H^{II}-5, H^{V}-3), 4.25 – 4.20 (m, 2H, H^{I}-2, H^{IV}-5), 4.11 – 4.05 (m, 2H, H^{I}-3, H^{III}-6_a), 4.05 – 3.95 (m, 4H, H^{I}-4, H^{III}-6_b, H^{IV}-6_a H^{IV}-6_b), 3.94 (dd, *J* = 3.2, 2.0 Hz, 1H, H^{III}-3), 3.93 – 3.89 (m, 2H, H-3, H^{III}-5), 3.86 (ddd, *J* = 22.2, 14.2, 7.0 Hz, 2H, H-5, H^{II}-6_a), 3.78 – 3.73 (m, 4H, H-6_a,H^{I}-5, H^{V}-5,H^{V}-4), 3.73 – 3.70 (m, 1H, H-4), 3.69 (dd, *J* = 10.6, 3.4 Hz, 1H, H^{II}-2), 3.67 – 3.62 (m, 3H, H^{V}-6_a, H^{I}-6_a, H^{I}-6_b), 3.62 – 3.59 (m, 1H, H^{II}-6_b), 3.55 (dd, *J* = 10.2, 4.8 Hz, 1H, H-6_b), 3.50 – 3.47 (m, 1H, H^{V}-6_b), 2.84 – 2.80 (m, 2H, CH_2, linker), 2.33 – 2.26 (m, 2H, CH_2, linker), 2.15 – 1.87 (m, 36H, Ac). ^{13}C NMR (201 MHz, CDCl3) δ 47.95, 47.83, 48.21, 47.57, 48.81, 46.78, 47.71, 48.26, 47.93, 48.34, 67.67, 68.42, 69.49, 68.74, 67.29, 71.76, 74.28, 95.93, 98.65, 97.63, 98.09, 73.04, 75.21, 95.39, 73.07, 73.33, 73.00, 75.22, 73.30, 66.94, 66.30, 78.21, 73.90, 66.66, 74.93, 61.46, 70.03, 73.04, 78.16, 74.18, 70.98, 71.22, 61.60, 116.51,

111.45, 106.09, 101.11, 100.60, 100.64, 100.59, 100.68, 100.66, 100.45, 109.71. HR MALDI-TOF MS: m/z: calcd for $C_{126}H_{137}F_{17}O_{43}$ $[M+Na]^+$: 2683.8193; found: 2683.8199.



1

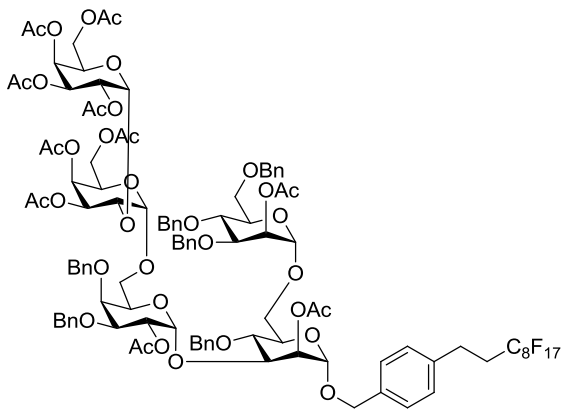
α-D-galactopyranosyl-(1→2)-*O*-α-D-galactopyranosyl)-(1→2)-*O*-α-D-galactopyranosyl)-(1→6)- -D-galactopyranosyl)-(1→3)-*O*-α-D-mannopyranosyl)-(1→6)-α-D-mannopyranoside (1).

Compound **25** (4 mg, 1.9 mol) was dissolve in methanol (3 mL). Fresh prepared NaOMe (0.1 mL, 1 M) was added to the resulting solution. After MALDI-TOF showed the completion of the reaction, the reaction was neutralized by Dowex® 50W X8-200 H^+ resin and the resin was removed by filtration and the filtrate was concentrated *in vacuo*. The resulting white solid was re-dissolved in methanol (3 mL). A catalytic amount of Pd/C and acetic acid (0.1 mL) was added to the reaction mixture subsequently under the atmosphere of hydrogen gas. The reaction was purged by argon gas after MALDI-TOF showed the completion of the reaction. The reaction was concentrated *in vacuo*. The resulting compound was purified by P2 column to obtain **1** (1.3 mg, 64% over two steps); ^{1}H NMR (800 MHz, D_2O) δ 5.31 (s, 1H,H^{III}-6), 5.07 (d, J = 4.0 Hz, 1H, H^{II}-1), 5.05 (s,

1H, H^{IV}-1), 5.01 (d, J = 1.2 Hz, 1H, H^{V}-1), 4.98 (d, J = 3.9 Hz, 1H, H^{VI}-1), 4.75 (d, J = 13.6 Hz, 1H, H^{I}-1), 4.22 – 3.49 (m, 30H); ^{13}C NMR (201 MHz, D_2O) δ 97.89, 94.49, 95.71, 94.28, 95.88, 99.25, 70.25, 69.83, 70.84, 69.53, 67.64, 64.60, 64.93, 70.68, 71.24, 67.86, 69.81, 69.11, 65.00, 72.36, 69.12, 69.03, 60.90, 60.91, 70.45, 60.92, 61.00, 60.99, 60.92, 60.95, 68.15, 72.17, 64.99, 66.65; HR MALDI-TOF MS: m/z: calcd for $C_{36}H_{62}O_{31}$ $[M+Na]^+$: 990.3275; found: 990.3284.

Fluorous supported synthesis of the GPI anchor moiety of *T. brucei.* F-SPE cartridge (FluoroFlash® SPE Cartridges, 10 g, 20 cc tube) was purchased from Fluorous Technologies, Inc. The compound with fluorous tag (200 mg-1 g) was loaded with minimum amount of mixture of water and DMF (9:1). 20% water in methanol and hexane were used as elution solvents. The general order of elution was 20% water in methanol (20 mL) ×3 and then hexane (20 mL) ×3. To obtain desired fluorous-tagged compound, acetone (20 mL) ×3 was employed. The desired compound was analyzed by TCL and MALDI-TOF, and then concentrated *in vacuo.*

For the F-SPE synthesis of **24**, each glycosylation reaction was performed twice and each protecting group removal reaction was performed once. No other purification methods except F-SPE were utilized. All the reaction conditions follow the solution phase synthesis. All the reactions were monitored by MALDI-TOF and TLC analysis.

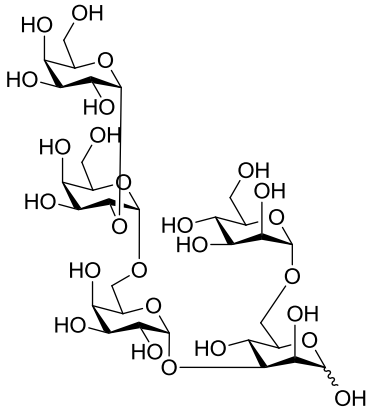


26

4-(1*H*,1*H*,2*H*,2*H*-Perfluorodecyl)-benzyl-*O*-(2,3,4,6-tetra-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl)-(1→2)(3,4,6-tri-*O*-α-D-galactopyranosyl)-(1→2)(1→6)-(3,4-di-benzyl-2-*O*-acetyl-4-*O*-benzyl-6-α-D-galactopyranosyl)-(1→3)(4,5,6-tri-benzyl-2,acetyl-α-D-mannopyranosyl)-(1→6)-2-acetyl-4-benzyl-6-levulinoyl-1-α-D-mannopyranoside (26).

Compound **26** was synthesized followed fluorous tag synthesis procedure of compound **25**. Each glycosylation reaction was performed twice and each protecting group removal reaction was performed once. No other purification methods except F-SPE were utilized. All the reaction conditions follow the solution phase synthesis. All the reactions were monitored by MALDI-TOF and TLC analysis. The resulting reaction crude was purified by LH-20 size exclusion chromatography (DCM: MeOH = 1:1, 0.2 mL/min) to afford compound **26** (6.7 mg, 17.1% for 12 step, 86.3% for each step), R_f = 0.24 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (500 MHz) δ (7.58-7.07, 34H, *H*Ar), 5.51 (dd, J = 10.9, 3.7 Hz, 1H, H^{II}-2), 5.47 (dd, J = 4.0, 3.3 Hz, 4H, H^{IV}-2,3, H^{III}-2,4), 5.37 (dd, J = 6.3, 3.4 Hz, 2H, F-tag CH_2), 5.35 (dd, J = 7.2, 3.8 Hz, 2H, H^{III-4}, H^{V}-2), 5.32 (d, J = 4.8

Hz, 4H, $2CH_2$), 5.24 – 5.17 (m, 3H, H^{III}-1, H^{II}-2, H^{II}-1), 5.13 (dd, J = 11.0, 3.6 Hz, 2H, H^{I}-1, H^{IV}-1), 5.07 – 4.93 (m, 5H, H^{V}-3,4, H^{IV}-4, H^{I}-3,H^{II}-4), 4.91 – 4.84 (m, 4H, $2CH_2$), 4.69 (ddd, J = 20.7, 12.1, 4.2 Hz, 5H, CH_2, H^{II}-3, H^{I}-4, H^{V}-5), 4.61 (d, J = 11.5 Hz, 1H, H^{III}-5), 4.53 (dd, J = 16.9, 11.6 Hz, 2H, H^{III}-6_a), 4.49 – 4.41 (m, 4H, H^{II}-5, H^{V}-6_a), 4.25 (t, J = 6.3 Hz, 1H, H^{IV}-5), 4.19 (dd, J = 9.6, 3.2 Hz, 1H, H^{I}-5), 4.17 – 4.12 (m, 1H, H^{I}-6_a), 4.09 (d, J = 9.8 Hz, 1H, H^{IV}-6_a), 4.08 – 4.03 (m, 1H, H^{II}-6_a), 3.97 (ddd, J = 14.1, 9.5, 6.3 Hz, 1H, H^{I}-6_a), 3.94 – 3.87 (m, 1H, H^{IV}-6_b), 3.82 (dt, J = 11.7, 8.2 Hz,1H, H^{II}-6_b), 3.80 – 3.66 (m, 2H, H^{V}-6_b, H^{III}-6_b), 2.84 – 2.80 (m, 2H, CH_2, linker), 2.33 – 2.26 (m, 2H, CH_2, linker), 2.15 – 1.87 (m, 30H, 10Ac). ^{13}C NMR (126 MHz, CDCl3) δ 129.73, 133.04, 128.34, 128.05, 128.11, 127.21, 127.90, 68.15, 68.52, 66.39, 99.91, 96.74, 72.46, 68.33, 74.68, 97.89, 96.23, 97.60, 74.80, 73.82, 72.10, 72.02, 68.73, 73.43, 74.72, 68.72, 71.61, 66.95, 66.84, 78.79, 61.31, 74.70, 70.22, 76.33, 61.46, 77.71, 74.17, 61.69, 68.26, 65.43, 71.55, 68.38, 65.42, 68.64, 21.05, 17.30, 20.75, 20.75, 20.79, 20.82, 20.69, 29.80. HR MALDI-TOF MS: m/z: calcd for $C_{109}H_{117}F_{17}O_{36}$ $[M+Na]^+$: 2347.6951; found: 2347.6948.



27

α-D-galactopyranosyl-(1→2)-*O*-α-D-galactopyranosyl)-(1→6)-α-D-galactopyranosyl)-(1→3)-*O*-α-D-mannopyranosyl)-(1→6)- α-D-mannopyranoside (27).

Compound **26** (5 mg, 2.1 mol) was dissolve in methanol (3 mL). Freshly made NaOMe (0.1 mL, 1 M) was added to the resulting solution. After MALDI-TOF showed the completion of the reaction, the reaction was neutralized by Dowex® 50W X8-200 H^+ resin and the resin was removed by filtration and the filtrate was concentrated *in vacuo*. The resulting white solid was re-dissolved in methanol (3 mL). A catalytic amount of Pd/C and acetic acid (0.1 mL) was added to the reaction mixture subsequently under the atmosphere of hydrogen gas. The reaction was purged by argon gas after MALDI-TOF showed the completion of the reaction. The reaction was concentrated *in vacuo*. The resulting compound was purified by P2 column to obtain **27** (1.6 mg, 71% over two steps); ^{1}H NMR (500 MHz, D_2O), 5.07 (d, J = 4.0 Hz, 2H, H^{II}-1, H^{IV}-1), 5.01 (d, J = 1.2 Hz, 1H, H^{V}-1), 4.98 (d, J = 3.9 Hz, 1H, H^{III}-1), 4.75 (d, J = 13.6 Hz, 1H, H^{I}-1), 4.22 – 3.49 (m, 25H); ^{13}C NMR (201 MHz, D_2O) δ 101.26, 95.86, 94.27, 94.35, 99.17, 70.19, 69.35, 69.85, 69.48, 69.60, 69.85, 69.20, 69.61, 60.64, 68.92, 63.51, 61.00, 61.01, 61.04, 61.03, 60.98, 61.03, 60.99, 72.51, 60.88; HR MALDI-TOF MS: m/z: calcd for $C_{30}H_{52}O26$ $[M+Na]^+$: 828.7128; found: 828.7147.

References

(1) (a)Cosgrove, D. J. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 850; (b)Huskens, J. *Curr. Opin. Chem. Biol.* **2006**, *10*, 537.

(2) (a)Dube, D. H.; Bertozzi, C. R. *Nature reviews* **2005**, *4*, 477; (b)Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855; (c)Dalziel, M.; Crispin, M.; Scanlan, C. N.; Zitzmann, N.; Dwek, R. A. *Science (New York, N.Y* **2014**, *343*, 1235681; (d)Hudak, J. E.; Bertozzi, C. R. *Chem. Biol.* **2014**, *21*, 16.

(3) Cummings, R. D. *Mol. BioSyst.* **2009**, *5*, 1087.

(4) (a)Hart, G. W.; Copeland, R. J. *Cell* **2010**, *143*, 672; (b)Cummings, R. D.; Pierce, J. M. *Chem. Biol.* **2014**, *21*, 1.

(5) (a)Boltje, T. J.; Buskas, T.; Boons, G. J. *Nat. Chem.* **2009**, *1*, 611; (b)Yasomanee, J. P.; Demchenko, A. V. *Trends Glycosci. Glyc.* **2013**, *25*, 13.

(6) (a)Codee, J. D. C.; Litjens, R.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chemical Society reviews* **2005**, *34*, 769; (b)Kaeothip, S.; Demchenko, A. V. *Carbohydr. Res.* **2011**, *346*, 1371; (c)Yang, L.; Qin, Q.; Ye, X. S. *Asian J. Org. Chem.* **2013**, *2*, 30.

(7) (a)Seeberger, P. H. *Chem Commun* **2003**, 1115; (b)Hsu, C. H.; Hung, S. C.; Wu, C. Y.; Wong, C. H. *Angew. Chem. Int. Ed.* **2011**, *50*, 11872.

(8) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837.

(9) Tang, S. L.; Pohl, N. L. *Org. Lett.* **2015**, *17*, 2642.

(10) (a)Jaipuri, F. A.; Pohl, N. L. *Org Biomol Chem* **2008**, *6*, 2686; (b)Ko, K. S.; Park, G.; Yu, Y.; Pohl, N. L. *Org. Lett.* **2008**, *10*, 5381; (c)Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162; (d)Zhang, F.; Zhang, W.;

Zhang, Y.; Curran, D. P.; Liu, G. *J. Org. Chem.* **2009**, *74*, 2594; (e)Liu, L.; Pohl, N. L. *Org. Lett.* **2011**, *13*, 1824; (f)Tanaka, H.; Tanimoto, Y.; Kawai, T.; Takahashi, T. *Tetrahedron* **2011**, *67*, 10011; (g)Zong, C.; Venot, A.; Dhamale, O.; Boons, G. J. *Org. Lett.* **2013**, *15*, 342; (h)Cai, C.; Dickinson, D. M.; Li, L. Y.; Masuko, S.; Suflita, M.; Schultz, V.; Nelson, S. D.; Bhaskar, U.; Liu, J.; Linhardt, R. J. *Org. Lett.* **2014**, *16*, 2240; (i)Hwang, J.; Yu, H.; Malekan, H.; Sugiarto, G.; Li, Y. H.; Qu, J. Y.; Nguyen, V.; Wu, D. Y.; Chen, X. *Chem Commun* **2014**, *50*, 3159; (j)Macchione, G.; de Paz, J. L.; Nieto, P. M. *Carbohydr. Res.* **2014**, *394*, 17; (k)Roychoudhury, R.; Pohl, N. L. B. *Org. Lett.* **2014**, *16*, 1156.

(11) Demchenko, A. V. *Synlett* **2003**, 1225.

(12) (a)Kim, J. H.; Yang, H.; Park, J.; Boons, G. J. *J. Am. Chem. Soc.* **2005**, *127*, 12090; (b)Park, J.; Boltje, T. J.; Boons, G. J. *Org. Lett.* **2008**, *10*, 4367; (c)Boltje, T. J.; Kim, J. H.; Park, J.; Boons, G. J. *Org. Lett.* **2011**, *13*, 284.

(13) Ferguson, M. A.; Homans, S. W.; Dwek, R. A.; Rademacher, T. W. *Science (New York, N.Y* **1988**, *239*, 753.

(14) Nagamune, K.; Nozaki, T.; Maeda, Y.; Ohishi, K.; Fukuma, T.; Hara, T.; Schwarz, R. T.; Sutterlin, C.; Brun, R.; Riezman, H.; Kinoshita, T. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 10336.

(15) (a)Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95; (b)Khiar, N.; Martinlomas, M. *J. Org. Chem.* **1995**, *60*, 7017; (c)Baeschlin, D. K.; Chaperon, A. R.; Green, L. G.; Hahn, M. G.; Ince, S. J.; Ley, S. V. *Chem. Eur. J.* **2000**, *6*, 172; (d)Ziegler, T.; Dettmann, R.; Duszenko, M. *Carbohydr. Res.* **2000**, *327*, 367; (e)Dettmann, R.; Ziegler, T. *Carbohydr. Res.* **2011**, *346*, 2348.

(16) van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, *17*, 4875.

(17) (a)Gaunt, M. J.; Yu, J. Q.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172; (b)Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169.

(18) (a)Fang, T.; Mo, K. F.; Boons, G. J. *J. Am. Chem. Soc.* **2012**, *134*, 7545; (b)Fascione, M. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem.-Eur. J.* **2012**, *18*, 321.

(19) Fascione, M. A.; Turnbull, W. B. *Beilstein J. Org. Chem.* **2010**, *6*, 19.

(20) Zhu, X. M.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900.

(21) Fascione, M. A.; Adshead, S. J.; Stalford, S. A.; Kilner, C. A.; Leach, A. G.; Turnbull, W. B. *Chem Commun* **2009**, 5841.

(22) Yu, B.; Tao, H. C. *Tetrahedron Lett.* **2001**, *42*, 2405.

(23) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.

(24) Boltje, T. J.; Zhong, W.; Park, J.; Wolfert, M. A.; Chen, W.; Boons, G. J. *J. Am. Chem. Soc.* **2012**, *134*, 14255.

(25) Crich, D.; Vinogradova, O. *J. Org. Chem.* **2007**, *72*, 3581

CHAPTER3:

THE SYNTHESIS OF THE HEXASACCHARIDE UNIT OF *CLOSTRIDIUM DIFFICILE*

Introduction

Clostridium difficile is a gram-positive bacterium that colonizes in the intestinal. It is the leading cause for the nosocomial diarrhea world wide[165]. The unbalance of intestinal flora led to overuse of antibiotics leaves the space for the dramatical growth of *C. difficile* that generates toxins and damages human tissues. The case of *Clostridium difficile* infection (CDI) is estimated over 500,000 per year in the USA, and over 14,000 deaths can be attributed to CDI[166]. The infectious agent of the CDI spread through healthcare facilities is the endospore of *C. difficile* that is notorious for the resistance of commonly used disinfectants[167]. Recently, a new strain named as ribotype 027 appeared with even higher virulence and more toxin generation[168]. The pathogenic strains of the *C.diffcile* produce two major toxins: toxin A and toxin B, which attack the G proteins on the cell surface resulting in cell death and cause severe diarrhea and severe inflammatory immune response resulting in tissue damage[169].

C. difficile is spread through oral-fecal route which can only be prevented by extremely strict antibiotic prescription policies. However, based on current hospital condition, such practice is almost impossible to maintain. Moreover, most of the

commonly used antibiotics are useless in the treatment for the CDIs since the drug resistance of the bacteria. The only effective antibiotics are vancomycin and metronidazole, and the failure rate is only 14.2% and 22.4% respectively[170]. The vaccine as another therapeutic method avoids the weakness of antibiotics. It neutralizes toxins and protects the human from severe C. difficile invasion[171-173]. However, vaccines aiming for neutralizing toxins cannot stop the colonization of the bacteria. In fact, the recurrence of CDIs is very common and affects about 30% people after recovery[174,175]. In certain cases, radical treatments such as resection surgery are becoming routine for the severe infection cases.

New treatment methods are still under research such as toxin antagonists, species-specific antibiotics and defined fecal transplantation. However, none of these treatments solve the problems of CDI colonization or transmission neither. So it is necessary to develop a solid and long effective method to control CDIs.

Glycans that are presented on the cell surface are a perfect target for bacteria treatment because of the significant role in bacteria-cell interactions[176,177]. Coutless carbohydrate based vaccines have been developed to against all kinds of pathogens. In the case of *C. difficile*, three major polysaccharides are found on the surface of *C. difficile*. However, polysaccharide II (PS II) is the only one present in all virulent strains, which suggests it is a potential target for the vaccine development that can introduce immunity to all strains. PS-II is a hexasaccharide repeating units that are linked through phosphate linkage (Figure 1).

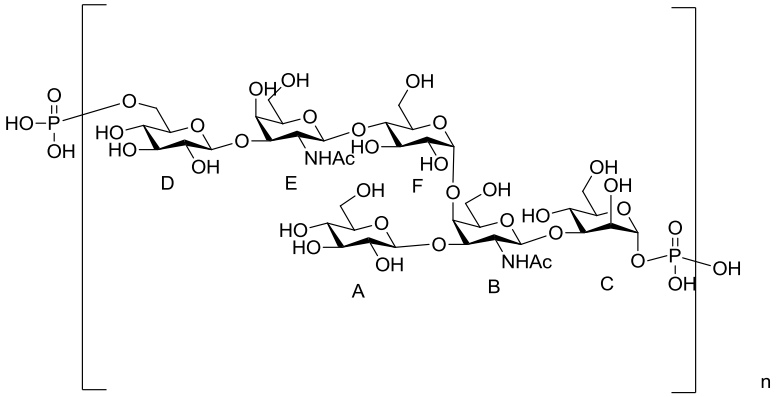


Figure 3.6 Structure of PS-II of *C. difficile*

In this project, we designed a synthetic vaccine that could not only neutralize the toxin generated by *C. difficile* but stop the colonization of the target bacteria. The first part of this project will be an exploration of a properly synthetic route for the hexasaccharide. In the meantime, two long-term orthogonal protecting groups will be installed. Because, in nature, the hexasaccharide forms repeating unit through phosphate linkages. In the second stage, the hexasaccharide can be coupled to each other through phosphate chemistry and the installed orthogonal protecting groups in a controllable manner. By changing the number of the repeating unit, the antigenicity will be evaluated. In the final step, the hexasaccharide and its repeating unit will conjugate to a carrier protein. In this case, we will choose a piece of the toxin protein from *C.difficile*. Finally, after conjugation with oligosaccharide antigens, a vaccine targeting on both bacteria and toxin will be obtained. In this chapter, the synthesis of PS-II hexasaccharide will be discussed.

The synthesis of oligosaccharide has been exploited for decades. However, such synthesis has never been an easy task because of the control of the newly generated stereocenter and the harsh reaction condition of glycosylation. For the structure of the repeating unit of *C.difficile* PS-II, the reducing end is the mannose **C** which is linked to a galactose amide **B** through β linkage. The galactose amide **B** then is linked with two glucose **A** and **F** at C-3 and C-4 position respectively. Glucose **F** is attached by a disaccharide chain made by galactose amide **E** and glucose **D** through β linkage. There are two phosphate groups are presented in the hexasaccharide repeating unit where one phosphate group locates at C-1 of the mannose **A** and the other at C-6 of the glucose **D**.

Among all the glycosylic linkage, the linkage between glucose **F** and galactose amide **B** is expected to be the most challenging one. Firstly, the C-4 hydroxyl group of the galactose amide **B** is an axial hydroxyl group which has limited reactivity and is hard to couple to. Moreover, the glycosylic linkage between **F** and **B** is the α linkage which is hard to control the outcome. In this case, the stereocontrol of the linkage largely depends on the anomeric effect which is normally not strong enough to generate an α exclusive product. Moreover, in most of the cases, the separation of the β isomer is very painful, and the yield of glycosylation is compromised. Two groups have reported the synthesis of *C.difficile* PS-II[178,179]. However, neither of them considered the installation of anomeric phosphate at mannose **C** and one paper even didn't consider the terminal phosphate at the glucose **D** and missing such functionality had been approved largely reduceing its antigenicity[180].

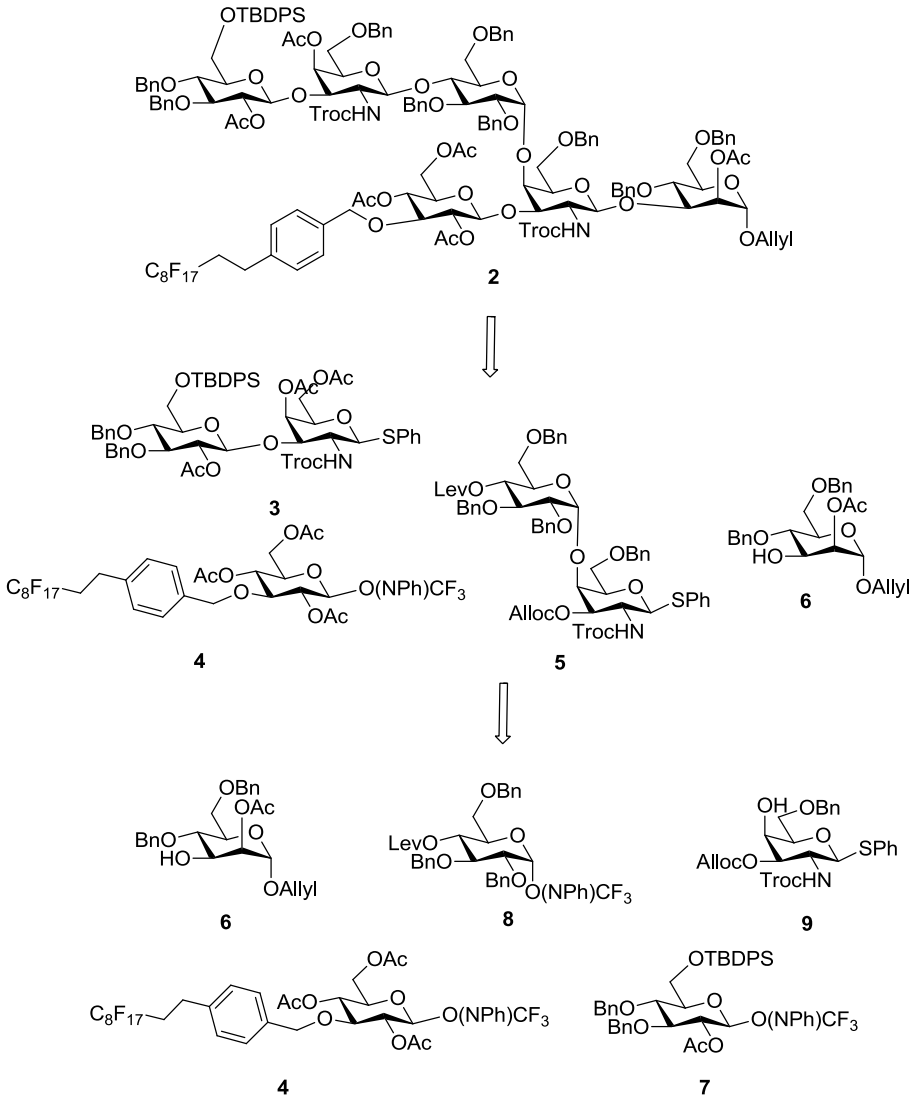


Figure 3.7 the first attempt of synthesis of PS-II

Recently, several reported have been established on fluorous tag-assisted glycosylation which accelerates the separation of glycosylation products[112]. Fluorous tags are functionalized perfluoroalkyl groups attached to substrates through covalent linkage. It is inert to most of the reaction conditions and barely affects the reaction results[181]. Since fluorine has a unique feature that it selectively and strongly interacts with the other fluorine element such as fluorous silica gel or solvent. In such way, it could fish out the

tagged molecule substrates from reaction mixture easily. For example, liquid-liquid extraction, silica gel-based fluorous solid-phase extraction, and high-performance liquid chromatography have been used for the separation of fluorous tagged molecules in recent years[112]. Compared with the conventional silica gel chromatography, the separation process is significantly simplified. On the other hand, compared with traditional solid phase synthesis, the reaction efficiency is largely improved because the fluorous tag can dissolve in the organic solvents and homogeneous phase reaction is more kinetically favored. In this project, we were trying to use C_8F_{17} as middle sized fluorous tag and fluorous silica cartridge as the mean of separation.

Result and discussion

In the very beginning, we designed a reasonable synthetic route based on the structure features above and previous literature reports[60,179]. The most complex glycosylic linkage will be established at the very first place. In such case, building block **8** and **9** will be coupled initially. Ether will be employed as a co-solvent with DCM to improve the stereoselectivity in this glycosylation. In the reaction, ether will interreact with oxocarbenium ion and occupy the β face of the anomeric center in such way the hydroxyl group from the acceptor can only attack from the α face. Moreover, the N-phenyl trifluoroacetamide will be employed as the leaving group based on our experience. With the building block **5** in hand, the next step is the removal of Alloc group selectively by palladium tetrakis and the building block **4** bearing a fluorous tag will be installed to form a trisaccharide product.

The fluorous tag was planned to install in the building block **4**. Moreover, since it is a benzyl type fluorous tag, it would donate electrons to increase the reactivity of the donor. After coupling with building block **5**, the trisaccharide will either couple with building block **6** through thiophenyl mediated glycosylation or act as an imidate glycosylation to obtain tetrasaccharide. After the selective removal of Lev group, the disaccharide **3** would be linked the acceptor to form hexasaccharide **2.** At last, the TBDPS and Allyl groups are selected as the long-lasting protecting groups which could be selectively removed at very final stage to install phosphate functionalities.

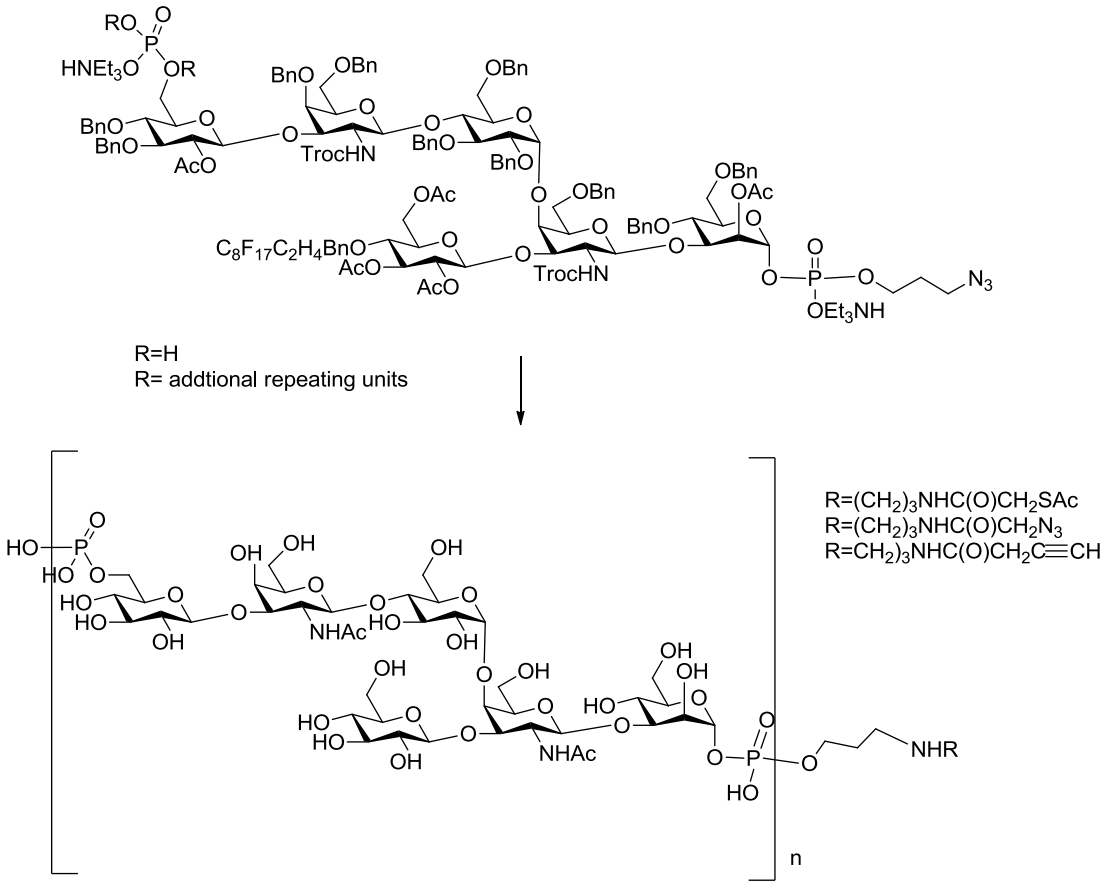


Figure 3.8 the final target and globe deprotection

With the hexasaccharide in hand, the Troc group will be transformed into acetyl under the Cu and Zn condition in first place. Then the allyl group at the anomeric center of the mannose **C** could be selectively removed by using palladium catalyst and acids. Moreover, a linker bearing azide group will be installed, and the phosphate coupling methods are planned to use either hydrogen phosphonate or phosphite trimester method[182,183]. At last, the TBDPS group of glucose **D** will be selectively removed by fluorine anion. The obtained hydroxyl group will be coupled with either a phosphate functionality or another hexasaccharide repeating unit through phosphate linkage.

A globe deprotection will perform to remove Bn and Ac. Moreover, the azide on the linker will be converted to an amine which can be functionalized with a chemical handle to couple with a carrier protein. The chemical handle has an azide or an alkyne group which clicks with a modified carrier protein to form site specific glycosylated protein.

The synthesis of C. difficile PS-II started with the block **5**. However, the very first glycosylation was hindered bt aglycon transfer. During the reaction, the thiophenyl group moved from acceptor **9** to the donor **8** generating thiophenyl modified donor in 92% yield. Such phenomenon happened due to two reasons. Firstly, the reactivity of the hydroxyl group is too small, instead reacted with a hydroxyl group, the donor attack the sulfur for high nucleophilicity. On the other hand, the electron donating group on the acceptor is too strong (arm and disarm effect) which increased the reactivity of the thiophenyl group. In our case, the aglycon transfer happened due to the C-4 hydroxyl group of acceptor **9** was an axil. Moreover, Bn group was an electron-donating group,

and the nitrogen atom of the galactose amine was less electron withdrawing than an oxygen atom.

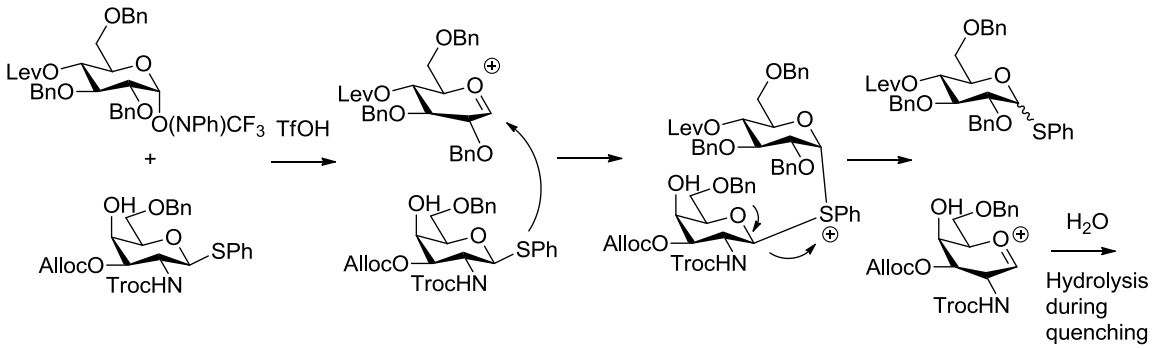
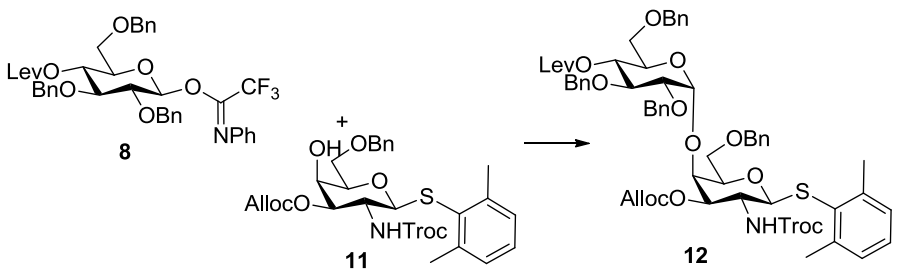


Figure 3.9 The proposed mechanism of aglycon transfer

To circumvent the phenomenon, a bulky type of thiophenyl group was employed to reduce the reactivity of sulfur atom by space hinderance. 2-6 dimethyl benzenethiol (DMBT) was a thiophenyl group with two additional methyl groups on the benzene ring which could generate stereo hindrance preventing the approaching of oxocarbenium ion[184]. Interestingly, after the installation of DMBT group, the molecule started to self-assemble to gel, especially when the C6 and C4 hydroxyl groups were occupied with the benzylidene protecting group. The gel barely dissolved in DCM and it was impossible to purify by silica gel chromatography. The unpurified product gel was used directly in the following protecting group manipulation reactions (2 steps). As we expected, the desired building block **11** was obtained in a low yield. A series of glycosylations were performed to identify the best reaction condition. During in the investigation, the traditional ether cannot conduct the desired α stereoselectivity.



	Solvent	**Catalyst**	**Time**	**Yield**	**Selectivity**
1	Et2O: DCM=1:2	TfOH	4min	10%	α:β = 3:1
2	Et2O: DCM=1:2	TfOH	4h	67%	α:β = 3:1
3	Et2O	TMSOTf	4h	61%	α:β = 3:1
4	Toluene	TfOH	4h	41%	α:β = 1:1
5	Thiophene: Toluene=1:2	TfOH	4h	52%	α:β = 15:1
6	Thiophene: Toluene=1:2	TBSOTf	4h	63%	α:β = 25:1

Table 3.1 reaction conditions for synthesis of building block **12**

In an aim to address the issue of stereoselectivity control, thiophene was employed as a co-solvent in the glycosylation[110]. Compared with ether, the sulfur in thiophene was much more nucleophilic than oxygen in TFH. Moreover, thiophene could

stabilize the oxocarbenium ion at the β position of the glycosylic donor in a more stable manner which further improved the α selectivity. Luckily, the disaccharide **12** was obtained in 63% yield and the α isomer as the major product.

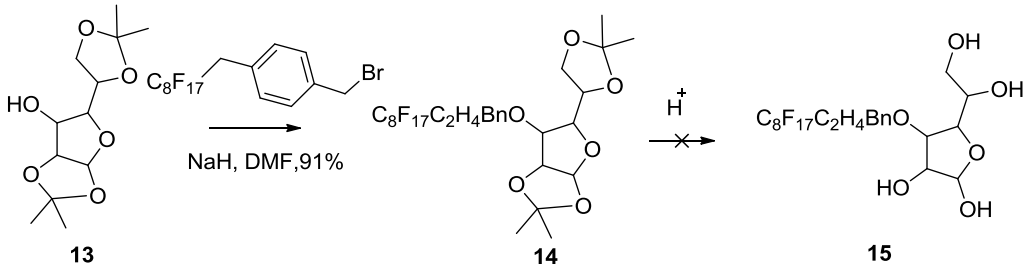


Figure 3.10 The synthesis of building block **15**

With the disaccharide **12** in hand, we moved to the next step which is synthesis building block **4** bearing a fluorous tag. However, the synthesis of the compound **4** is much more complicated than our expectation. The installation of the fluorous tag to the commercially available compound **13** is a convenient way to tag a carbohydrate substrate and the building block **14** can be obtained in 91% yield. However, when we were trying to unprotect the substrate **14**, surprisingly, only small portion of isopropylidene groups can be deprotected. Different acids with various concentrations (TFA, sulfonic acid, strong acidic resin, acetic acid; 1M to 10M) were tested and the NMR study was shown that the isopropylidene protecting C-2 and C-1 is the one can not be removed. Further increasing the amount of the acid resulted in the cleavage of the fluorous tag. Among the various conditions, the best yield of the desired compound **15** was only 24% under 50% TFA/MeOH. With compound **15** in hand, it was further transformed to donor **4**.

However, the glycosylation of donor **4** with acceptor **12** did not work well as we expected. MALDI-TOF mass spectrometry only detected a small amount of the product, most of the reaction crude were the hydrolyzed donor and the unreacted acceptor. The low yield of making both building block **4** and disaccharide **12** made us think about a re-design another synthetic route.

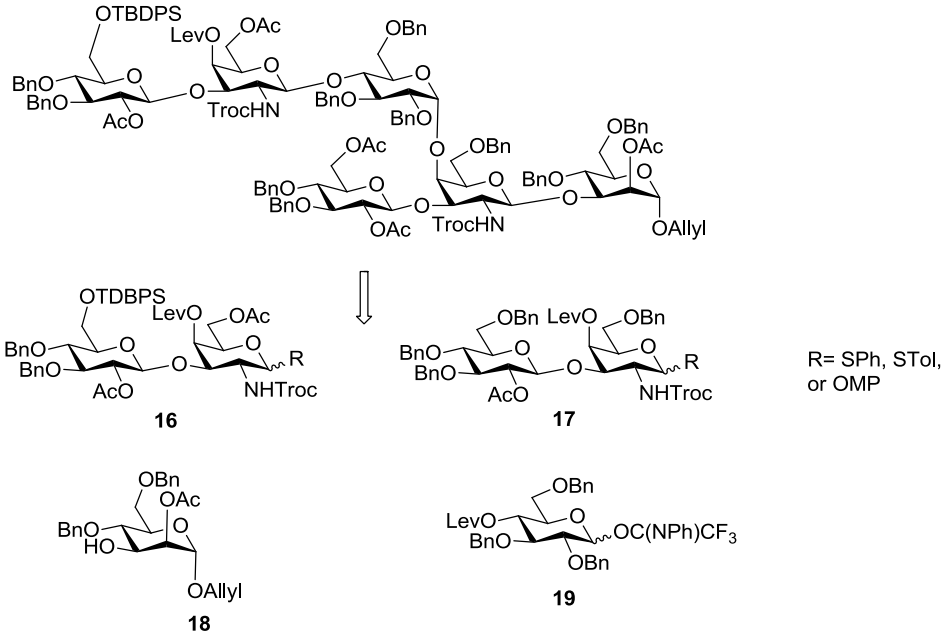


Figure 3.11 the investigation of galactose amine protecting groups for PS-II synthesis

In the new synthetic plan, we firstly decided to remove the feature of the fluorous tag and set exploring a solid synthetic route as the priority. Instead of synthesizing building block **12**, we decided to synthesize building block **16** in the first place as the literature reported[178]. In this synthetic route, the block **16** and **17** will share the same galactose amine building block making the synthesis more efficient. Then, building block **17** would transform to the trifluoro imidate donor and couple to building block **18** to

obtain the desired trisaccharide. Moreover, the Lev group will selectively be removed under hydrazine acetate condition. The building block **19** would attach to the trisaccharide forming tetrasaccharide as reported[178,179]. Lev group would be the orthogonal protecting group again to reveal the hydroxyl group and then it would be coupled with the donor derivated from building block **16** to form the desired hexasaccharide.

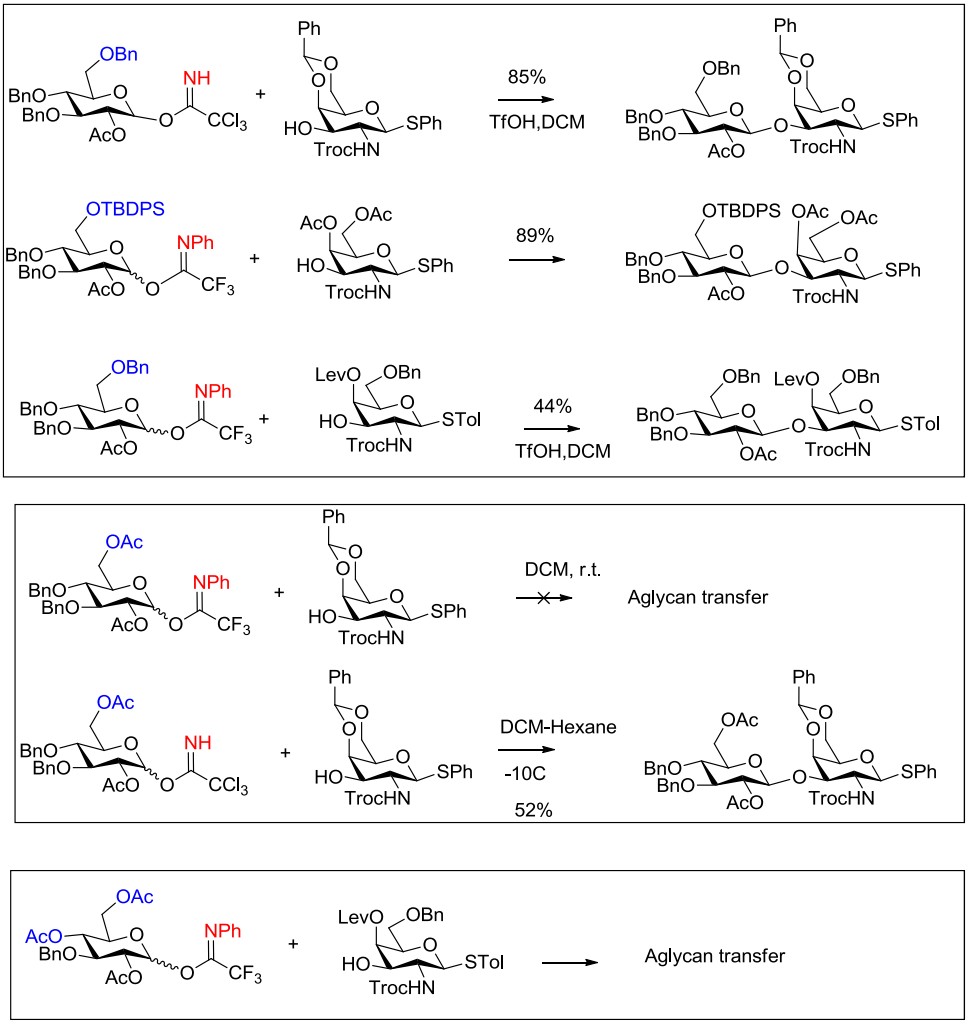


Figure 3.12 Synthesis of disaccharides from sulfur-based anomeric protecting groups

During the first attempt to synthesize building block **16** and **17**, we choose R as the SPh or STol and R' as the Troc for the reaction condition screening. The reaction that we chose SPh or STol as the anomeric protection group just because of the readily available starting material. As we expected, the unpleasant aglycon transfer could be suppressed initially when we installed the electron withdrawing protecting groups. However, the desired results were not repeatable if minor changes were made, for example, switch between SPh or STol, and trichloro imidate or trifluoro imidate. Based on such phenomenon, we then move to use OMP instead of sulfur based anomeric protecting groups. The disaccharide can be obtained very easily. However, the removal of the OMP did not generate the desired compound.

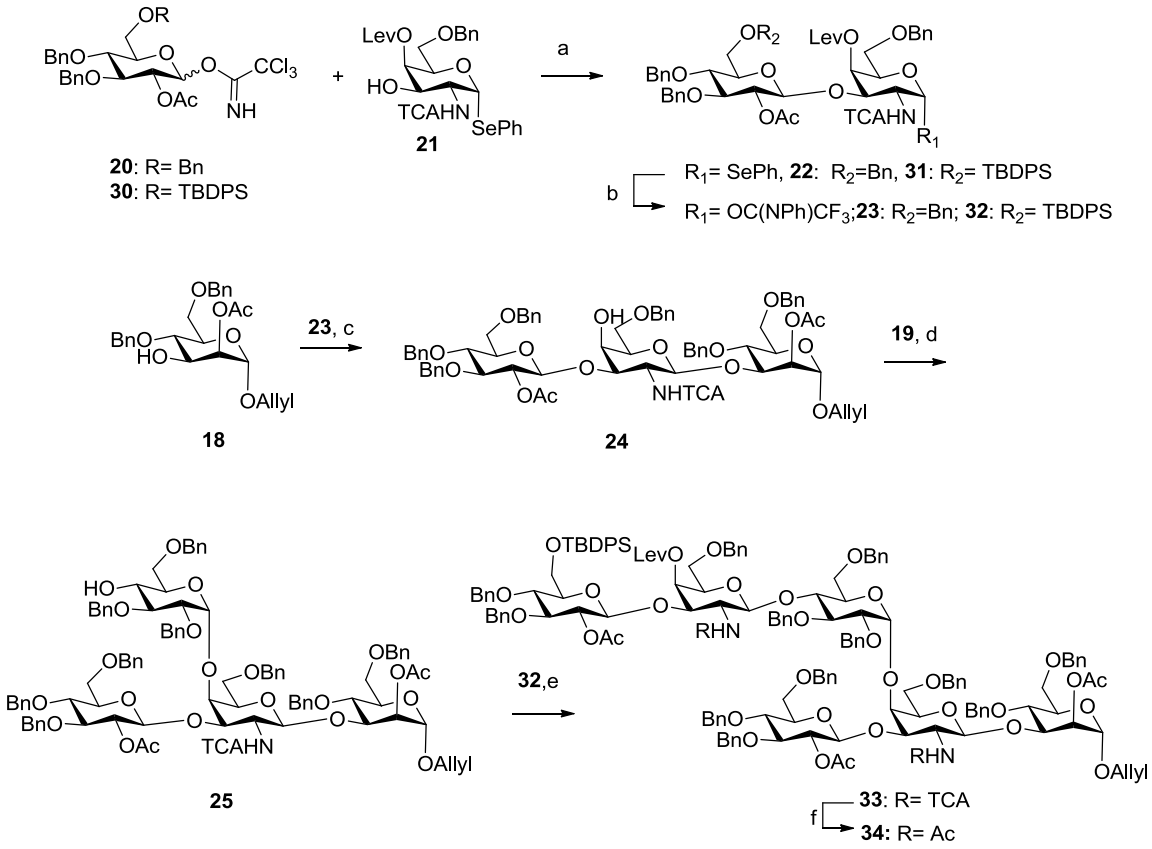


Figure 3.13 The assembly of the hexasaccharide from the PS-II of *C. difficile*

Reagents and conditions: a) TMSOTf, DCM, -10 ℃ to rt, 1 h, 22: 76%, 31: 91%; b) NIS, TFA, rt, 1h, then, 2,2,2-trifluoro-*N*-phenyl-acetimidoyl chloride, Cs_2CO_3, DCM, rt, 1 h,; c) TMSOTf, DCM, -10 ℃ to rt, 1.5 h, then, NH_2NH_2-AcOH, AllyOH, DCM, rt, 18 h, 74%; d) TMSOTf, DCM, -10 ℃ to rt, 3 h, then, NH_2NH_2-AcOH, AllyOH, DCM, rt, 18 h, 91%; e) TMSOTf, DCM, -10 ℃ to rt, 3 h, 87%; f) Cs_2CO_3, DMF, 75 ℃, 1.5 h, 67%.

Based on all trials and errors, we decided to use SePh as the anomeric protecting group which has been used quite often in galactose amine involved chondroitin sulfate synthesis[185]. Moreover, we also switched the traditional Troc protecting group to TCA group which had better stability under all chemical conditions and easier NMR

identification. The assembly of the hexasaccharide started with galactose amine derivative **21**. It coupled with either glucose **20** or glucose **30** which had a TBDPS group at C-6 under TMSOTf condition. Then, the disaccharide with SePh was tested for NIS/ TfOH catalyzed glycosylation with **18**. However, the MALDI-TOF showed this condition compromised the allyl group. So the disaccharide with SePh was then hydrolyzed and transformed to trifluorous imidate functionality. With the help of imidate mediated glycosylation, the trisaccharide was obtained in 74% yield. Moreover, the Lev orthogonal protecting group was selectively removed under hydrazine condition without further purification. Interestingly, the ally group was reduced to propyl group based on MALDI-TOF. As the literature reported[186], the problem is due to space hindrance around the Lev chemical environment, so instead of attacking Lev, hydrazine attacks allyl and performs the reduction reaction. This problem could be easily circumvented by adding additional ally alcohol as the scavenger reagent. With the trisaccharide **24** in hands, the donor **19** was coupled with the acceptor and followed by removal of Lev. In this glycosylation, ether was used to ensure the α selectivity and no β isomer was observed in the NMR study. The final hexasaccharide was accomplished by the glycosylation between donor **32** and acceptor **25** and it gave 87% yield after purification.

The next step was the removal of the TCA group which was notorious for resistance to all kind of chemical conditions. In this case, both reductive method mediated by AIBN and tributyltin hydrate[178], and base removal method mediated by NaOH or KOH did not work. Luckily, Cs_2CO_3 mediated transformation of TCA protected amine into an amine has been reported in the literature. The mechanism of this reaction is the TCA firstly lost a proton and rearranged to isocyanate. The isocyanate

reacted with water and then hydrolyzed to the desired amine product[187]. However, in the literature, the carbohydrate substrates always resulted in low yield due to the lost of ester type of protecting groups. The initial attempt to remove TCA by Cs_2CO_3 in DMF was carried out at 95 ℃ and overnight as literature reported. As we expected, the TCA was removed but the decomposition occurred and compromised the yield. After cooling the temperature to 75 ℃ and reducing the reaction time to 1 h, we found out that the TCA could be removed without interfering the carbohydrate backbone and protecting groups. The crude di-amine product was reacted with acetyl anhydride to obtain the desired compound **34**.

Conclusion

In this chapter, five possible synthetic routes to obtain hexasaccharide of PS-II from *C. difficile* was evaluated. Moreover, a reliable synthetic route to synthesize the hexasaccharide was established. The combination of Lev and Allyl orthogonal protecting groups was proved to be possible, but careful adjusting of chemical condition was necessary. A new method to remove TCA protecting group was established which could be potential for not only the PS-II synthesis but chondroitin sulfate and other amine containing complex carbohydrate synthesis. With the Allyl and TBDPS as the orthogonal protection groups, the hexasaccharide has a potential to be a repeating unit through phosphate chemistry. After the conjugation to the carrier protein, its antigenicity would be explored.

Experiment data

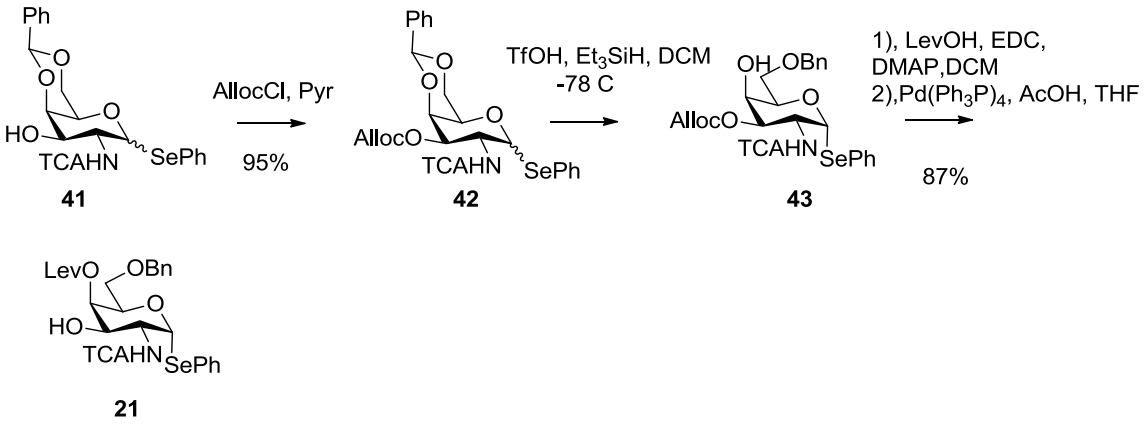
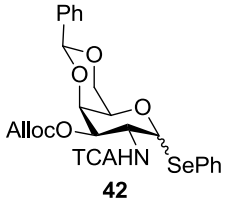


Figure 3.14, Synthesis of building block **21**

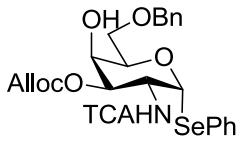
Phenyl 4, 6-O-benzyliden-3-O-allyloxycarbonyl-2-deoxy-2-trichloroacetamido-1-seleno-α/β-D-galactopyranoside (42)



Compound **41**[185] (5.00 g, 9.06 mmol) was dissolved in DCM (50 mL) and the resulting solution was cooled to -0 °C. Tetramethylethylenediamine (1.58 g, 13.59 mmol) and allyl chloroformate (1.20 g, 9.97 mmol) were added. The reaction mixture was stirred for 1 h and then quenched with water (10 mL). The organic phase was washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 40%, v/v) to give compound **42** (5.20 g, 95%). R_f

= 0.41 (EtOAc/Hexane, 40%, v/v); ^{1}H NMR (500 MHz,) δ 7.80-6.99 (m, 10H, Ar), 6.18-6.17 (d, 1H, H-1), 5.93-5.87 (m, 1H, C*H*, Allyl), 5.63 (s, 1H, CH), 5.38-5.27 (m, 2H, CH_2, Allyl), 5.03-4.95 (m, 2H, H-2, H-3), 4.65-4.64 (m, 2H, CH_2, Allyl), 4.49 (d, 1H, H-4), 4.32-4.29 (dd, 1H, H-6a), 4.19-4.12 (m, 2H, H6b, H-5), 3.98-3.88 (d, 1H, NH,); ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.50, 133.76, 126.26, 128.68, 128.66, 128.72, 88.11, 100.94, 100.62, 73.50, 51.43, 69.47, 73.26, 72.67, 68.88, 69.32, 65.77, 69.23, 68.20; HR MALDI-TOF MS: m/z: calcd for $C_{25}H_{24}Cl_3NO_7Se$ $[M+Na]^+$: 634.9783; found: 634.9787.

6-O-Benzyl-4-O-hydroxyl-3-O-allyloxycarbonyl-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside (43)

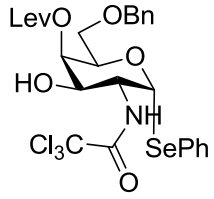


43

Compound **42** (5.20 g, 8.18 mmol) was dissolved in DCM (50 mL) and the resulting solution was cooled to -78 °C. Triethylsilane (2.58 g, 25.54 mmol) and TfOH (1.84 g, 12.27 mmol) were added. The reaction mixture was stirred for 1 h and then quenched with Et_3N/MeOH (10 mL 1/1). The mixture was then washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 40%, v/v) to give compound **42** (4.41 g, 95%). R_f = 0.45 (EtOAc/Hexane, 40%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) 1H NMR (500 MHz,) δ

7.57-7.22 (m, 10H, Ar), 6.07-6.06 (d, 1H, H-1), 5.39-5.28 (dd 2H, allyl), 4.91-4.85 (m, 2H, H-2, H-3), 4.66-4.54 (m, 4H, $2CH_2$, Bn, Allyl), 4.43-4.33 (m, 2H, H-4, H-5), 3.84-3.76 (m, 2H, H-6ab), 3.00 (s, 1H, OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.24, 129.29, 127.85, 130.07, 129.17, 87.69, 130.56, 119.59, 119.62, 119.62, 119.63, 51.47, 75.24, 51.42, 75.24, 51.41, 69.36, 73.70, 73.70, 73.69, 73.69, 71.87, 67.81, 69.22, 69.22; HR MALDI-TOF MS: m/z: calcd for $C_{25}H_{26}Cl_3NO_7Se$ $[M+Na]^+$: 636.9940; found: 636.9943.

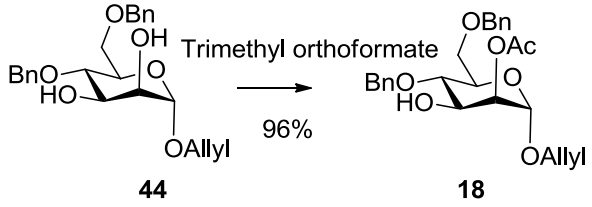
6-O-Benzyl-4-O-levulinic-3-O-hydroxyl-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside (21)



21

Compound **43** (4.40 g, 6.90 mmol) was dissolved in DCM (50 mL) and the resulting solution was cooled to 0 °C. Levulinic acid (1.20 g, 10.35 mmol), DMAP (0.17 g, 1.38 mmol) and EDC (2.65 g, 13.80 mmol) were added. The mixture was stirred for 3 h and then washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting reaction crude was re-dissolved in THF (20 ml) and palladium tetrakis (10 mg) was added to the reaction mixture and stirred for additional 1 h. Then, the reaction solution was washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was

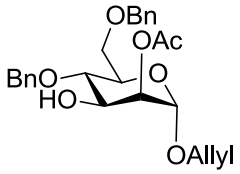
concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 50%, v/v) to give compound **42** (3.6 g, 80%). R_f = 0.41 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1H NMR (500 MHz,) δ 7.58-7.20 (m, 10H, Ar), 6.10-6.09 (d, 1H, H-1), 5.54-5.53 (dd, 1H, H-4), 4.61-4.33 (m, 4H, CH2, H-5, H-2), 3.91-3.79 (m, 1H, H-3) 3.61-3.59 (m, 2H, H-6ab), 3.15-3.14 (d, 1H, OH), 2.84-2.52 (m, 4H, CH_2CH_2, Lev) 2.20 (s, 3H, CH3, Lev). ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.49, 134.47, 129.30, 127.99, 130.25, 127.82, 128.00, 128.88, 129.08, 129.24, 129.38, 87.93, 88.55, 70.43, 73.91, 71.45, 73.73, 73.48, 73.49, 72.21, 53.88, 67.47, 69.66, 69.57, 68.27, 38.48, 28.16, 28.20, 28.20, 29.78, 21.10; HR MALDI-TOF MS: m/z: calcd for $C_{26}H_{28}Cl_3NO_7Se$ $[M+Na]^+$: 651.0096; found: 651.0091.



44 **18**

Figure 3.15, Synthesis of compound **18**

Allyl-*O*-(4,6-*O*-di-benzyl-2,3-hydroxyl)-1-α-D-mannopyranoside (18)



18

Compound **44** [Zegelaar-Jaarsveld, 1996 #149] (2.0 g, 5.00 mmol) was dissolved in DCM (20 mL) and the resulting solution was cooled to 0 °C. Trimethyl orthoformate (3.00 ml, 27.45 mmol)was added. The reaction mixture was stirred for 1 h and then concentrated *in vacuo*. The reaction crude was then re-dissolved in THF (10 ml) and 37% HCl (0.2 ml) was added to the solution and the reaction mixture was stirred for additional 30 min. At the last, the reaction mixture was washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 30%, v/v) to give compound **18** (2.0 g, 95%). R_f = 0.51 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.22 (m, 10H, Ar), 5.90-5.83 (m, 1H, Allyl,CH), 5.29-5.11 (m, 3H, Allyl-CH_2, H-2), 4.90 (s, 1H, H-1), 4.78-4.52 (m, 4H, 2×CH_2), 4.18-3.97 (m, 3H, Allyl-CH_2, H-3), 3.84-3.71 (m, 4H, H-6ab,H-4, H-5) 2.46 (s, 1H, OH), 2.15-2.14 (s, 3H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 127.99, 128.27, 128.09, 127.95, 127.29, 113.60, 110.18, 133.40, 117.66, 117.66, 117.65, 117.66, 72.65, 96.65, 96.78, 74.96, 74.95, 73.59, 73.57, 74.97, 74.29, 73.55, 67.60, 70.68, 67.89, 68.20, 68.83, 75.91, 68.54, 71.14, 75.97, 68.80, 68.81, 21.14; HR MALDI-TOF MS: m/z: calcd for $C_{25}H_{30}O_7$ $[M+Na]^+$: 422.1991; found: 422.1987.

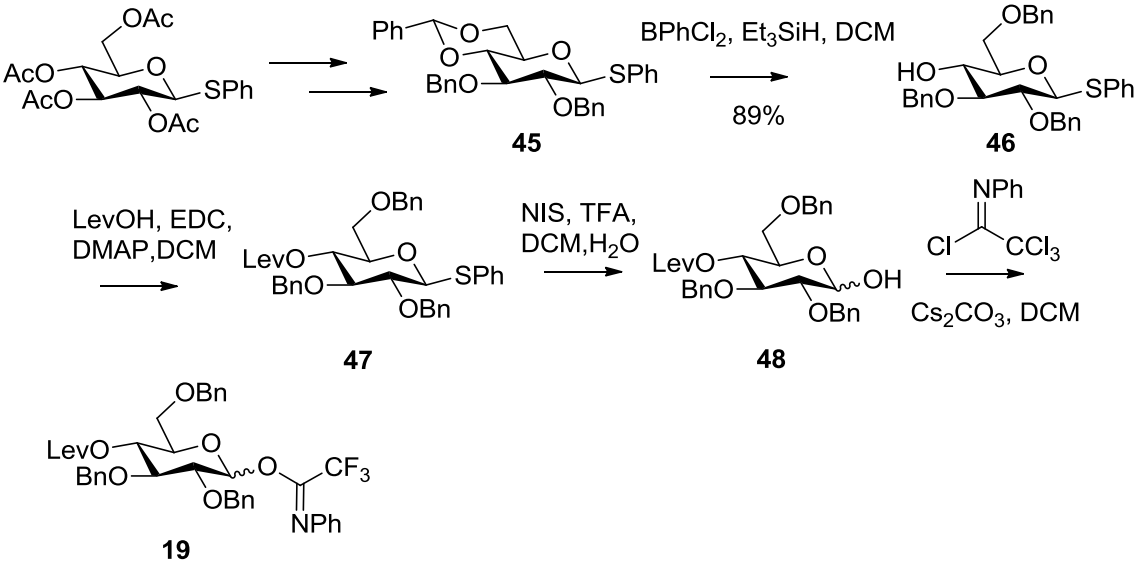
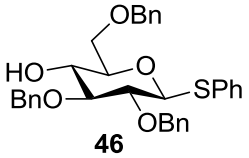


Figure 3.16 Synthesis of compound **19**

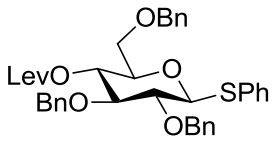
Thiophenyl-*S*-(2,3,6-*O*-tri-benzyl)-1-β-D-glucopyranoside (46)



Compound **45** (1.00 g, 1.85 mmol) was dissolved in DCM (40 mL) and the resulting solution was cooled to -78 °C. Triflic acid (170 µl, 0.92 mmol), Et_3SiH (0.89 ml, 5.55 mmol) were added. The reaction mixture was stirred for 3 h and then quenched with Et_3N/MeOH (10 mL 1/1). The mixture was then washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 40%, v/v) to give compound **46** (0.8 g, 80%). R_f = 0.37

(EtOAc/Hexane, 50%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62-7.26 (m, 15H, Ar), 4.97-4.58 (m, 7H, H-1, 3×CH_2), 3.85-3.77 (m, 2H, H-6ab), 3.70-3.51 (m, 4H, H-2, H-3, H-4, H-5), 2.67 (s, 1H, OH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 131.84, 128.25, 128.10, 127.91, 128.28, -9.62, 75.46, 185.78, -9.62, 75.47, 75.49, 75.50, -9.62, 75.39, -9.62, 75.40, 87.70, 87.70, 73.46, 73.50, 71.54, 82.06, 81.96, 70.43, 70.69, 71.47, 71.87, 71.75, 71.96, 71.98, 86.17, 86.16, 86.14, 79.97, 21.08; HR MALDI-TOF MS: m/z: calcd for $C_{33}H_{34}O_7S$ $[M+Na]^+$: 542.2126; found: 542.2128.

2,3,6-*O*-Benzyl-4-O-levulinic-1-thiolphenyl-β-D-galactopyranoside (47)

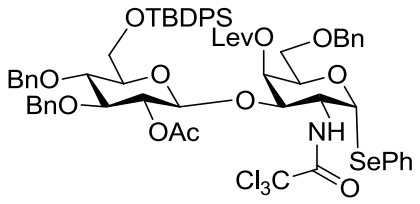


47

Compound **46** (1.00 g, 1.85 mmol) was dissolved in DCM (30 mL) and the resulting solution was cooled to 0 °C. Levulinic acid (0.43 g, 3.70 mmol), DMAP (23.2 mg, 0.19 mmol) and EDC (0.57 g, 3.70 mmol) were added. The reaction mixture was stirred for 3 h. The mixture was then washed with aq. saturated $NaHCO_3$ (20 mL), brine (20 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified by flash chromatography over silica gel (EtOAc/ Hexane, 0% to 40%, v/v) to give compound **47** (0.8 g, 67 %). R_f = 0.41 (EtOAc/Hexane, 50%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59-7.22 (m, 15H, Ar), 5.06-5.02 (m, 1H, H-4), 4.90-4.51 (m, 7H, 3×CH_2, H-1), 3.72-3.54 (m, 5H, H-2, H-3, H-5, H-6ab), 2.65-2.26 (m, 4H,

Lev), 2.14 (s, 3H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) 128.31, 131.82, 128.29, 128.04, 127.98, 127.76, 128.29, 71.03, 75.50, 75.50, 75.39, 75.40, 75.44, 87.55, 75.33, 87.56, 75.32, 75.38, 73.61, 73.46, 73.54, 83.97, 83.97, 83.97, 69.71, 77.56, 80.77, 80.71, 80.72, 69.31, 37.74, 37.75, 37.72, 37.79, 37.82, 27.89, 27.94, 29.81; HR MALDI-TOF MS: m/z: calcd for $C_{38}H_{40}O_7S$ $[M+Na]^+$: 640.2494; found: 640.2493.

3,4,6-tri-benzyl-2-*O*-acetyl-4-*O*-β-D-galactopyranosyl-(1→3)-6-*O*-Benzyl-4-*O*-levulinic-2-deoxy-2-trichloroacetamido-1-seleno-α-D-galactopyranoside (22)

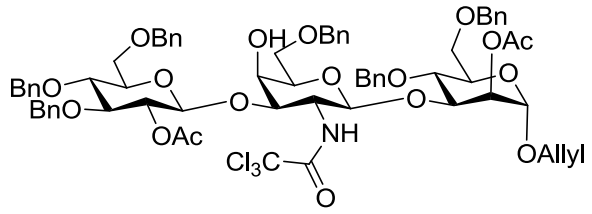


22

Compound **30** (1.99 g, 2.53 mmol) and **21** (1.50 g, 2.30 mmol) were dissolved in DCM (30 mL) and then flame dried molecular sieve (4Å) was added. The mixture was placed under an atmosphere of argon at room temperature, followed by cooling to -15 °C. TMSOTf (42 μL, 0.23 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature over a period of 1 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (10 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting white solid was purified silica gel chromatography (EtOAc/ Hexane, 0% to 50%, v/v) to afford compound **31** (2.25 g, 98%). R_f = 0.21 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz,

$CDCl_3$) δ 7.71-6.89 (m, 31H, Ar, N*H*), 5.86-5.85 (d, 1H, H^I-1), 5.58-5.57 (d, 1H, H^I-4), 5.19-5.18 (d, 1H, H^{II}-1), 4.94-4.91 (dd, 1H, H^{II}-2), 4.84-4.82 (d, 1H, C*H*CH), 4.73-4.50 (m, 6H, H^I-2, CHC*H*, $2CH_2$), 4.42-4.40 (t, 1H, H^I-5), 3.97-3.84 (m, 3H, H^{II}-3, H^{II}-6_{ab}), 3.76-3.68 (m, 3H, H^I-3, H^{II}-4, H^{II}-5), 3.64-3.56 (m, 2H, H^I-6_{ab}), 2.85-2.00 (m, 4H, Lev, CH_2CH_2), 1.61 (s, 3H, Lev or Ac), 1.09 (s, 3H, Lev or Ac), 1.08 (s, 9H, TBDPS); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.79, 135.60, 134.31, 129.57, 128.02, 128.01, 129.15, 127.46, 89.25, 65.28, 92.60, 72.05, 74.63, 75.01, 75.39, 75.48, 75.35, 51.43, 74.84, 75.28, 73.55, 71.86, 79.49, 62.60, 71.23, 71.33, 72.90, 77.59, 67.98, 29.86, 20.95, 27.08, 36.95, 17.35; HR MALDI-TOF MS: m/z: calcd for $C_{64}H_{70}Cl_3NO_{14}SeSi$ $[M+Na]^+$: 1273.2847; found: 1273.2849.

Allyl-*O*-(3,4,6-tri-benzyl-2-*O*-acetyl-*O*-β-D-glucopyranosyl)-(1→3)-(6-*O*-Benzyl-4-*O*-hydroxyl-2-deoxy-2-trichloroacetamido-*O*-β-D-galactopyranosyl)-(1→3)-4,6-*O*-di-benzyl-2acetyl-1-α-D-mannopyranoside (24)

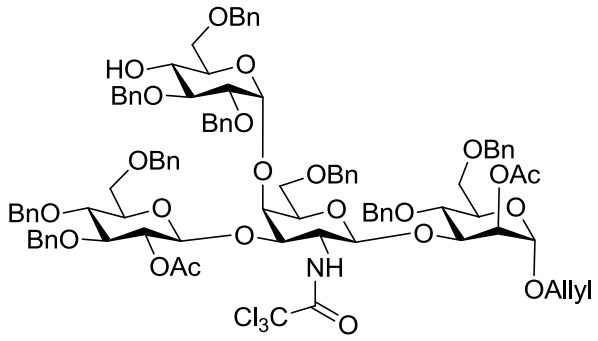


24

Compound **23** (385 mg, 0.33 mmol) and **18** (176 mg, 0.40 mmol) were dissolved in DCM (10 mL) and then flame dried molecular sieve (4Å) was added. The mixture was placed under an atmosphere of argon at room temperature, followed by cooling to -15 ℃.

TMSOTf (18 µL, 0.10 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature over a period of 1 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (10 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The crude product was re-dissolved in DCM (5 ml), AllylOH (0.1 ml, large excess) and NH_2NH_2-AcOH (45.9 mg, 0.49 mmol) were added to the reaction solution. The reaction was continued for 18h and quenched with Aceton (1 ml) and washed with saturated $NaHCO_3$ solution (10 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified silica gel chromatography (EtOAc/ Hexane, 0% to 40%, v/v) to afford compound **24** (321 mg, 74%). R_f =0.25 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.15 (m, 30H, Ar), 5.83-5.75 (m, 1H, CH, Allyl), 5.23-5.12 (m, 3H, H^{II}-1, Allyl-CH_2), 5.02-4.95 (m, 3H, CHC*H*, H^{II}-1, H^{I}-2), 4.85 (d, 1H, H^{III}-1), 4.80-4.32 (m, 13H, C*H*CH, 5*CH_2, H^{I}-1, H^{III}-3), 4.19-4.08 (m, 2H, H^{III}-4, Allyl-C*H*CH), 3.95-3.91 (dd, 1H, Allyl-CHC*H*), 3.85-3.41 (m, 14H, H^{II}-5, H^{II}-2, H^{II}-3, H^{I}-4, H^{III}-5, H^{II}-4, H^{I}-3, H^{I}-5, H^{II}-6_{ab}, H^{I}-6_{ab}, H^{III}-6_{ab}), 2.92 (s, 1H, OH), 2.13 (s, 3H, Ac) 1.97 (s, 3H, Ac); ^{13}C NMR (126 MHz, $CDCl_3$) δ 128.11, 128.15, 127.95, 129.82, 129.27, 127.99, 133.32, 117.92, 69.87, 117.87, 117.91, 117.91, 97.96, 72.84, 74.76, 74.75, 96.63, 74.97, 74.79, 74.60, 100.76, 75.04, 73.70, 75.04, 73.56, 73.45, 73.50, 73.55, 73.71, 74.62, 73.59, 74.52, 76.70, 76.94, 73.52, 73.49, 67.54, 68.17, 68.19, 73.95, 71.52, 55.95, 68.92, 77.68, 77.64, 73.44, 68.78, 68.64, 82.69, 82.66, 82.66, 74.88, 68.71, 21.23, 21.61, 19.76, 21.27, 19.43; HR MALDI-TOF MS: m/z: calcd for $C_{69}H_{76}Cl_3NO_{18}$ $[M+Na]^+$: 1311.4128; found: 1311.4131.

Allyl-*O*-(3,4,6-*O*-tri-benzyl-2-*O*-acetyl-*O*-β-D-glucopyranosyl)-(1→3)-[2,3,6-tri-*O*-benzyl-4-hydroxyl--α-D-glucopyranosyl-(6-*O*-benzyl-4-*O*-hydroxyl-2-deoxy-2-trichloroacetamido-*O*-β-D-galactopyranosyl)-(1→3)-4,6-*O*-di-benzyl-2acetyl-1-α-D-mannopyranoside (25)

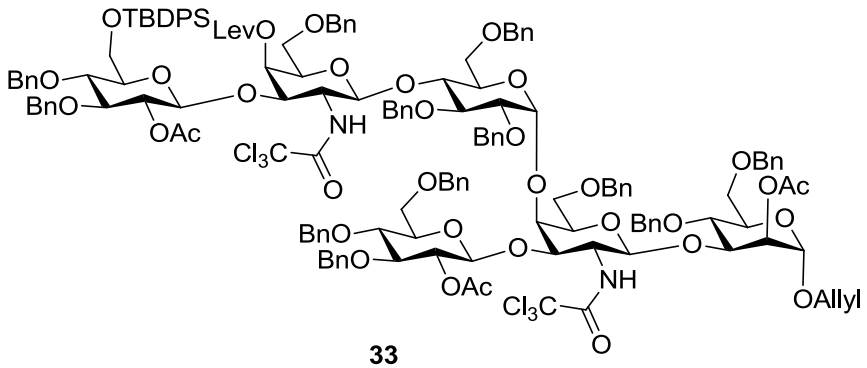


25

Compound **24** (302 mg, 0.23 mmol) and **19** (331 mg, 0.46 mmol) were dissolved in DCM/Et_2O (5 mL, v/v= 1:4) and then flame dried molecular sieve (4Å) was added. The mixture was placed under an atmosphere of argon at room temperature, followed by cooling to -45 ℃. TMSOTf (12 μL, 0.069 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature over a period of 3 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (1 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The crude product was re-dissolved in DCM (5 ml), AllylOH (0.1 ml, large excess) and NH_2NH_2-AcOH (45.9 mg, 0.49 mmol) were added to the reaction solution. The reaction was continued for 18h and quenched with Aceton (1 ml) and washed with saturated $NaHCO_3$ solution (10 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid

was purified silica gel chromatography (EtOAc/ Hexane, 0% to 50%, v/v) to afford compound **25** (361 mg, 74%). R_f = 0.34 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37-7.11 (m, 46H, Ar, NH), 5.81-5.75 (m, 1H, Allyl- CH), 5.22-5.11 (m, 3H, Allyl-CH_2, H^{III}-2), 50.5-5.04 (d, 1H, H^{IV}-1, J=3.01) 4.98-4.96 (d, 1H, H^{II}-1, J=8.2) 4.94-4.85 (m, 3H, CH_2, H^{III}-1) 4.80-4.70 (m, 3H, H^{I}-2, CH_2) 4.61-4.09 (m, 20H, 7CH_2, Allyl-C*H*CH, H^{IV}-5, H^{IV}-4, H^{III}-4, H^{II}-4, H^{I}-1), 3.95- 3.21 (m, 19H, Allyl-CHC*H*, H^{II}-2, H^{I}-4, H^{I}-5, H^{I}-3, H^{IV}-2, H^{IV}-3, H^{III}-5, H^{III}-3, H^{II}-5, H^{II}-3), 2.06 (s, 3H, Ac), 1.95 (s, 3H, Ac).^{13}C NMR (126 MHz, $CDCl_3$) δ 127.89, 130.14, 128.00, 77.35, 125.90, 127.95, 129.96, 127.77, 129.72, 133.26, 117.89, 70.16, 117.85, 117.87, 96.93, 98.75, 74.57, 96.66, 75.04, 77.77, 72.35, 94.83, 96.58, 72.99, 75.07, 74.97, 73.27, 73.50, 73.45, 73.37, 71.51, 100.99, 73.82, 70.82, 77.24, 72.20, 73.21, 73.15, 73.15, 68.44, 68.11, 68.36, 69.20, 74.08, 81.80, 71.52, 56.91, 69.15, 68.67, 73.93, 68.40, 82.15, 78.00, 79.45, 75.26, 67.77, 21.46, 21.17, 21.28, 29.84, 29.65; HR MALDI-TOF MS: m/z: calcd for $C_{96}H_{104}Cl_3NO_{23}$ $[M+Na]^+$: 1744.6098; found: 1744.6098.

Allyl-*O* 2-*O*-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→3)-[6-*O*-tertbutyldiphenylsilyl-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4-levulinic-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl- β-D-mannopyranoside (33)

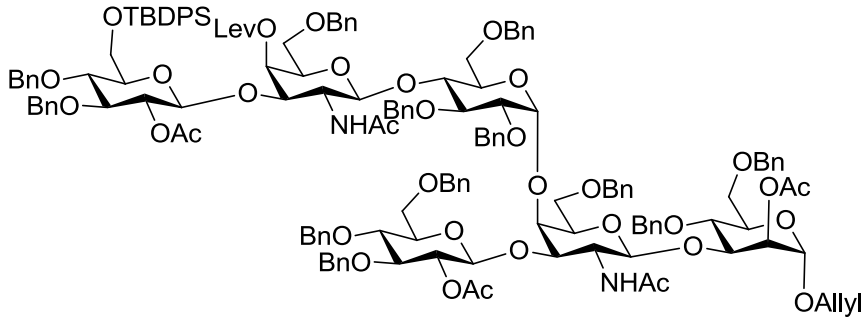


33

Compound **25** (300 mg, 0.17 mmol) and **32** (350 mg, 0.27 mmol) were dissolved in DCM (5 mL) and then flame dried molecular sieve (4Å) was added. The mixture was placed under an atmosphere of argon at room temperature, followed by cooling to -30 ℃. TMSOTf (12 μL, 0.052 mmol) was added drop wise and the reaction mixture was allowed to warm to room temperature over a period of 5 h. The reaction was quenched with aq. saturated $NaHCO_3$ solution (1 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified silica gel chromatography (EtOAc/ Hexane, 0% to 50%, v/v) to afford compound **33** (430 mg, 87%). R_f = 0.37 (EtOAc/Hexane, 30%, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67-6.98 (m, 65H, Ar), 5.82-5.77 (m, 1H, Allyl), 5.51-5.49 (m, 1H, H^V-4), 5.22-5.12 (m, 3H, Allyl-CH_2, H^{VI}-1), 5.04-5.01 (m, 1H, H^I-2), 4.99-4.96 (m, 1H,Bn-C*H*CH), 4.95-4.93 (m, 2H, H^{VI}-2, H^{IV}-1), 4.88-4.86 (m, 2H, H^{III}-2, H^V-1), 4.85-4.80 (m, 2H, H^I-1, Bn-CHC*H*), 4.75-4.28 (m, 39H, 17×CH_2, Bn- C*H*CH, H^{II}-1, H^{II}-1, H^V-3, H^I-3), 4.28-4.26 (m, 1H, H^{VI}-3), 4.20 (s, 1H, H^{IV}-4), 4.15-4.01 (m, 3H, Bn-CHC*H*, H^V-5, H^{II}-3), 3.94-3.52 (m, 17H, H^{IV}-5, H^{III}-5, H^I-4, H^{II}-4, H^{III}-4, H^{VI}-5, H^{III}-4, H^{VI}-4, H^{IV}-2, H^I-6ab, H^{II}-6ab, H^{III}-6ab, H^{IV}-6a, H^V-6ab), 3.51-3.32 (m, 7H, , H^{IV}-6b, H^{VI}-6ab, H^V-1, H^{II}-5, , H^{III}-3, , H^{IV}-3), 2.73-2.34 (m, 4H, Lev), 2.13-1.88 (m, 12H, CH_3), 1.65 (s, H_2O),

1.01 (s, 9H, TBDPS); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.85, 135.58, 128.23, 127.84, 127.94, 127.33, 64.89, 92.42, 70.42, 74.74, 72.19, 99.31, 73.36, 96.13, 72.56, 74.77, 99.10, 75.09, 97.99, 75.06, 75.10, 148.69, 11.69, 73.46, 73.46, 75.31, 74.89, 74.89, 75.14, 75.08, 75.05, 75.14, 73.51, 72.64, 100.73, 73.07, 74.90, 74.73, 72.80, 73.47, 74.79, 71.91, 74.76, 73.46, 75.37, 77.13, 77.85, 70.14, 70.38, 71.16, 63.09, 79.66, 68.35, 73.76, 62.26, 79.93, 71.33, 55.58, 68.51, 77.41, 74.20, 71.86, 68.90, 82.89, 74.98, 67.40, 71.94, 68.49, 67.38, 21.43, 29.79, 20.91, 25.00, 21.39, 29.71, 20.84, 26.87; HR MALDI-TOF MS: m/z: calcd for $C_{154}H_{168}Cl_6N_2O_{36}Si$ $[M+Na]^+$: 2861.9281; found: 2861.9285.

Allyl-*O* 2-*O*-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→3)-[6-*O*-tertbutyldiphenylsilyl-2-*O*-acetyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-4-levulinic-6-*O*-benzyl-2-deoxy-2-(2,2,2-acetamido)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-6-*O*-benzyl-2-deoxy-2-(2,2,2-acetamido)-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl- β-D-mannopyranoside (34)



34

Compound **33** (150 mg, 0.052 mmol) was dissolved in toluene (5 mL). The reaction solution was placed under an atmosphere of argon at room temperature, followed by heating to 75 ℃. Cs_2CO_3 (85 mg, 0.262 mmol) was added and the reaction mixture was stirred over a period of 1.5 h. The reaction was monitored by MALDI and then Ac_2O (1 ml) was added and the reaction mixture was stirred over 18 h. The reaction was then quenched with aq. saturated $NaHCO_3$ solution (1 mL). The organic phase was washed by brine (10 mL), dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The resulting yellow solid was purified silica gel chromatography (EtOAc/ Hexane, 0% to 80%, v/v) to afford compound **34** (101 mg, 72%). R_f = 0.33 (EtOAc/Hexane, 60%, v/v), HR MALDI-TOF MS: m/z: calcd for $C_{154}H_{174}N_2O_{36}Si$ $[M+Na]^+$: 2656.1649; found: 2656.1649.

Reference

(1) Knoop, F. C.; Owens, M.; Crocker, I. C. *Clinical Microbiology Reviews* **1993**, *6*, 251.

(2) Rupnik, M.; Wilcox, M. H.; Gerding, D. N. *Nature Reviews Microbiology* **2009**, *7*, 526.

(3) Loo, V. G.; Poirier, L.; Miller, M. A.; Oughton, M.; Libman, M. D.; Michaud, S.; Bourgault, A.-M.; Nguyen, T.; Frenette, C.; Kelly, M. *New England Journal of Medicine* **2005**, *353*, 2442.

(4) McDonald, L. C.; Killgore, G. E.; Thompson, A.; Owens Jr, R. C.; Kazakova, S. V.; Sambol, S. P.; Johnson, S.; Gerding, D. N. *New England Journal of Medicine* **2005**, *353*, 2433.

(5) Barbieri, J. T.; Riese, M. J.; Aktories, K. *Annual review of cell and developmental biology* **2002**, *18*, 315.

(6) Vardakas, K. Z.; Polyzos, K. A.; Patouni, K.; Rafailidis, P. I.; Samonis, G.; Falagas, M. E. *International journal of antimicrobial agents* **2012**, *40*, 1.

(7) (a)Fernie, D.; Thomson, R.; Batty, I.; Walker, P. *Developments in biological standardization* **1982**, *53*, 325; (b)Giannasca, P.; Zhang, Z.; Lei, W.; Boden, J.; Giel, M.; Monath, T.; Thomas Jr, W. *Serum antitoxin antibodies mediate systemic and mucosal protection from Clostridium difficile disease in hamsters. Infect. Immun*, *67*, 527; (c)Kim, P.-H.; Iaconis, J. P.; Rolfe, R. D. *Infection and immunity* **1987**, *55*, 2984.

(8) (a)Fekety, R.; McFarland, L. V.; Surawicz, C. M.; Greenberg, R. N.; Elmer, G. W.; Mulligan, M. E. *Clinical infectious diseases* **1997**, *24*, 324; (b)O'neill, G.; Beaman, M.; Riley, T. *Epidemiology and infection* **1991**, *107*, 627.

(9) (a)Cywes, C.; Wessels, M. R. *Nature* **2001**, *414*, 648; (b)Graveline, R.; Segura, M.; Radzioch, D.; Gottschalk, M. *International immunology* **2007**, *19*, 375.

(10) (a)Oberli, M. A.; Hecht, M.-L.; Bindschädler, P.; Adibekian, A.; Adam, T.; Seeberger, P. H. *Chemistry & biology* **2011**, *18*, 580; (b)Danieli, E.; Lay, L.; Proietti, D.; Berti, F.; Costantino, P.; Adamo, R. *Organic letters* **2010**, *13*, 378.

(11) Adamo, R.; Romano, M. R.; Berti, F.; Leuzzi, R.; Tontini, M.; Danieli, E.; Cappelletti, E.; Cakici, O. S.; Swennen, E.; Pinto, V. *ACS chemical biology* **2012**, *7*, 1420.

(12) Zhang, W. *Chemical reviews* **2009**, *109*, 749.

(13) Zhang, W. *Current opinion in drug discovery & development* **2004**, *7*, 784.

(14) Adamo, R.; Romano, M. R.; Berti, F.; Leuzzi, R.; Tontini, M.; Danieli, E.; Cappelletti, E.; Cakici, O. S.; Swennen, E.; Pinto, V.; Brogioni, B.; Proietti, D.; Galeotti, C. L.; Lay, L.; Monteiro, M. A.; Scarselli, M.; Costantino, P. *Acs Chemical Biology* **2012**, *7*, 1420.

(15) (a)Hogendorf, W. F.; Gisch, N.; Schwudke, D.; Heine, H.; Bols, M.; Pedersen, C. M. *Chemistry-A European Journal* **2014**, *20*, 13511; (b)Higson, A. P.; Ross, A. J.; Tsvetkov, Y. E.; Routier, F. H.; Sizova, O. V.; Ferguson, M. A.; Nikolaev, A. V. *Chemistry-A European Journal* **2005**, *11*, 2019.

(16) Li, Z.; Gildersleeve, J. C. *Journal of the American Chemical Society* **2006**, *128*, 11612.

(17) Park, J.; Kawatkar, S.; Kim, J.-H.; Boons, G.-J. *Organic letters* **2007**, *9*, 1959.

(18) Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H. *Angewandte Chemie International Edition* **2013**, *52*, 5858.

(19) Nitz, M.; Bundle, D. R. *The Journal of organic chemistry* **2001**, *66*, 8411.

(20) Nishikawa, T.; Urabe, D.; Tomita, M.; Tsujimoto, T.; Iwabuchi, T.; Isobe, M. *Organic letters* **2006**, *8*, 3263.

CONCLUSION

We established a set of strategies that integrated the orthogonal protecting groups, glycosyl donors modified by a (*S*)-phenylthiomethylbenzyl ether at C-2 and fluorous tag-assisted solution phase synthesis into the synthesis of *Trypanosoma brucei* hexasaccharide moiety. The rapid preparation of the complex branched carbohydrates with biological importance is available. The reaction conditions for C-2 auxiliary controlled the 1,2-*cis* galactosylation are investigated. Nap and Lev were installed as the orthogonal protecting groups to generate glycosyl acceptors and served as the branch spots. Once the glycosylation reaction is completed, the C-2 auxiliary can be selectively removed under acidic condition but kept the Lev and Nap groups, which made the immediate installation of 1,2-*cis* linkage possible. Also, we investigated the light fluorous tag assisted purification process by using fluorocarbons modified silica gel. An exploratory study was required to identify a potential problem during the synthesis. For example, the efficiency of auxiliary-mediated glycosylation is effected by the available space around hydroxyl group of the acceptor. In one case, we have to use a conventional donor to install the glycosylic donor. After the careful investigation of the oligosaccharide, a rapid carbohydrate assembly was performed without conventional silica gel chromatography.

In another part of this dissertation, the synthesis of hexasaccharide from *Clostridium difficile* was investigated. Five possible synthetic routes for the assembly of the oligosaccharide were tested. Ether was used in the control of the stereoselectivity. By

using phenyldiselenide as the anomeric protection group, the unfavored aglycon transfer was prevented. A new method to remove the trichloroacetyl amine protecting group by using cesium carbonate was established in the synthesis. Moreover, a reliable route to synthesize the hexasaccharide was confirmed.